Alma Mater Studiorum - Università di Bologna

SCUOLA DI SCIENZE

Dipartimento di Chimica Industriale"Toso Montanari"

Corso di Laurea Magistrale in

Chimica Industriale

Classe LM-71 - Scienze e Tecnologie della Chimica Industriale

SYNTHESIS OF NOVEL RIBOSE-BASED THIOIMIDATE *N*-OXIDES

Tesi di laurea sperimentale

CANDIDATO

Domenico Romano

RELATORE

Dott.ssa Mariafrancesca Fochi

Chiar.mo Prof. Arnaud Tatibouët

CORRELATORE

Prof.ssa Marie Schuler

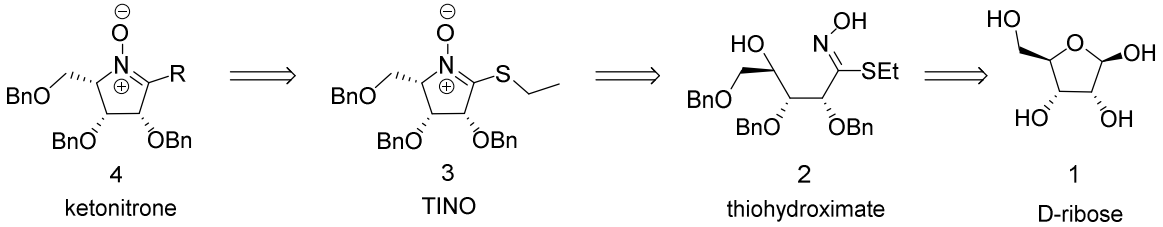
Sessione III

Anno Accademico 2014-2015

ABSTRACT

This work is based on the study of new synthetic paths to obtain thioimidate *N*-oxides (TINOs) from *D*-ribose and to study their reactivity with the purpose to obtain ketonitrones. TINOs, aren't well known molecules, but these enantiomerically pure backbones could be valuable intermediates in the synthesis of novel ketonitrones which are key intermediates in the synthesis of iminosugars. TINOs were discovered from the study of glucoraphanin, a particular glucosinolate, that unexpectedly cyclized into a TINO after desulfatation, by a spontaneous intramolecular Michael addition. The first part of this work was to synthetize the TINO **3** from *D*-ribose **1**. The key step was the desilylative cyclisation of a suitably functionalized thiohydroximate **2**. Based on precedent work developed in the laboratory, we could obtain the thiohydroximate from *D*-ribose. We then focused our studies on the cyclisation step trying to find the suitable substituents that could give the TINO in good yield by desilylative cyclisation. The second part of the project is to obtain ketonitrones **4** by palladium-catalyzed coupling reaction.

Lo scopo di questo lavoro è stato lo sviluppo della sintesi di tioimidati *N*-ossidi (TINO) a partire dal *D*-ribosio e lo studio della loro reattività con lo scopo di riuscire a sintetizzare chetonitroni. I TINO non sono molecole molto conosciute ma questi substrati enantiomericamente puri possono essere dei validi intermedi nella sintesi di chetonitroni che sono a loro volta alla base degli iminozuccheri. I TINO sono stati scoperti dallo studio della glucorafanina, un glucosinolato che dopo desolfatazione, tramite addizione di Michael intramolecolecolare ciclizzava dando un tioimidato *N*-ossido. La prima parte di questo lavoro è stata la sintesi della funzione TINO **3** a partire dal *D*-ribosio **1**. Lo step chiave è stato la ciclizzazione desililativa di un tioidrossimato **2** opportunamente funzionalizzato. Basandoci su precedenti lavori del gruppo di ricerca presso cui ho svolto il mio tirocinio, siamo riusciti ad ottenere il tioidrossimato a partire dal *D*-ribosio. Successivamente ci siamo concentrati sulla ciclizzazione cercando di trovare i sostituenti che ci permettessero di ottenere il TINO con una buona resa tramite ciclizzazione desililativa. La seconda parte del progetto è stata ottenere chetonitroni **4** tramite coupling catalizzato da palladio.



INTRODUCTION

GLUCOSINOLATES

Glucosinolates (GLs) are a class of natural organic compounds with a characteristic structure and biochemistry; they are secondary thiosaccharidic metabolites and they are mainly found in all the plants of the order of brassicaceae like broccoli, cabbage, cauliflower. The glucosinolates are responsible for the bitter and sharp taste of many common food such as mustard, radish, cress. About 120 glucosinolates are known; they occur in all parts of the plant; in general concentration depends on the tissue type but they are more concentrated in the seeds.

They are water soluble anions and they are remarkable for their structural homogeneity: they present a hydrophilic β-*D*-glucopyrano framework bearing an *O*-sulfated anomeric (*Z*)-thiohydroximate moiety, which gives to the molecule acidic properties, connected to a hydrophobic aglycon side chain[1] which is usually represented by R and which is responsible of the different biological activities of these compounds (FIGURE-1).

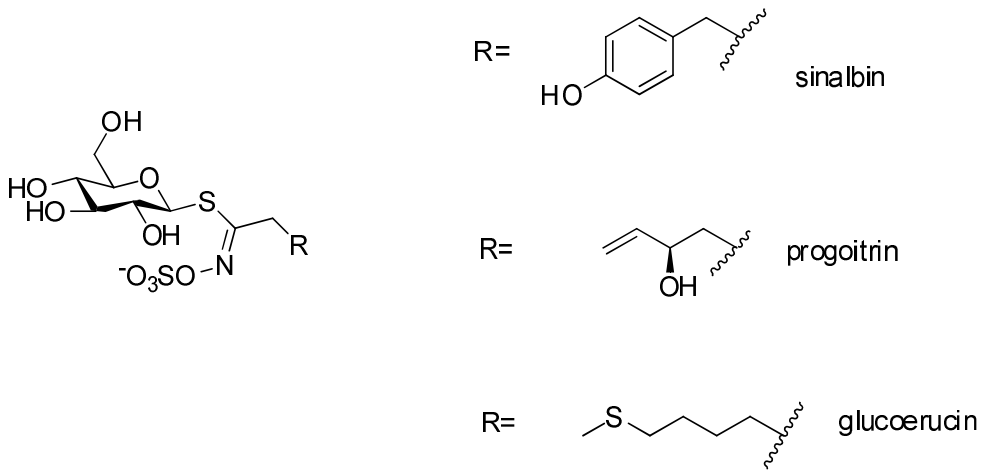


FIGURE-1

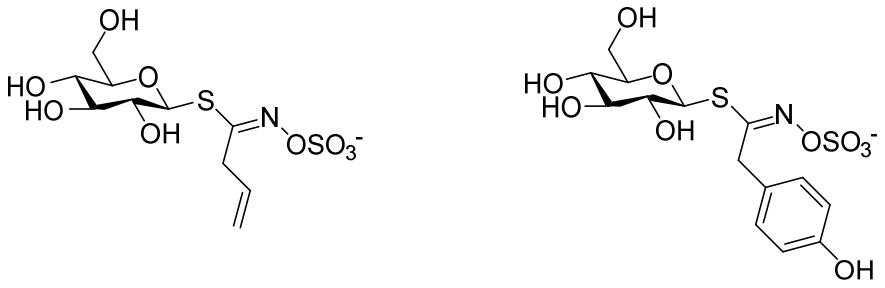
[1] Cerniauskaite, D.; Gueyrard, D.; Iori, R.; Rollin, P.; Tatibouët, A. *Synlett*, **2010**, No.5, 725-728

There are different way to classify GLs. One classification is based on their biosynthetic precursor standard amino acid[2]. The drawback of basing GL classification on amino acid precursor is that the biosynthetic precursor of a particular structure in a given plant species is often not known and cannot in all cases be predicted with reasonable certainty. So they are also classified in aliphatic, aromatic and indolic GLs[2]. Glucosinolate side chains, however, are characterized by a wide variety of chemical structures; but the most numerous are those containing either straight or branched carbon chains, which can contain also double bonds, hydroxyl or carbonyl groups or sulfur linkages in different oxidation states[3].

Systematic nomenclature of individual GLs is greatly simplified by the convention of naming the anionic structure (the central C as well as the connected, substituted S and N) "glucosinolate". Most natural GLs can be simply named by adding the name of the side chain as a radical to the word glucosinolate. This type of nomenclature is recommendable and generally accepted but some of the resulting names are elaborated so there is an abbreviation system based on side chain structures only. A number of GLs also carry trivial names coined before the core GL structure was elucidated; these names are composed of a prefix "gluco" combined with a base name derived from the original source plant and the suffix "in"[2] .

The first glucosinolates were isolated in the 1830s from black mustard seeds (sinigrin or 2-propenyl glucosinolate)(FIGURE-2A) and white mustard seeds (sinalbin or 4-hydroxybenzyl glucosinolate)(FIGURE-2B). The first general and also incorrect structure of these compounds, proposed at the end of the nineteenth century by Gadmer (1897), expected that the side chain was linked to the nitrogen atom instead of the carbon of the group NCS. This structure was believed correct till 1956 when it revealed itself unable to describe certain properties; so in 1957 Ettlinger and Lundeen proposed a new and correct structure and also described the first chemical synthesis of a glucosinolate. Finally the geometrical isomerism at the C=N bond was established to be *Z* by X-ray crystallography[3].

[2] Agerbirk, N.; Olsen, C. E., *Phytochemistry*, **2012**, 77, 16-45
[3] Fahey, J. W.; Zalcmann, A. T.; Talalay, P., *Phytochemistry*, **2001**, 56, 5-51



A B

FIGURE-2

GLs and/or their breakdown products have recently captured the interest of many studies because of their cancer chemopreventive qualities, but they have already been known for their bactericidal and fungicidal properties[3].

During the past years various research groups focused their studies on the isolation, characterization and synthesis of glucosinolates and their derivatives. The first attempts to isolate glucosinolates were based on high-voltage electrophoresis combined with paper chromatography, but this method was complicated and gave also low yields. Bjerg and Sorensen in 1987 and Peterka and Fenwick in 1988 obtained excellents results in isolating GLs by reversed phase (C-18) solid phase extraction or flash chromatographic reversed phase techniques; the problems of these methods were that the products were not very pure and just a few glucosinolates could be crystallized[3]. Glucosinolates can be extracted in a boiling solution of convenient polarity; the boiling solvent inactivates the myrosinase (a glycoprotein that hydrolyses GLs into a variety of active compounds) but can also thermically degrade the glucosinolate. The conditions to inactivate myrosinase may vary between the different glucosinolates but after several attempts and studies the appropriate conditions have been optimized as repeated brief extractions in boiling aqueous MeOH (MeOH:H_2O=7:3)[2].

To detect and characterize all the glucosinolates, the sulfate group is exploited in HPLC analysis to separate intact glucosinolates or their desulfo derivatives. The critical steps of both these methods is the use of eluent volumes that allow complete elution as both the glucosinolate and the desulfo-glucosinolate have different affinity to the different matrices of the ion exchange columns. Separation of intact glucosinolates as a group from other metabolites is simple and allows the detection of some glucosinolates by UV analysis but this method doesn't give quantitative elution. For this reason the most important analysis method is based on desulfo-glucosinolate derivatives[2]. This method provides for anion-exchange

column enzymatic desulfation treatment of plant extracts followed by HPLC detection of the desulfo-glucosinolate derivatives. The problem of this method is that the biological activity is compromised; after the desulfation they can't act as a substrate for myrosinase. During the past years some methods for the synthesis of glucosinolates have been developed but these compounds aren't routinely synthesized. The common accepted biosynthesis model involves three steps: side chain elongation, glucone biosynthesis and side chain modification[3].

MYROSINASE

Glucosinolates are involved in human and animal nutrition; they have impact on health as chemopreventive agents and antioxidants[1]. These properties are due to their hydrolysis products. When a plant is damaged or during mastication and ingestion we had what is called "the mustard oil bomb": an enzyme called myrosinase hydrolyses the GL into a variety of active compounds[4]. Myrosinase (FIGURE-3) is a glycoprotein that is physically segregated from its GL substrate. It has long been thought to be localized in particular "myrosin" cells but some studies suggest that this enzyme is localized in aqueous vacuoles. It has been isolated and characterized from several sources like cress, yellow and white mustard; recently it has also been cloned and sequenced and its X-ray structure has been mapped; different forms of this enzyme exists, also in the same plant[3].

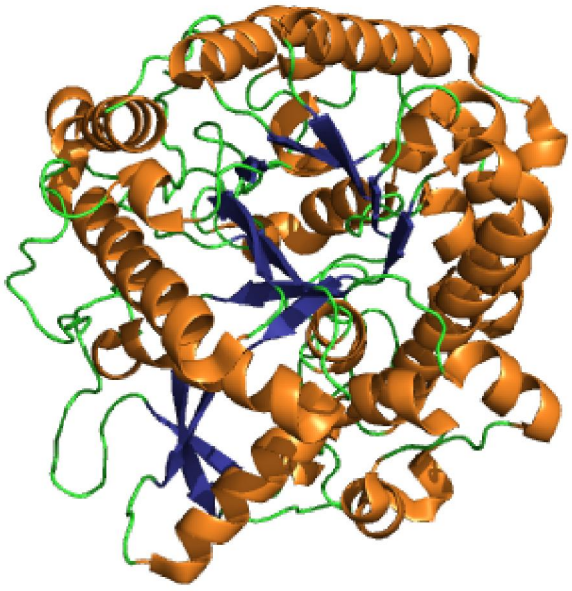
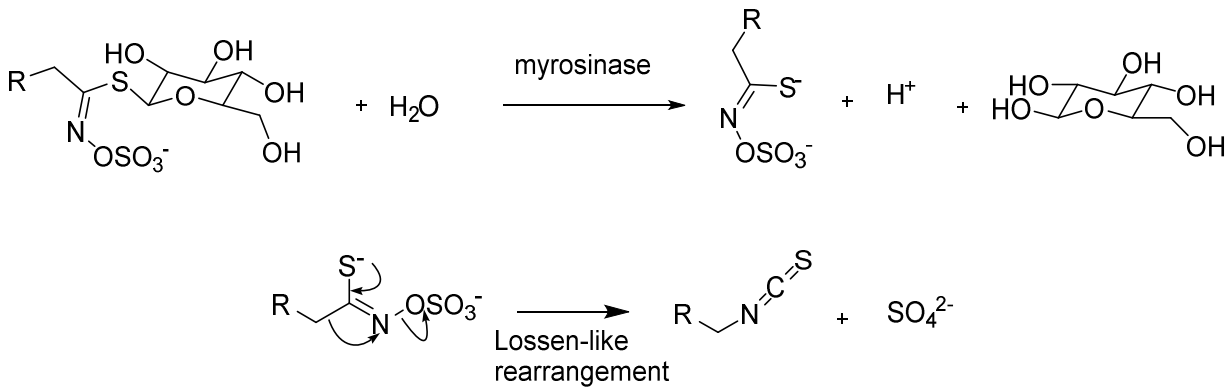


FIGURE-3

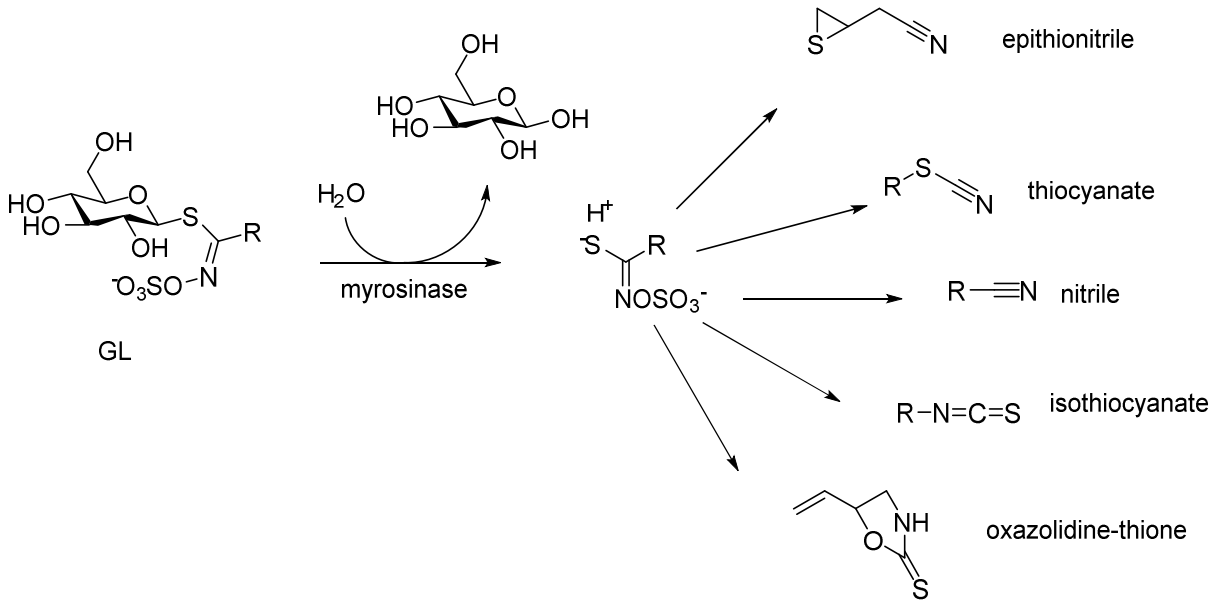
[4] Bones, M.; Rossiter, J. T., *Phytochemistry*, **2006**, 67, 1053-1067

Myrosinase hydrolyses the S-glucosyl bond thus giving glucose, hydrogen ion and an unstable aglucone. The loss of sulfate causes the Lossen-like rearrangement (SCHEME-1) into isothiocyanates which are sharp tasting and reactive electrophilic chemicals involved in plant defense. Variations in the GL hydrolysis due for example to herbivore specific proteins, structural characteristics of individual GLs or variations in reaction conditions, could lead to other different products[2].



SCHEME-1

The most common products obtained by a Lossen-like rearrangement are isothiocyanates, but there are also thiocyanates, nitriles, ephithionitriles, oxazolidines (SCHEME-2). The composition of the obtainable mixture depends on pH, metal ions and other protein elements[4].

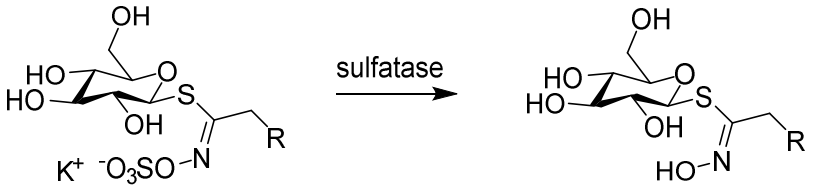


SCHEME-2

DISCOVERY OF THIOIMIDATE N-OXIDE

Glucoraphenin is one of the most popular thio-functionalised glucosinolate. It has chemical formula $C_{12}H_{22}NO_{10}SO_3$ and nowadays is popular because it can act as a bio-relevant redox couple in some neutraceutical applications. It is found in young sprout of broccoli and cauliflowers. During mastication and ingestion, it is hydrolyzed by myrosinase, producing sulforaphane. It is involved in the process of formation of a variety of antioxidant and detoxifying peptides and proteins; it is active against various human pathogens and for this reason it has been hypothesized that sulforaphane carries out anti cancer activity.

According to the EU official method ISO-9167-1 the analysis of glucosinolates in Brassicaceae vegetables is based on their enzymatic *O*-desulfation (SCHEME-3) and HPLC of their desulfo counterparts.

