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Glycosidase-Catalyzed Synthesis of Glycosyl Esters and Phenolic Glycosides of Aromatic Acids

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Abstract. Phenolic glycosides occur naturally in many plants and as such are often present in the human diet. Their isolation from natural sources is usually laborious due to their presence in complex matrices. Their chemical and enzymatic syntheses have been found complex, time-consuming, and costly, yielding only small amounts of glycosylated products. In quest of a convenient biocatalytic route to structurally complex phenolic glycosides, we discovered that the rutinosidase from *Aspergillus niger* not only efficiently converts hydroxylated aromatic acids (*e.g.* coumaric and ferulic acids) into the respective phenolic rutinosides, but surprisingly also catalyzes the formation of the respective glycosyl esters. We report here the results of a systematic study presenting the unique synthesis of naturally occurring glycosyl esters and phenolic glycosides accomplished by glycosidase catalysis. A panel of aromatic acids was tested as glycosyl acceptors and the crucial structural features required for the formation of glycosyl esters were identified. In the light of the present structure-activity relationship study, a plausible reaction mechanism was proposed. All the products were fully structurally characterized by NMR and MS.

Keywords: natural product; glycosylation; rutinosidase; carboxylic glycoside; coumaric acid; ferulic acid

Introduction

Hydroxyphenyl propenoic acids (hydroxycinnamic acids), e.g. ferulic acid(s), coumaric acids, sinapic acid, caffeic acid, etc., are common components of the human diet. These compounds also occur in plants in the form of various glycosides, such as glucosides, galactosides, rhamnosides, arabinosides, and rutinosides. As the diets rich in polyphenols have repeatedly been related to low incidence of cardiovascular, neurodegenerative, and oncological diseases, various food supplements containing these compounds are becoming increasingly popular among the general population. Some phenolic glycosides can quite easily be isolated from natural sources but most of them are not readily obtainable due to their occurrence in complex matrices.

Phenolic acids and their glycosides are frequently found as acyl moieties in esters of a plethora of polyphenols. Phenolic acids form esters either with phenolic OH groups or, in some cases, with the glycosidic moieties of polyphenol glycosides.[1] Acylation of polyphenol glycosides can be well accomplished (at their glycone moiety) using chemical or chemoenzymatic synthesis.[2] Glycosides of hydroxycinnamic acid derivatives were first described in the sixties of the last century[3] and later they were found to be ubiquitous in plants.[4a,b] Then 1-*O*-hydroxycinnamoyl-β-glucopyranoses were identified to be substrates of acyltransferases (known as serine carboxypeptidase-like acyltransferases) and

in plants they serve as activated acyl donors (besides acyl-CoA) for the biosynthesis of many secondary metabolites.[4c] They also play an important role in cell-wall lignification. There is a limited number of in-detail-described examples (with full spectral identification) showing that the carboxyl of the acid is involved in the formation of the glycosidic linkage (glycosyl ester). A series of ferulic acid glycosides, as e.g. 1-*O*-(*E*)- and (*Z*)-feruloyl-β-sophorose (tuberonoid A and B, respectively), was isolated from *Allium tuberosum* (garlic chives) leaves.[5] 1-*O*-(*E*)-Caffeoyl-β-rutinose (swertiamacroside) was isolated from the plant *Swertia macrosperma*;[6] a mixture of the (*E*)- and 1-*O*-(*Z*)-*p*-coumaroyl-β-rutinoses was isolated from an acetone extract of aspen knot wood.[7] Recently, 1-*O*-acyl-β-glucopyranose, which is a metabolite of the synthetic herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was described in wild radish (*Raphanus raphanistrum*).[8]

Glycosylated hydroxycinnamates are generally not easily accessible. Their isolation from plant material is tedious, time demanding and low-yielding. Kosuge and Conn described the glucosylation of coumaric acid after feeding it to higher plants such as *Meliototus alba* and *Hierochloe odorata*.[9] Alternatively, these compounds can be produced *in vitro*, exploiting either classical organic synthesis protocols or biocatalytic approaches. For instance a 5-step synthesis of the above-mentioned 1-*O*-feruloyl- and 1-*O*-β-sinapoyl-β-glucopyranoses yielded gram amounts of these compounds.[10]

Enzymatic methods are a straightforward route to the glycosylation of various natural products mostly due to their simplicity (single-step methods), stereo-, and regioselectivity.[11] Enzymatically produced glycosides are usually obtained under mild conditions in quite pure form as unprotected compounds, leading to their easy isolation using e.g. gel filtration or other chromatographic techniques. Glycosyltransferases, which are natural biosynthetic enzymes, are very selective, but they employ expensive and labile sugar nucleosides and as typically intracellular proteins are often rather unstable under *in vitro* conditions. This is illustrated, e.g., in the synthesis by sinapate glucosyltransferase from *Gomphrena globasa* (globe amaranth).[12] This method relied on a relatively expensive UDP-glucose and a not readily accessible enzyme, and as a result, only small amounts of 1-*O*-feruloyl- and 1-*O*-sinapoyl-β-glucopyranoses were prepared, probably due to product inhibition. In contrast to glycosyltransferases, extracellular glycosidases are readily available robust enzymes, operating under broad reaction conditions with much cheaper glycosyl donors. Glycosylation of carboxylic acids (e.g. benzoic acid) was described with sucrose phosphorylase from *Streptococcus mutans*; here the products were only α-glucopyranosides.[13] Glycosylation of caffeic acid with recombinant sucrose phosphorylase from *Bifidobacterium longum* was demonstrated in supercritical CO_2.[14] Authors claimed formation of 1-*O*-caffeoyl-α-glucopyranose based on MS studies only, no NMR data nor yields were given. Here it should be noted that sucrose phosphorylases have rather different reaction mechanism than glycosidases and they can produce only α-glucopyranosides, therefore they are not as flexible as glycosidases.

Glycosylation of phenolic OH groups is a rather challenging reaction especially for glycosidases.[11] Leloir glycosyltransferases that synthesize phenolic glycosides *in vivo* can be used for preparatory *in vitro* synthesis of phenolic glycosides as well;[15] on the other hand, there exist only a few examples of phenol glycosylation with non-Leloir glycosyltransferases such as phosphorylases.[16] These enzymes use sucrose as an inexpensive glycosyl donor; however, they typically synthesize only α-glucopyranosides.

Recently, we described a robust diglycosidase, which glycosylated a number of phenolic acceptors. This enzyme, the rutinosidase from *Aspergillus niger* (EC 3.2.1.168, CAZY GH 5_23), is capable of transferring rutinosyl (6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl) residue onto various alcohols, but most interestingly also to phenolic acceptors, which are generally difficult to glycosylate with glycosidases.[17] A major advantage of this transglycosylation reaction is the use of inexpensive and biocompatible rutin (**1**) as the glycosyl donor, while quercetin (**2**) as a byproduct of the reaction precipitates and can be easily removed from the reaction mixture by filtration. The produced rutinosides can be conveniently transformed *in situ via* a telescoping reaction with α-L-rhamnosidase to yield the respective β-glucopyranosides.[18] These biotransformations are of interest because certain industrial applications of carboxy group-containing compounds are limited by the strong sour smell and taste and also by their low solubility.[16a]

We present here an enzymatic glycosylation of a series of hydroxyphenyl propenoic acids, and we describe, besides phenolic OH glycosylation, probably the first *in vitro* glycosidase-catalyzed glycosylation at the carboxy moiety.

