Electronic Supplementary Information

Specific Targeting, Imaging and Ablation of Tumor-Associated Macrophages by Theranostic Mannose-AIEgen Conjugates

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1. Materials and Instrumentation and cell culture.

Materials

All the chemicals and reagents are commercially available and used without further purification. THF and DMF are distilled under nitrogen before use. 4-toluene sulfonyl chloride, 4-pentyn-1-ol, cesium carbonate, triethylamine, copper sulfate and sodium ascorbate are purchased from TCI. Concanavalin A (Con A), peanut agglutinin (PNA), bull serum albumin (BSA), wheat germ agglutinin (WGA), soybean agglutinin (SBA), DMSO, 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide, methanol, M-CSF from mouse and IL-4 from mouse are purchased from Sigma Aldrich. Proparyl α-D-mannopyranoside is purchased from Carbosynth. Fetal bovine serum (FBS) and

3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) are purchased from Invitrogen. PBS buffer (pH = 7.4, 10 mM) is prepared with pure water from a Millipore filtration system.

Instrumentation

^{1}H and ^{13}C NMR spectra is measured on Bruker ARX 400 NMR spectrometer using DMSO as solvent, and tetramethylsilane (TMS; δ = 0 ppm) was chosen as internal reference. Photoluminescence spectra are measured on PerkinElmer LS 55 spectrofluorometer and UV spectra are measured on Biochrom Libra S80PC double beam spectrometer. High-resolution mass spectra (HRMS) are measured on a GCT Premier CAB 048 mass spectrometer operating in MALDI-TOF mode. Particle sizes are measured using a Brookhaven ZetaPlus potential analyzer (Brookhaven instruments corporation, USA). Confocal lasing scanning microscopic (CLSM) images and fluorescence spectra are obtained on confocal microscope (Zeiss Laser Scanning Confocal Microscope: LSM 780 using ZEN 2009 software (Carl Zeiss).

Sample Preparation

The stock solution of probe was prepared at 1.0 mM in dimethyl sulfoxide (DMSO). Solution of Con A (1.0 mM), PNA (1.0 mM), BSA (1.0 mM), WGA (1.0 mM) and SBA (1.0 mM) were prepared with H_2O.

Titration

Different concentrations of proteins were added to PBS buffer (0.9 mL) and probe (1.0 μL, 1.0 μM) in a 1.5 mL centrifugal tube, the mixture was added to 1.0 mL by DMSO, and the final solution contains 1% DMSO. The mixture was vortex shaded

and placed at 25 ºC for 5 min before photoluminescence (PL) spectra was measured. The excitation wavelength was 445 nm.

Real-time PCR Analysis

Real-time quantitative polymerase chain reaction (qRT-PCR) analysis was carried out to determine the expression of genes. Briefly, 50-100 macrophage cells were harvested by self-made capillary. Then Single Cell Sequence Specific Amplification Kit (Vazyme, China) was used to apply the One-step mRNA reverse transcription. The obtained cDNA was adopted as the template for subsequent PCR amplification. The qRT-PCR reactions were performed by CFX96 (Bio-Rad, USA) and the SYBR Premix EX TaqTM kit (Takara, China). The relative gene expression values were calculated based on the comparative DDCT (threshold cycle) method, and normalized according to the housekeeping gene 18s ribosomal RNA. The primers used in this study were designed by Primer Premier 6 software (Premier Biosoft, USA).

Gene	NCBI Accession No.	Sequence
Arg-1	NM_017134	Forward 5'-TGGAGACCACAGTATGGCAAT-3' Reverse 5'-AGCGGAGTGTTGATGTCAGT-3'
iNOS	NM_012611.3	Forward 5'-TAGAGACGCTTCTGAGGTTCC-3' Reverse 5'-TCTTAGGGTCATCCTGTGTTGT-3'
TNF-α	NM_012675.3	Forward 5'-ATGTGGAACTGGCAGAGGAG-3' Reverse 5'-ACGAGCAGGAATGAGAAGAGG-3'
CD206	NM_001106123.2	Forward 5'-TGGAGCAGATGGAAGGTCTAC-3' Reverse 5'-GTGTCATAGTCAGTGGTGGTTC-3'
18S ribosome	BC126072.1	Forward 5'-CCTTCGCTATCACTGCCATTA-3' Reverse 5'-GCTATACTTCCCATCCTTCACG-3'

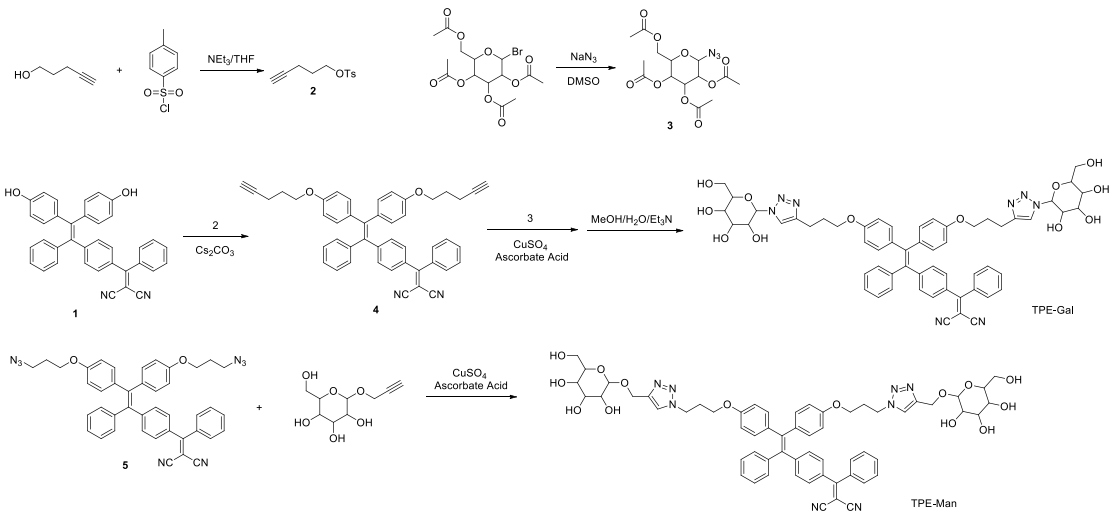
Photo-stability

The TPE-Man loaded macrophages and Alexa647 loaded macrophages were imaged by a confocal microscope (Zeiss laser scanning confocal microscope LSM 780) using ZEN 2009 software (Carl Zeiss). Conditions: for TPE-Man, excitation wavelength was 405 nm (100% laser power) and the emission filter was 550-700 nm; for Alexa647 anti CD206, excitation wavelength was 647 nm (100% laser power) and the emission filter was 650-700 nm.

