Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Functional native disulfide bridging enables delivery of a potent, stable and targeted antibody-drug conjugate (ADC)

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Sections Included

Conjugation Experiments General Remarks

Conjugation experiments were carried out in standard polypropylene micro test tubes 3810x at atmospheric pressure with mixing at 20 °C unless otherwise stated. Reagents and solvents were purchased from commercial sources and used as supplied. All buffer solutions were prepared with double-deionised water and filter-sterilised. Borate buffer was 25 mM Sodium Borate, 25 mM NaCl and 1 mM EDTA at pH 8.0 or pH 8.4. Phosphate-buffered saline (PBS) was 140 mM NaCl and 12 mM sodium phosphates at pH 7.4. Ultrapure DMF was purchased from Sigma-Aldrich and kept under dry conditions. Solutions of tris(2-carboxyethyl)phosphine hydrochloride (TCEP) 10 mM (2.87 mg/mL) were prepared in borate buffer. Filtration of particulates was carried out through Spin-X 0.22 µm cellulose acetate filters. Ultrafiltration was carried out in vivaspin 500 polyethersulfone (PES) membrane concentrators with a molecular weight cut-off (MWCO) of 10 kDa or in Amicon Ultra-15 low binding cellulose filters with 10 kDa MWCO. Centrifugation was carried out on an eppendorf 5415R fixed angle rotor centrifuge operating at 14000 rcf at 20 °C or in an eppendorf 5810 swing-bucket rotor centrifuge operating at 3220 rcf at 20 °C.

Trastuzumab is a chimeric IgG1 full length antibody directed against HER2. The antibody was obtained in its clinical formulation (Roche, lyophilised), dissolved in 10 ml sterile water and the buffer exchanged completely for borate buffer pH 8.0 *via* ultrafiltration (MWCO 10 kDa, Amicon). Concentration was determined by UV-vis absorbance (using ε_{280} = 215380 M^{-1} cm^{-1} for trastuzumab mAb), adjusted to 40 µM (5.88 mg/mL) and was stored in flash frozen aliquots at –20 °C. For experiments, aliquots were thawed and used immediately. ADC concentration was determined using the same extinction coefficient as for native trastuzumab (both MMAE and maleamic acid compounds were found to have negligible absorption at 280 nm). The following acronyms are used to describe antibody fragments based on their

constituent heavy and light chains: heavy-heavy-light (HHL), heavy-heavy (HH), heavy-light (HL, a.k.a. half antibody), heavy (H) and light (L) chains.

Analytical methods for antibody-drug conjugates

SDS-PAGE

Non-reducing glycine-SDS-PAGE at 12% acrylamide gels were performed following standard lab procedures. A 4% stacking gel was used and a broad-range MW marker (10-250 kDa, BioLabs) was co-run to estimate protein weights. Samples (3 µL at ~35 µM in total mAb) were quenched with maleimide (1 µL of a 12 mM solution in PBS, >100 eq.) and mixed with loading buffer (2 µL, composition for 6× SDS: 1 g SDS, 3 mL glycerol, 6 mL 0.5 M Tris buffer pH 6.8, 2 mg R-250 dye) and heated at 65 °C for 2 minutes. The gel was run at constant current (30-35 mA) for 40 min in 1× SDS running buffer. All gels were stained following a modified literature protocol[1] where 0.12 % of the Coomassie G-250 and the Coomassie R-250 dyes were added to the staining solution (5:4:1 MeOH:H_2O:AcOH). For reducing gel (using β-mercaptoethanol (BME) as reducing agent), samples (3 µL at ~35 µM in total mAb) were mixed with loading buffer (2 µL, composition for 4 x SDS: 0.8 mL BME, 0.8 g SDS, 4 mL glycerol, 2.5 mL 0.5 M Tris buffer pH 6.8, 2.5 mL H_2O, 2 mg R 250 dye). The gel was run at constant current (30-35 mA) for 40 min in 1× SDS running buffer. All gels were stained following a modified literature protocol1 where 0.12 % of the Coomassie G-250 and the Coomassie R-250 dyes were added to the staining solution (5:4:1 MeOH:H2O:AcOH). NGM conjugates (non-hydrolysed) are cleaved under reducing gel conditions, whereas NGM conjugates (hydrolysed) are not cleaved under reducing gel conditions.

Determination of fluorophore to antibody ratio (FAR)

UV-vis spectra were recorded on a Varian Cary 100 Bio UV-visible spectrophotometer, operating at 20 °C. Sample buffer was used as blank for baseline correction. Calculation of DyeAR (FAR) follows the formula below with ε_{280} = 215380 M^{-1} cm^{-1} for trastuzumab mAb, ε495 = 71000 M^{-1} cm^{-1} for Alexa Fluor 488® and 0.11 as a correction factor of the dye absorption at 280 nm.

$$\text{FAR} = \frac{(Abs_{495})/\varepsilon_{495}}{(Abs_{280} - 0.11 \times Abs_{495})/\varepsilon_{280}}$$

Alexa Fluor 488® Conjugate Serum Stability

Alexa Fluor 488® trastuzumab conjugates were diluted with 50% of human serum at a concentration of 0.1 mg/mL in sterile tubes. Sodium azide was added (final concentration 1 mM), and then the mixtures were split into 4 equal aliquots. One aliquot was immediately frozen at −80 °C. The remaining samples were transferred to a CO_2 incubator and kept at 37 °C. Aliquots were removed from the incubator after 48, 96 h and 168 h and transferred to a −80 °C freezer. After the final time point, samples were analysed by SEC-HPLC on a TSK gel G3000SWXL (7.8 mm x 30 cm) column connected to a Varian ProStar pump 500 coupled to a Varian ProStar 350 LC UV-vis and fluorescent detector. Each analysis was carried out for 30 min. at a flow rate of 0.5 mL/ min and using PBS pH 7.4 (0.1 M) containing 0.02% NaN_3 as mobile phase. Fluorescence was detected with an excitation wavelength of 495 nm and emission wavelength of 525 nm.

Hydrophobic Interaction Chromatography (HIC)

Samples of trastuzumab and ADCs (~35 μM) were diluted 2 times with water and injected (6-12 μL) onto a TSK-Gel Butyl-NPR 4.6 mm x 35 mm, 2.5 μm particle size column from Tosoh Bioscience, connected to an Agilent 1100 HPLC equipped with a diode array for UV-

vis detection. Samples were run with a step gradient from 100% buffer A (1.5 M ammonium sulfate, 25 mM sodium phosphates, pH 7) to 45% buffer B (25 mM sodium phosphates, 25% isopropanol (v/v), pH 7) over 52 minutes at a flow rate of 0.6 mL/min. The temperature was maintained at 20 °C for the duration of the run. Detection was by UV-vis absorbance at 280 nm.

Ellman's assay

Ellman's assay was carried out by mixing a 1 mM solution of 5,5'-dithio-*bis*-(2-nitrobenzoic acid) (Ellman's reagent) in PBS (10 μL) with the sample at ~35 μM (5 μL) and diluting with PBS (65 μL). The solution was incubated at 20 °C for 2 min and then absorption was measured at 280 nm (protein concentration) and 412 nm (2-nitro-5-thiobenzoic acid). A sample of 1 mM Ellman's reagent in PBS (10 μL) diluted with PBS (70 μL) was used as blank for baseline correction. Each sample in PBS was analysed in the absence and presence of Ellman's reagent, under identical concentration conditions. The absorption of maleimide at 412 nm in the absence of Ellman's reagent was subtracted from the absorption at 412 nm in the presence of Ellman's reagent. The sulfhydryl per protein ratio (SPR) was calculated as follows with ε_{412} = 14150 M^{-1} cm^{-1}:

$$\text{SPR} = \frac{(Abs_{412} - Abs_{412}(maleimide))/\varepsilon_{412}}{(Abs_{280})/\varepsilon_{280}}$$

Liquid chromatography mass spectrometry (LCMS)

Samples (22.9 μM) of albumin and its conjugate were diluted 5 times with water before LCMS analysis. LCMS was performed on samples using a Thermo Scientific uPLC connected to MSQ Plus Single Quad Detector (SQD). Column: Hypersil Gold C4, 1.9 μm 2.1 × 50 mm. Wavelength: 254 nm. Mobile Phase: 99:1 Water:MeCN (0.1% formic acid) to 1:9 Water: MeCN

(0.1% formic acid) gradient over 4 min. Flow Rate: 0.3 mL/min. MS Mode: ES+. Scan Range: m/z = 500-2000. Scan time: 1.5 s. Data obtained in continuum mode. The electrospray source of the MS was operated with a capillary voltage of 3.5 kV and a cone voltage of 50 V. Nitrogen was used as the nebulizer and desolvation gas at a total flow of 600 L/h. Ion series were generated by integration of the total ion chromatogram (TIC) over the 3.5-5.0 min range. Total mass spectra for protein samples were reconstructed from the ion series using the pre-installed ProMass software.

Enzyme-linked immunosorbent assay (ELISA)

Binding affinity to HER2 receptor was determined by ELISA. A maxisorp 96-well plate was coated for 2 h at 20 °C with HER2 (100 µL of a 0.25 µg/mL solution in PBS). One row of wells was coated with PBS only as a negative control. Next, each well was washed with PBS and blocked with a 3% BSA solution in PBS (200 µL) overnight at 4 °C. Then, the wells were washed with 0.1% Tween 20 in PBS, followed by PBS. Trastuzumab and NGM-MMAE-ADC **9** were diluted in PBS yielding the following concentrations: 30 nM, 10 nM, 3.33 nM, 1.11 nM, 0.37 nM and 0.12 nM. The dilution series was added, including PBS only and ADC at 30 nM in the absence of HER2 as negative controls. The plate was incubated for 2 h at 20 °C. Then, wells were washed and the detection antibody (100 µL of anti-human IgG, Fab-specific-HRP solution, 1:5000 in PBS) was added followed by incubation for 1 h at 20 °C. After another washing step, freshly prepared OPD solution (100 µL of 10 mg/20 mL OPD in phosphate-citrate buffer) was added to each well and the reaction was stopped by addition of 4 M HCl (50 µL). The colorimetric reaction was measured at 490 nm and the absorption was corrected by subtracting the average of negative controls. Each measurement was done in triplicates.

Conjugation Protocols

Conjugation of trastuzumab with maleimide 4 (conjugate 1)

The conjugate was prepared through an adaptation of a reported protocol.[2] To trastuzumab (22.9 μM, 300 μL, 0.0069 μmol) in borate buffer was added TCEP (22.9 mM, 0.6 μL, 2 eq.) and the reaction was incubated at 37 °C for 2 h under mild agitation. Next, maleimide **4** was prepared in dry DMF (9.16 mM) and added to the reduced trastuzumab (3 μL, 4 eq.). The concentration of DMF was corrected to 10% (v/v) and the reaction was incubated at 37 °C for 1 h. Afterwards, excess reagents were removed by ultrafiltration (10 kDa MWCO) with PBS to afford the modified trastuzumab ADC in PBS with yield 72%.

Conjugation of trastuzumab with NGM 5 (conjugates 2 and 3)

To trastuzumab (22.9 μM, 300 μL, 0.0069 μmol) in borate buffer was added TCEP (22.9 mM, 1.8 μL, 6 eq.) and the reaction was incubated at 37 °C for 2 h under mild agitation. Next, NGM **5** was prepared in dry DMF (9.16 mM) and added to the reduced trastuzumab (3.8 μL, 5 eq.). The concentration of DMF was corrected to 10% (v/v) and the reaction was incubated at 4 °C for 16 h. Afterwards, excess reagents were removed by ultrafiltration (10 kDa MWCO) with PBS to afford the modified trastuzumab conjugate in PBS with yield 80%. Hydrolysis of trastuzumab-NGM conjugate **2** to maleamic acid was promoted in BBS, pH 8 during 24 h at 20 °C. Stability of the conjugate was assessed in the presence of several reducing agents and characterized by SDS-PAGE.

Conjugation of human serum albumin to maleimide 4

Conjugate was prepared in a two step procedure. First, human serum albumin (HSA, 5 mg, 0.075 μmol) in 500 μL of PBS (pH 7.4, 5 mM EDTA) was pre-treated with dithiotreitol (DTT, 5 eq.) for 1h at 20 °C. Next, removed DTT by ultrafiltration (5 kDa MWCO) and added maleimide **4** (5 eq.), prepared in dry DMF (20 mM), to the reduced HSA (30 μM,

300 μL, 0.009 μmol). The excess reagent was removed by ultrafiltration with PBS (pH 7.4, no EDTA) to afford the modified albumin conjugate with yield 90%. This was reacted with Alexa Fluor 488® azide as a control for the serum stability study (see below).

Copper-catalysed Huisgen 1,3-dipolar cycloaddition to trastuzumab and albumin conjugates

To a solution of trastuzumab conjugate (50 μM, 50 μL, 0.0025 μmol) in PBS (pH 7.4, no EDTA) was added tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, 40 eq.) and CuSO4 (8 eq.). Next, added Alexa Fluor 488® azide in DMF (10 mM, 2.5 μL, 10 eq.), followed by sodium ascorbate (final concentration 5 mM) and the reaction mixture was incubated at 25 °C for 1 h. Excess reagents were removed by PD10 column and by ultrafiltration (GE Healthcare, 10 kDa MWCO) into fresh buffer (PBS with 5 mM EDTA). FAR values for conjugates: Conjugate **1** (with maleimide **4**) FAR 3.00. Conjugate **2** and **3** (with NGM **5**) FAR 3.91.

To a solution of albumin conjugate (50 μM, 50 μL, 0.0025 μmol) in PBS (pH 7.4, no EDTA) was added tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) (10 eq.) and CuSO4 (2 eq.). Following this, Alexa Fluor 488® azide in DMF (10 mM, 1.75 μL, 5 eq.) was added, followed by sodium ascorbate (final concentration 5 mM) and the reaction mixture incubated at 25 °C for 1 h. Excess reagents were removed by PD10 column and by ultrafiltration (GE Healthcare, 5 kDa MWCO) into fresh buffer (PBS with 5 mM EDTA). Conjugate of albumin gave fluorophore to albumin ratio of 1.00.

Conjugation of trastuzumab with NGM-MMAE 6

To trastuzumab (40 μM, 3 mL, 0.12 μmol) in borate buffer pH 8 was added TCEP (10 mM, 60 μL, 5 eq.) and the reaction was incubated at 37 °C for 3 h under mild agitation. Then, the

reduced trastuzumab solution was cooled to 4 °C. Next, NGM-MMAE **6** was prepared in dry DMF (10 mM, 60 µL, 5 eq.), diluted with DMF (340 µL) and borate buffer pH (600 µL) and immediately added to the reduced trastuzumab solution, thus giving a solution that was 10% (v/v) in DMF. The reaction was incubated at 20 °C for 5 min and at 37 °C for 40 min under mild agitation. Afterwards, excess reagents were removed by ultrafiltration (10 kDa MWCO) into borate buffer pH 8.4 and incubated at 20 °C over 72 h to effect hydrolysis of the maleimide unit to maleamic acid. Next, buffer swapped by ultrafiltration (10 kDa MWCO) into PBS to afford the modified trastuzumab NGM-MMAE-ADC **9** in PBS. Yield 83%, average DAR by HIC 3.89.

Supplementary Figures and Tables

SDS-PAGE gel and fluorescence spectra of trastuzumab conjugate 1

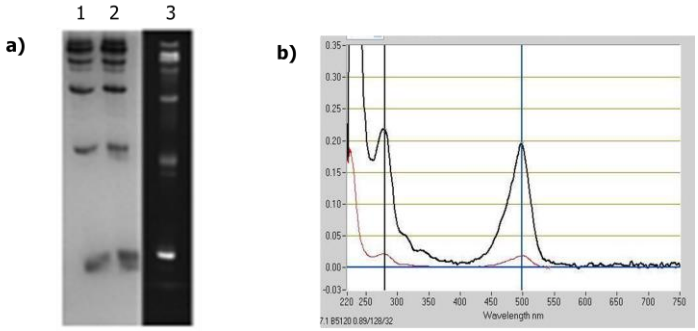


Figure S1 – **a)** SDS-PAGE gel of trastuzumab conjugate **1**. 1) partially reduced trastuzumab with TCEP (2 eq.). 2) conjugate **1** (no BME). 3) fluorescence of lane 2 under irradiation with UV-lamp (wavelength 254 nm). **b)** Fluorescence spectrum of trastuzumab conjugate **1**.