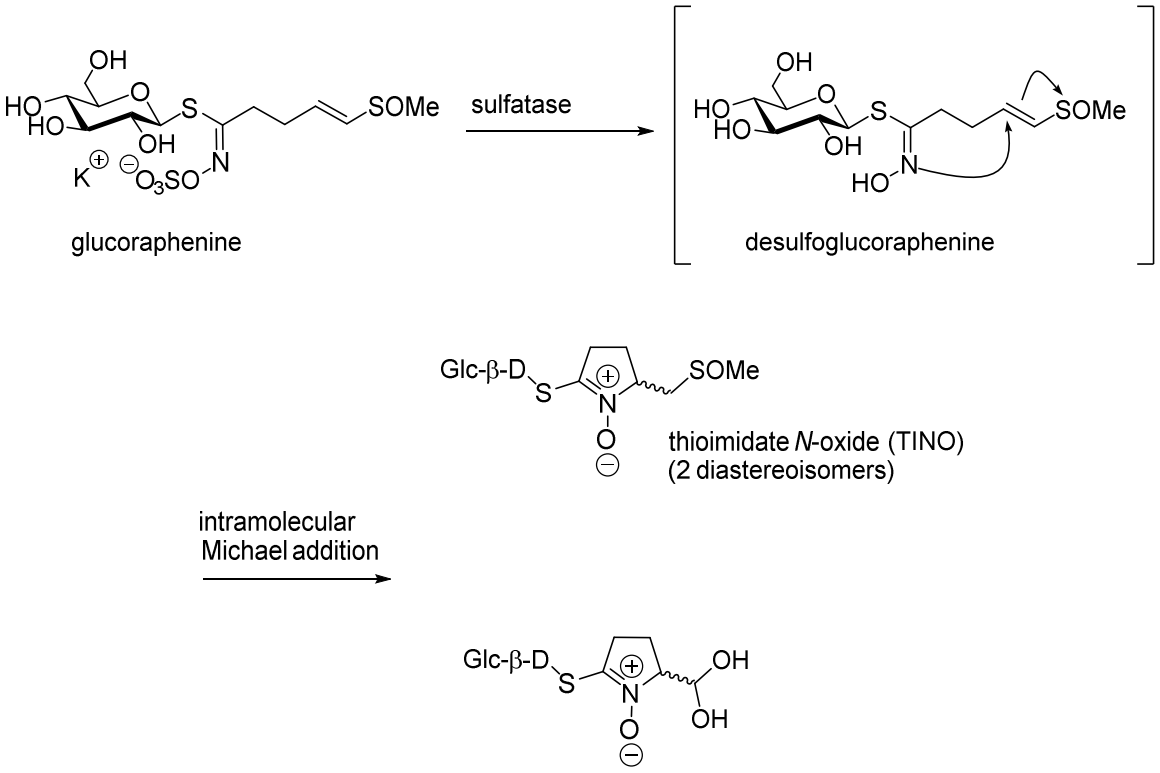


SCHEME-3

Desulfoglucoraphenin is not stable as glucoraphenin and the other desulfoglucosinolates. In water desulfoglucoraphenin undergoes degradation, giving three different products, as shown by HPLC analysis[5].

Desulfoglucoraphenin spontaneously undergoes intramolecular concerted Michael addition of the thiohydroximate moiety onto the vinyl sulfoxide acceptor producing the two diastereoisomers of the thioimidate *N*-oxide (TINO) and a third product that could result from an *in situ* Pummerer-type rearrangement of the TINO (SCHEME-4).

[5] Iori, R.; Barillari, J.; Gallienne, E.; Bilardo, C.; Tatibouët, A.; Rollin, P., *Tetrahedron Lett.*, **2008**, 49, 292-295


OH
HO
HO
O
S
SOMe
OH
K
O3SO
N
sulfatase
glucoraphenine
desulfoglucoraphenine
Glc-β-D
thioimidate N-oxide (TINO)
(2 diastereoisomers)
intramolecular
Michael addition

SCHEME-4

OBJECTIVE

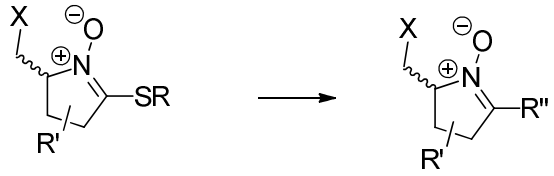
For the originality of its structure, the research group where I have trained, worked on the design of synthetic methods to obtain the thioimidate *N*-oxide function (TINO), to study and evaluate its reactivity and chemical potential[1].

Thioimidate-*N*-oxides (TINOs), aren't well known molecules, but these enantiomerically pure backbones could be valuable intermediates in the synthesis of novel ketonitrones which are key intermediates in the synthesis of iminosugars.

Nitrones are considered valuable synthetic intermediates and useful spin trapping reagents. They can be considered versatile 1,3 dipoles for the construction of nitrogen heterocycles which constitute the backbone of various biologically active compounds[6]. Nitrones are widely recognized as important building blocks in organic synthesis. They can easily have [3+2] cycloaddition with alkenes and alkynes dipolarophiles to form products such as isoxazolidines and 2,3-dihydroisoxazoles[7]. Nitrones are also excellent electrophiles for reactions with organometallic regents to give nitrogen-containing compounds[7]. In general nitrones are relatively stable, so they are easy to use in air and at room temperature and usually don't require *in situ* generation[8].

Cyclic nitrones undergo a wide range of reactions, like nucleophilic additions, 1,3 dipolar cycloadditions. Carbohydrate-based nitrones are also the key intermediates in the preparation of iminosugars with biological activities[9]. Cyclic nitrones have also emerged as theurapeutic molecules, spinning trap agents. Unfortunately nearly all the known synthetic paths leads to aldonitrones and with a limited range of substituents. That's why TINOs are object of recent studies: they are the key intermediate that could lead to ketonitrones[10] (SCHEME-5).

[6] Bartoli, G.; Marcantoni, E.;Petrini, M. *J.Org.Chem.*, **1992**, 57, 5834-5840
[7] Hood, T. S.; Huehls, C. B.; Yang, J. *Tetrahedron Lett.*, **2012**, 53, 4679-4682
[8] Kissane, M.; Maguire, A. R. *Chem.Soc.Rev.*, **2010**, 39, 845-883
[9] Marquès, S.; Schuler, M.; Tatibouët, A. *Eur.J.Org.Chem*, **2015**, 2411-2427
[10] Schleiss, J.; Rollin, P.; Tatibouët, A. *Angew. Chem. Int.Ed.*, **2010**,49,577-580



SCHEME-5

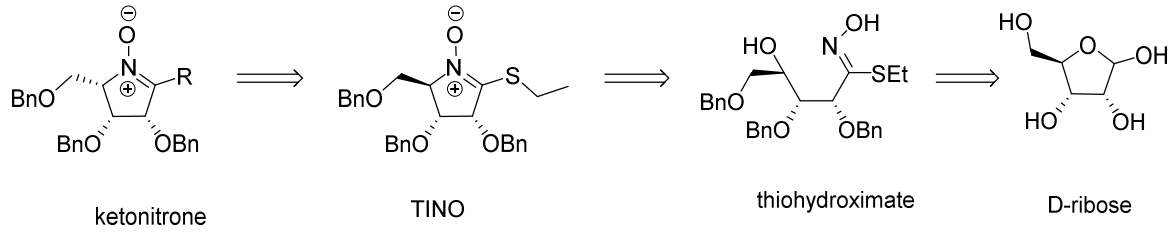
Iminosugars are analogues of pyranose and furanose in which there is a nitrogen instead of the endocyclic oxygen[11]. They have both chemical and biological stability; an important feature is that they are similar to carbohydrates but at the same time they are not processed by other carbohydrate-modifying systems[12]. For these reasons they are studied as an important class of potential therapeutic agents as anti-inflammatory, anti-viral or contraceptive agents. They are also known as glycosidase inhibitors[13]. Glycosidases are enzymes involved in many biological processes: intestinal digestion, post-translational processing of glycoproteins, lysosomal catabolism of glycoconjugates. So iminosugars, acting as glycosidase inhibitors in a reversible and competitive manner, could have beneficial effects in the treatment of diabetes, viral infections, cancer and lysosomal disease[14].

They are considered potential drug candidates also for their intrinsic properties: despite being small molecules, they share adequate water solubility; as said before they are chemically stable molecules but they are also metabolically stable, so, compared to most small polar molecules, iminosugars can be absorbed by the organism and excreted unchanged and can benefit from transport mechanisms designed for carbohydrates. They can also cross the blood-brain barrier[12].

The research group in which I did my internship encouraged by the synthetic potential of the TINOs, is working on the development of synthetic approaches to furanose and pyranose based derivatives and to the formation of cyclic ketonitrones through Liebeskind-Srogl cross-coupling reactions. The long term goal is to use these enantiomerically pure backbones in the synthesis of polyhydroxylated biologically active nitrogen-containing compounds.[9]

[11] Behr, J. B.,*Tetrahedron Lett.* **2009**, 50,4498-4501
[12] Horne, G.;Wilson, F. X.; Tinsley, J.;Williams, D. H.; Storer, R. *Drug Discovery Today*,**2011**,16, 107- 118
[13] Decroocq,C.;Stauffert,F.;Pamlard,O.;Oulaïdi,F.;Gallienne,E.;Martin,O.R.;Guillou,C.;Compain,P. *Bioorg. Med. Chem. Lett.*,**2015**,25,830-833
[14]Kato,A.;Hirokami,Y.;Kinami,K.;Tsuji,Y;Miyawaki,S.;Adachi,I.;Hollinshead,J.;Nash,R.J.;Kiappes,J.L.;Zitzman,N.;Cha,J.K.;Molyneux,R.J.;Fleet,G.W.J.;Asano,N. *Phytochemistry*,**2015**,111, 124-131

In this light, the main challenge of my project was the synthesis of novel ribose-based thioimidate *N*-oxides following the retrosynthetic path described in SCHEME-6



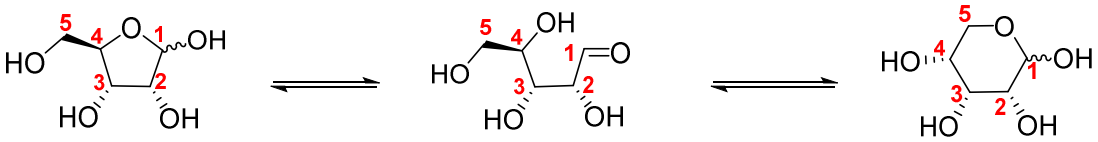
SCHEME-6

- ✓ The first target was the synthesis of TINO from *D*-ribose. To achieve this aim we planned the synthesis of various functionalized thiohydroximates that could afford the TINO *via* desilylative cyclisation. The finding of suitable substituents for the oxime function and for the secondary alcohol on C4 of the thiohydroximate was crucial for obtaining the TINO in good yield.
- ✓ Next we planned to explore the reactivity of the TINO in Liebeskind-Srogl cross-coupling reactions.

RESULTS AND DISCUSSION

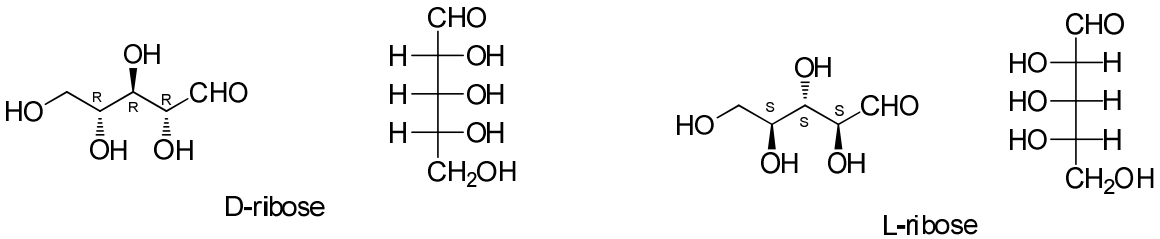
D-RIBOSE

We have chosen *D*-ribose as starting substrate to build thioimidate *N*-oxide (TINO) derivatives. Ribose is a pentose which contains four stereogenic centres, one of which posses particular characteristic since is a hemiacetal; this molecule is in equilibrium with diverse isomers such as an open-chain hydroxyl-aldehyde (SCHEME-7). The ring form is by far more stable. Thus any of the hydroxyl groups in position 4 or 5 could cyclize onto the aldehyde. In general there is competition between the six-membered and the five-membered rings; for *D*-ribose at 31°C in D_2O (deuterium oxide) the open chain form represents 0.05%, the five-membered oxygen heterocycle (called furanose) represent 80% and the six-membered oxygen heterocycle (called pyranose) count for 20%.



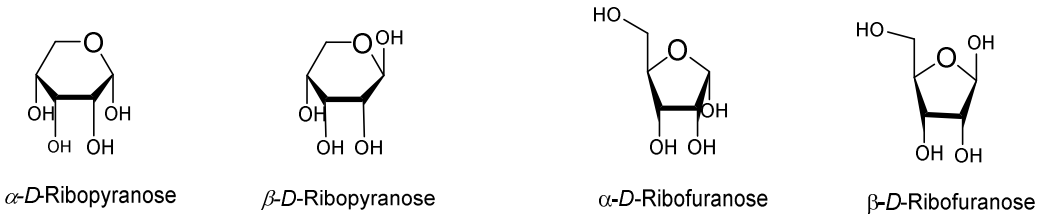
SCHEME-7

This *D*-aldopentose has all the hydroxyl groups on the same side in the Fischer projection. There are two enantiomers of the ribose: *D*-ribose and *L*-ribose. *D*-ribose is used in the metabolism and occurs in nature and all his stereogenic centres have the *R* configuration; *L*-ribose is not present in nature and all his stereogenic centres have the *S* configuration. (SCHEME-8)



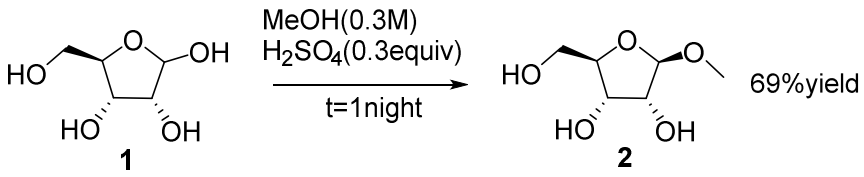
SCHEME-8

Both the five and the six membered rings can exist in two different anomeric forms α and β, depending on whether the new OH group lies above or below the average plane of the molecule. (SCHEME-9)



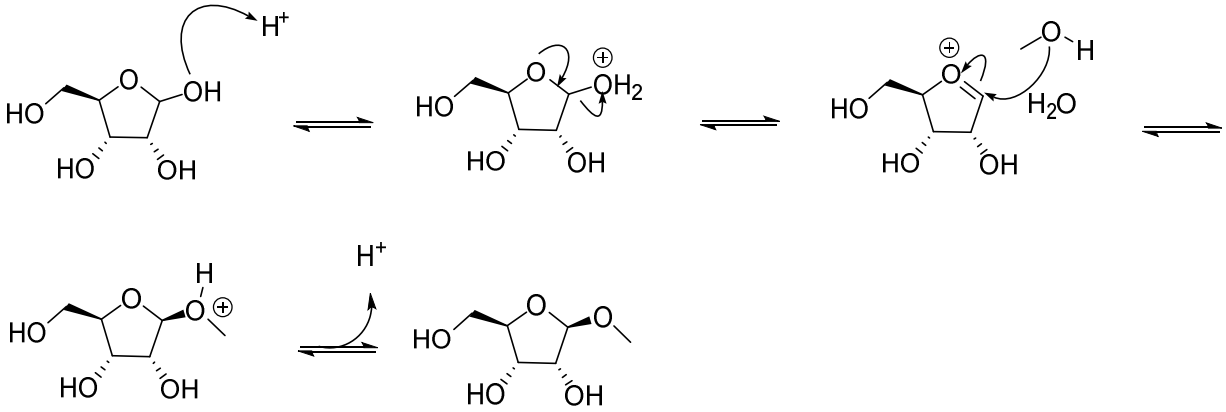
SCHEME-9

SELECTIVE PROTECTION OF C1 HYDROXYL GROUP[15]



SCHEME-10

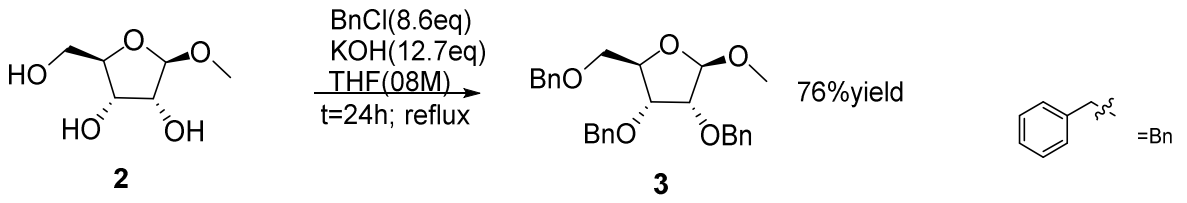
Starting from *D*-ribose **1** the first step was to protect selectively the hydroxyl group on C1 (SCHEME-10). We decided to do a Fischer glycosylation in the presence of methanol with the formation of an acetal under acid catalyzed conditions. This reaction is performed in an anhydrous environment, under Argon atmosphere and using concentrated H_2SO_4, which is added to a methanolic solution of *D*-ribose at 0°C. The yield of this reaction is of 69% after purification by chromatographic flash column. Anhydrous conditions and inert atmosphere are necessary because H_2O could displace the equilibrium of the reaction back toward the starting material. The methanol is incorporated on the anomeric position with a fairly good stereoselectivity for the β configuration; the ^{1}HNMR spectrum of the crude reaction mixture revealed the presence of only the β isomer, characterized by a very small coupling constant between H-1 and H-2 . This is quite unusual for *D*-pentoses where the α configuration is more favorable in the other series. The selectivity observed with the *D*-ribose could be attributed to the steric hindrance of the 2 and 3 hydroxy groups. (SCHEME-11)



SCHEME-11

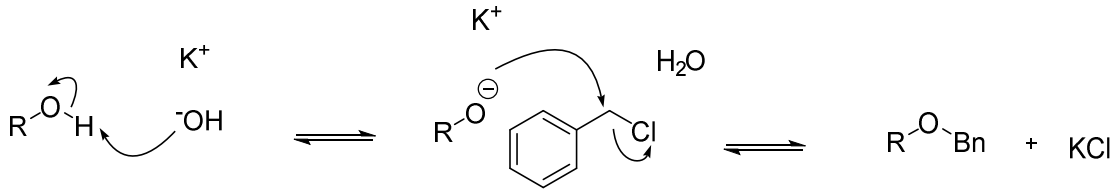
[15] Barker R.; Fletcher H. G. Jr; *J. Org. Chem.*, **1961**, 26, 4605-4609

PROTECTION OF HYDROXYL GROUPS WITH BENZYL PROTECTING GROUP[15]



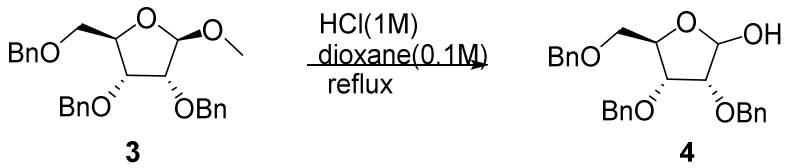
SCHEME-12

We decided to protect the other hydroxyl groups through a Williamson reaction with benzyl chloride and with KOH as a base (SCHEME-12). This step is a common benzyl protection approach that could be applied on almost every alcohols to form benzylethers. Product **3** was obtained in 76% yield after purification by flash chromatographic column.



