Supporting Information

Enzyme-Instructed Self-Assembly with Photo-Responses for Photo-Regulation of Cancer Cells

Zhengquan Zhou,[a] Xian Xie,[a] Qikun Yi,[a] Wencui Yin,[b] Adnan A. Kadi,[b] Jinbo Li[a,*] and Yan Zhang[a,*]

[a]*State Key Laboratory of Analytical Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China.*

[b]*Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Kingdom of Saudi Arabia.*

*Emails: njuzy@nju.edu.cn; jinboli@nju.edu.cn

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Materials and Methods

1. General materials and methods

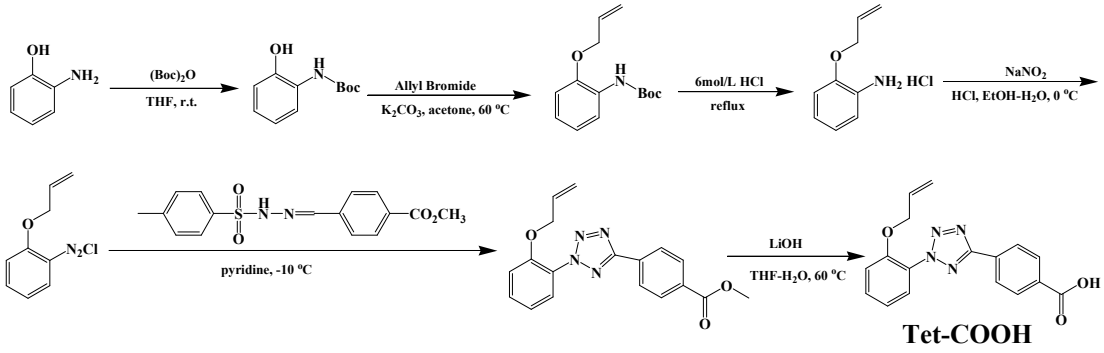
All chemicals and solvents were purchased from J&K chemicals (Shanghai, China) or Sigma-Aldrich (Shanghai, China). High glucose Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin were purchased from Life Technologies (Shanghai, China). MTT was purchased from Sigma-Aldrich (Shanghai, China). Alkaline Phosphatase Assay Kit was purchased from Beyotime Biotechnology (Shanghai, China).

^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were obtained on a 400 MHz Bruker AVANCE III-400 spectrometer. Chemical shifts are reported in δ (ppm) relative to the solvent residual peak. Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). MS and HRMS were performed on a SHIMADZU LCMS-2020 and Agilent 6550 iFunnel Q-TOF LC/MS; Transmission electron microscopy (TEM) images were taken on a JEM-2100 LaB6 transmission electron microscope (JEOL); HPLC was carried out on an Agilent 1200 LC (analytic) or Waters 2535 LC (preparative) with CH_3OH/H_2O (0.1% CF_3COOH) or CH_3CN/H_2O (0.1% CF_3COOH) as eluents.

2. Synthesis and Characterizations

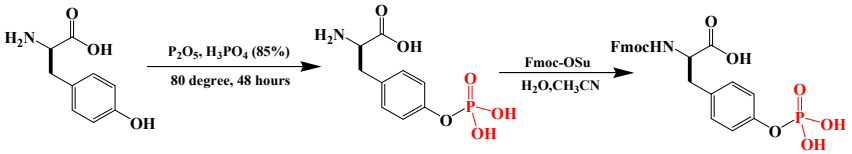
Synthesis of the tetrazoles

The tetrazoles were synthesized according to the literature procedure.[1, 2]



Synthesis of the Fmoc-D-Tyr-H_2PO_3

The Fmoc-D-Tyr-H_2PO_3 was synthesized according to the literature procedure.[3]



Fmoc-D-Tyr-H_2PO_3

Tetrazole-linked peptides

The tetrazole-inked peptides were synthesized using a standard solid phase peptide synthesis (SPPS) protocol by 2-chlorotrityl chloride resin (100–200 mesh and ~1.0 mmol • g^{-1}) and N-Fmoc-protected amino acids. The resin was swelled in dry dichloromethane (DCM) for 5 min, and then the first amino acid was loaded onto the resin at its C-terminal with Fmoc-protected amino acid (3 equiv.) and N,N-diisopropylethylamine (DIPEA) in DCM for 3 h. After wash with DCM (3 × 5 mL), the resin was treated with the blocking solution (DCM/MeOH/DIPEA, 8/1.5/0.5) for 20 min to deactivate the unreacted sites. Then the resins were treated with 20% piperidine (in DMF) for 40 min to remove the protecting group, followed by coupling Fmoc-protected amino acid (5 equiv.) to the free amino group on the resin using HOBt/HBTU as the coupling reagent. These two steps were repeated to elongate the peptide chain, which were carried out by the standard Fmoc SPPS protocol. Tet-COOH was also loaded using same protocol. The resin was washed with DMF for 3~5 times after each step. At the final step, the peptide was cleaved with 90% TFA (5 mL) for 2 h and then the resulted crude products were purified by reverse phase HPLC.

Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.07 (s, 1H), 8.22 (d, 2H), 8.13-8.07 (t, 3H), 7.76-7.73 (dd, J = 1.6 Hz, J = 7.6 Hz, 1H), 7.67 (dt, J = 1.6 Hz, J = 7.6 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.24-7.02 (m, 11H), 5.98-5.88 (m, 1H), 5.28-5.22 (dd, J = 2.0 Hz, J = 17.2 Hz, 1H), 5.19-5.16 (dd, J = 1.6 Hz, J = 10.8 Hz, 1H), 4.70 (d, J = 4.4 Hz, 2H), 4.59-4.53 (m, 1H), 4.43-4.37 (m, 1H), 3.82 (m, 2H), 3.58(s, 2H), 3.01-2.96 (m, 2H), 2.88-2.78 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 173.4, 171.2, 168.9, 166.1, 163.9, 152.7, 138.1, 136.3, 133.2, 130.2, 129.7, 129.5, 128.9, 128.4, 127.8, 126.8, 126.6, 126.2, 121.4, 120.0, 117.6, 115.0, 69.3, 54.1, 53.6, 41.9, 38.4, 36.7; ^{31}P NMR (162 MHz, DMSO-d_6) δ -5.76; MS (ESI+) *m/z*: 792.05 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{37}H_{36}N_7O_{10}PNa$ 792.3164 $[M+ Na]^+$; found 792.3172.