SDS-PAGE gel of NGM conjugates 2 and 3

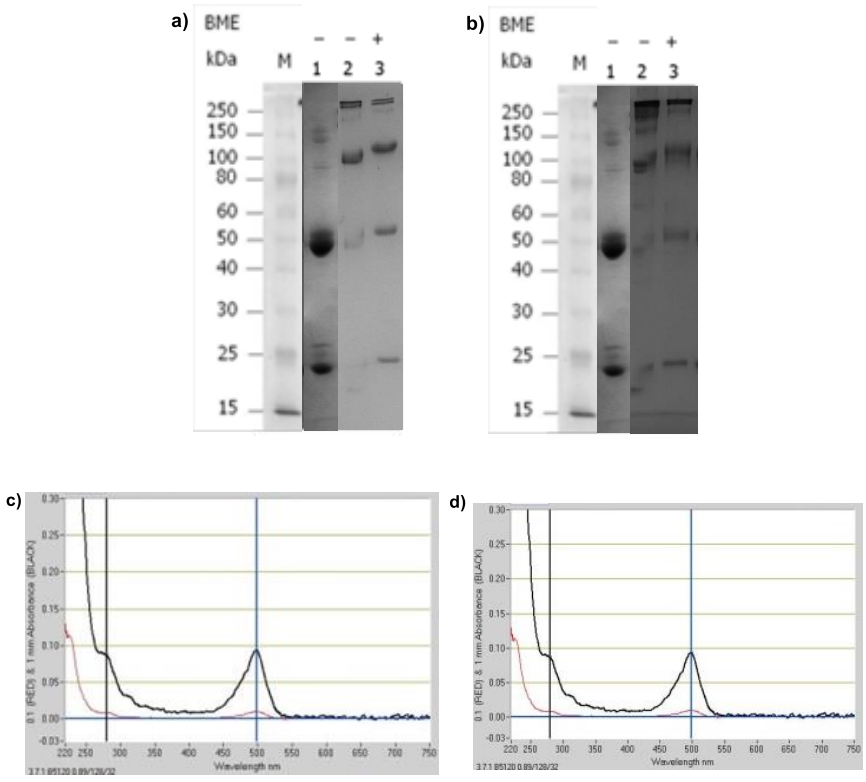
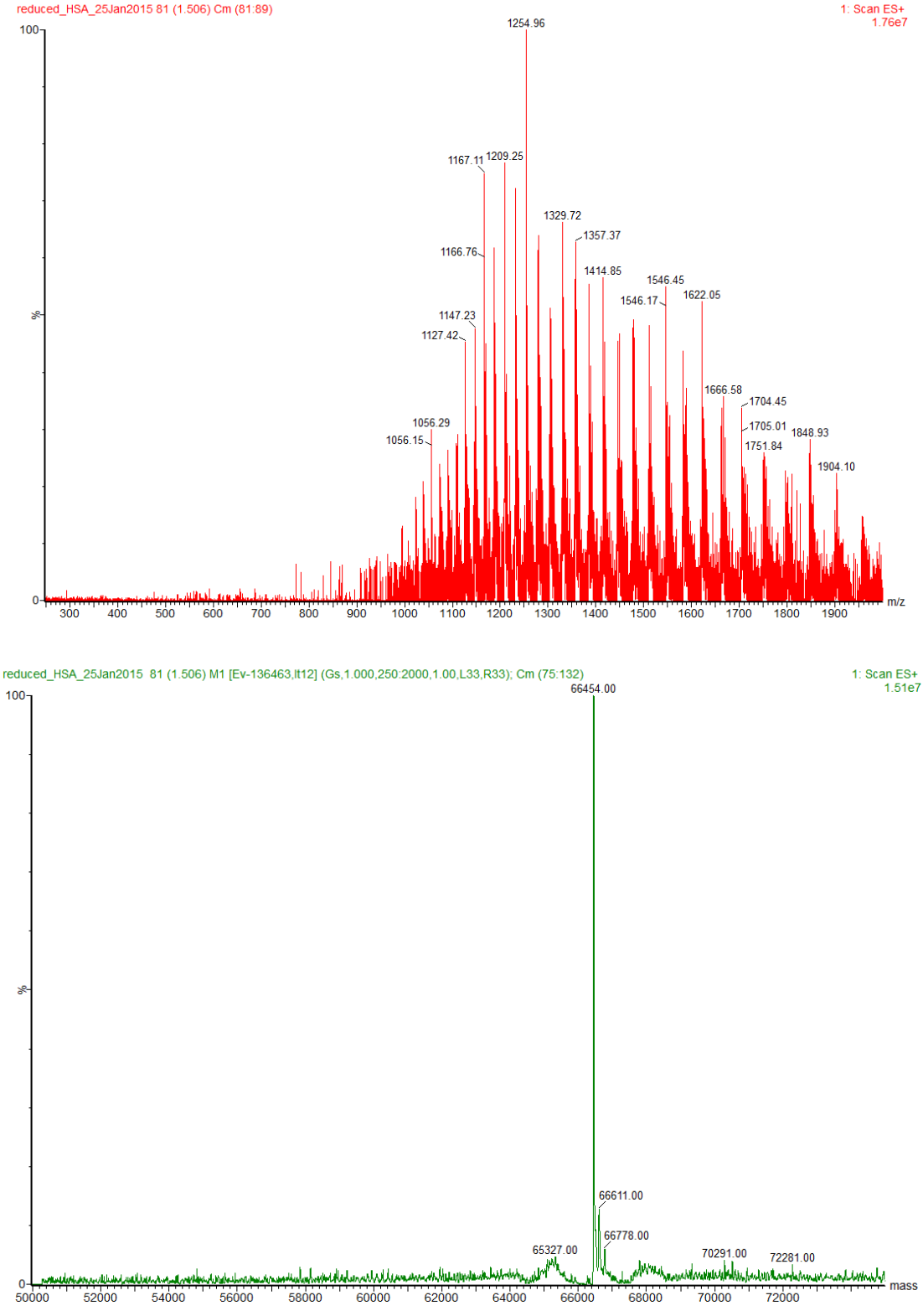


Figure S2 – SDS-PAGE gels: **a)** trastuzumab conjugate **2** (maleimide bridges). M) protein ladder. 1) reduced trastuzumab. 2) conjugate **2** (no BME). 3) conjugate **2** (with BME). **b)** trastuzumab conjugate **3** (maleamic acid bridges). M) protein ladder. 1) reduced trastuzumab. 2) conjugate **3** (no BME). 3) conjugate **3** (with BME). **c)** Fluorescence spectrum of trastuzumab conjugate **2**. **d)** Fluorescence spectrum of trastuzumab conjugate **3**.

LCMS data of albumin conjugate with maleimide 4

a)



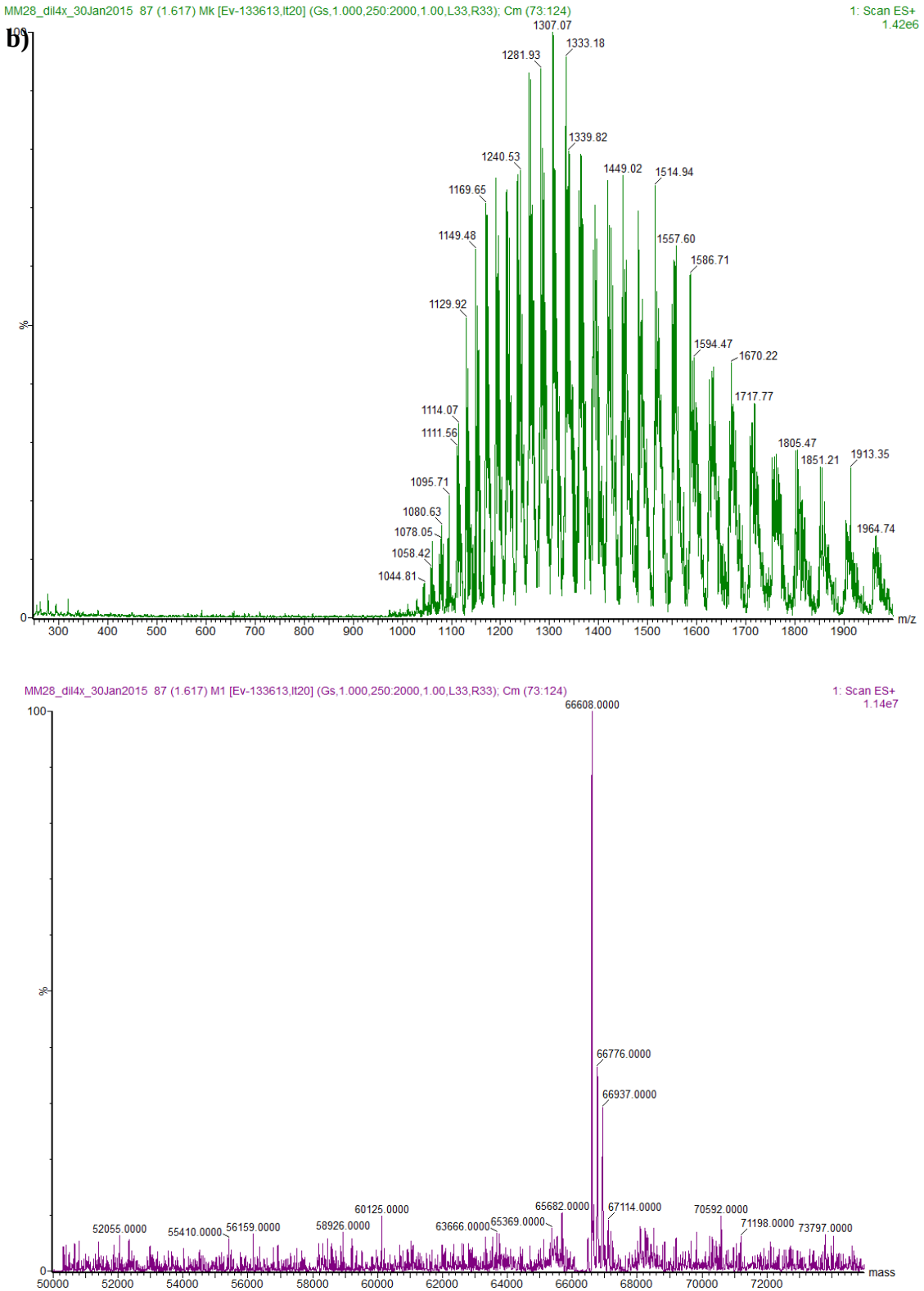
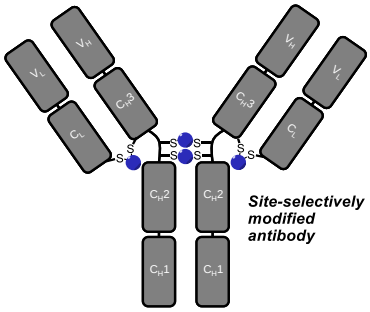
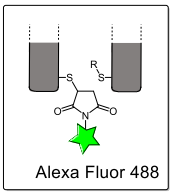


Figure S3 – ESI-MS data for albumin conjugation with maleimide **4**. Deconvoluted mass spectra of **a)** native albumin and **b)** albumin conjugate. This was reacted with Alexa Fluor 488® azide as a control for the serum stability study.

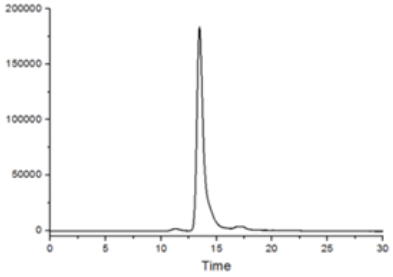
HPLC data for blood serum stability





Conjugate **1**

Time = 0 h



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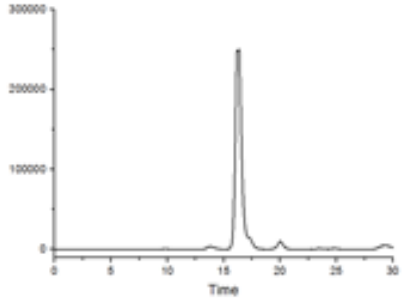

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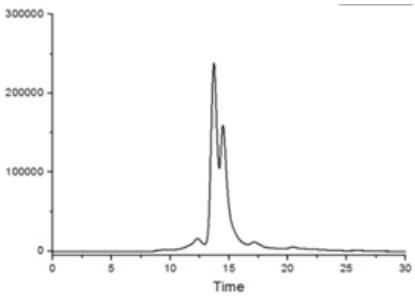
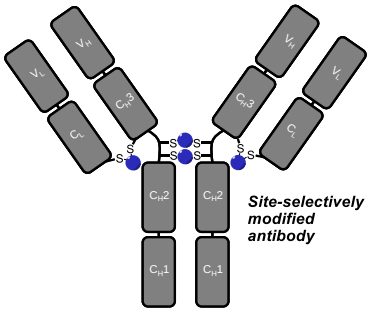
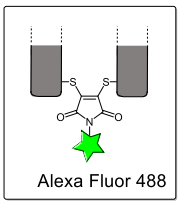


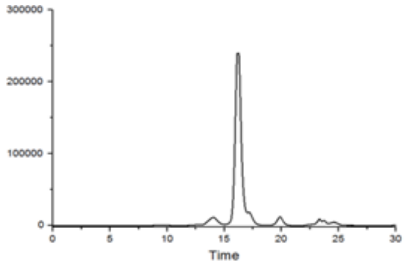


Micro-structure of each disulfide bond of modified antibody



Conjugate **2**

Time = 0 h



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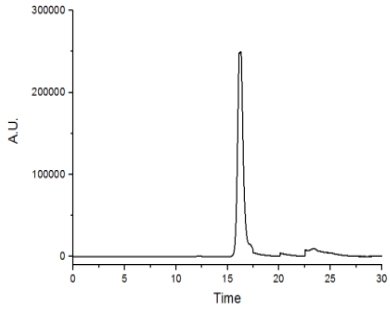

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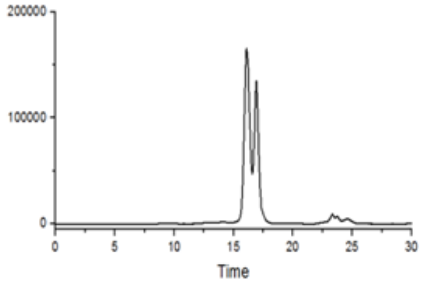
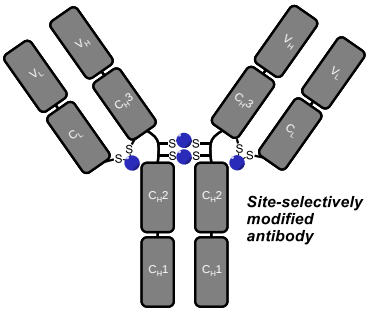
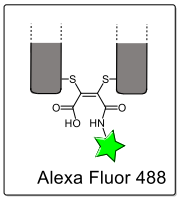




Micro-structure of each disulfide bond of modified antibody



Conjugate **3**

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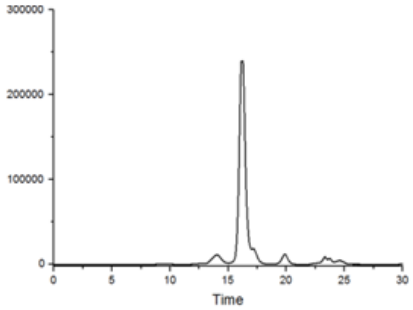

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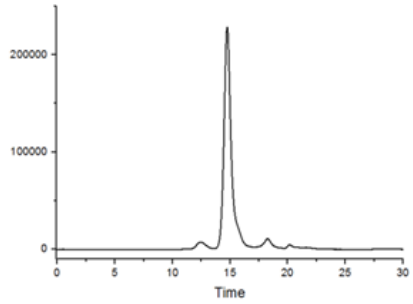

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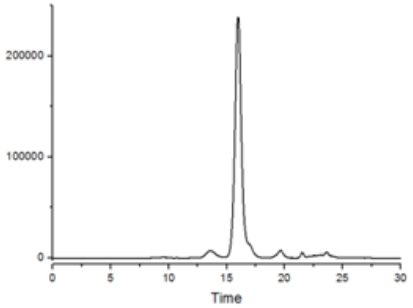
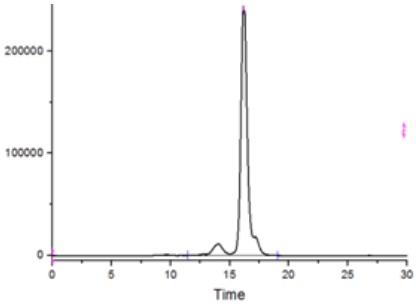


