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Synthesis of flavonol derivatives as probes of biological processes

Hiroko Tanaka,[a] Michelle M. Stohlmeyer,[a] Thomas J. Wandless[a,*] and Loverine P. Taylor[b]

[a]*Department of Chemistry, Stanford University, Stanford, CA 94305, USA*
[b]*School of Molecular Biosciences, Washington State University, Pullman, WA 99164, USA*

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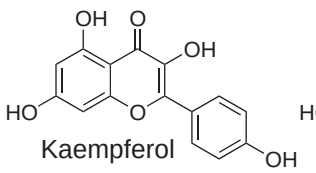
Abstract

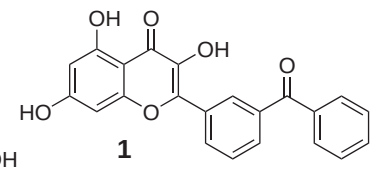
Two synthetic derivatives of the flavonol kaempferol were prepared in order to probe the biological mechanism of action of this natural product. Efficient synthetic routes to both a benzophenone-containing derivative and an amine-containing derivative are reported herein.

Flavonoids are phenolic secondary metabolites that are widely distributed throughout the plant kingdom. The average Western diet includes up to 2 g of flavonoids per day, and they have been identified as antitumor agents, antioxidants, and free radical scavengers.[1] In plants, flavonoids function as UV-protectants, pollinator attractants, and as signaling molecules between roots and nitrogen-fixing bacteria in the soil. An essential role in plant reproduction has been established for one class of flavonoid, the flavonols. Pollen from maize and petunia mutants lacking flavonols is unable to germinate. However, the mutant pollen is viable and the defect can be biochemically complemented by adding kaempferol at the time of pollination.[2]

In an effort to identify the biologically relevant receptors of the flavonols, we synthesized two derivatives of kaempferol at a site that is tolerant of modification.[3] The first flavonol derivative (**1**) possesses a benzophenone chromophore for use as a photoaffinity reagent.[4] The second derivative (**2**) substitutes an amino group for the kaempferol hydroxyl group at C4′ in order to facilitate the preparation of a solid-phase matrix for affinity chromatography. Both molecules are proving to be useful probes for understanding the biochemical basis for pollen germination.



Kaempferol



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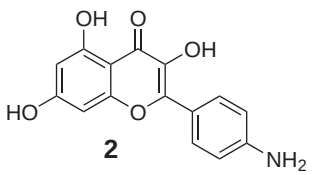

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* Corresponding author. Tel/fax: 650-723-4005; e-mail: wandless@stanford.edu

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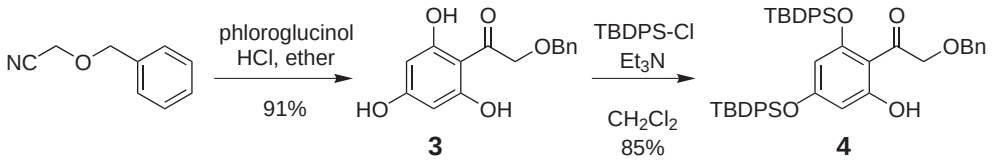
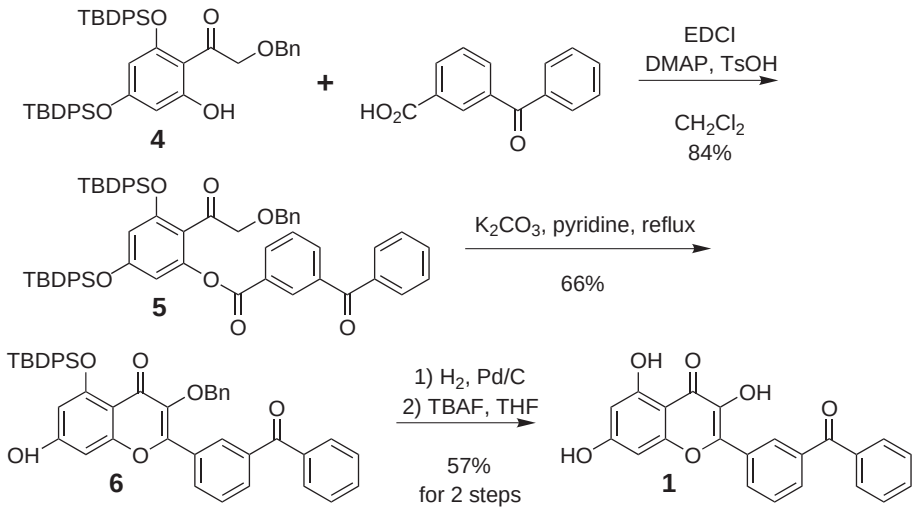
Phloroglucinol and benzyloxyacetonitrile were dissolved in ether and treated with HCl to provide, after hydrolytic workup, the ketone product **3** (Scheme 1).[5] Treatment of **3** with *tert*-butylchlorodiphenylsilane and triethylamine provided the bis-protected phenol **4** in good yield. This intermediate may be converted into either **1** or **2**, depending on the carboxylic acid used for the subsequent acylation. Previous flavonol syntheses often did not involve protection of the phenolic hydroxyl groups, which resulted in multiple acylations in subsequent steps.[6] We selectively protected two of the three available phenols of **3** to ensure that only one hydroxyl group is available for acylation.



