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SUPPORTING INFORMATION

Electronic Supporting Information

Biocatalytic CO_2 fixation initiates selective oxidative cracking of 1-naphthol under ambient conditions

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SUPPORTING INFORMATION

1. Experimental Procedures

1.1 Materials and methods

All analytical grade or higher quality chemicals and reagents were purchased from Macklin (Shanghai, China). HPLC solvents were purchased from JOHN LONG (Beijing, China). NADH was purchased from Solarbio (Tianjin, China). Ni Sepharose 6 Fast Flow resin was purchased from GE Healthcare (Boston, USA).

1.2 Construction of expression vector

The genes encoding the enzymes used in this study were synthesized and subsequently cloned into plasmid pRSFDuet1 by Genewiz (Beijing, China), including 2,3-dihydroxybenzoic acid decarboxylase from *Aspergillus oryzae* (2,3-DHBD_*Ao*)[1,2], 2,6-dihydroxybenzoic acid decarboxylase from *Rhizobium* sp. (2,6-DHBD_*Rs*)[3, 4], salicylic acid decarboxylase from *Trichosporon moniliifirme* (SAD_*Tm*)[5], 1-hydroxy-2-naphthoate dioxygenase from *Mycobacterium vanbaalenii* PYR-1 (1-HNDO_*Mv*)[6]. His-tag was appended in the C-terminal of the target gene for purification. *E. coli* BL21 Gold (DE3) was the host for the enzyme expression.

1.3 HPLC, NMR and ESI-MS analysis

HPLC analysis was performed to determine the amounts of 1-hydroxy-2-naphthoic acid (1-H2NA) and 2'-carboxybenzylzyl-pyruvic acid. The reaction samples (1 mL) were diluted by 1 mL acetonitrile and vigorous mixing. The supernatant from centrifugation (12,000 *g*, 10 min) was collected for HPLC analysis. HPLC (Thermo Dionex UltiMate 3000 equipped with DAD detector) analysis was carried out on an Ultimate XB-C18 column (4.6×250 mm, 5 µm, Welch Materials Inc., Shanghai, China) and eluted with an acetonitrile-sodium acetate (5 mM) gradient. The acetonitrile gradient was as follows: (1)analysis of 1-H2NA: 0 to 7 min, 5% (vol/vol), 0.6 mL/min (flow rate); 7 to 15 min, 5 to 80%, 0.8 mL/min; 15 to 22 min, 80%, 0.8 mL/min; 22 to 27 min, 80 to 5%, 0.6 mL/min; 27 to 30 min, 5%, 0.6 mL/min. (2) Analysis of 2'-carboxybenzylzyl-pyruvic acid: 0 to 7 min, 5%, 0.6 mL/min; 7 to 12 min, 5 to 45%, 0.8 mL/min; 12 to 24 min, 45 to 50%, 0.8 mL/min; 24 to 30 min, 50 to 5%, 0.6 mL/min. The absorbance of the eluent was monitored at 250 and 300 nm. ^{1}H-NMR, ^{13}C-NMR and ^{1}H-^{13}C Heteronuclear multiple bond coherence (HMBC) 2D-NMR spectra were recorded on a Bruker AVANCEIII spectrometer with a 5 mm BBO probe at 300 K. Mass spectra (ESI-MS) of 2'-carboxybenzylzyl-pyruvic acid was recorded on an Agilent 6230 TOF LC/MS.

1.4 Shake-flask expression and purification of 1-hydroxy-2-naphthoate dioxygenase and nonoxidative (de)carboxylases

Single clone was inoculated in LB liquid medium supplemented with Kanamycin (50 µg/mL) at 37°C. When optical density at 600 nm (OD_{600}) reached 0.7, gene expressions were induced by Isopropyl-b-D-thiogalactopyranoside (IPTG, 50 µM) at 20°C for 24 h. Subsequently, *E. coli* cells were harvested by centrifugation (4°C, 4,000 rpm, 15 min), suspended in lysis buffer (50mM NaH_2PO_4, 300 mM NaCl, and 10mM imidazole, pH8.0) and disrupted through high-pressure homogenization. The cell lysates were centrifugated (4°C, 12,000 rpm, 30 min) and then the supernatants (crude enzymes) were purified by Ni Sepharose 6 Fast Flow (GE Healthcare) containing column. Purified proteins in elution buffer were subsequently washed and concentrated by Amicon Ultra-4 10 kDa Centrifugal Filter Device (Merck Millipore, Schwalbach, Germany). The homogeneity of purified enzymes was detected by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The concentration of proteins was determined by BCA Protein Assay Kit (Sangon Biotech, Shanghai, China) with BSA as the standard.

1.5 Dioxygenase-catalyzed cracking of 1-hydroxy-2-naphthoic acid

For 1-HNDO_*Mv* -catalyzed cleavage of the substituted benzene ring in 1-hydroxy-2-naphthoic acid (1-H2NA), the 1 mL reaction mixture in 15 mL tube contained 1-H2NA (1-15 mM), N, N-dimethylformamide (DMF, 5%, v/v), K_2HPO_4–KH_2PO_4 buffer (pH 7.0, 100 mM) and purified 1-HNDO_*Mv* (0.5 mg/mL). The reaction mixture was incubated (220 rpm) at 30 °C. After 30 min incubation, the reaction was terminated by acetonitrile (MeCN, 1 mL) and vigorous mixing. The supernatant from centrifugation (12,000*g*, 10 min) was collected for HPLC analysis.

1.6 Kinetic characterization of three nonoxidative (de)carboxylases

For kinetic characterization, the final concentration of 2,3-DHBD_*Ao*, 2,6-DHBD_*Rs* and SAD_*Tm* was 1.41 µM, 35.31 µM and 5.41 µM, respectively. Carboxylases were exposed to different concentration of 1-naphthol (1.6, 3.2, 6.4, 12, 18 and 20 mM) and fixed concentration of $KHCO_3$ (200mM). The fluorescent properties of 1-H2NA, exiting and emission wavelength corresponding to 350 nm and 420 nm respectively, was used to determine the initial reaction rate of carboxylation. The initial-velocity data obtained was fitted to the equation $V= V_{max}[S]/([S]+K_m$. V is the initial velocity, V_{max} is the maximum velocity, [S] is the substrate concentration, and K_m is the Michaelis constant.