SCHEME-13

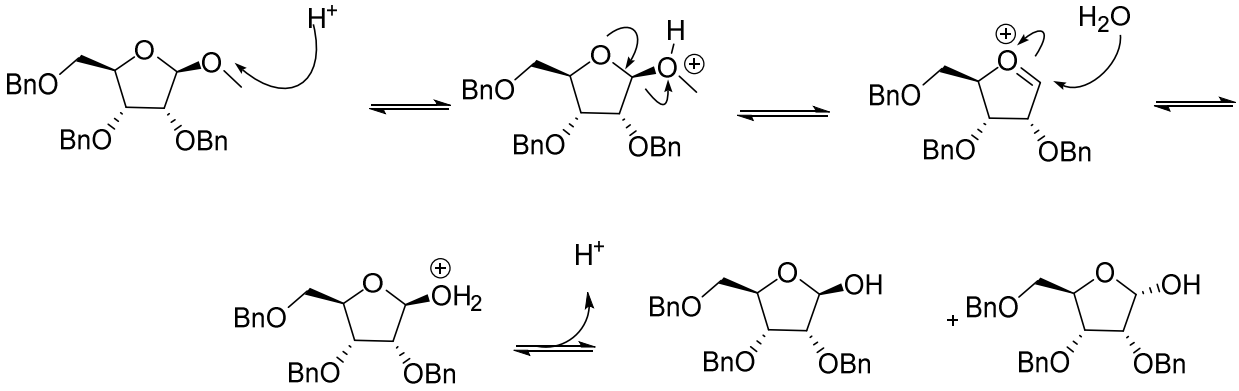
SELECTIVE DEPROTECTION OF C1 HYDROXYL GROUP[15]



3 **4**

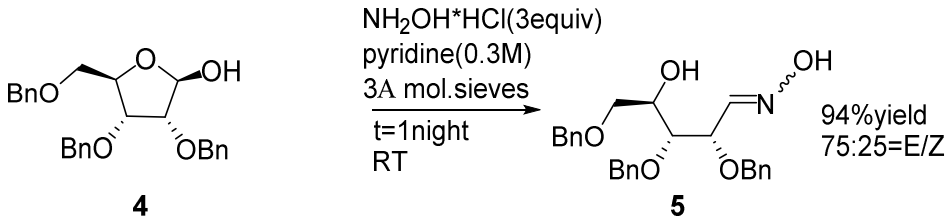
SCHEME-14

To obtain the oxime **5** in the next step, we deprotected the acetal **3** on the anomeric position with HCl 1M, performing this reaction in dioxane, under reflux and in normal atmosphere (SCHEME-14). The reaction was very efficient and gave a quantitative transformation. The purity of the compounds after classical treatment of the crude reaction (extraction and evaporation) was sufficient to avoid a purification through column chromatography. ^{1}H-NMR spectrum was quite difficult to analyse because of the presence of both α and β isomers, since the hydroxyl group on C-1 could again lie above and below the plane of the molecule. The mechanism is the reverse of the first Fischer reaction. (SCHEME-15)



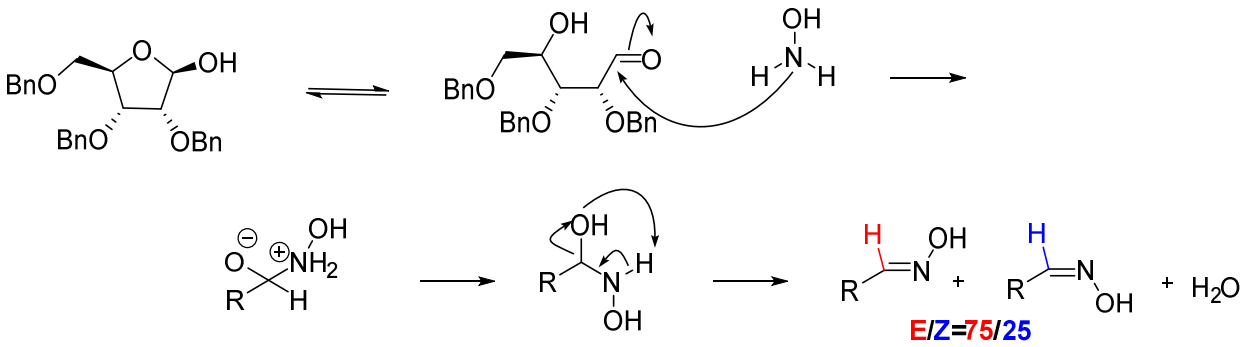
SCHEME-15

FORMATION OF OXIME



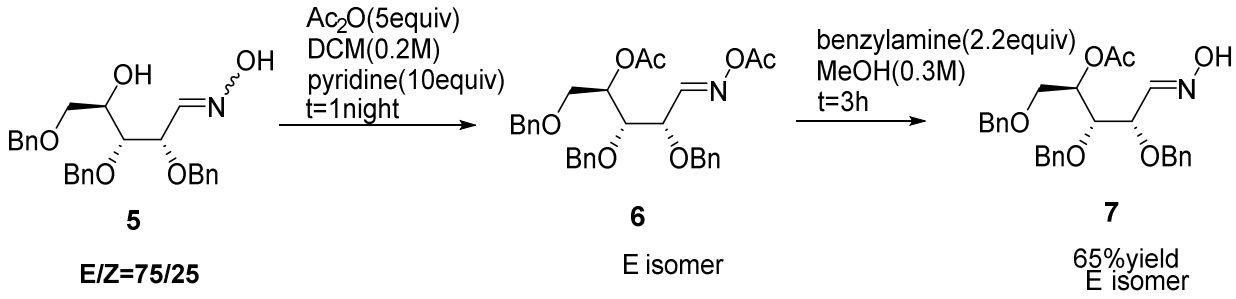
SCHEME-16

In this step we opened the ring to form an aldoxime by reaction with hydroxylamine hydrochloride and pyridine (SCHEME-16). The reaction was performed under Argon atmosphere and using dried 3Å molecular sieves (used to adsorb the water formed during the reaction). The yield of this step is 94% after purification by automatic chromatographic flash column. Before chromatographic column we did extraction with water and EtOAc but after evaporating the solvent there was still pyridine. We couldn't eliminate it by acid washing because we could deprotect benzyl groups, so the solution was to evaporate the solvent with the help of toluene (which makes an azeotrope with pyridine) until pyridine couldn't be detected anymore. In the following scheme (SCHEME-17) the possible mechanism of the reaction is described; we can see why we have to first deprotect the hydroxyl group of C1, to make possible the equilibrium between the open and the closed form of the *D*-ribose. We obtain the desired oxime **5** as a mixture of the two isomers E and Z with a ratio of E/Z=75/25. We gather this value by ^{1}H-NMR analysis, by making the ratio between the two doublet at 7.0 and 7.5 ppm; the signal at 7.5 ppm is due to the E isomer because the proton on C-1 is more deshielded by the oxigen of the hydroxyl group on the nitrogen.



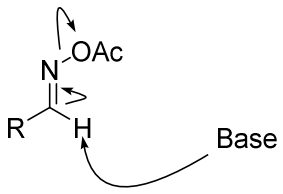
SCHEME-17

ACETYLATION OF THE NEW HYDROXYL GROUPS AND SELECTIVE DEPROTECTION



SCHEME-18

To prepare the thiohydroximate function, it was necessary to protect the secondary alcohol in position 4 and to have an unprotected aldoxime. The direct selective protection was not possible because the oxime reacts before the secondary alcohol. Therefore, a two steps sequence has been performed: first we protected both the hydroxyl groups and then deprotected selectively the acetylated aldoxime group using a slight excess of benzylamine in methanol (SCHEME-18). The total yield of this two steps was 65%. The per-acetylation of the starting molecule was checked by TLC and by ^{1}H-NMR and the selective deacetylation using benzylamine in MeOH was performed only once product **5** was completely converted into **6**. The second step was quite fast, indeed we just needed three hours to reach the completion of the reaction. Product **7** was purified by flash chromatographic column and was obtained just as E isomer. One of the fraction obtained was a side product identified as a nitrile which formation could occur either during the first step or the second step of the sequence where the basic conditions could lead to an elimination of the acetyl moiety. (SCHEME-19)

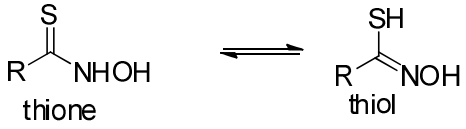


SCHEME-19

FORMATION OF THIOHYDROXIMATE

Formation of the thiohydroximate function is a key step toward the cyclisation to form the ring incorporating the TINO function.

Thiohydroxamic acids are a particular class of compounds because they contain four different contiguous atoms: S, C, N, O. The three electronegative atoms (S, N, O) give to thiohydroxamic acids interesting properties, that's why they play an important role in analytical and biological chemistry. For example they are used in nature to carry many different metal ions; or they can act as bidentate ligands thanks to S and O atoms. Thiohydroxamic acids exist in two different tautomeric forms: thione (*N*-hydroxythioamide) and thiol (*N*-hydroxythioimide)[16].(SCHEME-20)



SCHEME-20

From investigation of the infrared spectra it is evident that thiohydroxamic acids exist in thione form in the solid state but in the liquid state or in solution there is an equilibrium between the two tautomers[17].

However infrared spectroscopy may fail to distinguish the two tautomers; Raman spectroscopy instead is more accurate and after some studies it is possible to say that thione tautomer in aqueous solution is favoured[16].

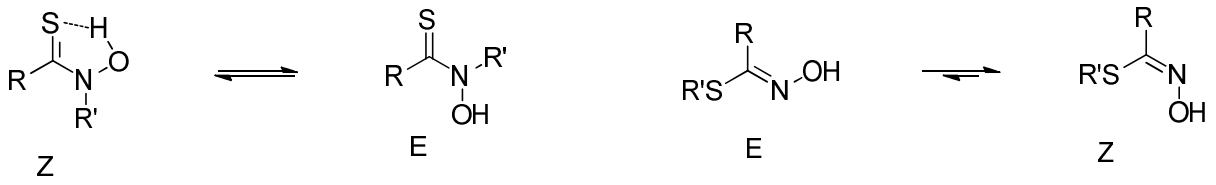
In the presence of alkylating agents, in aqueous solution we have a selective alkylation on the sulfur of the thiohydroxamic acid and if these agents are not in solution it is possible to have a dimerisation, with the formation of a S-S bond. This dimer could decompose to give isothiocyanates and 1,3-dialkyl-thioureas by a Lossen rearrangement.

Thiohydroxamic acids could be *N*- or *S*-alkylated and they have two different isomers: *E* and *Z*. (SCHEME-21)

[16] Kakkar R.; Dua A.; Zaidi S.; *Org. Biomol. Chem.*, **2007**, 5, 547-557

[17] Nagata K.; Mizukami S.; *Chem. Pharm. Bull.*, **1966**, 14, 1255-1262

With the *Z*-stereoisomer of the *N*-alkylated acids it has been observed a hydrogen bond between sulfur and hydroxy group; for the *S*-alkylated acids (thiohydroximates), the *Z* isomer is the preferred form.

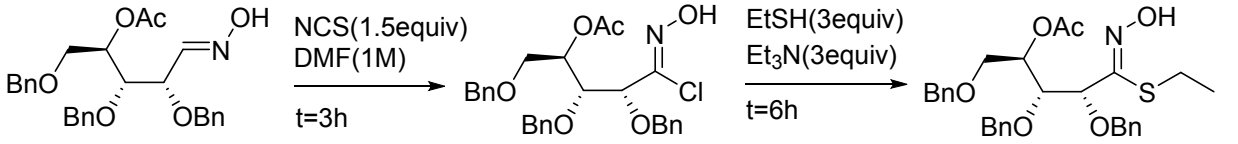


SCHEME-21

To introduce the thiohydroxymate function we adapted to our substrate a process previously developed by our research group but used on pyranoses. This function is introduced trough a one-pot, two-step sequence involving α-chlorination of the aldoxime with *N*-chlorosuccimide (NCS), followed by insertion, under basic conditions, of the ethylsulfanyl group. This process selectively afforded the *Z*-isomer.[9]

We first perform the reaction between the aldoxime **7** and NCS in anhydrous DMF; NCS was added at 0°C and the reaction was left for 3h protected from light. Then, we checked the end of the reaction by TLC; no more starting material remained so the mixture was cooled to 0°C and Et_3N and EtSH were added dropwise, at the same time and the reaction was left for 6h.(SCHEME-22)

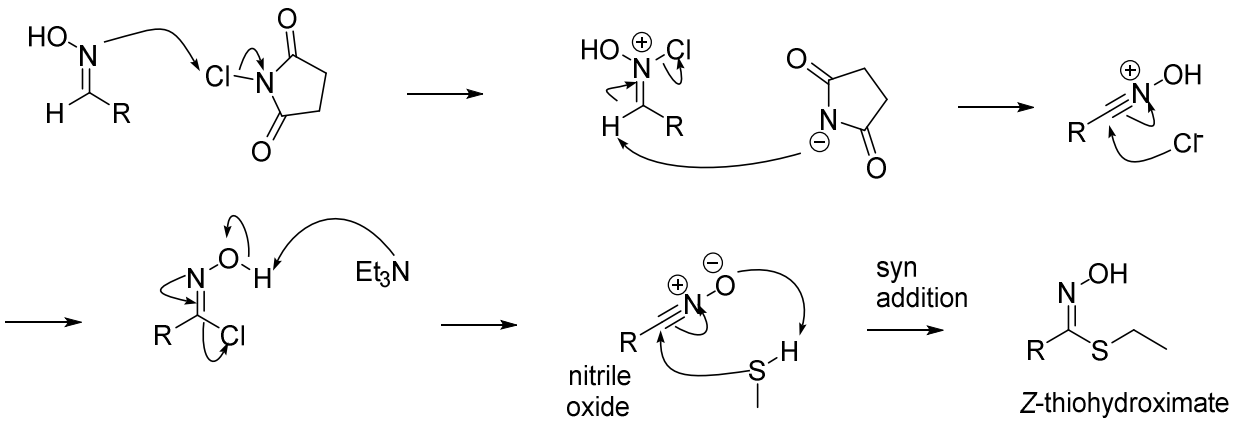
The end of the reaction was checked by TLC; the thiohydroximate **9** has the same R_f of the oxime but it is visible at UV whereas the starting material **7** is not. After column chromatography the thiohydroxymate was obtained in 88% yield.



7 **8** (hydroximoyl chloride) **9** 88%yeld

SCHEME-22

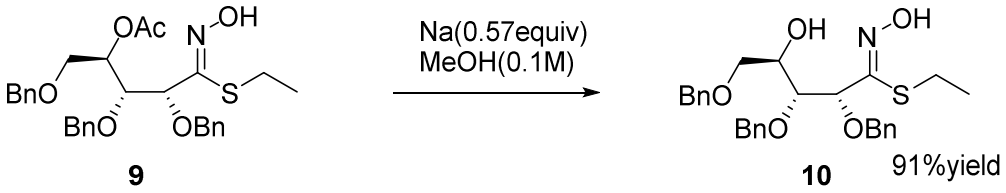
This is the hypothetic mechanism of the reaction:



SCHEME-23

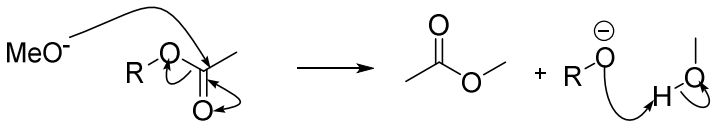
The hydroximoyl chloride by elimination of hydrogen and chloride gives a nitrile oxide, which by reaction with the thiol gives the thiohydroximate. Both the reactions to afford the products **8** and **9** pass through the same nitrile intermediate losing the E or Z configuration of the oxime/hydroximoyl chloride and we confirmed by ^{1}H-NMR that whatever was the configuration of the starting oxime, the final thiohydroximate **9** will always prefer the Z configuration.

DEACETYLATION OF C4 HYDROXYL GROUP



SCHEME-24

To perform the cyclisation, we had first to remove the acetyl protecting group. The deacetylation reaction was performed using sodium methoxide prepared with Na in anhydrous MeOH (SCHEME-24). The reaction was performed under Argon atmosphere at 0°C because the formation of sodium methoxide from sodium and methanol is a very exotermic reaction. After one night the reaction was completed and it was quenched adding Amberlist H^+ resin until neutral pH. Indeed, it was quite interesting to see that the thiohydroximate function could stand these basic and nucleophilic conditions without degradation. This is the hypothetic mechanism of the transesterification reaction:



SCHEME-25

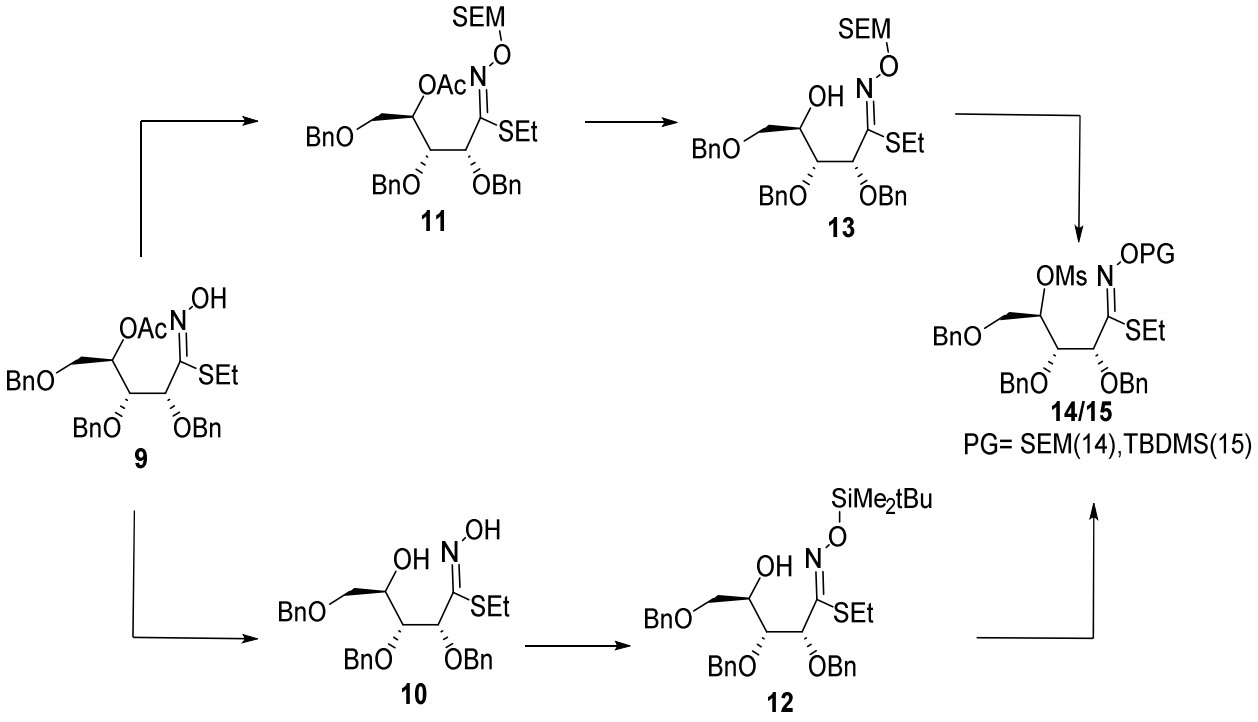
CYCLISATION STEP

Our research group has already experienced three different approaches of cyclisation: the direct mesylation activation, the Mitsunobu reaction and a desylilative cyclisation sequence. The first method has shown a complete disappearance of the starting material but it was observed the formation of a mixture of different products. Attempts to identify and isolate the desired molecule were difficult and even with mass spectrometry analysis it was not possible to determine the presence of the TINO structure in the mixture. Then different attempts have been explored utilizing the Mitsunobu reaction: only with a primary alcohol the cyclisation occurred, not with a secondary one. Associated with this method a problem of purification was frequently observed. The third approach, a desilylative cyclisation needed a sequence of three steps: first a selective silylation of the thiohydroximate group, then a mesylation of the secondary alcohol and the fluoride induced cyclisation.