Tet-G^{D}F^{D}Y

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (s, 1H,), 9.19 (s, 1H), 8.86 (s, 1H), 8.24 (d, 3H), 8.10-8.06 (t, 3H), 7.74 (d, J = 7.6 Hz, 1H), 7.67 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.24-7.15 (m, 5H), 7.02 (d, J = 7.6 Hz, 2H), 6.66 (d, J = 7.6 Hz, 2H), 5.98-5.88 (m, 1H), 5.28-5.23 (d, J = 17.2 Hz, 1H), 5.19-5.16 (d, J = 10.4 Hz, 1H), 4.70 (d, J = 2.8 Hz, 2H), 4.58-4.54 (m, 1H), 4.35-4.31 (m, 1H), 3.4-3.78 (m, 2H), 3.04-2.94 (m, 2H), 2.86-2.73 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 173.4, 171.4, 169.0, 166.2, 163.9, 156.4, 152.7, 138.2, 136.3, 133.2, 130.6, 129.8, 129.5, 128.9, 128.4, 128.0, 127.8, 126.9, 126.7, 126.2, 121.4, 117.6, 115.5, 115.0, 69.3, 54.5, 54.0, 42.9, 38.1, 36.5; MS (ESI+) *m/z*: 712.10 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{37}H_{35}N_7O_7Na$ 712.2653 $[M+ Na]^+$; found 712.2691.

Pyr-G^{D}F^{D}Y

Tet-G^{D}F^{D}Y (69 mg, 0.1 mmol) in 50 mL CH_3CN/PBS (1/1) was irradiated with a hand-held 302 nm UV lamp for 45 minutes. CH_3CN was removed and the aqueous layer was purified by HPLC (The eluents for HPLC was CH_3CN/H_2O (7/3) (0.1% CF_3COOH) to give a yellow powder (54 mg, 83 %).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.19 (s, 1H), 8.88 (d, 1H), 8.76 (t, J = 1.6 Hz, 1H), 8.44 (d, J = 8.4 Hz, 1H), 8.23 (d, 1H), 8.07 (d, 1 H), 8.02-7.97 (m, 2 H), 7.79-7.73 (m, 1H), 7.70-7.65 (m, 1H), 7.46-7.39 (m, 1H), 7.16-7.13(m, 3 H), 7.06-7.00(m, 4 H), 6.65 (d, 2H), 5.27-5.22 (m, 1H), 5.19-5.16 (m. 1H), 4.70 (d, 1H), 4.62-4.54 (m, 2H), 3.94-3.81 (m, 2H), 2.99-2.94 (dd, J = 4.0 Hz, J = 13.2 Hz, 2H), 2.78-2.69 (m, 3H), 2.59-2.55 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 173.4, 171.2, 168.9, 166.3, 156.3, 149.8, 144.8, 138.2, 134.9, 134.1, 133.8, 131.5, 130.6, 130.1, 129.7, 128.4, 128.2, 128.1, 126.7, 126.2, 121.9, 120.9, 120.2, 118.2, 117.9, 117.7, 117.4, 115.4, 114.1, 69.3, 56.6, 54.1, 42.9, 39.7, 38.1, 36.5; MS (ESI+) *m/z*: 684.55 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{37}H_{35}N_5O_7Na$ 684.3572 $[M+ Na]^+$; found 684.3621.

3. EISA and PIDA

Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3) (5 mM) was dissolved in 1x PBS (pH=7.4) in a glass vial by

ultrasonic, and ALP was added with a final concentration of 5 U/mL. The mixture was incubated at 37 ^{o}C and gel formed after 24 hrs' incubation. The formed gel was further subject to irradiation with an 8 W hand-held 302 nm UV lamp for 1-5 min.

4. TEM

TEM samples were prepared by direct dipping the sample onto the copper omentum and dried in air for 30 min.

5. CD spectra

CD spectra were acquired on a Jasco J-810 spectropolarimeter. A quartz cuvette with a sample thickness of 1 mm was used as the container for hydrogel or solution samples to be tested.

6. Rheology test

All rheological measurements were performed on a HAAKE RheoStress 6000 rheometer (Thermo Scientific) equipped with a UV measuring cell. The tests were carried out with a cone and a plate (19.992 mm diameter plate and 1° cone angle). The gap opening between the cone and the plate was set to be 0.051 mm. The dynamic frequency sweep test of the hydrogels was investigated at 1% strain. The in situ photo-degradation rate of the hydrogel was determined at a frequency of 1 rad s^{-1} and a strain of 1%. For the in situ photo-degradation test, optically thin gels (0.051 mm) were placed between a cone and a clear quartz plate (3 mm thickness). After full gelation, the sample was exposed to UV light by EXFO Omnicure S2000 200 W (40% output with a 302 nm filter) and degradation was quantified by monitoring the storage modulus G′ compared to the full gelation storage modulus G'_0 with constant exposure.

7. Cell culture

HeLa or 3T3 cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were maintained in a fully humidified incubator containing 5% CO_2 at 37 °C.

8. Measurement of relative ALP expression levels

ALP level was measured according to literature procedure.[3] HeLa and 3T3 cells were seeded in 96-well plate with cell density of 50,000 cells/well. After incubation at 37 ^{o}C for 4 h, medium was removed and cells were washed with 1xPBS buffer for three times. After adding 100 μL water, cells were incubated in a fully humidified incubator containing 5% CO_2 at 37 °C for 1 h. Then the cells were stored at -80 ºC for 1 h. Finally, the supernatant was collected after defrosting and relative ALP level was measured by using Alkaline Phosphatase Assay Kit.

