A selective and mild glycosylation method of natural phenolic alcohols

Mária Mastihubová* and Monika Poláková

Address: Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 38 Bratislava, Slovakia
Email: Mária Mastihubová* - chemjama@savba.sk
* Corresponding author

Keywords: diastereoselectivity; p-hydroxyphenylalkyl glycosides; mild promoters; natural products; 1,2-trans-glycosylation

Abstract
Several bioactive natural p-hydroxyphenylalkyl β-D-glucopyranosides, such as vanillyl β-D-glucopyranoside, salidroside and isoconiferin, and their glycosyl analogues were prepared by a simple reaction sequence. The highly efficient synthetic approach was achieved by utilizing acetylated glycosyl bromides as well as aromatic moieties and mild glycosylation promoters. The aglycones, p-O-acetylated arylalkyl alcohols, were prepared by the reduction of the corresponding acetylated aldehydes or acids. Various stereoselective 1,2-trans-O-glycosylation methods were studied, including the DDQ–iodine or ZnO–ZnCl₂ catalyst combination. Among them, ZnO–iodine has been identified as a new glycosylation promoter and successfully applied to the stereoselective glycoside synthesis. The final products were obtained by conventional Zemplén deacetylation.

Introduction
Arylalkyl (substituted benzyl, phenethyl and phenylpropenyl) glycosides having a free phenolic function at the para-position of the aglycone are substances widely occurring in plants. They exhibit numerous biological activities which are in many cases related to their structure–antioxidant activity relationship. 4-Hydroxy-3-methoxybenzyl β-D-glucopyranoside (vanillyl β-D-glucoside, 1) isolated from the exocarp of Juglans mandshurica Maxim showed antibacterial activity [1]. A wide range of pharmacological effects, e.g., anti-oxidant, anti-inflammatory, anticancer, hepatoprotective, cardioprotective, neuroprotective, antidiabetic, and antiviral activities [2-13] are reported for 4-hydroxyphenethyl β-D-glucopyranoside (salidroside, 2) which is known to be the main bioactive component of plants of the genus Rhodiola. 4-Hydroxy-3-methoxycinnamyl β-D-glucopyranoside (isoconiferin or citrusin D, 3) has been shown to exhibit a hypotensive effect [14] (Figure 1).

The activities of the above mentioned glycosides are primarily related to the structure of the aglycone. The glycosylation of the poorly soluble hydroxalkylphenols, such as 4-hydroxybenzyl, vanillyl, 4-hydroxyphenethyl and coniferyl alcohols, significantly increases their water solubility. Further it influences the
The total syntheses of compounds 1, 2 and 3 and their structurally related glycosides employing various chemical [9,17-23] or enzymatic [24-29] methods have been previously reported. The most frequently used protocol under Koenigs–Knorr conditions is represented by the reaction of an acetobromoglucose and (4-O-benzyloxyphenyl)alkyl alcohol catalysed by Ag salts [9,18]. The final removal of the benzyl protecting group from the phenolic function of the aglycone by catalytic reduction can be however problematic in the case of more complex molecules containing for example double bonds (e.g., arenarioside) [30]. Isoconiferin (3) has been prepared mainly by the trichloroacetimidate method [20,21] using 4-O-acetylated coniferyl alcohol as the acceptor. On the other hand, the Mizoroki–Heck reaction of 4-hydroxy-3-methoxyphenylboronic acid and peracetylated allyl β-D-glucoside has been used to synthesize 3 in 52% yield [22].

Enzymatic glycosylations of arylalkyl alcohols are easily accomplished, however, the glycosides have often been obtained in low to moderate yields (usually below 30%). Glucosidases from fruit seed meals are the most commonly used biocatalysts for reverse hydrolysis reactions carried out in organic media or ionic liquids as co-solvents [24-27]. The enzymatic transglucosylation using 4-nitrophenyl β-D-glucopyranoside as the donor and almond β-glucosidase as biocatalyst gave salidroside (2) in moderate yield [28].

Recently, we have published the enzymatic glycosylation of tyrosol (2-(4-hydroxyphenyl)ethanol) with cellobiose, lactose and melibiose as donors for the preparation of salidroside and its α- and β-galactoside analogues [31]. However, all transglycosylation reactions required a distinct pair of the disaccharide donor and the glycosidase for which the reaction conditions had to be optimized. The current paper deals with an efficient, safe and uniform chemical synthesis of various p-hydroxyaryllalkyl glycosides, including compounds 1–3.

