NOVEL BIOCATALYZED FLOW SYNTHESIS OF NATURE-INSPIRED PHENOLIC CARBONATE AND CARBAMATE DERIVATIVES AS ANTIRADICAL AND ANTIMICROBIAL AGENTS

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Green Chemistry allows the control of environmental hazards and pollution, reducing chemical waste and dangerous effects on workers' health [1]. Recently, the use of continuous biocatalysis has become widespread in API synthesis, stimulating the application of immobilized enzymes in packed bed reactors, allowing the overcoming of some practical problems connected to batch procedures, such as product inhibition, low productivity, scalability, biocatalyst stability and product and/or intermediate degradation [2].

During my Thesis, I worked on the development of two novel continuous synthetic protocols exploiting the combination of the flow chemistry and biocatalysis for the obtainment of carbonate and carbamate derivatives of natural phenolic compounds, characterized by increased lipophilicity compared to the parent compounds (Figure 1). As natural phenolic compounds, I selected tyrosol and hydroxytyrosol, mainly present in olive oil, due to their broad range of biological activities and their potential health benefits in particular as natural antioxidants [3].

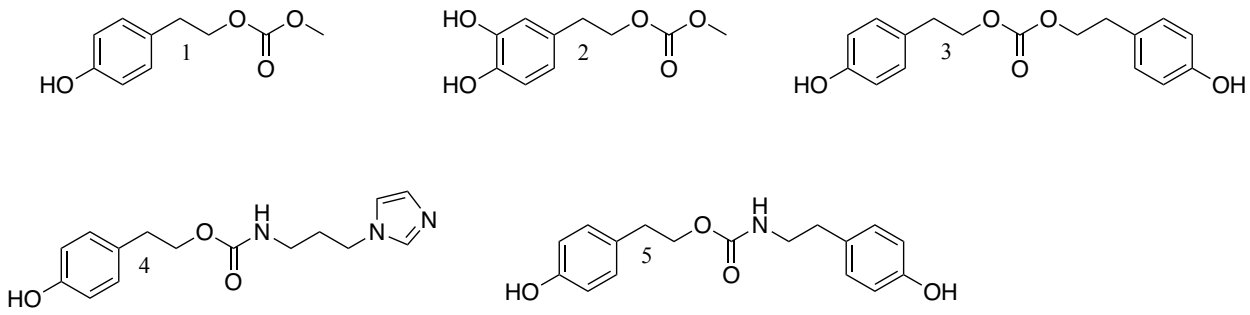

Figure 1: Chemical structures of the synthetized compounds.

It is noteworthy that phenols and catechols can transfer the hydrogen from the phenolic hydroxyl group to different reactive oxygen species, reducing the oxidative stress in cells (Figure 2) and, as reported in literature, several alkyl-carbonate derivatives of hydroxytyrosol possess an increased activity compared to the parent compound, heightening the molecule lipophilicity and dimensions [4]. In addition, different natural antimicrobial agents can be plant origin, such as tyrosol, whose mechanism is related to the ATP synthase inhibition, and hydroxytyrosol, whose activity depends on the bacterial strains [5].

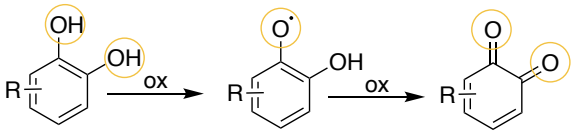

Figure 2: Catechol antioxidant action guaranteed by the hydrogen transfer and the electrons redistribution on the aromatic ring.

During my Thesis, first, I synthesized tyrosol and hydroxytyrosol carbonate derivatives (Figure 1, compounds **1**-**3**) exploiting an immobilized lipase from *Candida antarctica* (CaLB) as biocatalyst in an unconventional organic medium as dimethyl carbonate (DMC). The reaction was studied in batch and then moved to flow. The switch from a batch traditional set up to a continuous one allowed to reduce the reaction time, simplify the work-up and increase the yield: the former needed 6 h to bring the reaction to completeness, filtration under pressure and flash chromatography purification steps were necessary to isolate the pure product (**1**), and the best yield achieved was 93%; the latter needed only 10 min of residence time to bring the reaction to completeness, did not require manual downstream processes or purifications, giving a quantitative yield. The optimized conditions were then applied to the synthesis of compound **2**. Considering the hydroxytyrosol low availability in nature and its considerable price, I employed the biocatalyzed flow procedure previously developed by the research group for the oxidation of tyrosol into hydroxytyrosol [6]. Subsequently, hydroxytyrosol was used as starting reagent for the synthesis of the 3,4-dihydroxyphenetyl methyl carbonate (**2**).

