UNIVERSIDADE DA BEIRA INTERIOR
Faculdade de Ciências

Synthesis, characterization and anti-oxidant activity of Thio-imidate N-oxides (TIO) sugars

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Dedicatória

Dedico este trabalho ao meu avô Jerónimo Pinheiro Domingues

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Resumo

A função N-óxido de tio-imidato é uma função rara e original. O objectivo deste trabalho foi a síntese, caracterização e determinação da actividade antioxidante de um composto derivado da D-ribose, o N-óxido de tio-imidato, assim como de todos os compostos sintetizados.

A síntese do N-óxido de tioimidato **8**, foi efectuada por duas sequências diferentes de reacções a partir da D-ribose. A primeira sequência de reacções iniciou-se com a protecção dos grupos hidroxilo das posições 2 e 3 da D-ribose utilizando o grupo isopropilideno como grupo protector. A redução, seguida de clivagem oxidativa permite obter a 2,3-O-isopropilideno-L-eritrose (**2**) com 58% de rendimento, que após tratamento com cloreto de hidroxilamina originou a aldoxima **3** com 73% de rendimento. A protecção do grupo hidroxilo da posição 4 com o grupo *ter*-butildimetilsilílico foi efectuada por reacção da aldoxima **3** com cloreto de *ter*-butildimetilsililo, tendo-se obtido o éter sililico **4** com 19% de rendimento e o éter sililico **5** com 63% de rendimento. A introdução da função tio-hidroximato foi efectuada por reacção do composto **4** ou por reacção do composto **5** com etanotiol e tri-etilamina, originando o composto **6** com 63% e 18% de rendimento respectivamente. O grupo hidroxilo da posição 4 foi desprotegido por reacção com difluortrifenilsilicato de tetrabutilamónio (TBAT) dando origem ao composto **7**, com 40% de rendimento. A ciclização foi efectuada por reacção de Mitsunobu, obtendo-se o N-óxido de tioimidato (**8**) com 50% de rendimento na forma de um sólido cristalino branco.

Iniciou-se a segunda sequência reaccional com a protecção dos grupos hidroxilo 1, 2 e 3 da D-ribose, com metanol e acetona respectivamente, em meio ácido. A substituição nucleófilica do grupo hidroxilo desprotegido (posição 4) foi realizada com trifenilfosfina, iodo e imidazol, originando o desoxi-iodo açúcar **10** com 71% de rendimento. Seguidamente efectuou-se a abertura do anel de açúcar com butil lítio a -78ºC, obtendo-se aldeído **11**, que foi utilizado, sem ser isolado, para a reacção de obtenção do álcool **12,** tendo este sido obtido com 70% de rendimento. Este composto foi protegido com cloreto de terbutildimetilsililo, originando o composto **13**, com 70% de rendimento na forma de um líquido incolor.

Os compostos obtidos foram isolados e purificados por cromatografia em coluna. A análise da sua estrutura foi efectuada por espectroscopia de Infravermelho, de Ressonância Magnética Nuclear (RMN) de protão (^{1}H RMN) e de carbono treze (^{13}C RMN). Assim como por espectrometria de massa. Foram também determinados os pontos de fusão (dos compostos sólidos) e os poderes rotatórios específicos dos compostos isolados.

Foi realizada a determinação da actividade antioxidante para os compostos **3**, **5**, **6**, **7**, **8**, **10** e **11** utilizando o método do radical DPPH (2,2-difenil-1-picril-hidrazilo), baseado na capacidade

que este radical tem em reagir com doadores de hidrogénio para conhecimento da sua actividade antioxidante, utilizando como referência o antioxidante sintético comercialmente conhecido como Trolox.

Dos compostos analisados a aldoxima **3**, o desoxi-iodo-açúcar **10** e o éter sililico **13** apresentam actividade antioxidante. O composto **13** tem uma actividade antioxidante de 68%, seguido da aldoxima **3** com uma actividade antioxidante de 60% e por último o desoxi-iodo-açúcar **10** com uma actividade de 56%. Os restantes compostos analisados apresentam actividade antioxidante residual.

O dexosi-iodo-açúcar **10** apresenta uma concentração eficiente de 8084μg/mL para uma concentração inicial de 16500μg/mL e uma concentração eficiente de 8586μg/mL para uma concentração inicial de 12530μg/mL, o que demonstra ser um potencial antioxidante.

Foi possivel obter o N-óxido de tio-imidato pela primeira sequência de reacções, o seu ponto de fusão é 139-143 ºC, o seu poder rotatório é de 143 a sua actividade antioxidante é de 34%.

Palavras-chave

Hidratos de carbono, derivados de açúcar, N-óxidos de tio-imidate, desoxi-iodo-açúcares, actividade antioxidantes.

Abstract

The thioimidate N-oxide function is a rare and original function. The objective of this work is the synthesis of heterocyclic rings linked to the sugar moiety by a C-C bond and the characterization of obtained compounds. Regio and stereoselective branched-chain construction starting from D-ribose which led to the synthesis of thio-imidate N-oxide sugar derivates.

The synthesis of compounds was made by several reactions, starting from D-ribose. The protection of D-ribose by isopropylidene group allows us to obtain the 2,3-O-isopropylidene-ß-D-ribofuranose (**1**). The reduction and oxidative cleavage of the 2,3-O-isopropylidene-ß-D-ribofuranose allows to obtain the 2,3-O-isopropylideno-L-erithrose (**2**), the ring opening with hydroxylamine hydrochloride lead to aldoxime **3**. Protection of **3** with tert-butyldimethylsilyl group lead to compounds **4** and **5**. The introduction of the thiohydroximate function was made with ethanethiol and triethyhamine. The thiohydroximate cyclized to generate efficiently the thio-imidate N-oxide by Mitsunobu reaction.

The protection of D-ribose by methyl group and isopropylidene group lead to compound **9**. The ionic deoxygenation of the hydroxyl group in the compoud **9** allows obtaining the deosy-iodenesugar **10**. The deosyiodine-sugar after reductive elimination leads to aldehyde **11**. Compound **11** undergoes reduction to generate **12** which lead to compound **13**.

The obtained compounds were isolated and purified by column chromatography. The characterization of compounds was made by NMR analysis (^{1}H NMR, and ^{13}C NMR), infrared spectrum and by mass spectroscopy. The anti-oxidant activities were also evaluated for some obtained compounds by DPPH method.

Determination of antioxidant activity of some compounds was made by the method of DPPH radical (2,2-diphenyl-1-picrylhydrazyl) based on the ability of this radical has to react with hydrogen donor for knowledge of their antioxidant activity, using as a reference commercially product known as Trolox.

Compounds **3**, **10** and **13** show the antioxidant activities of 60%, 56% and 68% respectively. The remaining compounds just demonstrate residual antioxidant activity.

Keywords

Carbohydrates, sugar derivatives, thio-imidate N-oxides, deoxy-iodine-sugar, synthesis, anti-oxidant activities.

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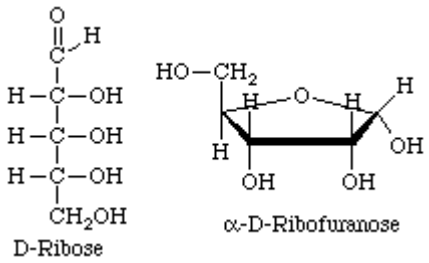
Acronym list

EA	Ethyl Acetate
PE	Ether Petrole
COSY	Spectroscopy Correlation
Éq	Equivalent(s)
MeOH	Methanol
EtOH	Ethanol
NMR	Nuclear Magnetic Resonnance
THF	Tetrahydrofurane
UV	Ultra-violet
Rf	Retention factor
DMSO	Dimethyl sulfoxide
BuOH	Buthanol
DMF	Dimethylformamide
NCS	*N*-Cholorosuccinimide
TBAT	Tetrabutylammonium triphenyldifluorosilicate
DEAD	Diethyl azodicarboxylate
Molecular Sieve	MS
Hz	Hertz
M.S	Mass spectroscopy
λ	Wavelength
RNA	Ribonucleic acid
ROS	Reactive oxygen species
r.t	Room temperature
Superoxide dismutase	SOD
Glutathione peroxidase	GPx
Catalase	Cat
AA	Antioxidant activity

Chapter 1

Introduction

The term “carbohydrate” was originally introduced in the 19th century because the molecular formulae of the simpler carbohydrates were show to be of the form $C_nH_{2n}O_n$ or $C_n(H2O)_n$ – hence “hydrate of carbon”. Many of these compounds were sweet, and the term “sugar” was also adopted. The carbohydrates are among the most constituents of plants and animals. These simple sugars or “monosaccharides” of known molecular weight were shown to be polyhydroxy-aldehydes or polyhydroxy-ketones, the interconversions are possible and are typical of the experiments carried out which provided evidence for these functionalities.[1]
Of the simple sugars, glucose and ribose are probably the most important. The former occurs as the monomeric unit in numerous polysaccharides (cellulose, starch and glycogen) but is also a common constituent of secondary metabolites. D-Ribose is key constituents of the essential ribonucleic acids (RNA).[1]



Picture 1 - Acyclic form and cyclic form of the D-ribose

Many natural products contain sugars in structurally modified forms. Those sugars have carboxylic acid groups while others have amino groups in place of specific hydroxyl groups or have undergone more substantial change in having branched instead of linear carbon chains in their structures. The combinations of these modifications produce compounds with specific properties. These modifications allow obtain planning antibiotics, anti-tumor compounds and anti-oxidant compounds. The synthesis and biosynthesis of these sugars are currently areas of active investigation.[2]

1. Protection of the Hydroxyl groups

Due to the multifunctional nature of the sugars, it becomes necessary to introduce protecting groups from hydroxyl functions when trying to perform a chemical reaction in a certain position of the molecule, thus preventing the remaining hydroxyl groups present in the molecule also react under the applied reaction conditions.[3]

The protection of hydroxyl groups has had a wide application in the chemistry of sugars, including nucleosides[4], nucleotides and also in the chemistry of steroids.[5]
The protecting group should present the following characteristic:[5]

- Ease of introduction;
- Stability under the required reaction conditions;
- Ease of removal;

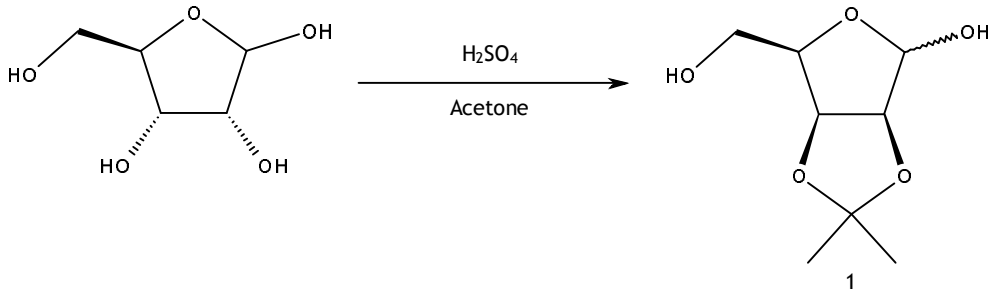
It must be also taken into account other considerations such as cost and toxicity of reagents used in the reaction, choosing always the most appropriate for the situation in cause. It is also necessary to ensure that the released protecting groups are stable under the reaction conditions used in the following steps.[3]

The sugars can be protected by conversion of hydroxyl groups in acetals, ketals, esters or ether as there is a wide variety of protecting groups, with different selectivity, allowing the formation of unwanted compounds.[3]

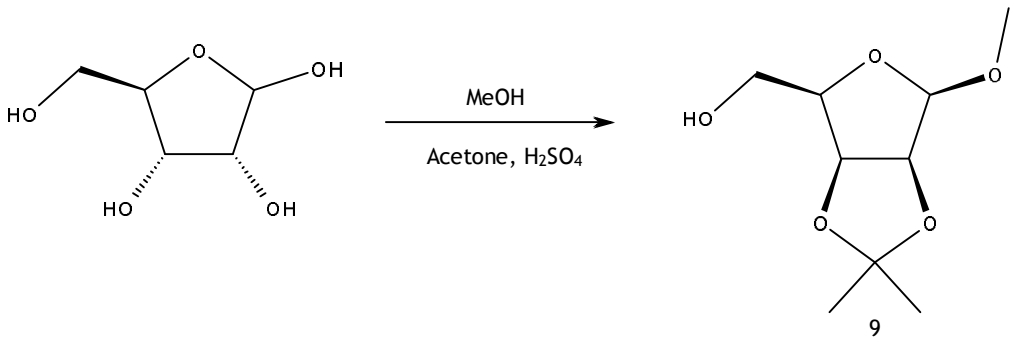
The protecting groups commonly used in chemistry of the sugars are the isopropylidene group, benzyl group, the trityl group, methyl group and tert-butyldimethylsilyl group. In this dissertation we will only use the isopropylidene group, methyl group and tert-butyldimethylsilyl group for protecting hydroxyl groups.[3]

1.1. Isopropylidene group

The most used acetal in protection of sugar is the one that results from the protection of two neighboring hydroxyl groups, and that is known as isopropylidene. Several reagents can be used for the formation of the isopropylidene group, such as acetone, pyridine, or methanol. The scheme 1 and 2 shows the protection of two neighboring hydroxyl groups.[2,6]



Scheme 1- D-ribose protection withe isopropylidene group.



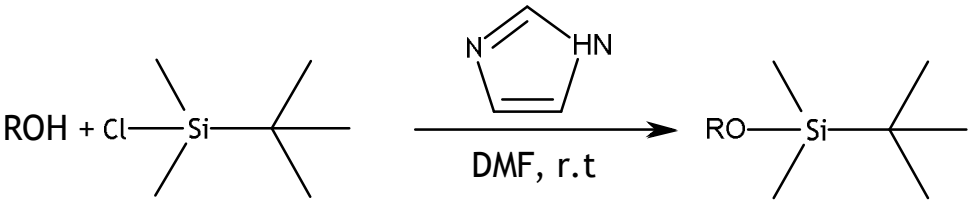
Scheme 2- D-ribose protection with isopropylidene goup and methyl group.

The isopropylidene group can be selectively introduced (protecting specific pairs of hydroxyl groups). In both cases, as shown in scheme 1 and 2 we will protect the hydroxyl groups 2 and 3. In the second scheme we can also observe the protection of the hydroxyl group of the anomeric carbon. The placement of a methyl group is also a possible and an easy way to protect an alcohol group. This protection was made simultaneously with the protection of hydroxyl groups 2 and 3.[6]

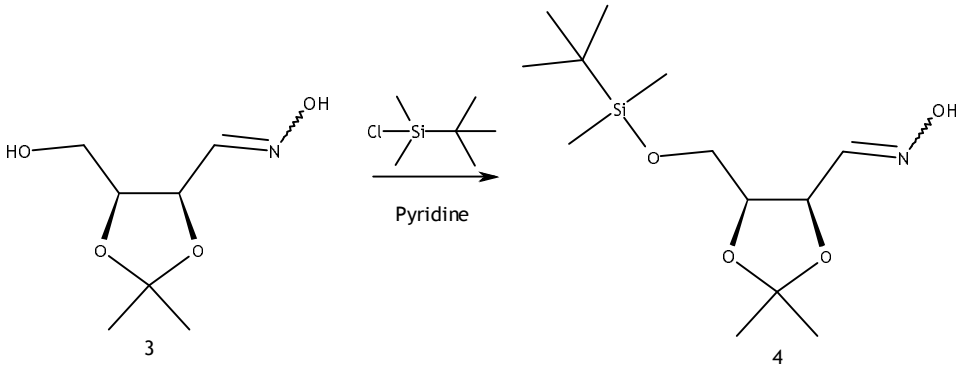
Removal of the isopropylidene group can be done in a simple way by performing a hydrolysis with an aqueous solution of a weak acid at room temperature. Acid hydrolysis breaks the isopropylidene group regenerating again two hydroxyl groups previously protected.[6]

1.2. Tert-butyldimethylsilyl group

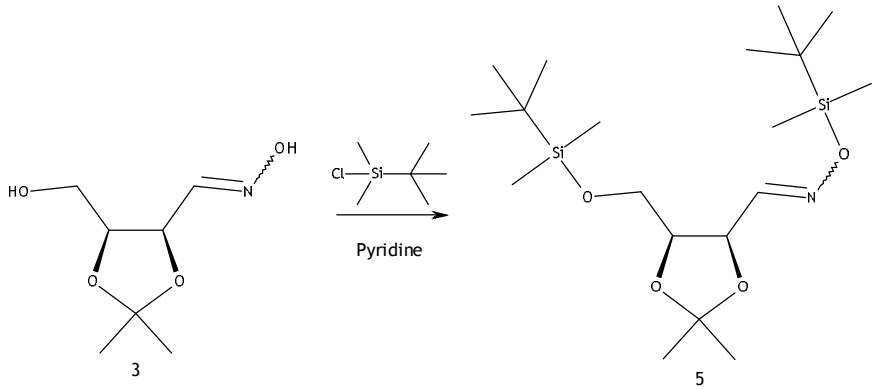
The hydroxyl group can also be protected by converting it to a silylated ether group, the most widely used protective agent is tert-butyldimethylsily. The tert-butyldimethylsilyl group is ca. 10^4 times more hydrolytically stable and holds more promise for such applications. The *tert*-butyldimethylsilyl chloride was used as a silylation agent. The tert-butyldimethylsilyl group can be added allowing the alcohol group to react with tert-butyldimethylsilyl in the presence of an aromatic amine (base) such as imidazole or pyridine according to the scheme 3. That procedure resulted in the conversion of various alcohols to *tert*-butyldimethylsilyl ethers in high yield.[7]



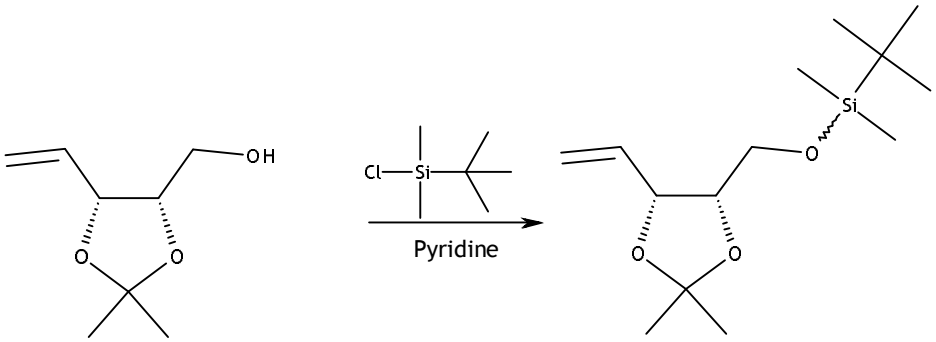
Scheme 3- Alcohol protection.



Scheme 4- Silylation of hydroxyl group in compound **3**.



Scheme 5- Silylation of hydroxyl group in compound **3**.



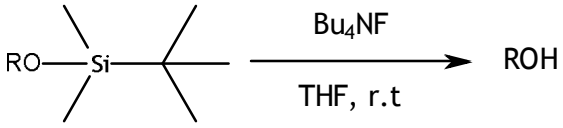
12 13

Scheme 6- Silylation of hydroxyl group in compound **12**.

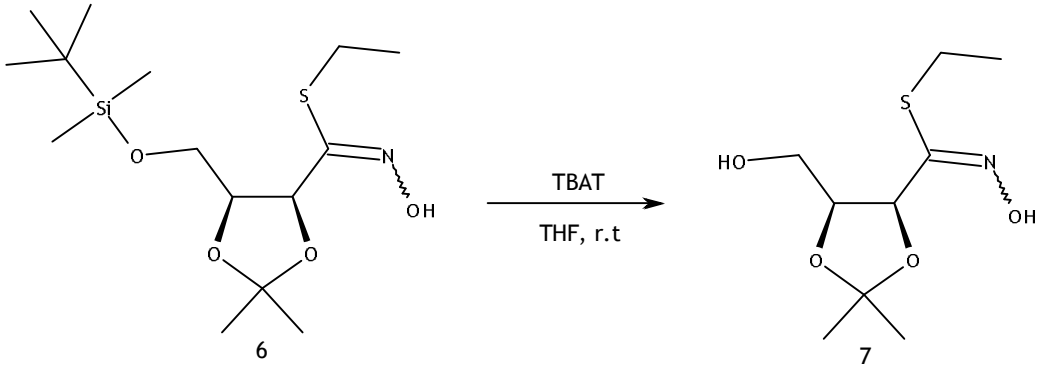
The conversion of an alcohol to a silylated ether makes it much more volatile. Given this fact the tert-butyldimethylsilyl group is also labile to be used as a protective group in many reactions.[8]

1.3. De-O-Silylation

The rapid cleavage of the silyl ethers to alcohols is given by treatment with tetra-n-butylammonium fluoride (TBAF) in THF at room temperature. Thus the tert-butyldimethylsilyl group can be removed according to the scheme 7. This is called the de-O-silylation.[7]



Scheme 7- De-o-Silylation.

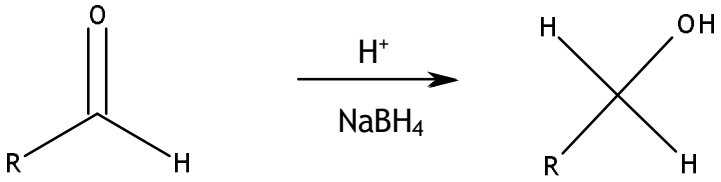


6 7

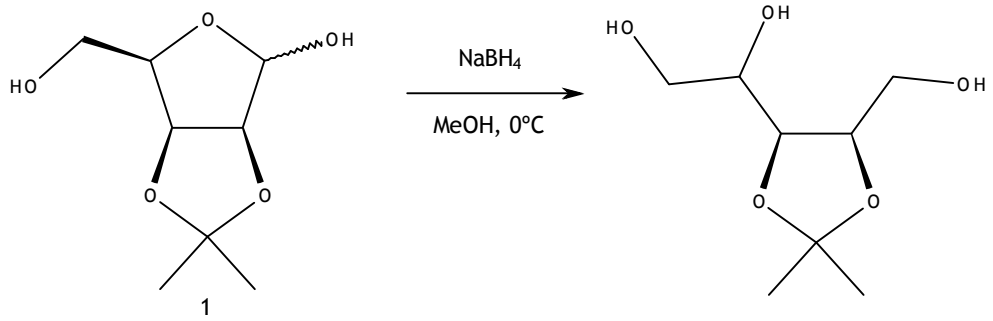
Scheme 8- Unprotection of the silyl ether **6**.

