Supporting Information

Pathways for vanillin production through alkaline aerobic oxidation of a phenolic lignin model compound, guaiacylglycerol-β-guaiacyl ether, in concentrated aqueous alkali.

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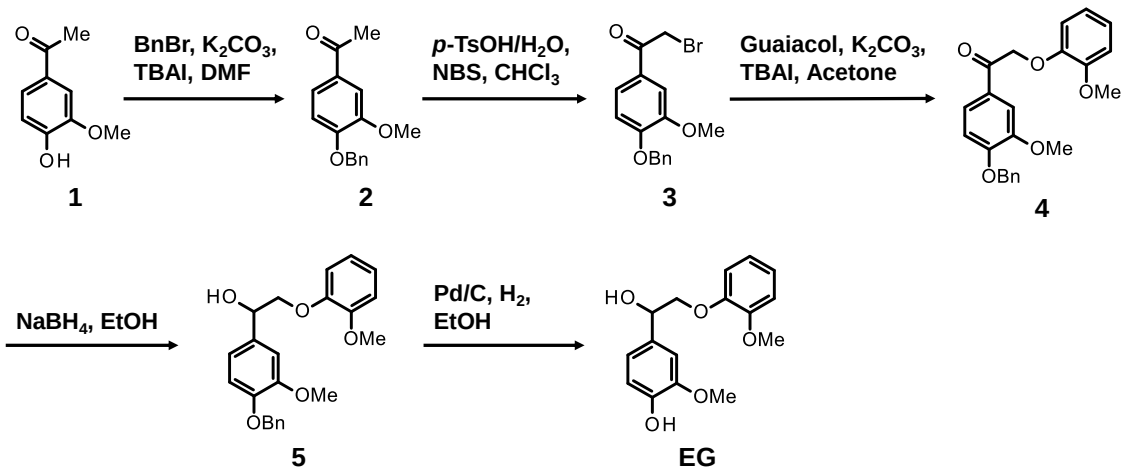
Contents

Detailed synthetic procedure of synthetic route for guaiacyl ethanediol-β-guaiacyl ether (EG).

General information: all synthetic reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. A mixture of *n*-hexane and ethyl acetate served as the eluent. Visualization of the spots was achieved using a 10 wt% phosphomolybdic acid/ethanol solution and ultraviolet light at a wavelength of 254 nm. Column chromatography was performed using CHROMATOREX PSQ100B silica gel from Fuji Silica Chemical, Ltd. The quantity of silica gel used was set at approximately 50 g for every 1 g of the crude mixture.

Nuclear magnetic resonance (NMR) spectra were acquired using a JNM-ECZ 400 S spectrometer operating at 400 MHz for ^{1}H measurements, at ambient temperature in $CDCl_3$. Chemical shifts (δ ppm) are reported relative to chloroform (δ_H = 7.26 ppm). The acquisition time for ^{1}H NMR was 2.73215 s, and the relaxation delay was set at 5 s. The number of ^{1}H NMR scans was 8. In the ^{1}H NMR spectra presented below, the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, ddd = double double doublet.

EG was synthesized following the synthetic route outlined in Scheme S1. The details of each step are presented below.



Scheme S1. Synthetic route for guaiacyl ethanediol-β-guaiacyl ether (**EG**).

1-[4-(Benzyloxy)-3-methoxyphenyl]ethanone (2). K_2CO_3 (3.31 g, 24 mmol), tetrabutylammonium iodide (TBAI) (738 mg, 2 mmol) and benzyl bromide (2.85 mL, 24

mmol) were added to a DMF solution (20 mL) of 1-(4-hydroxy-3-methoxyphenyl) ethanone (**1**) (3.32 g, 20 mmol) at room temperature. After stirred for 3 h at room temperature, the reaction mixture was extracted with ethyl acetate, washed with saturated $NaHCO_3$ aq. twice and brine, and dried over anhydrous Na_2SO_4. The solvent was concentrated to dryness *in vacuo*. The product mixture was recrystallized from ethanol to afford 1-[4-(benzyloxy)-3-methoxyphenyl]ethanone (**2**) as a white crystal (4.37 g, 85.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ =7.55-7.30 (m, 7H), 6.88 (d, *J* = 8.6 Hz, 1H), 5.22 (s, 2H), 3.93 (s, 3H), 2.53 (s, 3H).

