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Supporting information

Reductive Catalytic Fractionation of pine wood: Elucidating and quantifying the molecular structures in the lignin oil

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C. Experimental procedures

1. Chemicals and materials

All commercial chemicals were analytic reagents and were used without further purifications. 5 % Pd on Carbon (Pd/C, 12% metal dispersion, other characteristics can be found in this paper[1]), guaiacol (2-methoxyphenol, 98%), 4-n-propylguaiacol (<99%), tetrahydrofuran (>99%, stabilized with 250 ppm BHT), dmso-d_6 (99.9% atom D) N-methyl-N-(trimethylsilyl)trifluoroacetamide (>98.5%), 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (95%), chromium(III) acetylacetonate (97%), cholesterol (99%), chloroform-d (99.8% atom D), silica gel (pore size 60 Å, 70-230 mesh, 63-200 µm), anhydrous pyridine (99.8%) and 2-isopropylphenol (>98%) were purchased from Sigma Aldrich. 4-ethylguaiacol (98%), n-heptane (>99%), ethylacetate (99.5%), acetonitrile (99.9%) methanol (99.9%) were purchased from Acros organics. Dichloromethane (>99%) was purchased from Fischer Chemical Ltd. 4-propanolguaiacol (3-(4-hydroxy-3-methoxyphenyl)-1-propanol, >98%) was purchased from TCO chemicals. Isoeugenol (2-methoxy-4-propenylphenol, >98%) was purchased from Alfa Aesar. Pine was obtained from a local dealer (Aveve), milled and sieved to obtain a sawdust fraction with a size of 250-500 µm. Subsequently it was soxhlet extracted with an ethanol/toluene (1/2) mixture for 3h to remove most extractives.

2. Determination of the Klason lignin content

Product yields in lignin depolymerisation literature are typically based on the amount of acid insoluble lignin, also called Klason lignin, in the lignocellulose sample. The determination of the Klason lignin content of pine was based on a procedure from Lin & Dence. Triplicate samples of pre-extracted pine (1 g each) were transferred to 50 mL beakers after which 15 mL of a 72 wt% H_2SO_4 solution was added. The mixture was left at room temperature for 2 h while being continuously stirred with a magnetic rod. Afterwards the content of each beaker was transferred to a round-bottom-flask which already contained 300 to 400 mL of water. The beakers were rinsed and additional water was added until a H_2SO_4 concentration of 3 wt% was reached. The diluted solution was boiled for 4 h under reflux conditions, to maintain a constant volume and acid concentration. After filtration of the hot solution, a brown lignin precipitate was retained. 20 mL of this solution was kept aside for acid soluble lignin analysis. The precipitate was washed with hot water to remove any leftover acid and the obtained residue was dried at 80 °C overnight. The reported Klason lignin content of 24.6 wt% was determined relative to the oven dried substrate by averaging the measured weight of the residues.

3. Reductive Catalytic Fractionation

The RCF experiment was performed in a 2 L stainless steel batch reactor (Parr Instruments & Co.). 150 g of extracted pine sawdust (250-500 µm) was loaded into the reactor, together with 15.0 g Pd/C and 800 mL methanol. Subsequently, the reactor was sealed, flushed three times with N_2 (10 bar) and then pressurized with H_2 (30 bar at room temperature). Next, the reaction mixture was stirred (750 rpm) and simultaneously heated to 235 °C (~30 min. heating time). After the reaction time of 3h, the reactor was cooled and depressurized at room temperature. The reactor contents were quantitatively collected by washing the reactor with ethanol.

The solid pulp was separated by filtration and washed thoroughly with acetone. Next, the resulting filtrate was evaporated and a brown oil was obtained, which was subjected to a threefold liquid-liquid extraction using dichloromethane (DCM) and water. To obtain the RCF lignin oil, the DCM-extracted phase was dried and based on the weight of this oil, the degree of delignification of 49% is determined (relative to Klason lignin weight).

4. Fractionation

The RCF lignin oil was fractionated using a sequential extraction protocol. Approximately 15 g of lignin was threefold extracted at 80 °C for 0.5 h with 75 mL of solvent; respectively: 100% heptane, 80% heptane/20% EtoAc, 60% heptane/40% EtoAc, 40% heptane/60% EtoAc, 20% heptane/80% EtoAc, 100% EtoAc. After each extraction, the soluble fraction was decanted and any remaining extraction solvent in the residual fraction was removed by rotary evaporation prior to the next extraction step. The soluble extracts were concentrated by rotary evaporation and dried in an oven to determine its weight.

Silica gel column chromatography was performed with silica gel (60 Å), using binary acetone/heptane mixtures with varying gradients. The separation was monitored using TLC analysis.

5. GC analysis

The phenolic monomers of RCF were quantitatively analysed by GC. Therefore, a weighed amount of external standard (2-isopropylphenol; ~20 mg) was added to a GC-vial containing a weighed amount of lignin (~40 mg). Subsequently 0.3 mL of pyridine and 0.3 mL of N-methyl-N-(trimethylsilyl)trifluoroacetamide was added, next to 0.6 mL of acetonitrile. The vial was sealed and put in an oven at 80 °C for 30 min. Afterwards, the samples were analysed on a GC (Agilent 6890 series)

equipped with a HP5-column and a flame ionization detector (FID). The following operating conditions were used: injection temperature of 300 °C, column temperature program: 50 °C (2min), 15 °C min^{-1} to 150 °C, 10 °C min^{-1} to 220 °C and 20 °C min^{-1} to 290 °C (12min), with a detection temperature of 300 °C. The sensitivity factors of most of the monomers were obtained by calibration with commercial standards. The phenolic dimers were analysed in the same way as the monomers.

The identification of the monomer and dimer signals was performed with GC-MS using an Agilent 6890 series GC equipped with a HP1-MS capillary column and an Agilent 5973 series Mass Spectroscopy detector. The scanning range of the MS was set between 150 and 800 g/mol.

6. GPC analysis

The distribution of the molar mass of the lignin products was investigated using gel permeation chromatography – size exclusion (GPC-SEC). Therefore, a lignin sample was solubilized in THF (5 mg mL^{-1}) and subsequently filtered with a 0.2 µm PTFE membrane to remove any particulate matter to prevent plugging of the column. GPC-SEC analyses were performed at 40 °C on a Waters E2695 equipped with a PL-Gel 3 µm Mixed-E column with at length of 300 mm, using THF as a solvent with a flow of 1 mL min^{-1}. The detection was UV based at a wavelength of 280 nm. Calibration were based on calibration with commercial polystyrene standards of Agilent.

7. ^{1}H-^{13}C 2D HSQC NMR analysis

Approximately 70 mg of the lignin sample was dissolved in 0.6 mL DMSO-d_6 and loaded in an NMR tube. The two-dimensional ^{1}H-^{13}C HSQC NMR experiment was conducted at 298K using a Bruker Avance III HD 400 MHz console with a Bruker Ascend™ 400 Magnet, equipped with a 5 mm PABBO probe. A Bruker standard pulse sequence ('hsqcetgpsp.3') was used for semi-quantification with the following parameters: spectral width in F2 dimension (^{1}H) of 13 ppm using 2048 data points, a spectral width in F1 dimension (^{13}C) of 165 ppm, using 256 data points, a total of 16 scans were recorded with a 2s interscan delay (D1). The Bruker standard pulse sequence ('hsqcedetgp') was used for qualification with the following parameters: spectral width in F2 dimension (^{1}H) of 13 ppm using 1024 data points, a spectral width in F1 dimension (^{13}C) of 165 ppm using 256 data points. Depending on the sample, the amount of scans was varied between 2 and 16. Bruker's Topspin 4.0.2 software was used for data processing and volume integration. The spectra was processed in 2048 data points in the F2 and F1 dimension (with one level of linear prediction and 32 coefficients). The solvent peak of DMSO was used as the internal reference (δ_C/ δ_H: 39.5 ppm/2.49 ppm) following by manually phasing and automatic baseline correction.

8. ^{13}C-NMR

Lignin samples with a concentration of approximately 300 mg mL^{-1} and 0.01 M chromium (III) acetylacetonate were loaded in an NMR tube. The ^{13}C experiment was conducted at 298 K using a Bruker Avance II^{+} 600 MHz console with a Bruker 600 Ultrashield^{TM} magnet, equipped with a 5 mm PABBO probe. A Bruker standard pulse sequence was used and 20k scans were obtained with a d1 of 2s and an acquisition time of 1.4s. The spectra were processed in MestReNova using a Gaussian apodization of 10 Hz and multi-point baseline correction before its integration.

9. ^{13}P-NMR analysis

^{31}P-NMR measurements were performed in triplicate using a standard phosphitylation procedure.[2] A solvent solution (1.6 pyridine : 1 $CDCl_3$) was used to make stock solutions of the internal standard (cholesterol, 20 mg mL^{-1}) and relaxation agent (chromium acetylacetonate, 10 mg mL^{-1}). An amount of lignin (approximately 20 mg) was accurately weighed and 100 µL of the internal standard solution and 50 µL of the relaxation agent solution was added, next to 400 µL of solvent solution. Subsequently, 75 µL of 2-chloro-4,4,5,5-tetramethyl-,1,3,2-dioxaphospholane (TMDP) was added and the sample was thoroughly mixed before transferring them to the NMR-tube. ^{31}P-NMR spectra were obtained on a Bruker Avance III 400 MHz NMR using a standard phosphorous pulse program (256 scans, 5s interscan delay, O1P 140 ppm). The chemical shifts were calibrated by assigning the sharp peak of residual water + TMDP at 132.2 ppm and automatic baseline correction was applied.

Milled wood lignin

Milled wood lignin was isolated according to previously reported methods. Briefly, 10 g of soxhlet extracted pine was ball milled in a ZrO_2 jar using 9 ZrO_2 balls of 10 mm in a Retsch GmbH PM 100 planetary ball mill for 20 h at 500 rpm with intervals of 15 minutes to avoid overheating. Then 5 g of the obtained wood meal was extracted with 50 mL dioxane/water (9:1, v/v) for 24 h under stirring. Afterwards, the solvent was separated from the solids by centrifugation. This extraction was repeated twice. Subsequently, the solvent was evaporated under vacuum at 35 °C.

D. Additional data

1. Solvent Fractionation

The RCF lignin oil (15g) was extracted using a threefold sequential extraction protocol: the extraction with a certain solvent, Heptane, 80% Heptane/20% Ethylacetate (EtoAc), 60% Heptane/40% EtoAc, 40% Heptane/60% EtoAc, 20% Heptane/80% EtoAc, 100% EtoAc was performed 3 times (75 mL, 80 °C, 0.5h, stirring) before advancing with the next solvent (**Fig. S1)** with a different polarity **(Table S1).** The fractions were dried to determine their yields: F_{H100}: 10.7 wt%, $F_{H80/EtoAc20}$: 14.8 wt%, $F_{H60/EtoAc40}$: 33.9 wt%, $F_{H40/EtoAc60}$: 23.9 wt%, $F_{H20/EtoAc80}$: 12.7 wt%, $F_{EtoAc100}$: 4.0 wt%. Clearly, most of the lignin oil is extracted in the binary solvent mixtures with medium polarity.

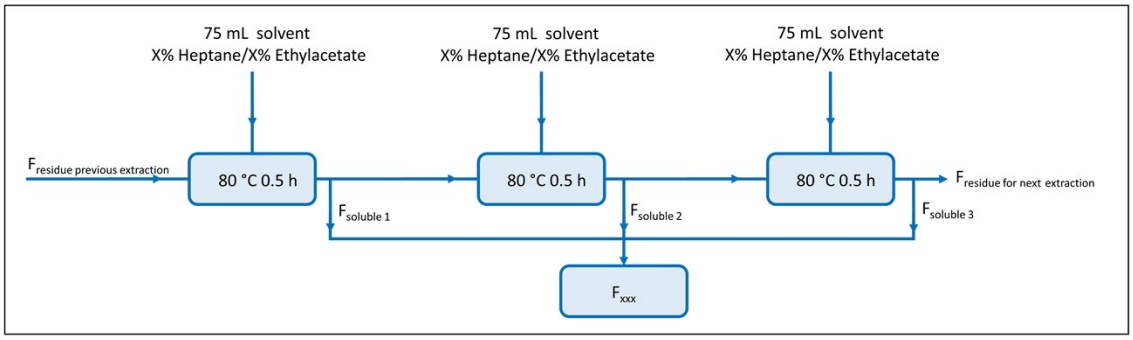


Fig S1. Graphical overview of one extraction step of the solvent fractionation procedure.

Table S1. Hansen solubility parameters of the binary solvent mixtures of heptane and ethyl acetate used in the solvent fractionation.

	δd ($MPa^{1/2}$)	δp ($Mpa^{1/2}$)	δh ($Mpa^{1/2}$)
F_{H100}	15.3	0	0
$F_{H80/EtoAc20}$	15.4	1.06	1.44
$F_{H60/EtoAc40}$	15.5	2.12	2.88
$F_{H40/EtoAc60}$	15.6	3.18	4.32
$F_{H20/EtoAc80}$	15.7	4.24	5.76
$F_{EtoAc100}$	15.8	5.3	7.2

2. GPC-SEC data

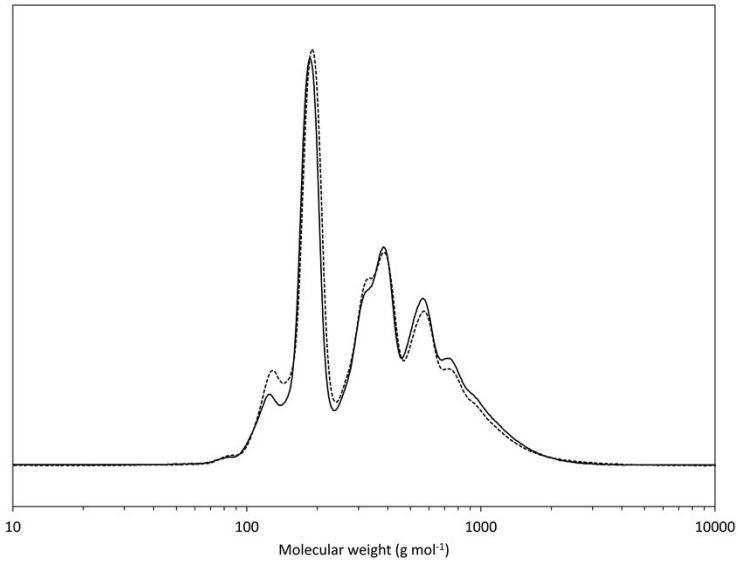


Fig S2. GPC chromatograms of the RCF lignin oil and the mass balanced RCF lignin oil (dotted line). The GPC profile of the mass balanced RCF lignin oil was obtained by multiplying the normalized GPC profile of each fraction with the respective fractional percentage.

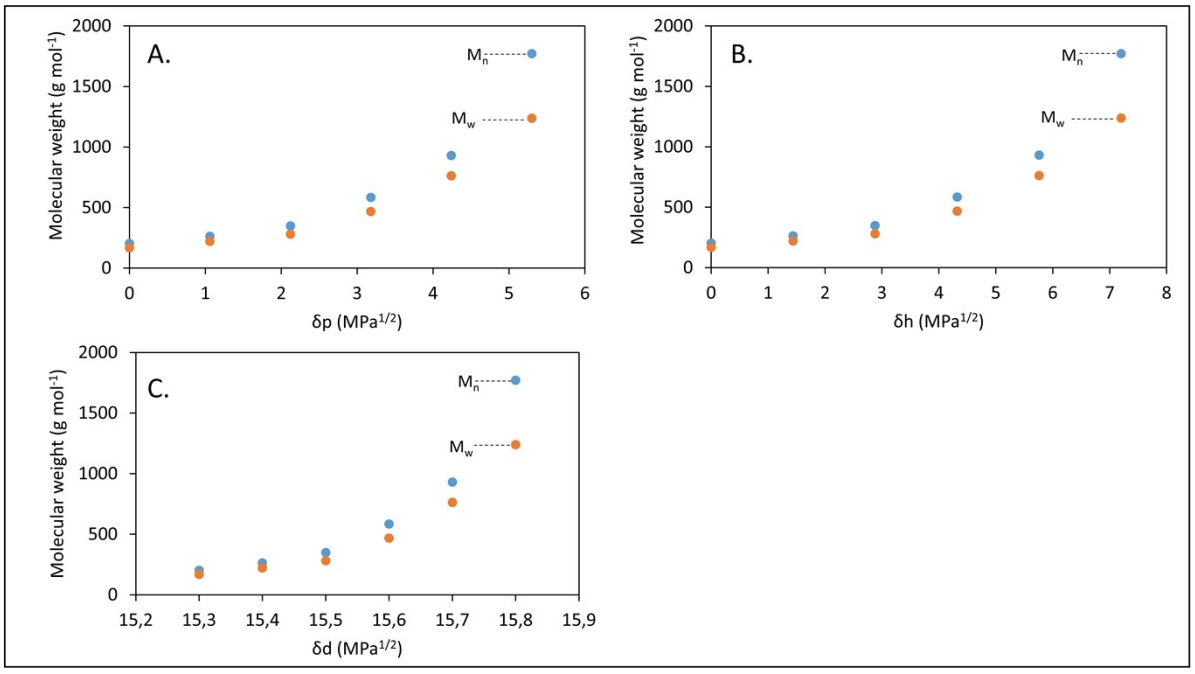


Fig S3. Number average molecular weight (M_n) and weight average molecular weight (M_w) in function of the Hansen solubility parameters of the 6 extraction solvents (a) M_w and M_n in function of dipolar intermolecular force δp (b) M_w and M_n in function of hydrogen bonds δh and (c) M_w and M_n in function of dispersion force δd.

Table S2. GPC quantification of the lignin oil and its derived fractions, run in triplicate.

		F_{H100}	F_{H80}	F_{H60}	F_{H40}	F_{H20}	F_{EA100}	F_{Oil}
Measurement 1	M_n (g mol^{-1})	169	225	288	478	769	1308	426
	M_w (g mol^{-1})	202	269	360	598	941	1895	675
	DI	1.20	1.20	1.25	1.25	1.22	1.45	1.58
Measurement 2	M_n (g mol^{-1})	168	222	279	465	759	1270	421
	M_w (g mol^{-1})	200	263	345	578	927	1818	667
	DI	1.19	1.18	1.24	1.24	1.22	1.43	1.59
Measurement 3	M_n (g mol^{-1})	168	217	276	460	759	1139	413
	M_w (g mol^{-1})	207	259	343	575	925	1600	659
	DI	1.23	1.19	1.24	1.25	1.22	1.24	1.60
Average	M_n (g mol^{-1})	168	221	281	468	762	1239	420
	stdev	1	4	6	9	6	89	7
	M_w (g mol^{-1})	203	264	349	584	931	1771	667
	stdev	4	5	9	13	9	153	8
	DI	1.21	1.19	1.24	1.25	1.22	1.37	1.59
	stdev	0.021	0.010	0.006	0.006	0.000	0.116	0.010

3. GC-data

Table S3. Detailed monomer composition and standard deviations between brackets of Fig 1.

	F_{H100}	F_{H80}	F_{H60}	F_{H40}	F_{H20}	F_{EA100}	F_{oil}	**Mass balance oil**
4-Propanolguaiacol	6.7 (0.35)	46.3 (0.84)	52.8 (0.16)	14.2 (0.06)	0.1 (0.00)	0 (0)	28.8 (0.24)	28.9
4-Propylguaiacol	21.1 (0.5)	0.46 (0.02)	0 (0)	0 (0)	0 (0)	0 (0)	2.5 (0.02)	2.3
4-(3-Methoxypropyl)guaiacol	10.1 (0.08)	2.8 (0.00)	0.3 (0.02)	0.3 (0.00)	0 (0)	0 (0)	1.7 (0.02)	1.7
4-Ethylguaiacol	3.8 (0.04)	0.4 (0.02)	0.1 (0.00)	0 (0)	0 (0)	0 (0)	0.5 (0.00)	0.5
Iso-eugenol	0.2 (0.02)	0.2 (0.05)	0.5 (0.05)	0.7 (0.00)	0 (0)	0 (0)	0.3 (0.03)	0.4
4-Methylguaiacol	3.1 (0.01)	0.6 (0.01)	0.1 (0.00)	0 (0)	0 (0)	0 (0)	0.5 (0.00)	0.1
Total monomers	45.2 (0.015)	51.2 (0.00)	53.8 (0.00)	15.2 (0.00)	0.1 (0.00)	0 (0)	34.4 (0.00)	33.8

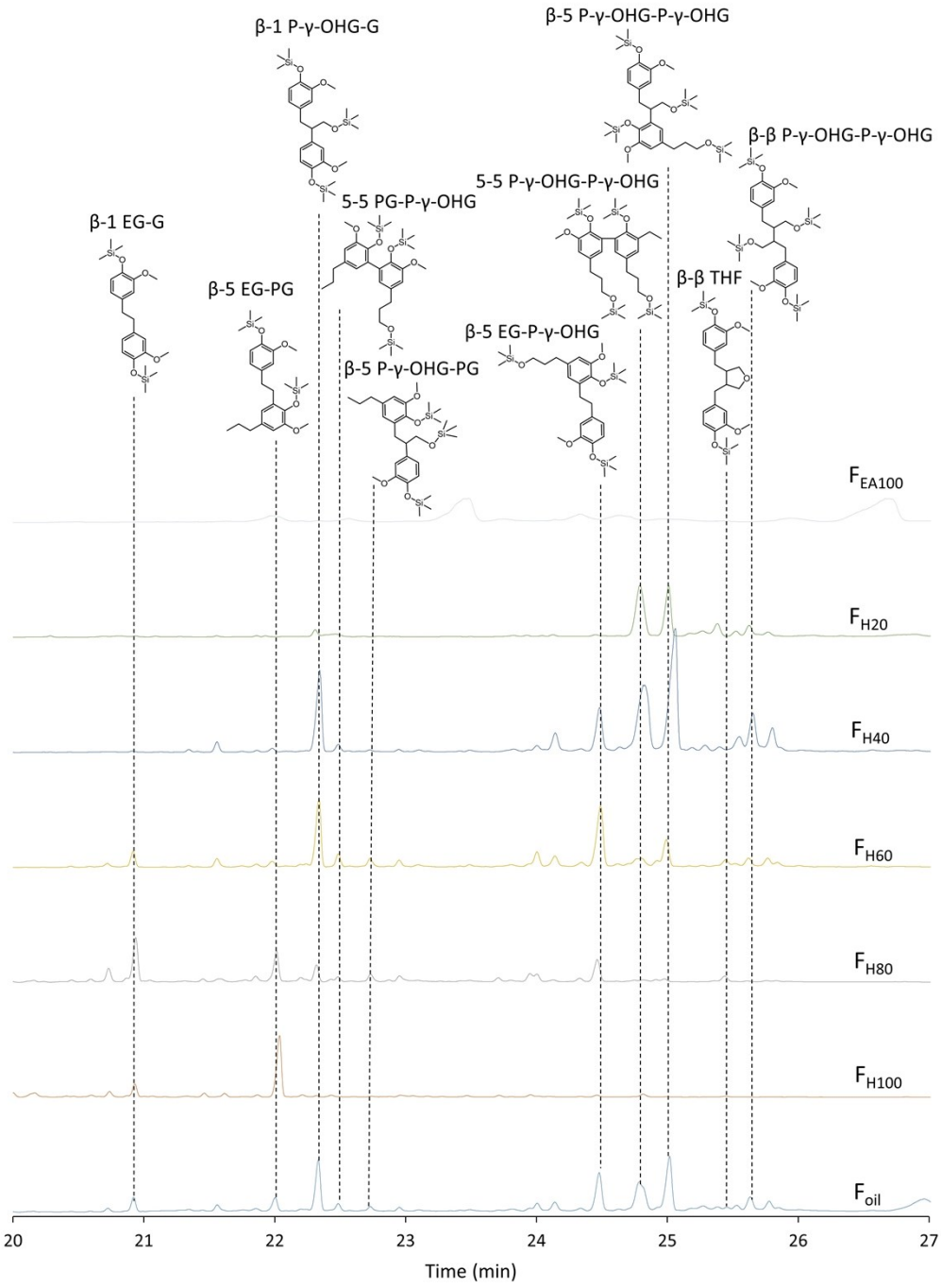


Fig S4. Identification of trimethylsilylated dimers in the different fractions and the complete oil in the GC-FID spectrum. The obtained MS-spectra were in accordance with literature. [3–6]

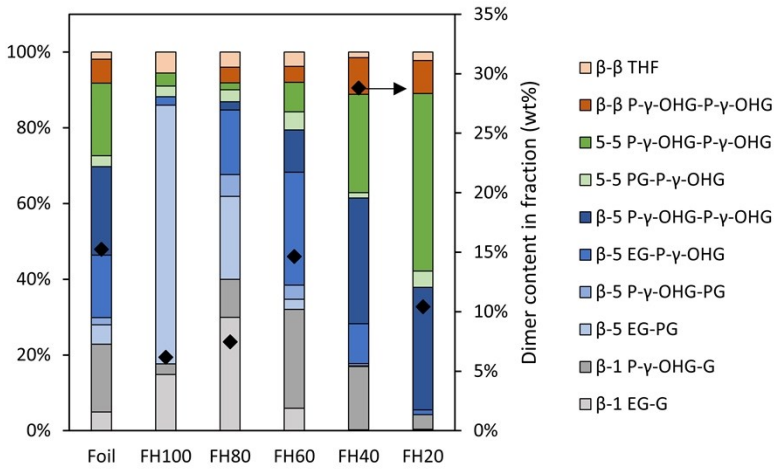


Fig S5. Relative distribution of the RCF dimers in the complete oil and the fractions as shown in **Fig S4.** The molecular structures of the respective dimers can be found in **Fig S4.** Important remark: Since the quantification is based on response factors calculated by the ECN method, the observed yields may deviate from the actual yields, as the error on ECN values on highly oxygenated compounds can be high. However, this is the best available method.

