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# Synthesis of various $\beta$ -D-glucopyranosyl and $\beta$ -D-xylopyranosyl hydroxybenzoates and evaluation of their antioxidant activities

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**Abstract:** Various  $\beta$ -D-glucopyranosyl and  $\beta$ -D-xylopyranosyl hydroxybenzoates were efficiently prepared from 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide (TAGB) or 2,3,4-tri-*O*-acetyl- $\alpha$ -D-xylopyranosyl bromide (TAXB), respectively, by amine-promoted glycosylation. Regioselective deacetylation of the resulting acetylated  $\beta$ -D-glucopyranosyl and  $\beta$ -D-xylopyranosyl hydroxybenzoates was investigated using Novozym 435 as a lipase catalyst. In the case of  $\beta$ -D-glucopyranosyl hydroxybenzoates, Novozym 435-catalyzed deacetylation is regioselective at C-4 and C-6 positions. On the other hand,  $\beta$ -D-xylopyranosyl hydroxybenzoates are deacetylated only at the C-4 position. Antioxidant activities of free hydroxybenzoic acids and the respective  $\beta$ -D-glucopyranosyl and  $\beta$ -D-xylopyranosyl hydroxybenzoates were evaluated by DPPH<sup>•</sup> radical scavenging as well as their inhibitory effect on autoxidation of bulk methyl linoleate. The  $\beta$ -D-xylopyranosyl protocatechoate, as well as quercetin and  $\alpha$ -tocopherol, show high antioxidant activity for the radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>). In bulk methyl linoleate, the antioxidant activities of  $\beta$ -D-glucopyranosyl and  $\beta$ -D-xylopyranosyl protocatechoates are higher than that of  $\alpha$ -tocopherol.

**Keywords:** antioxidant activity;  $\beta$ -D-glucopyranosyl hydroxybenzoate; hydroxybenzoic acid; lipase-catalyzed regioselective deacetylation; methyl linoleate.

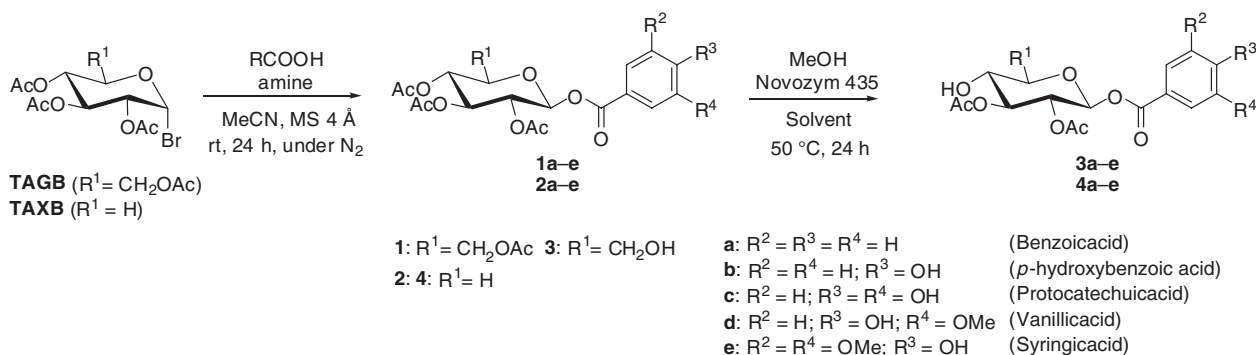
## Introduction

Hydroxybenzoic acid derivatives such as protocatechuic, vanillic, and syringic acids are widely distributed in plants and foods derived from natural products [1–6]. In recent years, there have been many reports on the biological activities of hydroxybenzoic acid derivatives. It has been reported that vanillic acid shows anticoagulant [7], anti-inflammatory [8], and nephroprotective effects [9], as well as estrogen-like [10] and antinociceptive activities [11]. Syringic acid has an antihyperglycemic effect [12] and antiangiogenic and antimicrobial activities [13, 14]. In many cases, these hydroxybenzoic acids exist predominantly as ester and glycoside forms with only lesser amounts of them being present in free carboxylic acid forms [15]. Many plants containing these hydroxybenzoic acid derivatives have been used as traditional medicines. *Conyza sumatrensis* leaves have been used for traditional treatment of malaria in Cameroon [16]. The stems of *Berchemia racemosa* are used in Japan for treatment of gall stones, liver diseases, neuralgia and stomach cramp [17]. *Conyza sumatrensis* contains various flavonoids as phenolic components [18]. The occurrence of  $\beta$ -D-glucopyranosyl syringoate in *Berchemia racemosa* has been confirmed by Inoshiri and coworkers [19]. Additionally, it is known that various Spanish *Cistus* species include  $\beta$ -D-glucopyranosyl vanilloate [20]. In traditional folk medicine, *Cistus* is used as anti-inflammatory, anti-ulcerogenic, wound healing, antimicrobial, cytotoxic and vasodilator remedies [21]. These activities of the plants containing hydroxybenzoic acid derivatives are related to their ability to act as antioxidants [22], metal ion chelators [23], radical scavengers [24] and inhibitors of prooxidant enzymes [25]. Many researchers have studied the antioxidant effects of hydroxybenzoic acid derivatives [22, 26–28]. We have previously reported preparation of  $\beta$ -D-glucopyranosyl and  $\beta$ -D-xylopyranosyl hydroxycinnamates using amine-promoted glycosylation and Novozym 435-catalyzed regioselective deacetylation, and evaluation of their antioxidant activities [29, 30].  $\beta$ -D-Glucopyranosyl feruloyl and synapinoates and  $\beta$ -D-xylopyranosyl caffeoyl show similar or higher antioxidant activities compared with  $\alpha$ -tocopherol.

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**Scheme 1** Glycosylation of hydroxybenzoic acid derivatives and deacetylation using Novozym 435.

In this study, we investigated the synthesis of various  $\beta$ -D-gluco- and  $\beta$ -D-xylopyranosyl hydroxybenzoates and described the antioxidant activities of hydroxybenzoic acids and their gluco- and xylopyranosates as well as some vanillic acid-related compounds such as *p*-hydroxybenzoic, protocatechuic and syringic acids and their glycosates. Their antioxidant activities were evaluated based on their inhibitory effects on the autoxidation of methyl linoleate in a bulk system and the radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH).

## Results and discussion

### Amine-promoted glycosylation of hydroxybenzoic acids and Novozym 435-catalyzed regioselective deacetylation

We have previously reported synthesis of  $\beta$ -D-glucopyranosyl hydroxycinnamates by amine-promoted glycosylation and Novozym 435-catalyzed regioselective deacetylation [29].  $\beta$ -D-Glucopyranosyl feruloyl and sinapinoates show high antioxidant activities compared with  $\alpha$ -tocopherol and quercetin. It is known that glycosyl benzoate analogues have various biological activities such as antioxidant [31], cytotoxic [32, 33] and enzyme inhibitory properties [34]. Therefore, we synthesized  $\beta$ -D-glucopyranosyl hydroxybenzoates by previous methods using various hydroxybenzoic acids such as vanillic acid and protocatechuic acid as an aglycone (Scheme 1).

$\beta$ -D-Xylopyranosyl hydroxybenzoates were also synthesized because  $\beta$ -D-xylopyranosyl hydroxycinnamates show higher antioxidant activities compared to the corresponding  $\beta$ -D-glucopyranosyl hydroxycinnamates. Glycosylation of each hydroxybenzoic acid was performed under the previously described conditions [29, 30]. As in the case of glycosylation of hydroxycinnamic acids, the yields of

$\beta$ -D-glucopyranosyl *p*-hydroxybenzoyl and protocatechates, which have phenolic hydroxyl groups, were lower than that of  $\beta$ -D-glucopyranosyl benzoate. Jover and co-workers have reported the relationship between chemical structures and acidity for benzoic acid derivatives [35]. The acidity of benzoic acid derivatives used in this work decreases in the order benzoic acid > syringic acid > protocatechuic acid > vanillic acid > *p*-hydroxybenzoic acid. On the other hand, the yields of  $\beta$ -D-glucopyranosyl hydroxybenzoates decrease in the order benzoic acid > vanillic acid > syringic acid > *p*-hydroxybenzoic acid > protocatechuic acid. It was expected that the yield of  $\beta$ -D-glucopyranosyl benzoate **1a** would be highest because the stability of benzoate anion is highest among these benzoic acid derivatives. Galland and co-workers performed the glycosylation of hydroxycinnamic acids such as ferulic and caffeic acids after methateification at carboxylic group [36]. It seemed that the decrease in yield is not proportional to the acidity of benzoic acids themselves because glycosylation of competing phenoxy anion and carboxylate anion takes place. Additionally, it can be assumed that the rate of glycosylation of the phenolic hydroxyl groups for protocatechuic acid is higher than that of *p*-hydroxybenzoic acid because protocatechuic acid has two phenolic hydroxyl groups.

$\beta$ -D-Xylopyranosyl hydroxybenzoates were synthesized using tri-*O*-acetyl- $\alpha$ -D-xylopyranosyl bromide (TAXB) as a saccharide donor (Scheme 1). Glycosylation was performed under previously described conditions that were used for the synthesis of  $\beta$ -D-xylopyranosyl hydroxycinnamates [30]. As in the case of the synthesis of  $\beta$ -D-glucopyranosates **1b** and **1c**, yields of products **2b** and **2c** are lower than that of  $\beta$ -D-xylopyranosyl benzoate **2a**. By contrast, reactions of vanillic and syringic acids give almost the same yields as  $\beta$ -D-xylopyranosyl benzoate **2a**. The decrease of the yields occurs for the same reason as in the case of synthesis of  $\beta$ -D-glucopyranosyl benzoate.