**Results and Discussion**

**Preparation of aglycones**

The synthesis of the appropriate aglycones 6a–c was commenced from readily available commercial p-hydroxyphenylcarbaldehydes 4a–c which are less expensive than the corresponding p-hydroxybenzyl alcohols (Scheme 1). The conventional acetylation of 4a–c with acetic anhydride in pyridine gave p-acetoxyphenylcarbaldehydes 5a–c in more than 98% yields. Subsequently the aldehyde function was reduced by NaBH₄ at pH 7–8, which was kept constant by the continuous addition of 85% H₃PO₄ to avoid phenolic acetyl-group cleavage. The p-acetoxybenzyl alcohols 6a–c were isolated in 85–95% yields.

**Scheme 1:** Reagents and conditions: a) Ac₂O, pyridine, rt, 10 h, >98%; b) NaBH₄, H₃PO₄, −5 °C, 85–95%.
Scheme 2: Reagents and conditions: a) Ac₂O, H₂SO₄, 5 °C to rt, 30 min, >94%; b) 1. NaBH₄, THF, 5 °C, 10 min, 2. I₂, 5 °C, 15 min, rt, 3 h, 84% for 9, 69% for 12.

Figure 2: Synthesized glycosyl donors.
known mild catalysts such as Ag$_2$O [33] (method A), the less frequently used ZnO–ZnCl$_2$ system [34] (method B) and the combination of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) with iodine (DDQ–I$_2$) [35] (method C). In addition ZnO–I$_2$ (method D) was successfully applied as a new promoter in the stereoselective 1,2-trans-glycoside synthesis (Table 1, entries 4, 9, 13, 17, 19 and 25). The selection of methods C and D was based on the common knowledge that iodine, either alone or in combination with other promoters such as salts of various metals (other than the traditional Koenigs–Knorr heavy metals), serves as an effective activator of disarmed glycosyl halides in the 1,2-trans-glycoside synthesis [35-38]. Despite the fact that the precise mechanism is not clear, it is assumed that the reaction of the glycosyl bromide promoted by iodine (through the formation of an iodobromonium ion) results in a carbohydrate-derived oxocarbonium ion that functions as the reactive intermediate [35].

In the first step, acceptor 6b and glucopyranosyl bromide 13 as the donor were selected and tested in the presence of the above mentioned promoters (see Table 1, entries 1–4) in order to identify the optimal glycosylation conditions in terms of yield and

---

**Scheme 3**: General reaction scheme for the synthesis of α-hydroxyphenylalkyl glycosides.

---

**Table 1**: Synthesis of 21α–c to 30 under various conditions.

| Entry | Donor | Acceptor | Method$^a$ | Mol. sieves | Temp. | Time (min) | Product | Yield (%) | α:β$^b$ |
|-------|-------|----------|------------|-------------|-------|------------|---------|-----------|---------|
| 1     | 13    | 6b       | A          | No          | rt    | 180        | 21b     | 57        | 0:1     |
| 2     | 13    | 6b       | B          | Yes         | rt    | 90         | 21b     | 46        | 0:1     |
| 3     | 13    | 6b       | C          | Yes         | rt    | 40         | 21b     | 68        | 0:1     |
| 4     | 13    | 6b       | D          | Yes         | rt    | 360        | 21b     | 56        | 0:1     |
| 5     | 13    | 6a       | C          | Yes         | rt    | 20         | 21a     | 78        | 0:1     |
| 6     | 13    | 6c       | C          | Yes         | rt    | 60         | 21c     | 63        | 0:1     |
| 7     | 13    | 9        | B          | Yes         | rt    | 90         | 22      | 49        | 0:1     |
| 8     | 13    | 9        | C          | Yes         | rt    | 110        | 22      | 61        | 0:1     |
| 9     | 13    | 9        | D          | Yes         | rt    | 360        | 22      | 63        | 0:1     |
| 10    | 13    | 12       | C          | Yes         | rt    | 15         | 23      | <5        | n.d.    |
| 11    | 14    | 12       | E          | Yes         | –$^d$ | 30         | 23      | 55        | 0:1     |
| 12    | 15    | 6b       | C          | Yes         | rt    | 20         | 25      | 68        | 0:1     |
| 13    | 15    | 6b       | D          | Yes         | rt    | 360        | 25      | 63        | 0:1     |
| 14    | 16    | 6b       | C          | Yes         | rt    | 30         | 26      | 70        | 1:0     |
| 15    | 17    | 6b       | C          | Yes         | rt    | 30         | 27      | 70        | 0:1     |
| 16    | 18    | 6b       | C          | Yes         | rt    | 30         | 28      | 50        | 0:1     |
| 17    | 18    | 6b       | D          | Yes         | rt    | 360        | 28      | 46        | 0:1     |
| 18    | 19    | 6b       | C          | Yes         | rt    | 20         | 29      | 66        | 1:0     |
| 19    | 19    | 6b       | D          | Yes         | rt    | 45         | 29      | 62        | 1:0     |
| 20    | 20    | 6b       | C          | Yes         | rt    | 20         | 30α/β   | 56        | 1:2.3   |
| 21    | 20    | 6b       | C          | No          | rt    | 25         | 30α/β   | 52        | 1:4     |
| 22    | 20    | 6b       | C$^c$      | No          | rt    | 25         | 30α/β   | 45        | 1:2.1   |
| 23    | 20    | 6b       | C          | No 4 °C     | rt    | 30         | 30α/β   | 78        | 1:4.7   |
| 24    | 20    | 6b       | B          | No 4 °C     | rt    | 90         | 30α/β   | 50        | 1:3.5   |
| 25    | 20    | 6b       | D          | No 4 °C     | rt    | 75         | 30β     | 72        | 0:1     |