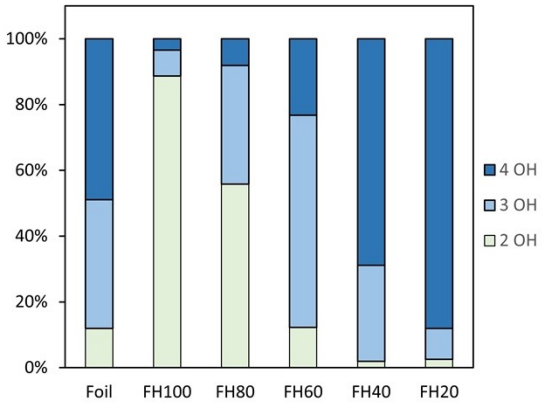


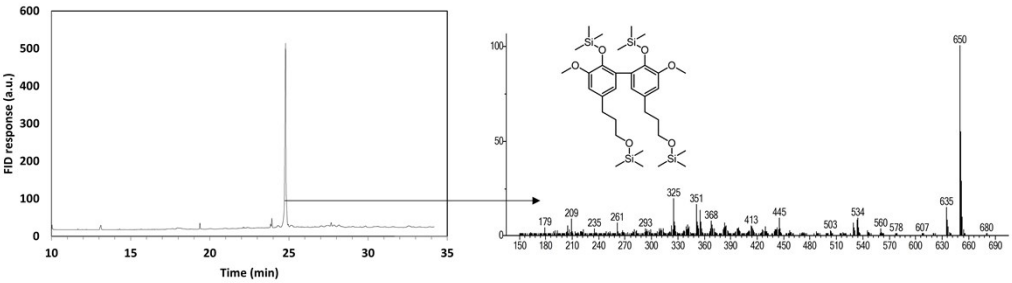
Fig S6. Relative distribution of dimers with 2, 3 or 4 OH-functionalities in the complete oil and the fractions.

In FH100 dimers β-1 EG-G and β-5 EG-PG account for more than 80% of the total observed dimers (Fig S5-S6). These dimers are only present for 55% in FH80 and 12% in FH60. Both dimers only contain 2 phenolic OH groups, in contrast to the other dimers, indicating a large selectivity for apolar dimers in this fraction. Dimers with 3 OH groups (2 phenolic and 1 aliphatic) are mainly extracted in FH80, FH60 and FH40 which are fractions resulting from slightly more polar solvents, in comparison to 100% heptane. Two dimers, β-1 P-γ-OHG-G and β-5 EG - P-γ-OHG make up the majority of this group. The last group of dimers contain 4 OH groups and are mainly extracted in the most polar solvents. Clearly, a few structural motifs are present in a high concentration. These are the β-1 E, β-5 E, β-1 γ-OH, β-5 γ-OH, β-β 2x γ-OH and 5-5 inter-unit linkage. Similar trends have been observed in literature.[6–8]

4. Isolation of RCF-dimers

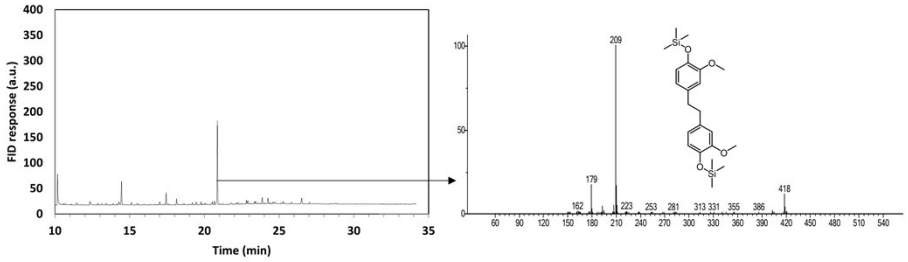
Dimer 6,6'-Dihydroxy-5,5'-dimethoxy[1,1'-biphenyl]-3,3'-dipropanol (5-5 PGOH-PGOH)

5,5'-Bis(3-hydroxypropyl)-3,3'-dimethoxy-2,2'-biphenyldiol (or 5-5 PGOH-PGOH) was obtained starting from 1.5 g of $F_{H40/EtoAc60}$.The sample was separated by silica gel chromatography eluting with a gradient of 40-100 % Acetone/ 60-0 % Heptane, resulting in 40 different fractions. In fraction 29 the dimer 5-5 PGOH-PGOH was the main product, as is shown by a combined GC-FID & MS analysis. The MS spectrum is in accordance with previous literature.[3,4]



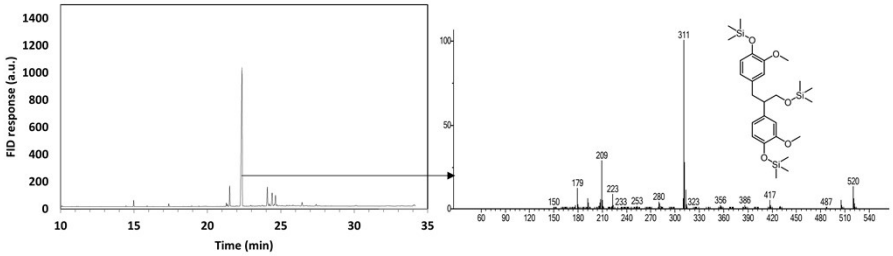
Dimer 1,2-Bis(4-hydroxy-3-methoxyphenyl)ethane (β-1 EG-G)

1,2-Bis(4-hydroxy-3-methoxyphenyl)ethane (or β-1 EG-G) was obtained starting from 1.0 g of $F_{H80/EtoAc20}$. The sample was separated by silica gel chromatography eluting with a gradient of 25-100 % Acetone/ 75-O % Heptane, resulting in 22 different fractions. In fraction 5 the dimer β-1 EG-G was the main product, as is shown by a combined GC-FID & MS analysis. The MS spectrum is in accordance with previous literature.[3–6]



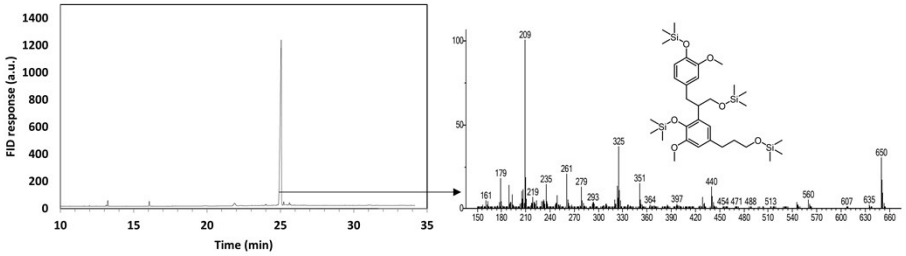
Dimer 4-Hydroxy-β-(4-hydroxy-3-methoxyphenyl)-3-methoxybenzenepropanol (β-1 POHG-G)

4-Hydroxy-β-(4-hydroxy-3-methoxyphenyl)-3-methoxybenzenepropanol (or β-1 POHG-G) was obtained starting from 1.5g of $F_{H40/EtoAc60}$. The sample was separated by silica gel chromatography eluting with a gradient of 40-100 % Acetone/ 60-0 % Heptane, resulting in 40 different fractions. In fraction 29 the dimer β-1 POHG-G was the main product, as is shown by a combined GC-FID & MS analysis. The MS spectrum is in accordance with previous literature.[3–5]



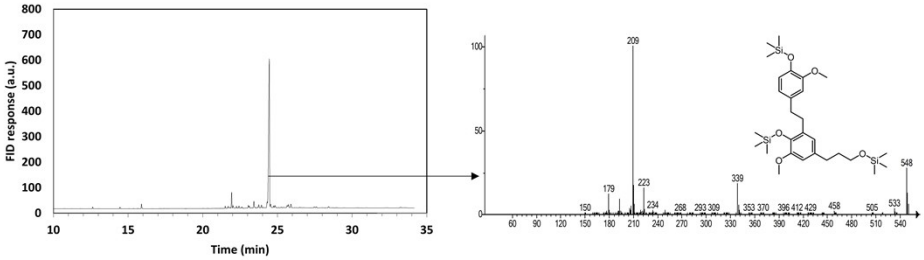
Dimer (-)-4-Hydroxy-β-[2-hydroxy-5-(3-hydroxypropyl)-3-methoxyphenyl]-3-methoxybenzenepropanol (β-5 PGOH-PGOH)

(-)-4-Hydroxy-β-[2-hydroxy-5-(3-hydroxypropyl)-3-methoxyphenyl]-3-methoxybenzenepropanol (or β-5 PGOH-PGOH) was obtained starting from 1.5 g of $F_{H40/EtoAc60}$. The sample was separated by silica gel chromatography eluting with a gradient of 40-100 % Acetone/ 60-0 % Heptane, resulting in 40 different fractions. In fraction 25 the dimer β-5 PGOH-PGOH was the main product, as is shown by a combined GC-FID & MS analysis. The MS spectrum is in accordance with previous literature.[3,4]



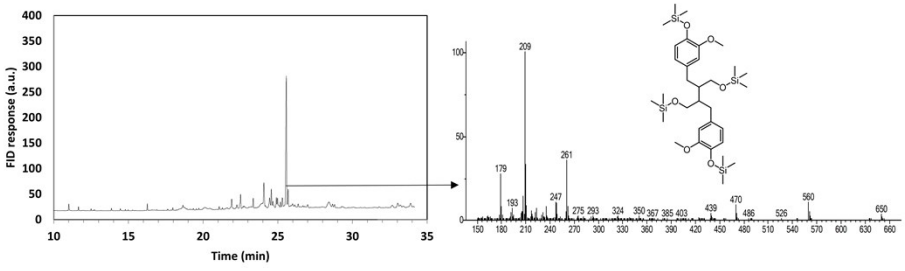
Dimer 4-Hydroxy-3-[2-(4-hydroxy-3-methoxyphenyl)ethyl]-5-methoxybenzenepropanol (β-5 EG-PGOH)

4-Hydroxy-3-[2-(4-hydroxy-3-methoxyphenyl)ethyl]-5-methoxybenzenepropanol (or β-5 EG-PGOH) was obtained starting from 1.5 g of $F_{H40/EtoAc60}$. The sample was separated by silica gel chromatography eluting with a gradient of 40-100 % Acetone/ 60-0 % Heptane, resulting in 40 different fractions. In fraction 14 the dimer β-5 EG-PGOH was the main product, as is shown by a combined GC-FID & MS analysis. The MS spectrum is in accordance with previous literature.[3–5]



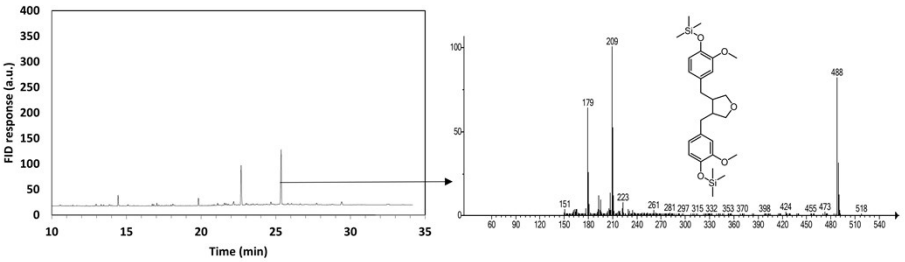
Dimer 2,3-Bis[(4-hydroxy-3-methoxyphenyl)methyl]-1,4-butanediol (β-β PGOH-PGOH)

2,3-Bis[(4-hydroxy-3-methoxyphenyl)methyl]-1,4-butanediol (or β-β PGOH-PGOH)was obtained starting from 1.5 g of $F_{H40/EtoAc60}$. The sample was separated by silica gel chromatography eluting with a gradient of 40-100 % Acetone/ 60-0 % Heptane, resulting in 40 different fractions. In fraction 21 the dimer β-β PGOH-PGOH was the main product, as is shown by a combined GC-FID & MS analysis. The MS spectrum is in accordance with previous literature.[6]



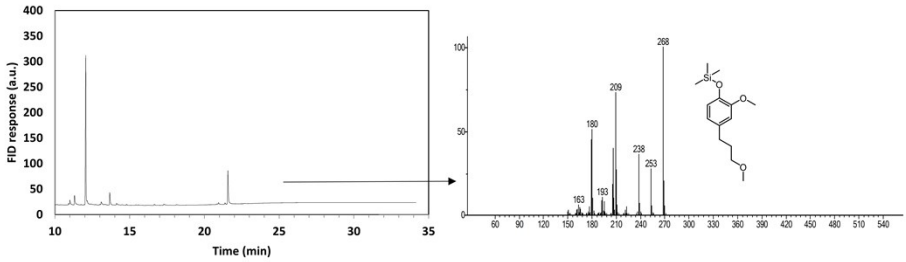
Dimer 4,4'-[(Tetrahydro-3,4-furandiyl)bis(methylene)]bis[2-methoxyphenol] (β-β THF)

4,4'-[(Tetrahydro-3,4-furandiyl)bis(methylene)]bis[2-methoxyphenol] (or β-β THF) was obtained starting from 1.5 g of $F_{H40/EtoAc60}$. The sample was separated by silica gel chromatography eluting with a gradient of 40-100 % Acetone/ 60-0 % Heptane, resulting in 40 different fractions. In fraction 7 the β-β THF was the main product, as is shown by a combined GC-FID & MS analysis. The MS spectrum is in accordance with previous literature.[5,9–12]



4-(3-methoxypropyl)guaiacol (End unit P-γ-O-Me)

4-(3-methoxypropyl)guaiacol (or P-γ-O-Me) was obtained starting from 1,0g of $F_{H80/EtoAc20}$. The sample was separated by silica gel chromatography eluting with a gradient of 25-100 % Acetone/ 75-0 % Heptane, resulting in 22 different fractions. In fraction 7 4-(3-methoxypropyl)guaiacol was the main product, as is shown by a combined GC-FID & MS analysis.



5. 2D HSQC NMR assignments & analysis

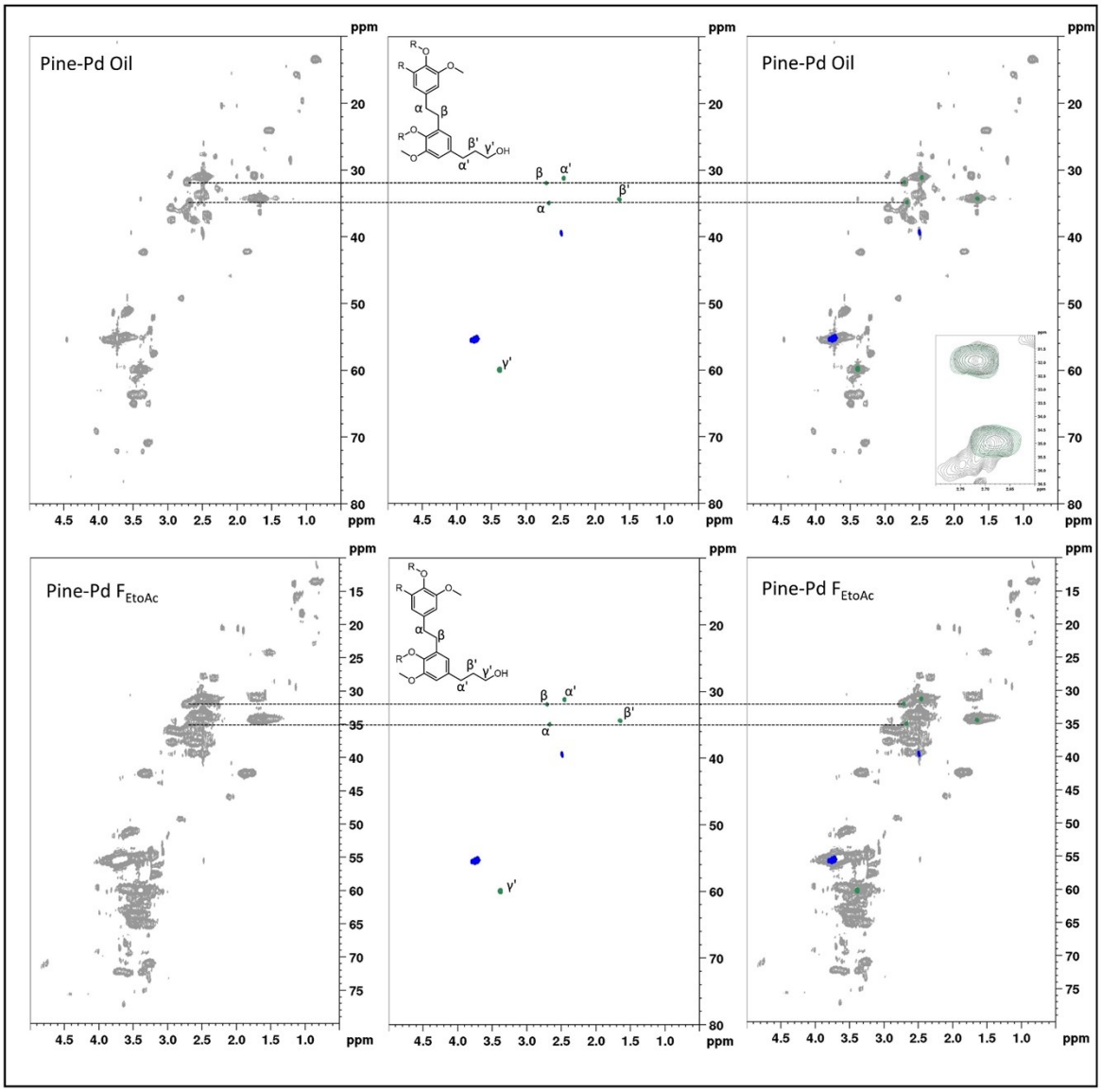


Fig S7. Assignment of β-5 ethyl inter-unit linkages in the 2D HSQC spectrum of RCF lignin oil and F_{EtoAc}. The 2D HSQC spectrum containing the β-5 ethyl inter-unit linkage is measured in 135 DEPT mode, the CH and CH_3 signals are colored in blue, the CH_2 in green. F_{EtoAc} is a fraction exclusively composed of RCF oligomers (absence of monomers and dimers), indicating the existence of this inter-unit linkage in RCF lignin oligomers. Horizontal lines are drawn to indicate the overlap between the β-5 ethyl linkage and the RCF lignin.

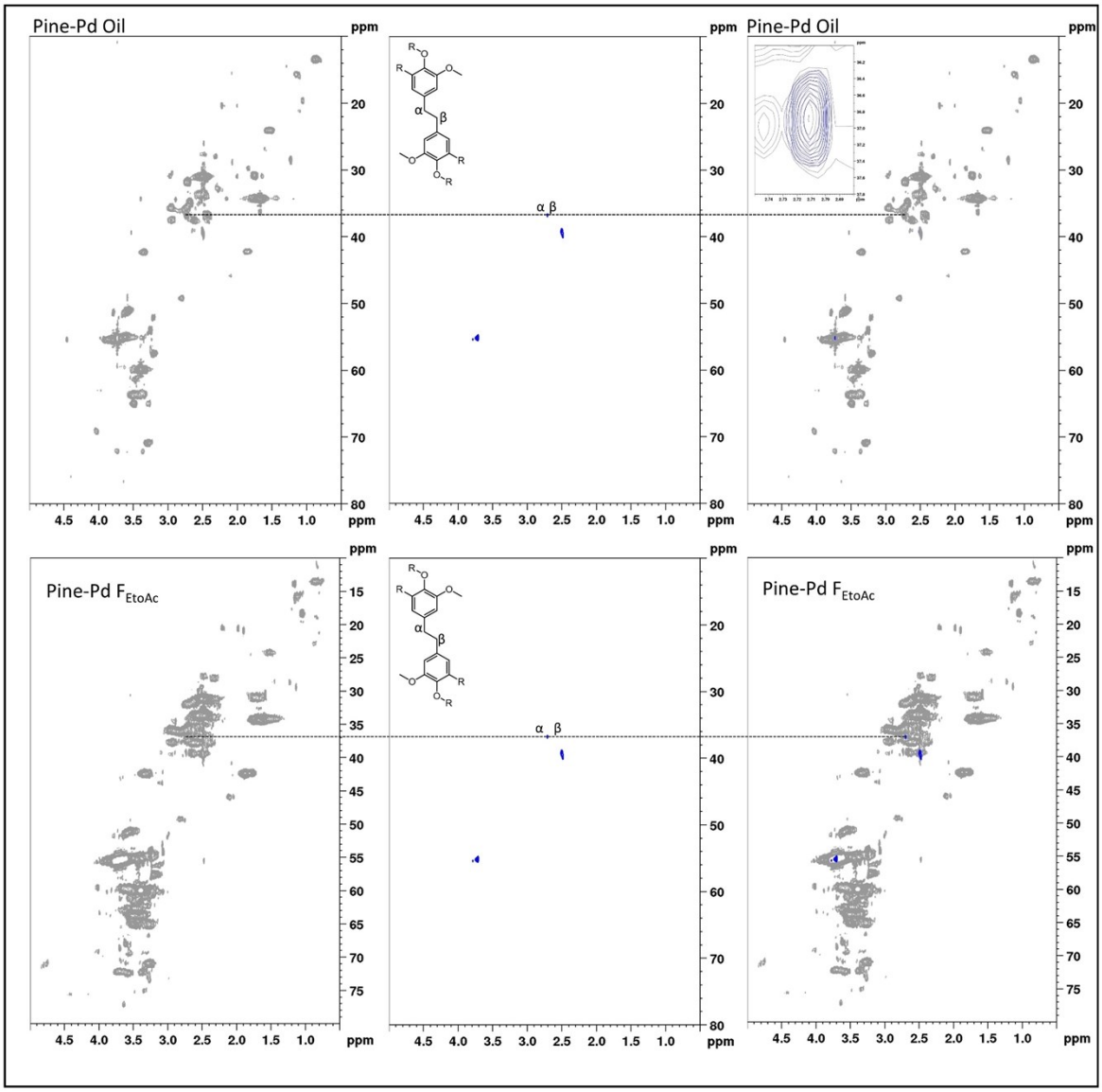


Fig S8. Assignment of β-1 ethyl inter-unit linkages in the HSQC spectrum of RCF lignin oil and F_{EtoAc}. The 2D HSQC spectrum containing the β-1 ethyl inter-unit linkage is measured in 135 DEPT mode, the CH and CH_3 signals are colored in blue, the CH_2 in green. F_{EtoAc} is a fraction exclusively composed of RCF oligomers (absence of monomers and dimers), indicating the existence of this inter-unit linkage in RCF lignin oligomers. Horizontal lines are drawn to indicate the overlap between the β-5 ethyl linkage and the RCF lignin.

