Supporting Information

Antibody Polymer Conjugates (APCs) for Active Targeted Therapeutic Delivery

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Contents: **Page No.**

Materials……………………………………………………………………………………S2

Synthesis of monomers…………………………………………………...………………...S2

Synthesis of polymer PEG-co-PDS, Polymer P1……………………………………………………S4

The protocol for APC preparation ………………………………………………………....……. S5

Characterization of APC via SDS-PAGE, Western Blot, UV-vis spectroscopy, and DLS...……………. S8

DAR calculation of APC for DM1 drug ....……………………………………………………...…S10

Synthesis of polymer PEG-*co*-PDS-*co*-NHBoc, Polymer P2………………………...………………. S12

Fluorophore conjugation to Polymer P2…………………………………………………...…. S12

Binding interaction evaluation using flow cytometry………………………………………......…. S13

Cytotoxicity evaluation using CellTiter-Glo luminescent assay…………………………………………S14

Synthesis of polymer PEG-*co*-NPC, Polymer P3……………………………………………………. S17

DAR calculation of APC for SN38 drug……………..…………………………..…………………. S18

Characterization of monomers………………………………………………………...…………S20

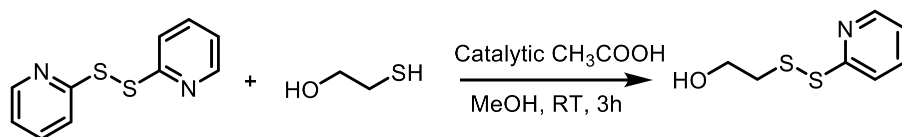
References…………………………………………………………………………………. S26

Materials:

2,2'-Dithiodipyridineand and di-*tert*-butyl pyrocarbonate were brought from Chem-Impex International. Methacryloyl chloride, 2-mercaptoethanol, poly(ethylene glycol) methacrylate (MW 500), 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid 3-azido-1-propanol ester were bought from Sigma-Aldrich and used without further purification. 2,2'-Azobis-(2-methylpropionitrile) was obtained from Sigma-Aldrich and recrystallized prior to use. Sulfo-Cyanine3-NH-ester was brought from Lumiprobe. *InVivo*Mab anti-human/rat HER2 (neu) and *InVivo*Mab mouse IgG2a isotype control, unknown specificity was brought from BioXCell. APC goat anti-mouse IgG (minimum x-reactivity antibody was brought from BioLegend. Mertansine (DM1) was brought from MedChemExpress. DBCO-PEG8-NHS ester was brought from Broadpharm. CellTitre-Glo 2.0 cell viability assay was brought from Promega. HER2/ErbB2 (D8F12) XP Rabbit mAb and EGF Receptor (D38B1) XP Rabbit mAb were brought from the cell signaling.

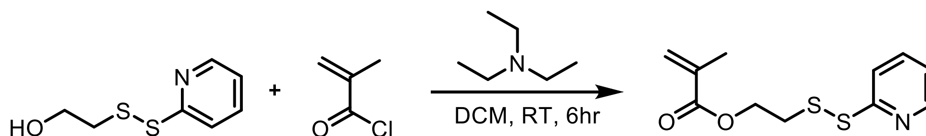
Synthesis of monomers:

Synthesis of pyridyl disulfide monomer, step 1:



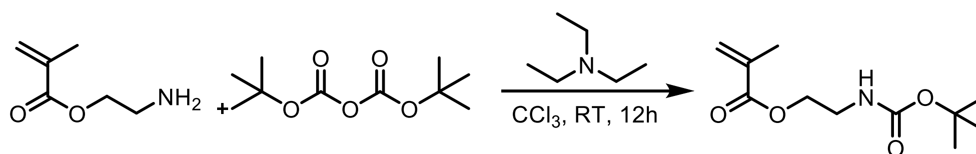
The pyridyl disulfide monomer (M1) was synthesized using previously established protocol[1-2] and compounds in both steps were characterized using ^{1}H, ^{13}C and ESI-MS. First, 15 g (68 mmol, 2 equiv) of 2,2'-dipyridylsulfide was dissolved in 75 mL of methanol in a 250 mL round bottom flask and 1 mL of acetic acid was added slowly to the stirring solution. After 15 minutes, 25 mL solution of 2.65 g (33 mmol, 1 equiv) of 2-mercaptoethanol in methanol was added dropwise to the reaction mixture and allowed stirring at ambient temperature. After 3 h of stirring, methanol was evaporated to obtain a yellow viscous liquid reaction mixture, to which diethylether was added and was swirled vigorously. The crystals formed during the process were filtered out and diethylether was evaporated to obtain the desired product mixture. Finally, the compound was purified using flash column chromatography using hexane/ethyl acetate eluent mixture. Yield: 58%.^{1}H NMR (400 MHz, $CDCl_3$) (δ ppm):8.52-8.53 (d, 1H), 7.58-7.62 (t, 1 H), 7.41-7.43 (d, 1H), 7.15-7.18 (t, 1H), 5.72-5.75 (t, 1H), 3.80-3.84 (dd, 2H), 2.96-2.98 (t, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): 159.09, 149.75, 137.01, 122.04, 121.58, 58.25, 42.77. ESI-MS (m/z) for $C_7H_9NOS_2$ expected $[M]^+$:187.01, obtained: $[M+Na]^+$: 210.08.

Synthesis of pyridyl disulfide monomer, step 2:



3 g (16 mmol, 1 equiv) of 2-(pyridine-2-yldisulfanyl)ethanol (product in step 1,) was dissolved in 12 mL of DCM and reaction mixture was cooled in an ice bath. Then, 1.94 g (19 mmol, 1.2 equiv) of triethylamine was added dropwise to this solution. And allowed string for 15 minutes. Followed by this, 10 mL DCM solution of 1.67 g (16 mmol, 1 equiv) of methacryloyl chloride was added dropwise to the reaction mixture and allowed stirring for 6 h at ambient temperature. The product was purified by washing the crude mixture with distilled water (3x50 mL), and with saturated brine solution (1x50 mL). Finally, it was dried over sodium sulfate anhydrous, and the organic layer was purified via flash column chromatography using hexane/ethyl acetate. Yield: 89 %. ^{1}H NMR (400 MHz, $CDCl_3$) (δ ppm): 8.48-8.49 (d, 1H), 7.70-7.72 (d, 1H), 7.62-7.66 (t, 1H), 7.09-7.13 (t, 1H), 6.14 (s, 1H), 5.60 (s, 1H), 4.40-4.43 (t, 2 H), 3.09-3.13 (t, 2H), 1.96 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): 167.05, 159.75, 149.69, 137.09, 135.96, 126.04, 129.85, 119.80, 62.4, 37.45, 18.28. ESI-MS (m/z) for $C_{11}H_{13}NO_2S_2$ expected $[M]^+$: 255.04, obtained: $[M+Na]^+$: 278.13.

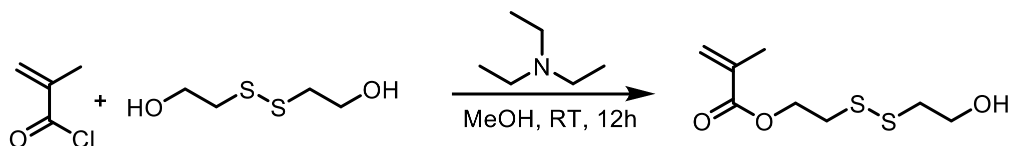
Synthesis of BOC protected amine monomer:



The boc-protected amine monomer (M3) was synthesized using previously established protocol[1] and compounds in both steps were characterized using ^{1}H, ^{13}C and ESI-MS. 500 mg (4 mmol, 1 equiv) of 2-aminoethyl methacrylate hydrochloride was dissolved in 10 mL chloroform in a 100 mL round bottom flask and 593.98 mg (5 mmol, 1.2 equiv) of triethylamine was added to the reaction media. After 15 minutes of stirring, 10 mL solution of 1.28 g (5 mmol, 1.2 equiv) of di-tert-butyl decarbonate in chloroform was added slowly to the solution and allowed stirring for 12 h of stirring at ambient temperature. To purify the product, chloroform was evaporated followed by redissolving the crude mixture in ethyl acetate. The mixture was washed in saturated sodium bicarbonate solution (1x50 mL), followed by saturated brine solution (2x50 mL). The organic layer was collected, dried over sodium sulfate anhydrous, concentrated, and purified by flash column chromatography using hexane/ethyl acetate. Yield: 89%. ^{1}H NMR (400 MHz, $CDCl_3$) (δ

ppm): 6.14 (s, 1H), 5.61 (s, 1H), 4.78 (s, 1H), 4.21-4.24 (t, 2H), 3.46-3.47 (d, 2H), 1.97 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): 173.25, 167.32, 155.76, 136.04, 125.95, 79.62, 64.08, 63.98, 45.99, 42.25, 39.71, 28.37, 18.32, 15.07. ESI-MS (m/z) for $C_{11}H_{19}NO_4$ expected $[M]^+$: 229.13, obtained: $[M+Na]^+$: 252.22.

Synthesis of 2-hydroxyethyl disulfide monomer (M4):



We synthesized the monomer M4 following previous established protocol in our group[4] and characterized them using ^{1}H, ^{13}C NMR and ESI-MS. To a solution of 4 g (26 mmol, 1 equiv) of 2-hydroxyethyl disulfide in 75 mL DCM, 5.4 mL (3.9 g, 39 mmol, 1.5 equiv) of triethylamine was added and allowed stirring for 15 minutes. Then the reaction mixture was cooled on ice for 10 minutes, followed by addition of 2.5 mL (26 mmol, 1 equiv) of methacryloyl chloride (in 75 mL DCM) through a dropping funnel over a period of 30 min. After complete addition, the ice bath was removed and allowed stirring for 12 h at ambient temperature. For purification, first the DCM was evaporated followed by redissolving the reaction crude in 50 mL ethyl acetate. Solution was washed with distilled (3x50 mL) and saturated NaCl (50 mL) water and finally, the organic layer was collected, dried over sodium sulfate anhydrous and isolated the product by flash column chromatography using hexane/ethyl acetate (75:25 v/v). Yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$) (δ ppm): 6.16 (d, 1H), 5.62 (d, 1H), 4.47-4.43 (t, 2H), 3.91-3.92 (d, 2H), 2.98-3.01 (t, 2H), 2.9-2.92 (t, 2H). 1.97 (d, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) (δ ppm): 172.12, 167.25, 142.92, 136.01, 126.12, 126.04, 112.56, 62.61, 62.50, 60.21, 41.67, 36.99, 20.70, 20.54, 18.29. ESI-MS (m/z) for $C_{11}H_{19}NO_4$ expected $[M]^+$: 222.04, obtained: $[M+Na]^+$: 245.12.

Synthesis of polymer PEG-*co*-PDS, Polymer P1:

The polymer P1 is a random copolymer of pyridyl disulfide (PDS) methacrylate (M1) and PEG methacrylate (M2), synthesized via RAFT polymerization. The PDS monomer was synthesized following our previous work[1-2] and PEG monomer was commercially available. To synthesize the polymer, 100 mg (0.392 mmol) of PDS monomer (M1), 784 mg (1.568 mmol) of PEG monomer (M2), and 13.18 mg (0.029 mmol) of 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid 3-azido-1-propanol ester were dissolved in 1200 µL of dry THF in a 7 mL glass vial. Then 96.7 µL (0.0058 mmol) of AIBN solution (10mg/mL in dry THF) was added into the solution mixture and sealed the glass vial tightly with septum and duct tape. Then the solution degassed by four cycles of freeze-pump-thaw and allowed the

polymerization at 65 °C for 18 h. After quenching the polymerization reaction, the crude mixture was dissolved in minimal amount of DCM and precipitated in diethyl ether three times. Finally, the polymer was dissolved in DCM, transferred in a reweighed 25 mL glass vial, and dried overnight via high vacuum. The obtained yield for the polymerization was 83%. The polymer was characterized by GPC and ^{1}H NMR. The molecular weight obtained by THF GPC was 17.4 kDa and PDI 1.65. The monomer composition of polymer P1 was determined by ^{1}H NMR and obtained ratio was n:33% and m:67%.