Time = 168 h



SDS-PAGE gel of NGM-MMAE-ADC 9

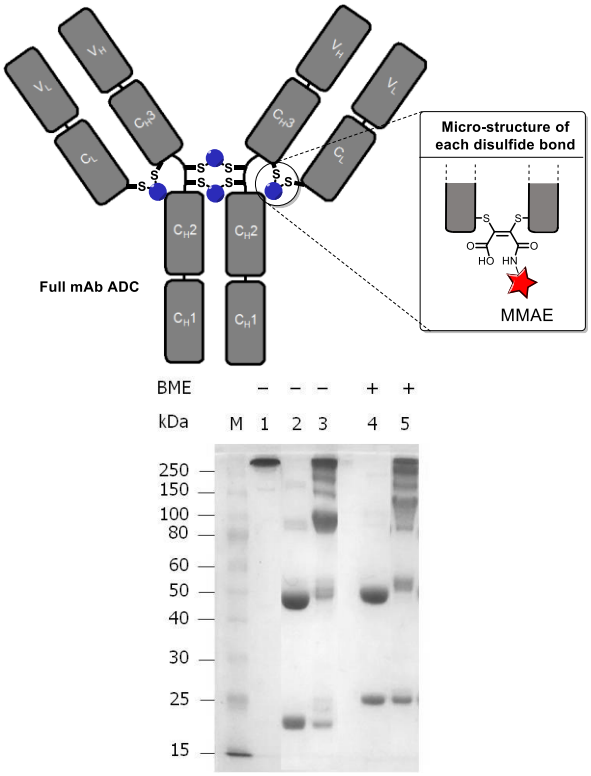


Figure S4 – SDS-PAGE gel of: M) protein ladder. 1) unmodified trastuzumab (no BME). 2) reduced trastuzumab prior to conjugation (no BME). 3) NGM-MMAE-ADC **9** (no BME). 4) unmodified trastuzumab (with BME). 5) NGM-MMAE-ADC **9** (with BME). The other major species in lane 3 at *ca.* 80-95 kDa may be attributed to site-selectively modified half-antibody, as reported previously.[2]

HIC analysis of NGM-MMAE-ADC 9

HIC analysis was carried out for NGM-MMAE-ADC **9**. Assignment of DAR peaks was established through comparison with control conjugate NGM-MMAE-ADC-CONTROL, prepared under a similar conjugate protocol, which provided a chromatogram composed of all DAR species between 0 and 5. Hydrolysis of maleimide to maleamic acid bridged disulfides in NGM-MMAE-ADC **9** was confirmed by incubation of the ADC with dithiothreitol (DTT, 100 eq.) and reanalysis by HIC. The marginal drop in average DAR indicates loss of less than 5% of MMAE loading per antibody, indicating that maleimides are effectively hydrolysed to maleamic acid disulfide bridges.

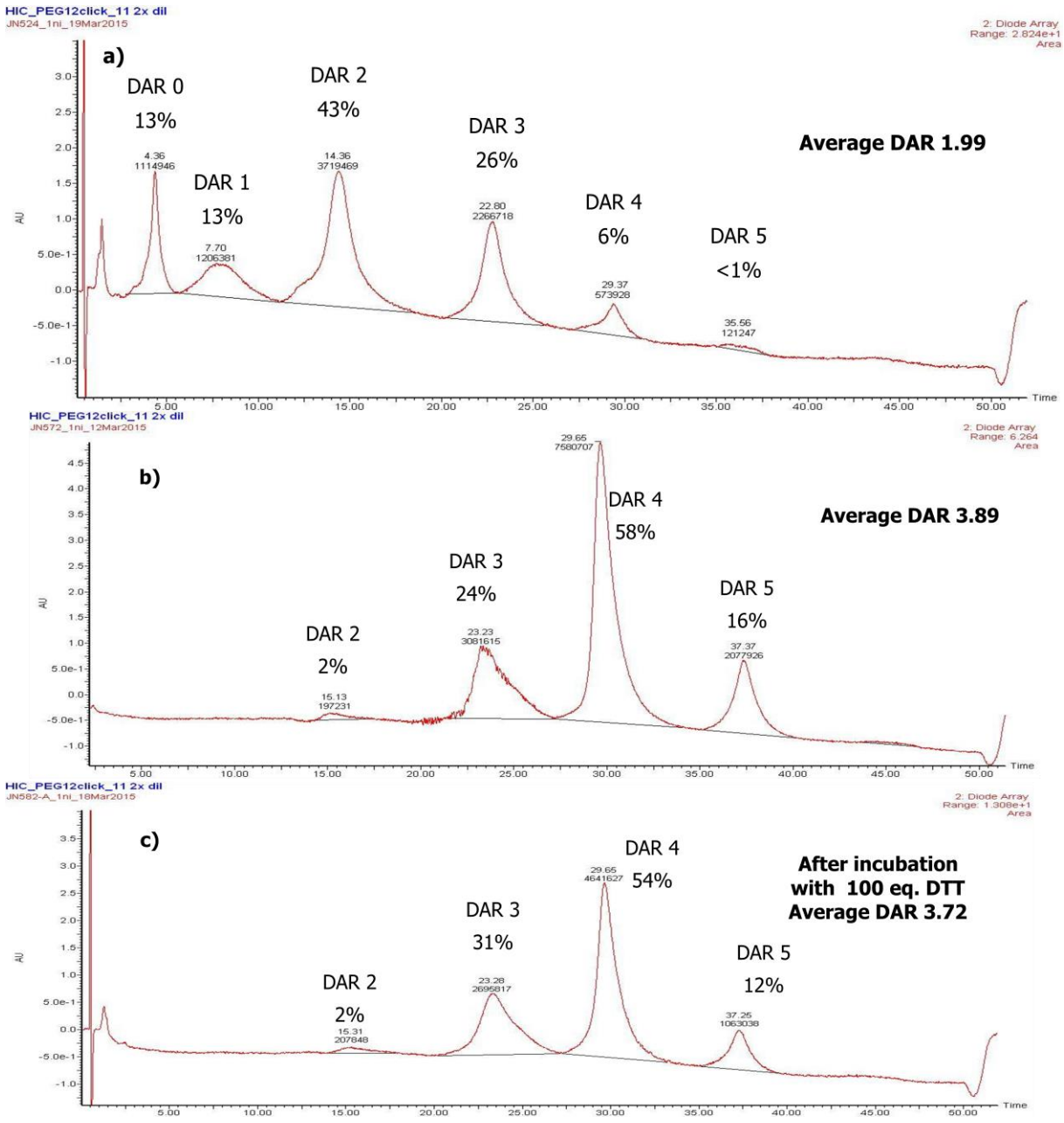


Figure S5 – HIC analysis for **a)** Control conjugate with NGM-MMAE-ADC-CONTROL displaying full range of DAR species between 0 and 5, **b)** NGM-MMAE-ADC **9** and **c)** NGM-MMAE-ADC **9** after incubation with DTT (100 eq.) to cleave any non-hydrolysed maleimide bridges.

Sulfhydryl per antibody (SAR) and corresponding estimated DAR from Ellman's analysis of NGM-MMAE-ADC 9.

Conditions	SAR	Estimated DAR (by Ellman's)*	DAR (by HIC)
Reduced prior to conjugation	7.73	n.a.	n.a.
After conjugation and purification	0.38	3.81	3.89

*Estimated DAR assumes non-free sulfhydryls are NGM-MMAE maleamic disulfide bridges.

HER2 ELISA of NGM-MMAE-ADC 9

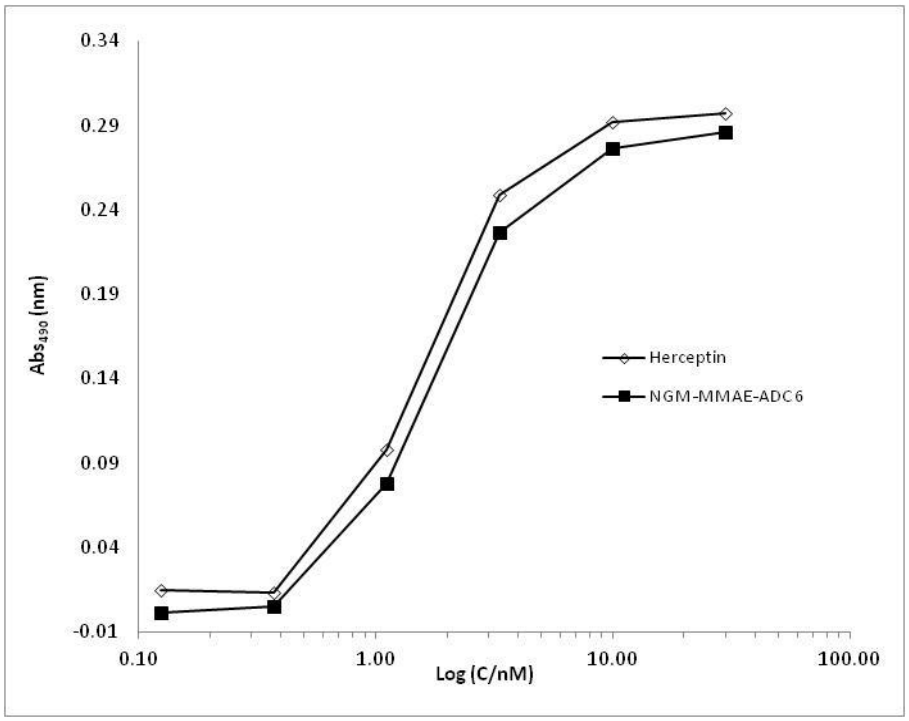


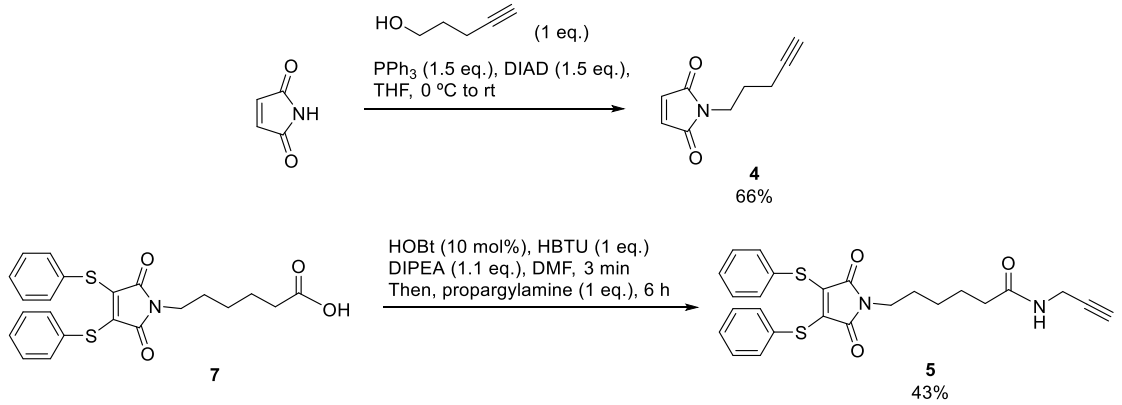
Figure S6 – Binding activity of NGM-MMAE-ADC **9** to HER2 compared with trastuzumab by ELISA assay.

Synthesis General Remarks

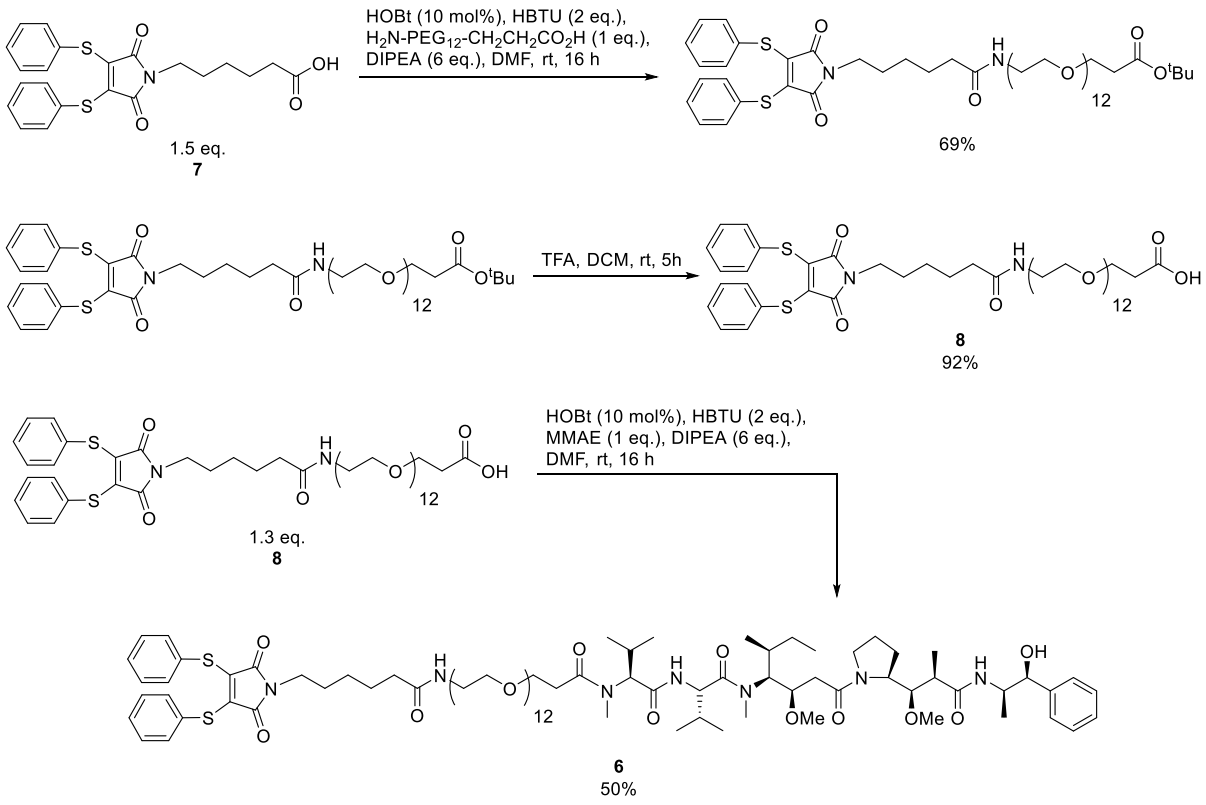
All reactions were carried out at atmospheric pressure with stirring at 20 °C unless otherwise stated. Reagents and solvents were purchased from Sigma Aldrich and Alfa Aesar and used as supplied. H_2N-PEG_{12}-$CH_2CH_2CO_2{}^tBu$ was purchased from Iris Biotech GmbH. Reactions were monitored by TLC analysis carried out on silica gel SIL G/UV254 coated onto aluminium plates purchased from VWR. Visualization was carried out under a UV lamp operating at 254 nm wavelength and by staining with a solution of potassium permanganate (3 g) and potassium carbonate (20 g) in 5% aqueous sodium hydroxide (5 mL) and water (200 mL) or with a solution of phosphomolybdic acid in ethanol (12 g/250 mL), followed by heating. Flash column chromatography was carried out with silica gel 60 (0.04-0.063 mm, 230-400 mesh) purchased from Merck, using solvents dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc) and petroleum ether 40 °C - 60 °C boiling range, purchased from Fisher Scientific. Nuclear magnetic resonance spectra were recorded in either $CDCl_3$ or MeOD-d_4 (unless another solvent is stated) on Bruker NMR spectrometers operating at ambient 20 °C probe. ^1H spectra were recorded at 400, 500 or 600 MHz and ^{13}C spectra were recorded at 100, 125 or 150 MHz, using residual solvents as internal reference. Where necessary, DEPT135, COSY, HMQC, HMBC and NOESY spectra have been used to ascertain structure. Data is presented as follows for ^1H: chemical shift in ppm (multiplicity, *J* coupling constant in Hz, nº of H, assignment on structure); and on ^{13}C: chemical shift in ppm (assignment on structure). Multiplicity is reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), quint. (quintet), sext. (sextet), oct. (octet), m (multiplet), br (broad), dd (doublet of doublet), dt (doublet of triplets), ABq (AB quartet). Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer operating in ATR mode. Melting points were measured on a Gallenkamp apparatus and are uncorrected. Experimental procedures for

all isolated compounds are presented. All yields quoted are isolated yields, unless otherwise stated, and when multiple products are obtained, data are presented in terms of order isolated.

