João Filipe Nunes da Costa e Silva Barateiro

Licenciado em Química Aplicada

Synthesis of sugar-biguanide hybrids to study their potential biological effects

Dissertação para obtenção do Grau de Mestre em
Química Bioorgânica

Orientador: Maria Teresa Barros, Associate Professor With Habilitation (retired), NOVA University of Lisbon

Fevereiro, 2021

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Acknowledgements

I would like to thank everyone who accompanied me on this journey. First, my teacher and advisor Maria Teresa Barros who accompanied me on this process and motivated me a lot to carry out this work and transmitted valuable knowledge throughout my academic life.

I am especially grateful to my family for having motivated me to persist, when things were more difficult this year, that was not (and still is not) easy for anyone. My mother, sister and Luís were saints for listening to my ramblings and frustrations that happened along the way. I appreciate them to no end.

To my friends who pulled me into a more positive state of mind, even sometimes without knowing it, when my family could not be present. To Cláudia, André, Vanessa, Sebastião, Pedro and Piedade who were there most of my way through this stage of my life and before, I thank them profusely. To Liliana, I just thank you for being there all the time for 12 years of my life.

I keep this journey forever in my heart, which although difficult, it will be impossible to forget.

Resumo

A síntese de híbridos açúcar-biguanida foi tentada começando a abordagem a partir de sais de dicianamida, pelo uso de meio ácido para ativar a dicianamida adicionando a amina para criar um composto de biguanida, a ser conectado a um açúcar por um anel triazole. Os princípios da Química Verde foram considerados para tentar manter a reação o mais "verde" possível e isso foi parcialmente alcançado na via de síntese da biguanida. RMN foi a principal ferramenta para monitorar o sucesso das reações. As reações com os açúcares já eram conhecidas na literatura, foram obtidos derivados de Galactose e Manose, foram obtidos também dimetil, β-naftil e imidazol cianoguanidinas, os rendimentos obtidos variaram, entre 20-60%. Os RMNs de carbono possibilitaram uma maneira rápida de saber se ocorreram as reações de guanidina, com o aparecimento de um sinal em torno de 160 ppm, o que facilita a identificação e não desperdiça tantos recursos.

Abstract

Synthesis of sugar-biguanide hybrids was attempted by starting the approach from dicyanamide salts, by use of acidic medium to activate the dicyanamide for amine addition to create a biguanide compound to be connected to a sugar by a triazole ring. Green Chemistry principles were considered to try and maintain the reaction as "green" as possible and that was partially achieved in the biguanide synthesis pathway. NMR was the primary tool for monitoring the success of the reactions. The reactions with the sugars were already known in literature, Galactose and Mannose derivatives were obtained, dimethyl, β-naphtyl and imidazole cyanoguanidine were obtained as well, the yields obtained varied, between 20-60%. The carbon NMRs allowed for a fast way to know if the guanidine reactions happened, with the appearance of a signal around 160 ppm, this facilitates identification and doesn't waste as much resources.

Índice

Abreviaturas

NMR/RMN – Nuclear Magnetic Ressonance/ Ressonância Magnética Nuclear

AMPK – Adenosine monophosphate kinase

GLUT – Glucose transporter protein

Kcal – Kilocalories

g – gram

ppm – parts per million

Hz – Hertz

$\nu_{máx}$ – Maximum wave length

UV – Ultra Violet

°C – Degrees Celsius

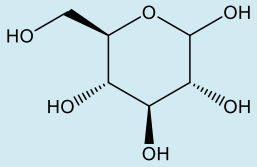
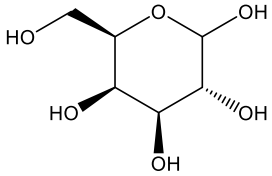
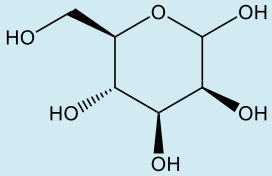
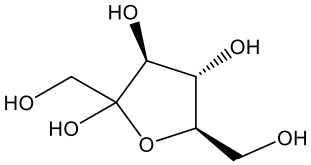
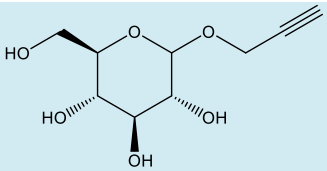
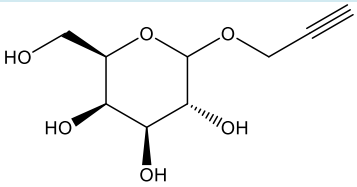
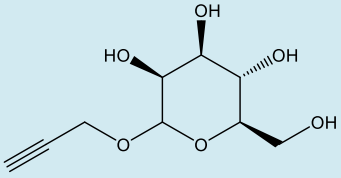
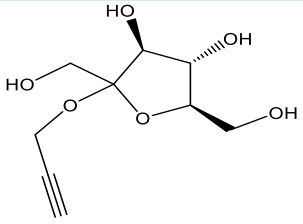
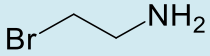
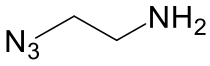
mol – mole

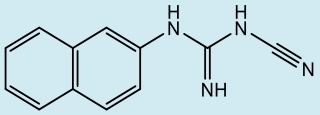
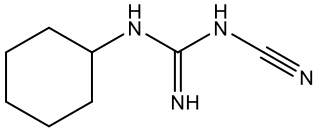
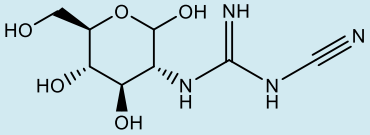
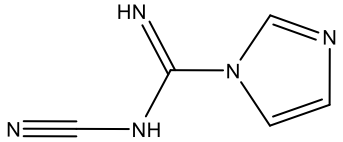
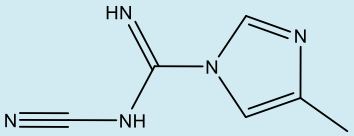
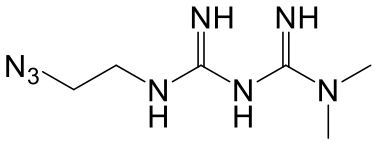
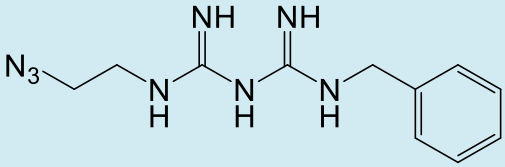
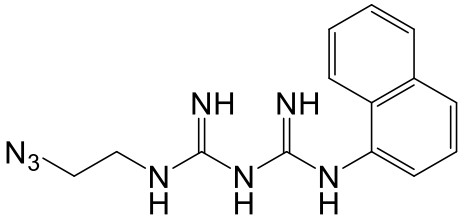
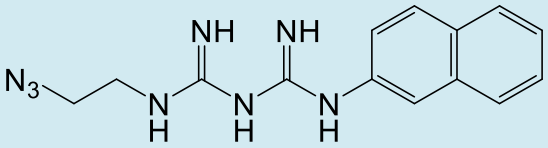
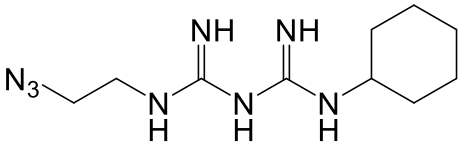
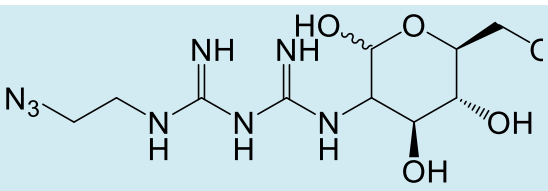
ml – mililiter

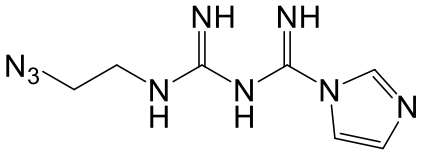
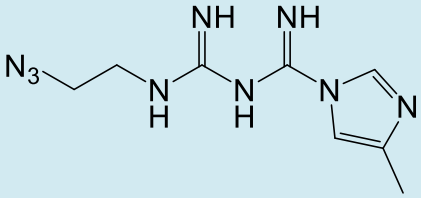
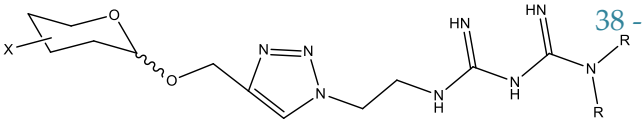
mg – miligram

M – Molar concentration

Compound List

	Compound	Number	Page number
Glucose		3	13
Galactose		4	13
Mannose		5	13
Fructose		6	13
Propargyl Glucose		7	43
Propargyl Galactose		8	44
Propargyl Mannose		9	43
Propargyl Fructose		10	44
Bromoethanamine		11	23
Azidoethanamine		12	23

β-Naphtylcyanoguanidine		25	31
Cyclohexylcyanoguanidine		26	31
glucocyanoguanidine		27	31
imidazolecyanoguanidine		28	31
4-methylimidazolecyanoguanidine		29	31
Azidoethyl Dimethylbiguanide		30	-
Azidoethyl Benzylbiguanide		31	-
Azidoethyl α-Naphtylbiguanide		32	-
Azidoethyl β-Naphtylbiguanide		33	-
Azidoethyl Cyclohexylbiguanide		34	-
Azidoethyl glucobiguanide		35	-

Azidoethyl imidazolebiguanide		36	-
Azidoethyl 4-methylimidazolebiguanide		37	-
Target molecule for a single arbitrary sugar		38 - 45	39

I. Introduction

I.1. Diabetes

Worldwide there are 415 million people who have diabetes, and it is estimated that an additionally of 193 million people are living with the undiagnosed disease. Diabetes was the nineth leading cause of death worldwide in 2019 (1.5 million people), with an increase of 70% since 2000. [1]

Diabetes is a chronic disease that occurs when there is a relative insulin deficiency caused by pancreatic malfunction. Insulin is a hormone that regulates blood sugar. Raised blood sugar leads to various serious complications, namely blindness, heart attacks, stroke, kidney failure and lower limb amputations. [2]

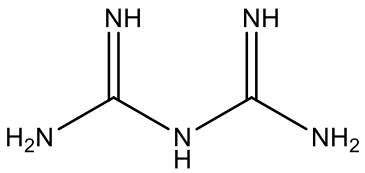
There are 4 types of diabetes:

- Type I: when not enough insulin is being produced by pancreas and requires daily administration of insulin. Neither the cause of type I diabetes nor the means to prevent it are known.
- Type II: when the body is ineffectively using insulin. This is the most common type of diabetes. It is caused by excess body weight and physical inactivity. An increased incidence in children has been observed in later years.
- Gestational diabetes: when blood glucose value is above normal but below those needed for the diagnosis of diabetes during pregnancy. Gestational diabetes increases the risk of either or both pregnancy and delivery complications.
- Impaired glucose tolerance and impaired fasting glycaemia: these two are intermediate conditions to type II diabetes, although the disease can be avoided in this stage if necessary actions are taken.

Type II diabetes can be prevented or at least delayed with a healthy diet, regular physical activity, managing a normal body weight and avoiding tobacco. Once the chronic disease is installed it can be treated to avoid or delay complications. [3] Treatment of diabetes involves maintaining a healthy lifestyle and lowering the blood sugar levels using insulin (type I and sometimes type II) or oral medication (type II), blood preasure control and foot care. [3] Biguanides, more specifically metformin, are used in the treatment of type II diabetes and will be introduced next.

I.2. Biguanides and metformin

Biguanides are an important class of compounds, based on the biguanidine molecule (Figure 1), and they have several applications such as antidiabetic, antimalarial, antiseptic and disinfectants. Metformin, for example, is the most common antidiabetic compound, while proguanil is an antimalarial agent.[4-7] Other molecules as buformin, phenformin, clorophenylbiguanide are also known biguanidines, but their use was discontinued because of side-effect agressiveness. Apart from the well-established antimalarial and antidiabetic effects, biguanide derivatives have shown antiviral and antimicrobial activity. [8-9]



1

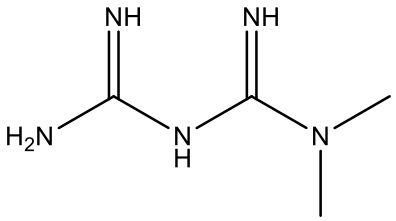
Figure 1 – Biguanidine, the base of biguanide class of compounds.

Although the mechanism of action of some of these biguanides is not fully understood, Holland and co-workers declared that tyrosine kinase is stimulated by metformin.[10] It is now being studied the possibility that adenosine monophosphate kinase (AMPK) is also activated. [11-13]

Another interesting application of biguanides is their derivatization and transformation into polymeric nanoparticles that can kill the methicillin-resistant *Staphylococcus aureaus*, responsible for drug-resistant infections. [14]

I.2.1. Metformin

Metformin (*N*,*N*-dimethylimidodicarbonimidicdiamide, Figure 2) is derived from goat's rue plant (Galega officinalis). [15, 16] It is the first choice to treat diabetes type II since the 1950's, especially for patients with obesity and/or hyperlipidemia. [17, 18] Metformin **2** has various antihyperglycemic effects, plus inhibition of intestinal glucose absorption, improvement of peripheral and hepatic insulin sensitivity, reduction of hepatic glucose production and enhancement of peripheral glucose utilization. [19] Metformin, like other biguanides, does not stimulate insulin secretion, and has favorable effects on dyslipidemia, vascular function, hypertension and fibrinolytic activity. [20, 21] Plus, there have been reports of anti-cancer activity mediated by metformin. Xinbing Sui et al. studied this activity by proposing that metformin could act either indirectly or directly on tumors. [22]



2

Figure 2 – Metformin, the most used antidiabetic compound.

Regarding metformin's mechanism of action, it is believed that it can decrease glucose blood levels by enhancing suppression of glycogenesis by insulin and reducing glucagon-stimulated gluconeogenesis. It can positively affect insulin receptor phosphorylation and tyrosine kinase activity. Also, it can increase translocation of glucose transporters GLUT-1 and GLUT-4, and prevent the development of insulin resistance in hepatocytes and adipocytes. [4]

Unfortunately, metformin is a highly basic antihyperglycemic agent that is fully protonated under physiological conditions, so it is slowly and incompletely absorbed from the upper intestine after it has been orally administrated. With kidney excretion, metformin suffers from variable bioavailability and causes uncomfortable gastrointestinal effects, such as abdominal discomfort, nausea, vomiting, diarrhea and metallic taste. [23] Additionally, since metformin is eliminated by kidneys, toxicity can occur if there is an accumulation due to insufficient renal clearance or if the compound is overdosed. Although new strategies have been developed to try improving metformin, no success has been observed. The work presented in this thesis is a step towards trying to make metformin less aggressive and toxic to patients.

Reitz and co-workers hypothesized that attaching the biguanide to a monosaccharide moiety could enhance specificity by targeting to the transport system. As such, appropriate monosaccharides are those capable to be recognized within the human body, and they are discussed in the next section. [24]

I.3. Carbohydrates

In the middle of the 21st century, the world still faces a high consumption of products of fossil origin, and with the forecast of their depletion in future times, the industry has to take measures to reduce the consumption of petrochemical derivatives. In this sense, the industry will have to take a more sustainable approach, which does not cause as much environmental pressure as the one that currently exists, using renewable natural products instead. [25]

A possible example would be the use of carbohydrates (Figure 3), which represent a large percentage of natural origin resources. Carbohydrates [general formula $C_n(H_2O)_n$)], in addition to their traditional use in food and paper production, among others, have a high application potential due to their diverse chemical functionality. The exploration of their possible selective reactivity can open a wide range of possible applications, which have not yet been properly explored. [26]

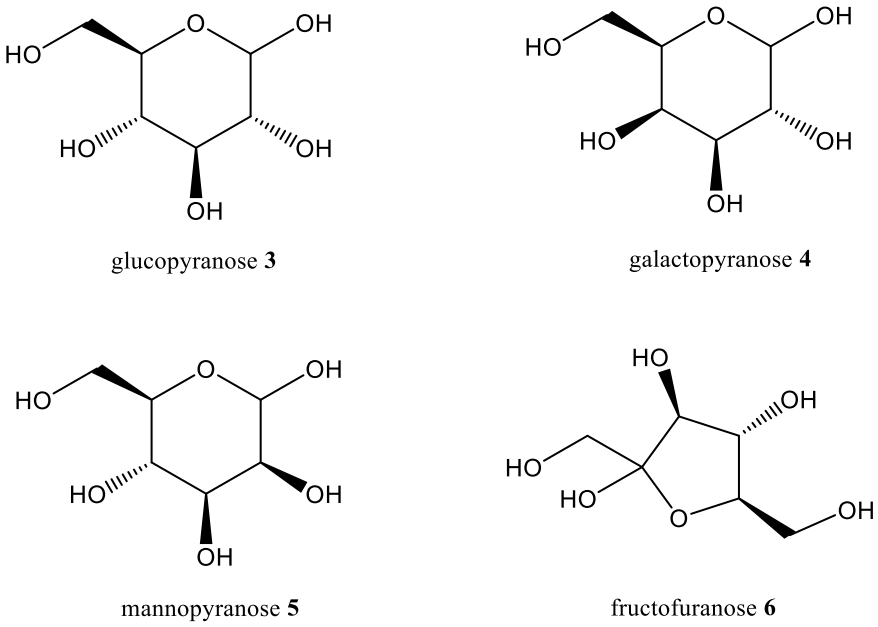


Figure 3 – Chemical structures of some D-monosaccharides in their predominant cyclic form.

