Supporting Information for

Enzyme-Instructed Self-Assembly of Small D-Peptides as A Multiple-Step Process for Selectively Killing Cancer Cells

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S1. Experimental materials and instruments

All the solvents and chemical reagents were used directly as received from the commercial sources without further purification. All of the products (**a-2p**, **b-2p**, **c-2p**, **a-1p**, **b-1p**, **c-1p**, **NBD-2p** and **NBD-1p**) were purified with Water Delta600 HPLC system, equipped with an XTerra C18 RP column and an in-line diode array UV detector, hydrophobic product **a**, **b** and **c** with flash chromatography. ^{1}H-NMR spectra were got on Varian Unity Inova 400, LC-MS spectra on a Waters Acouity ultra performance LC with Waters MICRO-MASS detector, rheological data on TA ARES G2 rheometer with 25 mm cone plate, TEM images on Morgagni 268 transmission electron microscope, confocal microscopy images on Leica TCS SP2 spectral confocal microscope or Marianas Spinning Disk confocal microscope.

S2. Synthesis and characterizations

We prepared the precursors and hydrogelators by solid phase synthesis in fair yields (70-80%) and reasonable scales (0.1-0.5 g). The standard solid-phase peptide synthesis (SPPS)[1] uses 2-chlorotriyl chloride resin (100-200 mesh and 0.3-0.8 mmol/g) and N-Fmoc-protected amino acids with side chains properly protected. Before that, we prepared NBD-COOH, which was directly used in SPPS, from NBD-Cl based on literature[2]. The following scheme (Figure S1) illustrates the synthetic procedure of **b-1p** and **NBD-1p**. The synthetic route of others is the same with that of **b-1p** and **NBD-1p**.

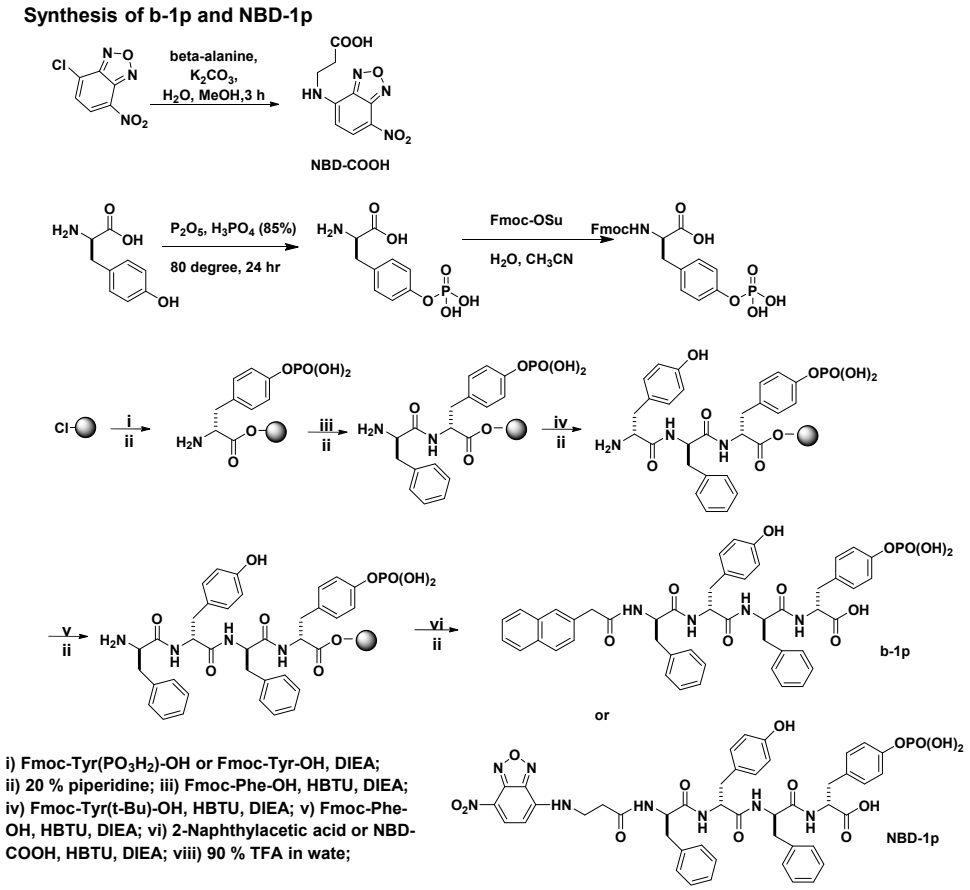
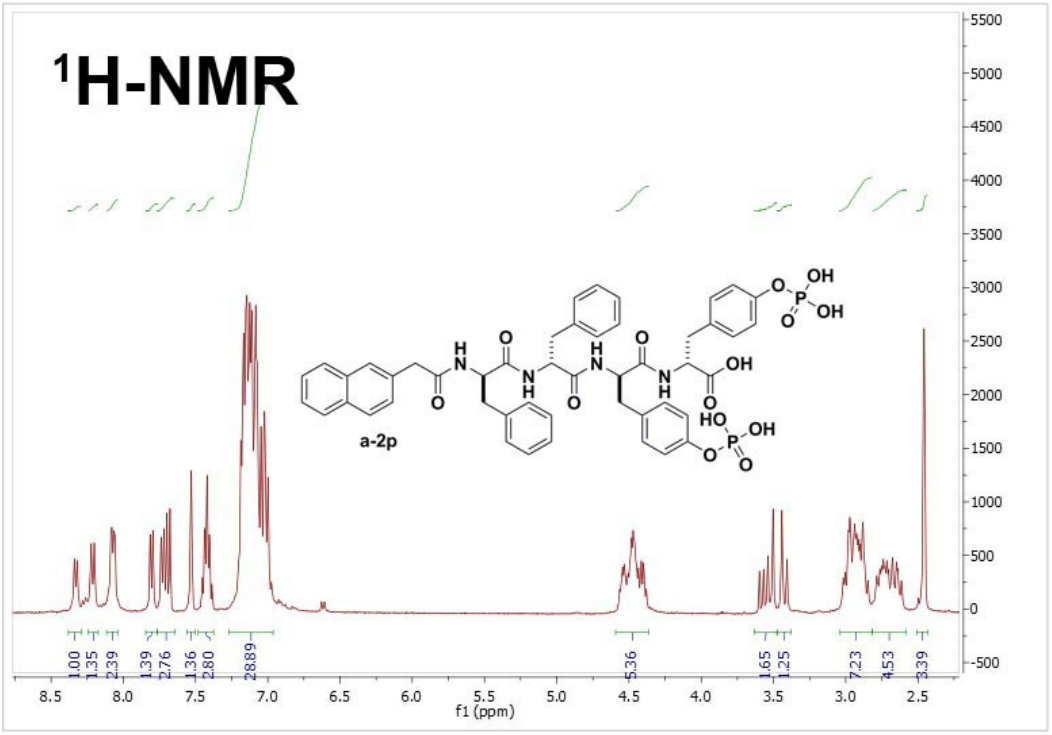
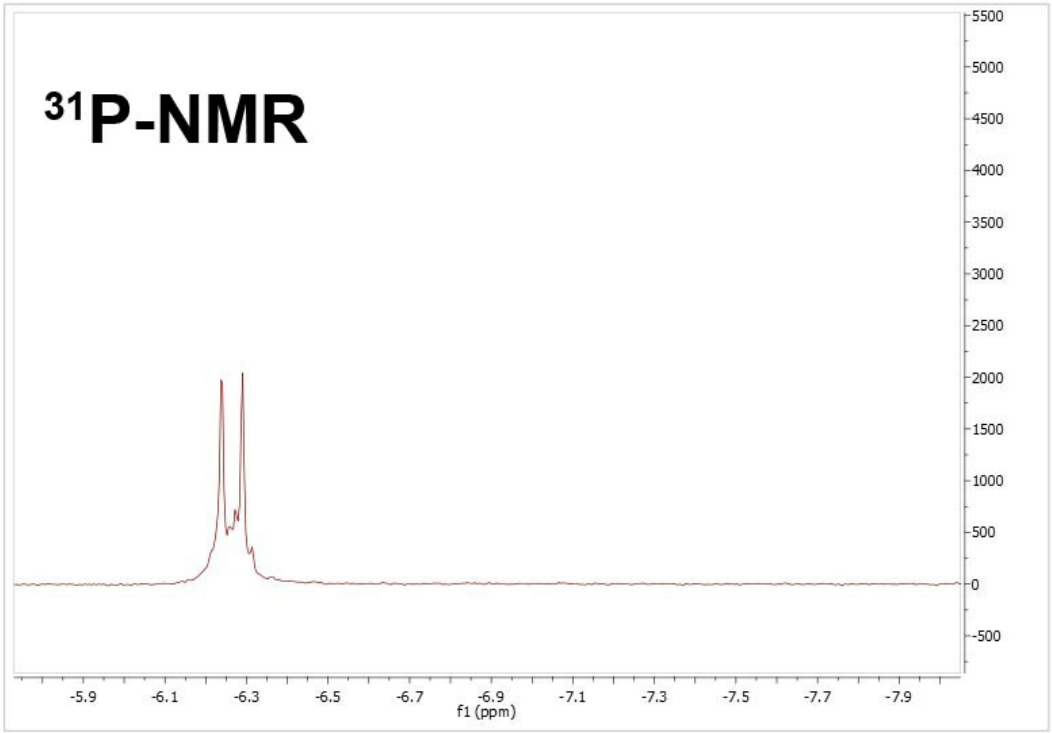
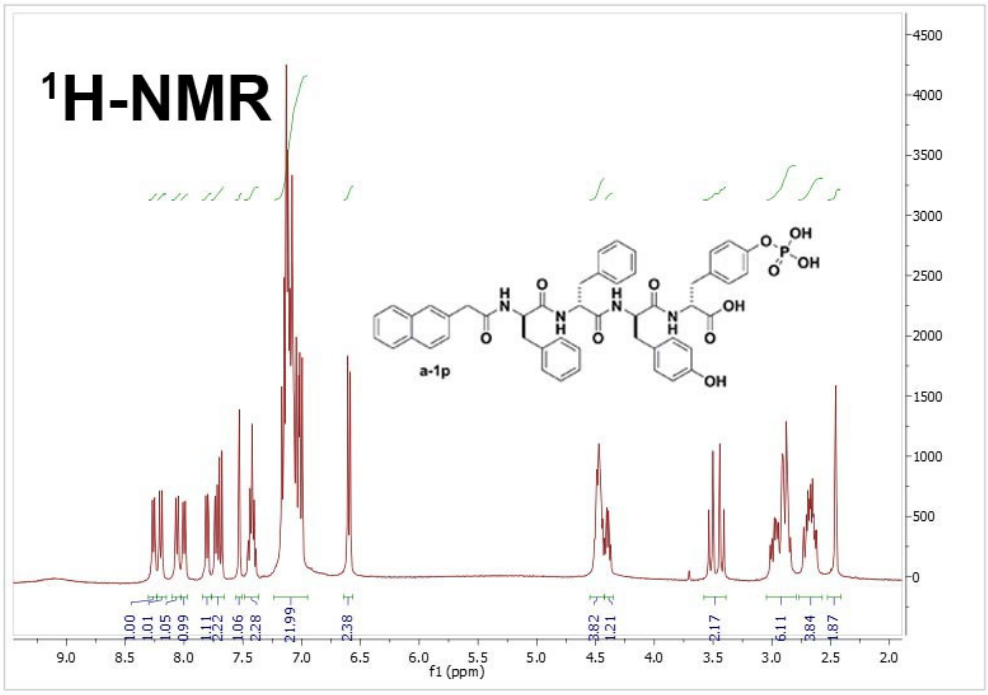