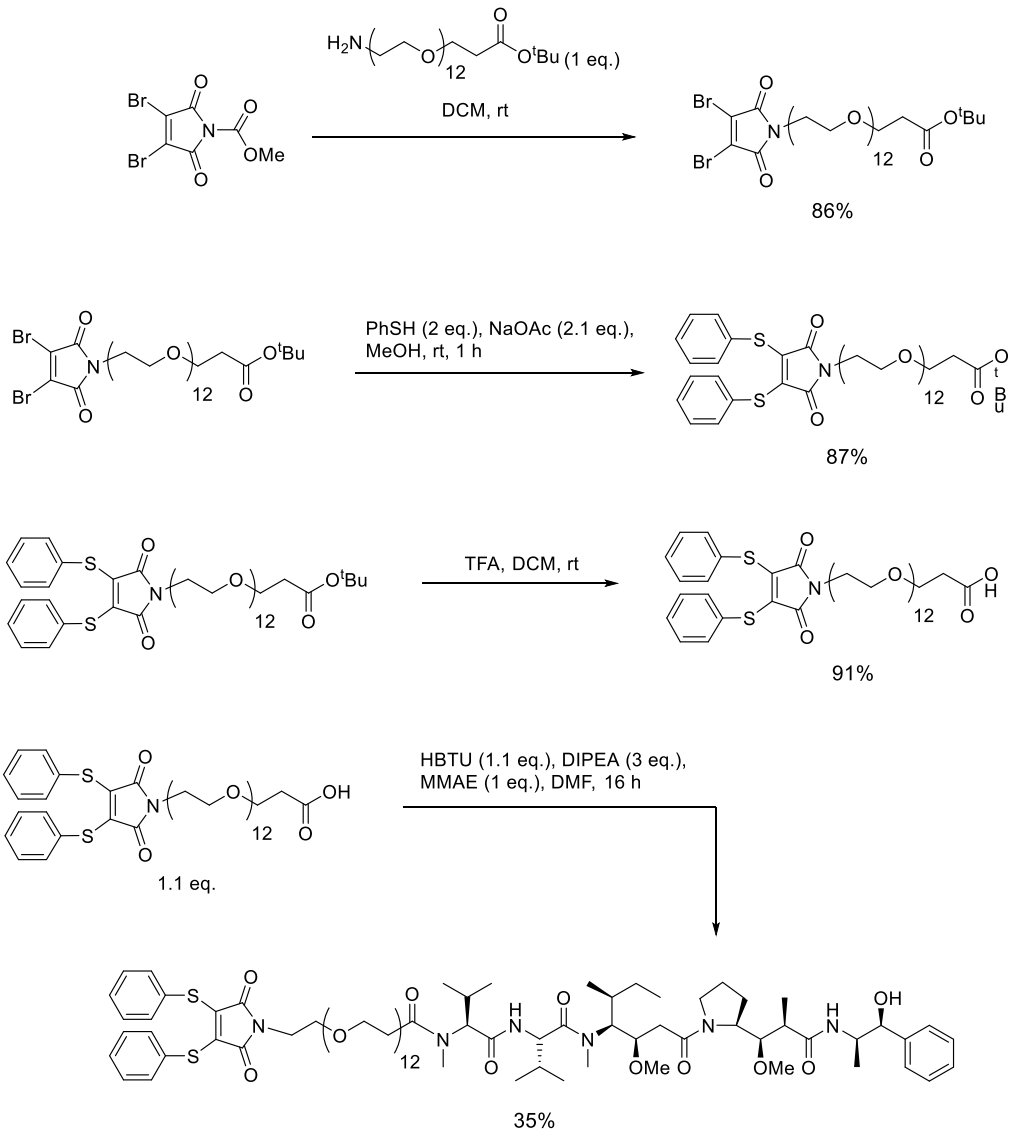
Synthesis of Compounds



Scheme S1 – Synthesis of maleimide **4** and NGM **5**.

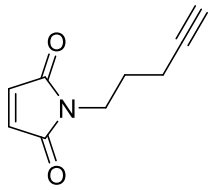


Scheme S2 – Synthesis of NGM-MMAE **6**.

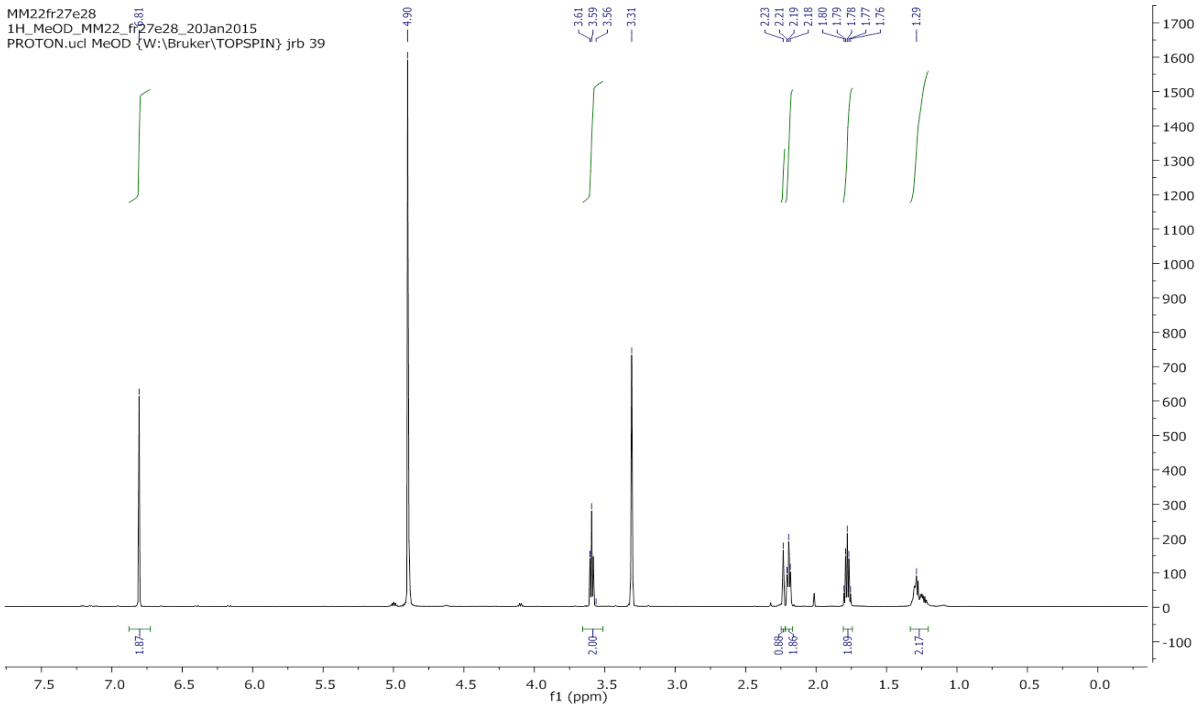


Scheme S3 – Synthesis of 3,4-dithiophenoyl-maleimide-*N*-PEG_{12}-CO-MMAE.

***N*-Pentyne-maleimide 4**



To a solution of maleimide (1g, 10 mmol), and pentyn-1-ol (1.02 mL, 11.0 mmol, 1.1 eq.), in THF (50 mL) at 0 ºC was added triphenylphosphine (3.93 g, 15 mmol, 1.5 eq.), followed immediately by dropwise addition of DIAD (2.95 mL, 15.0 mmol, 1.5 eq.). After stirring for 4 h at 0 ºC the reaction mixture was concentrated under vacuum (5 mL). Then, cold Et_2O (50 mL) was added and the resultant triphenylphosphine oxide precipitate was filtered off. Purification by column chromatography (0% to 15% EtOAc/petrol) yielded **4** as a white solid (1.10 g, 6.7 mmol, 66%). Characterisation data: m.p. 116-117 ºC. FTIR ν_{max} (cm^{-1}) 3489, 2913, 1784, 1720, 1597. ^{1}H NMR (MeOD-d4, 600 MHz) δ_H: 1.76-1.78 (quint., *J* = 6.0 Hz, 2H, C*H*$_2$), 2.18-2.21 (t, *J* = 6.0 Hz, 2H, C*H*$_2$), 2.23 (s, 1H, C*H*), 3.59 (t, *J* = 6.0 Hz, 2H, C*H*$_2$), 6.81 (s, 2H, C*H*); ^{13}C NMR (MeOD-d4, 150 MHz) δ_C: 16.7 (CH_2), 28.4 (CH_2), 37.9 (CH_2), 70.0 (CH), 84.1 (C), 135.4 (CH), 170.5 (CO). HRMS (ESI) calcd. for C_9H_9NO2 $[M]^+$ 163.1827, observed: 163.1855.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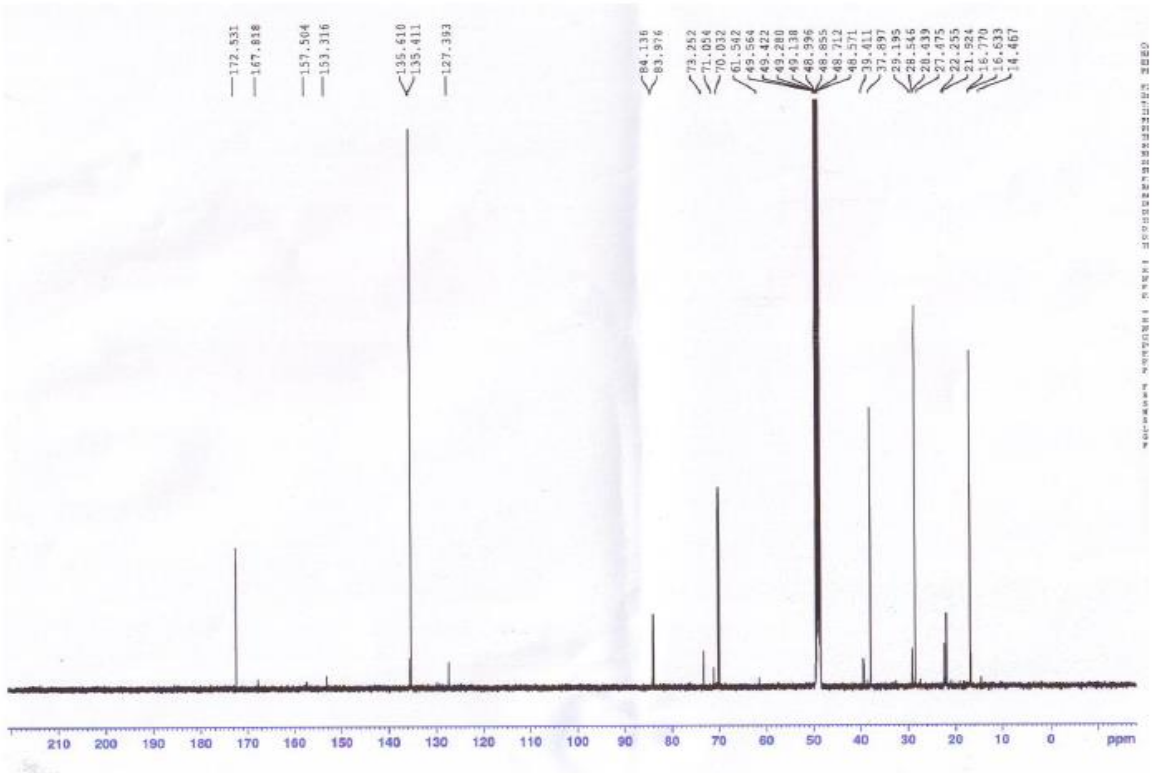
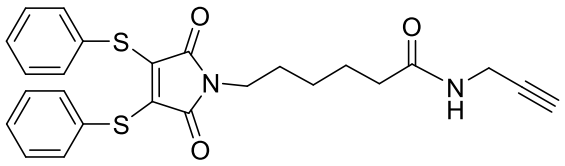
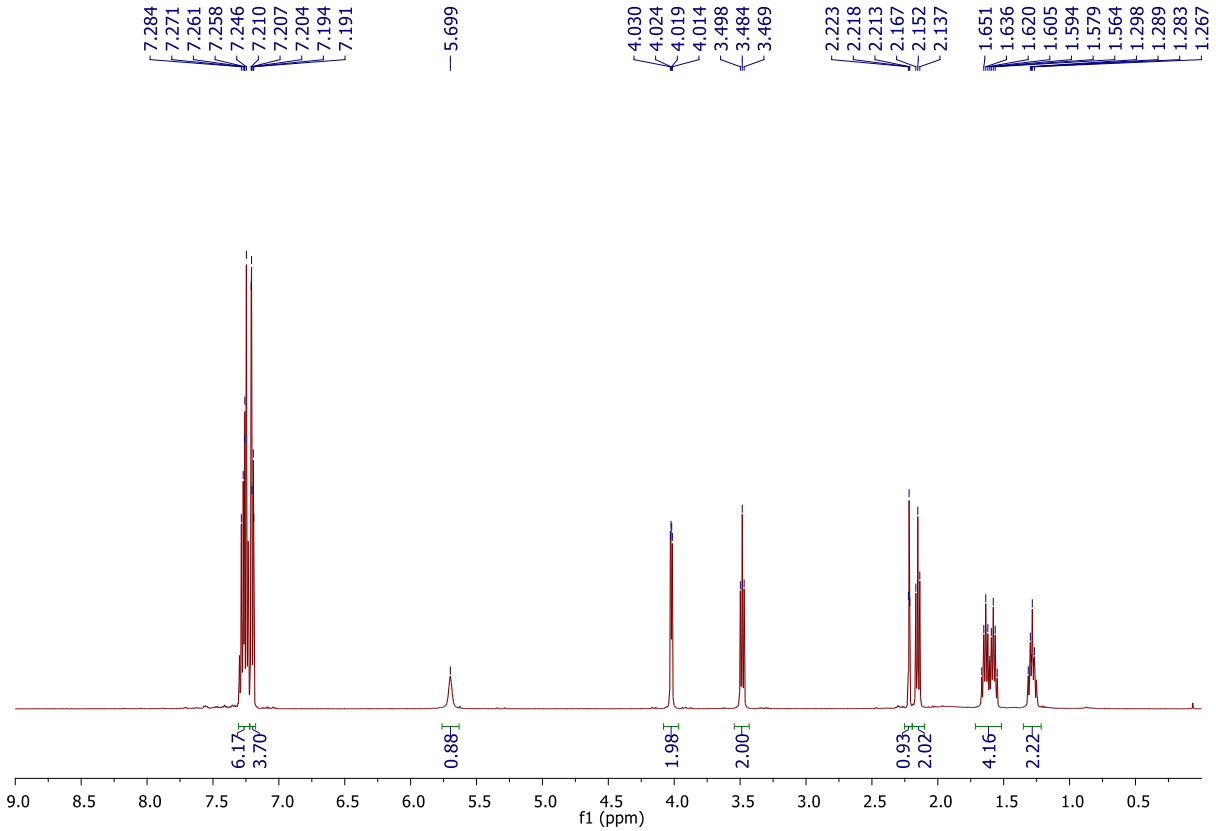
PROTON.ucl MeOD {W:\Bruker\TOPSPIN} jrb 39
f1 (ppm)


