Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting Information

Deciphering lignin heterogeneity in ball milled softwood; Unravelling the synergy between supramolecular cell wall structure and molecular events

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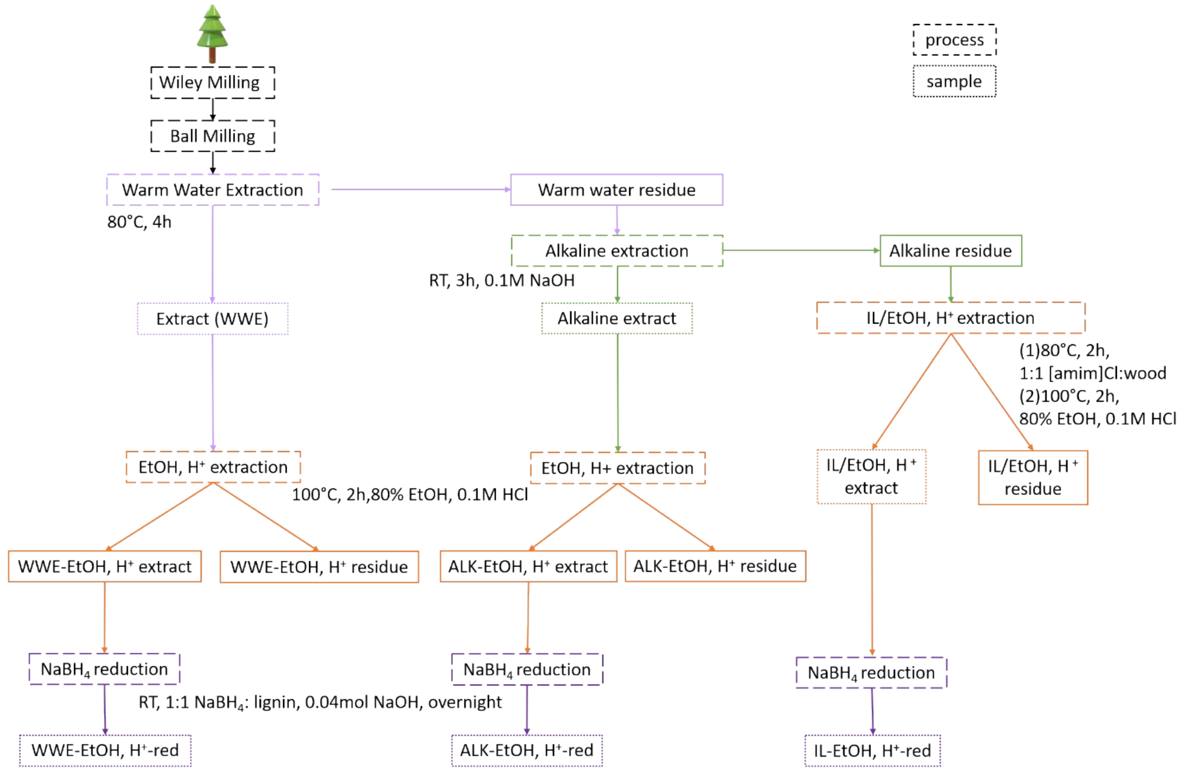


Figure S1: The extraction protocol.

For the relative determination of lignin and hemicelluloses in the extracted fractions, the following equations (S1&S2) were used. In the first one, for the determination of the hemicelluloses extracted in the fraction, the C2-Ar signal of the phenyl propane unit is integrated and set to 100 (y) and the region where the signals for the anomeric C1 appear is also integrated (χ). The ratio of the hemicelluloses to the total hemicelluloses and lignin ($\chi + y$) is multiplied with the molecular weight ratio of the phenyl propane unit (183 g×mol^{-1}) to the anhydro sugar (162 g×mol^{-1}). Afterwards, the value is multiplied with the percentage of wood extracted in this fraction. The result is divided by the theoretical amount of hemicelluloses in softwoods (0.3) to yield the %hemicelluloses on wood basis. For the second equation, the region where the anomeric carbon C1 of the hemicelluloses resonate is integrated first and set to 100 (χ) followed by the C2-Ar signal of the phenyl propane unit (y). The ratio of the later (y) to the total amount of lignin and hemicelluloses ($y + x$) is then multiplied with the ratio of the molecular weights of anhydro sugar to phenyl propane and the % of wood extracted. The result is divided by the theoretical amount of lignin in softwoods expressed as 0.27 to yield the percentage of lignin on wood basis.

$$\frac{\frac{\chi}{\chi + y} \times \frac{183}{162} \times \%\, of\ wood\ extracted}{0.3} = \%hemicelluloses\ on\ wood\ basis$$

$$\frac{\frac{y}{y + x} \times \frac{162}{183} \times \%\, of\ wood\ extracted}{0.27} = \%lignin\ on\ wood\ basis$$

Equations S1&S2: Determination of relative %hemicelluloses and %lignin on wood basis for the extracted fractions.

In Tables S1a-c, the reported amounts of lignin and hemicelluloses were calculated using the results from the HSQC NMR experiments and Equations S1&S2. Klason lignin, acid soluble lignin and carbohydrate analysis using the classical, acid hydrolysis protocol was performed for a few representative samples, in order to confirm the composition of the fractions. The process as well as the results are presented in Table S2c.

Table S1a: Mass balance of extracted material from warm water extraction as a function of milling time and atmosphere.

				Wood basis		Fraction	
	Warm water extraction	Average mass extracted (mg)	% wood extracted	% lignin	% hemicelluloses	% lignin	% hemicelluloses
Air milled	*1h ball milled*	18.7 ± 2.4	1.8	1.75	4.7	29.7	70.3
	2h ball milled	23.5 ± 3.3	2.3	1.15	7.3	15.3	84.7
	4h ball milled	39.0 ± 1.6	3.9	1.4	13.1	10.6	89.4
	8h ball milled	100.6 ± 9.8	10.0	3.5	33.7	10.6	89.4
	12h ball milled	121.0 ± 3.8	12.1	3.7	41.3	9.0	91.0
	18h ball milled	148.8 ± 9.5	14.9	5.3	50.0	10.8	89.2
	24h ball milled	145.3 ± 11.9	14.5	5.5	48.2	11.7	88.3
N2 milled	1h ball milled	26.0 ± 1.8	2.6	1.4	8.1	16.8	83.2
	2h ball milled	47.9 ± 6.5	4.8	2.2	15.5	14.4	85.6
	4h ball milled	82.1 ± 0.7	8.2	3.9	26.4	14.4	85.6
	8h ball milled	120.1 ± 3.3	12.0	4.4	40.1	11.2	88.8
	12h ball milled	146.7 ± 14.0	14.7	5.6	48.9	11.7	88.3
	18h ball milled	164.8 ± 11.2	16.5	5.9	55.4	10.9	89.1
	24h ball milled	179.5 ± 13.8	18.0	6.9	59.8	11.8	88.2

Table S1b: Mass balance of extracted material from mild alkaline extraction as a function of milling time and atmosphere.

				Wood basis		Fraction	
	Alkaline extraction	Average mass extracted (mg)	% wood extracted	% lignin	% hemicelluloses	% lignin	% hemicelluloses
Air milled	*1h ball milled*	14.7 ± 1.3	1.5	4.2	0.8	85.1	14.9
	2h ball milled	18.4 ± 0.1	1.8	5.0	1.0		
	4h ball milled	21.2 ± 0.8	2.1	5.9	1.2		
	8h ball milled	30.1 ± 3.8	3.0	8.1	2.0	82.2	17.8
	12h ball milled	53.6 ± 8.5	5.4	14.6	3.6		
	18h ball milled	70.0 ± 4.9	7.0	16.5	7.4	72.1	27.9
	24h ball milled	80.8 ± 15.8	8.1	21.3	6.0	80.3	19.7
N2 milled	1h ball milled	15.8 ± 0.6	1.5	4.4	0.6	90.0	9.8
	2h ball milled	19.0 ± 1.1	1.9	5.6	0.7		
	4h ball milled	33.1 ± 2.7	3.3	9.75	1.2		
	8h ball milled	52.1 ± 3.0	5.2	14.3	3.2	83.7	16.3
	12h ball milled	86.0 ± 9.7	8.6	22.5	6.6	79.8	20.2
	18h ball milled	110.7 ± 13.6	11.1	31.7	5.4	87.0	13.0
	24h ball milled	167.7 ± 1.4	16.8	49.4	6.6	89.6	10.4

Table S1c: Mass balance of extracted material from IL-EtOH/H^+ extraction as a function of milling time and atmosphere.

				Wood basis	Fraction
	IL-EtOH, H+ extraction	Average mass extracted (mg)	% wood extracted	% lignin	% lignin*
Air milled	*1h ball milled*	31.7 ± 4.5	3.2	11.8	100
	2h ball milled	41.0 ± 2.0	4.1	15.2	100
	4h ball milled	87.8 ± 2.3	8.8	32.6	100
	8h ball milled	97.3 ± 1.6	9.7	35.9	100
	12h ball milled	89.9 ± 27.4	9.0	33.4	100
	18h ball milled	76.9 ± 4.0	7.7	28.5	100
	24h ball milled	101.7 ± 8.9	10.2	37.8	100
N2 milled	1h ball milled	53.4 ± 12.9	5.3	19.6	100
	2h ball milled	57.4 ± 21.3	5.7	21.1	100
	4h ball milled	76.2 ± 0.8	7.6	28.1	100
	8h ball milled	59.3 ± 12.7	5.9	21.8	100
	12h ball milled	74.2 ± 6.2	7.4	27.4	100
	18h ball milled	102.1 ± 3.3	10.2	37.8	100
	24h ball milled	75.5 ± 1.6	7.6	28.1	100

* Carbohydrates below detection limit.

