

Supporting Information

Lignin Depolymerisation and Lignocellulose Fractionation by Solvated Electrons in Liquid Ammonia

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Materials and instrumentation

Reactants

Dichloromethane (DCM): (stab./amylene) AR, GC (on anhydrous basis min. 99.9%, Biosolve B.V.).

Ethanol: Absolute 99.8% (Nedcalco B.V.).

Ethyl acetate (EA): 0.05% H₂O, GC (on anhydrous basis): 99.8% (Biosolve B.V.).

Isopropanol: Reag. Ph. Eur. (99.5%) (Acros Organics™).

Liquid anhydrous ammonia (LAA): 5 kg in 10 L deep tube liquid/gas bottle, UN 1005 (GHC Gerling, Holz& Co Handel GmbH).

Phenol red: pH range 6.8-8.4 (TCI Europe N.V.).

Sodium (Na(s)): lumps in kerosene, > 99.8% (on sodium basis) (Sigma Aldrich B.V.).

All other reactants, solvents and pure compounds were purchased from Sigma Aldrich B.V.

Protobind™ 1000 lignin fractionation

Protobind™ 1000 lignin, a commercial soda lignin from mixed wheat straw and Sarkanda grass, was fractionated by 3 consecutive extraction steps with DCM (Figure S1). The HMW – and LMW fractions represented 76 and 23 wt% of the initial lignin, which were the weights collected of the combined fractions obtained after 3 filtration steps on paper and washing with DCM, removal of DCM by rotavaporation at 400 mbars/40°C and drying at 70°C.

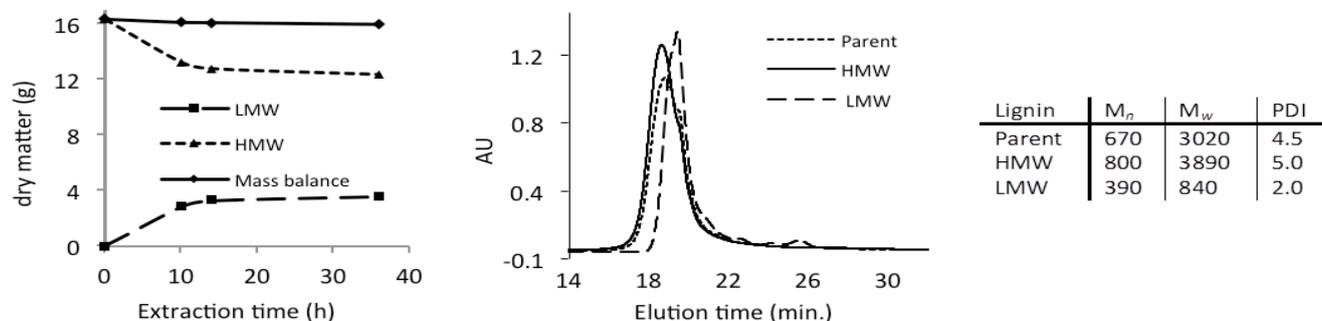


Figure S1. (Left) Fractionation of the parent lignin Protobind™ 1000 in a high – and low molecular weight fraction (HMW and LMW) by consecutive extraction with dichloromethane (DCM), (Middle) Chromatograms from SEC analysis of the isolated fractions and (Right) Corresponding molecular weights and polydispersities.

ICP

Around 10 mg of lignin was weighed in tin recipients and placed in an automatic analyser (vario EL cube, Elementar, Germany). The samples were analysed in duplicate and the mean value of the content of elements was calculated.

GC-MS

The GC-MS analyses were performed on a Agilent7890A (Agilent, Santa Clara, CA 95051, United States) coupled with an AccuTOF (JEOL, Tokyo, 196-8558, JAPAN) equipped with a HP-5MS capillary column (30m × 0.25mm i.d. and 0.1 μm film thickness; Agilent). Helium was used as carrier gas at a rate of 1 mL min⁻¹. The samples were injected with an auto-injector (Agilent 7693A) directly onto the column using septum-equipped programmable injector (SPI) system in split mode (15:1 ratio). The temperature of the injector during the injection was 230°C and the oven started at 50°C, held for 3 min, raised to

315°C at a rate of 15°C min⁻¹ and held for 5 min at 315°C. The temperature of the transfer line was set at 250 °C. The ionisation mode was EI (70 eV, 300 µA, 250 °C). The identification of the compounds was performed by comparison with pure standards, with MS data in the NIST library and with existing MS spectra.^[1] The response factors (RF) relative to a fixed concentration of anisole (IS) were determined by preparing dilution sets of phenol, ethylphenol, propylphenol, guaiacol, methylguaiacol, ethylguaiacol, vinylguaiacol, propylguaiacol, vanillyl alcohol, isoeugenol, vanillin, acetoguaiacone, guaiacylacetone, syringol, methylsyringol, syringaldehyde and acetosyringone in a concentration range from 0.02 to 0.90 mg/mL. The linear regression coefficients were high for phenols (r^2 0.991 - 0.995) and alkylphenols (r^2 0.990 - 0.998), but low for carbonyl containing phenols (r^2 0.974 - 0.988) and hydroxyl alkyl phenols (r^2 0.961 - 0.993). Although GC-FID is a much more appropriate analysis technique to quantify absolute amounts, the GC-MS calibration curves were still reasonable (Figure S2). The RF of compounds identified in the bio-oils that were not among the compounds described above, were calculated by the Effective Carbon Method (ECN)^[2] relative to a compound with an experimentally determined RF and with a similar structure in terms of methoxyl groups and side chain type, which is still more reliable than applying no response factor at all. For instance the RF of propylsyringol was calculated using the RF of methylsyringol, which was experimentally determined, as:

$$RF(\text{propylsyringol}) = RF(\text{methylsyringol}) \times ECN(\text{propylsyringol}) \div ECN(\text{methylsyringol}) = 0.8327 \times 8.25 \div 6.25$$

The monomer yields were expressed as carbon wt% on dry lignin, by calculating the wt% of carbon in all identified monomers and express it against the carbon wt% of the parent lignin as determined by ICP analysis (Table S1).

Direct Insertion Probe – Mass Spectrometry (DIP-MS)

The lignins and bio-oils were dissolved in 1,4 dioxane in the same concentration (\pm 10 mg/mL). Around 1 µL was transferred in a capillary quartz cup with some silica and left drying for 25 min. Then the cup was in placed in the probe, which was introduced in the ion source chamber at 200°C and 1.10⁻⁶ mbars. Then, the temperature of the probe was raised at 16 °C min⁻¹ until 490°C. The filament of the Electron Impact (EI) source was set at 70 eV, 300 µA, 200 °C. The MS analyser was identical to the one used for GC-MS analysis. MassCenter version 2.6.2b (JEOL, Japan) software was used to process the mass spectra.

