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Heteronuclear Single-Quantum Coherence Nuclear Magnetic Resonance (HSQC NMR) Characterization of Acetylated Fir (*Abies sachallnensis* MAST) Wood Regenerated from Ionic Liquid

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Supporting Information

ABSTRACT: An ionic liquid, 1-butyl-3-methylimidazolium chloride ([Bmim]Cl), was used to dissolve Japanese fir (*Abies sachallnensis* MAST) wood. Milled woods prepared by planetary ball-milling for 8 h dissolved completely in [Bmim]Cl at 100 °C in 2 h. The dissolved woods were then subjected to in situ acetylation, and the fully acetylated woods were regenerated from [Bmim]Cl. $^{1}H-^{13}C$ correlation heteronuclear single-quantum coherence (HSQC) nuclear magnetic resonance (NMR) experiments were successfully conducted with the acetylated woods in dimethyl sulfoxide (DMSO)-d_6. The acetylated lignin and polysaccharide signals dispersed reasonably well on the 2D spectra. Characterization of the NMR signals for the whole cell-wall components, including lignin, cellulose, and hemicelluloses, was achieved by comparison with isolated lignin and commercial cellulose and hemicelluloses (arabinoxylan, galactomannan, and glucomannan). The procedure used here is applicable for the characterization of cell-wall components in various plant biomasses.

KEYWORDS: ionic liquid, ball-milling, cell-wall component, NMR, *Abies sachallnensis* MAST

INTRODUCTION

The use of woody biomass is becoming an increasingly important means for reducing carbon dioxide emissions and petroleum consumption. For efficient use of cell-wall components, including cellulose, hemicellulose, and lignin, a better understanding of their chemical structures is important.

Much research has focused on characterizing the chemical structure of various kinds of plant cell walls. Usually, fine ball-milling, although somewhat degradative,[1] is required for common isolation and spectroscopic analysis of plant cell-wall components. Much effort has been devoted to establishing efficient solvent systems for the complete and nondegradative dissolution of whole plant cell walls. In particular, Ralph and co-workers[2−4] developed a bisolvent system of *N*-methylimidazole (NMI) and dimethyl sulfoxide (DMSO) to dissolve finely ball-milled plant cell walls and performed detailed structural studies on the acetylated[2] and nonacetylated[3] plant cell-wall solutions using high-resolution NMR spectroscopy. Their research has focused mainly on lignin structures. Recently, they used DMSO-d_6[5] or DMSO-d_6/pyridine-d_5[6] to characterize plant cell-wall gels by solution-state HSQC NMR spectroscopy and also assigned anomeric positions of polysaccharides. Matsumoto et al.[7] also found a new solvent system of lithium chloride and DMSO to dissolve finely milled wood and quantitatively determined the relationship between the degree of ball-milling and structural changes in lignin.

Ionic liquids show potential for use as environmentally friendly solvents for biomass refinery. Ionic liquids have many advantages, including tunable physicochemical properties, negligible vapor pressure, and thermal stability. In 2006, Rogers et al.[8] first used the ionic liquid 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) to dissolve wood chips. They partially dissolved hardwoods and softwoods in a [Bmim]Cl/DMSO system and characterized the regenerated cellulose materials by ^{13}C NMR spectroscopy. A revised version of their method, using the ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim]OAc), completely dissolved wood.[9] Kilpelanen and co-workers[10] later used [Bmim]Cl and 1-allyl-3-methylimidazolium chloride ([Amim]Cl) to dissolve wood. They reported that the solubilization efficiency of lignocellulosic materials in ionic liquids is in the following order: ball-milled wood powder > sawdust > thermomechanical pulp (TMP) fibers ≫ wood chips. They characterized acetylated Norway spruce samples recovered from [Amim]Cl by ^{1}H NMR spectroscopy. Recently, Holmes and co-workers[11] reported the impact of [Emim]OAc pretreatment on eucalyptus. They analyzed the structural changes of the pretreated biomass by HSQC NMR spectroscopy. Jiang and co-workers[12] also reported that a new bisolvent system of perdeuterated pyridinium ionic liquid and DMSO-d_6 can be used for direct dissolution and HSQC NMR analysis of plant cell walls without ball-milling. Although this method is rapid and promising, only major lignin structures have been analyzed, probably because of low signal intensities and severe signal overlaps for polysaccharides.

In studies to date on wood dissolution in ionic liquids, typical conditions for complete dissolution of wood are 80−130 °C with

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relatively long dissolution times of 8−24 h.[9,10] In this study, we adopted ball-milling in the hope of achieving milder dissolution conditions than those reported so far for complete dissolution of wood in ionic liquids, in order to avoid or decrease the possible degradation of cell-wall components during the dissolution processes. Furthermore, we explored the possible use of ionic liquids for the complete dissolution and HSQC NMR analysis of whole cell-wall components, including lignin, cellulose, and hemicelluloses. Particular attention was paid to the HSQC NMR characterization of polysaccharides other than the major lignin structures in the whole plant cell wall, which has not been conducted sufficiently in the reported NMR analyses of whole cell walls.

MATERIALS AND METHODS

Materials. The ionic liquids [Bmim]Cl (Merck, Darmstadt, Germany), [Amim]Cl (Sigma-Aldrich, St. Louis, MO), 1-butyl-3-methylimidazolium methylsulfate ([Bmim][$MeSO_4$]) (Sigma-Aldrich), and [Emim]OAc (Sigma-Aldrich) were used as received without any purification. An additional ionic liquid, 1-ethyl-3-methylimidazolium methylphosphonate ([Emim][(MeO)HPO_2]), was prepared according to the reported procedures.[13] To a THF solution of distilled *N*-ethylimidazole (49.8 mL), dimethyl phosphite (46.8 mL) in THF was added dropwise under argon at room temperature. The reaction mixture was stirred under reflux at 90 °C for 48 h. THF was removed, and the residual liquid was washed with diethyl ether and dissolved in dichloromethane. The resulting solution was passed through a column filled with neutral activated alumina. Dichloromethane was removed, and the residual liquid was dried under vacuum to give 1-ethyl-3-methylimidazolium methylphosphonate as a colorless liquid in 85% yield: ^{1}H NMR ($CDCl_3$) δ 1.58 (t, 3H, J = 7.4 Hz, N−CH_2CH_3), 3.54 (d, 3H, J = 11.8 Hz, POCH_3), 4.07 (s, 3H, N−CH_3), 4.37 (q, 2H, J = 7.4 Hz, N−CH_2CH$_3$), 6.90 (d, 1H, J = 589 Hz, P*H*), 7.65 (d, 2H, J = 9.9 Hz, NC*H*C*H*N), 10.5 (s, 1H, NC*H*N); ^{13}C NMR ($CDCl_3$) δ 15.0 (N−CH_2CH_3), 35.6 (N−OCH_3), 44.3 (N−CH_2CH$_3$), 49.9 (POCH_3), 121.3, 123.1 (NCHCHN), 137.8 (NCHN).

