SUPPORTING INFORMATION

Performance of Three Delignifying Pretreatments on Hardwoods: Hydrolysis Yields, Comprehensive Mass Balances, and Lignin Properties

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	Pretreatment	**Glucan** (% by mass)	**Xylan** (% by mass)	**Klason Lignin** (% by mass)	**Other or Unquantified** (% by mass)	**Mass Yield** (% by mass)
Hybrid Poplar	Untreated	44.0	15.6	24.0	16.4	100.0
	Cu-AHP 1st Stage	61.5	13.5	19.5	5.5	70.2
	Cu-AHP 2nd Stage	67.5	14.2	10.8	7.5	59.4
	[Ch][Lys]	61.1	8.8	17.8	12.2	67.8
	CELF	90.5	2.5	7.1	0.0	46.8
Eucalyptus	Untreated	32.8	14.4	30.0	22.8	100.0
	Cu-AHP 1st Stage	54.0	16.3	20.4	8.4	63.0
	Cu-AHP 2nd Stage	59.9	11.7	19.8	8.7	54.0
	[Ch][Lys]	55.6	8.6	22.5	13.3	53.4
	CELF	79.2	3.1	11.4	6.3	37.1

Table S1. Composition and mass yields of hybrid poplar and eucalyptus prior to and following pretreatment. Cu-AHP: Cu-catalyzed alkaline hydrogen peroxide pretreatment; [Ch][Lys]: cholinium lysinate pretreatment; CELF: co-solvent enhanced lignocellulosic fractionation.

δ_C/δ_H (ppm) lignin samples										Assignments
Eucalyptus					**Hybrid Poplar**					
a	b	c	d	e	f	g	h	i	j	
53.50 /3.03	53.50 /3.02	53.50 /3.02	53.53 /3.04	52.99 /2.99	53.61 /3.03	53.20 /3.03	53.20 /3.03	53.26 /3.04	52.84 /2.94	C_β–H_β in phenylcoumaran (β-5')
55.62 /3.71	55.70 /3.73	55.48 /3.70	55.65 /3.63	55.63 /3.69	55.58 /3.71	55.35 /3.72	55.57 /3.74	55.28 /3.72	55.41 /3.71	C–H in methoxy
59.87 /3.18	59.90 /3.18	59.44 /3.35	59.27 /3.24	59.16 /3.14	59.49 /3.35	59.06 /3.35	59.28 /3.37	59.42 /3.62	59.08 /3.35	Cγ–Hγ in β-O-4'
59.78 /3.62	59.75 /3.61	59.44 /3.68	59.71 /3.63	-	59.72 /3.62	59.51 /3.59	59.35 /3.71	-	59.30 /3.69	
-	62.94 /3.84	62.49 /3.38	-	63.06 /3.85	-	62.82 /3.85	-	-	62.88 /3.86	Cγ–Hγ in resinol (β-β')
71.03 /3.78	70.71 /3.74	71.17 /3.79	71.07 /3.77	-	71.01 /3.78	70.98 /3.73	71.18 /3.78	70.62 /3.77	-	Cγ–Hγ in phenylcoumaran (β-5')
71.08 /4.15	70.71 /4.13	71.02 /4.15	70.97 /4.16	-	-	70.70 /4.14	-	-	-	
71.75 /4.82	71.62 /4.82	72.08 /4.83	71.65 /4.79	-	71.75 /4.82	71.48 /4.83	71.62 /4.84	70.79 /4.72	71.63 /4.84	Cα–Hα in β-O-4'
83.27 /4.28	-	83.66 /4.25	83.35 /4.27	-	83.81 /4.26	83.30 /4.26	83.36 /4.30	83.29 /4.27	-	C_β–H_β in β-O-4' linked to a guaiacyl unit
-	85.03 /4.58	84.87 /4.62	85.0 /4.61	-	-	84.70 /4.62	84.79 /4.65	84.61 /4.60	-	Cα–Hα in β-5'
85.94 /4.07	85.94 /4.07	85.94 /4.07	85.90 /4.09	-	85.96 /4.08	85.46 /4.10	85.72 /4.10	85.49 /4.10	-	Cβ–Hβ in β-O-4' linked to a syringyl unit
-	-	-	-	-	-	86.53 /5.45	86.71 /5.45	86.63 /5.42	-	Cα–Hα in resinol (β-β')
103.89 /6.67	103.30 /6.58	103.96 /6.68	103.23 /6.59	103.96 /6.67	103.89 /6.67	103.70 /6.67	103.86 /6.69	103.09 /6.60	103.68 /6.70	$C_{2,6}$–$H_{2,6}$ in syringyl units (*S*)
106.14 /7.04	106.60 /7.19	106.50 /7.19	106.30 /7.30	106.17 /7.29	106.09 /7.03	105.91 /7.29	106.36 /7.20	105.98 /7.30	-	$C_{2,6}$–$H_{2,6}$ in oxidized syringyl units (*S*')
106.2 /7.22	106.09 /7.29	106.19 /7.29	106.81 /7.20	106.85 /6.48	106.02 /7.22	106.50 /7.20	-	106.46 /7.13	-	

