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Supporting information for the paper:

Lignin solubilisation and gentle fractionation in liquid ammonia

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1. Properties of ammonia and safety considerations

Table S1: Physical properties of liquid ammonia.[1]

Critical temperature (°C)	132
Critical pressure (atm)	112.3
Vapour pressure of liquid	$\mathrm{Log}_{10}\ P_{mm} = 9.95028$
Vapour pressure of solid	$\mathrm{Log}_{10}\ P_{mm} = 9.98379 - (1627.22/T)$
ΔH_f (cal/mole)	1351.6
ΔH_v (cal/mole)	55581
Refractive index (λ=5899 Å, 16°C)	1.325
Dielectric constant	22.7 (-50°C), 18.94 (5°C), 17.82 (15°C), 16.90 (25°C)
Density (kg/m^3)	725 (-70°C), , 702 (-50°C), , 681 (-33°C), , 652 (-10°C), 625 (10°C), 595 (30°C), 563 (50 °C), 506 (80 °C), 457 (100 °C)
Viscosity (centipoises)	0.254 (-33.5°C), 0.230 (-26°C), 0.183 (-10°C), 0.170 (-4°C), 0.162 (-8°C), 0.152 (10°C), 0.145 (15°C), 0.141 (20°C)

[1] Hayashi J, Sufoka A, Ohkita J, Watanabe S: Confirmation of existence of cellulose III(I), III(II), IV(I), and IV(II) by X-ray method. J *Polym Sci Pol Lett* 1975, **13**, 23-27.

CAUTION! Ammonia vapours have a sharp, irritating, pungent odour that acts as a warning of potentially dangerous exposure. The average odour threshold is 5 ppm, well below any exposure limit. Exposure to high concentrations of gaseous ammonia can result in lung damage and death.

For more information see
Risks statement: 10-23-34-50
Safety statement: 9-16-26-36/37/39-45-61

2. Comparison of the physical properties of ammonia and water

Table S2: Physical properties of ammonia and water.[2]

	NH_3	**H_2O**
Boiling point (°C)	-33.35	100
Freezing point (°C)	-77.7	0
Specific conductivity (ohm^{-1} cm$^{-1)}$	1×10^{-11}	4×10^{-8}
Dielectric constant	22.7 (-50°C)	81.7(18°C)
Viscosity (centipoises)	0.254 (-33°C)	1.00 (20°C)
Density (kg/dm^3)	681.4 (-33°C)	1000 (4°C)
Dipole moment (Debye)	1.49	1.84
Polarizability ($\times10^{24}$)	2.21	1.48
Autoprotolysis constant (25 °C)	1.9×10^{-30}	1×10^{-14}
Orientation effect (erg cm^6)	84	190
Dispersion effect (erg cm^6)	93	47

[2] Lagowski, J. J., *Synth. React. Inorg. Met.-Org. Chem.* 2007**,** **37,** 115-153**.**

Because of the low equilibrium constant, ammonia is also a thousand times less conductive than water. Ammonia also forms weaker and fewer hydrogen bonds than water, simply because nitrogen is less electronegative than oxygen, and has only one lone pair.

3. Experimental conditions

Unless otherwise noted, all chemicals were purchased from commercial sources and used as received. The ammonia bottle (99.98 wt% purity) was purchased from Linde Benelux. 250mg of lignin was transferred in a 50 mL stainless steel in-house-built autoclave (*vide infra*). The autoclave was flushed 3 times with argon to remove moisture and then 30 mL of liquid ammonia is added aat room temperature (experiment d1). The reaction mixture was then stirred magnetically for 24 h.The autogeneous ammonia pressure was 7-11 bars depending on the actual room temperature. At the end of the reaction, the autoclave was depressurized and hence the ammonia shifted to gas phase. In order to fully remove the ammonia residues, the autoclave was flushed for 20 min. with low-pressure nitrogen. This allowed the deposition of the low molecular weight products and the residual lignin. The solid residue was extracted with 50 mL dichloromethane (DCM), filtered, and the solvent was removed subsequently by rotavaporation. The extract residue was dried overnight under vacuum (< 100 mbars) at 40 °C and analysed by SEC and ICP (*vide infra)*. Identically to the ammonia treated lignins, 250 mg of P1000 soda lignin was extracted by 50 mL of DCM overnight (experiment b), together with a second DCM extraction of the residue with the same dry lignin:DCM ratio (experiment c).