Cellulose triacetate (Wako, Japan), xylan from oat spelts (arabinoxylan) (Tokyo Kasei, Japan), glucomannan (konjac extractives, Leorex LM) (Shimizu Chemical, Japan), locust bean gum from *Ceratonia siliqua* seeds (galactomannnan) (Sigma-Aldrich), and methyl α-D-galactopyranoside (Wako) were purchased and used for HSQC NMR analysis.

Preparation of Wood Samples. Japanese fir (*Abies sachallnensis* MAST) wood was ground in a microfine grinding mill with cutting grinding head MF-10 (IKA Works, USA) and fractionated by sieves. The fractionated wood meal (0.15−0.35 mm) was extracted with ethanol/benzene (1:2, v/v) for 6 h and dried under vacuum over P_2O_5. The extractive-free wood meal (3 g) was further ground in a planetary mono mill P-6 (Fritsch, Germany) under argon for 1, 2, 4, and 8 h using a 45 mL ZrO_2 jar with 18 balls. The milling frequency was 600 rpm. A 15 min pause was introduced after every 30 min of milling to prevent overheating.

Japanese fir milled wood lignin (MWL) was isolated from Japanese fir wood and purified according to a method similar to that used for the previous NMR analyses of lignins.[14,15] The MWL was acetylated with pyridine/acetic anhydride at room temperature in the dark for 24 h.

Dissolution of Ball-Milled Wood in Ionic Liquid. In a screw-cap test tube, 2 g of ionic liquid and 0.1 g of ball-milled fir wood were heterogeneously mixed, heated at 100 °C in an oil bath under constant magnetic stirring for a given time period, diluted with 8 mL of DMSO at 100 °C, and centrifuged at 3000 rpm for 30 min. The supernatant solution was transferred to pass a 1-G4 glass filter (5−10 μm).

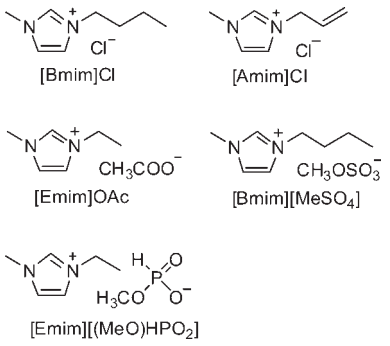

Figure 1. Structures of ionic liquids used.

Undissolved materials were washed with DMSO, acetone, and distilled water in that order. The residue was transferred to a 1-G4 glass filter, dried overnight at 105 °C, and weighed for determination of the residual amounts.

Acetylation of Ball-Milled Wood. For NMR analysis, milled fir wood was heated in [Bmim]Cl for 2 h at 100 °C and then acetylated in situ with 2 mL of pyridine/acetic anhydride (1/1, v/v) at room temperature in the dark for 24 h. The fully acetylated wood was regenerated by simply dropping into distilled water under rapid agitation. The obtained white solid materials were filtered, washed with distilled water, and dried for 24 h under vacuum over P_2O_5 at room temperature.

Infrared (IR) and NMR Spectra of Acetylated Wood. IR spectra were recorded on an FT-IR spectrometer (Spectrum 100) (Perkin-Elmer, USA) with attenuated total reflection (ATR) base. NMR spectra were recorded on a 500 MHz NMR spectrometer (Avance II) (Bruker, Germany) with a 5 mm BBI probe at 300 K using DMSO-d_6 as solvent. The Bruker standard pulse program hsqcetgpsi2 was used for HSQC experiments. For analysis of the aliphatic region, spectra were acquired from 6.8 to 2.0 ppm in F_2 (^{1}H) (acquisition time, 0.213 s; 1024 data points) and from 120 to 30 ppm in F_1 (^{13}C) (acquisition time, 11.3 ms; 256 increments). The relaxation delay was 1.44 s, and the number of scans was 128−512 for each of the 256 increments. Chemical shifts were referenced to DMSO-d_6 (2.50/39.5 ppm). An acetylated wood regenerated from [Bmim]Cl (60 mg) was dissolved in 0.5 mL of DMSO-d_6 with stirring for 1 h and then carefully transferred to an NMR sample tube.

RESULTS AND DISCUSSION

Dissolution of Ball-Milled Japanese Fir Wood in Various Ionic Liquids. Several alkyl-imidazolium ionic liquids, such as [Bmim]Cl,[16] [Amim]Cl,[17] [Emim]OAc,[9] and [Emim][(MeO)HPO_2],[13] have been reported to be excellent solvents for cellulose (Figure 1). [Bmim]Cl and [Amim]Cl are actually solids at room temperature. In contrast, [Emim]OAc and [Emim][(MeO)HPO_2] are halogen-free room temperature ionic liquids. It has also been reported that [Bmim][$MeSO_4$] can dissolve lignin.[18]

Before exploring the effect of ball-milling on wood dissolution, we examined the capability of these alkyl-imidazolium ionic liquids to dissolve milled fir wood powder prepared by ball-milling for 1 h. The amounts of residual ball-milled wood that dissolved in the ionic liquids at 100 °C are shown in Figure 2. [Bmim][$MeSO_4$] is ineffective in this dissolution task, although it is effective in dissolving lignin.[18] In contrast, good cellulose solvents are reasonably effective. [Amim]Cl and [Emim]-[(MeO)HPO_2] have fairly similar tendencies, and both are more

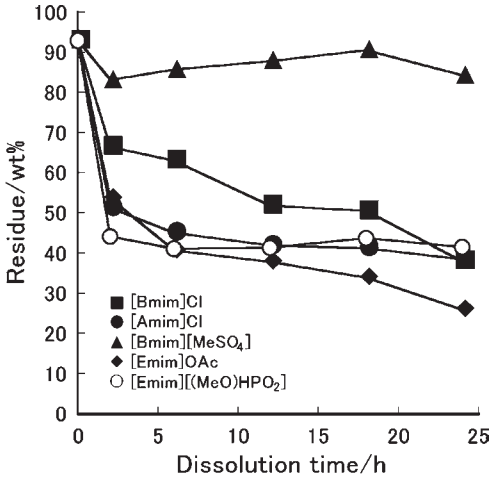

Figure 2. Dissolution of milled fir wood (1 h of ball-milling) in various ionic liquids at 100 °C.

