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Chemo-enzymatic flow synthesis of nature-inspired phenolic carbonates and carbamates as antiradical and antimicrobial agents

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ABSTRACT

A series of carbonate and carbamate derivatives of natural phenols, tyrosol and hydroxytyrosol, were synthetized through a chemo-enzymatic flow approach. First, the chemoselective synthesis of tyrosol and hydroxytyrosol carbonates was performed using immobilized lipase B from *Candida antarctica* as catalyst in *tert*-amyl alcohol, a non-conventional green solvent. Then, two selected carbonates were reacted in flow with the appropriate amine to obtain the desired carbamates. All the compounds were isolated in moderate to good yields. The carbonate and carbamate derivatives showed increased lipophilicity compared to the natural parent compounds, while maintaining similar radical scavenger properties. Moreover, their antimicrobial activity against four selected bacterial strains was evaluated and they showed improved activity in comparison with tyrosol and hydroxytyrosol.

1. Introduction

Tyrosol (Ty) and hydroxytyrosol (HTy) have attracted widespread attention because of their broad range of biological activities and their potential health benefits, including antioxidant, antimicrobial, anti-inflammatory, anticancer, and neuroprotective effects (Fig. 1) (Bertelli et al., 2020; Hu et al., 2014; Karković Marković et al., 2019; Wani et al., 2018). They are natural phenolic compounds present in olive oil and wine. Despite the high concentration in the olive fruit, only 2% of the initial concentration is found into virgin olive oil, while the remaining fraction is found in the olive mill wastewater (approximately 53%) and in the pomace (approximately 45%) (Caporaso et al., 2019; Çelik et al., 2021). As primary antioxidants, their ability to inactivate reactive oxygen species (ROS), mainly through hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms (Leopoldini et al., 2011), makes these molecules of great interest for food, nutraceutical, cosmetic and pharmaceutical applications. However, Ty and HTy show a low bioavailability and limited applications in lipidic media due to their hydrophilic character. Consequently, several studies on novel derivatives, in particular esters and ethers, with increased lipophilicity have been reported (Annunziata et al., 2022; Bernini et al., 2012, 2017; Sun et al., 2018). Lipophilic phenolic derivatives have shown good solubility in oils and emulsions, and are used as active ingredients in food and cosmetics as well as in several pharmaceutical preparations (Anankanbil et al., 2018; Arzola-Rodríguez et al., 2022; Wang et al., 2023). Chemical lipophilization is commonly achieved under drastic conditions of temperature and pH using strong acidic catalysts, resulting in low selectivity, formation of by-products, with consequent need for purification steps that generate large

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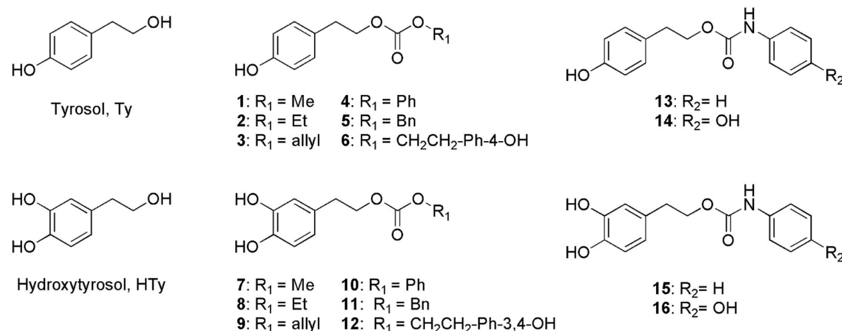


Fig. 1. Structures of tyrosol (Ty), hydroxytyrosol (HTy), and their carbonate (**1-12**) and carbamate derivatives (**13–16**).

amount of waste (Mendoza-Sanchez et al., 2019; Won Young et al., 2017; Zhong and Shahidi, 2011). In this context, biocatalysis represents a key enabling technology to perform the lipophilization of the molecules of interest: it allows the application of milder conditions and assures selectivity, minimizing side reactions and by-products formation (Liu et al., 2014; Mardani et al., 2022). Moreover, enzymatic reactions are more environmentally friendly because of the reduced amount of energy consumption and waste production. Enzymatic lipophilization can be performed by esterification using different lipases, proteases, esterases, and acyl transferases (Durand et al., 2013; Mardani et al., 2022; Zieniuk et al., 2022). In particular, lipases can act in a wide range of pH and temperature, proving high chemo- and stereo-selectivity.

