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An Efficient Total Synthesis of Resokaempferol 3-*O*-β-D-Glucoside

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Resokaempferol 3-*O*-β-D-glucoside, an artificial flavonol glycoside, was synthesized from 2,4-dihydroxyacetophenone in six steps and 40% overall yield via an efficient glycosylation method using NaH.

Flavonol glycosides are present in a wide variety of fruits, vegetables, and herbal medicines and show a broad range of biological and pharmacological activities.[1] Structure–activity studies of flavonol glycosides have shown that flavonol 3-*O*-monosaccharides are more potent than the corresponding flavonols without the sugar linkage at the 3-OH.[2] For example, quercetin 3-*O*-β-D-glucoside (Figure 1, **1a**) was more active than quercetin in the treatment of cancer, renal, and cardiovascular diseases, and in the inhibition of the 3C-like protease, which is responsible for viral replication in acute respiratory syndrome coronavirus (SARS-CoV 3CL).[3–6] Another example is kaempferol 3-*O*-β-D-glucoside (Figure 1, **1b**), which has profound antioxidant activity.[7] Recently, flavonol glycosides, but not flavonols, have been detected in human plasma in studies of the absorption and metabolism of flavonols and their glycosides.[8] Thus, flavonol 3-*O*-glycosides are worthy of further evaluation.

Resokaempferol (Figure 1) is an important, natural flavonol found in several plant species, such as *Ginkgo biloba L.* and pine needles, and shows moderate antioxidant activity.[9] Recently, our laboratory has synthesized a series of resokaempferol and it's derivatives. These compounds potently have antidiabetic activity, making them worthy of further evaluation.

To exploit this molecule in the development of effective antidiabetic agents, we hypothesized that the introduction of a monosaccharide at the 3-OH of resokaempferol would increase its antidiabetic activity both in vitro and in vivo. A series of resokaempferol 3-*O*-glycosides is required to assess this.

Although many flavonol *O*-glycosides have been successfully synthesized,[6,10–24] glycosylation at the 3-OH of 5-OH-protected or 5-deoxyflavonols, such as resokaempferol, is particularly difficult and has always been accomplished with low yields due to strong hydrogen bonding between the 3-OH and the 4-carbonyl group.[25–27] Here, we report the first total synthesis of resokaempferol 3-*O*-β-D-glucoside (Figure 1, **1c**) via a highly efficient glycosylation method, which paves the way for more efficient syntheses of various 3-*O*-glycosylated, 5-OH-protected or 5-deoxyflavonols and their analogs.

Resokaempferol derivative **2** (Scheme 1) was prepared prior to the synthesis of **1c**. For this purpose, the 4-OH of 2,4-dihydroxyacetophenone (**3**) was first selectively protected with a benzyl group to form **4**, which was then reacted with *p*-benzyloxybenzaldehyde to afford chalcone **5** in 88% yield. The Algar–Flynn–Oyamada reaction was chosen for oxidative cyclization. Treatment of **5** with 30% hydrogen peroxide (H_2O_2) and NaOH converted **5** into **2** in good yield (83%) after **2** was

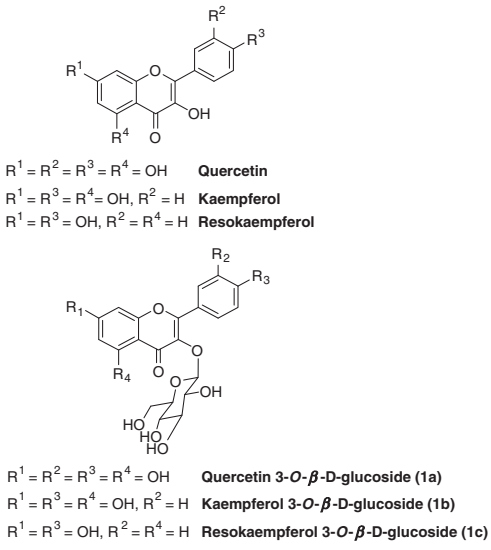
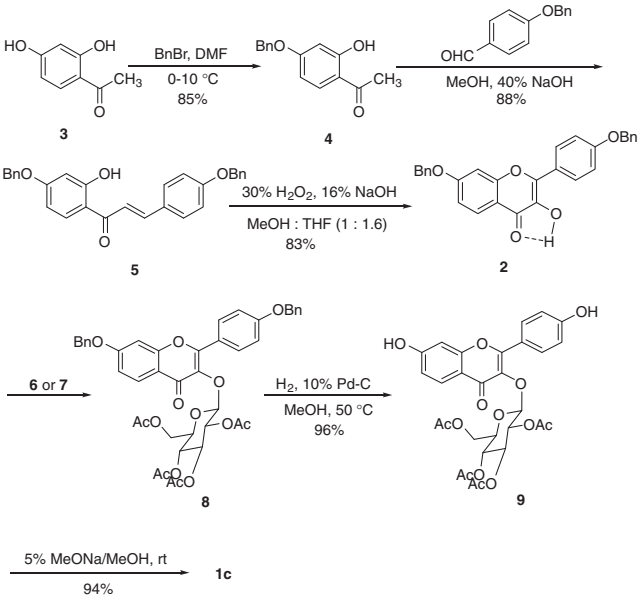
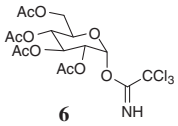
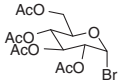


Figure 1. Typical natural flavonols and their 3-*O*-β-D-glucosides.