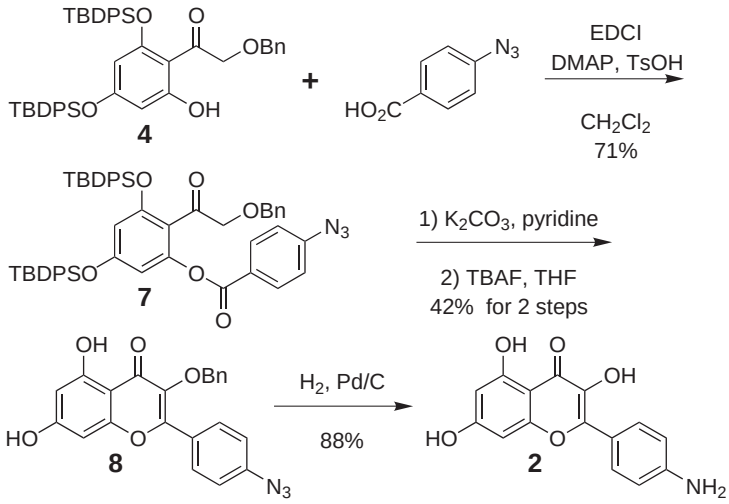
Scheme 1.

To prepare the benzophenone flavonol derivative, commercially available 3-benzoylbenzoic acid and phenol **4** were coupled using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in the presence of catalytic 4-dimethylaminopyridine and *para*-toluenesulfonic acid as shown in Scheme 2. These coupling conditions provide a good yield of ester **5**, which is suitably functionalized for ring closure. Intramolecular dehydrative condensation to provide flavonol **6** was achieved by treating ester **5** with potassium carbonate in refluxing pyridine.[7] Partial loss of the TBDPS protecting groups was observed, however these conditions consistently provide moderate to good yields of a mixture of **6** and the product lacking both TBDPS groups (**9**). The benzyl ether was removed by hydrogenolysis with care taken to prevent overreduction, and deprotection of the remaining TBDPS ether was achieved with tetrabutylammonium fluoride to provide the desired benzophenone-substituted flavonol **1** in 57% yield for the final two transformations.



Scheme 2.

Synthesis of the amino-substituted kaempferol **2** is shown in Scheme 3 and proceeds via a similar strategy to the benzophenone derivative outlined above. Phenol **4** was acylated with 4-azidobenzoic acid under the same conditions used for the benzophenone derivative to provide ester **7** in 71% yield. Potassium carbonate in refluxing pyridine transformed **7** into the protected flavonol with concomitant partial deprotection of the TBDPS ethers. Treatment of the resulting mixture with tetrabutylammonium fluoride in THF provided intermediate **8** in 42% yield through two steps. The benzyl ether was hydrogenolyzed and the azide group was reduced using 5% palladium on carbon under a hydrogen balloon to provide the desired amino-substituted kaempferol derivative **2** in 88% yield.



