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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 unless otherwise stated. Molecular probe **1a** and **1b** were purified using Water Delta600 HPLC system, equipped with an XTerra C18 RP column and an in-line diode array UV detector. The LC-MS spectrums were obtained on Waters Acquity Ultra Performance LC with Waters MICROMASS detector, ultraviolet-visible (UV) spectra on JASCO J-810 spectrophotometer, ^{1}H NMR spectra on Varian Unity Inova 400, TEM images on a Morgagni 268 transmission electron microscope, and confocal microscopy images on Zeiss LSM 880 confocal microscopy at the lens of 63× with oil.

S2. Synthesis and characterizations

Synthesis of compound 3. TBSCl (1 eq.) and Et_3N (1 eq.) were added at room temperature to a solution of 3-formyl-4-hydroxybenzoic acid in THF (4 to 10 mL/mmol). After 30 min, the solution was cooled to 0 ºC and treated with CBr_4 (3 eq.). Following by sequential addition of $HP(O)(OEt)_2$ (2 eq.) and Et_3N (3 eq.) in the dark, the reaction was stirred overnight in the dark with gradual warming to room temperature. The next day, most of the THF was removed on rotary evaporator, the concentrated solution was partitioned between Et_2O and 5% aq. $NaHCO_3$/Na_2SO_3, and the organic layer was discarded. The aqueous layer was acidified to pH = 2 and extracted with EtOAc for 3 times. The combined organic extracts were dried with Na_2SO_4, concentrated, and the residue was purified by silica gel flash chromatography to afford pure diethyl phosphate protected 3-formyl-4-hydroxybenzoic acid.[1] The diethyl phosphate protected 3-formyl-4-hydroxybenzoic acid (1 eq.), 2-amino-5-chlorobenzamide (1.2 eq.), and TsOH (0.06 eq.) were dissolved in dry EtOH (100 mL/mmol) and then refluxed for 5 h. After the reaction solution was cooled to room temperature for 2 h, water (20 mL/mmol) was added, and the precipitation was vacuum filtrated and washed with EtOH to afford compound **3** without further purification.[2]

Synthesis of compound 4. We used solid phase peptide synthesis (SPPS)[3] for the synthesis of compound **4**. As shown in Scheme S1b, we used 2-chlorotrityl chloride resin and N-Fmoc amino acids with side chain protecting groups for the synthesis. After loading Fmoc-Tyr(tBu)-OH as the first amino acid residue on the resin, and removing the Fmoc group, we sequentially added and deprotected Fmoc-Phe-OH, Fmoc-Phe-OH for obtaining the sequence of ^{D}Phe-^{D}Phe-^{D}Tyr on the resin. Then we used compound **3** to cap the N-terminal of the tripeptide. After cleaving the peptides from resin, we used compound **4** without further purification.

Synthesis of compound 1a. N,O-Bis(trimethylsilyl)acetamide (BSA) (10 eq.) was added at room temperature to a solution of **4** in DCM. After 30 min, the solution was cooled to 0 ºC and treated with TMSI (8 eq., dropwise addition). After 1 h at 0 ºC and an additional 2 h at room temperature, the reaction mixture was concentrated under vacuo. The residue was treated with a mixture of CH_3CN/TFA/H_2O (10/3/5) for 1 h at room temperature. This was followed by concentration under vacuo, and the resulting product was dissoved in DCM. After that, TMSBr was added dropwise to afford product **1a**, which was purified by reversed-phase high-performace liquid chromatography (HPLC).

Synthesis of compound 1b. Compound **4** (1 eq.), CH_3NH_2·HCl (1.5 eq.), HBTU (1.1 eq.), DIEA (2.5 eq.) were dissovled in DMF and stirred overnight. After that, DMF was removed. N,O-Bis(trimethylsilyl)acetamide (BSA) (10 eq.) was added at room temperature to a solution of **4** in DCM. After 30 min, the solution was cooled to 0 ºC and treated with TMSI (8 eq., dropwise addition). After 1 h at 0 ºC and an additional 2 h at room temperature, the reaction mixture was concentrated under vacuo. The residue was treated with a

mixture of $CH_3CN/TFA/H_2O$ (10/3/5) for 1 h at room temperature. This was followed by concentration under vacuo, and the resulting product **1b** was purified by reversed-phase high-performace liquid chromatography (HPLC).

S3. 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 (3 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.