ppm

3,4-Dithiophenoyl-maleimide-1-(*N*-propargyl)hexanamide 5



In a 10 mL round-bottom flask under nitrogen, 3,4-dithiophenoyl-maleimide-*N*-hexanoic acid **7** (42.8 mg, 100 μmol, 1 eq.), prepared as reported,[2] HOBt hydrate (1.26 mg, 10 μmol, 0.1 eq.) and HBTU (38 mg, 100 μmol, 1 eq.) were dissolved in DMF (1 mL) to give a yellow solution. Next, added DIPEA (19 μL, 110 μmol, 1.1 eq.) and the solution was stirred for 5 minutes at 20 ºC. Then, added propargylamine (6.4 μL, 100 μmol, 1 eq.) and the solution was stirred at 20 ºC for 6 hours. Then, added DCM (10 mL) and washed with 0.68 M AcOH:AcONa buffer pH 5 (10 mL) and aq. sat. LiCl solution (3×10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under vacuum to yield an orange oil which was purified by flash chromatography on silica with DCM:EtOAc (6:1 v/v) to afford **5** as an orange solid (19.9 mg, 43 μmol, 43%). Characterisation data: FTIR ν_{max} (cm^{-1}) 3300, 3276, 3057, 2934, 2858, 1704, 1641, 1527, 1392, 1359, 1187, 1046, 735, 685, 641. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 1.26-1.31 (quint., J = 7.5 Hz, 2H, CH_2), 1.55-1.67 (overlapped quint., J = 7.5 Hz, 4H, CH_2), 2.14-2.17 (t, J = 7.5 Hz, 2H, CH_2), 2.21-2.22 (t, J = 2.5 Hz, 1H, C*H*), 3.47-3.50 (t, J = 7.5 Hz, 2H, CH_2), 4.01-4.03 (dd, J = 5.5, 2.5 Hz, 2H, CH_2), 5.70 (br, 1H, N*H*), 7.19-7.22 (overlapped m, 4H, Ar*H*), 7.23-7.30 (overlapped m, 6H, Ar*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 24.9 (CH_2), 26.3 (CH_2), 28.2 (CH_2), 29.3 (CH_2), 36.2 (CH_2), 38.7 (CH_2), 71.7 (CH), 79.8 (C), 128.5 (ArCH), 129.1 (ArCH), 129.2 (ArC), 132.0 (ArCH), 135.8 (C), 167.0 (CO), 172.4 (CO). LRMS (NSI) 465 (100, $[M+H]^+$); HRMS (NSI) calcd. for $C_{25}H_{25}N_2O_3S_2$ $[M+H]^+$ 465.1301, observed: 465.1293.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4.014
3.498
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3.469
2.223
2.218
2.213
2.167
2.152
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1.651
1.636
1.620
1.605
1.594
1.579
1.564
1.298
1.289
1.283
1.267
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3.70
0.88
1.98
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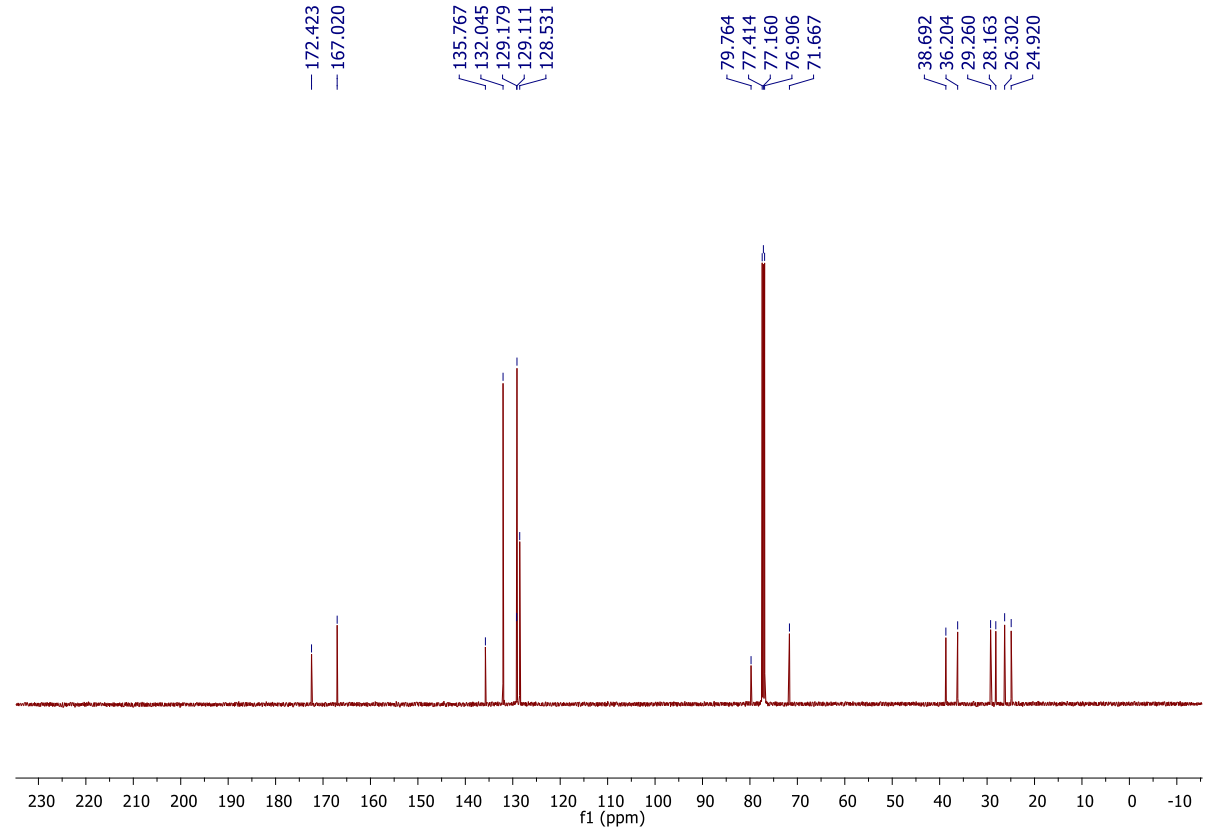
4.16
2.22
f1 (ppm)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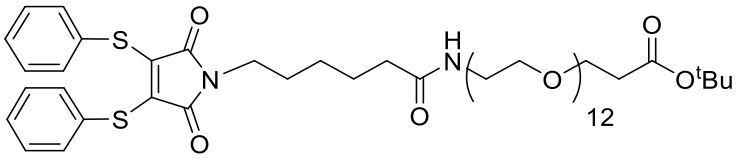
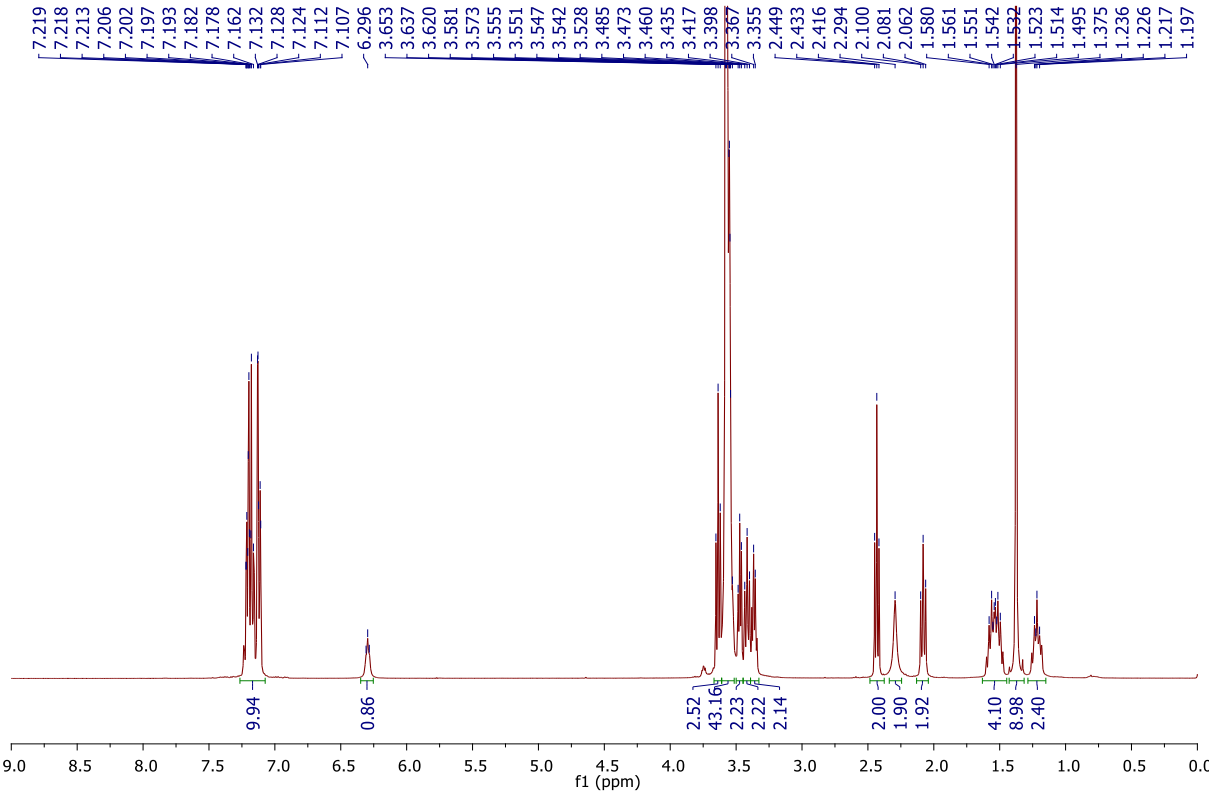
f1 (ppm)

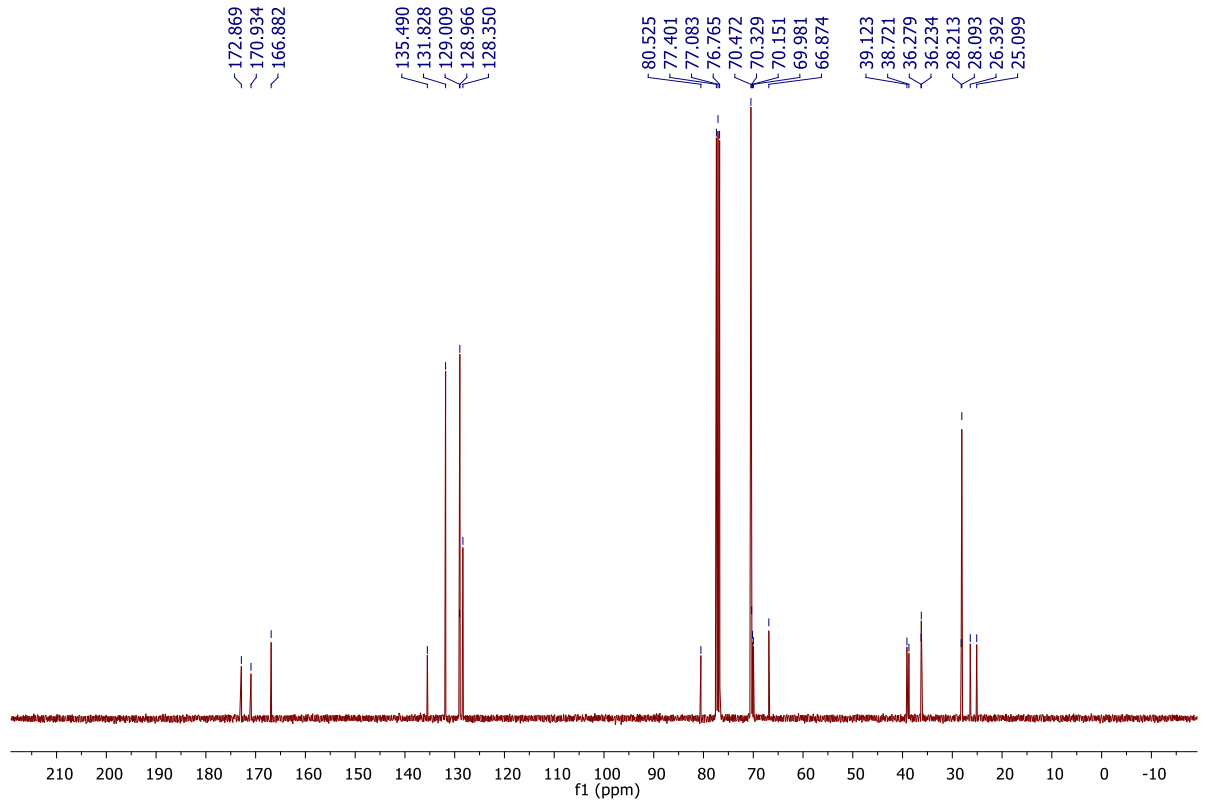
3,4-Dithiophenoyl-maleimide-*N*-hexanamide-PEG$_{12}$-*tert*-butyl ester



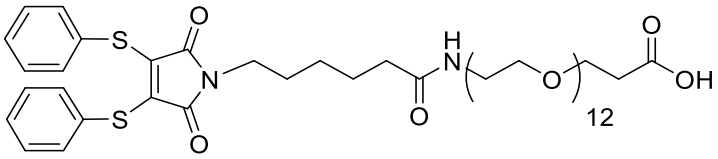
In a 10 mL round-bottom flask, oven dried and under nitrogen, 3,4-dithiophenoyl-maleimide-*N*-hexanoic acid **7** (32 mg, 75 μmol, 1.5 eq.), prepared as reported,[2] HOBt hydrate (0.68 mg, 5 μmol, 0.1 eq.) and HBTU (38 mg, 100 μmol, 2 eq.) were dissolved in DMF (0.5 mL). Then, added DIPEA (52 μL, 300 μmol, 6 eq.). The solution was stirred at 20 °C for 10 min. Next, added H_2N-PEG_{12}-$CH_2CH_2CO_2{}^tBu$ (34 mg, 50 μmol, 1 eq.) in DMF (0.5 mL) and the solution was stirred at 20 °C for 16 h. Then, concentrated under vacuum, added DCM (10 mL) and washed with 0.68 M acetates pH 5.0 (10 mL), aq. sat. $NaHCO_3$ (10 mL) and 40% aq. LiCl (3×10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under vacuum to yield an orange oil which was purified by flash chromatography on silica with DCM:EtOAc:MeOH (10:5:1 to 10:5:2 v/v) to afford 3,4-dithiophenoyl-maleimide-*N*-hexanamide-PEG_{12}-*tert*-butyl ester as an orange oil (37 mg, 34 μmol, 69%). Characterisation data: FTIR ν_{max} (cm^{-1}) 2866, 1704, 1668, 1533, 1441, 1397, 1365, 1249, 1097, 948, 842, 738, 688. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.16-1.26 (quint., J = 7.6 Hz, 2H, CH_2), 1.38 (s, 9H, CH_3), 1.49-1.61 (overlapped quint., J = 7.6 Hz, 4H, CH_2), 2.06-2.10 (t, J = 7.6 Hz, 2H, CH_2), 2.24-2.34 (br, 2H, CH_2), 2.42-2.46 (t, J = 7.2 Hz, 2H, CH_2), 3.35-3.38 (t, J = 4.8 Hz, 2H, CH_2), 3.39-3.43 (t, J = 7.6 Hz, 2H, CH_2), 3.48-3.46 (t, J = 4.8 Hz, 2H, OCH_2), 3.53-3.62 (overlapped multiplets, 44H, OCH_2), 3.62-3.65 (t, J = 6.8 Hz, 2H, OCH_2), 6.28-6.30 (t, J = 4.8 Hz, 1H, N*H*), 7.10-7.24 (overlapped m, 10H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 25.1 (CH_2), 26.4 (CH_2), 28.1 (CH_3), 28.2 (CH_2), 36.2 (CH_2), 36.3 (CH_2), 38.7 (CH_2), 39.1 (CH_2), 66.9 (OCH_2), 69.9 (OCH_2), 70.1 (OCH_2), 70.3 (OCH_2), 70.4-70.6 (overlapped OCH_2), 80.5 (C), 128.3 (ArCH), 128.9 (ArCH), 129.0 (ArC), 131.8 (ArCH), 135.5 (C), 166.9 (CO),

170.9 (CO), 172.9 (CO). LRMS (ESI) 1105 (100, $[M+Na]^+$) 1083 (48, $[M+H]^+$), ; HRMS (ESI) calcd. for $C_{53}H_{83}N_2O_{17}S_2$ $[M+H]^+$ 1083.5133, observed: 1083.5123.

