Chemical synthesis of *β-O*-4 type artificial lignin†

Takao Kishimoto,* Yasumitsu Uraki and Makoto Ubukata

Received 19th December 2005, Accepted 7th February 2006
First published as an Advance Article on the web 27th February 2006
DOI: 10.1039/b518005h

An artificial lignin polymer containing only the *β-O*-4 substructure was synthesized. The procedure consists of two key steps: 1) polycondensation of a brominated monomer by aromatic Williamson reaction; and 2) subsequent reduction of the carbonyl polymer. ^{13}C-NMR and HMQC spectra of the polymer were consistent with *β-O*-4 substructures in milled wood lignin isolated from Japanese fir wood. The weight average degree of polymerization (DP_w) ranged from 19.5 to 30.6, which is comparable to enzymatically synthesized artificial lignin from *p*-hydroxycinnamyl alcohols (dehydrogenation polymer, DHP) and some isolated lignins. Using this new lignin model polymer, it will now be possible to reinvestigate the properties and reactivity of the main lignin structure in terms of its polymeric character.

Introduction

Lignin is the second most abundant biopolymer after cellulose. Increasing attention has been paid to its potential use as a renewable raw material. However, despite much investigation in this area, most lignins produced as byproducts in the pulp industry are not used as raw materials and it is still a challenging issue to develop new utilization methods of lignin. This is partly because lignin is a complicated branched polymer.[1,2] The *β-O*-4 linkage is both the most abundant and the most important substructure in lignin (Fig. 1). It greatly affects the chemical and physical properties of lignin. In order to elucidate the property and reactivity of this complex biomacromolecule, *β-O*-4 type lignin model dimers are often used. These small model compounds are quite simple and are easy to characterize, but they cannot mimic the polymeric nature of lignin. It is becoming more important to use larger lignin model compounds. To address this problem, polymer-supported lignin model compounds[3] and a polymeric material of lignin model dimer[4] were synthesized. Unfortunately, their structures were unlike native lignin polymer. Oligomeric lignin model compounds were also synthesized by several groups.[5–7] Recently, a *β-O*-4 type oligomer was synthesized by addition polymerization,[8] but the degree of polymerization was limited to an oligomeric level in this case as well. Alternatively, synthetic lignin or artificial lignin has been enzymatically synthesized from *p*-hydroxycinnamyl alcohols (dehydrogenation polymer, DHP).[9] DHP resembles native lignin in many respects, but its structure is as complicated as isolated lignin. The preparation of syringyl type DHP specifically linked with *β-O*-4 bonds was reported,[10] but here, the unusually high phenolic content suggests that the DHP contains crosslinks between relatively short linear *β-O*-4 chains.

We recently reported a remarkably simple method for the synthesis of lignin related polymers composed exclusively of the *β-O*-4 substructure.[11] Although these polymers are *β-O*-4 type linear polymers with well-defined structures, they lack γ-CH_2OH groups that are crucial for lignin model polymer. Despite several attempts, introduction of the γ-CH_2OH into the intermediate *β-O*-4 type carbonyl polymer failed[11] due to the impracticality of adding formaldehyde completely into each monomer unit of the carbonyl polymer. To overcome this problem, we revised our strategy in the present investigation to utilize a monomer containing a three-carbon side chain instead of a two-carbon side chain. We subsequently succeeded in synthesizing an artificial lignin polymer composed exclusively of the *β-O*-4 substructure with three-carbon side chains. This artificial lignin polymer has a well-defined structure, and the structural changes caused by various treatments are much easier to analyze than DHP. The relationship between the structure and properties of lignin polymer can now be better understood by using this lignin model polymer. The properties of *β-O*-4 rich lignin in wood cell wall[12] can also be elucidated in more detail.

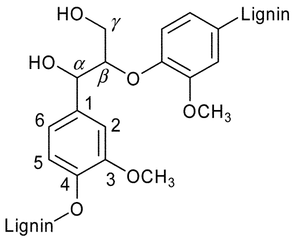

Fig. 1 The *β-O*-4 linkage in lignin.