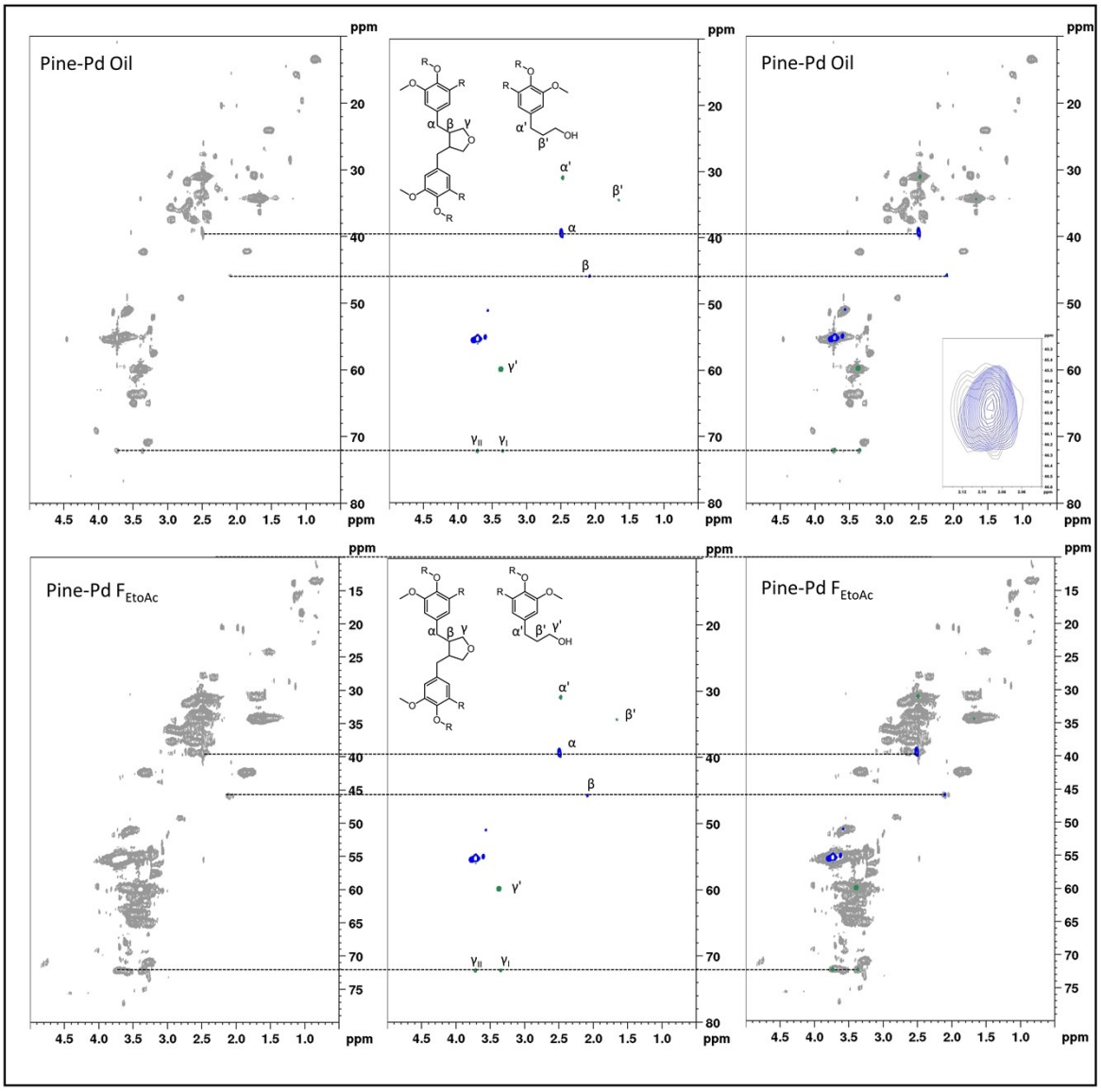


Fig S9. Assignment of β-β THF inter-unit linkages in the HSQC spectrum of RCF lignin oil and F_{EtoAc}. The 2D HSQC spectrum containing the β-β THF inter-unit linkage is measured in 135 DEPT mode, the CH and CH_3 signals are colored in blue, the CH_2 in green. F_{EtoAc} is a fraction exclusively composed of RCF oligomers (absence of monomers and dimers), indicating the existence of this inter-unit linkage in RCF lignin oligomers. Horizontal lines are drawn to indicate the overlap between the β-5 ethyl linkage and the RCF lignin.

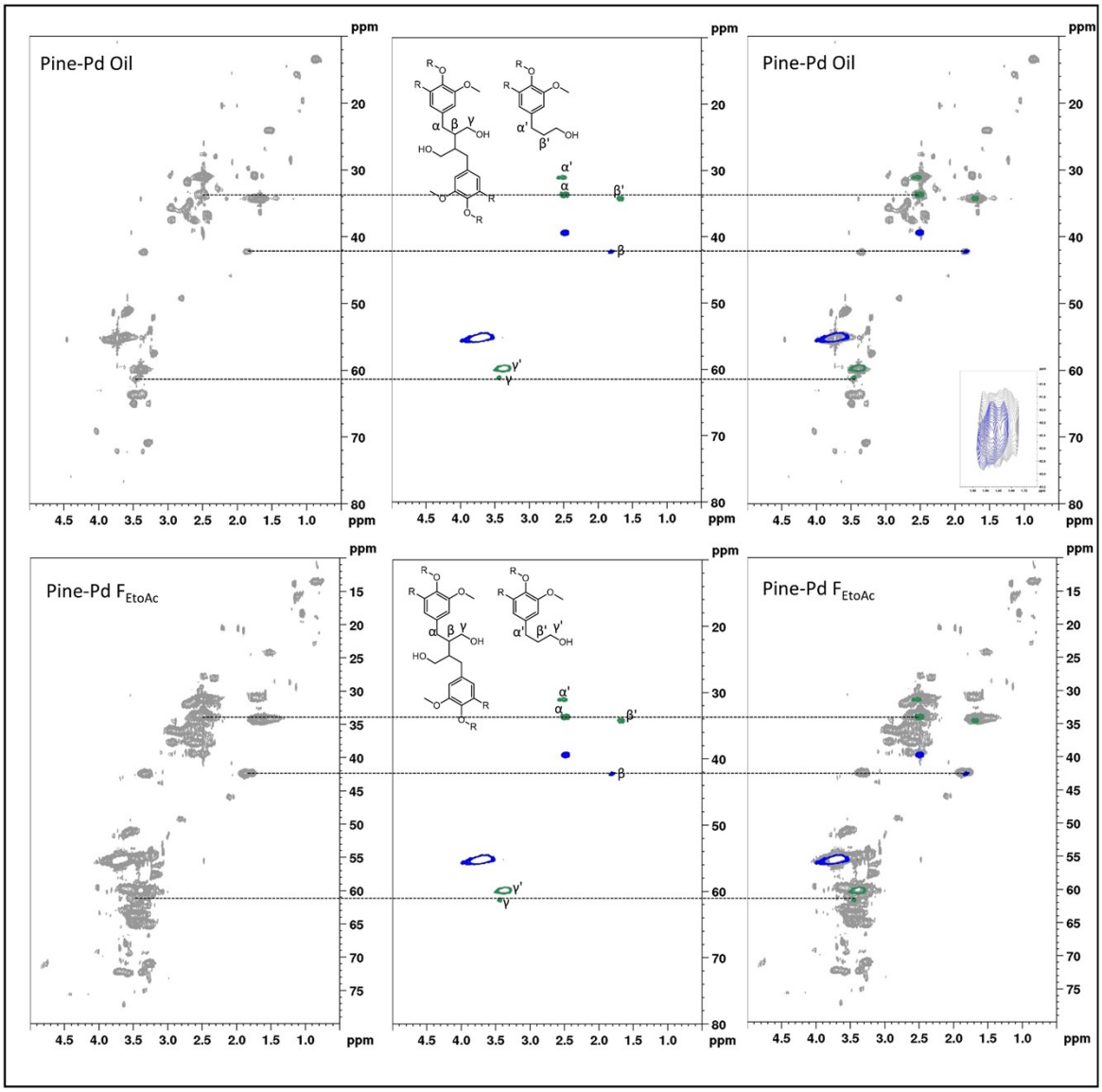


Fig S10. Assignment of β-β 2x γ-OH inter-unit linkages in the HSQC spectrum of RCF lignin oil and F_{EtoAc}. The 2D HSQC spectrum containing the β-β 2x γ-OH inter-unit linkage is measured in 135 DEPT mode, the CH and CH_3 signals are colored in blue, the CH_2 in green. F_{EtoAc} is a fraction exclusively composed of RCF oligomers (absence of monomers and dimers), indicating the existence of this inter-unit linkage in RCF lignin oligomers. Horizontal lines are drawn to indicate the overlap between the β-5 ethyl linkage and the RCF lignin.

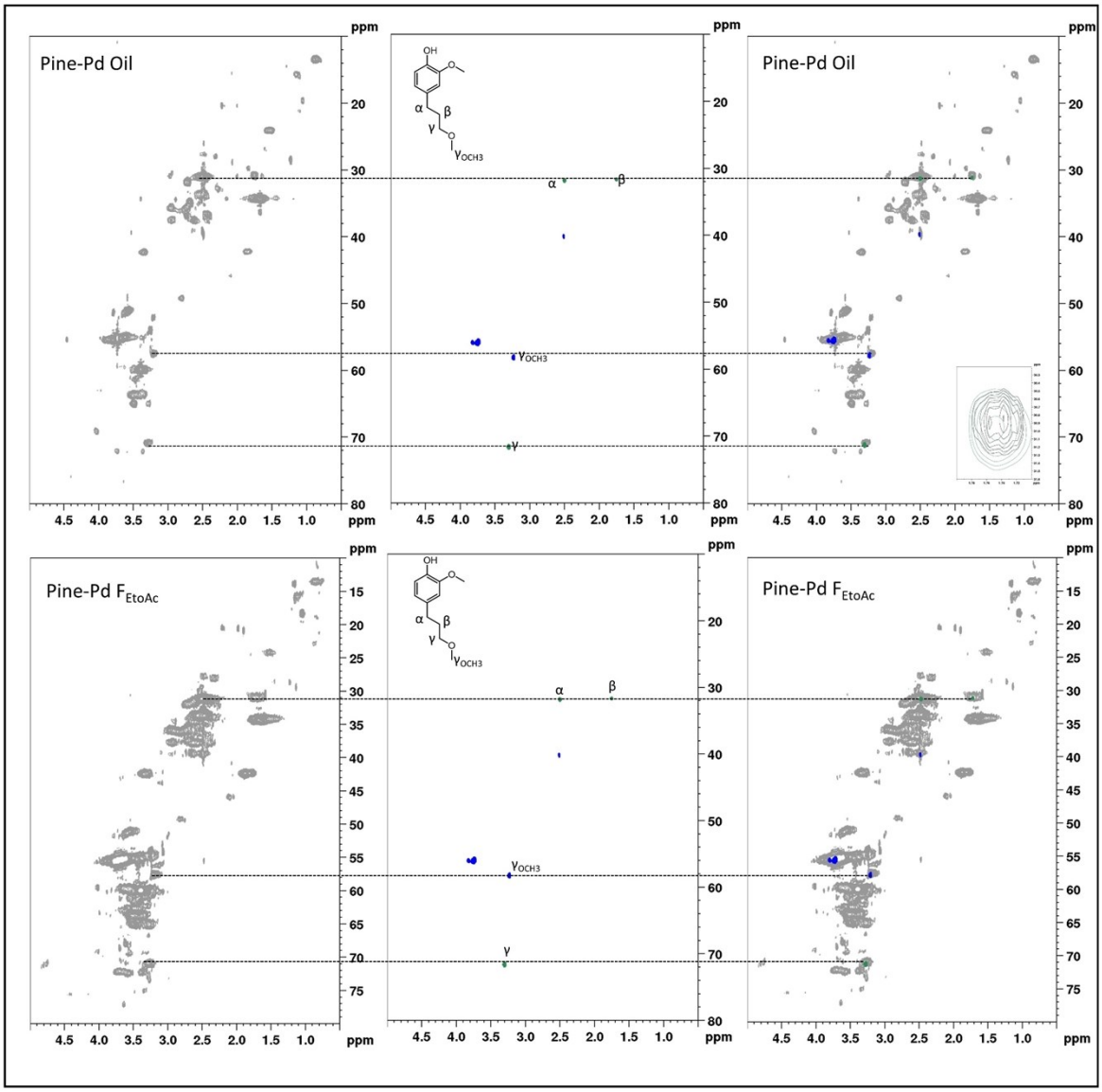


Fig S11. Assignment of 4-(3-methoxypropyl) end-units in the HSQC spectrum of RCF lignin oil and F_{EtoAc}. The 2D HSQC spectrum containing the 4-(3-methoxypropyl) end-units is measured in 135 DEPT mode, the CH and CH_3 signals are colored in blue, the CH_2 in green. F_{EtoAc} is a fraction exclusively composed of RCF oligomers (absence of monomers and dimers), indicating the existence of this end-unit in RCF lignin oligomers. Horizontal lines are drawn to indicate the overlap between the β-5 ethyl linkage and the RCF lignin.

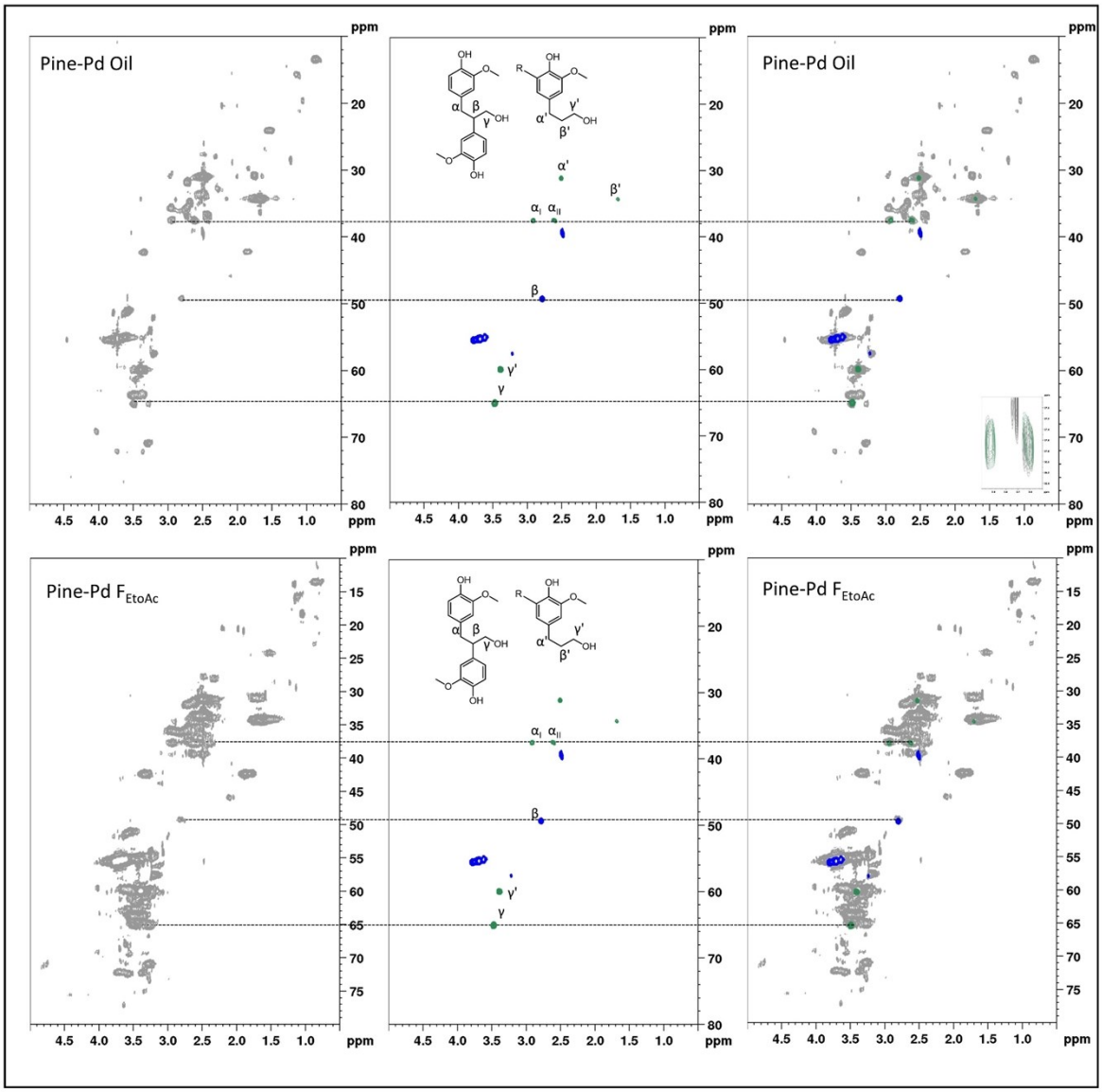


Fig S12. Assignment of β-1 γ-OH inter-unit linkages in the HSQC spectrum of RCF lignin oil and F_{EtoAc}. The 2D HSQC spectrum containing the β-1 γ-OH inter-unit linkage is measured in 135 DEPT mode, the CH and CH_3 signals are colored in blue, the CH_2 in green F_{EtoAc} is a fraction exclusively composed of RCF oligomers (absence of monomers and dimers), indicating the existence of this inter-unit linkage in RCF lignin oligomers. Horizontal lines are drawn to indicate the overlap between the β-5 ethyl linkage and the RCF lignin.

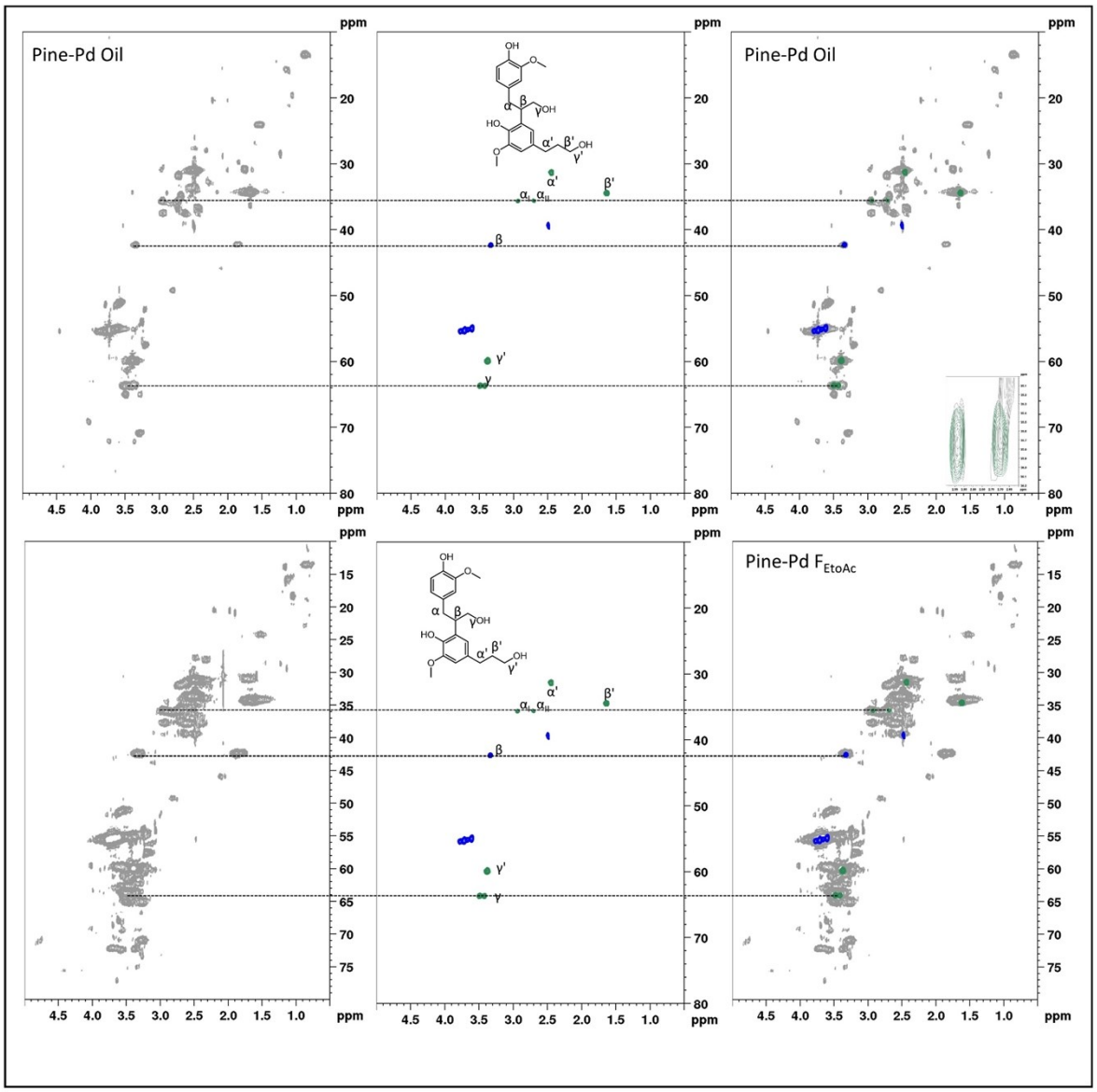
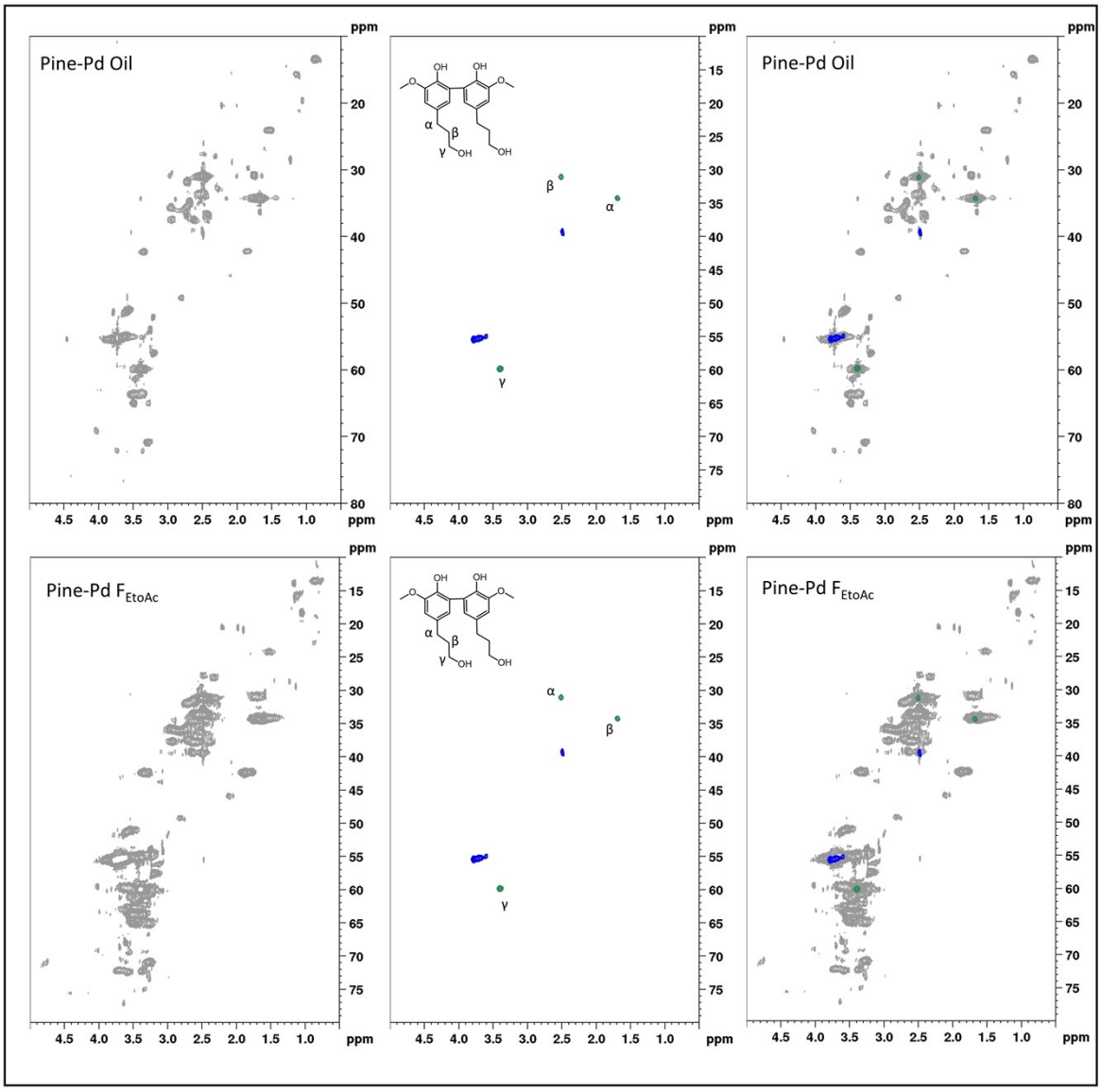