Figure S1. Synthesis route of **b-1p** and **NBD-1p**

The NMR spectra of precursors and hydrogelators.



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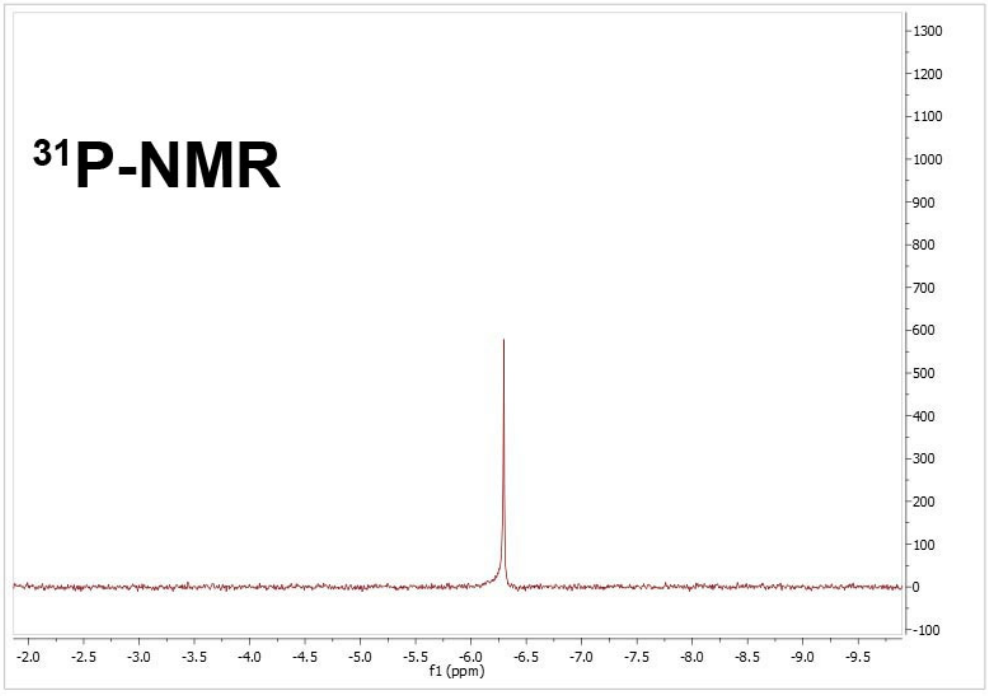
a-1p
f1 (ppm)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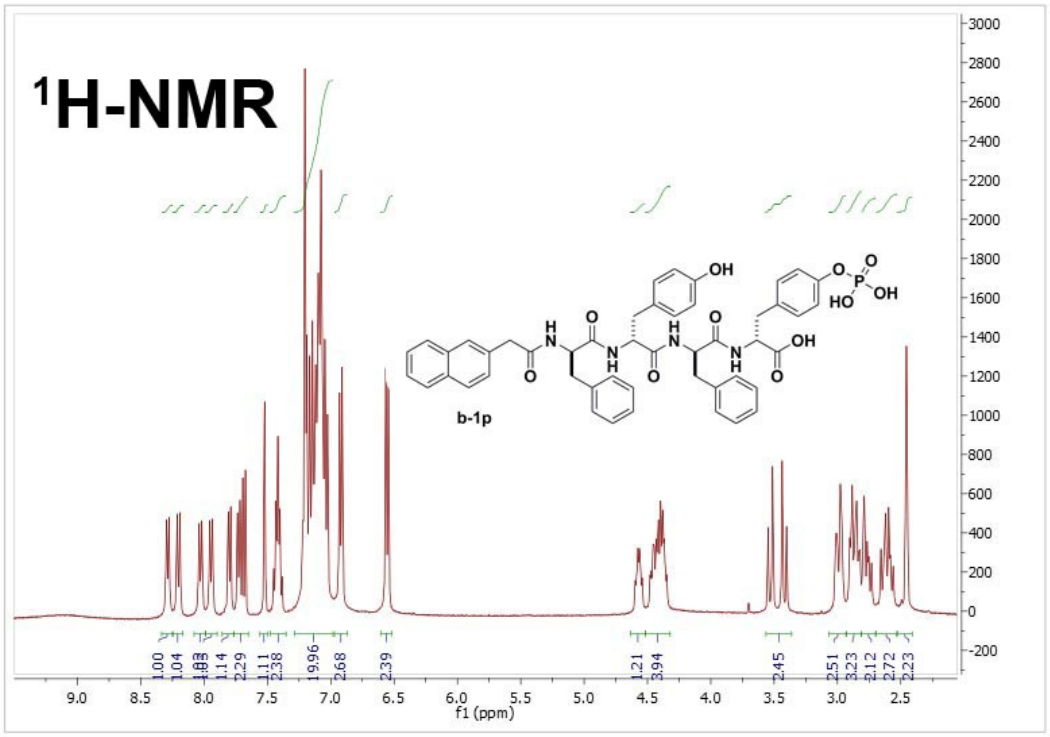
f1 (ppm)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1.03
1.03
1.14
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1.11
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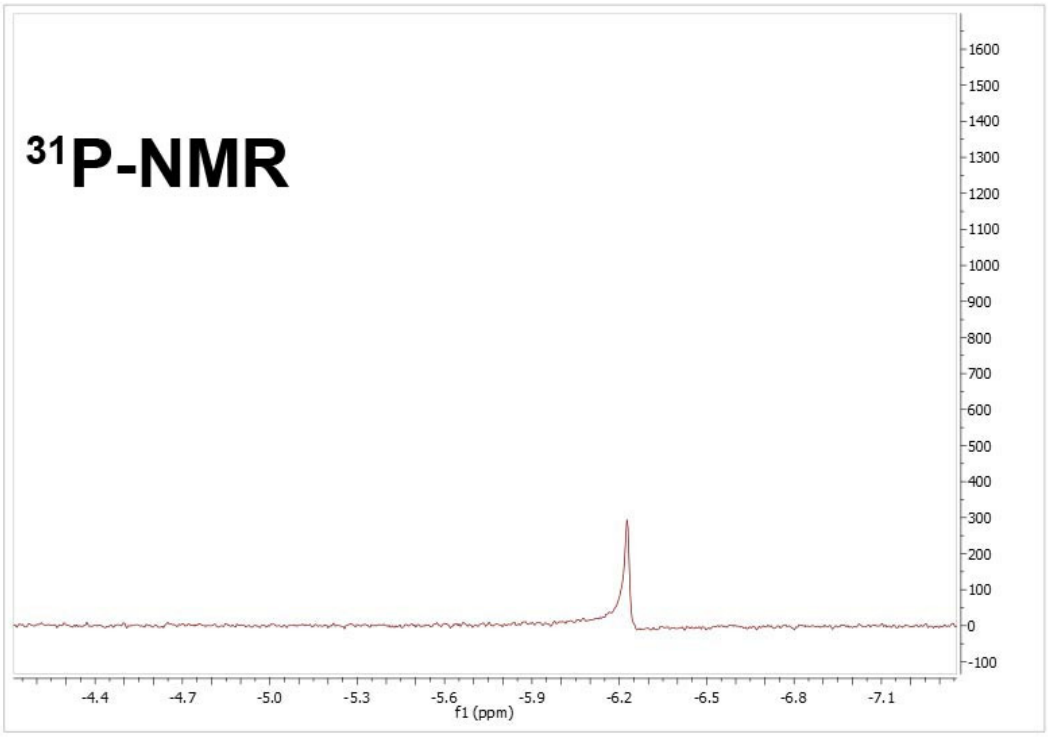
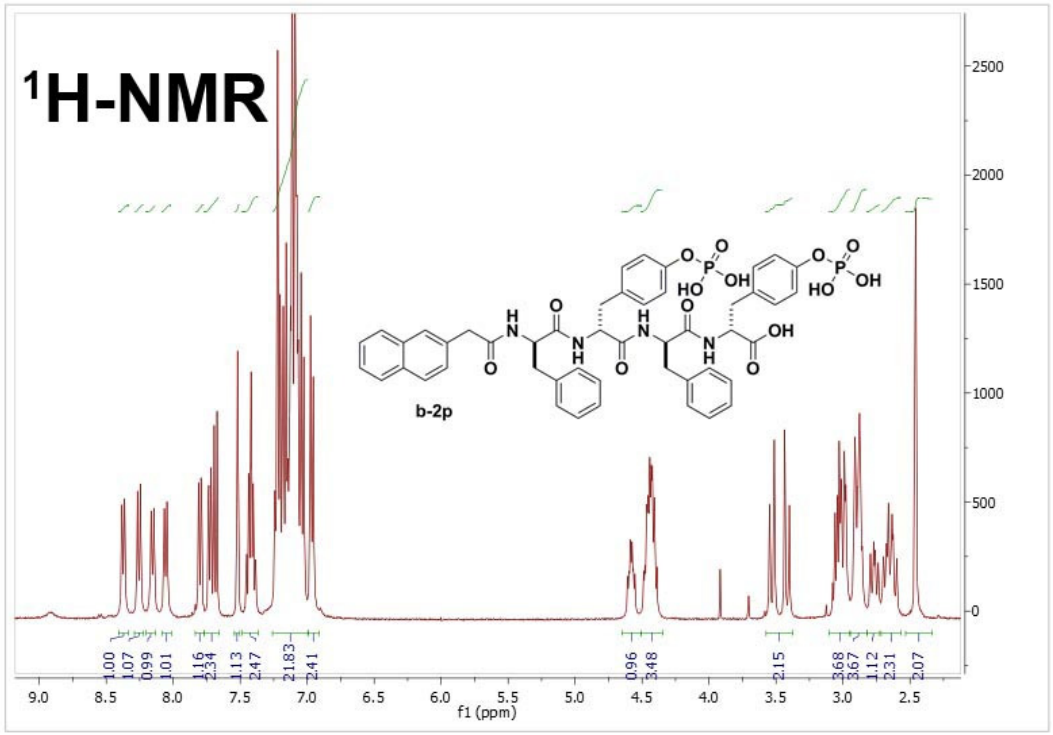
1.21
3.94
2.45
2.51
3.23
2.12
2.72
2.23
f1 (ppm)


