

# Supporting Information

## An in-depth Mechanistic Study and Process Understanding of p-hydroxyphenylglycine Synthesis with *in-situ* ATR-IR

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### 1 Experimental sections

#### ESI 1: Exploration of reaction sequence

##### Step 1: Reaction of phenol with glyoxylic acid

44.8 g distilled water and 29.6 g glyoxylic acid were added into a 250 mL four-mouth flask, then 18.8 g phenol was added into the reaction system. Raised the temperature to 28 °C and stirred for 22 h.

##### Step 2: Reaction of phenol with sulfamic acid

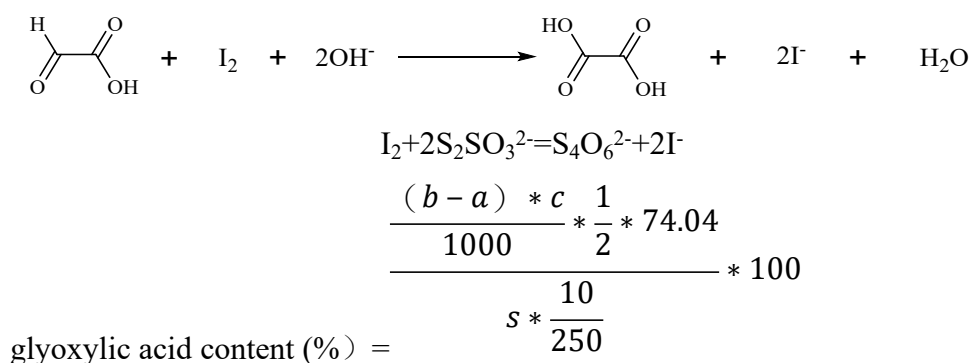
44.8 g distilled water and 21.9 g sulfamic acid were added into a 250 mL four-mouth flask, then 18.8 g phenol was added into the reaction system. Raised the temperature to 28 °C and stirred for 22 h.

##### Step 3: Reaction of sulfamic acid with glyoxylic acid

44.8 g distilled water and 29.6 g glyoxylic acid were added into a 250 mL four-mouth flask, then 21.9 g sulfamic acid was added into the reaction system. Raised the temperature to 28 °C and stirred for 22 h.

#### ESI 2: Redox titration of glyoxylic acid

Scheme S1. Principle of REDOX titration



Where:  $a$  is the amount of  $\text{Na}_2\text{S}_2\text{O}_3$  solution required by the reactant, mL;  $b$  is the amount of  $\text{Na}_2\text{S}_2\text{O}_3$  solution required for blank experiment, mL;  $c$  is the molar concentration of  $\text{Na}_2\text{S}_2\text{O}_3$  solution, mol/L;  $s$  is amount of reaction product, g.

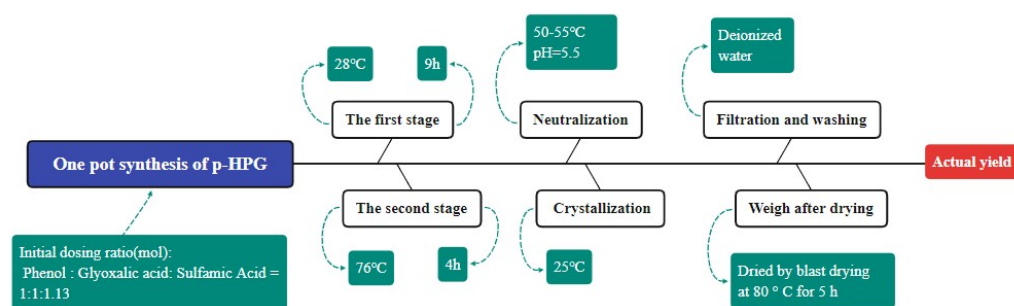
### ESI 3: Synthesis process of p-HPG

#### Step 1: The first stage (lower temperature stage)

44.8 g distilled water and 29.6 g glyoxylic acid were added into a 250 mL four-mouth flask, then 21.9 g sulfamic acid and 18.8 g phenol were added into the reaction system. Raised the temperature to 28 °C and stirred for 22 h.

