SUPPORTNG INFORMATION

Cell surface clicking of antibody-recruiting polymers to metabolically azide-labeled cancer cells

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Experimental

Materials

Unless otherwise stated, all chemicals were purchased from Sigma Aldrich. Pentafluorophenol was obtained from Fluorochem and used as such. 2, 2'-azobis(2-methylpropionitrile) (AIBN) was provided by Wako Chemicals and purified by recrystallization from diethyl ether prior to use. Biotin and Boc anhydride were obtained from TCI chemicals. Dibenzylcyclooctyne-EG_4-amine was purchased from Jena Bioscience. DNP polyclonal antibodies (labeled either with or without AF488 dye) were provided by Thermo Fischer Scientific. Jurkat T cells were obtained from ATCC. Cell culture medium and supplements, DPBS (+ Ca^{2+}; +Mg^{2+}), penicillin/streptomycin (100 x), and sodium pyruvate (100 x) were purchased from Life Technologies. Human Fc block was obtained from Miltenyi Biotec.

Instrumentation

All ^{1}H-, ^{13}C-, and ^{19}F-NMR spectra were recorded on a Bruker 300/400/500 MHz FT-NMR spectrometer. Chemical shifts (δ) were provided in ppm relative to TMS. Samples were prepared in chloroform-d, MeOH-d_4, or DMSO-d_6. Their signals were referenced to residual non-deuterated signals of the solvent.

ESI-MS spectroscopy was performed on a Waters LCT Premier XE TOF equipped with an electrospray ionization interface and coupled to a Waters Alliance HPLC system. Samples were infused in an acetonitrile:formic acid (1000:1) mixture at 0.1 mL/min.

Size exclusion chromatography (SEC) was performed on a Shimadzu 20A system, equipped with a 20A ISO-pump and a 20A refractive index detector (RID). Measurements were executed in tetrahydrofuran (THF), containing 1 % toluene as an internal standard. Calibration of the 2 PL 5 µm Mixed-D columns was done with polystyrene-standards obtained from PSS (Mainz).

UV-Vis spectra were recorded on a Shimadzu UV-1650PC spectrophotometer.

FACS analysis was performed using a BD Accuri C6 (BD Biosciences) and data was processed by FlowJo software.

Confocal microscopy images of 2D cell cultures were taken via a Leica DMI6000 B inverted microscope equipped with an oil immersion objective (Leica, 40 x, NA 1.40) and attached to an Andor DSD2 confocal scanner. Confocal microscopy images of the 3D spheroids were recorded on a Zeiss LSM710 confocal microscope equipped with a 20x objective. Images were processed using ImageJ software.

Biolayer interferometry experiments were performed using an Octet RED96 system (Pall FortéBio). For all experiments, Streptavidin coated sensors were used (Pall FortéBio) together with black flat bottom 96 well plates (Greiner).

Synthesis of pentafluorophenyl acrylate (PFPA).

Pentafluorophenyl acrylate was synthesized according to literature.[1] Pentafluorophenol (27.6 g, 149 mmol) was dissolved in a pre-dried round bottom flask of 500 mL equipped with stirring bar by the addition of 150 mL anhydrous dichloromethane (DCM) under inert atmosphere. The resulting clear solution was placed in an ice bath. Under continuous stirring, 1.05 equivalents of anhydrous triethylamine (21.8 mL, 156 mmol) was added dropwise to the cooled reaction mixture, followed by the dropwise addition of 1.03 equivalents of acryloyl chloride (12.8 mL, 153 mmol). After 30 min, the reaction was allowed to reach room temperature and stir for an additional 2 h. Monitoring of the reaction was performed by thin-layer chromatography (TLC; 80:20 Hexane:EtOAc; R_f = 0.70) until complete consumption of pentafluorophenol was observed. The reaction mixture was diluted with ethyl acetate, filtered and concentrated under vacuum. The resulting crude product (yellow oil) was purified by silica gel chromatography (80:20 Hexane:EtOAc) and yielded a colorless, clear oil that was characterized by ^{19}F-NMR and ^{1}H-NMR (vide infra) (79 % yield).

^{19}F-NMR (282 MHz, $CDCl_3$, **Figure S1**): δ (ppm): -152.7 (d; J = 16.9 Hz; 2F; o-C_6F_5); -158.1 (t; J = 21.6 Hz; 1F; p-C_6F_5); -162.5 (t; J = 19.2 Hz; 2F; m-C_6F_5).

^{1}H-NMR (500 MHz, $CDCl_3$, **Figure S2**), δ (ppm): 6.72 (dd; J = 17.4, 0.76 Hz, 1H; -CH=**CH_2**); 6.38 (dd; J = 17.2, 10.5 Hz; 1H; -**CH**=CH_2); 6.19 (dd; J = 10.5, 0.76 Hz; 1H; -CH=**CH_2**).

Synthesis of 2-(butylthiocarbonothioylthio)propanoic acid (PABTC).

The RAFT chain transfer agent 2-(butylthiocarbonothioylthio)propanoic acid (PABTC) was synthesized according to literature.[2] To a pre-dried 1 L uni-neck round bottom flask equipped with stirring bar and 150 mL anhydrous dichloromethane, 15.0 mL of 1-butanethiol (140 mmol, 1.00 Eq) and 21.2 mL of triethylamine (TEA, 152 mmol, 1.09 mmol) was added dropwise under inert atmosphere. This round bottom flask was placed in an ice bath on a stirring plate to cool the reaction to 0 °C. In a separate round bottom flask, 9.15 mL of carbondisulfide (152 mmol, 1.09 mmol) was dissolved in 150 mL anhydrous DCM under inert atmosphere. The content of the latter flask was dropped to the cooled reaction mixture containing 1-butanethiol and TEA. Upon addition, the reaction mixture developed a distinct yellow color. Once all reagent was added, the reaction mixture was allowed to warm to room temperature under continuous stirring. After 30 minutes, the content of a third round bottom flask containing 75.0 mL anhydrous DCM and 13.7 mL 2-bromopropanoic acid was added dropwise to the reaction mixture. After 2 h the reaction mixture was reduced under vacuum, diluted with cyclohexane and extracted subsequently with 10 % HCl aqueous solution,

deionized water and brine. All organic phases were collected and dried over sodium sulfate before being concentrated in vacuum. The obtained crude yellow crystals were afterwards purified by recrystallization from hexane (65 % yield) prior to characterization by ^{1}H-NMR, APT ^{13}C-NMR and ESI-MS.

^{1}H-NMR (500 MHz, $CDCl_3$, **Figure S3**), δ (ppm): 11.5 - 8 (br; 1H; **H**O-C=O-CH-); 4.88 (q; J = 7.35 Hz; 2H; HO-C=O-C**H**-); 3.38 (t; J = 7.40 Hz; 2H; -C=S-S-C**H$_2$**-CH_2-); 1.70 (m; 2H; -S-CH_2-C**H$_2$**-CH_2-CH_3); 1.64 (d; J = 7.35 Hz; 3H; -C=O-CH-(C**H$_3$**)-S-) 1.45 (sextet; J = 7.72 Hz; 2H; -S-CH_2-CH_2-C**H$_2$**-CH_3); 0.95 (t; J = 7.35 Hz; 3H; -S-CH_2-CH_2-CH_2-C**H$_3$**).

APT ^{13}C-NMR (125 MHz, $CDCl_3$, **Figure S4**), δ (ppm): 176.86 (2C; HO-(**C=O**)-CH(CH_3)-S-(**C=S**)-S-); 47.55 (1C; HO-(C=O)-**CH**(CH_3)-S-(C=S)-S-); 37.29 (-S-(C=S)-S-**CH$_2$**-CH_2-CH_2-CH_3); 30.02 (1C; -S-(C=S)-S-CH_2-**CH$_2$**-CH_2-CH_3); 22.20 (1C; -S-(C=S)-S-CH_2-CH_2-**CH$_2$**-CH_3); 16.73 (1C; HO-(C=O)-CH(**CH$_3$**)-S-(C=S)-S-); 13.72 (1C; (-S-(C=S)-S-CH_2-CH_2-CH_2-**CH$_3$**).

ESI-MS: m/z $[M+H]^+$ = 239.0228 (theoretical), found = 239.067
$[M+Na]^+$ = 261.0048 (theoretical), found = 261.044

Synthesis of (2,3,4,5,6-pentafluorophenyl)-2-(butylthiocarbonothioylthio) propanoate (PFP-PABTC).

The pentafluorophenyl containing RAFT CTA was synthesized according to a detailed protocol by Stenzel et al.[3] 3.5 g of 2-(butylthiocarbonothioylthio)propanoic acid (PABTC; 1 Eq; 5 mmol; as synthesized earlier, *vide infra*), 3 g of pentafluorophenol (1.1 Eq; 5.5 mmol) and 180 mg of DMAP were transferred in a pre-dried round bottom flask and purged with nitrogen. Anhydrous dichloromethane was added (150 mL) and the resulting yellow reaction mixture was stirred in an ice bath until all reagents were completely dissolved. 1.1 Equivalents of *N,N'*-diisopropylcarbodiimide (DIC; 850 µL) was added dropwise to the reaction mixture, which immediately developed an orange-red colour. After 10 minutes of reaction, the reaction mixture displayed precipitate formation, indicating the formation of urea. Monitoring via TLC (DCM, R_f = 0.80) indicated complete consumption of PABTC after 2 h of reaction. The reaction mixture was filtered, reduced under vacuum and purified by silica gel column chromatography (100 % DCM) before yielding a yellow-orange oil after concentration under vacuum (95 % yield). These purified yellow-orange oil was afterwards characterized by ^{1}H-NMR and ^{19}F-NMR.

^{1}H-NMR (500 MHz, $CDCl_3$, **Figure S5**), δ (ppm): 5.12 (q; J = 7.48 Hz; 2H; -O-C=O-C**H**-); 3.40 (m; 2H; -C=S-S-C**H$_2$**-CH_2-); 1.77 (d; J = 7.48 Hz; 3H; -C=O-CH-(C**H$_3$**)-S-); 1.71 (m; 2H; -S-CH_2-C**H$_2$**-CH_2-CH_3); 1.45 (m; 2H; -S-CH_2-CH_2-C**H$_2$**-CH_3); 0.94 (t; J = 7.32 Hz; 3H; -S-CH_2-CH_2-CH_2-C**H$_3$**).

^{19}F-NMR (470 MHz, $CDCl_3$, **Figure S6**), δ (ppm): -152.2 (d; J = 17.3 Hz; 2F; o-C_6F_5); -157.4 (t; J = 21.9 Hz; 1F; p-C_6F_5); -162.0 (t; J = 19.5 Hz; 2F; m-C_6F_5).

Synthesis of tert-butyl-N-[2-[2-(2-aminoethoxy)ethoxy]ethyl]carbamate

2,2'-(ethylenedioxy)bis(ethylamine) was first mono-Boc protected. 5 equivalents of 2,2'-(ethylenedioxy)bis(ethylamine) (8 mL; 54.8 mmol) were dissolved in 50 mL anhydrous DCM under inert atmosphere in a 250 mL round bottom flask equipped with stirring bar and dropping funnel. The round bottom flask was placed in an ice bath and stirred for 30 minutes prior to the addition of the Boc-anhydride. In a separate flask, 1 equivalent of Boc-anhydride (2.52 mL; 10.97 mmol) was dissolved in 20 mL anhydrous DCM and transferred to the dropping funnel. The latter solution was added dropwise to the 250 mL flask and left to react overnight. Afterwards, the turbid reaction mixture was reduced under vacuum and extracted 3 times with saturated $NaHCO_3$ solution with DCM. The organic phases were collected and dried over Na_2SO_4before being purified by flash chromatography (80:20:1 DCM:MeOH:NH_4OH). Yield = quantitative. The resulting pure product was analysed by ^{1}H-NMR.

^{1}H-NMR (400 MHz, $CDCl_3$, **Figure S7**) δ ppm: 5.13 (br s; 1H; -CH_2-CH_2-**NH**-(C=O)-O-C(CH_3)); 3.61 (s; 4H; NH_2-CH_2-CH_2-O-**CH_2**-**CH_2**-O-); 3.53 (dt; 4H; J = 7.02, 5.22 Hz; -**CH_2**-O-CH_2-CH_2-O-**CH_2**-); 3.31 (br q; 2H; J = 5.10 Hz; -CH_2-**CH_2**-NH-(C=O)-O-C(CH_3)); 2.88 (t; 2H; J = 5.19 Hz; -CH_2-**CH_2**-NH_2); 1.93 (s, 2H, -CH_2-CH_2-**NH_2**); 1.43 (s, 9H, -CH_2-NH-(C=O)-O-C(**CH_3**)).

Synthesis of tert-butyl-N-[2-[2-[2-(2,4-dinitroanilino)ethoxy]ethoxy]ethyl)carbamate.

3 g of tert-butyl-N-[2-[2-(2-aminoethoxy)ethoxy]ethyl]carbamate (12.1 mmol, 1.1 Eq) was dissolved by the addition of 50 mL anhydrous 1,4-dioxane under inert atmosphere in a 250 mL round bottom flask equipped with stirring bar. To this clear solution, 1.80 mL of TEA was added (13.2 mmol, 1.2 Eq) and the reaction was left stirring vigorously. In a separate flask, a stock solution of 2.20 g 2,4-dinitrophenyl-1-chloride in 50 mL anhydrous 1,4-dioxane was made and the latter solution was added to the reaction mixture in a dropwise fashion. The resulting reaction mixture immediately turned yellow and showed precipitate formation after 2 h of reaction at room temperature. TLC analysis indicated the almost complete consumption

of starting material after 24 h of reaction (DCM:MeOH 99:1). The turbid solution was diluted with EtOAc before being filtered and concentrated under vacuum. The resulting yellow crude was purified by flash chromatography (pure DCM to get rid of the starting material, afterwards 5% MeOH in DCM) to yield a clear yellow oil. (yield = 82 %). The resulting pure product was analysed by ^{1}H-NMR.

^{1}H-NMR (400 MHz, $CDCl_3$, **Figure S8**) δ ppm: 9.14 (d; J = 2.64 Hz; 1H; -C(NO_2)-**CH**=C(NO_2)-C(NH-CH_2-R)=CH-CH=); 8.82 (br s; 1H; -C(NO_2)-CH=C(NO_2)-C(**NH**-CH_2-R)=CH-CH=); 8.27 (dd; J = 9.61, 2.64 Hz; 1H; -C(NO_2)-CH=C(NO_2)-C(NH-CH_2-R)=CH-**CH**=); 6.95 (d; J = 9.42 Hz; 1H; -C(NO_2)-CH=C(NO_2)-C(NH-CH_2-R)=**CH**-CH=); 5.10 – 4.80 (m; 1H; -CH_2-CH_2-**NH**-(C=O)-O-C(CH_3)$_3$); 3.84 (t; 2H; -C(=R-)-NH-**CH_2**-CH_2-O-CH_2-CH_2-O-); 3.75 - 3.53 (m; 8H; -NH-CH_2-**CH_2**-O-**CH_2**-**CH_2**-O-**CH_2**-CH_2-NH-(C=O)-O-C(CH_3)$_3$); 3.33 (br m; 2H; -CH_2-**CH_2**-NH-(C=O)-O-C(CH_3)$_3$); 1.43 (s, 9H, -CH_2-NH-(C=O)-O-C**(CH_3)$_3$**).

