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Synthesis of novel potentially bioactive Pseudo-*C*-Nucleosides for the treatment or control of Bipolar Disorder

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Dissertação para obtenção do Grau de Mestre em
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Dedico este trabalho à minha família, aos que me acompanharam neste processo e aos que, infelizmente, não estão cá para ver os resultados.

<u>Olhar o invisível e o sonho</u>

Rebusco o tempo na linha que imaginei,
Pensei estar e nada encontrei; um vazio!
Foi tempo de conspiração no Olimpo
Que envolveu em névoa o intelecto sano.

O ar que rasguei no pulsar da vida sem dano
Foi triturado por Hefesto na raiva, dor e afinco,
Temperado por Hermes num tranquilo rio,
Límpido da névoa que já não vejo e, ... olvidei!

Esse Apolo que desceu na noite escura
Encabrestando a Fênix do pensamento;
Que moldou o sonho humano na procura
E a vontade em força de discernimento.

Enfuna jubilosos semblantes na descoberta
E envolve os sentidos na doce ambição.
Cumprido o dever mesmo que não se acerta,
Por ter seguido, O dão, no sublime da criação!

Manuel Baptista

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Maria Gomes de Pinho Baptista

Abstract

Carbohydrates are natural aliphatic polyhydroxy aldehydes and ketones with an empirical formula of $C_n(H_2O)_n$ that are distributed throughout our planet. This class of compounds have a central role in numerous physiological events, such as in cellular communication, and are part of the basic structure of some endogenous biomolecules. It also presents several chiral carbons that confers them stereogenic centres, which is an advantage for the selective interaction with the biological targets. In fact, carbohydrates are considered privileged scaffolds and can be linked to several pharmacophores, such as heterocycles, forming pseudo-*C*-nucleosides, which are composed by a heterocyclic ring attached to the carbon 4 of the furanose ring by a carbon-carbon bond. Therefore, they are largely used at the therapeutic level in the treatment of several illnesses. As an important example the association between carbohydrates and the treatment of bipolar disorder occurred through the discovery of an anticonvulsive compound, topiramate.

Bipolar Disorder (BD), also known as manic-depressive illness, affects between 1 to 4% of the world's population and is characterised by recurrent mood changes, named manic or depressive episodes. In a manic episode, the patient is extremely euphoric, while in depressive episodes the patient feels depressed and may present suicidal tendencies. Currently, the most effective drug on the market for the treatment of this disorder is Lithium Carbonate. However, this inorganic compound has very significant adverse effects. Despite a large group of studies that have been developed in this context, it has not yet been possible to clearly identify the pathological basis of this disease as well as the mechanism of action of Lithium Carbonate.

Thus, as presently there is a clear lack of available pharmacological alternatives for the treatment of this disease, the objective of this dissertation was to synthesise pseudo-*C*-nucleosides with potential interest for the treatment of BD and subsequent evaluation of cytotoxicity.

For this purpose, three different techniques to synthesise the pseudo-*C*-nucleosides were performed, the Click Chemistry, the cyclization to form pyrazolidin-3-ones and the cyclization to form 5-hydroxypyrazoles. From these three strategies seven different compounds, six of which are pyrazolidin-3-one derivatives and one 5-hydroxypyrazole, have been obtained.

Finally, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assays were performed to evaluate the cytotoxicity of the final compounds synthesised in two different cell lines, NHDF (Normal Human Dermal Fibroblasts) and N27 (Rat Mesencephalic Dopaminergic Neural Cells). In both cell lines, the compounds under study did not show relevant cytotoxicity. Thus, even if it was not possible to infer their potential for the treatment of BD, the results obtained for cytotoxicity are positive indicators to continue to develop tests to evaluate the activity of this compound for the treatment of this disorder.

Keywords

Resumo Alargado

Os açúcares são compostos naturais que se encontram disponíveis no nosso planeta. Estes compostos são constituídos por uma cadeia carbonada alifática com um grupo carbonilo, na forma de cetona ou de aldeído, e com um grupo hidroxilo ligado a cada um dos restantes carbonos. Acrescem ainda os compostos obtidos por reações de oxidação e de redução. São considerados derivados de glúcidos os compostos obtidos por substituição de um grupo hidroxilo por um halogéneo, um hidrogénio, uma cadeia carbonada, entre outras modificações.

Esta classe de compostos começou a ser estudada no século XVIII, mas a maior parte dos avanços de elucidação estrutural e funcional só aconteceram no século XIX, com os estudos desenvolvidos por Emil Fischer. No entanto, só a partir do século XX, e com os avanços da ciência e da tecnologia é que se conseguiu estudar esta classe de compostos no seu expoente máximo. Consequentemente, foram descobertos inúmeros processos fisiológicos nos quais os açúcares ou os seus derivados participavam e, por isso, começou a tentar-se desenvolver compostos derivados de glúcidos com propriedades farmacológicas. Este interesse surgiu, naturalmente, não só pela função dos glúcidos no organismo mas também devido à sua estrutura, que evidencia um elevado número de carbonos quirais e que, consequentemente, tem um elevado número de centros estereogénicos que permitem a interação seletiva com alvos biológicos. Contudo, devido à quantidade de grupos hidroxilo livres, os açúcares, por si só, não são moléculas adequadas para serem utilizados como fármacos, por se revelarem demasiado polares, e, portanto, as suas propriedades farmacocinéticas não são as mais apropriadas, tendo, por isso, de ser objeto de modificações estruturais. Por todos estes motivos, os açúcares são considerados *scaffolds* privilegiados e podem ser ligados a diferentes farmacóforos, como por exemplo anéis heterocíclicos, formando nucleósidos e seus derivados. Dentro dos derivados dos nucleósidos, estão incluídos os pseudo-*C*-nucleósidos, que são constituídos por um anel heterocíclico que se encontra ligado ao carbono 4 do anel de furanose através de uma ligação carbono-carbono.

Atualmente, já se encontram disponíveis vários fármacos com propriedades farmacológicas completamente distintas que têm na sua base estrutural um derivado de açúcar. Dentro das classes de fármacos representadas por, pelo menos, um composto glucídico estão os anticancerígenos, os antivirais, os agentes anti-Alzheimer e os anticonvulsivantes. Um composto importante que pertence à classe dos anticonvulsivantes é o topiramato. Este é constituído por um anel de piranose, com dois grupos isopropilideno, um ligado na posição 2 e 3 e outro na posição 4 e 5, e um grupo sulfamida ligado ao carbono 1. O topiramato bloqueia canais de sódio, potencia a ação do neurotransmissor inibitório GABA (ácido γ-aminobutírico) e é considerado um antagonista do neurotransmissor excitatório glutamato por atuação nos recetores AMPA (ácido α-amino-3-hidroxi-5-metil-4-isoxazolepropiónico) e cainato. Neste âmbito já existem estudos que confirmam a atividade deste composto no tratamento do

transtorno bipolar (BD, do inglês, *Bipolar Disorder*), sendo este um ponto-chave do presente trabalho de investigação.

O BD, também conhecido como transtorno maníaco-depressivo, afeta entre 1 a 4% da população mundial e carateriza-se por mudanças de humor recorrentes, denominadas como crises maníacas ou crises depressivas. As crises maníacas são, normalmente, fases em que a pessoa se apresenta extremamente eufórica em que o tratamento pode ser efetuado através de fármacos antipsicóticos, enquanto que as crises depressivas são fases em que a pessoa se sente deprimida, podendo, inclusivamente, apresentar tendências suicidas, e em que o tratamento pode ser efetuado através de fármacos antidepressivos.

Atualmente, o fármaco mais eficaz que se encontra disponível no mercado para o tratamento deste transtorno é o Carbonato de Lítio. Este é um composto inorgânico e origina efeitos adversos muito significativos. Quando o tratamento em monoterapia não resulta, este fármaco pode ser combinado com o ácido valpróico ou com agentes antioxidantes. Contudo, este tipo de estratégia não é a mais adequada, uma vez que cerca de 37% dos pacientes medicados têm, pelo menos, uma crise de mania ou de depressão em menos de 1 ano, elevando-se para cerca de 60% em menos de 2 anos. Efetuando uma análise objetiva dos dados disponíveis, poder-se-á concluir que os fármacos que se encontram disponíveis no mercado são inefetivos contra este transtorno.

Apesar dos estudos que se têm desenvolvido, ainda não foi possível identificar claramente as bases fisiopatológicas do BD. Contudo, sabe-se que existem alterações a nível de atividade de determinadas proteínas, sendo que a mais marcada é a hiperatividade na família das GSK-3 (Glicogénio Sintase Cinase 3), particularmente da GSK3B. Esta enzima é conhecida por estar envolvida no metabolismo energético celular e no desenvolvimento neuronal e, portanto, a sua hiperatividade desencadeia uma cascata de sinalização celular que pode originar danos neuronais irreparáveis e, inclusivamente, ser causa de morte neuronal.

Apesar de ser ainda parcialmente desconhecido, tem-se assumido que o mecanismo de ação do Carbonato de Lítio se baseia no desencadeamento de múltiplos sinais e cascatas intracelulares que ocorrem através da inibição ou da ativação de recetores membranares. A principal via de atuação envolve a inibição dos canais de cálcio associados aos recetores NMDA (*N*-metil-D-aspartato), que são recetores do neurotransmissor excitatório glutamato. A inibição destes recetores, neste caso, permite proteger, a nível neuronal, o cérebro da excitotoxicidade[1] provocada pelo excesso deste neurotransmissor.

Atualmente, por falta de alternativas eficazes, a indústria farmacêutica tem vindo a utilizar técnicas de HTS (do inglês, *High Throughput Screening*) para avaliar fármacos comercializados para outras doenças do sistema nervoso central, tais como a esquizofrenia e a epilepsia, na

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[1] Excitotoxicidade: capacidade do Glutamato e de outros compostos relacionados danificarem os neurónios como consequência da ativação excessiva dos recetores de glutamato.

tentativa de encontrar compostos potencialmente bioativos destinados ao tratamento do BD. Os resultados têm sido promissores, particularmente no caso dos fármacos utilizados para o tratamento da epilepsia, que têm demonstrado maior eficácia, uma vez que a maior parte deles também atua em vias igualmente relevantes no BD. Como já referido, um dos exemplos mais relevantes neste contexto é o fármaco glucosídico topiramato.

Assim sendo, existe clara necessidade de alargar as alternativas farmacológicas disponíveis para o tratamento desta doença, contribuindo para a melhoria da qualidade de vida daqueles que padecem desta doença. Além disso, estruturas glicosídicas com ação anticonvulsivante, podem ser um bom ponto de partida no desenvolvimento de compostos com esta atividade farmacológica. Desta forma, o objetivo desta dissertação foi sintetizar pseudo-*C*-nucleósidos, com potencial atividade para o tratamento do transtorno bipolar e posterior avaliação da citotoxicidade.

Assim, tendo em conta a estrutura base do topiramato, foram realizadas alterações estruturais que incluíram a passagem do anel glucídico de piranose para furanose e a ligação de anéis heterocíclicos em vez de uma cadeia carbonada. Os triazóis, as pirazolidin-3-onas e os 5-hidroxipirazóis foram os heterociclos escolhidos para serem ligados aos açúcares. A pertinência da escolha destes substituintes é justificada pela presença de átomos de azoto na sua estrutura, que está descrita na literatura como vantajosa para a atividade biológica requerida, bem como na, já existente, atividade reportada que as mesmas estruturas apresentam a nível de doenças neuropsiquiátricas.

Para a ligação do anel triazol ao açúcar, a técnica utilizada foi a *Click Chemistry*. No entanto, apesar de todas as tentativas realizadas não foi possível sintetizar nenhum composto através desta via. Foram utilizadas diversas estratégias, incluindo a proteção do grupo hidroxilo da posição 3 do anel de açúcar por três grupos protetores diferentes, benzilo, metilo e acetilo, bem como duas modificações estruturais que consistiram na substituição do grupo hidroxilo por iodo, num caso, e por hidrogénio no outro.

Para a síntese do derivado pirazolidin-3-ona, foi efetuada uma ciclização de um éster α, β-insaturado com a hidrazina monohidratada. Com este anel foram sintetizados dois conjuntos de três compostos, um par de isómeros, o *S* e o *R*, e um tautómero hidratado do heterociclo.
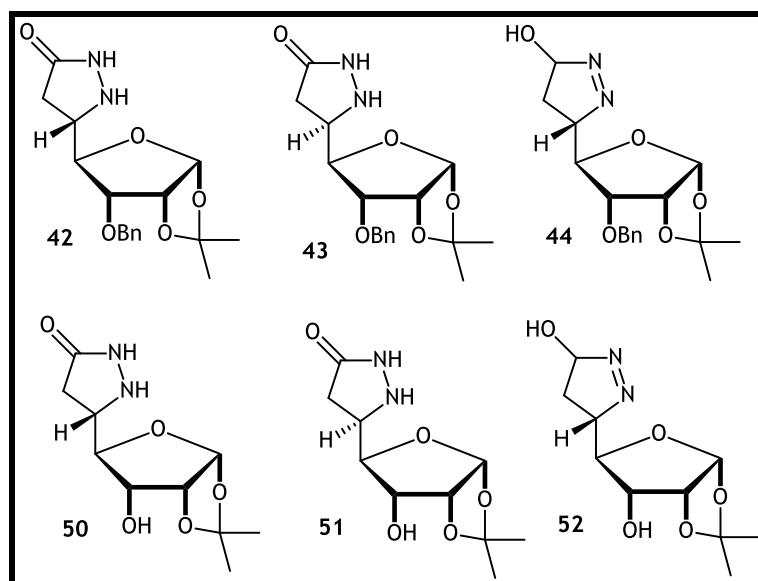
Figura i. Conjunto de compostos sintetizados por ciclização sob a forma de pirazolidin-3-ona.

Na figura i, estão representados na fila de cima os derivados de 5-(3-*O*-benzil-1,2-*O*-isopropilideno-α-D-eritrofuranos-4-*C*-il)-pirazolidin-3-ona e na de baixo de 5-(1,2-*O*-isopropilideno-α-D-eritrofuranos-4-*C*-il)-pirazolidin-3-ona. Em ambos os casos, os compostos mais à esquerda correspondem ao isómero **S**, denominados por 5-(*S*)-(3-*O*-benzil-1,2-*O*-isopropilideno-α-D-eritrofuranos-4-*C*-il)-pirazolidin-3-ona (**42**) e 5-(*S*)-(1,2-*O*-isopropilideno-α-D-eritrofuranos-4-*C*-il)-pirazolidin-3-ona (**50**), os do centro ao isómero **R**, denominados por 5-(*R*)-(3-*O*-benzil-1,2-*O*-isopropilideno-α-D-eritrofuranos-4-*C*-il)-pirazolidin-3-ona (**43**) e 5-(*R*)-(1,2-*O*-isopropilideno-α-D-eritrofuranos-4-*C*-il)-pirazolidin-3-ona (**51**), e os da direita ao tautómero hidratado, denominados por 5-(*S*)-(3-*O*-benzil-1,2-*O*-isopropilideno-α-D-eritrofuranos-4-*C*-il)-3-hidroxipirazolina (**44**) e 5-(*S*)-(1,2-*O*-isopropilideno-α-D-eritrofuranos-4-*C*-il)-3-hidroxipirazolina (**52**).

Para se obter o primeiro conjunto de compostos, **42-44**, foi realizada a proteção do grupo hidroxilo da posição 3 com um grupo benzilo, seguida da desproteção seletiva do grupo isopropilideno ligado por formação de um éter aos carbonos 5 e 6, deixando dois grupos hidroxilo livres em posição *cis*-vicinal. Posteriormente, foi realizada a clivagem oxidativa do diol *cis*-vicinal seguido do aumento da cadeia carbonada por reação de Wittig para obtenção do éster α,β-insaturado. Por fim, foi realizada a ciclização do sistema alceno-carbonilo com hidrazina, formando o anel de pirazolidin-3-ona e o tautómero 3-hidroxipirazolina, todos com um rendimento de 10%. No segundo conjunto de compostos, **50-52**, foram realizados os mesmos passos reacionais, excetuando o passo de proteção com o grupo benzilo. Os rendimentos obtidos também foram de 10% para cada um dos três compostos.

Quando o anel de ligação foi o 5-hidroxipirazol, foi feita uma ciclização de um β-enaminoéster com a hidrazina monohidratada. Por esta via apenas se conseguiu sintetizar um composto.
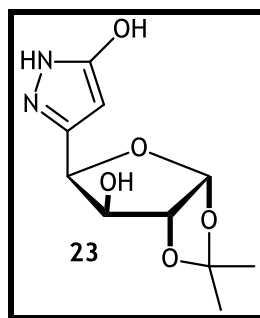
Figura ii. 3-(1,2-*O*-isopropilideno-α-D-treofuranos-4-*C*-il)-5-hidroxi-1*H*-pirazol (**23**)

Para se obter o composto **23** (Figura ii) foi realizada a desproteção seletiva do grupo isopropilideno ligado por formação de um éter aos carbonos 5 e 6, deixando dois grupos hidroxilo livres em posição *cis*-vicinal. Posteriormente, foi realizada a clivagem oxidativa do diol *cis*-vicinal seguida do aumento da cadeia carbonada por reação de Wittig para obtenção do éster α, ß-insaturado. Por fim, foi realizada a ciclização do sistema alceno-carbonilo, em micro-ondas, com a azida de sódio para a formação do ß-enaminoéster e posterior ciclização do sistema enamina-alceno-carbonilo com a hidrazina formando o anel de 5-hidroxipirazol com um rendimento de 24%.

Por fim, foram realizados ensaios MTT [Brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-Difeniltetrazólio] para avaliar a citotoxicidade dos compostos finais sintetizados em duas linhas celulares diferentes, a NHDF (do inglês, *Normal Human Dermal Fibroblasts*) e a N27 (do inglês, *Rat Mesencephalic Dopaminergic Neural Cells*). Em ambas as linhas celulares, os compostos em estudo não apresentaram citotoxicidade relevante. Consequentemente, mesmo não se podendo inferir sobre a potencialidade dos mesmos para o tratamento do BD, os resultados obtidos respeitantes à citotoxicidade são indicadores positivos para se continuar a desenvolver testes que avaliem a atividade destes compostos para o tratamento do BD.

Palavras-chave

Pseudo-*C*-nucleósidos, *Click Chemistry*, Pirazolidin-3-onas, 5-Hidroxipirazóis, Topiramato, Transtorno Bipolar.

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Acronyms and symbols Index

Ab	Antibiotic/antimycotic solution
Ac	Acetyl
AMP	Adenosine monophosphate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATCC	American Type Culture Collection
atm.	Atmosphere
ATR	Attenuated Total Reflectance
BD	Bipolar Disorder
BD I	Bipolar I Disorder
BD II	Bipolar II Disorder
BDNF	Brain-derived Neurotrophic Factor
BDNOS	Bipolar Disorder Not Otherwise Specific
Bn	Benzyl
CD	Cyclothymic Disorder
CNS	Central Nervous System
Cq	Quaternary Carbon
CuAAC	Copper (I)-catalyzed Azide-Alkyne Cycloaddition
d	Doublet
dd	Doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
DIBAL	Di-isobutyl Aluminum Hydride
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
Et	Ethyl
FBS	Fetal Bovine Serum
FTIR	Fourier Transform InfraRed
GABA	γ-aminobutyric acid
GFAP	Glial Fibrillary Acidic Protein
GSK-3	Glycogen Synthase Kinase 3
h.	Hour(s)
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HSQC	Heteronuclear Single Quantum Coherence

J	Coupling Constant
m	Multiplet
Me	Methyl
min.	Minute(s)
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide
MW	Microwave
NA	Not Applicable
NBDH	Number of Hydrogen Bond Donor protons
NHDF	Normal Human Dermal Fibroblasts
NMDA	*N*-methyl-D-aspartate
NMR	Nuclear Magnetic Resonance
N.O.	Total number of Nitrogen and Oxygen
N27	Rat Mesencephalic Dopaminergic Neural Cells
Obs.	Observations
PB	Propargyl Bromide
PCC	Pyridinium Chlorochromate
PDC	Pyridinium Dichromate
Ph	Phenyl
ppm	Parts per million
PSA	Polar Surface Area
Rf	Retention Factor
RPMI	Roswell Park Memorial Institute
r.t	Room Temperature
s	Singlet
SAR	Structure-Activity Relationship
S_N1	Nucleophilic Substitution unimolecular
S_N2	Nucleophilic Substitution bi-molecular
Sp	Antibiotic solution
t	Triplet
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
W	Watts
δ	Chemical Shifts
η	Yield
^{1}H NMR	Proton Nuclear Magnetic Resonance

5-FU   5-Fluorouracil

^{13}C NMR   Carbon-13 Nuclear Magnetic Resonance

Chapter I

Introduction

In this chapter, it is intended to introduce the characteristics and representation of the carbohydrates, as well as some of the reactions that can be performed to change them to synthesise new biologically active compounds, namely pseudo-*C*-nucleosides. All the reactions presented in this section are only the ones that were used to develop this work. It also explains why the carbohydrates derivatives are good drug candidates.

This work was developed based on a drug, topiramate, an anticonvulsant compound with a carbohydrate moiety which also shows to be able to control the typical recurrent crisis of the bipolar disorder when was administered to patients with that disorder. Some properties and the structure of this compound will be explained to justify the choice of the compounds developed in this work.

Finally, bipolar disorder, as well as the possible basis of this disease and the therapeutic approaches used today, will be briefly explained.

At the end of this introduction will be clear the lack of options that these patients deal with and the necessity to found new active compounds that can be used to control this disorder.

1. Carbohydrates

Carbohydrates are all the aliphatic polyhydroxy aldehydes and ketones, which presents $C_n(H_2O)_n$ as empirical formula and the respective compounds obtained by reduction and oxidation reactions, and is named a carbohydrate derivativee all the compounds obtained by the replacement of one or more hydroxyl groups by a hydrogen, a halogen, a nitrogen or a carbon chain. [1-3]

This group of compounds can be divided up into monosaccharides, which are the ones who cannot suffer hydrolysis into smaller subunits because they only have one subunit; and into oligosaccharides and polysaccharides, which are the ones who can split into smaller subunits by hydrolysis. The oligosaccharides have 2 to 9 subunits, and the polysaccharides have more than 10 subunits. [3]

In nature, the monosaccharides can be found in two different forms, the open chain structure or the cyclic structure. In the figures below, it is shown the two different forms of D-glucose (**1**), the most abundant carbohydrate in the planet Earth.
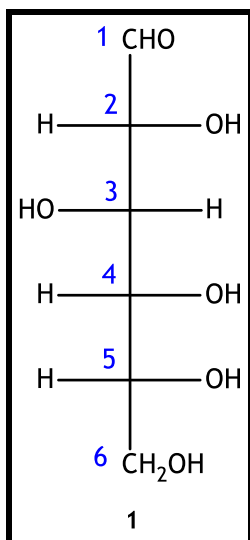
Figure 1. The open structure of D-glucose (**1**) and respective numbering (blue) of carbons.
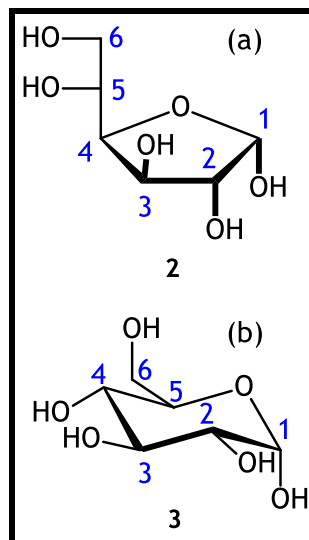
Figure 2. Cyclic structures adopted by D-glucose (**1**) and respective numbering (blue) of carbons. (a) α-D-glucofuranose (**2**); (b) α-D-glucopyranose (**3**).

1.1   Properties of Carbohydrates

Carbohydrates are well known by the sweet flavour and sweetish smell, but besides those physical characteristics, they also present specific chemical properties.

Due to their unique empirical formula, carbohydrates can suffer cyclization forming a hemiacetal by the reaction of the aldehyde present in C1 and the hydroxyl present in C4 to form a furanose ring or C5 to form a pyranose ring. The same can happen when the functional group is a ketone, but instead of a hemiacetal is formed a hemiketal. [1-3]

Once, almost all the carbons are linked to different substituents, the carbohydrates are molecules with several chiral carbons, which can be an advantage for synthetic purposes. Furthermore, the free hydroxyls groups present in their structure allow performing several reactions to obtain different carbohydrates derivatives, like amino sugars or deoxy sugars. [1-3]

It is also easy to link several monosaccharides together by glycosidic linkage due to the orientation of the hydroxyl groups to form oligosaccharides or polysaccharides or to link a monosaccharide to another type of compound, such as a heterocyclic ring or a steroid to form glycosidic compounds. Those monosaccharides with heterocyclic rings have been a useful type of compounds in the pharmaceutical industry. [1-3]

1.2   Type of projections used to represent Carbohydrates

Since the discovery of the carbohydrate structure, by Emil Fisher, several projections had been proposed to represent the carbohydrates. The first is known as Fisher projection and assumes that the cyclization reaction can occur to form a new chiral centre, named anomeric carbon,

and the cyclic structure can be formed by 5 or 6 atoms, named furanose or pyranose ring respectively. (Figure 3) [1,3]
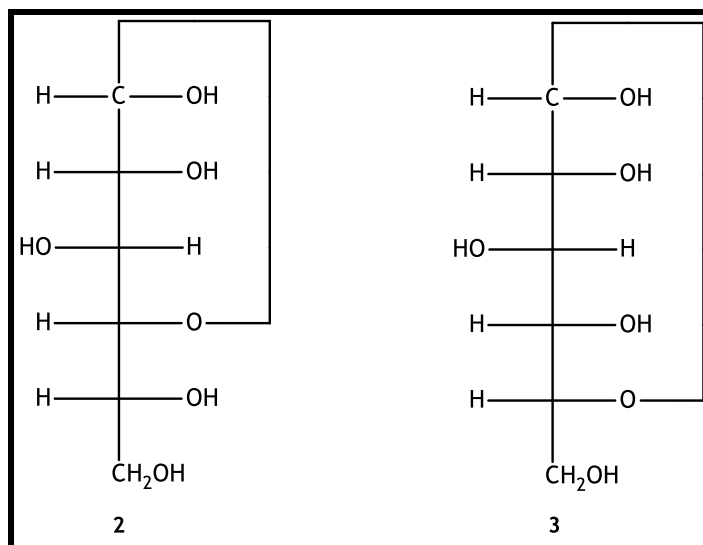
Figure 3. Fisher representation of α-D-glucofuranose (**2**) and α-D-glucopyranose (**3**).

However, this projection is not accurate since the cyclic representation is unrealistic, so others scientists proposed other ways to represent the cyclic structure of carbohydrates that are currently used. [1,3]

The most known and accepted nowadays is the Haworth projection, proposed by Norman Haworth, who assumed that the ring is viewed slightly from above and is oriented perpendicular to the plane of the paper. (Figure 4) [1,3]
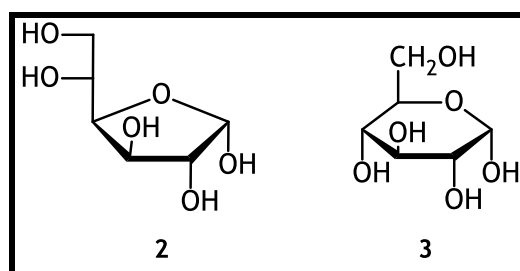
Figure 4. Haworth representation of α-D-glucofuranose (**2**) and α-D-glucopyranose (**3**).

Another projection is the one proposed by John Mills, which assumes that the hemiacetal rings are in the plan of the paper and the substituents are above or below that plan. If the substituent is below the plan, the representation is made with dashed bonds and if it is above with thickened bonds. (Figure 5) [3]
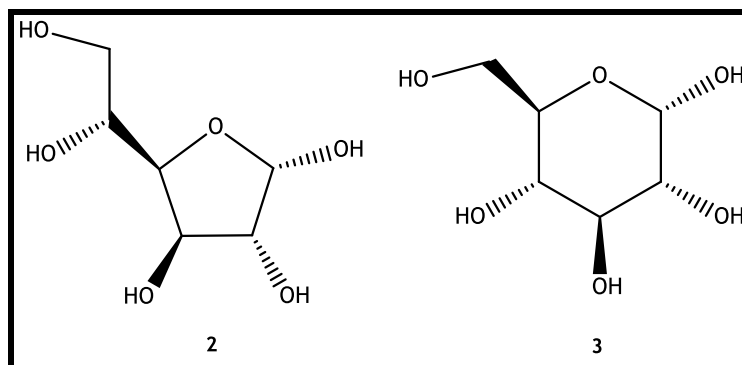
Figure 5. Mills representation of α-D-glucofuranose (**2**) and α-D-glucopyranose (**3**).

The Reeves projection, proposed by Richard Reeves, assumes that the ring has a nonplanar conformation, unlike the Haworth and Mills projections. (Figure 6) [3]
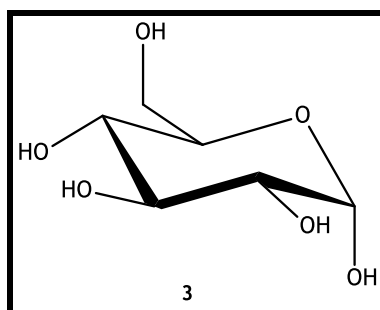
Figure 6. Reeves projection of α-D-glucopyranose (**3**).

During this Dissertation, the Reeves projection will be used when the carbohydrate ring is a pyranose, since this projection is the one that takes into account the less energetic conformation of a six-membered ring, and also can represent the position of the substituents in the plan; and Haworth's projection will be used when the carbohydrate ring is a furanose, since it is the best projection to see the spatial arrangement of the five-membered ring structure.

1.3 Reactions with Carbohydrates

Like many other classes of compounds, carbohydrates can suffer reactions to give derivatives, but due to the number of free hydroxyl groups present, there is a need for several regioselectivity strategies. [1,3]

The reactivity of the hydroxyl groups are different due to the type and the neighbourhood. The most reactive hydroxyl group is the linked to the anomeric carbon, which is the C1 in the case of compound **1**, followed by the primary hydroxyl and the hydroxyl groups linked to C2. Between the hydroxyls linked to the C3, C4 or C5 the ones in equatorial position are more reactive than the ones in axial positions. [1]

1.3.1   Protective Groups

There are several protective groups in Organic Chemistry, and they are used to protect a functional group. In carbohydrate chemistry, this type of strategy is the most important due to the number of free hydroxyl group, so we are looking for regioselective protective groups.

The main characteristics of a good protective group are that they should be cheap and ecological, react with good yields, be easily removed under suitable conditions and the ability to be removed without affecting subsequent reactions. [2]

1.3.1.1   Etheres as protecting groups

The most common ethers used as protecting groups are the benzyl ether, the trityl ether and the cyclic acetals, which includes the isopropylidene group. (Figure 7)
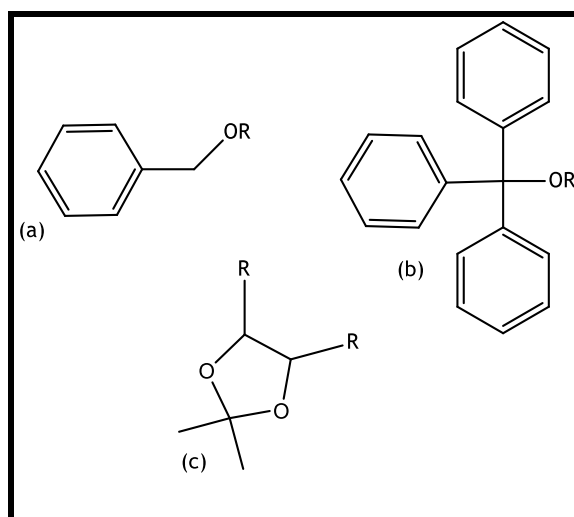
Figure 7. Common ethers protecting groups: (a) benzyl ether; (b) trityl ether; (c) isopropylidene.