Starting from *D*-ribose we decided to follow the cyclisation by a desilylative cascade reaction using a source of fluoride anion.

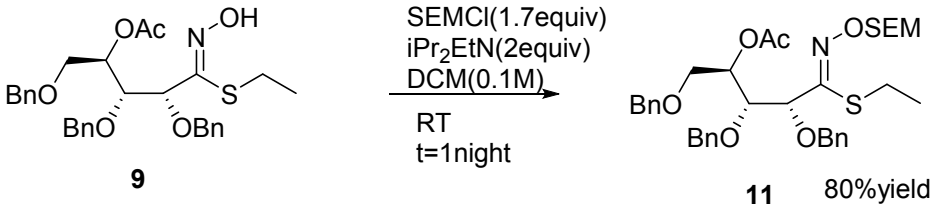
The first step was the selective silylation of the hydroxyl group of the thiohydroximate function. During our studies we have explored two different silyl derivatives to obtain the better conditions to prepare the TINO. We tried the 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl) and the *tert*-butyldimethylsilyl chloride (TBDMSCl).

The second step before the cyclisation was the transformation of the hydroxyl group on C4 in a better leaving group; we decided to activate it by reaction with methanesulfonyl chloride (MsCl). (SCHEME-26)



SCHEME-26

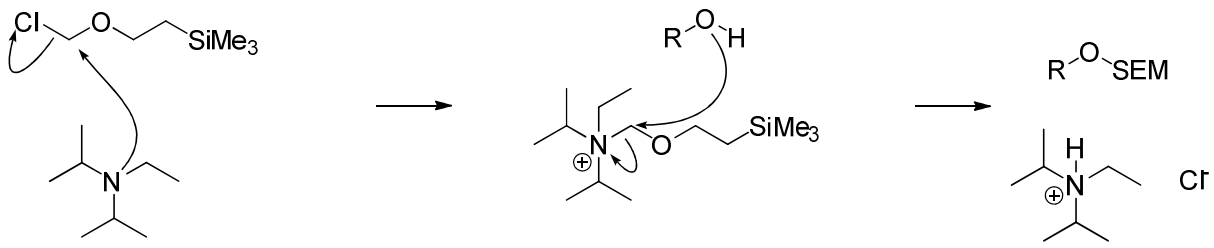
The first silylating reagent used was the 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl).



SCHEME-27

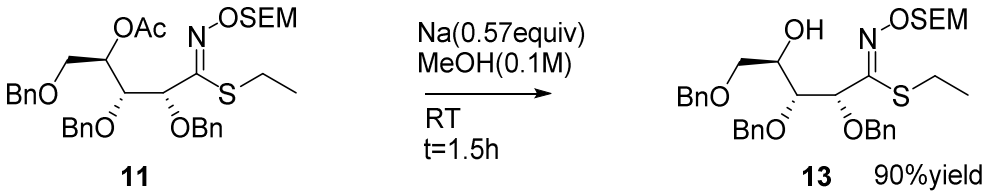
Starting from the mono-acetylated thiohydroxymate **9** we performed the reaction in redistilled DCM with *i*Pr_2EtN (1.5equiv) and SEMCl (1.2equiv), with temperature increasing from 0°C to room temperature, under Argon atmosphere. SEMCl was added slowly because its addition developed HCl. After one night, the starting material **9** was still present in the reaction mixture. Therefore, we performed again the reaction in the same conditions but this time using 1.7 equivalents of SEMCl and 2 equivalents of *i*Pr_2EtN. After one night, we obtained the expected product **11** in 80% yield after purification with chromatographic column (SCHEME-27).

This is the hypothetic mechanism of the reaction; the amine is very bulky.



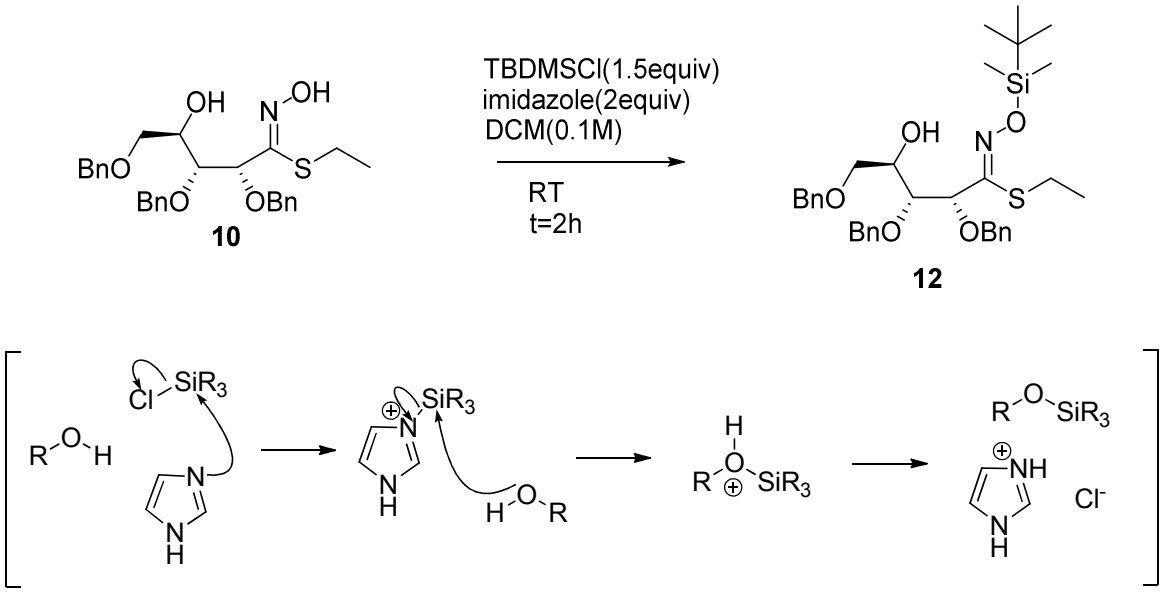
SCHEME-28

Then as described before in SCHEME-24 we deprotected the hydroxyl group on C4 but in this case, the reaction required a shorter reaction time (2.5h). (SCHEME-29)



SCHEME-29

We tried a different silyl containing protecting group, namely the *tert*-butyldimethylsilyl group. As described in SCHEME-30 we dissolved the thiohydroxymate **10** in redistilled CH_2Cl_2 and at 0°C, we added imidazole and TBDMSCl. After 2h, reaction is completed and the silylated product **12** was obtained in 70% yield.

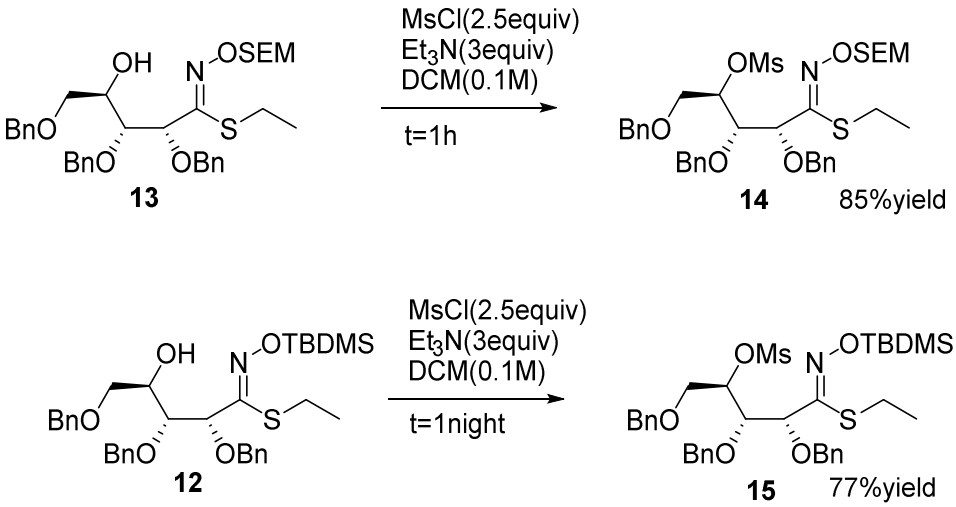


SCHEME-30

As previously described, to afford the cyclisation we had to transform the hydroxyl group on C4 in a better leaving group; we activated it by reaction with methanesulfonyl chloride (MsCl). As depicted in SCHEME-31, we performed the reaction in redistilled DCM, with Et_3N, under Argon atmosphere and in ice-salt bath.

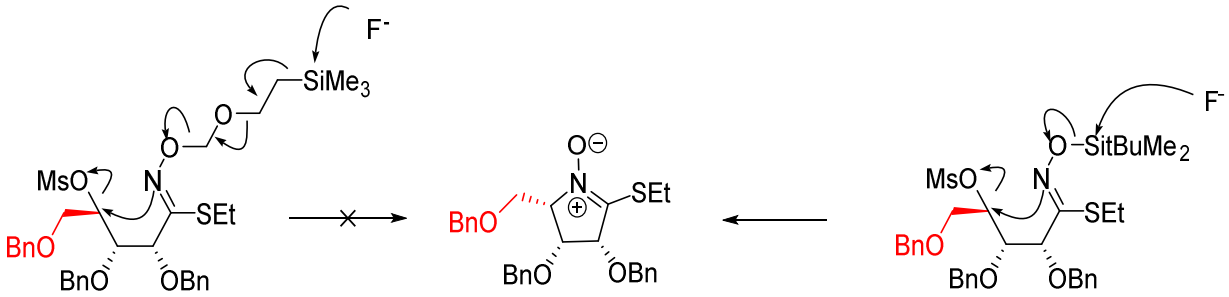
SCHEME-31

We developed this reaction starting from reagent **13** and using 1.5equivalents of MsCl and 2 equivalents of Et_3N; after 3h there was still the starting material, so we added another equivalent of both the reagents and we let the reaction running for 24 hours. We then quenched the reaction and purified the mixture by column chromatography but we obtained a mixture of undefined products. We tried again but using 2.5equivalents of MsCl and 3.0 equivalents of Et_3N; we ran the reaction just for one hour, to avoid degradation. This time the reaction run well and we obtained the mesylated product **14** in 85% yield so we used the same procedure to obtain the product **15** in 77% yield. (SCHEME-32)



SCHEME-32

The next step was the cyclisation. As described in SCHEME-33 it is a desilylative intramolecular cascade reaction activated by a source of fluoride anion; it is a SN2 reaction so we have inversion of configuration on the C4.



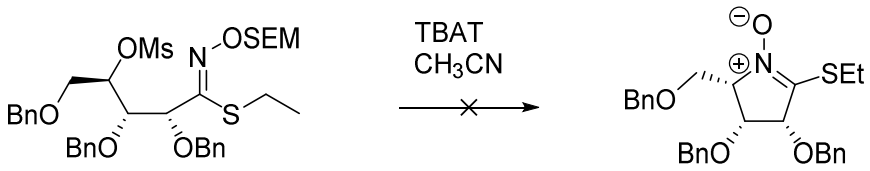
SCHEME-33

In the table,the cyclisation attempts are presented:

ENTRY	PG	SOURCE F^-	SOLVENT	CONDITIONS	RESULTS
1	SEM	TBAT (1.1equiv)	CH_3CN	RT-->reflux; t=50h	starting material
2	SEM	TBAT (1.1equiv)	THF	RT-->reflux; t=1night	starting material
3	SEM	TBAF (1.1equiv)	THF	RT-->reflux; t=50h	degradation
4	SEM	TBAF (1.1equiv)	THF;MS 4Å	RT-->reflux; t=50h;	degradation
5	SEM	TBAF (1.1equiv)	1,4-dioxane	reflux; t=7h	traces
6	SEM	TBAF (1.1equiv)	1,4-dioxane; MS4Å	reflux; t=4h	degradation
7	SEM	CsF (1.1equiv)	1,4-dioxane	reflux; t=4h	degradation
8	TBDMS	TBAT (1.1equiv)	CH_3CN; MS4Å	reflux; t=1h	53% + 23% desilyl. prod.
9	TBDMS	TBAT (1.1equiv)	CH_3CN; MS3A	reflux; t=4h	72%

Using as protecting group the 2-(trimethylsilyl)ethoxymethyl group we tried different solvents and conditions but nothing worked (see entries 1-7):

- We performed the reaction under Argon atmosphere in CH_3CN (0.1M) and using 1.1equivalents of TBAT (tetrabutylammonium diflourotriphenylsilicate) as source of F^- at room temperature (entry-1). After 1h there was just the starting material so we decided to heat under reflux. After 2h nothing was changed so we left the reaction for all the weekend at room temperature. We checked again the reaction by TLC but there was just the starting material so we stopped it and with extractions with EtOAc we recovered the starting reagent.



SCHEME-34

- We changed the solvent and we used THF (entry-2). Using TBAT as source of fluoride anion and under Argon atmosphere, we first ran the reaction at room temperature; after 2h there was still the starting material so we heated under reflux the reaction mixture. After 1h nothing was changed so we added another equivalent of TBAT and reaction was left under reflux overnight. We checked the reaction by TLC but there was just the starting material, so with extraction we recovered it. With ^{1}H-

NMR we also saw that there was still TBAT unreacted so the problem wasn't the equivalents of TBAT.

- We decided to change the source of F^- so we performed the reaction in THF(0.1M) with 1.1equivalents of TBAF(tetrabutylammonium fluoride), under Argon atmosphere (entry-3). After 1h at room temperature, nothing happened, so we started heating. After 2h we checked with TLC, but nothing changed at all so we left the reaction for all the weekend at room temperature. After the weekend, we purified the mixture by chromatographic column but we just had degradation, no product and we couldn't recover the starting material.

- We performed the reaction in THF, with TBAF in the presence of molecular sieves(4Å) (entry-4). After 1h at room temperature a new spot was visible on TLC, which was probably the desilylated but not cyclised product. We started to heat under reflux and after other 2h the color of the mixture changed, from colorless to brown, so we left the reaction for all the weekend at room temperature. Unfortunately we had degradation also in this attempt.

- We performed the reaction in dioxane, with TBAF and heating under reflux (entry-5). This time, by TLC, new spots were visible. After 7h, we decided to quench the reaction and after column chromatography, we obtained three fractions, analyzed by ^{1}H-NMR. One of the fraction was the cyclised product as confirmed also by mass spectroscopy. The problem was that there were just traces of the TINO.

- We repeated the reaction in the same conditions of entry-5 but we also added molecular sieves (4Å) (entry-6). After 4h we quenched the reaction but this time we observed degradation.

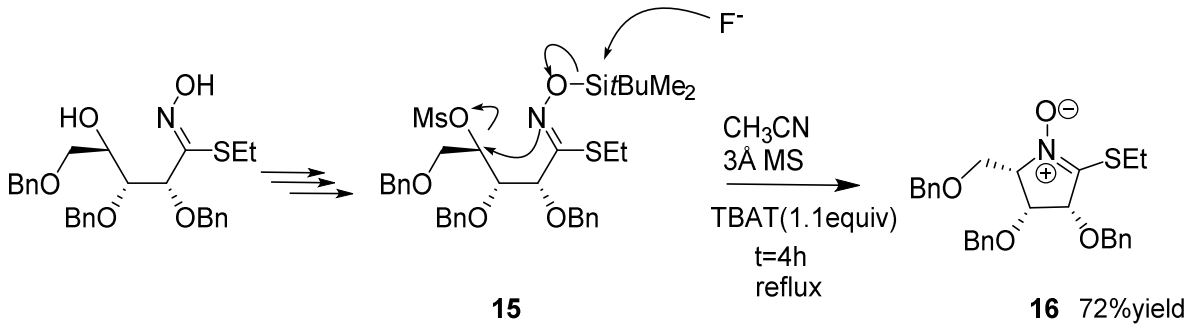
- We changed again the source of F^-, we used CsF in dioxane and the reaction was heated under reflux and under Argon atmosphere (entry-7). After 4h reaction was quenched; we did extraction and ^{1}H-NMR. We observed degradation.

At this point, we decided to use as silylated protecting group the *tert*-butyldimethylsilyl group.

As first attempt of cyclisation we decided to perform the reaction in anhydrous CH_3CN, heating under reflux, under Argon atmosphere, using as source of fluoride ion the TBAT in

the presence of 4Å molecular sieves (entry-8). After 1h we quenched the reaction and after purification by column chromatography we obtained two fractions identified by ^{1}H-NMR: one was the desired product in 53% yield, the other one was the desilylated product in 23% yield.

We finally obtained the TINO, so the next step was the optimization of the reaction conditions to convert the remaining desilylated product into the cyclised one. We ran again the reaction in more anhydrous conditions, using anhydrous CH_3CN, with 3Å molecular sieves , which are more suitable for this solvent, during 4h (entry-9). After purification by column chromatography, we obtained the TINO in 72% yield.(SCHEME-35)

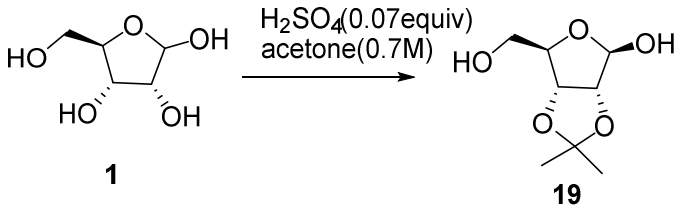


SCHEME-35

To reduce the number of steps in the synthesis of our TINO we decided to try another synthetic path to prepare the desired thiohydroximate precursors. In particular we found out that we could make direct protection of the hydroxyl groups on C-2 and C-3 with the isopropylidene group, without the need of purification, avoiding the two steps of selective protection and deprotection of the hydroxyl group on C-1.