Subsequently, deacetylation of glucopyranosates **1** was investigated (Scheme 1). We have reported

Novozym 435-catalyzed regioselective deacetylation of 2,3,4,6-tetraacetyl- $\beta$ -D-glucopyranosyl hydroxycinnamates [29]. Novozym 435 catalyzed deacetylation at C-4 and C-6 positions takes place regioselectively without methanolysis of a hydroxycinnamoyl group at the anomeric position. Since antioxidant activities of deacetylated  $\beta$ -D-glucopyranosyl cinnamates were improved, deacetylation of  $\beta$ -D-glucopyranosyl hydroxybenzoates **1a–e** was also investigated using Novozym 435 as a catalyst (Scheme 1). Methanolysis of **1** was performed in methyl *tert*-butyl ether (MTBE) for 24 h at 50°C. A mixture of MTBE/benzene, 3:1, was used as a solvent because  $\beta$ -D-glucopyranosyl *p*-hydroxybenzoyl, protocatechoyl and vanilloates **1b**, **1c** and **1d** are sparingly soluble in MTBE. In the case of all substrates, methanolysis progressed to yields in a range of 84–90%. As with  $\beta$ -D-glucopyranosyl hydroxycinnamates, methanolysis of the hydroxybenzoate moiety at the anomeric position was not observed. By using 2D NMR analysis, it was confirmed that acetyl groups at C-4 and C-6 positions were deacetylated regioselectively regardless of the difference in the benzoyl part. As with  $\beta$ -D-glucopyranosyl hydroxycinnamates, Novozym 435-catalyzed methanolysis of  $\beta$ -D-glucopyranosates **1** exhibits high regio- and functional group selectivity. Additionally, Novozym 435 shows high affinity toward all substrates because all deacetylated  $\beta$ -D-glucopyranosates **2** were obtained in high yield after 24 h.

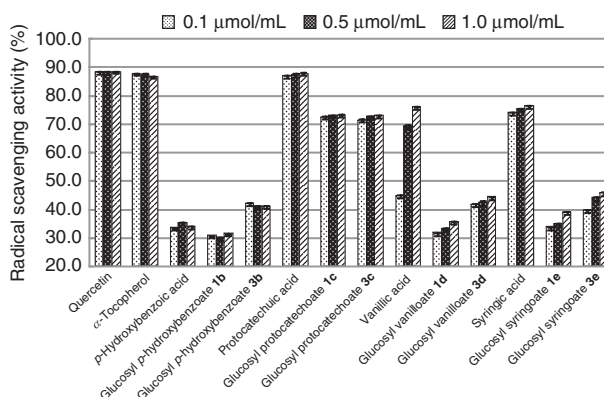
Methanolysis of  $\beta$ -D-xylopyranosyl hydroxybenzoates **2a–e** was also performed using Novozym 435. The solubility of compounds **2b,c** in MTBE is low, and a mixture of MTBE/benzene, 3/1, was used as a solvent. Novozym 435 catalyzed deacetylation of  $\beta$ -D-xylopyranosyl hydroxycinnamates proceeds regioselectively at the C-4 position [30]. Deacetylation of  $\beta$ -D-xylopyranosyl hydroxybenzoates **2** was also investigated using Novozym 435 in the expectation that it would show high regio- and functional group selectivity. Methanolysis progressed to about 80% yields for all reactions. The hydroxybenzoate moiety did not react, and deacetylation at the C-4 position was only observed. Novozym 435 also shows high regio- and functional group selectivity toward  $\beta$ -D-xylopyranosyl hydroxybenzoates **2** and  $\beta$ -D-glucopyranosates **1**.

Novozym 435 catalyzes methanolysis of acetyl groups at C-4 and C-6 positions for  $\beta$ -D-glucopyranosyl hydroxybenzoates **1** in MTBE. We have previously reported that deacetylation of  $\beta$ -D-gluco- and xylopyranosyl hydroxycinnamates increases antioxidant properties [29, 30]. For deacetylation at C-2 and C-3 positions, regioselective hydrolysis of  $\beta$ -D-glucopyranosyl benzoate **1a** was investigated using various lipases in pH 6 citrate buffer with MTBE as a co-solvent. To confirm that **1a** is not hydrolyzed

under mildly acidic conditions of pH 6, a citrate buffer solution of **1a** without lipase was stirred at 40°C for 24 h; the hydrolysis was not observed. In addition to Novozym 435, lipase AS Amano (from *Aspergillus niger*), lipase AK Amano (from *Pseudomonas fluorescens*), lipase AYS (from *Candida rugosa*), lipase PS (from *Burkholderia cepacia*), and PPL (from porcine pancreas) were used. All these lipases show no reactivity for deacetylation of **1a**. Deacetylation of  $\beta$ -D-xylopyranosyl benzoate **2a** at C-2 and C-3 positions was also investigated using the same conditions described above. All lipases except lipase AYS Amano showed little reactivity. Hydrolysis of a benzoate group at the C-1 position was confirmed by analysis of NMR spectra. By contrast, deacetylated compound **4a** was obtained by regioselective hydrolysis at the C-4 position for Novozym 435, lipase AK Amano and lipase PS Amano. Lipase can hydrolyze non-water soluble lipids and esterase catalyzes hydrolysis of water-soluble lipids [37]. We have previously reported PPL-catalyzed hydrolysis of *N*-methyl-5-acetoxalkanamides [38]. Although the reaction mixture is a suspension because the water solubility of *N*-methyl-5-acetoxalkanamides is poor, lipase-catalyzed hydrolysis progresses with high enantioselectivity. The reaction mixtures of  $\beta$ -D-glucopyranosyl and  $\beta$ -D-xylopyranosyl benzoates **1a** and **2a** are also heterogeneous but the water solubility of xylose is higher than that of glucose [39]. It can be assumed that  $\beta$ -D-xylopyranosyl benzoate **2a** shows slightly higher affinity for lipase compared with  $\beta$ -D-glucopyranosate **1a**.

## DPPH<sup>•</sup> radical scavenging activities

The antioxidant properties of hydroxybenzoyl acids and  $\beta$ -D-glucopyranosates **1b–e** were evaluated by the demonstration of DPPH<sup>•</sup> radical scavenging activity (Figure 1).



**Figure 1** DPPH<sup>•</sup> radical scavenging activities of free hydroxybenzoic acids and their  $\beta$ -D-glucopyranosates.

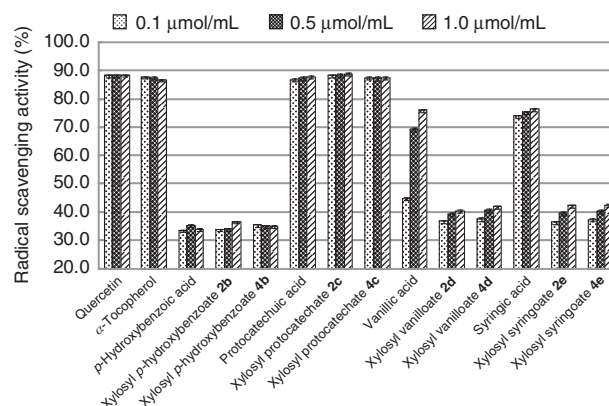
The activity decreases in the order of protocatechuic acid > syringic acid ≈ glucopyranosyl protocatechoates **1c** and **3c** > vanillic acid > glucopyranosyl syringoate **3e** > deacetylated glucopyranosyl vanilloate **3d** > deacetylated glucopyranosyl *p*-hydroxybenzoate **3b** > glucopyranosyl syringoate **1e** > glucopyranosyl vanilloate **1d** ≈ *p*-hydroxybenzoic acid > glucopyranosyl *p*-hydroxybenzoate **1b**.

There are many reports of DPPH<sup>•</sup> radical scavenging activities of hydroxybenzoic acids. Karamać and co-workers have reported that the activities decrease in the order syringic acid > protocatechuic acid > > vanillic acid > > *p*-hydroxybenzoic acid [40]. Fujimoto and co-workers have reported different results: vanillic acid > > protocatechuic acid > syringic acid [22]. Furthermore, Noipa and co-workers have reported the order of syringic acid > protocatechuic acid > vanillic acid [28]. Still, different results were obtained in this work. It can be suggested that the differences between our and other results are due to the measurement conditions. On the other hand, our results are in good agreement with Gadow's results [41]. Free protocatechuic acid shows almost the same high DPPH<sup>•</sup> radical scavenging activity as quercetin and  $\alpha$ -tocopherol. Antioxidants that have many phenolic hydroxyl groups show high antioxidant activities [42, 43]. Free syringic and vanillic acids show high radical scavenging activities compared with free *p*-hydroxybenzoic acid. *Ortho*-methoxy groups as electron-donating groups at the phenolic hydroxyl group improve antioxidant activity and stabilize phenoxy radicals [42, 43]. The radical scavenging activity of free vanillic acid is higher than that of free *p*-hydroxybenzoic acid. Syringic acid has two methoxy groups at the *ortho* positions of phenolic hydroxyl groups. The phenoxy radical of syringic acid is more stable than that of vanillic acid. It can be assumed that syringic acid shows almost the same activity as vanillic acid because the steric hindrance of syringic phenoxy radicals is larger than that of vanillic acid. Only vanillic acid among free hydroxybenzoic acids shows concentration dependency. These results are in good agreement with those of Shyamala [44] and Sang [45]. DPPH<sup>•</sup> radical scavenging activities of glucopyranosyl protocatechoates **1c** and **3c** are highest in glucopyranosyl hydroxybenzoates **1** and **3**. In the case of glucosyl *p*-hydroxybenzoyl, vanilloyl and syringoates, the corresponding deacetylated glucopyranosates **3b**, **3d** and **3e** show about 10% higher activities than acetylated glucopyranosates **1b**, **1d** and **1e**. Regioselective deacetylation at C-4 and C-6 positions increases water solubility and the increase of affinity to DPPH<sup>•</sup> free radicals increases the activity. All glucopyranosyl hydroxybenzoates except glucopyranosyl

*p*-hydroxybenzoate **3b** exhibit lower activities than the corresponding free hydroxybenzoic acids. Fukumoto and co-workers have suggested that the size of the steric hindrance of the sugar moiety reduces the antioxidant properties, and our results are in good agreement with their suggestion [22].

