ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Use of Bisulfite Processing To Generate High-β-O-4 Content Water-Soluble Lignosulfonates

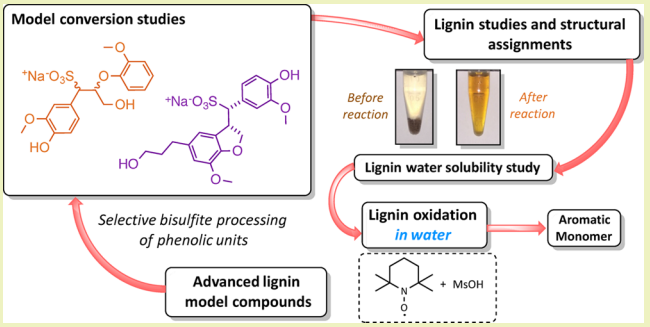
Daniel M. Miles-Barrett,[†] James R. D. Montgomery,[†] Christopher S. Lancefield,[†] David B. Cordes,[†] Alexandra M. Z. Slawin,[†] Tomas Lebl,[†] Reuben Carr,[‡] and Nicholas J. Westwood*[,†]

[†]School of Chemistry and Biomedical Sciences Research Complex, University of St Andrews and EaStCHEM, St Andrews, Fife KY16 9ST, Scotland, U.K.

[‡]Ingenza, Roslin BioCentre, Midlothian EH25 9PP, U.K.

Supporting Information

ABSTRACT: With lignin-first biorefineries likely to become a reality, controlled depolymerization of high-quality lignin streams to high-value products has become a priority. Using bisulfite chemistry, access to a high-β-O-4 content water-soluble lignosulfonate has been achieved, allowing follow-up procedures in water to be conducted. We show that phenolic β-O-4 units preferentially react under acidic bisulfite conditions, while nonphenolic β-O-4 units react much more slowly. Exploiting this improved chemical understanding and inherent selectivity, we have prepared a softwood lignosulfonate in which phenolic β-O-4 α-sulfonation has occurred, leaving significant native β-O-4 content. Use of an O-benzoylation protocol with lignin coupled with advanced two-dimensional nuclear magnetic resonance methods has allowed detailed analysis of this and other commercial and industrial lignosulfonates. Conversion of the native β-O-4 to benzylic, oxidized β-O-4 units was followed by a selective reductive cleavage to give a premium aromatic monomer in pure form.



KEYWORDS: *Biorefineries, Controlled depolymerization, Bisulfite chemistry, O-Benzoylation, Chemical feedstocks*

INTRODUCTION

With an ever-increasing demand for sustainable chemical feedstocks,[1−3] interest in the processing of lignocellulosic biomass, and one of its main components, lignin, has grown in recent years. A heterogeneous biopolymer, lignin is potentially a major source of renewable aromatic chemicals, yet it is predominantly used as a low-value fuel.[4,5] While its use for the generation of process energy is clearly important, an alternative route to aromatic feedstock chemicals may also prove to be economically viable with little adaptation of current industrial setups.

One approach to maximizing lignin's potential uses involves finding new ways to isolate technical lignins that are closer in structure to the protolignin *in planta* through milder extraction approaches (e.g., SEW,[6] SPORL,[7] ammonia,[8,9] and modified Organosolv,[10] including the OrganoCat[11] process) in a lignin-first biorefinery approach (Figure 1). These new methods are developed with the belief that they will be run on pilot to commercial scales in the near future.[12] With the production of lignins with high β-O-4 content potentially on the horizon, interest is peaking in how to process them to aromatic monomers.[13−17]

Sulfite chemistry, specifically in the context of pulping, has been used for more than a century as a way to access cellulose for use in paper production. Developments over 70 years ago saw the move from sulfite pulping as the major producer of papered goods to the Kraft process. However, sulfite pulping offers several benefits, in particular its ability to yield a water-soluble lignin component. Unfortunately, most current sulfite processes yield lignin components with low aryl-ether content

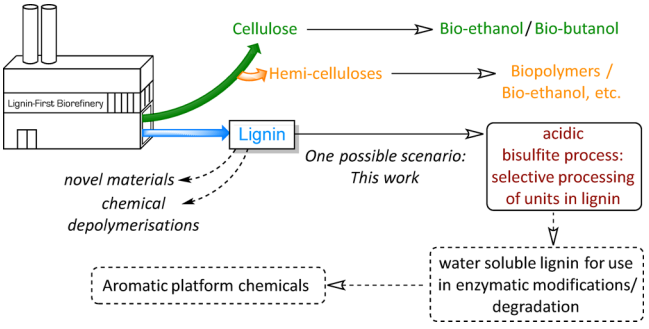


Figure 1. Proposed lignin stream utilization (from a lignin-first biorefinery) using bisulfite processing to produce water-soluble (enzyme accessible) lignins and lignosulfonates retaining a high β-O-4 content.

Received: October 25, 2016
Revised: December 11, 2016
Published: December 22, 2016

DOI: 10.1021/acssuschemeng.6b02566
ACS Sustainable Chem. Eng. 2017, 5, 1831−1839

because of the harsh conditions used (see lignin comparison in Figure S1). Little structural detail about these industrial lignosulfonates has been reported.

Proposed structures of lignins derived from acidic sulfite pulping are largely based on the detailed work of Gellerstedt and co-workers in the 1970−1980s (Figure 2).[18−22]

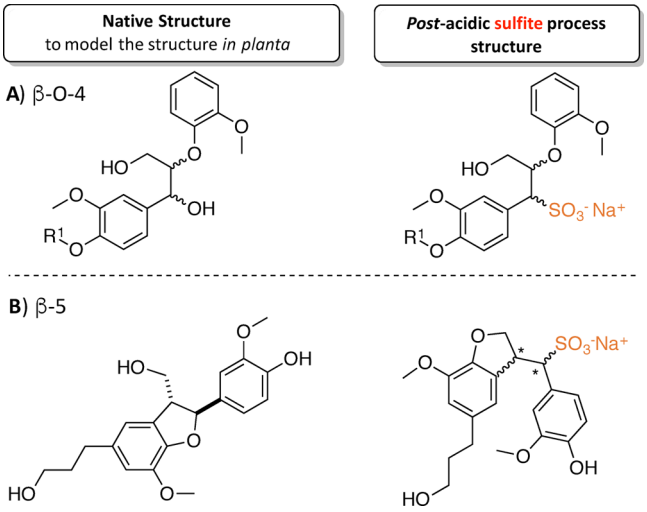


Figure 2. Proposed reactivity of common lignin units under sulfite processing. (A) Reaction of the β-O-4 unit. (B) Reactivity of the β-5 unit, reported by Gellerstedt et al. using acidic sulfite processing.[18,19,21,22] For reaction conditions, see refs 18−22. Reactivity under acidic bisulfite conditions was not explored in the early studies. Asterisks indicate stereochemistry was not assigned at this position in the original work. R^1 = H/OMe.

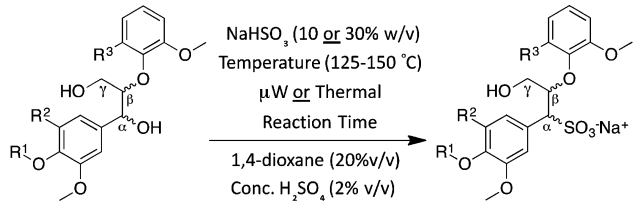
In Gellerstedt's work, model studies using the most commonly occurring linkages in lignin, the β-O-4 (Figure 2A) and the phenylcoumaran [β-5, Figure 2B(i)] were analyzed under acidic and neutral sulfite conditions. Resinol structures have also been studied but are present in relatively low abundance in most lignins.[12,23,24] The chemical transformations previously observed upon processing the lignin models were (a) α-sulfonation (Figure 2), (b) cleavage of β-O-4 linkages, and (c) condensation reactions. The down side to sulfite processing is that a high degree of condensation and cleavage of the most tractable linkage for lignin depolymerization chemistry often occurs.[13−16] Via the tuning of the conditions of bisulfite pulping, the ability to sulfonate lignins selectively at specific positions may yield lignosulfonates with higher β-O-4 content, while retaining the desirable solubility in water for greener and safer processes to be developed (Figure 1). Here we report the use of a microwave-assisted bisulfite process to produce water-soluble lignins that are reacted further in water.