#### Step 2: The second stage (higher temperature stage)

After the first step, raised the temperature to 76 °C and reacted for another 6 h.



$$\text{Yield} = \frac{p - \text{HPG mass after drying/g} \times \text{purity}}{\text{Theoretically deserved } p - \text{HPG quality/g}} \times 100\%$$

### ESI 4: $\text{BaSO}_4$ precipitation experiments

#### 1: $\text{BaSO}_4$ Precipitation produced in sulfamic acid-glyoxylic acid system along with time (corresponding to Fig. 2)

44.8 g distilled water and 29.6 g glyoxylic acid were added into a 250 mL four-mouth flask, then 21.9 g sulfamic acid was added into the reaction system. Raised the temperature to 28 °C and stirred for 22 h. Took samples every 2 h, added excess  $\text{BaCl}_2$  solution to the samples, and weighted the amount of  $\text{BaSO}_4$  precipitation produced. Pure sulfamic acid and glyoxylic acid were used as blank.

#### 2: Influence of temperature on $\text{BaSO}_4$ precipitation in different reaction solution system (corresponding to Fig. 5)

44.8 g distilled water and 29.6 g glyoxylic acid were added into a 250 mL four-mouth flask, then 21.9 g sulfamic acid was added into the reaction system. Heated up the mixture to different temperature to let each mixture react for 2 h. Then  $\text{BaCl}_2$  solution was added to each sample, and the amount of  $\text{BaSO}_4$  precipitation in the samples was weighted. Pure sulfamic acid and glyoxylic acid were used as blank.

### 3: BaSO<sub>4</sub> precipitation produced during the synthetic process of p-HPG with temperature stepped mode (corresponding to Fig. S6)

44.8 g distilled water and 29.6 g glyoxylic acid were added into a 250 mL four-mouth flask, then 21.9 g sulfamic acid and 18.8 g phenol were added into the reaction system. Raised the temperature to 28 °C and stirred the reaction liquid for 22 h. After the first step, raised the temperature to 76 °C and continued the reaction for 6 h. During the whole synthetic process, took samples every 2 h, added excess BaCl<sub>2</sub> solution, and weighted the amount of BaSO<sub>4</sub> precipitation in the samples. Pure sulfamic acid and glyoxylic acid were used as blank.

### ESI 5: Impurity verification experiment (corresponding to Fig. S8)

22.4 g distilled water and 14.8 g glyoxylic acid were added into a 100 mL three-mouth flask, then 9.4 g phenol was added into the reaction system. Raised the temperature to 76 °C and stirred the reaction liquid for 5 h.

### ESI 6: Experimental conditions of HPLC analysis

All the experimental conditions for HPLC tests were the same during the whole work.

Chromatographic column: C18 column (4.6 mm×250 mm, 5 μm)

Column temperature: 25 °C

Detection wavelength: 254 nm

Injection volume: 10 μL

Mobile phase:

A---Acetonitrile (HPLC);

B---Methanol (HPLC);

C---Water (Containing 0.1% phosphoric acid);

Gradient elution method was adopted (see the table below):

Time/min	Flow/(mL/min)	A/%	B%	C%
0.000	0.500	0.0	10.0	90.0
5.000	0.500	0.0	10.0	90.0
10.000	0.500	0.0	0.0	100.0
10.000	1.000	30.0	0.0	70.0
15.000	1.000	50.0	0.0	50.0
20.000	1.000	0.0	10.0	90.0
30.000	1.000	0.0	10.0	90.0

## 2 Supporting Figures

**Fig. S1** HPLC sample diagram of holding phenol and glyoxylic acid mixture at 28 °C

for 22 h.

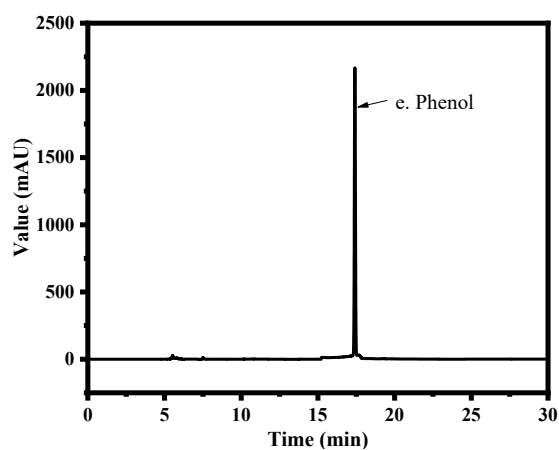


Fig. S1. HPLC sample diagram after holding phenol and glyoxylic acid mixture at 28 °C for 22 h.