2. Reduction

While the acid-base reactions can be characterized as proton transfer processes, reactions known as oxidation-reduction (or redox) are considered electron transfer reactions. A reaction involving the gain of electrons is called the reaction of reduction. The reduction is a chemical change that occurs where there is a decrease of oxidation number of a specific chemical species. When an element is oxidized, it acts as a reducing agent, since it donates electrons to another substance, causing its reduction. When, however, one element is reduced, it acts as an oxidizing agent because it accepts electrons, causing its oxidation. A simple way of reducing a compound is treating it with $NaBH_4$ and MeOH at 0ºC or $NaBH_4$ and EtOH at 0ºC. The $NaBH_4$ reduces aldehydes to primary alcohols. [9]

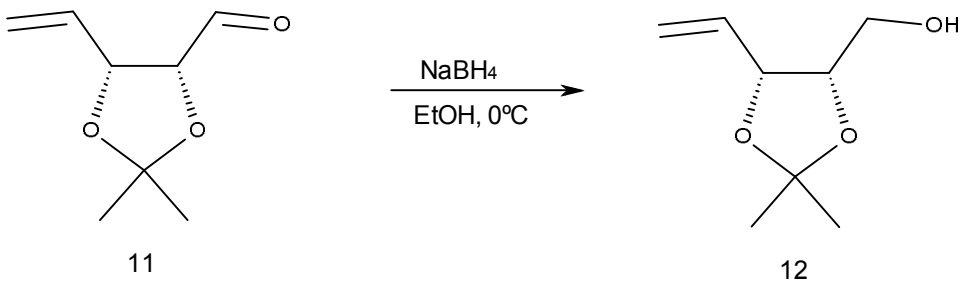


Scheme 9- Reduction of aldehydes to primary alcohols.



1

Scheme 10- Reduction of compound **1**.

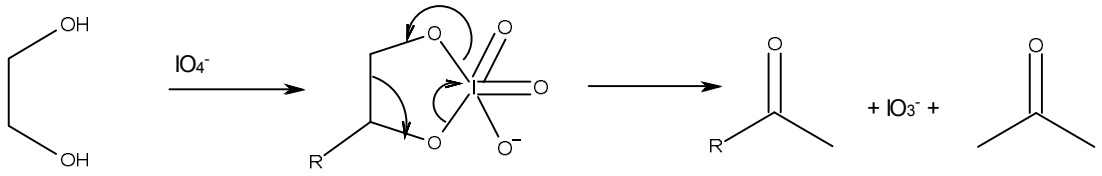


11 12

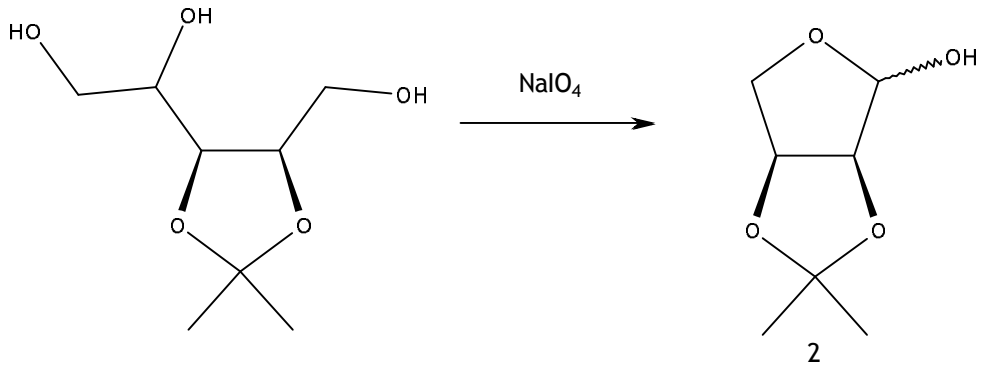
Scheme 11-Reduction of compound **11**.

3. Chain-shortening reactions: oxidative cleavage of α-diols

Several methods for cleaving the carbon chains of monosaccharides and thus producing 'lower' sugars. Reaction of α-diols with periodic acid H_5IO_6 or its salts (in this case with $NaIO_4$) gives rise to cyclic ester intermediates which cleave in a two-electron oxidation process to give two carbonyl products and iodate. This method was used to reduce the size of the carbon chain. The sodium periodate is often used in derivatives of carbohydrates. The proposed mechanism for oxidative cleavage is represented in Scheme 12.[10]



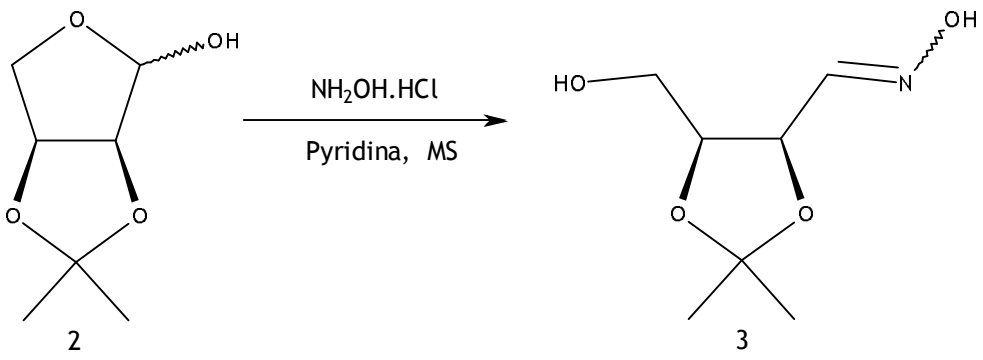
Scheme 12- Oxidative cleavage of α-diols.



Scheme 13- Chain – shortening of an intermediate compound.

4. Ring opening

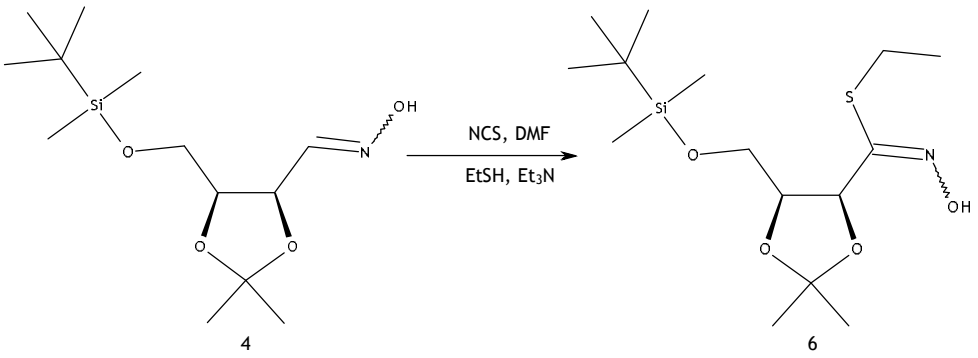
The ring opening is made using hydroxylamine hydrochloride, pyridine and activated molecular sieve. Thus we can transform the 2.3-O-isopropylidene-L-Erythrosis in 2.3-O-isopropylidene-L-oxime Erythrosis. [11,12]



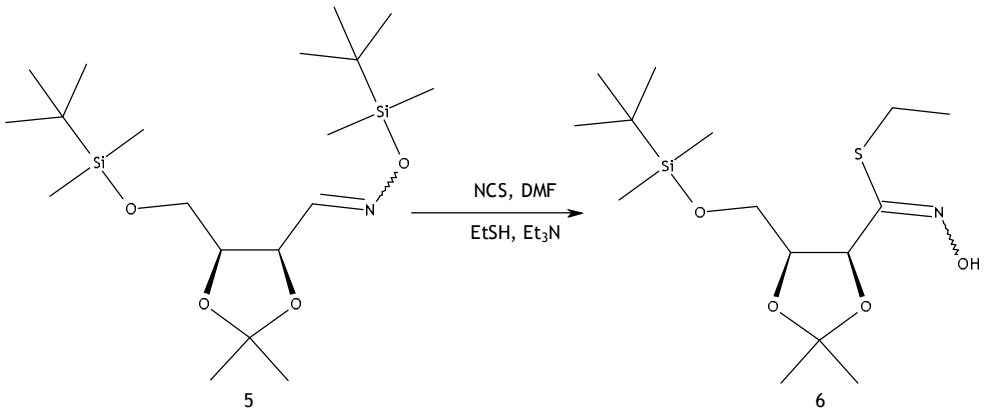
Scheme 14- Ring opening

5. Thiohydroximate function introduction

It is possible with relative ease the introduction of a thiohydroximate function, obtaining a good yield, a drawback of this procedure is the odour released during the addition of reagents, its easy propagation / dissipation and consequently its toxicity. Thus the thiohydroximate function can be introduced using NCS, DMF, ethanethiol and triethylamine.[13,14]



Scheme 15- Introduction of a thiohydroximate function.



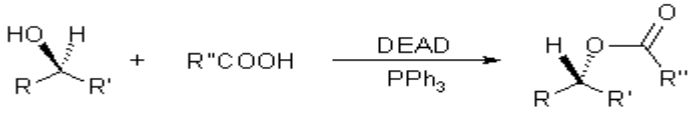
Scheme 16- Introduction of a thiohydroximate function.

6. Thioimidate N-oxides

The thioimidate N-oxide function is a rare and original function, of which little is known. The formation of thioimidate N-oxide can be controlled by avoiding direct attack to the electrophilic carbon. They chose to introduce the thiohydroximate function in one end of the chain and the activable group at the other end. There are two possibilites to convert thiohydroximate into a thioimidate N-oxide: halociclization using as activable group an alkenyl or a nucleophilic substitution under the conditions of the Mitsunobu reaction using as activable group an hydroxyl group.[15]

6.1. Mitsunobu reaction

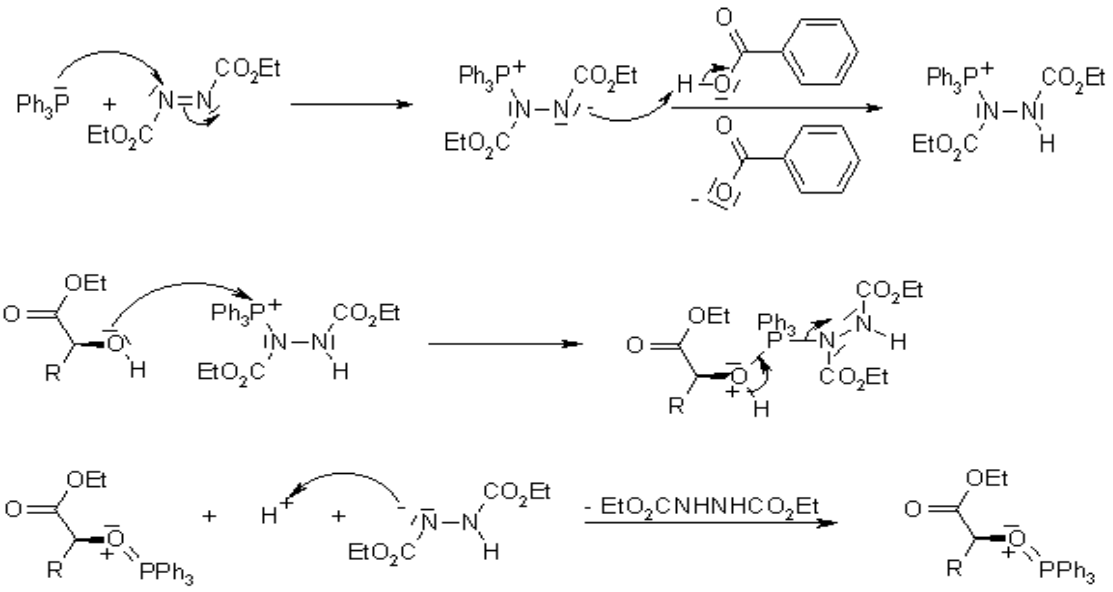
The Mitsunobu Reaction (scheme 17) allows the conversion of primary and secondary alcohols to esters, phenyl ethers, thioethers and various other compounds. Inter and intramolecular nucleophilic displacement of alcohols with inversion by means of diethyl azodicarboxylate (DEAD)-triphenylphosphine and a nucleophile. The nucleophile employed should be acidic, since one of the reagents (DEAD) must be protonated during the course of the reaction to prevent from side reactions. We were involved in the synthesis of cyclic sugar analogues.[15-23]



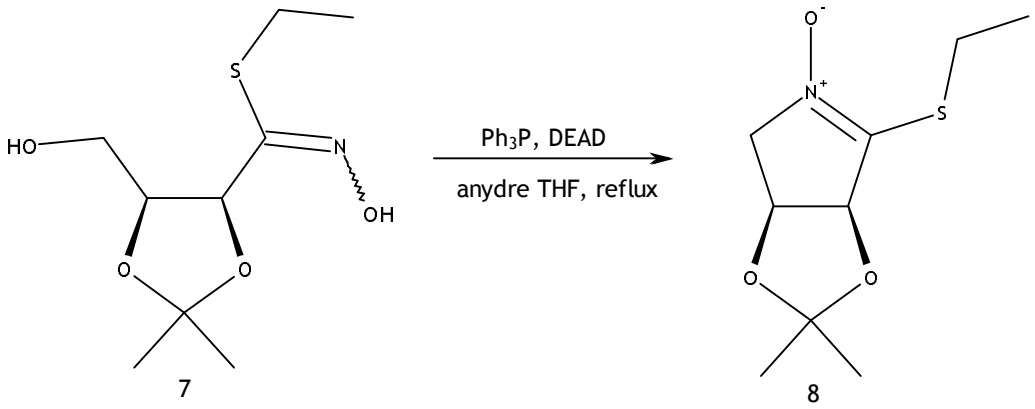
Scheme 17- Mitsunobu reaction.

The triphenylphosphine combines with DEAD to generate a phosphonium intermediate that binds to the alcohol oxygen, activating it as a leaving group. Substitution by the carboxylate, mercaptyl, or other nucleophile completes the process. The reaction proceeds with clean inversion, which makes the Mitsunobu Reaction with secondary alcohols a powerful method

for the inversion of stereogenic centers in natural product synthesis. The thioimidate N-oxide obtained is a stable compound, a thiohydroximate type with a double bond to improve the stability of the compound. Those compounds were stable at room temperature.The thioimidate N-oxide (TIO) function is a rare and original function. Imidates and thioimidates are important building blocks in organic synthesis, especially for heterocyclic compounds.[15-23]



Scheme 18- Synthesis scheme of Mitsunobu reaction.

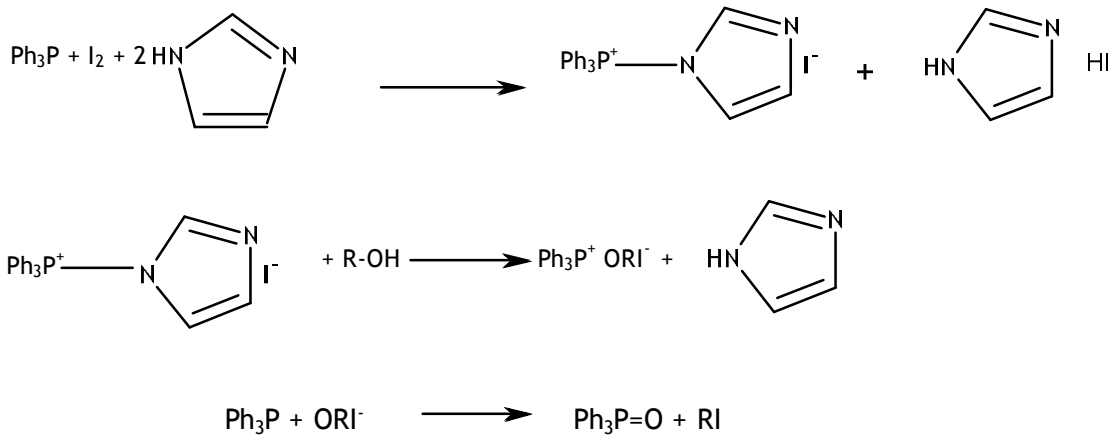


Scheme 19- Cyclization of compound **7**.

7. Ionic deoxygenation

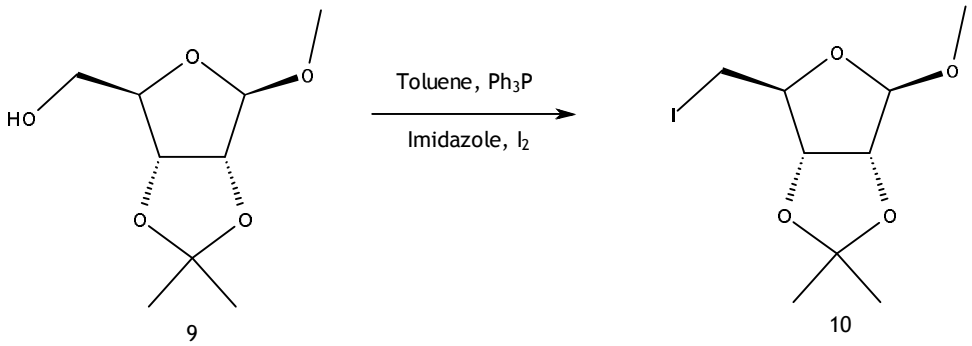
The ion deoxygenation always involves the formation of a deoxy-halogen-sugar. The deoxy-halogen-sugars are susceptible to nucleophilic attacks. The easiness of removability of a halide decreases in the order I> Br> Cl> F, in this work we will use the iodine.[2]

The method applied by Garegg Samuelsson consists essentially in a reaction between a free hydroxyl group and the triphenylphosphine / iodine / imidazole system using toluene as a solvent. The inversion of configuration occurs when there is replacement of the hydroxyl group by iodide ion. The same mechanism was proposed by the same researchers in 1980[24] which is presented in the scheme 20.[24]



Scheme 20- Method applied by Garegg Samuelsson.

The C-5 halogenation of the carbohydrate chain was carried out by this method resulting in a 5-iodo-deoxy-sugar.[24-28]

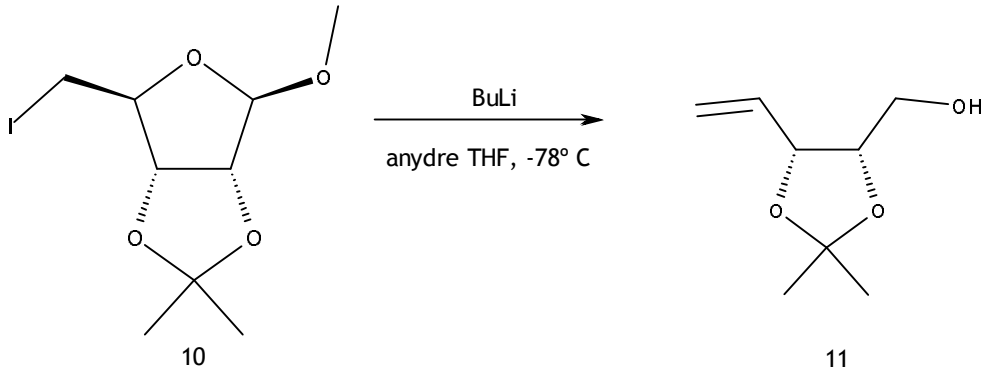


Scheme 21- Ionic deoxygenation of compound **9**.

8. Reduction of deoxy-sugars: reductive elimination

As the name implies reductive elimination involves the elimination or expulsion of a molecule from a transition metal complex. In the process of this elimination, the metal center is reduced by two electrons. In the simplest example below the metal goes from the x+2 to the x oxidation state and a coordinately unsaturated metal center is obtained.[25,29-31]

Reductive elimination is formally the microscopic reverse of oxidative addition, and it is not surprising that a series of reactions involving an oxidative addition, a rearrangement and then a reductive elimination form the basis for a variety of industrially important catalytic cycles.[25,29-31]



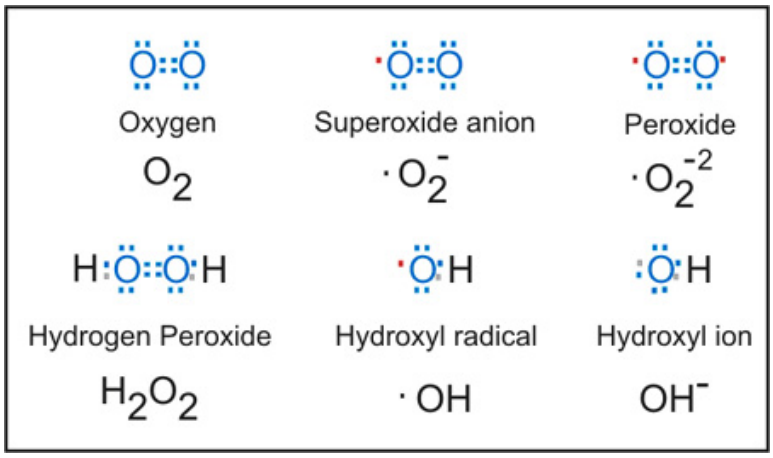
10 11

Scheme 22- Reductive elimination of compound **10**.

We tried to obtain the compound 11 by reacting compound 10 with zinc and methanol under reflux or zinc and anhydrous THF under reflux. In both cases it was possible to reach the desired aldehyde unsaured but the yield was so low that we chose to use the opening of the ring with butyllithium.[32]

9. Reactive oxygen species

Oxidation is a metabolic process that allows the cellular systems to convert potential energy of particles assimilate into usable energy and essential activities necessary for the cell. However, the metabolism of molecular oxygen in cells also causes the appearance of intermediates resulting from the partial reduction called by reactive oxygen species (ROS).[33]



Picture 2 - Reactive oxygen species

The imbalance between ROS production and detoxification mechanisms of these species in the human body, with a consequent increase in the concentration of free radicals in cells, leads to the development of a framework of oxidative stress[34], ie, the imbalance between oxidizing and anti-oxidants may result in the induction of cell damage by free radicals.[35] Thus the entropic control of biological processes at the cellular level requires a reducing environment that needs to be maintained with the use of numerous enzymatic anti-oxidants structures.[34]

9.1. Free radicals

Free radicals are produced naturally or by some biological dysfunction through oxidative and metabolic processes, and are often extremely useful, as in situations where there is need for activation of the immune system (macrophages use hydrogen peroxide to destroy bacteria and other foreign elements) in drug detoxification and relaxation[36]. The heightened production of free radicals leads to important changes at the molecular level that are associated with damage of biological macromolecules such as lipids, proteins and DNA, causing tissue alterations that involve a number of pathological processes such as cancer, atherosclerosis, neurodegenerative diseases and inflammatory diseases, cardiovascular, diabetes, cirrhosis, rheumatoid arthritis, etc.[37,38]

Free radicals are generated both by endogenous and exogenous sources. Free radicals generated by endogenous sources originate from biological processes that normally occur in the organism: [39]

- reduction of flavins and thiols, as a result of the activity of oxidases, cyclooxygenase, lipoxygenases, peroxidases and dehydrogenases;
- presence of transition metals within the cell;

- electron transport systems (mitochondria and endoplasmic reticulum membrane and plasma);
- Intermediate during drug detox;
- the synthesis of prostaglandins and during activation of leukocytes and platelets.