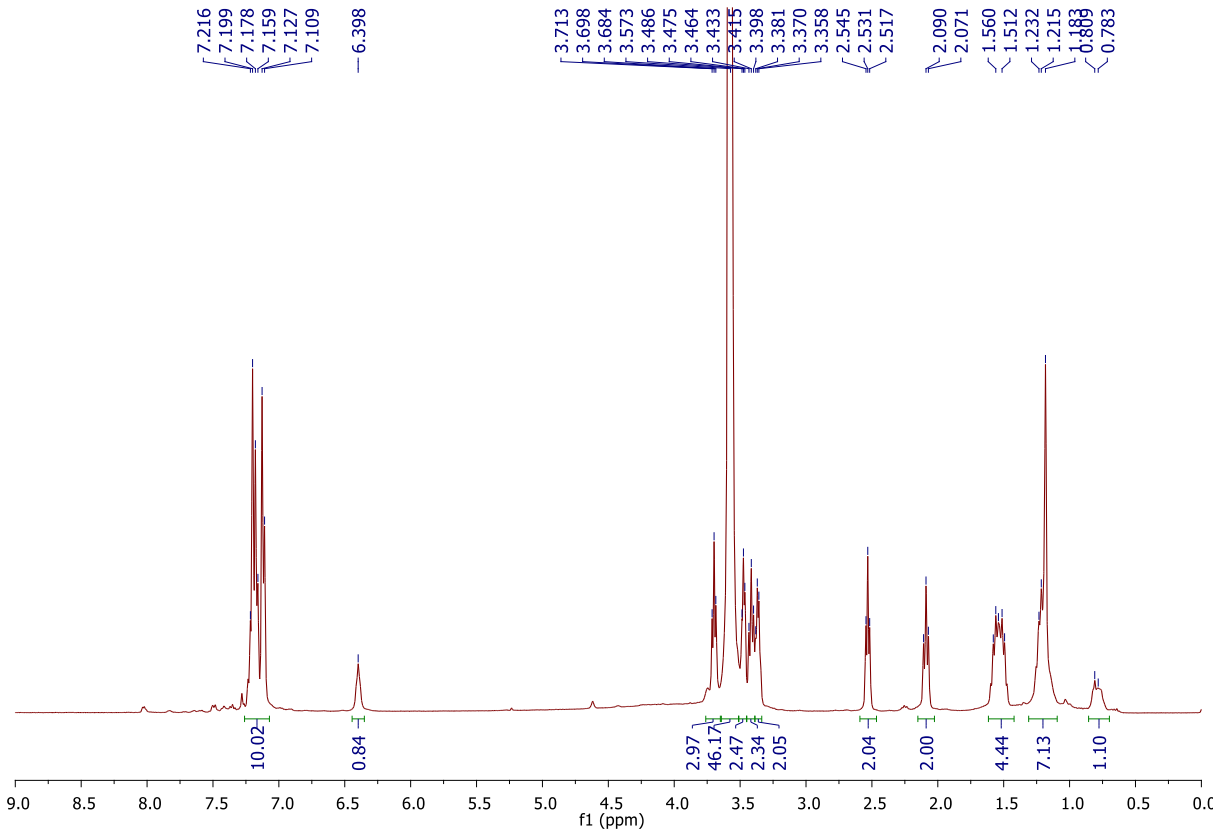




3,4-Dithiophenoyl-maleimide-*N*-hexanamide-PEG$_{12}$-acid 8



In a 10 mL round-bottom flask, 3,4-dithiophenoyl-maleimide-*N*-hexanamide-PEG$_{12}$-*tert*-butyl ester (30 mg, 28 μmol) was dissolved in DCM (0.43 mL). Then, added trifluoroacetic acid (0.43 mL, 5.6 mmol). The solution was stirred at 20 °C for 5 h. Then, concentrated under vacuum, redissolved in DCM (10 mL) and washed with 10 mM aq. HCl (10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under vacuum to afford **8** as an orange oil (26 mg, 25 μmol, 92%). Characterisation data: FTIR ν_{max} (cm^{-1}) 3336, 2864, 1704, 1535, 1441, 1398, 1351, 1248, 1094, 947, 844, 739, 670. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.16-1.26 (quint., J = 7.2 Hz, 2H, CH_2 overlapped with ^{t}BuOH), 1.55-1.60 (overlapped quint., J = 7.2 Hz, 4H, CH_2), 2.07-2.11 (t, J = 7.6 Hz, 2H, CH_2), 2.52-2.54 (t, J = 7.2 Hz, 2H, CH_2), 3.35-3.38 (t, J = 4.8 Hz, 2H, CH_2), 3.39-3.43 (t, J = 7.2 Hz, 2H, CH_2), 3.46-3.49 (t, J = 4.8 Hz, 2H, OCH_2), 3.53-3.62 (overlapped multiplets, 46H, OCH_2), 3.68-3.71 (t, J = 6.8 Hz, 2H, OCH_2), 6.36-6.44 (t, J = 4.8 Hz, 1H, N*H*), 7.10-7.25 (overlapped m, 10H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 25.1 (CH_2), 26.4 (CH_2), 28.2 (CH_2), 29.7 (CH_3 from ^{t}BuOH), 35.0 (CH_2), 36.3 (CH_2), 38.7 (CH_2), 39.1 (CH_2), 66.7 (OCH_2), 69.9 (OCH_2), 70.1 (OCH_2), 70.3 (OCH_2), 70.4-70.5 (overlapped OCH_2), 70.5 (OCH_2), 70.6 (OCH_2), 128.3 (ArCH), 128.9 (ArCH), 129.0 (ArC), 131.8 (ArCH), 135.5 (C), 166.9 (CO), 173.1 (CO), 173.5 (CO). LRMS (ESI) 674 (100, $[M\text{-}PEG_7CO_2]^+$) 1027 (1, $[M+H]^+$).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3.358
2.545
2.531
2.517
2.090
2.071
1.560
1.512
1.232
1.215
1.183
0.809
0.783
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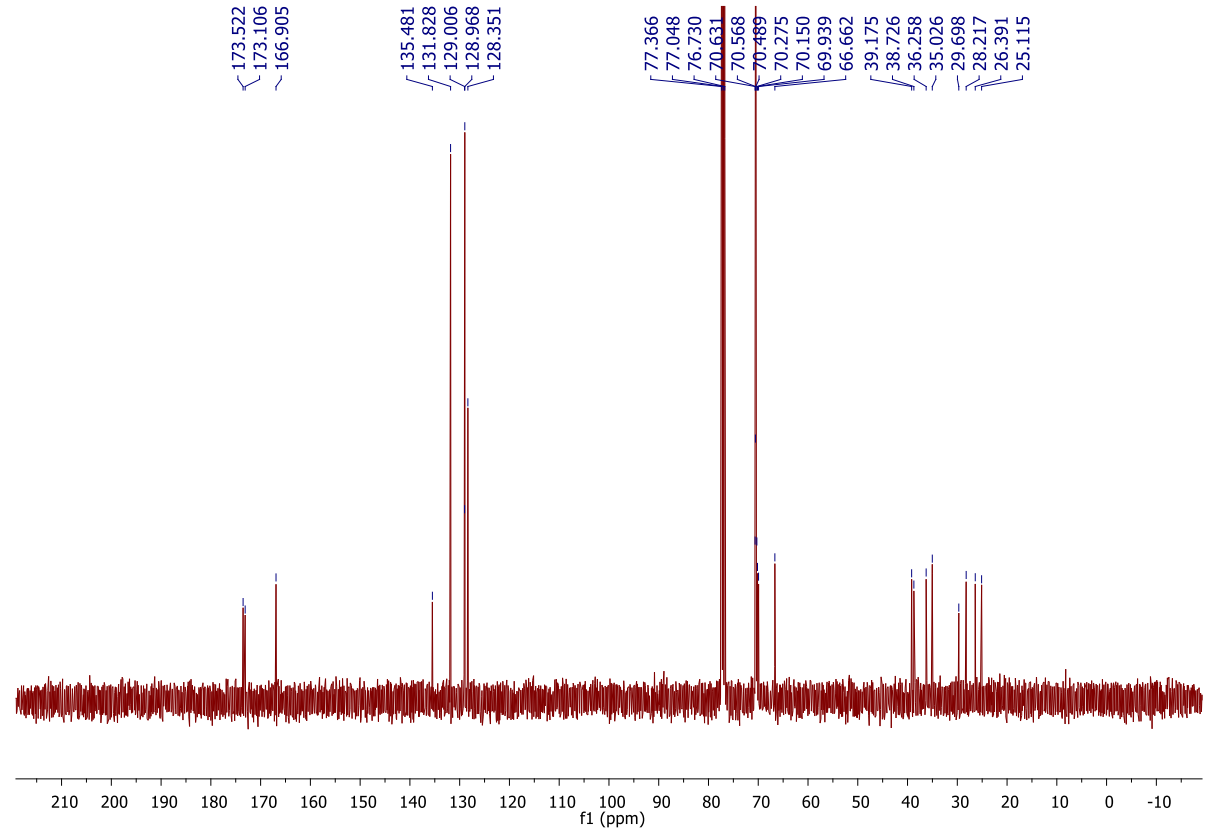
f1 (ppm)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70.275
70.150
69.939
66.662
39.175
38.726
36.258
35.026
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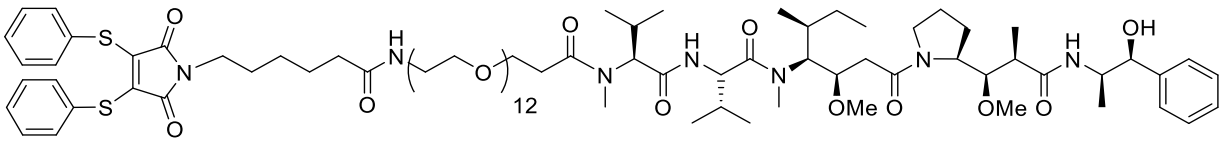
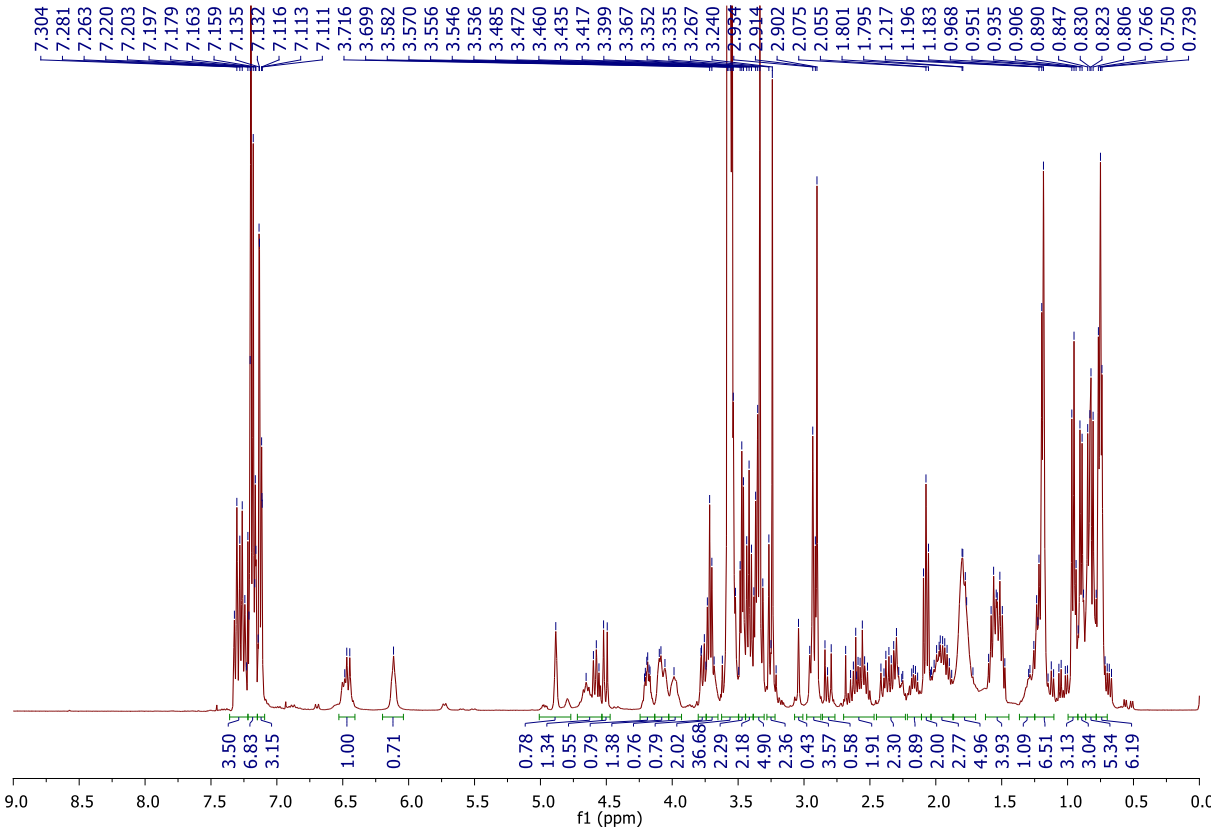
26.391
25.115
f1 (ppm)

3,4-Dithiophenoyl-maleimide-*N*-hexanamide-PEG$_{12}$-CO-MMAE 6

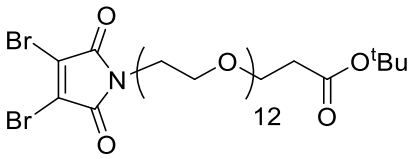


In a 10 mL round-bottom flask, oven dried and under nitrogen, compound **8** (10.8 mg, 10 µmol, 1.3 eq.) HOBt hydrate (0.2 mg, 1.5 µmol, 0.2 eq.) and HBTU (5.7 mg, 15 µmol, 2 eq.) were dissolved in DMF (0.5 mL). Then, added DIPEA (8 µL, 46 µmol, 6 eq.). The solution was stirred at 20 °C for 10 min. Next, added MMAE (5.5 mg, 7.7 µmol, 1 eq.) in DMF (0.5 mL) and the solution was stirred at 20 °C for 16 h. Then, concentrated under vacuum, added DCM (10 mL) and washed with 15% aq. citric acid (10 mL), aq. sat. $NaHCO_3$ (10 mL) and 40% aq. LiCl (2×10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under vacuum to yield an orange oil which was purified by flash chromatography on silica with a gradient from DCM:EtOAc:MeOH (5:5:1 v/v) to DCM:MeOH (10:1 v/v) to afford **6** as an orange oil (6.6 mg, 3.8 µmol, 50%). Characterisation data: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.71-0.78 (t, J = 6.4, 6H, MMAE), 0.78-0.88 (m, 6H, MMAE), 0.89-0.93 (m, 3H, MMAE), 0.93-1.00 (m, 3H, MMAE), 1.16-1.26 (m, MMAE overlapped with CH_2), 1.28-1.36 (m, MMAE), 1.46-1.61 (overlapped quint., J = 7.2 Hz, 4H, CH_2), 1.70-1.86 (m, MMAE), 1.86-2.03 (m, MMAE), 2.05-2.09 (t, J = 7.6 Hz, 2H, CH_2), 2.11-2.22 (m, MMAE), 2.24-2.45 (m, MMAE), 2.47-2.71 (m, MMAE overlapped with CH_2), 2.75-2.85 (m, MMAE), 2.88-2.97 (m, 3H, MMAE overlapped with PEG CH_2), 3.01-3.11 (m, MMAE), 3.21-3.27 (m, MMAE), 3.30-3.38 (m, MMAE overlapped with CH_2), 3.40-3.44 (t, J = 7.2 Hz, 2H, CH_2), 3.46-3.49 (t, J = 4.8 Hz, 2H, OCH_2), 3.50-3.62 (m, 2H, PEG OCH_2), 3.68-3.73 (t, J = 6.8 Hz, 2H, OCH_2), 3.73-3.80 (m, MMAE), 3.93-4.02 (m, MMAE), 4.02-4.14 (m, MMAE), 4.14-4.24 (m, MMAE), 4.49-4.52 (d, J = 11.2 Hz, 1H, MMAE), 4.53-4.60 (m, MMAE), 4.60-4.70 (m, MMAE), 6.15-6.25 (t, J = 5.2 Hz, 1H, NH), 6.40-6.55 (m,

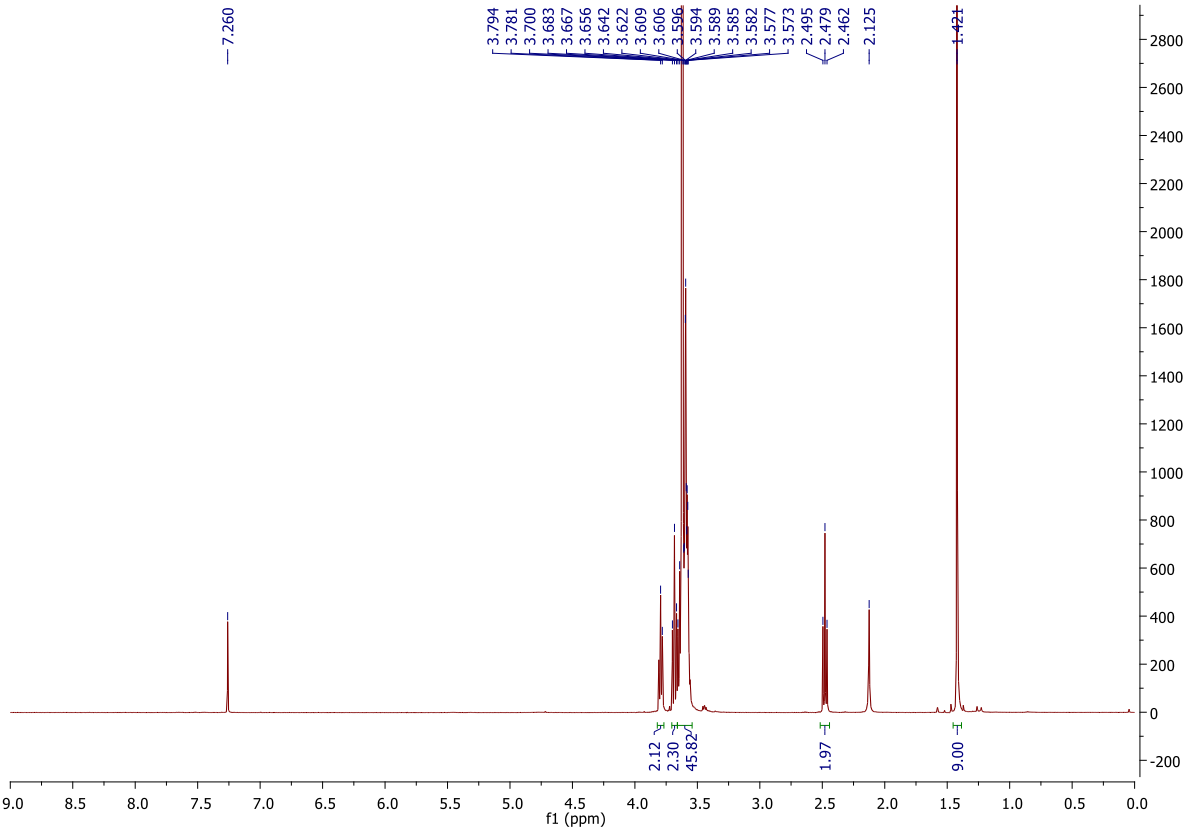
MMAE), 7.10-7.23 (overlapped m, 10H, Ar*H*), 7.23-7.34 (m, 5H, Ar*H* MMAE). LRMS (ESI) 864 (100, $[M+2H]^{2+}$), 1727 (5, $[M+H]^{+}$); HRMS (ESI) calcd. for $C_{88}H_{140}N_7O_{23}S_2$ $[M+H]^{+}$ 1726.9442, observed: 1726.9450.