**Fig. S2** HPLC sample diagram of holding phenol and sulfamic acid mixture at 28 °C for 22 h.

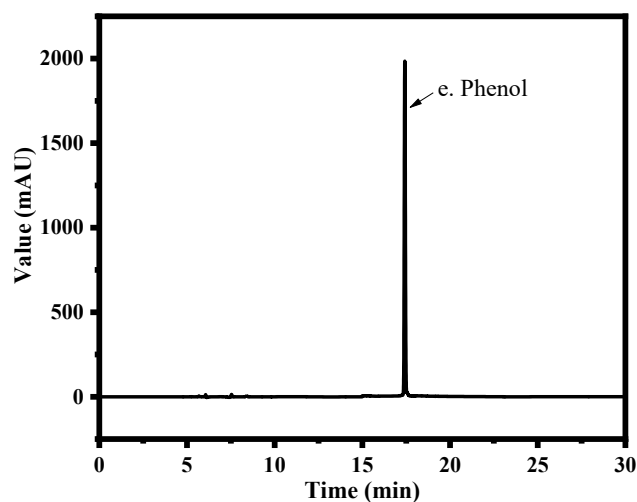


Fig. S2. HPLC sample diagram of holding phenol and sulfamic acid mixture at 28 °C for 22 h.

**Fig. S3** Infrared spectrum and HPLC diagram of the standards.

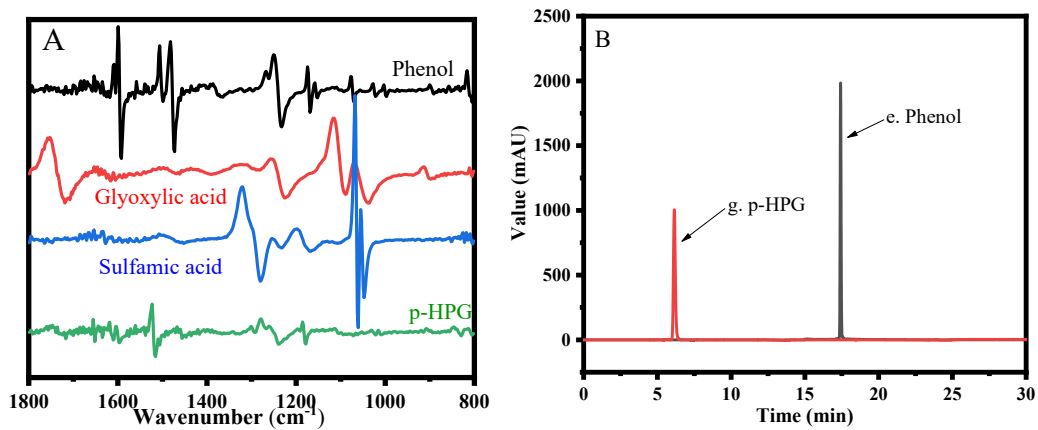


Fig. S3. (A) First derivative FT-IR spectra for standards; (B) HPLC plot.

**Fig. S4** Monitoring the reaction process of sulfamic acid-glyoxylic acid-phenol from low temperature to high temperature by HPLC.

The mixture of sulfamic acid, glyoxylic acid and phenol was reacted at 28 °C for 22 h and then was heated to 76 °C for another 6-hour reaction. HPLC results showed that a large amount of new intermediate (7.39 min, referred to **f**) was generated after reaction at 28 °C, and it was then transformed into p-HPG (6.12 min) when the temperature was raised to 76 °C.