At higher temperature and pressure, the same procedure was used as for experiments d1. However, after charging the ammonia and before heating, 45 bars of argon (experiment d2) or hydrogen (experiment d3) were charged in the reactor. Then the autoclave was heated to 85°C, the point at which a total pressure of 90 bars was reached as the sum of the partial ammonia and argon/hydrogen pressures. The reaction mixture was then stirred for 3h. At the end of the reaction, the autoclave was cooled with ice until 25 °C (52 bars) and then depressurized. Once a pressure of 7-11 bars was reached, the ammonia shifted to the gas phase. In order to fully remove ammonia, the autoclave was flushed for 20 min. with low pressure nitrogen. The solid residue was further worked up identically experiment d1.

Finally, we ran additional experiments with liquid ammonia upon heating, without previous addition of H_2 or Ar. During the course of these experiments, the volume of the liquid ammonia was not constant. When 30 mL liquid ammonia was heated, first the volume was reduced to ± 20 mL. After some time, an equilibrium between ammonia evaporation and condensation was reached, and from this moment the volume rose again due to the thermal expansion coefficient. This is the variation of the density in function of the temperature, which varies a lot in the case of liquid ammonia (Table S1). The original volume (30 mL) was reached at 120 °C.

4. In-house-built stainless steel autoclave setup for reactions in liquid ammonia

This autoclave was tested at 150 bars. It had two glass windows. The sealing was done by O-rings as follows: Teflex O-rings (Eriks) for the sealing of the autoclave and the windows. Autoclave valves: O-ring in Kalrez and seat in Teflon. Safety valve: O-ring in Kalrez and connected with a long ¼ tube (to cool down the gas if necessary). The in-flow valves and the out-flow valves are connected to the main head of the autoclave with copper O-rings. Besides the two valves on the autoclave on each site we will place a two-way ball valve. The gas bottle was connected by a DIN-6 gland and nut. No pressure reducer was used, but a deep tube was used to obtain at once liquid ammonia.

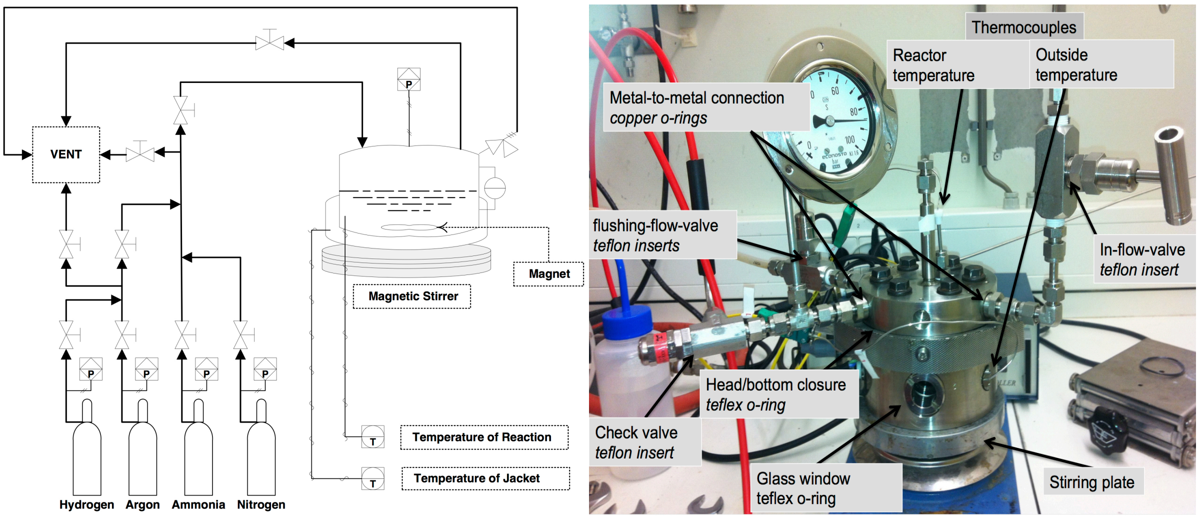

Fig. S1: Schematic and photo of the reactor set-up for working with liquid ammonia.

