# Preparation of synthetic lignins with superior NMR characteristics *via* isotopically labeled monolignols

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Synthetic lignins are particularly valuable for studying aspects of lignification, plant cell wall cross-linking, and lignin structure. If they are not too highly polymeric, they are soluble in normal lignin solvents and amenable to solution-state NMR studies. However, in the application of inverse-detected correlation experiments, particularly the popular HMQC and HMBC experiments, the spectra have annoying  $T_1$ -noise ridges. These artifacts make it difficult to locate correlation peaks that are near the methoxy signal in the proton dimension. One solution is to use gradient-enhanced NMR but that requires additional hardware that is not yet ubiquitous. An alternative is to produce monolignols in which the atoms of the methoxy group are NMR-invisible. We have accomplished this by preparing coniferyl and sinapyl alcohols using <sup>13</sup>C-depleted deuterated methyl iodide ( $^{12}C^2H_3I$ ). The methods, which incorporate steps simpler than have been used previously for labeled monolignols, are sufficiently low cost and straightforward that these monomers can be utilized for any synthetic lignins destined for NMR studies. The NMR spectra of lignins derived from these 'methoxy-less' monomers are markedly superior to their normal-monomer counterparts. Several popular NMR experiments are illustrated for synthetic lignins derived from normal *vs*. isotopically labeled coniferyl alcohol, along with some useful experiments that have not been seen in lignin-related publications to date.

#### Introduction

Despite its rather random and heterogeneous nature, the lignin polymer discloses many of its intimate structural details to diagnostic NMR experiments. <sup>13</sup>C NMR established itself early as a method for detailed structural characterization, aided by the almost exact agreement between chemical shifts of carbons in good low-molecular-mass model compounds and in the polymer.<sup>1,2</sup> Although this correspondence cannot generally be expected for proton NMR, good lignin model compounds and their counterpart units in the polymer also closely match proton NMR chemical shifts.<sup>3-5</sup> Despite the broad featureless 1D proton spectra, which have nevertheless allowed substantive interpretation,<sup>6</sup> 2D spectra, both homo- and hetero-nuclear, are informationally rich and strikingly useful.

The advent of inverse-detection, and the enabling of commercial spectrometers to easily carry out these experiments, provided a welcome sensitivity enhancement for the difficult lignin polymers, but not without some drawbacks. The major problems in the inverse experiments, particularly the popular HMBC<sup>7</sup> and HMQC<sup>8</sup> experiments, are related to  $T_1$ -noise artifacts which are particularly obstructive in lignins due to their intense methoxy signals. Methoxy resonances are relatively sharp and intense in both the proton domain (where the 3-6 methoxy protons per monolignol unit cause the methoxy peak to dominate proton spectra) and the carbon domain (where the narrow methoxy chemical shift range causes a similarly dominant methoxy carbon peak). The result in proton-detected 2D spectra is an almost complete obliteration of information from protons resonating near the methoxy. The problem can be somewhat minimized by using excessively long relaxation delays but only at the expense of valuable signal-to-noise in a given time. Although this problem can now be obviated by the use of pulsed field gradients on appropriately configured newer

instruments, an attractive solution for synthetic lignins is simple labeling that makes the methoxy NMR-invisible. Here we report on the synthesis of the two methoxylated monolignols, coniferyl and sinapyl alcohols, with the methoxy groups deuterated and <sup>13</sup>C-depleted. Such labeling greatly improves the appearance and interpretability of spectra as illustrated by comparison of 2D NMR spectra of synthetic lignins prepared from normal *vs.* the labeled coniferyl alcohol.

#### **Results and discussion**

An elegant solution to the methoxy region  $T_1$ -noise NMR artifact problem is possible for synthetic lignins, without requiring gradient NMR methods. The solution is to make the methoxy groups NMR-invisible, but normal in every other regard. This can be accomplished by replacing the normal methoxy carbon (which has ~1% natural abundance of NMR-active <sup>13</sup>C) by the <sup>12</sup>C isotope, which is not NMR-active, and replacing the protons with deuterons using a <sup>13</sup>C-depleted trideuteromethyl reagent.