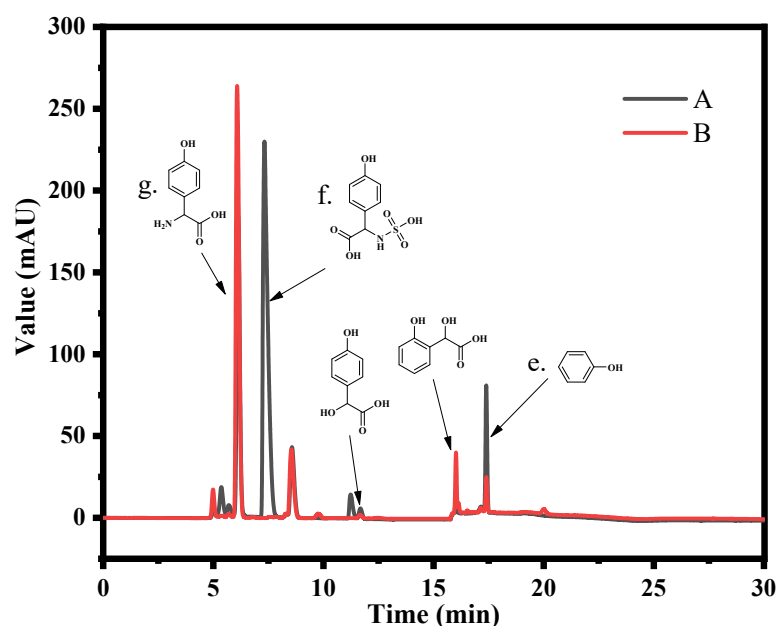
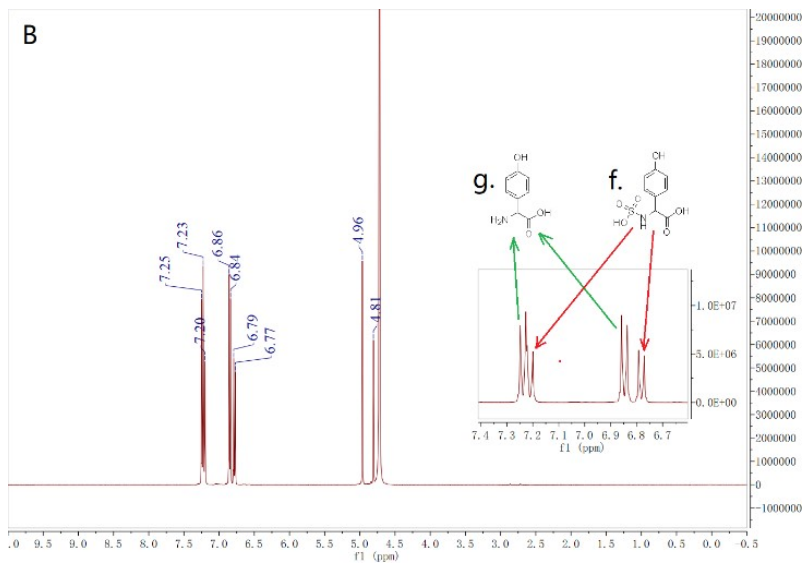
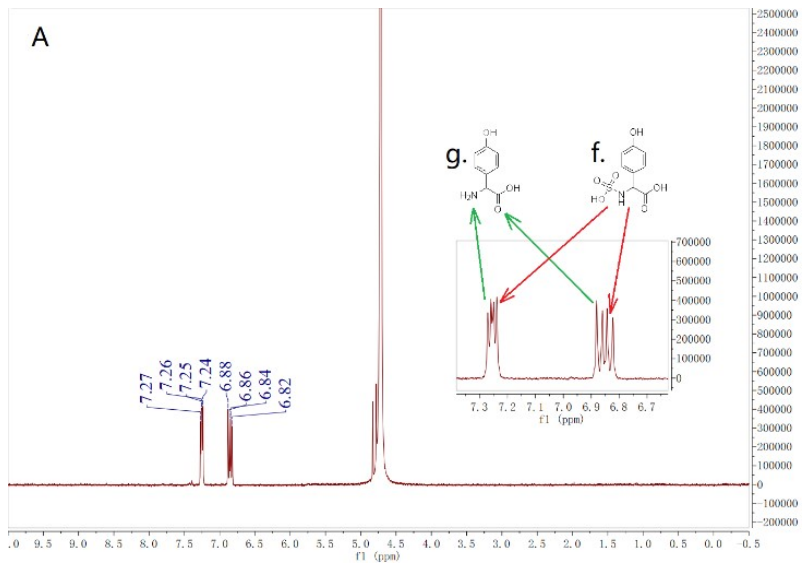


Fig. S4. HPLC sample map of sulfamic acid-glyoxylic acid-phenol reaction liquid at low temperature (black line, 28 °C) and then at high temperature (red line, 76 °C).