Results and Discussion

At the beginning we embarked on a project to test the transglycosylation capacity of the rutinosidase from *A. niger* with a variety of cinnamic acid derivatives and their analogues. The recombinant rutinosidase was prepared using a *Pichia pastoris* expression system.[17] Rutin was used as a universal glycosyl donor throughout all experiments. The primary screening was performed under the same conditions that proved to be effective for polyphenol rutinosylation.[18]

(*E*)-*p*-Coumaric acid (**3**) and (*E*)-ferulic acid (**4**) (Table 1) were tested as two pioneer substrates to examine the general feasibility of the transglycosylation concept. Both acceptors gave transglycosylation products in a good yield as clearly shown by TLC. After HPLC analysis and a detailed inspection of the corresponding ^{1}H NMR spectra, we

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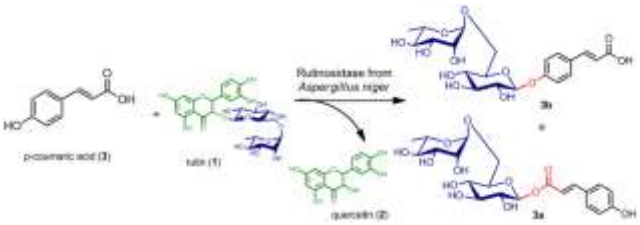
could observe that at least two glycosylation products were formed. Two peaks in proportions of about 1 : 3 and 1 : 2 were observed in each HPLC chromatogram for acceptors **3** and **4,** respectively. In both reactions the respective peaks had identical molecular masses (determined by LCMS) and were found to correspond to the respective *O*-monorutinosyl derivatives. This result was very surprising as both acceptors have only a phenolic group. Reactions such as *C*-glycosylation, oligoglycosylation and de-rhamnosylation were excluded by MS analysis. One remaining possibility, hitherto considered to be rather improbable, was the formation of glycosyl esters (Scheme 1), *i.e.* glycosylation of the OH group at the carboxyl moiety. As previously described, several glycosyl esters of phenolic acids (but not rutinosyl esters) were already isolated from natural sources.[4c,5,7] However, glycosylation of a carboxy group with glycosidases has never been described and it had always been considered highly improbable.



Scheme 1. Rutinosylation of *p*-coumaric acid (**3**) with rutinosidase from *Aspergillus niger* (the products of (*Z*)-configuration are not shown).

Glycosylation at the carboxy moiety yields esters, which, as such, are apt to hydrolyze (saponify) under alkaline conditions, while glycosides of alcohols including phenols are quite stable under these conditions. Conversely, it is well-known that all *O*-glycosides hydrolyze under acidic conditions. As a test of our hypothesis whether one of the products was a glycosyl ester, we incubated the glycosylation products of the enzymatic reactions with acceptors **3** and **4** under alkaline conditions (10% NaOH, r.t.). Indeed, in both cases one of the products hydrolyzed, yielding rutinose and the respective acid, whereas the other product remained intact. Under acidic conditions (1 N HCl, short boiling) all products hydrolyzed to yield the respective acid acceptor together with rhamnose and glucose, indicating complete cleavage of all glycosidic linkages in the molecules.

Advanced NMR methods allowed a detailed analysis of the mixture of glycosylated products of *p*-coumaric acid (**3**), pre-purified by extraction with Amberlite XAD-4 resin, and the structural determination of each component (Table S1, Supporting Information). Anomeric configurations of the rutinosyl moieties were determined from the magnitude of $J_{H1,\ H2}$ coupling constants; the 1→6 glycosidic linkage was proved by HMBC contact of the downfield shifted carbon C-6 of glucose with the anomeric proton H-1 of rhamnose. The position of the rutinosyl moiety was unambiguously determined using the HMBC correlations of the anomeric proton of glucose (Fig. S1d, Supporting Information). As a major product (about 79%) 4-*O*-rutinosyl (*E*)-*p*-coumaric acid (**3b**) was identified, in which the anomeric proton of glucose was correlated to the *para*-positioned aromatic carbon of the aglycone. Ten percent of the sample accounted for 1-*O*-(*E*)-*p*-coumaroyl-β-rutinose (**3a**) whose structure was unequivocally confirmed by the correlation of the glucose-based anomeric proton with the C=O at the carboxyl group (details of the HMBC spectrum are given in Fig. S1e in the Supporting Information). However, the sample contained two additional products, which were so far not observed even by chromatographic techniques (LC-MS); later we found that they were also inseparable using preparative HPLC. These products were glycosylated products of (*Z*)-*p*-coumaric acid, *i.e.* 4-*O*-rutinosyl (*Z*)-*p*-coumaric acid (**3c**, 6 %) and 1-*O*-(*Z*)-*p*-coumaroyl-β-rutinose (**3d**, 5 %), as determined by the respective coupling constants of the isolated olefinic protons in the aglycone (J = 15.9 ~ 16.0 Hz in (*E*)-*p*-coumaroyl; J = 12.9 Hz in (*Z*)-*p*-coumaroyl). It should be noted that all starting materials (cinnamic acid derivatives) were always uniformly of the *E*-configuration.

We were thus facing another enigma: "How the (*Z*)-isomers are formed during the reactions?" Control experiments – incubation of the respective (*E*)-acid under the same reaction conditions (without addition of the enzyme, under the same mild conditions) – confirmed that no (*E*)-(*Z*) isomerization occurred. Derivatives of cinnamic acid, such as e.g. coumaric or ferulic acid occur in nature both as (*E*)- and (*Z*)-isomers; they both were isolated e.g. from corn steep liquor[19]. Moreover, phenolic glycosides containing both (*E*)- and (*Z*)-*p*-coumaric acid were isolated from *Salix glandulosa*.[20] It is interesting to note that some simple glycosylated derivatives of (*Z*)-*p*-coumaric acid act as leaf-opening factors in nyctinastic plants (e.g. *Albizzia julibrissin* or *Mimosa*).[21] Isomerization between (*E*)- and (*Z*)-isomers of cinnamic acid derivatives occurs in nature typically under direct UV-A light. Mäkilä et al. demonstrated that (*E*)-*p*-coumaric acid was converted to the corresponding (*Z*)-isomer under UV-A radiation (one hour) and direct sunlight (one week) by 25 and 34%, respectively, when investigating the stability of these acids in black currant juice.[22] At preparatory scale, (*Z*)-cinnamates are readily accessible from the respective (*E*)-isomers, which are commercially available, by irradiation with a Pyrex-filtered high-pressure mercury lamp (λ_{em} >300 nm) or monochromatic light at 313 nm, resulting in the formation of a photostationary mixture consisting of about 67% (*E*)- and 33% (*Z*)-isomers.[23]

