Unveiling the heterogeneous structure of native lignin in bamboo cell walls via a novel isolation method for high-value applications

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Content

1. Material and methods

1.1 Preparation of double enzymatic lignin (MWL)

The raw materials were smashed into sawdust (40-60 mesh), dried in an oven at 60 ^{o}C, and then extracted with ethanol/benzene (1:2, v/v) using a Soxhlet extractor for 6 h. Finally, the extractive-free raw materials were air-dried and subjected to mill in a planetary ball mill (Fritsch GmbH, Idar-Oberstein, Germany) at a fixed frequency of 450 rpm for 5 h. To avoid high temperature destroying the subtle molecular structure of lignin, the ball-milling procedure was set a 10 min interval after every 10 min of milling. All chemicals used were purchased from Sigma Chemical Co. (Beijing, China), except for milled bamboo powder (20 g) was suspended in 80% dioxane with a solid-to-liquid ratio of 1:20 (g/mL) at 50 ^{o}C in the dark for 12 h under stirring. The mixture was filtered, then the residue was washed with the same solvents until the filtrate was clear. The above procedure was repeated twice. All the supernatants were concentrated under reduced pressure and then slowly dropped into three volumes of 95% ethanol to precipitate the hemicelluloses, which were separated by filtration. The mixed filtrates were concentrated again and then precipitated in acidic water (pH=2, adjust by aqueous HCl). After centrifugation and freeze-drying, the MWLs were obtained.

1.2 Preparation of double enzymatic lignin (CEL)

The ball-milled powder (5 g) was mixed with the desired amounts of sodium acetate buffer (pH 4.8) with a solid-to-liquid ratio of 1:20 (g/mL) and cellulase (35 FPU/g substrate). Then the mixture was incubated at 50 ^{o}C in a rotary shaker with a rotational

velocity of 150 rpm for 48 h. After enzymatic hydrolysis, the mixture was separated by centrifugation, and the hydrolyzed residue was washed with buffer solution and deionized water repeatedly. Finally, the freeze-dried residue was extracted with 80% aqueous dioxane to obtain CEL fractions, and the detailed extraction process was same as the aforementioned procedure of MWL.

1.3 Preparation of double enzymatic lignin (DEL)

The ball-milled powder (5 g) was mixed with the desired amounts of sodium acetate buffer (pH 4.8) with a solid-to-liquid ratio of 1:20 (g/mL) and cellulase (35 FPU/g substrate). Then the mixture was incubated at 50 ^{o}C in a rotary shaker with a rotational velocity of 150 rpm for 48 h. Next, the mixture was centrifuged and the residue was washed thoroughly with sodium acetate buffer (pH 4.8) to remove the hydrolyzed carbohydrates, and then freeze-dried. Finally, the dried residual solid was repeatedly subjected to ball-milling for 2 h and enzymatic hydrolysis again as above-mentioned processes. After washing with acidic water (pH 2.0) and freeze-drying, DEL sample was obtained. To increase the solubility of the lignin in tetrahydrofuran (THF) for the molecular weights determination by GPC technique, the acetylation of lignin was performed.

1.4 Acetylation of double enzymatic lignin (DEL)

About 30 mg of dry lignin was dissolved in 3 mL of a solution of dimethyl sulfoxide: 1-methylimidazole (2:1, v/v) and stirred without direct light at room temperature for 12

h. Acetic anhydride (1.0 mL) was added to the reaction mixture and continued reacting for 2 h. The reaction mixture was dropped slowly into 100 mL acid water (pH=2) to induce precipitation followed by centrifugation, the acetylated lignin was obtained.

1.5 Structure elucidation of the bamboo

The chemical compositions (%, w/w) of the cellulose-rich substrates were determined according to the NREL standard analytical method (NREL/TP-510-42618). The microstructural changes and surface characteristics of the different cellulose-rich substrates were analyzed with a scanning electron microscope (SEM) (S-3400N II, HITACHI Company, Japan) operating at 10 kV acceleration voltages. All samples were coated with gold prior to acquiring images. BET Brunauer-Emmett-Teller (BET) surface areas and Barrett-Joyner-Halenda (BJH) pore volumes of the substrates were recorded by analyzing of the nitrogen adsorption using a Kubo-X1000 surface area analyzer (Builder Electronic Technology co., Ltd., Beijing, China) after 8 h of degassing at 120 ^{o}C.

1.6 Characterization of the lignin fractions

The weight-average (M_w) and number-average (M_n) molecular weights of the lignin samples were determined by gel permeation chromatography (GPC) (Agilent 1200, Agilent Technologies, USA) with an ultraviolet detector (UV) at 240 nm. The column used was a PL-gel 10 mm mixed-B 7.5 mm i.d. column, which was calibrated with PL

polystyrene standards. NMR spectra of lignin samples were recorded on a Bruker AVIII 400 MHz spectrometer at 25 ^{o}C in DMSO-*d*6. For the quantitative ^{31}P NMR spectra, 20 mg of lignin was dissolved in 500 μL of a mixture of anhydrous pyridine and deuterated chloroform (at a volume ratio of 1.6:1, v/v) under stirring. This was followed by the addition 100 μL of cyclohexanol (10.85 mg/mL in anhydrous pyridine and deuterated chloroform 1.6:1, v/v) as an internal standard and 100 μL of chromium(III) acetylacetonate solution (5 mg/mL in anhydrous pyridine and deuterated chloroform 1.6:1, v/v) as the relaxation reagent. After that, the mixture was reacted with 100 μL of phosphitylating reagent (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholate, TMDP) for about 10 min and was transferred into a 5 mm NMR tube for subsequent ^{31}P NMR analysis.

1.7 Enzymatic hydrolysis

Enzymatic hydrolysis was carried out at 2% of different samples (w/v) in 10 mL of 50 mM sodium acetate buffer (pH 4.8) using shaking incubators (ZWYR-2102C) (Shanghai, China) at 150 rpm for 72 h. Cellulase (Cellic@ CTec2, 100 FPU/ml) was provided from Novozymes (Beijing, China) and employed at the activity of 15 FPU/g substrate for all the samples. The hydrolysis reaction solution of 200 μL was sampled at 0, 3, 6, 9, 12, 24, 48, 60, and 72 h. The intermittent sample was sealed and incubated in a boiling water bath for 5 min to terminate the cellulose hydrolysis reaction, and then centrifuged at 10,000 rpm for 5 min to obtain the supernatant. The supernatant (100 μL) was diluted by ultrapure water and filtered through a 0.22 μm filter prior to sugar

analysis by an HPAEC system with an integral amperometric detector and CarboPac PA100 (4*250 mm, Dionex) analytical column according to the literature. All the hydrolysis experiments were carried out in duplicates.