S4. Light scattering sample preparation

The static light scattering experiments were performed by 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 dissolved in PBS buffer. The addition of ALP to the solution of 1a and 1b for 24 h, we obtained corresponding enzymatic dephosphorylated samples. The SLS tests were carried out at room temperature, and the angles of light scattering we chose were 30º, 60º, 90º and 120º, respectively. The resulting intensity ratios are proportional to the amount of aggregates in the samples.

S5. Cell culture and cell viability assay

All cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The HeLa, HepG2, and MCF7 cells were propagated in Minimum Essential Media (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics in a fully humidified incubator containing 5% CO_2 at 37 ºC, Saos-2 cells in McCoy's 5A supplemented with 15% FBS and 1% antibiotics in a fully humidified incubator containing 5% CO_2 at 37 ºC, HS-5 and VCaP cells in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% antibiotics in a fully humidified incubator containing 5% CO_2 at 37 ºC, and PC-3 in Kaighn's Modification of Ham's F-12 Medium (F-12K) supplemented with 10% FBS and 1% antibiotics in a fully humidified incubator containing 5% CO_2 at 37 ºC.

Cells in exponential growth phase were seeded in a 96 well plate at a concentration of 1×10^4 cell/well, and were allowed to attach to the well for 24 h at 37 ºC, 5% CO_2. The culture medium was removed and 100 μL culture medium containing corresponding compounds (immediately diluted from fresh prepared stock solution) at gradient concentrations (0 μM as the control) was placed into each well. After culturing at 37 ºC, 5% CO_2 for 24h, 48h and 72h, each well was added with 10 μL of 5 mg/mL MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and the plated cells were incubated at dark for 4h. 100 μL 10% SDS with 0.01M HCl was added to each well to stop the reduction 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.

S6. Sample preparation for confocal microscopy

Live cell imaging: The HeLa cells in exponential growth were seeded in glass bottomed culture chamber at 10 × 10^4 cell/well, and were allowed for attachment for 24 h at 37 ºC, 5% CO_2. The culture medium was removed, and fresh culture medium containing **1a** and **1b** at certain concentrations was added. After incubation for certain time, cells were rinsed three times by PBS buffer and then kept in live cell imaging solution (Invitrogen Life Technologies A14291DJ) for imaging.

S7. Description of videos

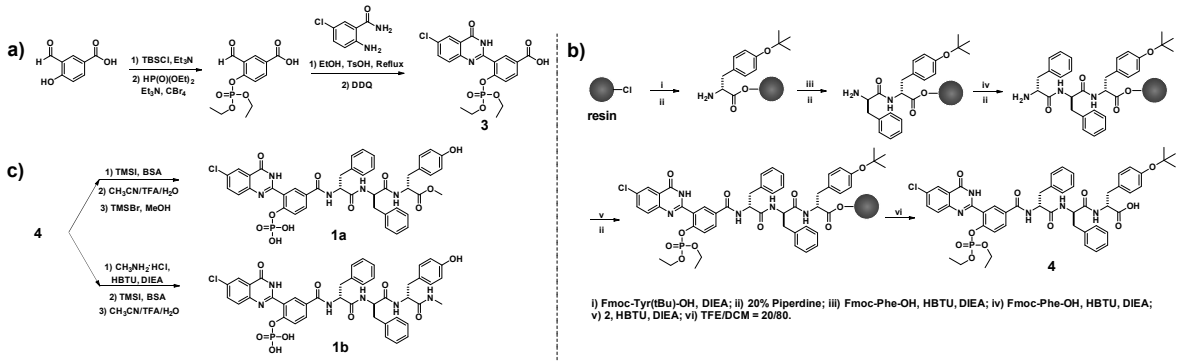
Video S1

Time-dependent fluorescence of 1a (250 μM) in live Saos-2 cells for 30 min.

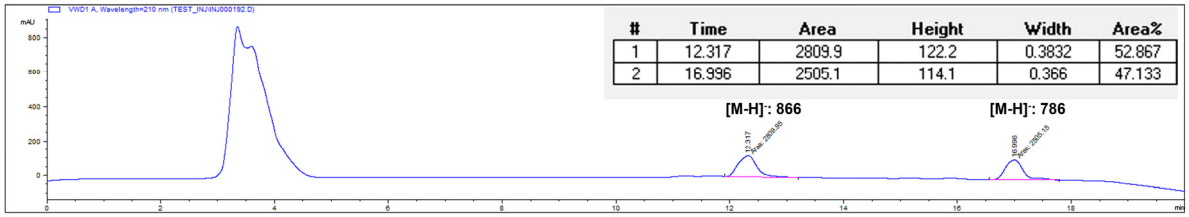
Video S2

Time-dependent fluorescence of 1a (250 μM) with the addition of DQB (5 μM) in live Saos-2 cells for 30 min. Saos-2 cells were pretreated with DQB for 2 h.

