

Supporting Information

Organic Transformation of Lignin into Mussel-inspired Glues: The Next Generation 2K Adhesive for Setting Corals Under Saltwater

Ching-Yi Choi,^a Francisco Lossada,^a Keven Walter,^a Tom Fleck-Kunde,^a Sascha Behrens,^b Thomas Meinelt,^b Jana Falkenhagen,^c Matthias Hiller,^d Hartmut Oschkinat,^d André Dallmann,^a Andreas Taden,^e and Hans G. Börner^a

^a Humboldt-Universität zu Berlin, Department of Chemistry, Laboratory for Organic Synthesis of Functional Systems, Brook-Taylor Straße 2, 12489 Berlin, Germany.

^b Institute of Freshwater Ecology and Inland Fisheries, Department of Ecophysiology and Aquaculture, Müggelseedamm 301, 12587 Berlin, Germany.

^c Bundesanstalt für Materialforschung und -prüfung, BAM, Department of Polymer Analysis, Richard-Willstätter-Straße 11, 12489 Berlin, Germany.

^d Leibniz-Forschungsinstitut für Molekulare Pharmakologie, FMP, Department of NMR assisted structural biology, Robert-Rössle-Straße 10, 13125 Berlin, Germany.

^e Henkel, Adhesives Technologies, Düsseldorf, Germany

Prof. Dr. Hans Börner
Humboldt-Universität zu Berlin
Laboratory for Organic Synthesis of Functional Systems
Brook-Taylor-Str. 2, 12489 Berlin
Germany

E-Mail: h.boerner@hu-berlin.de

Web: <http://www.boernerlab.de>

ORCID Publication ID:

Phone: +49 (0)30-2093 7348

Fax: +49 (0)30 2093-7500

Content

1.	Materials.....	3
2.	Instrumentation	5
3.	Synthesis and Analysis.....	9
3.1	Starting Material.....	9
3.2	Preparation of fractionated lignin.....	10
3.3	Preparation of demethylated lignin	13
3.4	Preparation of demethylated, oxidized lignin (DoxL)	16
3.5	General preparation of mono-thiol lignin functionalization for model reaction.....	18
4.	Material Testing	19
4.1	General procedure for adhesion tests	19
4.2	Leaching tests.....	21
5.	Experimental results.....	21
5.1	Quantitative analysis of functional groups after different lignin modifications steps	21
5.2	Thermal stability analysis	24
5.3	Thermal characterization.....	25
5.4	FTIR analysis of chemical transformation in lignin	26
5.5	Solid state ¹³ C MAS NMR analysis of lignin	27
5.6	UV/vis and ¹ H NMR analysis of leached content.....	28
5.7	Dry adhesion 2K protocol of lignin glues in different mass ratios	29
5.8	Underwater adhesion 2K protocol	32
6.	References	34

1. Materials

UPM BioPiva™ 300 lignin in 10 kg scale was purchased from UPM (Leuna, Germany). Sodium periodate (99.8%), tris-(2-carboxyethyl)-phosphine ($\geq 98\%$), acetic anhydride ($\geq 99\%$) and sodium chloride ($\geq 99\%$) were purchased from *Carl Roth GmbH & Co. KG* (Karlsruhe, Germany). Iodocyclohexane (98%), tributyl phosphine (98%) were purchased from *Sigma Aldrich® Chemie GmbH* (Seelze, Germany). 2-Chloro-1,3,2-dioxaphospholane (97%) was purchased from *Merck KGaA* (Darmstadt, Germany). Sodium acetate (99%) was purchased from *Acros Organics* (Geel, Belgium). Ethoxylated trimethylolpropane tri (3-mercaptopropionate) (THIOCURE® 333, ETTMP) ($\geq 99\%$) and polycaprolactone tetra (3-mercaptopropionate) (THIOCURE® 341, PCL4MP) ($\geq 90\%$) were purchased from *Bruno Bock Chemische Fabrik GmbH & Co. KG* (Marschacht, Germany). Ethanethiol ($> 98\%$), and octamethylcyclotetrasiloxane (D₄) (98%) were purchased from *TCI* (Tokyo, Japan). Pyridine anhydrous ($> 99.5\%$) was obtained from *Fisher Scientific GmbH* (Schwerte, Germany). Deuterated dimethyl sulfoxide (DMSO-d₆), deuterated chloroform (CDCl₃) and deuterated acetone (acetone-d₆) were obtained from *deuteron* (Kastellaun, Germany). *N*-Methyl-2-pyrrolidon (NMP) (peptide grade) was purchased from *Iris Biotech GmbH* (Marktredwitz, Germany), tetrahydrofuran (THF) ($\geq 99.8\%$ for HPLC) and *N,N*-dimethylformamide (DMF) ($\geq 99.8\%$, for peptide synthesis) were obtained from *VWR® chemicals* (Dresden, Germany). Loctite® 406 (monomer: ethyl cyanoacrylate), Loctite® 496 (monomer: methyl cyanoacrylate) and Pattex® Ultra Gel Matic instant adhesives (monomer: ethyl cyanoacrylate, with the addition of micro rubber particles) were purchased from *Henkel AG & Co.KGAA* (Düsseldorf, Germany). All chemicals were used as received unless otherwise noted.

AquaScape Construction Epoxy putty adhesive was purchased from *D-D The Aquarium Solution Ltd* (Ilford, United Kingdom).

Loctite[®] 406, Loctite[®] 496 and Pattex[®] Ultra Gel Matic instant adhesives were purchased from *Henkel AG & Co.KGAA* (Düsseldorf, Germany).

Ultrapure water was produced using SG LaboStar[®] TM 1-UV system from *SG water* (Hamburg, Germany). As ion exchanger, Evoqua Water Technologies Polisher HP2 module was inserted. Electric conductivity of Ultrapure water was 0,055 $\mu\text{S cm}^{-1}$.

For lap shear experiments aluminium plates (5005A, 80 × 25 × 1.5 mm with one-sided bore) were purchased from *ROCHOLL GmbH* (Eschelbronn, Germany). The test specimens used are made of an aluminum-magnesium alloy and are frequently applied in standard tests.

2. Instrumentation

Gel permeation chromatography (GPC):

A GPC system with WinGPC UniChrom 8.2 software (PSS GmbH) was used for the GPC analyses. The system was operated with an isocratic pump (HPLC COMPACT PUMP 3350, Bischoff) with a flow rate of 0.5 mL/min. DMSO (HPLC grade) was used as eluent with a salt addition of 0.075 M NaNO₃. A combination of four different columns was used. At first a pre-column (ABOA DMSO-Phil-P-250, 8 mm x 50 mm, 10² – 7·10⁴ g/mol, AppliChrom) was used followed by two columns (DMSO-Phil-P-250, 8 mm x 300 mm, 10² – 7·10⁴ g/mol, AppliChrom) and final separation column (DMSO-Phil-P-Multipore, 15 µm, 8 mm x 300 mm, 10²- 10⁶ g/mol, AppliChrom). A column oven heated the separation column to 60 °C (Thermostated Column Compartment). A refractive index detector (Shodex RI-501) and a photodiode array detector (SPD-M20A, Shimadzu Europe) were used to detect the fractions. The wavelength detector was operated at 280 nm. For calibration 10 pullulan standards (PSS GmbH, now Agilent) were used. Samples of 2 – 3 mg/mL concentration were filtered through a 0.2 µm syringe filter before injection.

Nuclear magnetic resonance spectroscopy (NMR):

Liquid-state ¹H, ¹³C, ³¹P NMR, and HSQC measurements were performed on a Bruker Avance II 500 MHz or 600 MHz spectrometer (*Bruker BioSpin GmbH*, Rheinstetten, Germany) in the given deuterated solvent.

Solid-state ¹³C {¹H} MAS NMR measurements were carried out using a wide bore spectrometer (Bruker Avance III) operating at 400 MHz and a 3.2 mm probe-head operating in double-mode (¹H, ¹³C). All measurements were done using a magic angle spinning (MAS) frequency of 15 kHz and the variable temperature (VT) was set to 285 K with a gas-flow of 535 L/h, which corresponds to a sample temperature of 293.8 K. The temperature was measured indirectly with a 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) sample (4% H₂O + DSS in D₂O) under the same conditions.¹ Additionally, all spectra were indirectly referenced to the proton frequency of the methyl-group of DSS (0 ppm). For each sample, 10 experiments with 1024 scans were recorded and then summed-up with the Bruker software Topspin 3.5pl7 (*Bruker BioSpin GmbH*, Rheinstetten, Germany).

Direct detected ¹³C-experiments were done using an initial carbon excitation pulse of 5 µs (50 kHz) followed by proton decoupling during acquisition with a pulse power of 80 kHz

(SPINAL 64 decoupling sequence).² The recycle delay between each scan was set to 10 s.

The experimental conditions for the cross polarization (CP) experiments were optimized with a ¹³C-labeled glycine sample.³ The energy for the initial proton excitation pulse was optimized to a value at which the pulse length was 3,125 μs (80 kHz). The magnetization transfer (cross polarization (CP)) between ¹H and ¹³C was carried out with a power of 60 kHz (¹H) and 52,5 kHz (¹³C) and a ramp of 50-100% (on the ¹³C-channel) for a duration of 2 ms. During the acquisition, protons were decoupled with a pulse power of 80 kHz (SPINAL 64 decoupling sequence). The recycle delay between each scan was set to 5 s.

The INEPT based experiments were done using a ¹H-¹³C INEPT transfer time of 1.4 ms.⁴ During the acquisition, protons were decoupled with a pulse power of 80 kHz (SPINAL 64 decoupling sequence). The recycle delay between each scan was set to 5 s.

Fourier transform infrared spectroscopy (FT-IR):

All measurements were carried out on a Bruker Vertex 70v FT-IR spectrometer (*Bruker Optik GmbH*, Ettlingen, Germany). Blank measurements were conducted before and after each sample.

UV-visible spectroscopy (UV/vis):

All measurements were performed on a Spectrometer UV-2501PC from *Shimadzu Deutschland GmbH*, Duisburg, Germany. The recorded spectra ranged from 200 to 800 nm.

Small-angle X-ray scattering (SAXS):

All measurements were performed in a polycarbonate flow-through capillary at 20 °C with a SAXess camera (*Anton Paar*, Graz, Austria) by Andreas Thünemann (BAM, Federal Institute for Materials Research and Testing). This camera was attached to a laboratory X-ray generator (PW3830, PANalytical) and operated with a fine focus glass X-ray tube at a voltage of 40 kV and a current of 40 (Cu- K_{α} , $\lambda = 0.1542$ nm).

Thermal gravimetric analysis (TGA):

All measurements were carried out on a Thermogravimetric Analyzer Pyris 1 (*Perkin Elmer*, Waltham, USA). Approximately 10 mg of each sample were weighed in a ceramic pan and heated from 30 °C up to 800 °C at a heating rate of 20 °C/min, under argon atmosphere with a flow rate of 20 mL/min.

Differential Scanning Calorimeter (DSC):

All measurements were performed on a Differential Scanning Calorimeter DSC 8000 (*Perkin Elmer*, Waltham, USA). Heating rate 10 °C/min. Samples of around 10 mg were measured in a punctured aluminum pan. T_g values were obtained from the second heating scan.

Mixing device:

All glue mixtures were homogenized with a SpeedMixer DAC 150 SP (*Hauschild*, Hamm, Germany).

Lap shear tests:

Shear tests have been carried out with a Texture Analyzer Ta.XT.plus100C (*Stable Micro Systems*, Godalming, United Kingdom) with a 100 kg force cell.

UV Ozone Cleaner:

All specimens were cleaned with ozone for 15 min using UVC-1014 (*NanoBioAnalytics*, Bürgel, Germany) prior to every shear test.

Ecotoxicity tests (DIN EN ISO 15088):

The direct toxicity was investigated by determining acute toxicity according to the DIN 15088 fish egg test. Cured and non-cured samples of DoxL₄₀/PCL4MP₆₀ were intensively ground and then dispersed into standardized dilution water for 7 days. The supernatant was mixed with distilled water in a dilution series, graded by integer volume ratios, to form dilution steps (G). After 48 h exposure of the fertilized fish eggs in the microtitration plates, the dilution level at which no toxic effect occurred is determined (G). At 26 °C, embryos hatch after 72 to 96 h. The test duration is 48 h. A solution of 3.7 mg/L reference substance 3,4-dichloroaniline with 10 fertilized fish eggs is used as positive control.

Standard dilution water according to ISO 7346-1 and ISO 7346-2.

