Preparative Monohydroxyflavanone Syntheses and a Protocol for Gas Chromatography-Mass Spectrometry Analysis of Monohydroxyflavanones

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We describe a facile efficient, and preparative approach for monohydroxyflavanone syntheses. Using this protocol, a hydroxyl is regio-selectively introduced at one carbon of a flavanone A- or B-ring per synthesis. The seven possible isomers were each synthesized from the corresponding monomethoxymethoxylated 2′-hydroxychalcones in acidic solution. These monohydroxyflavanones were characterized using a gas chromatography-mass spectrometry (GC-MS) system that incorporated a DB-5 capillary column. Ours is the first report of a preparative synthetic method during which a single hydroxyl can be selectively added to a flavanone A- or B-ring at any position. We are also the first to develop a procedure that separates the seven isomers by GC and characterizes the mass spectra of the isomers. Both the synthetic method and the GC-MS conditions may become important tools during future flavanone metabolism and oxidation studies.

Key words monohydroxyflavanone; GC-MS; chalcone; flavonoid

Flavonoids are phenolic compounds found in most plants. Since flavonoids are naturally present in fruits, vegetables, and tea, they are an integral part of the human diet.[1)] Ingested flavonoids are absorbed by the gastrointestinal tract and have been found in blood plasma.[2)] Flavonoids possess various biological and pharmacological properties, such as antioxidant activity,[3)] anti-HIV-1 activity,[4)] and anti-inflammatory activity.[5)] They seem to protect against cancer onset,[6)] vascular dementia,[7)] and coronary heart disease-related deaths.[8)] Flavonoids also modulate the activities of enzymes that metabolize drugs.[9)] Flavonoids form the largest class of phytoestrogens known. The binding affinity of a flavonoid for the estrogen receptor primarily depends on the positions of A- and C-ring hydroxyl groups.[10)] Recently, two interesting pharmacological properties of flavonoids have been reported. 1) The polymethoxyflavone, tangeretin, protects against the development of experimentally-induced Parkinson's disease in rats.[11)] 2) Other polymethoxyflavones inhibit the cellular excretion of certain drugs—a process mediated by P-glycoprotein.[12,13)] Cytochrome P450s can remove a flavonoid C-4′ methyl and can add a hydroxyl at the C-3′ position.[14,15)] To understand the diverse pharmacological effects of dietary flavonoids, their metabolic pathways must be known.

A previous study of ours[16)] showed that both rat and human cytochrome P450s convert flavanones to the corresponding 2,3-*trans*-flavanonols and flavones; whereas, only the human enzyme can convert the flavanone to isoflavone. We also recently reported a procedure for the specific deuteration of flavanones.[17)] With these deuterated flavanones as substrates, we determined that, most likely, the initial step in flavanone P450-catalyzed metabolism is abstraction of a hydrogen radical from the C-2 or -3 position of the flavanone skeleton.

After rats received flavanone *via* a stomach tube, the most common urinary metabolites found were those with the C-4 ketone reduced and those with hydroxyls added at positions C-3 and C-6.[18)] Another P450 metabolic study found that aromatic hydroxylation products, flavones, unusual quinol-type oxidation products, chromone derivatives, and flavan-4-ol derivatives were produced when several different flavanones were substrates.[19)] Therefore, although all flavonoids have a common three-ring skeleton, it appears that ring substituents determine the metabolic fate of a specific substrate.

If demethylated and hydroxylated flavonoid P450 metabolic products, amongst others, are to be unequivocally characterized, their physical properties must be compared with known standards. Previously, we reported a synthetic procedure for (±)-2,3-*trans*-flavanonols, which are hydroxylated at the C-3 position.[20,21)] Consequently, only those compounds could be unquestionably identified as P450 metabolic products during our previous study.[16)] In order to identify other metabolic monohydroxyflavanones, we have now developed procedures to synthetically produce these compounds and to identify them by GC-MS analysis.

Results and Discussion

Usually, the chemical synthesis of a flavanone involves cyclization of the corresponding chalcone in acidic solution. In turn, the chalcone is usually synthesized from derivatives of acetophenone and benzaldehyde by an aldol reaction. Monohydroxyflavanones have also usually been prepared by this route.[22)] However, because product yields are usually low, this method is not practical. While 5-hydroxyflavanone can be directly synthesized from acetophenone and benzaldehyde in the presence of silica gel, boric acid, and piperidine,[23)] other monohydroxyflavanones cannot be synthesized using analogous procedures. As mentioned above, we have prepared 2,3-*trans*-flavanonols (3-hydroxyflavanones), in high yields, from chalcone epoxides.[20,21)]

We have now applied this method to regio-selective monohydroxyflavanone syntheses and report that work here. Nine different monohydroxyflavanone isomers potentially exist. However, the monohydroxyflavanone that has a hydroxyl at position C-2 of ring C is unstable. Dehydration typically occurs and the corresponding flavone is produced. As noted, we have already reported a method for hydroxylation at position C-3 of ring C. Now, we report the synthesis of the other seven monohydroxyflavanones (compounds **4a**—**g**; Chart 1)

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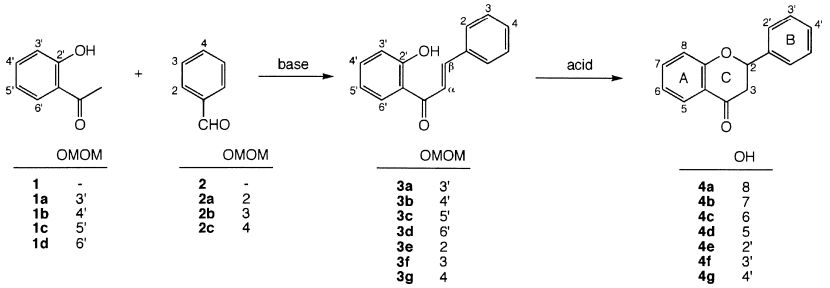


Chart 1. Synthetic Route of Monohydroxyflavanone **4a**—**g**

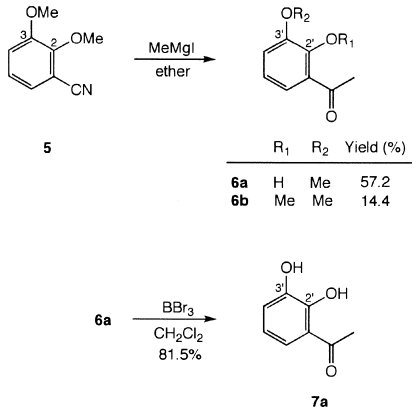


Chart 2. Synthesis of 2′,3′-Dihydroxyacetophenone **7a**

and also report conditions for GC-MS that separate the compounds and characterize their fragmentation patterns.

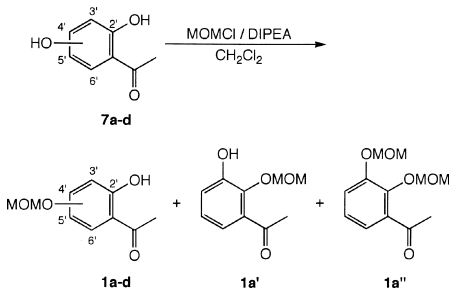
To regio-selectively synthesize these monohydroxyflavanones (Chart 1), we first protected the (non-C-2′) hydroxyl of an acetophenone derivative (**7a**—**d**) or the hydroxyl of a benzaldehyde derivative (**8a**—**c**) by reaction with methoxymethyl chloride (MOMCl) in the presence of *N*-ethyldiisopropylamine (DIPEA). During chalcone synthesis (in the presence of base) the hydroxyl remains protected; whereas, during the flavanone synthesis that follows, the methoxymethyl group is readily removed as a consequence of the acidic condition. When the aldol reaction (using acetophenone and benzaldehyde derivatives as reagents and producing a chalcone) is performed in basic solution—a condition for which the reaction proceeds smoothly—the acetophenone C-2′ hydroxyl need not be protected.

To produce monohydroxylated A-ring flavanones, protected monohydroxylated 2′-hydroxyacetophenones **1a**—**d**, the products of reaction of compounds **7a**—**d** with MOMCl, were the reagents. To produce monohydroxylated B-ring flavanones, protected monohydroxylated benzaldehydes **2a**—**c** were the reagents.

Baker *et al.*[24] reported the synthesis of 2′,3′-dihydroxyacetophenone, **7a**, (Chart 2). Applying this Grignard reaction, we found that 2,3-dimethoxybenzonitrile, **5**, was converted to 2′-hydroxy-3′-methoxyacetophenone, **6a**, (57.2% yield) and 2′,3′-dimethoxyacetophenone, **6b**, (14.4% yield). Perhaps Grignard reagent reacted with **6b** and deprotected the hydroxyl *ortho* to the carbonyl to produce **6a**.[25] Demethylation of **6a** by BBr_3 produced **7a** (81.5% yield).

