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# Synthesis and Evaluation as Biodegradable Herbicides of Halogenated Analogues of L-meta- Tyrosine

Julie Movellan<sup>a</sup>, Françoise Rocher<sup>a</sup>, Zohra Chikh<sup>a</sup>, Cécile Marivingt-Mounir<sup>a</sup>, Jean-Louis Bonnemain<sup>b</sup> and Jean-François Chollet<sup>a,\*</sup>

<sup>a</sup> *Institut de Chimie des Milieux et des Matériaux de Poitiers, Unité Mixte de Recherche CNRS 7285, Université de Poitiers, 40 avenue du Recteur Pineau, F-86022 Poitiers cedex, France*

<sup>b</sup> *Laboratoire Écologie et Biologie des Interactions, Unité Mixte de Recherche CNRS 7267, Université de Poitiers, 40 avenue du Recteur Pineau, F-86022 Poitiers cedex, France*

\* Corresponding author: Tel. fax: +33 5 49 45 39 65

E-mail address: jean.franc.chollet@univ-poitiers.fr

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**Abstract** *L-meta*-tyrosine is a herbicidal non protein amino acid isolated some years ago from fine fescue grasses and characterized by its almost immediate microbial degradation in soil (half life less than 24 hours). Nine mono- or dihalogenated analogs of this allelochemical have been obtained through a seven-step stereoselective synthesis from commercial halogenated phenols. Bioassays showed a large range of biological responses, from a growth root inhibition of lettuce seedling similar to that noted with *m*-tyrosine [2-amino-3-(2-chloro-5-hydroxyphenyl)propanoic acid or compound **8b**] to an increase of the primary root growth concomitant with a delay of secondary root initiation [2-amino-3-[2-fluoro-5-hydroxy-3-(trifluoromethyl)phenyl]propanoic acid or compound **8h**]. Compound **8b** was slightly less degraded than *m*-tyrosine in the non-sterilized nutritive solution used for lettuce development while the concentration of compound **8h** remained unchanged for at least two weeks. These data indicate that it is possible to manipulate both biological properties and degradation of *m*-tyrosine by halogen addition.

**Keywords** *Allelochemicals; Allelopathy; m-Tyrosine; Halogenated Analogues of L-meta-tyrosine; Herbicide; Sustainable agriculture*

## Introduction

Protection of crops against pests probably arose at the same time as agriculture itself. Modern crop protection developed quickly after the Second World War with the advent of organic chemistry and numerous active ingredients became available in various domains: organophosphate and organochlorine insecticides, dithiocarbamate fungicides or auxinic herbicides also called phytohormones. These compounds were widely used to meet the high demand for food after the war without adequate assessment of the risks that could result from the use of some molecules (Zadoks 1991). However, after many years, adverse effects on health or environment were often highlighted. Herbicides of the triazine family are leached and found in groundwater (Felding 1992b, a, Hall et al. 1991) while organochlorine insecticides may accumulate in the environment or in lipophilic parts of some organisms (Caro 1969, Mueller et al. 2008, Ntow et al. 2008, Smith & Gangolli 2002). Thus, intensive agriculture, as practiced for several decades in developed countries, is changing and in particular, the part concerning the protection of cultivated plants (Rabbinge & van Oijen 1997). Many phytopharmaceutical compounds were withdrawn from the market after adverse effects were demonstrated (Karabelas et al. 2009). The arsenal of agrochemical products is restricted and no major mode of herbicide action has been introduced to the market place for about two decades (Duke 2012). Therefore new strategies must emerge to meet the specific requirements for crop protection in a context of sustainable agriculture (Clark 2012, Dayan et al. 2012, Duke 2012, Epstein & Bassein 2003, Gosme et al. 2010, Jacobsen 1997, Tesio & Ferrero 2010, van Lenteren 2000).

Thus, in order to identify new molecules able to control weed growth, allelopathy may be a promising way and in particular, to develop new synthetic herbicide families (Bais et al. 2006, Birkett et al. 2001, Duke & Abbas 1995, Duke et al. 2005, Duke & Lydon 1993, Farooq et al. 2011, John et al. 2010, Macias 1995, Niemeyer 2009, Petroski & Stanley 2009, Vyvyan 2002). The definition of allelopathy has evolved over the years (Duke 2010, Molisch 1937, Rice 1984, Whittaker & Feeny 1971) and currently, allelopathy is defined by the International Allelopathy Society as any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agriculture and biological systems (Chou 2006). More restrictively, allelochemicals are substances that are produced by one plant. Phenolics compounds are an important and common family of plant allelochemicals in the ecosystem (Li et al. 2010, Wu et al. 2001). Recently, Bertin et al have isolated *L-meta*-tyrosine, a non protein amino acid exuded by the roots of fine fescue grasses,

especially *Festuca rubra* spp commutata (Bertin & Weston 2002, Bertin et al. 2007, Weston et al. 2006). This compound which is synthesized by hydroxylation of phenylalanine in *F. rubra* (Huang et al. 2012) exhibits herbicidal properties for a wide range of plant species (Bertin et al. 2007). More specifically *m*-tyrosine is a root growth inhibitor, contrary to its position isomers, tyrosine and ortho-tyrosine (Bertin et al. 2007). The mechanisms of *m*-tyrosine herbicidal activity are not known (Bertin et al. 2007). Furthermore, the concentrations required for significant root growth inhibition in the same plant species may vary considerably when filter paper bioassays are used (Bertin et al. 2007, Kaur et al. 2009) and may be higher than what would normally be found in *Festuca rubra* spp commutata rhizosphere (Kaur et al. 2009). This latter data and the rapid microbially loss of *m*-tyrosine in soil have raised a recent debate about the actual role of *m*-tyrosine in allelopathy (Bertin et al. 2009, Duke 2010, Kaur et al. 2009).

Here we report the synthesis in a stereo specific manner of nine mono- or polyhalogenated analogues of *L*-*meta*-tyrosine (Table 1). Using two bioassays methods, the phytotoxic activity of these compounds was then compared to that of *m*-tyrosine. Our data 1-support that *m*-tyrosine is an efficient allelopathic compound, 2-demonstrate for the first time that manipulation of both biological properties and degradation of *m*-tyrosine by halogen addition is possible and 3-point out two derivatives that may be used as tools to elucidate the mechanisms of the biological activity of *m*-tyrosine.

## Material and methods

### Synthesis

<sup>1</sup>H and <sup>13</sup>C FT-NMR spectra were measured with a Varian Model XL 400-MHZ spectrometer. Chemical shifts are reported as  $\delta$  in units of parts per million (ppm) relative to chloroform (<sup>1</sup>H 7.26 ppm, <sup>13</sup>C 77ppm) or water (<sup>1</sup>H 4.79 ppm). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), qd (quadruplet), dd (doublet of doublets), m (multiplet). Coupling constants are reported as a J value in Hertz (Hz). The number of protons (n) for a given resonance is indicated as nH, and is based on spectral integration values. Melting points were determined on an Electrothermal IA9200 apparatus and are uncorrected. Mass spectra were recorded using a Waters 3100 spectrometer in electro spray mode.

### *In vitro* bioassays

In a first approach, biological effect of each halogenated compound was assessed in comparison to *m*-tyrosine using filter paper bioassays with lettuce (Bertin et al. 2007). Ten

non-sterilized seeds (*Lactuca sativa* Var. Blonde de Paris) were placed in 9 cm Petri dishes on a filter paper impregnated with 1.5 mL of aqueous solutions of the tested compound at 0, 40, 80, 160, 320 or 640  $\mu\text{M}$  concentrations. To avoid evaporation, Petri dishes were sealed with Parafilm<sup>®</sup>. Two replicates of each assay were maintained under dim daylight conditions in a controlled environment ( $21 \pm 0.5^\circ\text{C}$ , HR  $90\% \pm 5\%$ ). Radicle length was measured 4 days later.