9. Confocal imaging

HeLa cells or 3T3 cells were seeded in glass bottomed culture chamber with 2x10^4 cells/well. The cells were cultured for 12 h in a fully humidified incubator containing 5% CO_2 at 37 °C. The culture medium was removed, and new culture medium containing 300 μM **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)** or **Tet-G^{D}F^{D}Y** was added. After incubation for different time points, cells were washed three times by 1xPBS buffer and stained with 5 μM DRAQ 5 for 10 min at room temperature. Then the cells were further washed three times by 1xPBS buffer. After that, the cells were exposed to light irradiation by a hand-held 8 W UV lamp emitting at 302 nm for 1-5 min. Prior to confocal imaging, the cells were further washed with PBS for three times to remove free molecules or broken nanofibers.

10. Cell viability assay

HeLa cells or 3T3 cells were seeded in a 96 well plate with a concentration of 5,000 cells per well in 100 μL culture medium. After 24 h, the medium was changed to 100 μL culture medium containing 100-500 μM **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)** or **Tet-G^{D}F^{D}Y**. After further culture for 24 hours, free precursors were removed through wash with PBS for three times and the medium was changed to normal culture medium. The cells were then exposure to light irradiation by a hand-held 302 nm UV lamp for 5 min. After further culture for 48 hours, cell viability was determined by MTT assay.

Reference:

[1] Ito, S.; Tanaka, Y.; Kakehi, A.; Kondo, K. *Bull. Chem. Soc. Jpn.* **1976**, 49, 1920-192

[2] He, M.; Li, J.; Tan, S.; Wang, R.; Zhang, Y. *J. Am. Chem. Soc.* **2013**, 135, 18718-18721

[3] Zhou, J.; Du, X.; Yamagata, N.; Xu, B. *J. Am. Chem. Soc.* **2016**, 138, 3813-3823

Supporting Figures

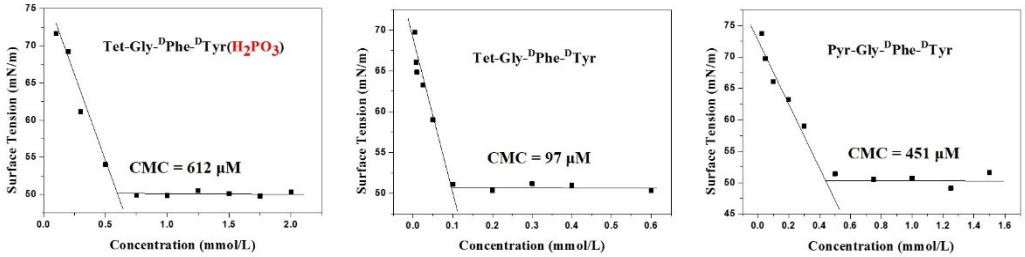


Figure S1. CMC determination of compound **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)**, **Tet-G^{D}F^{D}Y** and **Pyr-G^{D}F^{D}Y** by measuring the surface tension.

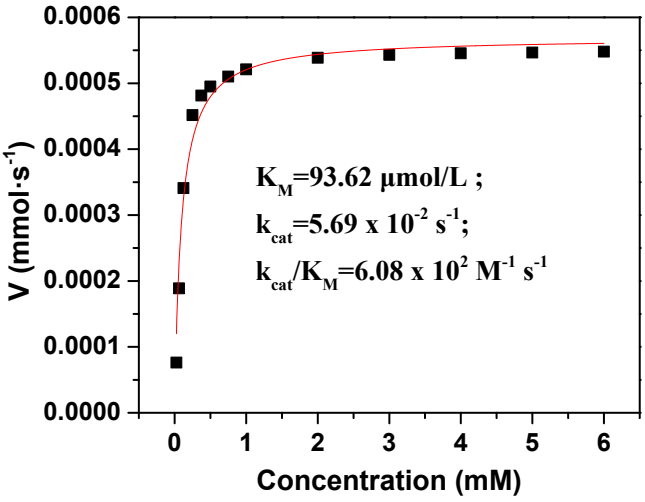


Figure S2. Michaelis-Menten plot of the hydrolysis of compound **Tet-Gly-^{D}Phe-^{D}Tyr (H_2PO_3)** by ALP (5 U/mL) in PBS buffer at 37 ^{o}C.

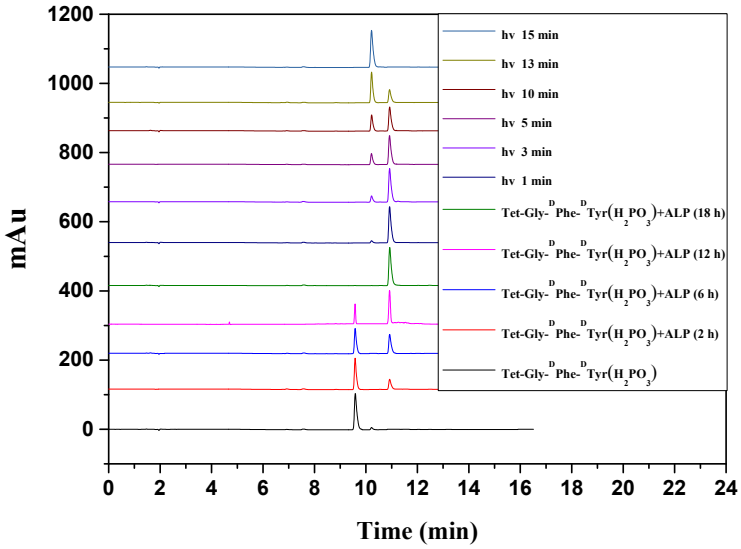


Figure S3. HPLC analysis of **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)** (5 mM) after treatment with 5 U/mL ALP at 37 °C for 2, 6, 12, 18 h respectively and further exposure to light irradiation by a hand-held 8 W UV lamp emitting at 302 nm for 1, 3, 5, 10, 13, 15 min respectively.

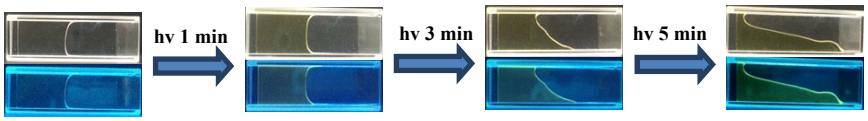


Figure S4. Optical and fluorescence images of hydrogel **Tet-G^{D}F^{D}Y** (3 mM) upon exposure to light irradiation by an 8 W hand-held 302 nm UV lamp for 1, 3, 5 min.