I.3.1. Glucose

Glucose **3** (Figure 3) is a simple sugar with the molecular formula $C_6H_{12}O_6$. Glucose is the most abundant monosaccharide[27], a subcategory of carbohydrates. Glucose is mainly made by plants and most algae during photosynthesis from water and carbon dioxide, using energy from sunlight, where it is used to make cellulose in cell walls. [28]

Regarding energy metabolism, glucose is the most important source of energy in all organisms. For that, glucose is stored as a polymer in plants (starch and amylopectin), and in animals (glycogen). In addition, glucose metabolites produce all non-essential amino acids, sugar alcohols such as mannitol and sorbitol, fatty acids, cholesterol and nucleic acids [29]. Furthermore, glucose is used as a building block in the glycosylation of proteins to form glycoproteins, glycolipids, peptidoglycans, glycosides and other substances.

The naturally occurring form of glucose is D-glucose, while L-glucose is produced synthetically in comparatively small amounts and is of lesser importance. Glucose is a monosaccharide containing six carbon atoms and an aldehyde group, and

is therefore an aldohexose. Glucose molecules can either exist in an open-chain (acyclic) form or as ring (cyclic). Glucose is naturally occurring and can be found in fruits and other parts of plants in its free state.

Industrial uses of glucose are the main use for the production of fructose and in the production of glucose-containing foods. In food industry and processing, it is used as a sweetener, humectant, to increase the volume and to create a softer mouthfeel. [30]

I.3.2. Galactose

Galactose **4** (Figure 3) is a simple sugar that is normally transformed in the liver before being used up as energy. This sugar is quite abundant in human diets and helps in a number of functions. The main dietary source of galactose is lactose from milk and yogurt, which is digested (hydrolyzed) to galactose **4** and glucose **3**. Foods containing small amounts of free galactose include low-lactose or lactose-free milk, certain yogurts, cheeses, creams, ice creams and other foods artificially sweetened with galactose. Plain natural foods (fruits, vegetables, nuts, grains, fresh meats, eggs, milk) usually contain less than 0.3 g galactose per serving.[31] Galactose has recently been reported to be beneficial in the management of a number of diseases, particularly those affecting brain function. The conversion of galactose to amino acids in the brain requires ammonia equivalents as a substrate. Galactose plays a potentially useful role in removing these neurotoxic compounds from the brain in patients suffering from hepatic encephalopathy or Alzheimer's disease.[32] Dementia is associated with dysfunction of the insulin-receptor system, followed by decreased glucose transport to brain cells and subsequent metabolism. As galactose is transported to the brain, it can act as an alternative source of energy owing to its metabolism to glucose. [33] Daily oral galactose administration has also been shown to be a promising new, non-toxic therapy for the treatment of resistant nephrotic syndrome. [34] Of course, things may be different for those who are intolerant or are not able to process galactose, like those with galactosaemia.

I.3.3. Mannose

While much of the mannose **5** (Figure 3) used in glycosylation is believed to be derived from glucose **3**, in cultured hepatoma cells (cancerous cells from the liver), most of the mannose for glycoprotein biosynthesis comes from extracellular mannose, and not glucose.[35] Many of the glycoproteins produced in the liver are secreted into the bloodstream, so dietary mannose is distributed throughout the body. [36] It is a C-2 epimer of glucose. Mannose is important in human metabolism, especially in the glycosylation of certain proteins. Several congenital disorders of glycosylation are

associated with mutations in enzymes involved in mannose metabolism.[37] Mannose is not an essential nutrient; it can be produced in the human body from glucose, or converted into glucose. Mannose provides 2-5 kcal/g of energy calories. It is partially excreted in the urine. D-Mannose is used as a nutritional supplement, packaged as "d-mannose", to prevent recurrent urinary tract infections. [38]

Although mannose has hydroxyl too, in the axial position and is therefore not recognized by glucose transporters, it is recognized by other specific transporters and can be used to the purpose of this work, metformin targeted delivery.

I.3.4. Fructose

Fructose **6** (Figure 3) has several properties, characteristics and origins, similarly to glucose **3**. Since it is one of the building blocks of sucrose (common table sugar), it can be obtained from it. Fructose also undergoes the Maillard reaction, non-enzymatic browning, with amino acids. Because fructose exists to a greater extent in the open-chain form than glucose does, the initial stages of the Maillard reaction occur more rapidly than with glucose. Therefore, fructose has various potential contributions to food products, namely to change palatability, nutritional effects, excessive browning, volume and tenderness reduction during cake preparation, and formation of mutagenic compounds.[39]

Fructose readily dehydrates to give hydroxymethylfurfural ("HMF"). This process, in the future, may become part of a low-cost, carbon-neutral system to produce replacements for petrol and diesel from plants.[40] Fructose is also a great alternative for dietary substitution in people suffering from diabetes, because this sugar is not only sweeter than either glucose or sucrose, but it can also be used in lower quantities in food. Fructose has a glycemic index of 23, compared with 100 for glucose and 60 for sucrose.[41] Fructose is also 73% sweeter than sucrose at room temperature, allowing diabetics to use less of it per serving. Fructose consumed before a meal may reduce the glycemic response of the meal.[42] Fructose-sweetened food and beverage products cause less of a rise in blood glucose levels than those manufactured with either sucrose or glucose does.[43]

I.4. Biological interest of novel triazole derivatives

The 1,2,3-triazole unit is relevant to medicinal chemistry and can act as a pharmacophore group, but it is also often used to link two or more compounds with potential interest in the intended purpose for a given molecule. In this case, our interest in the triazole ring is to link metformin and carbohydrate moieties to form the wanted derivatives.

The 1,2,3-triazole unit, known since the 19th century, is present, as a heterocyclic system, in many synthetic compounds of pharmacological and commercial interest. On the other hand, the triazole ring functions as a bioisomer of the amide group because it has similar physical and chemical properties (Figure 4). However, on the other hand, it appears that the triazole ring is more stable than amides, as there are no hydrolysis, oxidation or reduction reactions.[44] Thus, some research groups focus their studies on the replacement of amides by triazole rings in molecules where amidic bonds are crucial to biological activity, since it is very stable and has a chemical behavior similar to the amide function, found in nature.

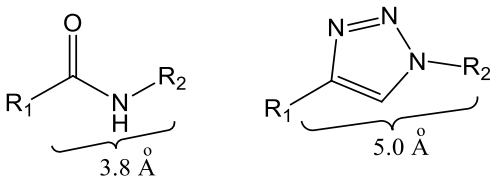
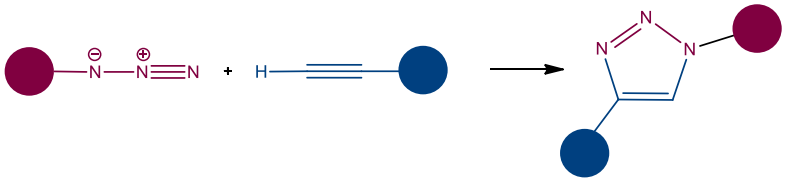


Figure 4 – Size comparison between an amide group and a triazole system. [44]

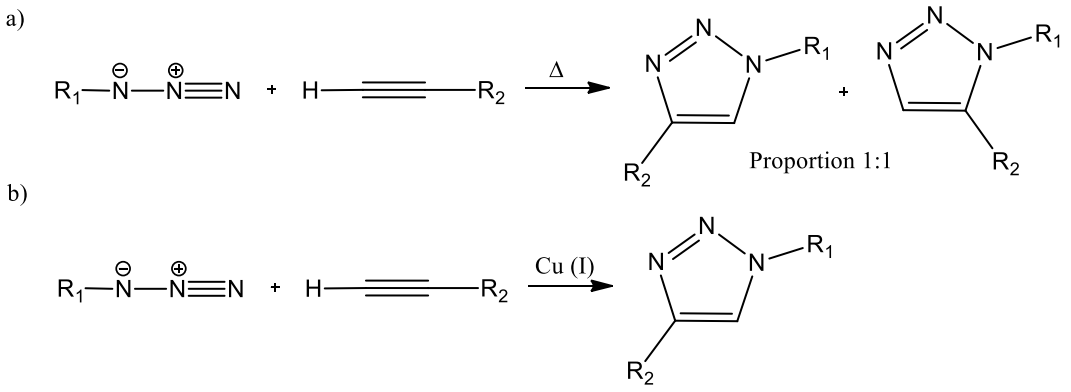
I.4.1. Triazole Formation Reaction: 1,3-Dipolar Cycloaddition

The 1,2,3-triazole heterocycle is only formed synthetically and does not occur in nature. [45] The most frequently used method is called "click chemistry", which basically consists of cycloaddition between azides and alkynes (Scheme 1).



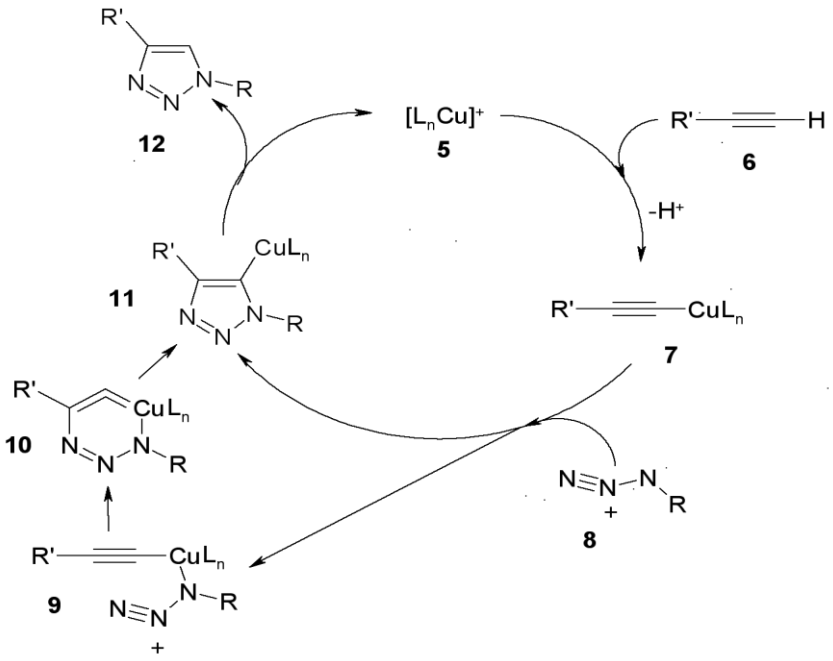
Scheme 1 - "Click" reaction between an azide and an alkyne forming a triazole ring.

Initially, the reaction developed by Huisgen, in 1967 [46], presented several problems, namely excessively long reaction times, high temperatures and low yields. In addition to these aspects, it led to the formation of a mixture of 1,4- and 1,5- triazole regioisomers as presented in Scheme 2a). Later, a different approach was described in the literature, involving a copper (I) catalyst, which selectively afforded 1,4 triazole product (Scheme 2b).



Scheme 2 – Comparison of the products obtained by a) Non-catalyzed alkyne-azide cycloaddition reaction, leading to a mixture of 1,4- and 1,5-triazole systems, and b) copper catalyzed formation of selective 1,4-triazole ring.

The selectivity observed using the copper (I) can be explained by the reaction mechanism that is presented in Scheme 3. In this synthesis process, a reaction between a dienophile and a diene takes place, giving rise to a five-membered ring, namely a triazole. This reaction is also called the Huisgen reaction. This reaction uses a catalyst to improve the overall yield, so it is not necessary to use drastic reaction conditions such as high temperatures. The most used catalyst is a copper compound (either I, or II plus a reducing agent). The regioselectivity observed is a result of the way copper (I) is inserted in the intermediate structure, leading to the formation of the final 1,4- product. [44]



Scheme 3 – Generalized representation of 1,4-triazole ring formation between an alkyne and an azide with a copper (I) catalyst. [44]

The mechanism involved is not fully understood and there is not yet a unanimous opinion on the nature of intermediaries. Some authors admit that after the formation of copper acetylides, complexation with the azide occurs. In this intermediate, copper makes the terminal nitrogen azide more electrophilic and the alkyne carbon more electrodeficient, thus promoting ring formation. However, other researchers admit the possibility of another type of metallic intermediate, and in particular some admit the possibility that cyclization occurs with two metallic centers. [47] Furthermore, other catalysts can be used, such as ruthenium that give 1,5-triazole derivatives. [48-50]

I.5. Principles of Green Chemistry

Since the 90's that a big effort has been made to improve the chemistry sector to diminish pollution to the environment and maleficents to human health. As such, new processess were developed to ensure safety. Green Chemistry (Figure 5) is a term developed to describe the evaluation, the planning and the development of new ways to produce, transform and use chemistry products, diminishing reaction times, residues and hazardous products.[51] Green Chemistry was organized by twelve principles, and each one will be briefly explained next.

Figure 5 – The twelve principles of green chemistry.

1. Prevention: it is better to prevent waste than treat it afterwards.
2. Atom economy: synthetic processes should maximize the incorporation of all reactants used to the final products.
3. Less dangerous chemical synthesis: synthetic pathways should avoid or minimize the use and the production of toxic substances either to people or to the environment.
4. Plan to safer chemicals: chemical products should be designed to have maximum desired effect and minimum toxicity.
5. Safer solvents and auxiliaries: the use of auxiliary substances should be avoided or should be innocuous when in use.
6. Design for energy efficiency: processes that require energy should be minimized regarding their environmental and economic impact. As much as one can, those synthetic processes should be performed at ambient temperature and pressure.
7. Use of renewable feedstocks: whenever viable, one should use renewable feedstocks instead of deplete the non-renewable ones.
8. Diminish derivatizations: unnecessary derivatizations should be avoided or minimized in order to prevent waste and the use of additional reactants.
9. Catalysis: whenever possible one should use selective catalysts in catalytic amounts.
10. Plan for degradation: chemical products should be planned to degrade in innocuous products after their function.
11. Real time analysis to prevent pollution: analytic methods should be developed to permit monitoring and controlling in real time the formation of dangerous products.
12. Inherently safer chemistry for accident prevention: substances used in chemical processes should be chosen in order to minimize accidents such as leaks, explosions and fires.

The work developed in this thesis took into account these principles of green chemistry in order to develop the safest chemistry possible for both the people and the environment.

II. Results, Discussion and Conclusions

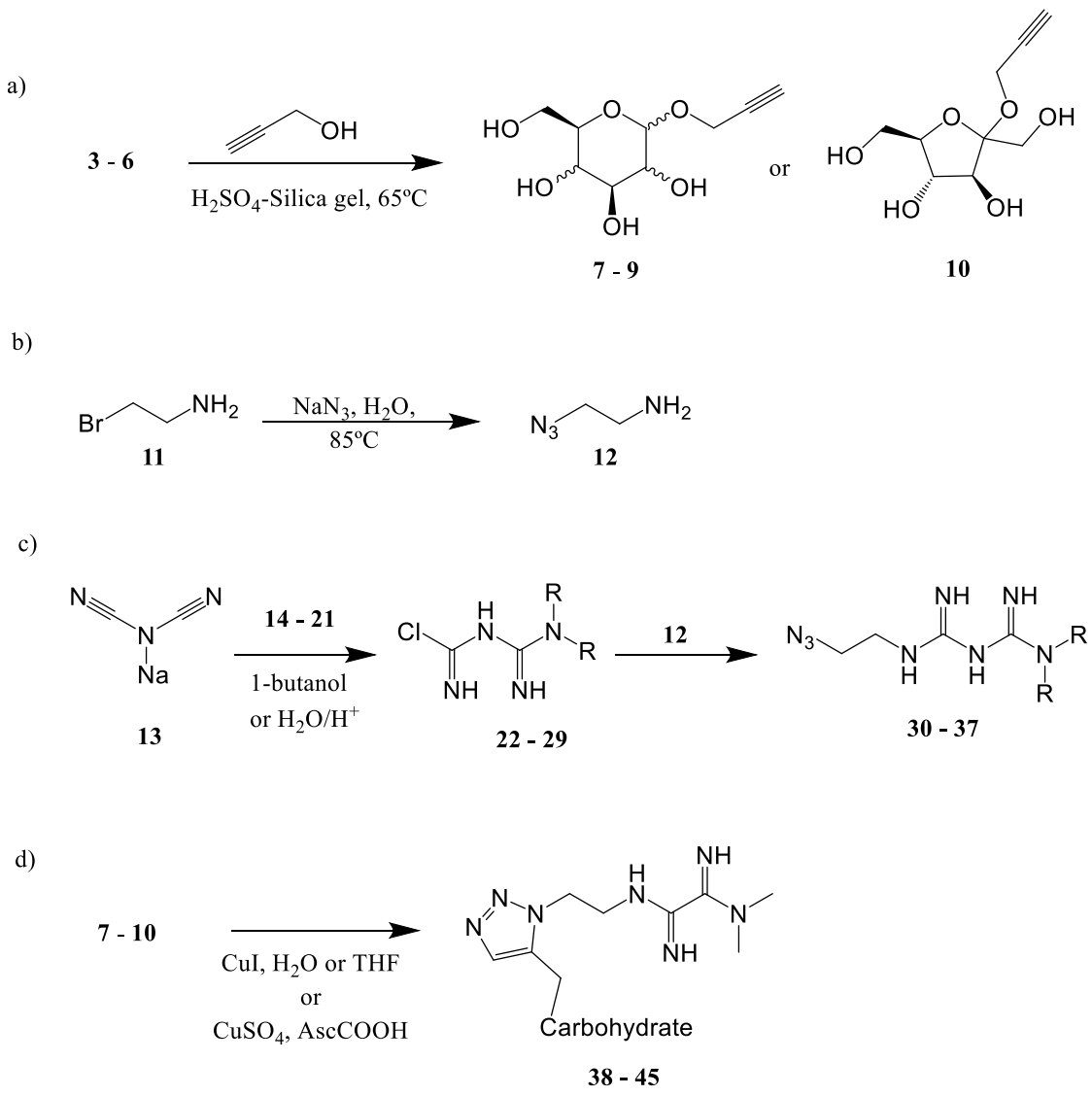
II.1. Main purpose

The purpose of this work was to synthetize a hybrid molecule to possibly treat and prevent type II diabetes. But due to the variety of biological effects that known biguanide compounds have other possibilities were considered as well not only diabetes. This final product was created with four building blocks:

- A carbohydrate unit – this will be attached to the final molecule with the anomeric hydroxyl group substituted with a propargyl group to form and effectively maintain closed the triazole ring;
- A dicyanamide compound to originate the cyanoguanidine, that will result in the biguanide compound;
- A linker, preferably short, but enough to distance the sugar from the biguanide structure to avoid stereochemical impediments, an azide on one side and an amine on the other;
- And finally, a triazole ring to unite all of the above, the sugar section with the biguanide section.