We also report new methods for analyzing the phenolic and nonphenolic β-O-4 content of lignin and lignosulfonate samples.

RESULTS AND DISCUSSION

At the outset of this work, we decided to revisit the elegant studies of Gellerstedt and Gierer on the reaction of the β-O-4 unit under conditions that mimicked those of sulfite processing.[18−21] Unlike Gellerstedt's study, we focused exclusively on acidic bisulfite processing. We extended the original sulfite studies by exploring the use of microwave irradiation as a heating method and including, in addition to the original G-G β-O-4 model compounds (Scheme 1, **1a** and **2a**), phenolic G-S (**1b**), S-S (**1c**), and S-G (**1d**) β-O-4 models to screen the majority of the variations in β-O-4 coupling possible in hardwood and softwood lignins.

Scheme 1. Conversion of β-O-4 Models 1a−d and 2a to the Corresponding α-Sulfonated Models 3a−d and 4a, Respectively[a]



[a]For synthesis of β-O-4 α-sulfonated model precursors (and corresponding products **3a−d** and **4a**), see Schemes S1−S4. For more details of the reaction conditions, see Tables 1 and 2.

Mock-Up of a Bisulfite Process. β-O-4 models **1a−d** and **2a** were prepared as mixtures of diastereomers using established methods (Schemes S1 and S2).[14,25] Two different heating methods were used, and the concentration of bisulfite salts varied. Encouragingly, quantitative conversion of **1a** under our selected microwave conditions occurred giving expected product **3a**[18,26] (Scheme 1) using either of the bisulfite concentrations (Table 1, entries 1 and 3). High but slightly lower conversions of **1a** to **3a** were also achieved when the reaction was performed in a sealed tube under standard heating conditions (entries 1 and 3). One current view is that microwave use leads exclusively to more efficient heating but that scalability should be considered a significant challenge.[27,28]

Table 1. Study of the Reactivity of β-O-4 Models 1a and 2a as a Function of Heating Method and Bisulfite Concentration

		conversion to SA[a] (%)		
entry	β-O-4 model	microwave	sealed tube	[$NaHSO_3$] [% (w/v)]
1	**1a**	>99 (to **3a**)	70 (to **3a**)	30
2	**2a**	0	0	30
3	**1a**	>99 (to **3a**)	87 (to **3a**)	10
4	**2a**	<5 (to **4a**)	<1 (to **4a**)	10

[a]Based on quantitative ^{1}H NMR analysis of the crude reaction mixture comparing the integrations of the peaks corresponding to the β-proton of the β-O-4 model (**1a** or **2a**) and the β-proton of the resulting α-sulfonate (**3a** or **4a**). Reaction conditions: 1,4-dioxane [20% (v/v)], H_2O, H_2SO_4 [2.0% (v/v)], 150 °C, 2 h. Microwave infrared temperature probe calibrated prior to use. Control sealed tube reactions with thermometers inside were used to ensure the desired internal temperature was achieved. See Figures S2 and S3 for NMR data.

In contrast, corresponding nonphenolic G-G model **2a** did not react using either heating method when a 30% (w/v) $NaHSO_3$ solution was used (Table 1, entry 2). When the percentage of bisulfite salts was decreased to 10% (w/v) (entry 4), low conversion of **2a** to **4a** (<5% for microwave heating and <1% for sealed tube) was observed. The dependence of the conversion of **2a** to **4a** on bisulfite salt concentration was

DOI: 10.1021/acssuschemeng.6b02566
ACS Sustainable Chem. Eng. 2017, 5, 1831−1839

probably due to a "salting out" effect that adversely affected the solubility of **2a** at higher bisulfite concentrations. While Gellerstedt reported that **2a** was converted to **4a**, no characterization of **4a** (now provided in the Supporting Information) or evidence of the relative rates of conversion of **1a** (phenol model) and **2a** (nonphenol model) was provided.[18,22]

On the basis of these initial results, microwave heating at 10% (w/v) bisulfite concentrations was selected for further study. Because the sulfite process is conducted over a range of temperatures in industry (125−150 °C),[29] often depending on the biomass used, the effect of temperature on substrate conversion was analyzed. **1a** was still quantitatively converted to **3a** at lower temperatures [125 and 135 °C (Table 2, entries 2 and 4, Figure S4); cf. 150 °C (Table 1, entry 3)].

Table 2. Microwave Conversion Study of 1a and 2a under Different Conditions[a]

entry	model	temp (°C)	time (h)	conversion (to SA)[b] (%)
1	**1a**	150	2	>99 (to **3a**)
2	**1a**	135	2	>99 (to **3a**)
3	**2a**	135	2	<5 (to **4a**)
4	**1a**	125	2	>99 (to **3a**)
5	**2a**	125	2	<5 (to **4a**)
6	**1a**	135	7	>99 (to **3a**)
7	**2a**	135	7	27[d] (to **4a**)
8	**1a**	180	0.5[c]	>99 (to **3a**)
9	**2a**	180	0.5[c]	18[d] (to **4a**)

[a]Reaction conditions: 10% (w/v) $NaHSO_3$, 1,4-dioxane [20% (v/v)], H_2SO_4 [2.0% (v/v)], H_2O, microwave heating. [b]Based on quantitative ^{1}H NMR analysis of the crude reaction mixture comparing the integrations of the peaks corresponding to the β-proton of the β-O-4 model (**1a** and **2a**) and the β-proton of resulting α-sulfonates **3a** and **4a** (Figures S3−S6). [c]A 10 min ramp time to temperature was used, and no 1,4-dioxane was used for solubilization. [d]Several degradation products were noted, including 3,4-dimethoxybenzaldehyde, a benzylic oxidized version of **2a** (compound **S10**), and a benzylic, oxidized γ-sulfonated version of **2a** (compound **S11**) (Schemes S5 and S6 and Figure S7a−c).

Nonphenolic model **2a** was converted even less effectively at lower temperatures as expected (Table 2, entries 3 and 5; cf. Table 1, entry 4). The reaction times used in sulfite pulping often reach 7 h. In our system, extending the reaction time had no detrimental effect on the reaction of **1a**, with no degradation of product **3a** being observed (Table 2, entry 6). For **2a**, conversion increased to 27% (entry 7). Reaction conditions were also selected to mimic those of the SPORL processing method developed by Gleisner et al. (entries 8 and 9).[7] Previous studies of SPORL processing[7] were conducted using microwaves but focused on analysis of the resulting cellulose and hemicellulose fractions.

The use of an increased temperature of 180 °C for 30 min[7] again led to quantitative conversion of **1a** to **3a**, but under these conditions, nonphenolic model **2a** reacted to give a variety of different products (Schemes S5 and S6 and Figure S6). It seems likely that the higher temperatures used in a SPORL process will cause other structural changes to the lignin polymer.

The role of additional methoxy substituents in the aromatic rings of the substrate was initially assessed using phenolic S-S β-O-4 model **1c** (Scheme 1). The two phenol models, G-G **1a** and S-S **1c**, were subjected to identical microwave conditions {10% (w/v) $NaHSO_3$, 1,4-dioxane [20% (v/v)], H_2SO_4 [2.0% (v/v)], H_2O, 135 °C, and microwaves} and conversions measured at early time points by ^{1}H NMR analysis (Table 3).