The exogenous sources that generate free radicals include tobacco, air pollution, organic solvents, toxins, alcohol, stress, anesthetics, pesticides, chemical additives, preservatives, hormones and radiation. [39]

Most free radicals are derived from the metabolism of molecular oxygen (O_2) used in the respiratory chain, which occurs in the inner membrane of mitochondria to produce energy (ATP), so called reactive species of oxygen metabolism, being the main: [37]

- superoxide radical (O_2^-), formed in the organism mainly through the electron transport chain or by action of phagocytic cells (neutrophils, monocytes, macrophages, and eosinophils) in defense against invading microorganisms in the metabolic pathway catalyzed by NADPH oxidase[40]. The O_2^- radical is formed by adding an electron in the O_2 molecule in its ground state, which can generate greater reactivity of other species such as hydrogen peroxide, hydroxyl radical and peroxinitrile.[37]

- hydroxyl radical ($OH^\bullet$) is the most reactive in biological systems, has a half-life 1^{0-9} and attacks molecules close to their place of training. Its formation *in vivo* may be related to the decomposition of peroxinitrile, hydrogen peroxide, and reactions catalyzed by transition metals, such as iron, the Fenton reaction, according to the scheme: [37]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^\bullet + OH^-$$

  Ions of transition metals can also catalyze the reaction between H_2O_2 and superoxide, leading to the production of hydroxyl radical, called the Haber Weiss reaction:[41]

$$H_2O_2 + OH^\bullet \rightarrow H_2O + O_2^- + H^+$$
$$H_2O_2 + O_2^- \rightarrow O_2 + OH^- + OH^\bullet$$

- Singlet oxygen ($^1O_2$): is the excited state of the molecular oxygen and has no unpaired electrons in the last layer. Can be produced by photochemical reactions or other radiation and reacts with a large number of biological molecules, including membrane lipids, initiating lipid peroxidation processes.[42]

- Hydrogen peroxide (H_2O_2): although it is not a free radical, due to the absence of unpaired electrons in the last layer, participates in the reaction that produces the radical $OH^\bullet$ [34.] Mitochondria are important sources of $O_2^{\bullet-}$ and the presence of this radical anion

superoxide can cause serious damage, it is converted enzymatically by the action of superoxide dismutase to H_2O_2, as the scheme:[37]

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow O_2 + H_2O_2$$

H_2O_2 can also be generated by spontaneous conversion of the resulting divalent reduction of O_2 in the third stage of respiration that occurs within the mitochondria, according to the scheme: [40]

$$O_2 \rightarrow O_2^- \rightarrow H_2O_2$$

The initiation of oxidation by free radicals is not yet clarified, being the hydroxyl radical ($OH^{\bullet}$) and the peroxinitrile radical ($NO_2^{\bullet}$) the radical initiators. It is also assigned an important role in anion superoxide radical (O_2^-) due to the possibility of generating hydroxyl radical ($OH^{\bullet}$).[43-45]

Once formed, the ROS goes to the microcirculation, and due to its high reactivity, they will perform at the level of different cellular structures (proteins and DNA) and also at the cell membrane (polyunsaturated fat).[43-45]

9.2. Lipid peroxidation

Lipid peroxidation has a great importance because it constitutes a process chain, ie, where the formation of a radical triggers the propagation of lipid peroxidative process. Lipid peroxidation is a reaction represented by the steps of initiation, propagation, and termination. These steps are presented below:[43-45]

Initiation: LH + OH• (or LO•) ———> L•+ H2O (ou LOH)
Propagation: L• + O2 ———> LOO•
Propagation: LH + LOO• ———> L•+ LOOH
Termination: LOO• + L. ———> LOOL
Termination: LOO• + LOO. ———> LOOL + O2

The above reaction begins with the kidnapping of hydrogen from the polyunsaturated fat acid (LH) of the cell membrane. This sequestration can be accomplished by OH• or LO• (alkoxyl radical), with consequent formation of L• (radical lipid). In the first equation of propagation, L• reacts rapidly with O_2, resulting in LOO• (peroxyl radical), which, in turn, kidnaps a new hydrogen from polyunsaturated fat acid, forming again in the second propagation equation, L•. The termination of lipid peroxidation occurs when the radicals (L• and LOO•) produced in the previous steps propagate to destroy themselves.[43-45]

The hydroxyl radical (OH•) is often recognized as the species and the most important initiator of lipid peroxidation. However, studies indicate that iron also plays a decisive role in the initiation of this process. Lipid peroxidation can be catalyzed by iron ions, by conversion of lipid hydroperoxides (LOOH) into highly reactive radicals (alkoxyl, and peroxyl LO•, LOO•) which, in turn, begins a new chain of reactions, called branching. These reactions, which can be fast or slow, depend on the valence of iron.[43-45]

Lipid peroxidation produces various reactive products including radicals lipids and malonic aldehyde. These final products, in addition to acting directly on other components of the cell membrane, can seep into the bloodstream by increasing the levels in blood and plasma. This increase is indicative of damage to cell membranes of organs or tissues, which may be responsible for triggering various pathological processes.[43-45]

9.3. Oxidation of proteins

Changing the properties of proteins and the formation of peroxides in amino acids can lead to degradation of proteins by fragmentation and crosslinking (cross-linking), which can result in their polymerization and inactivation, specifically in proteins that contain sulphydryl groups (-SH) groups and aromatic. The oxidation of proteins causes further change in its tertiary structure leading to protein aggregation and formation of amyloid plaques. The amino acid oxidation by free radicals induced changes in enzyme activity, there is also an impairment of antioxidant potential of cells and tissues.[43-45]

9.4. Oxidation of DNA

The DNA damages are the most important processes resulting from the occurrence of peroxidative process *in vivo*. The change in DNA by oxidative damage is one of the main causes responsible for the onset of carcinogenesis, either by activating proto-oncogenes or by inactivation of tumor suppressor genes. Inhibition of cell death program, apoptosis, may also be associated with oncogenesis and changes in DNA and may arise as a result of the action of free radicals that lead to increased cytoplasmic calcium concentration.[43-45]

The continuous production of free radicals during the metabolic processes lead to the development of many antioxidant defense mechanisms to limit intracellular levels of these species and prevent the induction of cell damage.[35]

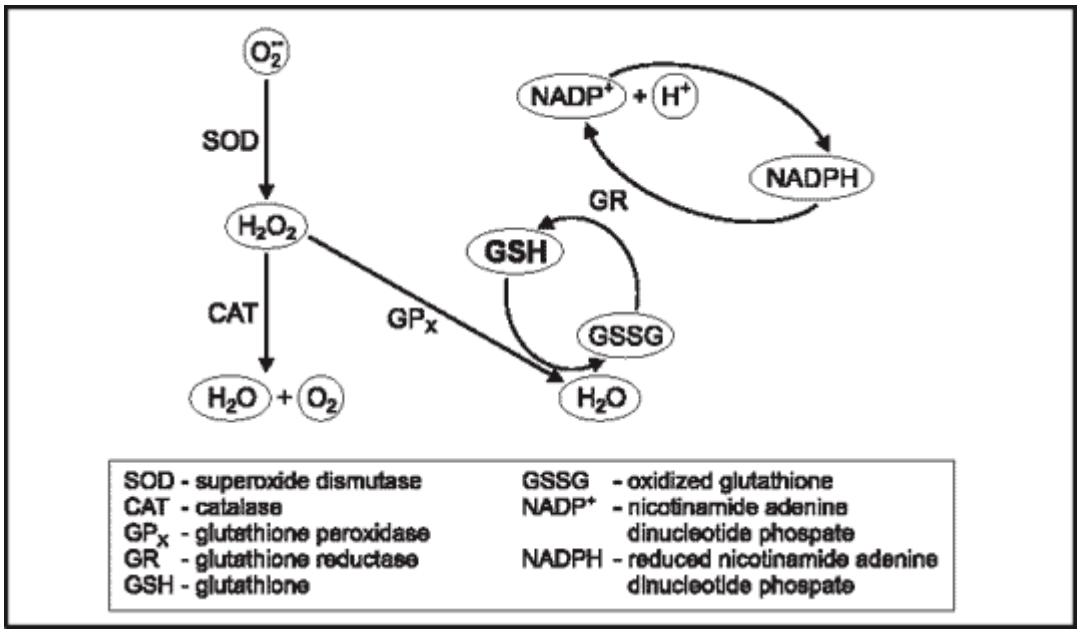
10. Antioxidants

The term antioxidant refers to any substance which, when present in low concentrations compared to the oxidizable substrate, delays or inhibits oxidation of this substrate effectively.[37]

The natural defense systems include a wide range of substances that act on three different levels of oxidation: (1) blocking the initiation step, because they prevent the generation of reactive species or kidnap them to prevent their interaction with targets cell, eg: antioxidant enzymes, tocopherols, bioflavonoids and carotenoids, (2) blocking the stage of progression of the kidnapping chain radical radical intermediates, eg: tocopherols (vitamin E), tocotrienols, flavonoids and synthetic antioxidants, and (3) repairing the injuries caused by ROS, this process is related to the removal of damaged DNA molecule and the reconstruction of damaged cell membranes.[46-48]

The two major antioxidant defenses in the body can be divided into two groups: enzymatic and non-enzymatic. The enzymatic antioxidant system is the first defense to attack by endogenous reactive oxygen species, preventing their formation or kidnapping them in order to prevent its interaction with cellular targets, ie, block the initiation step of radical chain. This system is formed mainly by the following antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat).[49]

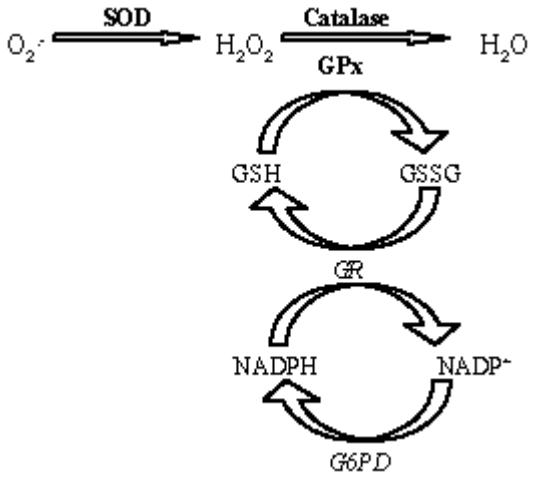
- The function of SOD is to catalyze the reaction of dismutation of anion superoxide radical, generating molecular oxygen and hydrogen peroxide. This is the main isoenzyme in extracellular fluids, but is also present in tissues.[50]



Picture 3 - Function of SOD

- Glutathione peroxidase is a selenium-dependent enzyme, cooperates with the Cat in removing hydroperoxides (ROOH). The GPx catalyzes the reduction of hydrogen peroxide and other organic peroxides at the expense of conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG). This enzyme is specific as the hydrogen donor (GSH), but can reduce various organic hydroperoxides, including lipid hydroperoxides [50]. In addition to its

central role in the activity of glutathione peroxidase, glutathione it is involved in several other antioxidant pathways, including the kidnapping of free radicals, ascorbate metabolism and detoxification of xenobiotics via glutathione transferase. [51]



Picture 4 - Function of gluathione peroxidase

- The Cat is found mainly in cellular peroxisomes and to some extent in the cytosol, directly decomposing hydrogen peroxide into water and molecular oxygen, which results from the dismutation of superoxide anion radical [51,52]. This enzyme is found in the blood, bone marrow, mucous, kidney and liver. [34]

These three enzymes are necessary for cell survival, even under normal conditions. They act through synergistic mechanisms to ensure the cell protection. However, this protection is achieved only when there is a feeling for maintaining a balance in their activity, which may be affected in the presence of ROS. [53]

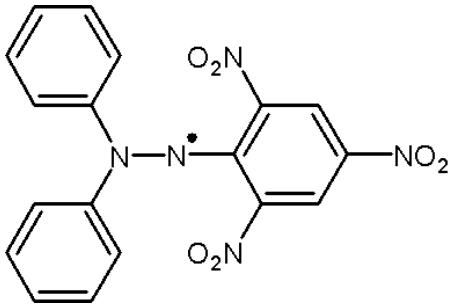
The non-enzymatic antioxidants, mostly need to be acquired by food. Fruits and vegetables are the main sources of these antioxidants. Non-enzymatic components of antioxidant defense include: trace elements (zinc, copper, selenium, etc.), vitamins (ascorbic acid, vitamin E, vitamin A, vitamin C, riboflavin), pyruvate, carotenoids (beta-carotene, lycopene and lutein), flavonoids and other compounds derived from plants. Some of the most important synthetic antioxidants include butyl hydroxyanisole (BHA) and butyl hydroxytoluene (BHT). [54]

The importance to the performance of common antioxidant in vivo depends on the following factors: types of free radicals formed, where and how these radicals are generated and optimum doses for protection. Thus it is possible that an antioxidant acts as a protective antioxidant in a system, but that fails to protect or even increase-induced lesions in other tissues or systems. [55]

Very recent studies indicate the existence of a metabolic specific signaling and regulation chain describing how oxidative stress is recognized within the system and translated into degeneration. This discovery gave a new impetus to research on substances or mixture of substances that allow the interruption of the cascade of metabolic reactions related to oxidative stress. [56]

10.1. Method of laboratory determination of antioxidant activity: DPPH Method

Various methods are used to determine the antioxidant activity of extracts and isolated chemical substances. One of the most frequently used methods is to evaluate the kidnapper free radical 2,2-diphenyl-1-hydrazine-picril (DPPH•) (picture 5) activity of purple coloration which absorbs 515 nm in a solution of methanol.



Picture 5 - DPPH

By action of an antioxidant (AH) or a radical species (R●), the DPPH• (picture 5) is reduced forming picril-diphenyl-hydrazine, in yellow, with consequent disappearance of the absorption, The same can be monitored by the decrease in absorbance. [57-59]

$$DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet}$$

$$DPPH^{\bullet} + R\bullet \rightarrow DPPH\text{-}R$$

From the results obtained determines the percentage of antioxidant activity or the percentage free radical scavenging and / or percentage of DPPH remaining in the reaction medium. The percentage of antioxidant activity (% AA) is the amount of DPPH consumed by the antioxidant. [57-58]

The quantity antioxidant needed to decrease the initial DPPH concentration by 50% is called effective concentration (EC_{50}), also called by inhibitory concentration (IC_{50}). The time required for the initial DPPH concentration decreases 50% is designated for time efficient

(TEC_{50}). The higher the consumption of DPPH by a sample, the lower the EC_{50} and the higher its antioxidant activity.[57-58]

This DPPH method is based, ultimately, in the ability of DPPH react with hydrogen donor substances.[57-58]

Chapter 2

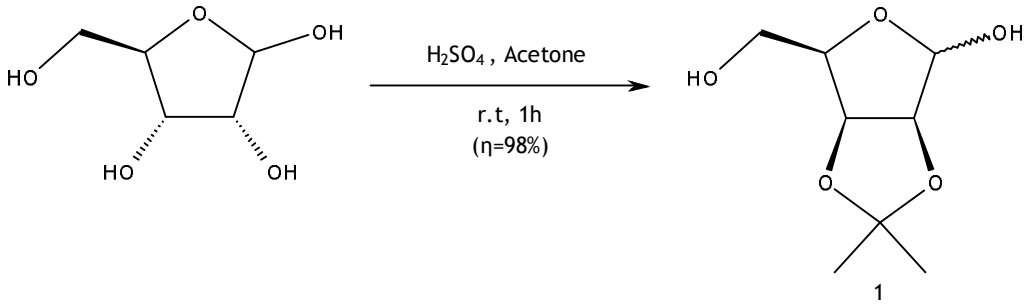
Results Discussion

The starting compound for the synthesis of all the heterocyclic compounds was the D-ribose transformed into 2,3-O-isopropylidene-ß-D-ribofuranose and 1-O-methyl-2,3-O-isopropylidene-ß-D-ribofuranose. These two compounds were used for the synthesis of its derivatives.

1. Preparation of isopropylidene-ß-D-ribofuranose

Compound **1** was obtained as indicated in the scheme 23. The starting compound, ie, D-ribose has three free hydroxyl groups in position 1,2 and 3 of the furanosidic ring.
To make the protection of any hydroxyl group you should pick up a protective group that is easy to introduce and easy to handle. To protect the hydroxyl group of positions 2 and 3 we used the isopropylidene group. This process involves the activation of acetone by concentrated sulfuric acid followed by D-ribose nucleophilic attack to the activated acetone.

The hydroxyl groups 2 and 3 are the less reactive present in the D-ribose, thereby protecting the hydroxyl groups of these two neighbours we will get a more stable conformation. Thus we turn D-ribose into isopropylidene-ß-D-ribofuranose with an income higher than that described in the literature. It was found that to obtain this income, which is close to 100%, it is necessary to flame the balloon to remove any remaining moisture. The product comes in the form of a colourless liquid.



Scheme 23- Protection of D-ribose white isopropylidene group.

By observing the thin layer chromatography it was concluded that it was not necessary to purify the obtained compound. We then performed the identification of the structure of compound **1** by the usual methods.

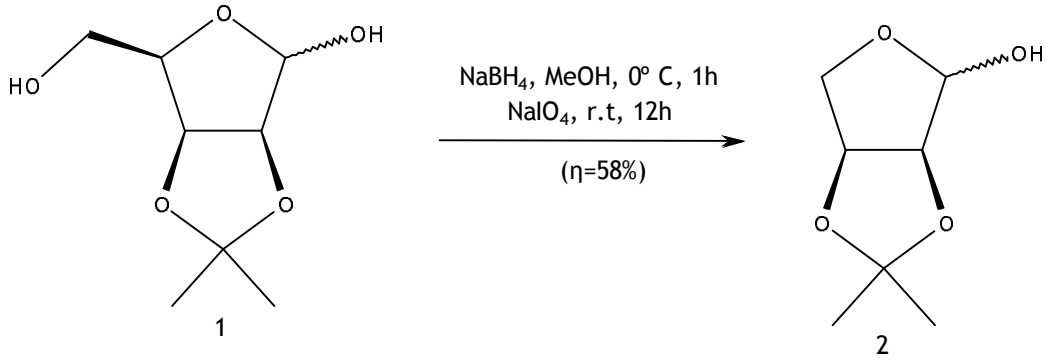
By ^{1}H-NMR spectrum it can be observed two signals at δ 1.27 and at δ 1.43, that represents two singlets, the integration curve of each singlet corresponds to three protons, that is, six protons in whole confirming the presence of the isopropylidene group.

By ^{13}C-RMN spectrum it can be observed a signal at δ 112.3 corresponding to single quaternary carbon present in the compound **1**, thus demonstrating the occurrence of reaction and the protection of hydroxyl groups 2 and 3 with the isopropylidene group.

By infrared spectrum it can be observed at 3374 a large band corresponding to the alcohol group. At 1063 it can be observed a band corresponding to cyclic ether. At 923 it can be observed a band corresponding to ketal (isopropylidene group).

2. Preparation of 2,3-O-isopropylidene-L-Erythrosis

The compound 2,3-O-isopropylidene-L-Erythrosis was obtained from compound **1** as indicated in the scheme 24, being the first step the reduction of the compound **1** with $NaBH_4$/MeOH at 0°C, followed by oxidative cleavage of the diol involving the breaking of a carbon-carbon bond resulting in a carbonyl compound thus reducing the size of the carbon chain. The reagent most commonly used as an oxidizing agent in this type of process is the sodium periodate ($NaIO_4$). The product comes in the form of a colorless liquid.



Scheme 24- Reduction and oxidative cleavage of α-diols.

By 1H-NMR spectrum it can be observed a signal at δ 4.24, represented by a broad singlet, the curve of integration is a proton. Across the spectrum of 13C-NMR, by exclusion, it can be confirm that this corresponds to the hydrogen of the alcohol group of the anomeric carbon.

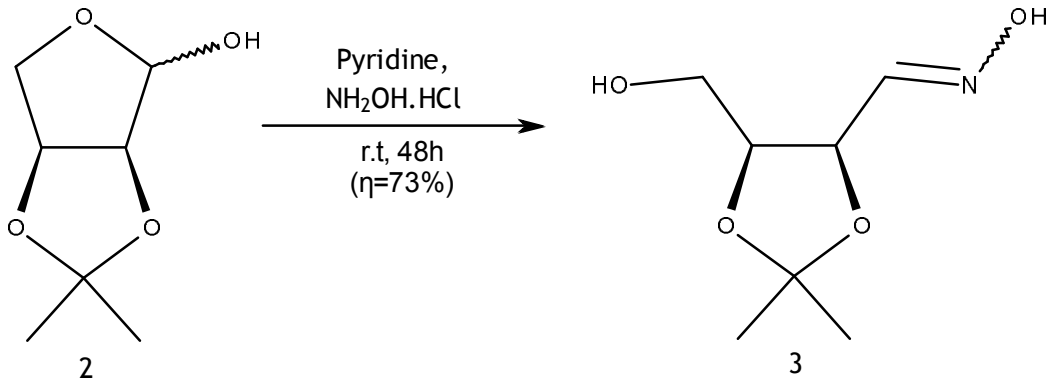
By 13C-NMR spectrum it can be observed that there are three tertiary carbons, one quaternary carbon and a secondary carbon. Compound **1** has four tertiary carbons, the presence of three tertiary carbons in the compound **2** confirms the reduction of the carbon chain. It can be also observed a signal at δ 112,4 corresponding to the quaternary carbon of the isopropylidene group.

By infrared spectrum it can be observed at 3411 cm^{-1} a large band corresponding to the alcohol group. At 1063 and at 906 cm^{-1} can be observed corresponding bands to cyclic ether. At 969 cm^{-1} can be observed a band corresponding to ketal (isopropylidene group).

The yield was lower than that described in the literature, perhaps due to loss of response during treatment or during purification of the obtained residue.

3. Preparation of 2,3-O-isopropylidene-L-oxime Erythrosis

Compound 2,3-O-isopropylidene-L-Erythrosis oxime was obtained from compound **2** as indicated in the scheme 25. In this procedure, the ring opening occurs through the use of hydroxylamine hydrochloride. The only drawback of this method is the smell released by pyridine. Pyridine is a toxic solvent that can damage the male reproductive system among other. Therefore you need the most attention, and wash all the material used by HCl to neutralize the odor of pyridine. Evaporate in the rotavapor, then you must verify if the product still shows traces of pyridine, if this odor is still present due to co-evaporate with toluene product. The product comes in the form of a pink/purple solid, and its melting point is between 46-51 ° C. The yield was slightly lower than that described in the literature.



Scheme 25- Ring opening of compoud **2**.

By observing the thin layer chromatography it was concluded that it was not necessary to purify the compound obtained. We then performed the identification of the structure of compound **3** by the usual methods.

By ^{1}H-NMR spectrum it can be observed that the product is an isomer. Thus it shows characteristic signs of the Z and E isomer. The compound **3** has the same number of quaternary carbons, tertiary and secondary than the compound **2** which makes it more difficult to explain the formation of product **3**.

Table 1-Comparison of chemical deviation of the ^{1}H-NMR spectrum of compound **2** and **3**.