110.81 /6.94	109.24 /7.10	110.86 /6.95	111.01 /6.98	110.99 /6.95	110.97 /6.98	110.67 /6.96	110.92 /6.97	110.76 /6.91	110.73 /6.98	C_2–H_2 in guaiacyl units (*G*)
-	-	-	110.23 /7.36	112.96 /7.19	-	-	111.85 /7.37	-	-	C_2–H_2 in oxidized guaiacyl units (*G'*)
114.55 /6.67	115.02 /6.67	114.92 /6.93	115.09 /6.73	114.61 /6.69	114.96 /6.76	114.29 /6.67	114.40 /6.69	114.73 /6.77	114.35 /6.73	$C_{3,5}$–$H_{3,5}$ in p-benzoate units (PB)
114.86 /6.92	114.95 /6.82	114.75 /6.92	115.15 /6.94	114.81 /6.92	114.69 /6.90	114.82 /6.95	114.96 /6.96	114.69 /6.87	114.78 /6.94	C_5–H_5 in guaiacyl units (*G*)
118.86 /6.75	117.97 /6.59	118.99 /6.80	118.74 /6.82	119.02 /6.77	118.90 /6.74	118.65 /6.77	118.86 /6.82	118.36 /6.75	118.74 /6.8	C_6–H_6 in guaiacyl units (*G*)
-	128.63 /7.20	-	-	128.43 /7.20	-	-	-	-	-	$C_{2,6}$–$H_{2,6}$ in *p*-hydroxylphenyl units (*H*)
131.47 /7.67	-	-	-	-	131.26 /7.64	131.02 /7.77	-	130.91 /7.64	130.92 /7.66	$C_{2,6}$–$H_{2,6}$ in *p*-benzoate units (PB)

Note: (-) not detected. (a) native lignin eucalyptus, (b) Pre-extraction eucalyptus, (c) Cu-AHP eucalyptus, (d) CELF eucalyptus, (e) [Ch][Lys] eucalyptus, (f) native lignin poplar, (g) Pre-extraction poplar, (h) Cu-AHP poplar, (i) CELF poplar, (j) [Ch][Lys] poplar

Table S2. Assignments of signals observed in $^{13}C/^{1}H$ 2D (HSQC) NMR spectra of lignins.

