Supplementary Information

Phosphobisaromatic motifs enable rapid enzymatic self-assembly and hydrogelation of short peptides

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Experiment materials and instruments

Materials

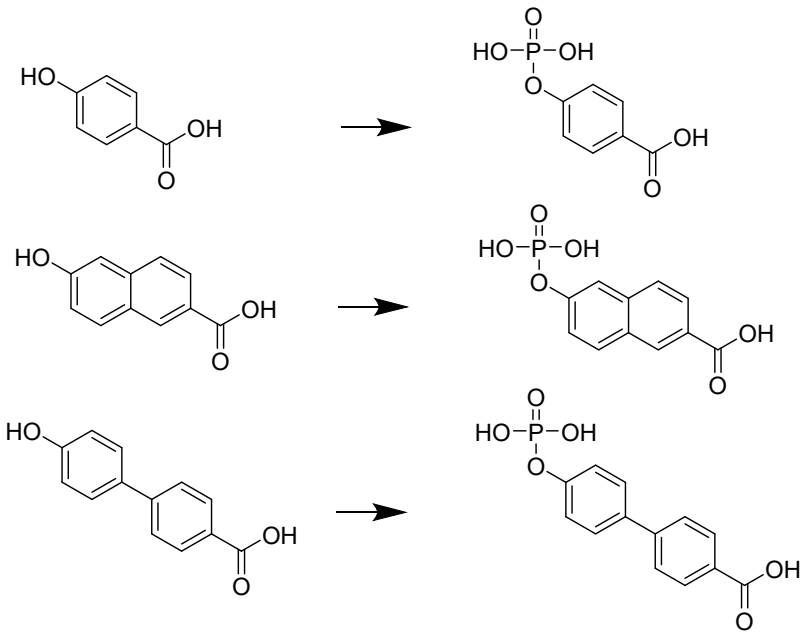
2-Cl-trityl chloride resin (1.0-1.2 mmol/g) and Fmoc-d-phenylalanine were obtained from GL Biochem (Shanghai, China). 4'-hydroxy-[1,1'-biphenyl]-4-carboxylic acid was obtained from 1PlusChem. 4-hydroxybenzoic acid was obtained from ACROS Organics. 6-hydroxy-2-naphthoic acid was obtained from Sigma-Aldrich. Other chemical reagents and solvents were obtained from Fisher Scientific; all chemical reagents and solvents were used as received from commercial sources without further purification; alkaline phosphatase was purchased from Biomatik.

Instruments

All precursors were purified with Agilent 1100 Series HPLC system equipped with a reverse phase C18 column. LC-MS was conducted on a Waters Acquity Ultra Performance LC with Waters MICRO-MASS detector. Rheology tests were obtained by a TA ARES-G2 rheometer at 25 ℃. TEM images were taken on a Morgagni 268 transmission electron microscope. SEM images were taken on a JEOL JSM-6060LV SEM model with an accelerating voltage of 15 kV.

Chemical Synthesis

Synthesis of phosphophenyl carboxylic acid, phosphonaphthoic acid, and phosphobiphenyl carboxylic acid



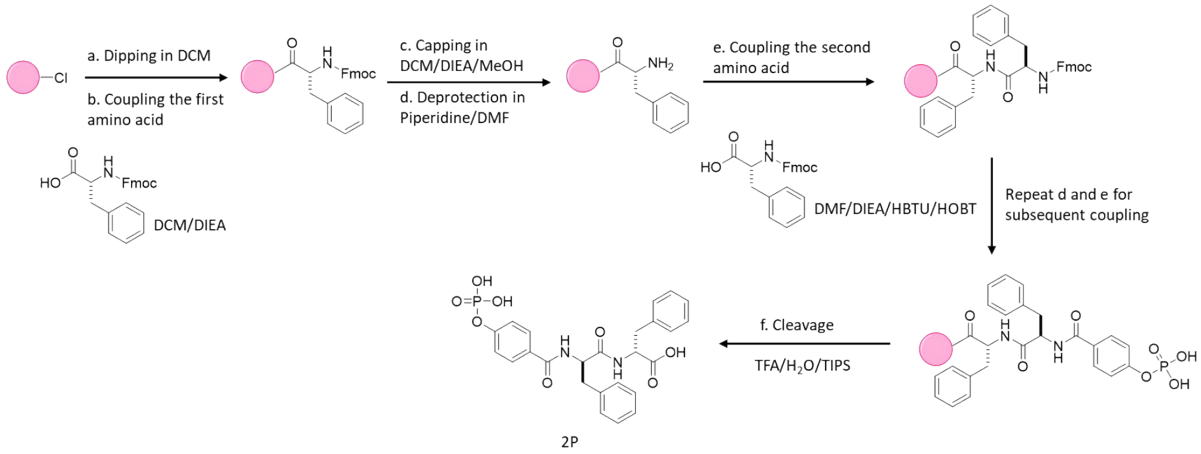
Scheme S1. The synthesis of phosphophenyl, phosphonaphthoic, and phosphobiphenyl group.