In Tables S2a-b the amount of residual lignin and hemicelluloses is calculated based on the theoretical 27% and 30% in softwoods respectively, by subtracting the amounts extracted in all the extraction steps. After drying the residue of the IL/EtOH, H^+ extraction it was not possible to re-disperse the material due to cellulose hornification, hence Klason lignin and sugar analysis was not possible with that method.

Klason lignin, acid soluble lignin (ASL) and carbohydrate analysis of the starting material, i.e. Wiley milled spruce and a few representative samples, were determined with acid hydrolysis.[1] In short, 100 mg of the lyophilized sample were mixed with 1.5 mL of 72% H_2SO_4 and placed in a vacuum desiccator for 80min, with occasional stirring. 42 mL of Milli-Q water were added to the mixture and the reaction bottles were sealed and placed in the autoclave at 125°C for 1h. Afterwards, the mixture was filtered through a pre-weighed glass fiber filter, which was then used for the gravimetric determination of Klason lignin. The hydrolysate was further diluted and analysed with High-Performance Anion-Exchange Chromatography equipped with Pulse Amperometric Detector (HPAEC-PAD) ICS 3000 (Dionex, ThermoScientific, USA). Acid soluble lignin was determined by measuring the absorbance of the diluted hydrolysate at 205nm and the Equation S3. The results are presented in Table S2c.

$$ASL\% = 100 \times \frac{A \times V \times f}{a \times b \times M} - 0.2$$

A= absorbance value at 205 nm
V= volume of hydrolysate (L)
f= dilution factor
α= absorptivity ($L \times g^{-1} \times cm^{-1}$). For softwood, α=128 $L \times g^{-1} \times cm^{-1}$
b= path length (cm)
M= dry weight of the sample (g)
0.2 is a correction value used for the absorbance of sugar degradation products at 205 nm

Equation S3: Determination of acid soluble lignin (ASL).

Table S2a: Total mass balance of lignin from all the steps of the extraction protocol on wood basis and residual lignin. Standard deviation for each fraction is reported in Tables S1a-c.

	% lignin extracted[1]	Warm water extraction	Alkaline extraction	IL/EtOH, H^+ extraction	Total lignin extracted	Residual lignin*
Air milled	*1h ball milled*	1.8	4.2	11.8	17.8	82.2
	2h ball milled	1.2	5.0	15.2	21.4	78.6
	4h ball milled	1.4	5.9	32.6	39.9	60.1
	8h ball milled	3.5	8.1	35.9	47.5	52.5
	12h ball milled	3.7	14.6	33.4	51.7	48.3
	18h ball milled	5.3	16.5	28.5	50.3	49.7
	24h ball milled	5.5	21.3	37.8	64.6	35.4
N2 milled	1h ball milled	1.4	4.4	19.6	25.4	74.6
	2h ball milled	2.2	5.6	21.1	28.9	71.1
	4h ball milled	3.9	9.8	28.1	41.8	58.2
	8h ball milled	4.4	14.3	21.8	40.5	59.5
	12h ball milled	5.6	22.5	27.4	55.5	44.5
	18h ball milled	5.9	31.7	37.8	75.4	24.6
	24h ball milled	6.9	49.3	28.1	84.3	15.7

[1] The %lignin is calculated on wood basis.

* Residual lignin was estimated after addition of the extracted lignin amounts and considering a theoretical 27% lignin in softwoods, as earlier discussed.

Table S2b: Total mass balance of extracted hemicelluloses from warm water extraction and mild alkaline extraction. Standard deviation for each fraction is reported in Tables S1a-c.

	% Hemicelluloses extracted[1]	Warm water extraction	Alkaline extraction	Total hemicelluloses extracted	Residual Hemicelluloses*****
Air milled	*1h ball milled*	4.7	0.8	5.5	94.5
	2h ball milled	7.3	1.0	8.3	91.7
	4h ball milled	13.1	1.2	14.3	85.7
	8h ball milled	33.7	2.0	35.7	64.3
	12h ball milled	41.3	3.6	44.9	55.1
	18h ball milled	50.0	7.4	57.4	42.6
	24h ball milled	48.2	6.0	54.2	45.8
N2 milled	1h ball milled	8.1	0.6	8.7	91.4
	2h ball milled	15.5	0.7	16.2	83.8
	4h ball milled	26.4	1.2	27.6	72.4
	8h ball milled	40.1	3.2	43.3	56.7
	12h ball milled	48.9	6.6	55.5	44.6
	18h ball milled	55.4	5.4	60.8	39.2
	24h ball milled	59.8	6.6	66.4	33.6

[1] The %hemicellulose is calculated on wood basis.

* Residual hemicelluloses were calculated by addition of the extracted amounts and considering a theoretical yield of 30% hemicelluloses for softwoods, as discussed above.

Table S2c: Klason lignin and sugar analysis of the starting material, i.e. Wiley milled spruce. The process is described in the Materials and Methods section. Monosugars detected but not quantified because they were below limit of detection are marked with <l.o.d in the corresponding cells in the Table.

	% Ara	**% Rha**	**% Gal**	**% Glc**	**% Xyl**	**% Man**	**%Total carbohydrates**	**%Klason lignin[1]**	**%ASL[1]**	**%Mass balance**
Wiley milled spruce	1.1	-	2.1	41	5.9	10.8	60.9	33.0	1.2	95.1
WWE 24h air	2.9	< l.o.d	3.9	10.1	12.7	26.4	56.0	6.9	2.5	65.4[2]
WWE 24h N2	3.0	< l.o.d	4.1	10.6	13.9	26.7	58.3	7.6	3.0	68.9[2]
Alkaline 24h	0.6	-	2.0	5.8	2.1	5.9	16.4	67.8	1.7	85.9
Alkaline 24h N2	0.6	-	2.0	4.9	2.1	5.9	15.5	69.8	1.5	86.8
IL 24h	< l.o.d	-	0.4	< l.o.d	< l.o.d	< l.o.d	0.4	74.5	3.0	77.9[3]
IL 24h N2	< l.o.d	-	0.4	< l.o.d	< l.o.d	< l.o.d	0.4	72.6	3.3	76.3[3]

[1] Includes extractives.

[2] Lower mass balance for WWE fractions for a number of reasons: acetyl decorations are not included as these are hydrolysed by the acid treatment, oxidized structures in carbohydrates created during ball milling are not analysed, galacturonic acids and glucuronic acids not analysed.

[3] Some residual ionic liquid present in sample and contributes to lower mass balance. This was also verified by NMR analysis.

Table S2d: Comparison between the hemicellulose and lignin amounts present in fractions of the protocol, analysed by acid hydrolysis as described in the text above and NMR.

	%Hemicelluloses		**%Lignin**	
	IC/HPAEC-PAD[1]	**NMR[2]**	**Klason lignin +ASL[1]**	**NMR[2]**
WWE 24h	56.0[3]	88.3	9.4	11.7
WWE 24h N2	58.3[3]	88.2	10.6	11.8
Alkaline 24h	16.4	19.7	69.5	80.3
Alkaline 24h N2	15.5	10.4	71.3	89.6
IL 24h	0.4	-	77.5	100*
IL 24h N2	0.4	-	75.9	100*

[1] This analysis gives the absolute values.

[2] This analysis is semi-quantitative and gives the relative values between lignin and hemicelluloses.

[3] Discrepancies when compared to NMR since some acetyl groups, uronic acids and possible oxidized moieties escaped the analysis but were included in the NMR analysis.

*Carbohydrates below detection limit.

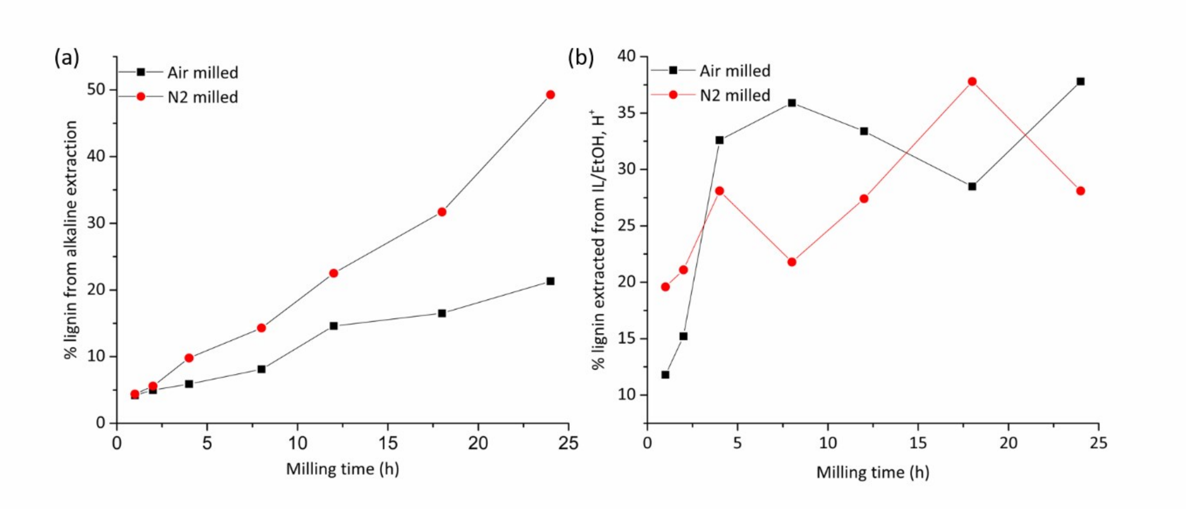


Figure S2: Lignin mass balance from (a) mild alkaline extraction and (b) IL/EtOH, H⁺ extraction.

