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Synthesis and structure elucidation of potential bioactive acetophenones

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Project I Report

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2018/2019

Faculdade de Farmácia da Universidade do Porto

Synthesis and structure elucidation of potential bioactive acetophenones

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SUMMARY

ABSTRACT

Biofouling, is a natural process in which certain marine species, including micro and macroorganisms, attach to natural or artificial underwater surfaces. Organotins as TBT were widely used in the past, but they had showed huge to environment. It is urgent to find new solutions to prevent biofouling without arming environment and our own health.

Acetophenones are building blocks to obtain chalcones. Chalcones are described as privilege structures, showing many different activities. Our group has already synthesized acetophenones with antifouling activity.

Four new acetophenones derivatives were synthesized in this work by diverse reactions as propargylation, “click chemistry” and sulfation. Microwave irradiation was used to performed sulfation and “click chemistry” reactions.

Different purification methods were applied in order to isolate the main product, namely flash column chromatography, liquid-liquid extraction and dialysis. Structural elucidation was stablished by infrared (IR) as well as by $_1$H NMR (proton nuclear magnetic resonance) and ^{13}C NMR (carbon nuclear magnetic resonance).

Keywords: biofouling, antifouling, eco-friendly, click chemistry, acetophenones, sulfation, SAR

ABBREVIATIONS AND SYMBOLS

δ - Chemical shift

^{13}C NMR - Carbon nuclear magnetic resonance

^{1}H NMR - Proton nuclear magnetic resonance

AF - Antifouling

CuAAC - Copper (I)-catalyzed azide-alkyne cycloaddiction

d - Doublet

DMA - *N*,*N*-Dimethylacetamide

DMF - *N,N*-Dimethylformamide

DMSO-d_6 - Dimethylsulfoxide-d_6

IR - Infrared

***J* -** Coupling constant

m – Multiplet

m.p.-melting point

MW - Microwave

NMR - Nuclear magnetic resonance

s - Singlet

S_N2 - Bimolecular nucleophilic substitution

t - Triplet

TBT - Tributyltin

TEA·SO$_3$ - Triethylamine sulfur trioxide adduct

THF - Tetrahydrofuran

TLC - Thin layer chromatography

1. INTRODUCTION

1.1. Marine biofouling

Biofouling, is a natural process in which certain marine species, including micro and macroorganisms, attach to natural or artificial underwater surfaces (**figure 1**). In the first minutes, a film composed by organic compounds as proteins is attached to the surface and some hours later unicellular algae (diatoms) and bacteria are settled in these surfaces. This complex aggregation of microorganisms and polymeric substances is known as microfouling. [1-3]

Bacterial species are able to secrete glue like substances that can anchor them to various materials including metals, medical implants and polymers.[1] This attachment occurs in distinguish phases: first, by an instantaneous and reversible attachment via hydrodynamic and electrostatic interactions, and then via an irreversible attachment that involves covalent bonding between the bacteria and substrate.[4-6] This bacterial film is the first step of the biofouling process, being followed by the settlement of other micro and macroorganisms to natural or artificial underwater surfaces.[2,4,6-8] The biofouling formed by those marine species represents high variability and can cause serious problems. It will not only represent material and economic loss for the marine sector, but also environmental and health issues, because it can influence aquaculture. [1,5,7,9]

Biofouling composition varies according the biotic factors as species availability, grazing and predation and also depends on abiotic factors such as salinity, temperature and tidal fluctuations.[2,4,8]Identification of the primary and secondary colonizers and understanding of co-occurrence of species can be important to the discovery of new chemical substances. These substances can be a new target to the development of new AF strategies.[2]

Material and economic loss in the marine sector happens because fouling increases the surface roughness, which subsequently results in increased fuel consumption and reduced maneuverability. Costs also result from the removal of biofilm and changing the damaged or corroded parts of ship hulls.[1,3,4,6,10]

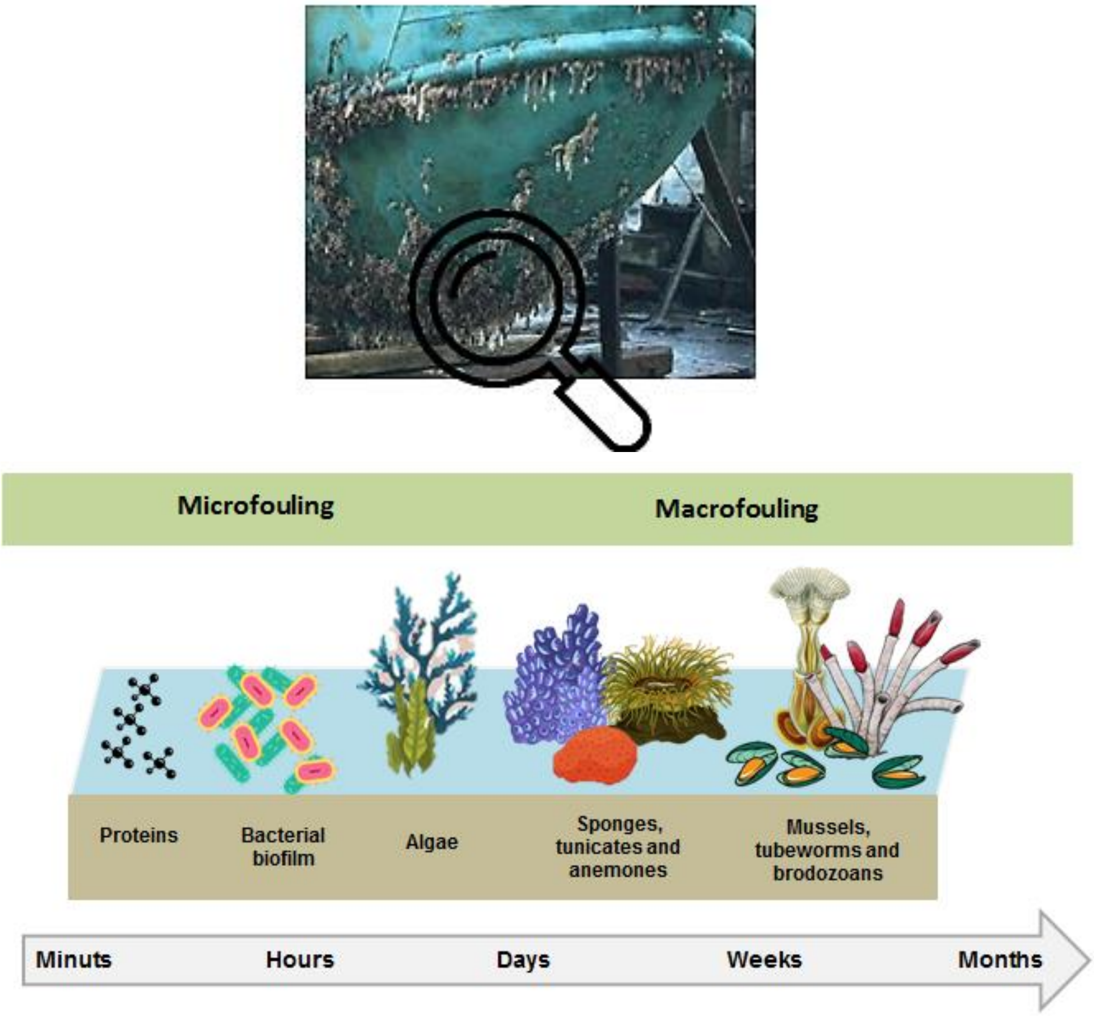


Figure 1-Biofouling process.