4-hydroxybenzoic acid, 6-hydroxy-2-naphthoic acid, or 4'-hydroxy-[1,1'-biphenyl]-4-carboxylic acid (1 equivalent) and PCl_5 (1 equivalent) were stirred at room temperature for 45 min. Subsequently, the reaction was brought to completion by sonication at 60 ℃ for 90 min. The ice-cooled reaction mixture was dissolved in 10 mL of acetone and 10 mL of benzene, and 1.4 mL (3

equivalent) of distilled water was added dropwise. After stirring at 0 °C for 30 min, 20 mL benzene was added. The reaction mixture was stirred at room temperature for 12 h. The precipitate was filtered off, washed with 20 mL benzene, and dried in high vacuum.

Solid phase peptide synthesis

a. 2-chlorotrityl chloride resin was weighed and dipped in methylene chloride (DCM) for 15 min.
b. The amino acids were weighed according to 1.2 mmol/g of resin. Dissolving the amino acid in DCM with the addition of 2.5 equivalent of N, N-Diisopropylethylamine (DIEA), then the resin is mixed with the solution well on a rocker for 1 h. Wash with DCM.
c. Add capping solution (DCM: MeOH: DIEA= 17: 2: 1) and react for 15 min. Wash with DCM first and then dimethylformamide (DMF).
d. Use 20% piperidine in DMF for 30 min to remove Fmoc group. Wash with DMF.
e. Load amino acid (1 equivalent), HBTU (1 equivalent), and DIEA (2.5 equivalent) in DMF for 40 min. Wash with DMF.
f. Use DCM to wash out DMF. Use 95% TFA, 2.5% triisopropyl silane (TIPS), 2.5% H_2O for 30 min for peptide cleavage.



Scheme S2. The synthesis scheme of **2P**. Same procedures were used to synthesize other short peptides.

Hydrogelation Experiment

Gelation experiments were carried out in 1.5 mL glass vials. 1 mg of each of the six precursors was first dissolved in 100 μL of 1x PBS buffer. The pH of the solution was carefully adjusted to 7.4 with 1 M NaOH (aq). Then extra PBS buffer was added to make the solutions with the volume of 200 μL and final concentration of 0.5 wt%. 1 μL of 0.2 U μL^{-1} ALP was added to make the final concentration of 1 U mL^{-1}. After incubation at room temperature for 24 h, the hydrogel was formed.

Rheology Experiment

Rheological tests were conducted on TA ARES-G2 rheometer, parallel-plate geometry with an upper plate diameter of 25 mm was used during the experiment, and the gap was 1 mm. Firstly, we make the solutions of **3P**, **5P** and **7P** with the concentration of 8 mM and volume of 400 μL. Then we add ALP into the solutions and mix with a pipette. Then, the samples were loaded into stage quickly, and we performed oscillation time-dependent strain: 1.0%, frequency:1 Hz), strain sweep

(0.1-100%) at 6.28 rad/s, frequency sweep test (0.1-200 rad/s).