Results and discussion

Synthesis of artificial lignin polymer

The synthesis of the *β-O*-4 type artificial lignin consists of two key steps: 1) polycondensation of a brominated three-carbon side chain monomer by aromatic Williamson reaction; and 2) subsequent reduction of the carbonyl polymer (Fig. 2). Inspired by the literature,[13] compound **3** was selected as the new monomer from several possible compounds. A similar, but benzylated,

---

Laboratory of Wood Chemistry, Research Group of Bioorganic Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan

† Electronic supplementary information (ESI) available: Synthesis and spectral data for acetylated lignin model compounds. See DOI: 10.1039/b518005h

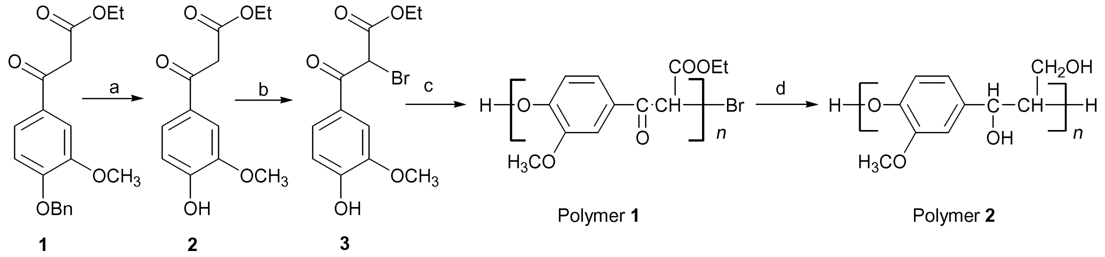

Fig. 2 Synthesis of artificial lignin polymer **2**. Reaction conditions: (a) Pd/C–H_2–EtOH–0 °C, (b) $CuBr_2$–HBr–EtOAc–reflux, (c) Cs_2CO_3–anhydrous DMF–rt, (d) $NaBH_4$–CH_3OH–rt, Bn = benzyl, Et = ethyl.

compound had been used to synthesize a *β*-*O*-4 type lignin model dimer. The coupling of the two aromatic moieties was carried out in 70% yield.

Compound **1** was synthesized from 4-hydroxy-3-methoxybenzaldehyde (vanillin) in five steps.[13] Debenzylation of compound **1** was carried out with palladium charcoal at 0 °C in 97.2% yield without reduction of the carbonyl group at the *α*-position. Bromination of compound **2** with $CuBr_2$ gave compound **3** in 79.7% yield after purification on silica gel.

Polymerization of compound **3** was carried out with cesium carbonate or potassium carbonate in anhydrous DMF. The reaction conditions were similar to those for *β*-*O*-4 type lignin related polymers with two-carbon side chains.[11] Because of the highly hygroscopic nature of cesium carbonate, the base was dried under vacuum at 110 °C before use. After reaction at room temperature for 24 h, polymer **1** was obtained in 93–97% yield. Contrary to the *β*-*O*-4 type two-carbon side chain polymer,[11] polymer **1** did not precipitate from solution during the polymerization of compound **3**.

Reduction of polymer **1** was problematic. Reduction with lithium aluminum hydride in anhydrous THF was unsuccessful because the reaction products could not be separated from a gelatinous precipitate of aluminium salts possibly due to the phenolic nature of the polymer. The best results were obtained with sodium borohydride in methanol at room temperature. A suspension of polymer **1** in methanol gradually became a clear solution and the reaction proceeded to completion. Polymer **2** was obtained in 34–45% yield. While DMSO also dissolved polymer **1**, gelation occurred during reduction with borohydride and resulted in incomplete reaction.

