Electronic Supplementary Material (ESI) for New Journal of Chemistry.
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Supporting information

Synthesis of Novel Lignin Model Compounds Labeled with Alkynyl and its potential application

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Contents

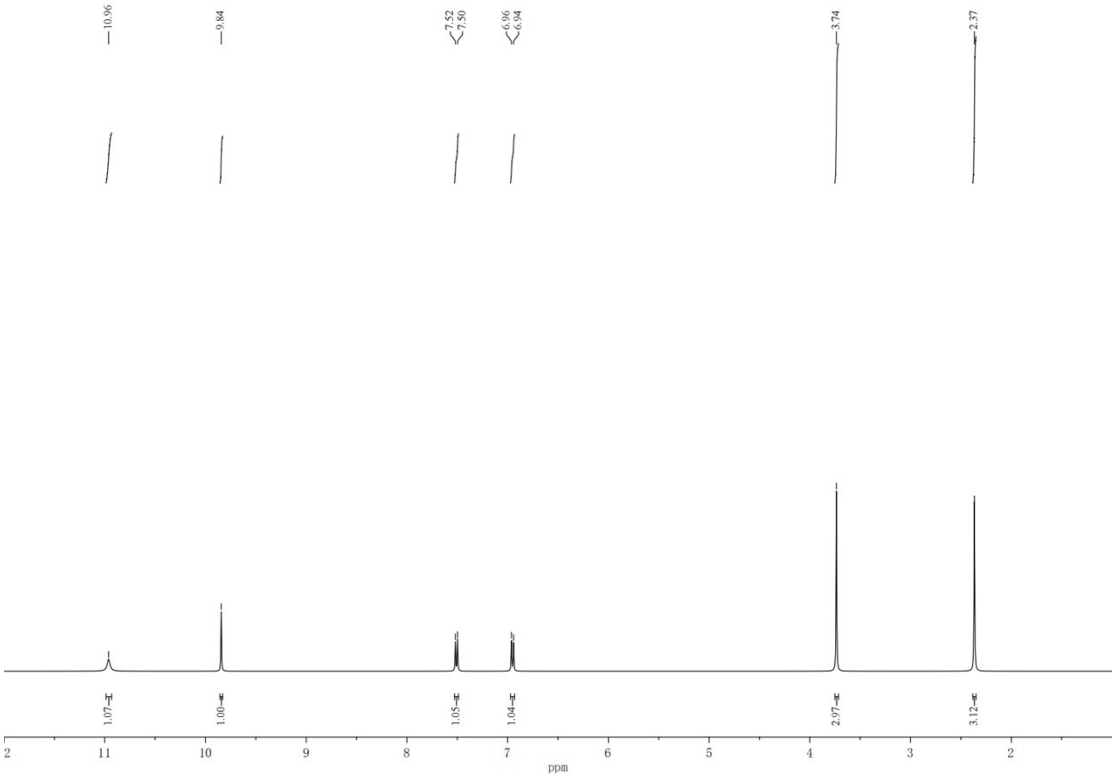
Synthetic procedures

Preparation of 2-O-propargyl coniferin

Tetrahydropyran (THP) was applied as the protecting group, which was stable and easy to fall off under certain conditions. Through the substitution reaction between phenolic hydroxyl at ortho position and bromopropylene, Alkynyl group was incorporated. Glycosidic bond was formed with fluoroglucose at 4th position. After reduction reaction with DIBAL-H, final target compound was obtained.

4-hydroxy-3- methoxy 2-O-acetyl Benzaldehyde (2)

Iodobenzene diethyl ester (3.6 mmol) was dissolved in 20 mL of acetic acid, which then was added dropwise with a constant pressure funnel into 30 mL of acetic acid in which 0.5 g (3.2 mmol) of Vanillin was completely dissolved [1]. The mixture was vacuum evaporated after 3 days of reaction. The target product was separated by chromatographic column (ethyl acetate/n-hexane, 3/7, v/v). After vacuum evaporation and dried for 12 hours, a yellow green solid was obtained (0.71 g, yield of 84%). ^{1}H NMR (600 MHz, DMSO-d6) δ 10.96 (s, 1H), 9.84 (s, 1H), 7.51 (d, J=8.6 Hz, 1H), 6.95 (d, J=8.6 Hz, 1H), 3.74 (s, 3H), 2.37 (s, 3H).