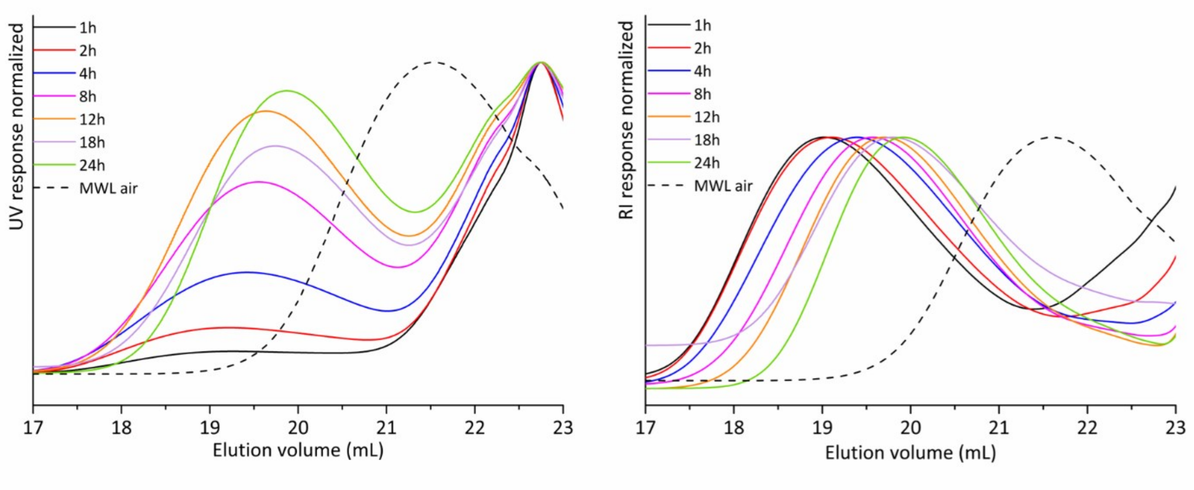


Figure S3a: DMSO SEC overlay of warm water extracts milled in air.

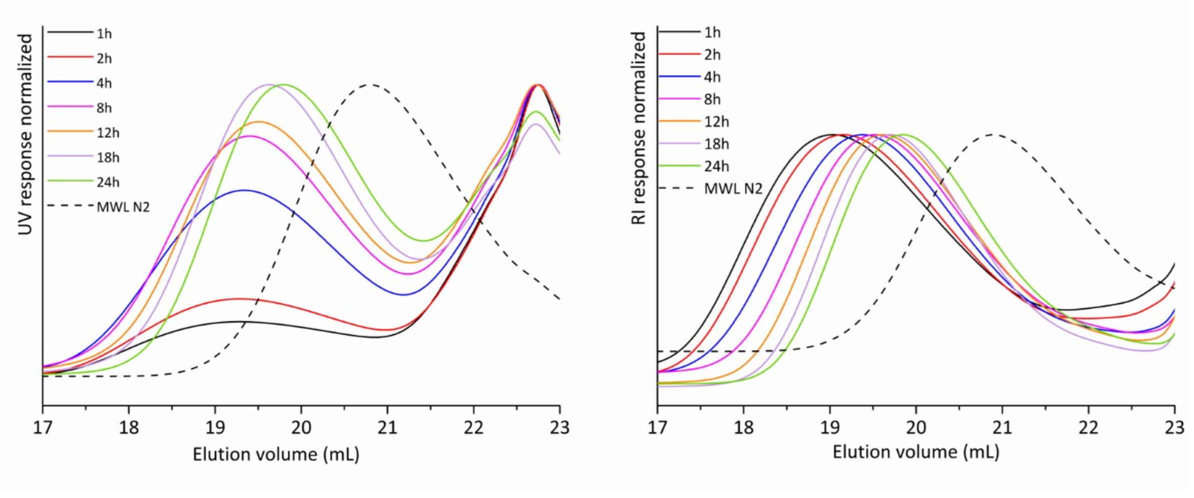


Figure S3b: DMSO-SEC overlay of warm water extracts milled in N2 atmosphere.

Table S3: Results of DMSO SEC using refractive index detector calibration with pullulan standards. The reported values correspond to the largest area peak.

Warm water

	extraction	Mn (g/mol)	Mw (g/mol)	PDI
Air milled	1h ball milled	8416	19690	2.34
	2h ball milled	7110	17771	2.50
	4h ball milled	5449	14342	2.63
	8h ball milled	4786	11805	2.47
	12h ball milled	3078	10051	2.27
	18h ball milled	2896	8524	2.94
	24h ball milled	3779	7676	2.03
N2 milled	1h ball milled	7309	18344	2.51
	2h ball milled	5707	16463	2.88
	4h ball milled	5496	14070	2.56
	8h ball milled	4965	11727	2.36
	12h ball milled	4643	10284	2.21
	18h ball milled	4576	9326	2.04
	24h ball milled	4075	8117	1.99

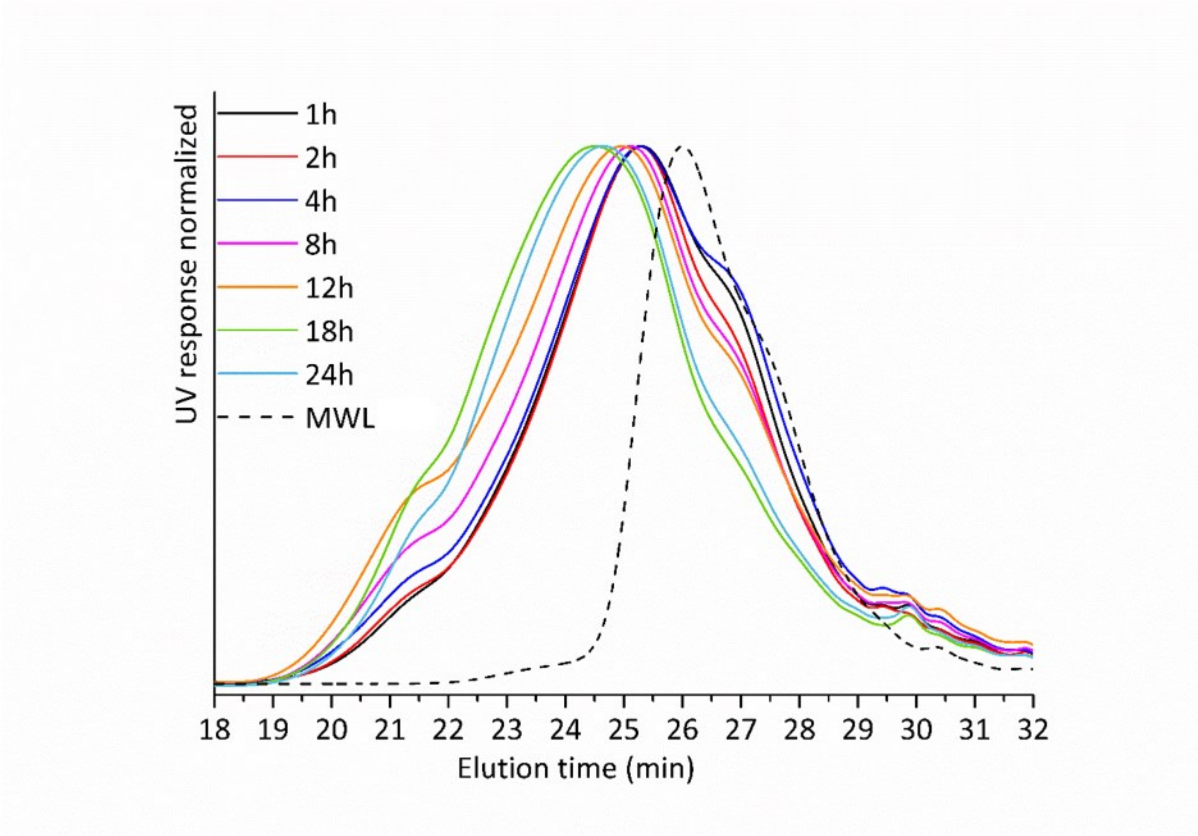


Figure S4a: THF SEC of IL/EtOH, H^+ fractions milled in air.