Size Exclusion Chromatography (SEC)

Lignin samples of 1 mg ml⁻¹ dissolved in 0.5 M NaOH were injected into two serial connected columns (4.6 × 30 cm), each with a manually packed column with ethylene glycolmethacrylate copolymer TSK gel Toyopearl, HW-75F and HW-55F respectively, and eluted with the same solvent, with the following conditions: flow 1 ml min⁻¹, column temperature 25°C, and detection at 280 nm. The standards used for calibration of the molar mass distribution consisted of sodium polystyrene sulfonates (M_w range: 891 Da to 976 kDa) and phenol.

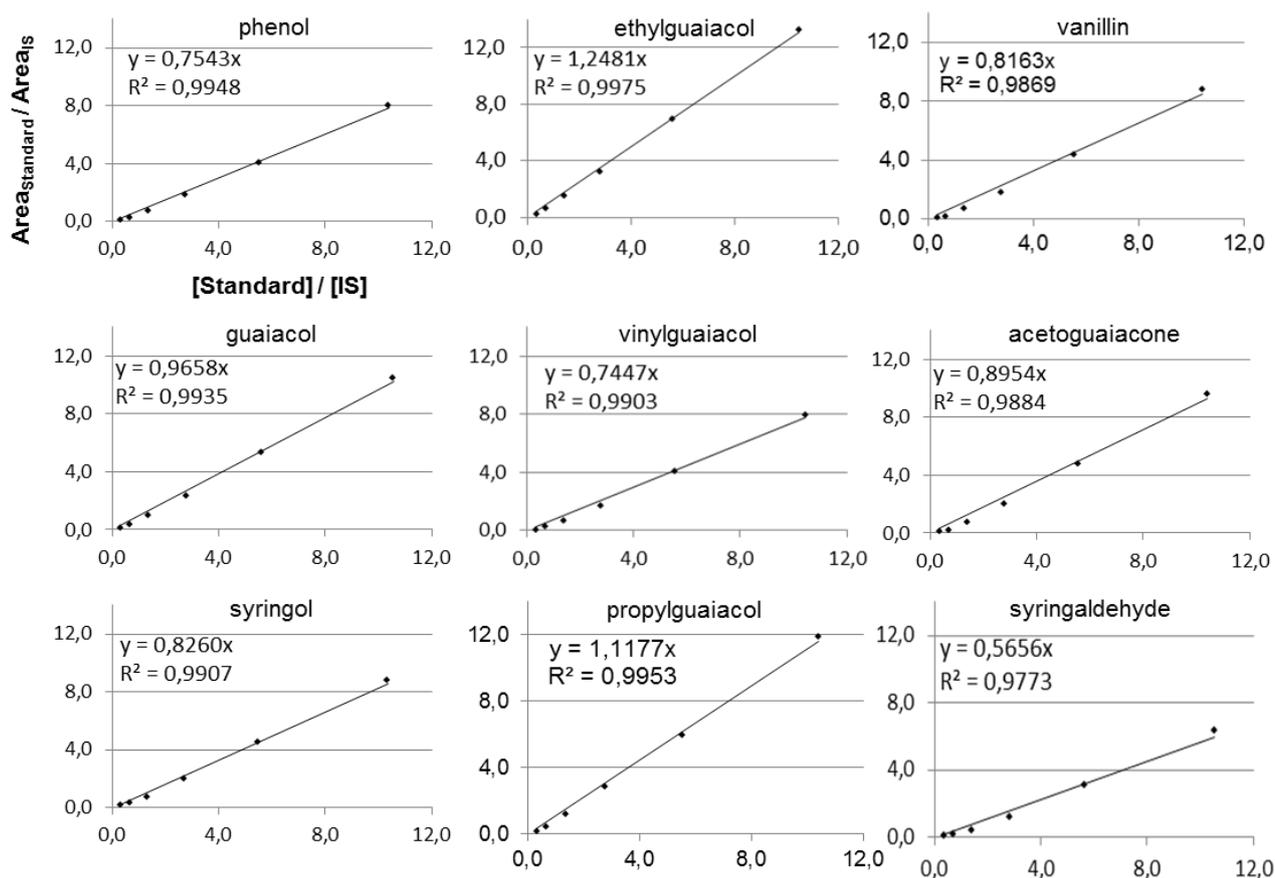


Figure S2. Calibration curves of dilution sets of phenolic monomers against a fixed concentration of anisole (IS) for determination of the relative response factors.

2D-NMR

Around 30 mg of sample was dissolved in 700 μL DMSO- d_6 . The NMR spectra were recorded at 25°C on a Bruker AVANCE II 500 MHz instrument equipped with a 5 mm BBI probe with z-gradient ($5 \text{ G cm}^{-1} \text{ A}^{-1}$). ^1H - ^{13}C Heteronuclear Single Quantum Coherence (HSQC) experiments used Bruker's "hsqcetgpsi2" pulse program with spectral widths of 5000 Hz (from 10 to 0 ppm) and 20843 Hz (from 165 to 0 ppm) for the ^1H - and ^{13}C dimensions. The number of collected complex points was 1024 for the ^1H -dimension with a recycle delay of 1.5 s. The number of transients was 32, and 256 time increments were always recorded in the ^{13}C dimension. The $^1J_{\text{CH}}$ used was 145 Hz. Processing used Gaussian and squared sine-bell apodization in both dimensions. Prior to Fourier transformation, the data matrixes were zero-filled up to 1024 points in the ^{13}C dimension. The central solvent peak was used as an internal reference (δ_{C} 39.5; δ_{H} 2.49). HSQC correlation peaks were assigned by comparing with the literature.^[3-6]

A semi-quantitative analysis of the volume integrals (uncorrected) of the HSQC correlation peaks was performed using Mnova 7 processing software. In the aliphatic oxygenated region, the relative abundances of side chains involved in the various inter-unit linkages were estimated from the $\text{C}_\alpha\text{-H}_\alpha$ correlations, except for substructures I and I', for which $\text{C}_\gamma\text{-H}_\gamma$ correlations had to be used. In the aromatic/unsaturated region, $\text{C}_6\text{-H}_6$ and/or $\text{C}_2\text{-H}_2$ correlations from H, G, and S lignin units and from *p*-coumarate (PCA) and ferulate (FA) were used to estimate their relative abundances. The PCA and FA integration volumes were not included for the calculation of the total aromatic units.

