Synthesis of highly deuterated coniferyl alcohol for silencing of NMR signals in the resulting dehydrogenative polymer

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Abstract
To establish a facile methodology for the elucidation of the lignin chain-growth mechanism, the preparation of monolignol that does not show NMR signals in the dehydrogenative polymer (DHP) was attempted. As a monolignol of which aliphatic moieties were deuterated, coniferyl alcohol-d7 was successfully synthesized from protocatechualdehyde and malonic acid via the modified Knoevenagel–Doebner reaction and the Luche reduction. The process achieved high to excellent deuteration efficiencies at the aimed positions (i.e., methoxy: > 99%D, α: > 99%D, β: 92%D, and γ: 98%D). DHP was prepared solely from coniferyl alcohol-d7, and its NMR spectra were compared with those from coniferyl alcohol. The results indicated that: (1) the deuterium atoms at methoxy group, α- and β-positions were highly retained even in the DHP, and their signals were effectively suppressed; (2) a part of the deuterium at γ-position was replaced with H through the reaction; (3) meanwhile, the formation of γ-CH2 was negligible. This study demonstrated that coniferyl alcohol-d7 could “silence” the majority of the signals even when converted to DHP. Highly deuterated monolignols can be a unique molecular tool that can differentiate the signals of interest from those derived from monolignols.

Keywords: Coniferyl alcohol, Deuteration, Lignin, Dehydrogenative polymer, HSQC

Introduction
Lignin, one of the major components of wood, is biosynthesized by random radical coupling of coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol, which are termed monolignols. Despite its importance in wood cell walls, the detailed chain-growth mechanism of lignin is poorly understood due to the complicated factors in its process [1–10]. The preparation of dehydrogenative polymer (DHP) is a powerful technique to reproduce the lignin biosynthesis in situ and is an extremely useful bottom-up method to elucidate the chain-growth mechanism of lignin [1, 2, 5–12]. For this purpose, Matsushita et al. prepared DHPs from monolignol dimers and demonstrated the involvement of radical mediator mechanisms based on their kinetics [11]. Moreover, by preparing DHPs in the presence of lignin–carbohydrate complex (LCC) model compound and monolignols, they carefully elucidated the chain-growth evolving from an LCC substructure [12]. The DHP preparation in the presence of such a precursor (here, we referred to it as “seed compound” to differentiate from “monolignol”) must be a very promising methodology for clarifying the details of the growth mechanism of lignin, because the seed compounds can be diversified.

Nuclear magnetic resonance (NMR) analysis, mainly heteronuclear single quantum coherence (HSQC), is one of the most straightforward structural analysis methods currently used for lignins and DHPs [13, 14]. The procedure would be much simpler if the above-mentioned DHP strategy was coupled with NMR analyses. However, it would be practically difficult, because the signals...
derived from “monolignol” can disturb the observation of signals of interest.

Deuterium (D) is a stable isotope of hydrogen and is one of the most readily available isotopic elements, because it is commercialized in a variety of forms as NMR solvents. Deuterium labeling of compounds is normally used to give an identifier to molecules for their “visibility.” The deuterium labeling of monolignols has also been widely applied for this purpose, especially as tracers in the biosynthetic studies of lignin, lignans, or a kind of volatile phytochemicals [15–24]. Another exploit of deuteration is the “invisibility” in NMR analyses. Since the deuteron has a 0.965% relative sensitivity to the proton and requires largely different operating frequencies, it can not be observed under 1H NMR experiments. In addition, the signals of the 13C bound to D are attenuated to negligible levels in 13C and HSQC measurements due to \( J_{\mathrm{CD}} \) splitting and the lack of the nuclear Overhauser effect. Therefore, if the highly deuterated monolignol was available, it would be useful as a “silent” monolignol in the NMR of the above-mentioned DHP experiments. A similar effort had been previously made by Ralph et al., but it only focused on methoxy groups of monolignols [25]. To enable more detailed and simplified observations, we conceived that a more exhaustive signal suppression, especially on all aliphatic signals, would be required. In this study, we first attempted the synthesis of coniferyl alcohol-\( \text{d}_7 \), of which aliphatic moieties were highly deuterated. Furthermore, the DHP was prepared solely from coniferyl alcohol-\( \text{d}_7 \) to validate if the intended “silencing” is really exerted.