1.7 Nonoxidative (de)carboxylase-catalyzed carboxylation of 1-naphthol

For enzymatic carboxylation of 1-naphthol, the 1 mL reaction mixture in 15 mL tube contained 1-naphthol (15 mM), $KHCO_3$ (200 mM), DMF (4% v/v), K_2HPO_4-KH_2PO_4 buffer (pH7.0, 100 mM) and purified nonoxidative (de)carboxylases (2,3-DHBD_*Ao*, 2,6-DHBD_*Rs*, SAD_*Tm*, 0.75 mg/mL). The reaction mixture was incubated (220 rpm) at 30°C. After 12 h incubation, the reaction was terminated by MeCN **(**1 mL) and vigorous mixing. The supernatant from centrifugation (12,000*g*, 10 min) was collected for HPLC analysis.

1.8 Conversion of 1-naphthol to 2'-carboxybenzylzyl-pyruvic acid by coupling nonoxidative (de)carboxylase with dioxygenase

Three nonoxidative (de)carboxylases (2,3-DHBD_*Ao*, 2,6-DHBD_*Rs* and SAD_*Tm*) were investigated to be coupled with 1-hydroxy-2-naphthoate dioxygenase (1-HNDO_*Mv*) for the conversion of 1-naphthol to 2'-carboxybenzylzyl-pyruvic acid. The 1 mL reaction mixture in 15 mL tube contained 1-naphthol (15 mM), $KHCO_3$ (200 mM), DMF (4% v/v), K_2HPO_4-KH_2PO_4 buffer (pH7.0, 100 mM), purified carboxylases (0.75 mg/mL) and 1-HNDO_*Mv* (0.5 mg/mL). The reaction mixture was incubated (220 rpm) at 30°C. After 12 h incubation, the reaction was terminated by MeCN **(**1 mL) and vigorous mixing. The supernatant from centrifugation (12,000*g*, 10 min) was collected for HPLC analysis.

1.9 Effects of bicarbonate concentration on the conversion of 1-naphthol to 2'-carboxybenzylzyl-pyruvic acid

To determine the effect of the bicarbonate concentration on conversion rate, the cascade reaction to convert 1-naphthol to 2'-carboxybenzylzyl-pyruvic acid was conducted using various concentrations of bicarbonate. For this, the 1 mL reaction mixture in 15 mL tube contained 1-naphthol (15 mM), DMF (4% v/v), purified 2,3-DHBD_*Ao* (0.75 mg/mL), purified 1-HNDO_*Mv* (0.5/mL mg), K_2HPO_4-KH_2PO_4 buffer (pH7.0, 100 mM), 45-150 mM $KHCO_3$, 30-100 mM NaH_2PO_4 (150 mM HCO_3^- and 100 mM $H_2PO_4^-$, 150 mM HCO_3^- and 60 mM $H_2PO_4^-$ 150 mM HCO_3^- and 30 mM $H_2PO_4^-$, 90 mM HCO_3^- and 100 mM $H_2PO_4^-$, 90 mM HCO_3^- and 60 mM $H_2PO_4^-$, 90 mM HCO_3^- and 30 mM $H_2PO_4^-$, 45 mM HCO_3^- and 30 mM $H_2PO_4^-$, 45 mM HCO_3^- and 60 mM $H_2PO_4^-$, 45 mM HCO_3^- and 100 mM $H_2PO_4^-$). The reaction mixture was incubated in a sealed tube (220 rpm) at 30°C. After 12 h incubation, the reaction was terminated by MeCN (1 mL) and vigorous mixing. The supernatant from centrifugation (12,000 *g*, 10 min) was collected for HPLC analysis.

1.10 Atmospheric CO_2 fixation initiated one-pot cascade conversion of 1-naphthol to 2'-carboxybenzylzyl-pyruvic acid

For one-pot enzymatic conversion of 1-naphthol to 2'-carboxybenzylzyl-pyruvic acid, a 20 mL-reaction in 50 mL tube was conducted with 1-naphthol (15 mM), DMF (4% v/v), K_2HPO_4-KH_2PO_4 buffer (pH 7.5, 100 mM), purified 2,3-DHBD_*Ao* (0.75 mg/mL) and 1-HNDO_*Mv* (0.5 mg/mL). Atmospheric CO_2 was slowly bubbled into the reaction mixture incubated at 30°C. After 12 h incubation, the reaction terminated by MeCN (20 mL) with vigorous mixing. The supernatant from centrifugation (12,000 *g*, 10 min) was collected for HPLC analysis.

1.11 Enzymatic synthesis, purification and characterization of 2'-carboxybenzylzyl-pyruvic acid

The 100 mL reaction mixture in 250 mL flask contained 1-naphthol (15 mM), $KHCO_3$ (100 mM), DMF (4% v/v), K_2HPO_4-KH_2PO_4 buffer (pH7.0, 100 mM), purified 2,3-DHBD_*Ao* (0.75 mg/mL) and 1-HNDO_*Mv* (0.5 mg/mL). The reaction mixture was incubated (220 rpm) at 30°C until full conversion of 1-naphthol was confirmed by thin-layer chromatography (TLC, silica gel 60 F254 plates, visualized by UV light irradiation, 254 nm). After 20 min incubation at 80°C to inactivate enzymes, the supernatant in reaction mixture was collected from centrifugation (12,000 *g,* 10 min), lyophilized and dissolved in the minimal volume of methanol. The target product, 2'-carboxybenzylzyl-pyruvate in methanol solution was precipitated by adding excess ethyl acetate. The precipitated 2'-carboxybenzylzyl-pyruvate was collected by centrifugation, washed by ethyl acetate and then dried at room temperature for the following characterization by ESI-MS (Fig. S1), ^{1}H-NMR (Fig. S2-3), ^{13}C-NMR (Fig. S4-5) and ^{1}H-^{13}C HMBC 2D-NMR (Fig. S6).