TEM sample preparation

a. Place sample solution on the grid (5 μL, sufficient to cover the grid surface).
b. Rinsing: ~10 sec later, place a large drop of the ddH_2O on parafilm and let the grid touch the water drop, with the sample-loaded surface facing the parafilm. Tilt the grid and gently absorb water from the edge of the grid using a filter paper sliver. (3 times)
c. Staining (immediately after rinsing): place a large drop of the UA (uranyl acetate) stain solution on parafilm and let the grid touch the stain solution drop, with the sample-loaded surface facing the parafilm. Tilt the grid and gently absorb the stain solution from the edge of the grid using a filter paper sliver.
d. Allow the grid to dry in air and examine the grid as soon as possible.

SEM sample preparation

The morphologies of the xerogels were characterized using scanning electron microscopy (SEM-JEOL JSM-6060LV) operating with an accelerating voltage of 5-30 kV. The xerogels were prepared by drying in an oven at 70 °C overnight. To minimize charging, the samples were coated with a thin layer of gold before the experiment.

Author Contributions

B.X. and M.Y. conceived the study. M.Y. performed the chemical synthesis, characterization and analyzed the experimental results. J.G. helped rheology experiment. H.H. and W.T. helped LCMS. N.H. helped the synthesis. K.G. performed SEM experiment. B.X. supervised the studies. B.X. and M.Y. wrote the manuscript with input from all authors.

Supplementary Figures

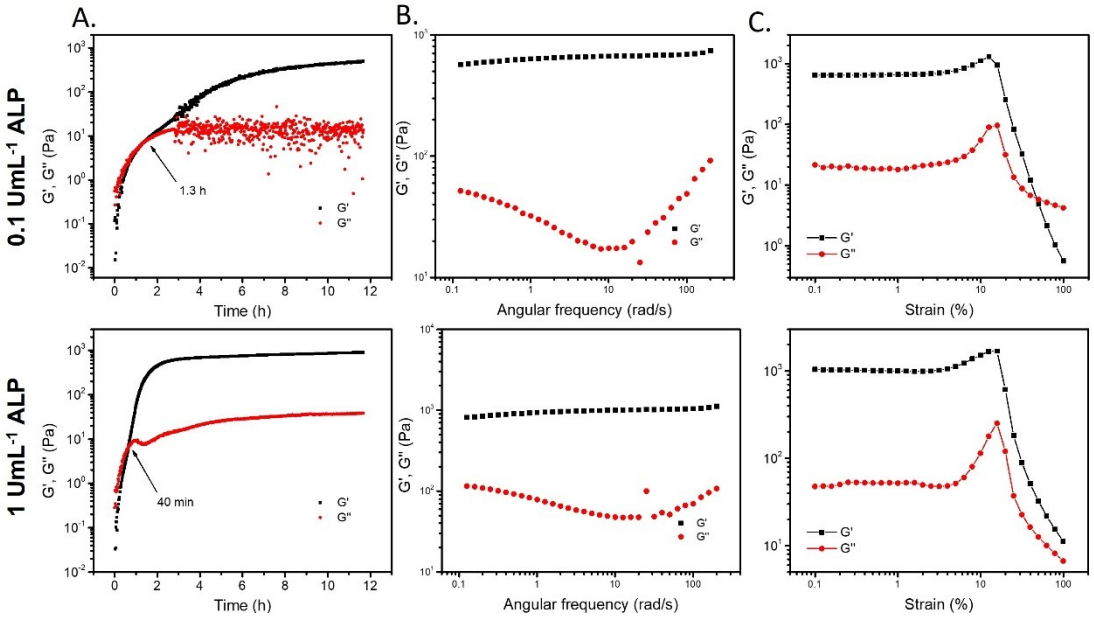


Figure S1. (A) Dynamic time sweeps of **1P** (8 mM) incubated with 0.1 UmL^{-1} or 1 UmL^{-1} ALP at a strain of 1% and frequency of 6.28 $rads^{-1}$. (B) Frequency sweeps of **1P** (8 mM) conducted after 24 h incubation with 0.1 UmL^{-1} or 1 UmL^{-1} ALP at the strain of 1%. (C) Dynamic strain sweeps of **1P** (8mM) conducted after 24 h incubation with 0.1 UmL^{-1} or 1 UmL^{-1} ALP at the frequency of 6.28 $rads^{-1}$.