S8. Supplemental figures

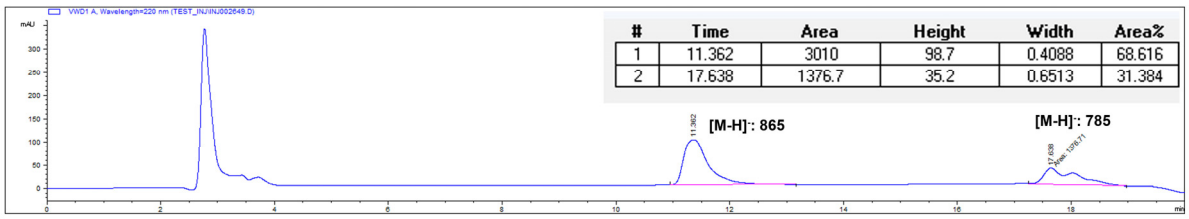


Scheme S1. The synthetic route of D-tripeptide conjugated quinazolinone derivatives. (a) Synthesis of ethyl protected phosphorylated quinazolinone derivative **3**. (b) Synthesis of D-tripeptides conjugated quinazolinone derivative **4** containing carboxylic acid groups at C-terminus by SPPS. (c) Synthesis of D-tripeptides conjugated quinazolinone derivatives **1a** and **1b** by replacing the carboxylic acid intermediates **4** with *N*-methylacetamide and methylacetate, respectively.



#	Time	Area	Height	Width	Area%
1	12.317	2809.9	122.2	0.3832	52.867
2	16.996	2505.1	114.1	0.366	47.133

Figure S1. The LC-MS spectrum shows the dephosphorylation of **1a** with the addition of ALP. The conversion of **1a** is 47% after 30 h. [**1a**] = 200 μM, [ALP] = 0.1 U/mL.



#	Time	Area	Height	Width	Area%
1	11.362	3010	98.7	0.4088	68.616
2	17.638	1376.7	35.2	0.6513	31.384

Figure S2. The LC-MS spectrum shows the dephosphorylation of **1b** with the addition of ALP. The conversion of **1b** is 31% after 30 h. [**1b**] = 200 μM, [ALP] = 0.1 U/mL.

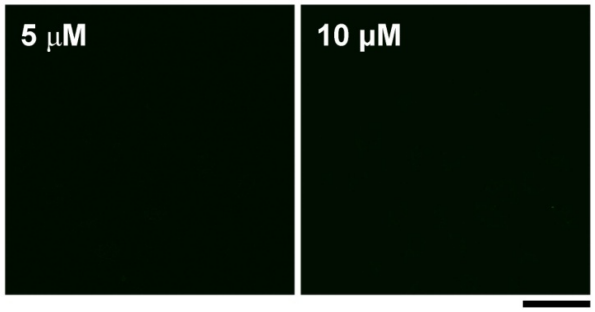


Figure S3. Fluorescent confocal microscopy images show the fluorescence emission in HeLa cells with the treatment of **1a** at low concentrations (5 μM and 10 μM) for 4 h, respectively. Scale bar = 50 μm.

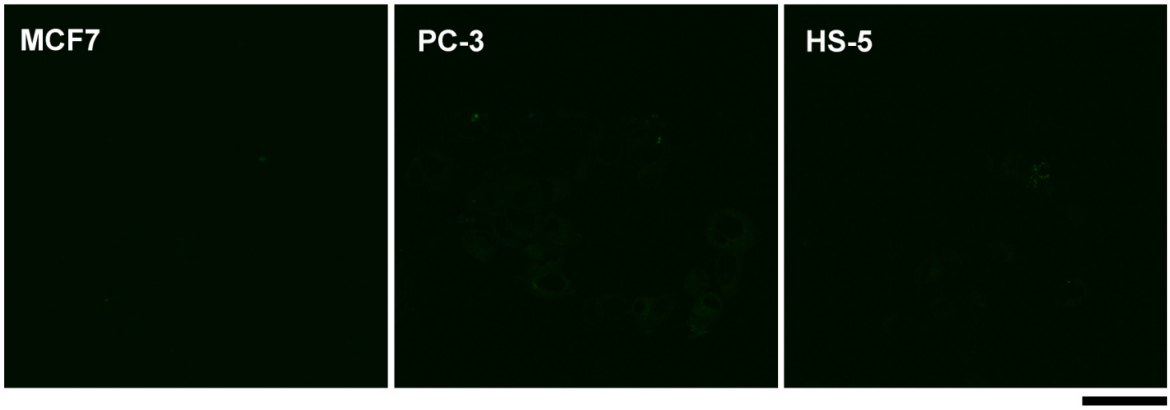


Figure S4. Fluorescent confocal microscopy images show the fluorescence in different cell line (MCF7, PC-3 and HS-5) with the treatment of **1a** at the concentration of 50 μM for 4 h, respectively. Scale bar = 50 μm.