Table 3. Conversion Comparison Study of Models 1a−1d for Short Time Periods To Assess the Effect on Conversion of the Different β-O-4 Units (G-G vs G-S vs S-S vs S-G)[a]

G-phenol models				S-phenol models			
entry	β-O-4 model	time (min)	% conversion to SA[b]	entry	β-O-4 model	time (min)	% conversion to SA[b]
1	**1a**	15	30 (to **3a**)	4	**1c**	15	7[c] (to **3c**)
2	**1a**	30	45 (to **3a**)	5	**1c**	30	12[c] (to **3c**)
3	**1b**	30	50 (to **3b**)	6	**1d**	30	32 (to **3d**)

[a]Reaction conditions: Table 2, entry 7 (different time in minutes). [b]Based on quantitative ^{1}H NMR analysis of the crude reaction mixture comparing the integrations of peaks corresponding to the native β-O-4 and the resulting α-sulfonates and their diastereoisomers (Figures S8−S14). Values reported here are averages of three repetitions; for standard error analysis, see Table S1. [c]Minor degradation products were observed in crude ^{1}H NMR spectra that may affect quantification. A repeat of each reaction was also conducted using an internal standard to ensure accuracy (Table S2). Results of these repeats showed little difference between figures reported in this table (Table S1).

Interestingly, a significant difference in reactivity between **1a** and **1c** was observed. After 15 min, 30% conversion of **1a** to **3a** occurred, which increased to 45% conversion after 30 min (entries 1 and 2). Analysis of S-S model **1c** showed much lower conversions to corresponding product **3c** of 7 and 12% after 15 and 30 min, respectively (entries 4 and 5; see also Table S1). Analysis after 30 min of the reactions of **1b** and **1d** showed, as expected, that **1a** and **1b** (entries 2 and 3, respectively) had similar conversion rates, both of which were faster than those of **1c** and **1d** (entries 5 and 6, respectively). These results are consistent with the formation of the benzylic cation being the rate-determining step in the conversion of **1** to **3** [Figure 3B(i), (ii)]. The "extensive condensation reactions yielding high molecular weight materials" of phenolic models reported by Gellerstedt were not, in general, observed under our conditions. In addition, information about the relative reactivity of the *erythro*:*threo* isomers of **1a** and **1c** was gained (Table S1). As expected, there was no correlation between the diastereomeric ratio of starting model **1a** or **1c** and resulting α-sulfonate **3a** or **3c**, respectively (Table S1).

In summary of this part of the study, the use of models **1a−d** and **2a** (Figure 3A) showed that (i) the microwave heating method led to excellent to good conversions of phenolic models **1a−d**, (ii) **1a−d** were efficiently converted to corresponding α-sulfonates **3a−d**, respectively, in line with previous literature reports,[18,26] (iii) nonphenolic **2a** was significantly less reactive than corresponding phenol model **1a**, suggesting the (less stable nonphenolic) intermediate benzylic cation likely forms more slowly [Figure 3B(iii)], as suggested by Gellerstedt[22] (because of the difference in activation energies of an order of magnitude required according to computational studies by Beckham et al.[30]), and (iv) G-G and G-S phenol models **1a** and **1b** reacted faster to give **3a** and **3b**, respectively, than the S-S and S-G phenol models **1c** and **1d** reacted to give **3c** and **3d**, respectively. This can be rationalized by additional methoxy substituents slowing quinone methide formation (Figure 3B). With this information and authentic samples of α-sulfonate products **3a−d** and **4a** in hand, our

DOI: 10.1021/acssuschemeng.6b02566
ACS Sustainable Chem. Eng. 2017, 5, 1831−1839

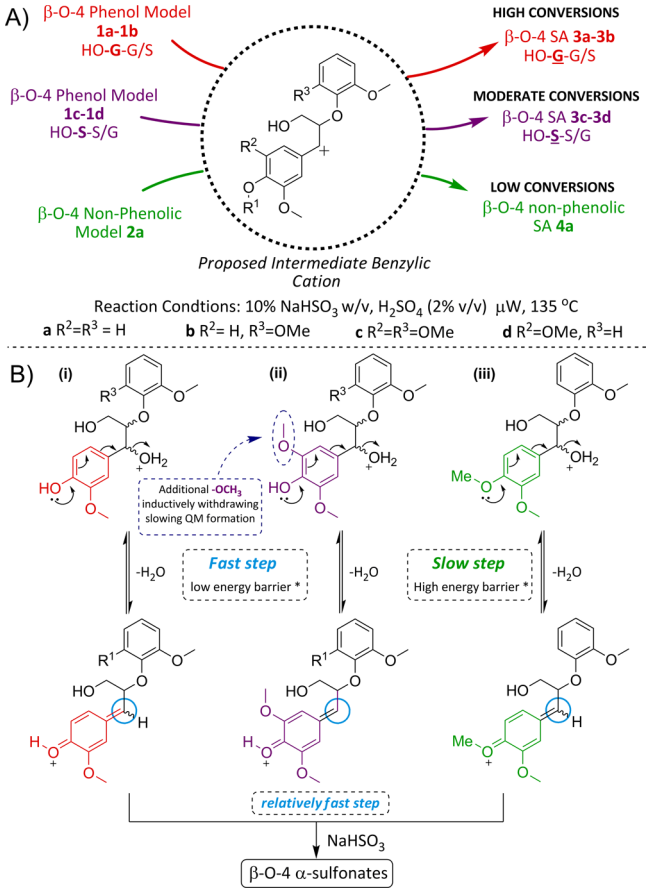


Figure 3. (A) Overview of the reactivity of β-O-4 models **1a**–**d** and **2a**. R^1 = H for phenol models; R^1 = Me for nonphenolic model. SA = sulfonates. (B) Proposed rationale for the observed difference in reactivity between different β-O-4 model compounds. Asterisks indicate Beckham et al. have provided computational evidence showing that for a HH phenol model to reach the corresponding carbocation under similar acidic conditions it required a ΔG of 1.0 kcal/mol whereas the corresponding HH nonphenolic model required a ΔG of 10.8 kcal/mol.[30]

studies turned to the analysis of current industrial lignosulfonate sources.

Presence of β-O-4 α-Sulfonate Units in Industrial Lignins. It has been proposed that there is a large degree of sulfonation of β-O-4 units under acidic sulfite conditions.[22] On the basis of previous reports[18−21] and through the use of **3a** and **3c** prepared here, it is possible to ascertain the chemical shifts of cross-peaks corresponding to α-sulfonated β-O-4 linkages in two-dimensional (2D) heteronuclear single-quantum coherence (HSQC) NMR spectra (Figure 4).

For example, the cross-peaks corresponding to the β-proton in diastereomeric mixture **3c** were overlapping at δ_H/δ_C 4.76–82.0 ppm [Figure 4A(ii) in d_6-dimethyl sulfoxide (d_6-DMSO)]. Upon comparison with the HSQC NMR analysis of an industrially sourced calcium lignosulfonate [Figure 4A(i)], it was clear that this lignosulfonate contained α-sulfonated β-O-4-derived units analogous in structure to **3c** [see Figure 4A(iii) for the overlay and Figure S15 for a more detailed discussion]. This was consistent with the proposal that this calcium lignosulfonate was generated from a source of biomass that was rich in S (and low in G) units. Comparison of the 2D HSQC analysis of an industrial (in d_6-DMSO) and a commercial (in d_4-methanol) sodium lignosulfonate with

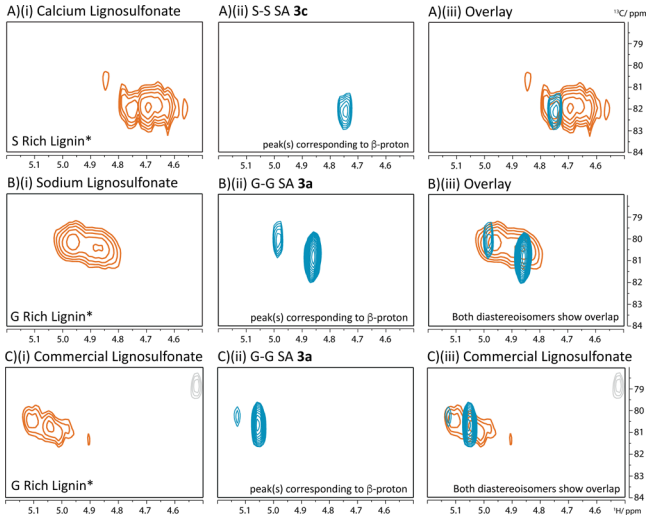


Figure 4. Comparison of the 2D HSQC spectra (δ_C/δ_H 78–84/4.5–5.2 ppm) of industrial and commercial lignins with α-sulfonated model compounds **3a** and **3c**. (A) In d_6-DMSO: industrial calcium lignosulfonate overlay with model **3c**. (B) In d_6-DMSO: industrial sodium lignosulfonate overlay with model **3a**. (C) In d_4-methanol: commercial sodium lignosulfonate overlay with **3a**. Asterisks indicate S rich and G rich lignins were assigned by analysis of the aromatic region of the 2D HSQC analysis of the lignosulfonates (Figures S15–S17).

