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Highly Regioselective Dehexanoylation in Fully Hexanoylated Flavonoids

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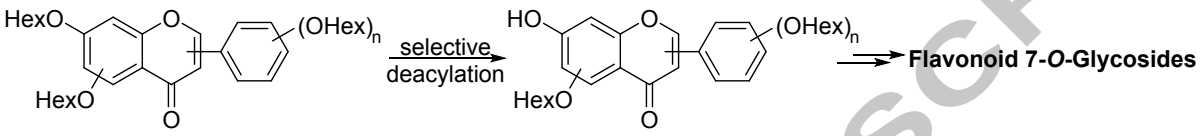
Abstract: Highly selective removal of the 7-*O*-hexanoyl group in fully hexanoylated flavones, isoflavones, flavanone, and flavonol was achieved under mild conditions using K_2CO_3 in a 1:1 mixture of CH_3OH–CH_2Cl_2. The resulting 7-OH flavonoids are valuable intermediates for the synthesis of flavonoid 7-*O*-glycosides via phase-transfer-catalyzed (PTC) glycosylation.



Keywords: Flavonoids; Dehexanoylation; Regioselectivity; Flavonoid glycosides; Phase transfer.

Introduction

Flavonoids are widely distributed in plants [1], fulfilling many important physiological functions. These compounds have also been shown to possess a broad spectrum of biological activities, such as antioxidant [2-3], anti-proliferative, anti-tumor [4-6], anti-microbial [7-8], estrogenic [9], acetylcholinesterase inhibitory [10], and anti-inflammatory [11] activities, and therefore been widely used in cancer, cardiovascular disease, neurodegenerative disorders, etc [12-14]. Unfortunately, issues remain unaddressed for flavonoids in terms of their druggability because an understanding has existed for some time that flavonoids tend to have low aqueous solubility and poor bioavailability. Many drugs derived from natural products are glycosides having a sugar moiety linked to the aglycone through an *O*-, or less commonly *C*-, glycosidic bond [15-16]. Therefore, glycosylation has been used more and more frequently as a practical approach to improve the solubility and, more importantly, the cellular uptake of flavonoids [17-21]. However, major challenges still remain for the synthesis of flavonoid glycosides that could replace those from plant sources: 1) effective protecting strategies are required to discriminate the polyphenols present in the flavonoid scaffold; 2) most flavonoids are not readily soluble in common organic solvents, which in turn causes low nucleophilicity of the phenolic hydroxyl groups [22-24].

The most widely employed protecting groups for flavonoids are benzyl and acyl groups. The introduction of benzyl-protecting groups in flavonoid derivatives usually requires a reductive removal which not only is not fully compatible with the C2-C3 double bond of many flavonoid structures but has the risk of the final products being strongly absorbed to the charcoal when using Pd-C as the catalyst and a slow reaction [25-26]. While a simple acetyl group that is commonly seen in carbohydrate chemistry seems ideal, it suffers from limited lipophilicity and stability as well as poor regioselectivity during deprotection [27]. Inspired by the work from Yu [28] and Botting [29] groups that use a hexanoyl ester-based protection strategy, we successfully achieved a facile synthesis of 7-*O*-β-D-glucopyranosyl-4'-*O*-α-L-rhamnopyranosyl apigenin starting from trihexanoyl apigenin (**1a**) and through protective manipulation of the hydroxyls and subsequent glycosylation [30]. In fact, the observed reactivity order of the hydroxyl groups of polyhydroxyflavones (Figure 1) toward nucleophilic substitution, 7-OH > 4'-OH > 5-OH, has been well documented before

[31-32]. In our current work, we further investigate the preferential reactivity of the 7-OH in the selective deacylation process and explore the substrate scope for its application on various peracylated flavonoids, aiming to provide an advantage over previous methods that use the foul-smelling thiophenol and achieve a facile route for various flavonoid 7-*O*-glycosides.