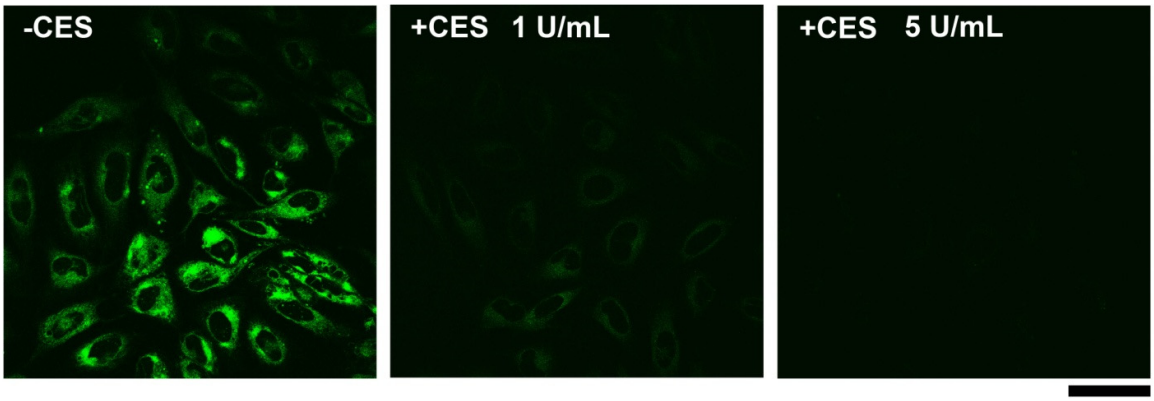


Figure S5. Fluorescent confocal microscopy images show the fluorescence in Saos-2 cells with the treatment of **1a** at the concentration of 50 μM with the addition of carboxylesterase (CES) at different concentrations (1 U/mL and 5 U/mL) for 4 h, respectively. Scale bar = 50 μm.

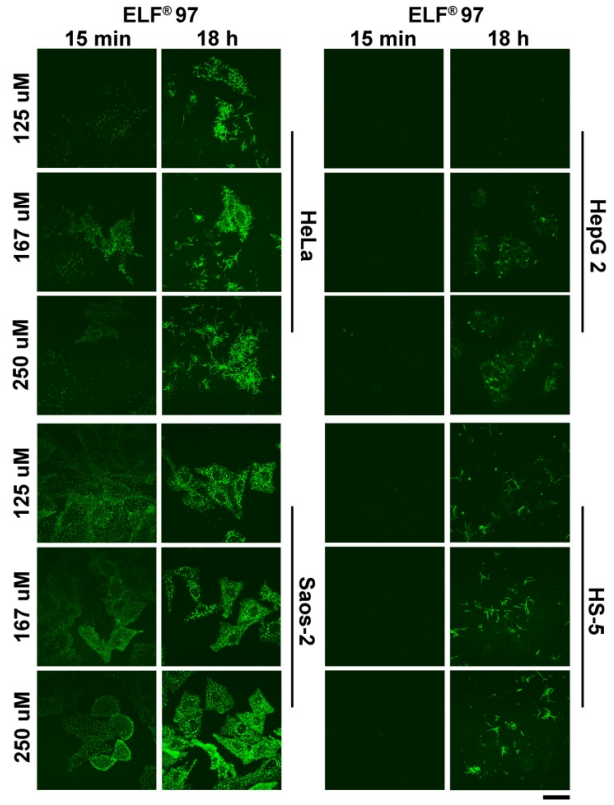


Figure S6. Fluorescent confocal microscopy images show green fluorescence in HeLa and Saos-2 cells, while low fluorescence in HepG2 and HS-5 cells. The images were taken according to the ELF®97 standard protocols. Cell were fixed by 4.0% formaldehyde and permeabilized by 0.2% tween-20. Fixed cells were treated with ELF®97 for 15 min and 18 h, respectively. Scale bar = 50 μm.

