Synthesis of β-*O*-4-type artificial lignin polymers and their analysis by NMR spectroscopy†

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We describe the synthesis and NMR spectroscopic analysis of three artificial lignin polymers containing only the β-*O*-4 substructure: syringyl-type homopolymer, *p*-hydroxyphenyl-type homopolymer and guaiacyl/syringyl-type heteropolymer. Using gel permeation chromatography, the weight-average degree of polymerization (DP_w) of the three polymers was determined as 19.2, 38.6, and 13.9, respectively. The polymers were prepared based on the synthetic methodology of guaiacyl-type homopolymer, and were fully characterized using ^{1}H-, ^{13}C-, and ^{1}H–^{13}C NMR spectroscopy of the acetylated and non-acetylated forms. The spectra of guaiacyl/syringyl-type heteropolymers were in good agreement with those of the β-*O*-4 substructure of milled wood lignin obtained from the hardwood of Japanese white birch.

Introduction

Lignin, a major cell wall component in woody plants, is the second most abundant biopolymer on earth after cellulose. The complex structure of lignin arises from three precursors – whereas the main constituent of softwood lignin is a guaiacyl (G) unit, hardwood lignin consists mainly of guaiacyl and syringyl (S) units. In addition to these units, herbaceous plants contain *p*-hydroxyphenyl (H) units.

To investigate the structure and property of this complex biopolymer, various lignin model compounds have been synthesized to date. We have synthesized artificial lignin polymers composed exclusively of a β-*O*-4 substructure,[1,2] which are linear with two- or three-carbon side chains and can be used as polymeric lignin models with well-defined structures. Such polymers have been found to be useful for the identification of characteristic ion peaks induced by time-of-flight secondary ion mass spectroscopic (ToF-SIMS) analysis of lignin.[3] A guaiacyl-type β-*O*-4 artificial lignin polymer with a three-carbon side chain was successfully applied for the identification of degradation products of pyrolysis-GC (gas chromatography) analysis of lignin.[4] As lignin models, our guaiacyl-type artificial lignin polymers have been found to be more appropriate than the common lignin model dimer, guaiacylglycerol guaiacyl ether.[4]

Modern ^{1}H–^{13}C-correlation 2-D NMR spectroscopic techniques such as heteronuclear multiple quantum coherence (HMQC) and heteronuclear single quantum coherence (HSQC) pulse sequences are often used for qualitative, and recently, quantitative analysis of lignins.[5–10] Typically, the chemical shifts are assigned by comparison with those obtained from lignin model dimers. Despite the numerous and readily available NMR spectral data for lignin model compounds,[11] ^{1}H- and ^{13}C-NMR spectral data for structures consisting of β-*O*-4 substructures[12–14] remain limited. In some cases, the chemical shifts of model compounds, especially those of aromatic carbons, differ significantly from those of isolated lignins. In some cases, the *p*-methoxyphenyl structure, such as veratrylglycerol-β-guaiacyl ether, is inadequate for non-phenolic β-*O*-4 structures. In contrast, β-*O*-4 type artificial lignin polymers that actually consist of non-phenolic structures should provide more accurate NMR spectral data.

Herein, we describe the synthesis of three artificial lignin polymers composed exclusively of the β-*O*-4 substructure: syringyl-type homopolymer, *p*-hydroxyphenyl-type homopolymer, and guaiacyl/syringyl-type heteropolymer. The polymers, both as the acetylated and non-acetylated forms, were fully analyzed using modern NMR techniques. The guaiacyl-type homopolymer, which was previously reported,[2] was re-analyzed in order to obtain additional NMR spectral data. The NMR spectra of the guaiacyl/syringyl-type heteropolymer, which was synthesized as an artificial hardwood lignin, were in good agreement with those of the β-*O*-4 substructure of milled wood lignin (MWL) from the hardwood of Japanese white birch (*Betula platyphylla* var. *japonica* Hara). We believe that the NMR spectral data of our artificial lignin polymers could prove to be useful for the NMR spectroscopic analysis of isolated lignins from softwoods, hardwoods, and herbaceous plants. Furthermore, the polymers could serve as appropriate polymeric lignin model compounds that possess both phenolic and non-phenolic structures.