The protocol for APC preparation:

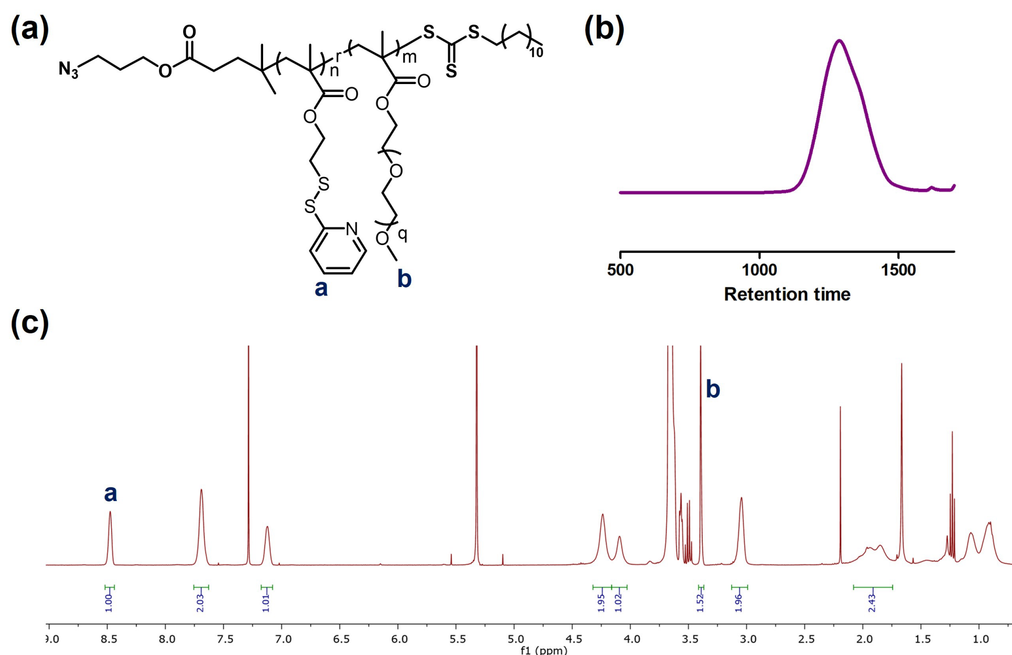


Figure S1: The chemical structure (a), GPC spectrum (b), and ^{1}H NMR spectrum of polymer PEG-*co*-PDS, Polymer P1.

Antibody linker conjugation: The APC preparation starts with antibody linker conjugations, and we employ lysine-NHS chemistry to conjugate linker onto the antibody (Figure S2).[2-3] We used anti-HER2, anti-EGFR, and IgG (isotype control) antibodies for our study and followed the same protocol in all cases. First, 500 ug of antibody (0.0033 µmole) was dissolved in 500 uL of buffer composed of 0.2 M Na_2HPO_4 and 0.1 M NaCl at pH 8.5 and was buffer exchanged at 4 °C using 50 kDa Amicon ultracentrifugation filter. After repeating the process two times, the antibody solution was spun down to 100 uL. The antibody solution was transferred to a 500 uL Eppendorf tube added the required volumes of NHS-PEG8-DBCO linker solutions (in DMSO), and put it on a shaker overnight maintaining 4 °C. The antibodies were incubated

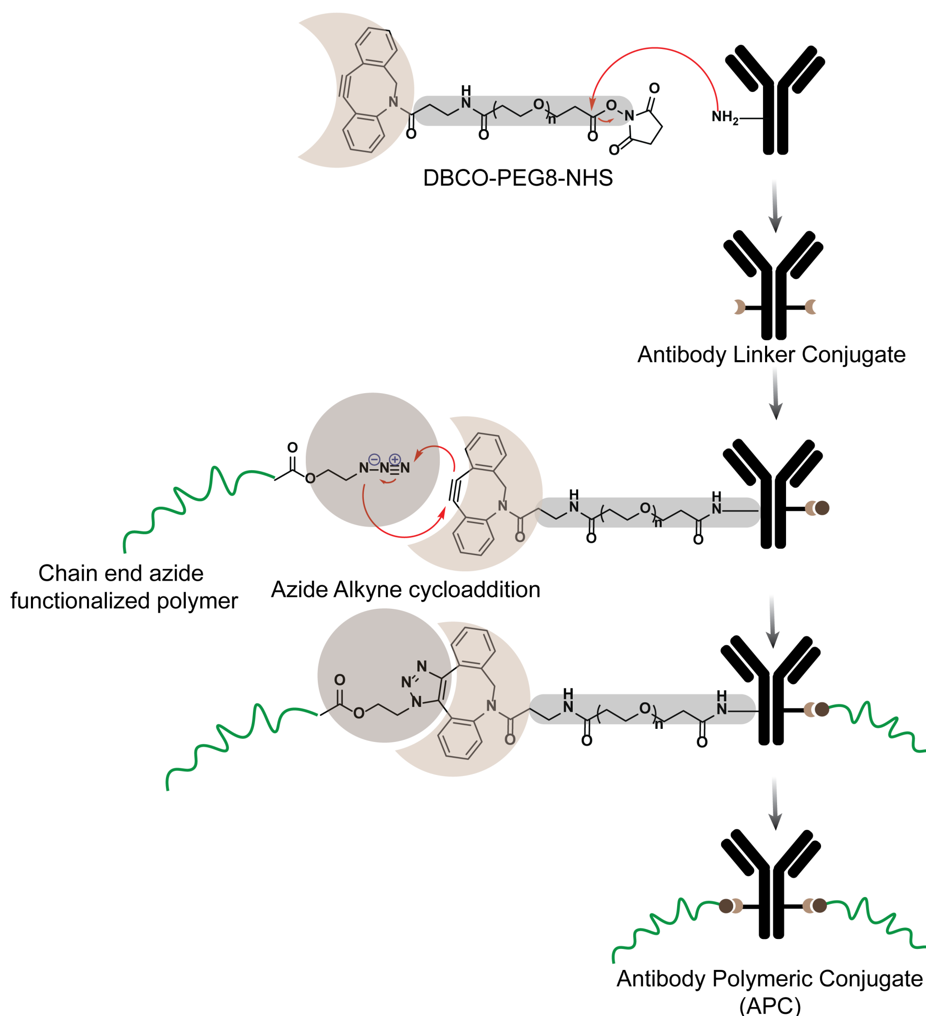


Figure S2: The detailed schematic representation of APC preparation.

with 7 (0.023 µmole) and 45 (0.1485 µmole) equivalents of the linker molecule to achieve a ligand to antibody ratio (LAR) of 2 and 5, respectively. After overnight incubation, the unreacted linker molecules were removed by ultracentrifugation using 50 kDa Amicon filter tube against PBS (pH 7.4). The linker

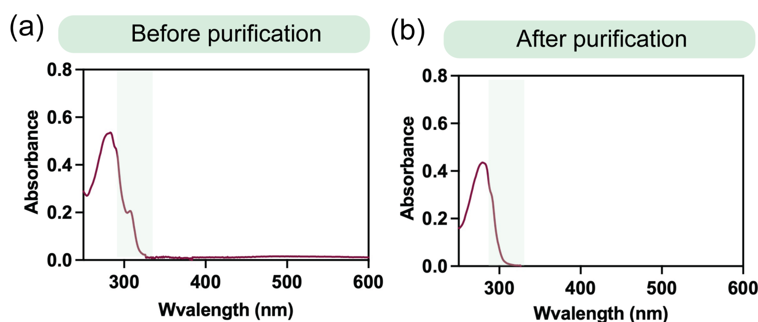


Figure S3: UV-vis spectra of physical mixture antibody and m-PEG-DBCO (a) before and (b) after purification using 50 kDa ultracentrifuge tube.

purification process was validated with an additional UV-Vis spectroscopy assay. The antibody was mixed with m-PEG-DBCO linker (without NHS reactive group) and absorbance was measured before and after the purification. As Figure S3 shows, we did not observe any absorbance corresponding to 310 nm confirms

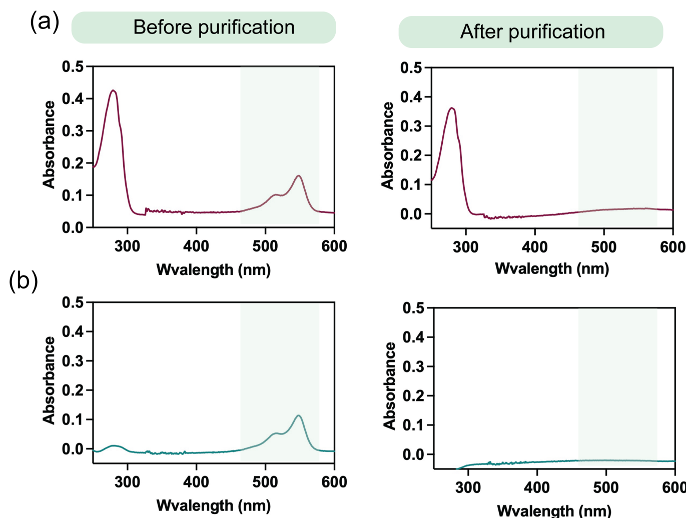


Figure S4: UV-vis spectra of (a) physical mixture antibody and fluorophore-tagged polymer and (b) fluorophore-tagged polymer before and after the purification using 100 kDa ultracentrifuge tube.

the successful purification of excess linker. To determine the LAR, the UV-Vis absorbance at 280 nm and 310 nm corresponding to the antibody and DBCO, respectively were used (Figure 2a, 2b). The molar extinction coefficient used for antibody and DBCO were 205000 and 12000 respectively.

Antibody polymer conjugation: The polymer was conjugated to antibody via copper-free strain-promoted azide-alkyne cycloaddition (SPAAC) reaction (Figure S2, Scheme 1). We have used anti-HER2, anti-EGFR, and IgG (isotype control) antibody linker conjugates for our study and followed the same protocol in all cases. For a typical reaction, 100 ug of the antibody linker conjugates was added 20 equivalents of polymer per linker in each antibody. The reaction was done in 500 uL of Eppendorf tube and reaction solution was incubated on a shaker maintaining 21 °C for 48 h. The total reaction volume was 100 uL which includes the antibody linker conjugates, polymer solution (2 mg/mL in PBS pH 7.4) and PBS (pH 7.4). To remove the excess polymers from the reaction solution, we used the 100 kDa Amicon ultracentrifuge tube where APC solution was washed with PBS five times. Finally, we spun down the solution volume to 100 uL and stored the conjugates at 4 °C. The ureacted free polymer chains purification was validated with UV-Vis spectroscopy assay. The fluorescently labeled polymer P2 with free antibody (without DBCO) and the absorbances were measured before and after purification. Figure R6 clearly demonstrates the successful purification of unreacted polymer. The fluorescently labelled free polymer was also measured in a similar way it also demonstrate the successful separation of polymer.

Characterization of APC via SDS-PAGE, Western Blot, UV-vis spectroscopy, and DLS:

SDS-PAGE gel run protocol: SDS-PAGE gel was used as a primary confirmation of a successful antibody polymer conjugations. For each separate gel run 5 ug of free antibody or antibody polymer conjugates were mixed with 10 uL gel loading buffer, and were loaded in Bio-Rad 4-15% precast polyacrylamide gel (10 wells). The gel was run in 1X SDS running buffer at a constant voltage of 130 V for 1 h. Finally, it was stained in Coomassie stain solution for 5 hours followed by destaining using 45% v/v methanol and 10% v/v acetic acid solution, as needed. Afterwards Bio-Rad ChemiDoc imaging system was used to image and analyze the gel (Figure 2d, Figure 6b).