Structure of artificial lignin polymer 2

^{13}C-NMR spectroscopy is a powerful tool to elucidate complicated lignin structures, and most of the signals in lignin have been assigned previously.[14] The ^{13}C-NMR spectrum of polymer **2** is shown in Fig. 3. The signals were assigned by comparison with phenolic and non-phenolic lignin model dimers (Fig. 4).[15] Polymer **2** exists as a mixture of many isomers which have asymmetric carbon atoms at *α* and *β* positions. The side chain signals at 59.8, 71.4 and 83.6 ppm were assigned to *γ*, *α* and *β* carbons and

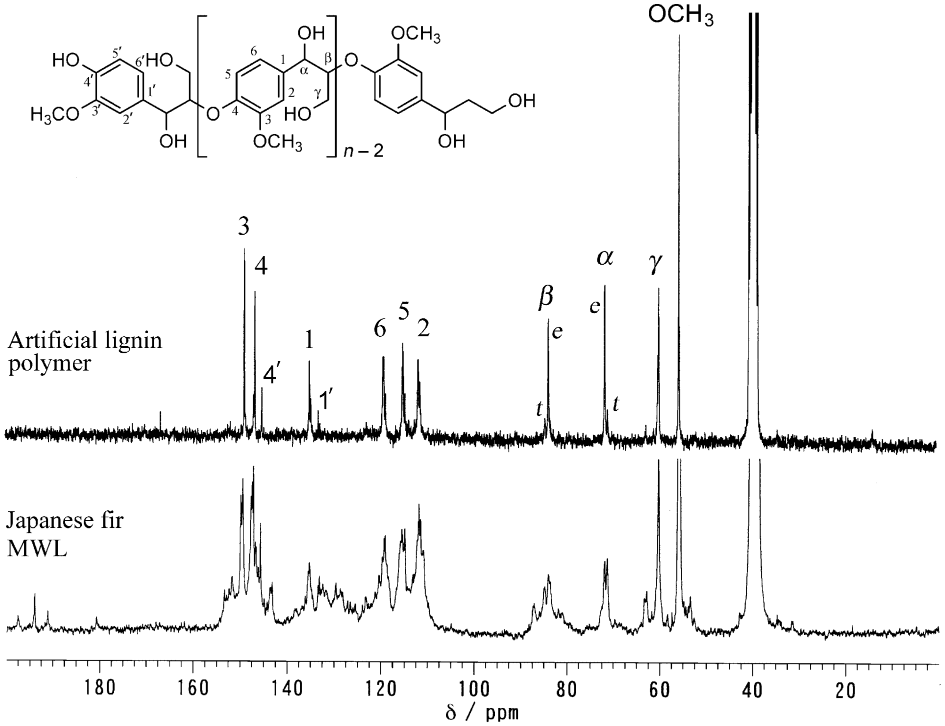

Fig. 3 ^{13}C-NMR spectra of *β*-*O*-4 type artificial lignin polymer **2** and Japanese fir MWL. 1′,4′: phenolic end units; *e*: *erythro*, *t*: *threo*; MWL: milled wood lignin. The structure of non-phenolic end units is uncertain.

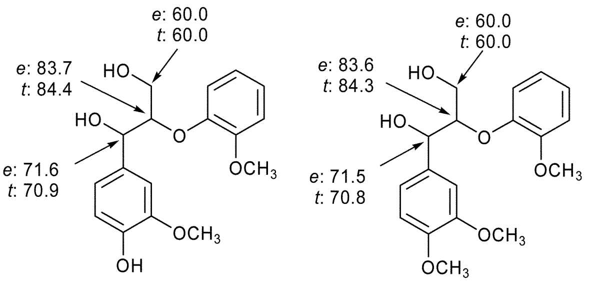

Fig. 4 ^{13}C-NMR spectral data in DMSO-d_6 for β-O-4 type lignin model compounds.[15]

occur at almost the same positions as those for the *erythro* form of veratrylglycerol β-guaiacyl ether (60.0, 71.5 and 83.6 ppm). Small resonances at 70.8 and 84.4 ppm correspond to the *threo* form of the β-O-4 substructure and correspond to those in the model dimer (70.8 and 84.3 ppm). Aromatic carbons were also assigned by comparison with lignin model dimers. Signals in polymer **2** were compared with those in milled wood lignin (MWL) obtained from Japanese fir (*Abies sachallnensis* MAST) wood. Softwood lignin mostly consists of guaiacyl lignin, which has only one methoxy group in each aromatic ring. MWL contains several substructures other than the β-O-4 substructure, but the amount of β-O-4 substructure is 41% in this specific sample.[16] As illustrated in Fig. 3, all signals in polymer **2** exist in MWL, and all correspond to the β-O-4 substructure.

