# Supporting Information for

## Dual Stimuli-Responsive Biotinylated Polymer-Drug Conjugate for Dual Drug Delivery

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### Experimental section

**Materials.** Thioglycolic acid (98%), benzoyl chloride (99%), anhydrous *N,N*-dimethylformamide (DMF, 99.8%), pyrene (98%), chlorambucil (CBL), doxorubicin (DOX), 4-(4-nitrobenzyl)pyridine (NBP), 2,2′-azobis(2-methylpropionitrile) (AIBN, 98%), 2-(4-hydroxyphenylazo)benzoic acid (HABA, ≥90%), 2,4-dimethylpyrrole (97%), boron trifluoride diethyl etherate ($BF_3$•$Et_2O$), 4-formylbenzoic acid (FBA, 97%), 2-hydroxyethyl methacrylate (HEMA, ≥99%, contains ≤50 ppm monomethyl ether hydroquinone as inhibitor), esterase [from porcine liver, lyophilized powder, >15 units/mg (U/mg) solid], poly(ethylene glycol) methacrylate [PEGMA, average molecular weight ($M_n$) = 500 g/mol, contains 900 ppm monomethyl ether hydroquinone as inhibitor], poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, average $M_n$ = 300 g/mol, contains 300 ppm BHT as inhibitor, 100 ppm MEHQ as inhibitor) and 4′,6-diamidino-2-phenylindole (DAPI) were obtained from Sigma-Aldrich and used as received except PEGMEMA, PEGMA, and AIBN. PEGMEMA and PEGMA were passed through the basic alumina ($Al_2O_3$) column to remove the inhibitors, and AIBN was

recrystallized in methanol. Lithium aluminium hydride ($LiAlH_4$) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC•HCl) were purchased from Spectrochem Pvt. Ltd., India and used without any purification. Dicyclohexylcarbodiimide (DCC, 99%), 4-dimethylaminopyridine (DMAP, 99%), trifluoroacetic acid (TFA, 99.5%), DMF (for HPLC & UV spectroscopy grade, 99.9%), triethylamine ($Et_3N$, 98%), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 98%), triton X-100 (molecular biology grade), 10X phosphate buffer saline (PBS, molecular biology grade), silica gel (100-200 mesh), avidin, water [high-performance liquid chromatography (HPLC) grade] and D-biotin were obtained from Sisco Research Laboratories (SRL) Pvt. Ltd., India and used as obtained. NMR solvents such as DMSO-$d_6$ (99% D), $CD_3OD$ (99.8% D), $CDCl_3$ (99.8% D), and $D_2O$ (99% D) were bought from Cambridge Isotope Laboratories, Inc., USA. Methacrylic acid (MA, stabilized with hydroquinone monomethyl ether), $Al_2O_3$, anhydrous sodium sulfate ($Na_2SO_4$, 99%), anhydrous sodium bicarbonate ($NaHCO_3$, 99.5%), sodium chloride (NaCl, 99%), hydrogen peroxide ($H_2O_2$, 30%), hexane (a mixture of isomers), dichloromethane (DCM), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), acetonitrile, methanol, diethyl ether, acetone and ethyl acetate were purchased from Merck, and used as obtained. Chain transfer agent, 4-cyano-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic (CDP) was prepared as reported elsewhere.[1] Human cervical cancer cell (HeLa) was obtained from the National Centre for Cell Science (NCCS, Pune, India) and human embryonic kidney cell (HEK 293T) was kindly gifted by Dr. Arnab Gupta (DBS, IISER Kolkata). Both the cells were maintained in a growth medium containing Dulbecco's Modified Eagle's medium (DMEM, Gibco, Invitrogen, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS, Gibco, Invitrogen, Thermo Fisher Scientific), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco, Invitrogen, Thermo Fisher Scientific) at 37 °C with 5% $CO_2$. Phalloidin 647 and trypsin were obtained from Abcam, USA, and Gibco, Invitrogen, Thermo Fisher Scientific, respectively. 3-

(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT) and *p*-formaldehyde (PFA) were acquired from HiMedia Laboratories Pvt. Ltd., India. 96-well, 24-well, and 6-well tissue culture plates were bought from Tarsons, India. Dialysis membranes with molecular weight cutoff (MWCO) 2000 g/mol (spectra/por® 6, nominal flat width-45 mm, diameter-29 mm, volume/length-6.4 mL/cm) and 6000-8000 g/mol (spectra/por® 1, nominal flat width-23 mm, diameter-14.6 mm, volume/length-1.7 mL/cm) were obtained from Spectrum Laboratories, USA.

**Instruments used for characterization**

*$^1$H Nuclear magnetic resonance (NMR) spectroscopy.* The $^1$H and $^{13}$C NMR spectroscopic characterization of monomers and polymers were carried out utilizing 400 MHz JEOL ECS NMR and 500 MHz Bruker Avance III NMR spectrometers. Tetramethylsilane (TMS) was used as an internal standard.

*Size exclusion chromatography (SEC).* The number average molar mass ($M_{n,SEC}$) and dispersity ($Đ$) of the polymers were determined using a Waters SEC instrument operating at 40 ºC and 0.8 mL/min DMF eluent flow rate. This instrument is composed of: (a) two 300 × 7.5 mm PolarGel-M analytical columns, (b) one 50 × 7.5 mm PolarGel-M guard column, (c) one Waters 2414 refractive index (RI) detector, and (d) one Waters 1515 HPLC pump. Poly(methyl methacrylate) (PMMA) standards were used to generate the calibration curve.

*UV-Vis spectroscopy.* Absorption spectra of the solution were collected using the Perkin Elmer Lambda 35 spectrophotometer instrument.

*High-resolution mass spectrometry (HRMS).* Bruker maXis impact MicrOTOF-Q II and Waters XeVO G2-XS QTof instruments were used to record the HRMS of the monomer. Before the experiment, the samples were prepared in either acetonitrile or methanol, and filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter.

*Fluorescence spectroscopy.* Emission spectra of the solutions were measured using the HORIBA Jobin Yvon Fluoromax-3 spectrofluorometer equipped with a 150 W xenon lamp power supply.

*Dynamic light scattering (DLS).* For measuring the hydrodynamic diameter ($D_h$) of the polymeric nanoparticle, the Malvern Zetasizer Nano ZS instrument (UK) was utilized. The instrument is outfitted with a 4 mV helium-neon laser with a wavelength of 630 nm and a 173º scattering angle.

*Field emission scanning electron microscopy (FE-SEM).* The surface topographic analysis of the aggregated nanoparticle was executed using the Carl Zeiss Sigma instrument.

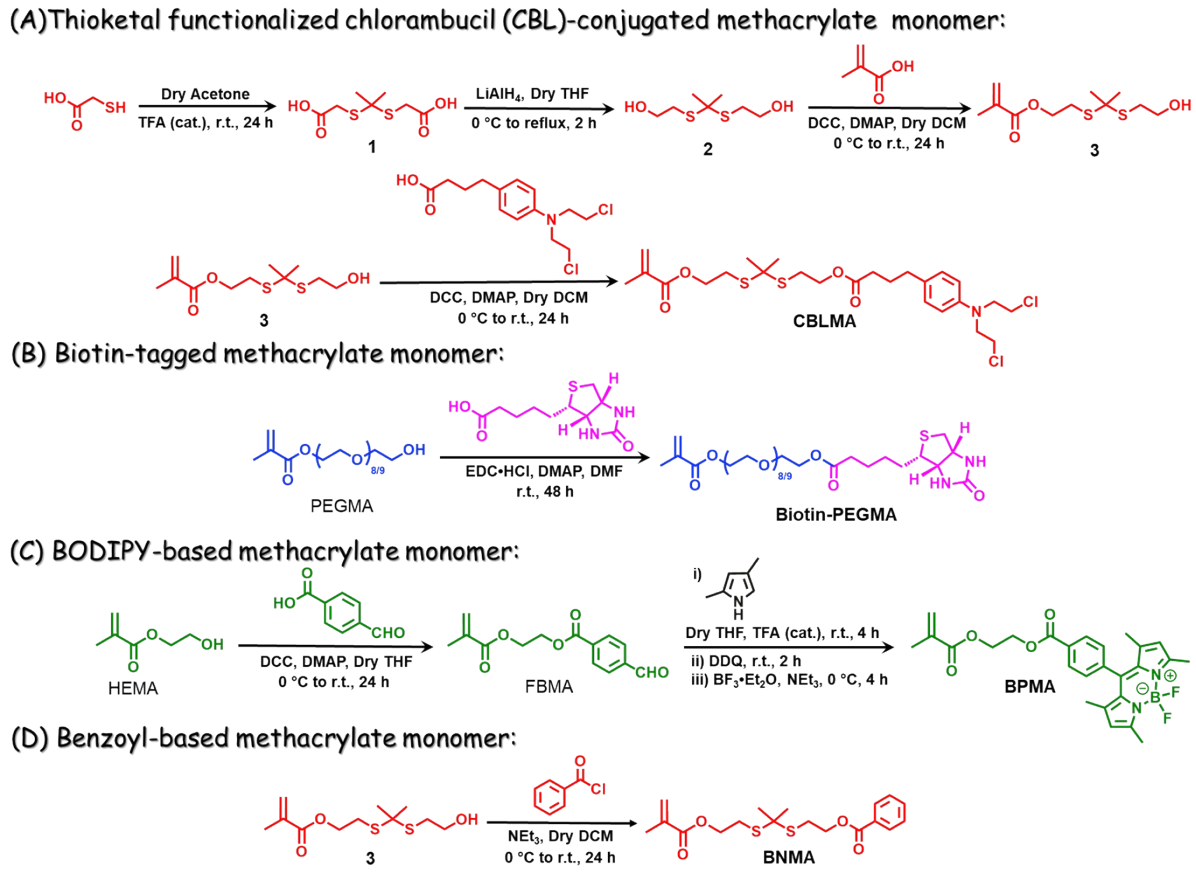
*Transmission electron microscopy (TEM).* The aggregated nanoparticle was visualized using the JEOL JEM 2100F electron microscope with 200 kV acceleration voltage.

*Isothermal calorimetry (ITC).* The protein-polymer interaction was investigated using the Malvern MicroCAL PEAQ-ITC instrument.

*Confocal laser scanning microscopy (CLSM).* CLSM images of the cells were captured by Leica SP8 (Leica Microscope, Wetzlar, Germany) with an oil immersion 63× objective lensusing LasX software. The images were processed using the FIJI software.

*Optical density.* The optical density of the cell samples was measured in the Biotek Epoch2 microplate spectrophotometer at 595 nm.

*Flow cytometry.* Flow cytometry analysis of the samples was executed using LSRFortessa, BD BioSciences instrument.




**Scheme S1.** Synthetic route of (A) **CBLMA**, (B) **Biotin-PEGMA**, (C) **BPMA**, and (D) **BNMA** monomers.

## Synthesis and experimental protocols

**Synthesis of 1.** Compound **1** in Scheme S1 was synthesized by following the previously mentioned procedure.[2] In a 100 mL round-bottomed flask (RBF), thioglycolic acid (2.30 g, 24.97 mmol) and acetone (0.89 g, 14.98 mmol) were added, followed by the addition of a catalytic amount of TFA (2.5 µL). Then, the reaction was kept under stirring conditions at room temperature (r.t.) for 24 hours (h). To promote the precipitation of the desired product, the reaction mixture was poured into a water-containing beaker. Next, the residue was washed with hexane and water 3 times each and dried under vacuum to obtain the white product. Yield = 68%. $^1$H NMR (400 MHz, $CD_3OD$, $\delta$ ppm, Fig. S1): 3.39 (HOOCC***H***$_2$-, 4H, s), 1.58 (-C(C***H***$_3$)$_2$-

, 6H, s). $^{13}$C NMR (126 MHz, $CD_3OD$, $\delta$ ppm, Fig. S2): 172.93, 56.53, 32.50, 29.45. HRMS (Fig. S3): Mass (*m*/*z*) calculated for $[M + Na]^+$ = 247.0075; observed = 247.0083.

**Synthesis of 2.** In a 250 mL RBF, compound **1** (1.00 g, 4.46 mmol) was dissolved in dry THF (100 mL) and placed in the ice water bath under stirring conditions (Scheme S1). Next, $LiAlH_4$ (1.69 g, 44.60 mmol) was slowly added with a small portion at a time to the reaction mixture and left for 1 h in ice-cold conditions. After 1 h, the reaction mixture was refluxed for 2 h. Then, the reaction mixture was quenched with water and the precipitated residue was filtered. The filtrate was washed with 3 × 100 mL aqueous NaOH solution and 3 × 100 mL brine solution, followed by drying over $Na_2SO_4$. Afterward, the organic part was concentrated under a vacuum and purified by silica column chromatography using 5% methanol/DCM to get a yellowish liquid. Yield = 55%. $^1$H NMR (400 MHz, $CDCl_3$, $\delta$ ppm, Fig. S4): 3.79 (HO***H*****$_2$**$CCH_2$-, 4H, t), 2.88 ($HOH_2CC$***H*****$_2$**-, 4H, t), 1.62 (-C(C***H***$_3)_2$-, 6H, s). $^{13}$C NMR (126 MHz, $CDCl_3$, $\delta$ ppm, Fig. S5): 61.30, 55.98, 33.45, 31.25. HRMS (Fig. S6): Mass (*m*/*z*) calculated for $[M + Na]^+$ = 219.0489; observed = 219.0483.