Set up for experiments with liquid ammonia

The set up used in all experiments is shown in Figure S3. In all experiments after the reaction time had elapsed and the reactor was cooled below 30°C, the stirring was stopped and valve 5 was opened gently and gradually to remove the liquid ammonia as a gas and trap it in a 750mL solution cooled with ice (strong exothermic reaction). The trap solution consisted of a 11% (m/v) HCl solution with 0.01% (m/m) phenol red as pH indicator. A second trap solution in series was installed to ensure that all the ammonia gas was trapped and neutralized completely. When all the ammonia was removed from the

autoclave, valve 4 was closed and valve 5 was opened. Valve 5 was then closed almost completely and valve 4 opened again gently. Valve 5 was then opened gradually until all ammonia present in the feeding line between valve 4 and the three-way valve 1-2-3 was removed. At the end of the ammonia gas removal, the colour of the trap solution started to change from dark red to yellow, and finally at a pH around 7.5 back to purple red (Figure S3). This way, the exact amount of HCl needed could be determined easily and the trap- and neutralization process could be followed up. Finally, an air or nitrogen flow was blown through the system during 10 min. before opening the autoclave.

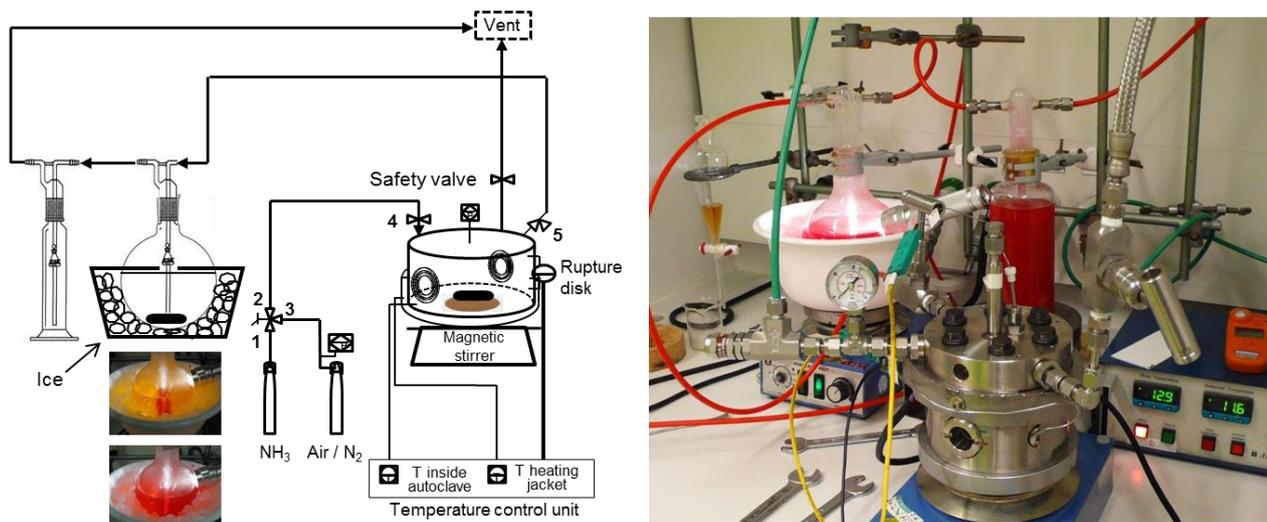


Figure S3. Scheme and image of the set-up used in the experiments with LAA.

Procedure for room-temperature depolymerisation of isolated lignins by metallic sodium in liquid ammonia.

In addition to the information in the article, a more detailed description of the experiments is given in this section. The THF was dried by a continuous distillation unit containing metallic sodium ($\text{Na}_{(s)}$) and benzophenone under an inert atmosphere. Pieces of $\text{Na}_{(s)}$ were transferred to petroleum ether, dried with paper and weighed off. To activate the $\text{Na}_{(s)}$ and facilitate the subsequent dissolution step, it was kept submerged in ethanol until a shinier surface was obtained (± 0.5 min.), cut into smaller pieces around 50 mg and dried on paper before adding to the autoclave. The autoclave was closed and purged 5 times with air at 2 bars, followed by the addition of liquid ammonia until reaching a total reaction volume of 30 mL. The solution was stirred gently, giving rise to a biphasic system with solvated electrons in the upper part and lignin at the bottom part (Figure S4a). The mixture was stirred gently until a homogenous black suspension was obtained (Figure S4b), and then stirred at 500 rpm. Independent from the amount of lignin and purging by air or nitrogen, the blue colour of solvated electrons disappeared after 2 h and a creamy light brown colour was observed (Figure S4c). After removing the ammonia, the autoclave was opened (Figure S4d) and 0.5 mL isopropanol was added gently to quench residual $\text{Na}_{(s)}$ and NaNH_2 . Then 60 mL water was added (Figure S4e). The dissolution was transferred to a beaker and 20 mL water was added extra to recover all residual matter from the autoclave. The pH of the aqueous phase was adjusted dropwise with 6M HCl from 12.0-12.5 to 2.0. The suspension was transferred quantitatively to a 250 mL centrifuge tube and stored at 4°C during 1 h. The precipitated lignin (Figure S4f) was recovered by centrifugation (9500 rpm, 40 min., 4°C) and dried to constant weight at 70°C. The aqueous supernatant was extracted with 3x30 mL EA. The extracts were combined, dried over MgSO_4 and rotavaporated at 40°C/210 mbars. Finally the extract was dried under a nitrogen current until reaching constant weight (EA1). The residual aqueous phase after this extraction was concentrated at 50°C/80 mbars until reaching a volume of ± 20 mL (pH ca. 0.9), which was further extracted with 2x15 mL EA (Figure S4g). The extract was dried identically (EA2). Finally, the combined extracts EA1 and EA2 were re-dissolved in 4 mL EA. Please note that the final dry extract was not re-dissolved completely (Figure S4h). Therefore, the suspension was mixed and filtered over a 0.2 μm polyvinylidene difluoride (PVDF) syringe filter (Figure S4i). The insoluble - and soluble fractions were determined gravimetrically after drying at 70°C and after drying with nitrogen gas, respectively. The insoluble fraction was lower than 10% in all cases (results not shown). The final soluble extract was then re-dissolved in 4 mL EA, from which a 250 μL aliquot was taken for GC-MS analysis, and added to 700 μL EA together with 50 μL internal standard (IS, 0.076 mg/mL anisole).