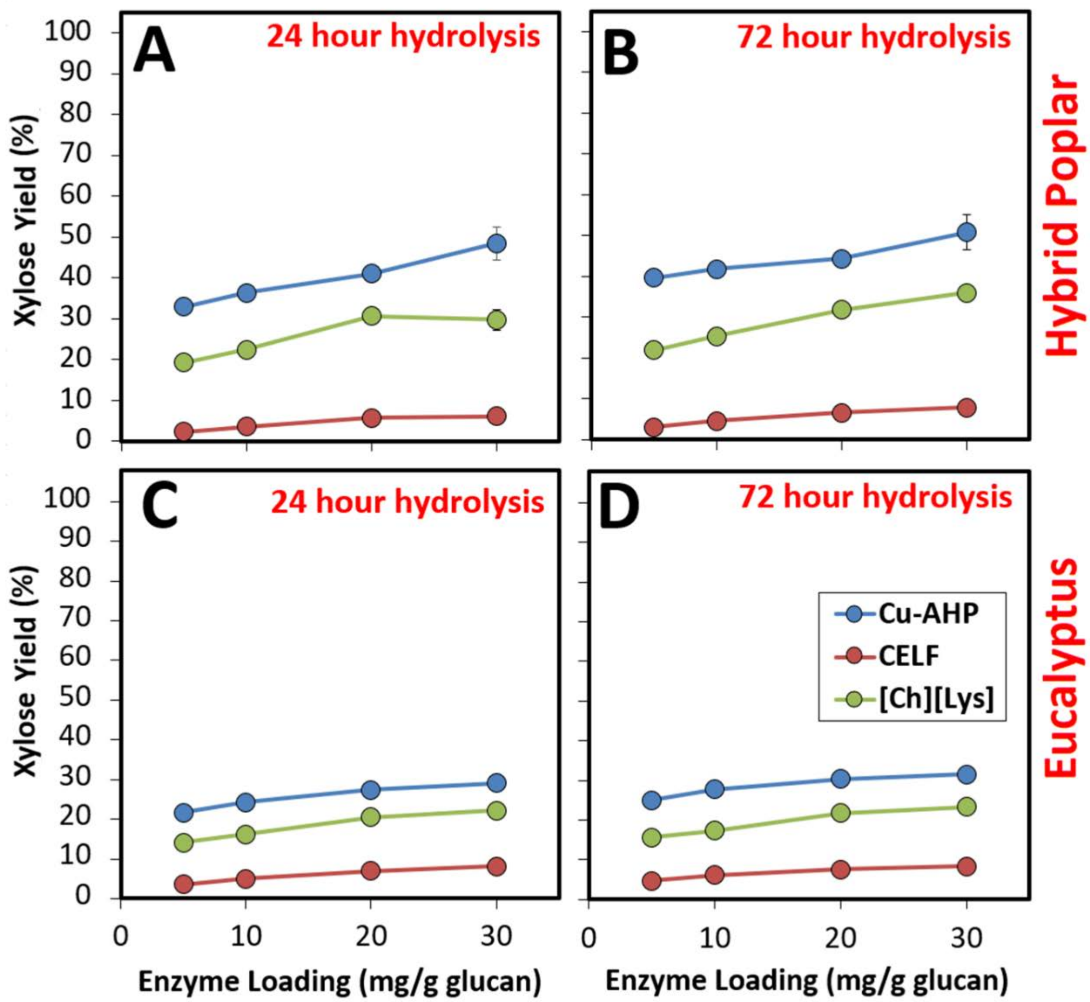
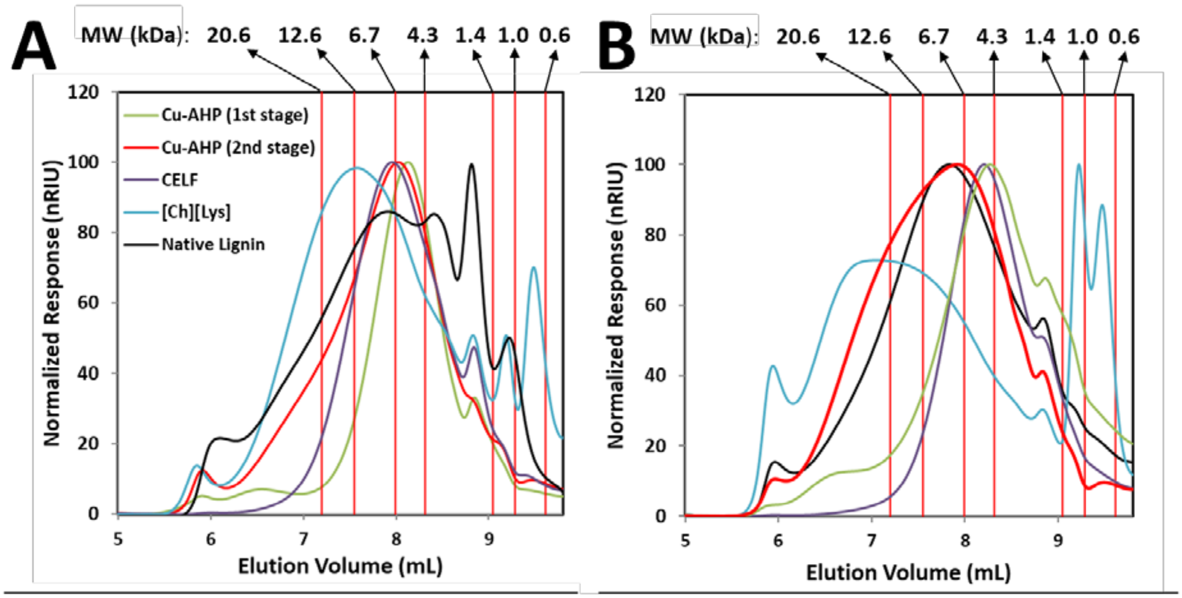