1-[4-(Benzyloxy)-3-methoxyphenyl]-2-bromoethanone (3). *N*-Bromosuccinimide (NBS) (2.94 g, 16.5 mmol) was added to a chloroform solution (50 mL) of **2** (3.84 mg, 15 mmol) and *p*-toluenesulfonic acid monohydrate (*p*-TsOH/H_2O) (285 mg, 1.5 mmol). After stirring over night at room temperature, the reaction mixture was extracted with ethyl acetate, washed with brine and dried over anhydrous Na_2SO_4. The solvent was concentrated to dryness *in vacuo*. The product mixture was recrystallized from ethanol to afford 1-[4-(benzyloxy)-3-methoxyphenyl]-2-bromoethanone (**3**) as a white crystal (4.63 g, 92.1 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.56-7.51 (m, 2H), 7.44-7.29 (m, 5H), 6.90 (d, *J* = 8.2 Hz, 1H), 5.24 (s, 2H), 4.38 (s, 2H), 3.94 (s, 3H).

1-[4-(Benzyloxy)-3-methoxyphenyl]-2-(2-methoxyphenoxy)ethanone (4). K_2CO_3 (2.07 g, 15 mmol) and compound **3** (3.35 g, 10 mmol) were added to an acetone solution (50 mL) of Guaiacol (1.23 mL, 11 mmol) at room temperature. The reaction solution was stirred for 3 h at room temperature. The reaction mixture was filtered and concentrated *in vacuo*. The product mixture was recrystallized from ethanol to afford 1-[4-(benzyloxy)-3-methoxy-phenyl]-2-(2-methoxyphenoxy)ethanone (**4**) as a crystal (3.15 g, 83.2 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.61-7.55 (m, 2H), 7.44-7.28 (m, 5H), 6.97-6.79 (m, 5H), 5.27 (s, 2H), 5.23 (s, 2H), 3.93 (s, 3H), 3.86 (s, 3H).

1-[4-((Benzyloxy)-3-methoxyphenyl]-2-(2-methoxyphenoxy)ethanol (5). $NaBH_4$ (409 mg, 10.8 mmol) was added to an ethanol solution (50 mL) of Compound **4** (3.40 g, 9 mmol) at room temperature. The reaction solution was stirred for 1 h at room temperature. An excess of NH_4Cl was added (pH = 5-6) and the reaction mixture was stirred for additional 0.5 h. The reaction mixture was extracted with ethyl acetate, washed with brine and dried over anhydrous Na_2SO_4. The solvent was concentrated to dryness *in vacuo*. The product was purified on a silica gel column with ethyl acetate/*n*-hexane (1/1, v/v) to afford 1-[4-(benzyloxy)-3-methoxyphenyl]-2-(2-methoxyphenoxy)ethanol (**5**) as a white crystal

(3.16 g, 92.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44-7.27 (m, 5H), 7.03-7.84 (m, 7H), 5.14 (s, 2H), 5.02(ddd, *J* = 9.1, 2.8, 1.8, 1H), 4.14 (dd, *J* =10.1, 3.2, 1H), 3.95 (dd, *J* = 10.3, 10.1 Hz, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.43 (d, *J* = 1.8 Hz, 1H)

Guaiacyl ethanediol-β-guaiacyl ether (EG). 5 % Palladium carbon (1.70 g, 10 mol%) was added to an ethanol solution (30 mL) of **5** (3.04 g, 8 mmol). After the atmosphere of the reaction system was replaced with H_2, the solution was stirred for 3h at room temperature. The reaction mixture was then filtered and concentrated to dryness *in vacuo*. The product mixture was recrystallized from ethanol to afford guaiacyl ethanediol-β-guaiacyl ether(**EG**) as a white crystal (1.67 g, 71.9 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.02-6.85 (m, 7H), 5.61(s, 1H), 5.02 (ddd, *J* = 9.1, 2.7, 2.2 Hz, 1H), 4.18 (dd, J = 10.1, 2.8 Hz, 1H), 3.94 (dd, *J* = 10.1, 9.6 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.45 (d, *J* = 2.2 Hz, 1H).

Table S1. Yields (mol%) of vanillin, guaiacol, and **EE**, and recovery (%) of the starting material after degradation of **GG** at 120°C for 240 min in 4.0 mol/L NaOH aq. under O_2 introduced via different replacement methods (blowing and glove box).

