UNIVERSIDADE DA BEIRA INTERIOR
Ciências

Synthesis and characterization of heterocyclic rings linked to sugar moieties

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Bioquímica

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Para os meus pais

Para a minha irmã

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Resumo

Este trabalho teve como objectivo a construção de cadeias ramificadas regio- e estereosselectivas, a parir da D-ribose e D-xilose, para sintetizar de N-óxidos de tio-imidatos derivados de açúcares.

Foram usados três métodos de síntese, dois deles a partir de açúcares, a D-ribose e a D-xilose, e o terceiro método foi iniciado por lactonas, nomeadamente a γ-valerolactona e a D-(-)-pantolactona. A síntese a partir da D-ribose deu origem a um N-óxido de tio-imidato, num rendimento global de 48%. A D-ribose, em acetona, reagiu durante uma hora com H_2SO_4 concentrado, adicionado a 0ºC, originou o composto 2,3-O-isopropilideno-ß-D-ribofuranose (**1**), com os grupos hidroxilo em C-2 e C-3 protegidos, num rendimento de 95%. Este composto em MeOH, reagiu com $NaBH_4$ durante uma hora e a 0ºC, de seguida evaporaram-se os solventes e adicionou-se t-BuOH/H_2O (3:2) e deixou-se reagir durante mais cinco minutos, e a 0ºC adicionou-se $NaIO_4$, e deixou-se reagir mais doze horas. Esta é uma reacção de clivagem oxidativa, e o composto obtido, num rendimento de 48%, foi 2,3-O-isopropilideno-L-eritrose (**2**). A oxima **3** foi obtida a partir deste, por reacção com $NH_2OH.HCl$ em piridina e peneiros moleculares durante doze horas, composto com isomeria geométrica (E e Z), sendo o rendimento desta reacção de 68%. A reacção seguinte foi a protecção dos grupos hidroxilos restantes, com TBDMS.Cl e piridina, foram obtidos dois compostos, o composto di- **4** e mono-sililado **5**, ambos com isomeria E e Z. Nesta reacção, que durou doze horas, o rendimento foi de 24% para o composto **4** e de 35% para o composto **5**. A partir do composto **5** ao reagir, em dois passos diferentes, com NCS e DMF durante quatro horas, e depois com EtSH e Et_3N durante mais doze horas, consegue-se introduzir o grupo SEt no C-1, levando ao composto **6**, num rendimento de 27%. A desprotecção do composto **6** foi conseguida pela reacção deste com TBAT e THF, durante doze horas, originando o composto **8**, sendo o rendimento da reacção de 38%. O fecho do anel, para dar origem ao N-óxido de tio-imidato **9**, foi feito com PPh_3, DEAD e THF anidro. Esta reacção foi feita sobre refluxo, e teve um rendimento de 4%.

A actividade do composto **9** foi testada pelo método do radical DPPH, e com uma concentração de 12500µg/ml a % de actividade anti-oxidante obtida foi de 32,42.

Usando D-xilose, como produto inicial, e reagindo com H_2SO_4 concentrado em MeOH durante 3h e sobre refluxo, conseguiu-se a protecção do hidroxilo primário, dando origem ao composto **14**, em ambas as formas, piranose e furanose. A protecção dos restantes grupos hidroxilo foi efectuada com adição de NaH, BnBr, $nBuNI_4$ em DMF, a 0ºC. Esta reacção durou quarenta e oito horas, e o rendimento foi de 30% para a forma piranose e 30% para a forma furanose. A desprotecção do grupo primário foi conseguida pela adição de AcOH, a 0ºC, ao composto **15**, seguidamente adicionou-se dioxano e H_2SO_4 1M e deixou-se reagir durante 16 horas sobre refluxo, dando origem ao composto **16** com um rendimento de 16%. A partir deste obtém-se a oxima **17**, por reacção de sódio com MeOH, durante dez minutos, e de seguida,

adicionou-se $NH_2OH.HCl$ e o composto **16**, e deixou-se reagir por doze horas sobre refluxo. Esta reacção teve um rendimento de 36%, e o rendimento global foi de 35%.

A % actividade anti-oxidante, para uma concentração de 17000µg/ml, foi de 24,83.

Partindo das lactonas, e em relação à γ-valerolactona obteve-se apenas o ácido hidroxâmico correspondente **7**, pois as protecções dos grupos hidroxilo não foram bem sucedidas. Para obter o composto **7**, fez-se reagir MeOH, $NH_2OH.HCl$ e KOH, de seguida filtrou-se, e adicionou-se a γ-valerolactona, e deixou-se reagir durante doze horas. Em relação à D-(-)-pantolactona, a obtenção do ácido hidroxâmico correspondente não foi conseguida, devido a uma reciclização do composto.

Todos 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 (^1H 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 o composto **16** 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.
Os restantes compostos analisados apresentam actividade antioxidante residual.

Palavras-chave

Açúcares, N-óxidos de tio-imidatos, actividade anti-oxidante

Abstract

The aim of this work was the synthesis of several compounds namely by the regio- and stereoselective branched-chain construction starting from D-ribose or D-xylose which led to the synthesis of thio-imidate N-oxides sugar derivatives. The synthesis of compounds was made by several reactions, either starting from two different sugars or by two different lactones.

Starting from D-ribose and making it react with H_2SO_4 in acetone, it's easy to convert it to 2,3-O-isopropylidene-β-D-ribofuranose (**1**). From this compound, and by reaction with $NaBH_4$ and $NaIO_4$ in MeOH, 2,3-O-isopropylidene-L-erythrose (**2**) was obtained. The conversion into the aldoxime (3) was achieved using hydroxylamine hydrochloride in pyridine. The hydroxyl groups were protected with TBDMSCl in pyridine, giving the di- and mono-silylated compounds, respectively, compounds **4** and **5**. The introduction of the SET group was achieved in a two step reaction by using NCS in DMF, and then by the addition of EtSH and Et_3N, leading to the obtainment of compound **6**. The de-O-silylation was performed using TBAT in THF. The ring-closing allowed the obtainment of the thioimidate N-oxide (9), and it was performed using PPh_3 and DEAD in anhydre THF. The global yield of all these reactions was 42%. When starting from D-xylose, and using H_2SO_4 in MeOH, the primary hydroxyl group was protected, giving compound **14**, in both the pyrano and furano form. The protection of the remaining hydroxyl groups was performed using NaH, BnBr and nBu_4NI in DMF. The deprotection of the primary hydroxyl group was achieved by using H_2SO_4 and diocane, in AcOH. The oxime **17** was obtained using Na, $NH_2OH.HCl$ in MeOH, in a global yield of 35%. The lactones used were γ-valerolactone and D-(-)-pantolactone. When starting from the latter it wasn't possible to obtain correspondent hydroxamic acid, but the same was not true to γ-valerolactone, with hydroxamic acid **7** obtained in 87%. The following steps were to protect the hydroxyl groups in this compound. However, these reactions weren't successful.

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

Keywords

Sugars derivatives, thioimidate N-oxides, antioxidant activity

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Acronims List

Ac_2O – acetic anhydride

AcOH – acetic acid

BnBr – benzyl bromide

DCM - dichlorometane

DEAD – diethyl azodicarboxylate

DMF – dimethylformamide

DMSO – dimethylsulfoxide d6

DPPH – 2,2-diphenyl-1-picrylhidrazyl

EA – ethyl acetate

eq - equivalents

Et_3N – triethylamine

EtSH – ethanethiol

GL - glucosinolates

MeOH – methanol

nBu_4NI – tetrabutyl ammonium iodide

NCS – N-chlorosuccinimide

NMR – nuclear magnetic resonance

MS – molecular sieves

PE – petroleum ether

PPh_3 – triphenylphosphine

TBAT – tetrabutylammonium difluorotriphenylsilicate

TBDMSCl – tert-Butylchlorodimethylsilane

THF – tetrahydrofuran

TIO – thioimidate N-oxide

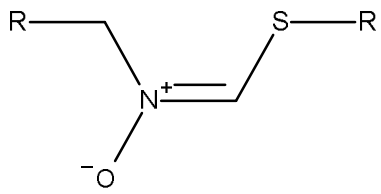
TLC – thin layer chromatography

Chapter 1

Introduction

General Introduction

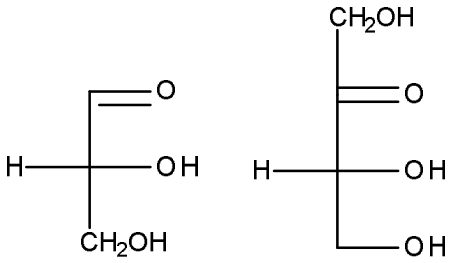
Sugars or saccharides are the most abundant bio-molecule on the planet. They are important in a number of biological roles and a major component in the human diet. Glucosinolates are a class of naturally occurring thioglycosides that play numerous important roles in living organisms. They are sulfur-containing secondary metabolites that display a structural homogeneity based on a hydrophilic ß-D-glucopyrano unit, an O-sulfated anomeric (Z)-thiohydroximate function connected to a rather hydrophobic side chain, the only structural variant, in which 120 different combinations have already been identified in the vegetable kingdom. They are present in various vegetables, namely the *Cruciferae* family. The glucosinolates are hydrolyzed by an enzyme called myrosinase (thioglucoside glycohydrolase E.C. 3.2.3.147), the only identified glycohydrolase able to break an anomeric carbon-sulfur bond [1]. As a defense mechanism the plant uses the relationship between the enzyme and the substrate, by production of bio-active compounds with a large activity spectrum, which include anti-fungi, anti-bacterial and insecticide activity. The principal degradation products of the glucosinolates are the isothyocianates, which are known for their efficacy as chemo-preventive agents. Glucosinolates can be extracted from vegetable sources and then refined by chromatography procedures, but the chemical synthesis approach seems to be a more general and efficient way to get access to glucosinolates in pure form. Functional groups are described as specific groups of atoms within molecules that are responsible for the characteristic chemical reactions of those molecules. The word moiety is often used as a synonym of functional group but, according to the IUPAC definition, a moiety is a part of a molecule that may include functional groups as substructures. The N-oxide thioimidate function is rare, and little can be found in literature about it [2].



Scheme 1 - The general structure of a thioimidate N-oxide

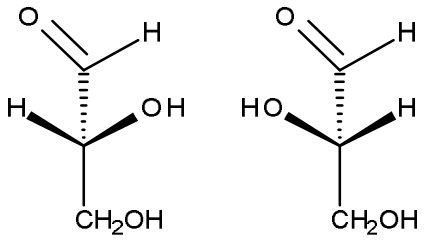
Carbohydrates

Carbohydrates are polihydroxy aldehydes, polihydroxy ketones or compounds that, by hydrolysis, can be transformed into the previous compounds. Their general formula is $C_x(H_2O)_y$ and contain ketones groups and aldehyde groups, but they exist mainly as hemi-aketals or aketals. The simplest carbohydrates are the ones that can be hydrolyzed into more simple compounds, and they are denominated monosaccharides; the ones that can be hydrolyzed in 2 monosaccharids molecules are called disaccharides; and the ones, that by hydrolysis, originate many monosaccharides molecules are polysaccharides. Monosaccharides can be subdivided: if they contain an aldehyde group, they are called aldoses; and if they are formed by a ketone group, they are ketoses; and depending on the number of carbon atoms, the monosaccharide is called triose (3 carbon atoms), tetrose (4), pentose (5), etc. As an example, an aldohexose, is as monosaccharide with 6 carbon atoms that contains an aldehyde group.



Scheme 2 – Aldoses (left) and ketoses (right)

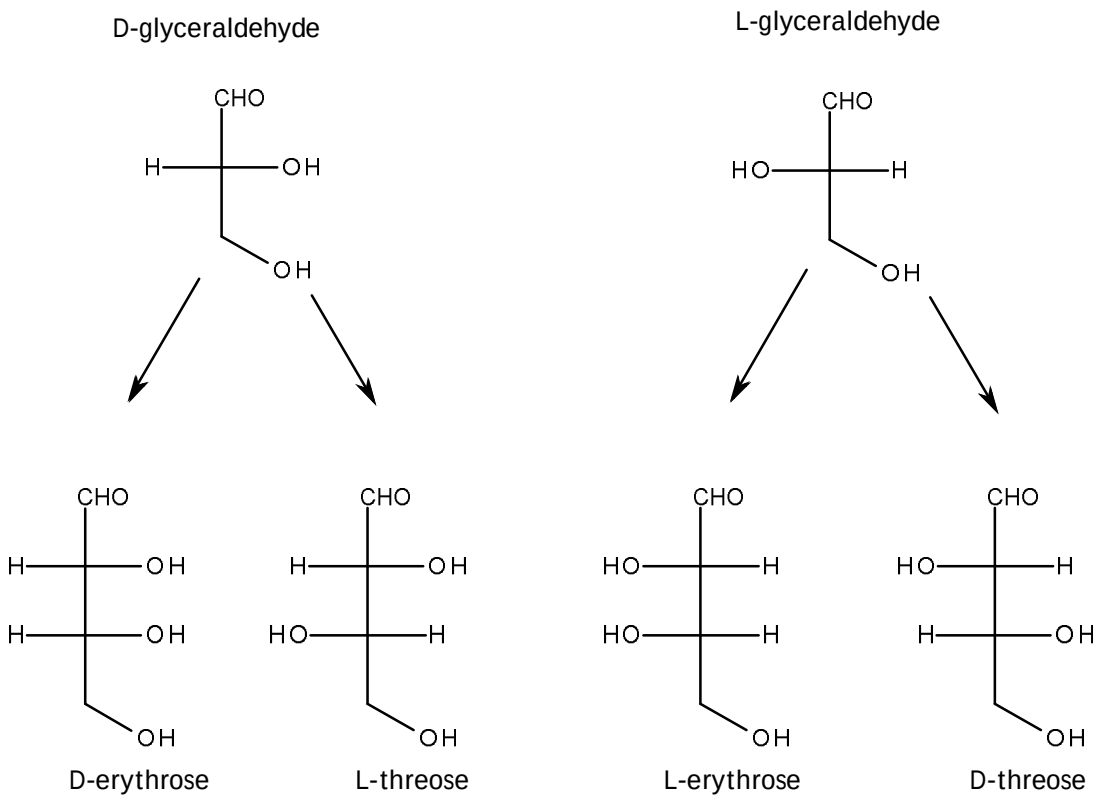
Glyceraldehyde, which is and aldotriose, is one of the simplest monosaccharides and exists in 2 enantiomeric forms. The middle carbon atom in glyceraldehyde is chiral, that is it bears four different substituents, and consequently has non-superimposable stereo isomers. Glyceraldehyde possesses enantiomeric (mirror-image) forms.



Scheme 3 – (D)-Glyceraldehyde (left) and (L)-Glyceraldehyde (right)

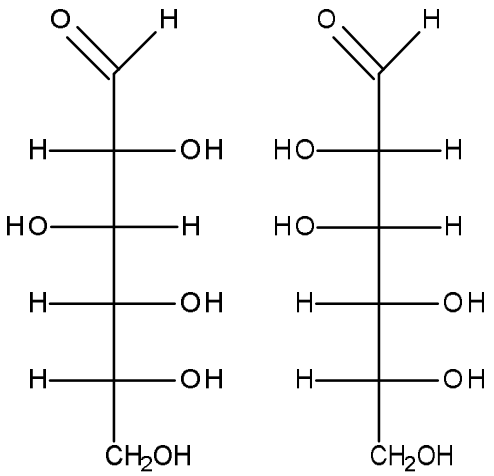
If the secondary OH is on the right we have D-glyceraldehyde, and vice-versa. For longer chains the D/L distinction is based on the orientation of the secondary OH furthest from the C=O, C5 in hexose. In this context D/L refers only to the configuration about this carbon atom and does not specify the optical activity of the sugar; the latter is denoted by +

(dextrorotatory) or – (levorotatory). N chiral centers yield 2^N isomers: tetroses have 2 chiral centers, which yield 4 stereoisomers.



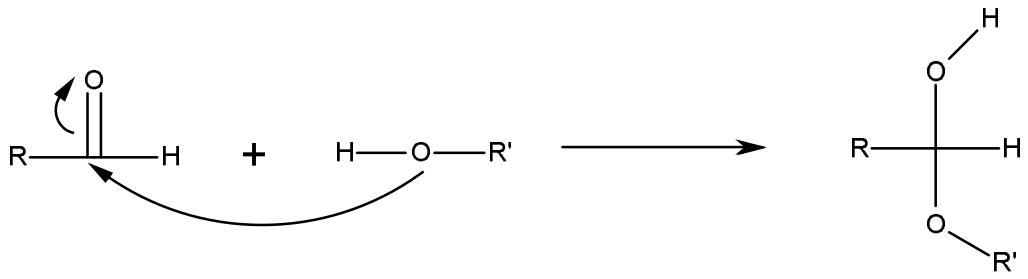
Scheme 4 – Tetroses

D-erythrose and L-erythrose are enantiomers, L-threose and D-erythrose are diastereomers and L-threose and D-threose are also enantiomers. D-glucose and D-mannose, which differ by the orientation of a –O at a single chiral center are called epimers.

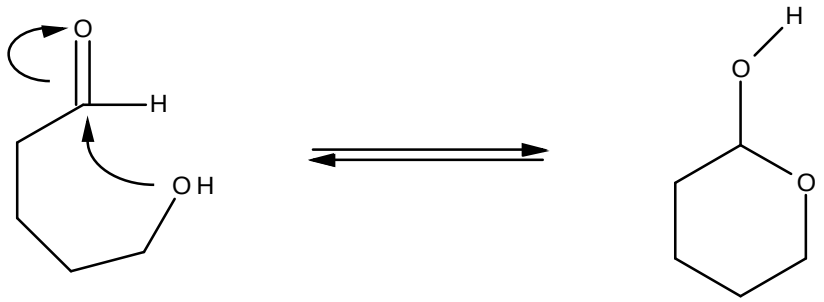


Scheme 5 – D-mannose (right) is C-2 epimer of D-glucose (left)

Carbohydrates have been so far represented in the linear form, but in reality the linear form is a minor species. In solution carbohydrates are usually closed rings. Ring closure occurs by attack of a secondary alcohol on the carbon of the electron deficient C=O. This attack can occur on either face of the planar CHO with the result that the –OH group that is created at C1 can be oriented in either of two directions (if the attack is on the left structure the –OH created will point to the left and vice-versa). The two forms that are formed are called anomers and the C bearing the C=O is the anomeric carbon. When the newly created –OH has the same orientation as the –OH that did the attacking (the two –OH's is *cis*) it's called the α-anomer, otherwise it's the ß-anomer. Usually the –OH group that does the attacking is located on C5 and a 6-membered ring (pyranoside) is formed. When the ring is closed another chiral center is formed, so the ring form has twice as many isomers as the linear form. The attack by the C4 -OH is less common, and leads to a 5-membered ring (furanoside). An important result of the formation of the cyclic hemiacetal is that the hydroxyl group created on C1, bearing the original aldehyde function can be oriented in either of two ways, so a new chiral center is created. Thus the α and ß anomers described are geometric forms of each other. They can be crystallized separately and can be distinguished by a measurement of optical activity. In the case of glucose, both α and ß forms are dextrorotatory, that when dissolved in water leads to the production of the mixture: α-glucopyranoside 40%, open form <0,1%, and ß-glucopyranoside 60%, a phenomenon called mutarotation, because the optical activity changes from that of the pure form observed immediately on dissolution to that of the equilibrium mixture hours later. Aldehydes and ketones react with alcohols to form hemiacetals. When the reaction is intermolecular the equilibrium is unfavorable and the amount of hemiacetal present is very small. However, when the aldehyde group and alcohol group are contained in the same molecule the intramolecular reaction is much more favorable and the hemiacetal is the predominant species present at the equilibrium.

