Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

A photo-degradable supramolecular hydrogel for selective delivery of microRNA into 3D-cultured cells

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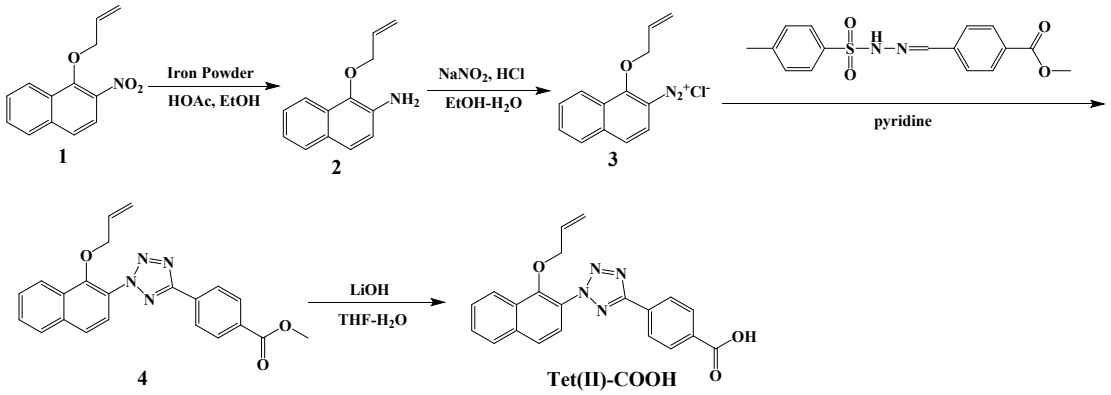
Table of contents

Synthesis and Characterization

Synthesis of the tetrazoles

The tetrazoleswere synthesized according to the literature procedure.[1]



Methyl-4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzoate (4)

2-allyloxy-4-nitronaphthalene 1 (1.15g, 5 mmol) was suspended in 12 mL EtOH, 6 mL H_2O and 12 mL HOAc. Iron dust (1.4 g, 25 mmol, 5 equiv.) was added and sonicated until full conversion was accomplished as monitored by TLC. The solvent was filtrated to remove the remaining iron dust. The mixture was extracted with ethyl acetate, and the organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the compound 3 was prepared by adding a cooling solution of sodium nitrite (0.32 g, 5.0 mmol) in 2 mL of water to a solution of the residue 2 (about 5.0 mmol) and 1.3 ml of concentrated hydrochloric acid in 8 ml of 50% ethanol below 0~ -5°C. The resulted solution of 3 was directly added dropwise over a period 15 minutes into a solution of methyl-4-((2-tosylhydrazono)methyl)benzoate (1.66 g, 5.0 mmol) in 30 ml pyridine at -20 ~ -25 °C. The reaction mixture was extracted with chloroform and water. The chloroform layer was washed with dilute hydrochloric acid and dried with Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 20:1) to give a light red powder methyl-4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzoate 4.(1.20 g, Yiled: 62 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (d, J = 8.4 Hz, 2H), 8.27 (q, J = 3.2 Hz, 1H), 8.20 (d, J = 8.4 Hz, 2H), 8.16 (q, J = 3.2 Hz, 1H), 8.02 (d, J = 8.8 Hz, 1H), 7.90 (d, J = 8.8 Hz, 1H), 7.79-7.74 (m, 2H), 6.01- 5.92 (m, 1H, $OCH_2C\mathbf{H}CH_2$), 5.32-5.27 (dd, J = 1.6 Hz, J = 17.2 Hz, 1H, $OCH_2CHC\mathbf{H}_2$), 5.19-5.15 (dd, J = 1.2 Hz, J = 10.4 Hz, 1H, $OCH_2CHC\mathbf{H}_2$), 4.56 (d, J = 5.6 Hz, 2H, $OC\mathbf{H}_2CHCH_2$), 3.92 (s, 3H, $COOC\mathbf{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 164.0, 149.1, 135.5, 133.5, 132.0, 131.1, 130.7, 129.0, 128.9, 128.4, 128.2, 127.4, 126.5, 125.6, 123.4, 118.8, 76.8, 52.9; MS (ESI+) m/z: 387 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{19}N_4O_3$: 387.2143 $[M+H]^+$; found 387.2149.

Methyl-4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzoic acid (Tet(II)-COOH)

Tetrazole 4 (1.16 g, 3 mmol,), lithium hydroxide (15 mmol, 0.63 g, 5 equiv) were suspended in 40 mL THF and 20 mL H_2O and the mixture was refluxed overnight. Then the reaction mixture

was extracted with ethyl acetate and water. The ethyl acetate layer was adjusted pH=1 by 2 mol/L hydrochloric acid. Then the mixture was again extracted with ethyl acetate and water and dried with Na_2SO_4. The solvent was removed under reduced pressure and the crude product was recrystallization with ethyl acetate to give a white powder. (1.07 g, Yiled: 96 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.21 (s, 1H, COO**H**), 8.33 (d, J = 8.4 Hz, 2H), 8.29-8.26 (q, J = 3.2 Hz, 1H), 8.18 (d, J = 8.4 Hz, 2H), 8.14 (q, J = 3.2 Hz, 1H), 8.02 (d, J = 8.8 Hz, 1H), 7.91 (d, J = 8.8 Hz, 1H), 7.79-7.75 (m, 1H), 6.01-5.91 (m, 1H, $OCH_2C\mathbf{H}CH_2$), 5.33-5.27 (dd, J = 1.6 Hz, J = 17.2 Hz, 1H, $OCH_2CHC\mathbf{H}_2$), 5.19-5.16 (dd, J = 1.2 Hz, J = 10.8 Hz, 1H, $OCH_2CHC\mathbf{H}_2$), 4.57 (d, J = 5.6 Hz, 2H, $OC\mathbf{H}_2CHCH_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 164.1, 149.1, 135.5, 133.5, 133.3, 130.8, 130.7, 128.9, 128.4, 128.2, 127.2, 126.4, 125.6, 123.3, 118.7, 76.8. MS (ESI+) m/z: 395 $[M+ Na]^+$; HRMS (ESI) calcd for $C_{21}H_{15}N_4O_3Na$: 394.1026 $[M+Na]^+$; found 394.1087.

Tetrazole-linked peptides.

The tetrazole-linked peptides were prepared by the solid-phase peptide synthesis and purified with the preparative HPLC. The purity of the peptide products was over 95%.

Tet(II)-GRGDS

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 2H, COO**H**), 8.87 (t, *J* = 5.8 Hz, 1H, N**H**$COCH_2NH$-$CH(CH_2COOH)CO$), 8.24 (d, *J* = 8.4 Hz, 2H), 8.22-8.18 (m, 2H), 8.14-8.07 (m, 3H, N**H**CHCO), 8.05 (t, *J* = 3.8 Hz, 2H), 8.03 (d, *J* = 2.0 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.8 (d, *J* = 8.8 Hz, 1H), 7.72-7.67 (m, 2H), 7.42-7.36 (m, 1H), 5.95-5.85 (m, 1H, $OCH_2C\mathbf{H}CH_2$), 5.26-5.20 (dd, *J* = 1.6 Hz, *J* = 17.2 Hz, 1H, $OCH_2CHC\mathbf{H}_2$), 5.12-5.08 (dd, *J* = 0.8 Hz, *J* = 10.4 Hz, 1H, $OCH_2CHC\mathbf{H}_2$), 4.90 (s, 1H), 4.51-4.45 (m, 3H), 4.31-4.26 (m, 1H), 4.24-4.19 (m, 1H), 3.98-3.84 (m, 3H), 3.68 (d, *J* = 6.0 Hz, 2H), 3.55-3.51 (m, 1H), 3.05 (t, *J* = 9.4 Hz, 2H), 2.57-2.54 (m, 2H, $CHC\mathbf{H}_2COOH$), 1.70-1.65 (m, 1H), 1.50-1.43 (m, 3H), 1.16 (s, 2H);^{13}C NMR (100 MHz, DMSO-d_6) 173.1, 172.4, 172.0, 169.6, 169.4, 168.8, 166.3, 164.2, 157.1, 149.1, 136.4, 135.4, 133.5, 129.4, 129.0, 128.9, 128.4, 128.2, 127.8, 127.1, 126.5, 125.6, 123.4, 123.3, 118.7, 76.8, 69.4, 65.4, 61.9, 55.1, 49.2, 40.9, 37.2, 29.7, 25.4, 23.5; MS (ESI+) *m/z*: 888 $[M+Na]^+$; HRMS (ESI) calcd.for $C_{38}H_{42}N_{12}O_{11}Na_2$ 888.2956 $[M+ Na]^+$; found 888.2963.