3. Synthesis and Analysis

3.1 Starting Material

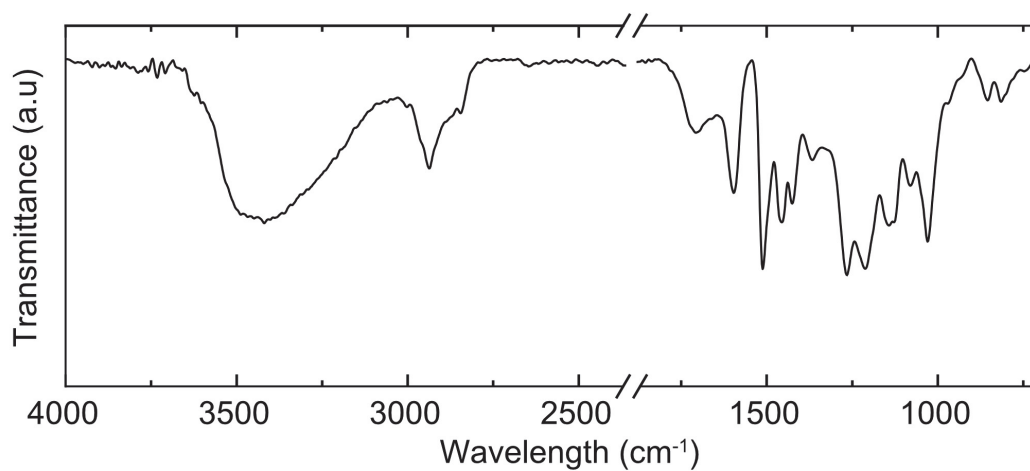
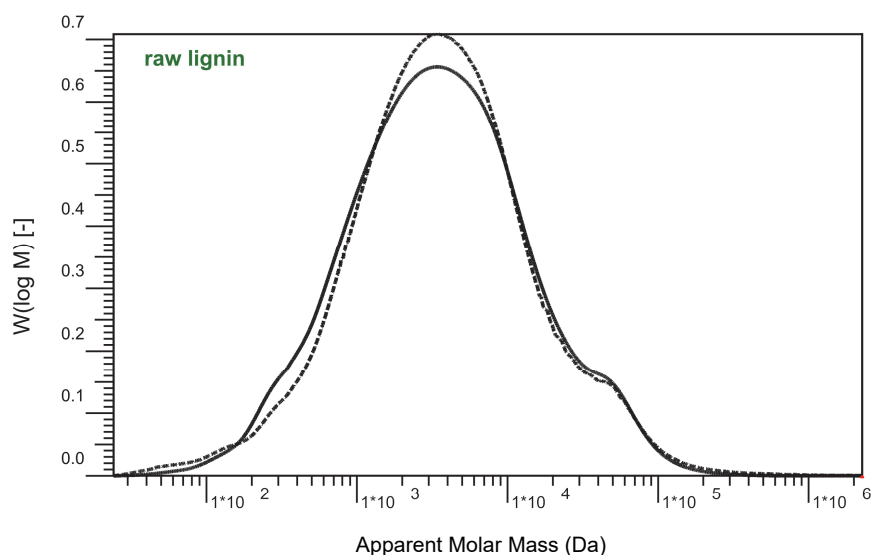


Figure S1. FTIR spectra for UPM BioPiva™ 300.



	<u>SPD-M20A</u>	<u>Shodex RI-501</u>	
Mn :	1.3820e3	1.3274e3	g/mol
Mw :	9.2361e3	1.0482e4	g/mol
Mz :	4.7838e4	1.2391e5	g/mol
Mv :	0.000000	0.000000	g/mol
D :	6.6834e0	7.8969e0	

Figure S2. GPC chromatogram of UPM BioPiva™ 300 in DMSO + 0.075 mM NaNO₃.

3.2 Preparation of fractionated lignin

The fractionated lignin was prepared by dissolving UPM Kraft lignin (50 g) in acetone (500 mL) and stirring for 2 h. After filtration the solvent was removed under reduced pressure and dried overnight under high vacuum (10^{-3} mbar). Recovered mass yield: 80%.

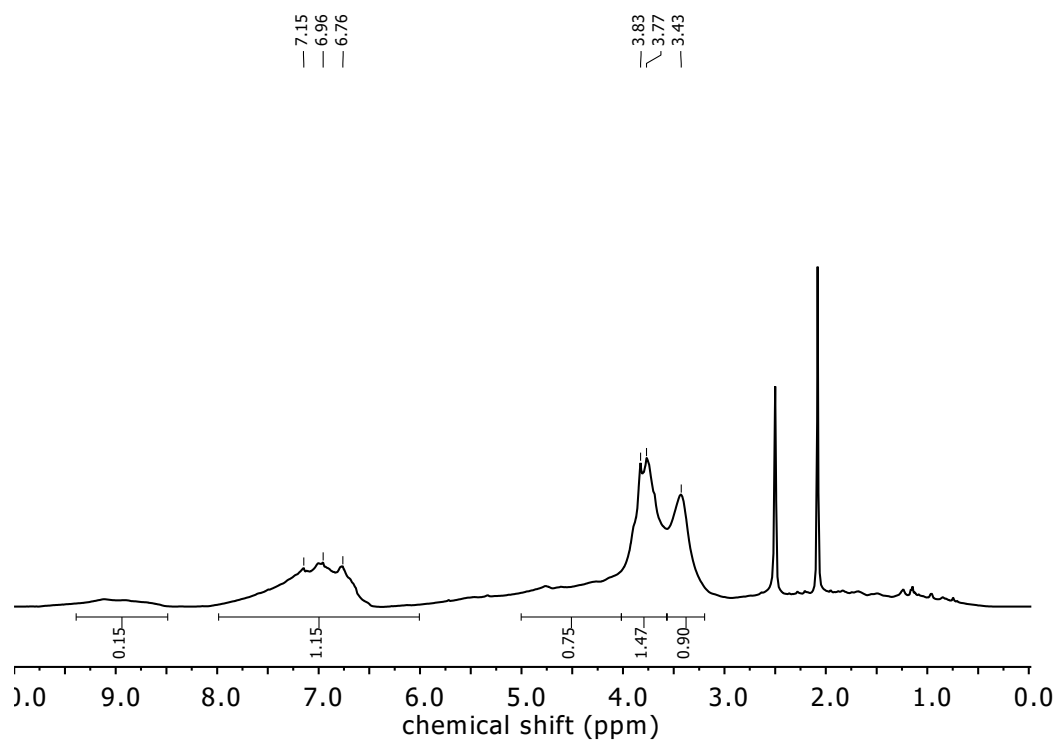


Figure S3. ^1H NMR spectrum of fractionated lignin measured in DMSO-d_6 at 500 MHz. Due to varying molecular size the NMR signal for the aromatic CH signals was referenced internally as integral = 1.

^1H NMR (500 MHz, DMSO-d_6): $\delta = 7.5 - 7.2$ (m, \mathbf{H}_{arom} in coumaryl), $7.2 - 6.7$ (m, \mathbf{H}_{arom} and $\mathbf{H}_{\text{vinyl}}$), $6.7 - 6.5$ (m, \mathbf{H}_{arom} in guaiacyl), $4.0 - 3.6$ (m, $\beta\text{-O-4-H}$ and $\beta\text{-5'-H}$), 3.4 (br s, OCH_3), $2.3\text{-}2.2$ (m, phenolic-OH), $1.3\text{-}1.1$ (m, aliphatic-OH) ppm.

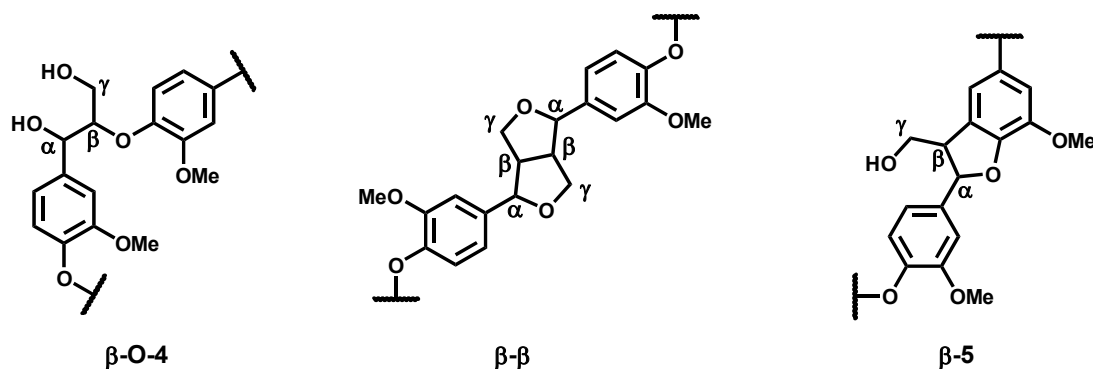


Figure S4. Representative structures of lignin.

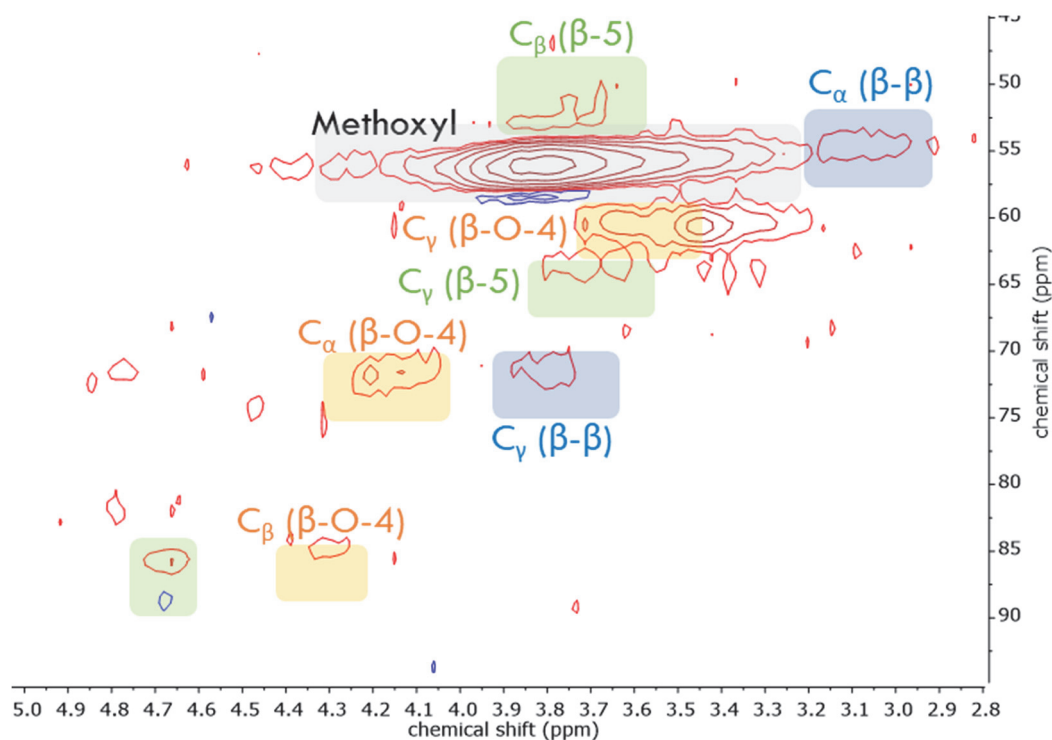


Figure S5. HSQC spectra of acetylated fractionated lignin. Different ether moieties are marked in different colours: β -aryl ether β -O-4 (orange), resinol β - β (blue) and phenylcoumaran β -5 (green).

The short range ^1H - ^{13}C correlation HSQC NMR spectra of acetylated fractionated lignin displayed typical lignin ether bonds such as β -O-4, β - β , β -5 linkages as well as methoxy moieties. Note, that this analysis method was used for qualitative structural clarification.

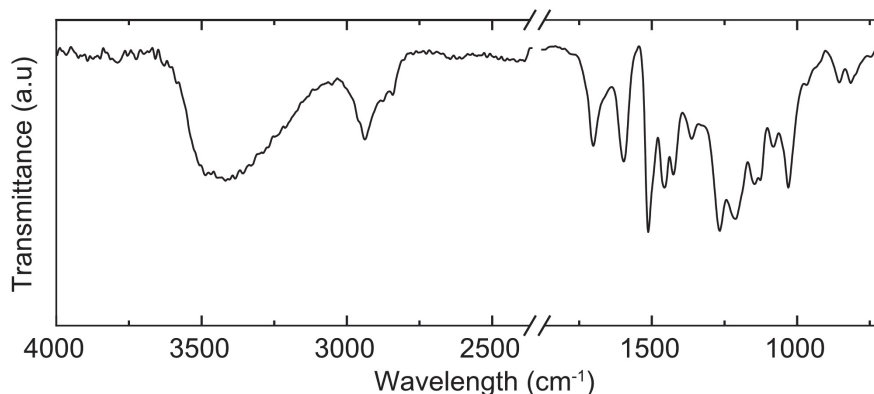
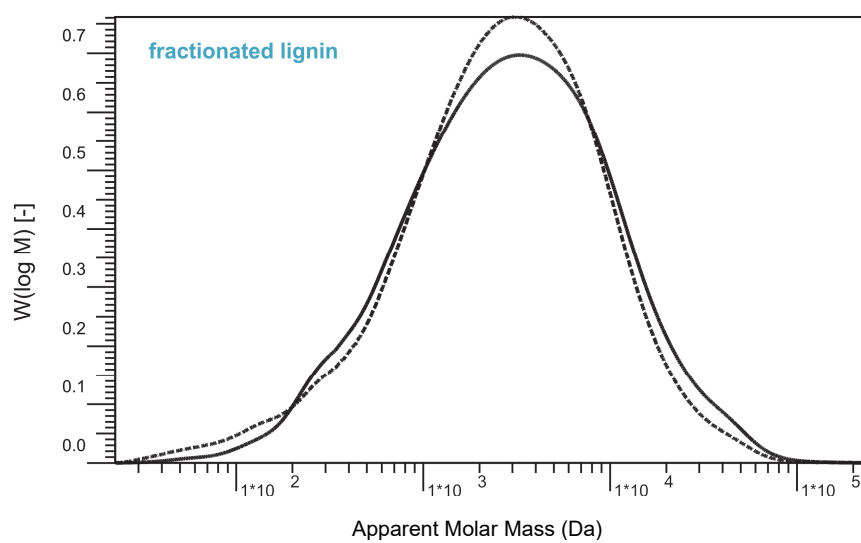


Figure S6. FTIR spectra for fractionated lignin.