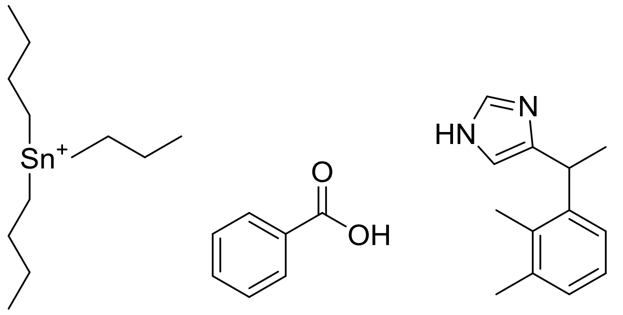
1.2. Antifouling strategies

Biofouling is prevented by the use of AF paints biocides.[11]

Arsenic and sulphur mixed with oils were used as coatings during the 5th century. In the 13–15th century it was tried some mixtures with oils, resins and tallow. Lead sheathing was abandoned in the late 17th century, as it was responsible for severe corrosion of the iron components of the ships.[4]Cooper was introduced as a different option in the beginning of 17th century. However, the reason for its AF effect was not known until the beginning of the 19th century, when it was discovered that was due to copper dissolution into seawater.[4]

In the middle of the 19th century, paints containing biocides – toxic compounds for the marine biological organisms– became the main focus of AF strategy. Later, these soluble coatings containing toxics were abandoned because of their uncontrollable dissolution rate.[4] Organometallic paint systems (comprising lead, arsenic, mercury) were introduced in 1960s, and tributyltin self-polishing coating (TBT-SPC) was developed in the 1970s.[3,4,10] TBT had great success because his rate of release can be controlled on a molecular level. This substance is covalently attached to the carboxylic group of the polymer, which is hydrolytically instable in slightly alkaline conditions (as in seawater).[10] TBT had toxicity associated to effects on non-target organisms, with extended ecologic and marine damage.[7,12,13] In fact, the use of TBT caused the local extinction of some marine organisms due to the inability of reproduction, as well as bioaccumulation of tin in fish, seals and even ducks.[3,4,13,14]

In order to prevent that to happen, organotins were banned in several countries by the International Maritime Organization (IMO) in 2008.[1,7,9,10,15] More recently, some biocides such as cooper, zinc and several organic compounds (benzoic acid, fullerene and medetomidine- **figure 2**) were introduced in the market to replace TBT (**figure 2**). [7,9,11] However, some studies demonstrated that these biocides are not readily degraded in the environment, releasing toxic and persistent organic pollutants affecting marine organisms and possibly human safety by consuming them.[1,9,12]



Tributyltin **Benzoic acid** **Medetomidine**

Figure 2- Antifouling compounds used in paints.

Currently, researchers work in two main approaches: biocide-release and non-biocide-release based AF coatings.[4]

Biocide-release based AF coatings apply the same principle as the TBT-based systems, but with non-toxic components. These coatings use additives dispersion in different polymeric binders capable of releasing the pigments into the seawater over time. Depending on the water solubility of polymeric binders, we can distinguish insoluble or soluble matrix coatings.[3,4]

The non-biocide-release based AF coatings approache is highly preferred since it is a greener alternative, compliant with the current environmental and health regulations. Within this approach two main strategies are persecuted: the detachment of settled biofoulants as water flow upon ship navigation and prevention of attachment of biofoulants.[4]

It has been made an effort to develop effective, nontoxic and environmentally safe and ecofriendly AF agents.[15] Ideally these new agents should be biodegradable and able to inhibit the settlement of certain biofouling species by acting in more specific targets, instead of inducing general toxicity.[4,6,7,12,14,16] The majority of AF natural products have been obtained from marine organisms such as sponges, corals, seaweeds and seagrass, but they can also be obtained from herbal plants. However, very low concentrations of these secondary metabolites can be obtained.[11,12,15,16]

1.3. New antifouling opportunities

Acetophenones can be used as building blocks to synthesize chalcones known as medicinal privileged structures. Chalcones are characterized by the presence of two aromatic rings, joined by a three carbon α,-βunsaturated carbonyl system (**figure 3**).[17]

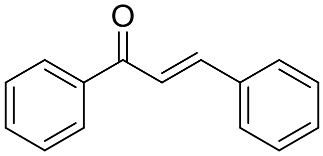
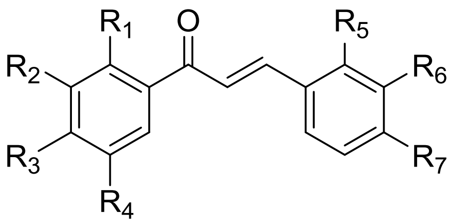


Figure 3-Chalcone Scaffold.

There are multiple biological activities described for chalcones. This privileged structure can act on various molecular targets, exerting many different actions, including not only several pharmacological activities, but other applications. Some of the described applications are as pesticide, analytical receptor for Fe(III) determination, fluorescent polymers organic brightening agent. [17] Chalcones have been used as anticorrosive agents, alone and with iodide ions to synergize the activity of the latter in acid-mediated corrosion of steel. This class of molecules is very well-known as antibacterial agents, and as they are small molecules, they have small half-life and may not persist for very long in ocean waters, being harmless to sea fauna and florafauna.[11]

Sivakumar, P.M. *et al* identified 47 synthesized chalcones with AF activity, being the most potent hydroxylated chalcones, as showed in **figure 4**.[11]



R_2=SMe; R_6=OH
R_2=SMe; R_5=OH
R3=OMe; R6=OH
R_2=SMe; R_7=OH

Figure 4-Hydroxyl substituted antifouling chalcones.

Previously a small library of acetophenones has been synthesized (**1-10**) and evaluated for their AF activity in Laboratório de Química Orgânica e Farmacêutica (LQOF) (**figure 5**).

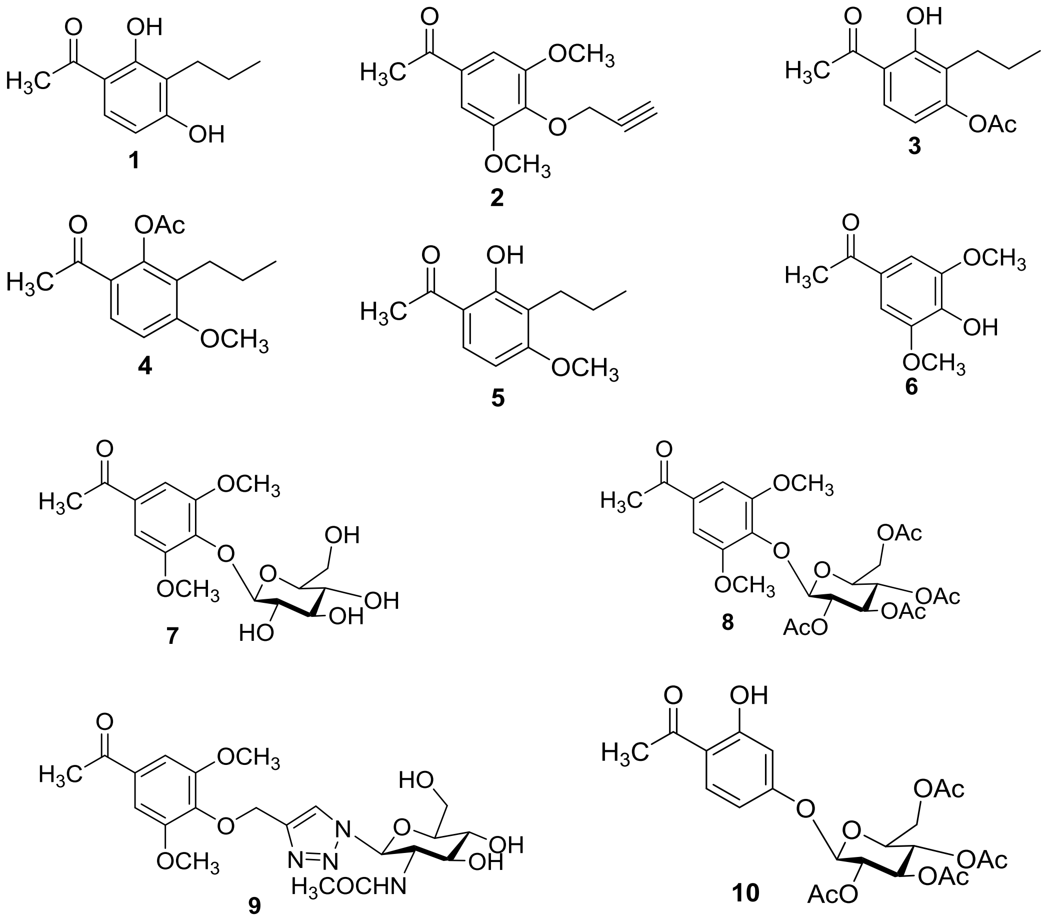


Figure 5-Acetophenones synthesized in LQOF with antifouling activity.

Compounds **3**, **4**, **5**, **6** and **9** showed the best activity against the settlement of the macrofouling species *Mytilus galloprovinciallis* larvae (**Table 1**). Interestingly, some of these compounds showed a therapeutic ratio (LC_{50}/EC_{50}) higher than 15.0, being therefore considered as non-toxic to this macrofouling species.[12] Preliminary toxicity assays also showed no toxicity or low toxicity to a nontarget organism, *Artemia salina*.

Table 1-Effect of acetophenones **1-10** in against the settlement of *Mytilus galloprovinciallis* larvae.