Cytotoxicity Assay

TAMs were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing penicillin (100 U/mL), 10% heat-inactivated fetal bovine serum (FBS), and streptomycin (100 μg/mL) and were maintained in humidified incubator at 37 ºC under 5% CO_2 environment. After removal of the medium, cells were incubated with various concentrations of probe in the dark or with white light irradiation (400-800 nm) for 8 min at the power density of 100 mW/cm^2, respectively. The cytotoxicity of the probe was assessed by MTT assay according to ISO 10993-5. For each independent experiment, the assays were performed in five replicates. And the statistic mean and standard derivation were utilized to estimate the cell viability.

2. **Synthetic procedures**



Scheme S1. The schematic synthetic procedure of TPE-Man and TPE-Gal.

Compound 1 was synthesized according to the procedures reported in literature.[1]

Synthesis of compound 2. To a solution of 4-pentyn-1-ol (1 mmol, 84.06 mg) and triethylamine (0.5 mL) in distilled THF (20 mL), 4-toluene sulfonyl chloride (1.5 mmol, 284.99 mg) was added dropwise under nitrogen at 0 ^{o}C. The reaction was stirred at room temperature overnight. The resulting solution was quenched with 5 mL water. The solvent was evaporated under reduced pressure. The crude product was extracted with DCM for 3 times and purified by silica gel chromatography with hexane/ethyl acetate (2:1) as eluent to afford the desired product (230 mg) as colorless oil. Yield = 70%. 1H NMR (400 MHz, DMSO): δ = 7.80 (d, 2H), 7.49 (d, 2H), 4.07 (t, 2H), 2.77 (t, 1H), 2.43 (s, 3H), 2.20-2.15 (m, 2H), 1.78-1.71 (m, 2H) ppm; 13C NMR (100 MHz, DMSO): δ = 144.9, 132.2, 130.1, 127.6, 82.7, 71.9, 69.3, 27.2, 21.0, 13.9 ppm; HRMS (ESI, m/z) Calcd. For [C12H14O3S]: 238.0664, found: 239.0734.

Synthesis of compound 3. To a solution of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (1 mmol, 411.2 mg) in DMSO (20 mL), sodium azide (5 mmol, 325.05 mg) was added. The reaction was stirred at room temperature overnight. The reaction was quenched with 10 mL saturated sodium bicarbonate solution. The crude product was extracted with DCM for 3 times and readily used in the next step.

Synthesis of compound 4. Compound **1** (0.1 mmol, 51.6 mg), compound **2** (0.3 mmol, 71 mg) and cesium carbonate (0.3 mmol, 977.46 mg) was heated to 50 ^{o}C in DMF (15 mL) and stirred for 15 minutes. After removing the solvent, the crude product was purified by silica gel chromatography with DCM as eluent to afford the desired product (50 mg) as red solid. Yield = 90%. 1H NMR (400 MHz, DMSO): δ = 7.79 (d, 4H), 7.65 (d, 1H), 7.63 (d, 1H), 7.56 (d, 1H), 7.48 (d, 4H), 7.42 (d, 1H), 7.26

(d, 1H), 7.17 (d, 1H), 7.15 (d, 1H), 7.09 (d, 1H), 7.08 (d, 1H), 6.88 (d, 1H), 6.85 (d, 1H), 6.79 (d, 1H), 6.73-6.68 (m, 2H), 4.10-4.06 (t, 4H), 2.78-2.76 (t, 2H), 2.20-2.14 (m, 4H), 1.78-1.70 (m, 4H) ppm; 13C NMR (100 MHz, DMSO): δ = 173.6, 157.8, 157.7, 148.3, 145.0, 143.1, 141.9, 137.9, 136.1, 135.6, 135.6, 133.8, 133.8, 132.6, 132.6, 132.4,131.0, 131.0, 130.3, 130.0, 128.6, 128.0, 127.5, 114.4, 114.3, 113.7, 113.6, 83.6, 82.6, 81.0, 71.9, 71.6, 65.7, 27.7, 27.2, 21.0, 14.4, 13.9 ppm; HRMS (ESI, m/z) Calcd. For [C46H36N2O2]: 648.2777, found: 649.2845.

Synthesis of TPE-Gal. To the solution of compound **4** (0.025 mmol, 16 mg) and compound **3**, copper sulfate (0.038 mmol, 6.1 mg) and sodium ascorbate (0.075 mmol, 15 mg) were added. The mixtures were stirred in DMF at room temperature overnight. Then, the solvent was removed, and mixtures were redissolved in methanol/water/triethylamine (8:1:1, v/v/v). The mixtures were stirred overnight at room temperature. The mixture was purified by HPLC to afford red solid powder. 1H NMR (400 MHz, DMSO): δ = 8.06-8.02 (d, 2H), 7.66-7.60 (t, 1H), 7.58-7.51 (t, 2H), 7.44-7.39 (d, 2H), 7.28-7.23 (d, 2H), 7.20-7.16 (d, 1H), 7.16-7.12 (d, 1H), 7.12-7.06 (d, 2H), 7.03-6.96 (d, 1H), 6.90-6.82 (m, 4H), 6.75-6.69 (m, 4H), 5.44-5.39 (m, 2H), 5.23-5.17 (d, 2H), 5.06-5.01 (d, 2H), 4.73-4.61 (m, 4H), 4.05-3.89 (m, 6H), 3.78-3.72 (s, 2H), 3.72-3.65 (m, 2H), 3.57-3.43 (m, 6H), 2.81-2.72 (m, 4H), 2.08-1.96 (m, 4H), 1.26-1.19 (s, 2H) ppm; HRMS (ESI, m/z) Calcd. For [C58H58N8O12]: 1058.4174, found: 1059.4282.