Method for O_2 replacement [a)]	Yield (mol%)			Recovery of **GG** (%)
	Vanillin	Guaiacol	**EE**	
Blowing	38.7	64.8	2.4	6.1
Glove box	39.4	66.3	2.2	4.8

a) The detailed procedure for the "blowing" method is described in the experimental section of the text. For the "glove box" method, the PFA tube was placed inside an acrylic vacuum glove box (VG400PC, AS ONE Co.), and the glove box was depressurized to 150 hPa. Pure oxygen (99.9%) was then introduced into the glove box until the internal pressure reached atmospheric levels. This procedure was repeated twice.

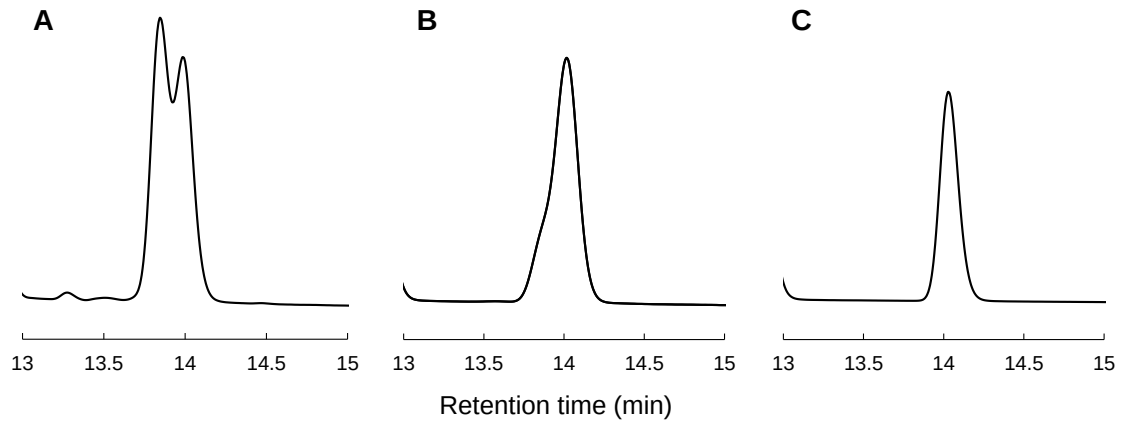


Figure S1. The vanillin peak in HPLC chromatogram of the reaction mixture obtained after the degradation of **GG**(A) and **EE**(B) in NaOD/D_2O at 120 °C for 240 min under O_2, as compared with the one of commercial vanillin (C). Detector: UV_{280nm}.

In the HPLC chromatogram of the reaction mixture obtained by alkaline aerobic oxidation of **GG** in NaOD/D_2O, two peaks were detected near the retention time of vanillin (13.8 min, 14.0 min, Figure S1A). Among these, the peak at 14.0 min matched the vanillin standard with its retention time. Additionally, when **GG** was oxidized under the same conditions using NaOH aq. as the reaction medium, only the peak of vanillin at 14.0 min was detected, and the peak at 13.8 min was not observed (see Figure 2 in the main text). These results strongly suggest that vanillin-D, resulting from the deuterated aldehyde group during the decomposition of **GG** in NaOD/D_2O (the discussion on the position of deuterium incorporation is referred to in **Aerobic oxidation of GG and EE in NaOD/D_2O** in the main text), and regular vanillin-H were distinctly separated at 13.8 min and 14.0 min, respectively.

Assuming that the peak at 13.8 min in Figure S1A corresponds to vanillin-D and the peak at 14.0 min corresponds to vanillin-H, the areas of each peak were determined by vertical integration. With the assumption of equal UV absorption for both, the molar ratio of vanillin-D to vanillin-H was calculated as 1.16. This value closely aligns with the $R_{D/H}$ values derived from the mass spectra shown in Table 2 (1.04 and 1.19). When **EE** was oxidatively decomposed in NaOD/D_2O, peaks corresponding to vanillin-D were identified as a shoulder (Figure S2B). The molar ratio of vanillin-D to vanillin-H in this case, determined using the same method as described above, was 0.15. This value shows a slight discrepancy with the $R_{D/H}$ values in Table 2 (0.25 and 0.27), likely due to limitations in accurately splitting the shoulder peak. Nevertheless, qualitatively, the formation of vanillin-D from **EE** is not as pronounced as in the case of **GG**.