31P-NMR
f1 (ppm)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1.07
0.99
1.01
1.16
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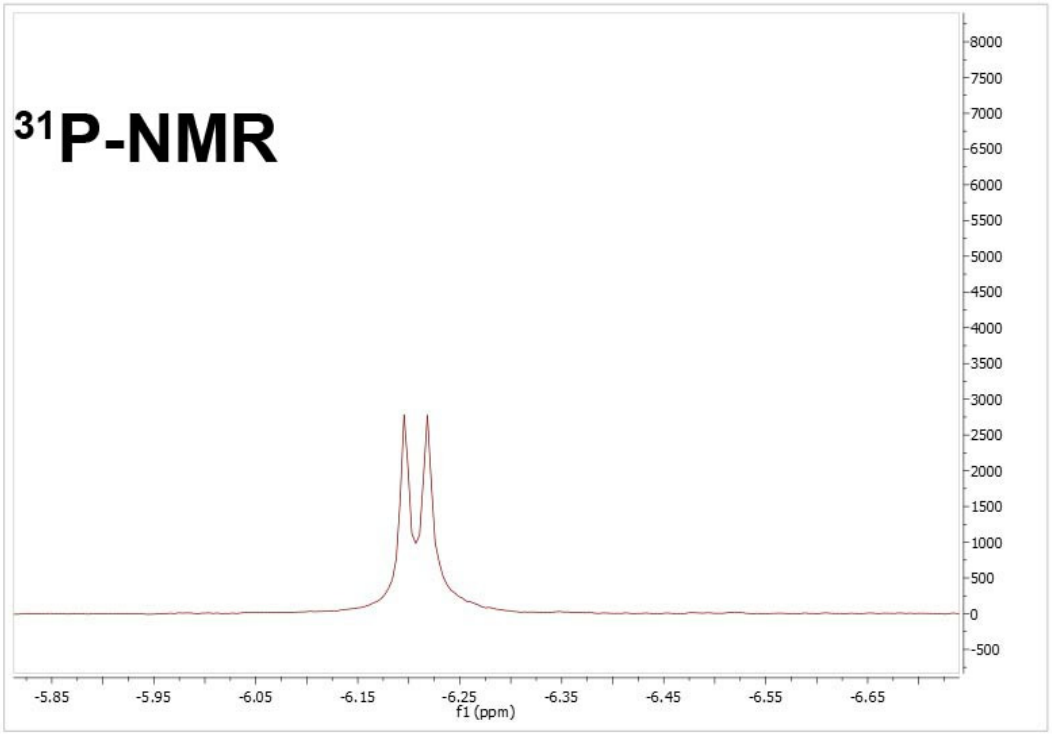
3.67
1.12
2.31
2.07
f1 (ppm)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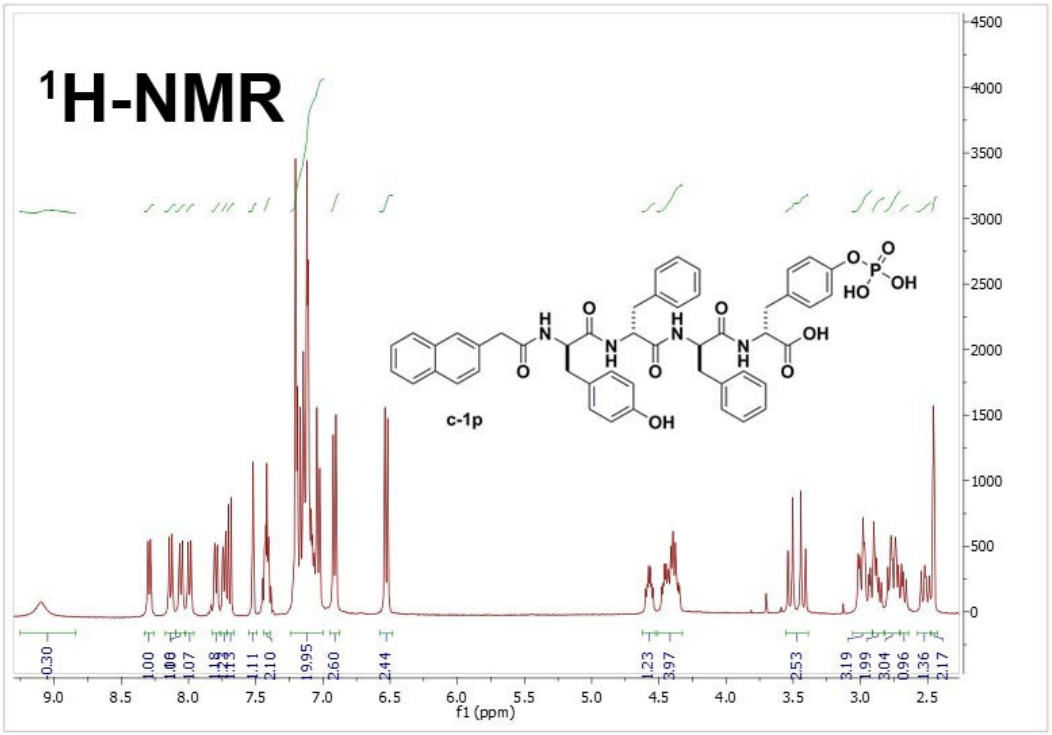
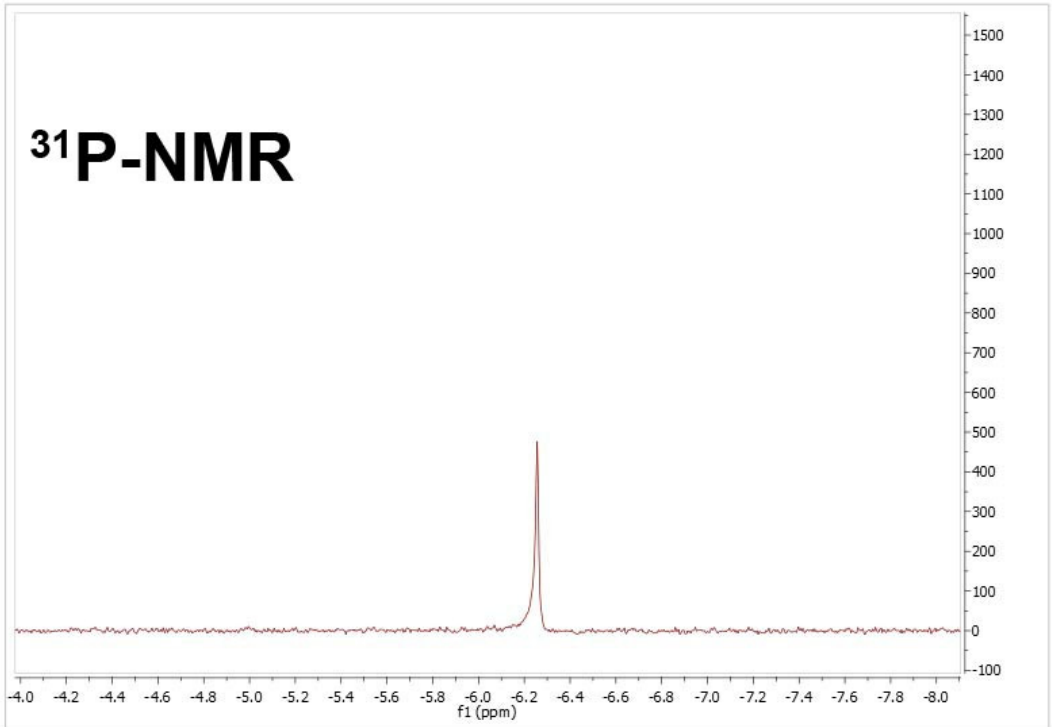
f1 (ppm)