Coniferyl alcohol **1a** with NMR-invisible methoxy groups was synthesized as shown in Scheme 1a, using the relatively cheap <sup>13</sup>C-depleted trideuteromethyl iodide as the key reagent. Other notable steps in the synthesis are the use of the tetrahydropyranyl group for the required regioselective phenol mono-protection of catechol **2**, and the direct conversion of bromoguaiacol **5** to vanillin **6** is achieved *via* a halogen–metal interchange and subsequent formylation using dimethylformamide.<sup>9</sup> These steps are considerable improvements over prior methods (directed *o*-hydroxylation following 2-chloro-5nitrobenzophenone protection <sup>10</sup> and the arduous route from bromide **5** *via* the acid through CO<sub>2</sub> addition to the anion, chlorination, and conversion of the acid chloride to the required aldehyde **6**).<sup>11</sup>



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Scheme 1 Synthetic schemes for compounds 1, and polymerization to synthetic lignins containing structures A–E and X. *Reagents and conditions*: a, 3,4-dihydro-2*H*-pyran, pyridinium toluene-*p*-sulfonate (PPTS), CH<sub>2</sub>Cl<sub>2</sub>, rt; b, <sup>12</sup>CD<sub>3</sub>I, K<sub>2</sub>CO<sub>3</sub>, acetone, 43 °C; c, (i) PPTS, EtOH, 55 °C, (ii) KBrO<sub>3</sub>, HBr, HOAc, rt; d, (i) ethyl vinyl ether (EVE), PPTS, CH<sub>2</sub>Cl<sub>2</sub>, rt, (ii) *n*-BuLi, DMF, THF, -78 °C; (iii) 2 M HCl, rt; e, (i) EVE, PPTS, CH<sub>2</sub>Cl<sub>2</sub>, rt; (ii) triethyl phosphonoacetate, NaH, THF, rt; (iii) AcOH, MeOH; f, (i) DIBAL, toluene, 0 °C; (iii) HCl, THF; g, PhCH<sub>2</sub>Cl, acetone, K<sub>2</sub>CO<sub>3</sub>, KI, reflux, overnight; h, (i) triethyl phosphonoacetate, NaH, THF, rt; (ii) AcOH, MeOH; (iii) HCl, THF; i, TMSI, CH<sub>3</sub>CN; j, peroxidase, H<sub>2</sub>O<sub>2</sub>, pH 6.5. Standard lignin numbering is used. Lignin structures are: A, β-aryl ether (β–O–4); B, phenylcoumaran (β–5); C, resinol (β–β); D, dibenzodioxocin (5–5 and β–O–4/α–O–4); E, α,β-diether (β–O–4/α–O–4); X, cinnamyl alcohols.

Specifically labeled sinapyl alcohol **1b** was prepared from commercial 3,4,5-trihydroxybenzaldehyde **8** by regioselective mono-benzylation (directed by the aldehyde group) followed by similar methylation and subsequent conversions, Scheme 1b. Removal of the benzyl group used TMSI; <sup>12</sup> normal hydrogenolysis is incompatible with the double bond.

As shown in Fig. 1, a variety of 2D NMR spectra of a synthetic lignin produced from the specialized coniferyl alcohol **1a** are markedly superior to an equivalent lignin made from normal coniferyl alcohol. The spectra also illustrate the value of spectral editing in 2D NMR experiments that has not

yet attracted much attention from lignin chemists. The DEPT-HMQC<sup>13</sup> experiments can aid assignment by selectively inverting (for example) -CH<sub>2</sub> resonances (Fig. 1C). The HMQC-TOCSY<sup>14</sup> experiment is still under utilized in plant cell wall research. It provides beautifully redundant correlations that greatly facilitate rapid assignment of lignin spectra, particularly in the sidechain region.

The freedom from rapid-pulsing artifacts in these spectra allows detailed assignments to be made; all of the major subunits are assigned in selected spectra of Fig. 1. Such assignments aid the characterization of real plant lignins.



Fig. 1 2D NMR spectra of synthetic lignins made using (left) normal coniferyl alcohol and (right) 'methoxy-less' coniferyl alcohol. A, HMQC; B, DEPT-HMQC with a  $\pi$ /3 editing pulse (CH's up, CH<sub>3</sub>'s and CH<sub>2</sub>'s down); C, DEPT-HMQC with a  $\pi$  editing pulse (CH's and CH<sub>3</sub>'s up, CH<sub>2</sub>'s down); D, HMQC-TOCSY; E, HMBC. Dashed lines are negative contours. Assignments: on HMQC, structure (A–E, X) followed (smaller size) by sidechain C–H pair (*e.g.* B<sub> $\beta\alpha$ </sub> is the structure **B** C- $\beta$ /H- $\beta$  correlation); on HMQC-TOCSY, structure followed by carbon excited in HMQC part followed by proton from TOCSY part (*e.g.* B<sub> $\beta\alpha$ </sub> is the structure **B** C- $\beta$ /H- $\alpha$  correlation); on HMBC, structure followed by carbon followed by coupled proton within 3-bonds (*e.g.* B<sub> $\beta\alpha$ </sub> is the structure **B** C- $\beta$ /H- $\alpha$  long-range correlation).

