

# Efficient mild high $\beta$ -O-4 organosolv lignin extraction in a flow setup yielding lignin with high $\beta$ -O-4 content

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## 1. Methodologies

### Yield correction for alcohol incorporation

To compensate the yield for the alcohol incorporation into the lignin, a correction factor is calculated. First the mass of the different monolignols is determined.

|   | No incorporation | EtOH | nPrOH | nBuOH |
|---|------------------|------|-------|-------|
| S | 226              | 254  | 268   | 282   |
| G | 196              | 224  | 238   | 252   |
| H | 166              | 194  | 208   | 222   |

Table S1: Mass of the monolignols with solvent incorporation.

For every monolignol an individual correction factor needs to be calculated. This is the relative mass of the lignin that belongs to the incorporated solvent, for example for a S unit with EtOH incorporation.

$$\text{Correction factor } S = \frac{\text{Mass (S } \beta' - \text{O} - 4)}{\text{Mass (S } \beta - \text{O} - 4)} - 1 = \frac{254}{226} - 1 = 0.124$$

|   | EtOH  | nPrOH | nBuOH |
|---|-------|-------|-------|
| S | 0.124 | 0.186 | 0.248 |
| G | 0.143 | 0.214 | 0.286 |
| H | 0.169 | 0.253 | 0.337 |

Table S2: Correction factors for determination of the corrected yield.

Together with the fractions S, G and H, the corrected yield can be calculated.

For example, for a lignin fraction extracted with EtOH:

Corrected yield (mg) =

$$\text{Experimental yield (mg)} * \left(1 - \frac{\beta' - \text{O} - 4}{100}\right) * (0.124 * \text{fraction S} + 0.143 * \text{fraction G} + 0.169 * \text{fraction H})$$

### Calculations of average value

$$\text{Average value} = \frac{\text{mass of fraction 1} * \text{corresponding value 1} + \text{mass of frac 2} * \text{corr value} + \dots}{\text{Sum of the total mass of the fractions}}$$

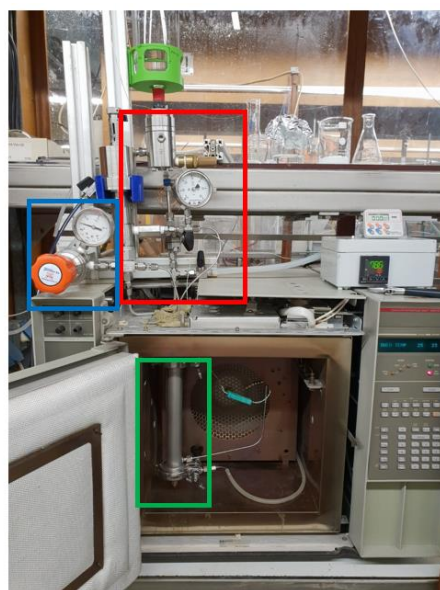
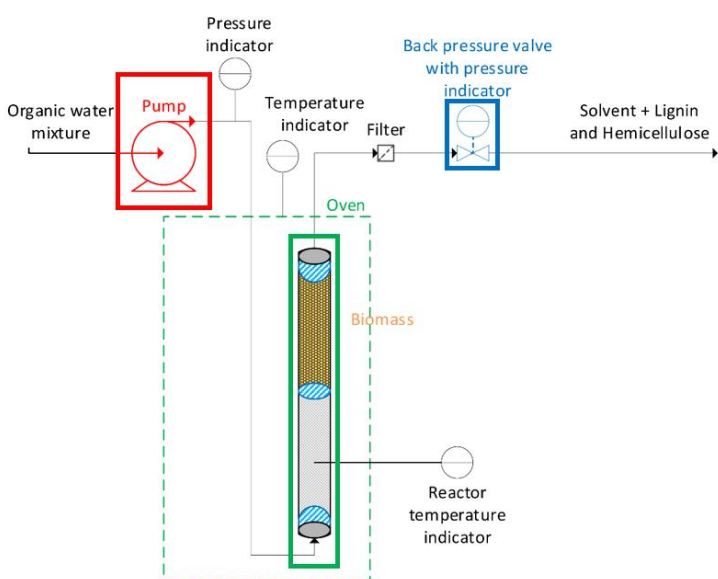
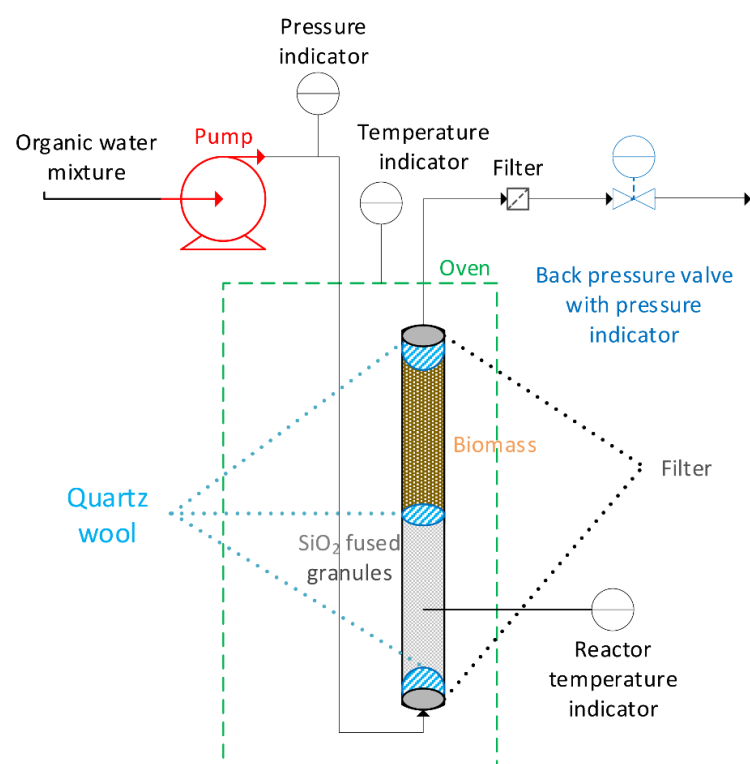
Formula has been applied to calculate the average condensation,  $\beta$ -O-4 content, H/G/S ratio and molecular weight.