**Synthesis of 3.** Compound **3** was synthesized by a typical DCC/DMAP esterification reaction (Scheme S1). In a 100 mL RBF, MA (172.00 mg, 2.00 mmol) was dissolved in 50 mL dry DCM, placed in the ice-water bath, and purged with dry $N_2$. After 30 min of purging, the solution of DCC (495.45 mg, 2.40 mmol) and DMAP (48.86 mg, 0.40 mmol) in 10 mL dry DCM was added to the RBF. When the milky appearance was observed, compound **2** (392.64 mg, 2.00 mmol) was added into it and left the reaction for 24 h under stirring conditions at r.t. Next day, *N,N*-dicyclohexylurea (DCU) was filtered by filtration and the organic layer was washed with 60 mL brine solution (3 times) followed by drying over anhydrous $Na_2SO_4$. Finally, the organic part was concentrated under vacuum and purified by column chromatography using 10% ethyl acetate (EtOAc)/hexane to get a transparent sticky liquid, compound **3**. Yield = 65%. $^1$H NMR (400 MHz, $CDCl_3$, $\delta$ ppm, Fig. S7): 6.15 (***H***HC=C($CH_3$)-,

1H, s), 5.61 (H***H***C=C($CH_3$)-, 1H, s), 4.34 (-OC***H***$_2$$CH_2$-, 2H, t), 3.81 (-$SCH_2$C***H***$_2$OH, 2H, q), 3.04 – 2.80 (-C***H***$_2$SC($CH_3$)$_2$C***H***$_2$-, 4H, m), 1.97 ($H_2$C=C(C***H***$_3$)-, 3H, t), 1.66 (-$CH_2$S(C***H***$_3$)$_2$$CH_2$-, 6H, s).$^{13}$C NMR (126 MHz, $CDCl_3$, $\delta$ ppm, Fig. S8): 167.30, 136.16, 126.03, 63.73, 61.61, 56.26, 33.78, 31.20, 29.17, 18.36. HRMS (Fig. S9): Mass (*m*/*z*) calculated for $[M + Na]^+$ = 287.0752; observed = 287.0749.