Fig S13. Assignment of β-5 γ-OH inter-unit linkages in the HSQC spectrum of RCF lignin oil and F_{EtoAc}. The 2D HSQC spectrum containing the β-5 γ-OH inter-unit linkage is measured in 135 DEPT mode, the CH and CH_3 signals are colored in blue, the CH_2 in green F_{EtoAc} is a fraction exclusively composed of RCF oligomers (absence of monomers and dimers), indicating the existence of this inter-unit linkage in RCF lignin oligomers. Horizontal lines are drawn to indicate the overlap between the β-5 ethyl linkage and the RCF lignin.


Pine-Pd Oil
Pine-Pd Oil
Pine-Pd F EtoAc
Pine-Pd F EtoAc
ppm
α
β
γ
OH

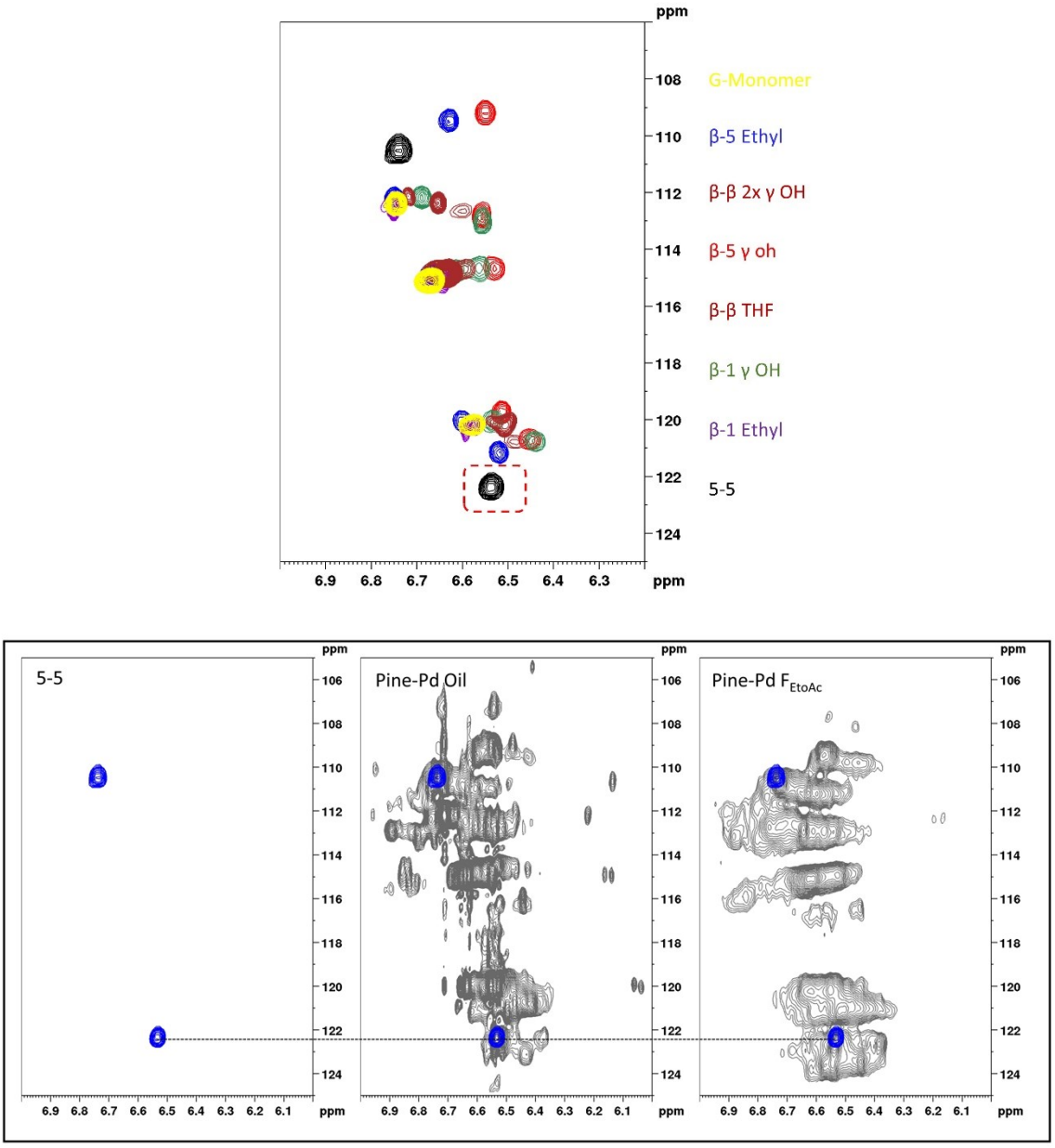


Fig S14. Assignment of 5-5 inter-unit linkages in the HSQC spectrum of RCF lignin oil and F_{EtoAc}. Clearly, the assignment is impossible in the aliphatic region. Therefore, the assignment is based on the aromatic G_6 position. An aromatic substitution on position 5 instead of an aliphatic (β-5) clearly influences the chemical shift of its neighboring C-H pair on position 6, as is evidenced by the figure on top. In RCF-lignin oil and F_{EtoAc} this difference is also observed. Besides, data obtained by ^{31}P-NMR shows similar results after integration of the area (Fig S23).

Table S4 ^{1}H and ^{13}C NMR assignments of diagnostic signals for structural units in lignin HSQC spectra. The cross peaks used in this study for quantification are indicated in bold, the integrals have to be multiplied with their respective factor to calculate the number of linkages per 100 G.

	Literature assignment	solvent	Source	Assignment in DMSO-d6	Factor
β-5 Propanol (β-5 γ-OH)	αI 37.06 / 2.87 ; αII 37.06/3.07 ; β 44.62 /3.46 ; γI: 65.46/3.73 ; γII 65.46 /3.65	Acetone-d6	[12]	αI 35.75 / 2.71 ; αII 35.75/2.94 ; **β 42.44 /3.33** ; γI: 63.85/3.49 ; γII 63.85 /3.42	1
β-5 Ethyl (β-5 E)	α: 36.47/2.79 ; β: 33.33/2.84	Acetone-d6	2 dimers in [12]	α: 34.947/2.68 ; **β: 32/2.72**	1
β-5 Propyl (β-5 P)	αI 43.14 / 2.63 ; αII 43.14/2.91 ; β 35.38 /3.41 ; γ: 20.06/1.15	Acetone-d6	2 dimers in [12]		-
β-1 Propanol (β-1 γ-OH)	αI 39.04 / 2.73 ; αII 39.04/3.04 ; β:51.14/2.92 ; γ: 66.69/3.68	Acetone-d6	[12]	αI 37.6 / 2.61 ; αII 37.6/2.92 ; **β:49.35/2.8** ; γ: 65.1/3.49	1
β-1 Ethyl (β-1 E)	α: 38.6/2.77 ; β: 38.6/2.77	Acetone-d6	[12]	**α: 36.9/2.71 ; β: 36.9/2.71**	0.25
β-β 2x γ-OH	α: 33.8/2.52 ; β: 42.3/1.87	DMSO-d6	[13]	α: 33.7/2.49 ; **β: 42.3/1.82**	0.5
β-β THF	α: 39.2/2.55 ; β: 46.5/2.17 ; γI: 73.3/3.97 ; γII: 73.3/3.53	$CDCL_3$	[8,10,11]	**β: 45.8/2.09** ; γI: 72.3/3.36 ; γII: 72.3/3.72	0.5
Cinnamaldehyde (J)	α: 153.64/7.58 ; β: 125.15/6.62 ; γ: 193.8/9.64	Acetone-d6	[14]		-
Cinnamylalcohol (X)	α: 128.6/6.49 ; β: 128.4/6.26 ; γ: 61.4/4.10	DMSO-d6	[13,14]		-
Dihydrocinnamylalcohol / 4-Propanol (P-γ-OH)	α: 32.62/2.48 ; β: 35.99/1.66 ; γ: 61.58/3.4	DMSO-d6	[14]	α: 31/2.48 ; **β: 34.3/1.66** ; γ: 60/3.39	0.5
5-5 Biphenyl	G6: 123.89/6.69	d-acetone	[12]	**G6: 122.5/6.53**	1
	G6: 122.73	DMSO-d6	[14]		
β-O-4 (A)	**α: 70.9/4.77** ; β: 84.1/4.30 ; γI: 59.9/3.60 ; γII: 59.9/3.26	DMSO-d6	[13]		1
β-5 Phenylcoumarane (B)	**α: 86.8/5.49** ; β: 53.2/3.47 ; γI: 62.9/3.62 ; γII: 62.9/3.73	DMSO-d6	[13]		1
β-β Resinol (C)	**α: 85.1/4.63** ; β: 53.6/3.07 ; γI: 70.9/4.16 ; γII: 70.9/3.76	DMSO-d6	[13]		0.5
Dibenzodioxocin (D)	α: 83.4/4.82 ; **β: 85.4/3.87**	DMSO-d6	[13]		1
End-Unit 4-Ethyl (E)	α: 28.8/2.6 ; β: 16/1.2	DMSO-d6	[7,15]	**α: 27.5/2.47** ; β: 15.7/1.14	0.5
End-unit 4-Methyl (M)	α: 21.6/2.34	Chemdraw		**α: 20.3/2.20**	0.33
End-unit 4-Propyl (P)	α: 36.6/2.42 ; β: 24.12/1.54 ; γ: 13.4/0.87	DMSO-d6	[7]	α: 36.7/2.42 ; **β: 24.1/1.54** ; γ: 61.4/4.10	0.5
End-unit 4-(3-methoxypropyl) (P γ-O-Me)	α: 32/2.52 ; β: 31/1.72; γ: 71.2/3.3; O-Me: 57.8/3.25	DMSO-d6	[15]	α: 31/2.49 ; **β: 31/1.75** ; γ: 70.9/3.3 ; Me: 57.3/3.23	0.5
Vanillic Acid	G2: 112.6/7.45 ; G6: 123.3/7.46	DMSO-d6	[13]		
Acetovanillone	G2: 111/7.45 ; G6: 123.2/7.46	DMSO-d6	[13]		
Vanillin	G2: 110.6/7.4 ; G6: 125.8/7.41	DMSO-d6	[13]		

Table S5. Quantification of structural motifs in lignin relative to the G_2 area in the 2D HSQC spectra.

		F_{H100}	F_{H80}	F_{H60}	F_{H40}	F_{H20}	F_{EA100}	F_{oil}	F_{oil} mass balance
β-β resinol α	**Processing I**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Processing II	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Processing III	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Average	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Standard deviation	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
β-β 2x γ-OH α	**Processing I**	0.00	0.00	0.00	6.00	12.80	14.00	4.60	3.62
	Processing II	0.00	0.00	0.60	6.10	12.90	15.20	3.70	3.91
	Processing III	0.00	0.00	0.60	5.80	13.62	14.26	3.46	3.89
	Average	**0.00**	**0.00**	**0.40**	**5.97**	**13.11**	**14.49**	**3.92**	**3.81**
	Standard deviation	0.00	0.00	0.35	0.15	0.45	0.63	0.60	0.16
β-β 2x γ-OH β	**Processing I**	0.00	0.00	0.50	4.60	11.90	12.60	2.90	3.28
	Processing II	0.00	0.00	0.54	5.14	11.92	15.18	3.16	3.53
	Processing III	0.00	0.00	0.70	4.86	12.42	14.80	3.36	3.57
	Average	**0.00**	**0.00**	**0.58**	**4.87**	**12.08**	**14.19**	**3.14**	**3.46**
	Standard deviation	0.00	0.00	0.11	0.27	0.29	1.39	0.23	0.15
β-β THF β	**Processing I**	0.00	0.32	0.56	1.28	1.54	1.12	0.78	0.78
	Processing II	0.00	0.32	0.60	1.60	1.58	1.84	1.06	0.91
	Processing III	0.00	0.36	0.64	1.50	1.60	1.22	1.06	0.88
	Average	**0.00**	**0.33**	**0.60**	**1.46**	**1.57**	**1.39**	**0.97**	**0.86**
	Standard deviation	0.00	0.02	0.04	0.16	0.03	0.39	0.16	0.07
β-β THF γ	**Processing I**	0.00	0.14	0.41	1.88	1.14	1.40	0.68	0.81
	Processing II	0.00	0.22	0.42	2.12	2.04	1.78	0.66	1.01
	Processing III	0.00	0.16	0.42	2.12	1.40	1.60	0.66	0.91
	Average	**0.00**	**0.17**	**0.42**	**2.04**	**1.53**	**1.59**	**0.67**	**0.91**
	Standard deviation	0.00	0.04	0.01	0.14	0.46	0.19	0.01	0.10

| | | F_{H100} | F_{H80} | F_{H60} | F_{H40} | F_{H20} | F_{EA100} | F_{oil} | F_{oil} mass balance |

β-1 γ-OH α	**Processing I**	0.00	0.51	2.12	5.67	4.11	3.14	3.28	2.80
	Processing II	0.00	0.51	2.37	6.01	4.42	4.00	3.35	3.04
	Processing III	0.00	0.46	2.32	5.62	3.79	2.83	2.79	2.79
	Average	**0.00**	**0.49**	**2.27**	**5.77**	**4.11**	**3.32**	**3.14**	**2.88**
	Standard deviation	0.00	0.03	0.13	0.21	0.32	0.61	0.31	0.14
β-1 γ-OH β	**Processing I**	0.00	0.38	1.84	3.23	2.07	1.65	1.99	1.78
	Processing II	0.00	0.39	1.95	3.62	2.02	2.01	2.20	1.92
	Processing III	0.00	0.48	2.12	3.48	2.22	2.11	2.29	1.99
	Average	**0.00**	**0.42**	**1.97**	**3.44**	**2.10**	**1.92**	**2.16**	**1.90**
	Standard deviation	0.00	0.06	0.14	0.20	0.10	0.24	0.15	0.11
β-1 E α.β	**Processing I**	0.85	1.69	0.84	0.95	0.71	0.86	1.08	0.98
	Processing II	0.93	1.76	0.77	0.77	0.63	0.69	1.03	0.91
	Processing III	1.02	1.74	0.87	0.94	0.90	0.84	1.03	1.03
	Average	**0.93**	**1.73**	**0.83**	**0.89**	**0.75**	**0.80**	**1.05**	**0.97**
	Standard deviation	0.09	0.04	0.05	0.10	0.14	0.09	0.03	0.06
β-5 Phenylcoumarane α	**Processing I**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Processing II	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Processing III	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Average	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	Standard deviation	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
β-5 γ-OH α	**Processing I**	0.00	0.41	1.53	8.44	12.25	10.72	4.22	4.58
	Processing II	0.00	0.40	1.69	8.58	11.76	12.86	4.09	4.69
	Processing III	0.00	0.42	1.33	7.69	10.35	10.17	3.78	4.07
	Average	**0.00**	**0.41**	**1.52**	**8.24**	**11.45**	**11.25**	**4.03**	**4.45**
	Standard deviation	0.00	0.01	0.18	0.48	0.99	1.42	0.23	0.33

		F_{H100}	F_{H80}	F_{H60}	F_{H40}	F_{H20}	F_{EA100}	F_{oil}	F_{oil} mass balance
β-5 γ-OH β	**Processing I**	0.00	0.20	1.26	7.53	10.45	8.55	3.90	3.93
	Processing II	0.00	0.24	1.34	8.15	10.67	10.12	4.07	4.20
	Processing III	0.00	0.23	1.39	7.81	11.01	10.00	4.02	4.17
	Average	**0.00**	**0.22**	**1.33**	**7.83**	**10.71**	**9.56**	**4.00**	**4.10**
	Standard deviation	0.00	0.02	0.07	0.31	0.28	0.87	0.09	0.15
β-5 E β	**Processing I**	4.81	2.76	3.04	3.31	2.65	3.93	3.11	3.24
	Processing II	5.27	3.27	3.24	3.57	2.34	3.20	3.57	3.42
	Processing III	5.06	3.08	3.43	3.46	2.48	3.83	3.15	3.46
	Average	**5.05**	**3.04**	**3.24**	**3.45**	**2.49**	**3.65**	**3.28**	**3.37**
	Standard deviation	0.23	0.26	0.20	0.13	0.16	0.40	0.25	0.12
β-O-4	**Processing I**	0.00	0.00	0.00	0.00	1.20	2.55	0.38	0.25
	Processing II	0.00	0.00	0.00	0.00	1.07	2.77	0.20	0.25
	Processing III	0.00	0.00	0.00	0.00	1.24	2.76	0.21	0.27
	Average	**0.00**	**0.00**	**0.00**	**0.00**	**1.17**	**2.69**	**0.26**	**0.26**
	Standard deviation	0.00	0.00	0.00	0.00	0.09	0.12	0.10	0.01
End-unit P γ-OH+ P γ-O-Me α	**Processing I**	32.70	65.50	62.00	40.10	28.60	24.00	50.00	48.39
	Processing II	34.60	66.73	64.53	42.59	29.85	28.76	53.21	50.57
	Processing III	33.28	65.02	64.22	40.53	30.50	27.28	52.43	49.61
	Average	**33.53**	**65.75**	**63.58**	**41.07**	**29.65**	**26.68**	**51.88**	**49.52**
	Standard deviation	0.97	0.88	1.38	1.33	0.97	2.44	1.67	1.10
End-Unit P γ-OH β	**Processing I**	12.00	58.50	58.20	36.50	27.10	24.00	46.50	42.80
	Processing II	12.98	60.00	61.16	40.46	28.21	25.20	49.34	45.26
	Processing III	12.48	59.63	62.07	38.82	29.88	25.46	49.17	45.29
	Average	**12.49**	**59.38**	**60.48**	**38.59**	**28.40**	**24.89**	**48.34**	**44.45**
	Standard deviation	0.49	0.78	2.02	1.99	1.40	0.78	1.59	1.43

		F_{H100}	F_{H80}	F_{H60}	F_{H40}	F_{H20}	F_{EA100}	F_{oil}	F_{oil} mass balance
End-Unit P γ-O-Me β	**Processing I**	18.50	4.00	1.30	1.70	2.30	3.00	2.82	3.83
	Processing II	18.18	4.01	1.42	2.48	1.43	3.67	2.94	3.94
	Processing III	18.19	4.04	1.29	2.27	2.19	3.25	2.93	3.93
	Average	**18.29**	**4.02**	**1.34**	**2.15**	**1.97**	**3.31**	**2.90**	**3.90**
	Standard deviation	0.18	0.02	0.07	0.40	0.47	0.34	0.07	0.06
End-Unit P γ-O-Me γ	**Processing I**	18.80	3.80	1.20	2.30	1.90	2.20	3.00	3.86
	Processing II	20.05	4.00	1.32	2.45	1.13	2.31	3.27	4.01
	Processing III	19.04	3.87	1.23	2.24	1.86	2.17	3.28	3.89
	Average	**19.30**	**3.89**	**1.25**	**2.33**	**1.63**	**2.23**	**3.18**	**3.92**
	Standard deviation	0.66	0.10	0.06	0.11	0.43	0.07	0.16	0.08
End-Unit P α	**Processing I**	31.70	3.40	2.30	2.70	1.40	1.10	2.80	5.54
	Processing II	33.50	3.59	1.81	2.08	1.83	2.68	3.14	5.57
	Processing III	31.96	3.71	1.74	1.57	1.43	1.87	3.08	5.19
	Average	**32.39**	**3.57**	**1.95**	**2.12**	**1.55**	**1.88**	**3.01**	**5.43**
	Standard deviation	0.97	0.16	0.31	0.57	0.24	0.79	0.18	0.21
End- Unit P β	**Processing I**	31.60	3.00	1.40	1.60	0.90	1.30	2.80	4.85
	Processing II	33.22	3.53	1.45	1.67	1.07	1.88	3.17	5.18
	Processing III	31.79	3.36	1.45	1.82	1.10	1.64	3.13	5.03
	Average	**32.20**	**3.30**	**1.43**	**1.70**	**1.02**	**1.61**	**3.03**	**5.02**
	Standard deviation	0.89	0.27	0.03	0.11	0.11	0.29	0.20	0.17
End-Unit 4-Propenol γ	**Processing I**	1.37	1.90	0.40	0.30	0.30	0.30	0.60	0.69
	Processing II	1.48	2.00	0.40	0.32	0.58	0.63	0.77	0.77
	Processing III	1.40	2.87	0.53	0.32	0.90	0.56	0.68	0.97
	Average	**1.42**	**2.26**	**0.44**	**0.31**	**0.59**	**0.50**	**0.68**	**0.81**
	Standard deviation	0.06	0.53	0.08	0.01	0.30	0.17	0.09	0.15

		F_{H100}	F_{H80}	F_{H60}	F_{H40}	F_{H20}	F_{EA100}	F_{oil}	F_{oil} mass balance
End-Unit E α	**Processing I**	5.30	1.20	0.50	1.10	0.70	0.80	0.90	1.30
	Processing II	4.30	1.20	0.95	1.19	0.74	1.14	1.14	1.38
	Processing III	4.19	1.51	1.00	1.13	0.76	1.19	0.99	1.43
	Average	**4.60**	**1.30**	**0.82**	**1.14**	**0.73**	**1.04**	**1.01**	**1.37**
	Standard deviation	0.61	0.18	0.28	0.05	0.03	0.21	0.12	0.06
End-Unit M α	**Processing I**	1.20	0.30	0.40	0.60	0.40	0.30	0.50	0.51
	Processing II	1.53	0.44	0.47	0.71	0.28	0.33	0.35	0.61
	Processing III	1.47	0.43	0.38	0.53	0.41	0.27	0.43	0.54
	Average	**1.40**	**0.39**	**0.42**	**0.61**	**0.36**	**0.30**	**0.43**	**0.55**
	Standard deviation	0.18	0.08	0.05	0.09	0.07	0.03	0.08	0.05
5--5	**Processing I**	2.10	2.40	4.70	16.14	26.40	26.22	11.30	10.43
	Processing II	2.07	2.49	4.39	17.80	23.45	28.08	10.43	10.43
	Processing III	1.95	2.10	4.45	16.41	26.04	26.80	10.97	10.33
	Average	**2.04**	**2.33**	**4.51**	**16.78**	**25.30**	**27.03**	**10.90**	**10.40**
	Standard deviation	0.08	0.20	0.16	0.89	1.61	0.95	0.44	0.06

Relative quantification of lignin-inter unit linkages by integrating cross peaks relative to the G_2 area in the ^{1}H-^{13}C HSQC NMR spectrum is a well-known method in literature.[13] However. the integration of some of the inter-unit linkages of RCF lignin has never been described in literature. Therefore, we integrated at least 2 C-H pairs (e.g. α and β) to check their similarity. In general, slightly lower values are obtained from better resolved signals, such as β β-5 γ-OH, β β-1 γ-OH and β-β 2x γ-OH. They have a clear separation from other cross peaks, probably due to less spectral overlap, resulting in lower integral values. Therefore, the signals with the best resolution were used to determine the relative abundancies, which can be found in **Table S6.**

Table S6. Quantification of the structural motifs of the complete RCF oil, its fractions and the mass balance over all fractions. Quantification is based on the signals determined in the previous section. Values between brackets are standard deviations.

