Supporting Information

Multifunctional, Histidine-tagged Polymers: Antibody Conjugation and Signal Amplification

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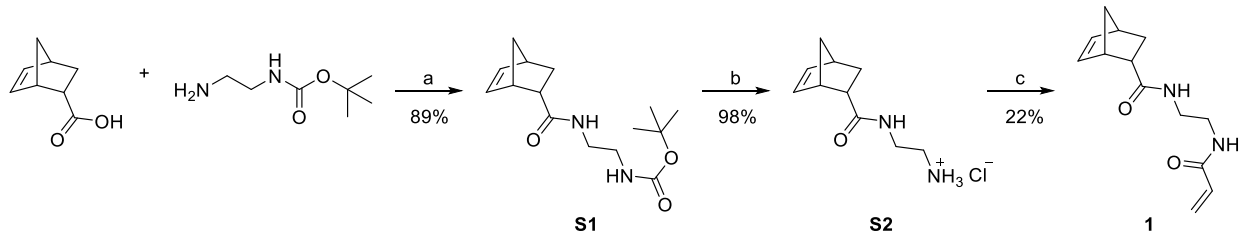
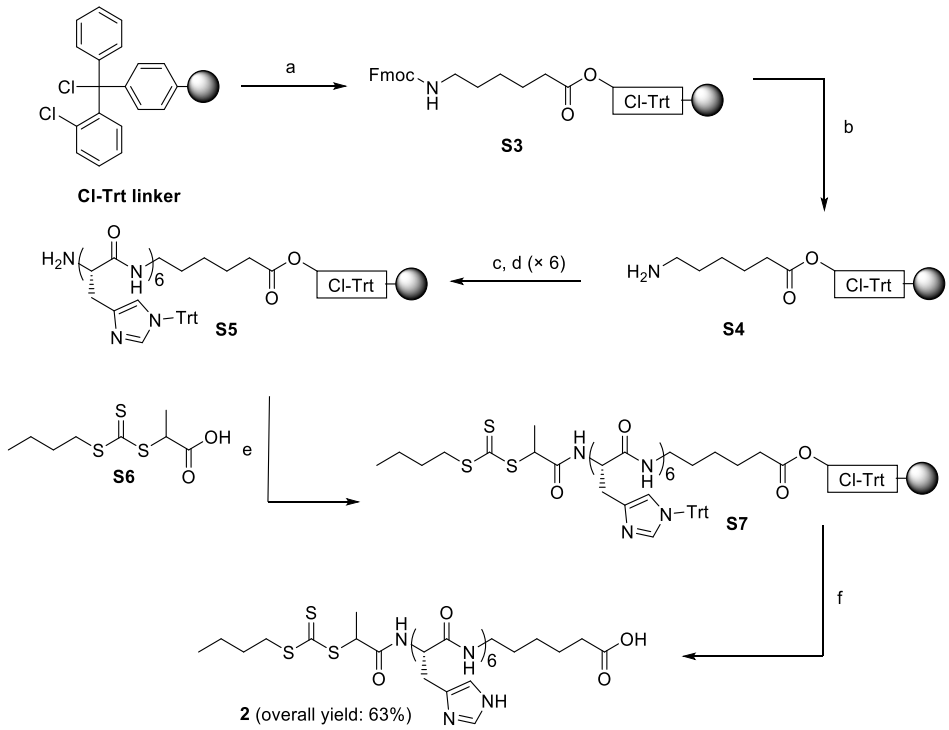
1. Supporting Figures and Tables

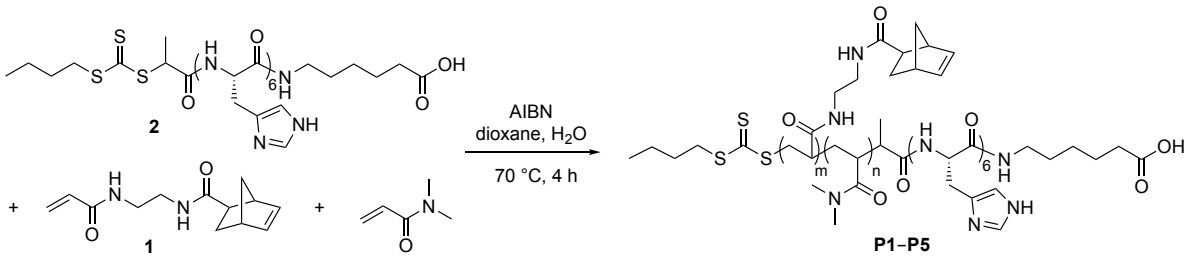


Scheme S1 Synthesis of the norbornene acrylamide monomer **1**. a) EDC, DMF, 18 h; b) 4 M HCl in dioxane, 18 h; c) acryloyl chloride, NEt_3, THF, 2 h.



Scheme S2 Solid-phase synthesis of Hexahistidine tagged RAFT agent **2** using a polystyrene resin functionalised with a 2-chlorotritylchloride linker (Cl-Trt). a) Fmoc-6-Ahx-OH, DIPEA, DMF, 1 h; b) and d) 20% piperidine in DMF, 2 × 10 min; c) Fmoc-His(Trt)-OH, DIC, Oxyma, DMF, 3 h; e) **S6**, DIC, Oxyma, DMF, 3 h; f) TFA/H_2O (95:5), 2 h. The yield was calculated based on a resin loading of 0.47 mmol/g (determined by a quantitative ninhydrin test after the Fmoc-6-Ahx-OH coupling and Fmoc group removal).

Table S1 Characterisation of the multifunctional polymers **P1–P5**.



	2:**1**:DMA theoretical [a]	**2**:**1**:DMA ^{1}H NMR [b]	Reactive monomer % [c]	Conversion [d]	M_n (kDa) theoretical	M_n (kDa) ^{1}H NMR	M_n (kDa) GPC	Đ
P1	1:10:100	1:8.0:101	7.3	90%	13	13	14	1.5
P2	1:4:40	1:3.3:57	5.4	91%	6.1	7.6	7.1	1.2
P3	1:25:250	1:21:240	8.0	89%	32	23	30	1.8
P4	1:4:100	1:2.8:107	2.6	96%	12	12	11	1.4
P5	1:25:100	1:18:89	16.8	78%	17	14	16	1.7

[a] The molar ratios of chain transfer agent **2** and norbornene acrylamide **1** and *N,N*-dimethylacrylamide (DMA) in the polymerisation mixture. The polymerisations were carried out with monomer concentrations of 0.44–2.75 M with 10 mM of **2**. [b] The ratios of **1**, **2** and DMA found in the synthesised polymers based on ^{1}H NMR analysis. [c] The percentage of the reactive norbornene unit in each polymer. [d] Calculated by ^{1}H NMR.

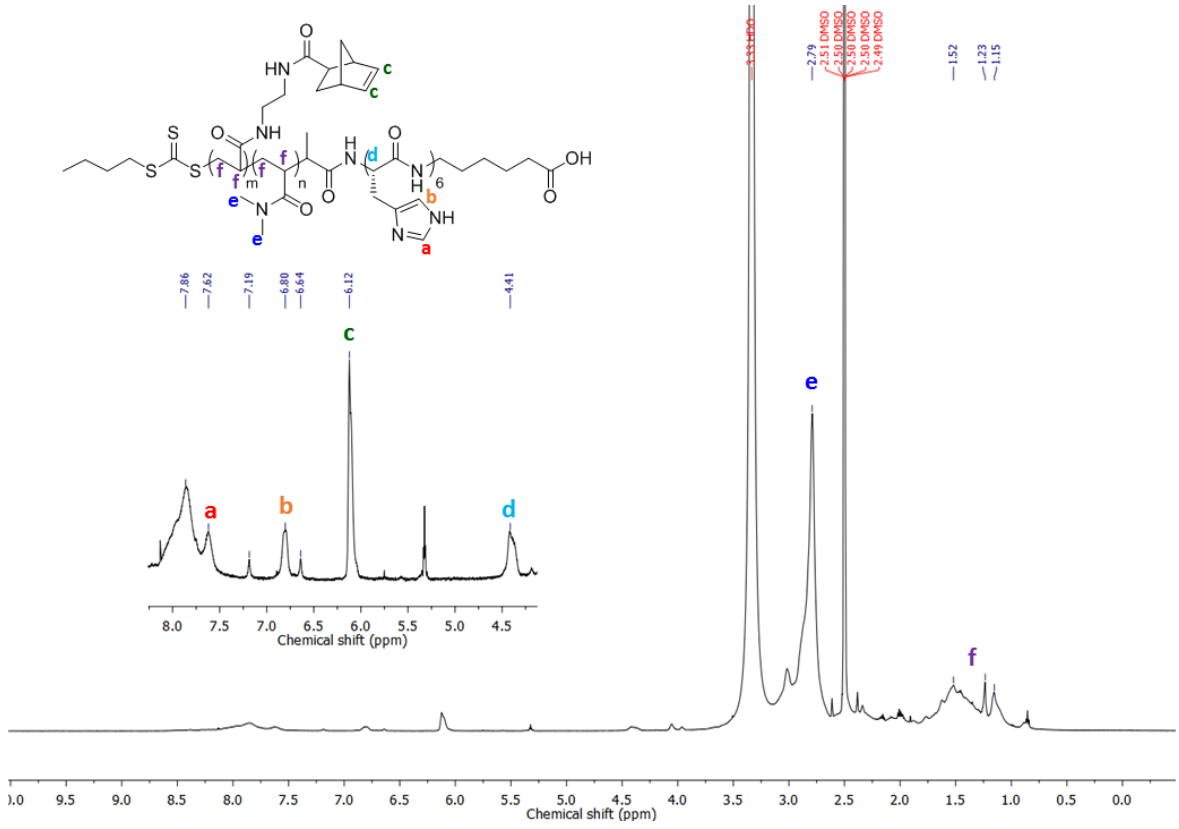
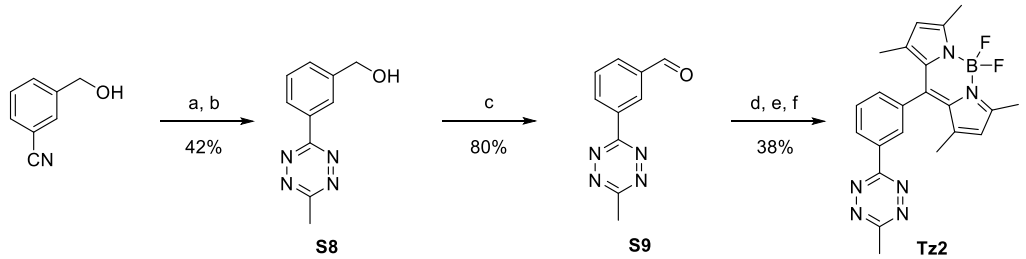
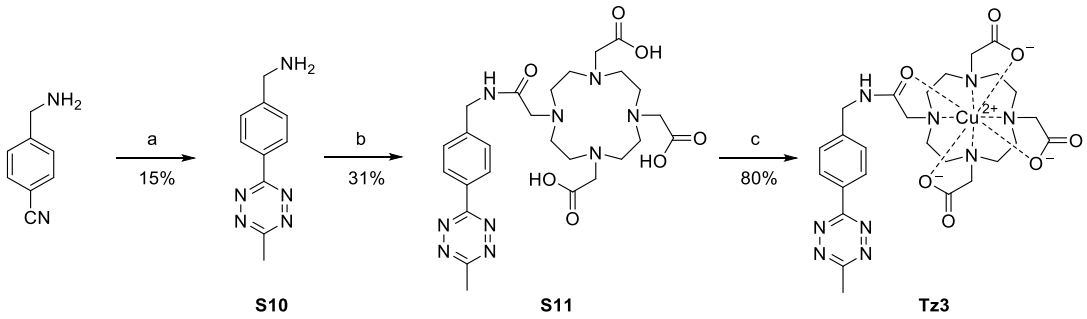


Fig. S1 ^{1}H NMR spectrum of polymer **P1** in d_6-DMSO. Resonances a, b and d (7.86, 6.90 and 4.41 ppm, respective) arise from the hexahistidine tag; c (6.12 ppm) and e (2.58–3.15 ppm) from the norbornene acrylamide and *N,N*-dimethylacrylamide building blocks, and f (0.68–1.85 ppm) from the polymer backbone.



Scheme S3 The efficient synthetic route developed to **Tz2** due to the unsuccessful literature route:[1] a) CH_3CN, $NH_2NH_2{\cdot}H_2O$, $Ni(OTf)_2$, 60 °C, 48 h; b) $PhI(OAc)_2$, CH_2Cl_2, 3 h; c) Dess-Martin periodinane, CH_2Cl_2, 2 h; d) 2,4-dimethylpyrrole, TFA, dry THF, 18 h; e) DDQ, dry THF, 4 h; f) NEt_3, $BF_3{\cdot}$ OEt_2, 0 °C, 24 h.



Scheme S4 Synthesis of the metal chelating tetrazine **Tz3**. a) CH_3CN, $NH_2NH_2 \cdot H_2O$, $Ni(OTf)_2$, 60 °C, 24 h;[2] b) DOTA-NHS ester, NEt_3, DMF, 2 h;[3] c) $CuCl_2$, NaOAc buffer (pH = 6.0), 40 °C, 30 min.