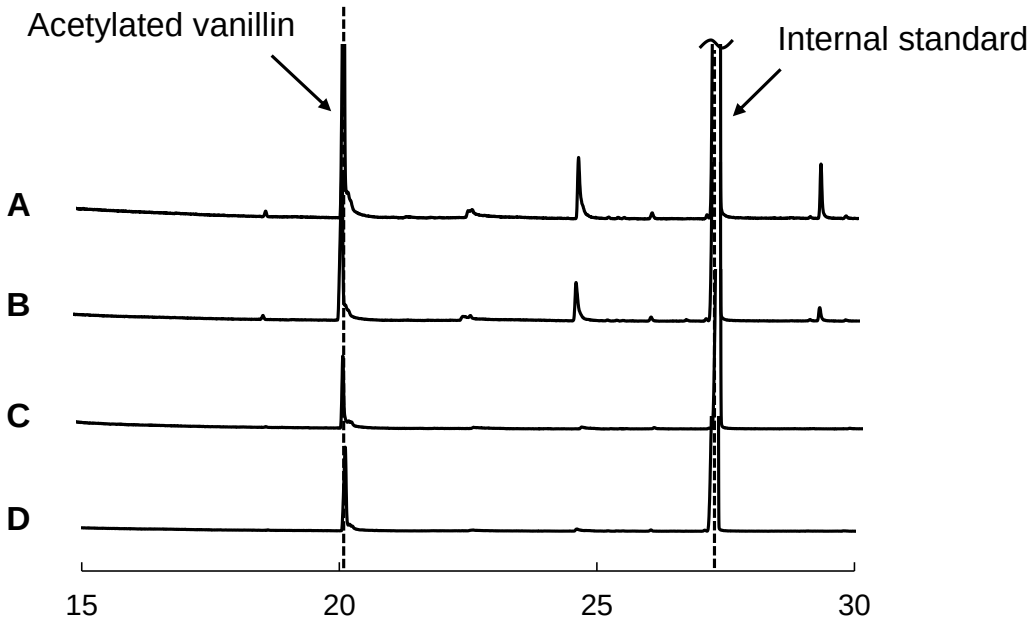


Figure S2. Total ion chromatogram of acetylated reaction mixture from the degradation of **GG** in NaOH aq. (A), **GG** in NaOD/D_2O (B), **EE** in NaOH aq. (C), and **EE** in NaOD/D_2O (D) at 120°C for 240 min under O_2.

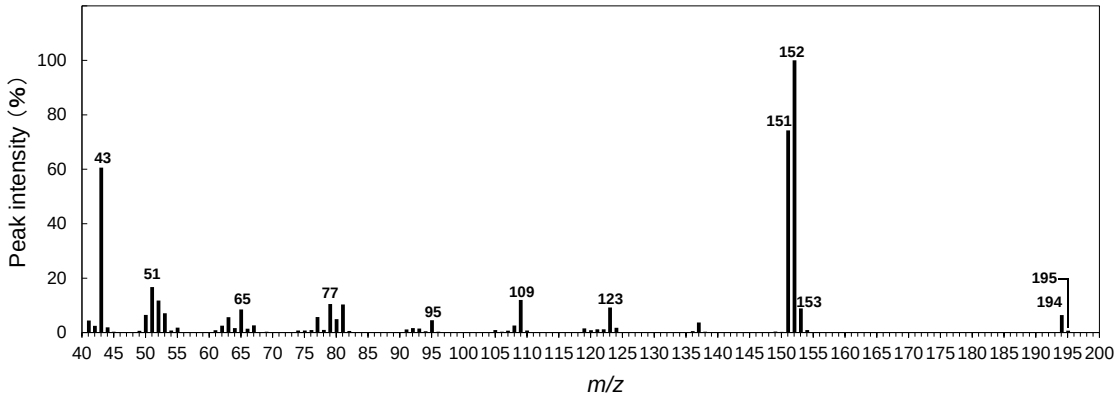


Figure S3. Mass spectra of acetylated vanillin detected at a retention time of 20.2 min in the GC/MS analysis of the reaction mixture obtained after the degradation of **GG** in 4.0 mol/L NaOH at 120°C for 240 min under O_2. The peak intensity is presented relative to that of the *m*/*z*=152 ion peak.