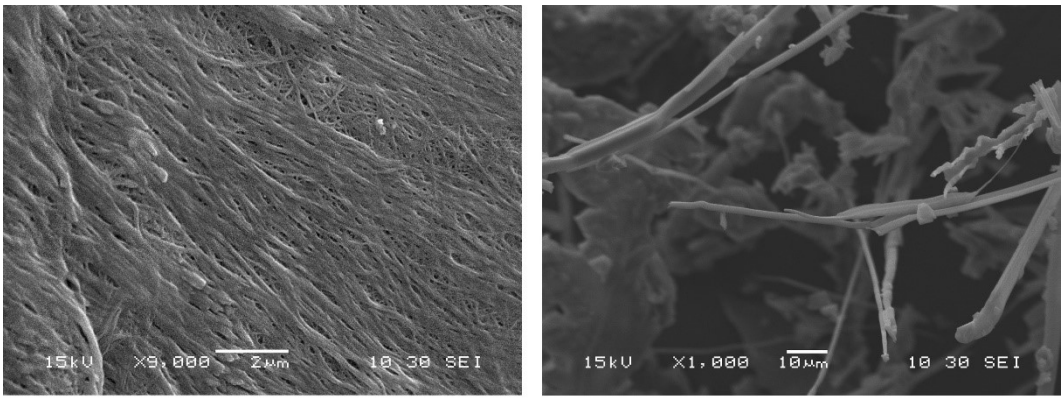


Figure S2. The SEM images of **3P** and **7P** at 8 mM after ALP treatment for over one week. The concentration of ALP is 0.1 UmL^{-1}.

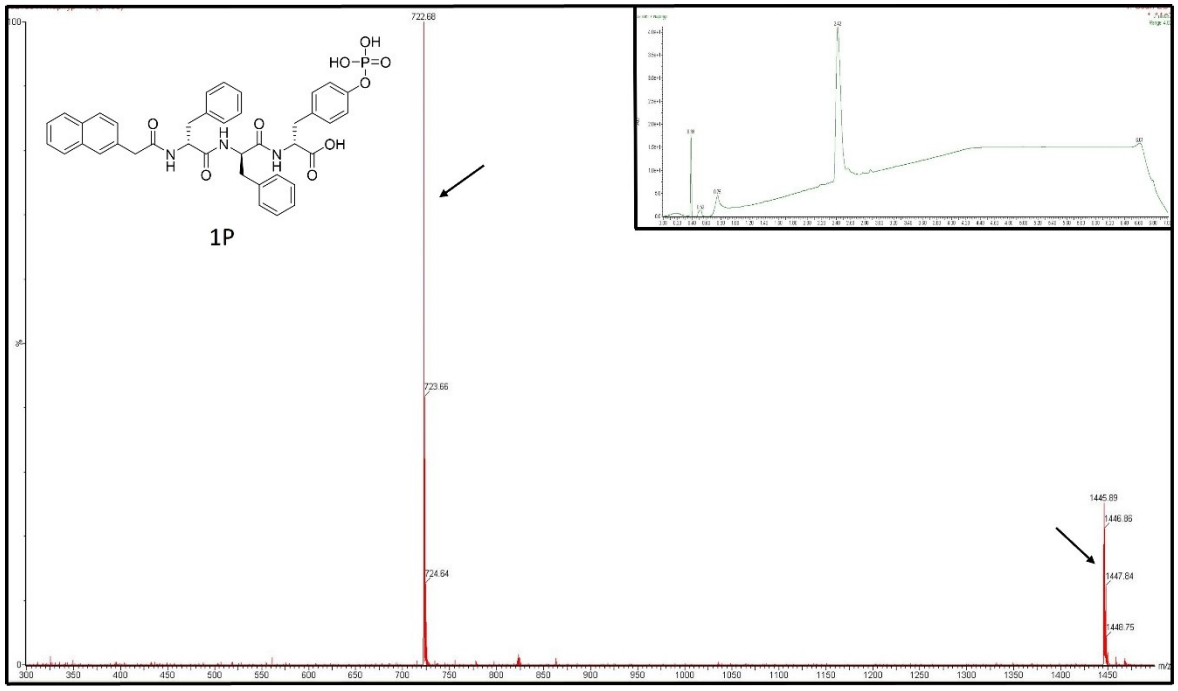


Figure S3. The LC spectrum of **1P** (inset) and its corresponding mass spectrum.