**Fig. S5** <sup>1</sup>HNMR characterization of the conversion of **f** to **g**

The reaction solution (including 68 % **f** and 10 % p-HPG) after the first stage (reaction at 28 °C) was separated by preparative liquid chromatography to obtain the enrichment solution containing only **f** and p-HPG. In order to avoid bringing in more impurities, the reaction liquid was not quenched. The column temperature of preparative liquid chromatography was 25 °C. The enrichment solution was freeze-dried then. The freeze-dried enrichment solution contained 55 % **f** and 45 % p-HPG (part of **f** converted to p-HPG during separation process). The freeze-dried concentrate was dissolved in heavy water and treated in batches. Among which, part A was directly characterized by <sup>1</sup>HNMR; Part B was heated to 76 °C for 2 h, and then cooled for <sup>1</sup>HNMR characterization; And meanwhile, an appropriate amount of pure p-HPG was added to

part A for nuclear magnetic characterization (labelled as C); Finally, the p-HPG standard was also characterized by  $^1\text{H}$ NMR (labelled as D).



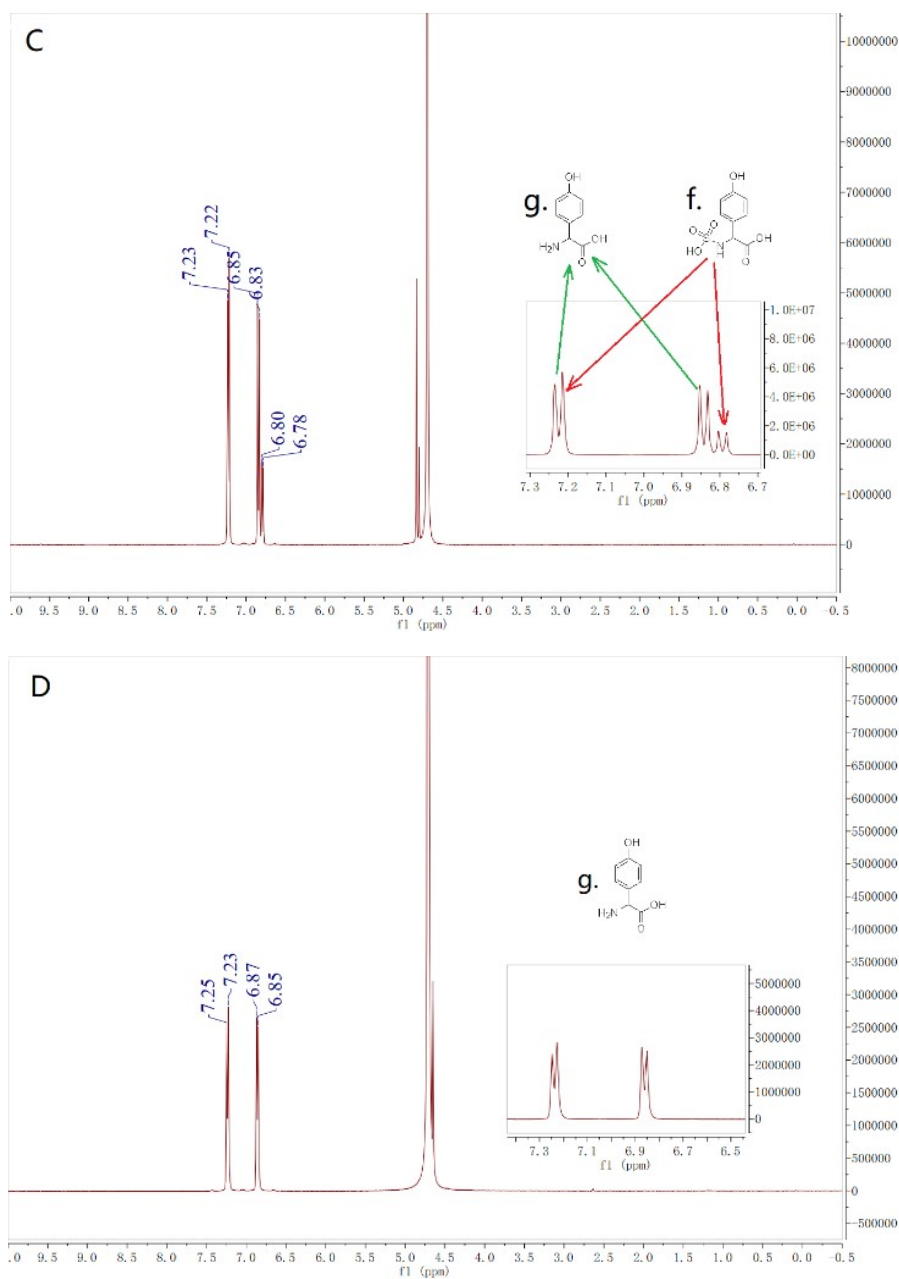


Fig. S5. <sup>1</sup>H NMR of enrichment solution treatment, (A) sample after lyophilization; (B) sample after high temperature treatment of A; (C) sample in which appropriate amount of pure p-HPG was added into A; (D) p-HPG standard.