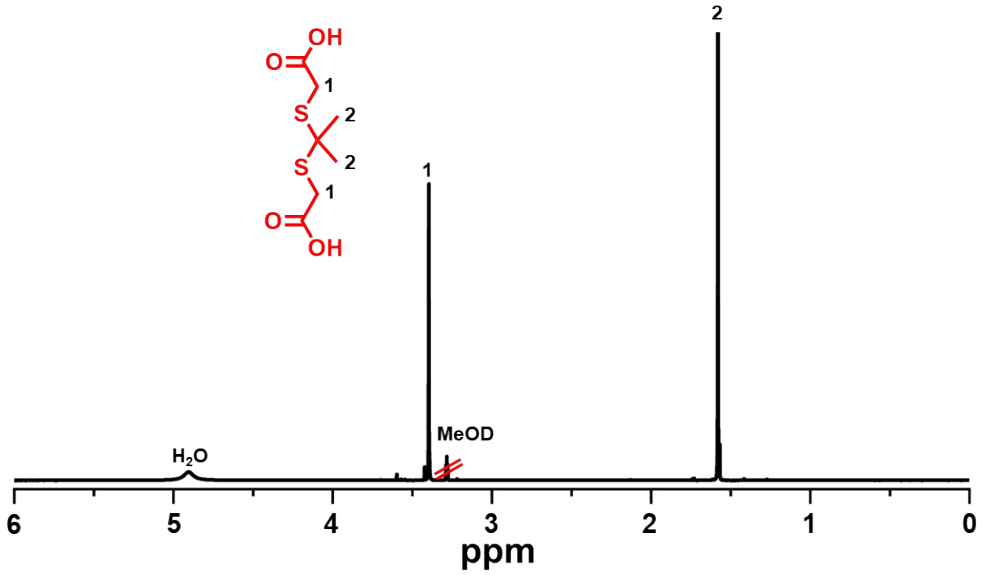



**Fig. S1** $^{1}$H NMR spectrum of compound **1**.

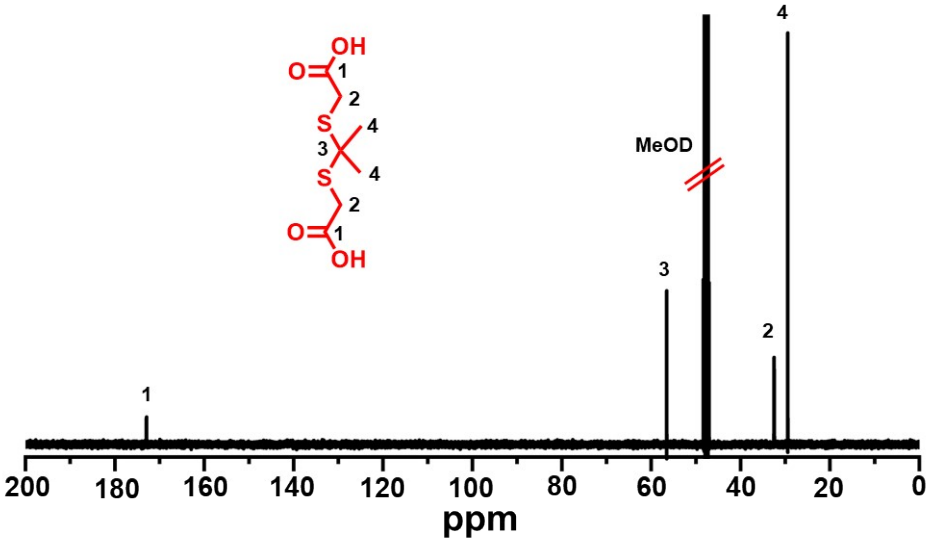



**Fig. S2** $^{13}$C NMR spectrum of compound **1**.

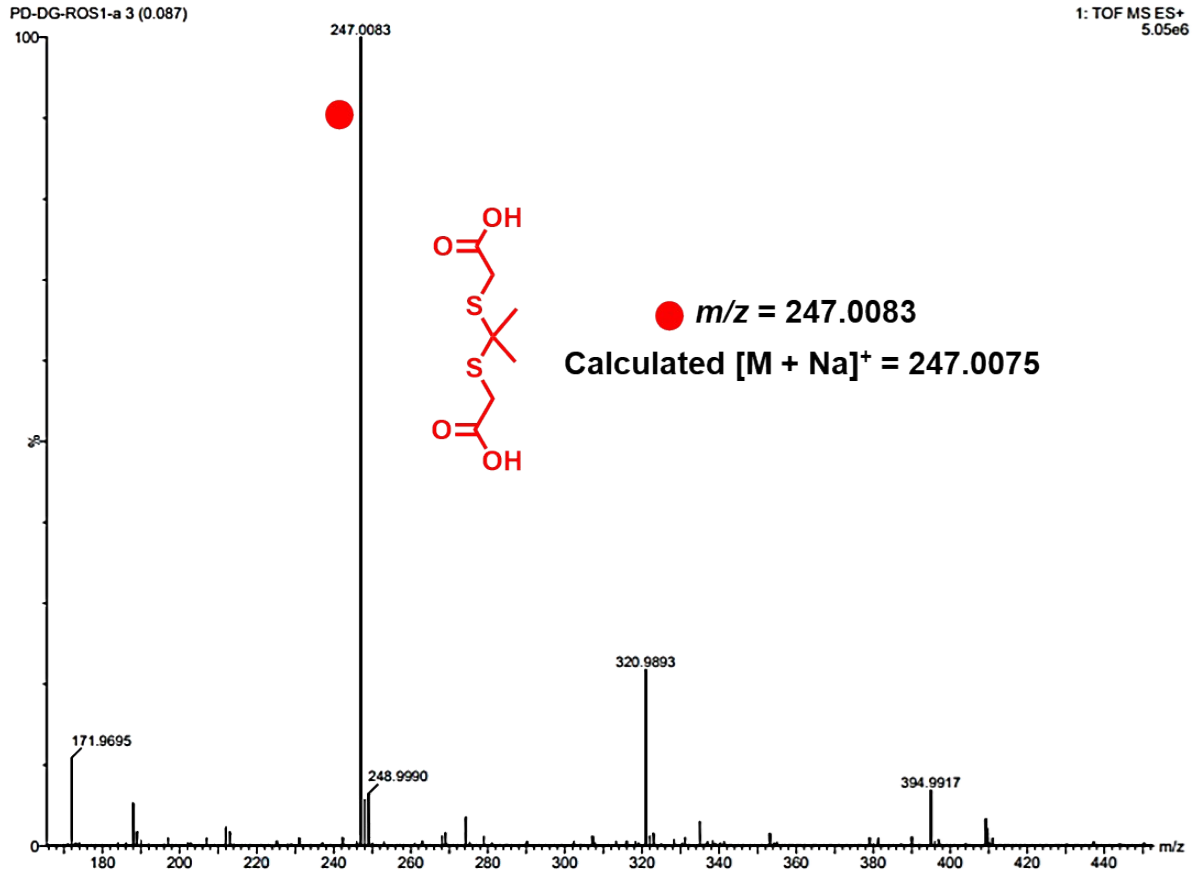



**Fig. S3** HRMS of compound **1**.

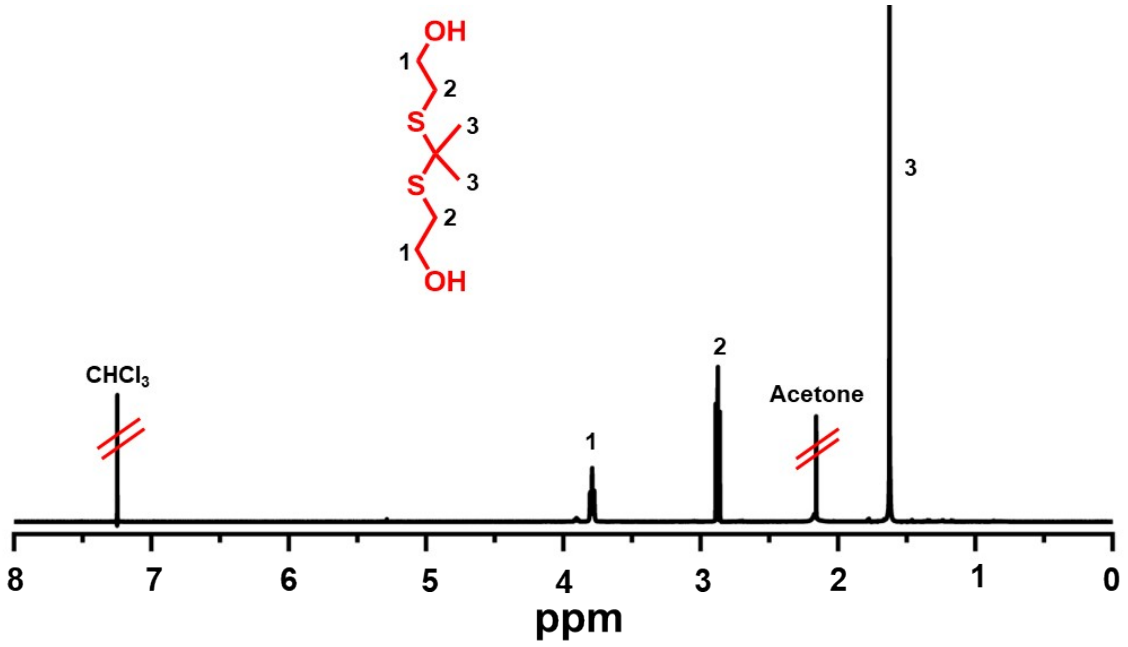



**Fig. S4** $^{1}$H NMR spectrum of compound **2**.

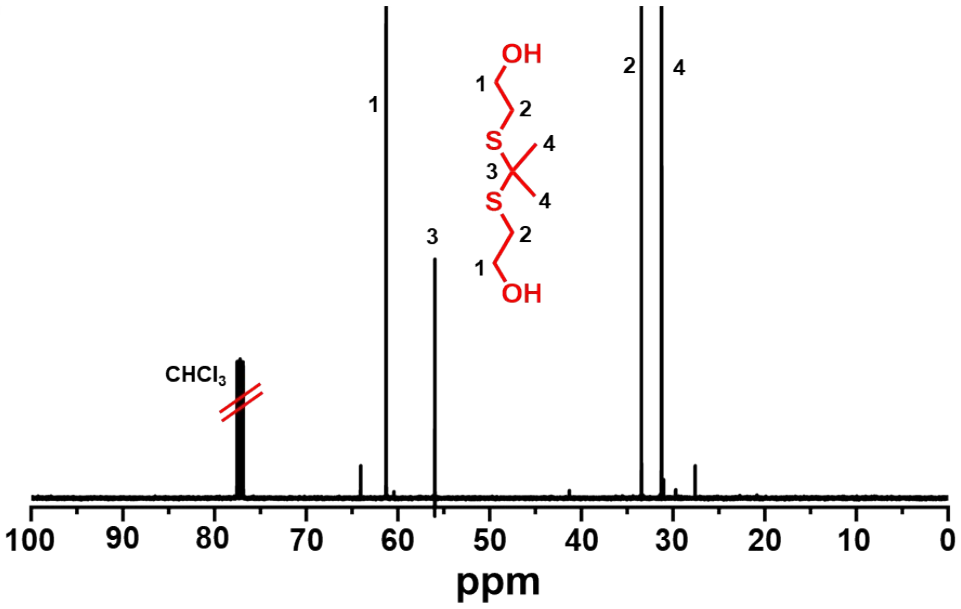



**Fig. S5** $^{13}$C NMR spectrum of compound **2**.

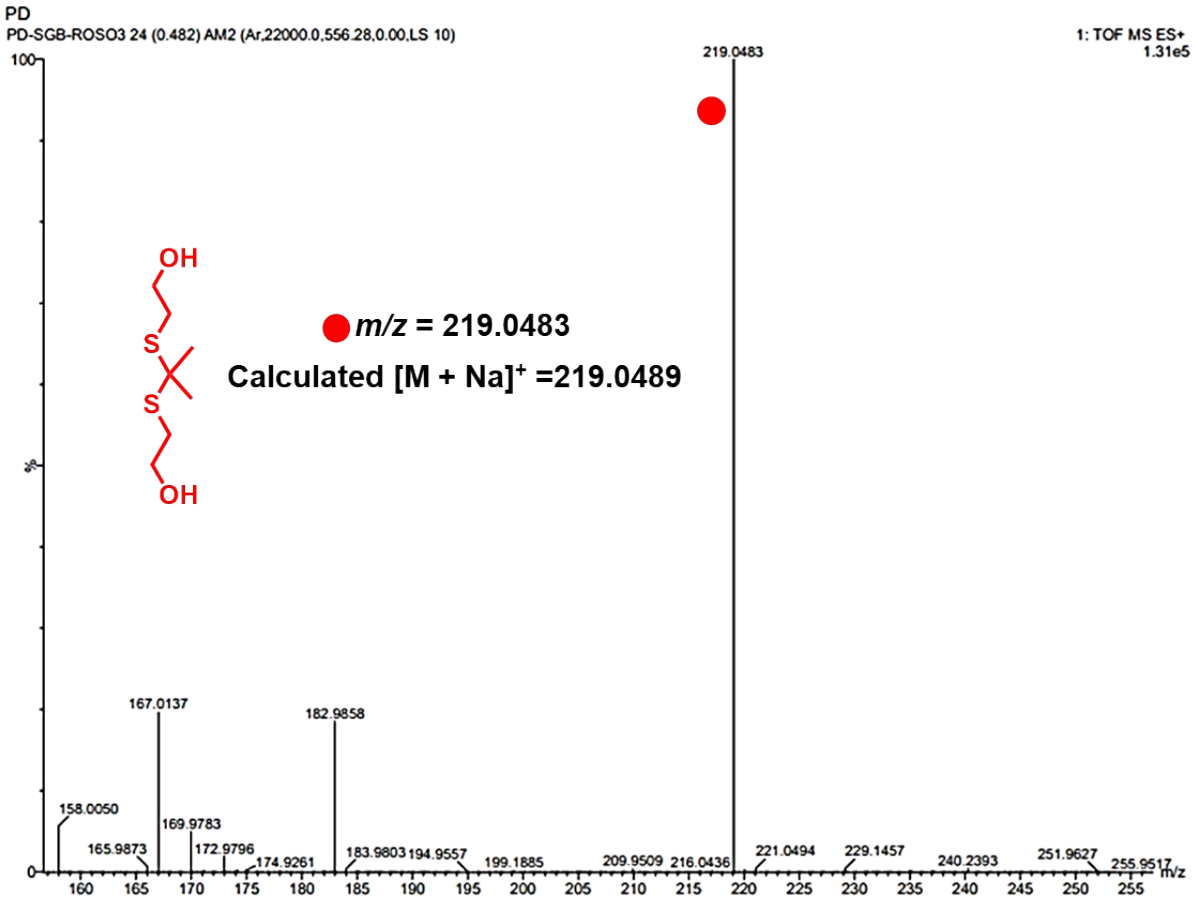



**Fig. S6** HRMS of compound **2**.

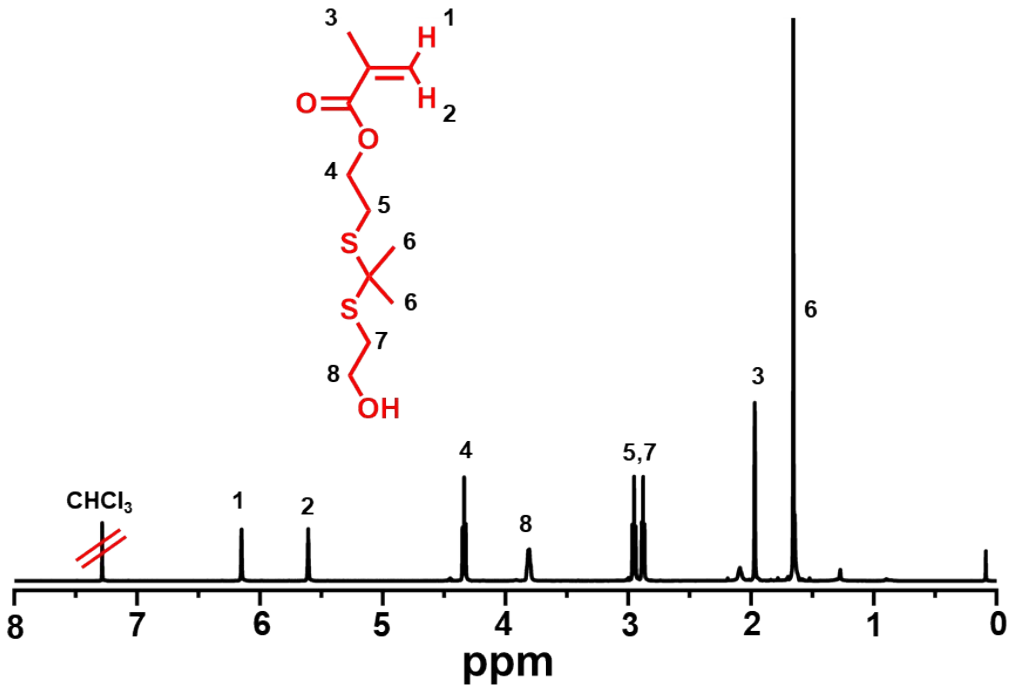



**Fig. S7** $^{1}$H NMR spectrum of compound **3**.

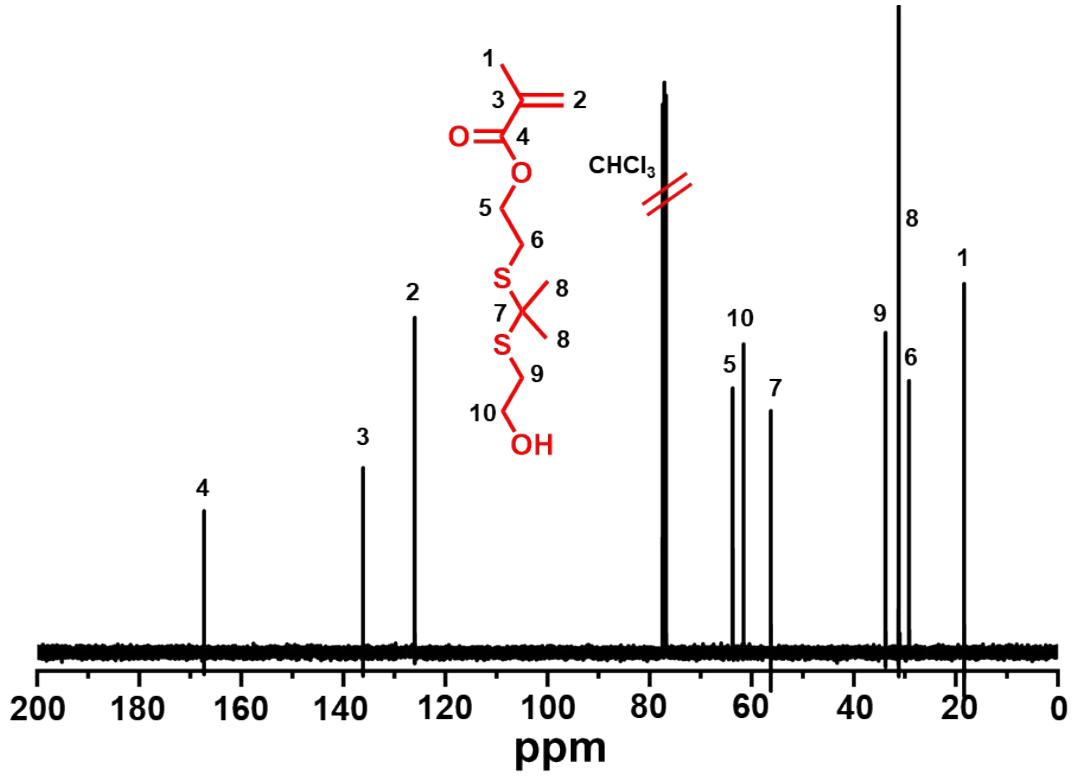



**Fig. S8** $^{13}$C NMR spectrum of compound **3**.

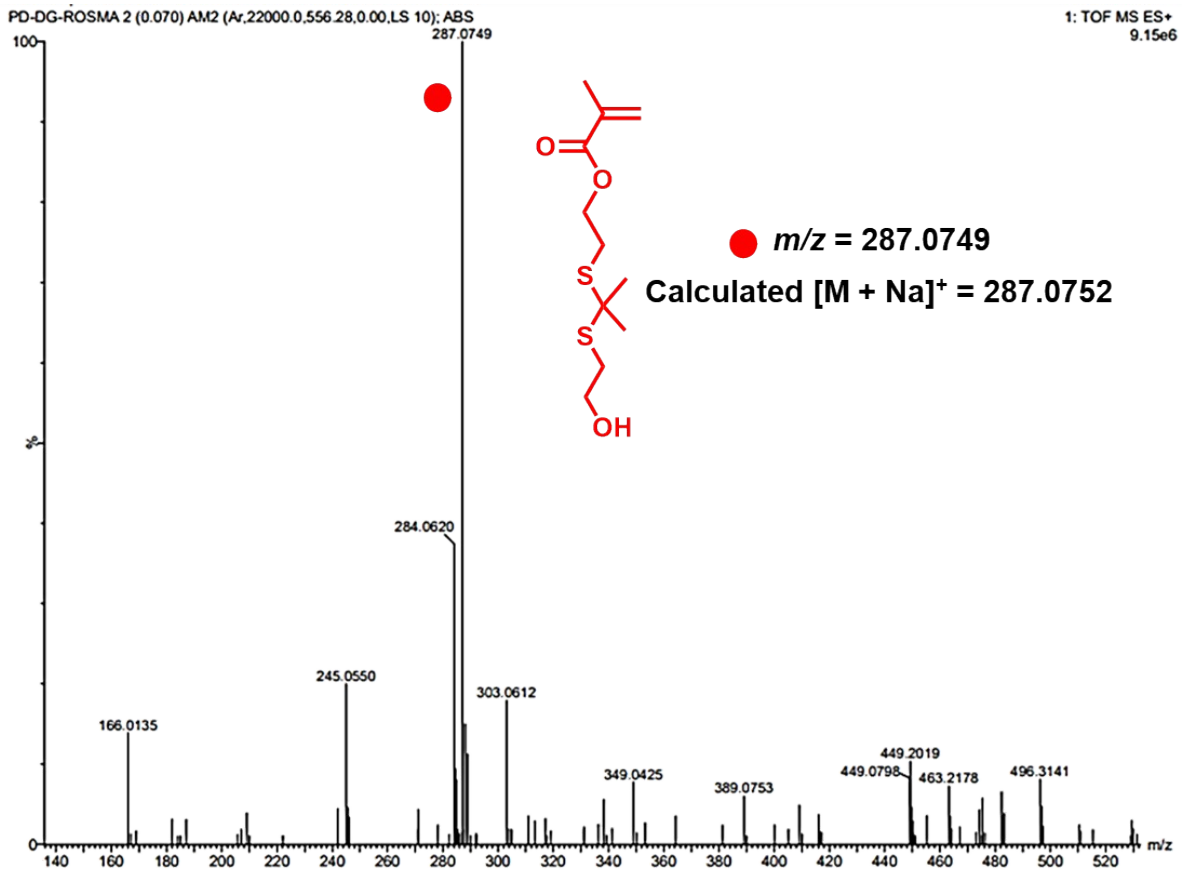



**Fig. S9** HRMS of compound **3**.

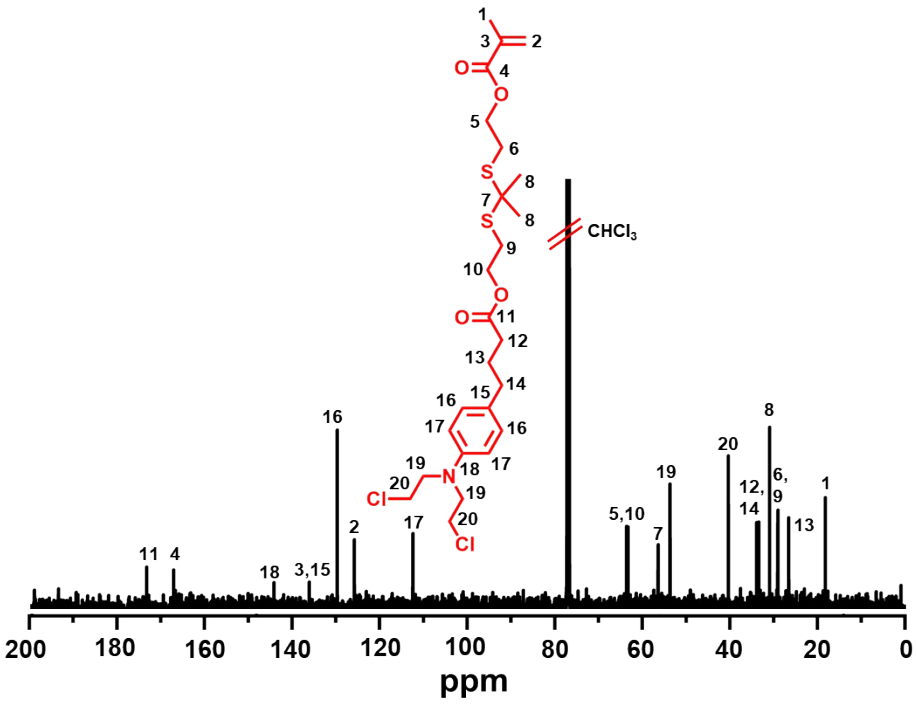



**Fig. S10** $^{13}$C NMR spectrum of **CBLMA**.

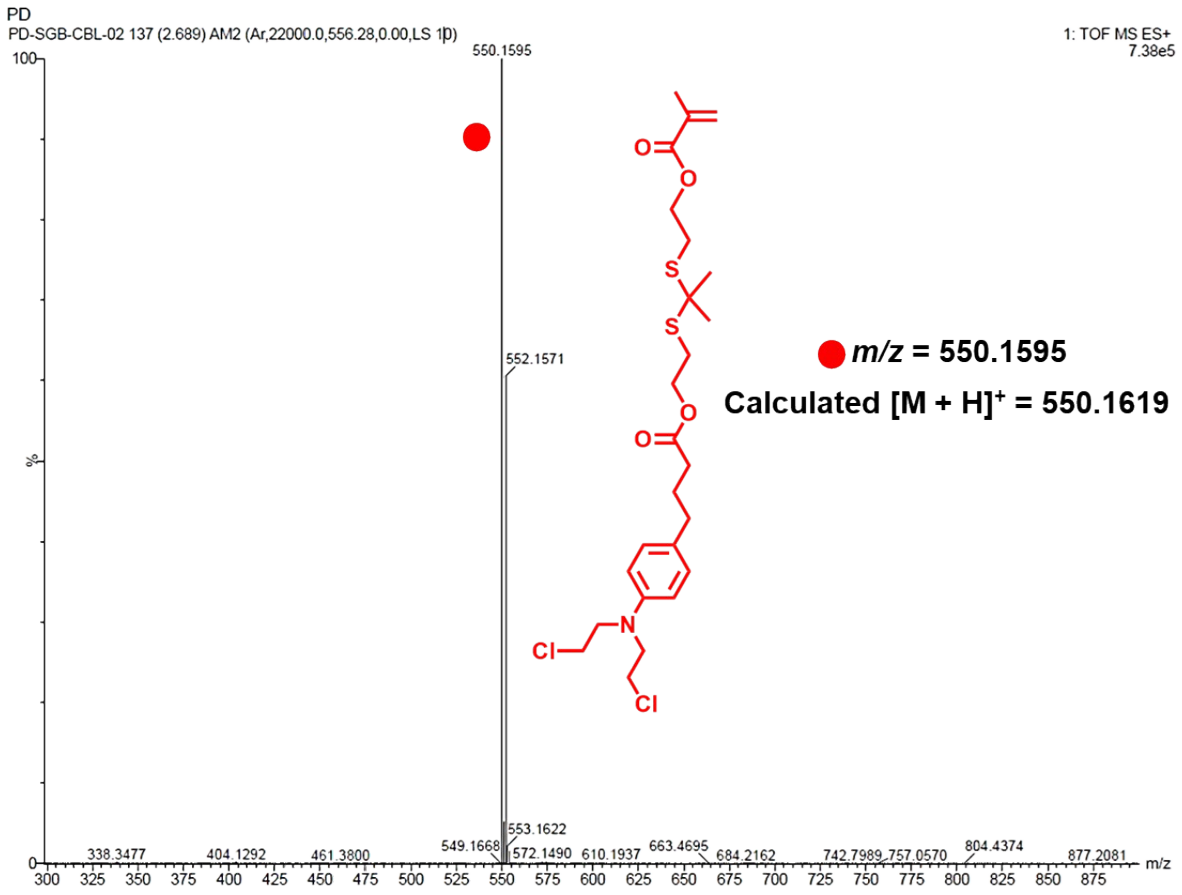



**Fig. S11** HRMS of **CBLMA**.

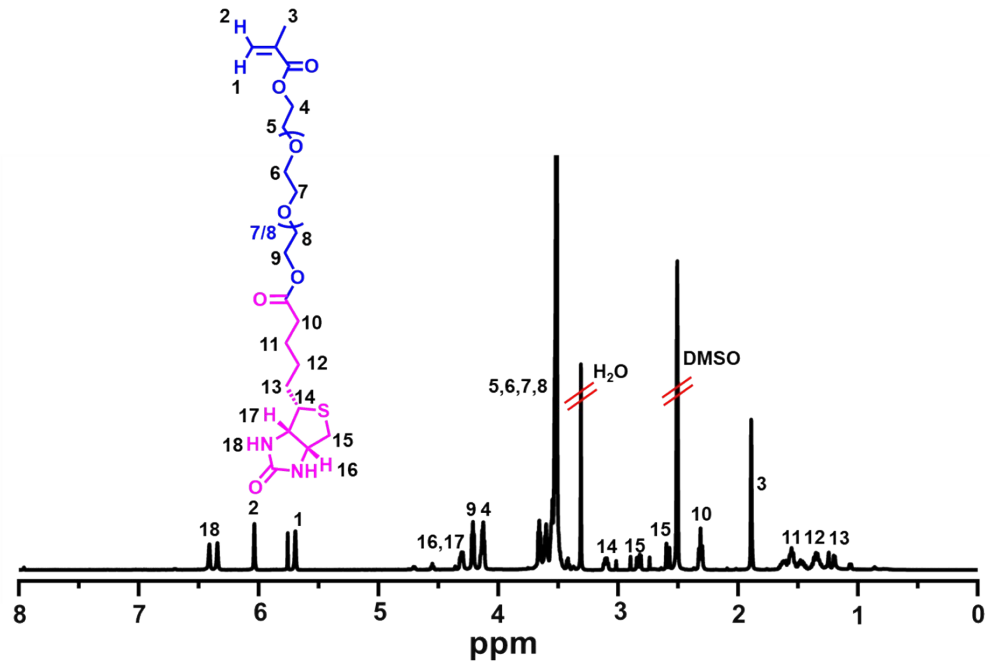



**Fig. S12** $^{1}$H NMR spectrum of **Biotin-PEGMA**.

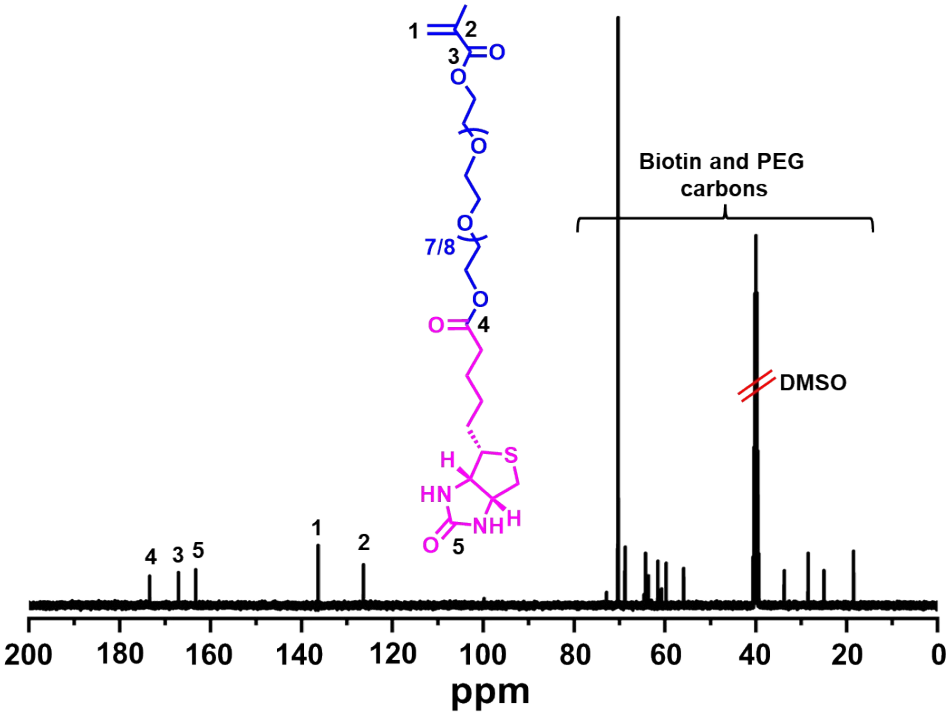



**Fig. S13** $^{13}$C NMR spectrum of **Biotin-PEGMA**.

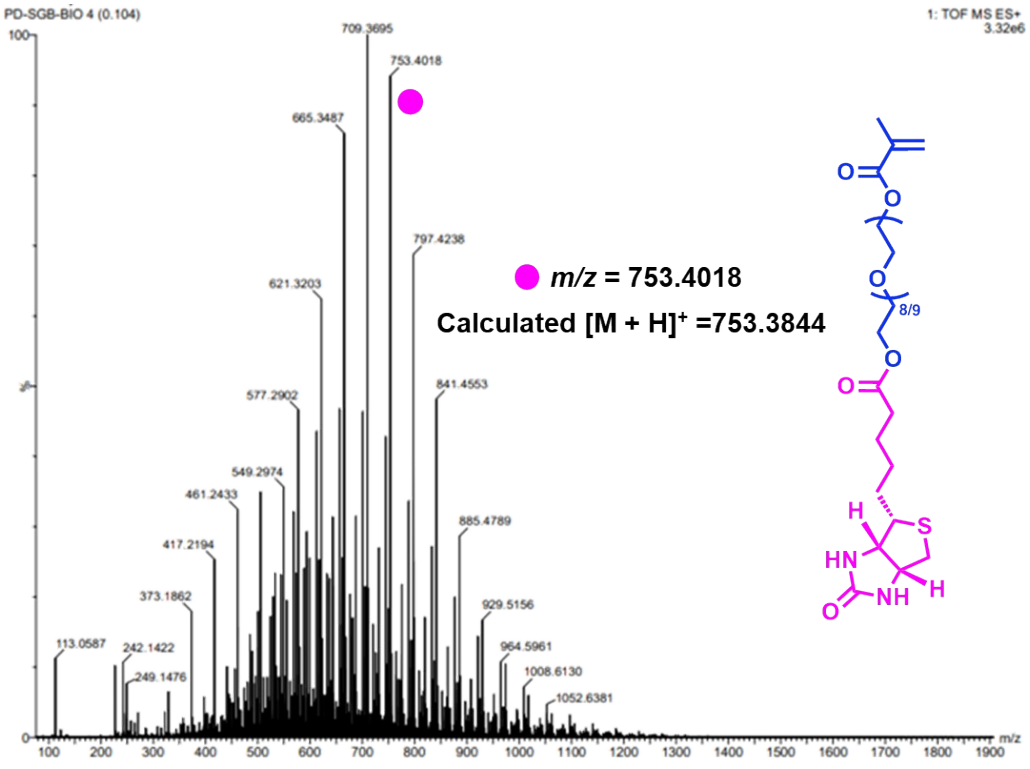