**Results and discussion**

**Synthesis of deuterated vanillin**

Coniferyl alcohol has been traditionally synthesized via the Knoevenagel–Doebner reaction of vanillin. Adopting the same strategy, this study first synthesized the vanillin, of which methoxy and formyl groups were substituted by deuterium (vanillin-\( \text{d}_4 \) in Scheme 1). A hydroxy group of protocatechualdehyde was selectively benzylated (1), and a \( \mathrm{CD}_3 \) group was introduced (2) using \((\text{H}_3\text{H})\text{methyl p-toluenesulfonate (CD}_3\text{OTs)} \) prepared from \( \text{CD}_3\text{OD} \). The H of the formyl group was substituted by deuterium under Geng’s umpolung condition with \( \text{N} \)-heterocarbenes [26]. The same conversion will also be accomplished via the derivatization to 1,3-dithiane and proton abstraction followed by D-quench, but the protection will have to be reconsidered. The deuteration efficiency in the formyl group was determined to be 98.5%D based on the residual 1H signal on NMR. As an attempt to verify whether an additional treatment can improve the deuteration efficiency, the obtained product was again subjected to the same reaction, and the value was successfully improved to > 99%D (3). The following debenzylation was first performed under conventional \( \text{H}_2\text{-Pd/C} \) condition in ethyl acetate (EtOAc), but the treatment interestingly reverted 20% of formyl-D to H. The capability of benzyl alcohol oxidation by \( \text{Pd}^{2+} \) species in the presence of an appropriate oxidant had been reported [27], and the contamination of \( \text{Pd}^{2+} \) in the commercial \( \text{Pd/C} \) reagent was experimentally proved [28]. Although the \( \text{H}_2\text{-Pd/C} \) is a commonly
known reductive condition of benzyl carbonyl, this may compete with the oxidation to benzyl carbonyl by \( \text{Pd}^{2+} \) in the reaction system. Such a mechanism would explain the conversion to \( \text{ArCHO} \) from \( \text{ArCDO} \) via \( \text{ArCDHOH} \). As an alternative strategy, trifluoromethanesulfonic acid (TfOH), rarely used in debenzyla-
tion, was selected as a deprotecting reagent for the reason that its deuterated form (TfOD) was commer-
cially available. Contrary to our anxiety, TfOH itself was highly effective in deprotecting the benzyl group without impairing the deuteration efficiency of the form-
myl group and without demethylating like other strong Lewis acids. The obtained vanillin-\( \text{d}_4 \) (4) exhibited only aromatic signals on \(^1\text{H NMR}\), a proper deuterated ion peak on mass spectrometry (MS), and the same smell as vanillin.

**Synthesis of ferulic acid under aniline-/piperidine-free condition**

In the Knoevenagel–Doebner reaction for monolignol syntheses, aniline or piperidine are commonly used as a catalytic reagent. However, it could be easily envisaged that their exchangeable H could impair the deutera-
tion efficiency of the product. Thus, we next attempted the development of the novel Knoevenagel–Doebner condition that can proceed without aniline or piperi-
dine. Ammonium sulfate, of which deuterated form ((ND\(_\text{4}\))\(_2\)SO\(_4\)) is commercially available, was selected as an alternative reagent, and the conditions were optimized using undeuterated vanillin and malonic acid. The yields were determined by gas chromatography–mass spec-
trometry (GC–MS) analyses, and the results are shown in Table 1. In the absence of (NH\(_4\))\(_2\)SO\(_4\), only 14% of the product, ferulic acid, was detected together with a quan-
tity of unreacted vanillin (Entry 1). The addition of 0.5 eq. (NH\(_4\))\(_2\)SO\(_4\) evidently promoted the reaction (35%, Entry 2), and the less amount of the pyridine further enhanced the yield (80%, Entry 4). The decreased (NH\(_4\))\(_2\)SO\(_4\) with increased pyridine did not result in a better result (27%, Entry 3). The optimization of the reaction temperature proved that 70 °C provides a near quantitative reac-
tion (Entries 4–6). The lower temperature might have an effect on preventing the thermal decomposition of malonic acid.