Rendering the normally intense methoxy groups NMRinvisible also dramatically improves homonuclear 2D experiments. Such isotopically labeled ligning have further value for studies using mass spectrometry, where the M+3 or M+6 ions (from the three deuterons in **1a** or the 6 deuterons in **1b**) allow use of these synthetic lignins as internal standards or markers for natural lignin studies.

#### Experimental

#### General

Reagents were all purchased from Aldrich Chemical Co. (Milwaukee, WI). Liquid reagents were distilled prior to use; solids were used as supplied. Light petroleum was the 40–60 °C boiling fraction. Reactions were monitored by thin layer chromatography (TLC) using Alugram Sil-G/UV254 plates (Macherey-Nagel); visualization was by UV light. Merck silica gel 60 (70–230 mesh) was used for flash chromatography. Melting points were on an Electrothermal digital mp apparatus and are uncorrected. High-resolution MS was on a Kratos MS-80RFA spectrometer.

NMR Spectra were taken on a Bruker AMX 360 MHz instrument (Bruker instruments, Billerica, MA) fitted with a 5 mm 4-nucleus (QNP) probe with normal geometry (proton coils furthest from the sample) using standard Bruker pulse programs. Samples were in 0.4 mL of acetone-d<sub>6</sub>, with the central solvent peak as internal reference ( $\delta_{\rm H}$  2.04,  $\delta_{\rm C}$  29.8). J Values are given in Hz. The carbon/proton designations are based on the standard lignin numbering system (Scheme 1). NMR assignments were authenticated by the usual complement of 1D and 2D NMR experiments. Benzyl group aromatic resonances are not reported. Phase-sensitive short-range 2D correlation spectra (HMQC,8 DEPT-HMQC13 and HMQC-TOCSY<sup>14</sup>) were acquired from ~10-140 ppm using 1k FIDs with 256 increments and 96-128 scans per increment. These acquisitions were for high S/N spectra and were run excessively long-remarkably good HMQC spectra with all correlations fully represented can be obtained in under 20 minutes from these samples (under 4 minutes using gradient selected experiments). The TOCSY sequence used an 80 ms mixing period. Apodization was by Gaussian multiplication, and the final matrix data size was 1k by 1k real points (corresponding to resolutions of 3.6 and 11.4 Hz per pt in the proton and carbon domains). Magnitude-mode long-range correlation (HMBC<sup>7</sup>) spectra were acquired from ~10-200 ppm using 2k FIDs with 256 increments and 480 scans per increment, and a longrange coupling delay of 100 ms (J = 5 Hz). Apodization was by Gaussian multiplication, and the final matrix data size was 1k by 1k real points (corresponding to resolutions of 3.6 and 17.4 Hz per pt in the proton and carbon domains).

#### Synthesis of labeled coniferyl alcohol 1a

**2-Tetrahydropyranyloxyphenol 3.** Catechol **2** (10 g, 91 mmol), 3,4-dihydro-2*H*-pyran (8.56 mL, 91 mmol) and pyridinium toluene-*p*-sulfonate (297 mg, 1.2 mmol) were dissolved in dichloromethane. The mixture was stirred at room temperature for 3 h, after which time TLC (CHCl<sub>3</sub>–EtOAc, 9:1) indicated that the reaction was complete. The solvent was removed *in vacuo* and the syrup dissolved in EtOAc, washed twice with sat. aq. NaHCO<sub>3</sub>, once with sat. aq. NaCl, dried over MgSO<sub>4</sub> and the solvent evaporated under vacuum to give compound **2** as a light yellow liquid (16.65 g, 86.2 mmol, 95%).