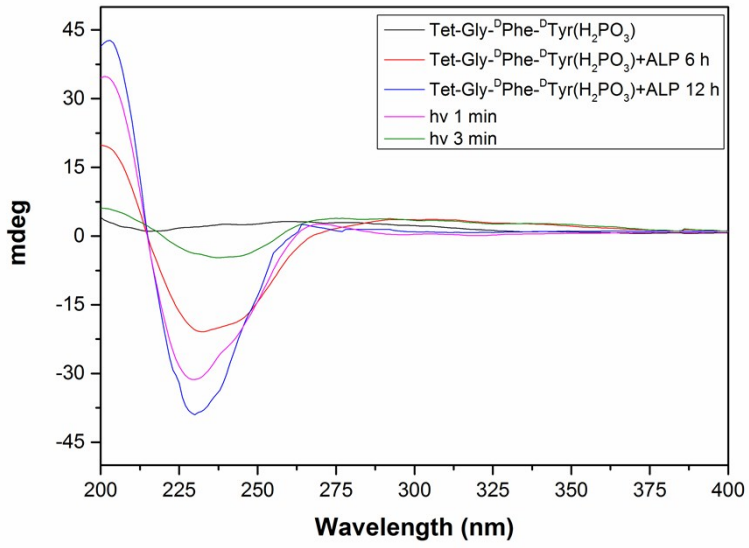


Figure S5. CD spectra of **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)** (5 mM) after treatment with 5 U/mL ALP

for 6, 12 h and then exposure to an 8 W hand-held UV lamp emitting at 302 nm for different time points (1, 3 min).

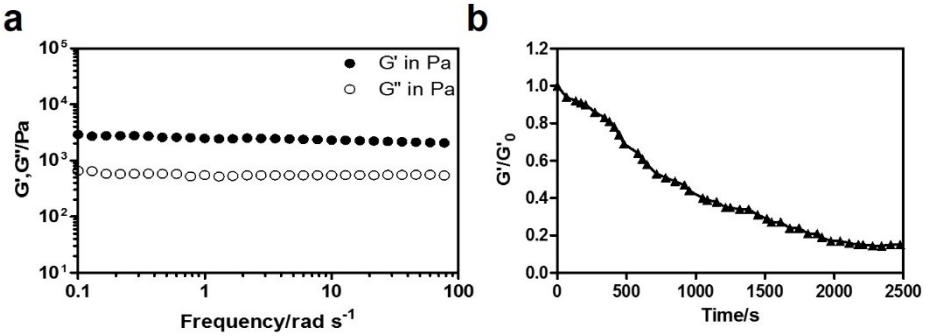


Figure S6. (a) Dynamic frequency sweep at 1% strain of **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)** (5 mM) after treatment with 5 U/mL ALP for 12 h. (b) In situ rheology data of the gel formed by EISA that was further exposure to the 302 nm UV light for different time points by EXFO Omnicure S2000 200 W (40 % output with a 302 nm filter). Data were plotted as the relative value of G' to the initial G'_0 before irradiation.

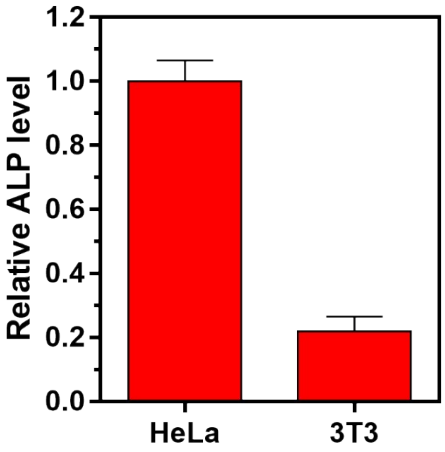


Figure S7. Relative ALP expression levels in HeLa and 3T3 cells. Data are shown as mean ± SEM (n=3).

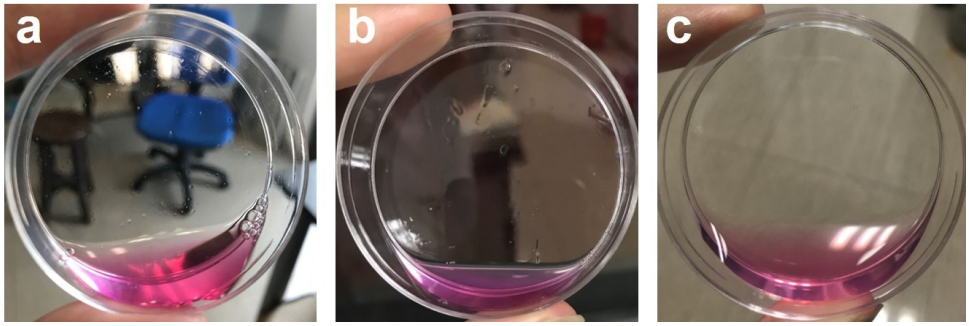


Figure S8. Optical images of HeLa cells after treatment with (a) 300 μM **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)**, (b) 500 μM **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)** or (c) 300 μM **Tet-G^{D}F^{D}Y** for 12 hours.

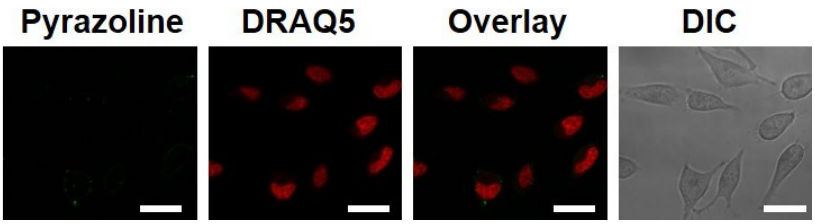


Figure S9. Confocal fluorescence images of HeLa cells after treatment with 300 μM **Tet-G^{D}F^{D}Y** for 24 h. Confocal images were taken after the cells were exposed to light irradiation by a hand-held 8 W UV lamp emitting at 302 nm for 1 min to generate fluorescent pyrazoline. Ex, 405 nm; Em, 450-550 nm. Nucleus were stained with DRAQ5. Scale bar = 25 μm.