1.8 The formulas for calculating the substructure content of lignin

$$S/G = \frac{I(S2,6) + I(S'2,6)}{2 \times I(G2)}$$

$$I(C9) = \frac{I(S2,6) + I(S'2,6)}{2} + I(G2) + \frac{I(H2,6)}{2}$$

$$\beta\text{-}O\text{-}4 = \frac{I(\beta\text{-}O\text{-}4)}{I(C9)} \times 100$$

$$FA = \frac{I(FA2)}{I(C9)} \times 100$$

$$PCE = \frac{I(PCE2,6)}{2 \times I(C9)} \times 100$$

1.9 Method for determining component content

1. Sample preparation and pretreatment (adapted for 40–60 mesh).

2. Sample drying: take the crushed bamboo samples (fibers F or parenchyma cells P) passing through a 40–60 mesh sieve, dry them in an oven at 105 °C for 24 hours, cool to room temperature, and store in a desiccator for later use (ensure sample moisture content < 10%).

3. Two step acid hydrolysis. First step: concentrated acid pretreatment. Accurately weigh 0.3 g (precision to 0.0001 g) of the pretreated sample into a graduated 250 mL pressure-resistant hydrolysis tube.

4. Add 3 mL of 72% (w/w) sulfuric acid solution, gently shake the hydrolysis tube

to fully mix the sample with the acid, and incubate in a constant temperature water bath at 30 °C for 60 minutes, shaking every 10 minutes to ensure uniform contact between the acid and the sample.

5. After incubation, dilute the hydrolysate with deionized water to a sulfuric acid concentration of 4% (w/w) with a total volume of approximately 86.73 mL, and shake well.

6. Second step: dilute acid reflux hydrolysis. Place the hydrolysis tube in an autoclave, and reflux hydrolyze at 121 °C and 103 kPa for 60 minutes.

7. After hydrolysis, cool naturally to room temperature, filter the hydrolysate into a 100 mL volumetric flask with quantitative filter paper, rinse the hydrolysis tube and filter paper multiple times with a small amount of deionized water, combine the washings into the volumetric flask, dilute to the mark, and shake well (designated as "hydrolyzed mother liquor").

8. Take 10 mL of the hydrolyzed mother liquor and filter through a 0.22 μm filter for later use (for sugar content analysis).

9. Quantitative analysis of sugars in hydrolysate.

9.1 Instrument and standard curve. (1) Instrument: high-performance anion exchange chromatography (HPAEC, e.g., Dionex CarboPac PA100 column as mentioned in the study) or high-performance liquid chromatography (HPLC) equipped with a refractive index detector. (2) Standard samples: glucose (corresponding to cellulose), xylose, arabinose, galactose, etc. (corresponding to hemicellulose). Prepare mixed standard solutions of different concentrations (0.1–10 mg/mL), draw a standard curve, and

calculate the regression equation.

9.2 Sample analysis. (1) Inject the filtered hydrolysate into the chromatograph, perform analysis according to the instrument operating conditions (column temperature 30 °C, mobile phase: NaOH solution or acetonitrile water mixture, flow rate 1.0 mL/min), and record the peak area of each monosaccharide. (2) Calculate the concentration of each monosaccharide in the hydrolysate based on the standard curve, and then convert it to the content of cellulose (calculated as glucose) and hemicellulose (calculated as xylose + arabinose + galactose, etc.) in the raw material.

10. Lignin content determination (klason lignin + acid soluble lignin).

10.1 Klason lignin (acid insoluble lignin) determination. (1) Transfer the filter residue (residual solid) after the second step hydrolysis into a pre-weighed crucible (recorded as m_1), dry in an oven at 105 °C to constant weight, and weigh the total mass (recorded as m_2). (2) Place the crucible in a muffle furnace, heat to 575 °C at a rate of 5 °C/min, ash for 4 hours, cool to room temperature, and weigh the ash mass (recorded as m_3). Calculation: Klason lignin content (%) = [(m_2-m_3) / Mass of pretreated sample] × 100%.

10.2 Acid soluble lignin determination. (1) Take the diluted solution after the first step hydrolysis (sulfuric acid concentration 4%), and measure the absorbance (A) at 205 nm with an ultraviolet visible spectrophotometer. (2) Calculation: Acid soluble lignin content (%) = (A × Dilution factor × 100) / (ε × b × Mass of pretreated sample), where ε is the molar absorption coefficient of lignin at 205 nm (usually 110 L/(g·cm)), and b is the cuvette path length (1 cm). Total lignin content = Klason lignin content +

Acid soluble lignin content.

11. Data calibration and result expression. (1) Sugar recovery calibration: due to possible sugar degradation during acid hydrolysis, perform a spiked recovery experiment (add samples to standard sugar samples with known content, repeat hydrolysis and analysis) to calculate the recovery rate and calibrate the determination results. (2) Result expression: report the mass fractions of cellulose (%), hemicellulose (%, total of each monosaccharide), and total lignin (%) based on the dried sample, retain two decimal places, and take the average value ± standard deviation of parallel experiments ($n \geq 3$).

1.10 Preparation of supercapacitor

1. Detailed carbonization procedure. The carbonization process of HEL samples was optimized to ensure uniform porous structure formation, with detailed parameters as follows: (1) Pretreatment of lignin precursor: HEL-F and HEL-P samples were dried at 60 °C for 12 hours to remove residual moisture, then ground into fine powder (particle size <100 μm) using an agate mortar. (2) KOH activation: HEL powder and anhydrous KOH (analytical grade, Macklin) were mixed at a mass ratio of 2:1 (lignin : KOH) in a glove box (N_2 atmosphere) to avoid moisture absorption. The mixture was thoroughly ground for 30 minutes to ensure homogeneous dispersion. (3) Carbonization-activation process: the mixed powder was loaded into a corundum crucible, placed in a tube furnace (GSL-1700X, Hefei Kejing Materials Technology Co., Ltd.), and heated under

high-purity N_2 flow (50 mL/min) with the following program. Ramp from room temperature to 400 °C at 5 °C/min, hold for 1 hour (to remove volatile impurities and promote KOH melting). Continue ramping to 800 °C at 5 °C/min, hold for 2 hours (key activation stage for pore formation). Cool naturally to room temperature under N_2 flow to prevent oxidation.