Results and discussion

Synthesis of artificial lignin polymers

The synthesis of β-*O*-4 type artificial lignin polymers was based on the synthetic methodology of the guaiacyl-type (G)

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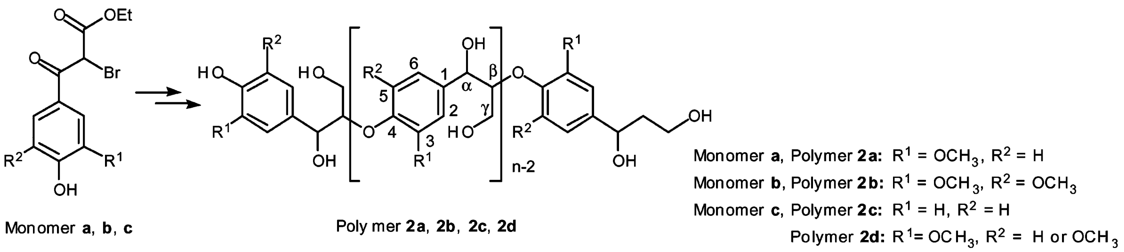

Scheme 1 Synthesis of β-*O*-4 type artificial lignin polymers.

homopolymer (polymer **2a**),[2] which involves the polycondensation of a brominated monomer and the subsequent reduction of the carbonyl polymer, as shown in Scheme 1. The three monomers, ethyl 2-bromo-3-(4-hydroxy-3-methoxyphenyl)-3-oxopropanoate (monomer **a**), ethyl 2-bromo-3-(4-hydroxy-3,5-dimethoxyphenyl)-3-oxopropanoate (monomer **b**), and ethyl 2-bromo-3-(4-hydroxyphenyl)-3-oxopropanoate (monomer **c**), were synthesized from the corresponding 4-hydroxyacetophenone derivatives, instead of the benzaldehyde derivative which was used in previous investigations.[2] 4-Hydroxyacetophenone derivatives were benzylated with benzyl chloride, coupled with ethyl carbonate[15,16] in the presence of NaH, debenzylated with Pd/C, and brominated with bromine. A typical synthetic procedure for monomers is available as ESI†, and details of the discussion was reported elsewhere.[17]

Syringyl-type (S) homopolymer (polymer **2b**) and *p*-hydroxyphenyl-type (H) homopolymer (polymer **2c**) were synthesized from monomers **b** and **c**, respectively. Guaiacyl/syringyl-type (G/S) heteropolymer (polymer **2d**) was synthesized from a 1 : 1 (mol/mol) mixture of monomers **a** and **b**. Polymerization was carried out using potassium carbonate in anhydrous DMF at rt for 24 h to afford polymers **1b–1d** with yields of 88%, 97%, and 90%, respectively. In contrast to the β-*O*-4 type polymers with two-carbon side chains,[1] precipitation of the resulting polymer was not observed during polymerization in the cases of polymers **1b–1d** with three-carbon side chains. Because of incomplete reduction of the carbonyl group of polymers **1b–1d** at rt in some cases, the reduction was carried out at 50 °C, in which polymers **1b–1d** were suspended in methanol, then treated with sodium borohydride. The crude products were purified by precipitation in ether, in order to remove the low molecular weight compounds, to afford polymers **2b–2d** with yields of 27%, 66%, and 18%, respectively. The low yield of polymer **2d** is attributable to its low molecular weight, as described below.

GPC analysis of artificial lignin polymers

The number-average and weight-average molecular weights of the acetylated form of the artificial lignin polymers were determined using gel permeation chromatography (GPC). Acetylation was performed using acetic anhydride and pyridine at rt. The degree of polymerization (*DP*) was calculated from the average molecular weight. The results, along with those of polymer **2a**, are summarized in Table 1. The weight-average degree of polymerization (DP_w) of polymer **2b** was 19.2, which is quite similar to that of polymer **2a**. Polymer **2c** exhibited the highest DP_w, which may be attributed to the accessibility of monomer **c** due to the absence of methoxy groups. The guaiacyl/syringyl heteropolymer **2d** exhibited a low *DP* – a detailed explanation, however, would require further investigations.