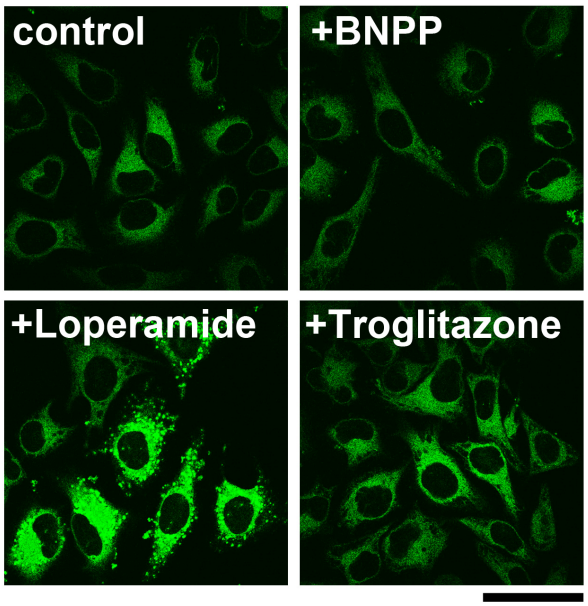


Figure S7. Fluorescent confocal microscopy images show fluorescence emission in live HeLa cells with the treatment of **1a** at the concentration of 50 μM with the addition of different esterase inhibitors (bis(p-nitrophenyl) phosphate (BNPP), loperamide and troglitazone) for 4 h, respectively. HeLa cells were pretreated with inhibitors for 2 h and then co-incubated for 4 h. [BNPP] = 100 μM, [loperamide] = 10 μM, [troglitazone] = 10 μM. Scale bar = 50 μm.

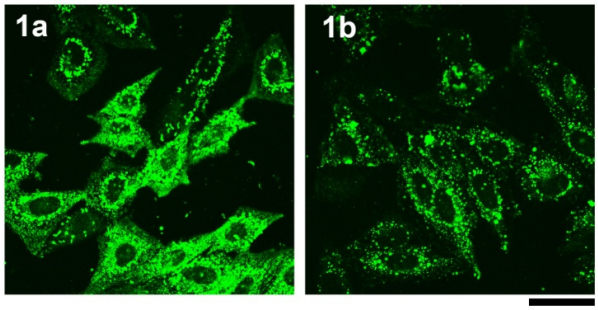


Figure S8. Fluorescent confocal microscopy images show bright fluorescence in fixed Saos-2 cells. Cell were fixed by 4.0% formaldehyde and permeabilized by 0.2% tween-20. Fixed cells were treated with **1a** and **1b** for 4 h, respectively. [**1a**] = [**1b**] = 50 μM. Scale bar = 50 μm.

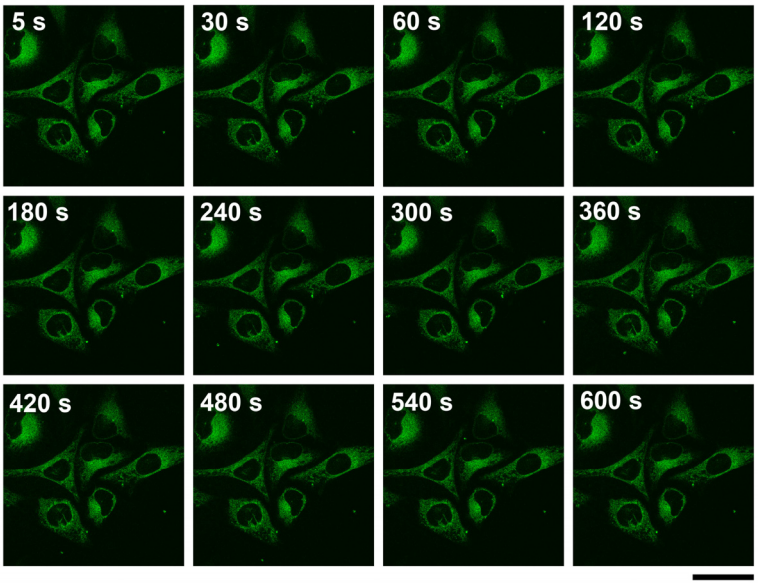


Figure S9. Fluorescent confocal microscopy images show the fluorescence changes little in HeLa cells with the increasing scanning times (5 s - 600 s). HeLa cells were incubated with **1a** for 4h. [**1a**] = 50 μM; scale bar = 50 μm.

