Glycero-sugars as chiral building blocks for the synthesis of bioactive compounds

Summary of the proposal

Our project aims at developing a library of bioactive molecules targeting antiviral, anticancer and anti *Trypanosoma cruzi* activities. Different aspects of this proposal are of importance. The first one is to design glycero-sugars derivatives with enhanced bioavailability and site selective targeting. Thus in the first part of the proposal carbohydrate moieties incorporating innovative glycero-glycosides as suitable synthons ready to be selectively functionalized. In this approach we will combine two renewable raw materials a glucal moiety (prepared from D-glucose) and glycerol carbonate using the knowledge developed from previous collaboration between the groups, to develop a library of glycosynthons ready to be linked to bioactive entities. The second part of the proposal is dedicated to the elaboration of closely related structures of lapachol and nor-lapachol which are natural naphthoquinones known for their potency in an important number of diseases (cancer, Chagas disease, etc...). A library of analogues incorporating reactive groups (such as alkynes for click chemistry) will be prepared and linked to the glycosynthons. The third part of the project aims at the development of nucleoside analogues. Nucleoside/Nucleotide analogues have been in clinical use for almost 50 years and have become cornerstones of treatment for patients with cancer or viral infections. These compounds are developed in order to overwork cellular metabolism with subsequent incorporation into DNA/RNA to inhibit cellular division and viral replication. The development of new bioactive analogues is based on the need to provide drugs with improved therapeutic potency as well as the need to overcome resistance mechanism. We will develop a set of alkynyl nucleosides able to be conjugated to the glycerosynthons. These are the challenges for the nucleoside/nucleotide antiviral approach to discovery potential drugs. The biological applications, through the different collaborations set up will show the patterns of bioactivities of the newly synthesized molecules: their antiviral, anticancer, antifungal, and anti-*Trypanosoma cruzi* activities. This project will deepen our ongoing collaborations toward the development of pharmacomodulation of natural compounds through valorisation of renewable materials in efficient green chemical processes.
Glycéro-Sucres synthons chiraux pour le développement de composés bioactifs

Summary in French

Notre projet est centré sur l’élaboration d’une bibliothèque de nouvelles molécules bioactives et est constitué de différentes étapes de recherche d’importance. La première étape d’importance est la conception de dérivés glycéro-sucre associant une biodisponibilité accrue ainsi qu’une amélioration des phénomènes d’interaction (reconnaissance, ciblage, sélectivité). Ces dérivés glycéro-glycosides seront issus de ressources renouvelables (D-glucose et glycérol) et seront les briques élémentaires sur lesquelles seront greffées sélectivement les entités bioactives. La chimie développée exploitera les méthodologies de synthèse issues du laboratoire hôte autour de la chimie du carbonate de glycérol et de la chimie des monosaccharides notamment des glycals. Le ciblage de la bioactivité sera apporté par diverses entités aromatiques. Les naphthoquinones naturelles ont montrées leur efficacité sur de nombreuses cibles thérapeutique (Maladies de Chagas, anticancéreux) et feront l’objet d’une partie de nos travaux de recherche. Cette seconde partie du projet est centrée sur les structures du Lapachol et nor-lapachol. Une bibliothèque de dérivés naphthoquinone sera élaborée portant notamment une fonction alcynyle qui permettra un couplage des dérivés sur les glycéro-glycosides principalement par click chemistry. Cette nouvelle famille de naphthoquinone sera testée pour leur activité biologique contre la maladie de Chagas et leur activité anticancéreuse. La troisième partie du projet est centrée sur l’incorporation de bases nucléiques et ainsi élaborer une famille d’analogues nucléosidiques. Les analogues nucléosides/nucléotides sont connus cliniquement pour leurs usages thérapeutiques dans le domaine des antiviraux et anticancéreux. Le développement de nouveaux composés thérapeutiques nécessite d’améliorer la combinaison efficacité / toxicité et de réduire les phénomènes de résistance. L’approche que le projet propose est de permettre une modulation de la partie glycéro-glycosides induisant une meilleure biodisponibilité et un meilleur ciblage des analogues nucléosidiques. Ce projet collaboratif permet d’approfondir notre collaboration entre glycochimie (Orléans) et pharmacomodulation (Recife); il offre l’opportunité d’utiliser les compétences de valorisation de molécules renouvelables et de molécules naturelles pour élaborer de nouvelles molécules à visée thérapeutiques.
1. Background

The desire to reduce society’s dependence on petroleum chemistry has directed researchers’ attention to the use of biomass as renewable resource. Among the different biomass products, a major attention has been focused on raw materials such as carbohydrates and vegetable oils which have been explored as sources of alternative energy like with the biodiesel® production as well as the formation of small molecules for solvent and fine chemistry.\(^1\) Carbohydrates are renewable molecules available in nature in large quantities and are enantiomerically pure. Our project follows this line of developing small molecules from renewable resource to access high value building-blocks for fine chemistry to target new pharmaceutically active molecules.