Tet(II)-GRDG

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.23 (s, 2H, COO**H**), 8.97 (t, *J* = 5.6 Hz, 1H, N**H**CH_2COOH), 8.56 (d, *J* = 7.2 Hz, 1H, N**H**$CH(CH_2CH_2CH_2NHC(NH)NH_2)CO$), 8.49 (d, *J* = 7.6 Hz, 1H, N**H**$CH(CH_2COOH)CO$), 8.31 (d, *J* = 8.4 Hz, 2H), 8.28 (t, *J* = 4.8 Hz, 1H), 8.16 (q, *J* = 3.2 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 2H), 8.07 (d, *J* = 8.8 Hz, 1H, N**H**CH_2CONH), 8.03 (d, *J* = 9.2 Hz, 1H), 7.93-7.88 (m, 1H), 7.79-7.76 (m, 2H), 7.18 (s, 2H, $CH_2CH_2CH_2NHC(NH)N\mathbf{H}_2$), 6.02-5.93 (m, 1H, $OCH_2C\mathbf{H}CH_2$), 5.33-5.28 (dd, *J* = 1.6 Hz, *J* = 17.2 Hz, 1H, $OCH_2CHC\mathbf{H}_2$), 5.19-5.16 (dd, *J* = 0.8 Hz, *J* = 10.4 Hz, 1H, $OCH_2CHC\mathbf{H}_2$), 4.57 (d, *J* = 5.6 Hz, 2H, $OC\mathbf{H}_2CHCH_2$), 4.55-4.51 (m, 2H, $NHC\mathbf{H}(CH_2CH_2CH_2NHC(NH)NH_2)CONHC\mathbf{H}(CH_2COOH)CONH$), 4.03-3.90 (m, 2H, $NHC\mathbf{H}_2COOH$), 3.79-3.70 (m, 2H, $NHC\mathbf{H}_2CONH$), 3.19-3.07 (m, 4H), 2.61-2.55 (m, 2H, $CHC\mathbf{H}_2COOH$), 1.85 (s, 2H, $CHC\mathbf{H}_2CH_2CH_2NHC(NH)NH_2$), 1.12 (d, *J* = 6.4 Hz, 2H, $CHCH_2C\mathbf{H}_2CH_2NHC(NH)NH_2$);^{13}C NMR (100 MHz, DMSO-d_6) 173.7, 172.7, 172.1, 169.9,

169.1, 166.3, 164.2, 157.4, 149.1, 136.5, 135.5, 133.5, 129.4, 129.0, 128.9, 128.4, 128.2, 127.0, 126.5, 125.6, 123.4, 123.3, 118.7, 76.9, 69.2, 50.9, 49.7, 43.1, 37.2, 29.3, 25.0, 23.6; MS (ESI+) *m/z*: 801 $[M+Na]^+$; HRMS (ESI) calcd.for $C_{35}H_{37}N_{11}O_9Na_2$ 801.2674 $[M+ Na]^+$; found 801.2691.

Tet(II)-GR^{D}GD^{D}S

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (s, 2H, COO**H**), 8.94 (t, *J* = 6.0 Hz, 1H, N**H**COCH$_2$NHCH(CH$_2$COOH)CO), 8.31 (m, 3H), 8.29-8.25 (m, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.17-8.14 (m, 2H), 8.12 (m, 2H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.91-7.87 (m, 2H), 7.77 (m, 2H), 7.49-7.45 (m, 2H), 6.02-5.91 (m, 1H, OCH$_2$C**H**CH$_2$), 5.33-5.27 (dd, *J* = 1.2 Hz, *J* = 17.2 Hz, 1H, OCH$_2$CHC**H**$_2$), 5.19-5.16 (d, *J* = 10.4 Hz, 1H, OCH$_2$CHC**H**$_2$), 4.98 (s, 1H), 4.70-4.65 (m, 1H), 4.57 (d, *J* = 5.6 Hz, 2H), 4.37-4.30 (m, 2H), 4.24 (m, 1H), 4.05 (m,1H), 3.98 (m,1H), 3.78 (m, 1H), 3.73-3.67 (m, 1H), 3.63-3.59 (m, 1H), 3.41-3.57 (t, *J* = 3.2 Hz, 2H), 2.74-2.68 (dd, 2H, *J* = 4.8 Hz, *J* = 16.4 Hz, CHC**H**$_2$COOH), 1.86 (s, 1H), 1.53 (m, 3H), 1.12-1.07 (m, 2H);^{13}C NMR (100 MHz, DMSO-d$_6$) 172.1, 171.2, 170.9, 169.5, 169.3, 169.1, 166.3, 164.2, 157.1, 149.1, 136.4, 135.4, 133.5, 129.4, 128.9, 128.4, 128.2, 127.7, 127.0, 126.5, 125.6, 123.4, 118.7, 76.8, 61.7, 55.2, 52.6, 49.8, 43.1, 42.3, 36.9, 36.6, 29.6, 25.4; MS (ESI+) *m/z*: 888 $[M+Na]^+$; HRMS (ESI) calcd.for $C_{38}H_{42}N_{12}O_{11}Na_2$ 888.2914 $[M+ Na]^+$; found 888.2955.

Pyr(II)-GRGDS

Tet(II)-GRGDS (84 mg, 0.1mmol) in 50 mL CH$_3$CN/PBS = 1:1 was irradiated with ahand-held 302 nm UV lamp for 20 minutes. CH$_3$CN was removed and the aqueouslayer was purified by HPLC(The eluents for HPLC was CH$_3$CN/H$_2$O (1‰ CF$_3$COOH) 7:3) to give a yellow powder (76 mg, 92 %).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (s, 1H, N**H**COCH$_2$NHCH(CH$_2$COOH)CO), 8.28 (s, 1H, N**H**CH(CH$_2$CH$_2$CH$_2$NHC(NH)NH$_2$)CO), 8.21 (s, 2H), 8.10 (d, *J* = 6.8 Hz, 1H, N**H**CH$_2$CONH), 8.02-7.95 (m, 2H), 7.83 (s, 2H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.45 (s, 2H), 7.40 (d, *J* = 6.8 Hz, 1H), 7.33 (s, 1H), 7.20 (s, 2H, CH$_2$CH$_2$CH$_2$NHC(NH)N**H**$_2$), 4.54 (s, 2H, NHC**H**(CH$_2$CH$_2$CH$_2$NHC(NH)NH$_2$)CONHC**H**(CH$_2$COOH)CONH), 4.28 (s, 2H, NHC**H**$_2$COOH), 3.96-3.87 (m, 2H, NHC**H**$_2$CONH), 3.72 (d, 3H), 3.61 (d, *J* = 8.0 Hz, 1H), 3.49 (s, 1H), 3.10 (s, 3H), 2.76 (s, 1H), 2.62 (s, 2H, CHC**H**$_2$COOH), 1.76 (S, 1H), 1.52 (m, 3H), 1.36 (s, 1H), 1.26-1.14 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d$_6$) 173.1, 172.4, 172.1, 169.6, 168.8, 166.5, 159.1, 158.8, 157.4, 150.2, 138.2, 134.9, 134.4, 132.8, 129.8, 128.4, 128.1, 126.3, 125.4, 124.8, 121.4, 120.7, 118.6, 118.4, 115.7, 73.1, 63.7, 61.9, 56.6, 55.2, 52.8, 49.2, 42.8, 42.0, 37.2, 35.5, 29.4, 25.4; MS (ESI$^+$) *m/z*: 860 $[M+Na]^+$; HRMS (ESI) calcd. for $C_{38}H_{42}N_{10}O_{11}Na_2$ 860.2834 $[M+ Na]^+$; found 860.2873.

Reference:

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

Supporting Figures

Figure S1. CD spectrum of Tet(II)-GRGDS gel (4 mg/mL).

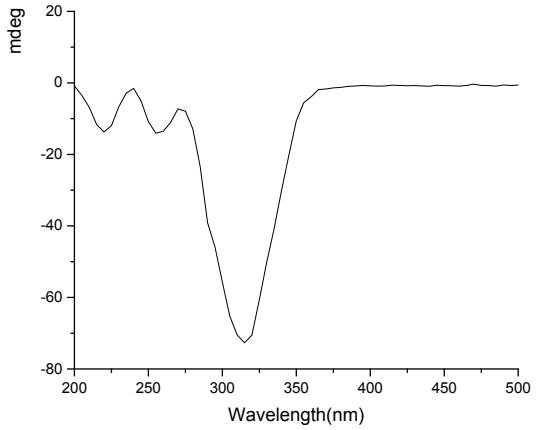


Figure S2. HPLC monitoring of the transformation of Tet(II)-GRGDS (1 mM in CH_3CN/PBS = 1:2) into Pyr(II)-GRGDS at different time points post light irradiation by a hand-held UV lamp. The eluents for HPLC was CH_3CN/H_2O (1‰ CF_3COOH) 7:3.