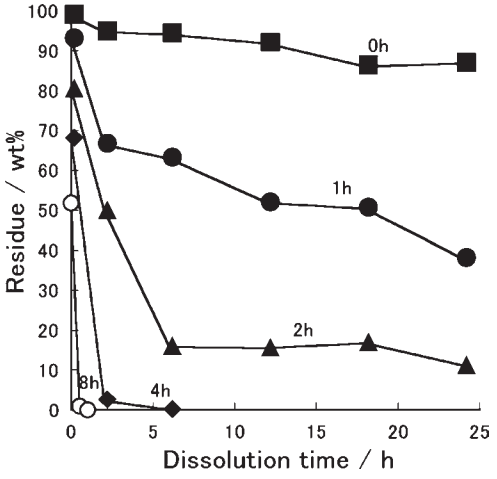

Figure 3. Dissolution of milled fir woods (0, 1, 2, 4, and 8 h of ball-milling) in [Bmim]Cl at 100 °C.

effective than is [Bmim]Cl, especially in the initial stage of dissolution. [Emim]OAc is the most effective among the ionic liquids tested in this study. These results indicate that dissolution of polysaccharides is essential for effective dissolution of wood in ionic liquids.

Effect of Ball-Milling on the Solubility of Japanese Fir Wood in [Bmim]Cl. Kubo et al. reported that a phenolic β-*O*-4-type lignin model compound decomposed gradually over an extended processing time to form an enol ether structure in [Bmim]Cl, [Amim]Cl, and [Emim]OAc.[19] Decomposition of the lignin model compound is evident in HPLC chromatograms even after treatment in [Emim]OAc at 120 °C for 1 h. In contrast, decompositions in [Bmim]Cl and [Amim]Cl seem to be negligible in the chromatograms under the same conditions. Thus, we decided to use [Bmim]Cl for further studies of the effect of ball-milling.

Japanese fir wood meal prepared by a cutting mill was finely ball-milled by a planetary ball mill for 1, 2, 4, and 8 h. The ball-milled woods were heated in [Bmim]Cl at 100 °C with stirring, and undissolved materials were filtered out. The amounts of residual milled wood resulting from the different ball-milling times are shown in Figure 3. Wood meal without ball-milling (0.15−0.35 mm) has limited solubility under the conditions used; only 15% of the wood dissolved even after heating for 24 h. Ball-milling was reasonably effective in increasing solubility, and solubility increased with increasing ball-milling time. Wood prepared by ball-milling for 4 h dissolved almost completely in 2 h, and no residue was observable to the naked eye. Wood prepared by ball-milling for 8 h dissolved completely in 30 min.

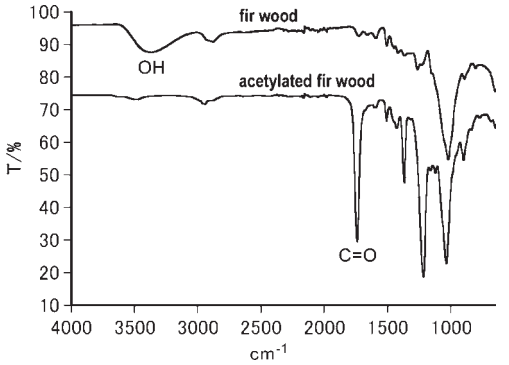

Figure 4. IR spectra of milled fir wood and acetylated fir wood regenerated from [Bmim]Cl.

The effect of ball-milling on dissolution can be attributed partly to the breakdown of the crystal structure of cellulose and to a decrease in the degree of polysaccharide polymerization.[2] Ball-milling also reportedly causes partial decomposition of lignin.[1,7]

Acetylation of Ball-Milled Wood in [Bmim]Cl. One advantage of ionic liquids is that they can be used as reaction media for chemical modification of wood-based lignocellulosic materials.[20] Acetylation of all cell-wall components was successfully conducted by in situ acetylation of the completely dissolved milled woods. Woods prepared by ball-milling for 4 and 8 h were heated at 100 °C in [Bmim]Cl for 2 h and then cooled to room temperature. Acetic anhydride and pyridine were added to the mixture for acetylation. The final solution was clear amber in color. The fully acetylated milled woods were regenerated and characterized by IR spectroscopy (Figure 4). A strong C═O stretch at 1741 cm^{-1} appeared and an OH stretch at 3356 cm^{-1} disappeared, indicating complete acetylation of the milled wood. The weights of the acetylated milled woods prepared by milling for 4 and 8 h were both 142% of the weights of the original milled woods. Direct acetylation of milled wood without dissolution in [Bmim]Cl was also tried, but resulted in incomplete acetylation.

$^{1}H-^{13}C$ HSQC Analysis of Acetylated Ball-Milled Fir Wood Regenerated from [Bmim]Cl. Acetylated milled wood prepared by ball-milling for 8 h dissolves completely in ordinary NMR solvents. In the two solvents DMSO-d_6 and $CDCl_3$, its solubility reaches 120 and 180 mg/mL, respectively. The concentrations were similar to those of acetylated cell walls reported for NMI/DMSO system[2] and were high enough to permit detailed analysis of whole cell-wall components by HSQC measurement on a 500 MHz NMR spectrometer. In contrast, the solubility of acetylated milled wood prepared by ball-milling for just 4 h is much lower, and only DMSO-d_6 gives adequate solubility for our purposes (50−60 mg/mL). These results suggest that ball-milling is necessary for sufficient dissolution of acetylated woods in NMR solvents.

