CHEMO-ENZYMATIC FLOW SYNTHESIS OF NATURE-INSPIRED PHENOLIC CARBONATES AND CARBAMATES AS ANTIRADICAL AND ANTIMICROBIAL AGENTS

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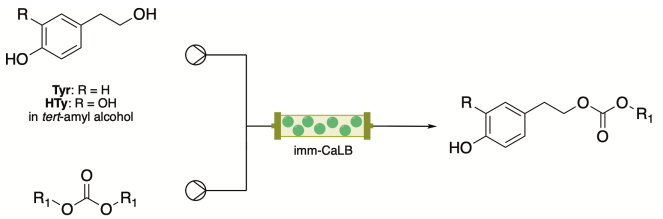
Background:
Tyrosol (**Ty**) and hydroxytyrosol (**HTy**) are natural phenolic compounds available in wine and olive oil and their potential health benefits as antioxidant, antimicrobial, anti-inflammatory, neuroprotective and anticancer agents, are object of interest for food, nutraceutical, cosmetic and pharmaceutical applications.[1] However, due to their solubility, metabolic/chemical stability and bioavailability issues, their applicability as active ingredients is still limited. With the aim to overcome this limitation, a growing interest has been devoted to the obtainment of more lipophilic derivatives.

Aim:
The work aimed at developing a scalable, sustainable, **chemo-enzymatic flow synthesis** of **lipophilic carbonate and carbamate derivatives** of Ty and HTy. Chemical lipophilization is commonly achieved under drastic conditions of temperature and pH using strong acidic catalysts, resulting in low selectivity, and by-product formation. Herein, first, a series of carbonates was obtained developing a biocatalytic flow protocol; second, starting from phenyl carbonates, tyramine and phenylethylamine, four carbamates were synthetized.

Methods:
Reagents and solvents were purchased from commercial suppliers. NMR spectra were recorded on a Varian Gemini 300 MHz. Continuous flow biotrasformations were performed using a R2^{+}/R4 flow reactor or Asia Flow Chemistry Syringe pumps (Syrris) equipped with an Omnifit ® glass column. Pressure was controlled by using back-pressure regulators. HPLC analyses were performed using a Waters 1525 Binary HPLC Pump, equipped with a Waters 2489 UV-vis detector, Waters C18 column µBondapack (10 µm, 125 Å), 254 nm. The DPPH radical-scavenging assay (Bio-quochem, Asturie, Spain) was performed using a spectrophotometer. Immobilized lipase B from *Candida antarctica* was purchased from Merck.

Results:
Initially, the synthesis of carbonates of Ty and HTy was investigated under flow conditions, exploiting the **chemoselective** reaction of the primary alcohol biocatalyzed by the commercially available immobilized lipase B from *Candida antarctica* (imm-CaLB) (**Scheme 1**).

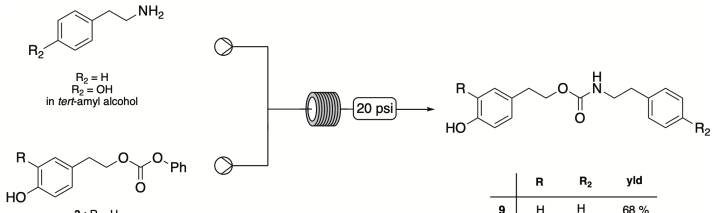


	R	R_1	yld		R	R_1	yld
1	H	Et	60 %	5	OH	Et	34 %
2	H	allyl	97 %	6	OH	allyl	40 %
3	H	Ph	80 %	7	OH	Ph	53 %
4	H	Bn	40 %	8	OH	Bn	10 %

Scheme 1: Flow reactor configuration for the synthesis of tyrosol and hydroxytyrosol carbonate derivatives[2].

Tert-amyl alcohol was selected as reaction organic solvent, able to solubilize polar compounds. Also, CPME, MeTHF, acetone, *tert*-butyl methyl ether and toluene were screened but solubility problems and no improvement in the conversion were obtained.
First, the reaction between Ty and diethyl carbonate was investigated, studying the effect of molar ratio (range: 1:3-1:6) of substrates and residence time (range: 30-120 min) on conversion. Ty concentration (0.1

M) and the temperature (T = 80 °C) have been previously optimized[3] and HTy was synthetized from Ty as previously reported[4].
Compounds **1, 2, 4, 5, 6** and **8** were obtained in moderate to good yields (molar ratio: 1:3, residence time: 60 min), pumping two stock solutions into a column reactor packed with imm-CaLB. Differently, due to the solubility issues in *tert*-amyl alcohol, compounds **3** and **7** were synthetized using one stock solution in which the molar ratio between Ty/HTy and diphenyl carbonate was 1:2.
Second, starting from the synthetized carbonates, the synthesis of Ty and HTy carbamate derivatives was investigated: ethyl carbonate **1**, allyl carbonate **2** and phenyl carbonate **3** were tested as reagents using 2-phenylethylamine as model nucleophile in a microwave reactor (small scale reactivity screening); only adopting phenyl carbonate derivative **3**, the desired carbamate was obtained.
The reaction between compound **3** and 2-phenylethylamine was adopted as model reaction for optimization in flow: the effects of stoichiometry (1:1, 1:1.5, 1:2) and residence time (range: 15-45 min) were evaluated, keeping the temperature constant at 110 °C and the whole system pressurized at 20 psi; the best conditions were obtained using a molar ratio 1:1.5 of the reagents and a residence time of 30 min, achieving compounds **9-12** (**Scheme 2**).



	R	R_2	yld
9	H	H	68 %
10	H	OH	74 %
11	OH	H	38 %
12	OH	OH	42 %

Scheme 2: Flow reactor configuration for the synthesis of compounds **9-12**[2].

Moreover, to reduce the manual handling and increase the protocol sustainability, a telescoped chemo-enzymatic process was developed for the synthesis of compound **9**, isolated in 45% overall yield.
cLogP and cLoGS calculated values demonstrated the increased lipophilicity of Ty and HTy derivatives respect to their parent compounds, making possible their application in lipid-rich matrices.
In addition, the synthetized compounds were evaluated both as **antiradical** and **antimicrobial agents**. As expected, the presence of the catechol moiety in HTy derivatives led to more efficient radical scavengers in comparison with Ty ones, and the derivatization of the natural compounds did not impact on their radical scavenger effect. Moreover, most of the new derivatives showed higher antimicrobial activity in comparison with Ty and HTy.

Conclusion:
An innovative chemo-enzymatic two-step flow protocol for the synthesis of new phenolic carbonates and carbamates was developed. A reproducible flow procedure to synthetize carbonates has been set-up using imm-CaLB as biocatalyst working in an unconventional organic medium as *tert*-amyl alcohol; then, a nucleophilic attack on compounds 3 and 7, using 2-phenylethylamine and tyramine, was performed to obtain the desired carbamates. Twelve more lipophilic compounds were synthetized in moderate to good yields without altering the biological properties of Ty and HTy.

References:

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