	F_{H100}	F_{H80}	F_{H60}	F_{H40}	F_{H20}	F_{EA100}	F_{oil}	F_{oil} mass balance

β-β resinol	0	0	0	0	0	0	0	0
β-β 2x γ-OH	0	0	0.6 (0.11)	4.9 (0.27)	12.1 (0.29)	14.2 (1.39)	3.1 (0.23)	3.5 (0.15)
β-β THF	0	0.3 (0.02)	0.6 (0.04)	1.5 (0.16)	1.6 (0.03)	1.4 (0.39)	1 (0.16)	0.9 (0.07)
Total β-β	**0**	**0.3 (0.02)**	**1.2 (0.14)**	**6.3 (0.43)**	**13.7 (0.32)**	**15.6 (1.69)**	**4.1 (0.38)**	**4.3 (0.22)**
β-1 γ-OH	0	0.4 (0.06)	2 (0.14)	3.4 (0.2)	2.1 (0.1)	1.9 (0.24)	2.2 (0.15)	1.9 (0.11)
β-1 E	0.9 (0.09)	1.7 (0.04)	0.8 (0.05)	0.9 (0.1)	0.8 (0.14)	0.8 (0.09	1 '0.03	1 (0.06)
Total β-1	**0.9 (0.09)**	**2.1 (0.08)**	**2.8 (0.17)**	**4.3 (0.13)**	**2.9 (0.24)**	**2.7 (0.22)**	**3.2 (0.13)**	**2.9 (0.14)**
β-5 Phenylcoumarane	0	0	0	0	0	0	0	0
β-5 γ-OH	0	0.2 (0.02)	1.3 (0.07)	7.8 (0.31)	10.7 (0.28)	9.6 (0.87)	4 (0.09)	4.1 (0.15)
β-5 E	5.1 (0.23)	3 (0.26)	3.2 (0.2)	3.5 (0.13)	2.5(0.16)	3.7 (0.4)	3.3 (0.25)	3.4 (0.12)
Total β-5	**5 (0.23)**	**3.3 (0.28)**	**4.6 (0.26)**	**11.3 (0.44)**	**13.2 (0.26)**	**13.21 (0.68)**	**7.3 (0.33)**	**7.5 (0.27)**
β-O-4	0	0	0	0	1.2 (0.09)	2.7 (0.12)	0.3 (0.1)	0.3 (0.01)
End-Unit P γ-OH	12.5 (0.5)	59.4 (0.78)	60.5 (2.02)	38.6 (1.99)	28.4 (1.4)	24.9 (0.78)	48.4 (1.6)	44.5 (1.43)
End-Unit P γ-O-Me	18.3 (0.18)	4 (0.02)	1.3 (0.07)	2.2 (0.4)	2 (0.47)	3.3 (0.34)	2.9 (0.07	3.9 (0.06
End- Unit P	32.2 (0.89)	3.3 (0.27)	1.4 (0.03)	1.7 (0.11)	1 (0.11)	1.6 (0.29)	3 (0.2)	5 (0.17)
End-Unit Xl	1.4 (0.06)	2.3 (0.53)	0.4 (0.08)	0.3 (0.01)	0.6 (0.3)	0.5 (0.17)	0.7 (0.09)	0.8 (0.15)
End-Unit E	4.6 (0.6)	1.3 (0.18)	0.8 (0.28)	1.1 (0.05)	0.7 (0.03)	1 (0.21)	1 (0.12)	1.4 (0.06)
End-Unit M	1.4 (0.18)	0.4 (0.08)	0.4 (0.05)	0.6 (0.09)	0.4 (0.07)	0.3 (0.03)	0.4 (0.08)	0.6 (0.05)
Total End-units	**70.4 (1.14)**	**70.6 (1.54)**	**64.9 (2.4)**	**44.5 (2.54)**	**33.1 (1.94)**	**31.6 (1.82)**	**56.4 (1.87)**	**56.1 (1.84)**
5--5	**2 (0.08)**	**2.3 (0.2)**	**4.5 (0.16)**	**16.8 (0.89)**	**25.3 (1.61)**	**27 (0.95)**	**10.9 (0.44)**	**10.4 (0.06)**
Sum	**78.4 (1.34)**	**78.7 (1.78)**	**78 (2.75**	**83.2 (4.32)**	**89.3 (3.62)**	**92.9 (5.04)**	**82.1 (2.23)**	**81.4 (2.40)**

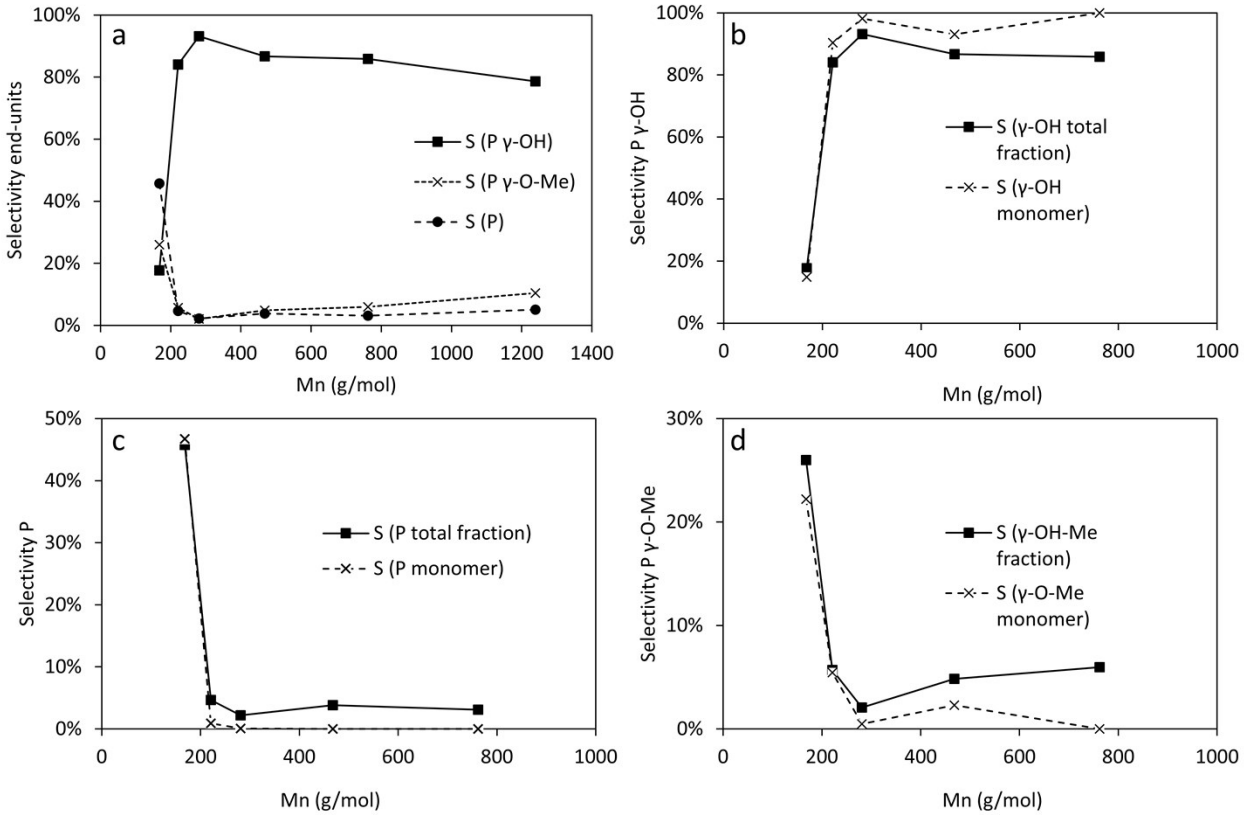


Fig S15. (a) comparison of the selectivity of 4-propanol (P γ-OH), 4-propyl (P) and 4-(3-methoxypropyl) (P γ-O-Me) end-units in all fractions as determined by 2D HSQC NMR. (b) comparison of the selectivity of 4-propanol (P γ-OH) end-units) in the monomers, determined by GC, and the complete oil, determined by 2D HSQC NMR (c) comparison of the selectivity of 4-propyl (P) end-units in the monomers, determined by GC, and the complete oil, determined by 2D HSQC NMR (d) comparison of the selectivity of 4-(3-methoxypropyl) (P γ-O-Me) end-units in the monomers, determined by GC, and the complete oil, determined by 2D HSQC NMR

It is apparent from **Fig 15a** that the selectivity to P γ-OH end-units decreases slightly with increasing molecular weight. To further examine this trend, the selectivities of the 3 main monomers are plotted next to the selectivities of the end-units of the total oil in **Fig S15b-d.** Since 4-propylguaiacol is almost completely extracted in F_{H100} (Table S3), suggesting that the signal of 4-propyl substitutents in the fractions with a Mn> 200 g mol^{-1} originates from dimers or oligomers. Clearly the selectivity to 4-propyl end-units does not increase with increasing molecular weight. On the other hand, the selectivity 4-(3-methoxypropyl) substitution increases (**Fig S15a)** whilst no monomers are observed at higher molecular weight (**Fig S15d)**. This observation, combined with the slight decrease to 4-propanol substitution, might indicate that methylation of the γ-OH group is more likely to happen on higher molecular weight RCF lignin.

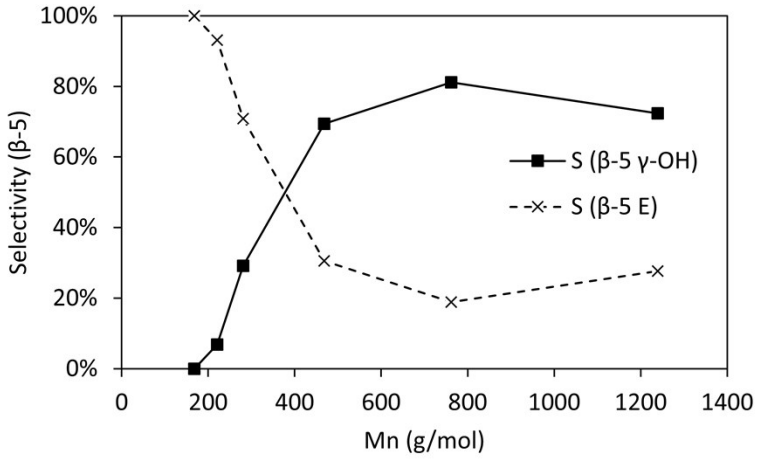


Fig S16. Selectivity profile of the β-5 linkages observed in RCF lignin, based on table S6.

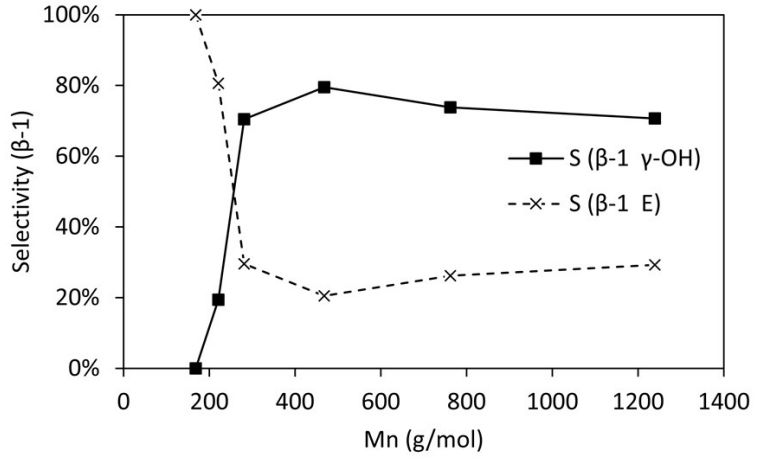


Fig S17. Selectivity profile of the β-1 linkages observed in RCF lignin, based on table S6.

Fig S18. Selectivity profile of the β-β linkages observed in RCF lignin, based on table S6.

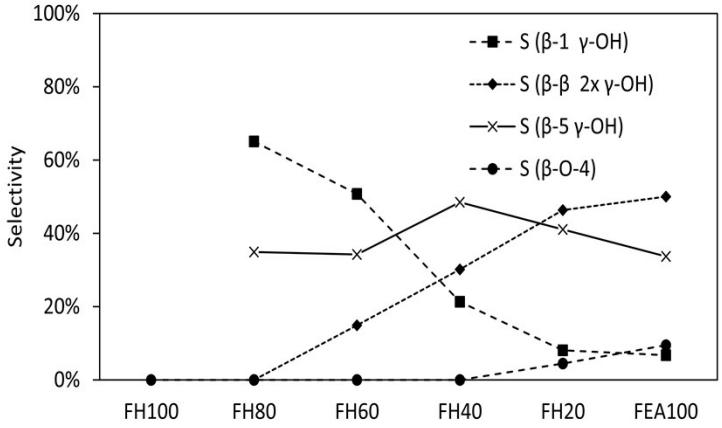


Fig S19. Selectivity profiles of the 4 inter-unit linkages; β-O-4, β-5, β-β, β-1; containing a γ-OH relative to each other.

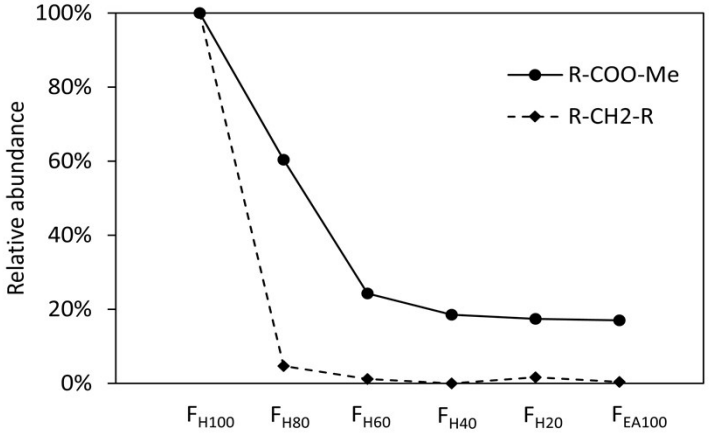


Fig S20. Relative abundancies of methylated carboxylic acids and the CH_2 signal of long chain alkyl substituents (R-CH_2-R).

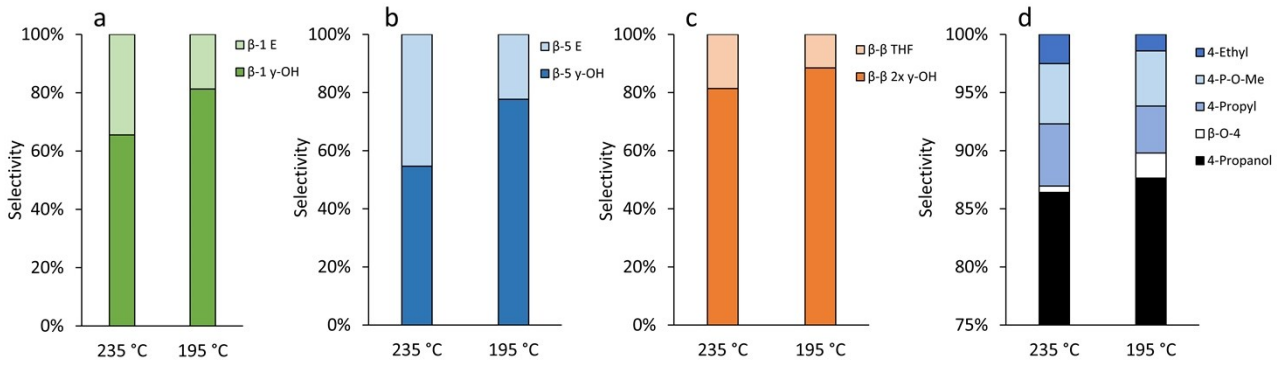
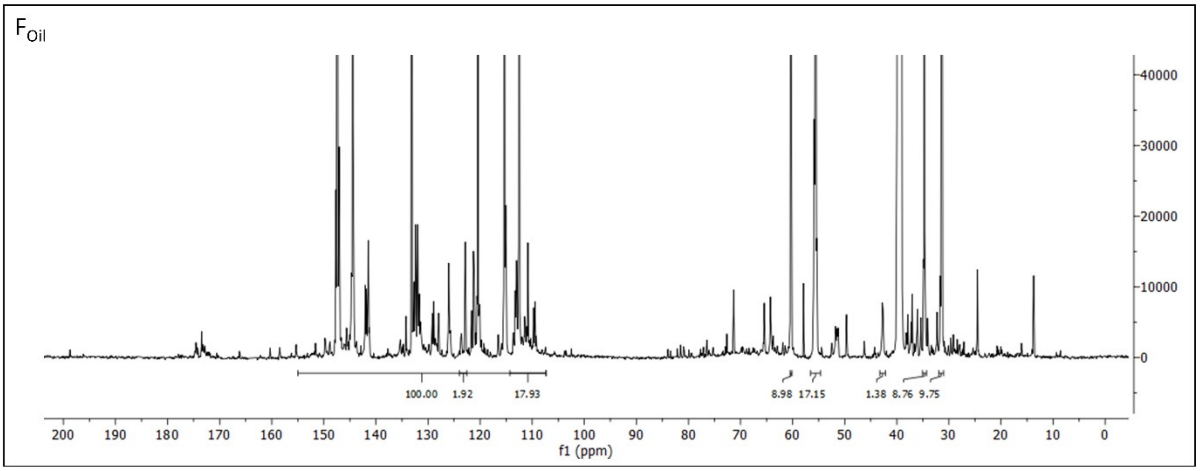
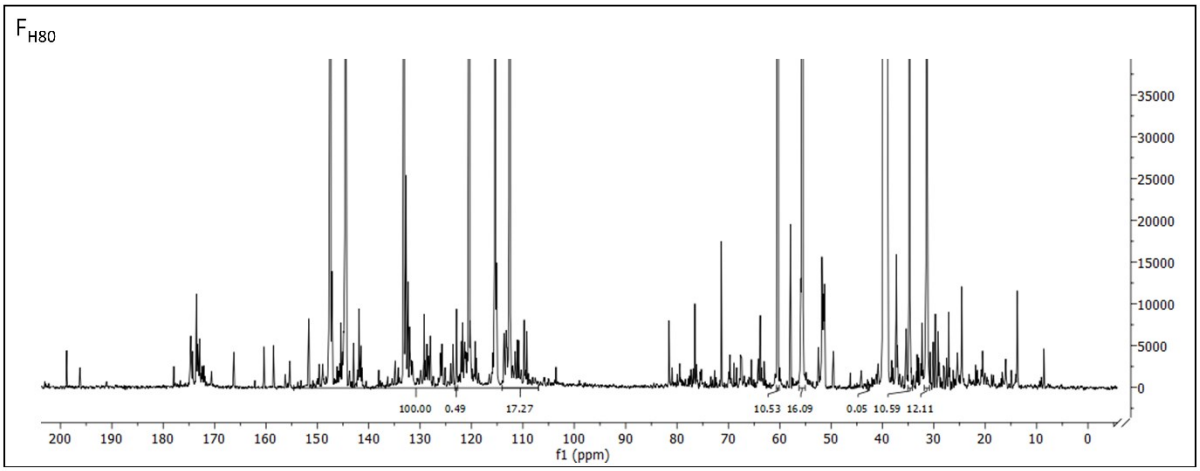


Fig S21. Selectivities of β-1 **(a)**, β-5 **(b)**, β-β **(c)**, and β-O-4 and end-units **(d)** at two different RCF reaction temperatures: 235 °C and 195 °C. The experiment at 235 °C is ran according to the materials and methods section, the experiment at 195 °C is ran in a 100 mL batch with 40 mL methanol, 2 g pine, 30 bar H_2, 0.2 g Pd/C for a reaction time of 3h. The delignification is lower at 195 °C(28 wt% relative to Klason Lignin), yet the total assignment in the 2D HSQC NMR spectrum is similar (83% vs 82%).