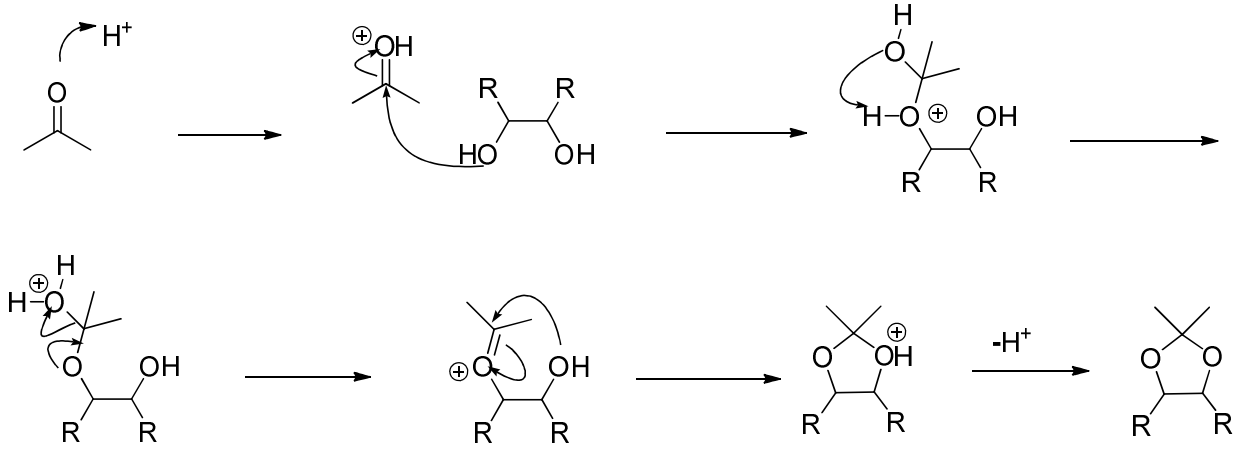
PROTECTION OF C2 AND C3 HYDROXYL GROUPS

Following the procedure reported in literature we tried the protection of OH-2 and OH-3 with an isopropylidene group. We performed the reaction adding H_2SO_4 to a slurry of *D*-ribose in dry acetone. After one hour and half the reaction mixture is neutralized by addition of calcium hydroxide ($Ca(OH)_2$). After filtration we obtained the 2,3-*O*-Isopropylidene-β-*D*-ribofuranose **19** in quantitative yield. (SCHEME-36)



SCHEME-36

In the scheme is reported the acid catalyzed procedure for the selective protection of syn diols as only C2 and C3 hydroxyl groups react. In SCHEME-37 the general mechanism for this reaction is described:



SCHEME-37

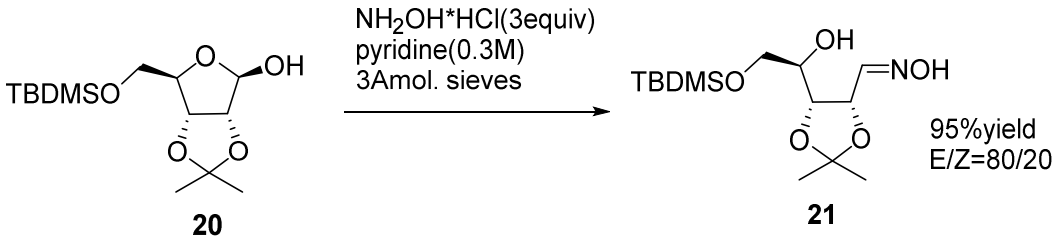
PROTECTION OF C5 HYDROXYL GROUP WITH TBDMSCl

The next step of the procedure reported in literature was the protection of the hydroxyl group on C5 with *tert*-butyldimethylsilyl chloride. Following the procedure reported in literature we added TBDMSCl to a solution of the reagent **19** and imidazole in anhydrous DMF. After extractions and purification by column chromatography we obtained the desired product **20** in 59% yield. The reaction mechanism is the same reported in SCHEME-30; just the solvent is different. (SCHEME-38)

SCHEME-38

FORMATION OF THE OXIME

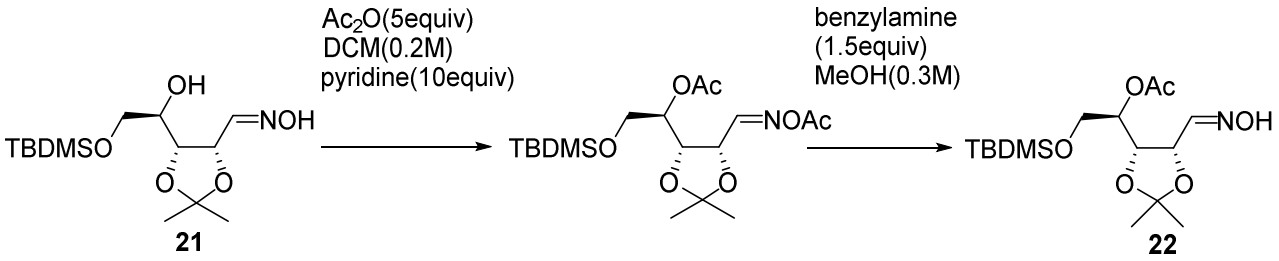
To open the carbohydrate ring and form the oxime we used the same procedure previously developed and described in SCHEME-16, so we performed the reaction in pyridine, under Argon atmosphere with hydroxylamine hydrochloride and 3Å molecular sieves. The reaction was very fast and was completed in just 2h. After purification by column chromatography the desired product **21** was obtained in 95% yield as a mixture of the two isomers E and Z in a 80/20 ratio. (SCHEME-39)



SCHEME-39

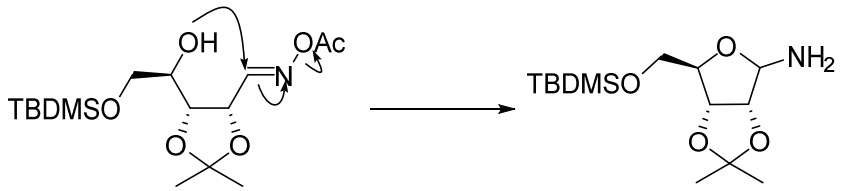
PROTECTION OF THE NEW HYDROXYL GROUPS AND SELECTIVE DEPROTECTION

Next step was the acetylation of both hydroxyl groups followed by the selective deprotection of the hydroxyl group of the aldoxime function. We tried to use the same procedure used for the product (**5** and described in SCHEME-18.



SCHEME-40

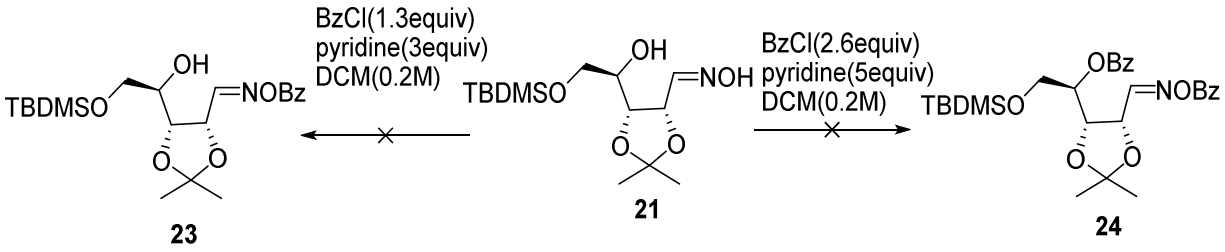
After purification by column chromatography we obtained different fractions; one of them was the desired monoacetylated product **22**, but in very little amount (20%yield). One of the fractions was the starting aldoxime (42%yield); another one was a cyclized product(38%yield) (its formation was probably due to the migration of the acetyl group from the secondary alcohol to the hydroxyl group of the oxime function)(SCHEME-41). We decided to repeat the reaction in the same conditions but using less equivalents of benzylamine (1equiv) in the selective deprotection step. Unfortunately the reaction didn't work; after purification by column chromatography we obtained the starting material (53%yield)and the ring closed product (30%yield).



SCHEME-41

We tried again the same protection procedure but this time we tried to do just the first step to see if we could obtain the diacetylated product but the reaction didn't work; we obtained a mixture of undefined products.

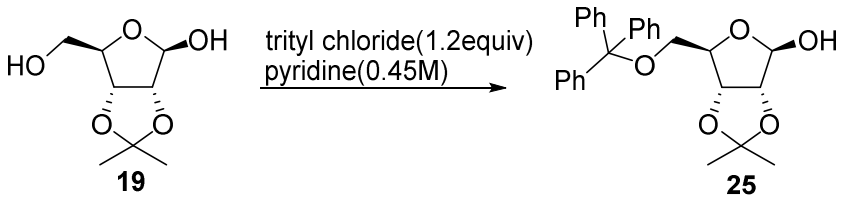
Therefore, we decided to change the protecting group and try benzoyl chloride instead of the acetic anhydride. We performed the reaction between starting material **21** and BzCl, with pyridine in distilled DCM at 0°C. At the same time, we performed two reactions with different amounts of BzCl and pyridine. In one test we used 2.6 equivalents of BzCl to protect both the hydroxyl groups; in the other test we used just 1.3equivalents of BzCl hoping in a selective protection of the hydroxyl group of the oxime. After thirty minutes we checked the absence of the starting material **21** by TLC but after purification we obtained three undefined fractions from both the two tests. (SCHEME-42)



SCHEME-42

PROTECTION OF C5 HYDROXYL GROUP WITH TRITYL CHLORIDE

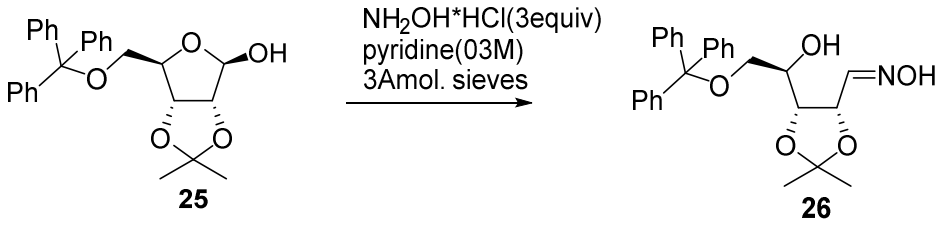
We thought that probably the use of trityl (triphenylmethyl) group could allow the use of TBDMS group to protect the oxime **21** instead of the Ac group. Therefore, we dissolved the reagent **19** in dry pyridine, then we added the trityl chloride and we left the reaction running under Argon atmosphere and at reflux for one night. We obtained the desired product **25** without purification by column chromatography in quantitative yield. (SCHEME-43)



SCHEME-43

FORMATION OF OXIME

The reaction described in SCHEME-16 worked also for reagent **25** and the desired aldoxime **26** was obtained by ring opening reaction with hydroxylamine and pyridine. The only problem was the purification of the reaction mixture because we weren't able to purify our product from the trityl impurities with chromatographic flash column. (SCHEME-44)



SCHEME-44

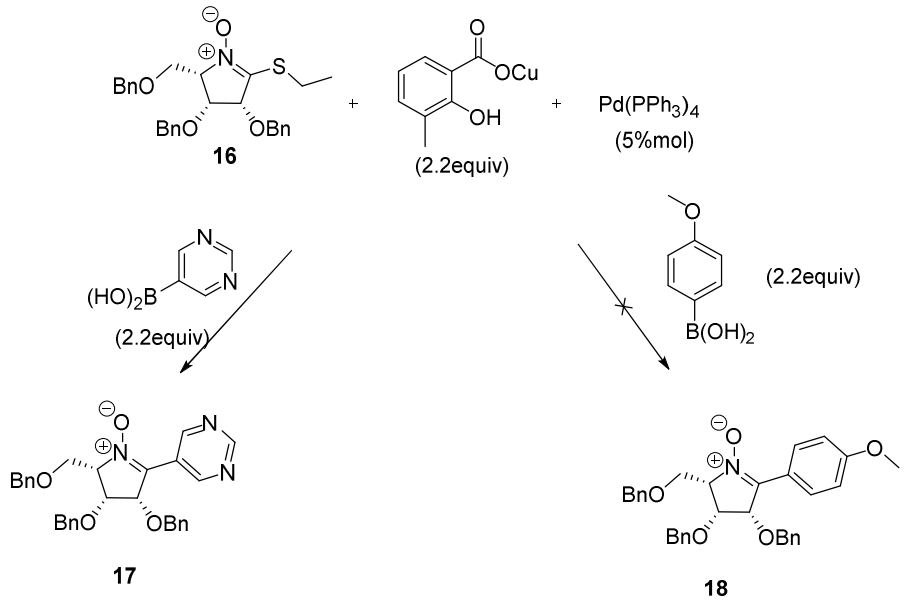
With this procedure in hand, we have the possibility to reduce the number of steps of all the synthesis of the TINO. Next targets are:

- The development of a way to purify product **26**;
- alternative protection/deprotection paths for the oxime **26**.

LIEBESKIND-SROGL CROSS COUPLING

The last focus of our studies was the synthesis of ketonitrones by palladium-catalyzed reaction; in particular we tried to synthetize a ketonitrone starting from TINO **16** by Liebeskind-Srogl coupling[18]. This is a desulfative cross-coupling process between an organosulfur compound and a boronic acid, it requires a catalytic amount of palladium(0) and a stoichiometric amount of a copper(I) carboxylate which acts as a thiophilic metal cofactor. The reaction is performed under inert atmosphere, both the Cu cofactor and the Pd catalyst required the absence of oxigen to avoid any undesired oxidation. Liebeskind-Srogl coupling doesn't need the addition of a base in contrast with the Suzuki-Miyaura cross-coupling where a base (carbonate such as Cs_2CO_3 or K_2CO_3) is essential[19]. Initially the Liebeskind-Srogl reaction was performed with thioesters and boronic acid; nowadays it has been extended to a variety of thioorganic reagents (in our work to a TINO) and to other organometallic substrates[19].

We tried the coupling over our substrate **16** using as boronic acid the pyrimidine-5-boronic acid and the 4-methoxyphenylboronic acid, as copper cofactor we used copper(I) 3-methylsalicylate and as palladium catalyst tetrakis(triphenylphosphine)palladium(0). The reaction was performed in distilled and degassed THF heating under reflux for 24h(SCHEME-45).

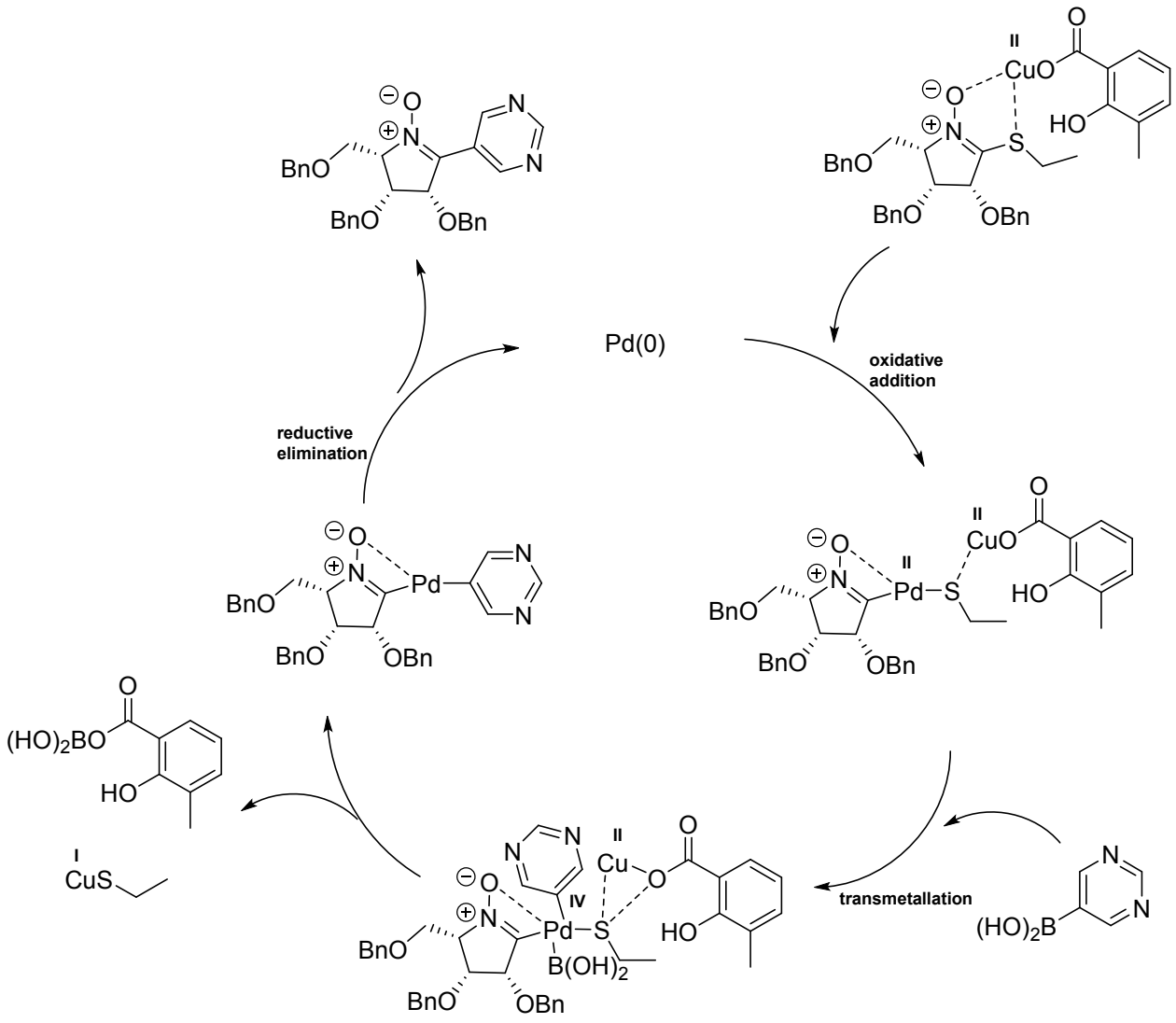


SCHEME-45

[18] Schleiss J.; Rollin P.; Tatibouët A.; *Angew. Chem. Int. Ed.*, **2010**, 49, 577-580
[19] Prokopcová H.; Kappe C. O.; *Angew. Chem. Int. Ed.*, **2009**, 48, 2276-2286

Just one of the two essays ran well; we had degradation in the reaction with 4-methoxyphenylboronic acid. Instead, we had the coupling with the pyrimidine-5-boronic acid as confirmed by ^{1}H-NMR and mass spectroscopy. Despite purification of the mixture by column chromatography a residue of triphenylphosphine oxide was visible both in the ^{1}H-NMR and mass spectra. We performed this reaction with a very small amount of reagent **16** so it was impossible to calculate the yield; so next steps would be an improvement of the purification procedure, the evaluation of the reaction yield and the use of other boronic acids.

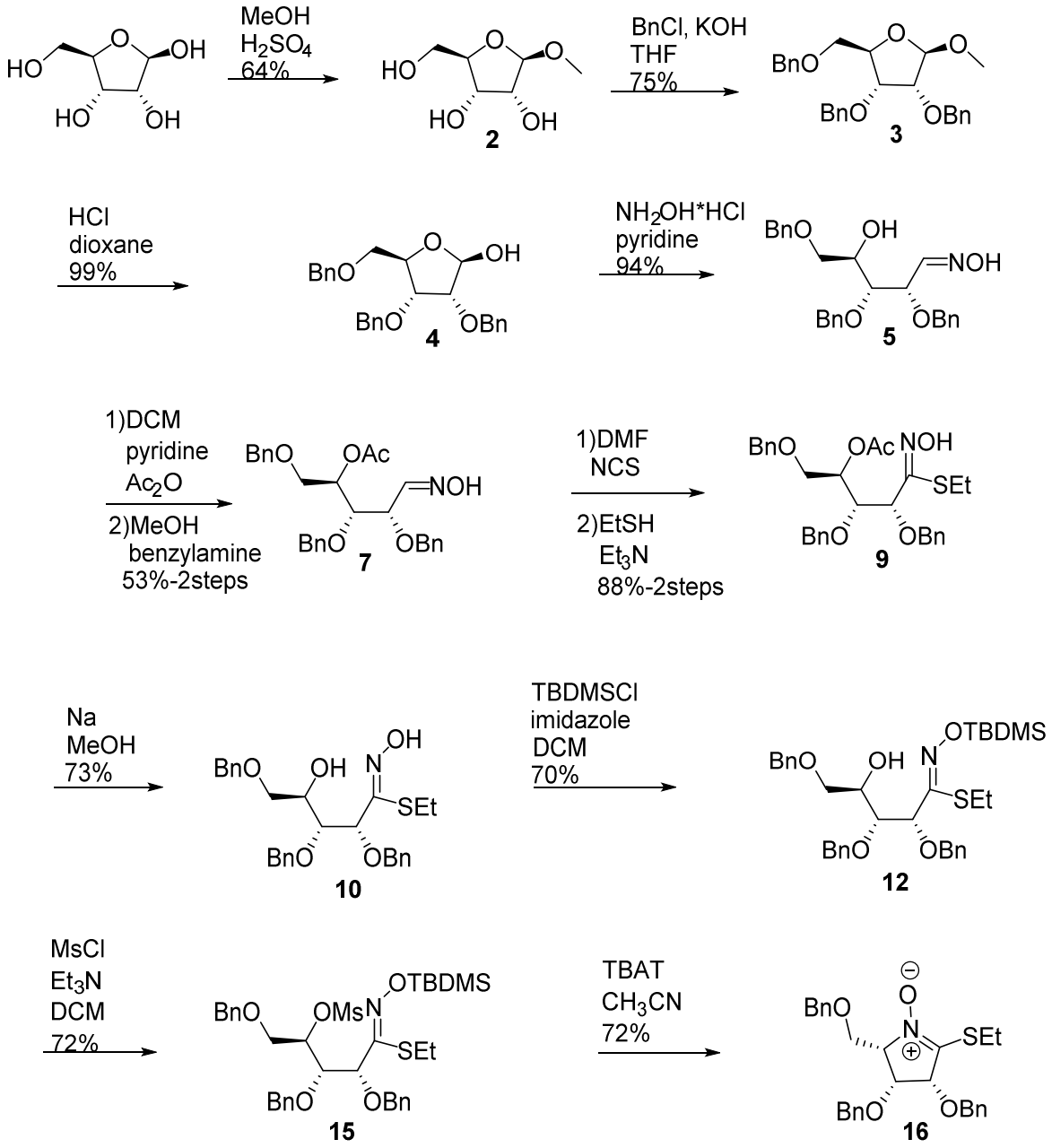
This is the hypothetic catalytic cycle:



SCHEME-46

CONCLUSIONS AND PERSPECTIVES

The main focus of this work was the synthesis of the thioimidate-*N*-oxide function starting from *D*-ribose. Basing our research on precedent studies made on different substrates, we obtained the desidered TINO by a twelve steps synthesis with a global yield of 5.5% (SCHEME-47).