The benzyl ethers can be formed by the reaction of a benzyl halide under basic conditions in the presence of a polar aprotic solvent, such as *N,N*-Dimethylformamide (DMF), with a free primary or secondary hydroxyl group. (Figure 8) This kind of protective group is very stable, and to undo a benzylation is necessary to perform a catalytic hydrogenation. [1-3]
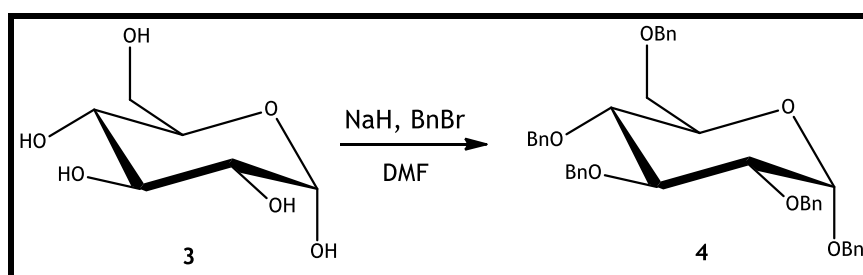
Figure 8. Reactional conditions to obtain 1,2,3,4,6-penta-*O*-benzyl-α-D-glucopyranose (**4**).

The trityl ethers, also known as triphenyl methyl ethers, are formed by the reaction of trityl chloride dissolved in pyridine with a free primary hydroxyl group, which makes this protective group one of the most selective. (Figure 9) This selectivity is due to the high stereochemical impairment of the trityl itself. This kind of protective group is used when there is a necessity to perform another type of reaction with other hydroxyl groups in the carbohydrate and to reverse this reaction is used mild acid treatment at low temperatures. [1,2]
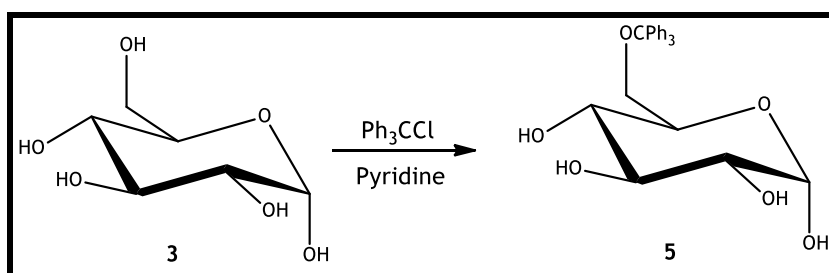
Figure 9. Synthesis of 6-*O*-trityl-α-D-glucopyranose (**5**).

The cyclic acetals, in which is included the isopropylidene group, also known as acetonide is one of the most used protective groups in carbohydrates. The isopropylidene formation is performed by the reaction of dry acetone and sulphuric acid with a carbohydrate with *cis*-vicinal diols, such as the hydroxyl groups linked to C1 and C2 in the case of α-D-glucofuranose (**2**). (Figure 10) At the end of the reaction is formed a new five-membered ring *cis*-fused to the carbohydrate ring. To take off an isopropylidene group are needed aqueous acid conditions. [1,2]
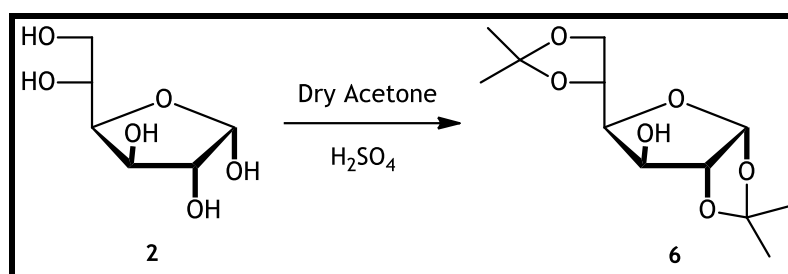
Figure 10. Synthesis of 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**6**).

1.3.1.2   Esters as protecting groups

The most common ester used as a protecting group, though not selective, is the acetyl ester and is formed under basic conditions, such as by the reaction of sodium acetate and acetic anhydride with the carbohydrate with free hydroxyl groups under reflux conditions. (Figure 11) For this reaction, the α-anomer is converted to the ß-anomer by mutarotation, because the last one is more nucleophilic. This protective group can be take off under acidic or basic conditions in methanol. [2,3]
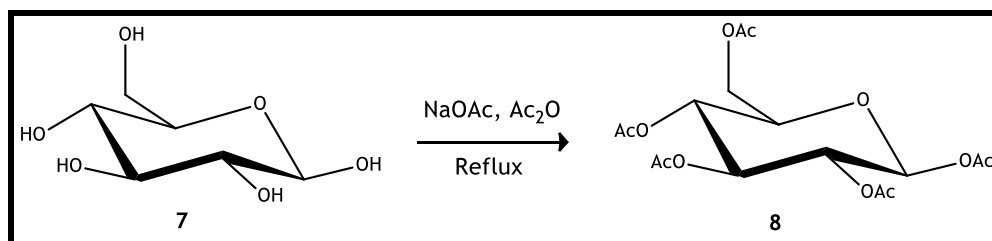
Figure 11. Synthesis of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**8**).

1.3.2   Oxidation-Reduction Reactions

Due to the high number of free hydroxyl groups, the oxidation-reduction reactions assume an important paper in the possibility of change carbohydrates structure. However, this type of reaction will affect all the free hydroxyl group unless the right conditions are used. So, it is easier to carry out this reaction after the protection of the free hydroxyl groups that aren't supposed to suffer oxidation-reduction.

In the case of oxidation reactions, the final functional group that the carbohydrate will present lies on the free hydroxyl group that suffers the reaction. If the free hydroxyl group is a primary hydroxyl group, then the oxidation will form an aldehyde or a carboxylic acid, depending on the oxidising agent, however, if the free hydroxyl group is a secondary hydroxyl group, then the oxidation will form a ketone. Besides the usual oxidation reactions, there is a particular type of oxidation that is performed with sodium periodate, which allows to cleavage *cis* 1,2-diol and form an aldehyde as a final product and is called oxidative cleavage. (Figure 12) [2]
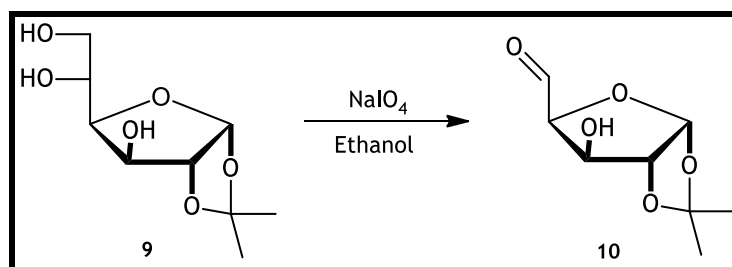
Figure 12. Synthesis of 1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**10**).

In addition to the oxidation reactions described above, this type of reaction can also be used in conjunction with a reduction to obtain epimers. Accordingly, it is necessary, first to oxidise the free hydroxyl group in which it is desired to change the orientation, and then the reduction of the carbonyl compound obtained, which is usually a ketone. At the end of these two steps, the epimer will normally be formed in high yield. [2]

In the case of compound **6**, the use of this strategy to obtain 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (**11**) is employed because the α-D-allose derivative is very expensive and this is a way to obtain the same compound in a more economical way. (Figure 13) [2]
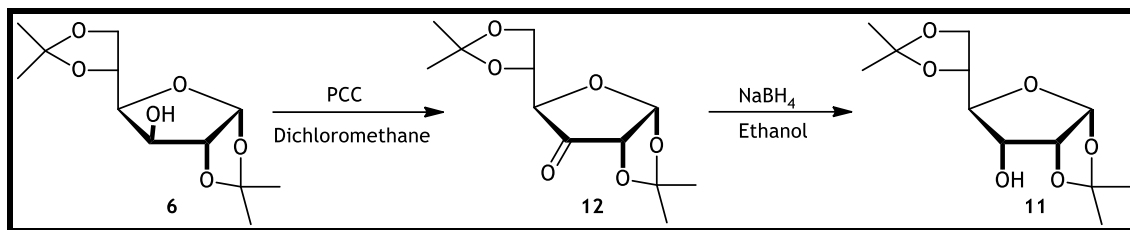
Figure 13. Synthesis of 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (**11**).

Another type of reduction reaction is the one used to obtain deoxy sugars, which are carbohydrates in which a hydrogen atom has replaced the hydroxyl group. One way to get the deoxy sugars is through a halogenation reaction and further reduction. (Figure 14) [1,2]
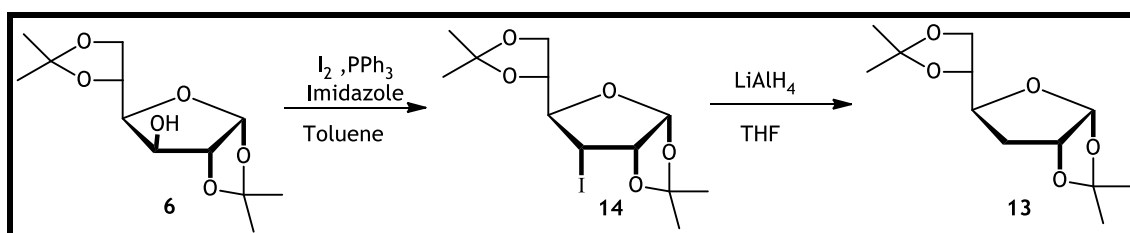
Figure 14. Synthesis of 3-deoxy-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**13**).

Another well-known reduction reaction applied in carbohydrates is the reductive amination, which consists in the reduction of an aldehyde or ketone into an amine under the appropriate conditions. This reaction presents better results when the starting compound is an aldehyde rather than a ketone. [4-6]

To perform a reductive amination is necessary, besides the aldehyde, the primary or secondary amine, and the *cis* 1,2-dichloroethene, a borohydride reagent more selective than the sodium borohydride, such as the cyanoborohydride. However, despite the remarkable way that the cyanoborohydride works in carbohydrate reduction, it presents toxic effects, so a less toxic reductive agent is preferred, such as sodium triacetoxyborohydride. This reductive agent works very effectively if the starting compound is an aldehyde. In the end, there is no aldehyde reduction as alcohol but only as an amine. (Figure 15) [4-6]
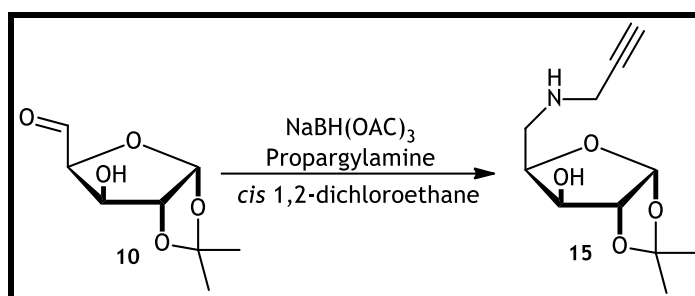
Figure 15. Reactional conditions to perform a reductive amination.

1.3.3   Microwave-assisted Reactions

With the advance of Medicinal and Organic Chemistry, there was a need to improve the yield of the reactions, the time spent, and the cost. So, it was adopted the microwave-assisted reactions, who made parallel and combinatory synthesis less expensive and time spending. This type of reactions usually takes no more than a few minutes and can be conducted with less solvent than the usual synthesis processes. [7,8]

The carbohydrates are good candidates for this type of technology because this kind of process is favoured by the interaction between the polar parts of the molecules and the electromagnetic field, which makes it less destructive than the usual heated synthetic procedures. [7,8]

One example of microwave-assisted reactions in carbohydrates are the halogenation reactions, for instance, the iodination reaction. (Figure 16) [7,8]
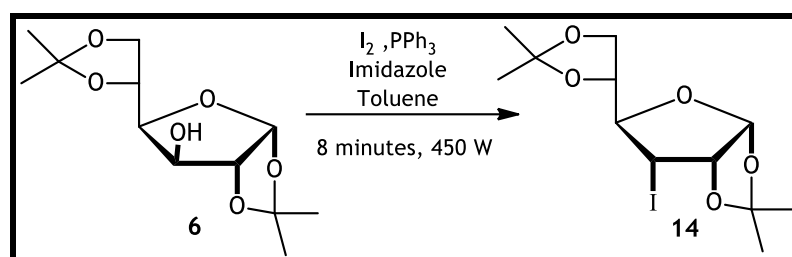
Figure 16. Reactional conditions to perform an iodination.

1.3.4   Wittig Reaction

A Wittig reaction consists in the reaction of an aldehyde or a ketone with a phosphorous ylide to obtain an alkene and the triphenylphosphine oxide. This type of reaction consists in two steps, the first is a nucleophilic substitution reaction and the second is an acid-base reaction. [9-12]

This kind of reaction is widely used because it is straightforward, convenient, and efficient since in the end there is no doubt of the localisation of the double bond. However, it is obtained the two isomers of the alkene, the *E* isomer, and the *Z* isomer. [9,10]

The phosphorous ylide is the type of ylide required for this reaction since this species has no charge and has a negative atom, the carbon, adjacent to a positive one, the phosphorous, which allows occurring the nucleophilic substitution reaction. Depending on the type of phosphorous ylide used it is possible to obtain not only alkenes but also α,ß-unsaturated esters, for example. (Figure 17) [9]
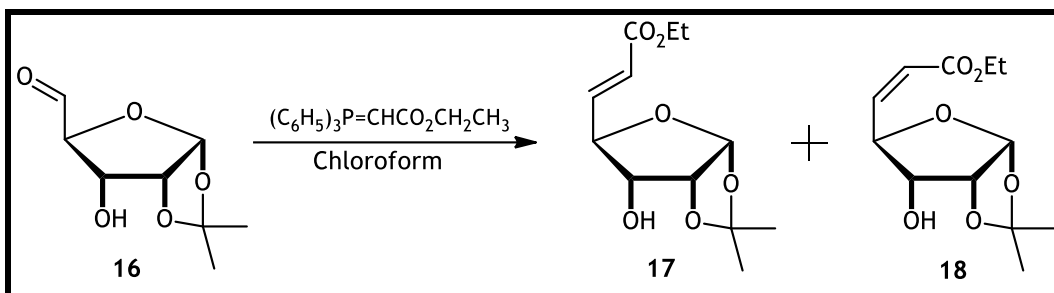
Figure 17. Reactional conditions to perform a Wittig to obtain both the isomers, ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**17**) and ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*Z*)-enfuranoate (**18**).

1.3.5   Reactions to obtain a 5-membered heterocyclic linked to the carbohydrate moiety

A strong interest has been developed in carbohydrate derivatives constituted by a sugar moiety linked to a heterocyclic due to the similarity found with endogenous molecules, so over the last few years, concepts like *C*-nucleosides and pseudo–*C*-nucleosides have been gaining another dimension. [13]

The difference between a *C*-nucleoside and a pseudo-*C*-nucleoside is in the carbon where the heterocyclic is linked. In the case of the *C*-nucleosides, a C-C bond at the position 1 of the carbohydrate links to the heterocyclic ring, whereas in the pseudo-*C*-nucleosides the carbohydrate is linked at the position 4 of furanosidic ring by a C-C bond. [13-15]

1.3.5.1   Click Chemistry

The Click Chemistry reaction is an exergonic process where the fusion of two unsaturated reagents is made, and it is obtained a five-membered heterocyclic ring. In the case of the cycloaddition of alkynes and azides, it is obtained a 1,4-disubstituted 1,2,3-triazole. [16-20] The azides are the most reliable family to introduce a nitrogen in a reaction, despite all the reservations in using this type of compounds. [20]

This type of reaction is known for being simple, have readily available starting compounds, the solvents needed are benign, for example, tert-butanol, and be simple to purify the final compound. This characteristic made this type of reaction convenient and also a quick reaction compared to others forms to obtain the same result. [18,21] Besides that, this reaction is also very flexible because it can be performed under a pH range, of 4 to 12, and a temperature range of 0°C to 160°C. Furthermore, it works better under aqueous systems such as water/*tert*-butanol. [22]

One way to perform a Click Chemistry is by a copper catalysis, more accurate a copper (I)-catalyzed azide-alkyne cycloaddition, also know as CuAAC. This catalysis is made by the reaction of a terminal alkyne with an azide. To obtain the 5-membered heterocyclic is also necessary to use a copper salt, such as copper sulphate pentahydrate and a reductant agent,

such as sodium ascorbate to be able to regenerate the copper, that works as a catalyst. (Figure 18) [20,23]
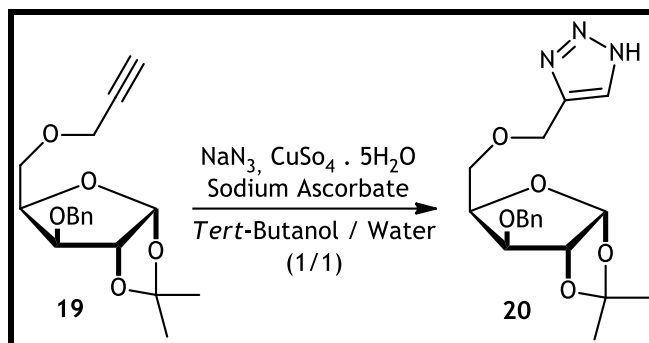
Figure 18. Synthesis of 4-(3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-methyl-α-D-xylofuranos-6-yl)-1*H*-1,2,3-triazole (**20**).

However, this approach is not environmental friendly because either the copper salts and the azide are toxic. Therefore, the green chemists try to discover new ways to overcome this obstacle, such as the incorporation of the copper salt into an organic polymer support, for example, the Amberlyst A-21. This support used in combination with a non-chelating solvent, such as dichloromethane, causes less quantity of copper in solution, and therefore final products with less metal to purify. [24,25]

1.3.5.2   Pyrazolidin-3-one synthesis

A pyrazolidinone is an oxo derivative of a pyrazolidine and is obtained by the condensation of an α,β–unsaturated ester, an α,β–unsaturated acid or an amide with hydrazine. As a result of this reaction, it is possible to obtain several tautomers due to the resonance of the ring. [26,27]

For example, in the case of the pyrazolidin-3-one, the reaction can be made by the condensation of an α,β–unsaturated ester with hydrazine hydrate. (Figure 19)
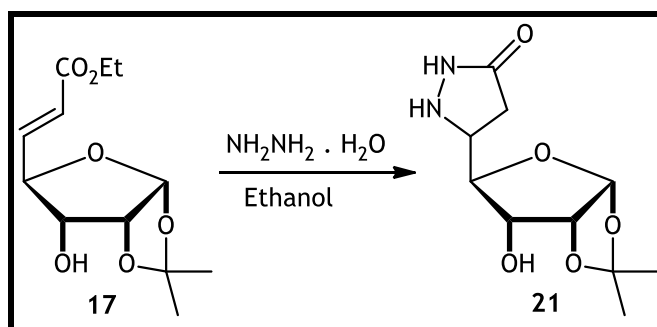
Figure 19. Synthesis of 5-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**21**).

1.3.5.3   5-Hydroxypyrazoles synthesis

A 5-hydroxypyrazole is obtained by the condensation of the hydrazine with a 1,3-dicarbonyl compound, or ester derivatives, such as the ß-enamino esters. [28,29] Likewise, the synthesis of pyrazolidin-3-one, this condensation also form tautomers. [30]

The advantage of use ß-enamino esters is that they are versatile intermediates for the synthesis of heterocyclic compounds containing nitrogen atoms and are useful and worldwide used building blocks for bioactive compounds. [31,32]

For example, in the case of the 5-hydroxypyrazole, the reaction can be made by the condensation of a ß-enamino ester with hydrazine hydrate in the presence of ethanol. (Figure 20)
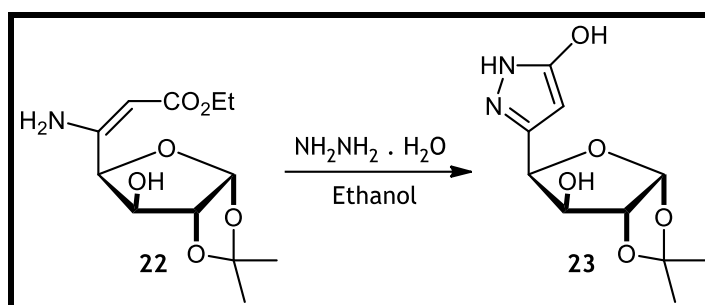
Figure 20. Synthesis of 5-(1,2-*O*-isopropylidene-α-D-threofuranos-4-*C*-yl)-5-hydroxy-1*H*-pyrazole (**23**).

2. Importance of Carbohydrates and its derivatives in Medicinal Chemistry

The major classes of naturally occurring compounds include proteins and peptides, nucleic acids, lipids, and carbohydrates. Due to the difficulty found in their study, carbohydrates have been neglected when compared with the other classes. However, more recently, carbohydrates have gained a new importance with the discovery of their action in the human body, being constituents of the cell wall and "fuel" for energy metabolism. In addition, they are involved in processes of transmission of biological information, and in the recognition and survival of cells, among many other important mechanisms to ensure the life. [2,33-35]

Therefore, carbohydrates won a new spotlight spot in drug discovery, not only because of their endogenous functions but also because of their unique structure, with multiple chiral carbons that are very important for the selective interaction with the biological target. However, carbohydrates are polar molecules mainly due to the number of free hydroxyl groups. Consequently, the idea of modifying a carbohydrate and create carbohydrate mimetics, with better stability, polarity and with functional groups linked to improving the interaction with the biological target, starts to rise. Therefore, nowadays carbohydrates are seen as useful scaffolds which can be linked to with several pharmacophores, for example, heterocyclic compounds, to generate multiple compounds with potential activity for the treatment of

several diseases. For instance, they can work as anticancer agents, as antibiotics and even as anticonvulsants. [33,34,36-40]

3. Topiramate

Topiramate (**24**) (Figure 21) is a drug developed for the treatment of epilepsy, which acts by inhibiting sodium channels, as well as by stimulating the GABA (γ-aminobutyric acid) neuroinhibitory effect of. Also, it is known to be a glutamate antagonist in non-NMDA (*N*-methyl-D-aspartate) receptors, which are the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and the kainate receptors. Due to these characteristics, studies were developed to evaluate the potential interest of this compound in the treatment of bipolar disorder (BD), which had very positive results. [39,41]

This drug is a carbohydrate derivative consisting of a pyranose ring, with two isopropylidene groups attached to in which one of them is linked to carbons 2 and 3 and the other to carbons 4 and 5 and a sulfamide group linked at the position 1. [39] (Figure 21)
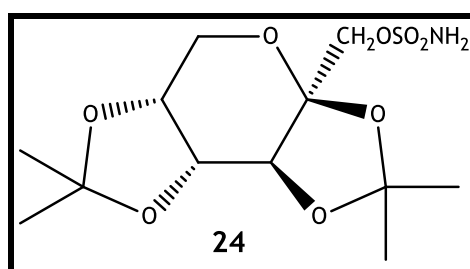
Figure 21. Topiramate (**24**). (adapted from [39])

Intensive studies on the structure-activity relationship (SAR) have already been developed, and it can be concluded that the presence of nitrogen atoms, a sulphonyl group and halogen atoms are advantageous for the biological activity of this and similar compounds. Therefore, considering the conclusions of the SAR study, the compounds that were synthesized in this dissertation were pseudo-*C*-nucleosides bearing, the furanose ring, since the structural studies with the pyranose ring were already extensive. The heterocyclic structures that were linked to the carbohydrate were triazole, pyrazolidin-3-one and 5-hydroxypyrazole, which are moieties present in compounds with activity in the treatment of diseases that affect the central nervous system (CNS). [27,40]

4. Bipolar Disorder

According to the Pharmacotherapy Handbook, Bipolar Disorder is a lifelong cyclic disorder, previously known as manic-depressive illness, which is characterised by recurrent extreme alternations in energy, mood, and behaviour. For a person to be diagnosed with this disorder, it is necessary to occur, a manic, hypomanic or a mixed episode, not caused by the use of

substances, another medical condition or psychiatric disorder. It is estimated that this disorder affects 1-4% of the world population. [42,43]

The BD can be divided into four types, the Bipolar I Disorder (BD I), the Bipolar II disorder (BD II), the Cyclothymic Disorder (CD) and the Bipolar Disorder Not Otherwise Specific (BDNOS), being the first two the most known. [44]

The diagnosis of BD I is only possible after the patient presents at least one manic or mixed episode accompanied by major depressive episodes, while the BD II is diagnosed when the patient presents at least one major depressive episode accompanied by at least one hypomanic episode. The CD is only diagnosed after two years of multiple periods of hypomanic symptoms and numerous periods of depressive symptoms, while the BDNOS is the term used when the symptoms are contradictory, or the symptoms presented by the patient do not meet criteria to be identified as one of the previously described. [44]

4.1 Characterization of the episodes types in Bipolar Disorder

Since the BD is characterised by several types of episodes with a small difference between them, it is necessary to distinguish the mania from the hypomania and the major depressive episodes. It is considered a major depressive episode when the patient presents a period of at least two weeks of depressive mood and loss of interest or pleasure in everything he does. The manic episode is characterised by a distinct period of at least one week in which is registered an abnormal persistent irritable and elevated mood associated with sense of grandiosity, decrease of the sleep time and distractibility while a hypomanic episode is defined by a distinct period of at least four days in which is registered an abnormal persistent irritable and elevated mood without delusions. [44]

4.2 Pathological basis of Bipolar Disorder

Despite all the attempts that have been made to disclose the pathological basis of BD, it has not yet been possible to clearly understand which originates this disorder. However, there are some signalling pathways known to be affected and some protein anomalies that will be presented. All the theories that exist in this context are based on the knowledge of the mechanisms of action of drugs used for the treatment of BD.

4.2.1 Neurotransmission imbalance

The neurotransmitters are on the basis of every emotion and action and, therefore, an impairment in the action of these molecules is enough to deregulate the person behaviour.

In BD, apparently, the dopaminergic and glutamatergic transmission is altered since there is an increase in the number of neuronal connections performed by these excitatory neurotransmitters. This change has been associated to the depressive phase of this disorder,

while in the manic phase the deregulation that occurs is in the muscarinic cholinergic transmission. In addition to these changes, some abnormalities in the prefrontal, hippocampus, and basal ganglia on the levels of myoinositol and *N*-acetyl-aspartate were also reported. This last effect associated with the change in the choline levels may cause alterations in the cell energy regulation and, consequently, glial loss. [45,46]

4.2.2 Progression of the disease

Another hypothesis reported suggests that a progression of this disease occurs over the years. This progression hypothesis is based on the observation of cognitive decline, progressive cerebral atrophy, and increased symptomatology. This set of symptoms is externalised in more frequent and closer mood cycles. [45-47]

This progression of the cerebral damage can be related to neuroinflammation and excitotoxicity processes, once there are levels of inflammatory markers, including GFAP (Glial Fibrillary Acidic Protein) and pro-inflammatory cytokines, which are elevated in patients suffering from this disorder. In addition, there is also evidence of a high glutamate/glutamine ratio, which can also lead to toxicity to neurons due to the elevated levels of glutamate. In fact, this neurotransmitter can trigger cascades of apoptotic cell signalling. (Figure 22) [45,46]
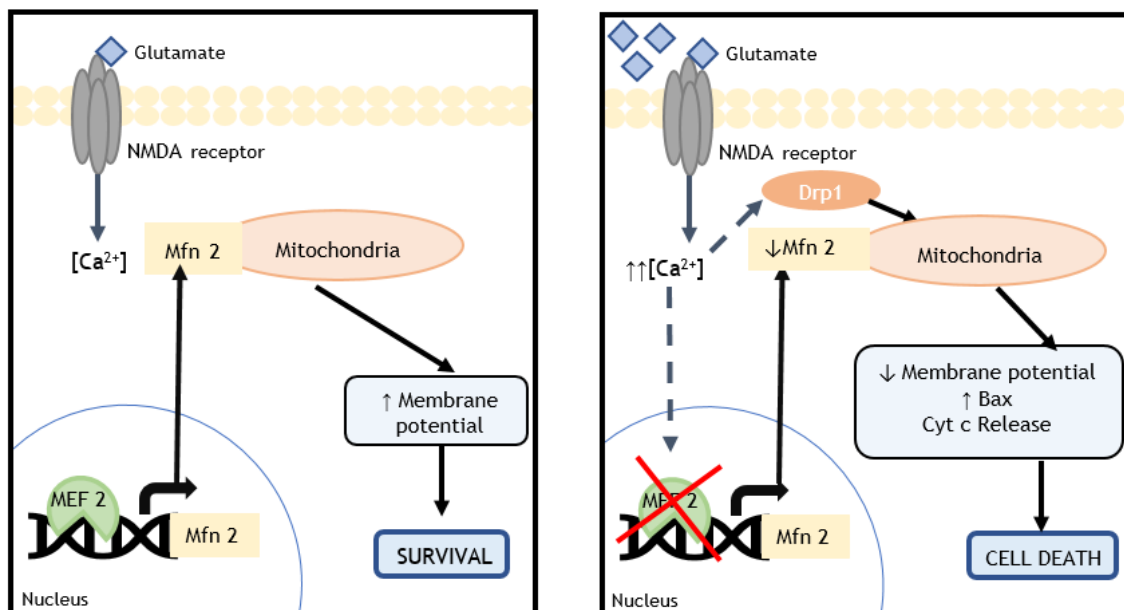
Figure 22. Mechanism of cell death by excitotoxicity. (adapted from [48])

In figure 22, at the left is represented the synaptic cleft in physiological conditions, while at the right is represented the mechanism of excitotoxicity provoked by the excess of glutamate in the synaptic cleft. In physiological conditions, the entrance of glutamate to cortical neurons is regulated, well as the levels of cytosolic calcium, leading to the transcription of the necessary genes for the perfect functioning of neurons. In excitotoxic conditions, as in BD, the increase of the entrance of glutamate into the neurons, leads to a toxic increasing of the cytosolic

calcium levels, and, consequently, to the degradation of the genes necessary to the functioning of the neurons, triggering signalling cascades, by the mitochondria, leading to the apoptosis of the neurons and, as a consequence, neuronal damage. [48]

The mitochondria are the main cell constituent responsible for the activation of caspases, which are apoptotic proteases. Besides that, a mitochondrial deregulation leads to an increase in the levels of free reactive oxygen and nitrogen species, and as a consequence to the propagation of the neuronal damage. [49,50]

4.2.3   Deregulation in neuroprotection

There is a serine/threonine kinase family, the GSK-3 (Glycogen Synthase Kinase 3), important in the neuroprotection, that seems to be deregulated in BD. [45]

The GSK-3B, which is the neuronal isoform present in the brain, is involved in several processes of the synapse plasticity, apoptosis, and gene transcription, and there is evidence that damage or malformation in this protein may be in the origin of mood disorders, such as BD. In this disorder, the GSK-3B is hyper activated leading to pro-apoptotic processes, via inactivation of the Wnt pathway, and to the inactivation of the cyclic AMP (Adenosine monophosphate), which leads to a deregulation in several biochemical processes, such as metabolic functions. Since the neuronal damage provoked is too extensive, neurotrophic factors, such as BDNF (Brain-derived Neurotrophic Factor), cannot compensate this process, and therefore brain defence mechanisms are not efficient in neuroprotection. [45]

4.3   Therapeutic approaches

Currently, there are four therapeutic approaches used for the treatment of BD, involving the use of Lithium Carbonate, antipsychotics and antidepressants, anticonvulsants, and combined therapy. Despite these approaches, about 37% of patients have a relapse in less than one year and 60% in fewer than two. [51,52]

The treatment with Lithium Carbonate, commonly known as Lithium, is the first line approach in BD. This compound has been shown to be effective in the two phases of this disorder, by maintenance and compensation of several biochemical processes, which are mostly deregulated. Despite all the advantages associated with the use of this drug, some adverse side effects have been worrying the medical community, so other therapeutic approaches are also considered with, potentially, fewer side effects. [45,51-53]

One example of the alternative approaches is the combination of antipsychotics and antidepressants. This combination is based on the individual effect of each of these classes of drugs. There is available information that confirms that when a patient presents an isolated manic crisis, the antipsychotics are the therapeutic approach that presents better results, and when presents an isolated depressive crisis, the antidepressants, mainly the tricyclic

antidepressants, present the best therapeutic results. However, globally, this alternative is not feasible, since there are phases in which the patient does not present any crisis, named euthymia, and for which the medication is not the most appropriate, because the administration of one of these classes during these periods can stimulate the appearance of the opposite mood leading to a crisis. [51,52]

Recently, anticonvulsants have been used for the treatment of BD, once, like Lithium, these drugs act mainly through sodium channels. The most commonly used anticonvulsant for the treatment of this disorder is valproic acid. This drug can be used individually or combined with lithium when monotherapy treatment does not work. [51,54]

The last approach consists of the combination of one of the therapeutic approaches described above with psychosocial therapy, such as cognitive-behavioural therapy, or even with antioxidants, to try to compensate the high level of radicals formed by the mitochondrial damage. [51,55,56]

None of the therapies presented above is completely effective and adequate for all patients, so it is necessary to continue to investigate and develop new molecules that have the potential to overcome this gap.