We speculated that for (*E*)-(*Z*) isomerization the formation of a quinone intermediate might be required (see Scheme 2). This requires a free phenolic OH group in *p*- or possibly also *o*-position as well as

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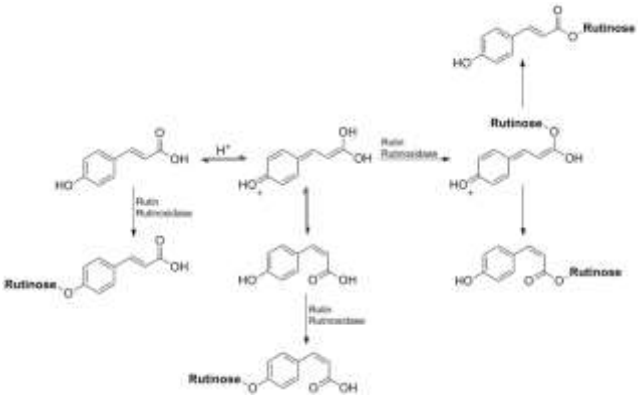
the presence of a conjugated 2,3-double bond and a carboxyl function.

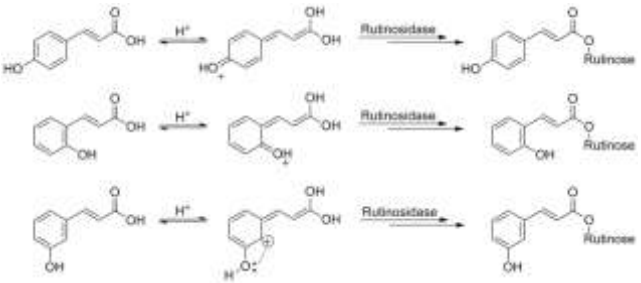


Scheme 2. Glycosylation of (*E*)-*p*-coumaric acid (**3**).

To support this hypothesis and to investigate the characteristics required for a particular phenolic acid substrate to be glycosylated, a large panel of aromatic acids bearing various structural features was tested (Table 1). As previously shown, **3** and **4** yielded both the (*E*) and (*Z*)-isomers of phenolic glycosides and glycosyl esters while *m*-coumaric acid (**5**) unable to form a quinone intermediate yielded both the phenolic glycosides and glycosyl esters exclusively in their original (*E*)-forms (**5a, b**). *o*-Coumaric (**6**) and caffeic acid (**7**), both of which are able to form *o*- or *p*-quinones, respectively, yielded only the (*E*)-isomers of the glycosylated products.

Transglycosylation in retaining glycosidases occurs by the mechanism depicted in Figure S45A (Supporting Information), where the acceptor alcohol approaches covalently bound glycosyl-enzyme intermediate and forms a transition state stabilized by the catalytic amino acid in the ionized state.[24] Acids are ionized and thus negatively charged and therefore they are repulsed electrostatically from the active site carboxylic acid (Glu) acting as acidobasic catalyst (see Figure S45B). This is probably the reason why most glycosidases do not glycosylate also phenols being weak acids. Rutinosidases seem to be rather special in its ability to glycosylate phenols.[25,26]

It appears that for the glycosylation of the carboxy group to occur, the complete structure of hydroxycinnamic acid is required. *p*-Hydroxyphenylacetic acid (**8**) lacking the conjugation of the carboxy function with the aromatic system yielded only the phenolic rutinoside **8a**. This was corroborated by the use of phenylacetic (**9**) and phenylpropionic (**10**) acids as acceptors where no glycosylation products were detected. Therefore, we propose that the formation of glycosyl esters is limited to the presence of a conjugated acid enriched in nucleophilicity *via* mesomeric resonances or inductive effects of a hydroxylated aromatic system (Scheme 3).



Scheme 3. Proposed mechanism of rutinosylation of hydroxycinnamic acids at the carboxy moiety.

Table 1. Screening of acceptors for rutinosylation (isolated yields).

Acceptor	Products
(*E*)-*p*-coumaric acid (**3**)	**3a** (3 %), **3b** (31 %), **3c** (1 %), **3d** (1 %)
(*E*)-ferulic acid (**4**)	**4a** (8 %), **4b** (6 %), **4c** (2 %)
(*E*)-*m*-coumaric acid (**5**)	**5a** (9 %), **5b** (46 %)
(*E*)-*o*-coumaric acid (**6**)	**6a** (1 %), **6b** (16 %)
(*E*)-caffeic acid (**7**)	**7a** (4 %), **7b** (8 %), **7c** (27 %)
p-hydroxyphenylacetic acid (**8**)	**8a** (12 %)
phenylacetic acid (**9**)	No product
phenylpropionic acid (**10**)	No product
(*E*)-cinnamic acid (**11**)	No product
p-methoxycinnamic acid (**12**)	No product
m-nitrocinnamic acid (**13**)	No product
sinapic acid (**14**)	No product
chlorogenic acid (**15**)	No product

Rut = 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl-(rutinosyl)

Moreover, the experiments performed with cinnamic acid (**11**) and with its two derivatives *p*-methoxycinnamic (**12**) and *m*-nitrocinnamic acid (**13**), in which the π-electronic density is enriched due to the presence of the electron donating methoxy group or reduced by the electron withdrawing nitro group, respectively, corroborated our hypothesis since no glycosyl esters were obtained.

Finally, sinapic (**14**) and quinic (**15**) acids, two sterically hindered substrates, were tested. Acid acceptor **14,** which was thought to be a potential substrate (in terms of our above-mentioned hypothesis), was not glycosylated at all, probably due to the steric hindrance of OH and/or due to hydrogen bond formation with adjacent methoxy groups; acid **15** bearing an esterified caffeoyl moiety was not substituted either.

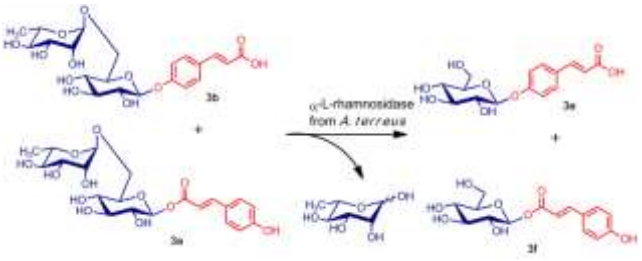
With regard to the above results we were interested to see whether, due to their particular nature, hydroxycinnamic acids can also yield phenolic or carboxylic glycosides with other glycosidases. *p*-Coumaric and ferulic acid, which gave the best transglycosylation yields with rutinosidase, were tested as substrates for glycosylation using a panel of β-*N*-acetylhexosaminidases, all retaining monoglycosidases. These enzymes were shown to afford high transglycosylation yields with various substrate types such as carbohydrates and/or aliphatic alcohols.[27] We tested seven synthetically potent β-*N*-acetylhexosaminidases from *Acremonium persicinum, Aspergillus oryzae, Fusarium oxysporum, Penicillium chrysogenum, P. oxalicum, Talaromyces flavus,* and *Trichoderma harzianum*. However, both aromatic acids strongly inhibited all β-*N*-acetylhexosaminidases tested (the glycosylation rate was reduced by more than 10 times) and despite the addition of higher amounts of enzyme no glycosylation products were observed using LCMS analysis.

