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ORIGINAL ARTICLE

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Synthesis of β-*O*-4 type oligomeric lignin model compound by the nucleophilic addition of carbanion to the aldehyde group

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Abstract A synthetic method for obtaining lignin oligomer that contains only the β-*O*-4 structure is described in detail. This method consists of three reaction steps: (1) the synthesis of *t*-butoxycarbonylmethyl vanillin (**2**), (2) the nucleophilic addition oligomerization of compound **2**, and (3) the reduction of the oligomeric β-hydroxyl ester. In the first step, compound **2** was synthesized from vanillin in 96.8% yield. In the second step, compound **2** was oligomerized with commercial lithium diisopropylamide (LDA) to obtain oligomeric β-hydroxyl ester (**3**) in 87.2% yield; the repeating units of this oligomer were joined only by β-*O*-4 linkages as confirmed by nuclear magnetic resonance (NMR) spectroscopy. In the third step, the oligomeric β-hydroxyl ester (**3**) was reduced with $LiAlH_4$ to give compound **4** in 42.4% yield. On the basis of NMR, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, and gel permeation chromatography analyses of compound **4**, it was concluded that compound **4** was an oligomeric lignin model compound containing only β-*O*-4 interunit linkages. The number average degree of polymerization (DPn) of obtained compound **4** was about 7.0 ($M_w/M_n = 1.42$). Using this oligomeric lignin model compound, conventional degradation and analytical methods will give new information.

Key words Lignin · β-*O*-4 Substructure · Synthesis · Oligomeric lignin model compounds

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Introduction

Lignin is composed of phenyl propane units with various interunit linkages. It has been found on the basis of many studies of lignin structure that the β-*O*-4 ether linkage is the predominant in lignins, making up about 50% of such interunit linkages.[1–3] Therefore, β-*O*-4 dimeric lignin model compounds, such as guaiacylglycerol-β-guaiacyl ether, have been widely utilized to study the chemical reactivity[4–6] and biodegradability of lignin.[7] However, lignin model compounds with high molecular weight have become increasingly important, because these compounds are more appropriate than dimeric compounds for mimicking the lignin structure. For this purpose, the synthesis of new β-*O*-4 type oligomeric and polymeric model compounds was investigated. Hitherto, trimeric,[8–10] tetrameric,[10,11] and hexameric[12] model compounds, which contained β-*O*-4 and 5–5′ linkages, have also been synthesized by a stepwise method or by a convergent synthetic method. The synthetic methods of these models, however, have many reaction steps and low yields. Thus, a novel synthetic method, which affords high molecular weight lignin model compounds in high yields and in fewer reaction steps, was proposed in a preliminary report.[13] This method consists of three reaction steps: (a) the preparation of monomer, *t*-butoxycarbonylmethyl vanillin (**2**), for oligomerization, (b) the intermolecular nucleophilic addition of compound **2**, and (c) the reduction of the oligomeric β-hydroxyl ester obtained (Fig. 1). In this article, we give detailed explanation and further investigate the synthetic method.

Experimental

Materials

Tetrahydrofuran (THF) was purified by distillation over potassium metal and used immediately. Lithium diisopropylamide (LDA) (2.0 M solution in heptane/THF/ethylbenzene) was purchased from Aldrich. Preparative

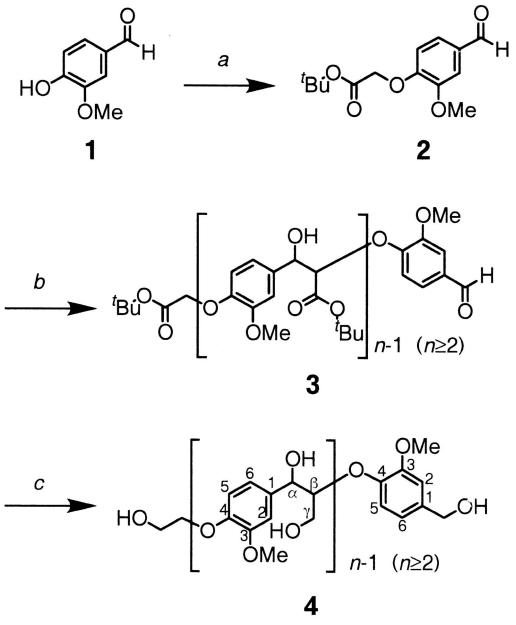

Fig. 1. Synthetic route for *β-O*-4 type oligomeric lignin model compound **4**

thin layer chromatography (PTLC) was performed on silica gel plates (Kieselgel 60 F_{254}, Merck, 2 mm × 20 cm × 20 cm). Wakogel C-200 (Wako) was used in silica gel column chromatography. The standard work-up for reaction mixtures consisted of extraction with ethyl acetate (EtOAc), washing with brine, drying over Na_2SO_4, and concentration of the organic extract in vacuo. The standard acetylation was performed with a mixture of dry acetic anhydride and pyridine (1/1, v/v) at 50°C overnight.