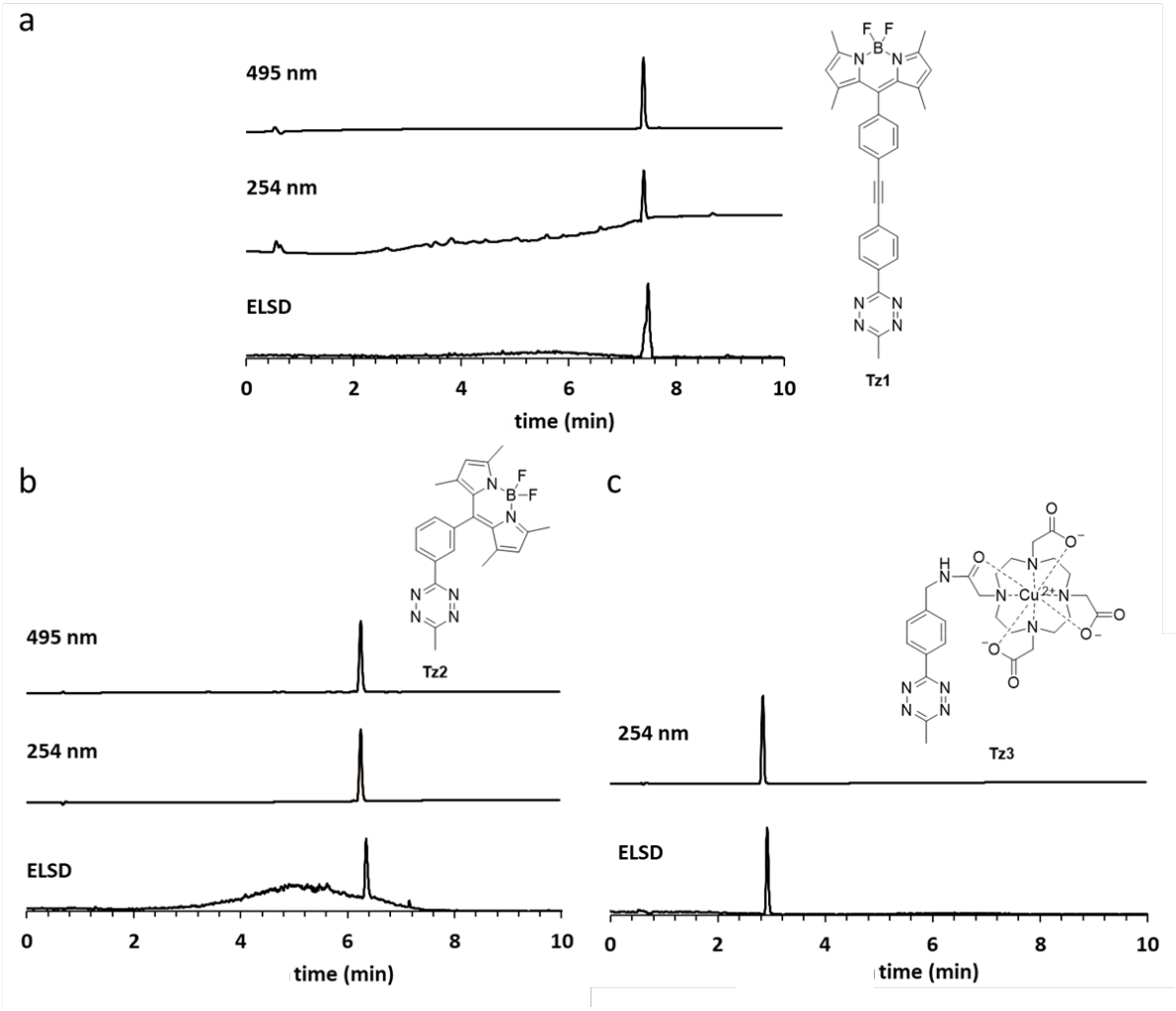


Fig. S2 HPLC traces of (a) **Tz1** (note slightly insoluble hence low intensity), (b) **Tz2** (taken immediately after column purification to minimise degradation) and (c) **Tz3** using ELSD and UV detectors with MeCN/H_2O as eluents.

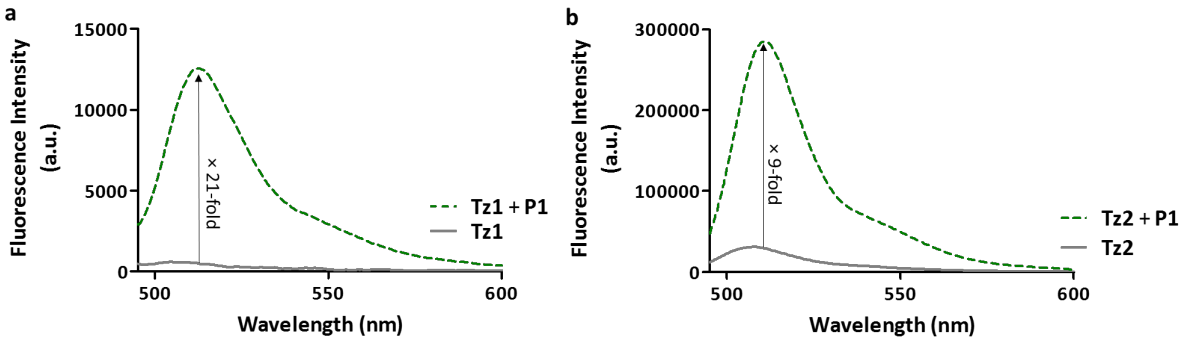


Fig. S3 Fluorescence spectra of (a) **Tz1** and (b) **Tz2** (both at 1 µM in PBS) before and after the addition of the polymer **P1** (12 µM for 30 min at 37 °C). Fluorescence measurements were carried out on a FluoroMax-3 fluorimeter (λ_{ex} = 488 nm) using a quartz cuvette.

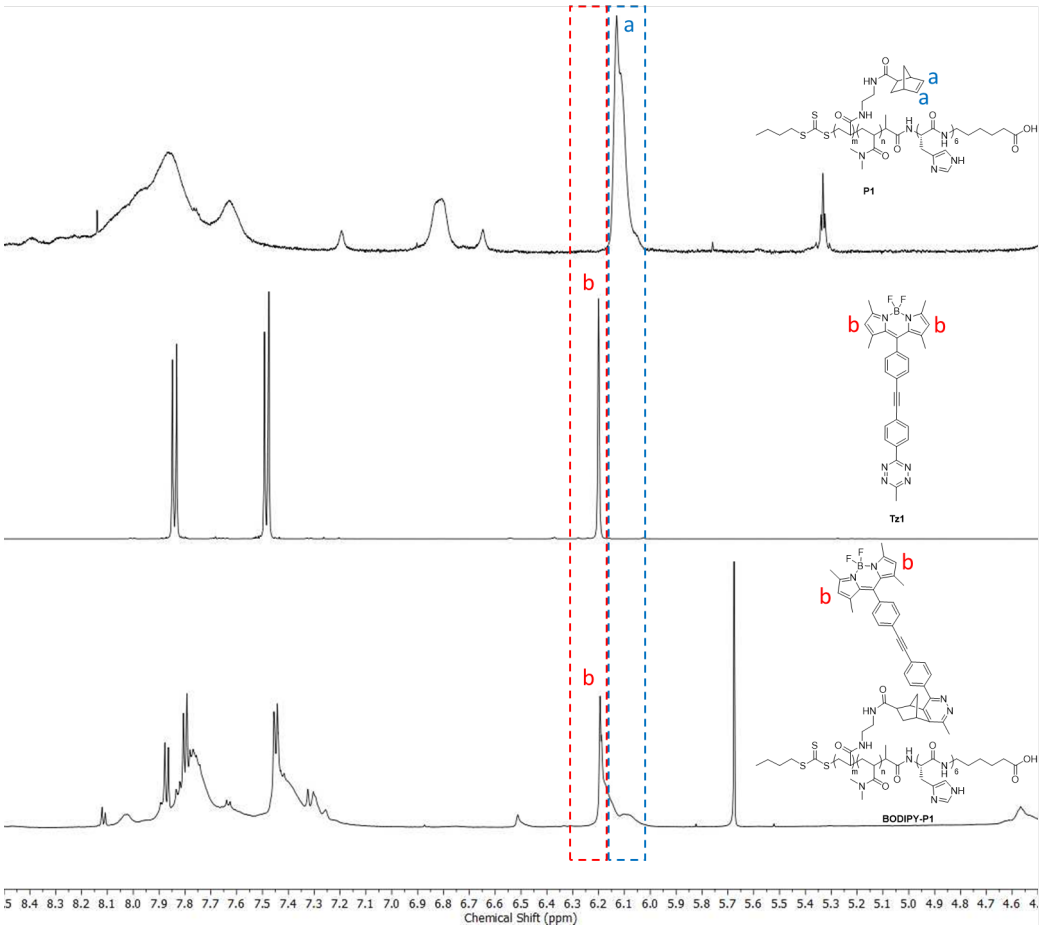


Fig. S4 [1]HNMR spectra of **P1**, **Tz1** and their resulting fluorescent polymer **BODIPY-P1**. **BODIPY-P1** was synthesised by incubation of **P1** (0.4 µM) with **Tz1** (5 µM) in PBS at 37 °C for 30 min followed by dialysis (molecular weight cut off 10 kDa). Full consumption of the norbornene (> 95% conversion shown by integration, see blue box for the loss of norbornene double bond) was observed, indicating the complete reaction.

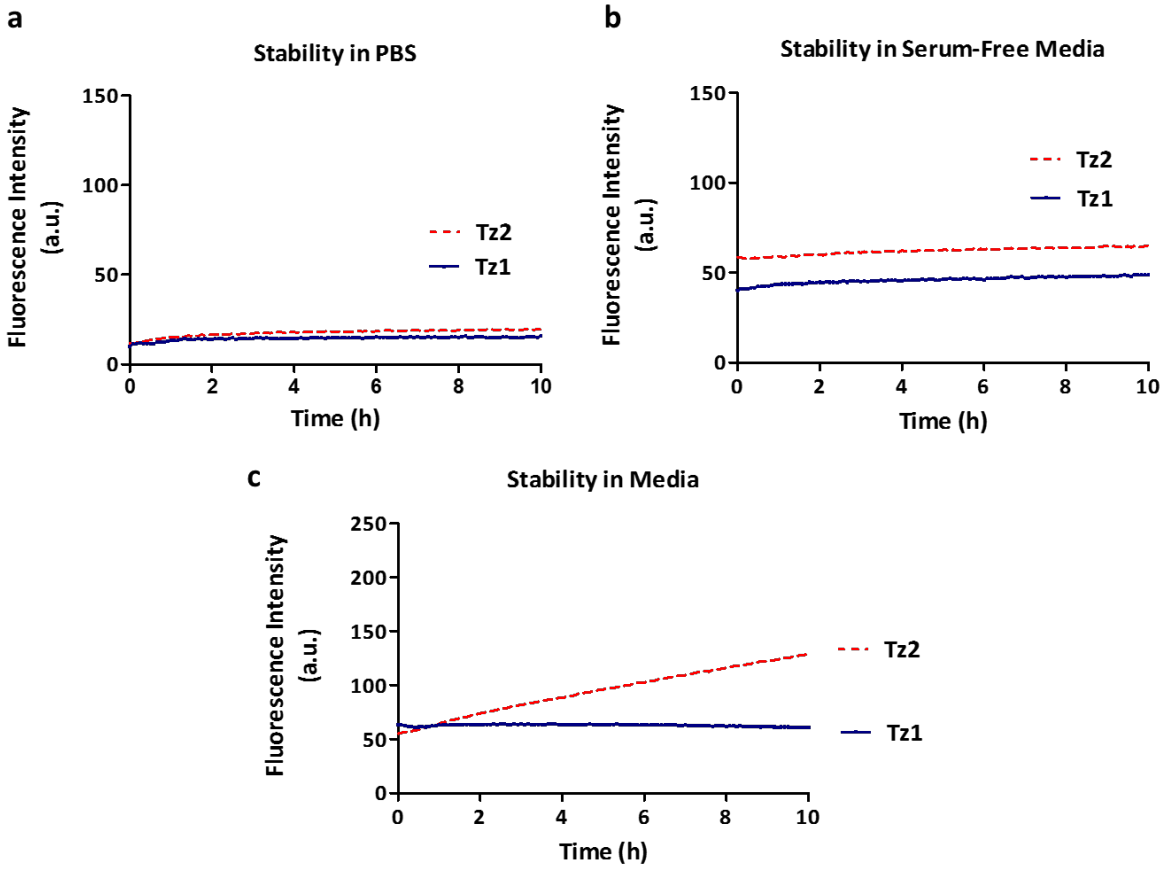


Fig. S5 Stability studies of **Tz1** and **Tz2**, both at 1 µM, (a) in PBS, (b) serum-free media and (c) cell culture media (with 10% FBS, *v*/*v*). The stability was monitored in black 96-well plates by measuring the time-dependent fluorescence intensity (BioTek HT Synergy multi-mode reader, λ_{ex} = 465–505 nm, λ_{em} = 508–548 nm), with readings every 5 min at 37 °C. The change in fluorescence intensity in serum containing cell culture media showed the lack of stability of **Tz2** compared to **Tz1**.

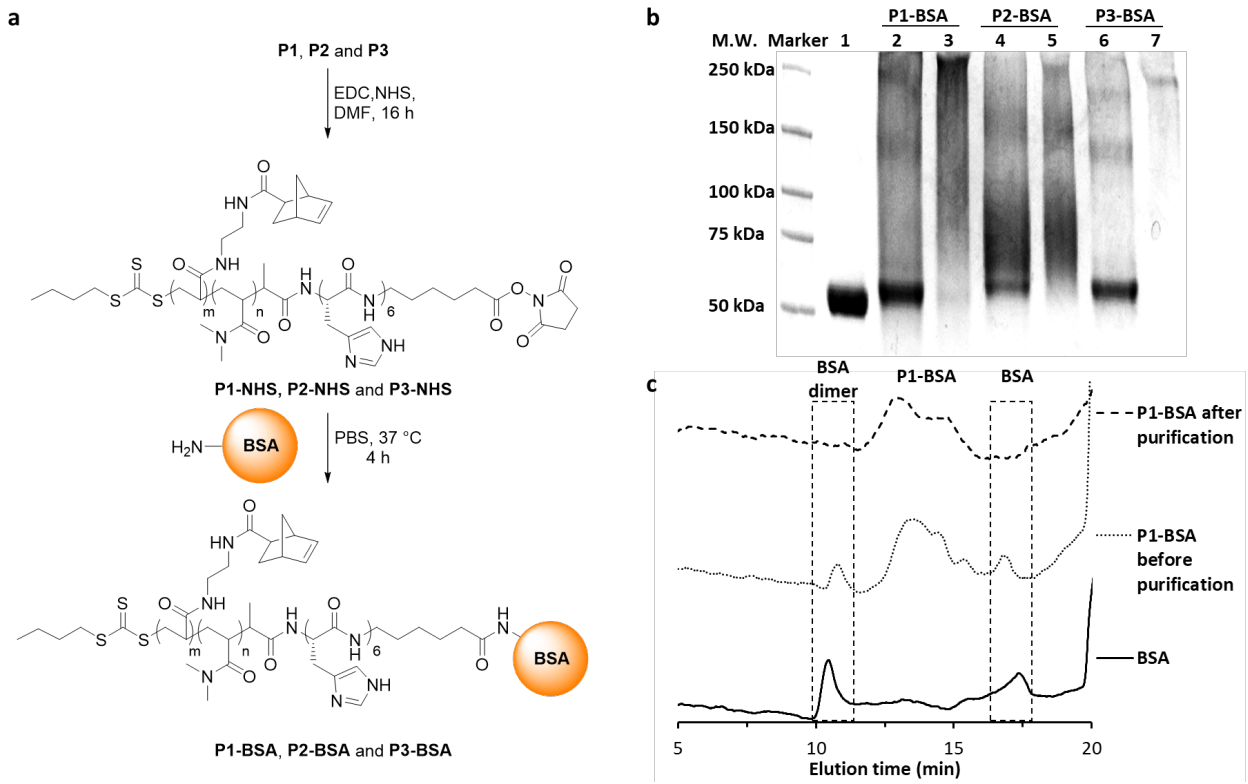


Fig. S6 (a) Polymer activation and conjugation to the model protein BSA. (b) SDS-PAGE of polymers conjugated to BSA before and after purification by metal-affinity chromatography. Precision Plus Protein™ Kaleidoscope™ Prestained 10–250 kDa Protein Standards were used as markers; Lane 1 BSA; Lane 2 **P1-BSA** (before purification); Lane 3 **P1-BSA** (after purification); Lane 4 **P2-BSA** (before purification); Lane 5 **P2-BSA** (after purification); Lane 6 **P3-BSA** (before purification); Lane 7: **P3-BSA** (after purification). Note, the smeary bands are indicative of BSA labelled with a series of polymers (with both varying numbers of attachment points and the polymers themselves being not mono-disperse), while the polymer will alter the protein mobility on the SDS-PAGE gel. (c) GPC traces of unmodified BSA (lower trace), **P1-BSA** before purification (middle trace) and **P1-BSA** after purification (top trace).