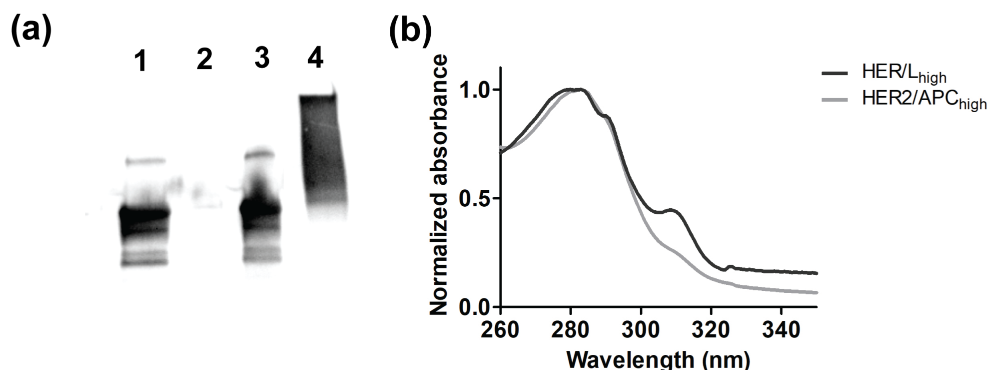


Figure S5: (a) Western blot image to characterize the antibody polymer conjugations. Lane 1: Free antibody, Lane 2: Free polymer, Lane 3: Mock reaction (Free antibody + polymer P1), Lane 4: APC (antibody polymer conjugates); (b) UV-vis spectrum of antibody linker conjugates (anti-HER2) and HER2/APC. The decrease in DBCO peak intensity at 310 nm upon click chemistry was used to quantify the antibody polymer conjugation percentage.

Western blot protocol: Western blot was used as an additional confirmation for a successful antibody polymer conjugation (Figure S5a). The Western blot protocol starts with SDS-PAGE gel run following the above-mentioned protocol, followed by the protein transfer to a polyvinylidene difluoride (PVDF) membrane. The transfer buffer composition was 25 mM Tris base, 0.192 M glycine and 200 mL methanol, and performed wet transfer for 1 h at 100 V maintaining iced conditions. Afterwards, the membrane was blocked with 5% milk in 1X TBS tween-20 for 1 h at room temperature followed by the incubation with HER2/ErbB2 primary antibody at 1:1000 dilution overnight maintaining 4 °C. Next day, the membrane was washed with 0.1% tween-20 three times, was incubated with secondary antibody for an hour at room temperature and washed again with 0.1% Tween-20 in PBS (pH 7.4). Finally, the membrane was incubated with Clarity™ Western ECL substrate (Bio-Rad) and was imaged using Bio-Rad ChemiDoc imaging system.

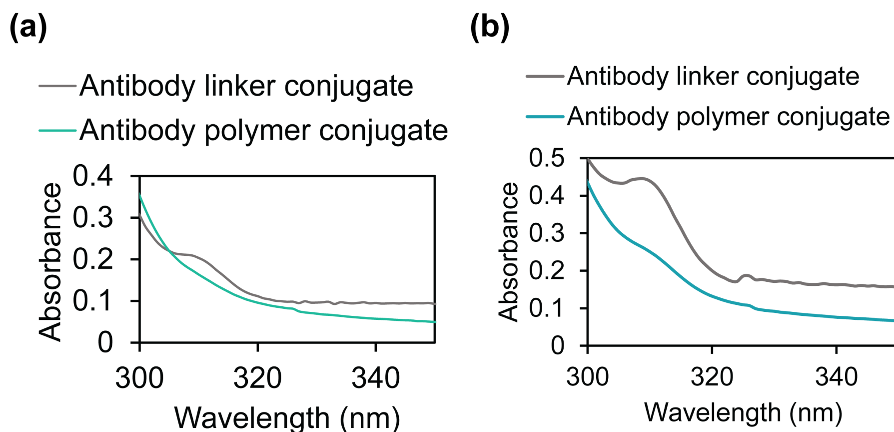


Figure S6: Decrease in peak intensity of DBCO functional group at 310 nm as a measure to quantify the number of polymer chains per antibody. (a) UV-vis spectrum of HER2/APC_{low}; (b) UV-vis spectrum of HER2/APC_{high}.

UV-vis measurement to quantify the number of polymers per antibody: As the appearance of DBCO peak intensity at 310 nm was measured to quantify the number of linkers per antibody, the decrease in this peak intensity was used to confirm the antibody polymer conjugation and quantify the number of polymer chains per antibody. Absorbance measurement of antibody linker conjugate was normalized as 100%. With respect to that, the decrease in moles of DBCO upon polymer conjugation was used to quantify the percentage conjugation (Figure S6). Finally, the percentage conjugation and LAR information was combined to

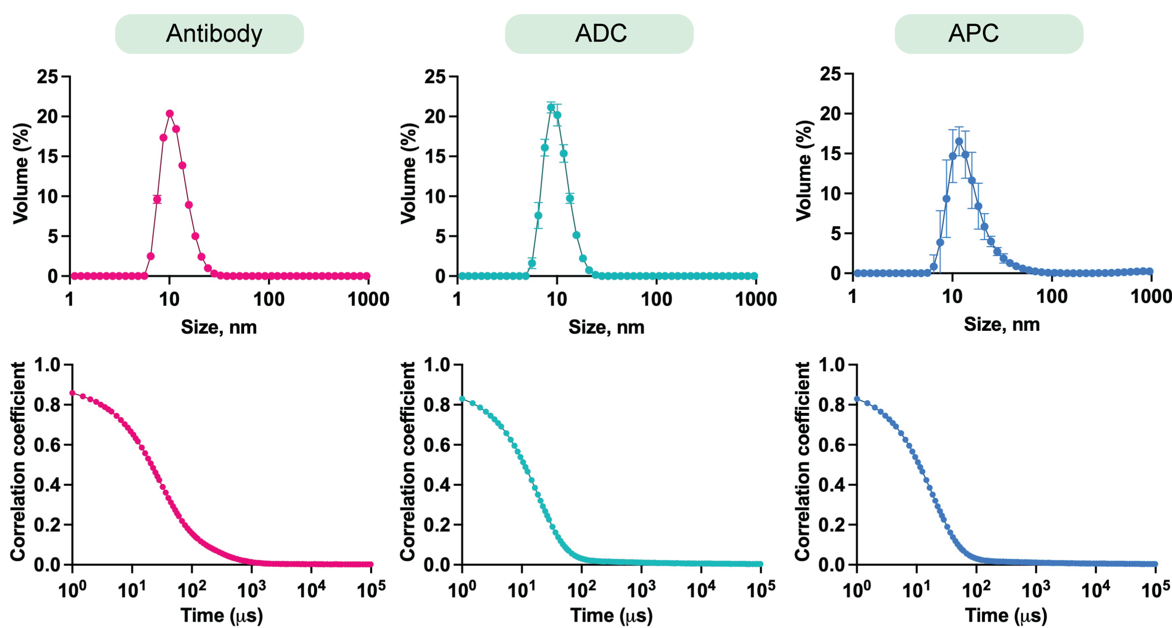
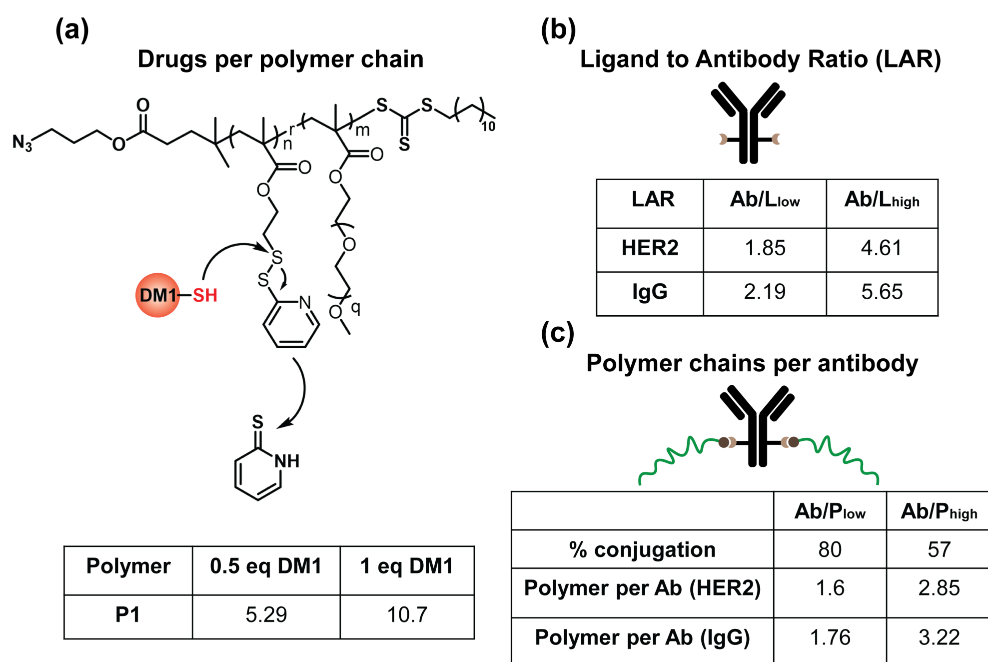


Figure S7: The DLS size and correlation profiles of antibody, ADC and APC.

determine the number of polymer chains per antibody. Figure S8C shows the percentage conversion for APC_{low} and APC_{high} system, and the respective numbers of polymer chains per antibody.

DLS measurement: DLS is widely used to measure the stability of conjugates. The size profile of APCs was tested using DLS and were compared with free antibody and ADC. A very similar size profile and correlation function of APCs (Figure S7) with other conjugates assures the stability.



Polymer	0.5 eq DM1	1 eq DM1
P1	5.29	10.7

LAR	Ab/L_{low}	Ab/L_{high}
HER2	1.85	4.61
IgG	2.19	5.65

	Ab/P_{low}	Ab/P_{high}
% conjugation	80	57
Polymer per Ab (HER2)	1.6	2.85
Polymer per Ab (IgG)	1.76	3.22

Figure S8: The quantification of drug to antibody ratio (DAR). (a) Quantification of drugs per polymer chains; (b) LAR of HER2/APC and IgG/APC (isotype control); (c) The percentage conjugation and the number of polymer chains per antibodies.

DAR calculation of APC for DM1 drug:

Quantitative measurements of (i) drugs per polymer chains, (ii) LAR, and (iii) polymer chains per antibody were combined to quantify the DAR of APC (Figure S5). Two equivalents of DM1 drug to PDS units in the polymer chain were used in the reaction and conjugated drug amount was quantified using the absorbance peak of 2-mercaptopyridine at 343 nm (Figure S5a, Figure 2f). The molar ratio of DBCO (310 nm) and antibody (280 nm) was used to quantify the linker to antibody ratio. 7 and 45 equivalents of linkers molecules per antibody were used to achieve the desired LAR in both cases. Decrease in DBCO absorbance intensity was used as the measure to quantify the number of polymers per antibody in both anti-HER2 and IgG (isotype) system. Finally, all these measurements were combined to determine the DAR of APCs. The following table shows the DAR of both HER2/APC and IgG/APC systems.

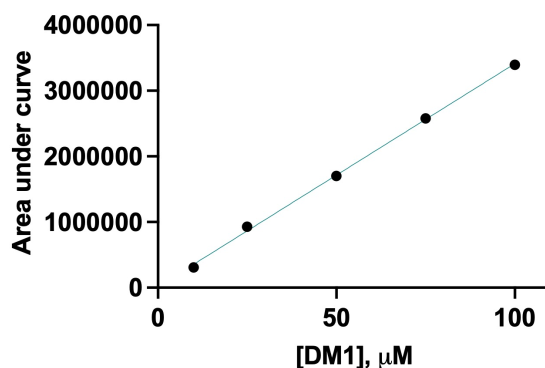


Figure S9: The HPLC calibration curve of DM1 drug.