Measurements

Nuclear magnetic resonance (NMR) spectroscopy (^{1}H and ^{13}C) was performed with a Varian INOVA 300 FT-NMR (300 MHz) spectrometer, in chloroform-*d* with tetramethylsilane (Me_4Si) as the internal standard. Chemical shifts (δ) and coupling constants (J) were given in parts per million (ppm) and hertz (Hz), respectively. ^{1}H-^{1}H correlated spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) were performed. Number averaged molecular weight of the oligomer was analyzed by gel permeation chromatography (GPC) in chloroform at 40°C. Calibration curves were obtained by using polystyrene standards (Shodex). A Shimadzu liquid chromatography injector (LC-10ATvp), a Shimadzu column oven (CTO-10Avp), a Shimadzu UV-Vis detector (SPD-10Avp), a Shimadzu refractive index detector (RID-10A), a Shimadzu communication bus module (CBM-10A), a Shimadzu LC workstation (CLASS-LC10), and Shodex column (K802, K802.5, and K805) were used. The flow rate was 1.0 ml/min. Mass spectra were also recorded on a REFLEX III™ MALDI-TOF (Bruker, Bremen, Germany) with a LSI VSL 337 nm nitrogen laser. The matrix used was 2,5-dihydroxybenzoic acid [10 mg/ml in methanol/dichloromethane (1/4, v/v)] and sinapic acid [22.4 mg/ml in acetonitrile/water (1/1, v/v)]. The standards used were Angiotensin II and insulin bovine [0.1 nmol/ml in methanol/dichloromethane (1/4, v/v)], 200 shots.

t-Butoxycarbonylmethyl vanillin (**2**)

To a solution of vanillin (**1**) (6.6 g, 43 mmol) in acetone, KI (0.7 g, 4.3 mmol) and K_2CO_3 (9 g, 65 mmol) were added. *t*-Butyl-2-bromoacetate (6.5 ml, 43 mmol) was then added at room temperature (rt). The solution was refluxed for 1.5 h. The reaction mixture was filtered to remove excess K_2CO_3 and the residue was washed with EtOAc. The combined filtrates and washings were treated with the standard work-up procedure to give a slightly yellow solid, which was crystallized from EtOAc/*n*-hexane (1/3, v/v) to give compound **2** as white crystals (11.18 g, 96.8%); m.p.: 86°–86.5°C; ^{1}H-NMR ($CDCl_3$): δ 1.48 (9H, s, –C(CH_3)$_3$), 3.96 (3H, s, OCH_3), 4.69 (2H, s, –CH_2COO^{t}Bu), 6.85 (1H, d, $J_{5,6}$ = 8.7, Arom-C_6-H), 7.43 (1H, d, $J_{5,6}$ = 8.7, Arom-C_5-H), 7.45 (1H, s, Arom-C_3-H), 9.87 (1H, s, CHO). ^{13}C-NMR ($CDCl_3$): δ 28.0 (–C(*C*H_3)$_3$), 56.1 (–OCH_3), 66.0 (–*C*H_2COO^{t}Bu), 82.9 (–*C*(CH_3)$_3$), 109.7–152.6 (aromatic carbons), 167.0 (–CH_2*C*OO^{t}Bu), 191.0 (–CHO); Anal. Calcd for $C_{14}H_{18}O_5$: C, 63.15%; H, 6.81%. Found: C, 63.18%; H, 6.80%.