On the other hand, the DPPH<sup>•</sup> radical scavenging activities of xylopyranosates **2** and **4** decrease in the order of xylopyranosyl protocatechoate **2c** > deacetylated xylopyranosyl protocatechoate **4c** ≈ protocatechuic acid > syringic acid > vanillic acid > xylopyranosyl syringoates **2e** and **4e** ≈ xylopyranosyl vanilloates **2d** and **4d** > xylopyranosyl *p*-hydroxybenzoates **2b** and **4b** ≈ *p*-hydroxybenzoic acid (Figure 2). Xylopyranosyl protocatechoate **2c** shows slightly higher activity compared with free protocatechuic acid. Deacetylated xylopyranosyl protocatechoate **4c** also shows comparatively high activity with free protocatechuic acid.

These activities are almost of the same strength as those of quercetin and  $\alpha$ -tocopherol. In the case of glucopyranosyl protocatechoates **1c** and **3c**, the activities decrease compared with that of free protocatechuic acid. The water solubility of xylose is higher than that of glucose [39]. It can be assumed that the activities of xylopyranosyl protocatechoates **2c** and **4c** increase compared with glucopyranosyl protocatechoates **1c** and **3c** because the affinities of xylopyranosates **2c** and **4c** with DPPH<sup>•</sup> radical are higher than those of glucopyranosates **1c** and **3c**. Whereas xylopyranosyl *p*-hydroxybenzoyl, vanilloyl and syringoates **2b**, **2d** and **2e** show about 30% activities, deacetylation increases the activities about 10%. In contrast, there are no significant differences between activities of xylopyranosyl vanilloyl and syringoates **2d** and **2e** and deacetylated xylopyranosates **4d** and **4e**. The activities of xylopyranosyl *p*-hydroxybenzoates **2b** and **4b** and free



**Figure 2** DPPH<sup>•</sup> radical scavenging activities of free hydroxybenzoic acids and their  $\beta$ -D-xylopyranosates.



*p*-hydroxybenzoic acid are similar. *p*-Hydroxybenzoic acid and its xylopyranosates show low activities compared with other hydroxybenzoic acid derivatives, and the sugar moiety does not affect activities. Xylopyranosyl protocatchoates **2c** and **4c** have the highest activities in this investigation.

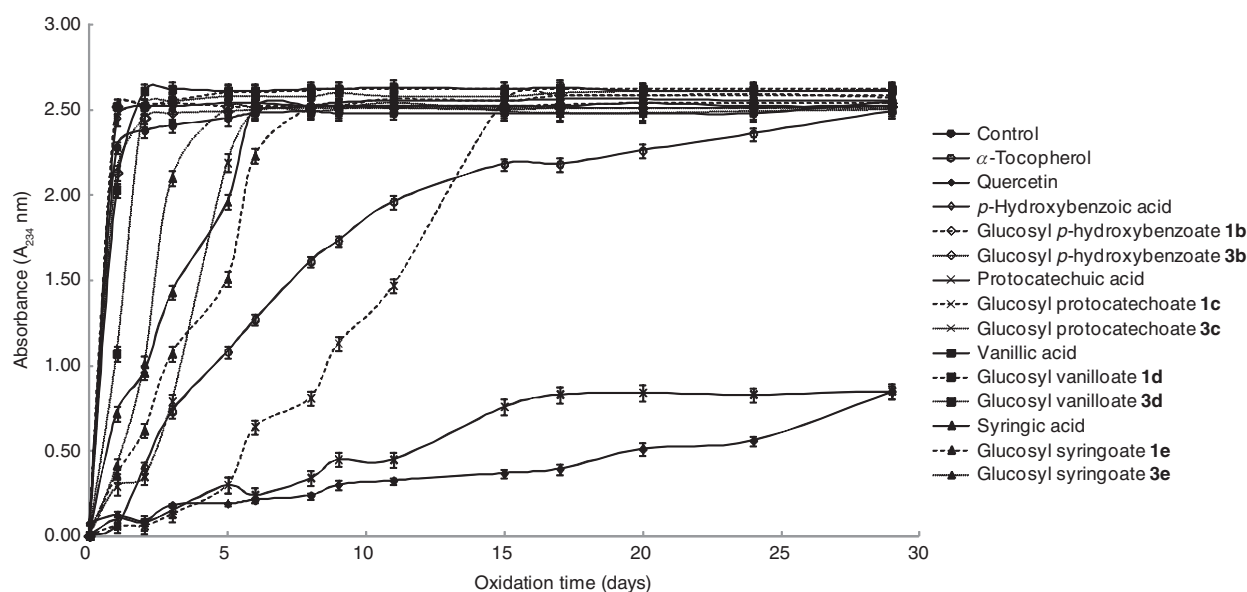
## Inhibitory effect on autoxidation of bulk methyl linoleate

Formation of hydroperoxides in bulk methyl linoleate at 40°C was estimated based on the absorbance at 234 nm generated during the initial oxidation stage (Figure 3). Modifications were made to the original method [46].

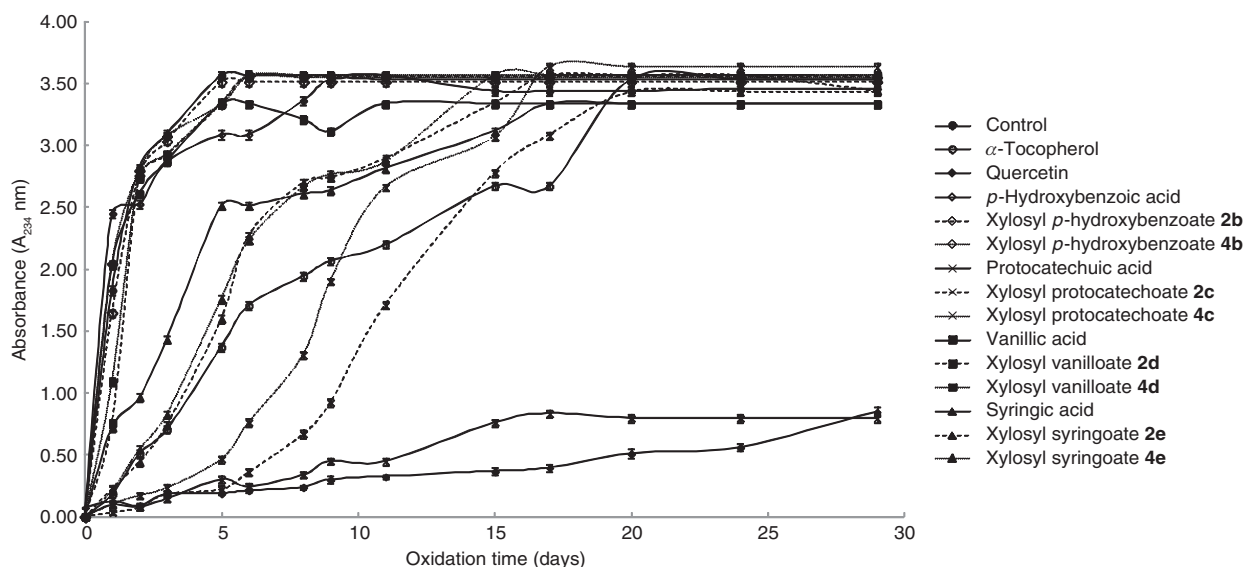
The antioxidant activity decreases in the order of protocatchuic acid > glucopyranosyl protocatchoate **1c** >  $\alpha$ -tocopherol > glucopyranosyl syringoate **1e** > deacetylated glucopyranosyl protocatchoate **3c** ≈ syringic acid > deacetylated glucopyranosyl syringoate **3e** > deacetylated glucopyranosyl vanilloate **3d** > vanillic acid ≈ glucopyranosyl vanilloate **1d** ≈ *p*-hydroxybenzoic acid ≈ glucopyranosyl *p*-hydroxybenzoates **1b** and **3b**. Free protocatchuic acid and glucopyranosyl protocatchoate **1c** show higher antioxidant activities than  $\alpha$ -tocopherol. The antioxidant activity of protocatchuic acid is higher than that of other hydroxybenzoic acids. Glucopyranosyl protocatchoate **1c** has high antioxidant activity among the glucosyl hydroxybenzoate synthesized in this investigation. Surprisingly, deacetylated glucopyranosyl

protocatchoate **3c** shows only the activity like free syringic acid. Comparing free hydroxybenzoic acids for DPPH radical scavenging activity, syringic and vanillic acids have relatively high activities. In contrast, these hydroxybenzoic acids have very low activities on the inhibition of autoxidation of methyl linoleate. Porter has advanced the so-called 'polar paradox' by the observation that polar antioxidants are more effective in nonpolar lipids, whereas nonpolar antioxidants are more effective in polar lipid emulsions [47]. The water solubility of hydroxybenzoic acid derivatives used in this investigation decreases in the order of protocatchuic acid > *p*-hydroxybenzoic acid > syringic acid > vanillic acid [48, 49]. The antioxidant activity of *p*-hydroxybenzoic acid is one of the lowest because there is only one phenolic hydroxyl group and no methoxy group to stabilize the phenoxy radical. Glycosylation causes the decrease in antioxidant activity compared with free hydroxybenzoic acids in almost all cases. A possible explanation for the decrease of antioxidant activities might be a decrease of polarity by glycosylation compared with free hydroxybenzoic acids.