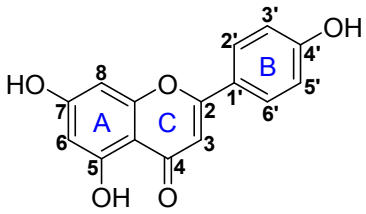
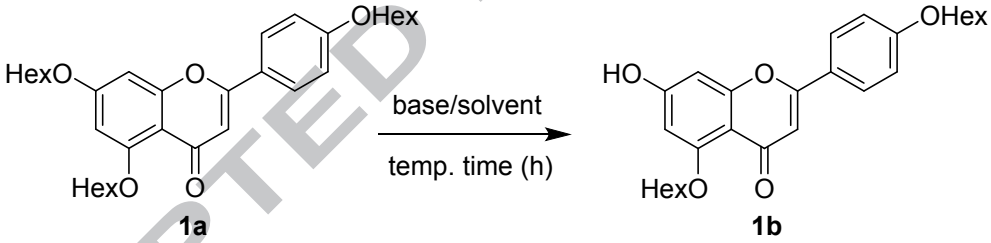


Figure 1. Structural formula of the model flavonoid skeleton, apigenin, showing rings A, B and C and the numbering.

Table 1. Reaction condition screening for the regioselective deprotection of the 7-*O*-hexanoyl group of apigenin (Hex = hexanoyl).



Entry	Base (0.5 eq.)	Reaction time (h)	Solvent	Temperature (℃)	Yield (%)
1	NaOH	24	CH_3CN	25	NR
2	NaOH	18	MeOH	0	28
3	NaOH	8	CH_3OH-CH_2Cl_2 (1:1)	25	48
4	KOH	8	Acetone	40	51
5	Et_3N	24	DMF	25	NR
6	K_2CO_3	12	Acetone	25	45
7	K_2CO_3	8	CH_3OH-CH_2Cl_2 (3:1)	25	68
8	K_2CO_3	4	CH_3OH-CH_2Cl_2 (1:1)	25	92

Results and Discussion

In the polyphenolic structure of flavonoids, the 7-hydroxyl group is known to be the most acidic because of the electron-withdrawing inductive effect of the pyrone carbonyl group at its *para* position. It therefore generates the most stable corresponding 7-phenolate which lays the foundation for the regioselective removal of the most electrophilic 7-*O*-acyl moiety [33-34]. Selective release of the 7-OH on the peracylated flavonoids had been achieved more than a decade ago using PhSH and a base catalyst in *N*-methyl pyrolidinone [28]. However, we decided to abandon this system because of the strict reaction conditions and aromatic thiols of repulsive and penetrating odor. Since PhS^- is the key reactive species in the transesterification/deprotection process, we envisioned the RO^- generated from a polar protic solvent (i.e. an alcohol) by a base with suitable basicity a promising alternative. Expectedly, in our model reaction with perhexanoylated apigenin (**1a**) (Table 1), both the solvent system and base played crucial roles. For bases used, NaOH and KOH proved to be too strong resulting in over-deprotection. A protic solvent component seems necessary as acetonitrile or DMF did not yield any dehexanoylation in the presence of NaOH or triethylamine. However, using a protic solvent (i.e. CH_3OH) alone or as a major component (i.e. CH_3OH-CH_2Cl_2 (3:1)) caused the loss of or decreased selectivity. The optimal reaction conditions, moderately basic K_2CO_3 (pK_a of its conjugate acid = 10.25) in a 1:1 mixture of CH_3OH-CH_2Cl_2, could remove the 7-*O*-hexanoyl group with excellent selectivity (92%) – no deprotection on the next reactive/electrophilic 4'-*O* position was observed. The reaction was performed at room temperature and reaction time determined by TLC monitoring.

To demonstrate the advantages of using the hexanoyl ester protection, we also employed tri-*O*-acetyl apigenin and used HPLC to monitor the progress of both deacetylation and dehexanoylation under the optimized condition for comparison (Figure 2). As shown, removal of the 7-*O*-hexanoyl was evidently much faster and cleaner with less byproduct(s): 1) at 30 min, >50% of the starting material tri-*O*-hexanoyl apigenin was converted to the desired 7-OH product and the conversion rate reached over 80% at 2 h; 2) upon completion at 4 h, regioselective release of 7-OH was accomplished to ~95% in dehexanoylation with minimum byproduct observed; in contrast, poor selectivity (<40%) was obtained from the deacetylation with >50% of over-deprotected dihydroxyl and trihydroxyl byproducts. These results indicated that the more lipophilic hexanoyl group is more amenable to selective

deprotection and could better facilitate the reactivity discrimination at the 7-*O* position.