β-Hydroxyl ether (**3**)

To a solution of *t*-butoxycarbonylmethyl vanillin (**2**) (2.66 g, 10 mmol) in anhydrous THF (40 ml), 5 ml of LDA solution (10 mmol) was added dropwise at −30°C over a period of 30 min. After additional stirring for 30 min at the same temperature, the stirring was continued for an additional 1.5 h at 0°C. After the resulting slightly yellow reaction mixture was neutralized with dry ice and 1N HCl, the reaction mixture was partitioned between EtOAc and water. The aqueous layer was extracted twice with 15 ml of EtOAc. The combined organic layers were washed with 15 ml of brine, dried over Na_2SO_4, and evaporated in vacuo to give crude yellow oil. The yellow oil was purified on a silica gel column eluted with dichloromethane to give pale yellow oil (**3**) (2.32 g, 90%); ^{1}H-NMR ($CDCl_3$): δ 1.23–1.46 (m, –C(CH_3)$_3$), 3.86–3.95 (m, –OCH_3), 4.56 (s, –CH_2COO^{t}Bu), 4.35–4.81 (m, Hβ), 4.98–5.20 (m, Hα), 6.72–7.43 (m, aromatic H), 9.85 (s, CHO); ^{13}C–NMR ($CDCl_3$): δ 27.7–28.0 (–C(*C*H_3)$_3$), 55.8–56.0 (–OCH_3), 66.4–66.5 (–*C*H_2COO^{t}Bu), 73.6–74.9 (Cα), 82.2–83.0 (–*C*(CH_3)$_3$), 81.8, 83.6–85.5 (Cβ), 110.0–126.1 (aromatic C-2, C-5, C-6), 131.3–134.8 (aromatic C-1), 146.8–147.4, 152.4 (aromatic C-4), 149.1–150.2 (aromatic C-3),

167.2–168.2 (Cγ and –CH_2COO^tBu), 190.9 (CHO); DPn = 7.2 (M_w/M_n = 1.36); Anal. Calcd for $(C_{14}H_{18}O_5)_n$: C, 63.15%; H, 6.81%. Found: C, 63.18%; H, 6.81%.

MALDI-TOF-MS of the acetate of compound **3** (matrix: 2,5-dihydroxybenzoic acid, positive ion mode) *m/z*: 1214.3 $[M + Na]^+$ (n = 4), 1522.3 (n = 5), 1830.7 (n = 6), 2139.0 (n = 7), 2447.3 (n = 8), 2755.2 (n = 9), 3063.2 (n = 10).

β-*O*-4 Oligomer (**4**)

Method A

To a solution of compound **3** (6.33 g, 23.8 mmol) in anhydrous THF (50 ml), lithium aluminum hydride ($LiAlH_4$) (3.62 g, 95.1 mmol) in anhydrous THF (50 ml) was added dropwise at 60°C over a period of 2 h. After additional stirring for 2 h at the same temperature, the reaction mixture was worked up by the standard method to afford slightly yellow syrup, which was purified on a short silica gel column eluted with methanol to give compound **4** (1.98 g, 42.4% yield). Compound **4** was treated by the standard acetylation method to give the acetate of compound **4**: ^{1}H-NMR ($CDCl_3$): δ 2.01–2.09 (m, –$COCH_3$), 3.81–3.86 (m, –OCH_3), 3.91–4.43 (m, Hγ), 4.21–4.23 (m, –C$H_2$$CH_2$OAc), 4.42–4.43 (m, –$CH_2CH_2$OAc), 4.60–4.63 (m, H$\beta$), 5.02–5.04 (m, –PhC$H_2$OAc), 6.00–6.07 (m, H$\alpha$), 6.83–7.05 (m, aromatic H); ^{13}C-NMR ($CDCl_3$): δ 20.8–21.0 (–COCH_3), 55.8–56.0 (–OCH_3), 62.4 (–$CH_2$$CH_2$OAc), 62.7–63.2 (C$\gamma$), 66.1 (–Ph$CH_2$OAc), 67.1 (–$CH_2$$CH_2$OAc), 73.9–74.6 (C$\alpha$), 79.8–80.2 (C$\beta$), 110.2–122.3 (aromatic C-2, C-5, C-6), 129.8–131.3 (aromatic C-1), 147.3–147.9 (aromatic C-4), 149.5–150.8 (aromatic C-3), 169.7–171.1 (–COCH_3); DPn = 7.0 (M_w/M_n = 1.42).

MALDI-TOF-MS of compound **4** (matrix: sinapic acid, positive ion mode) *m/z*: 1200.9 $[M + Na]^+$ (n = 6), 1396.8 (n = 7), 1593.2 (n = 8), 1789.2 (n = 9), 1985.1 (n = 10), 2180.8 (n = 11), 2377.7 (n = 12), 2572.8 (n = 13), 2769.2 (n = 14), 2966.7 (n = 15).