Synthesis of TPE-Man. To the solution of compound **5** (0.025 mmol, 17 mg) and Proparyl α-D-mannopyranoside (0.075 mmol, 16.3 mg), copper sulfate (0.038 mmol, 6.1 mg) and sodium ascorbate (0.075 mmol, 15 mg) were added. The mixtures were stirred in DMF at room temperature overnight. Then, the solvent was removed and the mixture was purified by HPLC to afford red solid powder. 1H NMR (400 MHz, DMSO): δ = 8.06-8.01 (d, 2H), 7.65-7.61 (t, 1H), 7.58-7.52 (t, 2H), 7.45-7.40 (d, 2H), 7.28-7.24 (d, 2H), 7.20-7.15 (d, 1H), 7.15-7.11 (d, 1H), 7.10-7.07 (d, 2H), 7.02-6.90 (d, 1H), 6.91-6.80 (m, 4H), 6.77-6.69 (m, 4H), 4.85-4.82 (d, 2H), 4.78-4.76 (d, 2H), 4.76-4.72 (d, 2H), 4.62-4.58 (d, 2H), 4.50-4.46 (t, 2H), 4.26-4.11 (m, 4H), 3.95-3.78 (m, 4H), 3.68-3.62 (m, 2H), 3.60-3.57 (m, 2H), 3.46-3.36 (m, 6H), 3.27-3.22 (m, 2H), 2.93-2.79 (m, 4H), 2.11-1.95 (m, 4H) ppm; HRMS (ESI, m/z) Calcd. For [C60H62N8O14]: 1118.4385, found: 1141.4266.

References

1. Hu, F.; Huang, Y.; Zhang, G.; Zhao, R.; Yang, H.; Zhang, D. *Anal. Chem.* **2014**, *86*, 7987-7995

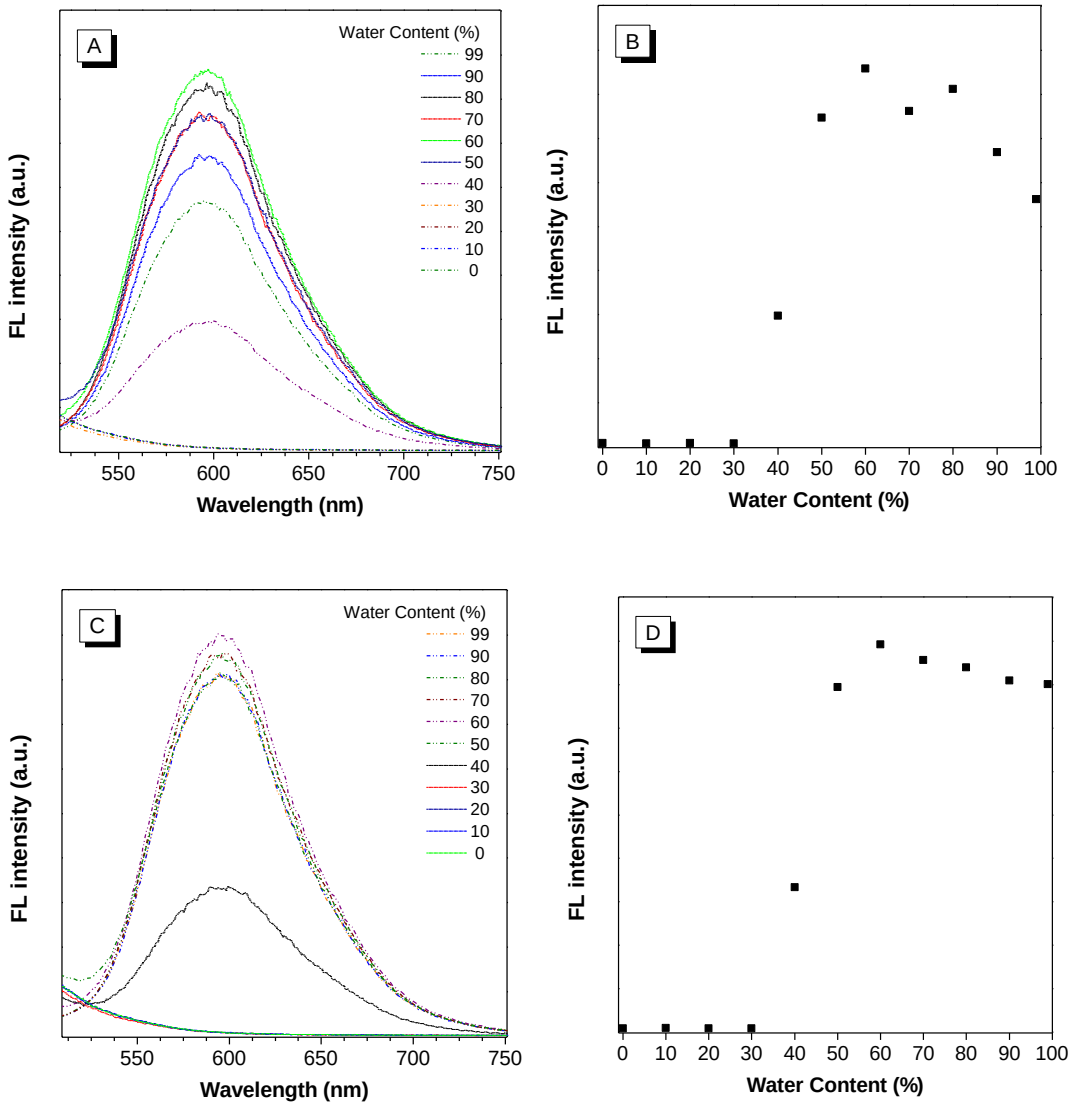
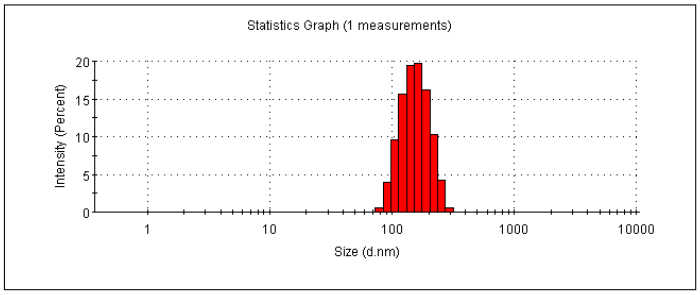


Figure S1. (A, C) Photoluminescence (PL) spectra of TPE-Man and TPE-Gal in DMSO/water mixtures with different water fractions (fw), respectively. (B, D) Plot of relative peak intensity (I/I_0) vs fw. Concentration of the probe: 10 μM；Excitation wavelength: 390 nm.

