Supplementary Information

Visualization of flax cell wall lignification using a novel click chemistry analogue of sinapyl alcohol

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Supplementary Methods

1.1 **FT-IR spectroscopy determination**

FT-IR spectroscopy (Nicolet 6700, Thermofisher Nicolet, America) was applied to scan the samples for 64 times from 4000 cm^{-1} to 500 cm^{-1}, with 2 cm^{-1} resolution.

1.2 **UV spectroscopy determination**

5 mg of lignin dehydrogenated polymer was dissolved in 10 mL of 95% (V/V) dioxane aqueous solution. Dilute 1 mL of the solution with distilled water to 10 mL. UV spectrophotometer (UV-2550, Shimadzu Co., Ltd., Japan) was employed to scan the solution at the wavelength of 200 nm~450 nm.

Supplementary Notes

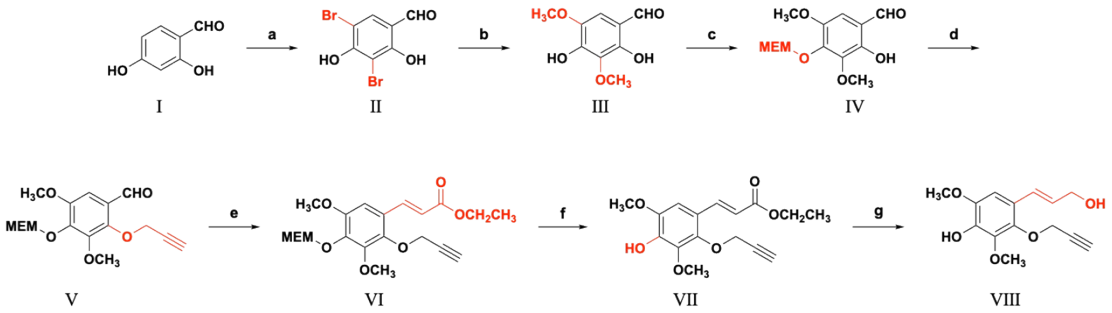

Fig.S1 Synthesis of 2-O-propargylsinapyl alcohol (2-O-PSA). (a) Bromination. (b) Methoxylation. (c) Protection with DIPEA and 2-methoxyethoxymethyl chloride. (d) Propargylation using propargyl bromide. (e)Wittig olefination with triethyl phosphonoacetate and sodium hydride. (f) Deprotection with trifluoroacetic acid, (g) Selective reduction with DIBAL-H

(1) 3,5-dibromo-2,4-dihydroxybenzene-1-carbaldehyde (II, CAS:116096-91-4)

Bromine (6.4 g, 40 mmol) was added dropwise tosolution of 2,4-dihydroxybenzaldehyde (2.8 g, 20 mmol) in ethanol (40 mL) at room temperature. After stirring for 30 minutes, the mixture was poured into distilled water (100 mL) and filtered. The filtrate was washed with water and residual bromine present in the aqueous phase was quenched with saturated sodium thiosulfate solution. Compound **2** was obtained as a white powder (5.9 g, 98%), and the physical and spectroscopic data were consistent with those reported previously[1]. m.p.: 188-189 ℃; ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.69 (s, 1H), 7.86 (s, 1H), 7.39 (s, 1H), 5.52 (s, 1H).