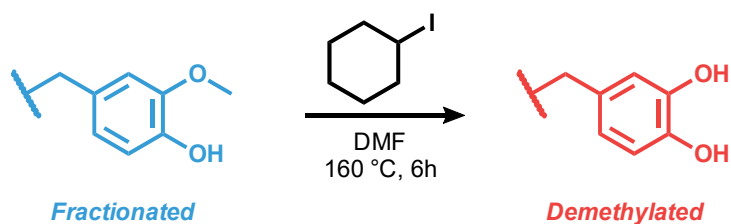
AT-FTIR (cm^{-1}): 813.90, 852.48, 970.13, 1027.99, 1080.06, 1124.42, 1145.64, 1211.21, 1265.21472, 1334.65, 1365.51, 1427.22, 1452.30, 1461.94, 1512.09, 1595.02, 1703.03, 2935.45, 3159.18, 3186.18, 3207.40, 3220.90, 3242.11, 3249.83, 3267.19, 3296.12, 3305.76, 3325.05, 3348.19, 3367.48, 3375.19, 3382.91, 3390.62, 3400.26, 3409.91, 3425.34, 3442.70, 3471.63, 3483.20, 3500.56, 3515.99.



	<u>SPD-M20A</u>	<u>Shodex RI-501</u>	
\overline{Mn} :	1.2321e3	1.0251e3	g/mol
\overline{Mw} :	5.9607e3	5.1789e3	g/mol
\overline{Mz} :	1.9804e4	1.6850e4	g/mol
\overline{Mv} :	0.000000	0.000000	g/mol
\overline{D} :	4.8377e0	5.0521e0	

Figure S7. GPC chromatogram of acetone fractionated lignin in DMSO + 0.075 mM NaNO₃.

3.3 Preparation of demethylated lignin



Demethylation of fractionated lignin was carried out starting from the modified procedure reported by Sawamura and co-workers.⁵ Fractionated lignin (10 g) was weighed in a two-neck round bottom flask, dissolved in DMF (150 mL) and stirred at ambient temperature until homogeneous conditions were obtained. Iodocyclohexane (ICH; 0.3 mol, 63.02 g, 38.8 mL) was passed through a small column of aluminium oxide (AlO₃) to remove the inhibitor prior to use, and then added dropwise to the lignin dispersion. Reflux condenser and escape chamber with aqueous ammonia were set to quench the released gas and side products. The reaction mixture was heated in an oil bath to 140 °C for 6 h, and then cooled down to ambient temperature (care should be taken with the under-pressure when cooling down). The reaction mixture was washed with *n*-hexane, and poured into 1 L water. The resulting precipitate was collected by filtration, washed several times with water, and lyophilized to afford the demethylated product. Recovered mass yield: 85%.

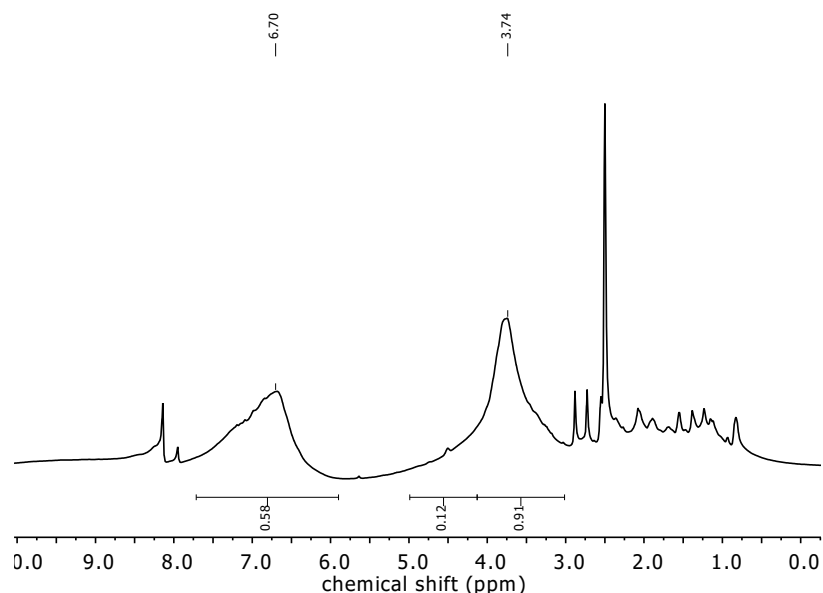


Figure S8. ¹H NMR spectrum of demethylated lignin measured in DMSO-d₆ at 500 MHz. Due to varying molecular size the NMR signal for the aromatic CH signals was referenced internally as integral = 1.

¹H NMR (500 MHz, DMSO-d₆) [δ in ppm]: 7.5 - 7.2 (**H_{arom}** in coumaryl), 7.2 - 6.7 (**H_{arom}** and **H_{vinyl}**), 6.7 - 6.5 (**H_{arom}** in guaiacyl), 4.0-3.6 (β -1'-**H**), 3.4 (**OCH₃**), 2.5-2.3 (phenolic-**OH**), 1.3-1.1 (aliphatic-**OH**).

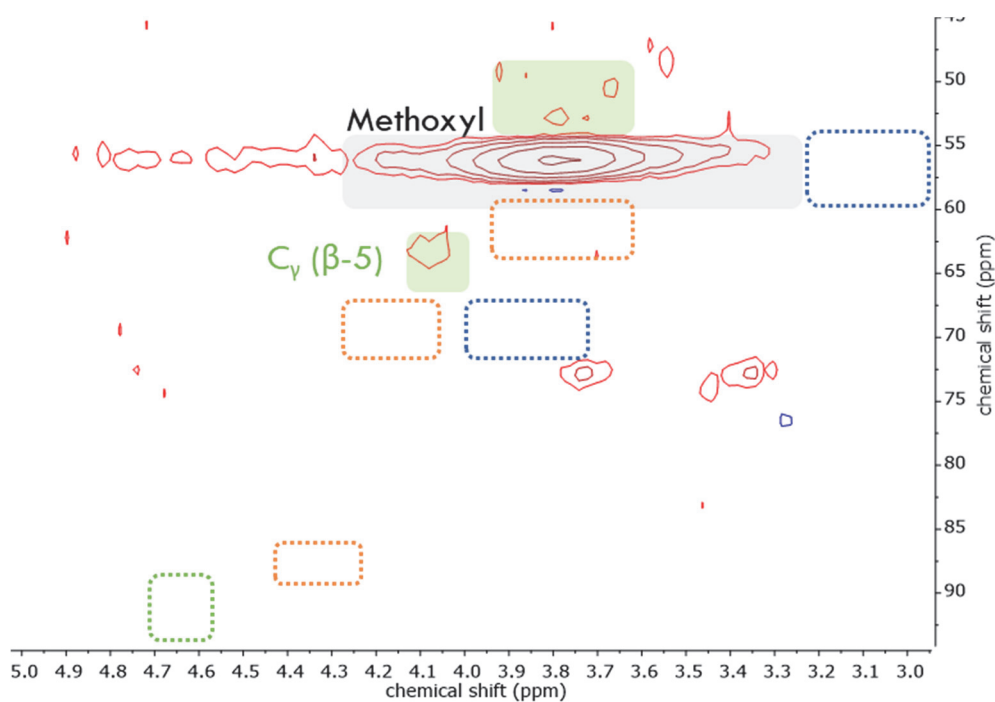


Figure S9. HSQC spectra of acetylated demethylated lignin. Different ether moieties are marked in different colours: β -aryl ether β -O-4 (orange), resinol β - β (blue) and phenylcoumaran β -5 (green).

The signals from the β -O-4, β - β , β -5 linkages were markedly depleted in the HSQC NMR spectrum of acetylated demethylated lignin. This suggests the cleavage of the β -aryl ether linkages, which, along with aromatic demethylation, possibly can contribute to the increase of free phenolic-OH groups in the lignin polymer. Note, that this analysis method was used for qualitative structural clarification.

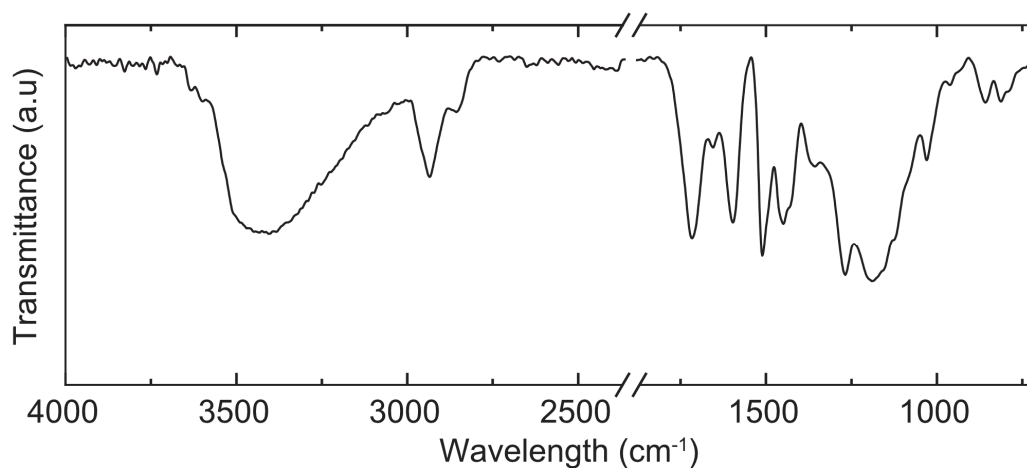


Figure S10. FTIR spectra for demethylated lignin.

AT-FTIR: 786.90, 796.55, 811.97, 860.19, 1027.99, 1037.63, 1130.21, 1199.64, 1267.14, 1359.72, 1434.94, 1448.44, 1510.16, 1596.95, 1718.45, 2933.52, 3197.75, 3211.25, 3.247.90, 3261.40, 3269.11, 3296.12, 3305.76, 3319.26, 3328.90, 3346.26, 3380.98, 3392.55, 3417.62, 3425.33, 3433.05, 3450.41, 3458.12, 3467.77, 3492.84 cm^{-1} .

The complementing analysis data obtained by IR and ^1H NMR confirmed the change in structure and successful modification. IR showed the strong decrease of methoxy groups but increase of hydroxy groups after demethylation.

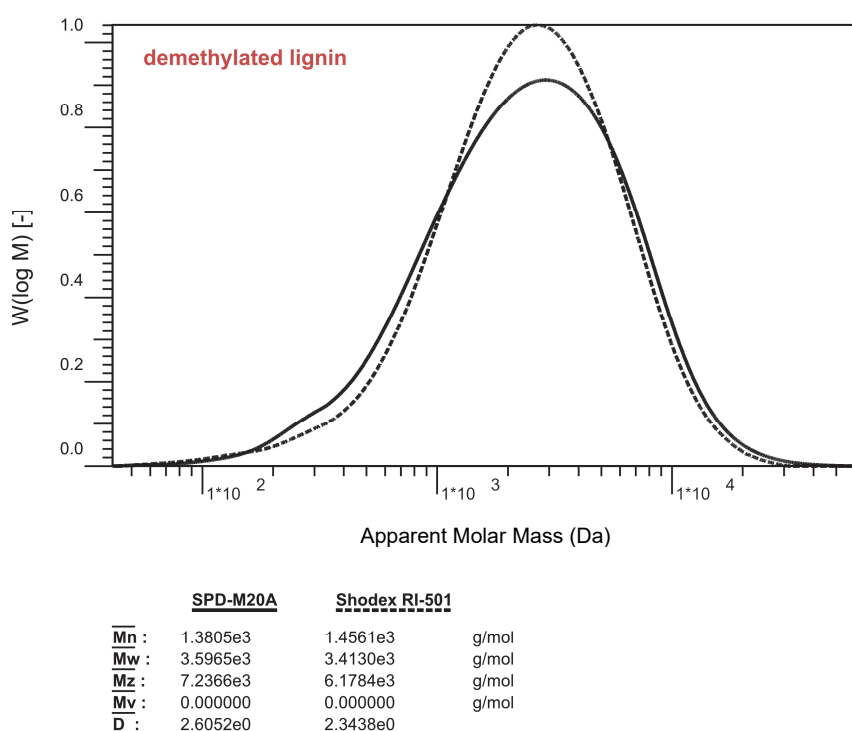
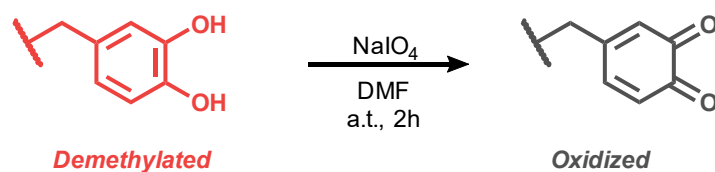


Figure S11. GPC chromatogram of demethylated lignin in DMSO + 0.075 mM NaNO_3 .

3.4 Preparation of demethylated, oxidized lignin (DoxL)



Dried demethylated lignin (5 g) was dissolved in DMF (30 mL) and sodium acetate buffer, (3 mL, pH 5, 0.1 M) was added. NaIO₄ (1.5 g, 30 w%) was added and the homogenous mixture was stirred at ambient temperature for 2 h. The reaction mixture was poured into 1 L water. The resulting precipitate was collected by filtration, washed several times with water, and lyophilized to afford the oxidized product. Recovered mass yield: 81%.