Table 1 GPC analysis of acetylated β-*O*-4 type artificial lignin polymers **2a–2d**

Polymer	Yield[a] (%)	M_n	M_w	DP_n	DP_w
2a[b] (G)	33	3390	5460	12.1	19.5
2b (S)	24	3690	5950	11.9	19.2
2c (H)	64	3700	9650	14.8	38.6
2d (G/S)	17	2550	4120	8.6	13.9

[a] Overall yields of polymers **2a–2d** from monomers **a–d**. [b] Data taken from ref. 2.

NMR spectroscopic analysis of artificial lignin polymers

Selected ^{1}H- and ^{13}C-NMR spectral data for the acetylated and non-acetylated forms of polymers **2a–2c** are summarized in Tables 2–5 (full spectral data are available as ESI†). The signals were assigned by HMQC and heteronuclear multiple bond coherence (HMBC) NMR spectra, and compared against those of dimer lignin model compounds, if available. In some cases, the *erythro*- and *threo*-forms were distinguishable and were read separately. Correlations between H_β and C_4, as shown by the HMBC spectra of polymers **2a–2d**, indicated the successful formation of β-*O*-4 linkages between the monomer units (data not shown). However, several minor signals could not be assigned for polymers **2a–2c** in Fig. 1. Some of them might be assigned to end units, but it is likely that some unexpected side-reactions took place at the non-phenolic end units, when polymers **1a–1d** were treated with $NaBH_4$. Further investigations are necessary to clarify this point.

The ^{13}C-NMR signals at 60.1, 71.5, and 86.7 ppm in syringyl-type polymer **2b** were assigned to the γ-, α-, and β-carbons, respectively in Fig. 1. The γ- and α-carbons (60.1 and 71.5 ppm) are comparable to those of the *threo*-form of syringylglycerol β-syringyl ether (60.2 and 71.6 ppm); the β-carbon (86.7 ppm) is also observed at a similar position (87.2 ppm).[11] The signals at 59.6, 72.2, and 86.0 ppm in polymer **2b** were assigned as the γ-, α-, and β-carbons, respectively, of the *erythro*-form. In the case of polymer **2b**, the *erythro*- and *threo*-forms exist in nearly equal amounts.

For *p*-hydroxyphenyl type polymer **2c**, the signals at 59.8, 71.2, and 83.1 ppm were assigned to the γ-, α-, and β-carbons of the *erythro*-form, respectively. The small resonance at 70.9 ppm was assigned to the α-carbon of the *threo*-form. In the case of polymer **2c**, the *erythro*-form is predominant. The difference in the chemical shifts of the side chain carbons between the *erythro*- and *threo*-forms of polymer **2c** is smaller than that of polymer

Table 2 Selected ^{1}H-NMR spectral data for β-*O*-4 type artificial lignin polymers in DMSO-d_6 (ppm)

	Proton assignment								
Polymer	α	β	γ	1	2	3	4	5	6
2a[a] (G, *erythro*)	4.75	4.28	3.47–3.63	—	7.02	—	—	6.94	6.85
2a (G, *threo*)	(4.75)[b]	(4.28)[b]	3.20, 3.47–3.63	—	(7.02)[b]	—	—	(6.94)[b]	(6.85)[b]
2b (S, *erythro*)	4.87	4.12	3.39, 3.73	—	6.70	—	—	—	6.70
2b (S, *threo*)	4.90	4.02	3.23, 3.65	—	6.78	—	—	—	6.78
2c (H, *erythro*)	4.73	4.29	3.50–3.66	—	7.25	6.85	—	6.85	7.25
2c (H, *threo*)	(4.73)[b]	(4.29)[b]	3.21, 3.50–3.66	—	(7.25)[b]	6.91	—	6.91	(7.25)[b]

[a] Data taken from ref. 2. [b] Chemical shifts in the *threo*-forms could not be read separately because of low intensities. The chemical shifts may or may not be the same as the *erythro*-forms.

