Carbohydrate Research 344 (2009) 1340–1346

Contents lists available at ScienceDirect

Carbohydrate Research

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

Note

A concise synthesis of glucuronide metabolites of urolithin-B, resveratrol, and hydroxytyrosol

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ARTICLE INFO

Article history:
Received 14 April 2009
Received in revised form 11 May 2009
Accepted 18 May 2009
Available online 22 May 2009

Keywords:
Resveratrol
Hydroxytyrosol
Urolithin-B
Glucuronic metabolites
Glycosylation reactions
Trichloroacetimidate donor

ABSTRACT

A simple and direct strategy to chemically synthesize *O*-β-D-glucuronides of urolithin-B **4**, resveratrol **5,** and the corresponding hydroxytyrosol derivatives **6**, **7** (as a regioisomeric mixture), and **8** is described. The critical glycosylation step has been optimized using a structurally simple phenol, urolithin-B, by modification of several reaction parameters (solvent, promoter, and glucuronide donor). Very high yields have been obtained in the first synthesis of the *O*-β-D-glucuronide of urolithin-B **4**. Extension of these reaction conditions was used for the synthesis of resveratrol-3-*O*-glucuronide **5** where a higher yield than previously reported was obtained by using the much more common trichloroacetimidate glucuronide donor. Finally, three *O*-β-D-glucuronides of hydroxytyrosol **6**, **7,** and **8** have been synthesized for the first time using chemical synthesis.

Polyphenols, plant secondary metabolites found in fruits, vegetables, and natural beverages, have been related to the lower incidence of certain types of cancer, cardiovascular diseases or inflammatory-related illnesses. Three of the most important dietary polyphenols are resveratrol, hydroxytyrosol, and ellagitannins. *trans*-Resveratrol ((*E*)-3,4′,5-trihydroxystilbene, **2**, Fig. 1) is found in plants, roots, peanuts, seeds, berries, and grapes. It has been shown that resveratrol exhibits an impressive array of antioxidative,[1] anticarcinogenic,[2] anti-inflammatory[3], and cardioprotective properties.[4] Hydroxytyrosol (3,4-dihydroxyphenylethanol, **3**), a orthodiphenolic antioxidant found in olives and olive oil, has been reported to show antibacterial,[5] anti-inflammatory[6], and anticancer activity,[7] inhibition of human LDL oxidation,[8] and prevention of platelet aggregation.[9] Ellagitannins, found in raspberries, pomegranates, and other fruits, have shown to inhibit angiogenesis[10] and possess anti-atherogenic properties.[11] They are metabolized in humans to ellagic acid, which is further metabolized by the human colonic microflora to dibenzopyran-6-one derivatives; mainly urolithins A and B (see Fig. 1 for urolithin-B, **1**).

Knowledge of the metabolic fate of these biologically relevant dietary phenols is of fundamental importance to know their real potential as health protecting agents or their possible use as drugs. This aspect is especially significant since different studies have pointed out that the biological activity of the metabolites of certain polyphenols is similar to or higher than that of the parent molecule.[12,13] The major metabolic pathway found in vivo for these dietary phenols is O-conjugation via glucuronidation and sulfation.

Preparation of 3-*O*-β-D- and 4′-*O*-β-D-glucuronide conjugates of *trans*-resveratrol has been reported by two different approaches. Learmonth[14] described the chemical synthesis of these two *O*-glucuronides, using a long route based on a Heck coupling of iodo-*O*-β-D-glucuronate derivatives with the corresponding styrenes. Wang et al.[15] prepared the corresponding glucuronides by direct coupling of resveratrol with the bromo glucuronide donor in a very low yield, probably due to the low solubility of resveratrol in organic solvents. The same authors also used a second route by silyl protection of the phenolic hydroxyl groups to finally couple a quite uncommon trifluoroacetamidite glucuronide donor in moderate yields. Biocatalyzed synthesis of hydroxytyrosol, tyrosol, homovanillic alcohol, and 3-(4′-hydroxyphenyl)propanol glucuronides has been developed.[16] However, enzymatic synthesis requires the use of either expensive glucuronyl transferases or liver microsomes followed by a final tedious purification of the mixtures obtained. Finally, to the best of our knowledge, the synthesis of urolithin glucuronic acid metabolites has not been described so far. In this work we report a simple and direct strategy to chemically synthesize *O*-β-D-glucuronides of urolithin-B **4**, resveratrol **5,** and the corresponding hydroxytyrosol derivatives **6**, **7** (as a regioisomeric mixture), and **8**.

First, we optimized the glycosylation reaction for a structurally simple, but biologically relevant phenol, such as urolithin-B (**1**). The preparation of the corresponding glucuronic derivative donors **9**–**11** (Scheme 1) is carried out from the commercially available D-glucurono-6,3-lactone.[17,18] The α/β diastereomeric mixtures of

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doi:10.1016/j.carres.2009.05.016

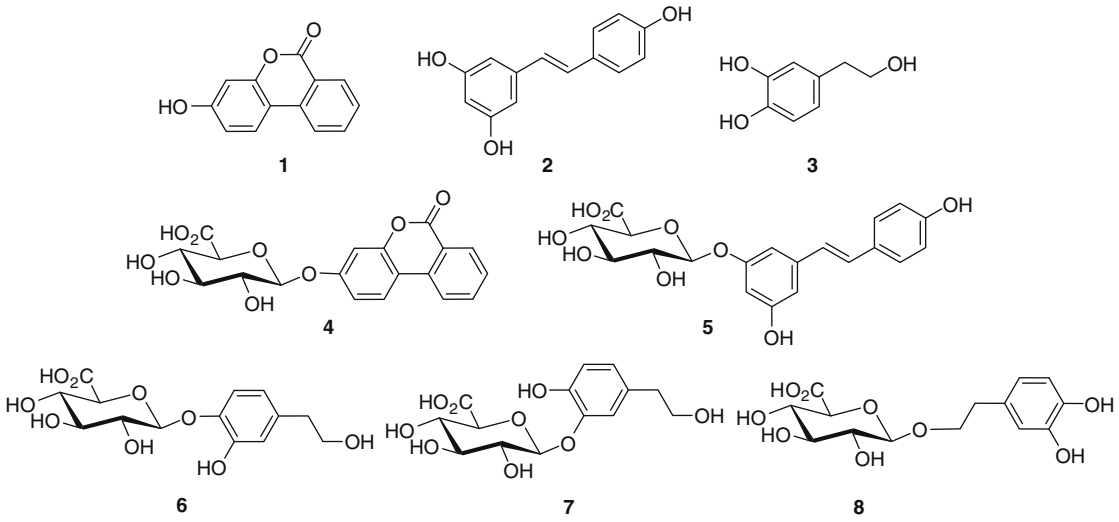
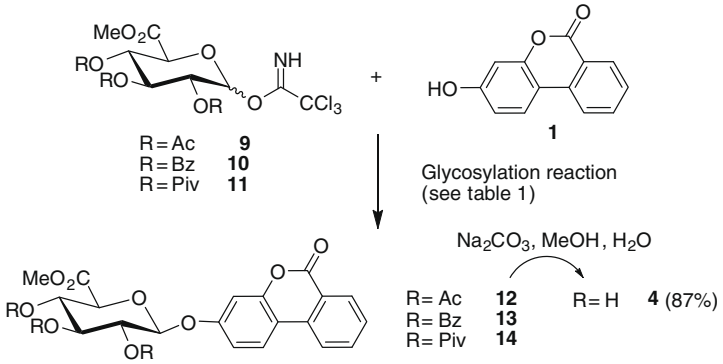