### Data representation

All the graphs are made with OriginPro 8.5. All data fits for the extraction efficiency are done with nonlinear curve fitting, as are most of the data fits for the  $\beta$ -O-4 content over time. If an insufficient fit was obtained a second order polynomial fit was used for fitting.

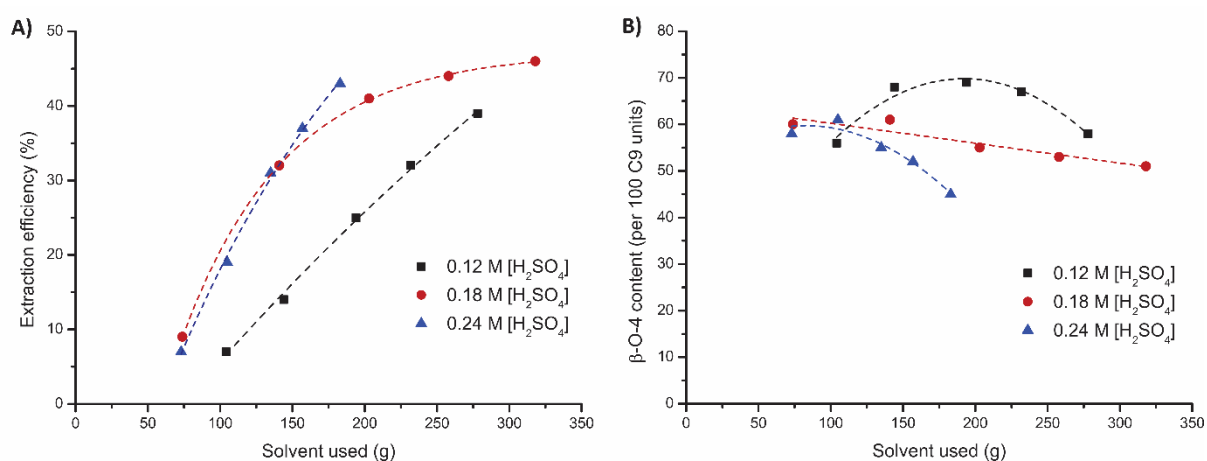
## Flow-through setup

The flow-through system consists of a glass solvent reservoir (800mL), pneumatic oscillatory pump (Williams; P250V225), pressure indicator (0-400 bar), GC oven (HP, 5890 series II), reactor (100 mL, cylindrical (length: 20 cm, diameter: 2,6 cm), SS 316L), Type K thermocouple with indicator, particulate filter (Swagelok, ¼ in. tube fitting, 2 micron pore size), adjustable back pressure regulator (Gommer B.V., BP-3 series, SS 316L) with pressure indicator (0-40 bar) and precision balance (Kern, PCB 2500-2). These units are connected by tubes and fittings (Swagelok, SS-316L, ½ and ¼ in.). Both openings of the reactor are closed with a glass filter (custom made, sintered, P2).