Method B

To a solution of compound **3** (1.39 g, 5.2 mmol) in anhydrous THF (15 ml), $LiAlH_4$ (594 mg, 15.6 mmol) in anhydrous THF (15 ml) was added dropwise at 60°C over a period of 2 h. After additional stirring for 2 h at the same temperature, the reaction mixture was cooled to 0°C. EtOAc (4.6 ml) was carefully added dropwise to the mixture, and a mixture of dry pyridine (3 ml) and dry Ac_2O (3 ml) was added subsequently. The reaction mixture was stirred overnight at 50°C. After water (3.5 ml) was added dropwise at rt, the reaction mixture was filtered. The filtrate was worked up by the standard method to afford the acetate of compound **4**. The residue was reacetylated by the standard acetylation method. The reaction mixture was centrifuged (3000 rpm × 10 min), and washed with EtOAc four times. The supernatant and washings were combined and worked up by the standard method to give the acetate of compound **4**. The yield of the acetate of compound **4** from the filtrate and from the residue was 91.4%

Results and discussion

Preparation of monomer (**2**)

In our basic strategy, it was expected that oligomeric or polymeric β-hydroxyl ester would be afforded by the intermolecular condensation with LDA of a compound having both an ester, which contains hydrogen at α-position of a carbonyl group, and an aldehyde group. In this method, *t*-butoxycarbonylmethyl vanillin (**2**), which was prepared from vanillin (**1**) in 96.8% yield, was selected as the starting material for the polymerization by nucleophilic addition of carbanion to the aldehyde group. The *t*-butoxycarbonyl group was selected as the ester group. This was because the pKa of the hydrogen at the α-position of the carbonyl group with the *t*-butoxy group would be larger than that with the ethoxy or the methoxy group, so that the resulting enolate anion from *t*-butoxycarbonylmethyl vanillin (**2**) would have higher reactivity than that from ethoxy or methoxy analogs.

Oligomerization of *t*-butoxycarbonylmethyl vanillin (**2**)

In the synthetic method for producing guaiacylglycerol-β-guaiacyl ether by the method of Nakatsubo et al.,[14] LDA solution in anhydrous THF was prepared from *n*-butyl lithium and diisopropyl amine. To the LDA solution, ethyl-2-methoxyphenoxyacetate in anhydrous THF was added dropwise at –74°C over a period of 1 h. Subsequently, benzyl vanillin in anhydrous THF was added at the same temperature over a period of 2.5 h. In this study, commercially available LDA (2.0 M solution in heptane/THF/ethylbenzene) was used because the preparation of LDA was tedious and time consuming. The LDA solution was added dropwise to the solution of compound **2** because the carbanion derived from compound **2** might easily be nucleophilically added to an aldehyde group. In this method, oligomerization was conducted at –30°–0°C, because compound **2** has low solubility in anhydrous THF below –40°C. The slight yellow precipitate appeared as soon as the LDA solution was dropped. After the reaction was stopped, the reaction products were extracted with ethyl acetate and purified on a short silica gel column to afford a yellow solid (**3**) in 87.2% yield.

The ^{1}H-NMR spectrum of compound **3**, the expected oligomeric β-hydroxyl ester, was assigned as follows: *t*-butyl group at δ 1.23–1.46 ppm, Hβ at δ 4.35–4.81 ppm, –CH_2COO^tBu at δ 4.56 ppm, Hα at δ 4.98–5.20 ppm, Aromatic-H at δ 6.72–7.42 ppm, and –CHO at δ 9.85 ppm. It was assumed that if compound **3** consisted β-hydroxyl esters, it could be converted to a α,β-unsaturated ester.[14] However, the corresponding Hα peaks of the expected enol ether did not appear. In addition, the methyl peak of acetyl groups at the α-position clearly appeared after acetylation of compound **3**. These results indicate that α,β-unsaturated ester was not produced. The HSQC NMR experiment of compound **3** indicated that the Hβ proton at δ 4.32–4.82 ppm correlated with the Cβ carbon at δ 81.8–85.5 ppm (shown by arrows in Fig. 2a). The HMBC NMR