Figure S1: Enzymatic hydrolysis xylose yields for pretreated solids of hybrid poplar (**A** and **B**) and eucalyptus (**C** and **D**) prepared by Cu-AHP, CELF, and [Ch][Lys] pretreatments as a function of enzyme loading (mg protein/g glucan in pretreated solids) and hydrolysis time. Hydrolysis was performed at a 10% (wt/vol) solids loading with the pH buffered at 5.0 for 24 or 72 h.



Lignin	M_N	M_W	PDI
Cu-AHP (1st stage)	6,000	9,100	1.5
Cu-AHP (2nd stage)	12,400	27,300	2.2
CELF	7,500	12,600	1.7
[Ch][Lys]	13,158	29,390	2.2
Native lignin	12,470	43,400	3.5

Eucalyptus

Lignin	M_N	M_W	PDI
Cu-AHP (1st stage)	5,800	13,000	2.2
Cu-AHP (2nd stage)	16,800	43,200	2.6
CELF	5,200	8,500	1.6
[Ch][Lys]	18,100	41,400	2.3
Native lignin	15,100	48,400	3.2

Hybrid Poplar

Figure S2: Size exclusion chromatography (SEC) elution profiles and estimated values for number average molar mass ($\overline{M}_N$), weight average molar mass ($\overline{M}_W$), and polydispersity index (PDI) for **(A)** eucalyptus and **(B)** hybrid poplar. SEC was performed using a Waters Ultrahydrogel™ 250 column with a mobile phase comprising a 80:20 (v/v) solution of 0.1 M $NaNO_3$:0.005 M NaOH/CH_3CN.