1.12 Activity measurement of 2,3-DHBD_*Ao* and 1-HNDO_*Mv* at different pH values

To investigate the catalytic activity of 2,3-DHBD_*Ao*, 0.5 mg/ml 2,3-DHBD_*Ao* was mixed with reaction buffer (100 mM KPi buffer, pH 6.5, 7.0 and 7.5) containing 10 mM 1-naphthol 100 mM of $KHCO_3$. The target product 1-H2NA was quantified (Ex=350 nm, Em= 420 nm) to evaluate the activity of 2,3-DHBD_*Ao*.

To investigate the catalytic activity of 1-HNDO_*Mv*, 0.1 mg/ml 1-HNDO was mixed with reaction buffer (100 mM KPi buffer, pH 6.5, 7.0 and 7.5) containing 0.2 mM 1-H2NA. Along with cleavage of 1-H2NA, the fluorescence reduction of 1-H2NA was measured (Ex=350 nm, Em= 420 nm) to evaluate the activity of 1-HNDO_*Mv*.

2. Results and Discussion (Supporting)

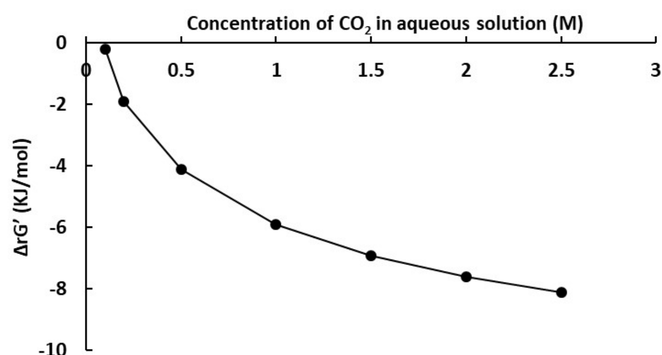


Fig. S1 Calculated Δ_rG' values with different concentration of CO_2 in aqueous solution with eQuilibrator (10 mM 1-naphthol, 0.1~2.5 M CO_2 (aq), 40 μM 1-hydroxy-2-naphthoate, pH=7.5, pMg=3, ionic strength=0. 25 M)

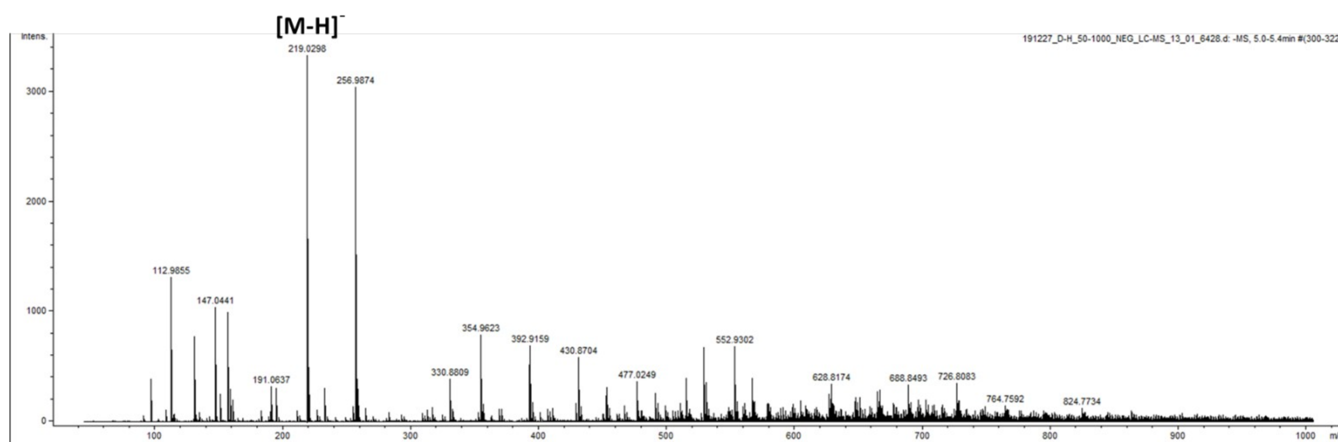


Fig. S2 The ESI-MS spectrum of 2'-carboxybenzylzyl-pyruvic acid ([M-H]$^-$=219).