Table 3 Selected ^{1}H-NMR spectral data for acetylated β-*O*-4 type artificial lignin polymers in $CDCl_3$ (ppm)

	Proton assignment								
Polymer	α	β	γ	1	2	3	4	5	6
2a[a] (G, *erythro*)	6.00	4.67	4.14, 4.37	—	7.03	—	—	6.85	6.90
2a (G, *threo*)	6.05	4.61	3.95, 4.24	—	(7.03)[b]	—	—	(6.85)[b]	(6.90)[b]
2b (S, *erythro*)	6.00	4.59	4.17, 4.42	—	6.61	—	—	—	6.61
2b (S, *threo*)	6.08	4.52	3.85, 4.31	—	6.61	—	—	—	6.61
2c (H, *erythro*)	5.98	4.73	4.14, 4.31	—	7.31	6.91	—	6.91	7.31
2c (H, *threo*)	6.01	(4.73)[b]	3.94, 4.20	—	7.34	6.98	—	6.98	7.34

[a] Data taken from ref. 2. [b] Chemical shifts in the *threo*-forms could not be read separately because of low intensities. The chemical shifts may or may not be the same as the *erythro*-forms.

Table 4 Selected ^{13}C-NMR spectral data for β-*O*-4 type artificial lignin polymers in DMSO-d_6 (ppm)

	Carbon assignment								
Polymer	α	β	γ	1	2	3	4	5	6
2a[a] (G, *erythro*)	71.4	83.6	59.8	135.0	111.7	148.9	146.7	115.0	119.1
2a (G, *threo*)	70.8	84.4	59.9	134.7	111.3	148.9	147.0	114.5	118.7
2b (S, *erythro*)	72.2	86.0	59.6	138.0, 138.1	104.3	152.1	134.2, 134.3[b]	152.1	104.3
2b (S, *threo*)	71.5	86.7	60.1	137.8	104.3	152.0	134.7, 134.8[b]	152.0	104.3
2c (H, *erythro*)	71.2	83.1	59.8	134.3	127.9	115.5	157.7	115.5	127.9
2c (H, *threo*)	70.9	83.1	59.9	134.1	127.7	115.4	158.0	115.4	127.7

[a] Data taken from ref. 2. [b] The *erythro*- and *threo*-forms were tentatively assigned.

Table 5 Selected ^{13}C-NMR spectral data for acetylated β-*O*-4 type artificial lignin polymers in $CDCl_3$ (ppm)

	Carbon assignment								
Polymer	α	β	γ	1	2	3	4	5	6
2a[a] (G, *erythro*)	73.9	79.7	62.4	131.3	111.9	150.6	147.0	118.3	119.7
2a (G, *threo*)	74.7	80.1	63.1	131.0	111.5	150.3	(147.0)[b]	(118.3)[b]	119.4
2b (S, *erythro*)	74.6, 74.7	80.7	62.8	133.2	104.5	152.8[c]	135.0, 135.3[c]	152.8[c]	104.5
2b (S, *threo*)	76.2	80.7	63.8	132.8	104.5	153.1, 153.2[c]	136.4, 136.6, 136.8[c]	153.1, 153.2[c]	104.5
2c (H, *erythro*)	73.8	78.1	62.3	129.5	128.9	116.6	158.2	116.6	128.9
2c (H, *threo*)	74.1	78.1	62.8	129.4	128.9	116.4	158.6	116.4	128.9

[a] Data taken from ref. 2. [b] Chemical shifts in the *threo*-forms could not be read separately because of low intensities. The chemical shifts may or may not be the same as the *erythro*-forms. [c] The *erythro*- and *threo*-forms were tentatively assigned.

2b. As shown in Tables 3 and 5, because the chemical shifts of the β-protons and carbons of polymer **2c** significantly differ from those of polymers **2a** and **2b** in the acetate forms, the presence of the *p*-hydroxyphenylglycerol-β-phenyl ether type substructure in herbaceous plants can be readily detected using 2-D NMR spectroscopy.

The ^{13}C-NMR spectra of polymer **2d** and milled wood lignin from Japanese white birch in DMSO-d_6 are shown in Fig. 2. The ^{13}C-NMR signals of polymer **2d** are basically the sum of polymers **2a** and **2b** with some exceptions. The side chain signals at 83.3, and 87.1 ppm do not exist in polymers **2a** nor **2b**. The signals at 83.3 and 87.1 ppm were assigned to the β-carbons of the