Compound	CH-1	CH-2	CH-3	CH_2-4	$C(CH_3)2$	*OH*
2	5.35	4.51	4.79	3.91-4.06	1.27 and 1.41	*4.24*
3	6.94(Z) and 7.44(E)	4.88(E) and 5.26(Z)	4.33-4.36(E) and 4.51-4.70(Z)	3.49-3.74	1.37 (Z) and 1.47(E)	-

The CH_2-4 compound **3** appears as a multiplet at δ 3.49-3.74 with an integration curve of 4 protons. At δ 4.33-4.36 it can be observed a multiplet corresponding to the CH-3 of the E-isomer and at δ 4.51-4.70 it can be also observed a multiplet corresponding to the CH-3 Z-isomer, both with an integration curve of 1 proton. At δ 4.88 it can be observed a triplet corresponding to the CH-2 and at δ 5.26 a large singlet corresponding to CH-2 Z-isomer. For the spectrum of compound **2** it can be observed that the signals corresponding to CH-3 appear soon after the CH_2-4 and before the signals corresponding to CH-2. In the compound **3** it can be observed that signals corresponding to CH-3 appear after the signals corresponding to CH-2 and before the signals corresponding to the CH_2-4, the reversing order of appearance shows that it was possible to obtain the compound **3**.

By 13C-NMR spectrum it can be observed two quaternary carbons at δ 109.6 and at δ 110.1 corresponding to the isopropylidene group of E and Z isomers, it cannot be known which isomer corresponds to each signal.

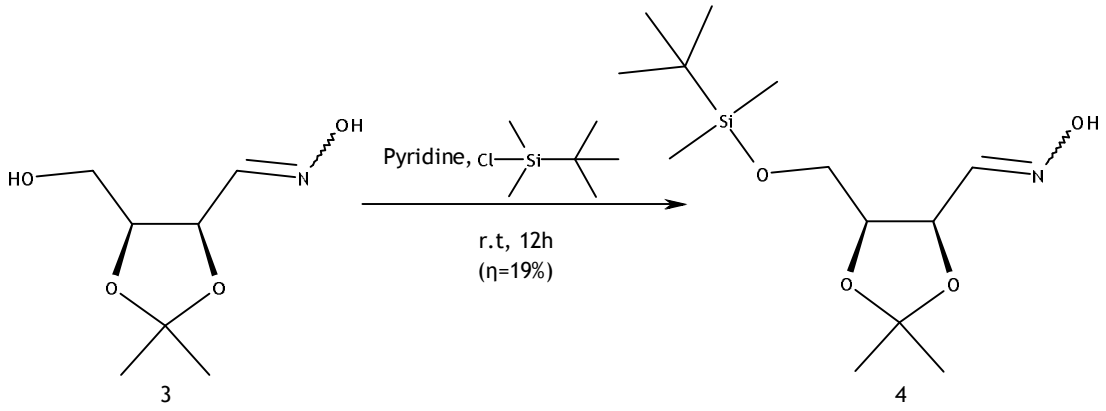
By infrared spectrum it can be observed at 3209 cm^{-1} a large band corresponding to the alcohol group. At 1438 cm^{-1} can be observed a band corresponding to C=N from the aldoxime. At 1043 cm^{-1} can be observed a band corresponding to N-O from the aldoxime. At 957 cm^{-1} can be observed a band corresponding to ketal (isopropylidene group).

In this case it is of great interest the mass spectrum as the compound **3** has the same number of carbons than compound **2**. Thus it can be observed at 176.0 the expected mass value plus the value of a H^+ at 198.0 it can be observed the expected mass value plus the value of the Na^+ ion, and at 214.0 the expected mass value plus the value of the K^+ ion. In that way it can be proved that the obtained product is in fact the 2,3-O-isopropylidene-L-erythrose oxime, that results from the furanosidic ring opening.

4. Preparation of 4-O-tert-butyldimethylsilyl-2 ,3-O-isopropylidene-L-oxime Erythrosis

The compound 4-O-tert-butyldimethylsilyl-2 ,3-O-isopropylidene-L-Erythrosis oxime was obtained from compound **3**. The alcohol function was protected with tert-butyldimethylsilyl

group with minor changes as described in the literature. The compound **4** is presented as a colorless liquid. The yield is low, but you can get the two isomers (E and Z).



Scheme 26- Protection of compound **3** with the tert-butyldimethylsilyl group.

By ^{1}H-RMN spectrum it can be observed a signal at δ 0.0, represented by a multiplet , with an integration curve of 6 protons. At δ 0.82 it can be observed a singlet, with an integration curve of 9 protons. These 15 protons correspond to the tert-butyldimethylsilyl protective group of the E-isomer. In a similar way at δ 0.10 and at δ 0.82 it can be founded the protons corresponding to the tert-butyldimethylsilyl group of the Z-isomer. In that way it can be proved the protection of the hydroxyl group 4 with the tert-butyldimethylsilyl group.

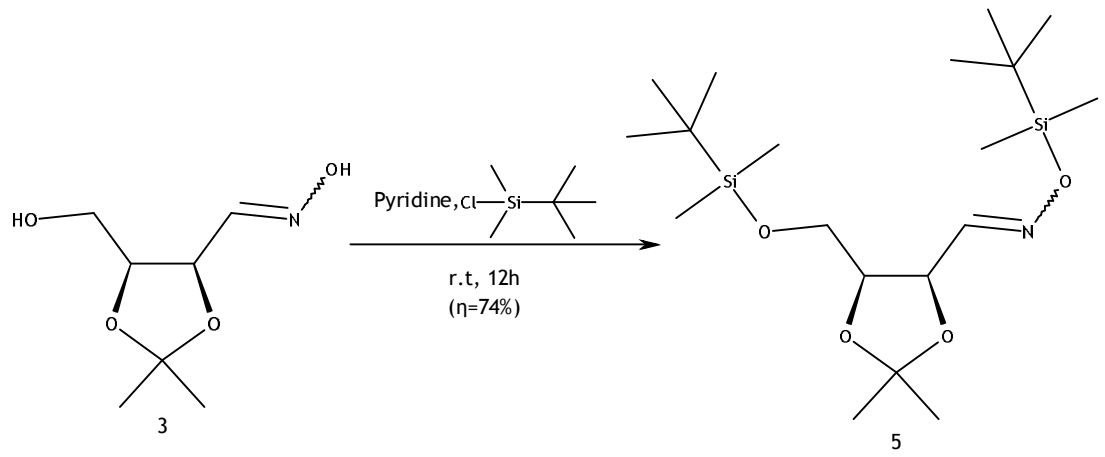
By the ^{13}C-RMN spectrum it can be observed 4 quaternary carbons, two of them corresponding to the E-isomer and other two to the Z-isomer. At δ 27.2 it can be observed the quaternary carbon from the tert-butyldimethylsilyl group from the E-isomer and at δ 18.1 for the Z-isomer.

By infrared spectrum it can be observed at 3192 cm^{-1} as a large band corresponding to the alcohol group. At 1374 cm^{-1} can be observed a band corresponding to tert-butyl. At 1046 cm^{-1} can be observed a band corresponding to N-O from the aldoxime. At 983 cm^{-1} can be observed a band corresponding to ketal (isopropylidene group). At 838 cm^{-1} can be observed a band corresponding to $Si(CH_3)_2$from the tert-butyldimthylsilyl group. At 791 cm^{-1} can be observed bands corresponding to Si-C from the tert-butyldimthylsilyl group.

By making the compound **3** react with 1.2 equivalents of tert-butyldimethylsilyl.Cl in pyridine it was able to obtain the compounds **4** and **5**. The compound **4** is obtained in a lower yield as the compund **5** (31.4%). The mono-silylated compound (**4**) is easily separated from the di-silyated compound (**5**) by silica gel column chromatography. The di-silyated compound is the first out of the column, and then the mono-silyated compound. The overall efficiency of this method is 50.4%.

5. Preparation of N-[tert-butyl (dimethyl) silyl] oxy-1-[(4R, 5S) - 5 - [[tert-butyl (dimethyl) silyl] oxymethyl] -2,2-dimethyl-1 ,3-dioxolan -4-yl] methanimine

The compound N-[tert-butyl (dimethyl) silyl] oxy-1-[(4R, 5S) -5 - [[tert-butyl (dimethyl) silyl] oxymethyl] -2,2-dimethyl-1 ,3-dioxolan -4-yl] methanimine was obtained from compound **3**. In this situation the two alcohol functions present in the compound **3** are going to be protected with tert-butyldimethylsilyl group. By increasing the number of equivalents of 1.2 to 2.5 it will allow protection of both alcohol groups. The protection of both alcohol groups enhances the yield of the reaction towards the protection of a single alcohol group with tert-butyldimethylsilyl, thus increasing the yield for the triple. The compound obtained is presented in the form of a pink / violet liquid.



Scheme 27- Protection of compound 3 with tert-butyldimethylsilyl group.

By 1H-NMR spectrum it can be observed a doublet at δ 0.04, with an integration curve of 12 protons. At δ 0.14 it can be observed a multiplet, with an integration curve of 12 protons. These 24 protons correspond to $Si(CH_3)_2$ from the mixture E+Z. At δ 0.88 and at δ 0.91 it can be observed 2 dublets with an integration curve of 36 protons, corresponding to $Si(CH_3)_3$ from the mixture E+Z. So the mixture E+Z have 60 protons in total, 30 for each isomer, demonstrating the formation of compound 5, ie, the protection of compound **3** with two tert-butyldimethylsilyl groups.

By the ^{13}C-RMN spectrum it can be observed 3 quaternary carbons, at δ 18.4 it can be observed the quaternary carbon corresponding to the isopropylidene group. At δ 109.3 and at 109.7 can be observed the quaternary carbons corresponding from the two tert-butyldimethylsilyl group, which corresponds to the di-silylated product.

By infrared spectrum it can be observed at 1251 cm^{-1} a band corresponding to Si-O-C from the silyl ether. At 834 cm^{-1} can be observed a band corresponding to 834 from the ter-butyldimethylsilyl group.

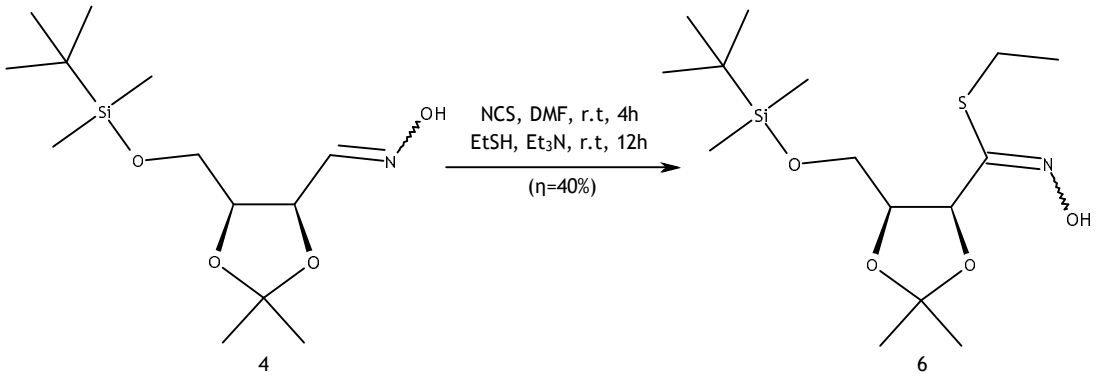
By making the compound 3 to react with 2.5 equivalents of tert butyldimethylsilyl.Cl in pyridine we get only the compound **5**, ie, the compound di-silylated. The yield is satisfactory and far superior to that obtained when using 1.2 equivalents of tert-butyldimethylsilyl.Cl.

6. Preparation of (Z)-4-O-tert-butyldimethylsilyl-2 ,3-O-isopropylidene-N-hydroxy-L-erythronimidothioate of s'ethyle

The ethylene- (Z)-4-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-N-hydroxy-L-erythro imidothioate compound was obtained from compound **3** and **4**. The introduction of the thiohydroximate function is a simple procedure to take. The major drawback of this procedure is the smell released by EtSH, the maximum attention was need since this compound is toxic.

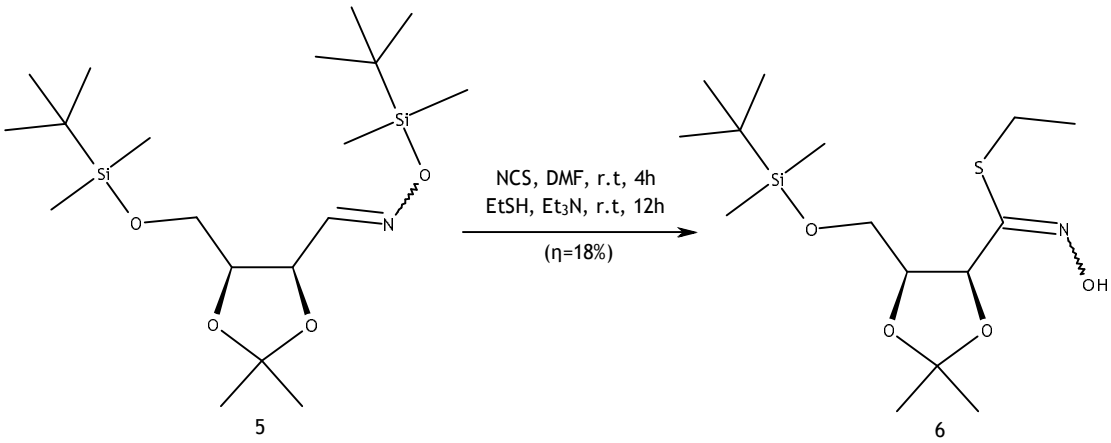
When the residue is evaporated in Rotavapor it is needed to add Javel water to the collection balloon of solvents to neutralize the smell. Only in this way is possible to evaporate the residue.

As mentioned the compound **6** can be obtained from compound **4** and **5** by the same procedure. The yield that is going to be obtained will be different, being compound **4** the one where it can be obtained a higher yield. Thus we can say that it is more satisfatory to obtain the compound di-silylated (**5**) but for the following reaction is more satisfatory to use the mono-silylated compound (**4**). The compound obtained is presented in the form of a yellow liquid.



4 6

Scheme 28- Thiohydroximate function introduction in compound **4**.



Scheme 29 Thiohydroximate function introduction in compound **5**.

By 1H-NMR spectrum the signals at δ 0.05 and at δ 0.87 are related to protons of the tert-butyldimethylsilyl group, its integration curve corresponds to 15 protons in its entirety. At δ 1.29 it can be observed a triplet, whose integration curve corresponds to 3 protons, accross the spectrum of ^{13}C-RMN it can be said that this signal is referring to $SCH_2\underline{C}H_3$. At δ 2.97-3.10 it can be observed a multiplet, its integration curve correspond to 2 protons, and accross the spectrum of ^{13}C-RMN it can be said that this signal is referring to $S\underline{C}H_2CH_3$. Through these 2 peaks it can be conformed the introduction of the thiohydroximate function. At δ 9.09 it can be observed a large singlet, whose integration curve corresponds to 1 proton, which accross the ^{13}C-RMN spectrum it can be said that it corresponds to the alcohol group present on the compound 6.

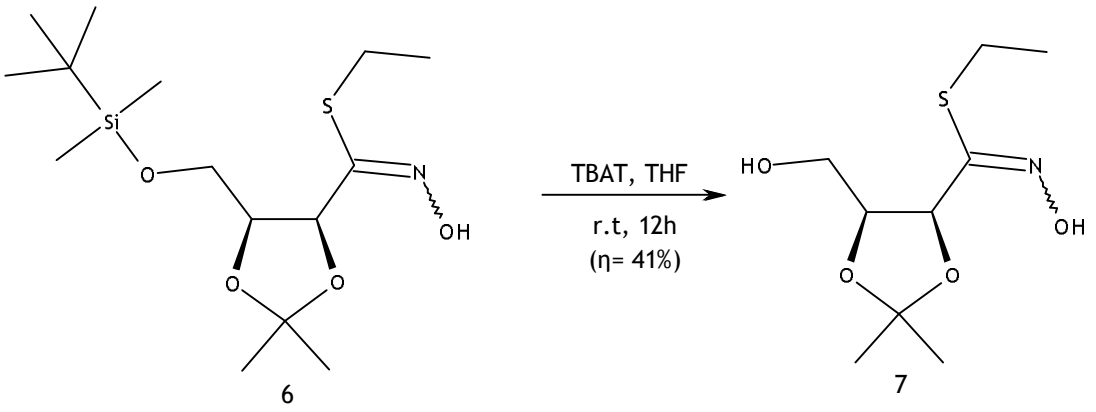
By the ^{13}C-RMN spectrum it can be observed 3 quaternary carbons at δ 18.6, δ 109.7 and at δ 149.8 that corresponds, respectively to $\underline{C}(CH_3)_3$, $\underline{C}(CH_3)_2$ and to C=N proving the introduction of the thiohydroximate function by the raise of a quaternary carbon in the ^{13}C-RMN spectrum.

By infrared spectrum it can be observed at 3288 a large band corresponding to the alcohol group. At 1601 it can be observed a band corresponding to C=N from the aldoxime. At 1078 it can be observed a strong band corresponding to Si-O-C from the silyl ether. At 835 it can be observed a band corresponding to $Si(CH_3)_2$ from the tert-butyldimthylsilyl group. At 667 it can be observed a band corresponding to C-S from the thiohydroximate function.

7. Preparation of (Z) -2,3-O-isopropylidene-N-hydroxy-L-erythronimidothioate of s'ethyle

Compound ethyle-(Z)-2,3-O-isopropylidene-N-hydroxy-L-erythronimidothioate was obtained from compound **6**. The unprotection of the hydroxyl group, ie, De-O-Silylation enables the

regeneration of the hydroxyl group by removing the protective group tert-butyldimethylsilyl. The compound obtained is presented in the form of a white solid, and its melting point is 88-94 ° C.



Scheme 30- De-O-Silylation of compound **6**.

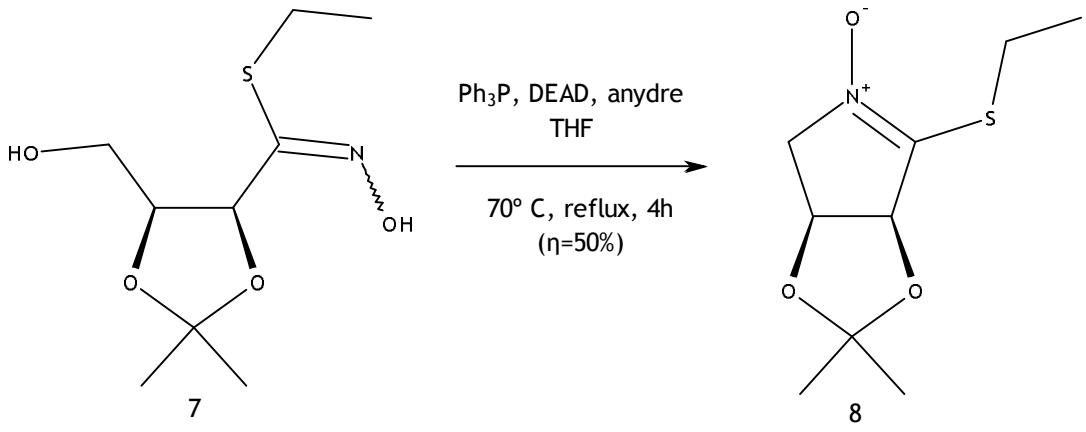
By the ^{1}H-RMN spectrum it can be observed a triplet at δ 1.33, with an integration curve of 3 protons, corresponding to $SCH_2\underline{CH_3}$. At δ 1.40 it can be observed a multiplet, with an integration curve of 2 protons, corresponding to $S\underline{CH_2}CH_3$. By the ^{1}C-RMN spectrum this attribution can be validated.

By the ^{1}C-RMN spectrum it can be observed the presence of 2 quaternary carbons, at δ 150.8 and at δ 158.0, corresponding to $\underline{C}(CH_3)_2$ and to C=N respectively. The disappearance of one quaternary carbon confirms the De-O-Silylation of compund **6**.

By infrared spectrum it can be observed at 3479 a strong band corresponding to the alcohol group. At 1688 it can be observed a band corresponding to ketal (isopropylidene group). At 670 it can be observed a band corresponding to C-S from the thiohydroximate function.

8. Preparation of (3S, 4S) -2 - (erythylthio) -3,4-isopropylidenedioxy-3 ,4-dihydro-5H-pyrrole-1-Oxyde

The compound (3S,4S)-2-(erythylthio)-3,4-isopropylidenedioxy-3,4-dihydro-5H-pyrrole-1-oxyde was obtained from the compound **7**. When applying the conditions used in the Mitsunobu reaction it will origin the thioimidate N-oxides through a nucleophilic substitution of an alcohol-activated terminal. The compound obtained is presented in the form of a white solid, its melting point is 139-143 ° C. The yield is approximately half that described in the literature.



Scheme 31- Cyclization of compound **7**.

Table 2 - Comparison of chemical deviation of the ^{1}H-NMR spectrum of compound **7** and **8**.

Compound	CH-2	CH-3	CH_2-4	$S\underline{C}H_2CH_3$	$SCH_2\underline{C}H_3$	N-OH	$C(CH_3)_2$
7	4.89	4.40	3.68 and 3.79	3.11	1.33	9.40	1.40 and 1.53
8	5.35	4.90	4.7 and 4.14	3.14	1.38	-	1.39 and 1.44

By the ^{1}R-RMN spectrum it can be observed at δ 1.38 a large triplet, with an integration curve of 3 protons, corresponding to $SCH_2\underline{C}H_3$. At δ 1.39 and 1.44 it can be observed 2 singlets, with an integration curve of 6 protons, corresponding to the isopropylidene group. At δ 3.14 it can be observed a quadroplet with an integration curve of 2 protons, corresponding to $S\underline{C}H_2CH_3$.
It can be observed disappearance of the peak corresponding to the function alcohol in relation to the spectrum of compound **7**. Thus proves the formation of the compound **8**.

Table 3 - Comparison of chemical deviation of the ^{13}C-NMR spectrum of compound **7** and **8**.