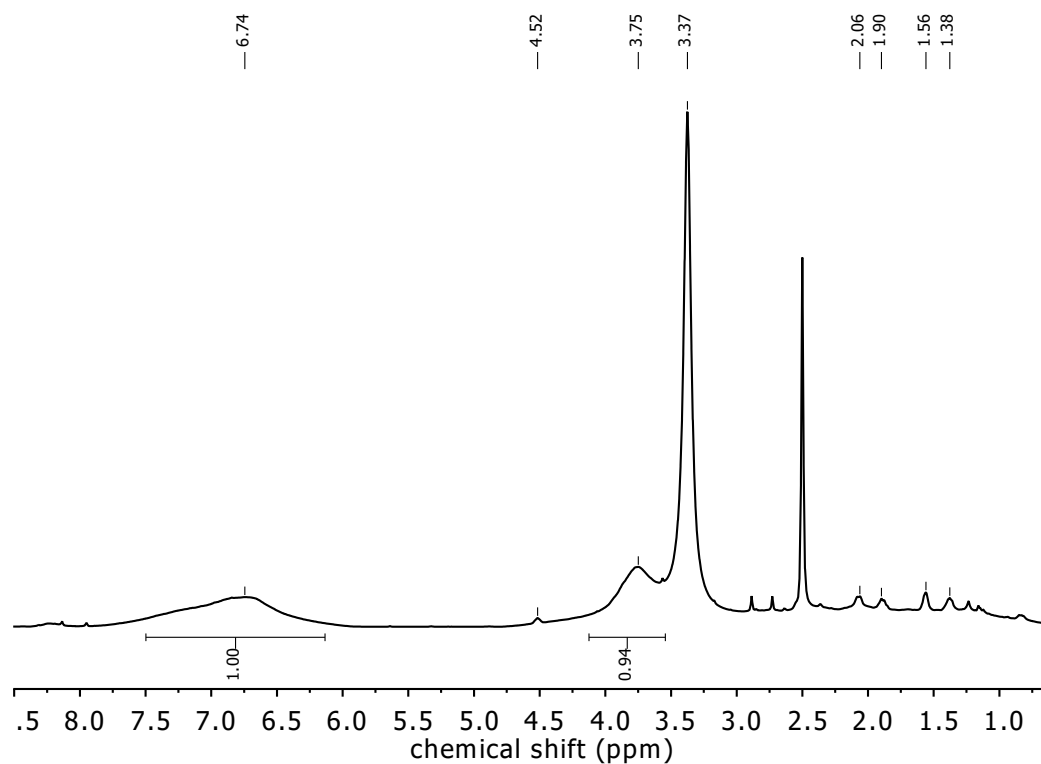


Figure S12. ¹H NMR spectrum of DoxL measured in DMSO-d₆ at 500 MHz. Due to varying molecular size the NMR signal for the aromatic CH signals was referenced internally as integral = 1.

¹H NMR (500 MHz, DMSO-d₆): δ = 7.5 - 7.2 (m, **H_{arom}** in coumaryl), 7.2 - 6.7 (m, **H_{arom}** and vinyl-H), 6.7 - 6.5 (m, **H_{arom}** in guaiacyl), 4.0 - 3.6 (m, β -1'-H), 3.4 (br s, OCH₃), 2.3-2.2 (m, phenolic-OH), 1.3-1.1 (m, aliphatic-OH) ppm.

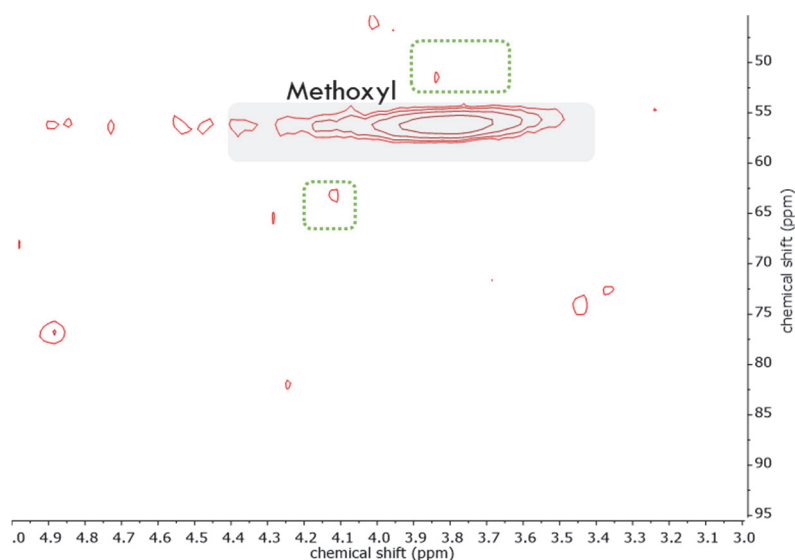


Figure S13. HSQC spectra of acetylated demethylated and oxidized lignin. Different ether moieties are marked in different colours: β -aryl ether β -O-4 (orange), resinol β - β (blue) and phenylcoumaran β -5 (green).

The signals from the β -O-4, β - β and β -5 linkages were almost fully depleted in the HSQC NMR spectrum of acetylated DoxL. Note that this analysis method was used for qualitative structural clarification.

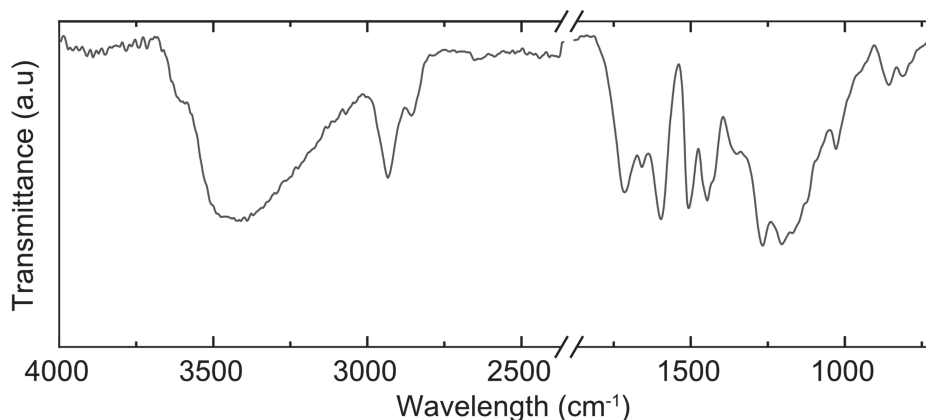


Figure S14. FTIR spectra for oxidized lignin.

AT-FTIR: 817.77, 856.33, 1027.99, 1037.63, 1126.35, 1153.35, 1205.43, 1267.14, 1334.65, 1355.86, 1429.15, 1450.37, 1461.94, 1504.37, 1596.95, 1660.59, 1720.38, 2933.52, 2939.31, 3247.90, 3259.47, 3269.11, 3278.76, 3305.76, 3319.26, 3328.90, 3334.69, 3348.19, 3365.55, 3373.26, 3380.98, 3390.62, 3406.05, 3417.62, 3436.91, 3442.70, 3458.13, 3469.70, 3481.27, 3506.34, 3517.91 cm^{-1} .

IR showed the strong decrease of hydroxy groups but an increase of vibrational bands of carbonyl-moieties after oxidation.

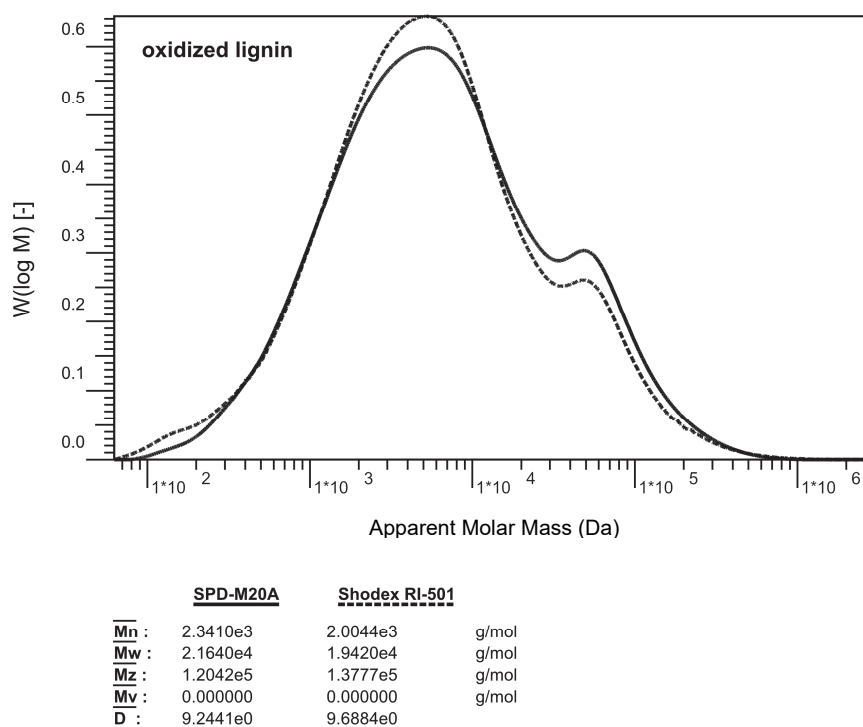
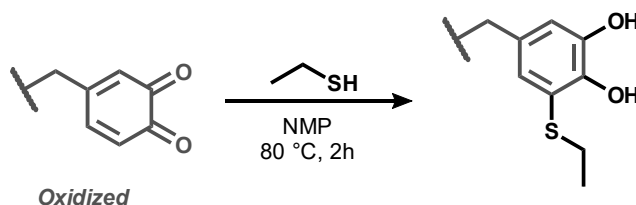


Figure S15. GPC chromatogram of oxidized lignin in DMSO + 0.075 mM NaNO₃.

3.5 General preparation of mono-thiol lignin functionalization for model reaction

In a typical model reaction DoxL (0.1 g) was dissolved in NMP (1 mL). The solution was treated with excess ethanethiol (30 eq. per catechol functionality) and was stirred at 80 °C for 2 h. The reaction mixture was poured into 50 mL water in case of 2-mercaptoethanol or ether in case of ethanethiol and centrifuged to separate the solid. Excessive washing was necessary to remove unreacted thiols After lyophilization the thiol-functionalized lignin was obtained.



4. Material Testing

4.1 General procedure for adhesion tests

Substrate preparation before gluing:

Aluminium substrates (25 mm×80 mm) were degreased with acetone and UV treatment.

2K adhesive homogenization:

The 2K adhesives mixtures were denoted as DoxL_x/ETTMP_y and DoxL_x/PCL4MP_y, where x and y correspond to the weight fraction given in wt.% of each component. For the preparation of the 2K adhesive systems, the DoxL and the hydrophobic (PCL4MP) or hydrophilic (ETTMP) multi-thiol were added to a 10 mL polypropylene container in the corresponding weight ratio. The container was then put into a SpeedMixer and the compounds were blended using a mixing ramp for 2 minutes and 40 seconds at maximum 3500 rpm resulting into a homogeneous viscous paste. The overall lignin content in the mixture was adjusted between 30 and 60 wt.%.

Dry adhesion application:

The 2K adhesive was applied to an aluminium substrate using 20 mg of adhesive per 40-60 mm² of surface area. A second substrate was then added on top of the applied adhesive resulting in an overlap between the two substrates. Both substrates were then fixated together and expelled glue was removed prior to curing in an oven at 60°C for 16 hours. Afterwards the samples were equilibrated at r.t. for 1h the adhesive strength was determined by lap shear tests, using DIN 53 281 norm specimens of 25 mm width but an overlap of 3-4 mm as determined for each sample. It should be noted that the overlap deviates from DIN EN 1465 norm experiments^[6] due to constrains of the measurement apparatus.



Figure S16. Exemplary picture of DoxL₄₀/PCL4MP₆₀ to showcase the methodology of preparing samples for lap shear experiments.

Underwater adhesion application:

For the underwater adhesion tests glues with DoxL₄₀/PCL4MP₆₀ weight ratio were mixed. The used water tanks were filled either with 599 mM NaCl-solution to simulate sea water conditions or saltwater from a living ecosystem tank. Three different application ways were then performed:

- 1) 2K glue was applied to an aluminium substrate using 20 mg of adhesive per 40 – 60 mm² of surface area. A second substrate was then added on top of the applied adhesive resulting in an overlap between the two substrates of 3 mm. Both substrates were then clamped together, cured at 60 °C for 12h and immersed into water tank filled
- 2) 2K glue was applied to an aluminium substrate using 20 mg of adhesive per 40 – 60 mm² of surface area. A second substrate was then added on top of the applied adhesive resulting in an overlap between the two substrates of 3 mm. Both substrates were then clamped together, putted into water tank and cured for 3 days at 40 °C. Afterwards the samples were equilibrated at r.t. for 1h.
- 3) 2K glue was applied to one of the aluminium substrates, which are already immersed into the water beforehand. The second substrate was then added on top of the applied adhesive resulting in an overlap between the two substrates of 3 mm. Both substrates were then clamped together and cured for 3 days at 40 °C. Afterwards the samples were equilibrated at r.t. for 1h.

4.2 Leaching tests

The adhesive materials DoxL₄₀/PCL4MP₆₀ and DoxL₄₀/ETTMP₆₀ were fully cured for 1d at 60 °C and were evenly freeze ground. 15 mL of THF, acetone, water or sea water were added, separately, into each leaching sample for 7 days. Additionally, reducing agent tributyl phosphine was added to organic solvent samples, whereas TCEP was added to water samples. The leached samples were removed and dried over 2 days in vacuum, and the final adhesive content was obtained by gravimetry from after and before the test. The leached extracts were analyzed by UV/vis and ¹H-NMR to determine the presence of small released traces of the adhesive.