The biological activities of *m*-tyrosine, **8b** and **8h** were also studied under non-sterilized hydroponic conditions. Shortened Pasteur pipettes were immersed in 15 mL centrifuge tubes filled with a nutrient solution composed of  $2 \text{ mmol.l}^{-1}$  of  $\text{KNO}_3$ ,  $1 \text{ mmol.l}^{-1}$  of  $\text{KH}_2\text{PO}_4$ ,  $1 \text{ mmol.l}^{-1}$  of  $\text{Ca}(\text{NO}_3)_2$ ,  $1 \text{ mmol.l}^{-1}$  of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $0.3 \mu\text{mol.l}^{-1}$  (molybdenum equivalent) of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ ,  $1 \mu\text{mol.l}^{-1}$  of  $\text{H}_3\text{BO}_3$ ,  $0.09 \mu\text{mol.l}^{-1}$  of  $\text{MnSO}_4$ ,  $\text{H}_2\text{O}$ ,  $0.12 \mu\text{mol.l}^{-1}$  of  $\text{NH}_4\text{NO}_3$ ,  $0.035 \mu\text{mol.l}^{-1}$  of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $18 \mu\text{mol.l}^{-1}$  of iron-EDDHA chelate 6% Fe. In these experiments, germination of lettuce seeds (*Lactuca sativa* Var. Blonde de Paris) occurs on an agar layer (4 mm high) at the top of each Pasteur pipette. Under these conditions, the radicle had to cross this layer to reach the nutritive solution. Unless stated otherwise, plants were grown at  $24 \pm 0.5^\circ\text{C}$  and  $60 \pm 5\%$  RH during the photoperiod (14 h,  $250 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ ).

## Results and discussion

### Synthesis of halogenated analogues of *m*-tyrosine

Several methods of synthesis for halogenated *meta*-tyrosine or tyrosine were previously proposed (Bovonsombat et al. 2008, Drain &Howes 1967, Kirk et al. 1986, Konkel et al. 2002). Fluorinated analogues and particularly 6-fluoro-*meta*-tyrosine (Table 1, **8d**) was synthesized to use it as a tracer for medical imaging (Konkel et al. 2002, VanBrocklin et al. 2004, Vasdev et al. 2001). Halogenated *m*-tyrosine derivatives were found to lower the blood pressure of mammals (Drain &Howes 1967). Finally, we chose the synthesis in seven steps proposed by Konkel et al (Kirk et al. 1986, Konkel et al. 2002) and among the nine products that were synthesized (Table 1), six were new (compounds **8a**, **8b**, **8e**, **8f**, **8h** and **8i**). The first six steps were performed with good to very good yields, whose median is between 65% (steps 2 and 4) and 95% (step 6). Only the last step (deprotection of the alpha-amino acid function) deserves to be optimized for some products, the yields ranging from 5% (**8g**) to 64% (**8c**) (median 42%). An interesting aspect of the proposed method is also the possibility of using crude products obtained at the end of reaction for the next step without further purifications.

### *Protection of the hydroxyl group of the phenolic derivatives (Fig. 1, 2a-i)*

To a solution of 2-chlorophenol (**1a**, 6.43 g, 50 mmol) in dimethylformamide (25 mL), were added imidazole (3.75 g, 55 mmol) and ter-butyldimethylsilyl chloride (TBDMSCl, 8.29 g, 55 mmol). The mildly exothermic reaction was placed in an iced-bath. After the solution was stirred overnight under nitrogen atmosphere, water (125 mL) was added and the mixture was extracted with pentane (3 x 50 mL). The organic layer was washed successively with water (50 mL), 10% sodium carbonate aqueous solution (3 x 50 mL), water (3 x 50 mL) and dried over anhydrous magnesium sulphate. Removal of solvent under reduced pressure gave **2a** (9.04 g, 37 mmol) which was used without further purification. The products **2b-i** were obtained following the same procedure. Yields and <sup>1</sup>H NMR spectra for compounds **2a-i** are given in the online resource 1 in the ESM.

### *Preparation of aldehydes (Fig. 1, 3a-i)*

To a solution of *tert*-butyl(2-chlorophenoxy)dimethylsilane (**2a**, 8.9 g, 36.7 mmol) in dry THF (25 mL) stirred under argon atmosphere and cooled to -78°C, was added dropwise a solution of *sec*-butyllithium in hexane (1.4 M, 29 mL, 40 mmol). After stirring for 1 hour, an excess (3 mL, 40 mmol) of distilled DMF was added dropwise at -78 °C. After 30 min, tetrabutylammonium fluoride (TBAF, 1 M in THF, 44.5 mL, 44.5 mmol) was added dropwise at the same temperature. After 1.5 hour, the reaction was allowed to warm to room temperature, and water (50 mL) was added. After removal of most of the THF under reduced pressure, aqueous sodium hydroxide solution (1 N, 25 mL) was added and the basic solution was washed with diethyl ether (3 x 50 mL). The organic layer was washed 3 times with aqueous sodium hydroxide solution (1 N, 25 mL). The combined aqueous layers were acidified with hydrochloric acid solution (3 M), and the product was extracted with diethyl ether (4 x 50 mL). After drying over anhydrous magnesium sulphate, removal of solvent under reduced pressure gave **3a** (2.25 g, 14.4 mmol) that was used without further purification. The products **3b-i** were obtained following the same procedure. Yields and <sup>1</sup>H NMR spectra for compounds **3a-i** are given in the online resource 2 in the ESM.

### *Benylation of the hydroxyl group (Fig. 1, 4a-i)*

To a solution of 2-chloro-3-hydroxybenzaldehyde (**3a**, 2.25 g, 16 mmol) in DMF (20 mL), potassium carbonate was added (2.30 g, 23 mmol). After 5 minutes of stirring, benzyl bromide (2.15 mL, 18 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 20 hours. Diethyl ether (30 mL) was added and the mixture was washed with

water (3 x 20 mL). The organic layer was dried over anhydrous magnesium sulphate and the removal of solvent under reduced pressure gave **4a** (3.33 g, 13.5 mmol) that was used without further purification. The products **4b-i** were obtained following the same procedure. Yields and <sup>1</sup>H NMR spectra for compounds **4a-i** are given in the online resource 3 in the ESM.

#### *Preparation of azalactones (Fig. 1, 5a-i)*

To a solution of 3-(benzyloxy)-2-chlorobenzaldehyde (**4a**, 3.2 g, 13 mmol) in acetic anhydride (25 mL) were added sodium acetate (1.23 g, 15 mmol) and hippuric acid (2.69 g, 15 mmol). The reaction mixture was stirred at 80 °C for 2 hours and cooled at room temperature and with an iced-bath. Cold ethanol (30 mL) was added and after 15 min, iced water (15 mL) was added. The orange precipitate was collected by filtration on Büchner and dried under vacuum to give **5a** (3.32 g, 8.53 mmol) that was used without further purification. The products **5b-i** were obtained following the same procedure. Yields and <sup>1</sup>H NMR spectra for compounds **5a-i** are given in the online resource 4 in the ESM.

#### *Ring opening of the azalactones (Fig. 1, 6a-i)*

(*Z*)-4-(3-(benzyloxy)-2-chlorobenzylidene)-2-phenyloxazol-5(4*H*)-one (**5a**, 3.23 g, 8.3 mmol) and sodium acetate (730 mg, 8.9 mmol) in MeOH (400 mL) were stirred at room temperature. After 1 hour, the solvent was removed under reduced pressure. The residue was dissolved in ethylacetate (200 mL) and washed with water (3 x 50 mL). The organic phase was dried over anhydrous magnesium sulphate and the solvent was evaporated to give **6a** (3.30 g, 7.82 mmol) that was used without further purification. The products **6b-i** were obtained following the same procedure. Yields and <sup>1</sup>H NMR spectra for compounds **6a-i** are given in the online resource 5 in the ESM.