In this context, and quite recently, glycerochemistry (chemistry of glycerol) for the creation of value-added chemicals has become attractive, partly from the manufacture of biodiesel, where glycerol is formed in large amounts and also from some aspect of the chemistry recently developed.\(^2\) Glycerol is a co-product of biodiesel and becomes an important by-product of the energy scheme inducing the necessity of its valorisation. With increasing investment in the production of biodiesel, glycerol is increasingly available and should become cheaper. It is considered to be produced for 2013 in the amount of an annual production of 250 thousand tons in Brazil\(^3\) and that world production is expected to reach 2 million tonnes.\(^4\) This level of production highlights some market problems for glycerol. In this regard, the need for constant technological innovations to achieve a balance between the production and consumption of this product from the biomass is crucial; this aspect leads to develop some specific field, chemistry glycerol. The use of glycerol requires large-scale solutions involving several molecular changes; a number of changes were already developed with glycerol.\(^2\) This area still requires further development leading to applications in the agrochemical and pharmaceutical fields.

Together with the application of raw materials for fine chemistry, the development of environmentally benign and clean synthetic methods for the synthesis of new chemical structures is an important approach for green chemistry. Several synthetic strategies have been developed recently to allow organic chemists to create a great variety of molecules in order to access novel and diverse chemical libraries of compounds with biological activity potential of them catalytic methods are prominent.\(^5\) From a synthetic point of view, 1,3-dipolar cycloaddition reaction between organic azides and terminal alkynes have been a straightforward method to assemble a large number of molecules, especially since Sharpless and Meldal have proposed the copper-catalyzed azide-alkyne cycloaddition (CuAAC).\(^6,7\) This protocol has permitted an easy access to molecular diversity of 1,2,3-triazoles and encouraged synthetic chemists to design projects based on this scaffold.\(^8\) This reaction gave the opportunity to synthesize molecules having new properties or biological activities, of which antitumoral,\(^9\) antifungal,\(^10\) antibiotic,\(^11\) among others could be found in the literature.\(^12\)
Our aim is the development and preparation of bioactive molecules combining three moieties: monosaccharide derivatives, a glycerol-linker and a heteroaromatic moiety develop from bioactive entities (Figure 1). These three entities will be linked through classical glycosidic bond and using click chemistry and incorporation of a triazole ring. The incorporation of a function such as the 1,2,3-triazole ring is based on the facts that a remarkable amount of bioactive molecules possess this heterocycle, its construction is made by a Huisgen reaction, very effective, so-called "click chemistry" and thus a very large structural modulation is possible.\cite{13,14} To achieve this goal we must prepare new building blocks for functionalization of interest around the glycerol.

Carbohydrates mediate numerous biological processes from cell –cell interactions, inflammation, fertility and development, or signal transduction. Glycopeptides, glycolipids, and various glycoconjugates are responsible of these effects mainly in recognition and internalization of carbohydrate residues by specific cell surface carbohydrate binding. Glycosyl diacylglycerols from simple monosaccharides and complex oligosaccharides, are the main constituents of the lipidic membrane of various cell types and are directly involved in cell recognition events.\cite{15} The literature describes the synthesis of various glycolipids (Figure 2a).\cite{16} Pozsgay and co-workers\cite{16} discovered that the antigenicity of the synthetic BBGL-2 glycolipids depends on the unsaturation of the lipid components, but is independent of the anomeric configuration; besides the presence of two fatty acids in the glycerol part is necessary. In Figure 2b, 4-carbamoylmethyl-1-glucosyl-1,2,3-triazoles were synthesized and evaluated as glycogen phosphorylase inhibitors revealing poor to moderate inhibitions toward this enzyme.\cite{17}

