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Electronic Supplementary Information

Rapid pretreatment of *Miscanthus* using the low-cost ionic liquid triethylammonium hydrogen sulfate at elevated temperatures

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Experimental Details

Fractionation of Biomass (also see Gschwend et al, 2016, JoVE, doi: 10.3791/54246)

An ionic liquid/water master-mix was prepared by adding 20 wt% of water to triethylammonium hydrogen sulfate followed by mixing until a colourless, homogenous, viscous solution was obtained. The water content was confirmed by Karl-Fischer titration in triplicate.

10±0.05 g of ionic liquid/water master-mix was weighed into a 15 ml glass pressure tube with silicone front seal (Ace Glass) and the exact weight recorded. Between 2.08 and 2.16 g of ground and sieved *Miscanthus* was added, the vials capped and the content mixed with a vortex shaker. The samples were placed into a preheated convection oven (OMH60 Heratherm Advanced Protocol Oven). After the pretreatment period, the tubes were taken out and left to cool at room temperature.

Ethanol (40 ml) was added to the cooled pretreatment mixture, mixed with a spatula and the suspension transferred into a 50 mL centrifuge tube. The tube was shaken for one minute and left at room temperature for at least 1 hour. The tube was shaken for 30 seconds and then centrifuged at 4000 rpm for 50 minutes. The supernatant was decanted carefully into a round bottom flask. The washing of the pulp was repeated three more times. The solid was transferred into a cellulose thimble and further washed by Soxhlet extraction with refluxing ethanol (150 mL) for 22 hours. The thimbles were left to dry on the bench overnight. The ethanol used for the Soxhlet extraction was combined with the previous washes and evaporated under reduced pressure at 40°C, leaving the dried ionic liquid/lignin mixture. To the dried ionic liquid/lignin mixture, 30 mL of water was added in order to precipitate the lignin. The suspension was then transferred into a 50 mL centrifuge tube, shaken for one minute and left at room temperature for at least 1 hour. The tube was centrifuged and the supernatant decanted and collected in a round bottom flask. This washing step was repeated twice more.

The air-dried pulp yield was determined by weighing the pulp that has been recovered from the cellulose thimbles. The oven-dried yield was determined as described for the untreated biomass. The lid of the centrifuge tube containing the lignin was pierced and the tube put into a vacuum oven overnight to dry at 40°C under vacuum. The dried lignin was weighed the next day in order to determine the lignin yield.

Compositional Analysis (according to NREL procedure NREL/TP-510-42620)

200-300 mg of air-dry biomass or recovered pulp was weighed out into a 100 ml pressure tube and the weight recorded. 3 mL of 72% sulfuric acid was added, the samples stirred with a Teflon stir rod and the pressure tubes placed into a preheated water bath at 30°C. The samples were stirred every 15 min for one hour, they were then diluted with 84 mL distilled water and the lids closed. The samples were autoclaved (Sanyo Labo Autoclave ML5 3020 U) for 1 h at 121°C and left to cool to close to ambient temperature. The samples were then filtered through filtering ceramic crucibles of a known weight. The filtrate was split into batches in 50 ml centrifuge tubes and the solid washed with distilled water. The crucibles were placed into a convection oven (VWR Venti-Line 115) at 105°C for 24±2 h. They were placed in a desiccator for 15 min before they were weighed and the weight recorded. The crucibles were placed into a muffle oven (Nabertherm + controller P 330) and ashed to constant weight at 575°C. The weight after ashing was recorded. The content of acid insoluble lignin (AIL) was determined according to equation 1. The content of one of the Falcon tubes was used for the determination of acid soluble lignin content (ASL) by UV analysis at 240 nm (equation 2) (Perkin Elmer Lambda 650 UV/Vis spectrometer).

$$\%AIL = \frac{Weight_{crucible + AIR} - Weight_{crucible + ash}}{ODW_{sample}} \cdot 100 \qquad \text{(eq. 1)}$$

$$\%ASL = \frac{A}{l \cdot \varepsilon \cdot c} \cdot 100 = \frac{A \cdot V_{filtrate}}{l \cdot \varepsilon \cdot ODW_{sample}} \cdot 100 \qquad \text{(eq. 2)}$$

where $Weight_{crucibles+AIR}$ is the weight of the oven-dried crucibles plus the acid insoluble residue, $Weight_{crucibles+ash}$ is the weight of the crucibles after ashing to constant temperature at 575°C, A is the absorbance at 240 nm, l is the pathlength of the cuvette in cm (1 cm in this case), ε is the extinction coefficient (12 L/g cm), c is the concentration in mg/mL, ODW is the oven-dried weight of the sample in mg and $V_{filtrate}$ is the volume of the filtrate in mL and equal to 86.73 mL.

To the contents of the other Falcon tube, calcium carbonate was added until the pH reached 5. The liquid was passed through a 0.2 µm PTFE syringe filter and submitted to HPLC analysis (Shimadzu, Aminex HPX-87P from Bio-Rad, 300 x 7.8 mm, with deashing columns, purified water as mobile phase at 0.6 ml/min, column temperature 85°C) for the determination of total sugar content. Calibration standards with concentrations of 0.1, 1, 2 and 4 mg/mL of glucose, xylose, mannose, arabinose and galactose were used. Sugar recovery standards were made as 10 mL aqueous solutions close to the expected sugar concentration of the samples and transferred to pressure tubes. 278 µL 72% sulfuric acid was added, the pressure tube closed and autoclaved and the sugar content determined as described above. The sugar recovery coefficient (SRC) was determined according to equation 3 and the sugar content of the analysed sample using equation 4:

$$SRC = \frac{c_{HPLC} \cdot V}{initial\ weight} \qquad \text{(eq. 3)}$$

$$\%Sugar = \frac{c_{HPLC} \cdot V \cdot corr_{anhydro}}{SRC \cdot ODW_{sample}} \cdot 100 \qquad \text{(eq. 4)}$$

where c_{HPLC} is the sugar concentration detected by HPLC, V is the initial volume of the solution in mL (10.00 mL for the sugar recovery standards and 86.73 mL for the samples), initial weight is the mass of the sugars weighed in, $corr_{anhydro}$ is the correction for the mass increase during hydrolysis of polymeric sugars (0.90 for C_6 sugars glucose, galactose and mannose and 0.88 for C_5 sugars xylose and arabinose) and ODW is the oven-dried weight of the sample in mg.

Saccharification Assay (according to NREL/TP-5100-63351)

100±10 mg (calculated on an ODW basis) air-dried biomass was placed into a Sterilin tube and the weight recorded. Three blanks were run with 100 µL of purified water in order to correct for sugar residues present in the enzyme solutions. 9.9 mL solution made from 5 mL 1M sodium citrate buffer at pH 4.8, 40 µL tetracycline solution (10 mg/mL in 70% ethanol), 30 µL cycloheximide solution (10 mg/mL in purified water), 4.71 mL purified water, 60 µL cellulase from *Trichoderma reesei* ATCC 26921 solution and 60 µL cellobiase from *Aspergillus niger* solution was added, the tubes closed and placed into an Stuart Orbital Incubator (S1500) at 50°C and 250 rpm.

Time point samples were taken after 4, 18, 48, and 96 hours and an end point sample after 168 hours. For time point samples, 500 µL of the saccharification mixture was removed from the Sterilin tubes using a 1 ml adjustable pipette with the tip cut off (representative amount of solids and liquids) and transferred to a microcentrifuge tube. The samples were centrifuged in a table top centrifuge at 4°C and 13.3 G for 10 min. The supernatant was pipetted into another microcentrifuge tube and frozen until analysis. Prior to analysis, samples in the tubes were shaken with a vortex shaker and centrifuged once more at 4°C and 13.3 G for 5 min and then transferred into HPLC vials. End point samples were obtained by filtering 1 mL of the saccharification mixture though a PTFE syringe filter. Samples were analysed on Shimadzu HPLC system with RI detector and an Aminex HPX-87P column (BioRad, 300 x 7.8 mm) with deashing columns and purified water as mobile phase (0.6 mL/min). The column temperature was 85°C and acquisition time was 40 min. Calibration standards with concentrations of 0.1, 1, 2 and 4 mg/mL of glucose, xylose, mannose, arabinose and galactose and 8 mg/mL of glucose were used.