Chapter II

Aims

Based on the information previously described, the general aim of this dissertation is to synthesise pseudo-*C*-nucleosides by means different synthetic approaches with potential interest in BD treatment and/or control. All of the designed compounds were developed taking into account the basic structure of topiramate; however, some changes were made to incorporate functional groups potentially advantageous to the biological activity. Besides that, the carbohydrate base has been modified from a pyranose to a furanose. In addition, the *in vitro* evaluation of the compounds synthesised to access their cytotoxicity was also performed.

Chapter III

Presentation and Discussion of Results

In this chapter, the results obtained during the pseudo-*C*-nucleosides synthesis will be presented and discussed in the first subchapter, followed by the presentation and discussion of the results achieved in the biological assay, in the second subchapter.

1. Synthesis of pseudo-*C*-nucleosides

During this subchapter, the obtained results from the synthetic pathways that led to the formation of new pseudo-*C*-nucleosides, as well as the attempts made, will be presented and discussed chronologically.

Therefore, this subchapter is divided into three different parts, in which the different synthetic techniques will be grouped. The first will be on Click Chemistry, the second on the synthesis of Pyrazolidin-3-ones and the last on the synthesis of 5-Hydroxypyrazoles.

1.1 Click Chemistry

The Click Chemistry was the first technique used to try to synthesise pseudo-*C*-nucleosides, using an inorganic azide and an alkyne carbohydrate derivative.

The first synthetic pathway, which has been carried out has compound **6** as the starting material. First, the benzylation of the free hydroxyl group linked to the C3 was made, followed by an acid hydrolysis of the isopropylidene group linked at C5 and C6. Subsequently, an oxidative cleavage and its reduction to alcohol were made. Finally, was performed a propargylation of the alcohol, to obtain an alkyne which was the precursor for the final reaction, the Click Chemistry, to obtain compound **20**. (Figure 23)
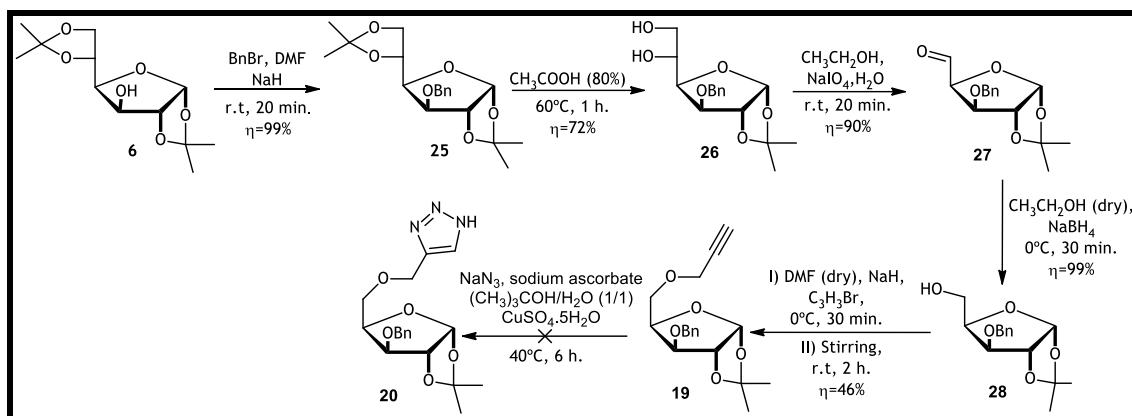
Figure 23. Synthetic pathway to obtain 4-(3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-methyl-α-D-xylofuran-6-yl)-1*H*-1,2,3-triazole (**20**).

Since this dissertation aims to synthesise pseudo-*C*-nucleosides and the starting compound is compound **6**, first, it was necessary to make a protection of the free hydroxyl group in position C3 of the furanosidic ring. For this protection, the benzyl group was chosen because is a very efficient protecting group in this case, since it is quite resistant to the following reactional conditions. For this reaction was used a fast technique with high yields. For this purpose, was used a benzyl halide, in this case, benzyl bromide, an apolar aprotic solvent, the DMF, and finally a hydride, the NaH, a Lewis base, which functioned as an alkaline agent and as an activator. These conditions allowed the deprotonation of the alcohol and the following nucleophilic substitution by S$_N$2. [2] This reaction formed the 3-*O*-benzyl-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**24**) with a yield of 99%. (Figure 24)
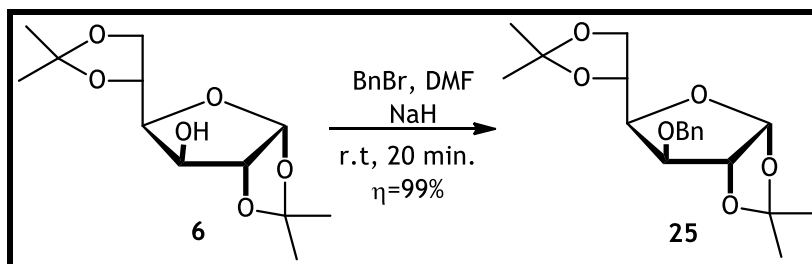
Figure 24. Synthesis of 3-*O*-benzyl-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**25**).

The second step of this synthetic pathway consists in the acid hydrolysis of the isopropylidene group bonded to C5 and C6 of compound **25** to be able to carry out the subsequent reactions, which are changes in the carbon chain linked to the furanose ring. To perform the selective deprotection was used a solution of 80% acetic acid at 60ºC for 1 hour or at room temperature overnight. After the workup of the reaction, the residue was purified, to obtained the 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**26**) in a yield of 72%. (Figure 25)
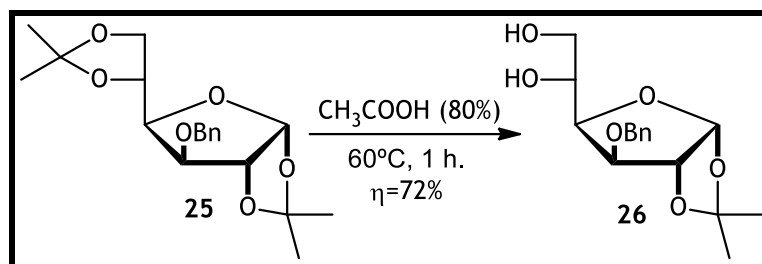
Figure 25. Synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**26**).

Then, an oxidative cleavage was made, in which a cleavage of the C-C bond, between carbons 5 and 6 occurred, decreasing the carbon chain on one carbon and gave a C5 linked aldehyde. For this reaction to occur it was necessary to have a *cis*-vicinal diol, as the free hydroxyl groups linked to C5 and C6 in compound **26**. To carry out this reaction is required sodium metaperiodate in ethanol and water and allowed to react for 20 minutes at room temperature. This reaction was made with light protection since the metaperiodate is sensitive to light and if there were no protection, there would be the possibility of not occurring the formation of the final product as well as having unwanted side products. With this reaction, the 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**27**) was formed with a 90% of yield. (Figure 26)
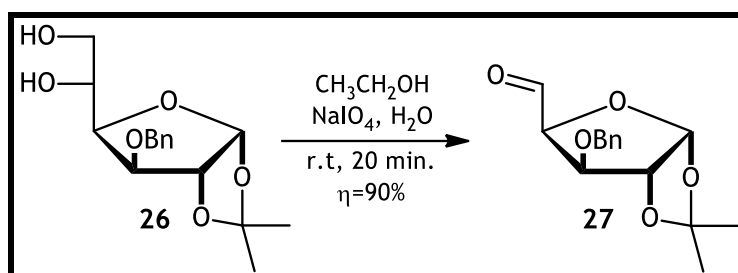
Figure 26. Synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**27**).

After that, the aldehyde **27** was reduced to a primary alcohol. To perform this reaction was used a reducing agent, the sodium borohydride, in anhydrous ethanol. This reaction is quite fast and very efficient and was formed the 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**28**) with a yield of 99%. (Figure 27)
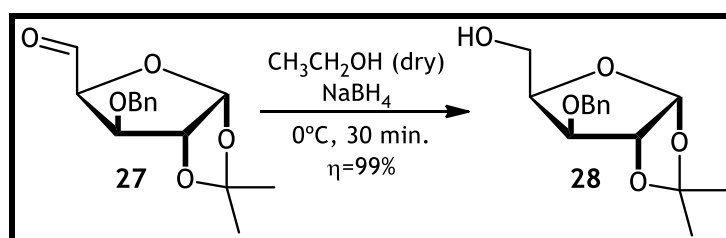
Figure 27. Synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**28**).

An alternative pathway tried to obtain compound **28,** had as starting compound the 1,2-*O*-isopropylidene-α-D-xylofuranose (**29**). From compound **29**, was made a tritylation of the primary hydroxyl linked to C5, followed by a benzylation of the free hydroxyl group of C3. Finally, was made a detritylation to obtained compound **28**. (Figure 28)
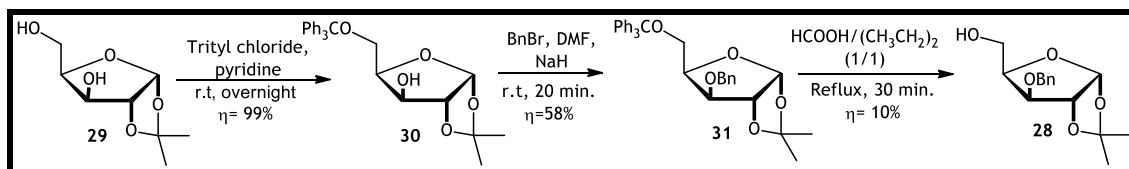
Figure 28. Alternative synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**28**).

The tritylation is a very selective protection reaction, via S_N1, in which a trityl halide, in this case, the trityl chloride, reacts with a primary alcohol, which in this case is the free hydroxyl linked to the C5 of compound **29**, under basic conditions. [2] This reaction formed the 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose (**30**) with a yield of 99%. (Figure 29)
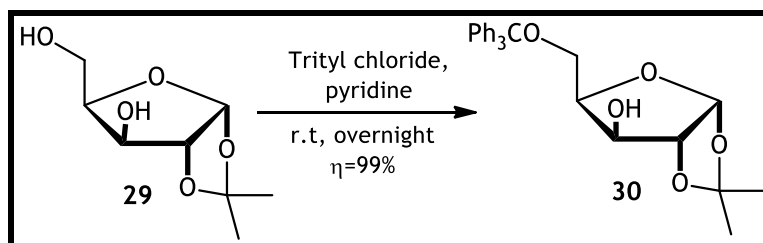
Figure 29. Synthesis of 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose (**30**).

Then, was made a benzylation of the compound **30,** to protect the free hydroxyl group linked to C3. At the end of the reaction, it was necessary to purify the compound. This reaction formed the 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose (**31**) with a yield of 58%. (Figure 30)
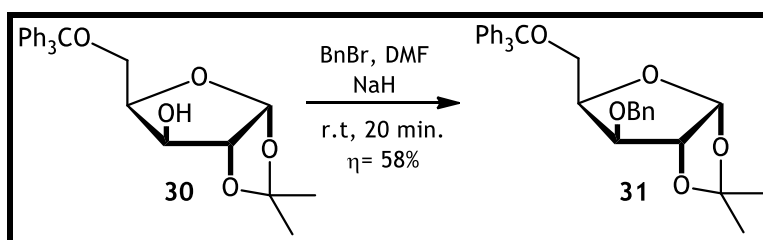
Figure 30. Synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose (**31**).

The final reaction of this alternative pathway was a detritylation reaction, in which acid conditions was used to take off the trityl group and obtain the primary alcohol **28**. (Figure 31) At the end of the reaction, it was necessary to purify the compound. The yield of this reaction was 10%, which is a very low yield, so this pathway was left aside.
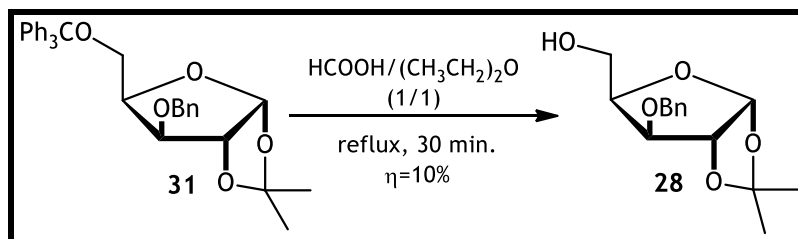
Figure 31. Synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**28**).

The lower yields obtained in the second pathway to get the alcohol **28** can be related to the fact that the α-D-xylose is a less reactive carbohydrate compared to α-D-glucose. Therefore, it is normal that even the simplest and higher yielding reactions have lower yields when the starting compound is a α-D-xylose derivative when compared to the same reaction having as starting compound a α-D-glucose derivative.

Subsequently, was formed 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-(2-propargyl)-α-D-xylofuranose (**19**) by a propargylation, which consists in the reaction of a primary alcohol, in this case, compound **28,** with a propargyl halide, in this case, propargyl bromide (PB). Two different procedures were used for this technique and are summarised in Table 1.

Table 1. Reactional conditions used to carry out the propargylation of compound **27** and the respective yields.

Reagents	Solvent	Time (I)	Temperature (I)	Time (II)	Temperature (II)	Yield
28 PB KOH 60%	Water	20 minutes	10-15ºC	48 hours	r.t	41%
28 PB NaH	Anhydrous DMF	30 minutes	0ºC	2 hours	r.t	46%

In the table, is described in the first row the method 1, and in the second the method 2. Briefly, in method 1, compound **28** and PB were dissolved in a solution of 60% KOH, and reacted for 20 minutes at 10-15ºC, and then left for 48 hours, with stirring, at r.t, while in method 2, compound **28** and PB were dissolved in anhydrous DMF, and react for 30 minutes at 0ºC, and then left for 2 hours, at r.t, with stirring. At the table, the time (I) and temperature (I) corresponds to the first part that is followed by the conditions described as time (II) and temperature (II).

Although the differences found in the protocols of both methods, the yields obtained were similar to each other. Comparing the reactional times of both approaches, it is notorious that the second approach is faster than the first, and the yield is slightly superior, so was adopted the second method when the synthetic pathway needed to be repeated. At the end of the reaction, it was obtained compound **19**, and it was necessary to purify the compound. (Figure 32)
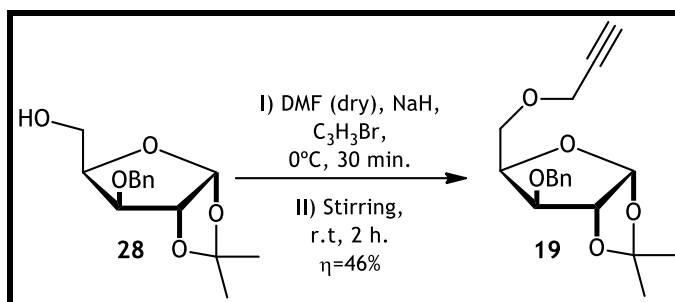
Figure 32. Synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-(2-propargyl)-α-D-xylofuranose (**19**).

The last reaction of this synthetic pathway was the Click Chemistry. To carry out this reaction is required a compound with a terminal alkyne, in this case, compound **19**, and sodium azide. In this case, the copper (I) compounds were used as a catalyst.

Two different procedures were used for this synthesis and are summarised in Table 2. In the first row is described the method 1 and in the second the method 2. Briefly, in method 1, compound **19**, NaN$_3$, sodium ascorbate and CuSO$_4$.5 H$_2$O was dissolved in tert -butanol/water (1/1) and reacted for 6 hours at 40ºC, whereas in method 2 the compound **19**, NaN$_3$, and CuI encapsulated in Amberlyst A-21, were dissolved in dichloromethane and reacted overnight at r.t.

Table 2. Reactional conditions used to perform the Click Chemistry of compound **19** and the respective yields.

Reagents	Polymeric support	Solvent	Time	Temperature	Yield
19 NaN$_3$ Sodium ascorbate CuSO$_4$.5 H$_2$O	NA	Tert-butanol Water (1/1)	6 hours	40ºC	10%
19 NaN$_3$ CuI	Amberlyst A-21	Dichloromethane	overnight	r.t	10%

However, despite the efforts to synthesise compound **20**, that was not possible, and instead, the final compound obtained, in both procedures, was (3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-propyl-α-D-xylofuranos-8-yl) diazene (**32**). At the end of the reaction, it was necessary to purify the compound. So, the yields presented in the table correspond to compound **31**.

Despite the large differences found in the two methods, the yield was the same as well as the final compound. (Figure 33) As the obtained compound was not the intended, this is, is not a pseudo-*C*-nucleoside, this synthetic pathway was left aside.
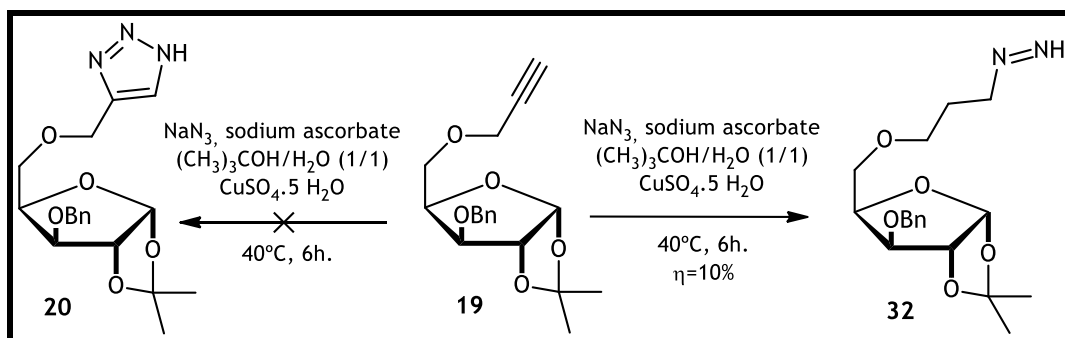
Figure 33. Synthesis of (3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-propyl-α-D-xylofuranos-8-yl) diazene (**32**), in which should be obtained 4-(3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-methyl-α-D-xylofuran-6-yl)-1*H*-1,2,3-triazole (**20**).

The hypothesis proposed for the triazole ring did not close is related to the protecting group chosen. The benzyl protecting group has in its constitution an aromatic ring, as such, that ring is in resonance. The fact that the aromatic ring is "up", which is the same side of the triazole ring, which also has resonance, may be the cause of not being able to obtain the desired final compound, but rather an aliphatic carbon chain with two terminal linked nitrogen atoms bonded to the carbohydrate moiety.

At the same time as the previous synthetic pathway was performed, a parallel synthetic route was attempted to obtain a compound like the **20**. In this case, instead of obtaining the terminal alkyne from the alcohol **28**, it was obtained from the aldehyde **27** via reductive amination. At the end of this pathway, it was supposed to obtain the 4-methyl–(5*H*-5-amino-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl)-1*H*-1,2,3-triazole (**33**). (Figure 34)
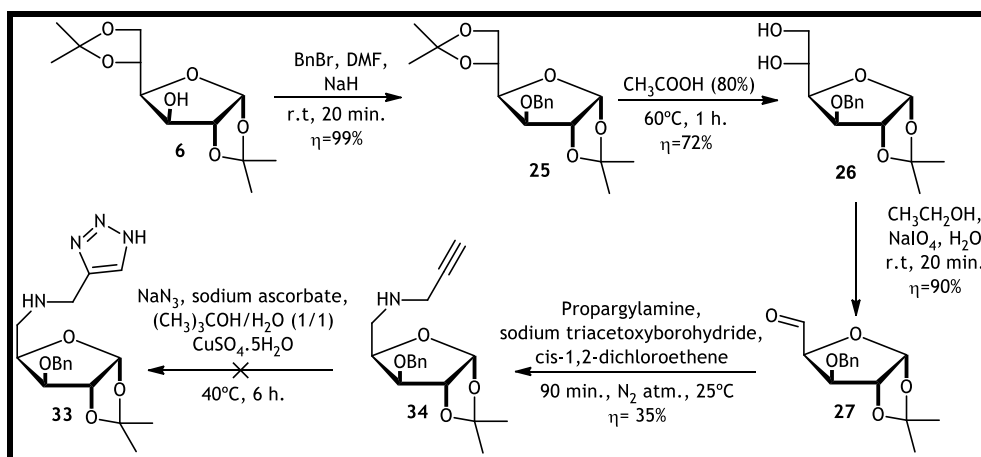
Figure 34. Synthesis of 4-methyl–(5*H*-5-amino-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl)-1*H*-1,2,3-triazole (**33**).

After the aldehyde **27** was obtained, a reductive amination was made, in which the carbonyl group was reduced to a secondary amine, since the starting amine was a primary one, in this case, the propargylamine. For the reaction to occur it was necessary to add a hydride similar to sodium borohydride, but specific for reductive amination such as sodium

triacetoxyborohydride. This reaction was performed under a nitrogen atmosphere to ensure that the aldehyde would only be reduced to the amine and not to alcohol. [4,5]

This reaction occurs in two steps; the first is the attack of the amine on the carbon in which the carbonyl group is attached, which gives rise to two intermediates, the hemiaminal, and the imine, which are in equilibrium. Subsequently, these intermediates undergo a reduction to give the secondary amine, 5*H*-5-amino-*N*-(2-propargyl)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**34**), with a yield of 35% after purification. (Figure 35)
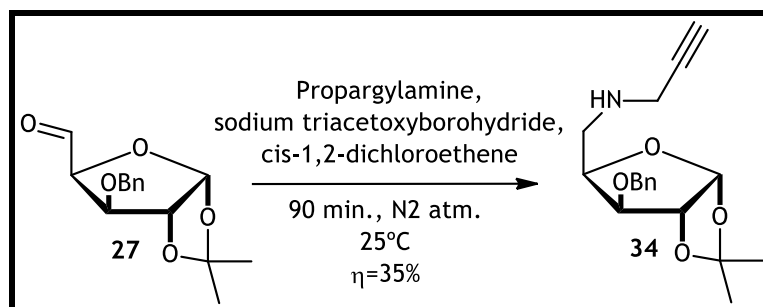
Figure 35. Synthesis of 5*H*-5-amino-*N*-(2-propargyl)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**34**).

It was used the method 1 referred in Table 2 to perform the Click Chemistry reaction, using as starting compound **34**. At the end of this reaction should have obtained compound **33**. However, the final pure compound was not obtained. (Figure 36) Therefore, this synthetic pathway was also left aside.
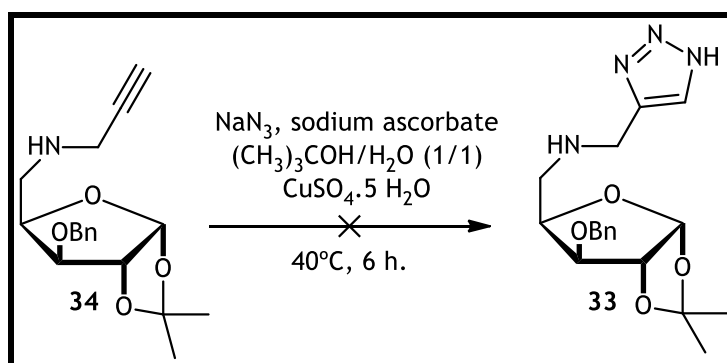
Figure 36. Click Chemistry reaction of 5*H*-5-amino-*N*-(2-propargyl)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**34**), in which should have been obtained 4-methyl-(5*H*-5-amino-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**33**).

As any of the desired pseudo-*C*-nucleosides was obtained with the benzyl protecting group by Click Chemistry, what was done next was to try to change the protecting group attached to C3. Within the protective groups that have been tried are the methyl and the acetyl.

The relevance of attempting to use the methyl protecting group compared to the benzyl is that the former is smaller and simpler and so does not have a stereochemical impairment effect as high as the latter.

Therefore, starting from compound **6** was made a methylation of the free hydroxyl group of C3. For this has been used diazomethane, which is a widely used methylating agent for the esterification of alcohols, carboxylic acids, and other classes of organic compounds. However, in the case of the alcohols, like compound **6**, diazomethane alone cannot perform the methylation, and therefore a catalyst, the boron trifluoride etherate, is needed. This catalyst increases the acidity of the compound and therefore facilitates the protective reaction. [57] At the end of this reaction should have obtained the 1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (**35**), however, the final compound could not be obtained, because it degraded easily. (Figure 37)
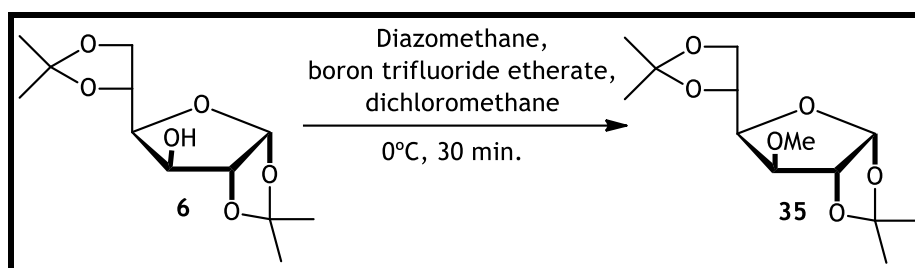
Figure 37. Methylation reaction of 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**6**), in which should have been obtained the 1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (**35**).

Thus, as the compound **35** degraded, it was necessary to replace the protecting group, and, instead of the methyl, the acetyl group was used. The acetyl protecting group is also a relatively small protecting group, but the acetylation instead of forming an ether, it forms an ester.

To carry out the acetylation was used acetic anhydride, which is a compound widely used for alcohol acetylation. In this case, the acetylation was carried out having as starting reagent compound **6**, which has a free hydroxyl group in C3. It is only necessary to use, besides the anhydride, anhydrous sodium acetate to ensure the basicity of the reaction. [57] At the end of the reaction, it was obtained the 3-*O*-acetyl-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**36**) with a yield of 99%. (Figure 38)
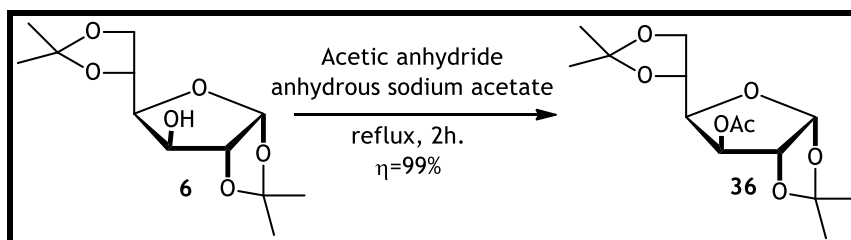
Figure 38. Synthesis of 3-*O*-acetyl-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**36**).

Then, it was performed an acidic hydrolysis of the isopropylidene group linked to C5 and C6 of compound **36**. At the end of the reaction, it was obtained the 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**37**) with a yield of 92%, and it was necessary to purify the compound. (Figure 39)
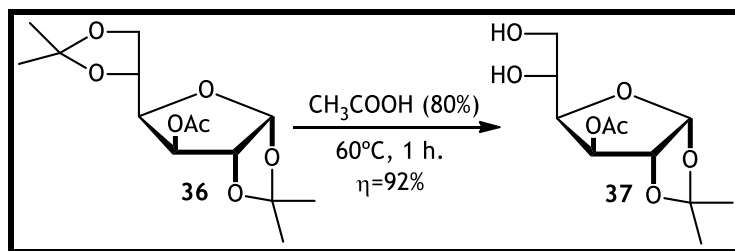
Figure 39. Synthesis of 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**37**).

Subsequently was made an oxidative cleavage of the *cis*-vicinal diol from compound **37** to obtain the aldehyde 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**38**) with a yield of 52%. (Figure 40)
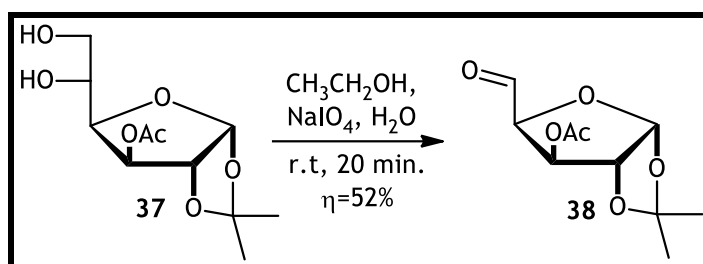
Figure 40. Synthesis of 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**38**).

Posteriorly, the aldehyde **38** was reduced to an alcohol, giving rise to 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**39**) with a yield of 99%. (Figure 41)
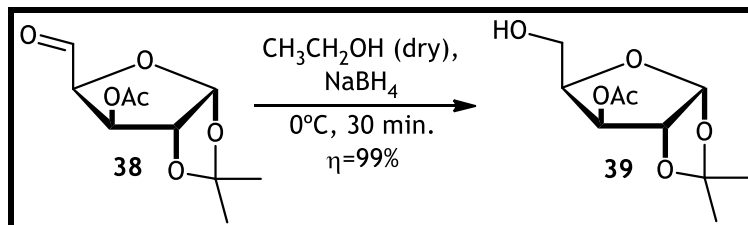
Figure 41. Synthesis of 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**39**).

Then was performed the propargylation of compound **39** to obtain the 3-*O*-acetyl-1,2-*O*-isopropylidene-5-*O*-(2-propargyl)-α-D-xylofuranose (**40**) but it was not possible to obtain that compound. (Figure 42)
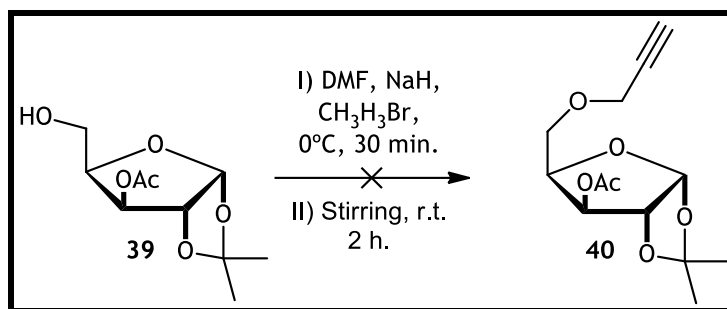
Figure 42. Propargylation reaction of 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**39**), in which should have been obtained the 3-*O*-acetyl-1,2-*O*-isopropylidene-5-*O*-(2-propargyl)-α-D-xylofuranose (**40**).