According to literature [7], the synthesis of compound **3** was difficult and far away from being environmentally friendly and sustainable. In fact, the first step of its synthesis is usually performed under inert atmosphere, adding triphosgene dropwise to the reactor vessel containing a tyrosol solution in anhydrous tetrahydrofuran. Secondly, tyrosol chloroformate was the substrate of tyrosol nucleophilic attack, obtaining *bis*(4-hydroxyphenetyl) carbonate (**3**). Considering the well-known triphosgene corrosivity and toxicity, non-phosgene strategies and catalytic carbonylation reactions could be highly attractive. Additionally, compound **3** has been recently described as monomer of a new class of biodegradable, aromatic polycarbonates deriving from tyrosol. Indeed, I exploited compound **1** as a starting material for the synthesis of compound **3** (Figure 3). *Tert*-amyl alcohol is an intermediate polar solvent that has been demonstrated to keep a high catalytic activity of lipase because of its large steric hindrance effect. Combined with its safety profile, low freezing point (in comparison to *t*-BuOH), and ability to solubilize polar compounds, it was chosen as unconventional medium for the biocatalyzed synthesis of the following compounds. The final flow protocol allowed the isolation of the desired compound **3** in two hours (vs 4 day in batch), maintaining the same isolated yield of 23% compared to batch.

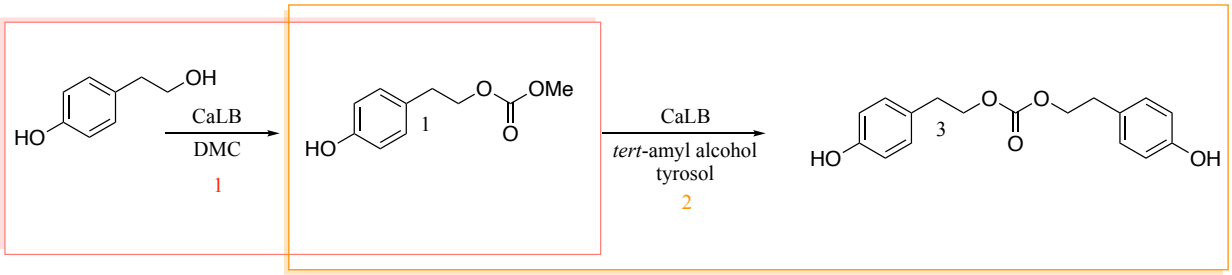

Figure 3: Synthesis of Ty carbonate derivatives.

Finally, the synthesis of the carbamate derivatives has been investigated. A novel protocol in *tert*-amyl alcohol has been designed to perform the CaLB-catalyzed carbonate aminolysis forming the carbamate derivatives **4** and **5** (Figure 4), exploiting amines with antioxidant and antifungal properties, *i.e.*, tyramine and 1-(3-aminopropyl) imidazole as nucleophiles [8]. The reaction gave the maximum conversion after 1 hour of residence time and I was able to isolate the desired product **4** and **5** in 19% and 26% yield, respectively.

In conclusion, thanks to the use of a commercially available immobilized biocatalyst in a packed bed reactor, reaction time, work-up efficiency and productivity were increased compared to the traditional batch synthesis. Five compounds were synthesized with biocatalyzed phosgene-free procedures, and, according to the biological results, the antimicrobial and antiradical activities of the parent compounds were left unchanged, improving at the same time their lipophilicity. As a result, it is reasonable to say that flow chemistry and biocatalysis are ideal partners for the design of novel, sustainable, efficient, scalable, and automated processes.

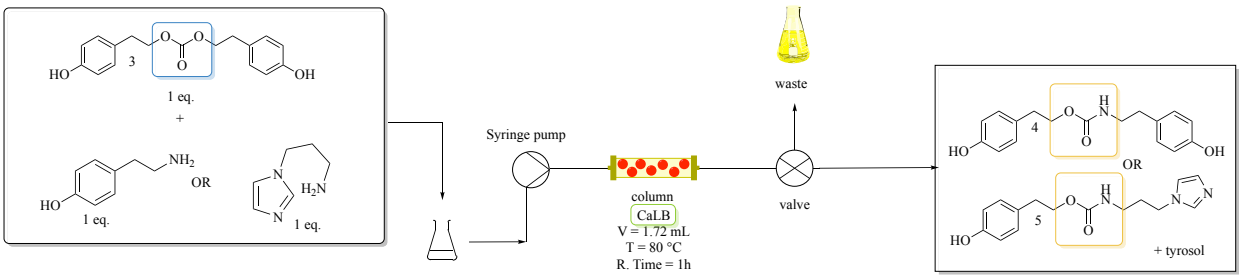

Figure 4: Flow set-up exploited for the carbamate derivative continuous biocatalyzed synthesis.

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