Additional Spectra and Numerical Values

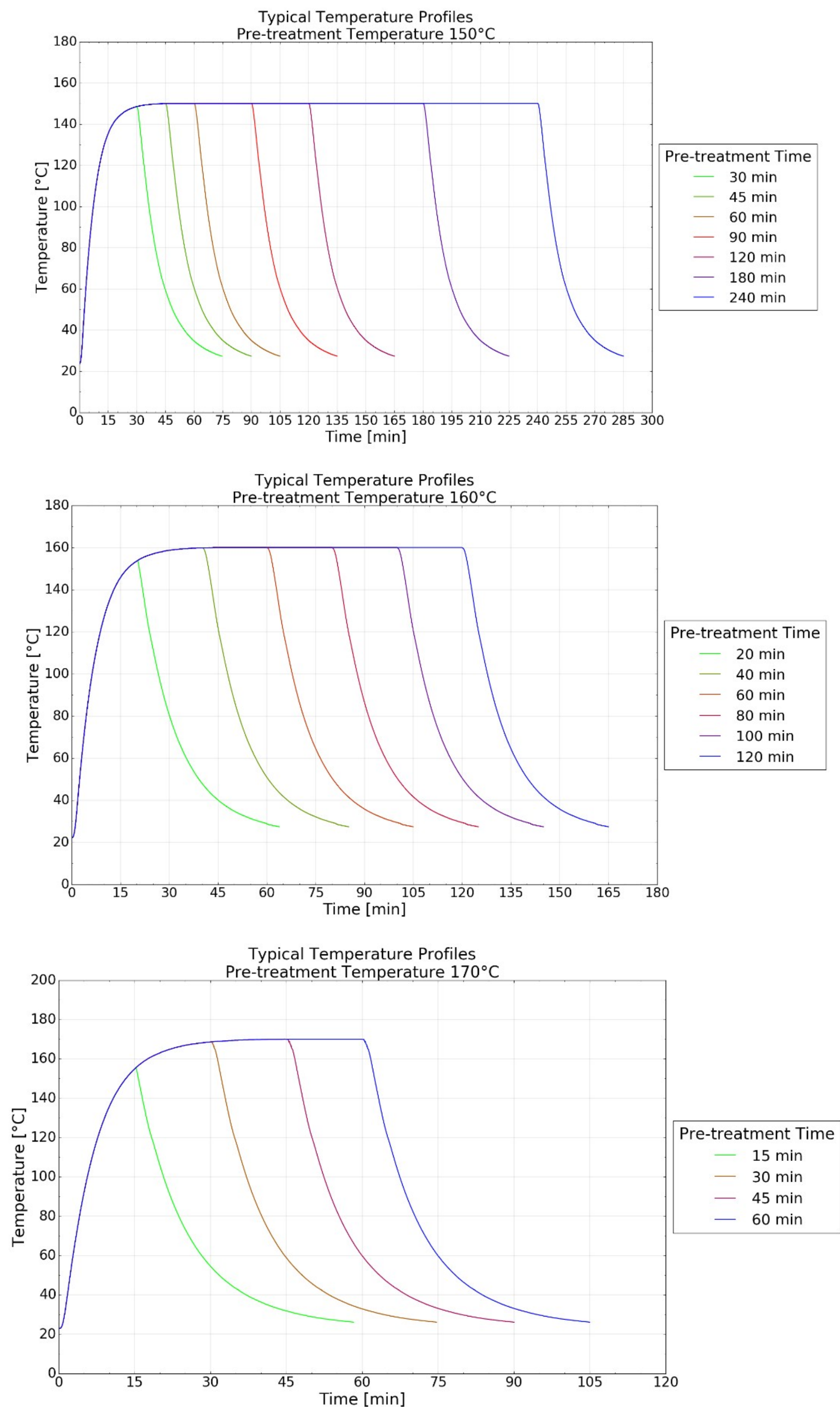


Fig. S1 Reaction medium temperature profiles inside 15 ml pressure tubes at an oven temperature of 150°C, 160°C and 170°C.

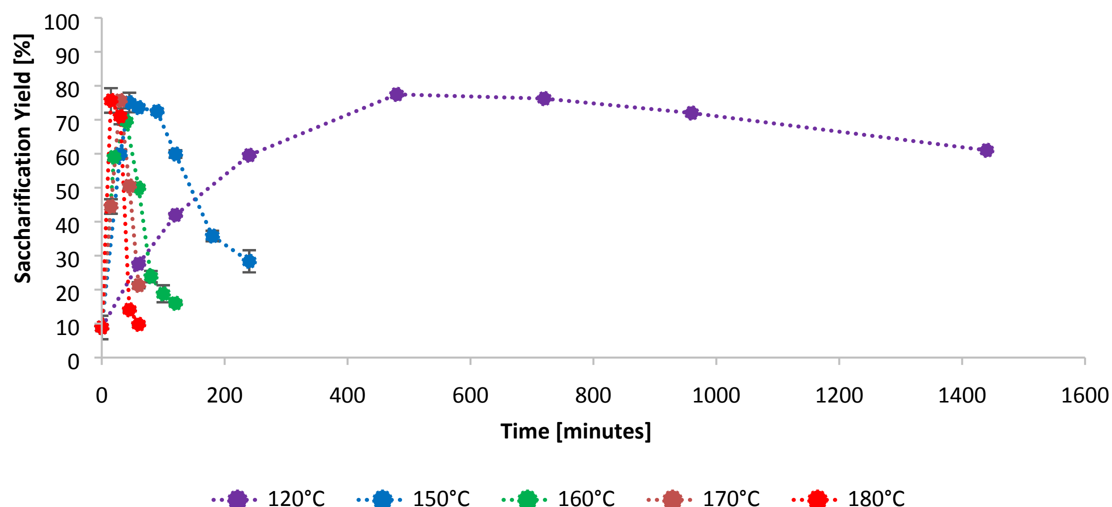


Fig. S2 Enzymatic glucose release from *Miscanthus* pulp fractionated at different temperatures, including 120°C.

Table S1 Pulp composition, as determined by compositional analysis, and lignin precipitate yield. *Miscanthus* was pretreated at a 1:5 g/g biomass to solvent ratio in [TEA][HSO_4] with 20% water.

		Pulp Composition (wt%)				Total pulp (wt%)	Lignin precipitate (wt%)
t (min)	**T (°C)**	**Glucan**	**Hemicelluloses**	**Lignin**	**Ash**		
Untreated *Miscanthus*		50.1	22.4	26.8	0.7	100.0	N/A
30	150	49.3	9.7	13.0	0.8	72.9	9.5
45	150	50.9	7.7	9.8	0.2	68.6	12.0
60	150	50.0	6.1	9.5	0.4	66.0	13.5
90	150	51.9	4.3	7.7	0.1	64.0	16.1
120	150	48.7	3.4	7.7	0.1	59.9	19.1
180	150	48.1	2.8	9.9	0.1	61.0	19.7
240	150	48.2	2.4	13.5	0.8	64.9	17.6
20	160	48.9	6.9	9.6	0.2	65.7	9.8
40	160	46.7	1.9	5.9	0.2	54.7	17.1
60	160	45.4	1.0	6.1	0.3	52.8	19.5
80	160	42.6	0.5	8.6	0.5	52.2	20.1
100	160	41.4	0.2	10.3	0.3	52.2	19.1
120	160	39.5	0.2	13.7	0.3	53.6	17.5
15	170	49.4	10.8	13.9	0.4	74.5	7.4
30	170	47.9	2.2	4.8	0.4	55.3	16.2
45	170	44.2	0.7	5.5	0.4	50.8	19.8
60	170	40.6	0.1	8.5	0.4	49.6	20.3
15	180	48.5	5.9	8.6	0.4	63.5	11.5
30	180	44.6	0.7	4.7	0.4	50.4	19.3
45	180	34.0	0.1	8.6	0.4	43.1	22.8
60	180	20.5	0.1	20.6	0.4	41.6	20.4

N/A: not applicable

Table S2 Enzymatic hydrolysis yields (glucose only) from *Miscanthus* pulp pretreated at 150°C (% of theoretical maximum)

PT time (min)	Day 1	Day 3	Day 5	Day 7
30	27.8	46.3	55.9	59.8
45	37.4	59.7	71.5	75.1
60	39.8	61.5	71.2	73.6
90	41.8	61.6	70.5	72.5
120	35.4	51.0	60.5	59.9
180	23.8	32.4	37.3	35.8
240	21.3	27.2	27.7	28.3

Table S3 HSQC NMR volume integral size (G_2+G_{2cond} was set to 100).

Temperature	**150°C**	**150°C**	**150°C**	**160°C**	**170°C**	**170°C**	**170°C**	**180°C**	**180°C**
Time	**45 min**	**60 min**	**90 min**	**40 min**	**15 min**	**30 min**	**45 min**	**15 min**	**30 min**
β-β'	7.6	6.5	4.3	4.3	9.6	5.3	3.2	7.8	3.4
β-*O*-4'	35.3	30.6	17.9	17.9	69.1	20.4	10.5	48.5	8.7
β-5'	19.3	18.5	13.6	13.6	19.1	15.8	8.1	19.4	8.2
S_{cond}	44.5	44.4	40.2	40.2	25.5	50.0	43.5	38.8	46.6
S	84.0	80.6	54.3	54.3	106.4	65.8	40.3	97.1	48.1
G_6	67.2	64.5	57.6	57.6	72.3	61.8	50.0	67.0	59.6
$G_{2,cond.}$	44.5	44.4	54.3	54.3	27.7	51.3	60.9	36.9	56.7
G_2	55.5	55.6	45.7	45.7	72.3	48.7	39.1	63.1	43.3
H	38.7	38.7	44.0	44.0	18.1	45.4	48.8	24.3	60.1
PCA	29.4	29.8	19.0	19.0	57.4	24.3	11.3	46.6	18.3

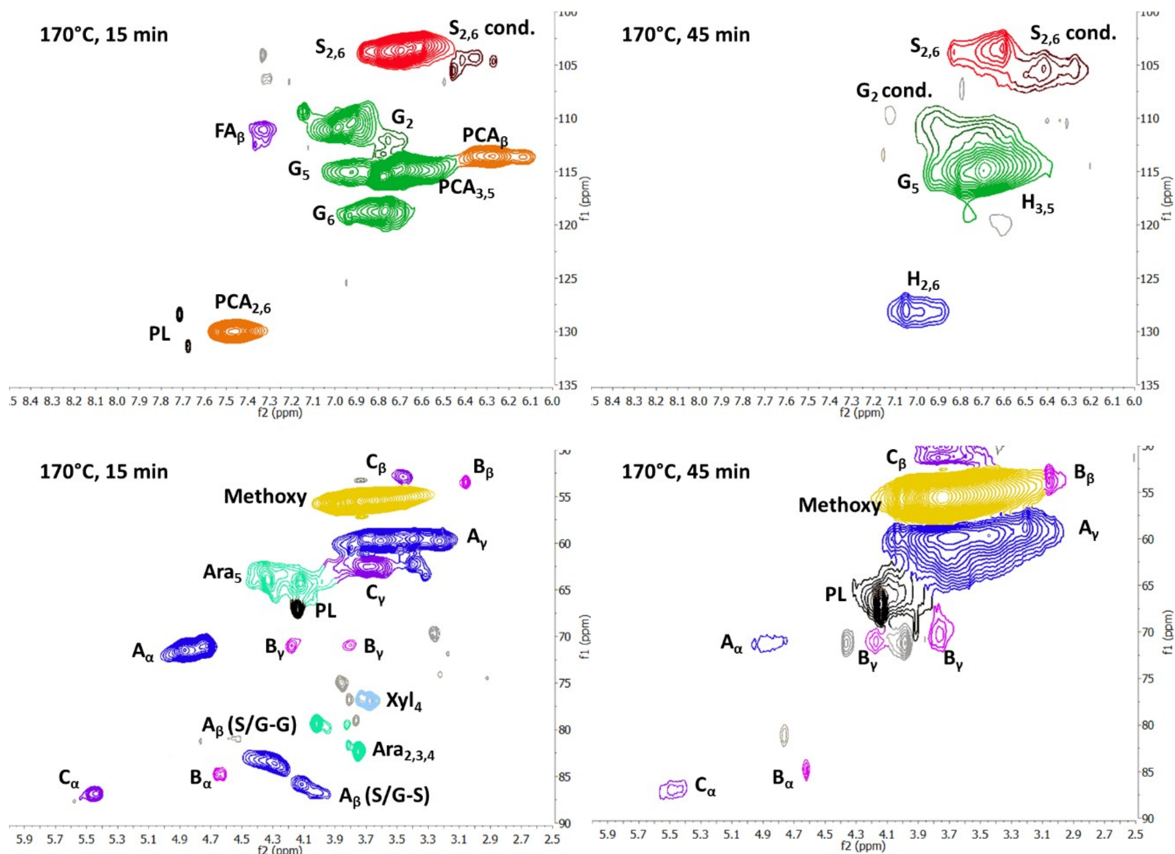


Figure S3 HSQC NMR spectra of *Miscanthus* lignin isolated after extraction with $[TEA][HSO_4]$ with a biomass to solvent ratio 1:5 g/g and a water content of 20 wt%. Aromatic region (top) and side chain region (bottom). These lignins were associated with suboptimal saccharifications yields (170°C, 15 min: undertreated, 170°C, 45 min: overtreated)