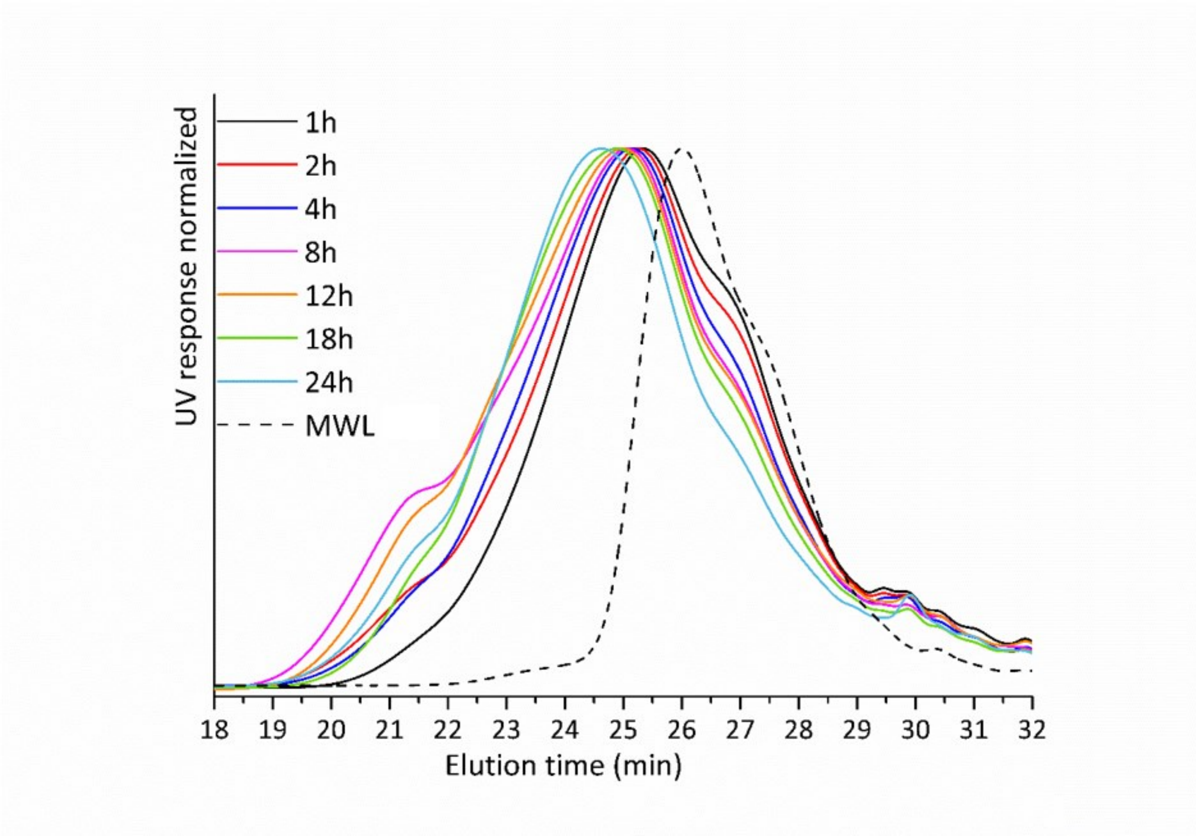


Figure S4b: THF SEC of the IL/EtOH, H^+ fractions milled in nitrogen atmosphere.

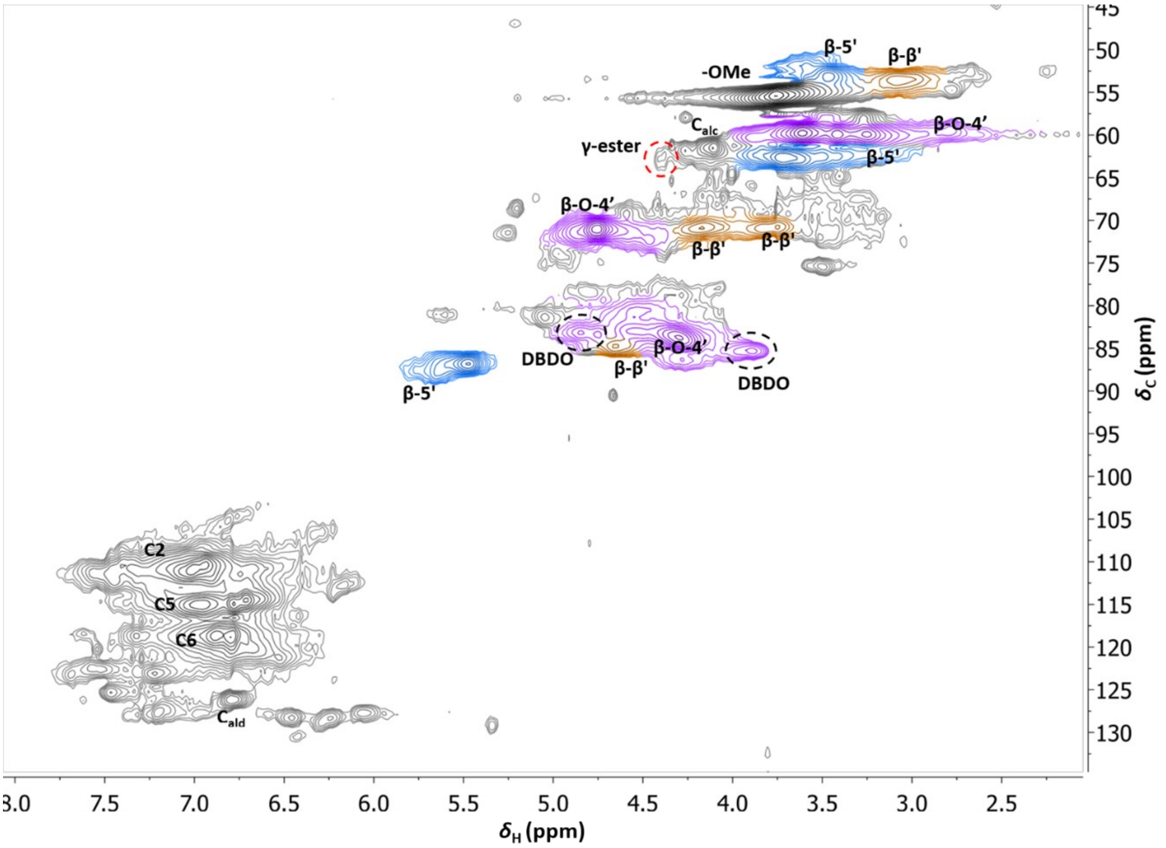


Figure S5: HSQC of MWL after purification for the removal of carbohydrates. The starting material was 18h ball milled wood in air. DMSO-d_6 was used for the NMR. Peak assignment can be found in Tables S7, S8.

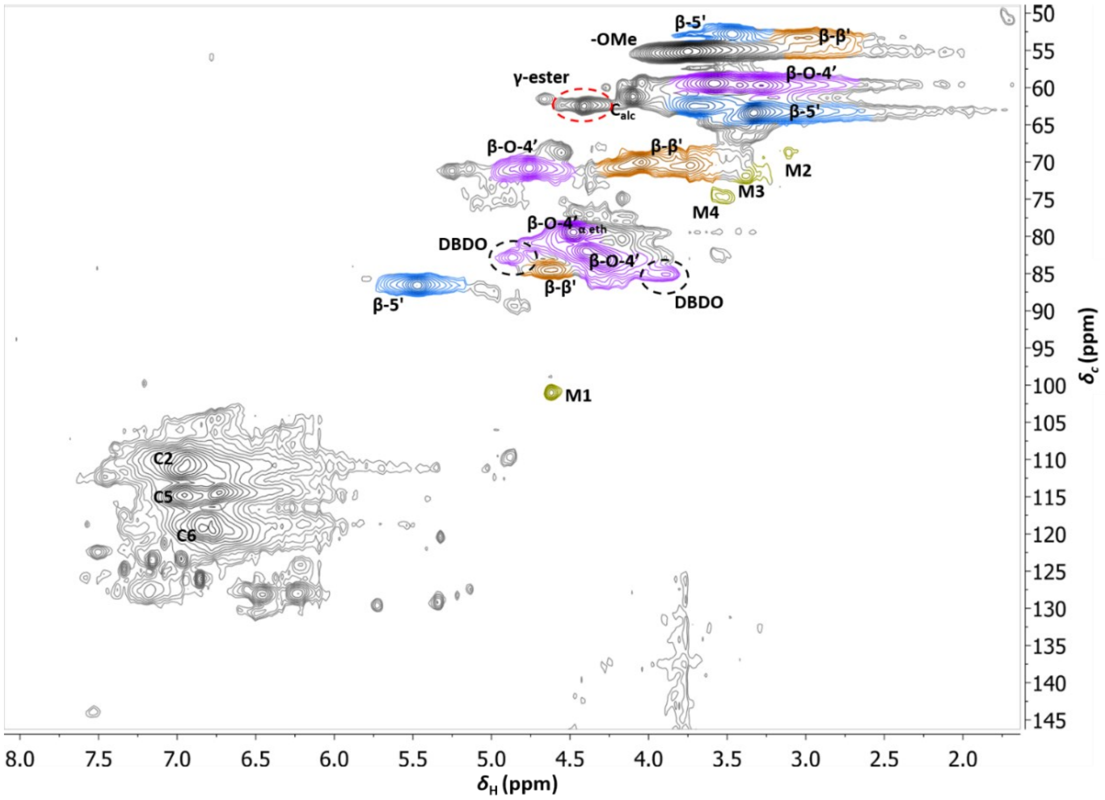


Figure S6a: HSQC of ALK-EtOH, H⁺- red sample. DMSO-d_6 was used for the NMR. Peak assignment can be found in Tables S7, S8.