We have to note that Katayama et al. prepared 4-*O*-rutinosides of vanillic, sinapic, ferulic, and caffeic acids using crude rutinosidase from tartary buckwheat (*Fagopyrum tataricum*) seeds, however no glycosylation of the carboxy moiety was described.[25] Although spectral characteristics of all compounds were given, overall yields were not provided. The resulting compounds were tested for antiviral activity against feline calicivirus, and it was found that rutinosylation, especially in the case of sinapic acid, improved antiviral activity.

Finally, we were interested whether the products of enzymatic glycosylation were substrates of the rutinosidase. Previously we found that some transglycosylation products, e.g. methyl rutinoside, were virtually resistant to rutinosidase hydrolysis. We have tested the hydrolytic cleavage of both glycosylation products of *p*-coumaric acid **3a** and **3b** using the rutinosidase under analogous conditions as the transglycosylation reactions, however in the absence of rutin, and indeed both rutinosides were hydrolyzed to yield acid **3** as determined by LC-MS analysis.

Enzymatic Derhamnosylation of Rutinosylated Aromatic Acids

To prove larger applicability of our enzymatic procedure we have tested the previously developed concept of a sequential two-enzyme synthesis of aryl glucopyranosides.[18] α-L-Rhamnosidase from *Aspergillus terreus*[28] was shown to selectively "trim" the rutinosyl unit to yield the respective β-glucopyranoside. Here, we have used a preparation containing both rutinosides **3a** (rutinosyl ester) and **3b** (phenolic rutinoside). After treatment by rhamnosidase we could observe a virtually quantitative conversion of rutinosides into respective β-glucopyranosides by LCMS analysis (see Figs. S41 and S42, Supporting Information; Scheme 4). Structures of the products were determined by UV spectra analysis and MS (loss of rhamnose). No free acid was observed in LCMS chromatogram, which demonstrates a clean and selective reaction.



Scheme 4. Enzymatic derhamnosylation of the products of enzymatic rutinosylation.

Conclusion

Many retaining glycosidases exhibit transglycosylation activities resulting in the facile synthesis of various glycosides, which greatly expands the use of these enzymes for synthetic applications. Typically, the great potential of enzymes in chemistry consists in their high selectivity, simple reaction conditions and the possibility of tuning their catalytic properties by selecting natural or generating artificial enzyme variants. Although mostly specific, enzymes may have promiscuous activities, and thus enable the exploration of novel reactions, possibly with surprising outcomes. Here we describe for the first time the enzymatic synthesis of glycosyl esters using a glycosidase as a catalyst. We present a detailed structure-activity study of a panel of aromatic acids as glycosyl acceptors and identify the crucial structural features required for this unique enzymatic activity as well as a plausible reaction mechanism. At the same time, this work highlights the rutinosidase from *Aspergillus niger* as a remarkable catalyst with unparalleled synthetic capabilities. Our study may serve as an initiation platform for future research in this uncharted area.

Experimental Section

Materials

Media components were from Oxoid (UK) or Carl-ROTH (DE). Rutin was from Alchimica (Prague, CZ) and other chemicals of the highest purity available were purchased from Sigma-Aldrich. All cinnamic acid derivatives used were of (*E*)-configuration.

Strains

An EasySelect *Pichia pastoris* KM71H Expression Kit was obtained from Invitrogen (US). This expression system employs methanol inducible AOX1 promoter, which is repressed by, e.g., glucose. The recombinant protein is produced as a fusion product into the growth medium in response to the secretion signal of the α-mating factor from *S. cerevisiae* included in the pPICZαA vector.
The culture of *Aspergillus niger* K2 CCIM is stored in the Collection of Microorganisms of the Institute of Microbiology of the Czech Academy of Sciences, Prague. The fungal strains producing β-*N*-acetylhexosaminidases (EC 3.2.1.52) originated from the Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague. The strains were cultivated and the enzymes were prepared as described previously.[29]

Structure Determination

NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer (600.23 MHz for ^{1}H, 150.93 MHz for ^{13}C) and a Bruker Avance III 700 MHz spectrometer (700.13 MHz for ^{1}H, 176.05 MHz for ^{13}C) in DMSO-d_6 at 30 °C using the manufacturer's software (data and the spectra *in extenso* in the Supporting Information). Individual spin systems of acceptors and monosaccharide units of rutinosyl were identified by COSY experiments, and then transferred to carbons by HSQC. The HMBC experiment enabled to assign quaternary carbons, connect partial structures, and thus determine the glycosidic linkage and the position of the aglycones.
Mass spectra in the negative ion mode were measured using LTQ Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an electrospray ion source. The samples were dissolved in methanol and introduced into the mobile phase flow (methanol/water 4:1; 100 µL/min) using a 5 µL loop. Spray voltage, capillary voltage, tube lens voltage and capillary temperature were 4.6 kV, -25 V, -125 V and 275 °C, respectively.

Analytical HPLC and LCMS

The HPLC and LCMS analyses were accomplished using the Shimadzu Prominence system consisting of a DGU-20A$_3$ mobile phase degasser, two LC-20AD solvent delivery units, a SIL-20AC cooling auto sampler, a CTO-10AS column oven, SPD-M20A diode array detector, and LCMS-2020 mass detector with single quadrupole equipped with an electrospray ion source (Shimadzu, Kyoto, Japan). The sample (ca 0.5 mg) was dissolved in mobile phase A (60 µL), filtered and analyzed on Chromolith Performance RP-18e column (100 × 3 mm, Merck, DE) equipped with Chromolith RP-18e (5× 4.6 mm) guard cartridge. Binary gradient elution was used: mobile phase A = 5 % acetonitrile in water, 0.1 % formic acid; mobile phase B = 80% acetonitrile in water, 0.1 % formic acid. Gradient used: start 10 % B, 30 % B for 0–3 min; 30 % B for 3–8 min; 10 % B 8-9 min, end at 15 min; flow rate 0.4 mL/min. temperature 25 °C, injection volume 1 µL; detection 200 nm (*p*-hydroxyphenylacetic acid), 330 nm (ferulic acid), 280 nm (*m*-coumaric acid), 285 nm (*o*-coumaric acid, caffeic acid). The MS parameters were as follows: ESI interface voltage, 4.5 kV; detector voltage, 1.15 kV; nebulizing gas flow, 1.5 mL/min; drying gas 15 mL/min; heat block temperature 200 °C; DL temperature 250 °C; SCAN mode 150-680 *m/z*. The data were processed by LabSolutions software ver. 5.75 SP2 (Shimadzu).