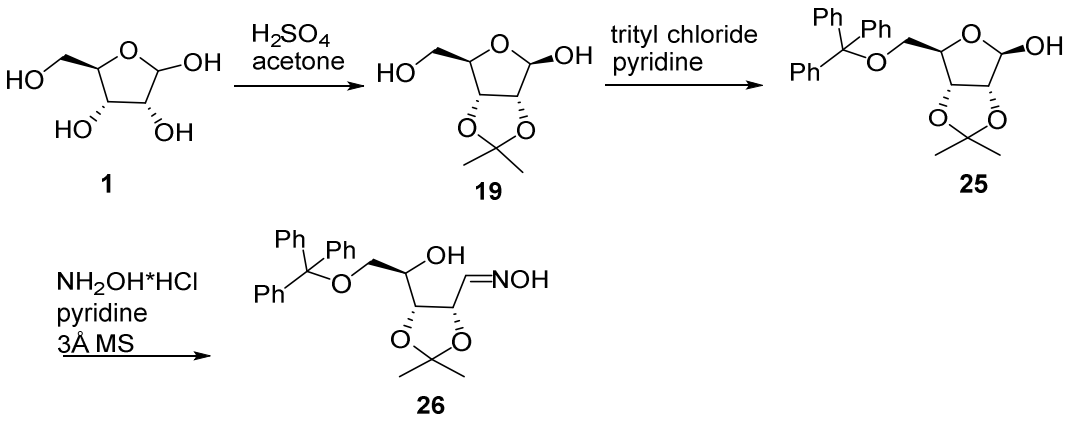
SCHEME-47

During our research:

- We developed a synthesis of thioimidate-*N*-oxides from *D*-ribose; in particular we worked on the cyclization step, which is the critical step of this synthesis, and we found out that it is better to perform it in CH_3CN, in anhydrous conditions, using as silylated substituent TBDMS and as source of F^- TBAT. These conditions allowed us to obtain the desidered TINO with a yield of 72% after 4h.

- We also tried two reactions of cross-coupling between the TINO and a boronic acid. Just one attempt worked well, using pyrimidine-5-boronic acid, but the yield still need improvement.

- We started a new synthetic path, using different protecting groups, for the TINO trying to reduce the number of steps.



SCHEME-48

Future work will focus on:

- the optimization of the synthesis, with the development of the second synthetic path, in order to reduce the total number of steps and increase the final yield.

- the evaluation of the generality of the studied methodology by using different starting materials.

- the exploration of the suitable conditions for the purification of the ketonitrone obtained in the reaction between the TINO and the pyrimidine-5-boronic acid.

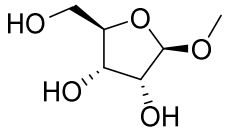
- The research of other boronic acids suitable for the cross-coupling reaction with TINO.

EXPERIMENTAL PART

General Methods

Flash silica column chromatographies were performed on silica gel 60N (spherical, neutral, 40-63 µm, Merck). Reactions were monitored by thin layer chromatography (TLC) on silica gel 60F254 precoated aluminium plates. Compounds were visualized under UV light and by charring with H_2SO_4 ethanolic solution spray (10%) or a solution of potassium permanganate. Solvent were dried by standards methods: THF was purified by a dry station (GT S100) immediately prior to use, dichloromethane was distilled from P_2O_5, methanol and *N*,*N*-dimethylformamide were dried with molecular sieves, pyridine and triethylamine were dried with potassium oxide. Molecular sieves were activated prior to use by heating for 4h at 500°C. All other commercially available solvents and reagents were used without further purification. All reactions were carried out under dry argon. Optical rotations were measured at 20°C with a Perkin-Elmer 341 polarimeter with a path length of 1 dm; values are given in deg dm^{-1} g^{-1} mL with concentration reported in g/100mL. ^{1}HNMR and ^{13}CNMR spectra were recorded with a Bruker Avance II 400 or Brucker DPX 250 spectrometers. Assignments are based on DEPT 135 sequence and on homo- and heteronuclear correlations. Chemical shifts are reported in parts per million (ppm) from tetramethylsilane as the internal standard. Coupling constants (J) are reported and expressed in Hertz (Hz), splitting patterns are designed as br (broad), s (singlet), d (doublet), dd (doublet of doublets), q (quartet), dt (doublet of triplets), m (multiplet). High-resolution mass spectra (HRMS) were obtained with a Maxis Bruker 4G instrument by the "Federation de Recherche" ICOA/CBM (FR2708) platform in the electrospray ionisation (ESI) mode. Infrared spectra of compounds were recorded with a Thermo Scientific Nicolet iS10 spectroscope.

Methyl β-D-ribofuranoside



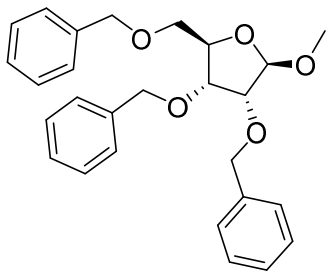
2
MW=164.07g/mol
$C_6H_{12}O_5$

D-ribose(5.05 g, $3.47*10^{-2}$ mol, 1 equiv) was dissolved in dry MeOH(0.3 M ≈ 100 mL) and left stirring for 20' in an ice bath. Then H_2SO_4 (0.5 mL, 9.38 mmol, 0.3 equiv.) was added. The reaction was warmed slowly to room temperature and then stirred overnight. When the reaction was finished, it was neutralized by addition of Et_3N (2.6 mL). After evaporation under reduced pressure, the product was purified by silica gel flash chromatographic column (EA/MeOH = 9.5/0.5). We obtained methyl β-*D*-ribofuranoside as a colorless oil in 69% yield.

R_f= 0.23 (EA/MeOH=9/1)

^{1}H-NMR (250MHz,CD_3OD) δ(ppm)= 4.75 (d, 1H, J_{1-2}=1Hz, H-1); 4.03 (dd, 1H, J_{3-2}=4.6Hz, J_{3-4}=6.91Hz,H-3); 3.99-3.90 (m,1H,H-4); 3.86 (dd,1H,J_{2-1}=1, J_{2-3}=4.6Hz,H-2); 3.72 (dd,1H,J_{5-4}=3.5Hz,J_{5a-5b}=11.7Hz,H-5_a); 3.54 (dd,1H,J_{5-4}=6.4Hz,J_{5b-5a}=11.7Hz,H-5_b); 3.35 (s,3H,OMe)

Methyl 2,3,5-tri-O-benzyl-β-D-ribofuranoside



3
MW= 434.21g/mol
$C_{27}H_{30}O_5$

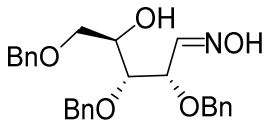
Methyl β-*D*-ribofuranoside (3.1 g, 0.02 mol, 1 equiv.) was dissolved in anhydrous THF(0.8 M, 23.5 mL) and then KOH(14 g, 0.25 mol, 12.7 equiv.) and BnCl(0.17 mol, 19.6 mL, 8.6 equiv.) were added. The reaction was heated under reflux, under Ar atmosphere for 24h. Then the reaction was quenched by addition of ice and water. Aqueous phase was extracted three times with EtOAc and the combined organic phases are washed with water and brine. After drying over $MgSO_4$, filtration and evaporation under vacuum, the product was purified by silica gel flash chromatographic column (PE/EA = 9/1). Methyl 2,3,5-tri-*O*-benzyl-β-*D*-ribofuranoside was obtained as a colorless sirup in 76% yield.

R_f= 0.27 (PE/EA=9/1)

^{1}H-NMR (400MHz, CD_3OD) δ(ppm)= 7.38-7.22 (m, 15H, H_{Ar}); 4.91 (bs, 1H, H-1); 4.65 (d, 1H, *J*=11.8Hz, CH_2Bn); 4.60 (d, 1H, *J*=11.8Hz, CH_2Bn); 4.56 (d, 1H, *J*=12.0Hz, CH_2Bn); 4.53 (d, 1H, *J*=11.7Hz, CH_2Bn); 4.51 (d, 1H, *J*=12.0Hz, CH_2Bn); 4.44 (d, 1H, *J*=11.7Hz, CH_2Bn); 4.25-4.18 (m, 1H, H-4); 4.03 (dd, 1H, J_{3-2}=4.7Hz, J_{3-4}=6.4Hz, H-3); 3.88 (dd, 1H, J_{2-1}=1.1Hz, J_{2-3}=4.7Hz, H-2); 3.59 (dd, 1H, J_{5-4}=3.7Hz, J_{5a-5b}=10.6Hz, H-5_a); 3.48 (dd, 1H, J_{5-4}=5.9Hz, J_{5b-5a}=10.6Hz, H-5_b); 3.28 (s, 3H, OMe)

^{13}C-NMR (100MHz, CD_3OD) δ(ppm)= 139.6 ($C_q$ar); 139.2 ($C_q$ar); 139.1 ($C_q$ar); 129.4 (CHar); 129.38 (CHar); 129.3 (CHar); 129.27 (CHar); 129.22 (CHar); 128.93 (CHar); 128.90 (CHar); 128.8 (CHar); 128.6 (CHar); 108.0 (C-1); 81.8 (C-4); 80.9 (C-2); 79.8 (C-3); 74.2(CH_2Bn); 73.45 (CH_2Bn); 73.4 (CH_2Bn); 72.61 (C-5); 55.6 (OCH_3)

2,3,5-tri-O-benzyl-D-ribofuranose oxime



5
MW=435.2g/mol
$C_{26}H_{29}NO_5$

Methyl 2,3,5-tri-*O*-benzyl-β-*D*-ribofuranoside (6.5 g, 0.015 mol, 1 equiv.) was dissolved in dioxane(0.1 M, 146.5 mL) and then HCl 1M(36.5 mL) was added. The mixture was heated under reflux for one night, then dioxane was evaporated. Aqueous phase was extracted with EtOAc and the combined organic phases were washed with NaOH 1N(to neutralize HCl), water and brine. The mixture was dried over $MgSO_4$, filtered and evaporated under reduced pressure.

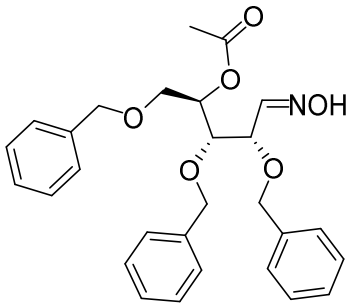
The resulting crude product (6.15 g, 0.015 mol, 1 equiv.) was dissolved, under Ar atmosphere, in dry pyridine (0.3 M, 50 mL). NH_2OH*HCl (3.2 g, 0.046 mol, 3 equiv.) was added and then two spoons of 3Å molecular sieves. The reaction mixture was stirred at RT for 5h. The mixture was first filtered through a celite pad, washed with EtOAc and then the solvent was evaporated. The organic phase was washed with water and brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resulting residue was then coevaporated three times with toluene. The product was purified by silica gel flash chromatographic column (PE/EA = 8/2 to 7/3). 2,3,5-tri-*O*-benzyl-*D*-ribofuranose oxime was obtained as a mixture of the two isomers E and Z (75:25=E:Z) with a yield of 94%.

R_f= 0.24 (PE/EA=8/2)

1H-NMR (400MHz, $CDCl_3$) δ(ppm)= data for the major E isomer 8.55 (s; 1H; NOH); 7.50 (d; 1H; $J_{1\text{-}2}$=8.3Hz; H-1); 7.38-7.24 (m; 15H; H_{Ar}); 4.85 (d; 1H; J=11.3Hz; H-3); 4.63-4.39 (m; 7H; CH_2Bn; H-2); 4.07 (bs; 1H; OH) 3.75 (bs; 2H; H-5); 3.49-3.41 (m; 1H; H-4)

^{13}C-NMR (100MHz, $CDCl_3$) δ(ppm)= 149.7 (C-1); 138.1 ($C_q$ar); 137.9 ($C_q$ar); 137.1 ($C_q$ar); 128.5 (CHar); 128.4 (CHar); 128.3 (CHar); 128.1 (CHar); 128.0 (CHar); 127.72 (CHar); 127.70 (CHar); 127.6 (CHar); 127.5 (CHar); 78.2 (CH_2Bn); 74.2 (C-3); 73.7 (C-2); 72.6 (C-4); 72.5 (CH_2Bn); 71.3 (CH_2Bn); 69.3 (C-5)

4-O-acetyl-2,3,5-tri-O-benzyl-D-ribofuranose oxime



7
MW=477.22g/mol
$C_{28}H_{31}NO_6$

1)To a solution of 2,3,5-tri-*O*-benzyl-*D*-ribofuranose oxime (2.5 g, 5.8 mmol, 1 equiv.) in anhydrous CH_2Cl_2 (0.2 M, 29 mL) was added dry pyridine (0.058 mol, 4.7 mL, 10 equiv.) and acetic anhydride (0.029 mol, 2.7 mL, 5 equiv.) at 0°C. Reaction mixture was stirred under Argon atmosphere all night at room temperature. The reaction is quenched by addition of water. The aqueous phase is extracted with EtOAc for three times; combined organic phases were washed with water and brine. The mixture was dried over $MgSO_4$, filtered and evaporated under reduced pressure.

2)The resulting crude bis-acetylated oxime (1 equiv.) was dissolved in anhydrous MeOH (0.3 M, 19 mL) and benzylamine (0.012 mol, 1.3 mL, 2.2 equiv.) was added at 0°C. After stirring under Argon atmosphere, at room temperature, for 3h the solvent was evaporated under reduced pressure. Then the product was isolated by silica gel flash chromatographic column (PE/EA = 8/2 to PE/EA = 7/3). 4-*O*-acetyl-2,3,5-tri-*O*-benzyl-*D*-ribofuranose oxime was obtained in a 65% yield as a colourless oil, as E isomer.

$[\alpha]_D$=+33 (20°C; c = 0.0105 g/100mL in $CHCl_3$)

R_f= 0.31(PE/EA=7/3)

^{1}H-NMR (250MHz,$CDCl_3$) δ(ppm)= 7.41 (d, 1H, $J_{1\text{-}2}$=7.9Hz, H-1); 7.37-7.24 (m, 15H, H_{Ar}); 5.23-5.13 (m, 1H, H-4); 4.74 (d; 1H; *J*=11.4Hz; CH_2Bn); 4.62 (m; 2H; CH_2Bn); 4.50 (d; 1H; *J*=12.0Hz; CH_2Bn); 4.40 (d; 1H; *J*=12.0Hz; CH_2Bn); 4.39 (d; 1H; *J*=11.4Hz; CH_2Bn); 4.13 (dd, 1H, $J_{2\text{-}3}$=4.5Hz, $J_{2\text{-}1}$=7.9Hz, H-2); 4.01 (dd, 1H, $J_{3\text{-}2}$=4.5Hz, $J_{3\text{-}4}$=6.1Hz, H-3); 3.71

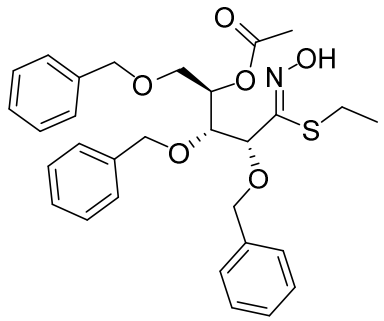
(dd, 1H, $J_{5\text{-}4}$=5.1Hz, $J_{5a\text{-}5b}$=10.8Hz, H-5_a); 3.62 (dd, 1H ,$J_{5\text{-}4}$=3.52Hz, $J_{5b\text{-}5a}$=10.85Hz, H-5_b); 2.02 (s, 3H, OMe)

^{13}C-NMR (100MHz,$CDCl_3$) δ(ppm)= 149.8 (C-1); 138.0 ($C_q$ar); 137.8 ($C_q$ar); 137.6 ($C_q$ar); 128.4 (CHar); 128.36 (CHar); 128.32 (CHar); 128.0 (CHar); 127.9 (CHar); 127.75 (CHar); 127.72 (CHar); 127.70 (CHar); 127.6 (CHar); 78.4 (C-3); 77.2 (C-2); 73.8 (CH_2Bn); 73.1 (CH_2Bn); 71.5 (C-4); 70.9 (CH_2Bn); 68.1 (C-5); 20.9 (CH_3)

IR (neat): ν (cm^{-1}) = 3306 (OH); 3027 (C_{sp2}-H ar); 2917(C_{sp3}-H); 1732.41 (C=O); 1246.79 (C-O); 1207.67 (N-O)

MS (ESI^+) m/z = 478.3 ($[M+H]^+$)

(Z)-S-Ethyl-4-O-acetyl-2,3,5-tri-O-benzyl-D-ribonimidothioate



9
MW=537.22g/mol
$C_{30}H_{35}NO_6S$

2,3,5-tri-*O*-benzyl-4-*O*-acetyl-*D*-ribofuranose oxime (1.5 g, 3.14 mmol, 1 equiv.), under Argon atmosphere, was dissolved in anhydrous DMF (0.1 M, 31.5 mL) and cooled down at 0°C. Then recristallized NCS (0.62 g, 4.71 mmol, 1.5 equiv.) was added and the ice bath was removed. The reaction was stirred at room temperature, protected from light, until full consumption of the starting material (2.5h). So the mixture was cooled down at 0°C and Et_3N (9.42 mmol, 1.3 mL, 3 equiv.) and EtSH (9.42 m mol, 0.7 mL, 3 equiv.) were added simultaneously and dropwise. After 1h the ice bath was removed and the reaction was left stirring for 3h. The reaction was quenched by addition of water and ice and diluted with ethyl acetate. The aqueous phase was extracted with EtOAc for three times and then the combined organic phases were washed with water and brine. After drying on $MgSO_4$, filtration and evaporation of the solvent, the mixture was purified by silica gel flash chromatographic column (PE/EA= 7/3). (*Z*)-*S*-ethyl-4-*O*-acetyl-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate was obtained as a light yellow oil with a yield of 88%.