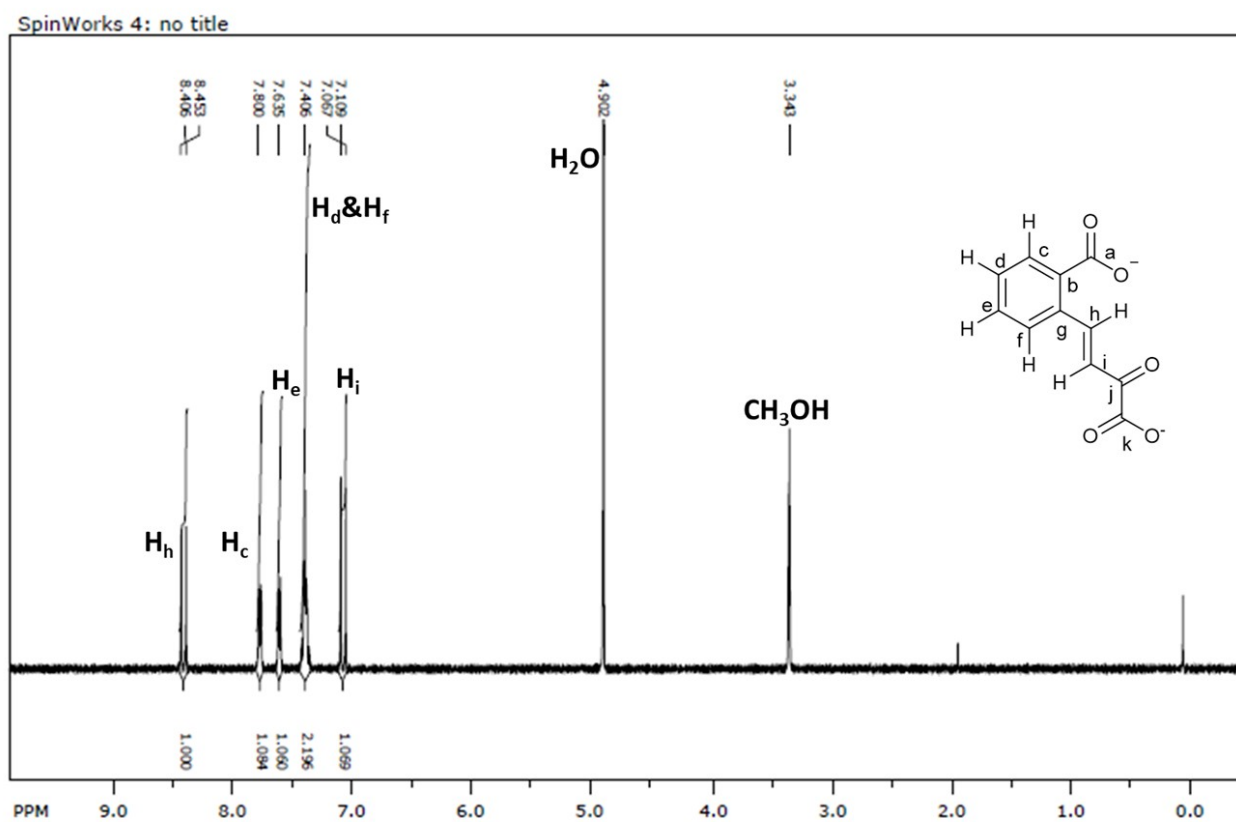


Figure S3 ^{1}H-NMR of 2'-carboxybenzylzyl-pyruvate (400 MHz, CD_3OD).

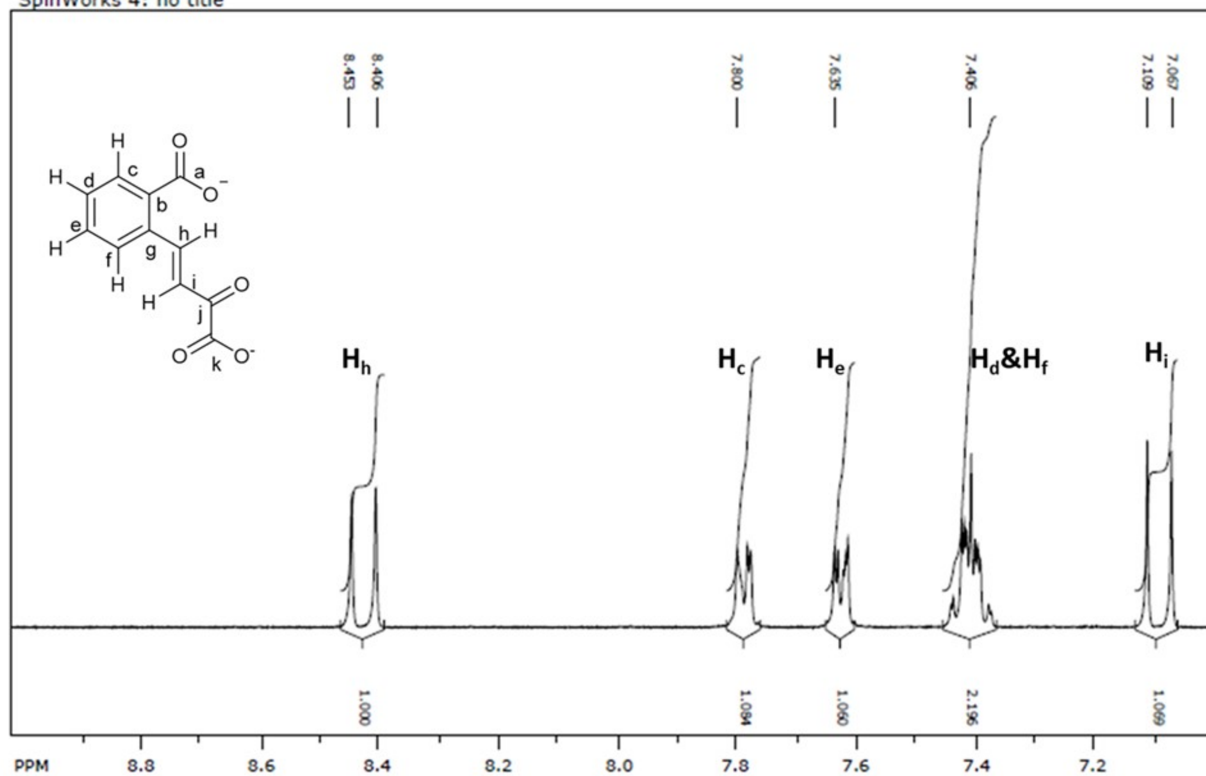


Fig. S4 ^{1}H-NMR of 2’-carboxybenzylzyl-pyruvate (zoom 7.0–9.0 ppm).