1H-NMR
c-1p
f1 (ppm)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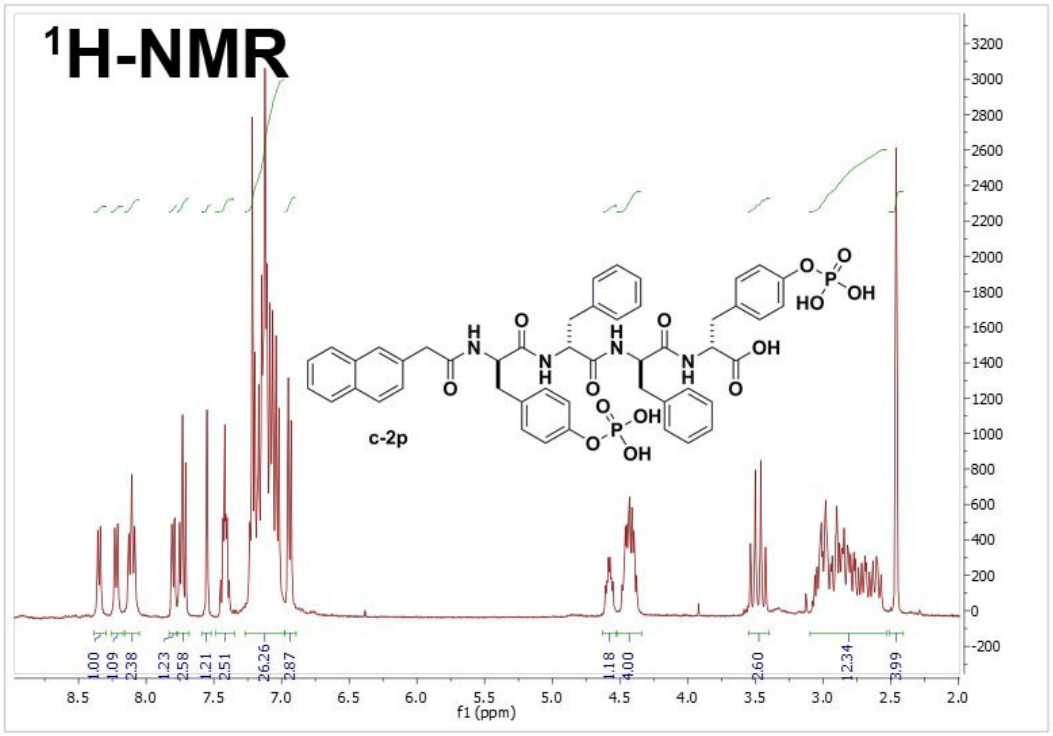
f1 (ppm)


1H-NMR
c-2p
1.00
1.09
2.38
1.23
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1.21
2.51
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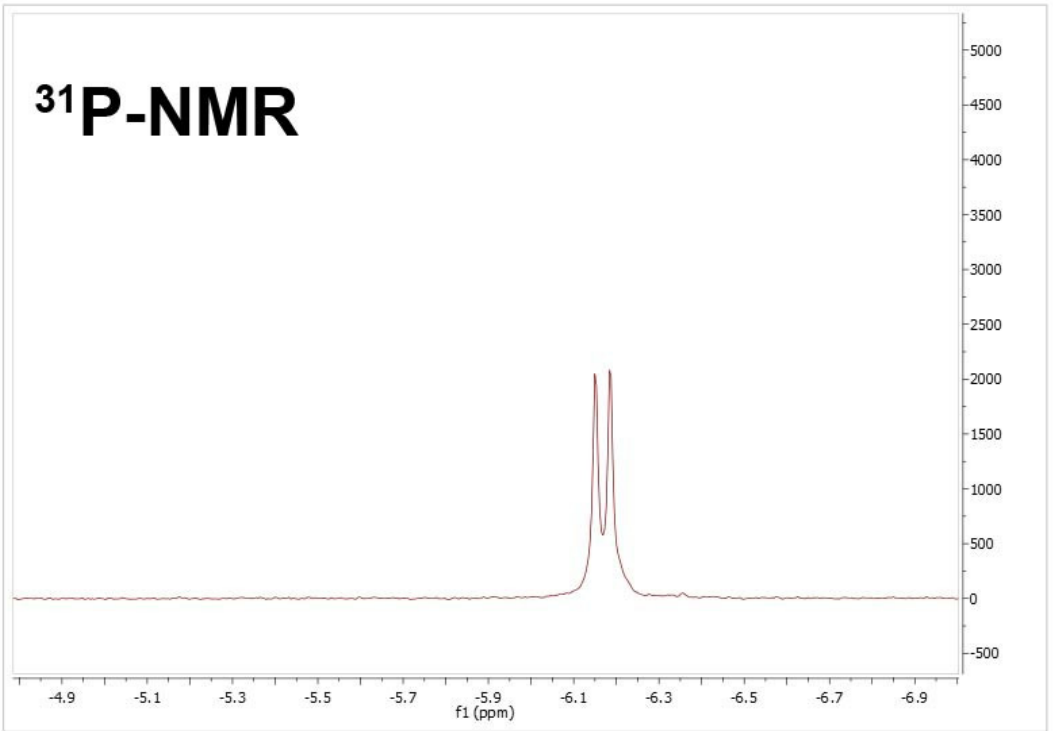
2.60
12.34
3.99
f1 (ppm)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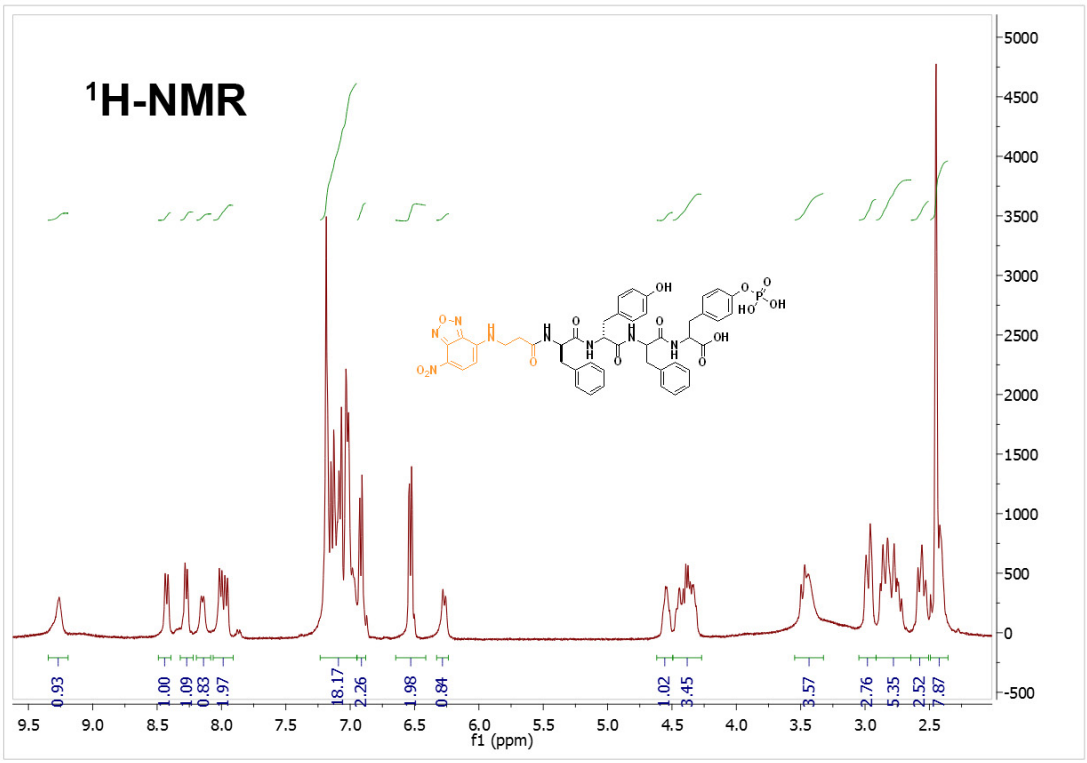
f1 (ppm)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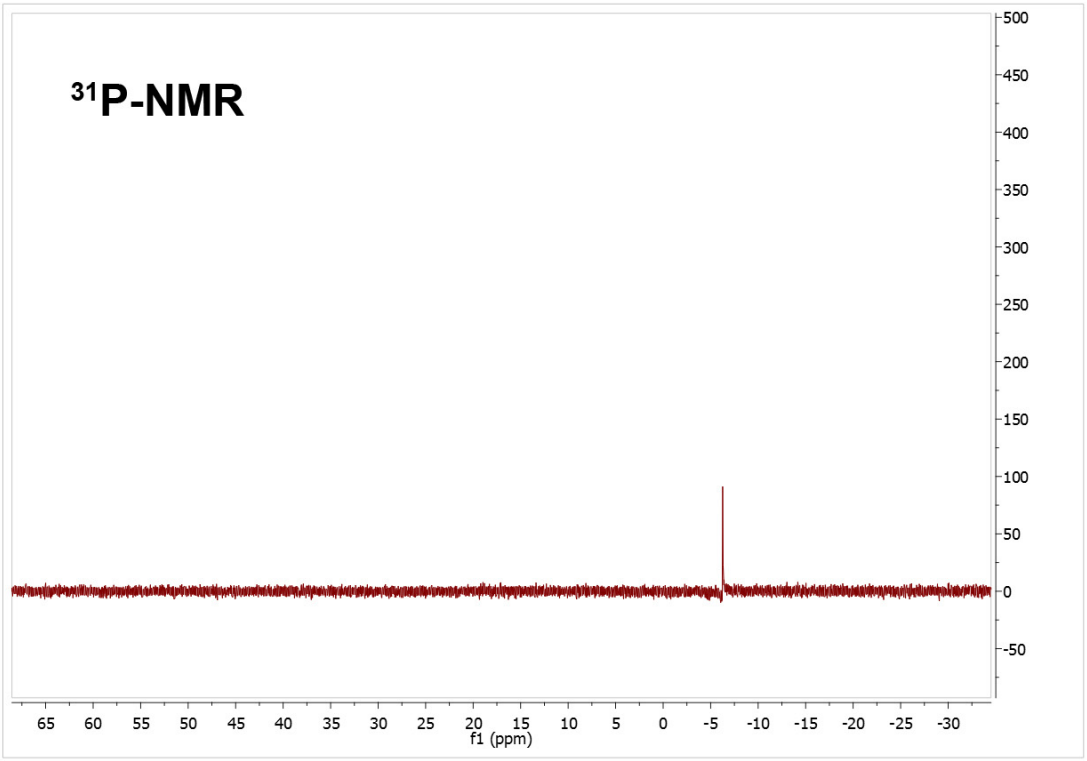
0.93
1.00
1.09
0.83
1.97
18.17
2.26
1.98
0.84
1.02
3.45
3.57
2.76
5.35
2.52
7.87
f1 (ppm)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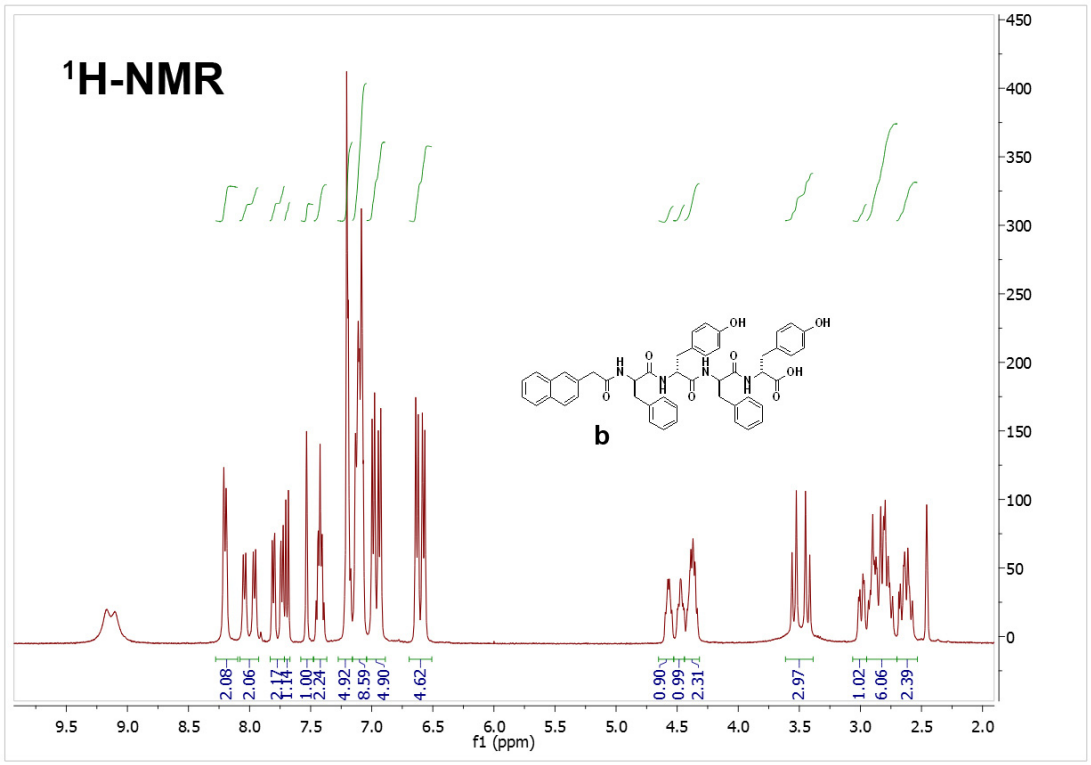
f1 (ppm)