Scheme 3 illustrates the synthetic pathway to link carbohydrates and biguanides to form a biguanide-sugar hybrid. Carbohydrates used were glucose, galactose, mannose and fructose and will be detailed later when the reactions are explored individually. Reactions a), b) and d) of Scheme 4 are reactions already described in the literature and proceeded as expected. For reaction c) a few synthetic hypotheses were made, and will be briefly explained next:

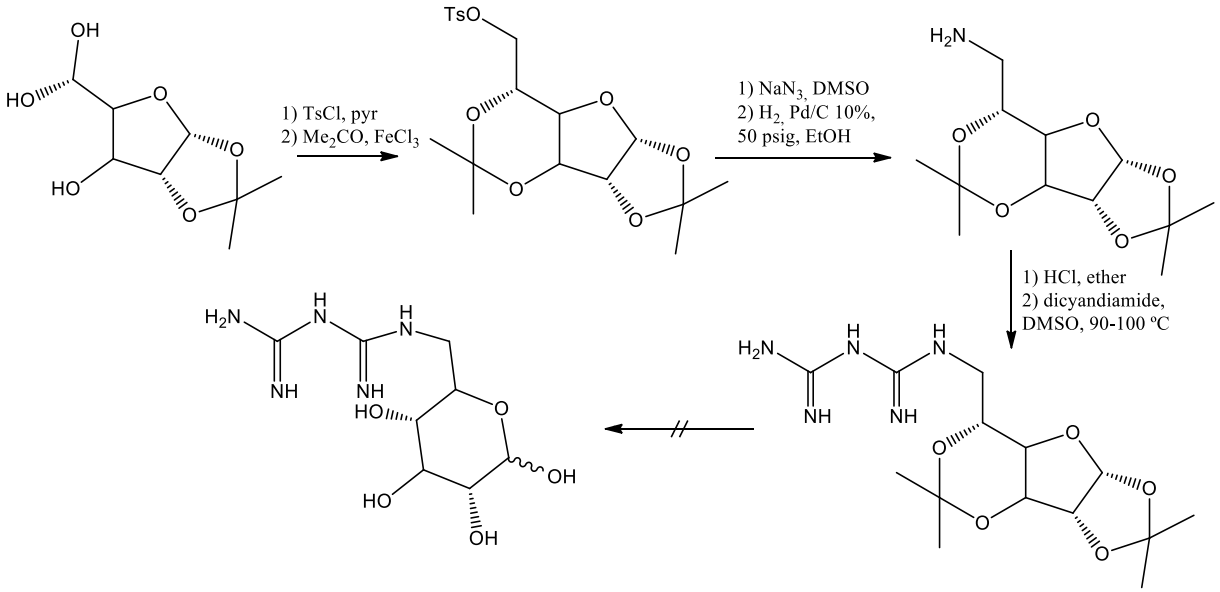
1. Direct insertion in the sugar molecule without spacing;
2. Addition of an amine group to the cyano group of dicyanamide in an acidic medium;
3. The same as the second, but in a very strong basic medium;
4. The use of a metal catalyst;
5. The use of an enzyme;
6. Microwave assisted organic synthesis.



Scheme 4 – Synthetic strategy used in this thesis to prepare products **38** - **45**.

Path 1 for reaction c): Direct insertion of a biguanide molecule to the sugar without spacing

Allen B. Reitz and co-workers[24] described a direct insertion of metformin **2** and phenformin to a carbohydrate (Scheme 5). Their results demonstrated hypoglycemic activity similar to metformin. Nevertheless, this approach to link the sugar to the biguanide moiety was discarded for the following reasons: several protection-deprotection steps, the use of dangerous solvents, and a possible stereochemical impediment due to biguanide size that can vary depending on the size of the molecule. Furthermore, Reitz and co-workers were unable to deprotect the furanose ring without hydrolyzing the biguanide moiety.

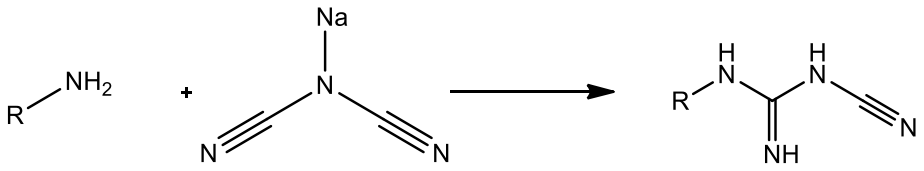


Scheme 5 – Reitz and et al pathway to link a carbohydrate and a biguanidine. [24]

Path 2 for reaction c): Addition of an amine to the cyano group of dicyanamide in an acidic medium

Bryan C. Redman and Daniel E. Nagy patented various dicyanamide salts reacting in an acidic medium, using the assumption that the acidification of the cyano group (activation) will turn it similar to an acyl chloride (scheme 6). This approach was chosen to create the biguanide derivative to connect with the sugar. These reactions are relatively clean and more of a "green" nature, the solvent is mainly water, chloridric acid salts are beneficial for general human health, and the purification process is precipitation of the solid product, which is the ideal purification method for a sustainable chemistry. On the other hand, it is important to note that this approach implicates a strict order in the way things are added to the reaction medium.

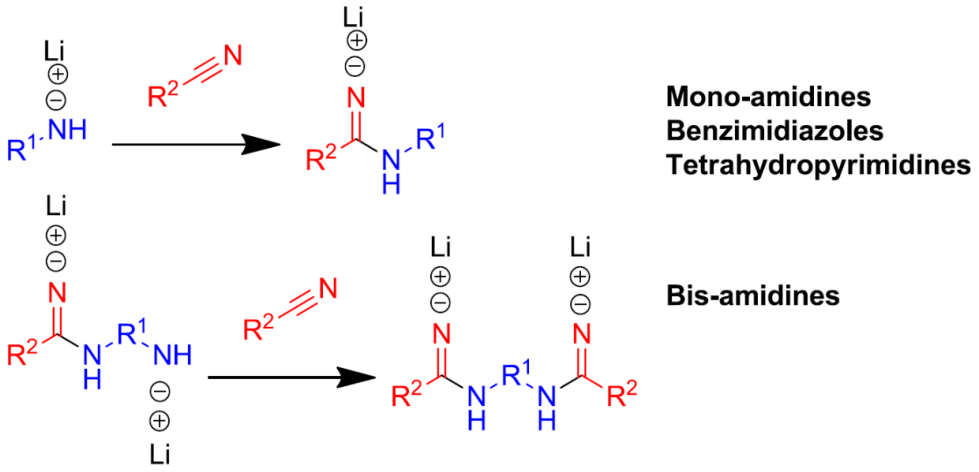
This path was the chosen hypothesis for the synthesis of the biguanide derivatives [reaction c) in Scheme 4].



Scheme 6 – Reaction between dicyanamide salts and amines.

Path 3 for reaction c): addition of an amine to the cyano group of dicyanamide in a very strong basic medium (masters essay)

This approach is the reverse way of the activation process referred in path 2. In this approach the amine is the one that is activated (scheme 7). Deprotonation of the desired amine increases it nucleophilic power, allowing it to attack the cyano group. This implies the use of a very strong (and dangerous) base, like buthyl lithium. The work-up type of this reaction needs an acidification step to eliminate the excess base. This approach was not chosen simply because the acidic way has more presumed advantages than the basic way.



Scheme 7: Activation of the amine to form a biguanide.

Path 4 for reaction c): Using a metal catalyst (soléne Fortun)

The use of metal catalysis in this type of reactions has been documented as a substituition of the acidic medium for a Lewis acid like iron chloride (III) or silicon compounds. However, the chosen Lewis acid can only be used in certain additions to the cyanoguanidine, because of side interactions with other functional groups which may prevent the formation of the desired biguanide product. In addition, this approach would cause the synthesis to have more steps and waste, so for the time being this approach was not chosen to perform reaction c) presented in Scheme 4.

Path 5 for reaction c): The use of an enzyme

Upon a great deal of investigation there were found no enzymes that seem to catalyze this type of reaction. As such, this approach was not considered for the lack of present information and time to experiment.

Path 6 for reaction c): The use of microwave radiation (stanislas Mayer)

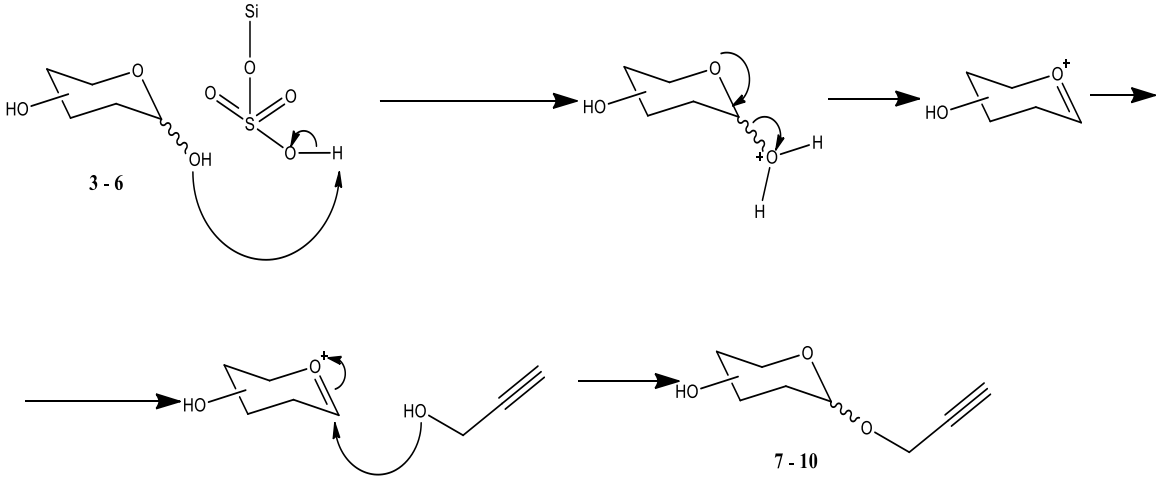
The use of microwave radiation is a good way of improving the reaction rates of most given reactions, comparing to the conventionally heated reactions, and it was considered to be used to ease the reactions time and conditions, although it is not a synthetic path *per se*. Nevertheless, this method was not used due to lack of appropriate and available equipment at the time.

With all the 6 previously described paths being studied and analyzed, the second one (path 2) was chosen to synthesize the biguanide derivatives, this is reaction c) in Scheme 4.

II.2. Results and discussion

II.2.1. Reaction a) carbohydrate propargylation using sulfuric acid supported on silica as catalyst

Carbohydrate propargylation did not needed protection-deprotection steps, which are very common in reactions involving sugars. Based on the Fisher glycosylation reaction, it was possible to attach a propargyl moiety in a simple and smooth manner, and the mechanism is presented in Scheme 8. The most reactive hydroxyl of the carbohydrate was the anomeric one for belonging to the hemiacetal. As such, in the presence of sulfuric acid, the hydroxyl was protonated and a molecule of water was liberated. The subsequent carbocation was stabilized by the pyranose oxygen lone pair of electrons. Next, the propargyl alcohol acted as nucleophile and attacked the carbocation, regenerating the original pyranose ring and inserting the desired propargyl group in the anomeric carbon. This reaction uses the propargyl alcohol both as solvent and reactant.



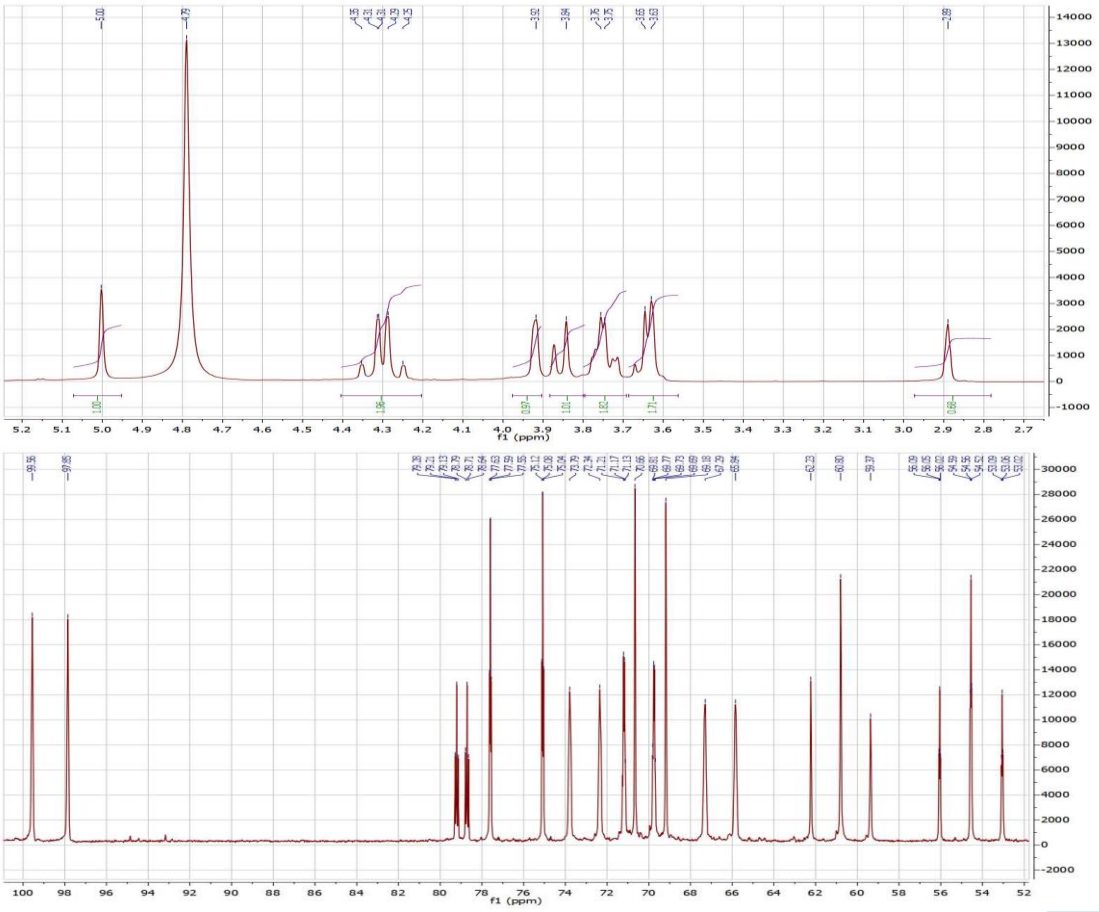
Scheme 8 – Fisher glycosylation using sulfuric acid supported on silica as the acidic catalyst.

II.2.1.1. Synthesis of propargyl glucose 7

Based on the reaction proposed by Bimalendu Roy and Balaram Mukhopadhyay, propargylation reaction was done in an acidic medium given by silica-trapped sulfuric acid to several carbohydrates. Due to the fact that in general sugars react with acid in a way that may destroy the sugar, glucose carbonized since the catalytic quantity of the acid trapped in silica was too much. This carbonization happened in less extent than fructose, as we will see further.