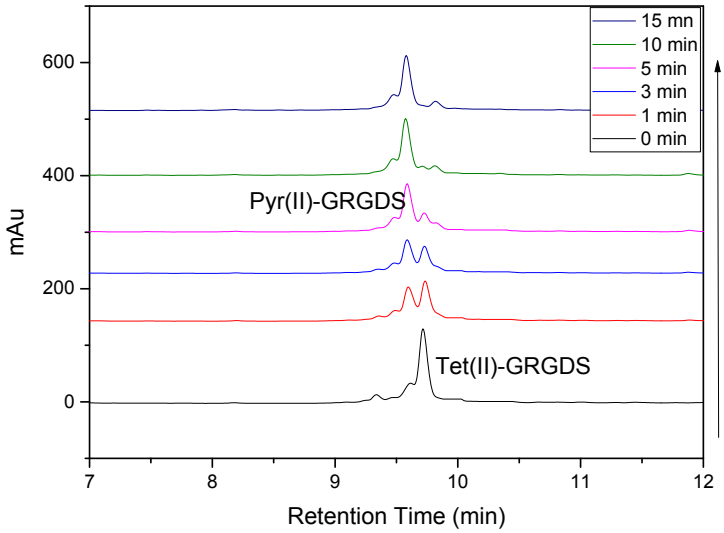


Figure S3. UV and fluorescence characterization of photo-click reaction mixtures of Tet(II)-GRGDS (1 mM in CH_3CN/PBS = 1:2) at different time points post light irradiation by a hand-held UV lamp emitting at 302 nm.

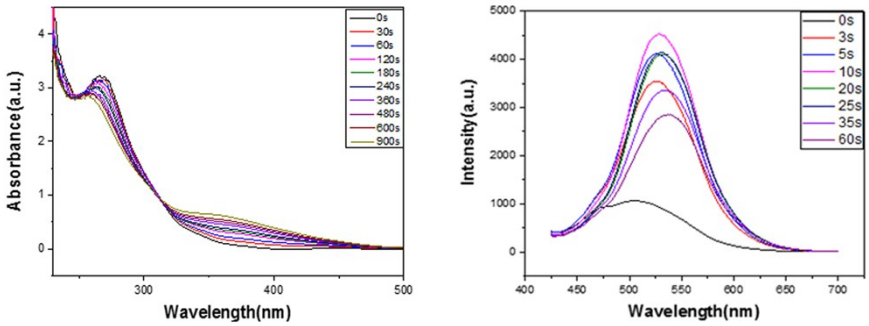


Figure S4. UV-vis (dotted line) and fluorescence (solid line) spectra of Tet(II)-GRGDS (25 μM) and Pyr(II)-GRGDS (25 μM) in CH3CN/PBS = 1:3. The excitation wavelength for the fluorescent spectra was 405 nm.

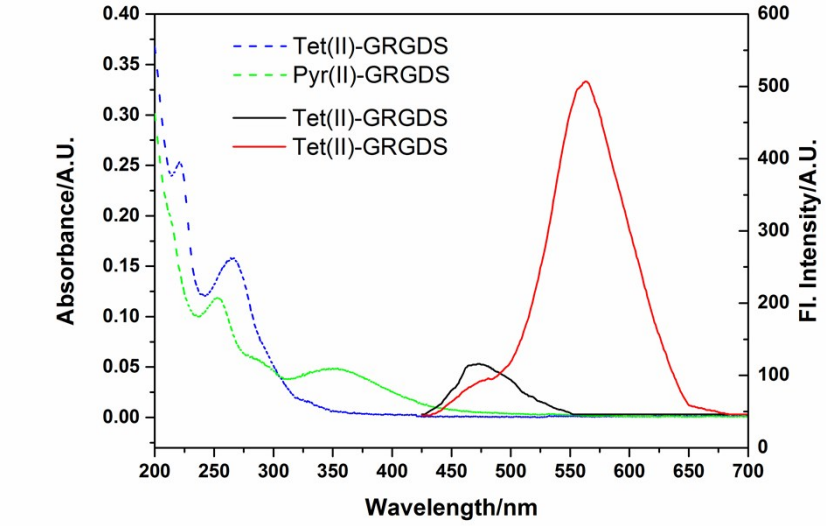


Figure S5. Fluorescent spectra of Tet(II)-GRGDS gel (4 mg/mL) at different time points post light irradiation by a hand-held UV lamp emitting at 302 nm. The excitation wavelength for the fluorescent spectra was 405 nm.

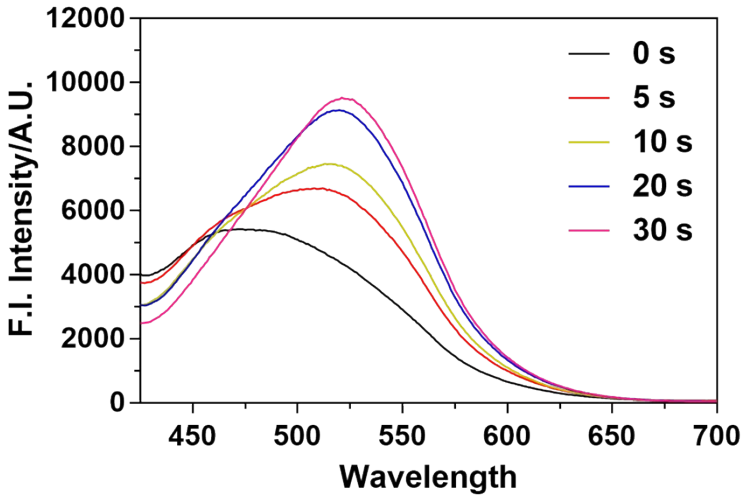


Figure S6. Characterization on the Tet(II)-GRGDS gel (4.0 mg/mL) upon exposure to an 8W hand-held UV lamp emitting at 302 nm for different time points using CD spectra.

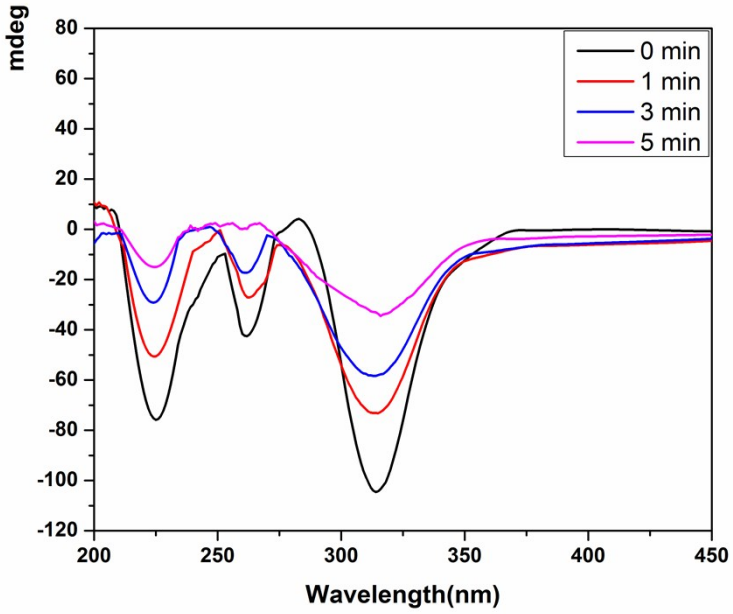


Figure S7. MTT tests on the cell viability of U87 cells after incubation with Tet(II)-GRGDS at different concentrations (10, 100, 500 μM) for 24 hours, 48 hours and 72 hours. Data are shown as mean ± SEM (n=3).

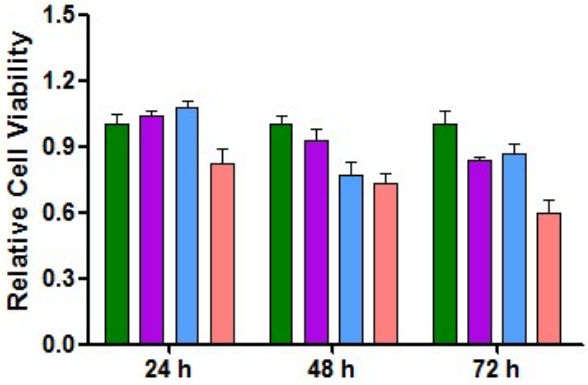


Figure S8. MTT tests on the viability of U87 cells with or without light irradiation by an 8W hand-held UV lamp emitting at 302 nm for 5 min. The cells were exposure to irradiation on day 0 and further cultured for 24 or 48 hours, followed by evaluation of cellular viability with MTT. Data are shown as mean ± SEM (n=3).