For synthesis of A-ring monohydroxyflavanones, 2′-hydroxyacetophenones **7a**—**d** that were also hydroxylated at positions C-3′, -4′, -5′ or -6′, were protected using MOMCl

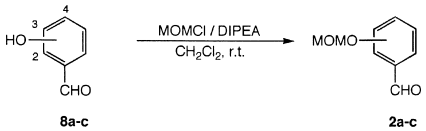
Table 1. Synthesis of 2′-Hydroxy-monomethoxymethoxyacetophenone **1a**—**d**



Substrate	OH	Temp.	Product	OMOM	Yield (%)
7a	3′	r.t.	**1a**	3′	72.4[a]
7b	4′	r.t.	**1b**	4′	96.8
7c	5′	reflux	**1c**	5′	72.8
7d	6′	r.t.	**1d**	6′	92.8

a) The yield of 3′-hydroxy-2′-methoxymethoxyacetophenone **1a′** was 7.0% and 2′,3′-dimethoxymethoxyacetophenone **1a″** was 16.0%.

Table 2. Synthesis of Monomethoxymethoxybenzaldehyde **2a**—**c**



Substrate	OH	Product	OMOM	Yield (%)
8a	2	**2a**	2	96.9
8b	3	**2b**	3	81.8
8c	4	**2c**	4	99.2

(Table 1). Protection of **7a** produced 2′-hydroxy-3′-methoxymethoxyacetophenone (**1a**, 72.4% yield), 3′-hydroxy-2′-methoxymethoxyacetophenone (**1a′**, 7.0% yield), and 2′,3′-dimethoxymethoxyacetophenone (**1a″**, 16.0% yield). Compound **7c**, 2′,5′-dihydroxyacetophenone, is insoluble in CH_2Cl_2 at room temperature; therefore, to solubilize **7c**, the reaction mixture was refluxed.

For synthesis of B-ring monohydroxyflavanones, MOM-protected monohydroxybenzaldehydes **2a**—**c** were synthesized using monohydroxybenzaldehydes **8a**—**c** as the starting materials. Compounds **2a**—**c** were obtained in good yields (Table 2).

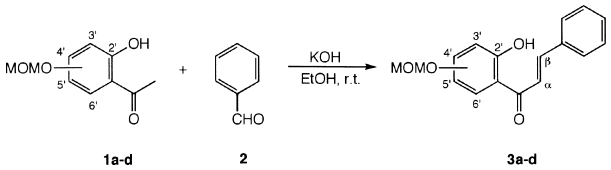
The 2′-hydroxy-monomethoxymethoxychalcones **3a**—**g** were synthesized from the protected acetophenones **1a**—**d**

and benzaldehyde, **2**, or from acetophenone, **1**, and the protected benzaldehydes **2a**—**c** by base-catalyzed aldol reactions (Tables 3, 4). During the synthesis of 2′-hydroxy-5′-methoxymethoxychalcone, **3c**, 6-methoxymethoxyflavanone, **4c′**, was formed in 26.4% yield as a side product. We assume that **4c′** was produced from the *p*-quinone of **3c**. Compound **4c′** was converted to **4c** in the presence of 12% HCl–MeOH (95.8% yield).

The 2′-hydroxy-monomethoxymethoxychalcones **3a**—**g** were cyclized and deprotected in acidic solution to produce the monohydroxyflavanones **4a**—**g**. For flavanones monohydroxylated at the A-ring (**4a**—**d**), the corresponding chalcones **3a**—**d** were treated with 17.8 or 25.8% HCl–MeOH at 100 °C (Table 5). Monohydroxyflavanones **4a**—**d** were obtained in good chemical yield. During cyclization and deprotection, the 2′-hydroxy-monohydroxychalcones **3a′**—**d′** were also produced. It was hard to isolate the dihydroxychalcone **3a′** because it absorbed tightly to silica gel. The dihydroxychalcone **3d′** was also difficult to isolate because it readily converted to **4d** in the presence of silica gel. With the exception of **3a′**, the other dihydroxychalcones (**3b′**—**d′**) could be converted to the monohydroxyflavanones **4b**—**d** using acid (data not shown).

To deprotect the B-ring hydroxyls and form the monohydroxyflavanones **4e**—**g**, monomethoxymethoxychalcones **3e**—**g** were also reacted with HCl–MeOH (Table 6). Both monohydroxyflavanones **4e**—**g** and dihydroxychalcones **3e′**—**g′** were produced when **3e**—**g** were reacted with HCl–MeOH. After isolation, the dihydroxychalcones **3e′**—**g′** were also converted to the monohydroxyflavanones **4e**—**g** by

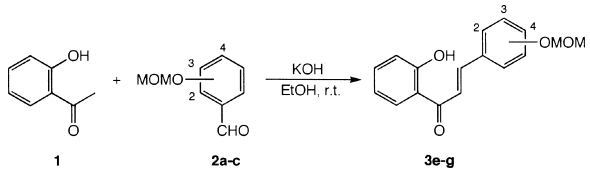
Table 3. Synthesis of 2′-Hydroxy-monomethoxymethoxychalcone **3a**—**d**



Substrate	OMOM	Product	OMOM	Yield (%)
1a	3′	**3a**	3′	91.0
1b	4′	**3b**	4′	69.2 (93.2)[a]
1c	5′	**3c**	5′	60.7[b]
1d	6′	**3d**	6′	90.0

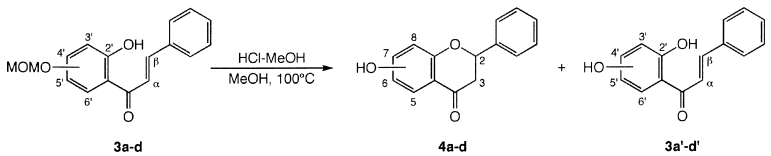
a) Based on recovered. *b*) The yield of 6-methoxymethoxyflavanone **4c′** was 26.4%.

Table 4. Synthesis of 2′-Hydroxy-monomethoxymethoxychalcone **3e**—**g**



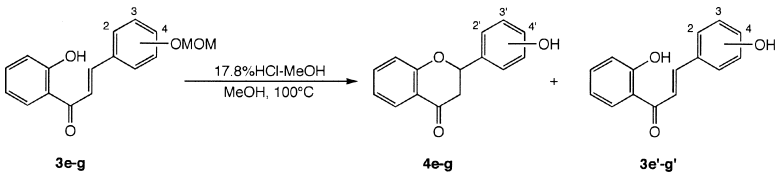
Substrate	OMOM	Product	OMOM	Yield (%)
2a	2	**3e**	2	81.2
2b	3	**3f**	3	73.6
2c	4	**3g**	4	81.3

Table 5. Synthesis of Monohydroxyflavanone **4a**—**d**



Substrate	OMOM	HCl–MeOH (%)	Flavanone	OH	Yield (%)	Chalcone	OH	Yield (%)
3a	3′	25.8	**4a**	8	74.1	**3a′**	3′	—
3b	5′	25.8	**4b**	7	71.4	**3b′**	4′	27.9
3c	5′	17.8	**4c**	6	91.1	**3c′**	5′	8.8
3d	6′	17.8	**4d**	5	82.2	**3d′**	6′	—

Table 6. Synthesis of Monohydroxyflavanone **4e**—**g**



Substrate	OMOM	Flavanone	OH	Yield (%)	Chalcone	OH	Yield (%)
3e	2	**4e**	2′	66.0	**3e′**	2	24.8
3f	3	**4f**	3′	81.8	**3f′**	3	14.0
3g	4	**4g**	4′	63.2	**3g′**	4	34.3