Preparative HPLC

Preparative HPLC was performed with the same Shimadzu Prominence system as described above; additionally, fraction collector FRC-10A (Shimadzu, Kyoto, Japan) was used. General procedure: the sample (10 mg) was dissolved in mobile phase (200 µL), centrifuged and injected (25 µL) in to the Chromolith SemiPrep (100 × 10 mm, Merck, DE) column equipped with Chromolith RP-18e (5× 4.6 mm) guard cartridge. The fractions (1.2 mL) were collected. Mobile phase: 10 % acetonitrile in water, 0.1 % formic acid, flow rate 1.2 mL/min, 25 °C, isocratic elution.

Cultivation Media

P. pastoris cultivation on agar plates was performed in YPD medium (Yeast Extract Peptone Dextrose Medium) [g/L]: yeast extract OXOID, 10; bacteriological peptone OXOID, 20; glucose LACHNER, CZ, 20.
The inoculum for *P. pastoris* cultivation was prepared in BMGY medium (Buffered Glycerol-Complex Medium) [g/L]: yeast extract, 10; peptone, 20; 100 mM potassium phosphate, pH 6.0; YNB, 13.4; biotin 0.0004; glycerol, 10.
Buffered Methanol-Complex Medium (BMMY) has the same composition as BMGY but instead of 1% (v/v) glycerol methanol (0.5%, v/v) is added.
For large-scale productions, minimal media were used. BMGH medium (Buffered Minimal Glycerol medium) [g/L]: 100 mM potassium phosphate pH 6.0; YNB, 13.4; biotin 0.0004; glycerol, 10) for overnight preculture. BMMH medium (Buffered Minimal Methanol medium) [g/L]: 100 mM potassium phosphate pH 6.0; YNB, 13.4; biotin 0.0004; methanol, 5) was used for the main culture.
Fed-batch fermentations were carried out in BSM medium (Basal Salt Medium) [g/L]: 85 % H_3PO_4, 26.7 mL; $CaSO_4$. 2 H_2O, 1.17; K_2SO_4, 18.2; $MgSO_4$. 7 H_2O, 14.9; KOH, 4.13; and glycerol, 40; and supplemented with 4.35 mL/L of PTM_1 (trace salts solution [g/L]: $CuSO_4$. 5 H_2O, 6; NaI, 0.08; $MnSO_4$. H_2O, 3; Na_2MoO_4 . 2 H_2O, 0.2; H_3BO_3, 0.02; $CoCl_2$, 0.5; $ZnCl_2$, 20; $FeSO_4$. 7 H_2O, 65; biotin, 0.2; H_2SO_4 conc. 9.2 g). Methanol added in fed-batch experiments was also supplemented with PTM_1 (1.2 mL/L pure methanol).
The production medium for *A. niger* cultivation of consisted of [g/L]: rutin, 5.0; KH_2PO_4, 15.0; NH_4Cl, 4.0; KCl, 0.5; yeast extract, 5.0; casein hydrolysate, 1.0; and 1.0 mL of trace element Vishniac solution[30] at pH 5.0. The pH of the medium was adjusted to 5.0. After sterilization, each flask was supplemented with 1.0 mL of sterile 10% $MgSO_4$ .7 H_2O (w/v).

Heterologous Expression of Rutinosidase in *Pichia pastoris*

The expression vector pPICZαA-RUT, obtained as described previously, was linearized with restriction endonuclease *Sac*I and then the prepared competent *P. pastoris* cells KM71H were transformed with the linearized expression vector by electroporation according to the manufacturer's instructions (EasySelect *Pichia* Expression Kit, Invitrogen).[17] The electroporated cells were grown at various concentrations under the selection pressure of zeocin (100 µg/mL) on YPD agar plates for 2 days at 28 °C.
Recombinant rutinosidase production was done according to the manufacturer's instructions: the colonies were inoculated into 100 mL of BMGY medium, pH 6.0, and incubated overnight with shaking at 28 °C. The cells were then collected by centrifugation (5000 × g, 10 min, 20 °C) and the pellet was resuspended in 30 mL of BMMY

medium in a 300 mL baffled flask. The production of rutinosidase was induced by the addition of methanol (0.5% v/v) every 24 h for 4 days. The flasks were incubated at 28 °C and 220 rpm.

Large Scale Enzyme Production

The inoculum was prepared in BMGY medium. Fed-batch fermentations were carried out in BSM medium. Methanol added in fed-batch experiments was also supplemented with PTM_1 (1.2 mL/L pure methanol).
The inoculum for the fermentation cultivation was prepared in 100 mL of BMGY medium. The fermentation was performed as described previously in 3-L laboratory fermenters (Brunswick BioFlo® 115, Eppendorf, DE).[31] Then, 1.5 L of BSM media supplemented with 6.53 mL of PTM_1 was inoculated with inoculum (OD_{600} approx. 10-12) to a concentration of 5 % v/v. The fermentation conditions were as follows: 30 °C, pH 5 maintained with ammonia solution (28-30 %), DO (dissolved oxygen) 20 % maintained by agitation cascade from 50 to 1000 rpm, aeration 0.66 vvm (volume of air per volume of medium per minute) with the addition of 200 µL of Struktol J650 (Schill + Seilacher "Struktol" GmbH, Hamburg, DE) as an antifoaming agent. After the complete consumption of glycerol (approx. 20 h), two methanol doses of 3 g/L were added in 20th and 31st h. After the utilization of the second dose, the agitation was set at 600 rpm, the agitation cascade was stopped and additional methanol (3 g/L) was added. The methanol feed was connected to the actual level of dissolved oxygen as described previously.[31] Whenever the level of DO rose above 20 %, the methanol feed was turned on by an automated program, and when the DO level rose above 30 %, signaling an excess of methanol and the inability of the culture to utilize it, the pump was turned off again.

Purification of Recombinant Rutinosidase

Recombinant rutinosidase was purified from the culture medium of *Pichia pastoris* after 6 days of cultivation with methanol induction. The cells were harvested by centrifugation (5000 × g, 10 min at 4 °C). The supernatant was dialyzed against 6 L of 10 mM sodium acetate buffer, pH 3.6, for 2 h (dialysis tubing cellulose membrane, Sigma-Aldrich, cut-off 10 kDa). The pH of the solution was then adjusted to 3.6 with 10 % acetic acid and filtered. This solution was loaded into a Fractogel EMD SO_3^- column (15 × 100 mm) in 10 mM sodium acetate buffer, pH 3.6. The protein was eluted using a linear gradient of 0 – 1 M NaCl (5 mL/min). Fractions were collected and then analyzed for rutinosidase activity using *p*-nitrophenol rutinoside as a substrate. The fractions containing rutinosidase activity were concentrated by ultrafiltration using cellulose membranes with a 10 kDa cut-off (Millipore, USA). The concentrated protein was then purified to homogeneity by gel filtration in a Superdex 200 10/300 GL column (10 × 300 mm, 10 mM citrate-phosphate buffer, pH 5.0, 150 mM NaCl).
Protein concentrations were determined by Bradford assay calibrated for BSA. The purity of recombinant rutinosidase was checked by 12 % SDS-PAGE.

