Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting Information

Lignin Extraction and Catalytic Upgrading from Genetically Modified Poplar

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1. Instrumentation and Characterization Conditions

HPLC-MS analysis

All HPLC separations for mass spectrometry analysis were performed on a Surveyor Plus HPLC system from Thermo Scientific consisting of a quaternary pump, an autosampler, a photodiode array (PDA) detector, and a Zorbax SB-C18 column. A non-linear gradient of water (A) and acetonitrile (B) was used as follows: 0.00 min, 95% A and 5% B; 10.00 min, 95% A and 5% B; 30.00 min, 40% A and 60% B; 35.00 min, 5% A and 95 %; 38.00min, 5% A and 95% B; 38.50 min, 95% A and 5% B; 45.00 min, 95% A and 5% B. Flowrate of the mobile phase was kept at 500 µL/min. PDA detector was set at the wavelength of 254 nm.

Mass spectrometric analysis (MS, MS^2 and MS^3) of HPLC eluent was performed using a Thermo Scientific linear quadrupole ion trap (LQIT) mass spectrometer equipped with an electrospray ionization (ESI) source. All mass spectrometry experiments were performed under negative ion mode. HPLC eluents were mixed via a T-connector with 1% sodium hydroxide water solution at a flow rate of 0.1 µL/min before entering the ESI source. Addition of sodium hydroxide facilitates deprotonation of the analytes. ESI source conditions were set as: 3.5 kV spray voltage; 50 (arbitrary units) sheath gas (N_2) flow and 20 (arbitrary units) auxillary gas (N_2) flow.

MS^3 analysis was performed using the data dependent scan function of the Thermo Xcalibur software. The most abundant ion formed upon ESI was isolated and subjected to collision-activated dissociation (CAD). The most abundant fragment ion was further selected for isolation and fragmentation. For all MS^3 experiments, an isolation window of 2 m/z units was used along with a normalized collision energy of 30 (arbitrary units).

HPLC-UV analysis

The liquid phase from lignin depolymerization reactions with methanol as solvent was analyzed with Agilent 1260 Infinity Quaternary High-Performance Liquid Chromatography (HPLC) system, using Zorbax Eclipse XDB-C18 Column (250 x 74.6mm) set at 30˚C. The chromatography apparatus is equipped with G1315D Diode Array Detector (DAD). A mixture of H_2O (A) and acetonitrile (B) were used as the mobile phase at a flow rate of 0.5 mL/min. Nonlinear gradient was used (80% A and 20% B from beginning to 5% A and 95 % B at 55.0 min). A fixed amount (400µL) of internal standard benzyl phenyl ether (10 mM) was added into each sample for the quantification purposes. Standard curves for all the aromatic products were made by comparison of the products to internal standard. All results were analyzed and quantified according to standard curves. Before analyzing by HPLC, the liquid samples were filtered through a 0.22µm cutoff syringe filter (2 5mm diameter).

Nuclear Magnetic Resonance (NMR)

All NMR spectra were collected on a Bruker Avance DMX500 spectrometer with a 11.74 Tesla standard-bore superconducting magnet operating at 500.13 and 125.77 MHz for ^1H and ^{13}C nuclei, respectively. C-H correlation spectra were recorded through a phase sensitive gradient enhanced 2D HSQC using echo-antiecho experiment (HSQCETGP experiment). Isolated lignin sample (20mg) was dissolved in 700µL mixture of 5:1 v/v DMSO-d_6/pyridine-d_5 solvent for each experiment.

Scanning Electron Microscopy (SEM)

SEM images were taken on an FEI Nova NanoSEM 650 high resolution instrument, equipped with a high stability Schottky field emission gun and a large specimen chamber. Oxford Inca x-ray EDX system was used as detector, back scattering detector for Z-imaging. Lignin samples were first coated with palladium metal at plasma discharge current 10mA for 100s under argon atmosphere. The instrument was vented to pressure less than 9×10^{-5} torr before use. Electron beam used for image taking was set to voltage at 7 keV.

Gel Permeation Chromatography (GPC)

Gel permeation chromatography (GPC) was carried out on a Waters (Millford, MA) chromatograph equipped with a Waters Alliance high performance liquid chromatography (HPLC) system pump (2695 Separation Module) and two Tosoh TSKgel Super HM-M columns. Detection was provided by a Waters 2414 differential refractometer, and N,N-dimethyl formamide with 0.1% LiBr was used as the mobile phase. Number average molecular weights (Mn) and weight average molecular weights (Mw) were calculated relative to linear polystyrene standards.