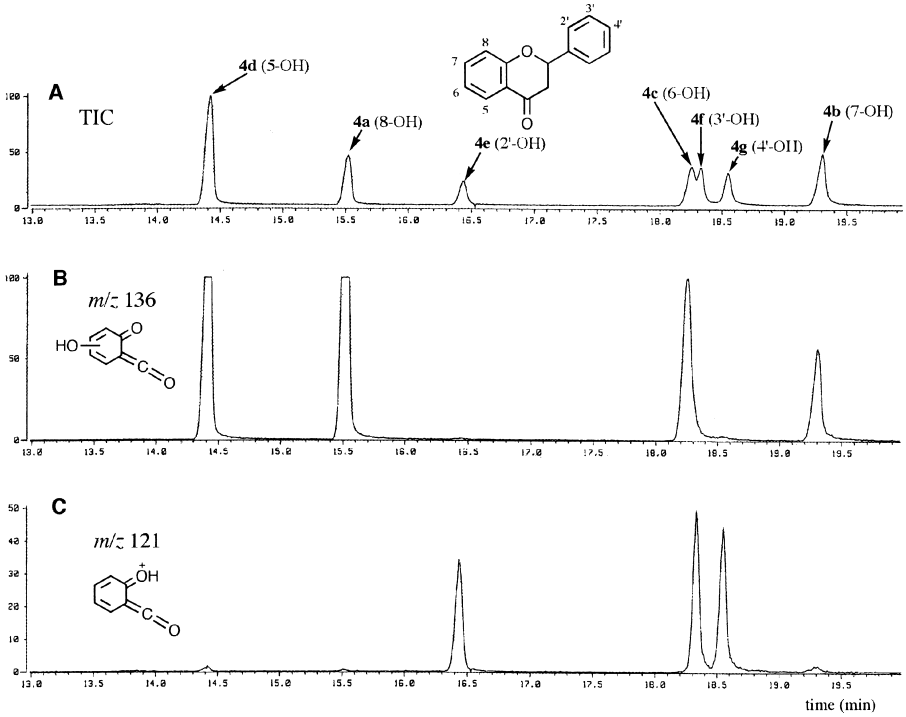


Fig. 1. Total Ion Chromatogram (TIC) and Mass Chromatogram of Monohydroxyflavanone **4a**—**g**

reaction with acid (data not shown.)

Although in this report, we have only shown that monohydroxyflavanones can be readily synthesized, we expect that similar procedures can be used to synthesize flavanones with multiple hydroxyl groups added to rings A and B.

Using GC in conjunction with a DB-5 capillary column, the seven monohydroxyflavanones, **4a**—**g**, were separated (Fig. 1A). Monohydroxyflavanones **4a**—**d**, which are hydroxylated at the A-ring, have a characteristic fragmentation ion with a *m/z* of 136 (Fig. 1B). For the B-ring monohydroxyflavanones, **4e**—**g**, the characteristic fragmentation ion *m/z* is 121 (Fig. 1C). We propose the structure of these fragmentation ions according to Van de Sande[26] and Nikolic.[19] Monohydroxyflavanone **4a**—**g** have also characteristic fragmentation ions with a *m/z* of 163 or 147. These fragmentation ions are due to the loss of phenyl radical from a flavanone skeleton. Therefore, observation of these fragmentation ions during characterization of flavanone metabolites or oxidation products will identify which ring is monohydroxylated. Retention times, in conjunction with mass fragmentation patterns, can be used to identify the flavanone hydroxylation site.

In conclusion, ours is the first report of a facile and efficient procedure for the preparative synthesis of flavanones monohydroxylated at any one specific site in the A or B ring and is also the first report of a GC-MS protocol that identifies all seven isomers.

Experimental

General Melting points were determined using a Yanagimoto micro melting apparatus and are reported as uncorrected values. IR spectra were acquired using a Hitachi 260-30 spectrometer.

^{1}H-NMR spectra were acquired using a Varian VXR-300 (300 MHz) or a Varian XL-400 (400 MHz) spectrometer. All NMR samples were dissolved in $CDCl_3$. Mass spectra were obtained using a JEOL-JMX-DX 505 H mass spectrometer (for low-resolution mass spectrometry) and a JEOL-JMS-AX505 HA mass spectrometer (for high-resolution mass spectrometry). For thin-layer chromatography, samples were chromatographed through silica gel 60 PF254 (Merck) and were detected in the presence of ultraviolet (u.v.) light at 254 and 365 nm. Column chromatography was performed using Wakogel C-200 silica gel. Elemental analyses were performed using a Yanaco CHN Coder MT-5. A 25.8% HCl–MeOH solution was prepared by bubbling HCl gas into MeOH. Other HCl–MeOH solutions were prepared by diluting this 25.8% HCl solution with MeOH.

Analysis by GC-MS GC-MS analysis was performed using a model JMS-AX505 HA mass spectrometer from Jeol (Tokyo, Japan), a gas chromatograph (5890 series II; Hewllet Packard, Palo Alto, CA, U.S.A.), a 30-m DB-5 capillary column (i.d., 0.25 mm; J & W Scientific, Palo Alto, CA, U.S.A.). The injection temperature, interface temperature, ionizing voltage, ionizing current, accelerating voltage, temperature of the ion source and the flow rate of helium were set at 250 °C, 280 °C, 70 eV, 300 mA, 3 kV, 250 °C and 30 ml/min respectively. The initial temperature of the column was 100 °C. After 1 min, it was raised at 15 °C/min to 220 °C. After 4 min at 220 °C, it was raised at 5 °C/min to 250 °C and then the temperature was maintained at 250 °C. One mM acetone solution (0.5 μl) of all monohydroxyflavanones mixture was subjected to analysis by GC-MS.

2′-Hydroxy-3′-methoxyacetophenone (6a) and 2′,3′-Dimethoxyacetophenone (6b) 2,3-Dimethoxybenzonitrile (**5**, 5.1041 g, 31.3 mmol) dissolved in anhydrous ether (30 ml) was added to a solution of methylmagnesium iodide, which had been prepared from magnesium (0.754 g), methyl iodide (4.0 ml, 64.3 mmol), and ether (20 ml). After 16 h reaction time, the mixture was refluxed for 2 h, decomposed by addition of dilute acetic acid, and extracted with CH_2Cl_2 (250 ml). The organic layer was isolated, washed with 70 ml saturated Na_2CO_3, dried over Na_2SO_4, and evaporated. The residue was a pale yellowish crystalline substance that was chromatographed over a column of silica gel in *n*-hexane and acetone (10 : 1) to purify **6a** (2.9747 g, yield 57.2%) and **6b** (0.8129 g, yield 14.4%).

2′-Hydroxy-3′-methoxyacetophenone (**6a**): Pale yellowish needles, *Rf*=0.24 (*n*-hexane : acetone=5 : 1); mp: 54 °C (EtOH) (lit.[27] 50—53.1 °C); IR ($CHCl_3$) cm^{-1}: 1638 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 12.558 (1H, d, *J*=0.5 Hz, 2-OH), 7.336 (1H, dd, *J*=1.5, 8.0 Hz, 6-H), 7.054 (1H, ddd, *J*=0.5, 8.0, 1.5 Hz, 4-H), 6.840 (1H, t, *J*=8.0 Hz, 5-H), 3.898 (3H, s, 3-OCH_3), 2.632 (3H, s, $COCH_3$); HR-EI-MS *m/z*: 166.0628 (Calcd for $C_9H_{10}O_3$: 166.0630); *Anal.* Calcd for $C_9H_{10}O_3$: C, 65.05, H, 6.07; Found: C, 64.95, H, 6.12.

2′,3′-Dimethoxyacetophenone (**6b**): Pale yellowish oil, *Rf*=0.31 (*n*-hexane : acetone=5 : 1); IR ($CHCl_3$) cm^{-1}: 1678 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.219 (1H, dd, *J*=2.2, 7.2 Hz, 6-H), 7.085 (1H, dd,

J=8.0, 7.2 Hz, 5-H), 7.043 (1H, dd, J=8.0, 2.2 Hz, 7-H), 3.905 (3H, s, 2-OCH_3), 3.892 (3H, s, 3-OCH_3), 2.627 (3H, s, $COCH_3$); HR-EI-MS *m/z*: 180.0787 (Calcd for $C_{10}H_{12}O_3$: 180.0786); *Anal.* Calcd for $C_{10}H_{12}O_3$: C, 66.65, H, 6.71; Found: C, 66.62, H, 6.85.

2′,3′-Dihydroxyacetophenone (7a) Under argon, 1 ml of 1.0 M BBr_3, dissolved in $(CHCl_2)_2$, was added to 2 ml 2′-hydroxy-3′-methoxyacetophenone (**6a**, 99.8 mg, 0.6 mmol) dissolved in CH_2Cl_2. The mixture was stirred at room temperature for 2.0 h. Water was added and the mixture was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (silica gel, *n*-hexane : acetone=10 : 1) to give **7a** (74.5 mg, yield 81.5%) as a pale yellowish prisms. *Rf*=0.21 (*n*-hexane : acetone=5 : 1); mp: 100—102 °C (benzene) (lit.[24] 97 °C); IR ($CHCl_3$) cm^{-1}: 1640 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 12.461 (1H, d, J=0.5 Hz, 2-OH), 7.287 (1H, dd, J=1.5, 8.0 Hz, 6-H), 7.132 (1H, ddd, J=0.5, 1.5, 8.0 Hz, 4-H), 6.823 (1H, dd, J=8.0 Hz, 5-H), 5.752 (1H, br, 3-OH), 2.632 (3H, s, $COCH_3$); HR-EI-MS *m/z*: 152.0471 (Calcd for $C_8H_8O_3$: 152.0473); *Anal.* Calcd for $C_8H_8O_3$: C, 63.15, H, 5.30; Found: C, 63.24, H, 5.31.

