Supporting Information (SI) for

The formation of lignin Alkyl-O-Alkyl ether structures via 1, 6-addition of aliphatic alcohols to the β-O-4-aryl ether quinone methides

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EXPERIMENTAL METHODS

General

Three types of phenolic β-O-4-aryl ether model compounds, guaiacylglycerol-β-syringyl ether (GSOH), guaiacylglycerol-β-guaiacyl ether (GGOH), and guaiacylglycerol-β-p-hydroxyphenyl ether (GHOH), were used as the starting material for the preparation of quinone methide (QM) compounds. They were from the Wood Chemistry Laboratory at the University of Tokyo, and corresponding NMR data can be found in below. All other organic and inorganic chemicals were reagent grade from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), Aladdin Reagent Co., Ltd. (Shanghai, China), or Cambridge Isotope Laboratories, Inc. (Tewksbury, USA) and used without further purification. Water in our experiment was distilled and passed through a Milli-Q water purification system. The naming of lignin structures was based on the traditional numbering system for lignin rather than the systematic IUPAC numbering scheme.[1]

Synthesis of β-O-4 linked model compounds
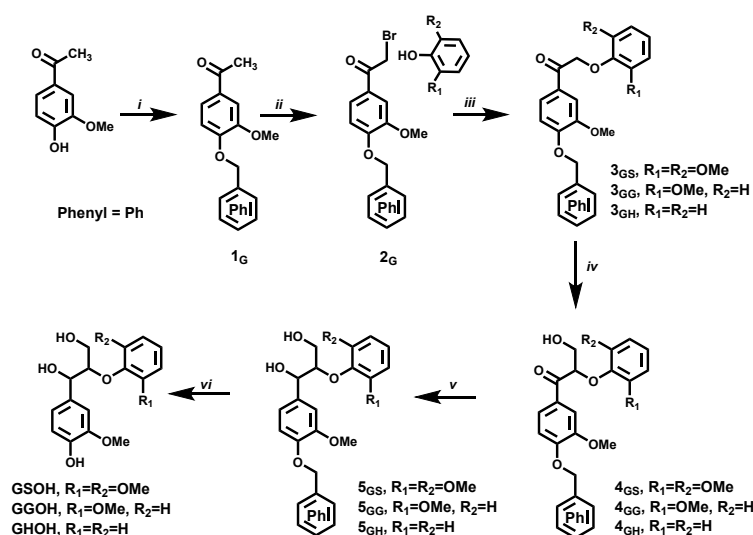
Scheme S1. Synthetic pathways of β-O-4 model GSOH, GGOH, and GHOH. Reagents and conditions were as follows: (*i*) BnCl, KI, K_2CO_3, anhydrous DMF, 90 °C, (*ii*) Br_2, EtOH, rt, (*iii*) K_2CO_3, acetone, 40 °C, (*iv*) HCHO, THF, K_2CO_3, 40 °C, (*v*) $NaBH_4$, THF-EtOH (v/v, 1:2), rt (mixture of *erythro* and *threo* isomers), (*vi*) 10% Pd/C, H_2, THF, rt (mixture of *erythro* and *threo* isomers).

The β-O-4 model GSOH, GGOH, and GHOH were synthesized according to Adler's method [2], corresponding synthetic pathways are shown in Scheme S1. It is emphasized here that the synthesis of the β-O-4 compounds, together with diastereomers separation and corresponding stereo-configuration determination, was performed in the Wood Chemistry Laboratory of the University of Tokyo during Xuhai's Ph.D study. Detailed experimental information can be found in his doctoral dissertation.[3] These related compounds also have been employed and described in previous reports.[4-6]

Synthesis of β-O-4-aryl ether QMs

Three β-O-4-aryl ether QM model compounds bearing Syringyl (S)-, Guaiacyl (G)-, and Hydroxyphenyl (H)-type nuclei on the β-etherified aromatic ring were synthesized from GSOH, GGOH, and GHOH compounds, respectively, according to Ralph's method.[7] They were named GS-QM, GG-QM, and GH-QM, respectively. In detail, the phenolic β-O-4-aryl ether compound (0.1 mmol) was dissolved in 1 mL of dichloromethane in a small vial with a screw cap. Around 0.2 mmol trimethylsilyl bromide was mixed into this solution and shaken for 90 s at room temperature to generate the benzyl bromide derivative. Subsequently, the saturated aqueous $NaHCO_3$ (1 mL) was added to this reaction mixture with shaking for 10 s. Finally, the organic layer containing QM products was collected, washed with saturated NaCl, and dried over Na_2SO_4. The resulting around 1mL of pale-yellow solution of QMs was used for the subsequent experiments without further purification.