Scheme 1. Synthetic route to resokaempferol 3-*O*-β-D-glucoside (**1c**).

Table 1. Studies on the glycosylation of the 3-OH of intermediate **2**

Entry	Donor	Activator	Solvent	Temp/°C	Time/h	Yield/%
1[a]	 **6**	TMSOTf	CH_2Cl_2	−78 to 0	5	0
2[a]	 **7**	Ag_2O	CH_2Cl_2	40	12	13
3[a]	**7**	K_2CO_3	DMF	20	10	0
4[a]	**7**	K_2CO_3	DMF	40	15	0
5[b]	**7**	K_2CO_3/TBAB	CH_2Cl_2/H_2O	20	4	trace
6[b]	**7**	K_2CO_3/TBAB	CH_2Cl_2/H_2O	50	4	15–17
7[b]	**7**	K_2CO_3/TBAB	CH_2Cl_2/H_2O	60	12	15–17

[a]The molar ratio of **2**:donor:activator is 2:3:3. [b]The molar ratio of **2**:**7**:K_2CO_3:TBAB is 2:3:6:6.

recrystallized from ethanol.[24] In the reaction, tetrahydrofuran was used as a cosolvent to enhance the solubility of **5** in methanol. Compound **2** was identified by ^{1}H NMR, ^{13}C NMR, and ESI-MS.[30]

Next, glucose was linked to the 3-OH of **2**. In a first attempt, glucopyranosyl trichloroacetimidate (**6**) was used because glycosyl trichloroacetimidate is a strong glycosyl donor and has been used successfully, together with activator trimethylsilyl trifluoromethanesulfonate (TMSOTf), in the glycosylation of phenols.[28] However, the reaction failed, leading to several complex products most likely due to decomposition of donor **6** or the resulting products under acidic conditions (Table 1, Entry 1). Glycosylation was then performed using glucopyranosyl bromide (**7**) under Koenigs–Knorr conditions;[10] the isolated product **8** was obtained with a mere 13% yield (Table 1, Entry 2). Moreover, the solubility of the reactants is poor in CH_2Cl_2. Because no or very little glycosylated product was formed under acidic conditions, the synthetic strategy was altered to basic conditions. It was anticipated that under basic conditions, the hydrogen bond between the 3-OH and the 4-carbonyl group would be broken, leading to the production of an oxygen anion with an enhanced nucleophilicity toward the anomeric center of the glycosyl donor. The glycosylation reaction between **2** and **7** was first performed in DMF in the presence of a weak base, K_2CO_3. However, no desired product was generated even after increasing the reaction time and temperature (Table 1, Entries 3 and 4, respectively). According to Li's reported reaction conditions,[24] glycosylation under phase-transfer-catalyzed (PTC) conditions was also evaluated (Table 1, Entries 5–7). In the presence of tetrabutylammonium bromide (TBAB), **8** was obtained in 15–17% yield when **2** and **7** were heated to 50–60 °C with K_2CO_3 in CH_2Cl_2 and water. This result is in agreement with Yamasaki's report.[27]

Encouraged by the successful application of K_2CO_3 in the glycosylation of **2** with glucosyl bromide **7**, we then investigated the glycosylation reaction using a strong Lewis base, sodium hydride (NaH), as the promoter. After several attempts, we found that solvent and temperature were two key factors determining the success of the reaction. Using DMF as the solvent improved the solubility of the reactants and gave the best results. When the reaction was performed at 0 °C for 15 h, **8** was obtained in 33% yield, which is better than that obtained when employing K_2CO_3 as a base (Table 2, Entry 1). Elevating the temperature to 10 and 15 °C increased the yield of **8** to 44% and 72%, respectively (Table 2, Entries 2 and 3). Interestingly, further increases in temperature to 20 and 25 °C, decreased the yield of **8** to 56% and 53%, respectively. This was likely due to decomposition of the glucosyl bromide **7** under strong basic conditions (i.e., elimination, to give the glycal; Table 2, Entries 4 and 5).

Table 2. NaH-promoted glycosylation of the 3-OH of **2** with **7**[a]

Entry	Temp/°C	Time/h	Yield/%
1	0	15	33
2	10	12	44
3	15	12	72
4	20	12	56
5	25	12	53

[a]Optimal reaction procedure: to an ice-cooled solution of **2** in DMF, 1.5 equiv of NaH (60% oil coated) was added. After stirring at 0 °C for 15 min, 1.5 equiv of **7** was added and the reaction was continued at 15 °C for 12 h.