Next, the substrate scope for the selective dehexanoylation was explored using various types of flavonoids (Table 2, entry **2-7**), including luteolin (**2**) and acacetin (**4**) (flavones), naringenin (**3**, a flavanone), daidzein (**5**) and genistein (**6**) (isoflavones), and quercetin (**7**, a flavonol). These commercially available flavonoids were first treated with hexanoyl chloride in DMF to afford the perhexanoylated substrates (**1a-7a**) in good yields (81-95%) after recrystallization from methanol. As shown in Table 2, highly selective dehexanoylation on the 7-*O* position was observed in all substrates (79-95%), including luteolin (**2a**) and quercetin (**7a**) that contain 5,7,3'4'-*O*-tetrahexanoyl and 3,5,7,3'4'-*O*-pentahexanoyl groups respectively. Therefore the preferential reactivity of the 7-OH was evidenced in all flavonoid structures used, yielding a broad substrate-scope strategy for fast and efficient protection-selective deprotection of flavonoids.

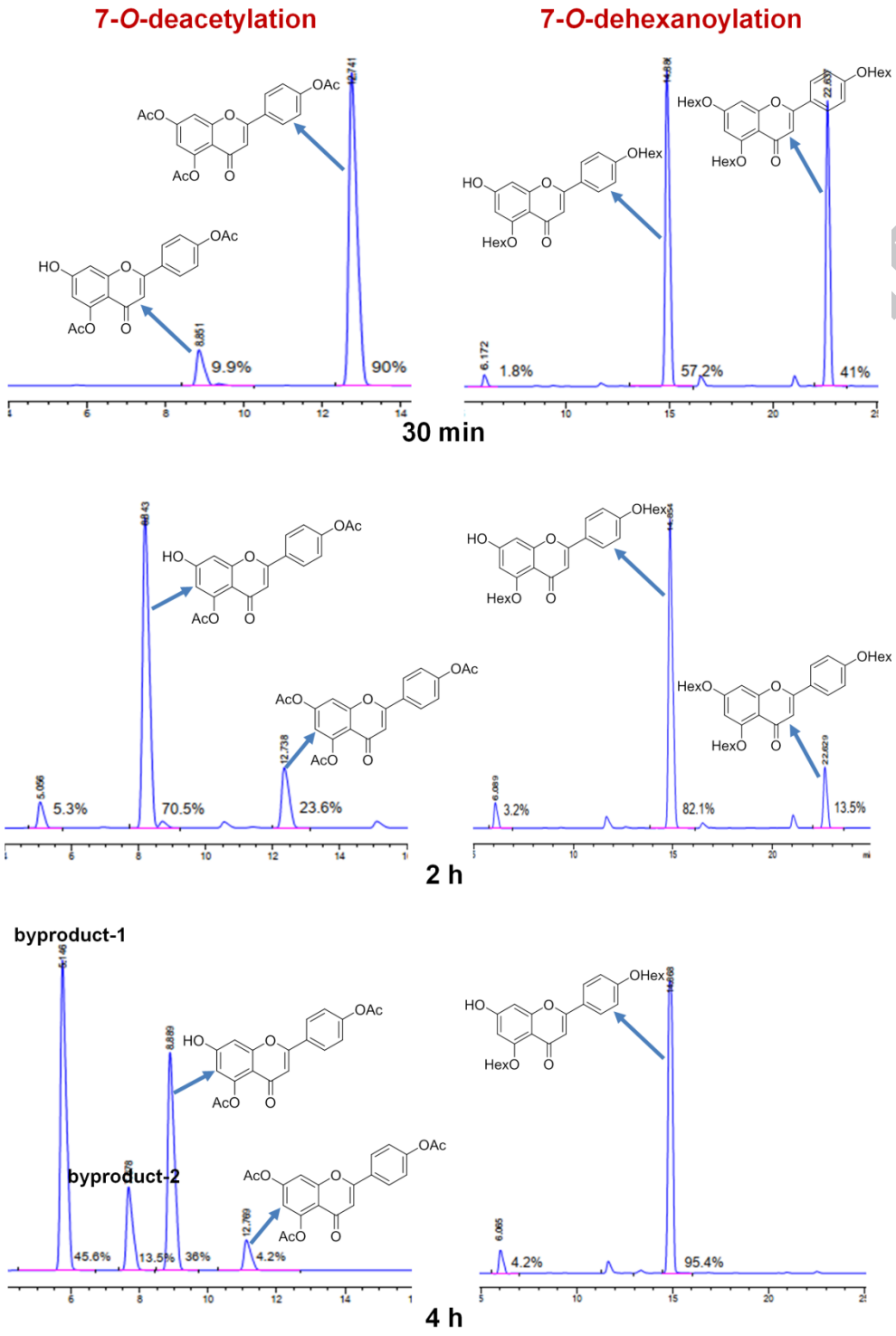
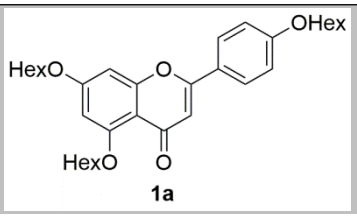
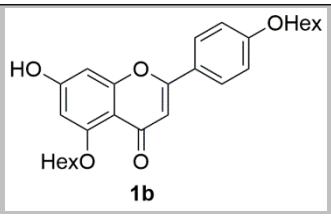
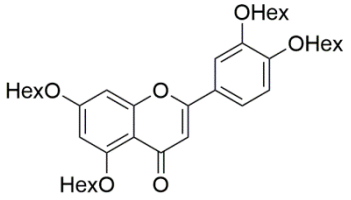
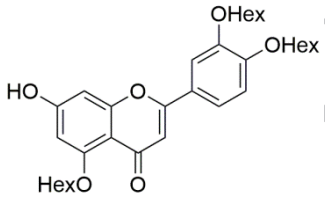
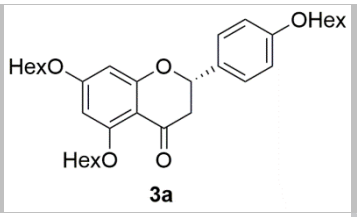
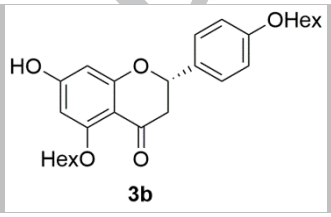
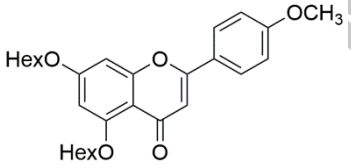
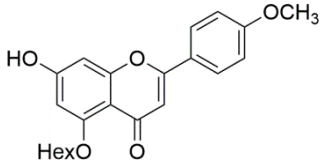
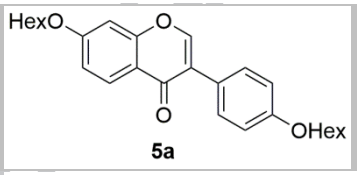
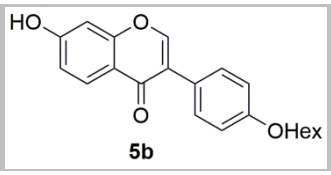
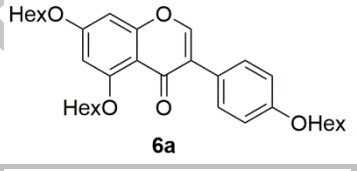
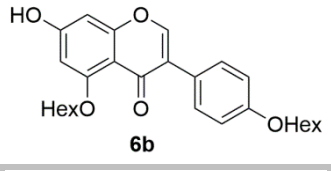
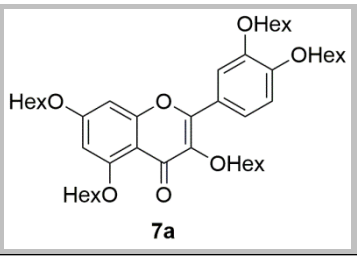
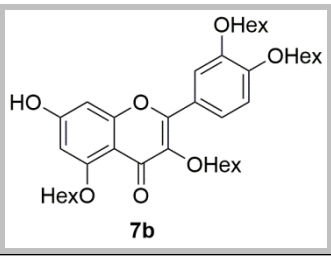


