Reversible protein affinity-labelling using bromomaleimide-based reagents

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General Experimental

All reagents were purchased from Aldrich or AlfaAesar and were used as received without further purification. Where described below petrol refers to petroleum ether (40-60 °C). All reactions were monitored by thin-layer chromatography (TLC) on pre-coated SIL G/UV254 silica gel plates (254 μm) purchased from VWR. Flash column chromatography was carried out with Kiesegel 60M 0.04/0.063mm (200-400 mesh) silica gel. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on a Bruker Avance 400 instrument operating at a frequency of 400 MHz for ^{1}H and 100 MHz for ^{13}C, a Bruker Avance 500 instrument operating at a frequency of 500 MHz for ^{1}H and 125 MHz for ^{13}C, and a Bruker Avance 600 instrument operating at a frequency of 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$ or CD_3OD (as indicated below). The chemical shifts (δ) for ^{1}H and ^{13}C are quoted relative to residual signals of the solvent on the ppm scale. ^{1}H NMR peaks are reported as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quintet), broad (br) or multiplet (m). Coupling constants (*J* values) are reported in Hertz (Hz) and are H-H coupling constants unless otherwise stated. Signal multiplicities in ^{13}C NMR were determined using the distortionless enhancement by phase transfer (DEPT) spectral editing technique. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 FTIR Spectrometer operating in ATR mode with frequencies given in reciprocal centimetres (cm^{-1}). Melting points were measured with a Gallenkamp apparatus and are uncorrected. Optical rotations were measured using a Perkin Elmer 343 polarimeter. Mass spectra were obtained on a VG70-SE mass spectrometer.

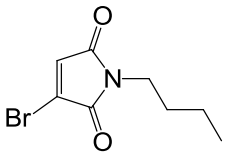
LC-MS was performed on protein samples using a Waters Acquity uPLC connected to Waters Acquity Single Quad Detector (SQD). Column: Acquity uPLC BEH C18 1.7μm 2.1 x 50 mm. Wavelength: 254 nm. Mobile Phase: 95:5 Water (0.1% Formic Acid): MeCN (0.1% Formic Acid). Gradient over 4 min (to 5:95 Water (0.1% Formic Acid): MeCN (0.1% Formic Acid). Flow Rate: 0.6 mL/min. MS Mode: ES^{+}. Scan Range: m/z = 85-2000. Scan time: 0.25 sec. 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. Total mass spectra for protein samples were reconstructed from the ion series using the MaxEnt 1 algorithm pre-installed on MassLynx software.

General procedure for the synthesis of bromomaleimide derivatives 1-4

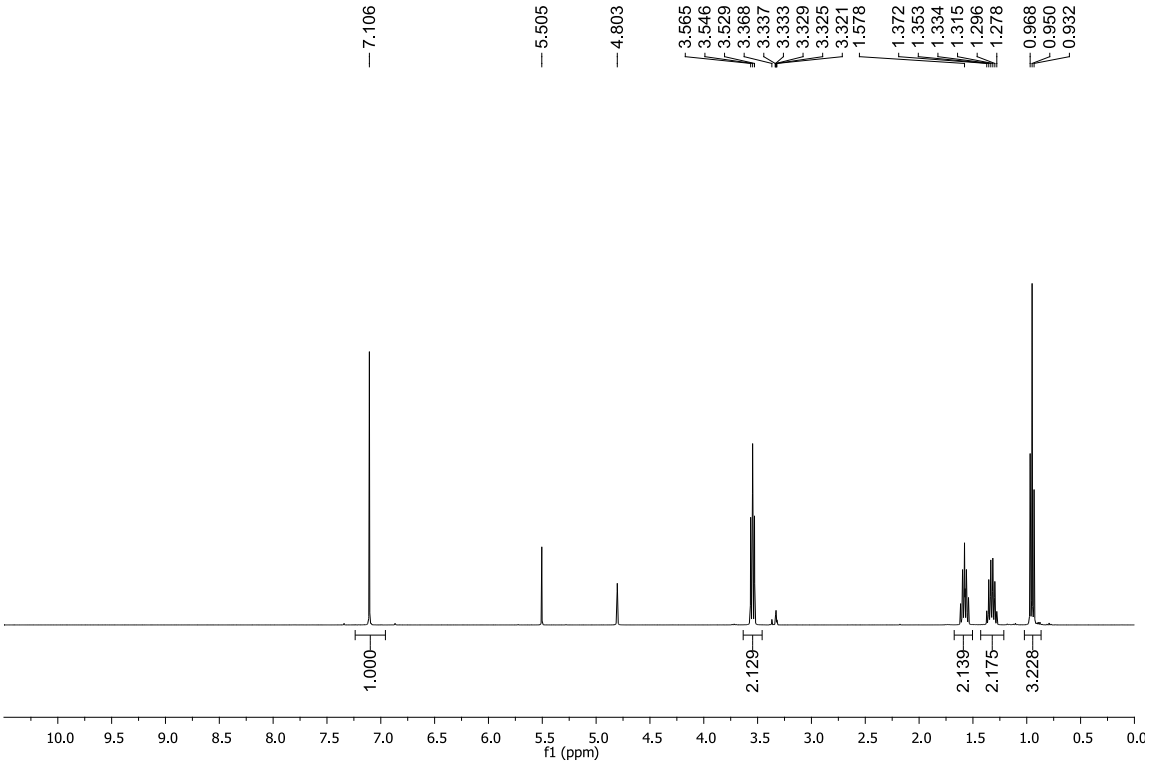
To a solution of amine (1 mmol) in AcOH (3 mL) was added 3-bromo-maleic anhydride (1 mmol) and the reaction mixture was heated under reflux for 3 h. After this time, the reaction mixture was