5. Native lignin solubilization experiments in liquid ammonia

125 mg of MWL from elephant grass was solubilized in 30 mL liquid ammonia at room temperature and autogeneous pressure (Fig S2a). This native grass lignin was solubilized instantaneously, but not completely (as seen from the presence of some residual lignin particles left in suspension). When the solution was heated, without the addition of H_2 or Ar, the colour changed from orange to green (Fig S2b). After heating at 120 °C and 88 bars (only NH_3) during 3 hours, the reactor was cooled with ice. During the cooling the colour of the solution changed again to its original colour (or similar). At this point the native lignin was completely solubilized (Fig S2c).

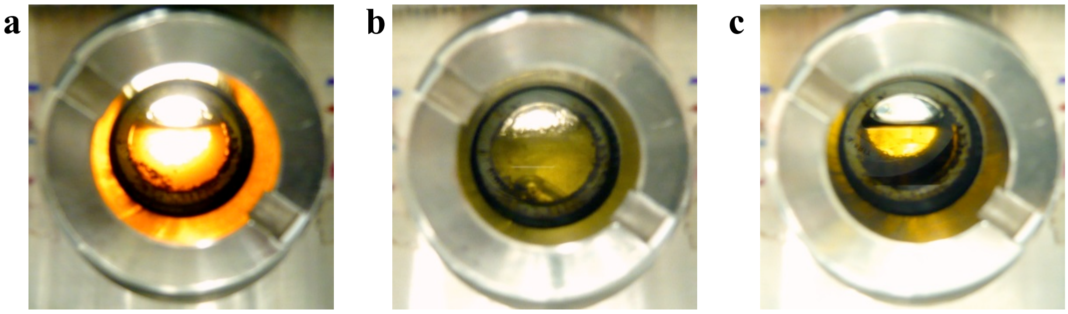

Fig. S2: Image of the reaction autoclave window (the light is shining through another window at the back of the autoclave), showing 125 mg native lignin (MWL) from elephant grass solubilized in 30 mL liquid ammonia at (**a**) room temperature and 7-11 bars ammonia pressure; (**b**) at 100°C and 60 bars ammonia pressure ; (**c**) at room temperature and 7-11 bars ammonia pressure after 3 hours heating at 120°C.

6. GC-MS analysis

After solubilisation of the dry extracts in DCM, monomeric and dimeric aromatic products were identified and quantified by GC-MS and GC-FID respectively (both Interscience). For the GC-MS analysis, a Restek RXI-5 MS column (30 m × 0.25 mm × 0.25 µm) was used with He as carrier gas with a temperature program starting for 2 min at 50 °C and a heating rate of 10 °C/min to 350 °C followed by an isothermal step of 3 min. Identification of the compounds was performed by comparison of MS data to the NIST library. The relative area of the peak of individual identified compounds in the extracts was calculated using Shimadzu software on the basis of GC-FID data. The peak areas were normalized to 100% and calculated as relative percentages.

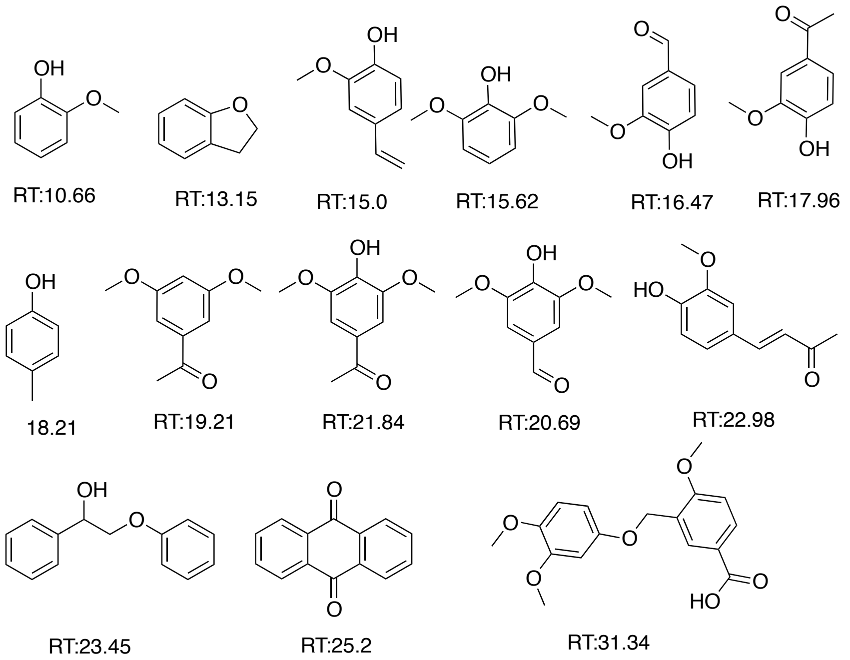

Fig. S3: Main compounds in the lignin depolymerisation extracts identified by GC/MS.