Herein, we develop a reproducible, green and scalable chemo-enzymatic flow synthesis to obtain a series of lipophilic carbonate and carbamate derivatives of Ty and HTy. A variety of methods to synthesize carbonates and carbamates are known, but they exploit toxic acylating agents and hazardous organic solvents (Ghosh and Brindisi, 2015). In this work, a chemo-selective biocatalyzed flow protocol was developed to obtain a set of carbonate derivatives (compounds **2–5** and **8–11**, Fig. 1). Then, four carbamate derivatives (compounds **13–16**), characterized by increased metabolic stability, were generated starting from phenyl carbonates **4** and **10** and using phenylethylamine and tyramine as nucleophiles. Tyramine was chosen because many natural and unnatural derivatives with interesting biological properties are known (Ayanlowo et al., 2020; Leonard et al., 2022). Indeed, phenylethylamine was used as model nucleophile. Then, the antiradical and antimicrobial properties of the obtained derivatives, together with the carbonates **1**, **6**, **7**, and **12** recently reported by us (Vicinanza et al., 2023), were investigated by comparison with the natural parent compounds Ty and HTy.

2. Materials and methods

Reagents and solvents were obtained from commercial suppliers and used without further purification. NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer using the residual signal of the deuterated solvent as internal standard. ^{1}H chemical shifts (δ) are expressed in ppm and coupling constants (J) in hertz (Hz). Continuous flow biotransformations were performed using a R2$^+$/R4 flow reactor or Asia Flow Chemistry Syringe pumps (Syrris) equipped with an Omnifit® glass column (6.6 mm i.d. × 100 mm length or 10 mm i.d. × 100 mm length). Pressure was controlled by using back-pressure regulators. TLC analyses were performed on commercial silica gel 60 F254 aluminium sheets; spots were further evidenced by spraying with a dilute alkaline solution of $KMnO_4$. HPLC analyses were performed using a Waters 1525 Binary HPLC Pump, equipped with a Waters 2489 UV–vis detector (Waters, Milford, MA). Waters C18 column μBondapack (10 μm, 125 Å), 254 nm (l). Injection volume: 10 μL. Flow rate: 1.0 mL min^{-1}. For the analyses of Ty and compounds **2**, **4**, and **13**, an isocratic method H_2O/acetonitrile (6:4 for Tyr and compound **2**, and 7:3 for compounds **4** and **13**, respectively) was used. Retention times: Ty = 3.8 min, **2** = 8.6 min, **4** = 12.4 min, **13** = 10.2 min. For the synthesis of carbonate **2**, the percentage of conversion was calculated on the basis of the depletion of tyrosol (substrate) and monitoring the formation of the carbonate product **2** (Conversion [%] = [product area/(product area + substrate area)] × 100). In the case of the synthesis of carbamate **13**, a correction factor (cf) of 0.6548 was used (Conversion [%] = [product area x cf/(product area x cf + compound **4** area)] × 100). The DPPH radical-scavenging assay (Bio-quochem, Asturie, Spain) was performed using a spectrophotometer (Eppendorf, Milan, Italy). Immobilized lipase B from *Candida antarctica* was purchased from Merck. HTy was synthetized from Ty as previously reported (Annunziata, et al., 2021). Compounds **1**, **6**, **7** and **12** were synthetized as previously reported (Vicinanza et al., 2023).

2.1. Continuous synthesis of Ty and HTy carbonate derivatives (2–5 and 8–11)

Two stock solutions were prepared as follows: a) a solution of Ty or HTy (0.2 M, 1.0 mmol) in *tert*-amyl alcohol (5.0 mL); b) a solution of the starting carbonate (0.6 M, 3.0 mmol) for the synthesis of compounds **2**, **3**, **5**, **8**, **9** and **11** in *tert*-amyl alcohol (5.0 mL). In the case of compounds **4** and **10**, one stock solution containing Ty or HTy (0.1 M, 0.5 mmol) and diphenyl carbonate (0.2 M, 1.0 mmol) was prepared. For the synthesis of all compounds, stock solution(s) were pumped through a column reactor packed with 600 mg imm-CaLB (volume: 2.6 mL) using syringe pumps with a total flow rate of 43.3 μL min^{-1} (residence time 60 min) at 80 °C. *tert*-Amyl alcohol was used as flow stream. The resulting crudes were collected, and the solvent was evaporated under reduced pressure. The crudes were purified by flash chromatography (cyclohexane/ethyl acetate 9:1 to 8:2 for compounds **2**–**4**, toluene/ethyl acetate 9:1 to 8:2 for **5**, cyclohexane/ethyl acetate 9:1 to 7:3 for **8**–**10**, toluene/ethyl acetate 98:2 to 95:5 for **11**).

