Food Chemistry 129 (2011) 1169–1178

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Preparation and antioxidant activity of tyrosyl and homovanillyl ethers

A. Madrona [a], G. Pereira-Caro [b], L. Bravo [c], R. Mateos [c], J.L. Espartero [a,*]

[a] Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, E-41012 Sevilla, Spain
[b] IFAPA, Centro Venta del Llano, Bailen-Motril, Km 18.5, E-23620 Mengíbar, Jaén, Spain
[c] CSIC, ICTAN-Instituto del Frío, C/José Antonio Novais 10, Ciudad Universitaria, E-28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form 29 March 2011
Accepted 20 May 2011
Available online 26 May 2011

Keywords:
Olive
Hydroxytyrosol
Tyrosol
Homovanillic alcohol
Alkyl ethers
Antioxidant activity
Lipophilic antioxidants
Rancimat
ABTS
FRAP
ORAC

ABSTRACT

Preparation of tyrosyl and homovanillyl lipophilic derivatives was carried out as a response to the food industry's increasing demand for new synthetic lipophilic antioxidants. Tyrosyl and homovanillyl ethers were synthesized in high yields by a three-step procedure starting from tyrosol (Ty) and homovanillic alcohol (HMV). The antioxidant activity of these new series of alkyl tyrosyl and homovanillyl ethers was evaluated by the Rancimat test in a lipophilic food matrix and by the FRAP, ABTS and ORAC assays and compared to free Ty and HMV as well as two antioxidants widely used in the food industry, butylhydroxytoluene (BHT) and α-tocopherol. The results pointed out the higher activity of homovanillyl series in comparison with tyrosyl series with all the assayed methods. However, while both synthetic series were less antioxidant than BHT and α-tocopherol in a lipophilic matrix after their Rancimat test evaluation, homovanillyl alkyl ethers showed the best reducing power and radical scavenging activity of all evaluated compounds. This batch of synthetic lipophilic compounds, derived from biologically active compounds such as Ty and HMV, provide interesting and potentially bioactive compounds.

1. Introduction

It is well known that lipid peroxidation decreases food's nutritive value and deteriorates its flavour and taste. Food industry attempts to prevent food oxidation using food additives, in order to improve its quality. In this sense, numerous phenolic antioxidants have the potential to be used in hydrophilic food matrices. Among natural polyphenols, olive oil phenols and, particularly, hydroxytyrosol, (2-(3′,4′-dihydroxyphenyl)ethanol, HTy, **1a**), has the capacity to protect against oxidative stress by scavenging radical species (Goya, Mateos, & Bravo, 2007; Rietjents, Bast & Haenen, 2007) and by inducing antioxidant enzymes (Martín et al., 2010). Furthermore, several studies have demonstrated that HTy has cardioprotective effects (De la Torre-Carbot et al., 2010; Rietjents, Bast, Vente & Haenen, 2007), anti-inflammatory (Bitler, Viale, Damaj, & Crea, 2005) and antiplatelet aggregation activity (Dell'Agli et al., 2008), largely related to its antioxidant properties.

In contrast, Ty (**1b**), another natural olive oil phenol, has significantly lower antioxidant capacity than HTy (Mateos, Domínguez, Espartero, & Cert, 2003) due to the absence of the *ortho*-diphenolic group in its chemical structure. Nevertheless, Ty exerts a protective effect against oxidative injury in cell models (Giovannini et al., 1999) and improves the intracellular antioxidant defence systems (Di Benedetto et al., 2007). In fact, one of the tyrosyl secoiridoid derivatives present in olive oil, oleocanthal, has shown anti-inflammatory activity similar to ibuprofen (Beauchamp et al., 2005). Furthermore, recent studies suggest that specifically *mono*-phenols as Ty or *p*-coumaric acid as well as *o*-diphenolic compounds inhibit homocysteine-induced endothelial cell adhesion, regardless their antioxidant activity, playing a key role in the control of several inflammation-associated processes (Manna, Napoli, Cacciapuoti, Porcelli, & Zappia, 2009).

Taking into account the high potential effectiveness as antioxidants of these virgin olive oil polyphenols and the great interest in the use of phytochemicals as biological ingredients for functional foods with enhanced nutritional value, HTy (**1a**) has already been used as a bioactive ingredient in tomato juice (Larrosa, Espín, & Tomás-Barberán, 2003) and fish products (Pazos, Alonso, Sánchez, & Medina, 2008), showing good results. To our knowledge, no applications has been studied for Ty (**1b**) or HMV (**1c**), which are present in the phenolic fraction of virgin olive oil, and may be effectively recovered from olive oil wastewaters, similarly to HTy (Fernández-Bolaños et al., 2005).

In response to the lack of antioxidants to be used in lipidic foods, new lipophilic derivatives have been investigated in the last

* Corresponding author.
E-mail address: jles@us.es (J.L. Espartero).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.05.098

years. In this sense, several chemical (Alcudia, Cert, Espartero, Mateos, & Trujillo, 2004; Appendino, Minassi, Daddario, Bianchi, & Tron, 2002; Bernini, Mincione, Barontini, & Crisante, 2008; Capasso, Sannino, De Martino, & Manna, 2006; Gordon, Paiva-Martins, & Almeida, 2001; Palozza et al., 2008; Tofani, Balducci, Gasperi, Incerpi, & Gambacorta, 2010; Torregiani, Seu, Minassi, & Appendino, 2005; Trujillo et al., 2006) or enzymatic (Alcudia et al., 2004; Bouallagui et al., 2011; Buisman et al., 1998; Grasso, Siracusa, Spatafora, Renis, & Tringali, 2007; Lucas et al., 2010; Mateos et al., 2008; Torres de Pinedo, Peñalver, & Morales, 2007; Torres de Pinedo, Peñalver, Pérez-Victoria, Rondón, & Morales, 2007; Torres de Pinedo, Peñalver, Rondon, & Morales, 2005) synthesis reactions of lipophilic esters derivatives of hydroxytyrosol, homovanillic alcohol and/or tyrosol esters have been reported and recently reviewed (Chillemi, Sciuto, Spatafora, & Tringali, 2010; Fernández-Bolanos, Lopez, Fernández-Bolanos, & Rodríguez-Gutiérrez, 2008). The antioxidant effects of the new series of esters derivatives, containing lipophilic acyl chains of different length with increasing lipophilicity, have been tested using different methods. Remarkable antioxidant capacity has been observed when the new compounds were tested in cell lines (Bouallagui et al., 2011; Grasso et al., 2007; Tofani et al., 2010) and in food matrices, such as oils and oil-in-water emulsions (Lucas et al., 2010; Mateos et al., 2008; Medina, Lois, Alcántara, Lucas, & Morales, 2009; Torres de Pinedo, Peñalver, & Morales, 2007; Torres de Pinedo, Peñalver, Pérez-Victoria et al., 2007; Trujillo et al., 2006). Having been evaluated using different methods, it may be concluded that these new esters derivatives possess slightly higher or comparable antioxidant activity than their respective precursors.

Recently, a new group of lipophilic hydroxytyrosyl derivatives, hydroxytyrosyl ethers (Madrona et al., 2009), with linear alkyl side chains of variable length, have been synthesised by our group. These new derivatives of HTy (**1a**) showed comparable or even higher antioxidant capacity than free HTy (Pereira-Caro et al., 2009) and higher bioavailability at both intestinal (Pereira-Caro et al., 2010) and hepatic levels (Pereira-Caro, Bravo, Madrona, Espartero, & Mateos, 2010). Taking into account the enhanced antioxidant properties of alkyl hydroxytyrosyl ethers in comparison with their precursor HTy (**1a**) and the promising biological activity described for Ty (**1b**), the synthesis of alkyl tyrosyl derivatives could be an interesting alternative to meet the food industry needs. Moreover, homovanillyl ethers have a great potential as phytochemicals, considering that their precursor homovanillic alcohol (HMV, **1c**) presents biological activity against oxidative kidney cell injury (Incani et al., 2010), and is one of the identified metabolites after human virgin olive oil intake (Caruso, Visioli, Patelli, Galli, & Galli, 2001; Miró-Casas et al., 2003; Visioli et al., 2000; Vissers, Zock, Roodenburg, Leenen, & Katan, 2002). Bearing this in mind, in the present work the synthesis of tyrosyl (**4h–n**) and homovanillyl ethers (**4o–u**) and their oxidative stability in lipid matrices spiked with these new synthetic compounds (**4h–u**) by Rancimat test, was carried out. Moreover, reducing power by FRAP assay and radical scavenging activity by ABTS and ORAC assays of tyrosyl and homovanilly ethers (**4h–u**) were also assessed.

2. Materials and methods

2.1. Materials

All solvents and reagents were of analytical grade unless otherwise stated. -Tocopherol, 2,6-di-*tert*-butyl-4-methylphenol (BHT) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were from Aldrich (Madrid, Spain). Benzyl bromide was from Fluka (Steinheim, Germany). Sodium hydroxide, sodium hydrogen phosphate and potassium dihydrogen phosphate were from Panreac (Madrid, Spain). Tyrosol (**1b**), homovanillic alcohol (**1c**) and the alkyl iodides (methyl, ethyl, *n*-propyl, *n*-butyl, *n*-hexyl, *n*-octyl and *n*-dodecyl iodides) were from Aldrich (Steinheim, Germany). Fluorescein, methylated β-cyclodextrin (RMCD), 2, 2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 2,2′-azino bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (98%), 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ) were from Sigma (Madrid, Spain).

NMR spectra were recorded on a Bruker Avance 500 spectrophotometer operating at 500.13 MHz (^{1}H) and 125.75 MHz (^{13}C). Samples were dissolved (0.1 mmol/ml) in hexadeuterated methylsulfoxide (DMSO-d_6), and spectra were recorded at 303 K. Chemical shifts are given in ppm with the residual solvent signals (2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C) as references. Elemental analyses were made on a Leco CHNS-932 apparatus. High-resolution EI, CI and FAB mass spectra were obtained on a Micromass AUTOSPECQ spectrometer.

2.2. Synthetic procedures

2.2.1. General procedure for alkylation of *2b* and *2c*

A mixture of **2b** (Ajao, Bird, & Chauhan, 1985) or **2c** (Battersby, Le Count, Garratt, & Thrift, 1961) (1 mmol), KOH (335 mg) and the corresponding alkyl iodide (3 mmol) in methylsulfoxide (12 ml) was stirred at room temperature until completion of reaction (thin layer chromatography, TLC). 3 M HCl (25 ml) was added and the mixture was extracted with $CHCl_3$ (3 × 25 ml). The organic phase was further washed with 2% $NaHSO_3$ (25 ml) and water (25 ml), dried over anhydrous Na_2SO_4, filtered and evaporated. The crude products were purified by flash column chromatography over silica gel yielding the desired products **3h–u**.

1-(Benzyloxy)-4-(2-methoxyethyl)benzene (**3h**): colourless liquid (85% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.90 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2*Ph* in pos. 6), 3.46 (t, J = 6.9 Hz, 2H, H_1), 3.21 (s, 3H, $H_{1'}$), 2.71 (t, J = 6.9 Hz, 2H, H_2); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.7 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.5 (C_5), 72.9 (C_1), 69.1 (CH_2Ph in pos. 6), 57.7 ($C_{1'}$), 34.4 (C_2). Elem. Anal. Calcd for $C_{16}H_{18}O_2$: C, 79.31; H, 7.49; found: C, 79.27; H, 7.27; HRMS, 242.1302 (2 ppm).

1-(Benzyloxy)-4-(2-ethoxyethyl)benzene (**3i**): colourless liquid (89% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.90 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2Ph in pos. 6), 3.49 (t, J = 7.1 Hz, 2H, H_1), 3.39 (q, J = 7.0 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.1 Hz, 2H, H_2), 1.07 (t, J = 7.0 Hz, 3H, $H_{2'}$); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.5 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.9 (C_5), 71.1 (C_1), 69.1 (CH_2Ph in pos. 6), 65.1 ($C_{1'}$), 34.7(C_2), 15.0 ($C_{2'}$). Elem. Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86; found: C, 79.46; H, 7.95; HRMS, 256.1465 (0.7 ppm).