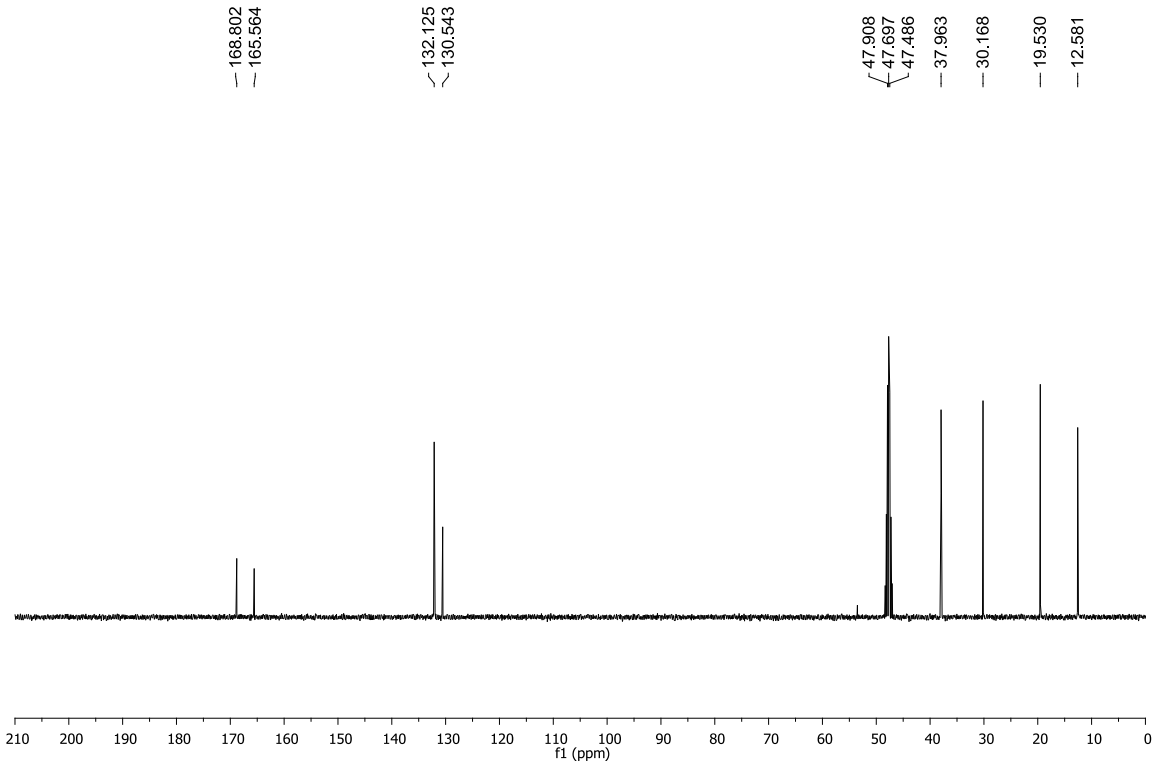
cooled to room temperature, toluene was added (3 mL), the solvents were removed *in vacuo* and the crude residue was purified as described below.

3-Bromo-1-(butyl)-pyrrole-2,5-dione 1

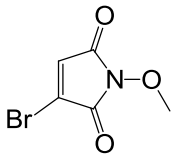


Purified by column chromatography (5% to 50% EtOAc/Petrol) gave 3-bromo-1-(butyl)-pyrrole-2,5-dione **1** as a yellow solid (166 mg, 0.72 mmol, 72%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.11 (s, 1H), 3.55 (t, J = 7.0 Hz, 2H), 1.62-1.54 (m, 2H), 1.37-1.28 (m, 2H), 0.95 (t, J = 7.0 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 168.8 (C), 165.7 (C), 132.1 (C), 130.5 (CH), 38.0 (CH_2), 30.2 (CH_2), 19.5 (CH_2), 12.6 (CH_3); LRMS (CI) 234 (100, $[M+H]^+$), 232 (100, $[M^{79}Br+H]^+$); HRMS (CI) calcd for $C_8H_{11}BrNO_2$ $[M^{79}Br+H]^+$ 231.9973, observed 231.9979.