**Fig. S6** Precipitation experiments

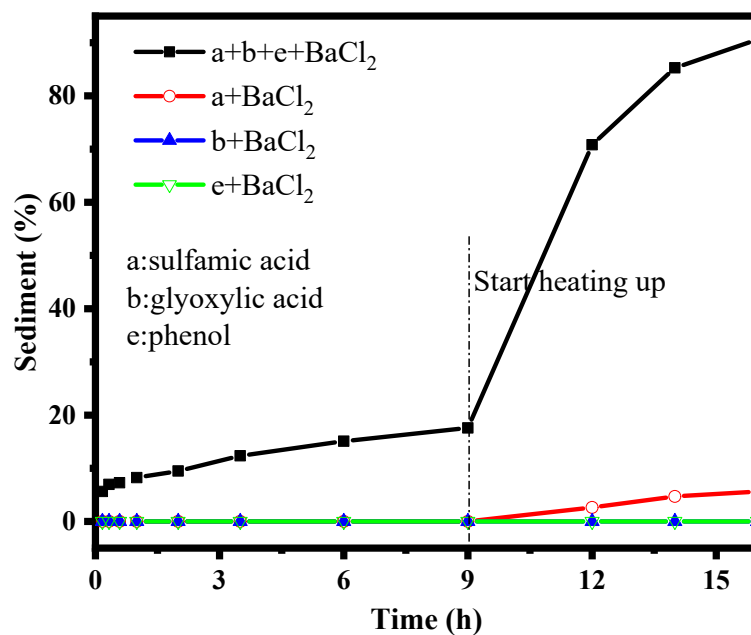


Fig. S6. Variation of precipitation amount with time during the reaction of sulfamic acid (a) - glyoxylic acid (b) - phenol (e) from low temperature to high temperature.

For sulfamic acid-glyoxylic acid-phenol system, the amount of BaSO<sub>4</sub> precipitation increased sharply with the increase of temperature, indicating that higher temperature favored the dissociation of sulfonate of f. In other words, higher temperature would accelerate the conversion of intermediate f to p-HPG.

**Fig.S7** HPLC monitoring of the reaction process of sulfamic acid-glyoxylic acid at 76 °C (reacting the mixture for 2 h then adding phenol).

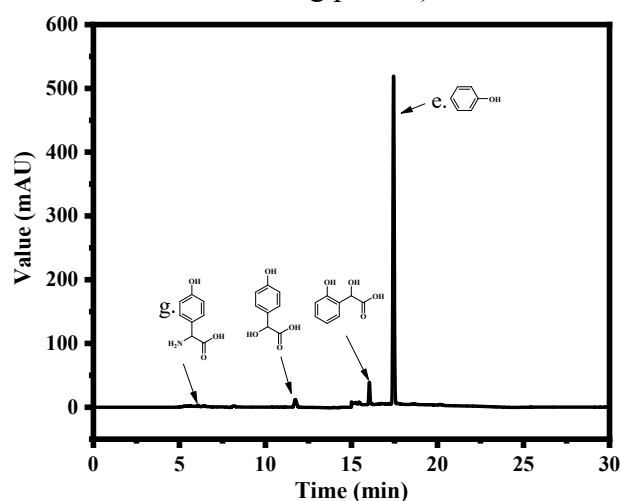


Fig.S7. HPLC monitoring of the reaction process of sulfamic acid-glyoxylic acid at 76 °C (reacting the mixture for 2 h then adding phenol).