A

Z-Average (nm):	172.7592	Derived Count Rate (kcps):	5518.10388954...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity Percent	Std Dev Intensity Percent	Size d.nm	Mean Intensity Percent	Std Dev Intensity Percent	Size d.nm	Mean Intensity Percent	Std Dev Intensity Percent	Size d.nm	Mean Intensity Percent	Std Dev Intensity Percent
0.4000	0.0		5.615	0.0		78.82	0.5		1106	0.0	
0.4632	0.0		6.503	0.0		91.28	3.9		1281	0.0	
0.5365	0.0		7.531	0.0		105.7	9.6		1484	0.0	
0.6213	0.0		8.721	0.0		122.4	15.6		1718	0.0	
0.7195	0.0		10.10	0.0		141.8	19.5		1990	0.0	
0.8332	0.0		11.70	0.0		164.2	19.7		2305	0.0	
0.9649	0.0		13.54	0.0		190.1	16.2		2669	0.0	
1.117	0.0		15.69	0.0		220.2	10.2		3091	0.0	
1.294	0.0		18.17	0.0		255.0	4.2		3580	0.0	
1.499	0.0		21.04	0.0		295.3	0.5		4145	0.0	
1.736	0.0		24.36	0.0		342.0	0.0		4801	0.0	
2.010	0.0		28.21	0.0		396.1	0.0		5560	0.0	
2.328	0.0		32.67	0.0		458.7	0.0		6439	0.0	
2.696	0.0		37.84	0.0		531.2	0.0		7456	0.0	
3.122	0.0		43.82	0.0		615.1	0.0		8635	0.0	
3.615	0.0		50.75	0.0		712.4	0.0		1.000e4	0.0	
4.187	0.0		58.77	0.0		825.0	0.0				
4.849	0.0		68.06	0.0		955.4	0.0				



B

Z-Average (nm):	182.9968	Derived Count Rate (kcps):	4279.32464144...
Standard Deviation:	0	Standard Deviation:	0
%Std Deviation:	0	%Std Deviation:	0
Variance:	0	Variance:	0

Size d.nm	Mean Intensity Percent	Std Dev Intensity Percent	Size d.nm	Mean Intensity Percent	Std Dev Intensity Percent	Size d.nm	Mean Intensity Percent	Std Dev Intensity Percent	Size d.nm	Mean Intensity Percent	Std Dev Intensity Percent
0.4000	0.0		5.615	0.0		78.82	0.5		1106	0.0	
0.4632	0.0		6.503	0.0		91.28	2.7		1281	0.0	
0.5365	0.0		7.531	0.0		105.7	6.7		1484	0.0	
0.6213	0.0		8.721	0.0		122.4	11.3		1718	0.0	
0.7195	0.0		10.10	0.0		141.8	15.3		1990	0.0	
0.8332	0.0		11.70	0.0		164.2	17.4		2305	0.0	
0.9649	0.0		13.54	0.0		190.1	16.9		2669	0.0	
1.117	0.0		15.69	0.0		220.2	13.9		3091	0.0	
1.294	0.0		18.17	0.0		255.0	9.3		3580	0.0	
1.499	0.0		21.04	0.0		295.3	4.6		4145	0.0	
1.736	0.0		24.36	0.0		342.0	1.3		4801	0.0	
2.010	0.0		28.21	0.0		396.1	0.0		5560	0.0	
2.328	0.0		32.67	0.0		458.7	0.0		6439	0.0	
2.696	0.0		37.84	0.0		531.2	0.0		7456	0.0	
3.122	0.0		43.82	0.0		615.1	0.0		8635	0.0	
3.615	0.0		50.75	0.0		712.4	0.0		1.000e4	0.0	
4.187	0.0		58.77	0.0		825.0	0.0				
4.849	0.0		68.06	0.0		955.4	0.0				

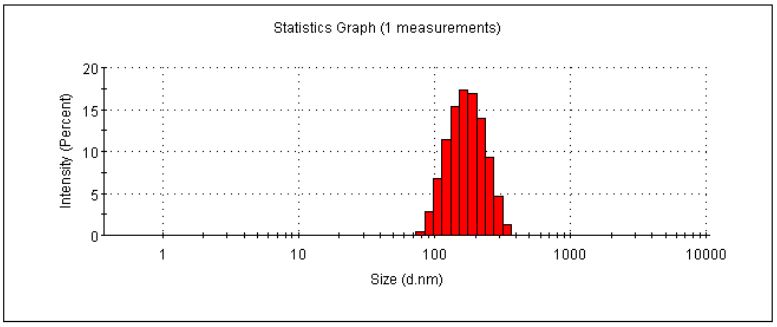


Figure S2. (A) Size distribution of TPE-Man in PBS buffer solution, containing 1% DMSO at 30 ℃. (B) Size distribution of TPE-Gal in PBS buffer solution, containing 1% DMSO at 30 ℃. Concentration of the probe: 10 μM