The dry $CDCl_3$ solution of the QMs (0.1M) was prepared in a separate experiment for the following NMR structural analysis in the same way, except using $CDCl_3$ containing tetramethylsilane as the reaction solvent instead of dichloromethane. The $CDCl_3$ solution of the crude QMs were transferred into an NMR tube and kept in liquid N_2 before the NMR measurement.

Conformational analysis on QMs by NMR

The ^1H and ^{13}C NMR experiment was performed on QMs in $CDCl_3$ solvent containing tetramethylsilane by a Bruker AVANCE III HD 700MHz spectrometer equipped with a QCI cryogenic probe using standard pulse programs. Processing the final spectrum was performed by EM window functions with Bruker's Topspin 3.0 (Windows) software. All chemical shifts were calibrated by setting the internal reference (tetramethylsilane) to 0 ppm. The structural elucidation and assignment to synthesized compounds were based on previous study.[3-6]

The NMR spectra of QMs are presented in Figure S1-3, and corresponding data are resulted in Table S1. As shown in Figure S1-3, geometric isomers of these QMs are identified based on the deshielding of 2-H in the *syn*-isomer compared with 2-H in the *anti*-isomer (e.g., H2 of *syn*, 6.58 vs. H2 of *anti*, 6.30 for GS-QM), and H6 in the *anti*-isomer compared with H6 in the *syn*-isomer (e.g., H6 of *syn*, 7.09 vs. H6 of *anti*, 7.51 for GS-QM) due to steric compression by the β-etherified aromatic ring. Furthermore, this steric compression on H2 (*syn*) and H6 (*anti*) induces an electron shift from these protons onto the corresponding carbon atoms, C2 (*syn*) and C6 (*anti*), resulting in a shielding effect as illustrated in Table 1B (e.g., C2 of *syn*, 104.6 vs. C2 of *anti*, 112.0, C6 of *syn*, 141.9 vs. C6 of *anti*, 133.3 for GS-QM). This effect is associated with protons forced to occupy a sterically crowded environment[8,9] and has been used for configuration determination of related structures.[10,11]