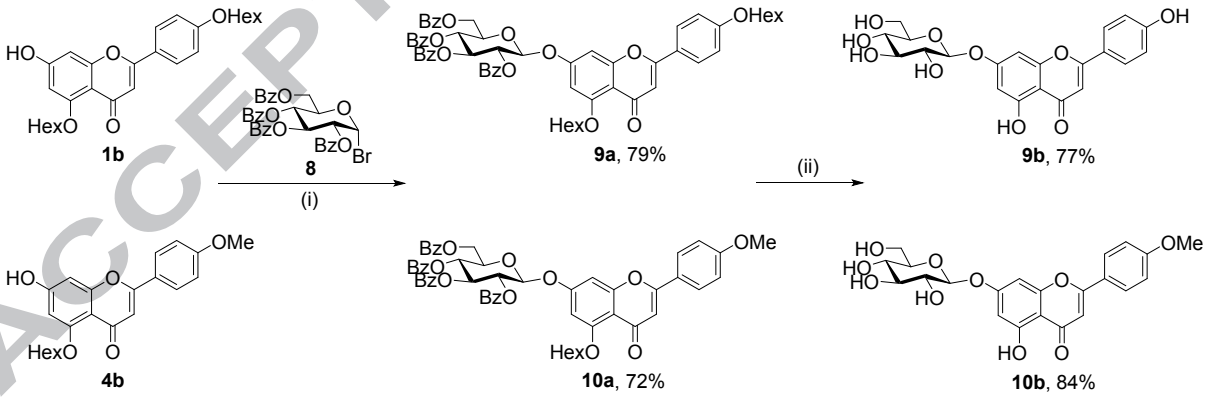
Figure 2. HPLC monitoring the deacylation progress, deacetylation *vs.* dehexanoylation.

Table 2. Selective dehexanoylation of the perhexanoylated flavonoids.

Entry	Substrate	Product	Yield
1	 **1a**	 **1b**	92%
2	 **2a**	 **2b**	83%
3	 **3a**	 **3b**	79%
4	 **4a**	 **4b**	95%
5	 **5a**	 **5b**	88%
6	 **6a**	 **6b**	93%
7	 **7a**	 **7b**	85%

The obtained 7-hydroxyl flavonoids (**1b-7b**) are key intermediates in the preparation of flavonoid *O*-glycosides as they often bear the sugar moiety at the 7-hydroxy position. It has been reported that among the over 500 flavone *O*-glycosides and over 100 isoflavone *O*-

glycosides recorded, the majority are 7-*O*-glycosides and among the over l000 flavonol glycosides recorded, roughly 40% contain a 7-*O*-glycosidic linkage [28,35]. In order to demonstrate the use of our newly synthesized 7-hydroxyl flavonoids for flavonoid *O*-glycosides, we chose 7-hydroxyl apigenin (**1b**) and 7-hydroxyl acacetin (**4b**) and carried out phase-transfer-catalyzed (PTC) glycosylation [32,36] under basic conditions with benzoylated glucosyl bromide (**8**) respectively (Scheme 1). Subsequent full deacylation of the coupling products (**9a**, **10a**) with sodium methoxide in CH_2Cl_2/CH_3OH afforded apigenin 7-*O*-glucoside (**9b**, Ref. [37]) and acacetin 7-*O*-glucoside (**10b**, Ref. [38]) (Scheme 1, see Supporting Information for the detailed procedure of synthesizing the glycosides). Apigenin 7-*O*-glucoside (apigetrin or cosmosiin, CAS: 578-74-5) can be found in dandelion coffee and in *Teucrium gnaphalodes* and also in a number of food items. Notably, recent studies show its antifungal activity and cytotoxic activity on colon cancer cells were more prominent compared to apigenin aglycone [39]. Acacetin 7-*O*-glucoside (tilianin, CAS: 4291-60-5) is a vasorelaxant agent with antihypertensive effect [40] and a potential anti-inflammatory agent which inhibit inducible nitric oxide synthase (iNOS) expression and production of NO [41]. Recently, potent cardioprotective effects of tilianin in rats have also been reported [42].



Scheme 1. Synthesis of apigenin 7-*O*-glucoside and acacetin 7-*O*-glucoside. Reagents and conditions: (i) $Bu_4N^+Br^-$, $CHCl_3$/aq. K_2CO_3 (0.24 M) (v:v, 1:1), 40 °C; (ii) CH_3ONa, CH_2Cl_2/CH_3OH, rt.