**Synthesis of coniferyl alcohol-\( \text{d}_7 \)**

To practice the devised Knoevenagel–Doebner reaction, malonic acid was also deuterated by treating malonic acid in D\(_2\)O at elevated temperature (Scheme 2a). The deuteration efficiencies were estimated on \(^1\text{H NMR}\).
Since the single treatment gave only 89.0%D in both the methylene and the carboxy groups, the treatment was repeated four times. While the 2nd treatment gave significantly improved deuteration efficiency (96.2%D), the value hit the ceiling in the 4th treatment (i.e., 97.2%D in the 3rd and 97.7%D in the 4th treatment, respectively).

The resultant malonic acid-\textit{d}_4 (5) and vanillin-\textit{d}_5 prepared from vanillin-\textit{d}_4 were treated under the optimized condition in the presence of (\textit{ND}_4)_2\text{SO}_4, and the reaction gave ferulic acid-\textit{d}_5 in an 82% yield (Scheme 2b). The deuteration efficiencies at the \(\alpha\)- and \(\beta\)-position of the product were >99%D and 92%D, respectively. As the same reaction under 5 mol% piperidine instead of (\textit{ND}_4)_2\text{SO}_4 resulted in a 33% yield with 86%D at the \(\beta\)-position (data not shown), the present condition was demonstrated to be highly advantageous. The 8% of H incorporated at the \(\beta\)-position was absolutely derived from the residual protium in malonic acid.

Ferulic acid-\textit{d}_5 (6) was subsequently reduced to coniferyl alcohol-\textit{d}_7 (7) by NaBD\textsubscript{4}, which is much less costly than LiAlD\textsubscript{4} or DIBAL-D, and stably supplied. The conditions were optimized to NaBD\textsubscript{4}, and the activation with (COCl\textsubscript{2}) followed by the Luche reduction in \(N\)-methylpyrrolidone (NMP) was found to give the best result (Scheme 2b). Briefly, the absence of CeCl\textsubscript{3} resulted in the formation of 24% 1,4-reduction product (\(\alpha\),\(\beta\)-dihydroconiferyl alcohol) in addition to 60% target, and the reaction without NMP resulted in 9% of the 1,4-reduction product and 41% of the target. In the activation step, (COCl\textsubscript{2}) was most successful compared to 1,1-carbonyldiimidazole (60% yield) or SOCl\textsubscript{2} (36% yield), probably due to the difference in reaction residues. The NMR spectra of coniferyl alcohol-\textit{d}_7 are displayed in Figs. 1 and 2 as a comparison with coniferyl alcohol. In Fig. 1b, aliphatic protons clearly disappeared, and the deuterium signals of corresponding positions could be observed in the 2H NMR spectrum. The deuterium efficiency at \(\gamma\)-position was 98%D, and the undesired 1,4-reduction product was not detected. The comparison of 13C NMR spectra also ensured the structure of coniferyl alcohol-\textit{d}_7 (Fig. 2). The signals of the deuterated carbons were significantly attenuated, which was especially evident in C\(\beta\): The C\(\beta\)-D triplet (126.4 ppm) is weaker than that of the residual C\(\beta\)-H singlet (126.7 ppm) despite the large difference in the presence (92% vs. 8%). As a result, highly deuterated coniferyl alcohol (methoxy: >99%D, \(\alpha\): >99%D, \(\beta\): 92%D, and \(\gamma\): 98%D) was successfully synthesized by modifying conventional strategy and using commercially accessible deuterium sources. The present study first achieved the deuteration of all aliphatic positions, including the new \(\alpha\)- and \(\beta\)-positions, while the deuteration of monolignols had been conducted mainly on their methoxy and/or \(\gamma\)-positions [12, 15, 16, 19–25, 29] and sometimes on the 2, 5, and 6 positions [11, 17, 18] to date.