The ^1H NMR spectra (Figure S1-3) of the QMs reveal that the ratio of *syn*- and *anti*-isomer is 1.8 for GS-QM, 2.0 for GG-QM, and 1.9 for GH-QM. The *syn*-isomer of QM, in which the OMe substituent is at the same side of the exocyclic methylene-substituent moiety, predominated and increase with the bulky volume of the methylene-substituent moiety. This is in agreement with the previous report that the preference for *syn*-isomer over the *anti*-one was presumably electronic rather than steric.[11]
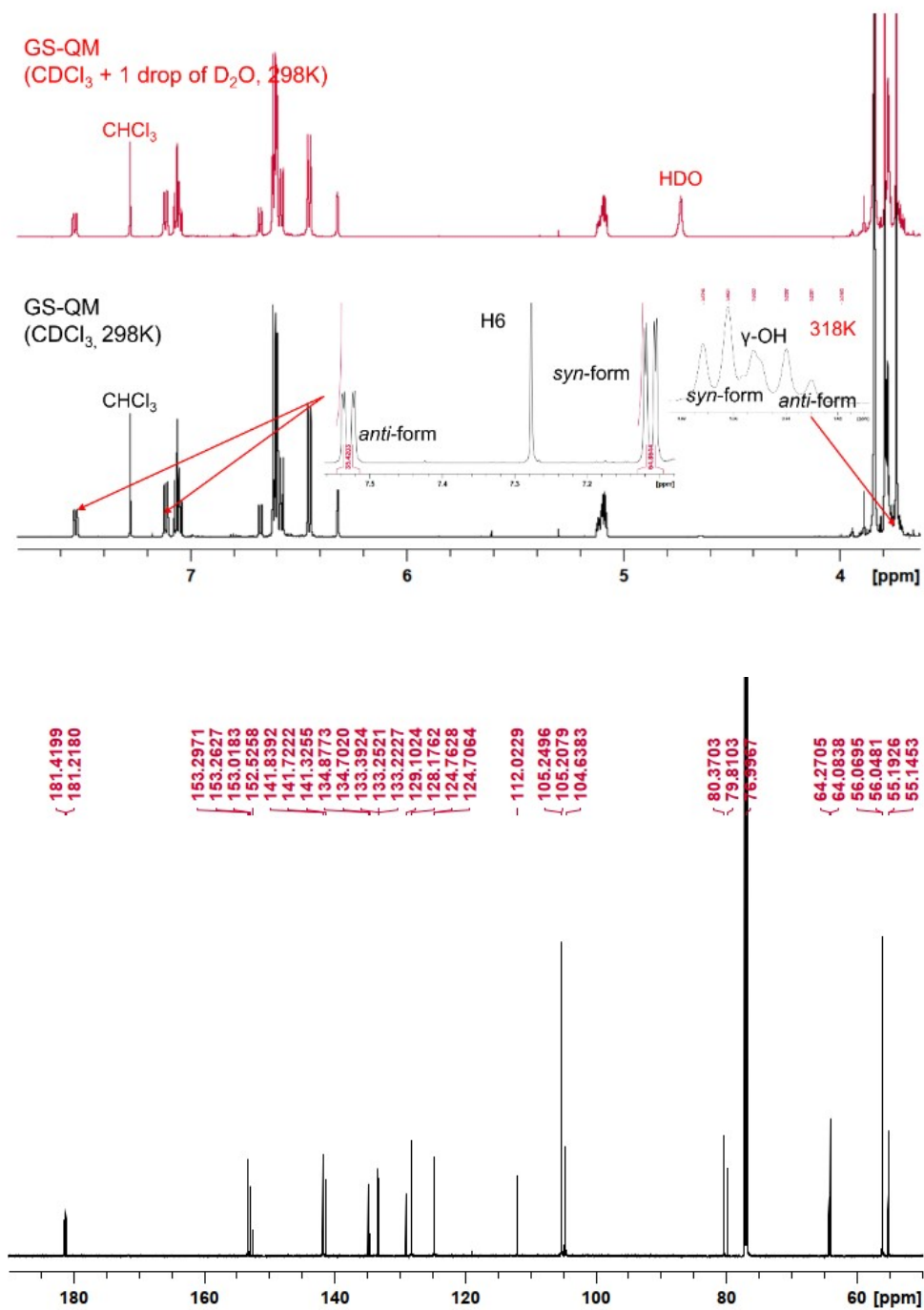
Figure S1. NMR spectra of GS-QM (mixture of *syn*- and *anti*-isomers) in CDCl₃, the upper is the ¹H NMR, the latter is the ¹³C NMR
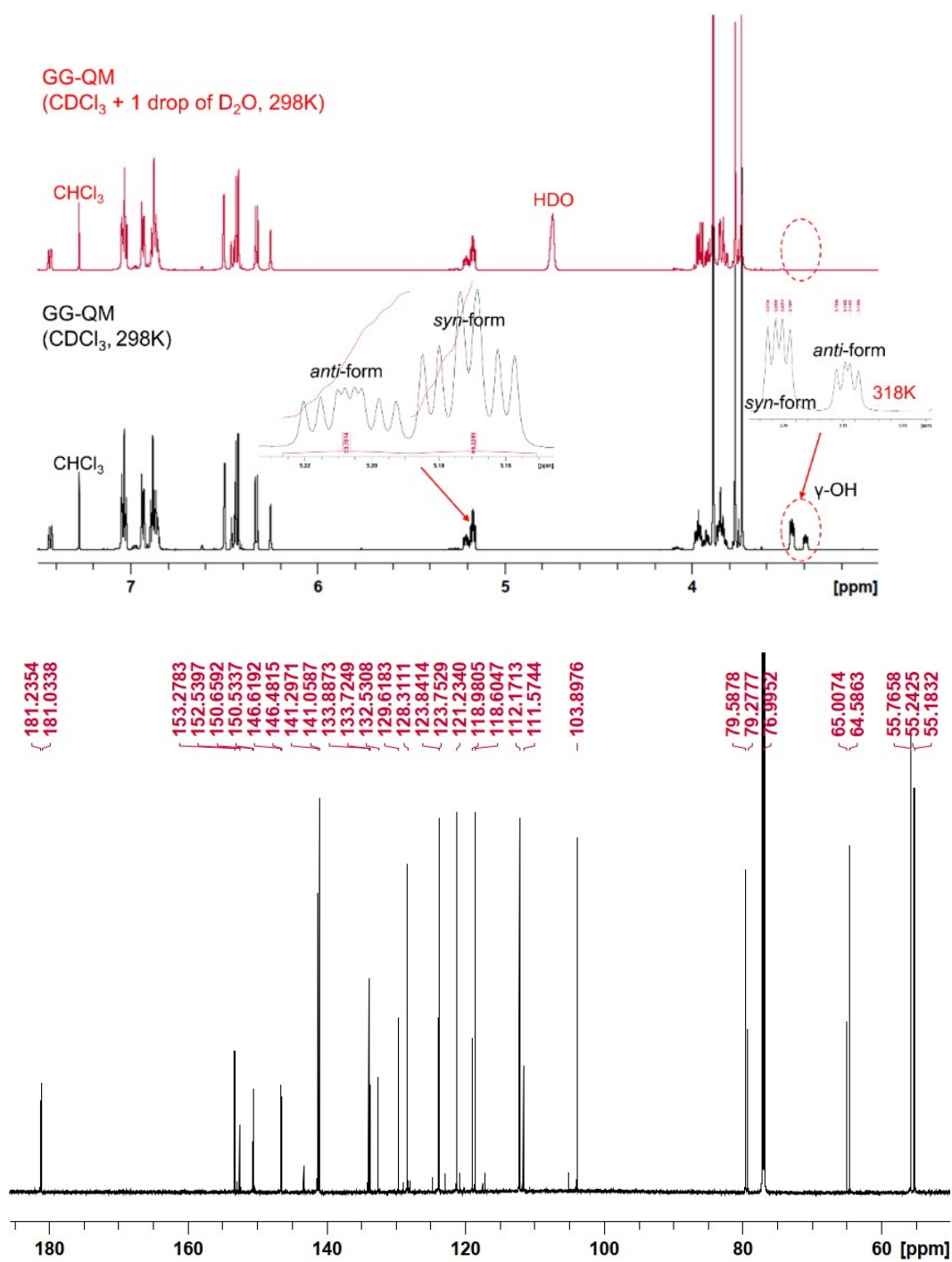
Figure S2. NMR spectra of GG-QM (mixture of *syn*- and *anti*-isomers) in CDCl₃, the upper is the ¹H NMR, the latter is the ¹³C NMR
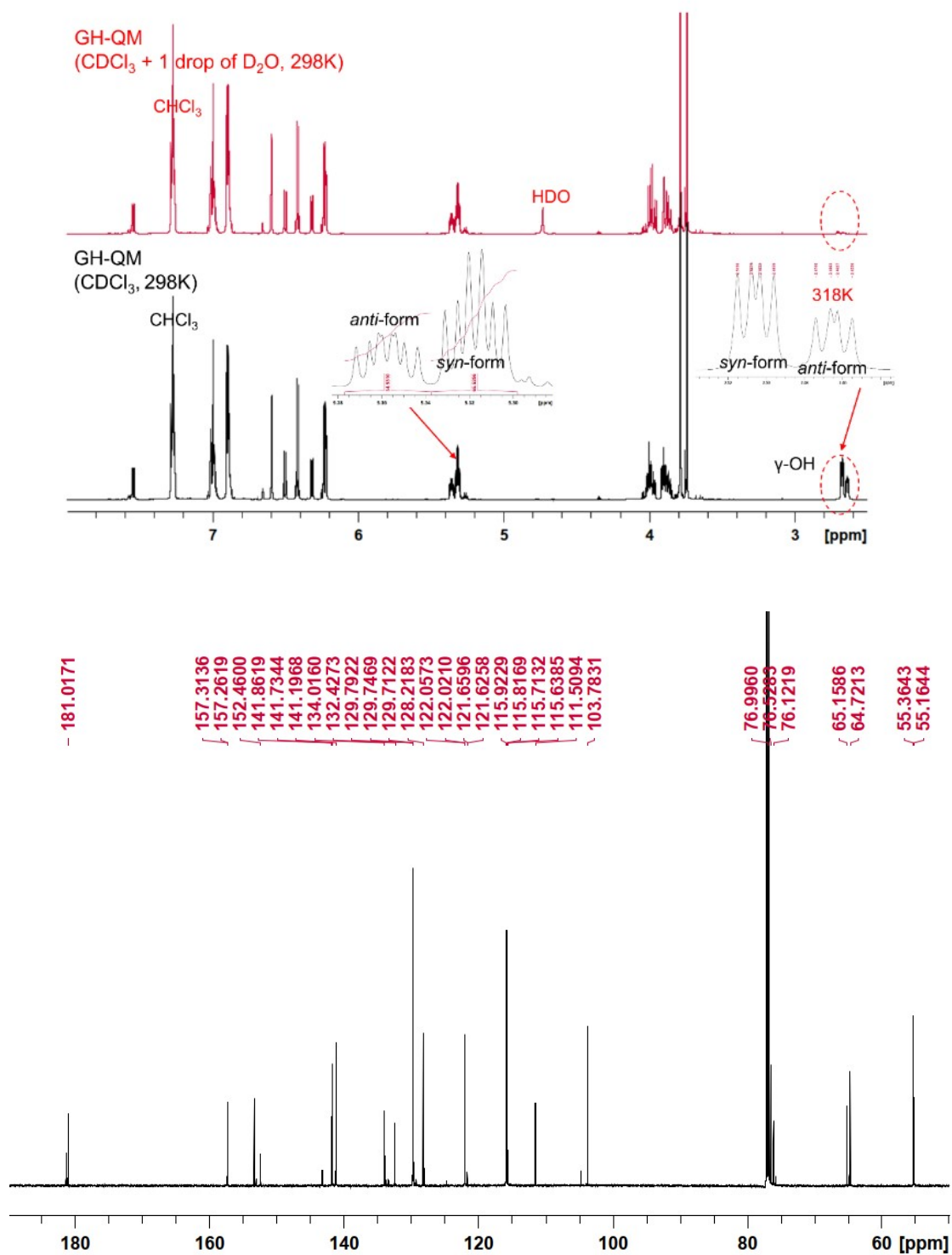
Figure S3. NMR spectra of GH-QM (mixture of *syn*- and *anti*-isomers) in CDCl₃, the upper is the ¹H NMR, the latter is the ¹³C NMR