II.2.1.2. Synthesis of propargyl mannose 9

Mannose propargylation was also performed, using the procedure presented for glucose. After purification with column chromatography (first with an eluent of ethyl acetate and then 10:10:1 ethyl acetate/acetone/water), a yellow oil was obtained. Because of that no melting point was measured, but instead the oil was submitted to proton and carbon NMR analysis. As observed by the proton NMR spectrum, a signal was present at 5.00 ppm corresponding to the anomeric hydrogen of the sugar, which is the most deshielded one for belonging to the hemiacetal. Another signal that could show us that the reaction was successful was the one between 4.40 – 4.20 ppm, corresponding to the CH_2 part of the propargyl section. The analysis of the coupled carbon NMR was necessary in order to identify which mannopyranose anomer was

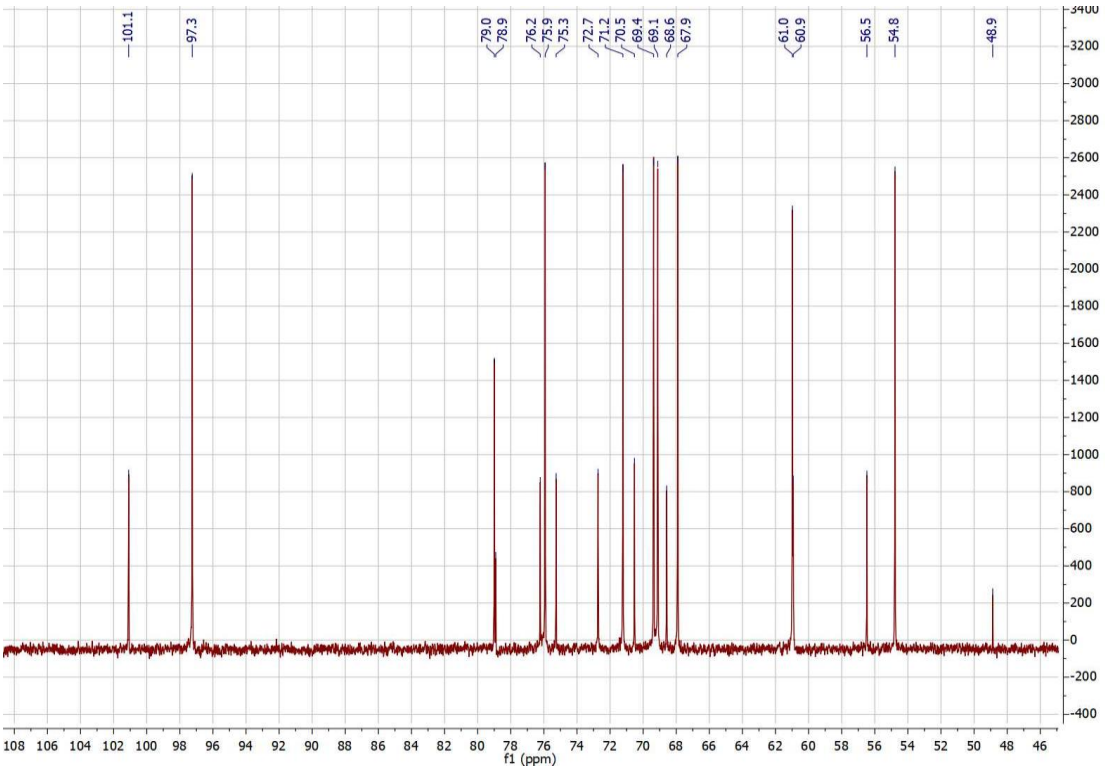


Spectrum 1 and 2 – Proton and Carbon NMR for propargylmannopyranose

present. That value showed to be J_{H-C} = 186 Hz, confirming the preparation of the α anomer.

II.2.1.3. Synthesis of propargyl galactose 8

Galactose propargylation proceeded as the previous reaction for mannose. It was obtained a yellowish oil that was submitted to proton and carbon NMR analysis. Proton NMR spectrum showed two anomeric protons, one singlet most likely corresponding to the α anomer at 5.04 ppm and one doublet at 4.50 ppm corresponding to β anomer with a J value of 7.9 Hz. Integration of these signals showed a mixture of α (5.04 ppm) and β (4.50) anomers with a ratio of 1:2 respectively. Carbon NMR spectrum presented two anomeric carbons at 101.1 and 97.3 ppm, corresponding to the anomers of the propargylated galactose. These results showed the formation of propargyl galactose as wanted.



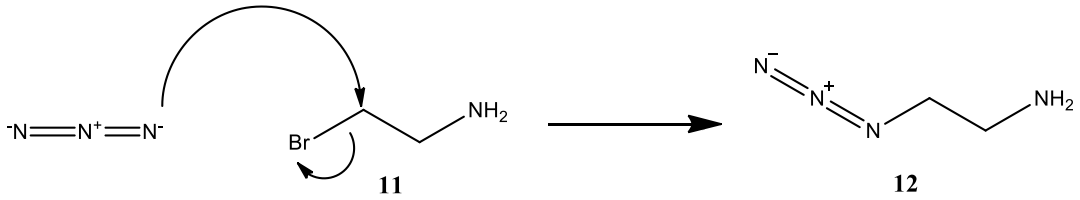
Spectrum 3 – Carbon NMR for propargylgalactopyranose

II.2.1.4. Synthesis of propargyl fructose 10

For the final carbohydrate propargylation, fructose was used. As it happened to glucose, fructose carbonized after the 24h reaction time. It seemed that fructose was much more reactive than the rest of the sugars used in this procedure. Opposing to glucose which showed a very darkened solution with carbon precipitate, fructose propargylation showed a precipitate appearing as a light dust. This was an almost clear solution which presented a carbon precipitate in the form of small rocks inside the reaction medium. With the results of this reaction it was possible to conclude that is possible that the quantity of the catalyst used for fructose propargylation needs to be adjusted. No further analysis were performed on the carbonized compounds.

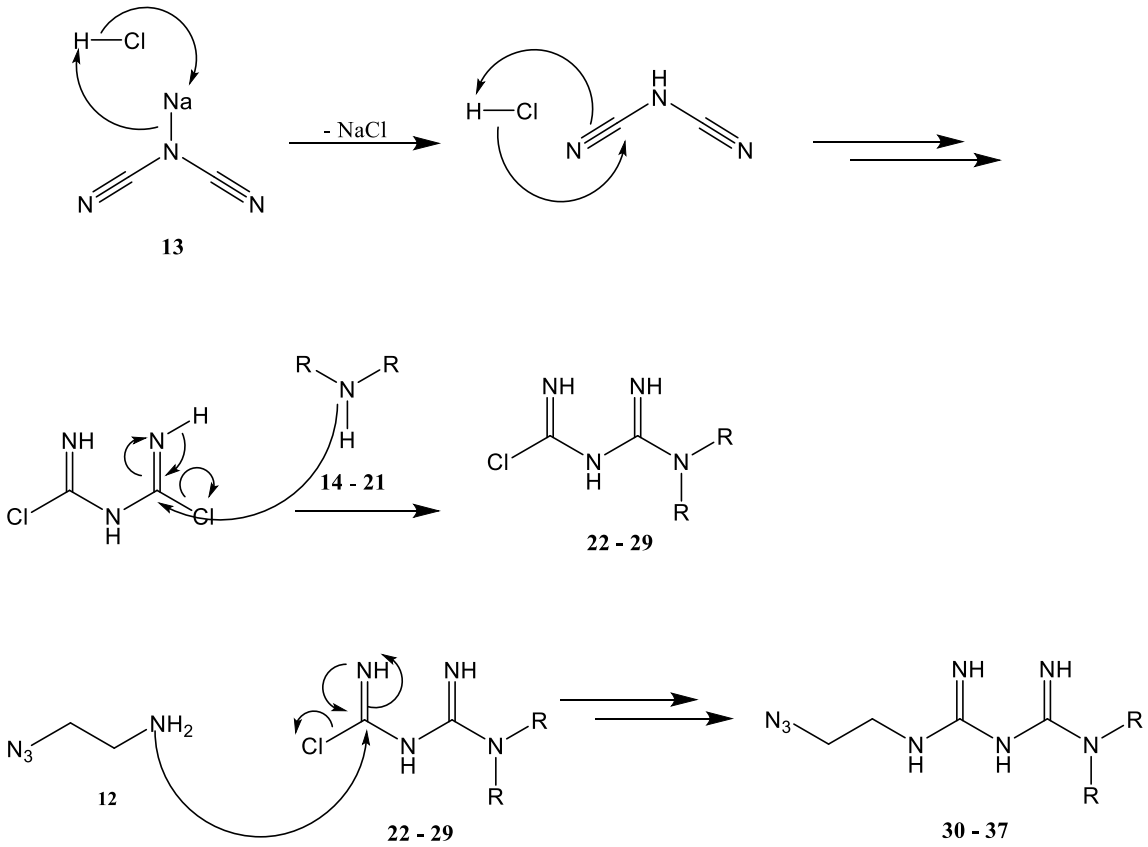
II.2.2. Reactions b) and c) addition of an amine to a cyano group in acidic medium, and addition of azide moiety

The second reaction performed in this work was a simple substitution of a bromine group by an azide. The carbon was electrophilic enough due to the influence of the bromine atom) to be attacked by the negative charged azide ion (Scheme 9). The most favorable mechanism should be SN_2 as presented.



Scheme 9 – Mechanism of a bromine substitution by an azide to afford product **12**.

The reaction c) of this thesis synthetic pathway (previously presented in Scheme 4) was the amine addition to **13**. This mechanism is presented in Scheme 10 and started by the sodium exchange by the proton in the amine **13**. Then, it was followed by two addition reactions in both cyano groups. Next, a double amine substitution was performed by SN_2 reaction. This process was based on the hypothesis that the dichloride product would have a similar reactivity to an acyl chloride, which was proved to be true.

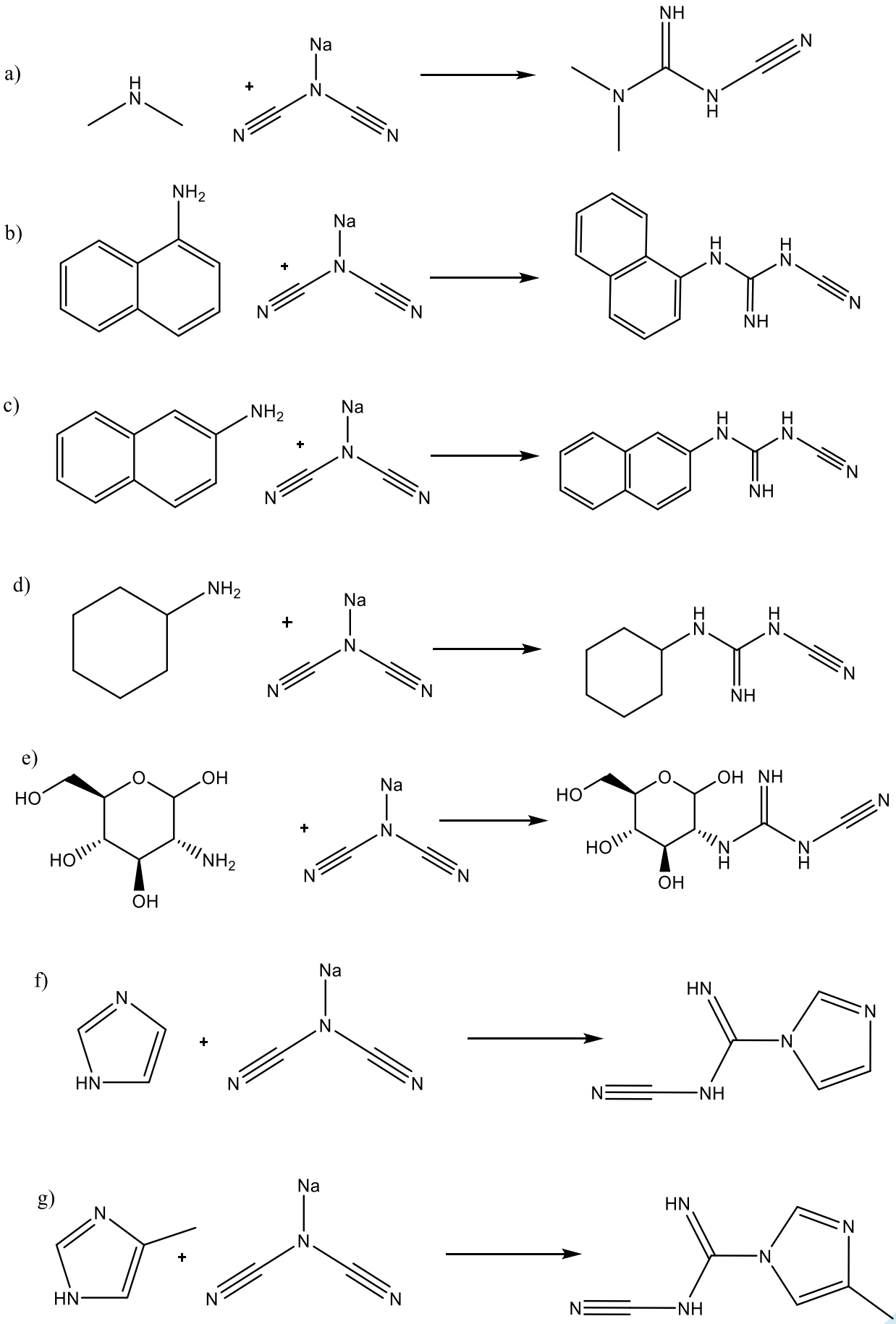


Scheme 10 – Mechanism for amine addiction (**14 – 21**) to compound **13** and subsequent double substitution to form compounds **30** - **37**. The mechanisms here presented are shown as concerted for simplification.

For this step several amines were selected to react with dicyanamide: dimethylamine **14**, benzylamine **15**, α- and β-naphtylamine **16** and **17**, cyclohexylamine **18**, glucosamine **19**, imidazole **20** and 4-methylimidazole **21** as presented in Scheme 11.

II.2.2.1. Synthesis of azidoethanamine 12

Formation of azidoethanamine was a simple bromine substitution of bromoethylamine by azide. The purpose of this molecule was to create space between the sugar part of the molecule and the biguanide. Since the product is volatile and reactive with a supposed risk of explosion or loss of the product, there was no concrete way to purify it out of the water. As such, and since most of further reactions are water based, the product was stored in the fridge. To prevent hazardous accidents no characterizations were made.



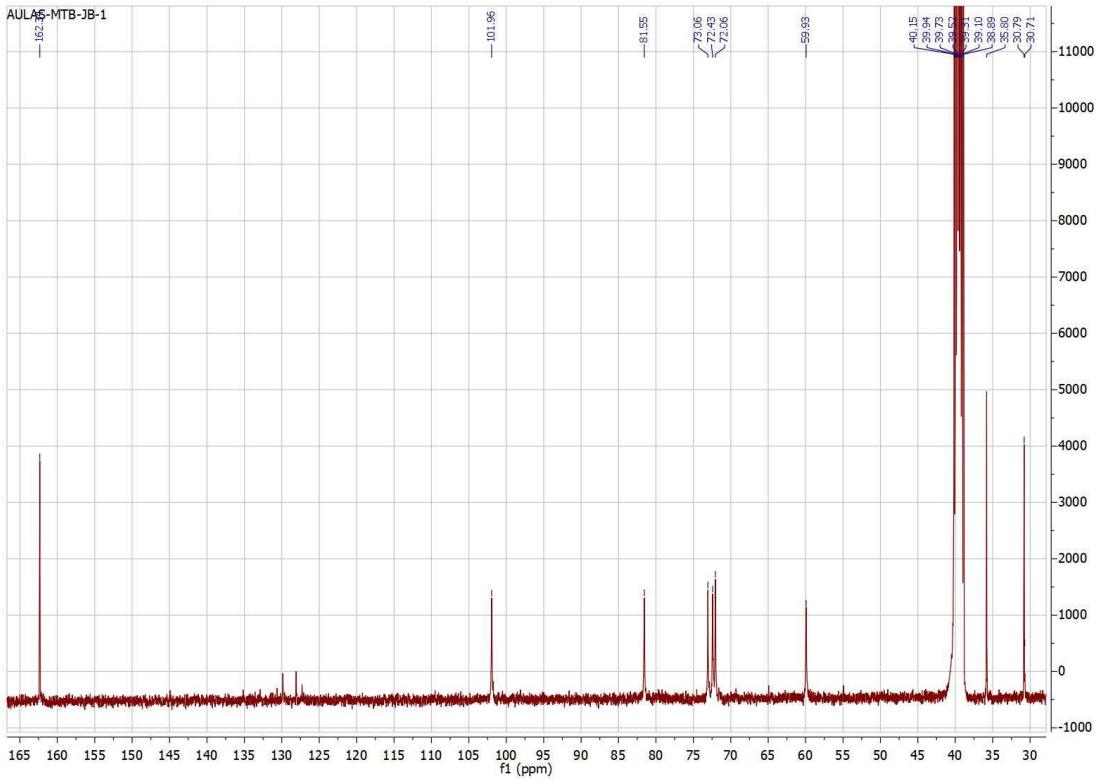
Scheme 11 – Amine addition to dicyanimide reactions performed in this thesis.