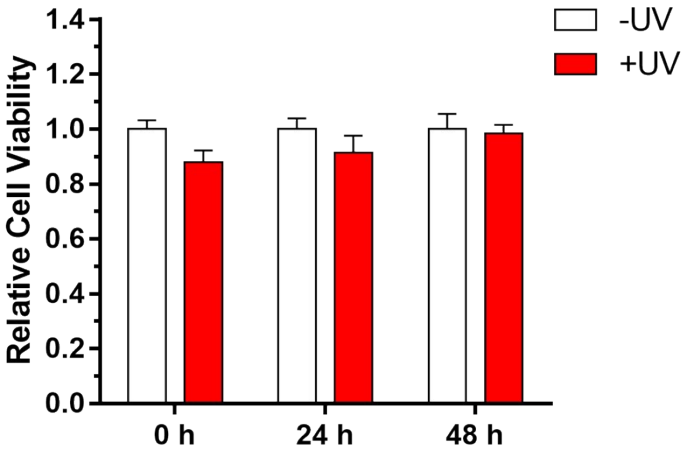


Figure S9. Confocal fluorescent images of U87 cells 3D cultured in 4 mg/mL Tet(II)-GRGDS gel with 80 pmole Cy3-miR-34a for 12 hours. Scale bar: 10 μm.

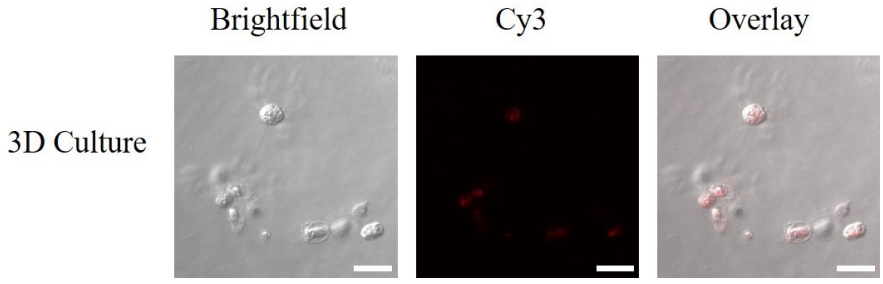


Figure S10. qRT-PCR analysis of miR-34a levels in U87 cells cultured in dish or in 4 mg/mL Tet(II)-GRGDS gel for 24 hours. The gel was degraded by light irradiation. Data are shown as mean ± SEM (n=3).

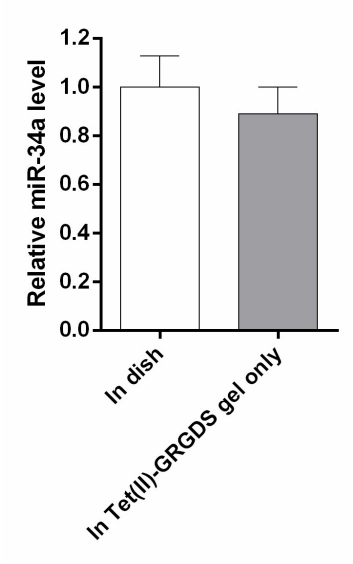


Figure S11. qRT-PCR analysis of miR-34a levels after U87 cells were 3D cultured in 4 mg/mL Tet(II)-GRGDS or 4 mg/mL Tet(II)-GRDG gel with 80 pmol miR-34a for 24 hours. The gels were degraded by proteinase K. Data are shown as mean ± SEM (n=3). *P < 0.05.

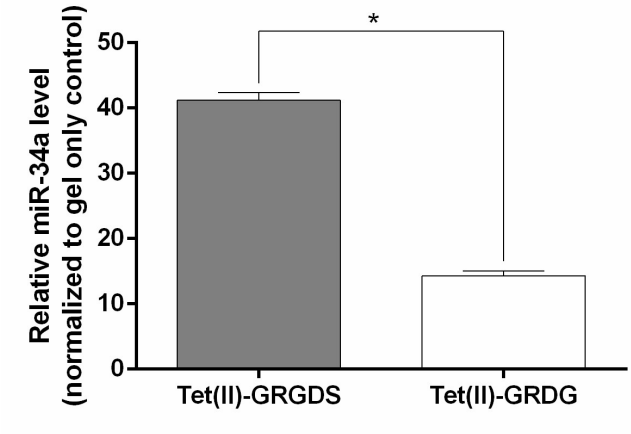


Figure S12. qRT-PCR analysis of miR-34a levels after U87, MCF-7, or 293T cells were 3D cultured in 4 mg/mL Tet(II)-GRGDS gel, 4 mg/mL Tet(II)-GRDG gel, 4 mg/mL Tet(II)-GR^{D}GD^{D}S gel, or mixed with 0.2 mg/mL Tet(II)-GRGDS or Pyr(II)-GRGDS with 80 pmol miR-34a for 24 hours. The gels were degraded by light irradiation. Data are shown as mean ± SEM (n=3).

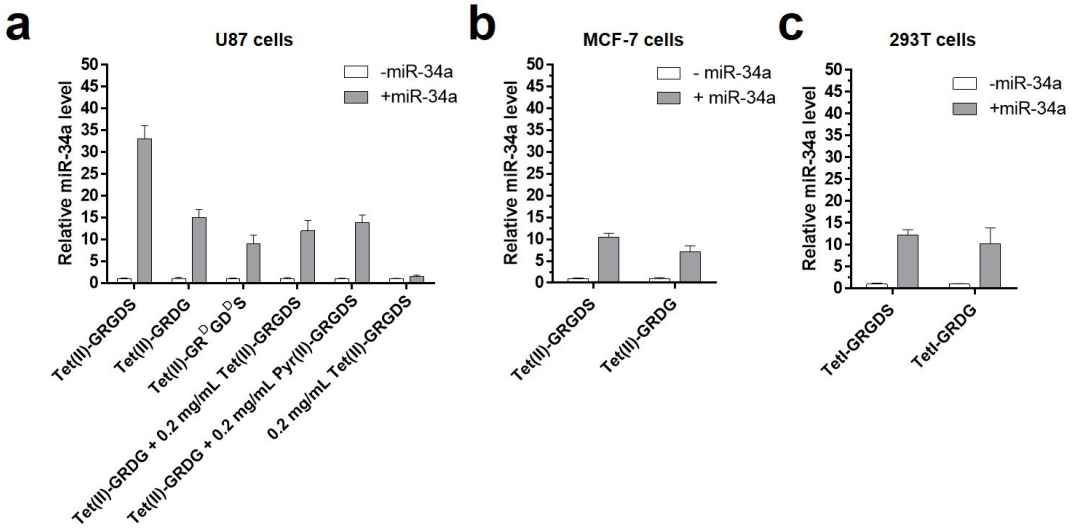


Figure S13. qRT-PCR analysis of miR-34a levels after U87 cells were 3D cultured in 4 mg/mL Tet(II)-GRGDS gel or mixed endocytosis inhibitors with 80 pmol miR-34a for 24 hours. The gels were degraded by light irradiation. Data are shown as mean ± SEM (n=3).

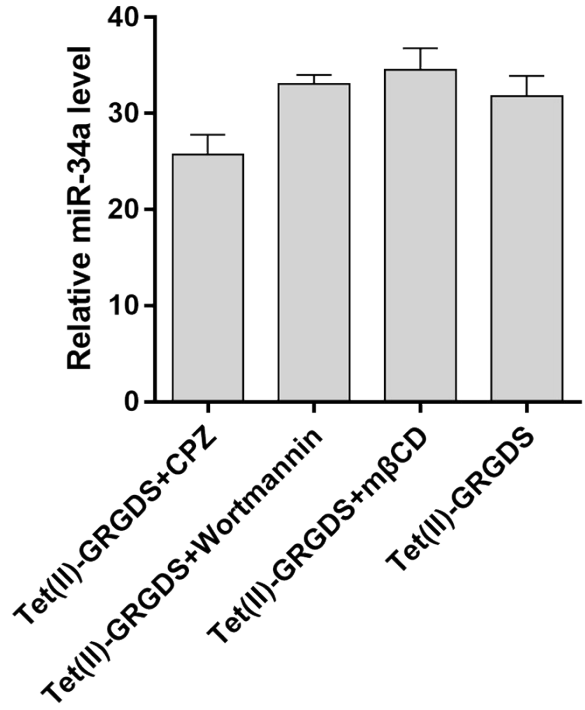


Figure S14. Confocal fluorescent images of U87 or MCF-7 cells 3D cultured in 4 mg/mL Tet(II)-GRGDS gel with 80 pmole Cy3-miR-34a for 12 hours. Lysosome was stained with LysoTracker. Scale bar: 10 μm.