Conclusion

In summary, we described here an efficient hexanoyl-based protection strategy for

flavonoids which represents a broad substrate-scope method for the preparation of 7-hydroxyl flavonoids. Hexanoate derivatives are more amenable to selective deprotection than other esters and the method involves mild conditions using potassium carbonate in methanol-dichloromethane. The resulting partially protected flavonoids were suitable substrates for glycosylation with glycosyl bromides and similar products have been shown to possess useful pharmacological properties. Further applications for the synthesis of these bioactive molecules are underway.

Experimental Section

1. General procedure for the synthesis of perhexanoylated flavonoid (1a-7a)

Flavonoid (**1** (5.40 g, 0.02 mol), **2** (5.72 g, 0.02 mmol), **3** (5.45 g, 0.02 mmol), **4** (5.69 g, 0.02 mmol), **5** (5.08 g, 0.02 mmol), **6** (5.40 g, 0.02 mmol), or **7** (6.04 g, 0.02 mmol)) was dissolved in DMF (50 mL); Et_3N (for **4**,**5**, 6.9 mL, 0.05 mol; for **1**,**3**,**6**, 9.7 mL, 0.07 mol; for **2**, 12.4 mL, 0.09 mol; for **7**, 15.3 mL, 0.11 mol) and DMAP (240 mg, 2 mmol) were added. The mixture was then cooled in an ice-bath, and hexanoyl chloride (for **4**,**5**, 6.9 mL, 0.05 mol; for **1**,**3**,**6**, 9.7 mL, 0.07 mol; for **2**, 12.4 mL, 0.09 mol; for **7**, 15.3 mL, 0.109 mol) was added and the reaction was allowed to slowly rise to room temperature in 30 minutes and stirred for an additional 4 h. After complete consumption of the flavonoid starting material as shown by TLC, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with 1 M HCl aqueous solution (100 mL), saturated aqueous $NaHCO_3$ (100 mL × 2), brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford the crude product (oil/solid), which was further purified by crystallization (methanol). Compound **1a-3a** (**1a**, 9.8 g, 87%, Ref. [30]; **2a**, 11.5 g, 85%; **3a**, 10.4 g, 92%) were obtained as a light yellow solid and **4a-7a** (**4a**, 8.4 g, 87%; **5a**, 8.6 g, 95%, Ref. [28]; **6a**, 9.1 g, 81%, Ref. [43]; **7a**, 13.1 g, 83%) as a white solid. Analytical data and NMR spectra see Supporting Information.

2. General procedure for the regioselective removal of the 7-*O*-hexanoyl (1b-7b)

Perhexanoylated flavonoid (**1a** (2.82 g, 5 mmol), **2a** (3.39 g, 5 mmol), **3a** (2.83 g, 5 mmol), **4a** (2.40 g, 5 mmol), **5a** (2.25 g, 5 mmol), **6a** (2.82 g, 5 mmol), or **7a** (3.96 g, 5 mmol)) was dissolved in CH_3OH–CH_2Cl_2 (1:1, 40 mL) and K_2CO_3 (345 mg, 2.5 mmol) was added slowly. The reaction was stirred at room temperature and monitored by TLC. After

complete consumption of the starting material, the reaction was cooled in an ice-bath and quenched with freshly prepared 1 M HCl/MeOH solution to a pH value of 8. The mixture was then concentrated under reduced pressure to give the crude product (yellow oil/solid). Further purification by silica gel column chromatography afforded **1b, 2b, 4b** (toluene/EtOAc = 5:1) as a white solid (**1b**, 2.14 g, 92%, Ref. [30]; **2b**, 2.41 g, 83%; **4b**, 1.81 g, 95%); **3b, 5b-7b** (CH_2Cl_2/acetone = 15:1 to 25:1) as a white solid (**3b**, 1.85 g, 79%; **5b**, 1.55 g, 88%, Ref. [28]; **6b**, 2.17 g, Ref. [43], 93%; **7b**, 2.95 g, 85%). Analytical data and NMR spectra see Supporting Information.

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Supplementary data: Supplementary data (full experimental details for the glycosides **9a**, **9b**, **10a**, **10b**, and characterization of compounds – analytical data and ^{1}H and ^{13}C NMR spectra) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tetlet.

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Highlights

- A hexanoyl-based protection strategy for flavonoids with broad substrate scope.
- Highly regioselective deprotection afforded various 7-hydroxyl flavonoids.
- A comparison between dehexanoylation and deacetylation.
- Phase-transfer-catalyzed glycosylation resulted in flavonoid 7-*O*-glycosides.