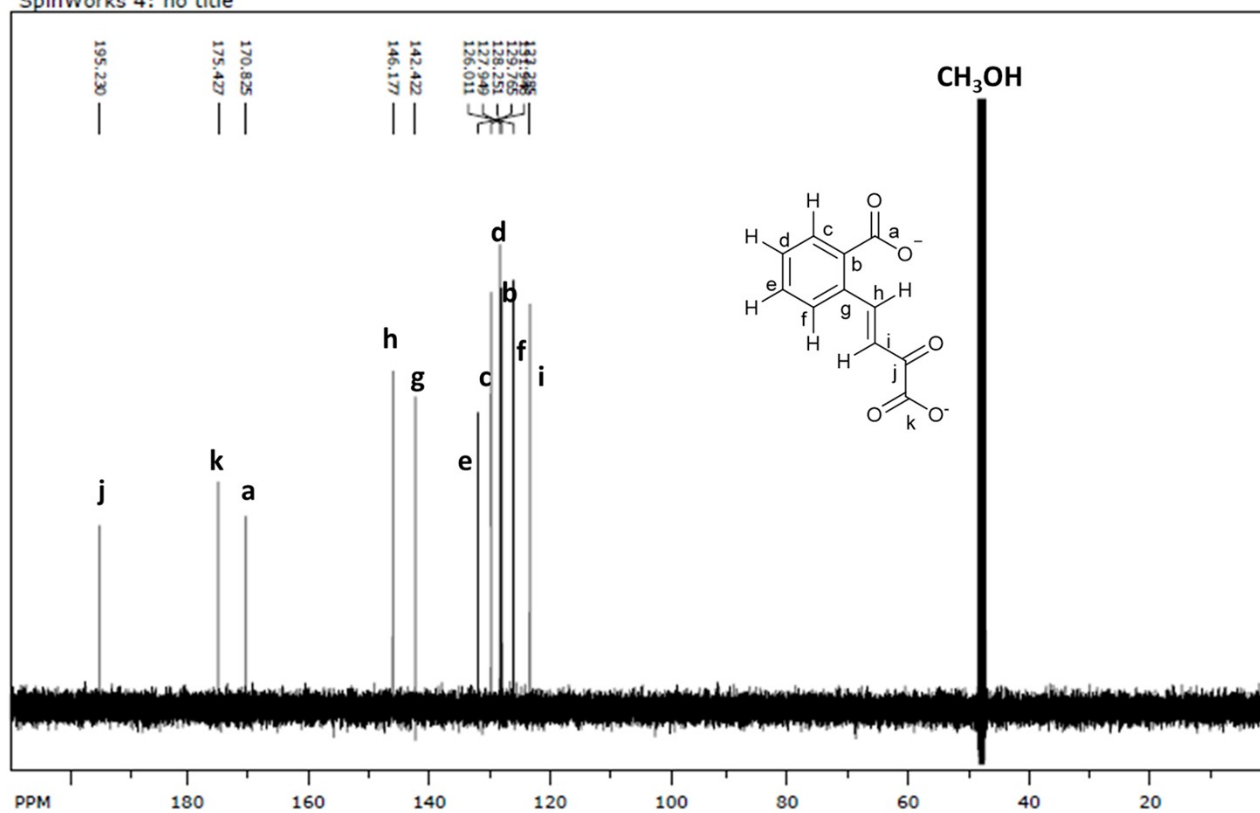


Fig. S5 ^{13}C-NMR of 2’-carboxybenzylzyl-pyruvate (100 MHz, CD_3OD).

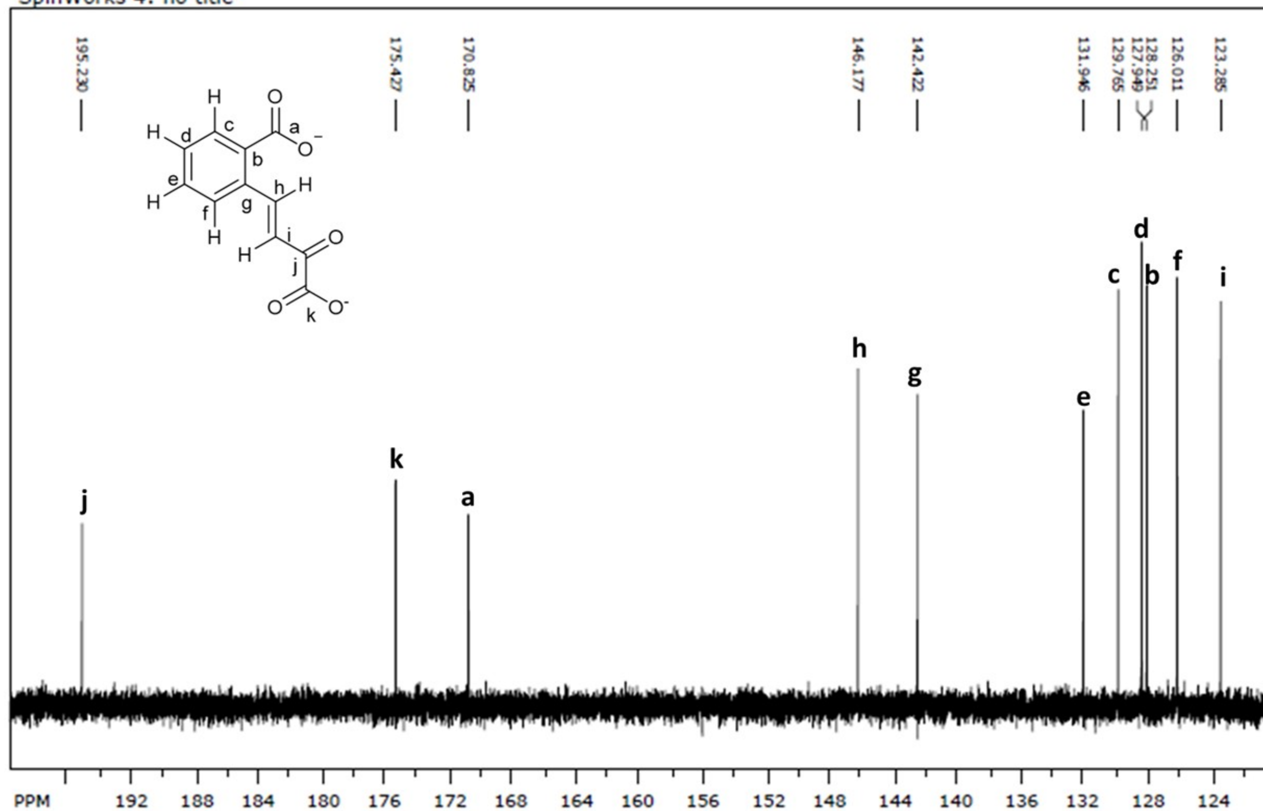


Fig. S6 ^{13}C-NMR of 2'-carboxybenzylzyl-pyruvate (zoom 120–200 ppm).