1-(Benzyloxy)-4-(2-propoxyethyl)benzene (**3j**): colourless liquid (89% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.90 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2Ph in pos. 6), 3.49 (t, J = 7.1 Hz, 2H, H_1), 3.31 (t, J = 6.6 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.1 Hz, 2H, H_2), 1.47 (m, 2H, $H_{2'}$) 0.83 (t, J = 7.4 Hz, 3H, $H_{3'}$); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.6 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.4 (C_5), 71.5 ($C_{1'}$), 71.0 (C_1), 69.1 (CH_2Ph in pos. 6), 34.6 (C_2), 22.3 ($C_{2'}$), 10.4 ($C_{3'}$). Elem. Anal. Calcd for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20; found: C, 80.17; H, 8.18; HRMS, 270.1630 (3.8 ppm).

1-(Benzyloxy)-4-(2-butoxyethyl)benzene (**3k**): colourless liquid (87% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.90 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2Ph in pos. 6), 3.49 (t, J = 7.0 Hz, 2H, H_1), 3.34 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.0 Hz, 2H, H_2), 1.44 (m, 2H, $H_{2'}$) 1.27 (m, 2H, $H_{3'}$), 0.84

(t, J = 7.4 Hz, 3H, $H_{4'}$); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.6 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.4 (C_5), 71.1 (C_1), 69.5 ($C_{1'}$), 69.1 (CH_2Ph in pos. 6), 34.6 (C_2), 31.2 ($C_{2'}$), 18.7 ($C_{3'}$), 13.6 ($C_{4'}$). Elem. Anal. Calcd for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51; found: C, 80.21; H, 8.68; HRMS, 284.1775 (0.5 ppm).

1-(Benzyloxy)-4-(2-hexyloxyethyl)benzene (**3l**): colourless liquid (73% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.89 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2Ph in pos. 6), 3.49 (t, J = 6.9 Hz, 2H, H_1), 3.33 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.70 (t, J = 6.9 Hz, 2H, H_2), 1.44 (m, 2H, $H_{2'}$) 1.22 (m, 6H, $H_{3'}$–$H_{5'}$), 0.84 (t, J = 7.1 Hz, 3H, $H_{6'}$); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.6 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.4 (C_5), 71.0 (C_1), 69.8 ($C_{1'}$), 69.0 (CH_2Ph in pos. 6), 34.6 (C_2), 31.0 ($C_{4'}$), 29.1 ($C_{2'}$), 25.2 ($C_{3'}$), 22.0 ($C_{5'}$), 13.8 ($C_{6'}$). Elem. Anal. Calcd for $C_{21}H_{28}O_2$: C, 80.73; H, 9.03; found: C, 80.73; H, 9.02; HRMS, 312.2091 (0.5 ppm).

1-(Benzyloxy)-4-(2-octyloxyethyl)benzene (**3m**): colourless liquid (75% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.89 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2Ph in pos. 6), 3.49 (t, J = 6.9 Hz, 2H, H_1), 3.33 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.70 (t, J = 6.9 Hz, 2H, H_2), 1.44 (m, 2H, $H_{2'}$) 1.22 (m, 10H, $H_{3'}$–$H_{7'}$), 0.84 (t, J = 7.1 Hz,3H, $H_{8'}$); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.6 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.4 (C_5), 71.0 (C_1), 69.8 ($C_{1'}$), 69.0 (CH_2Ph in pos. 6), 34.6 (C_2), 31.1 ($C_{6'}$), 29.1 ($C_{2'}$), 28.7 ($C_{4'}$), 28.6 ($C_{5'}$), 25.6 ($C_{3'}$), 22.0 ($C_{7'}$), 13.8 ($C_{8'}$). Elem. Anal. Calcd for $C_{23}H_{32}O_2$: C, 81.13; H, 9.47; found: C, 81.42; H, 9.75; HRMS, 340.2378 (7.1 ppm).

1-(Benzyloxy)-4-(2-dodecyloxyethyl)benzene (**3n**): colourless liquid (70% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.89 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2Ph in pos. 6), 3.48 (t, J = 6.9 Hz, 2H, H_1), 3.33 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.70 (t, J = 6.9 Hz, 2H, H_2), 1.44 (m, 2H, $H_{2'}$) 1.22 (m, 18H, $H_{3'}$–$H_{11'}$), 0.83 (t, J = 6.8 Hz, 3H, $H_{12'}$); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.6 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.4 (C_5), 71.0 (C_1), 69.8 ($C_{1'}$), 69.0 (CH_2Ph in pos. 6), 34.6 (C_2), 31.2 ($C_{10'}$), 29.0 ($C_{2'}$), 29.0–28.5 ($C_{4'}$, $C_{9'}$), 25.6 ($C_{3'}$), 22.0 ($C_{11'}$), 13.8 ($C_{12'}$). Elem. Anal. Calcd for $C_{27}H_{40}O_2$: C, 81.77; H, 10.17; found: C, 81.62; H, 10.10; HRMS, 396.3012 (4.1 ppm).

1-(Benzyloxy)-2-methoxy-4-(2-methoxyethyl)benzene (**3o**): colourless liquid (85% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 6.90 (d, J = 8.1 Hz, 1H, H_7), 6.85 (d, J = 2.0 Hz, 1H, H_4), 6.69 (dd, 1H, H_8), 5.02 (s, 2H, CH_2Ph in pos. 6), 3.74 (s, 3H, *OMe* in pos. 5), 3.49 (t, J = 7.0 Hz, 2H, H_1), 3.22 (s, 3H, $H_{1'}$), 2.71 (t, J = 7.0 Hz, 2H, H_2); ^{13}C-NMR δ ppm 149.0 (C_5), 146.1 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.9 (C_3), 128.3–127.5 (C_2–C_4, *Bn* group), 120.5 (C_8), 113.8 (C_7), 113.0 (C_4), 72.9 (C_1), 70.0 (CH_2Ph in pos. 6), 57.7 ($C_{1'}$), 55.5 (*OMe* in pos. 5), 34.8 (C_2). Elem. Anal. Calcd for $C_{17}H_{20}O_3$: C, 74.97; H, 7.40; found: C, 74.64; H, 7.15; HRMS, 272.1408 (−1.6 ppm).

1-(Benzyloxy)-2-methoxy-4-(2-ethoxyethyl)benzene (**3p**): colourless liquid (86% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 6.90 (d, J = 7.9 Hz, 1H, H_7), 6.86 (d, J = 1.9 Hz, 1H, H_4), 6.69 (dd, 1H, H_8), 5.02 (s, 2H, CH_2Ph in pos. 6), 3.74 (s, 3H, *OMe* in pos. 5), 3.52 (t, J = 7.0 Hz, 2H, H_1), 3.41 (q, J = 6.9 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.0 Hz, 2H, H_2), 1.08 (t, J = 6.9 Hz, 3H, $H_{2'}$); ^{13}C-NMR δ ppm 149.0 (C_5), 146.1 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.9 (C_3), 128.3–127.5 (C_2–C_4, *Bn* group), 120.5 (C_8), 113.8 (C_7), 113.0 (C_4), 70.8 (C_1), 70.0 (CH_2Ph in pos. 6), 65.1 ($C_{1'}$), 55.5 (*OMe* in pos. 5), 35.1 (C_2), 15.0 ($C_{2'}$). Elem. Anal. Calcd for $C_{18}H_{22}O_3$: C, 75.50; H, 7.74; found: C, 76.00; H, 7.64; HRMS, 286.1574 (1.8 ppm).

1-(Benzyloxy)-2-methoxy-4-(2-propoxyethyl)benzene (**3q**): colourless liquid (83% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 6.90 (d, J = 8.1 Hz, 1H, H_7), 6.86 (d, J = 1.8 Hz, 1H, H_4), 6.69 (dd, 1H, H_8), 5.02 (s, 2H, CH_2Ph in pos. 6), 3.74 (s, 3H, *OMe* in pos. 5), 3.51 (t, J = 7.0 Hz, 2H, H_1), 3.32 (t, J = 6.7 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.0 Hz, 2H, H_2), 1.48 (m, 2H, $H_{2'}$), 0.83 (t, J = 7.3 Hz, 3H, $H_{3'}$); ^{13}C-NMR δ ppm 149.0 (C_5), 146.1 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.9 (C_3), 128.3–127.5 (C_2–C_4, *Bn* group), 120.5 (C_8), 113.7 (C_7), 113.0 (C_4), 71.5 ($C_{1'}$), 71.1 (C_1), 70.0 (CH_2Ph in pos. 6), 55.5 (*OMe* in pos. 5), 35.1 (C_2), 22.4 ($C_{2'}$), 10.6 ($C_{3'}$). Elem. Anal. Calcd for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05; found: C, 75.97; H, 8.08; HRMS, 300.1724 (−0.5 ppm).

1-(Benzyloxy)-2-methoxy-4-(2-butoxyethyl)benzene (**3r**): colourless liquid (76% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 6.90 (d, J = 8.1 Hz, 1H, H_7), 6.85 (d, J = 1.9 Hz, 1H, H_4), 6.69 (dd, 1H, H_8), 5.02 (s, 2H, CH_2Ph in pos. 6), 3.74 (s, 3H, *OMe* in pos. 5), 3.51 (t, J = 6.9 Hz, 2H, H_1), 3.36 (t, J = 6.6 Hz, 2H, $H_{1'}$), 2.71 (t, J = 6.9 Hz, 2H, H_2), 1.45 (m, 2H, $H_{2'}$), 1.29 (m, 2H, $H_{3'}$), 0.85 (t, J = 7.4 Hz, 3H, $H_{4'}$); ^{13}C-NMR δ ppm 149.0 (C_5), 146.1 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.9 (C_3), 128.3–127.5 (C_{2-C4}, *Bn* group), 120.5 (C_8), 113.8 (C_7), 113.1 (C_4), 71.0 (C_1), 70.0 (CH_2Ph in pos. 6), 69.5 ($C_{1'}$), 55.5 (*OMe* in pos. 5), 35.1 (C_2), 31.2 ($C_{2'}$), 18.8 ($C_{3'}$), 13.6 ($C_{4'}$). Elem. Anal. Calcd for $C_{20}H_{26}O_3$: C, 76.40; H, 8.33; found: C, 76.45; H, 8.08; HRMS, 314.1881 (−0.3 ppm).

1-(Benzyloxy)-2-methoxy-4-(2-hexyloxyethyl)benzene (**3s**): colourless liquid (86% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 6.89 (d, J = 8.1 Hz, 1H, H_7), 6.85 (d, J = 1.8 Hz, 1H, H_4), 6.69 (dd, 1H, H_8), 5.02 (s, 2H, CH_2Ph in pos. 6), 3.74 (s, 3H, *OMe* in pos. 5), 3.51 (t, J = 7.0 Hz, 2H, H_1), 3.34 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.0 Hz, 2H, H_2), 1.45 (m, 2H, $H_{2'}$), 1.23 (m, 6H, $H_{3'}$–$H_{5'}$), 0.83 (t, J = 7.0 Hz, 3H, $H_{6'}$); ^{13}C-NMR δ ppm 149.0 (C_5), 146.1 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.9 (C_3), 128.3–127.5 (C_2–C_4, Bn group), 120.5 (C_8), 113.7 (C_7), 113.0 (C_4), 71.1 (C_1), 70.0 (CH_2Ph in pos. 6), 69.9 ($C_{1'}$), 55.5 (*OMe* in pos. 5), 35.1 (C_2), 29.1 ($C_{2'}$), 29.1 ($C_{4'}$), 25.3 ($C_{3'}$), 22.0 ($C_{5'}$), 13.8 ($C_{6'}$). Elem. Anal. Calcd for $C_{22}H_{30}O_3$: C, 77.16; H, 8.83; found: C, 77.27; H, 8.95; HRMS, 342.2190 (−1.4 ppm).