Synthesis of N-[2-[2-(2-aminoethoxy)ethoxy]ethyl]-2,4-dinitro-aniline (DNP-EG_2-amine).

To remove the Boc protecting group, 900 mg of the tert-butyl-N-[2-[2-[2-(2,4-dinitroanilino) ethoxy]ethoxy]ethyl)carbamate (2.17 mmol, 1 Eq, MW = 415.42 mg) was dissolved in 5 mL DCM in a round bottom flask equipped with stirring bar. Under continuous stirring, 5 mL trifluoroacetic acid was added dropwise to the reaction and the resulting reaction mixture was allowed to react for 2 h in an open vessel at room temperature. After 2 h, toluene was added prior to concentration under vacuum to evaporate all volatile side products/solvents. The resulted crude was analyzed by ^{1}H-NMR, APT ^{13}C-NMR and ESI-MS.

^{1}H-NMR (400 MHz, MeOH-d_4, **Figure S9**) δ ppm: 8.87 (d; J = 2.83 Hz; 1H; -C(NO_2)-**CH**=C(NO_2)-C(NH-CH_2-R)=CH-CH=); 8.87 – 8.80 (br m; 1H; -C(NO_2)-CH=C(NO_2)-C(**NH**-CH_2-R)=CH-CH=); 8.28 (ddd; J = 9.61, 2.83, 0.57 Hz; 1H; -C(NO_2)-CH=C(NO_2)-C(NH-CH_2-R)=CH-**CH**=); 7.81 (br s; 3H; -CH_2-CH_2-**NH_3^+**); 7.28 (d; J = 9.61 Hz; 1H; -C(NO_2)-CH=C(NO_2)-C(NH-CH_2-R)=**CH**-CH=); 3.75 – 3.55 (m; 10H; -NH-**CH_2**- **CH_2**-O- **CH_2**- **CH_2**-O-**CH_2**- CH_2-NH_3^+); 2.96 (m; 2H; -O- CH_2- **CH_2**-NH_3^+).

APT ^{13}C-NMR (100 MHz, MeOH-d_4, **Figure S10**) δ ppm: 148.32 (-C(NO_2)-CH=C(NO_2)-**C**(NH-CH_2-R)=CH-CH=); 134.98 (-**C**(NO_2)-CH=C(NO_2)-C(NH-CH_2-R)=CH-CH=); 129.96 (-C(NO_2)-CH=C(NO_2)-C(NH-CH_2-R)=**C**H-CH=); 129.65 (-C(NO_2)-CH=**C**(NO_2)-C(NH-CH_2-R)=CH-CH=); 123.56 (-C(NO_2)-**C**H=C(NO_2)-C(NH-CH_2-R)=CH-CH=); 115.69 (-C(NO_2)-CH=C(NO_2)-C(NH-CH_2-R)=CH-**C**H=); 69.63 (-NH-CH_2-CH_2-O-CH_2-CH_2-O-**C**H_2-CH_2-NH_2); 69.61 (-NH-CH_2-**C**H_2-O-CH_2-CH_2-O-CH_2-CH_2-NH_2); 68.21 (-NH-CH_2-CH_2-O-CH_2-**C**H_2-O-CH_2-CH_2-NH_2); 67.26 (-NH-

CH_2-CH_2-O-**C**H_2-CH_2-O-CH_2-CH_2-NH_2); 42.66 (-NH-**C**H_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-NH_2); 38.70 (-NH-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-**C**H_2-NH_2).

ESI-MS: m/z $[M+H]^+$ = 315.1299 (theoretical), found = 315.1295

Synthesis of N-Boc-N'-biotinyl-3,6-dioxaoctane-1,8-diamine.

In three separate round bottom flasks, biotin (500 mg, 2.05 mmol), HATU (1.1 Eq, 2.251 mmol, 856 mg, MW = 380.23 Da) and tert-butyl-2-(2-(2-aminoethoxy)ethoxy)ethylcarbamate (1.2 Eq, 2.456 mmol, 610 mg, MW = 248.31 Da) were weighed in. The biotin was dissolved by adding 30 mL anhydrous DMF to the biggest round bottom flask. Meanwhile, a dilution of the tert-butyl-2-(2-(2-aminoethoxy)-ethoxy)ethylcarbamate was made by adding 5 mL of DMF to the round bottom flask containing the amine. The solution was transferred to a syringe. The HATU powder was dissolved by the addition of 10 mL anhydrous DMF and adding this solution to the stirring solution of biotin in DMF. After this, 1.5 equivalents of TEA were added to the same vial (430 µL, 3.075 mmol). Upon addition of the TEA, the reaction turned slightly yellow. Afterwards, the amine solution was immediately added and the resulting mixture was mixed thoroughly. Immediately, a strong yellow colour was formed and TLC analysis (10 % MeOH in DCM) showed complete consumption of biotin within 10 minutes. The reaction mixture was extracted three times with a diluted $NaHCO_3$ solution against DCM, followed by extraction against brine. The organic phases were combined and dried over Na_2SO_4 before being reduced under vacuum. The crude product was purified by gradient flash chromatography (5 – 10 % MeOH in DCM) to ultimately obtain the purified product as an off-white solid (yield = 48.1 %). ESI-MS and ^{1}H-NMR analysis were performed on the purified product.

^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 7.00 – 6.39 (br s; 3H; -CH-**NH**-(C=O)-**NH-**CH- **+** CH_2-CH_2-CH_2-(C=O)-**NH**-); 5.38 – 4.85 (br s; 1H; -O-CH_2-CH_2-**NH**-(C=O)-O-); 4.52 (m; 1H; -NH-C**H**-(R)-CH_2-S-); 4.32 (dd; J = 7.81, 3.17 Hz; 1H; -NH-C**H**-(R)-$CH(R_2)$-S-); 3.61 (s; 4H; -NH-CH_2-CH_2-O-C**H₂**-C**H₂**-O-CH_2-CH_2-NH-(C=O)-O-); 3.59 – 3.52 (m; 4H; -NH-CH_2-C**H₂**-O-CH_2-CH_2-O-C**H₂**-CH_2-NH-(C=O)-O-); 3.44 (t; J = 4.88 Hz; 2H; -CH_2-CH_2-CH_2-NH-(C=O)-C**H₂**-CH_2-O-); 3.30 (t; J = 4.76 Hz; 2H; -O-CH_2-C**H₂**-NH-(C=O)-O-); 3.19 – 3.10 (m; 1H; -CH_2-S-C**H**(R_2)-CH(R)-); 2.91 (dd; J = 12.94, 4.88 Hz; 1H; -CH(R)-C**H₂**-S-$CH(R_2)$-); 2.8 – 2.72 (m; 1H; -CH(R)-C**H₂**-S-$CH(R_2)$-); 2.25 (t; J = 7.45 Hz; 2H; -CH_2-CH_2-C**H₂**-NH-(C=O)-CH_2-); 1.82 – 1.57 (m; 4H; -CH_2-S-CH(R)-C**H₂**-CH_2-C**H₂**-CH_2-(C=O)-NH-); 1.5 – 1.38 (m; 11H; -NH-(C=O)-O-C-(C**H₃)₃** + -CH_2-S-CH(R)-CH_2-C**H₂**-CH_2-CH_2-(C=O)-NH-).

ESI-MS: m/z $[M+H]^+$ = 475.2585 (theoretical), found = 475.2593
$[M+Na]^+$ = 497.2404 (theoretical), found = 497.2416
$[M+K]^+$ = 513.2144 (theoretical), found = 513.2152

Synthesis of N,N'-biotinyl-3,6-dioxaoctane-1,8-diamine.

To remove the Boc protecting group, 445 mg of the purified *N*-Boc-*N'*-biotinyl-3,6-dioxaoctane-1,8-diamine was weighed in a dry 50 mL round bottom flask and dissolved by the addition of 10 mL DCM. To this stirring solution, 10 mL of trifluoroacetic acid was added dropwise. The reaction mixture was allowed to stir at room temperature in an open vessel for 2 h, before TLC analysis indicated the complete conversion to the free amine compound. The reaction mixture was diluted and co-evaporated with toluene in order to remove the trifluoroacetic acid. This yielded the crude product as a translucent gum which was used as such without any further purification (yield = quantitative). ESI-MS and ^{1}H-NMR analysis were performed to characterize the purified product.

^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 8.35 – 7.98 (br s; 2H; -CH-**NH**-(C=O)-**NH-**CH-); 6.58 – 6.24 (br s; 1H; -CH_2-CH_2-CH_2-(C=O)-**NH**-CH_2-); 4.47 (dd; J = 7.81, 4.52; 1H; -NH-C**H**-(R)-CH_2-S-); 4.27 (dd; J = 7.81, 4.52 Hz; 1H; -NH-C**H**-(R)-CH(R_2)-S-); 3.70 – 3.50 (m; 10H; -(C=O)-NH-C**H$_2$**-C**H$_2$**-O-C**H$_2$**-C**H$_2$**-O-C**H$_2$**-CH_2-NH_2); 3.38 – 3.30 (m; 2H; -O-CH_2-C**H$_2$**-NH_2); 3.15 – 3.04 (m; 3H; -O-CH_2-CH_2-N**H$_2$** + -CH_2-S-C**H**(R_2)-CH(R)-); 2.86 (dd; J = 13.0, 8.06 Hz; 1H; -CH(R)-C**H$_2$**-S-CH(R_2)-); 2.73 – 2.64 (m; 1H; -CH(R)-C**H$_2$**-S-CH(R_2)-); 2.18 (t; J = 7.36 Hz; 2H; -CH_2-S-CH(R)-CH_2-CH_2-CH_2-C**H$_2$**-(C=O)-NH-); 1.72 – 1.48 (m; 4H; -CH_2-S-CH(R)-C**H$_2$**-CH_2-C**H$_2$**-CH_2-(C=O)-NH-); 1.44 – 1.33 (m; 2H; -CH_2-S-CH(R)-CH_2-C**H$_2$**-CH_2-CH_2-(C=O)-NH-).

ESI-MS: m/z $[M+H]^+$ = 375.2061 (theoretical), found = 375.2098
$[M+Na]^+$ = 397.1880 (theoretical), found = 397.2092

Synthesis of biotinylated chain transfer agent (biotin-PABTC).

1.5 equivalents of PFP-PABTC CTA (synthesized according to Vanparijs N, *Polymer Chemistry* **2015**, 6, 5602) (1.66 mmol, 670 mg) is weighed in a pre-dried 100 mL round bottom flask equipped with stirring bar. In a separate flask, a stock solution of the TFA salt of *N*,*N'*-biotinyl-3,6-dioxaoctane-1,8-diamine (1 Eq, 540 mg as TFA salt, 1.105 mmol) is made in 20 mL anhydrous DCM and 4 mL anhydrous MeOH. To this, 2.6 equivalents of TEA (400 µL) were added. The latter solution was added dropwise to the flask containing PFP-PABTC and the reaction was allowed to react for 2 h under inert atmosphere. TLC analysis (5% MeOH in DCM)

indicated the complete consumption of *N*,*N'*-biotinyl-3,6-dioxaoctane-1,8-diamine. The reaction mixture was reduced under vacuum before being purified immediately by flash chromatography (gradient 5% - 10 % MeOH in DCM). The resulting pure compound was further analyzed by ^{1}H-NMR, APT ^{13}C-NMR, COSY NMR (**Figure S13**) and ESI-MS (Yield = 78 %).

^{1}H-NMR (400 MHz, DMSO-d_6, **Figure S11**), δ (ppm): 8.40 (t; J = 5.49 Hz; 1H; -O-CH_2-CH_2-**NH**-(C=O)-CH(CH_3)-); 7.81 (t; J = 5.62 Hz; 1H; -CH_2-CH_2-CH_2-(C=O)-**NH**-); 6.41 (s; 1H; -**NH**-(C=O)-NH-CH(R)-CH_2-S-); 6.35 (s; 1H; -NH-(C=O)-**NH**-CH(R)-CH_2-S-); 4.72 (q; J = 7.00 Hz; -NH-(C=O)-C**H**(CH_3)-); 4.33 – 4.27 (m; 1H; -NH-C**H**(R)-CH_2-S-); 4.15 – 4.10 (m; 1H; -NH-C**H**(R)-CH(R_2)-S-); 3.50 (s; 4H; -(C=O)-NH-CH_2-CH_2-O-C**H_2**-C**H_2**-O-CH_2-CH_2-NH-(C=O)-); 3.44 – 3.34 (m; 6H; -(C=O)-NH-CH_2-C**H_2**-O-CH_2-CH_2-O-C**H_2**-CH_2-NH-(C=O)- + -CH(CH_3)-S-(C=S)-S-C**H_2**-CH_2-CH_2-CH_3); 3.25 - 3.15 (m; 4H; -(C=O)-NH-C**H_2**-CH_2-O-CH_2-CH_2-O-CH_2-**CH_2**-NH-(C=O); 3.12 – 3.05 (m; 1H; -CH_2-S-C**H**(R_2)-CH(R)-); 2.82 (dd; J = 12.45, 5.13 Hz; 1H; -NH-(C=O)-NH-CH(R)-CH(**H**)-S-); 2.57 (d; J = 12.45 Hz; 1H; -CH(R)-C**H_2**-S-CH(R_2)-); 2.06 (t; J = 7.32 Hz; 2H; -CH_2-CH_2-C**H_2**-(C=O)-NH-CH_2-); 1.67 – 1.43 (m; 9H; -CH_2-S-CH(R)-C**H_2**-CH_2-C**H_2**-CH_2-(C=O)-NH- + -CH(C**H_3**)-S-(C=S)-S-CH_2-C**H_2**-CH_2-CH_3); 1.40 – 1.23 (m; 4H; -CH_2-S-CH(R)-CH_2-C**H_2**-CH_2-CH_2-(C=O)-NH- + -S-(C=S)-S-CH_2-CH_2-C**H_2**-CH_3); 0.89 (t; J = 7.32 Hz; 3H; -S-(C=S)-S-CH_2-CH_2-CH_2-C**H_3**).