Figure 1. Chemical structure of dietary phenolics and their glucuronide metabolites.



Scheme 1. Synthesis of urolithin-B-*O*-β-D-glucuronides, protected **12**–**14**.

trichloroacetimidate donors **9**–**11** were used without further purification. Urolithin-B was prepared by the condensation of resorcinol and 2-bromobenzoic acid with copper sulfate as catalyst in alkaline medium (Hurtley reaction).[19] Glycosylation of urolithin-B acceptor **1** and glucuronosyl donor **11** was performed using TMSOTf as the promoter (Scheme 1 and Table 1, entry 1). The stereoselectivity of the reaction was very good and the β-diastereoisomer **14** could be isolated in moderate yields. Attempts to improve the yield of the reaction by modifying the solvent failed (Table 1, entries 2 and 3). Then, we considered the use of $BF_3{\cdot}OEt_2$, the most common promoter used in aromatic glycosylation.[20] Pleasingly, reaction of **1** with the glucuronosyl donor **11** produced compound **14** in much higher yield (78%, Table 1, entry 4). When the glucuronosyl donors **9** and **10** were reacted with urolithin-B (Table 1, entries 5 and 6), products **12** and **13** were obtained in very high yields (95% and 83%, respectively) with no sign of orthoester formation.

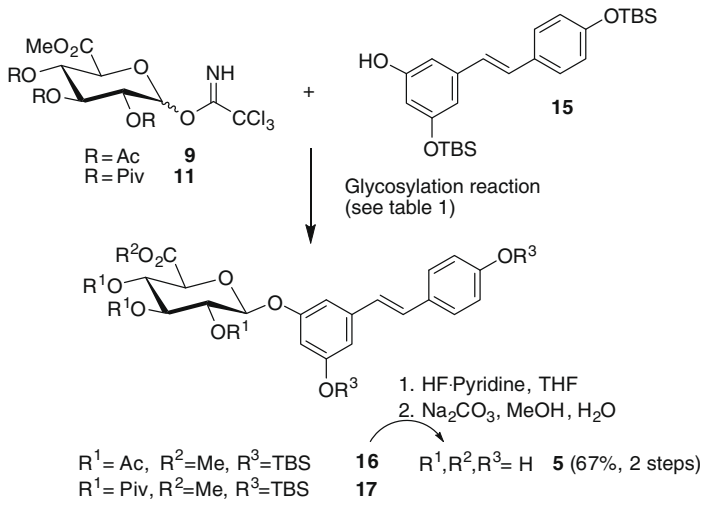
Table 1
Glycosylation reactions with glucuronic donors

Entry	Acceptor	Donor	Promoter	Solvent	Product	Yield[a] (%)
1	**1**	**11**	TMSOTf[d]	CH_2Cl_2[b]	**14**	45
2	**1**	**11**	TMSOTf[d]	THF[b] or Et_2O[b]	—	—
3	**1**	**11**	TMSOTf[d]	CH_3CN[c]	**14**	24
4	**1**	**11**	$BF_3{\cdot}OEt_2$[e]	CH_2Cl_2[b]	**14**	78
5	**1**	**9**	$BF_3{\cdot}OEt_2$[e]	CH_2Cl_2[b]	**12**	95
6	**1**	**10**	$BF_3{\cdot}OEt_2$[e]	CH_2Cl_2[b]	**13**	84
7	**15**	**11**	TMSOTf[d]	CH_2Cl_2[b]	**17**	54
8	**15**	**9**	$BF_3{\cdot}OEt_2$[e]	CH_2Cl_2[b]	**16**	71
9	**18**	**9**	$BF_3{\cdot}OEt_2$[e]	CH_2Cl_2[b]	**19**,**20**	51
10	**21**	**9**	$BF_3{\cdot}OEt_2$[e]	CH_2Cl_2[b]	**22**	18
11	**21**	**10**	$BF_3{\cdot}OEt_2$[e]	CH_2Cl_2[b]	**23**	84
12	**21**	**11**	$BF_3{\cdot}OEt_2$[e]	CH_2Cl_2[b]	**24**	53

[a] Yields of isolated products after purification.
[b] Glycosylation reactions were carried out with 1.5 equiv of donor and 1.0 equiv of acceptor, at −10 °C.
[c] Glycosylation reaction was carried out at 0 °C to room temperature for 16 h.
[d] 0.05 equiv of promoter was used.
[e] 0.25 equiv of promoter was added.

Deprotection of the acyl groups in compounds **12**–**14** was carried out using Na_2CO_3 in a mixture MeOH–H_2O to avoid the formation of the α,β-unsaturated acid side product and ring opening of the urolithin lactone that is observed when using KOH or NaOH in aqueous alcohols.[21] Reaction of the acetylated derivative **12** gave urolithin-B glucuronide **4** in high yield whereas a very slow reaction was observed for the benzoyl or pivaloyl derivatives **13** and **14**.