1-Tetrahydropyranyloxy-2-[<sup>12</sup>C, <sup>2</sup>H<sub>3</sub>]methoxybenzene 4. Compound 3 (2.00 g, 10.35 mmol) and powdered anhydrous  $K_2CO_3$  (2.29 g, 16.6 mmol) were dissolved in anhydrous acetone and <sup>12</sup>CD<sub>3</sub>I (0.71 mL, 11.4 mmol) was added. The mixture was stirred at 41 °C overnight. The reaction was checked for completeness by TLC (CHCl<sub>3</sub>–EtOAc, 9:1). The inorganic salts were filtered off, and the filtrate evaporated to give 4 as a syrup (1.77 g, 8.56 mmol, 83%).

**4-Bromoguaiacol, 4-bromo-2-[**<sup>12</sup>C, <sup>2</sup>H<sub>3</sub>]methoxyphenol **5.** Direct bromination with molecular bromine in HOAc is satisfactory, but we preferred to generate the bromine *in situ*.<sup>15</sup> Compound **4** (1.77 g, 8.56 mmol) was dissolved in EtOH and a catalytic amount of pyridinium toluene-*p*-sulfonate was added. The mixture was stirred at 55 °C for 3 h and evaporated at 35 °C *in vacuo* to afford a syrup (0.97 g, 7.63 mmol), which was dissolved in 20 mL glacial acetic acid. KBrO<sub>3</sub> (427 mg, 2.54 mmol) and HBr (2.58 mL of 30% HBr in HOAc, 12.97 mmol) were added. The mixture was stirred at room temperature for 4 h, diluted with 100 ml H<sub>2</sub>O, and then extracted twice with EtOAc. The organic phase was washed with sat. aq. NaHCO<sub>3</sub> to neutralize the solution, then washed with aq. NaCl, dried over MgSO<sub>4</sub>, and the solvent evaporated to give a syrup (1.21 g, 5.87 mmol, 77%).

Vanillin, 4-hydroxy-3-[<sup>12</sup>C, <sup>2</sup>H<sub>3</sub>]methoxybenzaldehyde 6. The phenol in 5 was first protected as its ethoxyethylidene derivative.11 Compound 5 (1.21 g, 5.87 mmol) and a catalytic amount of pyridinium toluene-p-sulfonate were dissolved in dichloromethane, and then ethyl vinyl ether (1.8 mL, 18.8 mmol) was added. The mixture was stirred at room temperature for 4 h, after which time TLC indicated that the reaction was complete. Work-up<sup>11</sup> gave the protected bromoguaiacol as a syrup (1.567 g, 5.63 mmol, 96%), which was then formylated.<sup>9</sup> Protected 5 was dissolved in dry THF under nitrogen and cooled to -78 °C, and n-BuLi (3.38 mL, 2.5 M in hexane, 16.9 mmol) added. The solution was stirred for 45 min at -78 °C, following which dry DMF (1.31 mL, 16.9 mmol) was added. Vigorous stirring was maintained for 2 h, then the solution was allowed to warm to room temperature, and the reaction quenched with 2 M HCl (5 mL). After an additional 1 h, the solvent was evaporated and the syrup was extracted with EtOAc, washed with sat. aq. NaHCO<sub>3</sub> and NaCl sequentially, dried over MgSO<sub>4</sub>, and the solvent evaporated under reduced pressure. Crystallization from acetone-H<sub>2</sub>O gave pure 6 as white needle crystals (706 mg, 4.56 mmol, 78%).

Ethyl ferulate, ethyl 4-hydroxy-3-[<sup>12</sup>C, <sup>2</sup>H<sub>3</sub>]methoxycinnamate 7a. The ethyl ferulate was made *via* a Horner–Emmons reaction as described previously, again following protection as the ethoxyethylidene derivative.<sup>11</sup> Thus, triethyl phosphonoacetate (0.90 mL, 4.54 mmol) was ionized with NaH (163 mg, 6.8 mmol) and the ethoxyethylidene derivative of **6** (1.03 g, 4.54 mmol) added to give **7a** (915 mg, 4.07 mmol, 90%) which was directly used in the next step without further purification.