Table S1. 175 MHz ^{13}C NMR of Quinone Methides in CDCl$_3$

Compounds		GS-QM		GG-QM		GH-QM	
		syn- isomer	*anti-* isomer	*syn-* isomer	*anti-* isomer	*syn-* isomer	*anti-* isomer
QM moiety	1	133.2	133.3	133.9	133.7	134.0	133.9
	2	104.6	112.0	103.9	111.6	103.8	111.5
	3	153.0	152.5	153.3	152.5	153.4	152.5
	4	181.2	181.4	181.0	181.2	181.0	181.2
	5	128.2	129.1	128.3	129.6	128.2	129.8
	6	141.8	133.4	141.3	132.5	141.2	132.4
	OMe	55.2	55.2	55.2	55.2	55.4	55.2
Aromatic ring	1′	124.8	124.7	123.8	123.8	122.1	122.0
	2′	105.3	105.2	112.2	112.2	129.8	129.7
	3′	153.3	153.3	150.5	150.7	115.8	115.9
	4′	134.9	134.7	146.6	146.5	157.3	157.3
	5′	153.3	153.3	118.6	119.0	115.6	115.7
	6′	105.3	105.2	121.2	121.2	129.8	129.7
	OMe	56.1	56.1	55.8	55.8	-	-
Side-chain	α	141.7	141.3	141.1	141.1	141.7	141.9
	β	80.4	79.8	79.6	79.3	76.5	76.1
	γ	64.1	64.3	64.6	65.0	64.7	65.2