Next, we moved to the structurally more complex phenolic derivative resveratrol **2**. We decided to explore different glycosylation conditions (Scheme 2) with the alcohol acceptor **15** (22% yield from resveratrol) used previously by Wang et al.[15] Our initial reaction conditions using glucuronosyl donor **11** in dry dichloromethane and using TMSOTf as promoter produced the protected compound **17** in a 54% yield (Table 1, entry 7). When using our optimized conditions, glucuronosyl donor **9** in dry dichloromethane and $BF_3{\cdot}OEt_2$ as the promoter, the corresponding protected res-

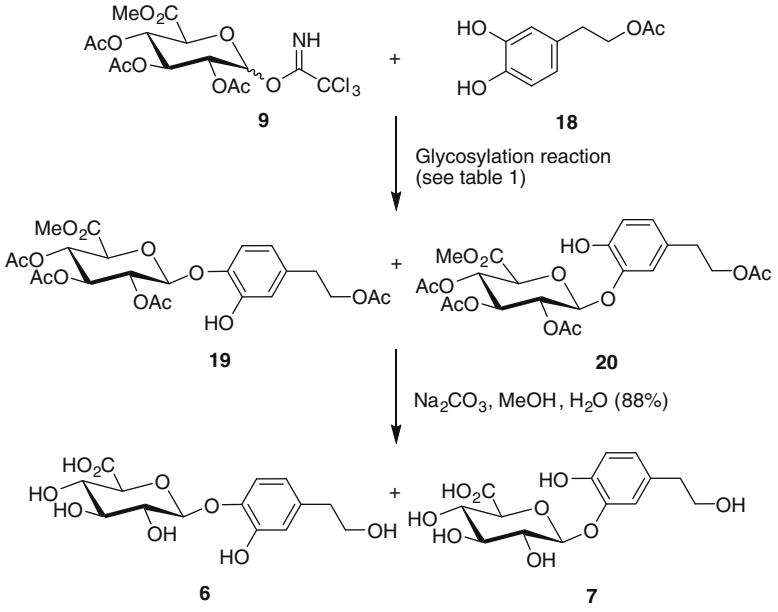


Scheme 2. Synthesis of protected resveratrol-3-*O*-β-D-glucuronide (**16**, **17**) and resveratrol-3-*O*-β-D-glucuronide (**5**).

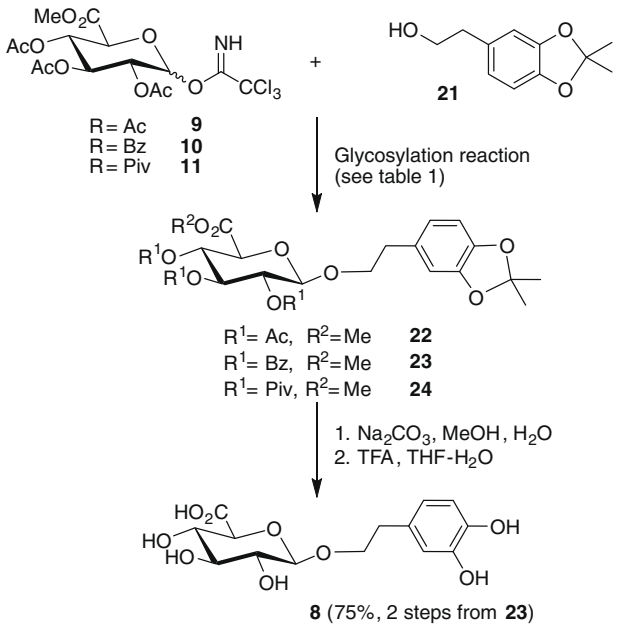
veratrol derivative **16** was obtained in a better yield than reported previously (71%; Table 1, entry 8). Resveratrol derivative **16** was deprotected in two steps (Scheme 2) by treatment with HF·Py in THF and basic hydrolysis resulting in compound **5** (67%).

Hydroxytyrosol **3** was prepared by reduction of 3,4-dihydroxyphenyl acetic acid with lithium aluminum hydride in 75% yield.[22] Then, enzymatic selective acetylation using the immobilized lipase Novozym 435® produced the glycosyl acceptor **18** (95% yield).[23,24] The glycosylation reaction of acceptor **18** with glucuronosyl donor **9** was performed using the same conditions described above (Scheme 3) to give compounds **19** and **20** in a regioisomeric mixture (51%; Table 1, entry 9). Deprotection of all the acetyl groups under basic conditions (Scheme 3) afforded a 1.7:1 regioisomeric mixture of 4- and 3-*O*-β-glucuronides of hydroxytyrosol **7** and **8**. The ratio between the regioisomers was calculated by ^{1}H NMR. A tri-*O*-acetyl glucuronide derivative of hydroxytyrosol (**22**) was prepared by glycosylation of the ketal-protected hydroxytyrosol **21**[23,24] with the glucuronosyl donor **9** in dry dichloromethane and $BF_3{\cdot}OEt_2$ as the promoter (Scheme 4). The low yield of the reaction (18%; Table 1, entry 10) might be explained due to the low reactivity of the glucuronosyl donor and the very reactive glycosyl acceptor **21**, which is very rapidly acetylated at the primary position. No improvement was observed when TMSOTf was used as the promoter or by changing the order of addition of the reagents. Better yields of the glycosylated products (**23** and **24**) could be obtained when the glycosylation reaction was carried out using the benzoyl and the pivaloyl-protected glucuronosyl donors **10** and **11** (84% and 53%; Table 1, entries **11** and **12**, respectively).

Deprotection of derivatives **22**–**24** was carried out in two steps. First, Na_2CO_3 was used to remove the acetyl groups and then, trifluoroacetic acid in a H_2O–THF mixture to remove the acetal group. The reaction of compounds **22** and **23** under these conditions produced the hydroxytyrosol glucuronide **8** in good yields (87% and 75%, respectively). In the case of the pivaloyl derivative **24**, no deprotection reaction was observed by treatment with Na_2CO_3 in aqueous methanol, and partial formation of the α,β-unsaturated



Scheme 3. Synthesis of hydroxytyrosol 4′-*O*-β-D-glucuronide (**6**) and hydroxytyrosol 3′-*O*-β-D-glucuronide (**7**).



Scheme 4. Synthesis of 2-(3′,4′-dihydroxyphenyl)ethanol-1-*O*-β-D-glucuronide (**8**).

acid side product was detected under reaction with NaOH in aqueous methanol.