	Antisettlement of *Mytillus galloprovincialis* larvae			*Artemia salina* ecotoxicity test
Compound	**EC_{50} (µM)**	**LC_{50}(µM)**	**Ratio (LC_{50}/EC_{50})**	**% of Lethality**
1	nd (75%I at 50 µM)	nd	Nd	nd
2	Nd (50%I at 50 µM)	nd	Nd	nd
3	16.32	>200	12.25	8% at 25 µM
4	19.36	>200	10.33	15% at 25 µM
5	4.97	>200	40.24	20% at 25 µM
6	13.94	nd	Nd	nd
7	>50 (35%I at 50 µM)	nd	Nd	nd
8	>50 (35%I at 50 µM)	nd	Nd	nd
9	20.68	nd	nd	<2% at 50 µM
10	Nd (75%I at 50 µM)	nd	Nd	nd

$EC_{50:}$ effective concentration, $LC_{50:}$ lethal concentration, Nd- not determined

In addition to these compounds, a benzaldehyde related with compound 6 was tested. Its activity was lower, with an inhibition of only 35%. Considering these results, we can presume that acetophenones can be a promising AF agents.

1.4. Aims and research plan

The aim of this work was to synthesize new acetophenones linked to sacharides with potential AF activity.

Polysaccharides are a promising material for AF coating, once their structure is highly hydrophilic, making possible to form water-storing hydrogels. Many recent studies showed that coatings with amphiphilic compounds have a high potential for inert surface coatings. That property can be acquired by hydrophilic polysaccharides after chemical modifications with hydrophobic molecules. [9] Flavonoids, xanthones, coumarins and other polyphenols with sulfated C-glysosyl and O-glycosyl units were synthesized in our group and promising results were disclosed.[7]

Acetophenones were planned to be linked to saccharides by a 1,2,3-triazole group in order to mimic potent triazole-based biocides.[7] There are many AF agents based on five-membered nitrogen heterocyclic compounds, in particular imidazoles and triazoles that are strong ligands for Cu^{2+} and Cu^{+}, which are also potent AF agents. Those AF agents are showed in **figure 5**, being medetomidine the most recognized for this activity.[10]

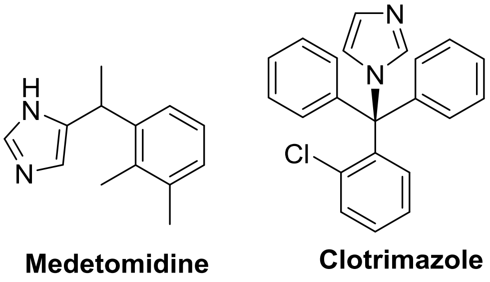
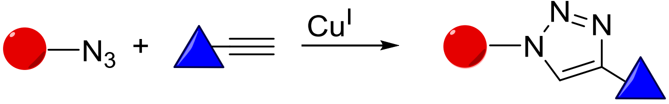


Figure 6- Triazole based biocides.

Taking this into account, we planned to synthesize a acetophenone and afterwards incorporate the glycosyl moiety, using 1, 2, 3-triazole ring as a linker between the acetophenone nucleus and the sugar moiety (**Scheme 1**).

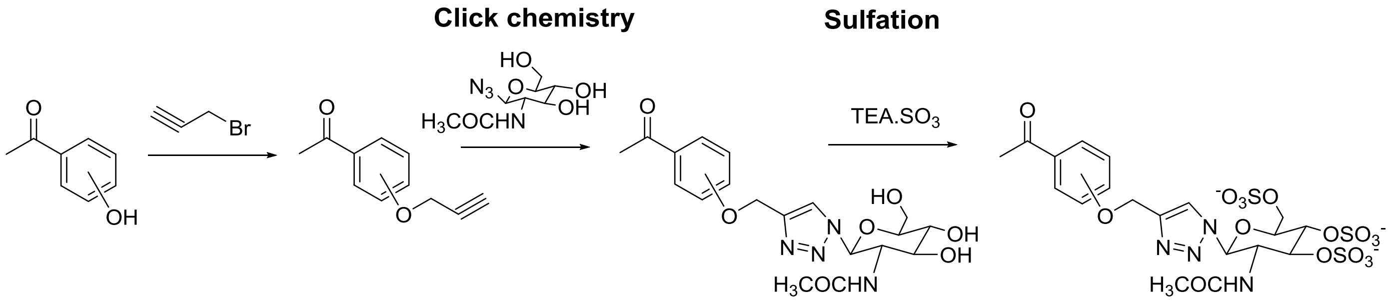
The 1,2,3-triazole core can be synthesized by reaction of an alkyne and an azide (**Scheme 1**).[18]



Scheme 1-Copper (I) catalyzed azide-alkyne cycloaddition reaction CuAAc (adapted from[18])

This reaction is an example of copper (I) catalyzed azide-alkyne cycloaddition reaction (CuAAc), the most recognized “click chemistry” reaction due to its reability, specificity, and biocompatibility.[19] It is widely used to synthesize organic compounds, drug discovery and of materials design.[19-21] Drug discovery approaches of Nature’s secondary metabolites often have slow and complex syntheses. “Click chemistry” simplifies compound synthesis. A click reaction must be of wide scope, giving consistently high yields with a variety of starting materials. It also must be easy to perform, be insensitive to oxygen or water, and use only readily available reagents. Reaction work-up and product isolation must be simple, without requiring chromatographic purification.[22,23]

Therefore, propargylated acetophenones had to be first synthesized (**Scheme 2**).



Scheme 2-Proposed research plan

Following, sulfation of the triazole-linked acetophenones was planned. Sulfation is part of the many metabolic strategies that Nature uses to prevent toxicity.[24] For example, there are some sulfated secondary metabolites, namely sulfated polyphenols, that have been emerged as AF agents.[7] Sulfated compounds evidenced potential as ecofriendly AF agents.[7] Additionally, bioactive sulfated compounds, are highly water soluble, suggesting a low bioaccumulation potential.[7]

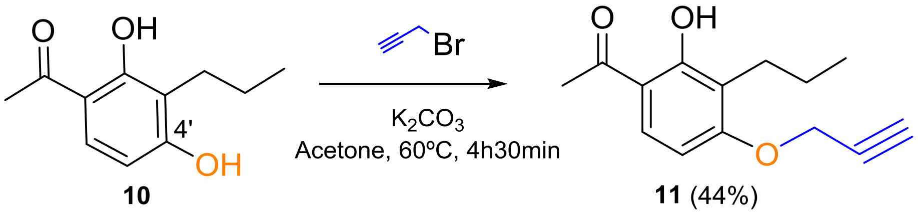
These molecular modifications were chosen since saccharides and sulfates have hydrophilic characteristics, reducing the bioaccumulation potential.

2. RESULTS AND DISCUSSION

2.1. Synthesis

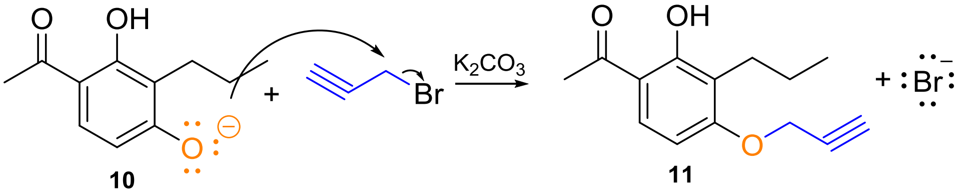
2.1.1. Propargylation of 2',4'-dihydroxy-3'-propylacetophenone

Compound **11** was obtained by the reaction of 2',4'-dihydroxy-3'-propylacetophenone (**10**) with propargyl bromide in acetone and in the presence of potassium carbonate, at 60 ºC for 4 hours and 30 minutes (**Scheme 3**).



Scheme 3-Synthesis of 2'-hydroxy-4'-(prop-2-yn-yloxy)-3'-propylacetophenone (**11**)

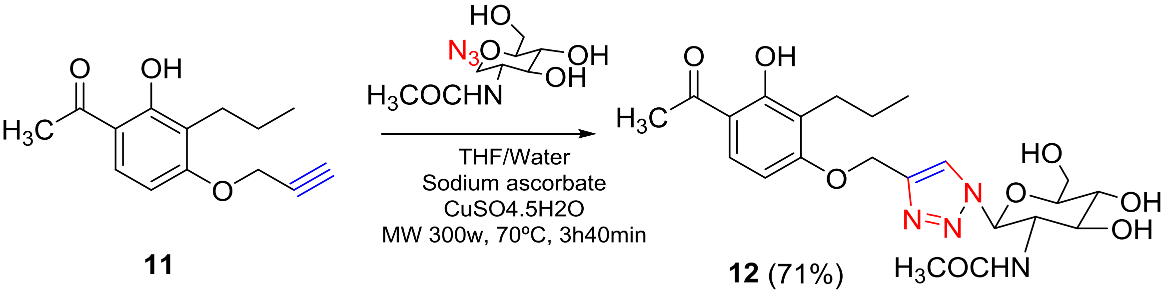
This reaction is a bimolecular nucleophilic substitution (S_N2). Acetone, an aprotic solvent, was selected since it will favour the reaction. In the presence of a base, phenol groups of 2',4'-dihydroxy-3'propylacetophenone (**10**) are deprotonated. A weak base (potassium carbonate) was used in order to only deprotonate the phenol group in 4' position. The resulted phenoxide acts as a Lewis base or nucleophile, and attacks the propargyl bromide once alkyl halides can act a Lewis acid or electrophile, suffering a halogen substitution (**Scheme 4**).