$^a$Method: A) Ag$_2$O; B) ZnO–ZnCl$_2$; C) DDQ–I$_2$; D) ZnO–I$_2$; E) TMSOTf. $^b$Anomeric ratios were determined by integration of the appropriate peaks in the $^1$H NMR spectra; n.d. – not determined. $^d$DCM was used as the solvent. $^d$Temperature −78 to 0 °C.
selectivity. In all cases, only the 1,2-trans-glycosylation product, β-glucoside 21b, was obtained. While method B (ZnO–ZnCl₂) performed in DCM instead of ACN afforded only a moderate yield (Table 1, entry 2, 46%) of 21b, the reactions in DCM promoted by Ag₂O (Table 1, entry 1, 57%) and ZnO–I₂ (Table 1, entry 4, 56%) gave comparably good yields. DDQ–I₂ in ACN (Table 1, entry 3, 68%) gave 21b in the highest yield, in addition to the exclusive selectivity and the shortest reaction time (Table 1, entry 5). Therefore this promoter was selected for the glycosylation reactions of acceptors 6a and 6c with bromide 13, affording compounds 21a and 21c with full β-selectivity.

Acetylated tyrosol 9 as glycosyl acceptor reacted only smoothly with 13 under all three studied reaction conditions (Table 1, entries 7–9) affording the acetylated salidroside 22 from moderate (ZnO–ZnCl₂, 49%) to good yields (DDQ–I₂, 61% and ZnO–I₂, 63%) with strict β-stereocontrol.

On the other side, the glycosylation of p-O-acetylated coniferyl alcohol 12 with bromide 13 failed under these conditions. Coniferyl aldehyde 24 was detected and isolated as a major product. For example, the DDQ–I₂-promoted reaction provided aldehyde 24 in 58% yield along with less than 5% of the desired product 23. This may be caused by the oxidative nature of the promoter and by the existence of a conjugated electronic push–pull system of coniferyl alcohol that is enhanced by the electron-withdrawing acetoxy group. On the contrary, the TMSOTf-promoted glycosylation [39] (method E) of coniferyl alcohol 12 with trichloroacetimidate 14 at low temperature was found to be more efficient and glycoside 23 was obtained in high yield with full β-selectivity as proved by NMR spectroscopy. The phenolic acetyl group remained intact under these conditions.

Six distinct acetylated glycosyl donors 15–20 were further examined to prove the feasibility of the method. D-Galacto-, D-manno- and methyl D-glucuronate-derived donors 15–17 originated from hexopyranoses. The pentosyl donors, which usually exhibit a diminished glycosylation selectivity, were represented by L-arabinofuranosyl bromide 19 and D-xylopyranosyl bromide 20.

The glycosylation of 6b with galactosyl, glucuronic acid methyl ester or lactosyl bromides 15, 17 and 18, proceeded also stereoselectively and the β-anomers of glycosides 25, 27 and 28 were isolated in good yields (50–68%) as the only products. The structures of all β-anomers (21a–e, 22, 23, 25, 27 and 28) were confirmed by the presence of a doublet of the anemic proton with characteristic vicinal interaction constant J₁₂ in the interval of 7.5–7.9 Hz in the ¹H NMR spectra. The glycosidic bond between galactose and glucose in lactosyl bromide 15 was not affected under the examined conditions. On the other hand, the reaction of D-mannosyl and L-arabinosyl bromides 16 and 19 with 6b afforded solely α-anomers 26 and 29 as the 1,2-trans-glycosylation products. The α-manno-configuration of glycoside 26 was confirmed by the characteristic coupling constant J₁C₁H₁ = 170.3 Hz. The α-configuration of the L-arabinofuranoside 29 was confirmed by ¹H NMR (broad singlet at 5.09 ppm) and ¹³C NMR spectra (C-1 at 104.6 ppm).