2. Post-treatment: The black carbonized product was immersed in 2.0 M HCl solution (100 mL per 1 g carbon) and stirred at 60 °C for 4 hours to remove residual KOH and inorganic impurities. The mixture was filtered and washed repeatedly with deionized water until the filtrate reached neutral pH (pH 6.8–7.2). The resulting LPC was dried at 105 °C for 12 hours and stored in a desiccator for subsequent use.

3. Ink preparation and electrode fabrication. (1) Ink component ratio: The electrode ink was prepared by mixing LPC (active material), acetylene black (conductive agent, Alfa Aesar), and polyvinylidene fluoride (PVDF, binder, Sigma-Aldrich) at a mass ratio of 8:1:1. (2) Dispersion process: 100 mg of the above mixture was added to 1 mL of N-methylpyrrolidone (NMP, solvent, analytical grade, Macklin) in a 5 mL centrifuge tube. The mixture was sonicated for 30 minutes (40 kHz, 150 W) to break agglomerates, then magnetically stirred at 600 rpm for 12 hours to form a homogeneous, viscous ink. (3) Electrode coating: The ink was uniformly coated onto a cleaned nickel foam current collector (1 cm × 1 cm, thickness 1.5 mm, Changsha Liyuan New Material Co., Ltd.) using a doctor blade with a gap of 200 μm. (4) Drying and pressing: The coated electrode was dried at 80 °C for 4 hours, then further dried at 120 °C under vacuum (0.01 MPa) for 12 hours to completely remove residual NMP. The dried electrode was

pressed at 10 MPa for 30 seconds using a tablet press (YP-15T, Shanghai Sanshi Testing Machine Co., Ltd.) to enhance the contact between active material and current collector.

4. Binder selection and specifications. (1) Binder type: Polyvinylidene fluoride (PVDF, average molecular weight ~534,000 g/mol) was selected for its excellent chemical stability, high adhesion, and compatibility with aqueous electrolytes (6 M KOH). (2) Binder dosage: The mass fraction of PVDF in the electrode was fixed at 10% (relative to total solid components), which balances adhesion strength and electron/ion transport efficiency—lower dosages led to poor electrode integrity, while higher dosages blocked pores and reduced conductivity.

5. Supercapacitor cell configuration. (1) Cell type: Symmetric two-electrode coin cells (CR2032, Tianjin Zhongnuo New Energy Technology Co., Ltd.) were assembled in a glove box (ambient atmosphere, relative humidity <10%). (2) Cell components: Working electrodes: Two identical LPC-coated nickel foam electrodes (1 cm × 1 cm) as cathode and anode. (3) Electrolyte: 6 M KOH aqueous solution (prepared with deionized water, resistivity ≥18.2 MΩ·cm), with a dosage of 100 μL per cell. (4) Separator: Cellulose-based separator (Whatman GF/D, thickness 260 μm, diameter 16 mm), soaked in 6 M KOH for 30 minutes before assembly to ensure full wetting. (5) Assembly sequence: Coin cell case → anode → separator → electrolyte (dropwise addition) → cathode → spacer → spring washer → coin cell cover. The cell was crimped using a coin cell crimping machine (MSK-110, Shenzhen MTI Corporation) with a pressure of 8 MPa to ensure tight contact between components.

6. Calculation of active material mass. The mass of active material (LPC) in each electrode was accurately calculated by the difference method. Step 1: Weigh the mass of the clean, dried nickel foam current collector (m_0), recorded to four decimal places using an analytical balance (FA2004N, Shanghai Jingmi Electronic Technology Co., Ltd.). Step 2: After ink coating, drying, and pressing, weigh the total mass of the electrode (m_1). Step 3: Calculate the total mass of solid components (LPC + acetylene black + PVDF) as ($m_{total} = m_1 - m_0$). Step 4: Derive the active material mass as ($m_{active} = m_{total} \times 0.8$) (since LPC accounts for 80% of the solid mixture). Quality control: The active material mass per electrode was controlled within 2.0–2.5 mg to ensure consistent testing conditions. Electrodes with mass deviations >5% were discarded.

1.11 Detailed protocols for GC–MS/GC-FID analysis and yield calculation

1. The monomer yield (wt%) was quantified via the internal standard method with n-decane as the internal standard (IS), following the protocol reported by Shuai et al. (*Science, 2017*) with minor adaptations to match our system. The complete workflow is as follows: (1) Post-reaction sample treatment. After hydrogen-free catalytic depolymerization, the reaction mixture was subjected to solid-liquid separation. The catalyst was thoroughly rinsed with ethanol and deionized (DI) water until the eluate was colorless to recover adsorbed monomers. The combined liquid fractions were concentrated under reduced pressure (40 °C, 0.08 MPa). (2) Extraction of monomers: The concentrated residue was extracted three times with dichloromethane (DCM, 20

mL) and DI water (40 mL) to transfer lignin monomers into the organic phase (DCM). The DCM extracts were combined, and a pre-calibrated volume of n-decane (IS, 10 mg/mL in DCM) was added to ensure a final IS concentration of 0.5 mg/mL.

2. Calibration curve and correction coefficient (η) determination. (1) Standard solutions of the five target monomers (H, G1, G2, S1, S2) were prepared in DCM at concentrations (0.1–1.0 mg/mL) matching the expected monomer concentration in reaction samples, each spiked with the same amount of n-decane (0.5 mg/mL). (2) Each standard solution was analyzed by GC-FID, and the peak area ratio (monomer/IS) was recorded. (3) The correction coefficient (η) for each monomer was calculated by comparing the experimentally measured peak area ratio with the theoretical ratio derived from the added monomer mass and its effective carbon number (ECN). The detailed calculation was as follows:

$$n_{decane} = \frac{W_{decane\ in\ sample}}{MW_{decane}}$$

$$n_{monomer} = \frac{A_{monomer\ in\ sample}}{A_{decane\ in\ sample}} \times n_{decane} \times \frac{ECN_{decane}}{ECN_{monomer}} \times \eta$$

$$W_{monomer} = n_{monomer} \times MW_{monomer}$$

$$Y_{monomer} = \frac{W_{monomer} \times V}{W_{extracted\ lignin}\ or\ W_{klason\ lignin}} \times 100\%$$

In the equations,

$W_{decane\ in\ sample}$ (mg): the weight of decane used as an internal standard in each analyzed sample;

MW_{decane} (mg mmol^{-1}): the molecular weight of decane (142 mg mmol^{-1});

n_{decame} (mmol): the molar amount of decane in each analyzed sample;