II.2.2.2. Synthesis of dimethylcyanoguanidine 22

This reaction was tested a few times, based on a U.S.Patent registered by Bryan C. Redman and Daniel E. Nagy[52], where various amines reacted with some form of dicyanamide (different salts). The reaction was done with two different solvents, water and n-butanol. The water version of this reaction was the one chosen, not only because it used greener solvents, but also involved lesser steps of purification namely the simple precipitation of the product followed by a recrystallization using ethanol.

This approach needed a specific order of compound addition, first dissolving the dicyanamide and acidifying it to facilitate the reactivity and avoid the formation of hydrochloride salts of the amine that's supposed to be nucleophilic.

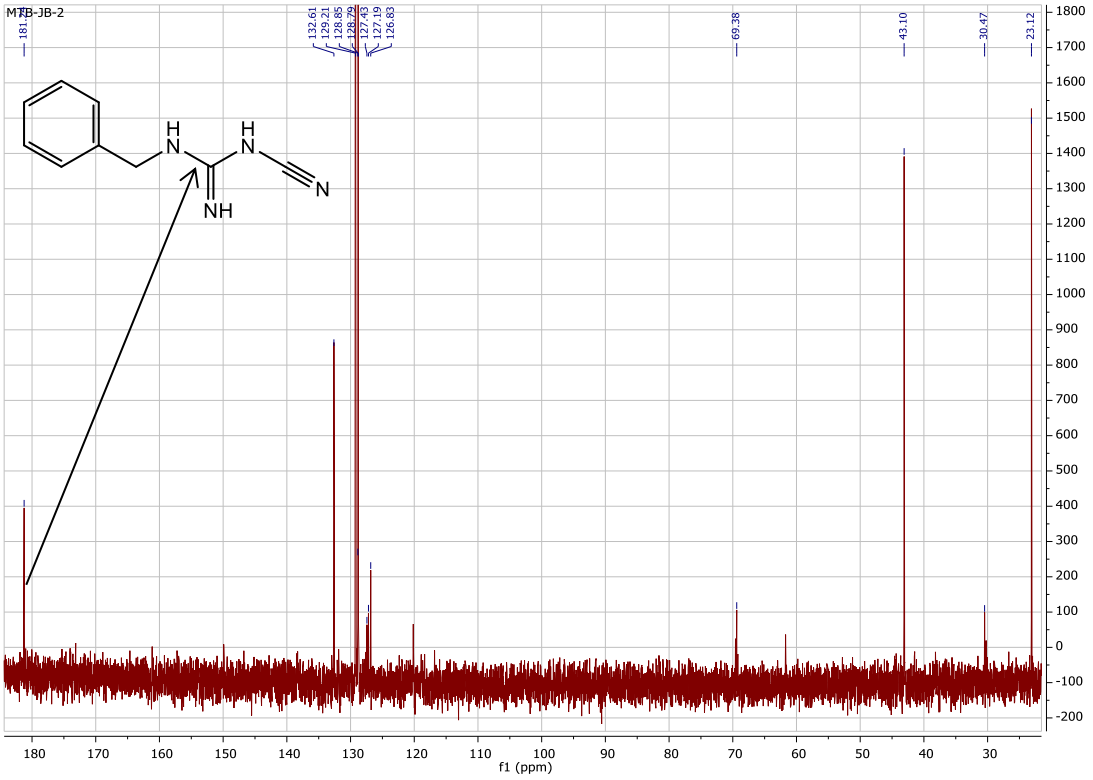
Cyanoguanidine products were only monitored with carbon NMR, because most of the molecules' hydrogens are connected to an nitrogen which are labile and not seen in the proton NMR analysis when protic deuterium solvents are used, such as D_2O. Carbon spectrum, on the other hand, shows a new signal around 160 ppm, corresponding to the C=N carbon, confirming the formation of the guanidine product. For the dimethylcyanoguanidine derivative this signal was observed at 162.3 ppm. At 101.9 ppm the carbon from C≡N was observed. It was also possible to observe the presence of impurities in the carbon NMR spectrum.



Spectrum 4 – Carbon NMR for dimethylcyanoguanidine

II.2.2.3. Synthesis of benzylcyanoguanidine 23

This reaction was one of the first reactions tested as well, although this one was made with sulfuric acid instead of chloridric acid, and n-butanol instead of water. Nevertheless, the principle is the same and the reaction mechanism supposedly happens similarly as well. In addition, a treatment with sodium hydroxide and acetic acid for initial reactant elimination and to neutralize the solution was made. The resultant solid was washed and recrystallized with water. It was then submitted to carbon NMR analysis. As observed by NMR of spectrum 5, the guanidine was formed but impurities could also be observed throughout the spectrum, probably from butanol. The carbon from the imine appeared in lower frequencies than dimethylcyanoguanidine, namely at 181.2 ppm, probably due to the inductive effect of the benzyl part. Cyano cabon was observed at 126.8 ppm, and aromatic carbons between 132.6 and 128.8 ppm. Vestigial acetic acid could also be present since a signal was observed at 23.1 ppm. This results from carbon NMR spectrum confirm the formation of benzylcyanoguanidine.

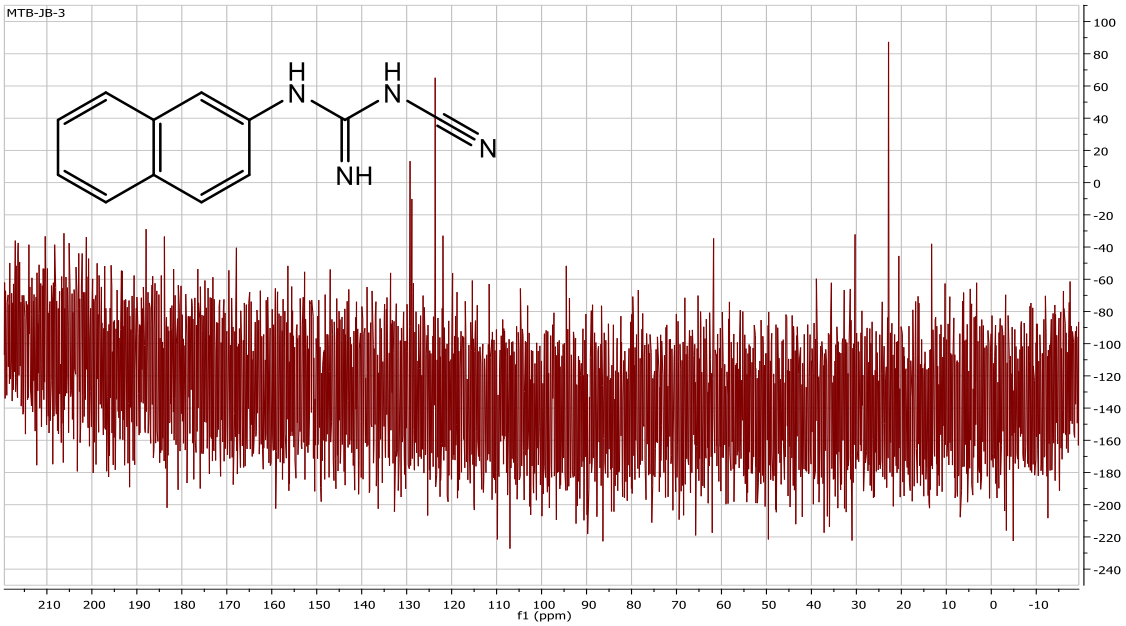


Spectrum 5 – Carbon NMR for benzylcyanoguanidine

II.2.2.4. Synthesis of α and β-naphtylcyanoguanidine 24/25

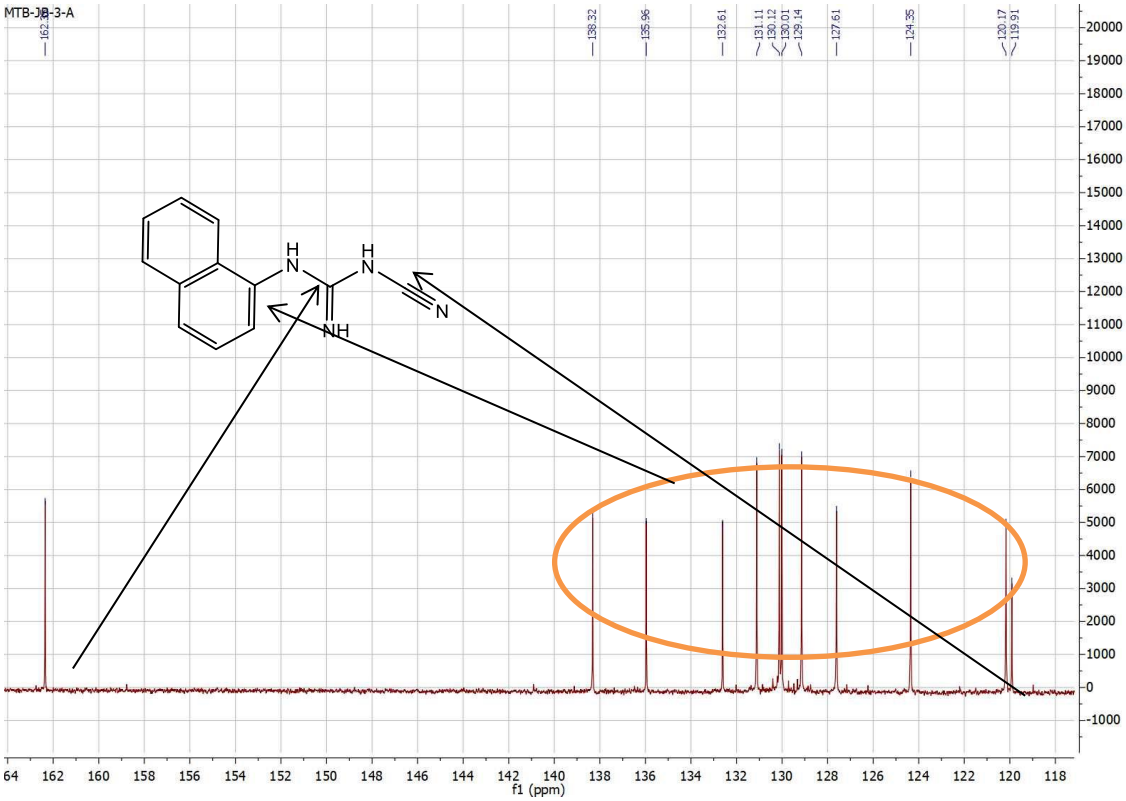
The formation of α and β naphtylcyanoguanidines was attempted using the procedure described previously.

Carbon spectra results of α-naphtylcyanoguanidine showed too much noise-to-signal ratio,, Nevertheless, and although it was difficult to read, no signal was shown in the area of 160 ppm for the imine carbon, meaning that this product was not formed. As such, and in the process of getting to know how to proceed with this type of reaction, a parameter was changed: to give more time for the acid dicyanamide to react.



Spectrum 6 – Carbon NMR for α-naphtylcyanoguanidine

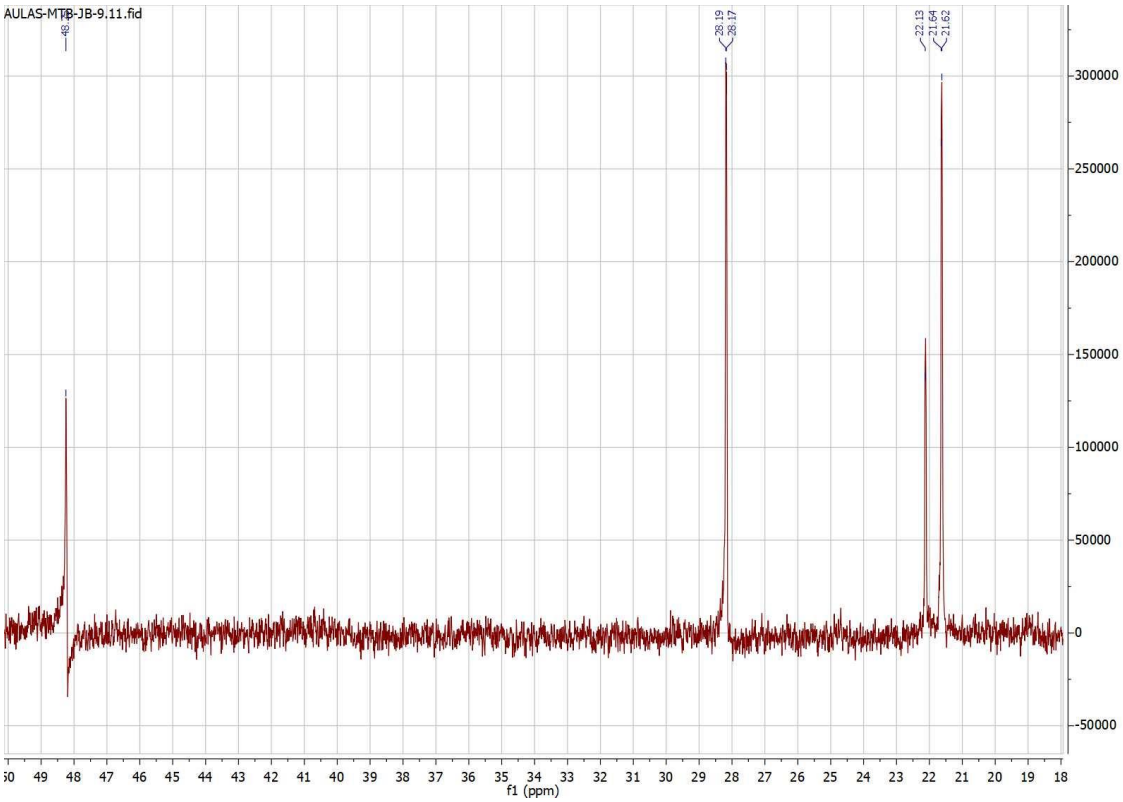
Giving more time to form β-naphtylcyanoguanidine, the NMR spectrum of the product is cleaner than the α product. A signal at 162.4 ppm was observed, confirming the formation of the imine bond and confirming the formation of the guanidine section. In addition cyanide carbon was observed at 120.2 ppm and aromatic and quaternary carbons were observed between 138.3 and 119.9 ppm from the aromatic ring. These results confirm the formation of the wanted product.



Spectrum 7 – Carbon NMR for β- Naphtylcyanoguanidine

II.2.2.5. Synthesis of cyclohexylcyanoguanidine 26

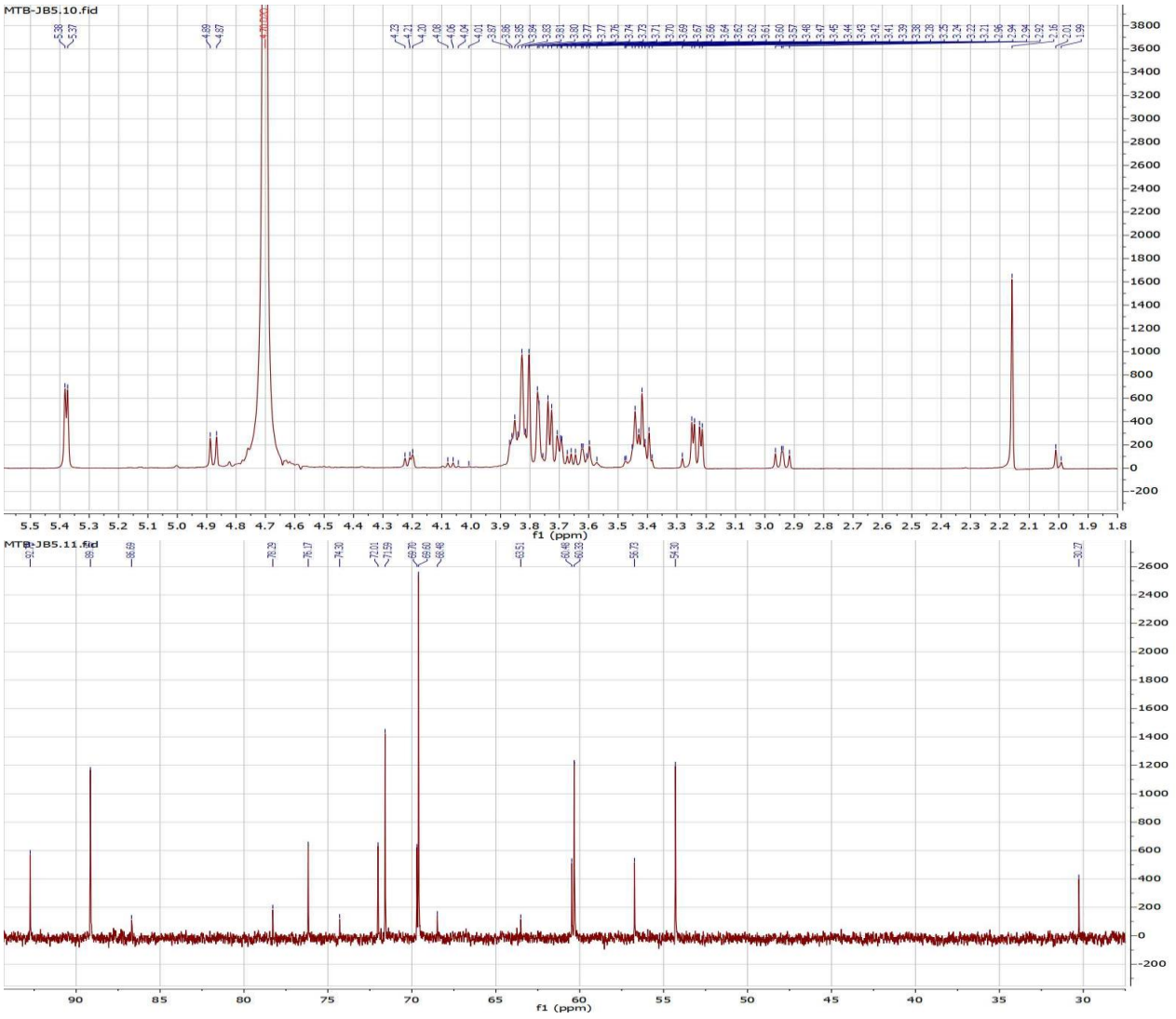
The reaction procedure used for the formation of β-naphtylcyanoguanidine was used to synthesize cyclohexylcyanoguanidine, briefly by the acidification of dicyanamide with chloridric acid, giving time to react, and then the addition of cyclohexylamine. After. 24h the mixture was evaporated and the solid was recrystallized in ethanol and then analysed by carbon NMR. As seen below no imine carbon was observed, but instead the starting amine, cyclohexylamine was observed at. 48.2 (CH) and between 28.2 and 21.6 (CH_2) ppm.