**Fig. S8** HPLC monitoring of the reaction process of glyoxylic acid-phenol at 76 °C for 5 h.

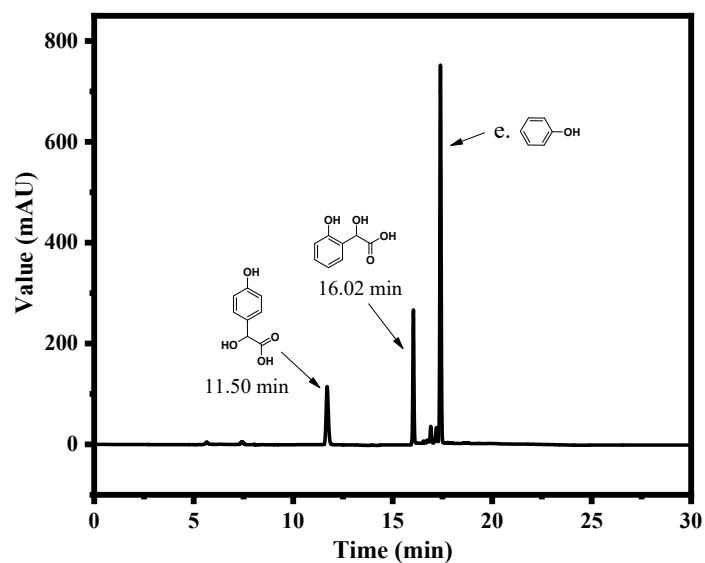


Fig. S8. HPLC monitoring of the reaction process of glyoxylic acid-phenol at 76 °C for 5 h.

**Fig. S9** The influence of temperature of the first stage on the product yield.

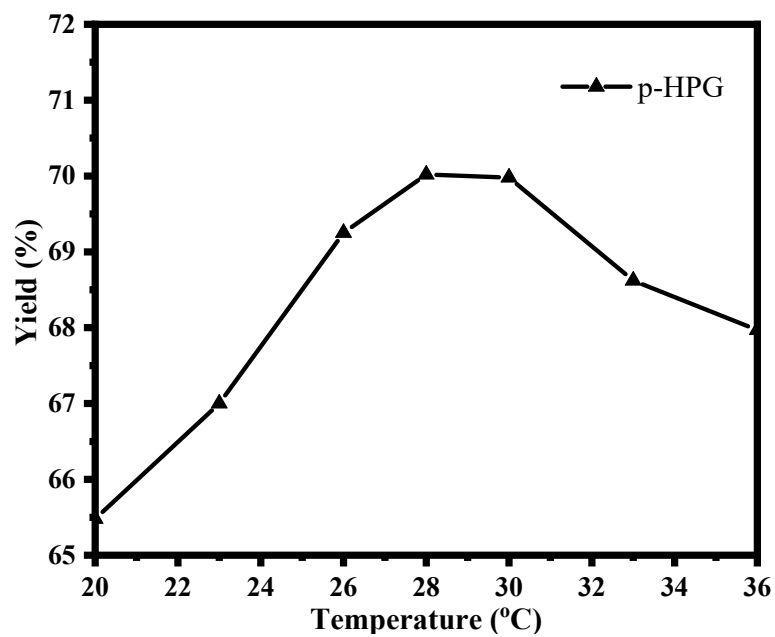


Fig. S9. The influence of temperature of the first stage on the product yield.

**Fig. S10** The influence of temperature of the second stage on the product yield.

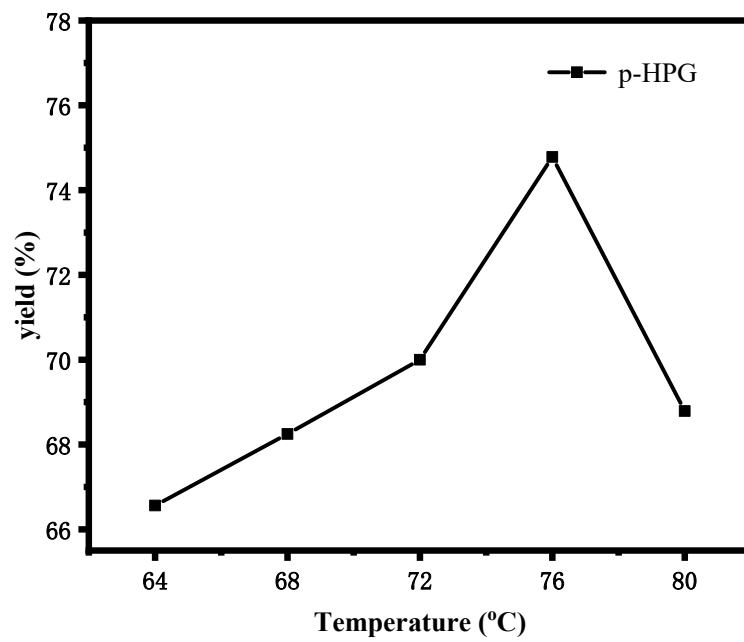


Fig. S10. The influence of temperature of the second stage on the product yield.