#### *Debenzylation and double bond saturation (Fig. 1, 7a-i)*

A solution of (*E*)-methyl-2-benzamido-3-(3-(benzyloxy)-2-chlorophenyl) acrylate (**6a**, 1.0 g, 2.37 mmol) in MeOH (100 mL) was hydrogenated over 10% Pd/C (205 mg) at 40 psi for 20 h. The palladium was then filtered on celite and the methanol was evaporated to give **7a** (0.79 g, 2.37 mmol) which was used without further purification. The products **7b-e** and **7g-i** were obtained following the same procedure. **7f** was obtained by hydrogenation at 60 °C under 80 psi during 20 h. Yields and <sup>1</sup>H NMR spectra for compounds **7a-i** are given in the online resource 6 in the ESM.



**Deprotection of the alpha-amino acid function to obtain L-m-tyrosine analogues (Table 1, Fig. 1, 8a-i)**

A solution of methyl 2-benzamido-3-(2-chloro-3-hydroxyphenyl) propanoate (**7a**, 2.89 g, 8.66 mmol) in hydrochloric acid (3 M, 125 mL) was refluxed for 24 h. The solution was concentrated to dryness and the residue was dissolved in water (60 mL) and washed with diethyl ether (3 x 20 mL). The aqueous phase was then filtrated and neutralized to pH 6 with an aqueous solution of sodium hydroxide 1M. The water was evaporated until the formation of a precipitate. The precipitate was filtrated and product was dried under reduced pressure to give **8a** (741 mg, 3.43 mmol). The products **8b-i** were obtained following the same procedure.

**8a**, 2-amino-3-(2-chloro-3-hydroxyphenyl)propanoic acid, yield: 40%; brown solid;  $^1\text{H}$  NMR  $\delta$  (ppm): 3.27-3.56 (ABX system,  $J_{AB} = 14$  Hz,  $J_{AX} = 9$  Hz,  $J_{BX} = 5.2$  Hz, 2H,  $\text{CH}_2$ ), 4.44 (m, 1H, CH), 6.97 (d,  $^3J = 7.5$  Hz, 1H, ArH), 7.04 (d,  $^3J = 8.1$  Hz, 1H, ArH), 7.33 (t,  $^3J = 7.7$  Hz, 1H, ArH);  $^{13}\text{C}$  NMR  $\delta$  (ppm): ( $D_2O/DCl$ ): 34.72 ( $\text{CH}_2$ ), 54.5 (CH), 116.1 ( $\text{Ar}_{\text{CH}}$ ), 122.9 (C-Cl), 127.9 (2  $\text{Ar}_{\text{CH}}$ ), 134.5 ( $\text{Ar}_{\text{Cq}}$ ), 173.7 (C-OH), 185.8 (COOH); Calculated  $[\text{M}]^+$  ( $\text{C}_9\text{H}_{10}\text{ClNO}_3$ )  $m/z = 215.03$ . Found: ESI+:  $[\text{M}+\text{H}]^+$   $m/z = 216.08$

**8b**, 2-amino-3-(2-chloro-5-hydroxyphenyl)propanoic acid; yield: 16%; grey solid;  $^1\text{H}$  NMR ( $D_2O/DCl$ )  $\delta$  (ppm): 3.11 (m, 1H,  $\text{CH}_2$ ), 3.33 (ddd,  $J = 19.7$  Hz,  $J = 14.7$  Hz,  $J = 5.6$  Hz, 1H,  $\text{CH}_2$ ), 4.04-4.06 (m, 1H, CH), 6.85-6.87 (m, 2H, ArH), 7.32 (dd,  $J = 17.6$  Hz,  $J = 8.3$  Hz, 1H, ArH);  $^{13}\text{C}$  NMR ( $D_2O$ )  $\delta$  (ppm): 33.6 ( $\text{CH}_2$ ), 53.7 (CH), 114.6 ( $\text{Ar}_{\text{CH}}$ ), 116.1 ( $\text{Ar}_{\text{CH}}$ ), 121.5 ( $\text{Ar}_{\text{CH}}$ ), 130.5 ( $\text{Ar}_{\text{Cq}}$ ), 150.2 (C-OH), 158.5 (C-Cl), 170.2 (COOH); Calculated  $[\text{M}]^+$  ( $\text{C}_9\text{H}_{10}\text{ClNO}_3$ )  $m/z = 215.03$ . Found: ESI+:  $[\text{M}+\text{H}]^+$   $m/z = 216.08$

**8c**, 2-amino-3-(2-fluoro-3-hydroxyphenyl)propanoic acid; yield: 64%; pink crystals;  $^1\text{H}$  NMR ( $D_2O$ )  $\delta$  (ppm): 3.15-3.36 (ABX syst.,  $J_{AB} = 14.4$  Hz,  $J_{AX} = 7.6$  Hz,  $J_{BX} = 4.8$  Hz, 2H,  $\text{CH}_2$ ), 4.11 (t, 1H,  $^3J = 5.6$  Hz, CH), 6.84 (t, 1H,  $^3J = 6.2$  Hz, 1H, ArH), 6.98 (dt, 1H,  $^4J = 1.7$  Hz,  $^3J = 8.3$  Hz, 1H, ArH), 7.05 (t, 1H,  $^3J = 7.8$  Hz, 1H, ArH);  $^{13}\text{C}$  NMR ( $D_2O$ )  $\delta$  (ppm): 33.1 ( $\text{CH}_2$ ), 57.9 (CH), 120.1 ( $\text{Ar}_{\text{CH}}$ ), 125.3 ( $\text{Ar}_{\text{CH}}$ ), 126.4 ( $\text{Ar}_{\text{Cq}}$ ), 127.7 ( $\text{Ar}_{\text{CH}}$ ), 146.2 (C-F), 146.3 (C-OH), 176.4 (COOH); Calculated  $[\text{M}]^+$  ( $\text{C}_9\text{H}_{10}\text{FNO}_3$ )  $m/z = 199.06$ . Found: ESI+:  $[\text{M}+\text{H}]^+$   $m/z = 200.14$

**8d**, 2-amino-3-(2-fluoro-5-hydroxyphenyl)propanoic acid; yield: 63%; beige solid;  $^1\text{H}$  NMR ( $D_2O$ )  $\delta$  (ppm): 3.22 (ABX syst.,  $J_{AB} = 14.6$  Hz,  $J_{AX} = 7.2$  Hz,  $J_{BX} = 5.4$  Hz, 2H,  $\text{CH}_2$ ), 4.42 (m, 1H, CH), 6.86 (m, 2H, ArH), 7.07 (m, 1H, ArH);  $^{13}\text{C}$  NMR ( $D_2O$ )  $\delta$  (ppm): 33.3 ( $\text{CH}_2$ ), 55 (CH), 119.3 ( $\text{Ar}_{\text{CH}}$ ), 120.7 ( $\text{Ar}_{\text{CH}}$ ), 124.3 ( $\text{Ar}_{\text{CH}}$ ), 124.4 ( $\text{Ar}_{\text{Cq}}$ ), 154.4 (C-OH), 157.1 (C-F),

173.5 (COOH); Calculated  $[M]^+$  ( $C_9H_{10}FNO_3$ )  $m/z = 199.06$ . Found: ESI+:  $[M+H]^+$   $m/z = 200.04$