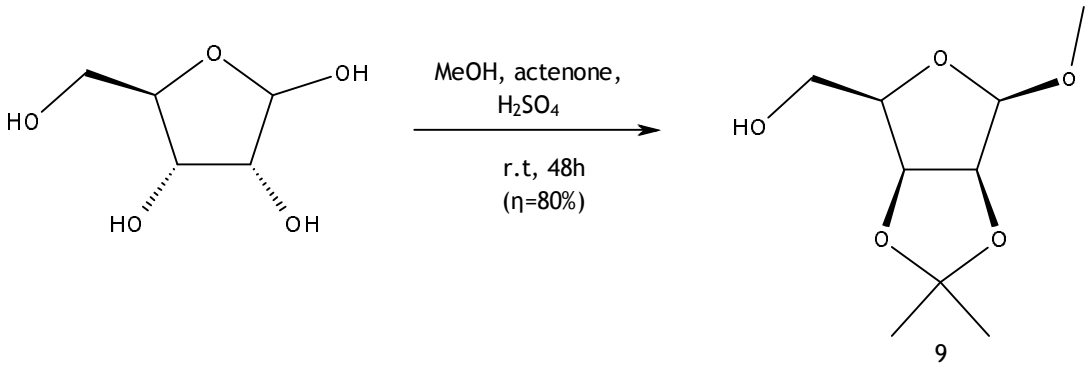
Compound	C-1	CH-2	CH-3	CH_2-4	$S\underline{C}H_2CH_3$	$SCH_2\underline{C}H_3$	$\underline{C}(CH_3)_2$	$C(\underline{C}H_3)_2$
7	158.0	75.2	78.8	61.8	25.8	15.2	150.8	25.5 and 27.2
8	144.0	81.8	73.2	66.6	23.6	15.7	112.9	26.2 and 27.3

By the ^{1}C-RMN spectrum it can be observed 2 quaternary carbons at δ 112.9 and at δ 144.0 corresponding to $\underline{C}(CH_3)_2$ and to C-1 respectively.

By infrared spectrum it can be observed at 1570 a band corresponding to C=N from the thio-imidate N-oxides. At 948 it can be observed a band corresponding to ketal (isopropylidene group). At 663 it can be observed a band corresponding to C-S from the thiohydroximate function.

9. Preparation of 1-O-methyl-2,3-O-isopropylidene-ß-D-ribofuranose

The compound 1-O-methyl-2,3-O-isopropylidene-ß-D-ribofuranose was obtained from the D-ribose. By making D-ribose to react with MeOH/acetone/H_2SO_2 concentrated the hydroxyl 1 group will be protected with the methyl group at the same time that it will be protected the neighbour hydroxyl 2 and 3 groups with the isopropylidene group. The compound is presented in the form of a yellow liquid.



Scheme 32- Protection of D-ribose with the isopropylidene group and the methyl group.

By the ^{1}H-RMN spectrum it can be observed at δ 1.28 a singlet, with an integration curve of 3 protons. At δ 1.44 it can be observed a singlet, with an integration curve of 3 protons. These two signals are referred to the protons from the isopropylidene group. At δ 3.39 it can be observed a singlet, with an integration curve of 3 protons corresponding to the metyl group. It can be also observed at δ 3.23 a dublet of a dublet corresponding to the only alcohol group existing in the compound **9**.

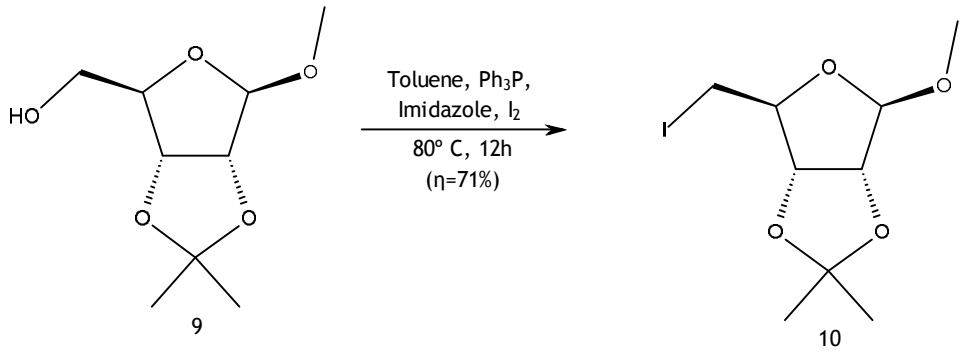
By the ^{1}C-RMN spectrum it can be observed a quaternary carbon at δ 112.2 corresponding to $\underline{C}(CH_3)_2$, ie, to the quaternary carbon resulting from the protection of the hydroxil 2 and 3 groups. At δ 55.6 it can be observed a primary carbon resulting from the protection of the

hydroxyl group with a methyl group. The compound presents also a secondary carbon at δ 64.1 and 4 tertiary carbons at δ 81.6, δ 85.9, δ 88.4, and at δ 110.1.

By infrared spectrum it can be observed at 3479 a large band corresponding to the alcohol group. At 1374 it can be observed a band corresponding to O-CH_3 from the methyl protecting group. At 1040 it can be observed a band corresponding to cyclic ether. At 960 it can be observed a band corresponding to ketal (isopropylidene group).

10. Preparation of methyl-5-deoxy-5-iodo-2,3-O-isopropylidene-ß-D-ribofuranoside

The methyl-5-deoxy-5-iodo-2,3-O-isopropylidene-ß-D-ribofuranoside compound was obtained from the compound 9. The deoxygenation reaction of a hydroxyl group was made in position 5. The transformation of the furanoside ring in its deoxygenated derivative ran a second nucleophilic substitution reaction involving the attack of a halogen atom to a free hydroxyl group. The compound obtained is presented in the form of a colorless liquid.



Scheme 33 – Ionic deoxygenation

Table 4 - Comparison of chemical deviation of the 1H-NMRspectrum

Compound	CH-1	CH-2	CH-3	CH-4	CH_2-5	OCH_3	$C(CH_3)_2$	OH
9	4.93	4.79	4.55	4.37	3.63	3.39	1.28 e 1.44	3.23
10	5.0	4.72	4.59	4.39	3.11 e 3.25	3.32	1.28 e 1.43	-

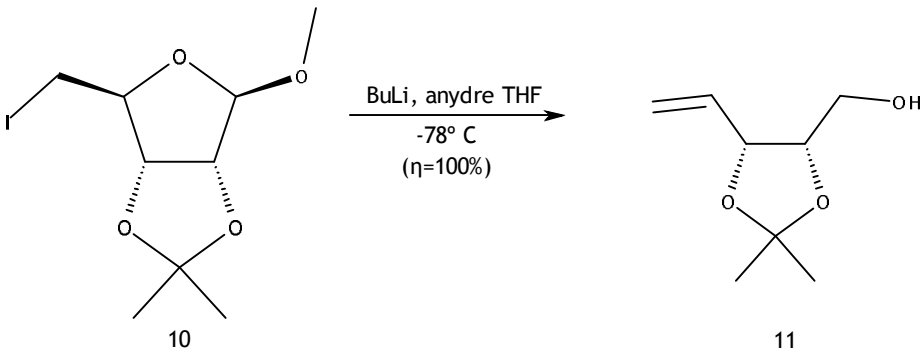
By the ^{1}H-RMN spectrum it can be observed the chemical deviations presented are similar to the one on compound **9**. The dissapearance of a peak corresponding to OH is confirmed by the ^{1}C-RMN spectrum. The disappearance of this hidroxyl group confirms the halogenation.

By infrared spectrum at 1373 it can be observed a band corresponding to O-CH_3 from the methyl protecting group. At 1063 it can be observed a band corresponding to cyclic ether. At 955 it can be observed a band corresponding to ketal (isopropylidene group).

The mass spectrum confirms the attainment of the deoxy-5-iodo-sugar.

11. Preparation of 4,5-dideoxy-2,3-O-isopropylidene-D-erythro-pent-4-enose

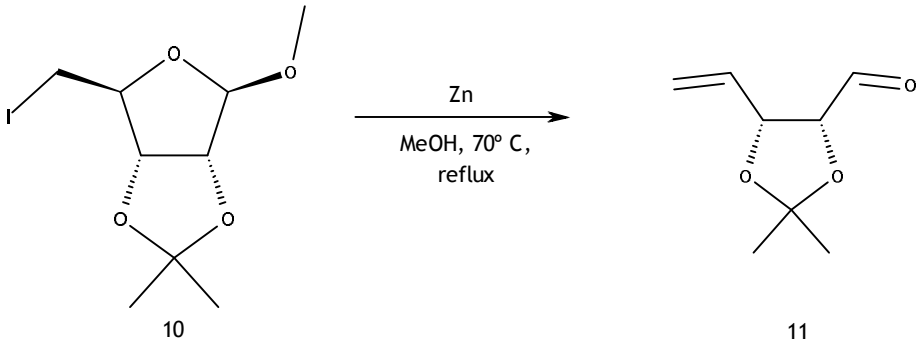
Compound 4,5-dideoxy-2,3-O-isopropylidene-D-erythro-pent-4-enose was obtained from the compound **10**. The eliminative reduction of the desoxi-5-iodade-sugar is carried out by the inclusion of the anhydrous THF and BuLi to the compound **10**. It is necessary to handle BuLi very carefully, because it reacts violently in contact with water, all material used to add BuLi must be anhydrous. The anhydrous conditions are fundamental to obtain a good yield. Compound **11** is extremely volatile, therefore the residue obtained is used in its raw form for the next reaction. Thus it is not necessary to treat the reaction, being the yield obtained in this step of 100%. The obtained compound is presented in a form of an orange solution.



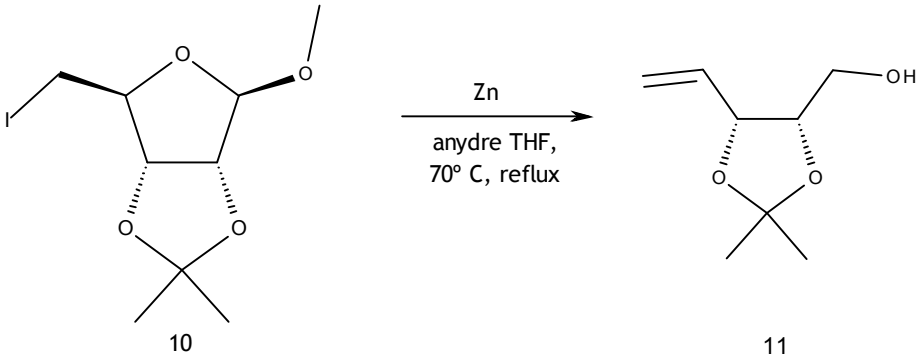
10 11

Scheme 34- Ionic deoxygenation of compound **10**.

It was tried to obtain the compound 4,5-dideoxy-2,3-O-isopropylidene-D-erythro-pent-4-enose from the compound **10** by procedures described in scheme 35 and 36.



Scheme 35- Cleavage redutively

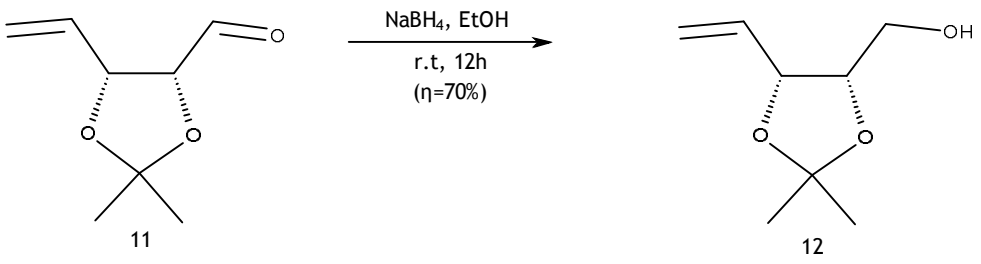


Scheme 36- Cleavage redutively

In both cases the yield is almost zero. This result may be due to the fact that the pentafuranose obtained is extremely volatile, and thus it may have lost when the residue evaporated in rotavapor. Another reason is the need to activate the zinc, so the zinc may not be activated efficiently, which will reduce the amount of zinc that reacts and consequently leads to obtain a yield lower than expected. Given these two factors it was tried to find a new method that would be more efficient to obtain pentafuranose, so we opted for the reductive elimination with BuLi at -78 ° C.

12. Preparation of 4,5-dideoxy-2,3-O-isopropylidene-D-erythro-pentitol

The compound 4,5-dideoxy-2,3-O-isopropylidene-D-erythro-pentitol was obtained from the compound **11** from reduction process. The obtained compound is presented in the form of a colorless liquid.



Scheme 37- Reduction of compound **11**.

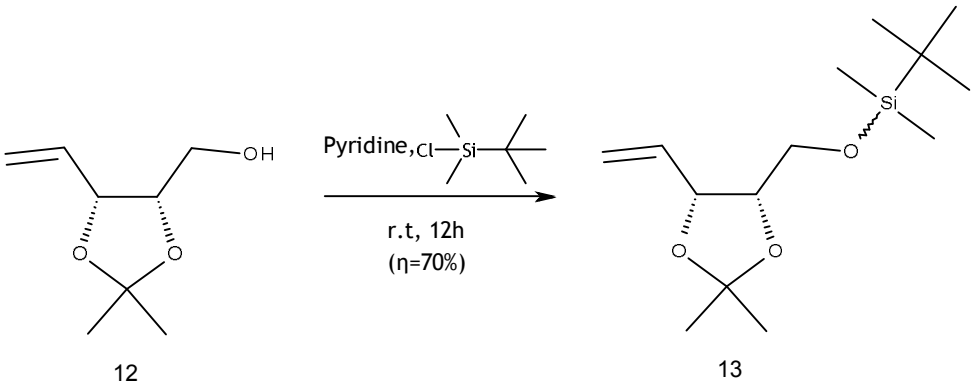
By the ^{1}H-RMN spectrum it can be observed at δ 1.37 and at δ 1.48 two singlets, with an integration curve of 6 protons corresponding to the isopropylidene group. At δ 2.12 it can be observed a large singlet, with an integration curve of 1 proton corresponding to OH. Thus it can be demonstrated the reduction of compound 11. At δ it can be observed a dublet of a dublet corresponding to CH_2-1, at δ 4.24 a quadruplet corresponding to CH-2, at δ 4.62 a triplet corresponding to CH-3, at δ 5.25 a dublet of a dublet corresponding to CH_2-5, at δ 5.33-5.43 a multiplet corresponding to CH_2-5' and at δ 5.79-5.88 a multiplet corresponding to CH-4. The signals obtained are the same as described in literature.

By the ^{13}C-RMN spectrum it can be observed 3 terciary carbons, at δ 78.4, δ 78.5 and at δ 133.2 corresponding respectively to CH-3, CH-2 and CH-4. It can be also observed 2 secondary carbons at δ 62.2 and at δ119.0 corresponding respectively to CH_2-1 and CH_2-5. Like CH_2-1 is related to a secondary carbon, it can be proved the dissapearance of the double connection in compound 11 and its reduction. The quaternary carbon from isopropylideno it can be observed at δ 109.0.

By infrared spectrum at 3421 it can be observed a large band corresponding to alcohol group. At 1645 it can be observed a band corresponding to C=C from the pentanose. At 925 it can be observed a band corresponding to ketal (isopropylidene group).

13. Preparation of tert-butyl-[[(4S,5R)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl]methoxy]-dimethyl-silane

The compound tert-butyl-[[(4S,5R)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl]methoxy]-dimethyl-silane was obtained from compound 12 from the protection of the hidroxyl 1 group with the tert-butyldimethylsilyl group. The obtained compound is presented in the form of a colorless liquid.



12 13

Scheme 38- Protection of compound **12** with the tert-butyldimethylsilyl group.

By the ^{1}H-RMN spectrum it can be observed at δ 0.05 a singlet, and at δ 0.88 a singlet with an integration curve of 15 protons, these 15 protons corresponds to the tert-butyldimethylsilyl group. At δ 1.36 and at δ 1.47 it can be observed two singlets with an integration curve of 6 protons corresponding to the other protective group present in the compound, the isopropylidene.

By the ^{13}C-RMN spectrum it can be observed the appearance of 2 secondary carbons at δ 18.5 and at δ 108.7 corresponding respectively to the quaternary carbon group of the tert-butyldimethylsilyl group and of the isopropylidene group. Thus it can be proved the protection of the hydroxyl 1 group.

By infrared spectrum at 924 it can be observed a band corresponding to ketal (isopropylidene group). At 835 cm^{-1} and at 775 cm^{-1} it can be observed bands corresponding to Si-C from the tert-butyldimethylsilyl group.

14. Determination percentage of antioxidant activiy

By the obtained results it can be verified that the compound with the greatest antioxidant activity is compound **13** followed by compounds **3** and **10**. Pentanose **13** has a double C-C link, this insaturated bond makes compound **13** reactive and a potential hydrogen donor substance. Regarding to the tert-buthyldimethylsilyl protective group it is not known if it raises the antioxidant activity of a substance.

Compound **3** has in its composition a nitrogen atom, the presence of this atom is very electronegative. The aldoximes in its generality are very reactives because they have nitrogen it its composition. In that way it was expected that aldoxime **3** presents a great antioxidant value.

Compound **10** has in its composition an halogen. Halogens are highly oxidants which make them have great reactivity. In that way the iodine makes compound **10** a hydrogen donor substance. This compound surprised by the positive as the obtained results were quite satisfactory, considering that its only necessary two chemical transformations to obtain it.

It can be seen that the introduction of the thiohidroxymate function raises the antioxidant activity of compound **6**. Since sulfur is a multivalent compound it was expected that thyohydroxymato would present a higher antioxidant activity. The unprotection of the alcohol function of compound **6** to obtain compoud **7**, ie, De-o-Silylation, increments in one unit the antioxidant activity. The conversion of the silylated ether in alcohol does not vary much the antioxidant activity.

The cyclization of compound **7** to obtain compound **8** has been increasing its stability, and consequently decreases its antioxidant activity, because it makes the compound less reactive. Yet it was expected that compound **7** had a greater antioxidant activity and consequently the thio-imidato N-oxides were still a good antioxidant.

Perhaps the DPPH method for determining antioxidant activity was not the most appropriate, as it needed a high concentration compared to the positive control, to be able to determine the antioxidant activity. The possible alternative would have been the linoleic ß-caroten/acid method. Or the compounds don't have antioxidante activities.

Chapter 3

Conclusion

In both methods used to obtain thioimidate N-oxide the research started with D-ribose. The first method, D-ribose was converted to aldoxime **3** in three steps with a 76% overall yield. After protection of D-ribose with the isopropylidene group the carbon chain is shortening by oxidative cleavage to obtain the compound **2**. The ring-opening of compound **2** originated acyclic aldoxime **3**. The acyclic aldoxime **3** was protected with one or two tert-butyldimethylsilyl group to obtain the compound **4** and **5**. The thiohydroximate function is easily introduced to obtain the compound **6**, it was proved that using **4-**O-tert-butyldimethylsilyl-2,3-O-isopropylidene-L-erythrose oxime **4** was more efficient. It is induced de-O-silylation to obtain the compound **7** followed by ring-closing used the Mitsunobu procedure to obtain the compound **8**. The thioimidate N-oxide **8** was obtained quantitatively, and it is presented in the form of a white solid. The thioimidate N-oxide (8) degrades when exposed to room temperature.

For the second method applied to D-ribose was converted into 4,5-dideoxy-2,3-O-isopropylidene-D-erythro-4-pentenose in four steps with a 80% overall yield. After the protection of D-ribose with an isopropylidene group and a methyl group it was obtained the compound **9**, the hydroxy group of the compound **9** was transformed into iodine compound **10**, the ring-opening of compound **10** originated the unsaturated aldehyde **11**. The unsaturated aldehyde **11** is reduced to compound **12**. The pentafuranose **12** obtained was protecting with tert-butyldimethylsilyl group to obtain the compound **13**. The tert-butyl-[[(4S,5R)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl]methoxy]-dimethyl-silane (**13**) was obtained, presenting in the form of a colorless liquid.

The determination of the antioxidant activity shows that compound **13** is a potential antioxidant, being its antioxidant activity of 67.5%. Compound **10** also shows a good percentage (56%) of antioxidant activity due to the presence of a halogen in its composition, the efficient concentration shows that compound **10** is a potential antioxidant. As expected aldoxime showed to be also a good antioxidant, with 60.3% of antioxidant activity.

Unfortunately it was not possible to advance the project due to lack of funding for the purchase of necessary materials for this investigation. In the future it is of great interest to continue with this project to finish the second reaction round in order to obtain the thioimidate N-oxides.

Chapter 4

Experimental part

1. General methods

Thin layer chromatography is used to check the progress of reactions and during purification. TLC plates (aluminum plates coated with silica gel Silica) proved / powered by UV light (λ= 254 nm) and immerse in a revealing and then heated to 100 ° C.

- Sulfuric acid (10%) in ethanol
- Phosphomolybdic acid (5%) in ethanol
- 5 g $KMnO_4$ in water

We can then calculate the Rf ie the ratio between the height of elution of the product and that of the eluent.

The synthesized compounds were purified by column chromatography on silica gel. To do this, we use silica gel SI 60 (40-63 µm) and chromatographic columns were eluted by gravity with the help of compressed air.

The products obtained were analyzed using different techniques:

Proton NMR spectra were recorded in DMSO and $CDCl_3$ with Bruker spectrometers type AV 250 (250 MHz) and Bruker AV 400 (400MHz) at ICOA. Correlations homonuclear 2-dimensional COSY type (*Correlated Spectrometry*) were performed to allow completion of the assignment of certain signals. Chemical shifts (δ) of the various signals are given in ppm, coupling constants are given in Hertz (Hz) and the multiplicity of signals is indicated on the spectra, using the abbreviations: s for singlet, sl for broad singlet, d for doublet, dd doublet of doublet, t for triplet, tl for trilpet broad, q for quadruplet and m for multiplet.

13C NMR spectra were recorded at 100 MHz in the same spectrometers that 1H NMR spectra. For the interpretation of certain signals, we also performed 2-dimensional heteronuclear correlation between the proton and carbon 13 of HSQC (^{1}H detection mode *Hetero Single-Quantum Correlation*)

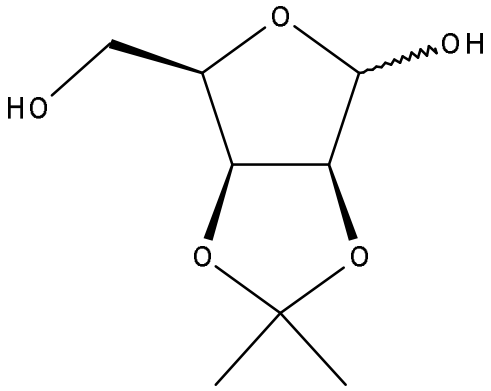
The results of mass specter were expressed as a percentage of the most intense peak as a function of mass / charge ratio (m / z).

The optical rotations (α) are determined at room temperature (20° C), using a polimeter, a sodium lamp at 589 nm and the length of the cell path is 1 dm.

Quantitative evaluation of antioxidant activity was done following the methodology described in the literature, with minor modifications, monitoring the consumption of DPPH free radical by the samples by measuring the decrease in the absorbance of solutions of different concentrations. These measurements were made in UV-Vis spectrophotometer at 515 nm, and the 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid (Trolox) as positive control.