5. Experimental results

5.1 Quantitative analysis of functional groups after different lignin modifications steps

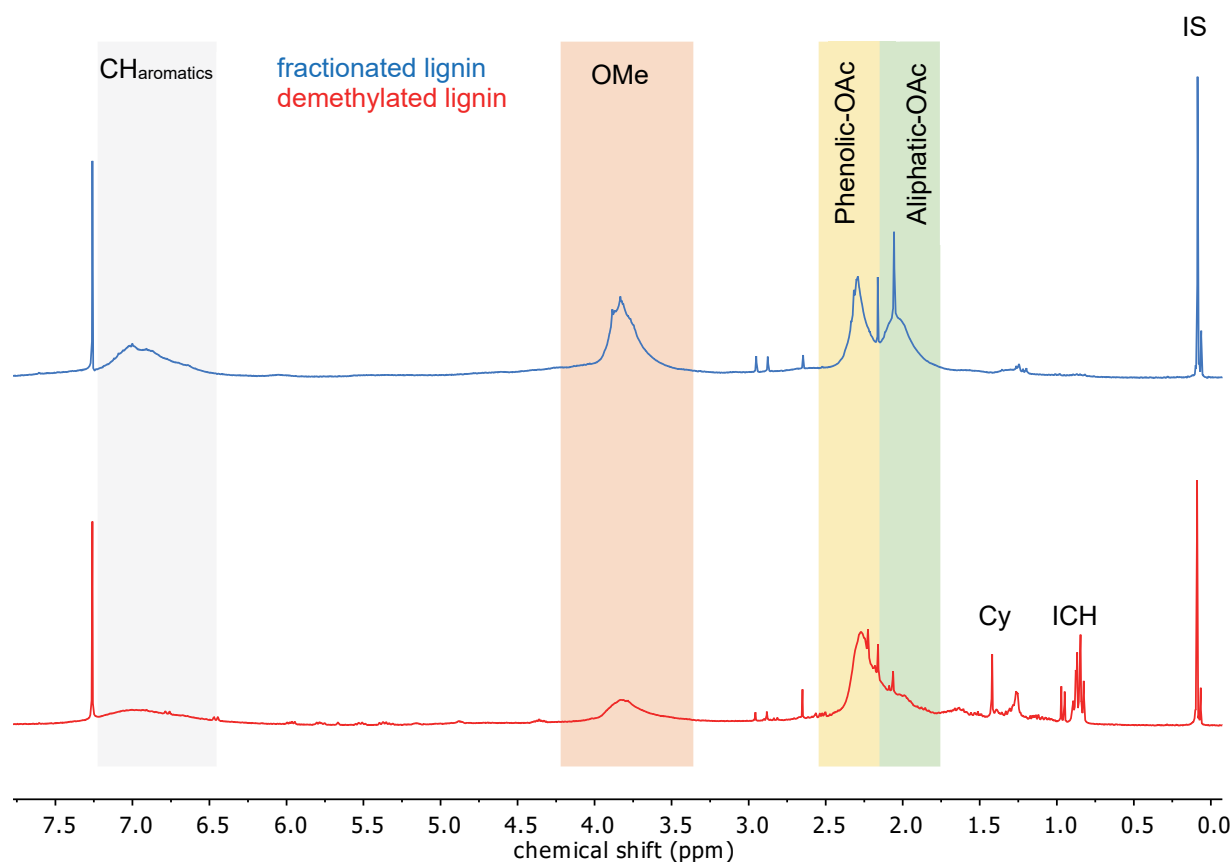


Figure S17. ¹H NMR of fractionated (blue) and demethylated (red) lignin after derivatization with acetate anhydride in CDCl₃. Reduction of the signal corresponding to OMe groups at (4.1 – 3.6 ppm) is clearly visible (light orange). Integration of the signals between 4.1 – 3.6 ppm for different batches was used for calculation of OMe content in lignin samples.

Table S1. Analysis of hydroxy pattern of the demethylated and fractionated lignin samples calculated from ¹H NMR after acetylation with acetic anhydride and using an internal standard.

Entry	OMe (mmol/g) ^a	Total OH (mmol/g) ^a	Ph-OH (mmol/g)	OMe/OH ratio	OMe/ molecule ^b	Ph-OH/ molecule ^b
Acetone fraction – Sample 1	3.80	6.38	2.82	0.60	23	17
Demethylation – Sample 1	2.30	8.77	5.17	0.26	14	31
Demethylation – Sample 2	2.45	7.13	4.08	0.34	15	24
Demethylation – Sample 3	1.93	7.34	4.12	0.26	12	25
Demethylation – Sample 4	2.24	7.37	4.34	0.30	13	26
Demethylation – Sample 5	2.97	6.99	4.15	0.43	18	25
Average	2.38	7.52	4.37	0.32	14	26

^a referring to 1 g of lignin, ^b functional groups inside 1 lignin macromolecule.

From the difference between the fractionated OMe and phenolic-OH concentration of lignin sample before and after demethylation the average number of catechols can be estimated. Since every demethylated OMe group is in theory in *ortho*-position to another phenolic OH-group, it can be indirectly converted to number of available catechols, which is around 1.4 to 1.6 mmol/g.

Table S2. Analysis of hydroxy pattern of demethylated lignin molecules calculated from ³¹P NMR after derivatization with CDP-Cl and using an internal standard – Referring to the first batch of UPM BioPiva™ 300 used for processing.

Entry	Aliphatic-OH (mmol/g)	Ph-OH (mmol/g)
Demethylation – Sample 1	1.19	4.56
Demethylation – Sample 2	1.12	4.12
Demethylation – Sample 3	1.01	3.56
Demethylation – Sample 4	1.14	3.89
Average	1.12	4.03

Table S3. Analysis of hydroxy pattern of demethylated lignin molecules calculated from ³¹P NMR after derivatization with CDP-Cl and using an internal standard – referring to the second batch of UPM BioPiva™ 300 used for processing.

Entry	Aliphatic-OH (mmol/g)	Ph-OH (mmol/g)
Demethylation – Sample 5	1.17	4.36
Demethylation – Sample 6	1.13	4.21
Demethylation – Sample 7	1.14	4.38
Demethylation – Sample 8	1.15	4.24
Average	1.15	4.30

The average phenolic OH-group content per gram of demethylated lignin is 4.16 ± 0.6 mmol/g. In general, each phenolic hydroxy group could belong to a catechol and a maximum of 2.08 ± 0.3 mmol/g catechols can be expected.

As model reaction DoxL was functionalized with ethanethiol according to the procedure in 3.5) to achieve TCCs. Using an internal standard (OMCTS, 70 mM) the amount of TCCs can be calculated quantitatively.

Table S4. Quantitative analysis of TCC groups after thiol-functionalization of lignin molecules calculated from ^1H NMR, using an internal standard. (Conditions: 66.6 mM demethylated lignin in DMF/acetate buffer (0.1 M, pH 5), r.t. °C, 2 h).

Entry	Periodate		TCC	
	Eq.	mmol/g lignin	mmol/g lignin	connections/molecule ^a
Sample 1	0.6	1.4	0.150	1
Sample 2	1	2.4	0.096	1
Sample 3	2	4.8	0.155	1

^a molecular weight from GPC measurements.

Table S5. Quantitative analysis of TCC groups after thiol-functionalization of lignin molecules calculated from ^1H NMR, using an internal standard. (Conditions: 66.6 mM demethylated lignin in DMF/acetate buffer (0.1 M, pH 5), 0 °C, 2 h).

Entry	Periodate		TCC	
	Eq.	mmol/g lignin	mmol/g lignin	connections/molecule ^a
Sample 4	0.6	1.4	0.541	3
Sample 5	1	2.4	0.556	3
Sample 6	1.5	3.6	0.641	4
Sample 7	2	4.8	0.563	3

^a molecular weight from GPC measurements.

According to procedure in 3.5) phenolic OH-group after thiol addition was determined by ^{31}P NMR analysis after derivatization with CDP-Cl and using an internal standard, resulting into 2.98 mmol phenolic-OH groups per gram lignin.

5.2 Thermal stability analysis

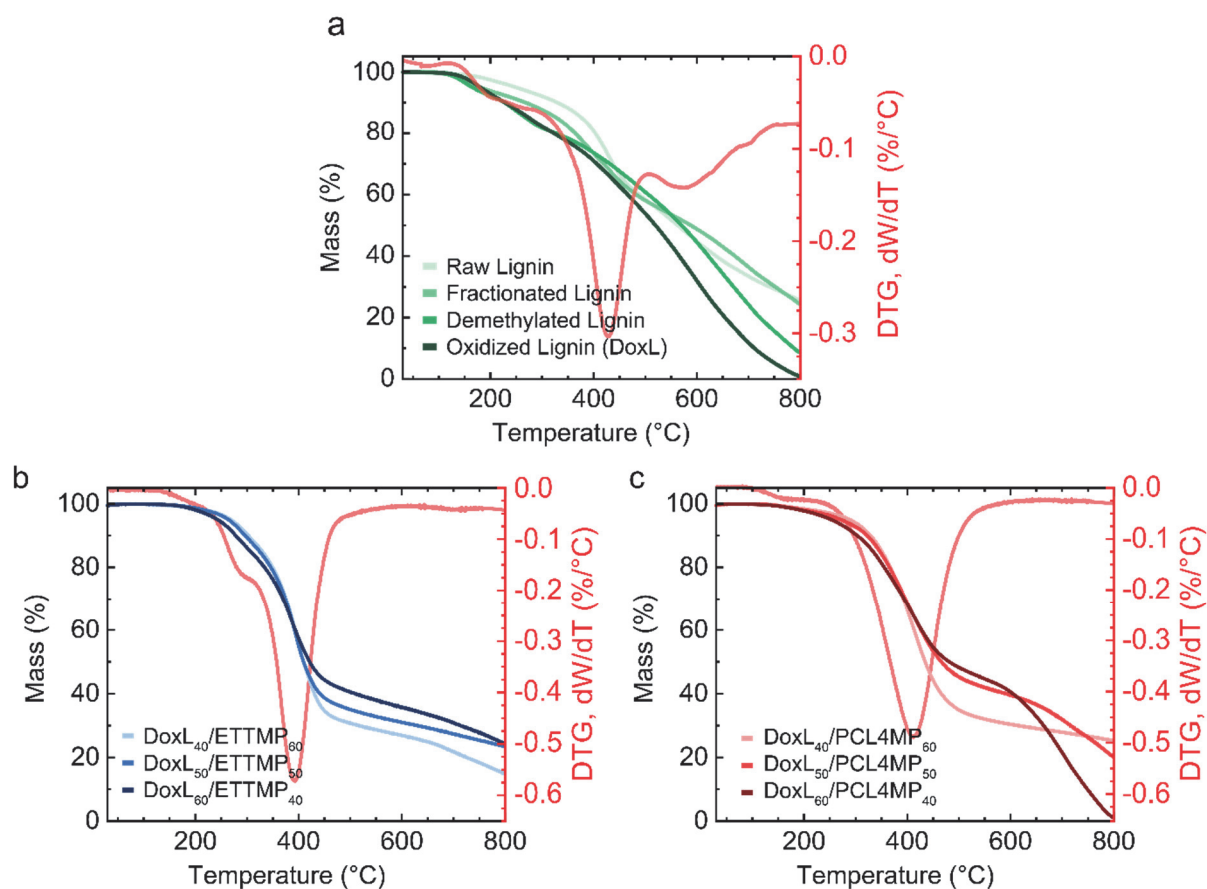


Figure S18. Thermogravimetric analysis data of the different processed and raw lignin samples (a), of the cured DoxL/ETTMP samples with different weight ratios, first derivative function of the weight loss for DoxL/ETTMP 40/60 is depicted in red on the right y-axis (b), of the cured DoxL/PCL4MP samples with different weight ratios, first derivative of DoxL₄₀/PCL4MP₆₀ is depicted in red on the right y-axis (c).

5.3 Thermal characterization

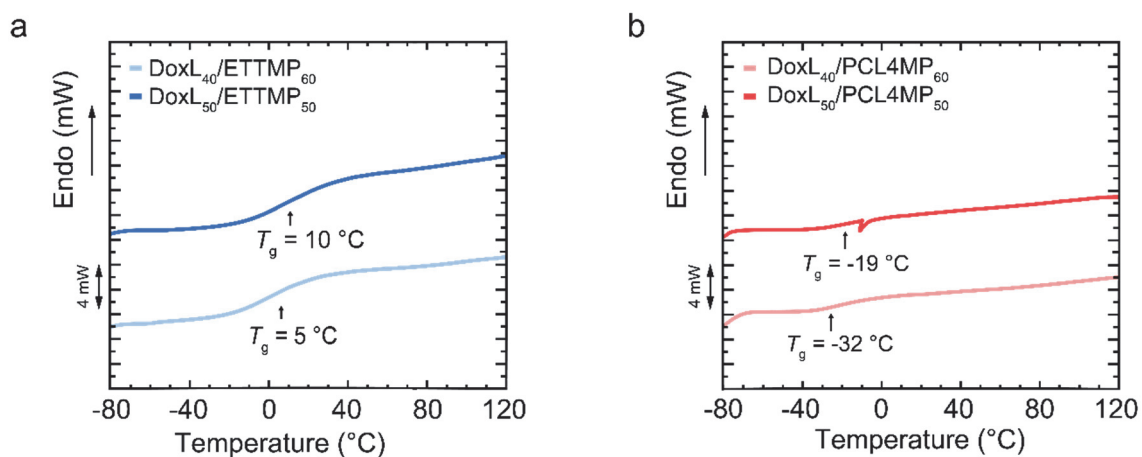


Figure S19. Differential scanning calorimetry analysis data of the cured DoxL/ETTMP samples with different weight ratios (a) and of the cured DoxL/PCL4MP samples with different weight ratios (b), T_g was determined using tangential evaluation.