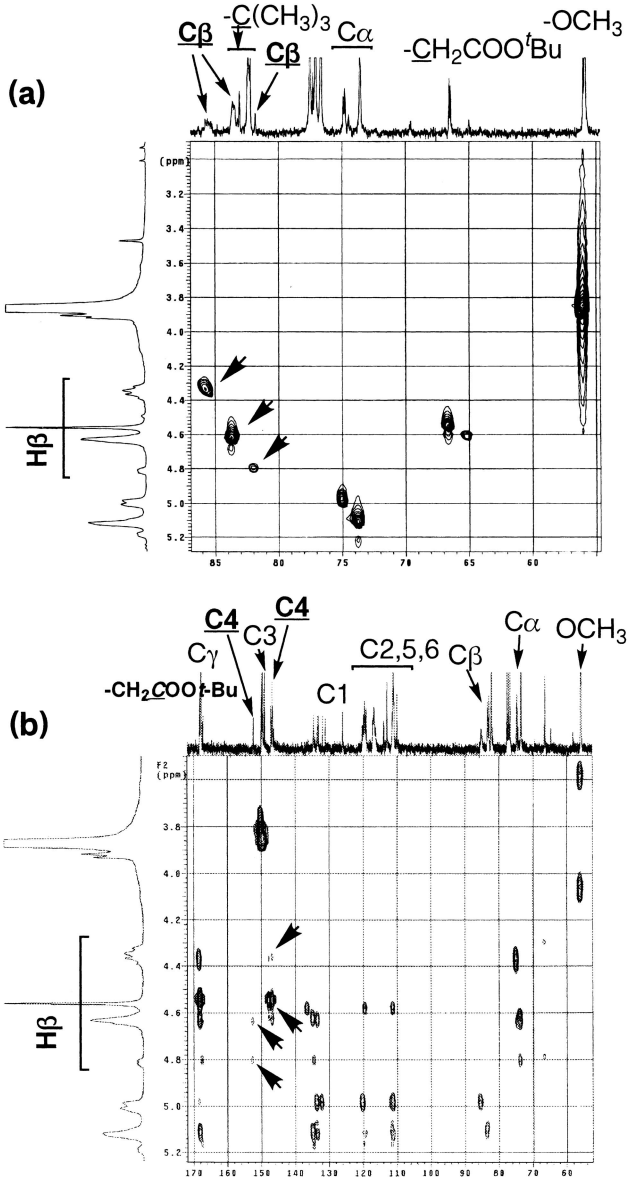

Fig. 2. **a** ^{1}H-^{13}C HSQC and **b** ^{1}H-^{13}C HMBC spectra of compound **3** ($CDCl_3$ solvent)

experiment of compound **3** indicated that there was a long-range correlation of the Hβ proton at δ 4.32–4.82 ppm with the C4 carbon at δ 146.8–152.4 ppm (shown by arrows in Fig. 2b). These results suggest that the β-*O*-4 linkages certainly exist in compound **3**. Thus, ^{1}H-NMR and ^{13}C-NMR data supported identification of the structure of compound **3** to be that of the expected polymeric β-hydroxyl esters. MALDI-TOF mass spectrometric analysis of the acetate of compound **3** indicates that compound **3** has repeating units that contain β-*O*-4 linkages. The number-average degree of polymerization (DPn) of compound **3** was calculated to be about 7.2 ($M_w/M_n = 1.36$) by the GPC analysis using polystyrene standards. Consequently, compound **3** was identified to be oligomers of the β-hydroxyl ester with repeating units joined exclusively by β-*O*-4 linkages.

The reaction mechanism of the oligomerization is proposed in Fig. 3. The proton at the α-position to the carbonyl group in compound **2** is attacked by LDA to afford carbanion, which attacks an aldehyde group of the monomer (**2**) to form the Cα-Cβ bond of a dimer. Subsequently, new carbanion is similarly produced by LDA from newly formed dimer and attacks an aldehyde group in another monomer (**2**). The same nucleophilic addition of carbanion to an aldehyde group is repeated to give oligomeric β-hydroxyl ester (**3**). Whether the addition occurs on the ester side or the aldehyde side is under investigation.

In order to obtain higher molecular weight β-hydroxyl ester, polymerization of compound **2** was conducted under various reaction conditions, which are summarized in Table 1. The polydispersity (M_w/M_n) of each resultant oligomer is almost the same, around 1.3. The monomer concentration and the temperature during addition of LDA have little influence for increase of DPn (entries 1, 2, 3, 7, and 8 in Table 1). On the other hand, the reaction time after dropping the LDA solution affects the DPn of the oligomer obtained (entries 1, 4, 5); long reaction time caused depolymerization. The highest DPn, approximately 7.2, was obtained under the conditions of entry 1.