2. Supplementary Figures and Tables

Figure S1. SEM images of organosolv lignin extracted from gene modified poplar species by different solvents.

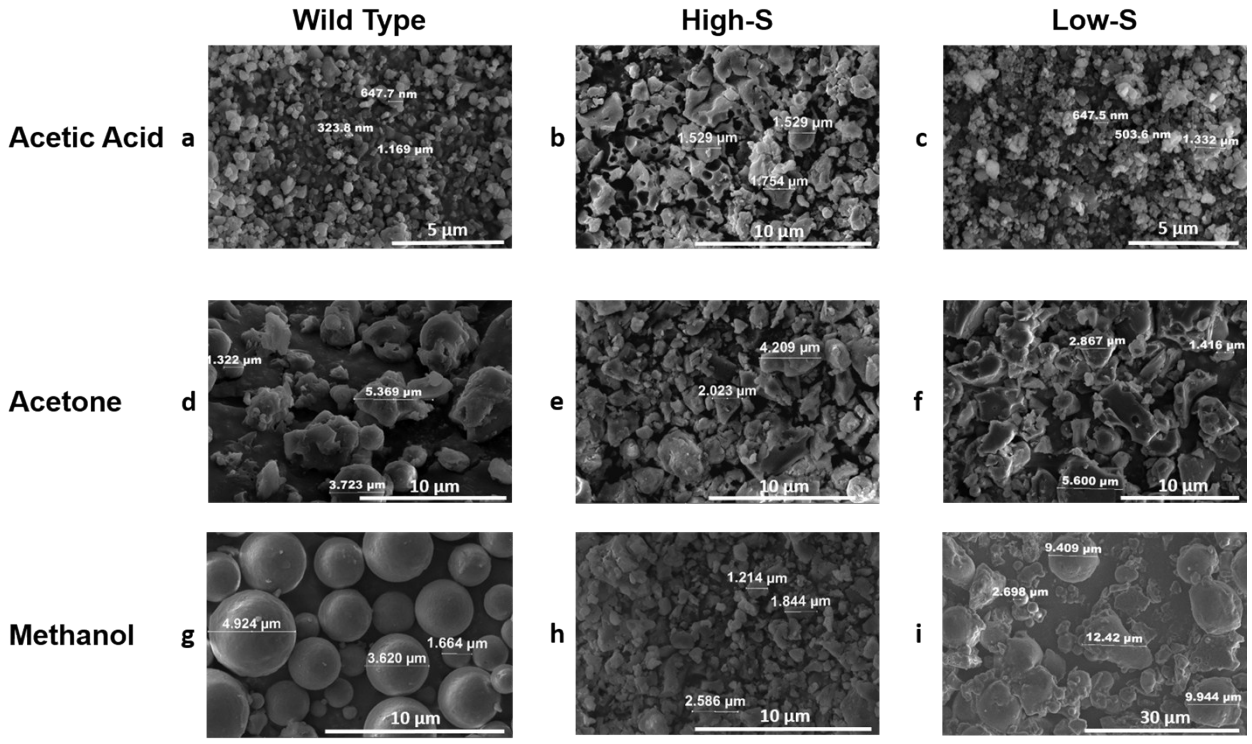
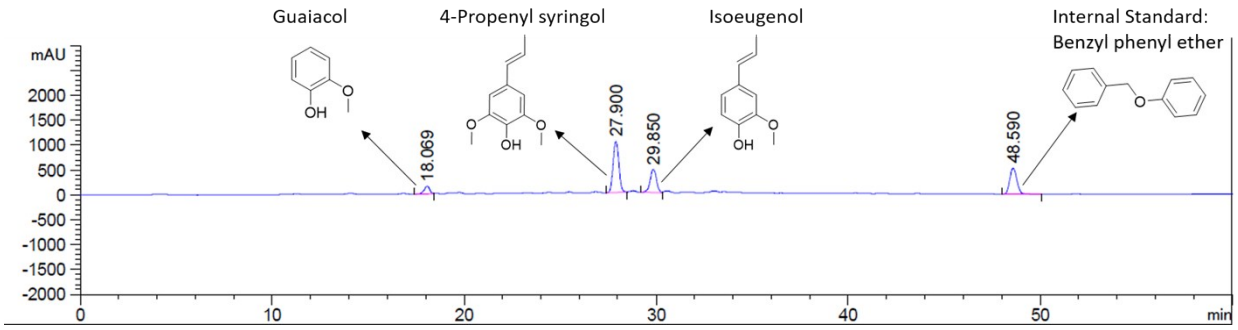