Enzymatic Glycosylation – General Procedure

The acid acceptor (1 equivalent) was dissolved in DMSO (90 mg/mL), the resulting solution was added to the crude rutinosidase medium (0.3 U/mL, 60 mL/g acceptor) and pH was adjusted to 3.0. Rutin (0.5 eq) was added followed by three additions (each 0.5 eq) of rutin every 90 minutes (final concentration of DMSO was 15 %, v/v). The resulting heterogeneous mixture was shaken (180 rpm, Thermoshaker, Eppendorf, D) at 40 °C for a total of 6-7 h. Reaction was monitored by TLC: the sample was diluted 5× with MeOH, analyzed on silica plates using AcOEt/MeOH/HCO_2H (4 : 1 : 0.05) as a mobile phase or on reverse phase plates (MeCN/H_2O, 4 : 6) and visualized under UV light and by Komarowski reagent (solution of *p*-hydroxybenzaldehyde in acidic MeOH, 15 mg/mL) or by charring with 5 % H_2SO_4 in EtOH.
This reaction setup was used (without reaction workup) for screening of the rutinosylation acceptors.
For the preparatory reaction, 300 mg of acceptor acid were used under the above conditions. When the reaction was completed, the reaction mixture was diluted with ca 20 mL of 50 mM citrate-phosphate buffer pH 4.5, centrifuged, supernatant was collected and the solids were resuspended in the same buffer volume and centrifuged again. Combined supernatants (pH 4.5) were extracted with AcOEt to remove the bulk of unreacted acceptor, rutin and quercetin. The aqueous phase was partially evaporated *in vacuo* to remove dissolved AcOEt. Non-ionic resin Amberlite XAD-4 (Sigma) was washed overnight with acetone, then washed extensively with water to remove all traces of organic solvents and filled into the column. The partially evaporated supernatant was loaded onto the column and washed with 4 column volumes of water. Then the resin was washed with MeOH to elute the glycosylation products and the methanol solution was evaporated to yield a crude mixture of products, which were separated by preparative HPLC.

***p*-Coumaric Acid (3) Rutinosylation**

(*E*)-*p*-Coumaric acid (**3**, 300 mg) was rutinosylated as described in the general procedure. The reaction was extracted by XAD-4 adsorption and the methanol eluate was evaporated to yield 170 mg (20 %) of the glycosylated products (mixture of glycosides). The above mixture of the products was analyzed by HPLC (LCMS) (see Figure S2 Supporting Information) and it showed two peaks of potential products with the same UV spectrum and *m/z*. This mixture was directly subjected to NMR analysis, which revealed the presence of (all possible) four rutinosides (see Tables S1a-d and Figures S1a-e, Supporting Information) in the following proportion (calculated from ^{1}H integration of the *meta*-proton signals of the aromatic moieties of respective aglycones in the ^{1}H NMR spectrum of this mixture): 4-*O*-rutinosyl (*E*)-*p*-coumaric acid (**3b**, 79 %), 1-*O*-(*E*)-*p*-coumaroyl-β-rutinose (**3a**, 10 %), 4-*O*-rutinosyl (*Z*)-*p*-coumaric acid (**3c**, 6 %), and 1-*O*-(*Z*)-*p*-coumaroyl-β-rutinose (**3d**, 5 %).
Preparative HPLC of the reaction mixture after XAD-4 workup (125.3 mg) yielded two fractions. 4-*O*-Rutinosyl (*E*)-*p*-coumaric acid (**3b**, 38.7 mg, 30.9 %) white powder, LCMS Fig. S3a-b, ^{1}H and ^{13}C NMR see Table S2, Fig. S5a-b; MS (ESI$^-$): ([M − H]$^-$, *m/z* 471.1); HRMS (ESI$^-$): calcd for $C_{21}H_{27}O_{12}$ 471.15080, measured 471.14974 (−2.26 ppm), see Fig. S4.
1-*O*-(*E*)-*p*-Coumaroyl-β-rutinose (**3a**, 3.9 mg, 3.1 %) white powder, LCMS (Fig. S6a-b). This preparation contained as an inseparable minority ca 25 % of 1-*O*-(*Z*)-*p*-coumaroyl-β-rutinose (**3d**) as determined by ^{1}H and ^{13}C NMR (see Table S3a,b, Figs. 8a,b). MS (ESI$^-$) spectrum: ([M − H]$^-$, *m/z* 471.1). HRMS (ESI$^-$): calcd for $C_{21}H_{27}O_{12}$ 471.15080, measured 471.14987 (-1.97 ppm), see Fig. S7.

Ferulic Acid (4) Rutinosylation

(*E*)-Ferulic acid (**4**, 300 mg) was rutinosylated as described in the general procedure to yield 162 mg (21 %) of the glycosylated products (mixture of glycosides). This mixture (45 mg) was analyzed by LCMS to show two peaks of products (Supporting Information, Fig. S9). They was separated by preparative HPLC to yield 4-*O*-rutinosyl (*E*)-ferulic acid (**4b**, 2.7 mg, 6 %), white powder, for HPLC see Fig. S10a,b, ^{1}H and ^{13}C NMR see Table S4 and Fig. 12Sa,b; MS (ESI$^-$): ([M − H]$^-$, *m/z* 501.2); HRMS (ESI$^-$): calcd for $C_{22}H_{29}O_{13}$ 501.16136; found 501.16046 (-1.80 ppm), Fig. S11 and 1-*O*-(*E*)-feruloyl-β-rutinose (**4a**, 3.7 mg, 8.2 %), white powder, HPLC see Fig. S13a,b, ^{1}H and ^{13}C NMR see Table S5a and Figures S15a,b; MS (ESI$^-$): ([M − H]$^-$, *m/z* 501.2); HRMS (ESI$^-$) calcd. for $C_{22}H_{29}O_{13}$ 501.1613; found 501.16136, see Fig. S14. The latter preparation contained ca 25% of inseparable minority 1-*O*-(*Z*)-feruloyl-β-rutinose (**4c**) as determined by ^{1}H and ^{13}C NMR (see Table S5b).

m-Coumaric Acid (5) Rutinosylation

(*E*)-*m*-Coumaric acid (**5**, 300 mg) was rutinosylated as described in the general procedure to yield 184 mg (21 %) of the glycosylated products (mixture of glycosides). This mixture (125.3 mg) was analyzed by LCMS to show two peaks of products (Fig. S16). They were separated with preparative HPLC to yield 4-*O*-rutinosyl (*E*)-*m*-coumaric acid (**5b**, 76.1 mg, 46 %) white powder, HPLC Fig. S17a,b, ^{1}H and ^{13}C NMR see Table S6 and Figs. S19a,b; MS (ESI^{-}): ([M − H]$^{-}$, *m/z* 471.1); HRMS (ESI^{-}): calcd for $C_{21}H_{27}O_{12}$ 471.15080, measured 471.14951 (-2.74 ppm), see Fig. S21 and 1-*O*-(*E*)-*m*-coumaroyl-β-rutinose (**5a**, 14.2 mg, 8.6 %), white powder, HPLC Fig. S20a,b, ^{1}H and ^{13}C NMR see Table S7 and Figs. S22a,b; MS (ESI^{-}): ([M − H]$^{-}$, *m/z* 471.1); HRMS (ESI^{-}): calcd for $C_{21}H_{27}O_{12}$ 471.15080, measured 471.14993 (-1.84 ppm), see Fig. S21. No (*Z*)-side product was observed in the NMR spectra.