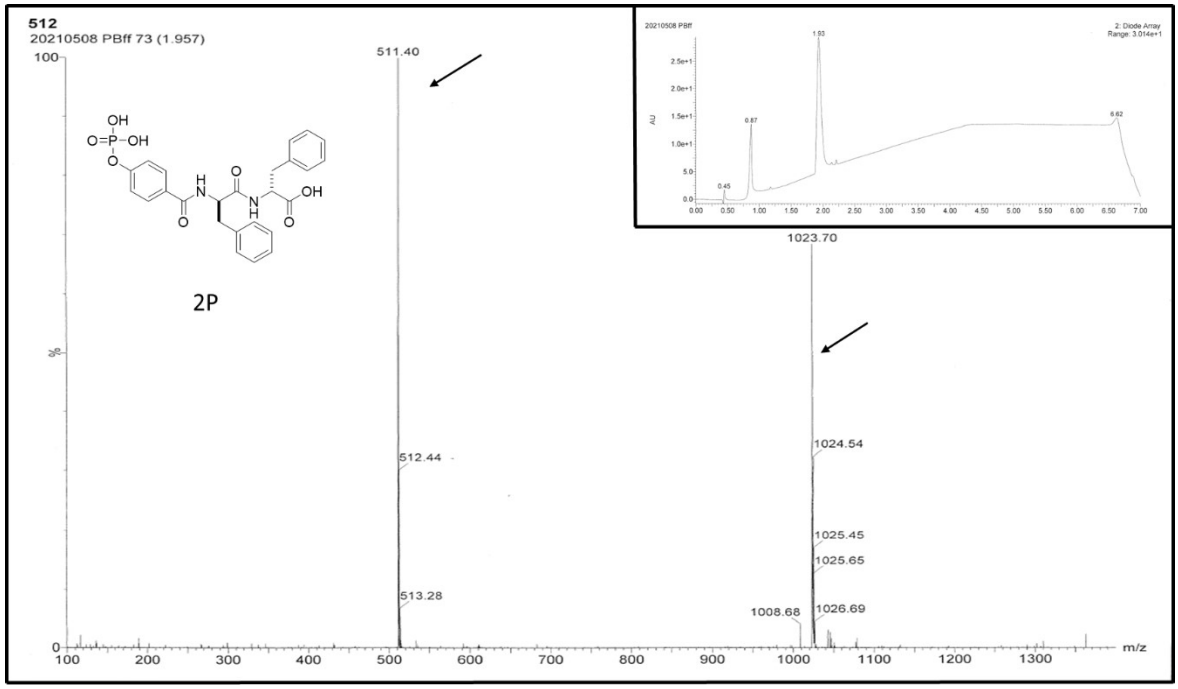


Figure S4. The LC spectrum of **2P** (inset) and its corresponding mass spectrum.