1H-NMR
b
2.08
2.06
2.17
1.14
1.00
2.24
4.92
8.59
4.90
4.62
0.90
0.99
2.31
2.97
1.02
6.06
2.39
f1 (ppm)

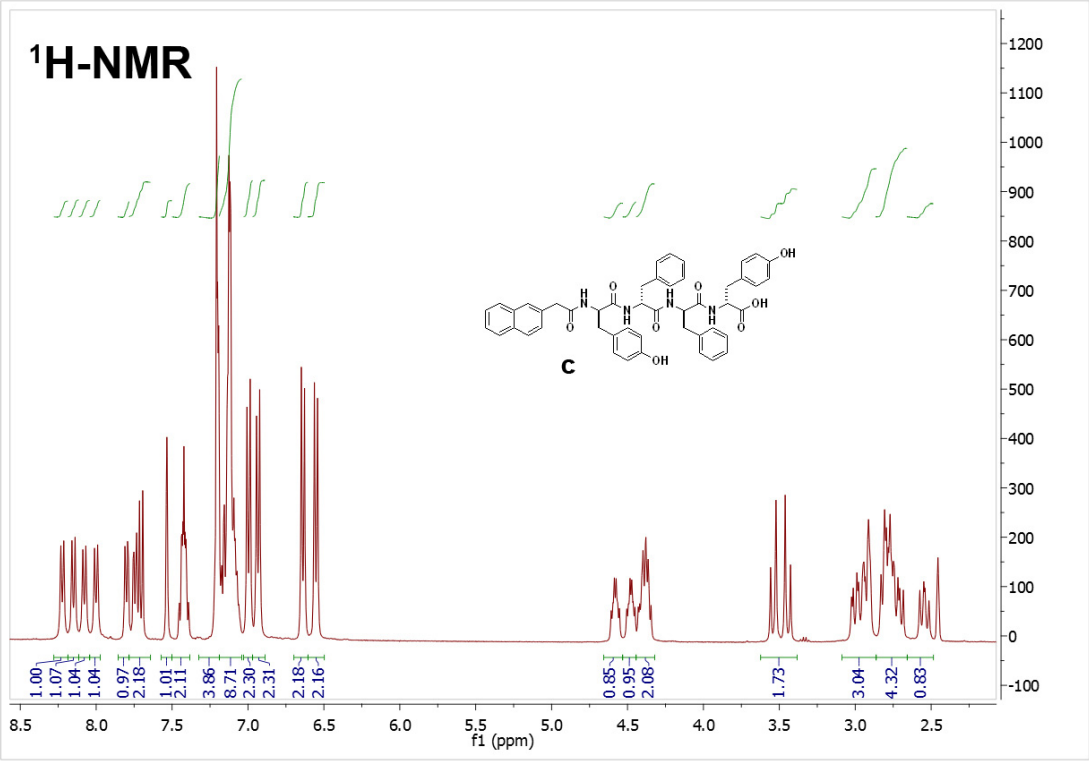


Figure S2. ^{1}H-NMR and ^{31}P-NMR spectra of compounds mentioned in main text.

LC-MS (ESI):

a-2p (m/z): $C_{48}H_{48}N_4O_{14}P_2$, calc. 966.26; observed (M+1)+ 967.08, (M-1)- 965.20.

b-2p (m/z): $C_{48}H_{48}N_4O_{14}P_2$, calc. 966.26; observed (M+1)+ 967.08, (M-1)- 965.20.

c-2p (m/z): $C_{48}H_{48}N_4O_{14}P_2$, calc. 966.26; observed (M+1)+ 967.08, (M-1)- 965.20.

a-1p (m/z): $C_{48}H_{47}N_4O_{11}P_1$, calc. 886.30; observed (M+1)+ 887.20, (M-1)- 885.32.

b-1p (m/z): $C_{48}H_{47}N_4O_{11}P_1$, calc. 886.30; observed (M+1)+ 887.27, (M-1)- 885.32.

c-1p (m/z): $C_{48}H_{47}N_4O_{11}P_1$, calc. 886.30; observed (M+1)+ 887.46, (M-1)- 885.58.

a (m/z): $C_{48}H_{46}N_4O_8$, calc. 806.92; observed (M+1)+ 807.58, (M-1)- 805.63.

b (m/z): $C_{48}H_{46}N_4O_8$, calc. 806.92; observed (M+1)+ 807.52, (M-1)- 805.57.

c (m/z): $C_{48}H_{46}N_4O_8$, calc. 806.92; observed (M+1)+ 807.45, (M-1)- 805.50.