1-(Benzyloxy)-2-methoxy-4-(2-octyloxyethyl)benzene (**3t**): colourless liquid (84% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 6.89 (d, J = 8.1 Hz, 1H, H_7), 6.85 (d, J = 1.9 Hz, 1H, H_4), 6.69 (dd, 1H, H_8), 5.02 (s, 2H, CH_2Ph in pos. 6), 3.74 (s, 3H, *OMe* in pos. 5), 3.51 (t, J = 6.9 Hz, 2H, H_1), 3.34 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.70 (t, J = 6.9 Hz, 2H, H_2), 1.45 (m, 2H, $H_{2'}$), 1.22(m, 10H, $H_{3'}$–$H_{7'}$), 0.84 (t, J = 7.1 Hz, 3H, $H_{8'}$); ^{13}C-NMR δ ppm 149.0 (C_5), 146.1 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.9 (C_3), 128.3–127.5 (C_2–C_4, *Bn* group), 120.5 (C_8), 113.7 (C_7), 113.1 (C_4), 71.0 (C_1), 70.0 (CH_2Ph in pos. 6), 69.8 ($C_{1'}$), 55.5 (*OMe* in pos. 5), 35.1 (C_2), 31.1 ($C_{6'}$), 29.1 ($C_{2'}$), 28.7 ($C_{4'}$), 28.6 ($C_{5'}$), 25.6 ($C_{3'}$), 22.0 ($C_{7'}$), 13.8 ($C_{8'}$). Elem. Anal. Calcd for $C_{24}H_{34}O_3$: C, 77.80; H, 9.25; found: C, 77.54; H, 9.35; HRMS, 370.2510 (0.6 ppm).

1-(Benzyloxy)-2-methoxy-4-(2-dodecyloxyethyl)benzene (**3u**): colourless liquid (64% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 6.89 (d, J = 8.2 Hz, 1H, H_7), 6.85 (d, J = 1.9 Hz, 1H, H_4), 6.68 (dd, 1H, H_8), 5.02 (s, 2H, CH_2Ph in pos. 6), 3.74 (s, 3H, *OMe* in pos. 5), 3.51 (t, J = 7.0 Hz, 2H, H_1), 3.34 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.0 Hz, 2H, H_2), 1.45 (m, 2H, $H_{2'}$), 1.22 (m, 18H, $H_{3'}$–$H_{11'}$), 0.84 (t, J = 7.0 Hz, 3H, $H_{12'}$); ^{13}C-NMR δ ppm 149.0 (C_5), 146.1 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.9 (C_3), 128.3–127.5 (C_2–C_4, *Bn* group), 120.5 (C_8), 113.8 (C_7), 113.1 (C_4), 71.0 (C_1), 70.0 (CH_2Ph in pos. 6), 69.8 ($C_{1'}$), 55.5 (*OMe* in pos. 5), 35.1 (C_2), 31.2 ($C_{10'}$), 29.1 ($C_{2'}$), 28.9–28.6 ($C_{4'}$, $C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$, $C_{9'}$), 25.6 ($C_{3'}$), 22.0 ($C_{11'}$), 13.8 ($C_{12'}$). Elem. Anal. Calcd for $C_{28}H_{42}O_3$: C, 78.83; H, 9.92; found: C, 78.73; H, 9.59; HRMS, 426.3148 (3.3 ppm).

2.2.2. General procedure for cleavage of benzyl protecting groups

A catalytic amount of palladium over charcoal (Pd–C) was added to a solution of the corresponding protected ethers (**3h–u**, 1 mmol) in THF (20 ml) and the mixture was hydrogenated at 4 bar with magnetic stirring. After 24 h at room temperature the catalyst was filtered off over Celite and solvent was evaporated in vacuum. Further purification by column chromatography yielded the desired compounds in each case (**4h–u**).

4-(2-Methoxyethyl)phenol (**4h**): colourless liquid (82% yield); ^{1}H-NMR δ ppm 9.09 (s, 1H, phenolic O*H*), 6.98 (d, J = 8.3 Hz, 2H, H_5), 6.64 (d, J = 8.3 Hz, 2H, H_4), 3.44 (t, J = 7.0 Hz, 2H, H_1), 3.21 (s, 3H, $H_{1'}$), 2.66 (t, J = 7.0 Hz, 2H, H_2); ^{13}C-NMR δ ppm 155.4 (C_6), 129.5 (C_4), 128.9 (C_3), 114.9 (C_5), 73.1 (C_1), 57.6 ($C_{1'}$), 34.4 (C_2). Elem. Anal. Calcd for $C_9H_{12}O_2$: C, 71.03; H, 7.95; found: C, 71.14; H, 7.60; HRMS, 152.0828 (6.1 ppm).

4-(2-Ethoxyethyl)phenol (**4i**): colourless liquid (81% yield); ^{1}H-NMR δ ppm 9.11 (s, 1H, phenolic O*H*), 6.99 (d, J = 8.5 Hz, 2H, H_5), 6.64 (d, J = 8.5 Hz, 2H, H_4), 3.46 (t, J = 7.2 Hz, 2H, H_1), 3.39(q, J = 6.9 Hz, 2H, $H_{1'}$), 2.65 (t, J = 7.2 Hz, 2H, H_2), 1.07 (t, J = 6.9 Hz, 3H, $H_{2'}$); ^{13}C-NMR δ ppm 155.4 (C_6), 129.5 (C_4), 128.9 (C_3), 114.9 (C_5), 71.1 (C_1), 65.1 ($C_{1'}$), 34.7 (C_2), 15.0 ($C_{2'}$). Elem. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49; found: C, 72.16; H, 8.13; HRMS, 166.0992 (−1.1 ppm).

4-(2-Propoxyethyl)phenol (**4j**): colourless liquid (87% yield); ^{1}H-NMR δ ppm 9.09 (s, 1H, phenolic O*H*), 6.99 (d, J = 8.4 Hz, 2H, H_5), 6.64 (d, J = 8.4 Hz, 2H, H_4), 3.47 (t, J = 7.2 Hz, 2H, H_1), 3.30 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.66 (t, J = 7.2 Hz, 2H, H_2), 1.47 (m, 2H, $H_{2'}$), 0.82 (t, J = 7.4 Hz, 3H, $H_{3'}$); ^{13}C-NMR δ ppm 155.4 (C_6), 129.5 (C_4), 128.9 (C_3), 114.9 (C_5), 71.4 ($C_{1'}$), 71.3 (C_1), 34.7 (C_2), 22.3 ($C_{2'}$), 10.4 ($C_{3'}$). Elem. Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95; found: C, 72.91; H, 8.96; HRMS, 180.1152 (0.9 ppm).

4-(2-Butoxyethyl)phenol (**4k**): yellowish liquid (86% yield); ^{1}H-NMR δ ppm 9.09 (s, 1H, phenolic O*H*), 6.99 (d, J = 8.5 Hz, 2H, H_5), 6.64 (d, J = 8.4 Hz, 2H, H_4), 3.46 (t, J = 7.2 Hz, 2H, H_1), 3.34 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.65 (t, J = 7.2 Hz, 2H, H_2), 1.44 (m, 2H, $H_{2'}$), 1.28 (m, 2H, $H_{3'}$), 0.84 (t, J = 7.4 Hz, 3H, $H_{4'}$); ^{13}C-NMR δ ppm 155.4 (C_6), 129.5 (C_4), 128.9 (C_3), 114.9 (C_5), 71.3 (C_1), 69.5 ($C_{1'}$), 34.7 (C_2), 31.2 ($C_{2'}$), 18.8 ($C_{3'}$), 13.7 ($C_{4'}$). Elem. Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.29; H, 9.34; found: C, 74.24; H, 9.38; HRMS, 194.1294 (6.6 ppm).

4-(2-Hexyloxyethyl)phenol (**4l**): yellowish liquid (85% yield); ^{1}H-NMR δ ppm 9.08 (s, 1H, phenolic O*H*), 6.98 (d, J = 8.5 Hz, 2H, H_5), 6.64 (d, J = 8.5 Hz, 2H, H_4), 3.46 (t, J = 7.1 Hz, 2H, H_1), 3.33 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.65 (t, J = 7.1 Hz, 2H, H_2), 1.44 (m, 2H, $H_{2'}$), 1.23 (m, 6H, $H_{3'}$–$H_{5'}$), 0.84 (t, J = 7.2 Hz, 3H, $H_{6'}$); ^{13}C-NMR δ ppm 155.4 (C_6), 129.5 (C_4), 128.9 (C_3), 114.9 (C_5), 71.3 (C_1), 69.8 ($C_{1'}$), 34.7 (C_2), 31.0 ($C_{4'}$), 29.1 ($C_{2'}$), 25.3 ($C_{3'}$), 22.0 ($C_{5'}$), 13.8 ($C_{6'}$). Elem. Anal. Calcd for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97; found: C, 75.80; H, 10.28; HRMS, 222.1623 (1.4 ppm).

4-(2-Octyloxyethyl)phenol (**4m**): colourless liquid (85% yield); ^{1}H-NMR δ ppm 9.08 (s, 1H, phenolic O*H*), 6.98 (d, J = 8.5 Hz, 2H, H_5), 6.64 (d, J = 8.5 Hz, 2H, H_4), 3.46 (t, J = 7.1 Hz, 2H, H_1), 3.33 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.65 (t, J = 7.1 Hz, 2H, H_2), 1.44 (m, 2H, $H_{2'}$), 1.22 (m, 10H, $H_{3'}$–$H_{7'}$), 0.84 (t, J = 7.1 Hz, 3H, $H_{8'}$); ^{13}C-NMR δ ppm 155.4 (C_6), 129.5 (C_4), 128.9 (C_3), 114.9 (C_5), 71.3 (C_1), 69.8 ($C_{1'}$), 34.7 (C_2), 31.2 ($C_{6'}$), 29.1 ($C_{2'}$), 28.7 ($C_{4'}$), 28.6 ($C_{5'}$), 25.6 ($C_{3'}$), 22.0 ($C_{7'}$) 13.8 ($C_{8'}$). Elem. Anal. Calcd for $C_{16}H_{26}O_2$: C, 76.75; H, 10.47; found: C, 76.45; H, 10.42; HRMS, 250.1926 (2.7 ppm).

4-(2-Dodecyloxyethyl)phenol (**4n**): colourless liquid (89% yield); ^{1}H-NMR δ ppm 9.08 (s, 1H, phenolic O*H*), 6.97 (d, J = 8.5 Hz, 2H, H_5), 6.64 (d, J = 8.5 Hz, 2H, H_4), 3.45 (t, J = 7.1 Hz, 2H, H_1), 3.32 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.65 (t, J = 7.1 Hz, 2H, H_2), 1.44 (m, 2H, $H_{2'}$), 1.22 (m, 18H, $H_{3'}$–$H_{11'}$), 0.84 (t, J = 7.0 Hz, 3H, $H_{12'}$); ^{13}C-NMR δ ppm 155.4 (C_6), 129.5 (C_4), 128.9 (C_3), 114.8 (C_5), 71.3 (C_1), 69.8 ($C_{1'}$), 34.7 (C_2), 31.2 ($C_{10'}$), 29.1 ($C_{2'}$), 28.9–28.6 ($C_{4'}$, $C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$, $C_{9'}$), 25.6 ($C_{3'}$), 22.0 ($C_{11'}$) 13.8 ($C_{12'}$). Elem. Anal. Calcd for $C_{20}H_{34}O_2$: C, 78.38; H, 11.18; found: C, 77.99; H, 11.02; HRMS, 306.2546 (4.2 ppm).