2′-Hydroxy-3′-methoxymethoxyacetophenone (1a), 3′-Hydroxy-2′-methoxymethoxyacetophenone (1a′), and 2′,3′-Dimethoxymethoxyacetophenone (1a″) To a solution of 2′,3′-dihydroxyacetophenone (**7a**, 0.9590 g, 6.3 mmol) and DIPEA (2.7 ml, 15.5 mmol) in CH_2Cl_2 (20 ml), MOMCl (0.7618 g, 9.5 mmol) was added. The mixture was stirred at room temperature for 1 h. Water was then added and the mixture was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, and evaporated. The residue was chromatographed over a column of silica gel in a 5 : 1 *n*-hexane : acetone mixture to separate **1a** (0.8909 g, yield 72.4%), **1a′** (0.0871 g, yield 7.0%), and **1a″** (0.2425 g, yield 16.0%).

2′-Hydroxy-3′-methoxymethoxyacetophenone (**1a**): Pale yellowish oil, *Rf*=0.28 (*n*-hexane : acetone=5 : 1); IR ($CHCl_3$) cm^{-1}: 1643 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 12.529 (1H, d, J=0.5 Hz, 2′-OH), 7.425 (1H, dd, J=1.5, 8.0 Hz, 6′-H), 7.340 (1H, ddd, J=0.5, 1.5, 8.0 Hz, 4′-H), 6.828 (1H, t, J=8.0 Hz, 5′-H), 5.245 (2H, s, 3′-OCH_2OCH_3), 3.527 (3H, s, 3′-OCH_2OCH_3), 2.635 (3H, s, $COCH_3$); HR-EI-MS *m/z*: 196.0744 (Calcd for $C_{10}H_{12}O_4$: 196.0736); *Anal.* Calcd for $C_{10}H_{12}O_4$: C, 61.22, H, 6.16; Found: C, 61.24, H, 6.21.

3′-Hydroxy-2′-methoxymethoxyacetophenone (**1a′**): Pale yellowish oil, *Rf*=0.10 (*n*-hexane : acetone=5 : 1); IR ($CHCl_3$) cm^{-1}: 1681 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.440 (1H, br, 3′-OH), 7.150—7.040 (3H, m, 4′,5′,6′-Hs), 5.075 (2H, s, 2′-OCH_2OCH_3), 3.625 (3H, s, 2′-OCH_2OCH_3), 2.558 (3H, s, $COCH_3$); HR-EI-MS *m/z*: 196.0737 (Calcd for $C_{10}H_{12}O_4$: 196.0736); *Anal.* Calcd for $C_{10}H_{12}O_4$: C, 61.22, H, 6.16; Found: C, 61.15, H, 6.25.

2′,3′-Dimethoxymethoxyacetophenone (**1a″**): Pale yellowish oil, *Rf*=0.21 (*n*-hexane : acetone=5 : 1); IR ($CHCl_3$) cm^{-1}: 1685 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.273 (1H, dd, J=2.0, 8.0 Hz, 4′-H), 7.208 (1H, dd, J=2.0, 8.0 Hz, 6′-H), 7.077 (1H, t, J=8.0 Hz, 5′-H), 5.210 (2H, s, 3′-OCH_2OCH_3), 5.148 (2H, s, 2′-OCH_2OCH_3), 3.510 (3H, s, 3′-OCH_2OCH_3), 3.500 (3H, s, 2′-OCH_2OCH_3), 2.637 (3H, s, $COCH_3$); HR-EI-MS *m/z*: 240.1005 (Calcd for $C_{14}H_{18}O_5$: 240.0998); *Anal.* Calcd for $C_{10}H_{12}O_4$: C, 59.99, H, 6.71; Found: C, 60.18, H, 6.67.

2′-Hydroxy-4′-methoxymethoxyacetophenone (1b), 2′-Hydroxy-5′-methoxymethoxyacetophenone (1c), and 2′-Hydroxy-6′-methoxymethoxyacetophenone (1d) Compounds **1b**—**d** were synthesized starting with the corresponding dihydroxyacetophenones **7b**—**d** using procedures similar to that described for the preparation of **1a**.

2′-Hydroxy-4′-methoxymethoxyacetophenone (**1b**): Pale yellowish oil, *Rf*=0.20 (benzene); IR ($CHCl_3$) cm^{-1}: 1632 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 12.605 (1H, s, 2′-OH), 7.648 (1H, d, J=9.0 Hz, 6′-H), 6.593 (1H, d, J=2.5 Hz, 3′-H), 6.547 (1H, dd, J=2.5, 9.0 Hz, 5′-H), 5.205 (2H, s, 4′-OCH_2OCH_3), 3.475 (3H, s, 4′-OCH_2OCH_3), 2.565 (3H, s, $COCH_3$); HR-EI-MS *m/z*: 196.0743 (Calcd for $C_{10}H_{12}O_4$: 196.0736), *Anal.* Calcd for $C_{10}H_{12}O_4$: C, 61.22, H, 6.16; Found: C, 61.36, H, 6.25.

2′-Hydroxy-5′-methoxymethoxyacetophenone (**1c**): The reaction mixture containing **7c** was refluxed for 1 h. Pale yellowish oil, *Rf*=0.23 (benzene); IR ($CHCl_3$) cm^{-1}: 1645 (C=O), ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 11.910 (1H, s, 2′-OH), 7.390 (1H, d, J=3.0 Hz, 6′-H), 7.224 (1H, dd, J=3.0, 9.0 Hz, 4′-H), 6.917 (1H, d, J=3.0 Hz, 3′-H), 5.125 (2H, s, 5′-OCH_2OCH_3), 3.497 (3H, s, 5′-OCH_2OCH_3), 2.614 (3H, s, $COCH_3$); HR-EI-MS *m/z*: 196.0745 (Calcd for $C_{10}H_{12}O_4$: 196.0736); *Anal.* Calcd for $C_{10}H_{12}O_4$: C, 61.22, H, 6.16; Found: C, 61.36, H, 6.36.

2′-Hydroxy-6′-methoxymethoxyacetophenone (**1d**): Pale yellowish oil, *Rf*=0.31 (benzene); IR ($CHCl_3$) cm^{-1}: 1625 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 13.097 (1H, s, 2′-OH), 7.325 (1H, dd, J=8.0, 8.5 Hz, 4′-H), 6.615 (1H, dd, J=1.0, 8.5 Hz, 3′-H), 6.595 (1H, dd, J=1.0, 8.0 Hz, 5′-H), 5.283 (2H, s, 6′-OCH_2OCH_3), 3.525 (3H, s, 6′-OCH_2OCH_3), 2.718 (3H, s, $COCH_3$); HR-EI-MS *m/z*: 196.0725 (Calcd for $C_{10}H_{12}O_4$: 196.0736), *Anal.* Calcd for $C_{10}H_{12}O_4$: C, 61.22, H, 6.16; Found: C, 61.02, H, 6.24.

2-Methoxymethoxybenzaldehyde (2a) To a solution of 2-hydroxybenzaldehyde (**8a**, 4.0084 g, 32.8 mmol) and DIPEA (17.1 ml, 98.2 mmol) in CH_2Cl_2 (45 ml), MOMCl (4.0678 g, 50.5 mmol) was added. The mixture was stirred at room temperature for 2 h. Water was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and evaporated. The residue was chromatographed over a column of silica gel in benzene to purify **2a** (5.2848 g, 96.9%) as a pale brownish oil, *Rf*=0.29 (benzene); IR ($CHCl_3$) cm^{-1}: 1685 (CHO); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.058 (1H, d, J=1.0 Hz, CHO), 7.843 (1H, dd, J=2.0, 8.0 Hz, 6-H), 7.531 (1H, ddd, J=2.0, 7.5, 8.5 Hz, 4-H), 7.218 (1H, dd, J=1.0, 8.5 Hz, 3-H), 7.082 (1H, ddd, J=1.0, 7.5, 8.0 Hz, 5-H), 5.305 (2H, s, 2-OCH_2OCH_3), 3.524 (3H, s, 2-OCH_2OCH_3); HR-EI-MS *m/z*: 166.0618 (Calcd for $C_9H_{10}O_3$: 166.0630); *Anal.* Calcd for $C_9H_{10}O_3$: C, 65.05, H, 6.07; Found: C, 64.75, H, 6.00.