**8e**, 2-amino-3-(2,4-dichloro-3-hydroxyphenyl)propanoic acid; yield: 45%; grey solid;  $^1H$  NMR ( $D_2O$ )  $\delta$  (ppm): 3.04-3.45 (ABX syst.,  $J_{AB} = 14$  Hz,  $J_{AX} = 7$  Hz,  $J_{BX} = 4.9$  Hz, 2H,  $CH_2$ ), 4.7-5.1 (m, 1H, CH), 6.8-6.92 (m, 1H, ArH), 7.10-7.30 (m, 1H, ArH); Calculated  $[M]^+$  ( $C_9H_9Cl_2NO_3$ )  $m/z = 249.00$ . Found: ESI+:  $[M+H]^+$   $m/z = 250.04$

**8f**, 2-amino-3-(2,6-dichloro-3-hydroxyphenyl)propanoic acid; yield: 10%; grey solid;  $^1H$  NMR ( $D_2O$ )  $\delta$  (ppm): 3.04-3.42 (ABX syst.,  $J_{AB} = 14$  Hz,  $J_{AX} = 6.9$  Hz,  $J_{BX} = 4.9$  Hz, 2H,  $CH_2$ ), 5.09-5.12 (m, 1H, CH), 6.82 (d,  $^3J = 8.8$  Hz, 1H, ArH), 6.93 (d,  $^3J = 8.8$  Hz, 1H, ArH),  $^{13}C$  NMR ( $D_2O$ )  $\delta$  (ppm): 36.2 ( $CH_2$ ), 55.9 (CH), 114.7 ( $Ar_{CH}$ ), 121.49 ( $Ar_{CH}$ ), 130.5 ( $Ar_{Cq}$ ), 154.4 (C-OH), 158.0 (C-Cl), 173.5 (COOH); Calculated  $[M]^+$  ( $C_9H_9Cl_2NO_3$ )  $m/z = 249.00$ . Found: ESI+:  $[M+H]^+$   $m/z = 250.04$

**8g**, 2-amino-3-(2,6-difluoro-3-hydroxyphenyl)propanoic acid; yield: 5%; grey solid;  $^1H$  NMR ( $D_2O$ )  $\delta$  (ppm): 3.21-3.33 (ABX syst.,  $J_{AB} = 14.5$  Hz,  $J_{AX} = 7.9$  Hz,  $J_{BX} = 5.9$  Hz, 2H,  $CH_2$ ), 3.93-3.95 (m, 1H, CH), 6.85-7.01 (m, 2H, ArH); Calculated  $[M]^+$  ( $C_9H_9F_2NO_3$ )  $m/z = 217.06$ . Found: ESI+:  $[M+H]^+$   $m/z = 218.25$

**8h**, 2-amino-3-[2-fluoro-5-hydroxy-3-(trifluoromethyl)phenyl]propanoic acid; yield: 90%; white solid;  $^1H$  NMR ( $D_2O$ )  $\delta$  (ppm): 3.19-3.42 (syst. ABX,  $J_{AB} = 14.5$  Hz,  $J_{AX} = 7.9$  Hz,  $J_{BX} = 5.9$  Hz, 2H,  $CH_2$ ), 4.06-4.09 (m, 1H, CH), 7.09-7.11 (m, 1H, ArH), 7.17-7.19 (m, 1H, ArH);  $^{13}C$  NMR ( $D_2O$ )  $\delta$  (ppm): 31.5 ( $CH_2$ ), 55.9 (CH), 119.4 ( $Ar_{CH}$ ), 122.5 ( $Ar_{CH}$ ), 133.6 ( $Ar_{Cq}$ ), 155.0 (C-OH), 149.1 (C-CF<sub>3</sub>), 157.2 (C-F), 173.5 (COOH); Calculated  $[M]^+$  ( $C_{10}H_9F_4NO_3$ )  $m/z = 267.05$ . Found: ESI+:  $[M+H]^+$   $m/z = 268.18$

**8i**, 2-amino-3-(6-chloro-2-fluoro-3-hydroxyphenyl)propanoic acid; yield: 32%; grey solid;  $^1H$  NMR ( $D_2O$ )  $\delta$  (ppm): 2.67-2.73 (dd,  $^3J = 9.5$  Hz,  $^4J = 9.45$  Hz, 1H, CH), 3.21-3.25 (dd,  $^4J = 4$  Hz,  $^3J = 12.1$  Hz, 2H,  $CH_2$ ), 6.68-6.72 (m, 1H, ArH), 6.81-7.01 (m, 2H, ArH, OH);  $^{13}C$  NMR ( $D_2O$ )  $\delta$  (ppm): 30.55 ( $CH_2$ ), 54.19 (CH), 116.27 ( $Ar_{CH}$ ), 120.66 ( $Ar_{CH}$ ), 123.70 (C-Cl), 125.18 (d,  $^2J_{C-F} = 12.6$  Hz,  $Ar_{Cq}$ ), 145.05 (d,  $^2J_{C-F} = 12.3$  Hz, C-OH), 149.25 (d,  $^1J_{C-F} = 240.7$  Hz, C-F), 170.08 (COOH); Calculated  $[M]^+$  ( $C_9H_9ClFNO_3$ )  $m/z = 233.03$  Found: ESI+:  $[M+H]^+$   $m/z = 234.25$

## Biological activity of compounds 8a-i

### *Filter paper bioassays*

After 4 days of treatment with *m*-tyrosine used at 640  $\mu\text{M}$  concentration, the lettuce root length was reduced by about 75% (Table 2). Adding a chlorine atom in the para position with respect to the hydroxyl group (compound **8b**) did not affect significantly this inhibition (Table 2). By contrast, addition of a fluorine atom on the same carbon (compounds **8d** and **8g**) led to a lack of biological activity of these xenobiotics and further addition of a trifluoromethyl group on the free adjacent position (compound **8h**) induced a clear increase of root growth under our experimental conditions (Table 2, Fig. 2). This increase was significant at concentrations  $\geq 160 \mu\text{M}$  while the root growth inhibition induced by *m*-tyrosine and compound **8b** were clearly significant at concentrations  $\geq 40$  and  $\geq 80 \mu\text{M}$  respectively (Fig. 2). According to previous filter bioassays, the concentration of *m*-tyrosine required to achieve 50% reduction of lettuce root growth vary from 10 – 20  $\mu\text{M}$  (Bertin et al. 2009, Bertin et al. 2007) to about 150  $\mu\text{M}$  (Kaur et al. 2009). This variability may be due to the fact that *m*-tyrosine is not equally phytotoxic towards the cultivars used or / and to some microbial degradation of this molecule around the seeds when they are not sterilized.

### *Hydroponic bioassays*

It is well known that *m*-tyrosine microbial degradation is high in soil, the half-life being estimated as less than 24 hours (Kaur et al. 2009). By contrast, in a medium unsuitable for bacterial growth such as ultrapure water, the degradation of *m*-tyrosine as well as compound **8b** was extremely low (21 and 17% respectively after 1.5 months in solution). In the nutritive medium used for lettuce growth, *m*-tyrosine and compound **8b** concentrations remained unchanged during four days then they dropped sharply, especially one week after the beginning of the experiment (Fig. 3). *m*-tyrosine and compound **8b** could not be detected at day 11 and day 14 respectively. If chloramphenicol – a broad spectrum antibiotic – was added at 0.25  $\text{g.L}^{-1}$  to the medium, the degradation of both compounds was considerably reduced. At day 11, *m*-tyrosine was detected at 129  $\mu\text{M}$  concentration (81% of the initial concentration) and **8b** was detected at 144  $\mu\text{M}$  concentration (90% of the initial concentration). These data indicate that bacterial degradation is slightly less efficient for compound **8b** than for *m*-tyrosine under our experimental conditions. Compound **8h** concentration remained unchanged during the whole experiment (Fig. 3). This does not mean that the latter is not degraded in soil taking into account the diversity and the high content of microorganisms in this compartment.