Table S2 The nomenclature of the synthesised polymer–protein conjugates.

Protein	Polymer	Conjugate	Yield[a]
BSA	**P1**	**P1-BSA**	42%
BSA	**P2**	**P2-BSA**	52%
BSA	**P3**	**P3-BSA**	15%
Herceptin	**P1**	**P1-Her**	72%
Herceptin	**P2**	**P2-Her**	45%
Herceptin	**P3**	**P3-Her**	88%
Herceptin	**P4**	**P4-Her**	73%
Herceptin	**P5**	**P5-Her**	56%

[a] Yields quantified by UV spectroscopy at λ = 280 nm

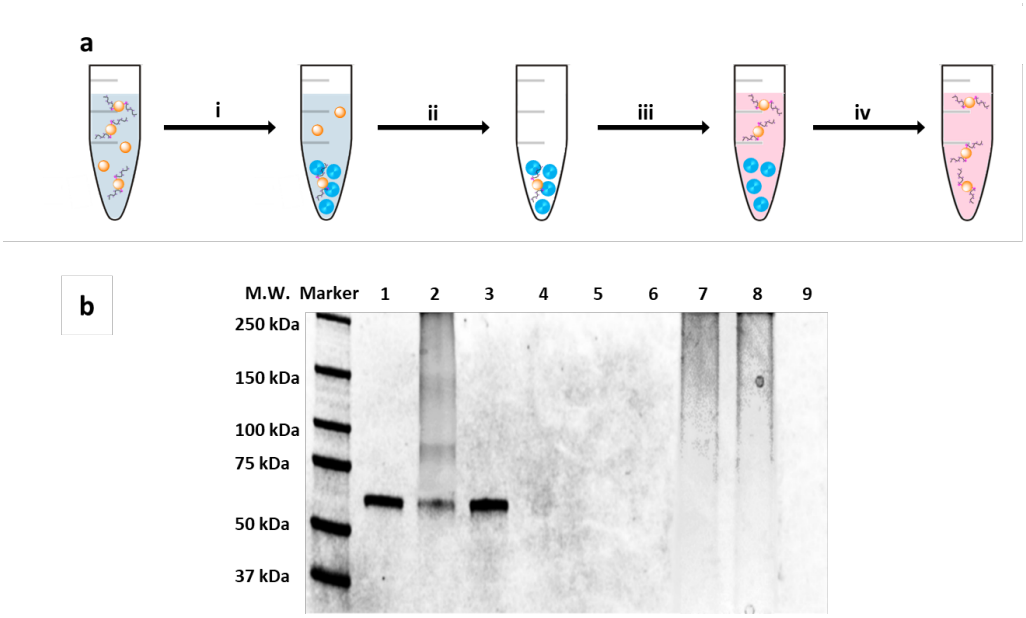


Fig. S7 (a) Isolation/purification of the polymer–protein conjugates. i) Conjugated proteins were bound to the Ni-charged metal-affinity chromatography resin;[4] ii) Unmodified proteins removed by washing; iii) Polymer–protein conjugates eluted with an elution buffer (50 mM sodium phosphate, 300 mM NaCl with either 500 mM imidazole or a gradient of 10–500 mM imidazole, pH = 8.0); iv) Removal of the beads gives the purified polymer–protein conjugates. (b) SDS-PAGE of polymer **P1** conjugated to BSA (**P1-BSA**) showing protein distributions following binding and elution from the Ni-charged metal-affinity chromatography resin using different concentrations of the imidazole elution buffer. Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards (10–250 kDa) were used as markers; Lane 1 unmodified BSA; Lane 2 **P1-BSA** before purification; Lane 3 column flow; Lane 4 elution buffer with 10 mM imidazole; Lane 5 elution buffer with 20 mM imidazole; Lane 6 elution buffer with 50 mM imidazole; Lane 7 elution buffer with 100 mM imidazole; Lane 8 elution buffer with 200 mM imidazole; Lane 9 elution buffer with 500 mM imidazole.

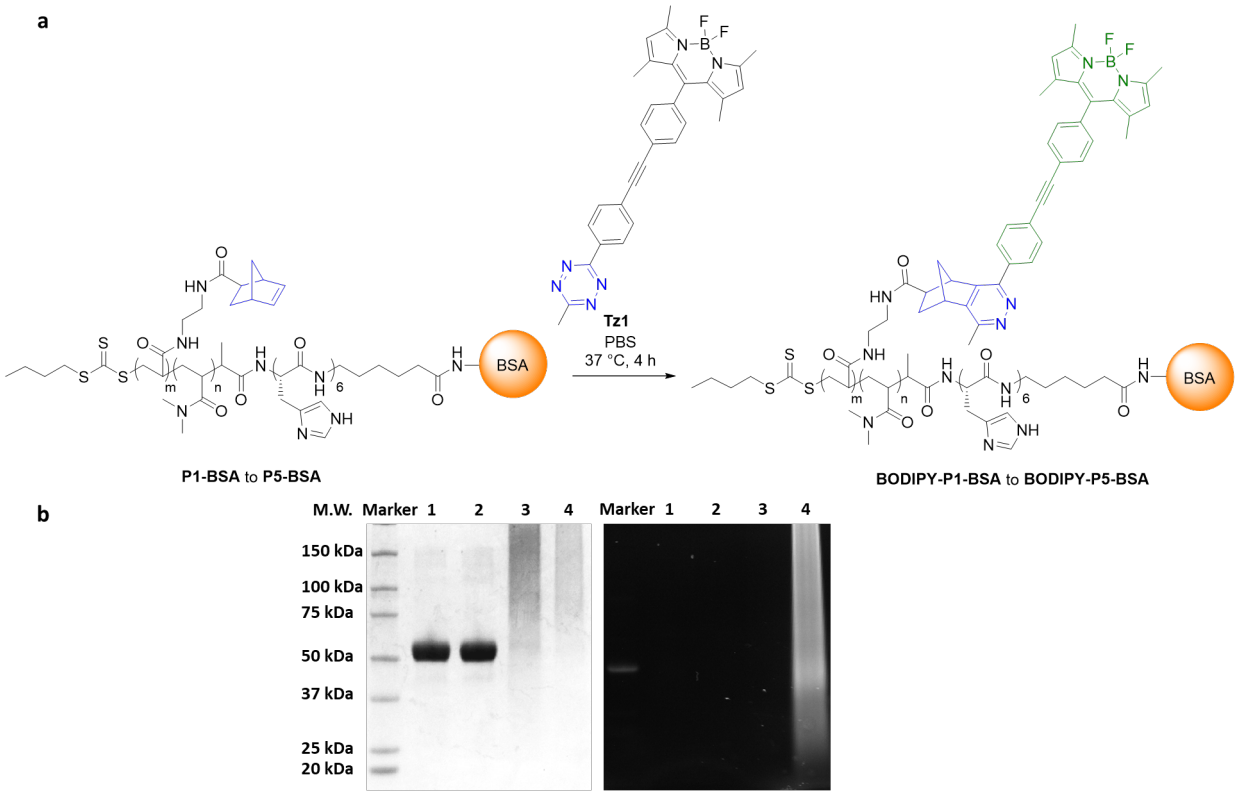


Fig. S8 (a) Polymer conjugated BSA (2.5 μM) was treated with **Tz1** (25 μM) to give **BODIPY-P1-BSA** with fluorescence "switch-on". Only one polymer chain is shown for clarity. (b) SDS-PAGE of the fluorescent BSA conjugate **BODIPY-P1-BSA**. Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards (10–250 kDa) were used as markers; Lane 1 BSA; Lane 2 BSA incubated with **Tz1**; Lane 3 **P1-BSA;** Lane 4 **P1-BSA** incubated with **Tz1.**

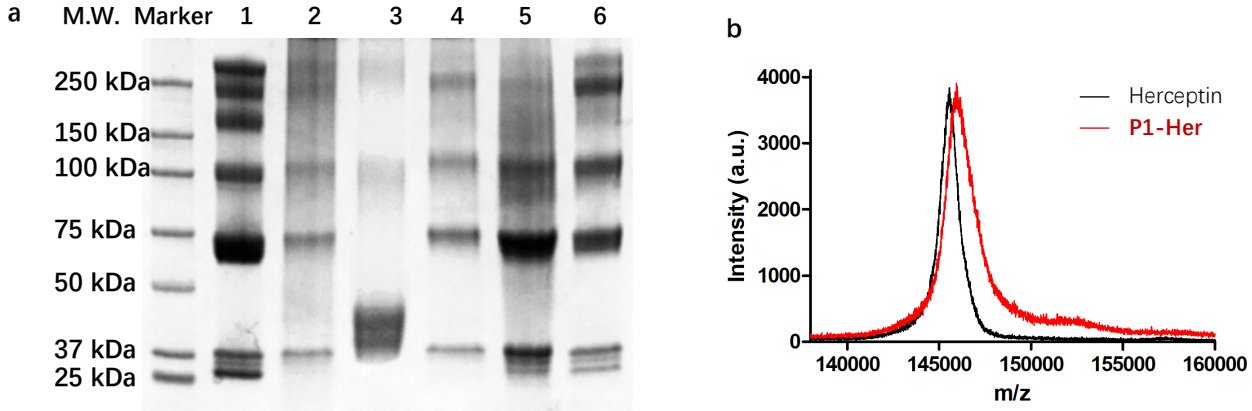
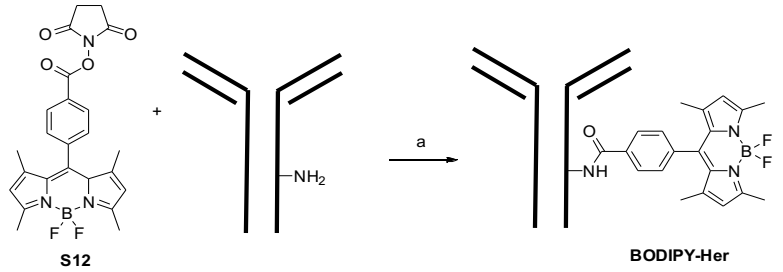


Fig. S9 (a) Reduced SDS-PAGE of purified polymer–antibody conjugates and the native antibody. Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards (25–250 kDa) were used as markers; Lane 1 Herceptin; Lane 2 **P1-Her**; Lane 3 **P2-Her**; Lane 4 **P3-Her**; Lane 5 **P4-Her**; Lane 6 **P5-Her**. (b) MALDI-TOF MS spectra of Herceptin and **P1-Her**. Herceptin has M_r of 145,531 and a peak width of 5,476, whereas **P1-Her** showed a maximum at M_r 146,139 and a peak width of 12,662. The presence of the large and polydisperse polymer chains on the antibody will alter the physico-chemical properties on the conjugates (compared to the native antibody or polymer) therefore prohibiting "copy number" analysis by MS.



Scheme S5 Synthesis of the fluorescently labelled antibody **BODIPY-Her** with a single available amine shown: a) PBS, 37 °C, 4 h. Only one antibody modification site is shown for clarity.

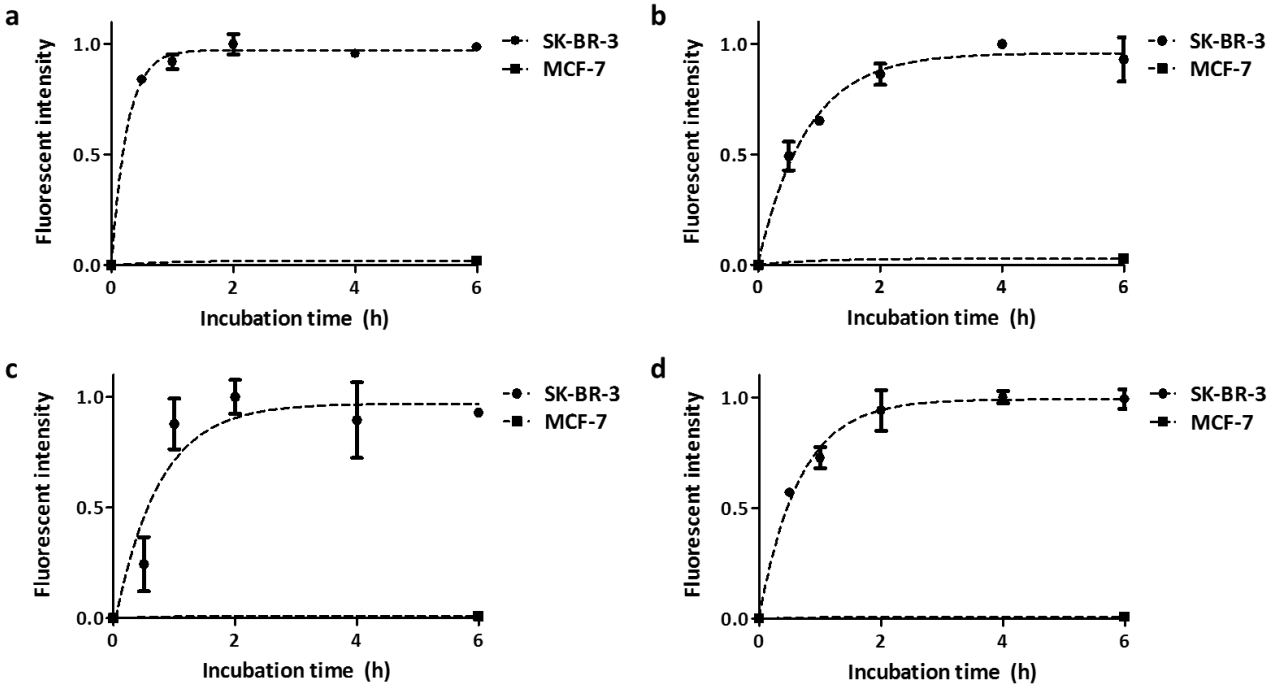


Fig. S10 Cell labelling was not altered by polymer conjugation compared to fluorophore labelled Herceptin. Labelling of (a) **BODIPY-Her**; (b) **BODIPY-P1-Her**; (c) **BODIPY-P2-Her** and (d) **BODIPY-P3-Her** to the HER2 expressing cell line SK-BR-3 and the non-HER2 expressing cell line MCF-7 (quantified by flow cytometry with antibody concentrations of 10 nM). Fluorescence intensity normalised to the intensity of fluorescence-saturated cells (6 h incubation, n = 3).