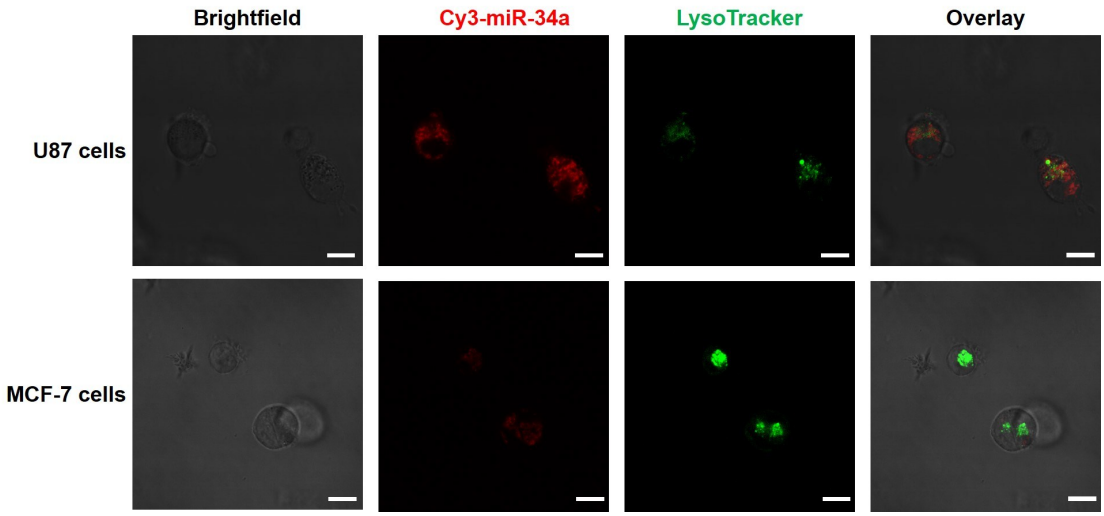
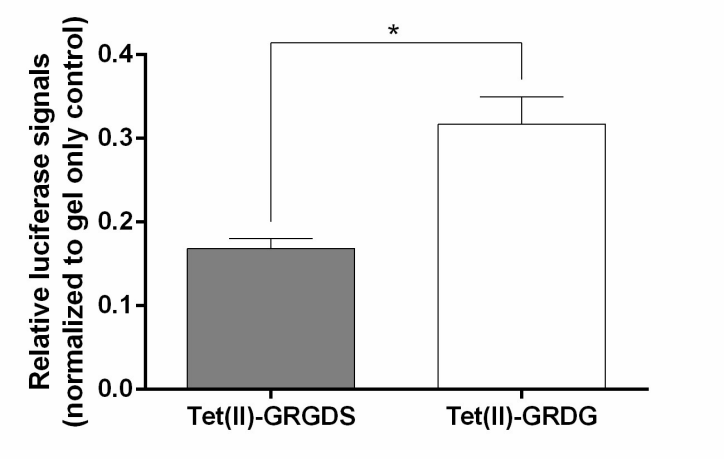
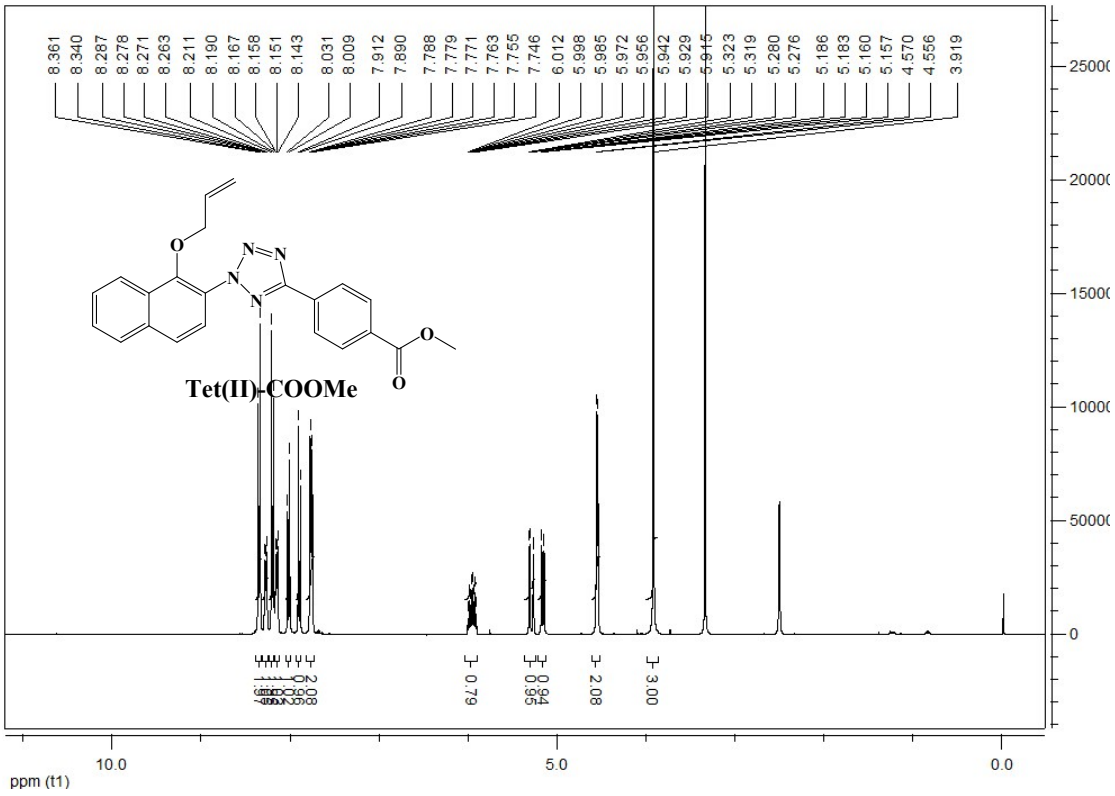


Figure S15. Relative luciferase signals from reporter U87 cells encapsulated with 80 pmol miR-34a in 4 mg/mL Tet(II)-GRGDS gel or 4 mg/mL Tet(II)-GRDG gel for 48 hours. The gels were degraded by proteinase K. Data are shown as mean ± SEM (n=3). *P < 0.05.