NBD-2p (m/z): $C_{45}H_{46}N_8O_{17}P_2$, calc. 1032.25; observed (M+1)+ 1033.20, (M-1)- 1031.67.

NBD-1p (m/z): $C_{45}H_{45}N_8O_{14}P$, calc. 952.28; observed (M+1)+ 953.43, (M-1)- 951.68.

S3. General procedures for hydrogel preparation.

Enzymatic gelation: We dissolved precursors (4 mg) into distilled water (700 □L), and adjusted pH of the solution, monitored by pH paper, carefully by adding 1M NaOH. After the pH of the solution reaches 7.4, we then added extra distilled water to make the final concentration of 0.5 wt%, followed by the addition of alkaline phosphatase (ALP).

S4. TEM sample preparation.

In this paper, we used negative staining technique to study the TEM images. We first glowed discharge the 400 mesh copper grids coated with continuous thick carbon film (~ 35 nm) prior to use to increase the hydrophilicity. After loading samples (4 μL) on the grid, we then rinsed grid by dd-water for twice or three times. Immediately after rinsing, we stained the grid containing sample with 2.0 % w/v uranyl acetate for three times. Afterwards, we allowed the grid to dry in air.

S5. Light scattering sample preparation

The static light scattering experiments were performed using an ALV (Langen, Germany) goniometer and correlator system with a 22 mW HeNe (λ = 633 nm) laser and an avalanche photodiode detector. All samples were filtered by using 0.22 μm filters after heating. The addition of ALP to the solution of precursors for 24 h, we obtained corresponding samples of hydrogelators. The SLS tests were carried out at room temperature, and the angles of light scattering we chose were 30, 60, 90, and 120°, respectively. The resulting intensity ratios are proportional to the amount of aggregates in the samples.

S6. Cell culture

All cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The HeLa cells were propagated in Minimum Essential Media (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics in a fully humidified incubator containing 5% CO_2 at 37°C. The HS-5 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with FBS to a final concentration of 10% and antibiotics, in a fully humidified incubator containing 5% CO_2 at 37°C. The A2780cis cells were propagated in RPMI-1640 medium, supplemented with 10% FBS, 2mM glutamine in a fully humidified incubator containing 5% CO_2 at 37°C. 1μM cisplatin was necessary every 2-3 passages. The SK-OV-3 cells were propagated in McCoy's 5A supplemented with 10% fetal bovine serum (FBS), and antibiotics, in a fully humidified incubator containing 5% CO_2 at 37°C. The T98G cells were propagated in Minimum Essential Media (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics in a fully humidified incubator containing 5% CO_2 at 37°C. The Saos-2 cells were propagated in McCoy's 5A supplemented with 15% fetal bovine serum (FBS), and antibiotics, in a fully humidified incubator containing 5% CO_2 at 37°C.

S7. Cell viability assay

Cells in exponential growth phase were seeded in a 96 well plate at a concentration of 1×10^4 cell/well. The cells were allowed to attach to the wells for 24 h at 37 ºC, 5% CO_2. The culture medium was removed and 100 μL culture medium containing compounds (immediately diluted from fresh prepared

stock solution of 10 mM) at gradient concentrations (0 μM as the control) was placed into each well. After culturing at 37 ºC, 5% CO2 for 48 h, each well was added by 10 μL of 5 mg/mL MTT ((3-(4, 5-DimethylthiazoL-2-yl)-2, 5-diphenyltetrazolium bromide), and the plated cells were incubate at dark for 4 h. 100 μL 10% SDS with 0.01M HCl was added to each well to stop the reduction reaction and to dissolve the purple. After incubation of the cells at 37 ºC for overnight, the OD at 595 nm of the solution was measured in a microplate reader. Data represent the mean ± standard deviation of three independent experiments.

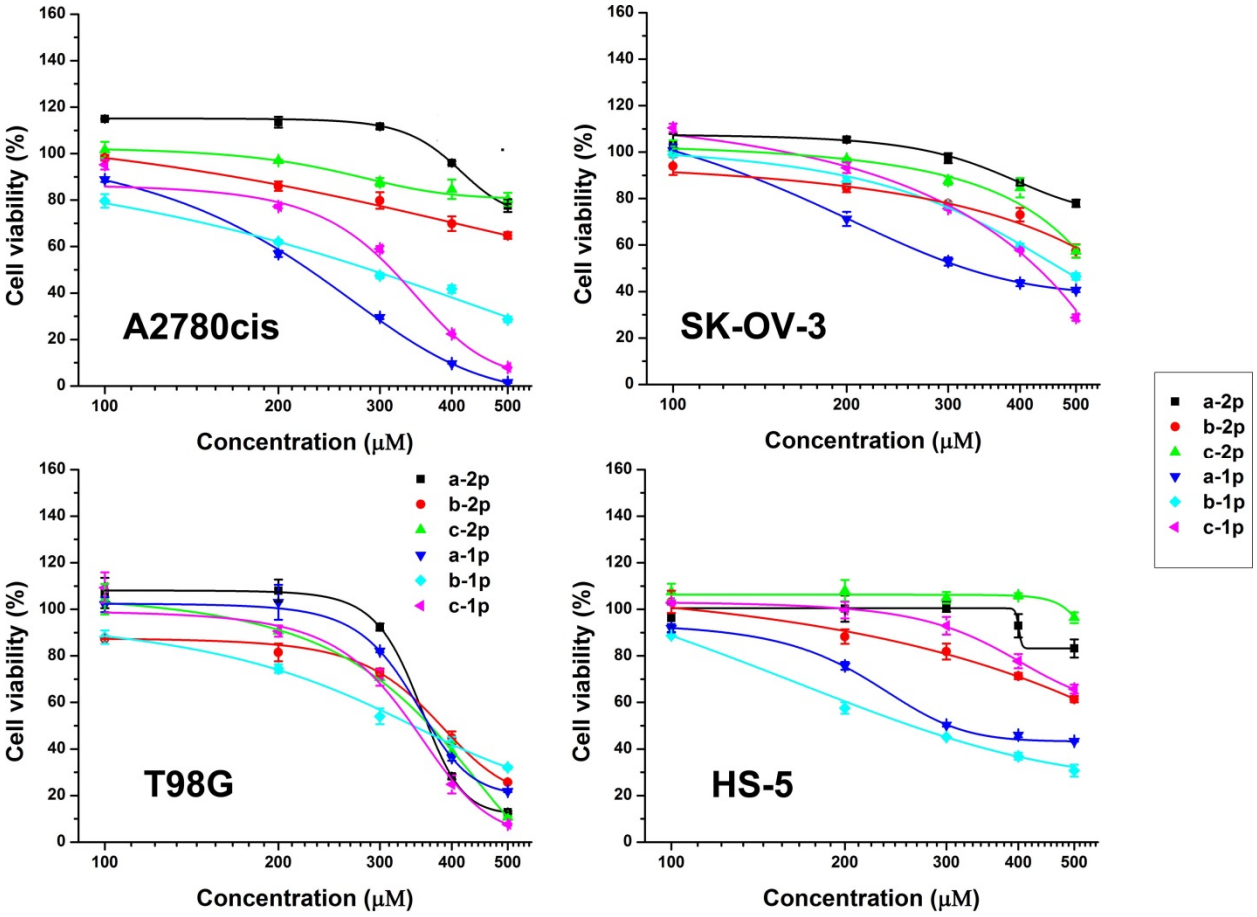


Figure S3. 48-hour cell viability of A2780cis, SK-OV-3, T98G, and HS-5 cells treated with different precursors at different concentration. The cell viability is determined my MTT cell viability assay.

S8. Relative ALP activity measurements

1. Plate HeLa, HS-5, Saos-2, A2780cis, T98G, and SK-OV-3 in 96-well plate with cell density of 50,000/well and allow 4-hour incubation for attachment.
2. Remove the medium and wash the cell with PBS buffer for 3 times.
3. Add 100 μL dd H2O water and incubate at 37 oC for one hour.
4. Store the plates at -80ºC for 1h.
5. Defrosted the samples, remove the solution from the culture wells and measured the ALP activity with pNPP assay.

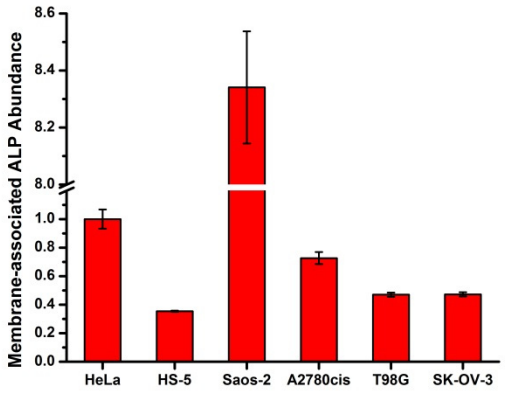


Figure S4. Membrane-associated ALP activities of different cell lines.

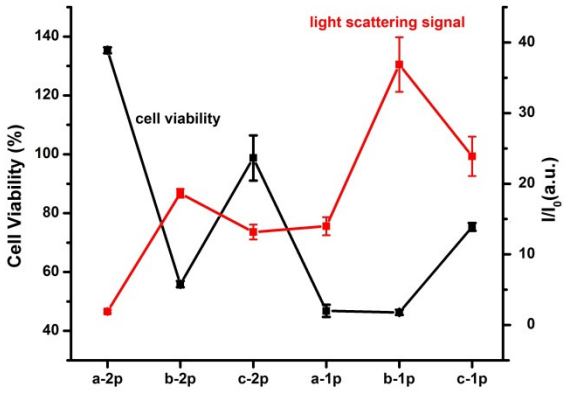


Figure S5. The trend of cell viabilities is largely opposite to that of the signal intensity of SLS. The cell viability is measured at the concentration of 200 μM on HeLa cell.