$n_{monomer}$ (mmol): the molar amount of monomer in each analyzed sample;

$A_{monomer\ in\ sample}$: the peak area of monomer in the GC-FID chromatogram;

$A_{decane\ in\ sample}$: the peak area of decane in the GC-FID chromatogram;

ECN_{decane}: the effective carbon number (10) of decane;

$ECN_{monomer}$: the effective carbon number of the lignin monomer molecule;

$W_{monomer}$ (mg): the molecular weight of a β-*O*-4-bonded monomer guaiacyl glycerol (196 mg $mmol^{-1}$) or a β-*O*-4-bonded syringyl glycerol (226 mg $mmol^{-1}$) in the analyzed sample;

$Y_{monomer}$: the yield of monomer based on the weight of extracted lignin or original (native) Klason lignin;

$W_{extracted\ lignin}$ (mg): the weight of extracted lignin;

W_{klason} *lignin* (mg): the weight of Klason lignin in the original biomass;

V (mL): the total volume of sample, 1 mL of which was used for GC analysis.

3. Conversion of FID signals to wt%. FID detects organic compounds based on carbon content, and the conversion of peak signals to mass percentage (wt%) involves two key steps. (1) Peak area integration: GC-FID chromatograms were integrated using Agilent OpenLab CDS software, with baseline correction applied to eliminate noise interference. The peak area of each monomer and the IS was recorded. (2) Mass calculation and wt% conversion: a. The mass of each monomer was calculated using the correction coefficient (η), peak area ratio, and IS mass (as shown in the yield formula above). b. Total monomer mass was the sum of the five target monomers' masses. c. Conversion to wt% was achieved by dividing the total monomer mass by the

initial lignin mass and multiplying by 100, ensuring alignment with the manuscript's reported yield units.

4. Carbon-number correction: application of effective carbon number (ECN). We explicitly confirm that carbon-number correction was applied via the effective carbon number (ECN) method, which is a standard approach for FID quantification of aromatic compounds with varying carbon contents. The rationale and details are as follows: (1) Rationale: FID response is proportional to the number of carbon atoms in the molecule. Since the target monomers (H: 8 C, G1/G2: 9 C, S1/S2: 10 C) and IS (n-decane: 10 C) differ in carbon number, ECN correction eliminates systematic bias caused by differential FID responses to carbon atoms in aromatic vs. aliphatic structures. (2) Implementation: The ECN of each monomer was determined based on its chemical structure (accounting for aromatic carbon reactivity), as reported in Shuai et al. (Science, 2017). This correction ensures that monomers with fewer carbon atoms (e.g., H) are not underestimated, and those with more carbon atoms (e.g., S2) are not overestimated.

2. Tables and Figures

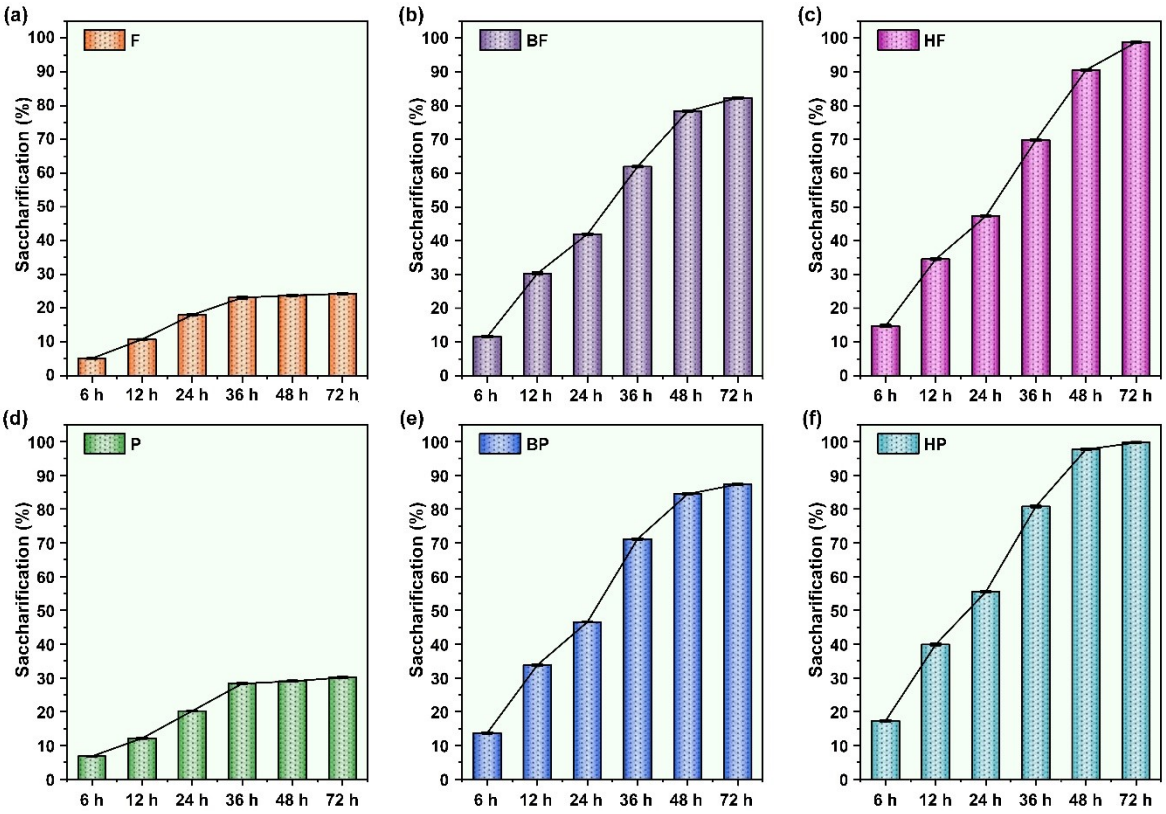

Fig. S1. Glucose yield of enzymatic saccharification of the raw and treated substrates.