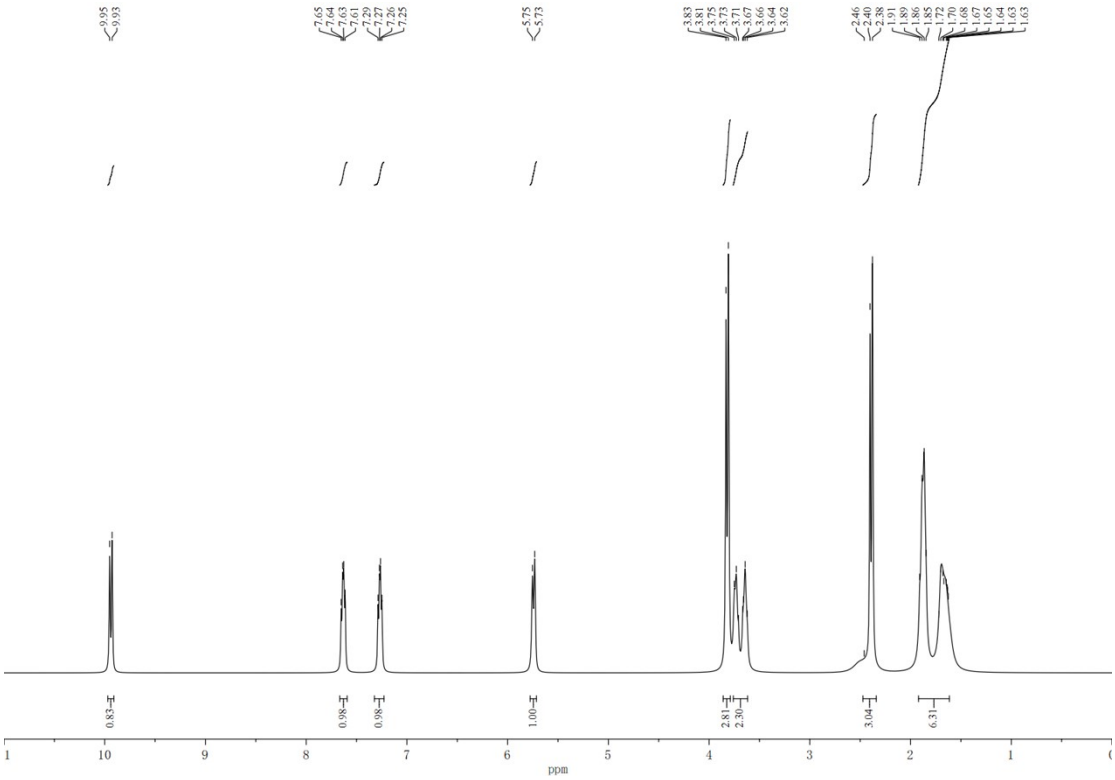


^{1}H-NMR Spectra of 4-hydroxy-3- methoxy 2-O-acetyl Benzaldehyde

4-O- tetrahydropyranyl-3- methoxy-2-O-acetyl Benzaldehyde (3)

Pyridinium *p*-toluenesulfonate (0.7 mmol) was dissolved in 50 mL of dichloromethane, then 2.94 g (14 mmol) of compound 2 was joined in [2]. 2.355 g (28 mmol) of 3,4-dihydro-2H-pyran was added by constant pressure funnel, which was stirred at room temperature for 1.5 hours. 150

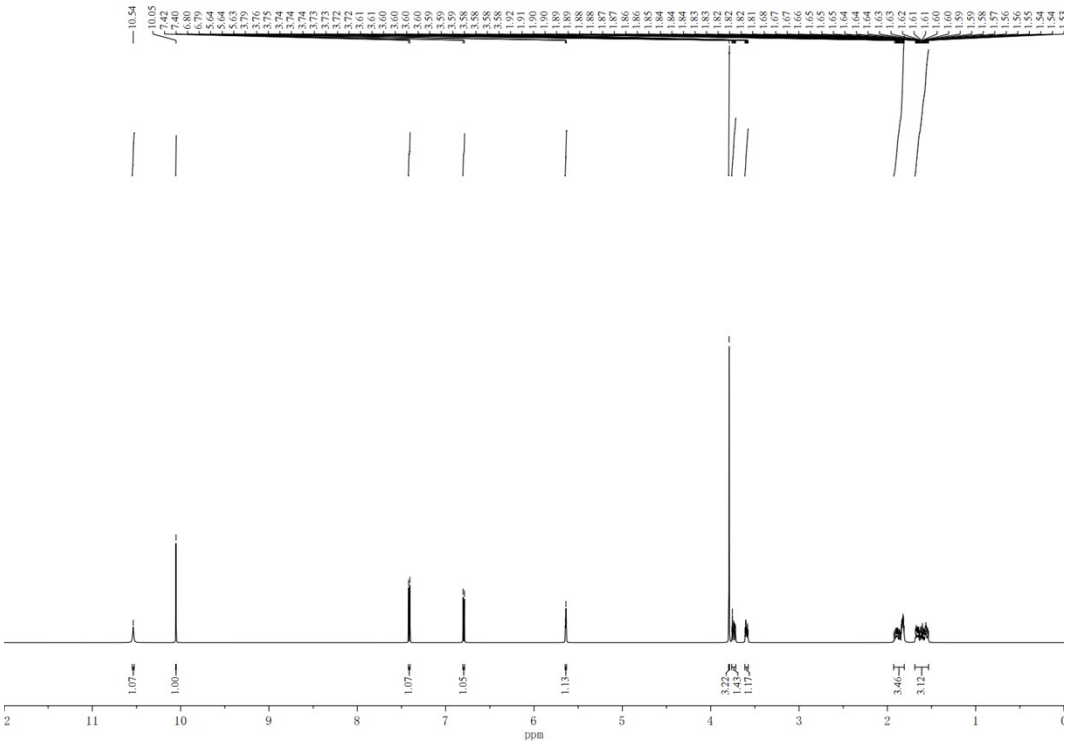
mL of ether was added for dilution, and washed three times with 100 ml of distilled water. The organic layer was dried over anhydrous sodium sulfate for 12 h. After filtration and vacuum evaporation, the target product was separated by chromatography (ethyl acetate/n-hexane, 1/5, v/v). 2.388 g of white product was obtained after vacuum evaporation and dried for 12 hours, with a yield of 58%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.94 (d, J=14.5 Hz, 1H), 7.67-7.59 (m, 1H), 7.32-7.23 (m, 1H), 5.74 (d, J=13.6 Hz, 1H), 3.82 (d, J=14.4 Hz, 3H), 3.76-3.62 (m, 2H), 2.39 (d, J=14.5 Hz, 3H), 1.92-1.61 (m, 6H).