Conjugaison between 1,4-naphthoquinone (1,4-NQ) and 1,2,3-triazole nucleus appeared to be an important pharmacophoric tool.\cite{18-20} These derivatives became attractive as bioactive compounds and
some approaches have been focused on the connection between them; for instance, the synthesis of 1,2,3-triazole linked to 1,4-NQ,\textsuperscript{[19]} which after biological assays showed potent anti-\textit{T. cruzi} activity.\textsuperscript{[20,21]} In another example, 2-aminomethyl-naphthoquinone alkynes were employed to afford new amino-naphthoquinone-sugars, which increased antitumor activity (Figure 3).\textsuperscript{[9]} Moreover Natural naphthoquinone are known to possess strong anticancer activity; the tumour-selective cytotoxic properties of the natural product, \(\beta\)-Lapachone is currently under clinical trials.\textsuperscript{[22]} This skeleton possess numerous biological activities because they may induce an oxidative stress which affect the redox potential of cells.\textsuperscript{[23]}

![Figure 3. Examples of 1,2,3-triazole-1,4-naphthoquinones](image3)

Our proposal starting with building blocks developed from raw materials (D-glucose and glycerol) and link to bioactive moieties (1,4-naphthoquinone nucleus and nucleoside bases (purines and pyrimidines)), will develop an innovative library of molecules that will be evaluated for their antiviral, antitumoral, antifungal and anti-\textit{T. cruzi} activities (Figure 4).

![Figure 4. Structural diversity from natural products and biological activities](image4)

2. **Scientific objectives**

We propose to combine innovative synthetic strategies (catalytic chemistry applied on renewable materials) for the development of new derivatives available raw materials. These protocols will furnish a variety of carbohydrates derived compounds as potential building blocks for promising biological activities. The chemistry we are using may be classified as eco-friendly, using renewable starting material, incorporating catalytic reaction to develop a family of bioactive compounds.

Our retrosynthetic plan (Figure 5) to access the biologically active molecules follow two key approaches. The last stage will be a pharmacomodulation using \textit{click chemistry} to incorporate the bioactive part of the molecule either the naphtoquinone, purines and pyrimidines rings. The second key
step is based on the glycosylation via the *Ferrier reaction* between a suitably functionalised glycerol and glycals.

![Diagram](image)

**Figure 5**: retrosynthetic approach

Thus our studies propose the glycosylation via Ferrier reaction between glycals and glycerol carbonate to gives $N$-, $S$-, and $O$-glycosides. Also, quinone derivatives 1,4-naphthoquinone (1,4-NQ) will be explored. Furthermore, we propose the combination of bio-based origin, i.e. lapachol and lawsone, with triazole-heterocyclic which does not occur in nature.

These new synthetic strategies will give us the opportunities to build libraries of molecules for biological explorations. In this project, the main biological aims are to study these molecules for their antiviral, anticancer, antifungal and anti-*Trypanosoma cruzi* properties.

### 3. Research plan

#### 3.1. **Task 1**: Glycero-Glycosides templates & click chemistry

One of the most important glycerol-derived synthon, is glycerol carbonate which have been explored for its versatile reactivities. This starting material will be modified to give access to the main glycerol-linkers as building blocks. A synthon studied in our group, tosylated glycerol 1,2-carbonate (TGC) will be used as the main starting material through previously described methodology (Scheme 1). The enantiopure form of this TGC could be access after enzymatic resolution of the glycerol carbonate. Reaction between AcSNa with TGC will allow the synthesis of 3-thio-GC after hydrolysis with MeOH/MeONa. 3-Amino-GC can be prepared from azide glycerol 1,2-carbonate. The $N$-Phthalimide derivative is another approach of the amine function for further modifications.
Scheme 1. Synthesis of O-, S-, N-glycerol 1,2-carbonates

Next, we are planning the conjugation between glycals (D-glucal, D-galactal, D-xylal) and glycerol carbonates via Ferrier reaction; then, azidation and subsequent click chemistry to obtain glyce-sugars conjuged with naphthoquinones, purines and pyrimidines bases (Scheme 2).

Scheme 2. Strategy for the synthesis of azido glyco-sugars, an example with D-glucal.

Ferrier protocol\[^{26}\] to preparing glycosyl glycerol derivatives can be applied, see Scheme 2.\[^{4}\] In general, some catalyst such as montmorillonite K-10, FeCl\(_3\), SbCl\(_5\), NbCl\(_5\) and BF\(_3\)·Et\(_2\)O are used. However, we obtained recent results using K-10/FeCl\(_3\) as an acidic catalyst since it is inexpensive, reusable and eco-friendly.\[^{27}\] Unpublished results from our research group showed that reaction between tri-O-acetyl-D-glucal and glycerol carbonate using BF\(_3\)·Et\(_2\)O as Lewis acid led to the formation of the intermediate glyco-sugar (X= O) and, after crystallization was obtained as a pure diastereoisomer in good yields with the stereogenic center fixed at position 2 of glycerol determined by X-ray as S-configuration.