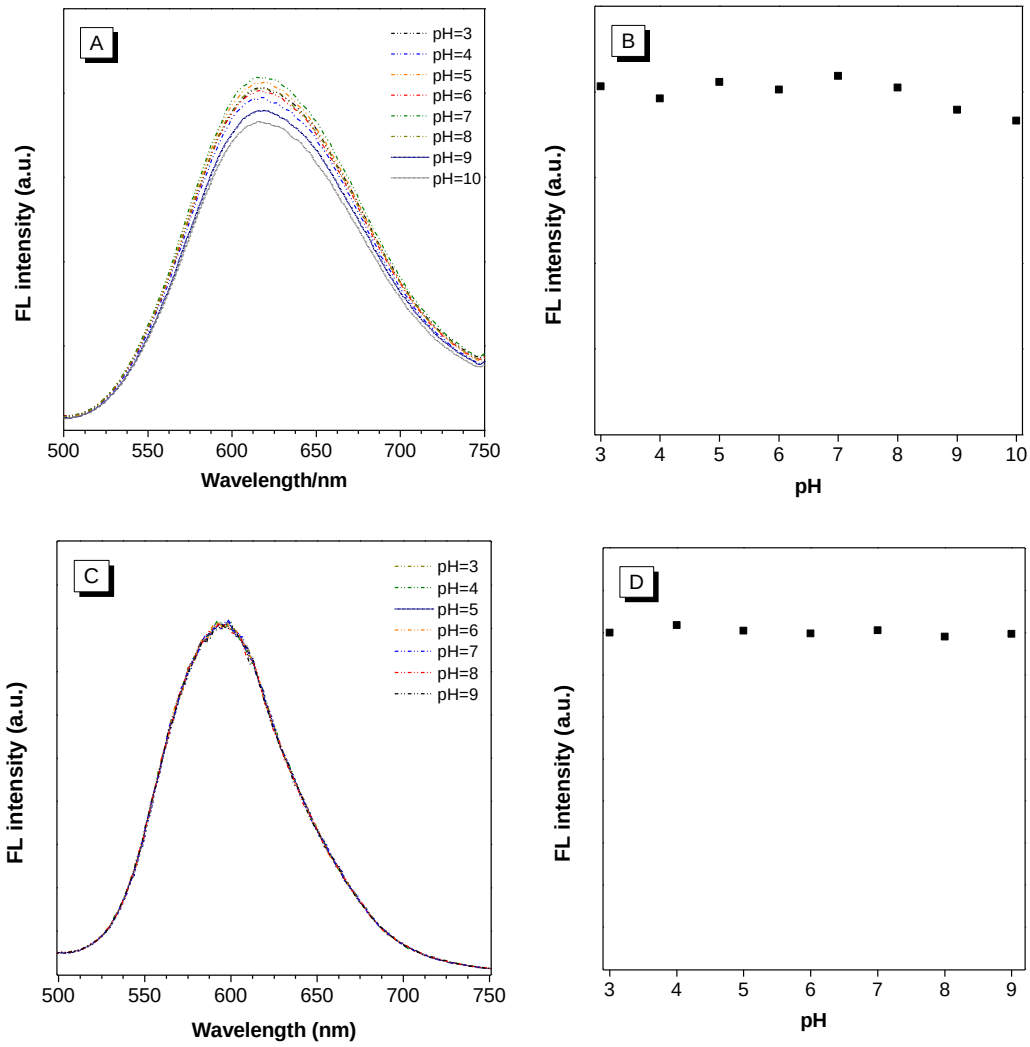


Figure S3. (A, C) Photoluminescence (PL) spectra of TPE-Man and TPE-Gal in PBS buffer solution, containing 1% DMSO, respectively. (B, D) Plot of FL peak intensity. Concentration of the probe: 10 μM; Excitation wavelength: 390 nm.

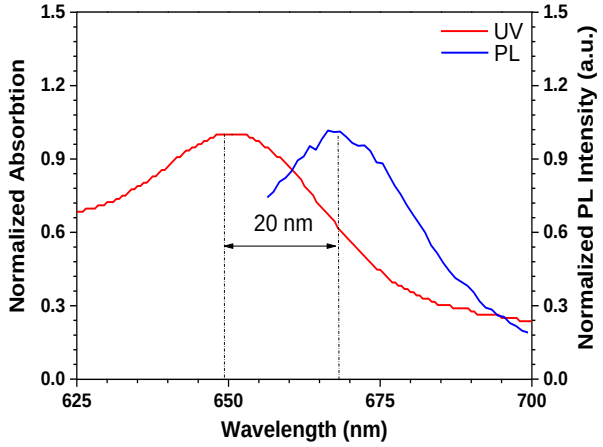


Figure S4. Absorption and emission spectra of Alexa647 used in immunostaining.

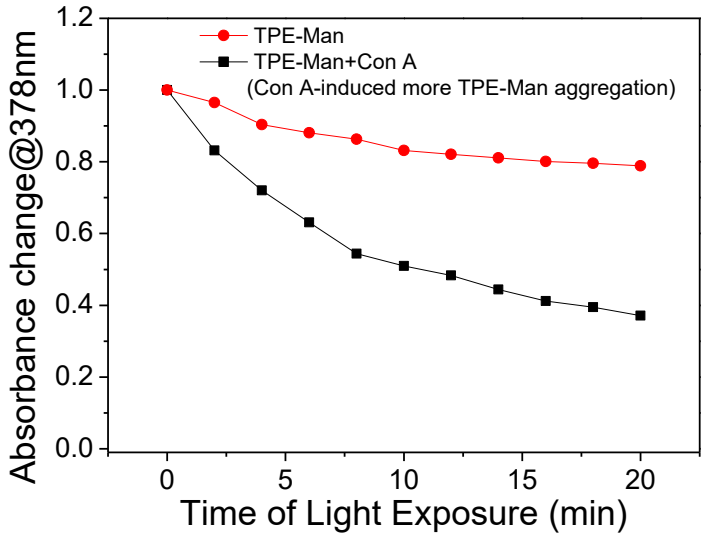


Figure S5. Time-dependent bleaching of ADPA ((anthracence-9,10-dipropionic acid disodium salt, 50 μM) by $^1O_2$ generated by TPE-Man (3 μM) incubated with and without Con A (40 μM). The samples were irradiated at 450 nm with an intensity of 12 mW/cm^2 .