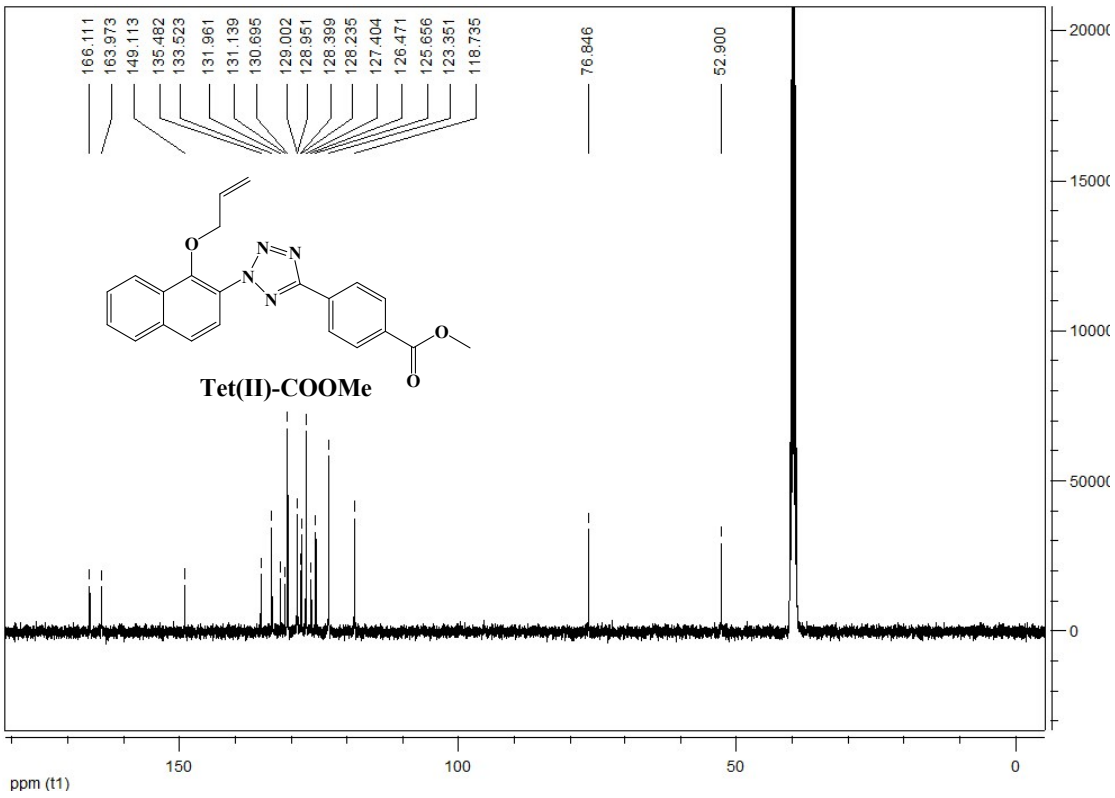


NMR spectra

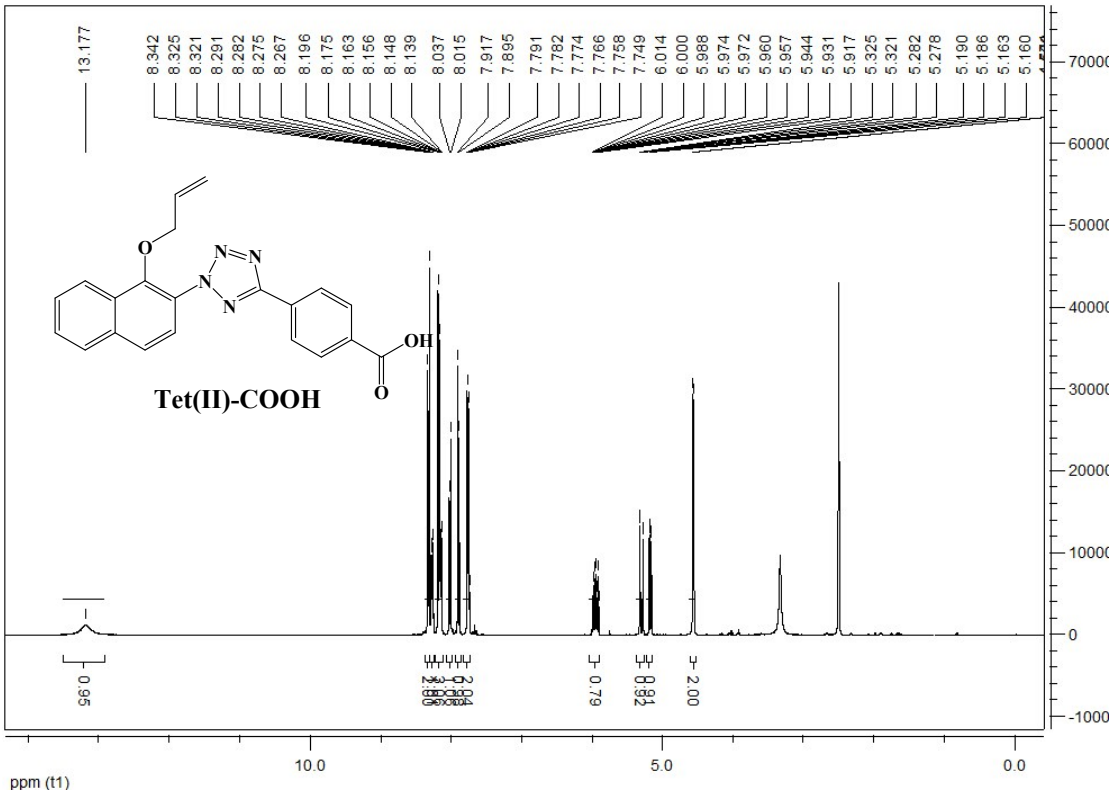
^{1}H NMR of Tet(II)-COOMe



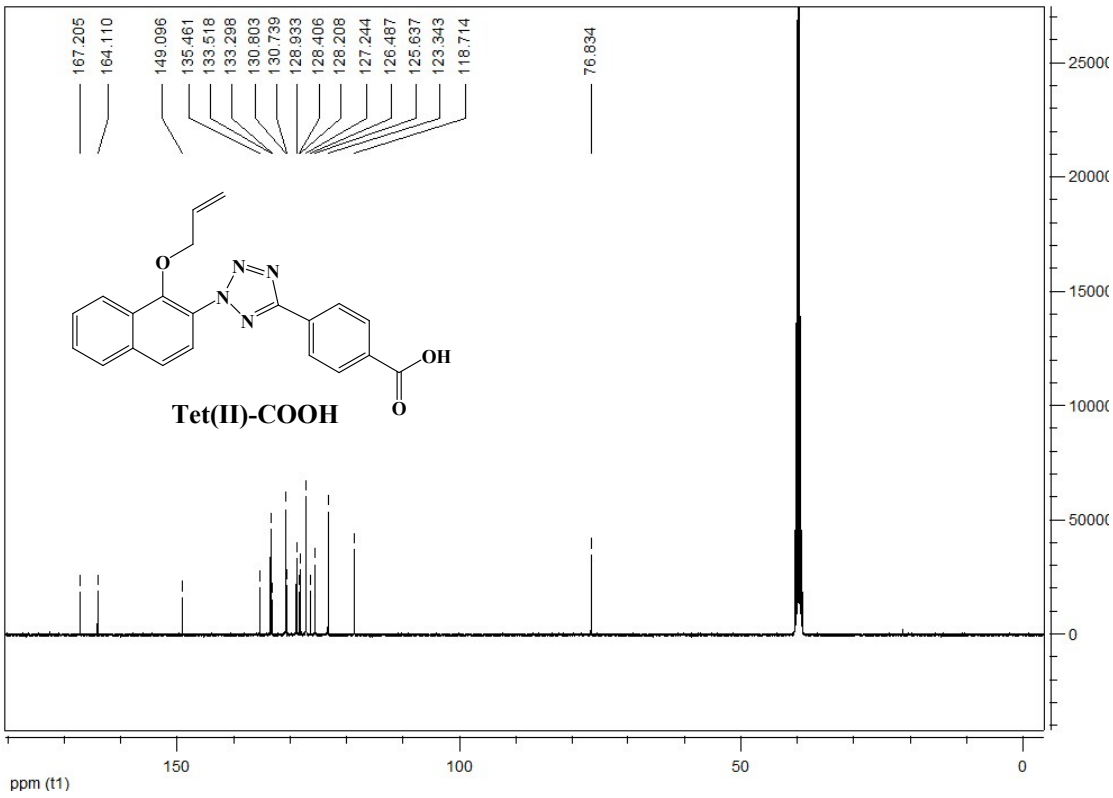
^{13}C NMR of Tet(II)-COOMe



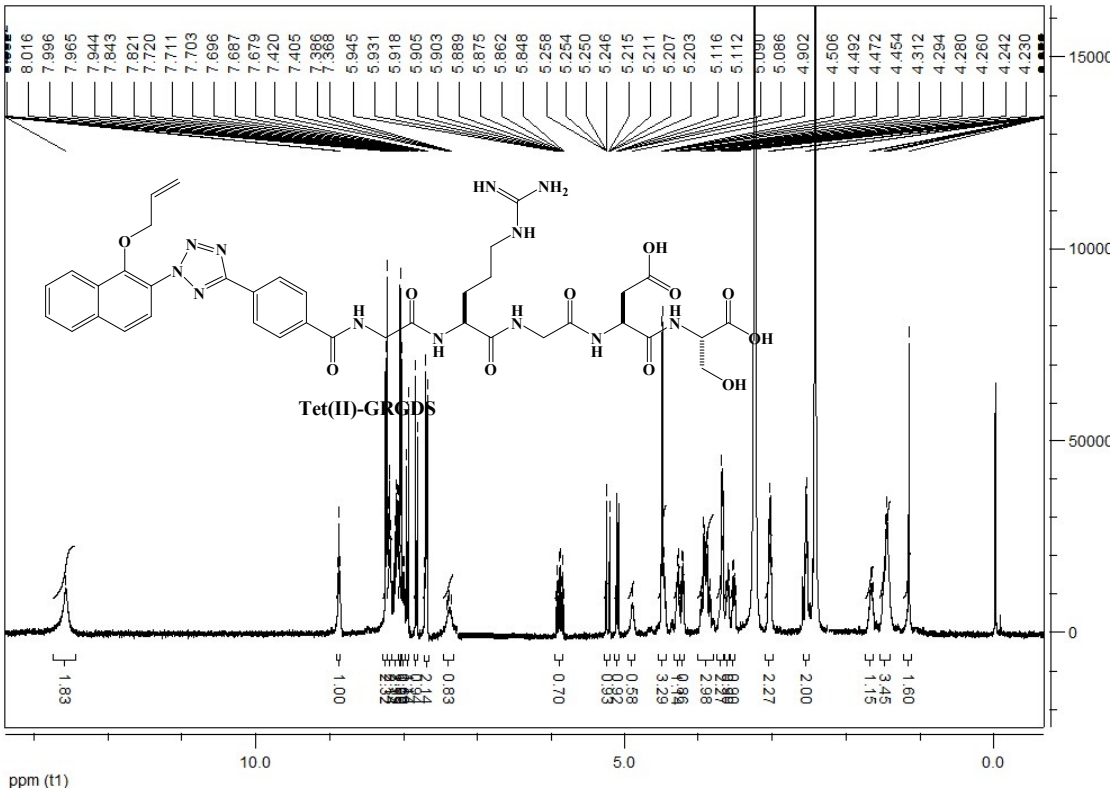
^{1}H NMR of Tet(II)-COOH