Table S2. 700 MHz ^{1}H NMR of Quinone Methides in CDCl$_3$

Compounds		GS-QM		GG-QM		GH-QM	
		syn- isomer	*anti-* isomer	*syn-* isomer	*anti-* isomer	*syn-* isomer	*anti-* isomer
QM moiety	2	6.60^s	6.32^d	6.50^d	6.25^d	6.60^d	6.22^d
	5	6.45^d	6.45^d	6.43^d	6.45^{dd}	6.41^d	6.50^{dd}
	6	7.11^{dd}	7.53^{dd}	7.04^{dd}	7.43^{dd}	7.00^{dd}	7.54^d
	OMe	3.74^s	3.79^s	3.73^s	3.77^s	3.79^s	3.74^s
	$J_{5,6}$	10	10	10	10	10	10
	$J_{2,6}$	2	2	2	2	2	2
Aromatic ring	1′	7.06^t	7.05^t	7.03^{dt}	7.03^{dt}	7.00^{dt}	7.00^{dt}
	2′	6.61^d	6.60^d	6.93^d	6.93^d	7.28^t	7.27^t
	3′	-	-	-	-	6.90^d	6.89^d
	5′	-	-	6.84-6.89^m	6.84-6.89^m	6.90^d	6.89^d
	6′	6.61^d	6.60^d			7.27^t	7.28^t
	OMe	3.84^s	3.84^s	3.89^s	3.89^s	-	-
	$J_{1′,2′}$	8	8	8	8	7	7
	$J_{2′,3′}$	-	-	-	-	8	8
	$J_{1′,6′}$	8	8	8	8	7	7
	$J_{5′,6′}$	-	-	-	-	8	8
Side-chain	α	6.58^d	6.66^d	6.32^d	6.43^d	6.23^d	6.32^d
	β	5.09^m	5.11^m	5.17^m	5.21^m	5.32^m	5.36^m
	γ1	3.78^d	3.78^d	3.84^{dd}	3.82^{dd}	3.89^{dd}	3.86^{dd}
	γ2	3.78^d	3.78^d	3.96^{dd}	3.92^{dd}	3.99^{dd}	3.96^{dd}
	$J_{α,β}$	8	9	8	10	8	8
	$J_{β,γ1}$	～0	～0	4	4	4	4
	$J_{β,γ2}$	～0	～0	7	7	7	7
	$J_{γ1,γ2}$	4	4	12	12	12	12

* superscript s, d, dd, t, dt, m means singlet, doublet, doublet of doublets, triplet, doublet of triplets and multiple

The developed Karplus relationships

The developed Karplus relationship[12] below was used to investigate the rotamer populations about the Cβ—Cγ bond in β-O-4 bonded QMs (conformation of the γ-hydroxymethyl group).

$$3J_{H\beta,H\gamma R} = 5.08 + 0.47\cos(\omega) + 0.90\sin(\omega) - 0.12\cos(2\omega) + 4.86\sin(2\omega) \quad (1)$$

$$3J_{H\beta,H\gamma S} = 4.92 - 1.29\cos(\omega) + 0.05\sin(\omega) + 4.58\cos(2\omega) + 0.07\sin(2\omega) \quad (2)$$

Hydrogen bond identification on QMs

The variable-temperature 1H NMR experiments were performed on NMR samples with a Bruker AVANCE III HD 700MHz spectrometer equipped with a QCI cryogenic probe from 288 to 318 K with a stepwise increase of 5K. The temperature was monitored with a thermocouple close to the sample tube. Samples were kept for at least 15 min at a given temperature to stabilize the temperature before collecting ^{1}H NMR data. The 65K data points were acquired from 18 to - 2 ppm, with an acquisition time of 2.32 sec, a 30° flip angle, 16 scans, and a 1.0 s relaxation delay. The same NMR spectra processing method as above. The Hydrogen-Deuterium exchange experiment confirmed the assignment of OH protons, i.e., the proton resonance of OHs disappeared while one drop of D_2O was mixed with the NMR samples.[13]