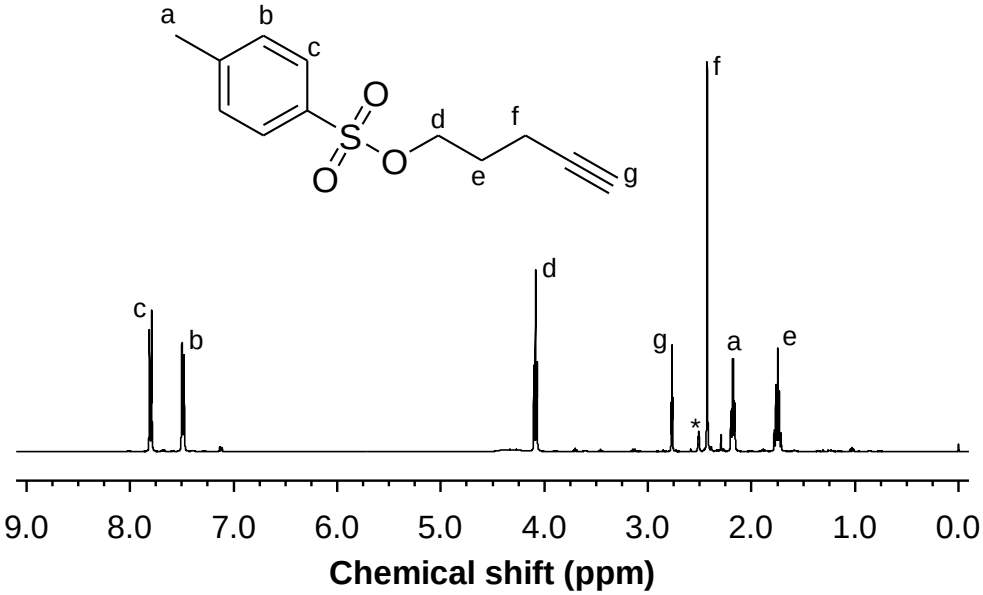


Figure S6. ^{1}H NMR spectrum of compound 2 in DMSO. The solvent peaks are marked with asterisks.

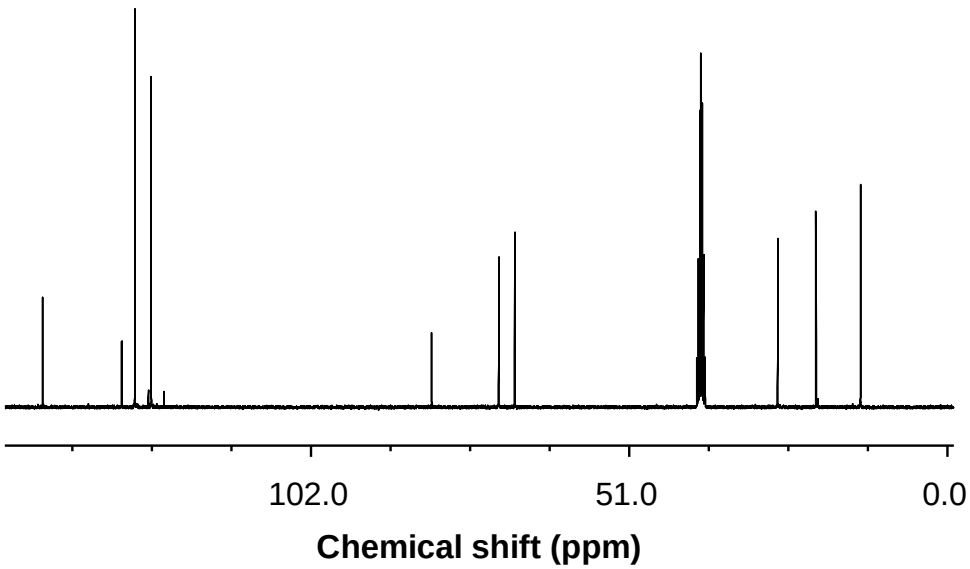


Figure S7. ^{13}C NMR spectrum of compound 2 in DMSO. The solvent peaks are marked with asterisks.

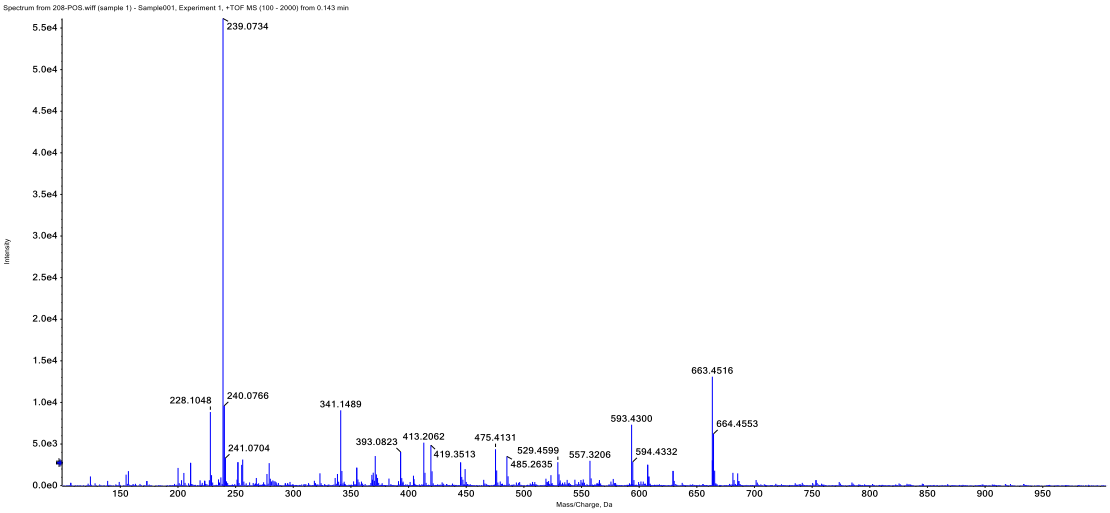


Figure S8. High resolution mass spectrum (HRMS) of compound 2.