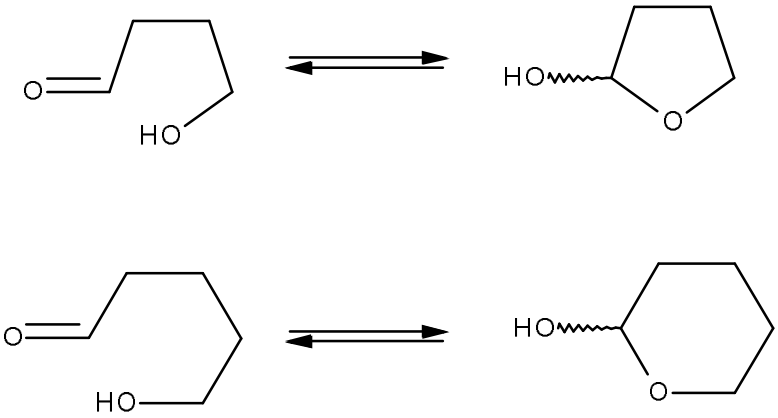


Scheme 6 – The intermolecular reaction



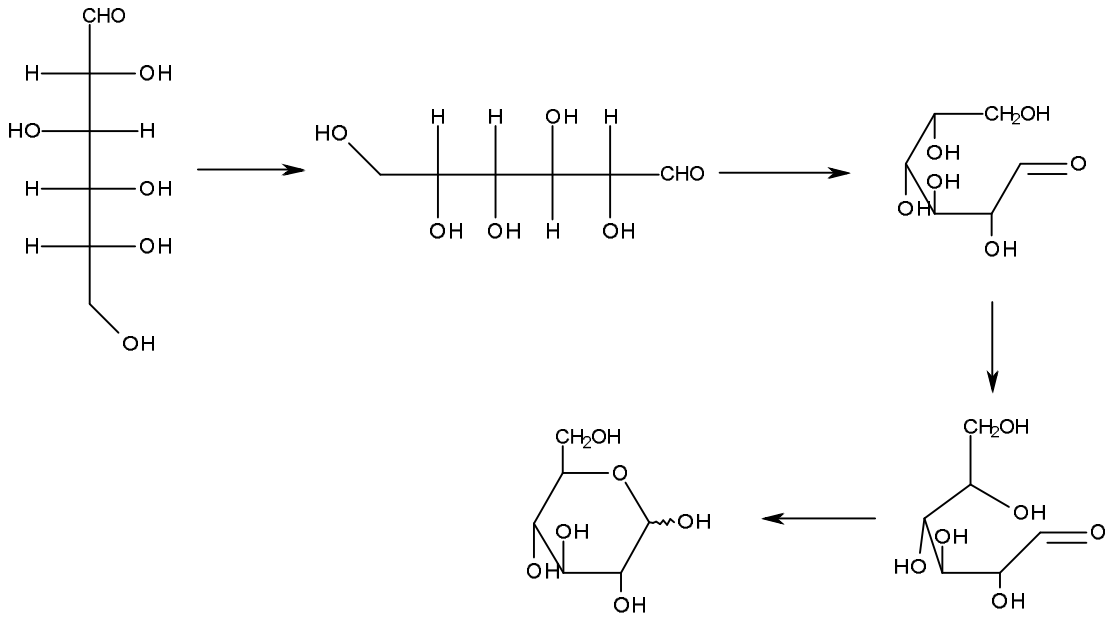
Scheme 7 – The intramolecular reaction

Because monosaccharides contain both and aldehyde group and alcohol group they exist predominantly in the form of cyclic hemiacetals (5- or 6-membered rings).

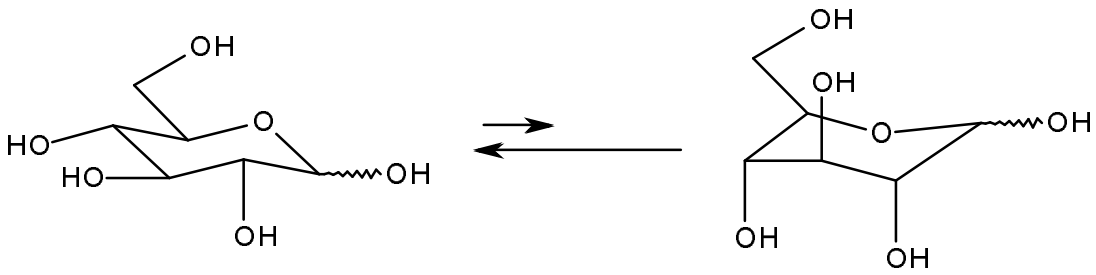


Scheme 8 – Cyclic hemiacetals: Furanose (up) and pyranose (down)

In the case of the cyclization of glucose there are five hydroxyl groups that might react with the aldehyde group. However 5- and 6-membered rings are much more stable than others.



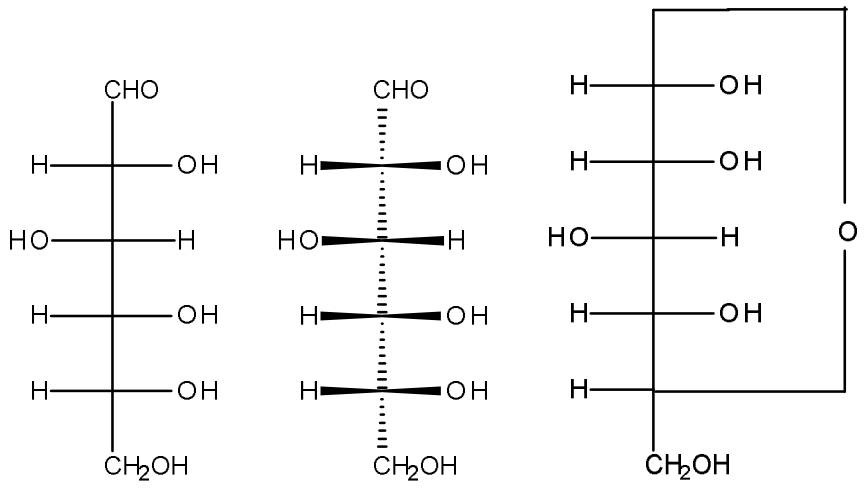
Scheme 9 – Cyclization of glucose



Scheme 10 – D-glucopiranose is more stable in the configuration on the left

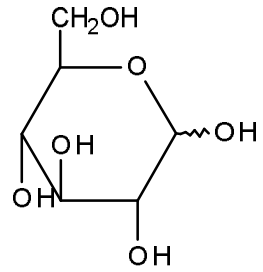
Representations of Carbohydrate Structure

There are several ways to draw the structure of carbohydrates. The simplest is the method introduced by Fischer which represents the sugar as a straight chain of carbon atoms with the lowest numbered at the top and the OH's of the secondary alcohol shown to the right or left. The horizontal lines are to be visualized as projecting out of the page; the vertical lines project into the page so that the carbon backbone has the overall profile of a banana, the top and bottom of which lies behind the plane of the page. The assignment of D or L depends on the orientation of the OH in the penultimate carbon and the orientation of the other OH's are relative to this one. As sugars exist mainly in the ring form, the Fischer representation is converted to the ring formation by drawing a "box" connecting the C bearing the keto function to the penultimate carbon.



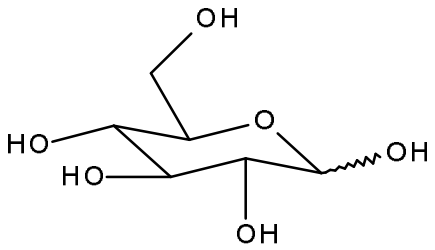
Scheme 11 – Fischer representation of D-glucose

An alternative is the Haworth representation which attempts to convey more three-dimensional information. The carbohydrate is drawn as a hexagon (or pentagon). The OH's are shown and lie either above or below the plane if the hexagon.



Scheme 12- Haworth representation of D-glucose

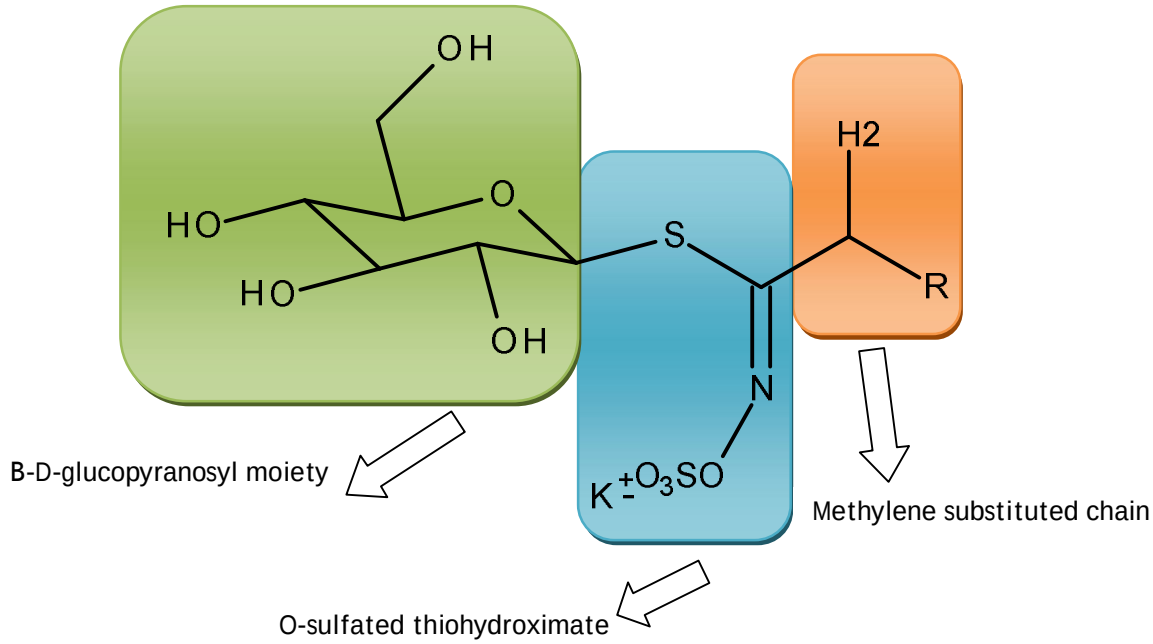
However the most realistic representation of the structure would show that the ring form is not planar. The predominant forms in solution are both "chair" forms, but a small percentage of the sugar molecules are present in the boat form [3].



Scheme 13 – "Chair" form of D-glucose

Glucosinolates

The glucosinolates are a class of secondary metabolites found in fifteen botanical families of dicotyledonous plants. Glucosinolates can be subdivided into three major classes, depending on the nature of their side chains, which may be derived from aliphatic, indolyl, or aralkyl α-amino acids. The skeleton of glucosinolates consists of a thioglycosides link to the carbon of a sulphonated oxime. The R group (side chain) and the sulphate group have anti stereochemical configuration. The R group is derived from amino acids and is highly variable. It can be aliphatic, aromatic or heterocyclic. The sulphate group imparts strongly acidic properties and thus the glucosinolates occur in nature as anions counterbalanced by a cation. The cation is usually potassium, being one of the most abundant cations in plant tissues.



Scheme 14 - Glucosinolates skeleton [1]

Generally, the concentration of GL's is higher in the seed, as opposed to the low levels find in the leaf, stem and root. Concentrations differ according to tissue type, physiological age, plant health and nutrition. Studies have shown that myrosinases are localized in vacuoles of specialized plant cells, called myrosin cells. Thus the two components of the system are separated until autolysis or tissue damage brings them into contact. The precise localization of glucosinolates is not known, but they have been reported to be stored in vacuoles.

Hydrolysis of glucosinolates

The enzymes catalyzing the hydrolysis of glucosinolates are known as myrosinases. The complexity of the myrosinase-glucosinolate system indicates an important role in the life cycle of plants. The function of this system may be diverse. The glucosinolates may be a sink

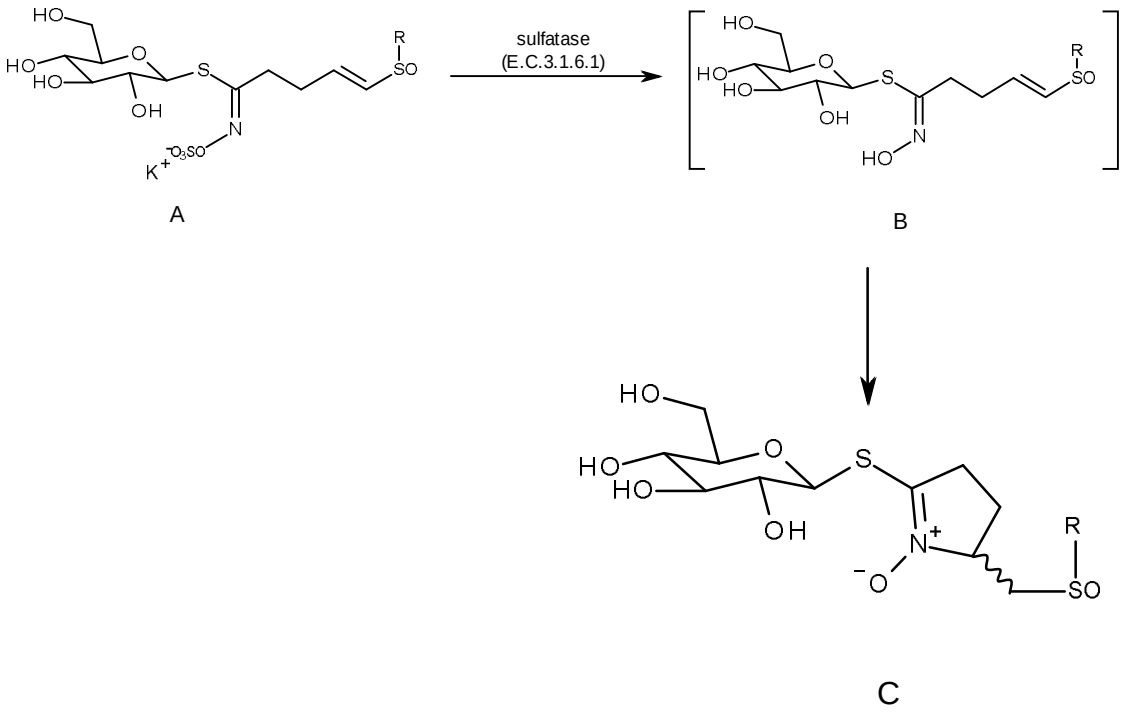
for nutrients like nitrogen and sulphur, while the products of hydrolysis may have important roles in the plant defense system against insect, fungi and microorganism infections.
Glucosinolates behave like bio-precursors to produce electrophilic isothiocianates, compounds that display a diversified and marked biological activity. The myrosinase-assisted hydrolysis cleavage of GL releases a labile aglycon chain which is converted into isothiocyanate through a Lossen-type rearrangement. When crushed plant tissue or seeds containing glucosinolates are added to water, myrosinases catalyze the hydrolytic cleavage of the thioglucosidic bond, giving D-glucose and a thiohydroximate-O-sulphonate (aglycone). The latter compound rearranges nonenzymatically with release of sulphate to give one of several possible products. The predominant product is dependent on the structure of the glucosinolate side chain and the presence of protein co-factors that modify the action of the enzyme. The most frequent fate of the unstable aglycone is to undergo rearrangement spontaneously via a proton independent Lossen rearrangement with a concerted loss of sulphate to yield an isothiocyanate, or a competing proton dependent desulphuration yielding a nitrile and elemental sulphur. Some glucosinolates also give rise to the formation of thiocyanates. [4]

Chemo-preventive activity of glucosinolates

They have the ability to block the carcinogenic potential of many particularly dangerous substances that can damage cell DNA, leading to cell damage that allows the growth of tumours. Glucosinolates stimulate our immune systems, and accelerate the elimination of carcinogens from our bodies, depriving them of a longer more destructive stay. In short, these vegetables prevent carcinogenic substances from causing the type of genetic damage that leads to the onset of cancer and the growth of cancerous tumours in the human body.

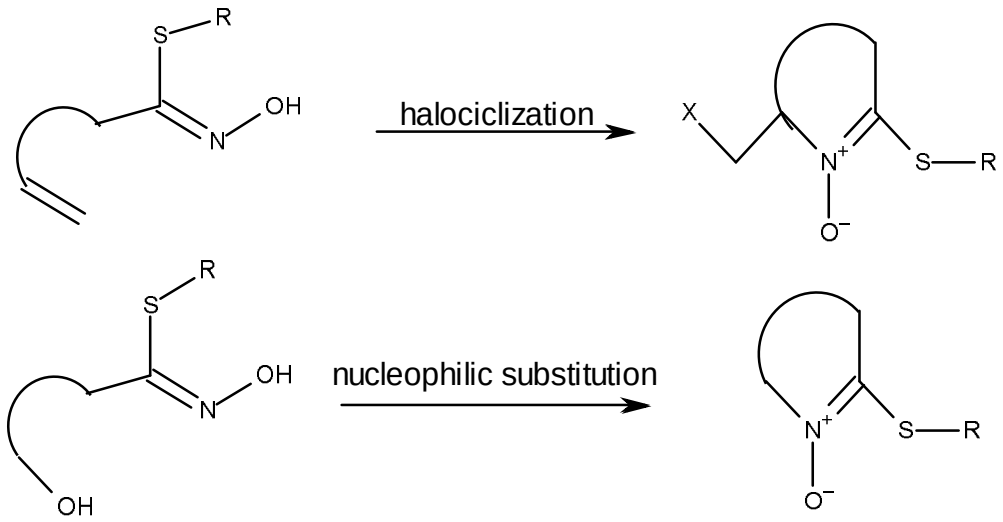
Thioimidate N-oxides

The thioimidate N-oxide function is a rare and original function, of which little is known. Glucoraphenin **A** displays a unique behavior: the desulfo-counterpart **B** is readily converted into a cyclic thioimidate N-oxide **C**, and that can be explained by intramolecular concerted Michael addition of the thiohydroximate moiety of the desulfo-counterpart onto the vinyl sulfoxide acceptor.



Scheme 15 - Glucosinolates structure and glucoraphenin desulfation B resulting in a cyclized TIO [4]

J.Schleiss *et al* designed preparative synthetic methods as a prerequisite to evaluate the chemical potential and reactivity scope of this rare function. 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, and they also used to ways to convert the thiohydroximate into a thioimidate N-oxide: halociclization, under the conditions developed by Grigg et Jäger, and using as the activable group an alkenyl; and nucleophilic substitution, under the conditions of the Mitsunobu reaction and using an hydroxyl as the activable group. [6-9]

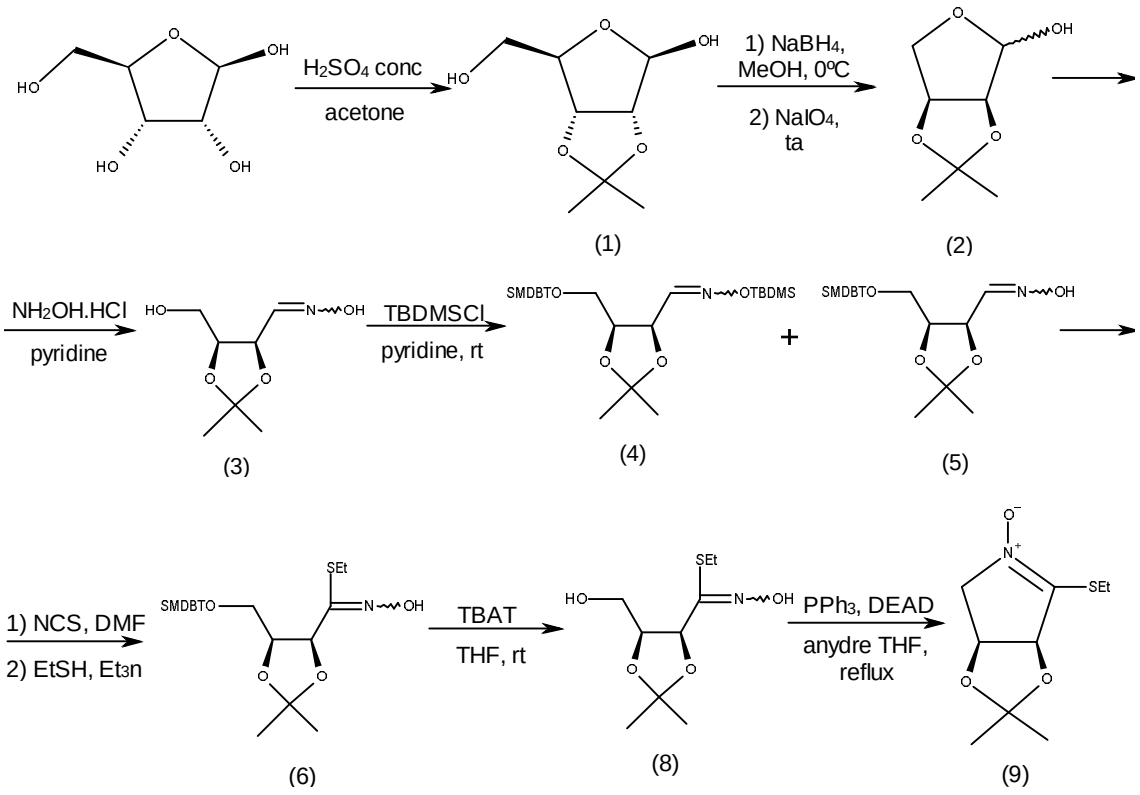


Scheme 16 – Two different routes to prepare a TIO

The compounds obtained from the enzymatic desulfation of the sugar ring were unstable and degradation occurred at room temperature. Using a carbohydrate template and performing halociclization showed compounds with some instability when kept at room temperature for a few days. When performing nucleophilic substitution the reaction had a low yield. Converting 2,3-O-isopropylidene-L-erythrose into the aldoxime, in a 4 step sequence, gave acess to the thiohydroximate and then de-O-sylilation with TBAT. To close the ring two methods were used, one involved a mesyl activation of the primary alcohol prior to application of basic conditions to induce intramolecular ciclization and the second method uses the Mitsunobu procedure. Then, starting from a group of lactones which were converted into hydroxamic acids, and protected in the form of bi-O-silylated derivatives, these compounds originated nitrile oxides, following a protocol developed by Carreira et al (formation of the triflated hydroximates, then condensation with ethanethiol, under basic conditions). To prevent the hydrolysis of the cyclic TIO into and N-hydroxylactam, a mild neutral reaction protocol should be used.