2-Methoxy-4-(2-methoxyethyl)phenol (**4o**): colourless liquid (78% yield); ^{1}H-NMR δ ppm 8.63 (s, 1H, phenolic O*H*), 6.76 (d, J = 1.9 Hz, 1H, H_4), 6.65 (d, J = 7.9 Hz, 1H, H_7), 6.58 (dd, 1H, H_8), 3.72 (s, 3H, *OMe* in pos. 5), 3.46 (t, J = 7.1 Hz, 2H, H_1), 3.22 (s, 3H, $H_{1'}$), 2.68 (t, J = 7.1 Hz, 2H, H_2); ^{13}C-NMR δ ppm 147.2 (C_5), 144.7 (C_6), 129.6 (C_3), 120.8 (C_8), 115.2 (C_7), 113.0 (C_4), 73.1 (C_1), 57.7 ($C_{1'}$), 55.5 (*OMe* in pos. 5), 34.9 (C_2). Elem. Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74; found: C, 66.33; H, 8.05; HRMS, 182.0949 (3.3 ppm).

2-Methoxy-4-(2-ethoxyethyl)phenol (**4p**): colourless liquid (80% yield); ^{1}H-NMR δ ppm 8.62 (s, 1H, phenolic O*H*), 6.77 (d, J = 1.9 Hz, 1H, H_4), 6.65 (d, J = 7.9 Hz, 1H, H_7), 6.58 (dd, 1H, H_8), 3.72 (s, 3H, *OMe* in pos. 5), 3.49 (t, J = 7.2 Hz, 2H, H_1), 3.41 (q, J = 7.0 Hz, 2H, $H_{1'}$), 2.67 (t, J = 7.2 Hz, 2H, H_2), 1.08 (t, J = 6.9 Hz, 3H, $H_{2'}$); ^{13}C-NMR δ ppm 147.2 (C_5), 144.7 (C_6), 129.6 (C_3), 120.8 (C_8), 115.2 (C_7), 113.0 (C_4), 71.0 (C_1), 65.1 ($C_{1'}$), 55.5 (*OMe* in pos. 5), 35.1 (C_2), 15.0 ($C_{2'}$). Elem. Anal. Calcd for $C_{11}H_{16}O_3$: C, 67.32; H, 8.22; found: C, 67.21; H, 8.30; HRMS, 196.1106 (3.3 ppm).

2-Methoxy-4-(2-propyloxyethyl)phenol (**4q**): colourless liquid (83% yield); ^{1}H-NMR δ ppm 8.66 (s, 1H, phenolic O*H*), 6.78 (d, J = 1.8 Hz, 1H, H_4), 6.64 (d, J = 7.9 Hz, 1H, H_7), 6.58 (dd, 1H, H_8), 3.72 (s, 3H, *OMe* in pos. 5), 3.49 (t, J = 7.2 Hz, 2H, H_1), 3.31 (t, J = 6.7 Hz, 2H, $H_{1'}$), 2.67 (t, J = 7.2 Hz, 2H, H_2), 1.48 (m, 2H, $H_{2'}$), 0.84 (t, J = 7.3 Hz, 3H, $H_{3'}$); ^{13}C-NMR δ ppm 147.2 (C_5), 144.7 (C_6), 129.6 (C_3), 120.9 (C_8), 115.2 (C_7), 113.0 (C_4), 71.5 ($C_{1'}$), 71.3 (C_1), 55.5 (*OMe* in pos. 5), 35.2 (C_2), 22.5 ($C_{2'}$), 10.4 ($C_{3'}$). Elem. Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63; found: C, 68.35; H, 8.65; HRMS, 210.1252 (−1.9 ppm).

2-Methoxy-4-(2-butyloxyethyl)phenol (**4r**): colourless liquid (81% yield); ^{1}H-NMR δ ppm 8.62 (s, 1H, phenolic O*H*), 6.77 (d, J = 1.9 Hz, 1H, H_4), 6.65 (d, J = 7.9 Hz, 1H, H_7), 6.58 (dd, 1H, H_8), 3.72 (s, 3H, *OMe* in pos. 5), 3.49 (t, J = 7.1 Hz, 2H, H_1), 3.35 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.67 (t, J = 7.1 Hz, 2H, H_2), 1.45 (m, 2H, $H_{2'}$), 1.29 (m. 2H, $H_{3'}$), 0.85 (t, J = 7.3, 3H, $H_{4'}$); ^{13}C-NMR δ ppm 147.2 (C_5), 144.7 (C_6), 129.6 (C_3), 120.8 (C_8), 115.1 (C_7), 113.0 (C_4), 71.2 (C_1), 69.5 ($C_{1'}$), 55.4 (*OMe* in pos. 5), 35.1 (C_2), 31.3 ($C_{2'}$), 18.8 ($C_{3'}$), 13.7 ($C_{4'}$). Elem. Anal. Calcd for $C_{13}H_{20}O_3$: C, 69.61; H, 8.99; found: C, 69.04; H, 9.30; HRMS, 224.1418 (2.5 ppm).

2-Methoxy-4-(2-hexyloxyethyl)phenol (**4s**): colourless liquid (86% yield); ^{1}H-NMR δ ppm 8.61 (s, 1H, phenolic O*H*), 6.77 (d, J = 1.9 Hz, 1H, H_4), 6.64 (d, J = 7.9 Hz, 1H, H_7), 6.58 (dd, 1H, H_8), 3.72 (s, 3H, *OMe* in pos. 5), 3.49 (t, J = 7.1 Hz, 2H, H_1), 3.34 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.66 (t, J = 7.1 Hz, 2H, H_2), 1.46 (m, 2H, $H_{2'}$), 1.24 (m. 6H, $H_{3'}$-$H_{5'}$), 0.84 (t, J = 7.1 Hz, 3H, $H_{4'}$); ^{13}C-NMR δ ppm 147.2 (C_5), 144.7 (C_6), 129.6 (C_3), 120.8 (C_8), 115.1 (C_7), 113.0 (C_4), 71.2 (C_1), 69.2 ($C_{1'}$), 55.4 (*OMe* in pos. 5), 35.1 (C_2), 29.1 ($C_{2'}$), 29.1 ($C_{4'}$), 25.3 ($C_{3'}$), 22.1 ($C_{5'}$), 13.8 ($C_{6'}$). Elem. Anal. Calcd for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59; found: C, 71.33; H, 9.59; HRMS, 252.1725 (-0.2 ppm).

2-Methoxy-4-(2-octyloxyethyl)phenol (**4t**): colourless liquid (79% yield); ^{1}H-NMR δ ppm 8.65 (s, 1H, phenolic O*H*), 6.77 (d, J = 1.7 Hz, 1H, H_4), 6.64 (d, J = 7.9 Hz, 1H, H_7), 6.57 (dd, 1H, H_8), 3.72 (s, 3H, *OMe* in pos. 5), 3.48 (t, J = 7.1 Hz, 2H, H_1), 3.34 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.66 (t, J = 7.1 Hz, 2H, H_2), 1.45 (m, 2H, $H_{2'}$), 1.22 (m. 10H, $H_{3'}$-$H_{7'}$), 0.84 (t, J = 7.0 Hz, 3H, $H_{8'}$); ^{13}C-NMR δ ppm 147.2 (C_5), 144.7 (C_6), 129.6 (C_3), 120.8 (C_8), 115.1 (C_7), 112.9 (C_4), 71.3 (C_1), 69.9 ($C_{1'}$), 55.4 (*OMe* in pos. 5), 35.1 (C_2), 31.2 ($C_{6'}$) 29.2 ($C_{2'}$), 28.7 ($C_{4'}$), 28.6($C_{5'}$), 25.7 ($C_{3'}$), 22.0 ($C_{7'}$), 13.8 ($C_{8'}$). Elem. Anal. Calcd for $C_{17}H_{28}O_3$: C, 72.82; H, 10.06; found: C, 72.78; H, 10.03; HRMS, 280.2033 (−1.9 ppm).

2-Methoxy-4-(2-dodecyloxyethyl)phenol (**4u**): colourless liquid (72% yield); ^{1}H-NMR δ ppm 8.62 (s, 1H, phenolic O*H*), 6.77 (d, J = 1.9 Hz, 1H, H_4), 6.64 (d, J = 7.9 Hz, 1H, H_7), 6.57 (dd, 1H, H_8), 3.72 (s, 3H, *OMe* in pos. 5), 3.48 (t, J = 7.1 Hz, 2H, H_1), 3.34 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.66 (t, J = 7.1 Hz, 2H, H_2), 1.45 (m, 2H, $H_{2'}$), 1.22 (m. 18H, $H_{3'}$-$H_{11'}$), 0.84 (t, J = 7.0 Hz, 3H, $H_{12'}$); ^{13}C-NMR δ ppm 147.2 (C_5), 144.7 (C_6), 129.6 (C_3), 120.8 (C_8), 115.1 (C_7), 113.0 (C_4), 71.2 (C_1), 69.8 ($C_{1'}$), 55.4 (*OMe* in pos. 5), 35.1 (C_2), 31.2 ($C_{10'}$) 29.1 ($C_{2'}$), 28.9–28.6 ($C_{4'}$,$C_{5'}$, $C_{6'}$, $C_{7'}$, $C_{8'}$, $C_{9'}$), 25.6 ($C_{3'}$), 22.0 ($C_{11'}$), 13.8 ($C_{12'}$). Elem. Anal. Calcd for $C_{21}H_{36}O_3$: C, 74.95; H, 10.98; found: C, 74.92; H, 10.76; HRMS, 336.2669 (1.4 ppm).

2.3. Evaluation of oxidative stability by the Rancimat Method

The oxidative stability was evaluated by an accelerated automated test using the Rancimat equipment (Model 743, Metrohm Co. Basel, Switzerland). A lipid matrix was obtained from virgin olive oil (VOO) of 'Arbequina' variety by purification through alumina (Yoshida, Kondo, & Kajimoto, 1992), according to the 'free solvent' procedure. The purified matrix, free of antioxidants, was stored at −18 °C under nitrogen atmosphere. Absence of polyphenols and tocopherols were checked by solid phase extraction and HPLC analysis with UV detector at 280 nm and C18 Column (Teckknokroma, 5 μm, 25 cm × 4,6 mm i.d.) (Mateos et al., 2001) and by HPLC analysis on a silica gel column (Lichrorb SI, 5 μm 25 cm × 4.0 cm i.d.) using a UV–Visible detector at 292 nm (Paquot & Hautfenne, 1992), respectively. The fatty acid composition of the matrix was C16:0 (15.0%), C16:1 (1.4%), C17:0 (0.1%), C17:1 (0.2%), C18:0 (1.8%), C18:1 (70.2%), C18:2 (10.0%), C18:3 (0.5%), C20:0 (0.4%), C20:1 (0.3%) and C22:0 (0.1%). Aliquots of the glyceridic matrix were spiked with increasing amounts of the new prepared compounds, ranging from 0.2 to 2.2 mmol/kg and then subjected to accelerated oxidation in a Rancimat apparatus at 80 °C. Results are expressed as induction time (IT) in hours corresponding to the stability of the lipid matrix evaluated. All determinations were carried out in duplicate.

2.4. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out according to the procedure described by Benzie and Strain (Benzie & Strain, 1996), with some modifications (Pulido, Bravo, & Saura-Calixto, 2000). The antioxidant potential of the synthesized compounds was estimated from their ability to reduce the ferric tripyridyltriazine (TPTZ–Fe^{III}) complex to its stable ferrous form (TPTZ–Fe^{II} complex). Briefly, the FRAP reagent contained 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, plus 2.5 ml of 20 mM $FeCl_3{\cdot}6H_2O$ and 25 ml of 0.3 M acetate buffer to a final pH of 3.6. This reagent was freshly prepared and warmed to 37 °C prior to its use. 900 μl of FRAP was mixed with 90 μl of distiled water and 30 μl of either test sample (ranging from 500 to 10000 μM for Ty (**1b**) and its derivatives (**4h–n**) and from 100 to 800 μM for HMV (**1c**) and its derivatives (**4o–u**)), standard, or methanol (as appropriate reagent blank), and the mixture was shaken. Readings at the absorption maximum at 595 nm were taken every 20 s, and the reaction was monitored up to 30 min at 37 °C, using a UV–Visible Varian (Cary 50 BIO, Holland) spectrophotometer, equipped with a thermostatted auto-cell-holder. The reading at 30 min was selected in each case for the calculation of FRAP values. Methanolic solutions of Trolox were used for calibration. The FRAP values are expressed as mM TE (Trolox Equivalent). All analyses were run in triplicate.