HPLC measurement to quantify the drugs per polymer chains: The dugs per polymer chains were further quantified by HPLC. The polymer solution (2 mg/mL) was incubated with 10 mM DTT solution for 1 h at 21 ºC. Then it was mixed with ACN to prepare a 1:1 ACN:water solution. Finally, the required volume of solution was injected into the HPLC column and the area under curve was used to quantify the drug to antibody ratio. A calibration curve (Figure S9) was plotted with free drug at different concentrations the obtained equation ($y=33960x+16303$; $R^2=0.9987$) was used to finally quantify the drug concentration. The calculated drug concentration via HPLC (*area under curve: 962710*) is determined to be 27.8 µM which is very close to the UV-Vis measurement (24.8 µM). The following tables show the comparative drug concentrations and DAR determined by UV-Vis and HPLC.

Table S1: The determined concentration of drug and number of drugs per polymer chains

	Concentration of drug (µM)	Drugs per polymer chains
UV-Vis	24.8	5.9
HPLC	27.8	6.4

Table S2: The DAR of APCs determined by UV-Vis and HPLC

	UV-Vis	HPLC
Ab/P_{low}	8.46	15.07
Ab/P_{high}	10.24	18.24

Flow binding interactions of APCs in comparison to respective antibody: The effect of polymer conjugation to antibody towards overall binding activity of APCs was compared with free antibody. The experiment was performed following flow cytometry protocol mentioned under materials and method section in main manuscript. The binding activity of both HER/$P_{low/high}$ and IgG/$P_{low/high}$ were tested in SKOV3 cells and were compared to their respective free antibody. As FigureS10 shows, the lower number of polymers antibody has minimum loss in binding activity and is dependent on the number of polymer chains per APCs.

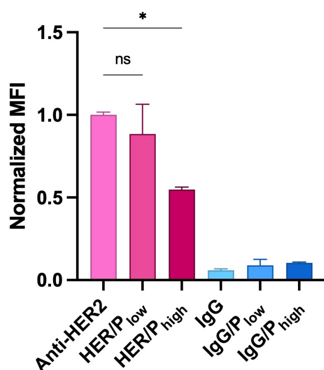


Figure S10: A comparative binding activity of APCs with anti-HER2 antibody in SKOV3 cell.

Synthesis of polymer PEG-*co*-PDS-*co*-NHBoc, Polymer P2:

The polymer P2 is a random copolymer of pyridyl disulfide (PDS) methacrylate (M1), PEG methacrylate (M2) and Boc protected amine (NHBoc) methacrylate (M3), synthesized via RAFT polymerizations. Monomer M3 was synthesized following our previous work[1] and followed the same polymerization protocol as mentioned before. To synthesize the polymer, 100 mg (0.392 mmol) of PDS monomer (M1), 424.65 mg (0.849 mmol) of PEG monomer (M2), 14.98 mg of NHBoc monomer (M3) and 8.04 mg (0.0179 mmol) of 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid 3-azido-1-propanol ester were dissolved in 1200 μL of dry THF in a 7 mL glass vial and 59 uL (0.00359 mmol) of AIBN solution (10mg/mL in dry THF) was added into the solution mixture. The reaction vial was sealed tightly with septum and duct tape, the solution was degassed by four cycles of freeze-pump-thaw and finally allowed the at 65 °C for 18 h. After quenching the reaction, crude mixture in minimal volume of DCM and was precipitated in diethyl ether three times. Afterwards, the polymer was dissolved in DCM, transferred in a reweighed 25 mL glass vial, and dried overnight via high vacuum. The yield of the polymerization reaction was 78%. The polymer was characterized using THF GPC and ^{1}H NMR. The molecular weight of was obtained by THF GPC was 22.98 kDa and PDI 1.63. The composition of NMR was determined by 1H NMR and obtained ratio was n:33% and m:61%, p:6%.

Fluorophore conjugation to polymer P2:

First, the amine functional handle in polymer P2 was deprotected using trifluoracetic acid (TFA) following the previous established protocol.[1] After confirming the deprotection (Figure S11d), Sulfo-Cyanine3 (Sulf-

Cy3) was conjugated to the polymer via NHS-amine chemistry. To conjugate the fluorophore, 100 mg polymer was dissolved in 2mL of DCM and 20 µL of triethylamine was added to the solution. After that, 2 equivalents of fluorophore were added targeting 2% conjugation to the polymer and allowed stirring overnight at ambient temperature. The unreacted fluorophore was dialyzed out using 3.5 kDa MWCO snakeskin dialysis tubing against DCM/MeOH. The dialyzing solvent was changed at a regular interval for 48 h until the solution became completely clear. Afterwards, the polymer was transferred to a glass vial, evaporated the solvent, and dried using high vacuum overnight. The fluorophore conjugation was confirmed using UV-vis spectroscopy (Figure 3e).

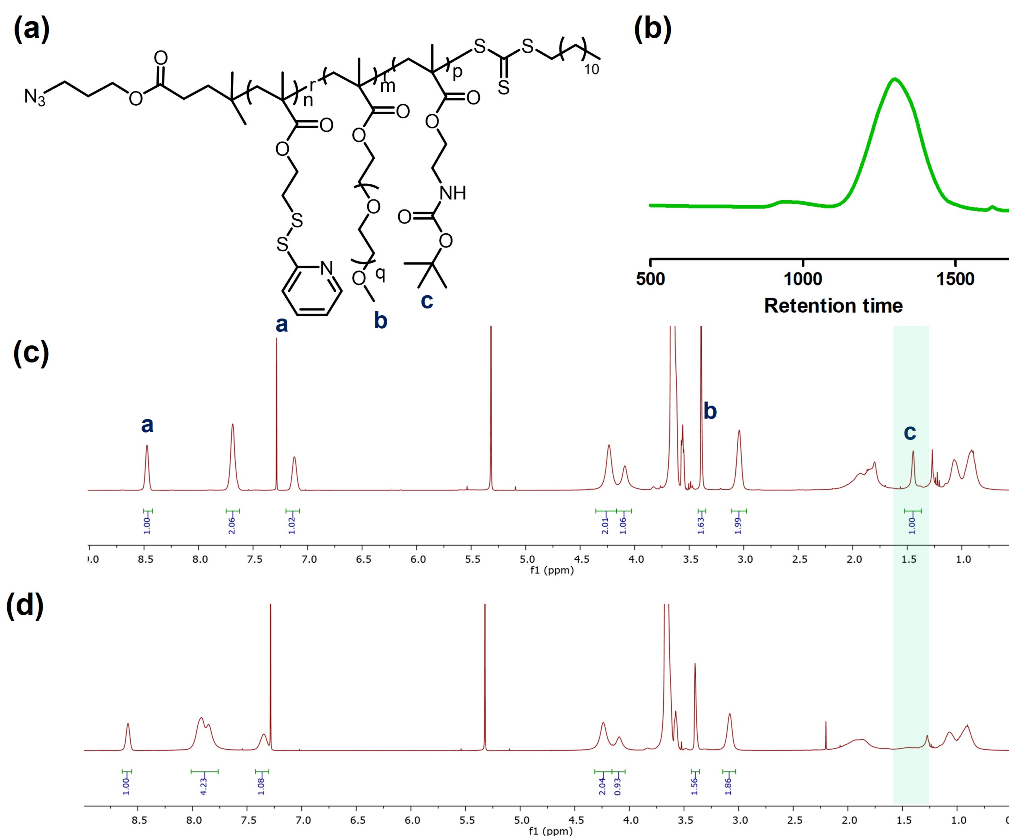


Figure S11: The chemical structure (a), GPC spectrum (b), and ^{1}H NMR spectrum of polymer PEG-*co*-PDS-*co*-NHBoc, Polymer P2 (c). (d) ^{1}H NMR spectrum of polymer P2 after deprotection of Boc functional group. The disappearance of Boc functional group is highlighted in light blue.

Binding interaction evaluation using flow cytometry:

The cellular binding interaction of APCs were evaluated using (*i*) a fluorophore tagged secondary antibody, (*ii*) fluorophore tagged polymer directly conjugated to antibody. HER2+ (SKOV3, SKBR3, BT474), HER2- (MCF10A) cells and EGFR+ (A431) cells were used for this study and the binding interactions were evaluated using fluorophore-tagged secondary antibody in all cells. For secondary antibody labelling

method, 0.2 million cells were taken in 500 μL Eppendorf tube maintaining 100 μL cell media, 5 μg of antibody/APC were added as needed and were incubated for 30 minutes at 37 °C maintaining 5% CO_2. Then cells were two times with cold PBS and were incubated with APC labelled secondary antibody following suggested protocol. Afterwards the cells were washed again with PBS and resuspended in FACS buffer flow cytometry analysis. The binding assay with fluorophore tagged APC in SKOV3 cell following same protocol except the incubation with secondary antibody. Finally, the samples were run on BD LSRFortessa using FACSDiva software and analyzed the data using FlowJo.

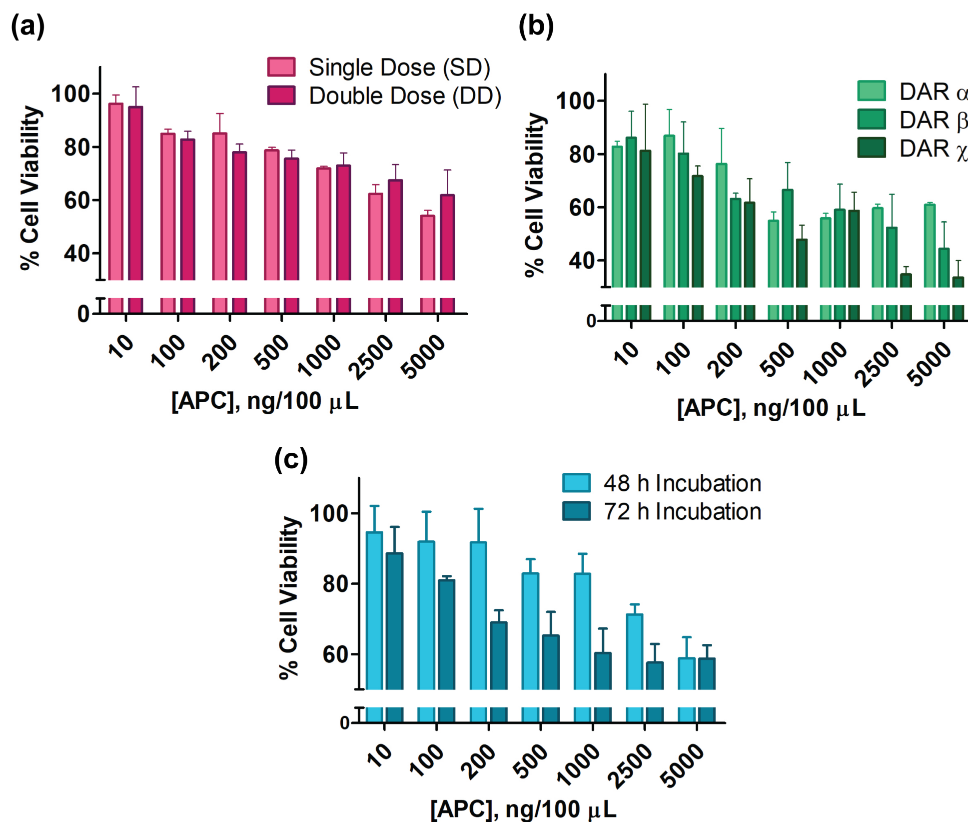


Figure S12: Cytotoxicity studies of APCs in SKOV3 cell at various conditions. (a) Effect the multiple dosing at 24 h interval on cytotoxicity; (b) Effect of increasing the DAR of APCs on cellular cytotoxicity (α: 30.49, β: 45.57, χ:60.98). (c) Effect of different incubation time on cellular cytotoxicity.