3,4-Dibromo-maleimide-*N*-PEG$_{12}$-*tert*-butyl ester



To a solution of *N*-methoxycarbonyl-3,4-dibromomaleimide (19 mg, 60 μmol), prepared as reported,[3] in DCM (10 mL) was added a solution of H_2N-PEG_{12}-$CH_2CH_2CO_2{}^tBu$ (40 mg, 60 μmol, 1.0 eq.) in DCM (1 mL). The mixture was stirred at 20 °C for 2 h. Then, concentrated under vacuum to yield a yellow oil that was purified by column chromatography on silica gel 60 in DCM:MeOH (15:1 v/v) to afford 3,4-dibromo-maleimide-*N*-PEG_{12}-*tert*-butyl ester as a light yellow oil (47.1 mg, 52 μmol, 86%). Characterisation data: FTIR ν_{max} (cm^{-1}) 2867, 1723, 1437, 1391, 1350, 1250, 1097, 948, 846, 827, 733. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.42 (s, 9H, C*H*$_3$), 2.46-2.49 (t, *J* = 6.8 Hz, 2H, C*H*$_2$), 3.55-3.66 (overlapped multiplets, 46H, OC*H*$_2$), 3.67-3.70 (t, *J* = 6.8 Hz, 2H, OC*H*$_2$), 3.78-3.81 (t, *J* = 5.2 Hz, 2H, C*H*$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 28.1 (CH_3), 36.2 (CH_2), 38.9 (CH_2), 66.9 (OCH_2), 67.5 (OCH_2), 70.0 (OCH_2), 70.5-70.6 (overlapped OCH_2), 80.5 (C), 129.4 (C), 163.8 (CO), 170.9 (CO). LRMS (NSI) 927 (50, $[M^{79}Br^{79}Br+NH_4]^+$), 929 (100, $[M^{81}Br^{79}Br+NH_4]^+$), 931 (50, $[M^{81}Br^{81}Br+NH_4]^+$); HRMS (NSI) calcd. for $C_{35}H_{65}N_2O_{16}Br_2$ $[M+NH_4]^+$ 927.2695, observed: 927.2690.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3.585
3.582
3.577
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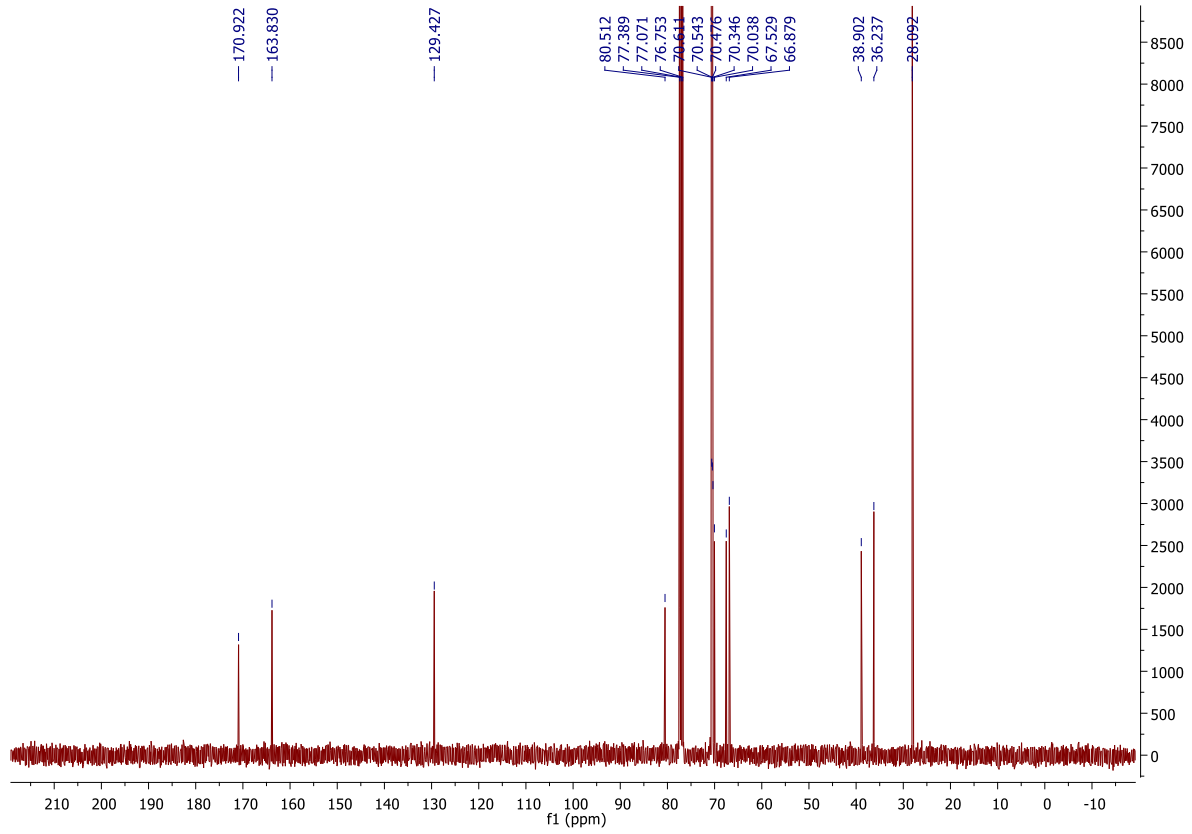
9.00
f1 (ppm)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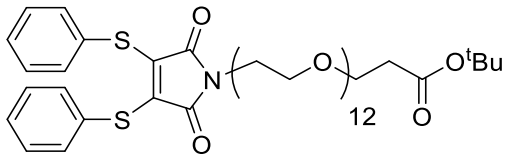
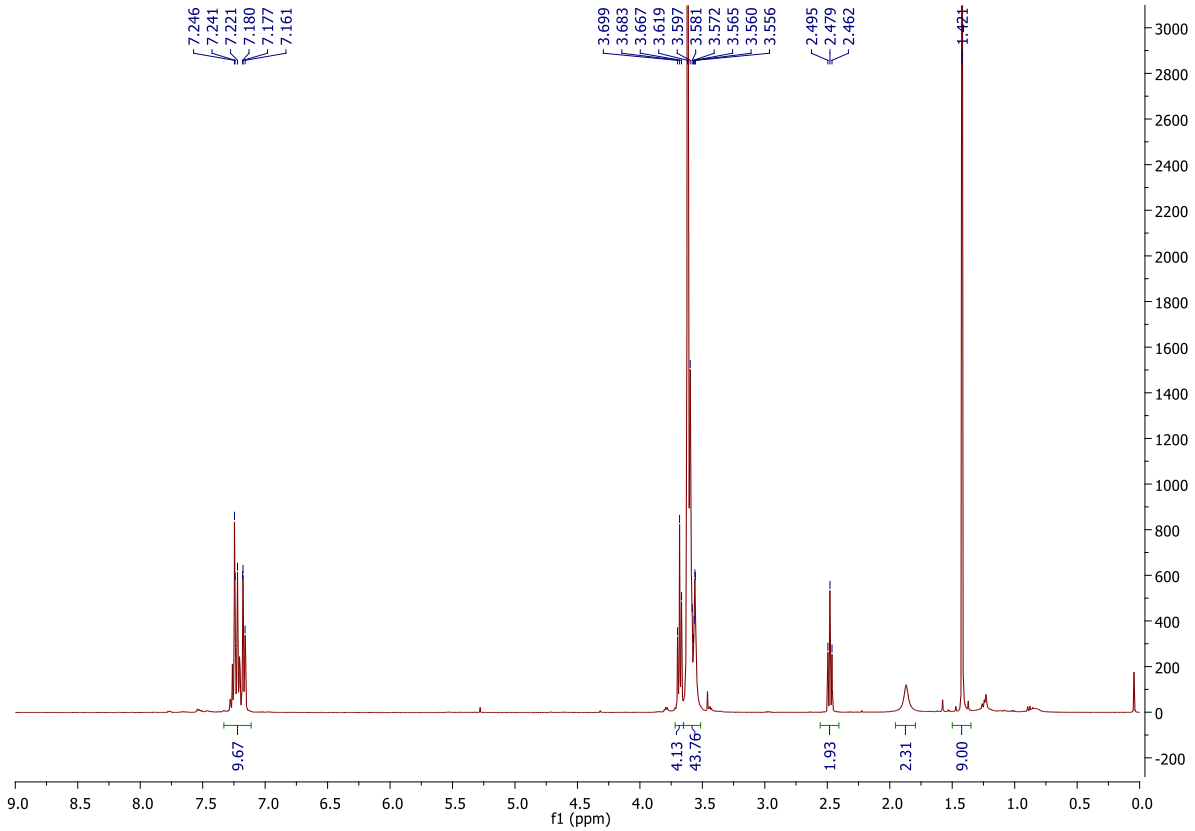
f1 (ppm)

3,4-Dithiophenoyl-maleimide-*N*-PEG$_{12}$-*tert*-butyl ester



In a 10 mL round-bottom flask, 3,4-dibromo-maleimide-*N*-PEG$_{12}$-*tert*-butyl ester (41 mg, 45 μmol) was dissolved in MeOH (1 mL). Then, added NaOAc (7.8 mg, 95 μmol, 2.1 eq.). Next, added thiophenol (9.3 μL, 91 μmol, 2.0 eq.), giving a yellow solution. The solution was stirred at 20 °C for 2h. Then, added DCM (10 mL) and sat. aq. $NaHCO_3$ (5 mL). Separated layers and further extracted aqueous layer with DCM (3×10 mL). The combined organic layer was dried ($MgSO_4$), filtered and concentrated under vacuum to yield an orange oil which was purified by flash chromatography on silica with DCM:MeOH (15:1 v/v) to afford 3,4-dithiophenoyl-maleimide-*N*-PEG$_{12}$-*tert*-butyl ester as an orange oil (38 mg, 39 μmol, 87%). Characterisation data: FTIR ν_{max} (cm^{-1}) 2867, 1710, 1441, 1392, 1254, 1096, 948, 846, 740, 688. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.42 (s, 9H, C*H*$_3$), 2.46-2.49 (t, *J* = 6.8 Hz, 2H, C*H*$_2$), 3.55-3.65 (overlapped multiplets, 46H, OC*H*$_2$), 3.67-3.70 (t, *J* = 6.8 Hz, 4H, OC*H*$_2$), 7.14-7.28 (overlapped m, 10H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 28.1 (CH_3), 36.2 (CH_2), 37.9 (CH_2), 66.9 (OCH_2), 67.6 (OCH_2), 69.8 (OCH_2), 70.4 (OCH_2), 70.5-70.6 (overlapped OCH_2), 80.5 (C), 128.3 (ArCH), 128.9 (ArCH), 129.0 (ArC), 131.8 (ArCH), 135.6 (C), 166.7 (CO), 170.9 (CO). LRMS (NSI) 987 (100, $[M+NH_4]^+$); HRMS (NSI) calcd. for $C_{47}H_{75}N_2O_{16}S_2$ $[M+NH_4]^+$ 987.4553, observed: 987.4542.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3.565
3.560
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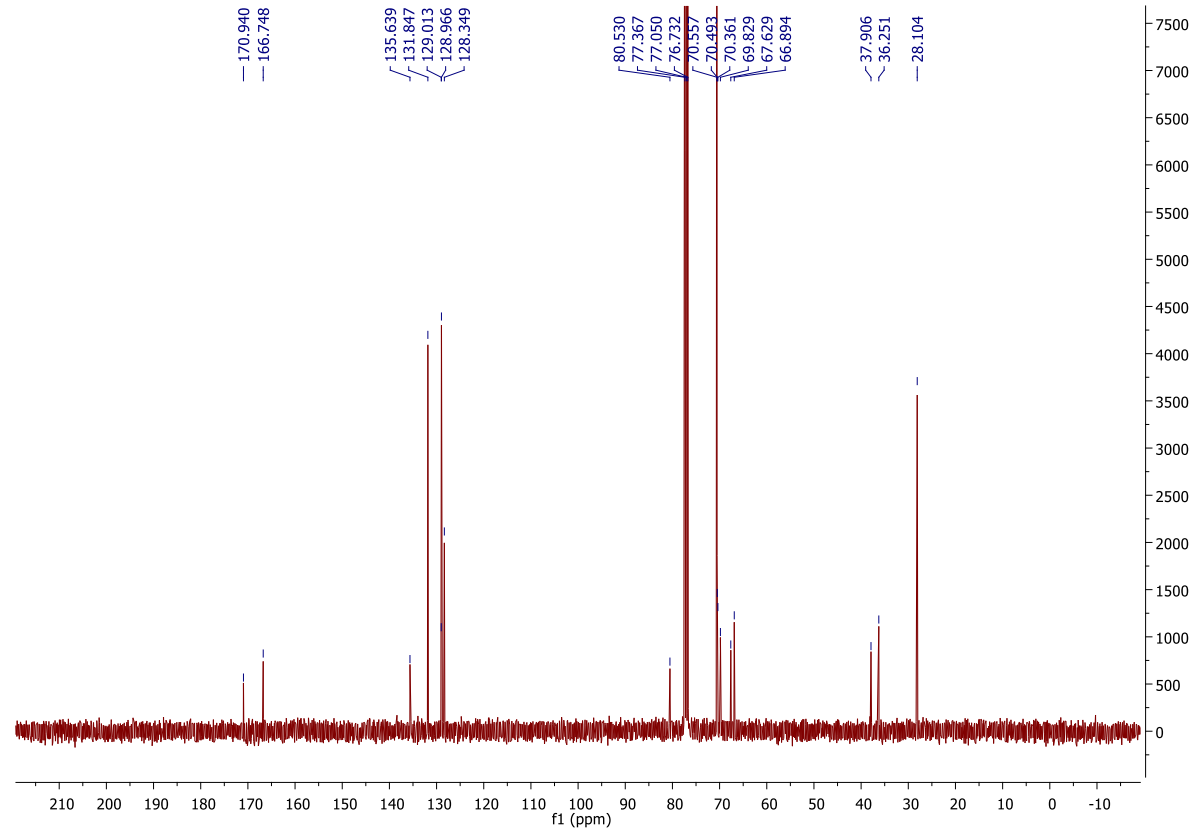
f1 (ppm)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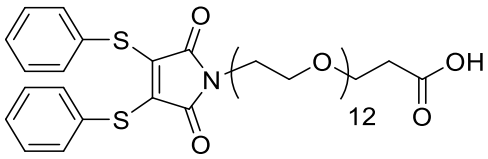
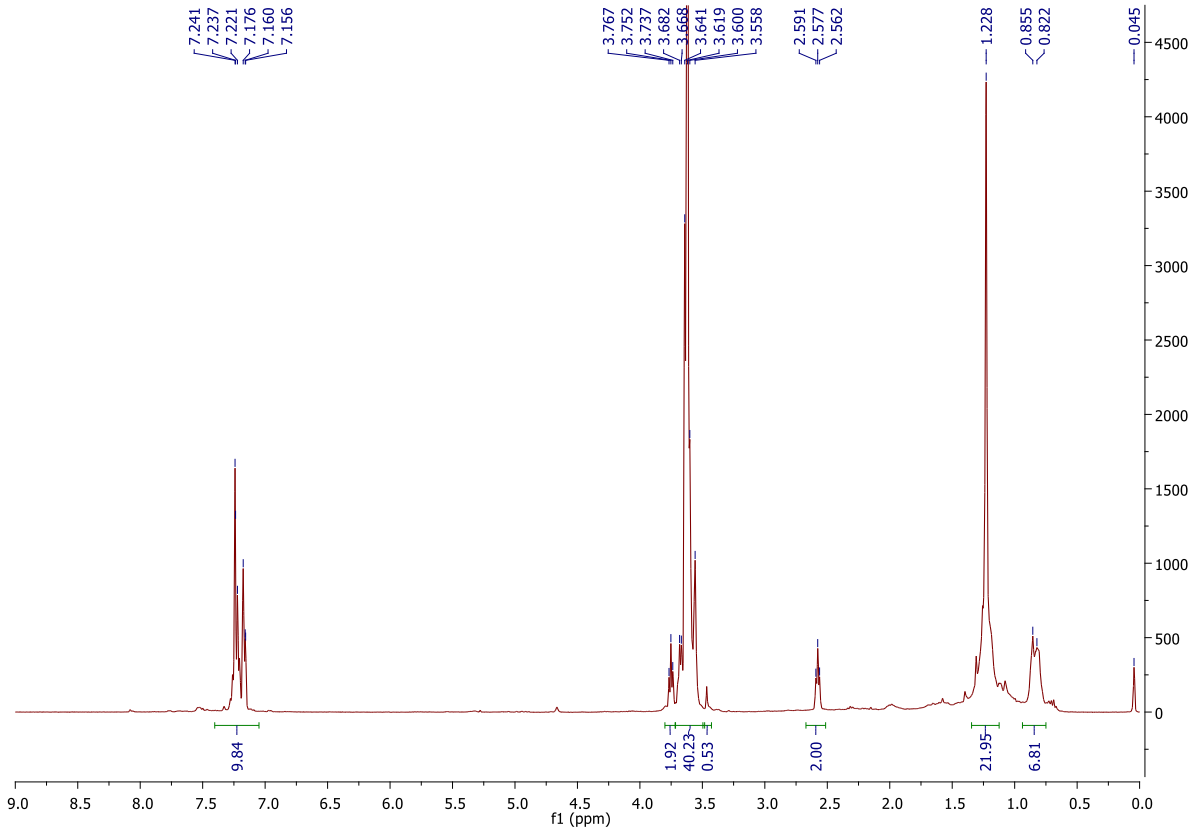
3,4-Dithiophenoyl-maleimide-*N*-PEG$_{12}$-acid