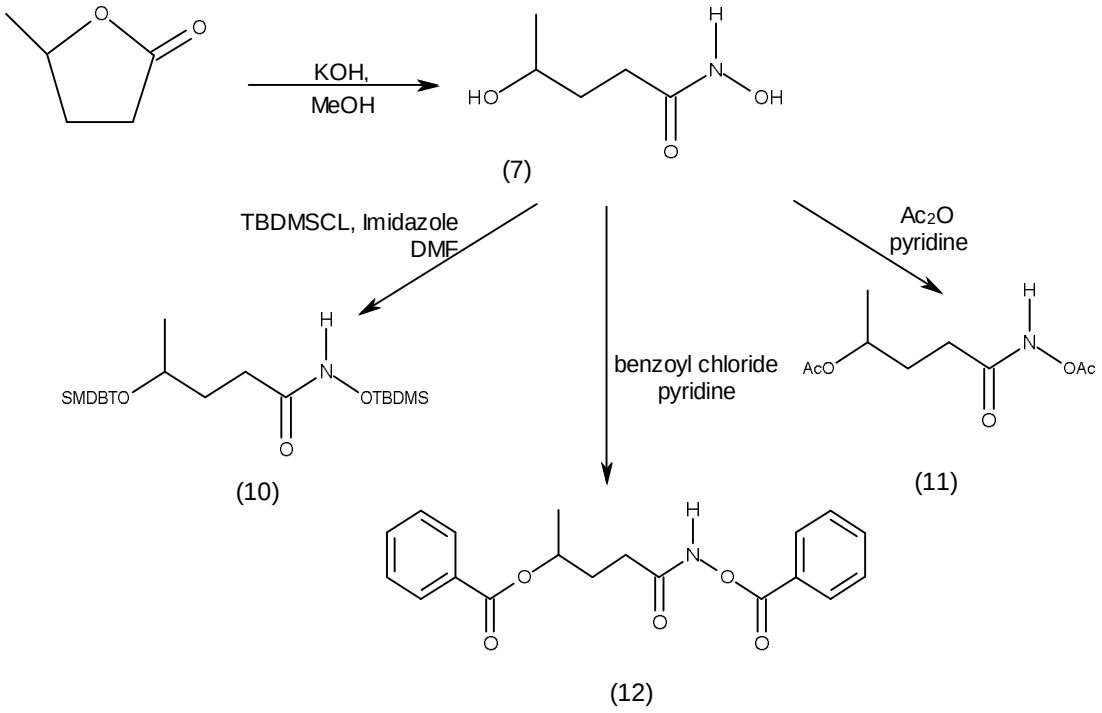
Synthesis of thioimidate N-oxides

Three paths of synthesis were tested to obtain TIO. The first path starts from a carbohydrate template. Starting from D-ribose, the latter can be converted to 2,3-*O*-isopropylidene-L-erythrose (1), which can be converted to the aldoxime (6), and in a four step sequence the thiohydroximate (8) was achieved. De-O-silylation was performed using TBAT, and then closing of the ring was performed using the Mitsunobu procedure:



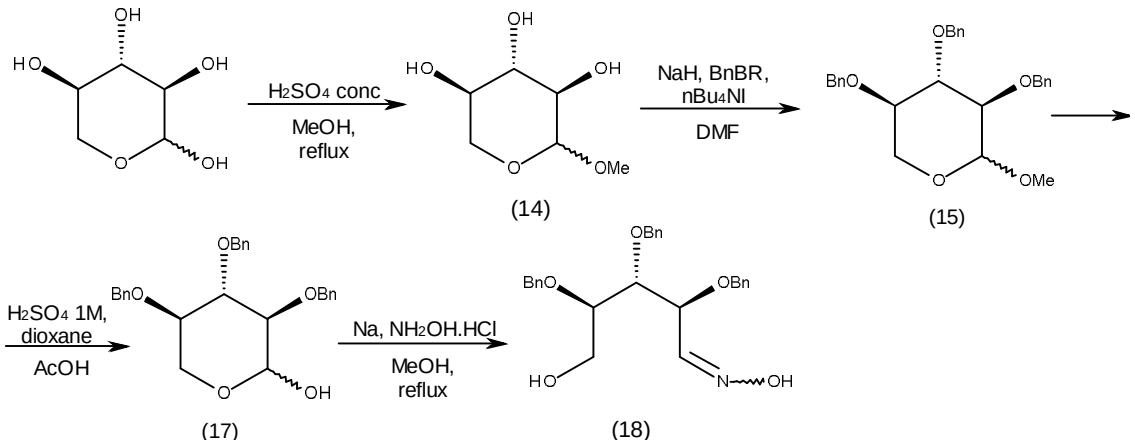
Scheme 17 - Synthesis path starting from D-ribose

In the second path the depart product is a lactone. γ-valerolactone was converted to a hydroxamic acid (7), which was readily protected by either TBDMSCl or Ac_2O or benzoyl chloride.



Scheme 18 - Synthesis starting from a lactone

This synthesis path wasn't further explored, and so we moved to the third path. Starting from D-xylose, the anomeric carbon was protected by a methyl group (14), and then protection to the others groups was performed using benzyl bromide (15). Deprotecting the anomeric carbon and then ring opening allowed the access to the aldoxime (18).



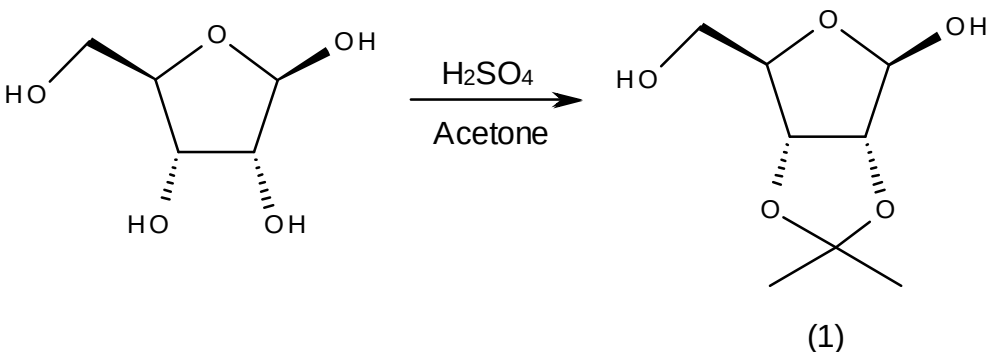
Scheme 19 - Synthesis starting from D-xylose

Hydroxyl group protection

Monossacharides contain several hydroxyl groups and to execute certain reactions in a specific location the protection of these groups is necessary. To protect a group the following characteristics are required: a) easily and selectively on; b) stable under the reaction conditions, and; c) easily and selectively off. Carbohydrate may be protected by conversion of hydroxyl groups in acetals, hemiacetals, esters and ethers. Protective groups exist in a big variety, and some are more selective than others, therefore we can achieve the desired compound [10].

Acetals

Acetals are useful in carbohydrate chemistry because they can protect two hydroxyl groups at the same time, and they are often selective for certains sorts of hydroxyl groups. When acetals are derived from aldehydes they generally prefer to be 6-membered rings, that is protection of 1,3-diols. The formation of isopropylidene can be achieved in acid conditions using acetone as solvent, taking as example (1) [11].



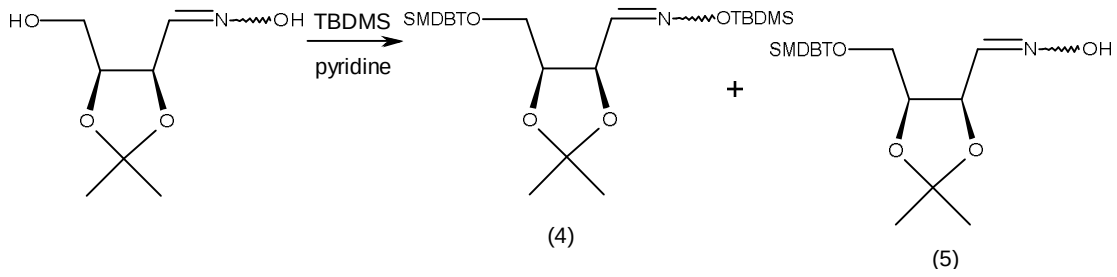
(1)

Scheme 20 – Hydroxyl group protection with isopropylidene

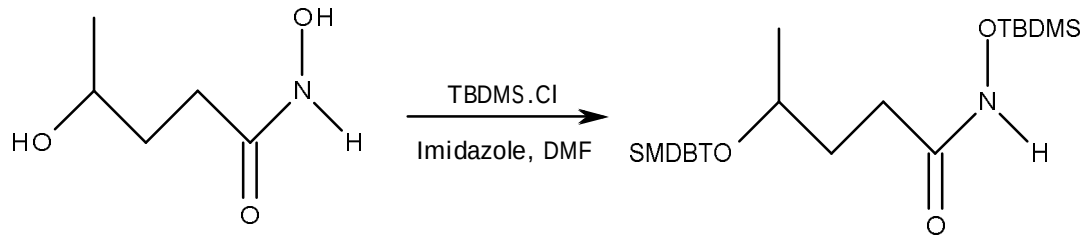
Isopropylidene are stable in basic conditions and cleaved by acidic conditions.

Silyl ether protecting groups

The silyl ether family is a large one and they are popular protecting groups because they are readily formed and cleaved under mild conditions and their relatively stability can be adjusted by simply varying the substituents on silicon. Using TBDMS in pyridine allows us to achieve compounds (4) and (5). The hydroxamic acid (7), originated from γ-valerolactone, was protected using TBDMS and Imidazole in DMF. In both cases the silicon is directly attached to the oxygen.



(4) (5)

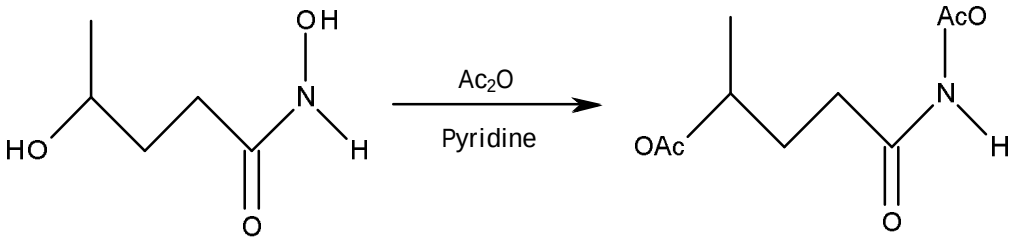


(7) (10)

Scheme 21 – Protection with TBDMS.Cl

O-Acylation

Using acetic anhydride in pyridine also allows the protection of the hydroxyl groups in compound (7) [12].

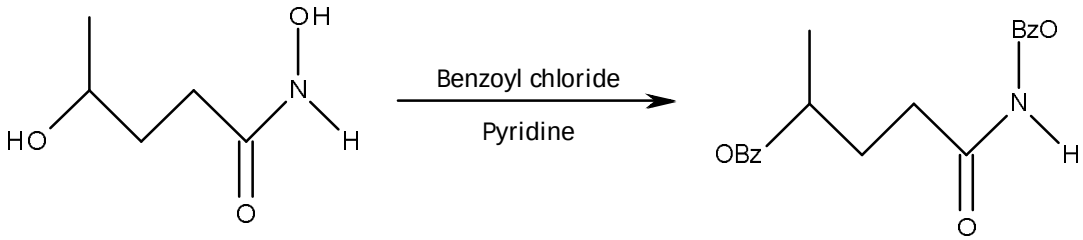


(7) (11)

Scheme 22 – O-acylation of hydroxyl groups

Benzoylation

When benzoyl chloride reacts with alcohols it originates esters. In this reaction benzoyl chloride in pyridine reacted with compound (7) and originated (12).

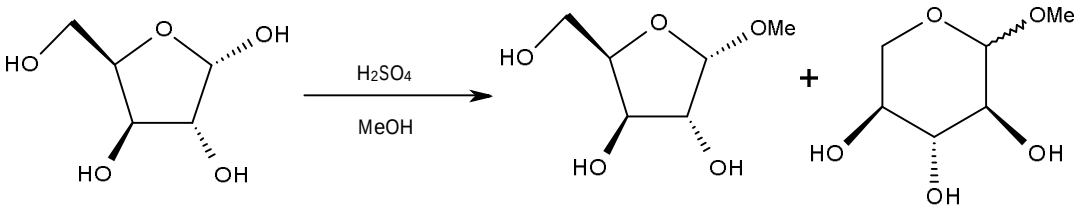


(7) (12)

Scheme 23 – Benzoylation of hydroxyl groups

Methylation

Methylation is an easy way to protect a hydroxyl group so that we don't want to react in the next reactions. It can be done using sulphuric acid in MeOH [3][13].

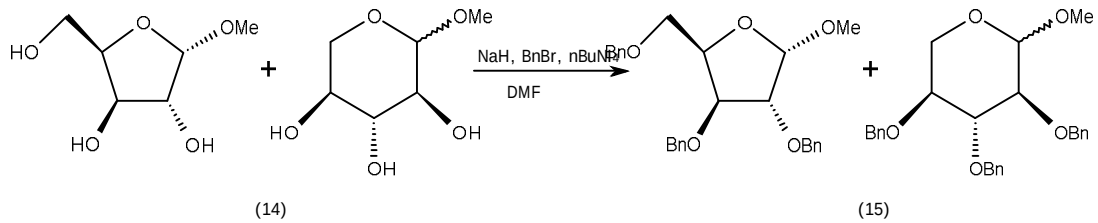


(14)

Scheme 24 – Methylation of primary hydroxyl group

Benzylation

Benzylation of (14) allowed the hydroxyl group protection of the remaining groups. Using NaH as a base, BnBr for the introduction of the benzyl group and $nBuNI_4$ as a catalyst, in DMF originated compound (15) [14].

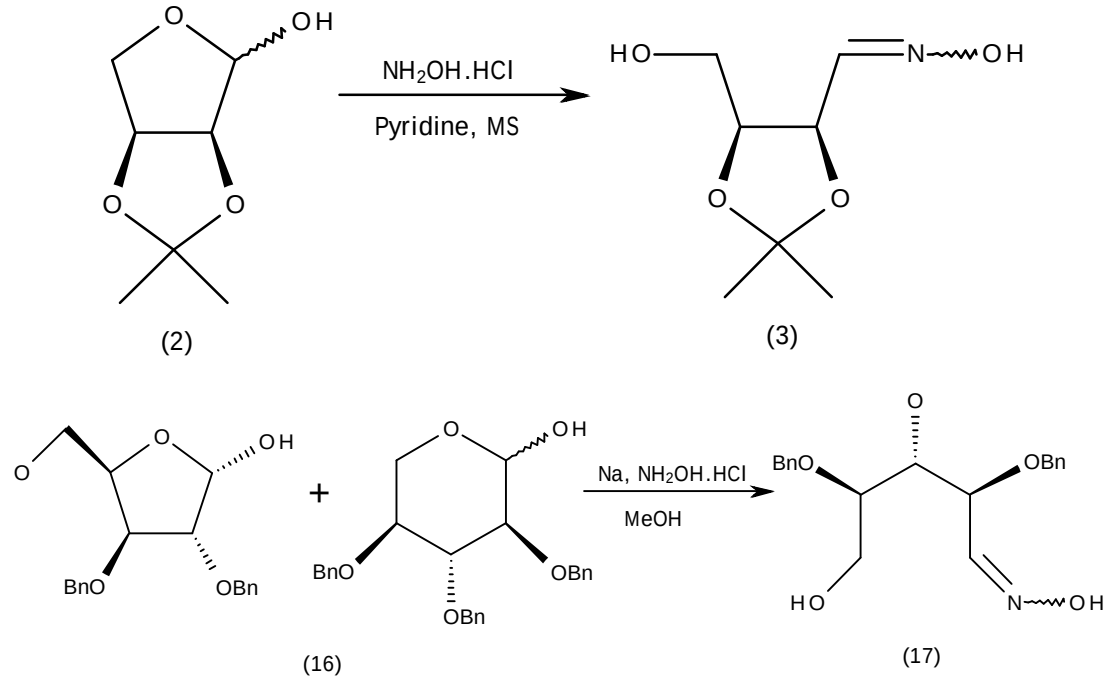


(14) (15)

Scheme 25 – Benzylation of hydroxyl groups

Aldoxime formation

An aldoxime is an oxime produced in the condensation reaction between an aldehyde and hydroxylamine, and exists always as two stereoisomers. When compound (2) was allowed to react with hydroxylamine hydrochloride in pyridine and with molecular sieves it originated the aldoxime (3) [15]. Compound (16) reacted with Na and hydroxylamine hydrochloride in MeOH and so we obtained aldoxime (17).



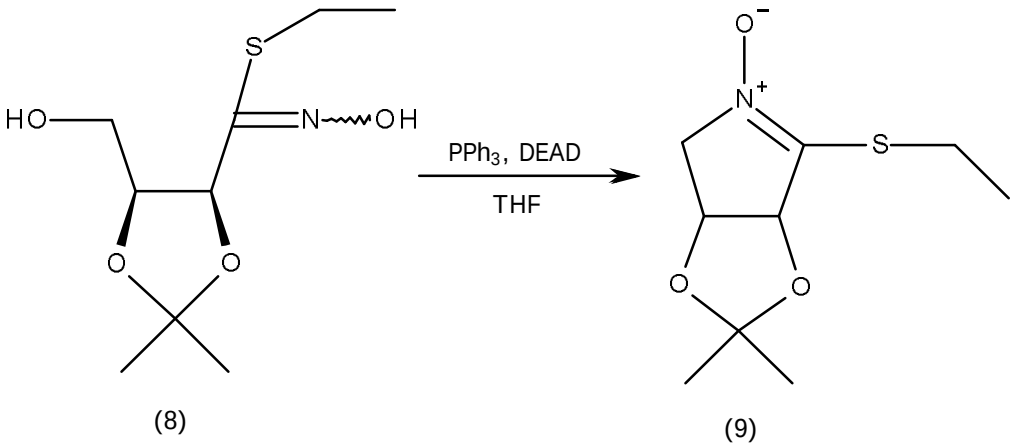
(2) (3)

(16) (17)

Scheme 26 – Aldoxime formation

Mitsunobu

The mitsunobu reaction is a reaction in which an alcohol reacts with PPh_3 and DEAD in THF. [16][17]

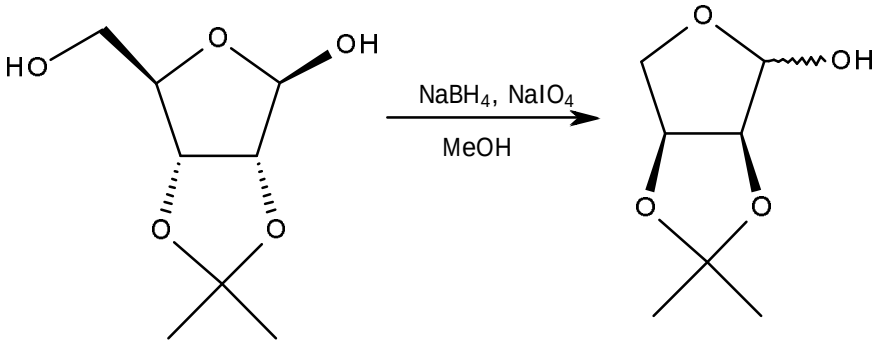


(8) (9)

Scheme 27 – Mitsunobu reaction

Oxidative cleavage

When $NaBH_4$ and $NaIO_4$ in MeOH react with compound (1) first occurs a reduction which is followed by a oxidative cleavage, originating compound (2) [18][19].

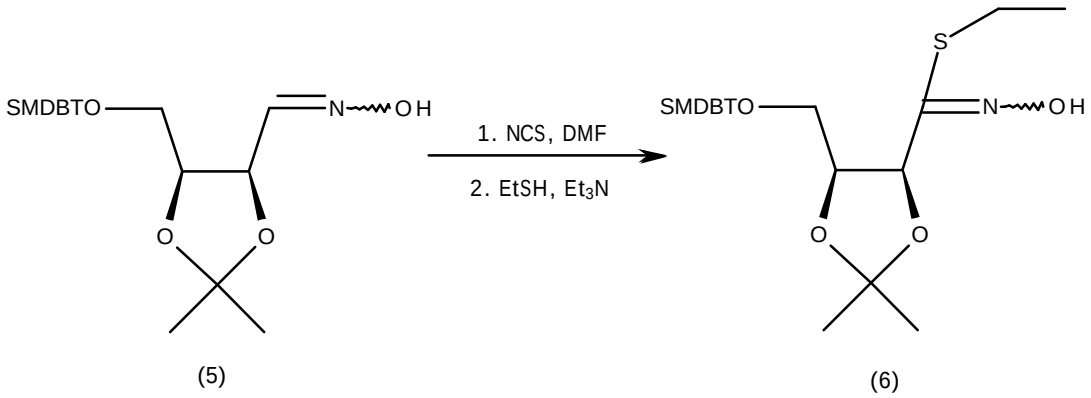


(1) (2)

Scheme 28 – Oxidative cleavage

Conversion to a thiohydroximate

The thiohyroximate is generated in a two step sequences, in which the intermediary is not isolated. Starting from compound (5), and using NCS, that is a mild oxidant, in DMF, these substances are allowed to react for 4 hours. After that EtSH and Et_3N are added and the reaction proceeds for 12h more hours, in order to produce compound (6).