Scheme 3.

The syntheses outlined above provided probe reagents **1** and **2** for biological characterization, and preliminary studies show that both kaempferol variants are biologically active. The benzophenone- and amino-substituted flavonols are both substrates for a pollen-specific flavonol galactosyltransferase (F3GalTase).[8] Further, the benzophenone derivative **1** can be photoactivated and covalently crosslinked to the F3GalTase protein making it a useful probe for catalytic residues. The amino-derivative induces flavonol-deficient pollen to germinate as efficiently as the endogenous kaempferol. An affinity matrix prepared from immobilized **2** is being used to identify kaempferol-binding proteins in germinating pollen. Further studies that utilize these kaempferol derivatives to probe the biology of pollination are currently in progress and will be reported in due course.

Selected experimental procedures

Transformation of **3** to **4**: Ketone **3** (1.0 g, 3.65 mmol; $R_f = 0.4$, 15% ethyl acetate in hexanes) was dissolved in 50 mL dichloromethane. Triethylamine (1.27 mL, 9.13 mmol) and *tert*-butylchlorodiphenylsilane (2.38 mL, 9.13 mmol) were added over 4 h, and the reaction stirred overnight at room temperature. Upon completion, the reaction solution was washed twice with 1% HCl. The organic layer was dried over $MgSO_4$, concentrated, and the product was purified from silica gel eluting with 6% ethyl acetate in hexanes to isolate 2.33 g (3.11 mmol, 85% yield)

of compound **4** as a clear oil (R_f=0.65, 20% ethyl acetate in hexanes). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.7–7.2 (m, 25H), 5.81 (d, 1H, J=2.2 Hz), 5.55 (d, 1H, J=2.2 Hz), 4.92 (s, 2H), 4.66 (s, 2H), 0.90 (s, 9H), 0.78 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 201.3, 166.1, 161.9, 158.8, 137.7, 135.2, 135.1, 135.0, 131.8, 131.1, 130.2, 129.9, 128.4, 128.1, 127.8, 127.7, 106.9, 104.5, 102.4, 75.8, 73.0, 26.6, 26.2, 19.3, 19.1.

Transformation of **4** to **5**: Phenol **4** (1.1 g, 1.47 mmol) was dissolved in 15 mL dichloromethane. 3-Benzoylbenzoic acid (452 mg, 2.0 mmol), DMAP (61 mg, 0.5 mmol), TsOH (95 mg, 0.5 mmol) and EDCI (478 mg, 2.2 mmol) were added in that order. Additional EDCI (956 mg, 4.4 mmol) was added over the course of 12 h. After 24 h, the reaction was diluted with dichloromethane, washed twice with 0.1 M HCl then once with 0.01 M HCl. The organic layer was dried over $MgSO_4$, concentrated, and the product was purified from silica gel eluting with 10% ethyl acetate in hexanes to provide 1.17 g of compound **5** (1.22 mmol, 84% yield) as a pale yellow oil (R_f=0.4, 20% ethyl acetate in hexanes). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.30 (dd, 1H, J=7.8 Hz, J=1.4 Hz), 8.07 (dd, 1H, J=7.8 Hz, J=1.4 Hz), 7.81 (dd, 2H, J=8.3 Hz, J=1.2 Hz), 7.62, (t, 2H, J=8.0 Hz), 7.6–7.1 (m, 27 H), 6.31, (d, 1H, J=2.1 Hz), 5.87 (d, 1H, J=2.1 Hz), 4.60 (s, 2H), 4.54 (s, 2H), 0.92 (s, 9H), 0.85 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 199.7, 195.5, 163.8, 157.6, 154.5, 149.0, 138.1, 137.5, 136.9, 135.2, 135.1, 134.8, 133.8, 132.9, 131.7, 131.6, 131.1, 130.1, 130.0, 129.8, 129.3, 128.8, 128.5, 128.3, 127.9, 127.8, 127.7, 117.2, 109.2, 108.1, 76.0, 73.2, 26.3, 26.2, 19.3, 19.2.