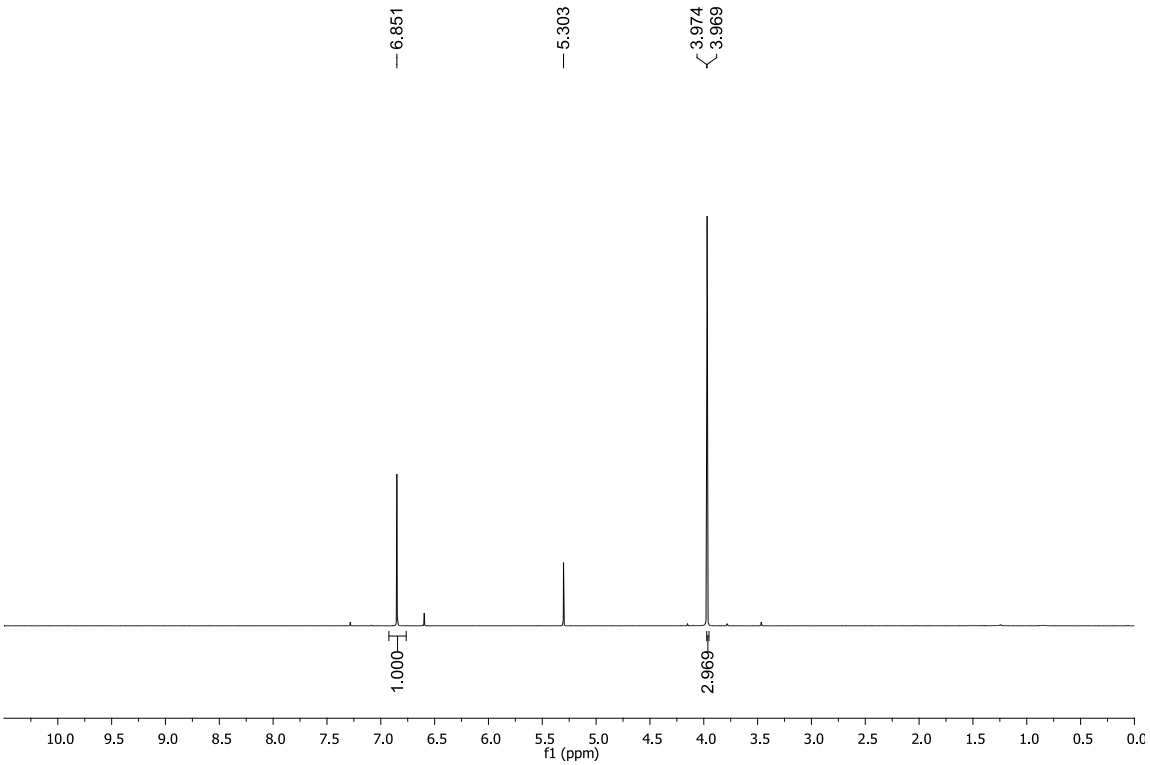


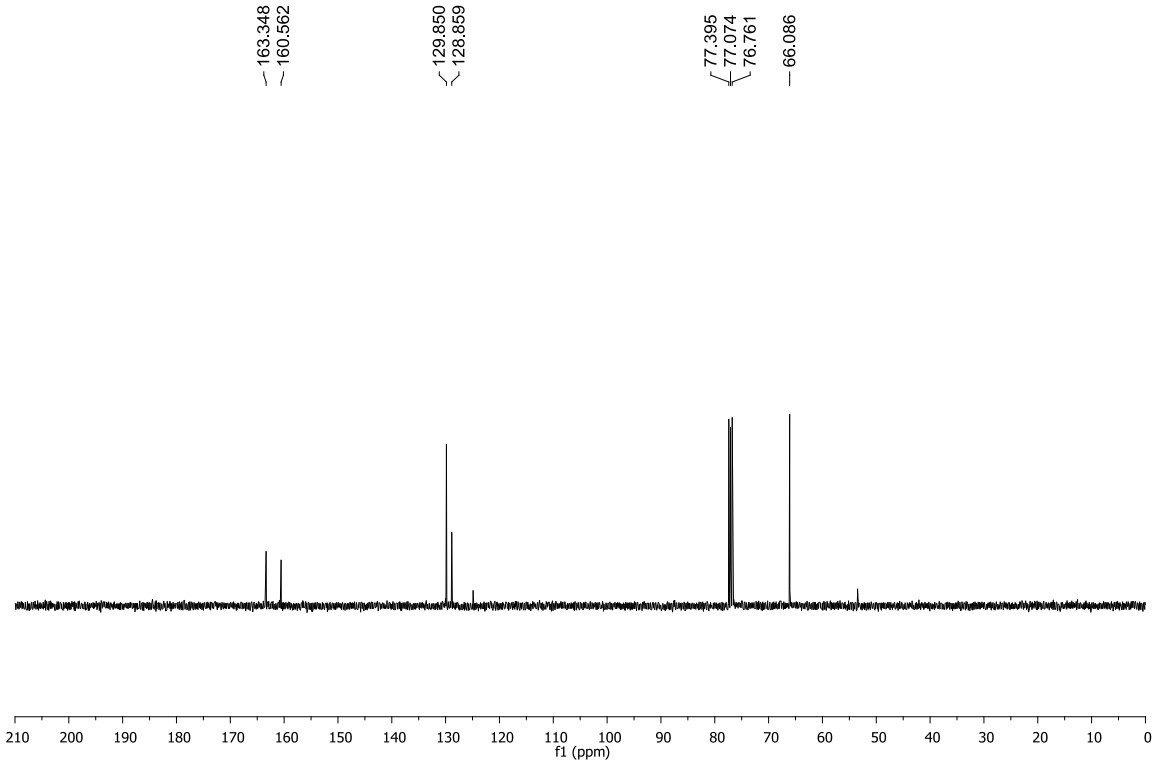


3-Bromo-1-methoxy-pyrrole-2,5-dione 2

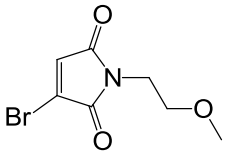


Purification by column chromatography (20% EtOAc/Petrol to neat EtOAc to 1% MeOH/CH_2Cl_2) gave 3-bromo-1-methoxy-pyrrole-2,5-dione **2** as a yellow oil (119 mg, 0.58 mmol, 58%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 (s, 1H), 3.97 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 163.3 (C), 160.6 (C), 129.9 (C), 128.9 (CH), 66.1 (CH_3); LRMS (CI) 206 (100, $[M^{81}Br+H]^+$), 204 (100, $[M^{79}Br+H]^+$); HRMS (CI) calcd for $C_5H_5O_3NBr$ $[M^{81}Br+H]^+$ 205.9453, observed 205.9447.