Figure S2. Aromatic products (HPLC-UV) after catalytic depolymerization of (a) organosolv lignin and (b) native lignin.

a



b

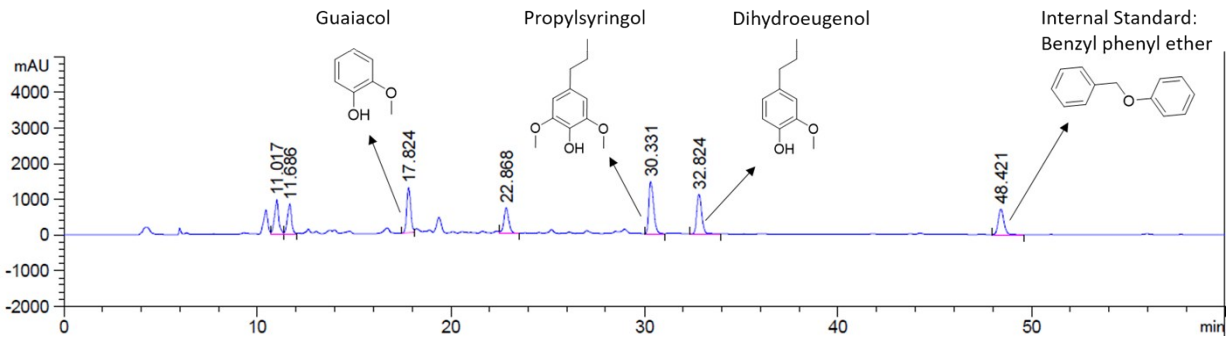
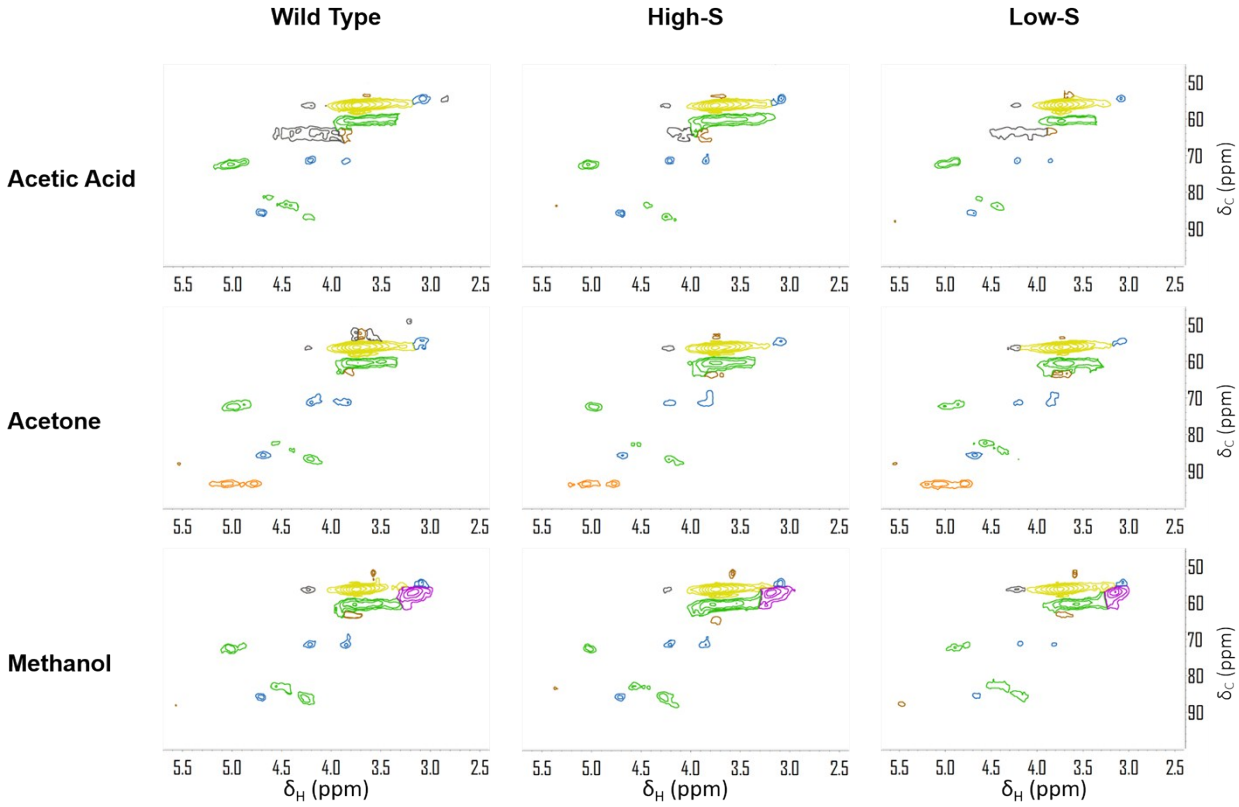
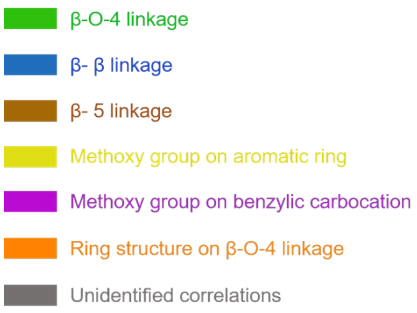