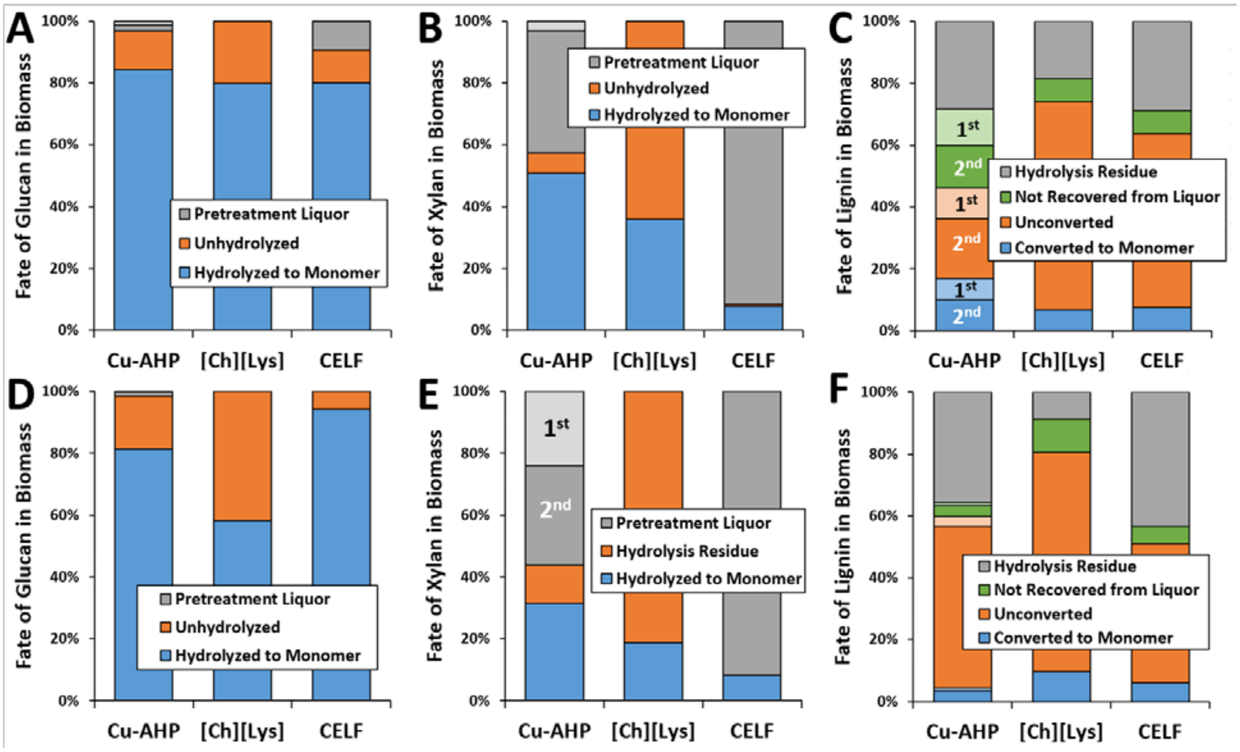
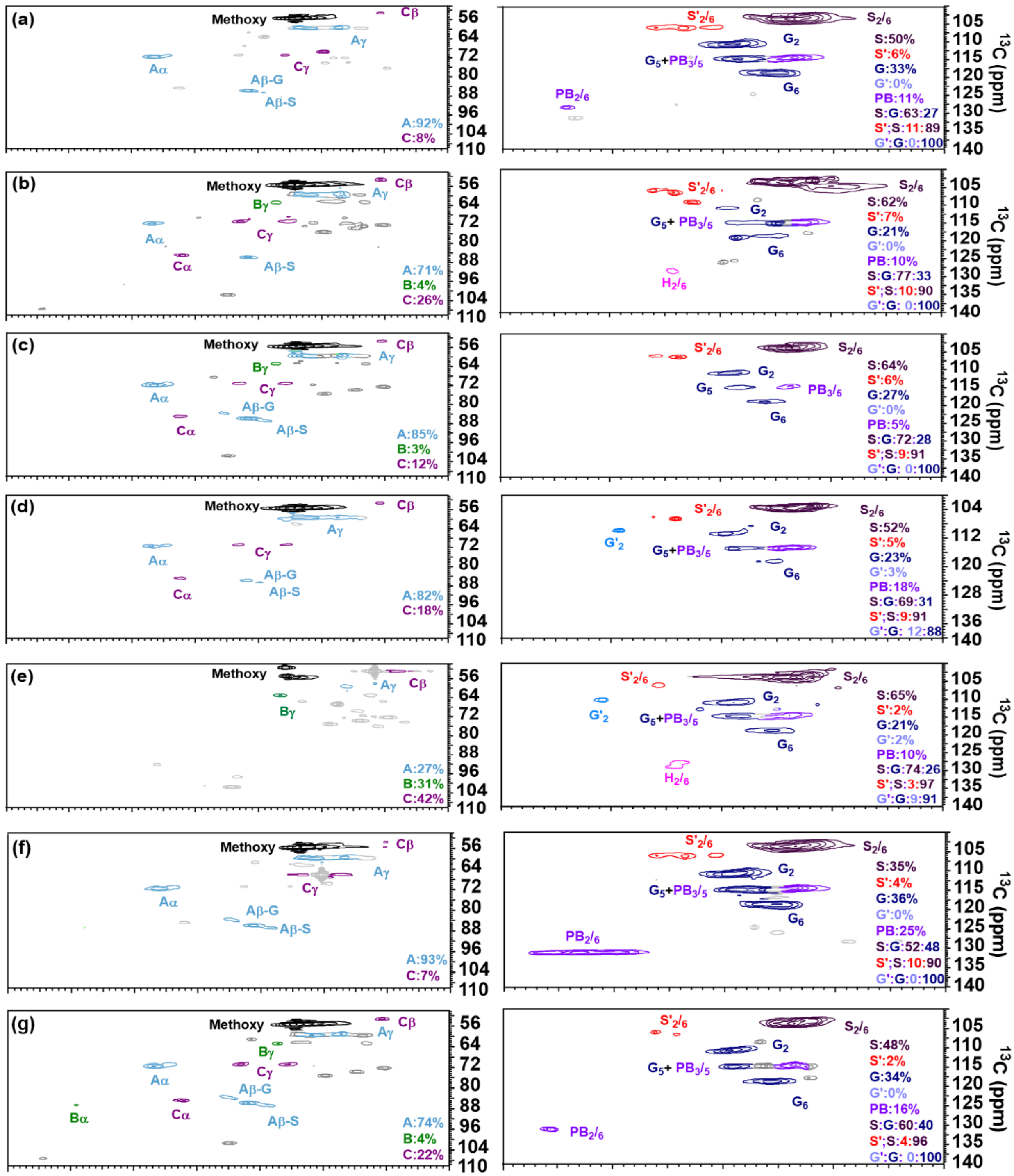