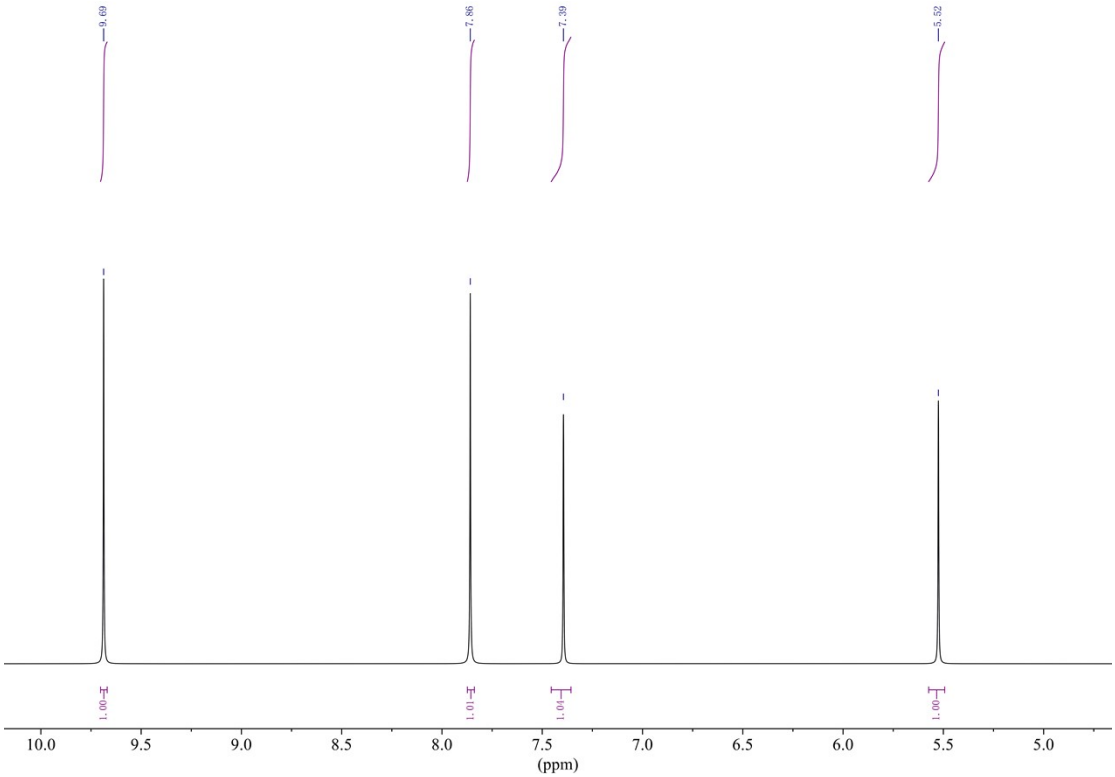

Fig.S2 ^{1}H-NMR spectrum of 3,5-dibromo-2,4-dihydroxybenzene-1-carbaldehyde (II)

(2) 2,4-dihydroxy-3,5-dimethoxybenzene-1-carbaldehyde (III, CAS:182427-46-9)

CuCl (0.5 g, 5 mmol) and NaOMe (21.6 g, 400 mmol)was added to solution of II (11.8 g, 40 mmol) dissolved in anhydrous methanol and anhydrous dimethylformamide (70 mL, 2:5) . The reaction mixture was heated at reflux at 100°C for 4 hours. The solvent was then evaporated in vacuum, distilled water (50 mL) was added and the pH was adjusted to 2 by concentrated HCl. After 15 min, the reaction mixture was transferred to a separatory funnel and the product was extracted with chloroform (5 × 20 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and the solvent was evaporated in vacuum. Compound **III** was obtained as a brown solid (5.5 g, 70%) with physical and spectroscopic data consistent with those previously reported[1]. m.p.: 88-89 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H), 10.10 (s, 1H), 9.95 (s, 1H), 7.00 (s, 1H), 3.76 (d, J = 16.0 Hz, 6H).