o-Coumaric Acid (6) Rutinosylation

(*E*)-*o*-Coumaric acid (**6**, 300 mg) was rutinosylated as described in the general procedure to yield 4-*O*-rutinosyl (*E*)-*o*-coumaric acid (**6b**, 138 mg, 16 %) yellowish powder; HPLC see Fig. S23a,b, ^{1}H and ^{13}C NMR see Table S8a and Fig. S25a,b; MS (ESI^{-}): ([M − H]$^{-}$, *m/z* 471.1); HRMS (ESI^{-}): calcd for $C_{21}H_{27}O_{12}$ 471.15080, measured 471.14978 (-2.16 ppm), see Fig. S24. This product contained 7 % of inseparable minority 1-*O*-(*E*)-*o*-coumaroyl-β-rutinose (**6a**) as inferred from NMR spectra; ^{1}H and ^{13}C NMR see Table S8b. No (*Z*)-configured product was observed in the NMR spectra.

Caffeic Acid (7) Rutinosylation

(*E*)-Caffeic acid (**7**, 300 mg) was rutinosylated as described in the general procedure. Reaction was worked up with XAD-4 adsorption and methanolic eluate was evaporated to yield 224 mg (28 %) of the glycosylated product, which after LCMS analysis showed three peaks having the UV spectrum and *m/z* of expected rutinosylated product (for HPLC see Fig. S16). This reaction mixture (109.3 mg) was separated with preparatory HPLC to yield, 4-*O*-rutinosyl (*E*)-caffeic acid (**7b**, 8.3 mg, 7.6 %) as a white powder; HPLC see Fig. S27a,b, ^{1}H and ^{13}C NMR see Table S9 and Figs. 29a,b; MS (ESI^{-}): ([M − H]$^{-}$, *m/z* 487.1); HRMS (ESI^{-}): calcd for $C_{21}H_{27}O_{13}$ 487.14571, measured 487.14478 (-1.93 ppm), see Fig. S28; 3-*O*-rutinosyl (*E*)-caffeic acid (**7c**, 29.9 mg, 27.3 %) as a white powder, HPLC see Fig. S30a,b, ^{1}H and ^{13}C NMR see Table S10 and Figs. S32a,b; MS (ESI^{-}): ([M − H]$^{-}$, *m/z* 487.1); HRMS (ESI^{-}): calcd for $C_{21}H_{27}O_{13}$ 487.14571, measured 487.14442 (-2.66 ppm), see Fig. S31; 1-*O*-(*E*)-caffeoyl-β-rutinose (**7a**, 4.5 mg, 4.1 %) as a white powder, for LCMS see Fig. S33a,b, for ^{1}H and ^{13}C NMR see Table S11 and Figs. S35a,b, MS ESI^{-}: ([M − H]$^{-}$, *m/z* 487.1); HRMS ESI^{-}: calcd for $C_{21}H_{27}O_{13}$ 487.14571, measured 487.14511 (-1.24 ppm), see Fig. S34.

p-Hydroxyphenylacetic Acid (8) Rutinosylation

p-Hydroxyphenylacetic acid (**8**, 300 mg) was rutinosylated as described in the general procedure to yield 145 mg (16 %) of the glycosylated product, which after LCMS analysis showed one peak of rutinosylated product (for HPLC see Fig. S36). This product (52.9 mg) was was further purified by preparative HPLC to yield 4-*O*-rutinosyl phenylacetic acid (**8a**, 6.6 mg, 12.4 %; r.t. 2.660 min) as a white powder, LCMS see Fig. S37a,b, ^{1}H and ^{13}C NMR see Table S12 and Figs. S39a,b; MS ESI(−): ([M − H]$^{-}$, *m/z* 459.1;); HRMS ESI(−): calcd for $C_{20}H_{27}O_{12}$ 459.15080, measured 459.14993 (-1.89 ppm), see Fig. S38.

Alkaline Hydrolysis

Aqueous solution of NaOH (10 % w/v, 3 mL) was added to the mixture of dry glycosylated products (32 mg) of ferulic acid (**4**) and left overnight at room temperature under stirring. Then the resulting mixture was analyzed by HPLC – see Fig. S40. Mixture of glycosides contains two product peaks (Fig. S40A as determined by UV scan, LCMS and by co-chromatography with isolated products **4a** and **4b**). After alkaline hydrolysis (Fig. S40B) product **4a** disappeared, while the phenolic glycoside **4b** remained intact and free acid **4** appeared. Liberated ferulic acid (**4**) in the alkaline milieu probably partially underwent oxidative oligomerization forming an unidentified peak (Fig. S40B).

Enzymatic Derhamnosylation of Rutinosides

Mixture (20 mg) of 4-*O*-rutinosyl (*E*)-*p*-coumaric acid (**3b**, 79 %), 1-*O*-(*E*)-*p*-coumaroyl-β-rutinose (**3a**, 10 %) was incubated with recombinant α-L-rhamnosidase from *Aspergillus terreus* (2.19 U/mL, 30.1 mg/mL)[26] in 4 mL of 50 mM citrate-phosphate buffer, pH 5, for 4 hours at 37 °C. Then, the reaction was terminated by enzyme denaturation (90 °C, 5 min) and the reaction mixture was analyzed by HPLC and LCMS (Figures S41 and S42, Supporting Information); a sample of the compound **3e** was isolated by preparatory HPLC to obtain clean NMR spectra (Table S13 and Figure S44, Supporting Information), compound **3f** was not isolated due to its paucity.

Glycosylation of *p*-Coumaric and Ferulic Acid with Hexosaminidases

Reaction mixtures comprised of (final. conc.) *p*-coumaric or ferulic acid (300 mM), 30 mM *p*-nitrophenyl β-*N*-acetylglucosaminide (30 mM), McIlvaine buffer pH 5 / 40 % (v/v) acetonitrile and the respective β-*N*-acetylhexosaminidase (1.5 U/mL). Fungal extracellular β-*N*-acetylhexosaminidases with a good transglycosylation potential[32] from the following sources were tested: *Acremonium persicinum* CCF 1850, *Aspergillus oryzae* CCF 1066, *Fusarium oxysporum* CCF 371, *Penicillium chrysogenum* CCF 1269, *P. oxalicum* CCF 2315, *Talaromyces flavus* CCF 2686, *Trichoderma harzianum* CCF 2687. Reactions were incubated at 35 °C and 850 rpm for 24 hours and monitored by TLC (propan-2-ol : H_2O : $NH_4OH_{aq.}$, 7:2:1) and LCMS. A control reaction containing only 30 mM *p*-nitrophenyl β-*N*-acetylglucosaminide (30 mM) donor in McIlvaine buffer pH 5 / 40 % (v/v) acetonitrile and the enzyme (0.15 U/mL) was run under the same conditions to verify the enzyme activity in the absence of acid acceptor.