6. ^{13}C NMR – 2D HSQC NMR analysis

The quantification of the 2D HSQC NMR for softwoods is often performed relative to the G_2 area of the NMR spectrum, assuming no substitution on position 2 of the aromatic moiety. In order to check the error on this internal standard, the G_2 area (107-114 ppm) was integrated, divided by the total aromatic area (107-155 ppm) and multiplied with 6. The G_2 area is a reliable standard, if the obtained value approaches 100%. For all three fractions – having a distinctly different M_w - the obtained value is slightly higher (3-7%). Based on this analysis the error in using the G_2 region as internal standard is ~5%, giving an under estimation for linkage abundance in HSQC analysis (**Table S7)**. When comparing the G_2 and methoxy region, both integrals should be equal, since each aromatic group is substituted by 1 methoxy. This condition is demonstrated in the three fractions. **(Table S7)**. Consequently, the G_2 area is a reliable standard for the relative quantification of RCF lignin by 2D HSQC NMR with minor errors.





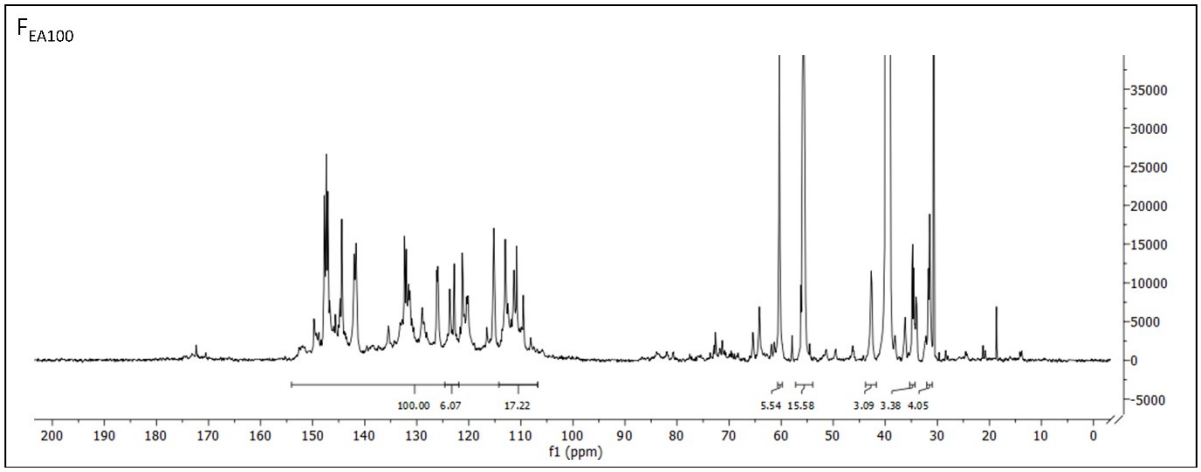


Figure S22. Quantitative ^{13}C-NMR spectra of F_{oil}, F_{H80} and F_{EA100}, including the integrated regions.

Table S7. Comparison of the q-^{13}C-NMR integration results of G_2-area (107-114 ppm) and methoxy area (55.0-56.4 ppm) relative to the integration result of the aromatic area (107-155 ppm).

	F_{Oil}	F_{H80}	F_{EA100}
G2-area	107.6%	103.6%	103.4%
Methoxy	102.9%	96.5%	93.5%

Table S8. Comparison of the quantification of 2D HSQC NMR and ^{13}C data of F_{oil}, F_{H80} and F_{EA100} for 5-5 units, 4-propanol end-units and the sum of β-5 γ-OH + β-β 2x γ-OH. The latter two are combined since the ^{13}C-NMR shifts of these units are almost identical.

	Oil		FH80		FEA100	
	^{13}C	2D HSQC Difference	^{13}C	2D HSQC Difference	^{13}C	2D HSQC Difference

5-5	10.9%	11.2%	-2.8%	2.8%	2.3%	17.9%	32.2%	27%	16.8%
β-5 γ-OH + β-β 2x γ-OH	7.1%	7.7%	-9.0%	0.3%	0.2%	14.3%	25.4%	23.8%	6.6%
4-Propanol	48.4%	46.5%	3.9%	61.3%	59.4%	3.1%	24.8%	24.9%	-0.3%

7. ^{31}P-NMR data

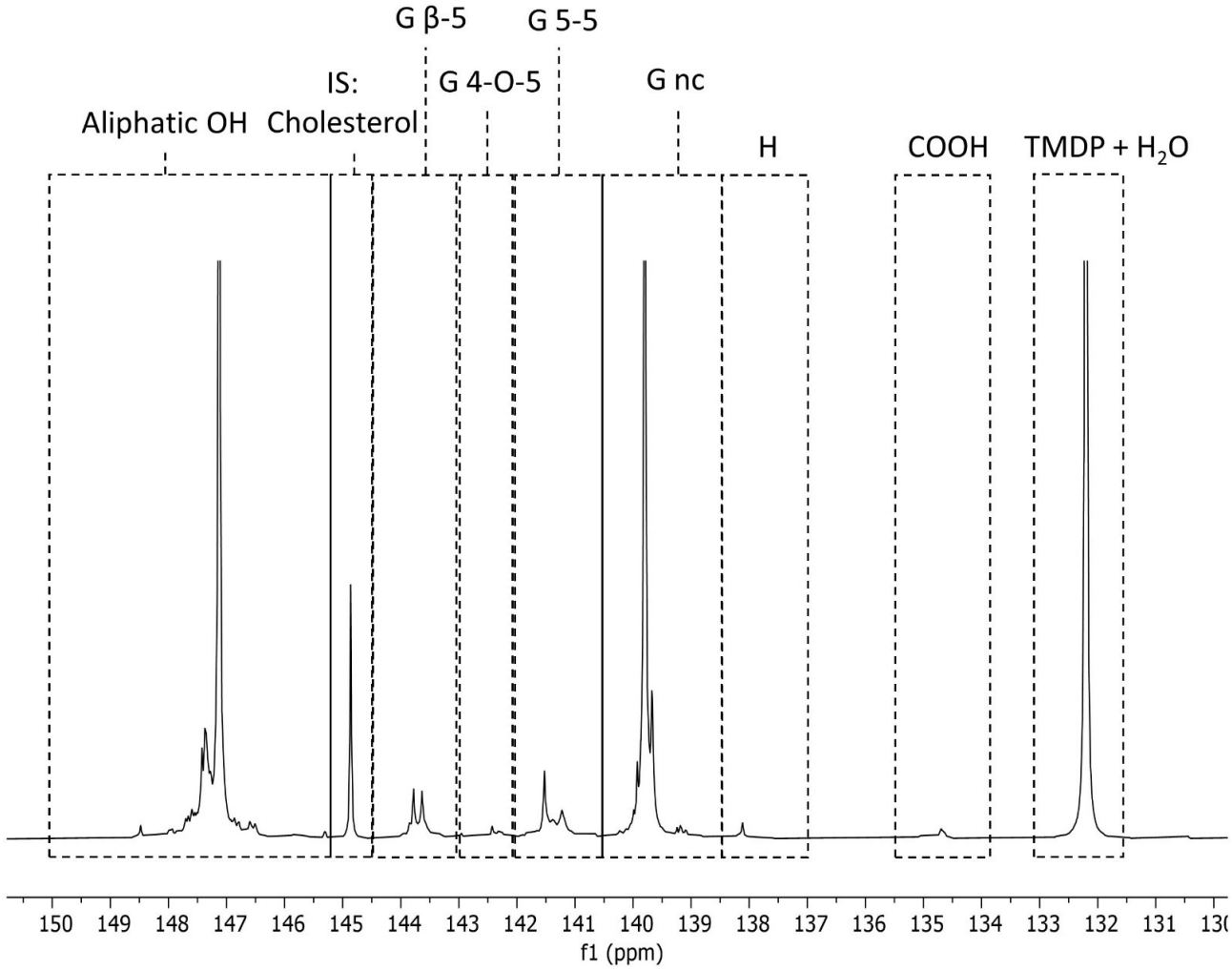


Fig .23. ^{31}P-NMR spectrum of phosphitylated RCF lignin oil derivatized with TMDP and with cholesterol as internal standard.

Table S9. ^{31}P-NMR results of RCF lignin fractions. The hydroxyl content (mmol OH g^{-1}) is quantified using phosphitylated cholesterol as internal standard. Measurements were performed in triplicate. COOH: Carboxylic acids; H: Hydroxyphenyl-units; G nc: non-condensed guaiacyl-units; G c: Condensed guaiacyl units (5-substituted).

		units in mmol OH g^{-1}										
		COOH	**H**	**G nc**	**G c**	**G c 5-5**	**G c 4-O-5**	**G c β-5**	**Aliphatic**	**∑ Phenolic**	**∑ Aliphatic**	**Total OH**
F_{oil}	**I**	0.07	0.10	3.36	1.30	0.66	0.13	0.51	4.30	4.76	4.30	9.12
	II	0.10	0.10	3.37	1.33	0.68	0.14	0.51	4.31	4.79	4.31	9.19
	III	0.09	0.11	3.38	1.31	0.67	0.13	0.50	4.28	4.79	4.28	9.16
	Average	**0.08**	**0.10**	**3.37**	**1.31**	**0.67**	**0.13**	**0.51**	**4.29**	**4.78**	**4.29**	**9.16**
	Stdev	0.013	0.004	0.010	0.012	0.008	0.003	0.003	0.011	0.015	0.011	0.030
F_{H100}	**I**	0.13	0.04	3.52	0.48	0.15	0.05	0.28	1.16	4.04	1.16	5.33
	II	0.11	0.04	3.58	0.51	0.17	0.05	0.29	1.20	4.14	1.20	5.45
	III	0.11	0.05	3.61	0.34	0.11	0.03	0.20	1.05	4.00	1.05	5.16
	Average	**0.12**	**0.04**	**3.57**	**0.44**	**0.15**	**0.04**	**0.26**	**1.14**	**4.06**	**1.14**	**5.31**
	Stdev	0.01	0.005	0.048	0.093	0.031	0.009	0.054	0.079	0.073	0.079	0.144
F_{H80}	**I**	0.07	0.11	4.27	0.46	0.18	0.04	0.23	4.02	4.84	4.02	8.92
	II	0.03	0.09	4.29	0.34	0.13	0.03	0.18	4.09	4.72	4.09	8.85
	III	0.08	0.11	4.33	0.43	0.16	0.04	0.23	4.19	4.88	4.19	9.15
	Average	**0.06**	**0.11**	**4.30**	**0.41**	**0.16**	**0.04**	**0.21**	**4.10**	**4.81**	**4.10**	**8.97**
	Stdev	0.02	0.012	0.032	0.062	0.025	0.009	0.029	0.088	0.083	0.088	0.158
F_{H60}	**I**	0.04	0.09	4.08	0.72	0.33	0.07	0.32	4.54	4.89	4.54	9.47
	II	0.05	0.09	3.94	0.68	0.30	0.06	0.32	4.40	4.71	4.40	9.16
	III	0.05	0.10	4.11	0.74	0.32	0.07	0.35	4.54	4.95	4.54	9.54
	Average	**0.05**	**0.09**	**4.04**	**0.71**	**0.32**	**0.07**	**0.33**	**4.49**	**4.85**	**4.49**	**9.39**
	Stdev	0.00	0.003	0.077	0.022	0.011	0.006	0.010	0.065	0.102	0.065	0.167
F_{H40}	**I**	0.05	0.09	2.62	1.87	0.99	0.17	0.71	4.31	4.59	4.31	8.95
	II	0.04	0.11	2.69	1.91	1.00	0.18	0.73	4.40	4.71	4.40	9.15
	III	0.05	0.12	2.62	1.88	0.98	0.18	0.72	4.31	4.61	4.31	8.97
	Average	**0.05**	**0.11**	**2.64**	**1.89**	**0.99**	**0.17**	**0.72**	**4.34**	**4.64**	**4.34**	**9.03**

	Stdev	0.01	0.010	0.034	0.015	0.008	0.002	0.008	0.041	0.053	0.041	0.088
	I	0.02	0.11	1.47	2.49	1.44	0.28	0.77	4.39	4.06	4.39	8.47
	II	0.03	0.11	1.41	2.37	1.37	0.27	0.74	4.20	3.89	4.20	8.12
F_{H20}	**III**	0.04	0.11	1.42	2.41	1.39	0.27	0.75	4.23	3.94	4.23	8.21
	Average	**0.03**	**0.11**	**1.43**	**2.42**	**1.40**	**0.27**	**0.75**	**4.27**	**3.97**	**4.27**	**8.27**
	Stdev	0.01	0.002	0.026	0.047	0.031	0.004	0.013	0.085	0.071	0.085	0.148
	I	0.09	0.15	1.22	2.16	1.18	0.32	0.66	4.00	3.53	4.00	7.62
	II	0.07	0.13	1.28	2.16	1.19	0.33	0.65	4.01	3.57	4.01	7.64
F_{EA100}	**III**	0.12	0.14	1.28	2.26	1.22	0.34	0.70	4.11	3.68	4.11	7.90
	Average	**0.09**	**0.14**	**1.26**	**2.19**	**1.19**	**0.33**	**0.67**	**4.04**	**3.59**	**4.04**	**7.72**
	Stdev	0.02	0.009	0.028	0.046	0.017	0.010	0.022	0.050	0.063	0.050	0.128
Mass balance	**Average**	0.06	0.09	3.25	1.21	0.62	0.12	0.47	4.01	4.56	4.01	8.62

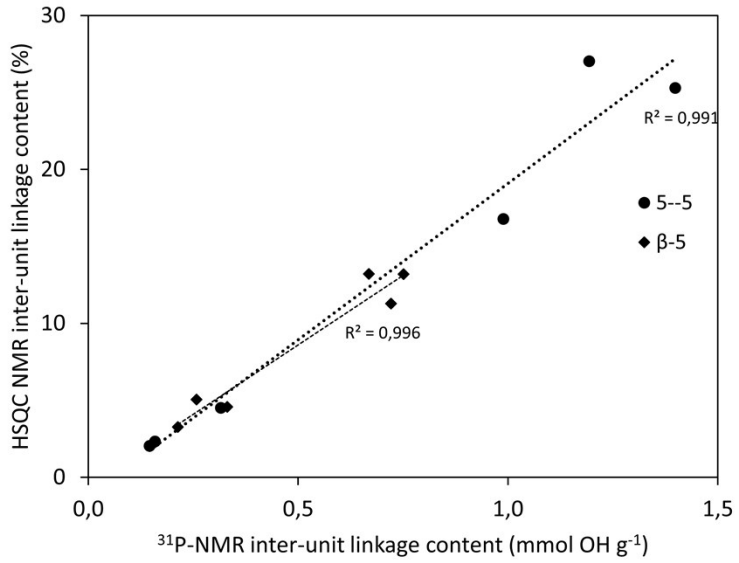


Fig S24. Correlation plot of the β-5 and 5-5 inter-unit linkages as determined by 2D HSQC NMR and ^{31}P NMR.

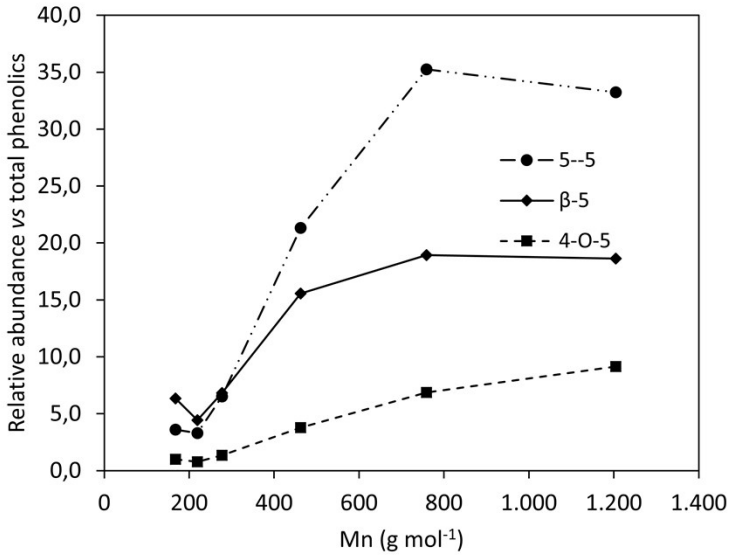


Fig S25. Relative abundancies of β-5, 5-5 and 4-O-5 inter-unit linkages. determined by ^{31}P NMR. The absolute abundancies are relative to the total number of phenolics (**Table S9)**.

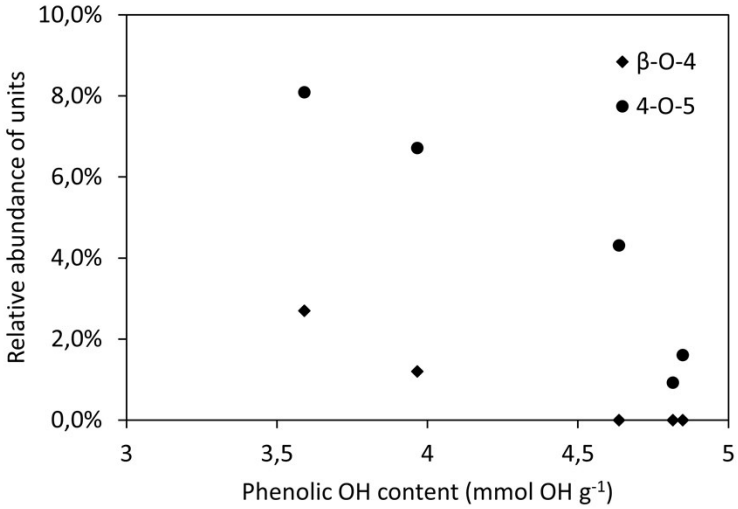


Fig S26. β-O-4 and 4-O-5 content relative to the amount of total phenolic OH. The β-O-4 content is determined by 2D HSQC NMR and the 4-O-5 content and total phenolic OH content are determined by ^{31}P-NMR. To obtain relative abundancies of 4-O-5, the phenolic 4-O-5 units were divided by the total number of phenolic OH. This creates an error, since it is not relative to the total number of aromatics. However this is the best possible option. It is apparent that the amount of free phenolic OH is inversely correlated with the amount of 4-O-5 and β-O-4 units. Both inter-unit linkages decreases the amount of free phenolic OH.

8. RCF reactions without catalyst

Table S10. Detailed monomer composition of RCF reactions performed according to the experimental procedures without a catalyst and with respectively nitrogen (30 bar) or hydrogen (30 bar) as gas.

	N_2	H_2
4-ethylguaiacol	0.0%	0.0%
4-PG	0.2%	0.2%
Iso-eugenol	1.5%	1.6%
4-propanalguiacol	0.4%	0.4%
4-propenolguaiacol	1.6%	1.8%
4-3-methoxypropylguaiacol	0.0%	0,0%
4-propanolguaiacol	0.3%	0.2%

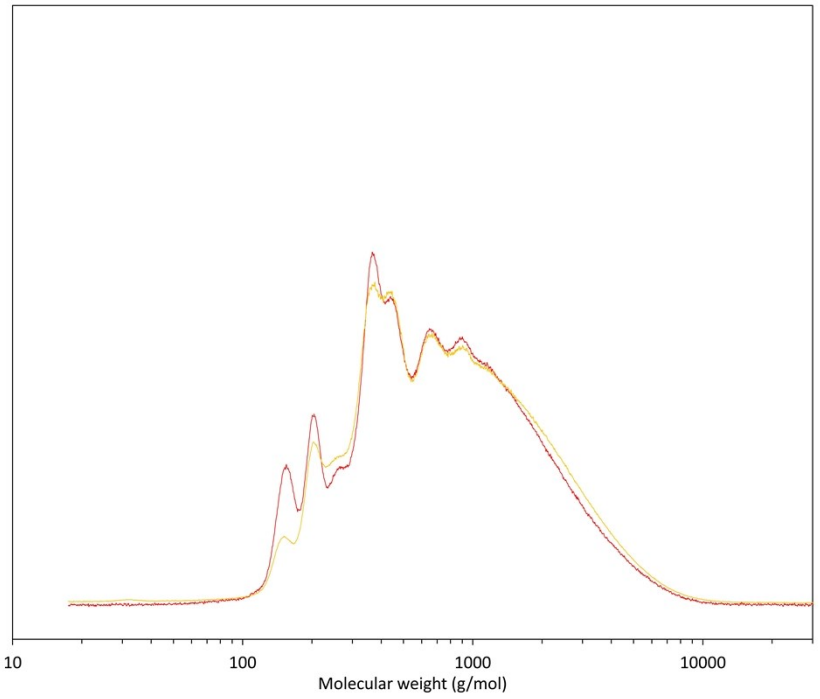


Fig S27. GPC profile of RCF reactions performed according to the experimental procedures without a catalyst and with respectively nitrogen (30 bar) – red line - or hydrogen (30 bar) – yellow line - as gas.

Table S11. Detailed 2D HSQC NMR integration of an RCF reactions performed according to the experimental procedures without a catalyst and with hydrogen (30 bar).

β-O-4	4.0%
β-β resinol	1.2%
β-β epiresinol	0.3%
β-β THF	0.6%
β-β 2x γ-OH	1.4%
β-1 stilbene	2.4%
β-5 phenylcoumarane	1.5%
β-5 stilbene	4.2%
4-propanol	5.0 %
4-(3-methoxypropyl)	2.0%
4-propenol	0.8%
4-propyl	-
4-ethyl	-
4-methyl	-
4-propenyl	2.6%

The results of these experiments don't provide any more information regarding a real mechanism of stabilization of the monomers as well as on the rearrangements that lignin undergoes while it is not stabilized. The amount of 4-propanol is similar as the amount of dihydroconiferylalcohol found in MWL, thus it is expected that these +- 5% of 4-propanols are present in native lignin. Since the results of both experiments (N_2 and H_2) are comparable, it can be concluded that the hydrogenation catalyst is necessary to activate the hydrogen, as was already suggested in previous RCF papers.