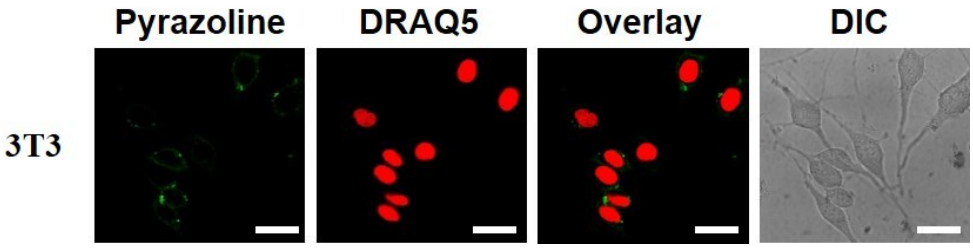


Figure S10. Confocal fluorescence images of 3T3 cells after treatment with 300 μM **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)** for 24 h. Confocal images were taken after the cells were exposed to light irradiation by a hand-held 8 W UV lamp emitting at 302 nm for 1 min to generate fluorescent pyrazoline. Ex, 405 nm; Em, 450-550 nm. Nucleus were stained with DRAQ5. Scale bar = 25 μm.

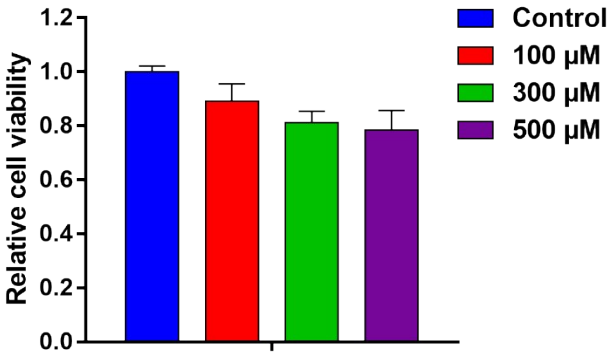


Figure S11. MTT test on the viability of HeLa cells after treatment with 100, 300, or 500 μM **Tet-G^{D}F^{D}Y** for 72 h. Data are shown as mean ± SEM (n=6).

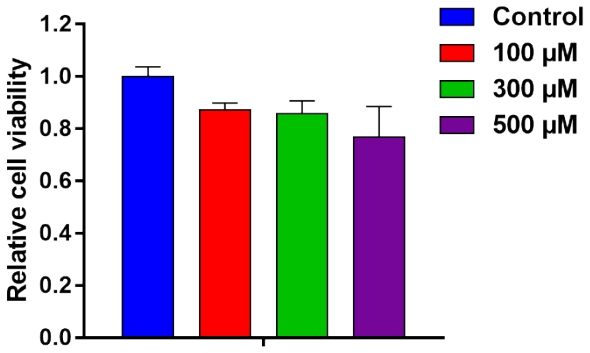


Figure S12. MTT test on the viability of 3T3 cells after treatment with 100, 300, or 500 μM **Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)** for 72 h. Data are shown as mean ± SEM (n=6).

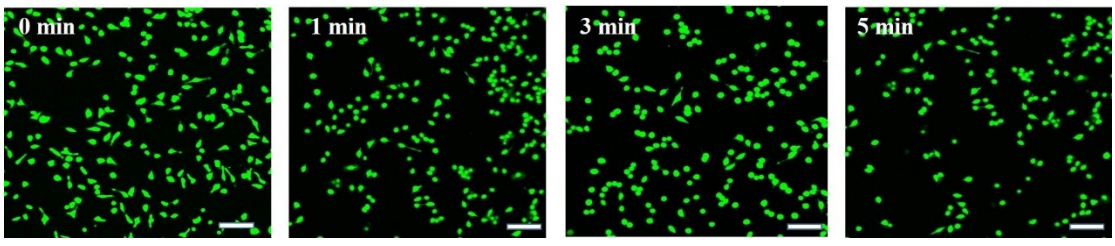
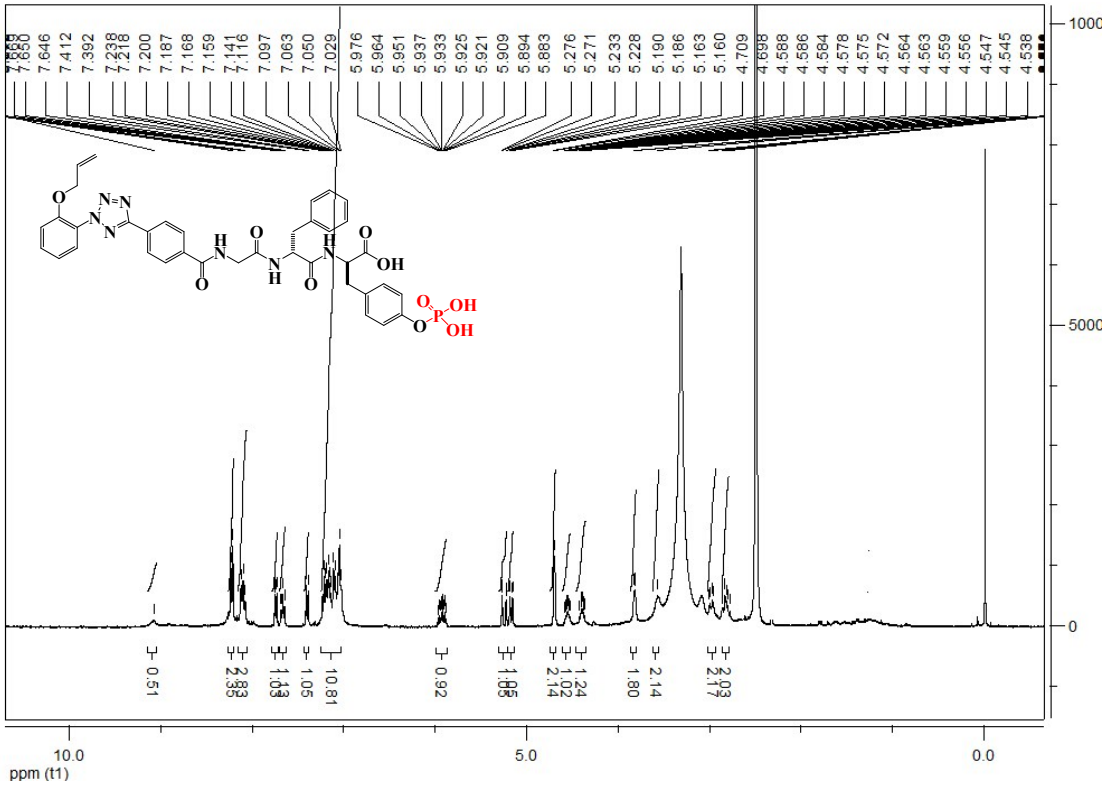