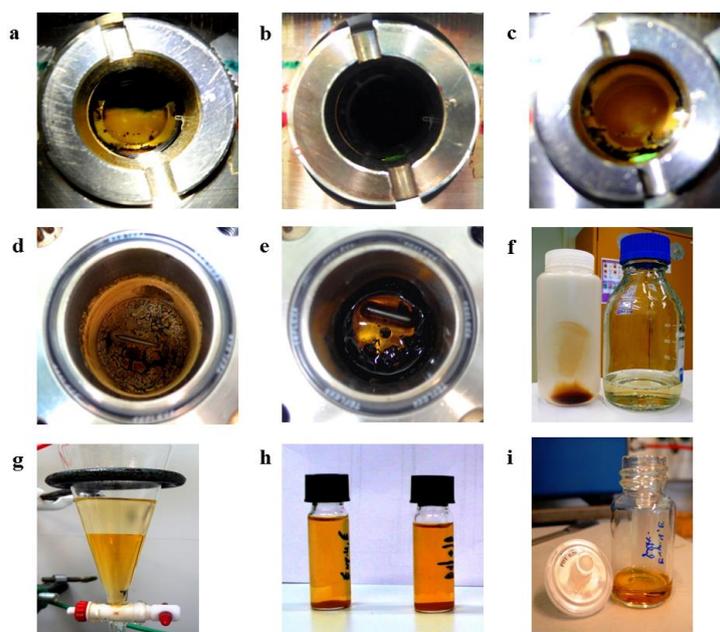


Figure S4. Images of (a) Blue colour revealing in the presence of solvated electrons in the upper LAA phase and lignin and white Na_2O solids in the bottom THF phase, (b) Black oil-like colour after mixing both phases observed in (a), (c) Reaction medium after 2.5 h revealing the absence of solvated electrons, (d) Residue after ammonia removal, (e) Dissolution in water of the dry residue after ammonia removal, (f) Separation of residual lignin from the aqueous phase at pH 2.0, (g) Second extraction step of the low molecular weight fractions from the aqueous phase with ethyl acetate (EA), (h) Isolated bio-oils re-dissolved in EA and (i) Separation of the EA soluble and insoluble bio-oil fractions.

In some cases the mass balance of the described experiments exceeded 100% (Table 3). This can occur when isopropanol (added after removing the ammonia) reacted with the organic sodium salt residue. In fact, two significant isopropanol derived compounds were present in the chromatograms (eluting before the IS). Note that they are not included in the monomer yield. Secondly, the AS yield was calculated correcting only for the amount of $\text{NaCl}_{(s)}$ as the by-product, but not the excess chlorides added (HCl). Mass balances smaller than 100% can be caused by loss of volatiles during vaporation at reduced pressure and drying, or by volatile solvent residues present in the sodium used in the experiments (diethyl ether and ethanol).

Elemental analysis

Table S1 shows the elemental composition of the parent lignins and from the residual lignins isolated from Indulin AT at high temperature (Table 1, entry 3) and with $\text{Na}_{(s)}$ at room temperature (Table 3, entry 1).

Table S1. Elemental analysis (as wt% on dry solids) of the parent lignins and residual lignin (RL) isolated from Indulin AT after 3 h in 30 mL liquid ammonia at 120 °C and after 6 h in 20mL liquid ammonia + 10mL THF with 75 wt% $\text{Na}_{(s)}$ at RT. The oxygen content was calculated as the difference of 100% with the C, H, N and S content.

Lignin	C	H	N	S	O
Indulin AT	62.6 ± 0.0	5.2 ± 0.0	0.9 ± 0.0	2.0 ± 0.0	29.3 ± 0.0
Indulin AT, RL, 3 h at 120°C	57.6 ± 0.0	5.8 ± 0.0	2.4 ± 0.0	1.8 ± 0.0	32.5 ± 0.0
Indulin AT, RL, 6 h at RT, 75% $\text{Na}_{(s)}$	57.1 ± 0.0	5.1 ± 0.0	0.4 ± 0.0	1.0 ± 0.0	36.4 ± 0.0
Protobind™ 1000 HMW	61.0 ± 0.1	6.0 ± 0.1	0.9 ± 0.0	1.1 ± 0.0	31.0 ± 0.2
Protobind™ 1000 LMW	66.0 ± 0.3	7.6 ± 0.1	0.2 ± 0.0	0.7 ± 0.0	25.6 ± 0.4
Wheat straw organosolv	63.4 ± 0.4	6.5 ± 0.1	0.9 ± 0.0	0.2 ± 0.0	28.8 ± 0.5
Poplar organosolv	65.0 ± 0.4	6.0 ± 0.6	0.2 ± 0.0	0.4 ± 0.2	28.4 ± 0.8
EG MWL	61.1 ± 0.3	6.4 ± 0.0	0.5 ± 0.0	0.0 ± 0.0	32.0 ± 0.3

High-temperature lignin depolymerization in liquid ammonia

Figure S5 shows the chromatograms of the experiments (Table 1, entry 1-5) with the corresponding numbers of identified lignin derived monomers, which are shown in Figure S6.