In conclusion, a short and efficient chemical synthesis has been developed for the stereoselective preparation of important protected glucuronide metabolites **12**–**14**, **16**, **17**, **19**, **20**, **22**, **23,** and **24**. The *O*-β-D-glucuronide conjugate of urolithin-B (**4**) has been synthesized for the first time. The major glucuronic acid metabolite of resveratrol, resveratrol 3-*O*-β-D-glucuronide, **5**, has been prepared using the much more common trichloroacetimidate glycosyl donor obtaining a better yield of the product than previously reported. Finally, three hydroxytyrosol metabolites **6**, **7** (as a regioisomeric mixture), and **8** have been synthesized following similar glycosylation reaction conditions. It is important to note that these phenolic glucuronides will help to elucidate their actual contribution to the reported biological functions in vivo in comparison with their parent compounds. These compounds will also find use in the more accurate determination of the metabolic and pharmacokinetic profiles of urolithins, resveratrol, and hydroxytyrosol.

1. Experimental

1.1. General methods

All chemicals were obtained from chemical suppliers and used without further purification, unless otherwise noted. All reactions were monitored by TLC on precoated Silica-Gel 60 plates F254, and detected by heating with Mostain (500 mL of 10% H_2SO_4, 25 g of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1 g $Ce(SO_4)_2{\cdot}4H_2O$). Products were purified by flash chromatography with Silica Gel 60 (200–400 mesh). NMR spectra were recorded on 300, 400 or 500 MHz NMR equipment, at room temperature for solutions in $CDCl_3$, D_2O or CD_3OD. Chemical shifts are referred to the solvent signal and are expressed in ppm. 2D NMR experiments (COSY, TOCSY, ROESY, and HMQC) were carried out when necessary to assign the corresponding signals of the new compounds. Sephadex G-25 then ion-exchanged with Dowex 50W was used in the purification of several glucuronic metabolites. Finally, samples were lyophilized to dryness three times from D_2O to deuterate all exchangeable protons.

1.2. Methyl-[1-(dibenzo[*b*,*d*]pyranyl-6-one)]-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate (12)

To a solution of trichloroacetimidate **9**[17] (450 mg, 0.943 mmol) and urolithin-B **1**,[19] (100 mg, 0.472 mmol) in anhydrous CH_2Cl_2 (6 mL) at −10 °C, $BF_3{\cdot}OEt_2$ (0.235 mmol) was added dropwise. After 5 h, TLC (hexane–EtOAc 2:1) showed the formation of a major product (R_f 0.23) and complete consumption of the starting material. The reaction was quenched by the addition of NEt_3 and concentrated in vacuo. The resulting residue was purified by flash column chromatography (hexane–EtOAc from 3:1 to 1:1) to afford **12** (238 mg, 95%) as a yellow glassy solid; $[\alpha]_D^{22}$ −29.5 (*c* 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, J = 8.1 Hz, H_{arom}), 7.98–7.88 (m, 2H, H_{arom}), 7.76, 7.75 (2t, J = 8.1 Hz, 2H, $_{Harom}$), 6.9–6.93 (m, 2H, H_{arom}), 5.40–5.26 (m, 4H, H-1, H-2, H-3, H-4), 4.29 (d, J = 8.7 Hz, H-5), 3.73 (s, 3H, OCH_3), 2.10, 2.05 (2s, 9H, $CH_3C{=}O$); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.0, 169.3, 169.2 (C=O), 161.01 (C=O lactone), 157.9, 152.1, 135.0, 134.4, 130.5, 128.3, 123.6, 121.3, 120.2, 114.1, 113.4, 105.0 (C_{arom}), 98.4 (C-1), 72.5, 71.7, 70.8, 69.0 (C-2, C-3, C-4, C-5), 53.0 (CH_3O), 20.6, 20.5 ($CH_3{-}C{=}O$). ESIMS: Calcd for $C_{26}H_{24}NaO_{12}$: 551.1165. Found: 551.1165.

1.3. Methyl-[1-(dibenzo[*b*,*d*]pyranyl-6-one)]-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate (13)

To a solution of trichloroacetimidate **10**[17] (100 mg, 0.150 mmol) and **1** (22 mg, 0.10 mmol) in anhydrous CH_2Cl_2 (3 mL) at −10 °C, $BF_3{\cdot}OEt_2$ (0.0375 mmol) was added dropwise. After 5 h, TLC (hexane–EtOAc 3:1) showed the formation of a major product (R_f 0.20) and complete consumption of the starting material. The reaction was quenched by the addition of NEt_3 and concentrated in vacuo. The resulting residue was purified by flash column chromatography (toluene–EtOAc, from 10:1 to 6:1) to afford **13** (60 mg, 84%) as a yellow glassy solid; $[\alpha]_D^{22}$ +31.0 (*c* 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, J = 8.1 Hz, H_{arom}), 8.01–7.01 (m, 21H, H_{arom}), 6.04 (t, J = 8.7 Hz, 1H, H-3), 5.92–5.84 (m, 2H, H-2, H-4), 5.62 (d, J = 6.7 Hz, H-1), 4.62 (d, J = 8.7 Hz, H-5), 3.68 (s, 3H, OCH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.0, 165.5, 165.2, 165.0 (C=O), 161.1 (C=O lactone), 152.1, 134.9, 134.6, 133.5, 133.4, 130.6, 129.9, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 123.9, 121.3, 120.3, 114.4, 113.5, 105.2 (C_{arom}), 98.7 (C-1), 73.0 (C-5), 71.1 (C-3), 70.9 (C-2), 69.4 (C-4), 53.0 (CH_3O). ESIMS: Calcd for $C_{41}H_{30}O_{12}$: 714.2. Found: 714.2.

1.4. Methyl-[1-(dibenzo[*b*,*d*]pyranyl-6-one)]-2,3,4-tri-*O*-pivaloyl-β-D-glucopyranosyluronate (14)

To a solution of trichloroacetimidate **11**[21,25] (260 mg, 0.43 mmol) and **1** (46.0 mg, 0.21 mmol) in anhydrous CH_2Cl_2 (6 mL) at −10 °C, $BF_3{\cdot}OEt_2$ (0.1075 mmol) was added dropwise. After 2 h we observed a complete consumption of the starting material. The reaction was quenched by the addition of NEt_3 and concentrated in vacuo. The resulting residue was purified by flash column chromatography (hexane–EtOAc, from 8:1 to 2:1) to afford **14** (108 mg, 78%) as a yellow glassy solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 8.1 Hz, H_{arom}), 8.00–7.96 (2d, J = 8.1 Hz, 2H, H_{arom}), 7.80, 7.54 (2t, J = 8.1 Hz, 2H, H_{arom}), 6.98 (s, 2H, H_{arom}), 5.50 (t, J = 9.1 Hz, 1H, H-4), 5.42–5.34 (m, 2H, H-2, H-3), 5.23 (d, J = 7.8 Hz, H-1), 4.29 (d, J = 9.1 Hz, H-5), 3.76 (s, 3H, OCH_3), 1.16 (s, 27H, $C(CH_3)_3$); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 177.0, 176.5, 176.4 (C=O), 166.6 (C=O lactone), 158.2, 152.1, 135.0, 134.5, 130.6, 128.4, 124.1, 121.3, 120.3, 114.1, 113.5, 105.0 (C_{arom}), 99.1 (C-1), 72.9 (C-5), 71.2, 70.4, 69.0 (C-2, C-3, C-4), 53.0 (CH_3O), 38.7 ($C(CH_3)_3$), 27.0 ($C(CH_3)_3$). ESIMS: Calcd for $C_{35}H_{42}NaO_{12}$: 677.2574. Found: 677.2569.