HSQC spectra of samples prepared by ball-milling for 8 h (Figure 5) and 4 h (Figure S1 of the Supporting Information) were taken in DMSO-d_6. The spectra are basically the same, but

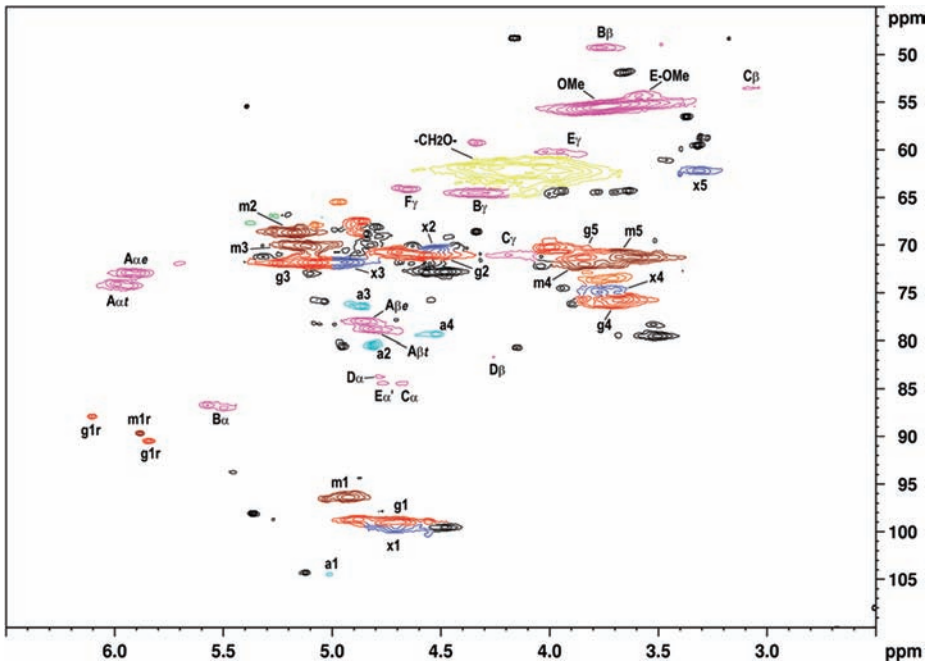

Figure 5. Aliphatic region of the HSQC spectrum of acetylated fir wood (8 h of ball-milling) regenerated from [Bmim]Cl. Solvent: DMSO-d_6. See Figures 6 and 7 for signal assignments.

some weak signals corresponding to lignin substructures are missing in the spectrum for the 4-h-milled sample, due to the lower concentration. This decreased sensitivity for shorter milling times can be offset using cryogenically cooled probes or higher field instruments.[2]

Using acetylated samples for NMR analysis of plant cell-wall polysaccharides causes the information of naturally acetylated hemicelluloses to be lost.[6] However, acetylation is still useful, because NMR signals for acetylated polysaccharides disperse well on the 2D NMR spectra, whereas signals for nonacetylated samples overlap.

Characterization of Cellulose. A lot of ^{1}H and ^{13}C NMR spectroscopic data for carbohydrates are available in the literature. HSQC NMR signals of cellulose acetate have been assigned in $CDCl_3$ [2] and acetone-d_6.[21] However, it is not easy to find spectroscopic data for peracetylated polysaccharides in DMSO-d_6. In general, chemical shifts are affected very much by the solvent used in NMR measurement. Thus, we used commercial cellulose triacetate and hemicelluloses to assign the signals corresponding to each monosaccharide residue in the complicated plant cell-wall polysaccharides mixture, the acetylated fir wood.

The chemical shifts of β-D-glucopyranose (β-D-Glcp) units of cellulose in DMSO-d_6 were closer to those in acetone-d_6 than those in $CDCl_3$. Especially, the H5 (g5) chemical shift in DMSO-d_6 was similar to that in acetone-d_6, but was quite different from that in $CDCl_3$. The signal at 4.67/99.1 ppm was assigned to anomeric H1/C1 (g1) (Figure 6A). The strong signals at 4.53/71.1, 5.07/71.9, 3.66/76.0, and 3.82/71.3 ppm were assigned to H2/C2 (g2), H3/C3 (g3), H4/C4 (g4), and H5/C5 (g5), respectively. The signals at 4.00/61.8 and 4.23/61.8 ppm were assigned to H_6/C_6 (g6). We also assigned signals to the reducing and nonreducing end groups in cellulose by comparison with cellobiose octaacetate (Figure 6B). Most of the signals corresponding to β-D-Glcp units were distinguishable from the other signals in the acetylated fir wood in DMSO-d_6.

Characterization of Hemicelluloses. The structures of hemicelluloses are more complicated than those of cellulose. Typical softwood hemicelluloses are composed of galactoglucomannan (20%) and arabinoglucuronoxylan (5−10%). The main chain of galactoglucomannan includes β-D-mannopyranose (β-D-Manp) and β-D-Glcp units, and parts of the hydroxyl groups at the C2 and C3 positions are acetylated naturally. The side chain contains an α-D-galactopyranose (α-D-Galp) unit that is easily cleaved by acids. The basic unit of arabinoglucuronoxylan is a β-D-xylopyranose (β-D-Xylp) unit, and the side chain contains a 4-*O*-methyl α-D-glucuronic acid (α-D-GlcpA) unit and an α-L-arabinofuranose (α-L-Araf) unit.

To assign the signals for softwood hemicelluloses in the HSQC spectra of the acetylated milled fir wood in DMSO-d_6, we used arabinoxylan from oat, konjac glucomannan, and locust bean gum (galactomannan). Arabinoxylan was ball-milled for 8 h, dissolved in [Bmim]Cl, and acetylated in situ according to the same method as for the milled fir wood. Konjac glucomannan and galactomannan were ball-milled for 8 h and directly acetylated.

The HSQC NMR signals for the β-D-Xylp units in acetylated oat arabinoxylan in DMSO-d_6 (Figure 6C) were determined by ^{1}H−^{1}H correlation spectroscopy (COSY). The signals at 4.70/99.5, 4.50/70.4, 4.93/71.9, and 3.75/75.0 ppm were assigned to H1/C1 (x1), H2/C2 (x2), H3/C3 (x3), and H4/C4 (x4), respectively. The signals at 3.85/62.2 and 3.29/62.2 ppm were assigned to H5/C5 (x5). These signals were detected clearly in the acetylated fir wood, although one of the H5/C5 signals overlapped other $-CH_2O-$ signals.