3-Methoxymethoxybenzaldehyde (2b) and 4-Methoxymethoxybenzaldehyde (2c) Compounds **2b** and **2c** were synthesized starting with the corresponding monohydroxybenzaldehydes **8b** and **8c** using procedures similar to that described for preparation of **2a**.

3-Methoxymethoxybenzaldehyde (**2b**): Colorless oil, *Rf*=0.33 (benzene); IR ($CHCl_3$) cm^{-1}: 1698 (CHO); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.975 (1H, s, CHO), 7.547 (1H, ddd, J=0.5, 1.0, 2.5 Hz, 2-H), 7.527 (1H, dt, J=1.0, 7.5 Hz, 6-H), 7.453 (1H, ddd, J=0.5, 7.5, 8.0 Hz, 5-H), 7.298 (1H, ddd, J=1.0, 2.5, 8.0 Hz, 4-H), 5.230 (2H, s, 3-OCH_2OCH_3), 3.489 (3H, s, 3-OCH_2OCH_3); HR-EI-MS *m/z*: 166.0616 (Calcd for $C_9H_{10}O_3$: 166.0630), *Anal.* Calcd for $C_9H_{10}O_3$: C, 65.05, H, 6.07; Found: C, 65.08, H, 6.18.

4-Methoxymethoxybenzaldehyde (**2c**): Colorless oil, *Rf*=0.22 (benzene); IR ($CHCl_3$) cm^{-1}: 1690 (CHO); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.900 (1H, s , CHO), 7.835 (2H, d, J=9.0 Hz, 2,6-Hs), 7.145 (2H, d, J=9.0 Hz, 3,5-Hs), 5.252 (2H, s, 4-OCH_2OCH_3), 3.490 (3H, s, 4-OCH_2OCH_3); HR-EI-MS *m/z*: 166.0629 (Calcd for $C_9H_{10}O_3$: 166.0630), *Anal.* Calcd for $C_9H_{10}O_3$: C, 65.05, H, 6.07; Found: C, 64.95, H, 6.05.

2′-Hydroxy-3′-methoxymethoxychalcone (3a) A mixture of 2′-hydroxy-3′-methoxymethoxyacetophenone (**1a**, 0.5761 g, 2.9 mmol), benzaldehyde (**2**, 0.4676 g, 4.4 mmol), and KOH (0.8431 g, 15.0 mmol) in EtOH (10 ml) was stirred at room temperature for 22.5 h. Then the mixture was neutralized by addition of 10% AcOH in EtOH. This mixture was evaporated and the residue was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (benzene : ethyl acetate=20 : 1) to give **3a** (0.7541 g, yield 91.0%) as an orange prisms. *Rf*=0.34 (benzene : ethyl acetate=20 : 1); mp: 76.5—77 °C (EtOH); IR ($CHCl_3$) cm^{-1}: 1638 (C=O), ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 13.115 (1H, d, J=0.5 Hz, 2′-OH), 7.940 (1H, d, J=15.5 Hz, β-H), 7.720—7.640 (2H, m, 2,6-Hs), 7.650 (1H, d, J=15.5 Hz, α-H), 7.620 (1H, dd, J=1.0, 8.0 Hz, 6′-H), 7.480—7.410 (3H, m, 3,4,5-Hs), 7.380 (1H, ddd, J=0.5, 1.0, 8.0 Hz, 4′-H), 6.853 (1H, dd, J=8.0 Hz, 5′-H), 5.280 (2H, s, 3′-OCH_2OCH_3), 3.550 (3H, s, 3′-OCH_2OCH_3); HR-EI-MS *m/z*: 284.1052 (Calcd for $C_{17}H_{16}O_4$: 284.1049); *Anal.* Calcd for $C_{17}H_{16}O_4$: C, 71.82, H, 5.67; Found: C, 71.94, H, 5.75.

2′-Hydroxy-4′-methoxymethoxychalcone (3b), 2′-Hydroxy-5′-methoxymethoxychalcone (3c) and 2′-Hydroxy-6′-methoxymethoxychalcone (3d) Compounds (**3b**—**d**) were prepared from the corresponding monomethoxymethoxylated derivatives of 2′-hydroxyacetophenone (**1b**—**d**) using procedures similar to that described for preparation of **3a**.

2′-Hydroxy-4′-methoxymethoxychalcone (**3b**): Yellow needles, *Rf*=0.36 (benzene); mp: 85.5 °C (EtOH); IR ($CHCl_3$) cm^{-1}: 1637 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 13.262 (1H, s, 2′-OH), 7.905 (1H, d, J=15.5 Hz, β-H), 7.855 (1H, d, J=9.0 Hz, 6′-H), 7.685—7.090 (2H, m, 2,6-Hs), 7.585 (1H, d, J=15.5 Hz, α-H), 7.465—7.400 (3H, m, 3,4,5-Hs), 6.653 (1H, d, J=2.5 Hz, 3′-H), 6.598 (1H, dd, J=2.5, 9.0 Hz, 5′-H), 5.231 (2H, s, 4′-OCH_2OCH_3), 3.495 (3H, s, 4′-OCH_2OCH_3); HR-EI-MS *m/z*: 284.1054 (Calcd for $C_{17}H_{16}O_4$: 284.1049); *Anal.* Calcd for $C_{17}H_{16}O_4$: C, 71.82, H, 5.67; Found: C, 71.62, H, 5.71.

2′-Hydroxy-5′-methoxymethoxychalcone (**3c**): Orange needles, *Rf*=0.41 (benzene); mp: 67.5 °C (EtOH); IR ($CHCl_3$) cm^{-1}: 1640 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 12.450 (1H, s, 2′-OH), 7.933 (1H, d, J=15.5 Hz, β-H), 7.700—7.650 (2H, m, 2,6-Hs), 7.598 (1H, d, J=15.5 Hz, α-H), 7.578 (1H, d, J=3.0 Hz, 6′-H), 7.480—7.430 (3H, m, 3,4,5-Hs), 7.280 (1H, dd, J=3.0, 9.0 Hz, 4′-H), 6.975 (1H, d, J=9.0 Hz, 3′-H), 5.167 (2H, s, 5′-OCH_2OCH_3); 3.526 (3H, s, 5′-OCH_2OCH_3); HR-EI-MS *m/z*: 284.1048 (Calcd for $C_{17}H_{16}O_4$: 284.1049); *Anal.* Calcd for $C_{17}H_{16}O_4$: C, 71.82, H,

5.67; Found: C, 71.86, H, 5.77.

6-Methoxymethoxyflavanone (**4c′**): Colorless needles, *Rf*=0.35 (benzene); mp: 96—97 °C (EtOH), IR ($CHCl_3$) cm^{-1}: 1683 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.558 (1H, d, *J*=3.0 Hz, 5-H), 7.510—7.360 (5H, m, 2′,3′,4′,5′,6′-Hs), 7.223 (1H, dd, *J*=3.0, 9.0 Hz, 7-H), 7.006 (1H, d, *J*=9.0 Hz, 8-H), 5.445 (1H, dd, *J*=3.0, 13.5 Hz, 2-H), 5.162 (2H, s, 6′-OCH_2OCH_3), 3.487 (3H, s, 6′-OCH_2OCH_3), 3.068 (1H, dd, *J*=13.5, 17.0 Hz, 3ax-H), 2.883 (1H, dd, *J*=3.0, 17.0 Hz, 3eq-H); HR-EI-MS *m/z*: 284.1050 (Calcd for $C_{17}H_{16}O_4$: 284.1049), *Anal.* Calcd for $C_{17}H_{16}O_4$: C, 71.82, H, 5.67; Found: C, 71.77, H, 5.64.

2′-Hydroxy-6′-methoxymethoxychalcone (**3d**): Orange needles, *Rf*=0.34 (benzene); mp: 52.5—53 °C (EtOH), IR ($CHCl_3$) cm^{-1}: 1633 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 12.863 (1H, s, 2′-OH), 7.907 (1H, d, *J*=15.5 Hz, α-H), 7.815 (1H, d, *J*=15.5 Hz, β-H), 7.650—7.590 (2H, m, 2,6-Hs), 7.460—7.390 (3H, m, 3,4,5-Hs), 7.348 (1H, dd, *J*=8.0, 8.5 Hz, 4′-H), 6.672 (1H, dd, *J*=1.0, 8.0 Hz, 3′-H), 6.613 (1H, dd, *J*=1.0, 8.5 Hz, 5′-H), 5.310 (2H, s, 6′-OCH_2OCH_3), 3.535 (3H, s, 6′-OCH_2OCH_3); HR-EI-MS *m/z*: 284.1039 (Calcd for $C_{17}H_{16}O_4$: 284.1049), *Anal.* Calcd for $C_{17}H_{16}O_4$: C, 71.82, H, 5.67; Found: C, 71.85, H, 5.83.