1.5. Sodium 1-(dibenzo[*b*,*d*]pyranyl-6-one)]-β-D-glucopyranosyl-uronic acid (4)

A suspension of ester **12** (40 mg, 0.075 mmol) in methanol (1 mL) was stirred at 20° C with a solution of Na_2CO_3 (22 mg, 0.204 mmol) in H_2O (0.5 mL). After 5 h, water (1 mL) was added, followed by addition of glacial acetic acid to adjust the pH to 6.2. The solvents were then removed and residue was purified by RP-C18 column eluting with buffer triethylammonium acetate 10 mM and CH_3CN (1:1). Fractions containing the desired product were freeze-dried three times affording compound **4** (29 mg, 83%). ^{1}H NMR (500 MHz, D_2O) δ 7.9 (d, J = 7.85 Hz, H_{arom}), 7.31 (t, J = 7.7 Hz, 1H, H_{arom}), 7.11 (t, J = 7.7 Hz, 1H, H_{arom}), 6.97 (d, J = 8.8 Hz, 1H, H_{arom}), 6.49 (d, 1H, J = 8.8 Hz, H_{arom}), 6.22 (s, 1H, H_{arom}), 4.91 (d, J = 7.7 Hz, 1H, H-1), 3.86 (d, J = 9.3 Hz, 1H, H-4), 3.67–3.56 (m, 3H, H-2, H-3, H-5); ^{13}C NMR (100.5 MHz, D_2O) δ 181.5 (C=O), 162.7 (C=O lactone), 157.6, 149.9, 135.5, 133.3, 128.9, 128.2, 123.6, 120.8, 117.2, 113.7, 111.7 (C_{arom}), 99.6 (C-1), 76.7 (C-5), 75.3, 72.7, 71.9 (C-2, C-3, C-4). ESIMS: Calcd for $C_{19}H_{15}Na_2O_9$: 433.0511. Found: 433.0493.

1.6. (*E*)-1-[3-*tert*-Butyldimethylsilyloxy-5-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranoside)phenyl]-2-(4′-*tert*-butyldimethylsilyloxy-phenyl) ethene methyl ester (16)

To a solution of trichloroacetimidate **9**[17] (1.77 g, 3.72 mmol) and resveratrol derivative **15**[15] (1.7 g, 3.72 mmol) in anhydrous CH_2Cl_2 (30 mL) at −10 °C, $BF_3{\cdot}OEt_2$ (0.93 mmol, 930 μL) was added. After 90 min, TLC showed the formation of a major product and consumption of the glycosyl donor. The reaction was then quenched by the addition of NEt_3 and concentrated in vacuo. The resulting residue was purified by flash column chromatography (hexane–EtOAc, from 4:1 to 1:1) to afford **16** (2.0 g, 71%) as a yellow glassy solid; $[\alpha]_D^{22}$ −19.5 (c 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (d, J = 8.0 Hz, H_{arom}), 6.75 (d, J = 16.0 Hz, H_{arom}), 6.64–6.46 (m, 6H, H_{arom}, =CH), 6.17 (m, 1H, =CH), 5.15–5.06 (m, 3H, H-3, H-4, H-2), 4.93 (d, J = 7.2 Hz, 1H, H-1), 3.99 (d, J = 8.1 Hz, 1H, H-5), 3.52 (s, 3H, OCH_3), 1.86, 1.82 (2s, 9H, CH_3C=O), 0.76 (3s, 18H, $C(CH_3)_3$), −0.10 (s, 12H, Si–$C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 169.4, 169.3, 166.9 (C=O), 157.7, 156.8, 155.7 (C_{qarom}), 139.9, 130.2, 129.2, 127.8, 127.7, 126.0, 120.3, 113.4 (C_{arom}), 108.1 (=CH), 99.1 (C-1), 72.6 (C-5), 71.9 (C-3), 71.0 (C-2), 69.1 (C-4), 53.0 (CH_3O), 25.7 ($C(CH_3)_3$), 20.6, 20.5 ($C(CH_3)_3$), 18.2 ($SiC(CH_3)_3$), −4.38 ($Si/CH_3)_2$). ESIMS: Calcd for $C_{39}H_{56}NaO_{12}Si_2$: 795.3234. Found: 795.3208.

1.7. (*E*)-1-[3-*tert*-Butyldimethylsilyloxy-5-*O*-(2,3,4-tri-*O*-pivaloyl-β-D-glucopyranoside)phenyl]-2-(4′-*tert*-butyldimethylsilyloxy-phenyl) ethene methyl ester (17)

To a solution of trichloroacetimidate **11** (100 mg, 0.165 mmol) and resveratrol derivative **15** (120 mg, 0.262 mmol) in anhydrous CH_2Cl_2 (2 mL) at −10 °C, TMSOTf (0.016 mmol, 3.0 μL) was added. After a 3-h period, TLC showed the formation of a major product and consumption of the glycosyl donor. The reaction was then quenched by the addition of NEt_3 and concentrated in vacuo. The resulting residue was purified by flash column chromatography (hexane–EtOAc from 10:1 to 8:1) to afford **17** (80 mg, 54%) as a yellow glassy solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 8.4 Hz, 2H, H_{arom}), 6.98 (d, J = 16.0 Hz, 1H, H_{arom}), 6.86–6.39 (m, 6H, H_{arom}, =CH), 5.48–5.33 (m, 3H, H-3, H-4, H-2), 5.14 (d, J = 7.8 Hz, 1H, H-1), 4.24 (d, J = 9.9 Hz, 1H, H-5), 3.76 (s, 3H, OCH_3), 1.23, 1.18, 1.17 (3s, 27H, $C(CH_3)_3$), 1.01, 1.00 (2s, 18H, $SiC(CH_3)_3$), 0.23, 0.20 (2s, 12H, Si–$C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.0, 176.5, 176.4, 166.8 (C=O), 157.9, 156.8, 155.7, 139.9, 130.3, 129.2, 128.0, 127.8, 126.1, 120.3, 113.4, 108.1, 107.9, 99.7 (C-1), 73.0 (C-5), 71.4 (C-3), 70.5 (C-2), 69.1 (C-4), 52.9 (CH_3O), 38.8 ($C(CH_3)_3$), 27.4, 27.3, 27.2 ($C(CH_3)_3$), 25.9, 25.7 ($SiC(CH_3)_3$), 18.3, 18.2 ($SiC(CH_3)3$), −4.19, −4.39 ($Si(CH_3)_2$). ESIMS: Calcd for $C_{48}H_{74}O_{12}Si_2$: 899.4797. Found: 899.4827.