Cytotoxicity evaluation using CellTiter-Glo luminescent assay:

Cytotoxicity of APCs/free antibodies/ADC: The toxicity study of APC, ADC or free antibodies were similar to that of free drug toxicity. 20000 cells were plated in 100 μL of cell media to 96-well plate and were incubated overnight at 37 °C maintaining 5% CO_2. Afterwards the old media was replaced with new media containing different concentrations of APC or free antibody or ADC, as needed. After incubating for 30 minutes, the media was removed, cells were washed with PBS and were incubated for 48 h with fresh media. Afterwards, 100 μL of CellTitre-Glo was added to each well and incubated for 10 minutes with

gentle shaking at room temperature. Finally, 100 μL of the solutions from each well were transferred to an opaque, white 96-well plate, and luminescence was using SpectraMax iD5 multiplate reader.

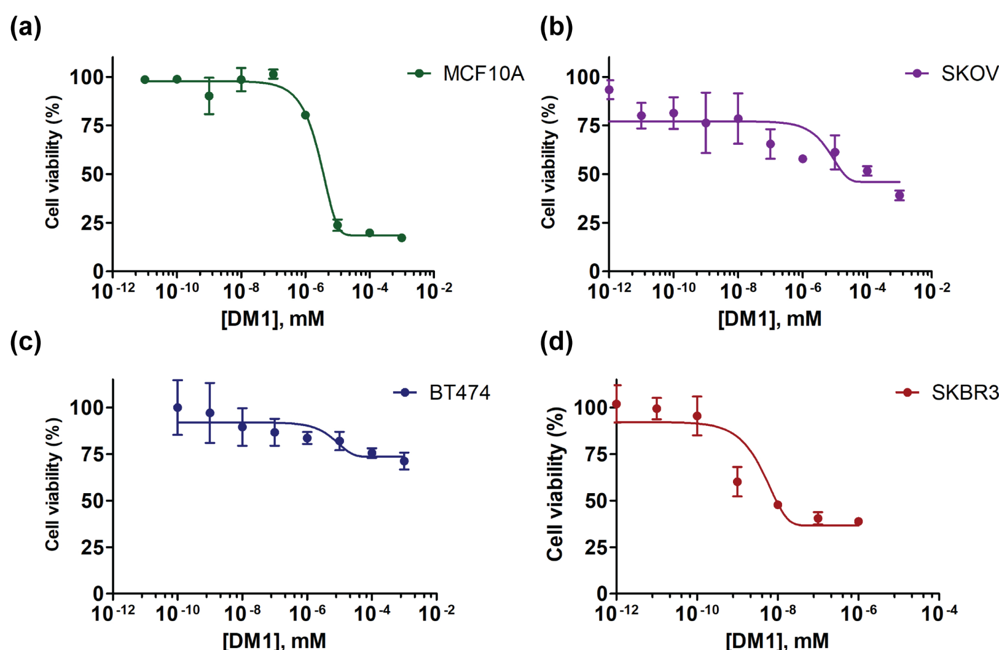


Figure S13: Free drug cytotoxicity of DM1 in (a) MCF10A; (b) SKOV3; (c) BT474; (d) SKBR3 cell lines.

Free drug toxicity: Free drug toxicity of DM1 drug were performed in SKOV3, MCF10A, BT474, SKBR3, and A431 cell lines, along with SN38 cytotoxicity in SKOV3 cell line. For the experiment, 20000 cells in 100 μL cell media were plated in 96-well plate and were incubated overnight at 37 °C maintaining 5% CO_2. Afterwards, old media was replaced with new media containing different concentrations of drugs and incubated for next 48 h. Then 100 μL of CellTitre-Glo was added to each well and incubated for 10 minutes

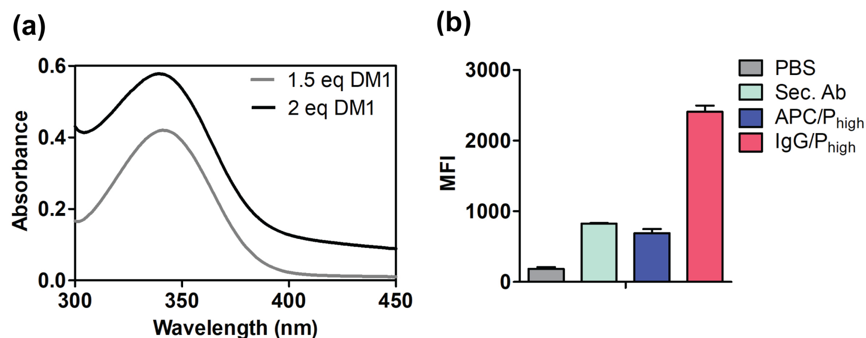


Figure S14: (a) UV-vis spectrum of drug conjugation to polymer P1 at higher equivalent of drugs; (b) Surface receptor binding of APCs in BT474 cell.

with gentle shaking at room temperature. Finally, 100 μL solution from each well were transferred to an opaque, white 96-well plate, and measured the luminescence using SpectraMax iD5 multiplate reader.

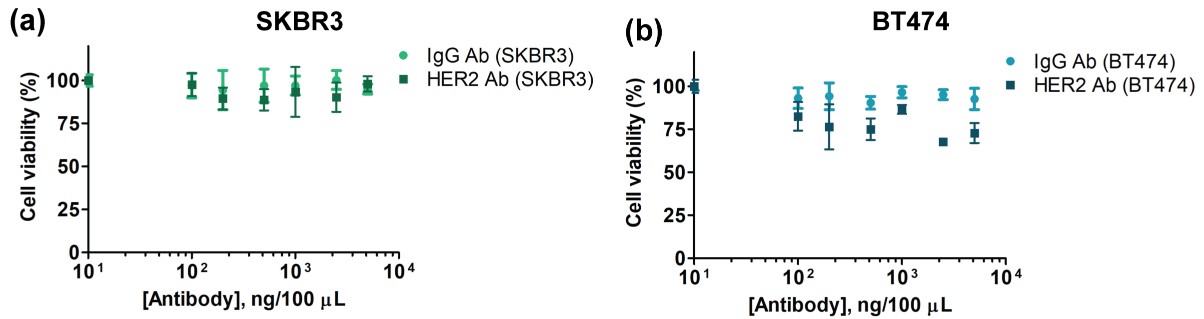


Figure S15: Free antibodies toxicity evaluation in (a) SKBR3 cell; (b) BT474 cells.

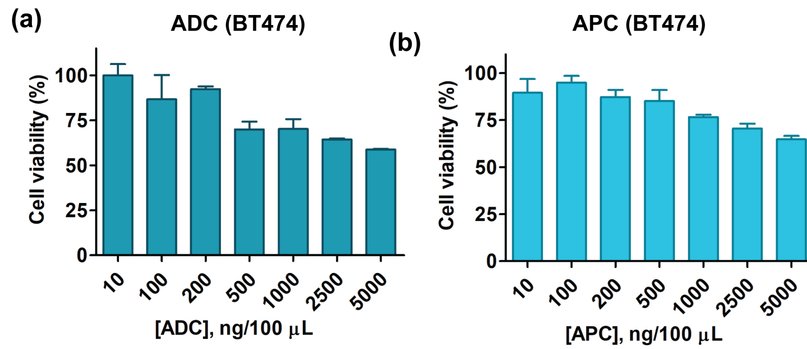


Figure S16: (a) ADC & (b) APC cellular cytotoxicity evaluation in BT474 cells.

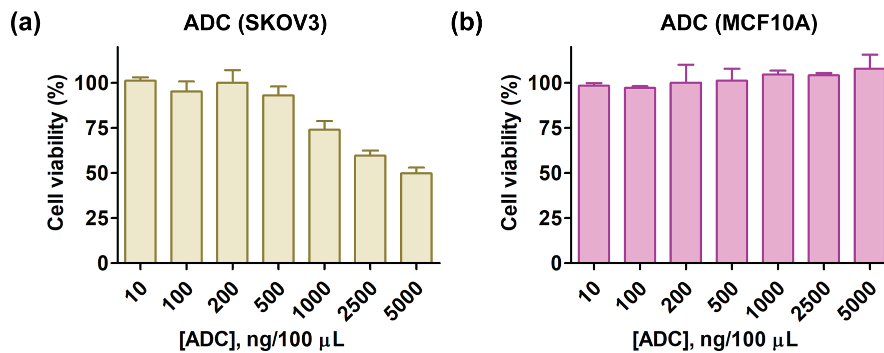


Figure S17: Cellular toxicity of ADC in (a) SKOV3 cell line and (b) MCF10A cell line.

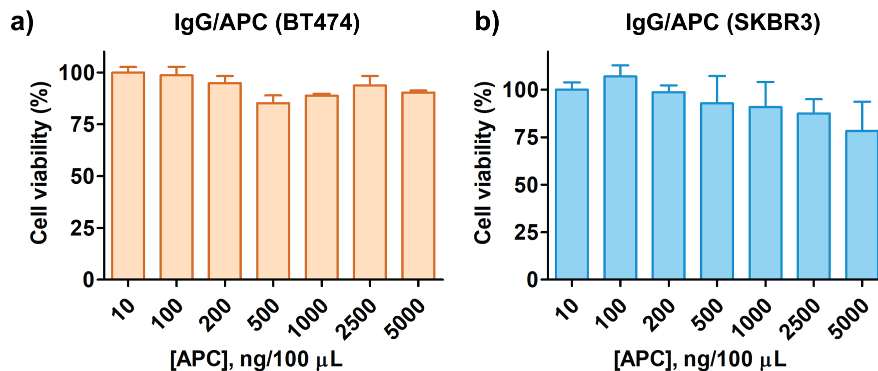


Figure S18: Cytotoxicity evaluation of IgG-APC in (a) BT474 cell & (b) SKBR3 cells.

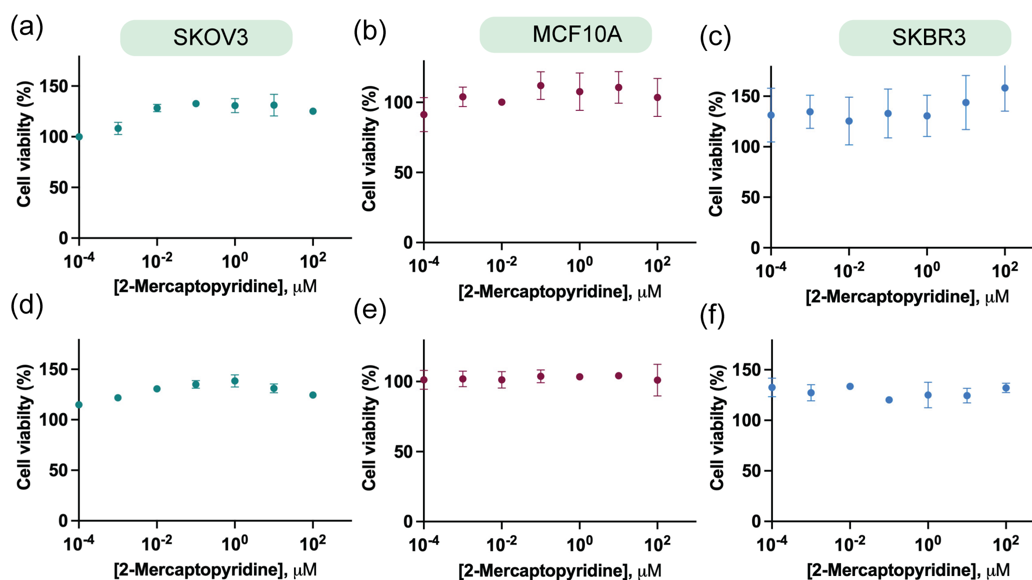


Figure S19: Cellular cytotoxicity of 2-mercaptopyridine in SKOV3, MCF10A, and SKBR3 cells upon 30 minutes treatment followed by 48 h incubation (a-c), and 48 h treatment (d-f).