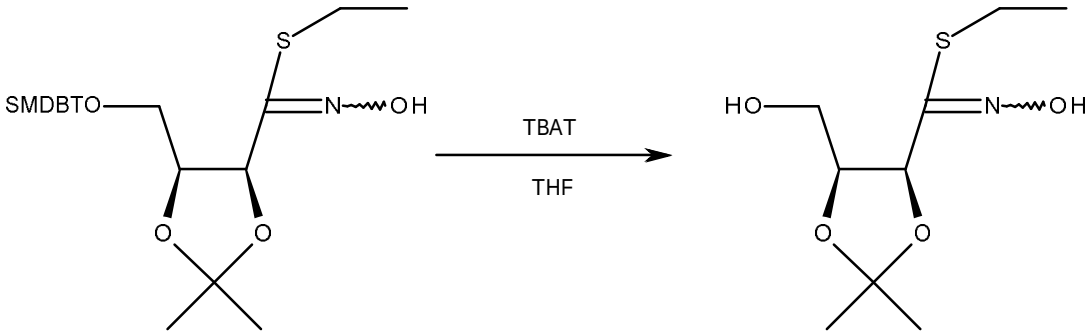


(5) (6)

Scheme 29 – Conversion to a thiohydroxamate

De-O-silylation

Using TBAT in THF allows the de-O-silylation of compound (6) originating compound (8).

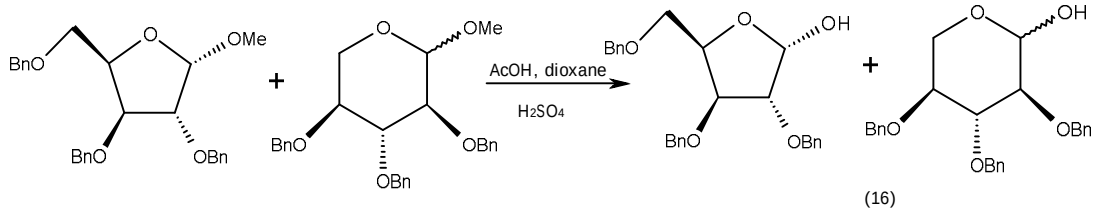


(6) (8)

Scheme 30 – De-O-silylation

Primary hydroxyl deprotection

When compound (15) is allowed to react with AcOH and dioxane in H_2SO_4 it originates compound (16), in which the primary hydroxyl group is deprotected.



(15) (16)

Scheme 31- Primary hydroxyl group protection

Antioxidant activity [24-28]

Redox reactions are considered electron transfer reactions. The term “oxidation” was originally used to represent element combinations with oxygen. Now it has a much wider

meaning, including reactions that do not involve oxygen. A reduction is a reaction in which electron gain is involved when an element is oxidized, and so it acts as a reducing agent, because it donates electrons to another substance, causing the reduction. In other hand, when an element is reduced it acts as an oxidizing agent, because it accepts electrons causing its oxidation [20].

Free radicals

A radical (often, but unnecessarily called a free radical) is an atom or group of atoms that have one or more unpaired electrons. Radicals can have positive, negative or neutral charge. They are formed as necessary intermediates in a variety of normal biochemical reactions, but when generated in excess or not appropriately controlled, radicals can cause damage on a broad range of macromolecules. Radicals have extremely high chemical reactivity, which explains not only their normal biological activities, but how they inflict damage on cells. [21]

Free radicals can be produced by various ways, such as:

- Enzymes: in redox reactions in the electron transport chain in mitochondria, endoplasmatic reticulum and plasmatic and cell membrane; as intermediaries during drug desintoxication, prostaglandin synthesis and during platelet and leucocyte activation.
- Environmental factors: light, UV radiation or ionizing radiation.
- Non-enzymatic processes: through self-oxidation of many substances (polyunsaturated fatty acids, hemoglobin, myoglobin, catecholamines), than can be stimulated by metallic ions (iron and copper) with redox capacity (ionizing or UV radiation and by photo-activated pigments).

Reactive oxygen species (ROS)

Oxygen has two unpaired electrons in separate orbitals in its outer shell. This electronic structure makes oxygen especially susceptible to radical formation, and so they represent the most important class of radical species generated in living systems.

Sequential reduction of molecular oxygen (equivalent to sequential addition of electrons) leads to formation of a group of reactive oxygen species. Molecular oxygen (O_2) has a unique electronic configuration and is itself a radical. The addition of one electron to dioxygen forms the superoxide anion radical ($O_2^{\bullet -}$). The hydroxyl radical ($OH^{\bullet}$) is the neutral form of the hydroxide ion, and it has a high reactivity, making it a very dangerous radical with a very short half-life *in vivo*. Adittionaly peroxyl ($ROO^{\bullet}$) radicals, also derived from oxygen, can also be formed.

Oxygen-derived radicals are generated constantly as part of normal aerobic life. They are formed in mitochondria as oxygen is reduced along the electron transport chain. Reactive oxygen species are also formed as necessary intermediates in a variety of enzyme reactions. Examples of situations in which oxygen radicals are overproduced in cells include:

- White blood cells, such as neutrophils specialize in producing oxygen radicals, which are used in host defense to kill invading pathogens.
- Cells exposed to abnormal environments, such as hypoxia or hyperoxia generate abundant and often damaging reactive oxygen species.
- Ionizing radiation, is well known to generate oxygen radicals within biological systems.The damaging effects of radiation are higher in well oxygenated tissues than in tissues deficient in oxygen.

Reactive nitrogen species (RNS)

Nitric oxide ($NO^{\bullet}$) is a small molecule that contains one unpaired electron on the anti-bonding $2\pi_y^*$ orbital and is, therefore, a radical. It is generated in biological tissues by specific nitric oxide synthases, and is an abundant reactive radical that acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation.

Oxidative stress

ROS as well as RNS are products of normal cellular metabolism and are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. Beneficial effects occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia. The harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress. Oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of pro-oxidant/antioxidant reactions in living organisms. The excess ROS can damage cellular lipids, proteins or DNA, inhibiting their normal function. Because of this oxidative stress has been implicated in a number of human diseases as well as in the aging process. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms and is achieved by mechanisms called “redox regulation”. This process protects living organisms from various oxidatives stresses and maintains “redox homeostasis” by controlling the redox status *in vivo*. How the oxidative process starts is still not clarified, but the $HO^{\bullet}$ radical and peroxynitrite radical ($ONOO^{\bullet}$) are the ones that initiate it. The $O_2^{\bullet -}$ radical also as an important part, due to the possibility of creation of the $HO^{\bullet}$, very reactive through the Haber-Weiss reaction:

$$H_2O_2 + O_2^{\bullet -} \rightarrow OH^- + HO^\bullet + O_2$$

This reaction only occurs in the presence of a transition metal (usually iron), which is reduced by $O_2^{\bullet -}$ and reacts with H_2O_2, in a reaction type Fenton:

$$O_2^{\bullet -} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$

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

Once formed, ROS travel to the microcirculation, and due to their high reactivity, act at different cellular structures (DNA and proteins) and also at the cellular membrane (polyunsaturated lipids). When an imbalance between ROS/free radicals formation and antioxidant defenses/organism repair mechanisms is verified, oxidative stress exists and can be disease associated.

Lipid peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process exists due to a free radical chain reaction mechanism. Usually it affects polyunsaturated fatty acids, because they contain multiple double bonds. As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination.

Initiation: Initiation is the step in which a fatty acid radical is produced. The most notable initiators in living cells are ROS, such as $OH^\bullet$ and HO_2, which combines with a hydrogen atom to make water and a fatty acid radical.

Propagation: The fatty acid radical is not a very stable molecule, so it reacts readily with O_2, thereby creating a peroxyl-fatty acid radical. This too is an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide, or a cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way.

Termination: When a radical reacts with a non-radical, it always produces another radical, which is why the process is called a chain reaction mechanism. The radical reaction stops when two radicals react and produce a non-radical species. This happens only when the concentration of radical species is high enough for there to be a high probability of collision of two radicals.

Usually many reactive products are formed, namely lipids radicals and malondialdehyde. These don not only act directly on cell membrane components, but can also infiltrate the blood stream, increasing the blood and plasma levels. This increase indicates damage in the cell membrane of organs or tissues that can be responsible by the triggering of many pathologic processes.

Protein oxidation

When the properties of a protein are altered and when amino acids peroxide formation occurs it can lead to protein degradation, by fragmentation and by cross-linking, which can result in polymerization and inactivation, specifically in proteins with SH and aromatic groups. Protein oxidation induces alteration in the tertiary structure which leads to protein aggregation and amyloids formation. Amino acid oxidation by free radicals leads to alterations in the enzymatic activity, with antioxidant compromise in cells and tissues.

DNA oxidation

DNA damage is one of the most important results of the peroxidative process *in vivo*. DNA alteration caused by oxidative damage is one of the main causes that trigger carcinogenese, either by proto-oncogenes activation, or by tumor suppressor genes inactivation. Apoptose inhibition may also be associated with oncogenese and with DNA alterations, and can appear as a consequence of free radicals action that leads to an increase of cytoplasmic calcium.

Antioxidants

Antioxidants can be defined as any substance that, present in relatively low concentrations (when compared to the one of the substrate), prevent or slow significantly substrate oxidation. For convenience, antioxidants were divided into two classes, primary or chain-breaking antioxidants and secondary or preventative antioxidants. Secondary or preventative antioxidants are compounds that retard the rate of oxidation. This may be achieved in a number of ways, including removal of substrate or singlet oxygen quenching. Primary antioxidants (AH) when present in trace amounts, may either delay or inhibit the initiation step by reacting with a lipid radical or inhibit the propagation step by reacting with peroxyl or alkoxyl radicals:

$$L^{\bullet} + AH \rightarrow LH + A^{\bullet}$$

$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet}$$

$$LO^{\bullet} + AH \rightarrow LOH + A^{\bullet}$$

The antioxidant free radical may further interfere with chain-propagation reactions by forming peroxy antioxidant compounds:

$$A^{\bullet} + LOO^{\bullet} \rightarrow LOOA$$

$$A^{\bullet} + LO^{\bullet} \rightarrow LOA$$

The activation energy of the above reactions increases with increasing A-H and L-H bond dissociation energy. Therefore, the efficiency of the antioxidant increases with decreasing A-H bond strength.

Living organisms have evolved different molecules that speed up termination by catching free radicals and, therefore, protecting the cell membrane. One important

such antioxidant is vitamin E. Other anti-oxidants made within the body include the enzymes superoxide dismutase, catalase, and peroxidase.

Antioxidant systems

The production of free radicals is controlled in humans by several antioxidant compounds, some of which may be from and endogenous origin and some can be originated from the diet. Antioxidants can stabilize or deactivate free radicals before they attack the biological targets in the cells. The human body has several control mechanisms of ROS production and mechanisms to limit and reaper tissue damage. The integrated antioxidant system has many components:

- Antioxidants that prevent ROS formation: Cu-ceruloplasmin, Cu-albumin, Fe-transferrin and Fe-myoglobin.
- Antioxidants that remove ROS, preventing chain reactions: enzymes, such as superoxide dismusate, glutathione peroxidase, glutathione redutase, catalase, metaloenzymes; and small molecules, like glutathione, vitamin C, tocopherol, bilirubin, uric acid, carothenoids and flavonoids.
- Repair enzymes: which include DNA repair enzymes.

However, the primary intracellular antioxidant defense is given to enzymatic antioxidants and non-enzymatic antioxidants.

Enzymatic Antioxidants

Three groups of enzymes play significant roles in protecting cells from oxidant stress:

Superoxide dismutases are enzymes that catalyze the conversion of two superoxides into hydrogen peroxide and oxygen. The benefit here is that hydrogen peroxide is substantially less toxic that superoxide. They accelerate this detoxifying reaction roughly 10,000-fold over the non-catalyzed reaction.

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

They are metal-containing enzymes that depend on bound manganese, copper or zinc for their antioxidant activity. In mammals, the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms predominant in cytoplasm. Interestingly, they are inducible enzymes, which mean that exposure of bacteria or vertebrate cells to higher concentrations of oxygen results in rapid increases in the concentration of SOD.

Catalase is found in peroxisomes in eucaryotic cells. It degrades hydrogen peroxide to water and oxygen, and hence finishes the detoxification reaction started by superoxide dismutase.

Glutathione peroxidase is a group of enzymes, the most abundant of which contain selenium. These enyzmes, like catalase, degrade hydrogen peroxide. They also reduce organic peroxides to alcohols, providing another route for eliminating toxic oxidants.

Non-enzymatic Antioxidants

Three non-enzymatic antioxidants of particular importance are:

Vitamin E (or tocopherol) is the major lipid-soluble antioxidant, and plays a vital role in protecting membranes from oxidative damage. Its primary activity is to trap peroxyl radicals in cellular membranes.

Vitamin C (or ascorbic acid) is a water-soluble antioxidant that can reduce radicals from a variety of sources. It also appears to participate in recycling vitamine E radicals. Vitamin C also functions as a pro-oxidant under certain circumstances.

Glutathione may be the most important intracellular defense against damage by reactive oxygen species. It is a tripeptide (glutamyl-cysteinyl-glycine). The cysteine provides an exposed free sulphydryl group (SH) that is very reactive, providing an abundant target for radical attack. Reaction with radicals oxidizes glutathione, but the reduced form is regenerated in a redox cycle involving glutathione reductase and the electron acceptor NADPH.

Relation between oxidative stress and pathologies

It is now common knowledge that oxidative stress is related to a wide number of pathologies namely:

- Cancer, because free radicals have initiation and promoting capacity, which are the two fundamental phases of cancer development.
- Cardiovascular diseases, free radicals excess can initiate aterosclerose by damaging the blood vessels.
- Diabetes Mellitus, free radicals contribute to pancreatic cell destruction in insulin-dependent diabetes mellitus.
- Inflammatory disease, free radicals may act indirectly as cellular messengers and trigger inflammatory response.
- Infertility, free radicals initiate lipid peroxidation and peroxide accumulation in the spermatozoid membrane, causing motility and viability reduction.
- Cataracts, superoxide and hydroxyl radicals cause damage to proteins and lipids in cell membranes that accumulate in the crystalline surface, causing opacity.
- Aging, due to free radicals production in mitochondria that accumulate with age.

- Neurologic diseases, the brain is rich in polyunsaturated fatty acids and iron, and poor in antioxidants, and it's also surrounded by spinal fluid, and it has none or slim capacity to iron chelation.
- Hepatic disease, high levels of short chain fatty acids increase iron captation by hepatocytes, and so increasing hydrogen peroxide production and free radicals production.
- Pulmonary disease, ROS and other toxic products produced by pulmonary cells and by neutrophilic cells activity, that accumulate in the lungs when pure oxygen is breathed, can possibly contribute to oxidative damage.

Measurement of antioxidant activity

The methods to evaluate antioxidant behavior can be clustered in two categories that reflect antioxidant activity importance in food or human bioactivity. In the case of food systems, the need consists in evaluating antioxidant efficacy in providing food protection against oxidative deterioration. A subcategory involves measuring antioxidant activity in food, especially fruits, vegetables and beverages, but with the goal of determining antioxidant load present in the diet and its antioxidant activity in vivo.

Antioxidants can act by several mechanisms, such as free radical trapping, peroxide decomposition and metallic ions chelation. Therefore, antioxidant activity can be measured and evaluated by various methods that look on different antioxidant activity chemical mechanisms.

According to the chemical reactions used, the antioxidant activity determination methods can be clustered into two classes: hydrogen atoms transfer (HAT) and electron transfer (ET). HAT methods measure the ability of an antioxidant to inactivate free radical by hydrogen atoms donation. ET methods measure the ability of a potential antioxidant to transfer an electron to the reduced radicals, metals or carbonyls.

One of the problems when determining antioxidant activity is that the antioxidant activity is variable depending on the method used. But it's known that the antioxidant mechanism in many biological matrixes is very complex and many other factors can intervene in such process.

DPPH method

The DPPH method is based on the study of the trapping activity of the stable free radical 2,2-diphenyl-1-pycril-hydrazyl (DPPH), which has a purple coloration, and absorbs at a specific wavelength (515-517nm). When an antioxidant (AH) or a radicalar species ($R^{\bullet}$) intervene, DPPH is reduced forming diphenylpycril-hydrazine, of yellow coloration, with consequent absortion disappearement, and this can be monitored by the decrease in absorbance. From

the obtained results the percentage of antioxidant activity and the percentage of remaining DPPH are determined.

The percentage of antioxidant activity corresponds to the quantity of DPPH consumed by the antioxidant. The efficient concentration (EC_{50}) is the necessary quantity of antioxidant that reduces the initial DPPH concentration in 50%.

Chapter 2

Experimental Part

General Methods

The **TLC** was used to verify the progress of the reactions and the progress of the product purifications. The TLC sheets (aluminium sheets covered by Silica Gel 60 F_{254}) where revealed with UV light (λ=254nm) and with immersion in one of the following visualization reagents, and then heated:

- Sugars: 800ml EtOH, 150ml water, 50ml H_2SO_4
- $KMnO_4$: 5g $KMnO_4$, 500ml water, 8,5ml AcOH, 33g K_2CO_3
- Phosphomolibdic acid: solution of 5% or 10% of phosphomolibdic acid in EtOH

The **Rf** can be calculated by the relation between the height of the elution product and the height of the eluent.

The products were purified by **column chromatography** in silica gel SI 60 (40-63 µm), and these columns were eluted by gravity with the help of compressed air.

The **proton NMR spectra** were registered in $CDCl_3$ or DMSO by Bruker Spectrometer (250MHz) and (400MHz), at the *Institut de Chimie Organique et Analytique*. The homonuclear correlations (COSY) were done to allow the complete attribution of some signals. The chemical shift (δ) of the signals is indicated in ppm, and the solvent was used as an internal reference. The coupling constants are indicated in Hz (Hertz) and the multiplicity of the signals is indicated by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quadruplet) and m (multiplet).

The **^{13}C NMR spectra** were registered at 100MHz in the same spectrometer used for the proton spectra. The heteronuclear correlations between the proton and the carbon (HSQC) were done to allow the correct interpretation of some signals.

To evaluate the **Antioxidant Activity** of some compounds, the latter were allowed to react with a stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) in a methanol solution. The reduction of DPPH$^{\bullet}$ was followed by monitoring the decrease in absorbance at a characteristic wavelength (515nm) during the reaction. DPPH$^{\bullet}$ absorbs at 515nm, but upon reduction by an antioxidant (AH) or a radical species (R$^{\bullet}$), the absorption disappears.

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

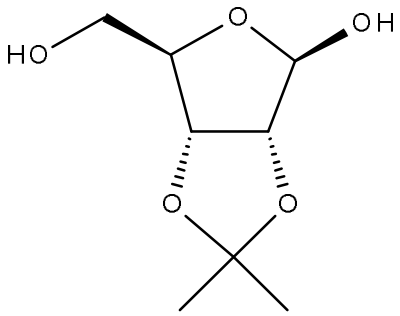
$$DPPH^{\bullet} + R^{\bullet} \rightarrow DPPH\text{-}R$$

Scheme -

These measurements were performed using a UV-Vis spectrophotometer in the 515nm wavelength, using as positive control trolox (6-hydroxy-2,5,7,8-tetramethylchromam-2-carboxylic acid).

General Procedures

(1) 2,3–*O*–isopropylidene–ß–D–ribofuranose [11][31]



190,19 g/mol

$C_8H_{14}O_5$

Procedure: To a solution of D-ribose (10g, 66,6mol, 1 eq) in acetone (100ml), was added H_2SO_4 (0,25ml, 4,5mmol), at 0ºC. The mixture was stirred for 1h, and then the $NaHCO_3$ was added, a 0ºC. The solvents were evaporated. The suspension was filtered through celite and with EA, and evaporated. Toluene was added, and the solvents were co-evaporated.