APT ^{13}C-NMR (100 MHz, DMSO-d_6, **Figure S12**), δ (ppm): 172.11 (-CH_2-CH_2-CH_2-(**C**=O)-NH-); 169.37 (-NH-(**C**=O)-NH-); 162.67 (-O-CH_2-CH_2-NH-(**C**=O)-CH(CH_3)-); 69.56 (-(C=O)-NH-CH_2-CH_2-O-**C**H_2-CH_2-O-CH_2-CH_2-NH-(C=O)-); 69.51 (-(C=O)-NH-CH_2-CH_2-O-CH_2-**C**H_2-O-CH_2-CH_2-NH-(C=O)-); 69.15 (-(C=O)-NH-CH_2-**C**H_2-O-CH_2-CH_2-O-CH_2-CH_2-NH-(C=O)-); 68.73 (-(C=O)-NH-CH_2-CH_2-O-CH_2-CH_2-O-**C**H_2-CH_2-NH-(C=O)-); 61.02 (-NH-**C**H(R)-CH(R_2)-S-); 59.17 (NH-**C**H(R)-CH_2-S-); 55.41 (-CH_2-S-**C**H(R_2)-CH(R)-); 49.84 (-NH-(C=O)-**C**H(CH_3)-); 39.84 (-CH(R)-**C**H_2-S-CH(R_2)); 38.96 (-(C=O)-NH-**C**H_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-NH-(C=O)-); 38.42 (-(C=O)-NH-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-**C**H_2-NH-(C=O)-); 36.06 (-S-(C=S)-S-**C**H_2-CH_2-CH_2-CH_3); 35.08 (CH_2-S-CH(R)-CH_2-CH_2-CH_2-**C**H_2-(C=O)-NH-); 29.58 (-S-(C=S)-S-CH_2-**C**H_2-CH_2-CH_3); 28.18 (CH_2-S-CH(R)-CH_2-CH_2-**C**H_2-CH_2-(C=O)-NH-); 28.03 (-CH_2-S-CH(R)-CH_2-**C**H_2-CH_2-CH_2-(C=O)-NH-); 25.24 (CH_2-S-CH(R)-**C**H_2-CH_2-CH_2-CH_2-(C=O)-NH-); 21.40 (-S-(C=S)-S-CH_2-CH_2-**C**H_2-CH_3); 18.15 (-NH-(C=O)-CH(**C**H_3)-); 13.41 (-S-(C=S)-S-CH_2-CH_2-CH_2-**C**H_3).

ESI-MS: m/z $[M+H]^+$ = 595.2111 (theoretical), found = 595.2114

$[M+Na]^+$ = 617.1930 (theoretical), found = 617.1917

$[M+K]^+$ = 633.1670 (theoretical), found = 633.1671

Synthesis of biotin-poly(pentafluorophenyl acrylate) (biotin-p(PFPA))

The polymerization reaction is exemplified for a polymer with a theoretical DP of 110. 2.47 mL PFPA (3.57 g, 15 mmol), 81.12 mg Biotin CTA (0.136 mmol) and 4.48 mg AIBN (0.027 mmol) were dissolved in 9.70 mL anhydrous 1,4-dioxane in a Schlenk vial to obtain a final monomer concentration of 1.2 M. The solution was degassed by 5 subsequent freeze-pump-thaw cycles and was placed inside a pre-heated oil bath of 80 °C while maintaining the Schlenk vial under vacuum. After 4 hours, the polymerization was quenched by cooling the vial in ice and exposing the reaction to oxygen. Conversion was calculated by ^{19}F-NMR spectra of the reaction mixture in $CDCl_3$. The reaction mixture was purified by triple precipitation into ice cold ethanol and dried in a vacuum oven. The resulting purified polymer was analyzed using THF-SEC to determine M_n, M_w and Đ. The theoretical M_n was calculated based on conversion determined by ^{19}F-NMR. SEC-traces are depicted above in **Figure S14**.

Trithiocarbonate end-group removal of biotin-p(PFPA).

The polymer was treated with an excess of ACVA according to literature (H. Willcock, *Polymer Chemistry* **2010**, 1, 149.). 1.4 g of the pre-dried Biotin-PABTC-p(PFPA) polymer (MW = 23 930 Da, 0.05850 mmol) was added to a Schlenk vial and dissolved in 10 mL anhydrous 1,4-dioxane. 30 equivalents of ACVA (492 mg, 1.76 mmol) were added to the same Schlenk vial and the vial was placed in a pre-heated oil bath of 80 °C without prior degassing cycles. The reaction was left overnight until the yellow solution turned colorless. The reaction was quenched by cooling down in ice and exposure to oxygen. The resulting polymer was purified by threefold precipitation in an ice cold mixture hexane:diethylether (3:1) and dried in a vacuum oven and was used as such for the following post-polymerization modification reactions. The polymer was analyzed using THF-SEC to determine M_n, M_w and Đ. SEC-traces are depicted in **Figure S14.** The removal of the trithiocarbonate end-group was verified by UV-Vis spectrophotometric analysis of 0.042 mM stock solutions of polymers before and after ACVA treatment (**Figure S14**).

DBCO-functionalization of biotin-poly(PFPA).

To pre-dried Schlenk vials equipped with stirring bar, 3 vials in total, 30 mg (1.256 µmol) biotin-p($PFPA_{100}$) was added and dissolved in 3 mL anhydrous tetrahydrofuran. All Schlenk vials were placed in a pre-heated oil bath of 40 °C under inert atmosphere. To the stirring solutions, either 2 or 5 equivalents of the DBCO-EG_4-amine was added as stock solution in DMSO, accompanied by 5 equivalents of TEA according to the DBCO-EG_4-amine added (2 µL for the polymer modified with 2 equivalents of DBCO-EG_4-amine; 5 µL for the polymer modified with 5 equivalents of DBCO-EG_4-amine). After overnight reaction at 40 °C, a 5-fold excess (relative to PFPA repeating units) of 2-ethanolamine and 10 equivalents of TEA were added. 1 mL DMSO was added to aid solubilization of the polymer. The mixture was allowed to react overnight at 40 °C. A control polymer was synthesized by omitting the addition of DBCO-amine. Finally, the reaction mixtures were transferred to dialysis membranes (Cut off: 3.5 kDa) and dialyzed extensively for 3 days against ultra-pure water. Afterwards, the resulting clear solutions were lyophilized to obtain dry polymers.

Synthesis of poly(PFPA).

For a polymerization reaction with a theoretical DP of 100, 3.29 mL PFPA (4.76 g, 20 mmol), 47.68 mg PABTC (0.200 mmol) and 6.57 mg AIBN (0.040 mmol) were dissolved in 6.71 mL anhydrous 1,4-dioxane in a Schlenk vial to obtain a final monomer concentration of 2 M. The solution was degassed by 5 subsequent freeze-pump-thaw cycles and placed inside a pre-heated oil bath of 80 °C while maintaining the Schlenk vial under vacuum. After 2 hours, the polymerization was quenched by cooling the vial in an ice bath and exposing the reaction to oxygen. The reaction mixture was purified by triple precipitation into ice-cold ethanol and dried in a vacuum oven. The resulting pure polymer was analyzed using THF-SEC to determine M_n, M_w and Đ. The theoretical M_n was calculated based on conversion determined by ^{19}F-NMR. SEC-traces are depicted in **Figure S14**.

Trithiocarbonate end-group removal of poly(PFPA)

Trithiocarbonate end-group removal is performed analogous to the reaction described above. The resulting pure polymer was dried for 24 h in a vacuum oven and was used as such for the following post-polymerization modification reactions. The polymer was analyzed by THF-SEC to determine M_n, M_w and Đ. SEC-traces are provided in **Figure S14**. The removal of the

trithiocarbonate end-group was verified by UV-Vis spectrophotometric analysis of 0.042 mM stock solutions of polymers before and after ACVA treatment (**Figure S14**).

DBCO/DNP-functionalization of poly(PFPA).

To a pre-dried 100 mL round bottom flask equipped with stirring bar, 200 mg (9.786 µmol) poly(PFPA) was added and dissolved in 20 mL anhydrous tetrahydrofuran. The flask was placed in a pre-heated oil bath of 40 °C under inert atmosphere. To this solution, 5 equivalents TEA (6.85 µL, 0.04893 mmol) and 1 equivalent (504 µL of a 10 mg/mL stock solution, 9.786 µmol) tetramethylrhodamine-cadaverine were added. The reaction mixture was allowed to react overnight protected from light.

After 24 hours, the reaction mixture was equally distributed over 4 different Schlenk tubes. 1 mL of anhydrous DMSO was added to each Schlenk vial to prevent precipitation. The polymers were either reacted with 5 equivalents of DBCO-EG_4-amine (640 µL of a 10 mg/mL stock solution in DMSO, 0.01223 mmol) yielding P2 (DBCO + DNP -), 5 equivalents of DNP-EG_2-amine (386 µL of a 10 mg/mL stock solution in DMSO, 0.01223 mmol) yielding P3 (DBCO - DNP +), or 5 equivalents of both yielding P4 (DBCO + DNP +), in presence of 5 equivalents of anhydrous triethylamine (TEA) per amine. As a control a polymer was modified with neither DBCO nor DNP to yield P1 (DBCO - DNP -). After 24 h reaction at 40°c in the dark, 5 equivalents of 2-ethanolamine and 10 equivalents of TEA per PFP unit were added to the reaction mixtures together with 1 mL DMSO to aid solubilization. After overnight reaction at 40 °C in the dark, the reaction mixtures were transferred to dialysis membranes (Cut off 3.5 kDa) and dialysed extensively for 3 days against ultra-pure water. Afterwards, the resulting clear solutions were lyophilized to obtain dry polymers. The ^{1}H-NMR spectra of all DBCO/DNP modified polymers are provided in **Figures S15**, **S16**, **S17** and **S18**. The chemical structures are provided in **Figure S19**.

Determination of optimal amount of Ac_4ManN_3 for in vitro metabolic cell labelling.

A 10 mM stock solution of Ac_4ManN_3 was prepared by adding 1.21 mL ethanol to 5.2 mg Ac_4ManN_3 and either 0; 10; 25 or 50 µL of this stock solution was added to four different cell culture flasks (T075, 75 cm^2). These will yield final concentrations of 0, 10, 25 and 50 µM Ac_4ManN_3 once 10 mL of cell culture medium is added. Ethanol was left to evaporate in a sterile laminar flow biohood.

To each cell culture flask, 10 mL Jurkat T cell suspension (200 000 cells/mL) was added and incubated for 48 h (5% CO_2, 37 °C). After 48 h, the cells were centrifuged for 5 minutes (200 G, 4°C), the supernatant was discarded and the cells were washed with 10 mL PBS (+Ca^{2+}, Mg^{2+}). The cells were seeded at 250 000 cells per mL in full cell culture medium (1 mL per Eppendorf). Next, each Eppendorf was pulsed with either 1, 10 or 20 µM dibenzoylcyclooctyne-EG_4-Fluor 545 dye (0.94 µL, 9.36 µL and 18.7 µL of a 1 mg/mL stock solution in DMSO, respectively) in triplicate. The samples were incubated for 1 h at 37 °C. Afterwards, the cells were centrifuged, washed with 1 mL of PBS (+Ca^{2+}, Mg^{2+}) and resuspended in 300 µL PBS (+Ca^{2+}, Mg^{2+}) for analysis by flow cytometry and confocal microscopy.

In vitro binding of DBCO-polymers to metabolically labeled cells.

Jurkat T cells were pre-incubated with 25 µM Ac_4ManNAz as described above. After 48 h, cells were pelleted and washed with 10 mL of PBS (+ Ca^{2+} and Mg^{2+}) before being added to Eppendorfs at a concentration of 200 000 cells/mL in full culture medium (1 mL per Eppendorf). In parallel, an equal amount of control Jurkat T cells that were not cultivated in the presence of Ac_4ManN_3 were pelleted, washed with 10 mL of PBS (+ Ca^{2+} and Mg^{2+}) and seeded in Eppendorfs at a concentration of 200 000 cells/mL in full culture medium (1 mL per Eppendorf). Next, 20 µM of biotin-functionalized polymers equipped with various amounts of DBCO units were added to the Eppendorfs and incubated at 37 °C for 2 h. Afterwards, cells were centrifugated, washed with 1 mL of PBS (+ Ca^{2+} and Mg^{2+}), followed by counterstaining with AF488-streptavidin (2 µL Streptavidin-AF488 per 100 µL PBS with 3% BSA) for 30 minutes on ice in the dark. Next, cells were centrifugated, washed with 1 mL of PBS (+ Ca^{2+} and Mg^{2+}), resuspended in 300 µL PBS (+ Ca^{2+} and Mg^{2+}) and analyzed by flow cytometry and confocal microscopy.

In vitro uptake experiment of DBCO/DNP modified polymers by* Ac_4ManN_3 *modified Jurkat T cells.

To investigate the *in vitro* uptake of DBCO/DNP modified polymers, Jurkat T cells were pre-incubated with 25 µM Ac_4ManN_3 as described earlier. 62.5 µL of a 10 mg/mL stock solution in ethanol was added to a cell culture flask (T175, 175 cm^2). The ethanol was left to evaporate in a sterile laminar flow biohood over weekend. To this flask, a 25 mL Jurkat T cell suspension

(200 000 cells/mL) was added, which gives rise to a final concentration of 25 μM Ac_4ManN_3 in the cell culture medium. The cells were incubated for 48 hours prior to pulsing.

After 48 h of incubation, the cells were pelleted and washed with 10 mL of PBS (+ Ca^{2+} and Mg^{2+}) before being seeded in Eppendorfs at a concentration of 250 000 cells/mL in full culture medium (1 mL per Eppendorf). In parallel, an equal amount of Jurkat T cells that were cultivated in the absence of Ac_4ManN_3 were pelleted, washed with 10 mL of PBS (+ Ca^{2+} and Mg^{2+}) and seeded in Eppendorfs at a concentration of 250 000 cells/mL (1 mL per Eppendorf). The polymers were added in triplicate to Eppendorfs of each cell line (Jurkat T cells with and without Ac_4ManN_3) containing 250 000 cells to yield final concentrations of 5, 10 and 20 μM. Stock solutions of the polymers were made in PBS (+ Ca^{2+} and Mg^{2+}) and adjusted to identical fluorescence prior to pulsing using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies) equipped with a Varian Cary temperature controller in 700 μL Micro Fluorescence cuvettes (Thorlabs). The presence of DNP had a minimal influence on the fluorescence (data not shown). All samples were incubated for 2 hours at 37 °C. Afterwards, the cells were pelleted, washed with 1 mL of PBS (+ Ca^{2+} and Mg^{2+}) and resuspended in 300 μL PBS (+ Ca^{2+} and Mg^{2+}) for analysis by flow cytometry and confocal microscopy. Finally, small aliquots of the samples were taken and visualized using a Leica DMI6000 B inverted microscope equipped with an oil immersion objective (Leica, 63 x, NA 1.40) and attached to an Andor DSD2 confocal scanner. All images were recorded with identical sensitivity settings and processed using ImageJ software.

In vitro analysis of antibody recruitment

Jurkat T cells were pre-incubated with 25 μM Ac_4ManN_3 as described earlier and non-treated cells were used as control. After 48 h of incubation, the cells were pelleted and washed with 10 mL of PBS (+ Ca^{2+} and Mg^{2+}) before being distributed in Eppendorfs at a concentration of 250 000 cells/mL in full culture medium (1 mL per Eppendorf).