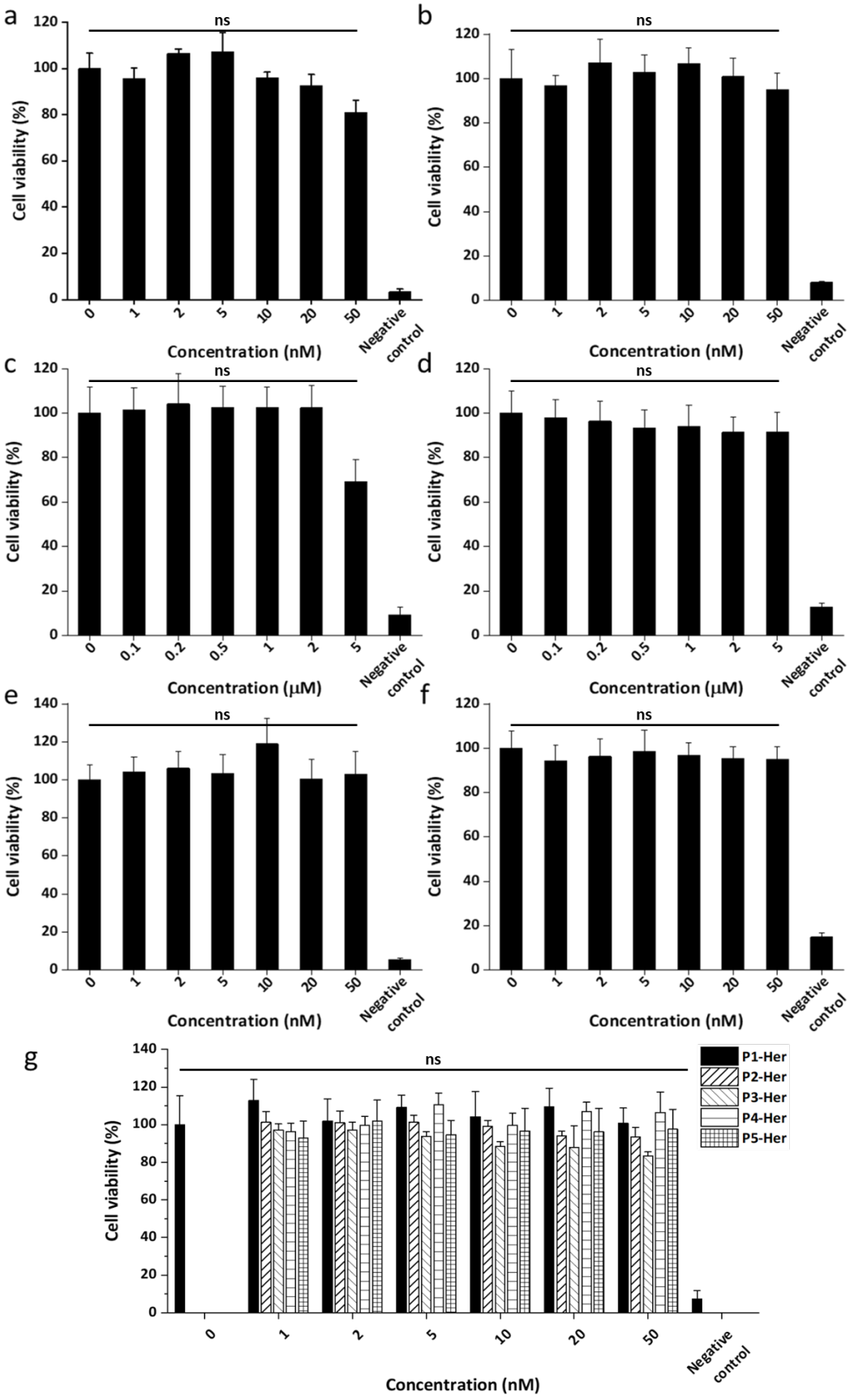


Fig. S11 SK-BR-3 cell viability against (a) Herceptin; (b) **BODIPY-Her**; (c) **Tz1;** (d) **Tz3**; (e) **Cu-P5-Her**; (f) **Cu-Her** and (g) **P1-Her**, **P2-Her**, **P3-Her**, **P4-Her** and **P5-Her** (MTT assay, n = 6). As a negative control, the cells were treated with 50% DMSO in DMEM. Data were analysed using one-way ANOVA with Dunnett post test (ns, not significant).

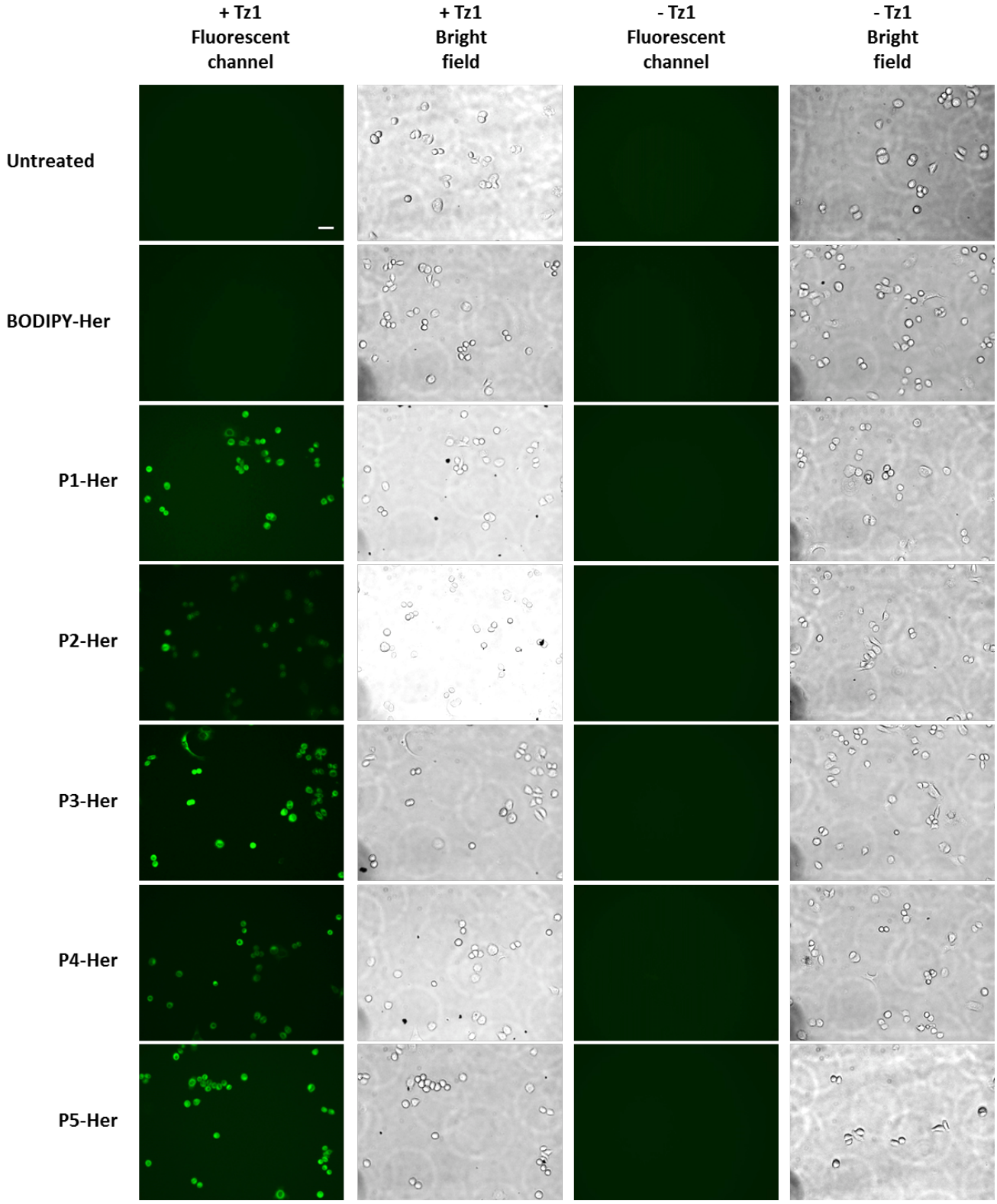
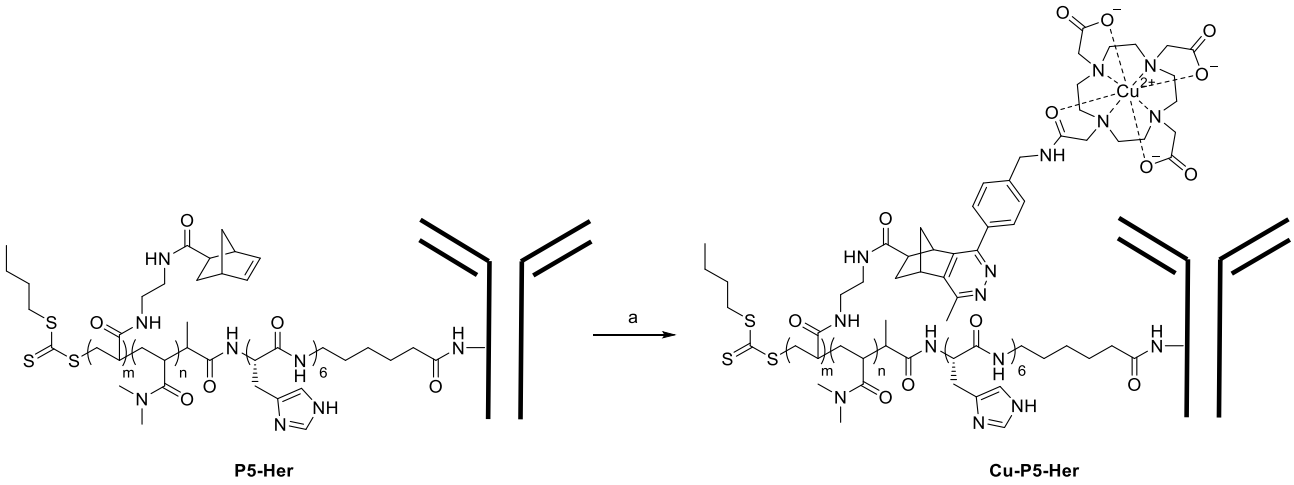
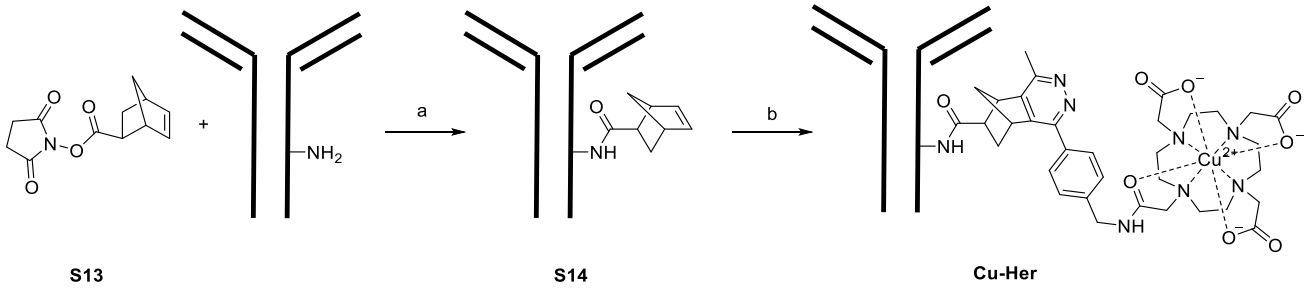


Fig. S12 Fluorescent and bright field images (Zeiss AxioVert 200M fluorescence Microscope, λ_{ex} = 447–494 nm, λ_{em} = 500–554 nm) of SK-BR-3 cells treated with the polymer–Herceptin conjugates (4 h at 10 nM) followed by addition of the tetrazine quenched fluorophore **Tz1** (30 min at 1 µM). Scale bar = 50 µm.



Scheme S6 Synthesis of the Cu(II) labelled polymer-antibody conjugate **Cu-P5-Her**: a) **Tz3**, PBS, 37 °C, 4 h. Only one antibody modification site is shown for clarity.



Scheme S7 Synthesis of the Cu(II) labelled antibody conjugate **Cu-Her**. a) PBS, 37 °C, 4 h; b) **Tz3**, PBS, 37 °C, 4 h. Only one antibody modification site is shown for clarity.

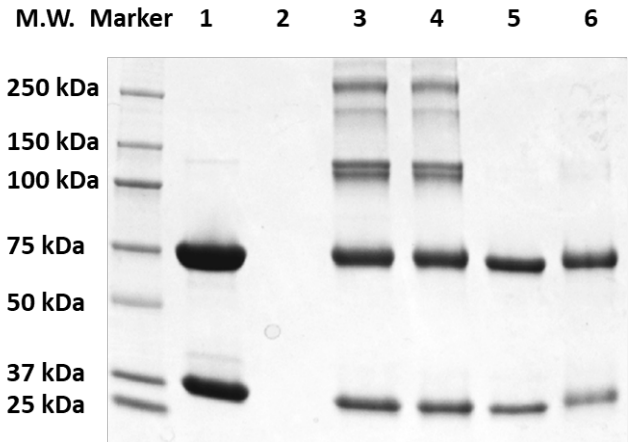


Fig. S13 SDS-PAGE of Cu(II) labelled antibody and Cu(II) labelled polymer–antibody conjugate. Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards (10–250 kDa) were used as markers; Lane 1 Herceptin; Lane 2 **Tz3**; Lane 3 **P5-Her**; Lane 4 **Cu-P5-Her**; Lane 5 **S14**; Lane 6 **Cu-Her**.