Yield = 95%

Rf = 0,7 (EA/PE 9:1)

^{1}H NMR (400MHz, $CDCl_3$) δ: 1,27 (s, 3H, CH_3iPr), 1,43 (s, 3H, CH_3iPr), 3,63-3,69 (m, 2H, CH_2-5), 4,34 (t, 1H, J=4Hz, J=2,8Hz, CH-4), 4,52 (d, 1H, J=6Hz, CH-3), 4,76 (d, 1H, J=6Hz, CH-2), 5,35 (s, 1H, CH-1)

^{13}C NMR (100MHz, $CDCl_3$) δ: 24,7 (CH_3iPr), 63,6 (CH_2-5), 81,7 (CH_2-2), 86,8 (CH_2-3), 87,7 (CH-4), 102,8 (CH-1), 112,3 ($\underline{C}(CH_3)_2$)

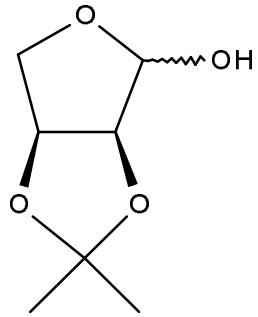
MS (IS): m/z = 173 $[M\text{-}OH]^+$, 213 $[M+Na]^+$, 403 $[2M+Na]^+$

IR : 867, 1036, 1159, 1209, 1376, 1458, 2940, 3369

$[\alpha]_D^{20}$ **=** -20 ($CHCl_3$)

IR : 778, 867, 923, 1000, 1036, 1063, 1159, 1210, 1241, 1325, 1376, 1458, 1642, 2941, 2986, 3374

(2) 2,3-*O*-isopropylidene-L-erythrose [18-19][31-32]



160,19 g/mol

$C_7H_{12}O_4$

Procedure: To a solution of **1** (2g, 10,5mmol, 1eq) in MeOH (20ml), $NaBH_4$ was added (0,598g, 15,8mmol, 1,5eq) and the mixture was stirred for 1h, at 0ºC. The solvents were evaporated, then it was added t-BuOH/H_2O (3:2) (18/12ml) and the mixture was stirred for 5min. The $NaIO_4$ was added (8,9g; 42mmol, 4eq), at 0ºC and the mixture was stirred for 12h. The resulting mixture was diluted with EA and filtered through celite and with EA. The organic phase was washed with a saturated solution of $NaHCO_3$ (50ml) and a saturated solution of NaCl, and dried with $MgSO_4$. The suspension was filtered and re-evaporated. The product was purified by column chromatography (PE/EA 7:3).

Yield = 48%

Rf = 0,66 (PE/EA 7:3)

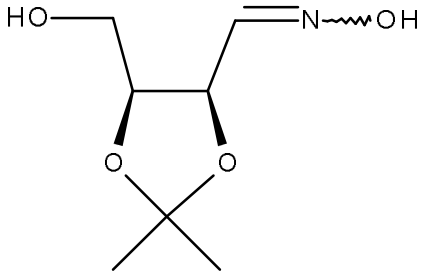
^{1}H NMR (250MHz, CDCl3) δ: 1,27 (s, 3H, CH_3iPr), 1,41 (s, 3H, CH_3iPr), 3,91-4,06 (m, 2H, CH_2-4), 4,24 (sl, 1H, OH), 4,51 (d, 1H, $J_{2,3}$=6Hz, CH-2), 4,79 (dd, 1H, J=6Hz, J=3,2Hz,CH-3), 5,35 (s, 1H, CH-1)

^{13}C NMR (100MHz, $CDCl_3$) δ: 24,8 ($\underline{C}H_3$iPr), 26,3 ($\underline{C}H_3$iPr), 71,9 (CH_2-4), 80,1 (CH-3), 85,3 (CH-2), 101,8 (CH-1), 112,4 ($\underline{C}(CH_3)_2$)

$[\alpha]_D^{20}$ **=** +50 ($CHCl_3$)

IR : 665, 761, 817, 855, 873, 907, 969, 985, 1042, 1063, 1097, 1161, 1208, 1331, 1375, 1459, 2940, 2085, 3411

(3) 2,3-*O*-isopropylidene-L-erythrose oxime [15]



E+Z

175,18 g/mol

$C_7H_{13}NO_4$

Procedure: To a solution of **(2)** (3,21g, 0,02mol, 1eq) in pyridine (45ml), the TM (2,5g) and hydroxylamine hydrochloride (4,2g, 0,06mol, 3eq) were added. The mixture was stirred for 12h. The resulting mixture was diluted with EA and filtered through celite and with EA. The organic phase was washed 3 times with a saturated solution of NaCl and dried with $MgSO_4$, and then filtered. The solvents were evaporated. Toluene was added and then the solvents were co-evaporated.

Yield = 68%

Rf = 0,3 (EA/PE 50:50)

^{1}H NMR (400MHz, $CDCl_3$) δ: 1,37 (s, 6H, $(CH_3)_2$iPr-Z), 1,47 (s, 6H, $(CH_3)_2$iPr-E), 3,49-3,74 (m, 4H, J_1= 5Hz, J_2=12Hz, $(CH\text{-}2)_2$-E+Z), 4,33-4,36 (m, 1H, CH-3-E), 4,51-4,7 (m, 1H, CH-3-Z), 4,88 (t, 1H, $J_{2\text{-}1}$=$J_{2\text{-}3}$=7,2Hz, CH_2-4-E), 5,26 (sl, 1H, CH_2-4-Z), 6,94 (sl, 1H, CH-1-Z), 7,44 (d, 1H, J=7,2Hz, CH-1-E)

^{13}C NMR (100MHz, $CDCl_3$) δ (E+Z mixture): 24,8 & 25,2 & 27,3 & 27,6 (CH_3iPr), 61 & 61,9 (CH_2-4), 72 (CH-2-Z), 74,9 (CH-2-E), 78,4 & 78,5 (CH-3), 109,6 & 110,1 ($\underline{C}(CH_3)_2$), 149 (CH-1-E), 151 (CH-1-Z)

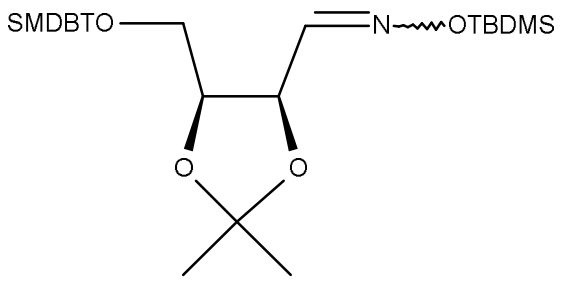
BP/MP: 46-51 ºC

MS (IS): m/z = 176 $[M+H]^+$, 198 $[M+Na]^+$, 214 $[M+K]^+$

$[\alpha]_D^{20}$ **=** -76 ($CHCl_3$)

IR : 698, 792, 837, 883, 909, 927, 957, 984, 1043, 1077, 1104, 1125, 1161,1218, 1253, 1337, 1377, 1415, 1438, 2937, 2989, 3082, 3209, 3383

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



E+Z

403,7 g/mol

$C_{19}H_{41}NO_4Si_2$

Procedure: To a solution of **(3)** (2,41g, 13,8mmol, 1eq) in pyridine (22ml), the TBDMSCl (2,5g, 16,6mmol, 1,2eq) was added. The mixture was stirred for 12h. The resulting mixture was diluted with EA and filtered through celite and with EA. The organic phase was washed with distillated H_2O, HCl 1M, a saturated solution of NaCl and dried with $MgSO_4$, and then filtered. The solvents were evaporated, and then toluene was added to eliminate the pyridine. The solvents were co-evaporated and the product was purified by column chromatography (PE/EA 95:5).

Yield = 24%

Rf = 0,71 (PE/EA 95:5)

^{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, $(CH_3)_2$iPr), 1,49 (d, 6H, $(CH_3)_2$iPr), 3,48 (dd, 1H, J_1=4,58Hz, J_2=11,02Hz, H-4b), 3,61-3,71 (m, 1H, H-4a), 4,24-4,28 (m, 1H, H-3-E), 4,39-4,4 (m, 1H, H-3-Z), 4,71 (dd, 1H, J_1=6,79Hz, J_2=8,11Hz, H-2-E), 5,24 (dd, 1H, J_1=7,26Hz, J_2=4,21Hz, H-2-Z), 7,08 (d, 1H, J_1= 4,29Hz, H-1-Z), 7,49 (d, 1H, J_1=8,19Hz, H-1-E)

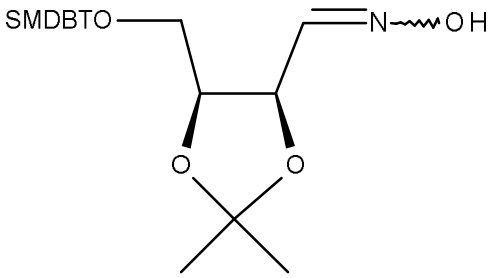
^{13}C NMR (100MHz, $CDCl_3$) δ (E+Z mixture): 5,18 & 5,21 ($Si(CH_3)_2$), 18,35 ($\underline{C}(CH_3)_2$iPr) 25,31 & 25,51 (($\underline{C}H_3)_2$iPr), 26,08 & 26,16 ($SiC(\underline{C}H_3)_3$), 27,31 & 27,85 (($\underline{C}H_3)_2$iPr), 61,7 (CH_2-4), 62,88 (CH_2-4), 72,1 (CH-2-Z), 75,2 (CH-2-E), 78,7 (CH-3-E), 79,11 (CH-3-Z), 109,33 ($\underline{C}(CH_3)_3$), 109,7 ($\underline{C}(CH_3)_3$), 152,2 (CH-1-E), 155,0 (CH-1-Z)

MS (IS): m/z = 404 $[M+H]^+$, 426 $[M+Na]^+$

$[\alpha]_D^{20}$ **=** -53 ($CHCl_3$)

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

(5) 4-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-L-erythrose oxime



E+Z

289,4 g/mol

$C_{13}H_{27}NO_4Si$

Procedure: To a solution of **(3)** (2,41g, 13,8mmol, 1eq) in pyridine (22ml), the TBDMSCl (2,5g, 16,6mmol, 1,2eq) was added. The mixture was stirred for 12h. The resulting mixture was diluted with EA and filtered through celite and with EA. The organic phase was washed with distillated H_2O, HCl 1M, a saturated solution of NaCl and dried with $MgSO_4$, and then filtered. The solvents were evaporated, and then toluene was added to eliminate the pyridine. The solvents were co-evaporated and the product was purified by column chromatography (PE/EA 95:5).

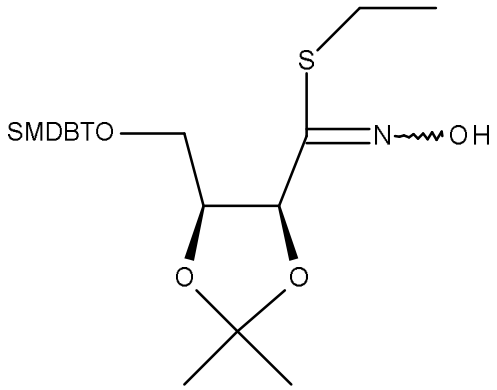
Yield = 35%

Rf = 0,313 (PE/EA 95:5)

^{1}H NMR (400MHz, $CDCl_3$) δ (E+Z mixture): 0,0 (m, 6H, $Si(CH_3)_2$), 0,10 (m, 6H, $Si(CH_3)_2$), 0,82 (s, 9H, $SiC(CH_3)_3$), 0,82 (m, 9H, $SiC(CH_3)_3$), 1,30 (s, 6H, $C(CH_3)_2$), 1,41 (s, 6H, $C(CH_3)_2$), 3,61 (m, 2H, CH-4), 3,61 (m, 2H, CH-4), 4,62 (dd, 1H, $J_{2,3}$=6,8Hz, CH-3), 4,67 (t, 1H, $J_{3,4}$=7,2Hz, CH-3), 5,19 (dd, 1H, CH-2), 5,22 (dd, 1H, CH-2), 6,84 (d, 1H, $J_{1,2}$=4,8Hz, CH=N), 7,36 (d, 1H, $J_{1,2}$=4,8Hz, CH=N)

^{13}C NMR (100MHz, $CDCl_3$) δ (E+Z mixture): -5.56 ($Si(\underline{C}H_3)_2$), -5.48 ($Si(\underline{C}H_3)_2$), -5.40 ($Si(\underline{C}H_3)_2$), -5.37 ($Si(\underline{C}H_3)_2$), 18.1 ($SiC(\underline{C}H_3)_3$), 18.2 ($SiC(\underline{C}H_3)_3$), 25.6 ($SiC(\underline{C}H_3)_3$), 25.8 ($C(\underline{C}H_3)_2$), 25.9 ($SiC(\underline{C}H_3)_3$), 25.9 ($C(\underline{C}H_3)_2$), 27.0 ($C(\underline{C}H_3)_2$), 27.1 ($\underline{C}(CH_3)_3$), 27.4 ($\underline{C}(CH_3)_3$), 27.5 ($C(\underline{C}H_3)_2$), 27,5 ($Si\underline{C}(CH_3)_3$), 27,6 ($\underline{C}(CH_3)_3$), 61.4 (CH_2-4), 62.5 (CH_2-4), 74.7 (CH-2), 74.9 (CH-2), 78.3 (CH-3), 78.5 (CH-3), 109.1 ($\underline{C}(CH_3)_2$), 109.5 ($\underline{C}(CH_3)_2$), 147.8 (N=CH), 150.3 (N=CH)

(6) (Z)-4-*O*-*tert*-butyldimethylsilyl-2,3-isopropylidene-N-hydroxy-L-erythroimidothioate S-ethyl



349,56g/mol

$C_{15}H_{31}NO_4SSi$

Procedure: To a solution of **(5)** (1,96g, 6,8mmol, 1eq) in DMF ([C]=0,25M), it was added NCS (1,36g, 10,2mmol, 1,5eq) and the mixture was stirred for 4h. The Et_3N (2,8ml, 20,4mmol, 3eq) and EtSH (1,5ml, 20,4mmol, 3eq) were added and the resulting mixture was stirred for 12h. The mixture was diluted with EA, and then the organic phase was washed 4 times with distillated H_2O, once with saturated NaCl, dried with $MgSO_4$ and then filtered. The solvents were evaporated. The product was purified by column chromatography (PE/EA 9:1).

Yield = 27%

Rf = 0,24 (PE/EA 9:1)

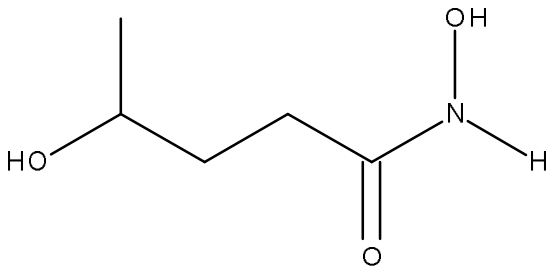
^{1}H NMR (400MHz, $CDCl_3$) δ: 0,05 (s, 3H, $SiCH_3$), 0,05 (s, 3H, $SiCH_3$), 0,87 (s, 9H, $(CH_3)_3C$), 1,29 (t, 3H, J=8,0Hz, CH_2CH_3), 1,37 (s, 3H, OCH_3iPr), 1,53 (s, 3H, OCH_3iPr), 2,97-3,1 (m, 2H,SCH_2), 3,73 (dd, 1H, $J_{4a,4b}$=10,6Hz, $J_{4b,3}$=6,2Hz, H-4b), 3,79 (dd, 1H, $J_{4a,4b}$=10,6Hz, $J_{4a,b}$=6,0Hz, H-4a), 4,37 (q, 1H, $J_{3,2}$=6,7Hz, H-2), 9,09 (sl, 1H, N-OH)

^{13}C NMR (100MHz, $CDCl_3$) δ: -5,2 ($SiCH_3$), -5,1 ($SiCH_3$), 15,1 (CH_3CH_2S), 18,6 ($C(CH_3)_3$), 25,3 (CH_3iPr), 25,8 (CH_2S), 26,2 ($(CH_3)_3C$), 27,1 (CH_3iPr), 62,6 (C-4), 77,3 (C-2), 79,1 (C-3), 109,7 ($C(CH_3)_2$), 149,8 (C=N)

MS (IS): m/z = 350 $[M+H]^+$, 372 $[M+Na]^+$, 388 $[M+K]^+$

IR : 667, 777, 835, 900, 992, 1078, 1161, 1214, 1252, 1378, 1462, 1601, 2857, 2930, 3288

(7) 4-hydroxypentanehydroxamic acid



133,146g/mol

$C_5H_{11}NO_3$

Procedure: At 0ºC, to a solution of MeOH (50ml) and $NH_2OH.HCl$ (17,4g, 0,25mol, 5eq), the KOH (14g, 0,25mol, 5eq) was added and the mixture was stirred for 10min. The resulting mixture was filtered and then it was added γ-valerolactone (4,8ml, 49,94mmol, 1eq). The mixture was stirred for 12h, and then the solvents were evaporated. The product was purified by column chromatography (EA/MeOH 9:1).

Yield = 87%

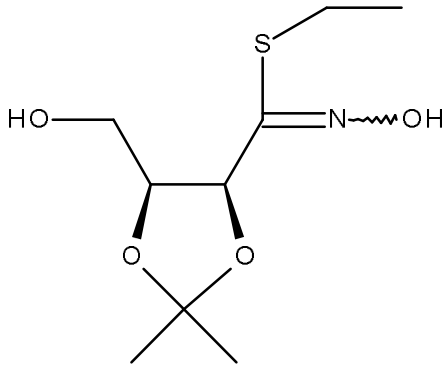
Rf = 0,24 (EA/MeOH 9:1)

^{1}H NMR (250MHz, DMSO) δ: 1,04 (d, 3H, $J_{5\text{-}4}$=6,1Hz, CH_3-5), 1,57-1,47 (m, 2H, CH_2-3), 1,99 (dd, 2H, J_1=7,5Hz, J_2=15,023Hz, CH_2-2), 3,51-3,57 (m, 1H, CH-4), 4,46 (d, 1H, OH), 8,65 (sl, 1H, NH), 10,34 (s, 1H, NOH)

$[\alpha]_D^{20}$ **=** +0,7 ($CHCl_3$)

IR : 904, 944, 1015, 1047,1085,1127,1181, 1376,1451, 1538, 1634, 1759, 2969, 3194

(8) (Z)-2,3-*O*-isopropylidene-N-hydroxi-L-erythronimidothioate de S-ethyl



235,3g/mol

$C_9H_{17}NO_4S$

Procedure: To a solution of **(6)** (0,65g, 1,86mmol, 1eq) in THF (11ml), TBAT (1,41g, 2,6mmol, 1,4eq) was added. The resulting mixture was stirred for 12h. The mixture was diluted with EA, and the organic phase was washed twice with distillated H_2O and once with a saturated solution of NaCl, and then dried with $MgSO_4$. The resulting mixture was filtered and then the solvents were evaporated. The product was purified through column chromatography (PE/EA 50:50).

Yield = 38%

Rf = 0,28 (PE/EA 5:5)

^{1}H NMR (400MHz, $CDCl_3$) δ: 1,33 (t, 3H, J= 7.6 Hz, SCH_3CH_2), 1,40 (s, 3H, CCH_3), 1,53 (s, 3H, CCH_3), 3,11 (m, 2H, SCH_3CH_2), 3.68 (m, 1H, CH_2-4b), 3,79 (m, 1H, CH_2-4a), 4,40 (q, 1H, $J_{2,3}$= $J_{3,4}$= 4.4, CH-3), 4,89 (d, 1H, $J_{2,3}$= 6.0, CH-2), 9,40 (s, 1H, NOH)

^{13}C NMR (100MHz, $CDCl_3$) δ: 15,1 ($\underline{C}H_3CH_2$), 25,4 ($\underline{C}(CH_3)_2$), 25,6 (CH_3CH_2), 27,3 ($C(\underline{C}H_3)_2$), 61,7 (CH_2-4), 76,9 (CH-3), 76,8 (CH-3), 109,5 ($C(\underline{C}H_3)_2$), 150,8 (C=N)

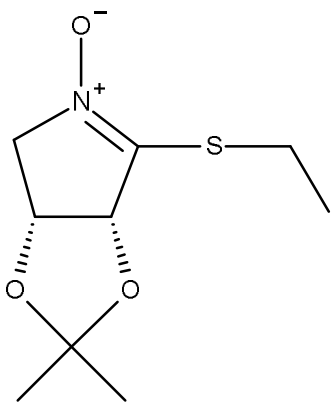
BP/MP: 88-94 ºC

MS (IS): m/z = 236 $[M+H]^+$, 258 $[M+Na]^+$, 274 $[M+K]^+$

$[\alpha]_D^{20}$ **=** +18 ($CHCl_3$)

IR : 670, 699,715, 736, 794, 811, 856, 894, 950, 929, 969, 1001, 1033, 1057, 1086, 1122, 1147, 1162, 1204, 1240, 1273, 1315, 1377, 1408, 1453, 1618, 1688, 2868, 2981, 3034, 3155, 3479

(9) (3S,4S)-2-(ethylthio)-3,4-isopropylidenedioxy-3,4-dihydro-5H-pyrrole-1-oxyde [16-17]



217,29/mol

$C_9H_{15}NO_3S$

Procedure: To a solution of **8** (0,17g, 0,72mmol, 1eq) in anydre THF, it was added PPh_3 (0,2g, 0,76mmol, 1,1eq) and DEAD (0,37ml, 2,376mmol, 3,3eq), and the resulting mixture was stirred for 4 hours, under reflux. The solvents were evaporated and the product was purified by column chromatography (EA 100% and EA/MeOH 9:1).