4-Hydroxyphenethyl ethyl carbonate (**2**). Yield: 60%; yellow oil; R_f (cyclohexane/ethyl acetate 6:4): 0.73; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.09–7.80 (m, 2H), 6.75–6.64 (m, 2H), 4.22 (t, J = 7.1 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 2.83 (t, J = 7.1 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 155.7, 155.3, 129.6, 128.2, 114.9, 68.3, 63.5, 33.9, 13.2.

4-Hydroxyphenethyl allyl carbonate (**3**). Yield: 97%; light yellow oil; R_f (cyclohexane/ethyl acetate 6:4): 0.74; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.10–6.79 (m, 2H), 6.73–6.67 (m, 2H), 5.90 (ddd, J = 16.9, 10.6, 5.6 Hz, 1H), 5.33 (dd, J = 16.9, 1.5 Hz, 1H), 5.22 (dd, J = 10.6, 1.5 Hz, 1H), 4.57 (dd, J = 5.6, 1.5 Hz, 2H), 4.24 (t, J = 7.1 Hz, 2H), 2.84 (t, J = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 155.7, 155.1, 131.9, 129.6, 128.1, 117.3, 114.9, 68.5, 67.9, 33.9.

4-Hydroxyphenethyl phenyl carbonate (**4**). Yield: 80%; white solid; R_f (cyclohexane/ethyl acetate 6:4): 0.63; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.38–7.22 (m, 5H), 7.21–7.09 (m, 2H), 6.75–6.70 (m, 2H), 4.35 (t, J = 7.0 Hz, 2H), 2.91 (t, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 155.9, 153.7, 151.4, 129.6, 129.1, 127.9, 125.6, 120.8, 114.9, 69.2, 33.8.

4-Hydroxyphenethyl benzyl carbonate (**5**). Yield: 40%; white solid; R_f (cyclohexane/ethyl acetate 6:4): 0.58; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.35–7.34 (m, 5H), 7.04–7.01 (m, 2H), 6.71–6.68 (m, 2H), 5.11 (s, 2H), 4.26 (t, J = 7.1 Hz, 2H), 2.84 (t, J = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 155.7, 155.2, 135.7, 129.6, 128.2, 128.1, 128.0, 127.8, 115.0, 69.0, 68. 6, 33.9.

3,4-Dihydroxyphenethyl ethyl carbonate (**8**). Yield: 34%; yellow oil; R_f (cyclohexane/ethyl acetate 6:4): 0.33; ^{1}H NMR (300 MHz, Methanol-d_4) δ 6.81–6.66 (m, 2H), 6.55–6.52 (m, 1H), 4.22 (t, J = 7.2 Hz, 2H), 4.12 (q, J = 5.2 Hz, 2H), 2.78 (t, J = 7.2 Hz, 2H), 1.25 (t, J = 5.2 Hz, 3H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 155.3, 144.7, 143.5, 128.9, 119.8, 115.6, 115.0, 68.3, 63.5, 34.1, 13.2.

3,4-Dihydroxyphenethyl allyl carbonate (**9**). Yield: 40%; yellow oil; R_f (cyclohexane/ethyl acetate 6:4): 0.49; ^{1}H NMR (300 MHz, Methanol-d_4) δ 6.70–6.66 (m, 2H), 6.55–6.51 (m, 1H), 5.90 (ddd, J = 16.7, 10.5, 5.0 Hz, 1H), 5.33 (dd, J = 16.7, 1.3 Hz, 1H), 5.19 (dd, J = 10.5, 1.3 Hz, 1H), 4.56 (dd, J = 5.0, 1.3 Hz, 2H), 4.23 (t, J = 7.1 Hz, 2H), 2.78 (t, J = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 155.7, 144.8, 143.6, 131.9, 128.9, 119.9, 117.3, 115.7, 115.0, 68.5, 67.9, 34.1.

3,4-Dihydroxyphenethyl phenyl carbonate (**10**). Yield: 53%; colourless oil; R_f (cyclohexane/ethyl acetate 6:4): 0.49; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.40–7.09 (m, 5H), 6.74–6.69 (m, 2H), 6.58–6.55 (m, 1H), 4.34 (t, J = 7.0 Hz, 2H), 2.85 (t, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 153.8, 151.3, 144.9, 143.7, 129.2, 128.7, 125.6, 120.8, 119.9, 115.7, 115.0, 69.2, 34.0.