Scheme 4-SN2 Reaction mechanism to obtain 2'-hydroxy-4'-(prop-2-yn-yloxy)-3'-propylacetophenone (**11**)

2.1.2. Addition of an azide glucosamide to propargylated acetophenone

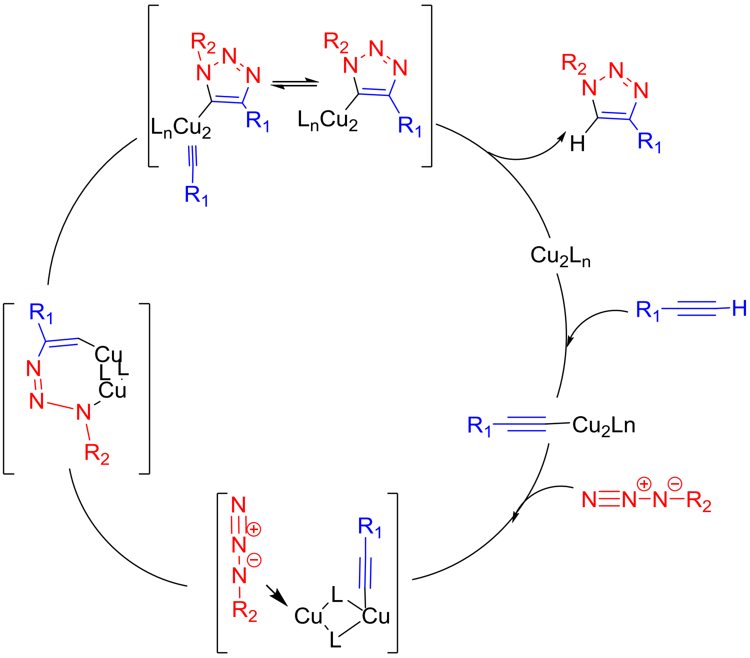
Compound **12** was synthesized by 1,3-dipolar cycloaddition[23] between 2-acetamido-2-deoxy-*β*-D- glucopyranosyl azide and 2-hydroxy-4-(prop-2-yn-yloxy)-3-propylacetophenone (**11**) under MW irradiation, at 70 °C (**Scheme 5**).



Scheme 5-Synthesis of 2'-hydroxy-3'-propyl-4'-(1-(1-(2-acetamido-2-deoxy- 3,4,6-tetrahydroxy-β-D- glucopyranosyl)-1H-1,2,3-triazole-4-yl)methoxy)acetophenone(**12**)

MW: microwave, THF: tetrahydrofuran

This cycloaddition process which resulted in the formation of a triazole ring (**Scheme 6**) was discovered in 1963. However, the copper catalyzed variant has improved its importance, since it assures decreasing reaction times and temperatures, while increases regioselectivity.[21] Another approach to accelerate the reaction has been the application of microwave (MW) irradiation, essential to achieve and maintain reaction internal heat. Chatkewitz, E. *et al* noted that higher yields were observed with increased power settings (300 W), but increasing irradiation time led to the decrease of the yield.[21]



Scheme 6-Reactional mechanism of CuAAc

L: ligand (Adapted from[25])

Cu (I) is easily oxidized to Cu(II) due to its thermodynamical instability and it can also be quickly disproportionated to Cu(II), which is catalytically inactive, or to Cu(0). In order to prevent that, it is common to use a combination of Cu(II) salts with a reducing agent, such as sodium ascorbate to generate Cu(I) species *in situ*.[20]

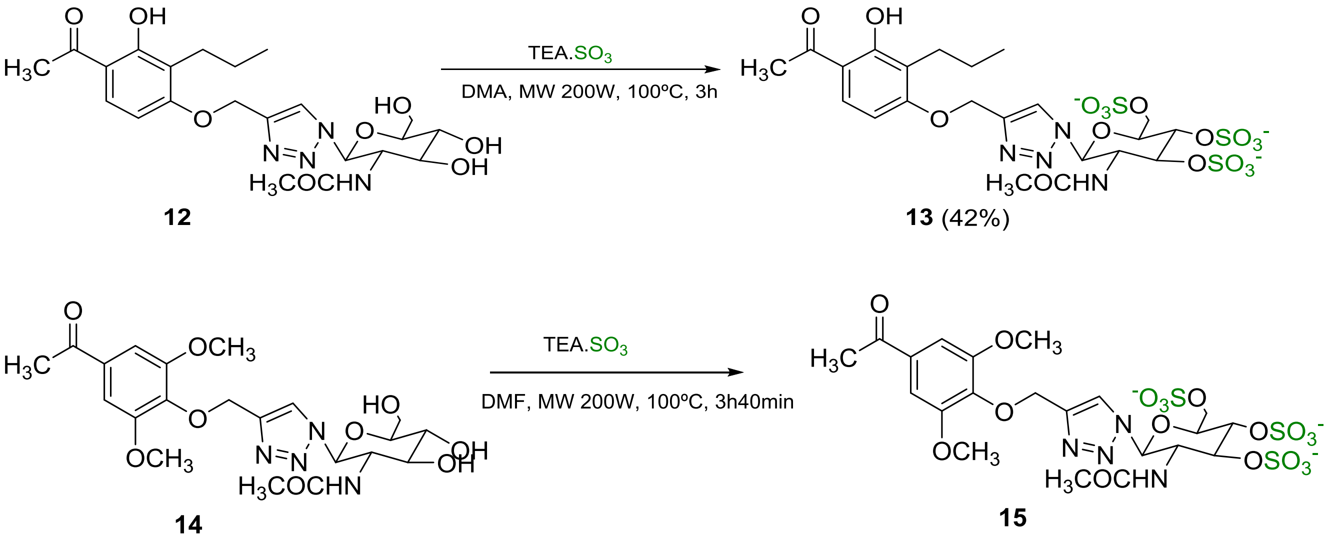
Certain Cu-binding ligands, as sulfide ones[19] also have an important role in the stabilization of copper(I) ions by avoiding oxidation or disproportionation, as well as making possible the formation of a soluble copper acetylide species, or even being responsible for the suppressing of side reactions as dimerization of alkynes and coupling of the copper-triazolyl intermediate with the alkyne.[26]

Water is frequently used as a solvent to this “click chemistry” reaction, along with other water-miscible organic solvents such as THF. [19] Furthermore, water is cheap, nonflammable, nontoxic, and environmentally safe.[10]

Purification of compound **12** was performed by liquid-liquid extraction with ethyl acetate followed by the extraction with water to remove any inorganic compound, such as copper or sodium ascorbate. From the aquose phase an impure solid was obtained which aws after purified by solubilization with methanol.

2.1.3. Sulfation of acetophenone triazole-linked to glucosamide

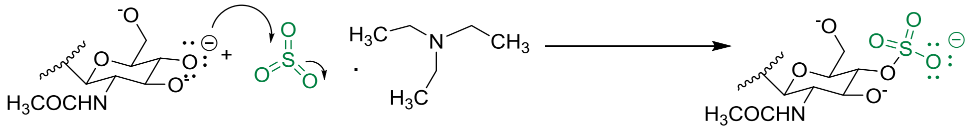
Compound **13** and compound **15** were obtained by the reaction of compound **12** and compound **14** respectively, with a sulfur trioxide-triethylamine complex (TEA·SO$_3$) adduct (**Scheme 7**).



Scheme 7-Synthesis of 2'-hydroxy-3'-propyl-4'-(1-(1-(2-acetamido-2-deoxy-β-D-glucopyranosyl-3,4,6-tetrasulfate)-1H-1,2,3-triazole4yl)methoxy)acetophenone (**13**) and 3',5'-dimetoxy-4'-(1-(1-(2-acetamido-2-deoxy-β-D-glucopyranosyl-3,4,6-tetrasulfate)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (**16**)

DMA: *N*,*N*-Dimethylacetamide; DMF **-** *N,N*-Dimethylformamide; TEA.SO$_3$: Triethylamine sulfur trioxide adduct

Those reactions are nucleophilic acyl substitutions.