Fig. 3. The proposed reaction mechanism of the oligomerization of compound **2** with lithium diisopropylamide (LDA)

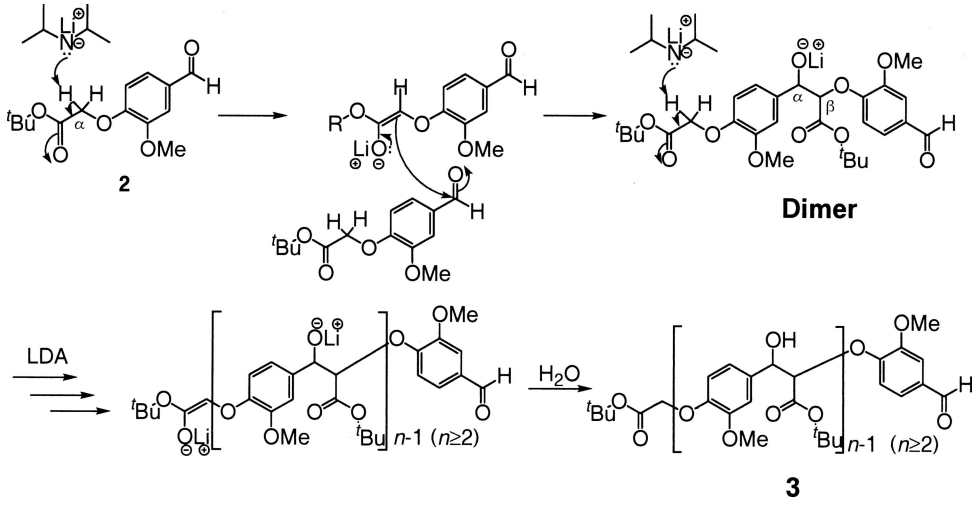

Table 1. Polymerization of *t*-butoxycarbonylmethyl vanillin (**2**) with lithium diisopropylamide (LDA)

Entry	Monomer concentration (mg/ml)[a]	Temperature during addition of LDA (°C)[b]	Temperature[c] (°C)	Time[d] (h)	Yield (%)	DPn[e]	M_w/M_n
1	67	–30	–30 → 0	0.5 → 1.5	90.4	7.21	1.36
2	33.3	–30	–30 → 0	0.5 → 1.5	82.4	6.92	1.28
3	133	–30	–30 → 0	0.5 → 1.5	76.2	7.00	1.38
4	67	–30	–30 → 0	0.5 → 0	79.3	6.77	1.28
5	67	–30	–30 → 0	0.5 → 12	86.5	5.31	1.49
6	67	–30	–30	0.5 → 1.5	79.3	6.56	1.27
7	67	0	0	0.5 → 1.5	85.0	6.61	1.37
8	67	–30	–30 → rt	0.5 → 1.5	78.1	7.00	1.34

DPn, Number average degree of polymerization; rt, room temperature
[a] Monomer (**1**)/solvent (anhydrous tetrahydrofuran)
[b] The temperature of monomer solution during the addition dropwise of LDA. The addition time was 30 min
[c, d] Reaction temperature and stirring time after addition of LDA: [–30° → 0°C / 0.5 h → 1.5 h] means that the reaction solution was stirred at –30°C for 0.5 h after dropping LDA, and then the stirring was continued at 0°C for an additional 1.5 h
[e] Molecular weight was calculated from gel permeation chromatography data using polystyrene standard in chloroform

Reduction of *β*-hydroxyl ester (**3**)

The oligomeric *β*-hydroxyl ester (**3**) was reduced with $LiAlH_4$ in anhydrous THF for 4 h at 60°C. The reaction mixture was neutralized with HCl and then filtered to separate the aluminum and lithium salts. The filtrate was extracted with ethyl acetate and subsequently purified to give compound **4** in 42.4% yield (method A). By the NMR analyses of compound **4**, it was found that the reaction proceeded quantitatively.

On the ^{1}H-NMR spectrum of the acetylated compound **4**, the peaks of both the *t*-butyl ester group and the aldehyde group disappeared, and the signals of methylene (δ 5.02–5.04 ppm) and ethylene protons (δ 4.21–4.23 and 4.42–4.43 ppm) of terminal structures appeared. These results indicate that the *t*-butyl ester group and the aldehyde group were completely reduced. The HSQC NMR experiment indicated that the Hβ proton at δ 4.60–4.63 ppm correlated with the Cβ carbon at δ 79.8–80.2 ppm (shown by an arrow in Fig. 4a). The HMBC NMR experiment indicated that there was a long-range correlation of the Hβ proton at δ 4.60–4.63 ppm with the C4 carbon at δ 147.3–147.9 ppm (shown by an arrow in Fig. 4b). All of these correlations confirmed that *β-O*-4 ether linkages were present. The mass spectrum of compound **4** was recorded on a MALDI-TOF mass spectrometer under the same conditions as those described by Paola et al. (Fig. 5).[15] On the MALDI-TOF mass spectrum of compound **4**, the signals, which were sodium adducted, of the main ions of each molecule were observed at *m/z* 2181 (n = 11), 2378 (n = 12), 2573 (n = 13), 2769 (n = 14), and 2967 (n = 15). The difference in molecular weight between consecutive main ions was 196, corresponding to the molecular weight of the repeating unit of compound **4**. The above results indicate that compound **4** is an oligomeric lignin model compound containing *β-O*-4 interunit link-