**Fig. S14** HRMS of **Biotin-PEGMA**.

**Synthesis of BODIPY-based methacrylate monomer (BPMA). BPMA** has been synthesized *via* two steps using the previous literature procedure (Scheme S1).[3] At first, FBMA was synthesized by a typical DCC/DMAP coupling reaction. In a 250 mL RBF, FBA (4.00 g, 26.60 mmol) was dissolved in 80 mL dry THF and placed in an ice-water bath under $N_2$ atmosphere. After 30 min of $N_2$ purging, DCC (6.58 g, 31.90 mmol) and DMAP (0.64 g, 5.32 mmol) were dissolved in 20 mL dry THF and added to the reaction mixture. After getting the milky white appearance in the reaction mixture, HEMA (4.16 g, 31.90 mmol) was added and left under stirring conditions at r.t. for 24 h. After completion of the reaction, DCU was filtered out and the organic layer was washed with 2 × 100 mL $NaHCO_3$ solution followed by 2 × 100 mL brine solution, and dried over anhydrous $Na_2SO_4$. Next, the organic part was concentrated under a rotary evaporator, and the crude product was purified by column chromatography using 10% EtOAc/hexane to get a transparent viscous liquid product.

In the second step, at first, FBMA (2.00 g, 7.68 mmol) and 2,4-dimethyl pyrrole (1.54 g, 16.18 mmol) were dissolved in dry THF (100 mL) in a 250 mL RBF and left for 30 min under nitrogen atmosphere. Then, TFA (3-4 drops) was added to it and the reaction mixture was stirred for 4 h at r.t. Next, DDQ (1.74 g, 7.68 mmol) in dry