model **3a** [mixture of *erythro* and *threo* isomers and hence two cross-peaks for the β-proton in **3a** (Figure 4B,C)] indicated that these two lignosulfonates also contain α-sulfonated β-O-4 units. This time, however, the biomass batches from which these lignins were liberated must have been rich in G in both cases {the overlay of the spectra obtained upon analysis of **3a** with that of the industrial sodium lignosulfonate provided an almost perfect overlap of the relevant cross-peaks [Figure 4B(iii)]}.

Interestingly, analogous cross-peaks observed upon 2D HSQC analysis of lignosulfonates have been highlighted, although not assigned in a publication by Sumerskii et al.[31] On the basis of the analysis presented here, we propose that Sumerskii's highlighted cross-peaks are consistent with the presence of α-sulfonated β-O-4 units in their lignosulfonates. The α- and γ-protons in industrial lignosulfonates are overlapped with other cross-peaks, making their assignment less simple (Figures S15–S17).

As expected, the cross-peaks discussed here were not observed when another industrial lignin sample, Kraft lignin, was analyzed (Figure S18). However, 70–75% of Kraft lignin (of the 2% used for niche applications) is converted to lignosulfonates using postsulfonation methods.[32,33] As our sample of Kraft lignin contained signals consistent with the presence of small quantities of native β-O-4 units (~12 β-O-4 per 100 C_9 units), we reasoned that some of these units may be at the end of lignin chains (phenolic) and may therefore be susceptible to α-sulfonation under our bisulfite processing conditions. Reaction of a sample of the Kraft lignin {10% (w/v) $NaHSO_3$, 1,4-dioxane [20% (v/v)], H_2SO_4 [2.0% (v/v)], H_2O, 135 °C, microwaves, 7 h} led to the production of a Kraft lignosulfonate (Figure S19) containing small amounts of β-O-4 units and α-sulfonated β-O-4 units. This incorporation of sulfonate groups led to the increased water solubility of the Kraft lignosulfonate (Figure 5A,B for a solubility study). The starting Kraft lignin was apparently insoluble in water (Figure

DOI: 10.1021/acssuschemeng.6b02566
ACS Sustainable Chem. Eng. 2017, 5, 1831−1839

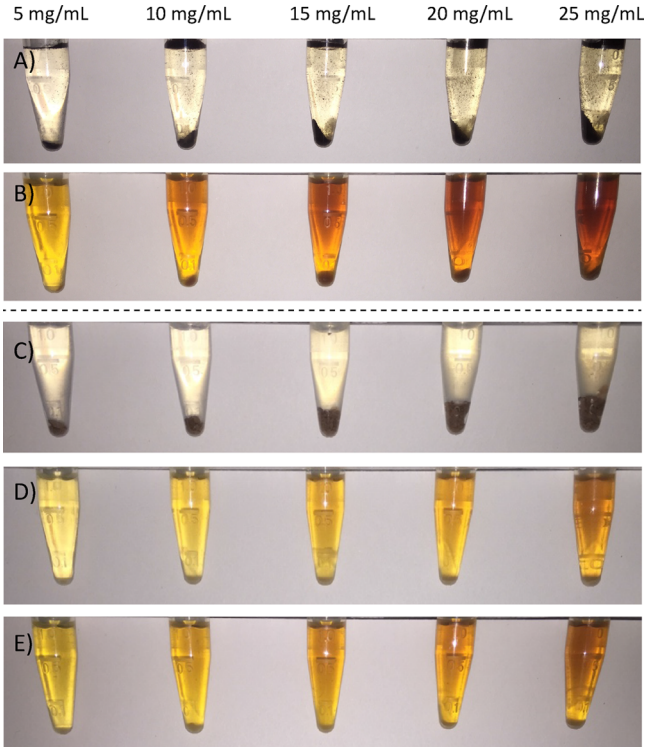


Figure 5. Solubility study: (A) attempt to dissolve starting Kraft lignin in deionized water, (B) Kraft lignosulfonate in deionized water, (C) DFL in deionized water, (D) DFSL in deionized water, and (E) DFSL in 50 mM Tris-HCl buffer (pH 8.0) and enzyme-compatible buffer. Samples were briefly vortexed and then centrifuged at 8000 rpm for 5 min. Note that small deposits seen in panels B, D, and E were subjected to NMR analysis and found to contain no lignin and are believed to be residual salts and/or inorganics in the mixture.

5A). Post-bisulfite processing, the Kraft lignosulfonate was significantly more soluble (Figure 5B).

Acidic Bisulfite Processing of Douglas Fir Dioxasolv Lignin. Inspired by the results obtained on postsulfonation of Kraft lignin (Figure 5A,B), we decided to treat an organosolv lignin under our bisulfite processing conditions as organosolv lignins may become more available from industry in the future.[3] In addition, the high β-O-4 content that is associated with mild organosolv lignins should allow a more detailed study of the effect of chemical modification of lignin (as opposed to lignin models) under bisulfite processing conditions. Finally, the controlled conversion of organosolv lignins to water-soluble lignins and/or lignosulfonates with high β-O-4 content could open the door to more facile enzymatic transformations of β-O-4 rich samples.

These ideas were explored further by preparing a sample of dioxasolv lignin from Douglas fir wood using a previously reported procedure.[34] A G rich biomass was selected as in the model studies G-G model **1a** performed best under the bisulfite reaction conditions [cf. S-containing models such as **1c** (Table 3)].

It should be noted that softwood species, e.g., pine, are generally considered unsuitable for acidic sulfite pulping because of a high degree of intramolecular condensation reactions that occur through G-aromatic units (specifically the G5 position).[22] 2D HSQC NMR analysis of the Douglas fir lignin (DFL) was consistent with that previously reported [β-O-4 content calculated to be ~28 per 100 C9 units (Figure S20)].[34] Mock bisulfite processing of DFL gave a Douglas fir sulfonated lignin (DFSL) in which several chemical transformations had taken place as assessed by comparing the 2D HSQC NMR analysis of the DFSL (Figure 6A and Figure S21)