Kinetic study on nucleophile addition to QMs

A fresh dichloromethane solution of QMs (ca. 1mL × 0.1 M, based on the assumption that a phenolic β-O-4-aryl ether model compound was transformed into its QM quantitatively), prepared as described above, was firstly diluted into 3 mM with certain anhydrous dioxane. Then, to the 6 mL of methanol and 6 mL of ethanol placed in a small vial, the diluted QMs solution (ca. 0.1mL × 3 mM) was separately added to start the nucleophilic addition reaction. This time point was marked as a reaction time of 0 s. Initial QMs concentrations were approximately 0.49×10^{-4} M. After immediate shaking of the reaction mixture for a few seconds to make the mixture homogeneous, an aliquot of the mixture (approximately 3 mL) was transferred into a quartz UV cell (1 cm square), which was placed in a UV-vis spectrometer (Jasco V-660, Jasco International Company Ltd., Tokyo, Japan) equipped with a circulating water bath. The temperature of all reacting solutions was controlled at 25 °C, and monitoring of the reaction progress was started at the reaction time of 20 s. Reactions were monitored by following the decay of quinone methide absorbance at $\lambda_{max} = 300-310$ nm every 1 s from the reaction times of 20 s to 60 min. The observed first-order rate constants k_{obsd} (s^{-1}) for reactions with half-lives ($\tau_{1/2}$) were obtained by least-squares fitting of an exponential function. The gram extinction coefficient of the QMs at λ_{max} was calculated using the initial UV absorbance of QMs ($Abs_{initial}$).

Diastereomer products analysis by ^{1}H NMR

All the dichloromethane solutions of the above freshly prepared QMs (approximately 1mL × 0.1M QMs) were quickly added to 2 mL of methanol and ethanol to start the nucleophilic addition reaction, respectively. The reaction mixture was maintained at 25 °C with vigorous stirring for 4 h. When the pale-yellow color of the QMs solution disappeared, the stirring was stopped, and 2 mL of dichloromethane was added to the reaction mixture to extract the products.

The resulting organic layer was washed twice with H₂O, once with brine, and dried over Na₂SO₄. After filtration, the filtrate was concentrated under a vacuum to afford the mixture of *erythro* and *threo* isomers of β-O-4 products as syrup. The structural identification and isomers ratio of β-O-4 products was determined with ¹H NMR in CDCl₃ solution based on NMR data of corresponding model compounds[14] (Figure S4-6).
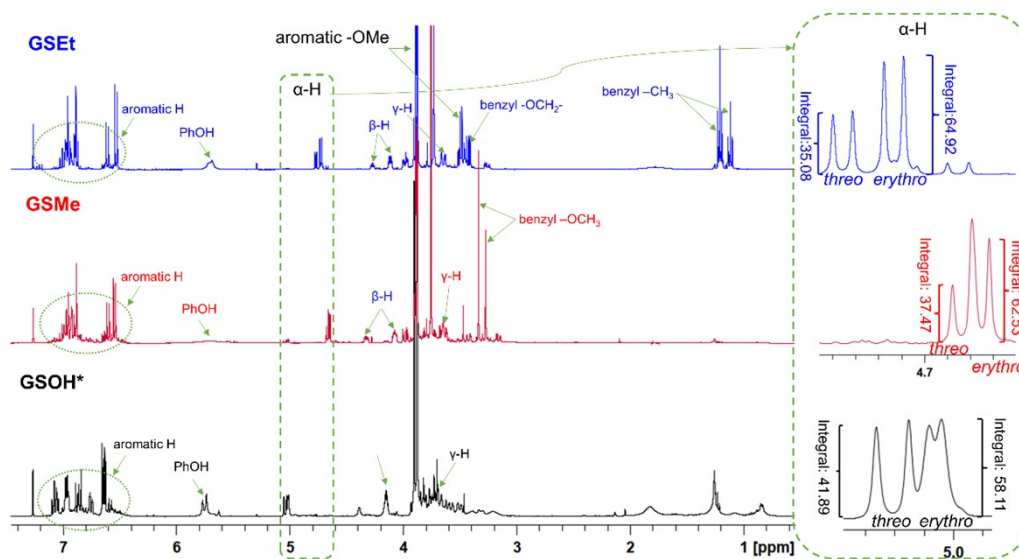