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References

[1] N. C. Veitch, I. Regos, G. C. Kite, D. Treutter, *Phytochemistry* **2011**, *72*, 423-429.

[2] E. Heřmánková-Vavříková, A. Křenková, L. Petrásková, C. S. Chambers, J. Zápal, M. Kuzma, K. Valentová, V. Křen, *Int. J. Mol. Sci.* **2017**, *18*, 1074.

[3] a) J. B. Harborne, J. J. Corner, *Biochem. J.* **1961**, *81*, 242-250; b) S. Asen, S. L. Emsweller, *Phytochemistry* **1962**, *1*, 169-174.

[4] a) B. Schuster, K. Herrmann, *Phytochemistry* **1985**, *24*, 2761-2764; b) D. Strack, N. Marxen, H. Reznik, H.-D. Ihlenfeld, *Phytochemistry* **1990**, *29*, 2175-

2178; c) C. Milkowsky, D. Strack, *Phytochemistry* **2004**, *65*, 517-524.
[5] S. H. Han, W. S. Suh, K. J. Park, K. H. Kim, K. R. Lee, *Arch. Pharm. Res.* **2015**, *38*, 1312–1316.
[6] H. M. Zhou, Y. L. Liu, *Acta Pharm. Sinica* **1990**, *25*, 123-126.
[7] M. Neacsu, V. Micol, L. Pérez-Fons, S. Willför, B. Holmbom, *Molecules* **2007**, *12*, 205-217.
[8] D. E. Goggin, G. L. Nealon, G. R. Cawthray, A. Scaffidi, M. J. Howard, S. B. Powles, G. R. Flematti, *J. Agric. Food Chem.* **2019**, *66*, 13378-13385.
[9] T. Kosuge, E. E. Conn, *J. Biol. Chem.* **1961**, *236*, 1617-1621.
[10] Y. Zhu, J. Ralph, *Tetrahedron Lett.* **2011**, *52*, 3729–3731.
[11] T. Desmet, W. Soetaert, P. Bojarová, V. Křen, L. Dijkhuizen, V. Eastwick-Field, A. Schiller, *Chem. Eur. J.* **2012**, *18*, 10786-10801.
[12] Y. Matsuba, Y. Okuda, Y. Abe, Y. Kitamura, K. Terasaka, H. Mizukami, H. Kamakura, N. Kawahara, Y. Goda, N. Sasaki, Y. Ozeki, *Plant Biotechnol.* **2008**, *25*, 369–375.
[13] K. Sugimoto, K. Nomura, H. Nishiura, K. Ohdan, T. Nishimura, H. Hayashi, T. Kuriki, *J. Biosci. Bioeng.* **2007**, *104*, 22–29.
[14] M. H. Shin, N.Y. Cheong, J. H. Lee, K. H. Kim, *Food Chem.* **2009**, *115*, 1028–1033.
[15] V. Křen, L. Martínková, *Curr. Med. Chem.* **2001**, *8*, 1313–1338.
[16] a) C. Goedl, T. Sawangwan, P. Wildberger, B. Nidetzky, *Biocat. Biotrans.* **2010**, *28*, 10-21; b) K. De Winter, L. Van Renterghem, K. Wuyts, H. Pelantová, V. Křen, W. Soetaert, T. Desmet, *J. Agric. Food Chem.* **2015**, *63*, 10131–10139.
[17] D. Šimčíková, M. Kotik, L. Weignerová, P. Halada, H. Pelantová, K. Adamcová, V. Křen, *Adv. Synth. Catal.* **2015**, *357*, 107-117.
[18] I. Bassanini, J. Krejzová, W. Panzeri, V. Křen, D. Monti, S. Riva, *ChemSusChem* **2017**, *10*, 2040-2045.
[19] T. Niwa, U. Doi, Y. Kato, T. Osawa, *J. Agric. Food Chem.* **2001**, *49*, 177-182
[20] C. S. Kim, O. W. Kwon, S. Y. Kim, S. U. Choi, J. Y. Kim, J. Y. Han, S. I. Choi, J. G. Choi, K. H. Kim, K. R. Lee, *J. Nat. Prod.* **2014**, *77*, 1955-1961.
[21] M. Ueda, Y. Sawai, Y. Shibazaki, C. Tashiro, T. Ohnuki, S. Yamamura, *Biosci. Biotechnol. Biochem.* **1998**, *62*, 2133-2137.
[22] L. Mäkilä, O. Laaksonen, A. L. Alanne, M. Kortesniemi, H. Kallio, B. Yang, *J. Agric. Food Chem.* **2016**, *64*, 4584-4598.
[23] M. L. Salum, C. J. Robles, R. Erra-Balsells, *Org. Lett.* **2010**, *12*, 4808-4811.
[24] P. Bojarová, V. Křen, *Trends Biotechnol.* **2009**, *27*, 199-209.
[25] S. Katayama, F. Ohno, Y. Yamauchi, M. Kato, H. Makabe, S. Nakamura, *J. Agric. Food Chem.* **2013**, *61*, 9617-9622.
[26] L. S. Mazzaferro, G. Weiz, L. Braun, M. Kotik, H. Pelantová, V. Křen, J. D. Breccia, *Biotechnol. Appl. Biochem.* **2019**, *66*, 53-58.
[27] K. Slámová, P. Bojarová, L. Petrásková, V. Křen, *Biotech. Adv.* **2010**, *28*, 682-693.
[28] D. Gerstorferová, B. Fliedrová, P. Halada, P. Marhol, V. Křen, L. Weignerová, *Process Biochem.* **2012**, *47*, 828-835.
[29] P. Fialová, L. Weignerová, J. Rauvolfová, V. Přikrylová, A. Pišvejcová, R. Ettrich, M. Kuzma, P. Sedmera, V. Křen, *Tetrahedron* **2004**, *60*, 693-701.
[30] W. Vishniac, M. Santer, *Bacteriol. Rev.* **1957**, *21*, 195–213.
[31] K. Markošová, L. Weignerová, M. Rosenberg, V. Křen, M. Rebroš, *Front. Microbiol.* **2015**, *6*, 1140.
[32] a) P. Bojarová, N. Kulik, M. Hovorková, K. Slámová, H. Pelantová, V. Křen, *Molecules* **2019**, *24*, 599; b) K. Slámová, R. Gažák, P. Bojarová, N. Kulik, R. Ettrich, H. Pelantová, P. Sedmera, V. Křen, *Glycobiology* **2010**, *20*, 1002-1009.

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FULL PAPER

Glycosidase-Catalyzed Synthesis of Glycosyl Esters and Phenolic Glycosides of Aromatic Acids

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