9. MWL NMR

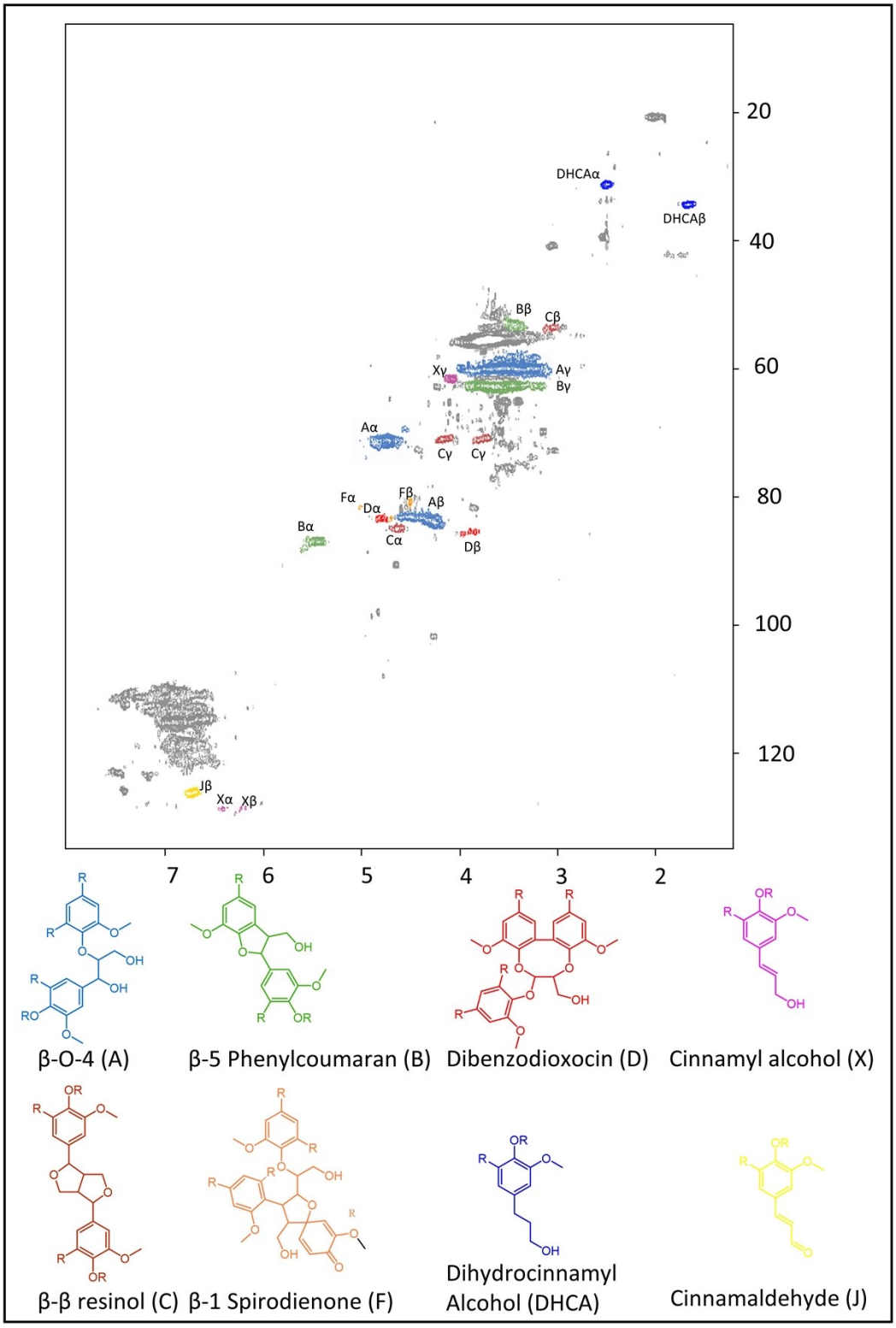


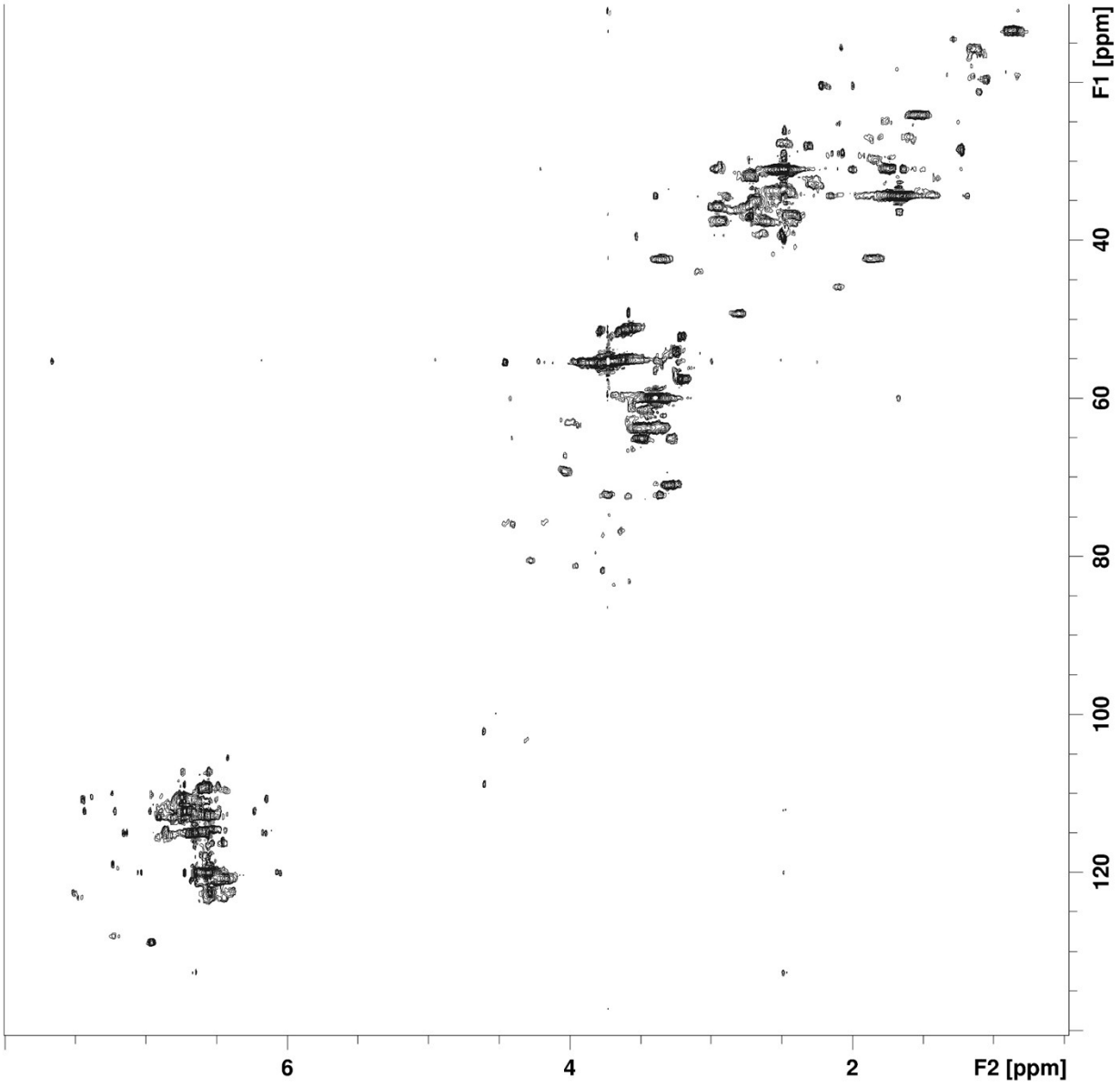
Fig S28. 2D HSQC spectrum of MWL of pine.

Table S12. Detailed integration results of MWL lignin, the complete RCF oil and the mass balanced RCF oil.

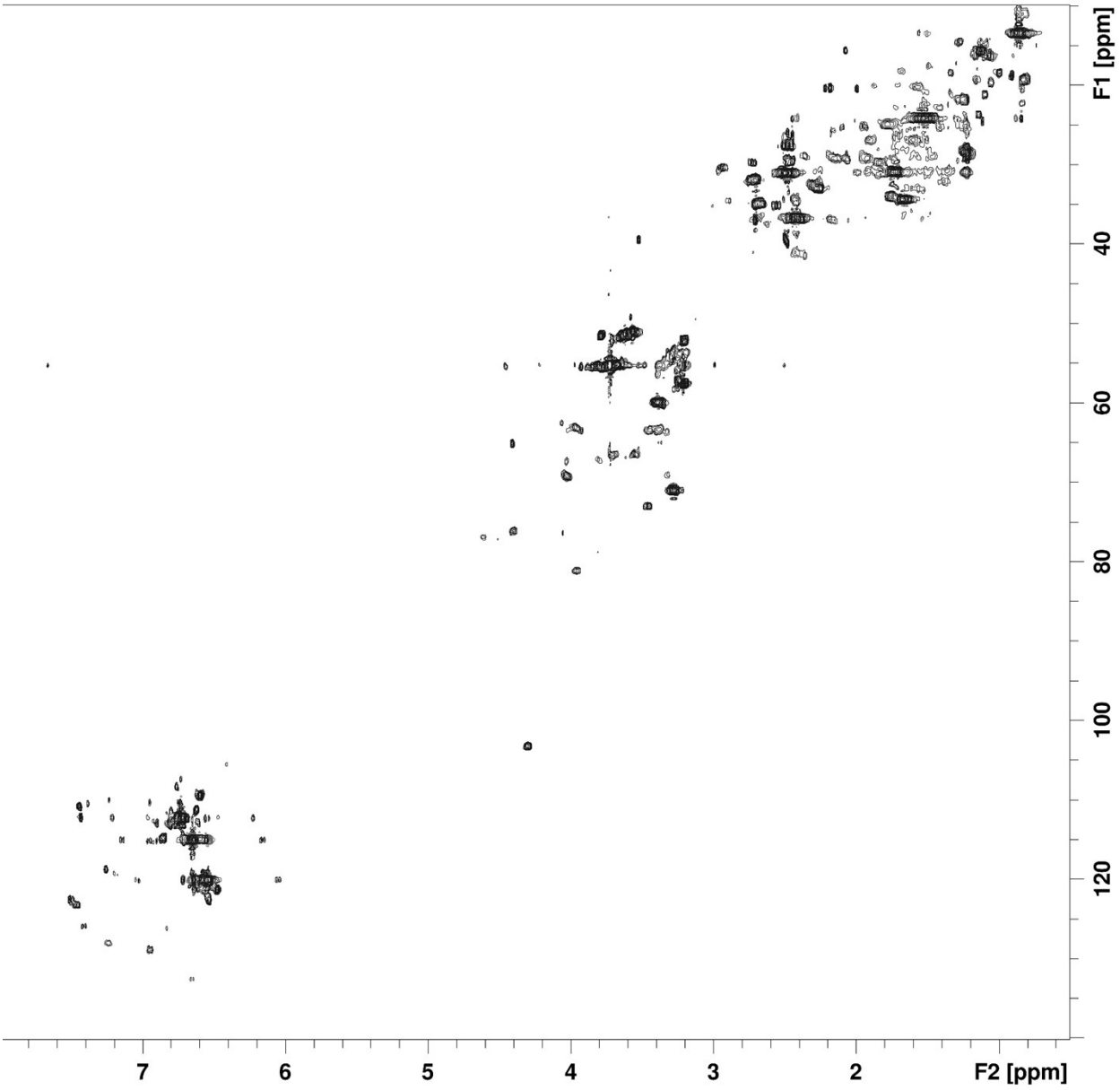
MWL units	MWL	RCF Oil	RCF Mb Oil	RCF units
β-O-4	35.4	0.7	0.8	X
X	6.3	48.3	44.5	P-γ-OH
J	5.8	3.0	5.0	P
DHCA	5.8	2.9	3.9	P-γ-O-Me
5-5 (D)	5.2	1.0	1.4	E
		0.4	0.6	M
Total β-O-4 + end-units	58.4	56.7	56.4	
β-5 (B)	8.7	3.3	3.4	β-5 E
		4.0	4.1	β-5 γ-OH
β-β (C)	3.9	3.1	3.5	β-β 2x γ-OH
		1.0	0.9	β-β THF
5-5 (D)	5.2	10.9	10.4	5-5
β-1 (F)	1.3	2.2	1.9	β-1 γ-OH
		1.0	1.0	β-1 E

10. Additional NMR spectra

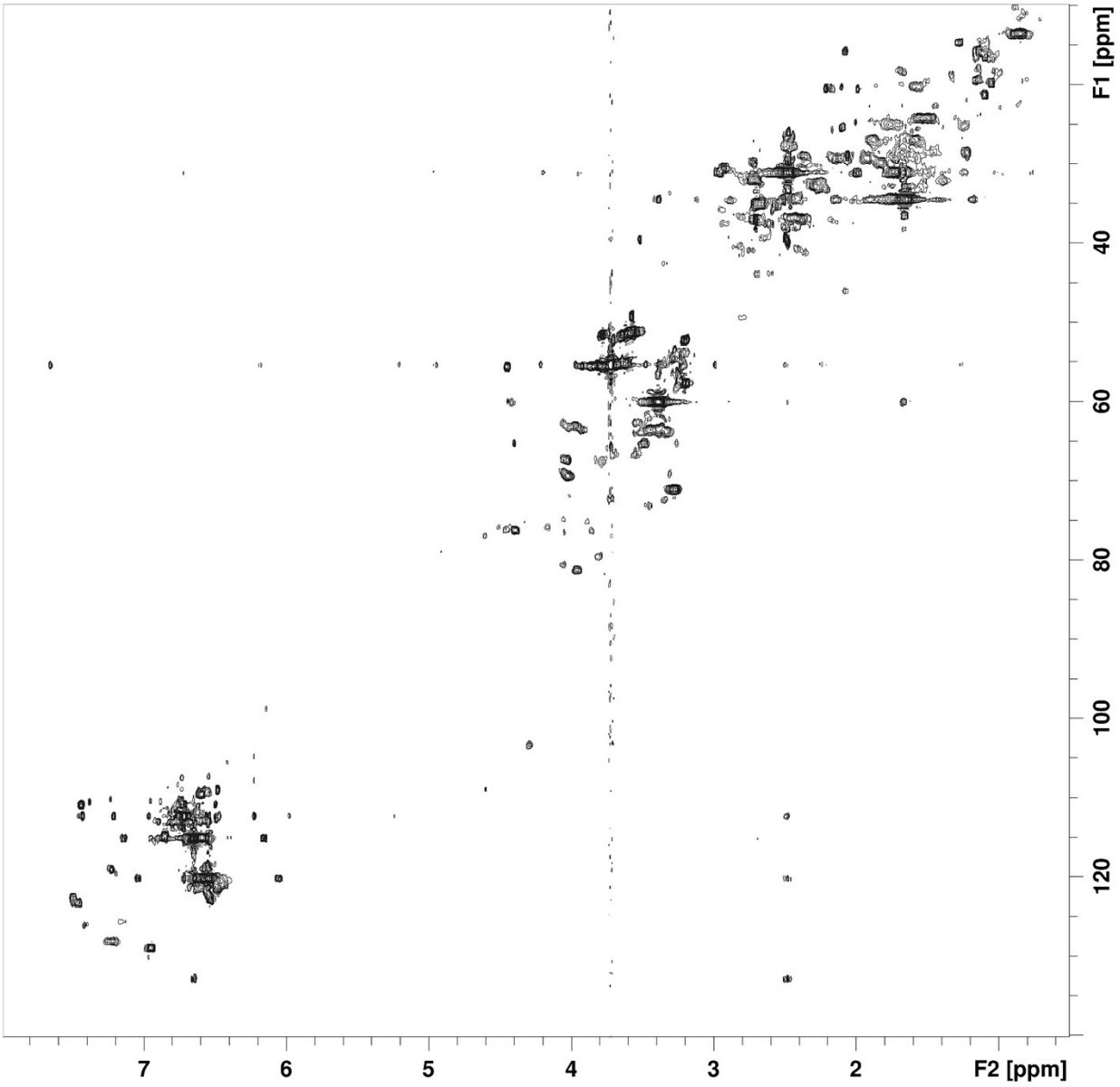
10.1. 2D HSQC NMR spectrum of the RCF lignin oil (F_{oil}). The NMR experiment was performed according to the conditions described in the materials and methods.



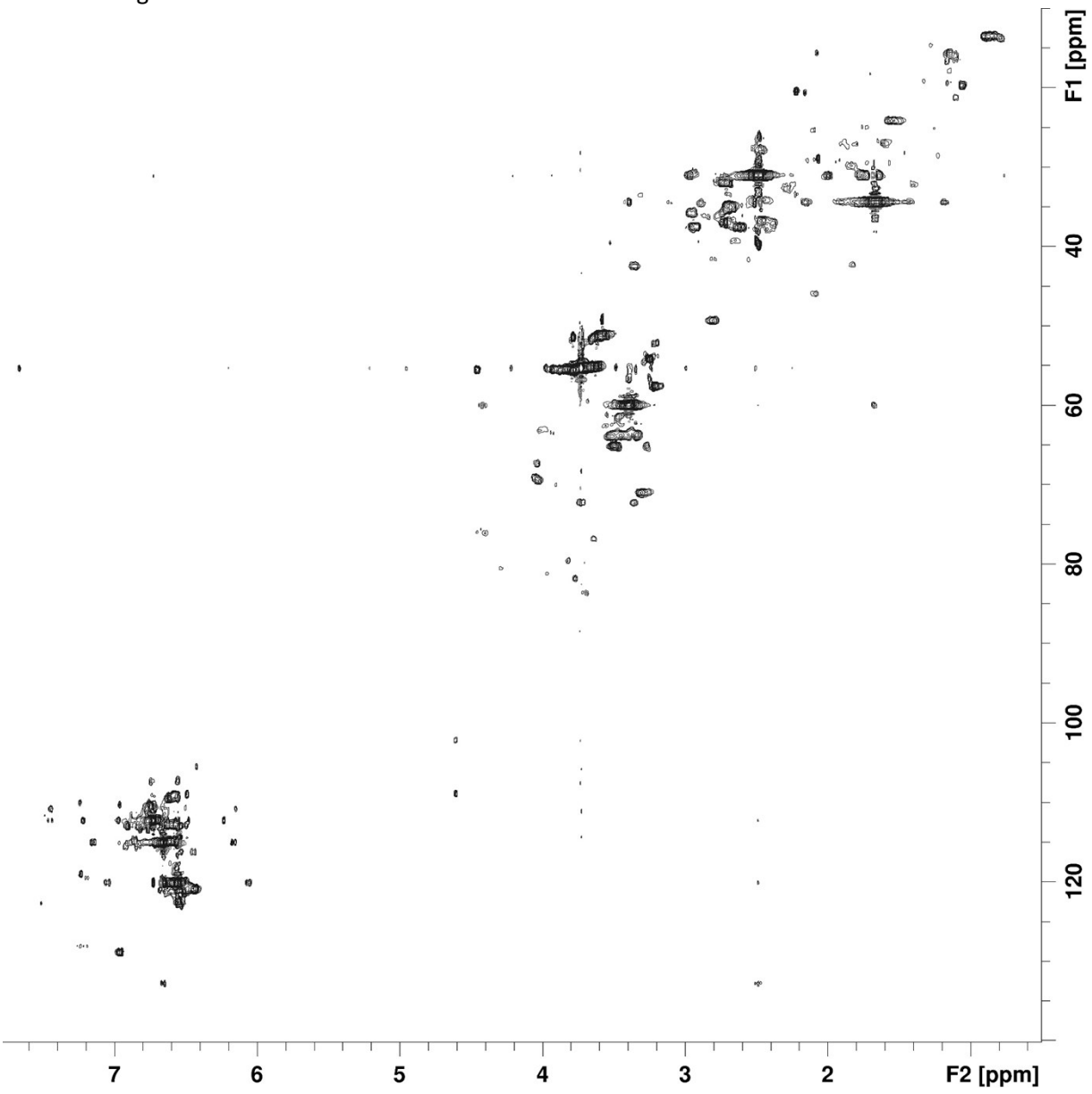
10.2. 2D HSQC NMR spectrum of the heptane soluble fraction (F_{H100}) of the RCF lignin oil. The NMR experiment was performed according to the conditions described in the materials and methods.



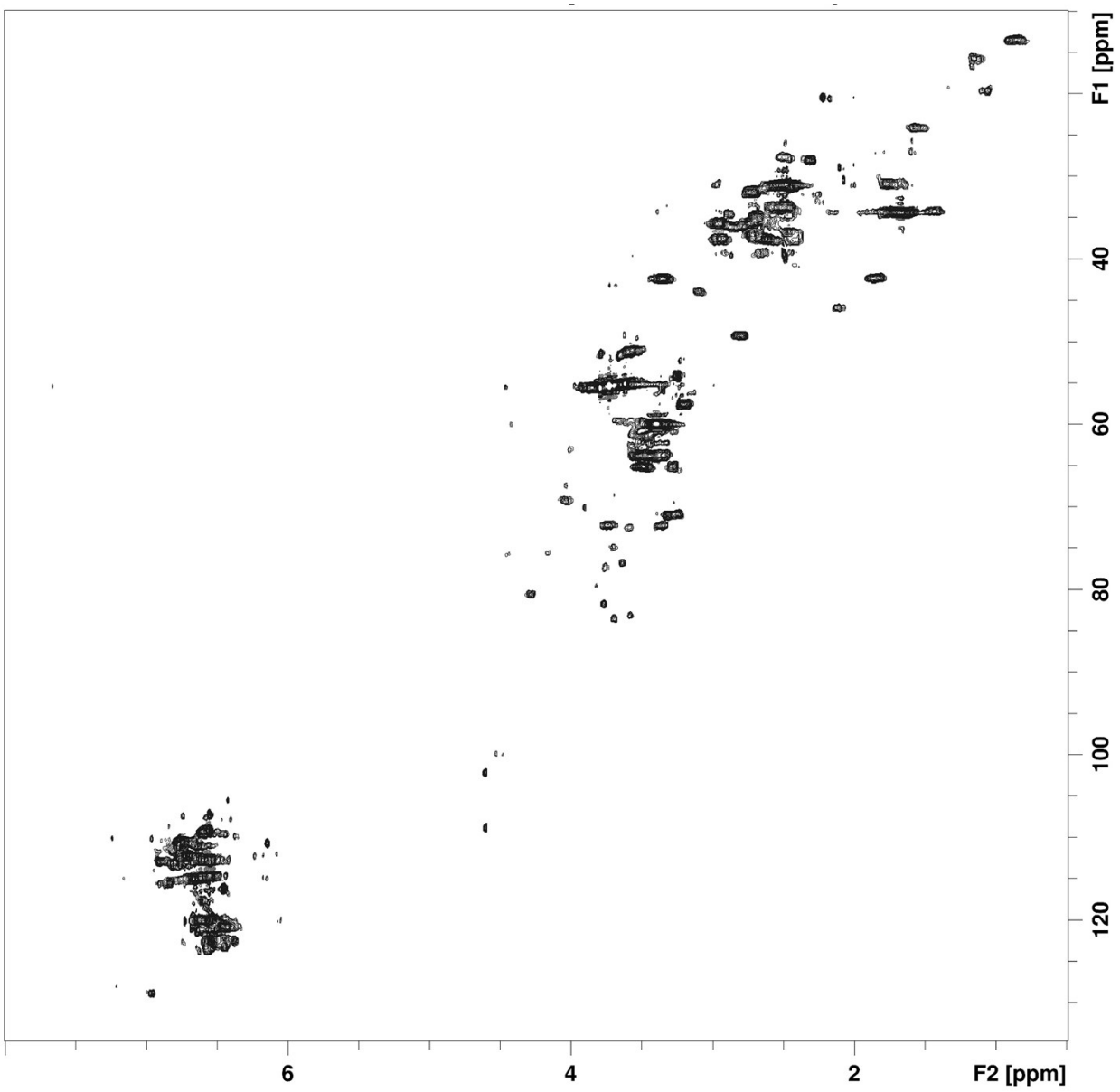
10.3. 2D HSQC NMR spectrum of the 80 % heptane/ 20 % ethyl acetate soluble fraction (F_{H80}) of the sequential extraction of the RCF lignin oil. The NMR experiment was performed according to the conditions described in the materials and methods.