2. Compounds description

2,3-O-isopropylidene-ß-D-ribofuranose (1)



$C_8H_{14}O_5$

M.W=190.19

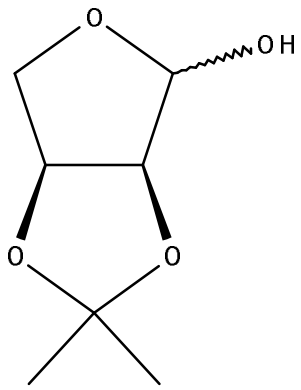
A solution of D-ribose (10g; 66,6mmol) was dissolved in 100mL of acetone at 0ºC. The H_2SO_4 (0,25mL; 4,5mmol) was added and was allowed to react for one hour at room temperature. The reaction was quenched with 14mL of $NaHCO_3$ to neutralize the solution. The solution was diluted with ethyl acetate, then filtered over celite then dried with $MgSO_4$ then evaporated in a rotary and co-evaporated with a toluene solution.

Yield: 98%
Rf = 0.7 (EA/ PE: 90/10)
M.S (IS): m/z= 213.0 $[M + Na]^+$, 173.0 $[M - OH]^+$, 403 $[2M + Na]^+$.
$[\alpha]_{20}$ = -20 ($CDCl_3$)
IR: 3366, 2940, 1456, 1376, 1209, 1159, 1063, 1035, 876.

^{1}H NMR (400MHz, $CDCl_3$) δ = 1.27 (s, 3H, $C(CH_3)_2$), 1.43 (s, 3H, $C(CH_3)_2$), 3.63-3.69 (m, 2H, CH_2-5), 4.34 (t, 1H, J= 2.8Hz, CH-4), 4.52 (d, 1H, $J_{3,2}$= 6Hz, CH-3), 4.76 (d, 1H, $J_{2,1}$= 6Hz, CH-2), 5.35 (s, 1H, CH-1).

^{13}C NMR (100 MHz, $CDCl_3$) δ = 24.7 ($C(\underline{C}H_3)_2$), 27.9 ($C(\underline{C}H_3)_2$), 63.6 (CH_2-5), 81.7 (CH-2), 86.8 (CH-3), 87.7 (CH-4), 102.8 (CH-1), 112.3 ($\underline{C}(CH_3)_2$).

2,3-O-isopropylidene-L-erythrose (2)



$C_7H_{12}O_4$

M.W=160.19

A solution of 2,3-O-isopropylidene-ß-D-ribofuranose (2.037g; 10.71mmol) was dissolved in 20mL of MeOH at 0ºc. The $NaBH_4$ (1.5eq; 0.607g; 16.06mmol) was slowly added and allowed to react for one hour and then evaporated in a rotary. The resulting residue was dissolved in a solution of 18/12mL $BuOH/H_2O$, it was slowly added $NaIO_4$ (4eq; 9.163g; 42.84mmol) and was allowed to react for 12 hours at room temperature. The solution was diluted with ethyl acetate and filtered through celite. The organic phase, was washed once with a saturated $NaHCO_3$ solution and once with a saturated NaCl solution, then dried with $MgSO_4$, filtered and evaporated in a rotary. The residue was purified by silica gel column chromatography using a mixture of PE/ EA (70:30) as eluent.

Yield: 58%

Rf = 0.66 (PE/ EA: 70/30)

M.S (IS): m/z= 183.0 [M + Na]+, 143.0 [M - OH]+.

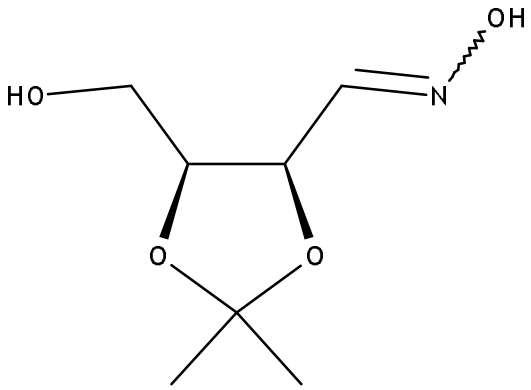
$[\alpha]_{20}$ = 50 ($CDCl_3$)

IR: 3411, 2985, 2941, 1459, 1375, 1331, 1208, 1161, 1097, 1063, 1042, 985, 969, 907, 873, 855, 817, 761, 665.

^{1}H NMR (250MHz, $CDCl_3$) δ = 1.27 (s, 3H, $C(CH_3)_2$), 1.41 (s, 3H, $C(CH_3)_2$), 3.91-4.06 (m, 2H, CH_2-4), 4.24 (ls, 1H, OH), 4.51 (d, 1H, $J_{2,3}$= 6Hz, CH-2), 4.79 (dd, 1H, $J_{3,2}$= 6Hz, J_{3-4}=3.2, CH-3), 5.35 (s, 1H, CH-1).

^{13}C NMR (100MHz, $CDCl_3$) δ = 24.8 ($C(\underline{C}H_3)_2$), 26.3 ($C(\underline{C}H_3)_2$), 71.9 (CH_2-4), 80.1 (CH-3), 85.3 (CH-2), 101.8 (CH-1), 112.4 ($\underline{C}(CH_3)_2$).

2,3-O-isopropylidene-L-erythrose oxime (3)



$C_7H_{13}NO_4$

M.W=175.18

A solution of 2,3-O-isopropylidene-L-erythrose (6.24g; 38.96mmol) was dissolved in 84mL of pyridine at room temperature. The hidrochloride hidroxylamine (3eq; 8.122g; 116.88mmol) and activated molecular tamis were added and are allowed to react for 48 hours at room temperature. The solution was diluted with ethyl acetate and filtered through celite, evaporated in a rotary and co-evaporated with a toluene solution.

Yield: 73%

Rf = 0.3 (EA/PE: 50/50)

M.S (IS): m/z= 176.0 $[M + H]^+$, 198.0 $[M + Na]^+$, 214.0 $[M + K]^+$.

$[\alpha]_{20}$ = -76 ($CDCl_3$)

AA = 60.3%

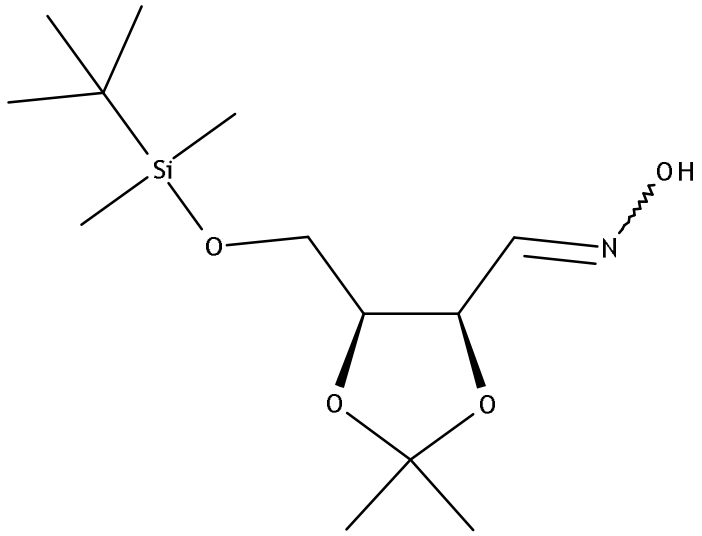
M.P: 46-51 ºC

IR: 3383, 3209, 3082, 2989, 2937, 1438, 1415, 1377, 1337, 1253, 1218, 1161, 1125, 1077, 1043, 984, 927, 908, 883, 837, 792, 698.

^{1}H NMR (250MHz, $CDCl_3$) δ = 1.37 (s, 6H, $C(CH_3)_2$) (Z)), 1.47 (s, 6H, $C(CH_3)_2$) (E)), 3.49-3.74 (m, 4H, J= 12Hz, J= 5Hz, CH_2-4), 4.33-4.36 (m, 1H, CH-3 (E)), 4.51-4.70 (m, 1H, CH-3 (Z)), 4.88 (t, 1H, J= 7.2Hz, CH-2 (E)), 5.26 (ls, 1H, CH-2 (Z)), 6.94 (ls, 1H, CH-1 (Z)), 7.44 (d, 1H, J= 7.2Hz, CH-1 (E)).

^{13}C NMR (100MHz, $CDCl_3$) δ = 24.8 ($C(\underline{C}H_3)_2$), 25.2 ($C(\underline{C}H_3)_2$), 27.3 ($C(\underline{C}H_3)_2$), 27.6 ($C(\underline{C}H_3)_2$), 61.0 (CH_2-4), 61.9 (CH_2-4), 61.9 (CH_2-4), 72.0 (CH_2-4), 74.9 (CH-2 (E)), 78.4 (CH-3), 78.5 (CH-3), 109.6 ($\underline{C}(CH_3)_2$), 110.1 ($\underline{C}(CH_3)_2$), 149.0 (CH-1 (E)).

4-O-tert-butyldimethylsislyl-2,3-O-isopropylidene-L-erythrose oxime (4)



$C_{13}H_{27}NO_4Si$

M.W= 289

A solution of 2,3-O-isopropylidene-L-erythrose oxime **(**4.989g; 28.48mmol) was dissolved in 45 mL of pyridine at room temperature. The tertbutyldimethylsilyl-Cl(1.2eq; 5.15g; 34.18mmol) was added and was allowed to react for 12 hours at room temperature. The solution was diluted with ethyl acetate. The organic phase was washed once with water, HCl (1M) solution and once with a saturated NaCl solution, was dried with $MgSO_4$, filtered and evaporated in a rotary. The residue was purified by silica gel column chromatography using a mixture of PE/EA (98:2) as eluent.

Yield: 19%
Rf: 0.31 (PE/EA: 95/5)
M.S (IS): m/z=290.1 $[M+H]^+$, 312.1 $[M+Na]^+$.
IR: 3192, 1440, 1374, 1254, 1220, 1078, 1046, 983, 883, 838, 791.

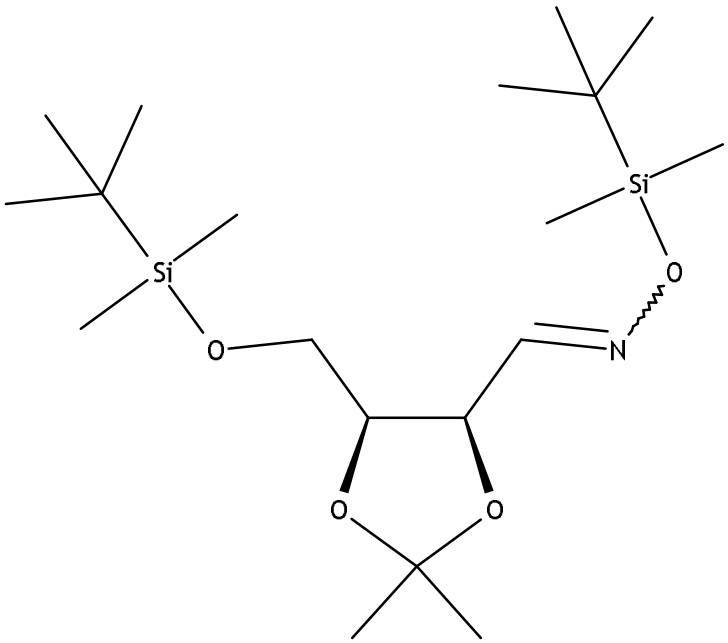
Classical signs of the E isomer: **^{1}H RMN** (400 MHz, CDCl3) δ = 0.0 (m, 6H, $Si(CH_3)_2$), 0.82 (s, 9H, $SiC(CH_3)_3$), 1.30 (s, 6H, $C(CH_3)_2$), 3.61 (m, 2H, CH-4), 4.62 (dd, 1H, $J_{2,3}$= 6.8 Hz, CH-3), 5.22 (dd, 1H, CH-2), 7.36 (d, 1H, $J_{1,2}$= 4.8 Hz, CH=N).

Classical signs of the Z isomer: **^{1}H RMN** (400 MHz, CDCl3) δ = 0.10 (m, 6H, $Si(CH_3)_2$), 0.82 (m, 9H, $SiC(CH_3)_3$), 1.41 (s, 6H, $C(CH_3)_2$), 3.61 (m, 2H, CH-4), 4.67 (t, 1H, $J_{3,4}$= 7.2 Hz, CH-3), 5.19 (dd, 1H, CH-2), 6.84 (d, 1H, $J_{1,2}$= 4.8 Hz, CH=N).

Classical signs of the E isomer: **^{13}C RMN** (100MHz, $CDCl_3$) δ = -5.56 ($Si(\underline{C}H_3)_2$), -5.48 ($Si(\underline{C}H_3)_2$), 18.1 ($Si\underline{C}(CH_3)_3$), 25.6 ($C(\underline{C}H_3)_2$), 25.9 ($C(\underline{C}H_3)_2$), 27.0 ($SiC(\underline{C}H_3)_3$), 27.2 ($SiC(\underline{C}H_3)_3$), 27.5 ($SiC(\underline{C}H_3)_3$), 61.4 (CH_2-4), 74.9 (CH-2), 78.3 (CH-3), 109.5 ($\underline{C}(CH_3)_2$), 147.8 (N=CH).

Classical signs of the Z isomer: **^{13}C RMN** (100MHz, $CDCl_3$) δ = -5.40 ($Si(CH_3)_2$), -5.37 ($Si(\underline{C}H_3)_2$), 18.2 ($SiC(\underline{C}H_3)_3$), 25.8 ($C(\underline{C}H_3)_2$), 25.9 ($C(\underline{C}H_3)_2$), 27.1 ($Si\underline{C}(CH_3)_3$), 27.4 ($Si\underline{C}(CH_3)_3$), 27.6 ($\underline{C}(CH_3)_3$), 62.5 (CH_2-4), 74.7 (CH-2), 78.5 (CH-3), 109.1 ($\underline{C}(CH_3)_2$), 150.3 (N=CH).

N-[tert-butyl(dimethyl)silyl]oxy-1-[(4R,5S)-5-[[tert-butyl(dimethyl)silyl]oxymethyl]-2,2-dimethyl-1,3-dioxolan-4-yl]methanimine (5)



$C_{19}H_{41}NO_4Si_2$

M.W=403

A solution of 2,3-O-isopropylidene-L-erythrose oxime (0.5g; 2.85mmol) was dissolved in 4.5mL of pyridine at room temperature. The tertbutyldimethylsilyl.Cl (2.5eq; 1.076g; 7.136mmol) was added and was allowed to react for 12 hours at room temperature. The solution was diluted with ethyl acetate. The organic phase was washed once with water and once with saturated NaCl solution, dried with $MgSO_4$, filtered and evaporated in a rotary. The residue was purified by silica gel column chromatography using a mixture of PE/ EA (98 :2) as eluent.

Yield: 74%

Rf =0.71 (PE/ EA: 95/5)

M.S (IS): m/z= 404.0 $[M + H]^+$, 426.0 $[M + Na]^+$.

$[\alpha]_{20}$ = -53 ($CDCl_3$)

AA = 23.2%

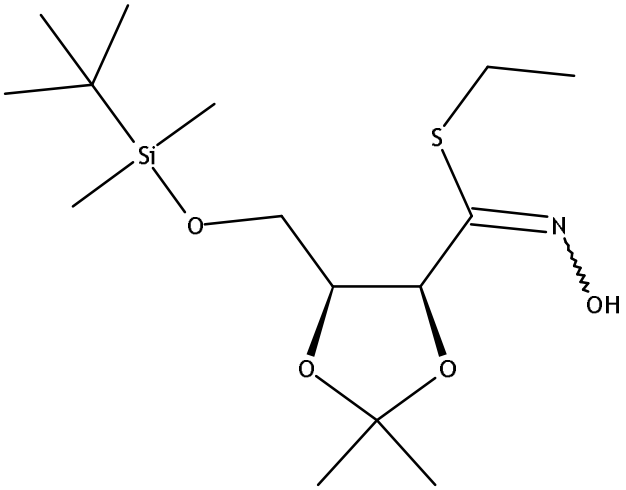
IR: 2955, 2930, 2888, 2858, 1471, 1380, 1251, 1216, 1143, 1094, 1007, 983, 936, 878, 834, 778, 722, 673.

^{1}H NMR (400MHz, $CDCl_3$) δ (E+Z mixture): 0,04 (d, 12H, $Si(CH_3)_2$), 0,14 (m, 12H, $Si(CH_3)_2$), 0,88 (d, 18H, $Si(CH_3)_3$), 0,91 (d, 18H, $Si(CH_3)_3$), 1,36 (d, 6H, $C(CH_3)_2$), 1,49 (d, 6H, $C(CH_3)_2$), 3,48 (dd, 1H, J_1=4,58Hz, J_2=11,02Hz, CH-4b), 3,61-3,71 (m, 1H, H-4a), 4,24-4,28 (m, 1H, CH-3 (E)),

4,39-4,4 (m, 1H, CH-3 (Z)), 4,71 (dd, 1H, J_1=6,79Hz, J_2=8,11Hz, CH-2 (E)), 5,24 (dd, 1H, J_1=7,26Hz, J_2=4,21Hz, CH-2 (Z)), 7,08 (d, 1H, J_1= 4,29Hz, CH-1 (Z)), 7,49 (d, 1H, J_1=8,19Hz, CH-1 (E))

^{13}C NMR (100MHz, $CDCl_3$) δ (E+Z mixture): 5,2 ($Si(CH_3)_2$), 5,2 ($Si(CH_3)_2$), 18,4 ($\underline{C}(CH_3)_2$) 25,3 ($C(\underline{C}H_3)_2$), 25,5 ($C(\underline{C}H_3)_2$), 26,1 ($SiC(\underline{C}H_3)_3$), 26,2 ($SiC(\underline{C}H_3)_3$), 27,3 ($C(\underline{C}H_3)_2$), 27,9 ($C(\underline{C}H_3)_2$), 61,7 (CH_2-4), 62,9 (CH_2-4), 72,1 (CH-2(Z)), 75,2 (CH-2 (E)), 78,7 (CH-3 (E)), 79,1 (CH-3 (Z)), 109,3 ($Si\underline{C}(CH_3)_3$), 109,7 ($Si\underline{C}(CH_3)_3$), 152,2 (CH-1 (E)), 155,0 (CH-1 (Z)).

(Z)-4-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-N-hydroxy-L-erythronimidothioate de s'ethyle (6)



$C_{15}H_{31}NO_4SSi$

M.W=349.12

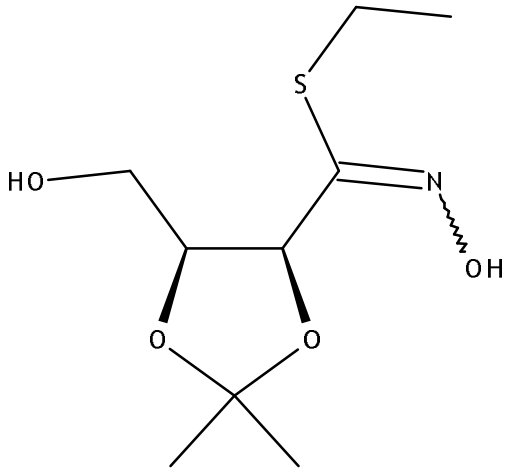
A solution of 4-O-tert-butyldimethylsislyl-2,3-O-isopropylidene-L-erythrose oxime (1.599g; 5.53mmol) was dissolved in 55mL of DMF at room temperature. The NCS (1.5eq; 1.108g; 8.295mmol) was introduced and was allowed to react for 4 hours at room temperature. The Et_3N (3eq, 2.31mL, 16.598mmol) and EtSH (3eq, 1.24mL, 16.598mmol) was added and was allowed to react for 12 hours at room temperature. The solution was diluted with ethyl acetate. The organic phase was washed four times with water and once with saturated NaCl solution, dried with $MgSO_4$, filtered and evaporated in a rotary. The residue was purified by silica gel column chromatography using a mixture of PE/ EA (90:10) as eluent.

Yield: 40%
Rf = 0.24 (PE/ EA: 90/10)
M.S (IS): m/z= 350.0 $[M + H]^+$, 372.0 $[M + Na]^+$, 388.0 $[M + K]^+$.
$[\alpha]_{20}$ = -46 ($CDCl_3$)
AA = 36.8%
IR: 3288, 2930, 2857, 1601, 1462, 1378, 1252, 1214, 1161, 1078, 992, 900, 835, 777, 668.

^{1}H NMR (400MHz, $CDCl_3$) δ = 0.05 (s, 3H, $Si(CH_3)_2$), 0.05 (s, 3H, $Si(CH_3)_2$), 0.87 (s, 9H, $SiC(CH_3)_3$), 1.29 (t, 3H, J= 8.0Hz, $CH_2\underline{CH_3}$), 1.37 (s, 3H, $C(CH_3)_2$), 1.53 (s, 3H, $C(CH_3)_2$), 2.97-3.10 (m, 2H, SCH_2), 3.73 (dd, 1H, $J_{4,\,4'}$= 10.6Hz, $J_{4',\,3}$= 6.2Hz, CH-4b), 3.79 (dd, 1H, $J_{4,\,4'}$= 10.6Hz, $J_{4,\,3}$= 6.0Hz, CH-4a), 4.37 (q, 1H,$J_{3,\,2}$= 6.7Hz, CH-3), 4.89 (d, 1H, $J_{2,3}$= 6.7 Hz, CH-2), 9.09 (ls, 1H, N-OH).

^{13}C NMR (100MHz, $CDCl_3$) δ = -5.2 ($Si(CH_3)_2$), -5.1 ($Si(CH_3)_2$), 15.13 ($\underline{C}H_3CH_2S$), 18.6 ($\underline{C}(CH_3)_3$), 25.3 ($C(\underline{C}H_3)_2$), 25.8 (CH_2S), 26.2 ($SiC(\underline{C}H_3)_3$), 27.1 ($C(\underline{C}H_3)_2$), 62.6 (CH_2-4), 77.3 (CH-2), 79.1 (CH-3), 109.72 ($\underline{C}(CH_3)_2$), 149.8 (C=N).

(Z)-2,3-O-isopropylidene-N-hydroxy-L-erythronimidothioate de s'ethyle (7)



$C_9H_{31}NO_4SSi$

M.W=349.12

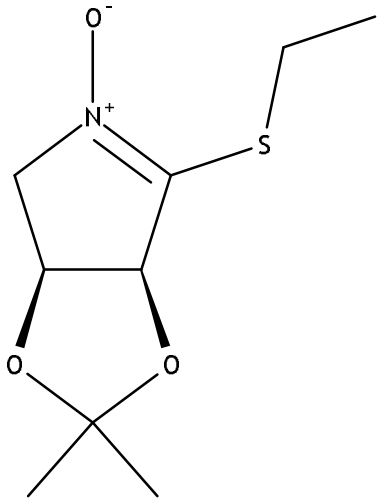
A solution of (Z)-4-O-tert-butyldimethylsislyl-2,3-O-isopropylidene-N-hydroxy-L-erythronimidothioate de s'ethyle (0.584g; 1.673mmol) was dissolved in 10mL of THF at room temperature. The TBAT (1.4eq; 1.108g; 2.342mmol) was added and was allowed to react for 12 hours at room temperature. The solution was diluted with ethyl acetate. The organic phase was washed twice with water and once with a saturated NaCl solution, dried with $MgSO_4$, filtered and evaporated in a rotary. The residue was purified by silica gel column chromatography using a mixture of EA /PE (50:50) as eluent.