Based on the result of the preliminary uptake experiment described above, the different polymer samples were added to the Eppendorfs at a final concentration of 20 μM. Stock solutions of the polymers were made in PBS (+ Ca^{2+} and Mg^{2+}). All samples were incubated for 2 hours at 37 °C. Afterwards, the cells were centrifuged, washed with 1 mL of PBS (+ Ca^{2+} and Mg^{2+}) and resuspended in 100 μL anti-DNP antibody staining solution and incubated on ice for 30 minutes in the absence of light. The staining solution contained 30 μL AF488-anti-DNP (2 mg/mL stock), 30 μL human Fc Block and 2940 μL 3% BSA in PBS (+ Ca^{2+} and Mg^{2+}).

After 30 minutes, the cells were centrifuged, washed with 300 µL PBS and resuspended in 300 µL PBS for analysis by flow cytometry and confocal microscopy.

4T1 tumor spheroids

The mouse breast cancer cell line 4T1 (ATCC) was maintained in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 mg/mL streptomycin, and 1 mg/mL G418 and incubated at 37 °C, 10% CO_2. Cells were grown in adherent conditions to 70% confluency, trypsinized, counted (Countess automatic cell counter, Invitrogen), and adjusted to 10^5 cells/mL. Cell suspensions of 5 mL were incubated at 37 °C, 10% CO_2 under continuous gyratory shaking (50 rpm) to allow spheroid formation. After 72 h, spheroids were treated with Ac_4ManN_3 and polymer in identical way as 2D cell cultures. Confocal microscopy image were recorded on a Zeiss LSM710 confocal microscope equipped with a 20x objective. Image analysis was performed with the ImageJ software package.

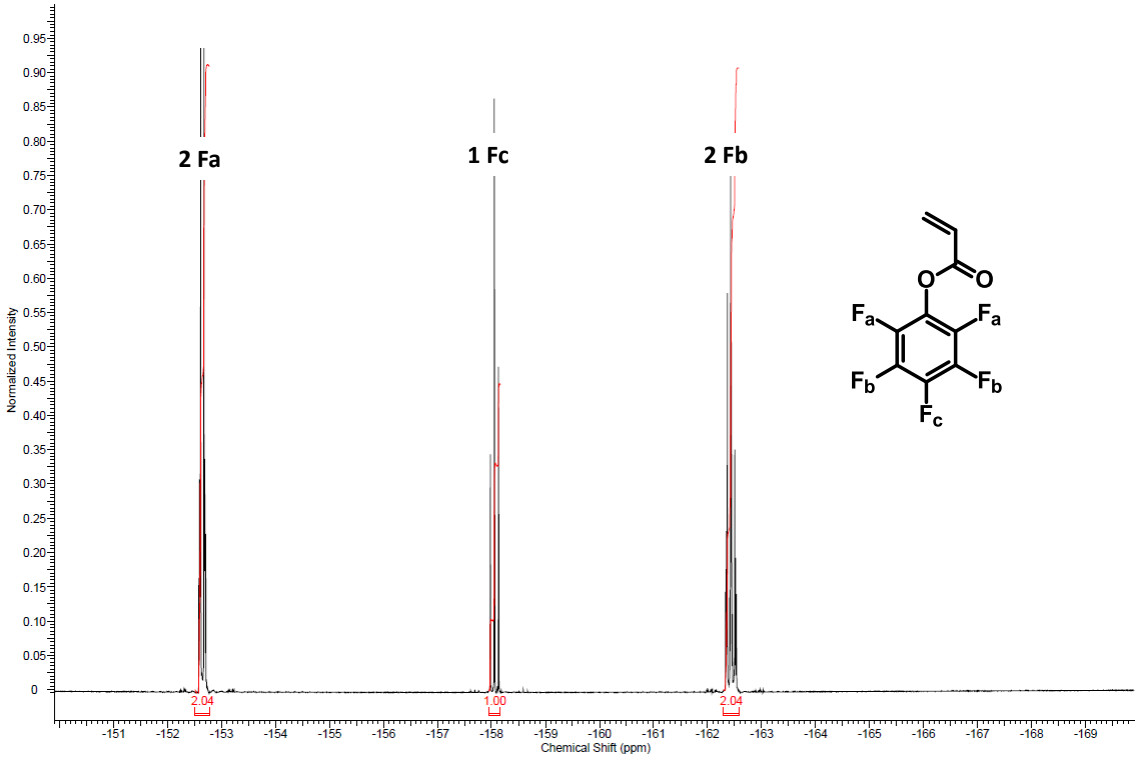

Figure S1| ^{19}F-NMR spectrum of pentafluorophenyl acrylate.

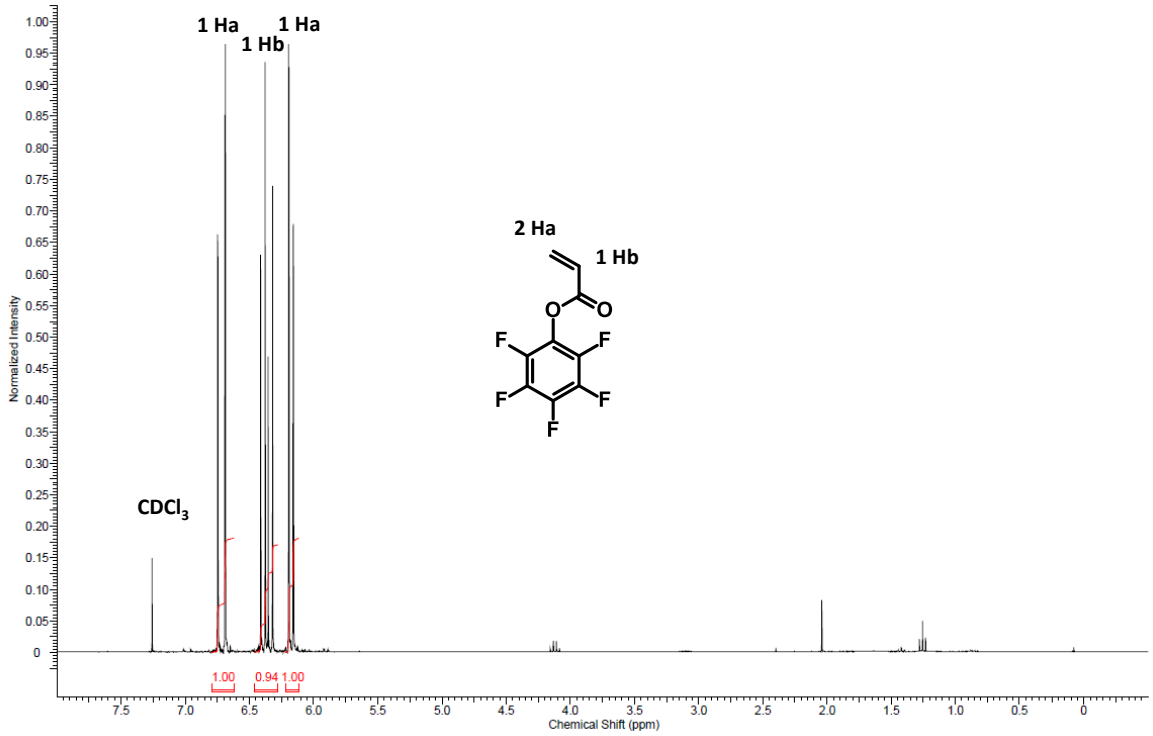

Figure S2| ^{1}H-NMR spectrum of pentafluorophenyl acrylate.

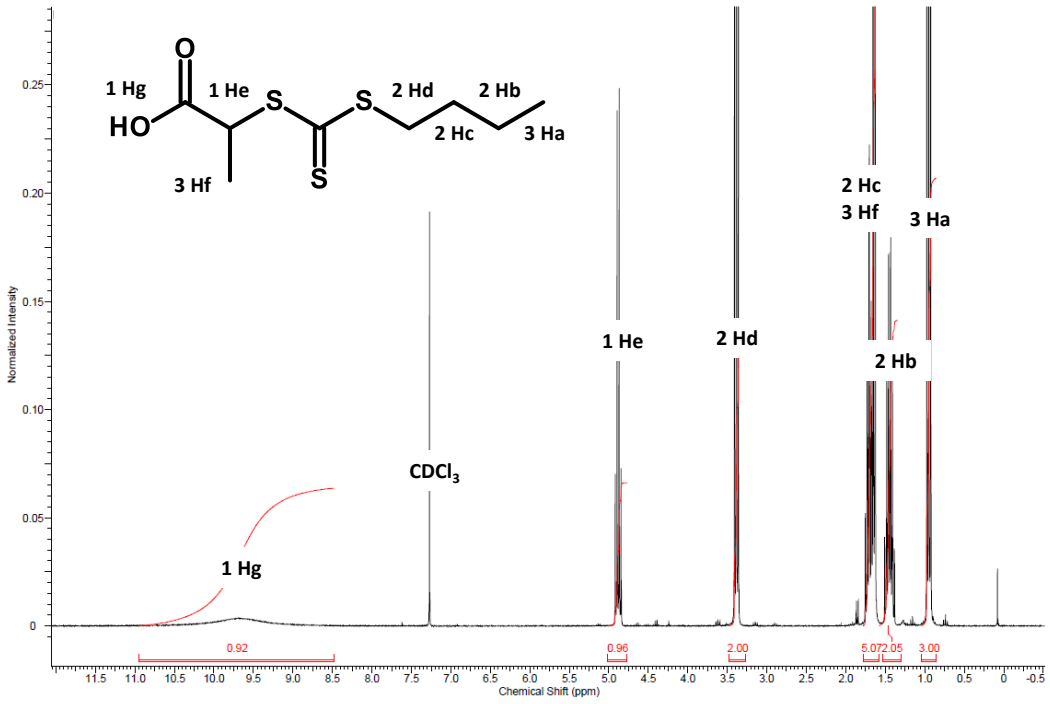

Figure S3| ^{1}H-NMR spectrum of 2-(butylthiocarbonothioylthio)propanoic acid.

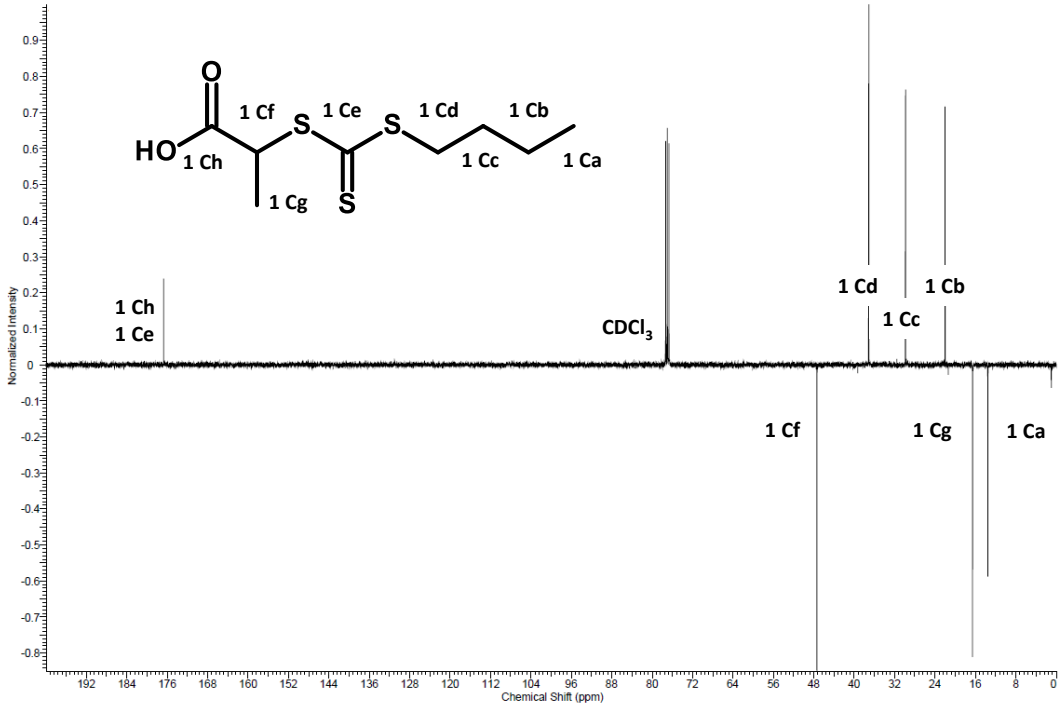

Figure S4| APT ^{13}C-NMR spectrum spectrum of 2-(butylthiocarbonothioylthio)propanoic acid.

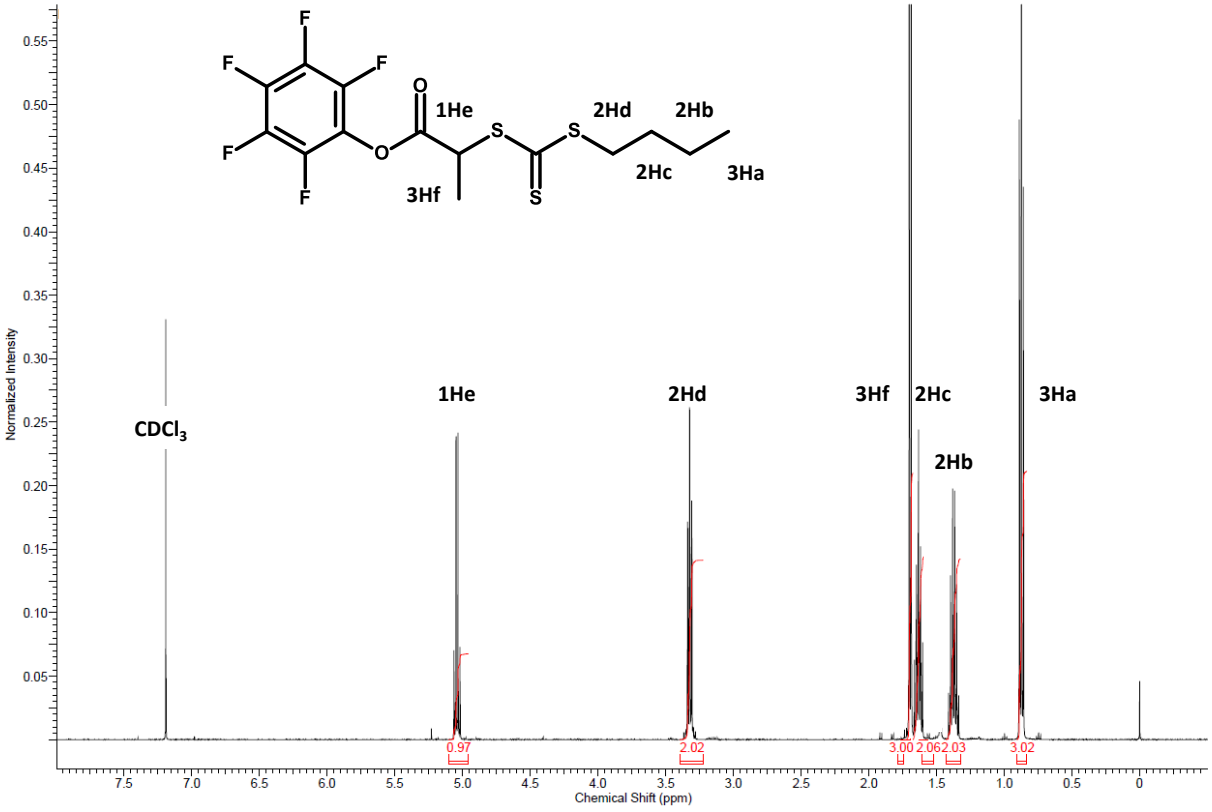

Figure S5| ^{1}H-NMR spectrum of (2,3,4,5,6-pentafluorophenyl)-2-(butylthiocarbonothioylthio) propanoate (PFP-PABTC).