Figure S3: Summary of the fate of the (A, D) glucan, (B, E) xylan, and (C, F) lignin for the three pretreatments for (A, B, C) hybrid poplar and (D, E, F) eucalyptus. Hydrolysis yields are for 30 mg/g glucan enzyme loading for 72 h.



(a)
Methoxy
Cβ
Aγ
Aα
Cγ
Aβ-G
Aβ-S
A:92%
C:8%
S'2/6
S2/6
G2
G5+PB3/5
G6
PB2/6
S:50%
S':6%
G:33%
G':0%
PB:11%
S:G:63:27
S';S:11:89
G':G:0:100
(b)
Methoxy
Cβ
Aγ
Bγ
Aα
Cγ
Cα
Aβ-S
A:71%
B:4%
C:26%
S'2/6
S2/6
G2
G5+ PB3/5
G6
H2/6
S:62%
S':7%
G:21%
G':0%
PB:10%
S:G:77:33
S';S:10:90
G':G: 0:100
(c)
Methoxy
Cβ
Aγ
Bγ
Aα
Cγ
Aβ-G
Aβ-S
Cα
A:85%
B:3%
C:12%
S'2/6
S2/6
G2
G5
PB3/5
G6
S:64%
S':6%
G:27%
G':0%
PB:5%
S:G:72:28
S';S:9:91
G':G: 0:100
(d)
Methoxy
Cβ
Aγ
Aα
Cγ
Aβ-G
Aβ-S
Cα
A:82%
C:18%
S'2/6
S2/6
G'2
G2
G5+PB3/5
G6
S:52%
S':5%
G:23%
G':3%
PB:18%
S:G:69:31
S';S:9:91
G':G: 12:88
(e)
Methoxy
Aγ
Cβ
Bγ
A:27%
B:31%
C:42%
S'2/6
S2/6
G'2
G2
G5+PB3/5
G6
H2/6
S:65%
S':2%
G:21%
G':2%
PB:10%
S:G:74:26
S';S:3:97
G':G:9:91
(f)
Methoxy
Cβ
Aγ
Cγ
Aα
Aβ-G
Aβ-S
A:93%
C:7%
S'2/6
S2/6
G2
G5+PB3/5
G6
PB2/6
S:35%
S':4%
G:36%
G':0%
PB:25%
S:G:52:48
S';S:10:90
G':G:0:100
(g)
Methoxy
Cβ
Aγ
Bγ
Cγ
Aα
Aβ-G
Aβ-S
Bα
Cα
A:74%
B:4%
C:22%
S'2/6
S2/6
G2
G5+ PB3/5
G6
PB2/6
S:48%
S':2%
G:34%
G':0%
PB:16%
S:G:60:40
S';S:4:96
G':G: 0:100
56
64
72
80
88
96
104
110
105
110
115
120
125
130
135
140
13C (ppm)