Instead of compound **40**, it was possible that the compound obtained was more complex and more apolar due to the rf obtained (rf =0.87 Ethyl Acetate/*n*-hexane (1/1)). It was made an InfraRed spectrum, and it was evident by the analysis that the ester group was not present, since the spectrum do not present the characteristic carbonyl band, but the Nuclear Magnetic Resonance (NMR) spectra were inconclusive. (Attachment 1) Therefore it was not possible to know the structure.

As the changes made relates to the protecting groups did not result, thence what was attempted to do was to modify the hydroxyl group to a halogen, in this case, iodine. To carry out the iodination reaction were used triphenylphosphine, iodine, and imidazole in 4/3/4 proportions compared to the alcohol **6**, which was used in ratio 1.

This reaction is a typical S_N2 reaction with inversion of the structure conformation, which is characteristic of both the reaction mechanism as well as the fact that the carbon where the iodine linked to is secondary. [2] This reaction was carried out by three different methods that are summarised in Table 3.

Table 3. Reactional conditions used to perform the iodination of compound **6** and the respective yields.

Reagents	Solvent	Time	Temperature	Potency	Workup	Yield
6 Imidazole $(C_6H_5)_3P$ Iodine	Toluene	16 hours	Reflux	NA	a)	16%
6 Imidazole $(C_6H_5)_3P$ Iodine	Toluene	16 hours	Reflux	NA	b)	16%
6 Imidazole $(C_6H_5)_3P$ Iodine	Toluene	8 minutes	NA	450 W	a)	50%

In the table, is described in the first row the method 1, in the second the method 2 and in the third the method 3. Briefly, in all the methods the compound 6, imidazole, triphenylphosphine and iodine are dissolved in toluene. In methods 1 and 2 the reaction time was 16 hours, while in method 3 was 8 minutes, since this reaction was performed by a microwave. It is also presented two different workup procedures named a) and b), in which: a) is after cool down is added ethyl acetate and acetone, for further filtration and concentration in vacuum; and b) is after cool down is added toluene and ice and was made an extraction, and the organic phase was washed with saturated solution of sodium thiosulphate for further concentration. Finally, was added diethyl ether to precipitate the triphenylphosphine oxide, and then was filtrated and concentrated in vacuum.

Despite the differences found in the first two methods the yields obtained were the same, leading to the conclusion that the difference at the workup did not affect the final yield of the reaction, although the fact that the workup b) precipitate the triphenylphosphine oxide and facilitates the purification step. However, the third method is a microwave-assisted reaction, which is a simpler, faster, almost solvent-free method that presents a good yield comparing to the other two, so the third method was adopted when the synthetic pathway needed to be repeated. At the end of the reaction, it was obtained 3-iodo-1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (**14**), and, it was necessary to purify the compound. (Figure 43)
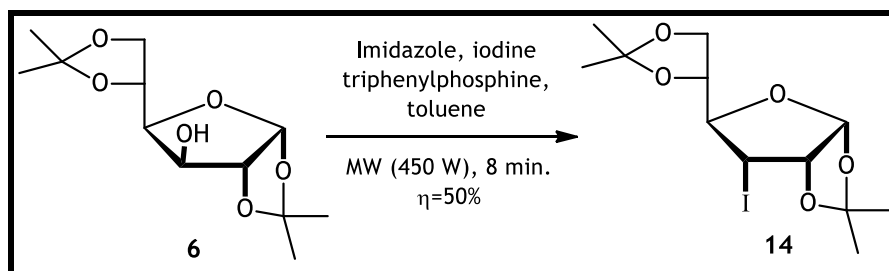
Figure 43. Synthesis of 3-iodo-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**14**).

Afterwards, was performed the acid hydrolysis of the isopropylidene group linked to C5 and C6 of compound **14** to obtain the 3-iodo-1,2-*O*-isopropylidene-α-D-glucofuranose (**41**), with a yield of 50%. (Figure 44)
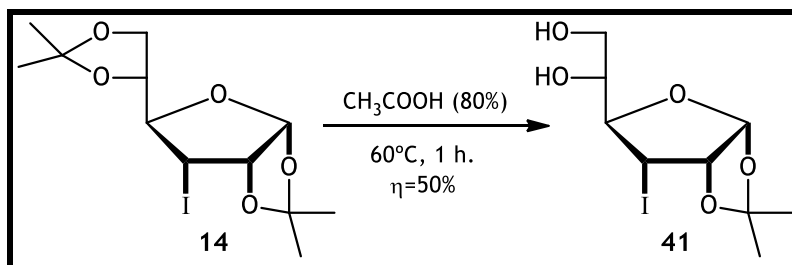
Figure 44. Synthesis of 3-iodo-1,2-*O*-isopropylidene-α-D-glucofuranose (**41**).

As the yields obtained in the two reactions necessary to obtain compound **41** from **6** were of 50%, at this moment, the mass of compound **41** is too low. Since it was needed to perform a series of reaction steps to obtain the triazole derivative, it was chosen to leave this reaction pathway aside.

Since the attempt to exchange the hydroxyl group for a halogen also didn't result, one more attempt was made, which consists in make a deoxy sugar. To this end, the iodination step described above was done to obtain the compound **14**. Subsequently, **14** was reduced using anhydrous THF and lithium aluminium hydride. This hydride is a widely used and potent reducing agent, which is therefore suitable for this reduction, in which a hydrogen atom will replace the iodine atom. The solvent used for this reaction must be anhydrous, once the hydride should not come into contact with water under risk of violent reaction. At the end of the reaction, it was obtained compound **13** with a yield of 16%, and it was necessary to purify the compound. (Figure 43)
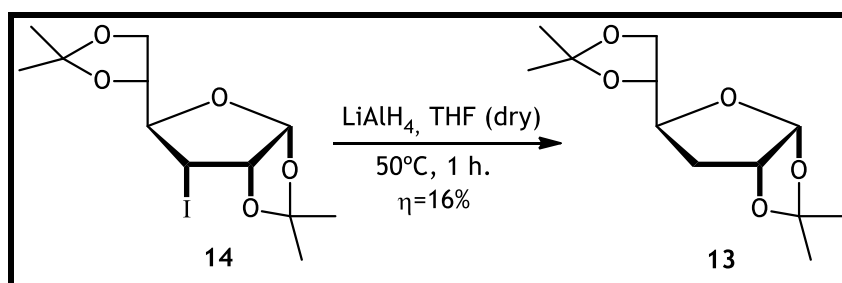
Figure 45. Synthesis of 3-deoxy-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**13**).

As the yield obtained in this reaction was too low and was necessary to perform a series of reaction steps to get the triazole derivative, it was chosen to leave this reaction pathway aside.

Despite all the attempts made, no triazole ring attached to the sugar moiety was obtained, for the reasons explained and discussed above.

As a suggestion for the future, the idea is to try to attempt the following synthetic pathway: starting from compound **6** obtain compound **38** following the procedures described below at the experimental part. After the obtention of the aldehyde **38**, perform a Schmidt reaction to obtain a nitrile. Subsequently, carry out a Pinner reaction, in which the nitrile reacts with an alcohol, in this case, the propargyl alcohol, to give an imino esther salt. This salt can be maintained for the final Click Chemistry reaction, or else, it also can be performed a substitution reaction to obtain a thioester or an ester, which can be subsequently subjected to the Click Chemistry reaction.

1.2 Pyrazolidin-3-ones

Pyrazolidin-3-ones was the second technique used to synthesise pseudo-*C*-nucleosides, using an α, β- unsaturated ester and hydrazine monohydrate.

The first synthetic pathway, which has been carried out, in this part, has compound **6** as the starting material. First, was made an oxidation of the free hydroxyl group linked to C3, followed by the reduction of the ketone. Posteriorly was performed the benzylation of the free hydroxyl group attached to C3 for further acid hydrolysis of the isopropylidene group linked to C5 and C6. Finally, was done an oxidative cleavage for subsequent Wittig reaction to obtain an α, ß-unsaturated ester which was the precursor for the final reaction to getting the two pyrazolidin-3-one isomers, named 5-(*S*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**42**) and 5-(*R*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**43**) and a tautomer, named 5-(*S*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-3-hydroxypyrazoline (**44**). (Figure 46)
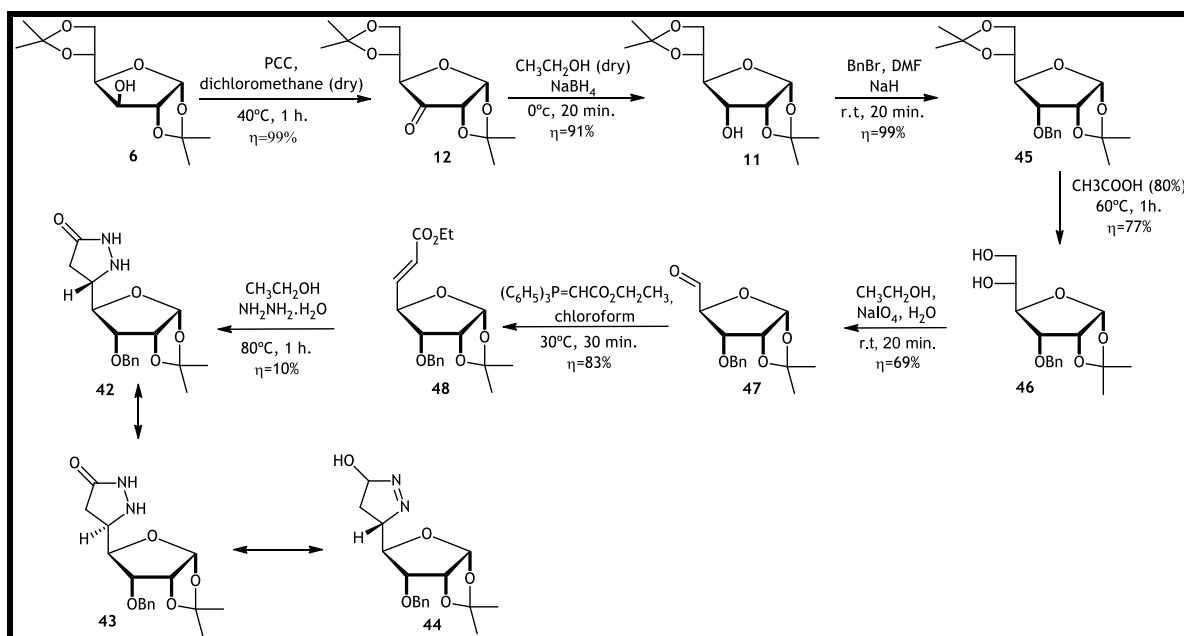
Figure 46. Synthetic pathway to obtain compounds 5-(*S*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**42**), 5-(*R*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**43**) and 5-(*S*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-3-hydroxypyrazoline (**44**).

To synthesise compound **11** from compound **6** is necessary to perform an oxidation reaction followed by a reduction, once this type of approach allows the inversion of the conformation. In this case was chosen to synthesise compound **11** in the laboratory since this alternative is cheaper than buy the reagent, and the yield is usually high.

First, was performed the oxidation of the free hydroxyl group linked to C3 of compound **6**. To accomplish this is necessary to use an oxidising agent, such as Pyridinium Chlorochromate (PCC), and anhydrous dichloromethane. The PCC is a derivative of the chromic acid utilised for the oxidation of primary and secondary alcohols to aldehydes or ketones, respectively. It was chosen PCC over Pyridinium Dichromate (PDC) because although they are serving for the same, the last is more toxic.[9] The use of anhydrous solvents to perform this reaction is essential since the ketone formed in contact with water forms the keto hydrate tautomer, which is not

reactive. At the end of the reaction, it was obtained 3-deoxy-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuran-3-ulose (**12**) with a yield of 99%. (Figure 47)
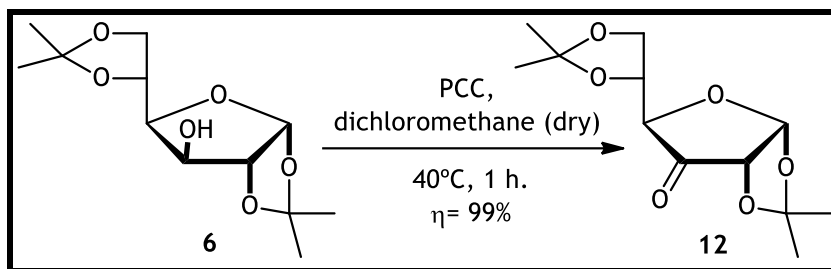
Figure 47. Synthesis of 3-deoxy-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuran-3-ulose (**12**).

Then, was performed the reduction of compound **12**, to obtain compound **11**, which is an epimer of compound **6**. To do so, it is necessary a reducing agent, like sodium borohydride and anhydrous ethanol. This reaction is a common reduction reaction to reduce a ketone to a secondary alcohol, like the one performed to reduce the aldehyde into a primary alcohol described above. At the end of the reaction, it was obtained compound **11** with a yield of 91%. (Figure 48)
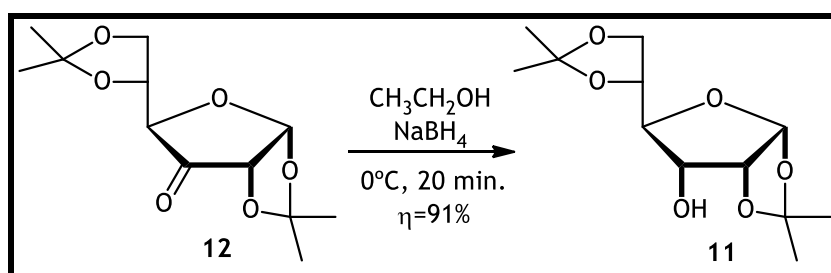
Figure 48. Synthesis of 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (**11**).

Afterwards, was made a benzylation of the compound **11**, to protect the free hydroxyl group linked to C3. This reaction formed the 3-*O*-benzyl-1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (**45**) with a yield of 99%. (Figure 49)
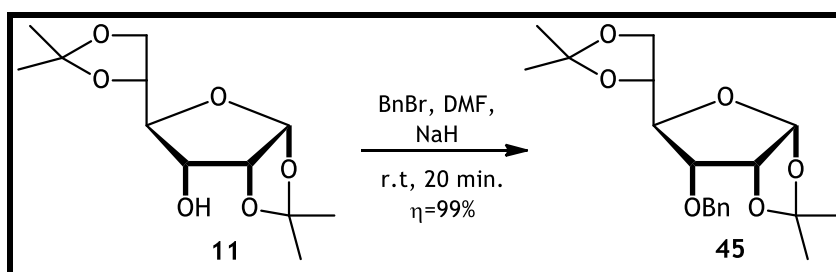
Figure 49. Synthesis of 3-*O*-benzyl-1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (**45**).

Subsequently was performed the acid hydrolysis of the isopropylidene group linked to C5 and C6 of compound **45** to obtain the 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-allofuranose (**46**), with a yield of 77%. (Figure 50)
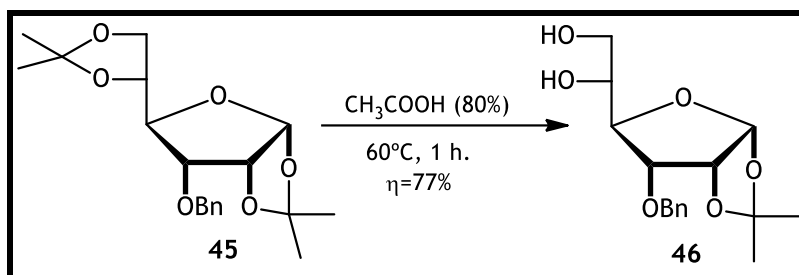
Figure 50. Synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-allofuranose (**46**).

Posteriorly was made an oxidative cleavage of the *cis*-vicinal diol from compound **46** to obtain the aldehyde 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribo-pentodialdo-1,4-furanose (**47**) with a yield of 69%. (Figure 51)
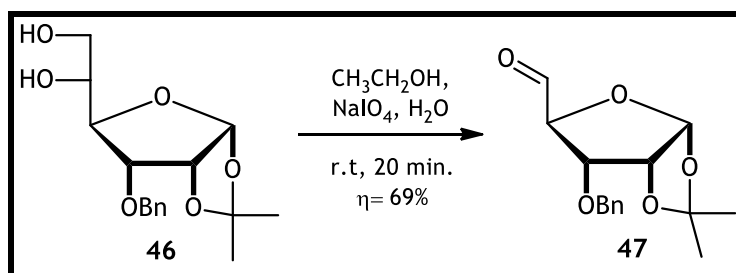
Figure 51. Synthesis of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribo-pentodialdo-1,4-furanose (**47**).

Then was performed a Wittig reaction, which is a reaction to increase the carbon chain between a ketone or an aldehyde and a phosphorus ylide, also known as, phosphorane that will give origin to the both isomers of an alkene and a triphenylphosphine oxide. In this case, the reagents were the aldehyde **47** and the (carbethoxymethylene)triphenylphosphorane. [9] Due to the structure of the phosphorane chosen, the final alkene obtained were the two isomers of an α, ß- unsaturated ester, named ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**48**) and ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*Z*)-enfuranoate (**49**). It was tried to isolate the both isomers, but that was not possible. The yield obtained for the mixture was 83%. (Figure 52)
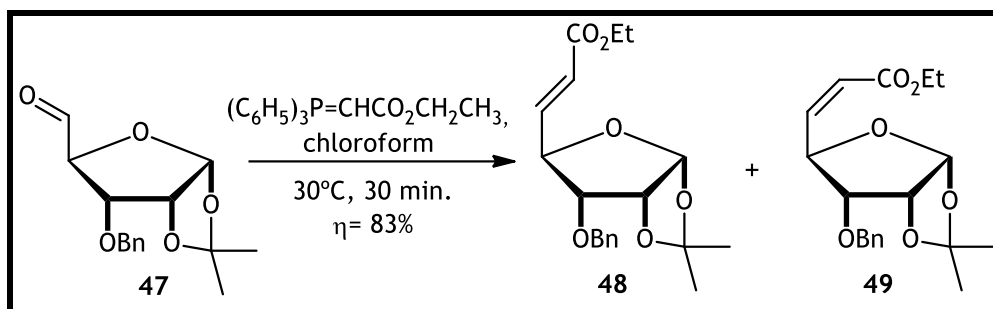
Figure 52. Synthesis of the isomers ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**47**) and ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*Z*)-enfuranoate (**48**).

Finally, was performed the reaction to obtain the pyrazolidin-3-one ring, in which only the *E* isomer of the α, β-unsaturated ester reacts due to the stereochemical impairment observed in the *Z* isomer. This reaction consists in the cyclization of the alkene-carbonyl system with the hydrazine monohydrate and consequent cleavage of the remaining carbon chain after the carbonyl carbon. At the end of this reaction where three different compounds were obtained, which are a pair of isomers: compound **42** and **43**; and a hydroxypyrazoline: compound **44**, with the yield of 10% for each one of them. (Figure 53)
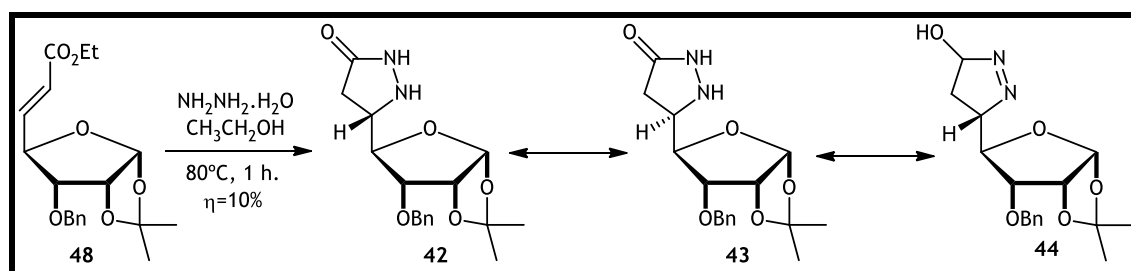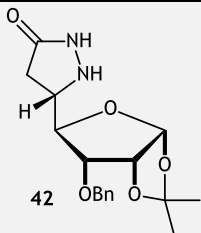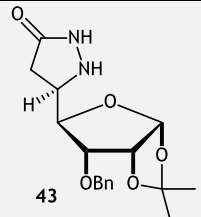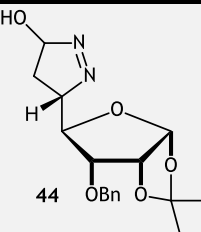
Figure 53. Synthesis of the pyrazolidin-3-one ring derivatives: 5-(*S*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**42**), 5-(*R*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**43**) and 5-(*S*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-3-hydroxypyrazoline (**44**).

The formation of the hydroxy tautomer can be related to the characteristics of the solvent used, once in the literature is described that the cyclization to form the pyrazolidin-3-one ring can lead to the formation of tautomers, but is also described that if the solvent used is anhydrous, there is only the formation of the principal product. [27,58] Therefore, as the solvent used was not anhydrous, it is plausible the formation of that tautomer.

The identification of the isomers was made based on the coupling constants (*J*) obtained in the Proton Nuclear Magnetic Resonance (¹H NMR) for the chemical shift (δ) correspondent to the proton of the chiral carbon of the pyrazolidin-3-one ring, named H-5. The *J* values are summarised in Table 4.

Table 4. Chemical shifts (δ in ppm) of the signals in ^{1}H NMR spectra of compounds **42**, **43** and **44** and the respective coupling constants (in Hertz).

Compound	H-5 (δ)	J
42	3.43 (dd)	6.80 7.58
43	3.42 (dd)	7.94 9.53
44	4.04 (dd)	6.72 7.45

The *J* value is influenced by the proximity of active nucleus, once its related to the coupling of two different active nuclei and the interaction between the various spin states. Therefore, if two active nuclei are nearby, then the *J* value is higher than if the nuclei are apart. So considering the spatial arrangement of the S isomer, it is possible to observe that the proton-linked to the chiral carbon of the heterocyclic ring is close to one of the protons of the adjacent carbon in the heterocyclic and apart from the other proton of the heterocyclic and from the proton of the carbohydrate moiety. So, looking to the coupling constants obtained in the spectra and comparing to the ones obtained for the R isomer, which is close to two protons and apart from one, it is clear that the coupling constants obtained for the S isomer have to be lower than the ones obtained for the R isomer. So, compound **42** must be the S isomer and compound **43** the R isomer. In the case of compound **44**, it was harder to identify the isomer present but looking to the coupling constants it is possible to see that the values are close to the ones of the compound **42**. Therefore as the coupling constants present values not so high as the ones associated with the R isomer, the compound **44** must be an S isomer.

The second synthetic pathway, which has been carried out, is similar to the first but without the benzylation of the free hydroxyl group attached to C3. In the final reaction, it was also obtained two pyrazolidin-3-one isomers and a tautomer. (Figure 54)
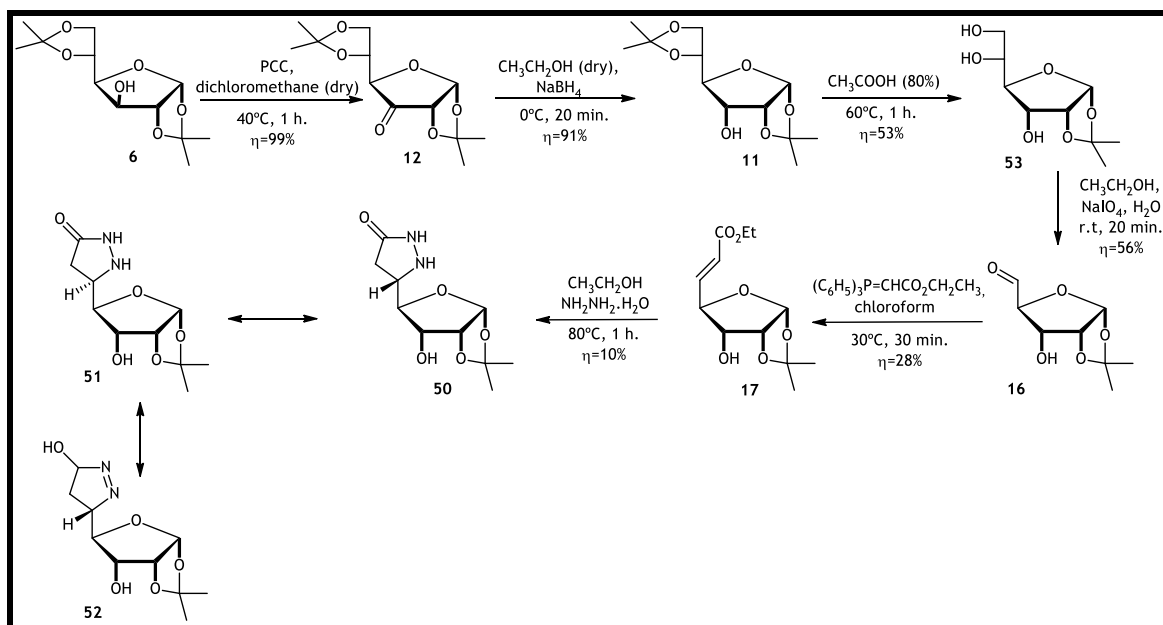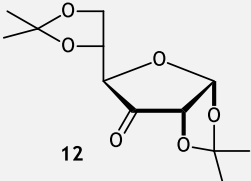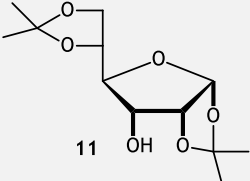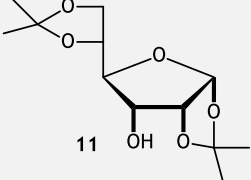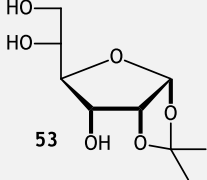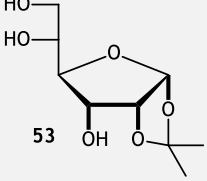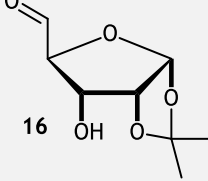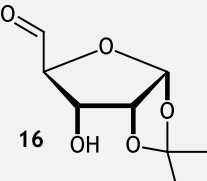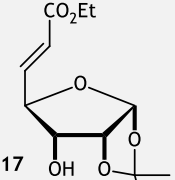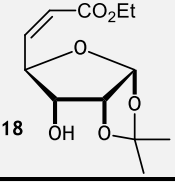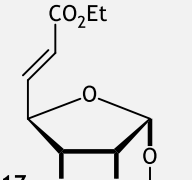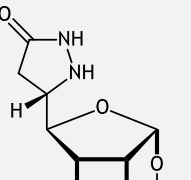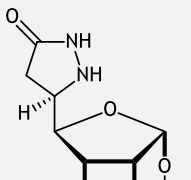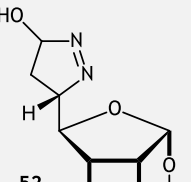
Figure 54. Synthesis of compounds 5-(*S*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**50**), 5-(*R*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**51**) and 5-(*S*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-3-hydroxypyrazoline (**52**).

Briefly, this pathway starts with compound **6** and first was made the epimer formation, which consists in the oxidation of the free hydroxyl group linked to C3 and further reduction, where is obtained the compound **11**. Then, was performed the acid hydrolysis of the isopropylidene group attached to the C5 and C6 of the furanose ring, to get the 1,2-*O*-isopropylidene-α-D-allofuranose (**53**) followed by the oxidative cleavage in which is formed compound **16**. Finally, was made the increase of the carbon chain, by a Wittig reaction and the cyclization to obtain compounds **50**, **51** and **52**.

Since the reactions performed, were all explained above, the starting compounds, the compounds obtained, the type of reaction and the yields are all summarised, in columns, in Table 5, and in each row, is represented a reaction step.
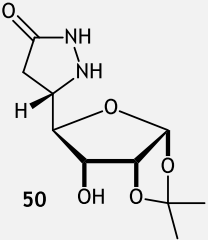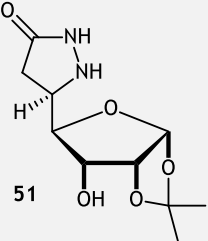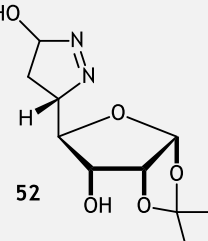
Table 5. Synthesis of compounds 5-(*S*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**50**), 5-(*R*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**51**) and 5-(*S*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-3-hydroxypyrazoline (**52**).

Starting compound	Obtained compound	Reaction	Yield
6	**12**	Oxidation	99%

Starting material	Product	Reaction	Yield
12	11	Reduction	91%
11	53	Acid hydrolysis	53%
53	16	Oxidative cleavage	56%
16	17 + 18	Wittig reaction	28%
17	50	Cyclization to obtain the pyrazolidin-3-one ring	10%
	51		10%
	52		10%

The identification of the isomers was also made based on the coupling constants obtained in ^{1}H NMR for the chemical shift correspondent to the proton of the chiral carbon of the pyrazolidin-3-one ring, named H-5. The *J* values are summarised in Table 6.
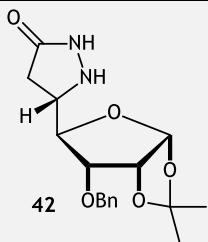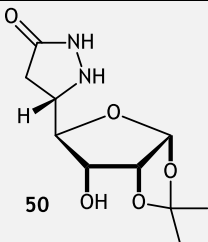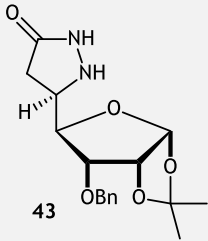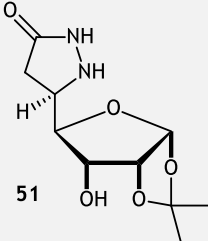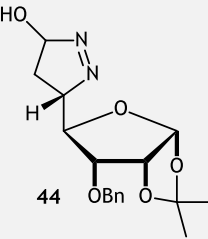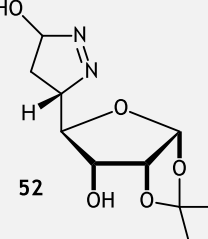
Table 6. Chemical shifts (δ in ppm) of the signals in ^{1}H NMR spectra of compounds **50**, **51** and **52** and the respective coupling constants (in Hertz).

Compound	H-5 (δ)	J
50	3.49 (dd)	2.81 6.98
51	3.48 (dd)	7.09 9.29
52	4.25 (dd)	2.69 4.89

Making the same considerations as above, the coupling constants obtained in the spectra of the S isomer compared to the ones obtained for isomer R must be lower. So, it is clear that the compound **50** must be the S isomer and the compound **51** the R isomer. In the case of compound **52**, the values of the coupling constants are close to the ones **50**. Therefore, it is clear that the coupling constants of the structure of compound **52** are low, so, the isomer present must be the S isomer.

Since the difference between the two sets of pyrazolidin-3-one compounds is the protection or not of the free hydroxyl group attached to the position 3 of the furanose ring, it makes perfect sense to compare the yields. Therefore, this information is summarised in Table 7, in which in the first row is presented the information for compound **42** and compound **50**; in the second row is present the information for compound **43** and **51**; and in the third row is present the information for compound **44** and compound **52**.