Figure S6| ^{19}F-NMR spectrum of (2,3,4,5,6-pentafluorophenyl)-2-(butylthiocarbonothioylthio) propanoate (PFP-PABTC).

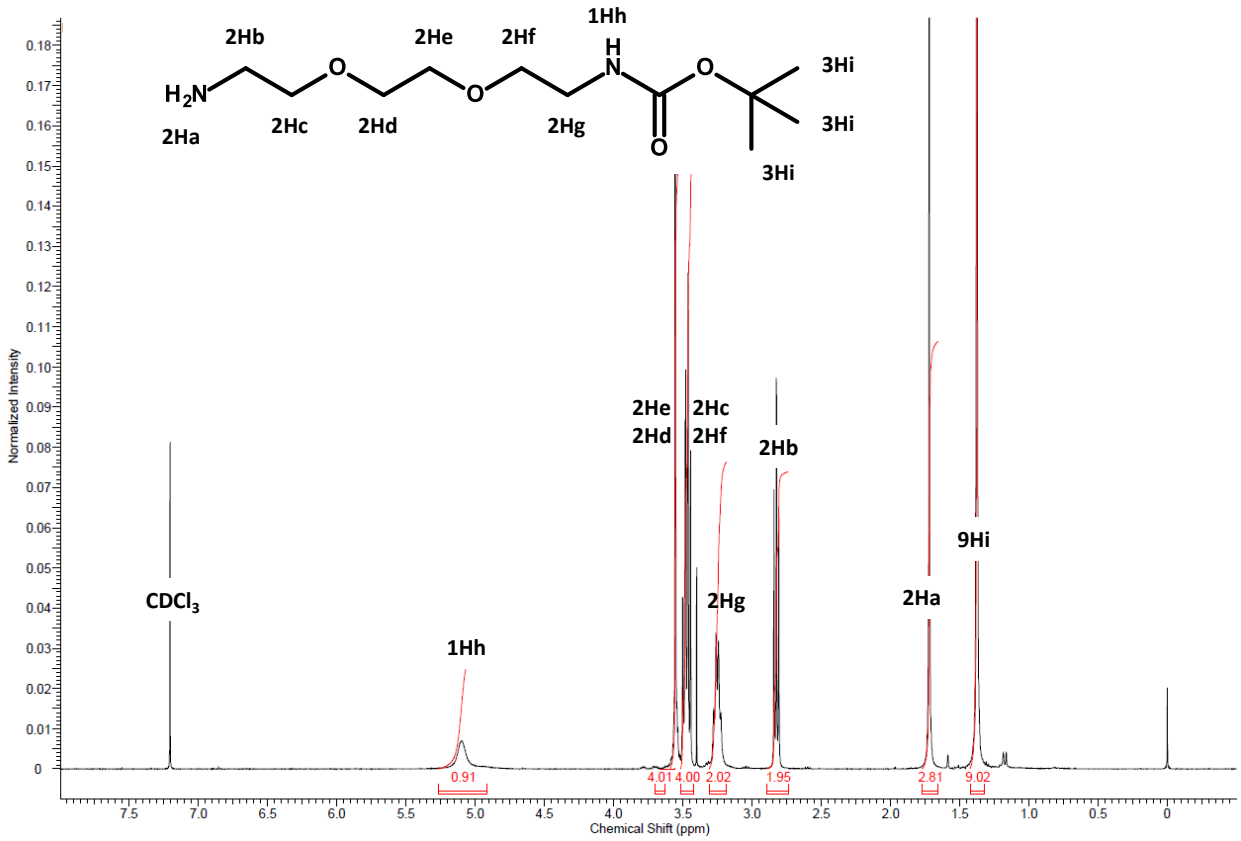

Figure S7| ^{1}H-NMR spectrum of tert-butyl-*N*-[2-[2-(2-aminoethoxy)ethoxy]ethyl]carbamate.

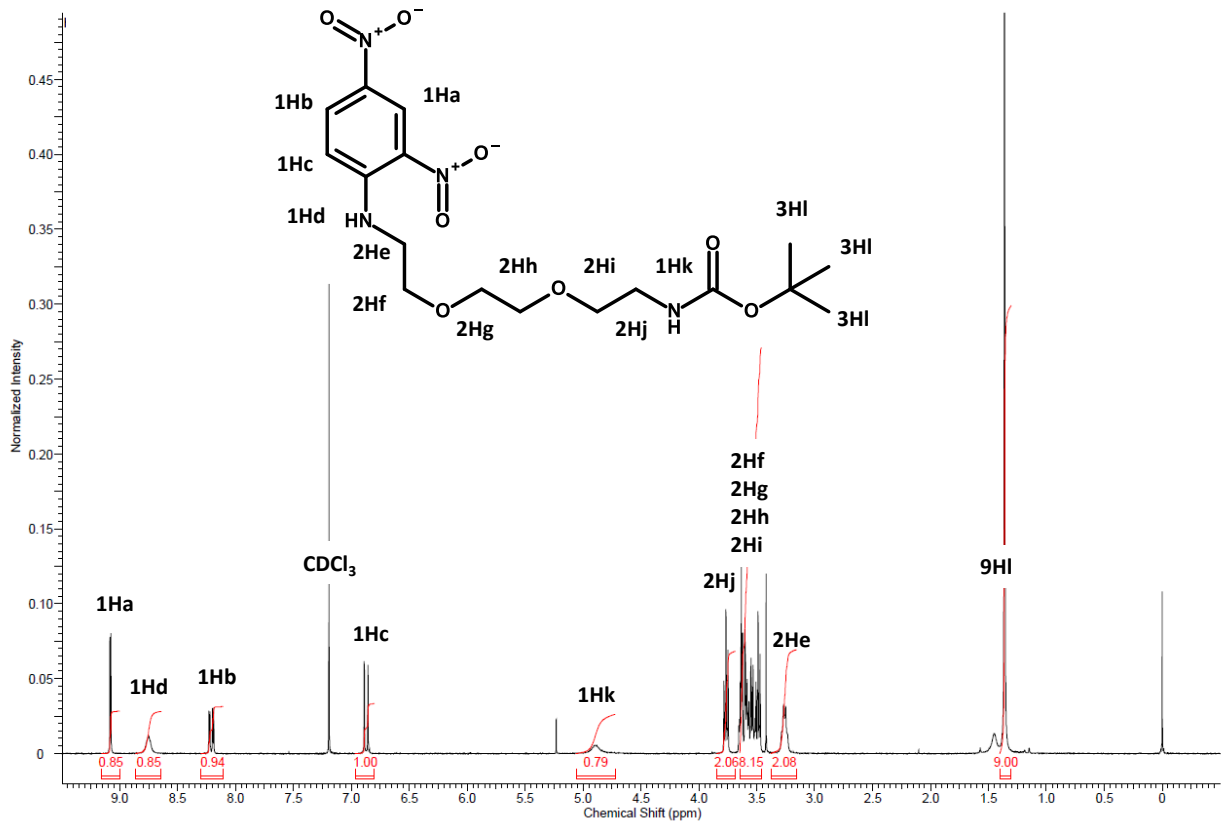

Figure S8| ^{1}H-NMR spectrum of tert-butyl-*N*-[2-[2-[2-(2,4-dinitroanilino)ethoxy]ethoxy]ethyl) carbamate.

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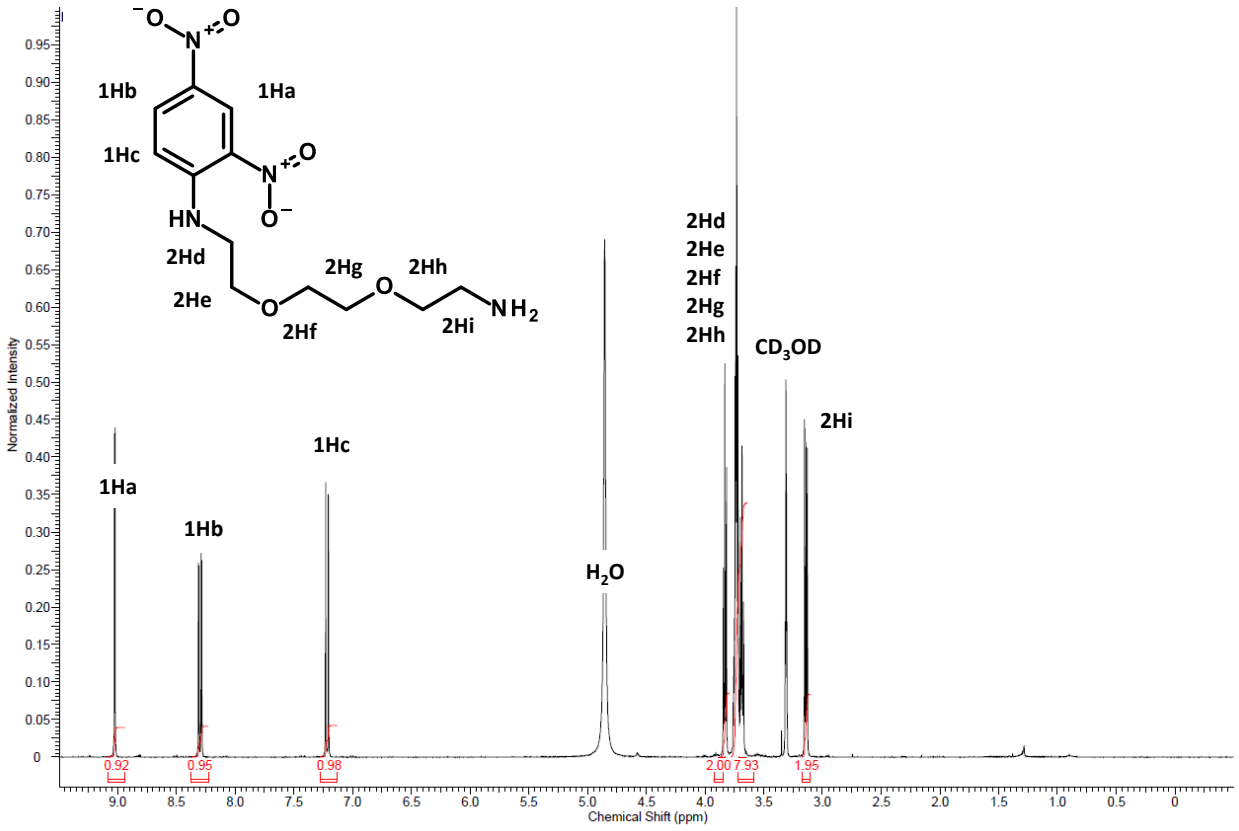

Figure S9| ^{1}H-NMR spectrum of N-[2-[2-(2-aminoethoxy)ethoxy]ethyl]-2,4-dinitro-aniline.

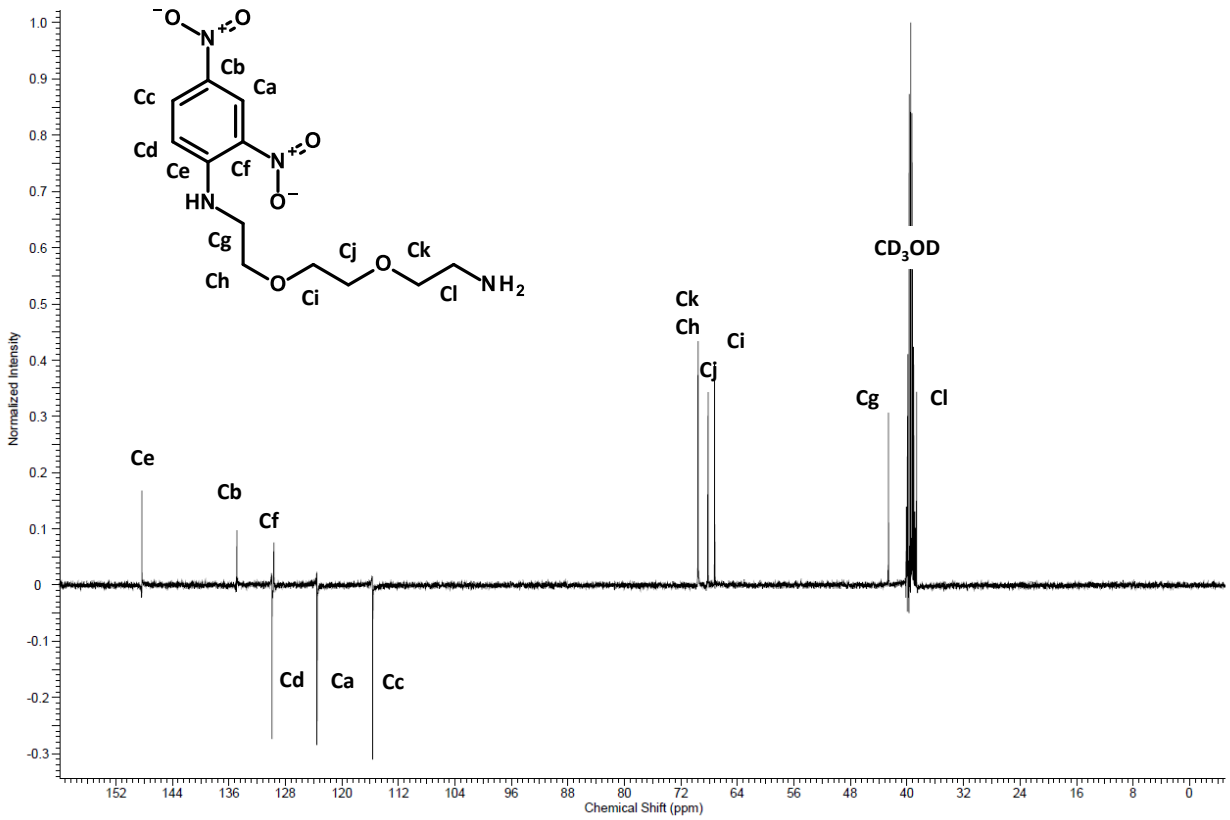

Figure S10| APT ^{13}C-NMR spectrum of N-[2-[2-(2-aminoethoxy)ethoxy]ethyl]-2,4-dinitro-aniline.