7. ICP analysis

Table S3: ICP measurements of the extracted low-molecular-weight fractions.

Entry	Sample	Solubilization/ extraction sequence	t (h)	p (bars)	T (°C)	Gas	DCM extractives wt% C	wt% H	wt% N	wt% O
1	b	DCM	-	-	-	-	70.5	8.4	0.2	20.7
2	d1	NH_3/DCM [1]	24	7	18	NH_3(l)	65.4	10.0	0.5	22.8
3	d2	NH_3/DCM [2]	3	90	85	Ar	63.3	9.0	0.4	24.1
4	d3	NH_3/DCM [3]	3	90	85	H_2	71.4	8.6	0.8	18.9
5	e1	DCM/NH_3/DCM [1]	24	7	18	NH_3(l)	74.6	10.1	1.0	13.5
6	e2	DCM/NH_3/DCM [3]	3	90	85	H_2	66.7	10.9	0.8	21.1
7	f	NH_3/DCM/NH_3/DCM [1]	24	7	18	NH_3(l)	65.2	9.6	0.9	22.1

[1] 7 bars autogenous NH_3 pressure,18 °C.
[2] 90 bars total pressure of Ar and NH_3, 85 °C .
[3] 90 bars total pressure of H_2 and NH_3, 85 °C.

8. NMR analysis

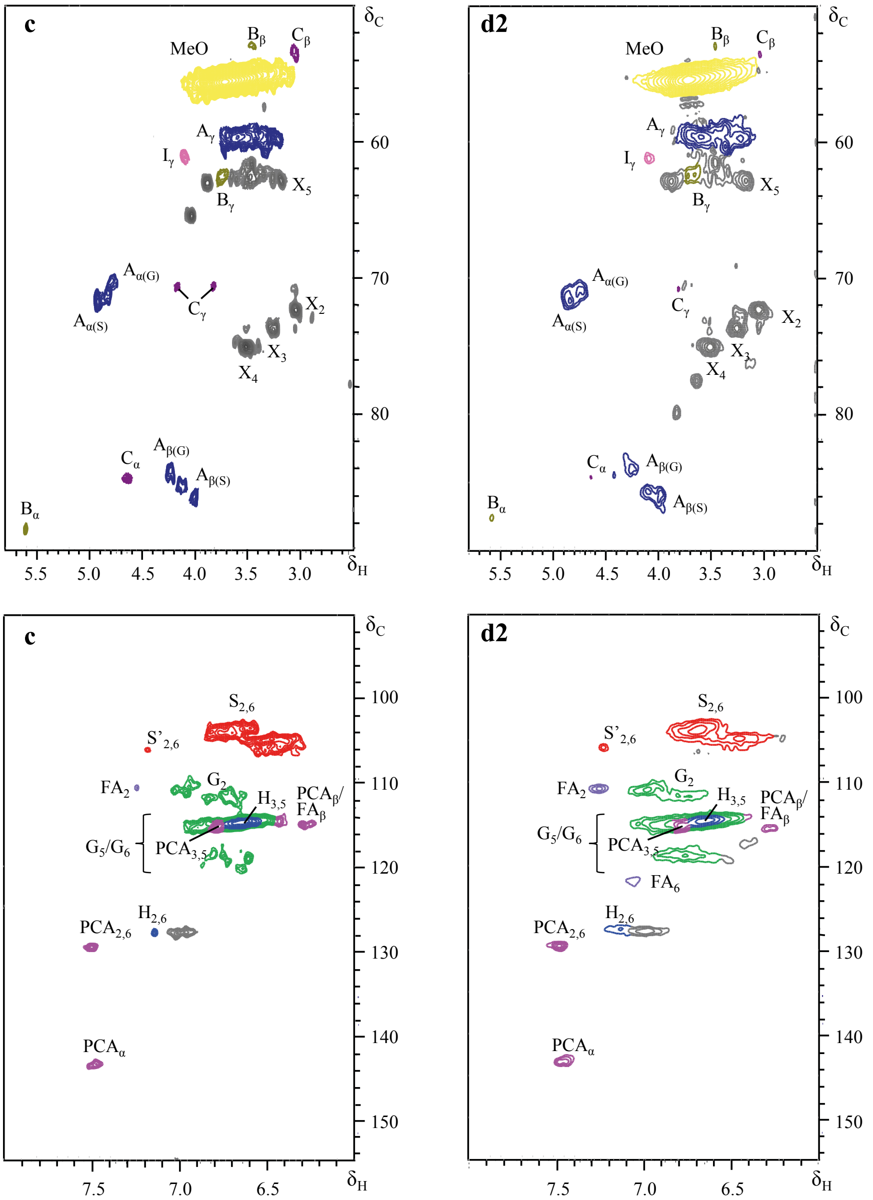

Fig. S4: Side chain (δ_C/δ_H 50–90/2.5–5.8) and aromatic/unsaturated (δ_C/δ_H 90–155/5.5–8.0) regions in the 2D HSQC NMR spectra of P1000 soda lignin after (**c**) DCM/DCM extraction and after (**d2**) extraction sequence NH_3/DCM (at 90 bars total pressure of NH_3 and argon and at 85 °C).

9. Model compound experiments

For the synthesis of both model compounds, first 4-benzyloxy-3-methoxy-2-(2-methoxyphenoxy)acetophenone was synthesised as an intermediate (Fig. S4).

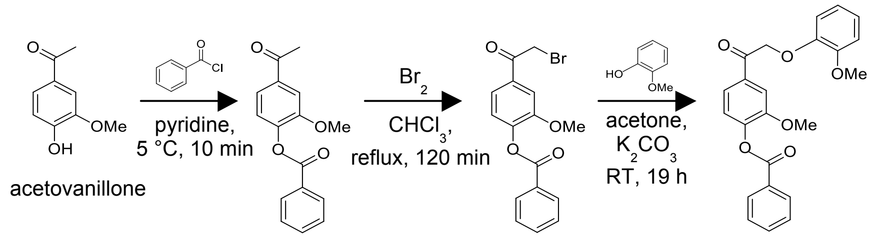

Fig. S5: Scheme of intermediate (4-benzyloxy-3-methoxy-2-(2-methoxyphenoxy)acetophenone) synthesis, based on the synthesis route from (E. Adler and E. Eriksoo, 1955, *Acta. Chem. Scand.*, **9 (2)**, 341-342).

9.1. Guaiacylglycerol-β-guaiacyl ether, GGGE