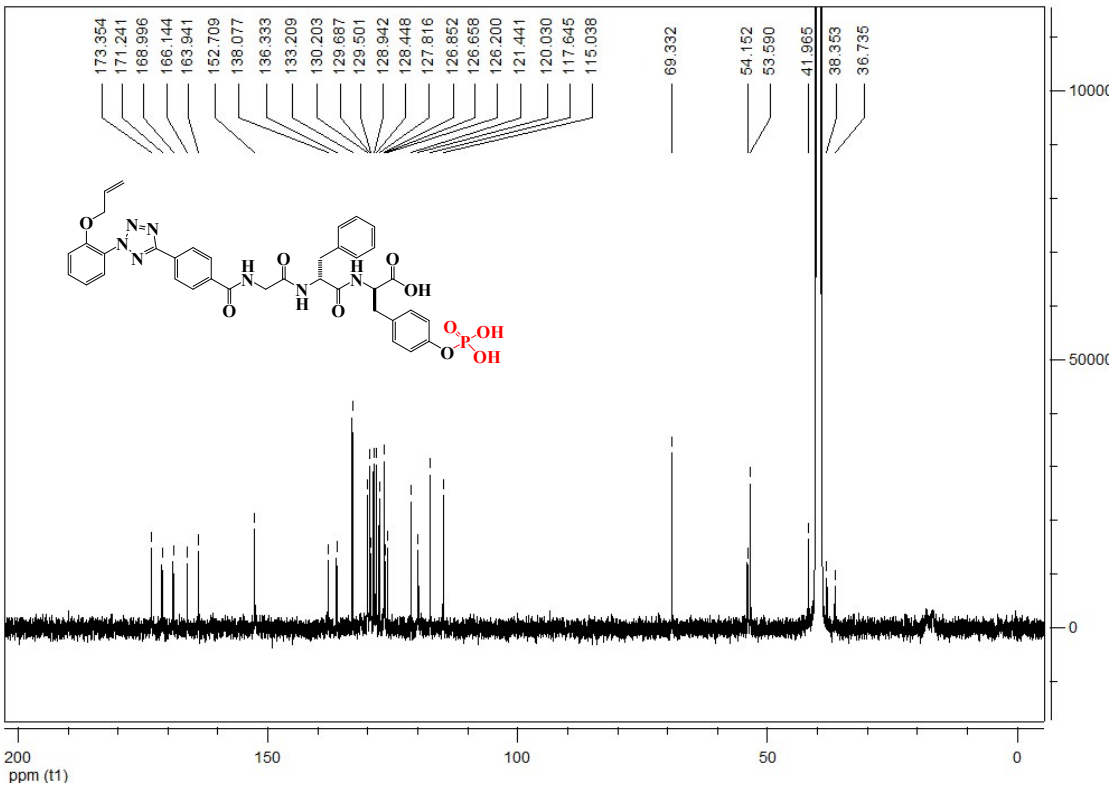
Figure S13. Confocal images of HeLa cells upon exposure to light irradiation for different time points (0 min, 1 min, 3 min, 5 min) by a hand-held 8 W UV lamp emitting at 302 nm and further culture 24 hours. Calcein AM and EthD-1 were used to respectively stain live cells and dead cells. Scale bar = 250 μm.

NMR Spectra

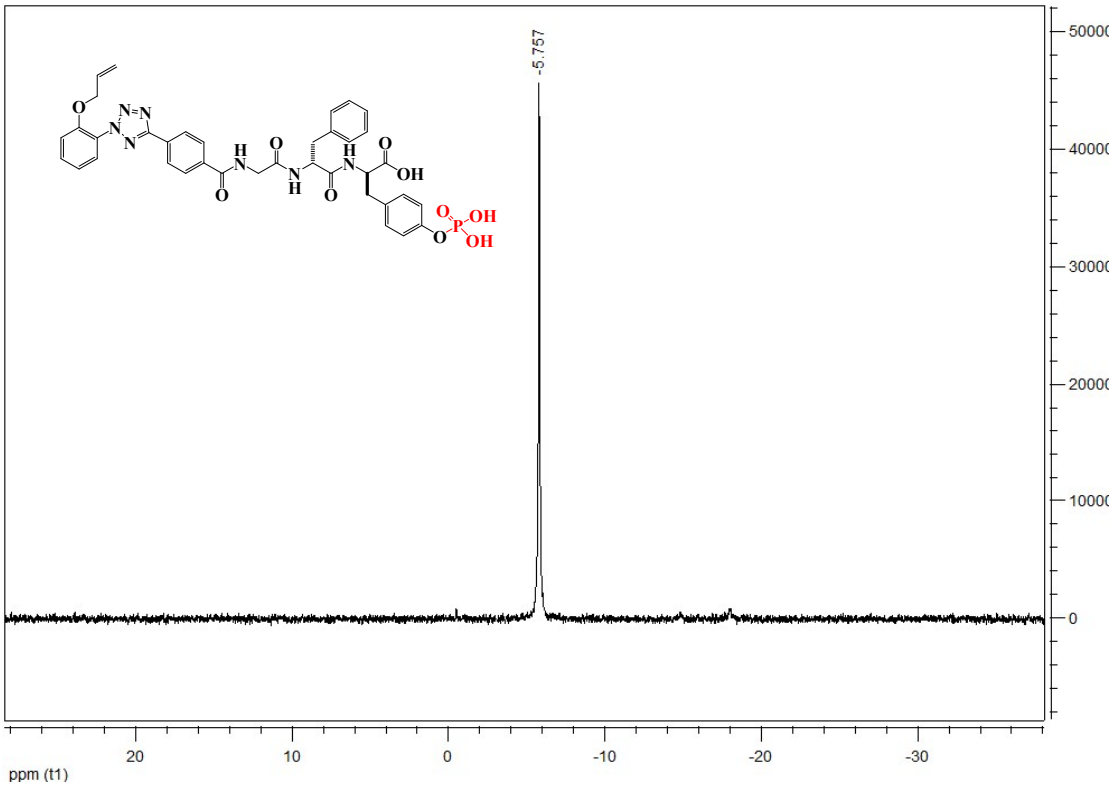
^{1}H NMR of Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)