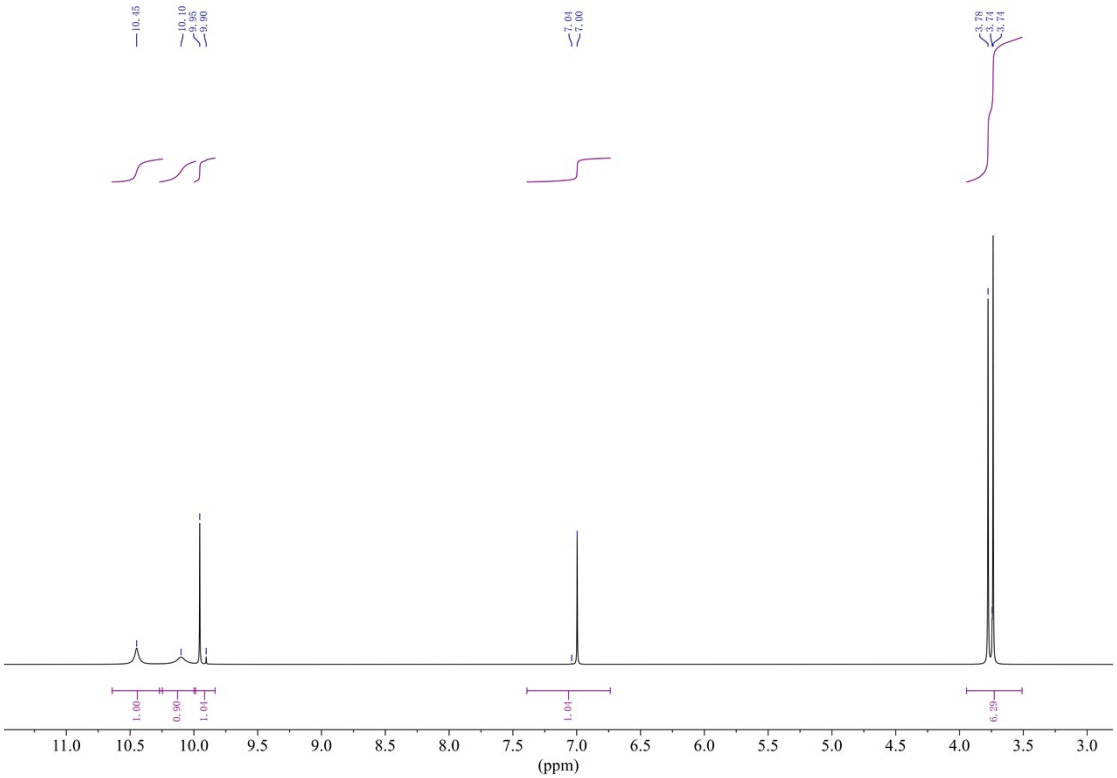

Fig. S3 ^{1}H-NMR spectrum of 2,4-dihydroxy-3,5-dimethoxybenzene-1-carbaldehyde (III)

(3) 4-(2,5-dioxahex-1-yloxy)-2-hydroxy-3,5-dimethoxybenzene-1-carbaldehyde (IV)

DIPEA (12.50 mL, 72.3 mmol) was added dropwise to compound III (14.31 g, 72.3 mmol) in DCM (100 mL) at -5°C over 5 minutes. The following mixture was stirred for 5 minutes before 2-methoxyethoxymethyl chloride (8.20 mL, 72.3 mmol) was added. The solution was stirred at -5°C for 6 hours. Water (50mL) was applied to quench the reaction and extracted with DCM (3 × 100 mL) at 0°C, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuum to obtain a yellow oil[2]. Flash purification chromatography (ethyl acetate/hexane 50%) yielded compound **IV** (14.9 g, 91%) as a colorless oil.^{1}H NMR (600 MHz, Deuterium Oxide) δ 12.41 (s, 1H), 12.36 (s, 1H), 9.26 (s, 1H), 7.45 (s, 2H), 6.07 – 6.03 (m, 2H), 5.99 (d, *J* = 8.3 Hz, 6H), 5.70 – 5.65 (m, 3H), 5.46 (d, *J* = 1.1 Hz, 2H).