Table 7. Comparison of the yields of the pyrazolidin-3-ones derivatives in the both synthetic pathways.

Compound	Yield	Compound	Yield
42	10%	**50**	10%
43	10%	**51**	10%
44	10%	**52**	10%

It is possible to verify, from the table above, that the yields obtained for the different isomers and the hydroxy tautomer are the same and are formed in an equal proportion, being 10% for each form.

The final yields of the pyrazolidin-3-ones derivatives are very low, but the compounds were pure, once it was carried out a purification step, in which the three products could be separated. Nevertheless, for the scope of this dissertation, the mass obtained was enough, because to perform the *in vitro* studies it was not needed more than 10 mg.

The last synthetic route, which has been attempted in this part, involves a structural modification comparing with the compounds presented above. So, instead of the heterocyclic being attached to the C4 of the furanose ring, it was tried to link the heterocyclic to the C3.

Therefore, to perform this synthetic pathway, the starting compound was the **29**. Then, was attempt to do a selective benzylation of the hydroxyl group attached to the position 5 of the furanose ring. To carry out this reaction the temperature should have been -80ºC, however, the desired temperature could not be reached, so the reaction was made at a higher temperature (-17ºC) which resulted in the benzylation of the two hydroxyl groups of the xylose, forming the 3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**54**) with a yield of 10 %. (Figure 55) [57]
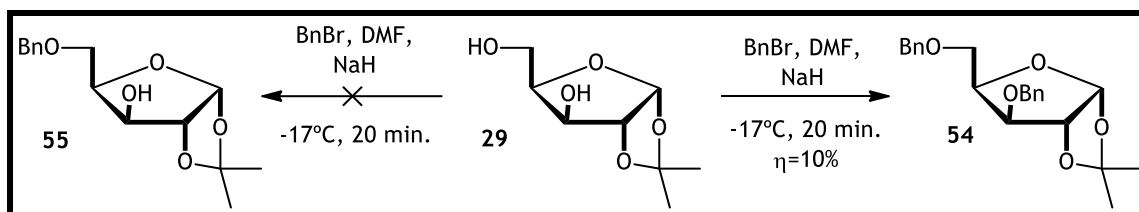
Figure 55. Synthesis of 3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**54**) in which 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**55**) should have been obtained.

Then, since the desired compound could not be obtained, once the hydroxyl group linked to C3 was also protected it was not possible to continue this synthetic pathway. However, the following steps consisted of an oxidation reaction of the free hydroxyl group at C3, followed by a Reformatsky reaction, which consists in the reaction of an aldehyde or ketone with a α-halo ester, which in this case it would be a bromoester. Subsequently, the ester would be reduced to an aldehyde using a proper hydride to avoid the reduction to alcohol. An example of a suitable hydride for this reaction is the Di-isobutyl Aluminum Hydride (DIBAL). Next, a Wittig reaction would be done, which allows to increase the carbon chain and to form a α, β-unsaturated ester and allows the final cyclization to form the pyrazolidin-3-one derivative.

1.3 5-Hydroxypyrazoles

5-Hydroxypyrazoles was the third and the last technique used to synthesise pseudo-*C*-nucleosides, using a β-enaminoester and hydrazine monohydrate.

The first synthetic pathway, which has been carried out, in this part, has compound **6** as the starting material. First, was performed an acid hydrolysis of the isopropylidene group linked to C5 and C6. Posteriorly, was done an oxidative cleavage for subsequent Wittig reaction to obtain an α, β- unsaturated ester. Finally, was made an addition to the ester of an amine group using the microwave, to do so was used sodium azide, which is a good reagent to add nitrogen atoms to compounds, forming a β-enaminoester, which was the precursor for the final reaction to obtain the 5-hydroxypyrazole. (Figure 56)
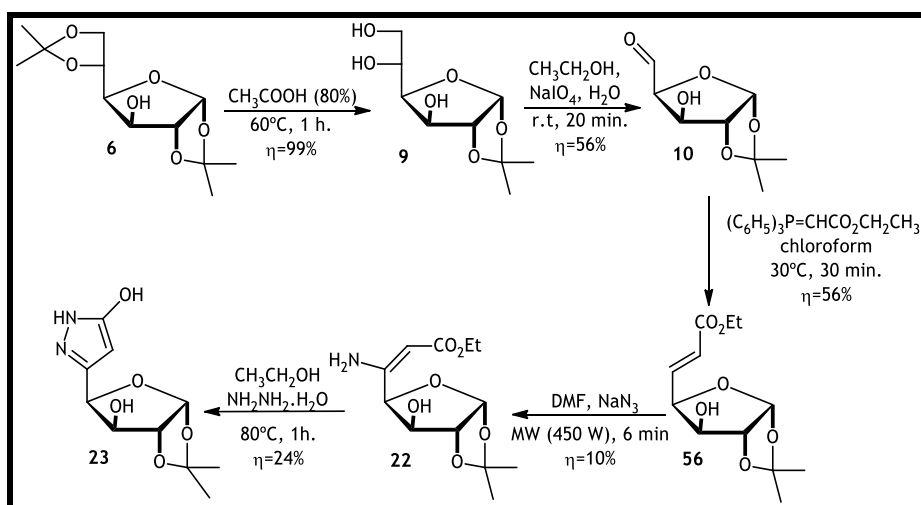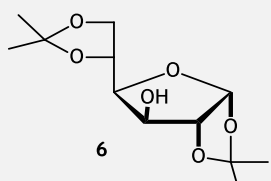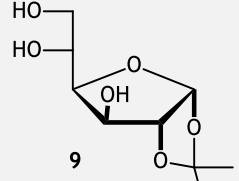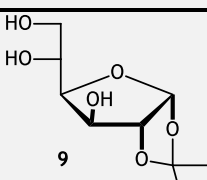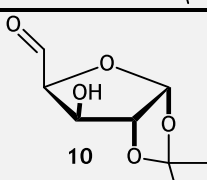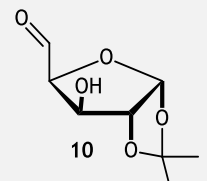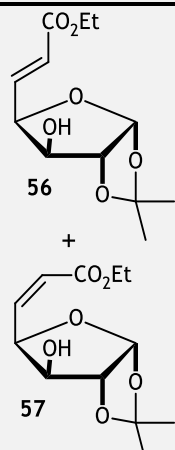
Figure 56. Synthetic pathway of 3-(1,2-*O*-isopropylidene-α-D-threofuranos-4-*C*-yl)-5-hydroxy-1*H*-pyrazole (**23**).

The synthetic pathway which has been carried out is similar to the present above, on the topic of the pyrazolidin-3-ones, until getting the α, ß-unsaturated ester. Briefly, this pathway started with compound **6** and was performed the acid hydrolysis of the isopropylidene group attached to the C5 and C6 of the furanose ring, to get the 1,2-*O*-isopropylidene-α-D-glucofuranose (**9**). Then, was made an oxidative cleavage followed by a Wittig reaction to obtain compounds ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*E*)-enfuranoate (**56**) and ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*Z*)-enfuranoate (**57**).

Since the reactions performed until getting the ester were explained above, the starting compounds, the compounds obtained, the type of reaction until that point and the yields are present in columns, and are all summarised in Table 8.

Table 8. Reactional pathway to get ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*E*)-enfuranoate (**56**) and ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*Z*)-enfuranoate (**57**) from 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**6**).

Starting compound	Compound obtained	Reaction	Yield
6	**9**	Acid hydrolysis	99%
9	**10**	Oxidative cleavage	56%
10	**56** + **57**	Wittig reaction	57%

Then, was made a reaction between the *E* isomer of the α, ß – unsaturated ester and the sodium azide to obtain a ß-enaminoester since the *Z* isomer cannot cyclize due to the stereochemical impairment. This strategy consists in the cyclization of the carbonyl with an amine, as an intermediate, and further rearrangement of the structure leading to an aliphatic compound. [58] This reaction was a microwave-assisted reaction, since this strategy is stereoselective,

obtaining almost exclusively the *E* isomer, which is the isomer required for the next step in this synthetic pathway. [58] In the final of this reaction was obtained Ethyl 5-amino-5,6-dideoxy-1,2-O-isopropylidene-α-D-xylo-hept-5-(E)-enfuranoate (**22**) in a 10% yield and was necessary to purify the product. (Figure 57)
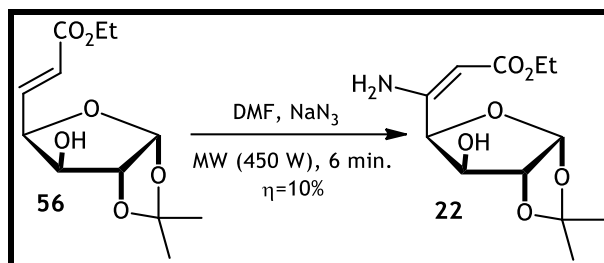
Figure 57. Synthesis of Ethyl 5-amino-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*E*)-enfuranoate (**22**)

Finally, was performed the cyclization of the system enamine-alkene-carbonyl with the hydrazine monohydrate and a consequent rearrangement where part of the carbon chain and the amine group of the ß-enaminoester are cleavage, allowing the formation of the aromatic heterocyclic with a free hydroxyl group linked to the C5. [58] The ß-enaminoester is the adequate starting compound for this reaction due to the nucleophilicity of the enamine and the electrophilicity of the enone. [59] In the final of this reaction was obtained compound **23** in a 24% yield and was necessary to purify the product. (Figure 58)
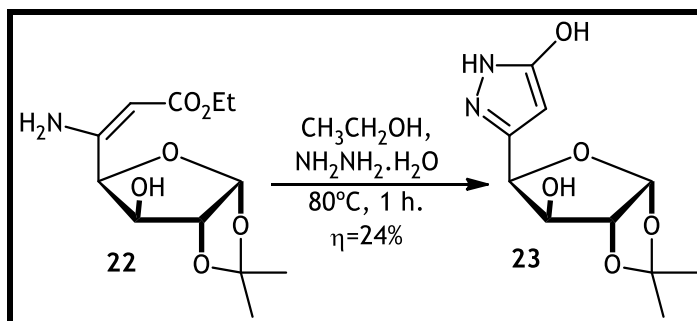
Figure 58. Synthesis of 3-(1,2-*O*-isopropylidene-α-D-threofuranos-4-*C*-yl)-5-hydroxy-1*H*-pyrazole (**23**).

The second synthetic pathway which has been carried out is similar to the first, but it was necessary to do the epimer formation first. At the end of this reactional pathway, it was formed the 3-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-5-hydroxy-1*H*-pyrazole (**58**). (Figure 59)
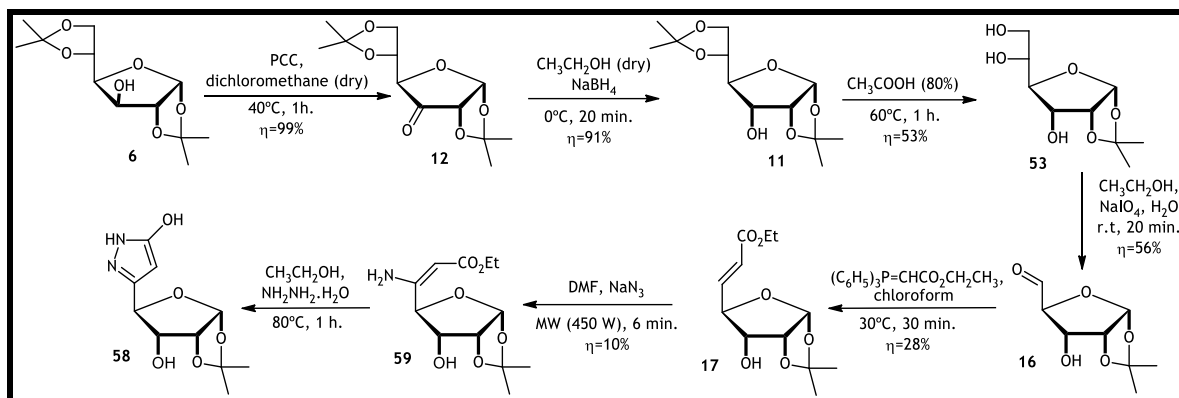
Figure 59. Synthetic pathway in which 3-(1,2-O-isopropylidene-α-D-erythrofuranos-4-C-yl)-5-hydroxy-1H-pyrazole (**58**) should have been obtained.

Briefly, this pathway starts with compound **6** and first was made the epimer formation, which consists in the oxidation of the free hydroxyl group linked to C3 and further reduction, where is obtained the compound **11**. Then, was performed the acid hydrolysis of the isopropylidene group attached to the C5 and C6 of the furanose ring, to get the 1,2-O-isopropylidene-α-D-allofuranose (**53**) followed by the oxidative cleavage in which is formed compound **16**. Posteriorly, was made the increase of the carbon chain, by a Wittig reaction, and then the formation of the ß-enaminoester, ethyl 5-amino-5,6-dideoxy-1,2-O-isopropylidene-α-D-ribo-hept-5-(E)-enfuranoate (**59**). Finally, the cyclization to obtain compound **56** was made. However, the final pure compound could not be obtained, even using chromatographic techniques.

Since the reactions performed were all explained above, the starting compounds, the compounds obtained, the type of reaction and the yields, expect the final cyclization step are presented in columns, are all summarised in Table 9.

Table 9. Reactional pathway to obtained the ethyl 5-amino-5,6-dideoxy-1,2-O-isopropylidene-α-D-ribo-hept-5-(E)-enfuranoate (**59**).

Starting compound	Compound obtained	Reaction	Yield
6	**12**	Oxidation	99%
12	**11**	Reduction	91%

11	**53**	Acid hydrolysis	53%
53	**16**	Oxidative cleavage	56%
16	**17** + **18**	Wittig reaction	28%
17	**59**	Formation of the β-enaminoester	10%
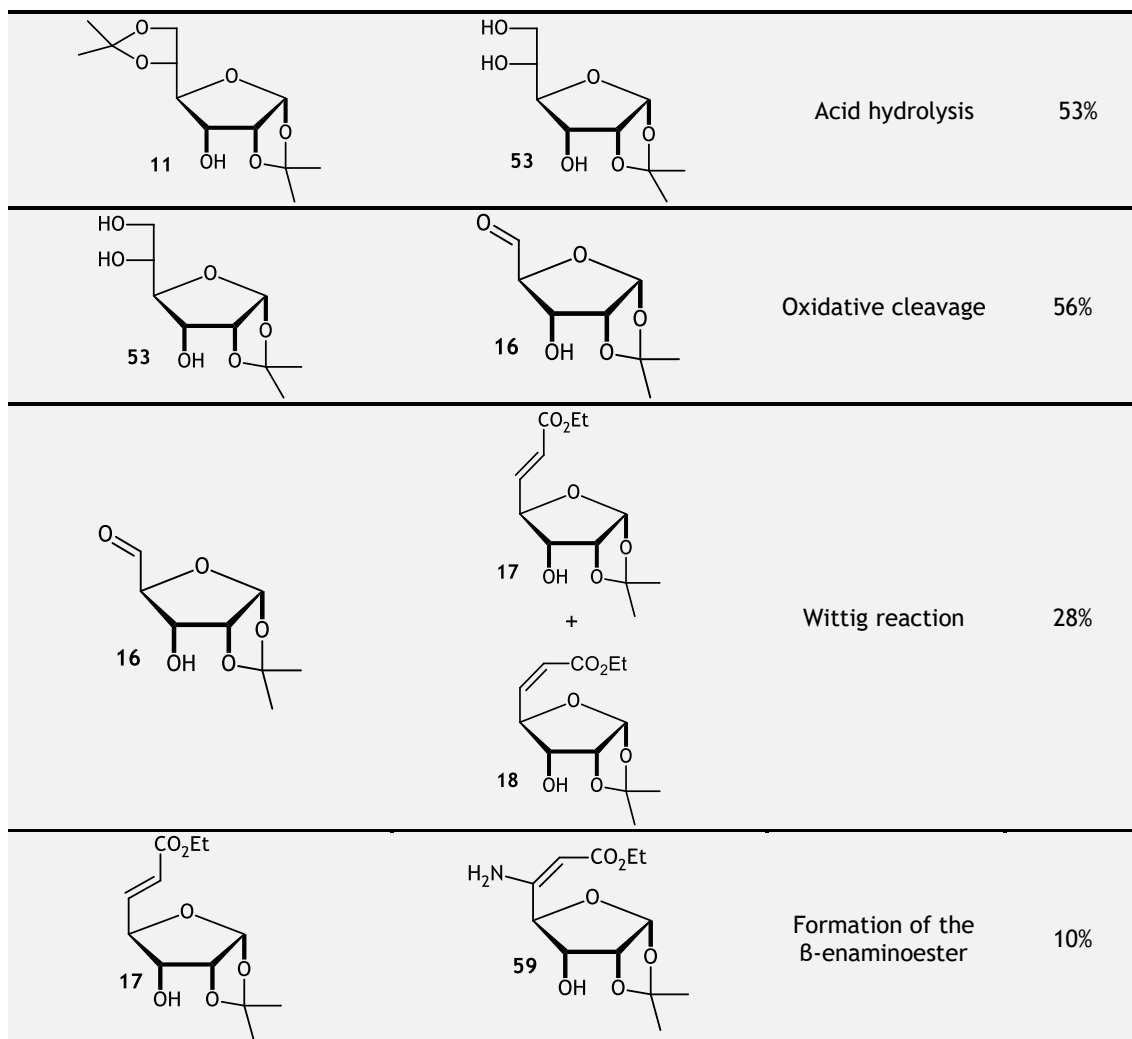
Unfortunately, only a 5-hydroxypyrazole derivative was obtained, and the final yield was low, but the compound was pure, once it was carried out a purification step, in which the product could be isolated. Nevertheless, for the scope of this dissertation, the mass obtained was enough, because to perform the *in vitro* studies does not need more than 10 mg.

In attachment 2 are presented the Lipinski's rule of five data for the final compounds **23**, **42-44** and **50-52**, predicted by MedChem Designer 3.1.0.30, in which every compound follows all the established parameters.

2. Biological assay

Biological assays include tests carried out on living organisms (*in vivo*) and on artificial environments (*in vitro*). Within the scope of this dissertation, it was only performed *in vitro* tests, which are part of the preclinical tests in the drug discovery process. This type of tests serves to access, mainly, aspects of toxicity, the efficacy and the mechanism of action, the SAR and some pharmacokinetics properties of compounds. [35]

In this work, a cytotoxicity assay was performed for the synthesised compounds on two different cell lines. The cell lines used were N27 (Rat Mesencephalic Dopaminergic Neural Cells) and the NHDF (Normal Human Dermal Fibroblasts). The first is a healthy dopaminergic rat cell line, which served to assess the cytotoxic effect of the compounds on neuronal cells, while the second is a healthy dermal cell line that was used to evaluate general cellular cytotoxicity in healthy cells in another part of the body, since it is intended that the compounds not present cytotoxicity in any part of the body.

For this, it was performed the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay, which is a colourimetric method to evaluate the cytotoxicity of compounds. This method is based on the MTT metabolization by mitochondrial reductases of the viable cells forming insoluble formazan crystals.
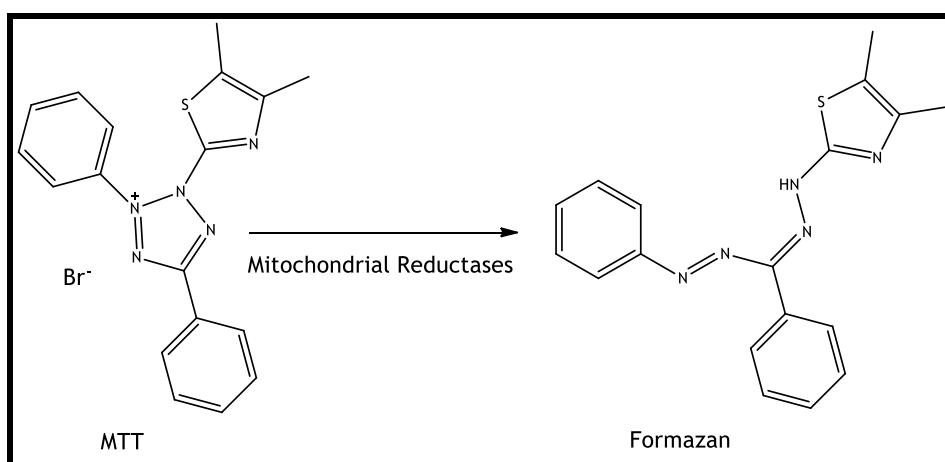
Figure 60. MTT reduction, mediated by mitochondrial reductases, forming formazan.

MTT is a yellow compound, and its metabolite is purple and therefore, after its solubilization, the absorbance at 570 nm, which is the wavelength at which the formazan crystals absorb, is measured. In this context, it is considered that the higher the absorbance read, the higher was the MTT metabolism and, consequently, the cell viability.

In the MTT assay cells were incubated for 72 hours in the presence of the compounds studied in a concentration of 30 μM.

In all the assays, 5-fluorouracil (5-FU) was used as the positive control. This compound is an analogue of the uracil and is used worldwide as an antineoplastic agent. This drug acts mainly by thymidylate synthetase inhibition, which is an enzyme responsible for the cell growth and function, causing cell death. Therefore, this compound is used as a positive reference, since its action is already studied and characterised in cell lines. [60]

The negative control, named "Control" in Graph 1, was constituted only by complete cell culture medium. For NHDF cells the medium was RPMI 1640 properly supplemented and complemented with FBS (Fetal Bovine Serum) and an antibiotic/antimycotic solution (Ab), and

for N27 cells the medium was RPMI 1640 medium complemented with FBS and an antibiotic solution (Sp).

The relative cell viability observed in both cell lines after exposition to 5-hydroxypyrazole **23** and to pyrazolidin-3-one derivatives **42**, **43**, **44**, **50**, **51** and **52** are summarised in Graph 1.
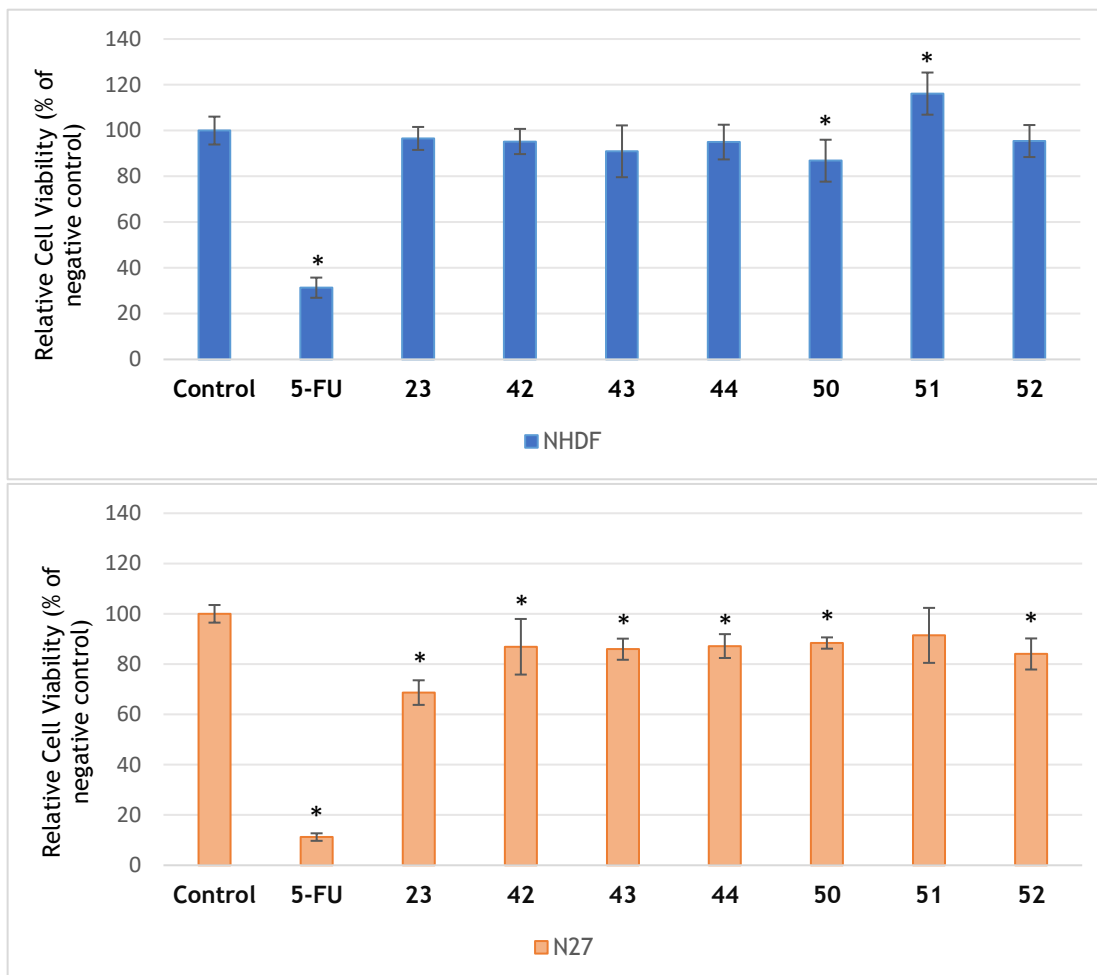
Graph 1. Results observed in the MTT assays screenings in NHDF (blue) and N27 (orange) cells after 72 hours of exposition to the compounds at 30 μM. The statistical significance of the data was determined by the Student's *t*-Test of the data in relation to the negative control. * p <0.05 indicates statistical significance.

The data from the Graph 1 are expressed as cell viability percentage (%) relative to the negative control and the values presented are the mean ± standard deviation representative of two independent assays. By analysing this graph, it is possible to verify that the final synthesised compounds always lead to values of cellular viability higher than the observed with 5-FU, and it is also possible to conclude that all the compounds studied do not present relevant cytotoxicity. In the NHDF cell line, it was observed that compound **50** leads to values of cell viability superior to the negative control, which means that this compound stimulates the proliferation of this cell line in these experimental conditions. On the other hand, compound **23** was the most cytotoxic for N27 cells.

These results are insufficient to infer about the potentiality of these compounds in the treatment of bipolar disorder. However, the low toxicity observed for these compounds in these assays are a positive indicator to continue and to deepen this study.

Chapter IV

Conclusions and Future Work

In this work, three different techniques to obtain pseudo-*C*-nucleosides were explored and applied. When the synthesis involved the Click Chemistry reaction, despite several attempts, which included changing the protecting group used, and modifying the substituents, mainly at the position 3 of the furanose ring, no pseudo-C-nucleoside was obtained. However, with the other two strategies used, which aimed to form pyrazolidine-3-ones and 5-hydroxypyrazoles, some of the desired final compounds have been achieved. In the case of pyrazolidine-3-ones, two sets of three compounds were obtained, being a pair of isomers and a hydroxy tautomer, all of them with a low yield. In the case of the third technique, only one 5-hydroxypyrazole, derived from the α-D-glucose could be obtained, again in a low overall yield.

Although some of the techniques attempted did not work, we were able to obtain seven different compounds for *in vitro* tests. Thus, MTT assays were performed to evaluate the cytotoxicity of the compounds in two different cell lines, the N27 and the NHDF. At the end of the assays, it was possible to state that the compounds do not present relevant cellular cytotoxicity and, therefore, although it cannot be inferred about their action in bipolar disorder, they seem to be safe to follow for additional tests.

As future work, it is proposed to synthesize carbohydrate derivatives with the sulfonyl group and bioisosters attached to the furanose ring and prepare other analogues of these compounds for accurate SAR studies to subsequently perform assays that allow to access the efficacy of the compounds as anti-BD agents, in appropriate models. Lastly, if the compounds pass all the standard *in vitro* tests, it will be important to test in *in vivo* suitable models to know the effects (for the treatment of the disease and side effect) of these compounds.

It is also important to test the effects of these compounds in another cell lines namely to evaluate their toxicological effects in another type of neuronal cells. Beyond that, it will be interesting to test those compounds in primary cultures of rat embryos to assess neurotrophic effects in damaging conditions to know if these compounds attract neurotrophic factors to control and try to fix the damage.

Chapter V

Experimental Part

1. General Data

All reagents and solvents used are analytically pure and were bought at Fisher, Merck, Aldrich or Acros Organics. When necessary, the solvents were dried and purified using the appropriate standard methods. [61]

All reactions were followed by thin layer chromatography (TLC). The TLC plates used are from Macherey-Nagel (60 G / UV_{254}), which are made of 2 mm aluminium and coated with silica gel. All plates were visualized using UV light (λ = 254 nm) and then immersed in a revealing solution depending on the synthesized compound. The revealing solutions were:
a) the vanillin revealing solution that is made by the addition of vanillin (3 g) to ethanol (100 mL) and subsequent addition of sulfuric acid (1.5 mL);
b) Hanessian's revealing solution, which is made by adding ammonium molybdate tetrahydrate (5 m/v) and cerium ammonium sulphate dihydrate (0.2% m/v) to a 6% aqueous solution of sulfuric acid;
c) and the phosphomolybdic acid revealing solution which is made by the addition of phosphomolybdic acid (5 g) to ethanol (100 ml). The plates revealed with the revealing solutions a) and c) were heated to 200°C and those revealed with revealing solution b) at 100°C. The eluent for the plates is referred to each case.

When necessary, chromatographic columns were performed to purify the compounds in which the stationary phase is silica gel 60A (40-60 μm), and the mobile phase varies to be suitable for the compounds and is referred to each case. The columns were, where possible, made under reduced pressure using an appropriate pump. Alternatively, whenever it was not feasible, it was made at ambient pressure.

The FTIR (Fourier Transform InfraRed) spectra were made in a Thermo Fisher Scientific Nicolet Is10 spectrophotometer obtained by ATR (Attenuated Total Reflectance) using Omnic 8.2 software. To get the spectra were performed 32 scans between 4000 cm^{-1} and 600 cm^{-1} with a resolution of 4 cm^{-1}.

NMR spectra were performed on a Brüker Avance III spectrometer where ^1H was acquired at 400.13 MHz and ^{13}C at 100.62 MHz. The internal standard used was tetramethylsilane (TMS), and spectra were processed in TopSpin 3.1 software. The solvent used was deuterated chloroform ($CDCl_3$, δ = 7.26 ppm for ^1H NMR and 77.16 ppm for ^{13}C NMR). In addition to these

two types of spectra, the DEPT (Distortionless Enhancement by Polarization Transfer) technique and the bidimensional HSQC (Heteronuclear Single Quantum Coherence) spectra were also made when necessary. All chemical shifts are in ppm and the coupling constants in Hertz (Hz). NMR spectra were made for all compounds, and only those not found in the literature are shown.

All the structures were drawn using the ChemDraw Ultra 12.0.2 software from CambridgeSoft.

All the compounds synthesised that were submitted to biological evaluation assays were pure according to the ^{1}H NMR and the ^{13}C NMR (carbon-13 Nuclear Magnetic Resonance) spectrum data.