2.5. ABTS assay

The free-radical scavenging capacity was measured using the ABTS decolouration method (Re et al., 1999) with some modifications. The method is based on the capacity of different components to scavenge the ABTS radical cation ($ABTS^{\cdot+}$) compared to a standard antioxidant (Trolox). Briefly, ABTS was dissolved in a 2.45 mM $K_2S_2O_8$ solution and stored in the dark at room temperature for 12–16 h, to get a 7 mM concentration of ABTS radical cation ($ABTS^{\cdot+}$) stock solution. The $ABTS^{\cdot+}$ stock solution was diluted with methanol to get an absorbance of 0.70 ± 0.02 at 730 nm. After the addition of 0.1 ml of sample (ranging from 200 to 2000 μM for Ty (**1b**) and its derivatives (**4h–n**) and from 100 to 800 μM for HMV (**1c**) and its derivatives (**4o–u**)), methanol as a blank, or Trolox standard to 3.9 ml of diluted $ABTS^{\cdot+}$ solution, absorbance readings were taken every 20 s at 30 °C over 6 min, using a UV–Visible Varian (Cary 50 BIO, Holland) spectrophotometer, equipped with a thermostatted auto-cell-holder. The percentage inhibition of absorbance versus time was plotted, and the area below the curve (0–360 s) was calculated. Methanolic solutions of known concentrations of Trolox were used for calibration. Results are expressed in mM TE (Trolox Equivalent). Each value is the average of three determinations.

2.6. ORAC assay

The oxygen radical scavenging capacity was measured by the lipophilic ORAC assay according to the method developed by Huang (Huang, Ou, Hampsch-Woodill, Flanagan, & Deemer, 2002) with some modifications. This assay is based on the fluorescence decay of a reference substance (fluorescein) after the addition of a peroxyl radical (AAPH), which acts as an initiator of the oxidative reaction. Ty (**1b**) and its derivatives (**4h–n**) from 5 to 50 μM, HMV (**1c**) and its derivatives (**4o–u**) from 5 to 40 μM and Trolox standard (6.25, 12.5, 25, 50, 75 and 100 μM) were dissolved in 7% methylated β-cyclodextrin (RMCD) in acetone/water (1:1, v/v) solution. Then, 25 μl of either trolox or test sample or solvent as blank were added to a 96-well microplate followed by the addition of 150 μl of fluorescein work solution (8.5×10^{-5} mM) prepared in 75 mM phosphate buffer (pH 7.4). The microplate reader (Bio-Tek, Winooski, VT, USA) was programmed to record every two minutes for 120 min at 485 and 528 nm excitation and emission wavelengths, respectively, the fluorescence after the addition of 30 μl of AAPH (153 mM) as peroxyl radical generator, prepared also in 75 mM phosphate buffer (pH 7.4). Limit of quantification was set at 5 μM. Each value is the average of four determinations. Final results were calculated according the following equation:

$$\mathrm{ORAC_{Value}} = \frac{(\mathrm{AUC_{Sample}} - \mathrm{AUC_{Blank}})}{(\mathrm{AUC_{Trolox}} - \mathrm{AUC_{Blank}})} \cdot \frac{[\mathrm{Trolox}]}{[\mathrm{Sample}]}$$

where AUC_{Sample} is the area under curve in the presence of the tested compounds; AUC_{Blank} is the area under curve of control; AUC_{Trolox} is the area under curve in the presence of Trolox, and [Trolox] and [Sample] are the molar concentrations of Trolox and tested compounds, respectively. ORAC values are expressed as micromoles of Trolox/micromoles of antioxidant compound.

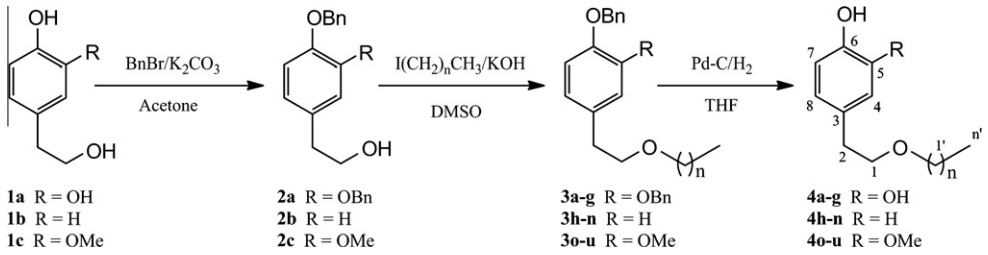
2.7. Statistical analysis

Results are expressed as means ± standard deviation (SD) of three measurements for the analytical determination. The data were subjected to a one-way analysis of variance (ANOVA) using Statistix 8.0. The level of significance was $p < 0.05$.

3. Results

3.1. Preparation and characterisation of tyrosyl (*4h–n*) and homovanillyl (*4o–u*) alkyl ethers

To carry out the synthesis of the new compounds, commercial tyrosol (**1b**) and homovanillic alcohol (**1c**) were used. Pure tyrosol and homovanillic alcohol were transformed into their benzyl derivatives, **2b** and **2c**, respectively, by reaction with benzyl bromide/potassium carbonate in acetone (Ajao et al., 1985; Battersbay et al., 1961). Further alkylation of the remaining free hydroxylic group with the corresponding alkyl iodides yielded the intermediate compounds, **3h–u**, in good to excellent yields. In the final step, desired alkyl tyrosyl and homovanillyl ethers, **4h–u**, were obtained in good overall yield by hydrogenolytical cleavage of the protecting benzyl groups (Scheme 1). Synthesized compounds were characterised by MS spectroscopy, as well as by their elemental analyses.



Scheme 1. Synthesis of new alkyl ethers.

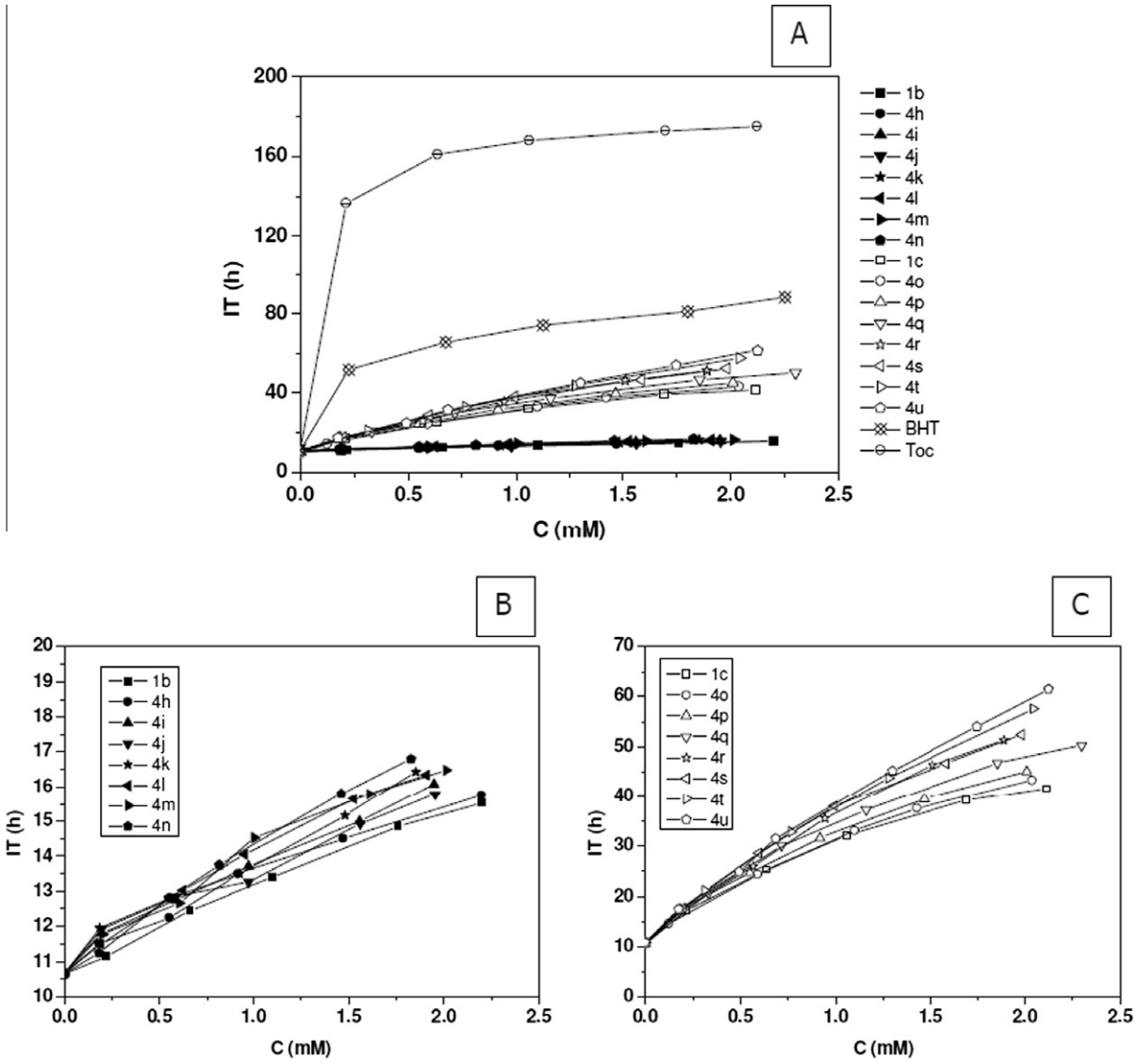


Fig. 1. Induction times (ITs) of lipid matrices spiked with the assayed compounds: (Panel A) tyrosol (**1b**), homovanillic alcohol (**1c**), tyrosyl derivatives (**4h–n**), homovanillyl derivatives (**4o–u**), α-tocopherol and BHT; (Panel B) tyrosol (**1b**) and tyrosyl derivatives (**4h–n**); (Panel C) homovanillic alcohol (**1c**) and homovanillyl derivatives (**4o–u**).

Their structures were unambiguously determined by NMR spectroscopy (see Experimental part).

3.2. Antioxidant activity in lipid matrices

The efficacy of the new synthesized compounds as antioxidants in food in comparison with two widely used synthetic antioxidants (α-tocopherol and BHT) was evaluated using the Rancimat method. The most standard temperature used for Rancimat analysis is 98 °C; although higher temperatures than 98 °C (100–120 °C) have been also reported to reduce the analysis time (Mateos, Uceda, Aguilera, Escuderos, & Beltrán, 2006). However, taking into account the low activity previously reported for free Ty (**1b**) (Mateos et al., 2003), and in order to find greater differences in the activity of the new synthesized compounds, an experimental temperature of 80 °C was selected for this determination. Fig. 1 (Panel A) shows the induction times (IT) corresponding to purified matrices of olive oil spiked with different concentrations of the new ethers (**4h–u**),

Ty (**1b**) or HMV (**1c**), BHT and α-tocopherol. Results indicate that all new prepared ethers maintain or even overcome the activity of their respective free biophenols (**1b** or **1c**) (Panel B and C). Besides, as expected, the homovanillyl alkyl ether series (**4o–u**) showed a better protecting capacity against oxidation in lipid matrices than the tyrosyl alkyl ether series (**4h–n**) although it did not reach the activity showed by α-tocopherol or BHT (Panel A).