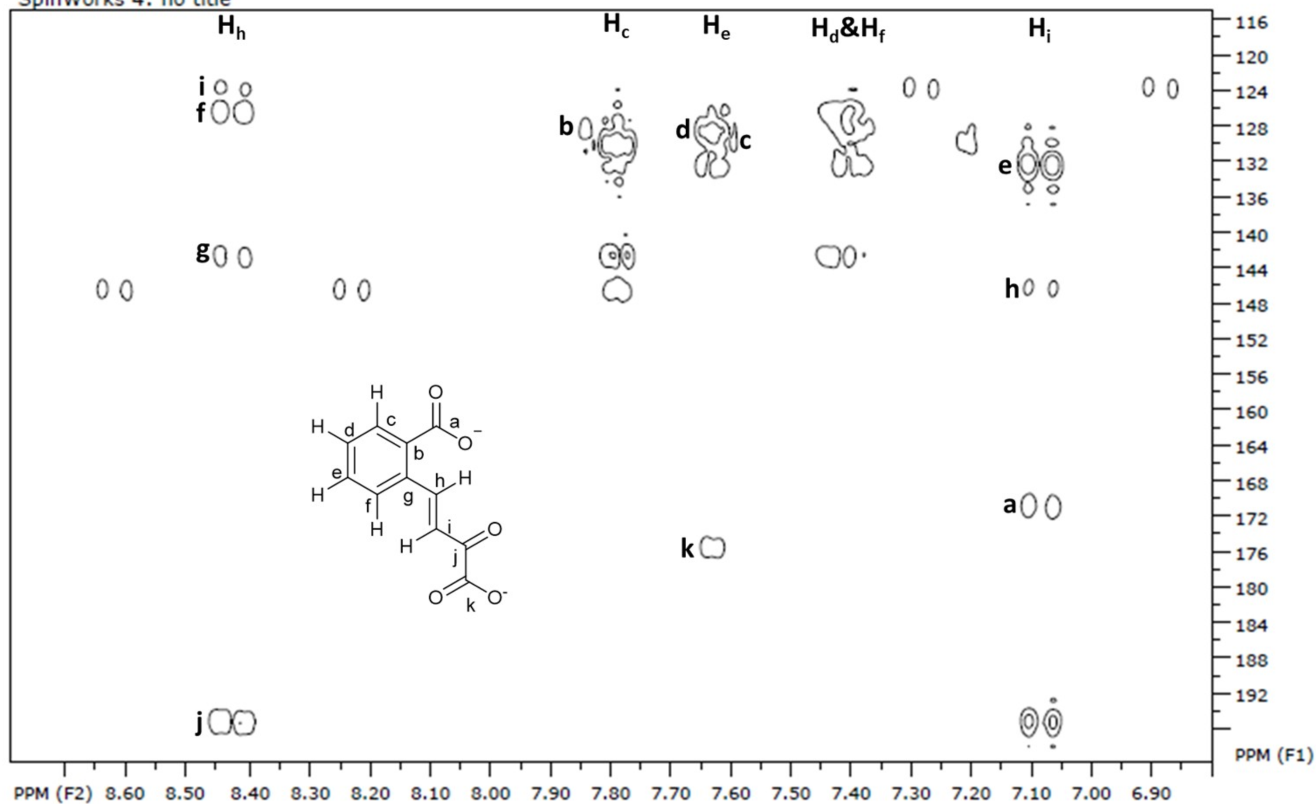


Fig. S7 ^{1}H-^{13}C HMBC of 2'-carboxybenzylzyl-pyruvate (400 MHz, CD_3OD).

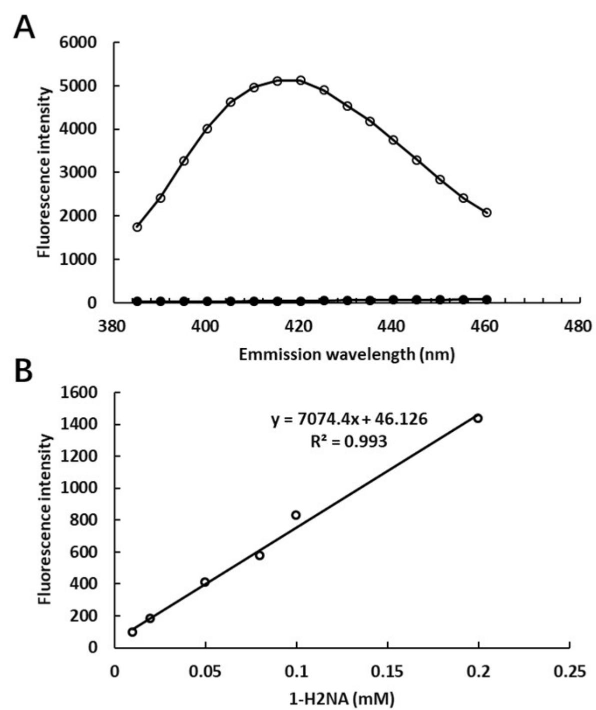


Fig. S8 A. The fluorescence spectrum of 1-naphthol (●) and 1-H2NA (○). B. Standard curve of 1-H2NA. E_x=350 nm and E_m=420 nm.