2′-Hydroxy-2-methoxymethoxychalcone (3e) A mixture of 2-methoxymethoxybenzaldehyde (**2a**, 50.2 mg, 0.30 mmol), 2′-hydroxyacetophenone (**1**, 40.8 mg, 0.30 mmol), and KOH (168.9 mg, 3.0 mmol) in EtOH (5 ml) was stirred at room temperature for 23 h and then neutralized by the addition of 10% AcOH in EtOH. This mixture was evaporated and the residue was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (benzene : *n*-hexane=2 : 1) to give **3e** (69.2 mg, yield 81.2%) as a yellow oil. *Rf*=0.31 (benzene : hexane=2 : 1); IR ($CHCl_3$) cm^{-1}: 1632 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.910 (1H, s, 2′-OH), 8.300 (1H, d, *J*=15.5 Hz, β-H), 7.930 (1H, dd, *J*=1.5, 8.0 Hz, 6′-H), 7.752 (1H, d, *J*=15.5 Hz, α-H), 7.696 (1H, dd, *J*=2.0, 7.5 Hz, 6-H), 7.501 (1H, ddd, *J*=1.5, 7.5, 8.0 Hz, 4′-H), 7.388 (1H, ddd, *J*=2.0, 7.5, 8.0 Hz, 4-H), 7.202 (1H, dd, *J*=1.0, 8.0 Hz, 3-H), 7.073 (1H, dt, *J*=1.0, 7.5 Hz, 5-H), 7.035 (1H, dd, *J*=1.0, 8.0 Hz, 3′-H), 6.950 (1H, ddd, *J*=1.0, 7.5, 8.0 Hz, 5′-H), 5.313 (2H, s, 2-OCH_2OCH_3), 3.530 (3H, s, 2-OCH_2OCH_3); HR-EI-MS *m/z*: 284.1044 (Calcd for $C_{17}H_{16}O_4$: 284.1049), *Anal.* Calcd for $C_{17}H_{16}O_4$: C, 71.82, H, 5.67; Found: C, 71.70, H, 5.74.

2′-Hydroxy-3-methoxymethoxychalcone (3f) and 2′-Hydroxy-4-methoxymethoxychalcone (3g) Compounds **3f** and **3g** were prepared starting with the corresponding monohydroxybenzaldehydes (**2b**, **c**) using procedures similar to that described for preparation of **3e**.

2′-Hydroxy-3-methoxymethoxychalcone (**3f**): Yellow needles, *Rf*=0.39 (benzene); mp: 78—78.5 °C (EtOH); IR ($CHCl_3$) cm^{-1}: 1639 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.794 (1H, s, 2′-OH), 7.928 (1H, dd, *J*=1.5, 8.0 Hz, 6′-H), 7.888 (1H, d, *J*=15.5 Hz, β-H), 7.640 (1H, d, *J*=15.5 Hz, α-H), 7.510 (1H, ddd, *J*=1.5, 7.5, 8.0 Hz, 4′-H), 7.362 (1H, dd, *J*=7.5, 8.5 Hz, 5-H), 7.335 (1H, dd, *J*=1.5, 2.5 Hz, 2-H), 7.315 (1H, ddd, *J*=1.0, 1.5, 7.5 Hz, 6-H), 7.128 (1H, ddd, *J*=1.0, 2.5, 8.5 Hz, 4-H), 7.036 (1H, dd, *J*=1.0, 8.0 Hz, 3′-H), 6.956 (1H, ddd, *J*=1.0, 7.5, 8.0 Hz, 5′-H), 5.234 (2H, s, 3-OCH_2OCH_3), 3.514 (3H, s, 3-OCH_2OCH_3); HR-EI-MS *m/z*: 284.1049 (Calcd for $C_{17}H_{16}O_4$: 284.1049), *Anal.* Calcd for $C_{17}H_{16}O_4$: C, 71.82, H, 5.67; Found: C, 71.65, H, 5.79.

2′-Hydroxy-4-methoxymethoxychalcone (**3g**): Yellow oil, *Rf*=0.36 (benzene); IR ($CHCl_3$) cm^{-1}: 1640 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.906 (1H, s, 2′-OH), 7.924 (1H, dd, *J*=1.5, 8.0 Hz, 6′-H), 7.904 (1H, d, *J*=15.5 Hz, β-H), 7.628 (2H, d, *J*=9.0 Hz, 2,6-Hs), 7.558 (1H, d, *J*=15.5 Hz, α-H), 7.495 (1H, ddd, *J*=1.5, 7.5, 8.5 Hz, 4′-H), 7.094 (2H, d, *J*=9.0 Hz, 3,5-Hs), 7.027 (1H, dd, *J*=1.0, 8.5 Hz, 3′-H), 6.944 (1H, ddd, *J*=1.0, 7.5, 8.0 Hz, 5′-H), 5.233 (2H, s, 4-OCH_2OCH_3), 3.497 (3H, s, 4-OCH_2OCH_3); HR-EI-MS *m/z*: 284.1040 (Calcd for $C_{17}H_{16}O_4$: 284.1049), *Anal*. Calcd for $C_{17}H_{16}O_4$: C, 71.82, H, 5.67; Found: C, 71.70, H, 5.81.

8-Hydroxyflavanone (4a) To a solution of 2′-hydroxy-3′-methoxymethoxychalcone (**3a**, 55.6 mg, 0.20 mmol) in MeOH (2.0 ml), 25.8% HCl–MeOH (2.0 ml) was added. The mixture was stirred at 100 °C for 2.0 h, neutralized with a saturated $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was extracted with water, dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (silica gel, benzene : ethyl acetate=20 : 1) to give **4a** (34.8 mg, yield 74.1%). In the same manner, the residue was purified using preparative TLC (silica gel, *n*-hexane : acetone=5 : 1) to give a trace of 2′,3′-dihydroxychalcone **3a′**.

8-Hydroxyflavanone (**4a**): Colorless needles, *Rf*=0.08 (*n*-hexane : acetone=5 : 1); mp: 204 °C (EtOH) (lit.[28] 192 °C); IR ($CHCl_3$) cm^{-1}: 1690 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.570—7.420 (5H, m, 2′,3′,4′,5′,6′-Hs); 7.485 (1H, dd, *J*=1.5, 8.0 Hz, 5-H), 7.163 (1H, dd, *J*=1.5, 8.0 Hz, 7-H), 6.973 (1H, t, *J*=8.0 Hz, 6-H), 5.585 (1H, s, 8-OH), 5.525 (1H, dd, *J*=3.0, 13.0 Hz, 2-H), 3.157 (1H, dd, *J*=13.0, 17.0 Hz, 3ax-H), 2.897 (1H, dd, *J*=3.0, 17.0 Hz, 3eq-H); HR-EI-MS *m/z*: 240.0805 (Calcd for $C_{15}H_{12}O_3$: 240.0786); *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.88, H, 5.15.

2′,3′-Dihydroxychalcone (**3a′**): Red needles, *Rf*=0.15 (*n*-hexane : acetone=5 : 1); mp: 155 °C (EtOH) (lit.[28] 151 °C); IR ($CHCl_3$) cm^{-1}: 1640 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.102 (1H, d, *J*=0.5 Hz, 2′-OH), 7.946 (1H, d, *J*=15.5 Hz, β-H), 7.710—7.650 (2H, m, 2,6-Hs), 7.650 (1H, d, *J*=15.5 Hz, α-H), 7.483 (1H, dd, *J*=1.5, 8.0 Hz, 6′-H), 7.480—7.425 (3H, m, 3,4,5-Hs), 7.169 (1H, ddd, *J*=0.5, 1.5, 8.0 Hz, 4′-H), 6.877 (1H, t, *J*=8.0 Hz, 5′-H), 5.801 (1H, s, 3′-OH); HR-EI-MS *m/z*: 240.0798 (Calcd for $C_{15}H_{12}O_3$: 240.0786), *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.89, H, 5.13.

7-Hydroxyflavanone (4b) To a solution of 2′-hydroxy-4′-methoxymethoxychalcone (**3b**, 50.2 mg, 0.18 mmol) in MeOH (1.0 ml), 25.8% HCl–MeOH (2.0 ml) was added. The mixture was stirred at 100 °C for 2.5 h, neutralized with a saturated $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was extracted with water, dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (silica gel, benzene : ethyl acetate=20 : 1) to give **4b** (30.3 mg, yield 71.4%) and 2′,4′-dihydroxychalcone (**3b′**, 11.5 mg, 27.9%).