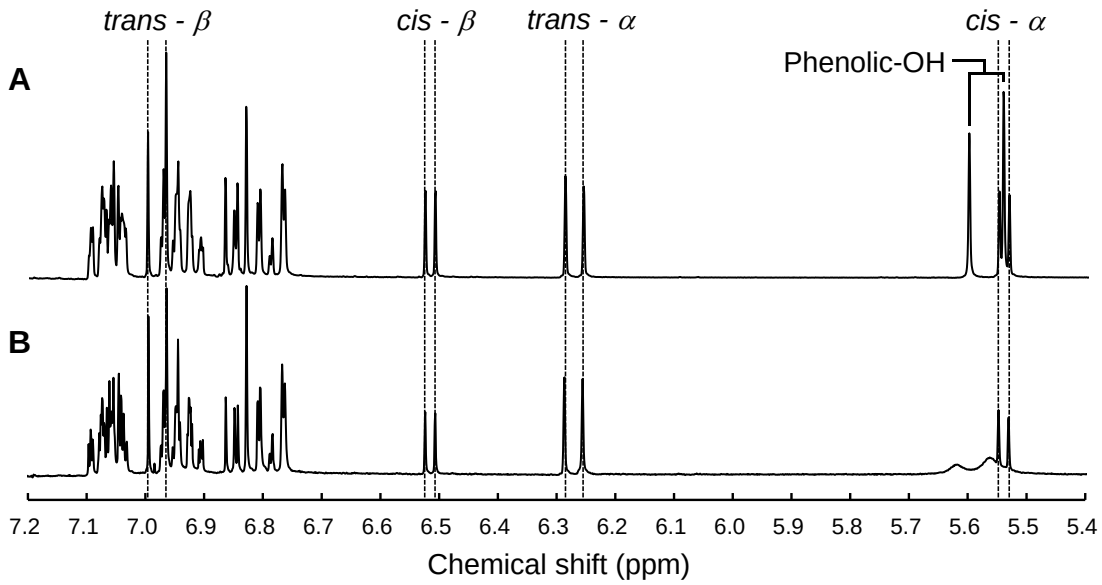


Figure S4. ^{1}H NMR (400 MHz, $CDCl_3$) spectra of **EE** obtained after the degradation of **GG** in NaOH aq. (A) and NaOD/D_2O (B) at 120ºC for 240 min under N_2. After the reaction, acetic acid was added to adjust the pH to 5-6. The reaction mixture was extracted with ethyl acetate, washed with brine and dried over anhydrous Na_2SO_4. The solvent was concentrated to dryness *in vacuo*. The product was purified on a silica gel column with ethyl acetate/*n*-hexane (1/2, v/v) to afford **EE**. In these spectra, the enlargement ratio was adjusted to equalize the intensity of signals at *trans* α-position in A and B.

In this study, **GG** was decomposed under standard reaction conditions, excluding an atmosphere of N_2, and the introduction of deuterium at the α-position of **EE** was investigated by examining the ^{1}H NMR spectrum of **EE** isolated from the decomposition products. From Figure S4, the ^{1}H NMR spectrum of **EE**, obtained from decomposition experiments in NaOH aq. and NaOD/D_2O, showed signals for the protons at *cis* α-position (δ = 5.54 ppm), *trans* α-position (δ = 6.28 ppm), *cis* β-position (δ = 6.52 ppm), and *trans* β-position (δ = 6.98 ppm) of **EE**. The signal of the proton at the *cis* α-position was close to that of the phenolic hydroxyl group, and the signal of the proton at the *trans* β-position was close to the C-H signal of the benzene ring. Accordingly, accurate integration of these signals was difficult. However, it was evident from this figure that there are no significant differences in intensity or shape between the signals of the protons at the α and β positions for both *cis* and *trans* forms. Based on this, we concluded that side chain deuteriation does not progress significantly during **EE** is formed from **GG**.

Additionally, the molar ratio of the *cis/trans* isomers was 0.74/1.00 in NaOH aq. and

0.48/1.00 in $NaOD/D_2O$ solution. Notably, in $NaOD/D_2O$ solution, the proportion of *trans* isomer formation was higher compared to NaOH aq. The reason for this is currently unknown.