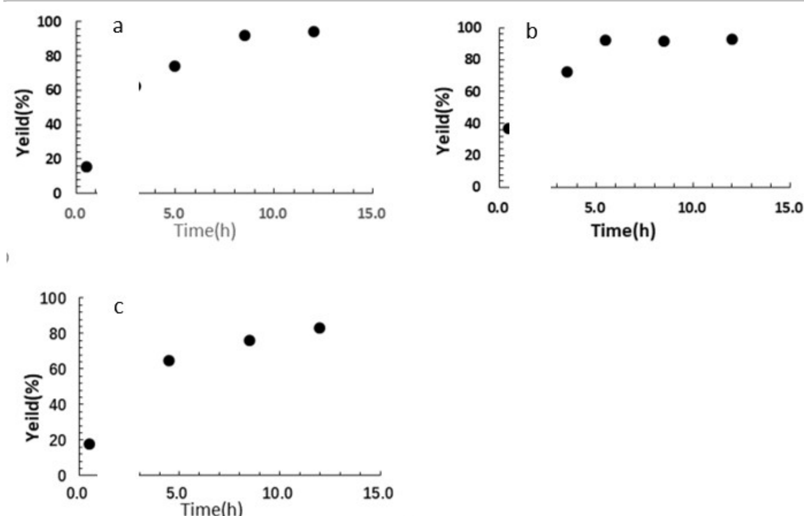


Fig. S9 Time course for the conversion of 1-naphthol to 2'-carboxybenzylzyl-pyruvic acid performed with different concentration of $KHCO_3$ and NaH_2PO_4 at 30°C for 12 h. (a): 90 mM HCO_3^- and 30 mM $H_2PO_4^-$, (b): 150 mM HCO_3^- and 30 mM $H_2PO_4^-$, (c): 45 mM HCO_3^- and 30 mM $H_2PO_4^-$.

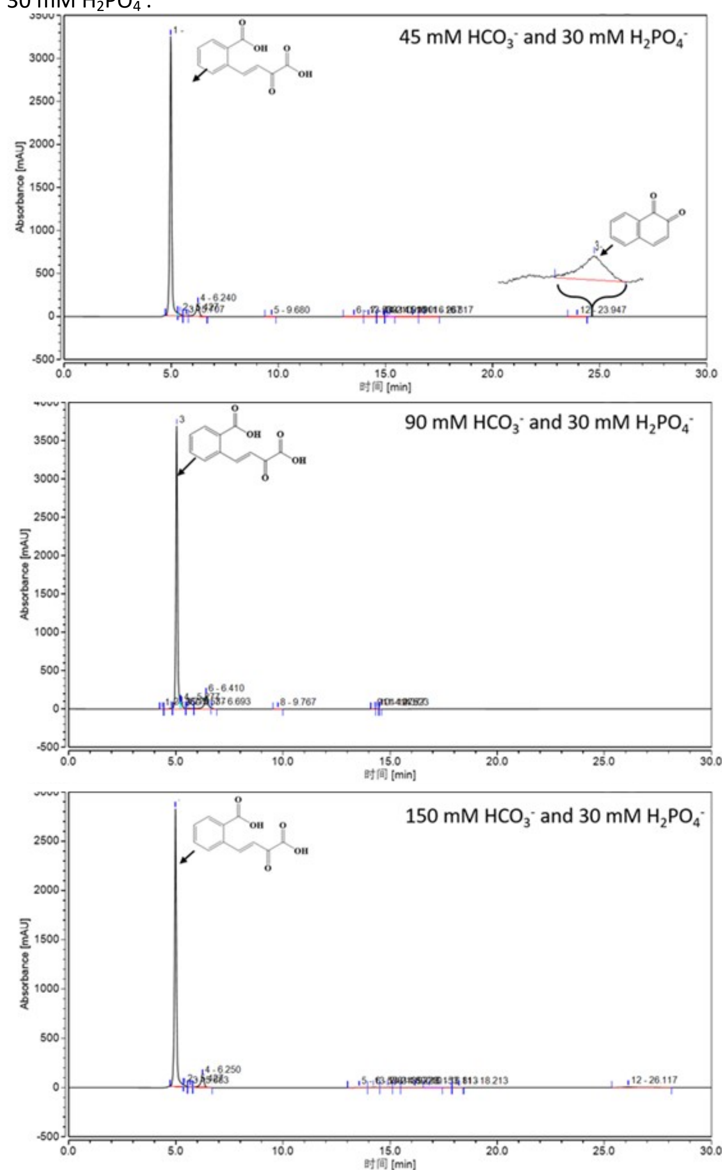


Fig. S10 HPLC analysis of the products of the coupled carboxylation-oxygenation reactions. Conversion of 1-naphthol to 2′-carboxybenzylzyl-pyruvic acid was performed with different concentration of $KHCO_3$ and NaH_2PO_4 at 30°C for 12 h.