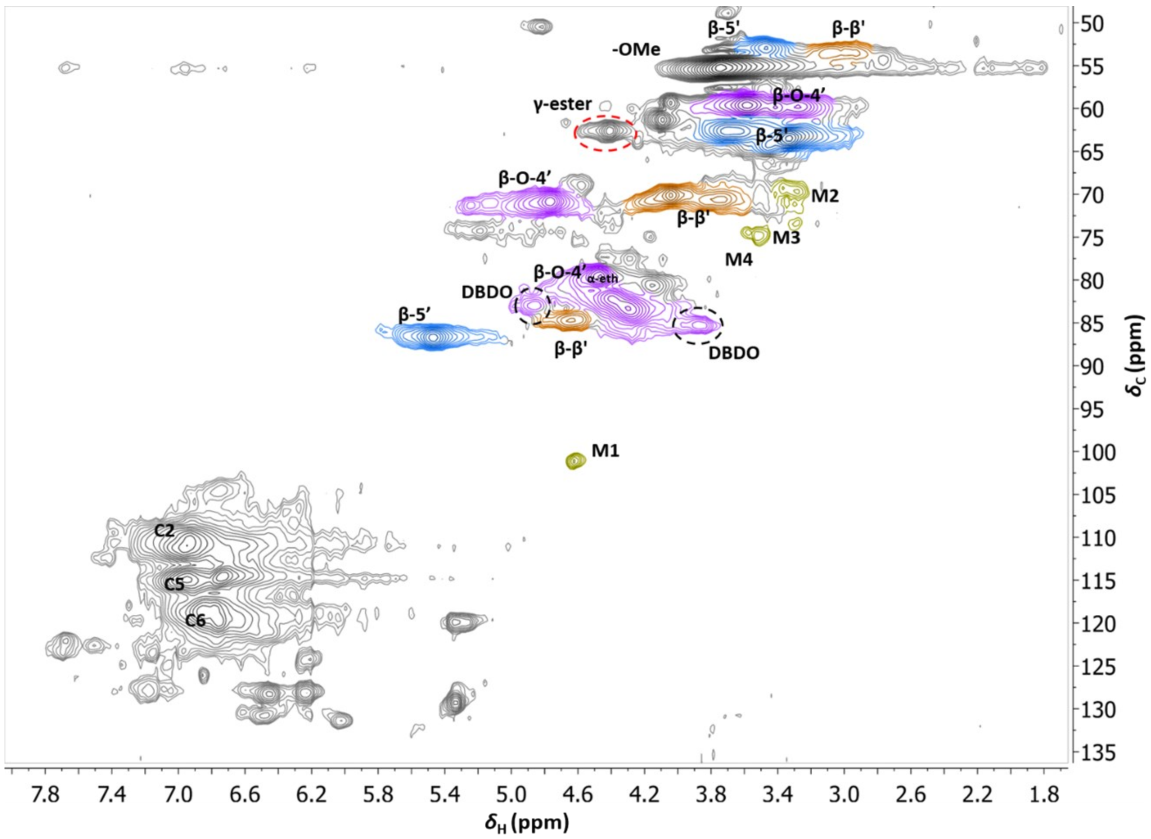


Figure S6b: HSQC of IL/EtOH, H⁺- red sample. DMSO-d_6 was used for the NMR. Peak assignment can be found in Tables S7, S8.

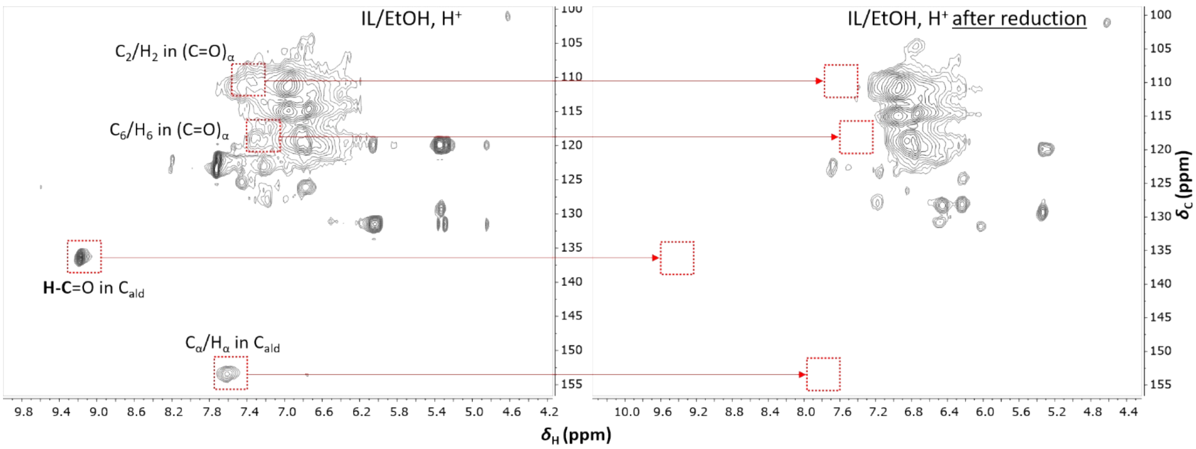


Figure S6c: HSQC of IL/EtOH, H^+ sample before and after reduction. The marked areas highlight the completion of the reduction as the aldehyde signals disappear. DMSO-d_6 was used for the NMR. Peak assignment can be found in Tables S7, S8.

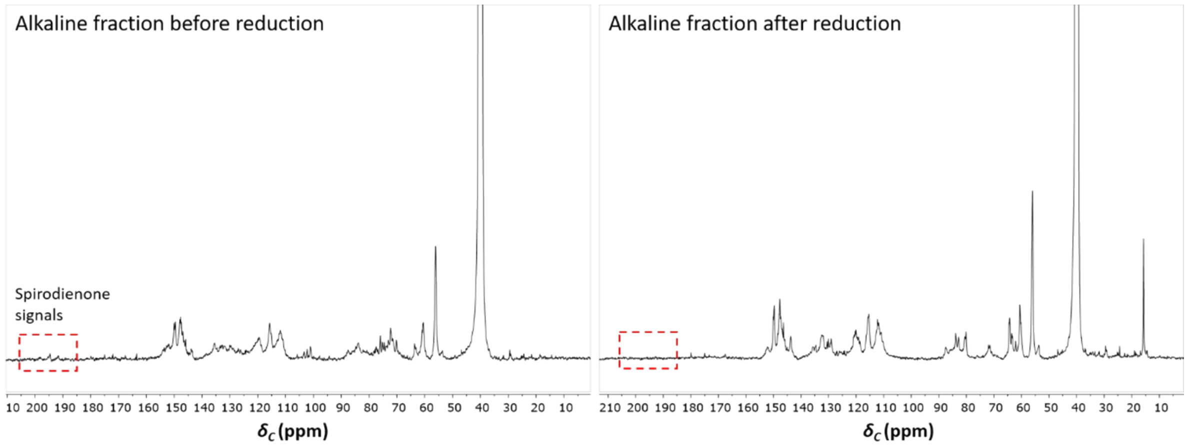


Figure S6d: ^{13}C-NMR spectra of alkaline fractions before and after reduction. The marked areas highlight the completion of the reduction as the spirodienone signals disappear. DMSO-d_6 was used for the NMR. Peak assignment can be found in Table S9.

In the following figures, S7a-b, the NMR spectra of dehydrogenation polymer lignin (DHP) produced in the presence of uronic acid or without the presence of uronic acid (reference sample) are presented. DHP was synthesized according to previous work.[2] Using a NE-1800 Eight Channel Programmable syringe pump, 20 mL coniferyl alcohol solution (34 mM in 50% acetone) and 20 mL of H2O2 (34 mM) were pumped into 20 mL solution (50 mg/L in 50% acetone) of horseradish peroxidase (HRP) type IV. The addition occurred under a constant speed of 250 μL/h, at room temperature, under slow stirring (150 rpm). After the addition, the mixture was stirred for additionally 4h. For the DHP produced in the presence of galacturonic acid, 0.2M of the later were dissolved in 20 mL of 50% acetone and the pH was adjusted at 6.5-7 before the addition of HRP. The coniferyl alcohol used for these experiments was reduced from coniferyl aldehyde. This process is described elsewhere.[3]