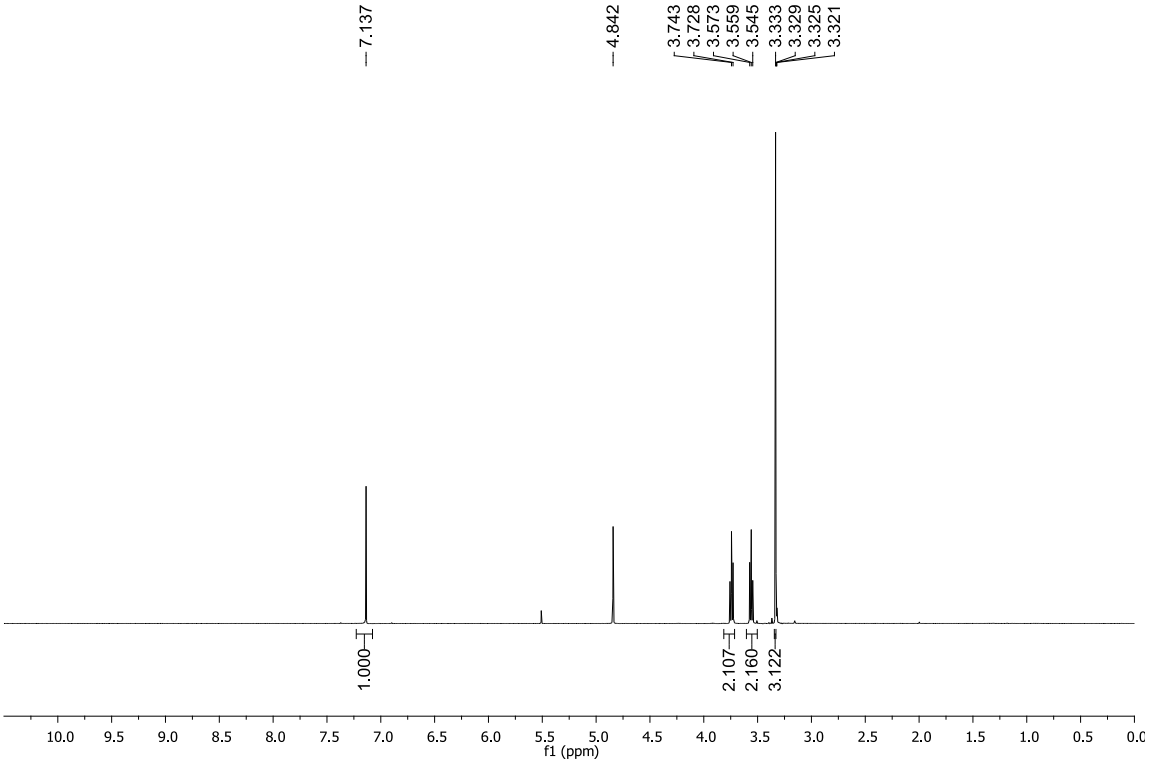


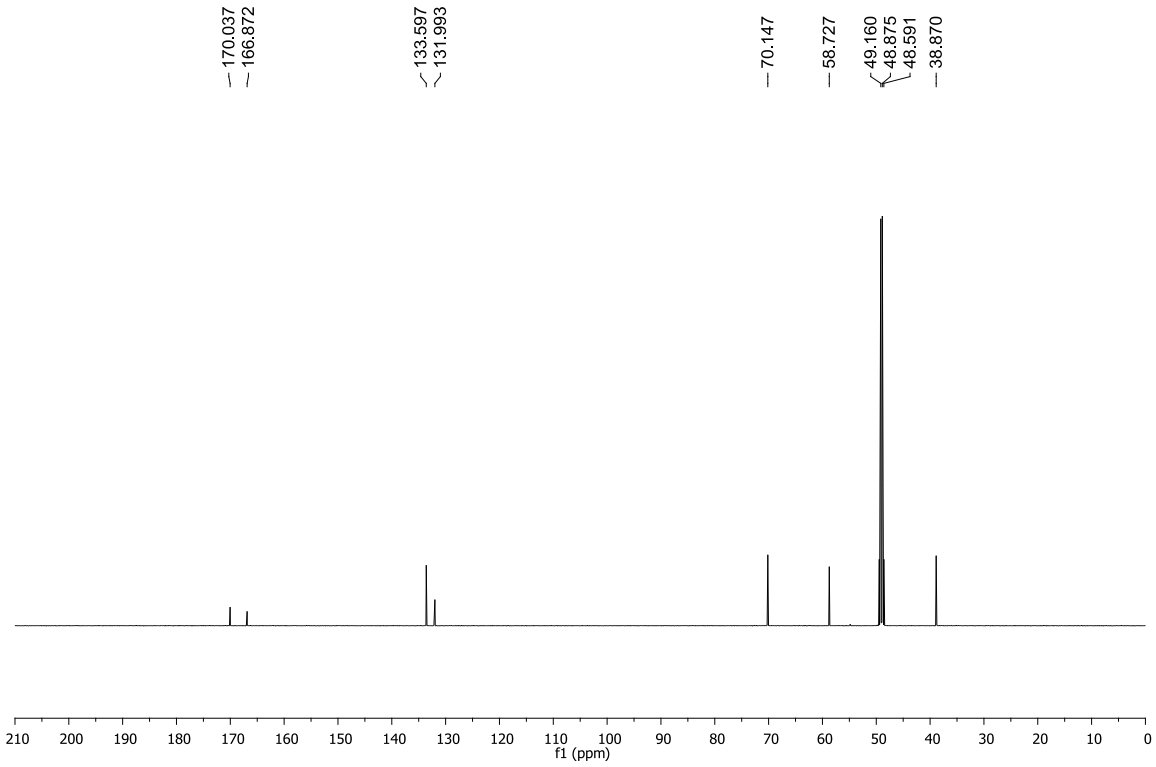


3-Bromo-1-(2-methoxy-ethyl)-pyrrole-2,5-dione 3

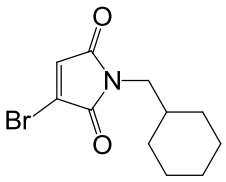


Purification by column chromatography (5% EtOAc/Petrol to neat EtOAc) gave 3-bromo-1-(2-methoxy-ethyl)-pyrrole-2,5-dione **3** as a yellow oil (126 mg, 0.54 mmol, 54%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.14 (s, 1H), 3.74 (t, J = 5.5 Hz, 2H), 3.56 (t, J = 5.5 Hz, 2H), 3.32 (s, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 170.0 (C), 166.9 (C), 133.6 (CH), 132.0 (C), 70.1 (CH_2), 58.7 (CH_3), 38.9 (CH_2); LRMS (CI) 236 (85, $[M^{81}Br+H]^+$), 234 (85, $[M^{79}Br+H]^+$), 204 (100, $[(M^{81}Br\text{-}OCH_3)+H]^+$), 202 (100, $[(M^{79}Br\text{-}OCH_3)+H]^+$); HRMS (CI) calcd for $C_7H_9BrNO_3$ $[M^{79}Br+H]^+$ 233.9766, observed 233.9772.