2D NMR spectra, especially HMQC and HSQC, are frequently used to elucidate lignin structures.[17–19] Signals of the main substructure in lignin are well resolved and assigned. Fig. 5 shows partial HMQC spectra of polymer **2** and Japanese fir MWL in side chain regions. The crosssignals at δ_C/δ_H 71.4/4.75, 83.6/4.28 and 59.8/3.15–3.26, 3.36–3.71 ppm in the HMQC spectrum of polymer **2** are assigned to α, β, and γ-positions in the β-O-4 substructure and are consistent with those in Japanese fir MWL. The HMBC spectrum of polymer **2** is shown in Fig. 6. The distinct crosspeak at δ_C/δ_H 146.7/4.28 ppm indicates the correlation between H_β and C_4 in polymer **2**. These results clearly indicate that polymer **2** has β-O-4 linkages between monomer units.

Characterization of acetylated polymer 2

The structure of polymer **2** was further characterized by analysis of the NMR spectrum of the acetyl derivative of polymer **2**. Acetylation was performed with acetic anhydride and pyridine at room temperature. ^{1}H and ^{13}C-NMR spectral data are shown in the Experimental section. The ^{1}H-NMR spectrum of the acetylated polymer **2** in CDCl$_3$ is shown in Fig. 7. The signals were assigned by comparison with β-O-4 type acetylated lignin model dimers.[15,20] Aliphatic acetyl protons at 2.00 ppm are assigned to γ-OAc in *erythro* form andα, γ-OAc in *threo* form. Aliphatic acetyl protons at 2.06 ppm are α-OAc in *erythro* form. Acethyl protons at 2.29 ppm correspond to aromatic OAc in phenolic end units.[20] The content of phenolic end units were roughly estimated to be around 8–10% from signal area, although some signals seem to overlap with acetyl protons. It is quite strange that there are no distinct signals corresponding to non-phenolic end unit. It is likely that some unexpected side-reactions took place at the end group, when polymer **1** was treated with NaBH$_4$. The structure of non-phenolic end unit is uncertain at this moment. Further chemical analyses such as thioacidolysis and pyrolysis–gas chromatography are necessary to clarify this point.

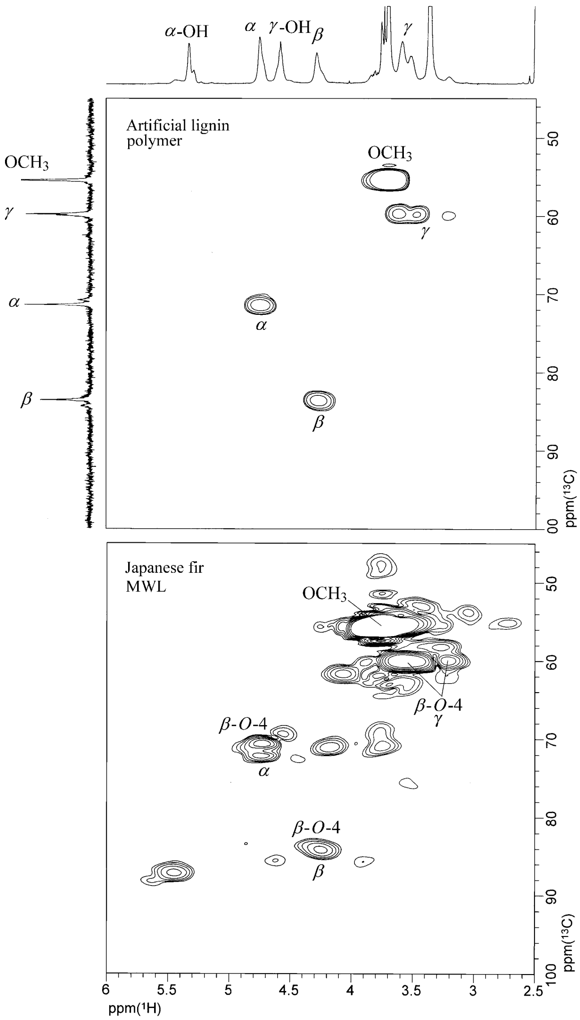

Fig. 5 HMQC spectra of β-O-4 type artificial lignin polymer **2** and Japanese fir MWL in DMSO-d_6.MWL: milled wood lignin.