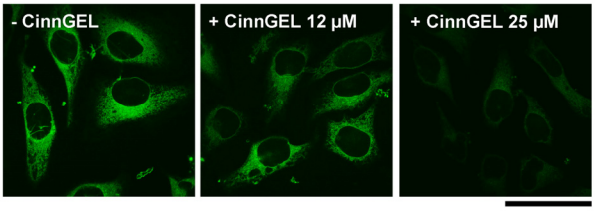


Figure S10. Fluorescent confocal images of HeLa cells with the treatment of **1a** plus different concentrations of PTP1B inhibitor (CinnGEL) for 4 h. Cells were pretreated with CinnGEL for 30 min before co-incubated with **1a** for 4 h. [**1a**] = 50 μM; scale bar = 50 μm.

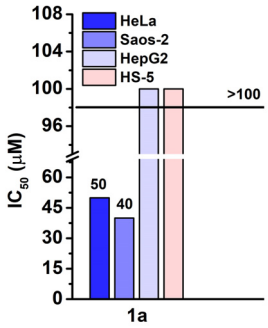


Figure S11. IC_{50} summary of **1a** and **1b** against HeLa, Saos-2, HepG2 and HS-5 cells for 24 h, respectively.

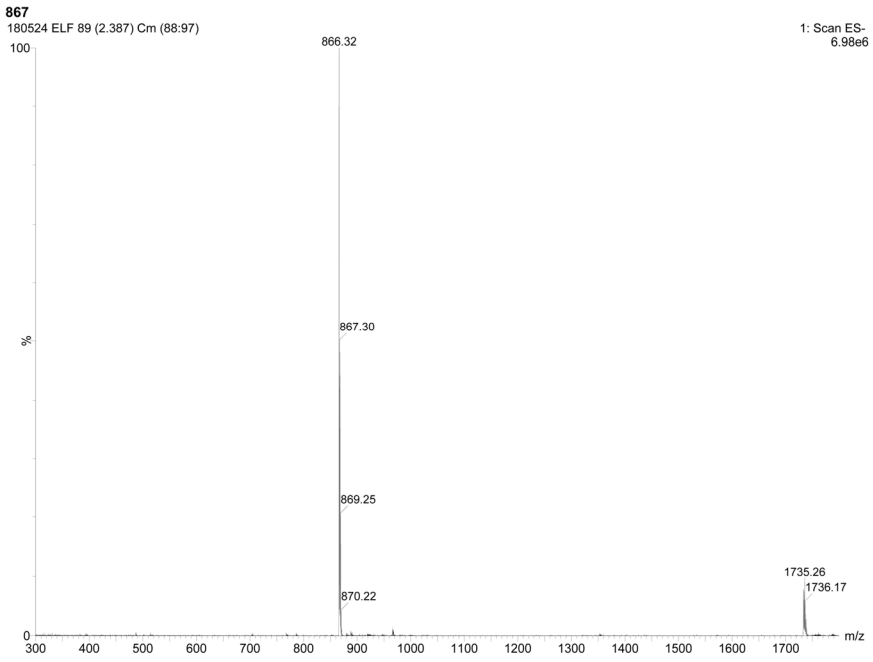


Figure S12. The Mass spectrum of **1a**.

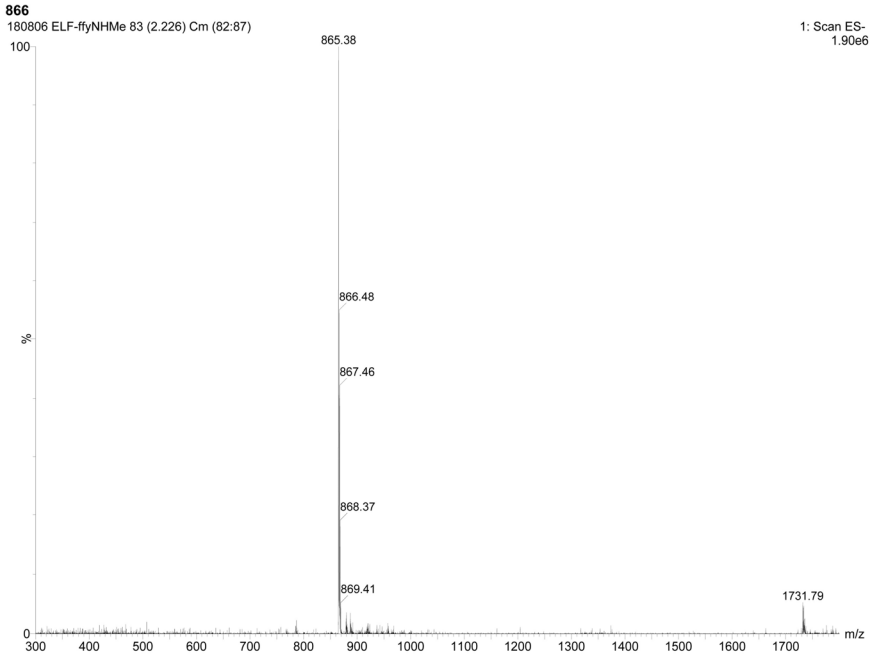


Figure S13. The Mass spectrum of **1b**.