3,4-Dihydroxyphenethyl benzyl carbonate (**11**). Yield: 10%; brown solid; R_f (toluene/ethyl acetate 7:3): 0.47; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.36–7.34 (m, 5H), 6.69–6.67 (m, 2H), 6.54–6.51 (m, 1H), 5.14 (s, 2H), 4.25 (t, J = 7.1 Hz, 2H), 2.79 (t, J = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 155.2, 144.9, 143.9, 135.8, 128.9, 128.1, 128.0, 127.2, 119.9, 115.6, 115.0, 69.0, 66.6, 34.1.

2.2. Continuous synthesis of Ty and HTy carbamate derivatives 13-16

Two stock solutions were prepared as follows: a) a solution of compound **4** or **10** (0.2 M, 0.8 mmol) in *tert*-amyl alcohol (4 mL); b) a solution of 2-phenylethylamine or tyramine (0.3 M, 1.2 mmol) in *tert*-amyl alcohol (4 mL). The stock solutions were pumped through a coil reactor (reactor volume: 2 mL) using syringe pumps with a total flow rate of 66.7 μL min^{-1} (R_t = 30 min) at 110 °C. The whole system was pressurized at 20 psi. *tert*-Amyl alcohol was used as flow stream. The resulting crudes were collected, and the solvent was evaporated under reduced pressure. The crudes were purified by flash chromatography (cyclohexane/ethyl acetate 9:1 to 7:3 for compounds **13** and **15**, petroleum ether/ethyl acetate 8:2 to 6:4 for **14**, dichloromethane/methanol 98:2 to 95:5 for **16**).

4-Hydroxyphenethyl phenethyl carbamate (**13**). Yield: 68%; yellow oil; R_f (cyclohexane/ethyl acetate 6:4): 0.55; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.27–7.13 (m, 5H), 7.03–7.00 (m, 2H), 6.73 - 6.70 (m, 2H), 4.13 (t, J = 7.0 Hz, 2H), 3.28 (t, J = 6.0 Hz, 2H), 2.76 (t, J = 6.0 Hz, 2H), 2.74 (t, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 156.0, 155.6, 139.1, 129.5, 128.8, 128.4, 128.1, 125.9, 114.8, 65.4, 42.0, 35.7, 34.3.

4-Hydroxyphenethyl 4-hydroxyphenethyl carbamate (**14**). Yield: 74%; colourless oil; R_f (petroleum ether/ethyl acetate 7:3): 0.12; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.14–6.89 (m, 4H), 6.75–6.63 (m, 4H), 4.13 (t, J = 7.0 Hz, 2H), 3.23 (t, J = 7.6 Hz, 2H), 2.78 (t, J = 7.0 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 157.6, 155.5, 155.4, 129.9, 129.6, 129.4, 128.8, 114.8, 65.4, 42.2, 34.9, 34.3.

3,4-Diydroxyphenethyl phenethyl carbamate (**15**). Yield: 38%; yellow oil; R_f (cyclohexane/ethyl acetate 6:4): 0.3; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.28–7.16 (m, 5H), 6.69–6.65 (m, 2H), 6.54 - 6.51 (m, 2H), 4.13 (t, J = 7.1 Hz, 2H), 3.30 (t, J = 6.0 Hz, 2H), 2.74 (t, J = 7.1 Hz, 2H), 2.71 (t, J = 6.0 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 157.1, 144.8, 143.4, 139.1, 129.5, 128.4, 128.0, 125.9, 119.8, 115.6, 115.0, 65.4, 42.0, 35.7, 34.5.

3,4-Dihydroxyphenethyl 4-hydroxyphenethyl carbamate (**16**). Yield: 42%; brown viscous oil; R_f (dichloromethane/methanol 95:5): 0.24; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.01–6.99 (m, 2H), 6.70–6.52 (m, 5H), 4.13 (t, J = 7.4 Hz, 2H), 3.23 (t, J = 7.1 Hz, 2H), 2.73 (t, J = 7.4 Hz, 2H), 2.65 (t, J = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, Methanol-d_4) δ 157.7, 155.4, 144.8, 143.4, 129.9, 129.5, 129.4, 119.9, 115.6, 114.9, 65.4, 42.3, 34.9, 34.5.