**Figure S1.** Schematic representation of the developed flow-through setup (**top**) and a picture of the setup with the most important features highlighted (**bottom**).

## Extraction efficiency and $\beta$ -O-4 content vs Solvent used



**Figure S2.** Influence of the different  $\text{H}_2\text{SO}_4$  concentrations on the mild organosolv extraction, (80:20 EtOH/ $\text{H}_2\text{O}$ , 120 °C, 5 hours) on **A)** extraction efficiency (corrected for alcohol incorporation as determined by 2D HSQC) and **B)** total  $\beta$ -O-4 content as determined by 2D HSQC, plotted against solvent used.

## Extraction results

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 1157       | 13                                       | 69                            | 17                        | 8/44/48         | 0                  | 61                              | 28                        | 33                         | 874                  | 1530                 |
| 2            | 1691       | 31                                       | 138                           | 25                        | 2/34/64         | 0                  | 66                              | 24                        | 42                         | 982                  | 2230                 |
| 3            | 1103       | 43                                       | 206                           | 16                        | 1/31/68         | 2.5                | 60                              | 17                        | 43                         | 1080                 | 2690                 |
| 4            | 585        | 49                                       | 272                           | 9                         | 0/31/69         | 4                  | 62                              | 16                        | 45                         | 1110                 | 2970                 |
| 5            | 268        | 52                                       | 339                           | 4                         | 0/31/69         | 5                  | 55                              | 16                        | 38                         | 1060                 | 2730                 |

**Table S1.** Extraction efficiency and lignin properties of Entry 1 (EtOH/H<sub>2</sub>O 80:20, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]). <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 663        | 7                                        | 104                           | 6                         | 7/49/44         | 0                  | 56                              | 38                        | 18                         | 1100                 | 1920                 |
| 2            | 668        | 14                                       | 144                           | 16                        | 3/40/57         | 1                  | 68                              | 28                        | 40                         | 1320                 | 2390                 |
| 3            | 1073       | 25                                       | 194                           | 22                        | 1/35/64         | 0                  | 69                              | 25                        | 44                         | 1420                 | 2970                 |
| 4            | 684        | 32                                       | 232                           | 18                        | 0/32/68         | 1                  | 67                              | 24                        | 43                         | 1500                 | 3560                 |
| 5            | 711        | 39                                       | 278                           | 15                        | 0/31/69         | 3                  | 58                              | 17                        | 41                         | 1730                 | 4280                 |

**Table S2.** Extraction efficiency and lignin properties of Entry 2 (EtOH/H<sub>2</sub>O 80:20, 0.12 M [H<sub>2</sub>SO<sub>4</sub>]) \*Different walnut source. <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 829        | 9                                        | 74                            | 11                        | 6/45/49         | 2                  | 60                              | 27                        | 33                         | 1250                 | 2300                 |
| 2            | 2078       | 32                                       | 141                           | 31                        | 1/34/65         | 2                  | 61                              | 18                        | 42                         | 1390                 | 3660                 |
| 3            | 892        | 41                                       | 203                           | 14                        | 0/32/68         | 4                  | 55                              | 18                        | 36                         | 1580                 | 4580                 |
| 4            | 292        | 44                                       | 258                           | 5                         | 0/32/68         | 5.5                | 53                              | 12                        | 41                         | 1680                 | 4790                 |
| 5            | 159        | 46                                       | 318                           | 3                         | 0/31/69         | 10                 | 51                              | 14                        | 37                         | 1870                 | 5720                 |