Scheme 8-Reaction mechanism of sulfation

Sulfur trioxide is a electron acceptor or Lewis acid that can be combined with electron donors or Lewis bases, to form coordination compounds, also known as “adducts” or “complexes.”[27] These complexes with organic strong bases as trimethylamine (NMe3), and triethylamine (NEt3) or with weak bases such as DMF or pyridine (Py) have been used for sulfating many scaffolds.[27] Those kind of reaction required an polar aprotic solvents as pyridine, DMF,DMSO or DMA .[24,28] Scaffolds containing acidic OH groups seem to be better sulfated with SO_3 complexes in the presence of weaker bases such as pyridine and DMF. Sulfation using sulfur trioxide complexes have increased their importance in chemical synthesis due to the stability, ease to handle and mild acid character of the reagents.[28]The stability of the complex usually varies directly with the strength of the base used as their reactivity varies inversely to the strength of the base used. When the adduct is employed for sulfonating an organic compound, the SO_3 is released and the base forms the salt of the new sulfonic acid. Basic strength is not the only factor determining the reactivity of an SO3 complex. Trimethylamine, although equal in basic strength to triethylamine yields a complex which is more stable and less reactive

A MW-based protocol increased the rate of sulfation of phenolic structures. Persulfation of small polyphenolic scaffold is usually difficult due to anionic overcrowding as well as reduced stability of highly sulfated products to high temperatures.[24] Optimal results were achieved when the sulfur trioxide adduct was used at approximately 6–10 times molar proportion per phenolic group in the presence of MW irradiation at 100 ºC.[24]

2.2. Structural Elucidation

2.2.1. 2’-hydroxy-4’-(prop-2-yn-yloxy)-3’-propylacetophenone (12)

The structure of 2’-hydroxy-4’-(prop-2-yn-yloxy)-3’-propylacetophenone (**12, figure 7**), was elucidated by Infrared (IR) and ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR).

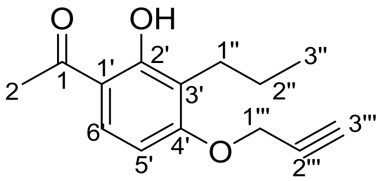


Figure 7-Structure of 2’-hydroxy-4’-(prop-2-yn-yloxy)-3’-propylacetophenone (**12**).

The IR spectrum of compound **12** (Erro! A origem da referência não foi encontrada.**7**) revealed characteristic bands at 3425 cm^{-1} (O-H fenol) and 2125 cm^{-1} (C≡C), confirming the successful propargylation in one of the phenols of compound **11**.

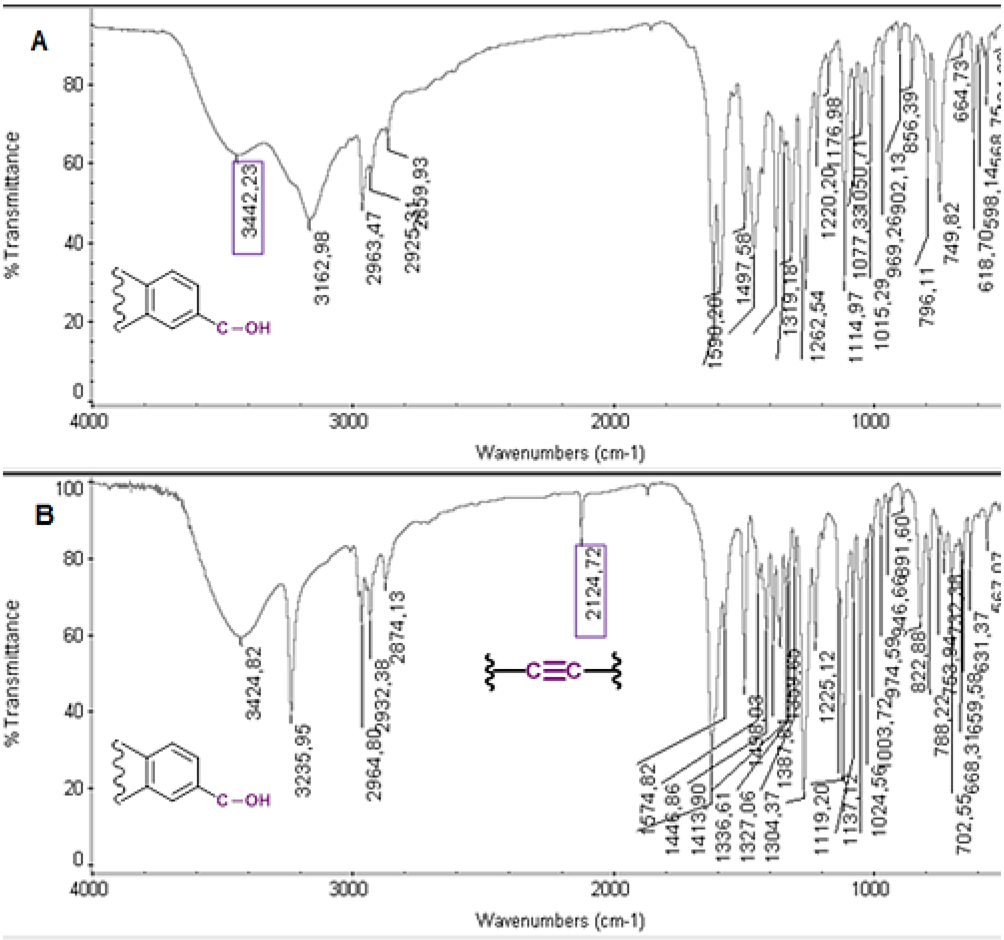


Figure 8-IR spectrum (KBr, cm-1) of compound 11(**A**) and compound 12 (**B**).

The ^{1}H and ^{13}C NMR data of compound **12** is represented in **Table 2**.

Table 2-^{1}H and ^{13}C NMR chemical shifts (δ) of **compound 12** ($CDCl_3$).

^{1}H	δ (ppm)
2	2.57 (s)
2’	12.73 (s)
5’	6.57 (d, J=9.0)
6’	7.61 (d, J=9.0)
1’’	2.64 (t, J=7.6)
2’’	1.61-1.48 (m)
3’’	0.94 (t, J=7.4)
1’’’	4.48 (d, J=2.4)
3’’’	2.52 (t, J=2.3)

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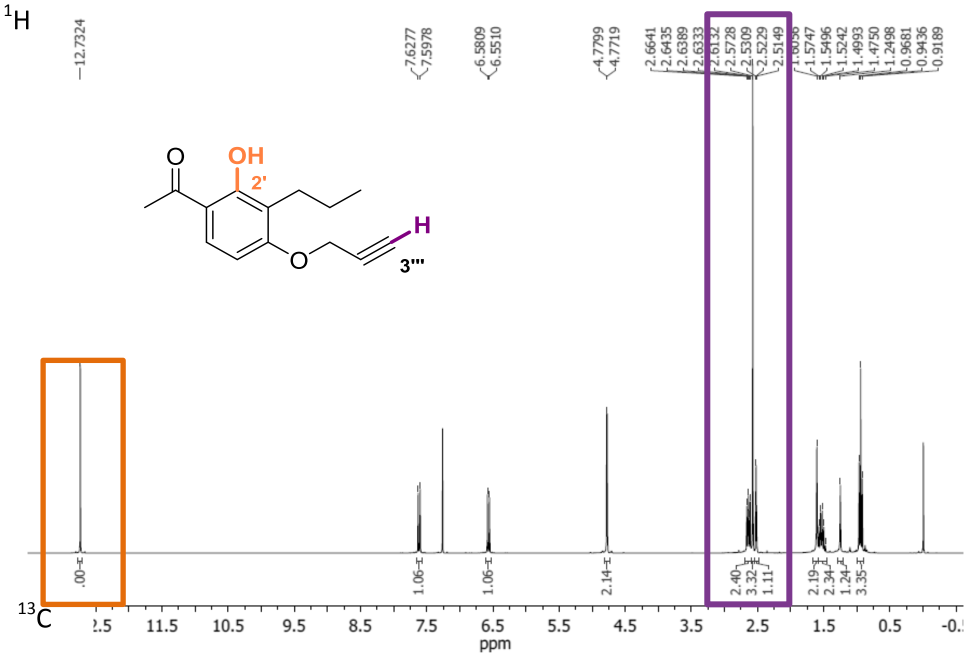
^{13}C	δ (ppm)
1	203.2
2	26.5
1’	114.8
2’	162.3
3’	119.3
4’	161.4
5’	103.3
6’	129.9
1’’	24.5
2’’	22.1
3’’	14.3
1’’’	56.1
2’’’	78.3
3’’’	76.0

The **figure 8** shows the ^{1}H NMR and the^{13}C NMR spectrum of compound 12.