The signals for the α-L-Araf units in acetylated oat arabinoxylan in DMSO-d_6 were determined by analogy with the reported spectroscopic data for acetylated arabinoxylo-oligosaccharides in $CDCl_3$.[22] The signals at 5.00/104.5, 4.80/80.6, 4.85/76.6, and 4.51/79.3 ppm were assigned to H1/C1 (a1), H2/C2 (a2), H3/C3 (a3), and H4/C4 (a4), respectively. The signals at 4.32/62.4

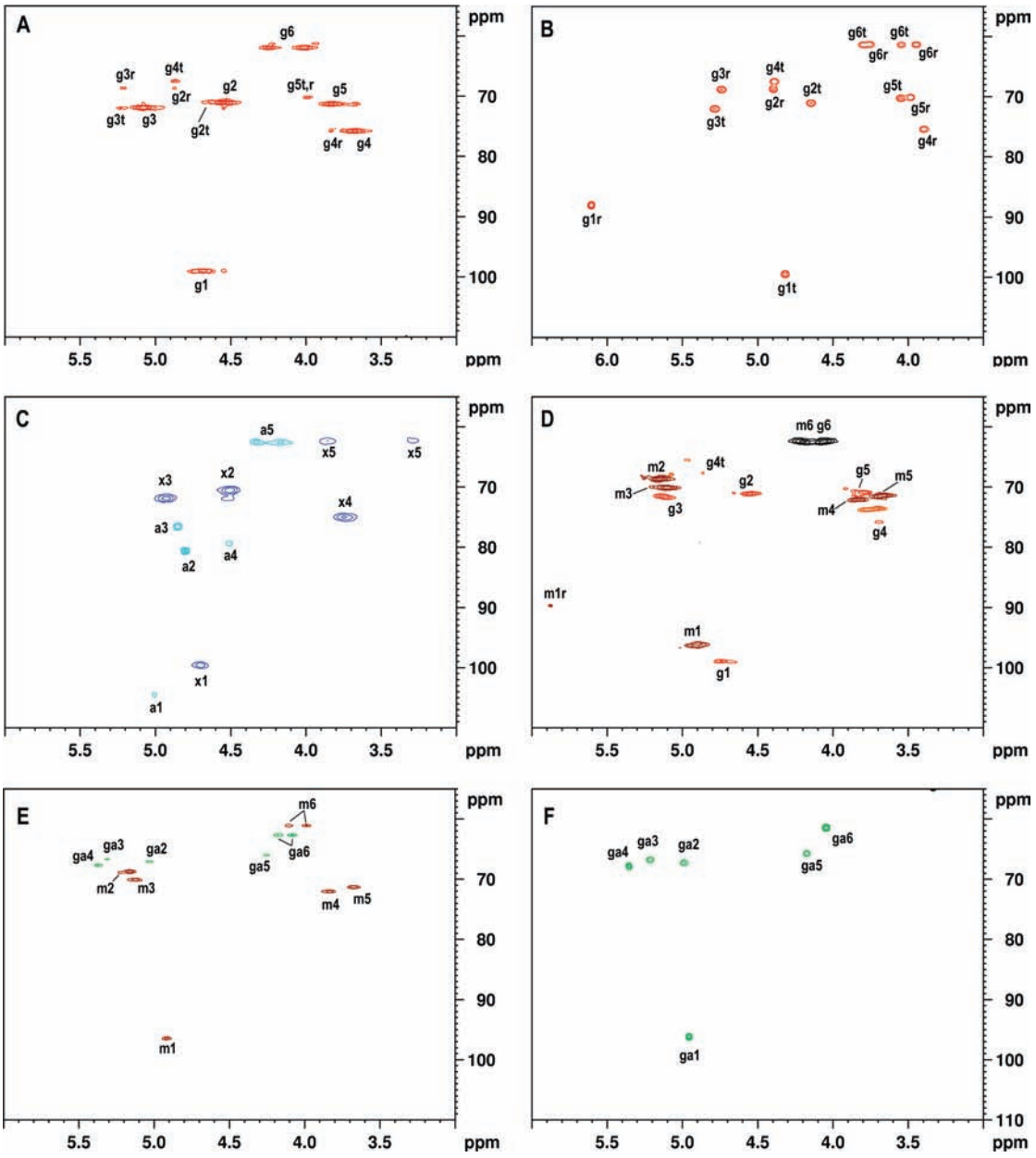

Figure 6. HSQC spectra of peracetylated carbohydrate model compounds in DMSO-d_6: (A) cellulose; (B) cellobiose; (C) arabinoxylan from oat; (D) konjac glucomannan; (E) locust bean gum (galactomannan); (F) methyl α-D-galactopyranoside. g, β-D-glucopyranose (red); x, β-D-xylopyranose (blue); a, α-L-arabinofuranose (light blue); m, β-D-mannopyranose (brown); ga, α-D-galactopyranose (green) units (glucomannan signals that are present in the acetylated fir wood but unassigned are shown in orange); r, reducing end units; t, nonreducing end (terminal) units.

and 4.16/62.4 ppm were assigned to H5/C5 (a5). A distinct H3−H4 correlation was observed in the ^{1}H−^{1}H COSY spectrum of acetylated oat arabinoxylan in DMSO-d_6, which supports these assignments. The signals for the α-L-Araf unit were detected clearly in the HSQC spectrum of the acetylated fir wood, except for the H5/C5 (a5) signals, which overlapped other −CH_2O− signals in the acetylated fir wood.

The α-D-GlcpA unit is one of the side-chain structures in the softwood arabinoglucuronoxylan. However, we could not identify signals for the unit, because NMR data for a proper acetylated model compound in DMSO-d_6 were not available. Further studies are necessary to clarify whether the units are present in acetylated fir woods recovered from [Bmim]Cl.