**Table S3.** Extraction efficiency and lignin properties of Entry 3 (EtOH/H<sub>2</sub>O 80:20, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]) \*Different walnut source. <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 672        | 7                                        | 73                            | 9                         | 8/47/45         | 0                  | 58                              | 33                        | 25                         | 980                  | 1680                 |
| 2            | 1050       | 19                                       | 105                           | 33                        | 1/39/60         | 0                  | 61                              | 19                        | 42                         | 1180                 | 2740                 |
| 3            | 1133       | 31                                       | 135                           | 38                        | 1/31/68         | 7                  | 55                              | 14                        | 41                         | 1510                 | 3680                 |
| 4            | 575        | 37                                       | 157                           | 26                        | 0/30/70         | 7                  | 52                              | 12                        | 40                         | 1600                 | 4130                 |
| 5            | 501        | 43                                       | 183                           | 19                        | 0/30/70         | 10.5               | 45                              | 8                         | 37                         | 1510                 | 3900                 |

**Table S4.** Extraction efficiency and lignin properties of Entry 4 (EtOH/H<sub>2</sub>O 80:20, 0.24 M [H<sub>2</sub>SO<sub>4</sub>]) \*Different walnut source. <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 729        | 8                                        | 87                            | 8                         | 7/44/49         | 0                  | 51                              | 27                        | 24                         | 961                  | 1800                 |
| 2            | 1373       | 23                                       | 141                           | 25                        | 2/32/66         | 2.5                | 55                              | 27                        | 28                         | 783                  | 1600                 |
| 3            | 1027       | 34                                       | 196                           | 19                        | 0/31/69         | 5                  | 48                              | 22                        | 25                         | 804                  | 1680                 |
| 4            | 181        | 36                                       | 231                           | 5                         | 0/33/67         | 6.5                | 51                              | 22                        | 29                         | 815                  | 1880                 |

**Table S5.** Extraction efficiency and lignin properties of Entry 5 (EtOH/H<sub>2</sub>O 50:50, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]) <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 1046       | 11                                       | 55                            | 19                        | 4/46/50         | 0                  | 62                              | 10                        | 52                         | 1130                 | 2520                 |
| 2            | 2197       | 35                                       | 114                           | 37                        | 1/31/68         | 9.5                | 53                              | 5                         | 48                         | 1320                 | 3710                 |
| 3            | 1360       | 50                                       | 172                           | 23                        | 0/23/77         | 20.5               | 32                              | 0                         | 32                         | 1150                 | 3280                 |
| 4            | 528        | 56                                       | 231                           | 9                         | 0/31/69         | 22                 | 9                               | 0                         | 9                          | 1160                 | 3040                 |
| 5            | 141        | 57                                       | 290                           | 2                         | 0/28/72         | 25.5               | 11                              | 0                         | 11                         | 1210                 | 3050                 |

**Table S6.** Extraction efficiency and lignin properties of Entry **6** (EtOH/H<sub>2</sub>O 95:5, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]) <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage S<sub>condensed</sub> of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 1095       | 12                                       | 84                            | 13                        | 7/44/49         | 0                  | 58                              | 9                         | 49                         | 1030                 | 2290                 |
| 2            | 1991       | 33                                       | 147                           | 31                        | 3/33/64         | 0                  | 72                              | 19                        | 53                         | 1110                 | 2660                 |
| 3            | 1071       | 45                                       | 200                           | 20                        | 1/30/69         | 0                  | 65                              | 11                        | 53                         | 1390                 | 4690                 |
| 4            | 726        | 53                                       | 258                           | 13                        | 1/30/69         | 2.5                | 64                              | 9                         | 55                         | 1460                 | 5810                 |
| 5            | 400        | 57                                       | 321                           | 6                         | 0/27/73         | 2                  | 66                              | 7                         | 59                         | 1400                 | 5690                 |

**Table S7.** Extraction efficiency and lignin properties of Entry **7** (nPrOH/H<sub>2</sub>O 80:20, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]) <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage S<sub>condensed</sub> of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 1926       | 21                                       | 59                            | 33                        | 4/39/57         | 0                  | 68                              | 5                         | 63                         | 1310                 | 4500                 |
| 2            | 2472       | 48                                       | 113                           | 45                        | 0/25/75         | 8.5                | 57                              | 2                         | 55                         | 1400                 | 6650                 |
| 3            | 2260       | 72                                       | 207                           | 24                        | 0/27/73         | 20                 | 41                              | 0                         | 41                         | 1320                 | 5440                 |
| 4            | 132        | 74                                       | 253                           | 3                         | 0/32/68         | 17                 | 32                              | 0                         | 32                         | 1360                 | 4980                 |