Spectrum 8 – Carbon NMR for cyclohexylcyanoguanidine

II.2.2.6. Synthesis of glucosaminecyanoguanidine 27

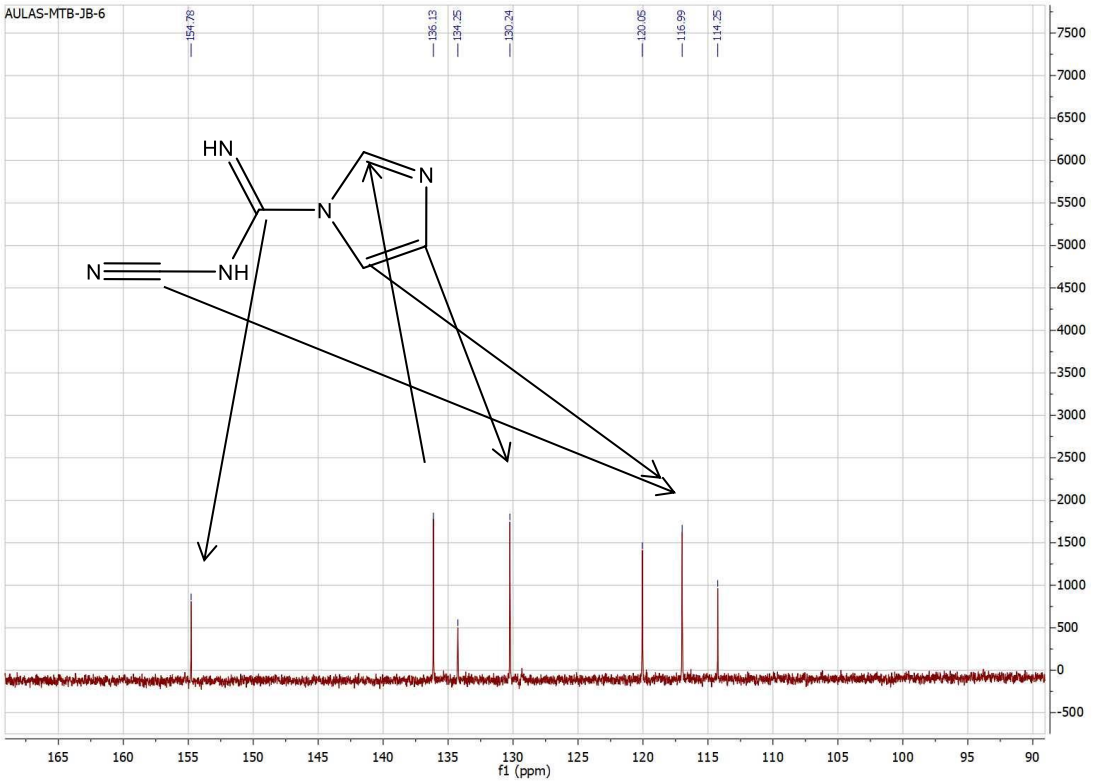
Synthesis of glucosaminecyanoguanidine was not successfull and only the starting amine was observed by carbon NMR. It was possible to observe excess signals in the carbon NMR meaning there were two different anomers of the glucosamine. Proton spectrum was able to help identify the two anomers; the anomeric proton of the α anomer was present as a doublet at 5.38 ppm with a J coupling of 3.5 Hz, and the b anomer present as a doublet at 4.88 ppm and a J coupling of 8.5 Hz. Nevertheless no product was observed.



Spectrum 9 and 10 – Proton and Carbon NMR for Glucocyanoguanidine

II.2.2.7. Synthesis of imidazolecyanoguanidine 28

This synthesis followed the before mentioned protocol. And as it can be seen in the following carbon NMR spectrum a signal appeared at 154.8 ppm, meaning that a guanidine structure was formed (C=N). With the formation of this group the two carbons of the double bond from imidazole are no longer magnetically equal, however one of them is probably overlapping with the signal from the initial reagents that remained after purification, most likely at 114.3 ppm.



Spectrum 11 – Carbon NMR for imidazolcyanoguanidine

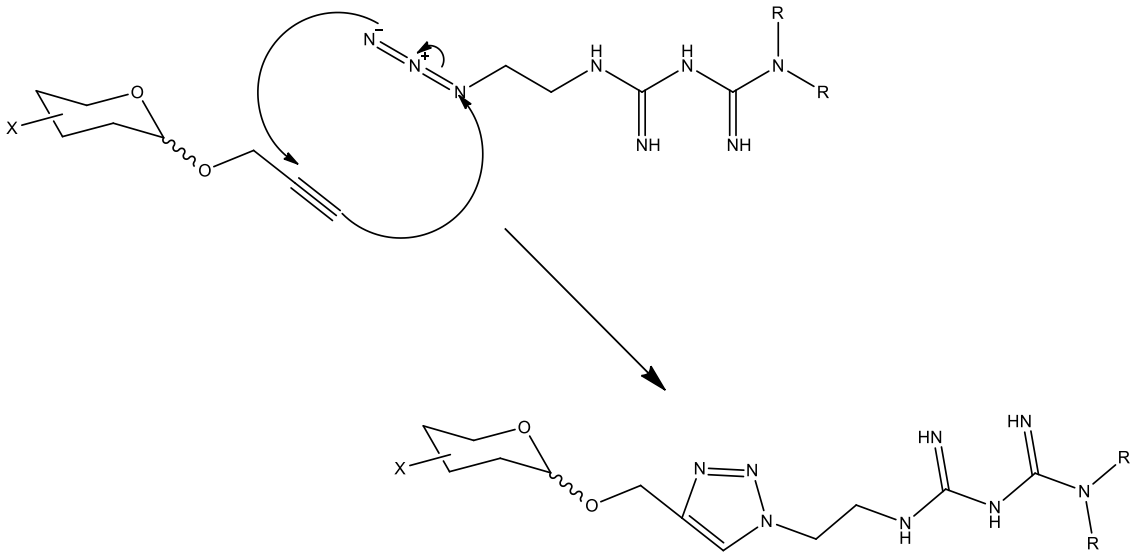
II.2.2.8. Synthesis of methylimidazolecyanoguanidine 29

Methylimidazolecyanoguanidine synthesis was attempted using the procedure described earlier, however when the water evaporated, a substance like a gum-like solid was obtained, but it was impossible to dissolve in several solvents. Therefore recrystallization was not possible and no analyses were made.

The next step of this synthetic process would be the reaction between the prepared cyanoguanidines and azidoethanamine, but that attempt was not possible due to the pandemic restrictions and unrelated personal health issues.

II.2.3. Reaction d): copper catalyzed triazole cycloaddition

Triazole formation via copper catalytic cycle was previously presented in Scheme 3. Here, a more simplified way is shown to demonstrate the way this reaction occurs (Scheme 12). This reaction is based in a cycloaddition formed by the interaction of a mono-substituted alkyne with an azide group. The reaction occurs naturally but at very slow reaction rates. The addition of copper in the reaction medium accelerates the interaction and makes the "click" happen faster. Both attacks, by the alkyne and the azide, occur simultaneously but only in the direction presented by the arrows in this simplified version.



Scheme 12 – Simplified mechanism of triazole cycloaddition formation.

The reaction above was not achieved just like the second wave of amine additions but it will be mentioned below in the experimental part of this essay, because it was a known process and it was meant to be done, but as mentioned above there were special circumstances that cut the time available for experimentation.

III. Experimental

III. 1. Analytic Methods

III.1.1. Nuclear Magnetic Resonance (NMR)

NMR is a technique that explores the magnetic properties of a given atomic nucleus and it provides information on the structure, dynamics and chemical environment of molecules. Often this type of spectroscopy is used by chemists and biochemists for the investigation of organic molecules. The type of spectra is varied, namely on the type of nuclei which can be proton, carbon, nitrogen, phosphorus, among others and the dimensions used, namely 1D (for those nucleus) and 2D such as correlation spectroscopy (COSY), heteronuclear multiple-bond correlation (HMBC), heteronuclear multi-quantum coherence (HMQC), among others. Preparation of samples is usually made with 5 to 20 mg of the pure compound, and a deuterated solvent that dissolves the sample completely.

NMR spectra were obtained at 400 MHz for proton and 101 MHz for carbon, on a BRUKER ARX 400 spectrometer, using $CDCl_3$, D_2O or DMSO-d6 as solvent. Chemical shifts (δ) are expressed in ppm (parts per million). The data obtained from the proton spectra (NMR) are presented using the following order: deuterated solvent, chemical deviation of each signal (δ), spin multiplicity, number of protons, coupling constant (*J*, in Hz) and attribution in the molecule whenever possible. The data obtained for the carbon spectra are presented in the following order: deuterated solvent, chemical deviation (δ), allocation to the molecule whenever possible. The characteristic signal of each solvent was used as an internal reference, with the exception of deuterated chloroform where tetramethylsilane (TMS) was used as an internal reference.

III.1.2. Infrared Sprectroscopy (IR)

Infrared spectroscopy is a technique that explores the vibration of bonds between atoms in the infrared radiation range. This allows the detection of functional groups of the molecule under study.

The infrared spectra were plotted on a Bruker spectrophotometer, model Tensor 27 and treated in the OPUS 6.0 software. The solid samples were analyzed in potassium bromide (KBr) tablets. In the description of each spectrum, the bands with the highest intensity and the most characteristic are mentioned. The data are presented in the following order: sample support (KBr), frequency of the maximum absorption

band (ν_{max} in cm^{-1}), type of band and assignment to a functional group in the molecule whenever possible.

III.1.3. Polarimetry (Optical Rotation)

Polarimetry allows the detection of the specific optical rotation of a molecule, when it is hit with a beam of polarized light. When reaching the chiral centers of the molecule the polarized light will rotate to the left or to the right. The following equation allows the calculation of this value:

$$[\alpha]_{\lambda}^{T} = \frac{\alpha_{observed}}{l \; x \; c}$$

Equation 1 – Equation to calculate the value of $[\alpha]$, being that l is the width of the sample container, and c is the concentration of the sample, expressed in mg/100 ml.

III.2. Other Methodologies

The reactants and solvents used were supplied by Panreac, Cambridge Isotope Laboratories, Inc, Sigma Aldrich, among others (table CR). Some starting compounds were synthesized in the laboratory where the execution of this experimental project took place. All possible reactions were followed by thin layer chromatography (TLC) on 60G / UV254 Macherey-Nagel silica gel plates, with 0.20 mm thickness and aluminum support. After elution, as indicated in each synthesis, the plates were observed under ultraviolet light at 254 nm (Vilber Lourmat) and later developed in an iodine chamber or with a methanolic solution of sulfuric acid.

The compounds were purified in a chromatographic column, using Merck silica gel and by preparative plate chromatography, using silica gel with UV254 indicator, with a thickness of 1 mm. The eluents used are indicated in the various stages of the synthesis described throughout the experimental part of this thesis.

The solvents were evaporated under reduced pressure on a rotary evaporator Büchi R-210 with water bath B-491 and vacuum pump V-700 with vacuum module V-801. Traces of solvent were removed using an Edwards vacuum pump.

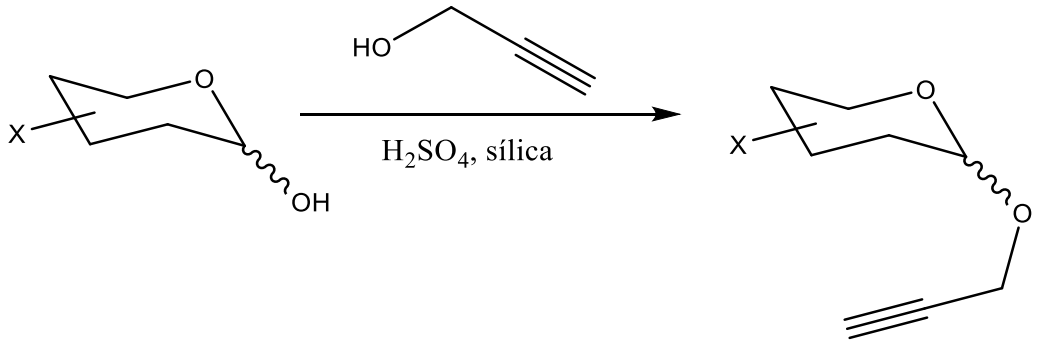
Weighting was done on a Sartorius BL210S scale with four decimal places and ± 0.1 mg of precision.

Melting points were measured on an Electrothermal Melting Point Apparatus.

	Molecular Mass (g/mol)	Purity (%)	Melting Point (ºC)	Boiling Point (ºC)	Density	Brand
Deuterium Oxide		99.9	0	100	1	CIL
Sodium Dicyanamide	89.03	96	-	-	-	Aldrich
Cyclohexylamine						
Imidazole		99	88-90	-	-	Merck
Chloridric Acid	36.46	37	-35	42	1.19	Fluka
Sulfuric Acid		99				Merck
Silica Gel	-	-	-	-	-	Carlo Erba
α-Naphtylamine	143.18	-	50	-	-	Merck
β-Naphtylamine	143.18	-		-	-	Merck
D-Mannose	180.16	99		-	-	Aldrich
D-(+)-Glucose Hydrate	198.17	98		-	-	Riedel-de Haen
D-(+)-Galactose	180.16	98	168-170	-	-	Sigma-Aldrich
D-(-)-Fructose	180.16	98		-	-	Aldrich
Bromoethylamine(bromidric acid)	204.89	99	170-175	-	-	Aldrich
D-(+)-Glucosmine hydrochloride	215.63	98		-	-	TCI
Diethylamine hydrochloride	81.55	98	170-171	-	-	Fluka
Ethanol	46	99.8		78-79	0.79	Riedel-Da Haen
Sodium Azide				-	-	Aldrich
Benzylamine						
4-Methyl-imidazole				-	-	
Propargyl Alchool						
N-Butanol	74.123	99	-89.8	117.7	0.81	Sigma-Aldrich

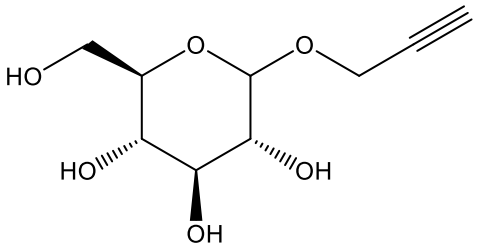
Table 1 – Reactants used and their characteristics.

III.3.1. General Method for carbohydrate propargylation



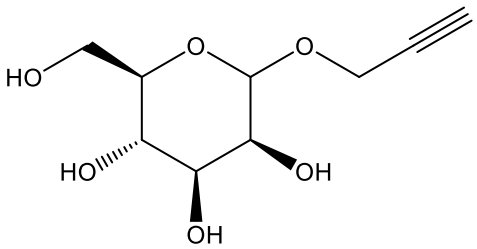
The desired monosaccharide was dissolved in propargyl alcohol and a catalytic amount of sulfuric acid supported in silica was added. The mixture was heated during 24h at 80°C. Then, the reaction flask was allowed to stir at room temperature for 24h. The crude mixture was purified by flash column chromatography using 100:100:1 and then 10:10:1 of ethyl acetate/acetone/water eluent.

III.3.1.1. Synthesis of propargylglucose 7



Glucose **3** (5.55 mmol, 1.00 g), propargyl alcohol (1.6 ml, 27.8 mmol) and sulfuric acid supported on silica (29 mg) reacted together using the general procedure described in III.3.1. The reaction carbonized due to excess of catalytic sulfuric acid.