Yield: 41%
Rf = 0.3 (EA /PE: 50/50)
M.S (IS): m/z= 236.0 $[M + H]^+$, 258.0 $[M + Na]^+$, 274.0 $[M + K]^+$.
$[\alpha]_{20}$ = 18 ($CDCl_3$)
M.P: 88-94 °C
AA = 37.7 %
IR: 3356, 2992, 1688, 1451, 1375, 1269, 1223, 1208, 1056, 998, 895, 859, 806, 740.

^{1}H NMR(400MHz, $CDCl_3$) δ = 1.33 (t, 3H, J= 7.6 Hz, $SCH_2\underline{CH_3}$), 1.40 (s, 3H, $C(CH_3)_2$), 1.53 (s, 3H, $C(CH_3)_2$), 3.11 (m, 2H, $S\underline{CH_2}CH_3$), 3.68 (m, 1H, CH_2-4b), 3.79 (m, 1H, CH_2-4a), 4.40 (q, 1H, $J_{2\text{-}3}= J_{3\text{-}4}$= 4.4, CH-3), 4.89 (d, 1H, $J_{2\text{-}3}$= 6.0, CH-2), 9.40 (s, 1H, N-OH).

^{13}C NMR (100MHz, $CDCl_3$) δ = 15.2 ($SCH_2\underline{C}H_3$), 25.5 ($C(\underline{C}H_3)_2$), 25.8 ($S\underline{C}H_2CH_3$), 27.2 ($C(\underline{C}H_3)_2$), 61.8 (CH_2-4), 75.2 (CH-2), 78.8 (CH-3), 150.8 ($\underline{C}(CH_3)_2$), 158.0 (C=N).

(3S,4S)-2-(erythylthio)-3,4-isopropylidenedioxy-3,4-dihydro-5H-pyrrole-1-oxyde (8)



$C_9H_{15}NO_3S$

M.W=217.29

A solution of (Z)-2,3-O-isopropylidene-N-hydroxy-L-erythronimidothioate de s'ethyle (0.16g; 0.68mmol) was dissolved in 11mL of anhydrous THF at 70º C, in reflux. The Ph_3P (1.1eq; 0.196g; 0.748mmol) and DEAD (3.3eq, 0.35mL, 2.24mmol) were introduced and was allowed to react for 4 hours at 70º C, in reflux. It was evaporated in a rotary. The residue was purified by silica gel column chromatography using a mixture of EA / MeOH (90:10) as eluent.

Yield: 50%

Rf = 0.05 (EA/MeOH: 90/10)

M.S (IS): m/z= 218.0 $[M + H]^+$, 240.0 $[M + Na]^+$, 244 $[M + NH_4]^+$, 256.0 $[M + K]^+$.

$[\alpha]_{20}$ = 143 ($CDCl_3$)

M.P: 139-143 ºC

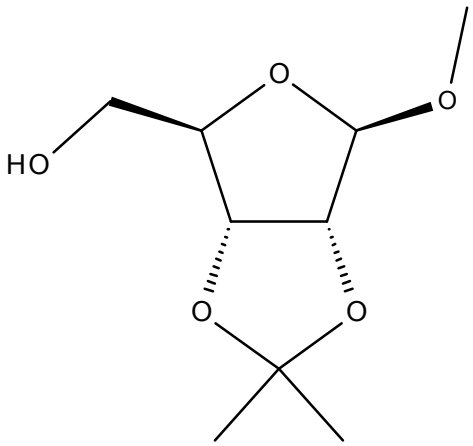
AA = 32.4%

IR: 2975, 1730, 1570, 1451, 1423, 1383, 1327, 1300, 1277, 1261, 1235, 1205, 1154, 1077, 1022, 989, 968, 948, 899, 865, 836, 802, 765, 706, 663.

^{1}H NMR (400MHz, $CDCl_3$) δ = 1.38 (lt, 3H, J= 7.5 Hz, $SCH_2\underline{CH_3}$), 1.39 (s, 3H, $C(CH_3)_2$), 1.44 (s, 3H, $C(CH_3)_2$), 3.14 (q, 2H, $S\underline{CH_2}CH_3$), 4.07 (d, 1H, $J_{4\text{-}4'}$= 14.7 Hz, CH-4'), 4.14 (dd, 1H, $J_{4\text{-}4'}$= 14.7 Hz, $J_{4\text{-}3}$= 5.19, CH-4), 4.90(lt, 1H, J= 6.41 Hz, CH-3), 5.35 (d, 1H, CH-2).

^{13}C NMR (100MHz, $CDCl_3$) δ = 15.7 ($SCH_2\underline{C}H_3$), 23.6 ($S\underline{C}H_2CH_3$), 26.2 ($C(\underline{C}H_3)_2$), 27.3 ($C(\underline{C}H_3)_2$), 66.6 (CH_2-4), 73.2 (CH-3), 81.8 (CH-2), 112.9 ($\underline{C}(CH_3)_2$), 144.0 (C-1).

1-O-methyl-2,3-O-isopropylidene-ß-D-ribofuranose (9)



$C_9H_{16}O_5$

M.W=204.22

To a solution of D-ribose (10g, 66,6mmol) in dry acetone (50.0mL) and dry methanol (50.0mL), was slowly added sulfuric acid con. (5.0mL). The stirred mixture was kept for 48h at room temperature, and the reaction was quenched with $NaHCO_3$ to neutralize the solution. The mixture was filtered and concentrated to a reduced volume. The resulting residue was dissolved with water (200mL) and washed with ethyl acetate (3×150mL). After drying over anhydrous magnesium sulfate, the solvent was evaporated.

Yield: 80%

Rf = 0.79 (EA/PE: 90/10)

M.S (IS): m/z= 205.0 $[M + H]^+$.

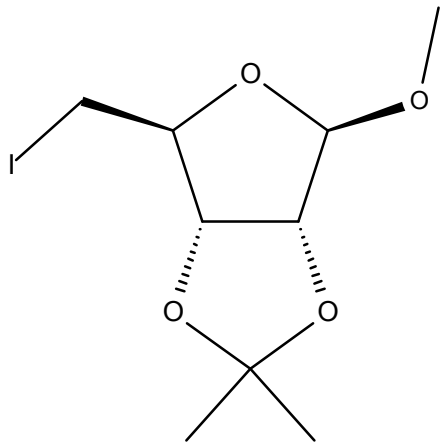
$[\alpha]_{20}$ = -63 ($CDCl_3$)

IR: 3454, 2987, 2940, 2835, 1457, 1374, 1272, 1240, 1209, 1160, 1088, 1040, 1008, 960, 868, 774.

^{1}H NMR (400MHz, $CDCl_3$) δ = 1.28 (s, 3H, $C(CH_3)_2$), 1.44 (s, 3H, $C(CH_3)_2$), 3.23 (dd, 1H, OH), 3.39 (s, 3H, OCH_3), 3.54-3.63 (m, 2H, CH_2-4), 4.37 (ls, 1H, CH-4), 4.55 (d, 1H, $J_{3,2}$= 4.0 Hz, CH-3), 4.79 (d, 1H, CH-2), 4.93 (s, 1H, CH-1).

^{13}C NMR (100MHz, $CDCl_3$) δ= 24.8 ($C(\underline{C}H_3)_2$), 26.5 ($C(\underline{C}H_3)_2$), 55.6 (OCH_3), 64.1 (CH_2-5), 81.6 (CH-2), 85.9 (CH-3) 88.4 (CH-4), 110.1 (CH-1), 112.2 ($\underline{C}(CH_3)_2$).

Methyl-5-deoxy-5-iodo-2,3-O-isopropylidene-ß-D-ribofuranoside (10)



$C_9H_{15}IO_4$

M.W=314.12

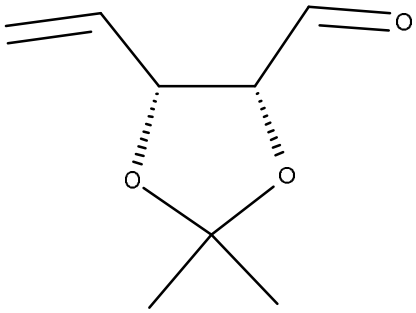
A solution of 1-O-methyl-2,3-O-isopropylidene-ß-D-ribofuranose (1g, 4.897mmol) was dissolved in 16.3 mL of toluene at 80ºC with Ph_3P (2,565g, 9,79mmol, 2eq.) iodine (1,741g, 6,856mmol, 1,4eq) and imidazole (0,664g, 9,79mmol, 2eq.). The solvent was evaporated. The solution was diluted with ethyl acetate. The organic phase was washed twice with a saturated $Na_2S_2O_3$ solution and once with a saturated NaCl solution, dried with $MgSO_4$, filtered and evaporated in a rotary. The residue was purified by silica gel column chromatography using a mixture of PE/ EA (95:5) as eluent.

Yield: 71%
Rf = 0.77 (PE/EA: 95/5)
M.S (IS): m/z= 315.0 $[M + H]^+$, 337.0 $[M + Na]^+$.
$[\alpha]_{20}$ = -54 ($CDCl_3$)
AA = 56%
IR: 2987, 2936, 2833, 1441, 1373, 1271, 1238, 1209, 1160, 1091, 1063, 1016, 982, 955, 925, 867, 818, 796.

^{1}H NMR (400MHz, $CDCl_3$) δ = 1.28 (s, 3H, $C(CH_3)_2$), 1.43 (s, 3H, $C(CH_3)_2$), 3.11 (t, 1H, $J_{5\text{-}5'}$= 9.99 Hz, CH_2-5'), 3.25 (dd, 1H, $J_{5\text{-}5'}$= 9.99 Hz, $J_{5\text{-}4}$= 6.07 Hz, CH_2-5), 3.32 (s, 3H, O-CH_3), 4.39 (dd, 1H, CH-4), 4.59 (d, 1H, $J_{3\text{-}2}$= 5.91 Hz, CH-3), 4.72 (d, 1H, CH-2), 5.0 (s, 1H, CH-1).

^{13}C NMR (100MHz, $CDCl_3$) δ = 6.9 (CH_2-5), 25.1 ($C(\underline{C}H_3)_2$), 26.5 ($C(\underline{C}H_3)_2$), 55.3 (O-CH_3), 83.1 (CH-2), 85.4 (CH-3), 87.5 (CH-4), 109.7 (CH-1), 112.7 ($\underline{C}(CH_3)_2$).

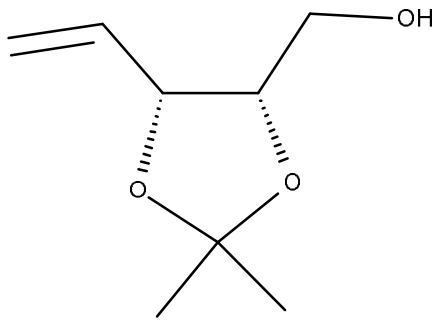
4,5-dideoxy-2,3-O-isopropylidene-D-erythro-pent-4-enose (11)



$C_8H_{12}O_3$
M.W= 156.18

A solution of methyl-5-deoxy-5-iodo-2,3-O-isopropylidene-β-D-ribofuranoside (1g, 3.183mmol) was dissolved in 6.4 mL of anydre THF at -78ºC. The BuLi (3.4eq; 1mL; 10.824mmol) was added and was allowed to react for 2 hours at -78ºC. The reaction mixture is used directly in next reaction.

4,5-dideoxy-2,3-O-isopropylidene-D-erythro-pentitol (12)



$C_8H_{14}O_3$

M.W= 158.19

A solution of 4,5-dideoxy-2,3-O-isopropylidene-D-erythro-pent-4-enose was dissolved in 4.8 mL of EtOH at 0ºC. The $NaBH_4$ (2 eq; 0.072g; 1.91mmol) was added and was allowed to react for 12 hours at room temperature. The solution was diluted with a saturated NaCl solution. The organic phase was washed three times with ethyl acetate, dried with $MgSO_4$, filtered and evaporated in a rotary. The residue was purified by silica gel column chromatography using a mixture of PE/ EA (80:20) as eluent.

Yield: 70%

Rf = 0.13 (PE/EA: 80/20)

M.S (IS): m/z = 159.0 $[M + H]^+$, 181.0 $[M + Na]^+$, 197.0 $[M + K]^+$.

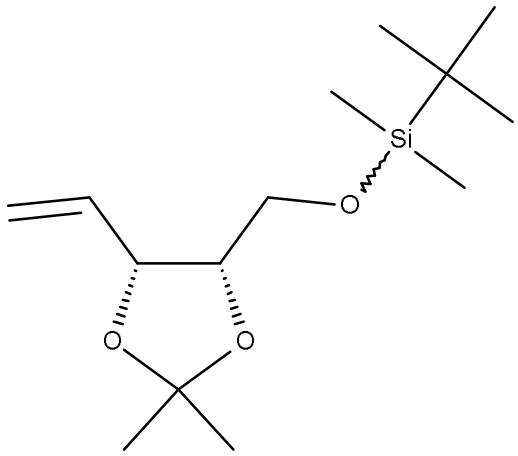
$[\alpha]_{20}$ = 40 ($CDCl_3$)

IR: 3421, 2987, 2936, 1645, 1374, 1247, 1213, 1165, 1113, 1044, 995, 925, 875, 790.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.37 (s, 3H, $C(CH_3)_2$), 1.48 (s, 3H, $C(CH_3)_2$), 2.12 (ls, 1H, OH), 3.55 (dd, 2H, J= 5.8Hz, J= 1Hz, CH_2-1), 4.24 (q, 1H, J =6 Hz, CH-2), 4.62 (t, 1H,J =6.8, CH-3), 5.25 (dd, 1H, J= 10.2Hz, J =1 Hz, CH_2-5), 5.33-5.34 (m, 1H, CH_2-5), 5.79-5.88 (m, 1H, CH-4).

13C NMR (100MHz, CDCl3) δ = 25.4 ($C(\underline{C}H_3)_2$), 27.9 ($C(\underline{C}H_3)_2$), 62.2 (CH_2-1), 78.4 (CH-3), 78.5 (CH-2), 109.0 ($\underline{C}(CH_3)_2$), 119.0 (CH_2-5), 133.2 (CH-4).

Tert-butyl-[[(4S,5R)-2,2-dimethyl-5-vinyl-1,3-dioxolan-4-yl]methoxy]-dimethyl-silane (13)



$C_{14}H_{28}O_3Si$

M.W= 275.5

A solution of 4,5-dideoxy-2,3-O-isopropylidene-D-erythro-pentitol(0.1246g, 0.7876mmol) was dissolved in 3mL pyridine at room temperature. The tertbutyldimethylsilyl-Cl (1.2eq; 0.1419g; 0.945mmol) was added and was allowed to react for 12 hours at room temperature. The solution was diluted with ethyl acetate. The organic phase was washed once with water and once with a saturated NaCl solution, was dried with $MgSO_4$, filtered and evaporated in a rotary. The residue was purified by silica gel column chromatography using a mixture of PE/EA (95:5) as eluent.

Yield : 70%
Rf = 0.72 (PE/EA: 90/10)
M.S (IS): m/z= 277.0 $[M + Na]^+$.
$[\alpha]_{20}$ = 0.3 ($CDCl_3$)
AA = 67.5%
IR: 2987, 2930, 2858, 1471, 1372, 1251, 1215, 1094, 1049, 1007, 924, 835, 775, 667.

1H NMR (400MHz, CDCl3) δ = 0.05 (s, 6H, $Si(CH_3)_2$), 0.88 (s, 9H, $SiC(CH_3)_3$), 1.36 (s, 1H, $C(CH_3)_2$9, 1.47 (s, 1H, $C(CH_3)_2$), 3.61 (d, 2H, J =5.2 Hz, CH_2-1), 4.19 (q, 1H, J =12.4 Hz, J =6Hz, CH-3), 6.62 (t, 1H, J =6.8Hz, CH-2), 5.21 (dd, 1H, J = 10.4Hz, J =1.2 Hz, CH_2-5 (Z)), 5.35 (d, 1H, J =1.4Hz, CH_2-5 (E)), 5.83-5.92 (m, 1H, CH-4).

^{13}C NMR (100MHz, $CDCl_3$) δ = -5.2 ($Si(\underline{C}H_3)_2$), -5.2 ($Si(\underline{C}H_3)_2$), 18.5 ($Si\underline{C}(CH_3)_3$), 25.6 ($C(\underline{C}H_3)_2$), 26.1 ($SiC(\underline{C}H_3)_3$), 28.0 ($C(\underline{C}H_3)_2$), 62.5 (CH_2-1), 78.7 (CH-3), 78.9 (CH-2), 108.7 ($\underline{C}(CH_3)_2$), 118.0 (CH_2-5), 133.9 (CH-4).

3. Determination of antioxidant activity

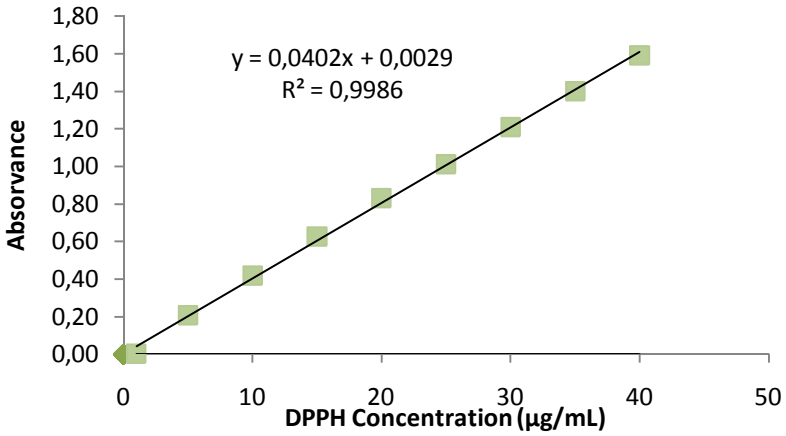
3.1 Construction of calibration curve

First were prepared 50 mL of stock solution of DPPH in methanol at a concentration of 60 mg/mL, kept cool and protected from light. Dilutions were made 40, 35, 30, 25, 20, 16, 10, 5, 1 mg/mL.

The calibration curve was constructed from the 515 nm absorbance values of all solutions (1 to 40 mg/mL), measured in glass vials with 1 cm optical path and having a "White" a methanol solution.

The absorbance measurements were made in triplicate and at intervals of 1 minute between each reading.

The equation of the curve of DPPH was Abs= 0,0402C + 0,0029, where C is the concentration of DPPH in the middle, Abs is the absorbance measured at a wavelength of 515 nm and the correlation coefficient R = 0,9986.



Graphic 1 - Calibration curve

3.2 Reading measures the absorbance in the samples and positive control

Solutions of positive control (Trolox) were diluted in methanol at concentrations of 250, 200, 150, 100, 50 and 25 µg /mL. The measures the absorbance of the mixtures reactional (0.3 mL of the positive control and 2.7 mL of DPPH) were made to 515 nm in 1, 2 and 3 minutes, the given one minute to complete one hour. The mixture of methanol (2.7mL) and methanolic solution of the positive control (0.3 mL) was used as white. All the absorbance readings at different concentrations were performed in triplicate.

From the equation of the calibration curve and absorbance values at the time of 60 minutes for each concentration tested, we determined the percentage of remaining DPPH (% $DPPH_{REM}$), according to the equation (1):

$$\%DPPH_{REM} = [DPPH]_{T=t} / [DPPH]_{T=0} \times 100 \quad \textbf{(1)}$$

where $[DPPH]_{T=t}$ is the concentration of DPPH in the middle after the reaction with methanolic solution of the compounds and $[DPPH]_{T=0}$ is the initial concentration of DPPH, or 40 µg/mL .

The effective concentration, amount of antioxidant needed to decrease the initial DPPH concentration by 50% (EC_{50}) was determined from a first-order exponential curve, the abscissa obtained by placing the sample concentrations (µg/mL) or control positive and on the ordinate, the percentage of remaining DPPH (% $DPPH_{REM}$).
The absorbance values at all concentrations tested, at the time of one hour, were also converted to percentage antioxidant activity (AA) determined by the equation:

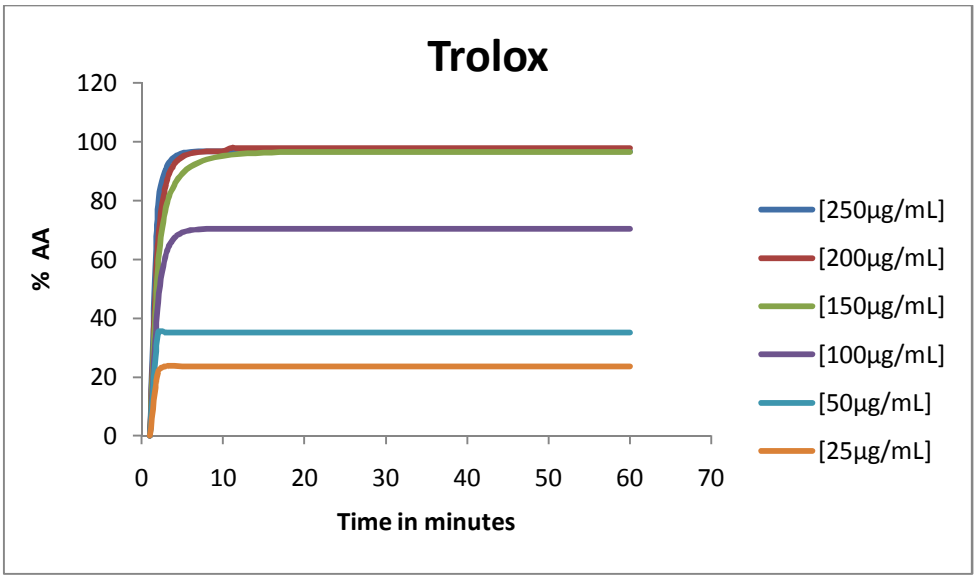
The absorbance values at all concentrations tested, at the time of one hour, were also converted to percentage antioxidant activity (AA) determined by the equation (2):

$$AA\% = \{[Abscontrol - (Abssample - Abswhite)] \times 100\} / Abscontrol \quad \textbf{(2)}$$

Abscontrol where is the initial absorbance of the methanolic solution of DPPH and Abssample is absorbance of the reaction mixture (DPPH + sample) .

3.2.2. Positive control

For positive control it was determined its antioxidant activity over one hour, as well as the remaining DPPH percentage and the efficient concentration. The obtained results are presented in the graphics 2, 3 and 4.