As with glucopyranosyl hydroxybenzoates **1** and **3**, antioxidant activities of xylopyranosyl hydroxybenzoates **2** and **4** were also investigated using methyl linoleate (Figure 4). The antioxidant activity decreases in the order of protocatchuic acid > xylopyranosyl protocatchoate **2c** > deacetylated xylopyranosyl protocatchoate **4c** >  $\alpha$ -tocopherol > xylopyranosyl syringoates **2e** and **4e** > syringic acid > *p*-hydroxybenzoic acid ≈ vanillic acid > xylopyranosyl vanilloates **2d** and **4d** ≈ xylopyranosyl



**Figure 3** Inhibition of formation of hydroperoxide in bulk methyl linoleate by free hydroxybenzoic acids and their  $\beta$ -D-glucopyranosates.



**Figure 4** Inhibition of formation of hydroperoxide in bulk methyl linoleate by free hydroxybenzoic acids and their  $\beta$ -D-xylopyranosates.

*p*-hydroxybenzoates **2b** and **4b**. In addition to free protocatechuic acid, xylopyranosyl protocatechoates **2c** and **4c** have higher antioxidant activities than  $\alpha$ -tocopherol.

Xylopyranosyl syringoates **2e** and **4e** show activities that are similar to that of  $\alpha$ -tocopherol for as long as 5 days. Although glycosylation decreases the antioxidant activity of protocatechuic acid, deacetylated xylopyranosyl protocatechoate **4c** is more active than the corresponding glucopyranosate **3c**. Moreover, antioxidant activities of xylopyranosyl syringoates **2e** and **4e** are better compared with free syringic acid. Xylose exhibits 1.5 times higher water solubility than glucose [39]. It can be suggested that the water solubility of xylopyranosates is higher than that of glucopyranosates. For this reason, some xylopyranosyl hydroxybenzoates show higher antioxidant activities than the corresponding free hydroxyl benzoic acids and glucopyranosates. We previously reported a similar investigation of hydroxycinnamic acids derivatives [29, 30]. In almost all cases, hydroxybenzoic acid derivatives show lower antioxidant activities than hydroxycinnamic acid derivatives. The phenoxy radical of cinnamic acids is stabilized because these acids have an  $\alpha,\beta$ -unsaturated C-C double bond in the phenylpropane structure. These results are in good agreement with related literature reports [46, 50].

## Conclusions

Various  $\beta$ -D-glucosyl- and  $\beta$ -D-xylopyranosyl hydroxybenzoates **1** and **2** were efficiently synthesized by

amine-promoted glycosylation. Deacetylation of products **1** and **2** was investigated using Novozym 435 as a lipase catalyst until the reaction progressed with over 80% yields in all cases.  $\beta$ -D-Glucopyranosyl hydroxybenzoates **1** are regioselectively deacetylated at C-4 and C-6 positions in the presence of Novozym 435. Regioselective deacetylation at the C-4 position exclusively was confirmed for  $\beta$ -D-xylopyranosyl hydroxybenzoates **2**. Novozym 435 is most effective for regioselective deacetylation among various lipase catalysts used in this investigation. In the case of DPPH $^{\cdot}$  radical scavenging activities, the activities of  $\beta$ -D-glucopyranosyl hydroxybenzoates **1** and **3** decrease compared with the corresponding free hydroxybenzoic acids. In contrast,  $\beta$ -D-xylopyranosyl protocatechoates **2c** and **4c** show almost the same high antioxidant activities as quercetin and  $\alpha$ -tocopherol. Moreover, the antioxidant activities of  $\beta$ -D-glucopyranosyl and  $\beta$ -D-xylopyranosyl protocatechoates **1c**, **2c** and **4c** in inhibition of autoxidation of bulk methyl linoleate are higher than that of  $\alpha$ -tocopherol.

## Experimental

$^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (126 MHz) spectra were recorded on a JNM-ECA-500 spectrometer with tetramethylsilane as the internal standard using  $\text{CDCl}_3$  and  $\text{DMSO}-d_6$  as solvents. Structural determination of all compounds was performed using COSY, HMQC and HMBC NMR techniques. ESI-MS spectra were taken on an AccuTof GCv 4G (JEOL, Tokyo, Japan) mass spectrometer. IR spectra were obtained in KBr pellets on an FT-IR JASCO 460 plus spectrometer. Melting points were determined on an MP-500D instrument and are uncorrected.

Optical rotations were determined with a JASCO P-1010 polarimeter. Lipase AS Amano, lipase AK amano, lipase AYS amano and lipase PS Amano were purchased from Wako Pure Chemical Industries, Ltd. Japan. Porcine pancreas lipase (PPL) was obtained from Nacalai Tesque Inc., Japan.

### General procedure for glycosylation of hydroxybenzoic acids

A mixture of 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide (TAGB, 411 mg, 1.0 mmol) or 2,3,4-tri-*O*-acetyl- $\alpha$ -D-xylopyranosyl bromide (TAXB, 339 mg, 1.0 mmol), a hydroxybenzoic acid (2.0 mmol for **1**, 3.0 mmol for **2**) and *i*-Pr<sub>2</sub>NEt (388 mg, 3.0 mmol for **1**, 129 mg, 1.0 mmol for **2**) and 4 Å molecular sieves (1 g) in CH<sub>3</sub>CN (5 mL) was stirred for 24 h at room temperature under argon. Progress of the reaction was monitored by thin layer chromatography. Upon the completion of the reaction, the mixture was concentrated under reduced pressure, the residue was neutralized with aqueous NaHCO<sub>3</sub> and extracted with AcOEt. The extract was washed with brine, dried over anhydrous MgSO<sub>4</sub>, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with hexane/AcOEt.

**2,3,4,6-Tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl benzoate (1a)** Yield 84%; colorless solid; mp 140–141°C; *R*<sub>f</sub> 0.55 (hexane-AcOEt, 1:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –24.71 (c 1.0, THF); IR: 2951 (C-H), 1737 (C=C-C=O), 1452 (Ar, C=C), 1251 (C-O-C=O), 1066 (C-O-C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.02 (m, 12H, CH<sub>3</sub>, OAc at C-2, C-3, C-4 and C-6), 3.95 (m, 1H, H-5), 4.14 (d, *J*=12.6 Hz, 1H, H-6a), 4.33 (dd, *J*=12.6 Hz and 4.6 Hz, 1H, H-6b), 5.21 (m, 1H, H-4), 5.36 (m, 2H, H-2, H-3), 5.94 (m, 1H, H-1), 7.46 (t, *J*=7.4 Hz, 2H, Ph), 7.61 (t, *J*=7.4 Hz, 1H, Ph), 8.05 (d, *J*=17.4 Hz, 2H, Ph); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  20.5 (2CH<sub>3</sub>, OAc at C-2 and C-4), 20.6 (CH<sub>3</sub>, OAc at C-3 and C-6), 61.4 (C-6), 67.9 (C-4), 70.1 (C-2), 72.7 (C-3, C-5), 92.3 (C-1), 128.3 (Ph), 128.5 (Ph), 130.1 (Ph), 134.0 (Ph), 164.5 (O-(C=O)-Ph), 169.3 (C=O, OAc at C-2), 169.4 (C=O, OAc at C-3), 170.0 (C=O, OAc at C-4), 170.6 (C=O, OAc at C-6). HRMS. Calcd for C<sub>21</sub>H<sub>24</sub>O<sub>11</sub>, [M]<sup>+</sup>: *m/z* 452.1319; found: *m/z* 468.1331.

**2,3,4,6-Tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl *p*-hydroxybenzoate (1b)** Yield 49%; colorless solid; mp 131–132°C; *R*<sub>f</sub> 0.45 (hexane-AcOEt, 1:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –79.45 (c 1.0, THF); IR: 3270 (O-H), 2935 (C-H), 1754 (C=C-C=O), 1519 (Ar, C=C), 1237 (C-O-C=O), 1087 (C-O-C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.06 (m, 12H, CH<sub>3</sub>, OAc at C-2, C-3, C-4 and C-6), 4.03 (dd, *J*=12.6 Hz and 2.3 Hz, 1H, H-6a), 4.20 (dd, *J*=12.6 Hz and 4.6 Hz, 1H, H-6b), 4.27 (ddd, *J*=9.7 Hz, 4.6 Hz and 2.3 Hz, 1H, H-5), 5.03 (t, *J*=9.7 Hz, 1H, H-4), 5.11 (dd, *J*=8.6 Hz and 9.7 Hz, 1H, H-2), 5.51 (t, *J*=9.7 Hz, 1H, H-3), 6.12 (d, *J*=8.6 Hz, 1H, H-1), 6.88 (m, 3H, Ph), 7.78 (m, 2H, Ph); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  20.6 (2CH<sub>3</sub>, OAc at C-2 and C-4), 20.7 (CH<sub>3</sub>, OAc at C-3 and C-6), 61.2 (C-6), 67.9 (C-4), 70.1 (C-2), 71.3 (C-5), 71.6 (C-3), 91.3 (C-1), 131.4 (Ph), 115.6 (Ph), 163.4 (C(=O)-Ph), 169.3, 169.5, 169.8, 169.9 (C=O, OAc at C-2, C-3, C-4 and C-6). HRMS. Calcd for C<sub>21</sub>H<sub>24</sub>O<sub>12</sub>, [M]<sup>+</sup>: *m/z* 468.1268; found: *m/z* 468.1281.