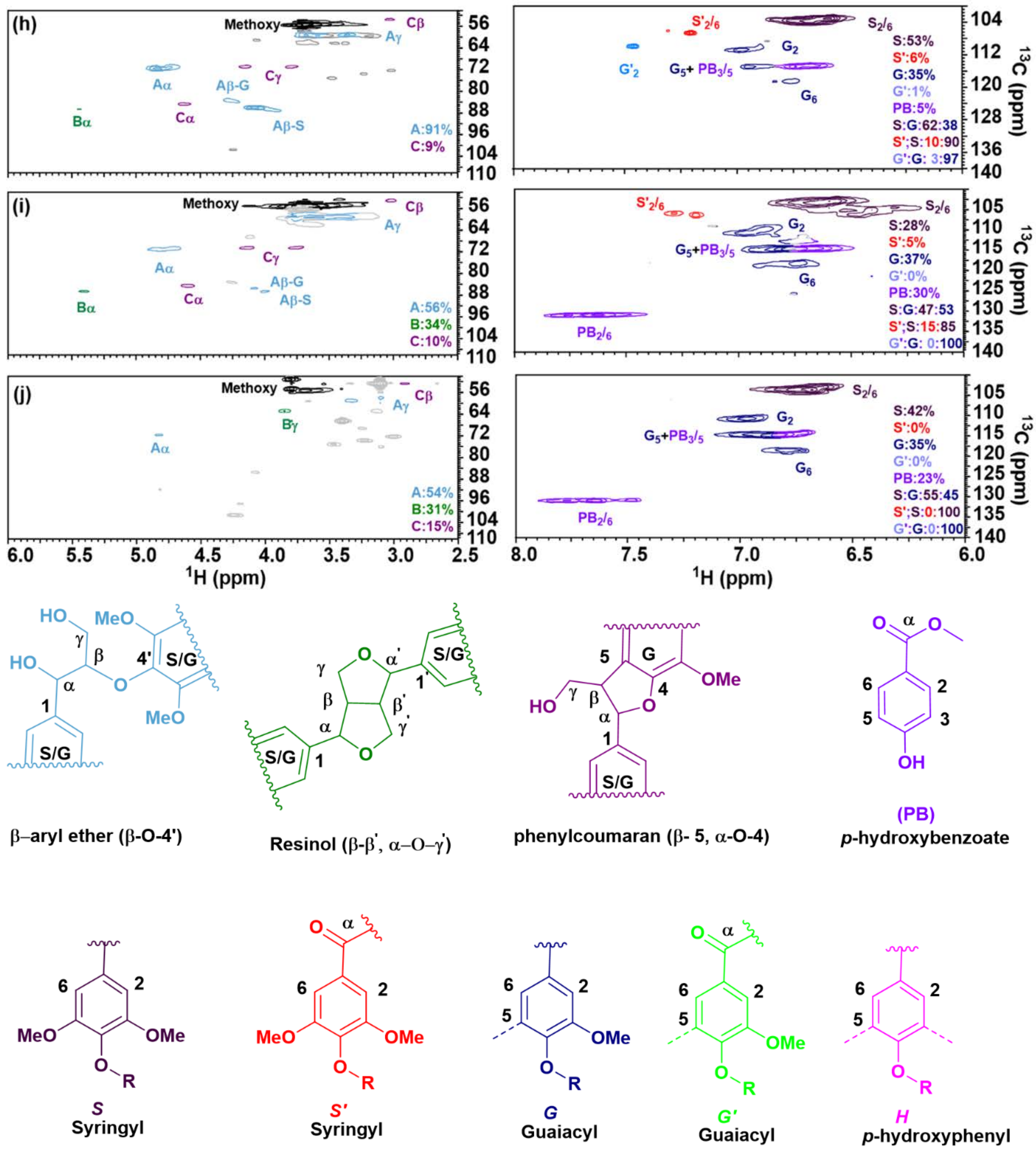


Figure S4: $^{13}C/^{1}H$ 2D (HSQC) NMR spectra of (a) native cellulolytic eucalyptus lignin, (b) eucalyptus 1st-stage Cu-AHP lignin, (c) eucalyptus 2nd-stage Cu-AHP lignin, (d) eucalyptus CELF lignin, (e) eucalyptus [Ch][Lys] lignin, (f) native cellulolytic poplar lignin, (g) poplar 1st-stage Cu-AHP lignin, (h) poplar 2nd-stage Cu-AHP lignin, (i) poplar