^{1}H-NMR Spectra of 4-O- tetrahydropyranyl-3- methoxy-2-O-acetyl Benzaldeh

4-O- tetrahydropyranyl-3- methoxy-2-hydroxy Benzaldehyde (4)

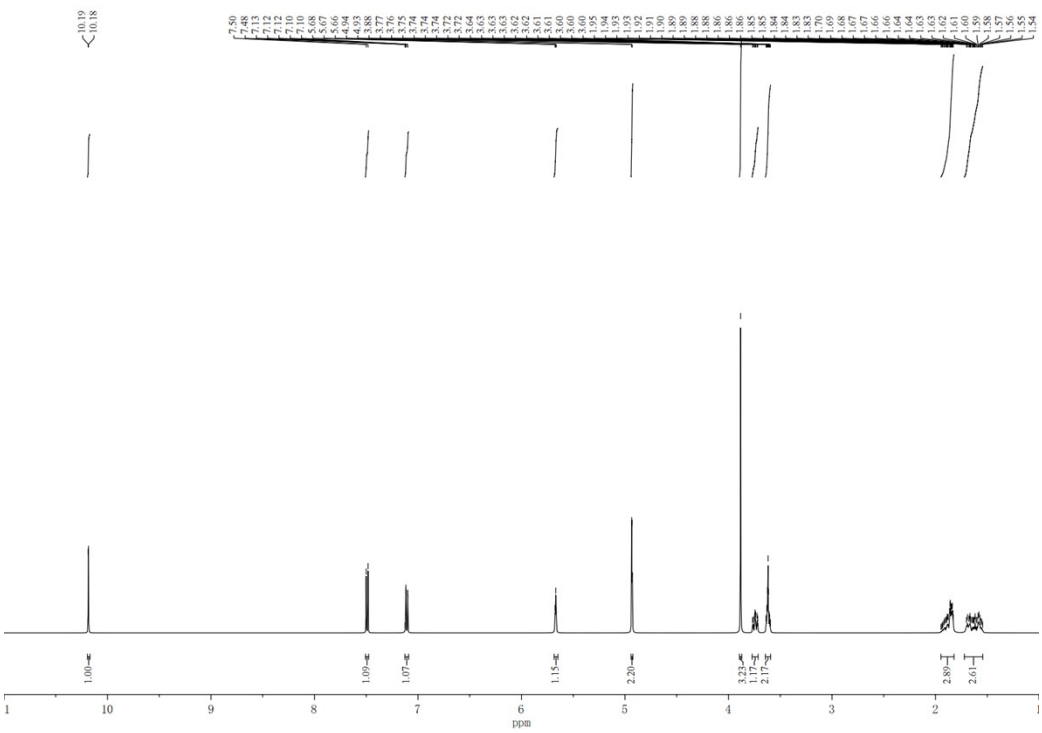
Compound 3 (4 mmol) was dissolved in 5 mL of tetrahydrofuran and 15 mL of methanol. Then 0.16 g (4 mmol) of sodium hydroxide was added. After stirring at room temperature for 5 h, 40 mL of ethyl acetate and 30 mL of water was added. Water layer was washed with 20 mL of ethyl acetate by three times. Organic layers were collected and dried over anhydrous sodium sulfate for 12 h. After filtration and vacuum evaporation, the liquid was separated by column chromatography (ethyl acetate/n-hexane, 1/8, v/v). The target product was evaporated under reduced pressure and vacuum dried for 12 hours to obtain 0.788 g of the product, with a yield of 78.17%. ^{1}H NMR (600 MHz, DMSO-d6) δ 10.54 (s, 1H), 10.05 (s, 1H), 7.41 (d, J=8.8 Hz, 1H), 6.80 (d, J=8.9 Hz, 1H), 5.64 (t, J=3.1 Hz, 1H), 3.79 (s, 3H), 3.76-3.72 (m, 1H), 3.60 (dtd, J=11.0, 4.0, 2.0 Hz, 1H), 1.93-1.81 (m, 3H), 1.69-1.53 (m, 3H).



^{1}H-NMR Spectra of 4-O- tetrahydropyranyl-3- methoxy-2-hydroxy Benzaldehyde

4-O- tetrahydropyranyl-3- methoxy-2-O-propargyl Benzaldehyde (5)

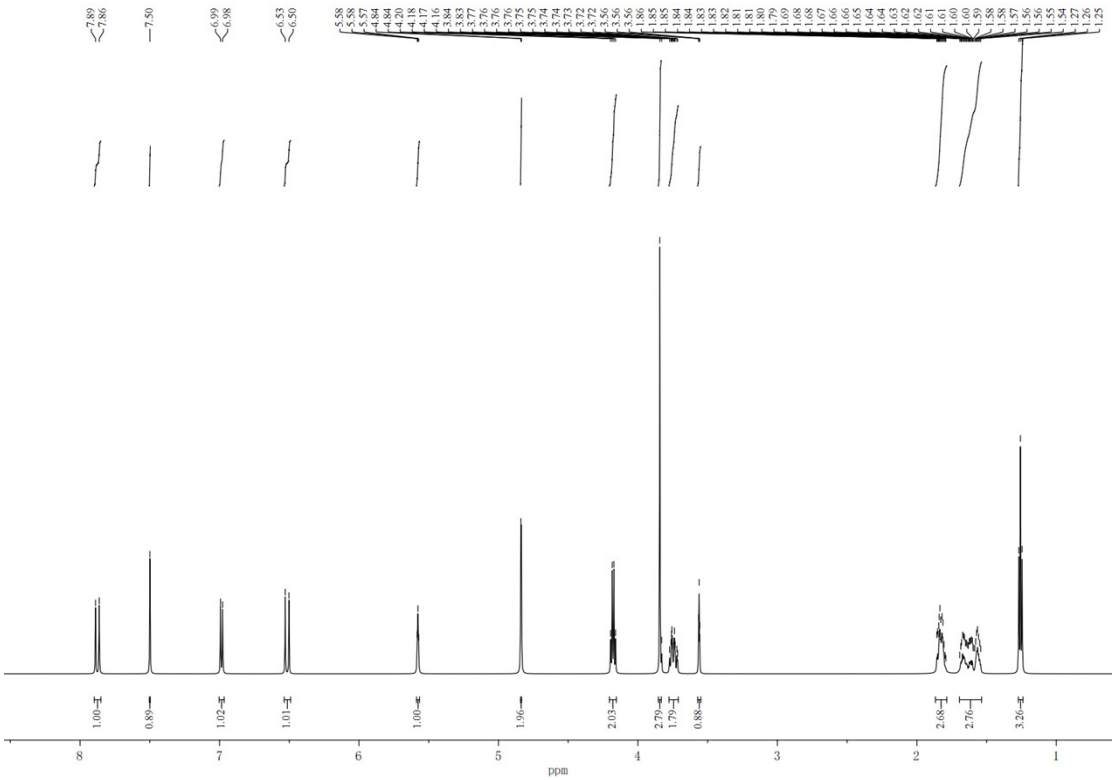
Compound 4 (10 mmol) was dissolved in 5 mL of DMF. Then anhydrous potassium carbonate (12 mmol) was added. The liquid was changed from dark green to grayish green after stirring for 20 minutes [3]. 1.3086 g (11 mmol) of bromopropylene was added dropwise by constant pressure funnel. Then, 30 mL of ethyl acetate was added. After filtration and vacuum evaporation, the liquid was separated by column chromatography (ethyl acetate/n-hexane, 1/3, v/v). 2.28 g of white solid was yielded (78.75%). ^{1}H NMR (600 MHz, DMSO-d6) δ 10.18 (d, J=0.9 Hz, 1H), 7.49 (d, J=8.8 Hz, 1H), 7.11 (dd, J=8.9, 0.8 Hz, 1H), 5.67 (t, J=2.9 Hz, 1H), 4.93 (d, J=2.4 Hz, 2H), 3.88 (s, 3H), 3.74 (ddd, J=11.4, 9.9, 3.4 Hz, 1H), 3.64-3.59 (m, 2H), 1.95-1.82 (m, 3H), 1.72-1.54 (m, 3H).