Coniferyl alcohol, 4-hydroxy-3-[ $^{12}C$ ,  $^{2}H_3$ ]methoxycinnamyl alcohol 1a. Compound 7a was reduced as has been described for the preparation of coniferyl alcohol from ethyl ferulate. <sup>16</sup> Thus, 7a (915 mg, 4.07 mmol) was reduced with DIBAL (11.4 mL of 1.5 M solution in toluene, 17.1 mmol) and worked up to give crude 1a as an oil (730 mg, 3.99 mmol, 98%). Crystallization from CH<sub>2</sub>Cl<sub>2</sub>–light petroleum gave 1a as colorless plates (540 mg, 2.95 mmol, 73%);  $\delta_H$  3.69 (1H, t, J 5.7,  $\gamma$ -OH), 4.18 (2H, td,  $J \sim 5.6$ , 1.6, H- $\gamma$ ), 6.21 (1H, dt, J 15.9, 5.6, H- $\beta$ ), 6.49 (1H, dt, J 15.9, 1.6, H- $\alpha$ ), 6.75 (1H, d, J 8.1, H-5), 6.84 (1H, dd, J 8.1, 2.0, H-6), 7.04 (1H, d, J 2.0, H-2), 7.53 (1H, s, Ar-OH);  $\delta_C$  63.4 ( $\gamma$ ), 109.9 (2), 115.5 (5), 120.6 (6), 128.0 ( $\beta$ ), 130.2 (1), 130.5 ( $\alpha$ ), 147.2 (4) 148.5 (3); *m*/*z* 183.0967 (69%) (C<sub>10</sub>H<sub>9</sub><sup>2</sup>H<sub>3</sub>O<sub>3</sub> requires 183.0975).

#### Synthesis of labeled sinapyl alcohol 1b

**4-Benzyloxy-3,5-dihydroxybenzaldehyde 9.** 3,4,5-Trihydroxybenzaldehyde **8** (3.0 g, 17.4 mmol) was dissolved in anhydrous acetone. Benzyl chloride (2.0 mL, 17.4 mmol) was added, followed by anhydrous K<sub>2</sub>CO<sub>3</sub> (4.0 g, 29 mmol) and a cat. amount of KI. The mixture was refluxed overnight and completeness was checked by TLC (EtOAc–light petroleum, 2:1). The inorganic salts were filtered off and the filtrate was diluted with water and extracted with EtOAc (3 × 100 mL), washed with sat. aq. NaCl, dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated under reduced pressure to give a yellow solid. Flash chromatography (EtOAc–light petroleum 2:1) afforded **9** as light yellow crystals (3.78 g, 15.5 mmol, 89%):  $\delta_{\rm H}$  5.22 (2H, s, CH<sub>2</sub>OPh), 6.97 (2H, s, H-2/6), 8.53 (2H, s, 3,4-OHs), 9.77 (1H s, α);  $\delta_{\rm C}$  74.6 (ArOCH<sub>2</sub>), 109.7 (2/6), 133.6 (1), 140.1 (4), 152.1 (3/5), 191.7 (α).

**4-Benzyloxy-3,5-bis([^{12}C, ^{2}H\_{3}]methoxy)benzaldehyde 10.** Compound **9** (0.98 g, 8.0 mmol) and anhydrous K<sub>2</sub>CO<sub>3</sub> (2.43 g, 17.6 mmol) were dissolved in anhydrous acetone and  $^{12}CD_{3}I$  (1.0 mL, 15.95 mmol) was added. The mixture was refluxed overnight and the inorganic salts filtered off. The filtrate was concentrated *in vacuo* to give **10** as a syrup (2.1 g, 7.6 mmol), which was directly used in next step without further purification. TLC-purification was used before characterizing the compound. Compound **10**:  $\delta_{\rm H}$  5.09 (2H, s, CH<sub>2</sub>OPh), 7.21 (2H, s, H-2/6), 9.88 (1H, s,  $\alpha$ );  $\delta_{\rm C}$  75.1 (ArOCH<sub>2</sub>), 107.3 (2/6), 133.1 (1), 143.1 (4), 154.9 (3/5), 191.6 ( $\alpha$ ).

Ethyl 4-benzyloxy-3,5-bis([<sup>12</sup>C, <sup>2</sup>H<sub>3</sub>]methoxy)cinnamate 11. The ethyl sinapate was made *via* a Horner-Emmons reaction as described previously.<sup>11</sup> Thus, triethyl phosphonoacetate (1.7 mL, 8.4 mmol) was ionized with NaH (273.6 mg,11.4 mmol) in dry THF (15 mL) and compound 10 (2.1 g, 7.6 mmol) added. Work-up gave 11 as a light yellow solid (2.58 g, 7.1 mmol), which was directly used in the next step without further purification. Compound 11:  $\delta_{\rm H}$  1.28 (3H, t, *J* 7.1, CH<sub>3</sub>CH<sub>2</sub>O), 4.20 (2H, q, *J* 7.1, CH<sub>3</sub>CH<sub>2</sub>O), 5.01 (2H, s, CH<sub>2</sub>OPh), 6.49 (d, 1H, *J* 15.9, H-β), 7.02 (2H, s, H-2/6), 7.59 (1H, d, *J* 15.9, H-α);  $\delta_{\rm C}$  14.6 (CH<sub>3</sub>CH<sub>2</sub>O), 60.6 (CH<sub>3</sub>CH<sub>2</sub>O), 75.1 (ArOCH<sub>2</sub>), 106.8 (2/6), 118.3 (β), 131.0 (1), 140.0 (4), 145.2 (α), 154.8 (3/5), 167.1 (γ).