Figure S3. Two dimensional HSQC-NMR spectra on the side chain region (δ_C/δ_H 45-100/2.5-5.5 ppm) of organosolv lignin from different treatments.





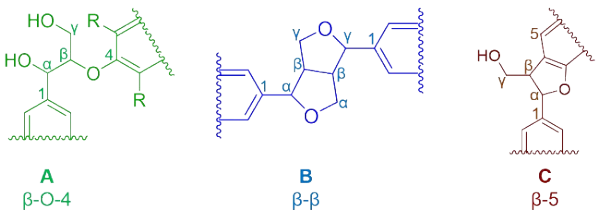


Figure S4. Two dimensional HSQC-NMR spectra on the aromatic region (δ_C/δ_H 95-140/6.0-8.0 ppm) of organosolv lignin from different treatments.

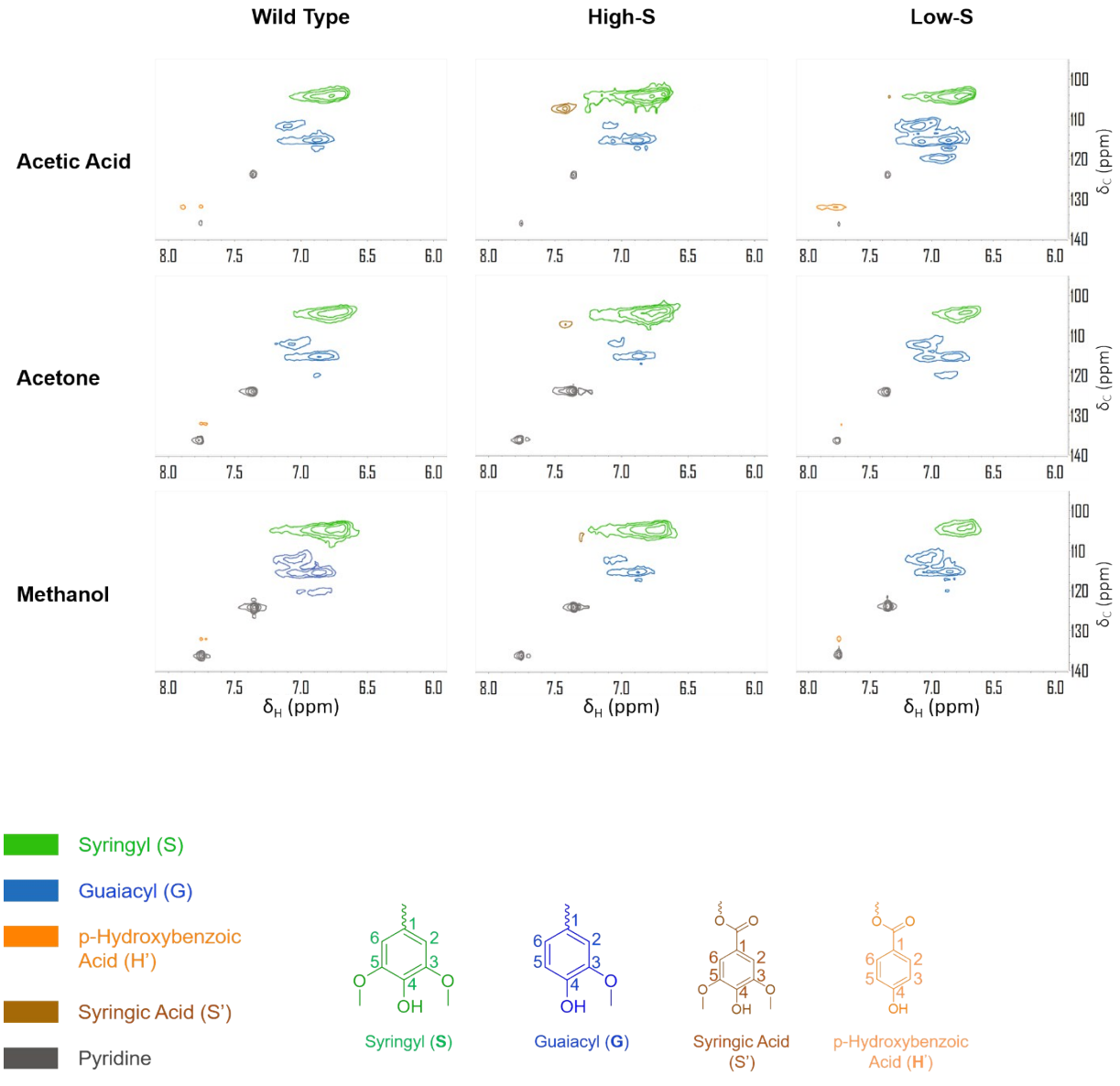
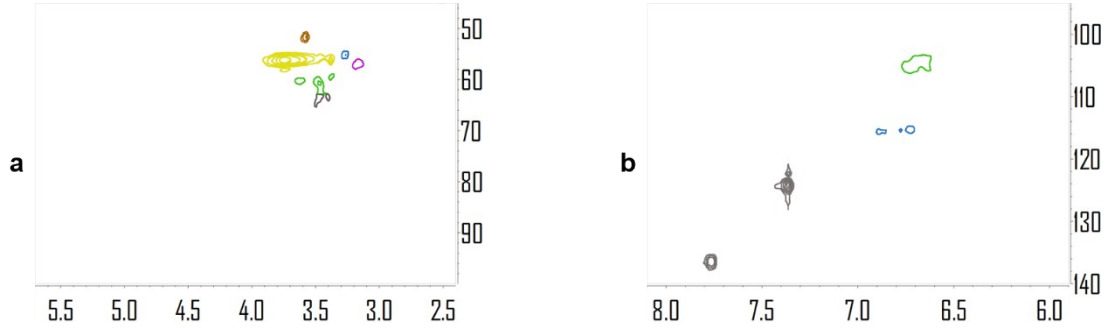


Figure S5. Two dimensional HSQC-NMR spectra of unconverted lignin[a] after catalytic depolymerization of lignin (CDL) reaction, (a) side chain region and (b) aromatic region.



[a] Unconverted lignin from MeOH - wild type poplar organosolv lignin is used as an example.

Table S1. Composition analysis of gene modified poplar species.[1,2]

Substrates	Glucan[a] %	Xylan[a] %	Acid Soluble Lignin[b] %	Acid Insoluble Lignin[b] %	Total Lignin[b] %	Others[c] %	Mass Balance%
Wild Type Poplar	43.4	21.2	5.9	15.5	21.4	12.1	98.1
High-S Poplar	44.5	21.6	5.6	16.7	22.3	6.5	94.9
Low-S Poplar	44.5	22.0	4.6	16.8	21.4	10.3	98.2

[a] Cellulose content was reported as glucan, hemicellulose content was reported as xylan.

[b] Acid soluble lignin (ASL), acid insoluble lignin (AIL) and total lignin content were determined by standard NREL procedures[1].

[c] Including acetyl, ash and other water and ethanol extractives.

Table S2. Relative ratio of S-lignin in poplar substrates.