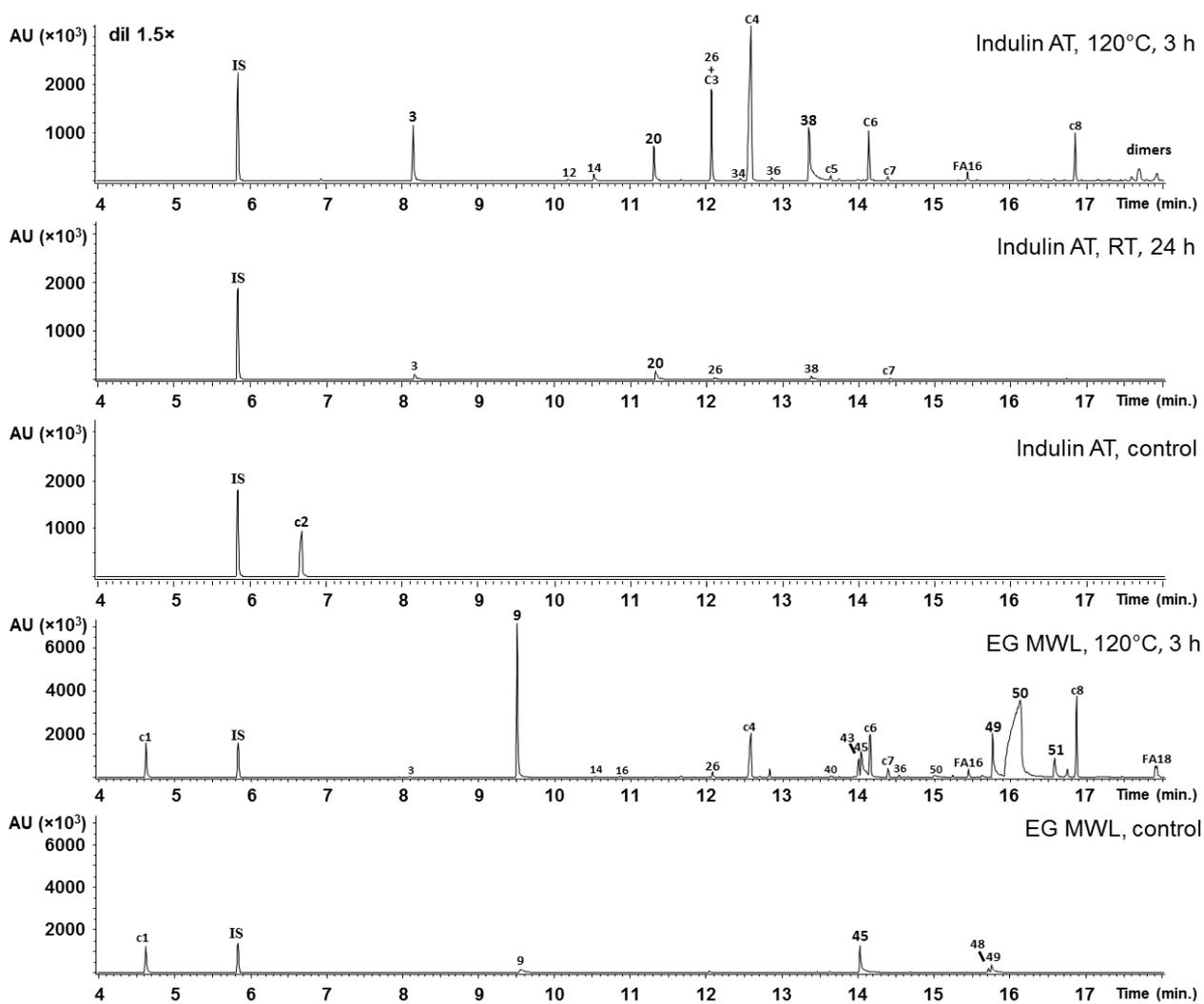


Figure S5. Chromatograms of the bio-oils isolated with DCM from Indulin AT after 3 h treatment in 30mL liquid ammonia at 120°C, after 24 h at RT and in the control experiment (DCM extraction only), and after 3 h at 120°C from EG MWL and in the control experiment. FA16 and FA18 are decanoic and octa(di)deca(e)noic acid respectively. Compounds c are contaminants, e.g. pentadecane (c3), dodecanoic acid (c4), heptadecane (c5), dodecanamide (c6) and hexadecanamide (c8). Mass spectrum based deconvolution was used to estimate the content of compound 26 (partial overlap with c3).

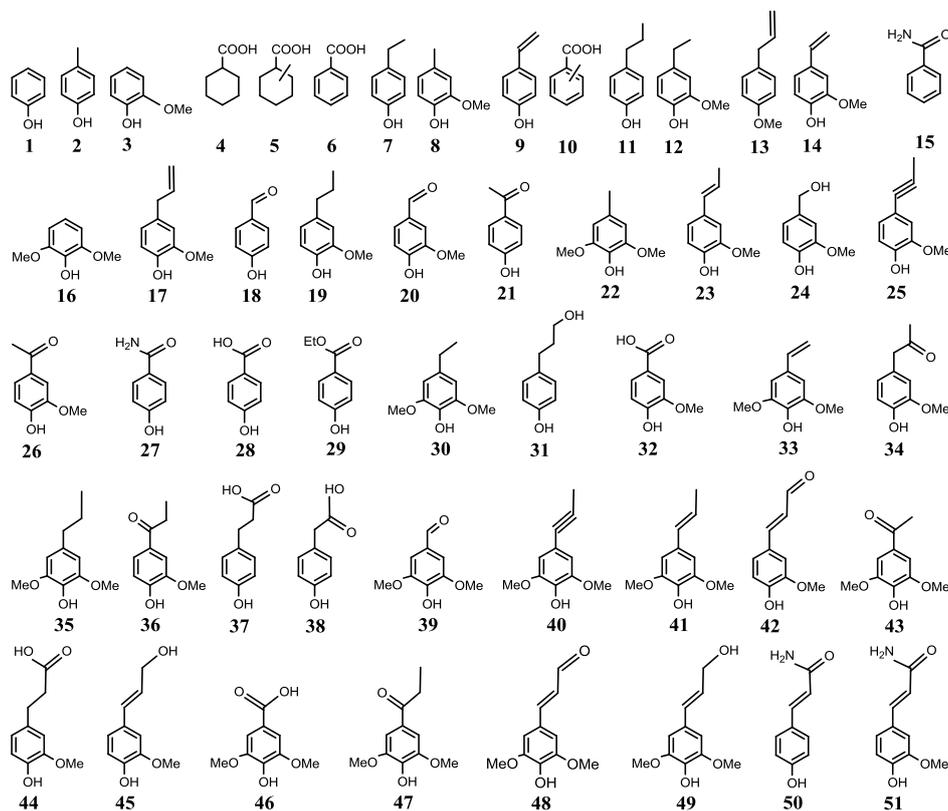


Figure S6. Lignin derived monomers identified in the bio-oils isolated from Indulin AT, Protobind™ 1000 HMW, wheat straw - and poplar organosolv lignin, EG MWL and poplar wood fibre. The compound numbers correspond to the ones used in Figures S5, S7a, S7b, S7c and S7d. The identification of compounds 25 and 40 are tentative, and based on the fact that they also are produced upon depolymerisation by pyrolysis.³

Depolymerization of isolated lignins and lignins *in situ* by metallic sodium in liquid ammonia at room temperature

Figure S7a shows the chromatograms of the bio-oils isolated from Protobind™ 1000 HMW with 75% and with 0% Na_(s) (Table 3, entry 3-4). Figure S7b shows the chromatograms of the bio-oils isolated from Indulin AT (entry 1-2) and EG MWL (entry 9-10). Figure S7c shows the chromatograms of the bio-oils isolated from wheat straw organosolv lignin (entry 5-6). Figure S7d shows the chromatogram of the bio-oils isolated from poplar organosolv lignin (entry 7-8), together with the ones from the bio-oil isolated from the lignin *in situ* (poplar wood fibre) (Table 5). Figure S8a, b and c show the SEC chromatograms of the parent lignins, RL and bio-oils isolated from wheat straw organosolv lignin, poplar organosolv lignin and EG MWL, respectively. Figure S8d shows the SEC chromatograms of the lignin-rich fraction (L) and bio-oil isolated in the experiments with lignin *in situ* (poplar fibre). Figure S9 shows the DIP chromatograms of the same samples used for SEC analysis and the corresponding mean EI-MS spectra of selected time intervals.