THF (40 mL) was added under ice-cold conditions and stirred at r.t. for another 2 h. Afterward, $BF_3$•$Et_2O$ (16 mL) and $Et_3N$ (24 mL) were added under ice-cold conditions and kept for 4 h. By checking the thin layer chromatography (TLC), the formation of the desired product was confirmed. Then, salt was removed by filtration, and the organic layer was washed with 3 × 100 mL brine solution, and dried over anhydrous $Na_2SO_4$. Next, the organic part was concentrated under a rotary evaporator, and the crude product was purified by column chromatography using 30% EtOAc/hexane to get an orange solid product. Yield = 34%. $^1$H NMR (400 MHz, $CDCl_3$, $\delta$ ppm, Fig. S15): 8.19 – 8.13 (-C=OAr***H***-, 2H, d), 7.43 – 7.36 (-C=OAr***H***-, 2H, d), 6.14 (***H***HC=C($CH_3$)-, 1H, s), 5.97 (pyrrole aromatic protons, 2H, s), 5.59 (H***H***C=C($CH_3$)-, 1H, s),

4.64 – 4.57 (-OC***H***$_2$CH$_2$OC=O-, 2H, m), 4.54 – 4.47 (-OCH$_2$C***H***$_2$OC=O-, 2H, m), 2.54 (ArC***H***$_3$, 6H, s), 1.94 (H$_2$C=C(C***H***$_3$)-, 3H, d), 1.34 (ArC***H***$_3$, 6H, s). $^{13}$C NMR (126 MHz, $CDCl_3$, $\delta$ ppm, Fig. S16): 167.23, 165.78, 156.13, 142.94, 140.21, 136.02, 131.00, 130.58, 128.57, 126.25, 121.61, 63.11, 62.41, 18.36, 14.36. HRMS (Fig. S17): Mass (*m*/*z*) calculated for $[M + H]^+$ = 481.2110; observed = 481.2136.