Under our experimental conditions, the lettuce root growth was dramatically and similarly inhibited by both *m*-tyrosine and compound **8b** at 40  $\mu\text{M}$  (initial concentration) and was reduced by about 65% in presence of these two compounds at 10  $\mu\text{M}$  (initial concentration) (Fig. 4). Our data support those of Bertin et al (2007) (Bertin et al. 2007), which clearly suggest that *m*-tyrosine is a potent inhibitor of plant development. In addition they show that compound **8b**, a less soluble (Table 1) and less degradable molecule, can exhibit the same deleterious properties on root development at these two low concentrations. The root growth was not reduced by both *m*-tyrosine and compound **8b** at the lowest concentration used (2.5  $\mu\text{M}$ ) (Fig. 4). However, it is likely that this initial concentration dropped quickly due to root uptake in addition to microbial degradation, and this may concern all the experiments conducted with higher concentrations. By contrast, in soil, the *m*-tyrosine flux occurs in a dynamic system with exudation from fescue roots on the one hand, microbial degradation, adsorption on soil constituents and receiving plant uptake on the other hand (Duke 2010). The mechanism of *m*-tyrosine uptake by plant tissues is not known but it is possible that a pH-dependent carrier system is involved in addition to diffusion taking into account the low specificity of several amino-acid carrier systems (Chen et al. 2001, Chollet et al. 1997, Deletage-Grandon et al. 2001, Fischer et al. 1995).

Long-term experiments suggest that the deleterious effects induced by high concentrations of *m*-tyrosine and compound **8b** are irreversible. After 14 days of post-germination, the growth of the roots exposed initially to 640  $\mu\text{M}$  *m*-tyrosine or compound **8b** remained completely inhibited despite the microbial degradation occurring in the nutritive solution (Fig. 5 A, B and C). The brownish coloration of root tips probably due to oxidation of phenolic compounds by extracellular peroxidases suggests a necrotic state of the apical meristem. Similarly, the shoot development was stopped. Such a herbicidal effect is not necessary in field. In this regard, the dwarf germinations, which are induced by concentrations as low as 10  $\mu\text{M}$  (Fig. 4), are not competitive for light and must be dramatically affected by soil drought taking into account the poor development of their root system. The complementary data with compound **8h** from these long-term experiments support and extend those from filter paper bioassays (Table 2, Fig. 2). Treatments with this moderate hydrophilic (Table 1) and stable analogue under our experimental conditions (Fig. 3) led to an increase of primary root growth (Fig. 5D). Furthermore, this response was concomitant with a clear delay of the secondary root emergence and a shoot growth inhibition (Fig. 5, compare A and D). By contrast, *m*-tyrosine promotes lateral root elongation in *Arabidopsis* and some lettuce isolates (Bertin et al. 2007).

## Conclusion

Nine halogenated *m*-tyrosine derivatives, among which six are new, have been obtained through a seven-step stereoselective synthesis from commercial halogenated phenol. Filter paper bioassays are an easy method to get preliminary information to evaluate the putative allelochemical properties of these compounds but they need to be completed by more suitable approaches such as hydroponic bioassays. Our experiments support previous data (Bertin et al. 2007). *m*-tyrosine is an efficient allelopathic agent but cannot be used in field because of its high microbial degradation (Bertin et al. 2009, Kaur et al. 2009). Our data indicate that it is possible, by halogen addition, to manipulate: i/ the biological properties of *m*-tyrosine, from similar (compound **8b**) to contrary (compound **8h**) properties on root growth, ii/ the stability of these compounds in non-sterilized conditions. Compound **8b** is slightly less degraded than *m*-tyrosine while compound **8h** remained stable for at least two weeks under our experimental conditions. Finally, these two halogenated derivatives, which induced deleterious but contrary effects on seedling development, may be tools to elucidate the mechanisms of the biological activity of *m*-tyrosine. Our investigation is a complementary approach to opposite strategies consisting to confer resistance to exogenously added *m*-tyrosine (Huang et al. 2010).

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## Figure legends

**Fig. 1** General reaction scheme

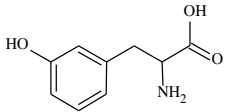
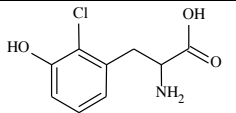
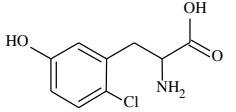
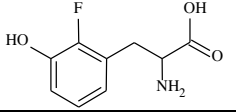
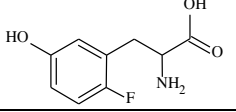
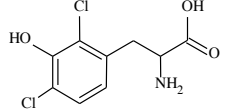
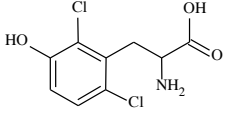
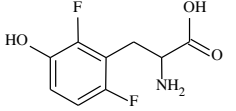
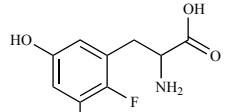
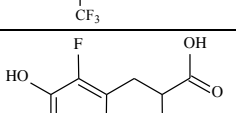
**Fig. 2** Effect of *m*-Tyr, **8b** and **8h** at various concentrations on lettuce (*L. sativa* var *Bonde Parisienne*) seedling root growth (filter paper bioassays). Radicle and shoot length were measured 4 days after sowing. The Kruskal-Wallis test was used to assess statistically significant differences in comparison to control. (\*\*\*  $P < 0.001$  ; \*\*  $P < 0.01$  ; NS, not significant). For box plots,  $n = 20$

**Fig. 3** Time-course changes over an-18-day period of *m*-Tyr, **8b** and **8h** concentrations in the nutrient solution used for lettuce growth. The initial concentration of the products was 160  $\mu\text{M}$ . Dark conditions, temperature  $20 \pm 1^\circ\text{C}$ . Mean of 3 assays

**Fig. 4** Effect of *m*-Tyr and **8b** at various concentrations on lettuce (*L. sativa* var *Bonde Parisienne*) seedling root growth (hydroponic experiments). Radicle and shoot length were measured 6 days after sowing. The Kruskal-Wallis test was used to assess statistically significant differences in comparison to control. (\*\*\*  $P < 0.001$  ; NS, not significant). For box plots,  $n = 25$  for control and  $n = 9$  for the other experiments