^{1}H-NMR Spectra of 4-O- tetrahydropyranyl-3- methoxy-2-O-propargyl Benzaldehyde

4-O- tetrahydropyranyl-3-methoxy-2-O-propargyl Cinnamic acid ethyl ester (6)

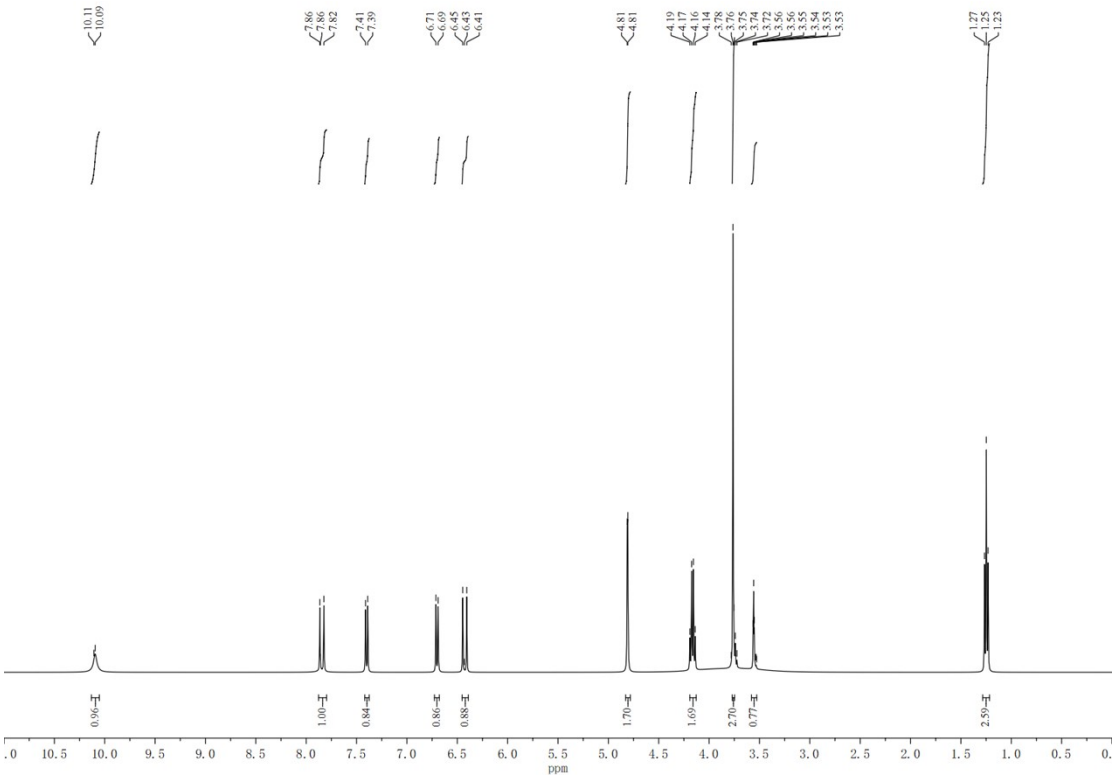
Triethyl phosphorylacetate (29 mmol) was joined with 50 mL of THF at 0 ℃ under N_2 atmosphere. Then 1.1 g NaH was added and stirred for 15 min, then 7.543 g (26 mmol) of compound 5 was added. After stirring for 11 h, 25 mL of water was added to quench the reaction [4]. 25 mL of saturated $NaHCO_3$ was applied to organic layer to separate the solution. The water layer was washed twice with 150 ml tert-Butyl methyl ether. The organic layer was combined, and dried over anhydrous sodium sulfate overnight. After filtration and vacuum evaporation, the liquid was separated by chromatographic column (ethyl acetate/n-hexane, 1/5, v/v). The target product was vacuum evaporated and dried overnight to yield 5.82 g of white solid (yield of 62.2%.) ^{1}H NMR (600 MHz, DMSO-d6) δ 7.88 (d, J=16.1 Hz, 1H), 7.50 (s, 1H), 6.99 (d, J=8.9 Hz, 1H), 6.52 (d, J=16.1 Hz, 1H), 5.58 (t, J=3.1 Hz, 1H), 4.84 (d, J=2.4 Hz, 2H), 4.18 (q, J=7.1 Hz, 2H), 3.84 (s, 3H), 3.78-3.71 (m, 2H), 3.56 (t, J=2.4 Hz, 1H), 1.83 (dtt, J=13.0, 9.0, 4.1 Hz, 3H H), 1.69-1.54 (m, 3H), 1.26 (t, J=7.1 Hz, 3H).