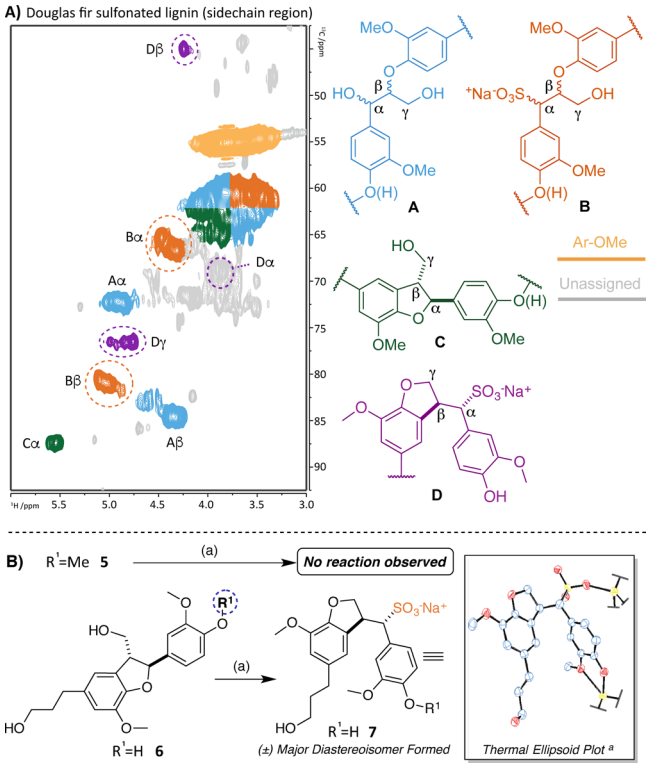


Figure 6. (A) 2D HSQC NMR analysis of the DFSL side chain region (in d_4-methanol, δ_C/δ_H 90–50/6.0–3.0 ppm). See Figure S21 for more detail. All other assignments (e.g., β-O-4 and β-5) are based on previous lignin NMR studies.[24,35−37] 2D HSQC NMR analysis has been conducted semiquantitatively using the G2 aromatic cross-peak as the internal reference[38−40] (see the Supporting Information for NMR acquisition details). Stereochemistry of **D** assigned on the basis of the model compound study. (B) Study of the reactivity of β-5 model compounds **5** and **6** under acidic bisulfite conditions. [a] Indicates a thermal ellipsoid plot shown at 30% ellipsoid probability with hydrogens omitted for the sake of clarity. Crystallographic analysis (see the Supporting Information)[41] showed a one-dimensional polymer formed by the bridging-coordination of Na^+ ions. The figure has been cropped for the sake of clarity. (a) 1,4-Dioxane [20% (v/v)], $NaHSO_3$ [10% (w/v)], H_2SO_4 [2% (v/v)], H_2O, 135 °C, 7 h, and microwaves.

with that of the starting DFL (Figure S20). (i) A significant percentage of the β-O-4 units were converted to the corresponding β-O-4 α-sulfonates [27% of linkage content (Figure 6A), new cross-peaks at 5.05/81.0 and 4.39/66.4 ppm].

(ii) Chemical transformation of several other lignin units also occurred. For example, rearrangement and α-sulfonation of the β-5 unit to give an α-sulfonated β-5 structure **D** [17% of linkage content (Figure 6A,B)] were observed. Reaction of β-5 nonphenolic model **5** under acidic bisulfite conditions led to no observable change as determined by ^{1}H NMR (Figure S22a). Interestingly, treatment of β-5 phenolic model **6** under identical reaction conditions led to formation of a major diastereoisomer of β-5 α-sulfonate **7** (Figure 6B, Figure S22b, and Scheme S8), the structure of which was confirmed by X-ray crystallographic analysis. Comparison of the 2D HSQC NMR

DOI: 10.1021/acssuschemeng.6b02566
ACS Sustainable Chem. Eng. 2017, 5, 1831−1839

spectrum of β-5 α-sulfonate **7** with that of the DFSL confirmed the presence of this unit within DFSL (Figure S23; see also 2D HSQC-TOCSY NMR analysis in Figures S24−S26 for further evidence of the presence of this structure in DFSL)

(iii) A broadening in the cross-peaks associated with the G-aromatic signals was also observed (Figure S21; cf. Figure S20). The 2D HSQC NMR analysis of our DFSL was also comparable with those of the industrially sourced lignosulfonates (Figure 4 and Figures S14−S17), but it retained a much higher β-O-4 content [37% of linkage content (Figure S21)]. Interestingly, when the preparation of DFSL was repeated using a shorter reaction time (3.5 h; cf. 7 h), the degree of conversion of the β-O-4 units to the α-sulfonated β-O-4 unit was very similar. This could be explained by the relatively rapid processing of the phenolic β-O-4 units and the much slower reaction of β-O-4 units in the middle of the lignin chains (nonphenolic) as seen in the model studies.

Additional evidence to support this came from the attempted bisulfite processing of DF lignin that had previously been methylated on the phenolic oxygens. The preparation of methylated DF lignin (Me-DFL) was achieved using TBAF and methyl iodide[42] (Figure S27). Treatment of the Me-DFL under our sulfite processing conditions for 7 h led to negligible formation of the β-O-4 α-sulfonates (Figure S28).

NMR Tools for Analyzing the Content of Phenolic to Nonphenolic β-O-4 Units in Lignins and Lignosulfonates. An alternative method for exploring the relative conversions of phenolic and nonphenolic β-O-4 units was developed. Exhaustive benzoylation of the hydroxyl groups using benzoic anhydride in pyridine in models **1a**, **2a**, **3a**, and **4a** gave **8**−**11**, respectively (Figure 7A). 2D HSQC NMR analysis of **8** and **9** [α-proton (Figure 7B); α- and β-protons (Figure 7D)] and **10** and **11** [β-proton (Figure 7D)] allowed the identification of diagnostic signals for each compound that could then be compared with those of benzoylated lignin/lignosulfonate samples. Benzoylation of the starting DFL was achieved under analogous conditions to the models, and 2D HSQC NMR analysis showed that, as expected, this lignin contained both phenolic and nonphenolic β-O-4 units (Figure 7C and Figure S29).

Because of the insolubility of DFSL in pyridine, a methodology initially reported for the analysis of whole cell walls was adapted for their benzoylation.[36] The lignosulfonate was solubilized in DMSO and *N*-methyl imidazole and then benzoylated using excess benzoic anhydride (Figure S31). For both DFL and DFSL, full hydroxyl group derivatization under the benzoylation conditions was confirmed by the ^{31}P NMR derivatization technique using phosphitylating reagent 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane[43] (Figures S30 and S32).

Interestingly, upon 2D HSQC NMR analysis of the benzoylated DFSL (Bz-DFSL), signals corresponding to the α- and β-protons of the nonphenolic β-O-4 linkages (Figure 7D, highlighted by the overlay with the signals from model **8**) were present. In addition, phenolic α-sulfonated β-O-4 linkages were present in the Bz-DFSL sample (Figure 7D, overlay with the signal for the β-proton of model **11**). However, no cross-peaks that corresponded to the benzoylated native phenolic β-O-4 linkage in the Bz-DFSL could be observed (Figure 7D, no cross-peaks that overlay with the signal for the α- or β-protons of model **9**). This result supported the idea that all of the native phenolic β-O-4 units in the starting DFL had been converted to the corresponding α-sulfonated phenolic β-O-4 units in

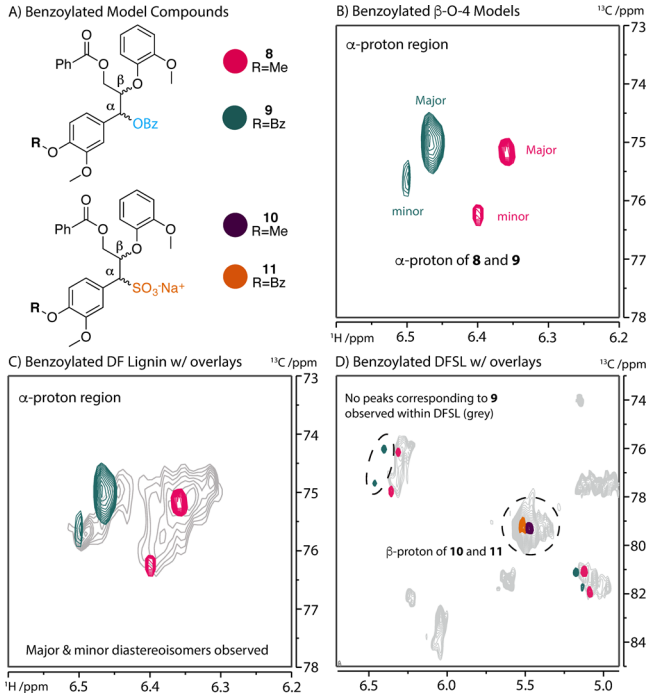


Figure 7. (A) Model compounds synthesized to observe differences in the phenol:nonphenol ratio (Scheme S9 for synthesis); 2D HSQC NMR analysis of benzoylated samples. (B) Benzoylated β-O-4 models (**8** and **9**), α-proton region, both diastereoisomers present (δ_C/δ_H 73−78/6.2−6.6 ppm) (in $CDCl_3$). (C) Benzoylated DF lignin (δ_C/δ_H 73−78/6.2−6.6 ppm) overlay with the α-proton region of benzoyl models **8** and **9** (in $CDCl_3$). (D) Benzoylated DFSL (δ_C/δ_H 73−85/4.9−6.7 ppm) overlay with the α-proton region of benzoyl models **8** and **9** and the β-proton region of benzoyl models **10** and **11** (in d_4-methanol).