Synthesis of polymer PEG-*co*-NPC, Polymer P3:

The polymer P3 is a random copolymer of 2-hydroxyethyl disulfide methacrylate monomer (M4), PEG methacrylate (M2) synthesized via RAFT polymerizations and post polymerization modified to conjugated 4-nitrophenol carbonate group. We synthesized monomer M4 following our previous work[3] and followed same polymerization protocol as mentioned above. To synthesize the polymer, 100 mg (0.449 mmol) of monomer M4, 524.9 mg (1.04 mmol) of PEG monomer (M2) and 9.3 mg (0.0207 mmol) of 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid 3-azido-1-propanol ester were dissolved in1200 uL of dry THF in a 7 mL glass vial. Then 68 uL (0.0041 mmol) of AIBN solution (10mg/mL in dry THF) was added into the solution mixture and sealed the glass vial tightly with septum and duct tape. Then the

solution was degassed by four cycles of freeze-pump-thaw allowed the polymerization at 65 °C for 18 h. After quenching the reaction, the polymerization mixture was dissolved in minimal volume of DCM , and purified by precipitation in diethyl ether three times. Afterwards, the polymer was dissolved in DCM, transferred in a reweighed 25 mL glass vial, and dried overnight via high vacuum. The yield of the polymerization reaction was 81%. The polymer was characterized using THF GPC and ^{1}H NMR. The molecular weight of was obtained by THF GPC was 26.8 kDa and PDI 1.55. After that, the polymer was modified with 4-nitrophenol carbonate (NPC) group via post-polymerization reaction with 4-ntrophenyl chloroformate. 100 mg of polymer was dissolved in 2 mL DCM and 2 excesses of 4-ntrophenyl carbonate molecule were added to the solutions. After that pyridine was added to the reaction medium and allowed stirring overnight at room temperature. Next, the DCM was evaporated, and the polymer was precipitated diethyl ether three times. Finally, the polymer was transfterred to a glass vial, evaporated the DCM and dried overnight in high vacuum. The composition of NMR was determined by ^{1}H NMR and obtained ratio was n:27% and m:73%.

DAR calculation of mAb-APC for SN38 drug:

The DAR calculation for SN38 drug system was done following same protocol as mentioned for the DM1 drug. First, the number of 4-nitrophenol carbonate (NPC) group on polymer chain was estimated by ^{1}H

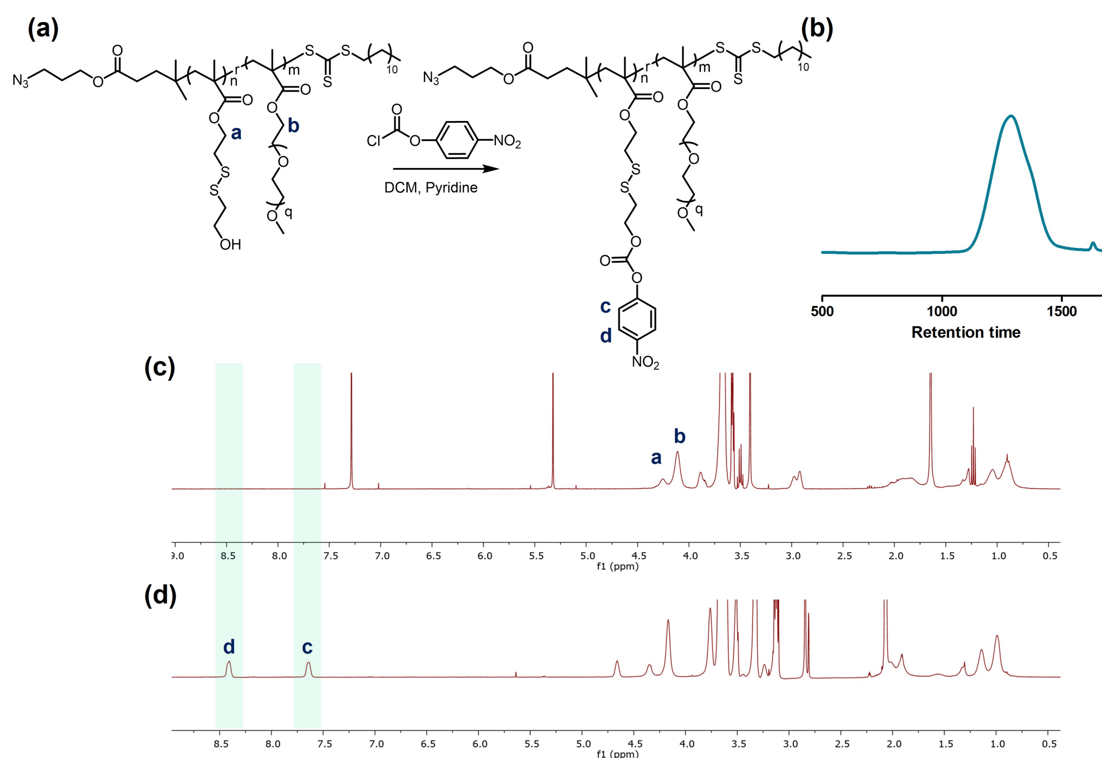


Figure S20: (a) Synthetic scheme of PEG-*co*-NPC polymer, P3; (b) GPC spectrum of polymer P3; (c) & (d) ^{1}H NMR spectra of polymer after conjugating the nitrophenol functional group.

NMR spectrum which was 32 NPC groups per polymer (Figure S11d). Then percentage conjugation of SN38 to polymer was calculated via UV-vis spectroscopy (Figure 5b) which was used to quantify the number of drugs per polymer chain. Anti-HER2 and isotype IgG antibodies were used and previously calculated number per antibody (Figure S5c) were combined to determine the overall DAR of SN38 system.

Table S3: The table summarizes the calculated DAR of APC for SN38 drug.

<table>
<tr><th></th><th>% Conjugation of SN38 in polymer chain (Figure 5b)</th><th>Drugs per polymer chain</th><th>Polymers per antibody</th><th>DAR</th></tr>
<tr><td>HER2/APC</td><td rowspan="2">56.45</td><td rowspan="2">18.02</td><td>2.85</td><td>51.35</td></tr>
<tr><td>IgG/APC</td><td>3.22</td><td>58.02</td></tr>
</table>

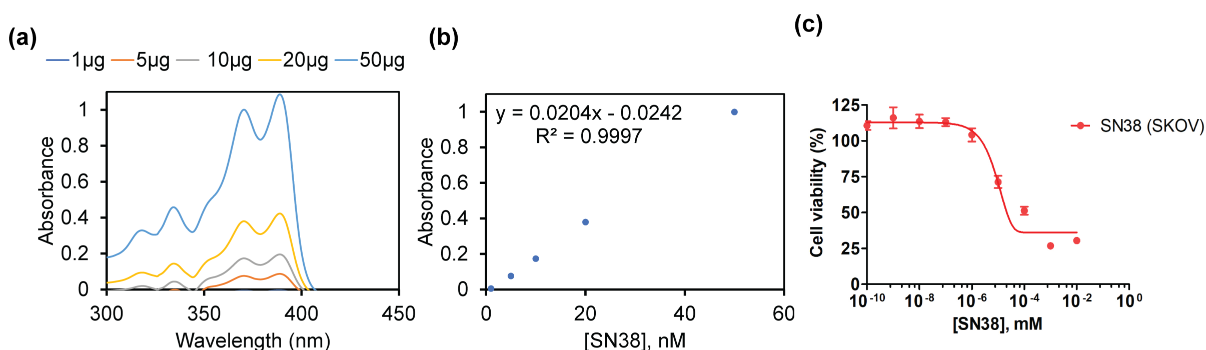


Figure S21: (a) UV-vis absorbance of SN38 at different concentration; (b) Standard curve of SN38 drug; (c) Bar graph showing the determined percentage conjugation of SN38 to the polymer; (d) Free SN38 drug toxicity in SKOV3 cell.

Characterizations of monomers:

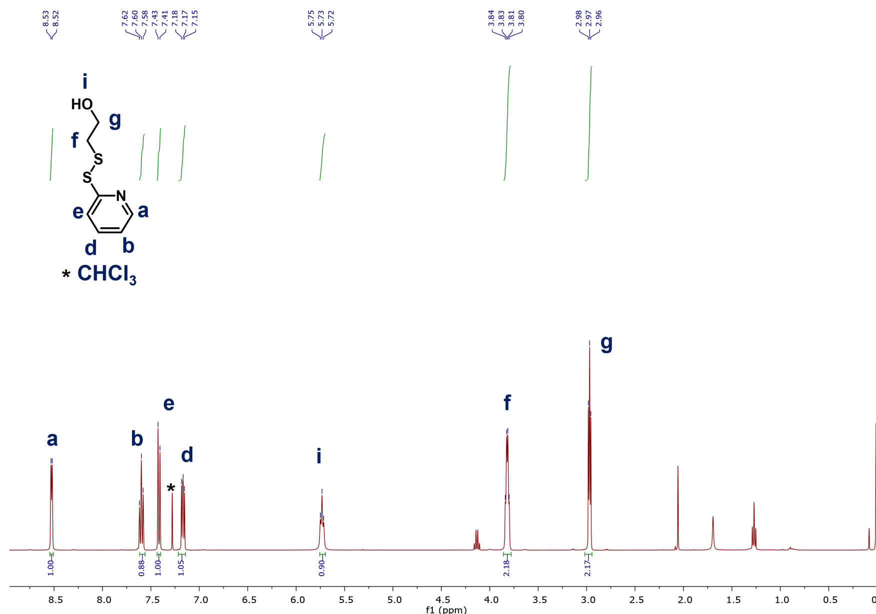


Figure S22: ^{1}H NMR (400 MHz) of monomer synthesis compound 1 in $CDCl_3$.

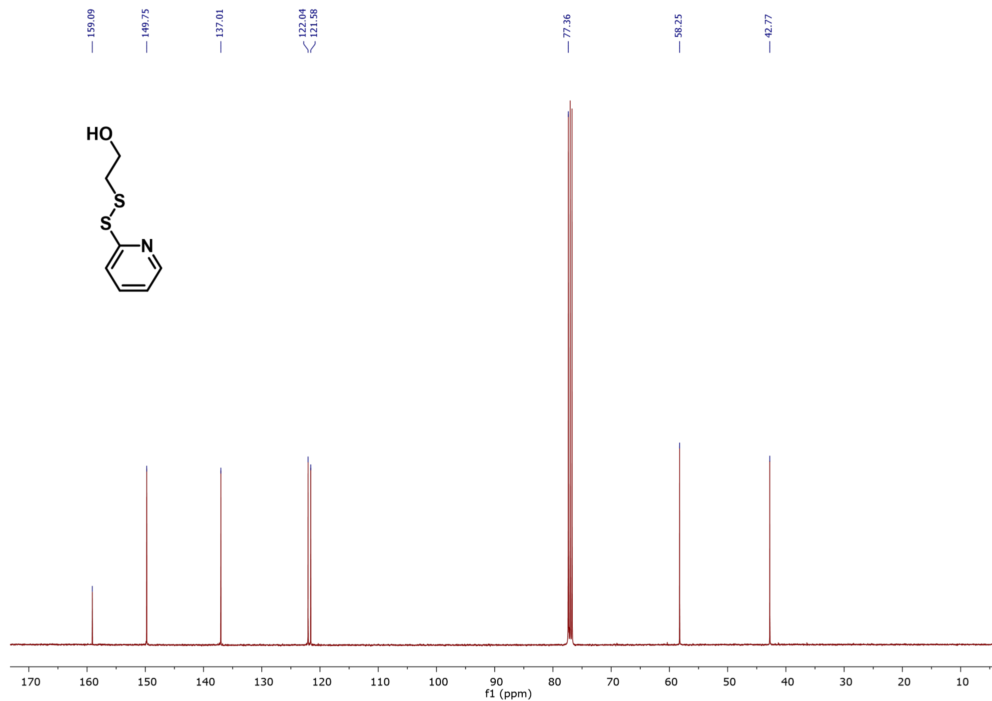


Figure S23: ^{13}C NMR (100 MHz) of monomer synthesis compound 1 in $CDCl_3$.