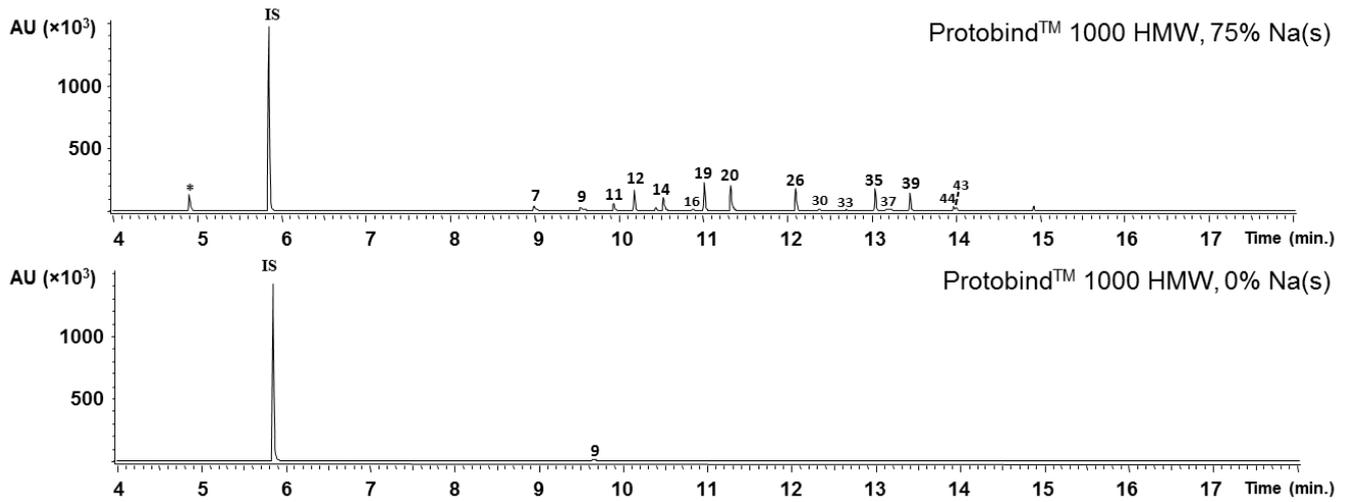


Figure S7a. Chromatograms of the bio-oil isolated from Protobind™ 1000 HMW after 6 h with 75% Na_(s) in 20mL liquid ammonia + 10mL THF at RT, and of the bio-oil isolated in the control experiment (0% Na_(s)).

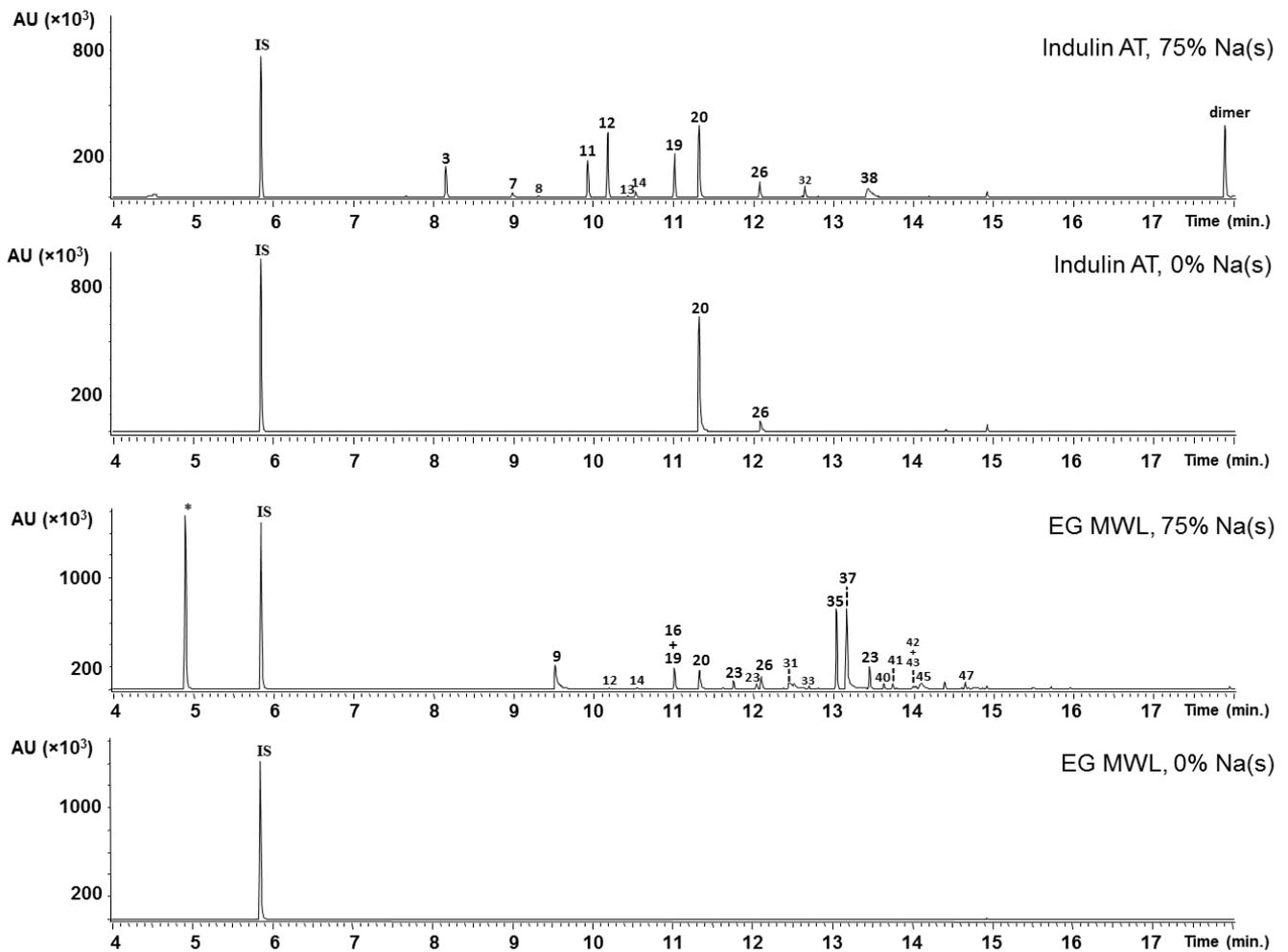


Figure S7b. Chromatograms of the bio-oils isolated from Indulin AT and EG MWL after 6 h with 75% Na_(s) (wt% on dry lignin) in 20mL LAA+ 10mL THF at RT, and of the bio-oils isolated in the control experiment (0% Na_(s)).