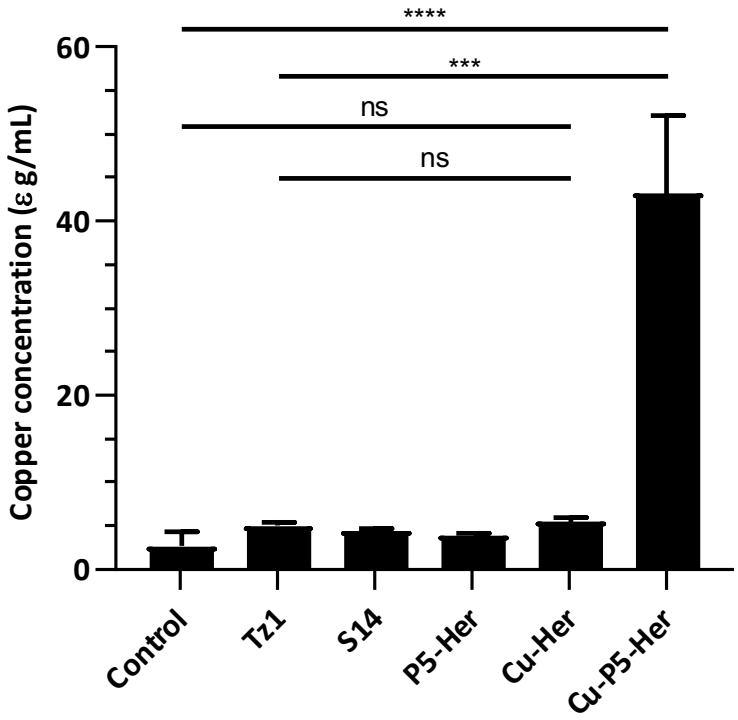


Fig. S14 Cellular copper concentrations quantified using inductively coupled plasma mass spectrometry (ICP MS) (n = 6). 5×10^4 SK-BR-3 cells were treated with **Tz3** (1 µM), **S14** (10 nM), **P5-Her** (10 nM), **Cu-Her** (10 nM) and **Cu-P5-Her** (10 nM) and washed with PBS (untreated cells were used as a control). The harvested cells were lysed by sonication in 0.1% NaOH solution and diluted to 5 mL for ICP MS analysis. Data were analysed using one-way ANOVA with Dunnett post test (ns, not significant, *** $P < 0.001$, **** $P < 0.0001$).

2. Experimental

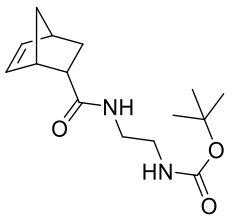
2.1 General Information

DOTA-NHS ester was purchased from CheMatech (France). All other chemicals were purchased from Acros Organics, Alfa Aesar, Fisher Scientific or Sigma Aldrich, and used as received. 2-Chlorotrityl polystyrene resin (100–200 mesh) was purchased from GL Biochem (Shangai) Ltd. TLC plates were purchased from Merck. 4–20% Mini-PROTEAN TGX precast protein gels, 2× Laemmli Sample Buffer, and Ni-charged Profinity™ IMAC Resin were purchased from Bio-Rad. Dialysis membrane (molecular weight cut off 7000 Da) was purchased from Medicell Membranes Ltd. Reverse phase chromatography was carried out on a Biotage Isolera™ Spektra One system equipped with a Biotage® SNAP Ultra C18 column.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVA500 spectrometer (500 and 125 MHz, respectively) or on a Bruker AVA600 (600 and 150 MHz, respectively) at 298 K in deuterated solvents. The residual non-deuterated solvent resonances were used as a reference for ^{1}H NMR experiments with $CHCl_3$ (δ_H = 7.26 ppm), CD_3SOCHD_2 (δ_H = 2.50 ppm), and HDO (δ_H = 4.79 ppm). The deuterated solvent resonances were used as reference for ^{13}C NMR experiments with $CDCl_3$ (δ_C = 77.2 ppm) and $(CD_3)_2SO$ (δ_C = 39.5 ppm). Analytical HPLC was performed on an Agilent Technologies 1100 modular HPLC system coupled to a multiwavelength and PL-ELSD-1000 detector and equipped with a Phenomenex Kinetex® 5µm XB-C18 100 Å column (50 × 4.6 mm), with a flow rate of 1 mL/min, eluting with 95% water for 5 min, then to 95% CH_3CN over 10 min (both with 0.1% HCO_2H). High Resolution MS were performed on a Bruker microTOF focus II mass spectrometer. GPC was performed on an Agilent 1100 GPC equipped with PLgel MIXED-C columns (2×10^2–2×10^6 g/mol, 5 mm) and an RI detector, eluting with DMF containing 0.1 % *w*/*v* LiBr at 60 °C at 1 mL/min or an Agilent 1100 GPC equipped with an PL aquagel-OH 30 column (8 µm) and one PL aquagel-OH MIXED-H column (8 µm) using H_2O as an eluent at 40 °C at 1 mL/min. Molecular weights obtained were relative to narrow dispersity polymethyl methacrylate or poly ethylene glycol standards. Labelling measurements involving fluorescence readout were performed on a FluoroMax-3 Jobin Yvon Div using a quartz cuvette.

2.2 Synthesis of the norbornene acrylamide monomer

Exo-2-*tert*-butoxycarbamatoethyl-carboxamidonorborn-5-ene S1[5]



S1

To a solution of *N*-Boc-ethylenediamine (3.6 g, 23 mmol) and *exo*-5-norbornenecarboxylic acid (2.8 g, 20 mmol) in DMF (50 mL), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (4.2 g, 22 mmol) was added and the reaction mixture was stirred overnight. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography (eluting with EtOAc/DCM, 2:8) to give compound **S1** as a white solid (5.0 g, 89%).

^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm 7.83 (t, *J* = 5.7 Hz, 1H), 6.77 (t, *J* = 5.7 Hz, 1H), 6.14 (dd, *J* = 5.6, 2.8 Hz, 1H), 6.10 (dd, *J* = 5.7, 3.0 Hz, 1H), 3.07 (m, 2H), 2.98 (t, *J* = 6.4 Hz, 2H), 2.85–2.79 (m, 2H), 2.04–1.98 (m, 1H), 1.81–1.74 (m, 1H), 1.62 (m, 1H), 1.38 (s, 9H), 1.20–1.11 (m, 2H).

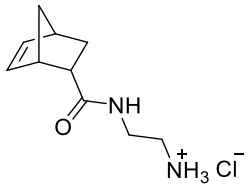
^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm 175.2, 156.1, 138.2, 136.7, 78.1, 47.3, 46.1, 43.5, 41.4, 30.2, 28.7.

HRMS (ESI) for $C_{15}H_{24}N_2O_3$ $[M+H]^+$: *calcd.*: 281.1860; *found*: 281.1859.

R_f = 0.10 (DCM/EtOAc, 9:1).

Data in agreement with the literature.[5]

***Exo*-2-aminoethyl-carboxamidonorborn-5-ene S2[5]**



S2

To a solution of **S1** (3.6 g, 12.8 mmol) in 1,4-dioxane (5 mL), 4 M HCl in 1,4-dioxane (25 mL) was added and the mixture was stirred overnight. The solvent was removed *in vacuo* to give the product as a white solid (2.3 g, 98%).

^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm 6.17–6.09 (m, 2H), 3.35–3.28 (m, 2H), 2.88–2.82 (m, 4H), 2.10–2.05 (m, 1H), 1.83–1.62(m, 2H), 1.23–1.15 (m, 2H).

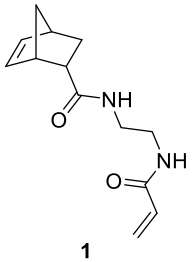
^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm 175.8, 138.3, 136.7, 47.2, 46.2, 43.6, 41.5, 39.1, 37.1, 30.3.

HRMS (ESI) for $C_{10}H_{16}N_2O$ $[M+H]^+$: *calcd.*: 181.1335; *found*: 181.1330.

R_f = 0.16 (9:1, CH_2Cl_2/CH_3OH).

Data in agreement with the literature.[5]

***Exo*-2-acrylamidoethyl-carboxamidonorborn-5-ene 1**



1

To a solution of **S2** (2.3 g, 13mmol) and triethylamine (2.7 mL, 19 mmol) in anhydrous THF (20 mL), acryloyl chloride (1.2 mL, 14 mmol) in anhydrous THF (10 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 2 h at room temperature, concentrated *in vacuo* and purified by column chromatography (eluting with EtOAc/DCM, 4:6) to give **1** as a white solid (0.8 g, 22%).

^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm 8.11 (t, *J* = 5.1 Hz, 1H), 7.91 (t, *J* = 5.3 Hz, 1H), 6.19 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.13 (dd, *J* = 5.5, 2.8 Hz, 1H), 6.11 (dd, *J* = 5.5, 2.8 Hz, 1H), 6.07 (dd, *J* = 17.1, 2.2 Hz, 1H), 5.58 (dd, *J* =

10.2, 2.2 Hz, 1H), 3.21–3.08 (m, 4H), 2.82 (m, 1H), 2.80 (m, 1H), 2.01 (m, 1H), 1.79 (m, 1H), 1.62 (m, 1H), 1.16 (m, 2H).

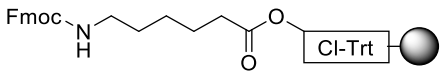
^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm 175.3, 165.3, 138.2, 136.8, 132.3, 125.5, 47.3, 46.1, 43.5, 41.4, 39.0, 38.9, 30.2.

HRMS (ESI) for $C_{13}H_{18}N_2O_2$ $[M+H]^+$: *calcd.*: 235.1447; *found*: 235.1445.

R_f = 0.49 (DCM/MeOH, 9:1).

2.3 Synthesis of the hexahistidine tagged RAFT agent

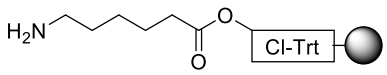
(Fmoc-amino)hexanoic acid linker bound resin S3



S3

In an SPE filter cartridge (12 mL, fitted with a polyethylene frit with 20 µm pores, Sigma-Aldrich), thionyl chloride (40 µL, 0.55 mmol) was added to preswollen (in anhydrous DCM) 2-chlorotrityl chloride resin (500 mg, reported loading 0.95 mmol/g) in anhydrous DCM under a N_2 atm, and the reaction mixture was stirred for 1 h. The solvent was drained and the resin was washed with anhydrous DCM (3 × 5 mL) and anhydrous DMF (3 × 5 mL). The re-activated resin was swollen in anhydrous DCM for 10 min, followed by addition of Fmoc-Ahx-OH (237 mg, 1.8 mmol) and DIPEA (275 µL, 1.7 mmol) in anhydrous DMF (5 mL), and shaken for 1 h. The resin was washed with anhydrous DCM (3 × 5 mL) and anhydrous DMF (3 × 5 mL), and then treated twice with DCM/MeOH/DIPEA (80:15:5, 5 mL) and washed with DCM (3 × 5 mL) and DMF (3 × 5 mL).

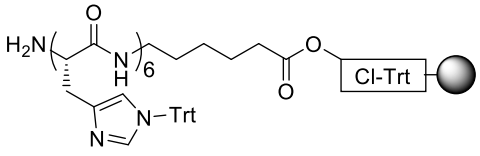
6-Aminohexanoic acid linker bound resin S4



S4

To the resin **S3** (500 mg, pre-swollen in DCM), piperidine (5 mL, 20% *v*/*v* in DMF) was added and the resin was shaken for 2 × 10 min. The solvent was drained and the resin was washed with DCM (3 × 5 mL), DMF (3 × 5 mL), MeOH (3 × 5 mL) and diethyl ether (3 × 5 mL). After the first coupling and deprotection, the resin loading was 0.47 mmol/g based on a quantitative ninhydrin test.

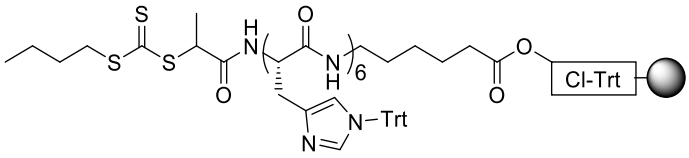
Hexahistidine tag resin S5



S5

Fmoc-His(Trt)-OH (930 mg, 1.5 mmol) and ethyl cyano(hydroxyimino) acetate (Oxyma) (213 mg, 1.5 mmol) were dissolved in DMF (5 mL) and stirred for 10 min. *N*,*N*′-Diisopropylcarbodiimide (232 µL, 1.5 mmol) was added and stirred for further 2 min. The mixture was added to resin **S4** (500 mg, pre-swollen in DCM) and stirred for 3 h. The solution was drained and the resin was washed with DCM (3 × 5 mL) and DMF (3 × 5 mL). The resulting resin was swollen in DCM, drained, and piperidine (5 mL, 20% *v*/*v* in DMF) was added and shaken for 2 × 10 min before the solvent was drained and the resin was washed with DCM (3 × 5 mL), DMF (3 × 5 mL), MeOH (3 × 5 mL) and Et_2O (3 × 5 mL). This procedure was repeated six times to generate the hexahistidine tag moiety. The coupling reactions were monitored by a ninhydrin test.[6]

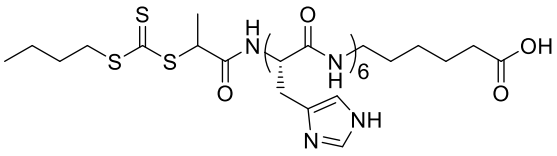
Hexahistidine tagged RAFT agent bound resin S7



S7

2-{[(Butylsulfanyl)carbonothioyl]sulfanyl} propanoic acid **S6**[7] (358 mg, 1.5 mmol), and ethyl cyano(hydroxyimino) acetate (213 mg, 1.5 mmol) were dissolved in DMF (5 mL) and stirred for 10 min. *N*,*N*′-diisopropylcarbodiimide (232 µL, 1.5 mmol) was added and stirred for further 2 min. The mixture was added to resin **S5** (500 mg, pre-swollen in DCM) and reaction mixture was shaken for 3 h. The solution was drained and the resin was washed with DCM (3 × 5 mL) and DMF (3 × 5 mL).