7-Hydroxyflavanone (**4b**): Colorless needles, *Rf*=0.10 (benzene : ethyl acetate=20 : 1); mp: 194—197 °C (EtOH) (lit.[29] 189—190 °C); IR ($CHCl_3$) cm^{-1}: 1678 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.863 (1H, d, *J*=9.0 Hz, 5-H), 7.490—7.360 (5H, m, 2′,3′,4′,5′,6′-Hs), 6.554 (1H, dd, *J*=2.0, 9.0 Hz, 6-H), 6.474 (1H, d, *J*=2.0 Hz, 8-H), 5.950 (1H, br, 7-OH), 5.467 (1H, dd, *J*=3.0, 13.0 Hz, 2-H), 3.049 (1H, dd, *J*=13.0, 17.0 Hz, 3ax-H), 2.843 (1H, dd, *J*=3.0, 17.0 Hz, 3eq-H); HR-EI-MS *m/z*: 240.0824 (Calcd for $C_{15}H_{12}O_3$: 240.0786); *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 75.03, H, 5.19.

2′,4′-Dihydroxychalcone (**3b′**): Yellow needles, *Rf*=0.15 (benzene : ethyl acetate=20 : 1); mp: 148—152 °C (EtOH/*n*-hexane) (lit.[29] 150 °C); IR ($CHCl_3$) cm^{-1}: 1635 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.365 (1H, s, 2′-OH), 7.889 (1H, d, *J*=15.5 Hz, β-H), 7.840 (1H, d, *J*=9.0 Hz, 6′-H), 7.670—7.635 (2H, m, 2,6-Hs), 7.575 (1H, d, *J*=15.5 Hz, α-H), 7.455—7.415 (3H, m, 3,4,5-Hs), 6.440 (1H, dd, *J*=2.5, 9.0 Hz, 5′-H), 6.435 (1H, d, *J*=2.5 Hz, 3′-H), 5.800 (1H, br, 4′-OH); HR-EI-MS *m/z*: 240.0795 (Calcd for $C_{15}H_{12}O_3$: 240.0786), *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.81, H, 5.24.

6-Hydroxyflavanone (4c) 1) To a solution of 2′-hydroxy-5′-methoxymethoxychalcone (**3c**, 51.2 mg, 0.18 mmol) in MeOH (1.0 ml), 17.8% HCl–MeOH (2.0 ml) was added. The mixture was stirred at 100 °C for 1.0 h, neutralized with a saturated $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was extracted with water, dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (silica gel, benzene : ethyl acetate=10 : 1) to give **4c** (39.4 mg, 91.1%) and 2′,5′-dihydroxychalcone (**3c′**, 3.8 mg, 8.8%).

2) To a solution of 6-methoxymethoxyflavanone (**4c′**, 33.1 mg, 0.12 mmol) in MeOH (0.5 ml), 12.0% HCl–MeOH (0.5 ml) was added. The mixture was stirred at room temperature for 0.5 h, after which an additional 0.5 ml of 12.0% HCl–MeOH was added. The mixture was stirred at 50 °C for an additional 0.5 h, then neutralized with a saturated $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was extracted with water, dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (silica gel, benzene : ethyl acetate=10 : 1) to give **4c** (28.4 mg, yield 95.8%).

6-Hydroxyflavanone (**4c**): Pale yellowish needles, *Rf*=0.11 (benzene : ethyl acetate=20 : 1); mp: 225 °C (EtOH) (lit.[23] 220 °C); IR ($CHCl_3$) cm^{-1}: 1685 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.500—7.350 (5H, m, 2′,3′,4′,5′,6′-Hs), 7.328 (1H, d, *J*=3.0 Hz, 5-H), 7.075 (1H, dd, *J*=3.0, 9.0 Hz, 7-H), 6.975 (1H, d, *J*=9.0 Hz, 8-H), 5.436 (1H, dd, *J*=3.0, 13.5 Hz, 2-H), 4.982 (1H, br, 6-OH), 3.065 (1H, dd, *J*=13.5, 17.5 Hz, 3ax-H), 2.873 (1H, dd, *J*=3.0, 17.5 Hz, 3eq-H); HR-EI-MS *m/z*: 240.0809 (Calcd for $C_{15}H_{12}O_3$: 240.0786); *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 75.01, H, 5.18.

2′,5′-Dihydroxychalcone (**3c′**): Orange needles, *Rf*=0.18 (benzene : ethyl acetate=20 : 1); mp: 179—180 °C (EtOH) (lit.[23] 170 °C); IR ($CHCl_3$) cm^{-1}: 1642 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.346 (1H, s, 2′-OH), 7.915 (1H, d, *J*=15.5 Hz, β-H), 7.685—7.640 (2H, m, 2,6-Hs), 7.582 (1H, d, *J*=15.5 Hz, α-H), 7.460—7.430 (3H, m, 3,4,5-Hs), 7.390 (1H, d, *J*=3.0 Hz, 6′-H), 7.062 (1H, dd, *J*=3.0, 9.0 Hz, 4′-H), 6.937 (1H, d, *J*=9.0 Hz, 3′-H), 4.900 (1H, br, 5′-OH); HR-EI-MS *m/z*: 240.0800 (Calcd for $C_{15}H_{12}O_3$: 240.0786), *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.95, H,

5.27.

5-Hydroxyflavanone (4d) To a solution of 2′-hydroxy-6′-methoxymethoxychalcone (**3d**, 2.2771 g, 8.0 mmol) in MeOH (10.0 ml), 17.8% HCl–MeOH (2.0 ml) was added. The mixture was stirred at 100 °C for 3.5 h, neutralized with a saturated $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was extracted with water, dried over Na_2SO_4, and evaporated. The residue was chromatographed over a column of silica gel in benzene to purify **4d** (1.5825 g, yield 82.2%) as a colorless needles. *Rf*=0.55 (benzene); mp: 64.5—65 °C (EtOH) (lit.[30] 62—63 °C); IR ($CHCl_3$) cm^{-1}: 1640 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 11.682 (1H, s, 5-OH), 7.490—7.360 (5H, m, 2′,3′,4′,5′,6′-Hs), 7.390 (1H, dd, *J*=8.0, 8.5 Hz, 7-H), 6.547 (1H, dd, *J*=1.0, 8.5 Hz, 6-H), 6.517 (1H, dd, *J*=1.0, 8.0 Hz, 8-H), 5.465 (1H, dd, *J*=3.0, 13.0 Hz, 2-H), 3.151 (1H, dd, *J*=13.0, 17.5 Hz, 3ax-H), 2.898 (1H, dd, *J*=3.0, 17.5 Hz, 3eq-H); HR-EI-MS *m/z*: 240.0800 (Calcd for $C_{15}H_{12}O_3$: 240.0786); *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.79, H, 5.02.

2′-Hydroxyflavanone (4e) To a solution of 2′-hydroxy-2-methoxymethoxychalcone (**3e**, 68.7 mg, 0.24 mmol) in MeOH (4.0 ml), 17.8% HCl–MeOH (0.1 ml) was added. The mixture was stirred at 100 °C for 15 min, neutralized with a saturated $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was extracted with water, dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (silica gel, benzene) to give **4e** (38.3 mg, yield 66.0%) and 2,2′-dihydroxychalcone (**3e′**, 14.4 mg, yield 24.8%).

2′-Hydroxyflavanone (**4e**): Colorless needles, *Rf*=0.14 (benzene : ethyl acetate=20 : 1); mp: 170 °C (EtOH) (lit.[31] 165—165.5 °C); IR ($CHCl_3$) cm^{-1}: 1690 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.968 (1H, ddd, *J*=0.5, 2.0, 8.0 Hz, 5-H), 7.537 (1H, ddd, *J*=2.0, 7.0, 8.5 Hz, 7-H), 7.363 (1H, dd, *J*=1.5, 7.5 Hz, 6′-H), 7.270 (1H, ddd, *J*=1.5, 8.0, 8.5 Hz, 4′-H), 7.102 (1H, ddd, *J*=1.0, 7.0, 8.0 Hz, 6-H), 7.088 (1H, ddd, *J*=0.5, 1.0, 8.5 Hz, 8-H), 6.990 (1H, ddd, *J*=1.0, 7.5, 8.5 Hz, 5′-H), 6.918 (1H, dd, *J*=1.0, 8.0 Hz, 3′-H), 6.268 (1H, br, 2′-OH), 5.778 (1H, dd, *J*=3.0, 13.0 Hz, 2-H), 3.156 (1H, dd, *J*=13.0, 17.0 Hz, 3ax-H), 3.022 (1H, dd, *J*=3.0, 17.0 Hz, 3eq-H); HR-EI-MS *m/z*: 240.0793 (Calcd for $C_{15}H_{12}O_3$: 240.0786); *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.75, H, 5.14.