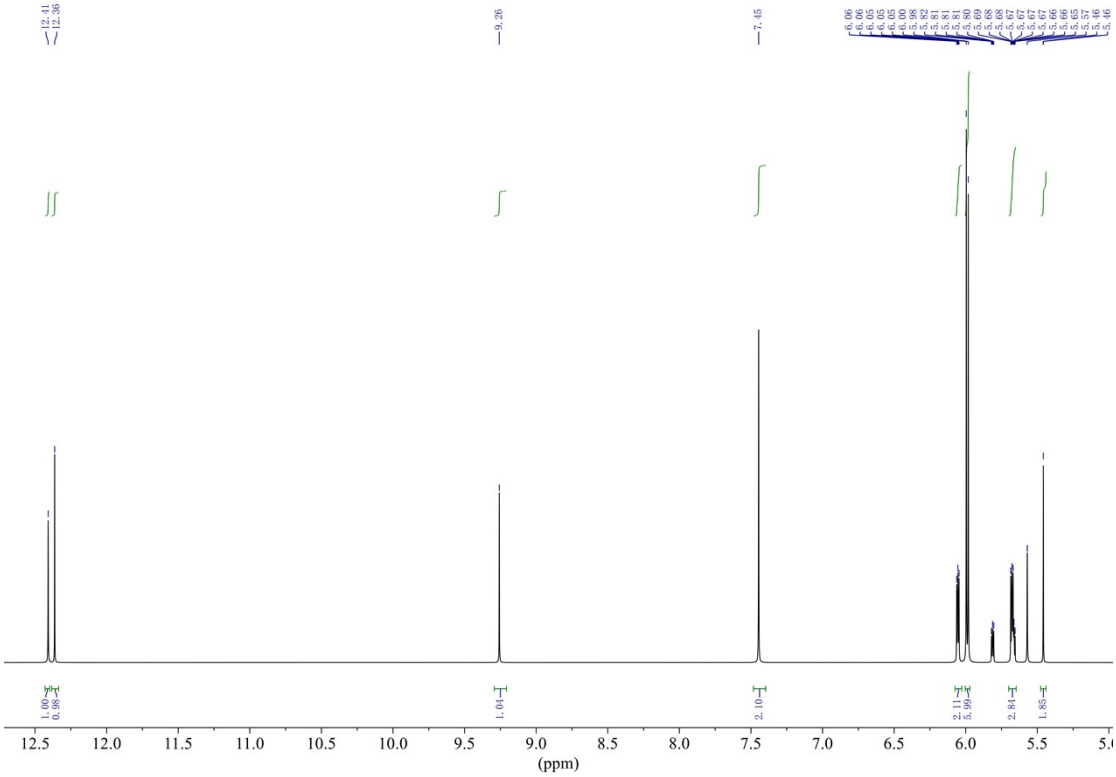

Fig.S4 ^{1}H-NMR spectrum of 4-(2,5-dioxahex-1-yloxy)-2-hydroxy-3,5-dimethoxybenzene-1-carbaldehyde (IV)

(4) 4-(2,5-dioxahex-1-yloxy)-3,5-dimethoxy-2-(prop-2-ynyloxy)benzene-1-carbaldehyde (V)

Compound IV (2.86 g, 10 mmol) was dissolved in 5 mL of DMF and anhydrous K_2CO_3 (12.0 mmol) were added to the solution at room temperature. It was then stirred for 20 minutes, propargyl bromide (11.0 mmol) was added, and the mixture was stirred at 45°C for 6 hours. After the reaction was completed, it was extracted with ethyl acetate (30 mL), the organic phase was washed with water (3×50 mL), and then dried over $MgSO_4$. Removal of the solvent yielded compound **V**[3]. m.p.: 101-130℃ ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 7.08 (s, 1H), 5.24 (s, 2H), 4.86 (d, J = 2.4 Hz, 2H), 3.94 – 3.79 (m, 8H), 3.64 (t, J = 2.4 Hz, 1H), 3.50 – 3.42 (m, 2H), 3.23 (s, 3H).

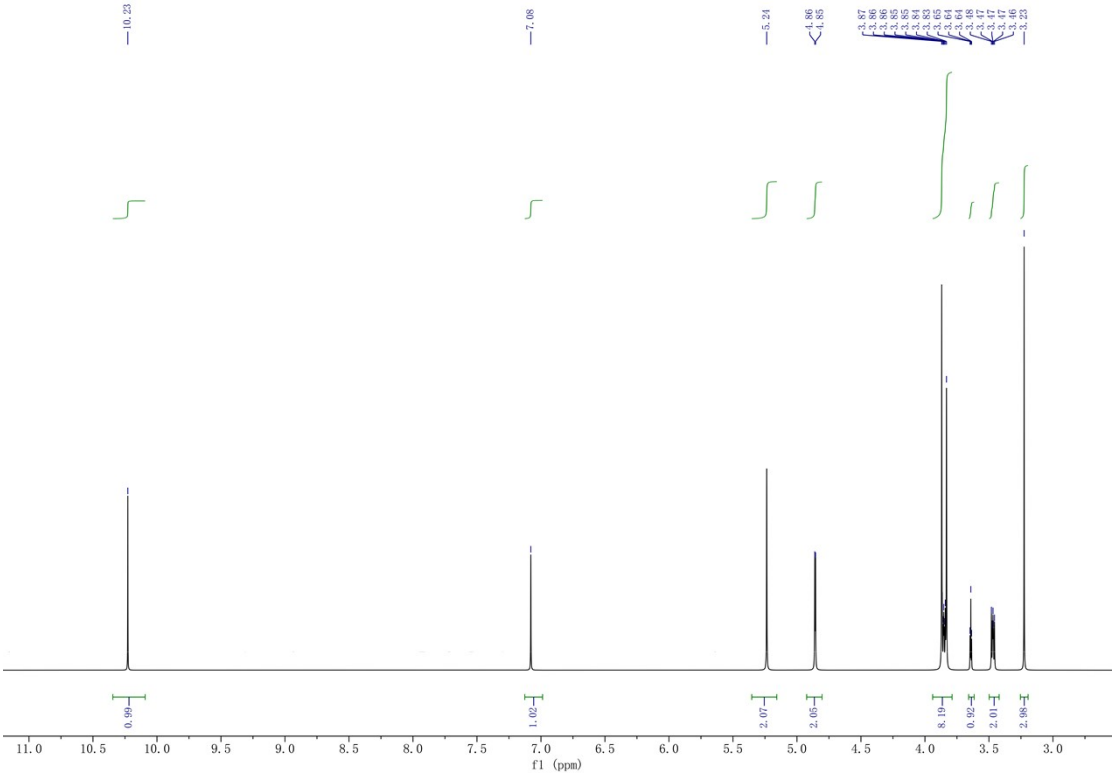

Fig.S5 ^{1}H-NMR spectrum of 4-(2,5-dioxahex-1-yloxy)-3,5-dimethoxy-2-(prop-2-ynyloxy)benzene-1-carbaldehyde (V)