**2,3,4,6-Tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl protocatechoate (1c)** Yield 29%; colorless solid; mp 65–66°C; *R*<sub>f</sub> 0.33 (hexane-AcOEt, 1:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –10.7 (c 1.0, THF); IR: 3423 (O-H), 2964 (C-H), 1750 (C=C-C=O), 1604 (Ar, C=C), 1521 (Ar, C=C), 1222 (C-O-C=O), 1070 (C-O-C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.06 (m, 12H, CH<sub>3</sub>, OAc at C-2, C-3, C-4 and C-6), 3.97

(ddd, *J*=9.2 Hz, 4.6 Hz and 2.3 Hz, 1H, H-5), 4.17 (dd, *J*=12.6 Hz and 2.3 Hz, 1H, H-6a), 4.33 (dd, *J*=12.6 Hz and 4.6 Hz, 1H, H-6b), 5.20 (t, *J*=9.2 Hz, 1H, H-4), 5.35 (m, 2H, H-2 and H-3), 5.88 (d, *J*=9.2 Hz, 1H, H-1), 6.92 (d, *J*=8.0, 1H, Ph), 7.56 (m, 2H, Ph); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  20.6 (CH<sub>3</sub>, OAc at C-2 and C-4), 20.7 (CH<sub>3</sub>, OAc at C-3 and C-6), 61.6 (C-6), 68.0 (C-4), 70.3 (C-2), 72.6 (C-3, C-5), 92.2 (C-1), 115.1 (Ph), 116.7 (Ph), 124.6 (Ph), 164.3 (O-C(=O)-Ph), 169.7, 170.1, 170.2, 171.0 (C=O, OAc at C-2, C-3, C-4 and C-6). HRMS. Calcd for C<sub>21</sub>H<sub>24</sub>O<sub>13</sub>, [M]<sup>+</sup>: *m/z* 484.1216; found: *m/z* 484.1215.

**2,3,4,6-Tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl vanilloate (1d)** Yield 73%; colorless solid; mp 111–112°C; *R*<sub>f</sub> 0.33 (hexane-AcOEt, 1:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –30.1 (c 1.0, THF); IR: 3427 (O-H), 2944 (C-H), 1752 (C=C-C=O), 1595 (Ar, C=C), 1515 (Ar, C=C), 1218 (C-O-C=O), 1035 (C-O-C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.06 (m, 12H, CH<sub>3</sub>, OAc at C-2, C-3, C-4 and C-6), 3.95 (m, 2H, H-5 and OCH<sub>3</sub> at Ph), 4.14 (dd, *J*=12.6 Hz and 2.3 Hz, 1H, H-6a), 4.34 (dd, *J*=12.6 Hz and 4.2 Hz, 1H, H-6b), 5.20 (m, 1H, H-4), 5.35 (m, 2H, H-2 and H-3), 5.88 (m, 1H, H-1), 6.95 (d, *J*=8.0 Hz, 1H, Ph), 7.53 (d, *J*=1.7 Hz, 1H, Ph), 7.65 (dd, *J*=8.0 Hz and 1.7 Hz, 1H, Ph); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  20.6 (CH<sub>3</sub>, OAc at C-2, C-3, C-4 and C-6), 61.5 (C-6), 67.9 (C-4), 70.2 (C-2), 72.6 (C-3, C-5), 92.2 (C-1), 125.1 (Ph), 114.4 (Ph), 112.1 (Ph), 164.1 (O-C(=O)-Ph), 169.3, 169.4, 170.1, 170.6 (C=O, OAc at C-2, C-3, C-4 and C-6). HRMS. Calcd for C<sub>22</sub>H<sub>26</sub>O<sub>13</sub>, [M]<sup>+</sup>: *m/z* 498.1373; found: *m/z* 498.1398.

**2,3,4,6-Tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl syringoate (1e)** Yield 71%; colorless solid; mp 67–68°C; *R*<sub>f</sub> 0.28 (hexane-AcOEt, 1:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –39.1 (c 1.0, THF); IR: 3439 (O-H), 2944 (C-H), 1755 (C=C-C=O), 1515 (Ar, C=C), 1223 (C-O-C=O), 1076 (C-O-C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.06 (m, 12H, CH<sub>3</sub>, OAc at C-2, C-3, C-4 and C-6), 3.91 (m, 7H, H-5 and 2OCH<sub>3</sub> at Ph), 4.14 (dd, *J*=12.6 Hz and 2.3 Hz, 1H, H-6a), 4.35 (dd, *J*=12.6 Hz and 4.6 Hz, 1H, H-6b), 5.21 (m, 1H, H-4), 5.36 (m, 2H, H-2 and H-3), 5.87 (d, *J*=8.0 Hz, 1H, H-1), 7.33 (s, 2H, Ph); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  20.6 (CH<sub>3</sub>, OAc at C-2, C-3, C-4 and C-6), 56.5 (CH<sub>3</sub>, OCH<sub>3</sub> at Ph), 61.5 (C-6), 67.8 (C-4), 70.3 (C-2), 72.4 (C-3, C-5), 92.2 (C-1), 107.1 (Ph), 164.1 (-O-(C=O)-Ph), 169.3 (C=O in OAc at C-2), 169.4 (C=O, OAc at C-3), 170.0 (C=O, OAc at C-4), 170.6 (C=O, OAc at C-6). HRMS. Calcd for C<sub>23</sub>H<sub>28</sub>O<sub>14</sub>, [M]<sup>+</sup>: *m/z* 528.1479; found: *m/z* 528.1451.

**2,3,4-Tri-*O*-acetyl- $\beta$ -D-xylopyranosyl benzoate (2a)** Yield 70%; colorless solid; mp 119–120°C; *R*<sub>f</sub> 0.63 (hexane-AcOEt, 1:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –52.9 (c 1.0, THF); IR: 2949 (C-H), 1755 (C=C-C=O), 1602 (Ar, C=C), 1244 (C-O-C=O), 1088 (C-O-C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.06 (m, 9H, CH<sub>3</sub>, OAc at C-2, C-3 and C-6), 3.64 (dd, *J*=12.6 Hz and 8.2 Hz, 1H, H-5a), 4.24 (dd, *J*=12.6 Hz and 4.6 Hz, 1H, H-5b), 5.18 (ddd, *J*=8.2 Hz, 7.8 Hz and 4.6 Hz, 1H, H-4), 5.22 (dd, *J*=8.2 Hz and 6.4 Hz, 1H, H-2), 5.29 (t, *J*=8.2 Hz, 1H, H-3), 5.97 (d, *J*=6.4 Hz, 1H, H-1), 7.46 (m, 2H, Ph), 7.60 (m, 1H, Ph), 8.05 (m, Ph); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  20.6 (CH<sub>3</sub>, OAc at C-2, C-3 and C-4), 62.5 (C-5), 68.0 (C-4), 69.1 (C-2), 70.3 (C-3), 92.5 (C-1), 128.5 (Ph), 129.6 (Ph), 133.5 (Ph), 164.5 (O-(C=O)-Ph), 169.3, 169.6, 169.8 (C=O, OAc at C-2, C-3 and C-4). HRMS. Calcd for C<sub>18</sub>H<sub>20</sub>O<sub>9</sub>, [M]<sup>+</sup>: *m/z* 380.1107; found: *m/z* 380.1096.

**2,3,4-Tri-*O*-acetyl- $\beta$ -D-xylopyranosyl *p*-hydroxybenzoate (2b)** Yield 51%; colorless solid; mp 205–206°C; *R*<sub>f</sub> 0.43 (hexane-AcOEt, 1:1); [ $\alpha$ ]<sub>D</sub><sup>25</sup> –68.3 (c 1.0, THF); IR: 3372 (O-H), 3000 (Ar, C-H), 1755 (C=C-C=O), 1619 (Ar, C=C), 1518 (Ar, C=C), 1241 (C-O-C=O), 1152 (C-O-C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.06 (m, 9H, CH<sub>3</sub>, OAc at C-2, C-3 and C-4), 3.95 (ddd, *J*=9.6 Hz, 4.6 Hz and 2.3 Hz, 1H, H-4), 4.13 (dd, *J*=12.6 Hz and 2.3 Hz, 1H, H-5a), 4.32 (dd, *J*=12.6 Hz and 4.6 Hz, 1H, H-5b), 5.35

(m, 2H, H-2 and H-3), 5.90 (d,  $J=9.2$  Hz, 1H, H-1), 6.74 (s, 1H, OH at Ph) 6.87 (m, 2H, Ph), 7.93 (m, 2H, Ph);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  20.6 ( $\text{CH}_3$ , OAc at C-2, C-3 and C-4), 61.6 (C-5), 69.9 (C-2), 72.6 (C-3 and C-4), 92.5 (C-1), 115.5 (Ph) 132.6 (Ph), 164.2 (O-(C=O)-Ph), 169.6, 169.8, 170.2, (-C=O in OAc at C-2, C-3 and C-4). HRMS. Calcd for  $\text{C}_{18}\text{H}_{20}\text{O}_{10}$ ,  $[\text{M}]^+$ :  $m/z$  396.1057; found:  $m/z$  396.1062.