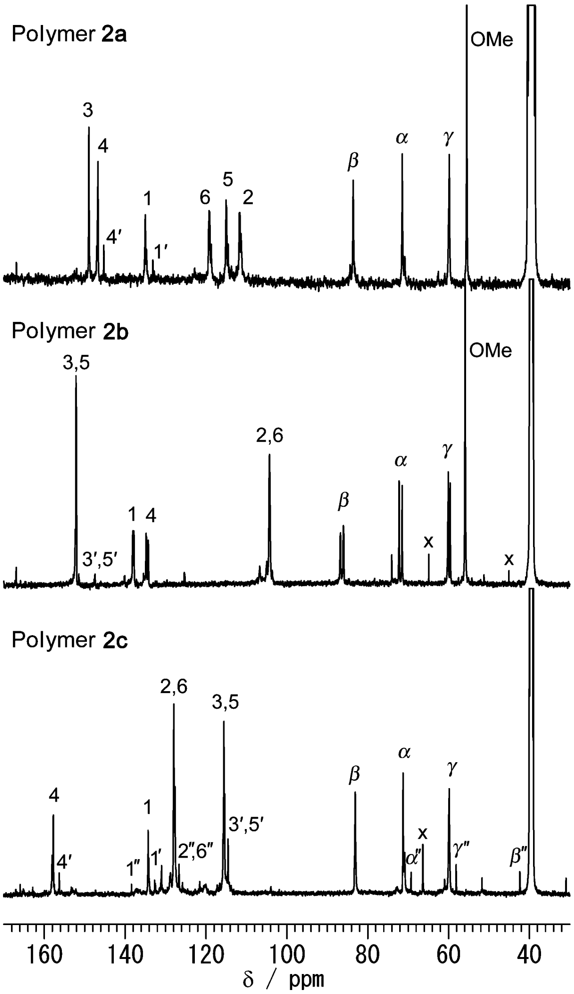

Fig. 1 ^{13}C-NMR spectra of β-*O*-4 type artificial lignin polymers **2a–2c** in DMSO-d_6. 1′, 3′, 4′, 5′: phenolic end units; 1″, 2″, 6″, α″, β″, γ″: non-phenolic end units; x: solvents.

syringylglycerol-β-guaiacyl (SG) and guaiacylglycerol-β-syringyl (GS) ether-type substructures, respectively.[13] While MWL contains, in addition to the β-*O*-4 substructure, various other substructures, the ^{13}C-NMR spectra of polymer **2d** and birch MWL in DMSO-d_6 are strikingly similar. Although the signals that correspond to the guaiacyl unit, especially those of the β-carbons, are weaker for birch MWL than for polymer **2d**, major carbon signals in polymer **2d** correspond to those of β-*O*-4 substructures of the hardwood lignin.

In contrast to the significant differences between the chemical shifts of some aromatic carbons of the dimer models and those of native lignins, the chemical shifts of artificial lignin polymer **2d** are quite similar to those of the isolated birch MWL, as described above. For example, a signal that corresponds to the guaiacyl aromatic carbon at the 5-position of the *erythro*-form of polymer **2d** appears comparably to that of the MWL (115.2 ppm). In contrast, the signal of the *erythro*-form of the veratrylglycerol-β-guaiacyl ether is observed at 111.6 ppm.[14] These results indicate that, in respect to NMR spectroscopic analyses, our artificial lignin polymers can serve as lignin models that are more accurate than the common lignin model dimers.

As shown in the ^{1}H-NMR spectra of the acetylated polymers **2a–2d** in $CDCl_3$ (Fig. 3), distinct NMR signals were observed for the side chain of each polymer. Syringyl aromatic protons were readily distinguishable from other aromatic protons in the β-*O*-4 structures. Although we expected that, for polymer **2d**, the bulky syringyl monomer **b** would be more difficult to polymerize than the guaiacyl monomer **a**, the syringyl/guaiacyl ratio of 49 : 51, which was calculated using the peak areas of syringyl (C2-H and C6-H, 6.57–6.70 ppm) and guaiacyl aromatic protons (C2-H, C5-H and C6-H, 6.78–7.08 ppm), indicated that monomers **a** and **b** were incorporated in nearly equal amounts.

Conclusions

Three artificial lignin polymers containing only the β-*O*-4 substructure were successfully synthesized and fully analyzed using NMR spectroscopy. The resulting NMR spectral data could benefit NMR spectroscopic analyses of isolated lignins; specifically, the spectra of guaiacyl/syringyl-type heteropolymer **2d** were in good agreement with those of the β-*O*-4 substructure in milled wood lignin from Japanese white birch. Our artificial lignin polymers would serve as appropriate polymeric lignin models in the investigations of reaction mechanisms during the various processes, such as pulping, bleaching, and biodegradation.