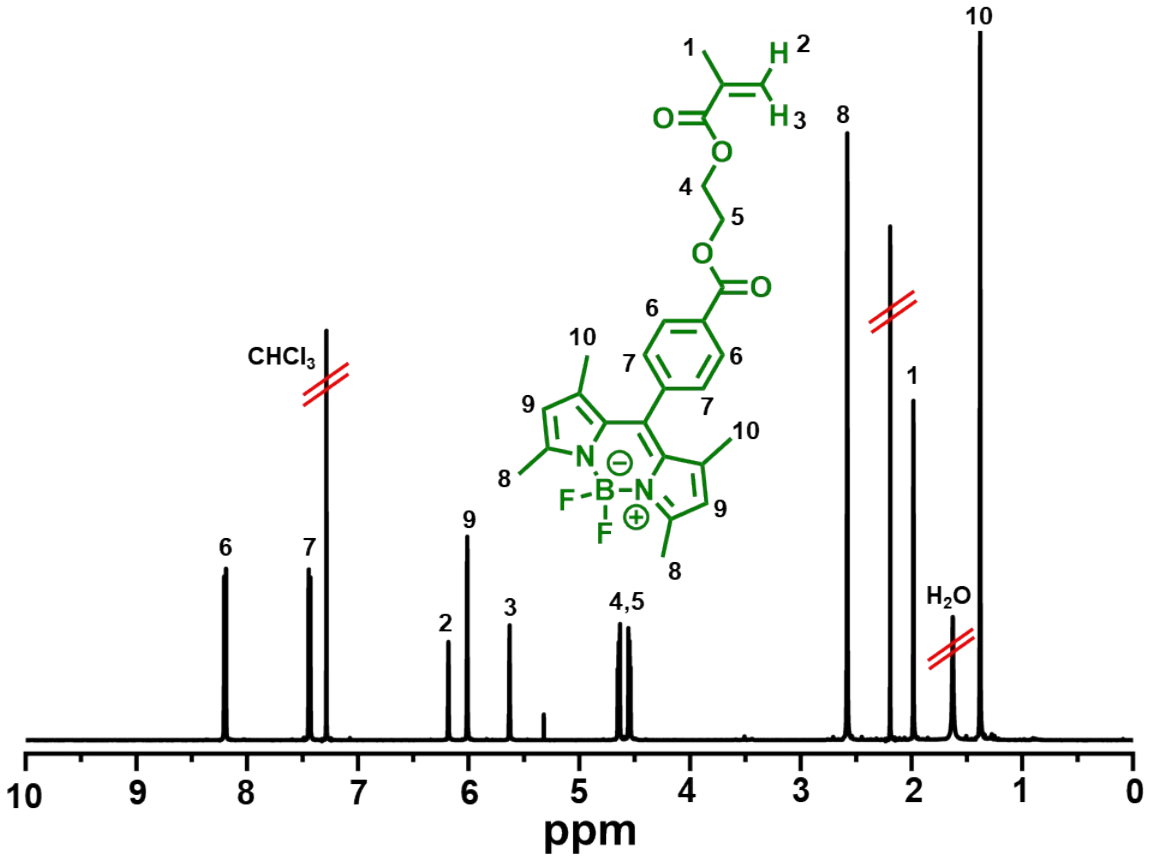



**Fig. S15** $^1$H NMR spectrum of **BPMA**.

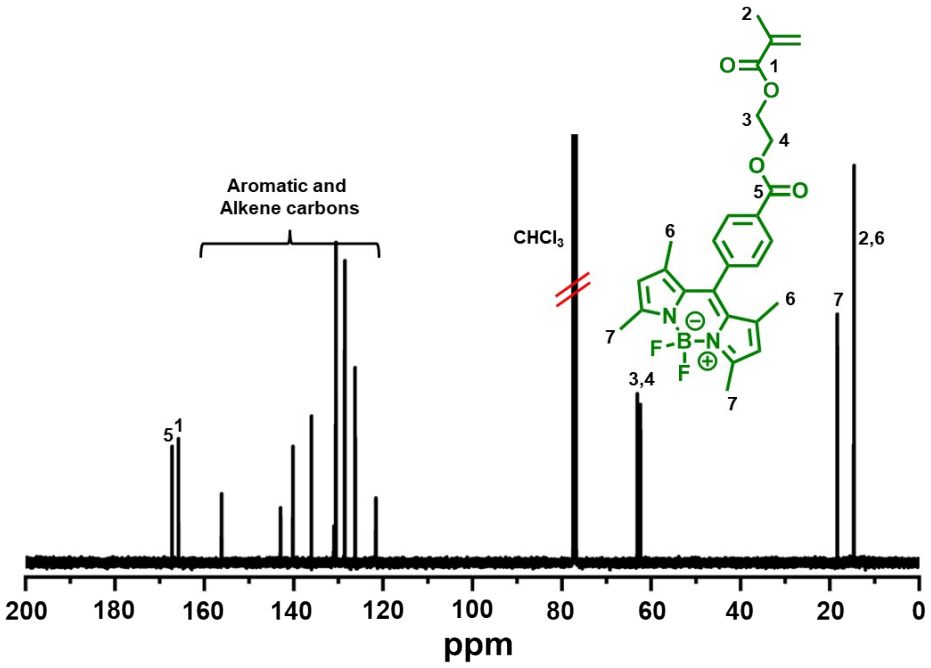



**Fig. S16** $^{13}$C NMR spectrum of **BPMA**.

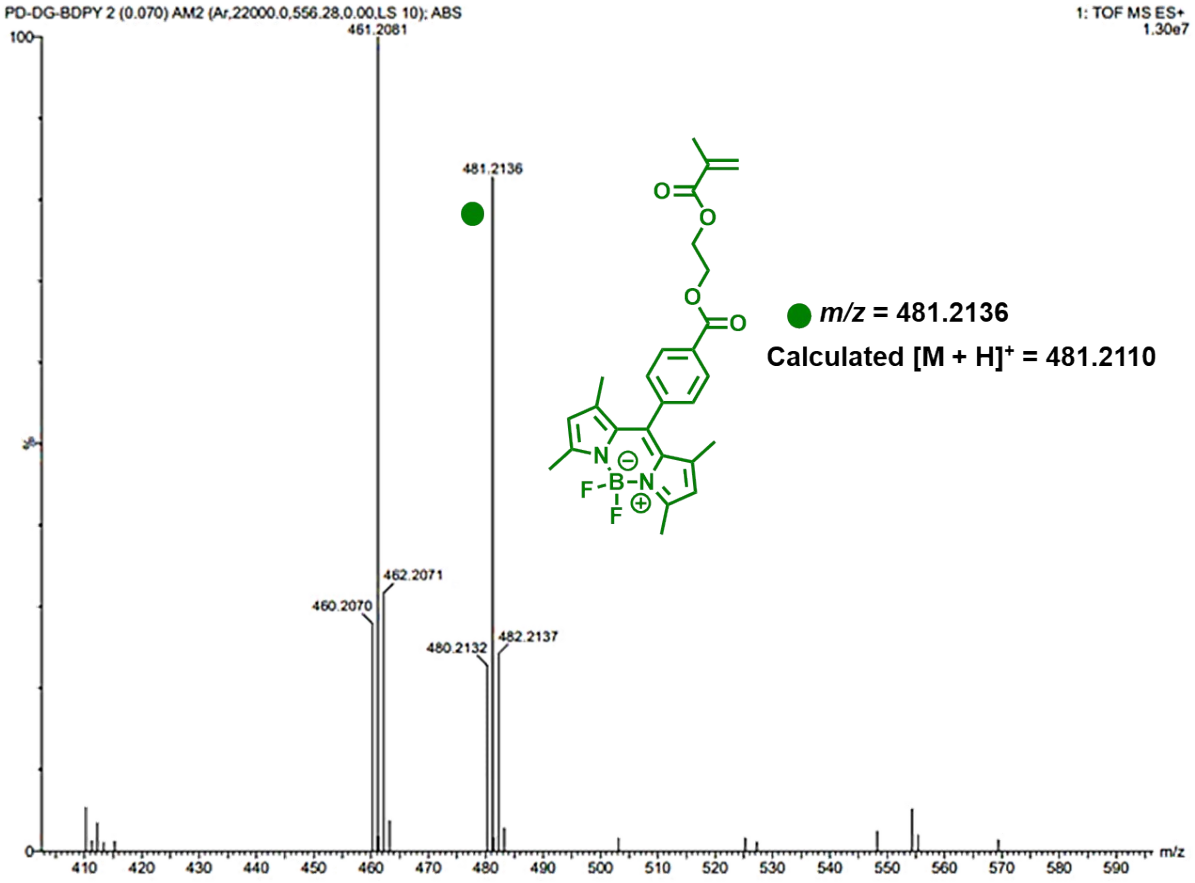



**Fig. S17** HRMS of **BPMA**.

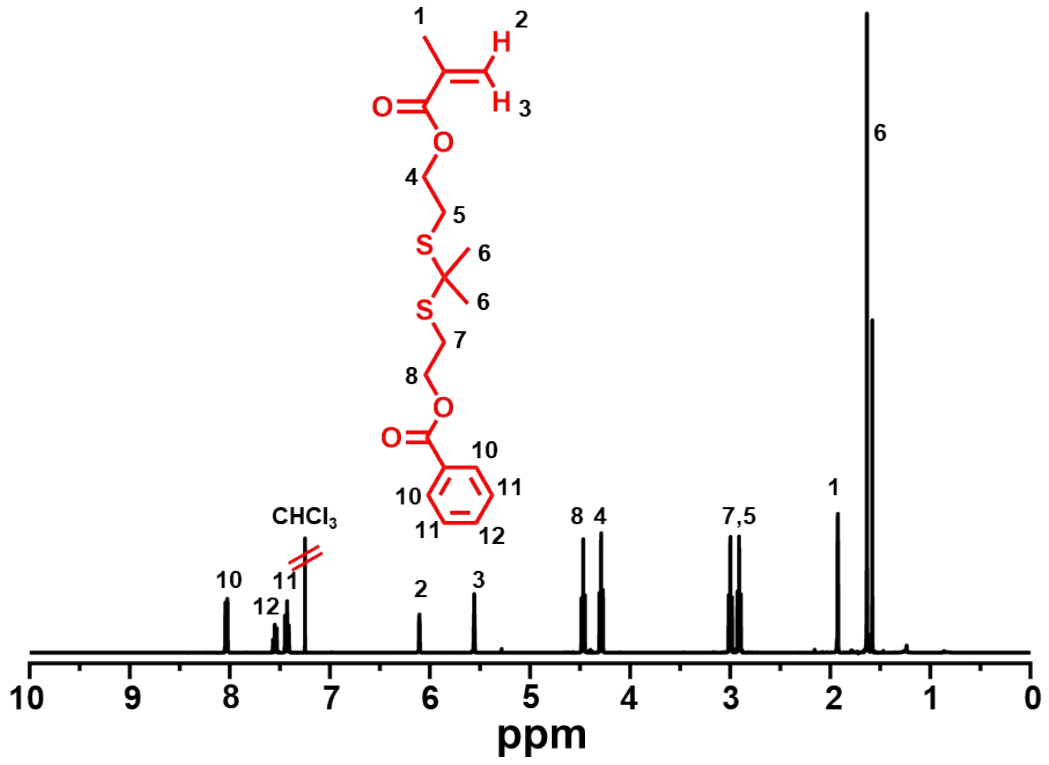



**Fig. S18** $^{1}$H NMR spectrum of **BNMA**.

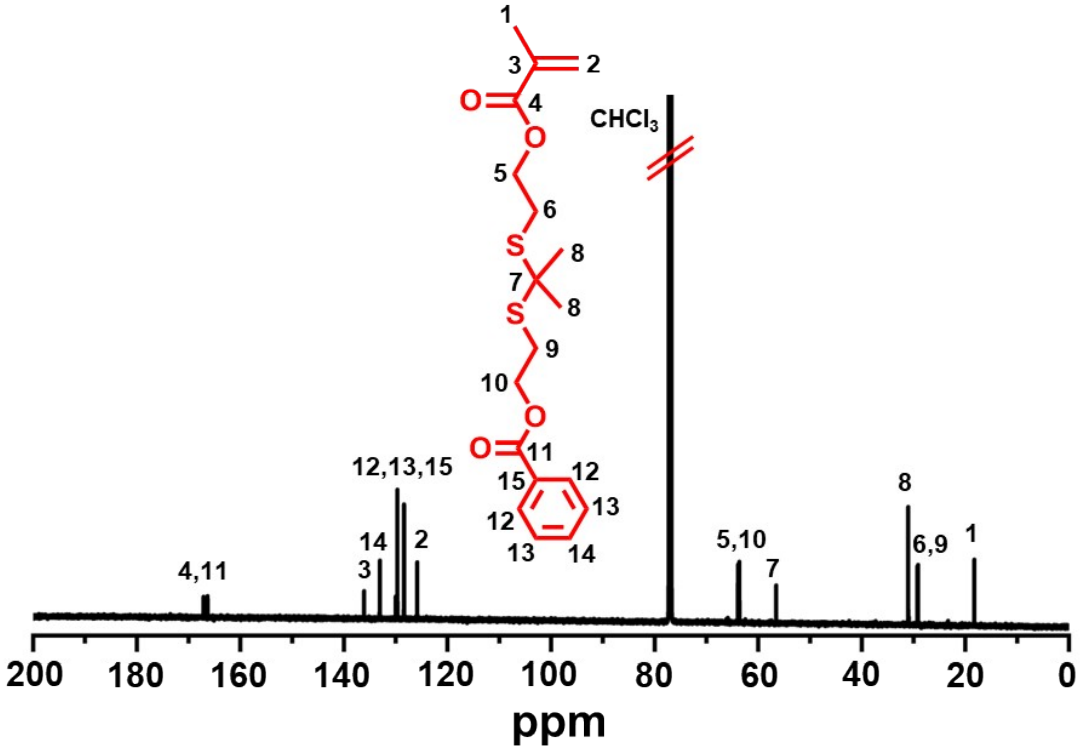



**Fig. S19** $^{13}$C NMR spectrum of **BNMA**.

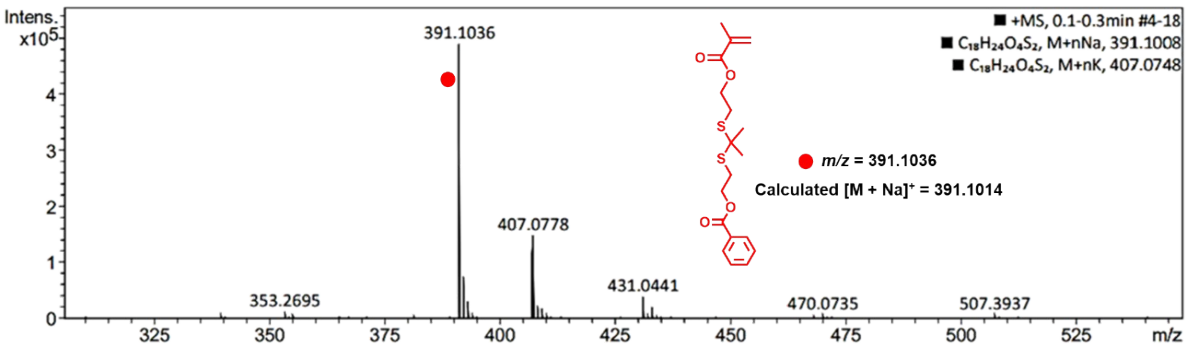



**Fig. S20** HRMS of **BNMA**.

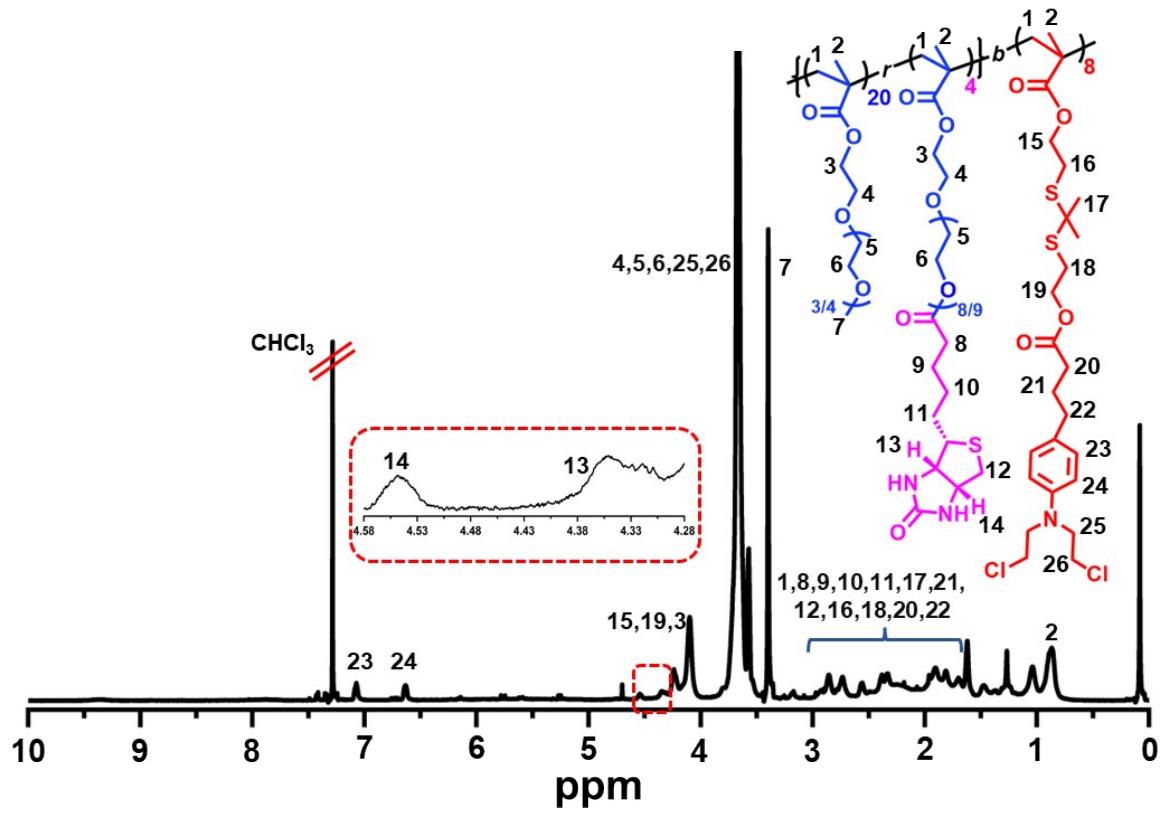



**Fig. S21** $^{1}$H NMR spectrum of **DP1**.

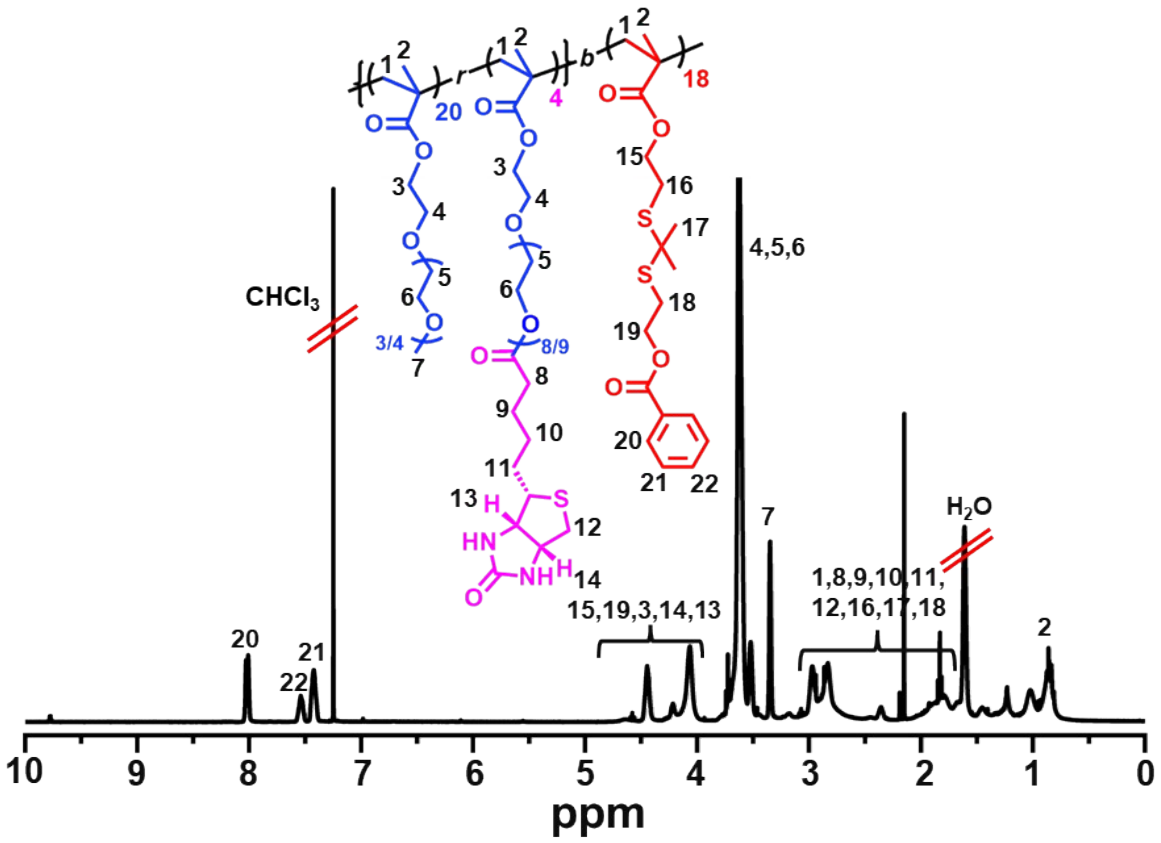
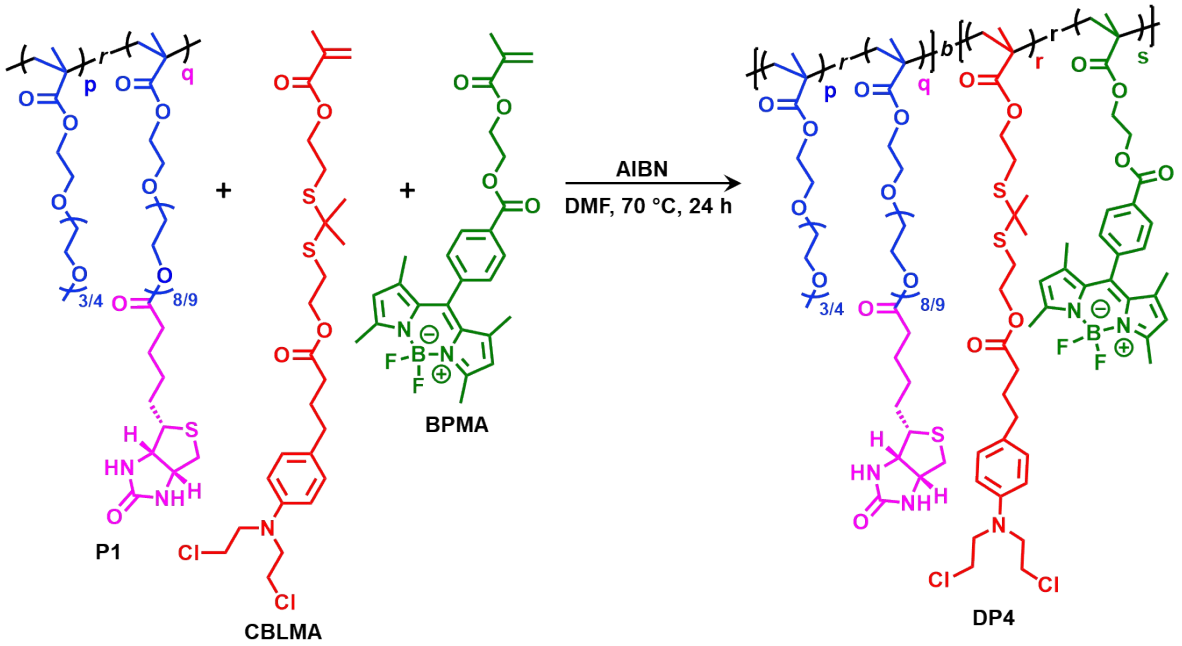



**Fig. S22** $^{1}$H NMR spectrum of **DP3**.




**Scheme S2.** Synthetic scheme of **DP4** copolymer.

**Synthesis of DP4.** For the synthesis of **DP4** (shown in Scheme S2), **CBLMA** (200 mg, 0.363 mmol), **BNMA** (35.0 mg, 0. 072 mmol), **P1** (128 mg, 0.014 mmol), and AIBN (0.47 mg, 0.003 mmol) were dissolved in 1.5 mL DMF in a 20 mL septa-sealed glass vial equipped with a magnetic stirring bar. Then, the glass vial was purged with dry $N_2$ for 15 min and placed in a 70 °C preheated polymerization block for 24 h. After 24 h, the vial was placed in an ice-water bath, and exposed to air to quench the polymerization. Initially, DMF was eliminated by washing three times with hexane and then the air-dried polymer was dissolved in a small quantity of acetone and precipitated with hexane. This process was repeated four times, and the polymer was then vacuum-dried for 12 h. The polymer was characterized by $^1$H NMR spectroscopy (Fig. S23) and SEC analysis (Fig. S24).