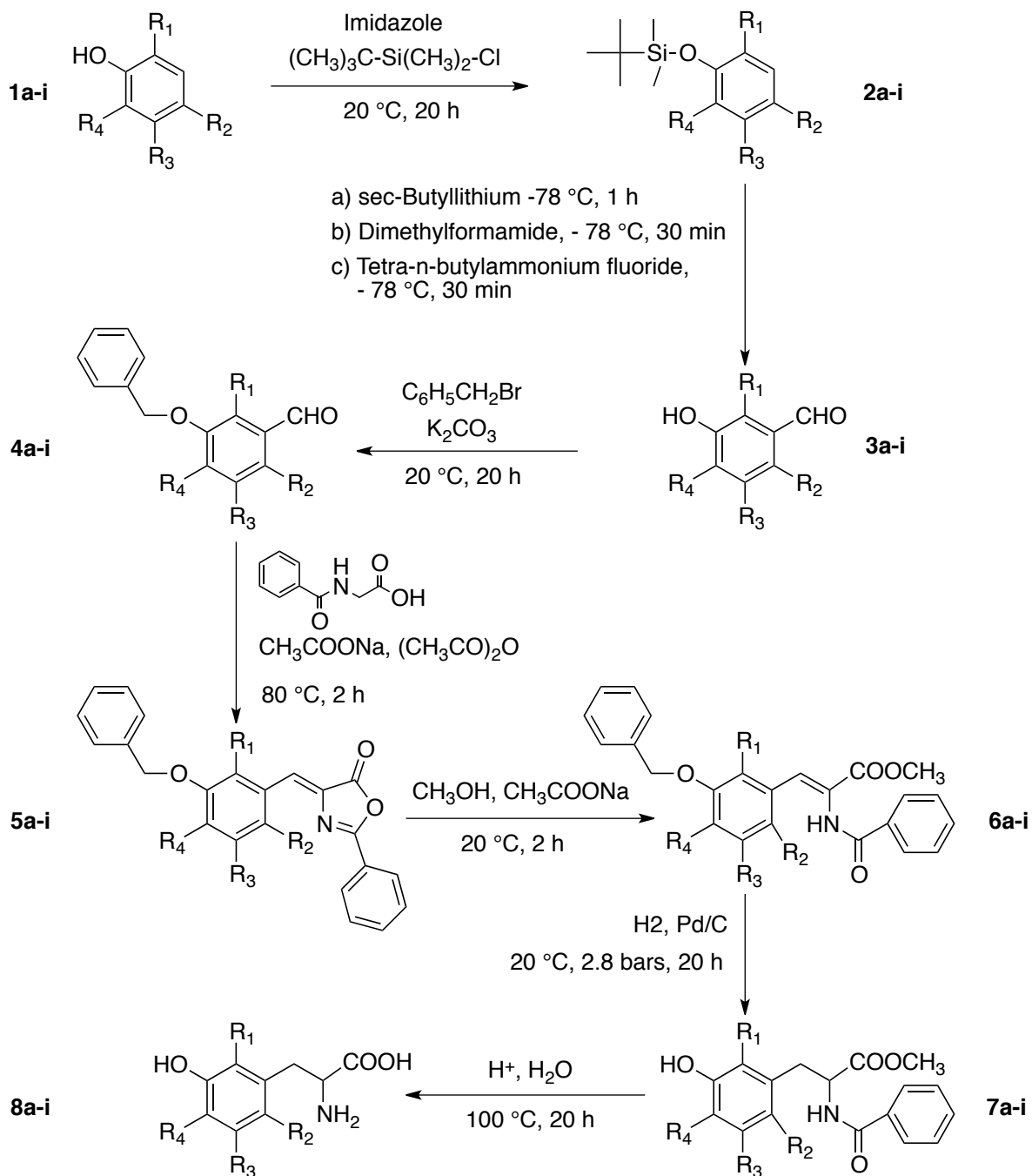
**Fig. 5** Long-term effect (14 days) of *m*-tyrosine (B), **8b** (C) and **8h** (D) used at 640  $\mu\text{M}$  concentration on lettuce seedling growth. Seedlings were grown under dim daylight conditions in a controlled environment ( $21 \pm 0.5^\circ\text{C}$ , HR  $90 \pm 5\%$ ) for the first week after sowing and then at  $24 \pm 0.5^\circ\text{C}$  and 60% RH during the photoperiod (14 h, 250  $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ ). Two arrows localize each Pasteur pipette. A: control

**Table 1.** Structure and physicochemical properties of m-tyrosine and halogenated analogs. For all products,  $2.1 < pK_{a1} < 2.2$ , Polar Surface Area (PSA) =  $83.55 \text{ \AA}^2$  and number of Hydrogen Bond Donors (HBD) = 4. MW = Molecular Weight. Mp = Melting point, decomp. = decomposition. All properties were computed using ACD Log D Sol Suite v.12.02 software except melting points that were experimentally determined.

Product	Structure	MW	Mp (°C)	Halogen ratio	Log D			Water solubility (mg.ml <sup>-1</sup> )		
					pH 4.0	pH 6.0	pH 8.0	pH 4.0	pH 6.0	pH 8.0
<b>m-Tyr</b>		181.19	275-281 decomp.	0.00	-2.95	-2.94	-2.97	10.65	10.5	11.35
<b>8a</b>		215.63	273-277 decomp.	0.07	-2.16	-2.16	-2.33	1.21	1.2	1.86
<b>8b</b>		215.63	249-251 decomp.	0.07	-1.96	-1.96	-2.02	1.08	1.07	1.25
<b>8c</b>		199.18	235-239	0.07	-2.76	-2.76	-2.88	31.19	30.89	42.31
<b>8d</b>		199.18	271-278 decomp.	0.07	-2.75	-2.74	-2.79	30.39	30.03	33.82
<b>8e</b>		250.08	221-239 decomp.	0.13	-1.56	-1.6	-2.38	0.15	0.17	2.15
<b>8f</b>		250.08	90-130 decomp.	0.13	-1.18	-1.18	-1.51	0.12	0.12	0.29
<b>8g</b>		217.17	238-241 decomp.	0.13	-2.61	-2.61	-2.76	27.07	26.88	39.09
<b>8h</b>		267.18	248-255 decomp.	0.22	-0.97	-0.97	-1.09	2.75	2.73	3.65
<b>8i</b>		233.62	264-271 decomp.	0.13	-1.76	-1.76	-2.02	7.92	7.91	15.5

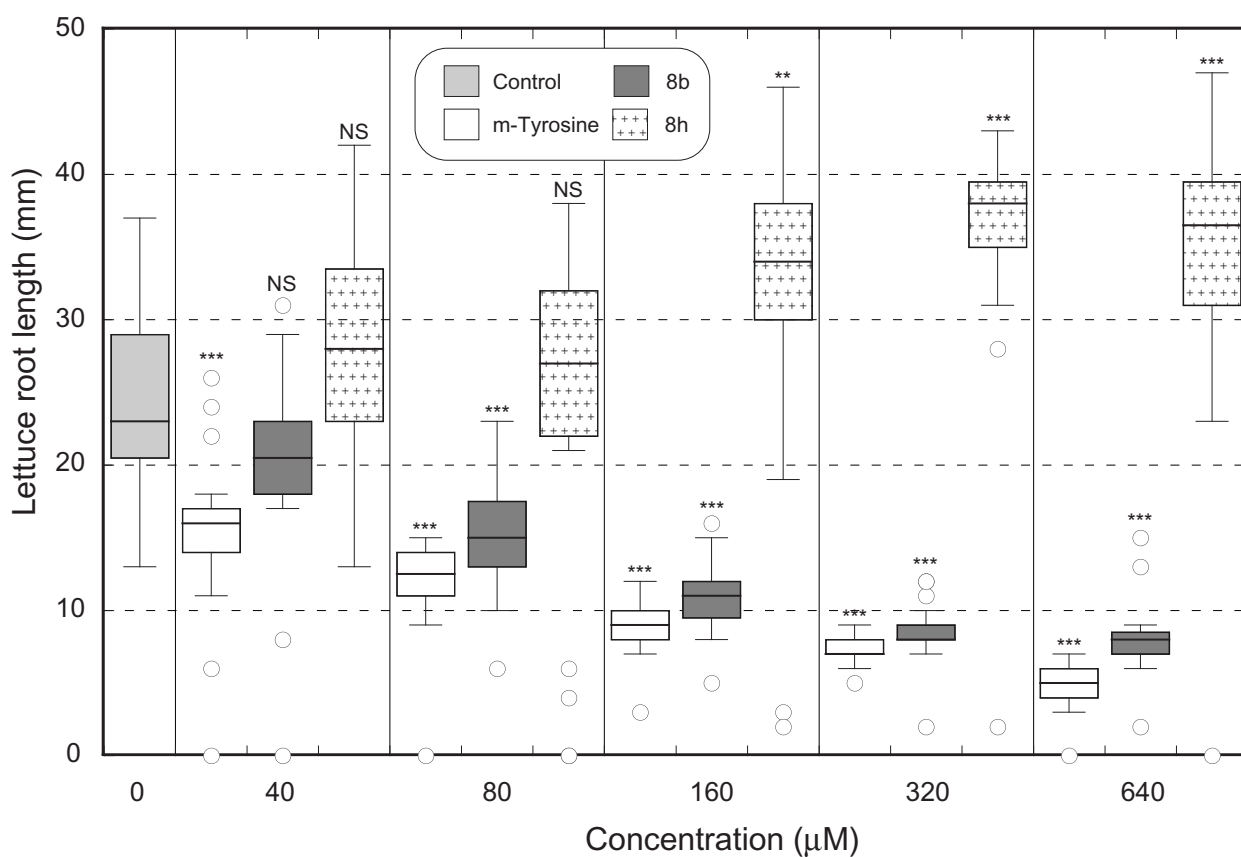
**Table 2.** Short time effect of halogenated products at 640  $\mu$ M concentration on lettuce root elongation in filter paper bioassays. For each experiment (A-F), one or two halogenated compounds were tested simultaneously with m-Tyr and control (without any product). Root length of seedlings was measured 4 days after placing seeds in control, m-tyrosine and m-tyrosine derivatives treatments. Results are expressed as the percentage of root growth inhibition in comparison to the control of the same experiment (A,B,C,D,E or F), taking into account the median of the main root length of 20 seedlings. The Kruskal-Wallis test was used to assess statistically significant differences in comparison to control (\*\* $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ , (NS) non significant).