**Table S8.** Extraction efficiency and lignin properties of Entry **8** (nBuOH/H<sub>2</sub>O/1,4-dioxane 80:15:5, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]) <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage S<sub>condensed</sub> of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 1860       | 19                                       | 58                            | 32                        | 6/41/53         | 0                  | 58                              | 20                        | 38                         | 1130                 | 2061                 |
| 2            | 2010       | 39                                       | 125                           | 30                        | 2/32/66         | 6.5                | 55                              | 14                        | 41                         | 1379                 | 3042                 |
| 3            | 950        | 48                                       | 185                           | 16                        | 0/32/68         | 10.5               | 53                              | 12                        | 41                         | 1365                 | 2963                 |
| 4            | 380        | 52                                       | 242                           | 7                         | 0/32/68         | 11.5               | 50                              | 10                        | 40                         | 1413                 | 3200                 |
| 5            | 100        | 53                                       | 301                           | 2                         | 0/26/74         | 13.5               | 43                              | 10                        | 33                         | 1408                 | 3250                 |

**Table S9.** Extraction efficiency and lignin properties of Entry **9** (nEtOH/H<sub>2</sub>O/1,4-dioxane 80:15:5, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]) <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage S<sub>condensed</sub> of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 1165       | 12                                       | 44                            | 26                        | 5/42/53         | 0                  | 63                              | 19                        | 44                         | 1138                 | 2157                 |
| 2            | 2581       | 35                                       | 95                            | 51                        | 1/33/66         | 9.5                | 59                              | 14                        | 45                         | 1351                 | 3224                 |
| 3            | 1402       | 52                                       | 148                           | 26                        | 0/32/68         | 18.5               | 46                              | 10                        | 36                         | 1339                 | 3302                 |
| 4            | 516        | 58                                       | 203                           | 9                         | 0/26/74         | 24                 | 42                              | 4                         | 38                         | 1374                 | 3479                 |
| 5            | 110        | 59                                       | 247                           | 3                         | 0/27/73         | 24.5               | 39                              | 4                         | 35                         | 1295                 | 3032                 |

**Table S10.** Extraction efficiency and lignin properties of Entry **10** (nPrOH/H<sub>2</sub>O/1,4-dioxane 80:15:5, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]) <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage S<sub>condensed</sub> of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 0.5          | 179        | 1                                        | 28                            | 6                         | 15/49/36        | 0                  | 59                              | 21                        | 38                         | 778                  | 1350                 |
| 1            | 2116       | 12                                       | 61                            | 64                        | 8/44/48         | 0                  | 62                              | 13                        | 49                         | 962                  | 1760                 |
| 1.5          | 4498       | 37                                       | 86                            | 179                       | 2/32/66         | 6                  | 59                              | 6                         | 53                         | 1180                 | 2950                 |
| 2            | 3920       | 58                                       | 129                           | 92                        | 0/30/70         | 15                 | 40                              | 3                         | 37                         | 1190                 | 3350                 |
| 2.5          | 1623       | 67                                       | 164                           | 46                        | 0/36/64         | 14                 | 36                              | 1                         | 35                         | 1170                 | 3200                 |
| 3            | 764        | 71                                       | 194                           | 26                        | 0/32/68         | 17.5               | 31                              | 0                         | 31                         | 1170                 | 5990                 |
| 3.5          | 469        | 74                                       | 215                           | 22                        | 0/29/71         | 18                 | 30                              | 0                         | 30                         | 1160                 | 2950                 |
| 4            | 661        | 77                                       | 247                           | 20                        | 0/36/64         | 16                 | 18                              | 0                         | 18                         | 1120                 | 2740                 |
| 4.5          | 517        | 80                                       | 279                           | 17                        | 0/34/66         | 18                 | 15                              | 0                         | 15                         | 1100                 | 2730                 |
| 5            | 452        | 82                                       | 313                           | 23                        | 0/32/68         | 18                 | 12                              | 0                         | 12                         | 1080                 | 2650                 |