(5) ethyl (2*E*)-3-[4-(2,5-dioxahex-1-yloxy)-3,5-dimethoxy-2-(prop-2-ynyloxy)phenyl]prop-2-enoate (VI)

NaH (300 mg, 7.50 mmol) was added to a solution of triethylphosphonoacetate solution (1.68 g, 7.50 mmol) in dried and degassed THF (20 mL) under a nitrogen atmosphere at -5°C. After stirring the mixed solution for 20 minutes, compound V (1.32g, 5.00mmol) was added. After the mixed solution was stirred for 20 minutes, compound 5 dissovled in THF (1.32 g, 5.00 mmol) was added dropwise. After the reaction is completed, 25 mL of distilled water was added. The aqueous layer was separated, then 25 mL of saturated sodium bicarbonate solution was added, to collect the organic layer. Then methyl tert-butyl ether (3 × 30 mL) was added to extract the aqueous layer, and the collected organic layer was dried over anhydrous sodium sulfate overnight[4]. The organic layer was then filtered, the solvent was evaporated under reduced pressure, and compound **VI** was collected by column chromatography (1.28 mg, 90%) as a white solid. m.p.: 90-98℃. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.02 (d, *J* = 16.1 Hz, 1H), 6.82 (s, 1H), 6.41 – 6.36 (m, 1H), 5.25 (s, 2H), 4.71 (d, *J* = 2.3 Hz, 2H), 4.27 (q, *J* = 7.1 Hz, 2H), 3.99 – 3.95 (m, 2H), 3.90 (d, *J* = 1.1 Hz, 3H), 3.84 (s, 3H), 3.59 – 3.55 (m, 2H), 3.37 (d, *J* = 1.1 Hz, 3H), 2.49 (q, *J* = 1.9, 1.3 Hz, 1H), 1.36 – 1.32 (m, 3H).

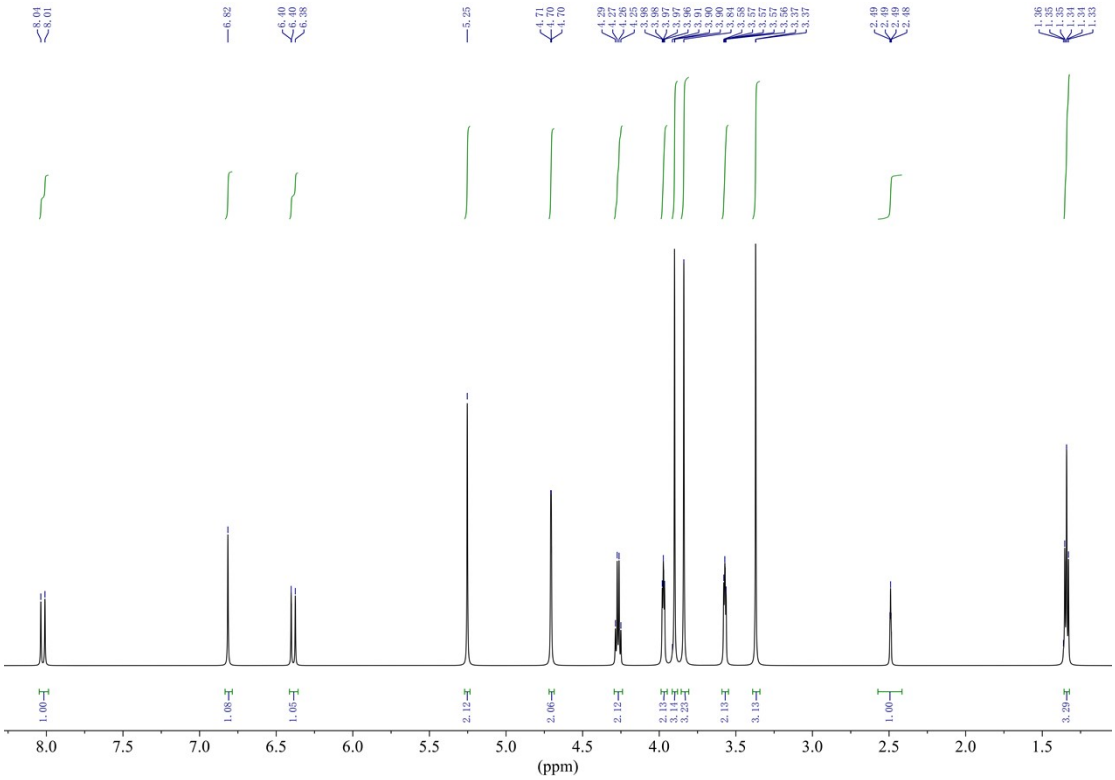

Fig.S6 ^{1}H-NMR spectrum of ethyl (2*E*)-3-[4-(2,5-dioxahex-1-yloxy)-3,5-dimethoxy-2-(prop-2-ynyloxy)phenyl]prop-2-enoate (VI)