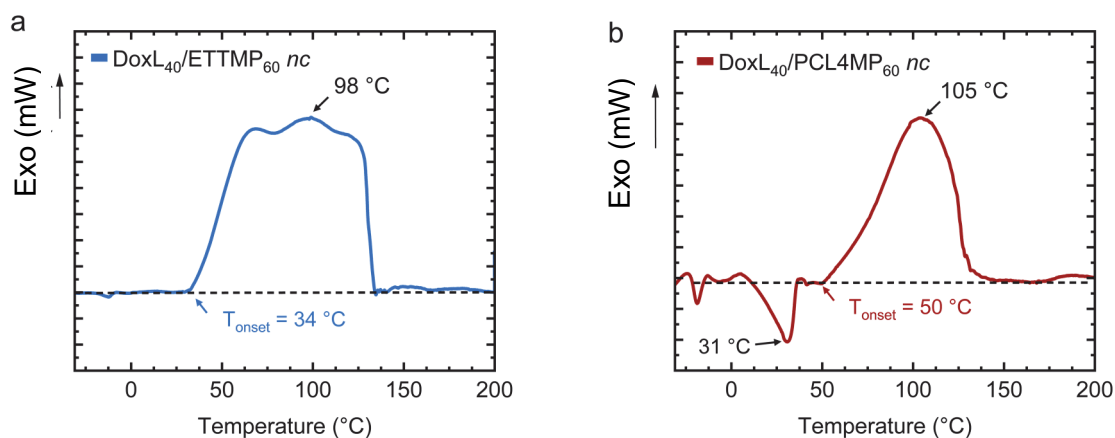


Figure S20. Differential scanning calorimetry analysis data of the non-cured (nc) DoxL₄₀/PCL4MP₆₀ sample (a) and of the non-cured DoxL₄₀/ETTMP₆₀ sample (b) T_{onset} was determined using tangential evaluation. Exothermic peak at 98 °C of non-cured DoxL₄₀/ETTMP₆₀. Exothermic peak at 105 °C of non-cured DoxL₄₀/PCL4MP₆₀ and endothermic peak at 31 °C as phase transition of PCL4MP. Heating curve: start at 40 °C, cool down to -40 °C (10 °C/min), equilibrate for 5 mins, heat to 200 °C (10 °C/min), cool to -40 °C (10 °C/min), heat to 200 °C (10 °C/min) and cool to 40 °C (10 °C/min).

5.4 FTIR analysis of chemical transformation in lignin

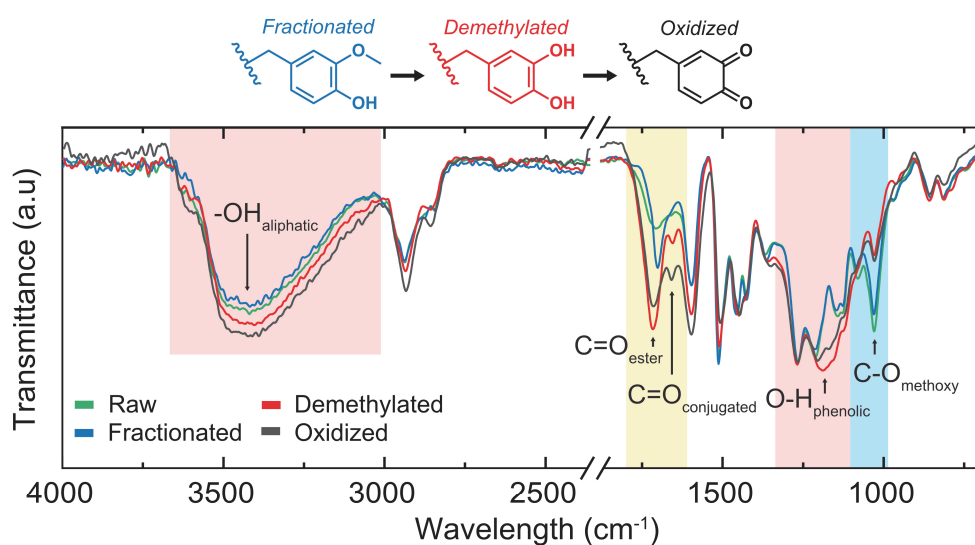


Figure S21. FTIR spectra for UPM BioPiva 300®, fractionated lignin, demethylated lignin and oxidized lignin (DoxL).

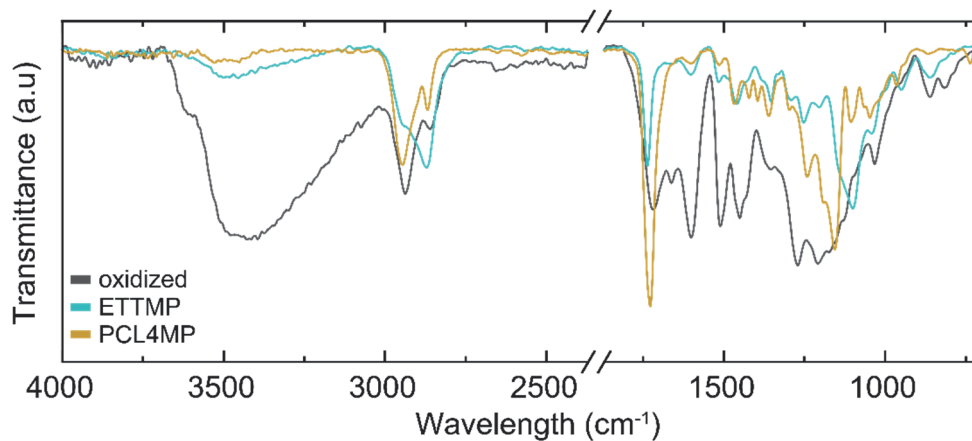


Figure S22. FTIR spectra for DoxL, pure ETTMP and PCL4MP.

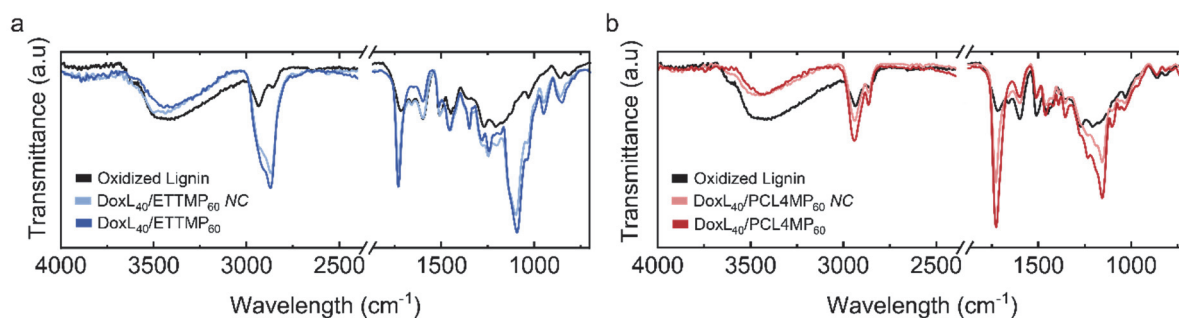


Figure S23. FTIR spectra for hydrophilic DoxL₄₀/ETTMP₆₀ before curing (NC) and after curing (a), and hydrophobic DoxL₄₀/PCL4MP₆₀ before (NC) and after curing (b). Oxidized lignin is added as reference for comparison (black spectrum).

5.5 Solid state ^{13}C MAS NMR analysis of lignin

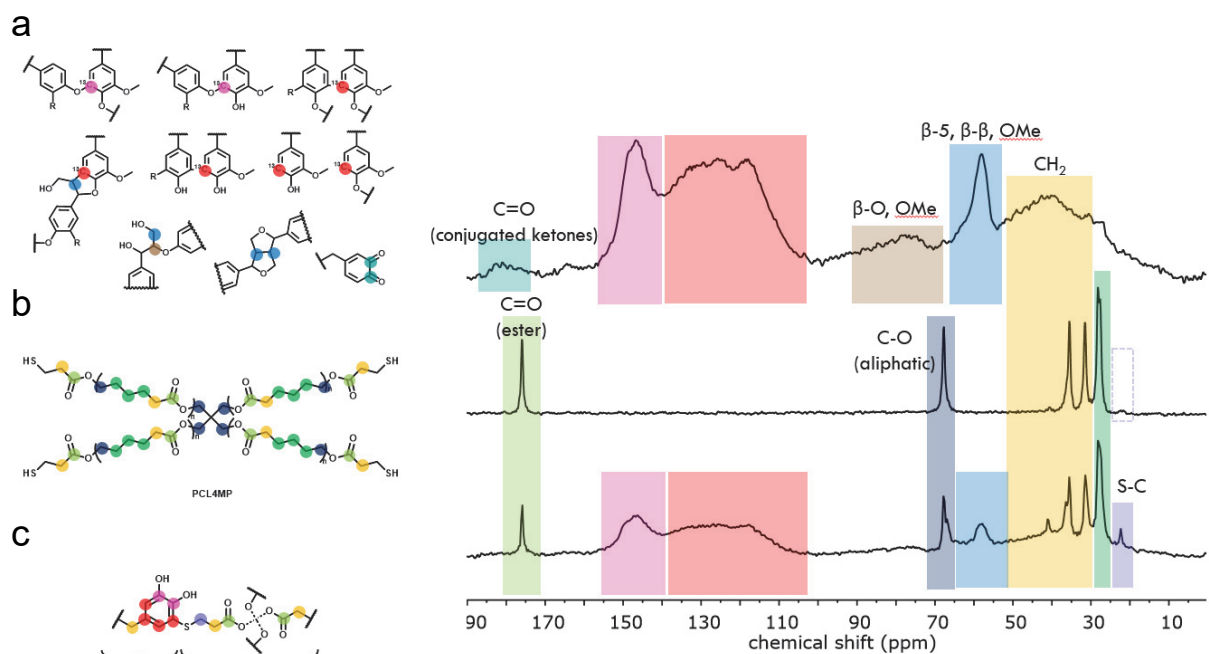


Figure S24. Solid-state hp.cp ^{13}C -NMR spectra of DoxL (a), PCL4MP (b) and the non-cured 2K adhesive (c). Rotor size: 3.2 mm, MAS frequency (sample rotation): 15 kHz, temperature: 293.8 K, hc.CP experiments with 10k scans.

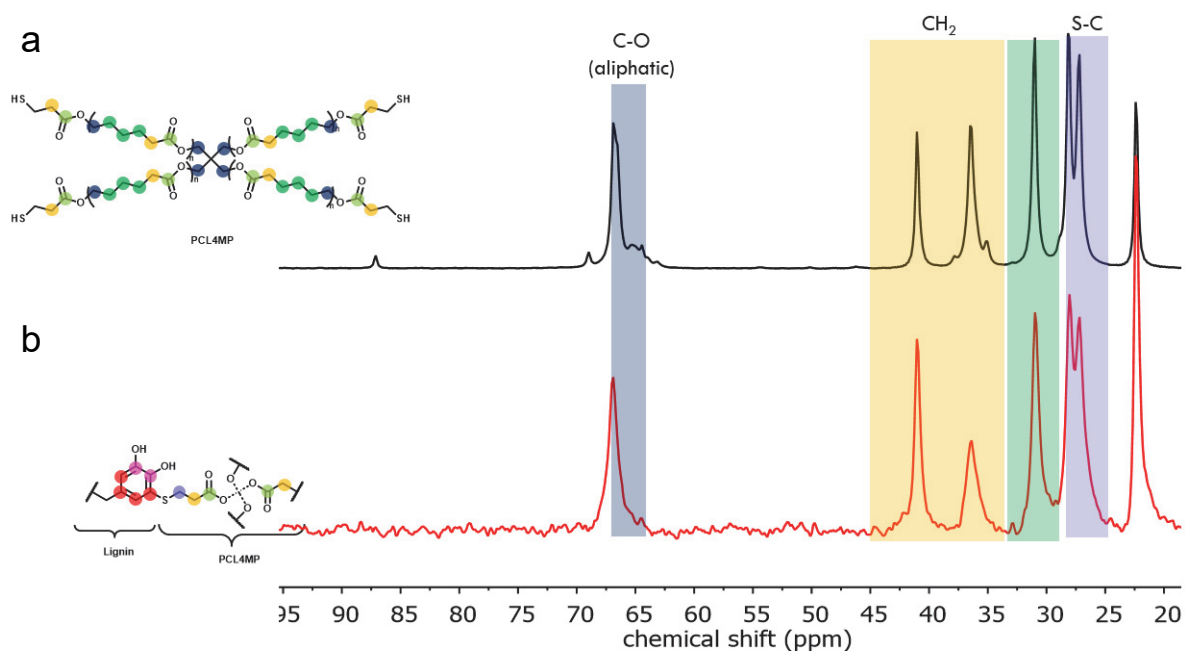


Figure S25. Solid-state INEPT ^{13}C -NMR spectra of PCL4MP (a) and the non-cured 2K adhesive (b). Rotor size: 3.2 mm, MAS frequency (sample rotation): 15 kHz, temperature: 293.8 K, INEPT experiments with 10k scans.

5.6 UV/vis and ^1H NMR analysis of leached content

The corresponding supernatants of DoxL₄₀/PCL4MP₆₀ were measured *via* UV-vis spectroscopy, distinct absorption bands at ≈ 300 nm (THF) and 265 nm (acetone) are observable, indicating the partial dissolving of simply to the lignin network attached parts. If reducing agents are added to the leaching solutions the absorption increased by the same concentration. The UV spectrum of the supernatant of DoxL₄₀/PCL4MP₆₀ indicating no dissolving of the cured system under aqueous conditions, unlike in DoxL₄₀/ETTMP₆₀ where the absorption of the multithiol is apparent. In ^1H NMR studies both extracts showing almost undetectable amount of lignin.