Preparation and NMR analyses of deuterated DHP

To examine whether the NMR signals are really “silenced”, DHP was prepared from coniferyl alcohol-\textit{d}_7 (DHP-\textit{d}) and compared with that from coniferyl alcohol (DHP-\textit{h}). Both reactions were performed by adding horseradish peroxidase and H\textsubscript{2}O\textsubscript{2} buffer solutions to the monolignol solutions in an aqueous (H\textsubscript{2}O) buffer. The yields were essentially the same (DHP-\textit{h}: 56.8 mg DHP-\textit{d}: 63.1 mg from ca. 100 mg starting materials), which reflects the comparable reactivity of deuterated monolignol. The exchangeable protons of the products were replaced with D using CD\textsubscript{3}OD, and the products were analyzed on 1H and edited HSQC NMR (Fig. 3). The panels Fig. 3a, b are the spectra of DHP-\textit{h}, and c–f are those of DHP-\textit{d}. In the panels, Fig. 3a–d, the same vertical magnifications were used for 1H NMR, and the same thresholds were applied for HSQC spectra. The spectra in panels e and f were “deeper” slices of c and d. Here, in-phase signals
are shown in blue and anti-phase signals in red. The typical cross-peaks for lignin substructures, namely, aryl ethers (A and A'), β–β (B), and β–5 (C), were observed in the aliphatic region of DHP-\textit{H} (Fig. 3b). A cross-peak at 3.1/56 ppm, designated “U” in Fig. 3b, is a specific signal arisen in the present condition and can be possibly derived from β-1 substructure, though it was difficult to conclude clearly due to the lack of enough examples for this structure. By contrast to Fig. 3b, hardly any \textsuperscript{1}H and HSQC signals were observed in Fig. 3d, except those of residual water (3.42 ppm) and cinnamyl alcohol-γ (CAγ, 4.07 ppm). By lowering the slice to the level at which excessive aromatic signals were rendered (Fig. 3e), a part of the aliphatic signals became observable in the HSQC (Fig. 3f). A subtle difference in the aromatic region (6.5–7.5 ppm) of \textsuperscript{1}H NMR spectra will stem from the minor signals that resonate at δH 6.60–6.85/δC 122–130 (unrendered in Fig. 3a), which will probably be related signals to β-proton of cinnamaldehyde. The intensities of cross-peaks Aβ, A'β, Bβ, and Cβ in Fig. 3f were approximately 4, 7, 15, and 17% of those in Fig. 3b, respectively. The increments in Bβ and Cβ from the original 8% β-H may suggest a partial exchange from β-D to H, but it would be more reasonable to consider these differences within the margin of error, given the inexplicable decrements in Aβ and A'β. The same silencing was likewise observed in the unidentified signal (U in Fig. 3b). Rather, the reversions to H were obvious at the γ-positions. This is typically seen in the signals, CAγ (4.07 ppm in Fig. 3d) and cinnamaldehyde-H (8.8–9.1 ppm, not displayed) in \textsuperscript{1}H NMR. The intensities of the corresponding cross-peaks in DHP-\textit{d}, calculated with the cross-peak of H-6/C-6 as a reference, were 13% and 49% of those in DHP-\textit{H}, respectively. In addition, all the cross-peaks of γ-positions, including CAγ, appeared as in-phase signals and no anti-phase signals were observed in Fig. 3f.

These results indicated that a part of D at γ-position was replaced by H, but this reversion was limited only to the formation of γ-CDH, without γ-CH_{2}. This type of substitution had already been reported in the tracer feeding experiments on \textit{Eucalyptus camaldulensis} or \textit{Ginkgo biloba} L. [15, 16]. Our results newly demonstrated that DHP preparation with deuterated monolignols can provide extremely simplified NMR spectra, which can be a powerful methodology for analyzing the structural alteration of the undeuterated seed structures. The increments in Bβ and Cβ from the original 8% β-H may suggest a partial exchange from β-D to H, but it would be more reasonable to consider these differences within the margin of error, given the inexplicable decrements in Aβ and A'β. The same silencing was likewise observed in the unidentified signal (U in Fig. 3b). Rather, the reversions to H were obvious at the γ-positions. This is typically seen in the signals, CAγ (4.07 ppm in Fig. 3d) and cinnamaldehyde-H (8.8–9.1 ppm, not displayed) in \textsuperscript{1}H NMR. The intensities of the corresponding cross-peaks in DHP-\textit{d}, calculated with the cross-peak of H-6/C-6 as a reference, were 13% and 49% of those in DHP-\textit{H}, respectively. In addition, all the cross-peaks of γ-positions, including CAγ, appeared as in-phase signals and no anti-phase signals were observed in Fig. 3f.