The NMR signals corresponding to galactoglucomannan in the fir wood are rather complicated, because the main chain contains both β-D-Manp and β-D-Glcp units. To assign the signals, we used konjac glucomannan and locust bean gum (galactomannan) (Figure 6D,E). We assigned signals for the β-D-Glcp units in konjac glucomannan by comparison with the signals for cellulose. These signals overlapped those of cellulose in the acetylated fir wood. We assigned the β-D-Manp signals at 4.92/96.3, 5.16/68.6, 5.11/70.0, 3.83/72.0, and 3.68/71.3 ppm in both glucomannan and galactomannan to H1/C1 (m1), H2/C2 (m2), H3/C3 (m3), H4/C4 (m4), and H5/C5 (m5), respectively, by ^{1}H−^{1}H COSY spectra. All of these signals were detected in the acetylated fir wood. The other unassigned

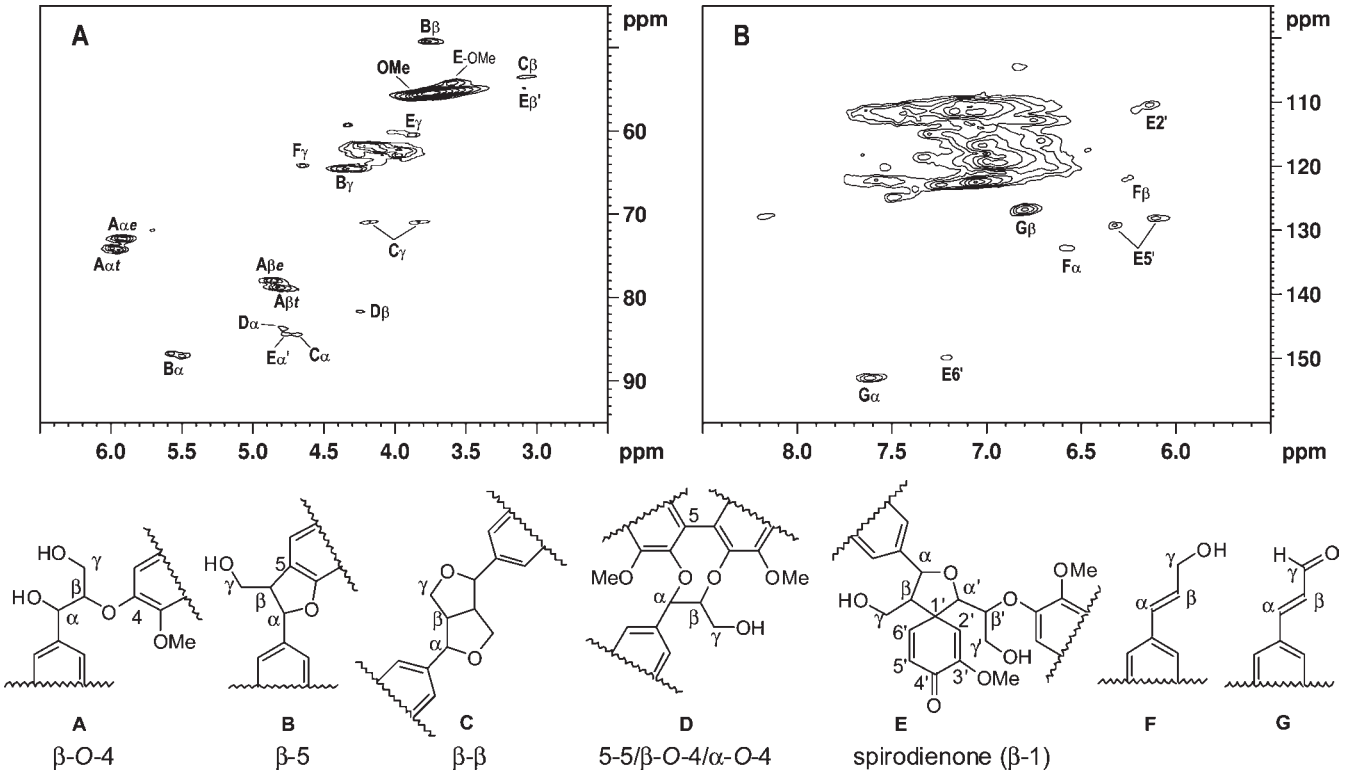

Figure 7. HSQC spectra of acetylated softwood lignin (fir MWL) in DMSO-d_6: (A) aliphatic region; (B) aromatic region.

signals in konjac glucomannan (orange color) may correspond to the β-D-Manp or β-D-Glcp units in β-D-Glcp−β-D-Manp or β-D-Manp−β-D-Glcp substructures. These signals were also detected in the acetylated fir wood. We used methyl α-D-galactopyranoside as a model compound to assign the α-D-Galp units in galactomannan (Figure 6F). The α-D-Galp units in galactoglucomannan were also visible in the acetylated fir wood.

There remain some unassigned signals, which may correspond to unidentified polysaccharide signals or artifacts produced during the processes. Further studies are necessary to clarify these points. However, the main polysaccharide signals, including hemicelluloses and cellulose end groups in DMSO-d_6, were assigned. The β-D-Glcp, β-D-Manp, β-D-Xylp, α-D-Galp, and α-L-Araf units assigned in this study are the main monosaccharide units not only in softwood polysaccharide but also in hardwoods and herbaceous plants. The procedures used in this study are therefore applicable without serious problems for the characterization of polysaccharides in such plant biomasses.

Characterization of Lignin. The HSQC spectrum of acetylated authentic lignin isolated from fir wood (MWL) was used for assignments of the aliphatic and aromatic regions of lignin in the acetylated fir wood (Figure 7A,B). Unless otherwise noted, the assignments were based on a compiled spectroscopic database for lignin model compounds[23] and 2D spectra of isolated lignins.[24]