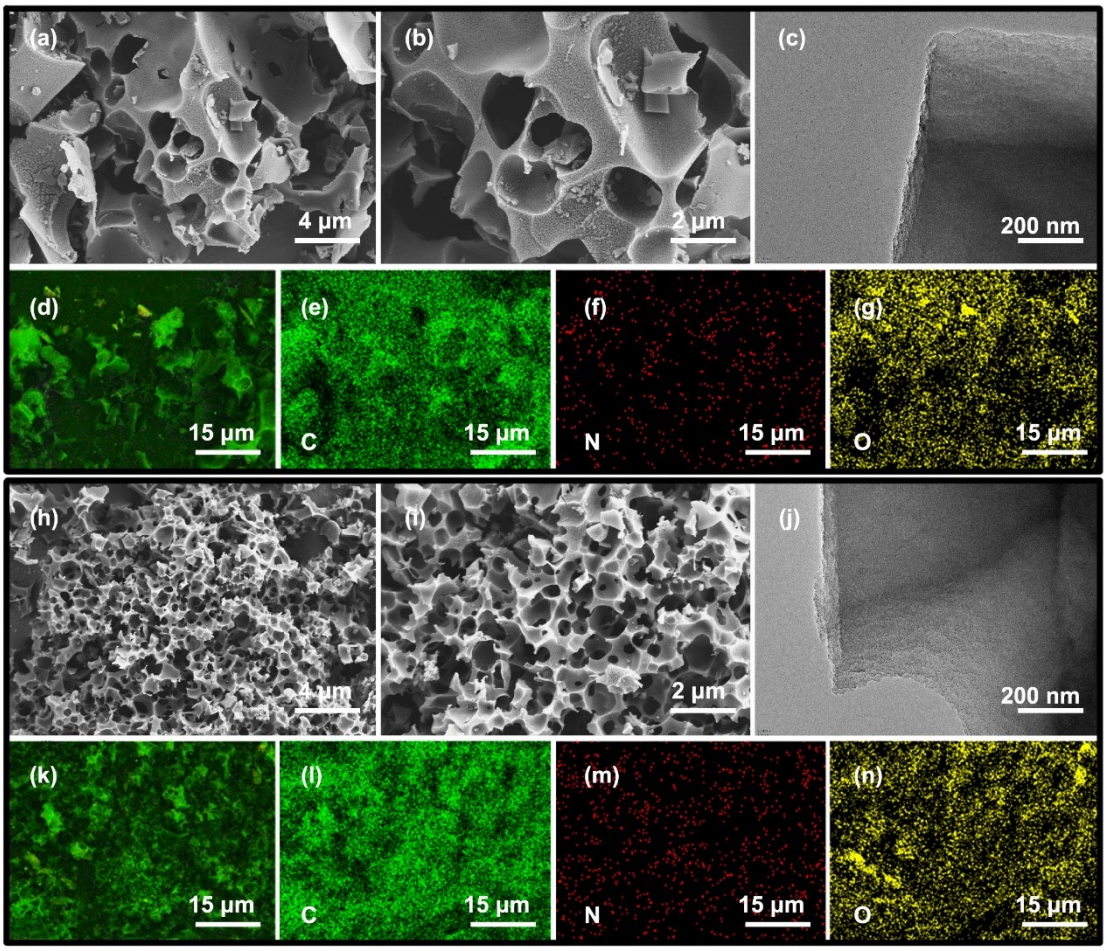

Fig. S2. SEM images: (a, b) LPC-F, (h, i) LPC-P; TEM image: (c) LPC-F, (j) LPC-P; Energy-dispersive X-ray spectroscopy (EDS) element mapping: (d, e, f, g) LPC- F, (k, l, m, n) LPC-P.

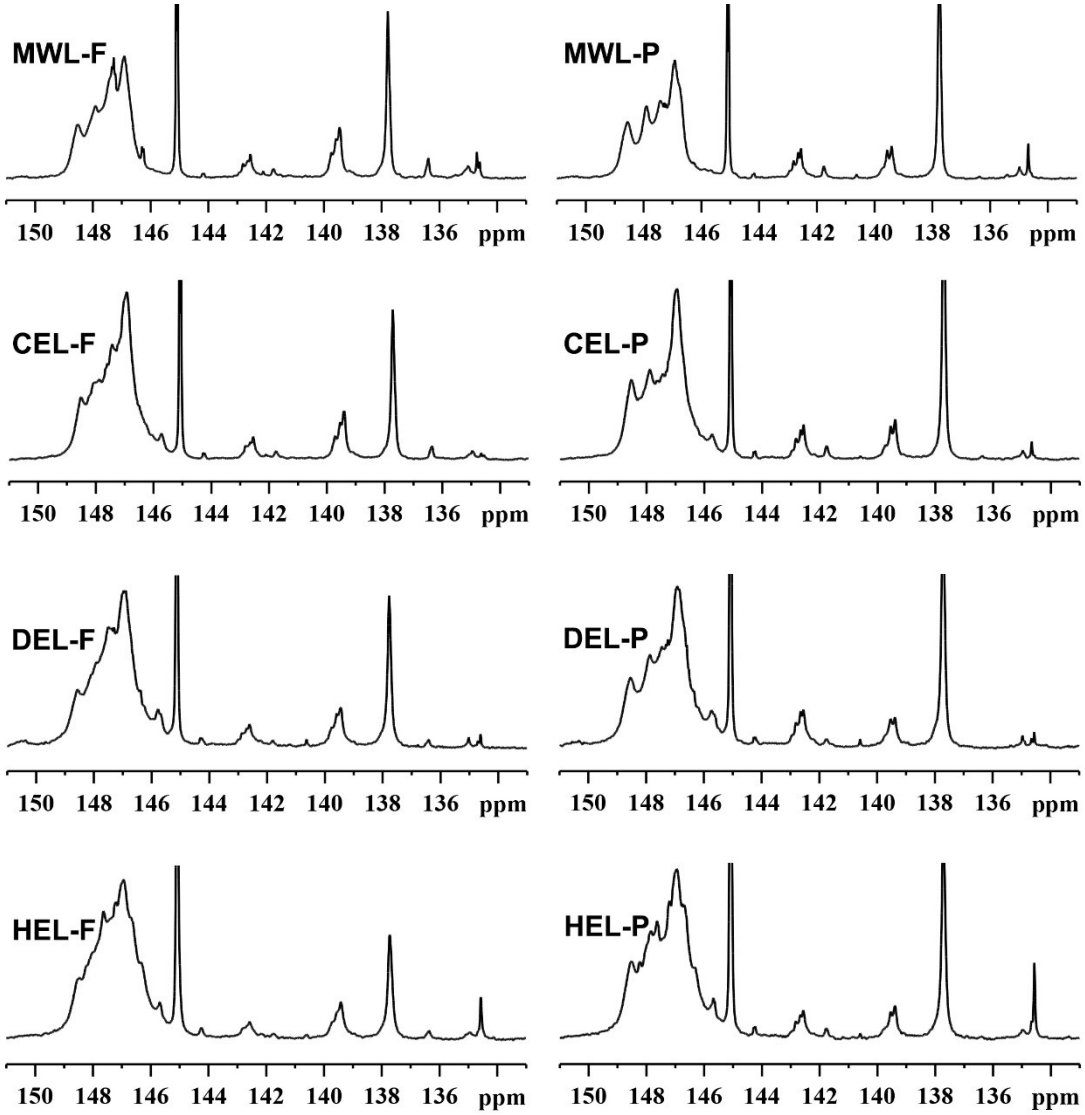

Fig. S3. ^{31}P NMR spectra of the native lignin.