^{1}H-NMR Spectra of 4-O- tetrahydropyranyl-3-methoxy-2-O-propargyl Cinnamic acid ethyl ester

4- hydroxy-3- methoxy-2-O-propargyl Ethyl Cinnamic acid (7)

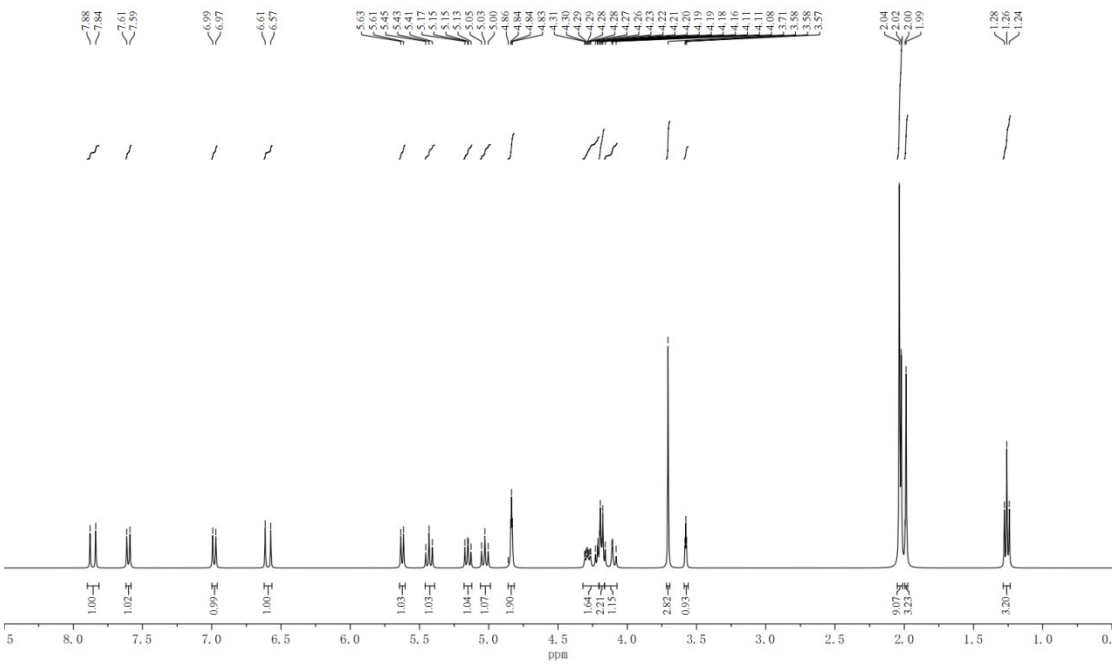
Pyridinium *p*-toluenesulfonate (0.1 mmol) was dissolved in 8 mL ethanol, then 0.36 g of compound 6 was added. After reacting at 55 ℃ for 3 hours, the mixture was vacuum evaporated [5]. Chromatography column (ethyl acetate/n-hexane, 1/8, v/v) was applied to separate the mixture. The target product was vacuum evaporated and dried to obtain a white solid of 0.114 g (yield of 41%). ^{1}H NMR (600 MHz, DMSO-d6) δ 10.09 (s, 1H), 7.84 (d, J=16.1 Hz, 1H), 7.40 (d, J=8.7 Hz, 1H), 6.70 (d, J=8.7 Hz, 1H), 6.43 (d, J=16.1 Hz, 1H), 4.81 (d, J=2.5 Hz, 2H), 4.16 (q, J=7.1 Hz, 2H), 3.76 (s, 3H), 3.56 (t, J=2.4 Hz, 1H), 1.25 (t, J=7.1 Hz, 3H).



[1]H-NMR Spectra of 4-hydroxy-3- methoxy-2-O-propargyl Ethyl Cinnamic acid

4-(2,3,4,6)-O-Acetyl-β-D-glucoside -3- methoxy-2-O-propargyl Cinnamic acid ethyl ester (8)