In a 250 mL three necked round bottom flask, equipped with a reflux condenser, 12.8 mmole of the intermediate (4-benzyloxy-3-methoxy-2-(2-methoxyphenoxy)acetophenone) is dissolved in dimethylsulfoxide (DMSO, 60 mL). To the resulting red solution aqueous formaldehyde (56.9 mmole) and K_2CO_3 (12.8 mmole) were added. After 10 min the reaction mixture was poured into demineralized water (100 mL) and acidified with a 5% HCl solution until pH was 6, and extracted with ethyl acetate (EtOAc, 2 × 50 mL). The combined organic layers were washed with demineralized water (2 × 50 mL) and brine (50 mL), dried with $MgSO_4$ and concentrated on the rotary evaporator. The residue was a red viscous oil and was dissolved in 75 mL tetrahydrofurane (THF). Then, in a 500 mL three necked round bottom flask under N_2 atmosphere equipped with a reflux condenser, inlet, dropping funnel and a thermometer, $LiAlH_4$ (79.0 mmole) was placed and cooled to 0 ºC, followed by the addition of 100 mL of THF, and then the hydroxymethylated intermediate in 75 mL THF. The reaction mixture is allowed to rise to room temperature. After 21 h a saturated Na_2SO_4 solution was added until no temperature increase was observed. The inorganic salts were filtered off and the filtrate was acidified with an NH_4Cl solution until pH 6. The organic layer was separated and the water layer is extracted with EtOAc (3×50 mL). The combined organic layers were washed with demi-water (2×50 mL) and brine (50 mL), dried with $MgSO_4$ and concentrated on the rotary evaporator, obtaining a red viscous oil. The residue was purified by column chromatography (250 g silica, eluent: EtOAc:hexane 3:7 – 1:1). After concentration a yellow viscous oil was obtained with 68% yield (8.7 mmole) with a purity of 95% (determined by ^{1}H NMR). The substance solidified after a few days in the freezer.