Substrate	% S-lignin[a]
Wild-Type	64
High-S	82
Low-S	34

[a] Lignin composition as determined by DFRC (derivatization followed by reductive cleavage) analysis.

Mass balance analysis for catalytic depolymerization of organosolv lignin.

To perform mass balance analysis, methanol solvent in the final product mixture was fully evaporated under vacuum. 5 mL of H_2O was used to precipitate the recovered lignin. The H_2O phase was then extracted with 5 mL of toluene three times. After extraction, all monomeric phenolic compounds went to organic phase and oligomers as well as recovered organosolv lignin remained in aqueous phase. After separation of two layers, solvent was removed under vacuum in order to analyze the desired components.

Table S3. Mass balance calculation after catalytic depolymerization of organosolv lignin.[a]

Substrates[b]	Toluene soluble aromatics%	Water precipitated oligomer & unconverted organosolv lignin%	Total%[c]
AA-Wild type	20	72	92
AA-High-S	21	67	88
AA-Low-S	25	52	87
Acetone-Wild type	35	57	92
Acetone-High-S	31	65	96
Acetone-Low-S	28	64	92
Methanol-Wild type	60	36	96
Methanol-High-S	63	28	91
Methanol-Low-S	56	29	85

[a] Organosolv lingin (50 mg) with 10wt% Ni/C catalyst in 20 mL of MeOH, at 225 °C and 35 bar H_2 for 12 h.

[b] Organosolv lignin samples were named as the solvent used for extraction followed by the specific poplar species. For example, AA-Wild type means wild type poplar being extracted by acetic acid/formic acid solvent, Methanol-Wild type means wild type poplar being extracted by methanol, etc.

[c] Total mass balance is calculated based on initial organosolv lignin substrate.

Table S4. Assignments on the common carbon-proton correlations of organosolv lignin in HSQC-NMR spectra (a) side chain region and (b) aromatic region.

a

Carbon-proton correlations	Assignments[I]
71.0-72.0/4.9-5.1	A_α
83.0-84.0/4.3-4.5	A_β (H,G)[II]
86.0-87.0/4.1-4.3	A_β (S)[III]
60.0-61.0/3.4-3.7	A_γ
84.0-85.0/4.6-4.7	B_α
53.0-54.0/3.0-3.1	B_β
71.0-72.0/3.8; 71.0-72.0/4.2	B_γ
86.8-87.1/5.5-5.6	C_α
53.0-54.0/3.5-3.6	C_β
61.6-62.5/3.7-3.8	C_γ

[I] For the assignments on carbon-proton correlations for the side chain region, A is used to represent β-O-4 linkage, B is used to represent β-β linkage, C is used to represent β-5 linkage. A_α is for the α position of β-O-4 linkage, B_α for the α position of β-β linkage, C_α for the α position of β-5 linkage, etc.

[II] β position of β-O-4 linkage in H and G type lignin.

[III] β position of β-O-4 linkage in S type lignin.

b

Carbon-proton correlations	Assignments[I]
128.0-129.0/7.2-7.3	$H_{2,6}$
114.0-115.0/6.7-6.9	$H_{3,5}$
110.0-111.0/7.0-7.1	G_2
114.0-115.0/6.7-6.9	G_5
118.0-119.0/6.8-6.9	G_6
103.0-104.0/6.6-6.8	$S_{2,6}$
130.0-131.0/7.7-7.8	$H'_{2,6}$[II]
105.0-106.0/7.3-7.4	$S'_{2,6}$[III]

[I] For the assignments on carbon-proton correlations for the aromatic region, H is used to represent H type lignin, G is used to represent G type lignin, S is used to represent S type lignin. $H_{2,6}$ for the 2 and 6 positions of H lignin, G_2 for the 2 position of G lignin, $S_{2,6}$ for the 2 and 6 positions of S lignin, etc.

[II] Oxidized H unit (p-Hydroxybenzoic Acid).

[III] Oxidized S unit (Syringic Acid)

3. References

1. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass*; Technical Report: NREL/TP-510-42618, Issue Date, 08/03/2013; National Renewable Energy Laboratory: Golden, CO, 2012.

2. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of sugars, byproducts, and degradation products in liquid fraction process samples;* Technical Report: NREL/TP-510-42623, Issue Date, 12/08/2006; National Renewable Energy Laboratory: Golden, CO, 2006.