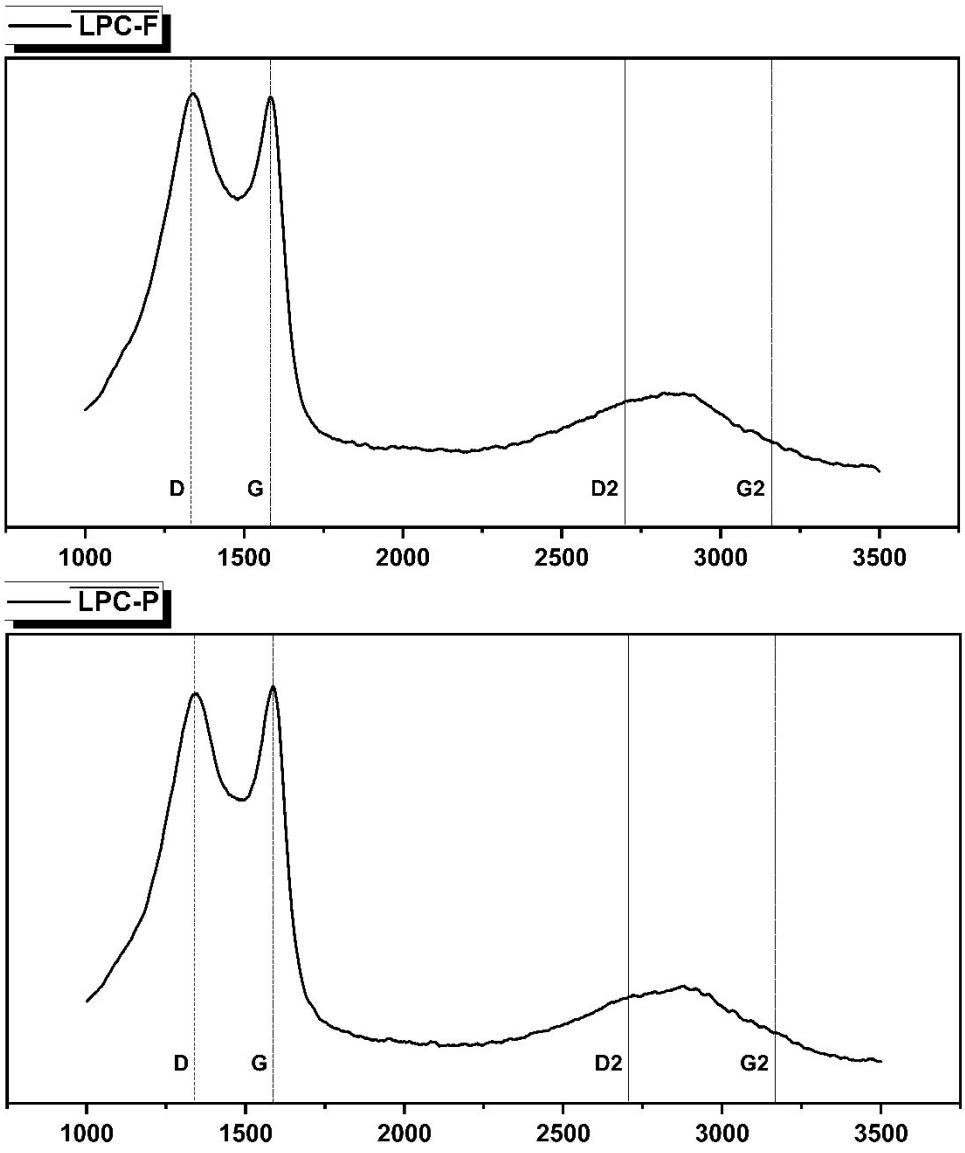

Fig. S4. Raman spectra of lignin-derived porous carbon (LPCs).

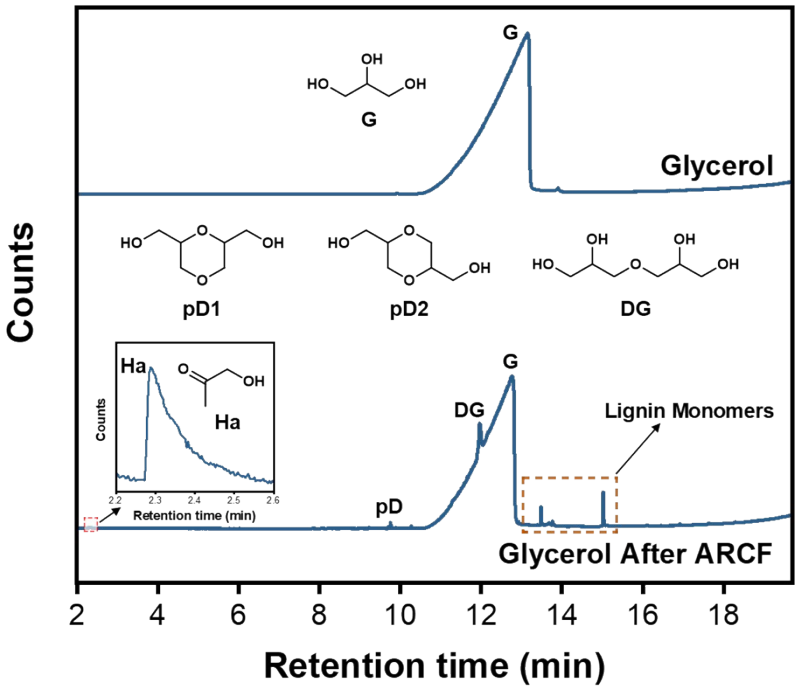

Fig. S5. GC-MS characterization of solvent transformation products following hydrogen-free depolymerization.