The number- and weight-average molecular weights of the acetylated polymer **2** were determined by gel permeation chromatography (GPC), and were $M_n = 3440$ and $M_w = 5990$, respectively. The number and weight average degrees of polymerization were calculated to be $DP_n = 12.3$ and $DP_w = 21.4$, respectively. The average molecular weight of polymer **2** was comparable to enzymatically synthesized artificial lignin (DHP) and some isolated lignins.[16,21]

Effects of base and catalyst on the polymerization

Effects of base and catalyst on the polymerization are summarized in Table 1. While both potassium carbonate and cesium carbonate were effective, cesium iodide increased both the yield and degree of polymerization and the DP_w of polymer **2** reached 30.6. The catalyst works better in these experiments than with the β-O-4 type two-carbon side chain polymer.[11] This is most likely due to a lack of precipitate formation during polymerization as was observed previously with experiments using the two-carbon side chain monomer. The molecular weight distribution of acetylated polymer **2** is shown in Fig. 8. Addition of cesium

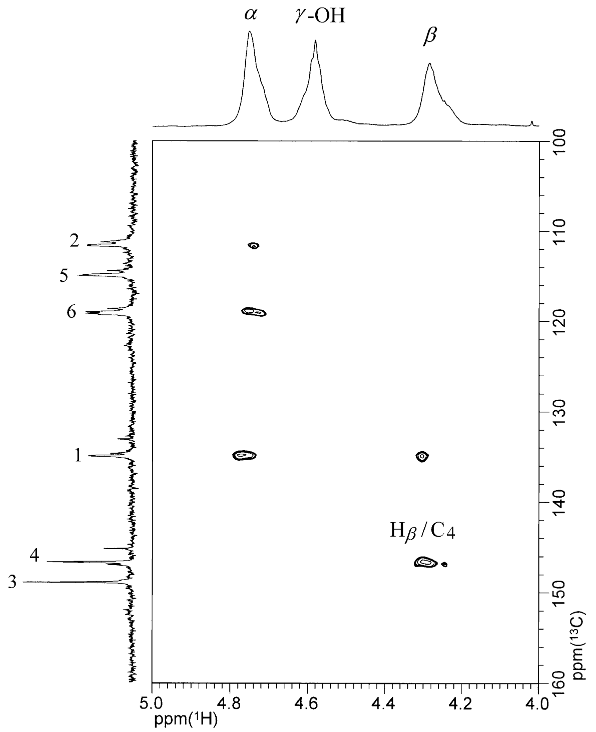

Fig. 6 HMBC spectrum of *β*-*O*-4 type artificial lignin polymer **2** in DMSO-d_6.

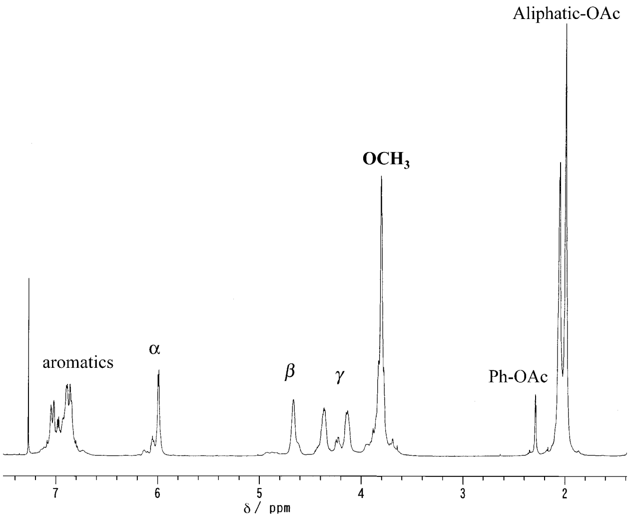

Fig. 7 ^{1}H-NMR spectrum of acetylated *β*-*O*-4 type artificial lignin polymer **2** in $CDCl_3$.

iodide exhibited bimodal distribution, and a shoulder appeared at a higher molecular weight. This peak suggests that the presence of cesium iodide accelerated the coupling between growing polymer chains, although further investigations are necessary in order to clarify this point.