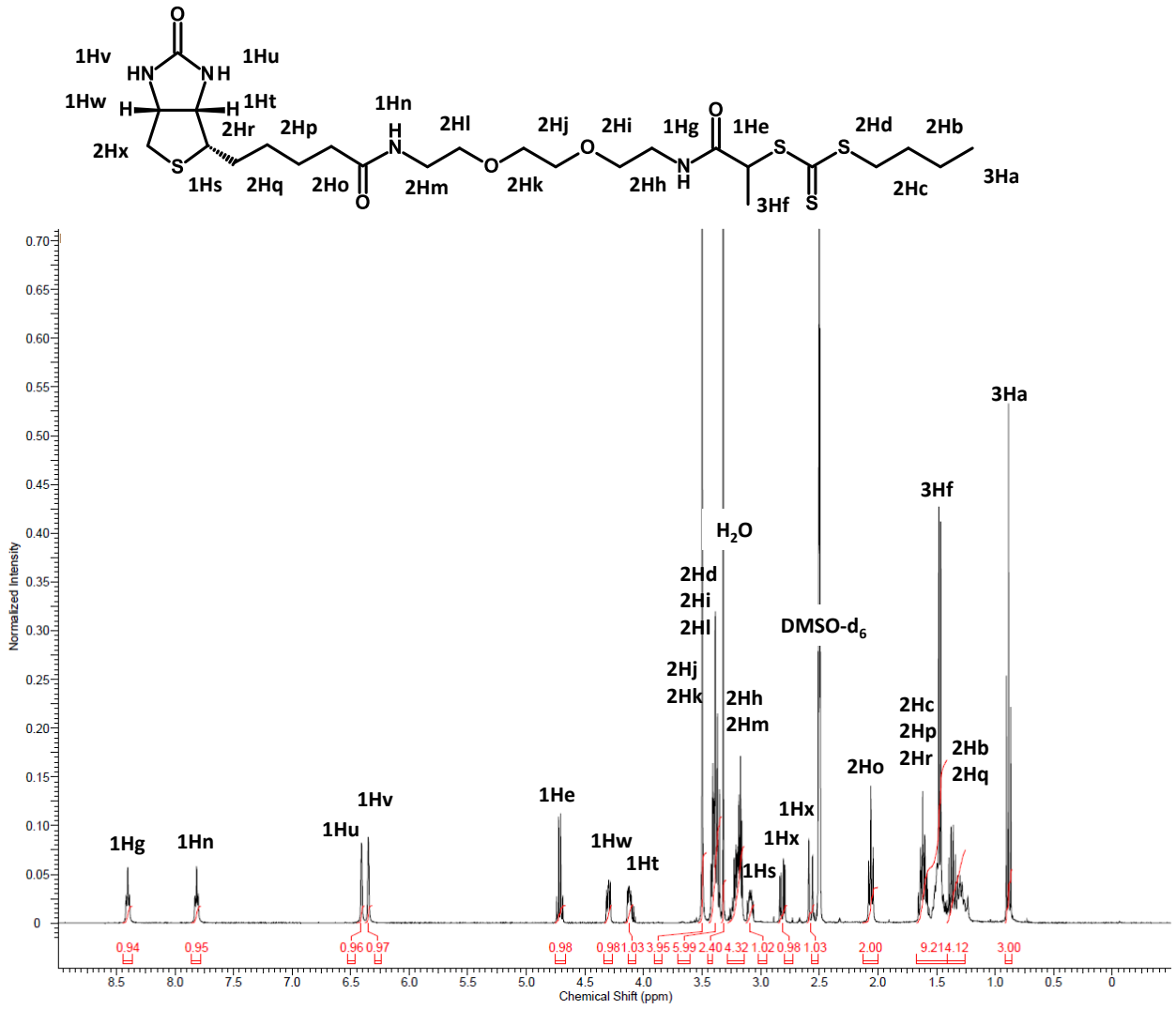

Figure S11| ^{1}H-NMR spectrum of Biotin-CTA.

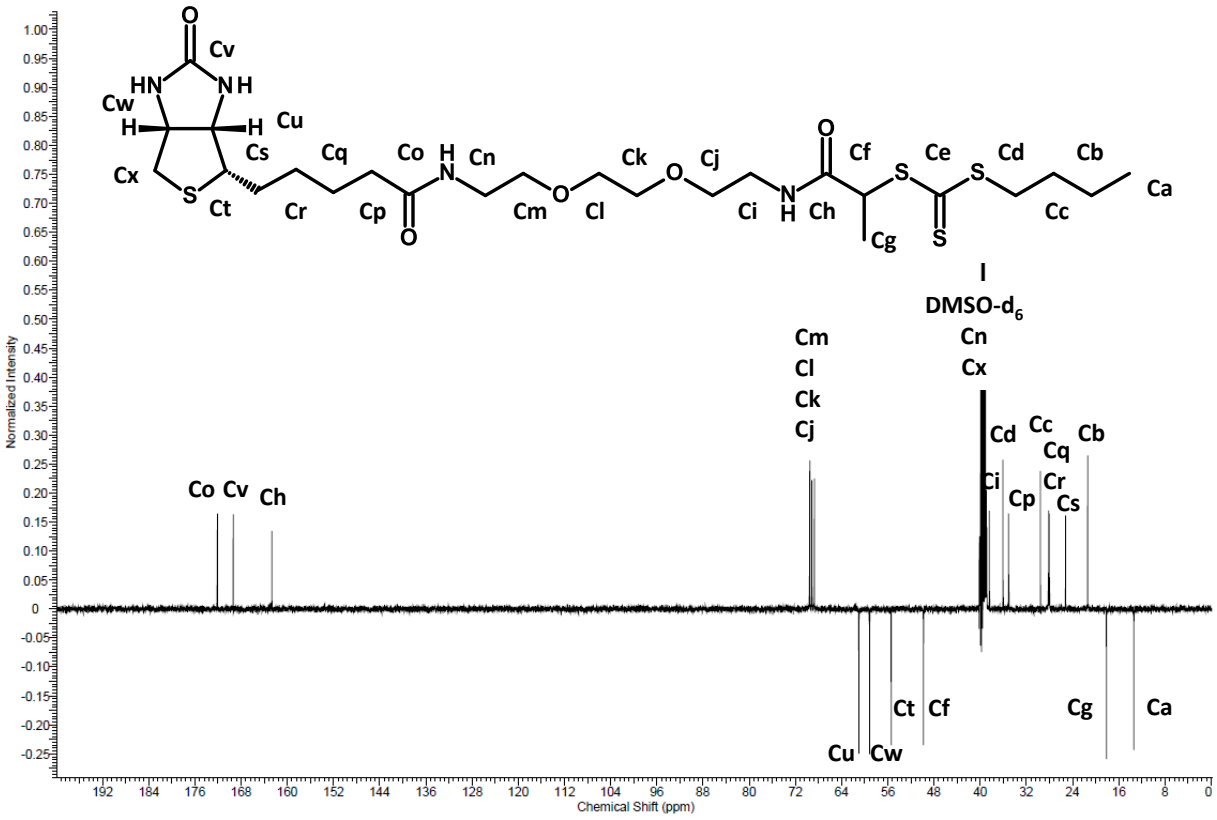

Figure S12| APT ^{13}C-NMR spectrum of Biotin-CTA.

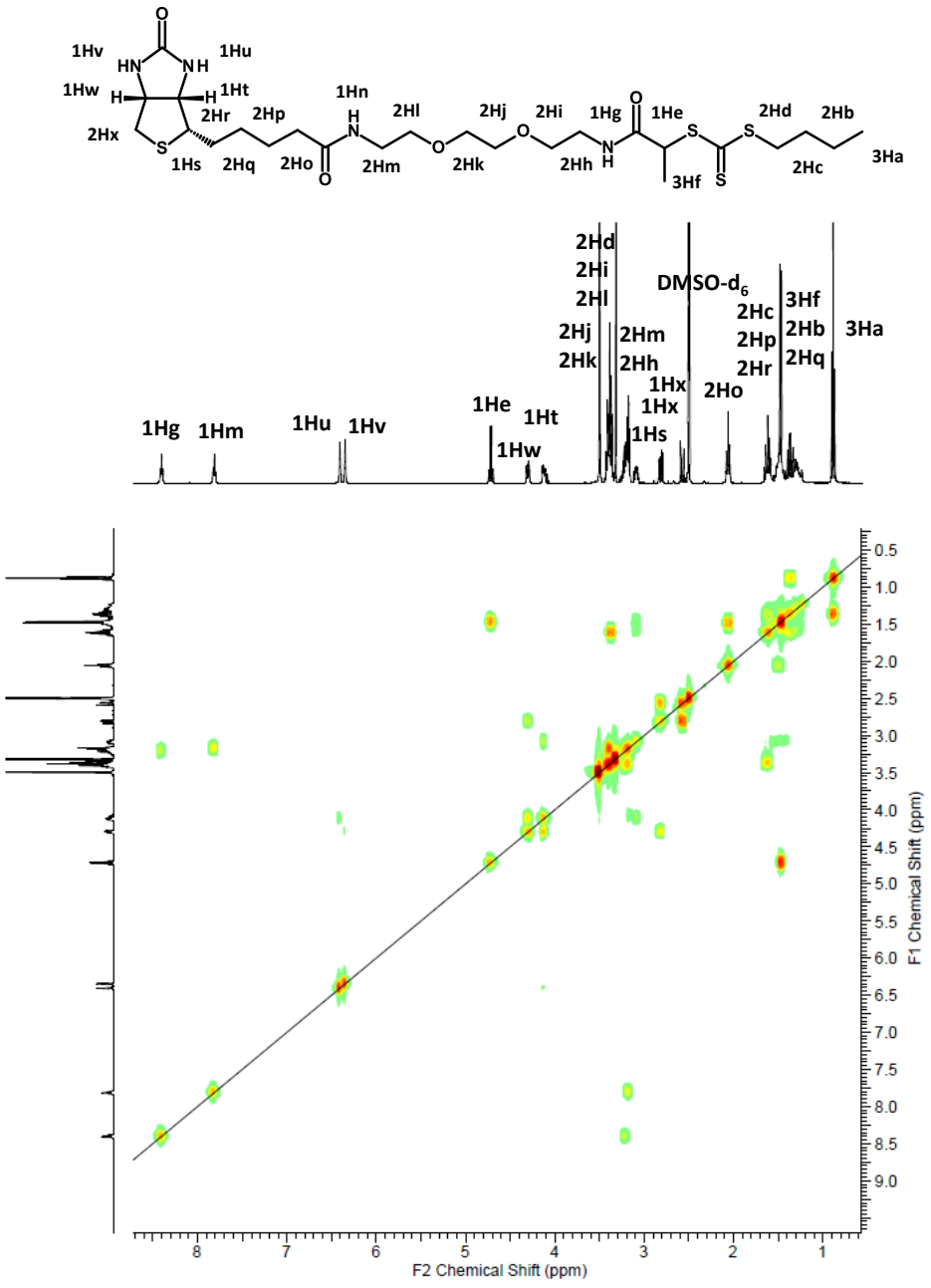

Figure S13| COSY spectrum of Biotin-CTA.

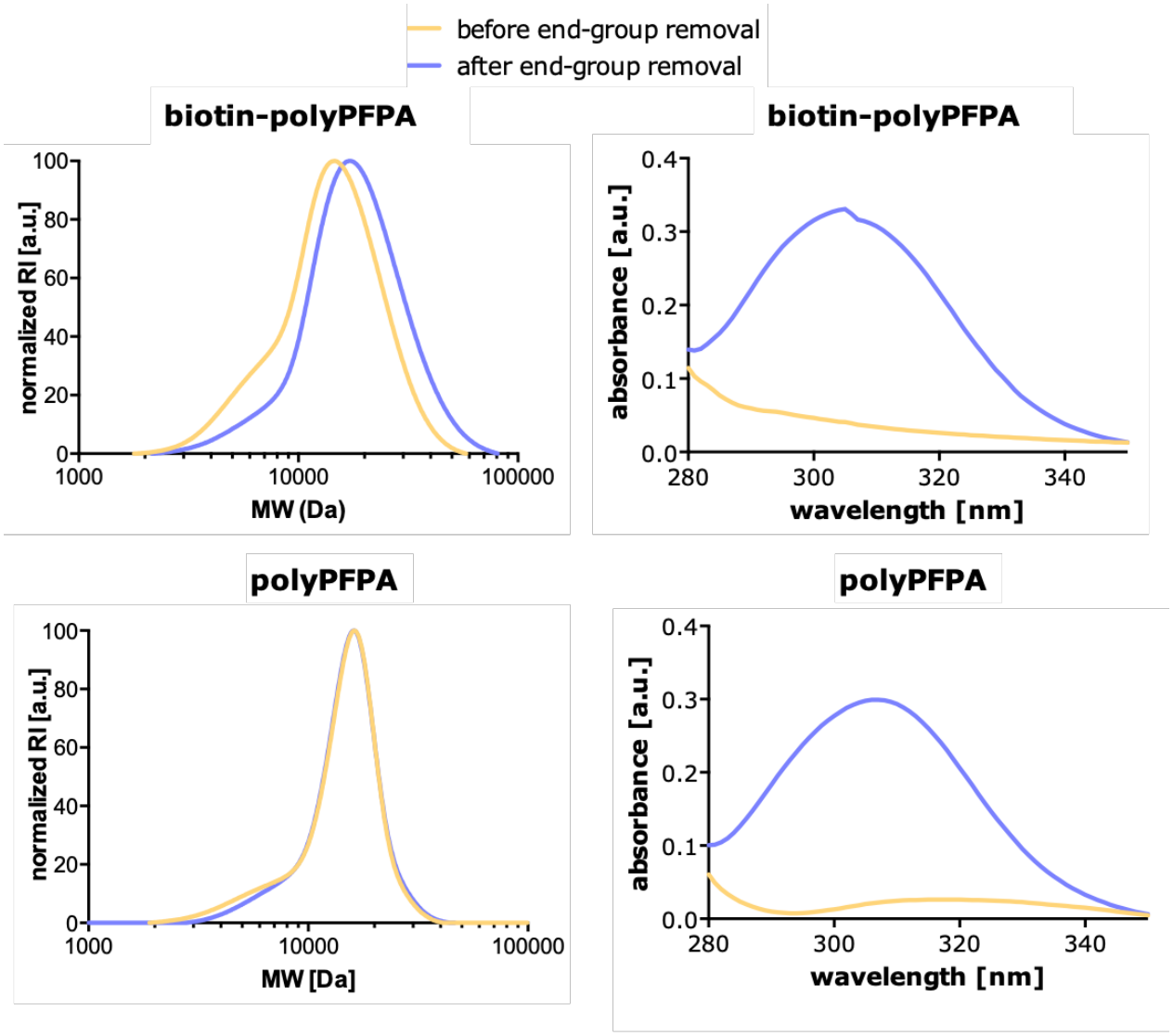

Figure S14| SEC traces and UV-vis spectra of polymers before and after trithiocarbonate end-group removal.