1.8. (*E*)-1-(5-Hydroxy-3-*O*-β-glucuronopyranosidophenyl)-2-(4′-hydroxyphenyl)-ethene (*trans*-resveratrol-3-*O*-β-glucuronide) (5)

To a solution of **16** (700 mg, 0.9 mmol) in dry THF (15 mL) was added (HF)·Py (2.5 mL) at room temperature. The reaction was stirred for 6 h and was then diluted with EtOAc (50 mL) and NH_4Cl sat (5 mL) was added. The organic phase was washed with NH_4Cl sat (2 × 25 mL). The solvents were then removed in vacuo and the crude was purified by flash column chromatography (hexane–EtOAc from 4:1 to 1:1) to afford the corresponding desylilated compound **5** (390 mg, 80%). The desylilated product (100 mg, 0.183 mmol) and Na_2CO_3 (52 mg, 0.49 mmol) were dissolved in a solution of methanol (4 mL) and H_2O (2 mL). The reaction mixture was stirred at room temperature for 16 h. After this period, water (1 mL) was added, followed by addition of glacial acetic acid to adjust the pH to 6.2. The solvents were then removed and residue was purified by sephadex G-25 eluting with H_2O–MeOH (9:1). Fractions containing the desired product were freeze-dried affording compound **5** (49 mg, 83%); $[\alpha]_D^{22}$ −17.5 (c 1 in $CHCl_3$); ^{1}H NMR (400 MHz, D_2O) δ 7.19 (d, J = 8.0 Hz, 2H, H_{arom}), 6.80 (d, J = 16.4 Hz, CH=CH), 6.66 (d, J = 8.0 Hz, 2H, H_{arom}), 6.58 (d, J = 16.4 Hz, 1H, CH=CH), 6.49 (d, J = 10.0 Hz, 2H, H_{arom}), 6.30 (s, 1H, H_{arom}), 4.89 (d, J = 7.2 Hz, 1H, H-1), 3.77 (d, J = 8.9 Hz, 1H, H-5), 3.56–3.52 (m, 3H, H-3, H-4, H-2); ^{13}C NMR (100.5 MHz, D_2O) δ 175.5 (C=O), 163.1, 162.7, 161.3 158.3, 140.1, 129.0 (C_{arom}), 128.2 (=CH), 126.3, 124.3, 117.5 (=CH), 109.6, 104.4, 103.3, 100.1 (C-1), 76.1 (C-5), 75.3 (C-3), 72.8 (C-2), 71.7 (C-4). ESIMS: Calcd for $C_{20}H_{19}O_8$ (M−17): 387.10. Found: 387.00.

1.9. 2-[3′-Hydroxy-4′-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)phenyl] EtOAc (19) and 2-[4′-hydroxy-3′-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate) phenyl] EtOAc (20)

To a solution of trichloroacetimidate **9**[17] (366 mg, 0.76 mmol) and 2-(3,4-dihydroxyphenyl)-EtOAc **18**[23,24] (200 mg, 1.02 mmol) in anhydrous CH_2Cl_2 (6 mL) at −10 °C, $BF_3{\cdot}OEt_2$ (25 μL, 0.19 mmol) was added dropwise. After 2 h, TLC (hexane–EtOAc 2:1) showed the formation of a new product and complete consumption of the glycosyl donor. The reaction was neutralized with NEt_3 and concentrated in vacuo. The resulting residue was purified by flash column chromatography (hexane–EtOAc from 3:1 to 1:1) to afford a regioisomeric mixture of **19** and **20** (205 mg, 51%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89–6.80 (m, 3H, H_{arom}), 6.63 (m, 1H, H_{arom}), 6.19 (m, 2H, H_{arom}), 5.37–5.25 (m, 6H, H-2a, H-2b, H-3a. H-3b, H-4a, H-4b), 5.03, 5.02 (2d, J = 7.6 Hz, 2H, H-1a, H-1b), 4.23–4.17 (m, 6H, H-5a, H-5b, 2 × CH_2), 3.74 (s, 6H, CH_3O), 2.84–2.80 (m, 4H, 2 × CH_2), 2.09–2.06 (m, 24H, CH_3C=O); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 171.1, 171.0 ($COOCH_3$), 170.0, 169.8, 169.7 169.4, 166.8, 143.9, 142.8, 135.4, 130.0, 125.7, 120.6, 118.3, 118.0, 117.0, 116.5, 101.4, 100.1 (C-1a, C-1b), 72.4, 71.5, 71.4, 71.2, 71.1, 69.0, 68.9, 64.8, 64.7, 53.1, 34.5, 34.2, 20.9, 20.6, 20.5, 20.4. ESIMS: Calcd for $C_{23}H_{28}NaO_{13}Na$: 536.1. Found: 536.8

1.10. 2-[3′-Hydroxy-4′-β-D-glucopyranosyluronic acid)phenyl] ethanol (6) and 2-[4′-hydroxy-3′-β-D-glucopyranosyluronic acid) phenyl] ethanol (7)