Conclusions

The artificial lignin polymer composed exclusively of the *β*-*O*-4 substructure was synthesized by a polycondensation method. The molecular weight of the polymer was comparable to DHP and some isolated lignins. Its structure is also well-defined and much simpler than DHP which is structurally as complex as isolated lignins. Cesium iodide effectively increased both yield and degree of polymerization with the DP_w of the polymer reaching 30.6. It will now be possible to reinvestigate the properties and reactivity of the main lignin structure by using this new lignin model polymer instead of small molecular weight lignin model dimers.

Table 1 Effect of base and catalyst on the polymerization of compound **3**

Base/catalyst	Yield[a] (%)	M_n	M_w	DP_n	DP_w
K_2CO_3	32.8	3390	5460	12.1	19.5
Cs_2CO_3	35.4	3440	5990	12.3	21.4
Cs_2CO_3–CsI	43.5	4420	8570	15.8	30.6

[a] Overall yields of polymer **2** from compound **3**.

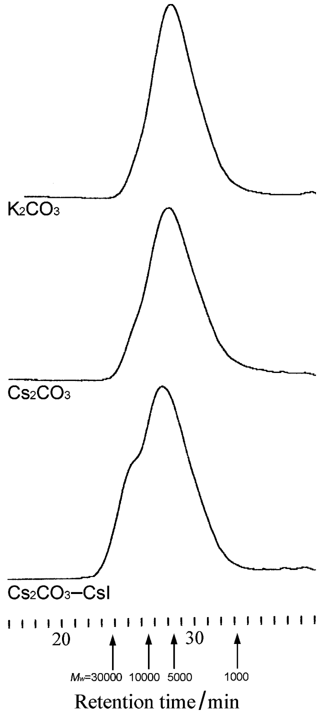

Fig. 8 GPC profile of acetylated *β*-*O*-4 type artificial lignin polymer **2**.

Experimental

Measurements

^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JNM EX-270 FT-NMR (270 MHz) or a Bruker AMX500 FT-NMR (500 MHz) spectrometer. HMQC and HMBC NMR spectra were recorded with a Bruker AMX500 FT-NMR (500 MHz) spectrometer. Chemical shifts (*δ*) and coupling constants are reported in *δ*-values (ppm) and Hz, respectively. Exponential multiplication with line broadening of 5.0 and 2.0 Hz was applied for processing of ^{13}C-NMR spectra of MWL and polymer **2**, respectively. Average molecular weights were analyzed with acetyl derivatives of polymer **2** by gel permeation chromatography (GPC) in tetrahydrofuran. A HITACHI Liquid Chromatograph L-6200 with a UV detector, L-4000 (Hitachi, 280 nm), and a RI detector (JASCO RI-2031 Plus) was used. Shodex GPC packed columns

KF-803l and 802 (30 cm × 8.0 mm) were connected in series and molecular weight was calibrated with polystyrene standard (tetrahydrofuran, flow-rate: 0.5 mL min^{-1}, 40 °C).

Compound 2. A stirred solution of compound **1** (2.835 g) in ethanol (60 mL) was treated with 10% palladium carbon (300 mg) under H_2 at 0 °C for 2.5 h. The reaction mixture was filtered and concentrated to dryness *in vacuo.* The product was purified on a silica gel column with ethyl acetate–hexane (1 : 2, *v*/*v*) to afford a syrup (2.00 g, 97.2%). ^{1}H-NMR ($CDCl_3$): δ 1.26 (t, 3H, J = 7.0, OCH_2CH_3), 3.94 (s, 2H, Cβ–H), 3.95 (s, 3H, OCH_3), 4.21 (q, 2H, J = 7.0, OCH_2CH_3), 6.95 (d, 1H, J = 8.1, C5–H), 7.50 (dd, 1H, J = 8.1, J = 1.8, C6–H), 7.54 (d, 1H, J = 1.8, C2–H).