Experimental

Measurements

^{1}H- and ^{13}C-NMR spectra were recorded using a JEOL JNM EX-270 FT-NMR (270 MHz), a Bruker AMX500 FT-NMR (500 MHz), or a JEOL FT-NMR ECA600 (600 MHz) spectrometer. HMQC and HMBC NMR spectra were recorded using a Bruker AMX500 FT-NMR (500 MHz) or a JEOL FT-NMR ECA600 (600 MHz) spectrometer. Chemical shifts (δ) are reported in ppm. The ^{13}C-NMR spectra were processed using exponential multiplication with 3 Hz line broadening. The average molecular weights of the acetyl derivatives of polymers **2a–2d** were determined using gel permeation chromatography (GPC) in tetrahydrofuran using a HITACHI Liquid Chromatograph L-6200, equipped with UV (L-4000 Hitachi, 280 nm) and RI (JASCO RI-2031 Plus) detectors, and Shodex GPC columns (KF-803L and 802, 30 cm × 8.0 mm, connected in series) (tetrahydrofuran, 0.5 mL min^{-1}, 40 °C). The molecular weights were calibrated using polystyrene standards.

Polymerization

To a 25 mL flask containing monomer **b** (0.97 g, 2.79 mmol), which was dried at rt overnight under vacuum, was added finely powdered dry K_2CO_3 (0.58 g, 4.19 mmol) in anhydrous DMF (5 mL). After 24 h under a nitrogen atmosphere at rt, the reaction mixture was poured into ice-water (120 mL), then adjusted to pH 2–3 using 2 M HCl to precipitate the polymer. The polymer was isolated by filtration, washed with water, and dried over P_2O_5 under vacuum to give polymer **1b** (650 mg, 88%).

Similarly, polymers **1c** (693 mg, 97%) and **1d** (724 mg, 90%) were synthesized from monomer **c** (1.0 g, 3.48 mmol) and a mixture of monomers **a** (510 mg, 1.6 mmol) and **b** (560 mg 1.6 mmol), respectively.

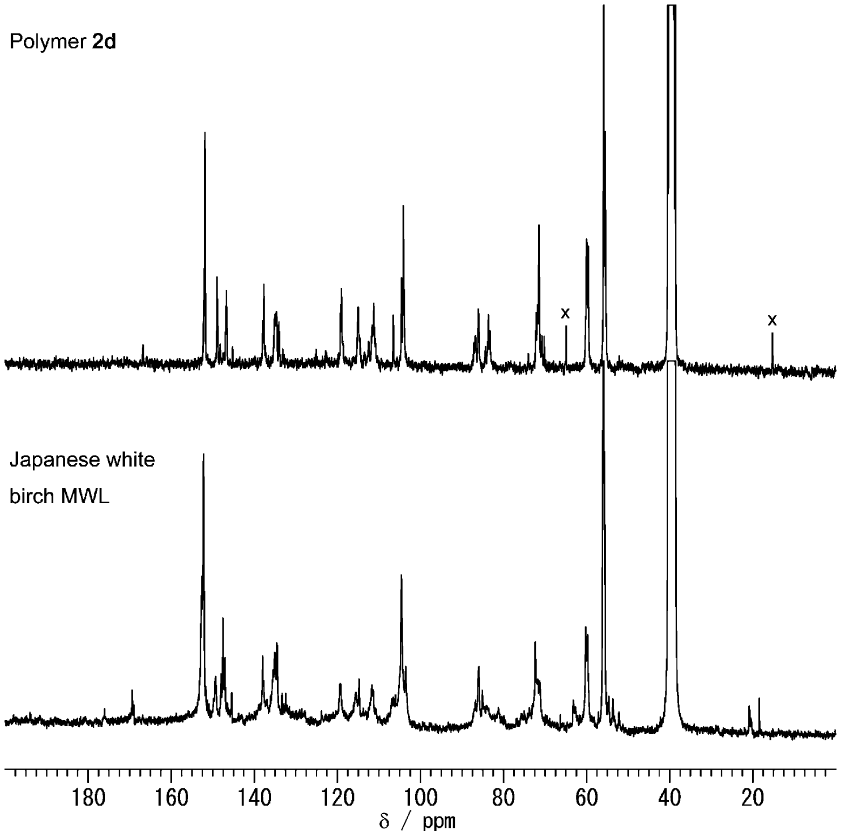

Fig. 2 ^{13}C-NMR spectra of β-*O*-4 type artificial lignin polymer **2d** and milled wood lignin (MWL) from Japanese white birch in DMSO-d_6.