A solution of the regioisomeric mixture of **19** and **20** (60 mg, 0.11 mmol) in methanol (2 mL) was stirred at room temperature

with a solution of Na_2CO_3 (22 mg, 0.204 mmol) in H_2O (0.5 mL). After 16 h., water (1 mL) was added, followed by addition of glacial acetic acid to adjust the pH to 6.2. The solvents were then removed and residue was purified by sephadex G-25 eluting with H_2O–MeOH (9:1). Fractions containing the desired product mixture were freeze-dried affording compounds **6** and **7** (32 mg, 88%) as a 1.7:1 regioisomeric mixture; ^{1}H NMR (500 MHz, D_2O) δ 7.00–6.70 (m, 6H, H_{arom}), 4.97, 4.94 (2d, J = 7.0 Hz, 2H, H-1, H-1′), 3.77–3.75 (m, 2H, H-3, H-3′), 3.76–3.52 (m, 10H, H-4, H-4′, H-5, H-5′, CH_2, H-2, H-2′), 2.68–2.64 (m, 4H, CH_2); ^{13}C NMR (75 MHz, D_2O) δ 181.1 175.0 (C=O), 143.8, 143.0, 135.1, 131.9, 124.3, 121.3, 117.4, 117.0, 116.9, 116.4 (C_{arom}), 101.3, 101.0 (C-1, C-1′), 76.3, 75.2, 72.6, 71.7, 62.5, 62.4, 46.5, 37.0. ESIMS: Calcd for $C_{14}H_{15}O_9$ (M^{3-}): 327.1. Found: 327.0.

1.11. 2-Ethyl-*O*-(2,2-dimethylbenzo[1,3]dioxol-5-yl)methyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate (22)

To a solution of trichloroacetimidate **9**[17] (986 mg, 0.76 mmol) and the hydroxytyrosol derivative **21**[26] (200 mg, 1.02 mmol) in anhydrous CH_2Cl_2 (8 mL) at −10 °C, $BF_3{\cdot}OEt_2$ (66 μL, 0.51 mmol) was added dropwise. After a 2-h period the reaction was neutralized with NEt_3 and concentrated in vacuo. The resulting residue was purified by flash column chromatography (toluene–EtOAc, from 6:1 to 3:1) to afford **22** (100 mg, 18%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.41–6.33 (m, 3H, H_{arom}), 5.01–4.98 (m, 2H, H-3, H-4), 4.82–4.79 (m, 1H, H-2), 4.30 (d, J = 7.7 Hz, 1H, H-1), 3.89–3.77 (m, 2H, H-5, CH_2), 3.53 (s, 3H, CH_3O), 3.39 (m, 1H, CH_2), 2.58–2.52 (m, 2H, CH2), 1.83, 1.79 (2s, 9H, CH_3C=O), 1.42 (s, 6H, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1 ($COOCH_3$), 169.3, 169.2, 169.7 167.2 (C=O), 147.3, 145.9 (C_{qarom}), 131.4, 121.2, 117.6, 109.2, 107.9, 100.7 (C-1), 72.6, 72.0, 71.1, 69.5, 52.9, 35.5, 29.7, 25.8, 20.6, 20.5, 20.4. ESIMS: Calcd for $C_{24}H_{30}O_{12}Na$: 533.2. Found: 533.2.

1.12. 1′-(Methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyluronate)-2′[(3′,4′-isopropylidene)phenyl] ethanol (23)

To a solution of trichloroacetimidate **10** (680 mg, 1.02 mmol) and the hydroxytyrosol derivative **21** (90 mg, 0.46 mmol) in anhydrous CH_2Cl_2 (4 mL) at −10 °C, $BF_3{\cdot}OEt_2$ (66 μL, 0.51 mmol) was added dropwise. After 1 h, the reaction was neutralized with NEt_3 and concentrated in vacuo. The resulting residue was purified by flash column chromatography (toluene–EtOAc from 20:1 to 6:1) to afford **23** (270 mg, 84%); $[\alpha]_D^{22}$ +25.2 (*c* 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89–7.28 (m, 15H, H_{arom}), 6.57–6.40 (m, 3H, H_{arom}), 5.92 (t, J = 9.3 Hz, 1H, H-3), 5.72 (t, J = 9.6 Hz, 1H, H-4), 5.09 (dd, J = 7.5 and 9.3 Hz, 1H, H-2), 4.90 (d, J = 7.5 Hz, 1H, H-1), 4.36 (d, J = 9.6 Hz, 1H, H-5), 4.15 (m, 1H, OCH_2), 3.74–3.71 (m, 4H, OCH_2, CH_3O), 2.82–2.77 (m, 2H, $PhCH_2$), 1.64, 1.63 (2s, 6H, $C(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.4, 165.6, 165.2, 165.0 (C=O), 147.3, 145.8 (C_{qarom}), 133.4, 133.3, 133.2, 131.2, 129.8, 129.0, 128.9, 128.8, 128.7, 128.4, 128.3, 128.2, 125.3, 121.2, 117.5, 109.1, 107.9, 101.0 (C-1), 72.9, 72.1, 71.1, 70.2, 52.9, 35.6, 25.8. ESIMS: Calcd for $C_{39}H_{36}O_{12}Na$: 719.2104. Found: 719.2097.

1.13. 2-Ethyl-*O*-(2,2-dimethylbenzo[1,3]dioxol-5-yl)methyl-2,3,4-tri-*O*-pivaloyl-β-D-glucopyranosyluronate (24)

To a solution of trichloroacetimidate **11** (1.6 g, 2.70 mmol) and the hydroxytyrosol derivative **21** (350 mg, 1.80 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C, $BF_3{\cdot}OEt_2$ (170 μL, 1.35 mmol) was added dropwise. After 1 h, the reaction was neutralized with NEt_3 and concentrated in vacuo. The resulting residue was purified by flash column chromatography (toluene–EtOAc from 20:1 to 6:1) to afford **24** (620 mg, 53%); $[\alpha]_D^{22}$ −10.5 (*c* 1 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.64–6.57 (m, 3H, H_{arom}), 5.37 (t, J = 9.6 Hz, 1H, H-3), 5.26 (t, J = 9.6 Hz, 1H, H-4), 5.09 (dd, J = 8.0 and 9.6 Hz, 1H, H-2), 4.59 (d, J = 8.0 Hz, 1H, H-1), 4.07 (d, J = 9.6 Hz, 1H, H-5), 4.03 (m, 1H, OCH_2), 3.75 (s, 3H, CH_3O), 3.67–3.61 (m, 1H, OCH_2), 2.80–2.77 (m, 2H, $PhCH_2$), 1.67, 1.66 (2s, 6H, $C(CH_3)_2$), 1.21, 1.17, 1.13 (3s, 27H, $C(CH_3)_3$); δ (100 MHz, $CDCl_3$); 170.0 ($COOCH_3$), 169.2, 169.7 167.2 (C=O), 147.0, 145.0 (C_{qarom}), 130.9, 121.2, 117.6, 109.1, 107.9, 101.0 (C-1), 72.9, 71.6, 71.1, 69.4, 52.7, 38.7, 35.8, 27.1, 27.0, 26.9, 25.8. ESIMS: Calcd for $C_{33}H_{48}O_{12}Na$: 659.3102. Found: 659.3043.