Furthermore, the double bond of the carbohydrate ring could be functionalised to give access to various series of monosaccharide, such as D-glucose and D-mannose, enhancing the library of molecules and incorporating some specific functionalities (Scheme 2). These modifications will add the possibility of a selective transport and thus site selective properties of the bioactive molecule.\[^{28}\]

*Click Chemistry to incorporate and create heterocyclic rings*
Using the cyclic carbonate a second step of glycerol functionalization will be explored by introduction of nucleophiles mainly with the objective of incorporating the azido group, in view of pharmacomodulation applying the click reaction (Scheme 3). Preliminary results showed that the glycer-azido-sugar could be obtained in 44% yield over a two steps sequence. Furthermore, the reactivity of the azido group has been explored over a short range of alkynes proved very efficient (results not yet published). Thus the formation of a library base on bioactive molecules is at hand.

In the course of our studies, we will explore the synthesis of a series of glycoheterocycles, to investigate the reactivity to form the triazole ring and explore the formation of various heterocycles using the glycerol [e.g. (S)-glycero-sugar] side-chain as a template. To achieve this goal, we planned intramolecular versions of the azide-alkyne [3+2] cycloadditions (Scheme 3). Tandem reaction will be also employed as an extension of click chemistry protocol via intramolecular C-H arylation to access polycycles systems.

\[
\begin{align*}
\text{O} & \quad \text{O} & \quad \text{O} & \quad \text{OH} & \quad \text{N_3} & \quad \text{OH} \\
\text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{N} & \quad \text{OH}
\end{align*}
\]

**Scheme 3.** CuAAC reaction onto azido derivatives from O-glycero-D-glucosyl-enosides

3.2. **Task 2 : Natural naphthoquinones modulations for click chemistry**

Therefore, based on the 1,4-naphthoquinone natural scaffolds, we could expand our strategies to prepare new derivatives from nor-lapachol and lapachol and exploring their molecular diversity after coupling with carbohydrates. Using the Kopanski method, it is possible to prepare nor-lapachol from the condensation between lawsone and isobutyraldehyde. Then, it is transformed into corresponding methylated products (Scheme 4). Numerous modification have been introduce on lapachol and norlapachol derivatives, none to our knowledge have been with incorporation of carbohydrate moieties adding specific solubility and targeting properties.
Scheme 4. Synthesis of lawsone and lapachol derivatives

Based on the structure of lapachol and nor-lapachol, two approaches will be designed to combine glycerocarbohydrate derivatives and applied the click chemistry protocol (Scheme 5).

Scheme 5. Modifications on lapachol and nor-lapachol structures toward ready to use naphthoquinone.

On both templates the double bond could be modify and replace by a triple bond. This triple bond, needed to perform the click-chemistry, could be incorporated in place of the hydroxyl group either through a direct alkylation or using the ability of the naphthoquinone ring to undergo a nucleophilic Michael type addition.

Having in hands both family of molecules (glycerol-carbohydrates and aromatic molecules). The direct cycloaddition will be realised (Scheme 6) on unprotected carbohydrates. These approaches will lead to a library of closely related natural naphthoquinone conjugated to carbohydrate template.
3.3. **Task 3: Nucleoside/Nucleotide analogues targeting viruses**

A high proportion of human and animal diseases are caused by viruses, from the common cold to poliomyelitis, hepatitis, and many others.\[^{35}\] Nucleoside and nucleotide are involved in several cellular processes, for instance DNA and RNA biosynthesis. On the other hand, synthetic nucleoside/nucleotide analogues are compounds developed in order to overwork cellular metabolism with subsequent incorporation into DNA/RNA to inhibit cellular division and viral replication;\[^{36}\] furthermore, can inhibit essential enzymes, such as human and viral polymerases, kinases, DNA methyltransferases, purine/pyrimidine nucleoside phosphorylase and thymidylate synthase. The development of new bioactive analogues is based on the need to provide drugs with improved potency therapeutic as well as the need to overcome resistance mechanism, besides to improve the balance between efficacy and toxicity; these are the challenges for the nucleoside/nucleotide antiviral approach to discovery potential drugs. The presence of nucleobases in antiviral drugs for chemotherapy has been highlighted; for example, acyclovir (Herpes - HSV), AZT (HIV) and tenofovir (Hepatitis B – HBV) (Figure 5).