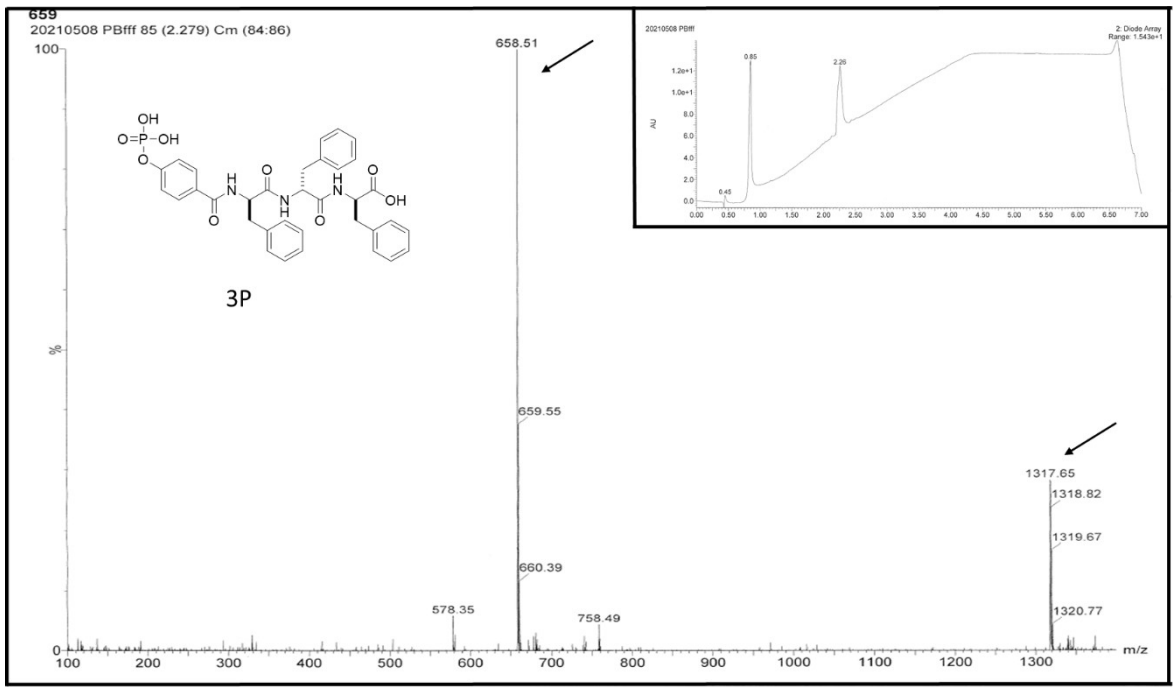


Figure S5. The LC spectrum of **3P** (inset) and its corresponding mass spectrum.

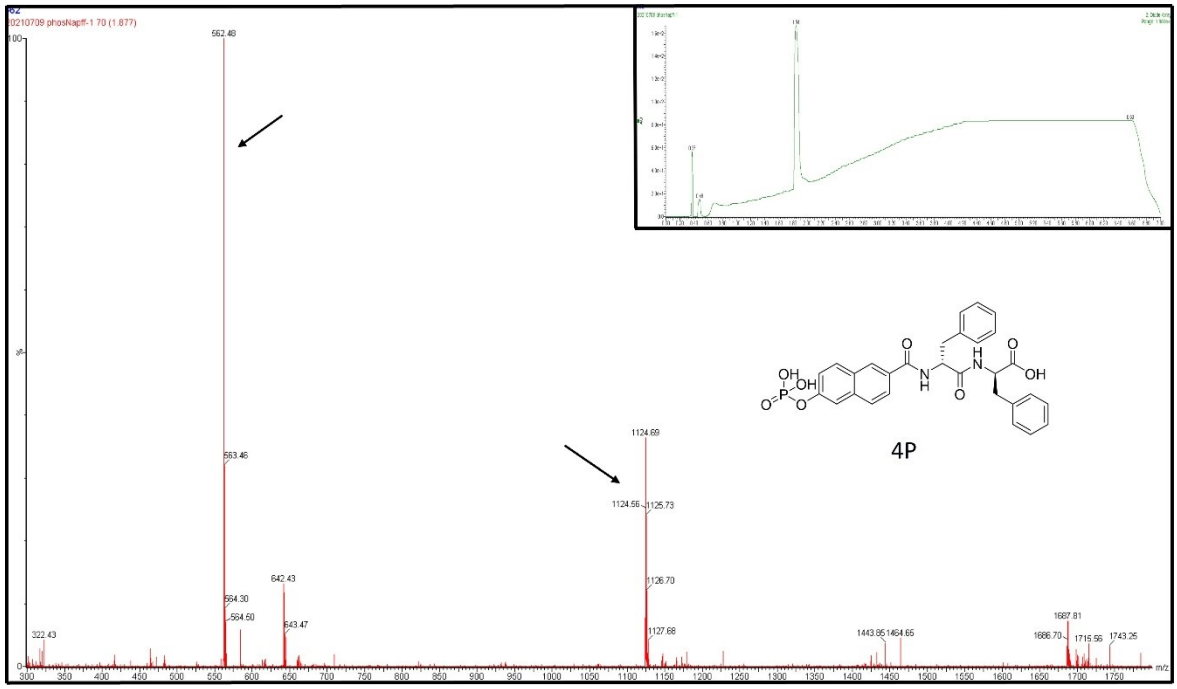


Figure S6. The LC spectrum of **4P** (inset) and its corresponding mass spectrum.