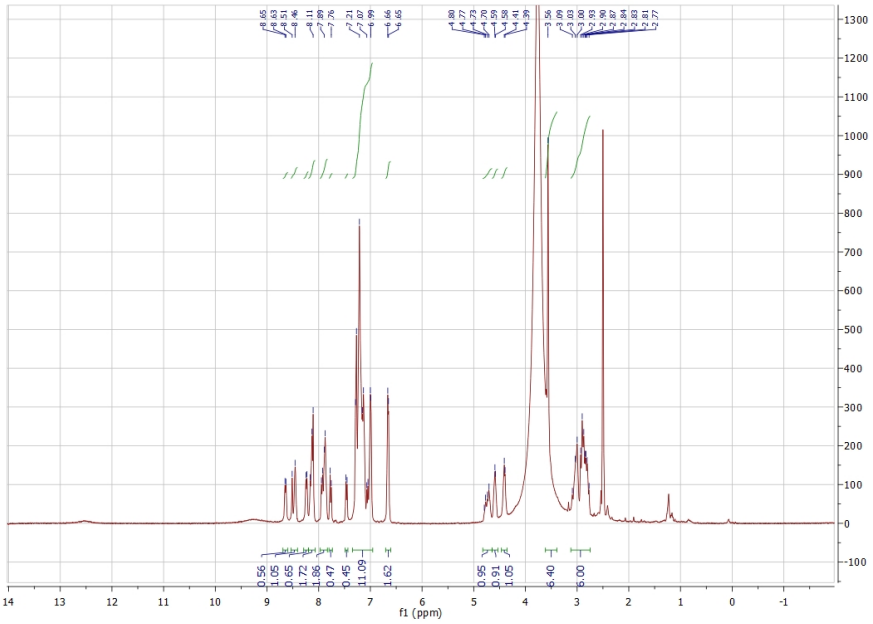


Figure S14. ^{1}H NMR of **1a** in DMSO-d_6.

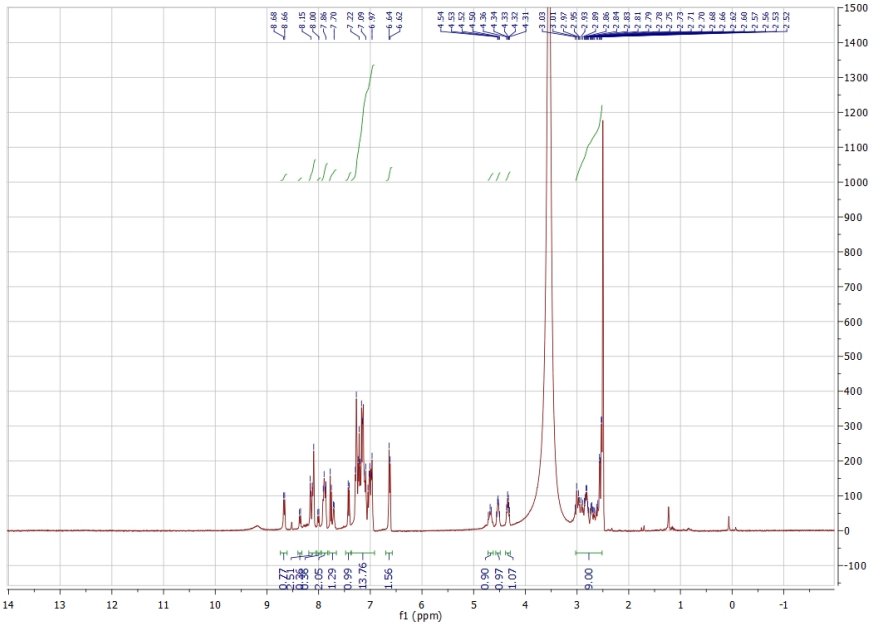


Figure S15. ^{1}H NMR of **1b** in DMSO-d_6.