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Fig. 3 (See legend on previous page.)
dimethyl sulfoxide (DMSO) as the internal standards. Coupling constants (J) are given in Hz. Mass spectra were acquired with FI mode on a JEOL JMS-T100GCV equipment. All MS and NMR spectra on AVANCE Neo were measured at the GC–MS and NMR Laboratory, Faculty of Agriculture, Hokkaido University.

4-(Benzyloxy)-3-hydroxybenzaldehyde (1)
To a solution of 3,4-dihydroxybenzaldehyde (7.37 g, 53.4 mmol) in 100 mL of MeCN, NaHCO3 (5.83 g, 1.3 eq.) was added at room temperature. A suspension of 0.1 eq.) was added to the mixture, which was stirred at room temperature. After the overnight reaction, the mixture was stirred at 0 °C. After gas generation stopped, 20.6 g of TsCl (ca. 44 mmol) in 100 mL of MeCN, NaH (1.76 g, ca. 4.4 mmol) was added to a THF (8.35 g, 36.6 mmol) solution of CD3OD (3.24 g, 90 mmol) portionwise at room temperature. The crude product was purified by silica gel column chromatography (hexane then EtOAc:hexane = 1:2). After evaporation of the combined target fractions, the product was recrystallized from EtOAc (×3) to give a total of 8.50 g of the 4-O-benzylated product as a pale-yellow solid (70%).

4-(Benzyloxy)-3-(2H)methoxybenzaldehyde (2)
Sodium hydride (5.6 g, ca. 127 mmol) was added to a THF (100 mL) solution of CD3OD (3.24 g, 90 mmol) portionwise at 0 °C. After gas generation stopped, 20.6 g of TsCl (1.2 eq.) was added at the same temperature. After the reaction was completed, 100 mL of Et2O and cold water were added to the mixture. The water layer was recovered and again washed with Et2O (×2). The combined organic layer was washed with aqueous sat.NaHCO3 and brine. The solution was dried over anhydrous Na2SO4, and the solvent was removed in vacuo. The purification by flash silica gel column chromatography eluting with EtOAc:hexane = 1:2 afforded 5.29 g of crude product, which deuteration efficiency was 98.5%D at the formyl group. This could be estimated by the comparison with an integration value of H-5 (7.10 ppm in 1H NMR). The same treatment was repeated for the recovered product with half amounts of the reagents, which gave 5.18 g of product with >99%D of deuteration efficiency in the formyl group.

The crude product was dissolved in 50 mL of CH2Cl2, and 2.0 mL of TIOH (22.7 mmol) was added dropwise to the solution at −20 °C. After the completion of the reaction, 2.3 mL of 10 M aqueous NaOH was added to the mixture. The mixture was partitioned between 1 M aqueous HCl and EtOAc (×3). The combined organic layer was neutralized with brine and dried over anhydrous Na2SO4. After removal of the solvent, the crude product was purified with flash silica gel column chromatography eluting with EtOAc:hexane = 1:2 to give 1.65 g of vanillin-d4 (50% from 2). 1H NMR (CDCl3, 500 MHz): δ 7.04 (1H, d, J = 8.4 Hz, H-5), 7.42–7.45 (2H, m, H-2 and H-6); 13C NMR (CDCl3, 125 MHz): δ 55.2 (OCD3, m, J = 22 Hz), 108.8 (C-2), 114.3 (C-5), 127.4 (C-6), 129.7 (t, J = 3 Hz, C-1), 147.1 (C-3), 151.7 (C-4), 190.6 (t, J = 27 Hz, formyl). HR–FI–MS: m/z = 245.11358 [M]+ calcd. for C15H142H3O3, found 245.11358.

4-Hydroxy-3-(2H)methoxybenzene(2H)carbaldehyde (4, Vanillin-d4)
Compound 2 (5.20 g, 21.2 mmol) was dissolved in 16.6 mL of toluene. To the solution, 40 mL of D2O, 2.94 g of K2CO3 (1.0 eq.) and 897 mg of 1,3-bis(2,5-disopropyl)phenylimidazolium chloride ([bis(dipp)Im]Cl, 0.1 eq.) were added. The reaction was allowed to stir at 40 °C overnight and then partitioned with EtOAc (×2). The water layer was acidified with 1 M aqueous HCl and washed with EtOAc. The combined organic layer was washed with 1 M aqueous HCl, aqueous sat.NaHCO3 and brine. The solution was dried over anhydrous Na2SO4, and the solvent was removed in vacuo. The purification by flash silica gel column chromatography eluting with EtOAc:hexane = 1:2 afforded 5.29 g of crude product, of which deuteration efficiency was 98.5%D at the formyl group. This could be estimated by the comparison with an integration value of H-5 (7.10 ppm in 1H NMR). The same treatment was repeated for the recovered product with half amounts of the reagents, which gave 5.18 g of product with >99%D of deuteration efficiency in the formyl group.