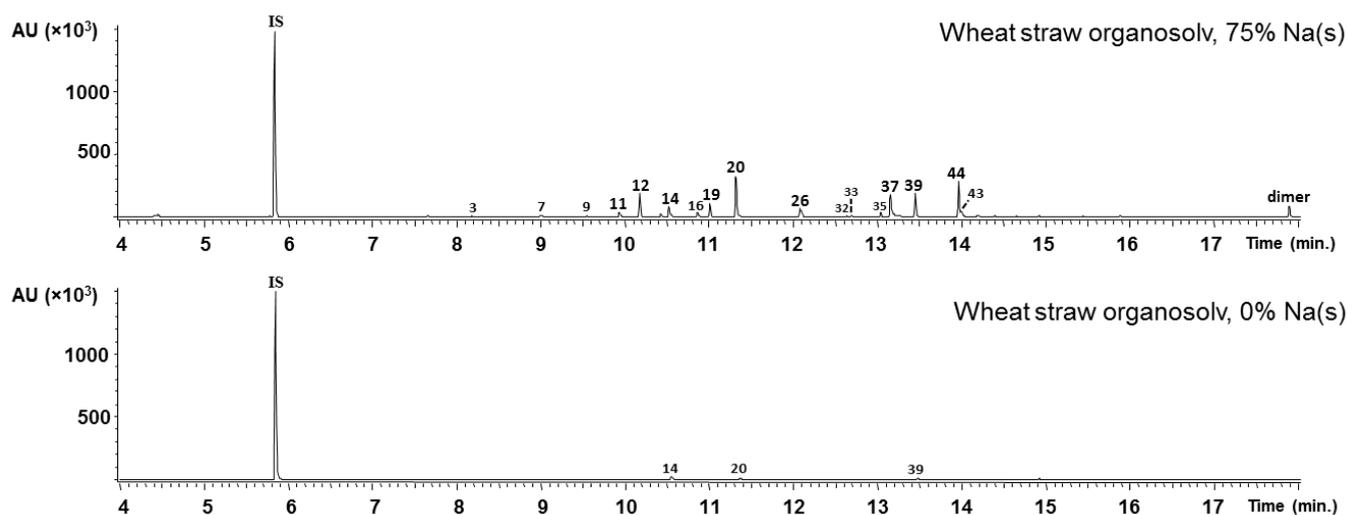


Figure S7c. Chromatograms of the bio-oils isolated from wheat straw organosolv lignin after 6 h with 75% and 0% Na(s) at 20 °C.

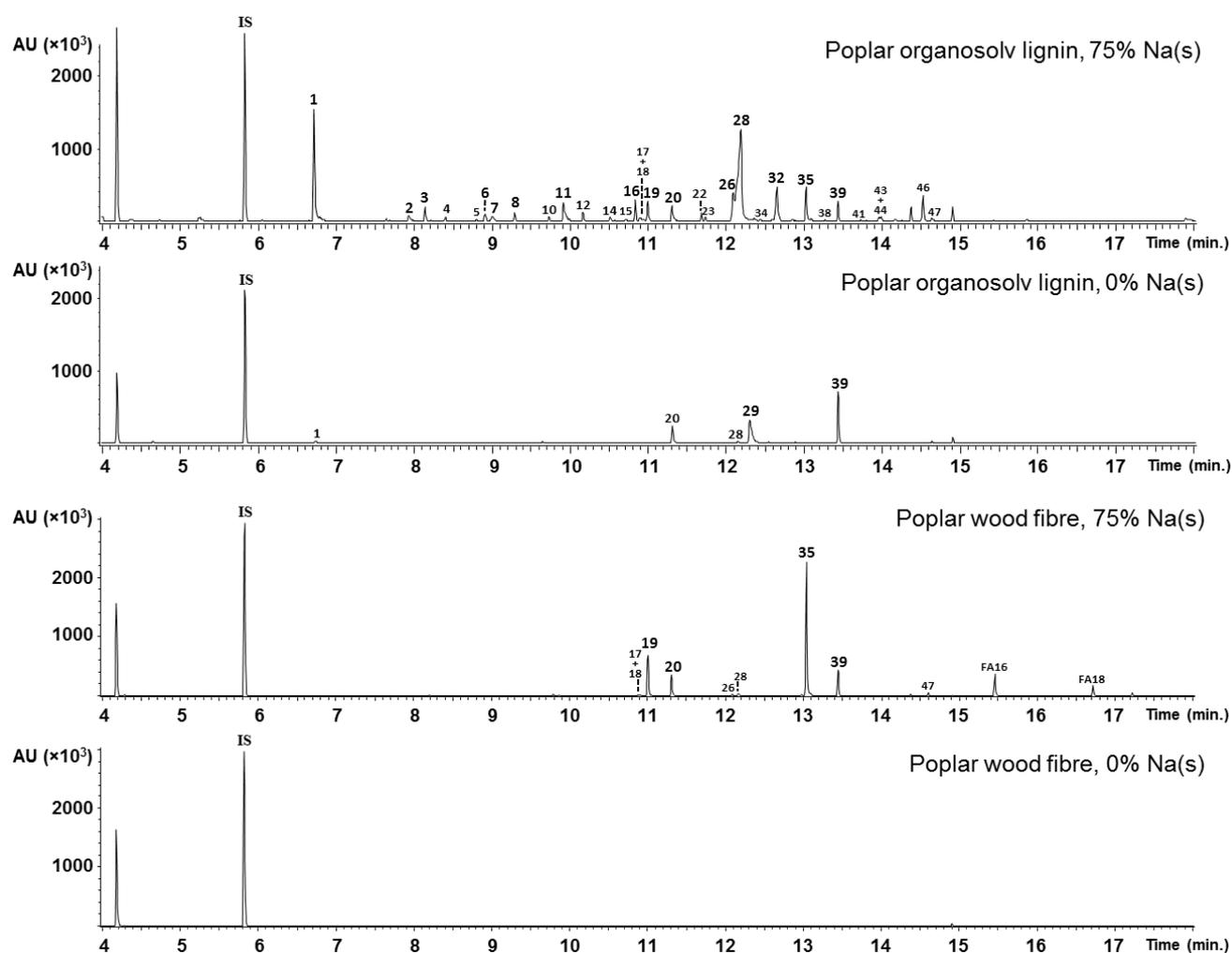


Figure S7d. Chromatograms of the bio-oil isolated from poplar organosolv lignin after 6 h with 75% and 0% Na(s) at 20 °C, of the bio-oil isolated from poplar wood fibre with 75% (Na(s)/NH₃ treatment) and 0% (NH₃ + NaOH(s) treatment, control) Na(s) after 3 h in liquid ammonia at 20 °C followed by alkaline extraction during 1 h/80 °C. In the control, an equimolar amount of NaOH(s) was added to the fibre suspension for alkaline extraction.