After the production of **8**, the remaining synthesis of **1c** was quite straightforward. As shown in Scheme 1, the benzyl groups in **8** were first removed by 10% Pd/C-catalyzed hydrogenation to afford compound **9** in a yield of 96%, which was used directly in the next step with no further purification. Treatment of **9** with 5% sodium methoxide in methanol removed all of the acetyl groups in **9** and gave **1c** in 94% yield. **1c** was identified by ^{1}H NMR, ^{13}C NMR, and ESI-MS.[29,30]

In conclusion, resokaempferol 3-*O*-β-D-glucoside (**1c**) was synthesized from inexpensive, 2,4-dihydroxyacetophenone (**3**) in six steps and 40% overall yield. A glycosylation method mediated by NaH was developed to break the hydrogen bond between the 3-OH and the 4-carbonyl group of resokaempferol, thereby allowing for the efficient introduction of a glucose moiety. Currently, using this method, a series of flavonol 3-*O*-glycosides have been prepared and studies on their biological activities are underway.

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29 Spectroscopic data for respresentative library members: **2**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.16 (s, 2H), 5.18 (s, 2H), 7.07 (dd, $J = 8.9$ Hz, 2.0 Hz, 1H, C^6–*H*), 7.10 (d, $J = 2.2$ Hz, 1H, C^8–*H*), 7.14 (d, $J = 8.9$ Hz, 2H, $C^{3'}$–*H*, $C^{5'}$–*H*), 7.37–7.56 (m, 10H), 8.16 (d, $J = 8.9$ Hz, 1H, C^5–*H*), 8.21 (d, $J = 8.9$ Hz, 2H, $C^{2'}$–*H*, $C^{6'}$–*H*); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 69.3, 70.0, 101.2, 114.8, 115.3, 123.8, 126.1, 127.8, 127.9, 128.4, 128.5, 136.1, 136.6, 137.8, 144.8, 156.2, 159.3, 162.5, 172.0; ESI-MS (m/z): $[M + Na]^+$ calcd for $C_{29}H_{22}O_5Na$ 473.1; found 472.9. **8**: $[\alpha]_D^{24.2}$ −0.78 (*c* 0.15, acetone); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.88 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.13 (s, 3H), 3.64–3.70 (m, 1H), 3.98–4.01 (m, 2H), 5.18 (s, 2H), 5.19 (s, 2H), 5.17–5.30 (m, 3H), 5.75 (d, $J = 7.5$ Hz, 1H, $C^{1''}$–*H*), 6.90 (d, $J = 2.1$ Hz, 1H, C^8–*H*), 7.00–7.10 (m, $J = 8.6$ Hz, 2H, $C^{3'}$–*H*, $C^{5'}$–*H*; $J = 9.3$ Hz, 2.0 Hz, 1H, C^6–*H*), 7.39–7.50 (m, 10H), 8.05 (d, $J = 8.4$ Hz, 2H, $C^{2'}$–*H*, $C^{6'}$–*H*), 8.14 (d, $J = 9.3$ Hz, 1H, C^5–*H*); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.9, 21.0, 21.4, 61.2, 68.7, 70.3, 70.8, 71.8, 71.9, 73.1, 99.1, 101.3, 114.7, 115.3, 118.3, 123.5, 127.2, 127.7, 127.8, 128.4, 128.7, 128.9, 129.0, 131.1, 135.7, 135.9, 136.7, 157.0, 157.1, 160.9, 163.4, 169.8, 170.2, 170.3, 170.6, 173.5; ESI-MS (m/z): $[M + H]^+$ calcd for $C_{43}H_{41}O_{14}$ 781.2; found 781.3. **1c**: $[\alpha]_D^{24.0}$ −3.38 (*c* 0.9, MeOH); ^{1}H NMR (methanol-d_4, 300 MHz): δ 3.20–3.70 (m, 6H), 5.19 (d, $J = 7.2$ Hz, 1H, $C^{1''}$–*H*), 6.90–6.92 (m, 4H, C^6–*H*, C^8–*H*, $C^{3'}$–*H*, $C^{5'}$–*H*), 8.00 (d, $J = 8.4$ Hz, 1H, C^5–*H*), 8.10 (d, $J = 8.4$ Hz, 2H, $C^{2'}$–*H*, $C^{6'}$–*H*); ^{13}C NMR (methanol-d_4, 150 MHz): δ 59.6, 68.3, 72.8, 75.2, 75.4, 100.2, 101.5, 113.1, 113.4, 114.5, 120.0, 124.9, 129.4, 134.4, 155.8, 156.0, 158.5, 161.8, 173.3; ESI-MS (m/z): $[M - H]^-$ calcd $C_{21}H_{19}O_{10}$ 431.1; found 431.0.
30 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