2.3. Telescoped continuous synthesis of compound 13

A stock solution were prepared as follows: a) a solution of Ty (0.1 M, 0.4 mmol) and diphenyl carbonate (0.2 M, 0.8 mmol) in *tert*-amyl alcohol (4 mL); b) a solution of 2-phenylethylamine (0.1 M, 0.4 mmol) in *tert*-amyl alcohol (4 mL). The stock solution a) was pumped through a column reactor packed with 450 mg imm-CaLB using a syringe pump with a flow rate of 33.3 μL min^{-1} (R_t = 60 min) at 80 °C. After the residence time, the exiting flow was mixed in a T junction with the stock solution b) that was pumped by a second syringe pump (flow rate: 33.3 μL min^{-1}). The outlet flow (total flow rate of 66.6 μL min^{-1}) was directed into a 2 mL coil reactor (R_t = 30 min) at 110 °C. The whole system was pressurized at 20 psi. *tert*-Amyl alcohol was used as flow stream.

The resulting crude was collected, and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography (cyclohexane/ethyl acetate 9:1 to 7:3). The overall yield was 45%.

2.4. DPPH radical-scavenging assay

Measurement of 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical-scavenging activity was performed using a commercial kit (Bioquochem, Asturie, Spain) following manufacturer's instructions. Briefly, samples were appropriately dissolved in dimethyl sulfoxide (DMSO) and mixed with the DPPH solution provided by the kit. Trolox at different concentrations (from 0 to 500 μM) was used to build the standard curve by plotting % inhibition (y-axis) vs. μM Trolox (x-axis). The antioxidant activity was determined by measuring absorbance at 517 nm by spectrophotometer (Eppendorf, Milan, Italy) after incubation in dark for 5 min and calculating the corresponding percentage of inhibition by using the standard curve. The assays were performed in independent duplicates. The results are expressed as means and errors are within 10 %.

2.5. Bacterial strains and culture conditions

Escherichia coli ATCC 25922 (*Ec*), *Salmonella enterica* subsp. *enterica* ser. Enteritidis ISM 8324 (*Se*), *Pseudomonas aeruginosa* IMV 1 (*Pa*) and *Staphylococcus aureus* ATCC 6538 (*Sa*) were used for the evaluation of antibacterial activity of the compounds. Stocks of the previously identified bacteria were thawed and then they were streaked onto blood agar plates (Tryptic Soy Agar + 5% sheep blood [Microbiol, Italy]) and incubated at 37 °C for 24 h under aerobic condition to obtain single colonies for antimicrobial tests.

2.6. Determination of the minimum inhibitory concentration

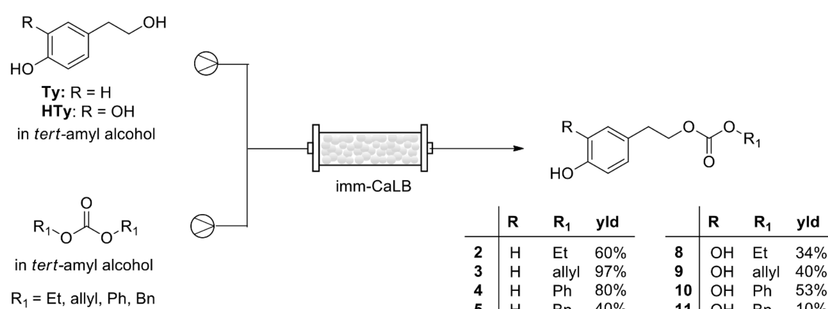
The Minimum Inhibitory Concentration (MIC) was determined using the microdilution assay, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute (CLSI, 2018) Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S15. Clinical and Laboratory Standards Institute, Wayne). Briefly, after their isolation on blood agar, all the previous cited strains were grown on Tryptic Soy Broth (TSB, Oxoid, Milan, Italy) and 3 or 4 isolated colonies were suspended in fresh sterile saline solution (9 gr/L NaCl) to reach an initial concentration of 1.5 x 10^8 CFU/mL (equivalent to 0.5 MacFarland standard); then a 1:100 diluted cell suspension in sterile saline solution was obtained (1.5 x 10^6 CFU/mL). One hundred microliters of the diluted suspension for each strain were dispensed into each well of a 96-well microtiter plate containing 100 μL of Mueller Hinton Broth (Oxoid, Milan, Italy). The strains were exposed to 2-fold dilution series of each derivative (dissolved in DMSO). After incubation for 24 h at 37 °C aerobically, the MICs were determined as the lowest dilution of molecules able to inhibit visible bacterial growth. Assays were performed in triplicate.

2.7. Calculation of selected properties of tested compounds

Selected properties of the compounds (i.e., molecular weights, cLogP, cLogS) were calculated with OSIRIS DataWarrior. Calculated properties are summarized in Table S1.