(6) ethyl (2*E*)-3-[4-hydroxy-3,5-dimethoxy-2-(prop-2-ynyloxy)phenyl]prop-2-enoate (VII)

Trifluoroacetic acid (0.6 ml) was added to compound VI (19.7 mg, 0.05 mmol) in CH_2Cl_2 (0.6 ml) at 0 °C under Ar atmosphere. After stirring continuously for 24 hours at room temperature, the solvent was evaporated, and the residue was purified by chromatography with CH_2Cl_2-EtOAc (1:1) to obtain compound **VII** (15.4 mg, 90%) as a white solid[5]. m.p.: 88-93℃ ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.49 (s, 1H), 7.88 (d, J = 16.1 Hz, 1H), 7.12 (s, 1H), 6.55 (d, J = 16.0 Hz, 1H), 4.72 (d, J = 2.5 Hz, 2H), 4.17 (q, J = 7.1 Hz, 2H), 3.82 (s, 3H), 3.76 (s, 3H), 3.54 (t, J = 2.4 Hz, 1H), 1.25 (t, J = 7.1 Hz, 3H).

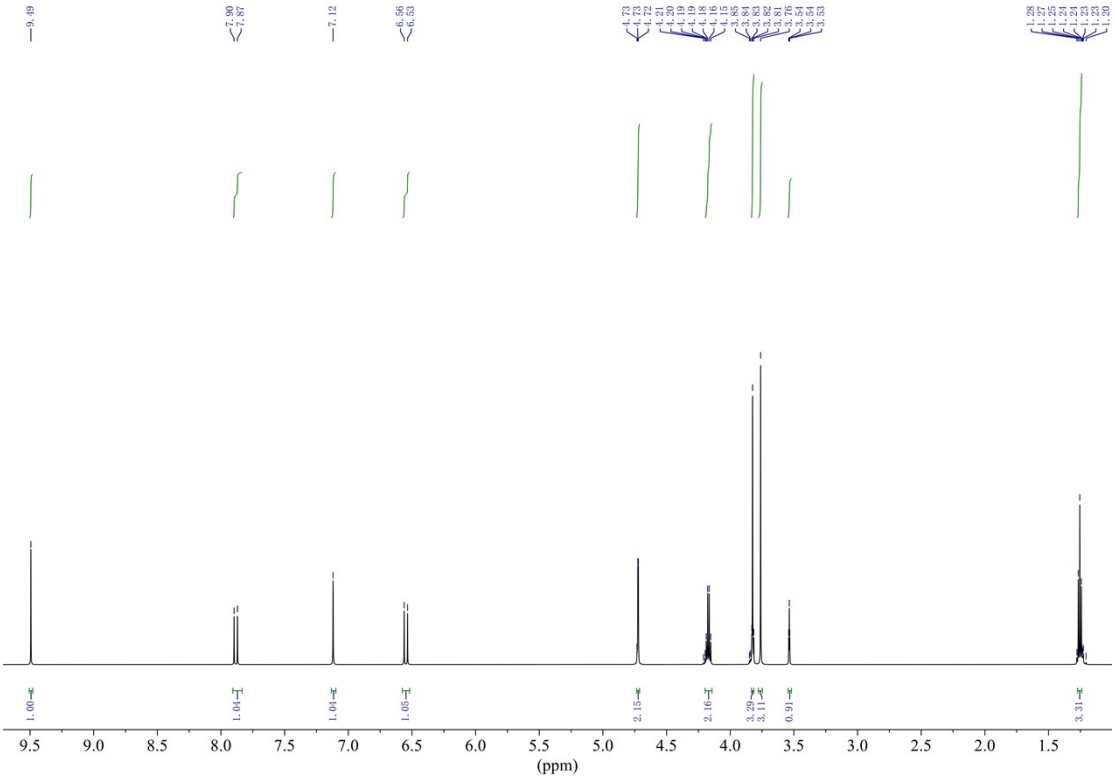

Fig.S7 ^{1}H-NMR spectrum of ethyl (2*E*)-3-[4-hydroxy-3,5-dimethoxy-2-(prop-2-ynyloxy)phenyl]prop-2-enoate (VII)