2. Synthesis techniques description

2.1 General technique to add a benzyl group to a free hydroxyl group

A carbohydrate derivative with a free hydroxyl group at C3 (3.84 mmol) was dissolved in DMF (1.6 mL) with stirring, and after BnBr (0.91 mL, 7.65 mmol) was added dropwise. After, the reaction mixture was placed in an ice bath, and NaH (0.31 g, 12.96 mmol) was added and reacted for 20 minutes at room temperature. The end of reaction was confirmed by TLC. [62]

To neutralise the hydride was added methanol (0.6 mL) dropwise, and the solution was then concentrated. Finally, an extraction was performed with dichloromethane (3 x 25 mL), and the organic phase was dried with NaSO$_4$ and then concentrated to dryness. [62]

i. 3-O-benzyl-1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (25)

Starting compound: 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (**6**) (1 g, 3.84 mmol)

Revealing Solution: a)

ŋ = 99% (1.332 g)

Rf = 0.86 Ethyl Acetate/ n-Hexane (1/1)

^{1}H NMR and ^{13}C NMR: according to literature. [63]

FTIR-ATR (cm^{-1}): 1650 (C=C)

25

MF: C$_{19}$H$_{26}$O$_6$
MW: 350.406 g/mol
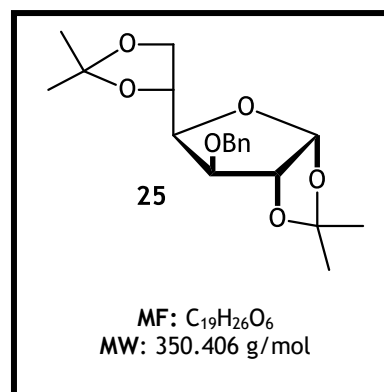
ii. 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose (31)

Starting compound: 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose (**30**) (1 g, 2.31 mmol)

Revealing Solution: a)

To purify the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/3) as eluent.

η = 58% (0.701 g)

Rf = 0.80 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR and ^{13}C NMR: according to literature. [63]

FTIR-ATR (cm^{-1}): 1600 (C=C)

MF: $C_{34}H_{34}O_5$
MW: 522.631 g/mol
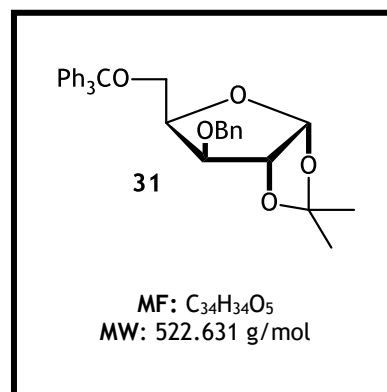
iii. 3-*O*-benzyl-1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (45)

Starting compound: 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (**11**) (1 g, 3.84 mmol)

Revealing Solution: a)

η = 99% (1.332 g)

Rf = 0.79 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR and ^{13}C NMR: according to literature. [64]

FTIR-ATR (cm^{-1}): 1663 (C=C)

MF: $C_{19}H_{26}O_6$
MW: 350.406 g/mol
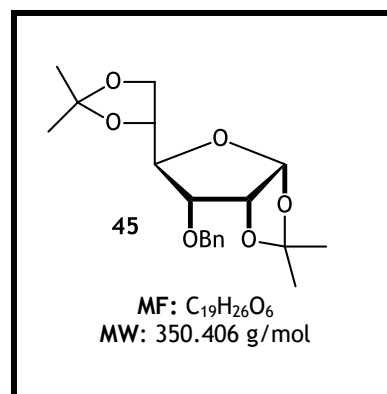
iv. 3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (54)

Starting compound: 1,2-*O*-isopropylidene-α-D-xylofuranose (**29**) (1 g, 5.26 mmol)

Alteration to the protocol: Instead of doing the reaction at the room temperature, it was performed at -17°C

Revealing Solution: a)

η = 10% (0.195 g)

Rf = 0.75 Ethyl Acetate/ *n*-Hexane (1/1)

MF: $C_{22}H_{26}O_5$
MW: 370.439 g/mol
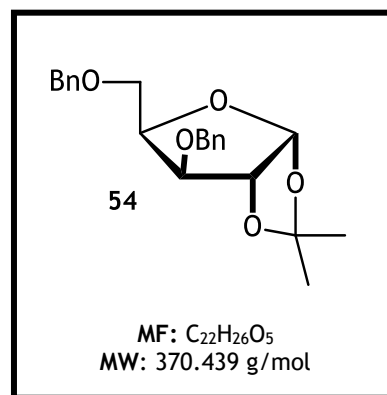
^{1}H NMR (400.1 MHz, CDCl$_3$), δ (ppm): 7.34 - 7.17 (m, 10H, C$_6$H$_5$); 4.72 (d, *J* = 4.16 Hz, 1H, H-1); 4.63 (d, *J* = 4.77 Hz, 1H, H-4); 4.54 - 4.47 (m, 4H, CH$_2$Ar); 3.81 (d, *J* = 2.08 Hz, 1H, H-2); 3.67 (m, 1H, H-3); 1.39 - 1.26 (s, 6 H, CH$_3$ of the isopropylidene group).

^{13}C NMR (100.6 MHz, CDCl$_3$), δ (ppm): 137.79, 137.32 (Cq, C$_6$H$_5$); 128.58, 128.40, 128.20, 128.01, 127.85, 127.68 (CH, C$_6$H$_5$); 111.77(Cq, $\underline{C}$(CH$_3$)$_2$); 105.14 ($\underline{C}$H, C-1); 82.34 ($\underline{C}$H, C-4); 81.75 ($\underline{C}$H, C-2); 79.6 ($\underline{C}$H, C-3); 78.8 (Cq, CH$_2$$\underline{C}$≡CH); 74.70 ($\underline{C}H, CH_2$C≡$\underline{C}$H); 72.07 ($\underline{C}H_2$Ar); 67.39 ($\underline{C}H_2$, C-5); 58.63 ($\underline{C}H_2$, $\underline{C}$H$_2$C≡CH); 26.82, 26.35 ($\underline{C}$H$_3$ of the isopropylidene group).

FTIR-ATR (cm^{-1}): 1635 (C=C).

2.2 General technique for acidic hydrolysis (deprotection of C5 and C6)

A solution of acetic acid 80% (20 mL) was added to a carbohydrate with an isopropylidene group linked to C5 and C6 (2.85 mmol) and reacted with stirring at 60°C for 1 hour or overnight with stirring at room temperature. The end of the reaction was confirmed by TLC. [62]

Then, the solution was concentrated using *n*-hexane as a co-solvent. [62]

i. 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (26)

Starting compound: 3-*O*-benzyl-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**25**) (1 g, 2.85 mmol)

Revealing Solution: a)

To purify the residue a silica gel column chromatography was made, using a mixture of Ethyl Acetate/*n*-Hexane (1/1) as eluent.

** η = 72% (0.637 g)**

Rf = 0.25 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR and ^{13}C NMR: according to literature. [63]

FTIR-ATR (cm^{-1}): 3421 (O-H), 1723 (C=C).

26

MF: C$_{16}$H$_{22}$O$_6$
MW: 310.342 g/mol

ii. 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (37)

Starting compound: 3-*O*-acetyl-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**36**) (1 g, 3.31 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/1) as eluent.

η = 92% (0.799 g)

Rf = 0.27 Ethyl Acetate/ *n*-Hexane (1/1)

37

MF: C$_{11}$H$_{18}$O$_7$
MW: 262.256 g/mol

¹H NMR and ¹³C NMR: according to literature. [65]

FTIR-ATR (cm⁻¹): 3431 (O-H), 1725 (C=O).

iii.   3-iodo-1,2-_O_-isopropylidene-α-D-glucofuranose (41)

Starting compound: 3-iodo-1,2:5,6-Di-_O_-isopropylidene-α-D-glucofuranose (**14**) (1 g, 2.70 mmol)

Revealing Solution: a)

η = 50% (0.446 g)

¹H NMR and ¹³C NMR: according to literature. [63]

Rf = 0.23 Ethyl Acetate/ _n_-Hexane (1/1)

MF: $C_9H_{15}IO_5$
MW: 330.117 g/mol

iv.   3-_O_-benzyl-1,2-_O_-isopropylidene-α-D-allofuranose (46)

Starting compound: 3-_O_-benzyl-1,2:5,6-Di-_O_-isopropylidene-α-D-allofuranose (**45**) (1 g, 2.85 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/_n_-Hexane (1/1) as eluent.

η = 77% (0.682 g)

Rf = 0.19 Ethyl Acetate/ _n_-Hexane (1/1)

¹H NMR and ¹³C NMR: according to literature. [64]

FTIR-ATR (cm⁻¹): 3423 (O-H), 1617 (C=C).

MF: $C_{16}H_{22}O_6$
MW: 310.342 g/mol

v.   1,2-_O_-isopropylidene-α-D-allofuranose (53)

Starting compound: 1,2:5,6-Di-_O_-isopropylidene-α-D-allofuranose (**11**) (1 g, 3.84 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/ _n_-Hexane (6/1)

η = 53% (0.448 g)

MF: $C_9H_{16}O_6$
MW: 220.220 g/mol

Rf = 0.13 Ethyl Acetate/ *n*-Hexane (6/1)

^{1}H NMR and ^{13}C NMR: according to literature. [64]

FTIR-ATR (cm^{-1}): 3427 (O-H).

vi.   1,2-*O*-isopropylidene-α-D-glucofuranose (9)

Starting compound: 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**6**) (1 g, 3.84 mmol)

Revealing Solution: a)

η = 99% (0.837 g)

Rf = 0.15 Ethyl Acetate/ *n*-Hexane (6/1)

^{1}H NMR and ^{13}C NMR: according to literature. [66]

FTIR-ATR (cm^{-1}): 3423 (O-H).

MF: $C_9H_{16}O_6$
MW: 220.220 g/mol

2.3 General technique for oxidative cleavage (aldehyde formation)

To a solution of carbohydrate derivative with free hydroxyl groups at C5 and C6 (3.22 mmol) in ethanol (2 mL) was added a solution of sodium periodate (1.711 g, 8 mmol) in water (35 mL). ML) and react with stirring at room temperature for 20 minutes isolated from light. The end of the reaction was confirmed by TLC. [62]

Then ethanol (340 mL) was added to precipitate all the inorganic salts formed, and after that, a filtration of the solution was performed which was then concentrated at low temperature (20-25°C). Finally, an extraction was carried out with dichloromethane (3 x 40 mL), and the organic phase was dried over NaSO$_4$ and then concentrated to dryness. [62]

i. 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (27)

Starting compound: 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**26**) (1 g, 3.22 mmol)

Revealing Solution: a)

η = 90% (0.807 g)

Rf = 0.61 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR and ^{13}C NMR: according to literature. [15]
FTIR-ATR (cm^{-1}): 1715 (C=O), 1654 (C=C).

MF: $C_{15}H_{18}O_5$
MW: 278.300 g/mol

ii. 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (38)

Starting compound: 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**37**) (1 g, 3.81 mmol)

Revealing Solution: a)

η = 52% (0.456 g)

Rf = 0.39 Ethyl Acetate/ *n*-Hexane (1/1)

MF: $C_{10}H_{14}O_6$
MW: 230.215 g/mol

^{1}H NMR (400.1 MHz, CDCl₃), δ (ppm): 9.65 (s, 1H, H-5); 5.52 (d, *J* = 3.55 Hz, 1H, H-3); 5.33 (d, *J* = 4.04 Hz, 1H, H-1); 4.75 (d, *J* = 3.30 Hz, 1H, H-4); 4.61 (d, *J* = 3.42 Hz, 1H, H-2); 2.03 (s, 3H, C$\underline{H}_3$ of the acetyl group); 1.51 - 1.35 (s, 6 H, C$\underline{H}_3$ of the isopropylidene group).

^{13}C NMR (100.6 MHz, CDCl₃), δ (ppm): 197.12 ($\underline{C}$H, HC=O); 170.47 (Cq, O$\underline{C}$=OCH₃); 112.53 (Cq, $\underline{C}$(CH₃)₂); 105.44 ($\underline{C}$H, C-1); 83.37 ($\underline{C}$H, C-4); 82.76 ($\underline{C}$H, C-2); 80.76 ($\underline{C}$H, C-3); 26.83, 26.67 ($\underline{C}$H₃ of the isopropylidene group); 20.86 ($\underline{C}$H₃ of the acetyl group).

FTIR-ATR (cm^{-1}): 1736 (C=O esther), 1664 (C=O aldehyde).

iii. 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribo-pentodialdo-1,4-furanose (47)

Starting compound: 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-allofuranose (**46**) (1 g, 3.22 mmol)

Revealing Solution: a)

η = 69% (0.618 g)

^{1}H NMR and ^{13}C NMR: according to literature. [15]
Rf = 0.40 Ethyl Acetate/ *n*-Hexane (1/1)

MF: $C_{15}H_{18}O_5$
MW: 278.300 g/mol

iv. 1,2-*O*-isopropylidene-α-D-ribo-pentodialdo-1,4-furanose (16)

Starting compound: 1,2-*O*-isopropylidene-α-D-allofuranose (**53**) (1 g, 4.54 mmol)

Revealing Solution: a)

η = 56% (0.479 g)

^{1}H NMR and ^{13}C NMR: according to literature. [65]

Rf = 0.14 Ethyl Acetate/ *n*-Hexane (1/1)

MF: $C_8H_{12}O_5$
MW: 188.178 g/mol

v. 1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (10)

Starting compound: 1,2-*O*-isopropylidene-α-D-glucofuranose (**9**) (1 g, 4.54 mmol)

Revealing Solution: a)

η = 56% (0.479 g)

^{1}H NMR and ^{13}C NMR: according to literature. [58]

Rf = 0.25 Ethyl Acetate/ *n*-Hexane (1/1)

10

MF: $C_8H_{12}O_5$
MW: 188.178 g/mol

2.4 General technique for aldehyde reduction

An aldehyde (3.59 mmol) was dissolved in 8 mL of dry ethanol at 0°C. Sodium borohydride (0.204 g, 5.39 mmol) was then added slowly and react at 0°C for 1 hour. The end of the reaction was confirmed by TLC. Finally, the solution was concentrated to the dryness.[67]

i. 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (28)

Starting compound: 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**27**) (1 g, 3.59 mmol)

Revealing Solution: a)

η = 99% (0.997 g)

Rf = 0.45 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR and ^{13}C NMR: according to literature. [68]

FTIR-ATR (cm^{-1}): 3473 (O-H), 1640 (C=C).

28

MF: $C_{15}H_{20}O_5$
MW: 280.316 g/mol

ii. 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose (39)

Starting compound: 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**38**) (1 g, 4.34 mmol)

Revealing Solution: a)

η = 99% (0.998 g)

Rf = 0.68 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR and ^{13}C NMR: according to literature. [65]

FTIR-ATR (cm^{-1}): 3450 (O-H), 1712 (C=O).

39

MF: $C_{10}H_{16}O_6$
MW: 232.230 g/mol

2.5 General technique for adding an alkyne to an alcohol

Method 1: A primary alcohol (3.57 mmol) was dissolved in 1.2 mL of water. Then, a solution of KOH 60% (0.904 g, 16.12 mmol) was added at 10-15°C followed by the addition of propargyl bromide in toluene (0.82 mL, 9.21 mmol) dropwise and react at 10-15°C for 20 minutes. After that, the reaction was placed at room temperature to finish the reaction. The end of the reaction was confirmed by TLC. [69]

After that, it was added 6 mL of acetone to the mixture and was made a separation of the layer acetone/toluene. Then, to the acetone layer was added 100 mL of ethanol to precipitate the propargyl bromide that did not react and was dissolved in this solvent. Finally, the solution was washed with water and ethanol and concentrated. [69]

Method 2: A primary alcohol (3.57 mmol) was dissolved in 8 mL of dry DMF at 0°C. Then was added NaH (0.600 g, 24.99 mmol) and propargyl bromide in toluene (2.86 mL, 32.13 mmol) dropwise and react at 0°C for 30 minutes. Subsequently, the solution was allowed to warm until reach the room temperature and was stirring for 2 hours. The end of the reaction was confirmed by TLC. [67]

Finally, the residue was diluted with water, and an extraction with diethyl ether (3 x 30 mL) was made, then the organic phase was dried over $MgSO_4$ and was concentrated to the dryness. [67]

i. 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-(2-propargyl)-α-D-xylofuranose (19)

Starting compound: 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**28**) (1 g, 3.58 mmol)
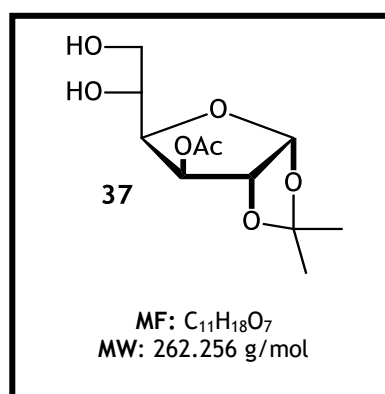
Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/5) as eluent.

η Method1 = 41% (0.466 g)

η Method2 = 46% (0.522 g)

Rf = 0.86 Ethyl Acetate/ *n*-Hexane (1/1)

19

MF: $C_{18}H_{22}O_5$
MW: 318.364 g/mol

¹H NMR (400.1 MHz, CDCl₃), δ (ppm): 7.36 - 7.26 (m, 5H, C₆H₅); 5.94 (d, *J* = 3.67 Hz, 1H, H-1); 4.69 (m, 1H, H-4); 4.60 – 4.52 (m, 2H, CH₂Ar); 4.38 (d, 1H, H-2); 4.22 - 4.17 (s, 2H, CH₂C≡CH); 3.97 (d, *J* = 2.93 Hz, 1H, H-5a); 3.89 (m, 1H, H-3); 3.72 (m, 1H, H-5b); 2.69 (s, 1H, CH₂C≡CH); 1.61 - 1.47 (s, 6 H, CH₃ of the isopropylidene group).

^{13}C NMR (100.6 MHz, CDCl$_3$), δ (ppm): 137.53 (Cq, C$_6$H$_5$); 128.48, 127.94, 127.67 (CH, C$_6$H$_5$); 111.77(Cq, C(CH$_3$)$_2$); 105.14 (CH, C-1); 82.34 (CH, C-4); 81.75 (CH, C-2); 79.6 (CH, C-3); 78.8 (Cq, CH$_2$C≡CH); 74.70 (CH, CH$_2$C≡CH); 72.07 (CH$_2$Ar); 67.39 (CH$_2$, C-5); 58.63 (CH$_2$, CH$_2$C≡CH); 26.82, 26.35 (CH$_3$ of the isopropylidene group).

FTIR-ATR (cm^{-1}): 2372 (C≡N), 1690 (C=C), 1072 (O-C).

2.6   General technique for reductive amination

An aldehyde (3.59 mmol) and propargylamine (0.23 mL, 3.59 mmol) were dissolved in 1,2-dichloroethane (13 mL). After was added sodium triacetoxyborohydride (1.066 g, 5.03 mmol) and left to react at 25°C for 1 hour in a nitrogen atmosphere. The end of the reaction was confirmed by TLC. [5]

Then the mixture was washed with a saturated solution of NaHCO$_3$ and extracted with ethyl acetate. Finally, the solution was dried with MgSO$_4$ and was concentrated to the dryness. [5]

i.   5*H*-5-amino-*N*-(2-propargyl)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (34)

Starting compound: 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**27**) (1 g, 3.59 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/1) as eluent.

MF: C$_{18}$H$_{23}$NO$_4$
MW: 317.380 g/mol

ƞ = 35% (0.399 g)

Rf = 0.22 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR (400.1 MHz, CDCl$_3$), δ (ppm): 7.40 - 7.28 (d, *J* = 8.9 Hz, 5H, C$_6$H$_5$); 5.95 (d, *J* = 3.79 Hz, 1H, H-1); 4.65 (d, *J* = 12.07 Hz, 2H, CH$_2$Ar); 4.49 (d, *J* = 3.98 Hz, 1H, H-2); 4.32 (d, *J* = 6.62 Hz, 1H, H-4); 3.91 (d, *J* = 4.03 Hz, 1H, H-3); 3.31 (d, *J* = 3.67 Hz, 2H, CH$_2$C≡CH); 3.08 (dd, *J* = 4.52 Hz, 1H, H-5a); 2.94 (dd, *J* = 5.14 Hz, 1H, CH$_2$C≡CH); 2.23 (t, *J* = 6.11 Hz, 1H, H-5b); 2.00 (s, 1H, NH) ; 1.51 - 1.28 (s, 6 H, CH$_3$ of the isopropylidene group).

^{13}C NMR (100.6 MHz, CDCl$_3$), δ (ppm): 137.49 (Cq, C$_6$H$_5$); 128.53, 128.05, 127.80 (CH, C$_6$H$_5$); 111.69 (Cq,C(CH$_3$)$_2$); 104.98 (CH, C-1); 82.36 (CH, C-3); 81.97 (CH, C-2); 81.48 (Cq,CH$_2$C≡CH); 79.28 (CH, C-4); 71.91 (CH, CH$_2$C≡CH); 71.81 (CH$_2$Ar); 46.86 (CH$_2$, C-5); 38.24 (CH$_2$, CH$_2$C≡CH); 26.76, 26.34 (CH$_3$ of the isopropylidene group).

FTIR-ATR (cm^{-1}): 3307 (N-H), 2168 (C≡C), 1606 (C=C), 1378 (C-N).

2.7 General technique for Click Chemistry

Method 1: To a carbohydrate with a terminal alkyne (3.14 mmol) was added sodium azide (0.306 g, 4.71 mmol) and was dissolved in 138 mL tert-butanol/water (1/1) at room temperature. Then the sodium ascorbate (0.355 g, 1.79 mmol) and the copper sulphate pentahydrate (3.026 g, 12.12 mmol) were added and was left to react with stirring at 40°C for 6 hours. The end of the reaction was confirmed by TLC. [67]

Subsequently, the solution was left to cool until reach the room temperature and was concentrated. Finally, the residue was diluted with 30 mL of water, and an extraction with chloroform (3 x 30 mL) was made, then the organic phase was dried over $MgSO_4$ and was concentrated to the dryness. [67]

Method 2: Dry Amberlyst A-21 (1 g, 4.8 mmol) was added to a solution of copper (I) iodide (0.381 g; 2.0 mmol) in acetonitrile (15 mL) and let to react with slow stirring for 17 hours. The solvent was filtered, and the resin was washed with acetonitrile (2 x 15 mL), dichloromethane (2 x 15 mL) and dry in vacuum at 40°C. [70]

Then, to a solution of a carbohydrate with a terminal alkyne (3.14 mmol) in dichloromethane (34 mL) was added sodium azide (0.243 g; 3.73 mmol) and the activated resin (0.5 mmol/g; 0.314 mmol). The suspension was left to react with stirring overnight. The catalyser was filtered, and the compound was concentrated to the dryness. [70]

i. (3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-propyl-α-D-xylofuranos-8-yl)diazene (32)

Starting compound: 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-(2-propargyl)-α-D-xylofuranose (**19**) (1 g, 3.14 mmol)

Revealing Solution: b)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/2) as eluent.

MF: $C_{18}H_{26}N_2O_5$
MW: 350.409 g/mol

η Method1 = 10% (0.110 g)

η Method2 = 10% (0.110 g)

Rf = 0.29 Ethyl Acetate/ *n*-Hexane (1/1)

1H NMR (400.1 MHz, CDCl₃), δ (ppm): 7.39 - 7.25 (m, 5H, C₆H₅); 4.77 (d, *J* = 9.54 Hz, 1H, H-1); 4.63 – 4.56 (d, *J* = 10.15 Hz, 2H, CH₂Ar); 4.14 (m, 1H, H-2); 3.91 - 3.85 (m, *J* = 6.62 Hz, 4H, OCH₂CH₂CH₂N); 3.64 (m, 1H, H-4); 3.12 (m, 2H, H-3); 2.48 (m, 2H, OCH₂CH₂CH₂N); 1.30 - 1.24 (s, 6 H, CH₃ of the isopropylidene group).

^{13}C NMR (100.6 MHz, CDCl$_3$), δ (ppm): 137.04 (Cq, C$_6$H$_5$); 128.70, 128.25, 127.76 (CH, C$_6$H$_5$); 111.81 (Cq,$\underline{C}$(CH$_3$)$_2$); 105.11 ($\underline{C}$H, C-1); 82.82 ($\underline{C}$H, C-2); 82.48 ($\underline{C}$H, C-4); 80.03 ($\underline{C}$H, C-3); 71.93 ($\underline{C}$H$_2$Ar); 69.80 (CH$_2$, O$\underline{C}$H$_2$CH$_2$CH$_2$N); 64.85 ($\underline{C}$H$_2$, C-5); 61.03 (CH$_2$, OCH$_2$$\underline{C}H_2CH_2$N); 45.45 ($\underline{C}H_2$, OCH$_2CH_2$$\underline{C}H_2$N); 26.82, 26.33 ($\underline{C}H_3$ of the isopropylidene group).

FTIR-ATR (cm^{-1}): 3288 (N-H) 1644 (C=C).

2.8 General technique to add a trityl group to a primary alcohol

A solution of carbohydrate derivative with a free primary hydroxyl group (5.26 mmol) and trityl chloride (1.673 g, 6 mmol) in pyridine (7.9 mL) was left to react overnight at room temperature. The end of the reaction was confirmed by TLC. [71]

Then the mixture was spilt in ice and water and was left to warm until reach the room temperature. Finally, an extraction with chloroform (3 x 50 mL) was done, and the organic phase was washed with saturated solution of ammonium chloride (2 x 50 mL) and with water (2 x 50 mL), then dried with NaSO$_4$ and was concentrated to the dryness. [71]

i. 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose (30)

Starting compound: 1,2-*O*-isopropylidene-α-D-xylofuranose (**29**) (1 g, 5.26 mmol)

Revealing Solution: a)

η = 99% (2.252 g)

^{1}H NMR and ^{13}C NMR: according to literature. [65]

Rf = 0.76 Ethyl Acetate/ *n*-Hexane (1/1)

30

MF: C$_{27}$H$_{28}$O$_5$
MW: 432.508 g/mol
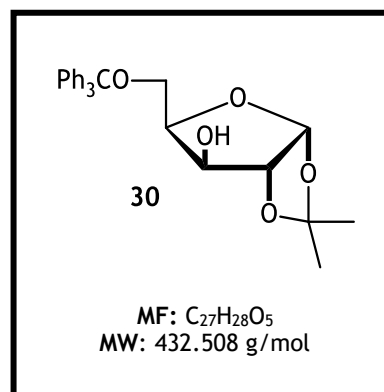
2.9 General technique to take off the trityl group (deprotection of the primary alcohol)

A carbohydrate derivative protected with a trityl group (1.91 mmol) was dissolved in a mixture of formic acid/diethyl ether (1/1) (16 mL) and was left to react at 80°C for 30 minutes. The end of the reaction was confirmed by TLC. [63]

Then the solution was diluted with diethyl ether, neutralised with NaHCO$_3$, and washed with brine solution and water. Finally, the solution was dried with MgSO$_4$ and was concentrated to the dryness. [63]

i. 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (28)

Starting compound: 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose (**31**) (1g, 1.91 mmol)

Revealing Solution: a)

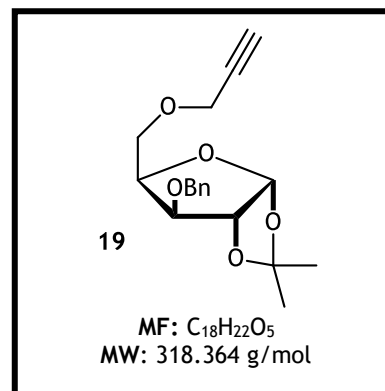To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/1) as eluent.

ɳ = 10% (0.054 g)

Rf = 0.45 Ethyl Acetate/ *n*-Hexane (1/1)

1H NMR and 13C NMR: according to literature. [68]

FTIR-ATR (cm^{-1}): 3473 (O-H), 1640 (C=C).

28

MF: $C_{15}H_{20}O_5$
MW: 280.316 g/mol
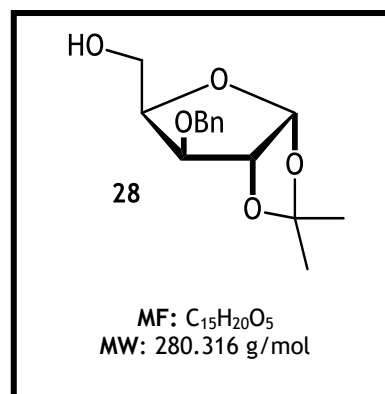
2.10 General technique for methylation of a free hydroxyl group at C3 (3-*O*-methylation)

A solution of boron trifluoro etherate (0.2 mL) was added to a solution of a carbohydrate derivative with a free hydroxyl group at C3 (3.84 mmol) in dichloromethane (2 mL). While keeping the temperature at 0°C, a solution of diazomethane in ether was added dropwise until a fainted yellow colour was observed. The end of the reaction was confirmed by TLC. [72]

Then, after 30 minutes at 0°C a white solid was filtered off, and the filtrate was washed with a solution of 10% of sodium hydrogen carbonate and with water. Finally, the mixture was concentrated to the dryness. [72]

2.11 General technique for acetylation of a free hydroxyl group at C3 (3-*O*- acetylation)

A carbohydrate derivative with a free hydroxyl group at C3 (3.84 mmol) was slowly added, under stirring, to a mixture of sodium acetate anhydrous (5.238 g, 7.68 mmol) and acetic anhydride (0.73 mL, 7.68 mmol). Then, the mixture was heated under reflux for 120 minutes. The end of the reaction was confirmed by TLC. [73]

Finally, an extraction with dichloromethane (3 x 25 mL) was done, and the organic phase was dried with NaSO$_4$ and was concentrated to the dryness. [73]

i. 3-*O*-acetyl-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (36)

Starting compound: 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**6**) (1 g, 3.84 mmol)

Revealing Solution: a)

η = 99% (1.150 g)

Rf = 0.76 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR and ^{13}C NMR: according to literature. [65]

FTIR-ATR (cm^{-1}): 1747 (C=O).

MF: $C_{14}H_{22}O_7$
MW: 302.320 g/mol
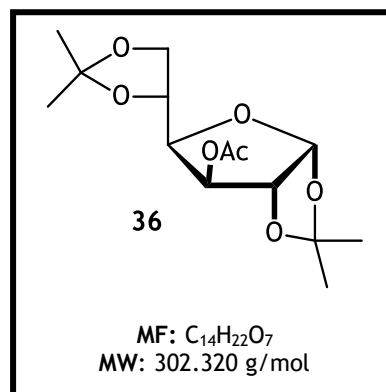
2.12 General technique for adding an Iodine to a carbon with a free hydroxyl group at C3

Method 1: To a solution of a carbohydrate derivative with a free hydroxyl group at C3 (3.84 mmol) in toluene (40 mL) was added imidazole (1.048 g,15.4 mmol), triphenylphosphine (4.039g, 15.4 mmol) and iodine (2.919 g, 11.5 mmol), and was left to react at reflux for 16 hours with stirring. The end of the reaction was confirmed by TLC. [64]

Then, the mixture was left to cool and was added ethyl acetate, and the balloon was washed with acetone. Finally, the solid was filtered off, and the filtrate was concentrated to the dryness. [64]

Method 2: To a solution of a carbohydrate derivative with a free hydroxyl group at C3 (3.84 mmol) in toluene (40 mL) was added imidazole (1.048 g,15.4 mmol), triphenylphosphine (4.039g, 15.4 mmol) and iodine (2.919 g, 11.5 mmol), and was left to react at reflux for 16 hours with stirring. The end of the reaction was confirmed by TLC. [74]

Then, toluene (50 mL) and crushed ice (100 g) was added to the mixture and was left to warm. After an extraction was made and the organic phase was washed with a saturated solution of sodium thiosulfate and was concentrated. Finally, was added diethyl ether to precipitate the triphenylphosphine oxide and the mixture was filtered, and the filtrate was concentrated to the dryness. [74]

Method 3: To a solution of a carbohydrate derivative with a free hydroxyl group at C3 (3.84 mmol) in toluene (40 mL) was added imidazole (1.048 g,15.4 mmol), triphenylphosphine (4.039 g, 15.4 mmol) and iodine (2.919 g, 11.5 mmol). The mixture was put in a microwave with a potency of 450 W for 8 minutes. The reaction was controlled every 2 minutes by TLC.