Product	Experiment	Inhibition of root growth (%)
m-Tyr	D	76.7 ***
<b>8a</b>		34.9 **
m-Tyr	A	82.9 ***
<b>8b</b>		75.6 ***
m-Tyr	F	78.3 ***
<b>8b</b>		65.2 ***
m-Tyr	A	82.9 ***
<b>8c</b>		61.0 ***
m-Tyr	D	76.7 ***
<b>8d</b>		2.3 (NS)
m-Tyr	C	75.8 ***
<b>8e</b>		33.3 *
m-Tyr	E	70.5 ***
<b>8f</b>		53.8 ***
m-Tyr	B	90.7 ***
<b>8g</b>		7.0 (NS)
m-Tyr	C	75.8 ***
<b>8h</b>		-51.5 **
m-Tyr	F	78.3 ***
<b>8h</b>		-58.7 ***
m-Tyr	E	70.5 ***
<b>8i</b>		48.7 ***

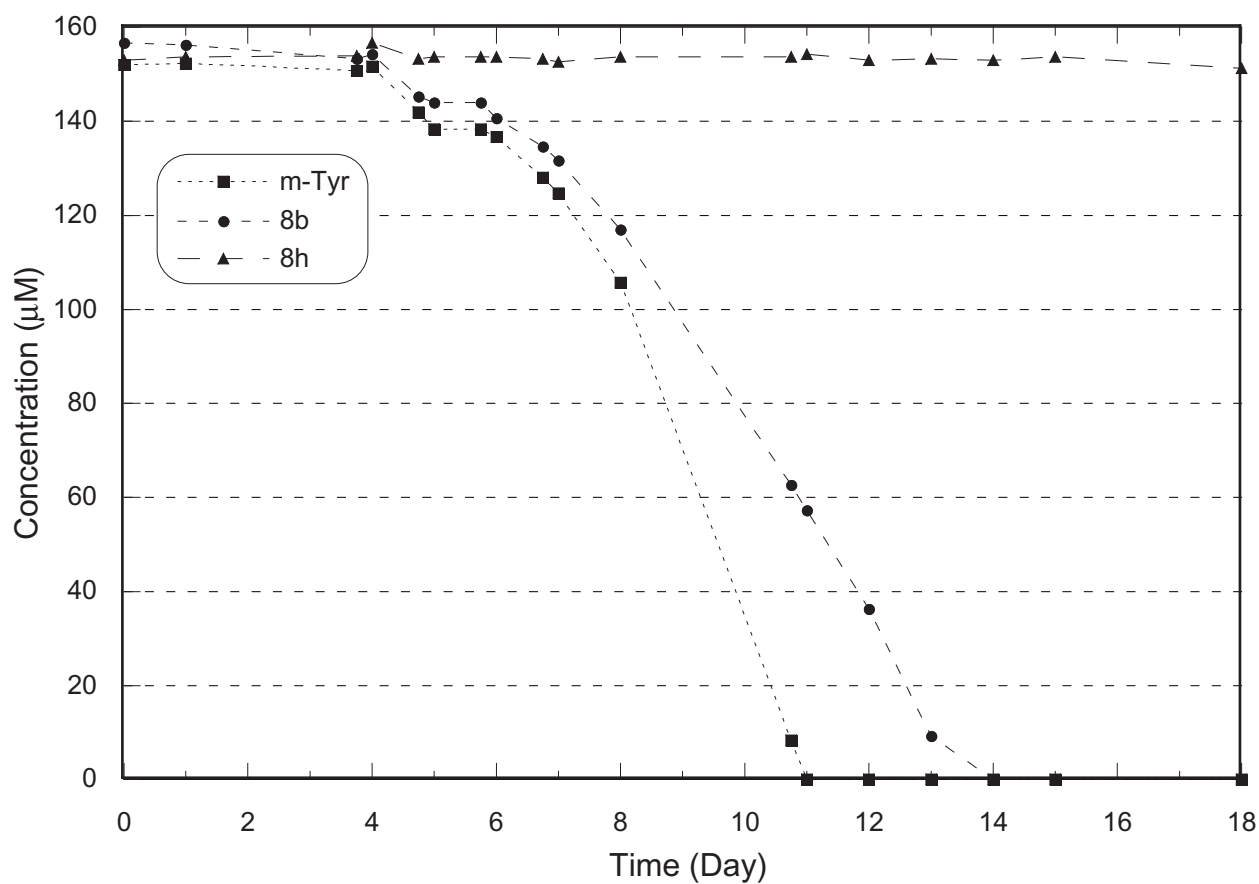


	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
<b>8a</b>	Cl	H	H	H
<b>8b</b>	H	Cl	H	H
<b>8c</b>	F	H	H	H
<b>8d</b>	H	F	H	H
<b>8e</b>	Cl	H	H	Cl
<b>8f</b>	Cl	Cl	H	H
<b>8g</b>	F	F	H	H
<b>8h</b>	H	F	CF <sub>3</sub>	H
<b>8i</b>	F	Cl	H	H