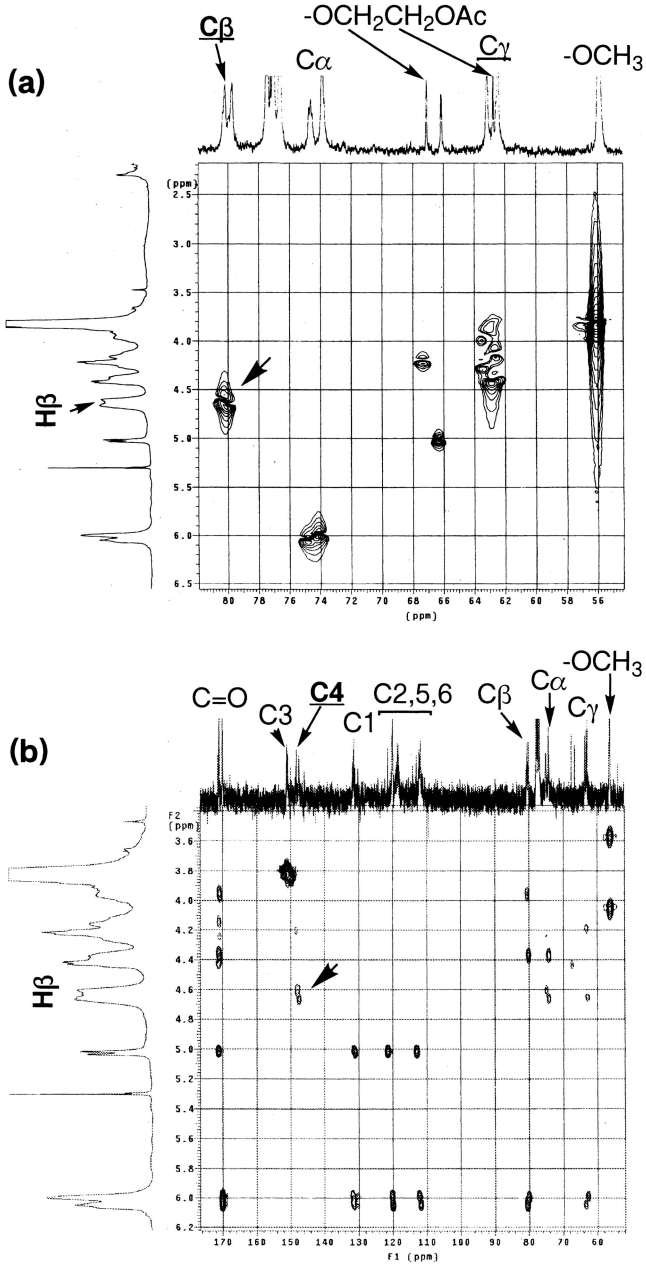

Fig. 4. a ^{1}H-^{13}C HSQC and **b** ^{1}H-^{13}C HMBC spectra of acetylated compound **4** ($CDCl_3$ solvent)

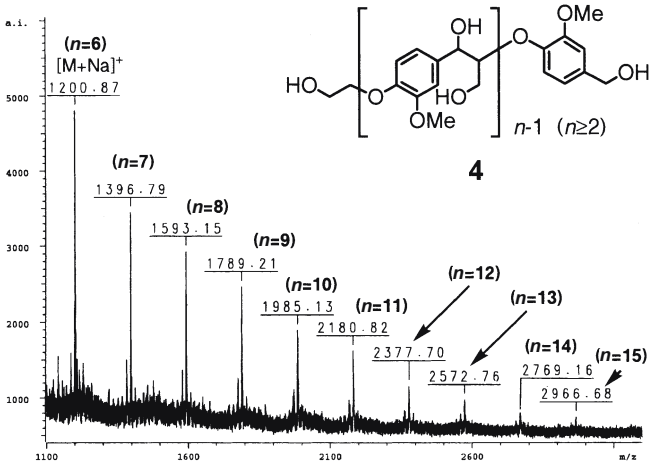

Fig. 5. Matrix-assisted laser desorption ionization time-of-flight mass spectrum of oligomeric β-O-4 lignin model compound **4**

ages. The DPn of compound **4** was determined by GPC to be about 7.0 ($M_w/M_n = 1.42$).