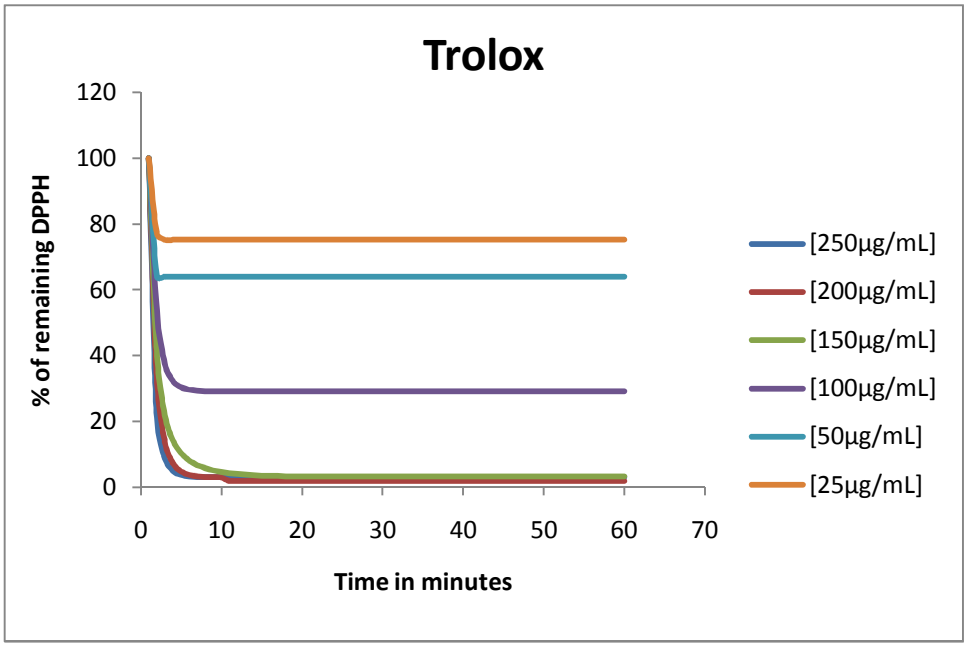


Graphic 2 - Antioxidant activity for the positive control

The maximum values obtained for the different concentrations for the antoxidant activity, in percentage, are presented in table 5.

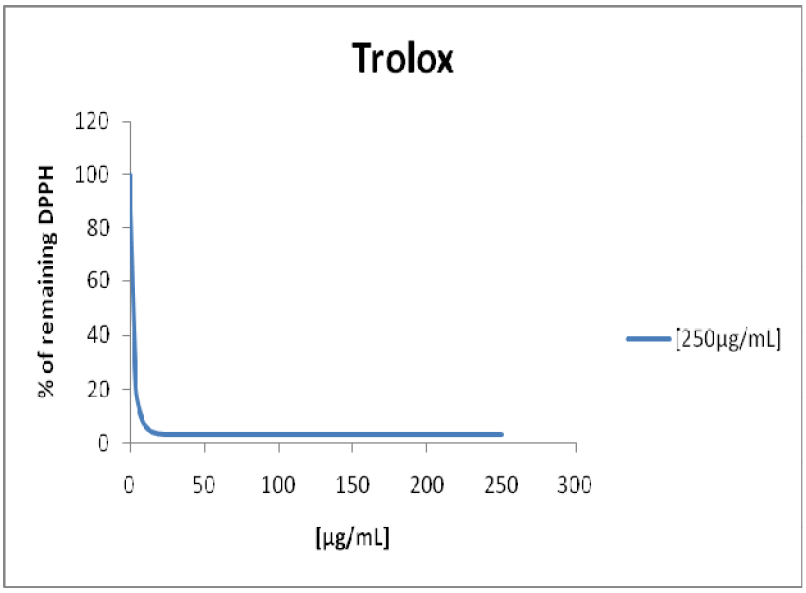
Table 5 - Maximum %AA obtaining for the posite control.

	250µg/mL	200µg/mL	150µg/mL	100µg/mL	50µg/mL	25µg/mL
maximum %AA	96,73%	97,78%	96,46%	70,33%	35,06%	33,75%

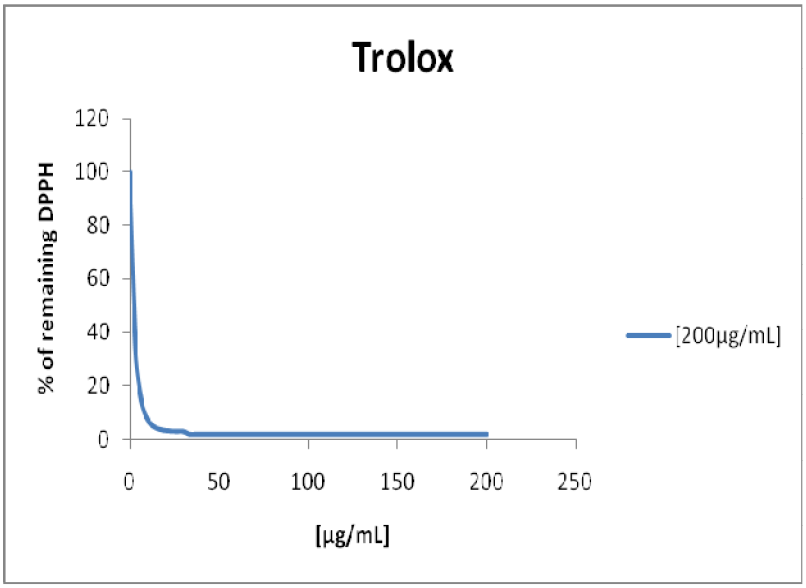


Graphic 3 - % of remaining DPPH for the positive control.

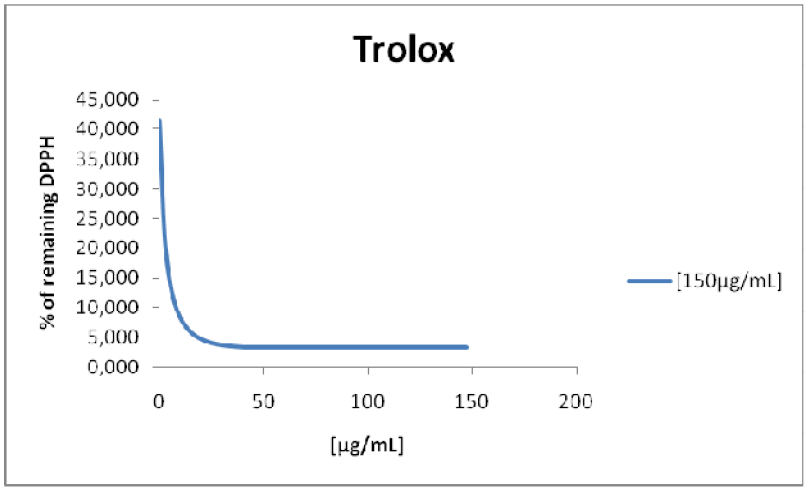
The efficient concentration values were determined graphically.



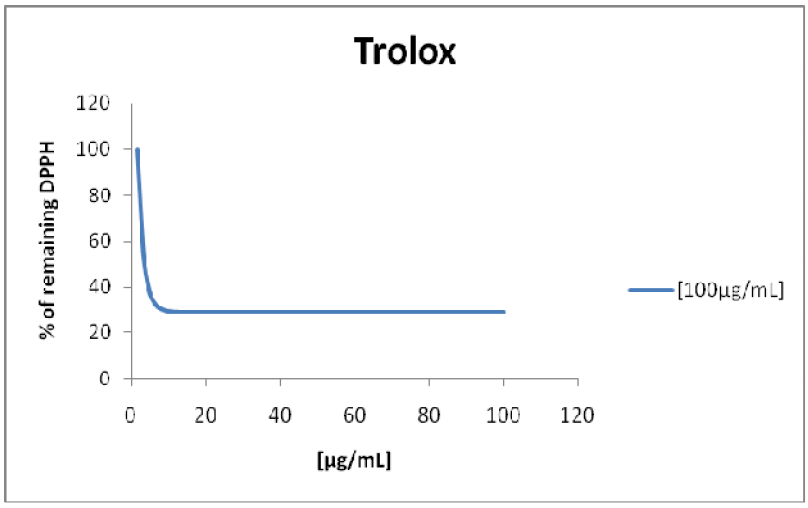
Graphic 4 - Efficient concentration for the positive control



Graphic 5 - Efficient concentration for the positive control



Graphic 6 - Efficient concentration for the positive control



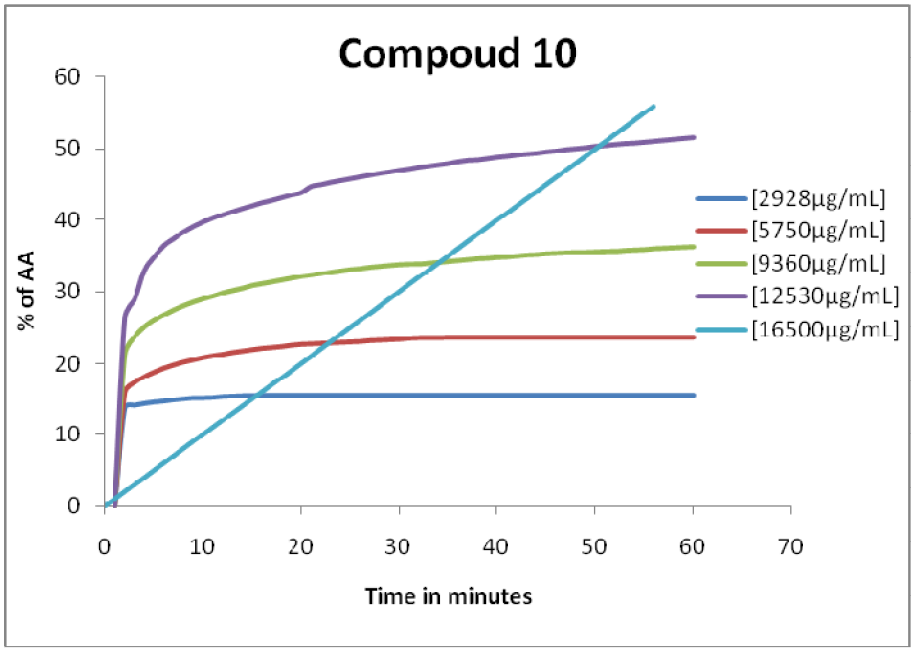
Graphic 7 - Efficient concentration for the positive control

Table 6 - Efficient concentration for the positive control

	250µg/mL	200µg/mL	150µg/mL	100µg/mL
EC_{50}(µg/mL)	1,5	2,5	3,2	4,1

3.2.3 Compound 10

For compound **10** it was determined the antioxidant activity, remaining DPPH percentage and the efficient concentration.

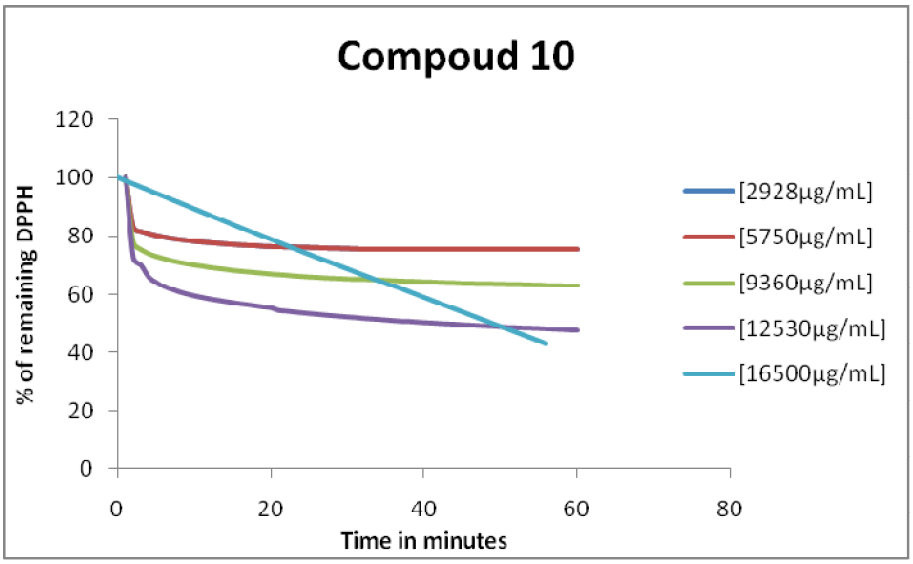


Graphic 8 - Percentage of antioxidant activity for the compound **10**

The maximum values obtained for the different concentrations for the antioxidant activity, in percentage, are presented in table 7.

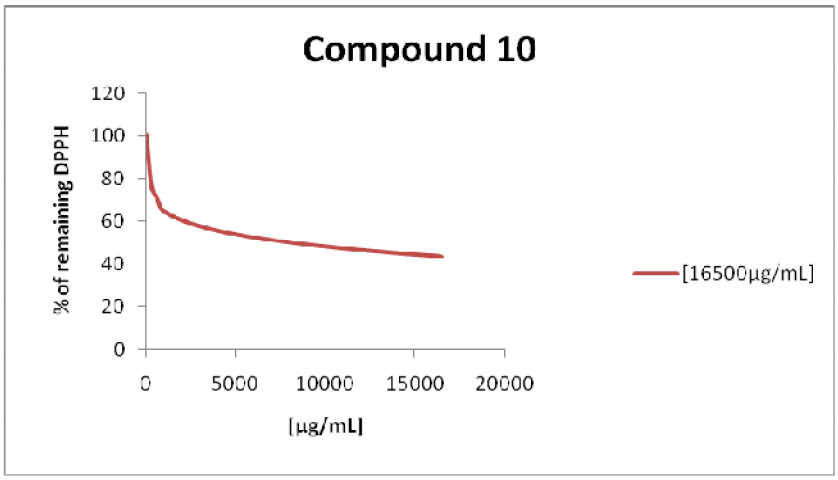
Table 7 - Maximum %AA obtaining for the compound **10**

	16500µg/mL	**12530µg/mL**	**9360µg/mL**	**5750µg/mL**	**2928µg/mL**
maximum %AA	55,99%	51,59%	36,2%	23,6%	15,4%

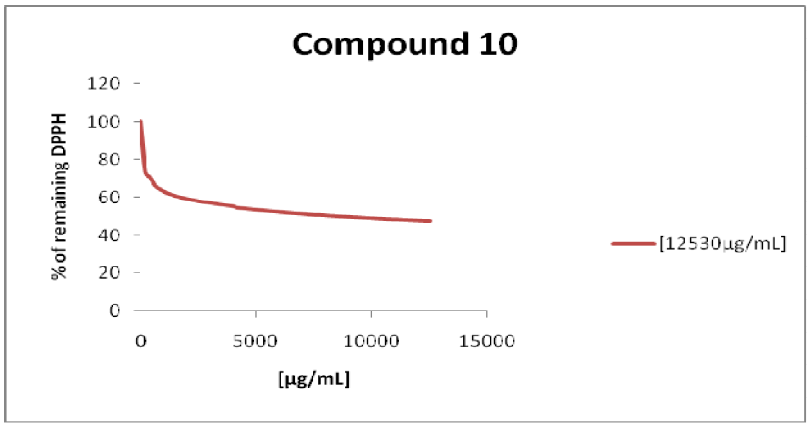


Graphic 9 - Remaining DPPH for the compound **10**

The efficient concentration values were determined graphically.



Graphic 10 - Efficient concentration for the compond **10**.



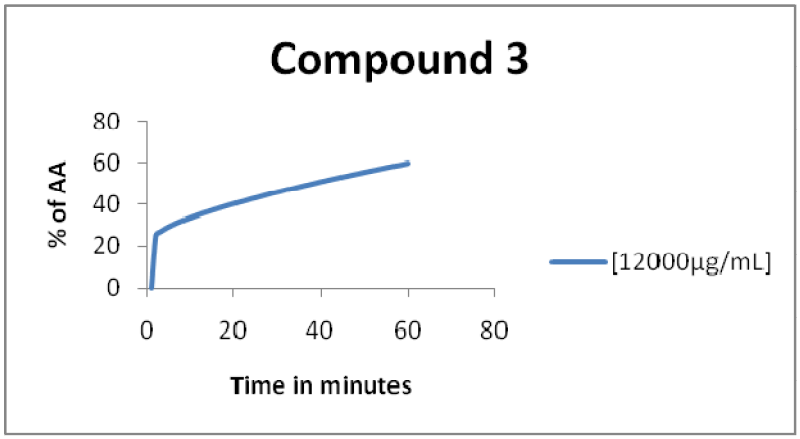
Graphic 11 - Efficient concentration for the compound 10.

Table 8 - Efficient concentration for the compound **10**

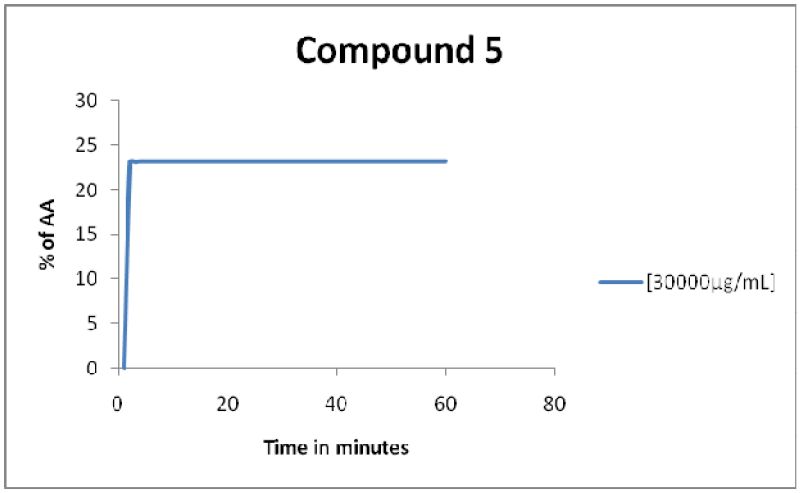
	16500µg/mL	**12530µg/mL**
EC_{50}(µg/mL)	8084	8586

3.2.4 Compound 3, 5, 6, 7, 8 and 13

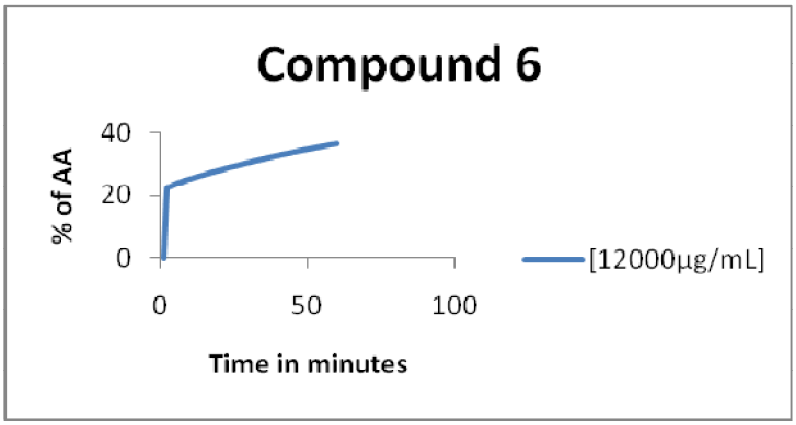
For compounds **3**, **5**, **6**, **7**, **8** e **13** it was determined the percentage of the antioxidant activity.



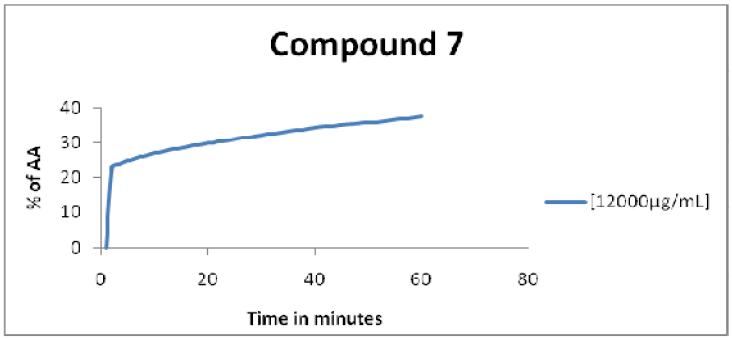
Graphic 12 - % of antioxidant activity for the compound **3**



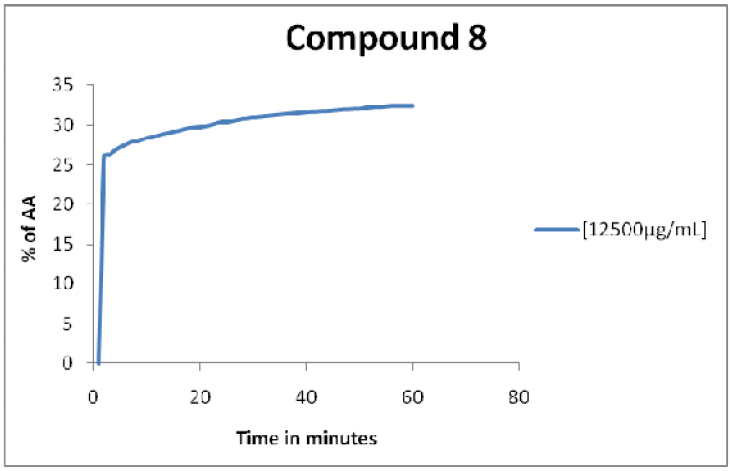
Graphic 13 - % of antioxidant activity for the compound **5**



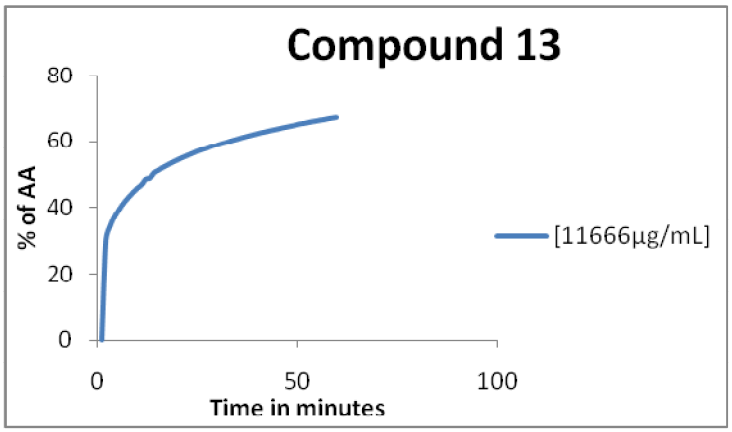
Graphic 14 - % of antioxidant activity for the compound **6**



Graphic 15 - % of antioxidant activity for the compound **7**



Graphic 16 - % of antioxidant activity for the compound **8**



Graphic 17 - % of antioxidant activity for the compound **13**

In table 9 it is presented the percentage of the antioxidant activity for the different compounds for a specific concentration.

Table 9 - % of antioxidant activity

	Compound 3	**Compound 5**	**Compound 6**	**Compound 7**	**Compound 8**	**Compound 10**	**Compound 13**
maximum % of AA	60,33	23,19	36,76	37.70	32,42	55,99	67,5

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