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(2,2-2H)Propane(2H)dioic acid (Malonic acid-d4, 5)
Malonic acid (2.08 g, 20.0 mmol) was dissolved in 6.0 mL of D2O, and the solution was allowed to stir overnight at 80 °C. The solution was frozen, and the solvent was removed by freeze-drying. Deuteration efficiency was evaluated by 1H NMR: Forty-two point zero milligrams of the product were dissolved in 0.45 mL of DMSO-d6 containing 2.2 mg of benzaldehyde as an internal standard.
standard. The integration values of CH$_3$ at 3.22 ppm and COOH at 12.57 ppm were compared with those of intact malonic acid, respectively. The treatment and analysis were repeated four times, and 1.73 g of white solid was obtained (80%).

**GC–MS analyses for the optimization of reaction conditions**

A portion of the reaction mixture equivalent to 1.0 mg of the starting material was sampled and diluted with 800 µL of EtOAc. The resultant solution was mixed with 200 µL of N,O-bis(trimethylsilyl)trifluoroacetamide and filtered with a 0.45 µm PTFE membrane filter. The sample was allowed to stand for 1 h at room temperature. One microliter of each solution was analyzed on GC-2010/QP-2010 system (Shimadzu, Kyoto, Japan) under the following conditions: Column: BPX5 30 m, φ0.25 mm, df 1.0 µm (SGE Analytical Science, Melbourne, VIC, Australia); Injection unit: 250 °C; Column oven: 80 °C for 3.0 min, 40 °C/min for 6.0 min then 320 °C for 4.0 min; Carrier gas: helium; Interface: 250 °C; Ion source: 250 °C. Total ion chromatograms (TIC) were obtained, and the yields were calculated simply based on the area of each compound per total of areas. Retention times of trimethylsilyl-derivatized compounds were as follows: Vanillin: 8.17 min; α, β-dihydroconiferyl alcohol: 8.81 min; Coniferyl alcohol: 9.22 min; Ferulic acid: 9.73 min.

**[(E)-3-Hydroxy-3-(H$_3$)methoxyphenyl]2,3-($^3$H$_2$)prop-2-enic acid (6, Ferulic acid-$d_5$)**

Prior to the reaction, the phenolic H of 4 was exchanged for D by repeating the addition and evaporation of CD$_3$OD three times to obtain vanillin-$d_5$.

To 2.4 mL of pyridine, 168 mg of (ND$_4$)$_2$SO$_4$ (0.5 eq.) and 520 mg of malonic acid-$d_4$ were added, and which was stirred for 10 min at room temperature. To the mixture was added 377.2 mg of vanilline-$d_5$ (2.40 mmol) and the reaction was allowed to stir at 70 °C overnight. Pyridine was azetropically removed with toluene, and the precipitates were washed with Milli-Q water three times. The residues were freeze-dried to give DHPs (56.8 mg from coniferyl alcohol; 63.1 mg from coniferyl alcohol-$d_5$).

**4-[(E)-3-Hydroxy(1,2,3,3-($^3$H$_2$)prop-1-enyl)-2-($^4$H$_3$)methoxyphenol (7, Coniferyl alcohol-$d_6$)**

Ferulic acid-$d_5$ (347.3 mg, 1.74 mmol) was dissolved in 5.0 mL of THF. To the solution, one drop of anhydrous DMF and 150 µL of (COCl)$_2$ (1.0 eq.) were added in this order at 0 °C. Anhydrous CeCl$_3$ (429 mg, 1.0 eq.) was suspended in 10 mL of NMP, which was sonicated for 15 min, and 146 mg of NaBD$_4$ (2.0 eq.) was added to the suspension. At ~20 °C, the acid chloride solution was added dropwise to the reductant suspension. The reaction was then allowed to stir overnight at room temperature. The solvents were removed under reduced pressure, and the resultant residue was resuspended in MeOH containing AcOH (minimum quantity for neutralization). The crude product was directly subjected to silica gel column chromatography (CHCl$_3$, then MeOH:CHCl$_3$ = 10:90). Careful evaporation of the target fractions gave 224.5 mg of coniferyl alcohol-$d_5$ (65%) as a mixture with 6% NMP. 