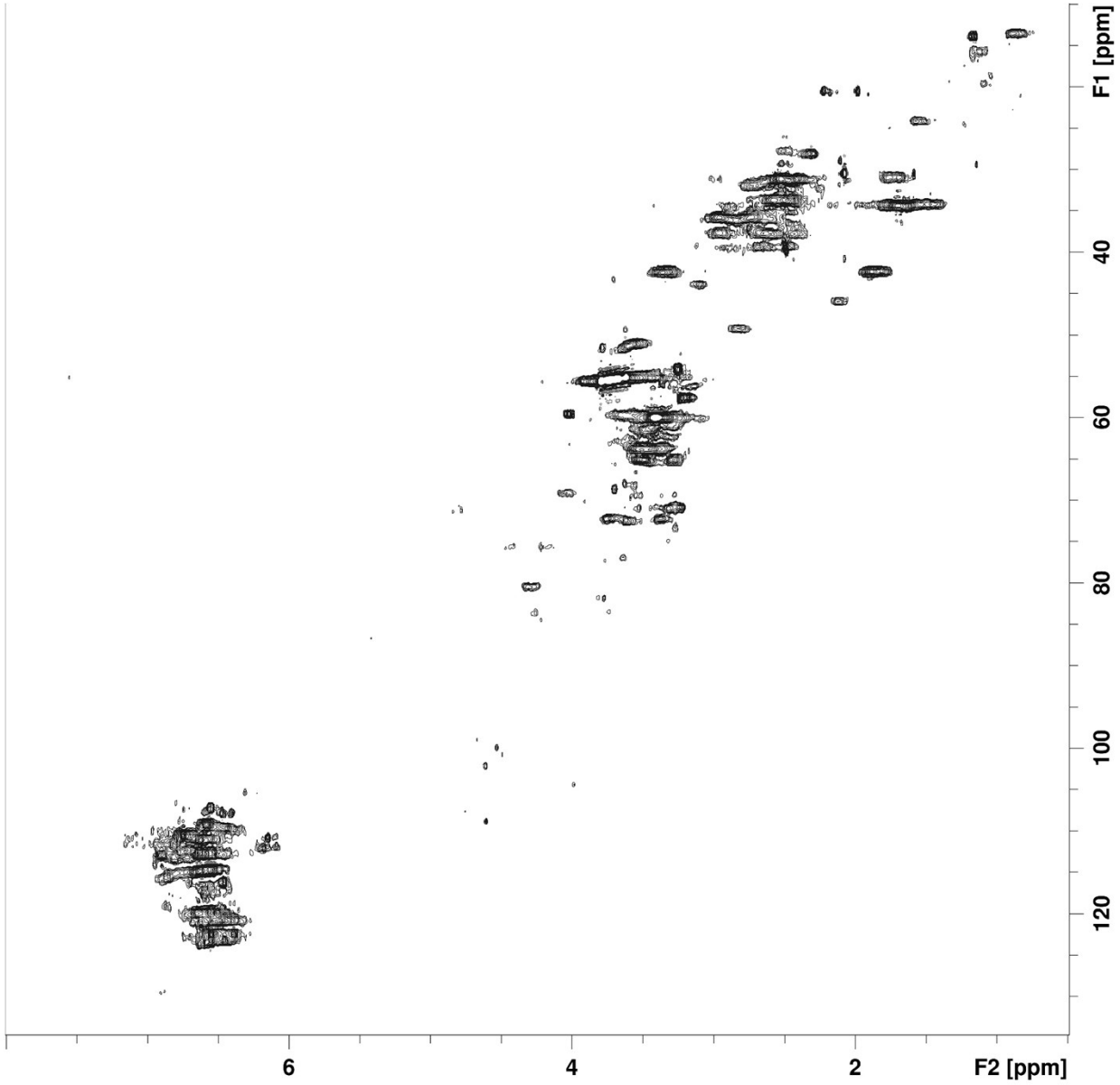
10.4. 2D HSQC NMR spectrum of the 60 % heptane/ 40 % ethyl acetate soluble fraction (F_{H60}) of the sequential extraction of the RCF lignin oil. The NMR experiment was performed according to the conditions described in the materials and methods.



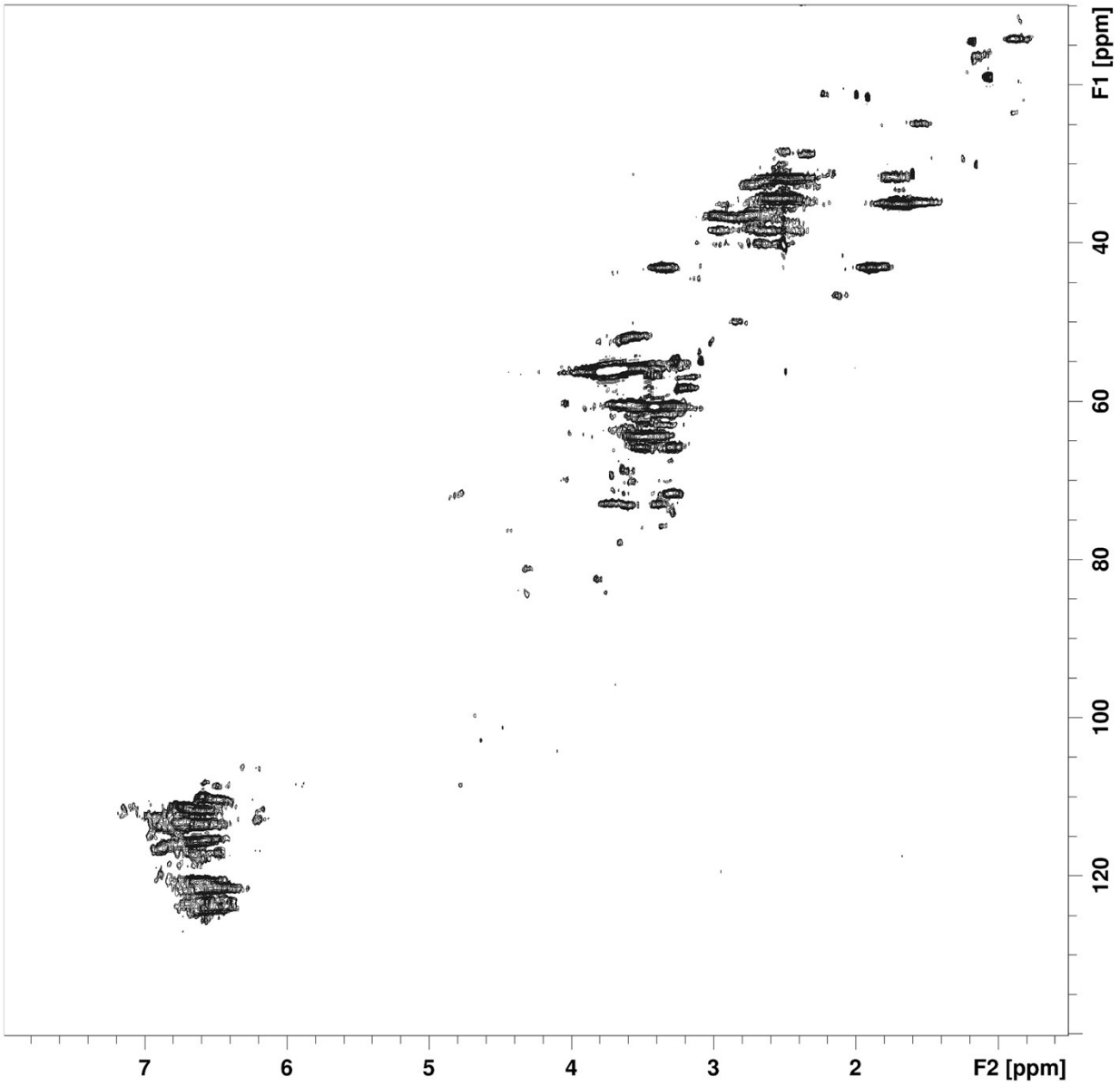
10.5. 2D HSQC NMR spectrum of the 40 % heptane/ 60 % ethyl acetate soluble fraction (F_{H40}) of the sequential extraction of the RCF lignin oil. The NMR experiment was performed according to the conditions described in the materials and methods.



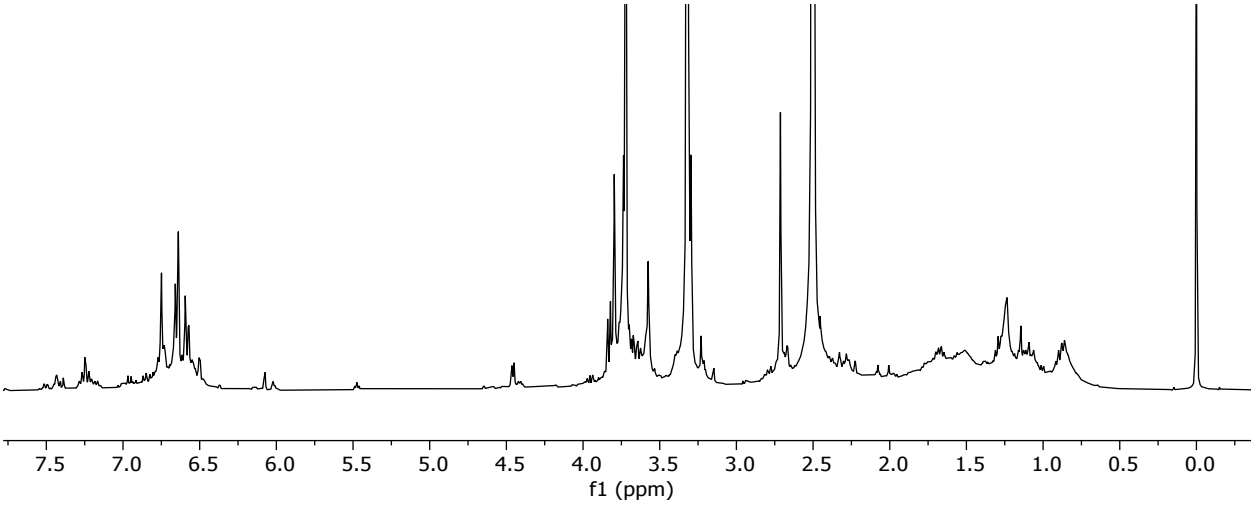
10.6. 2D HSQC NMR spectrum of the 20 % heptane/ 80 % ethyl acetate soluble fraction (F_{H20}) of the sequential extraction of the RCF lignin oil. The NMR experiment was performed according to the conditions described in the materials and methods.



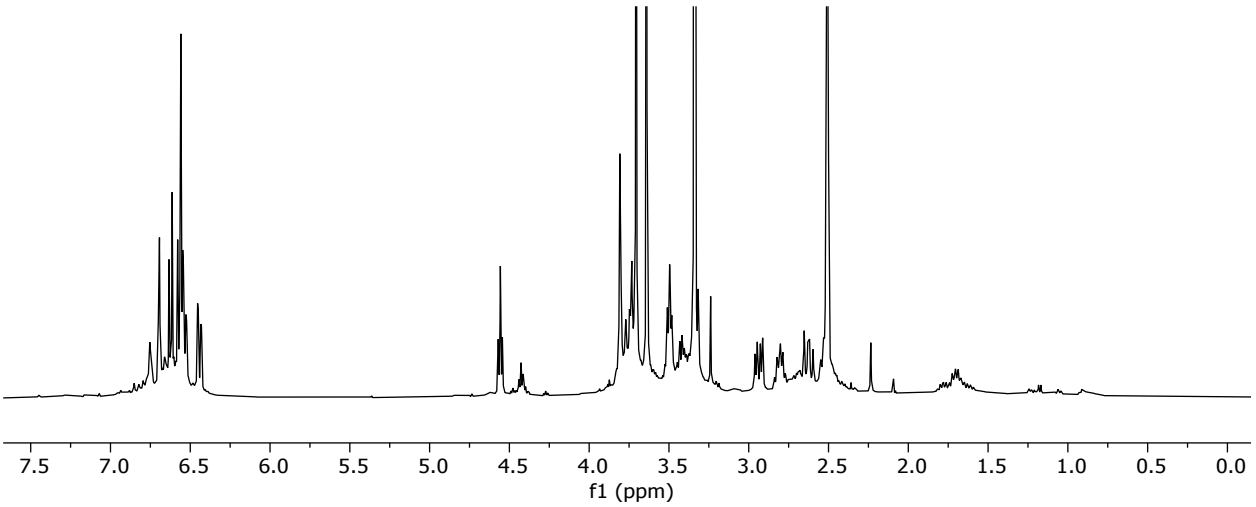
10.7. 2D HSQC NMR spectrum of the 100 % ethyl acetate soluble fraction (F_{EA100}) of the sequential extraction of the RCF lignin oil. The NMR experiment was performed according to the conditions described in the materials and methods.



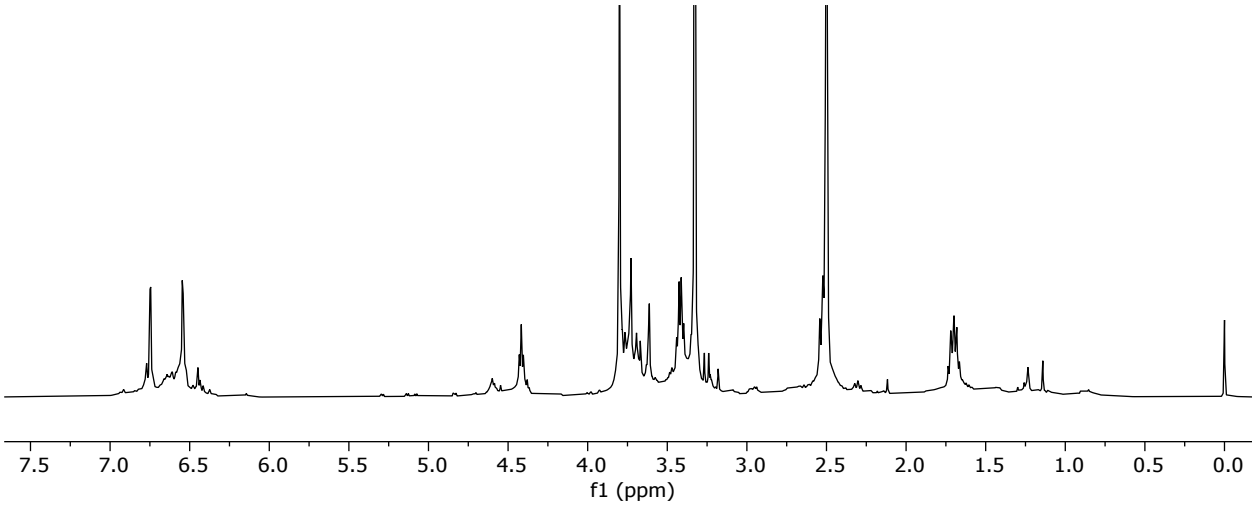
9.8 ^{1}H-NMR of the β-1 E dimer enriched fraction



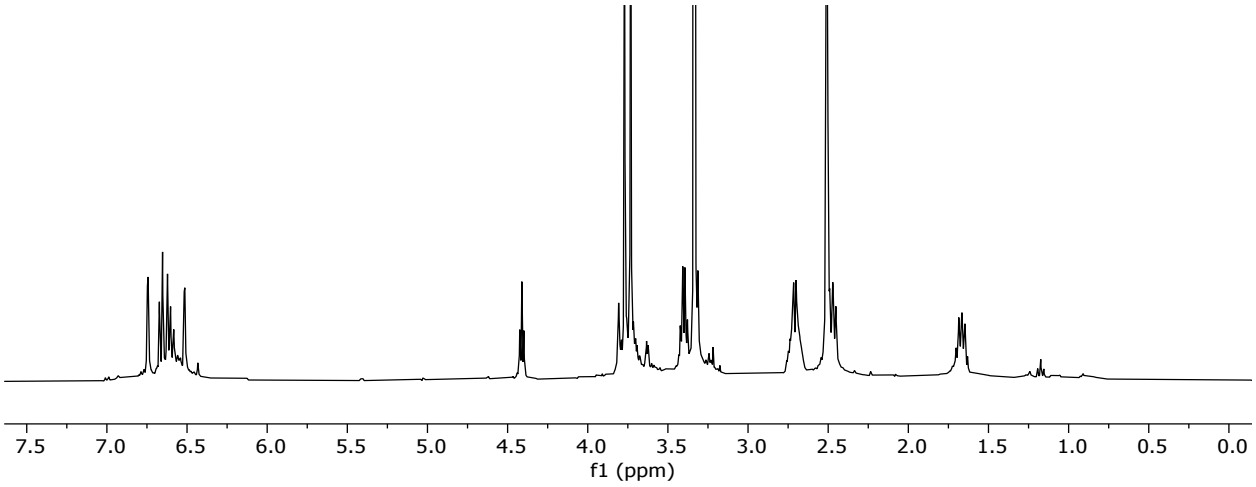
9.9 ^{1}H-NMR of the β-1 γ-OH dimer enriched fraction



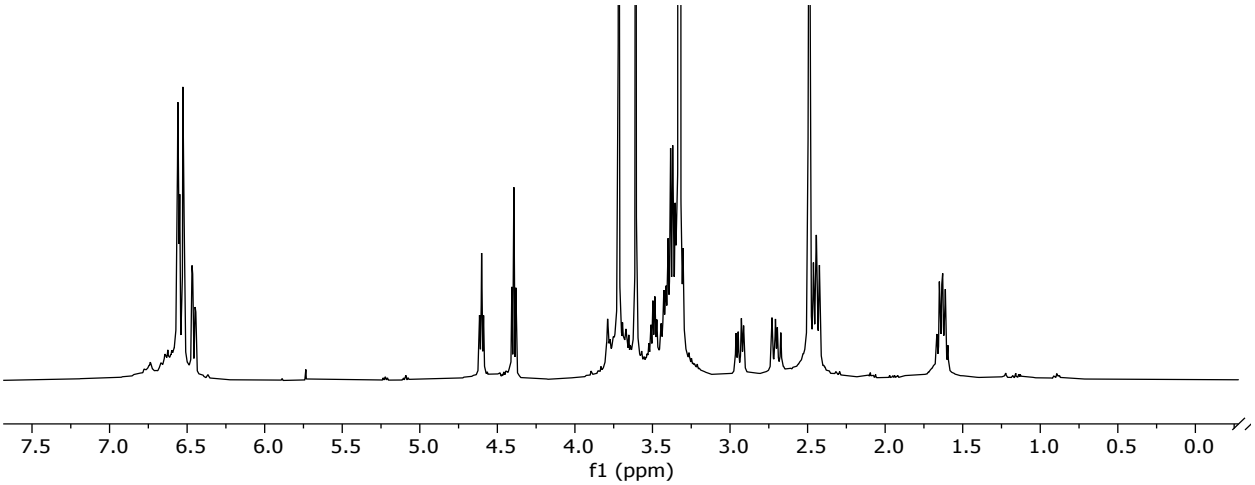
9.10 ^{1}H-NMR of the 5-5 dimer enriched fraction



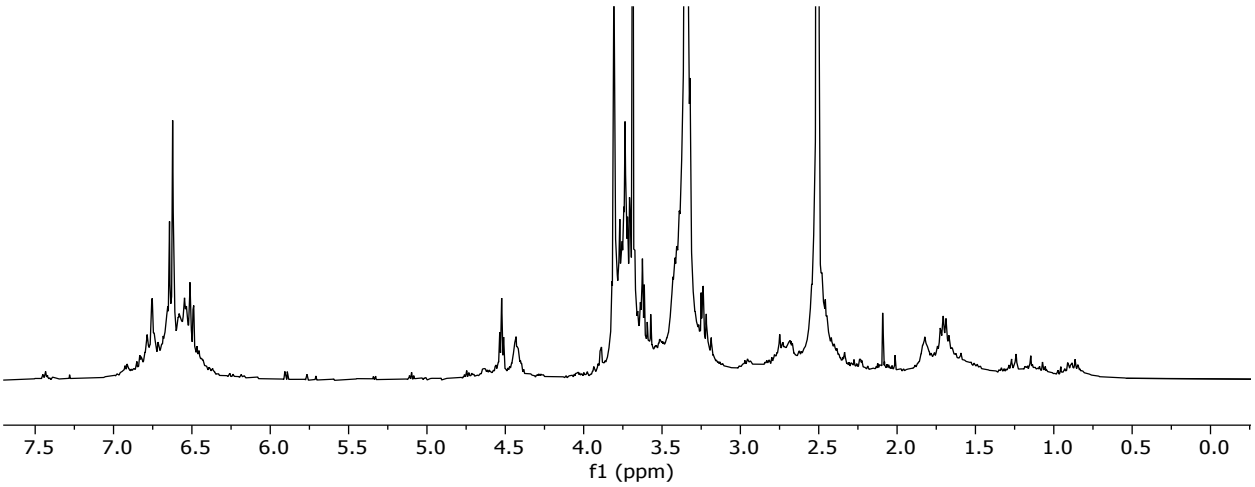
9.11 ^{1}H-NMR of the β-5 E enriched fraction



9.12 ^{1}H-NMR of the β-5 γ-OH dimer enriched fraction



9.13 ^{1}H-NMR of the β-β 2x γ-OH dimer enriched fraction



11. References

1 X. Liu, S. Feng, Q. Fang, Z. Jiang and C. Hu, *Molecular Catalysis*, 2020, **495**, 111164.

2 X. Meng, C. Crestini, H. Ben, N. Hao, Y. Pu, A. J. Ragauskas and D. S. Argyropoulos, *Nature Protocols*, 2019, **14**, 2627–2647.

3 T. Renders, S. Van den Bosch, T. Vangeel, T. Ennaert, S.-F. Koelewijn, G. Van den Bossche, C. M. Courtin, W. Schutyser and B. F. Sels, *ACS Sustainable Chemistry & Engineering*, 2016, **4**, 6894–6904.

4 W. Schutyser, S. den Bosch, T. Renders, T. De Boe, S.-F. Koelewijn, A. Dewaele, T. Ennaert, O. Verkinderen, B. Goderis, C. M. Courtin and B. F. Sels, *Green Chem.*, 2015, **17**, 5035–5045.

5 J. C. del Río, J. Rencoret, A. Gutiérrez, L. Nieto, J. Jiménez-Barbero and Á. T. Martínez, *Journal of Agricultural and Food Chemistry*, 2011, **59**, 11088–11099.

6 E. M. Anderson, M. L. Stone, R. Katahira, M. Reed, W. Muchero, K. J. Ramirez, G. T. Beckham and Y. Román-Leshkov, *Nature Communications*, 2019, **10**, 2033.

7 S. den Bosch, W. Schutyser, R. Vanholme, T. Driessen, S.-F. Koelewijn, T. Renders, B. De Meester, W. J. J. Huijgen, W. Dehaen, C. M. Courtin, B. Lagrain, W. Boerjan and B. F. Sels, *Energy Environ. Sci.*, 2015, **8**, 1748–1763.

8 S. den Bosch, W. Schutyser, S.-F. Koelewijn, T. Renders, C. M. Courtin and B. F. Sels, *Chem. Commun.*, 2015, **51**, 13158–13161.

9 H. Pan and L. N. Lundgren, *Phytochemistry*, 1995, **39**, 1423–1428.

10 T. Sicilia, H. B. Niemeyer, D. M. Honig and M. Metzler, *Journal of Agricultural and Food Chemistry*, 2003, **51**, 1181–1188.

11 T. Wukirsari, H. Nishiwaki, A. Hasebe, Y. Shuto and S. Yamauchi, *Journal of Agricultural and Food Chemistry*, 2013, **61**, 4318–4325.

12 K. Saito, A. Kaiho, R. Sakai, H. Nishimura, H. Okada and T. Watanabe, *Journal of Agricultural and Food Chemistry*, 2016, **64**, 9152–9160.

13 J.-L. Wen, S.-L. Sun, B.-L. Xue and R.-C. Sun, *Materials*, 2013, **6**, 359–391.