3. Results and discussion

First, the chemoselective reaction of the primary alcohol of Ty and HTy with symmetric carbonates was studied under flow conditions exploiting commercially available immobilized lipase B from *Candida antarctica* (imm-CaLB) (Scheme 1); the substrates (i.e., diethyl carbonate, diphenyl carbonate, dibenzyl carbonate and diallyl carbonate) were selected among the commercially available symmetric carbonates to evaluate different reactivities depending by the leaving group and to obtain products with increased lipophilicity without excessively increasing the molecular weight. The biotransformation was performed in *tert*-amyl alcohol, which was selected for its safety profile, low freezing point compared to *t*-BuOH, and its ability to solubilize polar compounds. Moreover, imm-CaLB has already shown good stability in this unconventional medium (Wang et al., 2016; Zhao 2020). We carried out the reaction using Ty and diethyl carbonate to investigate the effect of (i) the molar ratio of substrates (1:3, 1:6), and (ii) the residence time (range: 30–120 min) on the conversion, which was evaluated by HPLC. The concentration of Ty was 0.1 M and the temperature was set at 80 °C, as previously optimized (Vicinanza et al., 2023). After a first set of experiments, we pinpointed the following conditions: a 1:3 ratio between Ty (0.2 M in *tert*-amyl alcohol) and diethyl carbonate (0.6 M in *tert*-amyl alcohol), and a residence time of 60 min (see



	R	R1	yld
2	H	Et	60%
3	H	allyl	97%
4	H	Ph	80%
5	H	Bn	40%
8	OH	Et	34%
9	OH	allyl	40%
10	OH	Ph	53%
11	OH	Bn	10%

Scheme 1. Flow reactor configuration for the synthesis of Ty and HTy carbonate derivatives.

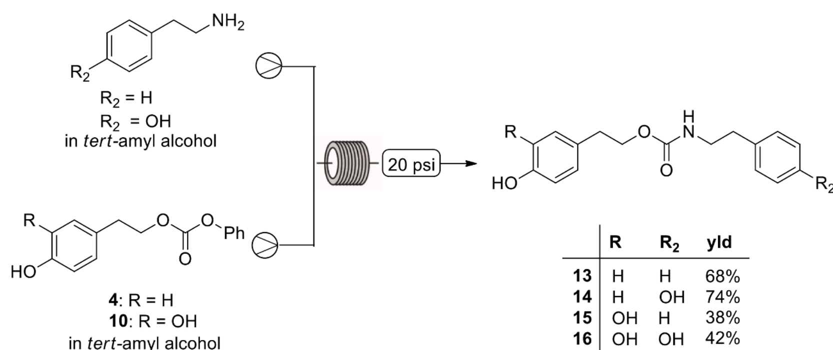
Table S2). Other green non-nucleophilic solvents (i.e., CPME, MeTHF, acetone, *tert*-butyl methyl ether and toluene) were screened. Toluene did not dissolve Ty, so it was abandoned, whereas the other solvents were exploited in the model biotransformation, but no improvement in the conversion was achieved. Therefore, the synthesis of compounds **2**, **3**, **5**, **8**–**11** was performed in *tert*-amyl alcohol at 80 °C in 60 min of residence time (Scheme 1). In the case of diphenyl carbonate, it was necessary to decrease the concentration due to solubility issues in *tert*-amyl alcohol. Indeed, one stock solution containing Ty or HTy (0.1 M) and diphenyl carbonate (0.2 M) was prepared and pumped through the bioreactor. Di-*tert* butyl carbonate was also used but no reaction was observed. Tyrosol carbonates were isolated in moderate to good yields (40–97%), whereas the yields of HTy derivatives were generally lower (10–53%). To improve the yield of HTy derivatives, an increase of the residence time up to 180 min was tested, but it led to the formation of a complex mixture without increasing the yield, probably due to a partial decomposition.

For the synthesis of the desired carbamates **13–16**, methyl carbonate (**1**), ethyl carbonate (**2**), allyl carbonate (**3**) and phenyl carbonate (**4**) were tested using 2-phenylethylamine (PEA) as model nucleophile in a microwave reactor for preliminary small scale reactivity screening (T = 100 °C, 30 min). Methyl carbonate (**1**), ethyl carbonate (**2**) and allyl carbonate (**3**) did not react, therefore, phenyl carbonate derivatives **4** and **10** were selected as starting materials and different experiments were performed in flow changing residence time (R_t, range: 15–45 min) and stoichiometry (1:1, 1:5, 1:2, Table S3). Two stock solutions, containing respectively compound **4** as starting material and PEA in *tert*-amyl alcohol, were mixed in a T-piece and flowed at different flow rates into a coil reactor (V = 2 mL), keeping the temperature constant at 110 °C and the whole system pressurized at 20 psi (Scheme 2). The molar conversions (*c*) were evaluated by HPLC.