3.3. Ferric-reducing antioxidant power (FRAP)

The reducing capacities of **4h–u** in comparison with their precursors, **1b** and **1c**, and two synthetic antioxidants widely used, α-tocopherol and BHT, were determined by the FRAP assay. The results summarised in Table 1 are expressed as mM TE (Trolox equivalent). The reducing capacities of Ty (**1b**) and its ethers (**4h–n**) was significantly lower than those shown by HMV (**1c**) and its derivatives (**4o–u**) and the references α-tocopherol and BHT. In fact, HMV and its derivatives showed to be the most active compounds studied. Exceptions were the higher homologous ethers of this serie, compounds **4t** and **4u**, which showed lower reducing capacities than BHT. Comparison of the results obtained for the two synthetic series of ethers (Table 1) with their respective free phenols, Ty (**1b**) and HMV (**1c**), revealed a contradictory trend. While tyrosyl ethers (**4h–n**) showed significantly lower reducing activities than their natural precursor, Ty (**1b**), homovanillyl ethers (**4o–u**) showed a variable behaviour depending on side-chain length, with higher activity than the parent compound HMV (**1c**) for those less lipophilic ethers (methyl (**4o**), ethyl (**4p**) and propyl (**4q**)), similar for the butyl homovanillyl ether (**4r**) and lower activity for the more lipophilic hexyl, octyl and, specially, dodecyl homovanillyl ethers (**4s–u**).

3.4. The ABTS assay

The radical-scavenging activities of the evaluated compounds (**4h–u**), Ty (**1b**), HMV (**1c**), BHT and α-tocopherol are summarised in Table 2. Results are expressed as mM TE (Trolox equivalent). The order of the scavenging activities towards the ABTS radical was HMV and its derivatives (**1c**, **4o–u**) > α-tocopherol > tyrosol and its derivatives (**1b**, **4h–m**) > BHT > dodecyl tyrosyl ether (**4n**). In addition, trends on the antioxidant activity observed within each series were similar to those described above for the ferric reducing activity.

3.5. The ORAC assay

The antioxidant capacity of Ty (**1b**), HMV (**1c**) and their derivatives (**4h–u**), BHT and α-tocopherol determined by ORAC assay is reported in Table 3. Also, slopes and intercepts of linear function obtained after plotting net AUC versus the concentration are summarised in Table 3. As expected, HMV and its derivatives (**1c**, **4o–u**) exerted higher protection than Ty and its derivatives (**1b**, **4h–m**) against the loss of fluorescence provoked by free radicals. Regarding the commonly used antioxidants, BHT possessed the lowest ORAC value of all assayed compounds, while α-tocopherol showed similar radical scavenging activity in comparison with Ty (**1b**) and its derivatives (**4h–m**) but lower than HMV (**1c**) and its derivatives (**4o–u**). Furthermore, the behaviour observed within each series was similar to that described above for the ferric reducing activity and for radical-scavenging activities by ABTS assay.

Table 1
Reducing antioxidant power evaluated by the FRAP assay of tyrosol (**1b**), homovanillic alcohol (**1c**), tyrosyl derivatives (**4h**–**n**), homovanillyl derivatives (**4o**–**u**), α-tocopherol and BHT. Each value is the mean of triplicate measurements ± standard deviations. Results are expressed as mM TE (Trolox Equivalents). All values within a column with different superscript letters are significantly different, $p < 0.05$.

Compound	mM TE	Compound	mM TE
Ty (**1b**)	0.31 ± 0.03^{c}	HMV (**1c**)	$1.44 \pm 0.02^{f,g}$
Methyl_Ty (**4h**)	0.16 ± 0.01^{b}	Methyl_HMV (**4o**)	1.47 ± 0.03^{g}
Ethyl_Ty (**4i**)	0.18 ± 0.03^{b}	Ethyl_HMV (**4p**)	1.60 ± 0.03^{h}
Propyl_Ty (**4j**)	0.16 ± 0.02^{b}	Propyl_HMV (**4q**)	1.61 ± 0.03^{h}
Butyl_Ty (**4k**)	0.16 ± 0.02^{b}	Butyl_HMV (**4r**)	$1.41 \pm 0.03^{e,f}$
Hexyl_Ty (**4l**)	$0.15 \pm 0.02^{a,b}$	Hexyl_HMV (**4s**)	1.37 ± 0.02^{d}
Octyl_Ty (**4m**)	0.11 ± 0.02^{a}	Octyl_HMV (**4t**)	1.05 ± 0.02^{c}
Dodecyl_Ty (**4n**)	$0.13 \pm 0.02^{a,b}$	Dodecyl_HMV (**4u**)	0.87 ± 0.02^{b}
BHT	1.32 ± 0.05^{e}	BHT	1.32 ± 0.05^{d}
α-Tocopherol	0.80 ± 0.04^{d}	α-Tocopherol	0.80 ± 0.04^{a}

Table 2
Radical-scavenging capacity evaluated by the ABTS assay of tyrosol (**1b**), homovanillic alcohol (**1c**), tyrosyl ethers (**4h–n**), homovanillyl ethers (**4o–u**), α-tocopherol and BHT. Each value is the mean of triplicate measurements ± standard deviations. Results are expressed as mM TE (Trolox Equivalents). All values within a column with different superscript letters are significantly different, $p < 0.05$.

Compound	mM TE	Compound	mM TE
Ty (**1b**)	0.39 ± 0.01^{e}	HMV (**1c**)	1.29 ± 0.01^{e}
Methyl_Ty (**4h**)	$0.30 \pm 0.01^{c,d}$	Methyl_HMV (**4o**)	1.37 ± 0.01^{g}
Ethyl_Ty (**4i**)	0.37 ± 0.01^{e}	Ethyl_HMV (**4p**)	1.32 ± 0.01^{f}
Propyl_Ty (**4j**)	0.31 ± 0.01^{d}	Propyl_HMV (**4q**)	1.49 ± 0.02^{h}
Butyl_Ty (**4k**)	$0.29 \pm 0.01^{b,c}$	Butyl_HMV (**4r**)	1.33 ± 0.01^{f}
Hexyl_Ty (**4l**)	$0.30 \pm 0.01^{c,d}$	Hexyl_HMV (**4s**)	1.27 ± 0.01^{e}
Octyl_Ty (**4m**)	$0.30 \pm 0.01^{c,d}$	Octyl_HMV (**4t**)	1.06 ± 0.01^{c}
Dodecyl_Ty (**4n**)	0.18 ± 0.01^{a}	Dodecyl_HMV (**4u**)	1.13 ± 0.01^{d}
BHT	0.27 ± 0.01^{b}	BHT	0.27 ± 0.01^{a}
α-Tocopherol	1.01 ± 0.02^{f}	α-Tocopherol	1.01 ± 0.02^{b}

4. Discussion

An increasingly strict regulation of the use of additives in foods, particularly within the European Union, is leading towards significant reductions in the number of antioxidants available for use as food preservatives. In particular, it cannot be ruled out a limitation in the number of the already few lipophilic antioxidants available for use in foods because concerns on the safety of current synthetic compounds such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluane), TBHQ (tertiary butylhydroquinone) and PG (propyl gallate) may lead to them being banned. Currently available natural lipophilic antioxidants are tocopherols (e.g. vitamin E), beta-carotene, in some cases, tocotrienols, and phospholipids (e.g. lecithin) in combination with phenolic antioxidants. For this reason, there is major interest by the food industry for the development of new lipophilic antioxidants for use as functional ingredients for incorporation into lipid food matrices such as fats and oils, processed foods, margarines that contain animal fats, milk fat, or frozen fish or fish oil among others (Chillemi et al., 2010; Pokorny, 1991, 2007). This is a challenge for food scientists since most phytochemicals with antioxidant activity are of hydrophilic nature. In order to find useful alternatives to synthetic additives, two series of potentially biologically active compounds, tyrosyl and homovanillyl ethers with linear alkylic side chains of variable length from 1 to 12 carbon atoms, **4h–u** (Scheme 1) have been synthesized, starting from two characteristic phenolic compounds of virgin olive oil as Ty (**1b**) and homovanillic alcohol (**1c**) by a straight three-steps procedure, similar to that previously described (Madrona et al., 2009) for the synthesis of hydroxytyrosyl ether derivatives (**4a–g**). Thus, protection of the phenolic OH by reaction with BnBr and K_2CO_3 in acetone, followed by the introduction of the alkyl side chain after reacting with the corresponding alkyl iodide in presence of KOH, and cleavage of protecting benzyl group by hydrogenolysis, constituted the main steps involved in the

Table 3
Radical-scavenging capacity evaluated by ORAC assay of tyrosol (**1b**), homovanillic alcohol (**1c**), tyrosyl ethers (**4h–n**), homovanillyl ethers (**4o–u**), α-tocopherol and BHT. ORAC values are expressed as μmol Trolox/μmol tested compound. Linear range used to plot Net AUC versus concentration, slopes and intercepts of the linear functions are also indicated. Each value is the mean of quadruplicate measurements ± standard deviations. All values with different superscript letters are significantly different, $p < 0.05$.

Compound	ORAC value	Conc. Range (μM)	Slope	Intercept	R^2
Ty (**1b**)	0.79 ± 0.04^{f}	5–20	0.2945	1.6529	0.9908
Methyl_Ty (**4h**)	$0.57 \pm 0.06^{b,c}$	5–20	0.2622	0.6876	0.9988
Ethyl_Ty (**4i**)	0.47 ± 0.05^{b}	5–20	0.2239	0.5062	0.9936
Propyl_Ty (**4j**)	$0.64 \pm 0.05^{c,d}$	5–20	0.1630	2.0843	0.9980
Butyl_Ty (**4k**)	$0.69 \pm 0.05^{d,e}$	5–20	0.2517	1.4854	0.9980
Hexyl_Ty (**4l**)	$0.62 \pm 0.02^{c,d}$	5–20	0.2357	1.2597	0.9986
Octyl_Ty (**4m**)	0.69 ± 0.03^{e}	20–40	0.3352	2.0080	0.9945
Dodecyl_Ty (**4n**)	0.68 ± 0.03^{e}	20–50	0.2217	5.1970	0.9998
HMV (**1c**)	$1.15 \pm 0.04^{i,j}$	5–40	0.4597	2.1122	0.9920
Methyl_HMV (**4o**)	1.36 ± 0.01^{l}	5–40	0.4692	3.2223	0.9903
Ethyl_HMV (**4p**)	1.25 ± 0.04^{k}	5–40	0.4758	2.4999	0.9968
Propyl_HMV (**4q**)	$1.33 \pm 0.04^{k,l}$	5–40	0.5334	2.4077	0.9981
Butyl_HMV (**4r**)	$1.09 \pm 0.04^{h,i}$	5–40	0.4014	2.3145	0.9992
Hexyl_HMV (**4s**)	1.18 ± 0.02^{j}	10–40	0.4337	2.5589	0.9961
Octyl_HMV (**4t**)	1.04 ± 0.05^{h}	10–40	0.2039	3.9922	0.9994
Dodecyl_HMV (**4u**)	0.92 ± 0.02^{g}	10–40	0.2125	3.2015	0.9998
BHT	0.12 ± 0.01^{a}	50–400	0.0425	2.9380	0.9989
α-Tocopherol	$0.63 \pm 0.06^{c,d,e}$	12.5–100	0.2174	3.7231	0.9963

preparation of tyrosyl and homovanillyl alkyl ethers with good yield.

Then, their potential antioxidant activities were evaluated by different methods and compared with their precursors Ty (**1b**) and HMV (**1c**), and others two controls traditionally used as food antioxidants: butylated hydroxytoluene (BHT) and α-tocopherol. The selected methods to evaluate their antioxidant capacity were, as it was explained above, the Rancimat test and the FRAP, ABTS and ORAC assays.