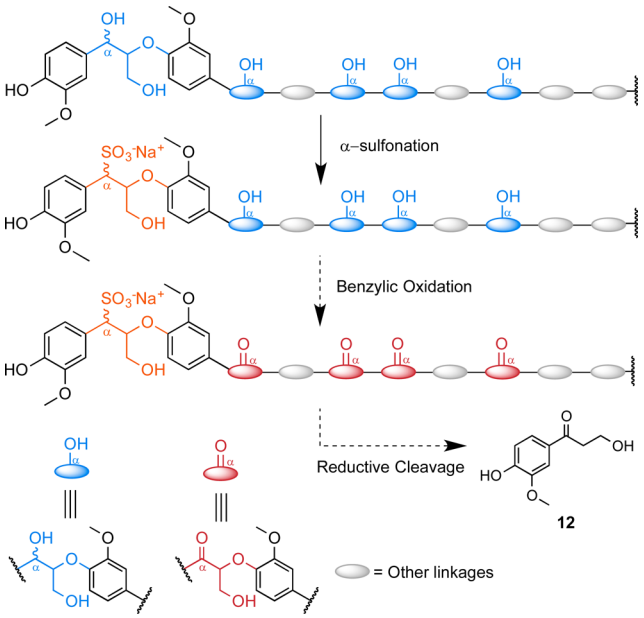
contrast to the nonphenolic β-O-4 units that had, at most, only partially reacted. In other words, the remaining native β-O-4 units in the DFSL sample are nonphenolic and hence in the middle of lignin chains (e.g., Scheme 2).

Whether any of the nonphenolic units had reacted to give the corresponding nonphenolic α-sulfonated units was more difficult to ascertain because of the overlap of the signal for the β-protons of the phenolic and nonphenolic α-sulfonated linkages (Figure 7D, overlay of signals for the β-protons of models **10** and **11** at δ_H/δ_C 5.5/78 ppm). Further work was conducted using 2D HSQC-TOCSY (as used in previous work by Ralph and co-workers[44]) as a way to ascertain whether any nonphenolic β-O-4 units in DFL had been converted to the corresponding α-sulfonated unit. The outcome of this showed that a small percentage of nonphenolic β-O-4 units had reacted (Figures S33−S36 for more detail and discussion), though quantification was not possible using this method. Armed with a developed understanding of the positioning of the α-sulfonated units being primarily on polymer end groups within DFSL, we focused on the depolymerization of lignosulfonates to monomers.

Lignosulfonate Oxidation and Depolymerization. The differential reactivity observed for the β-O-4 unit depending on whether they are phenolic (chain terminating) or nonphenolic (within the chain) provides the opportunity to explore benzylic oxidation of β-O-4 units[a] in water-soluble lignosulfonates, something that, to the best of our knowledge, has not been studied in detail before. A solubility study of DFSL in water was

DOI: 10.1021/acssuschemeng.6b02566
ACS Sustainable Chem. Eng. 2017, 5, 1831−1839

Scheme 2. Proposed Route for Oxidation and Cleavage of β-O-4 Linkages within DFSL To Yield Monomers Based on Our Previously Described Literature Method[14]



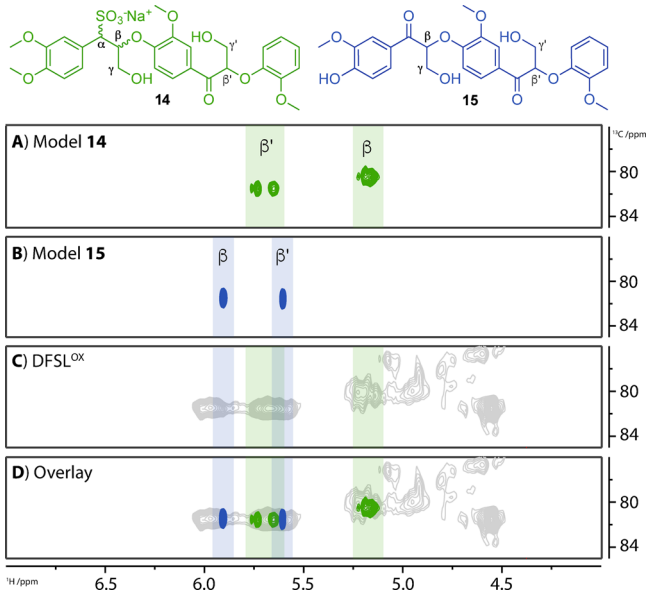


Figure 8. 2D HSQC NMR analysis (700 MHz, d_6-DMSO) of (A) model **14**, (B) model **15**, (C) and DFSL^{OX} and (D) an overlay of panels A–C. See Figure S37 for a full spectrum of DFSL^{OX}.

conducted and showed that in contrast to the starting DFL, DFSL was highly soluble in water (Figure 5C–E).

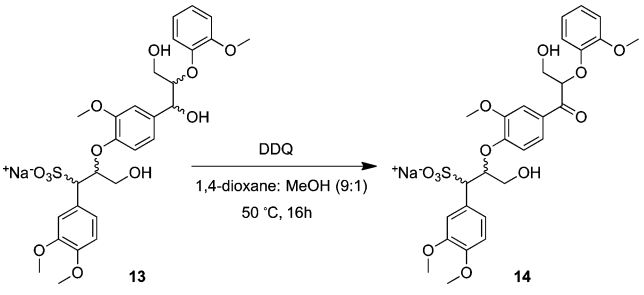
Scheme 2 outlines the planned experiment in which the organosolv lignin (DFL) was first selectively sulfonated to give DFSL, which was then oxidized to give DFSL^{OX} (using the literature nomenclature[13]). We decided to use a TEMPO-based oxidant in water to achieve this selective oxidation. Subsequent depolymerization of DFSL^{OX} was expected to yield ketone **12**, which has previously been reported to be isolable from organosolv lignins.[14]

Initial studies focused on the use of novel α-sulfonated model compound **13** [diastereomeric mixture (see Scheme S10 for synthesis)] that was successfully oxidized to corresponding benzylic ketone **14** (Scheme 3). With evidence that this oxidation was feasible, oxidation of DFSL to give DFSL^{ox} was attempted using *in situ*-generated TEMPO-derived oxoammonium salt in water.[25]

Evidence that supports the presence of oxidized β-O-4 units in DFSL^{OX} came from 2D HSQC analysis (Figure 8), with cross-peaks corresponding to native β-O-4^{OX} units being present (^{1}H/^{13}C 5.9/81.5 ppm and ^{1}H/^{13}C 5.65/81.5 ppm) and a new cross-peak present at ^{1}H/^{13}C 5.2/80.5 ppm corresponding to an α-sulfonated oxidized β-O-4 linkage adjacent to a native β-O-4^{OX} unit (see the comparison with model compound **14** in Figure 8D). Having achieved the chemical oxidation of DFSL to DFSL^{OX} achieved, we then attempted zinc-mediated reductive cleavage according to our literature procedure.[14]

Reaction was observed, and a sample of pure keto-alcohol **12** was isolated in 2.1 wt % yield (from a 4.1 wt % mixture of compounds) (Figure S38). To the best of our knowledge, this is the first time this monomer has been prepared directly from a lignosulfonate.

Scheme 3. Oxidation of Model Trimer 13 to Model Trimer 14



CONCLUSIONS

A controlled method for converting water-insoluble lignins into water-soluble lignosulfonates has been developed that makes use of a highly selective acid-catalyzed reaction of bisulfite with β-O-4 and β-5 phenolic end groups in lignin. Studies of model systems were an essential part of developing the underlying chemical knowledge. Through a detailed analysis of several industrial lignins and corresponding model systems, we have also been able to assign cross-peaks within the 2D HSQC spectra of industrial lignosulfonates.