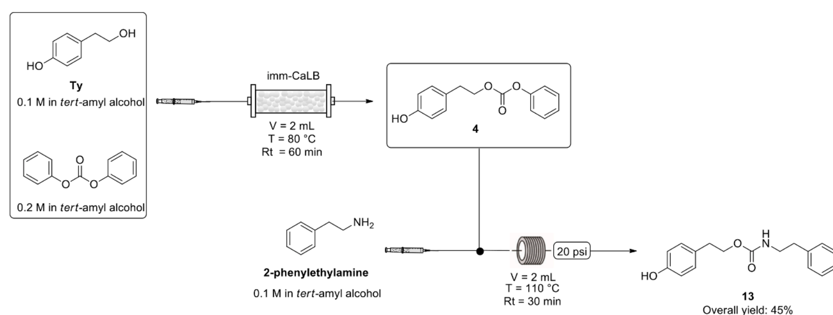
An unsatisfactory conversion (c = 40%) was achieved using a stoichiometric ratio between compound **4** and PEA (Table S3, entry 1) in a 30-min residence time. Therefore, a 1:1.5 ratio (**4**/PEA) was tested (Table S3, entries 2–4), selecting 30 min as the best reaction residence time. A 1:2 ratio was also evaluated (Table S3, entry 5) with no significant increase in the molar conversion. The selected conditions (1:1.5 ratio, R_t: 30 min, T = 110 °C) were then successfully applied to the synthesis of compounds **13**–**16**, using **4** and **10** as starting materials and 2-phenylethylamine and tyramine as nucleophiles in moderate to good isolated yields (38–74%).

In addition, to reduce the manual handling and increase the sustainability of the protocol, we developed a telescoped chemoenzymatic process exploiting the optimized conditions (Scheme 3). This process was successfully applied to the synthesis of carbamate **13**, that was isolated in 45% overall yield after solvent evaporation and column chromatography. The two-step protocol allowed to avoid the isolation and purification of intermediate **4**, reducing time and cost of the procedure. The system was run for 8 h and the outcome was evaluated by collecting a sample every hour and analysing it by HPLC. A constant conversion was maintained over the time.

Calculated cLogP and cLogS values (Table S1) indicate that the obtained carbonates and carbamates should dissolve better in the lipid phase than Ty and HTy, making possible their application in lipid-rich matrices.



Scheme 2. Flow reactor configuration for the synthesis of carbamates **13–16**.



Scheme 3. Telescoped chemo-enzymatic flow process for the synthesis of carbamate **13**.

Since phenolic compounds can act as free radical scavengers due to their ability to donate a hydrogen radical forming aryloxy radicals, we evaluated the efficiency of compounds **1–16** as radical scavengers performing a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Table 1) (Kedare and Singh, 2011).

The lipophilic carbamates and carbonates possess similar radical scavenger properties in comparison with the parent natural compounds Ty and HTy (Table 1), demonstrating that their conversion into carbonate and carbamate derivatives does not have a negative impact on this important biological property. As expected, the presence of the catechol moiety in HTy and its derivatives leads to more efficient radical scavengers in comparison with Ty and its derivatives. Indeed, the HTy series showed similar radical scavenger properties at concentrations 10-fold lower than the corresponding Ty series.

Finally, compounds **1–16** were investigated in a panel of Gram-negative and Gram-positive bacteria to assess their antibacterial properties (Table 2).

Most of the new derivatives showed higher antimicrobial activity in comparison with Ty and HTy. Carbonate **2** showed a MIC (mM) more than 6-fold lower against *S. aureus* compared with Ty, whereas carbamate **13** possesses a MIC of 0.11 mM against *S. aureus* and *S. enterica* subsp. *enterica* ser. Enteritidis, about 17-fold lower than the parent compound Ty. Symmetric carbonate **12** and carbamate **16** resulted the most active of the series against *S. aureus* with a MIC of 0.05 mM, about 8-fold lower than HTy. Interestingly, compound **16** showed a good selectivity against the Gram positive *S. aureus* over the tested Gram negative bacteria. Although the mechanism underlying the antimicrobial action of phenolic compounds is not completely understood, it has been hypothesized that they could inhibit key enzymes involved in bacterial growth and multiplication, or affect membrane permeability (Rempe et al.,