III.3.1.2. Synthesis of propargylmannose 9

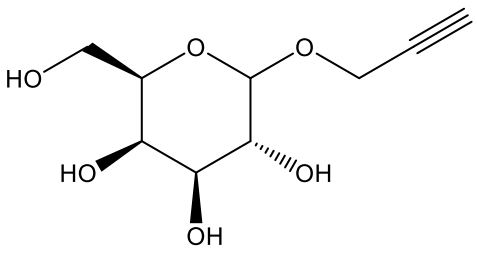


Mannose **5** (5.55 mmol, 1.00 g), propargyl alcohol (27.8 mmol, 1.6 ml) and sulfuric acid supported on sílica (29 mg) reacted together as described in general procedure III.3.1. affording 38 % of propargylated mannopyranose as an yellowish oil (0.2 mmol, 0.464 g).

^{1}H NMR (400 MHz, D_2O) δ 5.00 (s, 1H, H-1α), 4.40 – 4.20 (m, 2H, H-1'), 3.92 (s, 1H), 3.89 – 3.81 (m, 1H), 3.81 – 3.69 (m, 2H), 3.69 – 3.57 (m, 2H), 2.89 (s, 1H, C-3').

^{13}C NMR (101 MHz, D_2O) δ 98.7 (C-1α), 79.0, 76.3, 73.1, 70.5, 69.9, 66.6, 60.8, 54.6 (C-1').

III.3.1.3. Synthesis of propargylgalactose 8



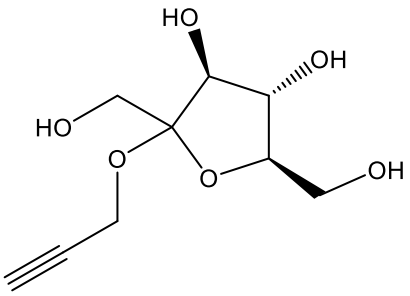
Galactose **4** (5.55 mmol, 1.00 g), propargyl alcohol (1.6 ml, 27.8 mmol) and sulfuric acid supported on silica (29 mg) reacted together using the general procedure III.3.1. affording 45% of propargylated galactopyranose as an yellowish oil (0.25 mmol, 0.549 g, 2:1 α/β ratio).

$[a]_D^{16} = +110.0\,°\,(\mathrm{C} = 1.4, \mathrm{MeOH})$.

^{1}H NMR (400 MHz, D_2O) δ 5.04 (s, 2H, H-1α), 4.50 (d, J = 7.9 Hz, 1H, H-1β), 4.43 – 4.39 (m, 1H), 4.26 (dd, J = 5.8, 2.2 Hz, 4H), 3.92 (s, 2H), 3.91 – 3.83 (m, 3H), 3.81 – 3.74 (m, 5H), 3.72 – 3.63 (m, 7H), 3.59 (dd, J = 9.9, 3.3 Hz, 1H), 3.50 – 3.41 (m, 1H), 2.86 – 2.80 (m, 1H).

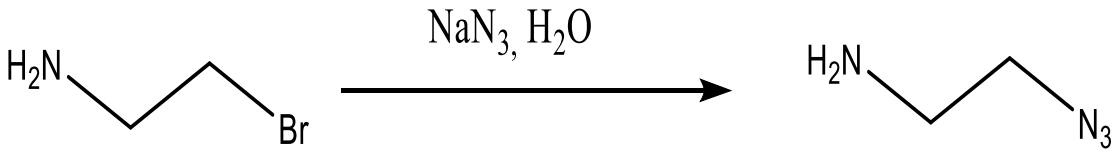
^{13}C NMR (101 MHz, D_2O) δ 101.1 (C-1β), 97.3 (C-1α), 79.0, 78.9, 76.2, 75.9, 75.3, 72.7, 71.2, 70.5, 69.4, 69.1, 68.6, 67.9, 60.9, 60.9, 56.5, 54.8

III.3.1.4. Synthesis of propargylfructose 10



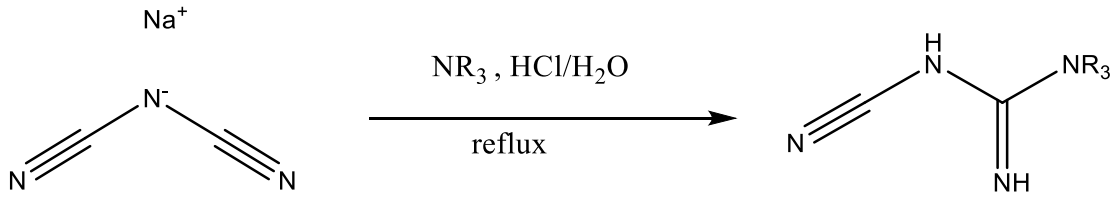
Fructose **6** (5.55 mmol, 1.00 g), propargyl alcohol (1.6 ml, 27.8 mmol) and sulfuric acid supported on silica (29 mg) reacted together using the general procedure described in III.3.1. The reaction carbonized due to excess of catalytic sulfuric acid.

III.3.2. 2-azidoethanamine 12



Bromoethylamine **11** (8.62 mmol, 1.00 g) was dissolved in water (10 ml) using magnetic stirring, and sodium azide (25.8 mmol, 1.68 mmol) was slowly added. After 24h the mixture flask was stored at -5 °C until further use.

III.3.3. General method for synthesis III



Sodium dicyanamide **13** was dissolved in a 1 M solution of chloridric acid at least 3 equivalents or higher. The desired amine after the dicyanamide reacts a few minutes with the acid. The mixture was heated to 100°C maximum and let it reflux until the next day. In some cases a precipitate was formed on the following day or with the addition of cold ethanol/acidic water to make the product precipitate, which was then filtered and dried. It was stored to make a similar reaction with the previous product to obtain an azidobiguanide.

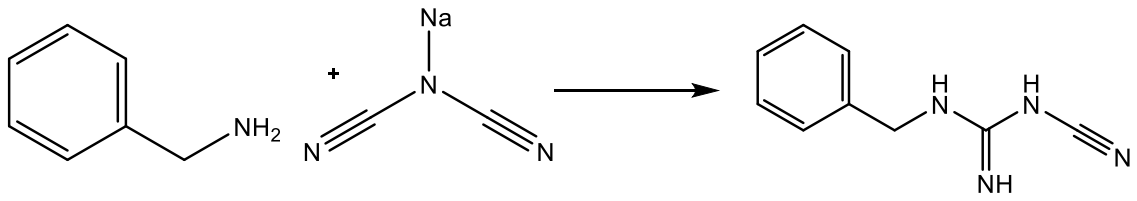
III.3.3.1. Synthesis of dimethylcyanoguanidine 22

To an aqueous solution of dimethylamine **14** (22.7 mmol, 1g) and sodium dicyanamide **13** (22.7 mmol, 2.02 g) was added, slowly, a solution of HCl 1M (22.7 mmol, 22.7 ml) to the mixture, already heated at 100 °C. The solid remained purple for the duration of the observed reaction time (24h). The crude product, after being recovered, was recrystallized from acetone, giving a colorless crystalline solid (6.805 mmol, 0.763g), affording 29.9% of the product. This solid's melting point was measured, but the solid decomposed. The product was submitted to carbon NMR analysis.

^{1}H NMR (400 MHz, D_2O) δ 3.74 (s, 3H, CH_3), 2.76 (s, 3H, CH_3).

^{13}C NMR (101 MHz, DMSO-d6) δ 162.3 (C=N), 101.9 (C≡N), 81.6, 73.1, 72.4, 72.1, 59.9, 35.8 (CH_3), 30.8 (CH_3).

III.3.3.2. Synthesis of benzylcyanoguanidine 23

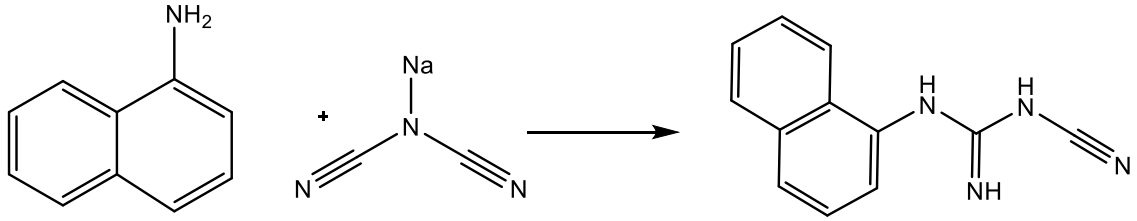


The sulfuric acid, diluted with 21% of the total butanol was added slowly, to a solution of benzylamine **15** (9.33 mmol, 1.02 ml) in the remaining butanol, being the total butanol in the final solution 8.91 ml. Sodium dicyanamide **13** (10.45 mmol, 0.93 g) in aqueous solution was added to the mixture, and then it was heated to 100 °C, for 7h. During the reaction time, water was added in small amounts, during which it distilled. After this time, a large amount of water was added to the solution and then distilled azeotropically. The crude product was then treated with both diluted NaOH and acetic acid solution to remove the residual amine. The solid was recovered, washed with cold water and then recrystallized from hot water. The product was submitted to a carbon and hydrogen NMR analysis. Due to stickiness of the solid no melting point was measured.

^{1}H NMR (400 MHz, D_2O) δ 7.49 (s, 4H, aromatic), 4.20 (s, 2H, CH_2), 1.93 (s, 2H, CH_3 acetic acid).

^{13}C NMR (101 MHz, D_2O) δ 181.2 (C=N/ O-C=O), 132.6 (Cq aromatic), 129.2 (CH aromatic), 128.9 (CH aromatic), 128.8 (CH aromatic), 126.8 (C≡N), 43.1 (CH_2), 23.1 (CH_3 acetic acid).

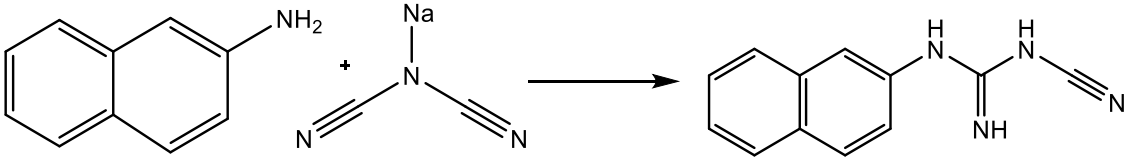
III.3.3.3. Synthesis of α-naphtylcyanoguanidine 24



To an aqueous solution of α-naphtylamine **16** (6.98 mmol, 1g) and sodium dicyanamide **13** (6.98 mmol, 0.621 g) was added, slowly, a solution of HCl 1M (6.98 mmol, 6.98 ml) to the mixture, already heated at 100 °C. The solid remained purple for the duration of the observed reaction time (24h). The crude product, after being recovered, was recrystallized from acetone, affording 20% of a colorless crystalline solid(1.398 mmol, 0.294 g). This solid's melting point was measured, but the solid decomposed. The product was submitted to carbon NMR analysis.

Degradation point = 170 °C.

III.3.3.4. Synthesis of β-naphtylcyanoguanidine 25



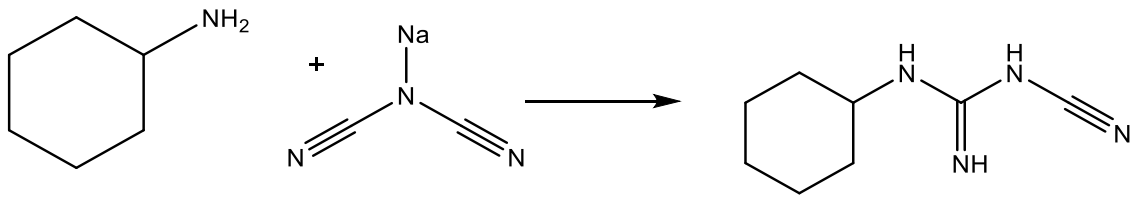
The difference between this reaction and the former one presented in III.3.3.3. is that the sodium dicyanamide was acidified previously, and when this solution was agitated for a few minutes, the clear solution turned milky. This was then added to the aqueous solution of β-naphtylamine **17** previously heated to 100 °C. The rest of the procedure was done exactly as described in III.3.3.2. The product was submitted to carbon NMR analysis and the melting point was measured. The β-naphtyl-cyanoguanidine obtained melted at 235 °C. Afforded 46.7% an opaque grey solid (3.258 mmol , 0.45 g).

Melting point = 235 °C.

^{1}H NMR (400 MHz, DMSO) δ 9.33 (s, 1H, NH), 7.94 (d, *J* = 2.2 Hz, 1H, aromatic), 7.89 – 7.80 (m, 4H, aromatic), 7.54 – 7.35 (m, 4H, aromatic), 7.12 (s, 2H, aromatic).

^{13}C NMR (101 MHz, DMSO) δ 162.4 (C=N), 138.3 (Cq), 135.9 (Cq), 132.6 (Cq), 131.1 (CH), 130.1 (CH), 130.0 (CH), 129.1 (CH), 127.6 (CH), 124.4 (CH), 120.2 (C≡N), 119.9 (CH).

III.3.3.5. Synthesis of cyclohexylcyanoguanidine 26

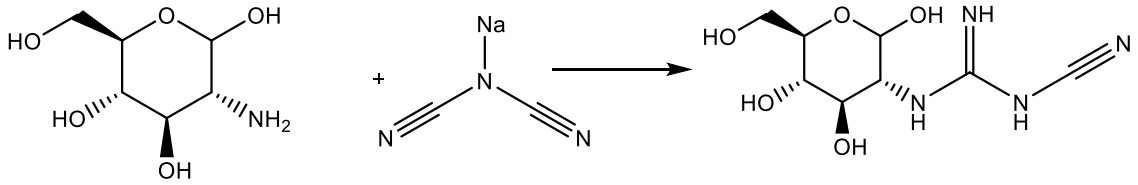


An acidified aqueous solution of sodium dicyanamide **13** (5.62 mmol. 0.50 g) was added to cyclohexylamine **18** (5.62 mmol, 0.64 ml). This mixture was then refluxed for a 24h period. Then, a recrystallization from aqueous ethanol was done to recover the pure cyclohexylcyanoguanidine.

^{1}H NMR (500 MHz, DMSO-d6) δ 3.29 – 3.09 (m, 1H, CH), 2.16 – 1.94 (m, 2H, CH_2), 1.91 – 1.76 (m, 2H, CH_2), 1.75 – 1.61 (s, 1H, CH_2-α), 1.55 – 1.25 (m, 4H, 2xCH_2), 1.28 – 1.12 (m, 1H, CH_2-β).

^{13}C NMR (126 MHz, DMSO-d6) δ 48.2 (CH), 28.2 (CH_2), 22.1 (CH_2), 21.6 (CH_2).

III.3.3.6. Synthesis of glucosaminecyanoguanidine 27

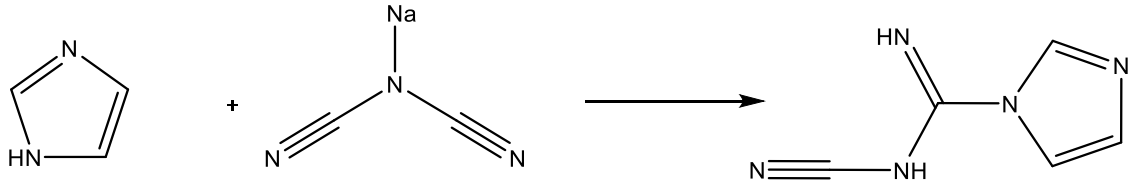


An acidified aqueous solution of sodium dicyanamide **13** (5.62 mmol, 0.50 g) was added to glucosamine hydrochloride **19** (5.62 mmol, 1.00 g). This mixture was then refluxed for a 24h period, but no products were observed.

^{1}H NMR (400 MHz, D_2O) δ 5.38 (d, *J* = 3.5 Hz, 2H, $H_{anom-\alpha}$), 4.88 (d, *J* = 8.5 Hz, 1H, $H_{anom-\beta}$), 3.90 – 3.68 (m, 10H), 3.63 – 3.54 (m, 1H), 3.47 – 3.37 (m, 4H), 3.23 (dd, *J* = 10.6, 3.6 Hz, 2H), 2.16 (s, 2H).

^{13}C NMR (101 MHz, D_2O) δ 92.7 (C_{anom}), 89.1 (C_{anom}), 86.7, 78.3, 76.2, 74.3, 72.0, 71.6, 69.7, 69.6, 68.5, 63.5, 60.5, 60.3, 56.7, 54.3, 30.3.

III.3.3.7. Synthesis of imidazolecyanoguanidine 28

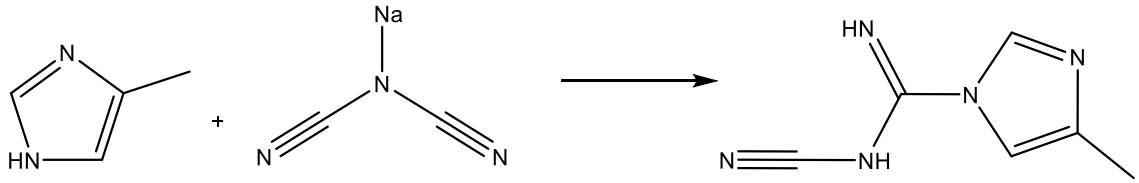


An acidified aqueous solution of sodium dicyanamide **13** (5.62 mmol, 0.50 g) was added to imidazole **20** (5.62 mmol, 0.382 g). This mixture was then refluxed for a 24h period. Then, a recrystallization from aqueous ethanol was done, affording 60.1% of the pure imidazolecyanoguanidine (3.38 mmol, 0.458 g) as an orange hard wax.