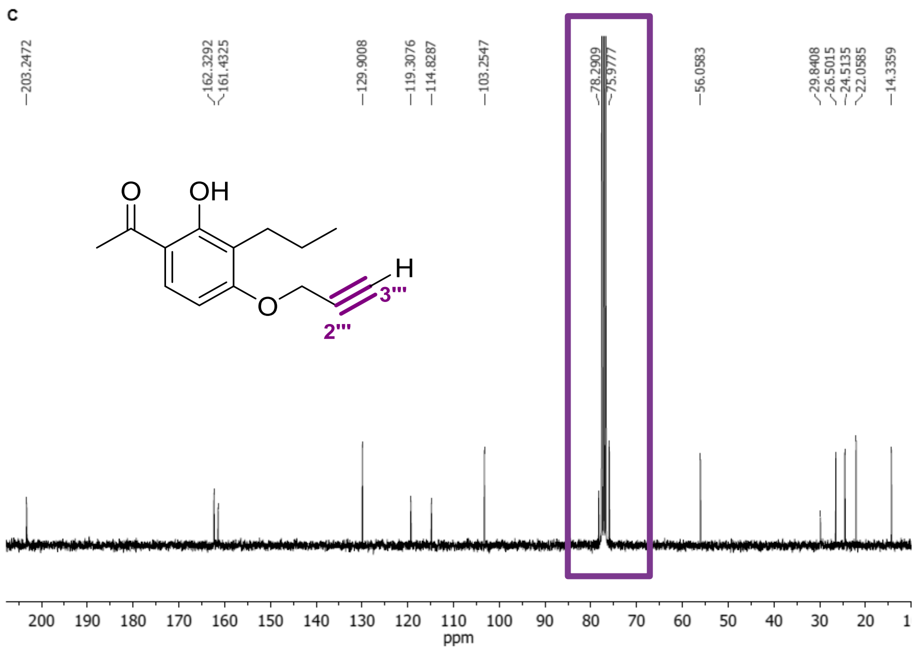


Figure 9-^{1}H (CDCl$_3$, 300.13 MHz) and ^{13}C (CDCl$_3$, 75.47 MHz) NMR spectrum of compound **12.**

The ^{1}H NMR spectrum of acetophenone **12** showed one singlet at δ 12.73 ppm corresponding to a hydroxyl group at the *ortho* position to the carbonyl group, two duplets at δ 6.57 and 7.61ppm, corresponding to the aromatic protons at 5’ and 6 position, and one signal at δ 2.1-2.4ppm due to the methyl protons of the ketone group. Moreover, it showed multiplet between δ 1.61-1.48ppm, corresponding to the protons at 2’’, a triplet at δ 0.94 ppm that corresponds to the proton linked to the alkyne, at 3’’ and other triplet at δ 2.64ppm, corresponding to the 1’’ protons.

The presence of a signal at δ 12.73 ppm allowed to confirm that the hydroxyl group at C-2 was not propargylated, as expected. Additionally, a triplet at δ 2.52 ppm was assigned to the proton at C-3’’ and a δ 4.48 corresponding to the protons of 4’-OCH_2,

Acetophenone ^{13}C NMR spectrum typically showed characteristic signals of aromatic carbons (δ between 162.3 and 103.3 ppm), a ketone carbon (between 205-220) and aliphatic carbons (δ between 14.3 and 78.3 ppm). Among the signals between 162.3 and 103.3 ppm the signals of oxygenated aromatic carbon atoms at positions 2’ and 4’ should be deshielded due to the electronegativity effect (signals at δ 161.4 and 162.3 ppm). The ketone carbon, C1 with δ 203.2 is highly deprotected, not only by the deshielded due to the electronegativity, but also to the polarity of the carbonyl. Alkynes typically occur δ range 70-85, here C-2’’’ and C-3’’ carbons appeared at 78.3 and 76.0. Those carbons are highly deprotected due the triple linkage.

2.2.2. 2’-hydroxy-3’-propyl-4’-(1-(1-(2-acetamido-2-deoxy- 3,4,6 tetrahydroxy-*β*-D- glucopyranosyl)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (13)

The structure of 2’-hydroxy-3’-propyl-4’-(1-(1-(2-acetamido-2-deoxy- 3,4,6 tetrahydroxy-β-D- glucopyranosyl)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (**13, figure 10**), was elucidated by Infrared (IR) and ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR).

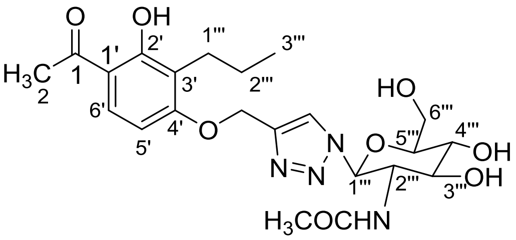


Figure 10-Structure of 2’-hydroxy-3’-propyl-4’-(1-(1-(2-acetamido-2-deoxy- 3,4,6 tetrahydroxy-β-D- glucopyranosyl)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (**13**).

The IR spectrum of compound **13** (**figure10**) revealed a band at 3292 cm^{-1}, corresponding to the N-H of the acetamide. A band typical of a C≡C bond was not observed in the IR spectrum of compound **13**. Those data confirmed the success of the reaction.

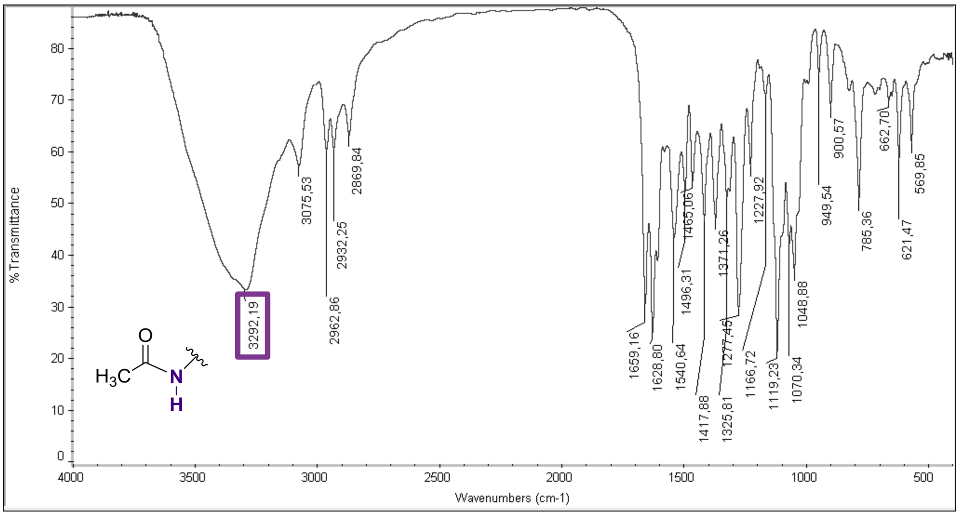


Figure 11-IR (KBr, cm^{-1}) spectrum (KBr, cm-1) of compound **13.**

The ^{1}H NMR spectrum of compound 1**3** is showed in **figure 12**.

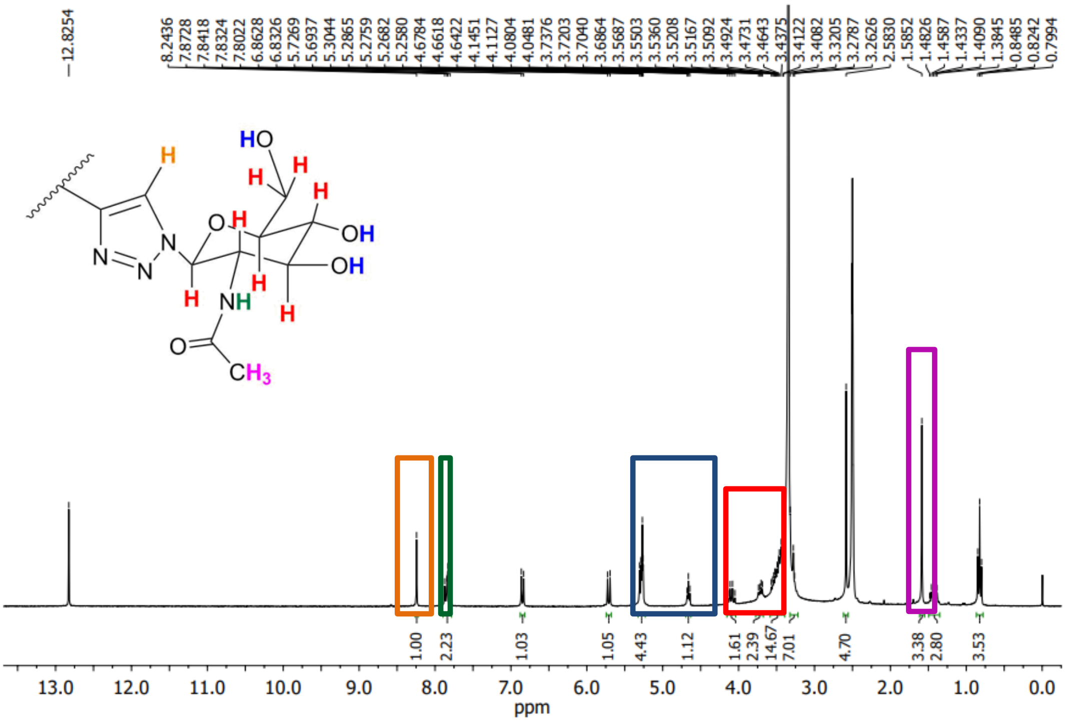


Figure 12- ^{1}H NMR spectrum of compound 13.