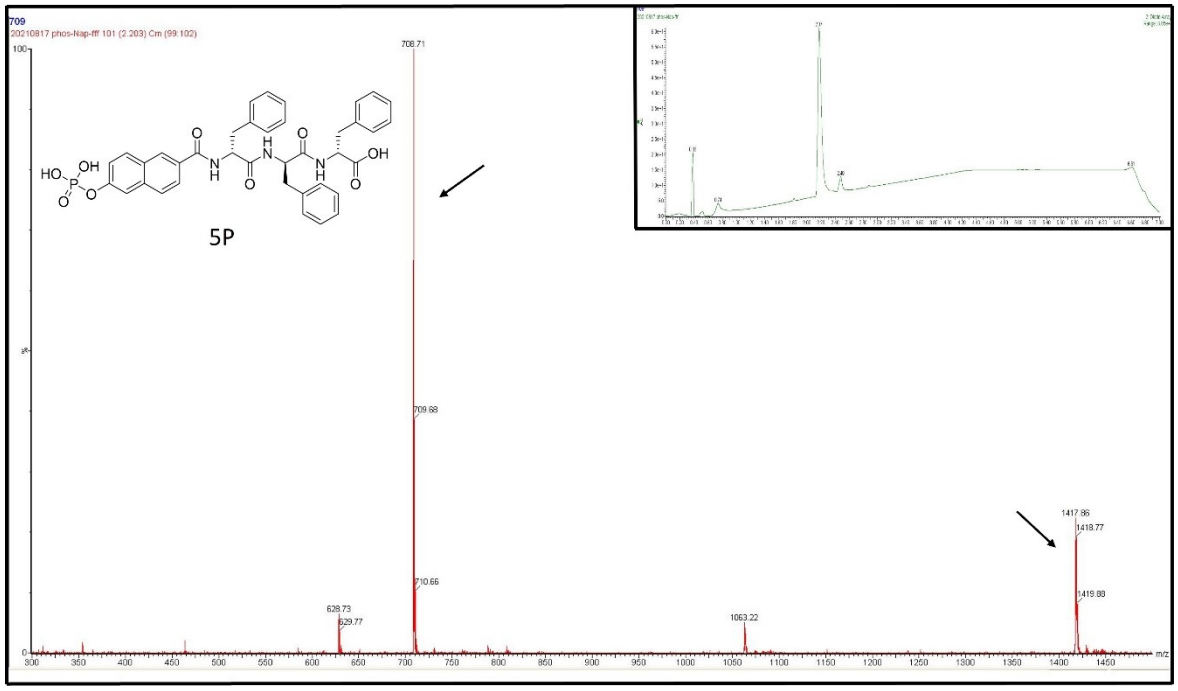


Figure S7. The LC spectrum of **5P** (inset) and its corresponding mass spectrum.