Lignin substructures, including β-aryl ether (β-*O*-4; **A**), phenylcoumaran (β-5; **B**), resinol (β−β; **C**), dibenzodioxocin (5−5/β-*O*-4/α-*O*-4; **D**), spirodienone (**E**), cinnamyl alcohol (**F**), and cinnamyl aldehyde structures (**G**),were observed in the HSQC spectrum of acetylated fir wood recovered from [Bmim]Cl. The signals for the *erythro* and *threo* forms of the β-*O*-4 structures were resolved relatively well. The $\mathbf{A}_\alpha$ signals at 5.92/72.9 and 5.97/74.1 ppm were assigned to the *erythro* and *threo* forms, respectively, and the $\mathbf{A}_\beta$ signals at 4.87/77.9 and 4.82/78.7 ppm were assigned to the *erythro* and *threo* forms, respectively. These assignments were produced by comparison with our β-*O*-4-type artificial lignin polymers.[25,26] Almost equal amounts of the *erythro* and *threo* forms of the β-*O*-4 structures were present in the acetylated fir wood. The β-5 correlations were observed at 5.53/86.8 ppm ($\mathbf{B}_\alpha$), 3.77/49.3 ppm ($\mathbf{B}_\beta$), and 4.35/64.5 ppm ($\mathbf{B}_\gamma$). The β−β signals were at 4.69/84.4 ($\mathbf{C}_\alpha$), 3.09/53.5 ($\mathbf{C}_\beta$), 3.83/70.9 ($\mathbf{C}_\gamma$), and 4.17/70.9 ppm ($\mathbf{C}_\gamma$). The signals for dibenzodioxocin were rather weak, but clearly evident at 4.78/83.7 ppm ($\mathbf{D}_\alpha$) and 4.25/81.6 ppm ($\mathbf{D}_\beta$). Signals for spirodienone structures were also detected. The signals at 3.08/54.8, 4.79/84.3, and 3.59/54.3 ppm were tentatively assigned to $\mathbf{E}_\beta'$, $\mathbf{E}_\alpha'$ and **E**-OMe in the spirodienone structures by analogy with the reported spectroscopic data in acetone-d_6.[27]

The aromatic region of the HSQC spectrum of acetylated milled fir wood is shown in Figure 8. Softwood lignins such as fir lignin consist mainly of the guaiacyl unit. Strong signals for H2/C2, H5/C5, and H6/C6 in the guaiacyl unit can be seen. The existence of cinnamyl alcohol (**F**) and cinnamyl aldehyde (**G**) end groups is evident in the acetylated fir wood. The signals at 6.57/132.8 and 6.25/121.9 ppm correspond to $\mathbf{F}_\alpha$ and $\mathbf{F}_\beta$, respectively, in the cinnamyl alcohol moieties. The signals at 7.62/159.9 and 6.80/126.8 ppm correspond to $\mathbf{G}_\alpha$ and $\mathbf{G}_\beta$, respectively, in the cinnamyl aldehyde structures. The signals of guaiacyl-type spirodienone structures **E** were also assigned on the basis of the reported spectroscopic data.[25] At lower counter levels, the weak signals at 6.15/110.6 ($\mathbf{E}_2'$), 6.31/129.2 ($\mathbf{E}_5'$), and 6.06/128.1 ($\mathbf{E}_5'$) ppm were assigned to $\mathbf{E}_2'$ and $\mathbf{E}_5'$.

Note that most of the main lignin structures were identified in the acetylated fir wood regenerated from [Bmim]Cl. In particular, resinol (**C**), dibezodioxocin (**D**), and spirodienone

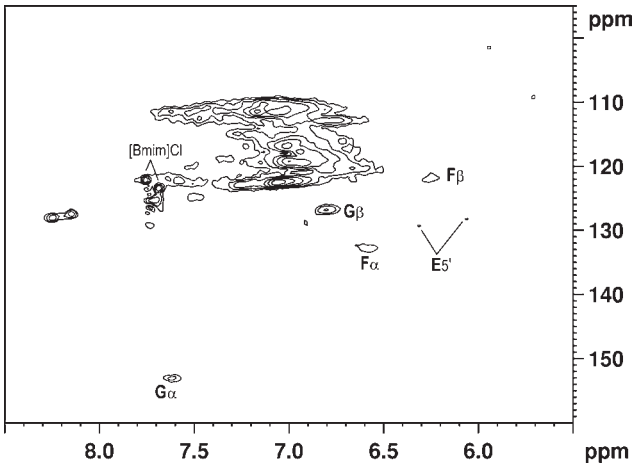

Figure 8. Aromatic region of the HSQC spectrum of acetylated fir wood regenerated from [Bmim]Cl. Solvent: DMSO-d_6. See Figure 7 for signal assignments.

structures (**E**) were detected, indicating that detailed analysis of lignin structures in plant cell walls is possible with the procedure used in this investigation without isolation of lignin.

Finally, we can conclude that ball-milling is effective for the complete dissolution of Japanese fir wood in [Bmim]Cl. The whole cell-wall components that dissolved in [Bmim]Cl, including lignin, cellulose, and hemicelluloses, were regenerated as their acetate forms and analyzed by HSQC NMR spectroscopy in DMSO-d_6. The acetylated lignin and polysaccharide signals dispersed reasonably well on the 2D spectra, which made it possible to characterize most of the signals. The similar 2D spectra of acetylated cell walls have been obtained by another cell-wall dissolution method, the NMI/DMSO system,[2] for which only lignin and cellulose have been characterized. In this study, particular attention was paid to the characterization of polysaccharides other than the major lignin structures, and the main polysaccharide signals, including cellulose, galactoglucomannan, and arabinoxylan, were assigned by comparison with commercial cellulose and hemicelluloses. Although there remain some unassigned signals, which may correspond to unidentified polysaccharide signals or artifacts produced during the processes, the procedure used here can be applied to the analysis of cell-wall components in various plant biomasses. It can also be used to characterize the structural changes of cell-wall components caused by various biomass-refinery processes.

■ ASSOCIATED CONTENT

Supporting Information. Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

[Bmim]Cl, 1-butyl-3-methylimidazolium chloride; HSQC, heteronuclear single-quantum coherence; NMR, nuclear magnetic resonance; NMI, *N*-methylimidazole; [Emim]OAc, 1-ethyl-3-methylimidazolium acetate; [Amim]Cl, 1-allyl-3-methylimidazolium chloride; TMP, thermomechanical pulp; ([Bmim][$MeSO_4$]), 1-butyl-3-methylimidazolium methylsulfate; [Emim][(MeO)HPO_2], 1-ethyl-3-methylimidazolium methylphosphonate; MWL, milled wood lignin; ATR, attenuated total reflection; β-D-Glcp, β-D-glucopyranose; β-D-Manp, β-D-mannopyranose; α-D-Galp, α-D-galactopyranose; β-D-Xylp, β-D-xylopyranose; α-D-GlcpA, 4-*O*-methyl α-D-glucuronic acid; α-L-Araf, α-L-arabinofuranose; COSY, correlation spectroscopy.

■ REFERENCES

(1) Fujimoto, A.; Matsumoto, Y.; Chang, H.-M.; Meshitsuka, G. Quantitative evaluation of milling effects on lignin structure during the isolation process of milled wood lignin. *J. Wood Sci.* **2005**, *51*, 89–91.