Hexahistidine tagged RAFT agent 2



2

The resin **S7** (500 mg, pre-swollen in DCM) was shaken in TFA/water (95:5, *v*/*v*, 5 mL) for 2 h. The filtrate was collected and the resin was washed with TFA/water (3 × 5 mL). The solutions were combined and evaporated *in vacuo*. The crude product was purified by reverse phase column chromatography using a gradient of acetonitrile (5% to 95%) and water as the eluent (170 mg, 63%).

^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm 8.97–8.73 (m, 6H), 8.68 (m, 1H), 8.59–8.38 (m, 3H), 8.30 (m, 1H), 8.13 (m, 1H), 7.42–7.08 (m, 6H), 4.81–4.41 (m, 7H), 3.34 (t, *J* = 7.2 Hz, 2H), 3.16–2.81 (m, 14H), 2.18 (t, *J* = 7.4, 2H), 1.61 (m, 2H), 1.54–1.28 (m, 8H), 1.28–1.13 (m, 3H), 0.88 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (150 MHz, DMSO-d_6): δ/ppm 223.1, 174.9, 170.6, 170.5, 170.4, 170.4, 170.2, 170.1, 170.0, 159.6, 159.3, 159.1, 158.9, 134.4, 134.3, 130.3, 130.2, 130.0, 129.9, 120.5, 118.5, 117.3, 117.2, 117.1, 116.5, 114.6, 52.6, 52.5, 52.5, 52.3, 50.1, 49.8, 39.0, 38.7, 36.6, 35.8, 34.1, 30.0, 29.0, 27.6, 26.4, 26.3, 25.5, 24.7, 24.6, 21.8, 18.5, 18.0, 13.9.

HRMS (ESI) for $C_{50}H_{67}N_{19}O_9S_3$ $[M+H]^+$: *calcd.*: 1174.4604; *found*: 1174.4599.

HPLC (ELSD) t_R = 1.49 min (purity > 98%).

2.4 Synthesis of polymers P1 – P5 and their NHS esters

Hexahistidine tagged poly(*exo*-2-acrylamidoethyl-carboxamidonorborn-5-ene-*co*-*N*,*N*'-dimethylacrylamide) P1–P5

N,*N*'-dimethylacrylamide (103 µL, 41 µL, 258 µL, 103 µL and 103 µL for **P1** to **P5**, respectively), norbornene acrylamide **7** (23 mg, 9.4 mg, 59 mg, 9.4 mg, 59 mg for **P1** to **P5**, respectively), RAFT agent **6** (12 mg, 10 µmol) and 2,2'-azobis(2-methylpropionitrile) (0.16 mg, 1.0 µmol) were dissolved in 1,4-dioxane (1 mL) and degassed by freeze-pump-thaw cycling. The polymerisation solution was stirred at 70 °C under an Ar atm for 4 h and subsequently quenched by freezing in liquid nitrogen and thawed in the presence of air. The solvent was removed *in vacuo* and the resulting polymer was purified by dialysis (molecular weight cut off 30 kDa) against deionised water for 3 days (water changed every 12 h). Representative NMR data are reported for **P1**. See Table 1 for full GPC data and polymer compositions.

^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm 8.32–7.45, 6.81, 6.11, 4.42, 3.15–2.58, 1.85–0.68.

GPC (DMF) M_n = 14 kDa, Đ = 1.49.

Polymer NHS esters P1-NHS – P5-NHS

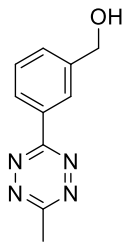
Polymers **P1** to **P5** (1 equiv., 10 mg) and 10 equiv. of *N*-hydroxysuccinimide (0.88 mg, 1.5 mg, 0.50 mg, 0.93 mg, 0.80 mg) were dissolved in anhydrous DMF and stirred for 5 min, followed by addition of 10 equiv. *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (1.5 mg, 2.5 mg, 0.83 mg, 1.5 mg, 1.3 mg for **P1** to **P5** respectively). The reaction mixture was stirred under a N_2 atm for 16 h and the product precipitated from Et_2O (50 mL). The polymer active esters were re-dissolved in a minimum amount of DMF and precipitated from Et_2O a further 3 times before drying *in vacuo*. Representative NMR data is reported for **P1-NHS**. See Table 1 for full GPC data and polymer compositions.

^{1}H NMR (600 MHz, DMSO-d_6): δ/ppm 7.96, 6.45–5.78, 4.59, 3.18–2.65, 1.87–0.62.

GPC (DMF) Mn = 16 kDa, Đ = 1.65.

2.5 Synthesis of Tz2

(3-(6-Methyl-1,2,4,5-tetrazin-3-yl)phenyl)methanol 3[8]



S8

3-(Hydroxymethyl)benzonitrile (200 mg, 1.5 mmol), $Ni(OTf)_2$ (268 mg, 0.75 mmol), acetonitrile (3.9 mL, 75 mmol), and hydrazine monohydrate (727 µL, 75 mmol) were mixed in a microwave vial and sealed. The reaction mixture was stirred for 48 h at 60 °C. After cooling to 0 °C, water (10 mL) was added and the mixture

was extracted with DCM (2 × 20 mL). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in anhydrous DCM (5 mL), $PhI(OAc)_2$ (725 mg, 2.3 mmol) was added, and the reaction mixture was stirred for 3 h. The solvent was evaporated *in vacuo* and the crude product was purified by flash column chromatography (eluting with hexane/EtOAc, 7:3) to give the compound **S8** as a pink solid (127 mg, 42%).

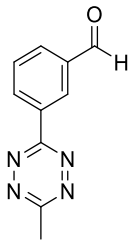
^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm 8.58 (m, 1H), 8.51 (d, *J* = 7.7 Hz, 1H), 7.65–7.59 (m, 2H), 4.84 (s, 2H), 3.10 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ/ppm 167.5, 164.2, 142.3, 132.2, 131.2, 129.7, 127.3, 126.4, 65.1, 21.3.

HRMS (ESI) for $C_{10}H_{11}ON_4$ $[M+H]^+$: *calcd.*: 203.0927 ; *found*: 203.0928.

Data in agreement with the literature.[8]

3-(6-Methyl-1,2,4,5-tetrazin-3-yl)benzaldehyde S9[8]



S9

Compound **S8** (127 mg, 0.62 mmol) and Dess–Martin periodinane (291 mg, 0.69 mmol) were dissolved in DCM (10 mL) and the reaction mixture was stirred 2 h at room temperature. The mixture was filtered and the filtrate was concentrated *in vacuo*. The crude product was purified by flash column chromatography (eluting with hexane/EtOAc, 7:3) to give compound **S9** as a pink solid (99 mg, 80%).

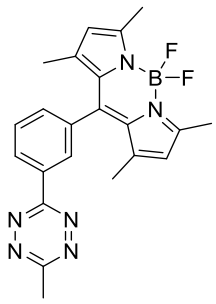
^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 10.18 (d, *J* = 1.7 Hz, 1H), 9.11 (td, *J* = 1.7, 0.5 Hz, 1H), 8.87 (ddd, *J* = 7.8, 1.8, 1.2 Hz, 1H), 8.17–8.15 (m, 1H), 7.79 (ddd, *J* = 8.4, 7.8, 0.5 Hz, 1H), 3.14 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm 191.5, 168.0, 163.5, 137.4, 133.4, 133.0, 132.6, 130.1, 130.0, 21.4.

HRMS (ESI) for $C_{10}H_9ON_4$ $[M+H]^+$: *calcd.*: 201.0771 ; *found*: 201.0779.

Data in agreement with the literature.[8]

Tetrazine Tz2



Tz2

Compound **S9** (99 mg, 0.49 mmol) and 2,4-dimethylpyrrole (102 µL, 0.99 mmol) were dissolved in anhydrous THF (10 mL) under an Ar atm. TFA (10 µL) was added and the reaction mixture was stirred at room temperature for 18 h. The mixture was cooled to 0 °C and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 225 mg, 0.99 mmol) in anhydrous THF (6 mL) was added dropwise, and mixture was stirred at room temperature for 5 h under an Ar atm. Triethylamine (1.5 mL, 10 mmol) was added under Ar at 0 °C and the reaction was stirred for additional 30 min. Then, $BF_3{\cdot}OEt_2$ (1.8 mL, 15 mmol) was added dropwise at 0 °C and the mixture stirred at room temperature for 18 h. The solvent was removed *in vacuo* and the residue was dissolved in DCM (30 mL) and washed with NH_4Cl (2 × 20 mL) and brine (2 × 20 mL). After evaporation, the crude product was purified by flash column chromatography (eluting with toluene/hexane, 4:1) to give compound **Tz2** as red crystals (79 mg, 38%).

^{1}H NMR (500 MHz, $CDCl_3$): δ/ppm 8.72 (ddd, *J* = 8.0, 1.8, 1.2 Hz, 1H), 8.59 (d, *J* = 1.8 Hz, 1H), 7.75 (t, *J* = 7.8 Hz, 1H), 7.58 (ddd, *J* = 7.6, 1.8, 1.2 Hz, 1H), 6.00 (s, 2H), 3.11 (s, 3H), 2.57 (s, 6H), 1.44 (s, 6H).

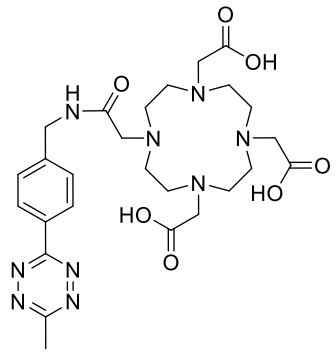
^{13}C NMR (125 MHz, $CDCl_3$): δ/ppm 167.8, 163.8, 156.2, 143.0, 140.3, 136.4, 133.1, 132.4, 131.5, 130.3, 128.5, 127.8, 121.7, 21.4, 15.0. 14.8.

HRMS (ESI) for $C_{22}H_{22}{}^{11}BF_2N_6$ $[M+H]^+$: *calcd.*: 419.1962; *found*: 419.1962.

Data in agreement with the literature.[1]

2.6 Synthesis of Cu(II) labelled tetrazine Tz3

DOTA tagged tetrazine S12[3]



S11

DOTA tagged tetrazine **S11** was synthesised according a published procedure [3] with modification. Briefly, amino tetrazine **S10[2]** (10 mg, 50 µmol) was dissolved in anhydrous THF (2 mL) and DIPEA (13 µL, 75 µmol) was added, and the solution was degassed by freeze-pump-thaw cycles (× 3). DOTA NHS ester (41.6 mg, 55 µmol) was added in one portion and the reaction mixture was stirred under an Ar atm for 24 h. The solvent was removed *in vacuo* and crude product was purified by reverse phase column chromatography using a gradient of acetonitrile (5% to 95%) with water as the eluent (10 mg, 31%).

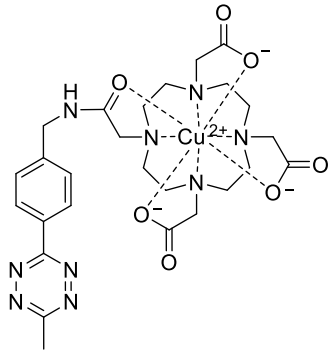
^{1}H NMR (500 MHz, CD_3OD): δ/ppm 8.51 (d, *J* = 8.4 Hz, 2H), 8.16 (s, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 4.52 (s, 2H), 3.80 – 3.72 (m, 4H), 3.64 – 3.38 (m, 12H), 3.05 (s, 11H).

^{13}C NMR (125 MHz, CD_3OD): δ/ppm 173.5, 170.9, 168.6, 167.3, 163.9, 144.0, 130.7, 128.1, 127.5, 56.3, 56.1, 55.4, 53.3, 51.2, 50.6, 48.8, 48.6, 42.6, 19.6.

HRMS (ESI) for $C_{26}H_{37}N_9O_7$ $[M+H]^+$: *calcd.*: 586.2728; *found*: 586.2743.

Data in agreement with the literature.[3]

Cu(II) loaded tetrazine Tz3



Tz3

To **S11** (1.7 mM, 1 mL) in 0.2M NaOAc buffer (pH = 6.0), $CuCl_2$ aqueous solution (170 mM, 10 µL) was added and the reaction mixture was shaken at 40 °C for 30 min. The mixture was loaded on a reverse phase chromatography column and the product eluted using a gradient of acetonitrile (5% to 95%) in water to give **Tz3** as a pink powder (0.8 mg, 80%).

^{1}H NMR (500 MHz, D_2O): δ/ppm 8.49 (m, 2H), 7.89 (m, 2H), 4.99 (s, 2H), 3.03 (s, 3H).

^{13}C NMR (125 MHz, D_2O): δ/ppm 169.9, 167.3, 164.1, 130.6, 128.7, 128.4, 36.3, 20.1.

HRMS (ESI) for $C_{26}H_{35}CuN_9O_7$ $[M+Na]^+$: *calcd.*: 671.1869; *found*: 671.1848.

2.7 Synthesis of the polymer–protein conjugates

P1-BSA, P2-BSA and P3-BSA

To BSA (0.9 mg, 14 nmol) in PBS (100 µL, pH = 7.4), 10 eq. polymer active ester **P1-NHS**, **P2-NHS**, or **P3-NHS** (1.8 mg, 1.0 mg, or 3.2 mg, respectively) were added and the mixture was stirred at 37 °C for 4 h. The solution was diluted with PBS (5 mL) before being concentrated to 100 µL using an Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa, 30 kDa, 100 kDa for **P1-BSA**, **P2-BSA** and **P3-BSA**, respectively) and the dilution and concentration were repeated 10 times to remove any unreacted polymer. The protein conjugate solutions were stored at 4 °C.

P1-Her – P5-Her

To Herceptin (20 mg/mL, 137 µM, 20 µL) in PBS, 10 eq. of the polymer active esters **P1-NHS** to **P5-NHS** (0.4 mg, 0.2 mg, 0.7 mg, 0.4 mg, 0.4 mg, respectively) were added. The solutions were stirred at 37 °C for 4 h, subsequently diluted with PBS (5 mL, pH = 7.4), and concentrated to 100 µL using an Amicon Ultra-15 centrifugal filter (molecular weight cut off 100 kDa), and the dilution and concentration were repeated for 10 times to remove any unreacted polymer. The protein–polymer conjugate solutions were stored at 4 °C.