The yield of compound **4** was low. This seems to occur because not all of the oligomeric products were extracted in EtOAc. When the residue obtained after filtration was extracted with MeOH or dioxane/water (9/1, v/v), residual compound **4** was obtained with aluminum and lithium salts. It was difficult to remove these salts completely by silica gel column chromatography or dialysis. When the subsequent acetylation of the $LiAlH_4$ reaction mixture before extraction with EtOAc (method B) was carried out, the acetylated compound **4** was obtained in 91.4% yield. However, the sodium acetate could not be completely removed after deacetylation of acetylated compound **4** with sodium methoxide. It is necessary to improve the work-up method of this reduction with $LiAlH_4$.

Conclusions

A synthetic method for β-*O*-4 type oligomeric lignin model compound has been described. The key points of this method are the selection *t*-butoxycarbonylmethyl vanillin (**2**) as the monomer for polymerization and the intermolecular polymerization of compound **2** with LDA. Consequently, the β-*O*-4 oligomeric lignin model compound was synthesized in 35.8% overall yield in only three steps from vanillin (**1**) by the continuous nucleophilic addition oligomerization of carbanion to the aldehyde group and subsequent reduction.

The present synthetic method can be conveniently applied to synthesize various types of lignin oligomers or polymers. For example, co-oligomers containing guaiacyl, syringyl, and *p*-hydroxylphenyl nuclei may be created by the use of a mixture of the corresponding starting monomers.

References

1. Lai YZ, Sarkanen KV (1971) Isolation and structural studies. In: Sarkanen KV, Luwding CH (eds) Lignins – occurrence, formation, structure, reactions. Wiley, New York, pp 195–240
2. Higuchi T, Tanahashi M, Nakatsubo F (1973) Acidolysis of bamboo lignin III. Estimation of arylgrycerol-β-aryl ether groups in lignins. Wood Res 54:9–18
3. Lundquist K, Lundtrren R (1972) Acid degradation of lignin part VII. The cleavage of ether bonds. Acta Chem Scand 26:2005–2023
4. Chang HM, Allan GG (1971) Oxidation. In: Sarkanen KV, Luding CH (eds) Lignins – occurrence, formation, structure and reactions. Wiley, New York, pp 433–446
5. Ralph J, Grabber JH (1993) Dimeric β-ether thioacidolysis products resulting from incomplete ether cleavage. Holzforschung 50:425–428
6. Lu F, Ralph J (1997) Derivatization followed by reductive cleavage (DFRC method), a new method for lignin analysis of DFRC monomers. J Agric Food Chem 45:2590–2592
7. Tien M, Kirk T (1983) Lignin-degrading enzyme from the hymenomycete *Phamerochaete chrysosporium*. Science 26:2005–2023
8. Namba H, Nakatsubo F, Higuchi T (1980) Synthesis of trimeric lignin model compounds composed of β-*O*-4 and β-1 structures. Mokuzai Gakkaishi 26:426–431
9. Nakatsubo F (1981) Enzymic dehydrogenation of *p*-coumaryl alcohol and syntheses of oligolignols. Wood Res 67:59–118
10. Simone C-B, Lucia B, Alberto B (1998) Synthesis of oligomeric mimics of lignin. J Chem Soc Perkin Trans 1 19:3207–3217
11. Hyatt A (1987) Synthesis of some tetrameric lignin model compounds containing β-*O*-4 and 5,5′-interunit linkages. Holzforschung 41:363–370
12. Iikka K, Asta T-W, Hannu P, Jorma M, Gösta B (1994) Synthesis of hexameric lignin model compounds. Holzforschung 48:381–386
13. Katahira R, Kamitakahara H, Takano T, Nakatsubo F (2003) Novel synthesis of β-*O*-4 type oligomeric lignin model compounds by nucleophlic addition of carbanion to the aldehyde group. J Wood Sci 49:553–554
14. Nakatsubo F, Sato K, Higuchi T (1975) Synthesis of guaiacylglycerol-β-guaiacyl ether. Holzforschung 29:165–168
15. Paola B, Guido CG, Seraglia R, Traldi P, Camarero S, Martinez AT (1996) Matrix-assisted laser desorption/ionization mass spectrometry of natural and synthetic lignin. Rapid Commun Mass Spectrom 10:1144–1147