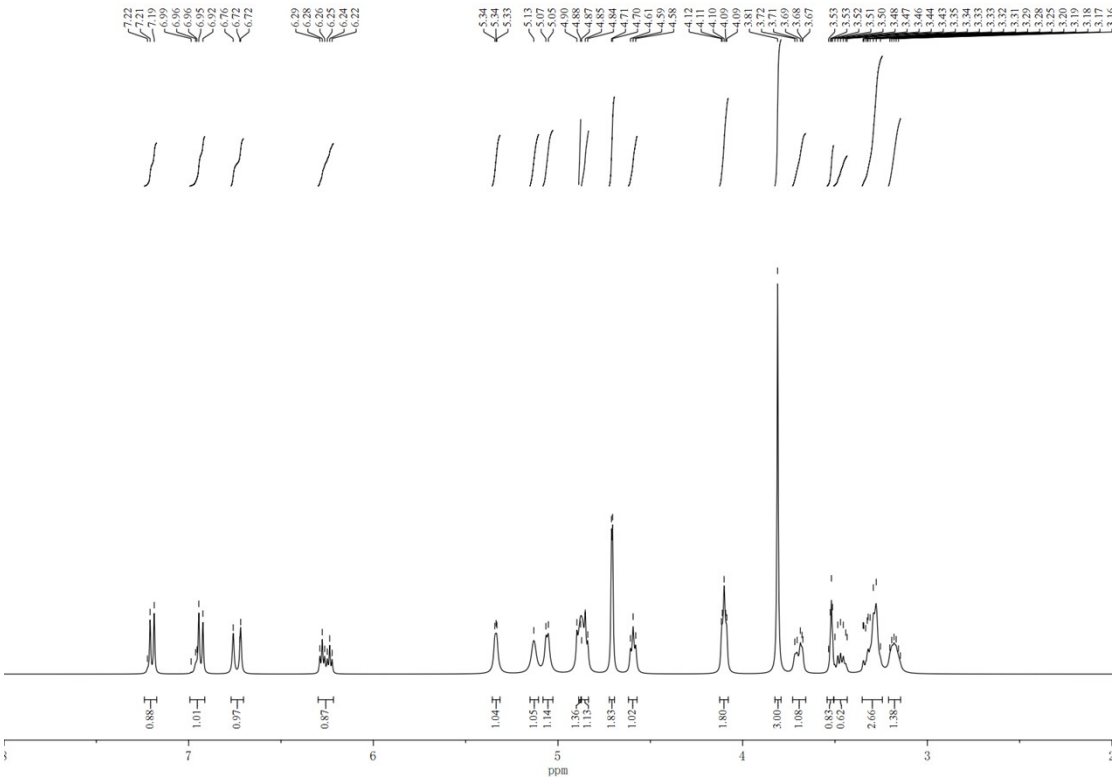
Compound 7 (0.9 mmol) and 2,3,4,6-tetra-O - Acetyl group-α-D-glucopyranosyl fluoride (1.8 mmol)was put into solution containing 3 mL dichloromethane and 18 mL chlorobenzene. Then 738 mg 2,6-di-tert-butyl-4-methylpyridine and 111 μL1,1,3,3-tetramethylguanidine was added. After the color of liquid turning to yellow green, 480 μL Boron trifluoride diethyl etherate was joined in and reacted at room temperature for 1h [6]. 24 mL saturated sodium bicarbonate was added to quench the reaction. Then the water layer was washed three times by 24 mL ethyl acetate, which then was dried over anhydrous sodium sulfate for 12 hours. After filtration and vacuum evaporation, the mixture was purified by chromatography column (ethyl acetate/n-hexane, 1/1, v/v). The target product was vacuum evaporated and dried for 12 hours to yield 497 mg of white solid (yield of 94%). ^{1}H NMR (600 MHz, DMSO-d6) δ 7.86 (d, J=16.2 Hz, 1H), 7.60 (d, J=8.9 Hz, 1H), 6.98 (d, J=8.9 Hz, 1H), 6.59 (d, J=16.2 Hz, 1H), 5.62 (d, J=7.9 Hz, 1H), 5.43 (t, J=9.6 Hz, 1H), 5.15 (dd, J=9.8, 7.9 Hz, 1H), 5.03 (t, J=9.7 Hz, 1H), 4.84 (t, J=2.5 Hz, 2H), 4.32-4.21 (m, 2H) 4.19 (dd, J=6.5, 2.8 Hz, 2H), 4.16-4.07 (m, 1H), 3.71 (s, 3H), 3.58 (t, J=2.4 Hz, 1H), 2.03 (d, J=5.4 Hz, 9H), 1.99 (s, 3H), 1.26 (t, J=7.1 Hz, 3H).



^{1}H-NMR Spectra of 4-(2,3,4,6)-O-Acetyl-β-D-glucoside -3- methoxy-2-O-propargyl Cinnamic acid ethyl ester

2-O-propargyl coniferin (9)

Compound 8 (6 mmol) was put into 200 mL toluene under nitrogen at -5 ℃. 80 mL DIBAL-H (1.5 M in toluene) was added by constant pressure funnel, and the reaction was continued for 1 hour until the liquid was almost colorless and transparent [7]. Ethanol was then added until no bubbles observed. The reaction liquid was vacuum evaporated at 50 ℃, then 100 mL of water was added. After filtration at 90 ℃, the solid was washed three times with 50 mL boiling water to obtain the yellow green filtrate. The target product was separated by chromatographic column (ethyl acetate/acetone/water, 10/10/1, v/v/v), followed by vacuum evaporation and dried overnight to yield a white solid of 1.85 g (yield of 78%). ^{1}H NMR (600 MHz, DMSO-d6) δ 7.20 (d, J=8.9 Hz, 1H), 6.94 (t, J=7.9 Hz, 1H), 6.74 (d, J=16.2 Hz, 1H), 6.26 (dt, J=16.1, 5.2 Hz, 1H), 5.36-5.31 (m, 1H), 5.13 (s, 1H), 5.06 (d, J=5.3 Hz, 1H), 4.88 (s, 1H), 4.85 (t, J=6.6 Hz, 1H), 4.71 (d, J=2.5 Hz, 2H), 4.59 (t, J=5.7 Hz, 1H 4.12-4.08 (m, 2H), 3.81 (s, 3H), 3.73-3.66 (m, 1H), 3.52 (t, J=2.4 Hz, 1H), 3.46 (dq, J=8.3, 4.5, 2.9 Hz, 1H), 3.30 (dd, J=15.3, 4.2 Hz, 3H), 3.18 (dt, J=9.5, 4.6 Hz, 1H).