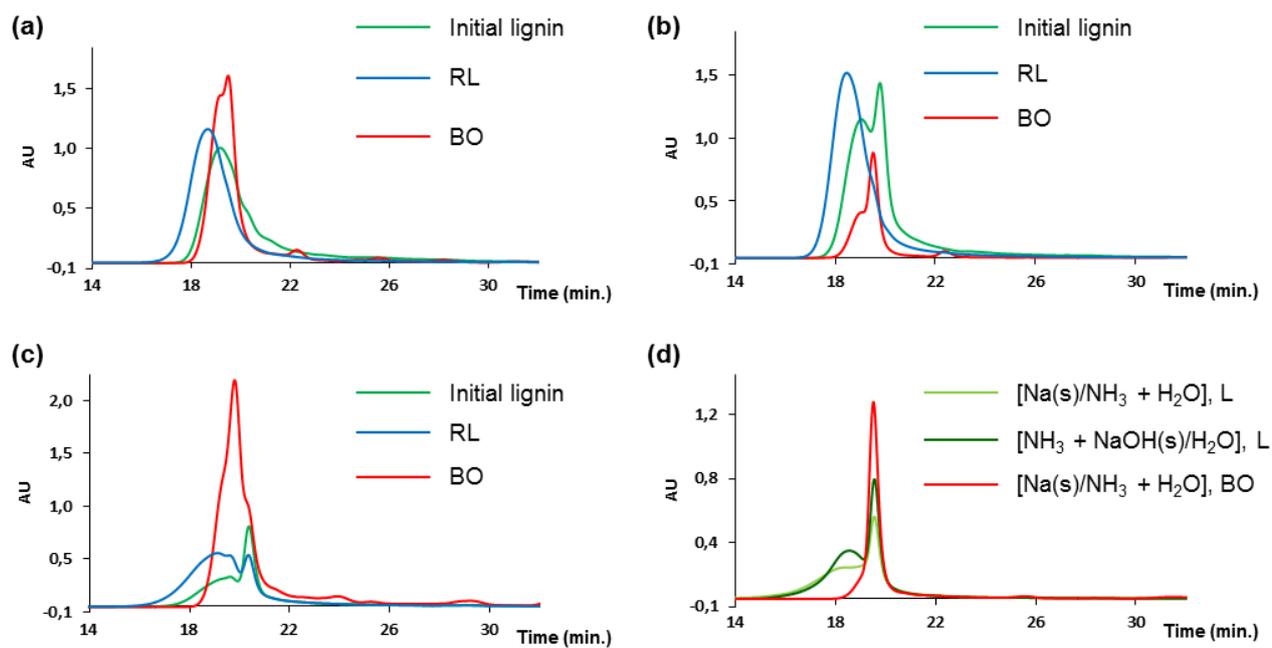


Figure S8. SEC chromatograms of the initial lignins, RL and bio-oils isolated after 6 h with 75% Na_(s) (wt% on dry lignin) in 20mL liquid ammonia + 10mL THF at RT from **(a)** wheat straw organosolv lignin, **(b)** poplar organosolv lignin, **(c)** EG MWL and **(d)** of the bio-oil and lignin-rich fractions (L) isolated from poplar wood fibre in the experiments [Na_(s)/NH₃ + H₂O] and [NH₃ + NaOH_(s)/H₂O].

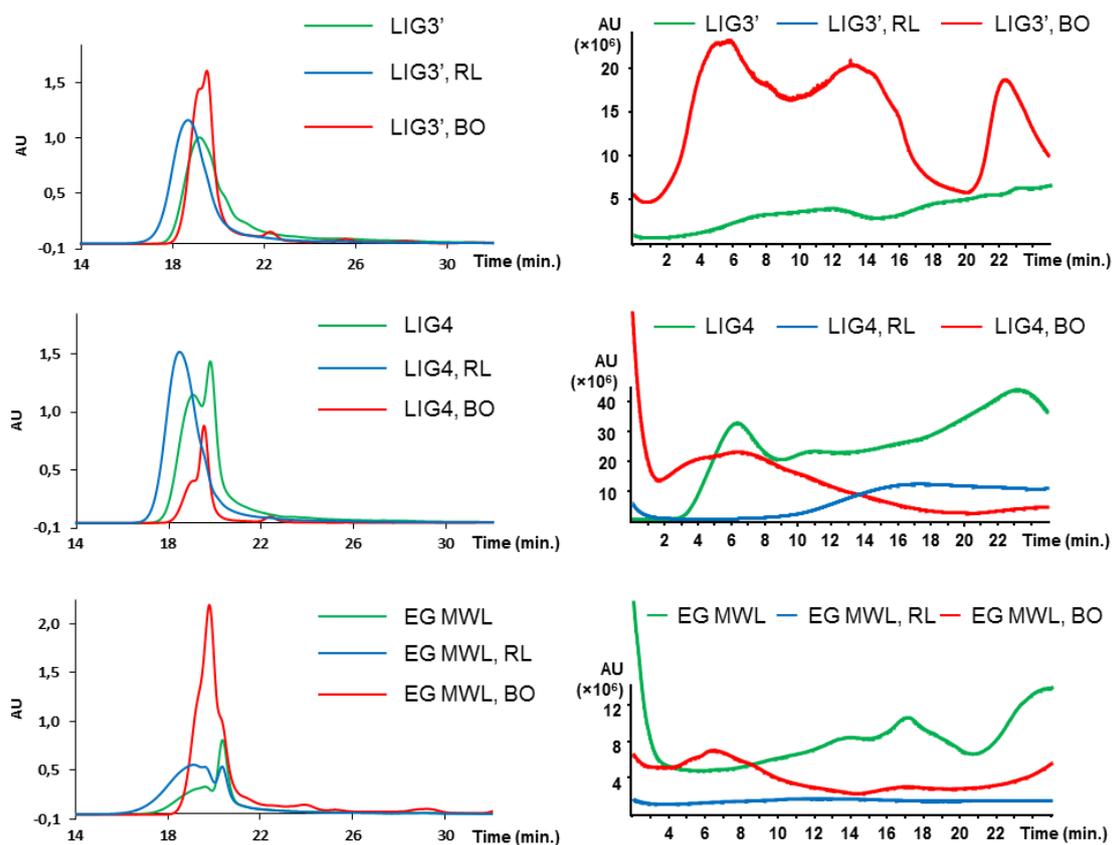


Figure S9. (Left) Direct Insertion Probe (DIP) chromatograms from initial lignin (green), residual lignin (blue) and bio-oil (red) isolated with 75% Na_(s) in 20mL liquid ammonia + 10mL THF at RT, from **(a)** wheat straw organosolv, **(b)** poplar organosolv and **(c)** EG MWL, and **(right)** mean EI-MS spectra calculated in several time intervals from the volatile fractions produced during the DIP analysis of **(a)** wheat straw and **(c)** EG MWL.

References

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