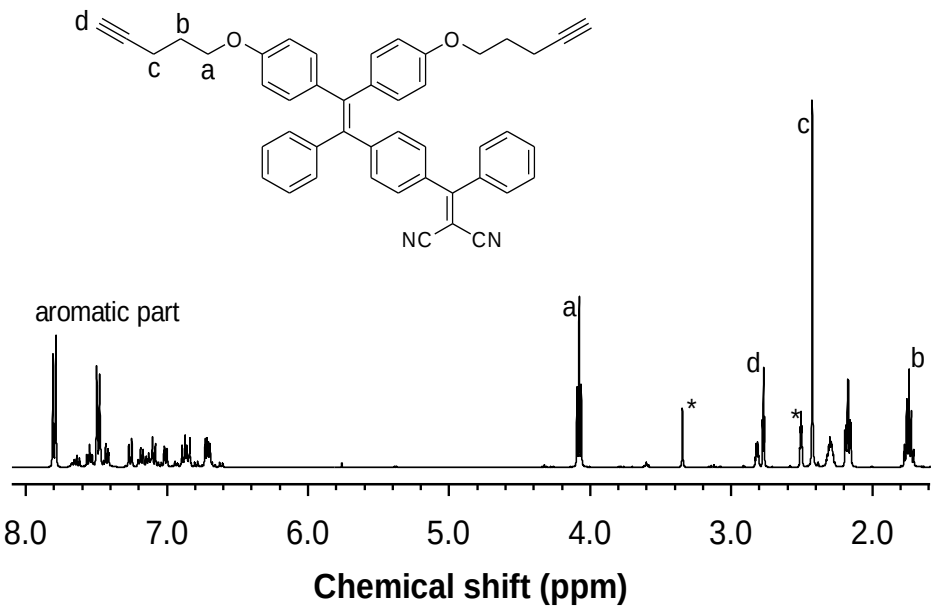


Figure S9. ^{1}H NMR spectrum of compound 4 in DMSO. The solvent peaks are marked with asterisks.

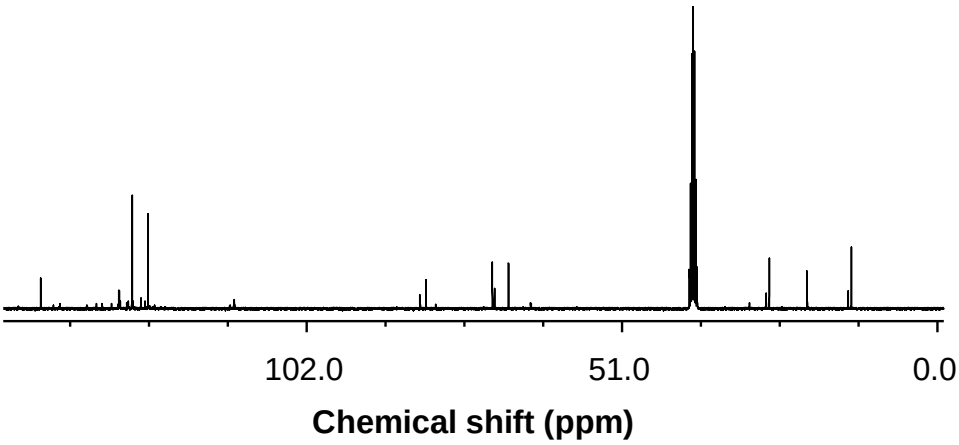


Figure S10. ^{13}C NMR spectrum of compound 4 in DMSO. The solvent peaks are marked with asterisks.

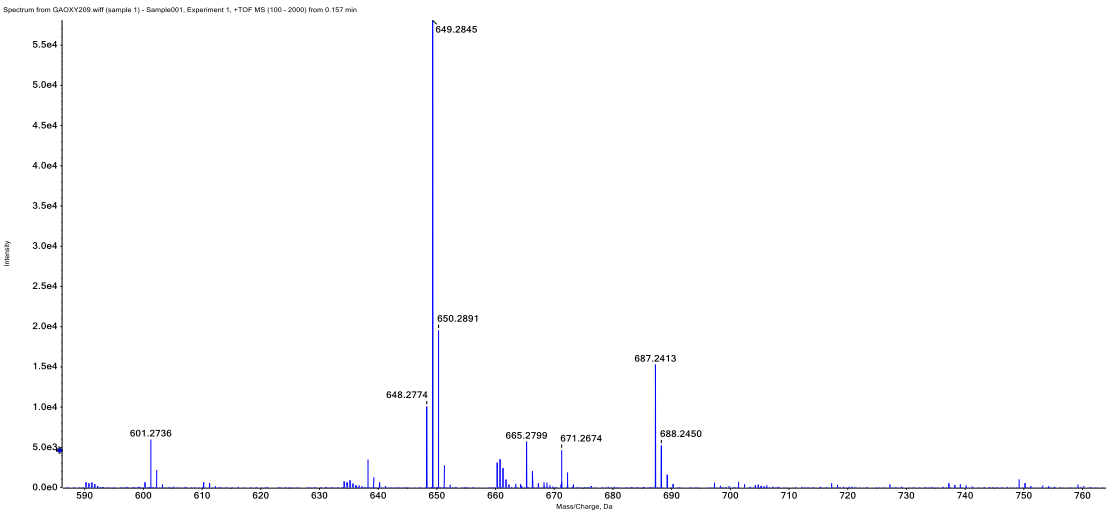


Figure S11. High resolution mass spectrum (HRMS) of compound 4.

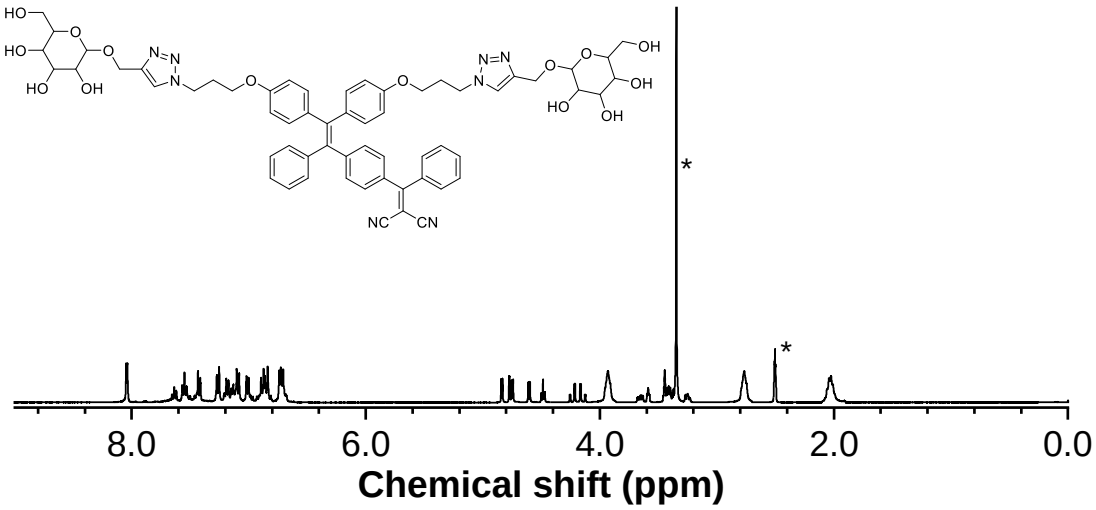


Figure S12. 1H NMR spectrum of TPE-Man in DMSO. The solvent peaks are marked with asterisks.