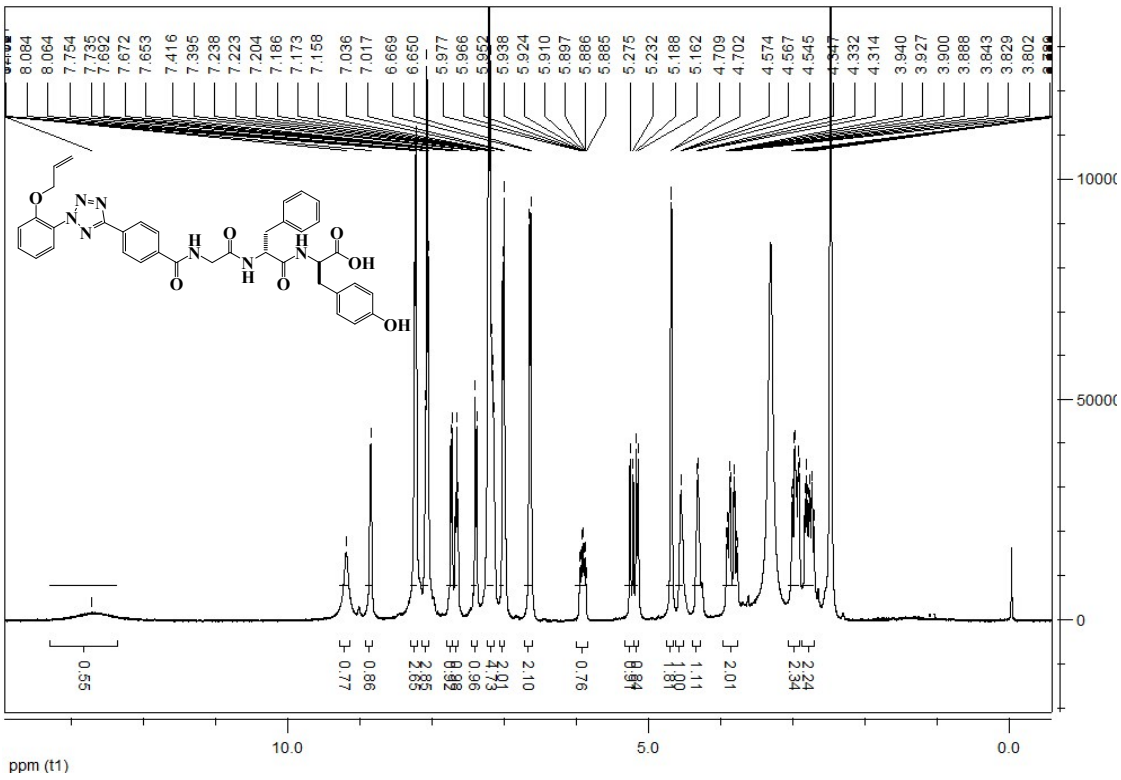
^{13}C NMR of Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)



^{31}P NMR of Tet-Gly-^{D}Phe-^{D}Tyr(H_2PO_3)



^{1}H NMR of Tet-G^{D}F^{D}Y



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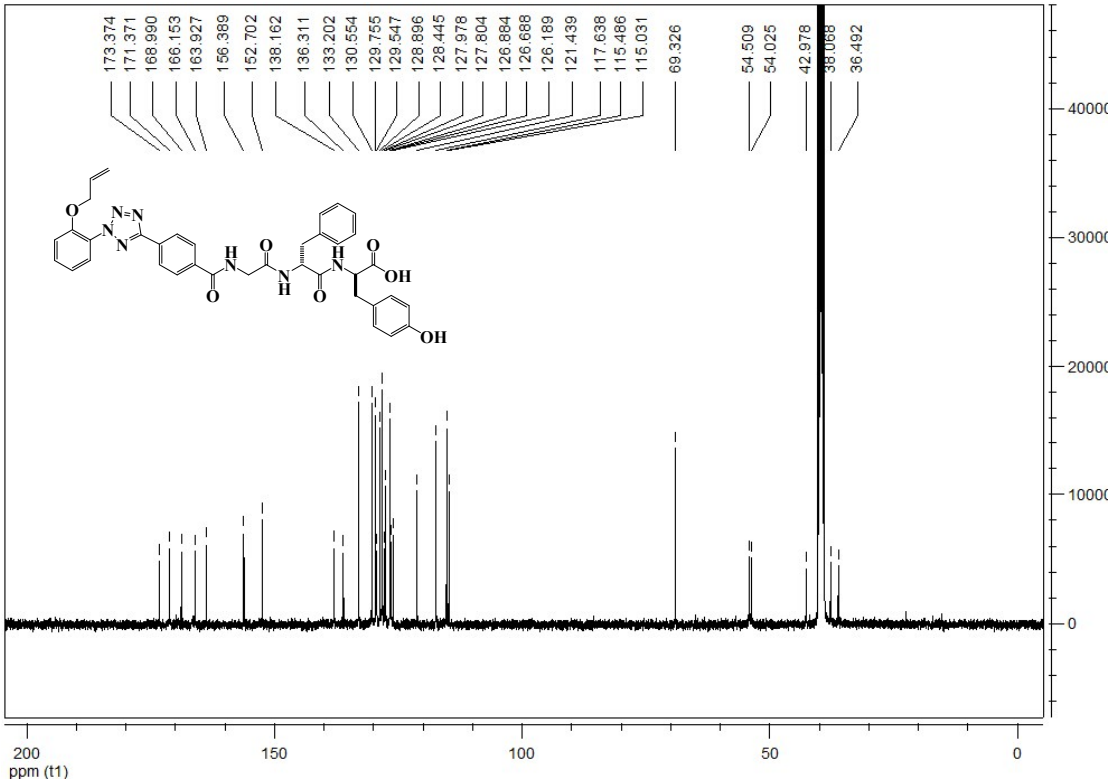