In a 5 mL round-bottom flask, 3,4-dithiophenoyl-maleimide-*N*-PEG$_{12}$-*tert*-butyl ester (9.7 mg, 10 μmol) was dissolved in DCM (0.4 mL). Then, added trifluoroacetic acid (0.4 mL, 5.2 mmol). The solution was stirred at 20 °C for 5 h. Then, concentrated under vacuum, redissolved in DCM (10 mL) and washed with water (3×10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under vacuum to afford 3,4-dithiophenoyl-maleimide-*N*-PEG$_{12}$-acid as an orange oil (8.3 mg, 9.1 μmol, 91%). Characterisation data: FTIR ν_{max} (cm^{-1}) 3481, 2915, 2868, 1711, 1442, 1394, 1349, 1249, 1096, 948, 845, 742, 689, 595. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 2.56-2.59 (t, J = 6.4 Hz, 2H, CH_2), 3.55-3.70 (overlapped multiplets, 48H, OCH_2), 3.74-3.77 (t, J = 6.4 Hz, 2H, OCH_2), 7.15-7.27 (overlapped m, 10H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 35.0 (CH_2), 37.9 (CH_2), 66.7 (OCH_2), 67.6 (OCH_2), 69.8 (OCH_2), 70.2 (OCH_2), 70.5-70.7 (overlapped OCH_2), 128.3 (ArCH), 128.9 (ArCH), 129.0 (ArC), 131.8 (ArCH), 135.6 (C), 166.8 (CO), 173.2 (CO). LRMS (ESI) 937 (100, $[M+Na]^+$), 914 (10, $[M+H]^+$); HRMS (ESI) calcd. for $C_{43}H_{64}NO_{16}S_2$ $[M+H]^+$ 914.3667, observed: 914.3657.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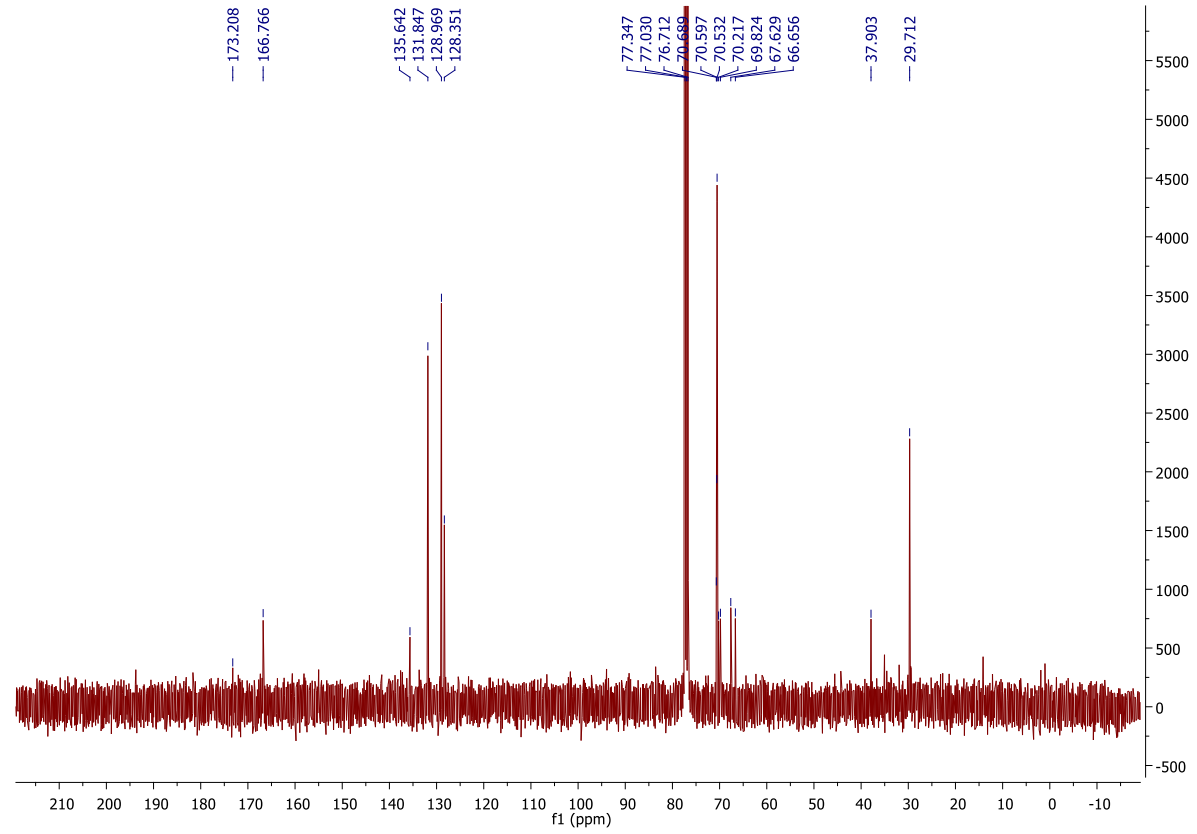
f1 (ppm)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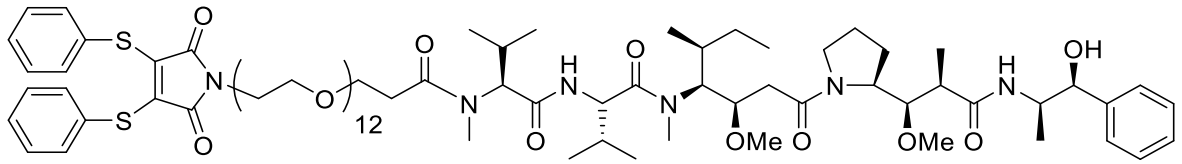
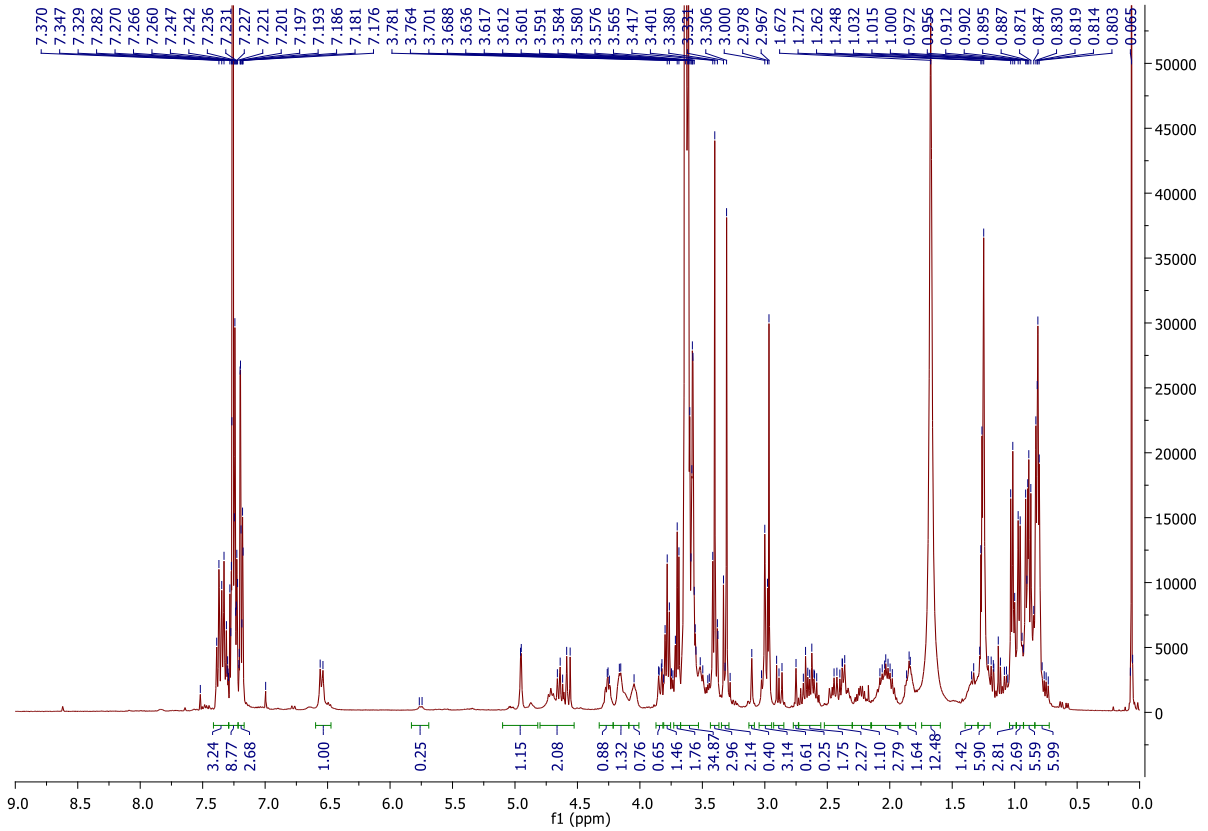
f1 (ppm)

3,4-Dithiophenoyl-maleimide-*N*-PEG$_{12}$-CO-MMAE



In a 10 mL round-bottom flask, oven dried and under nitrogen, 3,4-dithiophenoyl-maleimide-*N*-PEG$_{12}$-acid (6.2 mg, 6.4 µmol, 1.1 eq.) and HBTU (2.4 mg, 6.4 µmol, 1.1 eq.) were dissolved in DMF (0.5 mL). Next, added DIPEA (3 µL, 17 µmol, 3 eq.). The solution was stirred at 20 °C for 10 min. Then, added MMAE (4.2 mg, 5.8 µmol, 1 eq.) in DMF (0.5 mL) and the solution was stirred at 20 °C for 16 h. Then, concentrated under vacuum , added DCM (10 mL) and washed with 15% aq. citric acid (10 mL), aq. sat. $NaHCO_3$ (10 mL) and 40% aq. LiCl (2×10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under vacuum to yield an orange oil which was purified by flash chromatography on silica with a gradient from DCM:EtOAc:MeOH (5:5:1 v/v) to DCM:MeOH (10:1 v/v) to afford 3,4-dithiophenoyl-maleimide-*N*-PEG$_{12}$-CO-MMAE as an orange oil (3.3 mg, 2.0 µmol, 35%). Characterisation data: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.72-0.80 (t, *J* = 6.4, 6H, MMAE), 0.80-0.91 (m, 6H, MMAE), 0.95-0.97 (d, *J* = 6.4, 3H, MMAE), 1.00-1.03 (m, 3H, MMAE), 1.22-1.28 (m, 6H, MMAE), 1.30-1.39 (m, MMAE), 1.66-1.80 (m, MMAE), 1.84-1.86 (m, MMAE), 1.95-2.10 (m, MMAE), 2.18-2.28 (m, MMAE), 2.34-2.48 (m, MMAE), 2.55-2.76 (m, MMAE), 2.86-2.91 (m, MMAE), 2.94-3.04 (m, 3H, MMAE overlapped with PEG CH_2), 3.01-3.11 (m, MMAE), 3.28-3.33 (m, MMAE), 3.37-3.42 (m, MMAE), 3.48-3.54 (m, MMAE), 3.56-3.59 (m, 2H, PEG O*C*H_2), 3.55-3.65 (overlapped multiplets, O*C*H_2), 3.69-3.72 (t, *J* = 6.8 Hz, 2H, O*C*H_2), 3.76-3.80 (t, *J* = 6.8 Hz, 2H, O*C*H_2), 3.78-3.86 (m, MMAE), 4.01-4.08 (m, MMAE), 4.08-4.19 (m, MMAE), 4.21-4.30 (m, MMAE), 4.55-4.80 (m, MMAE), 4.94-4.96 (d, *J* = 2.4 Hz, 1H, MMAE), 6.54-6.56 (d, *J* = 8.8 Hz, 1H, MMAE), 7.17-7.28 (overlapped m, 10H, Ar*H*), 7.29-7.40 (m, 5H, Ar*H* MMAE).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0.40
3.14
0.61
0.25
1.75
2.27
1.10
2.79
1.64
12.48
1.42
5.90
2.81
2.69
5.59
5.99
f1 (ppm)

Cell Lines

Breast cancer cell lines SKBR-3 (HER2-positive) and MCF-7 (HER2-negative) were purchased from ATCC. SKBR-3 cells were maintained at 37 °C, 5% CO_2 in McCoy's 5A medium (Lonza, UK) supplemented with 10% foetal bovine serum (Life Technologies, UK). MCF-7 cells were maintained at 37 °C, 5% CO_2 in Minimum-Essential Medium supplemented with 10% foetal bovine serum (Sigma-Aldrich, UK).

Toxicity Assays

Cells were seeded in 96-well plates at 7 x 10^3 cells/well and allowed to attach for 24 h. Serial dilutions of trastuzumab and NGM-MMAE-ADC **9** were added to the cells at concentrations ranging from 0-70 nM in complete growth medium. After 72 h, medium was replaced with fresh medium and cells were incubated for 3 days, following which, cell viability was measured using the MTT cell proliferation assay (Sigma-Aldrich, UK) according to manufacturer's instructions. Cell viability was plotted as percentage of untreated cells.

References

1. G. Candiano, M. Bruschi, L. Musante, L. Santucci, G. M. Ghiggeri, B. Carnemolla, P. Orecchia, L. Zardi and P. G. Righetti, *Electrophoresis*, 2004, **25**, 1327-1333.
2. F. F. Schumacher, J. P M. Nunes, A. Maruani, V. Chudasama, M. E. B. Smith, K. A. Chester, J. R. Baker and S. Caddick, *Org. Biomol. Chem.*, 2014, **12**, 7261-7269.
3. L. Castañeda, Z. V. F. Wright, C. Marculescu, T. M. Tran, V. Chudasama, A. Maruani, E. A. Hull, J. P. M. Nunes, R. J. Fitzmaurice, M. E. B. Smith, L. H. Jones, S. Caddick and J. R. Baker, *Tetrahedron Lett.*, 2013, **54**, 3493-3495.