Yield = 4%

Rf = 0,6 (PE/EA 5:5)

^{1}H NMR (400MHz, $CDCl_3$) δ: 1,38 (tl, 3H, J_1=5,7Hz, SCH_2CH_3), 1,39 (s, 3H, CH_3iPr), 1,44 (s, 3H, CH_3iPr), 3,14 (q, 2H, SCH_2CH_3), 4,07 (d, 1H, $J_{4\text{-}4'}$=14,7Hz, CH-4_a), 4,14 (dd, 1H, $J_{4\text{-}4'}$=14,7Hz, $J_{4\text{-}3}$=5,19Hz, CH-4_b), 4,9 (tl, 1H, J_1=6,41Hz, 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 ($S\underline{C}H_2CH_3$), 26,1 (CH_3iPr), 27,3 (CH_3iPr), 66,6 (CH_2-4), 73,2 (CH-3), 81,8 (CH-2), 112,9 ($\underline{C}(CH_3)_2$), 144,0 (C-1)

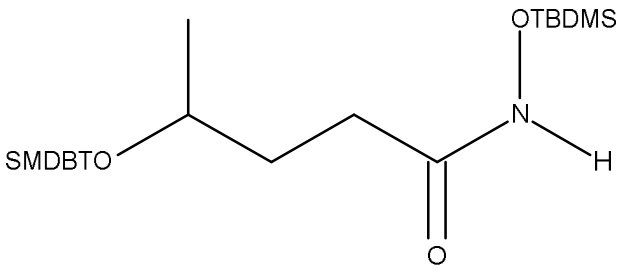
BP/MP: 139-143 ºC

MS (IS): m/z = 218 $[M+H]^+$, 240 $[M+Na]^+$, 256 $[M+K]^+$, 244 $[M+NH_4]^+$

$[\alpha]_D^{20}$ **=** +144 ($CHCl_3$)

IR : 663, 706, 745, 802, 836,865, 899, 948, 968, 989, 1022, 1077, 1154, 1205, 1235, 1261, 1277, 1300, 1327, 1383, 1423, 1451, 1570, 1730, 2975

(10) 4-(*tert*-butyldimethylsilyloxy)-pentanhydroxamate *tert*-butyldimethylsilyl



361,68g/mol

$C_{17}H_{39}NO_3Si_2$

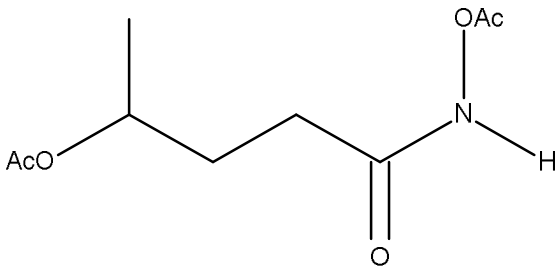
Procedure: To a solution of **(7)** (5,8g, 0,0436mmol, 1eq), in DMF (44ml), it was added imidazole (8,9g, 0,131mol, 3eq) and TBDMSCl (16g, 0,105mol, 2,4eq) and the mixture was stirred for 12 hours. The resulting mixture was diluted with EA, and the organic phase was washed with distillated H_2O and with a saturated solution of NaCl, and dried with $MgSO_4$. The mixture was filtered and the solvents were evaporated. The product was purified by column chromatography (PE/EA 95:5).

Yield = 42%

Rf = 0,42 (PE/EA 9:1)

MS (IS): m/z = 362 $[M+H]^+$, 384 $[M+Na]^+$

(11) 4-(acetoxy)-pentanhydroxamate acetyl



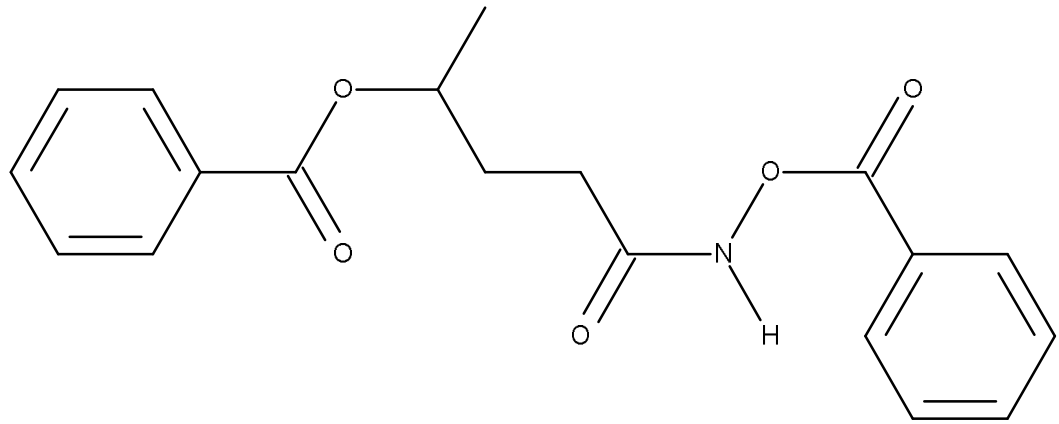
214,2g/mol

$C_9H_{15}NO_5$

Procedure: A solution of **7** (0,26g, 1,9mmol, 1eq) in pyridine (6ml) was stirred for 10 min, at -5ºC. To the resulting mixture it was added Ac_2O (460µl, 4,8mmol, 2,5eq), and then it was stirred for 4h, at room temperature. The resulting mixture was transferred to another flask with the help of toluene, and the solvents were co-evaporated. The organic phase was washed 3 times with EA, once with a saturated solution of NaCl, and then dried with $MgSO_4$. The resulting mixture was filtered and the solvents were evaporated.

Rf = 0,5 (EA/MeOH 9:1)

(**12**) 5-(benzoyloxyamino)-5-oxopentan-2-yl benzoate



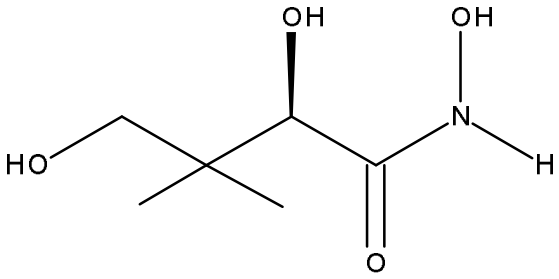
341,363g/mol

$C_{19}H_{19}NO_5$

Procedure: A solution of (**7**) (0,3g, 2,25mmol, 1eq) in pyridine (6ml) was stirred for 10mins, at -5ºC. Then the benzoyl chloride (630µl, 5,4mmol, 2,5eq) was added, and the resulting mixture was stirred for 4h, at room temperature. The mixture was transferred to another flask with the help of toluene, and the solvents were co-evaporated. The organic phase was washed 3 times with EA, once with a saturated solution of NaCl, and then dried with $MgSO_4$. The resulting mixture was filtered and the solvents were evaporated. The organic phase was washed twice with saturated $NaHCO_3$, once with a saturated solution of NaCl, and dried with $MgSO_4$. The mixture was filtered and the solvents were evaporated. The product was purified by column chromatography (PE/EA 95:5).

Rf = 0,92 (DCM/EA 98:2)

(**13**) (R)-N-2,4-trihydroxybutanamide



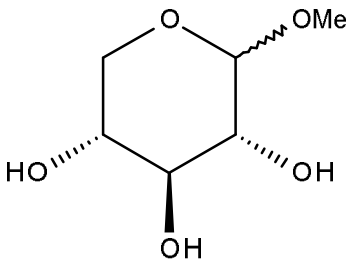
162,1651g/mol

$C_6H_{12}NO_4$

Procedure: To a solution of MeOH (16ml) and $NH_2OH.HCl$ (5,34g, 76,84mmol, 5eq), it was added KOH (4,31g, 76,84mmol, 5eq) and the mixture was stirred for 10min, at 0ºC. The resulting mixture was filtered and then the D-(-)-pantolactone (2g, 15,368mmol, 1eq) was added, and the mixture was stirred for 12h. It was added toluene and the solvents were co-evaporated. Acetone was added, the resulting mixture was filtered and then the solvents were evaporated.

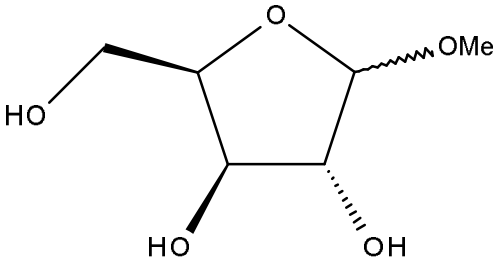
Rf = 0,37 (EA/MeOH 9:1)

(14) Methyl-α,ß-D-xylopyranoside; Methyl- α,ß-D-xylofuranoside [13]



164,068g/mol

$C_6H_{12}O_5$



164,068g/mol

$C_6H_{12}O_5$

Procedure: To a solution of D-xylose (10g, 66,6mmol, 1eq) in MeOH (100ml) was added concentrated H_2SO_4 (0,25ml). The resulting mixture was stirred for 3h, under reflux. To neutralize it was added solid $NaHCO_3$, and then the solution was filtered through celite. The solvents were evaporated. The product was used in the next reactions without purification.

Rf = 0,45 (DCM/MeOH 9:1)

^{1}H NMR (400MHz, DMSO) δ: (α+ß mixture) 3,29 (6H, s, OMe), 3,52-3,65 (4H, m, CH_2-5), 3,77-3,94 (2H, m, CH-2), 4,0-4,1 (2H, m, CH-3), 4,6 (2H, s, CH-4), 4,69-4,76 (2H, m, CH-1)

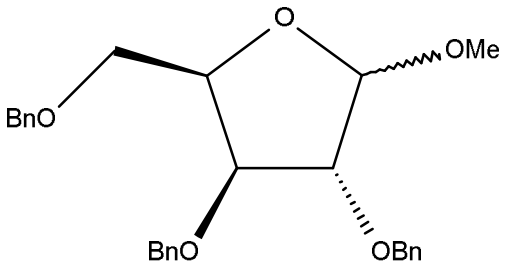
^{13}C NMR (100MHz, DMSO) δ: (α+ß mixture) 54,6 & 54,8 (OMe), 60,6 & 60,7 (C-5), 75,0 & 71,2 (C-2), 77,5 & 80,8 (C-3), 78,8 & 82,5 (C-4), 102,2 & 109,4 (C-1)

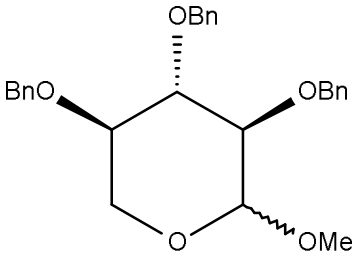
MS (IS): m/z = 187 $[M+Na]^+$, 351 $[2M+Na]^+$

$[\alpha]_D^{20}$ **=** +1,6 ($CHCl_3$)

IR : 767, 892, 1019,1194, 1357, 1448, 2839, 2936, 3363

(15) Methyl 2,3,4-tri-*O*-benzyl-α,ß-L-xylofuranoside; Methyl 2,3,4-tri-*O*-benzyl-α,ß-L-xylopyranoside [14]





434,209g/mol

$C_{27}H_{30}O_5$

Procedure: At 0ºC, to a solution of **(14)** (2g, 12,109mmol, 1eq) in DMF (50ml) it was added NaH (1,94g, 48,44mmol, 4 eq), nBu_4NI (0,4473g, 1,2109mmol, 0,1eq) and BnBr (6ml, 48,44mmol, 4eq). The resulting mixture was stirred for 48h, at room temperature. At 0ºC, it was added MeOH (10ml) and distillated H_2O (50ml). The organic phase was washed 3 times with EA, once with distillated H_2O, once with a saturated solution of NaCl and then dried with $MgSO_4$. The solvents were evaporated. The product was purified by column chromatography (PE/EA 95:5).

Yield = 30% (furanoside), 30% (pyranoside)

Rf = 0,59 (furanoside), 0,47 (pyranoside) (PE/EA 9:1)

^{1}H NMR (400MHz, $CDCl_3$) δ: (α+ß mixture) 3,33 (6H, s, OMe), 3,77-3,79 (2H, m, CH-3), 3,98 (2H, s, CH-2), 4,24-4,25 (2H, m, CH-4), 4,43-4,67 (12H, m, $(OCH_2Bn)_3$), 4,71-4,74 (4H, m, CH2-5), 4,91 (2H, s, CH-1), 7,28-7,34 (H-ar)

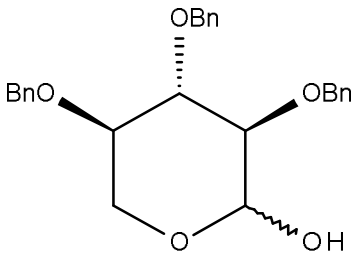
^{13}C NMR (100MHz, $CDCl_3$) δ: (α+ß mixture) 55,9 (OMe), 70,0 & 73,6 & 73,7 (OCH_2Bn), 72,13 (C-5), 80,3 (C-3), 81,7 (C-4), 87,1 (C-2), 108,4 (C-1) 127,8-128,6 (C-ar), 137,8-139,0 (C_{IV})

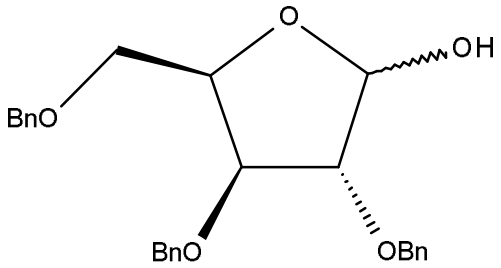
MS (IS): m/z = 435 $[M+H]^+$, 457 $[M+Na]^+$

$[\alpha]_D^{20}$ **=** -9 ($CHCl_3$)

IR : 696,734, 819, 909, 952, 1027, 1055, 1091, 1199, 1244, 1365, 1435, 1496, 1605, 2911, 3030, 3062, 3337

(16) 2,3,4-tri-*O*-benzyl-D-xylopyranose; 2,3,4-tri-*O*-benzyl-D-xylofuranose





(α+ß)

420,194g/mol

$C_{26}H_{28}O_5$

Procedure 1: At 0ºC, to a solution of **15** (1,6g, 3,633mmol, 1eq) in AcOH (5ml) was added dioxane (5ml) and H_2SO_4 1M (0,278ml). The resulting mixture was stirred for 16h, under reflux. To the solution it was added PE and distillated H_2O, and then the solution was filtered. The resulting mixture was washed with PE. The solvents were evaporated, and then the product was diluted in AE and washed with a saturated solution of NaCl, dried with $MgSO_4$ and then filtered. The solvents were evaporated. The product was purified by column chromatography (PE/EA 8:2).

Yield = 10%

Procedure 2: At 0ºC, to a solution of **(15)** (1,6096g, 3,7042mmol, 1eq) in AcOH (5ml) was added dioxane (5ml) and H_2SO_4 1M (0,278ml). The resulting mixture was stirred for 20h, under reflux. The mixture was diluted with EA and distillated H_2O. The organic phase was extracted 3 times with EA, and then washed with distillated H_2O, with a saturated solution of $NaHCO_3$ and with a saturated solution of NaCl, then dried with $MgSO_4$ and filtered. The solvents were evaporated. The product was purified by column chromatography (PE/EA 8:2).

Yield = 44%

Rf = 0,23 (PE/EA 8:2)

^{1}H NMR (400MHz, $CDCl_3$) δ: 3,48 (dd, 2H, J=6,6Hz, J=10,42Hz, CH_2-5ß), 3,6-3,7 (m, 2H, CH_2-5α), 3,9 (sl, 1h,CH-2α), 3,9 (t, 1H, J=4,6Hz, CH-2ß), 4,02 (dd, 1H, J=2,6Hz, J=5,4Hz, CH-3α), 4,15 (t, 1H, J=5,4Hz,CH-3ß), 4,23 (q, 1H, J=5,2Hz, J=11,6Hz, CH-4α), 4,3-4,34 (m, 1h, CH-4ß), 4,45-4,64 (m, 12H, $(CH_2)_3$Ph α/ß), 5,13 (d, 1H, J=2Hz, CH-1α), 5,32 (d, 1H, J=4,4Hz, CH-1ß), 6,28 (d, 1H, J=6,8Hz, OH-ß), 6,51 (d, 1H, J=5,6Hz, OH-α), 7,27-7,37 (m, 30H, Ph_3 α/ß)

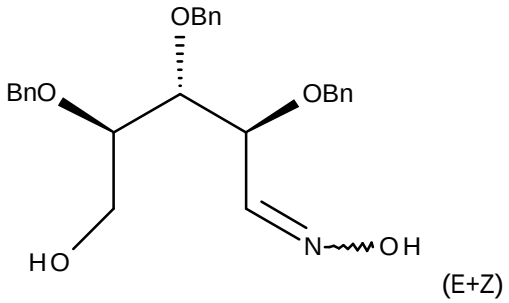
^{13}C NMR (100MHz, $CDCl_3$) δ: 69,5 (C-5α), 69,7 (C-5ß), 71,5 & 71,6 & 71,7 (($\underline{C}H_2Ph)_3$), 75,8 (C-4ß), 78,7 (C-4α), 81,7 (C-3ß), 82,0 (C-3α), 83,2 (C-2ß), 87,4 (C-2α), 94,6 (C-1α), 101,5 (C-1ß), 127,8 & 127,9 & 128,0 & 129,5 %128,6 (30C, CPh α/ß), 138,4 & 138,6 & 138,7 &138,9 (6C, C_{IV}Ph α/ß)

MS (IS): m/z = 421 $[M+H]^+$, 443 $[M+Na]^+$

$[\alpha]_D^{20}$ **=** +14 ($CHCl_3$)

IR : 1059, 1446, 1498, 2854, 2916, 3030, 3067, 3431

(17) 2,3,4-tri-*O*-benzyl-D-xylose oxime



(E+Z)

435,5g/mol

$C_{26}H_{29}NO_5$

Procedure: At 0ºC, to MeOH (10ml) is added Na (0,109g, 4,7584mmol, 8eq), and the resulting mixture is stirred for 10mins. Then the $NH_2OH.HCl$ (0,33g, 4,7584mmol, 8eq) and **(16)** are added, and the resulting mixture is stirred for 12 hours, under reflux. To the mixture it was added a saturated solution of NaCl. The organic phase was washed 3 times with DCM, dried with $MgSO_4$ and then filtered. The solvents were evaporated. Then the organic phase was washed with distillated H_2O and with a saturated solution of NaCl, dried with $MgSO_4$ and then filtered. The solvents were evaporated. The product was purified by column chromatography (PE/EA 1:1).

Yield = 36%

Rf = 0,5 (PE/EA 1:1)

^{1}H NMR (400MHz, $CDCl_3$) δ: 2,55 (s, 1H, OH), 3,36-3,46 (m, 4H, CH_2-5 E/Z), 3,72 (dd, 1H, J=2,4Hz, J=6,4Hz, CH-3E), 3,79 (dd, 1H, J=3,2Hz, J=5,6Hz, CH-3Z), 3,95 (sl, 1H, CH-4Z), 4,26 (dd, 1H, J=6,4Hz, J=8Hz, CH-4E), 4,39-4,44 (m, 6H, $(OCH_2Ph)_3$ E), 4,51-4,56 (m, 2H, OCH_2Ph Z), 4,6-4,64 (m, 2H, OCH_2PH Z), 4,75-4,8 (m, 2H, OCH_2Ph Z), 4,88 (t, 1H, J=7,6Hz, CH-2E), 5,0 (t, 1H, J=6,2Hz, CH-2Z), 6,9 (d, 1H, J=6,8Hz, CH-1Z), 7,26-7,31 (m, 30H, Ph_3 E/Z), 7,39 (s, 1H, CH-1E), 7,46 (d, 1H, J=8Hz, NOH)

MS (IS): m/z = 436 $[M+H]^+$, 458 $[M+Na]^+$

IR : 696, 734, 819, 935, 1027, 1064, 1209, 1252, 1359, 1393, 1453, 1496, 2866, 3030, 3330

Antioxidant activity

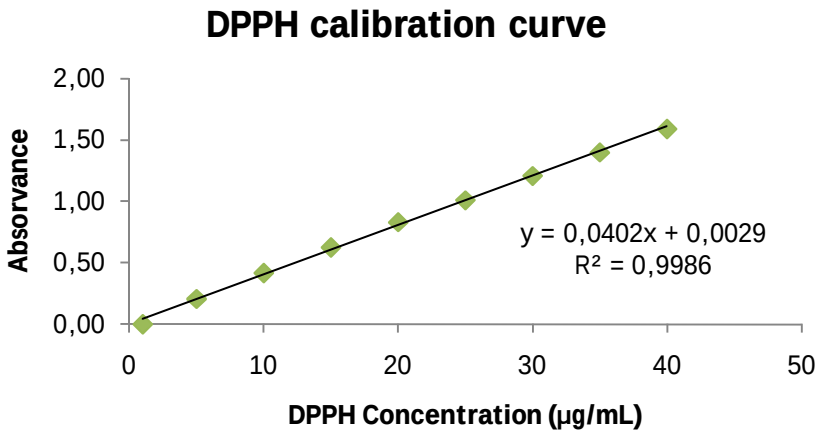
DPPH Calibration curve construction

First of all, it was prepared 50ml of a stock solution of DPPH in methanol (60µg/ml), and it was kept under refrigeration and protected from light. 40, 35, 30, 25, 20, 15, 10, 5 and 1µg/ml dilutions were done. The calibration curve was constructed from the absorbance values at 515nm of every concentration (1 to 40µg/ml). The absorbance measurements were performed in triplicate and at every minute, until it reached a plateau. The equation of the calibration curve obtained was

$$Abs_{515nm} = 0,0402 * [DPPH] + 0,0029,$$

were [DPPH] corresponds to the DPPH concentration in the medium, Abs_{515nm} is the absorbance measured at 515nm and the correlation coefficient is R=0,998.