Then, the mixture was left to cool and was added ethyl acetate, and the balloon was washed with acetone. Finally, the solid was filtered off, and the filtrate was concentrated to the dryness.

i. 3-Iodo-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (14)

Starting compound: 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**6**) (1 g, 3.84 mmol)

Revealing Solution: a)

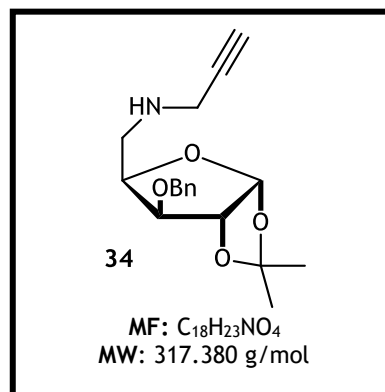To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/2) as eluent.

14

MF: $C_{12}H_{19}IO_5$
MW: 370.181 g/mol

η Method 1= 16% (0.228 g)

η Method 2= 16% (0.228 g)

η Method 3= 50% (0.711 g)

^{1}H NMR and ^{13}C NMR: according to literature. [64]

Rf = 0.59 Ethyl Acetate/ *n*-Hexane (1/1)
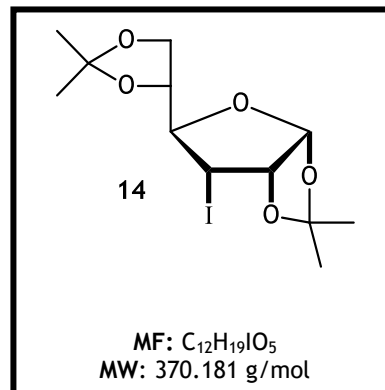
2.13 General technique for obtaining a deoxy sugar at C3

To a solution of a carbohydrate derivative with a halogen at C3 (2.70 mmol) in dry THF, was added lithium aluminium hydride and was left to react for 1 hour at 50°C. The end of the reaction was confirmed by TLC. [64]

Then, the mixture was cooled using an ice bath and solution of THF 70% was added, followed by the addition of water. The mixture was left for 20 minutes with stirring at room temperature, and after was filtered over silica gel and concentrated. [64]

Finally, an extraction with dichloromethane (3 x 25 mL) was done, and the organic phase was dried with $NaSO_4$ and was concentrated to the dryness. [64]

i. 3-deoxy-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (13)

Starting compound: 3-Iodo-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**14**) (1 g, 2.70 mmol)
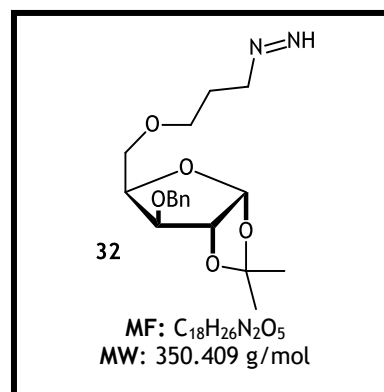
Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/2) as eluent.

η = 16% (0.106 g)

¹H NMR and ¹³C NMR: according to literature. [64]

Rf = 0.38 Ethyl Acetate/ *n*-Hexane (1/1)

MF: $C_{12}H_{20}O_5$
MW: 244.284 g/mol
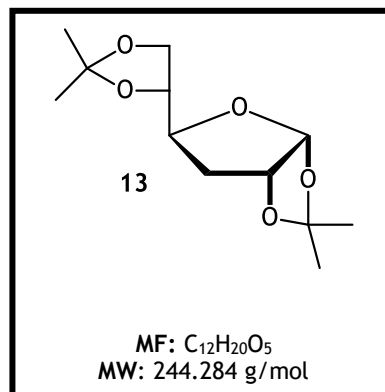
2.14 General technique for oxidation of tertiary alcohols

To a solution of molecular sieves 4Å (4.84 g) and PCC (2.459 g, 11.53 mmol) in dry dichloromethane (20 mL) was added a solution of a carbohydrate derivative with a free tertiary hydroxyl group (3.84 mmol) in dry dichloromethane (2 mL) and was left to react at 40°C for 1 hour. The end of the reaction was confirmed by TLC. [64]

Then the mixture was left to cool and was added to diethyl ether (300 mL) under vigorous stirring. Finally, the mixture was filtered, the filtrate was passed through fluorisil (20 g) becoming colourless and was concentrated to the dryness. [64]

i. 3-deoxy-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuran-3-ulose (12)

Starting compound: 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**6**) (1 g, 3.84 mmol)

Revealing Solution: a)

η = 99% (0.982 g)

¹H NMR and ¹³C NMR: according to literature. [64]

Rf = 0.43 Ethyl Acetate/ *n*-Hexane (1/1)

MF: $C_{12}H_{18}O_6$
MW: 258.268 g/mol
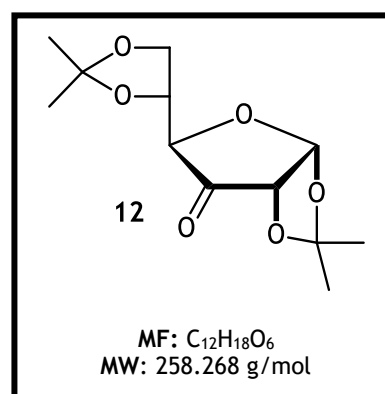
2.15 General technique for reduction of ketones linked to the furanose ring

To dry ethanol (29 mL), at 0°C, was added a carbohydrate derivative with a ketone linked to the furanose ring (3.87 mmol) and sodium borohydride (0.329 g, 8.69 mmol), and was left to react for 20 minutes with stirring. The end of the reaction was confirmed by TLC. [64]

Finally, an extraction with chloroform (3 x 25 mL) was done, and the organic phase was dried with $NaSO_4$ and was concentrated to the dryness. [64]

i. 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (11)

Starting compound: 3-deoxy-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuran-3-ulose (**12**) (1 g, 3.87 mmol)

Revealing Solution: a)

η = 91% (0.917 g)

Rf = 0.45 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR and ^{13}C NMR: according to literature. [64]

FTIR-ATR (cm^{-1}): 3469 (O-H).

MF: $C_{12}H_{20}O_6$
MW: 260.284 g/mol
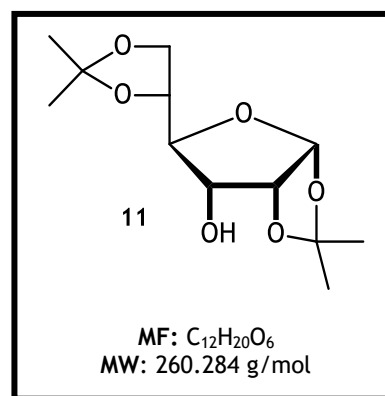
2.16 General technique for Wittig Reaction (formation of α, β-unsaturated esters)

To (carbethoxymethylene)triphenylphosphorane (2.539 g, 7.29 mmol) was added an aldehyde (3.59 mmol) and chloroform (35.9 mL), and was left to react at 30°C for 30 minutes with stirring. The end of the reaction was confirmed by TLC. [58]

Finally, the mixture was concentrated to the dryness. [58]

i. **Ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (48) and Ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*Z*)-enfuranoate (49)**

Starting compound: 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribo-pentodialdo-1,4-furanose (**47**) (1 g, 3.59 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/1) as eluent and were obtained the two isomers together.

η = 83% (1.039 g)

^{1}H NMR and ^{13}C NMR: according to literature. [58]

Rf = 0.78 Ethyl Acetate/ *n*-Hexane (1/1)

48

MF: $C_{19}H_{24}O_6$
MW: 348.390 g/mol

49

MF: $C_{19}H_{24}O_6$
MW: 348.390 g/mol

ii. Ethyl 5,6-dideoxy-1,2-O-isopropylidene-α-D-ribo-hept-5-(E)-enfuranoate (17) and Ethyl 5,6-dideoxy-1,2-O-isopropylidene-α-D-ribo-hept-5-(Z)-enfuranoate (18)

Starting compound: 1,2-O-isopropylidene-α-D-ribo-pentodialdo-1,4-furanose (**16**) (1 g, 5.31 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/n-Hexane (2/1) as eluent and were obtained the two isomers together.

η = 28% (0.384 g)

Rf = 0.75 Ethyl Acetate/ n-Hexane (2/1)

MF: $C_{12}H_{18}O_6$
MW: 258.268 g/mol

MF: $C_{12}H_{18}O_6$
MW: 258.268 g/mol

¹H NMR (400.1 MHz, CDCl₃), δ (ppm): 7.37 (d, J = 4.65 Hz, 1H, $\underline{H}$C=CHCO₂Et); 6.12 (d, J = 15.77 Hz, 1H, HC=C$\underline{H}$CO₂Et); 5.49 (d, J = 3.18 Hz, 1H, H-4); 4.82 (d, J = 5.09 Hz, 1H, H-1); 4.20 (m, 1H, H-3); 4.18 – 4.15 (m, 2H, CO₂C$\underline{H}_2$CH₃); 3.75 (m, 1H, H-2); 1.40 – 1.37 (s, 6 H, C$\underline{H}_3$ of the isopropylidene group); 1.26 (t, J = 7.09 Hz, 3H, CO₂CH₂C$\underline{H}_3$).

¹³C NMR (100.6 MHz, CDCl₃), δ (ppm): 165.99 (Cq,C=O); 128.55 (CH, H$\underline{C}$=CHCO₂Et); 122.33 (CH, HC=$\underline{C}$HCO₂Et); 112.02 (Cq, $\underline{C}$(CH₃)₂); 105.30 ($\underline{C}$H, C-1); 79.63 ($\underline{C}$H, C-2); 78.44 ($\underline{C}$H, C-4); 75.02 ($\underline{C}$H, C-3); 60.55 (CH₂, CO₂$\underline{C}$H₂CH₃); 26.72, 26.58 ($\underline{C}$H₃ of the isopropylidene group); 14.12 (CH₃, CO₂CH₂$\underline{C}$H₃).

FTIR-ATR (cm⁻¹): 3279 (O-H), 1660 (C=C), 1715 (C=O).
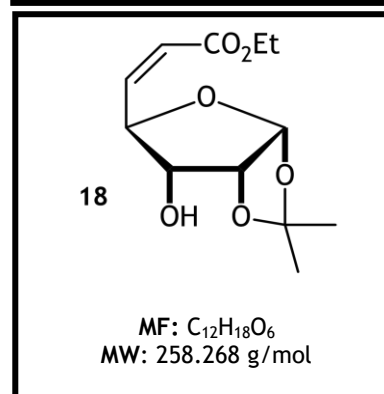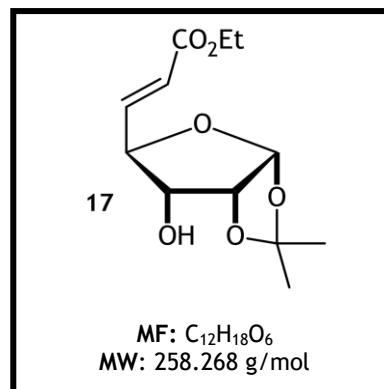
iii. Ethyl 5,6-dideoxy-1,2-O-isopropylidene-α-D-xylo-hept-5-(E)-enfuranoate (56) and Ethyl 5,6-dideoxy-1,2-O-isopropylidene-α-D-xylo-hept-5-(Z)-enfuranoate (57)

Starting compound: 1,2-O-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**10**) (1 g, 5.32 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/n-Hexane (1/1) as eluent and were obtained the two isomers together.

η = 57% (0.782 g)

Rf = 0.60 Ethyl Acetate/ n-Hexane (1/1)

¹H NMR and ¹³C NMR: according to literature. [58]

FTIR-ATR (cm⁻¹): 3459 (O-H), 1662 (C=C), 1716 (C=O).

56

MF: $C_{12}H_{18}O_6$
MW: 258.268 g/mol

57

MF: $C_{12}H_{18}O_6$
MW: 258.268 g/mol
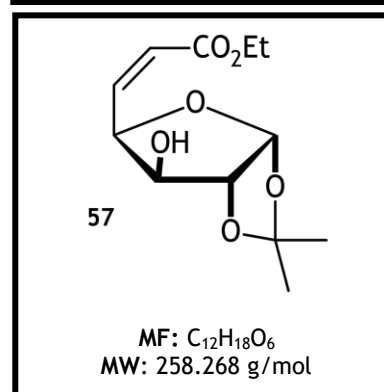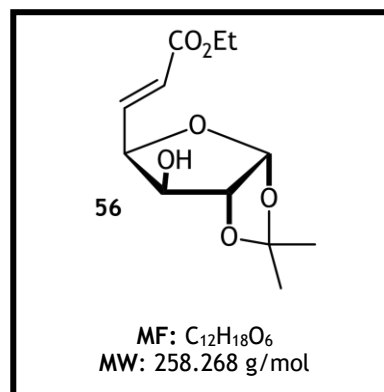
2.17 General technique for closing the ring as a pyrazolidin-3-one

To a solution of α, β-unsaturated ester (3.87 mmol) in ethanol (3.87 mL) was added hydrazine monohydrate (0.213 g, 4.26 mmol), and was left to react at 80°C for 1 hour with stirring. The end of reaction was confirmed by TLC. [58]

Finally, the mixture was concentrated to the dryness. [58]

i. **5-(*S*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (42), 5-(*R*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (43) and 5-(*S*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-3-hydroxypyrazoline (44)**

Compound **42**

Starting compound: Ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**48**) (1 g, 2.87 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/1) as eluent.

η = 10 % (0.096 g)

Rf = 0.58 Ethyl Acetate/ *n*-Hexane (4/1)

42

MF: $C_{17}H_{22}N_2O_5$
MW: 334.367 g/mol

¹H NMR (400.1 MHz, CDCl₃), δ (ppm): 8.01 (s, 1H, N$\underline{H}$C=O); 7.70 - 7.25 (m, 5H, C₆$\underline{H}$₅); 5.75 (m, 1H, H-1'); 4.78 (m, 1H, H-2'); 4.57 (m, 2H, C$\underline{H}$₂Ar); 3.54 (m, 1H, H-4'); 3.89 (m, 1H, H-3'); 3.43 (dd, *J* = 7.58, 6.80 Hz, 1H, H-5); 2.96 (m, 2H, H-4); 2.87 (m, 1H, N$\underline{H}$); 1.58 - 1.37 (s, 6 H, C$\underline{H}$₃ of the isopropylidene group).

¹³C NMR (100.6 MHz, CDCl₃), δ (ppm): 169.96 (Cq,C=O); 128.45 (Cq, C₆H₅); 128.13, 128.07, 128.03 (CH, C₆H₅); 113.79 (Cq, $\underline{C}$(CH₃)₂); 104.14 ($\underline{C}$H, C-1'); 79.98 ($\underline{C}$H, C-5); 78.35 ($\underline{C}$H, C-3'); 76.77 ($\underline{C}$H, C-4'); 72.45 ($\underline{C}$H, C-2'); 72.07 ($\underline{C}$H₂Ar); 31.41 ($\underline{C}$H₂, C-4); 26.91, 26.54 ($\underline{C}$H₃ of the isopropylidene group).

FTIR-ATR (cm⁻¹): 3292 (O-H), 3150 (N-H), 1733 (C=O), 1660 (C=C), 1242 (C-N).

Compound **43**

Starting compound: Ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**48**) (1 g, 2.87 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/1) as eluent.

η = 10 % (0.096 g)

Rf = 0.38 Ethyl Acetate/ *n*-Hexane (4/1)

43

MF: $C_{17}H_{22}N_2O_5$
MW: 334.367 g/mol
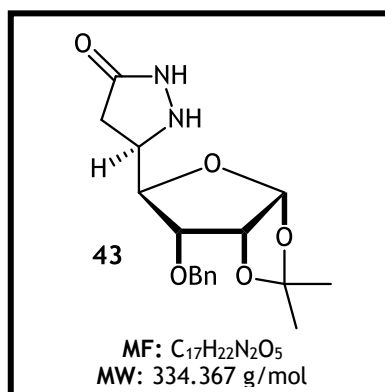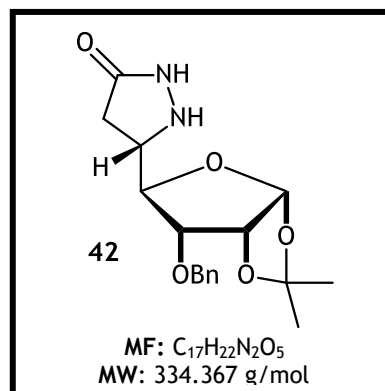
¹H NMR (400.1 MHz, CDCl₃), δ (ppm): 8.03 (s, 1H, N$\underline{H}$C=O); 7.45 - 7.24 (m, 5H, C₆$\underline{H}$₅); 5.74 (m, 1H, H-1'); 4.80 (m, 1H, H-2'); 4.67(m, 2H, C$\underline{H}$₂Ar); 4.09 (m, 1H, H-4'); 3.90 (m, 1H, H-3'); 3.42 (dd, J = 9.53, 7.94 Hz, 1H, H-5); 2.91 - 2.85 (m, 2H, H-4); 1.79 (m, 1H, N$\underline{H}$); 1.63 - 1.34 (s, 6 H, C$\underline{H}$₃ of the isopropylidene group).

¹³C NMR (100.6 MHz, CDCl₃), δ (ppm): 170.10 (Cq,C=O); 128.55 (Cq, C₆H₅); 128.17, 128.12, 128.04 (CH, C₆H₅); 112.78 (Cq, $\underline{C}$(CH₃)₂); 104.10 ($\underline{C}$H, C-1'); 79.96 ($\underline{C}$H, C-5); 78.40 ($\underline{C}$H, C-3'); 76.74 ($\underline{C}$H, C-4'); 72.49 ($\underline{C}$H, C-2'); 72.12 ($\underline{C}$H₂Ar); 30.73 ($\underline{C}$H₂, C-4); 26.88, 26.50 ($\underline{C}$H₃ of the isopropylidene group).

FTIR-ATR (cm⁻¹): 3292 (O-H), 3150 (N-H), 1733 (C=O), 1660 (C=C), 1242 (C-N).

Compound **44**

Starting compound: Ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**48**) (1 g, 2.87 mmol)
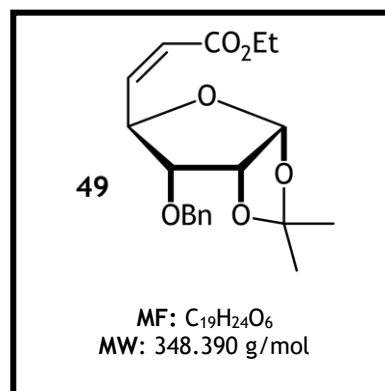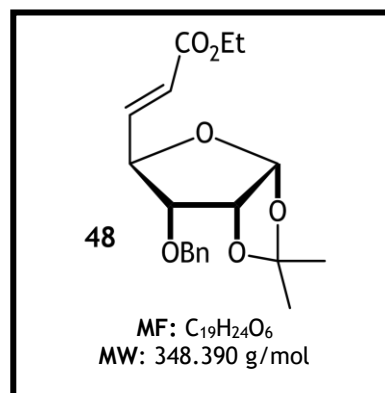
Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/1) as eluent.

MF: C₁₇H₂₂N₂O₅
MW: 334.367 g/mol

η = 10 % (0.096 g)

Rf = 0.16 Ethyl Acetate/ *n*-Hexane (4/1)

¹H NMR (400.1 MHz, CDCl₃), δ (ppm): 7.41 - 7.29 (m, 5H, C₆$\underline{H}$₅); 5.70 (dd, J = 7.33 Hz, 1H, H-3) ; 4.78 (t, J = 10.70 Hz, 1H, H-1'); 4.58 (m, 2H, C$\underline{H}$₂Ar); 4.15 (m, 1H, H-4'); 4.04 (dd, J = 7.45, 6.72 Hz, 1H, H-5); 3.94 (m, 1H, H-2'); 3.38 (m, 1H, H-3'); 2.54 - 2.46 (d, J = 4.03 Hz, 1H, H-4); 1.37 - 1.23 (s, 6 H, C$\underline{H}$₃ of the isopropylidene group).

FTIR-ATR (cm⁻¹): 3234 (O-H), 1660 (C=C), 1251 (C-N).

ii. **5-(*S*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (50), 5-(*R*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (51) and 5-(*S*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-3-hydroxypyrazoline (52)**

Compound **50**

Starting compound: Ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**17**) (1 g, 3.87 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (4/1) as eluent.

η = 10 % (0.095 g)

Rf = 0.30 Ethyl Acetate/ toluene (4/1)

¹H NMR (400.1 MHz, CDCl₃), δ (ppm): 7.55 (s, 1H, N<u>H</u>C=O); 4.93 (d, *J* = 7.95 Hz, 1H, H-1'); 4.13 (m, 1H, H-2'); 4.05 (d, *J* = 6.97 Hz, 1H, H-4'); 3.67 (m, 1H, H-3'); 3.49 (dd, *J* = 6.98, 2.81 Hz, 1H, H-5); 2.76 (m, 2H, H-4); 2.36 (d, *J* = 7.70 Hz, 1H, N<u>H</u>); 1.35 - 1.31 (s, 6 H, C<u>H</u>₃ of the isopropylidene group).

FTIR-ATR (cm⁻¹): 3362 (O-H), 3100 (N-H), 1710 (C=O), 1253 (C-N).

Compound **51**

Starting compound: Ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**17**) (1 g, 3.87 mmol)

Revealing Solution: a)

To purified the residue, a silica gel column chromatography, was made using a mixture of Ethyl Acetate/*n*-Hexane (4/1) as eluent.

η = 10 % (0.095 g)

Rf = 0.24 Ethyl Acetate/ toluene (4/1)

¹H NMR (400.1 MHz, CDCl₃), δ (ppm): 7.26 (s, 1H, N<u>H</u>C=O); 4.96 (m, 1H, H-1'); 4.56 (m, 1H, H-2'); 3.74 (m, 1H, H-3'); 3.62 (m, 1H, H-4'); 3.48 (dd, *J* = 9.29, 7.09 Hz, 1H, H-5); 2.51 - 2.36 (m, 2H, H-4); 1.35 - 1.31 (s, 6 H, C<u>H</u>₃ of the isopropylidene group).

FTIR-ATR (cm⁻¹): 3362 (O-H), 3100 (N-H), 1710 (C=O), 1253 (C-N).
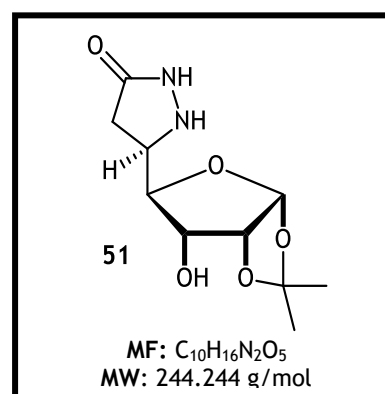
Compound **52**

Starting compound: Ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**17**) (1 g, 3.87 mmol)

Revealing Solution: a)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (4/1) as eluent.

η = 10 % (0.095 g)

Rf = 0.10 Ethyl Acetate/ toluene (4/1)

MF: $C_{10}H_{16}N_2O_5$
MW: 244.244 g/mol

^{1}H NMR (400.1 MHz, CDCl$_3$), δ (ppm): 5.77 (d, *J* = 4.03 Hz, 1H, H-3); 4.72 (d, *J* = 6.84 Hz, 1H, H-1'); 4.56 (m, 1H, H-4'); 4.25 (dd, *J* = 4.89, 2.69 Hz, 1H, H-5); 4.14 (m, 1H, H-2'); 3.94 (m ,1H, H-3'); 2.29 - 2.02 (m, 2H, H-4); 1.51 - 1.25 (s, 6 H, C$\underline{H}_3$ of the isopropylidene group).

^{13}C NMR (100.6 MHz, CDCl$_3$), δ (ppm): 113.07 (Cq, $\underline{C}$(CH$_3$)$_2$); 103.49 ($\underline{C}$H, C-1'); 78.80 ($\underline{C}$H, C-3); 76.71 ($\underline{C}$H, C-4'); 75.99 ($\underline{C}$H, C-2'); 74.31 ($\underline{C}$H, C-3'); 59.21 ($\underline{C}$H, C-5); 30.19 ($\underline{C}$H$_2$, C-4); 26.39, 26.28 ($\underline{C}$H$_3$ of the isopropylidene group).

FTIR-ATR (cm^{-1}): 3249 (O-H), 1246 (C-N).
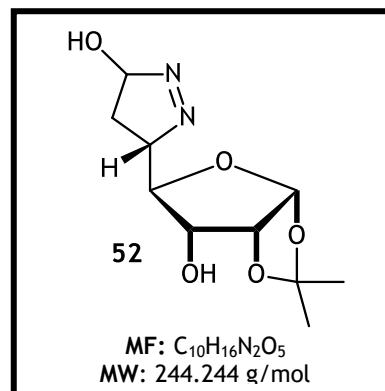
2.18 General technique for making a ß-enaminoester from a α,ß-unsaturated ester

To a solution of α,ß- unsaturated ester (1mmol) in DMF (1 mL), was added sodium azide (0.325 g, 5 mmol), and the mixture was heated in a microwave with a potency of 450 W for 6 minutes. The reaction was controlled every 2 minutes by TLC. [58]

Then, the mixture was concentrated, and a dark precipitate was formed. The mixture was filtrated and washed with ethyl acetate and concentrated to the dryness. [58]

i. Ethyl 5-amino-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*E*)-enfuranoate (22)

Starting compound: Ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*E*)-enfuranoate (**56**) (1 g, 3.87 mmol)

Revealing Solution: b)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/3) as eluent.

η = 10 % (0.106 g)

Rf = 0.81 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR (400.1 MHz, CDCl$_3$), δ (ppm): 8.06 (s, 2H, NH$_2$); 5.78 (s, 1H, NH$_2$C=C$\underline{H}$); 4.77 (d, *J* = 4.65 Hz, 1H, H-1); 4.45 (d, *J* = 7.83 Hz, 1H, H-4); 4.25 (m, 2H, C$\underline{H}_2$CH$_3$); 4.13 (d, *J* = 3.18 Hz, 1H, H-3); 3.85 (d, *J* = 3.06 Hz, 1H, H-2); 1.37 - 1.34 (s, 6 H, C$\underline{H}_3$ of the isopropylidene group); 1.26 (m, 3H, CH$_2$C$\underline{H}_3$).

MF: C$_{12}$H$_{19}$NO$_6$
MW: 273.282 g/mol
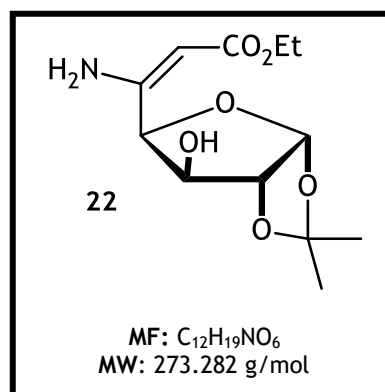
ii. Ethyl 5-amino-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (59)

Starting compound: Ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate (**17**) (1 g, 3.87 mmol)

Revealing Solution: b)

To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/*n*-Hexane (1/3) as eluent.

η = 10 % (0.106 g)

Rf = 0.80 Ethyl Acetate/ *n*-Hexane (1/1)

^{1}H NMR (400.1 MHz, CDCl$_3$), δ (ppm): 8.01 (s, 2H, N$\underline{H}_2$); 5.29 (s, 1H, NH$_2$C=C$\underline{H}$); 4.68 (d, *J* = 8.56 Hz, 1H, H-1); 4.44 (d, *J* = 6.72 Hz, 1H, H-4); 4.23 (m, 2H, C$\underline{H}_2$CH$_3$); 3.99 (d, *J* = 6.98 Hz, 1H, H-3); 3.87 (d, *J* = 4.15 Hz, 1H, H-2); 1.39 - 1.35 (s, 6 H, C$\underline{H}_3$ of the isopropylidene group); 1.26 (m, 3H, CH$_2$C$\underline{H}_3$).

MF: C$_{12}$H$_{19}$NO$_6$
MW: 273.282 g/mol
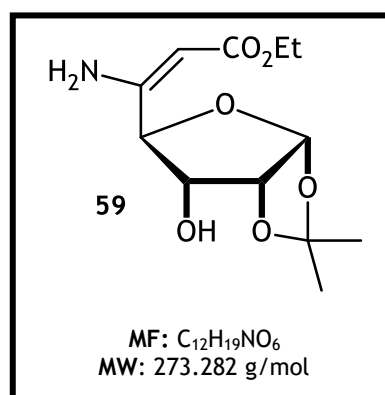
2.19 General technique for closing the ring as a 5-hydroxypyrazole

To a solution of ß-enaminoester (1 mmol) in ethanol (2 mL) was added hydrazine monohydrate (0.58 mL, 1.2 mmol), and the mixture was worm at 80°C to react for 1 hour. The end of the reaction was confirmed by TLC. [58]

Then, the mixture was concentrated and dissolved in chloroform to precipitate hydrazine crystals. Finally, the mixture was dried with Na_2SO_4 and was concentrated to the dryness. [58]

i. **5-(1,2-O-isopropylidene-α-D-threofuranos-4-C-yl)-5-hydroxy-1H-pyrazole (23)**

Starting compound: Ethyl 5-amino-5,6-dideoxy-1,2-O-isopropylidene-α-D-xylo-hept-5-(E)-enfuranoate (**22**) (1 g, 3.66 mmol)

Revealing Solution: a)
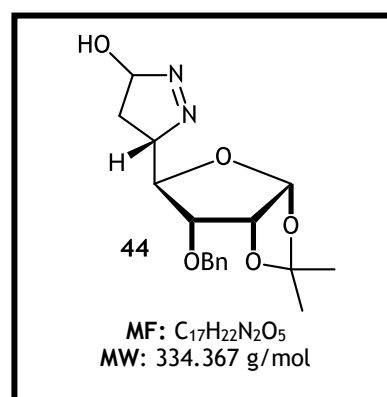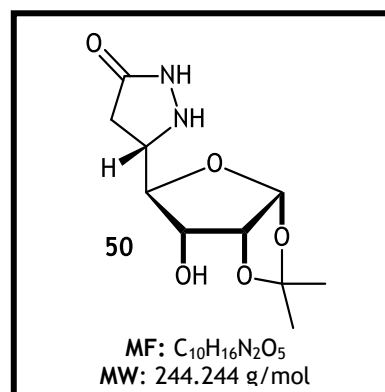
To purified the residue was made a silica gel column chromatography using a mixture of Ethyl Acetate/n-Hexane (1/1) as eluent.

MF: $C_{10}H_{14}N_2O_5$
MW: 242.229 g/mol

ɳ = 24 % (0.213 g)

Rf = 0.26 Ethyl Acetate/ n-Hexane (3/1)

¹H NMR (400.1 MHz, CDCl₃), δ (ppm): 12.71 (s, 1H, N<u>H</u>); 5.35 (s, 1H, H-4); 5.10 (d, J = 7.46 Hz, 1H, H-1); 4.28 (d, J = 6.85 Hz, 1H, H-4'); 3.97 (m, 1H, H-2'); 3.88 (m, 1H, H-3'); 1.34 - 1.32 (s, 6 H, C<u>H</u>₃ of the isopropylidene group).