Compound 3. To a stirred solution of compound **2** (2.00 g, 8.4 mmol) and $CuBr_2$ (5.09 g, 22.8 mmol) in ethyl acetate (30 mL), a small amount of 25% HBr in acetic acid was added *via* capillary. The reaction mixture was refluxed for 6 h. The reaction mixture was diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dryness *in vacuo.* The product was purified on a silica gel column with chloroform to afford crystals (2.13 g, 79.7%). ^{1}H-NMR ($CDCl_3$): δ 1.26 (t, 3H, J = 7.1, OCH_2CH_3), 3.96 (s, 3H, OCH_3), 4.28 (q, 2H, J = 7.1, OCH_2CH_3), 5.63 (s, 1H, Cβ–H), 6.97 (d, 1H, J = 8.6, C5–H), 7.56–7.59 (m, 2H, C2–H, C6–H).

Polymerization of compound 3. In a typical experiment, finely powdered Cs_2CO_3 (830 mg, 2.36 mmol) was placed in a 10-mL flask and dried at 110 °C under vacuum. Compound **3** (500 mg, 1.57 mmol) in anhydrous DMF (2.5 mL) was added to the flask. The solution was kept under nitrogen atmosphere at room temperature for 24 h. The reaction mixture was poured into ice water (100 mL) and the pH of the solution was adjusted to 3 with 2 M HCl to precipitate the polymer. The polymer was filtered, washed with water, and dried over P_2O_5 under vacuum to give polymer **1** (360 mg, 97 mol%). Polymer **1**: ^{13}C-NMR ($CDCl_3$): δ 14.1 (CH_3), 56.0 (OCH_3), 62.6 (CH_2), 81.8 (β), 112.9, 115.5, 124.0, 128.9, 149.8, 150.9, 165.8 (γ), 188.7 (α).

Reduction of polymer 1. To a stirred suspension of polymer **1** (300 mg) in CH_3OH (5 mL) was added $NaBH_4$ (385 mg) portionwise carefully at 0 °C. The reaction mixture was kept at room temperature for 24 h. The suspension gradually became a clear solution. Acetic acid was added to the solution to decompose excess $NaBH_4$. The reaction mixture was poured into acidified water (100 mL, pH 2). The obtained precipitate was filtered, washed with water, and dried. The precipitate was dissolved in 1,2-dichloroethane–ethanol (2 : 1, *v*/*v*, 5 mL), and poured into diethyl ether (100 mL) to remove low molecular weight compounds. The precipitate was filtered and then dried *in vacuo* to give polymer **2** (113 mg). Polymer **2**: ^{13}C-NMR (DMSO-d_6): *erythro*: δ 55.5 (OCH_3), 59.8 (γ), 71.4 (α), 83.6 (β), 111.7 (2), 115.0 (5), 119.1 (6), 133.1 (1′), 135.0 (1), 145.2 (4′), 146.7 (4), 148.9 (3); *threo*: δ 70.8 (α), 84.4 (β), 111.3 (2), 114.5 (5), 118.7 (6), 134.7 (1), 147.0 (4); 1′, 4′: phenolic end units.

Acetylation of polymer 2. Polymer **2** (30 mg) was acetylated with acetic anhydride–pyridine (2 : 1, *v*/*v*, 3 mL) at room temperature for 21 h. The reaction mixture was diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dryness *in vacuo.* The molecular weight of acetylated polymer **2** was analyzed by GPC. Polymer **2** (acetate): ^{1}H-NMR ($CDCl_3$): δ 2.00, 2.06, 2.29 (s, $OCOCH_3$), 3.81 (s, OCH_3), 3.95 (Cγ–H, *threo*), 4.14 (Cγ–H, *erythro*), 4.24 (Cγ–H, *threo*), 4.37 (Cγ–H, *erythro*), 4.67 (Cβ–H), 6.00 (Cα–H, *erythro*), 6.05 (Cα–H, *threo*), 6.80–7.08 (aromatics); ^{13}C-NMR ($CDCl_3$): *erythro*: δ 20.8, 21.0 ($OCOCH_3$ × 2), 55.9 (OCH_3), 62.4 (γ), 73.9 (α), 79.7 (β), 111.9 (2), 118.3 (5), 119.7 (6), 131.3 (1), 147.0 (4), 150.6 (3), 169.5 (α-$OCOCH_3$), 170.5 (γ-$OCOCH_3$); *threo*: δ 63.1 (γ), 74.7 (α), 80.1 (β).