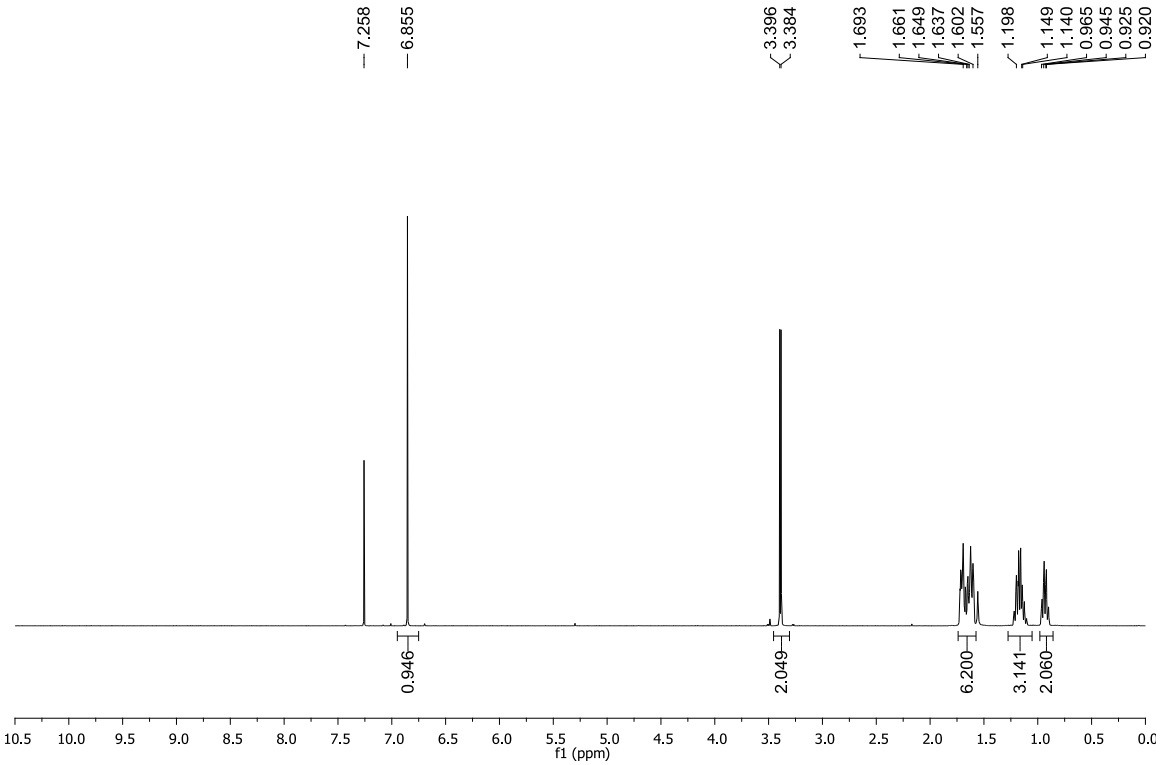


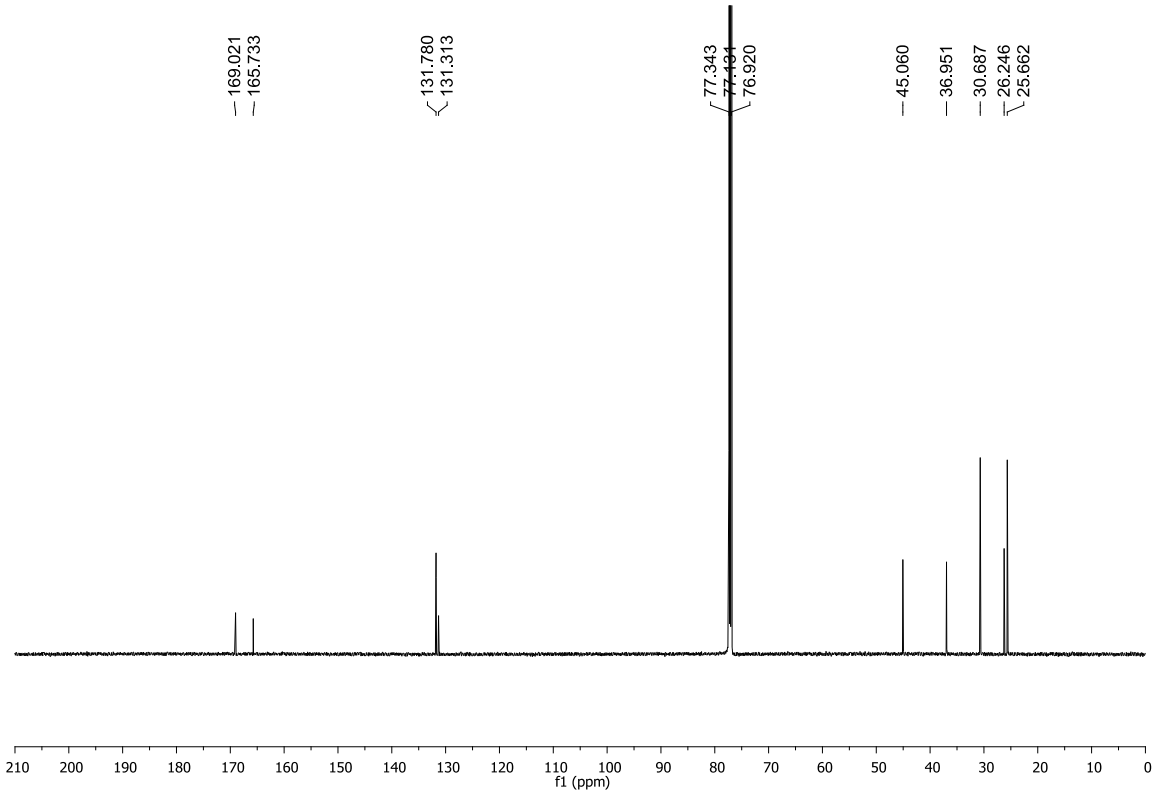


3-Bromo-1-(cyclohexylmethyl)-pyrrole-2,5-dione 4



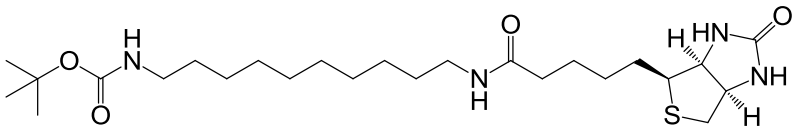
Purification by column chromatography (5% to 20% EtOAc/Petrol) gave 3-bromo-1-(cyclohexylmethyl)-pyrrole-2,5-dione **4** as a yellow solid (163 mg, 0.60 mmol, 60%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.85 (s, 1H), 3.39 (d, J = 7.0 Hz, 2H), 1.72-1.60 (m, 6H), 1.22-1.10 (m, 3H), 0.98-0.88 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.0 (C), 165.7 (C) 131.8 (CH), 131.3 (C), 45.1 (CH_2), 37.0 (CH), 30.7 (CH_2), 26.3 (CH_2), 25.7 (CH_2); LRMS (EI) 273 (65, $[M^{81}Br]^{+\cdot}$), 271 (65, $[M^{79}Br]^{+\cdot}$), 190 (100, $[(M^{81}Br\text{-}C_6H_{11})]^{+}$), 188 (100, $[(M^{79}Br\text{-}\ C_6H_{11})]^{+}$); HRMS (EI) calcd for $C_{11}H_{14}BrNO_2$ $[M^{79}Br]^{+\cdot}$ 271.0202, observed 271.0206.