**Figure 5. Chemical structures of some antiviral drugs**

Nucleoside analogues have been in clinical use for almost 50 years and have become cornerstones of treatment for patients with cancer or viral infections. The approval of several additional drugs over the past decade demonstrates that this family still possesses strong potential.\[^{36}\] Herein we focus on their
antiviral activities against various virus strains, such as Respiratory Syncytial Virus (RSV), Influenza A Virus (IAV), Hepatitis C Virus (HCV) and Dengue (DENV), including studies about Zika virus.

Strategy to prepare chiral building blocks is based on purine/pyrimidine structures and glycerol carbonate or glycidol (racemic or enantiopure), see Scheme 8. Enzymatic resolution of glycerol carbonate (e.g. Lipase source P. Fluorescens) could be used to prepare enantiopure glycerol carbonate [(R)-GC and (S)-GC]. Then, once obtained tosylated glycerol carbonate using TsCl/DMAP/pyridine, the nucleobases will be installed to form N-glycerol carbonate (R- or S-NGC), after deprotonation with NaH/DMF at room temperature (N-alkylation). Hydrolysis of (R)- or (S)-NGC affords the enantiopure (R or S) N-glycero-purines and N-glycero-pyrimidines (NGPs). The racemic mixture can also be prepared and spontaneous crystallization will be attempted. To determine the absolute configuration, we will synthesis the corresponding enantiopure compounds from the chiral glycidol. Symmetric compounds, bis-N-glycero purine/pyrimidine, will also be synthesized from reaction between glycerol and 2 equiv of TsCl, and subsequent N-alkylation with nucleobases.

**Scheme 8.** Strategy from glycerol and nucleobases

The synthetic strategy is built on the separation of pure diatereoisomer carbonate glycero-sugar (CGS), as previously described. Preliminary experiment have shown that the azido-glycero-sugar (AGS) could be obtained after azidation (NaN$_3$/DMF) and hydrolysis with K$_2$CO$_3$/MeOH in good yields (70%) (see also Scheme 3). The alkenyl-nucleobases can be prepared after reaction between nucleobases and propargyl bromide, and then reacted with azido-glycero-sugar (AGS) to afford the 1,2,3-triazole nucleoside.
analogues (Scheme 9). In addition, carbonate glycero-sugar (CGS) can react with nucleobases followed by hydrolysis. Alternatively, the compounds R- or S-NGP prepared in the Scheme 8 can react with tri-O-acetyl-D-glucal and after hydrolysis provide nucleoside analogues that could be tested.

Scheme 9. Chemical strategies from glycero-sugars and alkynyl-nucleobases

4. Outcomes and Significance

The chemistry that will be developed will offer three main outcome:

The first part, will develop the knowledge of sustainable chemistry with glycerol carbonate linked to carbohydrate to access a library of enantiopur glycero-glycoside which will be used as advanced synthons for selective functionalisation such as CuAAC Click chemistry. This first part will give the partner a strong knowledge in glycerol-glycoside that could be applied to various fields of research notably by developing glycolipid analogues.

The next two orientations of the project target bioactive molecules. Formation of a library of natural product analogues of lapachol and nor-lapachol will be develop as conjugated forms with the glycerol-glycosides using click chemistry. A panel of hydrophilic molecules will be obtain to combine the carbohydrate moiety properties and the known activity of naphthoquinones especially for their anticancer activities and anti T. Cruzi. This part of the project will give both partners a sound knowledge of the impact of a glycoside moiety on the lapachol and nor-lapachol activities in collaboration with

On a third part, we will adapt the knowledge on glycerol chemistry to develop new nucleoside-family in order to develop libraries of bioactive molecules targeting anti-virus properties. This third part will be done in close collaboration with Dr. Lindomar Jose Pena at the Virology Department at Fundação Oswaldo Cruz (Fiocruz/PE).
The different tasks of the project will be managed as described in the previous figure.

In this project, the biological applications, through the different collaborations set up will show the patterns of bioactivities of the newly synthesized molecules: their antiviral, anticancer, antifungal and anti-*Trypanosoma cruzi* activities, following a proposed Chart 1.