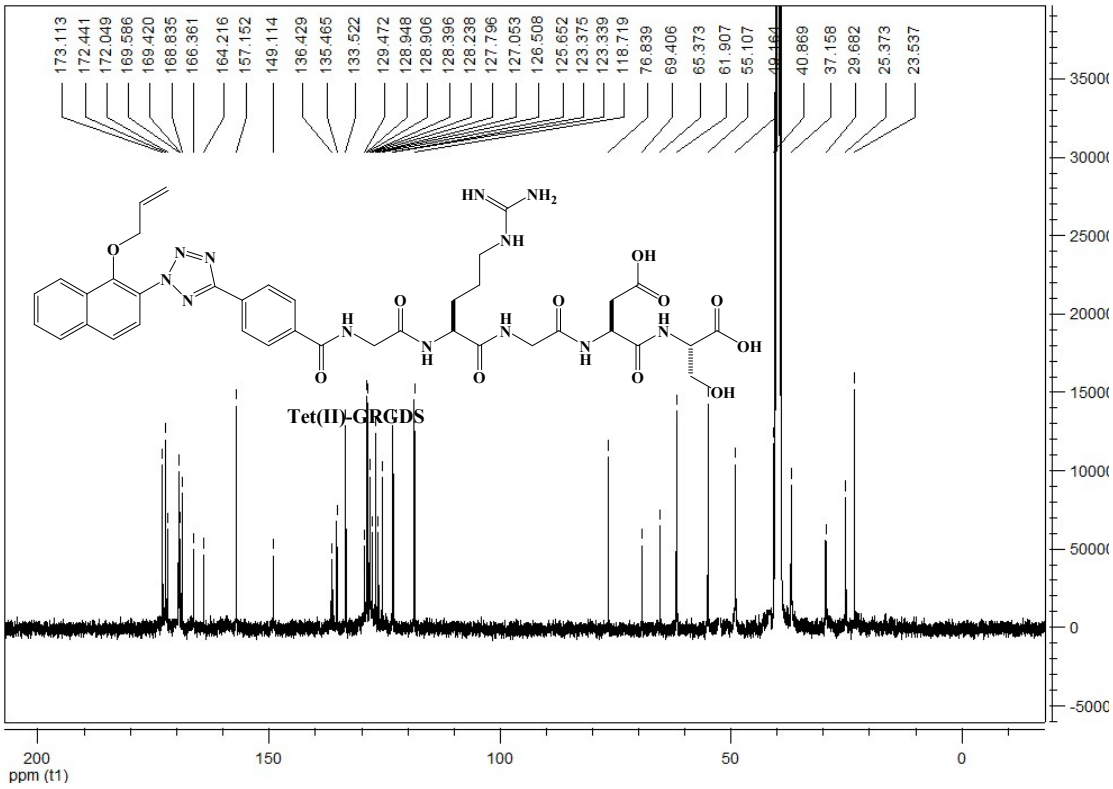
^{13}C NMR of Tet(II)-COOH



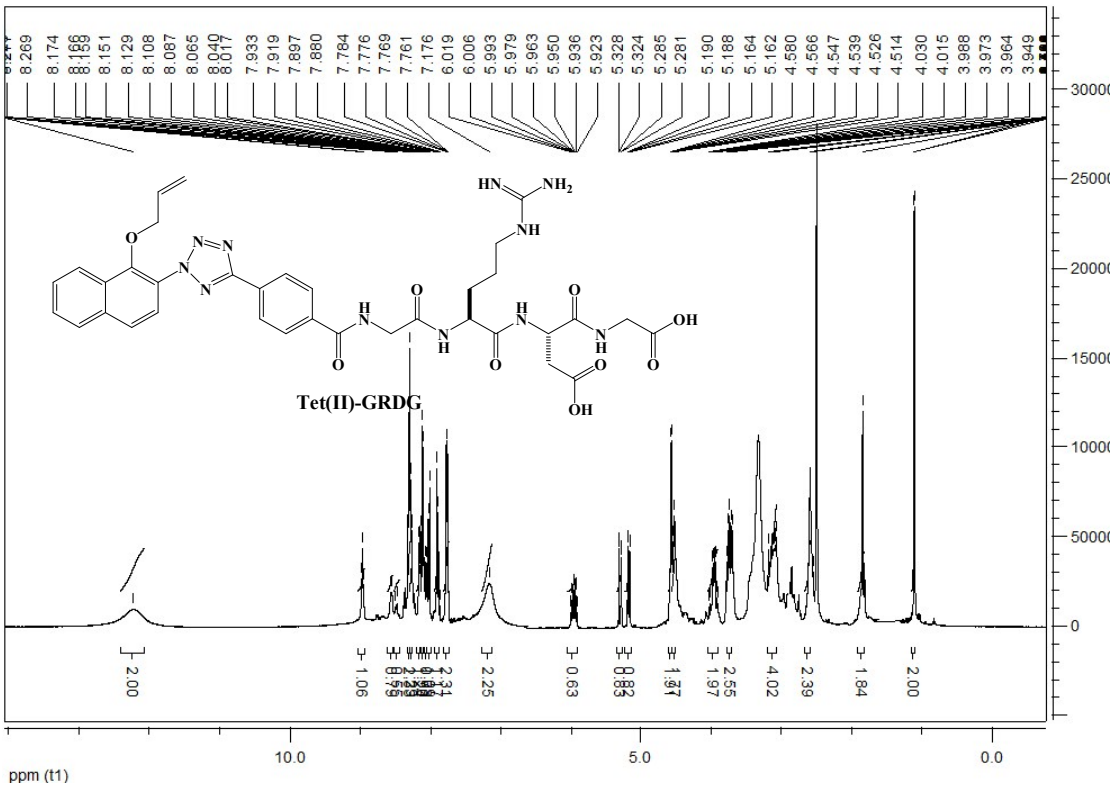
^{1}H NMR of Tet(II)-GRGDS



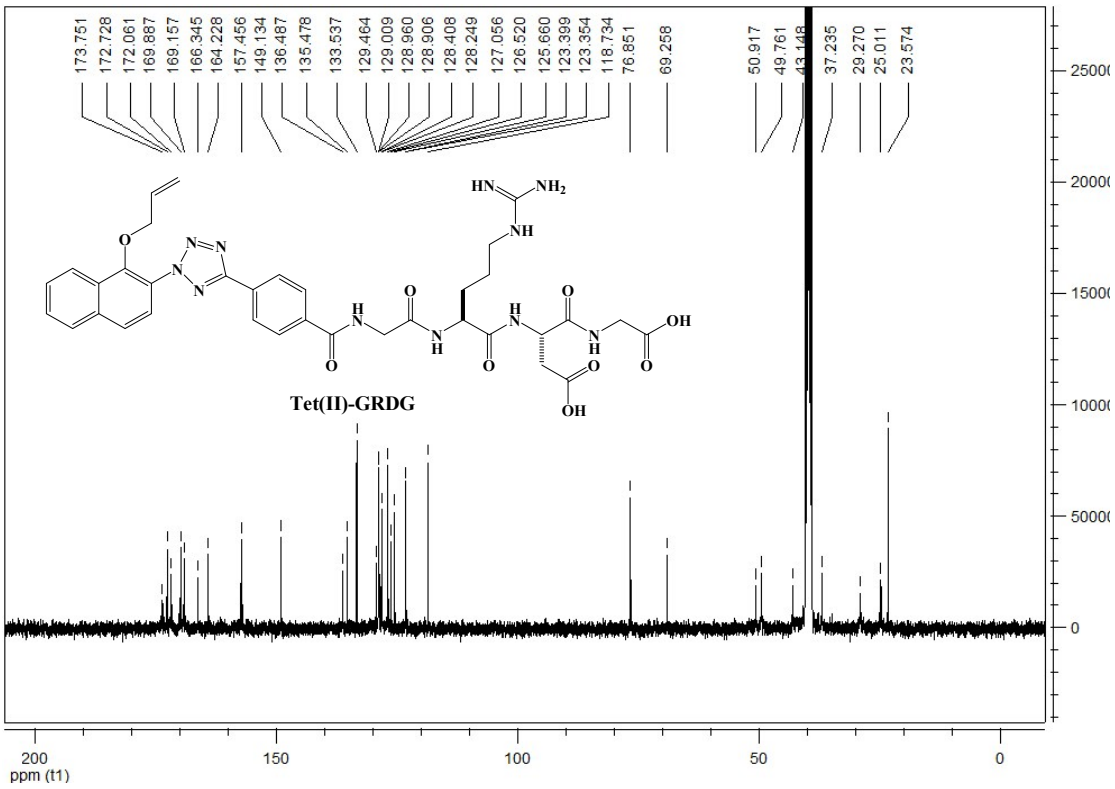
^{13}C NMR of Tet(II)-GRGDS



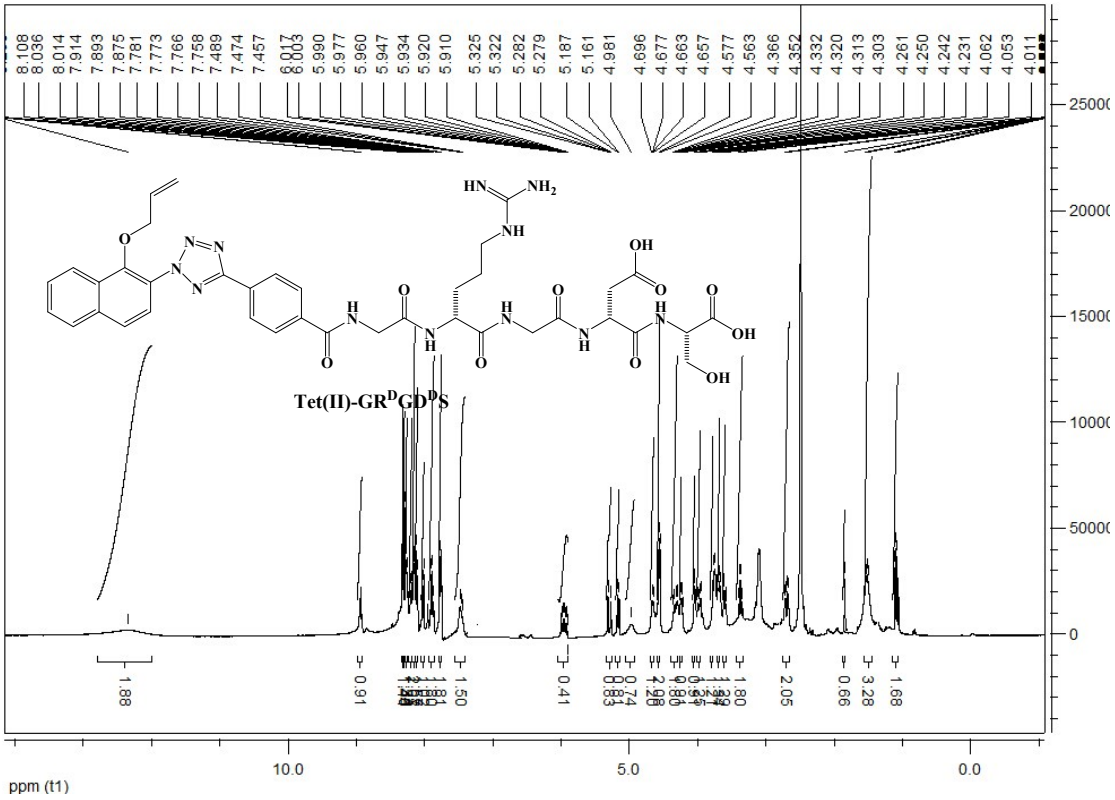