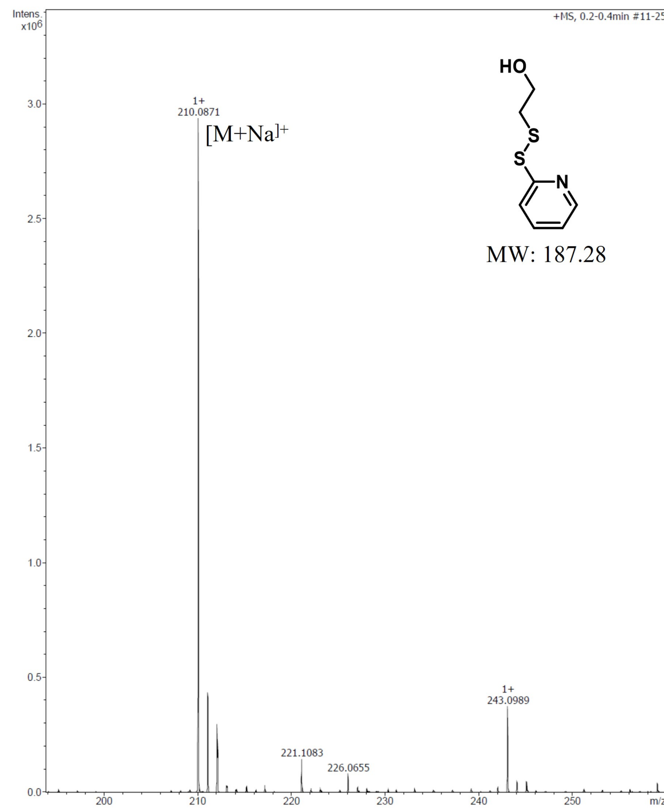


Figure S24: ESI-MS analysis of monomer synthesis compound 1 in methanol.

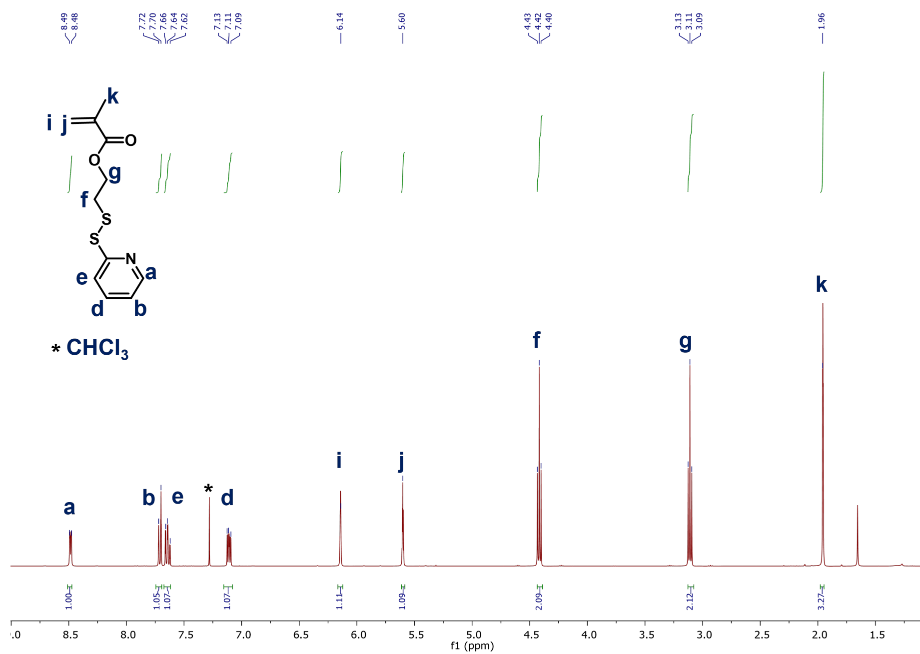


Figure S25: ^{1}H NMR (400 MHz) of pyridyl disulfide monomer (M1) in $CDCl_3$.

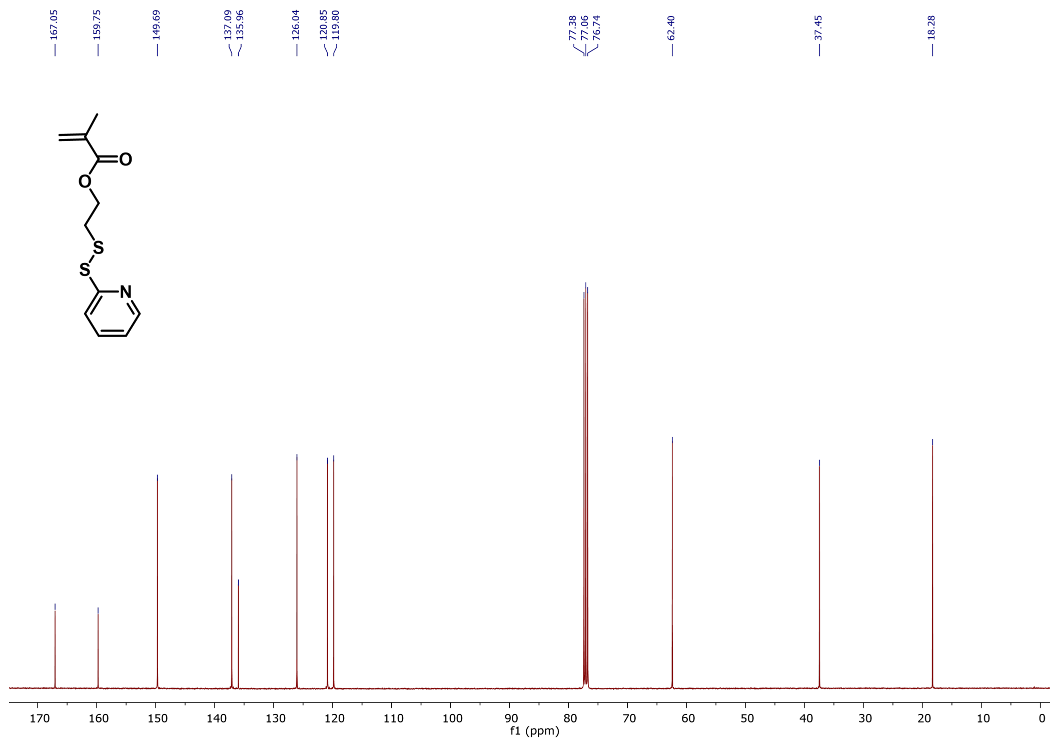


Figure S26: ^{13}C NMR (100 MHz) of pyridyl disulfide monomer (M1) in $CDCl_3$.

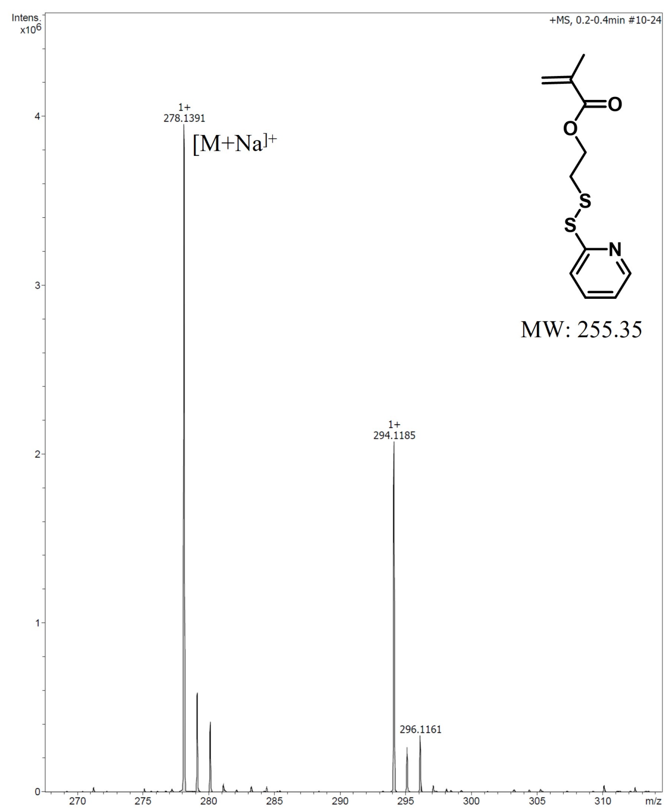


Figure S27: ESI-MS analysis of pyridyl disulfide monomer (M1) in methanol.

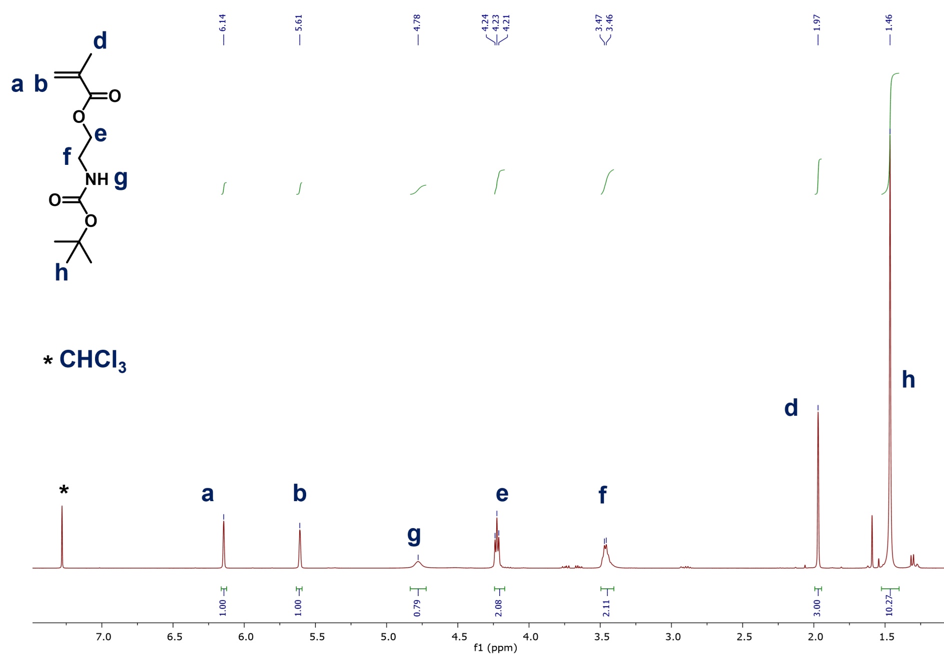


Figure S28: ^{1}H NMR (400 MHz) of Boc amine monomer (M3) in $CDCl_3$.