Reduction of polymers 1b–1d

To a stirred suspension of polymer **1b** (602 mg) in CH_3OH (10 mL) was carefully added $NaBH_4$ (683 mg) portionwise, while maintaining the reaction temperature at 50 °C. The suspension gradually turned into a clear solution. After 24 h, the reaction mixture was treated with acetic acid to decompose excess $NaBH_4$, then poured into 0.5 M HCl (200 mL). The resulting precipitate was isolated by centrifuge, washed with water, lyophilized, then dissolved in 1,2-dichloroethane–ethanol (2 : 1, v/v, 5 mL) or 1,4-dioxane (5 mL). The resulting solution was poured into diethyl ether (100 mL) to remove low molecular weight compounds. The resulting precipitate was isolated by filtration, then dried *in vacuo* to give polymer **2b** (158 mg, 31%).

Similarly, polymers **2c** (336 mg, 66%) and **2d** (114.8 mg, 18%) were synthesized from polymers **1c** (630 mg) and **1d** (625 mg), respectively.

Polymer 2d. ^{1}H-NMR (DMSO-d_6): 3.21 (Cγ-H*t*), 3.36, 3.48–3.84 (Cγ-H), 3.71, 3.73, 3.75 (OCH_3), 4.01 (S, Cβ-H*t*), 4.10 (S, Cβ-H*e*), 4.27 (G, Cβ-H), 4.77 (G, Cα-H), 4.84 (GS or SG, Cα-H), 4.89 (S, Cα-H), 6.70 (S, C2-H*e*, C6-H*e*), 6.75 (S, C2-H, C6-H), 6.79 (S, C2-H*t*, C6-H*t*), 6.87 (G, C6-H), 6.94 (G, C5-H), 7.03 (G, C2-H);^{13}C-NMR (DMSO-d_6): δ 55.6, 55.9 (OCH_3), 59.6, 59.9, 60.0 (γ), 70.8 (G, α*t*), 71.5 (S, α*t*; G, α*e*), 71.8, 71.9 (SG or GS, α), 72.2 (S, α*e*), 83.3 (SG, β), 83.7 (G, β*e*), 84.4 (G, β*t*), 86.1 (S, β*e*), 86.8 (S, β*t*), 87.1 (GS, β), 104.3, 104.7 (S, 2, 6), 111.4 (G, 2*t*), 111.7 (G, 2*e*), 114.8 (G, 5*t*), 115.2 (G, 5*e*), 118.8 (G, 6*t*), 119.1 (G, 6*e*), 134.3 (S, 4*e*), 134.8 (S, 4*t*; G, 1), 135.0, 135.2, 135.3 (G, 1), 137.8 (S, 1*t*), 138.1 (S, 1*e*), 145.4 (G, 4′), 146.9 (G, 4*e*), 147.0 (G, 4*t*), 149.2 (G, 3), 152.1, 152.4 (S, 3, 5). 4′: phenolic end units; *e*: *erythro*; *t*: *threo*.

Acetylation of polymers 2b–2d

Polymer **2b** (65 mg) was acetylated using acetic anhydride and pyridine (1 : 1, v/v, 4 mL) at rt. After allowing the reaction to proceed for 24 h, it was diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4, and concentrated to dryness *in vacuo*. The resulting polymers were used without further purification. Polymers **2c** and **2d** were acetylated following similar procedures.