^{1}H NMR (400 MHz, DMSO) δ 9.15 (NH), 8.38 (s, 1H, N-CH-N), 7.80 (s, 1H), 7.34 (s, 1H, CH), 7.08 (s, 1H, CH).

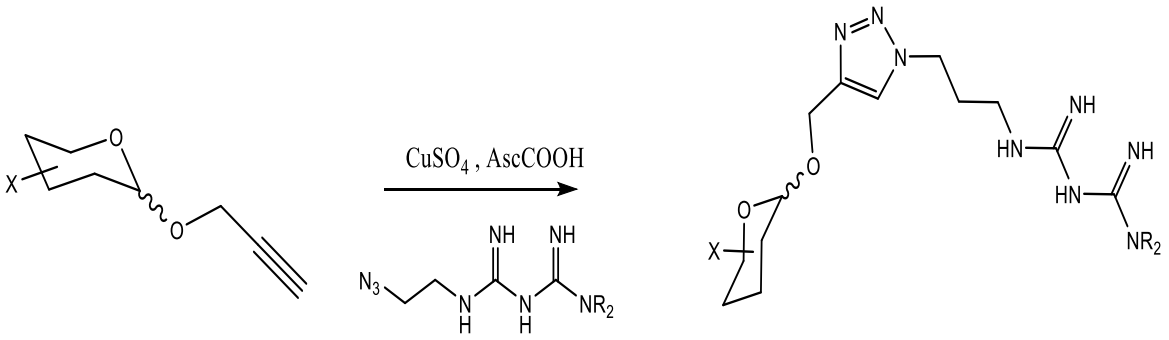
^{13}C NMR (101 MHz, DMSO) δ 154.8 (C=N), 136.1 (N-C=N), 134.3 (C=N-C=C), 130.2, 120.1 (C≡N), 116.9 (C=N-C=C), 114.3.

III.3.3.8. Synthesis of methyl-imidazolecyanoguanidine 29



An acidified aqueous solution of sodium dicyanamide **13** (5.62 mmol, 0.50 g) was added to 4-Methylimidazole **21** (5.62 mmol, 0.461 g). This mixture was then refluxed for a 24h period. Then, the mixture was evaporated and formed a sticky gum-like substance that prevented dissolution with any solvent, so a recrystallization was not performed.

III.3.4. General method for synthesis IV



Dissolve the sugar from I in a solution of copper sulfate 5% and next add a solution of the same molarity ascorbic acid let it stir for a few minutes and then add the azidobiguanide obtained from III and let it stir for 24 hours. The crude mixture would then be filtered and purified by column flash chromatography with an eluent of 100:100:1 and then 10:10:1 (the same as I), supposedly.

IV. Conclusions

This thesis had the purpose to synthesize biguanide derivatives potentially to treat and prevent type II diabetes. In addition, it was intended that the final molecules would be tested for biological activities that the biguanide compounds are known for.

To prepare the biguanide derivatives four carbohydrates were submitted to propargylation reaction under acidic conditions using sulfuric acid supported on sílica. The four carbohydrates used were glucose, galactose, mannose and fructose. Both glucose and fructose propargylation reactions resulted in degradation, while galactose and mannose were successfully propargylated with yields of 45 and 38%, respectively. NMR analysis showed a formation of anomers ratio of 2:1 α/β. A second attempt for glucose and fructose propargylation should be tested in the future, either using a lesser amount of catalyst, reducing the reaction time, in a lower temperature, or a combination of the previous conditions.

Next, formation of azidoethanamine was accomplished by bromine substitution by azide. This product was intended to be a linker between the carbohydrate part of the molecule and the biguanide. Since the product is volatile and reactive with a supposed risk of explosion or loss of the product, no purification or characterizations were made.

The third class of reactions was amine addition to dicyanamide, and several amines were chosen: dimethylamine, benzylamine, α- and β-naphtylamine, cyclohexylamine, glucosamine, imidazole and methylimidazole. For a-naphtylamine derivative no C=N carbon signal was observed by the carbon NMR spectrum, which displayed too much noise-to-signal ratio. In the future this spectrum should be obtained increasing the number of scans, or concentrating more the sample. For both cyclohexyl- and glucosamine derivatives the reaction showed to be unsuccessful. Reaction with methylimidazole afforded a gum-like substance that did not dissolve in any solvent tested. On the other hand, derivatives from dimethylamine, benzylamine, β-naphtylamine, and imidazole were obtained with yields ranging from 46.7 to 60%.

The last step, which would be the triazole formation was not attempted due to external forces. In fact, this thesis was a difficult process to achieve with special circumstances of the world (COVID-19) hitting most of it and stopping it for a long time, which made a dent in the amount of work that was meant to be done, in addition to health situations that I had to go through (not related to Covid), which shortened it even more so some things were prioritized over others.

As a future prospect I hope that this work can be continued in a way to achieve the goals proposed as stated above, on the purpose section. Reactions need to be optimized in order to increase yields and obtain products that were not possible to

synthesize. In addition, more data needs to be acquired such as IR, polarimetry and 2D NMR spectra to better explain some of the data already established above. The amine additions were performed to be as "green" as possible. Finally, the end products that would be obtained would be tested in regards to their biological activity.

V. Bibliography

[1] World Health Organization: WHO Reveals Leading Causes of Death and Disability Worldwide, https://www.who.int/news/item/09-12-2020-who-reveals-leading-causes-of-death-and-disability-worldwide-2000-2019, last visited on 25-08-2021.

[2] Chatterjee, S.; Khunti, K.; Davies, M. J. "*Type 2 diabetes*", **2017**, The Lancet, 389:10085, 2239-2251.

[3] World Health Organization: Fact Sheet About Diabetes, https://www.who.int/news-room/fact-sheets/detail/diabetes, last visited on 25-08-2021.

[4] Wang, G. S.; Hoyte, C. "*Review of Biguanide (Metformin) Toxicity*", **2019**, J. Intensive Care Med., 34(11-12), 863-876.

[5] Campbell, R. K.; White, J. R.; Saulie, B. A. "*Metformin: A New Oral Biguanide*", **1996**, Clin. Ther., 18, 360-371.

[6] Uchiyama, H.; Okamoto, A.; Sato, K.; Yamada, T.; Murakami, S.; Yoneda, S.; Kajita, Y.; Tegoshi, T.; Arrirono, N. "*Quinine- Resistant Severe Falciparum Malaria Effectively Treated with Atovaquone and Proguanil Hydrochloride Combination Therapy*", **2004**, Intern. Med. (Tokyo, Jpn), 43, 624-627.

[7] Bharatam V. P.; Patel S. D.; Iqbal P., "*Pharmacophoric Features of Biguanide Derivatives: An Electronic and Structural Analysis*", **2005**, J. Med. Chem., 48, 7615-7622.

[8] Thomas, L.; Russell, A. D.; Maillard, J. Y. "*Antimicrobial Activity of Chlorhexidine Diacetate and Benzalkonium Chloride Against Pseudomonas aeruginosa and its Response to Biocide Residues*", **2005**, J. Appl. Microbiol., 98, 533-543.

[9] Pinelli, A.; Trivulzio, S.; Pojaga, G.; Rossoni, G. "*Effects of 2-guanidine-4-methylquinazoline on Gastric Acid Secretion in Rats*", **1996**, Pharmacol. Res., 34, 225-230.

[10] Holland, W.; Morrison, T.; Chang, Y.; Wiernsperger, N.; Stith, B. J. "*Metformin (Glucophage) Inhibits Tyrosine Phosphatase Activity to Stimulate the Insulin Receptor Tyrosine Kinase*", **2004**, Biochem. Pharmacol., 67, 2081-2091.

[11] Zou, M. H.; Kirkpatrick, S. S.; Davis, B. J.; Nelson, J. S.; Wiles, W. G.; Schlattner, U.; Neumann, D.; Brownlee, M.; Freeman, M. B.; Goldman, M. H. "*Activation of the AMP-activated Protein Kinase by the Anti-diabetic Drug Metformin in vivo*", **2004**, J. Biol. Chem., 279, 43940-43951.

[12] Zhou, G.; Myers, R.; Li, Y.; Chen, Y.; Shen, X.; Fenyk-Melody, J.; Wu, M.; Ventre, J.; Doebber, T.; Fujii, N.; Musi, N.; Hirshman, M. F.; Goodyear, L. J.; Moller, D. E. Role of AMP-Activated Protein Kinase in Mechanism of Metformin Action. J. Clin. Invest. 2001, 108, 1167-1174.

[13] Hawley, S. A.; Gadalla, A. E.; Olsen, G. S.; Hardie, D. G. "*The Antidiabetic Drug Metformin Activates the AMP-Activated Protein Kinase Cascade via an Adenine Nucleotide-Independent Mechanism*", **2002**, Diabetes, 51, 2420-2425.

[14] Li, J.; Zhong, W., Zhang, K., Wang, D., hu, J., Chan-Park, M. B. "*Biguanide-derived Polymeric Nanoparticles Kill MRSA Biofilm and Suppress Infection* In Vivo" **2020**, ACS Appl. Mater. Interfaces, 12:19, 21231-21241.

[15] Hadden, D. R. Goat's rue-French lilac-Italian fitch-Spanish sainfoin: Gallega officinalis and metformin: the Edinburgh connection. J. R. Coll. Physicians Edinburgh **2005**, 35, 258–260.

[16] Bailey, C. J. Traditional plant medicines as treatments for diabetes. Diabetes Care **1989**, 12, 553–564.

[17] DeFronzo, R. A. Pharmacologic therapy for type 2 diabetes mellitus. Ann. Intern. Med. **1999**, 131, 281–303.

[18] Nathan, D. M. Initial management of glycemia in type 2 diabetes mellitus. N. Engl. J. Med. **2002**, 347, 1343–1349.

[19] Davidson, M. B.; Peters, A. L. An Overview of Metformin in the Treatment of Type 2 Diabetes Mellitus. Am. J. Med. 1997, 102, 99–110.

[20] Kirpichnikov, D.; McFarlane, S. I.; Sowers, J. R. Metformin: An Update. Ann. Intern. Med. **2002**, 137, 25–33.

[21] Strack, T. Metformin: A Review. Drugs Today **2008**, 44, 303–314.

[22] Xinbing Sui, Yinghua Xu, Xian Wang, Weidong Han, Hongming Pan and Mang Xiao, Metformin: A Novel but Controversial Drug in Cancer Prevention and Treatment. Mol. Pharmaceutics, **2015**, 12, 3783−3791

[23] Je Ho Ryu, Jung A Lee, Shinae Kim, Young Ah Shin, Jewon Yang, Hye Young Han, Hyun Joo Son, Yong Hyuk Kim, Joon Ho Sa, Jae-Sun Kim, Jungeun Lee, Jeeyeon Lee and Hyeung-geun Park, Discovery of 2-((R)-4-(2-Fluoro-4-(methylsulfonyl)phenyl)-2methylpiperazin-1-yl)-N-((1R,2s,3S,5S,7S)-5-hydroxyadamantan-2yl)pyrimidine-4-carboxamide (SKI2852): A Highly Potent, Selective, and Orally Bioavailable Inhibitor of 11β-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1). J. Med. Chem. **2016**, 59, 10176−10189

[24] Reitz, A.B; Tuman, R. W.; Marchione, C. S.; Jordan Jr, A. D.; Bowden, C. R.; Maryanoff, B. E. "*Carbohydrate Biguanides as potential hypoglycemic agents*" **1989**, J. Med. Chem, 32;9, 2110-2116.

[25] Alexei V. Demchenko, "*Stereoselective Chemical 1,2-cis O-Glycosylation: From 'Sugar Ray' to Modern Techniques of the 21st Century"* , **2003**, Synlett nº 9, 1225–1240

[26] F.W.Lichtenthaler, Wiley-VCH **2006**, Vol.2, pp. 3 – 6.

[27] Domb, Abraham J.; Kost, Joseph; Wiseman, David (1998-02-04). *Handbook of Biodegradable Polymers*, 275.

[28] Kenji Kamide: *Cellulose and Cellulose Derivatives*. Elsevier, **2005**, p. 1.

[29] Peter C. Heinrich: *Löffler/Petrides Biochemie und Pathobiochemie.* Springer-Verlag, **2014**, 27.

[30] P. J. Fellows: *Food Processing Technology. Woodhead Publishing*, **2016**, p. 197.

[31] IDF Factsheet 002/2017-03, *"Reasons why galactose is good for you"*, **2017.**

[32] Waisbren SE et al. J Inherited Metab Dis., **2012**, 35:279–286.

[33] Berry GT et al.1995; 2004. *"Endogenous synthesis of galactose in normal men and patients with hereditary galactosaemia"*. Lancet 1995, **1995;2004**, 346: 1073–4

[34] Berry GT et al.*"The rate of de novo galactose synthesis in patients with galactose-1-phosphate uridyltransferase deficiency"*. Mol Genet Metab., **2004**, 81(1):22–30

[35] Alton, G.; Hasilik, M.; Niehues, R.; Panneerselvam, K.; Etchison, J. R.; Fana, F.; Freeze, H. H. *"Direct utilization of mannose for mammalian glycoprotein biosynthesis"*, **1998**, Glycobiology. 8:3, 285–295.

[36] Davis, J. A.; Freeze, H. H. *"Studies of mannose metabolism and effects of long-term mannose ingestion in the mouse"*, **2001**, Biochimica et Biophysica Acta (BBA) - General Subjects. 1528 (2–3), 116–126.

[37] Freeze, H. H.; Sharma, V. *"Metabolic manipulation of glycosylation disorders in humans and animal models"*, **2010**, Seminars in Cell & Developmental Biology. 21:6, 655–662.

[38] Porru, D.; Parmigiani, A.; Tinelli, C.; Barletta, D.; Choussos, D.; Di Franco, C.; Rovereto, B. *"Oral D-mannose in recurrent urinary tract infections in women: A pilot study"*, **2014**, Journal of Clinical Urology. 7:3, 208–213

[39] Dills, WL "*Protein fructosylation: Fructose and the Maillard reaction*", **1993**, Journal of Clinical Nutrition, 58, 779–787.

[40] Ravich, W. J.; Bayless T. M.; Thomas, M. *"Fructose: incomplete intestinal absorption in humans*", **1983**, Gastroenterology, 84.

[41] Glycemic Index Testing and Research, University of Sydney (Australia) Glycemic Index Research Service (SUGiRS). Retrieved 30 April 2021.

[42] Patricia M. Heacock; Steven R. Hertzler; Bryan W. Wolf, *"Fructose Prefeeding Reduces the Glycemic Response to a High-Glycemic Index, Starchy Food in Humans*", **2002**, Journal of Nutrition, 132, 2601–2604

[43] EFSA Panel on Dietetic Products, Nutrition and Allergies, *"Scientific Opinion on the substantiation of health claims related to fructose and reduction of post-prandial glycemic responses (ID 558) pursuant to Article 13(1) of Regulation (EC) No 1924/2006*", **2011**, EFSA Journal, 9, 2223.

[44] P. J. Fellows: *Food Processing Technology. Woodhead Publishing*, **2016**, p. 197.

[45] A Stepwise Huisgen Cycloaddition Process: Copper(II)-Catalyzed Regioselective Ligation of Azides and Terminal Alkyne, Angew. Chem. Int. Ed. **2002**, 41, p. 2596 – 2599

[46] Hartmuth C. Kolb Dr., M. G. Finn Prof., K. Barry Sharpless Prof. Angew. Chem. Int. Ed. **2001**, vol. 40, # 11, p. 2004–2021

[47] Huisgen, R.; Szeimies, G.; Moebius, L.; *Chem. Ber.* **1967**, *100*, 2494

[48] Binder, W. H.; Sachsenhofer, R.; *Macromol. Rapid Commun.* **2008**, *29*, 952

[49] Xiao-jun Wang, Kanwar Sidhu, Li Zhang, Scot Campbell, Nizar Haddad, Diana C. Reeves, Dhileepkumar Krishnamurthy and Chris H. Senanayake Organic Letters, **2009**, vol. 11 # 23 p. 5490 – 5493

[50] Wuming Yan, Qiaoyi Wang, Yunfeng Chen, Jeffrey L. Petersen and Xiaodong Shi Organic Letters, **2010**, vol. 12, # 15 p. 3308 – 3311

[51] Paul Anastas and Nicolas Eghbali, *"Green Chemistry: Principles and Practice"*, **2010,** Chem. Soc. Rev 39, 301-312

[52] Bryan C. Redman and Daniel E. Nagy, *"Preparation of Substituted Cyanoguanidine"*, **1948**, US Patent 2,455,807