Figure S4 ¹H NMR spectra of products (GSOH, GSMe, GSEt) from nucleophilic addition of GSQM, the ratio of *erythro* and *threo* form of GSOH was 58:42, GSMe was 62:38, GSEt was 65:35, which was determined by the integration of the signals of α-H, *the data was published in previous report.[6]
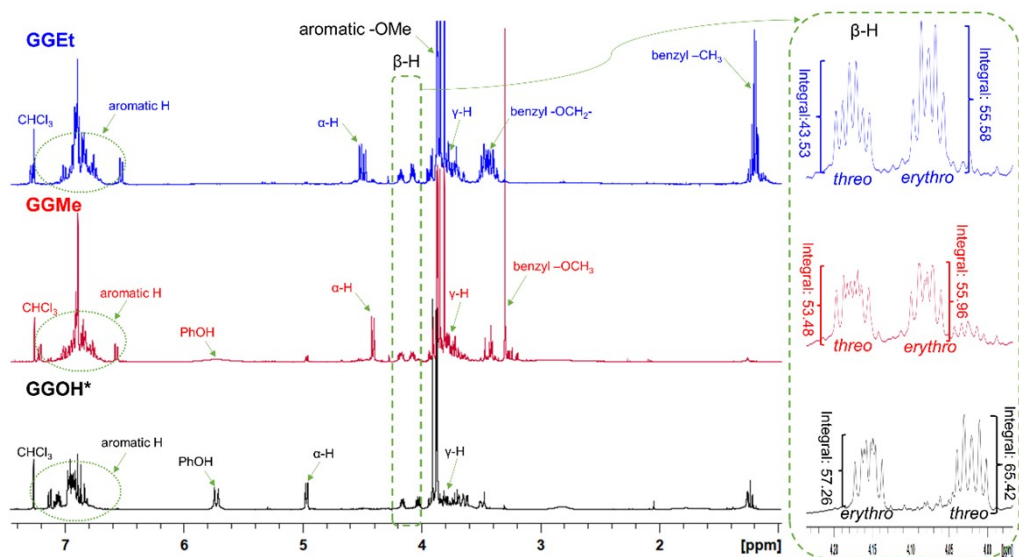

Figure S5 ¹H NMR spectra of products (GGOH, GGMe, GGEt) from nucleophilic addition of GGQM, the ratio of *erythro* and *threo* form of GGOH was 48:52, GGMe was 52:48, GGEt was 56:44, which was determined by the integration of the signals of β-H, *the data was published in previous report.[6]
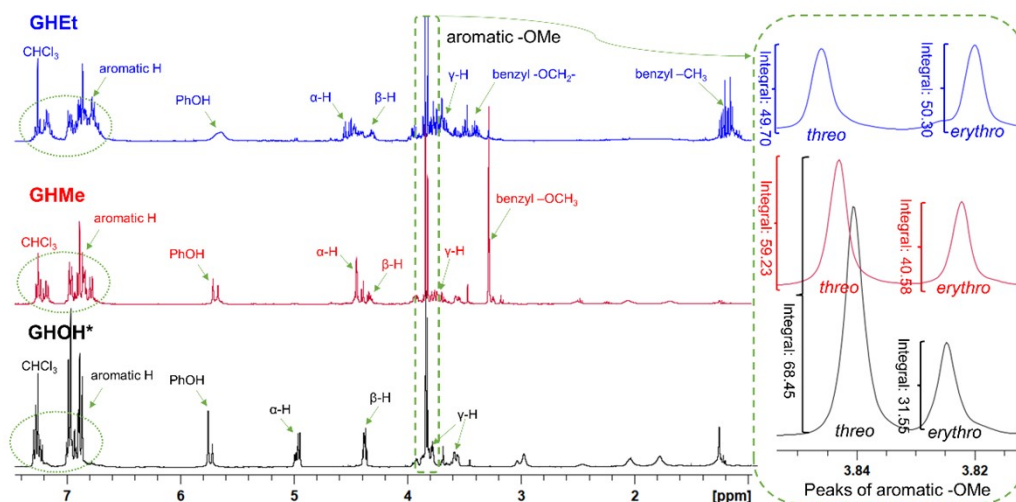
Figure S6 ^{1}H NMR spectra of products (GHOH, GHMe, GHEt) from nucleophilic addition of GGQM, the ratio of *erythro* and *threo* form of GHOH was 31:69, GHMe was 40:60, GHEt was 50:50, which was determined by the integration of the signals of OMe, *the data was published in previous report.[6]

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