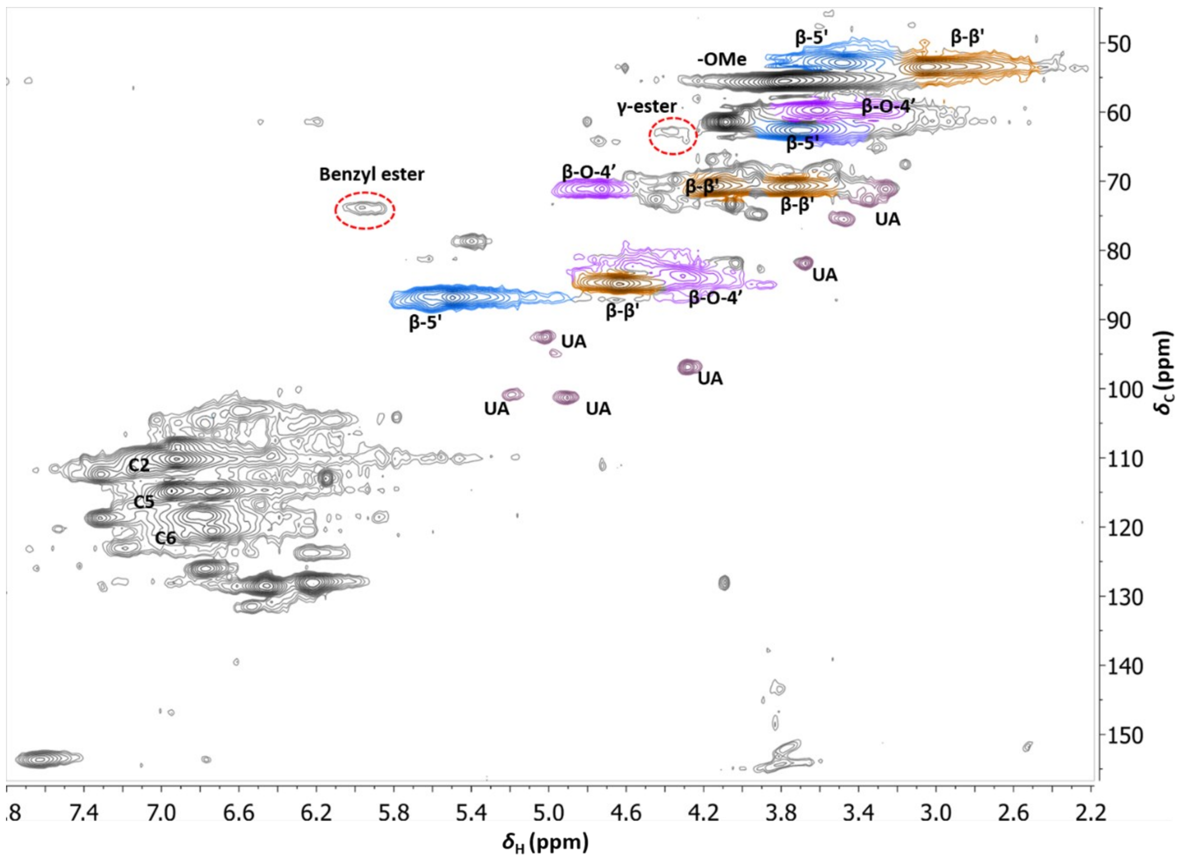
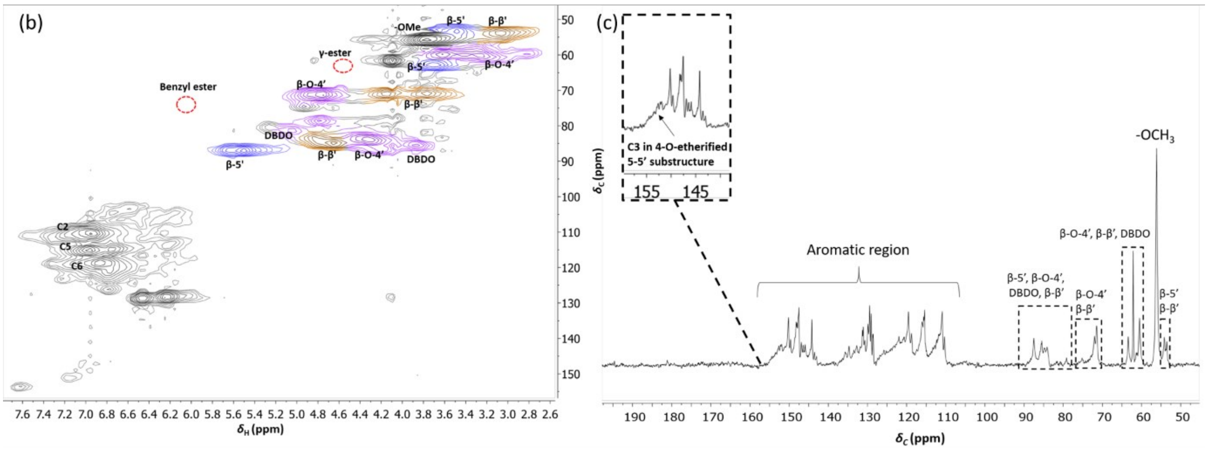


Figure S7a: HSQC of DHP produced in the presence of uronic acid. DMSO-d_6 was used for the NMR. Peak assignment can be found in Tables S7, S8.



Figures S7b-c: HSQC of reference DHP. DMSO-d_6 was used for the NMR. Peak assignment can be found in Tables S7, S8.

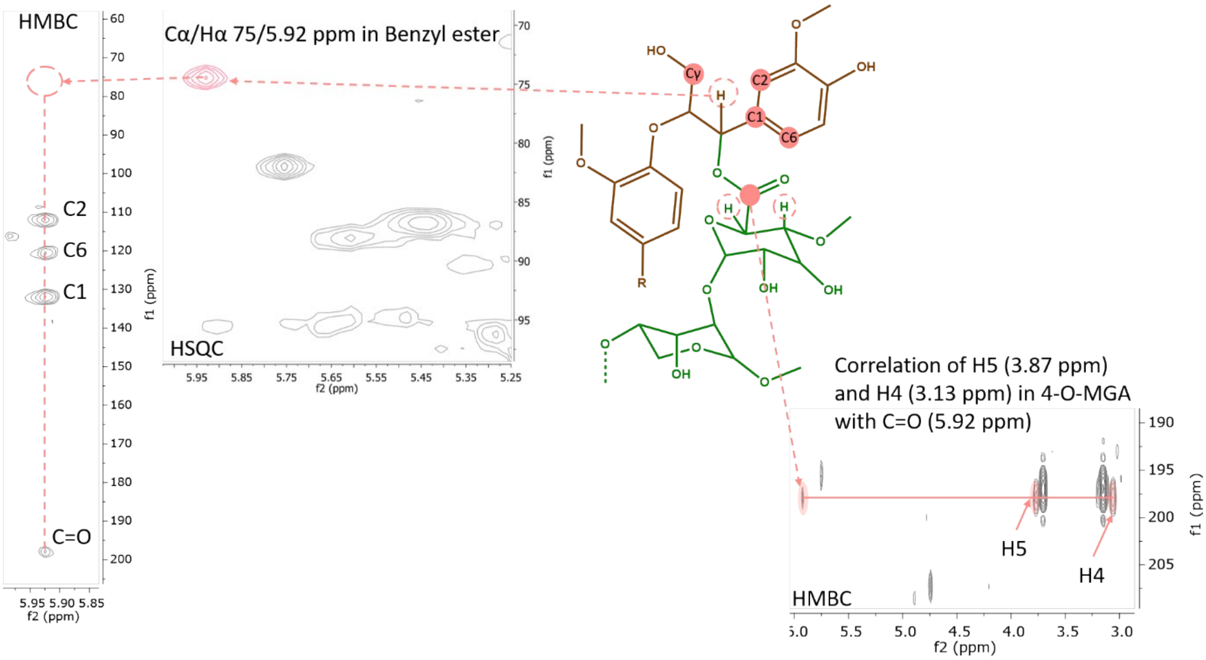


Figure S8: Evidence of benzyl ester structures between lignin and arabinoglucuronoxylan in wood isolates. Solvent for the NMR was DMSO-d_6.

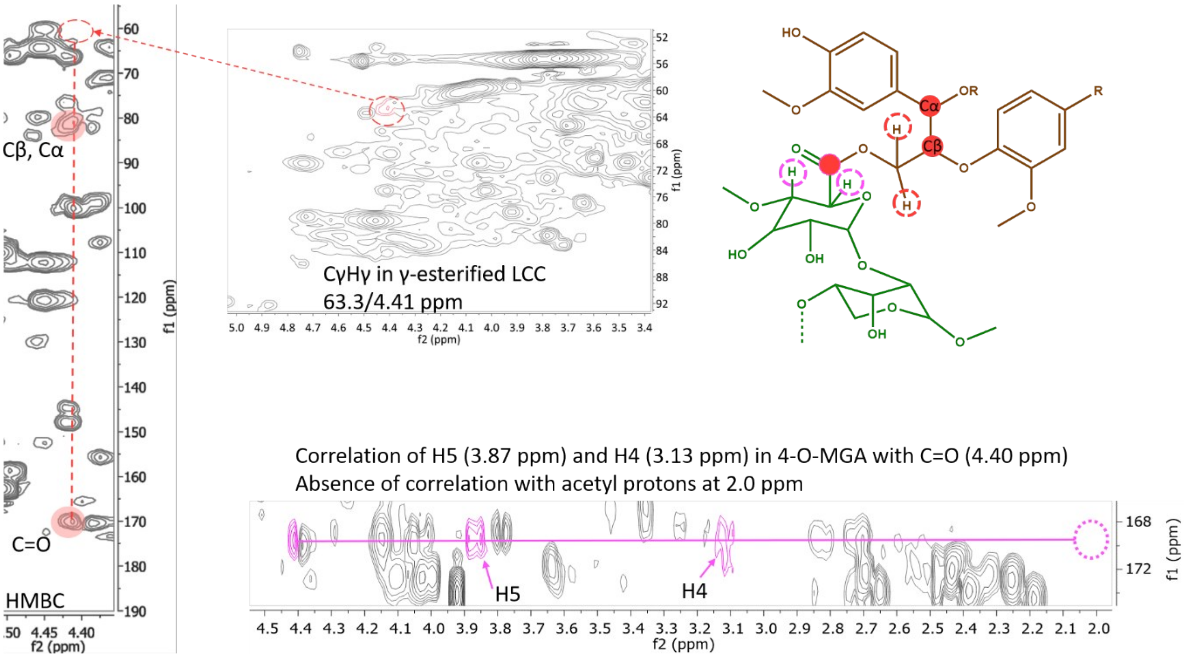


Figure S9: Evidence for the γ-ester LCC in lignin in wood isolates. Solvent for the NMR was DMSO-d_6.