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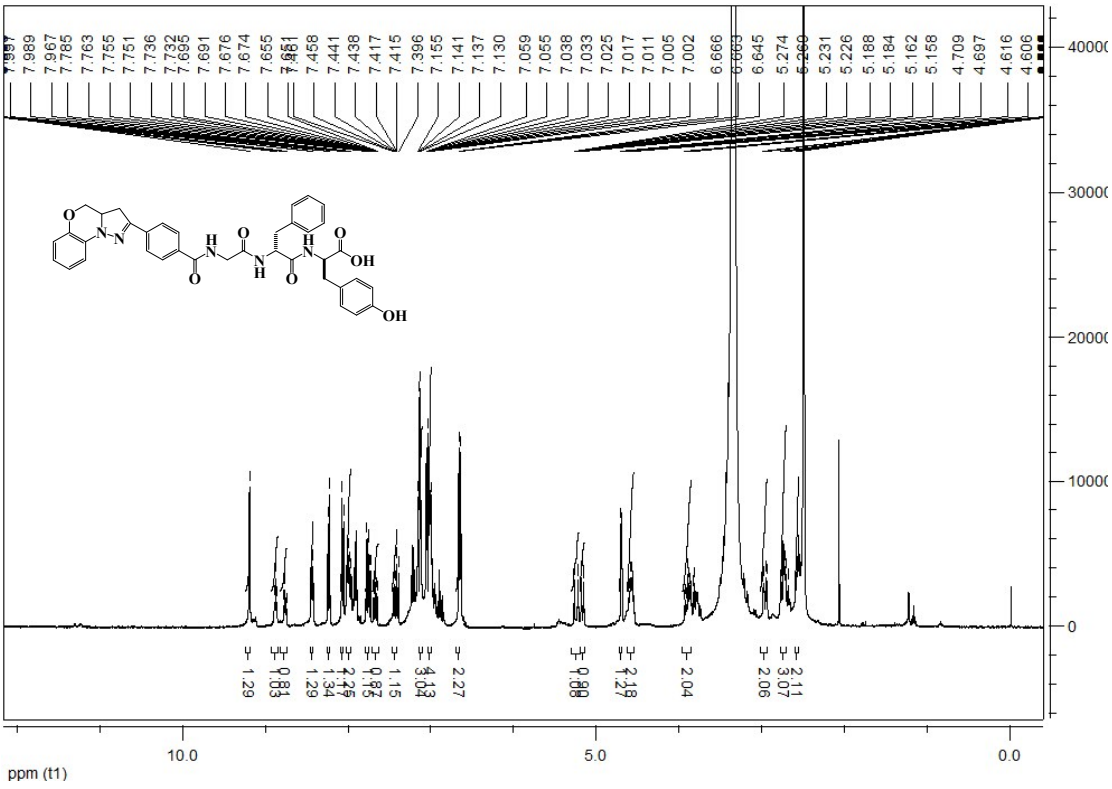

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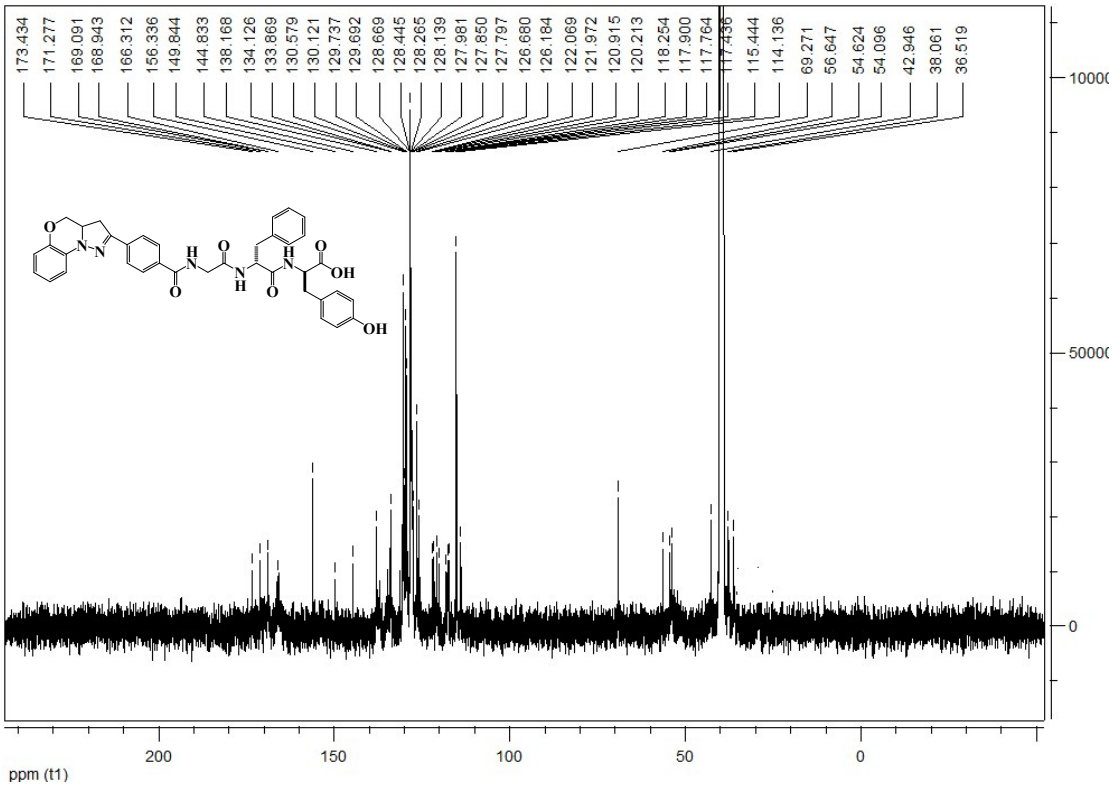
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