In contrast to the above mentioned results, the reaction of D-xylosyl bromide 20 with 6b did not proceed stereoselectively. An anemic mixture of vanillyl xylosides 30α/β in a ratio varying from 1:2.3 to 1:4 was obtained when the glycosylation was promoted with DDQ–I₂ in either DCM or ACN at room temperature (Table 1, entries 20–22). The same reaction performed at 4 °C provided again a mixture of xylosides 30α/β (Table 1, entry 23) but with a slightly higher selectivity (30α/β in ratio 1:4.7). Therefore, to improve the selectivity, other promoters were also examined at low temperature. The use of ZnO–ZnCl₂ in DCM (Table 1, entry 24) led again to a mixture of xylosides 30α/β. In contrast, the glycosylation promoted by ZnO–I₂ in DCM (Table 1, entry 25) was the only condition affording 30 as pure β-anomer (72%). The lack of selectivity at room temperature can be explained by a higher thermodynamic stability of the α-xlyopyranosides compared to the corresponding β-xlyopyranosides. Apparently, anomerisation of the kinetically formed β-anomer easily takes place under mild reaction conditions at ambient temperature [40], but it is suppressed by decreased temperature in combination with an appropriate promoter (ZnO–I₂). Moreover, this new promoter was successfully applied to the reactions of the corresponding acceptor and four other glycosyl bromides, i.e., D-glucosyl (Table 1, entries 4 and 9), D-galactosyl (Table 1, entry 13), lactosyl (Table 1, entry 17) and L-arabinosyl (Table 1, entry 19). The glycosylation reactions of the latter donors catalysed by ZnO–I₂ gave comparable yields and were completely stereoselective similarly to other promoters, although they required at least a 2-times longer reaction time.

In the final step, the removal of the acetyl groups under Zemplén conditions proceeded smoothly and the desired target glycosides 1–3, 31a,b and 32–37 were isolated in high yields (Figure 3).

**Conclusion**

The glycosylation methods studied in this work represent a simple and convenient approach to bioactive natural p-hydroxyphenylalkyl glycosides and their analogues. The mild reaction conditions with exclusive stereoselectivity can be used as an alternative to the common Koenigs–Knoefel or Heflerich glycosyla-
tion. In many cases, the DDQ–I$_2$-promoted reaction provided products in a stereoselective way and in the highest yields. It is noteworthy that ZnO–I$_2$ is a new glycosylation promoter, which was found to well activate also less reactive disarmed tetra-$O$-acetyl-$\alpha$-D-glycopyranosyl bromides, to give stereoselectively only the 1,2-trans glycosides in good to high yields. These conditions were efficiently used in the stereoselective xyloside synthesis that is not trivial. All used glycosylation conditions were compatible with acetyl protective groups of the phenolic function. The coupling reaction and deprotection were achieved in two steps, thus providing the rapid access to the targeted glycosides.

**Supporting Information**

Supporting Information File 1
Experimental procedures and analytical data.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-12-51-S1.pdf]

**Acknowledgements**

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-846-12, the Scientific Grant Agency of the Ministry of Education of Slovak Republic and Slovak Academy of Sciences (VEGA) under the project No. 2/0138/12 and the Research & Development Operational Programmes funded by the ERDF („Centre of Excellence on Green Chemistry Methods and Processes”, CEGreenI, Contract No. 26240120001 as well as „Amplification of the Centre of Excellence on Green Chemistry Methods and Processes“, CEGreenII, Contract No. 26240120025). The authors thank I. Uhliariková for NMR measurements, S. Bekešová and S. Vlčková for mass measurements.

**References**

1. Shi, A.-H.; Huang, J.-W.; Liu, Y.-H.; Yuan, K. Asian J. Chem. 2013, 25, 3361–3365. doi:10.14233/ajchem.2013.13725
2. Zhang, J.; Liu, A.; Hou, R.; Zhang, J.; Jia, X.; Jiang, W.; Chen, J. Eur. J. Pharmacol. 2009, 607, 6–14. doi:10.1016/j.ejphar.2009.01.046