[1]H-NMR Spectra of 2-O-propargyl coniferin

Synthesis of G-type DHP

According to the published method [8], 500 mg of Coniferin, 425 mg Coniferin and 75 mg 2-O-propargyl Coniferin, 375 mg Coniferin and 125 mg 2-O-propargyl Coniferin, 250 mg Coniferin and 250 mg 2-O-propargyl Coniferin, 500 mg 2-O-propargyl coniferin was dissolved in 36 mL of 0.2 M acetic acid-sodium acetate buffer, respectively. 12.3 mg (From almonds，7.55 u/mg，Fluka) of β-Glucosidase, 14.4 mg (Type Ⅱ: from *Aspergilus niger*，21,200 units/g ，Sigma) glucose oxidase, 1.2 mg (TypeⅡ: from Horseradish，181 purpurogallin units/mg, Sigma) peroxidase was added under sterile conditions into 14 mL 0.1 M buffer, and then sterile air was introduced. Lignin monomer solution was added dropwise by constant flow pump within 24 hours. Then, 12.3mg U β- Glucosidase, 14.4mg glucose oxidase, and 1.2mg peroxidase were added and reacted for 94 hours, followed by centrifugation to collect the precipitate (8000 r/min). The precipitate was washed 6 times by distilled water to remove water-soluble impurities such as coniferin, enzymes, and carbohydrate with lower molecular weight. After freeze-drying, it was dissolved in 10 mL of CH_2Cl_2/C_2H_5OH (2/1, v/v). The final product G-DHP was obtained after precipitate in 100 mL ether, during which impurities such as coniferyl alcohol and dimers formed during the reaction process coule be removed (G-DHP 112 mg, 41.04%; 15% G_{alk}-DHP 108 mg, yield 41.02%; 25% G_{alk}-DHP 142 mg, yield 53.96%; 50% G_{alk}-DHP 101 mg, yield 38.38%; 100% G_{alk}-DHP 103 mg, yield 39.14%).

Click Reaction of Lignin Dehydrogenation Polymer

MS basic culture medium (2.2 M MS saltand 0.6 M 2-Morpholinoethanesulfonic Acid) was prepared in sterile conditions, and then CuAAC solution (containing 1 μM Azide-fluor 545, 1 mM $CuSO_4$ and 1 mM ascorbic acid) was prepared [9]. 20 mg DHP (G-DHP, 25% G_{alk}-DHP, 100% G_{alk}-DHP) was dissovled in 4 mL of CuAAC solution and kept for 1 hour in dark. Excess CuAAC solution was removed by centrifugation and washed with water (20 mL) four times. A purple red labeled DHP solid was obtained after freeze drying.

Analytical methods

NMR determination

About 20 mg of DHPs in DMSO-d_6 (0.5 mL) was characterized using a Bruker Avance III 600 MHz spectrometer at 298 K. Spectral width of 11 ppm with 2048 data points was applied, with 1-s delay for ^{1}H-NMR.The spectral width of 11 ppm in F2 (^{1}H) with 2048 data points, 190 ppm in F1 (^{13}C) with 256 data points were applied for 2D-HSQC. The scanning delay is 1 second, with 56 scans.

FT-IR

1 mg of DHP and 10 mg of anhydrous KBr (analytically pure) was mixed and grinded to powder. The sample was scanned 64 times with a scanning wavelength range of 4000 cm^{-1} to 500 cm^{-1} and a resolution of 2 cm^{-1}.

Laser confocal Raman spectroscopy detection

Raman spectra were collected using a Thermo Nicolet Almega XR laser confocal Raman spectrometer. Raman spectroscopy was collected within the range of 250 cm^{-1} to 3500 cm^{-1}, and scanned 512 times.