The blank used for the calibration curve was 3ml of MeOH.



Graphic 2.1 – DPPH calibration curve

Compound samples absorbance measurements

The antioxidant activity of compound (9) was measured using the following dilutions: 550, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, and 25µg/ml of a stock solution (894µg/ml) in methanol. The measurements were performed in triplicate and every minute until it reached a plateau. The blank used was 2,7ml of MeOH and 0,3ml of methanolic compound solution. The compound measurements were performed using 2,7ml of DPPH. Firstly, the dilutions between 250-25µg/ml were tested, and then the concentration range tested was 550-300µg/ml. With the first range of concentrations (25-550µg/ml) tested no conclusive results could be drawn, so a even higher concentration was tested (12500µg/ml). The antioxidant activity of compound (17) was also tested, in a concentration of 17000µg/ml.

Table 1 – Antioxidant activity of compound (9)

t(min)	[]=12500µg/mL	%antioxidant activity	t(min)	[]=12500µg/mL	%antioxidant activity
1	1,176	26,07028352	**44**	1,084	31,8539008
2	1,173	26,25887974	**45**	1,083	31,9167662
3	1,164	26,82466838	**46**	1,082	31,97963161
4	1,158	27,20186082	**47**	1,082	31,97963161
5	1,153	27,51618784	**48**	1,081	32,04249701
6	1,148	27,83051487	**49**	1,081	32,04249701
7	1,146	27,95624568	**50**	1,079	32,16822782
8	1,143	28,14484189	**51**	1,078	32,23109323
9	1,139	28,39630351	**52**	1,077	32,29395863
10	1,137	28,52203432	**53**	1,077	32,29395863
11	1,134	28,71063054	**54**	1,076	32,35682404
12	1,131	28,89922676	**55**	1,075	32,41968944
13	1,129	29,02495757	**56**	1,075	32,41968944
14	1,126	29,21355378	**57**	1,075	32,41968944
15	1,124	29,33928459	**58**	1,075	32,41968944
16	1,121	29,52788081	**59**	1,075	32,41968944
17	1,119	29,65361162	**60**	1,075	32,41968944
18	1,118	29,71647702			
19	1,117	29,77934243			
20	1,115	29,90507324			
21	1,113	30,03080405			
22	1,11	30,21940026			
23	1,107	30,40799648			
24	1,107	30,40799648			
25	1,105	30,53372729			
26	1,103	30,6594581			
27	1,101	30,78518891			
28	1,1	30,84805432			
29	1,098	30,97378513			
30	1,096	31,09951594			
31	1,095	31,16238134			
32	1,094	31,22524675			
33	1,093	31,28811215			
34	1,092	31,35097756			
35	1,091	31,41384296			
36	1,09	31,47670837			
37	1,089	31,53957377			
38	1,088	31,60243918			
39	1,087	31,66530458			
40	1,086	31,72816999			
41	1,086	31,72816999			
42	1,085	31,79103539			
43	1,085	31,79103539			

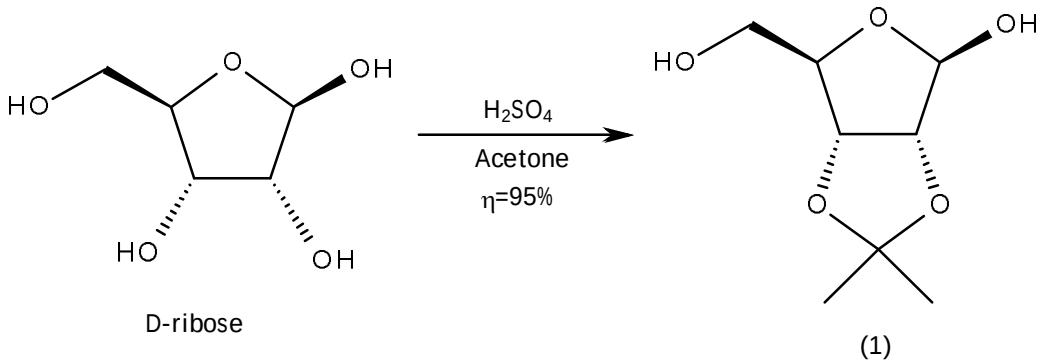
Table 2 – Antioxidant activity of compound (17)

t(min)	Abs	%Antioxidant Activity	t(min)	Abs	%Antioxidant Activity
1	1,589	12,113	**44**	1,407	22,179
2	1,587	12,223	**45**	1,404	22,345
3	1,582	12,500	**46**	1,400	22,566
4	1,571	13,108	**47**	1,397	22,732
5	1,562	13,606	**48**	1,394	22,898
6	1,554	14,049	**49**	1,392	23,009
7	1,548	14,381	**50**	1,389	23,175
8	1,543	14,657	**51**	1,386	23,341
9	1,538	14,934	**52**	1,382	23,562
10	1,533	15,210	**53**	1,379	23,728
11	1,529	15,431	**54**	1,376	23,894
12	1,524	15,708	**55**	1,373	24,060
13	1,519	15,985	**56**	1,371	24,170
14	1,516	16,150	**57**	1,368	24,336
15	1,512	16,372	**58**	1,364	24,558
16	1,508	16,593	**59**	1,362	24,668
17	1,503	16,869	**60**	1,359	24,834
18	1,499	17,091			
19	1,495	17,312			
20	1,491	17,533			
21	1,487	17,754			
22	1,483	17,976			
23	1,480	18,142			
24	1,476	18,363			
25	1,472	18,584			
26	1,469	18,750			
27	1,465	18,971			
28	1,461	19,192			
29	1,457	19,414			
30	1,454	19,580			
31	1,451	19,746			
32	1,447	19,967			
33	1,444	20,133			
34	1,440	20,354			
35	1,436	20,575			
36	1,433	20,741			
37	1,430	20,907			
38	1,426	21,128			
39	1,422	21,350			
40	1,419	21,515			
41	1,417	21,626			
42	1,413	21,847			
43	1,410	22,013			

Chapter 3

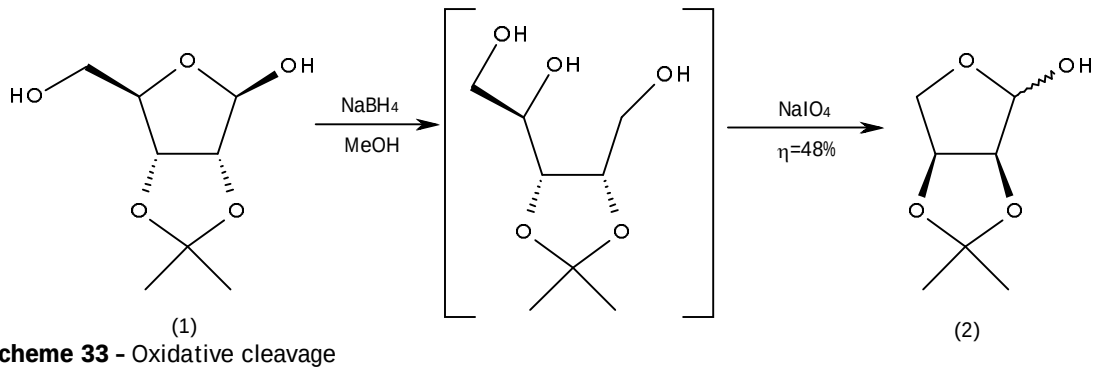
Results Discussion

The first path of synthesis started with D-ribose led to the obtainment of compound (9) in a global yield of 42%. Starting from D-ribose and using concentrated H_2SO_4 and acetone gave compound (1) in a 95% yield. It was easy to see that the reaction worked because the isopropylidene groups have a characteristic chemical shift that appears, as two singlets, at 1,27ppm and 1,43ppm. With the ^{13}C NMR spectrum it's also visible that the isopropylidene group was formed, with a peak at 112,3ppm.



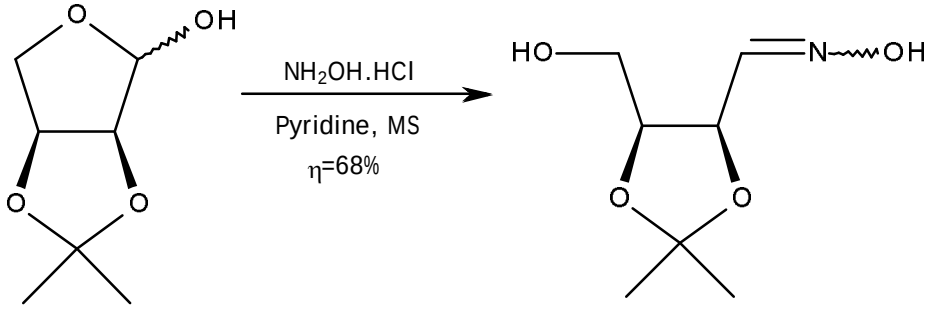
Scheme 32 - Hydroxyl group protection with isopropylidene

The reaction that leads to compound (2) is composed by 2 steps, in which the intermediary isn't isolated. Using $NaBH_4$ and $NaIO_4$, which are reductive compounds, in MeOH, there is a reduction followed by an oxidative cleavage. The cleavage happens in the carbon at position number four and a CH_2OH is "lost". The formation of compound (2) is verifiable by the disappearing of the multiplet that in the previous compound corresponded to the CH_2-5, with a chemical shift between 3,63-3,69ppm. The anomeric carbon is mostly in β position. In the carbon spectrum, when compared with the previous compound, there is a disappearance of peak at 63,6ppm that corresponded to the CH_2-5.



Scheme 33 – Oxidative cleavage

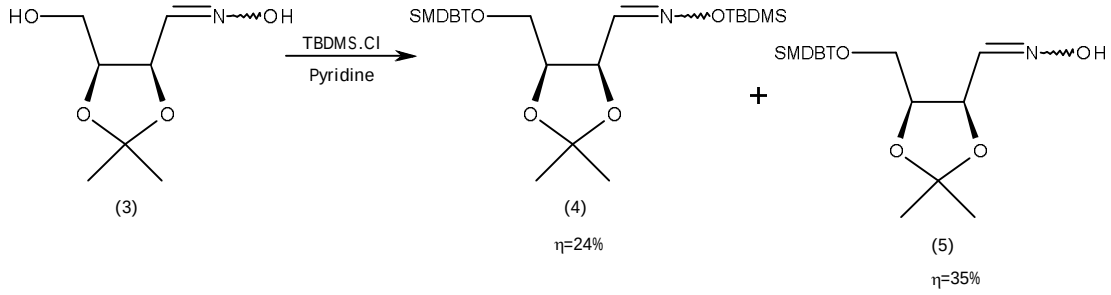
Compound (3) is obtained as a mixture of E and Z isomers. The reaction starts from the addition of NH2OH.HCl and pyridine to compound (2). The oxime formation can be verified because the CH-1 of both the isomers appear as a doublet at 6,94ppm (Z) and 7,44 (E), due to the proximity to the NOH group, that is very electronegative which means it attracts the proton, increasing its chemical shift. The CH-1 of the starting compound of this reaction appeared as a singlet at 5,35ppm and the OH group appeared as a large singlet at 4,24ppm.



(2) (3)

Scheme 34 – Aldoxime formation

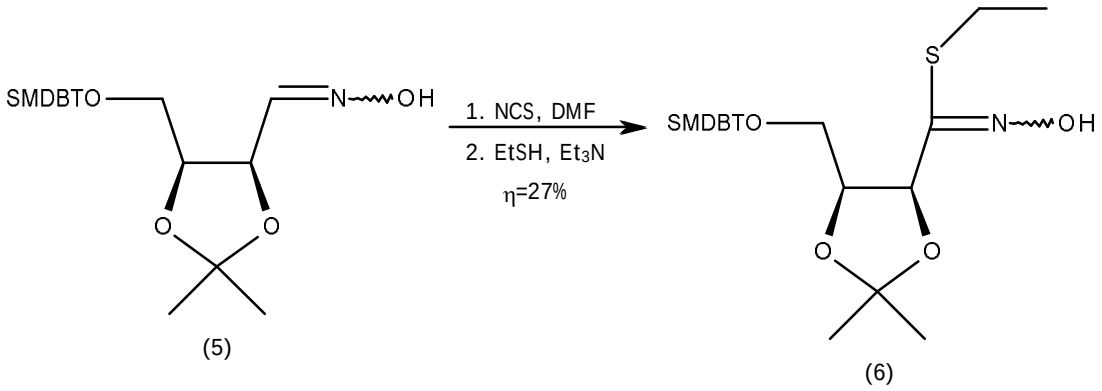
Compounds (4) and (5) correspond to the di- and mono-silylated compounds, respectively, which both have E and Z isomers, derived from the reaction between (3) and TBDMS.Cl in pyridine. From the proton spectrum of compound (4), which is a mixture of E and Z isomers, we can see the protons correspondent to the TBDMS. The protons corresponding to the two $Si(CH_3)_2$ groups (from both isomers), appear as one doublet and one multiplet, respectively, at 0,04 and 0,14ppm, and both peaks integrate for 12 protons each. The protons that correspond to the $Si(CH_3)_3$ from both isomers, appear as two doublets at 0,88 and 0,91ppm, and the peaks integrate for 18 protons each. As for the carbon spectrum the $Si(CH_3)_2$ groups appear at 5,16ppm and the $Si(CH_3)_3$ groups appear at 26,2ppm. Compound (5) only has one TBDMS group, and by the analysis of the proton spectrum it's visible that the two CH_3 groups, from both isomers, attached to Si appear at 0,0 and 0,1ppm, and the $C(CH_3)_3$ appear at 0,82 and 0,82, as a singlet and as a multiplet. The C=N groups, from both isomers, appear at 6,84 and 7,36ppm. These groups have such high chemical shift values because the nitrogen element is very electro negative, and these types of elements always have higher chemical shifts.



(3) (4) η=24% (5) η=35%

Scheme 35 – Protection with TBDMS.Cl

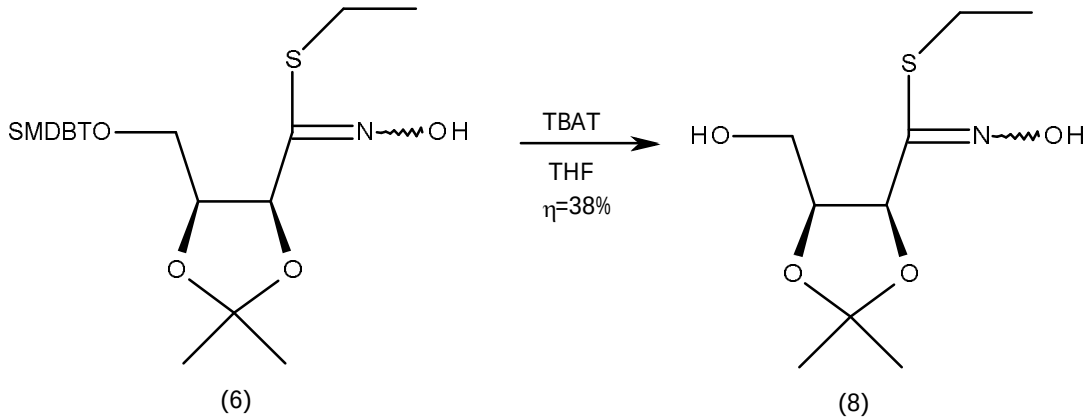
Compound (6) was obtained from a two-step reaction, in which firstly (5) reacted with NCS in DMF, and 4 hours later, EtSH and Et_3N were added. From the proton spectrum we can see that the reaction worked, because there is evidence that a S-CH_2-CH_3 group was added to the previous compound. The peak corresponding to the new CH_3 appears as a triplet at 1,23ppm, and the CH_2 appears as a multiplet between 2,97-3,1ppm. The NOH group is still present as showed by the presence of a large singlet at 9,09ppm, and with the carbon spectrum the double bond N=C as a peak at 149,8ppm. The new S-CH_2-CH_3 group appears as peak at 15,1ppm. In this reaction different reagents were added in two different times, and so that might be one of the reasons that could explain the low yield obtained, because if the reaction was not finished before the addition of EtSH and Et_3N, the depart product won't be totally transformed into the intermediary. The two last reagents, in order to form the desired final compound, would have to react only with the intermediary, and so if some depart product still exists it reduces the reaction yield.



(5) (6)

Scheme 36 – Conversion to a thiohydroxamate

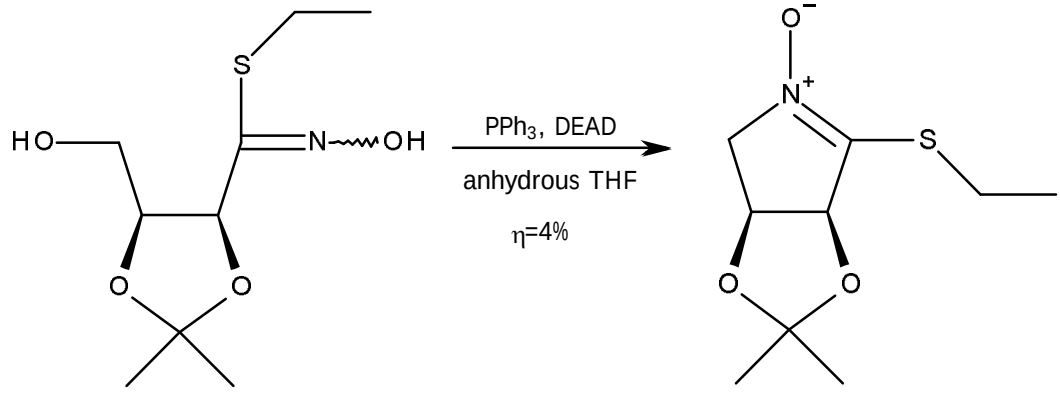
The de-O-sylilation of (6) was performed using TBAT in THF. With the analysis of the proton spectra we can see that the TBDMS characteristic peaks disappeared, and so the reaction was successful. The peaks that disappear could previously be found at 0,05, 0,05 and 0,87ppm, and corresponded to the all the protons in the TBDMS group. The two protons of C-4 also have new chemical shifts, because now there is an OH group, which when compared in size to the TBDMS group, and so they have a new structural arrangement.The OH group also has a high electronegativity value and so the displaces the peaks to higher values. Compound (8) was obtained in a 38% yield.



(6) (8)

Scheme 37 – De-O-silylation

The final compound in this synthesis path was obtained, in an extremely low yield, from the reaction between (8) and PPh_3 and DEAD in anhydrous THF. The synthesis of **(9)** was achieved in a yield of 4%. This reaction was under reflux, and if the temperature wasn't the correct one at all times, this could have led to a not totally finished reaction, which means some of the depart product hadn't been totally converted to compound **(9)**, lowering the reaction yield. With the analysis of proton spectrum we can see the disappearance of the peak correspondent to the NOH group, previously at 9,4ppm, because the N has now formed a bond with the oxygen, and has no bond with hydrogen. The OH group at C-4 disappeared and so the two hydrogens in that carbon now have different chemical shifts, because oxygen is very electronegative it attracts the hydrogens increasing the chemical shift. When the OH is no longer there the chemical shifts of both H-4 protons is lower, and the peaks appear at 4,07ppm, as a doublet, and at 4,14ppm, as a doublet doublet. This last proton is a doublet doublet because it couples with the other proton in the C-4, and with the proton in the C-3.

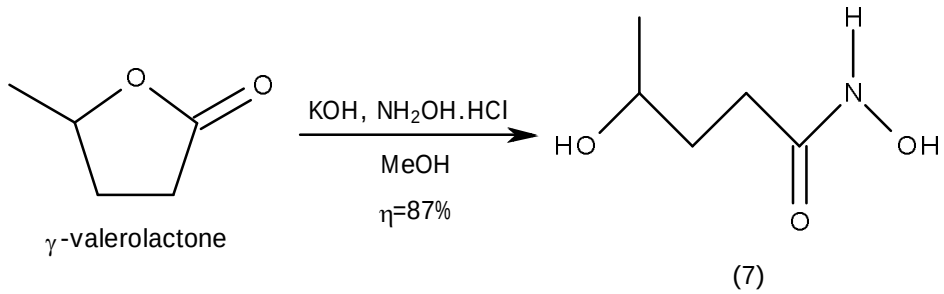


(8) (9)

Scheme 38 – Ring-closing

A second path of synthesis, using lactones, and using firstly γ-valerolactone, was initiated. When MeOH, $NH_2OH.HCl$ and solid KOH reacted with the lactone, it gave us access to the 4-hydroxypentanehydroxamic acid (7). The conclusion that the reaction worked can be drawn

from the analysis of the proton spectrum, where we can see that the NOH group and the NH, at one end of the chain, appear as singlets at 10,34ppm and at 8,65ppm, respectively. The other end of the chain, the OH group, appears as a doublet at 4,46ppm.