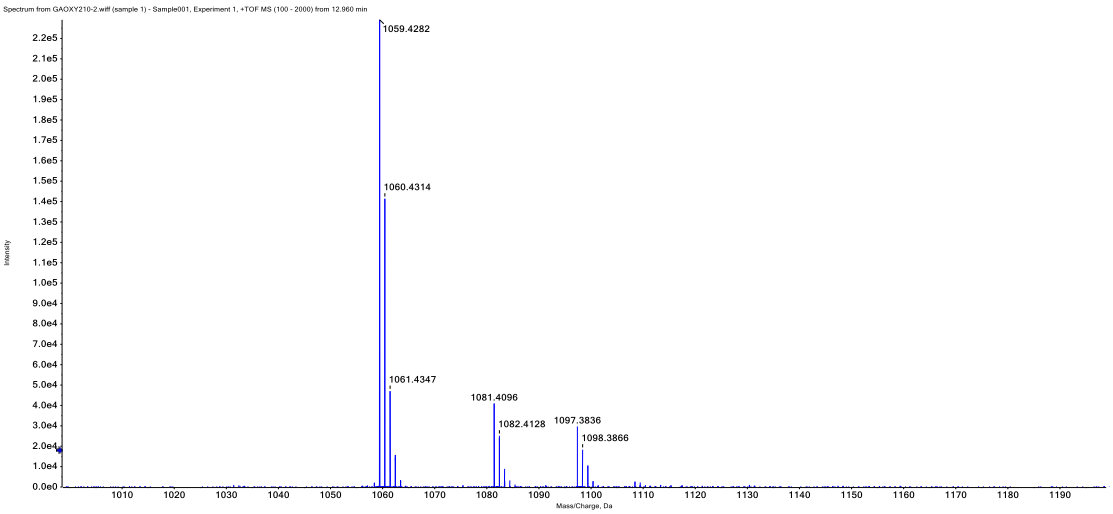


Figure S13. High resolution mass spectrum (HRMS) of TPE-Man

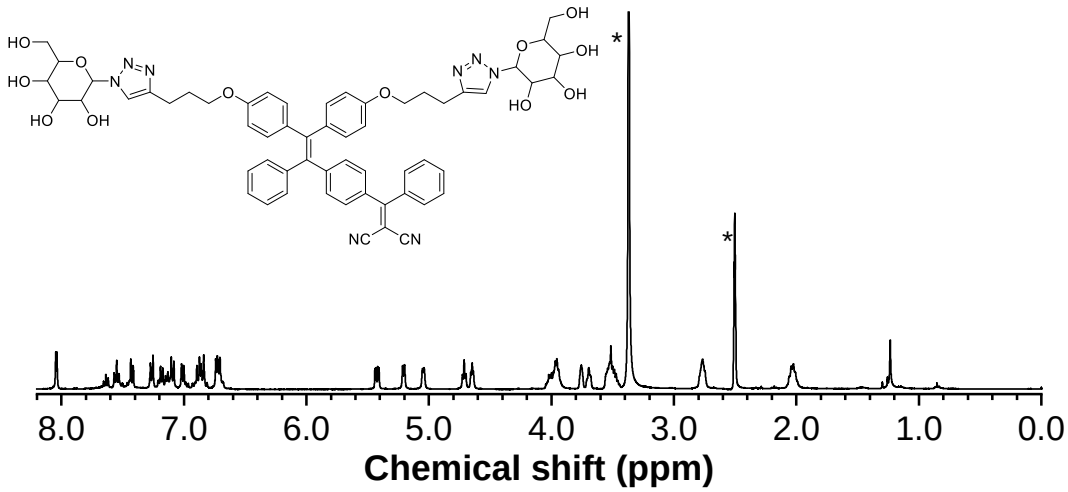


Figure S14. ^{1}H NMR spectrum of TPE-Gal in DMSO. The solvent peaks are marked with asterisks.

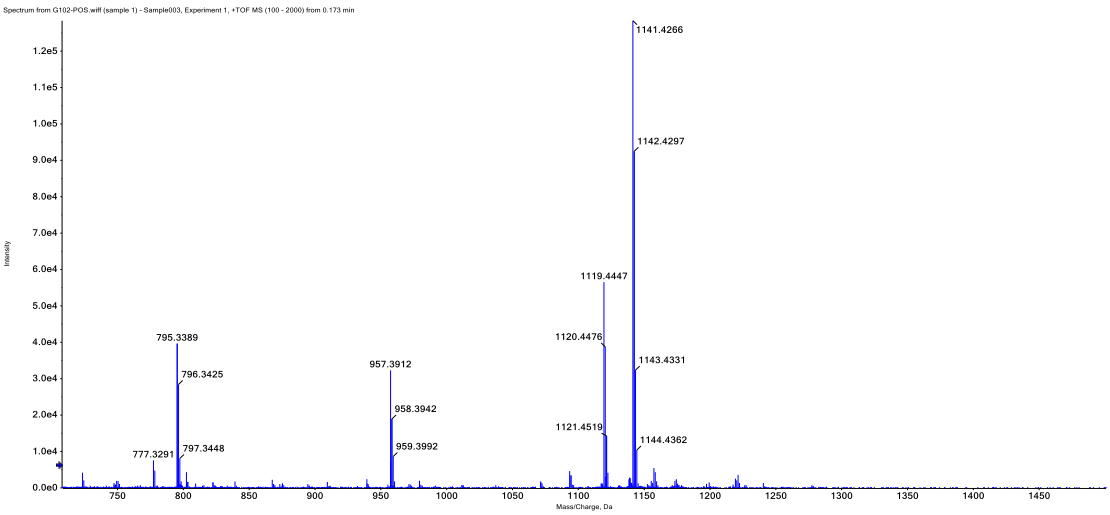


Figure S15. High resolution mass spectrum (HRMS) of TPE-Gal.