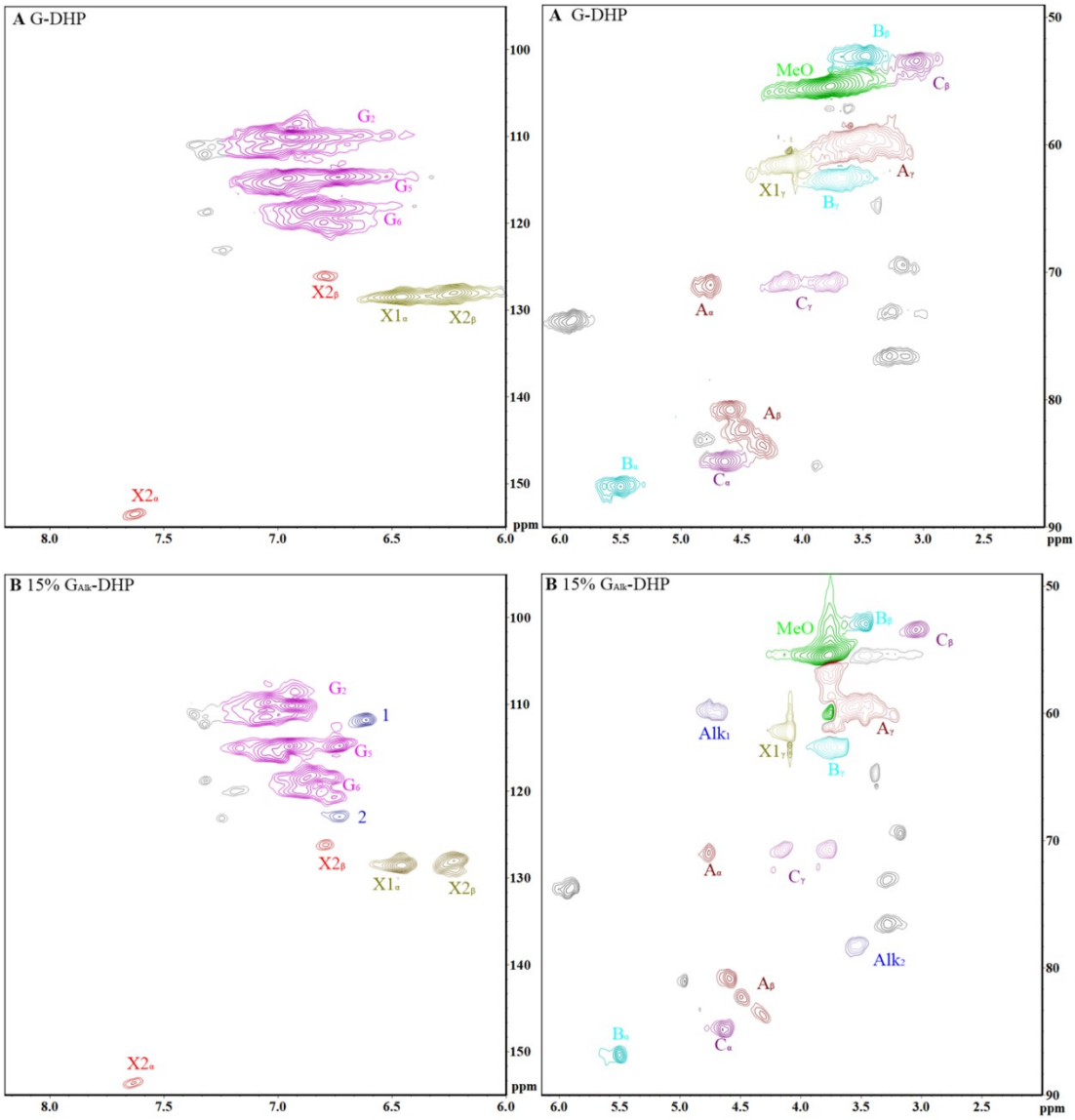
Fluorescence intensity detection

5 mg of DHP (unlabeled G-DHP, G-DHP, 25% G_{alk}-DHP, and 100% G_{alk}-DHP) was dissolved in DMSO. F-7000 fluorescence spectrophotometer was applied to determine the fluorescence intensity (Hitachi Koki Co., Ltd. Tokyo, Japan).

Gel permeation chromatographic (GPC) determination

Gel permeation chromatography was performed on an Agilent 1200 HPLC system (Agilent Technologies, Inc, Santa Clara, CA) with tetrahydrofuran (THF) as mobile phase, which was described in the previous study [10].

Supplementary Figures



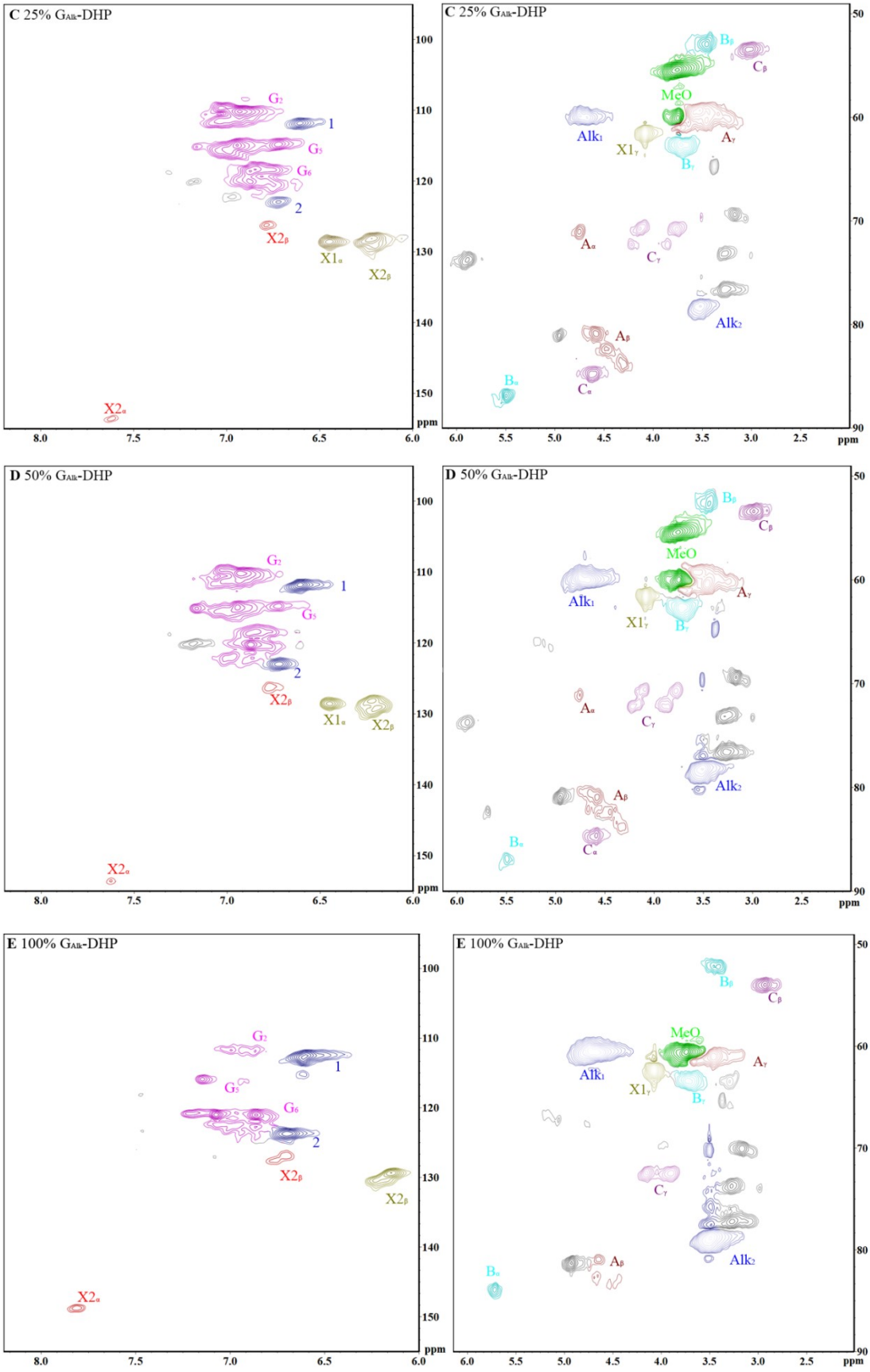


Fig.S1 2D-HSQC spectra of Lignin Dehydrogenation Polymer

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