**2,3,4-Tri-*O*-acetyl- $\beta$ -D-xylopyranosyl protocatechuate (2c)** Yield 44%; colorless solid; mp 124–125°C;  $R_f$  0.3 (hexane-AcOEt, 1:1);  $[\alpha]_D^{25}$  –55.0 (c 1.0, THF); IR: 3367 (O-H), 2964 (C-H), 1728 (C=C-C=O), 1605 (Ar, C=C), 1533 (Ar, C=C), 1213 (C-O-C=O), 1052 (C-O-C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.06 (m, 9H,  $\text{CH}_3$ , OAc at C-2, C-3 and C-4), 3.62 (dd,  $J=12.4$  Hz and 8.2 Hz, 1H, H-5a), 4.22 (dd,  $J=12.4$  Hz and 5.0 Hz, 1H, H-5b), 5.04 (ddd,  $J=8.7$  Hz, 8.2 Hz and 5.0 Hz, 1H, H-4), 5.21 (dd,  $J=8.7$  Hz and 6.9 Hz, 1H, H-2), 5.30 (dd,  $J=8.7$  Hz and 8.2 Hz, 1H, H-3), 5.89 (d,  $J=6.9$  Hz, 1H, H-1), 6.91 (m, 1H, Ph), 7.56 (m, 2H, Ph);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  20.6 ( $\text{CH}_3$ , OAc at C-2, C-3 and C-4), 62.5 (C-5), 68.3 (C-4), 69.6 (C-2), 70.7 (C-3), 92.5 (C-1), 115.0 (Ph), 116.7 (Ph), 124.4 (Ph), 164.4 (-C(=O)-C-), 170.0, 170.1, 170.2 (-C=O in OAc at C-2, C-3 and C-4); HRMS:  $m/z$   $[\text{M}]^+$  Calcd for  $\text{C}_{18}\text{H}_{20}\text{O}_{11}$ : 412.1006; found: 412.1030.

**2,3,4-Tri-*O*-acetyl- $\beta$ -D-xylopyranosyl vanilloate (2d)** Yield 73%; colorless solid; mp 91–92°C;  $R_f$  0.48 (hexane-AcOEt, 1:1);  $[\alpha]_D^{25}$  –14.2 (c 1.0, THF); IR: 3353 (O-H), 3000 (Ar, C-H), 1732 (C=C-C=O), 1595 (Ar, C=C), 1519 (Ar, C=C), 1249 (C-O-C=O), 1054 (C-O-C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.06 (m, 9H,  $\text{CH}_3$ , OAc at C-2, C-3 and C-4), 3.62 (dd,  $J=12.6$  Hz and 8.6 Hz, 1H, H-5a), 3.95 (s, 3H,  $\text{OCH}_3$  at Ph), 4.22 (dd,  $J=12.6$  Hz and 5.2 Hz, 1H, H-5b), 5.04 (dd,  $J=8.6$  Hz and 5.2 Hz, 1H, H-4), 5.23 (dd,  $J=8.6$  Hz and 6.9 Hz, 1H, H-2), 5.30 (t,  $J=8.6$  Hz, 1H, H-3), 5.90 (d,  $J=6.9$  Hz, 1H, H-1), 6.17 (s, 1H, OH at Ph) 6.95 (d,  $J=8.0$  Hz, 1H, Ph), 7.54 (d,  $J=1.7$  Hz, 1H, Ph), 7.65 (dd,  $J=8.6$  Hz and 1.7 Hz, 1H, Ph);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  20.6 ( $\text{CH}_3$ , OAc at C-2, C-3 and C-4), 62.8 (C-5), 68.4 (C-4), 69.5 (C-2), 70.8 (C-3), 92.6 (C-1), 112.0 (Ph), 114.3 (Ph), 124.9 (Ph), 164.2 (O-(C=O)-Ph), 169.4, 169.7, 169.8, (C=O, OAc at C-2, C-3 and C-4). HRMS. Calcd for  $\text{C}_{19}\text{H}_{22}\text{O}_{11}$ ,  $[\text{M}]^+$ :  $m/z$  426.1162; found:  $m/z$  426.1177.

**2,3,4-Tri-*O*-acetyl- $\beta$ -D-xylopyranosyl syringoate (2e)** Yield 71%; colorless solid; mp 103–104°C;  $R_f$  0.33 (hexane-AcOEt, 1:1);  $[\alpha]_D^{25}$  –51.8 (c 1.0, THF); IR: 3439 (O-H), 2948 (C-H), 1752 (C=C-C=O), 1610 (Ar, C=C), 1515 (Ar, C=C), 1217 (C-O-C=O), 1039 (C-O-C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.06 (m, 9H,  $\text{CH}_3$ , OAc at C-2, C-3 and C-4), 3.60 (dd,  $J=12.0$  Hz and 8.6, Hz, 1H, H-5a), 3.94 (s, 6H,  $\text{OCH}_3$  at Ph), 4.21 (dd,  $J=12.0$  Hz and 5.2 Hz, 1H, H-5b), 5.06 (dd,  $J=8.6$  Hz and 5.2 Hz, 1H, H-4), 5.27 (dd,  $J=8.6$ , 6.9 Hz, 1H, H-2), 5.33 (t,  $J=8.6$  Hz, 1H, H-3), 5.85 (d,  $J=6.9$  Hz, 1H, H-1), 6.04 (s, 1H, OH at Ph), 7.33 (s, 2H, Ph);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  20.6 ( $\text{CH}_3$ , OAc at C-2, C-3 and C-4), 56.3 ( $\text{OCH}_3$  at Ph), 63.0 (C-5), 68.7 (C-4), 70.0 (C-2), 71.4 (C-3), 93.2 (C-1), 107.4 (Ph), 164.3 (O-(C=O)-Ph), 169.4, 169.8 (C=O, OAc at C-2, C-3 and C-4). HRMS. Calcd for  $\text{C}_{20}\text{H}_{24}\text{O}_{12}$ ,  $[\text{M}]^+$ :  $m/z$  456.1268; found:  $m/z$  456.1291.

## General procedure for Novozym 435-catalyzed regioselective deacetylation

A solution of 2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl hydroxybenzoate **1a-e** (1.0 mmol) or 2,3,4-tri-*O*-acetyl- $\beta$ -D-xylopyranosyl hydroxybenzoates **2a-e** (1.0 mmol), MeOH (384 mg, 12.0 mmol), and Novozym 435 (0.8 g) in *tert*-butyl methyl ether (MTBE, 10 mL) was stirred for 24 h at 50°C. The progress of the reaction was monitored with thin layer chromatography. Upon completion of the reaction, Novozym

435 was filtered off the mixture and MTBE was then removed under reduced pressure. The residue was subjected to flash chromatography ( $\text{CHCl}_3/\text{MeOH}$ , 9:1) to give the partially deacetylated product **3a-e** or **4a-e**.

**2,3-Di-*O*-acetyl- $\beta$ -D-glucopyranosyl benzoate (3a)** Yield 85%; colorless solid; mp 136–137°C;  $R_f$  0.48 ( $\text{CHCl}_3/\text{MeOH}$ , 10:1);  $[\alpha]_D^{25}$  –54.7 (c 1.0, THF); IR: 3437 (O-H), 3069 (Ar, C-H), 1750 (C=C-C=O), 1601 (Ar, C=C), 1236 (C-O-C=O), 1028 (C-O-C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  2.06 (m, 6H,  $\text{CH}_3$ , OAc at C-2 and C-3), 3.68 (m,  $J=9.2$ , 4.6, 2.3 Hz, 1H, H-4), 3.87 (m, 2H, H-6a, H-6b), 3.96 (m, 1H, H-3), 5.21 (t,  $J=9.2$  Hz, 1H, H-5), 5.26 (dd,  $J=10.3$  Hz and 9.2 Hz, 1H, H-2), 5.93 (d,  $J=9.2$  Hz, 1H, H-1), 7.46 (t,  $J=8.0$  Hz, 2H, Ph), 7.60 (m, 1H, Ph), 8.02 (m, 2H, Ph);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  20.6 ( $\text{CH}_3$ , OAc at C-2 and C-3), 61.5 (C-6), 68.9 (C-4), 70.2 (C-2), 75.5 (C-3), 76.5 (C-5), 92.7 (C-1), 128.5 (Ph), 130.1 (Ph), 134.0 (Ph), 147.6 (-C(=O)-C-Ph), 164.5 (C(=O)CH=CH-), 169.5 (C=O, OAc at C-2), 171.2 (C=O, OAc at C-3). HRMS. Calcd for  $\text{C}_{17}\text{H}_{20}\text{O}_9$ ,  $[\text{M}]^+$ :  $m/z$  368.1107; found:  $m/z$  368.1125.

**2,3-Di-*O*-acetyl- $\beta$ -D-glucopyranosyl *p*-hydroxybenzoate (3b)** Yield 88%; colorless solid; mp 131–132°C;  $R_f$  0.33 ( $\text{CHCl}_3/\text{MeOH}$ , 10:1);  $[\alpha]_D^{25}$  –31.0 (c 1.0, THF); IR: 3379 (O-H), 3014 (Ar, C-H), 1732 (C=C-C=O), 1610 (Ar, C=C), 1517 (Ar, C=C), 1241 (C-O-C=O), 1086 (C-O-C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  2.06 (m, 6H,  $\text{CH}_3$ , OAc at C-2 and C-3), 3.58 (m, 3H, H-4, H-6a and H-6b), 3.70 (d,  $J=12.0$  Hz, 1H, H-5), 4.99 (t,  $J=9.2$  Hz, 1H, H-2), 5.18 (t,  $J=9.2$  Hz, 1H, H-3), 5.88 (d,  $J=8.0$  Hz, 1H, H-1), 6.91 (d,  $J=7.4$  Hz, 2H, Ph), 7.19 (d,  $J=7.4$  Hz, 2H, Ph);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  20.6 ( $\text{CH}_3$  in OAc at C-2 and C-3), 55.9 ( $\text{OCH}_3$  at Ph), 59.8 (C-5), 67.1 (C-6), 70.5 (C-2), 74.3 (C-3), 77.2 (C-4), 92.0 (C-1), 115.5 (Ph), 131.7 (Ph), 163.6 (-C=O in OAc at C-2), 169.5 (-C=O in OAc at C-3). HRMS. Calcd for  $\text{C}_{17}\text{H}_{20}\text{O}_{10}$ ,  $[\text{M}]^+$ :  $m/z$  384.1057; found:  $m/z$  384.1072.