Table S4 Hydroxyl group content in selected isolated lignins (in mmol OH per gram of lignin). *Miscanthus* was pretreated at a 1:5 g/g biomass to solvent ratio in 80% $[TEA][HSO_4]$ with 20% water.

t (min)	T (°C)	aliphatic	S	G	H	acid
45	150	1.77	1.01	1.09	0.65	0.02
60	150	1.27	1.02	1.14	0.59	0.02
90	150	1.30	1.38	1.51	0.75	0.16
40	160	1.33	1.78	1.80	0.94	0.16
15	170	3.04	0.66	0.98	0.80	0.09
30	170	1.66	2.13	2.17	1.11	0.12
45	170	2.04	4.38	4.37	2.17	0.74
15	180	2.54	1.22	1.33	0.85	0.01
30	180	0.92	1.89	1.89	0.95	0.19

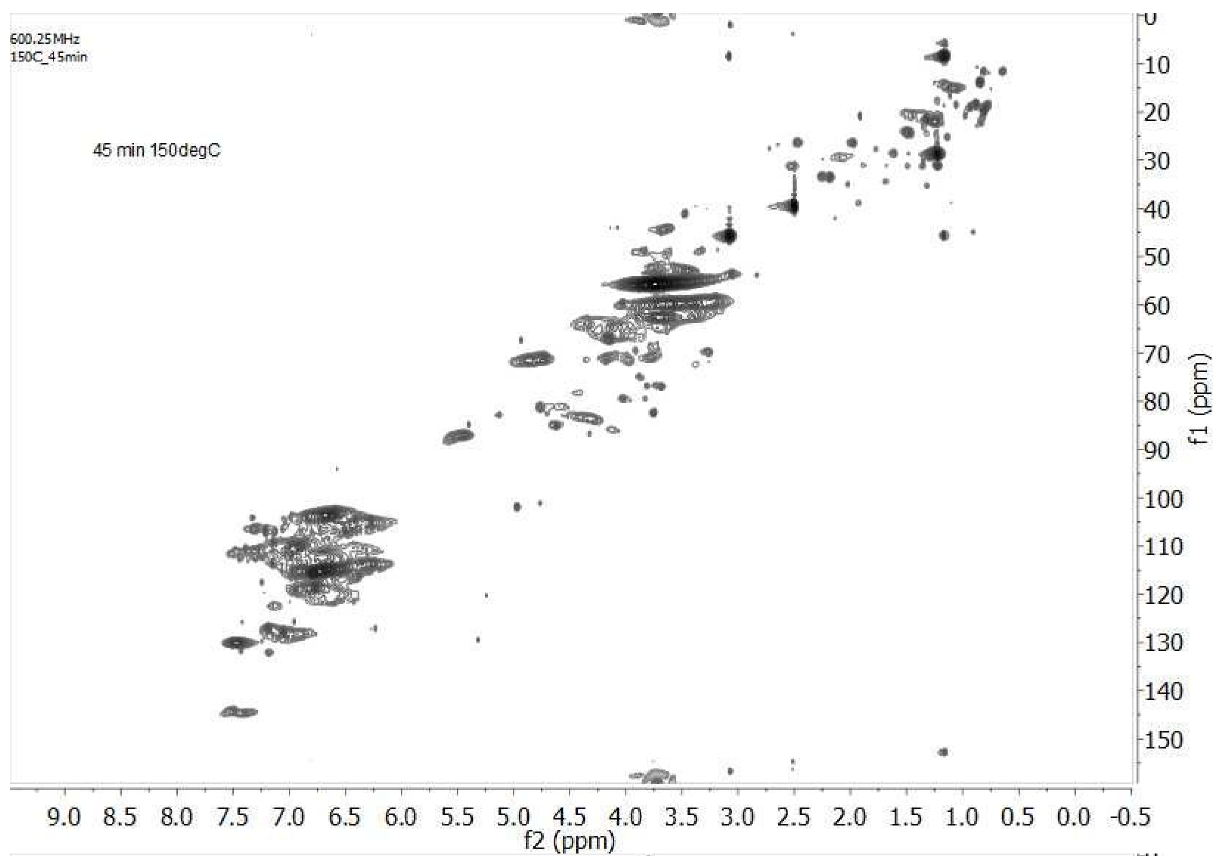
S: syringyl, G: guaiacyl, H: *p*-hydroxyphenyl, acid: carboxylic acid

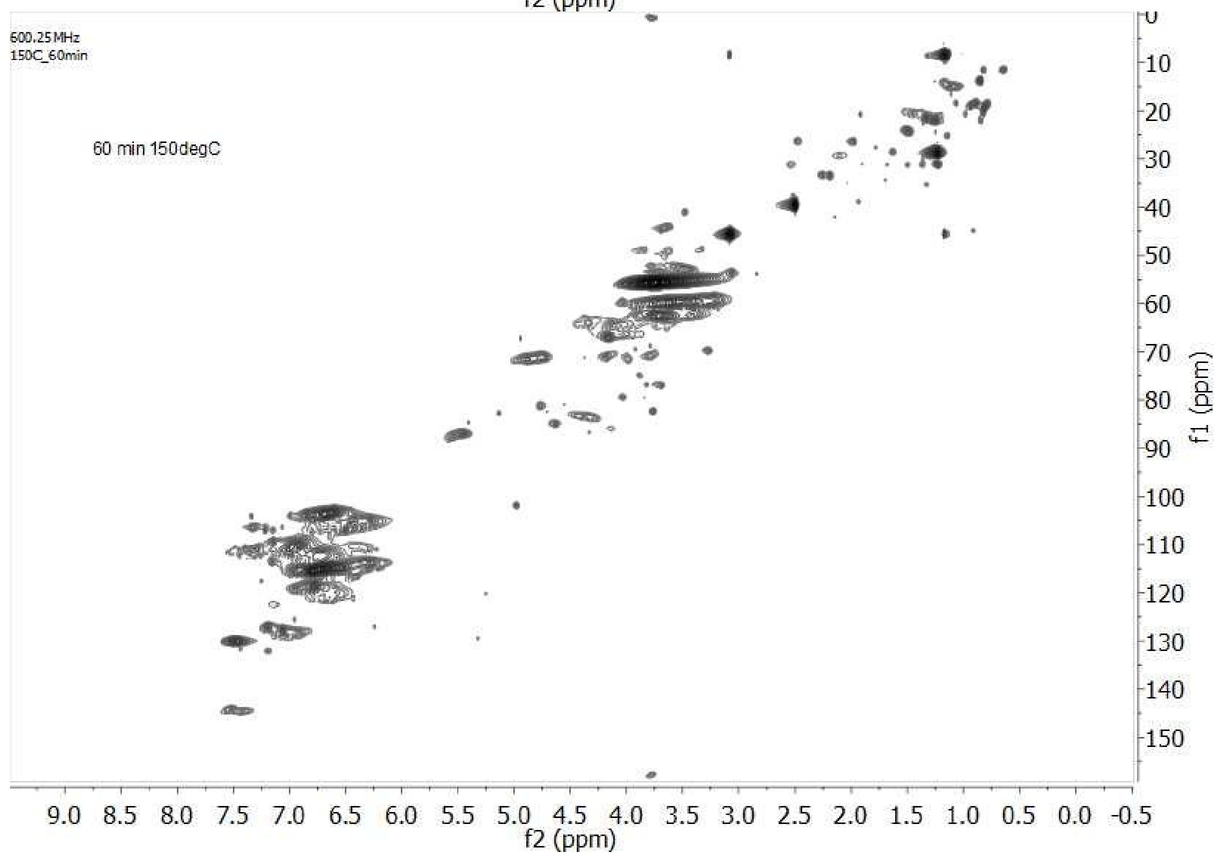
Table S4 Elemental analysis results of isolated lignins. *Miscanthus* was pretreated at a 1:5 g/g biomass to solvent ratio in 80% $[TEA][HSO_4]$ with 20% water.