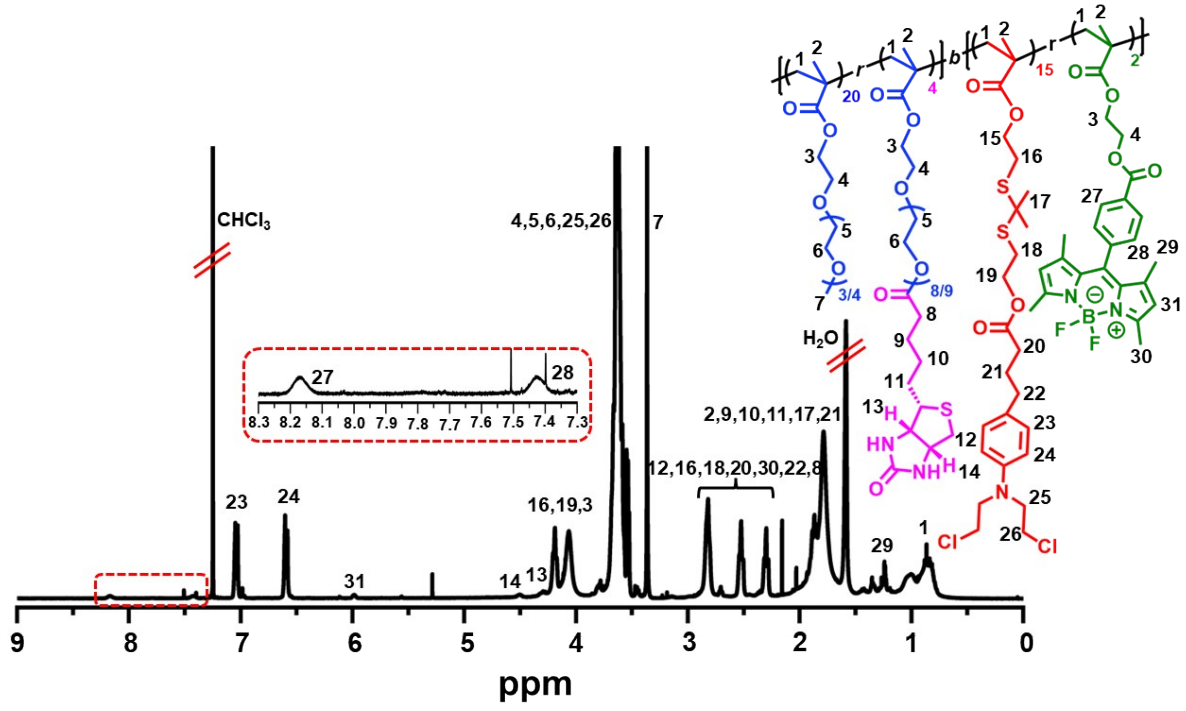



**Fig. S23** $^1$H NMR spectrum of **DP4**.

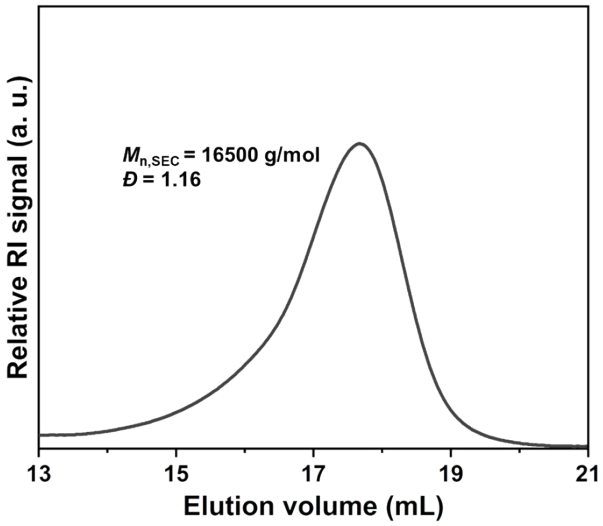



**Fig. S24** SEC RI trace of **DP4**.

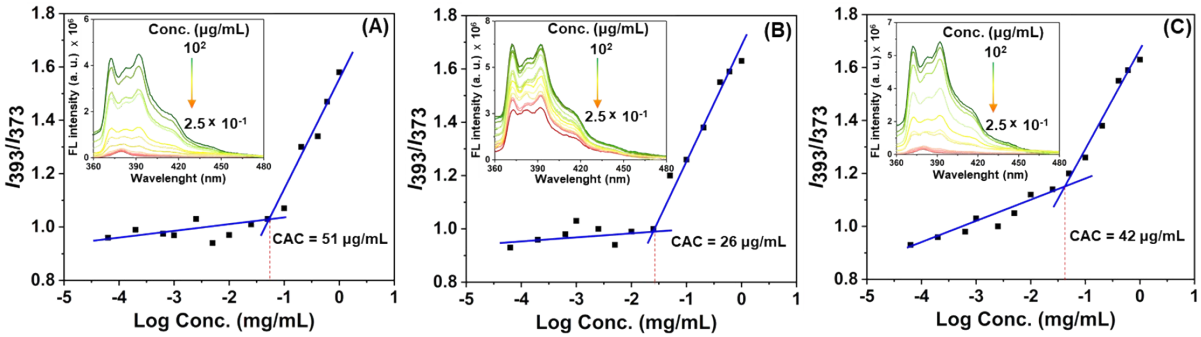



**Fig. S25** CAC determination of polymers: (A) **DP1**, (B) **DP2**, and (C) **DP3**.

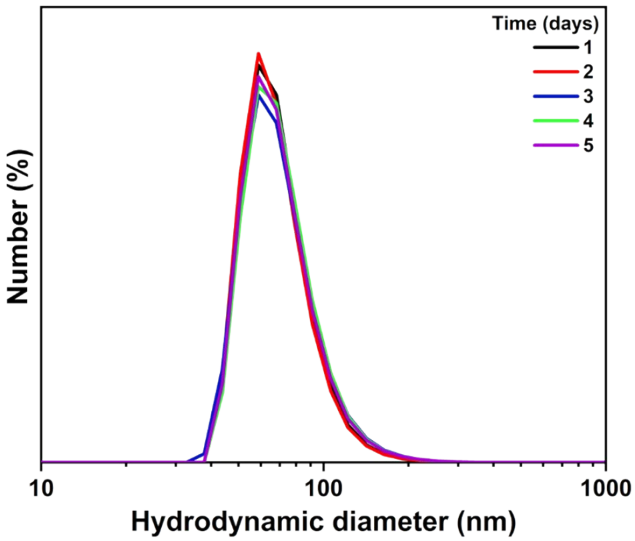



**Fig. S26** Stability of **DP2** polymeric nanoaggregates in PBS (pH 7.4) for 5 days.

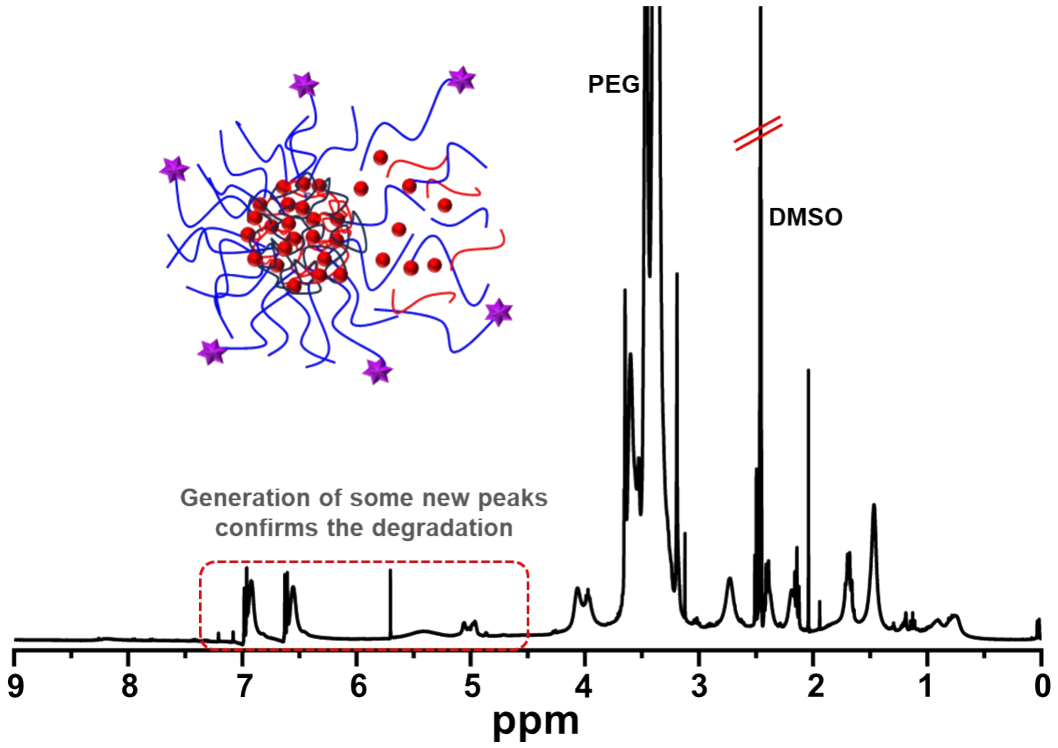



**Fig. S27** $^{1}$H NMR spectrum of **DP2** after degradation with the treatment of esterase (10 U/mL).

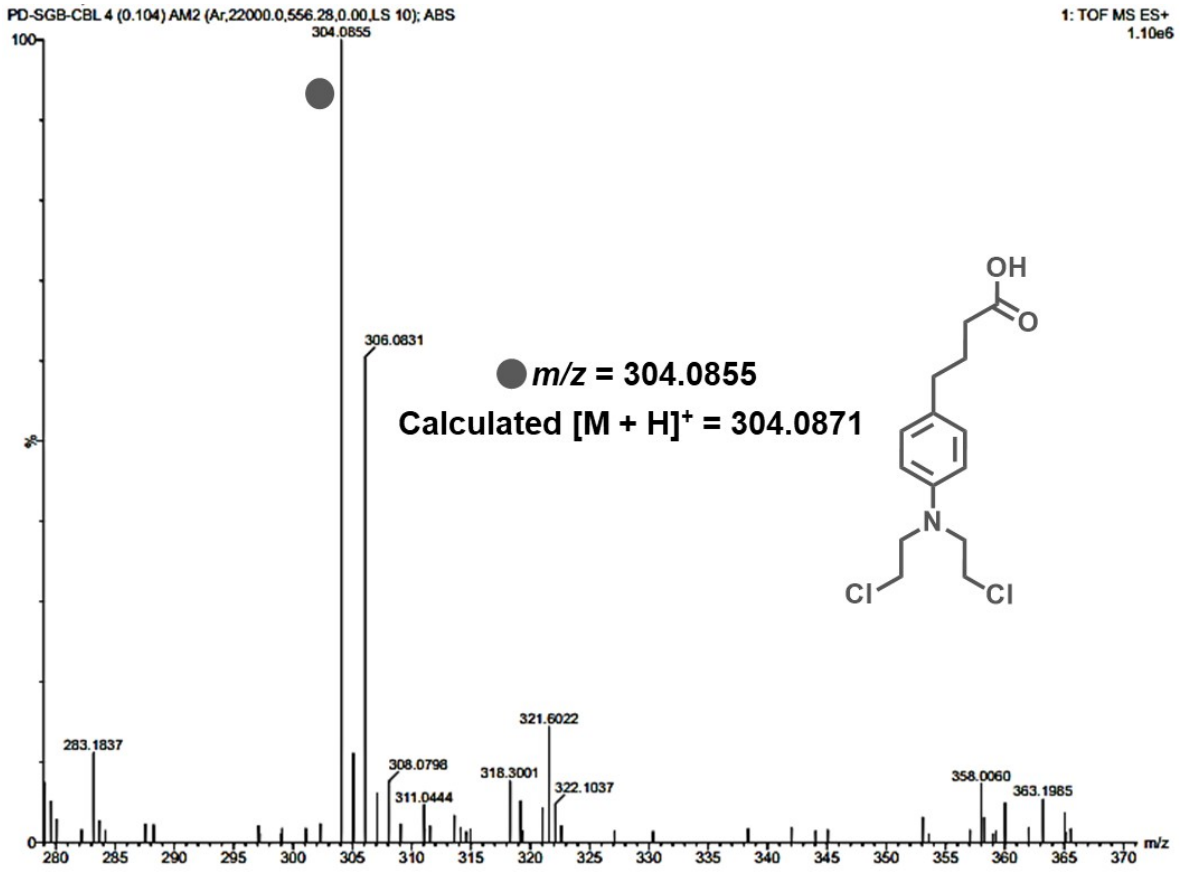



**Fig. S28** HRMS of the degradation product of **DP2** with the treatment of esterase (10 U/mL).

**Avidin-HABA assay.** The binding interaction between the biotinylated polymer (**DP2)**, a model protein (avidin), and a second chromophore, HABA was employed. This assay was carried out by following a previously reported protocol.[4] 24.2 mg of HABA was suspended in 10 mL of water and subsequently, 100 µL of NaOH solution was added to dissolve HABA. The avidin solution was prepared by dissolving 5.0 mg of avidin in 10 mL of PBS. In a typical experiment, the HABA-avidin complex at a 4:1 ratio was made in PBS, and the complex formation was monitored at 505 nm. To the above solution, the different concentrations of biotinylated polymer **DP2** were added and observed the change in the absorbance maxima at 505 nm.

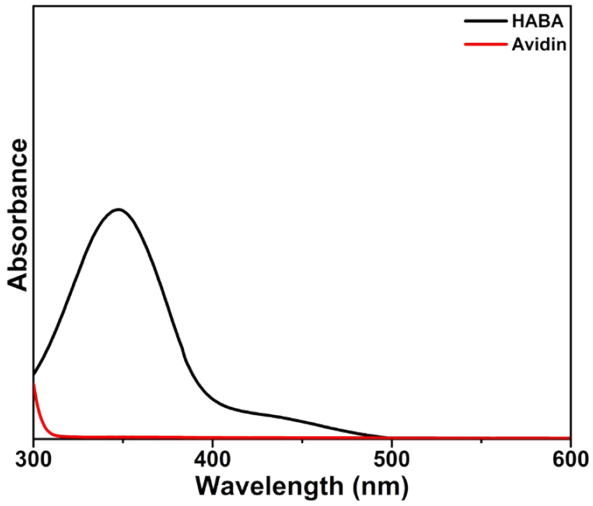



**Fig. S29** UV-Vis spectra of HABA and avidin.

**Isothermal calorimetry (ITC) assay.** The thermodynamic parameters of the non-covalent interaction between avidin (cell surface membrane proteins) and biotinylated polymer (**DP2**) were evaluated using the ITC measurement. The Hamilton syringe was loaded with **DP2** solution and titrated into the sample cell containing avidin solution. Overall, 19 successive injections were designed for each set of experiments, and 2 µL of polymer solution (1 mM) was injected into the sample cell containing avidin. The time duration between two successive injections was 10 s with 4 min intervals between every injection. After the experiment, the heat

of interaction, as well as thermodynamic parameters were determined by using Microcal PEAQ-ITC software.

**Tryptophan fluorescence assay.** The avidin-biotin interaction between the model protein avidin and biotinylated polymer **DP2** was determined by measuring the characteristic change of intrinsic tryptophan fluorescence of avidin. In a typical procedure, the intrinsic fluorescence of avidin (100 μg/mL) was recorded upon treatment with **DP2** (concentrations of **DP2** ranging from 10 –100 μg/mL) with an excitation wavelength of 295 nm.

**NBP alkylation assay.** The alkylating property of nitrogen mustard, CBL can be examined by reacting with a model DNA base pair, NBP resulting in an intense purple color.[5] At first, solutions of different concentrations of **DP2** (0.5 to 5 mg/mL) were prepared in Milli-Q water followed by the addition of 60 μL 0.5 M NBP in acetone. The reaction mixture was then heated to 100 °C for 20 min and cooled to r.t. After that, an acetone solution of $Et_3N$ was added to the reaction mixture and stirred for 5 min to develop an intense purple color and UV-Vis absorbance spectra were measured.