(2) Lu, F.; Ralph, J. Non-degradative dissolution and acetylation of ball-milled plant cell walls: high-resolution solution-state NMR. *Plant J.* **2003**, *35*, 535–544.

(3) Yelle, D. J.; Ralph, J.; Frihart, C. R. Characterization of non-derivatized plant cell walls using high-resolution solution-state NMR spectroscopy. *Magn. Reson. Chem.* **2008**, *46*, 508–517.

(4) Zhang, A.; Lu, F.; Sun, R.-C.; Ralph, J. Isolation of cellulolytic enzyme lignin from wood preswollen/dissolved in dimethyl sulfoxide/*N*-methylimidazole. *J. Agric. Food Chem.* **2010**, *58*, 3446–3450.

(5) Kim, H.; Ralph, J.; Akiyama, T. Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d_6. *Bioenerg. Res.* **2008**, *1*, 56–66.

(6) Kim, H.; Ralph, J. Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d_6/pyridine-d_5. *Org. Biomol. Chem.* **2010**, *8*, 576–591.

(7) Wang, Z.; Yokoyama, T.; Chang, H.; Matsumoto, Y. Dissolution of beech and spruce milled wood in LiCl/DMSO. *J. Agric. Food Chem.* **2009**, *57*, 6167–6170.

(8) Fort, D. A.; Remsing, R. C.; Swatloski, R. P.; Moyna, P.; Moyna, G.; Rogers, R. D. Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-*n*-butyl-3-methylimidazolium chloride. *Green Chem.* **2007**, *9*, 63–69.

(9) Sun, N.; Rahman, M.; Qin, Y.; Maxim, M. L.; Rodriguez, H.; Rogers, R. D. Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. *Green Chem.* **2009**, *11*, 646–655.

(10) Kilpelainen, I.; Xie, H.; King, A.; Granstrom, M.; Heikkinen, S.; Argyropoulos, D. S. Dissolution of wood in ionic liquid. *J. Agric. Food Chem.* **2007**, *55*, 9142–9146.

(11) Çetinkol, Ö. P.; Dibble, D. C.; Cheng, G.; Kent, M. S.; Knierim, B.; Auer, M.; Wemmer, D. E.; Pelton, J. G.; Melnichenko, Y. B.; Ralph, J.; Simmons, B. A.; Holmes, B. M. Understanding the impact of ionic liquid pretreatment on eucalyptus. *Biofuels* **2010**, *1*, 33–46.

(12) Jiang, N.; Pu, Y.; Samuel, R.; Ragauskas, A. J. Perdeuterated pyridinium molten salt (ionic liquid) for direct dissolution and NMR analysis of plant cell walls. *Green Chem.* **2009**, *11*, 1762–1766.

(13) Fukaya, Y.; Hayashi, K.; Wada, M.; Ohno, H. Cellulose dissolution with polar ionic liquids under mild conditions: required factors for anions. *Green Chem.* **2008**, *10*, 44–46.

(14) Kishimoto, T.; Ueki, A.; Sano, Y. Delignification mechanism during high-boiling solvent pulping. Part 3. Structural changes in lignin analyzed by ^{13}C-NMR spectroscopy. *Holzforschung* **2003**, *57*, 602–610.

(15) Kishimoto, T.; Ueki, A.; Takamori, H.; Uraki, Y.; Ubukata, M. Delignification mechanism during high-boiling solvent pulping. Part 6: Changes in lignin structure analyzed by ^{1}H−^{13}C correlation 2-D NMR spectroscopy. *Holzforschung* **2004**, *58*, 355–362.

(16) Swarovski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D. Dissolution of cellulose with ionic liquids. *J. Am. Chem. Soc.* **2002**, *124*, 4974–4975.

(17) Wu, J.; Zhang, J.; Zhang, H.; He, J.; Ren, Q.; Guo, M. Homogeneous acetylation of cellulose in a new ionic liquid. *Biomacromolecules* **2004**, *5*, 266–268.

(18) Pu, Y.; Jiang, N.; Ragauskas, A. J. Ionic liquid as a green solvent for lignin. *J. Wood Chem. Technol.* **2007**, *27*, 23–33.

(19) Kubo, S.; Hashida, K.; Yamada, T.; Hishiyama, S.; Magara, K.; Kishino, M.; Ohno, H.; Hosoya, S. A characteristic reaction of lignin in ionic liquids; glycelol type enol-ether as the primary decomposition product of *β*-*O*-4 model compound. *J. Wood Chem. Technol.* **2008**, *28*, 84–96.

(20) Xie, H.; King, A.; Kilpelainen, I.; Granstrom, M.; Argyropoulos, D. S. Thorough chemical modification of wood-based lignocellulosic materials in ionic liquids. *Biomacromolecules* **2007**, *8*, 3740–3748.

(21) 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.

(22) Ito, T.; Yamai, M.; Nishio, T.; Oku, T. Carbon-13 NMR spectra of peracetylated derivatives of methyl L-arabinofuranoside and oligosaccharides related to arabinoxylan. *J. Carbohydr. Chem.* **1994**, *13*, 491–498.

(23) Ralph, S. A.; Ralph, J.; Landucci, L. L. NMR Database of Lignin and Cell Wall Model Compounds, 2004; available at URL http://ars.usda.gov/Services/docs.htm?docid=10491 (accessed Jan 30, 2011).

(24) Ralph, J.; Landucci, L. L. NMR of lignins. In *Lignin and Lignans: Advances in Chemistry*; Heitner, C., Dimmel, D. R., Schmidt, A. J., Eds.; CRC Press: Boca Raton, FL, 2010; pp 138−222.

(25) Kishimoto, T.; Uraki, Y.; Ubukata, M. Chemical synthesis of *β*-*O*-4 type artificial lignin. *Org. Biomol. Chem.* **2006**, *4*, 1343–1347.

(26) Kishimoto, T.; Uraki, Y.; Ubukata, M. Synthesis of *β*-*O*-4 type artificial lignin polymers and their analysis by NMR spectroscopy. *Org. Biomol. Chem.* **2008**, *6*, 2982–2987.

(27) Zhang, L.; Gellerstedt, G. NMR observation of a new lignin structure, a spiro-dienone. *Chem. Commun.* **2001**, *24*, 2744–2745.