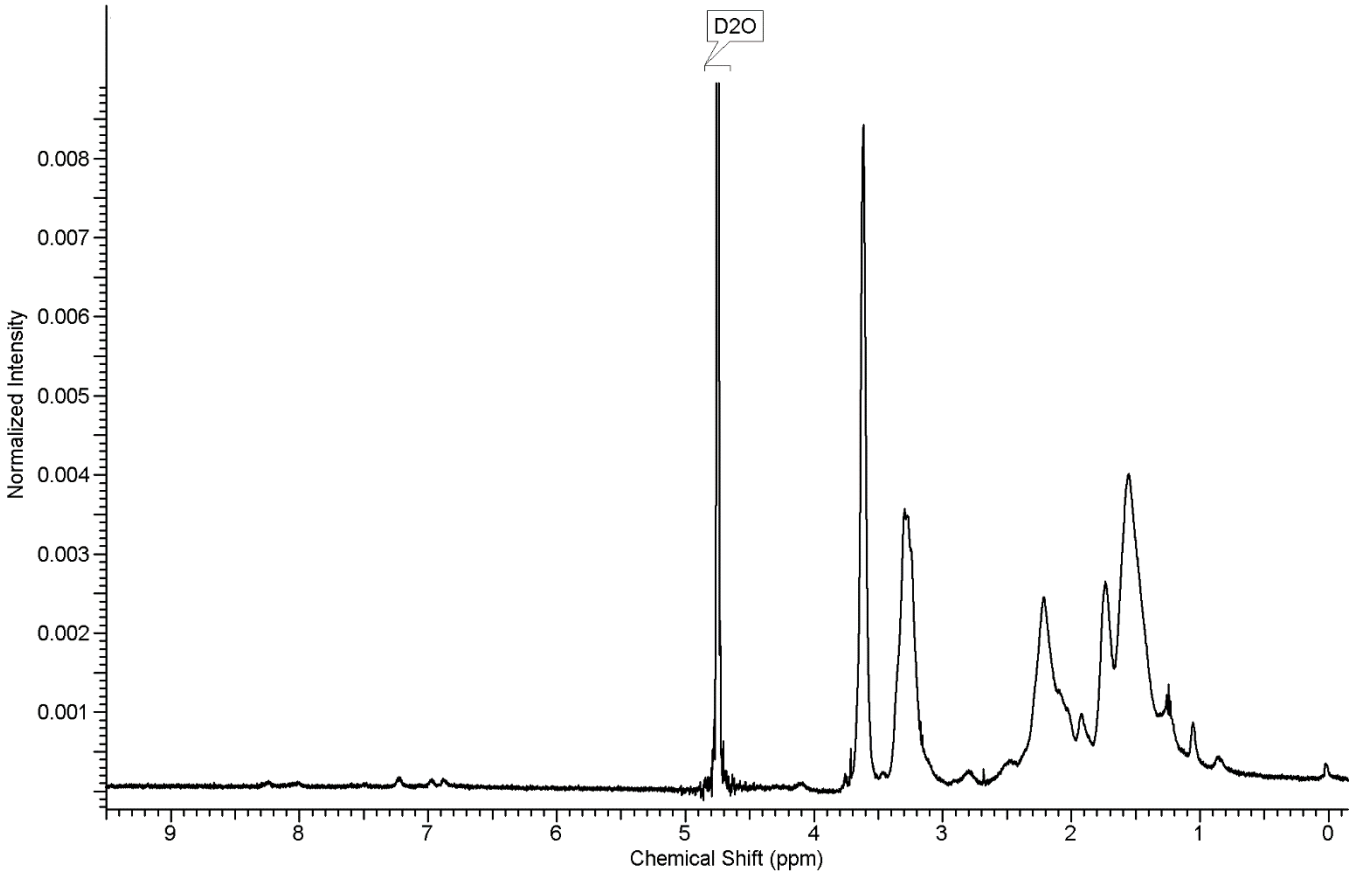

Figure S15| ^{1}H-NMR spectrum of control polymer.

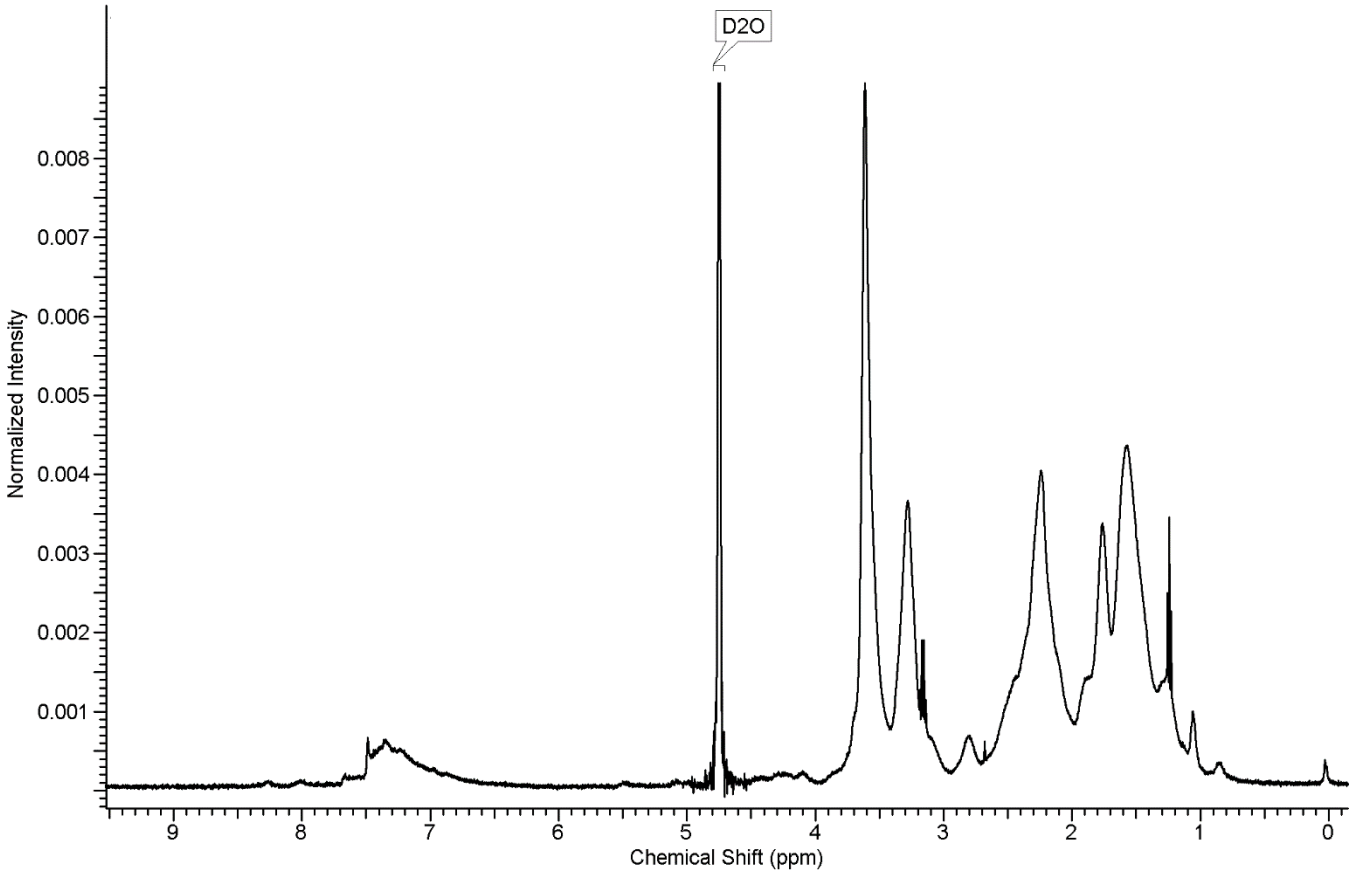

Figure S16| ^{1}H-NMR spectrum of DBCO polymer.

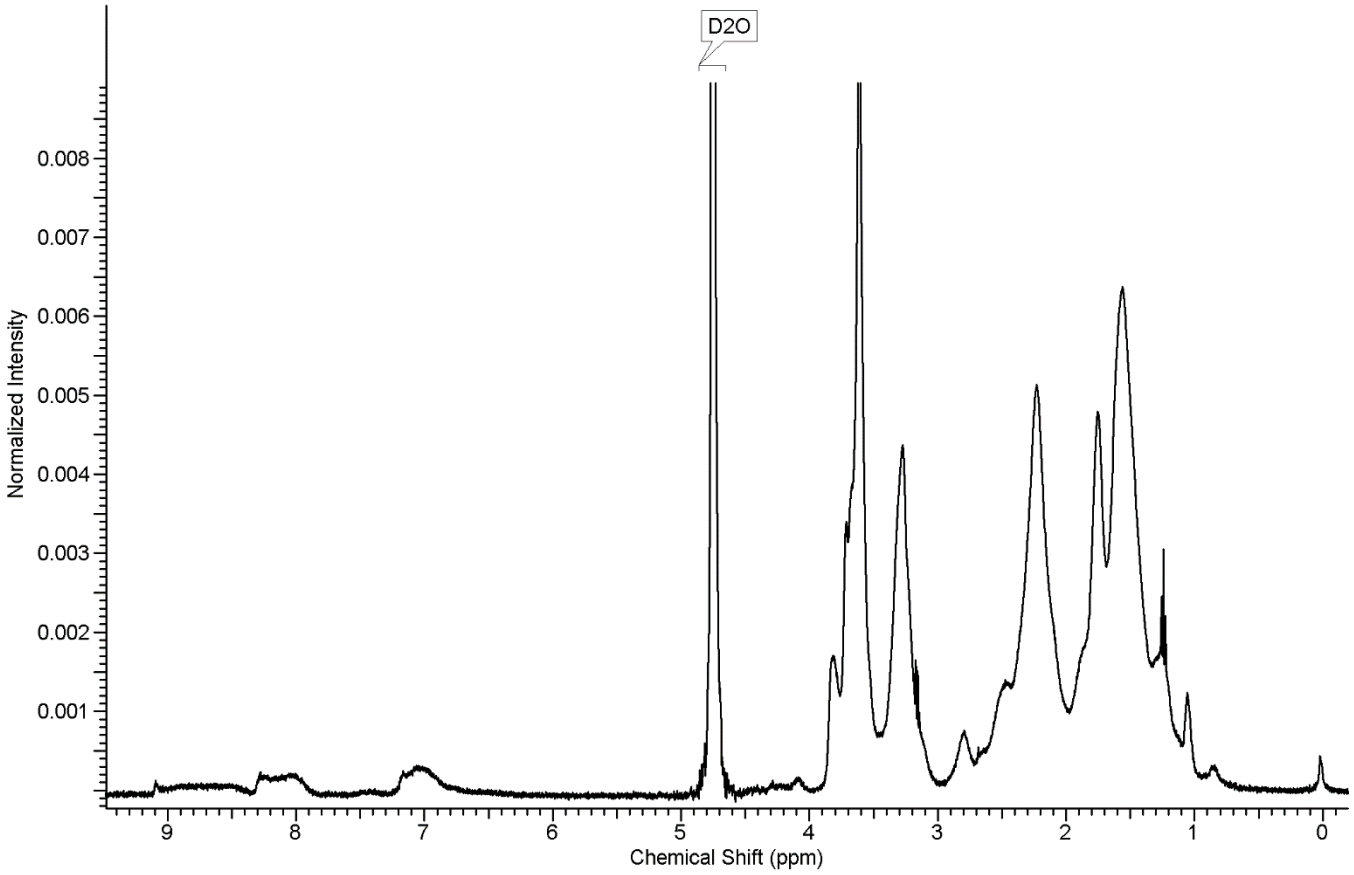

Figure S17| ^{1}H-NMR spectrum of DNP polymer.

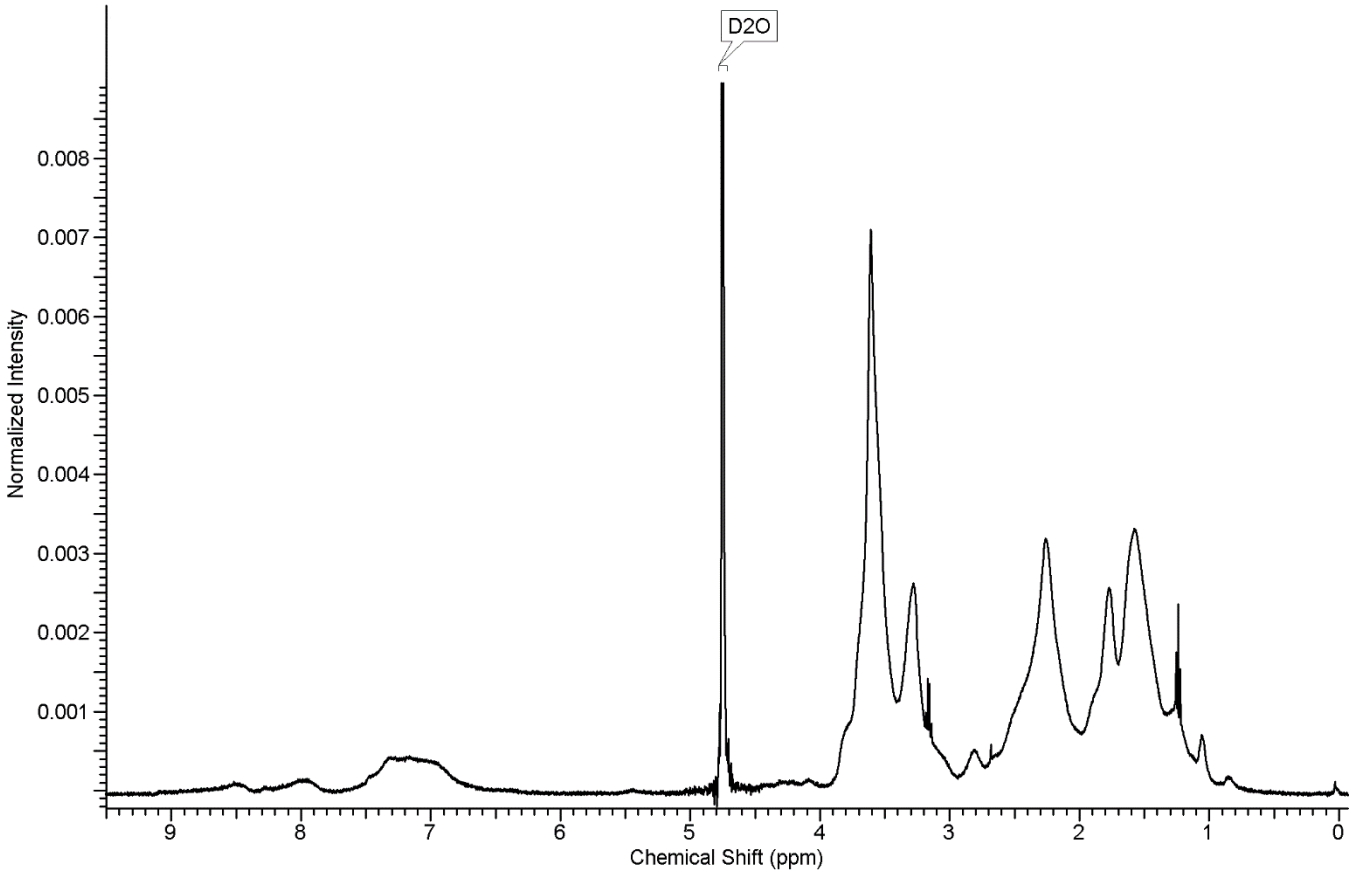

Figure S18| ^{1}H-NMR spectrum of DBCO/DNP polymer.