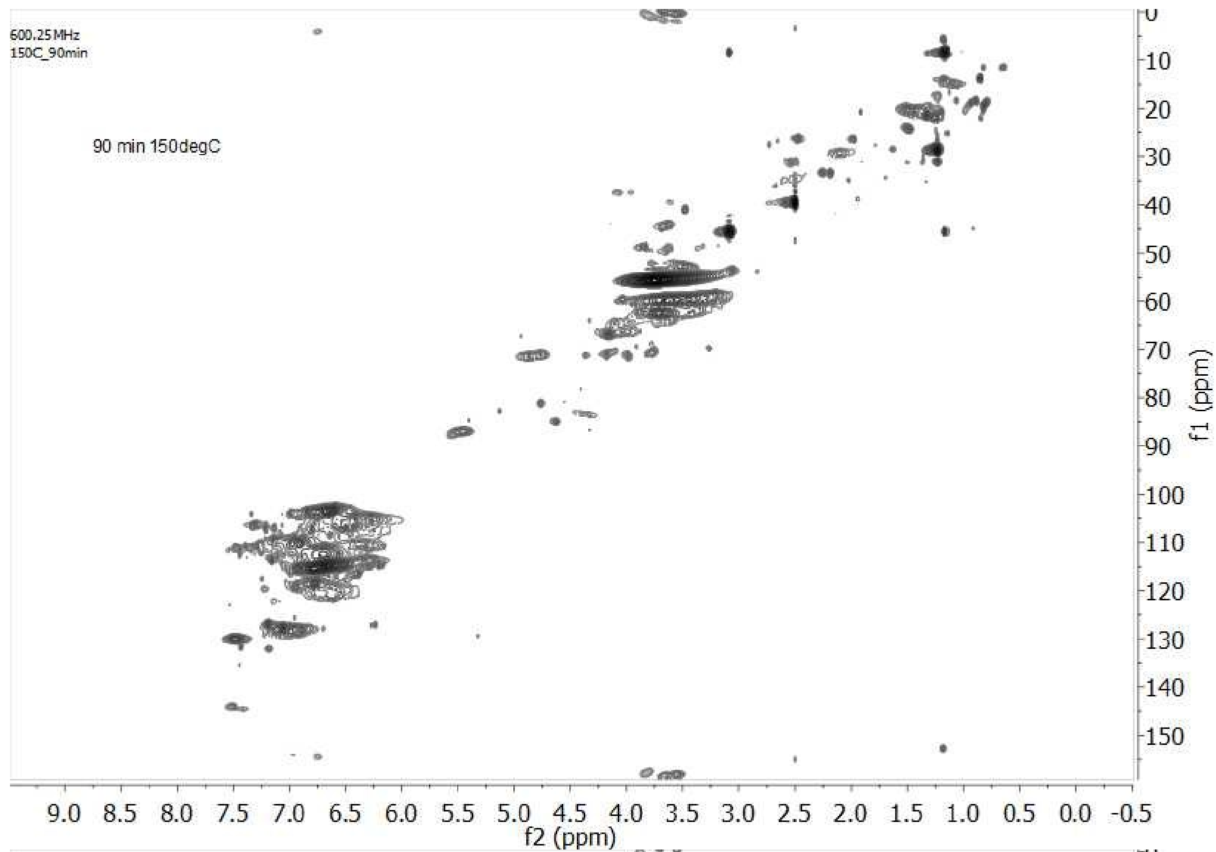
t (min)	T (°C)	C%	H%	N%	S%	O%[a]
45	150	61.5	6.1	BDL	0.8	31.7
60	150	62.5	5.9	BDL	0.5	31.1
90	150	63.3	5.7	BDL	0.4	30.5
15	170	60.0	6.1	BDL	0.4	33.5
30	170	62.8	5.8	BDL	0.5	31.0
45	170	64.3	5.6	BDL	0.4	29.8
15	180	60.7	6.0	BDL	0.5	32.8
30	180	63.7	5.6	BDL	0.5	30.2

C: carbon, H: hydrogen, N: nitrogen, S: sulfur, O: oxygen content, [a] by difference, BDL: Below detection limit.

HSQC-NMR raw spectra:



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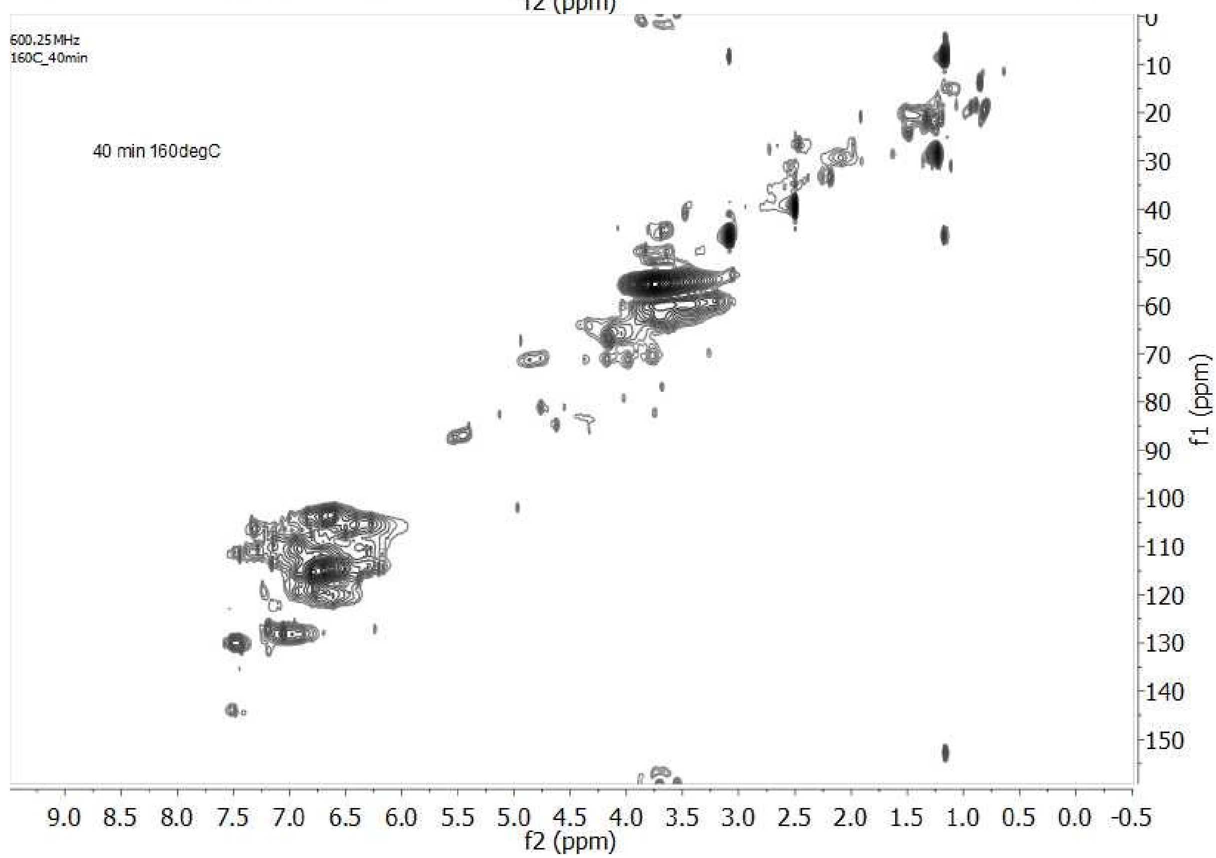
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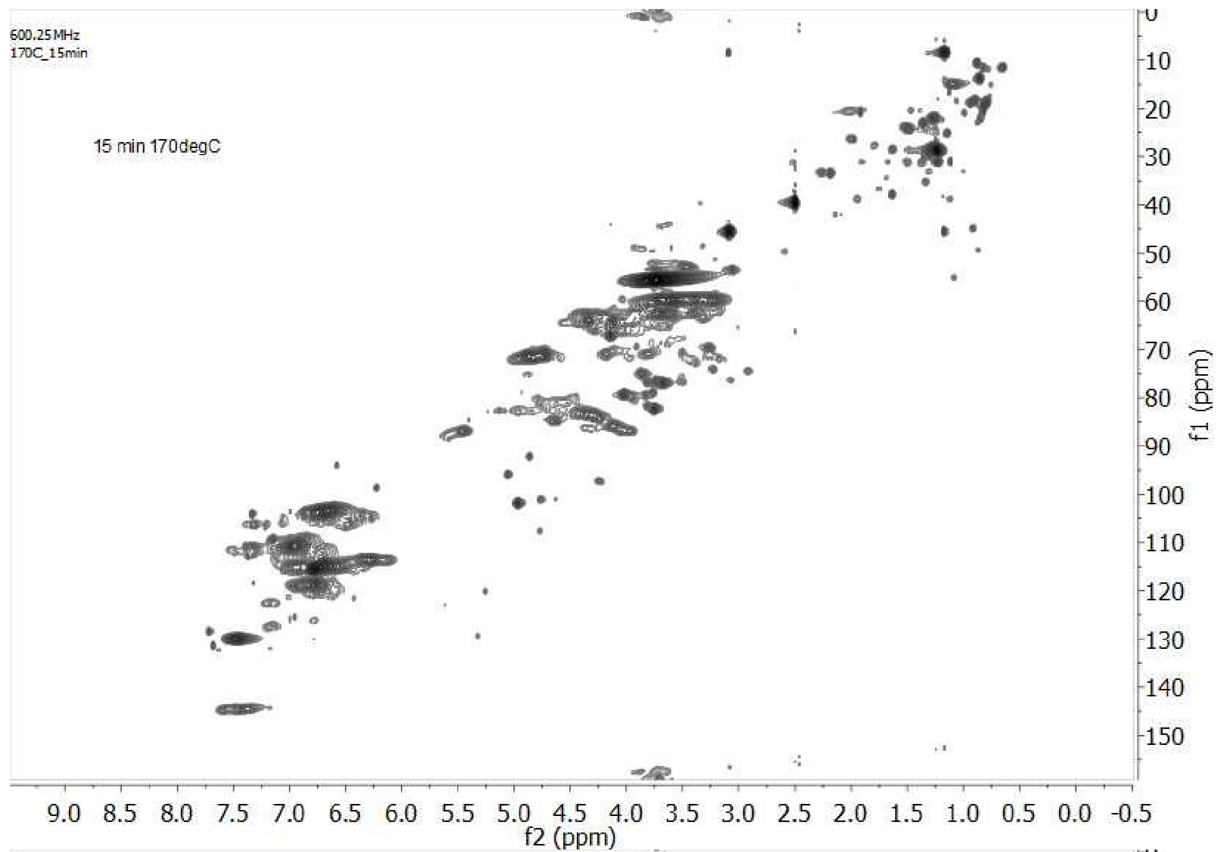
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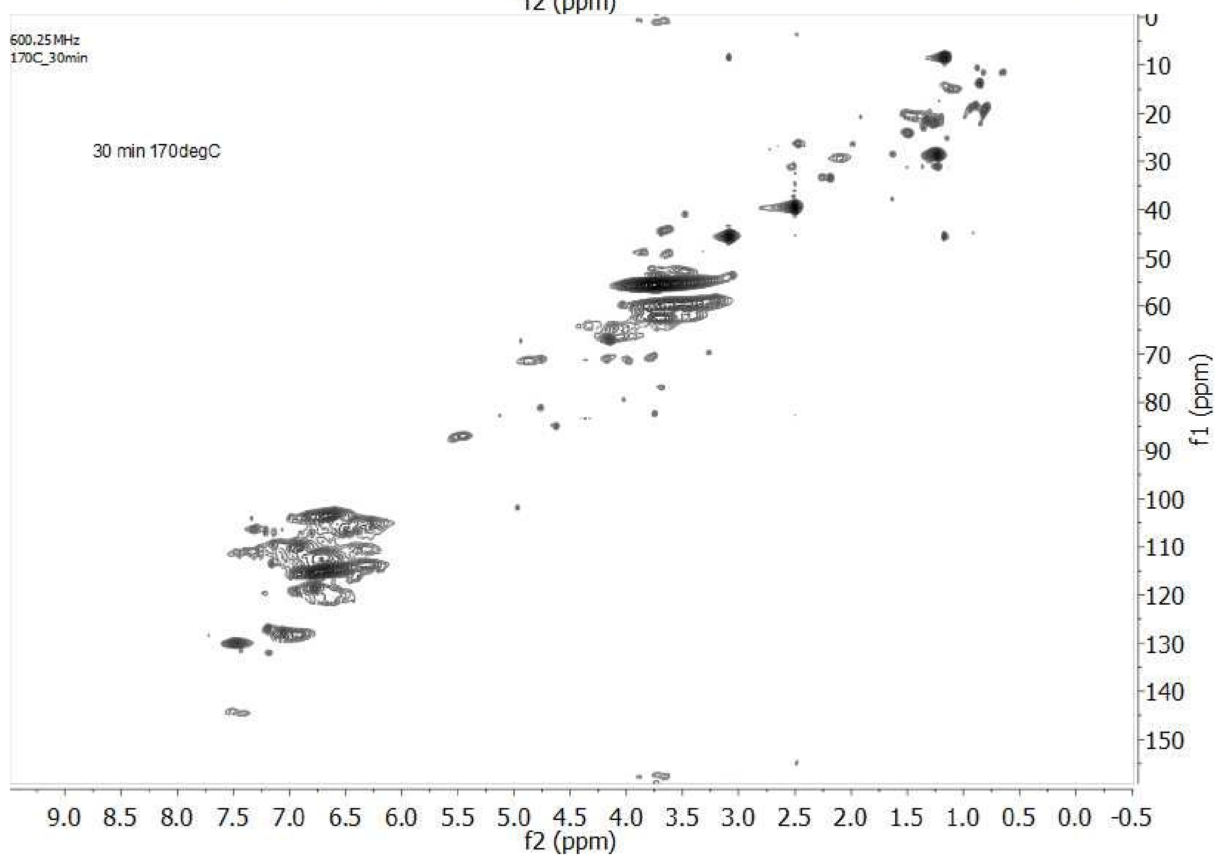