**Chart 1.** Expected biological activities

<table>
<thead>
<tr>
<th>Compounds from Scheme 3</th>
<th>Biological activities a</th>
<th>Class of compound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antiviral [38-42]</td>
<td>Glycero-D-glycosyl-enosides X</td>
</tr>
<tr>
<td></td>
<td>Anticancer [6]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antifungal [32]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-<em>T. cruzi</em> [10,21]</td>
<td></td>
</tr>
<tr>
<td>From Scheme 6</td>
<td>Amino-1,4-NQ glycero-sugars</td>
<td>X</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------</td>
<td>---</td>
</tr>
<tr>
<td>From Scheme 7</td>
<td>N-glycero-purines and N-glycero-pyrimidines</td>
<td>X</td>
</tr>
<tr>
<td>From Scheme 8</td>
<td>Nucleoside analogues</td>
<td>X</td>
</tr>
</tbody>
</table>

*The biological methodologies are described by us and co-workers in the references [6], [10], [21], and [37].

**Cytotoxicity assays and evaluation of the antiviral activity of the test compounds** [38-42]

**Cells and virus:** Vero cells will be used for the propagation and antiviral screening of compounds against Dengue and Zika virus. Cells will be grown in minimal essential medium (MEM) containing 10% fetal bovine serum, penicillin, streptomycin and amphotericin B at concentrations of 100 U/mL, 100 and 2.5 μg/mL, respectively. The virus strains used are available at the Virology Department at Fiocruz/PE. Viral stocks will be titrated by TCID<sub>50</sub> method and/or pfu and will be stored in a freezer at -80 °C until use.

**Cytotoxicity assays:** Cell viability will be measured by the XTT colorimetric method as described by Pena et al [42] using the Cell Proliferation Kit II (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer’s protocol. Briefly, 200 μL aliquots containing varying concentrations of the substances to be tested will be sequentially dispensed in microtiter plates. Cell controls and vehicle will be included. After 24, 48 and 72 hours of incubation, the culture media are removed and 1 mg/mL of working solution (freshly prepared before use) is added to each well and the plate is incubated for another two hours at 37 °C and read in a Benchmark microplate reader (Bio-Rad Laboratory, Mississauga, Ont., Canada) with a 450 nm optical filter. The fraction of viable cells was calculated by subtracting the optical density fraction of treated cells from the untreated cells. Each experiment will have eight replicates per treatment and will be repeated independently three times. **Evaluation of antiviral activity:** To evaluate the antiviral activity of substances, the protocols established for DENV and ZIKV (Moghaddam, Teoh et al. 2014) will be used.
Briefly, Vero cells will be infected with 100 TCID\textsubscript{50} of each virus and then incubated at 37 °C with 5% CO\textsubscript{2} for two hours, whereupon the inoculum will be removed. Cell control (cells and medium) and virus control (cell medium, viruses) will be also included. The compounds will be added as follows: a) before viral inoculation (1hr contact cell / extract); b) after viral inoculation (extracts added after viral inoculation and maintained) and; c) added before and after viral inoculation. In all treatments, increasing concentrations of each test substance will be used until it reaches the critical concentration 50% (CC\textsubscript{50}) each as set forth above. After five days of incubation, the culture media will be removed and the viability of the cells determined by observation of cytopathic effect will be scored to determine the effective concentration 50% (EC\textsubscript{50}), which is the concentration of the substance capable of inhibiting the virus-induced cytopathic effect by 50%. With these data, it will be possible to determine the therapeutic index or selectivity index (SI = CC\textsubscript{50} / EC\textsubscript{50}), which reflects the relative efficacy of the experimental drug to inhibit viral replication compared to their ability to induce cytotoxicity and cell death. It is desirable to have a high selectivity index that produces maximum activity maximum and minimum cellular toxicity (FDA 2006).

References


5. **Significance of the proposal**

Different aspects of this proposal are of importance; the first one is to design glycero-sugars derivatives with enhanced bioavailability and site selective targeting. Carbohydrate are entities of choice to enhance water solubility and bioavailability of a drug, adding the possibility to target specific recognition process either through passive or active transport of membranes with glucose derivatives or specific lectines recognitions.

Thus in the first part of the proposal, we want to design the carbohydrate moieties with a sequence of reactions aiming to prepare unprecedented glycero-carbohydrate which could behave as suitable synthons ready to be selectively functionalized. In this approach we will combine two renewable synthons D-glucose which can readily give access to the glucal part and the glycerol carbonate which could be modified from known technology developed within the Orleans’ team.