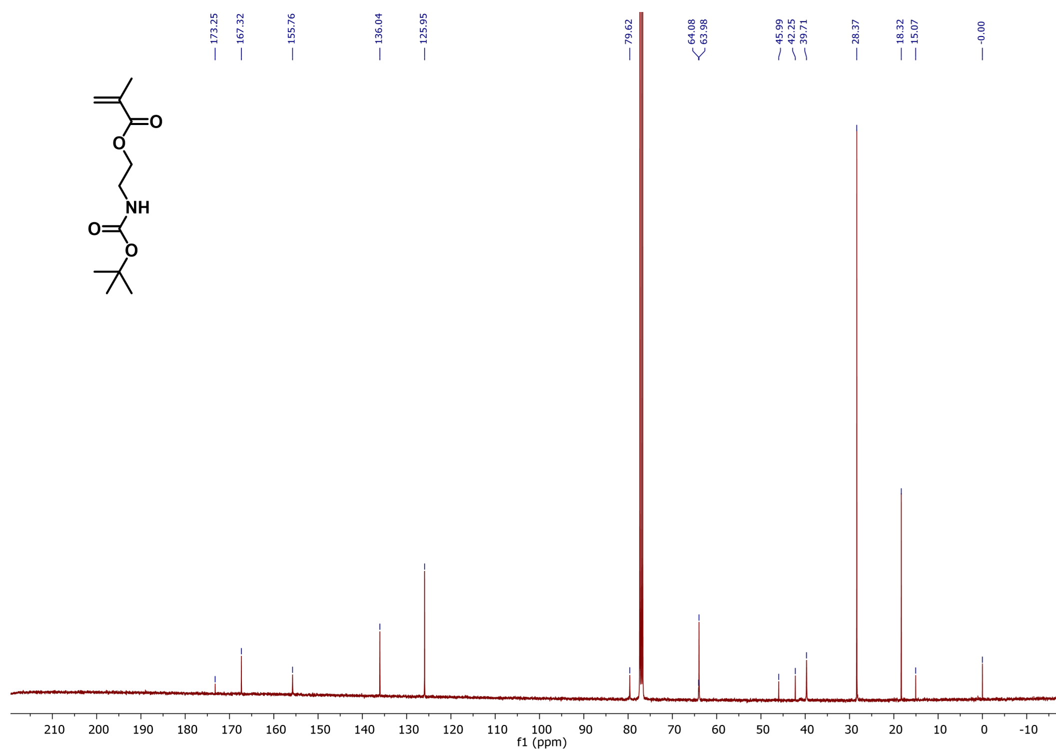


Figure S29: ^{13}C NMR (100 MHz) of Boc amine monomer (M3) in $CDCl_3$.

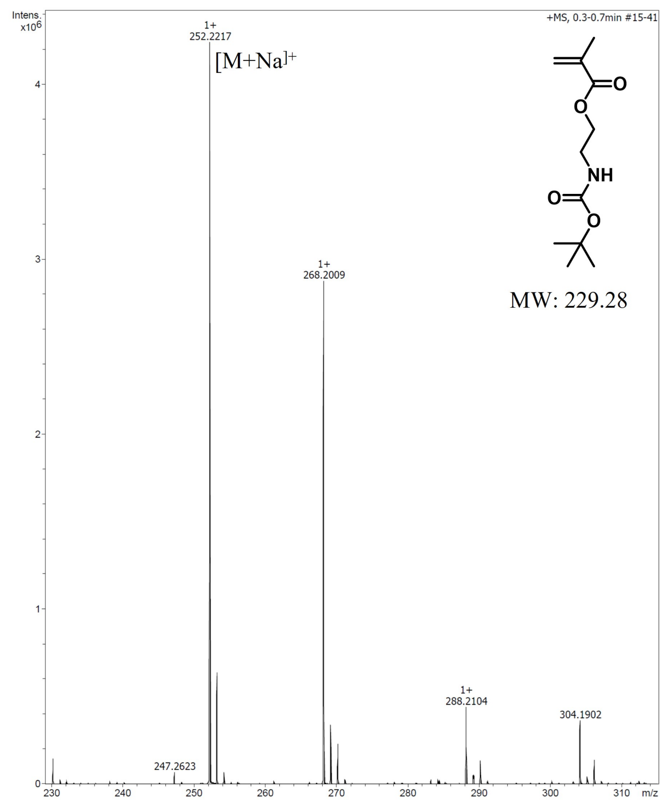


Figure S30: ESI-MS analysis of Boc amine monomer (M3) in methanol.

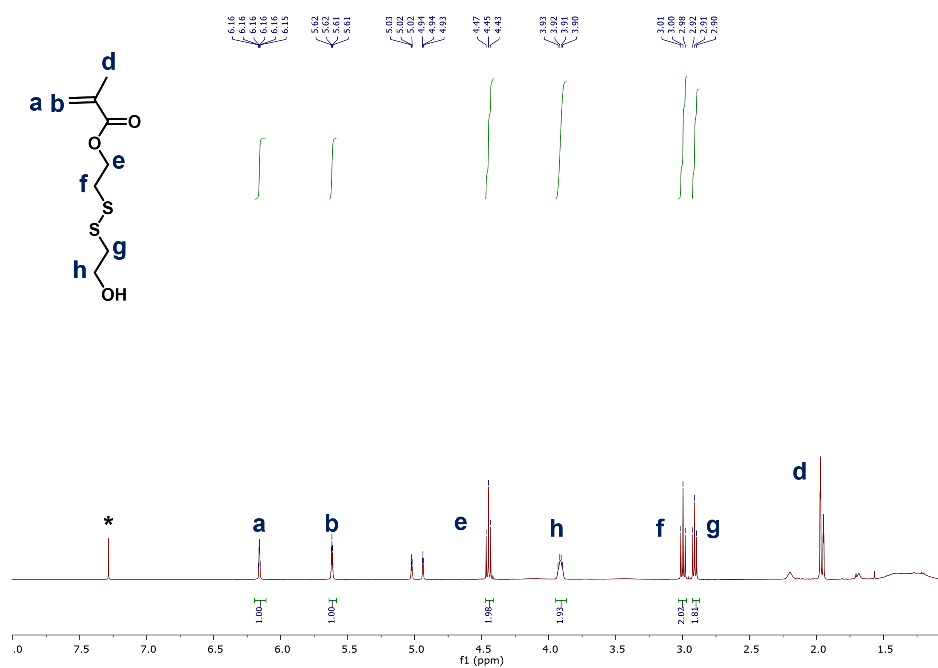


Figure S31: ^{1}H NMR (400 MHz) of 2-hydroxyethyl disulfide monomer (M4) in $CDCl_3$.

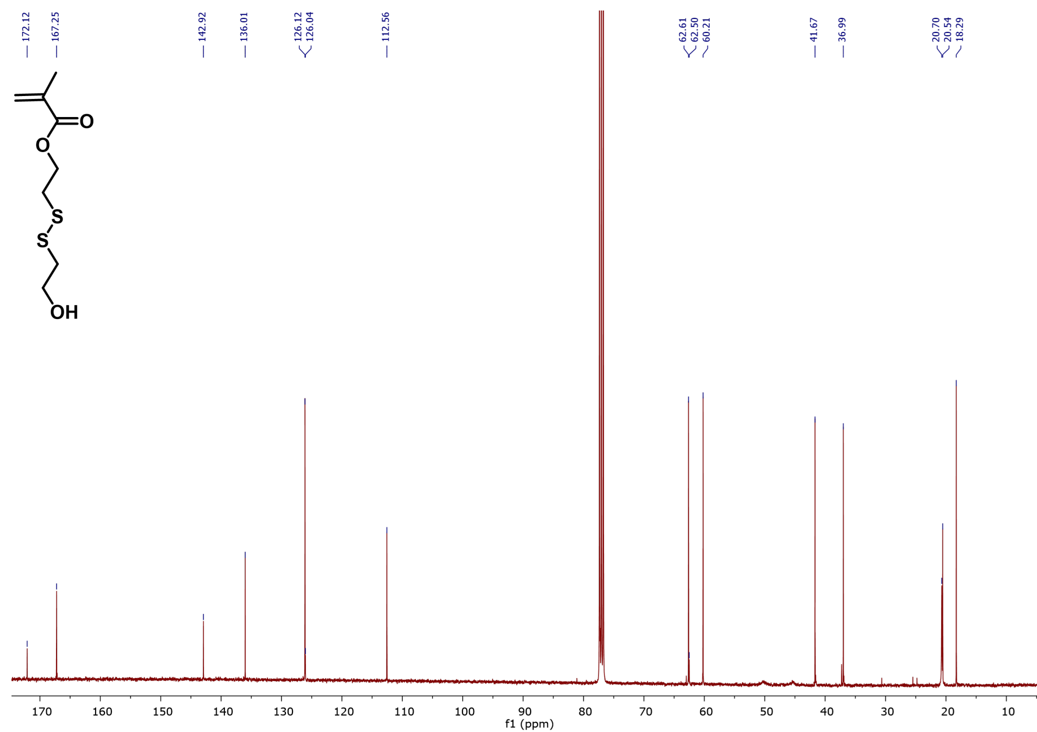


Figure S32: ^{13}C NMR (100 MHz) of 2-hydroxyethyl disulfide monomer (M4) in $CDCl_3$.

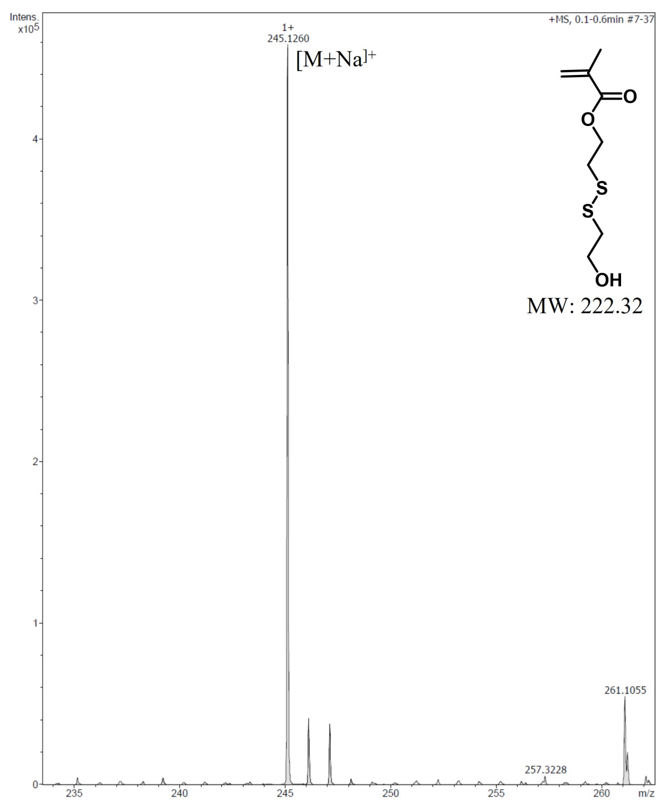


Figure S33: ESI-MS analysis of 2-hydroxyethyl disulfide monomer (M4) in methanol.

References:

1. Kanjilal, P.; Dutta, K.; Thayumanavan, S. Thiol-Disulfide Exchange as a Route for Endosomal Escape of Polymeric Nanoparticles. *Angew. Chem., Int. Ed.* **2022**, *61*, e202209227
2. Singh, K.; Canakci, M.; Kanjilal, P.; Williams, N.; Shanthalingam, S.; Osborne, B. A.; Thayumanavan, S. Evaluation of Cellular Targeting by Fab′ vs Full-Length Antibodies in Antibody-Nanoparticle Conjugates (ANCs) Using CD4 T-Cells. *Bioconjugate Chem.* **2022**, *33*, 486-495.
3. Canakci, M.; Singh, K.; Munkhbat, O.; Shanthalingam, S.; Mitra, A.; Gordon, M.; Osborne, B. A.; Thayumanavan, S. "Targeting CD4+ cells with anti-CD4 conjugated mertansine-loaded nanogels." *Biomacromolecules* **2020**, *21*, 2473-2481.
4. Dutta, K.; Kanjilal, P.; Das, R.; Thayumanavan, S. Synergistic Interplay of Covalent and Non-Covalent Interactions in Reactive Polymer Nanoassembly Facilitates Intracellular Delivery of Antibodies. *Angew. Chem., Int. Ed.* **2021**, *133*, 1849-1858.