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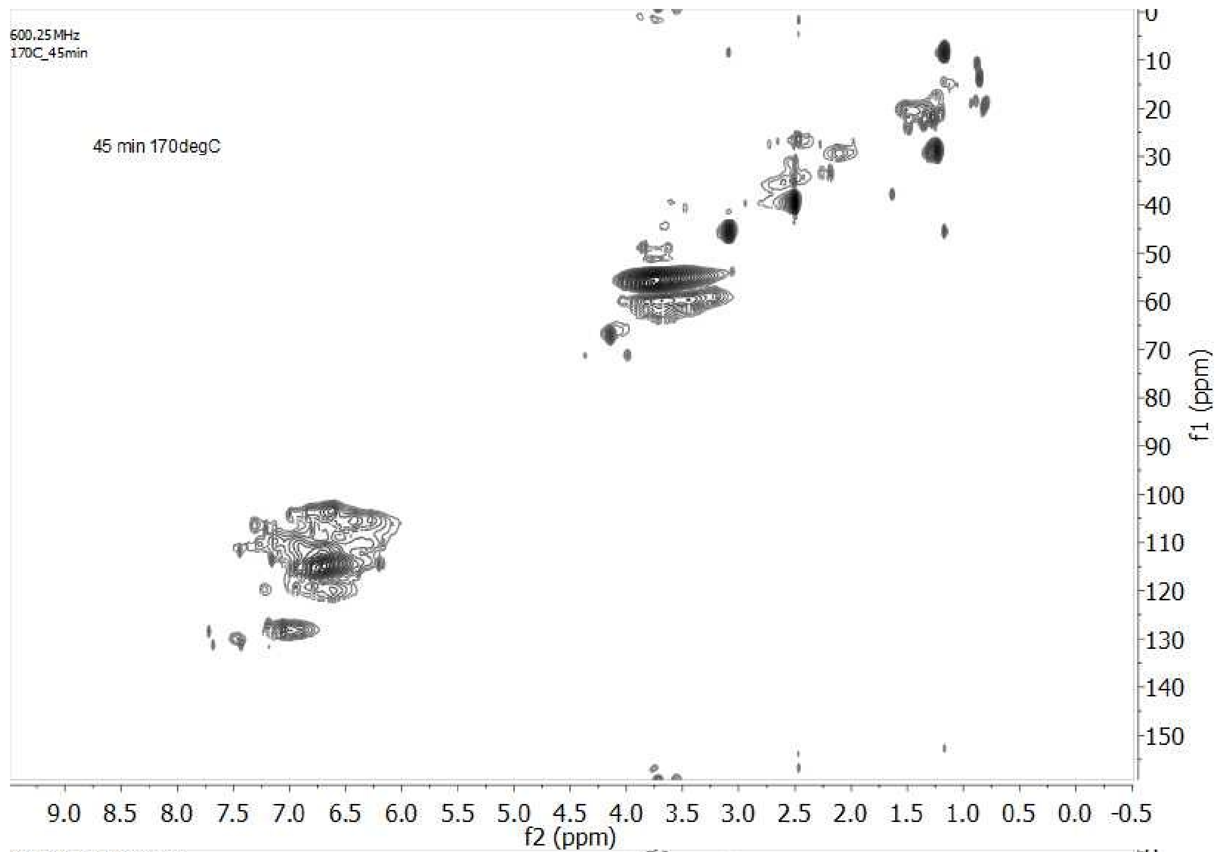
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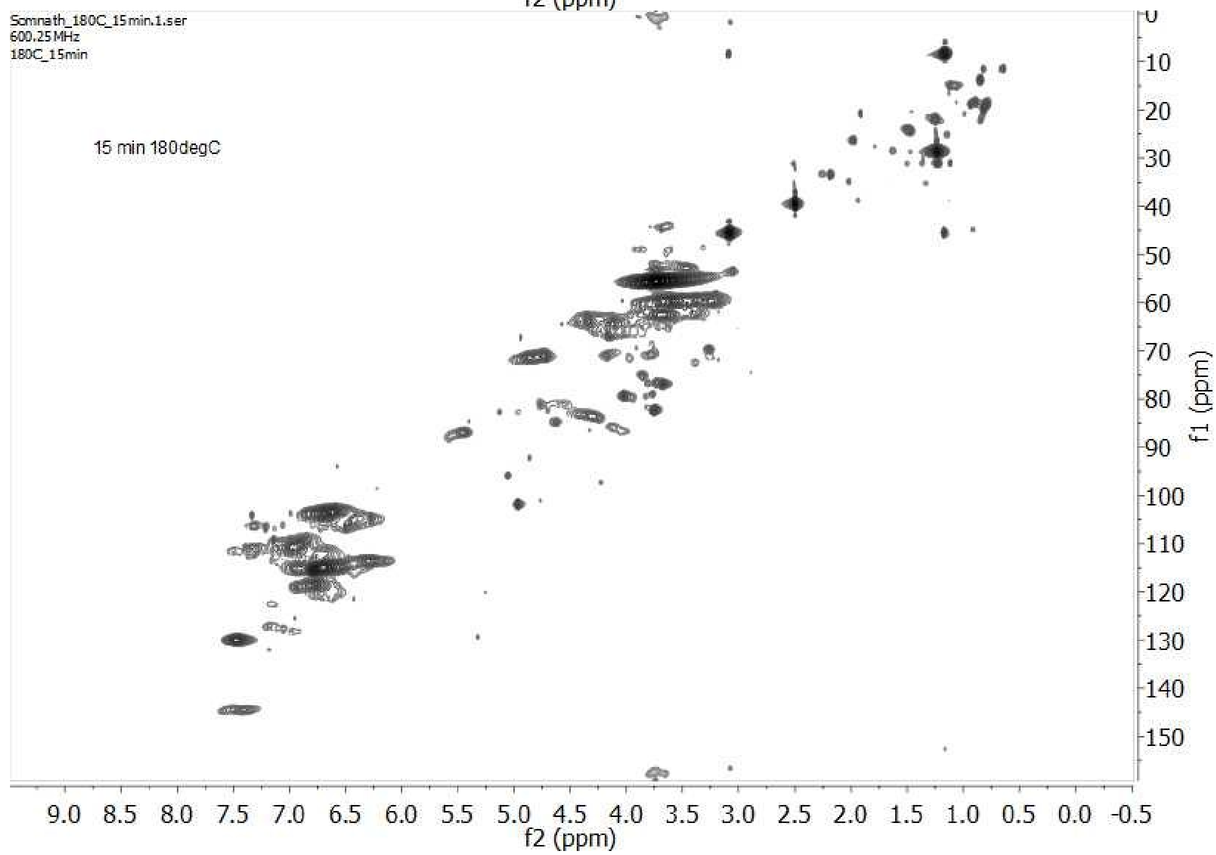
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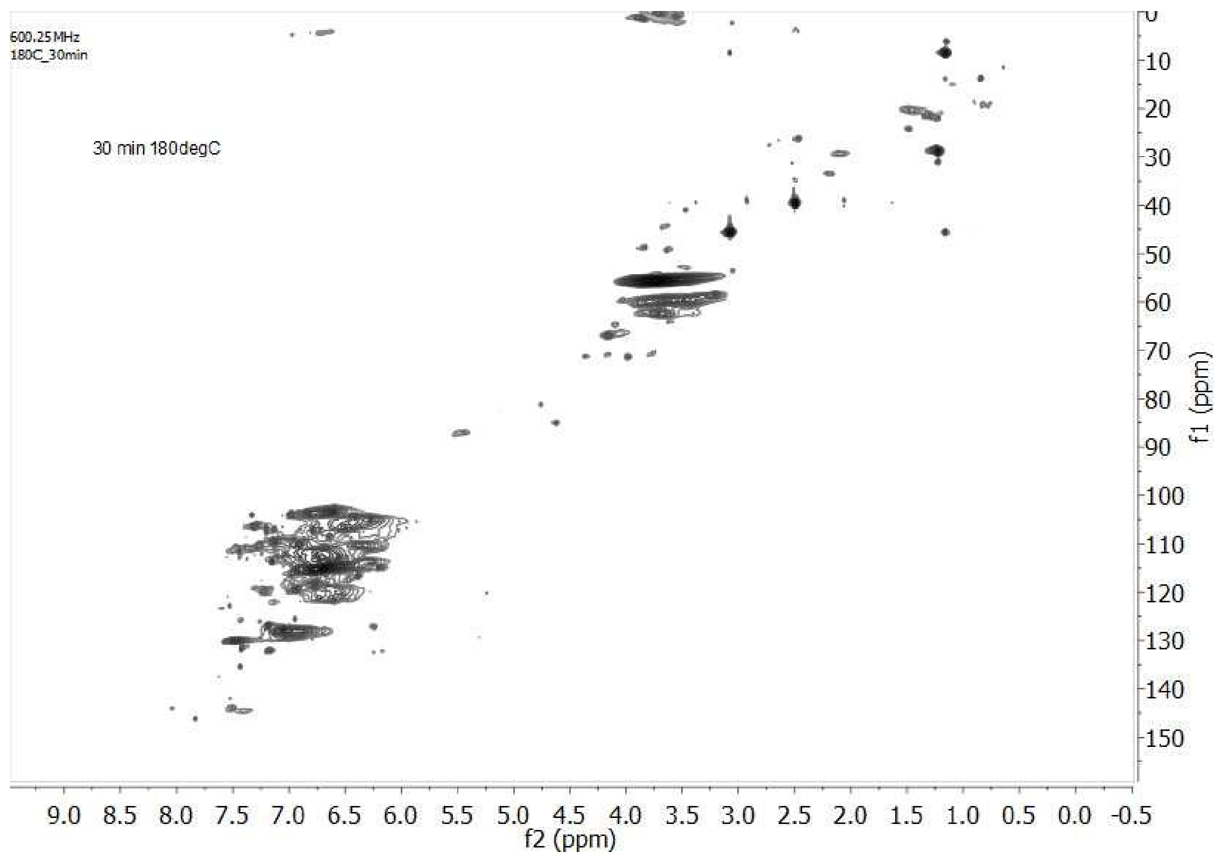