Polymer **2d** (acetate): ^{1}H-NMR ($CDCl_3$): δ 1.91, 1.94, 2.01, 2.06, 2.07, 2.08, 2.13 (s, α,γ-OCOCH_3), 2.29, 2.32 (s, Ph-OCOCH_3), 3.75, 3.79 (s, OCH_3), 3.86 (S, Cγ-H*t*), 4.17 (G, S, Cγ-H*e*), 4.21 (G, Cγ-H*t*), 4.29 (S, Cγ-H*t*), 4.37 (G, Cγ-H*e*), 4.42 (S, Cγ-H*e*), 4.54 (S, Cβ-H*t*), 4.59 (G, Cβ-H*t*, S, Cβ-H*e*), 4.67 (G, Cβ-H*e*), 6.00 (G, S, Cα-H*e*), 6.08 (G, S, Cα-H*t*), 6.60, 6.67 (S, 2, 6), 6.80–7.10 (G, aromatics); ^{13}C-NMR ($CDCl_3$): 20.8, 21.0 (*α*,γ-OCO*C*H_3), 55.9, 56.1 (OCH_3), 62.5 (G, γ*e*), 62.8 (S, γ*e*), 63.2 (G, γ*t*), 63.9 (S, γ*t*), 74.0 (G, α*e*), 74.6, 74.7 (G, α*t*, S, α*e*), 74.4, 76.0 (α), 76.3 (S, α*t*), 79.8 (G, β*e*), 80.2 (G, β*t*), 80.7 (S, β), 104.4, 104.8 (S, 2, 6), 111.7 (G, 2*t*), 112.1 (G, 2*e*), 118.4 (G, 5), 119.9 (G, 6), 131.2 (G, 1*t*), 131.5 (G, 1*e*), 132.0, 132.2, 132.4 (GS or SG, 1), 132.7 (S, 1*t*), 133.2 (S,

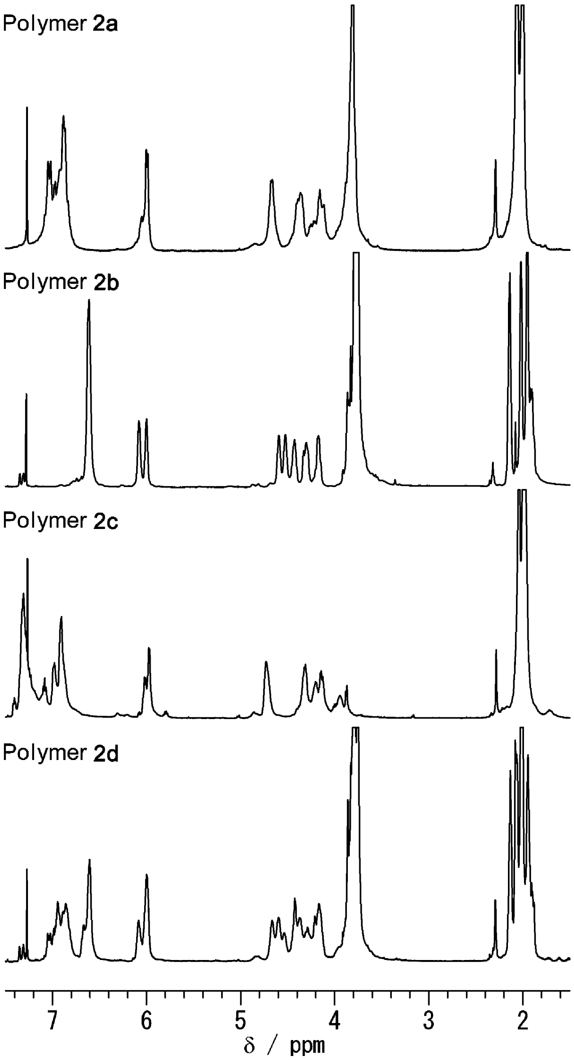

Fig. 3 ^{1}H-NMR spectra of acetylated β-*O*-4 type artificial lignin polymers **2a–2d** in $CDCl_3$.

1*e*), 135.1, 135.3, 136.4, 136.7, 136.8 (S, 4), 147.0, 147.3 (G, 4), 150.6, 150.8 (G, 3), 152.8, 153.1 (S, 3, 5), 168.8 (Ph-$OCOCH_3$), 169.7 (α-O$COCH_3$), 170.6, 170.7 (γ-O$COCH_3$). *e*: *erythro*; *t*: *threo*.

Preparation of milled wood lignin (MWL)

Milled wood lignin (MWL) was prepared from wood meal (<40 mesh) from Japanese white birch according to the standard procedure with dry-milling under nitrogen for 2 d, and purification by the method of Lundquist.[18] The Klason lignin content of the resulting MWL was 82.8%. Sugar content, which was measured by the standard alditol acetate method using gas chromatography, was 4.6%.

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