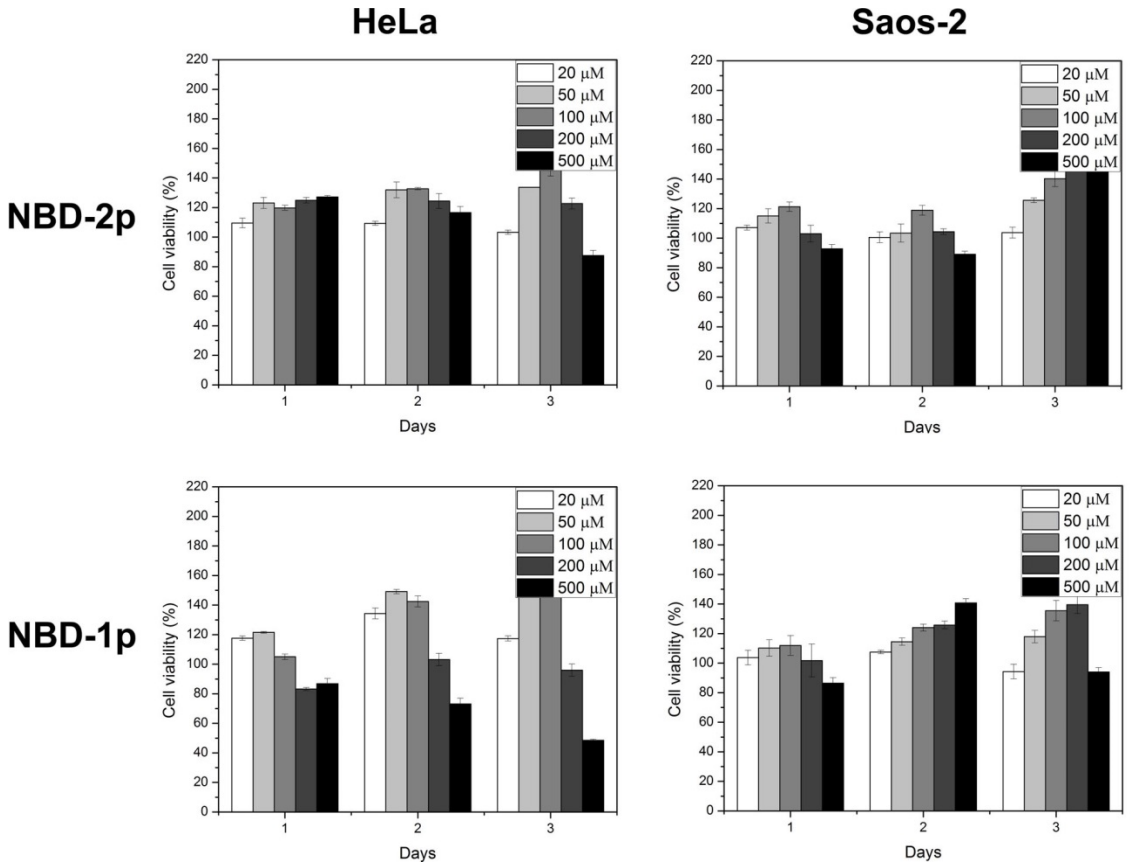


Figure S6. Cell viability of HeLa and Saos-2 cells treated with **NBD-2p** and **NBD-1p** at different concentration.

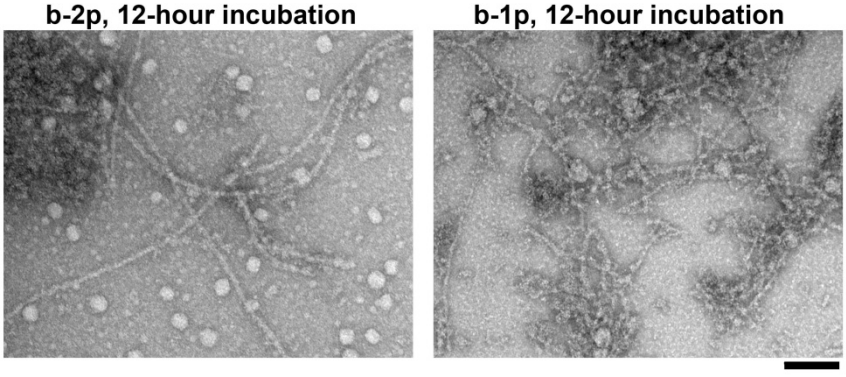


Figure S7. TEM images of the nanofibrils formed on the cell membrane after 12 h incubation of **b-2p** (500 μM) or **b-1p** (500 μM) with HeLa cells. The scale bar is 100 nm.

S9. Sample preparation for confocal microscopy

Life cell imaging: HeLa/Saos-2 cells in exponential growth phase were seeded in glass bottomed culture chamber at 1×10^5 -- 1×10^5 cell/well. The cells were allowed for attachment for 12 h at 37 ºC, 5% CO_2. The culture medium was removed, and new culture medium containing **b-2p**/**1p** and **NBD-2p/1p** at 500 μM was added. After incubation for certain time, cells were stained with 1.0 μg/ml Hochst 33342 for 5 or 10 min at 37 ºC in dark. After that, cells were rinsed three times by PBS buffer, and then kept in the live cell imaging solution (Invitrogen Life Technologies A14291DJ) for imaging.

Antibody staining:

- Seed cell (200,000 cell/3cm confocal dish) and allow attachment (overnight)
- Wash by PBS buffer 3X and 4% formaldehyde fixed (15 min)
- Wash by PBS buffer 3X and incubated in 1.0 %BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions.
- Wash by PBS buffer 3X and incubated with the antibody (1/100) (e.g., ALPP and ALPL) overnight at +4°C.
- Wash by PBS buffer 3X and the secondary antibody (green) was ab150077 Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at 2μg/ml (1/1000) for 1h.
- Hoechst 33342 was used to stain the cell nuclei (blue).
- Wash by PBS buffer 3X and mount for imaging.

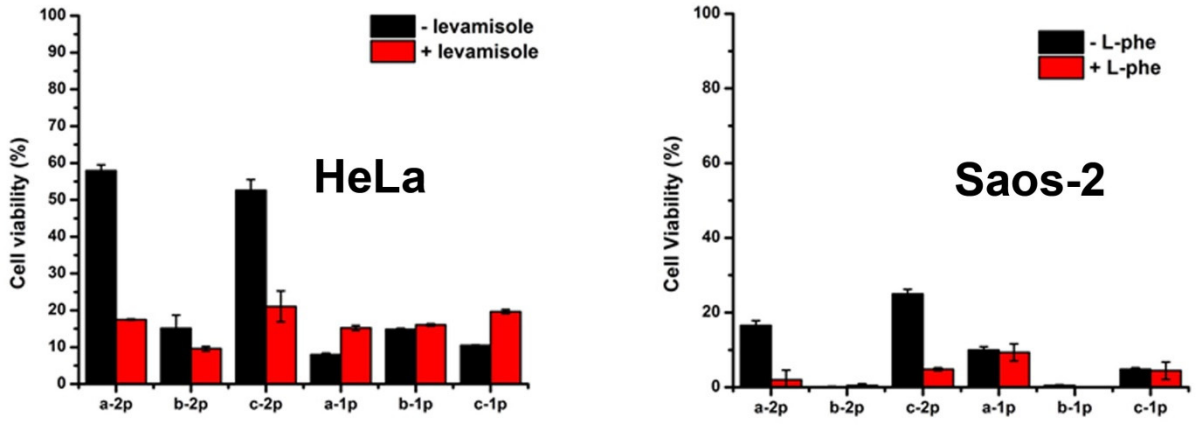


Figure S8. Viability of HeLa and Saos-2 cells incubated with six precursors (500 μM) with or without different phosphotase inhibitors for 48 h; [L-phe] = 3 mM; [levamisole] = 1 mM.