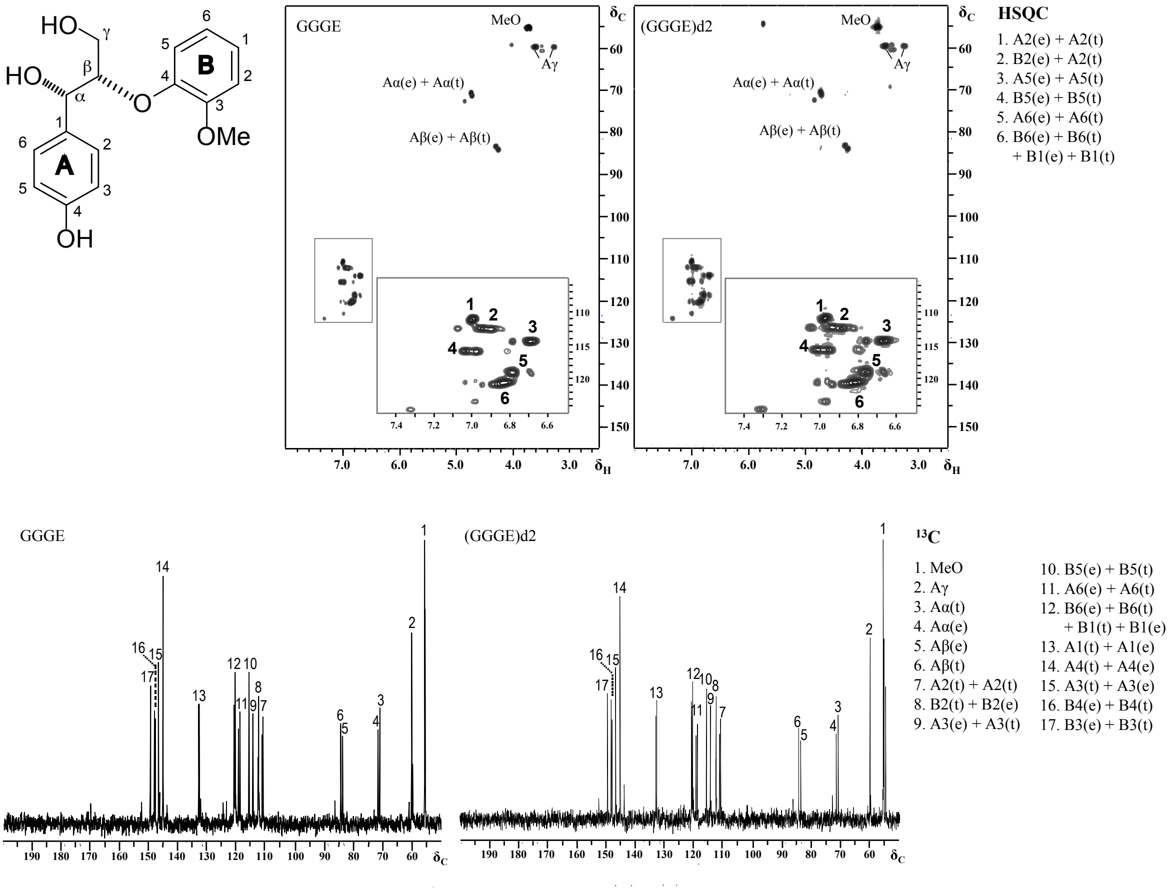

Fig. S6: HSQC spectra (δ_C/δ_H 50−155/2.5−8.0) and ^{13}C spectra of the synthesised model compound guaiacylglycerol-β-guaiacyl ether (GGGE) as a mixture of *threo* and *eryhtro* diastereomers, and after after the NH_3/DCM treatment at 85 °C and 90 bars (NH_3 + Ar) (identical to the conditions of experiment d2 with the P1000 soda lignin).

9.2. 2-(2-methoxyphenoxy)acetoguaiacone (MPAG)

In a 500 mL three necked round bottom flask, equipped with a reflux condenser and a thermometer, 37.9 mmole of the intermediate (4-benzyloxy-3-methoxy-2-(2-methoxyphenoxy)acetophenone) was suspended in 200 mL ethanol. The suspension was heated to 50 ºC and 60 mL of 1 N NaOH was added. Next, the reaction mixture was heated to reflux. After 40 min, the mixture was cooled to room temperature, and poured into 200 mL demineralized water and acidified with a 5% HCl solution until pH 6. The water layer was extracted with a EtOAc (2×100 mL and 1×80 mL). The combined organic layers were washed with demineralized water (80 mL) and brine (2×80 mL), and concentrated on the rotary evaporator. The dark brown viscous residue was purified by column chromatography (50 g silica and eluent: EtOAc:hexane 1:1). The fractions were treated with activated 0.8 g carbon at room temperature, and after filtering over Celite the colour was still light yellow. After concentration on the rotary evaporator a yellow viscous oil was obtained with 83% yield and 73% purity, which partly solidified after a few days in yellow cristals (melting point 67-69 ºC) with 49% yield and 94% purity (determined by ^{1}H NMR).