An overall evaluation of the described results showed the higher antioxidant capacity for homovanillic alcohol (**1c**) and its alkyl ether derivatives (**4o–u**) in comparison with Ty (**1b**) and its derivatives (**4h–n**). In the particular case of the Rancimat test (Fig. 1), comparison of IT values with those previously reported for HTy (**1a**) and its alkyl derivatives (**4a–g**) (Pereira-Caro et al., 2009) pointed out the higher influence of the *ortho*-diphenolic structure on the antioxidant capacity present in the hydroxytyrosyl series, in comparison with the monophenolic (**1b**, **4h–n**) and 3-methoxy-4-hydroxyphenolic series (**1c**, **4o–u**), respectively. However, the higher activity described above for homovanillyl ethers in comparison with tyrosyl derivatives indicated the positive influence of the methoxy group in *ortho* position to the hydroxylic group, in agreement with the described stabilization of the phenoxyl radical by ortho substitutions with electron-donating groups as alkyl or methoxy groups, among others (Chimi, Cillard, Cillard, & Rahmani, 1991; Pokorny, 1987). Finally, it is important to remark that, in spite of the higher activity of homovanillyl series (**1c**, **4o–u**) versus tyrosyl series (**1b**, **4h–n**) showed in the Rancimat test, it did not reach the activity of the references evaluated, BHT and α-tocopherol, significantly more active than the two synthetic series (**4h–u**) and their references (**1b** and **1c**), due to their chemical structures with methyl group in position *para* to the functional hydroxylic group in the chromanol ring of vitamin E and phenolic group of BHT, fundamental to the hydrogen donating ability implied in the lipidic peroxidation evaluated by this test.

Regarding the activity showed within each series by the Rancimat test (Fig. 1b and c), all the alkyl derivatives were slightly more active than their respective precursors, Ty (**1b**) or HMV (**1c**), and, in the specific case of homovanillyl series (**1c**, **4o–u**), a direct relationship between lipophilic nature and antioxidant activity can been observed (Fig. 1c). In this sense, the results obtained here were contrary to the polar paradox by which in non-polar medium the most potent antioxidant should be the most polar one. Besides, these results were in disagreement with those previously reported for the hydroxytyrosyl series (Pereira-Caro et al., 2009), whose alkyl derivatives (**4a–g**) resulted less actives than free hydroxytyrosol and no relationship was found between the length and nature of the alkyl side chain and the stability of lipid matrices.

On the other hand, the reducing and radical-scavenging capacities of the new synthesized compounds (**4h–u**) depicted in Tables 1–3, were significantly higher for homovanillyl derivatives (**4o–u**) in comparison with tyrosyl ones (**4h–n**). This great difference of activity between both series was somehow surprising taking into account that 3-methoxy-4-hydroxyphenolic series presents the *ortho*-diphenolic structure blocked by a methoxy group that could affect their antioxidant activity as indeed occurred in the Rancimat analysis, where HTy (**1a**) was substantially more antioxidant than Ty (**1b**) and HMV (**1c**). Furthermore, FRAP and ABTS activities of HMV (**1c**) (1.44 and 1.29 mM Trolox Equivalent, respectively) were somewhat higher than that described for HTy (1.39 and 0.84 mM Trolox Equivalent, respectively), previously reported by our group (Pereira-Caro et al., 2009). However, ORAC activity of HMV (**1c**) was significantly lower than in the case of HTy (1.15 and 2.28 as μmol Trolox/μmol tested compound, respectively).

Recently, the radical-scavenging activity of HTy (**1a**), Ty (**1b**), and HMV (**1c**), as well as some hydroxytyrosyl and HMV esters determined by the DPPH method has been reported (Grasso et al., 2007). Apart from the higher antioxidant capacity of lipophilic hydroxytyrosyl analogues in comparison with the homovanillyl series or free Ty (**1b**), substitution of the hydroxylic function in the aromatic ring by a methoxy group provoked a drastic decrease in the antioxidant activity, being HMV (**1c**) and its esters even less active than free Ty (**1b**). These results agreed with that reported by Torres de Pinedo, Peñalver, & Morales, 2007; Torres de Pinedo, Peñalver, Pérez-Victoria et al., 2007, where *ortho*-diphenolic alcohols (protocatechuyl alcohol, hydroxytyrosol, dihydrocaffeoyl alcohol and caffeoyl alcohol) showed better radical-scavenging activity evaluated by DPPH assay than did the corresponding monomethoxylated cathecolic alcohols (vanillyl alcohol, homovanillyl alcohol, dihydroconifeyl alcohol and coniferyl alcohol). However, these same authors evaluated the activity of new synthetic lipophilic antioxidants derivatives of HTy in comparison with HMV derivatives by ABTS assay observing as methoxylated-cathecolic antioxidants were significantly better radical scavengers than *ortho*-diphenolic lipophilic antioxidants. This behaviour is

extensive to hydroxycinnamic acids (caffeic, ferulic and chlorogenic acids) after their evaluation by ABTS assay, where substitution of the 3-hydroxyl group of caffeic acid by a methoxyl group in ferulic acid enhanced the antioxidant capacity (Rice-Evans, Miller, & Paganga, 1996). In line with this results, Gómez-Ruiz, Leake, and Ames (2007) found that ferulic acid was more active than caffeic acid evaluated by ABTS assay while their scavenging activity evaluation by ORAC assay showed a contrary tendency (Dávalos, Gómez-Cordovés, & Bartolomé, 2004; Gómez-Ruiz et al., 2007), confirming the results presented in the present work.

In this sense, although ABTS, ORAC and DPPH have been classified as radical-scavenging activity methods in which free radicals react with a H-atom donor such as phenolics compounds, DPPH and ORAC seems to be more selective than ABTS in the reaction with H-donors and it does not react with aromatic acids containing only one OH-group (Von Gadov, Joubert, & Hansmann, 1997), while ABTS is reduced in the presence of compounds with aromatic OH-groups, independently of their real antioxidant power (Roginsky & Lissi, 2005).

On the other hand, the behaviour of the radical scavenging and reducing activities of these new lipophilic synthetic compounds (**4h–u**) in comparison with their precursors (**1b** and **1c**) was antagonistic. While tyrosyl ethers (**4h–n**) were less active than their reference Ty (**1b**), homovanillyl ethers (**4o–u**) showed a non-linear evolution of antioxidant capacity with the enhanced lipophilic nature, with a parabolic relationship between the alkyl chain length and the antioxidant activity. This behaviour agrees with that previously described for tyrosyl and hydroxytyrosyl esters (Mateos et al., 2008) and hydroxytyrosyl ethers (Pereira-Caro et al., 2009). It is interesting to remark that the characteristic non-linear evolution of antioxidant capacity of homovanillyl ethers (**4o–u**) with the enhanced lipophilic nature are partially out of the popular polar paradox and in accordance with the results reported by Laguerre et al., after the evaluation of chlorogenate esters (Laguerre et al., 2009) and rosmarinate esters (Laguerre et al., 2010) of variable acylic chain length. Besides, in order to explain the non-linear hypothesis of the antioxidant activity, evaluation of surface-active properties of lipophilic antioxidants Ty (**1b**) and hydroxytyrosol fatty acid esters in oil-in-water emulsion was carried out (Lucas et al., 2010), providing a useful tool in the rational design of antioxidants used in oil-in-water emulsions.

In conclusion, these results provide a batch of new derivatives of Ty (**1b**) and HMV (**1c**) in which the enhanced lipophilic nature is attractive for their possible commercialisation as lipophilic derivatives in the food industry. Besides, despite the variable antioxidant activity observed after their evaluation by the Rancimat test and the FRAP, ABTS and ORAC assays, where homovanillyl ethers (**4o–u**) showed the best activity as reducing agents and radical scavengers, it is important to remark on the interesting biological activity described for their precursors, Ty (**1b**) and HMV (**1c**), potentially extrapolated to their alkyl derivatives (**4h–u**) presented herein.

Acknowledgements

This work was supported by projects AGL2007-66373/ALI and CSD2007-00063 as part of the Consolider-Ingenio Program from the Spanish Ministry of Science and Innovation (CICYT), Grant RTA2007-000036-00-00 from the Research National Institute of Food, Agriculture and Technology (INIA) and a contract (110105090014) with CSIC-IFAPA. G.P.-C. is a predoctoral fellow of the National Research Institute of Food, Agriculture and Technology (INIA).

References

Ajao, J. F., Bird, C. W., & Chauhan, Y. P. (1985). The evaluation of a biogenetically based approach to the synthesis of octahydro-1h-benzofuro[3, 2-e]isoquinolines. *Tetraedron Letters, 41*, 1367–1372.

Alcudia, F., Cert, A., Espartero, J. L., Mateos, R., & Trujillo, M. (2004). Method of preparing hydroxytyrosol esters, esters thus obtained and use of same. PCT WO 2004/005237.

Appendino, G., Minassi, A., Daddario, N., Bianchi, F., & Tron, G. C. (2002). Chemoselective esterification of phenolic acids and alcohols. *Organic Letters, 4*, 3839–3841.

Battersby, A. R., Le Count, D. J., Garratt, S., & Thrift, R. I. (1961). Synthetic applications of 1,2-dihydroisoquinolines: Synthesis of (±)-coreximine. *Tetraedron, 14*, 46–53.

Beauchamp, G. K., Keast, R. S. J., Morel, D., Lin, J., Pika, J., Han, Q., et al. (2005). Ibuprofen-like activity in extra-virgin olive oil. *Nature, 437*, 45–46.

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Bernini, R., Mincione, E., Barontini, M., & Crisante, F. (2008). Convenient synthesis of hydroxytyrosol and its lipophilic derivatives from tyrosol or homovanillyl alcohol. *Journal of Agricultural and Food Chemistry, 56*, 8897–8904.

Bitler, C. M., Viale, T. M., Damaj, B., & Crea, R. (2005). Hydrolyzed olive vegetation water in mice has anti-inflammatory activity. *Journal of Nutrition, 135*, 1475–1479.

Bouallagui, Z., Bouaziz, M., Lassoued, S., Engaser, J. M., Ghoul, M., & Sayadi, S. (2011). Hydroxytyrosol acyl esters: Biosynthesis and activities. *Applied Biochemistry and Biotechnology, 163*, 592–599.

Buisman, G. J. H., Van Helteren, C. T. W., Kramer, G. F. H., Veldsink, J. W., Derksen, J. T. P., & Cuperus, F. P. (1998). Enzymatic esterifications of functionalized phenols for the synthesis of lipophilic antioxidants. *Biotechnology Letters, 20*, 131–136.

Capasso, R., Sannino, F., De Martino, A., & Manna, C. (2006). Production of triacetylhydroxytyrosol from olive mill waste waters for use as stabilized bioantioxidant. *Journal of Agricultural and Food Chemistry, 54*, 9063–9070.

Caruso, D., Visioli, F., Patelli, R., Galli, C., & Galli, G. (2001). Urinary excretion of olive oil phenols and their metabolites in humans. *Metabolism Clinical and Experimental, 50*, 1426–1428.

Chillemi, R., Sciuto, S., Spatafora, C., & Tringali, C. (2010). Hydroxytyrosol lipophilic analogues: Synthesis, radical scavenging activity and human cell oxidative damage protection. In V. R. Preedy & R. R. Watson (Eds.), *Olives and olive oil in health and disease prevention* (pp. 1233–1243). New York: Academic Press.

Chimi, H., Cillard, J., Cillard, P., & Rahmani, M. (1991). Peroxyl radical scavenging activity of some natural phenolic antioxidants. *Journal of the American Oil Chemists Society, 68*, 307–312.

Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of the oxygen radical absorbance capacity (ORAC-Fluorescein) assay. *Journal of Agricultural and Food Chemistry, 52*, 48–54.

De la Torre-Carbot, K., Chavez-Servin, J. L., Jaúregui, O., Castellote, A. I., Lamuela-Raventós, R. M., Nurmi, T., et al. (2010). Elevated circulating LDL phenol levels in men who consumed virgin rather than refined olive oil are associated with less oxidation of plasma LDL. *Journal of Nutrition, 140*, 501–508.