Natural naphthoquinone have shown their potency in an important number of diseases (cancer, Chagas disease, etc...). Thus on the second part of the proposal, we aim at the elaboration of closely related structure of lapachol and nor-lapachol to develop a library of bioactive entities together with the flexibility of the incorporated reagent (alkynes) which could give numerous chemical opportunities to go beyond carbohydrate conjugation and explore various other type of functionalization on these modified natural naphthoquinone.

The biological applications, through the different collaborations set up will show the patterns of bioactivities of the newly synthesized molecules: their antiviral, anticancer, antifungal, and anti-*Trypanosoma cruzi* activities.
This collaborative project will deepen our ongoing collaboration toward the development of pharmacomodulation of natural compounds through valorisation of renewable materials in efficient green chemical processes. Moreover, the project will draw a biological impact overview of the glycerol-glycosides with their stereochemistry.

6. **Innovation in the proposal**

The innovations of the proposal are partly described in the previous point, where the development of the two types of families of molecules has never been described in the literature. The approaches studied may offer opportunities in developing other approach of modulations. The key point in our project is the possibility to modulate strongly the glycerol-glycoside stereochemistry by using both chirality on the glycerol moiety and further modulating the stereofunctionalisation of the carbohydrate backbone. This aspect will give our collaboration a strong knowledge on the relation between biological properties and stereochemistry of the glycerol-glycoside entity.

7. **Interdisciplinary aspects**

The opportunity to carry out the proposed project will be of benefit to the researchers involved and to the Region Centre and the European Scientific Community in general. In addition, as the project is in a research area that currently has a high profile, there are excellent prospects for high quality and highly prominent research publications and conference presentations to result from the work, therefore strengthening the reputation of Region Centre research. This project also has the potential to set several precedents that will lead to long term scientific excellence within the collaboration of the University of Orléans and the Federal Rural University of Pernambuco. Initially, success of this project would provide the opportunity for a high quality researcher to do research at the ICOA. This is especially important as the objective of this institute, and the STUDIUM is to bring together top researchers. In the long term, this will encourage other high quality researchers from Brazil to pursue research at ICOA and in Region Centre. This increased pool of high quality researchers, along with their transnational mobility should therefore contribute to the overall enhancement of the quality of research in our Region and the development of interdisciplinary research. The skills developed and acquired within this project, along with the resulting knowledge, could be potential areas for a future transnational collaborations between the researcher and host. These exchanges could enhance the research projects developed in Glycosciences and Medicinal Chemistry both at the University of Orléans and the Federal Rural University of Pernambuco through international collaborations and joint projects.

8. **Collaborations between laboratories or partnerships with industry**

This project is in collaboration between the two laboratories from the Federal Rural University of Pernambuco and University of Orleans. This collaboration will develop new skills in glycochemistry as well as in medicinal chemistry. Both skills could give the opportunity to develop further collaborations in terms of biological applications with our collaborators notably with Dr. Lindomar Jose Pena at the Virology Department at Fundação Oswaldo Cruz (Fiocruz/PE), together with industrial partnership. The chemical
Methodologies for the valorisation of glycerol could have a strong impact for industrial applications: for the producers of glycerol, the potential of innovative molecules with original applications could be seen within the formulation processes in various domains such as Cosmetics and Pharmaceuticals which are two important domains in Region Centre.

9. Potential for intellectual property and/or industrial applications

This project as part of an interdisciplinary project aims at the dissemination of results by publications in the best journals possible targeting Angewandte Chemie if the methods of synthesis showed impressive results. It is also obvious that if the works lead to very innovative results, the protection of the method of synthesis of compounds or the biological impact (on plants) will be envisaged by the deposit of patents (this later aspect will be prepared with the CNRS dedicated services to patent preparations and licensing to specific companies). Furthermore, the scientific results will be disseminated through meeting at both national and international conferences.