**DHP preparation**

Monolignols (coniferyl alcohol: 100.2 mg; coniferyl alcohol-$d_5$: 107.1 mg as a mixture with NMP) were separately dissolved in 1.0 mL of acetone, and to the solutions were added 49 mL of phosphate-buffered saline (PBS, pH 6.1, 50 mM phosphate). The solutions containing 0.5 mg of horseradish peroxidase (Fujifilm Wako Pure Chemicals Co. Ltd., Osaka, Japan) in 25 mL PBS and 4.0 mmol H$_2$O$_2$ in 25 mL PBS were added dropwise to the monolignol solutions over 3 h, and the reaction was continued for another 13 h. The resulting suspensions were centrifuged, and the precipitates were washed with Milli-Q water three times. The residues were freeze-dried to give DHPs (56.8 mg from coniferyl alcohol; 63.1 mg from coniferyl alcohol-$d_5$).

**NMR analyses**

The obtained DHP products were treated by the addition/evaporation of CD$_3$OD three times. Fifty milligrams of samples were dissolved in 0.45 mL of DMSO-$d_6$, and the complete dissolution of the samples was visually confirmed. The edited HSQC spectra were obtained.
using 256 scans using the standard Bruker pulse program (hsqcedetgpsp.3) with the following parameters for acquisition: TD = 2048 (F2), 192 (F1); SW = 11.1082 ppm (F2), 209.9969 ppm (F1); SW = 5555.556 Hz (F2); 26,411.512 Hz (F1); IN_F = 37.8623 ms; AQ = 0.1843200 s (F2), 0.00036348 s (F1); FIDRES = 5.425347 Hz (F2), 275.119934 Hz (F1); FW = 240,000,000,000 Hz. The resulting data were processed with the TopSpin 4.1.3. The thresholds in Fig. 3a–d were set as follows: Base level 1.4E10 (Positive) and −1.4E10 (Negative); Level increment 1.200 (Positive and Negative); Number of levels 16. Threefold magnification in Fig. 3b was obtained at the result. The thresholds in Fig. 3e, f were set as follows: Base level 1.4E9 (Positive) and −1.4E9 (Negative); Level increment 1.200 (Positive and Negative); Number of levels 16.

### Abbreviations

AcOH: Acetic acid; Br: Benzyl; CA: Cinnamyl alcohol; DHP: Dehydrogenative polymer; disp: 2,5-Diisopropylphenyl; DME: N,N-Dimethylformamide; DMSO: Dimethyl sulfoxide; DQAC: Ethyl acetate; G: Gás chromatography; HSQC: Heteronuclear single quantum coherence; Im: Imidazolium; LCC: Lignin–carbohydrate complex; MS: Mass spectrometry; NMP: N-Methylpyrrolidone; NMR: Nuclear magnetic resonance; PBS: Phosphate-buffered saline; TF: Trifluoromethanesulfonyl; THF: Tetrahydrofuran; TIC: Total ion chromatogram; Ts: p-Toluenesulfonyl.

### Acknowledgements

This study was financially supported by JSPS KAKENHI (Grant-in-Aid for Scientific Research (C) Grant Number 19K06161 and Grant-in-Aid for Scientific Research (A) Grant Number 21H04730).

### Author contributions

KS conceived and planned the experiments. MT and KS carried out the experiments. KS wrote the manuscript. KS and YU supervised the project. All authors read and approved the final manuscript.

### Funding

JSPS KAKENHI (Grant-in-Aid for Scientific Research (C) Grant Number 19K06161 and Grant-in-Aid for Scientific Research (A) Grant Number 21H04730).

### Availability of data and materials

All data generated or analyzed during this study are included in this published article. The spectral data of NMR are available from the corresponding author on reasonable request.

### Declarations

#### Competing interests

The authors declare that they have no competing interests.

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Received: 11 May 2022 Accepted: 1 July 2022 Published online: 12 August 2022

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