Dell'Agli, M., Maschi, O., Galli, G. V., Fagnani, R., Dal Cero, E., Caruso, D., et al. (2008). Inhibition of platelet aggregation by olive oil phenols via cAMP-phosphodiesterase. *British Journal of Nutrition, 99*, 945–951.

Di Benedetto, R., Vari, R., Scazzocchio, B., Filesi, C., Santangelo, C., Giovannini, C., et al. (2007). Tyrosol, the major extra virgin olive oil compound, restored intracellular antioxidant defences in spite of its weak antioxidative effectiveness. *Nutrition, Metabolism and Cardiovascular Diseases, 17*, 535–545.

Fernández-Bolaños, J., Heredia, A., Rodríguez, G., Rodríguez, R., Jiménez, A., & Gillen, R. (2005). Methods for obtaining purified hydroxytyrosol from products and by-products derived from the olive tree. US 6849,770 B2.

Fernández-Bolanos, J. G., Lopez, O., Fernández-Bolanos, J., & Rodríguez-Gutiérrez, G. (2008). Hydroxytyrosol and derivatives: Isolation, synthesis, and biological properties. *Current Organic Chemistry, 12*, 442–463.

Giovannini, C., Straface, E., Modesti, D., Coni, E., Cantafora, A., & De Vincenzi, M. (1999). Tyrosol, the major olive oil biophenol, protects against oxidized LDL-induced injury in $Caco_2$ cells. *Journal of Nutrition, 129*, 1269–1277.

Gómez-Ruiz, J. A., Leake, D. S., & Ames, J. M. (2007). In vitro antioxidant activity of coffee compounds and their metabolites. *Journal of Agricultural and Food Chemistry, 55*, 6962–6969.

Gordon, M. H., Paiva-Martins, F., & Almeida, M. (2001). Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. *Journal of Agricultural and Food Chemistry, 49*, 2480–2485.

Goya, L., Mateos, R., & Bravo, L. (2007). Effect of the olive oil phenol hydroxytyrosol on human hepatoma HepG2 cells. Protection against oxidative stress induced by tert-butylhydroperoxide. *European Journal of Nutrition, 46*, 70–78.

Grasso, S., Siracusa, L., Spatafora, C., Renis, M., & Tringali, C. (2007). Hydroxytyrosol lipophilic analogues: Enzymatic synthesis, radical scavenging activity and DNA oxidative damage protection. *Bioorganic Chemistry, 35*, 137–152.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Deemer, E. K. (2002). Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. *Journal of Agricultural and Food Chemistry, 50*, 1815–1821.

Incani, A., Deiana, M., Corona, G., Vafeiadou, K., Vauzour, D., Dessi, M. A., et al. (2010). Involvement of ERK, Akt and JNK signalling in H_2O_2-induced cell injury

and protection by hydroxytyrosol and its metabolite homovanillic alcohol. *Molecular Nutrition and Food Research, 54*, 788–796.

Laguerre, M., Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M.C., Baréa, B., Weiss, J., Decker, E.A., & Villeneuve, P. (2010). Relationship between hydrophobicity and antioxidant ability of 'Phenolipids' in emulsion: a parabolic effect of the chain length of rosmarinate esters. *Journal of Agricultural and Food Chemistry*, *58*, 2869-2876 (b).

Laguerre, M., Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M. C., Baréa, B., Weiss, J., et al. (2009). Chain length affects antioxidant properties of chlorogenate esters in emulsion: The cutoff theory behind the polar paradox. *Journal of Agricultural and Food Chemistry, 57*, 11335–11342.

Larrosa, M., Espín, J. C., & Tomás-Barberán, F. A. (2003). Antioxidant capacity of tomato juice functionalised with enzymatically synthesised hydroxytyrosol. *Journal of the Science of Food and Agriculture, 83*, 658–666.

Lucas, R., Comelles, F., Alcantara, D., Maldonado, O. S., Curcuroze, M., Parra, J. L., et al. (2010). Surface-active properties of lipophilic antioxidants tirosol and hydroxytyrosol fatty acid esters: A potencial explanation for the nonlinear hipótesis of the antioxidant activity in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 58*, 8021–8026.

Madrona, A., Pereira-Caro, G., Mateos, R., Rodriguez, G., Trujillo, M., Fernandez-Bolanos, J., et al. (2009). Synthesis of hydroxytyrosyl alkyl ethers from olive oil waste waters. *Molecules, 14*, 1762–1772.

Manna, C., Napoli, D., Cacciapuoti, G., Porcelli, M., & Zappia, V. (2009). Olive oil phenolic compounds inhibit homocysteine-induced endothelial cell adhesion regardless of their different antioxidant activity. *Journal of Agricultural and Food Chemistry, 57*, 3478–3482.

Martín, M. A., Ramos, S., Granado-Serrano, A. B., Rodríguez-Ramiro, I., Trujillo, M., Bravo, L., et al. (2010). The olive oil phenol Hydroxytyrosol induces antioxidant/detoxificant enzymes activity and Nrf2 translocation via ERKs and PI3K/AKT pathways in HepG2 cells. *Molecular Nutrition and Food Research, 54*, 1–11.

Mateos, R., Domínguez, M. M., Espartero, J. L., & Cert, A. (2003). Antioxidant effect of phenolic compounds, α-tocopherol, and other minor components in virgin olive oil. *Journal of Agricultural and Food Chemistry, 51*, 7170–7175.

Mateos, R., Espartero, J. L., Trujillo, M., Ríos, J. J., León-Camacho, M., Alcudia, F., et al. (2001). Determination of phenols, flavones, and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *Journal of Agricultural and Food Chemistry, 49*, 2185–2192.

Mateos, R., Trujillo, M., Pereira-Caro, G., Madrona, A., Cert, A., & Espartero, J. L. (2008). New lipophilic tyrosyl esters. Comparative antioxidant evaluation with hydroxytyrosyl esters. *Journal of Agricultural and Food Chemistry, 56*, 10960–10966.

Mateos, R., Uceda, M., Aguilera, M. P., Escuderos, M. E., & Beltrán, G. (2006). Relationship of rancimat method values at varying temperatures for virgin olive oils. *European Food Research and Technology, 223*, 246–252.

Medina, I., Lois, S., Alcántara, D., Lucas, R., & Morales, J. C. (2009). Effect of lipophilization of hydroxytyrosol on its antioxidant activity in fish oils and fish oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 57*, 9773–9779.

Miró-Casas, E., Covas, M. I., Farré, M., Fito, M., Ortuño, J., Weinbrenner, T., et al. (2003). Hydroxytyrosol disposition in humans. *Clinical Chemistry, 49*, 945–952.

Palozza, P., Simone, R., Picci, N., Buzzoni, L., Ciliberti, N., Natangelo, A., et al. (2008). Design, synthesis, and antioxidant potency of novel α-tocopherol analogues in isolated membranes and intact cells. *Free Radical Biology & Medicine, 44*, 1452–1464.

Paquot, C., & Hautfenne, A. (1992). *Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. Method 2, 432. Standard methods for the analysis of oils, fats and derivatives. IUPAC, revised and enlarged* (7th ed.). Oxford, UK: Blackwell Scientific Publications.

Pazos, M., Alonso, A., Sánchez, I., & Medina, I. (2008). Hydroxytyrosol prevents oxidative deterioration in foodstuffs rich in fish lipids. *Journal of Agricultural and Food Chemistry, 56*, 3334–3340.

Pereira-Caro, G., Bravo, L., Madrona, A., Espartero, J. L., & Mateos, R. (2010). Uptake and metabolism of new synthetic lipophilic derivatives, hydroxytyrosyl ethers, by human hepatoma HepG2 cells. *Journal of Agricultural and Food Chemistry, 58*, 798–806.

Pereira-Caro, G., Madrona, A., Bravo, L., Espartero, J. L., Alcudia, F., Cert, A., et al. (2009). Antioxidant activity evaluation of alkyl hydroxytyrosyl ethers, a new class of hydroxytyrosyl derivatives. *Food Chemistry, 115*, 86–91.

Pereira-Caro, G., Mateos, R., Saha, S., Madrona, A., Espartero, J. L., Bravo, L., et al. (2010). Transepithelial Transport and Metabolism of New Lipophilic Ether Derivatives of Hydroxytyrosol by Enterocyte-like Caco-2/TC7 Cells. *Journal of Agricultural and Food Chemistry, 58*, 11501–11509.

Pokorny, J. (1987). Major factors affecting the antioxidant of lipids. In H. Chan (Ed.), *Autoxidation of unsaturated lipids* (pp. 141–206). London: Academic Press.

Pokorny, J. (1991). Natural antioxidants for food use. *Trends in Food Science and Technology, 16*, 223–227.

Pokorny, J. (2007). Are natural antioxidants better – and safer – than synthetic antioxidants? *European Journal of Lipid Science and Technology, 109*, 629–642.

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry, 48*, 3396–3402.

Re, R., Pellegrini, N., Proteffente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolouration assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*, 933–956.

Rietjens, S. J., Bast, A., & Haenen, G. R. M. M. (2007). New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol. *Journal of Agricultural and Food Chemistry, 55*, 7609–7614.

Rietjens, S. J., Bast, A., Vente, J., & Haenen, G. R. M. M. (2007). The olive oil antioxidant hydroxytyrosol efficiently protects against the oxidative stress-induced impairment of the NO response of isolated rat aorta. *American Journal of Physiology Heart and Circulatory Physiology, 292*, 1931–1936.

Roginsky, V., & Lissi, E. A. (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry, 92*, 235–254.

Tofani, D., Balducci, V., Gasperi, T., Incerpi, S., & Gambacorta, A. (2010). Fatty acid hydroxytyrosyl esters: Structure/antioxidant activity relationship by ABTS and in cell-culture DCF assays. *Journal of Agricultural and Food Chemistry, 58*, 5292–5299.

Torregiani, E., Seu, G., Minassi, A., & Appendino, G. (2005). Cerium(III) chloride-promoted chemoselective esterification of phenolic alcohols. *Tetrahedron Letters, 46*, 2193–2196.

Torres de Pinedo, A., Peñalver, P., & Morales, J. C. (2007). Synthesis and evaluation of new phenolic-based antioxidants: Structure–activity relationship. *Food Chemistry, 103*, 55–61.

Torres de Pinedo, A., Peñalver, P., Pérez-Victoria, I., Rondón, D., & Morales, J. C. (2007). Synthesis of new phenolic fatty acid esters and their evaluation as lipophilic antioxidants in an oil matrix. *Food Chemistry, 105*, 657–665.

Torres de Pinedo, A., Peñalver, P., Rondon, D., & Morales, J. C. (2005). Efficient lipase-catalyzed synthesis of new lipid antioxidants based on a catechol structure. *Tetrahedron, 61*, 7654–7660.

Trujillo, M., Mateos, R., Collantes de Teran, L., Espartero, J. L., Cert, R., Jover, M., et al. (2006). Lipophilic hydroxytyrosyl esters. Antioxidant activity in lipid matrices and biological systems. *Journal of Agricultural and Food Chemistry, 54*, 3779–3785.

Visioli, F., Galli, C., Bornet, F., Mattei, A., Patelli, R., Galli, G., et al. (2000). Olive oil phenolics are dose-dependently absorbed in humans. *FEBS Letters, 468*, 159–160.

Vissers, M. H., Zock, P. L., Roodenburg, A. J., Leenen, R., & Katan, M. B. (2002). Olive oil phenols are absorbed in humans. *Journal of Nutrition, 132*, 409–417.

Von Gadov, A., Joubert, E., & Hansmann (1997). Comparison of the antioxidant activity of aspalathin with that of other plant phenols of Roobies tea (*Aspalathus linearis*), -tocopherol, BHT, and BHA. *Journal of Agricultural and Food Chemistry, 44*, 632–638.

Yoshida, H., Kondo, I., & Kajimoto, G. (1992). Participation of free fatty acids in the oxidation of purified soybean oil during microwave heating. *Journal of American Oil Chemists Society, 69*, 1136–1140.