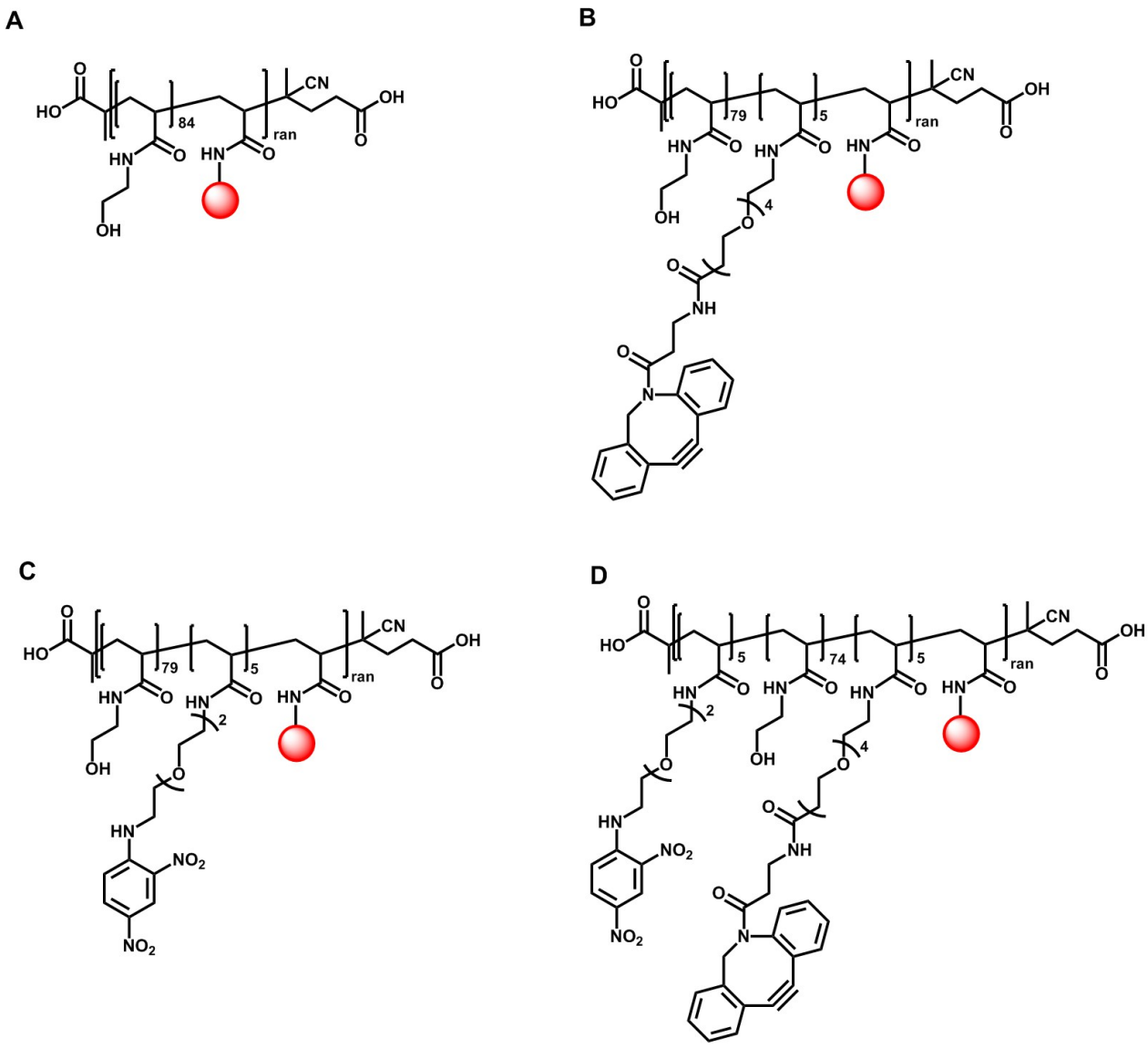

Figure S19| Chemical structures of synthesized DNP/DBCO polymers: control polymer (**A**), DBCO-polymer (**B**), DNP-polymer (**C**) and DBCO/DNP-polymer (**D**).

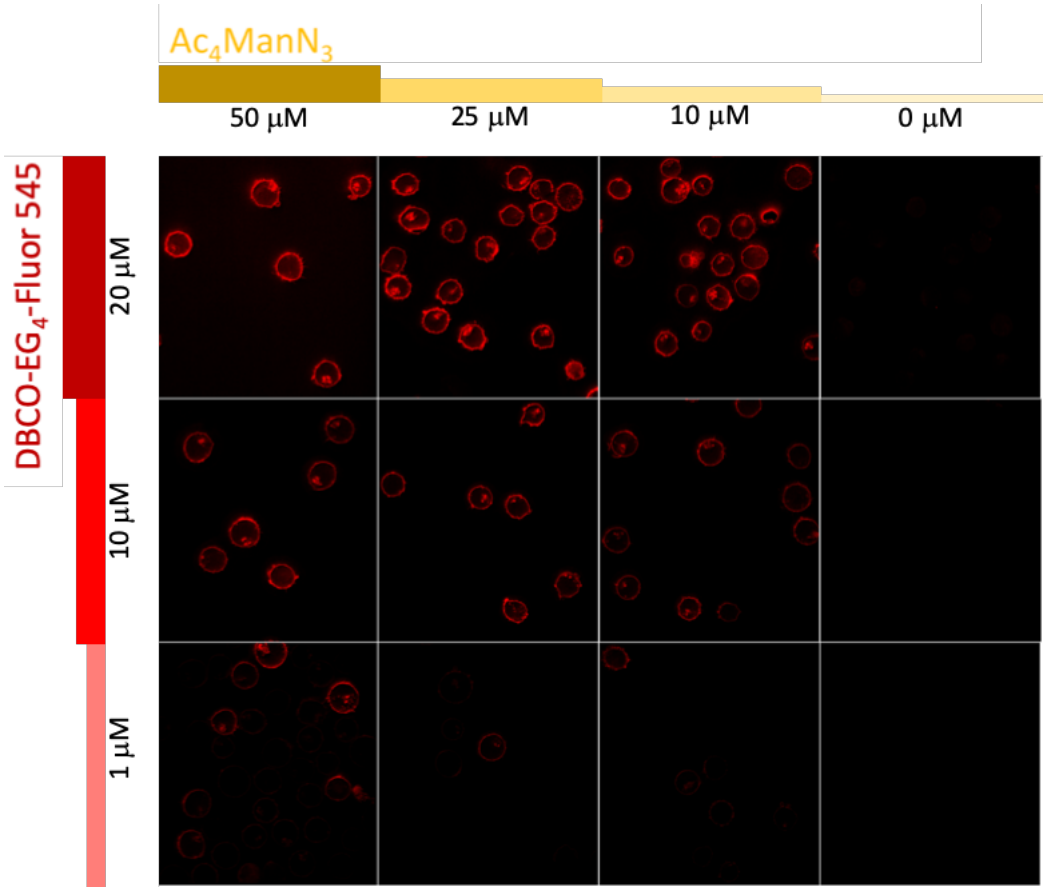

Figure S20| Confocal microscopy showing dose-dependent metabolic labeling and SPAAC conjugation of Jurkat T cells.

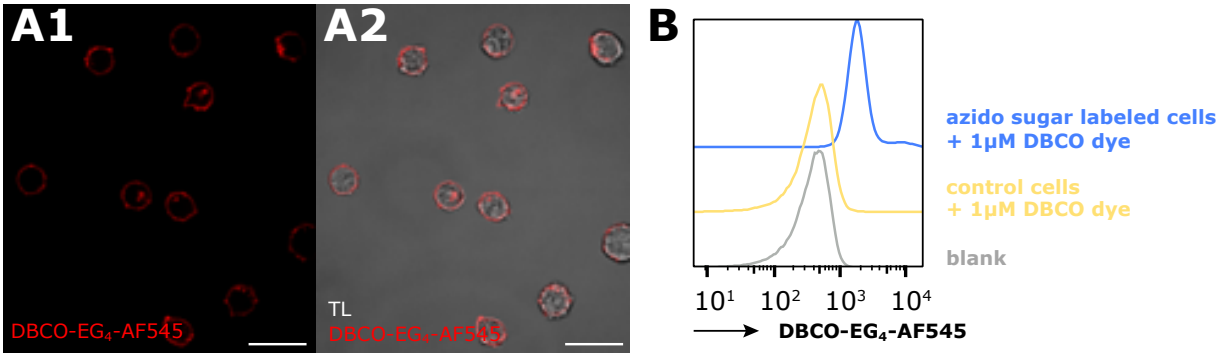

Figure S21| Confocal microscopy (A) and flow cytometry analysis (B) of Jurkat T cells metabolically labeled with the azido sugar Ac_4ManN_3, followed by SPAAC conjugation to DBCO-EG_4-eFluor 545 (red fluorescence). Scale bar represents 20 micron. As controls for flow cytometry blank cells and non-azide labeled cells were used.

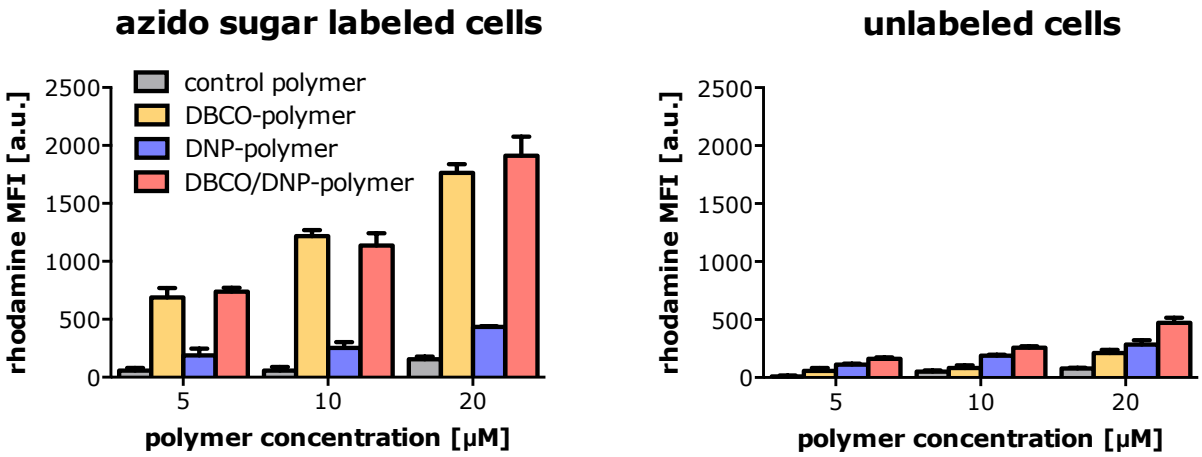

Figure S22| Flow cytometry analysis of polymer binding to metabolically azido sugar labeled (A) and unlabeled (B) cells.

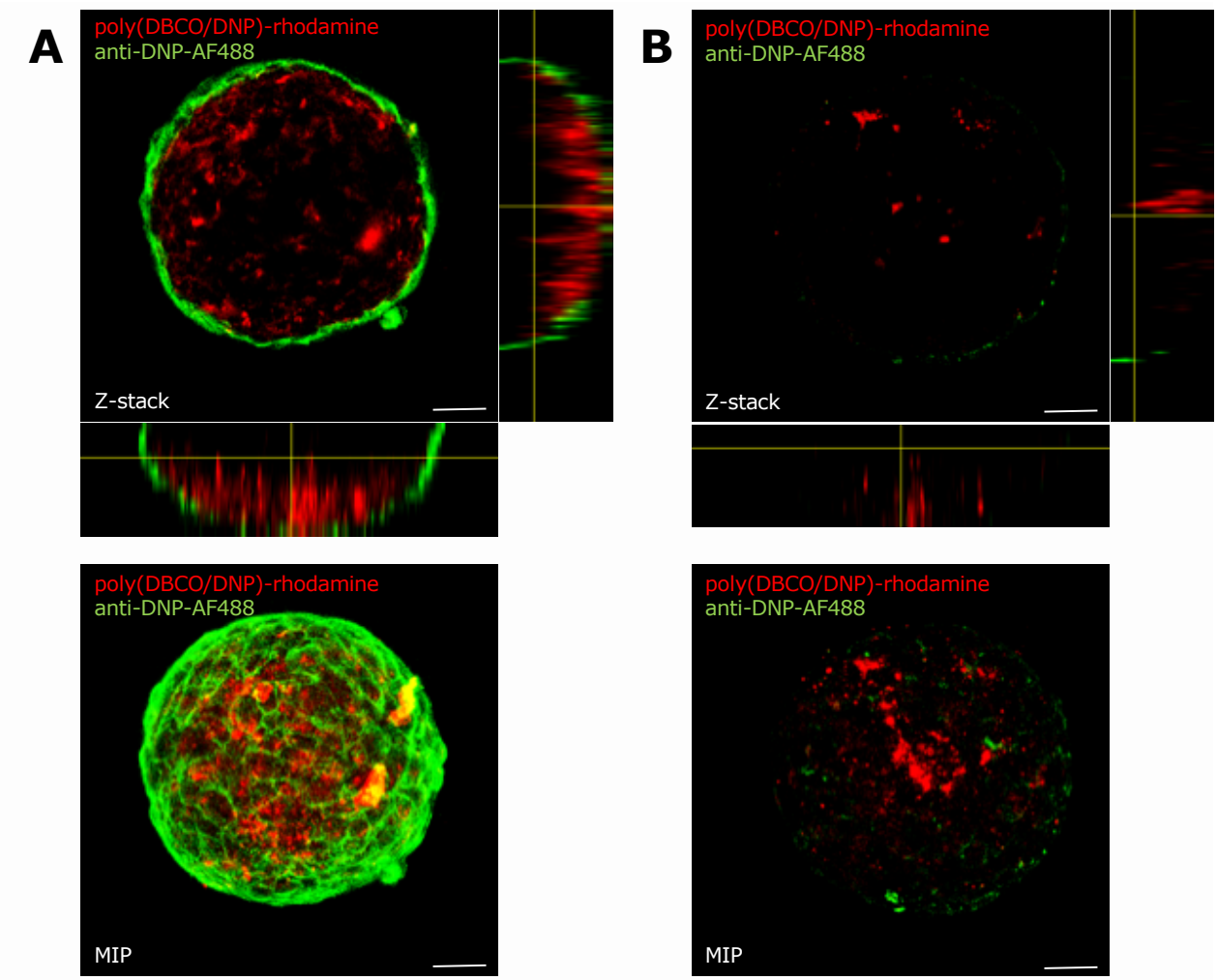

Figure S23| Confocal microscopy images (z-stacks (upper row panels) and maximum intensity projections (lower row panels)) of 4T1 mouse breast cancer spheroids, cultured with (**A**) or without (**B**) azido sugar and treated with DCBO/DNP-polymer (red fluorescence) and AF488-anti-DNP (green fluorescence). Scale bar represents 50 micron.