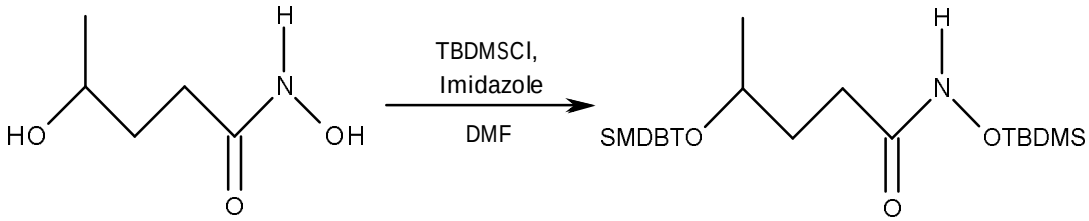


γ-valerolactone

(7)

Scheme 39 – Hydroxamic acid formation

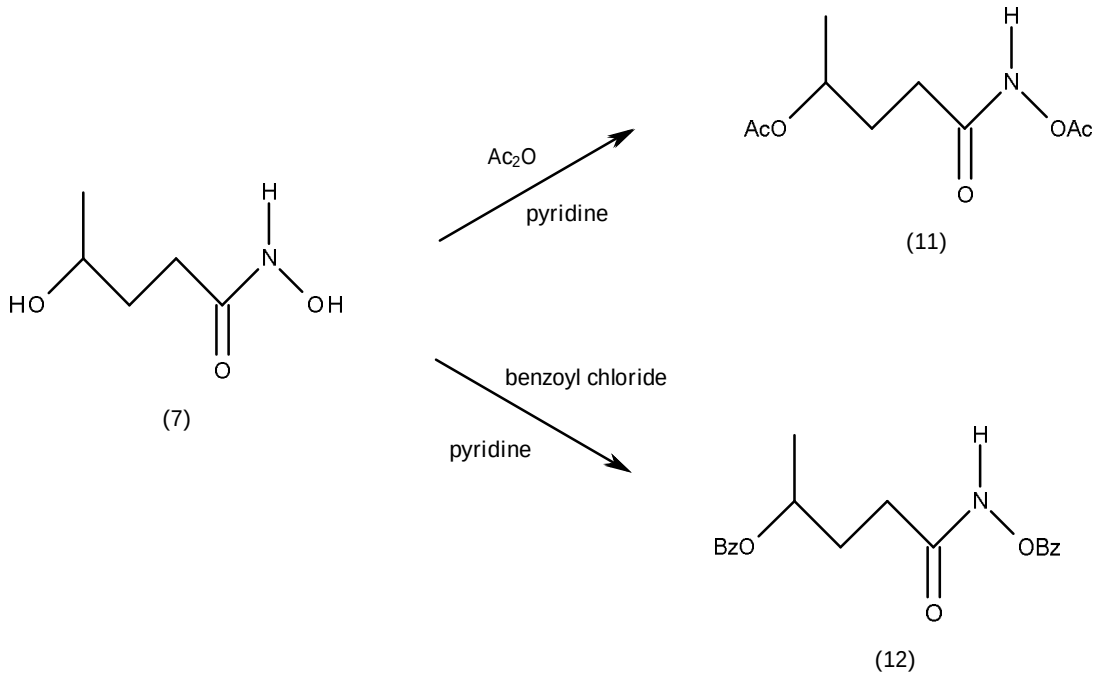
The following reactions, using (7) as a depart product, were all hydroxyl group protections, but they were not successful. Using TBDMS.Cl and imidazole in DMF, the objective was to protect the hydroxyl groups with silyl ethers and the desired compound was compound (10). One reason that could explain the non obtainment of said compound might be the degradation occurred during purification by column chromatography. Another explanation might be that the compound degraded while it wasn't used, because it was stored at room temperature.



(7) (10)

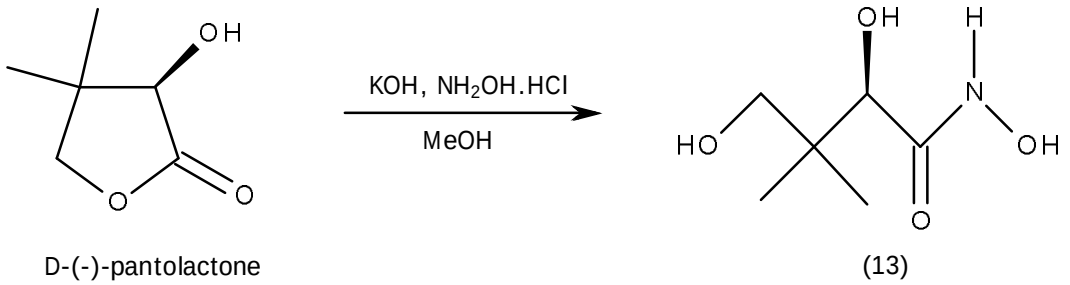
Scheme 40 – Protection with TBDMS.Cl

Next, we tried an acylation, using acetic anhydride (Ac_2O) in pyridine, and a benzoylation, using benzoyl chloride in pyridine. None of this reactions were successful because the depart product (7) was not proper.



Scheme 41 – Protection with Ac_2O and benzoyl chloride

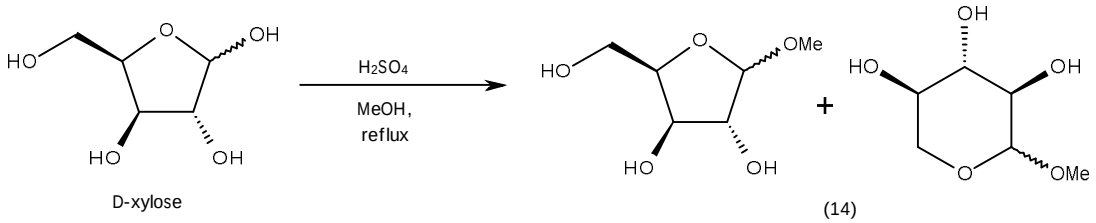
The obtainment of another hydroxamic acid, from D-(-)-pantolactone was also tried, using the same method that allowed the synthesis of compound (7). Unfortunately this reaction was also not successful. The desired compound (13) was not obtained due to a re-ciclization of the compound into the depart product. After 12hours of stirring, we could see by TLC that a compound different from the depart product had been formed, but after treatment with toluene and acetone, a re-ciclization into the depart product occurred, and that was the only compound present in the NMR analysis.



D-(-)-pantolactone (13)

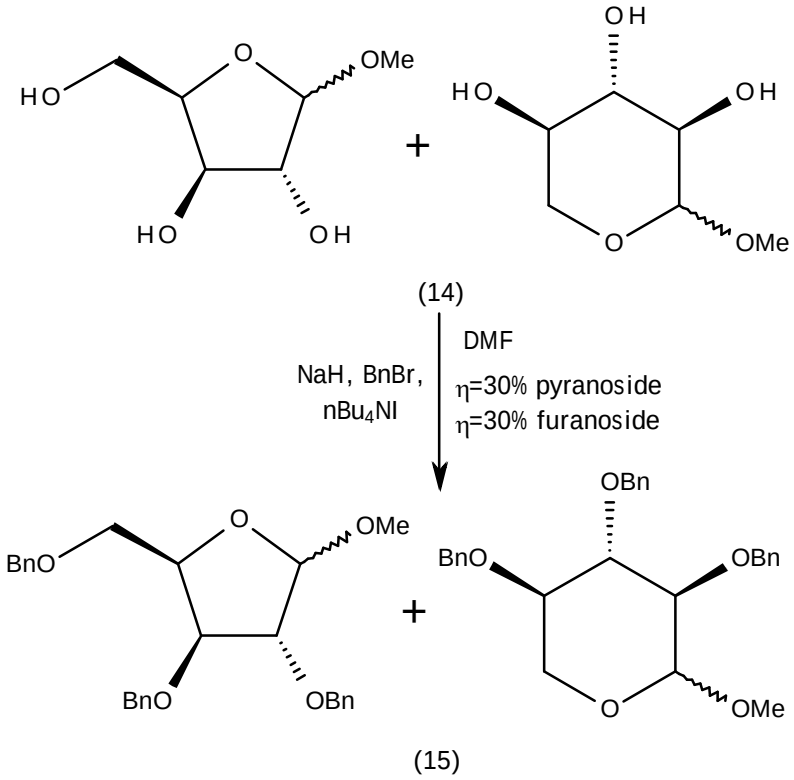
Scheme 42 – Conversion to a hydroxamic acid

The third path of synthesis had as the depart product D-xylose. Starting from the latter and using H_2SO_4 in MeOH, in a reaction stirred under reflux, compound (14) was obtained. This compound was obtained as mixture of the xylofuranoside and xylopyranoside forms, and also the anomeric carbon was in both α and β positions. With the analysis of the proton NMR, we can see that the methylation occurred and it appears as a singlet at 3,29ppm. In the carbon spectrum the OMe appears as a peak at 3,29ppm.



Scheme 43 – Primary hydroxyl group protection

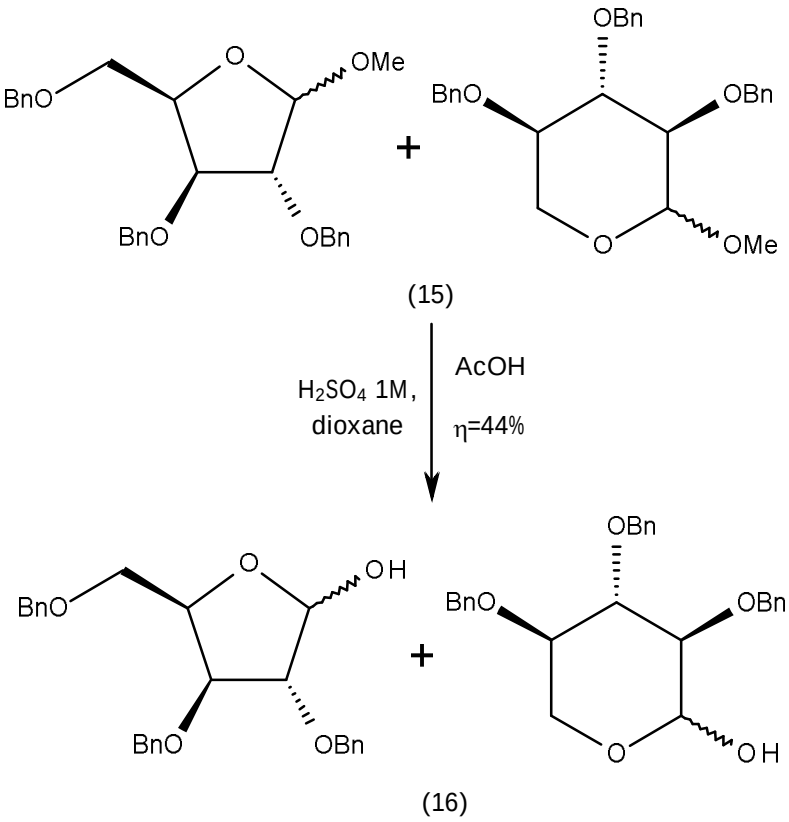
The benzylation of the remaining hydroxyl groups was achieved by making a reaction between compound (14) and NaH, BnBr and nBu_4NI in DMF. The compound (15) was o btained in a 30% yield for the furanoside form and also 30% for the pyranoside form. The peaks correspondent to the CH_2 groups of the benzylated OH groups appear as a multiplet between 4,43 and 4,67ppm, and the aromatic protons appear at 7,28-7,34pp. With the analysis of the carbon NMR spectrum we can see the 3 CH_2 groups, belonging to the benzyl, at 70, 73,6 and 73,7ppm. The aromatic carbons appear between 127,8 and 128,6ppm.



Scheme 44 – Protection with benzyl bromide

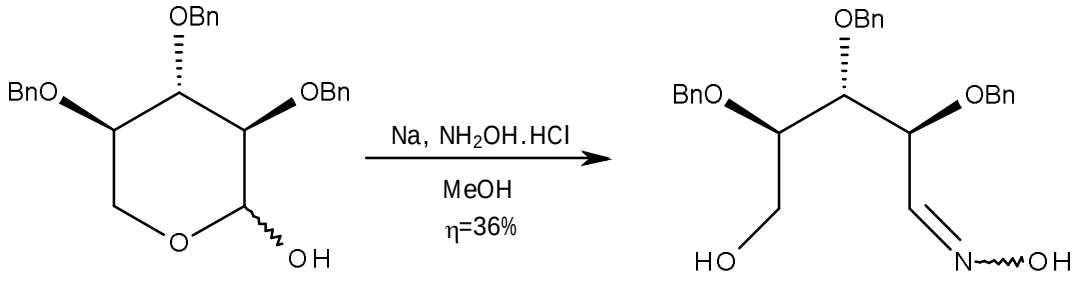
The deprotection of the primary hydroxyl group was performed using H_2SO_4 1M and dioxane in acetic acid (AcOH), and compound (16) was obtained. The first treatment was dilution with PE and H_2O, after 16 hours of stirring under reflux. This was followed by a filtration and a wash with PE. The solvents were evaporated, and then a new dilution with AE and wash with a saturated solution of NaCl, dried with $MgSO_4$ and filtration. This treatment gave (16) in a low yield, 10%, and so a new treatment was experimented. After 20 hours stirring, under reflux, the mixture was diluted with EA and H_2O, and then extracted with EA, washed with

H_2O, a saturated solution of $NaHCO_3$ and a saturated solution of NaCl, dried with $MgSO_4$. This second treatment gave (16) in a 44% yield. The success of this reaction can be seen, in the proton spectrum, by the appearance of two doublets, at 6,28 (β form) and 6,51ppm (α form), that represent the OH at the anomeric position. The disappearance of a peak at 55,9ppm, in the carbon spectrum, which in the previous compound represented the OMe in the anomeric position and the appearance of two peaks at 94,6 and 101,5ppm correspondent, respectively, to C-1α amd C-1β, also allows us to come to the conclusion that the deprotection of the primary hydroxyl group was completed.



Scheme 45 – Deprotection of the primary hydroxyl group

The formation of the oxime was achieved by the reaction between and Na in MeOH, firstly, and then by the addition of (16) and $NH_2OH.HCl$. Compound (17) was obtained in a36% yield, and as two isomers, E and Z. The success of this reaction can be seen by the disappearance of the peaks at 3,48 and 3,6-3,7ppm, that previously corresponded to the CH_2-5 (α and β), and now is now a OH group, that appears as a singlet at 2,55 ppm. The OH group at C-1, previously appeared at 6,51 (α form) and 6,28ppm (β form), and is now substituted by the NOH group that appears as a doublet at 7,46ppm. The protons in the C-1, in the proton spectrum of (16) appeared at 5,13 (α) and at 5,32 (β), and now they appear as a doublet at 6,9ppm (Z isomer) and as a singlet at 7,39pmm (E isomer).



(16) (17)

Scheme 46 – Aldoxime formation

The antioxidant activity of compound (9) was tested using the DPPH method. In this method the compound reacts with DPPH in a methanol solution. The reduction of DPPH$^{\bullet}$ was followed by the decrease in absorbance at 515nm. With the first range of concentrations (25-550µg/ml) tested no conclusive results could be drawn, so a even higher concentration was tested (12500µg/ml). The % antioxidant activity of compound (9) and (17) was calculated from the formula $\%AA=[(Abs_{control}-Abs_{sample})*100]/Abs_{control}$, and the value obtained for (9) in a concentration of 12500µg/ml was 32,42% of antioxidant activity, and for (17), in a concentration of 17000µg/ml, was 24,83%.

Chapter 4

Conclusions

In this work the objective was the synthesis of N-oxides thio-imidate derived from sugars, and the subsequently the evaluation of its antioxidant activity.

The first path of synthesis, starting from D-ribose, gave compound **9** in a global yield of 38%. The evaluation of antioxidant activity of the final compound was tested, and the % of antioxidant activity obtained, for a concentration of 12500µg/ml, was 32,42%, which is very low.

The second path, using lactones as substrates, was not successful. When starting from γ-valerolactone, the hydroxamic acid was synthesized, but from that no compound could be obtained. The steps that followed the obtainment of the hydroxamic acid were all hydroxyl group protections, using TBDMSCl, Ac_2O and benzoyl chloride, but not one of this reactions were successful. Using D-(-)-pantolactone as a depart product, the corresponding hydroxamic acid could not be obtained, due to a re-ciclization into the depart product.

The third path started from D-xylose, and from that and in 4 reactions, the oxime derivative was the last compound obtained. The antioxidant activity of this compound was not tested because the compound degraded easily.

The oxime **17** was obtained in a global yield of 35%.

The antioxidant activity obtained for compound **17** was 24,83%.

Bibliography

[1] Rollin P., Tatibouët A., Glucosinolates: the synthetic approach, *C.R. Chimie*, **2010**

[2] Coates R.M., Firsan S.J., *J. Org. Chem.*, **1986**, 51, 5198.5209

[3] Morrison R., Boyd R., *Organic Chemistry*, 15th edition, Calouste Gulbenkian Foundation, Lisbon, **2009**

[4] Rollin P., Tatibouët A., Glucosinolates: the synthetic approach, *C.R. Chimie*, **2010**

[5] Osbourn A. *The Plant Cell*, **1996**, 8, 1821-1831

[6] Iori R., Barillari J., Galliene E., Billardo C., Tatibouët A., Rollin P., *Tetrahedron Letters*, **2008**, 49, 292,295

[7] Schleiss J., Cerniauskaite D., Gueyrard D., Iori R., Rollin P., Tatibouët A., *SYNLETT*, **2010**, 5, 725-728

[8] Grigg R., Hadjisoteriou M., Kennewell P., Markandu J., Thornton-Pett M.S., *J. Chem. Soc., Chem Commum*, **1992**, 1388

[9]Jäger V., Bierer L., Doug H.-Q., Palmer A.M., Shaw D., Frey W.J., *Heterocycl Chem*, **2000**, 27,455

[10] Greene T. W., Wuts P. G. M., *Protective Groups In Organic Synthesis*, 3rd edition, John Wiley & Sons, New York, **1991**

[11] Palmer A.M., Jäger V., *Eur J Org Chem*, **2001**, 1293-1308

[12] Wey S-J, Augustyniak M.E., Cochran E.D., Ellis J.L, Fang X., Garvey D.S., Janero D.R., Letts L.G., Martino A.M., Melim T.L., Murty M.G., Richardson S.K., Schroeder J.D., Selig W.M., Trocha A.M., Wexler R.S., Young D.V., Zemtseva I.S., Zifcak B.M., *J Med Chem*, **2007**, 50, 6367-6377

[13] Barker R., Fletcher Jr.H.G., *J Org Chem,* **1961**, 26, 4605-4609

[14] Desvergnes S., Py S., Valleé Y., *J Org Chem*, **2005**, 70, 1459-1462

[15] Tajanbabu T.V., Nugent W.A., Taber D.F., Fagan P.J., *J Am Chem Soc,***1988**, 7128-7135

[16] Mitsunobu O., Yamada Y., *Bull. Chem. Soc. Japan*, **1967**, 40, 2380-2382

[17] Mitsunobu O., *Synthesis*, **1981**, 1-28

[18] Hudlicky T., Luna H., Price J.D., Rulin F., *J. Org. Chem.*, **1990**, 55, 4683-4687

[19] Kaskar B., Heise G.L., Michalak R.S., Vishnuvajjala B.R., *Synthesis,* **1990**, 1031-1032

[20] Chang R., *Chemistry,* 5th edition, McGraw Hill, Amadora, **1994**

[21] http://www.rice.edu/~jenky/sports/antiox.html, May 2011

[22] Miller H.E., Rigelhof F., Marquart L., Prakash A., Kanter M., *Cereal Foods World*, **2000**, 45(2), 59-63

[23] Miller H.E., Rigelhof F., Marquart L., Prakash A., Kanter M., *J. Am. Coll. Nutr.,* **2000**, 19(3), 312S-319S

[24] Antolovich M, Prenzler P.D., Patsalides E., McDonald S., Robards K., *Analyst, Royal Society of Chemistry*, **2002**, 127, 183-198

[25] Sousa C.M. de M., Rocha e Silva H., Vieira-Jr G.M., Ayres M.C.C., da Costa C.L.S., Araújo D.S., Cavalcante L.C.D., Barros E.D.S., Araújo P.B. de M., Brandão S., Chaves M.H., *Química Nova*, **2007**, 30, 351-355

[26] Brand-Williams W., Cuvelier M.E., Berset C., **1995**, 28, 25-30

[27] Çam M. *et al*, *Food Chemistry*, **2009**, 112, 721-726

[28] Valko M, Leibfritz D., Moncol J., Cronin M.T.D., Mazur M., Telser J., *The International Journal of Biochemistry & Cell Biology*, **2007**, 39, 44-84

[29] Jadhav, S.J., Nimbalkar, S.S., Kulkarni, A.D., Madhavi, D.L., *Food Antioxidants: Technological, Toxicological and Health perspectives*, Marcel Dekker, New York, **1996**, 5-64

[30] Argyropoulos, N.G., Panagiotidis, T.D., Gallos, J.K., *Tetrahedron: Asymmetry*, **2006**, 17, 829

[31] Gulla, M., Bierer, L., Schmidt, S., Redcliffe, L., Jäger, V.Z., *Naturforsch., B: Chem. Sci*, **2006**, 61, 471

[32] Cicchi, S., Marradi, M., Vogel, P., Goti, A., *J Org Chem*, **2006**, 71, 161