$[\alpha]_D$=+56 (20°C; c = 0.0096 g/100mL in $CHCl_3$)

R_f=0.35 (PE/EA=7/3)

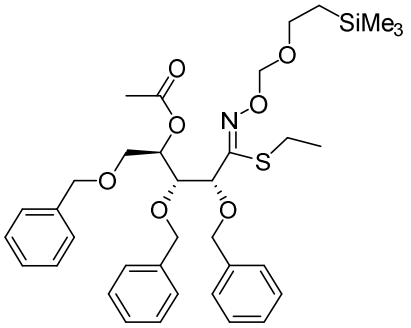
^{1}H-NMR (400MHz,$CDCl_3$) δ(ppm)= 8.54 (s, 1H, NOH); 7.34-7.21 (m, 15H, H_{Ar}); 5.59-5.49 (m, 1H, H-4); 4.66 (d, 1H, *J*=11.0Hz, CH_2Bn); 4.63 (d, 1H, *J*=11.5Hz, CH_2Bn); 4.59 (d, 1H, *J*=11.1Hz, CH_2Bn); 4.46 (d, 1H, *J*=12.1Hz, CH_2Bn); 4.38 (d, 1H, *J*=12.0Hz, CH_2Bn); 4.34 (d, 1H, *J*=11.4Hz, CH_2Bn); 4.33 (d, 1H, $J_{2\text{-}3}$=8.6Hz, H-2); 4.07 (dd, 1H, $J_{3\text{-}2}$=8.6Hz, $J_{3\text{-}4}$=2.9Hz, H-3); 3.68 (dd, 1H, $J_{5\text{-}4}$=6.9Hz, $J_{5a\text{-}5b}$=10.7Hz, H-5_a); 3.62 (dd, 1H, $J_{5\text{-}4}$=4.4Hz, $J_{5b\text{-}5a}$=10.7Hz, H-5_b); 3.02 (m, 2H; S-CH_2); 2.00 (s, 3H, CH_3OAc); 1.18 (t, 3H, *J*=7.42Hz, SCH_2-CH_3)

^{13}C-NMR (100MHz,$CDCl_3$) δ(ppm)= 170.4 (C=O); 152.6 (C=N); 138.1 ($C_q$ar); 137.8 ($C_q$ar); 136.9 ($C_q$ar); 128.4 (CHar); 128.32 (CHar); 128.3 (CHar); 128.24 (CHar); 128.2 (CHar); 127.8 (CHar); 127.7 (CHar); 127.6(CHar); 127.5 (CHar) ; 79.9 (C-2); 78.8 (C-3); 73.9 (CH_2Bn); 72.9 (CH_2Bn); 72.3 (C-4); 70.9 (CH_2Bn); 68.2 (C-5); 25.4 (S-CH_2); 21.1 (CH_3OAc); 15.1 (SCH_2-CH_3).

IR (neat): ν (cm^{-1}) = 3302 (O-H); 3029 (C_{sp2}-H ar); 2870 (C_{sp3}-H); 1740 (C=O); 1587 (SC=N); 1231 (C-O); 696 (S-C)

MS (ESI^+) m/z = 538.3 ($[M+H]^+$)

S-Ethyl-O-(2-(trimethylsilylethoxy)methyl)-(Z)-4-O-acetyl-2,3,5-tri-O-benzyl-D-ribonimidothioate



11
MW=667.30g/mol
$C_{36}H_{49}NO_7SSi$

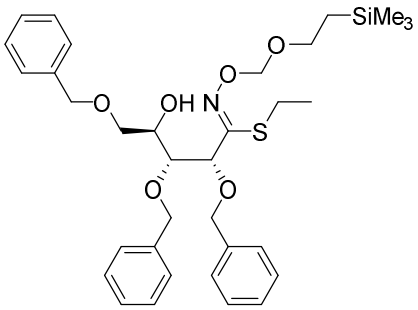
(*Z*)-*S*-Ethyl-4-*O*-acetyl-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate (0.31 g, 0.577 mmol,1 equiv.), was dissolved in anhydrous CH_2Cl_2 (0.1 M, 6 mL), under Argon atmosphere at 0°C. Then iPr_2EtN (1.15 m mol, 0.2 mL, 2 equiv.) and SEMCl (0.98 mmol, 0.17 mL, 1.7 equiv.) were added dropwise. For 10' the mixture was left at 0°C, then reaction was stirred all night at room temperature. The solvent was evaporated under reduced pressure and the product was isolated by silica gel flash chromatographic flash column (EP/EA = 8/2). *S*-ethyl-*O*-(2-(trimethylsilylethoxy)methyl)-(*Z*)-4-*O*-acetyl-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate was obtained as a colorless oil in a 80% yield.

Rf= 0.6 (PE/EA=8/2)

^{1}H-NMR (400MHz,$CDCl_3$) δ(ppm)= 7.44-7.18 (m, 15H, H_{Ar}); 5.60-5.52 (m, 1H, H-4); 5.28 (d, 1H, *J*=7.4Hz, OCH_2O); 5.22 (d, 1H, *J*=7.4Hz, OCH_2O); 4.66 (d; 1H; *J*=10.9Hz; CH_2Bn); 4.63-4.58 (m; 2H; CH_2Bn); 4.45 (d; 1H; *J*=12.0Hz; CH_2Bn); 4.42-4.32 (m; 2H; CH_2Bn); 4.35 (d; 1H; J_{2-3}=2.6Hz; H-2); 4.14 (dd, 1H, J_{3-2}=2.6Hz, J_{3-4}=8.84Hz, H-3); 3.82-3.68 (m, 3H, H-5_a, OCH_2-); 3.62 (dd; 1H; J_{5-4}=4.3, J_{5b-5a}=10.8Hz; H-5_b); 3.05 (m, 2H, SCH_2); 2.03 (s, 3H, CH3OAc); 1.20 (t, 3H, *J*=7.48Hz; SCH_2CH_3); 0.95 (t, 2H, *J*=8.38Hz; Me_3SiCH_2-); 0.01 (s; 9H; Me3Si)

^{13}C-NMR (100MHz,$CDCl_3$) δ(ppm)= 170.2 (C=O); 152.9 (C=N); 138.1 ($C_q$ar); 137.9 ($C_q$ar); 137.1 ($C_q$ar); 128.4 (CHar); 128.3 (CHar); 128.2 (CHar); 128.1 (CHar); 127.8 (CHar); 127.6 (CHar); 127.58 (CHar); 127.53 (CHar); 97.4 (OCH_2O); 79.7 (C-2); 78.6 (C-3); 74.0 (CH_2Bn); 72.9 (CH_2Bn); 72.4 (C-4); 70.6 (CH_2Bn); 68.1 (C-5); 66.4 (OCH_2-); 25.1 (SCH_2-); 21.3 (CH_3OAc); 18.0 (Me_3SiCH_2-); 14.7 (SCH_2CH_3); -1.6 (Me3Si)

S-Ethyl-O-(2-(trimethylsilylethoxy)methyl)-(Z)-2,3,5-tri-O-benzyl-D-ribonimidothioate



13
MW=625.29g/mol
$C_{34}H_{47}NO_6SSi$

S-Ethyl-*O*-(2-(trimethylsilylethoxy)methyl)-(*Z*)-4-*O*-acetyl-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate (0.18 g, 0.27 m mol, 1 equiv.), under Argon atmosphere, was dissolved in dry MeOH(0.1 M, 2.7 mL) and cooled down at 0°C for 10'. Then Na(0.004 g, 0.154 mmol, 0.57 equiv.) was added and reaction was stirred with temperature increasing from 0°C to RT. After 1.5h reaction mixture was quenched by addition of resin Amberlite® IR-H form until neutral pH. After filtration and evaporation under reduced pressure the crude residue was purified by silica gel flash chromatographic column (PE/EA = 9/1 to PE/EA = 8.5/1.5). *S*-Ethyl-*O*-(2-(trimethylsilylethoxy)methyl)-(*Z*)-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate was obtained as a light yellow oil in a 93% yield.

Rf= 0.5 (PE/EA=8/2)

$[\alpha]_D$=+56 (20°C; c = 0.0102 g/100mL in $CHCl_3$)

^{1}H-NMR (400MHz,$CDCl_3$) δ(ppm)= 7.44-7.19 (m, 15H, H_{Ar}); 5.27 (d, 1H, *J*=7.4Hz, OCH_2O); 5.23 (d ,1H, *J*=7.4Hz, OCH_2O); 4.72 (d, 1H, *J*=11.0Hz, CH_2Bn); 4.68 (d, 1H, *J*=11.4Hz, CH_2Bn); 4.66 (d, 1H, *J*=11.4Hz, CH_2Bn); 4.50 (bs, 2H, CH_2Bn); 4.44 (d, 1H, J_{2-3}=7.9Hz, H-2); 4.41 (d, 1H, *J*=11.0Hz, CH_2Bn); 4.14-4.09 (m, 1H, H-4); 4.02 (dd, 1H, J_{3-4}=4.8Hz, J_{3-2}=7.9Hz, H-3); 3.77-3.71 (m, 2H, OCH_2-); 3.63 (dd, 1H, J_{5-4}=6.7Hz, J_{5a-5b}=10.0Hz, H-5_a); 3.55 (dd, 1H, J_{5-4}=3.0Hz, J_{5b-5a}=10.0Hz, H-5_b); 3.15-3.01 (m, 2H, SCH_2); 1.21 (t ,3H, *J*=7.5Hz, SCH_2CH_3); 1.00-0.92 (m, 2H, Me_3SiCH_2-); -0.01 (s, 9H, $SiMe_3$)

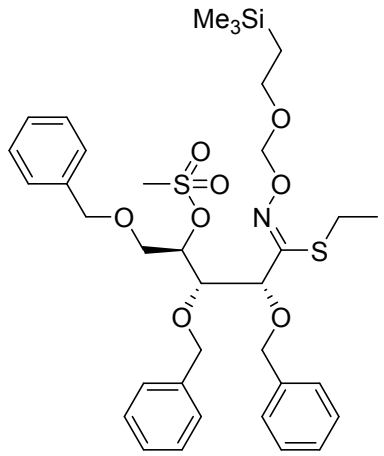
^{13}C-NMR (100MHz,$CDCl_3$) δ(ppm)= 153.2 (C=N); 138.15 ($C_q$ar); 138.12 ($C_q$ar); 136.8 ($C_q$ar); 128.5 (CHar); 128.4 (CHar); 128.3 (CHar); 128.2 (CHar); 128.1 (CHar); 128.0 (CHar); 127.8 (CHar); 127.7 (CHar); 127.6 (CHar); 97.5 (OCH_2O); 80.8 (C-2); 79.6 (C-3);

74.4 (CH_2Bn); 73.5 (CH_2Bn); 71.8 (C-4); 71.1 (CH_2Bn); 70.7 (C-5); 66.5 (OCH_2-); 25.6 (SCH_2-); 18.0 (Me_3SiCH_2-); 14.8 (SCH_2CH_3); -0.47 ($SiMe_3$)

IR (neat): ν (cm^{-1}) = 3466 (OH); 3030 (C_{sp2}-H ar); 2870 (C_{sp3}-H); 1567 (SC=N); 1248 (C-O); 1208 (N-O); 695 (S-C)

MS (ESI^+) m/z = 626.3 ($[M+H]^+$)

S-Ethyl-O-(2-(trimethylsilylethoxy)methyl)-(Z)-2,3,5-tri-O-benzyl-4-O-methanesulfonyl-D-ribonimidothioate



14
MW=703.27g/mol
$C_{35}H_{49}NO_8S_2Si$

S-Ethyl-*O*-(2-(trimethylsilylethoxy)methyl)-(*Z*)-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate (0.17 g, 0.272 mmol, 1 equiv.) was dissolved in anhydrous DCM (0.1 M, 2.7 mL)and cooled down to -50°C with ice-salt bath under Ar atmosphere. Then Et_3N (0.816 mmol, 0.11 mL, 3 equiv.) and MsCl (0.68 m mol, 0.05 mL, 2.5 equiv.) were added. The mixture was stirred until full consumption of the starting material (1h); then the reaction was quenched by addition of ice and water. The aqueous phase was extracted three times with DCM and the combined organic phases were then washed with water and brine. After drying on $MgSO_4$, filtration and evaporation of the solvent, the product was isolated by silica gel flash chromatographic flash column chromatographic (PE/EA = 8/2). *S*-Ethyl-*O*-(2-(trimethylsilylethoxy)methyl)-(*Z*)-2,3,5-tri-*O*-benzyl-4-*O*-methanesulfonyl-*D*-ribonimidothioate was obtained as a light yellow oil in a 85% yield.

$[\alpha]_D$=+51.2 (20°C; c = 0.0099 g/100mL in $CHCl_3$)

Rf= 0.57 (PE/EA=8/2)

^{1}H-NMR (400MHz,$CDCl_3$) δ= 7.43-7.20 (m, 15H, H_{Ar}); 5.30-5.21 (m, 3H, H-4, OCH_2O); 4.77 (d, 1H, *J*=10.9Hz, CH_2Bn); 4.67 (d, 1H, *J*=11.6Hz, CH_2Bn); 4.65 (d, 1H, *J*=10.9Hz, CH_2Bn); 4.42 (bs, 2H, CH_2Bn); 4.37 (d, 1H, *J*=11.6Hz, CH_2Bn); 4.27-4.22 (m, 2H, H-2, H-3); 3.81-3.48 (m, 3H, H-5_a, OCH_2-); 3.52 (dd, 1H, J_{5-4}=2.9Hz, J_{5b-5a}=11.2Hz, H-5_b); 3.16-2.9

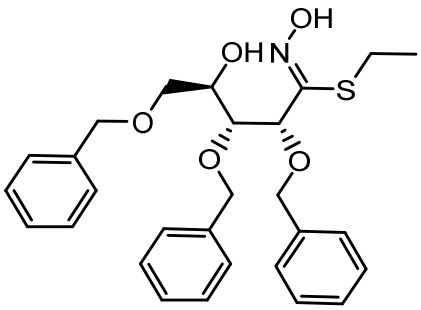
6(m, 2H, SCH_2), 3.00 (s, 3H, SO_2CH_3); 1.21 (t, 3H, *J*=7.1Hz, $SCH_2\mathbf{CH_3}$); 0.99-0.90 (m, 2H, $Me_3Si\mathbf{CH_2}$-); -0.01 (s, 1H, $SiMe_3$)

^{13}C-NMR (100MHz,$CDCl_3$) δ= 152.3 (C-1); 137.6 ($C_q$ar); 137.5 ($C_q$ar); 136.6 ($C_q$ar); 128.51 (CHar); 128.50 (CHar); 128.4 (CHar); 128.3 (CHar); 128.2 (CHar); 128.1 (CHar); 127.8 (CHar); 127.7 (CHar); 127.6 (CHar); 97.4 (-OCH2O-); 82.6 (C-4); 79.5 (C-3); 79.2 (C-2); 74.5 (CH_2Bn); 73.2 (CH_2Bn); 70.5 (CH_2Bn); 68.2 (C-5); 66.4 (-OCH_2-); 38.3 (SO_2CH_3); 25.0 (SCH_2-); 17.8 (Me_3Si-$\mathbf{CH_2}$-); 14.6 (SCH_2-$\mathbf{CH_3}$); -1.6 ($SiMe_3$)

IR (neat): ν (cm^{-1}) = 3031 (C_{sp2}-H ar); 2872.02 (C_{sp3}-H); 1567.15 (SC=N); 1248.35 (C-O); 1208.21 (N-O); 696.23 (S-C)

MS (ESI^+) m/z = 704.4 ($[M+H]^+$)

(Z)-S-Ethyl-2,3,5-tri-O-benzyl-D-ribonimidothioate



10
MW=495.21g/mol
$C_{28}H_{33}NO_5S$

(*Z*)-*S*-Ethyl-4-*O*-acetyl-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate (1.7 g, 3.1 mmol, 1 equiv.) under Argon atmosphere, was dissolved in dry MeOH(0.1M, 31 mL) and cooled down at 0°C for 10'. Then Na(0.04 g, 1.76 mmol, 0.57 equiv.) was added and the reaction was stirred with the temperature increasing from 0°C to RT. After one night the reaction was quenched by addition of resin Amberlyte IR-H until neutral pH. After filtration and evaporation of the solvent (*Z*)-*S*-ethyl-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate was obtained as a colorless oil with a yield of 91%. The product was pure enough to be used without any further purification in the next step.

$[\alpha]_D$=+83 (20°C; c = 0.0097 g/100mL in $CHCl_3$)

R_f=0.3 (PE/EA=8/2)

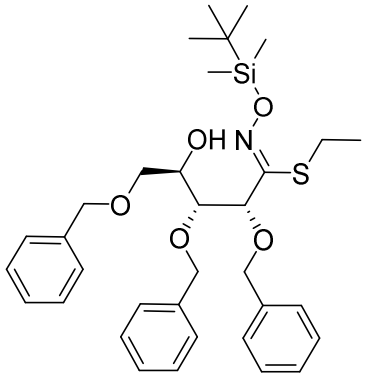
^{1}H-NMR (400MHz,$CDCl_3$) δ= 8.17 (s, 1H, NOH); 7.48-7.21 (m, 15H, H_{Ar}); 4.75 (d, 1H, J=11.0Hz, CH_2Bn); 4.66 (d, 1H, J=11.3Hz, CH_2Bn); 4.64 (d, 1H, J=11.3Hz, CH_2Bn); 4.49 (s, 2H, CH_2Bn); 4.42 (d, 1H, J=7.1Hz, H-2); 4.39 (d, 1H, J=11.6Hz, CH_2Bn); 4.12-4.04 (m, 1H, H-4); 3.96-3.91 (m, 1H, H-3); 3.61-3.54 (m, 2H, H-5); 3.18 (s, 1H, OH); 3.09 (q, 2H; *J*=7.5Hz, SCH_2); 1.21 (t, 3H, *J*=7.5Hz, SCH_2-CH_3)

^{13}C-NMR (100MHz,$CDCl_3$) δ= 152.3 (C=N); 137.9 ($C_q$ar); 137.8 ($C_q$ar); 137.1 ($C_q$ar); 128.5 (CHar); 128.4 (CHar); 128.3 (CHar); 128.16 (CHar); 128.15 (CHar); 127.9 (CHar); 127.7 (CHar); 127.6 (CHar); 81.0 (C-2); 79.9 (C-3); 74.5 (CH_2Bn); 73.5 (CH_2Bn); 71.3 (CH_2Bn); 71.2 (C-4); 71.1 (C-5); 25.8 (SCH_2); 15.2 (CH_3)

IR (neat): ν (cm^{-1}) = 3281 (OH); 3029 (C_{sp2}-H ar); 2867 (C_{sp3}-H); 1603 (SC=N); 1257 (C-O); 1208 (N-O); 695 (S-C)

MS (ESI$^+$) m/z = 496.3([M+H]$^+$)

S-Ethyl-O-tertbutyldimethylsilyl-(Z)-2,3,5-tri-O-benzyl-D-ribonimidothioate



12
MW=609.29g/mol
$C_{34}H_{47}NO_5SSi$

(*Z*)-*S*-Ethyl-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate (0.3 g, 0.605 mmol, 1 equiv.) was dissolved in anhydrous CH_2Cl_2 (0.1 M, 6.05 mL); at 0°C imidazole (0.08 g, 1.21 mmol, 2 equiv.) and TBDMSCl (0.14 g, 0.307 mmol, 1.5 equiv.) were added. The reaction was stirred at RT for 2h, then it was quenched by addition of water. The aqueous phase was extracted with DCM for three times and then the combined organic phases were washed with water and brine. After drying on $MgSO_4$, filtration and evaporation of the solvent, the mixture was purified by silica gel flash chromatographic column (PE/EA = 9/1). *S*-ethyl-*O*-tertbutyldimethylsilyl-(*Z*)-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate was obtained as a colorless oil in a 70% yield.