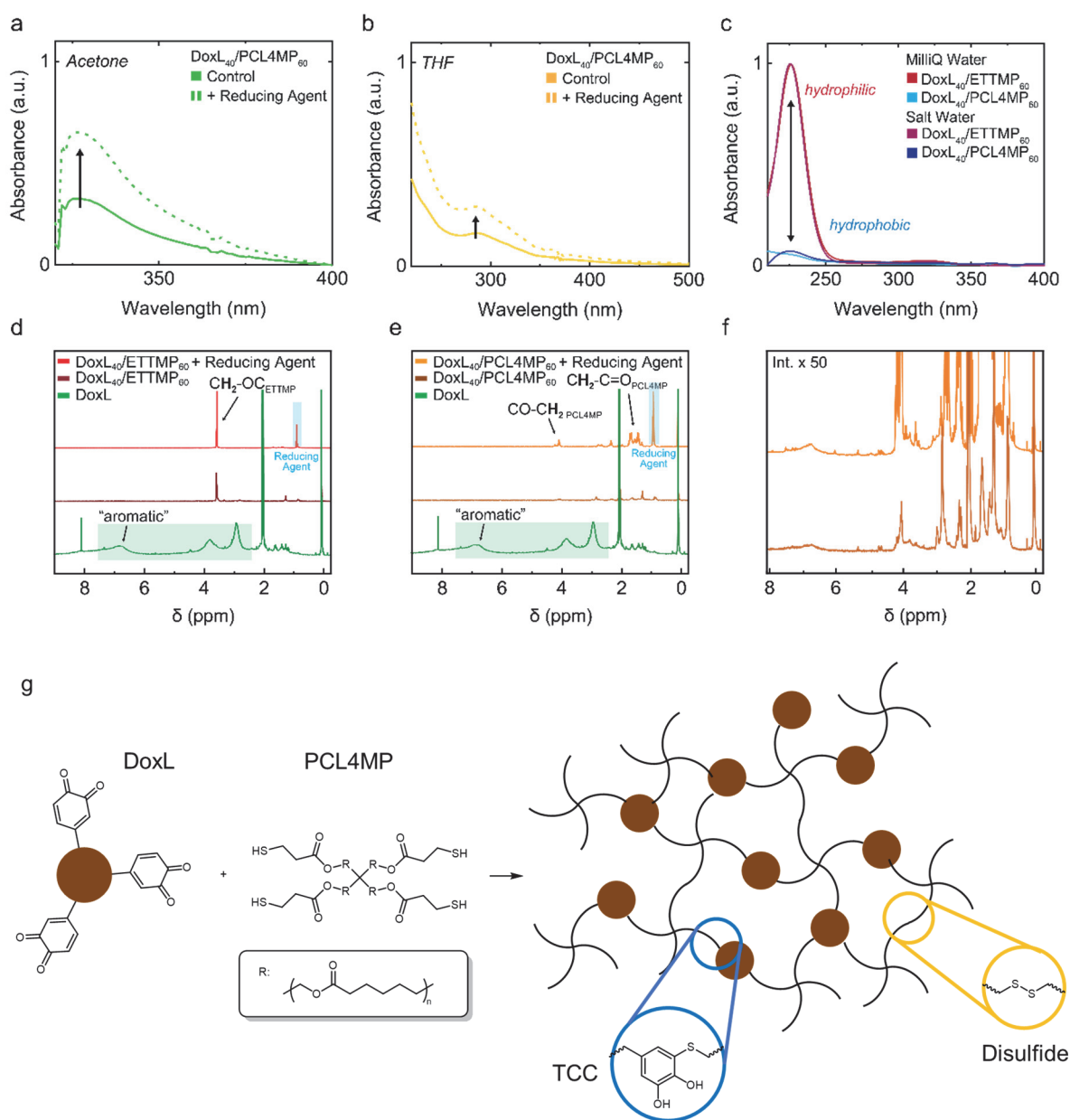


Figure S26. UV-vis spectra of supernatant from leaching tests of the 2K systems in acetone (diluted 1:5) (a), in THF (1:50) (b) and in water and saltwater without dilution (c). Superimposed ^1H NMR spectra of the supernatant from the leaching test of DoxL₄₀/ETTMP₆₀ (d) and DoxL₄₀/PCL4MP₆₀ (e) in acetone-d₆. The section of the ^1H NMR spectrum of DoxL₄₀/PCL4MP₆₀ was displayed in 50x enlargement to showcase the minor presence of lignin (f). Idealized chemical structure showing the cross-linking motifs expected in 2K DoxL/PCL4MP adhesive (g).

5.7 Dry adhesion 2K protocol of lignin glues in different mass ratios

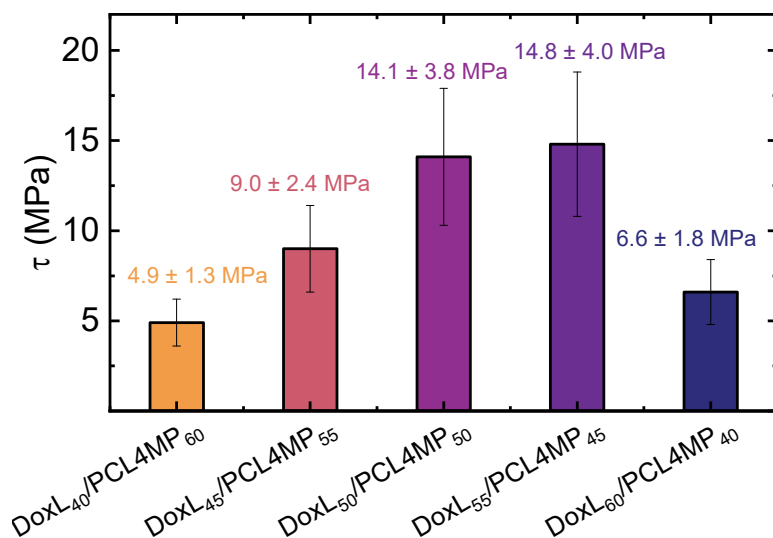


Figure S27. Dry adhesion tests for DoxL₄₀₋₆₀/PCL4MP₆₀₋₄₀ figured as bar chart. Curing conditions: 60 °C, overnight.

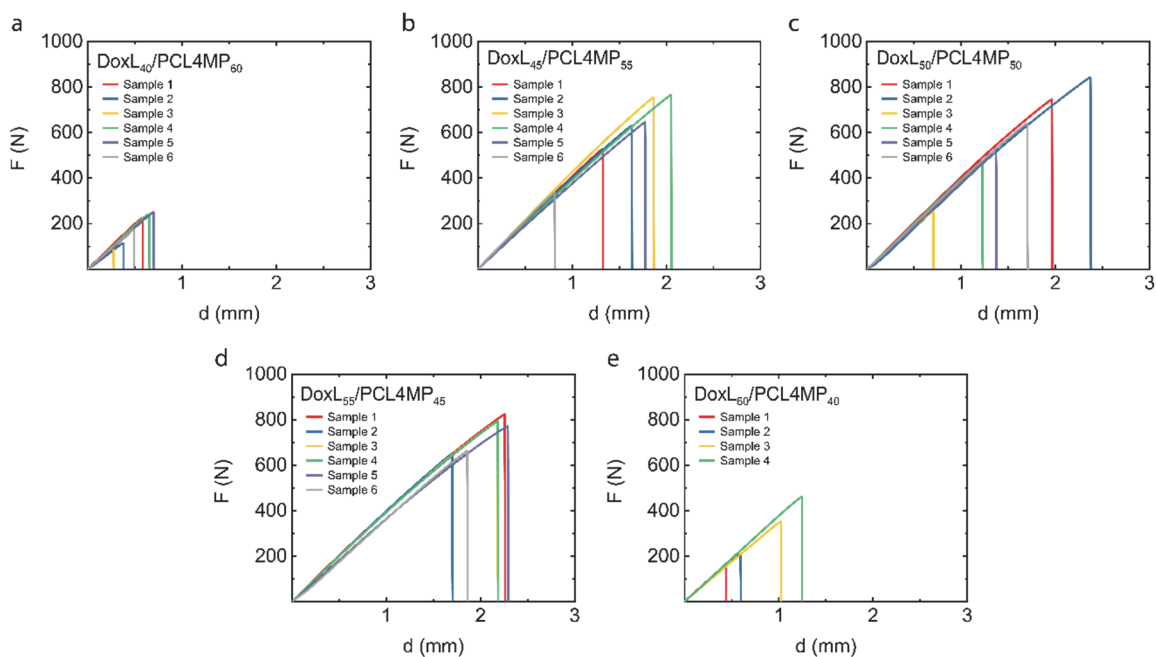


Figure S28. Dry adhesion tests for DoxL₄₀/PCL4MP₆₀ (a), DoxL₄₅/PCL4MP₅₅ (b), (c) DoxL₅₀/PCL4MP₅₀ (c), DoxL₅₅/PCL4MP₄₅ (d) and DoxL₆₀/PCL4MP₄₀ (e) figured as force-distance curves. Curing conditions: 60 °C, overnight.

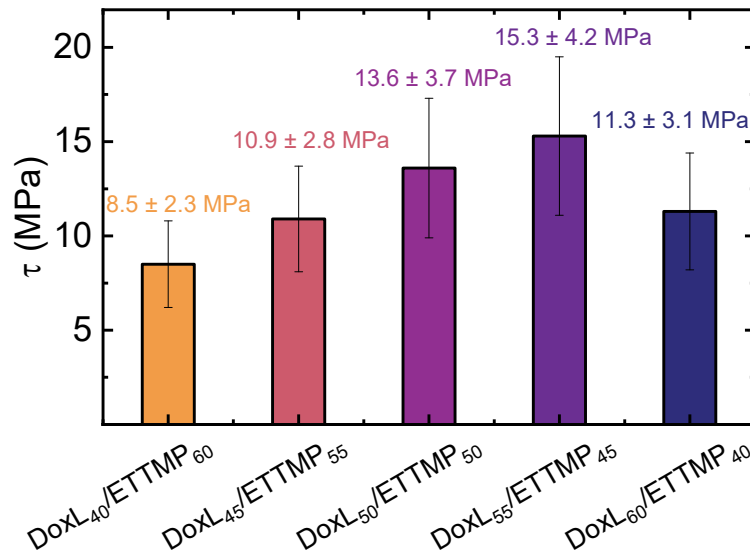


Figure S29. Dry adhesion tests for DoxL₄₀₋₆₀/ETTTP₆₀₋₄₀ figured as bar chart. Curing conditions: 60 °C, overnight.

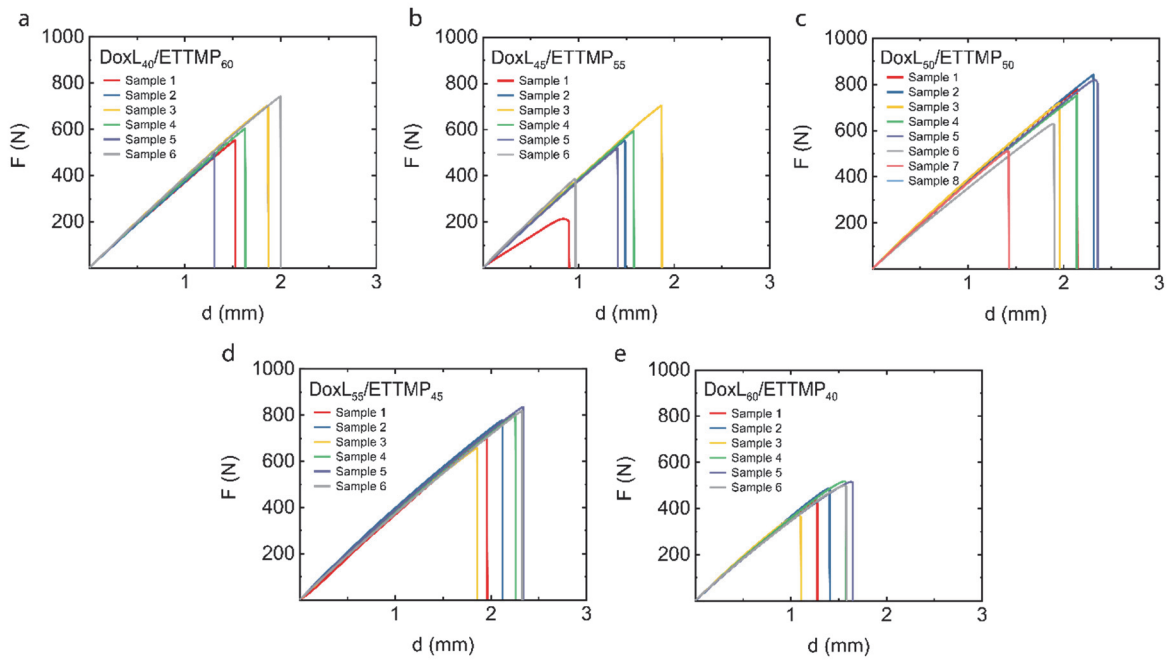


Figure S30. Dry adhesion tests for DoxL₄₀/ETTTP₆₀ (a), DoxL₄₅/ETTTP₅₅ (b), (c) DoxL₅₀/ETTTP₅₀ (c), DoxL₅₅/ETTTP₄₅ (d) and DoxL₆₀/ETTTP₄₀ (e) figured as force-distance curves. Curing conditions: 60 °C, overnight.