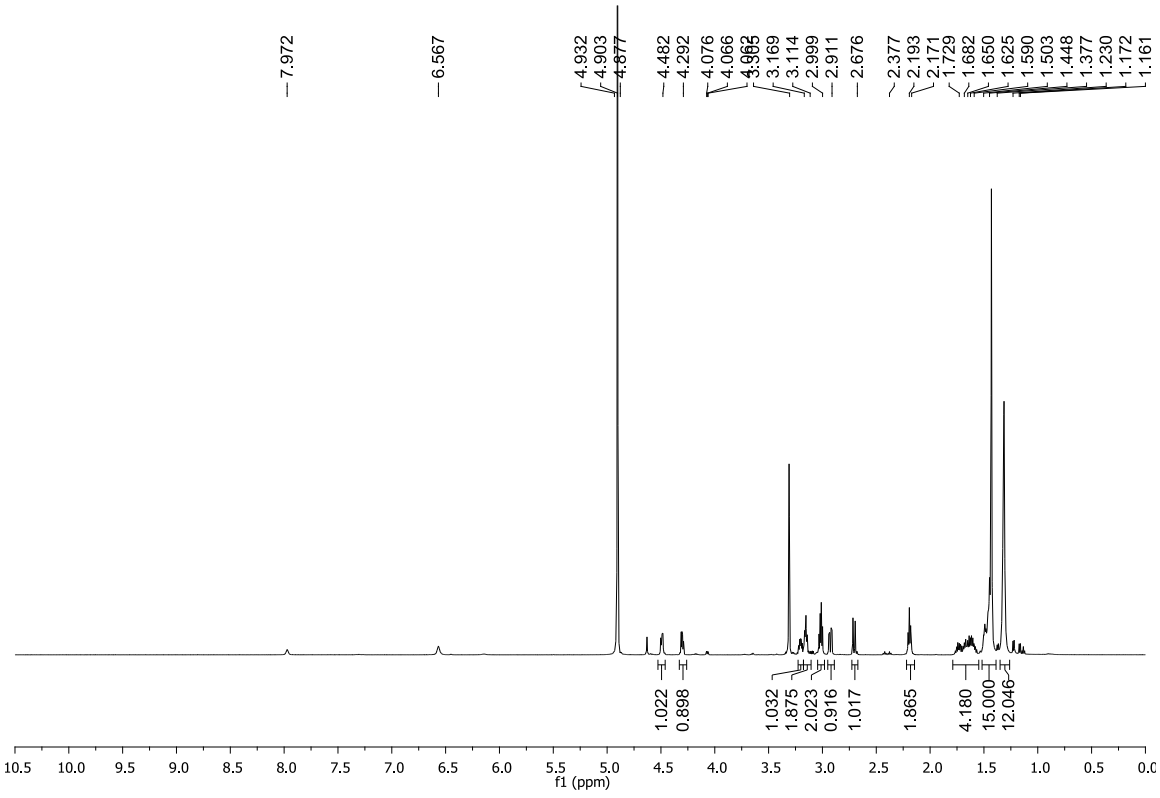


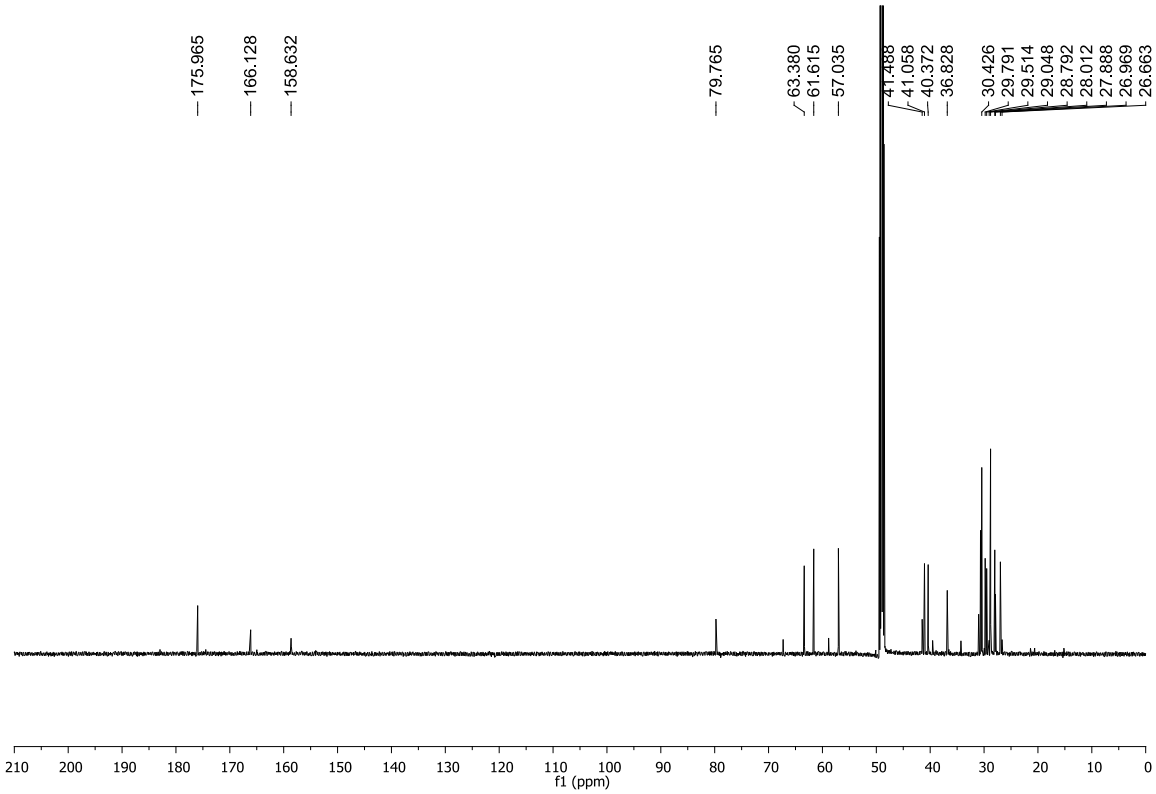
Synthesis of biotin-decane-maleimide reagent 6