**2,3-Di-*O*-acetyl- $\beta$ -D-glucopyranosyl protocatechuate (3c)** Yield 84%; colorless solid; mp 95–96°C;  $R_f$  0.10 ( $\text{CHCl}_3/\text{MeOH}$ , 20:1);  $[\alpha]_D^{25}$  –57.9 (c 1.0, THF); IR: 3423 (O-H), 2941 (C-H), 1733 (C=C-C=O), 1604 (Ar, C=C), 1521 (Ar, C=C), 1221 (C-O-C=O), 1033 (C-O-C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  1.91 (s, 3H,  $\text{CH}_3$ , OAc at C-3), 2.06 (s, 6H,  $\text{CH}_3$ , OAc at C-2), 3.54 (m, 3H, H-4, H-6a and H-6b), 3.68 (d,  $J=12.0$  Hz, 1H, H-5), 4.93 (dd,  $J=10.3$  Hz and 8.6 Hz, 1H, H-2), 5.13 (m, 1H, H-3), 5.89 (d,  $J=8.6$  Hz, 1H, H-1), 6.82 (d,  $J=8.6$  Hz, 1H, Ph), 7.27 (dd,  $J=2.3$  Hz and 8.6 Hz, 1H, Ph), 7.31 (d,  $J=2.3$  Hz, 1H, Ph);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  20.6 ( $\text{CH}_3$ , OAc at C-2 and C-3), 59.9 (C-5), 67.2 (C-6), 70.6 (C-2), 74.7 (C-3), 77.3 (C-4), 91.6 (C-1), 116.4 (Ph), 118.9 (Ph), 122.2 (Ph), 145.1 (C(=O)-C-Ph), 163.7 (C=O, OAc at C-2), 169.4 (C=O, OAc at C-3). HRMS. Calcd for  $\text{C}_{17}\text{H}_{20}\text{O}_{11}$ ,  $[\text{M}]^+$ :  $m/z$  400.1005; found:  $m/z$  400.1008.

**2,3-Di-*O*-acetyl- $\beta$ -D-glucopyranosyl vanilloate (3d)** Yield 89%; colorless solid; mp 127–128°C;  $R_f$  0.30 ( $\text{CHCl}_3/\text{MeOH}$ , 10:1);  $[\alpha]_D^{25}$  –71.2 (c 1.0, THF); IR: 3295 (O-H), 3021 (Ar, C-H), 2974 (C-H), 1752 (C=C-C=O), 1608 (Ar, C=C), 1519 (Ar, C=C), 1223 (C-O-C=O), 1032 (C-O-C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  1.93 (s, 3H,  $\text{CH}_3$ , OAc at C-3), 2.06 (s, 3H,  $\text{CH}_3$ , OAc at C-2), 3.58 (m, 3H, H-4, H-6a and H-6b), 3.70 (d,  $J=11.5$  Hz, 1H, H-5), 3.82 (s, 6H,  $\text{OCH}_3$  at Ph), 4.97 (t,  $J=8.6$  Hz, 1H, H-2), 5.17 (t,  $J=8.6$  Hz, 1H, H-3), 5.89 (d,  $J=8.6$  Hz, 1H, H-1), 6.90 (d,  $J=8.6$  Hz, 1H, Ph), 7.41 (d,  $J=1.7$  Hz, 1H, Ph), 7.43 (dd,  $J=1.7$  Hz and 8.6 Hz, 1H, Ph);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  20.6 ( $\text{CH}_3$ , OAc at C-2 and C-3), 55.5 ( $\text{OCH}_3$  at Ph), 59.9 (C-5), 67.2 (C-6), 70.7 (C-2), 74.5 (C-3), 77.4 (C-4), 91.9 (C-1), 115.4 (Ph), 119.0 (Ph), 123.9 (Ph), 147.4 (C(=O)-C-Ph), 163.6 (C=O in OAc at C-2), 169.3 (C=O in OAc at C-3). HRMS.  $m/z$   $[\text{M}]^+$  Calcd for  $\text{C}_{18}\text{H}_{22}\text{O}_{11}$ ,  $[\text{M}]^+$ :  $m/z$  414.1162; found:  $m/z$  414.1183.



**2,3-Di-O-acetyl-β-D-glucopyranosyl syringoate (3e)** Yield 90%; colorless solid; mp 159–160°C;  $R_f$  0.73 (CHCl<sub>3</sub>/MeOH, 10:1);  $[\alpha]_D^{25}$  –41.5 (c 1.0, THF); IR: 3312 (O-H), 3009 (Ar, C-H), 2995 (C-H), 1743 (C=C–C=O), 1604 (Ar, C=C), 1516 (Ar, C=C), 1062 (C–O–C=O) cm<sup>–1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 2.06 (m, 6H, CH<sub>3</sub>, OAc at C-2 and C-3), 3.58 (m, 1H, H-4, H-6a and H-6b), 3.70 (d, *J* = 12.0 Hz, 1H, H-5), 3.81 (s, 6H, OCH<sub>3</sub> at Ph), 4.99 (t, *J* = 9.2 Hz, 1H, H-2), 5.18 (t, *J* = 9.2 Hz, 1H, H-3), 5.88 (d, *J* = 8.0 Hz, 1H, H-1), 7.19 (s, 2H, Ph); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 20.6 (CH<sub>3</sub> in OAc at C-2 and C-3), 55.9 (OCH<sub>3</sub> at Ph), 59.8 (C-5), 67.1 (C-6), 70.5 (C-2), 74.3 (C-3), 77.2 (C-4), 92.0 (C-1), 107.0 (Ph), 147.6 (C(=O)C-Ph), 163.6 (C=O, OAc at C-2), 169.4 (C=O, OAc at C-3). HRMS. Calcd for C<sub>19</sub>H<sub>24</sub>O<sub>12</sub>, [M]<sup>+</sup>: *m/z* 444.1268; found: *m/z* 444.1243.

**2,3-Di-O-acetyl-β-D-xylopyranosyl benzoate (4a)** Yield 83%; colorless solid; mp 149–150°C;  $R_f$  0.43 (CHCl<sub>3</sub>/MeOH, 20:1);  $[\alpha]_D^{25}$  –70.1 (c 1.0, THF); IR: 3411 (O-H), 2950 (C-H), 1738 (C=C–C=O), 1601 (Ar, C=C), 1492 (Ar, C=C), 1069 (C–O–C=O) cm<sup>–1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 1.93 (s, 3H, CH<sub>3</sub> in OAc at C-3), 2.03 (s, 3H, CH<sub>3</sub> in OAc at C-2), 3.93 (dd, *J* = 5.7 Hz and 14.9 Hz, 1H, H-5a), 3.58 (ddd, *J* = 5.7 Hz, 10.3 Hz and 14.9 Hz, 1H, H-4), 3.93 (dd, *J* = 5.7 Hz and 10.3 Hz, 1H, H-5b), 4.99 (dd, *J* = 8.2 Hz and 10.3 Hz, 1H, H-2), 5.11 (t, *J* = 9.2 Hz, 1H, H-3), 5.90 (d, *J* = 8.2 Hz, 1H, H-1), 7.55 (t, *J* = 8.0, 10.3 Hz, 2H, Ph), 7.69 (t, *J* = 8.0 Hz, 1H, Ph), 7.92 (dd, *J* = 1.7 Hz and 8.0 Hz, 1H, Ph); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 20.6 (CH<sub>3</sub>, OAc at C-2 and C-3), 65.8 (C-5), 66.8 (C-4), 70.2 (C-2), 74.0 (C-3), 92.7 (C-1), 128.9 (Ph), 129.2 (Ph), 134.0 (Ph), 163.9 (C(=O)C-Ph), 169.2 (C=O, OAc at C-2), 169.4 (C=O, OAc at C-3). HRMS. Calcd for C<sub>16</sub>H<sub>18</sub>O<sub>8</sub>, [M]<sup>+</sup>: *m/z* 338.1002; found: *m/z* 338.1016.