Ethyl sinapate, ethyl 4-hydroxy-3,5-bis([<sup>12</sup>C, <sup>2</sup>H<sub>3</sub>]methoxy)cinnamate 7b. Compound 11 (2.58 g, 7.1 mmol) was dissolved in anhydrous CH<sub>3</sub>CN (50 mL) and iodotrimethylsilane (5.1 mL, 35 mmol) was added under nitrogen. The mixture was stirred at 50 °C for 3 h, after which time TLC (EtOAc-hexane, 1:1) indicated that the reaction was complete. The reaction was quenched with methanol (4.5 mL, 3 equiv.), taken up in  $H_2O$ (100 mL) and extracted with EtOAc ( $3 \times 100$  mL) and the combined organic layer was dried over MgSO4 and concentrated in vacuo to afford a brown yellow solid, which was purified by flash chromatography (EtOAc-hexane, 1:1) to give 7b as colorless plate crystals (1.31 g, 4.9 mmol, 70%);  $\delta_{\rm H}$  1.27 (3H, t, J 7.1, CH<sub>3</sub>CH<sub>2</sub>O), 4.18 (2H, q, J 7.1, CH<sub>3</sub>CH<sub>2</sub>O), 6.40 (1H, d, J 15.9, H-β), 7.00 (2H, s, H-2/6), 7.56 (1H, d, J 15.9, H-α), 7.69 (1H, s, 4-OH); δ<sub>C</sub> 14.6 (CH<sub>3</sub>CH<sub>2</sub>O), 60.5 (CH<sub>3</sub>CH<sub>2</sub>O), 106.8 (2/6), 116.2 ( $\beta$ ), 126.2 (1), 139.4 (4), 145.8 ( $\alpha$ ), 148.9 (3/5), 167.4 ( $\gamma$ ).

Sinapyl alcohol, 4-hydroxy-3,5-bis([ $^{12}C$ ,  $^{2}H_3$ ]methoxy)cinnamyl alcohol 1b. Compound 7b was reduced as has been described for the preparation of normal sinapyl alcohol from ethyl sinapate.<sup>16</sup> Thus, compound 7b (1.31 g, 4.9 mmol) was reduced with DIBAL (13 mL of 1.5 M solution in toluene, 19.6 mmol) and worked up to give 1b as an oil (1.05 g, 4.8 mmol). Crystallization from CH<sub>2</sub>Cl<sub>2</sub>-hexane gave pure 1b as pale yellow needles (864 mg, 4.0 mmol, 82%);  $\delta_{\rm H}$  3.78 (1H, t, *J* 5.5,  $\gamma$ -OH), 4.20 (2H, td, *J* 5.5, 1.5, H- $\gamma$ s), 6.24 (1H, dt, *J* 15.8, 5.5,  $\beta$ ), 6.49 (1H, dt, *J* 15.8, 1.5,  $\alpha$ ), 6.71 (2H, s, H-2/6), 7.20 (1H, s, ArOH);  $\delta_{\rm C}$  63.4 ( $\gamma$ ), 104.8 (2/6), 128.4 ( $\beta$ ), 129.1 (1), 130.6 ( $\alpha$ ), 136.7 (4), 148.8 (3/5); *mlz* 216.1270 (89%) (C<sub>11</sub>H<sub>8</sub><sup>2</sup>H<sub>6</sub>O<sub>4</sub> requires 216.1269), 173.1099 (100%).

## Preparation of a synthetic lignin from labeled coniferyl alcohol 1a

The synthetic lignins were prepared from coniferyl alcohol using horseradish peroxidase at pH 6.5, as previously described.<sup>17,18</sup> NMR Spectra here are of acetylated samples, 95 mg, in 400  $\mu$ l acetone-d<sub>6</sub>.

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