Table 1
Investigation of free radical scavenging capacity of our novel series of compounds.[c]

Compound	Concentration (mM)	% Inhibition
Ty	0.1	45
1	0.1	46
2	0.1	55
3	0.1	45
4	0.1	43
5	0.1	35
6	0.1	54
13	0.1	46
14	0.1	55
HTy	0.01	54
7	0.01	53
8	0.01	57
9	0.01	49
10	0.01	55
11	0.01	54
12	0.01	67
15	0.01	44
16	0.01	61

[a]DPPH assays were conducted in duplicate. The results are expressed as means and errors are within 10 %.

Table 2
Minimum inhibitory concentrations (MIC) of tested compounds.

Compound	*Sa* MIC μg/mL (mM)	*Ec* MIC μg/mL (mM)	*Se* MIC μg/mL (mM)	*Pa* MIC μg/mL (mM)
Ty	256 (1.86)	256 (1.86)	256 (1.86)	128 (0.93)
1	128 (0.65)	128 (0.65)	128 (0.65)	128 (0.65)
2	32 (0.15)	128 (0.60)	64 (0.30)	128 (0.60)
3	64 (0.29)	128 (0.58)	128 (0.58)	128 (0.58)
4	64 (0.24)	64 (0.24)	32 (0.12)	128 (0.48)
5	128 (0.48)	128 (0.48)	64 (0.24)	128 (0.48)
6	128 (0.42)	128 (0.42)	128 (0.42)	128 (0.42)
13	32 (0.11)	128 (0.44)	32 (0.11)	128 (0.44)
14	128 (0.42)	128 (0.42)	128 (0.42)	128 (0.42)
HTy	64 (0.42)	128 (0.84)	128 (0.84)	128 (0.84)
7	64 (0.30)	128 (0.60)	128 (0.60)	128 (0.60)
8	32 (0.14)	64 (0.28)	32 (0.14)	128 (0.56)
9	32 (0.13)	128 (0.52)	32 (0.13)	64 (0.26)
10	32 (0.12)	64 (0.24)	32 (0.12)	64 (0.24)
11	32 (0.12)	64 (0.24)	16 (0.06)	128 (0.48)
12	16 (0.05)	128 (0.40)	32 (0.10)	128 (0.40)
15	> 256 (> 0.84)	> 256 (> 0.84)	128 (0.42)	128 (0.42)
16	16 (0.05)	> 256 (> 0.80)	> 256 (> 0.80)	> 256 (> 0.80)

[a]*Sa* = *S. aureus*, *Ec* = *E. coli*, *Se* = *S. enterica* subsp. *enterica* ser. Enteritidis, *Pa* = *P. aeruginosa*. All the tests were performed in triplicate.

2017), therefore, more lipophilic compounds (Table S1) may display increased activity due to a better cell penetration or membrane interaction.

4. Conclusions

Lipophilization of phenolic derivatives represents an efficient strategy to obtain amphiphilic compounds that can be used as multi-functional additives (e.g., antiradical, antimicrobial, antiviral, bacteriostatic) for pharmaceutical, nutraceutical and cosmetic applications. In this work, we developed an innovative chemo-enzymatic two-step flow protocol for the synthesis of a series of phenolic carbonate and carbamate derivatives selectively reacting the primary alcohol of a phenolic natural compound and preserving the phenolic group(s) responsible for their antioxidant activity. The chemoselective synthesis of Ty and HTy carbonates was performed using immobilized CaLB as biocatalyst in *tert*-amyl alcohol as solvent, and the biocatalyst showed excellent stability in this unconventional medium. The protocol was developed using Ty and diethyl carbonate as model substrates, and then applied to the synthesis of the desired Ty carbonates **2–5** and HTy carbonates **8–11**, that were isolated in moderate to good yields. Afterwards, the obtained phenyl carbonates **4** and **10** were reacted under heating in a reactor coil with tyramine and phenylethylamine to obtain the desired carbamates **13–16**. Moreover, a telescoped two-step process was easily set-up, allowing a reduction of manual handling, time and costs. All lipophilized compounds were tested as antimicrobial agents and radical scavengers, showing comparable or even increased activities in respect with parent compounds with increased applicability as preservative agents in lipid-rich media for pharma, nutraceuticals and cosmetic formulation.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scp.2024.101542.

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