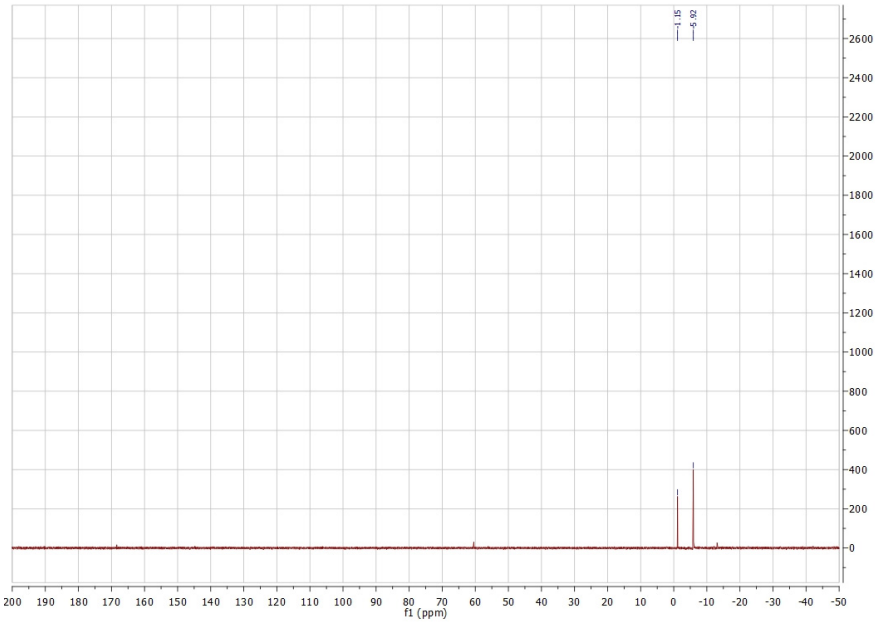


Figure S16. ^{31}P NMR of **1a** in DMSO-d_6.

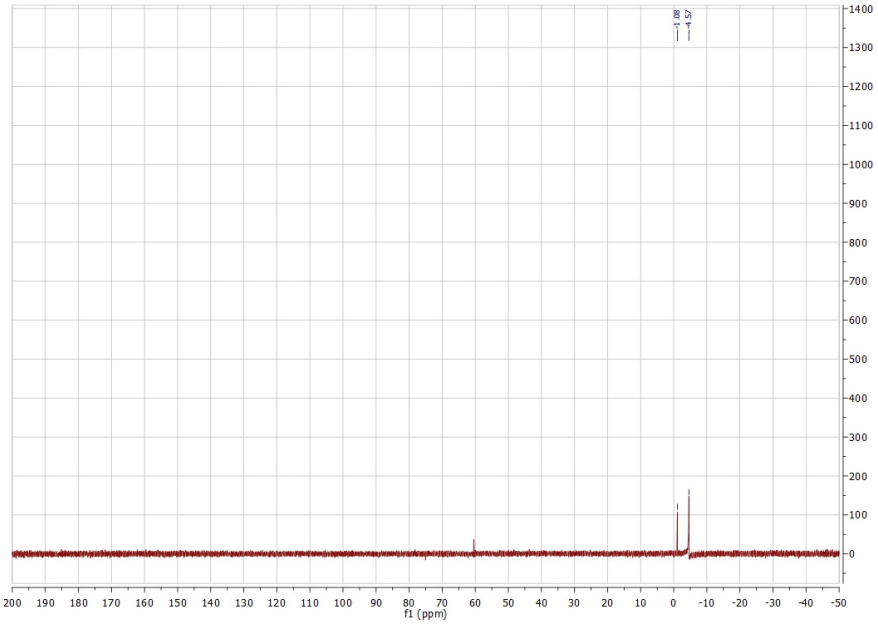


Figure S17. ^{31}P NMR of **1b** in DMSO-d_6.

[1] A. K. Szardenings, M. F. Gordeev, D. V. Patel, *Tetrahedron lett.* **1996**, *37*, 3635-3638.
[2] H.-W. Liu, K. Li, X.-X. Hu, L. Zhu, Q. Rong, Y. Liu, X.-B. Zhang, J. Hasserodt, F.-L. Qu, W. Tan, *Angew. Chem. Int. Ed.* **2017**, *56*, 11788-11792.
[3] C. Weng, D. Peter, *Fmoc Slild Phase Peptide Synthesis: A Practical Approach*, Oxford: Oxford University Press, **2000**.