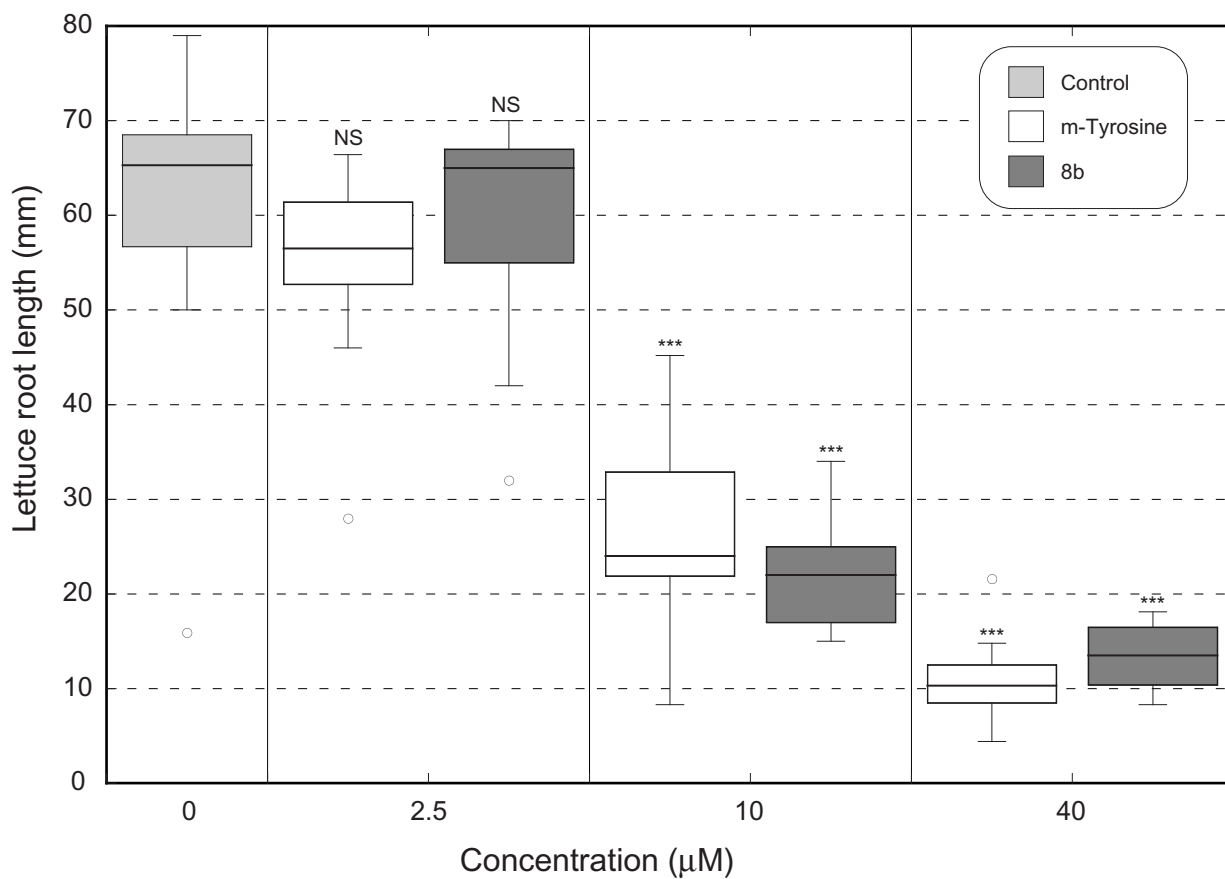
**Figure 1:** General reaction scheme



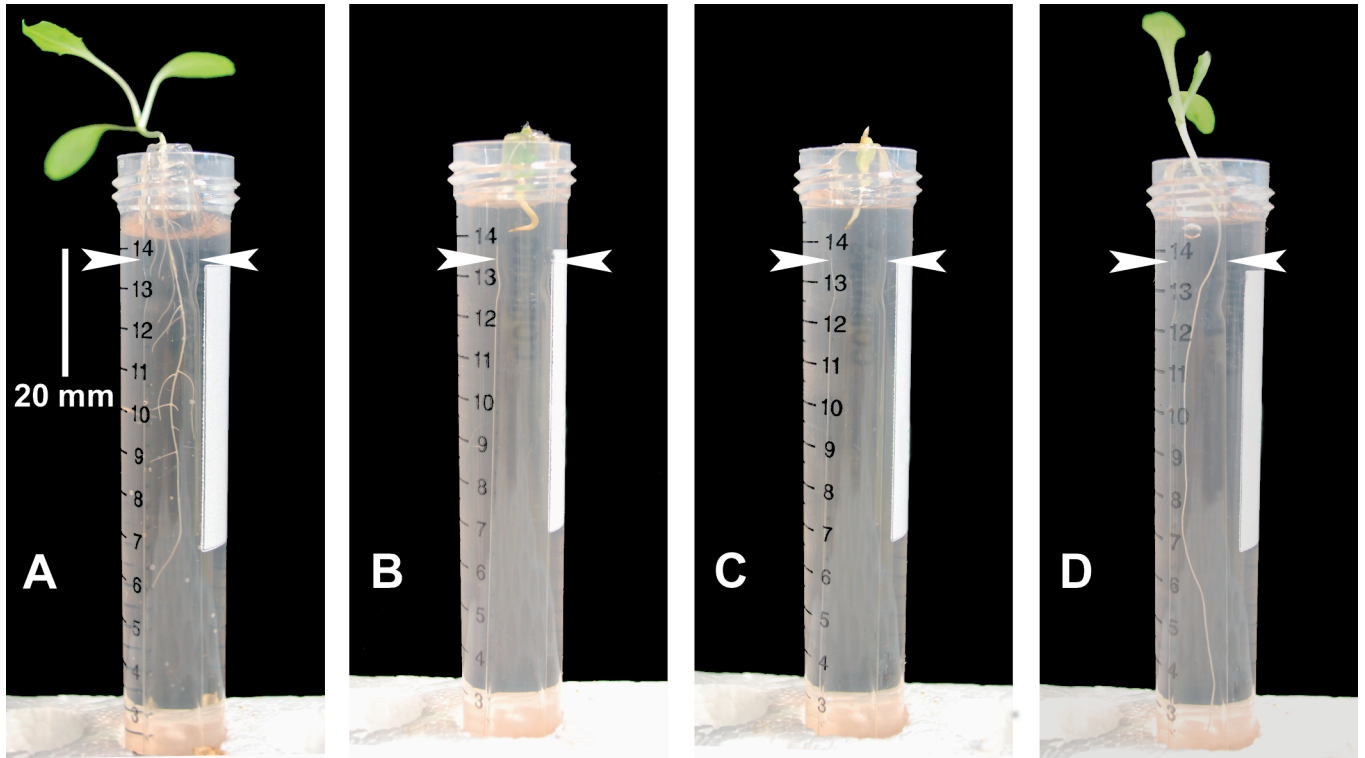
**Figure 2:** Effect of m-Tyr, **8b** and **8h** at various concentrations on lettuce (*L. sativa* var Bonde Parisienne) seedling root growth (filter paper bioassays). Radicle and shoot length were measured 4 days after sowing. The Kruskal-Wallis test was used to assess statistically significant differences in comparison to control. (\*\*\*)  $P < 0.001$  ; \*\*  $P < 0.01$  ; NS, not significant). For box plots,  $n = 20$ .



**Figure 3** : Time-course changes over a 18 day-period of m-Tyr, **8b** and **8h** concentrations in the nutrient solution used for lettuce growth. The initial concentration of the products was 160 µM. Dark conditions, temperature  $20 \pm 1$  °C. Mean of 3 assays.



**Figure 4:** Effect of m-Tyr and **8b** at various concentrations on lettuce (*L. sativa* var Bonde Parisienne) seedling root growth (hydroponic experiments). Radicle and shoot length were measured 6 days after sowing. The Kruskal-Wallis test was used to assess statistically significant differences in comparison to control. (\*\*\*)  $P < 0.001$  ; NS, not significant). For box plots,  $n = 25$  for control and  $n = 9$  for the other experiments.



**Figure 5:** Long-term effect (14 days) of m-Tyrosine (B), **8b** (C) and **8h** (D) used at 640  $\mu\text{M}$  concentration on lettuce seedling growth. Seedlings were grown under dim daylight conditions in a controlled environment ( $21 \pm 0.5^\circ\text{C}$ , HR  $90 \pm 5\%$ ) for the first week after sowing and then at  $24 \pm 0.5^\circ\text{C}$  and 60% RH during the photoperiod (14 h,  $250 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Two arrows localize each Pasteur pipette. A: control.