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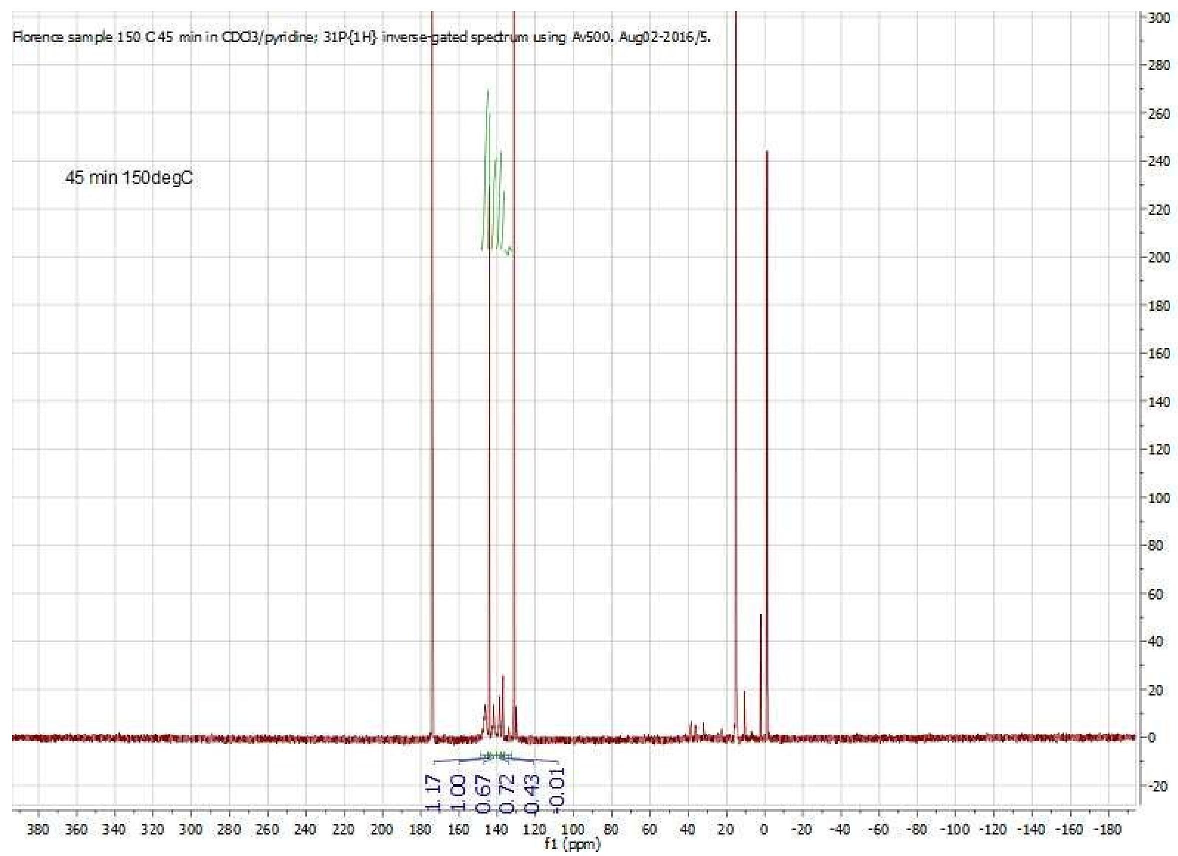
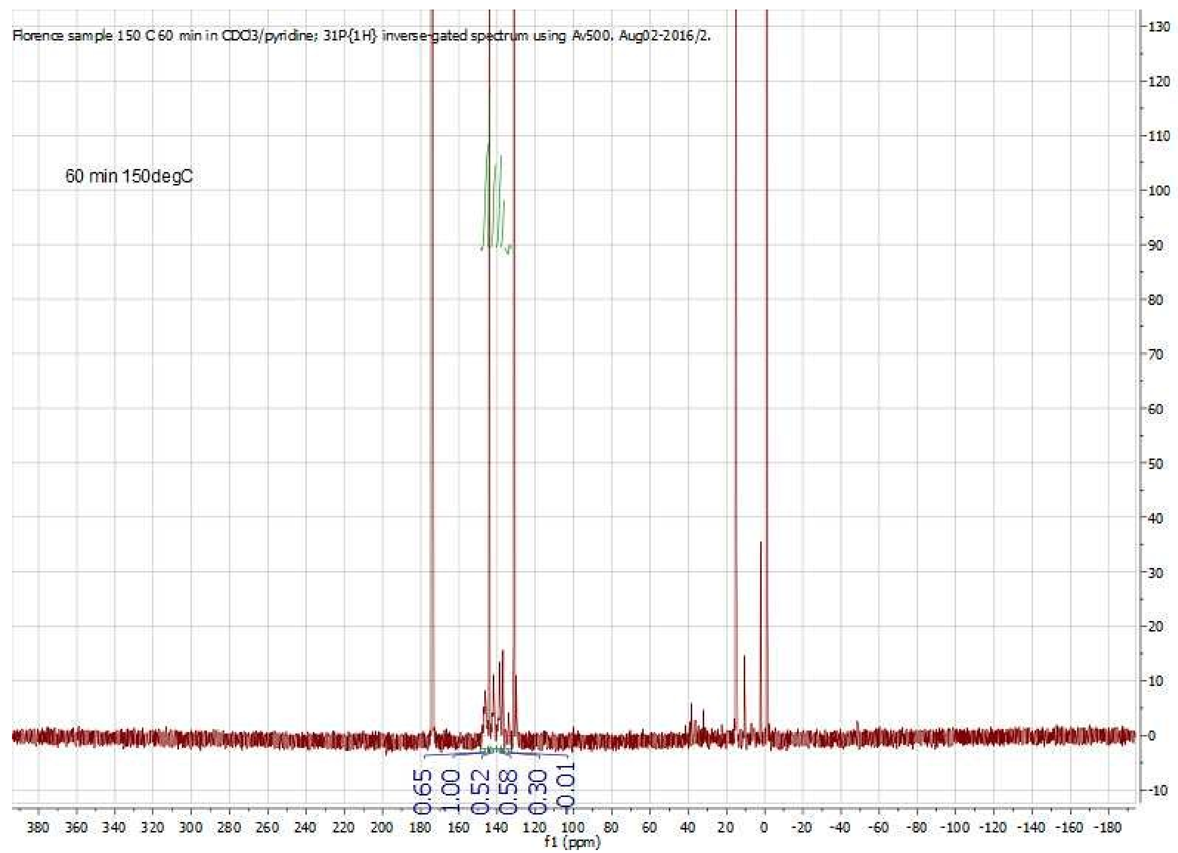
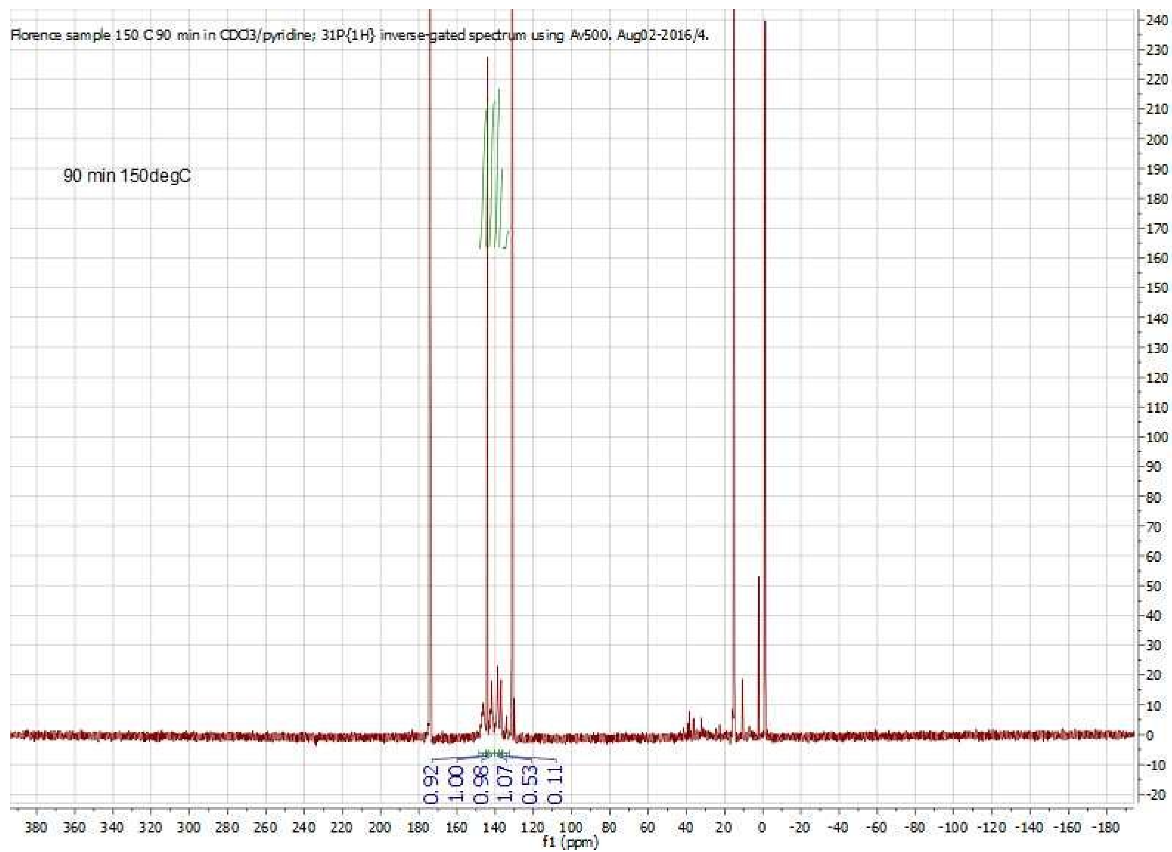
^{31}P NMR-spectra used for hydroxyl group analysis