^{1}HNMR of Tet(II)-GRDG



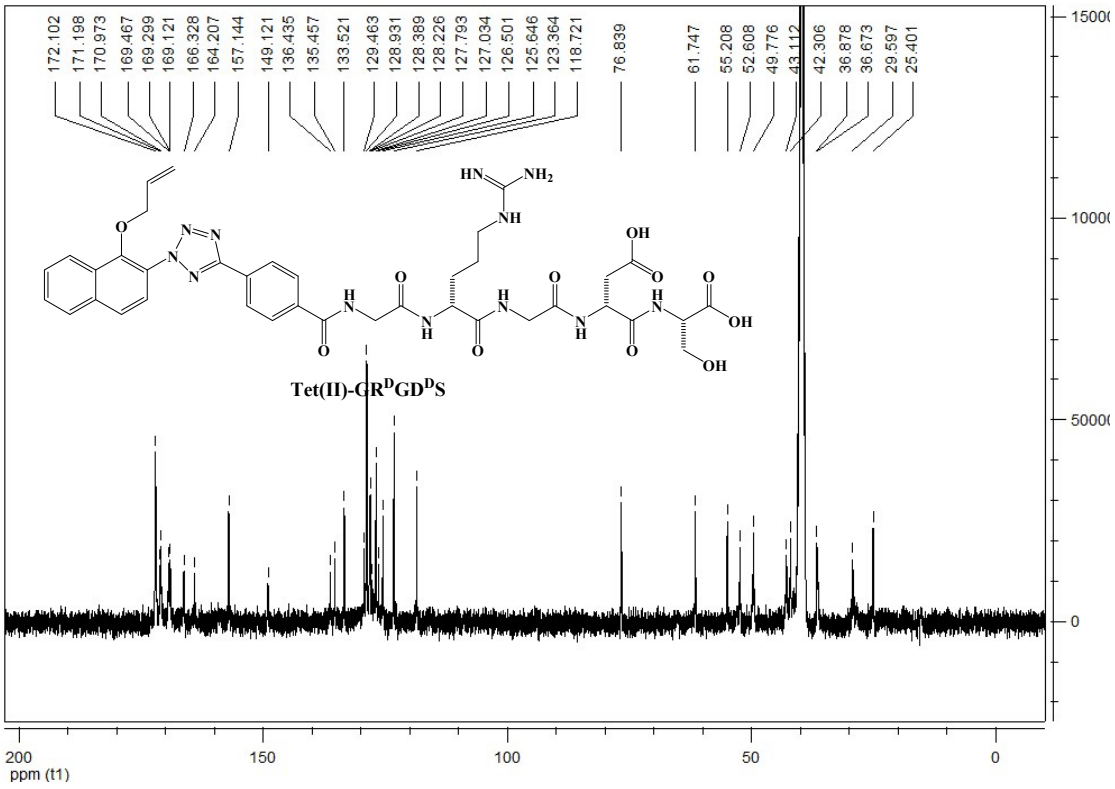
^{13}C NMR of Tet(II)-GRDG



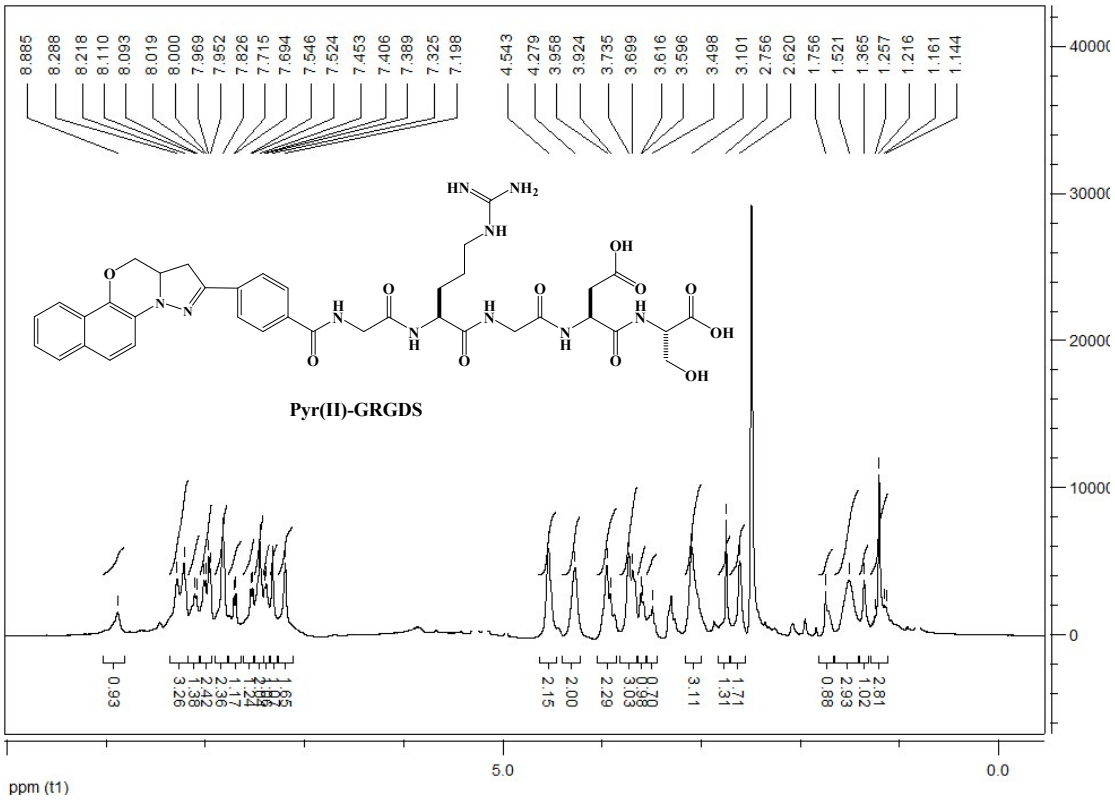
^{1}H NMR of Tet(II)-GR^{D}GD^{D}S



^{13}C NMR of Tet(II)-GR^{D}GD^{D}S



^{1}H NMR of Pyr(II)-GRGDS



^{13}C NMR of Pyr(II)-GRGDS