To determine the extent of alkylation of **DP2**, at first the calibration curve of free CBL (concentrations: 0.1-5 mg/mL)-NBP adduct was prepared. Next, by comparing the absorbance value of the **DP2**-NBP adduct with the calibration curve, the extent of alkylation of CBL in **DP2** was calculated by using the following equation;

Extent of alkylation = (Concentration of CBL derived from standard curve/Concentration of CBL in **DP2**) × 100%

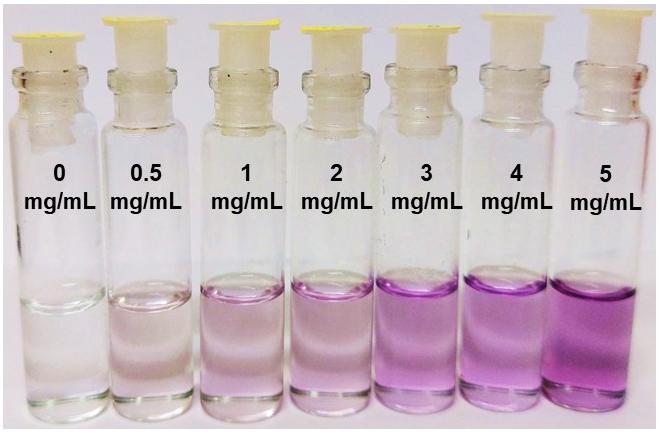



**Fig. S30** Photographic images of alkylated NBP-CBL adducts at different concentrations of **DP2**.

**Assessment of the cell cytotoxicity of DP2@DOX.** To determine the cell cytotoxicity and the target-specific response of the free CBL, free DOX, **DP2**, DOX-loaded **DP2** (**DP2**@DOX), DOX-loaded CBL free **DP3** polymer (**DP3**@DOX), a cocktail of two individual drug-carried polymers (**DP2** + **DP3**@DOX) and a cocktail of two free drugs (CBL + DOX), we chose to use human HeLa (representative of biotin receptor-positive cells) and HEK 293T (representative of biotin receptor-negative cells) cells,[6] and performed standard MTT assay as per the method described previously with minor modifications.[7] In brief, human HeLa and HEK 293T cells were seeded in the clear-bottom 96-well tissue culture plates with a density of $1.5 \times 10^4$ cells/well and maintained in the growth medium at 37 °C, under 5% $CO_2$ pressure, respectively. After 80% confluency, each type of cell was incubated with different concentrations of drug [for free CBL and **DP2** (CBL concentrations ranging from 3.9 to 500 µg/mL), for free DOX and **DP3**@DOX (DOX concentrations ranging from 0.15 to 20 µg/mL), for **DP2**@DOX, **DP2** + **DP3**@DOX and CBL + DOX (CBL concentrations ranging from 3.9 to 500 µg/mL and DOX concentrations ranging from 0.15 to 20 µg/mL)] for 24 h at 37 °C, under 5% $CO_2$ pressure. After the incubation, cells were washed with 1X PBS and supplemented with 100 µL of fresh growth medium containing MTT dye (final concentration

100 µg/mL). Finally, the medium was replaced with DMSO to dissolve the formazan crystal, and the absorbance was measured at 595 nm using a microplate reader. The percentage of cell viability was calculated according to the following equation (1),

$$\% \text{ Cell viability} = (A_{Tr}/A_{Un}) \times 100 \quad (1)$$

Where, $A_{Tr}$ is the optical absorbance of the drug-treated cells, and $A_{Un}$ is the optical absorbance of the untreated cells. $IC_{50}$ value was calculated from dose-response curves of the cell viability *vs* concentration graphs, plotted using GraphPad Prism 8.

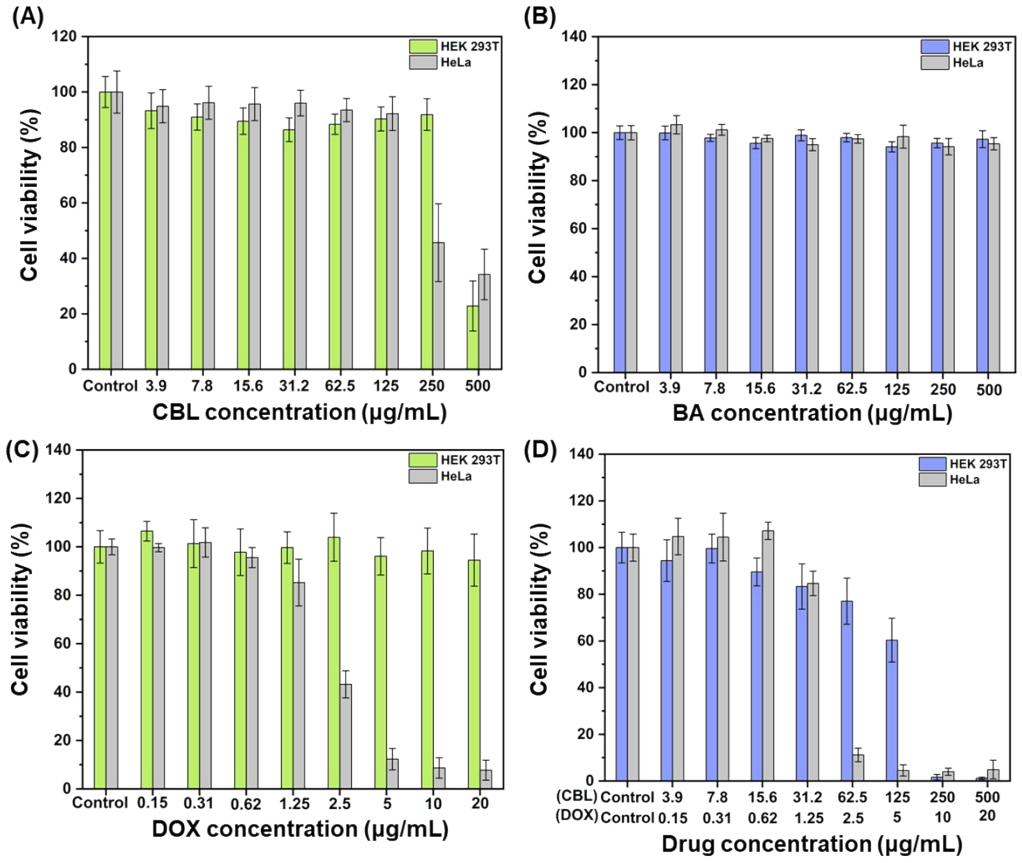



**Fig. S31** Cell viability of (A) free CBL, (B) **DP3**, (C) free DOX, and (D) cocktail of CBL and DOX (CBL + DOX) in HEK 293T and HeLa cells.

**Hemolysis assay.** The hemolytic efficacy of **DP2** was assessed using chicken blood red blood cells (RBCs) following standard hemolysis protocol.[8] Briefly, the chicken blood was collected in blood-collecting tubes coated with ethylenediaminetetraacetic acid (EDTA). Then, RBCs were isolated from plasma by centrifuging at 8000 rpm for 15 min. Next, the isolated RBC was washed with 1X PBS five times and 5% RBC solution (v/v) was prepared in 1X PBS. Next, different concentrations (12 – 1600 μg/mL prepared in 1X PBS) of polymer solutions were incubated with RBC solutions at 37 °C for 2 h. The RBC solution with 1% triton X-100 served as a positive control, while 1X PBS was a negative control. After the incubation, the samples were centrifuged at 8000 rpm for 5 min, and the visible RBC pellet at the bottom of the microcentrifuge tube (Fig. S32) indicates polymer biocompatibility. The optical absorbance of the supernatant was measured at 540 nm using a microplate reader. The experiment was performed in triplicates. The percentage of hemolysis was calculated according to the following equation (2),

$$\% \text{ Hemolysis} = (A_{\text{Sample}} - A_{\text{Neg}})/(A_{\text{Pos}} - A_{\text{Neg}}) \times 100 \qquad (2)$$

Where, $A_{\text{Sample}}$ is the optical absorbance of the **DP2**-treated RBC, and $A_{\text{Neg}}$ and $A_{\text{Pos}}$ are the optical absorbances of the RBCs treated with PBS (negative control) and 1% triton X-100 (positive control), respectively.

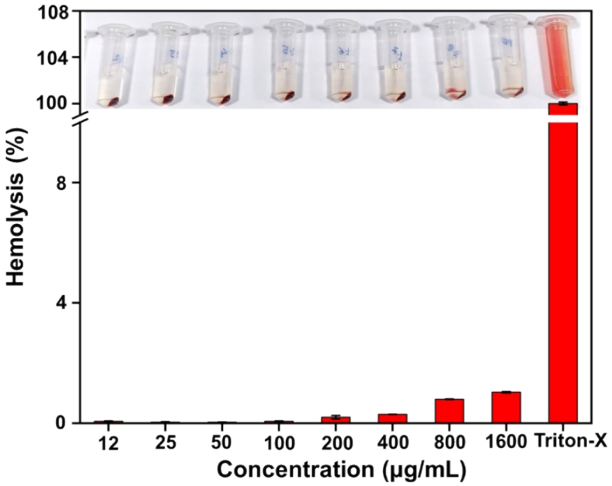



**Fig. S32** Hemolysis assay of **DP2**.

**Analysis of cell surface receptor-specific cellular uptake of DP4@DOX in HeLa and HEK293T cells. Confocal Laser Scanning Microscopy.** To monitor and visualize the biotin receptor-specific cellular uptake of **DP4** and **DP4**@DOX, CLSM was performed. Briefly, human HeLa and HEK 293T cells ($1 \times 10^6$ cells/well) were separately grown on coverslips in a 6-well tissue culture plate. For this study, cells were treated with **DP4**, **DP4**@DOX, and DOX (control) and incubated for 4 h. After the incubation, cells were washed and then fixed with 4% PFA for 15 min at r.t. After fixation, the cells were washed with PBS, and actin filaments of the cell membrane and nuclei were stained with phalloidin 647 and DAPI, respectively. The CLSM images were captured using 405 nm laser for DAPI stained nuclei, 488 nm laser for BODIPY tagged **DP4**, 552 nm laser for DOX, and 638 nm laser for phalloidin 647 stained membrane actin filaments. The images were analyzed using FIJI software.

**Flow cytometric analysis.** To determine the effects of biotin receptor-specific cellular uptake of **DP4**@DOX, a comparative flow cytometric analysis was performed as per the method described elsewhere with some modifications.[9] Briefly, human HeLa and HEK 293T cells ($1 \times 10^5$ cells/well) were seeded separately in the 24-well cell culture plate. Confluent monolayers of the cells were incubated with **DP4**@DOX (concentration of DOX and CBL is

1 μg/mL and 40 μg/mL, respectively) for 4 h at 37 °C with 5% $CO_2$. Untreated cells and cells treated with only DOX (concentration of DOX: 1 μg/mL) served as control. After the incubation, the cells were washed thrice with 1X PBS and treated with 0.25% trypsin solution to detach the cells from the cell culture plate surface. Next, the growth medium was added, washed, and fixed with 4% PFA. After the fixation, cells were washed thrice and resuspended in 1X PBS, and the fluorescence intensity of DOX and **DP4**@DOX was measured by flow cytometry. The mean fluorescence intensity (MFI) was calculated for all treatments and compared to the control cells (cells without treatment but processed similarly). The gating method for the quantification of fluorescence intensity of BODIPY and DOX in **DP4**@DOX is demonstrated in Fig S33. Cells were gated on the basis of FSC-A and SSC-A. From the P1 cell population using FSC-A and FSC-H, we chose the singlet cell population (P2) and quantified the fluorescence intensity of BODIPY using a 488 nm laser and DOX using a 561 nm laser.

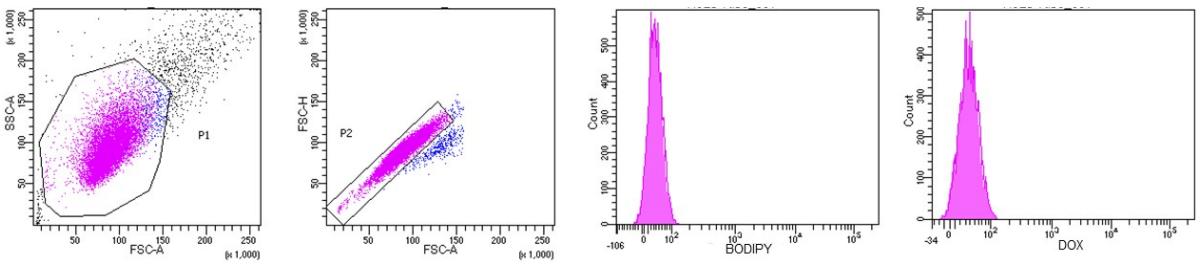



**Fig. S33** Gating method of flow cytometric analysis.

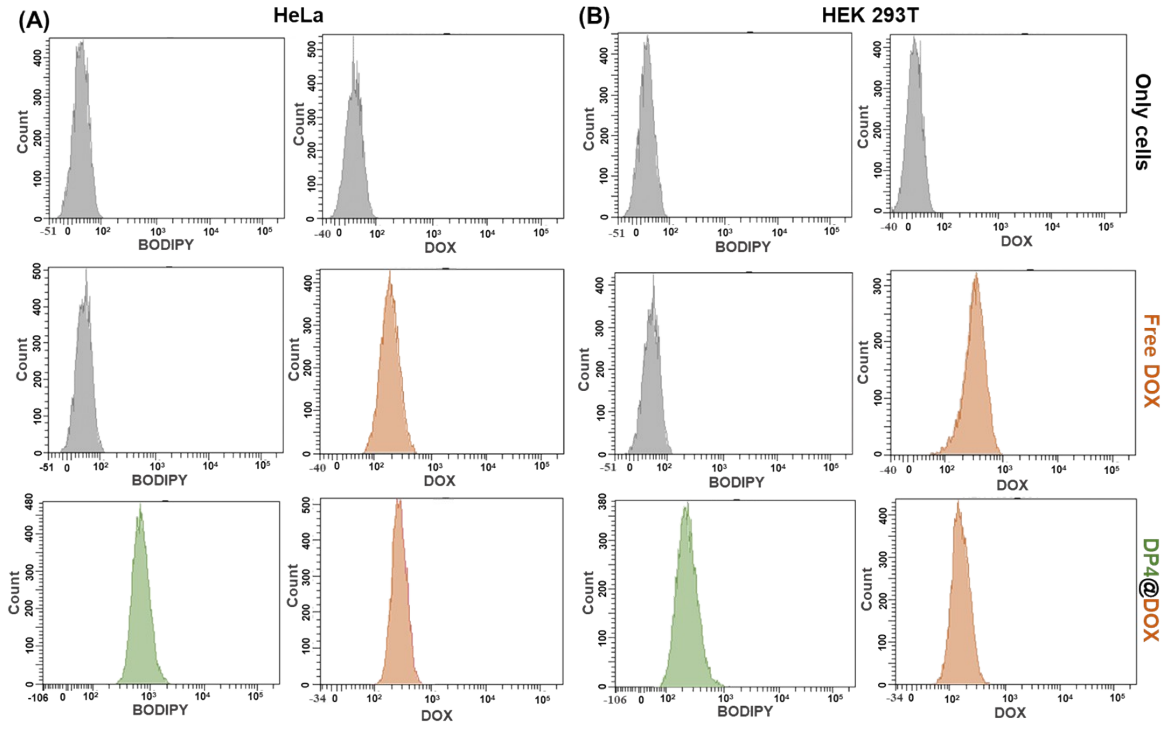



**Fig. S34** (A) and (B) *In vitro* flow cytometry data of HeLa and HEK 293T cells, respectively. Control cells (first row), cells treated with free DOX (second row), and **DP4**@DOX (third row).

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