This microwave approach has been applied to a softwood lignin to yield a water-soluble lignosulfonate.[b] A detailed 2D NMR study, following O-benzoylation of the sample, has shown that the majority of the β-O-4 content within a sulfonated Douglas fir lignin did not undergo a reaction with only β-O-4 end groups being sulfonated. Subsequent selective oxidation of the remaining β-O-4 units followed by reductive cleavage allowed isolation of a pure sample of an important aromatic monomer.

While it has been shown that the benzylic oxidation can be conducted using water-soluble chemical oxidants, the presence of the water-solubilizing sulfonate groups should allow this reaction and other reactions to be performed enzymatically in the future, potentially offering a greener, cheaper approach to lignin valorization.[12,45−47] The remaining lignin left after the selective removal of aromatic monomers may also prove to be

DOI: 10.1021/acssuschemeng.6b02566
ACS Sustainable Chem. Eng. 2017, 5, 1831−1839

suitable for standard lignosulfonate uses (e.g., additives) or may find new applications in lignin-derived materials chemistry.[c]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.6b02566.

Full synthetic procedures and analytical data for all synthesized compounds, further characterization of all novel compounds along with associated spectra, and lignin characterization (2D HSQC NMR analysis) (PDF)

Crystallographic data (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: njw3@st-andrews.ac.uk.

ORCID

Nicholas J. Westwood: 0000-0003-0630-0138

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by EPSRC grant (EP/1518175), the Industrial Biotechnology Innovation Centre (IBioIC) (DMB Ph.D. studentship), and an EPSRC Doctoral Prize Fellowship (C.S.L.). We also acknowledge the EPSRC UK National Mass Spectrometry Facility at Swansea University for mass spectrometry analysis. We thank Prof. Kamer at the University of St Andrews for use of microwave equipment and Professor Ronald de Vries for the industrial lignosulfonates.

■ ADDITIONAL NOTES

[a]It should be noted that chemical oxidation of benzylic alcohols of phenolic G-aromatic units has proven to be difficult under some oxidation conditions.[14] The lack of free phenolic G benzylic alcohols in DFSL should make the full chemical oxidation of DFSL simpler.

[b]Waste produced during this process is likely to include 1,4-dioxane, $SO_{2(g)}$ generated from $NaHSO_3$ and H_2SO_4, acidified H_2O, and an excess of $NaHSO_3$.

[c]The residual $DFSL^{OX}$ postreductive cleavage is difficult to analyze through routine NMR and GPC experiments. Further work on the material properties of this residual lignin is currently ongoing.

■ REFERENCES

(1) Zakzeski, J.; Bruijnincx, P. C. a; Jongerius, A. L.; Weckhuysen, B. M. The catalytic valorization of lignin for the production of renewable chemicals. *Chem. Rev.* **2010**, *110* (6), 3552−3599.

(2) Bruijnincx, P. C. a; Weckhuysen, B. M. Biomass conversion: Lignin up for break-down. *Nat. Chem.* **2014**, *6* (12), 1035−1036.

(3) Bozell, J.; Holladay, J.; Johnson, D.; White, J. Top Value Added Chemicals from Biomass-Volume II: Results of Screening for Potential Candidates from Biorefinery Lignin. Report PNNL-16983; Pacific Northwest National Laboratory: Richland, WA, 2007; Vol. *II* (October).

(4) Tomani, P. The Lignoboost Process. *Cellul. Chem. Technol.* **2010**, *44*, 53−58.

(5) Berlin, A.; Balakshin, M. Industrial Lignins: Analysis, Properties, and Applications. In *Bioenergy Research: Advances and Applications*, 1st ed.; Gupta, V., Tuohy, M., Kubicek, C., Saddler, J. N., Xu, F., Eds.; Elsevier: Amsterdam, 2014; Chapter 18.

(6) Iakovlev, M.; Pääkkönen, T.; Van Heiningen, A. Kinetics of SO2-ethanol-water pulping of spruce. *Holzforschung* **2009**, *63* (6), 779−784.

(7) Zhu, J. Y.; Pan, X. J.; Wang, G. S.; Gleisner, R. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour. Technol.* **2009**, *100* (8), 2411−2418.

(8) Strassberger, Z.; Prinsen, P.; van der Klis, F.; van Es, D. S.; Tanase, S.; Rothenberg, G. Lignin solubilisation and gentle fractionation in liquid ammonia. *Green Chem.* **2015**, *17*, 325−334.

(9) da Costa Sousa, L.; Foston, M.; Bokade, V. V.; Azarpira, A.; Lu, F.; Ragauskas, A.; Ralph, J.; Dale, B. E.; Balan, V. Isolation and Characterization of New Lignin Streams Derived from Extractive Ammonia (EA) Pretreatment. *Green Chem.* **2016**, *18*, 4205−4215.

(10) Zhao, X.; Cheng, K.; Liu, D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Appl. Microbiol. Biotechnol.* **2009**, *82* (5), 815−827.

(11) Grande, P. M.; Viell, J.; Theyssen, N.; Marquardt, W.; Domínguez de María, P.; Leitner, W. Fractionation of lignocellulosic biomass using the OrganoCat process. *Green Chem.* **2015**, *17* (6), 3533−3539.

(12) Rinaldi, R.; Jastrzebski, R.; Clough, M. T.; Ralph, J.; Kennema, M.; Bruijnincx, P. C. A.; Weckhuysen, B. M. Paving the Way for Lignin Valorisation: Recent Advances in Bioengineering, Biorefining and Catalysis. *Angew. Chem., Int. Ed.* **2016**, *55*, 8164−8215 A very recent report has described the DMR-EH process that also produces a high-quality lignin (see ref 46).

(13) Rahimi, A.; Ulbrich, A.; Coon, J. J.; Stahl, S. S. Formic-acid-induced depolymerization of oxidized lignin to aromatics. *Nature* **2014**, *515* (7526), 249−252.

(14) Lancefield, C. S.; Ojo, O. S.; Tran, F.; Westwood, N. J. Isolation of Functionalized Phenolic Monomers through Selective Oxidation and C-O Bond Cleavage of the *β*-O-4 Linkages in Lignin. *Angew. Chem., Int. Ed.* **2015**, *54* (1), 258−262.

(15) Deuss, P. J.; Scott, M.; Tran, F.; Westwood, N. J.; De Vries, J. G.; Barta, K. Aromatic Monomers by in Situ Conversion of Reactive Intermediates in the Acid-Catalyzed Depolymerization of Lignin. *J. Am. Chem. Soc.* **2015**, *137* (23), 7456−7467.

(16) Lahive, C. W.; Deuss, P. J.; Lancefield, C. S.; Sun, Z.; Cordes, D. B.; Young, C.; Tran, F.; Slawin, A. M. Z.; de Vries, J. G.; Kamer, P. C. J.; et al. Advanced model compounds for understanding acid catalyzed lignin depolymerization: identification of renewable aromatics and a lignin-derived solvent. *J. Am. Chem. Soc.* **2016**, *138* (28), 8900−8911.

(17) Jastrzebski, R.; Constant, S.; Lancefield, C. S.; Westwood, N. J.; Weckhuysen, B. M.; Bruijnincx, P. C. A. Tandem Catalytic Depolymerization of Lignin by Water-Tolerant Lewis Acids and Rhodium Complexes. *ChemSusChem* **2016**, *9* (16), 2074−2079.

(18) Gellerstedt, G.; Gierer, J.; Buchardt, O.; Norin, T. The Reactions of Lignin during Neutral Sulphite Cooking. Part I. The Behaviour of beta-Arylether Structures. *Acta Chem. Scand.* **1968**, *22*, 2510−2518.

(19) Gellerstedt, G.; Gierer, J.; Reinhammar, B.; Nielsen, P. H. The Reactions of Lignin During Neutral Sulphite Cooking. Part II. The Behaviour of Phenylcoumaran Structures. *Acta Chem. Scand.* **1968**, *22* (6), 2029−2031.