$[\alpha]_D$=+69 (20°C; c = 0.0103 g/100mL in $CHCl_3$)

R_f=0.2 (PE/EA=9/1)

^{1}H-NMR (400MHz,$CDCl_3$) δ(ppm)= 7.28 (m, 15H, H_{Ar}); 4.67 (d, 1H, *J*=11.0Hz, CH_2Bn); 4.65 (d, 1H, *J*=11.2Hz, CH_2Bn); 4.62 (d, 1H, *J*=10.8Hz, CH_2Bn); 4.54-4.46 (m, 2H, CH_2Bn); 4.40 (d, 1H, $J_{2\text{-}3}$=7.8Hz, H-2); 4.36 (d, 1H, *J*=11.4Hz, CH_2Bn); 4.10-4.04 (m, 1H, H-4); 4.00 (dd, 1H, $J_{3\text{-}4}$=7.4Hz, $J_{3\text{-}2}$=7.8Hz, H-3); 3.62 (dd, 1H, $J_{5\text{-}4}$=6.4Hz, $J_{5a\text{-}5b}$=10.0Hz, H-5_a); 3.55 (dd, 1H, $J_{5\text{-}4}$=2.9Hz, $J_{5b\text{-}5a}$=10.0Hz, H-5_b); 3.15-3.10 (m, 2H, S-CH_2); 2.94 (d, 1H, *J*=3.3Hz,OH); 1.2 (t, 3H, *J*=7.4Hz, SCH_2-**CH_3**); 0.97 (s, 9H, Si-*t*Bu); 0.21 (s, 3H, $SiCH_3$); 0.18 (s, 3H, $SiCH_3$)

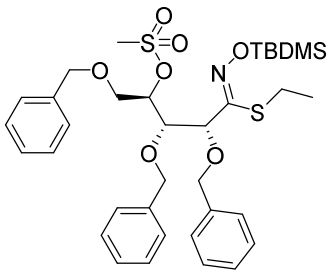
^{13}C-NMR (100MHz,$CDCl_3$) δ= 155.7 (C=N); 138.2 ($C_q$ar); 138.1 ($C_q$ar); 137.0 ($C_q$ar); 128.4 (CHar); 128.35 (CHar); 128.31 (CHar); 128.2 (CHar); 127.9 (CHar); 127.8 (CHar);

127.6 (CHar); 127.5 (CHar); 81.1 (C-2); 79.6 (C-3); 74.3 (CH_2Bn); 73.4 (CH_2Bn); 72.0 (C-4); 70.9 (CH_2Bn);70.7 (C-5); 26.1 (CH_3-tBu); 25.3 (SCH_2); 18.1 (C_qtBu); 15.0 (SCH_2-**CH_3**)

IR (neat): ν (cm^{-1}) = 3030 (C_{sp2}-H ar); 2856 (C_{sp3}-H); 1561 (SC=N); 1250 (C-O); 1208 (N-O); 695 (S-C)

MS (ESI^+) m/z = 610.4 ($[M+H]^+$)

S-Ethyl-O-tertbutyldimethylsilyl-(Z)-2,3,5-tri-O-benzyl-4-O-methanesulfonyl-D-ribonimidothioate



15
MW=687.27g/mol
$C_{35}H_{49}NO_7S_2Si$

S-Ethyl-*O*-tertbutyldimethylsilyl-(*Z*)-2,3,5-tri-*O*-benzyl-*D*-ribonimidothioate (0.648 g; 1.06 mmol; 1 equiv.) was dissolved in anhydrous DCM (0.1M; 10.6 mL) under Argon atmosphere and cooled down in ice-salt bath. Et_3N (3.18 mmol; 0.44 mL;3 equiv.) and then MsCl (2.65 mmol; 0.21 mL; 2.5 equiv.) were added. The reaction was stirred at RT overnight and then quenched by addition of ice and water. The aqueous phase was extracted with DCM for three times and then the combined organic phases were washed with water and brine. After drying over $MgSO_4$, filtration and evaporation of the solvent, the mixture was purified by silica gel flash chromatographic column (PE/EA = 9/1 to 5/5). Purification gave the desired *S*-ethyl-*O*-*tert*butyldimethylsilyl-(*Z*)-2,3,5-tri-*O*-benzyl-4-*O*-methanesulfonyl-*D*-ribonimidothioate in a 77% yield.

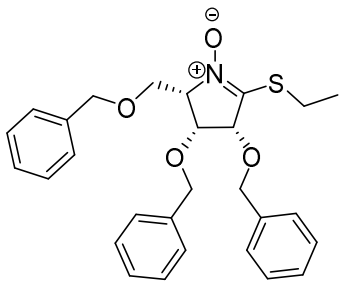
R_f=0.3 (PE/EA=9/1)

^{1}H-NMR (400MHz,$CDCl_3$) δ(ppm)= 7.40-7.25 (m, 15H, H_{Ar}); 5.28-5.23 (m, 1H, H-4); 4.75 (d, 1H, *J*=11.0Hz, CH_2Bn); 4.62 (d, 1H, *J*=11.3Hz, CH_2Bn); 4.58 (d, 1H, *J*=11.0Hz, CH_2Bn); 4.42 (d, 1H, *J*=12.4Hz, CH_2Bn); 4.38 (d, 1H, *J*=12.4Hz, CH_2Bn); 4.31 (d, 1H, *J*=11.6Hz, CH_2Bn); 4.28-4.20 (m, 2H, H-3, H-2); 3.79-3.72 (m, 1H, H-5_a); 3.54-3.48 (m, 1H, H-5_b); 3.10-3.02 (m, 2H, SCH_2); 2.96 (s, 3H, SO_2CH_3); 1.21 (t, 3H, *J*=8.4Hz, $SCH_2\mathbf{CH_3}$); 0.96 (s, 9H, Si*t*Bu); 0.20 (s, 3H, SiMe); 0.19 (s, 3H, $SiMe_3$).

^{13}C-NMR (100MHz,$CDCl_3$) δ= 154.8 (C=N); 137.7 ($C_q$ar); 137.5 ($C_q$ar); 136.7 ($C_q$ar); 128.6 (CHar); 128.44 (CHar); 128.42 (CHar); 128.2 (CHar); 128.0 (CHar); 127.8 (CHar); 127.7 (CHar); 127.6 (CHar); 82.7 (C-4); 79.6 (C-3); 79.2 (C-2); 74.5 (CH_2Bn); 73.2 (CH_2Bn);

70.3 (CH_2Bn); 68.3 (C-5); 38.5 (SO_2CH_3); 26.2 (*t*Bu); 25.1 (SCH_2-); 15.2 (SCH_2CH_3); -5.01 ($SiMe_3$)

S-Ethyl-2,3,5-tri-O-benzyl-4-deoxy-L-lyxono-1,4-imidothioate-N-oxide



16
MW=477.20g/mol
$C_{28}H_{31}NO_4S$

S-Ethyl-*O*-tertbutyldimethylsilyl-(*Z*)-2,3,5-tri-*O*-benzyl-4-*O*-methanesulfonyl-*D*-ribonimidothioate (0.2 g; 0.29 mmol; 1 equiv.) was dissolved in anhydrous CH_3CN(0.1M; 2.9 mL); then TBAT (0.17 g; 0.319 mmol; 1.1 equiv.) and molecular sieves (3Å) were added. The reaction mixture was heated at reflux under Argon atmosphere for 4h. Water was added and the aqueous layer was extracted three times with EA. Then the combined organic layers were washed with water and brine, dried over $MgSO_4$ and then the solvent was evaporated. Purification by chromatographic column (EP/EA=5/5 to 2/8) gave the desired *S*-ethyl-2,3,5-tri-*O*-benzyl-4-deoxy-*L*-lyxono-1,4-imidothioate-*N*-oxide with a yield of 72%.

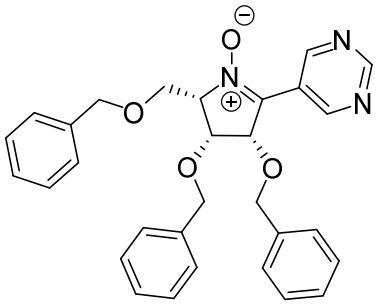
Rf = 0.25 (PE/EA=2/8)

^{1}H-NMR (250MHz,$CDCl_3$) δ(ppm)= 7.37-7.20 (m, 15H, H_{Ar}); 4.89 (d, 1H, *J*=11.3Hz, CH_2Bn) ; 4.81 (d, 1H, *J*=11.6Hz, CH_2Bn); 4.72 (d, 1H, $J_{2\text{-}3}$=6.1Hz, H-2); 4.69 (d, 1H, *J*=11.6Hz, CH_2Bn); 4.67 (d, 1H, *J*=11.3Hz, CH_2Bn); 4.63 (d, 1H, *J*=11.8Hz, CH_2Bn); 4.57 (d, 1H, *J*=11.8Hz, CH_2Bn); 4.40 (t, 1H, $J_{3\text{-}2}$=$J_{3\text{-}4}$= 6.1Hz, H-3); 4.30-4.27 (m, 1H, H-4); 4.30-4.24 (m, 1H, H-5_a); 4.05 (dd, 1H, $J_{5\text{-}4}$=3.9Hz, $J_{5b\text{-}5a}$=10Hz, H-5_b); 3.01-2.85 (m, 2H, S-CH_2-); 1.23 (t, 3H, *J*=6.9Hz, S-CH_2**CH_3**)

^{13}C-NMR (100MHz,$CDCl_3$) δ= 142.2 (C=N); 137.9 ($C_q$ar); 137.4 ($C_q$ar); 137.3 ($C_q$ar); 128.5 (CHar); 128.4 (CHar); 128.3 (CHar); 128.1 (CHar); 128.0 (CHar); 127.9 (CHar); 127.7 (CHar); 127.6 (CHar); 78.7 (C-2); 74.8 (C-4); 74.1 (C-3); 73.4 (CH_2Bn); 73.1 (CH_2Bn); 72.8 (CH_2Bn); 67.2 (C-5); 23.9 (SCH_2-); 15.5 (SCH_2**CH_3**)

HRMS (ESI^+): m/z for $C_{28}H_{32}NO_4S$ ($[M+H]^+$): 478.2000 found 478.2042

1,4-Anhydro-2,3,5-tri-O-benzyl-1,4-dideoxy-1-imino-1-(5-pyrimidiny)-L-lyxitol-N-oxide



17
MW=495.22g/mol
$C_{30}H_{29}N_3O_4$

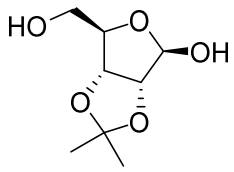
S-Ethyl-2,3,5-tri-*O*-benzyl-4-deoxy-*L*-lyxono-1,4-imidothioate-*N*-oxide (0.065 g, 0.136 mmol, 1equiv.) was dissolved in anhydrous degassed THF (0.1 M, 1.36 mL). Under Argon atmosphere copper(I) 3-methylsalicylate (0.064 g, 0.299 mmol , 2.2equiv.) and pyrimidine-5-boronic acid (0.037 g, 0.299 mmol, 2.2 equiv.) were added in the reaction flask. The previous solution of starting material in THF was added and the reaction mixture was evacuated and backfilled with dry Argon for three times. Then the catalyst $Pd(PPh_3)_4$ ($7.86*10^{-3}$ g, $6.8*10^{-6}$ mol, 5%mol) was added. The reaction mixture was heated under reflux for 24h and then the solvent was evaporated. The desired product was obtained after purification by silica gel column chromatography (PE/EA=3/7) but it was contaminated with triphenylphosphine oxide. This reaction was just a test, it was performed with a very little amount of reagent so it was not possible determining the yield of the product.

^{1}H-NMR: (400MHz,$CDCl_3$) δ(ppm)= 9.39 (s, 1H, H_{pyr}); 9.11 (s, 1H, H_{pyr}); 7.35-7.16 (m, 15H, H_{Ar}); 6.96 (s, 1H, H_{pyr}); 5.05 (d, 1H, $J_{2\text{-}3}$=5.2Hz, H-2); 4.92 (d, 1H, *J*=11.1Hz, CH_2Bn) ; 4.80 (d, 1H, *J*=11.5Hz, CH_2Bn); 4.70-4.59 (m, 3H, CH_2Bn); 4.55 (d, 1H, *J*=11.6Hz, CH_2Bn); 4.5-4.46 (m, 1H, H-3); 4.45-4.38 (m, 1H, H-4); 4.23 (dd, 1H, $J_{5\text{-}4}$=6.4Hz, $J_{5a\text{-}5b}$=10.1Hz, H-5_a); 4.14-4.05 (m, 1H, H-5_b)

^{13}C-NMR (100MHz,$CDCl_3$) δ= 154.8 (C_{pyr}); 136.76 (C_{pyr}) 132.1 ($C_q$ar); 132.02 ($C_q$ar); 132.0 ($C_q$ar); 131.9 ($C_q$ar); 128.66 (CHar); 128.63 (CHar); 128.58 (CHar); 128.46 (CHar); 128.44 (CHar); 128.40 (CHar); 128.0 (CHar); 127.9 (CHar); 77.2 (C-2); 75.8 (C-4); 73.7 (CH_2Bn); 73.44 (C-3); 73.4 (CH_2Bn); 73.3 (CH_2Bn); 67.5 (C-5)

MS (ESI^+) m/z = 496.3 ($[M+H]^+$)

2,3-O-Isopropylidene-β-D-ribofuranose

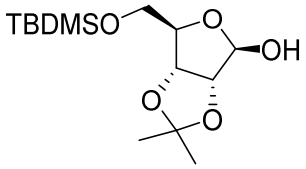


19
MW=190.08g/mol
$C_8H_{14}O_5$

Conc. H_2SO_4 (2.42 mmol, 0.13 mL, 0.07 equiv.) was added to a slurry of *D*-ribose (5 g, 0.035 mol,1 equiv.) in dry acetone (0.7 M, 52 mL) at RT. A clear solution was obtained after 30' and stirring was continued for 1h. When all the *D*-ribose was reacted, the pH of the solution was adjusted to 7 by addition of $Ca(OH)_2$. The resulting salts were removed by filtration through a pad of celite and the clear filtrate was evaporated under reduced pressure to afford a light yellow viscous oil (6.65 g), which was used without any further purification in the next step.

^{1}H-NMR (250MHz,CD_3OD) δ (ppm) = 5.39 (bs, 1H, H-1); 4.82 (d, 1H, J_{2-3}=5.9Hz, H-2); 4.56 (d, 1H, J_{3-2}=5.9Hz, H-3); 4.4-4.38 (m, 1H, H-4); 3.79-3.68 (m, 2H, H-5,); 1.45 (s, 3H, CH_3); 1.29 (s, 3H, CH_3)

2,3-O-Isopropylidene-5-O-tertbutyldimethylsilyl-β-D-ribofuranose



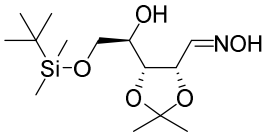
20
MW=304.17g/mol
$C_{14}H_{28}O_5Si$

To a solution of 2,3-*O*-isopropyldiene-β-*D*-ribofuranose (6.65 g, 0.035 mol, 1 equiv.) and imidazole (6.67 g ,0.098 mol, 2.8 equiv.) in anhydrous DMF (2.6 M, 13.3 mL) was added TBDMSCl (5.73 g, 0.038 mol, 1.1 equiv.) in one portion. The resulting solution was stirred for 2h and it was subsequently diluted in H_2O. The aqueous layer was extracted three times with EtOAc. The combined extracts were washed with water and dried over $MgSO_4$. The solvent was evaporated and the thick syrup was purified by silica gel flash column chromatographic (EP/EA=9/1 to 8/2). The product was obtained with a yield of 59% (α/β=20/80).

Rf = 0.6 (PE/EA=7/3)

^{1}HNMR (250MHz,$CDCl_3$) δ(ppm) = 5.30 (d, 1H, $J_{1\text{-}OH}$=11.7Hz, H-1); 4.76 (d, 1H, $J_{OH\text{-}1}$=11.6Hz, OH); 4.72 (d, 1H, $J_{2\text{-}3}$=5.8Hz, H-2); 4.53 (d, 1H, *J*=5.9Hz, H-3); 4.40-4.36 (m, 1H, H-4); 3.8 (dd, 1H, $J_{5\text{-}4}$=2.1Hz, $J_{5a\text{-}5b}$=11.0Hz, H-5_a); 3.76 (dd, 1H, $J_{5\text{-}4}$=2.5Hz, $J_{5b\text{-}5b}$=11Hz, H-5_b) ; 1.51 (s, 3H, $C(CH_3)_2$); 1.35 (s, 3H, $C(CH_3)_2$); 0.95 (s, 9H, $SiC(CH_3)_3$); 0.17 (s, 3H, $SiCH_3$); 0.16 (s, 3H, $SiCH_3$)

2,3-O-Isopropylidene-5-O-tertbutyldimethylsilyl-D-ribofuranose oxime



21
MW=319018g/mol
$C_{14}H_{29}NO_5Si$

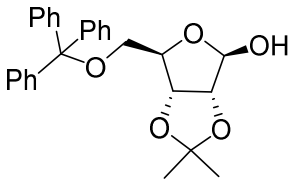
Starting material (6.3 g, 0.021 mol, 1 equiv.) was dissolved in dry pyridine (70 mL,0.3 M) under Argon atmosphere. Then NH_2OH*HCl (4.3 g,0.062 mol, 3 equiv.) was added and then two spoons of 3A molecular sieves. The reaction mixture was stirred at RT for 2h then filtered through a pad of celite and the solvent evaporated under reduced pressure. The aqueous phase was extracted with EtOAc three times and then all the organic layers were washed with water and brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by silica gel flash chromatographic column (PE/EA=7/3). The product was obtained with a yield of 90% as a mixture of the two isomers Z and E (E/Z=80/20).

Rf = 0.26 (PE/EA=7/3)

^{1}H-NMR (400MHz,CD_3OD) δ(ppm), data for the major E isomer = 7.84 (bs, 1H, NOH); 7.50 (d, 1H, $J_{1\text{-}2}$=7.3Hz, H-1); 4.78 (dd, 1H, $J_{2\text{-}3}$=6.3Hz, $J_{2\text{-}1}$=7.6Hz, H-2); 4.18-4.10 (m, 1H, H-3); 3.85-3.65(m, 3H, H-5_a, H-5_b, H-4); 2.81 (bs, 1H, OH); 1.48 (s, 3H, $C(CH_3)_2$); 1.37 (s, 3H, $C(CH_3)_2$); 0.91 (s, 9H, Si-*t*Bu); 0.08 (s, 6H, $Si(CH_3)_2$)

^{13}CNMR (100MHz,$CDCl_3$) δ(ppm)= 148.5 (C-1); 109.8 (Cq isopr); 77.2 (C-3); 75.2 (C-2); 69.3 (C-4); 64.3 (C-5); 27.8 (CH_3 isopr); 25.39 (CH_3 isopr); 25.9 (CH_3 *t*bu); -5.81 ($Si(CH_3)_2$)

2,3-O-Isopropylidene-5-O-triphenylmethyl-β-D-ribofuranose



25
MW=432.19g/mol
$C_{27}H_{28}O_5$

2,3-*O*-isopropylidene-β-*D*-ribofuranose (5 g, 0.026 mol, 1 equiv.) was dissolved in dry pyridine (0.45 M, 58 mL); then triphenyl methyl chloride (8.79 g,0.031 mol,1.2 equiv) was added. The mixture, under Argon atmosphere, was heated under reflux for one night. Then the pyridine was coevaporated with toluene. The product was used without any further purification in the next step.

^{1}H-NMR(250MHz,$CDCl_3$) δ(ppm)= 7.37-7.17 (m, 15H, H_{Ar}); 5.30 (d, 1H, *J*=9.1Hz, H-1); 4.76 (d, 1H, J_{2-3}=5.5Hz, H-2); 4.63 (d, 1H, J_{3-2}=5.9Hz, H-3); 4.36-4.29 (m, 1H, H-4); 3.4 (dd, 1H, J_{5-4}=3.6Hz ,J_{5a-5b}=10.3Hz, H-5_a); 3.3 (dd, 1H, J_{5-4}=3.7Hz, J_{5b-5a}=10.3Hz, H-5_b); 3.84 (d, 1H, J_{OH-1}=9.2, OH); 1.4 (s, 3H, CH_3); 1.31 (s, 3H, CH_3)