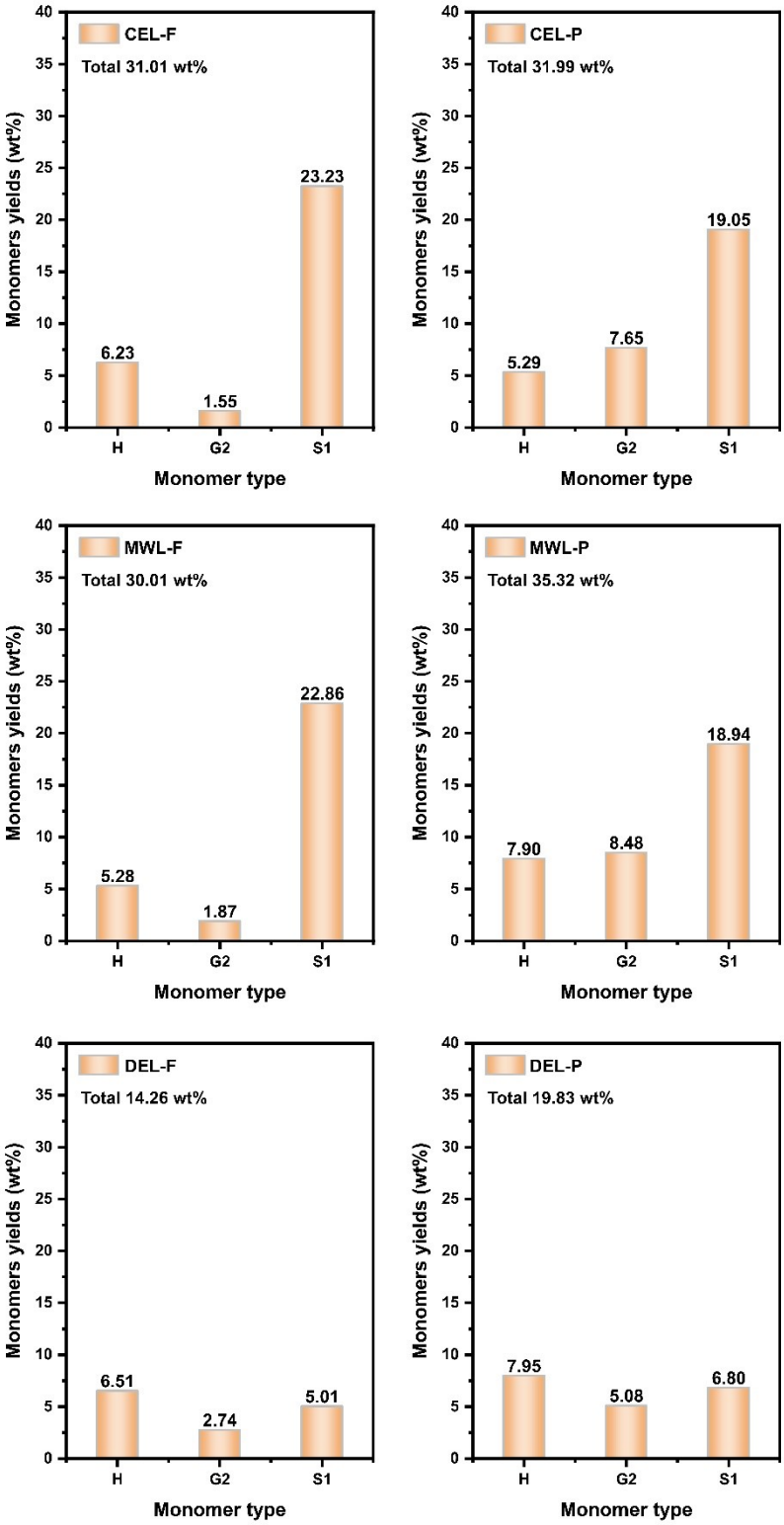

Fig. S6. Hydrogen-free catalytic degradation of native lignin (MWL, CEL and DEL) at the optimal temperature (220°C).

Table S1. Chemical composition of bamboo fiber and parenchyma cells (%).

	Ara [a]	Gal	Glu	Xyl	Man	GluA	GalA	AL	KL
F	0.61±0.11	0.52±0.10	43.36±0.25	22.23±0.17	N.D. [b]	0.66±0.10	N.D.	3.53±0.05	22.53±0.11
P	0.77±0.13	3.00±0.14	35.52±0.23	23.43±0.11	N.D.	0.72±0.10	N.D.	4.47±0.06	24.78±0.09

[a] Ara: araban, Gal: galactan, Glu: glucan, Xyl: xylan, Man: mannan, GalA: Galacturonic acid; GluA: Glucuronic acid, AL: acid soluble lignin, KL: klason lignin;

[b] N.D.: not detected.

Table S2. The yield and carbohydrates analysis of the lignin preparations (%).

	Yield [a] (%)	Ara [b]	Gal	Glu	Xyl	Total
MWL-F	15.82±0.12	0.81±0.02	0.13±0.01	1.31±0.07	5.07±0.13	7.22
MWL-P	17.74±0.21	0.75±0.03	0.12±0.01	1.02±0.08	4.47±0.09	6.36
CEL-F	20.25±0.14	0.91±0.03	0.01±0.01	1.34±0.09	5.27±0.12	7.53
CEL-P	20.53±0.11	0.65±0.02	0.01±0.01	1.07±0.07	4.33±0.10	6.06
DEL-F	97.46±0.15	0.99±0.04	0.21±0.02	1.32±0.08	6.09±0.10	8.61
DEL-P	98.15±0.22	1.20±0.02	0.20±0.02	1.74±0.06	4.66±0.11	7.80
HEL-F	99.21±0.13	1.01±0.04	0.20±0.03	1.37±0.06	4.45±0.08	7.03
HEL-P	99.57±0.14	1.02±0.03	0.20±0.02	1.67±0.07	3.82±0.09	6.71

[a] The yield is calculated based on the Klason lignin.

[b] Ara: araban, Gal: galactan, Glu: glucan, Xyl: xylan, Man: mannan, GalA: Galacturonic acid, GluA: Glucuronic acid.

Table S3. Molecular weights of the lignin samples (g/mol).

Sample	MWL-F	MWL-P	CEL-F	CEL-P	DEL-F	DEL-P	HEL-F	HEL-P
M_w	7100±30	7200±20	7300±30	7500±20	7100±20	7400±20	7200±20	7400±10
M_n	3900±10	3900±10	4200±10	4200±10	3800±10	4000±10	4000±10	3900±10
ĐM	1.82	1.84	1.74	1.78	1.86	1.85	1.80	1.90

Table S4. Quantification of the native lignin fractions by quantitative 2D-HSQC NMR techniques.