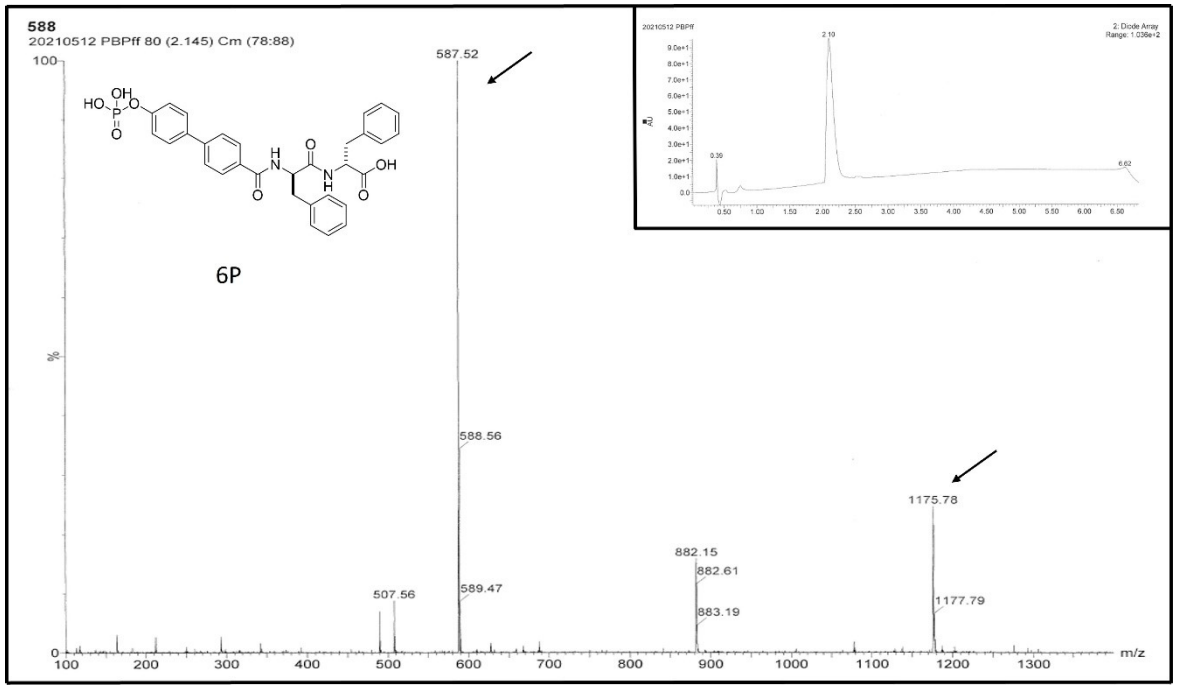


Figure S8. The LC spectrum of **6P** (inset) and its corresponding mass spectrum.

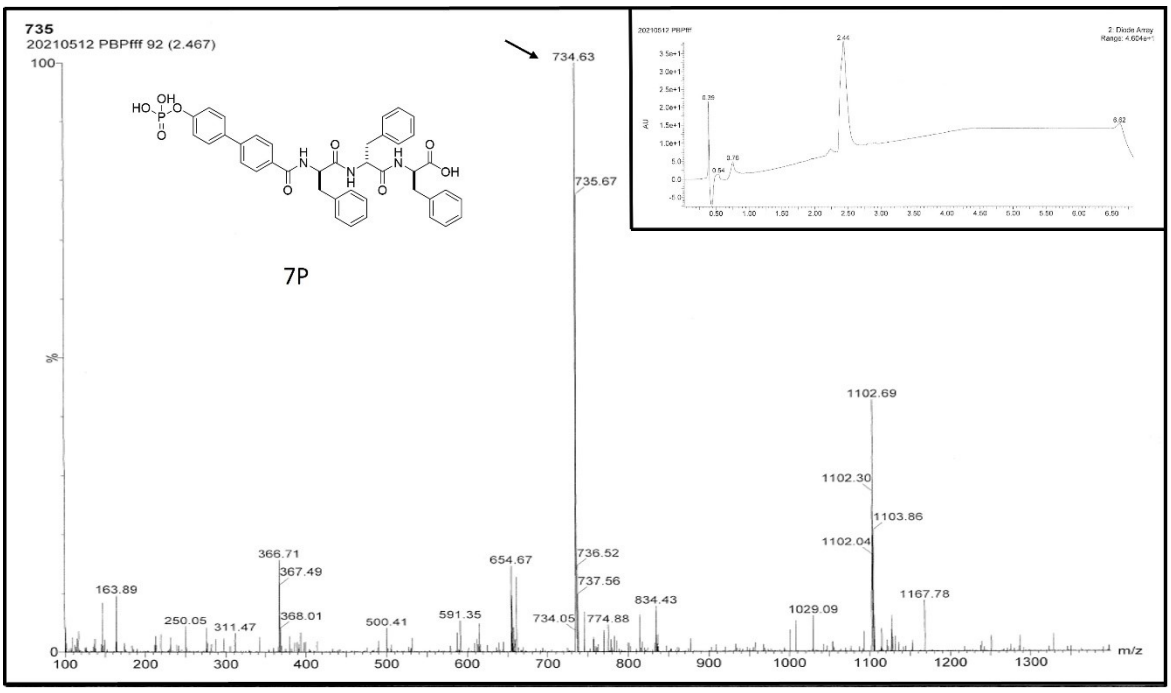


Figure S9. The LC spectrum of **7P** (inset) and its corresponding mass spectrum.