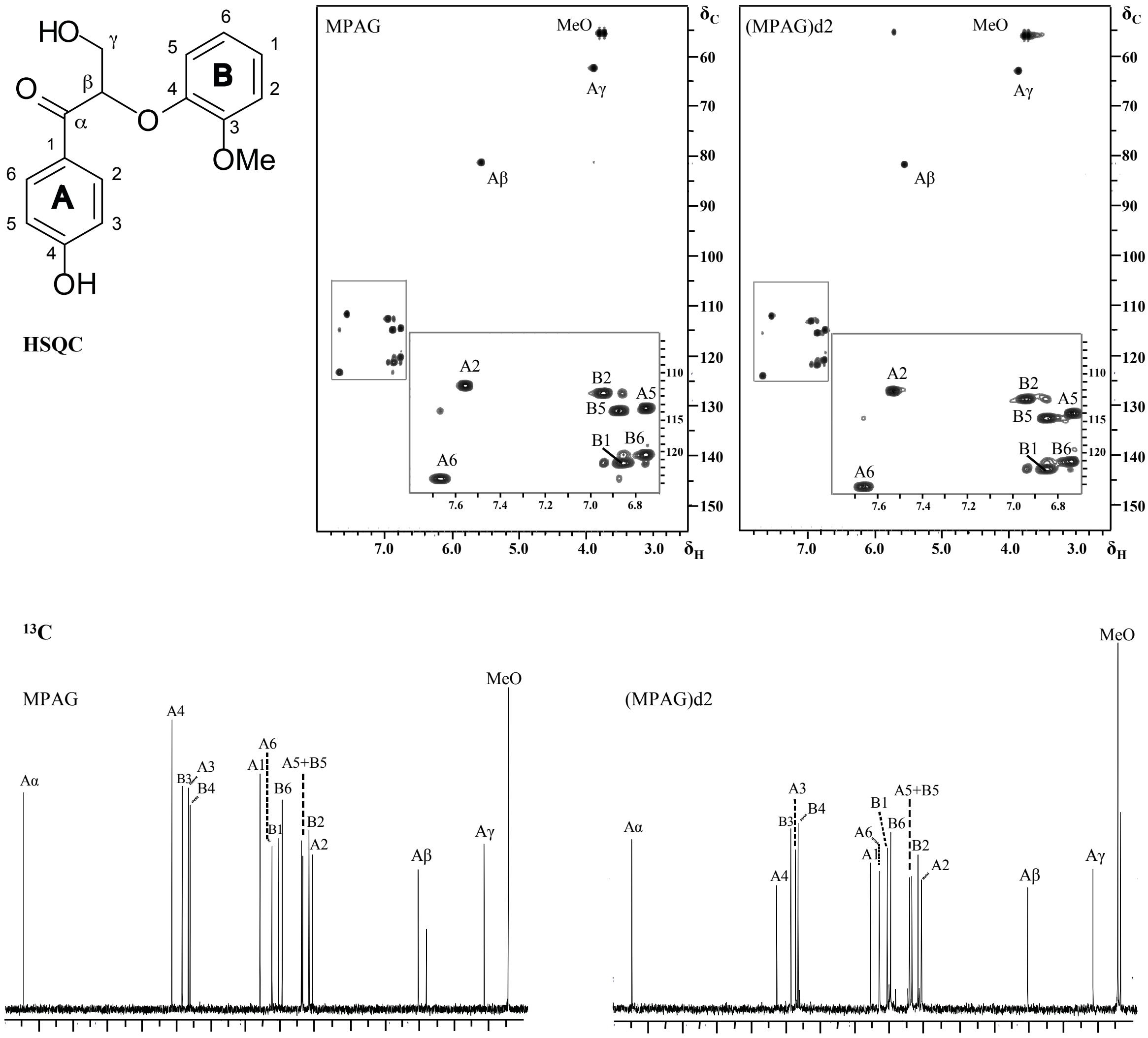

Fig. S7: HSQC spectra (δ_C/δ_H 50−155/2.5−8.0) and ^{13}C spectra of the synthesised model compound 2-(2-methoxyphenoxy)acetoguaiacone (MPAG), and after after the NH_3/DCM treatment at 85 °C and 90 bars (NH_3 + Ar) (identical to the conditions of experiment d2 with the P1000 soda).