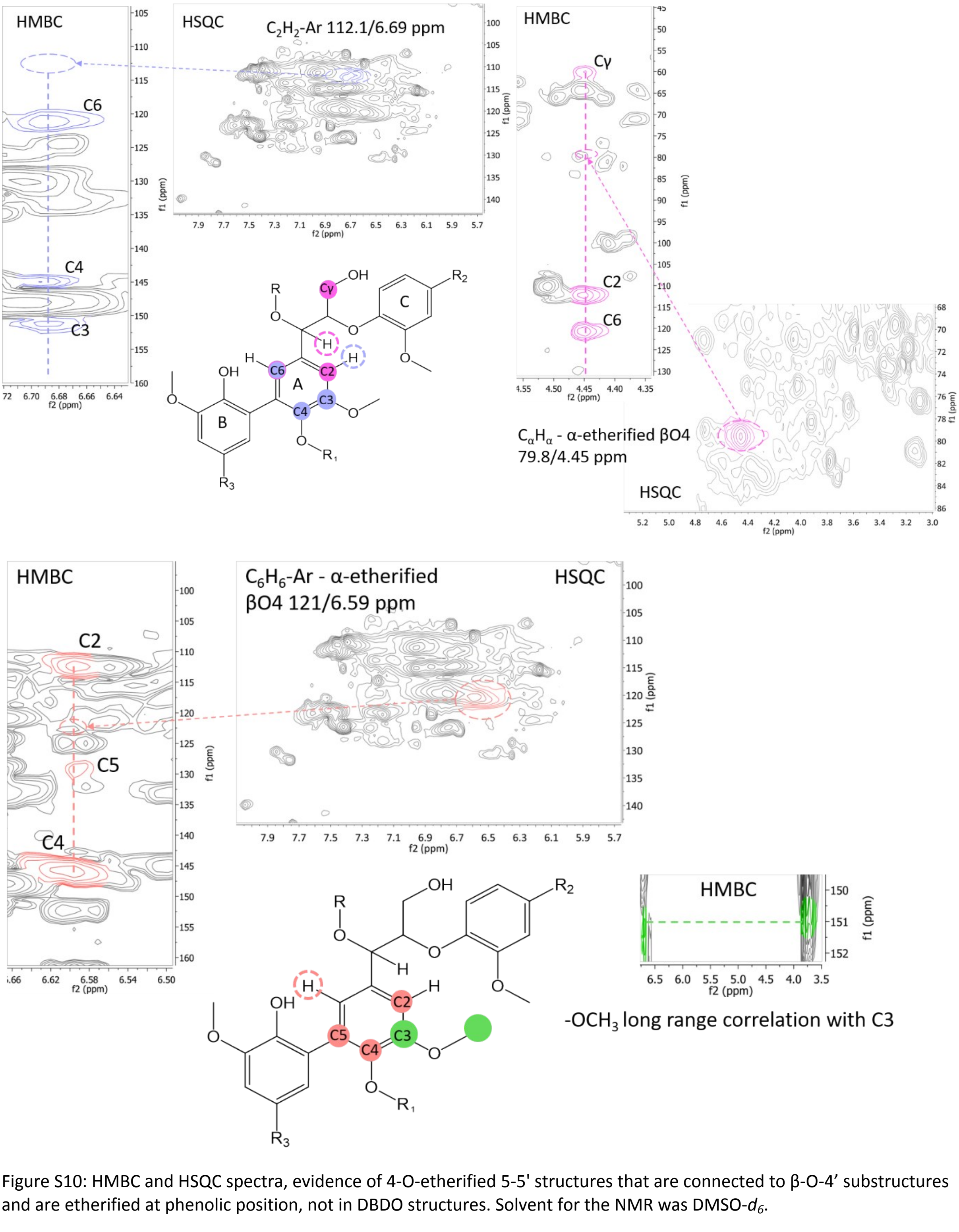


Figure S10: HMBC and HSQC spectra, evidence of 4-O-etherified 5-5' structures that are connected to β-O-4’ substructures and are etherified at phenolic position, not in DBDO structures. Solvent for the NMR was DMSO-d_6.

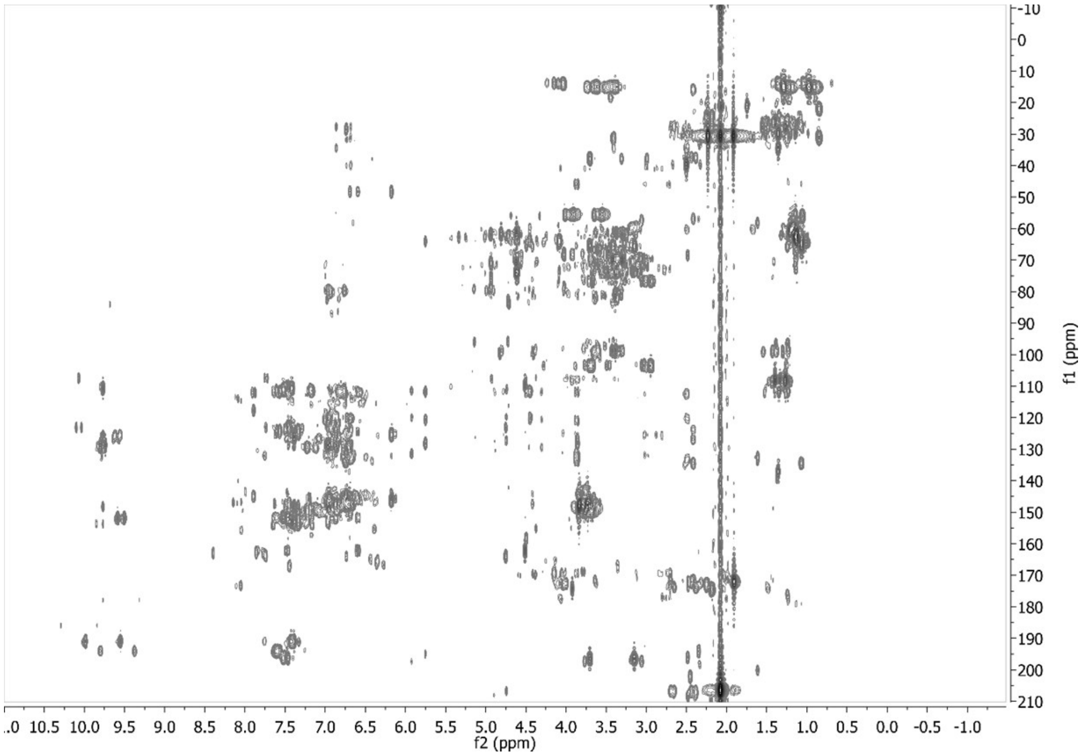


Figure S11: HMBC of lignin from warm water extract after mild acidolysis in ethanol and extraction with ethyl acetate. Solvent for the NMR was DMSO-*d_6*.

Table S4: Comparison of lignin inter-unit linkages from HSQC and quantitative ^{13}C NMR experiments.

	%β-O-4’		β-5’	
	HSQC-NMR*	^{13}C-NMR	HSQC-NMR	^{13}C-NMR
ALK-EtOH, H^{+}-red air milled	40	42	10	11
ALK-EtOH, H^{+}-red N2 milled	46	47	13	12
IL/EtOH, H^{+}-red air milled	41	46	13	12
IL/EtOH, H^{+}-red N2 milled	44	47	13	13

*With HSQC-NMR, the total amount of β-O-4’ is presented as described in the main paper.

Table S5: Quantification of 4-O-etherified 5-5’ substructures with 13C-NMR.

	% 5-5’ etherified
ALK-EtOH, H^{+}-red air milled	23
ALK-EtOH, H^{+}-red N2 milled	23
IL/EtOH, H^{+}-red air milled	26
IL/EtOH, H^{+}-red N2 milled	24
DHP*	25

*DHP was not reduced before NMR analysis, hence the signal corresponding to 5-5’ etherified bond overlaps with α- and γ-carbonyls and spirodienone structures. Using the HSQC and ^{13}C NMR data, the integral values for α-carbonyls (3%), coniferyl aldehyde (2%), spirodienone (2%) and DBDO (3%) structures were subtracted from the integral value for C3 in 4-O-etherified 5-5’ substructures.

Table S6: Most common lignin inter-unit linkages in all fractions. The amounts are calculated from the HSQC spectra, after normalization of C2-Ar integral to 100. Some of the samples were combined for the NMR analysis due to small amounts extracted and are presented in the same cell.