FTIR-ATR (cm⁻¹): 3315 (O-H), 3150 (N-H), 1674 (C=N), 1256 (C-N).
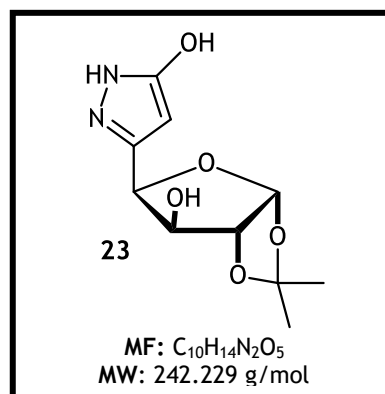
3. Biological assay technique description

The biological assay performed aimed to evaluate the cytotoxicity of the final synthesised compounds. The results were compared with the obtained for 5-FU, the positive control and with the negative control (absence of compounds). The cell lines used were NHDF and N27, brought to American Type Culture Collection (ATCC). The cell culture medium RPMI (Roswell Park Memorial Institute), the reagents and the supplements were bought from Sigma-Aldrich. Each assay was performed in quadruplicate and independently repeated.

3.1 Cell cultures

The NHDF cells were maintained in RPMI 1640 medium supplemented with 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 2 mM L-Glutamine, and 1 mM Sodium Pyruvate

and complemented with 10% of FBS and 1% of Ab (10,000 U/mL penicillin G, 100 mg/mL streptomycin and 25 μg/mL amphotericin B.

The N27 cells were kept in RPMI 1640 medium complemented with 10% of FBS and 1% Sp (10,000 U/mL penicillin G and 100 mg/mL streptomycin).

Both cell lines were maintained in 75 cm^2 culture flasks and incubated at 37ºC in a humified atmosphere with 5% of CO_2, and the medium was renewed every 2/3 days. When the cells reached a confluence of 90-95%, approximately, a mild trypsinisation was performed to detach them, using an aqueous solution of trypsin (0.5 g/L) and EDTA (Ethylenediaminetetraacetic acid) (0.02 g/L). Before every experiment, the cells were counted using trypan-blue as a stain and by means of a hemocytometer.

3.2 Compounds solutions preparation

The compounds were all dissolved in DMSO (Dimethyl sulfoxide) in a 10 mM concentration and were stored at 4ºC. From these stock solutions, working solutions of the compounds were prepared, at a concentration of 30 μM, by doing suitable dilutions in the complete cell culture medium. The final solvent concentration in the assays was less than 1% (V/V), with no significant effect on cell viability (data not shown).

3.3 Cell viability assay (MTT assay)

The cell viability was evaluated by the quantification of the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), according to procedures described in the literature. [75,76] After trypsinisation and cells counting, they were adequately diluted and seeded in a 96-well plate at a concentration of 2 x 10^4 cells/mL and incubated at 37ºC in a humified atmosphere with 5% CO_2 for 48 hours. After the cell adhesion to the plate, they were treated with the compounds (concentration 30 μM) and incubated for 72 hours. Untreated cells were used as a negative control and 5-FU as a positive control. At the end of the incubation, the cell culture medium was removed, then the cells were washed with phosphate saline buffer [NaCl (137 mM), KCl (2.7 nM), Na_2HPO_4 (10 nM) and KH_2PO_4 (1.8 nM)]. Posteriorly, incomplete culture medium (without FBS and the antibiotic) containing MTT solution (5 mg/ mL in phosphate buffered saline) was added and incubated at 37ºC for 4 hours. Finally, the supernatant was removed, and the formazan crystals were dissolved in DMSO. Whenever needed dilutions were performed to obtain absorbance values in the linearity of the assay. The absorbance was recorded on a Bio-Rad xMark™ microplate spectrophotometer at 570 nm. The extent of cell death was expressed as percent of cell viability in comparison with the negative control, after the background subtraction.

3.4 Statistics

The treatment of the data obtained in the biological evaluation assays, as well as all the statistical studies was performed with the Microsoft Excel 2016 software. In all the graphs, the values presented are the averages of the results of the assays associated with the standard deviation, and the statistical significance analysis was performed by the Student's t-test in comparison with the corresponding negative control. A $p < 0.05$ was considered statistically significant.

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Communications and Publications

1. Poster presentations inside the scope of this dissertation

1.1 Abstract submitted for a poster presentation in "V Ciclo de Conferências da Faculdade de Ciências da Universidade da Beira Interior."

Síntese de pseudo-*C*-nucleósidos, via *Click Chemistry*, com potencial actividade para o tratamento de transtorno maníaco-depressivo

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Os transtornos maníaco-depressivos são atualmente tratados geralmente com duas classes diferentes de fármacos, os antipsicóticos e os antidepressivos. Este tipo de transtorno, também conhecido como doença bipolar, é caraterizado por dois tipos diferentes de crises, as crises maníacas, na qual é utilizada a primeira classe de fármacos, e as crises depressivas, na qual é utilizada a segunda. No entanto, existe uma falha no mercado para o tratamento deste transtorno, pelo que a indústria farmacêutica está a testar compostos utilizados para o tratamento de outras patologias neurológicas como potenciais fármacos para esta patologia.

Os derivados de açúcares têm, ao longo do tempo, ganho terreno na indústria farmacêutica, por serem compostos biocompatíveis e terem atividades farmacológicas com interesse em diversas doenças, nas quais se incluem as doenças neurológicas. O objetivo deste trabalho centra-se no desenho e síntese de compostos derivados de glúcidos, nomeadamente os pseudo-*C*-nucleósidos, com potencial atividade para o tratamento do transtorno bipolar.

Para a síntese dos pseudo-*C*-nucleósidos partiu-se de compostos comercialmente disponíveis, como a D-glucose e a D-xilose. Quando o composto de partida foi a D-glucose procedeu-se à proteção dos grupos hidroxilos livres. De seguida, foi feita a hidrólise do grupo isopropilideno da posição 5 e 6 e posteriormente fez-se uma clivagem oxidativa para se obter um aldeído. Partindo do aldeído foram realizados dois percursos diferentes, um dos quais envolveu a redução a álcool e o outro a redução a amina. Em ambos os compostos foi introduzido um alcino terminal, que após *Click Chemistry*, originou dois pseudo-*C*-nucleósidos diferentes.

Os compostos obtidos serão submetidos a ensaios *in vitro* para avaliar a sua atividade biológica.

Palavras-chave: *Pseudo-C-nucleósidos, Transtorno bipolar, Click Chemistry*.

1.2 Abstract submitted for a poster presentation in "19th European Symposium on Carbohydrate – EUROCARB 2017."

Synthesis of pseudo-C-nucleosides, with potential activity for the treatment of Manic-Depressive Disorder

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Among neuropsychiatric disorders, manic-depressive disease, also known as bipolar disorder, is a disabling, severe and chronic illness in which the patient presents recurrent episodes of two opposing mood states, mania and depression.[1-4] This disorder is currently controlled by different classes of drugs, namely antipsychotics and antidepressants. Antipsychotics can be used in maniac episodes and antidepressants in depressive episodes. However, due to the lack of efficacy and side effects of current drugs, there is a clear need of other molecules for the treatment of this disorder, so the pharmaceutical industry is testing compounds used to treat other neurological conditions as potential drugs for this pathology.[5-7]

Sugar derivatives have gained ground in the pharmaceutical industry because they usually are biocompatible compounds and have pharmacological activities with interest in several diseases, which include neurological disorders.[7,8] The objective of this work is the design and synthesis of carbohydrate derivatives, namely pseudo-C-nucleosides, of potential interest for the treatment of bipolar disorder, for *in vitro* biological evaluation.

The synthesis of the pseudo-C-nucleosides started with commercially available compounds, such as D-glucose and D-xylose. Starting from D-glucose, free hydroxyl groups in positions 1 and 2 and 5 and 6 were protected. Subsequently, the isopropylidene group of position 5 and 6 was hydrolysed, and oxidative cleavage was later performed to obtain an aldehyde. Starting from the aldehyde two different routes were carried out, one of which involved the reduction to alcohol and the other a reduction to the amine. In both compounds, a terminal alkyne was introduced, which after Click Chemistry, gave rise to two different pseudo-C-nucleosides. The *in vitro* biological evaluation of the cell proliferation effects on various cell lines, including neuronal cells, of these new pseudo-C-nucleosides will also be presented.

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1.3 Abstract submitted for a poster presentation in "12ª Reunião do Grupo de Glúcidos – GLUPOR 12."

Synthesis of 5-Hydroxypyrazoles with potential activity for the treatment of Manic-Depressive Disorder

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Manic-depressive illness, also known as bipolar disease, is a neuropsychiatric disorder in which the patient has recurrent episodes of two opposing mood states, depression and mania. This disorder is characterised by being a disabling, grave and chronic illness, whose available medical treatment is not the most suitable, and usually to treat manic crisis are used antipsychotic drugs and to treat depressive crisis are used antidepressant drugs. [1,2,3,4] Due to the lack of efficacy of the treatment and the various side effects, it is crucial to create new molecules with potential to treat this disorder. Thus, the pharmaceutical industry is testing compounds present in chemical libraries that are used to treat other neurological diseases as possible drugs for the treatment of epilepsy. [5,6,7]

Sugar derivatives, over time, have gained ground in the pharmaceutical industry because of their biocompatibility and the interesting pharmacological activities that they have shown for the treatment of several illnesses, including neurological diseases. [7,8]

This work aims to design and synthesise carbohydrate derivatives, including 5-hydroxypyrazoles, with potential interest in the treatment of bipolar disorder and further *in vitro* evaluation.

The synthesis of the 5-hydroxypyrazoles started with a commercially available compound, the 1,2:5,6-Di-O-isopropylidene-α-*D*-glucofuranose and several reactions were performed until an aldehyde at the position 5 of the furanose ring was obtained. After that, by a Wittig reaction an α, ß-unsaturated ester was obtained and subsequently by addition reaction the corresponding ß-enaminoester was obtained as the final precursor to the reaction in which the ring is closed to give the 5-hydroxypyrazoles.

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2. Oral presentations inside the scope of this dissertation

2.1 Abstract submitted for an oral presentation in "2nd Symposium of the FibEnTech Research Unit: Fiber Materials and Environmental Technologies."

Synthesis of carbohydrate derivatives with potential activity for the treatment of bipolar disorder

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Bipolar disease, also known as manic-depressive illness, is a serious, incapacitating, and chronic neuropsychiatric disorder in which the patient has recurrent episodes of two opposing mood states, depression and mania. The available medical treatment for this disorder is not the most appropriate, once it is usually used antipsychotic drugs to treat maniac disease and antidepressant drugs to treat depressive crisis [1,2,3,4]. Because of the lack of efficacy of the treatment available and the various side effects, it is crucial to creating new molecules with potential for the treatment of this disorder. Thus, the pharmaceutical industry is testing compounds present in chemical libraries that are used to treat other neurological diseases [5,6,7].

Carbohydrate derivatives, over the last few years, have gained ground in the pharmaceutical industry because of their biocompatibility and the interesting pharmacological activities that they have shown for the treatment of several illnesses, including neurological diseases [7,8].

This work aims to design and synthesise carbohydrate derivatives, namely pseudo-*C*-nucleosides, with potential interest in the treatment of bipolar disorder and further *in vitro* evaluation.

The pseudo-C-nucleosides synthesised were Pyrazolidin-3-ones and 5-hydroxypyrazoles.

The synthesis of the Pyrazolidin-3-ones started with a commercially available compound, the 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose. Several reactions were performed until an aldehyde group at the position 5 of the furanose ring was obtained. Then, was obtained an α,β-unsaturated ester by a Wittig reaction, which is the final precursor to the reaction in which the ring is closed to give the pyrazolidin-3-one. The route for the synthesis of the 5-hydroxypyrazoles is similar the described above. The synthetic pathway is the same until the

α,ß-unsaturated ester was obtained. After that, an addition reaction to getting the ß-enaminoester was made, in which the final precursor is ring closed to get the 5-hydroxypyrazole.

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3. Papers published in minute book inside the scope of this dissertation

3.1 Paper submitted for the "Book of Proceedings-Atas" in "2nd Symposium of the FibEnTech Research Unit: Fiber Materials and Environmental Technologies."

Synthesis of carbohydrate derivatives with potential activity for the treatment of bipolar disorder

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Abstract

The aim of this work is to design and synthesise carbohydrate derivatives, namely pseudo-*C*-nucleosides, with potential interest in the treatment of bipolar disorder.

Bipolar disease, also known as manic-depressive illness, is a serious, incapacitating, and chronic neuropsychiatric disorder in which the patient has recurrent episodes of two opposing mood states, depression and mania. The available medical treatment for this disorder is not the most appropriate, once antipsychotic drugs are usually used to treat maniac disease and antidepressant drugs to treat depressive crisis.

The synthesised pseudo-*C*-nucleosides with potential interest for the treatment of this manic-depressive disorder included pyrazolidine-3-ones and 5-hydroxypyrazoles. The prepared compounds were analysed by ^{1}H- and ^{13}C- Nuclear Magnetic Resonance (NMR) and will be subject of further in vitro biological evaluation.

Introduction

Carbohydrates have a central role in numerous physiological events, such as in cellular communication, and are part of the basic structure of some endogenous biomolecules, which makes them usually biocompatible and good starting materials for the synthesis of new compounds with potential biological activity [1]. This class of compounds presents several chiral carbons that confers them stereogenic centres, which is an advantage for the selective interaction with the biological targets [2]. In fact, carbohydrates are considered privileged scaffolds and can be linked to several pharmacophores, such as heterocycles. Therefore, they

are largely used at the therapeutic level in the treatment of several illnesses, including in central nervous system pathologies [2-4].

Among the neuropsychiatric diseases, one of the most challenging is bipolar disease also known as manic-depressive disorder. This is a disabling, chronic and severe disease, in which the patient has recurrent episodes of two opposite mood states - mania and depression [4-8]. It is known that, between other neurobiological changes involved in this disease, a GSK3β hyperactivity occurs, which leads to apoptosis of neuronal cells causing reductions of neurons and glial cells. Hence, it is important to develop a compound presenting at least the ability to inhibit the GSK3β phosphorylation and having neurotrophic effects to reduce this loss of neuronal cells [6].

With this study, we aimed to synthesise carbohydrate derivatives, namely pseudo-*C*-nucleosides, with potential interest for the treatment of the manic-depressive disorder, for further *in vitro* evaluation.

Results and Discussion

The pseudo-*C*-nucleosides were synthesised from a commercially available compound, 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**1**).
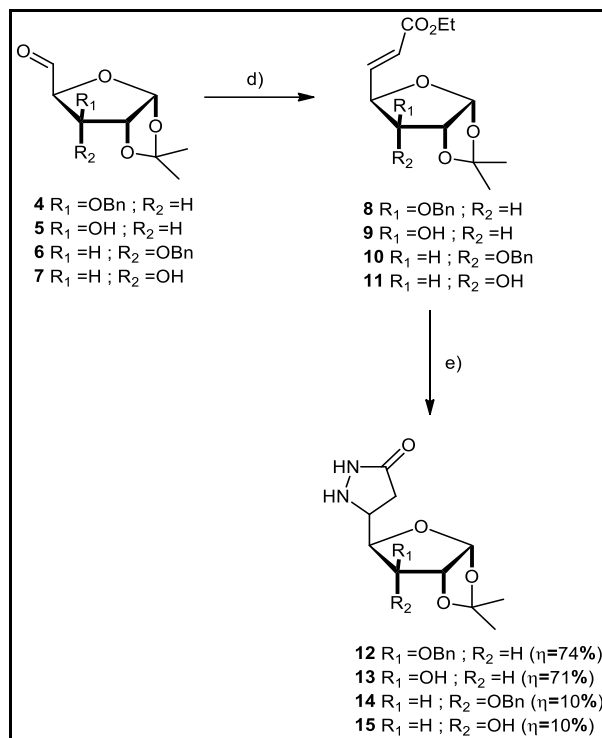
Of these, 5-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-threofuranos-4-*C*-yl)-pyrazolidine-3-one (**12**) was synthesised from compound **1**. For this, a benzylation of the hydroxyl group at C3, followed by acidic hydrolysis of the isopropylidene in C5 and C6 of the furanosidic ring and further oxidative cleavage was performed (Scheme I).
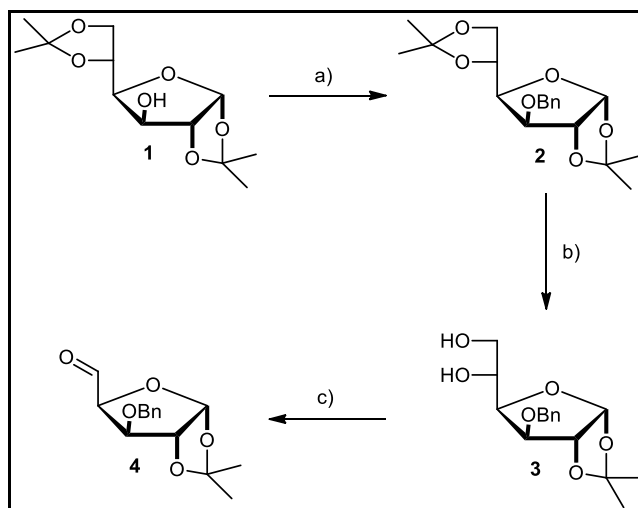
Scheme I- Reactional pathway to obtain the aldehyde 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose (**4**) from 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**1**). Reactional conditions: a) BnBr, DMF, NaH, r.t., 20 min.; b) CH₃COOH 80%, 60°C, 1 h.; c) NaIO₄, ethanol, water, r.t., 20 min.

Then, a Wittig reaction allowed the preparation of an α,β-unsaturated ester, which is the precursor for the final reaction, in which was obtained the pyrazolidine-3-one linked to the C4

of the furanose ring. For obtaining 5-(1,2-*O*-isopropylidene-α-D-threofuranos-4-*C*-yl)-pyrazolidine-3-one (**13**) the same synthetic pathway was followed but without the step of the benzylation of the hydroxyl group linked to C3 (Scheme II).

4 R$_1$ =OBn ; R$_2$ =H
5 R$_1$ =OH ; R$_2$ =H
6 R$_1$ =H ; R$_2$ =OBn
7 R$_1$ =H ; R$_2$ =OH

8 R$_1$ =OBn ; R$_2$ =H
9 R$_1$ =OH ; R$_2$ =H
10 R$_1$ =H ; R$_2$ =OBn
11 R$_1$ =H ; R$_2$ =OH

12 R$_1$ =OBn ; R$_2$ =H (η=74%)
13 R$_1$ =OH ; R$_2$ =H (η=71%)
14 R$_1$ =H ; R$_2$ =OBn (η=10%)
15 R$_1$ =H ; R$_2$ =OH (η=10%)

Scheme II- Synthetic pathway of the pyrazolidine-3-one derivatives and yields. Reactional conditions: d) (Carbethoxymethylene)triphenylphosphorane, Chloroform, 30°C, 30 min; e) Ethanol, Hydrazine monohydrate, 80°C, 1h. [9].

Compounds 5-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**14**) and 5-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one (**15**) were obtained from the precursor 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (**17**), which was prepared from compound **1** by an oxidation of the free hydroxyl linked to C3 and posterior reduction to obtain (Scheme III). The further reactions were the same described for compounds **12** and **13**, respectively (Scheme II).

The difference between the final yields of these compounds can be explained by the fact that the precursor 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose, is less reactive than the homologous glucose derivative.
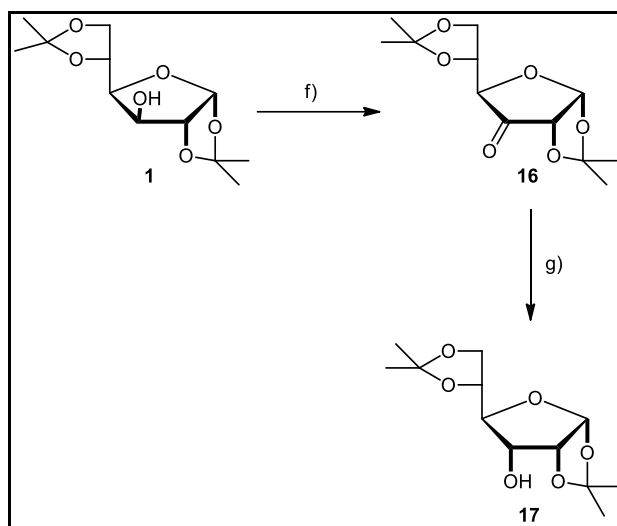
Scheme III- Synthetic pathway to compound **17** from compound **1**. Reactional conditions: f) PCC, anhydrous dichloromethane, 40°C, 1 h.; g) Anhydrous ethanol, sodium borohydride, 0°C, 20 min

Compounds 3-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-threofuranos-4-*C*-yl)-5-hydroxypyrazole (**21**), 3-(1,2-*O*-isopropylidene-α-D-threofuranos-4-*C*-yl)-5-hydroxypyrazole (**22**) and 3-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-5-hydroxypyrazole (**23**), were obtained by the same procedure applied to synthesise compounds **12**, **13** and **14**, respectively, until the α, β-unsaturated esters were prepared. After that, an addition reaction was performed to obtain a β-enaminoester, which is the precursor for the final reaction, in which was obtained the 5-hydroxypyrazole linked to the C4 of furanose ring (Scheme IV).
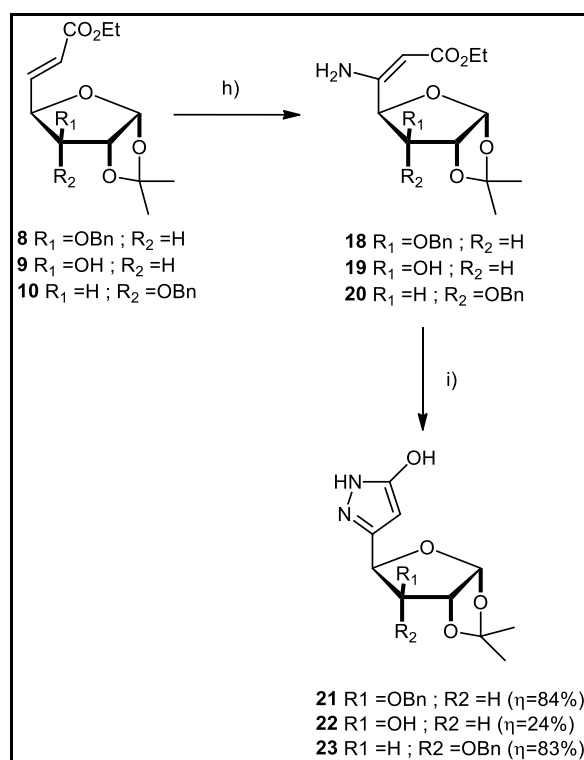
Scheme IV- Synthetic pathway of the 5-hydroxypyrazole derivatives and yields. Reactional conditions: h) DMF, NaN$_3$, MW (450 W), 6 min; i) Ethanol, Hydrazine monohydrate, 80°C, 1 h. [9].

In the case of 5-hydroxypyrazoles **21-23**, the benzylated derivatives were obtained in superior yields than without any protecting group attached to C3.

At this moment, *in vitro* cytotoxicity assays are being performed.

Conclusions

The pseudo-*C*-nucleosides **12**, **13**, **21** and **23** were obtained in good yields when compared with compounds **14**, **15** and **22,** which were obtained with very low yield.

As future work, we pretend to optimise the synthetic pathways for the pyrazolidine-3-one derivatives, whose precursor is the 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose, to posterior *in vitro* biological evaluation, in which is expected to confirm the potential interest to control the maniac-depressive disorder. If the compounds present good *in vitro* results in cell assays, we pretend to study the effects of these compounds in *in vivo* assays.

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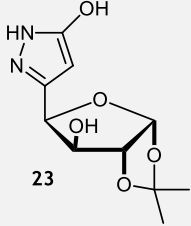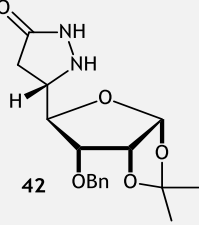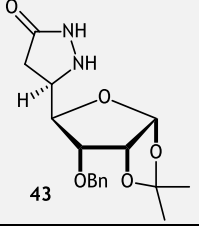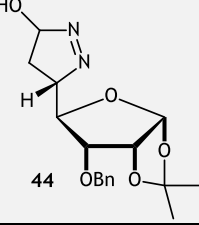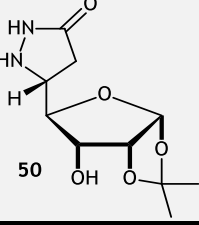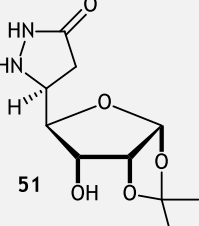
Attachments

1. **InfraRed spectrum of the compound obtained instead of compound 39**
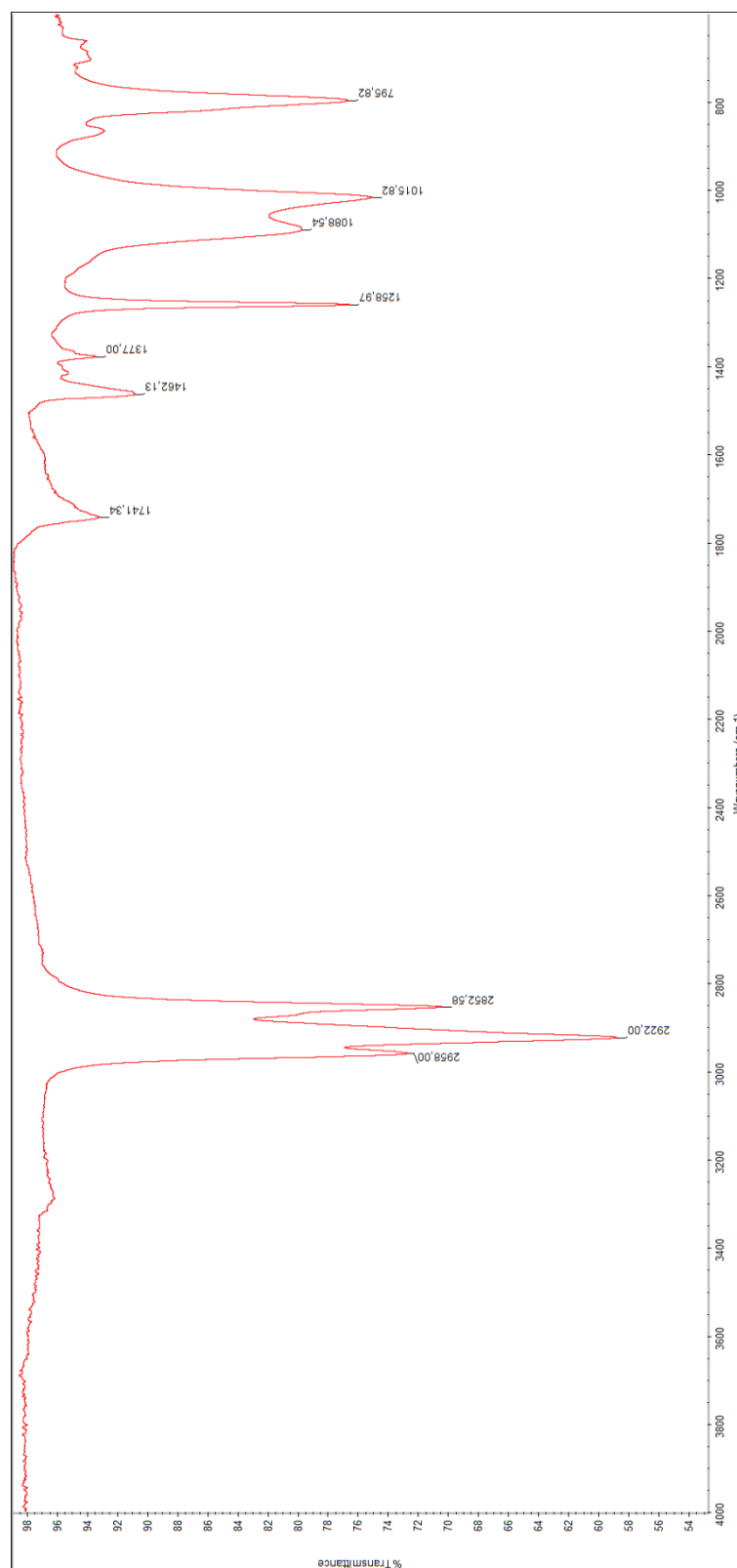
2. Lipinski's rule of five data for the final compounds 23, 42-44 and 50-52

Table 10. Data related to Lipinski's rule of five and the value of the Polar Surface Area (PSA) predicted by MedChem Designer 3.1.0.30 2014 free trial. In the first column are represented the final compounds, in the second the molecular weight, in the third the log P, in the fourth the Number of Hydrogen Bond Donor protons (NBDH), in the fifth the total number of Nitrogen and Oxygen atoms (N.O.), in the sixth the PSA and in the seventh the observations (Obs.) that are related with the fulfil of Lipinski's Rule of five.

Compound	Molecular Weight	LogP	NBDH	N.O.	PSA (Å^2)	Obs.
23	242.233	-0.560	3	7	96.830	Follow Rule of 5
42	244.249	-1.417	3	7	89.050	Follow Rule of 5
43	244.249	-1.417	3	7	89.050	Follow Rule of 5
44	244.249	-1.417	2	7	92.870	Follow Rule of 5
50	334.374	0.656	2	7	78.050	Follow Rule of 5
51	334.374	0.656	2	7	78.050	Follow Rule of 5

	334.374	1.991	1	7	81.870	Follow Rule of 5
52						

3. Guideline of the synthesised compounds

Table 11. Guideline of the synthesised compounds. In the first column are represented the numbers of the compounds, and in the second the name of the compound.

Compound	Name
9	1,2-*O*-isopropylidene-α-D-glucofuranose
10	1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose
11	1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose
12	3-deoxy-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuran-3-ulose
13	3-deoxy-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose
14	3-Iodo-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose
16	1,2-*O*-isopropylidene-α-D-ribo-pentodialdo-1,4-furanose
17	Ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate
18	Ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*Z*)-enfuranoate
19	3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-(2-propargyl)-α-D-xylofuranose
22	Ethyl 5-amino-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*E*)-enfuranoate
23	5-(1,2-*O*-isopropylidene-α-D-threofuranos-4-*C*-yl)-5-hydroxy-1*H*-pyrazole
25	3-*O*-benzyl-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose
26	3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose
27	3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose
28	3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose
30	1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose
31	3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-trityl-α-D-xylofuranose
32	(3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-propyl-α-D-xylofuranos-8-yl)diazene
34	5*H*-5-amino-*N*-(2-propargyl)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose
36	3-*O*-acetyl-1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose
37	3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose

38	3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylo-pentodialdo-1,4-furanose
39	3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-xylofuranose
41	3-iodo-1,2-*O*-isopropylidene-α-D-glucofuranose
42	5-(*S*)-(3-O-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one
43	5-(*R*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one
44	5-(*S*)-(3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-3-hydroxypyrazoline
45	3-*O*-benzyl-1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose
46	3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-allofuranose
47	3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribo-pentodialdo-1,4-furanose
48	Ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate
49	Ethyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*Z*)-enfuranoate
50	5-(*S*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one
51	5-(*R*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-pyrazolidin-3-one
52	5-(*S*)-(1,2-*O*-isopropylidene-α-D-erythrofuranos-4-*C*-yl)-3-hydroxypyrazoline
53	1,2-*O*-isopropylidene-α-D-allofuranose
54	3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose
56	Ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*E*)-enfuranoate
57	Ethyl 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-xylo-hept-5-(*Z*)-enfuranoate
59	Ethyl 5-amino-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-ribo-hept-5-(*E*)-enfuranoate