(20) Gellerstedt, G.; Gierer, J.; Lüning, B.; Liaaen-Jensen, S.; Lamvik, A.; Sunde, E.; Sørensen, N. A. Reactions of Lignin during Neutral Sulfite Pulping. Part III. The Mechanism of Formation of Styrene-beta-sulphonic Acid Structures. *Acta Chem. Scand.* **1970**, *24*, 1645−1654.

(21) Gellerstedt, G.; Gierer, J.; Liminga, R.; Nilsson, K.; Nimmich, W. Reactions of Lignin During Neutral Sulfite Pulping. Part IV. The Behaviour of Structural Elements of the Pinoresinol Type. *Acta Chem. Scand.* **1972**, *26*, 701−714.

(22) Gellerstedt, G. Reactions of Lignin During Sulfite Pulping. *Sven. Papperstidning, Nord. Cellul.* **1976**, *79* (16), 537−543.

(23) Vanholme, R.; Demedts, B.; Morreel, K.; Ralph, J.; Boerjan, W. Lignin biosynthesis and structure. *Plant Physiol.* **2010**, *153* (3), 895−905.
(24) Constant, S.; Wienk, H. L. J.; Frissen, A. E.; de Peinder, P.; Boelens, R.; van Es, D. S.; Grisel, R. J. H.; Weckhuysen, B. M.; Huijgen, W. J. J.; Gosselink, R. J. A.; Bruijnincx, P. C. A. New insights into the structure and composition of technical lignins: a comparative characterisation study. *Green Chem.* **2016**, *18* (9), 2651−2665.
(25) Rahimi, A.; Azarpira, A.; Kim, H.; Ralph, J.; Stahl, S. S. Chemoselective metal-free aerobic alcohol oxidation in lignin. *J. Am. Chem. Soc.* **2013**, *135* (17), 6415−6418.
(26) Lutnaes, B. F.; Myrvold, B. O.; Lauten, R. a; Endeshaw, M. M. 1H and 13C NMR data of benzylsulfonic acids-model compounds for lignosulfonate. *Magn. Reson. Chem.* **2008**, *46* (3), 299−305.
(27) de la Hoz, A.; Diaz-Ortiz, A.; Moreno, A. Microwaves in organic synthesis. Thermal and non-thermal microwave effects. *Chem. Soc. Rev.* **2005**, *34*, 164−178.
(28) Monteil-Rivera, F.; Huang, G. H.; Paquet, L.; Deschamps, S.; Beaulieu, C.; Hawari, J. Microwave-assisted extraction of lignin from triticale straw: Optimization and microwave effects. *Bioresour. Technol.* **2012**, *104*, 775−782.
(29) Bjorsvik, H.; Minisci, F. Fine Chemicals from Lignosulfonates. 1. Synthesis of Vanillin by Oxidation of Lignosulfonates. *Org. Process Res. Dev.* **1999**, *3*, 330−340.
(30) Sturgeon, M. R.; Kim, S.; Lawrence, K.; Paton, R. S.; Chmely, S. C.; Nimlos, M.; Foust, T. D.; Beckham, G. T. A Mechanistic investigation of acid-catalyzed cleavage of aryl-ether linkages: Implications for lignin depolymerization in acidic environments. *ACS Sustainable Chem. Eng.* **2014**, *2* (3), 472−485.
(31) (a) Sumerskii, I.; Korntner, P.; Zinovyev, G.; Rosenau, T.; Potthast, A. Fast Track for Quantitative Isolation of Lignosulfonates from Spent Sulfite Liquors. *RSC Adv.* **2015**, *5*, 92732−92742. (b) Khokarale, S. G.; Le-That, T.; Mikkola, J.-P. Carbohydrate Free Lignin: A Dissolution−Recovery Cycle of Sodium Lignosulfonate in a Switchable Ionic Liquid System. *ACS Sustainable Chem. Eng.* **2016**, *4* (12), 7032−7040.
(32) Macfarlane, A.; Mai, M.; Kadla, J. Bio-based chemicals from biorefining: Lignin conversion and utilisation. In *Advances in Biorefineries*, 1st ed.; Waldron, K. W., Ed.; Woodhead Publishing Ltd.: Cambridge, U.K., 2014; Chapter 20.
(33) Sakke, B.; Lehnen, R. Lignin. *Ullmann's Encyclopedia of Industrial Chemistry*; Wiley: New York, 2012.
(34) Lancefield, C. S.; Westwood, N. J. The synthesis and analysis of advanced lignin model polymers. *Green Chem.* **2015**, *17* (11), 4980−4990.
(35) Heitner, C.; Dimmel, D. R.; Schmidt, J. A. *Lignin and Lignans*; CRC Press: Boca Raton, FL, 2010.
(36) Mansfield, S. D.; Kim, H.; Lu, F.; Ralph, J. Whole plant cell wall characterization using solution-state 2D NMR. *Nat. Protoc.* **2012**, *7* (9), 1579−1589.
(37) Wen, J. L.; Sun, S. L.; Xue, B. L.; Sun, R. C. Recent advances in characterization of lignin polymer by solution-state nuclear magnetic resonance (NMR) methodology. *Materials* **2013**, *6* (1), 359−391.
(38) Zhang, L.; Gellerstedt, G. Quantitative 2D HSQC NMR determination of polymer structures by selecting suitable internal standard references. *Magn. Reson. Chem.* **2007**, *45*, 37−45.
(39) Sette, M.; Lange, H.; Crestini, C. Quantitative HSQC Analyses of Lignin: A Practical Comparison. *Comput. Struct. Biotechnol. J.* **2013**, *6* (7), 1−7.
(40) Liitia, T. M.; Maunu, S. L.; Hortling, B.; Toikka, M.; Kilpelainen, I. Analysis of Technical Lignins by Two- and Three-Dimensional NMR Spectroscopy. *J. Agric. Food Chem.* **2003**, *51*, 2136−2143.
(41) CCDC 1507323 (7) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
(42) Lu, F.; Ralph, J. Non-degradative dissolution and acetylation of ball-milled plant cell walls: High-resolution solution-state NMR. *Plant J.* **2003**, *35* (4), 535−544.
(43) Granata, A.; Argyropoulos, D. S. 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, a Reagent for the Accurate Determination of the Uncondensed and Condensed Phenolic Moieties in Lignins. *J. Agric. Food Chem.* **1995**, *43*, 1538−1544.
(44) Ralph, J.; Landucci, L. L. *NMR of Lignins*; Heitner, C., Dimmel, D. R., Schmidt, J. A., Eds.; CRC Press (Taylor & Francis Group): Boca Raton, FL, 2010.
(45) Sainsbury, P. D.; Hardiman, E. M.; Ahmad, M.; Otani, H.; Seghezzi, N.; Eltis, L. D.; Bugg, T. D. H. Breaking Down Lignin to High-Value Chemicals: The Conversion of Lignocellulose to Vanillin in a Gene Deletion Mutant of Rhodococcus jostii RHA1. *ACS Chem. Biol.* **2013**, *8*, 2151−2156.
(46) Salvachúa, D.; Katahira, R.; Cleveland, N. S.; Khanna, P.; Resch, M. G.; Black, B. A.; Purvine, S. O.; Zink, E. M.; Prieto, A.; Martínez, M. J.; Martínez, A. T.; Simmons, B. A.; Gladden, J. M.; Beckham, G. T. Lignin depolymerization by fungal secretomes and a microbial sink. *Green Chem.* **2016**, *18*, 6046−6062.
(47) Salvachúa, D.; Karp, E. M.; Nimlos, C. T.; Vardon, D. R.; Beckham, G. T. Towards lignin consolidated bioprocessing: simultaneous lignin depolymerization and product generation by bacteria. *Green Chem.* **2015**, *17* (11), 4951−4967.

DOI: 10.1021/acssuschemeng.6b02566
ACS Sustainable Chem. Eng. 2017, 5, 1831−1839