1.14. 2-Ethyl-*O*-(3′,4′dihydroxyphenyl)methyl-β-D-glucopyranosyluronate (8)

A solution of compound **23** (120 mg, 0.17 mmol) in methanol (6 mL) was stirred at room temperature with a solution of Na_2CO_3 (110 mg, 1.02 mmol) in H_2O (2.0 mL). After 4 days, water (1 mL) was added, followed by addition of glacial acetic acid to adjust the pH to 6.2. The solvents were then removed and residue was used for the next step without further purification. The latter crude was dissolved in THF–H_2O (1:1, 2 mL) and TFA (3 mL) was then added. The reaction mixture was stirred at room temperature for 48 h. Solvents were then removed in vacuo and the residue was purified by Sephadex G-25 eluting with H_2O–MeOH (9:1) and RP-C18 eluting with H_2O–CH_3CN (from 100:0 to 70:30). Fractions containing the desired product were freeze-dried affording compound **8** (42 mg, 75%). ^{1}H NMR (300 MHz, D_2O) δ 6.59–6.44 (m, 3H, H_{arom}), 4.19 (d, J = 7.8 Hz, 1H, H-1), 3.79–3.74 (m, 1H, CH_2), 3.62–3.52 (m, 2H, CH_2, H-5), 3.31–3.20 (m, 2H, H-3, H-4), 3.05–2.99 (m, 1H, H-2), 2.56–2.51 (m, 2H, CH_2); δ (75 MHz, D_2O); 163.2, 162.7, 143.8, 142.2, 131.3, 121.1, 116.6, 116.1, 102.1 (C-1), 75.2, 72.7, 71.3, 71.1, 34.3. ESIMS: Calcd for $C_{14}H_{15}O_9$ (M^{3-}): 327.0733. Found: 327.0408.

Acknowledgments

We would like to thank JAE-Doc program (R.L.) and Intramural Frontier Projects (200680F0132) from CSIC for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.05.016.

References

1. Tadolini, B.; Juliano, C.; Piu, L.; Franconi, F.; Cabrini, L. *Free Radical Res.* **2000**, *33*, 105–114.
2. Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W.; Fong, H. H.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. *Science* **1997**, *275*, 218–220.
3. Das, S.; Das, D. K. *Inflamm. Allergy Drug Targets* **2007**, *6*, 168–173.
4. Hao, H. D.; He, L. R. *J. Med. Food* **2004**, *7*, 290–298.
5. Capasso, R.; Evidente, A.; Schivo, L.; Orru, G.; Marcialis, M. A.; Cristinzio, G. *J. Appl. Bacteriol.* **1995**, *79*, 393–398.
6. de la Puerta, R.; Ruiz Gutierrez, V.; Hoult, J. R. *Biochem. Pharmacol.* **1999**, *57*, 445–449.
7. Owen, R. W.; Giacosa, A.; Hull, W. E.; Haubner, R.; Wurtele, G.; Spiegelhalder, B.; Bartsch, H. *Lancet Oncol.* **2000**, *1*, 107–112.
8. Visioli, F.; Bellomo, G.; Montedoro, G.; Galli, C. *Atherosclerosis* **1995**, *117*, 25–32.
9. Petroni, A.; Blasevich, M.; Salami, M.; Papini, N.; Montedoro, G. F.; Galli, C. *Thromb. Res.* **1995**, *78*, 151–160.
10. Heber, D. *Cancer Lett.* **2008**, *269*, 262–268.
11. Aviram, M.; Volkova, N.; Coleman, R.; Dreher, M.; Reddy, M. K.; Ferreira, D.; Rosenblat, M. *J. Agric. Food Chem.* **2008**, *56*, 1148–1157.
12. Koga, T.; Meydani, M. *Am. J. Clin. Nutr.* **2001**, *73*, 941–948.
13. Tribolo, S.; Lodi, F.; Connor, C.; Suri, S.; Wilson, V. G.; Taylor, M. A.; Needs, P. W.; Kroon, P. A.; Hughes, D. A. *Atherosclerosis* **2008**, *197*, 50–56.
14. Learmonth, D. A. *Bioconjugate Chem.* **2003**, *14*, 262–267.
15. Wang, L. X.; Heredia, A.; Song, H.; Zhang, Z.; Yu, B.; Davis, C.; Redfield, R. *J. Pharm. Sci.* **2004**, *93*, 2448–2457.

16. Khymenets, O.; Joglar, H.; Clapés, P.; Parella, T.; Covas, M.-I.; de la Torre, R. *Adv. Synth. Catal.* **2006**, *348*, 2155–2162.
17. Fischer, B.; Nudelman, A.; Ruse, M.; Herzig, J.; Gottlieb, H. E.; Keinan, E. *J. Org. Chem.* **1984**, *49*, 4988–4993.
18. Schmidt, R. R.; Michel, J.; Roos, M. *Liebigs Ann. Chem.* **1984**, *1984*, 1343–1357.
19. Ito, H.; Iguchi, A.; Hatano, T. *J. Agric. Food Chem.* **2008**, *56*, 393–400.
20. Jacobsson, M.; Malmberg, J.; Ellervik, U. *Carbohydr. Res.* **2006**, *341*, 1266–1281.
21. Brown, R. T.; Scheinmannb, F.; Stachulski, A. V. *J. Chem. Res.-S.* **1997**, 370–371.
22. Capasso, R.; Evidente, A.; Avolio, S.; Solla, F. *J. Agric. Food Chem.* **1999**, *47*, 1745–1748.
23. Torres de Pinedo, A.; Peñalver, P.; Rondón, D.; Morales, J. C. *Tetrahedron* **2005**, *61*, 7654–7660.
24. Grasso, S.; Siracusa, L.; Spatafora, C.; Renis, M.; Tringali, C. *Bioorg. Chem.* **2007**, *35*, 137–152.
25. Rao, P. N.; Rodriguez, A. M.; Miller, D. W. *J. Steroid Biochem.* **1986**, *25*, 417–421.
26. Gambacorta, A.; Tofani, D.; Bernini, R.; Migliorini, A. *J. Agric. Food Chem.* **2007**, *55*, 3386–3391.