Purification for polymer–protein conjugates

The polymer–protein conjugates were purified using an immobilised metal-affinity chromatography resin following the manufacturer's instructions. Briefly, Ni-charged Profinit IMAC Resin slurry (500 µL) was transferred to a SPE filter cartridge (12 mL with a polyethylene frit with 20 µm porosity, Sigma-Aldrich) and washed with deionised water (3 × 5 mL) and drained. The polymer–protein conjugates (20 µL, 14 µM) were solvent exchanged into the washing buffer (50 mM sodium phosphate, 300 mM NaCl, 5 mM imidazole, pH = 8.0) using Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa), concentrated to 200 µL, and added to the immobilised metal-affinity chromatography resin. The resin slurry was shaken for 30 min, the solvent drained, and the resin washed with washing buffer (3 × 5 mL) and then treated with elution buffer (3 × 5 mL, 50 mM sodium phosphate, 300 mM NaCl and either 500 mM imidazole (pH = 8.0) or a gradient concentration of 10–500 mM imidazole, pH = 8.0). The solution was collected by filtration before being concentrated to 100 µL using an Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa) and washed with PBS (pH = 7.4) 10 times. The protein-conjugate yields were determined by measuring the absorbance of the protein samples at 280 nm using a NanoDrop® ND-1000 UV-Vis Spectrophotometer and calibrated with 5 concentrations of the native corresponding protein solutions (1 µL sample volume, n = 3). The protein–polymer solutions were stored at 4 °C.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed on a Bio-Rad Laboratories Mini-Protean 3 Cell System using a 4–20% Mini-PROTEAN TGX precast protein gels, run at 100 V and 95 mA for 90 min. Samples were dissolved in PBS (10 µL, 1.37 µM, pH = 7.4), mixed with 2× Laemmli Sample Buffer (10 µL)[9] (with β-mercaptoethanol) and heated at 95 °C for 5 min before loading. Staining was carried out with Coomassie blue (0.1% *w*/*v* in 1:4:5 acetic acid/water/MeOH for 2 h). Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standards, 10–250 kDa (Bio-Rad) were used as markers. Bright field and fluorescent images (λ_{ex} = 365 nm, λ_{em} = 520 ± 30 nm) of gels were taken using a Universal Hood II Gel Doc System (Bio-Rad).

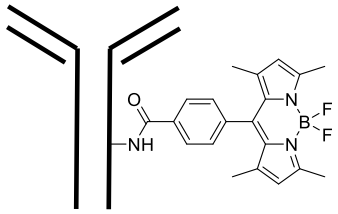
2.8 In vitro fluorescence "switch on" of Tz1 and Tz2

Solutions of **Tz1** and **Tz2** (1 µM in PBS from a freshly prepared 10 mM stock in DMSO) were prepared. The fluorescence spectra were recorded using a fluorimeter using a quartz cuvette (λ_{ex} = 488 nm). Polymer **P1** (10 mM in PBS) was added to the above mentioned solution (to give a final concentration of 12 µM) and incubated at 37 °C for 30 min and the fluorescence spectra were directly recorded.

2.9 *In vitro* BODIPY labelling of polymer–antibody conjugates

To the polymer antibody conjugates **P1-Her**, **P2-Her** and **P3-Her** in pH 7.4 PBS (10 µL, 2.7 µM, pH = 7.4), **Tz1** in DMSO (1 µL, 274 µM) was added and the mixtures were shaken at 37 °C for 4 h. The mixtures were diluted with PBS (5 mL) and concentrated to 100 µL using an Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa), and the dilution and concentration were repeated for 10 times to give **BODIPY-P1-Her**, **BODIPY-P2-Her** and **BODIPY-P3-Her**.

2.10 Synthesis of the BODIPY labelled antibody BODIPY-Her



BODIPY-Her

BODIPY-NHS **S12**[10] in DMSO (2 µL, 14 mM) was added to Herceptin in PBS (pH = 7.4, 137 µM, 20 µL), and the mixture was shaken at 37 °C for 4 h. The mixture was diluted with PBS (5 mL) and concentrated to 100 µL using an Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa), and the dilution and concentration were repeated for 10 times. The labelled-antibody solutions were stored at 4 °C.

2.11 Herceptin binding

Binding of Herceptin–polymer conjugates **BODIPY-P1-Her**, **BODIPY-P2-Her** and **BODIPY-P3-Her** were determined by measuring the rate of fluorescence intensity increase on individual cells over time by flow cytometry, and compared to **BODIPY-Her**[10]. SK-BR-3 cells were seeded in 24-well plates (5×10^4 cells/well in 500 µL DMEM) 24 h prior to the experiments. **BODIPY-Her**, **BODIPY-P1-Her**, **BODIPY-P2-Her** or **BODIPY-P3-Her** (all at 10 nM) were added to the cells and incubated for different period of time (0.5, 1, 2, 4 and 6 h). The cells were washed with PBS (3 × 1 mL, pH = 7.4), harvested with trypsin/EDTA (0.25% trypsin, 1 mM EDTA), and the fluorescence intensity was analysed by flow cytometry, Becton Dickinson (BD) FACSAria™, laser excitation at 488 nm and emission filter of 530/30 nm). The fluorescence intensities were normalised to the intensity of saturated cells. Each of the antibody samples was incubated with MCF-7 cells as a negative control.

2.12 Cell viability

Cell viability was evaluated using an MTT assay. Briefly, SK-BR-3 cells were seeded in a 96-well plate (1×10^4 cells/well) and incubated overnight. The cells were treated with the desired compound at different concentrations in DMEM for 24 h. The media was removed and the cells were washed with PBS (× 3) and incubated with 100 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (1 mg/mL) for 4 h at 37 °C. Then 100 µL of MTT solubilisation solution (10% Triton-X 100 in 0.1 N HCl in isopropanol) was added to each well and the plate was shaken horizontally for 30 min to dissolve the formazan crystals. The absorbance at 570 nm was measured on a multimode plate reader and the cell viability was calculated compared to untreated cells.

2.13 Fluorescence “switch on” and amplification

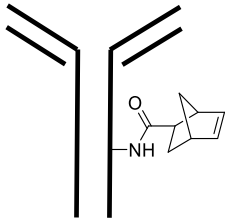
SK-BR-3 cells were seeded on 24-well plates (5×10^4 cells/well in 500 µL DMEM) 24 h prior to the experiments. **BODIPY-Her** (10 nM) or the polymer antibody conjugates **P1-Her**, **P2-Her**, **P3-Her**, **P4-Her** and **P5-Her** (10 nM)

were added to the cells and incubated for 4 h (n = 3). The cells were washed with PBS (3 × 1 mL, pH = 7.4) and treated with **Tz1** (1 μM) for 30 min before washing with PBS (3 × 1 mL, pH = 7.4). The cells were harvested with trypsin/EDTA (0.25% trypsin, 1 mM EDTA) and analysed by flow cytometry (Becton Dickinson (BD) FACSAria™, laser excitation at 488 nm and emission filter of 530/30 nm). For confocal microscopy, the cell nuclei were stained with Hoechst 33342 (blue, $\lambda_{ex/em}$ = 353/483 nm), the plasma membrane stained with CellMaskTM Deep Red (red, $\lambda_{ex/em}$ = 649/666 nm) and the cells were imaged using a Zeiss LSM 880 Airyscan confcocal microscope with a 40x / 1.3 oil immersion objective.

Fold increases of the fluorescence intensities of the cells were determined (defined as the value of the average fluorescence intensity of the cell population treated first with the polymer–Herceptin conjugates and then **Tz1** divided by the average fluorescence intensity of the cell population treated with **BODIPY-HER)**.

2.14 Synthesis of Cu(II) labelled antibody Cu-Her

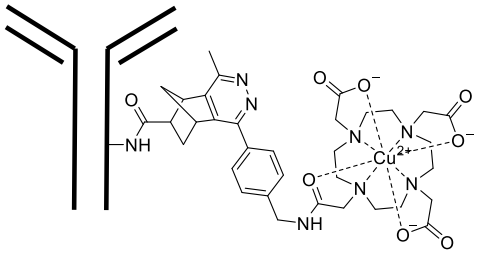
Norbornene labelled antibody S14



S14

To Herceptin in PBS (pH = 7.4, 137 μM, 20 μL), norbornene NHS ester **S13**[11] in DMSO (2 μL, 14 mM) was added and the mixture was stirred at 37 °C for 4 h. The mixture was diluted with PBS (5 mL) and concentrated to 100 μL using an Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa), and the dilution and concentration were repeated for 10 times. The norbornene labelled antibody solutions were stored at 4 °C.

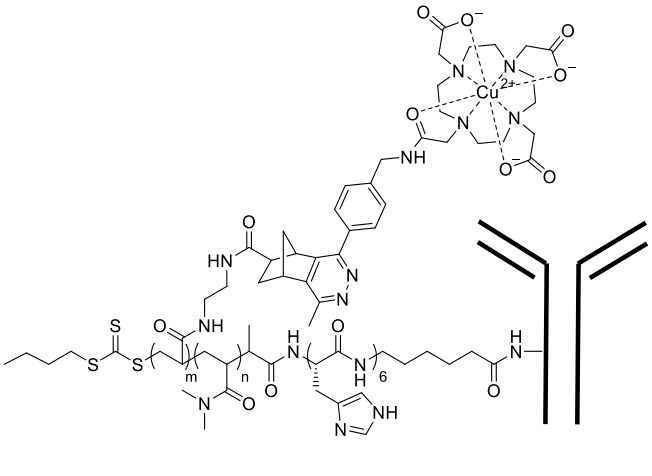
Cu(II) loaded antibody Cu-Her



Cu-Her

Tz3 (1.36 mM, 40 μL) in PBS was added to **S14** (27.4 μM, 20 μL) in PBS and the solution shaken at 37 °C for 4 h. The mixture was diluted with PBS (5 mL) and concentrated to 100 μL using an Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa), and the dilution and concentration were repeated for 10 times. The Cu(II) loaded antibody solutions were stored at 4 °C prior to use.

2.15 Synthesis of Cu(II) labelled polymer–antibody conjugate Cu-P5-Her



Cu-P5-Her

To a solution of **P5-Her** (27.4 μM, 20 μL) in PBS, **Tz3** (1.36 mM, 40 μL) in PBS was added and the solution was shaken at 37 °C for 4 h. The mixture was diluted with PBS (5 mL) and concentrated to 100 μL using an Amicon Ultra-15 centrifugal filter (molecular weight cut off 30 kDa), and the dilution and concentration were repeated for 10 times. The Cu(II) loaded polymer–antibody solutions were stored at 4 °C.

2.16 Quantification of copper loading in cells

SK-BR-3 cells were seeded in 24-well plates (5×10^4 cells/well in 500 μL DMEM) 24 h prior to the experiments. **Tz3** (1 μM), **S14** (10 nM), **P5-Her** (10 nM), **Cu-Her** (10 nM) or **Cu-P5-Her** (10 nM) were added to the media and incubated at 37 °C for 4 h (n = 6). The cells were washed (3 × PBS) and harvested with trypsin/EDTA into aqueous NaOH (0.1%, *w*/*v*, 2 mL) followed by sonication for 10 min. The solutions were diluted to 5 mL (with water) and filtered through 0.45 μm filters and analysed on an Agilent 7500ce ICP-MS system.

3. NMR Spectra

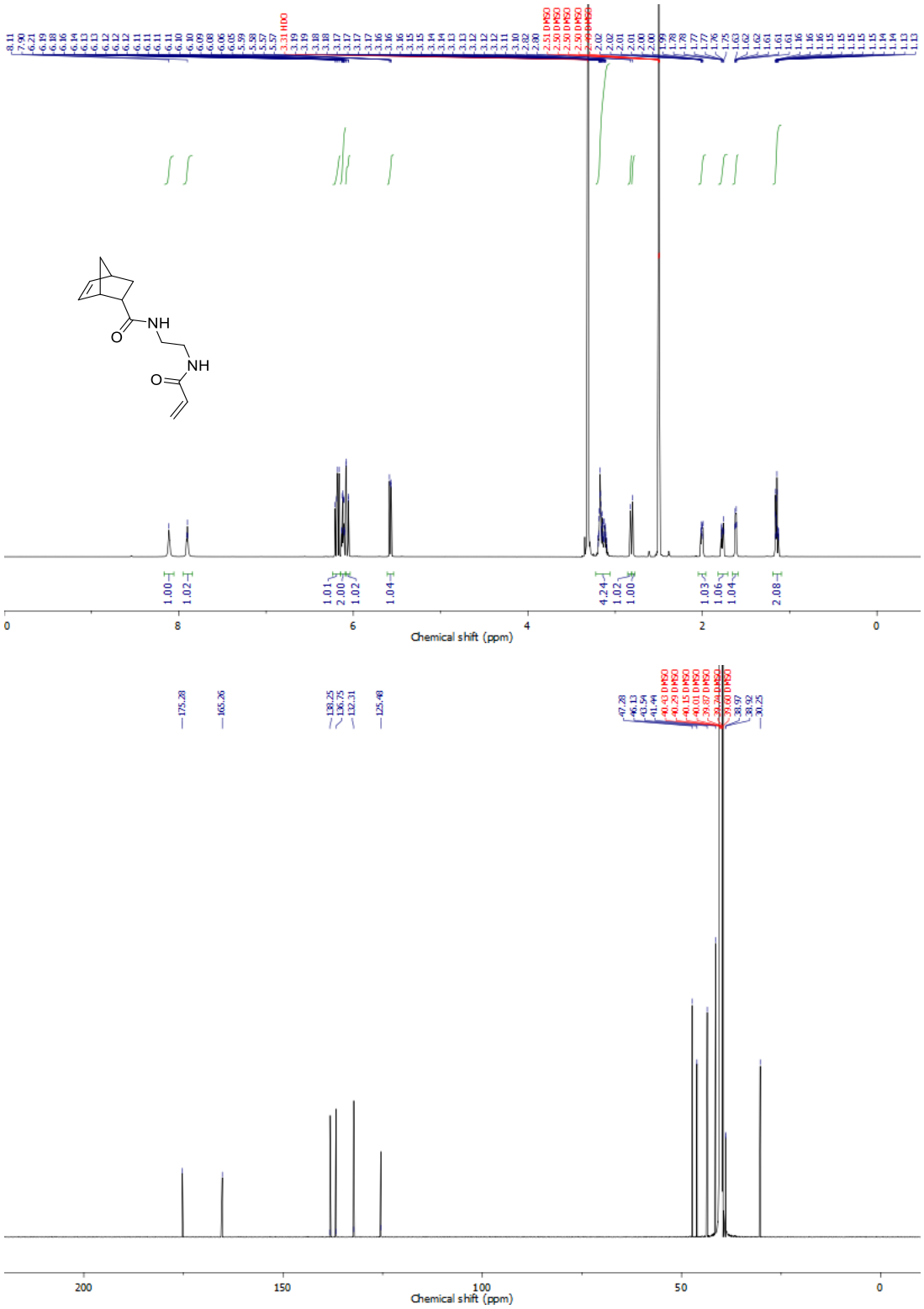


Figure S15. ^{1}H and ^{13}C NMR spectra of compound **1** recorded in DMSO-d_6 at 600 MHz and 150 MHz, respectively.