**Table S11.** Extraction efficiency and lignin properties of Entry **11** (EtOH/H<sub>2</sub>O 95:5, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], 40 gram loading). <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 246        | 3                                        | 62                            | 4                         | 0/85/15         | 0                  | 58                              | 10                        | 48                         | 1090                 | 2590                 |
| 2            | 570        | 11                                       | 117                           | 10                        | 0/82/18         | 0                  | 66                              | 5                         | 61                         | 1410                 | 3670                 |
| 3            | 392        | 17                                       | 169                           | 8                         | 0/82/18         | 0                  | 53                              | 4                         | 49                         | 1370                 | 3580                 |
| 4            | 189        | 19                                       | 229                           | 3                         | 0/88/12         | 0                  | 44                              | 4                         | 40                         | 1340                 | 3360                 |
| 5            | 72         | 20                                       | 290                           | 1                         | 0/100/0         | 0                  | 37                              | 2                         | 35                         | 1350                 | 3310                 |

**Table S12.** Extraction efficiency and lignin properties of Entry **12** (EtOH/H<sub>2</sub>O 95:5, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], Spruce/poplar). <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 896        | 9                                        | 57                            | 16                        | 0/100/0         | 35                 | 25                              | 0                         | 25                         | 912                  | 2140                 |
| 2            | 963        | 18                                       | 112                           | 18                        | 0/100/0         | 0                  | 60                              | 0                         | 60                         | 1240                 | 3230                 |
| 3            | 579        | 24                                       | 178                           | 9                         | 0/100/0         | 25                 | 38                              | 0                         | 38                         | 1330                 | 3420                 |
| 4            | 338        | 27                                       | 236                           | 6                         | 0/100/0         | 100                | 0                               | 0                         | 0                          | 1360                 | 3260                 |
| 5            | 147        | 29                                       | 292                           | 3                         | 0/100/0         | 100                | 6                               | 0                         | 6                          | 1330                 | 2810                 |

**Table S13.** Extraction efficiency and lignin properties of Entry **13** (EtOH/H<sub>2</sub>O 95:5, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], Cedar wood). <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 212        | 4                                        | 50                            | 4                         | 0/37/63         | 0                  | 60                              | 16                        | 44                         | 1380                 | 3090                 |
| 2            | 1118       | 22                                       | 106                           | 20                        | 0/19/81         | 10                 | 57                              | 9                         | 48                         | 1510                 | 3950                 |
| 3            | 678        | 33                                       | 162                           | 12                        | 0/14/86         | 22                 | 38                              | 1                         | 37                         | 1290                 | 3060                 |
| 4            | 464        | 41                                       | 218                           | 8                         | 0/15/85         | 30                 | 19                              | 0                         | 19                         | 1170                 | 2620                 |
| 5            | 171        | 44                                       | 274                           | 3                         | 0/12/88         | 42                 | 13                              | 0                         | 13                         | 1120                 | 2380                 |

**Table S14.** Extraction efficiency and lignin properties of Entry **14** (EtOH/H<sub>2</sub>O 95:5, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], Beech wood). <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Entry | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|-------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 15    | 2112       | 46                                       | 83                            | 25                        | 0/36/64         | 13                 | 28                              | 8                         | 20                         | 978                  | 2450                 |
| 16    | 1760       | 38                                       | 90                            | 20                        | 0/35/65         | 11                 | 38                              | 16                        | 22                         | 1180                 | 2610                 |
| 17    | 1832       | 40                                       | 80                            | 23                        | 0/42/58         | 24                 | 10                              | 0                         | 10                         | 968                  | 2070                 |
| 18    | 1777       | 39                                       | 84                            | 21                        | 3/36/61         | 2                  | 60                              | 9                         | 51                         | 1140                 | 3360                 |
| 19    | 1985       | 43                                       | 83                            | 24                        | 1/34/65         | 12                 | 44                              | 2                         | 42                         | 1220                 | 3800                 |
| 20    | 320        | 9                                        | 80                            | 4                         | 0/84/16         | 12                 | 30                              | 2                         | 28                         | 982                  | 2280                 |
| 21    | 1294       | 28                                       | 80                            | 16                        | 0/100/0         | 16                 | 8                               | 1                         | 7                          | 971                  | 2160                 |
| 22    | 586        | 19                                       | 80                            | 8                         | 0/18/82         | 27                 | 26                              | 0                         | 26                         | 958                  | 2000                 |