10. Experience of the host laboratory in the field of the proposal

Expertise in Glycerol chemistry

The team in Orléans could be seen as one of the most innovative group in valorisation of glycerol and glycerol chemistry over the last years in France in terms of molecular modification. Our group has introduced glycerol carbonate for fine chemistry through three recent publications:


Expertise in carbohydrate chemistry

Numerous publications related to carbohydrate chemistry has been made by the group in Orléans. Glycochemistry is the central topic of the team of Professor A. Tatibouet together with the sulfur chemistry; Among the publications a more specialised manuscript on Ferrier I reaction has been published: Fernandes, A.; Dell’Olmo, M.; Tatibouët, A.; Imberty, A.; Philouze, C.; Rollin, P. Dramatic Effect of PSE Clamping on the Behavior of D-Glucal under Ferrier I Conditions. *Tetrahedron Lett.* **2008**, **49**, 3484–3488.
11. Resources and environment of the host laboratory available for the research

The resources in the chemistry research laboratory are fitting for a laboratory of international stature. Particular emphasis is placed on NMR and mass spectrometry. The NMR facility in the Institut de Chimie Organique et Analytique is composed of two FT instruments with proton operating frequencies ranging between 250 and 400 MHz, which are capable of running most experiments of interest to the research chemist. Access through the Research Federation to the 500 MHz NMR facilities of the Centre de biophysique moléculaire (CBM-Orléans)- UPR 4301 is also possible. Similarly the Mass Spectrometry could be accessible in the Institute (ICOA) and with the federation (CBM) (Instruments: Micromass AutoSpec-oaTof, Ionisation modes: EI and CI (high and low resolution); Micromass LCT (DP 0.31), Ionisation mode: Electrospray (high resolution), Micromass GCT (DP 0.06), Ionisation modes: EI and CI (high resolution, dedicated GC/MS); etc) is world class. In addition there is a shared Chemistry resource in X-ray crystallography, permitting rapid and easy acquisition of structural data.

At both the laboratory and University levels the library facilities are excellent, with increasing emphasis on electronic access to journals and databases. All researchers have direct and instantaneous access to computer terminals that provide full IT including WP, spreadsheet, modelling, internet and database facilities such as PDB, Sci-finder, Reaxys. An intensive seminar programme regularly attracts eminent organic chemists to present their work in Orléans. Therefore, in terms of practical arrangements, the host Institute and laboratory can fully meet the requirements necessary for the execution of the research project presented herein.

12. Track record of the external applicant


**Major Area:** Exact and Earth Sciences.

**Articles Published in Scientific Journals (last 5 years)**

(de Oliveira RN or Oliveira RN, Univ Fed Pernambuco or Univ Fed Rural Pernambuco or Univ Fed Rural Pernambuco; h-index = 8; times cited = 174, at Web of Science).


List of relevant publications (five from above list)

5. NASCIMENTO, W. S., et al. Synthesis of 2-{1H-1,2,3-triazol-1-yl}-1,4-naphthoquinones conjugates from 2-azido-1,4-naphthoquinone and terminal alkynes. Synthesis (Stuttgart), p.3220-3224, 2011.

Book Chapter

13. **Laboratory host scientist**

Dr Arnaud TATIBOUET, Professor, 02 juillet 1968, French, Insitut de Chimie Organique et Analytique (ICOA UMR7311) Université d’Orléans, Pole Chimie, Rue de Chartres, BP6759, F-45067 Orléans Cedex 02. Tel 0238494854 /arnaud.tatibouet@univ-orleans.fr

14. **Financial support to the fellowship**

- Operating cost: host laboratory will provide fund for usual laboratory consumables.
  
  Associate with this proposal, the collaboration will be reinforced by the strong implication within the team of Orléans to the chemistry with Dr Marie Schuler (Maitre de conférences) and will be associated a Master 2 student.

- Salary: none

- Event costs: none

15. **Collaborators**

Lindomar Pena (Fiocruz-PE): This collaboration will develop new skills in medicinal chemistry. Then, the opportunity to develop further collaborations in terms of biological applications at the Virology Department at Fundação Oswaldo Cruz (Fiocruz/PE). Dr. Pena will contribute to cytotoxicity assays and evaluation of the antiviral activity of the test compounds.

Celso Amorim Camara (UFRPE): He will contribute to Natural Naphthoquinones modulations for click chemistry, furnishing lapachol, nor-lapachol, lawsone and beta-lapochone for preparation of derivatives.

Adilson Beatriz (UFMS): Prof Beatriz will contribute to prepare enantiopure form of the glycerol derivatives via enzymatic resolution of the glycerol carbonate.

Rossana Cordeiro (UFC): contribute to antifungal activities.

16. **Reviewers**

Dr Maria Isabel Guerreiro Costa Ismael jismael@ubi.pt Professeur Associé, Présidente du Département de Chimie, Université : Universidade da Beira Interior. Faculdade de Ciências Exactas, Rua Marquês D’Ávila e Bolama 6201-001 Covilhã http://www.ubi.pt/Entidade/Departamento_de_Quimica.

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