**2,3-Di-O-acetyl-β-D-xylopyranosyl *p*-hydroxybenzoate (4b)** Yield 85%; colorless solid; mp 202–203°C;  $R_f$  0.23 (CHCl<sub>3</sub>/MeOH, 20:1);  $[\alpha]_D^{25}$  –190.8 (c 0.5, THF); IR: 3411 (O-H), 3000 (Ar, C-H), 2942 (C-H), 1725 (C=C–C=O), 1618 (Ar, C=C), 1518 (Ar, C=C), 1078 (C–O–C=O) cm<sup>–1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 1.92 (s, 3H, CH<sub>3</sub>, OAc at C-3), 2.03 (s, 3H, CH<sub>3</sub>, OAc at C-2), 3.51 (t, *J* = 11.5 Hz, 1H, H-5a), 3.75 (m, 1H, H-4), 3.91 (dd, *J* = 5.7 Hz and 11.5 Hz, 1H, H-5b), 4.96 (t, *J* = 8.6 Hz, 1H, H-2), 5.09 (t, *J* = 8.6 Hz, 1H, H-3), 5.84 (d, *J* = 8.6 Hz, 1H, H-1), 6.87 (d, *J* = 8.6 Hz, 2H, Ph), 7.77 (d, *J* = 8.6 Hz, 2H, Ph); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 20.6 (CH<sub>3</sub>, OAc at C-2 and C-3), 65.7 (C-5), 66.9 (C-4), 70.2 (C-2), 74.2 (C-3), 92.3 (C-1), 115.5 (Ph), 131.7 (Ph), 163.6 (C(=O)C-Ph), 169.1 (C=O, OAc at C-2), 169.4 (C=O, OAc at C-3). HRMS. Calcd for C<sub>16</sub>H<sub>18</sub>O<sub>9</sub>, [M]<sup>+</sup>: *m/z* 354.0951; found: *m/z* 354.0965.

**2,3-Di-O-acetyl-β-D-xylopyranosyl protocatechoate (4c)** Yield 80%; colorless solid; mp 78–79°C;  $R_f$  0.13 (CHCl<sub>3</sub>-MeOH, 20:1);  $[\alpha]_D^{25}$  –68.5 (c 0.5, THF); IR: 3423 (O-H), 2988 (C-H), 1732 (C=C–C=O), 1605 (Ar, C=C), 1524 (Ar, C=C), 1080 (C–O–C=O) cm<sup>–1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 1.92 (s, 3H, CH<sub>3</sub> in OAc at C-3), 2.02 (s, 3H, CH<sub>3</sub> in OAc at C-2), 3.49 (t, *J* = 10.8 Hz, 1H, H-5a), 3.73 (dd, *J* = 5.7 Hz and 10.8 Hz, 1H, H-4), 3.89 (dd, *J* = 4.6 Hz and 10.8 Hz, 1H, H-5b), 4.93 (dd, *J* = 8.6 Hz and 9.2 Hz, 1H, H-2), 5.06 (t, *J* = 9.2 Hz, 1H, H-3), 5.81 (d, *J* = 8.6 Hz, 1H, H-1), 6.82 (d, *J* = 8.6 Hz, 1H, Ph), 7.27 (dd, *J* = 1.7 Hz and 8.6 Hz, 1H, Ph), 7.31 (d, *J* = 1.7 Hz, 1H, Ph); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 20.6 (CH<sub>3</sub>, OAc at C-2 and C-3), 65.8 (C-5), 66.9 (C-4), 70.3 (C-2), 74.3 (C-3), 92.3 (C-1), 115.4 (Ph), 116.4 (Ph), 122.3 (Ph), 163.8 (C(=O)C-Ph), 169.1 (C=O, OAc at C-2), 169.5 (C=O, OAc at C-3). HRMS. Calcd for C<sub>16</sub>H<sub>18</sub>O<sub>10</sub>, [M]<sup>+</sup>: *m/z* 370.0900; found: *m/z* 370.0921.

**2,3-Di-O-acetyl-β-D-xylopyranosyl vanilloate (4d)** Yield 88%; colorless solid; mp 40–41°C;  $R_f$  0.33 (CHCl<sub>3</sub>/MeOH, 20:1);  $[\alpha]_D^{25}$  –75.9 (c 1.0, THF); IR: 3437 (O-H), 3018 (Ar, C-H), 2941 (C-H), 1734 (C=C–C=O), 1595 (Ar, C=C), 1515 (Ar, C=C), 1082 (C–O–C=O) cm<sup>–1</sup>; <sup>1</sup>H NMR

(DMSO-*d*<sub>6</sub>): δ 1.95 (s, 3H, CH<sub>3</sub>, OAc at C-3), 2.03 (s, 3H, CH<sub>3</sub>, OAc at C-2), 3.52 (dd, *J* = 9.7 Hz and 10.3 Hz, 1H, H-5a), 3.75 (m, 1H, H-4), 3.81 (s, 3H, OCH<sub>3</sub> at Ph), 3.91 (dd, *J* = 5.2 Hz and 10.3 Hz, 1H, H-5b), 4.98 (dd, *J* = 8.0 Hz and 9.7 Hz, 1H, H-2), 5.10 (t, *J* = 9.7 Hz, 1H, H-3), 5.81 (d, *J* = 8.0 Hz, 1H, H-1), 6.89 (d, *J* = 8.6 Hz, 1H, Ph), 7.40 (d, *J* = 2.3 Hz, 1H, Ph), 7.43 (dd, *J* = 2.3 Hz and 8.6 Hz, 1H, Ph); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 20.6 (CH<sub>3</sub>, OAc at C-2 and C-3), 55.5 (OCH<sub>3</sub> at Ph), 65.8 (C-5), 66.9 (C-4), 70.3 (C-2), 74.1 (C-3), 92.5 (C-1), 112.8 (Ph), 115.3 (Ph), 123.9 (Ph), 163.6 (C(=O)C-Ph), 169.3 (C=O, OAc at C-2), 169.4 (C=O, OAc at C-3). HRMS. Calcd for C<sub>17</sub>H<sub>20</sub>O<sub>10</sub>, [M]<sup>+</sup>: *m/z* 384.1057; found: *m/z* 384.1081.

**2,3-Di-O-acetyl-β-D-xylopyranosyl syringoate (4e)** Yield 82%; colorless solid; mp 144–145°C;  $R_f$  0.30 (CHCl<sub>3</sub>/MeOH, 20:1);  $[\alpha]_D^{25}$  –97.1 (c 1.0, THF); IR: 3480 (O-H), 2985 (C-H), 1732 (C=C–C=O), 1615 (Ar, C=C), 1516 (Ar, C=C), 1119 (C–O–C=O) cm<sup>–1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 1.95 (s, 3H, CH<sub>3</sub>, OAc at C-3), 2.03 (s, 3H, CH<sub>3</sub>, OAc at C-2), 3.53 (t, *J* = 10.8 Hz, 1H, H-5a), 3.76 (m, 1H, H-4), 3.82 (s, 6H, CH<sub>3</sub> at Ph), 3.92 (dd, *J* = 5.7 Hz and 10.8 Hz, 1H, H-5b), 5.00 (dd, *J* = 8.0 Hz and 9.2 Hz, 1H, H-2), 5.20 (t, *J* = 9.2 Hz, 1H, H-3), 5.80 (d, *J* = 8.0 Hz, 1H, H-1), 7.12 (s, 2H, Ph); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 20.6 (CH<sub>3</sub>, OAc at C-2 and C-3), 65.9 (C-5), 67.0 (C-4), 70.3 (C-2), 74.0 (C-3), 92.7 (C-1), 107.2 (Ph), 163.7 (C(=O)C-Ph), 169.3 (C=O, OAc at C-2), 169.4 (C=O, OAc at C-3). HRMS. Calcd for C<sub>18</sub>H<sub>22</sub>O<sub>11</sub>, [M]<sup>+</sup>: *m/z* 414.1162; found: *m/z* 414.1183.

## Lipase screening for deacetylation of 1a and 2a

Lipase (0.1 g) (AS from *Aspergillus niger*, AK from *Pseudomonas fluorescens*, AYS from *Candida rugosa*, PS from *Burkholderia cepacia* and PPL from porcine pancreas) was added to a mixture of **1a** (45 mg, 0.1 mmol) or **2a** (38 mg, 0.1 mmol) in pH 7 citrate buffer (10 mL) and MTBE (2.5 mL), and the mixture was stirred at 40°C for 24 h. The progress of the reaction was monitored with thin layer chromatography. After stirring, lipase was filtered off and the organic phase was extracted with AcOEt. The combined extracts were washed with brine, dried over anhydrous MgSO<sub>4</sub> and concentrated under reduced pressure. The crude product was purified by flash chromatography (CHCl<sub>3</sub>/MeOH, 9:1).

## DPPH<sup>•</sup> radical scavenging activity

The antioxidant activity of β-D-glycopyranosyl hydroxybenzoates was estimated by measuring their free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) as free radical according to the literature report [51]. The reaction mixture (10 mL) comprised of freshly made 0.15 mM DPPH<sup>•</sup> in ethanol (7000 μL), different concentrations of each β-D-glycopyranosyl hydroxybenzoates (1, 5 and 10 μmol) in 300 μL DMSO and tris-HCl buffer (pH 7.4, 100 mM). The reaction mixture was kept for 30 min in a water bath at 25°C under dark and optical density was measured at 517 nm. DPPH<sup>•</sup> has an unpaired electron, which gave purple color, and when this electron is balanced, the color is lost. The compounds which can give an electron to the DPPH<sup>•</sup> bleaching the reagent, which is monitored as the decrease in absorbance of the DPPH<sup>•</sup> solution. All analyses were carried out in triplicate.

## Inhibitory effect on autoxidation of bulk methyl linoleate

To 1 g of methyl linoleate, 25 μL of the acetone solution of the test compound was mixed in a 50-mL vial, and the mixture was agitated

under ultrasonic wave for 30 s. A 25  $\mu$ L aliquot of acetone without sample was added for control. After purging the acetone with nitrogen, the mixture was placed in an oven at 40°C in the dark. Final concentration of each sample was 0.05  $\mu$ mol/g. An aliquot was dissolved in ethanol, and the conjugated diene absorbance was measured at 234 nm with an UVmini-2400 UV-vis spectrophotometer every 24 h at 20°C. All tests were run in triplicate.

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