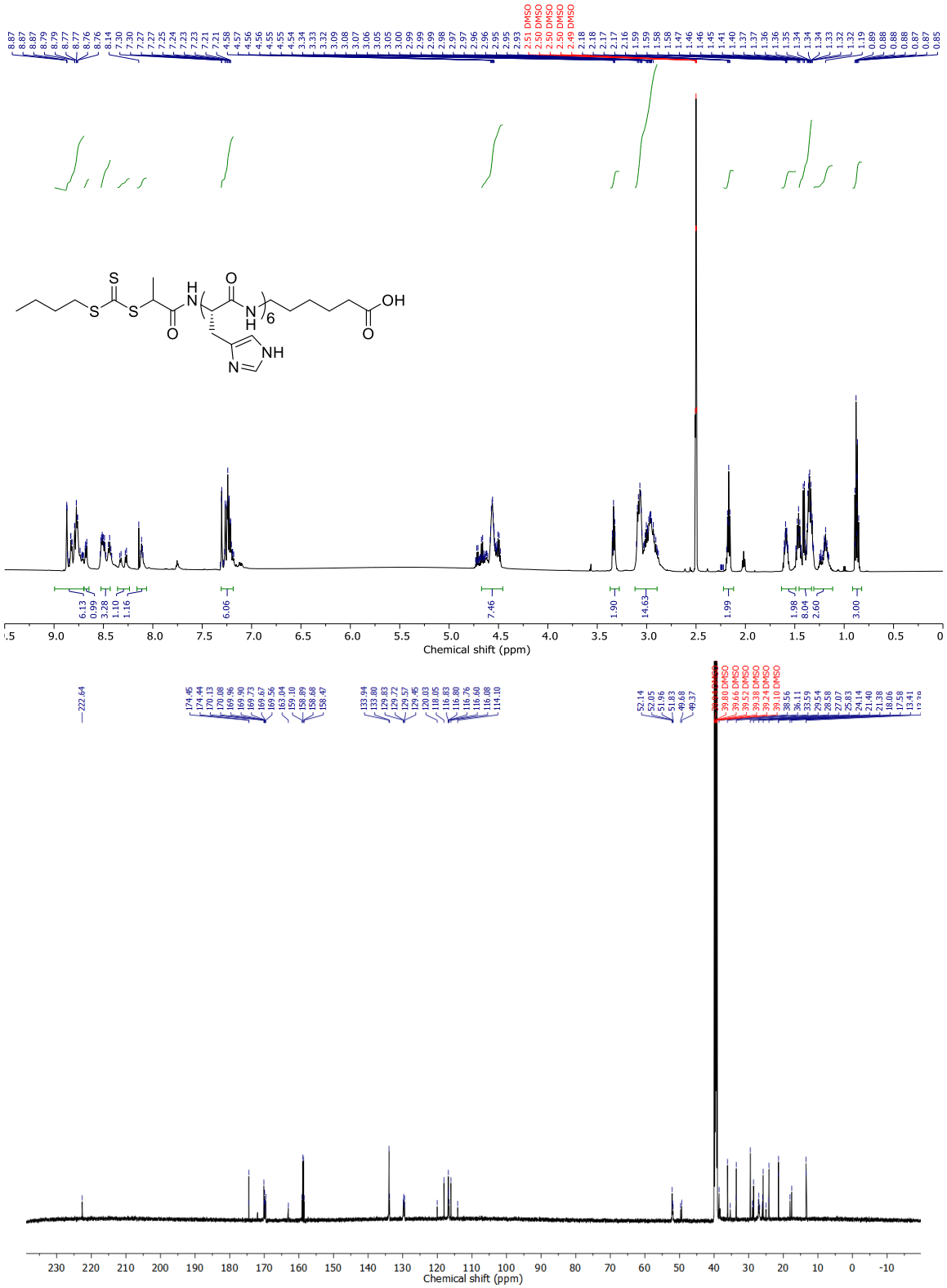


Figure S16. ^{1}H and ^{13}C NMR spectra of compound **2** recorded in DMSO-d_6 at 600 MHz and 150 MHz, respectively.

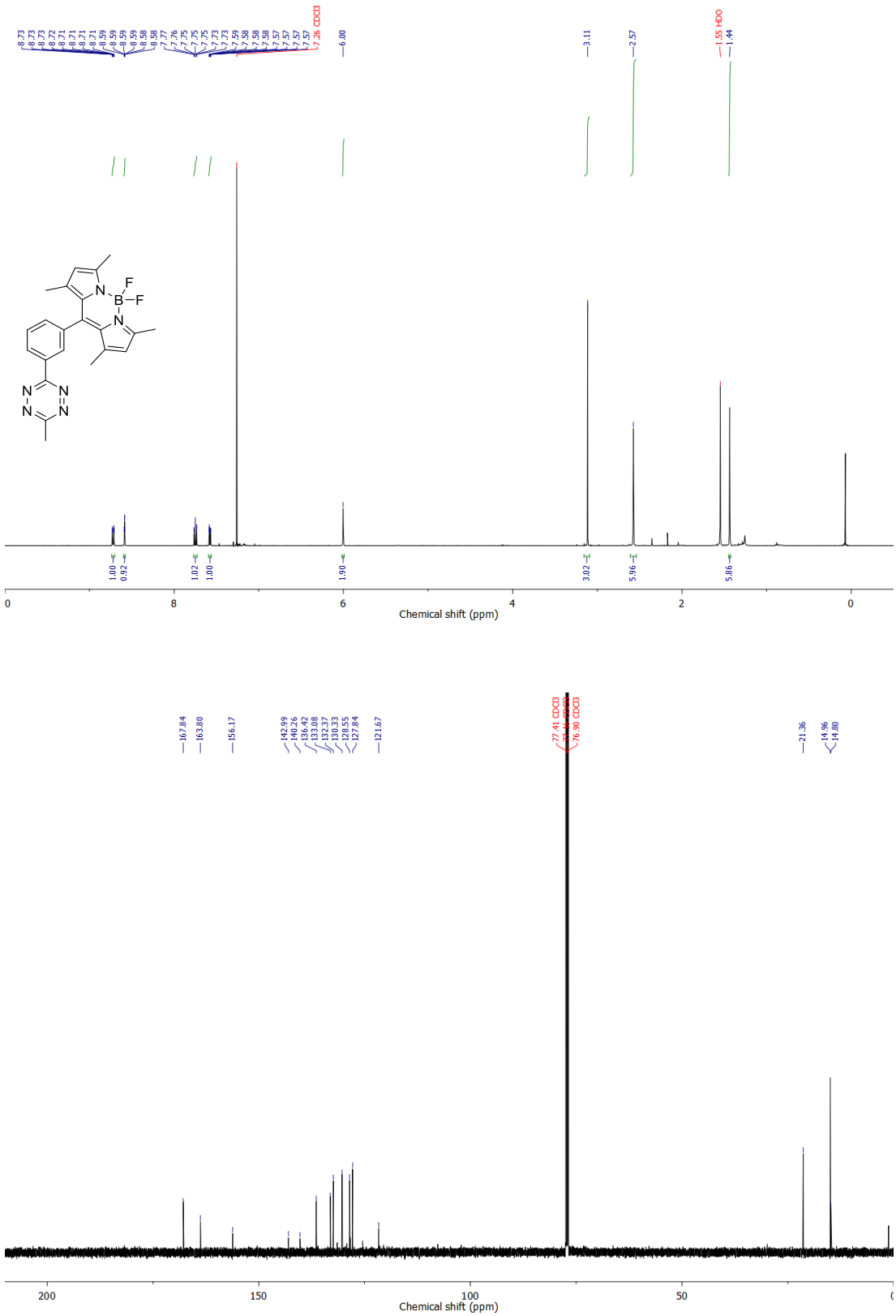


Figure S17. ^{1}H and ^{13}C NMR spectra of compound **Tz2** recorded in $CDCl_3$ at 500 MHz and 125 MHz, respectively

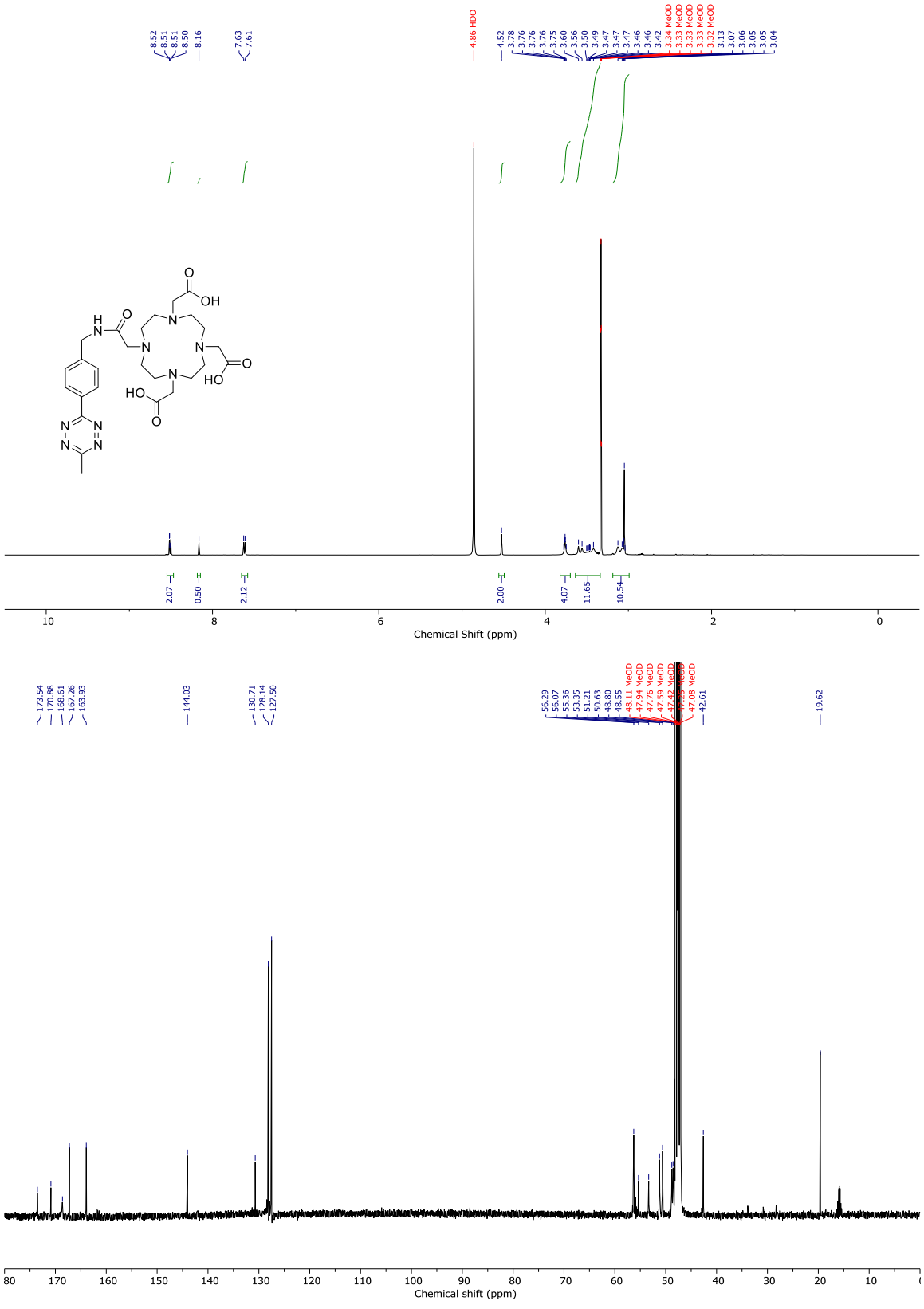


Figure S18. ^{1}H and ^{13}C NMR spectra of compound **S11** recorded in CD_3OD at 500 MHz and 125 MHz, respectively.

4. References

1 J. C. Carlson, L. G. Meimetis, S. A. Hilderbrand, R. Weissleder, *Angew. Chem. Int. Ed.*, 2013, **52**, 6917.
2 R. Sen, D. Gahtory, J. Escorihuela, J. Firet, S. P. Pujari, H. Zuilhof, *Chem. Eur. J.*, 2017, **23**, 13015.
3 H. L. Evans, L. Carroll, E. O. Aboagye, A. C. Spivey, *J. Labelled Compd. Radiopharm.*, 2014, **57**, 291.
4 J. Schmitt, H. Hess, H. G. Stunnenberg, *Mol. Biol. Rep.*, 1993, **18**, 223.
5 F. M. Pfeffer, P. E. Kruger, T. Gunnlaugsson, *Org. Biomol. Chem.*, 2007, **5**, 1894.
6 E. Kaiser, R. Colescott, C. Bossinger, P. Cook, *Anal. Biochem.*, 1970, **34**, 595.
7 C. J. Ferguson, R. J. Hughes, B. T. Pham, B. S. Hawkett, R. G. Gilbert, A. K. Serelis, C. H. Such, *Macromolecules*, 2002, **35**, 9243.
8 A. Wieczorek, P. Werther, J. Euchner, R. Wombacher, *Chem. Sci.*, 2017, **8**, 1506.
9 S. Cannon-Carlson, J. Tang, *Anal. Biochem.*, 1997, **246**, 146.
10 A. B. Nepomnyashchii, A. J. Pistner, A. J. Bard, J. Rosenthal, *J. Phys. Chem. C*, 2013, **117**, 5599.
11 L. E. Strong, L. L. Kiessling, *J. Am. Chem. Soc.*, 1999, **121**, 6193.