2.2.3. 2’-hydroxy-3’-propyl-4’-(1-(1-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl-3,4,6-tetrasulfate)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (14)

The structure of 2’-hydroxy-3’-propyl-4’-(1-(1-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl-3,4,6-tetrasulfate)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (**14, figure 13**) was elucidated by IR.

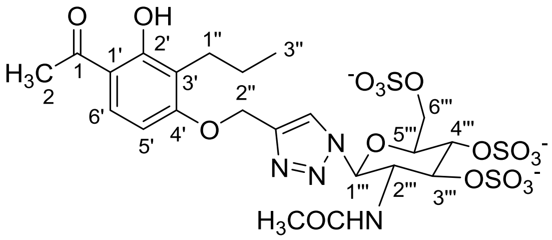


Figure 12- Structure of 2’-hydroxy-3’-propyl-4’-(1-(1-(2-acetamido-2-deoxy-β-D-glucopyranosyl-3,4,6-tetrasulfate)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (**14**).

The IR spectrum of **compound 14** (**figure 14**) revealed a band at 1264 cm^{-1} corresponding to S=O. Another typical band of sulfates appeared at 1048cm^{-1} (S-O) and at 804 cm^{-1} a bond corresponding to C-O-S.

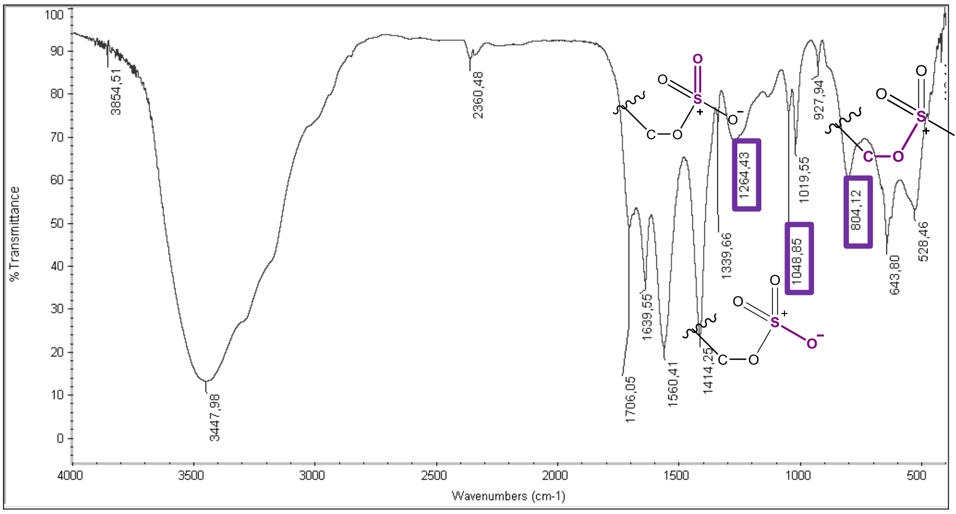


Figure 13- IR (KBr, cm^{-1}) spectrum of **compound 14.**

The structure of this compound will be further elucidated by NMR

2.2.4. 3’,5’-dimetoxy-4’-(1-(1-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl-3,4,6-tetrasulfate)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (16)

The structure of 3’,5’-dimetoxy-4’-(1-(1-(2-acetamido-2-deoxy- -*β*-D-glucopyranosyl-3,4,6-tetrasulfate)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (**16, figure 15**) was elucidated by IR.

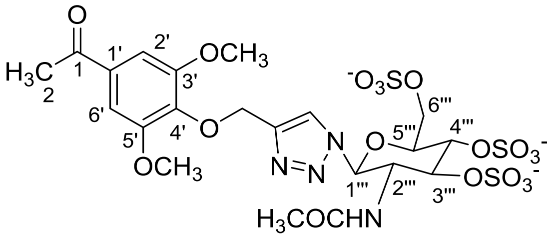


Figure 14- Struscture of 3’,5’-dimetoxy-4’-(1-(1-(2-acetamido-2-deoxy-β-D-glucopyranosyl-3,4,6-tetrasulfate)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (**16**).

The IR spectrum of **compound 15 (figure 16 A)** revealed a band at 3354cm^{-1}, corresponding to the N-H of the acetamide and IR spectrum of

compound 16 (**figure 16 B**) sulfates typical bands at 1412 cm^{-1} corresponding to S=O and at 824 cm^{-1}(C-O-S).

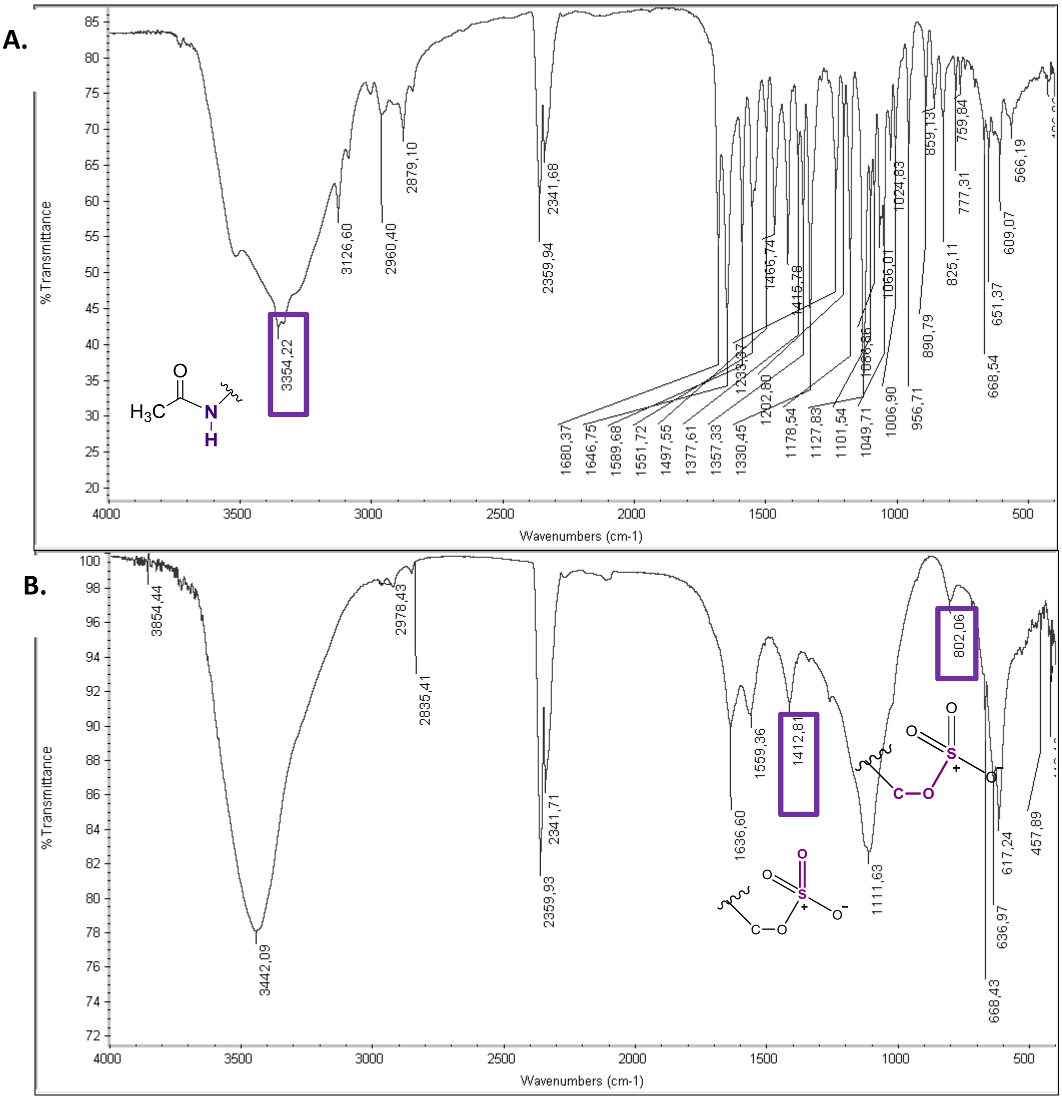


Figure 15- IR (KBr, cm^{-1}) spectrum of compound 15 (**A**) and 16 (**B**).

This compound will be further elucidated by NMR.

3. EXPERIMENTAL

3.1. General methods and materials

Commercial reagents were purchased from Sigma-Aldrich and Synthose. Solvents were evaporated using rotary evaporated under reduced pressure (Buchi Waterchath B-480).