10. ^{31}P-NMR

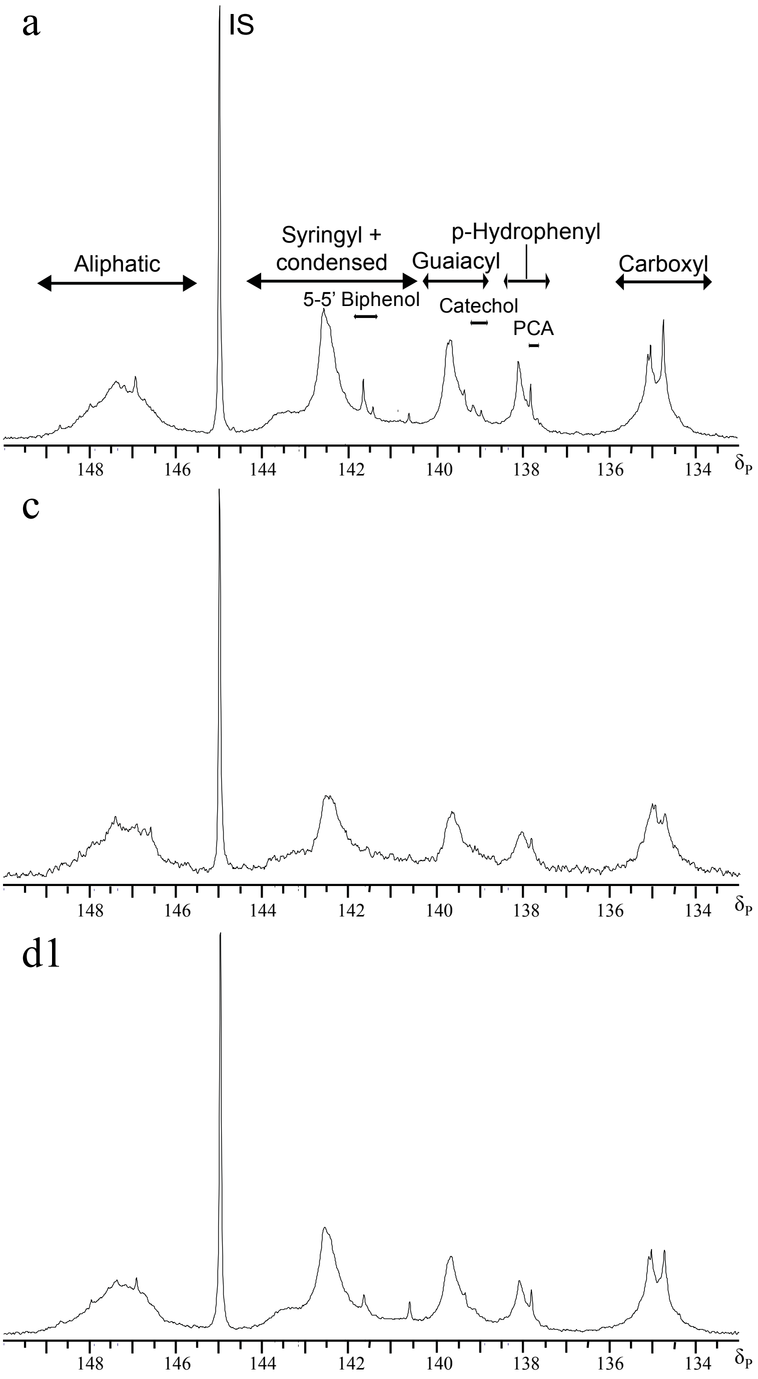

Fig. S8: ^{31}P-NMR spectra (δ_P 150−133 ppm) of the (**a**) P1000 soda lignin(**c**) DCM/DCM extracted lignin and (**d1**) the lignin after the NH_3/DCM treatment at 18 °C and autogeneous NH_3 pressure (7-11 bars).