**Table S15.** Extraction efficiency and lignin properties of all batch experiments. <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

**Entry 15:** Walnut, 80:20 EtOH/H<sub>2</sub>O, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], 120 °C, 5 hours

**Entry 16:** Walnut, 50:50 EtOH/H<sub>2</sub>O, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], 120 °C, 5 hours

**Entry 17:** Walnut, 95:5 EtOH/H<sub>2</sub>O, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], 120 °C, 5 hours

**Entry 18:** Walnut, 80:20 nPrOH/H<sub>2</sub>O, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], 120 °C, 5 hours

**Entry 19:** Walnut, 80:15:5 nBuOH/H<sub>2</sub>O/1,4-dioxane, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], 120 °C, 5 hours

**Entry 20:** Pre-paper, 80:20 EtOH/H<sub>2</sub>O, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], 120 °C, 5 hours

**Entry 21:** Cedar wood, 80:20 EtOH/H<sub>2</sub>O, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], 120 °C, 5 hours

**Entry 22:** Beech wood, 80:20 EtOH/H<sub>2</sub>O, 0.18 M [H<sub>2</sub>SO<sub>4</sub>], 120 °C, 5 hours

## Reproducibility

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 1157       | 13                                       | 69                            | 17                        | 8/44/48         | 0                  | 61                              | 28                        | 33                         | 874                  | 1530                 |
| 2            | 1691       | 31                                       | 138                           | 25                        | 2/34/64         | 0                  | 66                              | 24                        | 42                         | 982                  | 2230                 |
| 3            | 1103       | 43                                       | 206                           | 16                        | 1/31/68         | 2.5                | 60                              | 17                        | 43                         | 1080                 | 2690                 |
| 4            | 585        | 49                                       | 272                           | 9                         | 0/31/69         | 4                  | 62                              | 16                        | 45                         | 1110                 | 2970                 |
| 5            | 268        | 52                                       | 339                           | 4                         | 0/31/69         | 5                  | 55                              | 16                        | 38                         | 1060                 | 2730                 |

**Table S16.** Extraction efficiency and lignin properties of Entry 1 in appendix A (EtOH/H<sub>2</sub>O 80:20, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]). <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).

| Time (hours) | Yield (mg) | Extraction efficiency (%) <sup>a,b</sup> | Solvent used (g) <sup>b</sup> | Solvent efficiency (mg/g) | H/G/S ratio (%) | Cond. <sup>c</sup> | Total $\beta$ -O-4 <sup>d</sup> | $\beta$ -O-4 <sup>d</sup> | $\beta'$ -O-4 <sup>d</sup> | Mn (Da) <sup>e</sup> | Mw (Da) <sup>e</sup> |
|--------------|------------|------------------------------------------|-------------------------------|---------------------------|-----------------|--------------------|---------------------------------|---------------------------|----------------------------|----------------------|----------------------|
| 1            | 1304       | 16                                       | 52                            | 25                        | 6/41/53         | 0                  | 62                              | 21                        | 42                         | 921                  | 1940                 |
| 2            | 1782       | 37                                       | 108                           | 32                        | 0/33/67         | 1                  | 63                              | 18                        | 45                         | 1090                 | 2840                 |
| 3            | 907        | 48                                       | 166                           | 16                        | 0/31/69         | 8                  | 49                              | 14                        | 35                         | 1140                 | 3280                 |
| 4            | 288        | 52                                       | 226                           | 5                         | 0/31/69         | 9.5                | 48                              | 12                        | 35                         | 1280                 | 3640                 |
| 5            | 39         | 52                                       | 285                           | 1                         | 0/34/66         | 7.5                | 44                              | 12                        | 32                         | 1050                 | 3340                 |

**Table S17.** Extraction efficiency and lignin properties of a redo of extraction 1 (EtOH/H<sub>2</sub>O 80:20, 0.18 M [H<sub>2</sub>SO<sub>4</sub>]). <sup>a</sup> Corrected for alcohol incorporation as determined by 2D-HSQC, <sup>b</sup> Cumulative values, <sup>c</sup> expressed as percentage  $S_{\text{condensed}}$  of the total S content <sup>d</sup> linking motifs per 100 C9 units as determined by 2D-HSQC (1.3 correction factor applied), <sup>e</sup> determined by GPC (THF).