2,2′-Dihydroxychalcone (**3e′**): Yellow needles, *Rf*=0.23 (benzene : ethyl acetate=20 : 1); mp: 167 °C (EtOH) (lit.[31] 160—161 °C); IR ($CHCl_3$) cm^{-1}: 1632 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.914 (1H, s, 2′-OH), 8.188 (1H, d, *J*=15.5 Hz, β-H), 7.940 (1H, dd, *J*=1.5, 8.0 Hz, 6′-H), 7.855 (1H, d, *J*=15.5 Hz, α-H), 7.610 (1H, dd, *J*=1.5, 8.0 Hz, 6-H), 7.498 (1H, ddd, *J*=1.5, 7.5, 8.5 Hz, 4′-H), 7.298 (1H, ddd, *J*=1.5, 7.5, 8.0 Hz, 4-H), 7.032 (1H, dd, *J*=1.0, 8.0 Hz, 3′-H), 6.998 (1H, ddd, *J*=1.0, 7.5, 8.0 Hz, 5-H) 6.942 (1H, ddd, *J*=1.0, 7.5, 8.0 Hz, 5′-H), 6.857 (1H, dd, *J*=1.0, 8.0 Hz, 3-H), 5.810 (1H, br, 2-OH); HR-EI-MS *m/z*: 240.0793 (Calcd for $C_{15}H_{12}O_3$: 240.0786), *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 75.08, H, 5.20.

3′-Hydroxyflavanone (4f) To a solution of 2′-hydroxy-3-methoxymethoxychalcone (**3f**, 56.7 mg, 0.20 mmol) in MeOH (1.0 ml), 17.8% HCl–MeOH (2.0 ml) was added. The mixture was stirred at 100 °C for 1 h, neutralized with a saturated $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was extracted with water, dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (silica gel, benzene : ethyl acetate=20 : 1) to give **4f** (39.2 mg, yield 81.8%) and 2′,3-dihydroxychalcone (**3f′**, 6.7 mg, yield 14.0%).

3′-Hydroxyflavanone (**4f**): Colorless needles, *Rf*=0.14 (benzene : ethyl acetate=20 : 1); mp: 143—144 °C (EtOH) (lit.[32] 141—144 °C); IR ($CHCl_3$) cm^{-1}: 1685 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.933 (1H, dd, *J*=2.0, 8.0 Hz, 5-H), 7.519 (1H, ddd, *J*=2.0, 7.5, 8.5 Hz, 7-H), 7.298 (1H, dd, *J*=7.5, 8.0 Hz, 5′-H), 7.060 (1H, ddd, *J*=1.0, 7.5, 8.0 Hz, 6-H), 7.058 (1H, dd, *J*=1.0, 8.5 Hz, 8-H), 7.021 (1H, ddd, *J*=1.0, 2.0, 7.5 Hz, 6′-H), 6.993 (1H, dd, *J*=2.0, 2.5 Hz, 2′-H), 6.857 (1H, ddd, *J*=1.0, 2.5, 8.0 Hz, 4′-H), 5.444 (1H, dd, *J*=3.0, 13.0 Hz, 2-H), 5.360 (1H, br, 3′-OH), 3.065 (1H, dd, *J*=13.0, 17.0 Hz, 3ax-H), 2.898 (1H, dd, *J*=3.0, 17.0 Hz, 3eq-H); HR-EI-MS *m/z*: 240.0783 (Calcd for $C_{15}H_{12}O_3$: 240.0786); *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.86, H, 5.08.

2′,3-Dihydroxychalcone (**3f′**): Yellow needles, *Rf*=0.20 (benzene : ethyl acetate=20 : 1); mp: 172 °C (EtOH) (lit.[32] 165—166.5 °C); IR ($CHCl_3$) cm^{-1}: 1640 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.792 (1H, s, 2′-OH), 7.918 (1H, dd, *J*=1.5, 8.0 Hz, 6′-H), 7.860 (1H, d, *J*=15.5 Hz, β-H), 7.631 (1H, d, *J*=15.5 Hz, α-H), 7.510 (1H, ddd, *J*=1.5, 7.5, 8.5 Hz, 4′-H), 7.313 (1H, t, *J*=8.0 Hz, 5-H), 7.243 (1H, ddd, *J*=1.0, 2.0, 8.0 Hz, 6-H), 7.140 (1H, dd, *J*=2.0, 2.5 Hz, 2-H), 7.038 (1H, dd, *J*=1.0, 8.5 Hz, 3′-H), 6.953 (1H, ddd, *J*=1.0, 7.5, 8.0 Hz, 5′-H), 6.923 (1H, ddd, *J*=1.0, 2.5, 8.0 Hz, 4-H), 5.240 (1H, br, 3-OH); HR-EI-MS *m/z*: 240.0770 (Calcd for $C_{15}H_{12}O_3$: 240.0786); *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.80, H, 5.31.

4′-Hydroxyflavanone (4g) To a solution of 2′-hydroxy-4-methoxymethoxychalcone (**3g**, 50.0 mg, 0.18 mmol) in MeOH (1.0 ml), 17.8% HCl–MeOH (2.0 ml) was added. The mixture was stirred at 100 °C for 1.0 h, neutralized with a saturated $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was extracted with water, dried over Na_2SO_4, and evaporated. The residue was purified using preparative TLC (silica gel, benzene : ethyl acetate=20 : 1) to give **4g** (26.7 mg, yield 63.2%) and 2′,4-dihydroxychalcone (**3g′**, 14.5 mg, yield 34.3%).

4′-Hydroxyflavanone (**4g**): Colorless needles, *Rf*=0.12 (benzene : ethyl acetate=20 : 1); mp: 191 °C (EtOH) (lit.[33] 189—190 °C); IR ($CHCl_3$) cm^{-1}: 1690 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.932 (1H, ddd, *J*=0.5, 2.0, 8.0 Hz, 5-H), 7.506 (1H, ddd, *J*=2.0, 7.0, 8.5 Hz, 7-H), 7.368 (2H, d, *J*=9.0 Hz, 2′,6′-Hs), 7.050 (1H, ddd, *J*=1.0, 7.0, 8.0 Hz, 6-H), 7.038 (1H, ddd, *J*=0.5, 1.0, 8.5 Hz, 8-H), 6.895 (2H, d, *J*=9.0 Hz, 3′,5′-Hs), 5.424 (1H, dd, *J*=3.0, 13.5 Hz, 2-H), 5.120 (1H, br, 4′-OH), 3.100 (1H, dd, *J*=13.5, 17.5 Hz, 3ax-H), 2.864 (1H, dd, *J*=3.0, 17.5 Hz, 3eq-H); HR-EI-MS *m/z*: 240.0809 (Calcd for $C_{15}H_{12}O_3$: 240.0786); *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.72, H, 5.04.

2′,4-Dihydroxychalcone (**3g′**): Orange needles, *Rf*=0.17 (benzene : ethyl acetate=20 : 1); mp: 167 °C (EtOH) (lit.[33] 157—158 °C); IR ($CHCl_3$) cm^{-1}: 1637 (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 12.925 (1H, s, 2′-OH), 7.922 (1H, dd, *J*=1.5, 8.0 Hz, 6′-H), 7.894 (1H, d, *J*=15.5 Hz, β-H), 7.595 (2H, d, *J*=9.0 Hz, 2,6-Hs), 7.540 (1H, d, *J*=15.5 Hz, α-H), 7.494 (1H, ddd, *J*=1.5, 7.5, 8.5 Hz, 4′-H), 7.028 (1H, dd, *J*=1.0, 8.5 Hz, 3′-H), 6.942 (1H, ddd, *J*=1.0, 7.5, 8.0 Hz, 5′-H), 7.313 (1H, t, *J*=8.0 Hz, 5-H), 7.243 (1H, ddd, *J*=1.0, 2.0, 8.0 Hz, 6-H), 7.140 (1H, dd, *J*=2.0, 2.5 Hz, 2-H), 6.923 (1H, ddd, *J*=1.0, 2.5, 8.0 Hz, 4-H), 5.430 (1H, br, 4-OH); HR-EI-MS *m/z*: 240.0770 (Calcd for $C_{15}H_{12}O_3$: 240.0786); *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99, H, 5.03; Found: C, 74.80, H, 5.31.

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