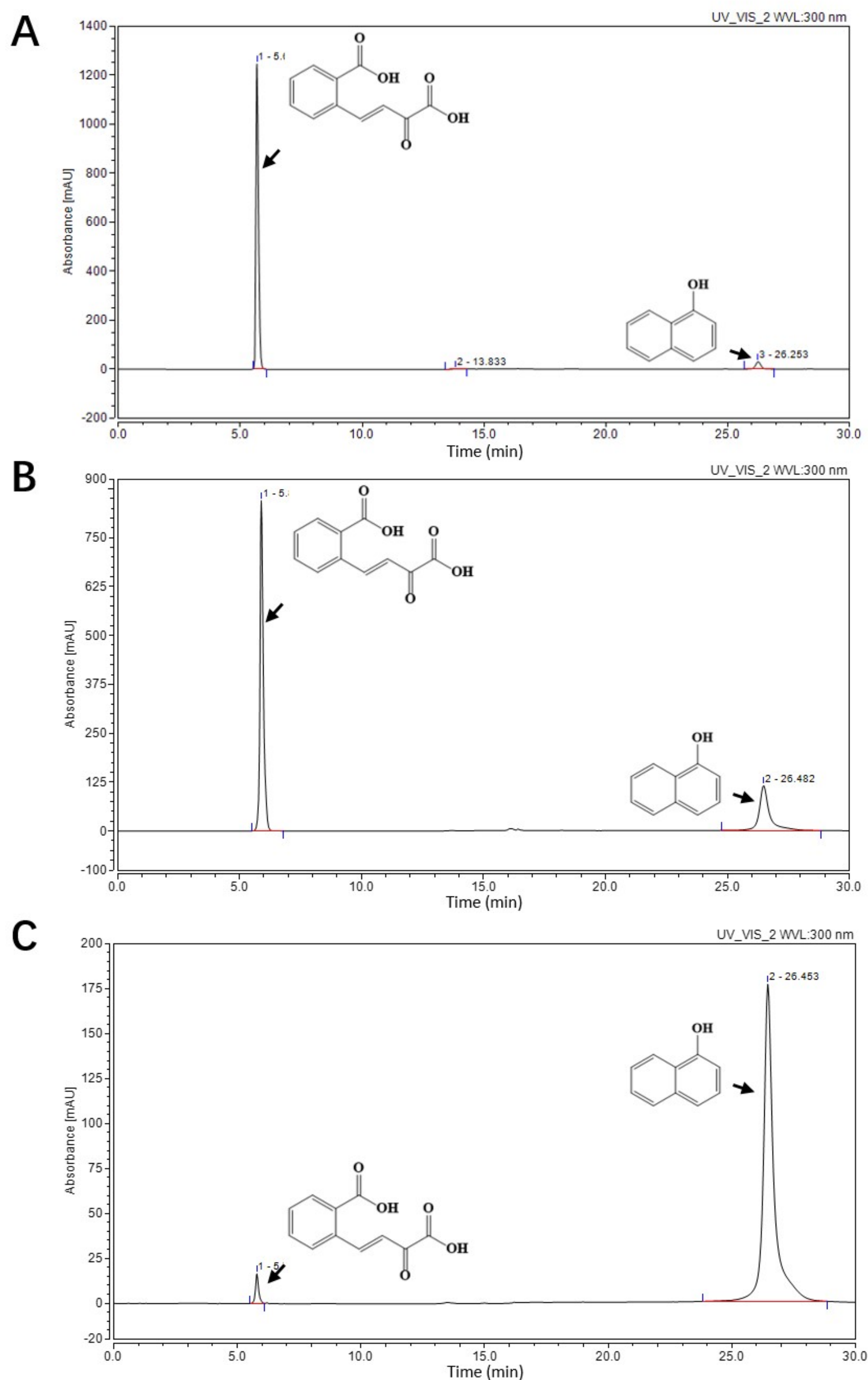


Fig. S11 HPLC analysis of conversion of 1-naphthol in presence of CO_2 with bubbling (A), CO_2 without bubbling (B)and air (C) without bubbling.

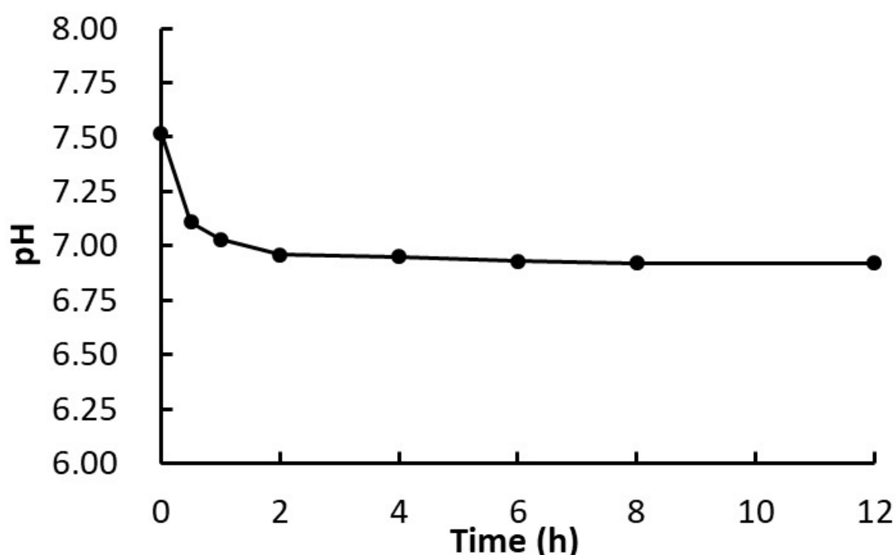


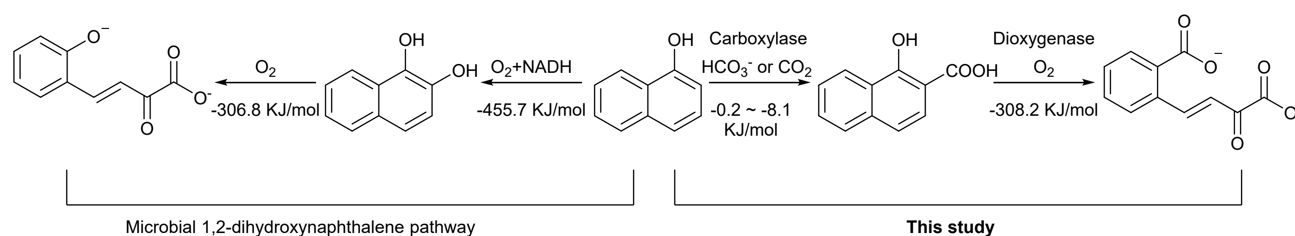
Fig. S12 The pH changed of the reaction solution One-pot conversion of 1-naphthol to 2′-carboxybenzyl-pyruvic acid with atmospheric CO_2 at 30°C.

Table S1 The activity of 2,3-DHBD_*Ao* at varied pH values

pH value	pH6.5	pH7.0	pH7.5
Reaction rate（mM/min）	0.0842	0.0818	0.0544

Table S2 The activity of 1-HNDO_*Mv* at varied pH values

pH value	pH6.5	pH7.0	pH7.5
Reaction rate（mM/min）	0.1127	0.0809	0.0795



Scheme S1 Comparison of the thermodynamics of 1-hydroxy-2-naphthoic acid route (this study) and the reported microbial 1,2-dihydroxynaphthalene pathway. The $\Delta_r G'$ value (reaction Gibbs energy) was calculated by using eQuilibrator (10 mM substrate, 100 mM CO_2 (O_2), 40 µM product, 1 mM NADH only for enzymatic hydroxylation, pH=7.5, pMg=3, ionic strength=0. 25 M)

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