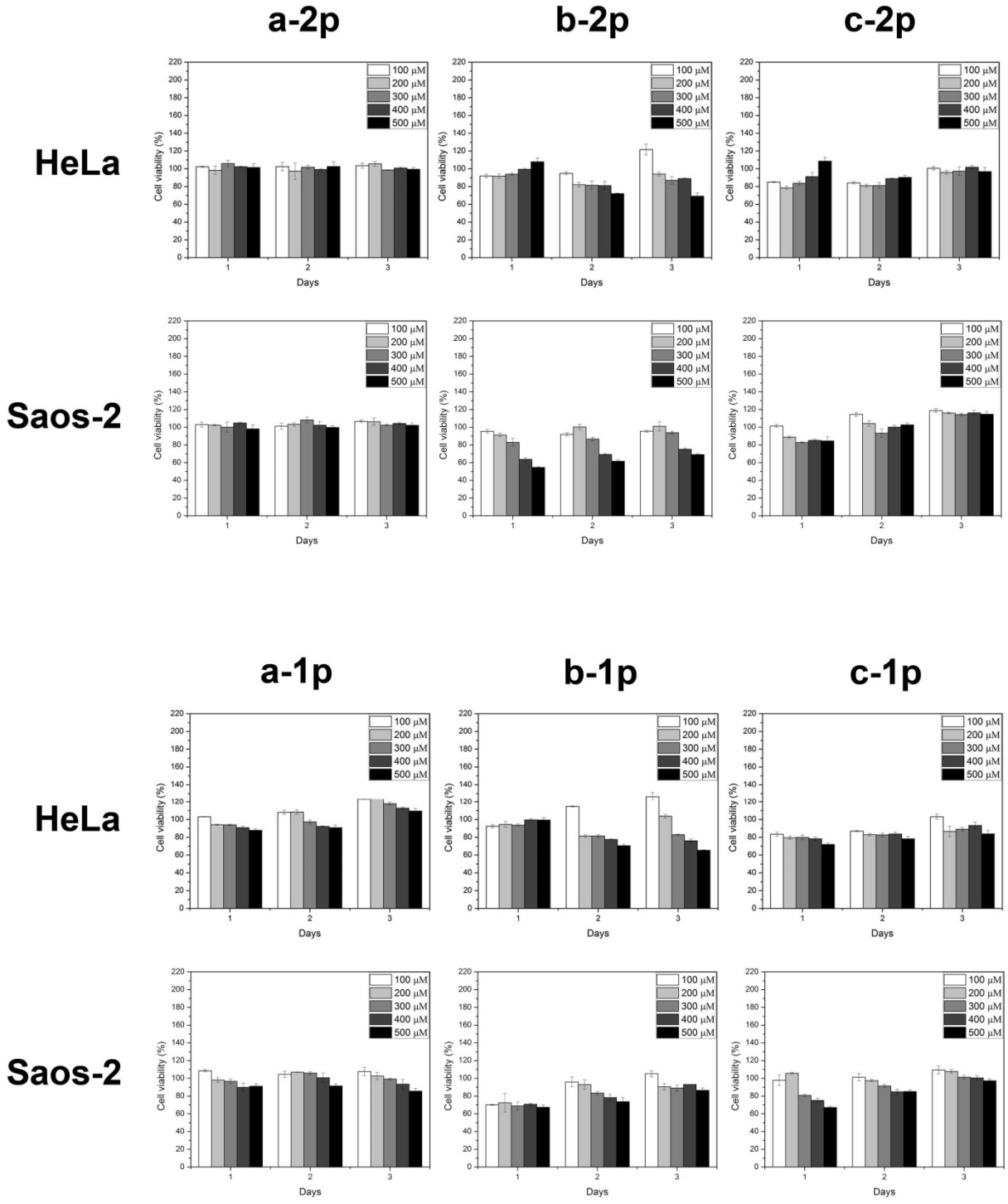


Figure S9. Cell viability of HeLa and Saos-2 cells treated by different precursors together with ALP (5U/mL). The toxicity is completely eliminated.

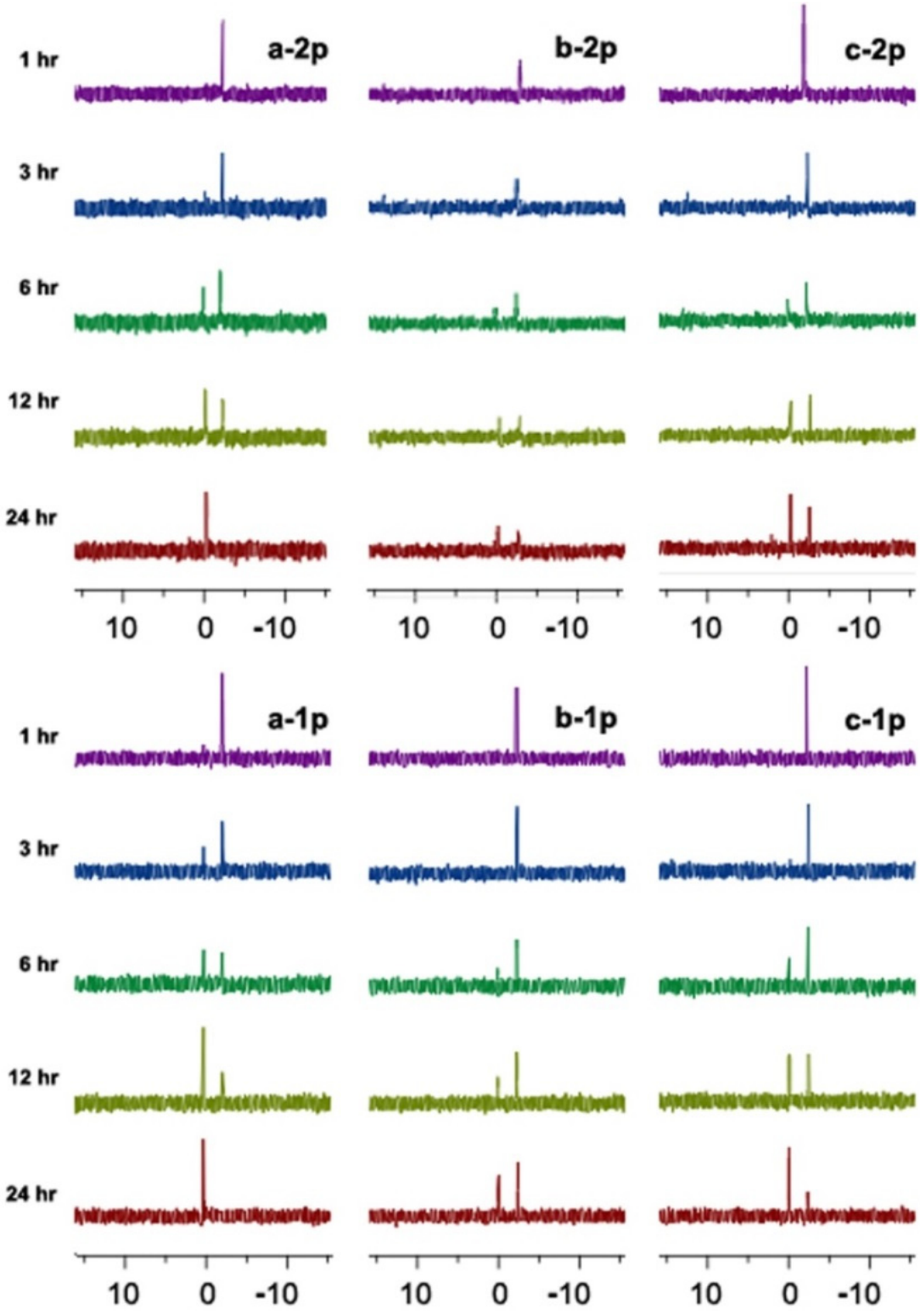


Figure S10. ^{31}P NMR shows the conversion of 0.5 wt % of **a-2p**, **b-2p**, **c-2p**, **a-1p**, **b-1p** and **c-1p** catalyzed by the phosphatase (0.05 U/mL) in Tris buffer at different time points. pH = 7.4.

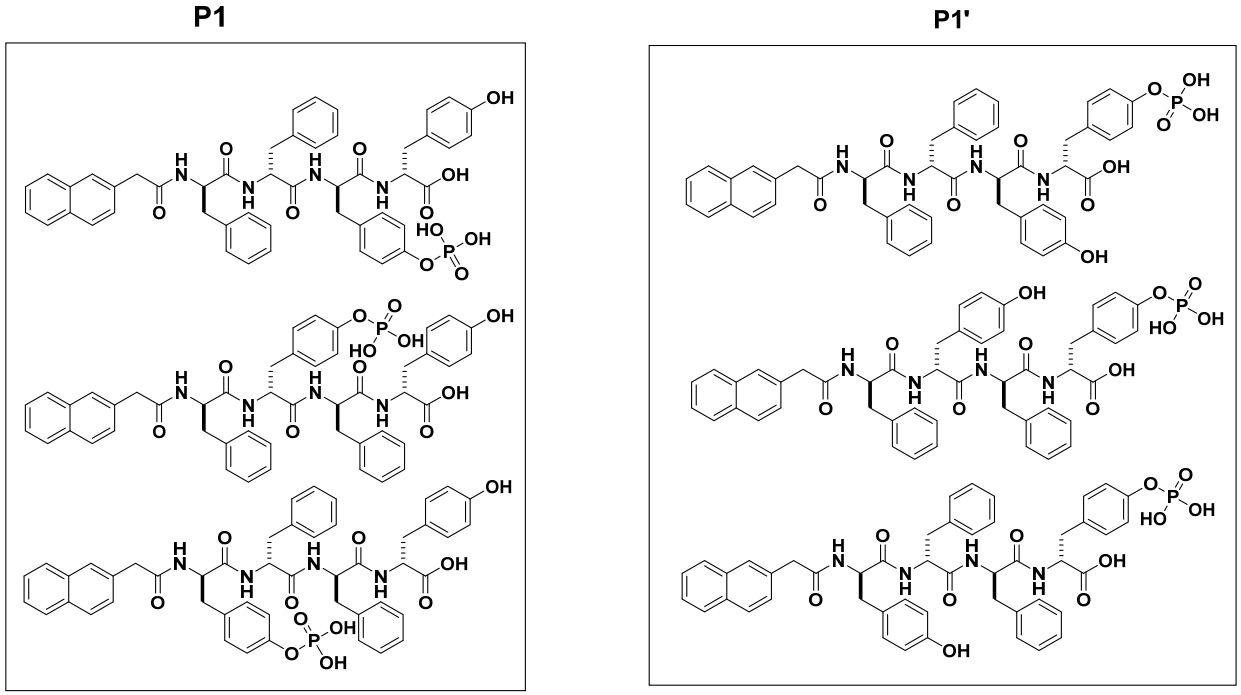


Figure S11. Chemical structures of intermediates **P1** and **P1'**. P1 means only one phosphate remains.