MW reactions were performed in reaction vessels, opened or closed, usig a MicroSYNTH 1600 synthesizer from Milestone (ThermoUnicam, Portugal). Reactions were monitored by TLC carried out in plates of silica gel (60 F254 Merck). Compounds were visually detected by absorbance at 254 and/or 365 nm, H_2SO_4 in 20% MeOH followed by heat activation or $FeCl_3$ 10% MeOH solution.

Melting point was determined by Omron Köfter microscope and are uncorrected.

Purification of compounds was performed either by flash chromatography using Fluka silica gel 60 (0.04 – 0.063 nm) or liquid-liquid extration. IR spectrum were measured on an ATI Mattson Genesis series FTIR spectrophotometer, in KBr microplates (cm^{-1}). ^1H and ^{13}C NMR spectrum were performed in the Department of Chemistry of the University of Aveiro and were taken in DMSO-d_6 or $CDCl_3$ at room temperature on Bruker Avance 300 instruments (300.13 for ^1H and 75.47 MHz for ^{13}C). Chemical shifts are expressed as δ (ppm) values relative to tetramethysilane as an internal reference. Coupling constants (*J*) are reported in hertz (Hz). Assignment abbreviations are the following: singlet (s), doublet (d), triplet (t), multiplet (m), and doublet of doublets (dd).

3.2. Synthesis

3.2.1. Synthesis of 2’-hydroxy-4’-(prop-2-yn-yloxy)-3’-propylacetophenone (12)

To a solution of 2,4-dihydroxy-3’-propylacetophenone (0.5 g, 2.57mmol) and potassium carbonate (0.355 g, 2.57 mmol) in anhydrous acetone (10 mL), propargyl bromide (2 eq/OH; 572µL, 5.14 mmol) was added. The mixture was kept under conventional heating (60 °C), for 4h30min. The reaction was controlled by TLC using hexane:ethyl acetate (7:3) as mobile phase. The reaction mixture was filtered to eliminate potassium carbonate. The filtrate was purified by flash column chromatography, using hexane:ethyl acetate (9.5:0.5).

White solid (0.261g, 44% yield). m.p.: 49.9-51.3 ºC (ethyl acetate) IR (KBr): 3425 (phenolic OH), 2125 (C≡C) cm^{-1}. ^{1}H NMR (CDCl3, 300 MHz)δ: 12.73 (1H, s, 2’-OH), 7.61 (1H, d, J= 9.0 Hz, H-6’), 6.57 (1H, d, J=9.0 H-5’), 4.48 (2H, d, J=2.4, 4’-OC*H₂*),2.64 (2H, t, J= 7.6, H-1’’) 2.57 (3H, s, H-2), 2.52 (1H, t, J= 2.3Hz, H-3’’’), 1.61-1.48 (2H, m, H-2’’), 0.94 (3H, t, J=7.4, H-3’’) ppm. ^{13}C NMR ($CDCl_3$, 75.47 MHz)δ: 203.2 (C-1), 162.3 (C-2’), 161.4 (C-4’), 129.9 (C-6’), 119.3 (C-3’), 114.8 (C-1’), 103.3 (C-5’), 78.3 (C-2’’’), 76.0 (C-3’’’), 56.1 (C-1’’’), 26.5 (C-2), 24.5 (C-1’’), 22.1 (C-2’’), 14.3 (C-3’’).

3.2.2. 2’-hydroxy-3’-propyl-4’-(1-(1-(2-acetamido-2-deoxy- 3,4,6 tetrahydroxy-β-d-glucopyranosyl)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (13)

To a solution of 2’-hydroxy-4’-(prop-2-yn-yloxy)-3’-propylacetophenone (0.20 g, 0.9 mmol) and 2-acetamido-2-deoxy-β-D-glucopyranosyl azide (0.20 g, 0.9 mmol) in THF (40 mL), a solution of sodium ascorbate (0.85 g, 4.3 mmol) and $Cu_2SO_4 \cdot 5H_2O$ (1.08 g, 4.3 mmol) in water (20 mL) was added. The mixture was kept under MW irradiation (300W), in a closed vessel, at 70 °C, for 3h.40min. The reaction was controlled by TLC using hexane: etyle acetate (8:2) as mobile phase.

The obtained reaction mixture was filtered to eliminate insoluble inorganic salts and then extracted twice with ethyl acetate. From the aqueous phase an insoluble material came out, which was filtered and washed with methanol affording a pure white solid.

White solid (0.29 g, 71% yield). m.p.: 228 ºC (methanol). IR (KBr): 3292 (N-H aketamide) cm^{-1} .^{1}H NMR (DMSO-d$_6$, 300.13 MHz) δ: 12.83 (1H, s, O*H*-phenol), 8.24 (1H,s, *H*-triazole), 7.86 (1H, d, J=9.3, N*H*-acetamide), 7.82 (1H,d,J= 9.1,H-6''), 6.85 (1H,d, J=9.1, H-5''), 5.71 (1H, d, J=10.0, H-1'''), 5.30-5.26 (4H,m, OC*H*$_2$ -triazole and O*H*-glucose), 4.68-4.64 (1H, m, O*H*-glucose), 4.15-4.01 (2H, q ,H-2'''), 3.74-3.69 (2H, q, H-6'''), 3.55-3.27 (3H-glucose under water) 2.65-2.50 (5H, s, C*H*$_3$ –ketone and H-1''), 1.59 (3H,s, C*H*$_3$-acetamide), 1.48-1.38 (2H,m, H-2'') 5,0.82 (3H, t, J=7.3, H-3'') ppm

3.2.3. 2'-hydroxy-3'-propyl-4'-(1-(1-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl-3,4,6-tetrasulfate)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (14)

To a solution of 2'-hydroxy-3'-propyl-4'-(1-(1-(2-acetamido-2-deoxy- 3,4,6 tetrahydroxy-*β*-D- glucopyranosyl)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (0.20g, 0.42mmol) in dimethylacetamide ,triethylamine sulfur trioxide adduct (1.36g, 7.5mmol) was added . The mixture was keptunder MW irradiation (200W) for 3 hours,in open vessel at 100 ºC. The reaction was controlled by TLC using chloroform: methanol (8:2) as mobile phase. The reaction mixture was basified by adding triethylamine until neutral pH. Then cold diethyl ether(100 mL) was added, resulting in the formation of a brown oil. To promote de formation of more oil, the solution was kept at 4 ºC for 2h30min. The ether was rejected and the oil solubilized in 7mL of 30% sodium acetate. Then cold ethanol was added, forming a solid. Once more, to promote the formation of the solid, the solution was kept at 4 ºC for 30 minutes. The resulting solid was filtrated and the solvent evaporated. The final solid was submitted to a dialysis for 2 hours.

Orange solid (1.26 g, 48% yield). m.p.: 210ºC (Tdec) (ultra pure water) IR (KBr): 3447(N-H aketamide), 1264 (S=O), 1048 (S-O) and 804 (C-O-S) cm^{-1}. .

3.2.4 3’,5’-dimetoxy-4’-(1-(1-(2-acetamido-2-deoxy-*β*-D-glucopyranosy-3,4,6-tetrasulfate)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (16)

To a solution of 3’,5’-dimetoxy-4’-(1-(1-(2-acetamido-2-deoxy-3,4,6-tetrahidroxy-βD-glucopyranosyl)-1H-1,2,3-triazole-4yl)methoxy)acetophenone (0.20g,0.42mmol) in dimethylformamide triethylamine sulfur trioxide adduct (0.60g, 3.36mmol) was added. The reaction was keptunder MW irradiation (200w), in open vessel, at 100 ºC, for 3h40min.

Reaction was controlled by TLC using chloroform:methanol (7:3) as mobile phase.

The treatment used for the formation of oil was the same described for the previous sulfation reaction.

White solid (0,31g, 100%) m.p.: 214-215 ºC (ultra pure water) IR (KBr): 3442(N-H aketamide), 1412 (S=O), and 802 (C-O-S) cm^{-1} .

4. CONCLUSION

In this project, a new scaffold was selected to synthesize antifouling compounds.

Four new acetophenone derivatives were synthesized for the first time through several molecular modifications: propargylation, “click chemistry” cycloaddition and sulfation. Both, conventional and MW heating were used.

Compounds purification involved different processes as liquid-liquid extraction, flash column chromatography and dialysis. Purity was verified by TLC with different mobile phases and melting point.

Structural elucidation was made by infrared and ^{1}H and ^{13}C NMR.

These newly synthesized acetophenones will be further tested for the inhibition of *Mytilus galloprovinciallis* larvae settlement and for the toxicity of non-toxic organisms as to *Artemia salina* in order to assess their potential as future alternative to toxic antifouling products in use.

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