CELF lignin, and (j) poplar [Ch][Lys] lignin. All NMR spectra were recorded in DMSO-d_6 solvent and signals at δ= 2.5 ppm for ^{1}H NMR and δ= 39.50 ppm for ^{13}C NMR were considered as reference peaks to assign the 2D (HSQC) NMR signals.

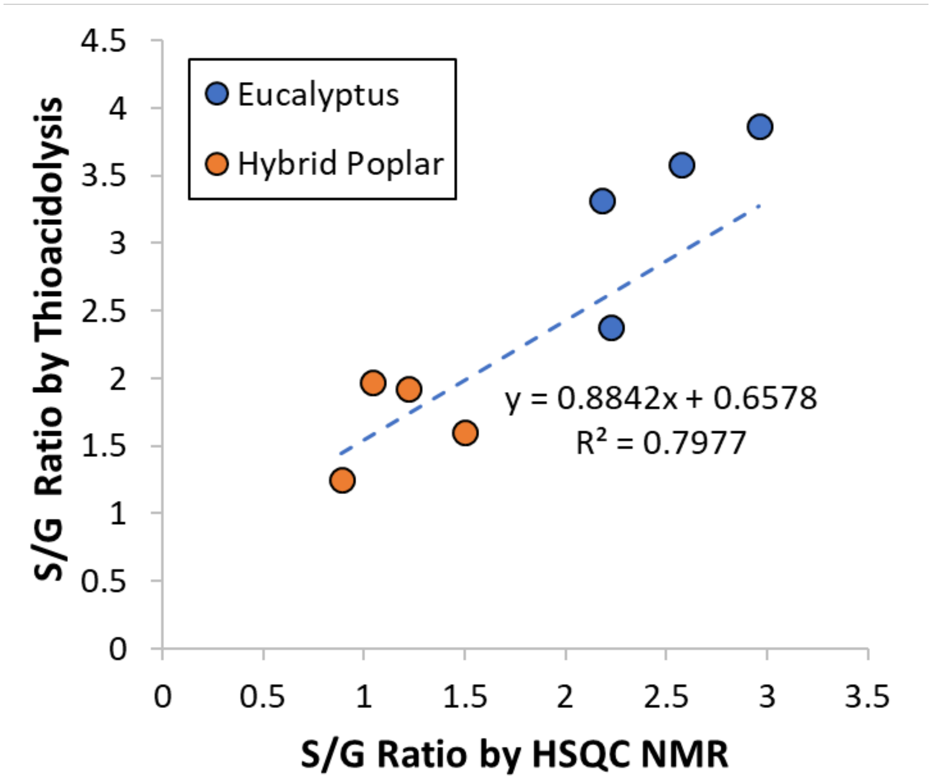


Figure S5: Comparison of S/G ratios as determined by quantitative thioacidolysis versus (semi-quantitative) 2-D HSQC NMR.