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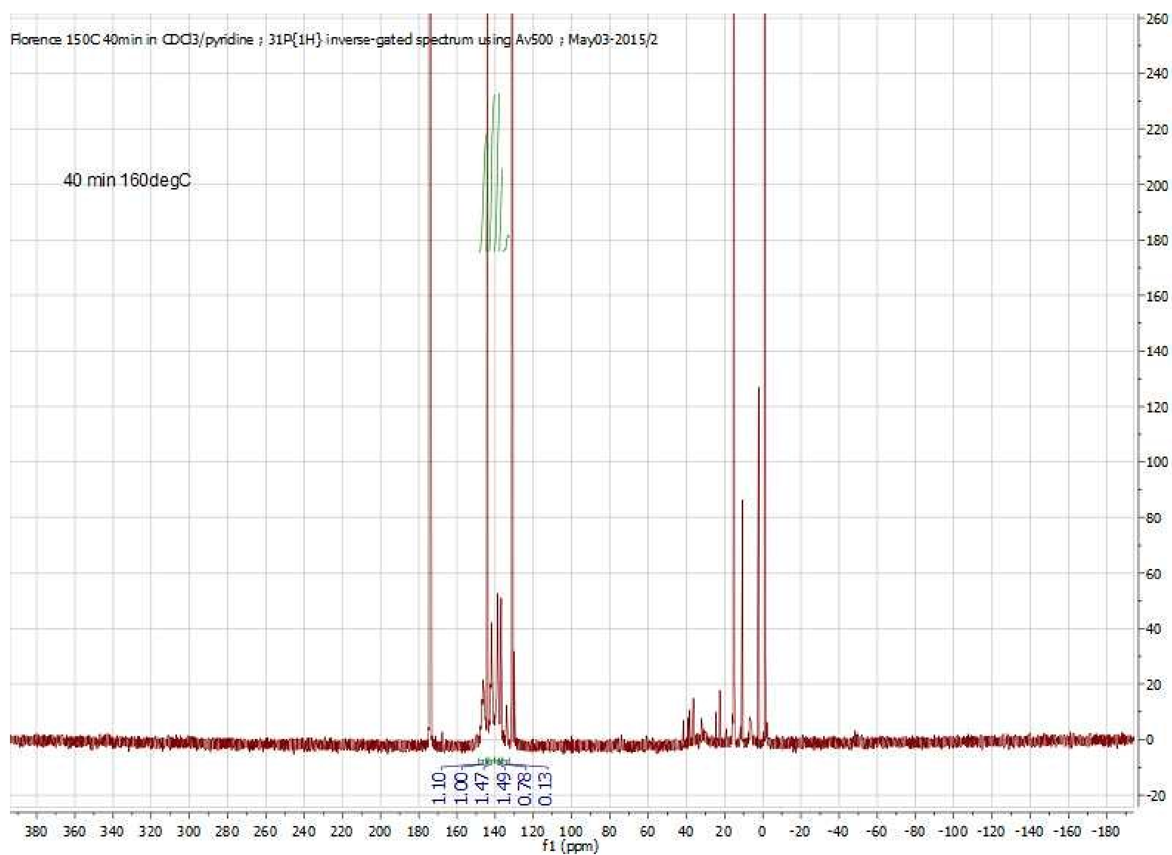
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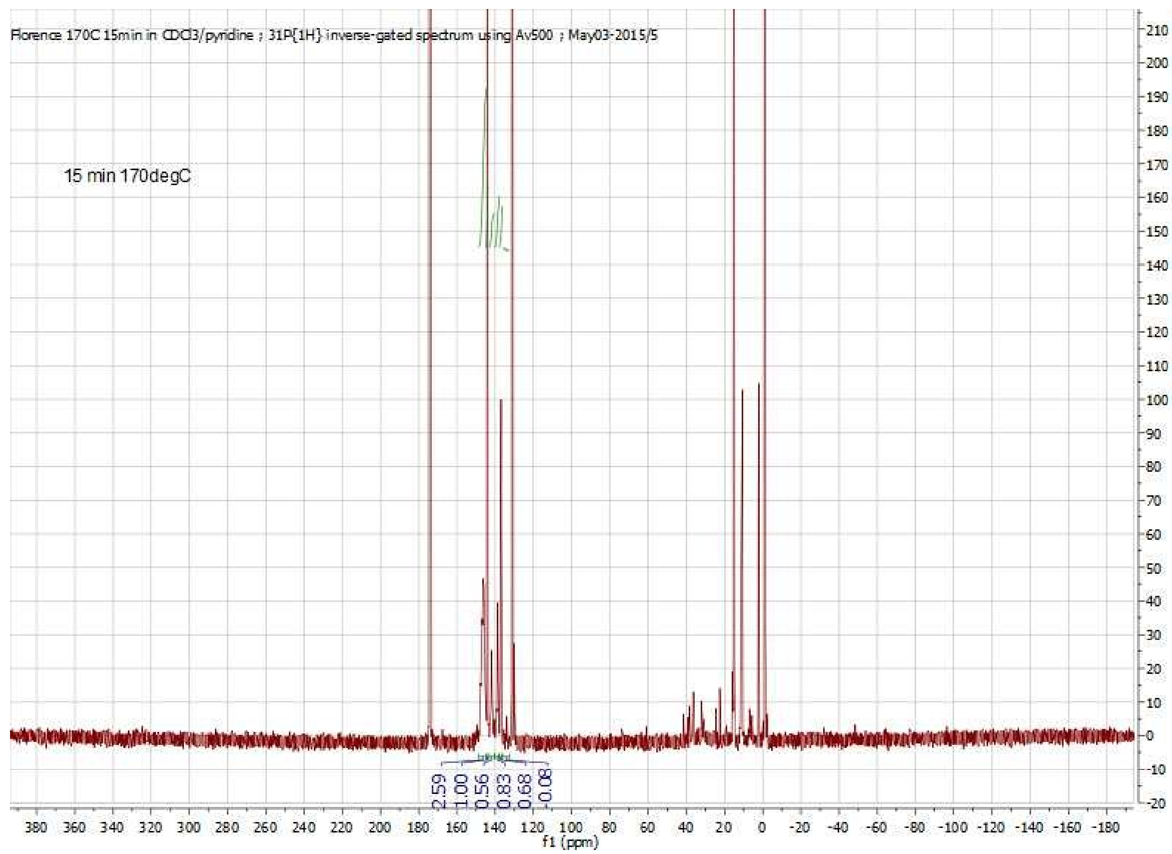
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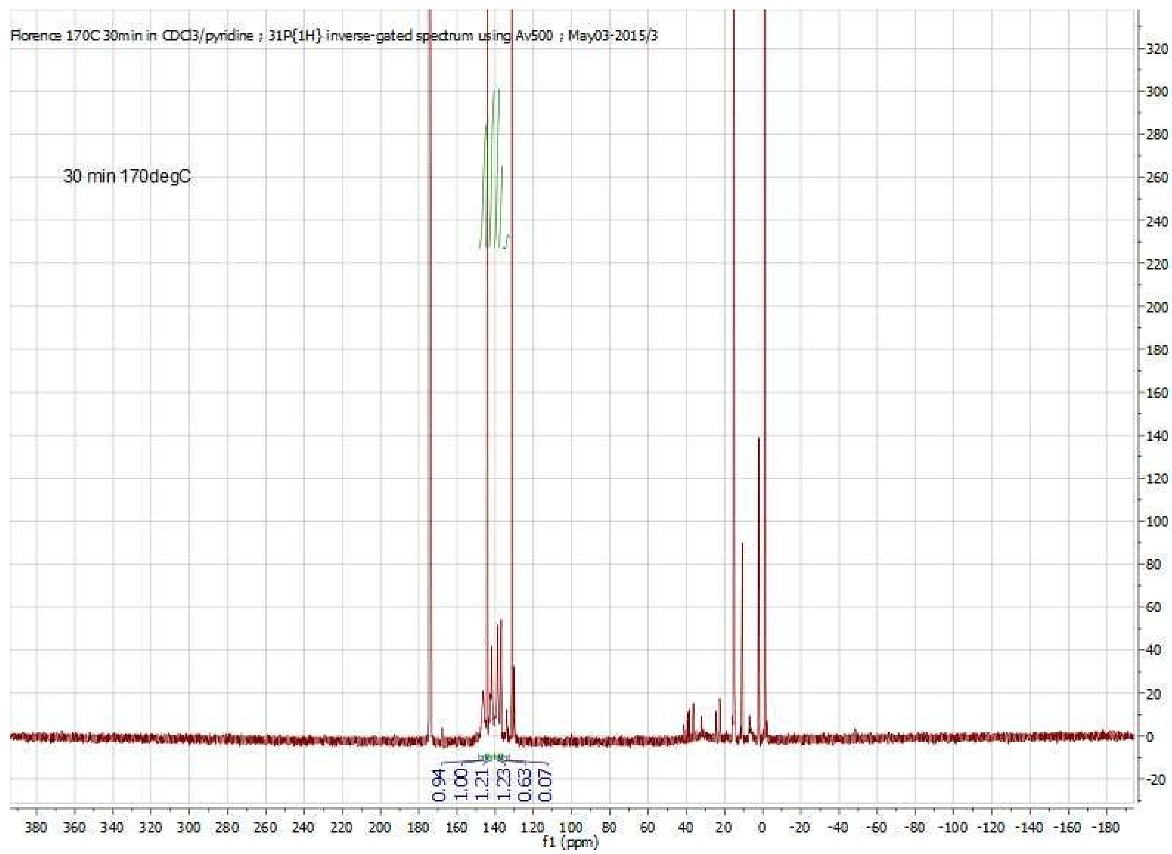
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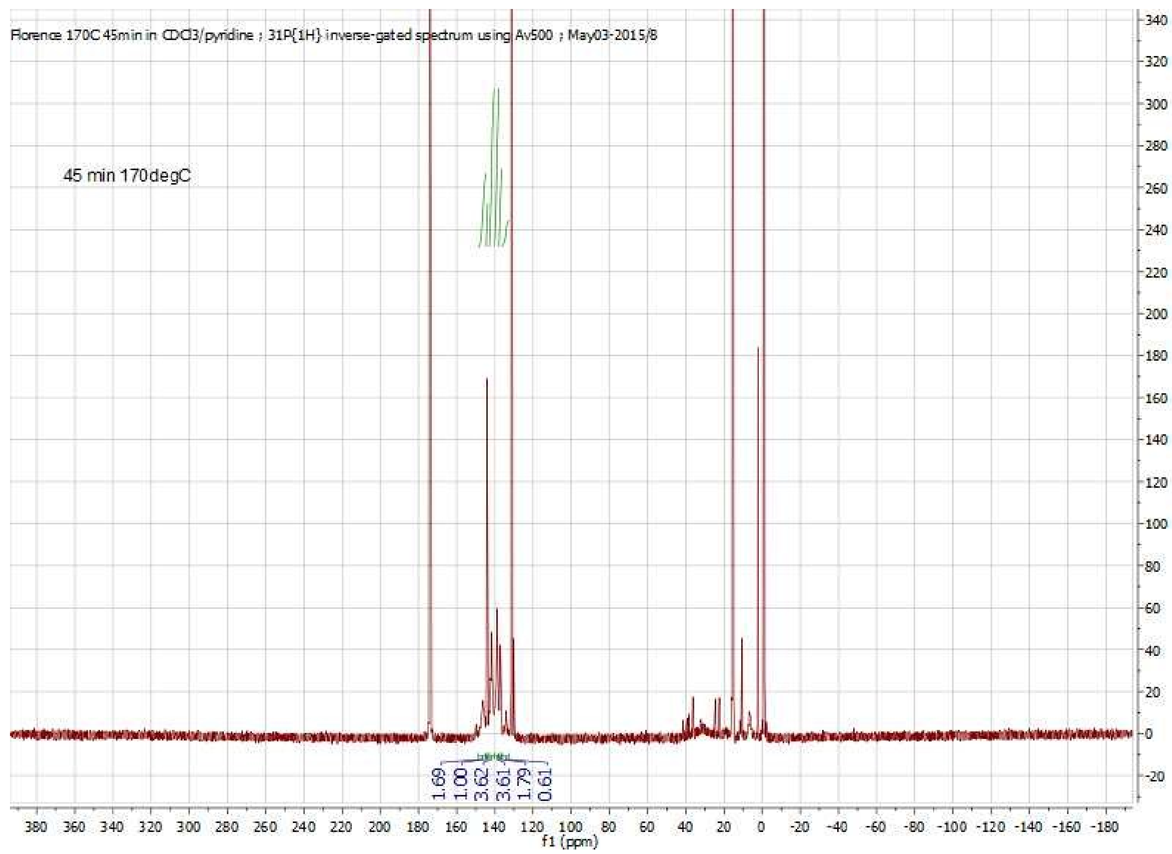
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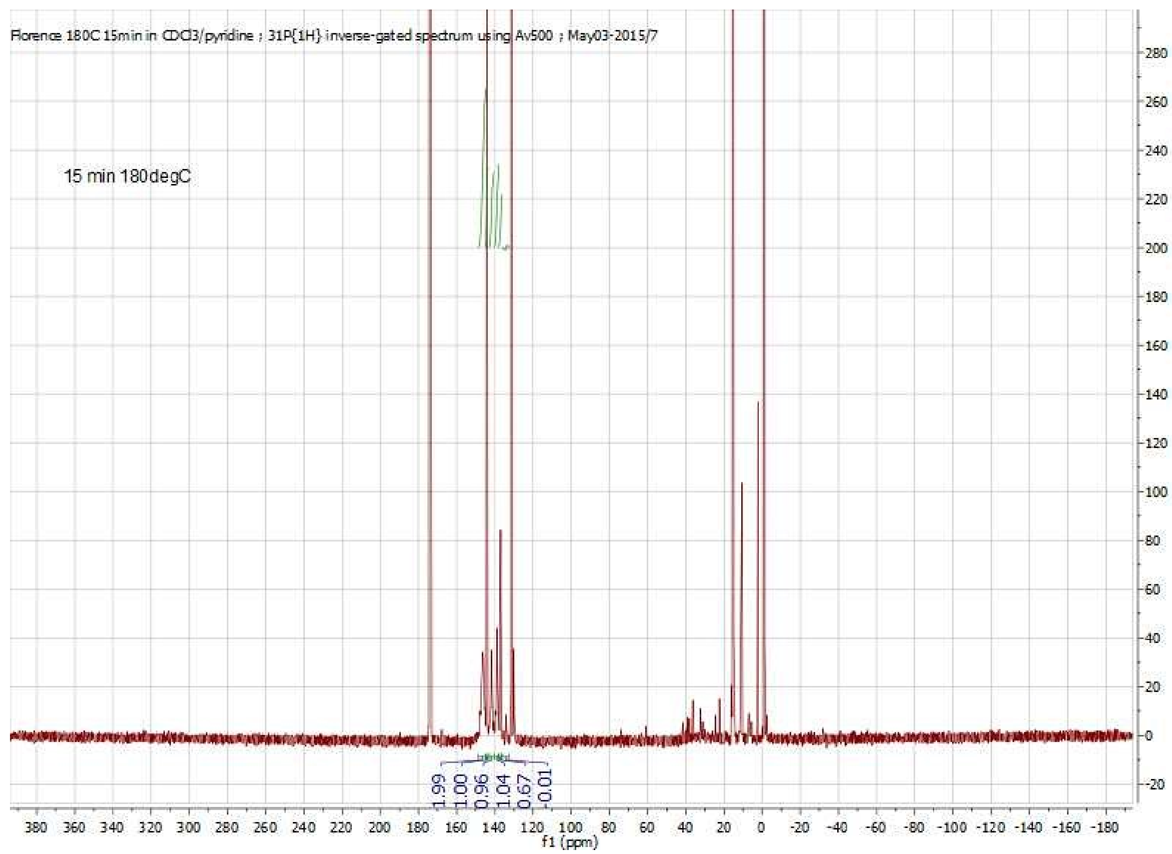
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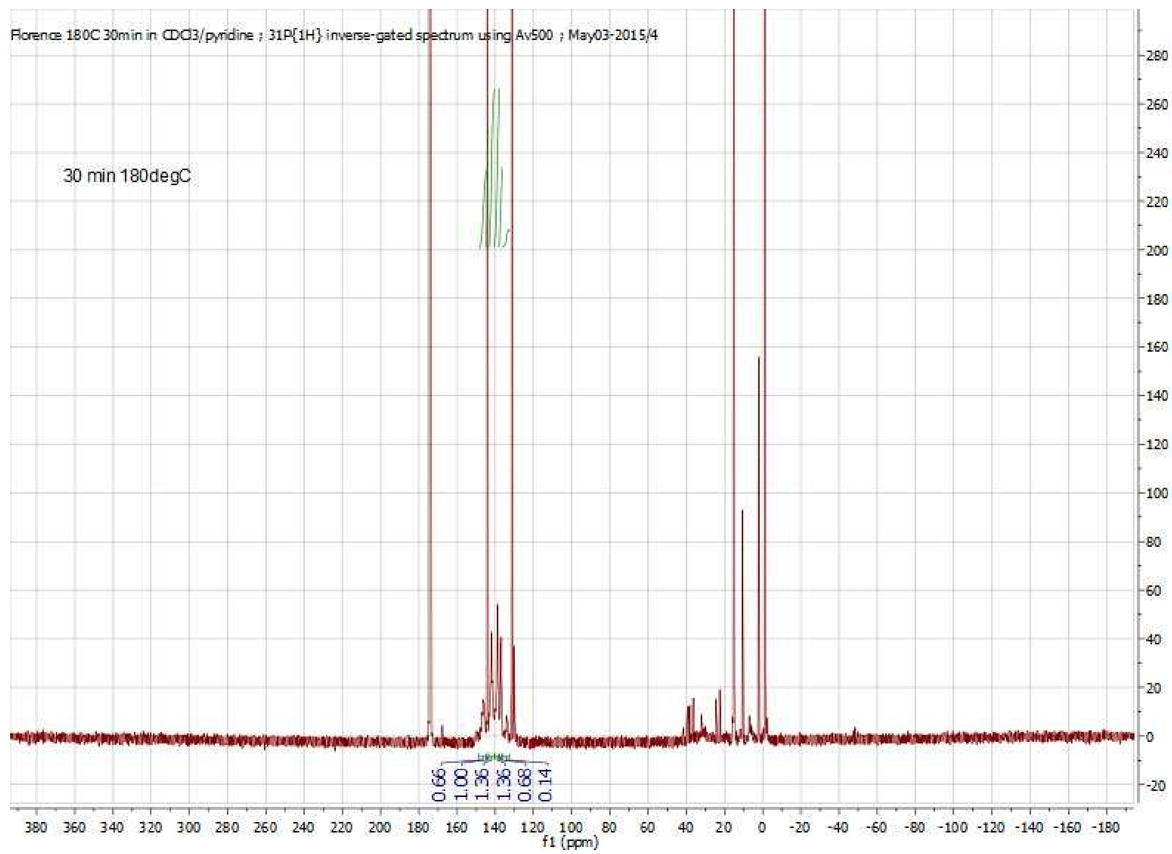
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