(7) 4-[(1*E*)-3-hydroxyprop-1-enyl]-2,6-dimethoxy-3-(prop-2-ynyloxy)phenol (VIII)

DIBAL-H (37 mL, 1.0 M in CH_2Cl_2 , 37.0 mmol) was added to solution of compound VII (3.2 g, 16.82 mmol) in anhydrous CH_2Cl_2 (50 mL) under argon at 0°C. The mixture was stirred for 2 hours. The mixture was quenched with 2M HCl (50 mL) at 0°C and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography using Hexanes/EtOAc (4:1) as eluent to afford compound **VIII** (2.37 g, 95%) as a yellow solid[6]. m.p.: 111-132℃. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.88 (s, 1H), 6.83 (s, 1H), 6.73 (d, J = 16.1 Hz, 1H), 6.24 (dt, J = 16.0, 5.4 Hz, 1H), 4.80 (s, 1H), 4.59 (d, J = 2.5 Hz, 2H), 4.12 – 4.08 (m, 2H), 3.78 (s, 3H), 3.75 (s, 3H), 3.50 (s, 1H).

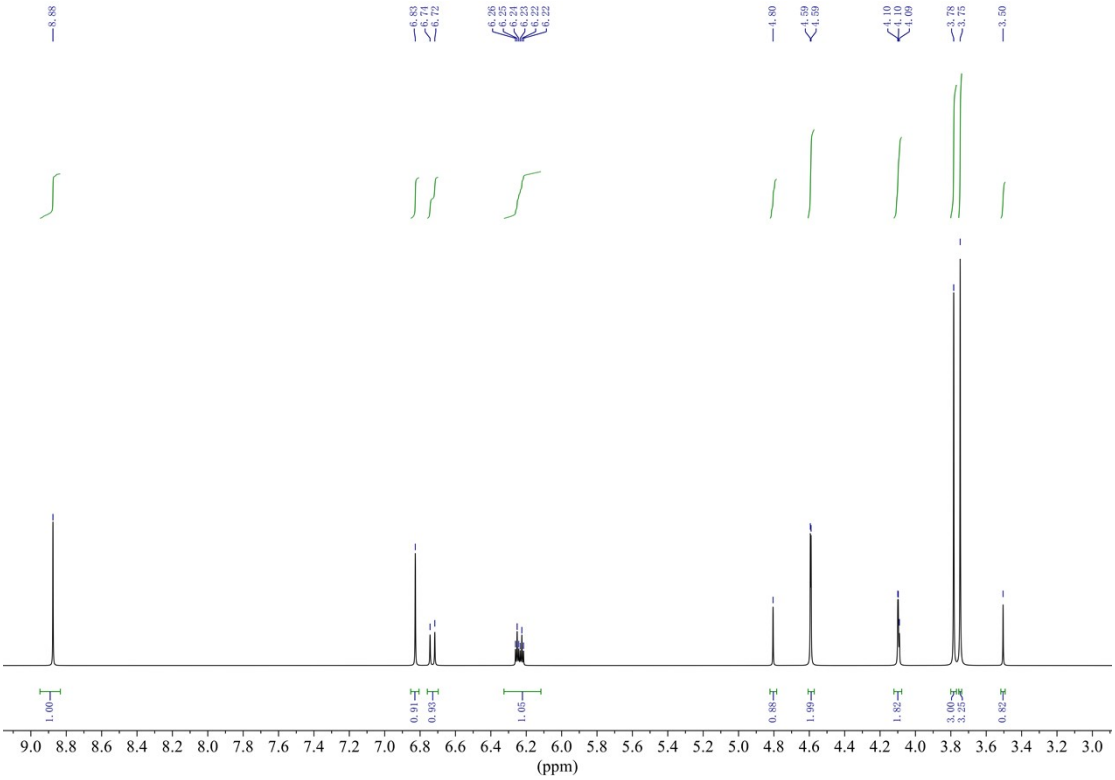

Fig.S8 ^{1}H-NMR spectrum of 4-[(1*E*)-3-hydroxyprop-1-enyl]-2,6-dimethoxy-3-(prop-2-ynyloxy)phenol (VIII)