References

1 K. V. Sarkanen, in *Lignins – Occurrence, Formation, Structure and Reaction*, New York, 1971, p. 150.
2 J. Ralph, K. Lundquist, G. Brunow, F. Lu, H. Kim, P. F. Schatz, J. M. Marita, R. D. Hatfield, S. A. Ralph and J. H. Christensen, *Phytochem. Rev.*, 2004, **3**, 29–60.
3 I. Kilpelainen and G. Brunow, *Holzforschung*, 1994, **48**, 222–225.
4 B. Cathala, B. Saake, O. Faix and B. Monties, *J. Chromatogr., A*, 2003, **1020**, 229–239.
5 J. Ralph, R. M. Ede and A. L. Wilkins, *Holzforschung*, 1986, **40**, 23–30.
6 I. Kilpelainen, A. Tervila-Wilo, H. Perakyla, J. Matikainen and G. Brunow, *Holzforschung*, 1994, **48**, 381–386.
7 S. Cofi-Baffoni, L. Banci and A. Brandi, *J. Chem. Soc., Perkin Trans. 1*, 1998, 3207–3217.
8 R. Katahira, H. Kamitakahara, T. Takano and F. Nakatsubo, *J. Wood Sci.*, 2003, **49**, 553–554.
9 K. Freudenberg, *Angew. Chem.*, 1956, **68**, 508.
10 L. Landucci, *J. Wood Chem. Technol.*, 2000, **20**(3), 243–264.
11 T. Kishimoto, Y. Uraki and M. Ubukata, *Org. Biomol. Chem.*, 2005, **3**, 1067–1073.
12 K. Hori and G. Meshitsuka, in *Lignin: Historical, Biological, and Materials Perspectives, ACS Symp. Ser. 742*, 2000, pp. 172–185.
13 G. E. Miksche, J. Gratzl and M. Fried-Matzka, *Acta Chem. Scand.*, 1966, **38**, 2010–1043.
14 C. L. Chen, in *Lignin and Lignan Biosynthesis, ACS Symp. Ser. 697*, 1998, pp. 255–275.
15 S. A. Ralph, J. Ralph and L. L. Landucci, http://www.dfrc.wisc.edu/software.html, 2004.
16 T. Kishimoto, A. Ueki and Y. Sano, *Holzforschung*, 2003, **57**(6), 602–610.
17 J. Ralph,; J. M. Marita, S. A. Ralph, R. D. Hatfield, F. Lu, R. M. Ede, J. Peng, S. Quideau, R. F. Helm, J. H. Grabber, H. Kim, G. Jimenez-Monteon, Y. Zhang, H.-J. G. Jung, L. L. Landucci, J. J. Mackay, R. R. Sederoff, C. Chapple and A. M. Boudet, in *Advances in Lignocellulosics Characterization.*, ed. D. S. Argyropoulos and T. Rials, TAPPI Press, Atlanta, 2001, pp. 55–108.
18 S. Heikkinen, M. Toikka, P. Karhunen and I. Kilpelainen, *J. Am. Chem. Soc.*, 2003, **125**, 4362–4367.
19 T. Kishimoto, A. Ueki, H. Takamori, Y. Uraki and M. Ubukata, *Holzforschung*, 2004, **58**, 355–362.
20 M. Hauteville, K. Lundquist and S. von Unge, *Acta Chem. Scand. Ser. B*, 1986, **40**, 31–35.
21 W. G. Glasser, V. Dave and C. E. Frazier, *J. Wood Chem. Technol.*, 1993, **13**(4), 545–559.