Transformation of **5** to **6**: Anhydrous potassium carbonate (72.2 mg, 0.522 mmol) was placed in a 5-mL round bottom flask and compound **5** (94.8 mg, 0.101 mmol) was added in 1 mL pyridine. The mixture was refluxed for 1 h. After cooling, saturated aqueous ammonium chloride was added and the organic products were extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. The product was purified from silica gel (eluting with a gradient of 5% ethyl acetate to 17% ethyl acetate in hexanes) to afford 21.3 mg (0.030 mmol, 30% yield) of partially deprotected compound **6** (R_f=0.6, 25% ethyl acetate in hexanes) and 17.0 mg (0.037 mmol, 36% yield) of compound **9** (R_f=0.2, 25% ethyl acetate in hexanes). **^{1}H NMR** of **6** (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 8.07 (d, 1H, J=8 Hz), 7.87 (d, 1H, J=8 Hz), 7.77 (d, 2H, J=8 Hz), 7.71 (d, 4H, J=8 Hz), 7.59 (t, 1H, J=8 Hz), 7.53 (t, 1H, J=8 Hz), 7.47–7.38 (m, 8H), 7.20 (br, 5H), 6.31 (d, 1H, J=2 Hz), 6.26 (d, 1H, J=2 Hz), 5.18 (s, 2H), 1.11 (s, 9H). **^{1}H NMR** of **9** (400 MHz, $CDCl_3$+DMSO-d_6) δ 8.17 (s, 1H), 8.02 (d, 1H), 7.74 (d, 1H), 7.64 (d, 2H), 7.48–7.41 (m, 2H), 7.34–7.30 (m, 2H), 7.08 (br, 5H), 6.25 (s, 1H), 6.18 (s, 1H).

Transformation of **6** to **1**: Compound **6** (251 mg, 0.367 mmol) was taken up in 16 mL ethyl acetate and 36.8 mg of 5% palladium on carbon was added. The mixture was placed under a hydrogen balloon for 2 h. Filtration and concentration afforded 247 mg of the crude product (R_f=0.7, 33% ethyl acetate in hexanes). This product was dissolved in 3 mL THF, and tetrabutylammonium fluoride (0.4 mL, 0.4 mmol, 1 M solution in THF) was added at 0°C. The reaction was complete within 30 min, and the reaction mixture was purified using silica gel (eluent: 7% ethyl acetate in hexanes, then 17% ethyl acetate in hexanes, then 17% ethyl acetate in hexanes with 1% TFA, then 33% ethyl acetate in hexanes with 1% TFA). Compound **1** (138 mg, 0.369 mmol, 57% yield over two steps) was obtained as a yellow solid (R_f=0.3, 33% ethyl acetate in hexanes). **^{1}H NMR** of **1** (400 MHz, DMSO-d_6) δ 11.01 (s, 1H), 10.02 (s, 1H), 8.66 (s, 1H), 8.51 (d, 1H, J=8.5 Hz), 7.94–7.80 (m, 6H), 7.70 (t, 2H, J=7.6 Hz), 6.55 (d, 1H, J=1.9 Hz), 6.32 (d, 1H, J=1.9 Hz). **^{13}C NMR** (100 MHz, acetone-d_6) δ 177.3, 165.8, 162.7, 158.3, 139.1, 138.8, 138.5, 133.9, 132.7, 132.2, 132.1, 131.1, 130.0, 130.0, 129.7, 104.7, 99.7, 94.9.

^{1}H NMR of **2** (400 MHz, DMSO-d_6) δ 7.92 (s, 1H), 7.87 (d, 2H, $J=8.9$ Hz), 6.59 (d, 2H, $J=8.9$ Hz), 6.25 (d, 1H, $J=2.1$ Hz), 6.11 (d, 1H, $J=2.1$ Hz), 2.55 (br, 2H). **^{13}C NMR** (100 MHz, DMSO-d_6) δ 173.7, 162.0, 159.0, 154.3, 149.3, 146.3, 133.0, 127.5, 115.8, 111.5, 101.1, 96.4, 91.6.

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