Sample	β-*O*-4[a]	β-*O*-4[b]	β-β	β-5	PCE	S/G[c]
MWL-F	52.45	85.35%	6.39	2.61	22.92	1.43
MWL-P	55.96	100%	N.D.[d]	N.D.	44.16	4.08
CEL-F	55.92	85.40%	5.74	3.82	23.75	1.40
CEL-P	59.31	100%	N.D.	N.D.	45.50	3.80
DEL-F	58.53	87.86%	4.31	3.78	29.45	1.76
DEL-P	69.59	100%	N.D.	N.D.	49.74	4.67
HEL-F	62.74	89.14%	4.54	3.10	29.12	1.58
HEL-P	70.86	100%	N.D.	N.D.	48.65	4.82

[a] Result expressed per 100 Ar based on quantitative 2D-HSQC spectra.

[b] Result expressed the relative content of lignin linkages (normalization method).

[c] S/G ratio obtained by this equation: S/G ratio = $0.5\times I(S_{2,6})/I(G_2)/0.5I(H_{2,6})$.

[d] N.D., not detected.

Samples	C (%)	H (%)	O (%)	C_{900} formula	Degree of unsaturation
MWL-F	60.11	5.81	34.08	$C_{900}H_{1044}O_{382}$	379
MWL-P	58.34	6.02	35.64	$C_{900}H_{1114}O_{412}$	344
CEL-F	60.02	5.76	34.22	$C_{900}H_{1037}O_{385}$	383
CEL-P	59.51	6.06	34.43	$C_{900}H_{1100}O_{390}$	351
DEL-F	56.08	5.68	38.24	$C_{900}H_{1095}O_{461}$	354
DEL-P	55.42	5.73	38.85	$C_{900}H_{1116}O_{473}$	343
HEL-F	59.89	5.91	34.20	$C_{900}H_{1066}O_{386}$	368
HEL-P	60.12	6.13	33.78	$C_{900}H_{1101}O_{379}$	350

Table S5. Elemental analysis and C_{900} empirical formula of the lignins.

Table S6. Quantification of the lignin fractions by quantitative ^{31}P-NMR spectroscopy (mmol/g).

Sample	Aliphatic OH	Syringyl OH	Guaiacyl OH		*p*-Hydroxy-phenyl OH	Carboxylic group	Total phenolic OH
			C[a]	NC[b]			
MWL-F	4.06	0.31	0.10	0.51	0.71	0.21	1.63
MWL-P	3.96	0.37	0.08	0.34	0.96	0.18	1.75
CEL-F	4.75	0.29	0.08	0.45	0.64	0.11	1.46
CEL-P	4.30	0.36	0.08	0.33	0.94	0.11	1.71
DEL-F	3.72	0.34	0.07	0.35	0.55	0.10	1.31
DEL-P	3.51	0.42	0.08	0.26	0.76	0.12	1.52
HEL-F	3.71	0.31	0.07	0.31	0.43	0.16	1.12
HEL-P	3.40	0.32	0.07	0.24	0.61	0.20	1.24

[a]C = condensed 5-substitued lignin.

[b]NC = non-condensed.

Table S7. BET surface area (m^2/g) and BJH volume (cm^3/g) of the original and the pretreated substrates.

Sample	BET surface area	BJH pore volume
F	1.77	0.006
P	1.95	0.016
BF	1.93	0.013
BP	2.41	0.021
HF	10.25	0.076
HP	13.85	0.097

Table S8. Assignments (ppm) of ^{13}C-^{1}H cross signals in 2D-HSQC NMR spectra of lignin fractions.

Label	δ_C/δ_H (ppm)	Assignments
C_β	53.1/3.46	C_β-H_β in phenylcoumaran substructures (C)
B_β	53.5/3.05	C_β-H_β in β-β (resinol) substructures (B)
OCH_3	56.4/3.70	C-H in methoxyls (OMe)
D_β	59.8/2.75	C_β-H_β in spirodienones (D)
A_γ	59.9/3.35-3.80	C_γ-H_γ in c-hydroxylated β-*O*-4′ substructures (A)
A'_γ	63.0/4.36	C_γ-H_γ in γ-acylated β-*O*-4′ substructures (A′)
C_γ	62.2/3.76	C_γ-H_γ in β-β′ resinol substructures (C)
I_γ	61.2/4.10	C_γ-H_γ in cinnamyl alcohol end-groups (I)
B_γ	71.0/3.79-4.16	C_γ-H_γ in β-β resinol substructures (B)
A_α	71.8/4.86	C_α-H_α in β-*O*-4′ substructures (A)
D_α	81.0/5.10	C_α-H_α in spirodienones (D)
C_α	86.8/5.45	C_α-H_α in phenylcoumaran (C)
B_α	84.8/4.66	C_α-H_α in β-β resinol (B)
A_β(G)	83.4/4.38	C_β-H_β in β-*O*-4 substructures linked to a G unit (A)
A_β(S)	85.8/4.12	C_β-H_β in β-*O*-4 linked to S (A, *erythro*)
A_β(S)	86.7/4.00	C_β-H_β in β-*O*-4 linked to S (A, *threo*)
$T'_{2,6}$	103.9/7.34	$C'_{2,6}$-$H'_{2,6}$ in tricin (T)
T_6	98.9/6.23	C_6-H_6 in tricin (T)
T_8	94.2/6.60	C_8-H_8 in tricin (T)
$S_{2,6}$	103.9/6.70	$C_{2,6}$-$H_{2,6}$ in syringyl units (S)
$S'_{2,6}$	106.3/7.32	$C_{2,6}$-$H_{2,6}$ in oxidized S units (S′)
G_2	110.8/6.97	C_2-H_2 in guaiacyl units (G)
G_5	114.5/6.70	C_5-H_5 in guaiacyl units (G)
G_6	119.0/6.78	C_6-H_6 in guaiacyl units (G)
$H_{2,6}$	127.7/7.17	$C_{2,6}$-$H_{2,6}$ in H units (H)
$PCE_{2,6}$	130.2/7.48	$C_{2,6}$-$H_{2,6}$ in *p*-coumarate (PCE)
PCE_7	144.8/7.51	C_7-H_7 in *p*-coumarate (PCE)
PCE_8	113.7/6.24	C_8-H_8 in *p*-coumarate (PCE)
FA_2	110.7/7.35	C_2-H_2 in ferulate (FA)
FA_6	123.1/7.20	C_6-H_6 in ferulate (FA)
FA_7	123.1/7.20	C_7-H_7 in ferulate (FA)