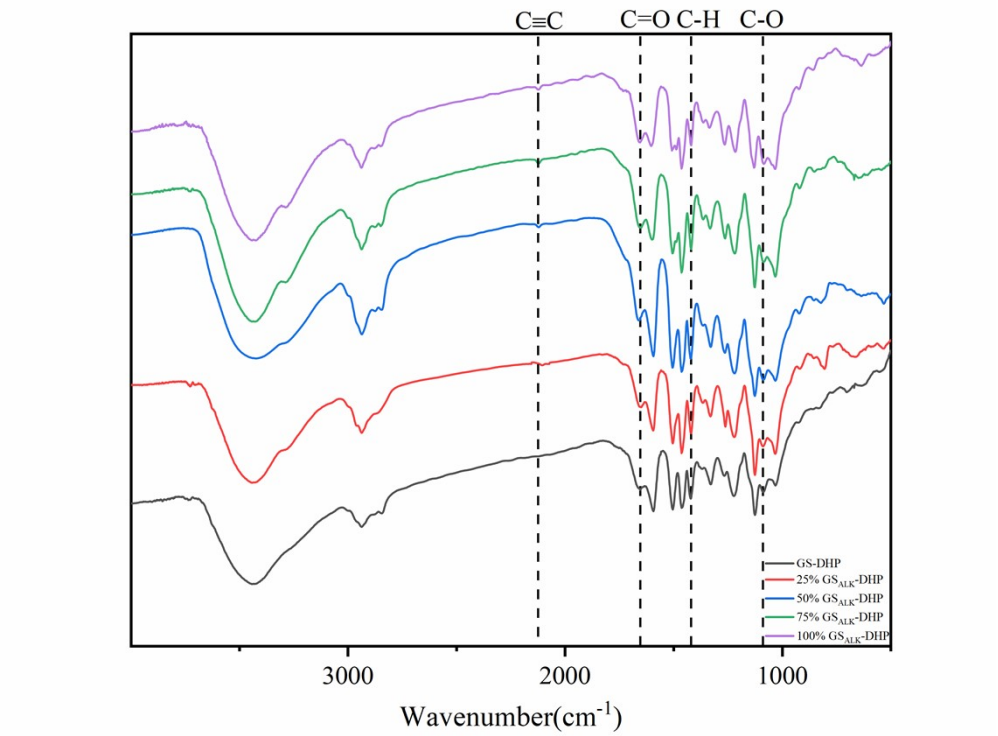

Fig. S9 Infrared spectra of Dehydrogenation Polymers (DHPs) with varying compositions.

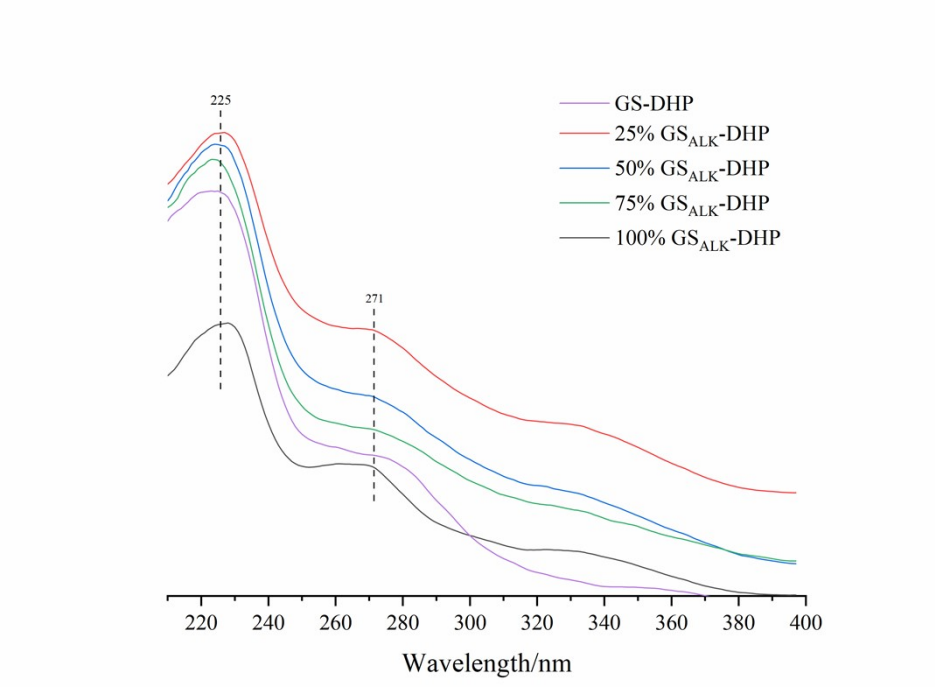

Fig. S10 Ultraviolet (UV) spectra of Dehydrogenation Polymers (DHPs) with different monomer ratios.

References

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