			%β-O-4’ *	%β-5’	%β-β’
Warm water extracts	Air milled	*1h ball milled*	15	5	n.a
		2h ball milled	18	4	n.a
		4h ball milled	12	3	n.a
		8h ball milled	16	3	n.a
		12h ball milled	18	4	n.a
		18h ball milled	23	4	n.a
		24h ball milled	16	4	n.a
	N2 milled	*1h ball milled*	17	4	n.a
		2h ball milled	18	5	n.a
		4h ball milled	26	4	n.a
		8h ball milled	24	4	n.a
		12h ball milled	20	5	n.a
		18h ball milled	23	4	n.a
		24h ball milled	22	5	n.a
Alkaline extracts					
	Air milled	*1h ball milled* *2h ball milled* *4h ball milled*	41	12	3
		8h ball milled *12h ball milled*	46	12	3
		18h ball milled	41	11	3
		24h ball milled	35	11	3
	N2 milled	*1h ball milled* *2h ball milled* *4h ball milled*	40	12	2
		8h ball milled	44	13	3
		12h ball milled	42	13	3
		18h ball milled	42	13	3
		24h ball milled	44	12	3
IL/EtOH, H^+ extracts	Air milled	*1h ball milled* *2h ball milled* *4h ball milled*	45	12	3
		8h ball milled *12h ball milled*	47	13	3
		18h ball milled	48	12	3
		24h ball milled	46	12	3
	N2 milled	*1h ball milled* *2h ball milled*	42	12	2
		4h ball milled	49	13	2
		8h ball milled	47	12	3
		12h ball milled	45	12	2
		18h ball milled	49	15	2
		24h ball milled	40	12	2

*For the alkaline and IL/EtOH, H^+ fractions, the total β-O-4′ content is reported and calculated as described in the main paper.

Table S7: ^{13}C-^{1}H correlations of the main lignin inter-unit linkages and LCC identified in 2D HSQC spectra. DMSO-d_6 was used as solvent.

	δ_C (ppm)	**δ_H (ppm)**	Description
	71.4	4.71	C_α/H_α in γ-hydroxylated β-O-4′
	83.7	4.28	C_β/H_β in γ-hydroxylated β-O-4′ in G-units
	59.9	3.42/3.85	C_γ/H_γ in β-O-4′
	85.7	4.3	C_β/H_β in γ-hydroxylated β-O-4′ in S-units
	81.0	4.62	C_γ/H_γ in γ-acylated β-O-4′
	85.6	4.33	C_β/H_β in β-O-4′ in Benzyl Ether structure
	61.2	3.79	C_γ/H_γ in β-O-4′ in Benzyl Ether structure
	79.6	4.48	C_α/H_α in α-O-ethylated β-O-4′
	82.9	4.86	C_α/H_α in Dibenzodioxin
	85.5	3.88	C_β/H_β in Dibenzodioxin
	60.4	3.42	C_γ/H_γ in Dibenzodioxin
	79.4	4.11	C_β/H_β in Spirodienone
	86.8	5.47	C_α/H_α in Phenylcoumaran
	52.9	3.47	C_β/H_β in Phenylcoumaran
	62.5	3.70	C_γ/H_γ in Phenylcoumaran
	84.5	4.65	C_α/H_α in Resinol
	53.5	3.10	C_β/H_β in Resinol
	71.0	3.78/4.15	C_γ/H_γ in Resinol
	110.5	7.35	C_2/H_2 in $(C=O)_\alpha$ in G-units
	122.6	7.5-7.7	C_6/H_6 in $(C=O)_\alpha$ in G-units
	105.5	7.0-7.2	$C_{2,6}/H_{2,6}$ in $(C=O)_\alpha$ in G-units
	153.4	7.61	C_α/H_α in Cinnamyl aldehyde
	126.1	6.76	C_β/H_β in Cinnamyl aldehyde
	59.5	4.04	C_γ/H_γ in Cinnamyl alcohol
	55.3	3.71	C/H in methoxy group in G-, S-units
	104.4	6.72	C_2/H_2 and C_6/H_6 in S-units
	110.8	6.96	C_2/H_2 in G-units
	114.7	6.73	C_5/H_5 in G-units
	119.6	6.76	C_6/H_6 in G-units
	112.1	6.69	C_2/H_2 in 4-O-etherified 5-5′ substructures
	120.6	6.59	C_6/H_6 in 4-O-etherified 5-5′ substructures
	127.9	7.19	C_2/H_2 and C_6/H_6 in H-units
	101.9	4.92	Phenyl Glycoside in G-units
	74.5	5.92	C_α/H_α in Benzyl ester
	63.3	4.41	C_γ/H_γ in γ-esterified LCC
	62.6	4.23/3.82	γ-acetyl esters in lignin

Table S8: Main ^{13}C-^{1}H correlations of carbohydrate bonds identified in 2D HSQC. The solvent used was DMSO-d_6.

	δ_C (ppm)	δ_H (ppm)	Description
	107.8	4.75	C1/H1 in β-L-arabinopyranoside
	83.3	3.76	C2/H2 in β-L-arabinopyranoside
	84.6	3.98	C4/H4 in β-L-arabinopyranoside
	62.3	3.36/3.44	C5/H5 in β-L-arabinopyranoside
	101.6	4.36	C1/H1 in β-D-glucopyranoside
	74.8	2.88	C2/H2 in β-D-glucopyranoside
	76.7	3.06	C3/H3 in β-D-glucopyranoside
	80.1	3.34	C4/H4 in β-D-glucopyranoside
	70.3	3.18	C5/H5 in β-D-glucopyranoside
	60.0	3.56	C6/H6 in β-D-gluco-/mannopyranoside
	101.5	4.36	C1/H1 in β-D-xylopyranoside
	72.6	3.02	C2/H2 in β-D-xylopyranoside
	74.9	3.30	C3/H3 in β-D-xylopyranoside
	75.3	3.45	C4/H4 in β-D-xylopyranoside
	62.9	3.13/3.78	C5/H5 in β-D-xylopyranoside
	75.0	4.78	C3/H3 in 3-O-acetyl-β-D-xylopyranoside
	71.4	4.49	C2/H2 in 2,3-O-acetyl-β-D-xylopyranoside
	72.9	4.97	C3/H3 in 2,3-O-acetyl-β-D-xylopyranoside
	99.2	4.67	C1/H1 in 2,3-O-acetyl-β-D-xylopyranoside
	73.7	4.45	C2/H2 in 2-O-acetyl-β-D-xylopyranoside
	100.0	4.44	C1/H1 in 2-O-acetyl-β-D-xylopyranoside
	66.8	3.28	C4/H4 in β-D-xylopyranoside of xylans non reducing end
	65.3	2.98/3.61	C5/H5 in β-D-xylopyranoside of xylans non reducing end
	103.4	4.14	C1/H1 in β-D-xylopyranoside of xylans non reducing end
	92.3	4.84	C1/H1 in α-D-xylopyranoside of xylans reducing end
	97.3	4.20	C1/H1 in β-D-xylopyranoside of xylans reducing end
	69.7	3.24	C2/H2 in α-D-xylopyranoside of xylans reducing end
	100.3	4.51	C1/H1 in β-D-mannopyranoside
	74.5	2.87	C2/H2 in β-D-mannopyranoside
	76.5	3.06	C3/H3 in β-D-mannopyranoside
	79.1	3.36	C4/H4 in β-D-mannopyranoside
	76.9	3.60	C5/H5 in β-D-mannopyranoside
	70.8	5.21	C2/H2 in 2-O-acetyl β-D-mannopyranoside
	73.1	4.80	C3/H3 in 3-O-acetyl β-D-mannopyranoside
	63.7	4.03/4.26	C6/H6 in 6-O-acetyl β-D-mannopyranoside
	93.5	4.87	C1/H1 in α-D-mannopyranoside of mannans reducing end
	93.8	4.55	C1/H1 in β-D-mannopyranoside of mannans reducing end
	105.3	4.23	C1/H1 in α-D-galactopyranoside
	96.8	5.11	C1/H1 in 4-O-methyl-α-D-Glucuronic Acid
	81.4	3.07	C4/H4 in 4-O-methyl-α-D- Glucuronic Acid
	91.9/95.5	5.18/4.98	C1/H1 in Galacturonic acid

Table S9: Peak assignments for the most common lignin inter-unit linkages ^{13}C NMR spectra. Due to signal overlap, not all bonds reported in Table S8 can be reported. The solvent used was DMSO-d_6.

δ_C (ppm)	Description
55.5	Methoxy group in G- units
71.4	C_α in γ-hydroxylated β-O-4’
83.4	C_β in γ-hydroxylated β-O-4’ in G-units
60.0	C_γ in β-O-4’
80.9	C_γ in γ-acylated β-O-4’
85.5	C_β in β-O-4’ in Benzyl ether structure
79.7	C_α in α-O-ethylated β-O-4’
82.5	C_α in Dibenzodioxin
85.5	C_β in Dibenzodioxin
79.7	C_β in Spirodienone
87.0	C_α in Phenylcoumaran
53.1	C_β in Phenylcoumaran
62.7	C_γ in Phenylcoumaran
84.6	C_α in Resinol
53.7	C_β in Resinol
71.0	C_γ in Resinol
59.5	C_γ in Cinnamyl alcohol
110.7	C_2 in G-units
114.7	C_5 in G-units
111.9	C_2 in 4-O-etherified 5-5’ substructures
120.6	C_6 in 4-O-etherified 5-5’ substructures
74.5	C_α in Benzyl ester

References

1. SCAN, Carbohydrate composition, 2009, SCAN-CM, 71, 09
2. N. Giummarella, C. Gioia, M. Lawoko. *Green Chemistry*. 2018, **20**, 2651-62.
3. M. Jawerth, M. Lawoko, S. Lundmark,C. Perez Berumen, M. Johansson, *RSC Advances*, 2016, **6,** 96281-8.