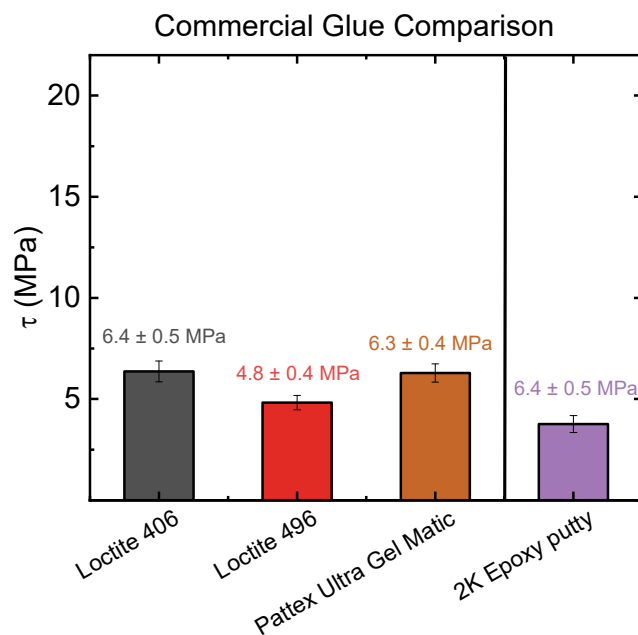


Figure S31. Dry adhesion tests for commercial glues, covering Pattex® and Loctite® product families and epoxy-based coral's glue figured as bar chart. Application and curing in accordance with the manufacturer's instructions.

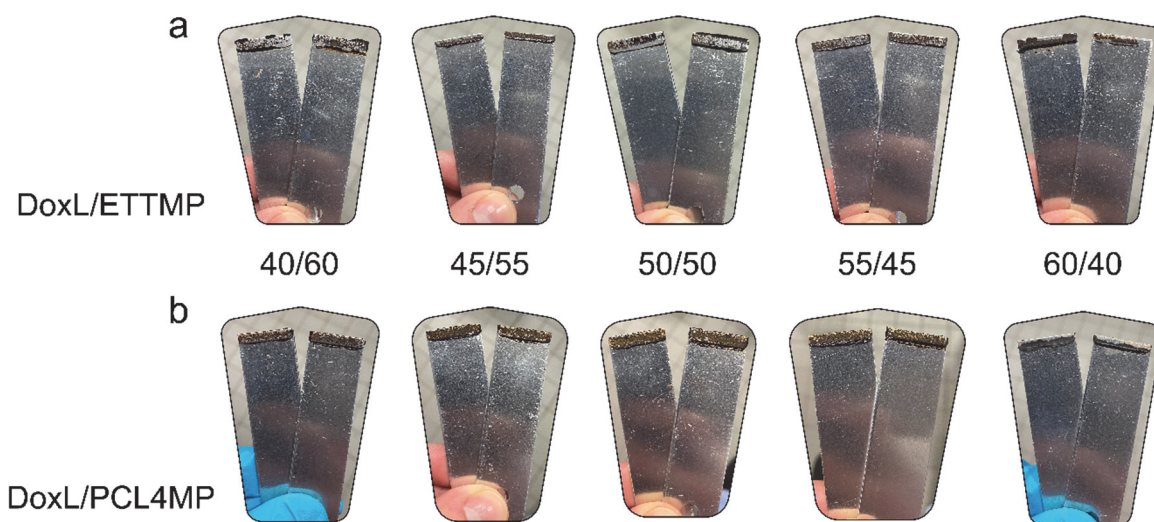


Figure S32. Image of rupture DoxL/ETTMP (a) and (b) DoxL/PCL4MP (b) for different weight ratios. Cohesive and adhesive failure is observed for lignin/thiol weight ratios of 40/60, 45/55, 50/50 and 55/45. Adhesive failure is only observed for a lignin/thiol weight ratio of 60/40.

5.8 Underwater adhesion 2K protocol

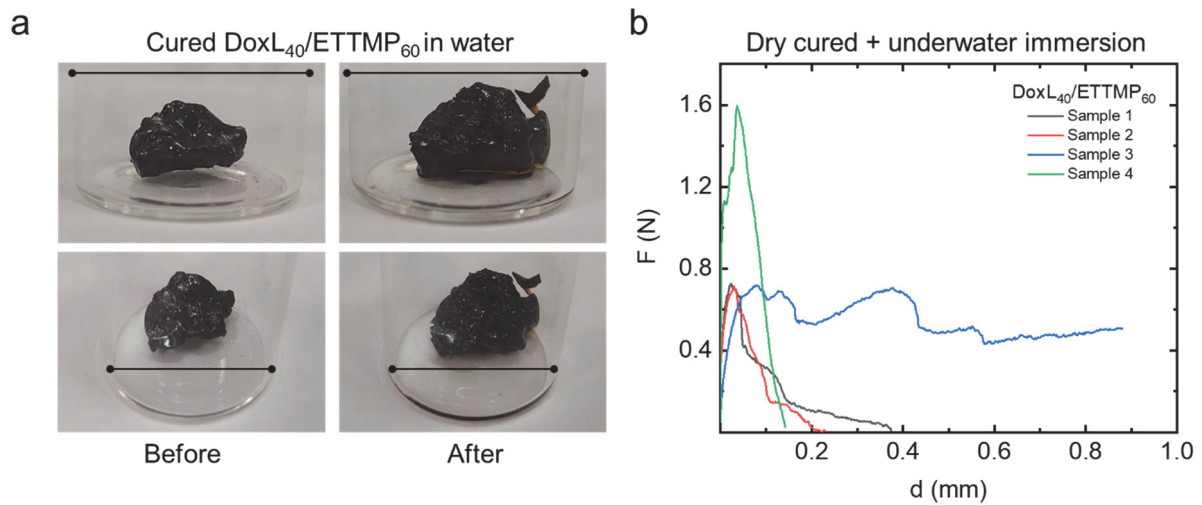


Figure S33. Comparison of cured DoxL₄₀/ETTMP₆₀ before and after leaching in water for 7 days (a) and adhesion test of dry cured DoxL₄₀/ETTMP₆₀ after immersion in water.

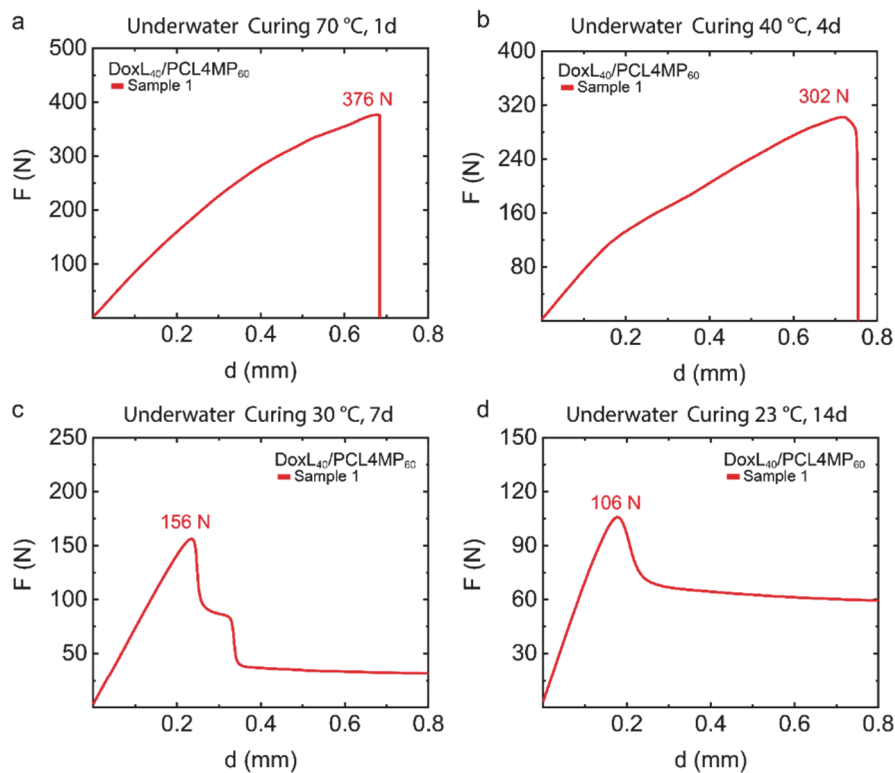
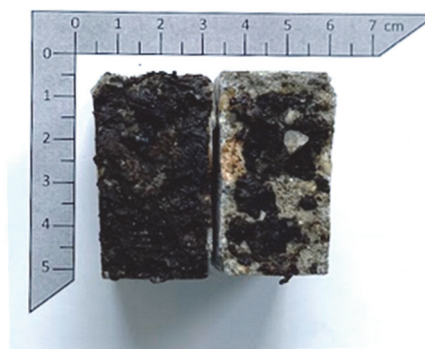


Figure S34. Initial underwater adhesion tests of glued fractured concrete stones using DoxL₄₀/PCL4MP₆₀ as adhesive and cured under saltwater conditions at 70 °C for 1 day (a), 40 °C for 4 days (b), 30 °C for 7 days (c) and 23 °C for 14 days (d), figured as force distance curves. Note that used test specimens are not standardized therefore a bonding area could not be determined.

a Underwater Curing 70 °C, 1d



b Underwater Curing 40 °C, 4d



c Underwater Curing 30 °C, 7d



d Underwater Curing 23 °C, 14d

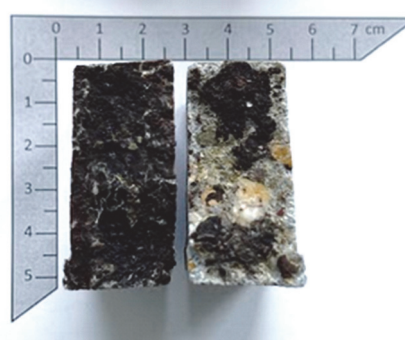


Figure S35. Image of fracture patterns of glued concrete stones using DoxL₄₀/PCL4MP₆₀ as adhesive and cured under saltwater conditions at 70 °C for 1 day (a), 40 °C for 4 days (b), 30 °C for 7 days (c) and 23 °C for 14 days (d). Ruler was displayed with as a reference.

The live coral gluing project underwent internal examination by the animal welfare officer and was classified as a "project not requiring approval under the Animal Welfare Act." (internal label: StN-HU-01/22) A notification in accordance with the Animal Welfare Laboratory Animal Ordinance (*Tierschutz-Versuchstierverordnung, TierSchVersV*) was therefore not required.

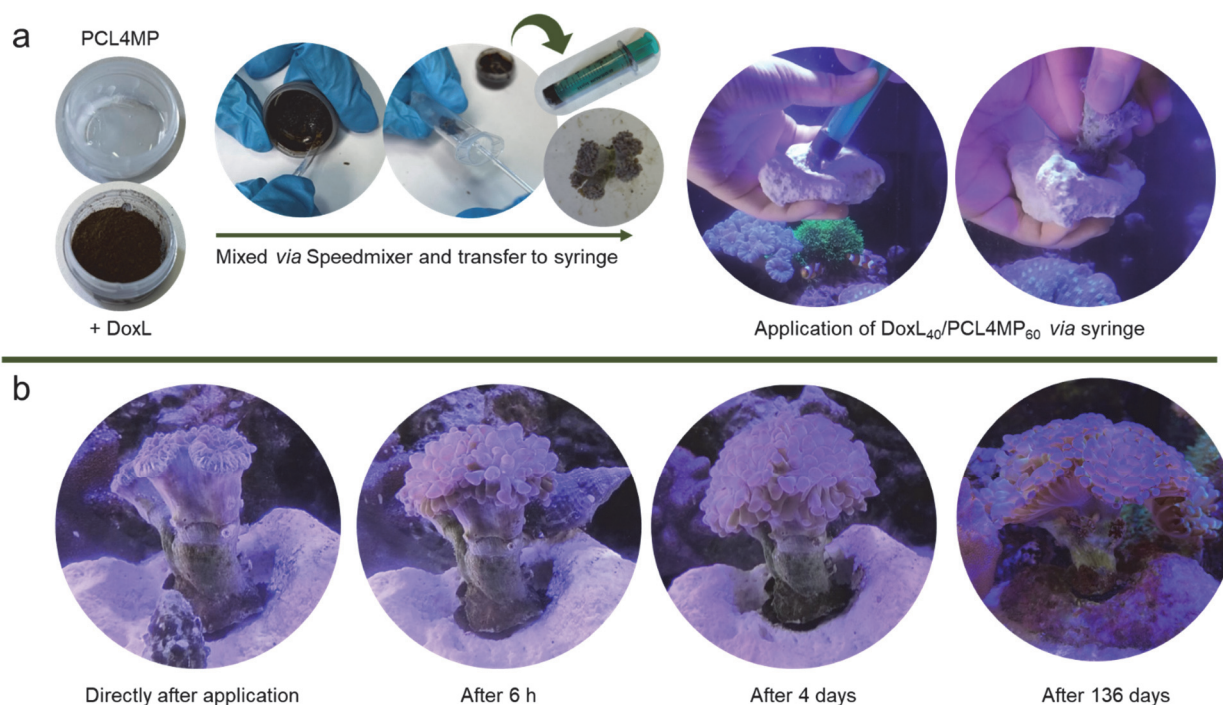


Figure S36: Underwater bonding of living coral with DoxL₄₀/PCL4MP₆₀. Illustration of the procedure to prepare the 2K adhesive and the application of the living coral on aragonite stone (a). Images of the coral after direct setting, after 6 h and after 4 days and 136 days (b).

6. References

- 1 J. Cavanagh, *Protein NMR spectroscopy: principles and practice*, Academic press, **1996**.
- 2 B. Fung, A. Khitrin, K. Ermolaev, *Journal of magnetic resonance* 2000, **142**, 97-101.
- 3 A. Pines, M. Gibby, J. Waugh, *The Journal of Chemical Physics* 1972, **56**, 1776-1777.
- 4 A. Bax, G. A. Morris, *Journal of Magnetic Resonance (1969)* 1981, **42**, 501-505.
- 5 K. Sawamura, Y. Tobimatsu, H. Kamitakahara, T. Takano, *ACS Sustain. Chem. Eng.* 2017, **5**, 5424-5431.
- 6 M. Rasche, *Ahäsion* 1990 **11**, 36-43.