***tert*-Butyl (10-(5-((4*S*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido) decyl)carbamate**

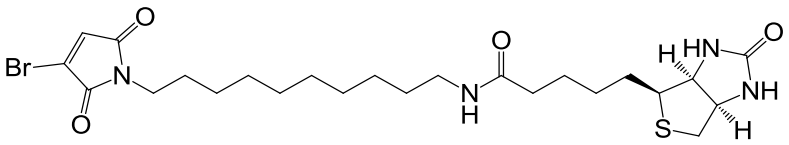


A solution of biotin (0.59 g, 2.42 mmol), HBTU (0.79 g, 2.10 mmol) and DIPEA (0.45 mL, 2.60 mmol) in DMF (15 mL) was stirred for 20 min at 21 °C before being added dropwise to a solution of *tert*-butyl (4-aminodecyl)carbamate [1] (400 mg, 1.61 mmol) in DMF (10 mL). The reaction mixture was stirred for 2 h at 21 °C. After this time, the DMF was removed *in vacuo* and the crude residue purified by column chromatography (2% to 10% MeOH/CH_2Cl_2) to yield tert-butyl (10-(5-((4*S*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)decyl)carbamate as a white solid (641 mg, 1.29 mmol, 80%): ^{1}H NMR (600 MHz, CD_3OD) δ 4.49 (dd, J = 5.0, 7.0 Hz, 1H), 4.30 (dd, J = 5.0, 7.0 Hz, 1H), 3.22-3.19 (m, 1H), 3.16 (dt, J = 2.5, 7.0 Hz, 2H), 3.01 (q, J = 7.0 Hz, 2H), 2.93 (dd, J = 5.0, 12.5 Hz, 1H), 2.70 (d, J = 12.5 Hz, 1H), 2.19 (t, J = 7.0 Hz, 2H), 1.76-1.57 (m, 4H), 1.51-1.41 (m, 15H), 1.35-1.28 (m, 12H); ^{13}C NMR (150 MHz, CD_3OD) δ 176.0 (C), 166.1 (C), 158.6 (C), 79.8 (C), 63.4 (CH), 61.6 (CH), 57.0 (CH), 41.5 (CH_2), 41.0 (CH_2), 40.4 (CH_2), 36.8 (CH_2), 31.0 (CH_2), 30.7 (CH_2), 30.6 (CH_2), 30.4 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 28.8 (CH_3), 28.0 (CH_2), 27.9 (CH_2), 27.0 (CH_2); IR (neat) 3305, 2937, 1692 cm^{-1}; LRMS (ES$^+$) 521 (100, $[M+Na]^+$); HRMS (ES$^+$) calcd for $C_{25}H_{46}N_4O_4NaS$ $[M+Na]^+$ 521.3137, observed 521.3126.



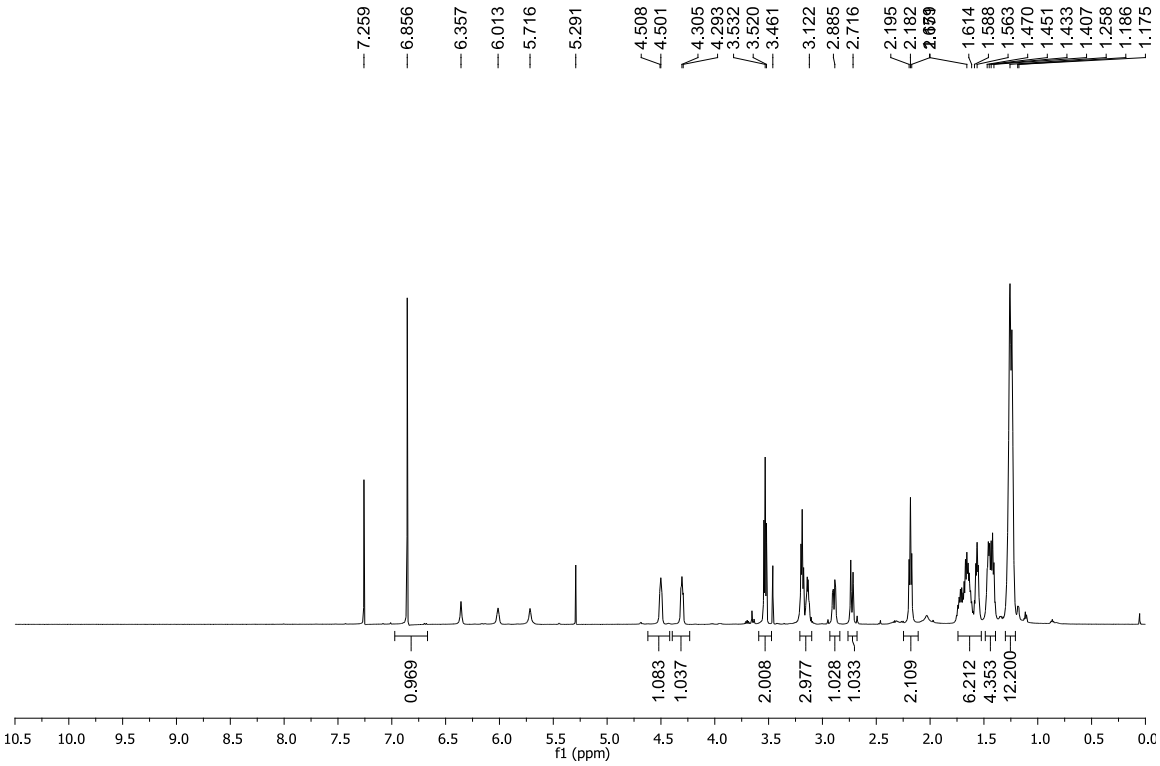


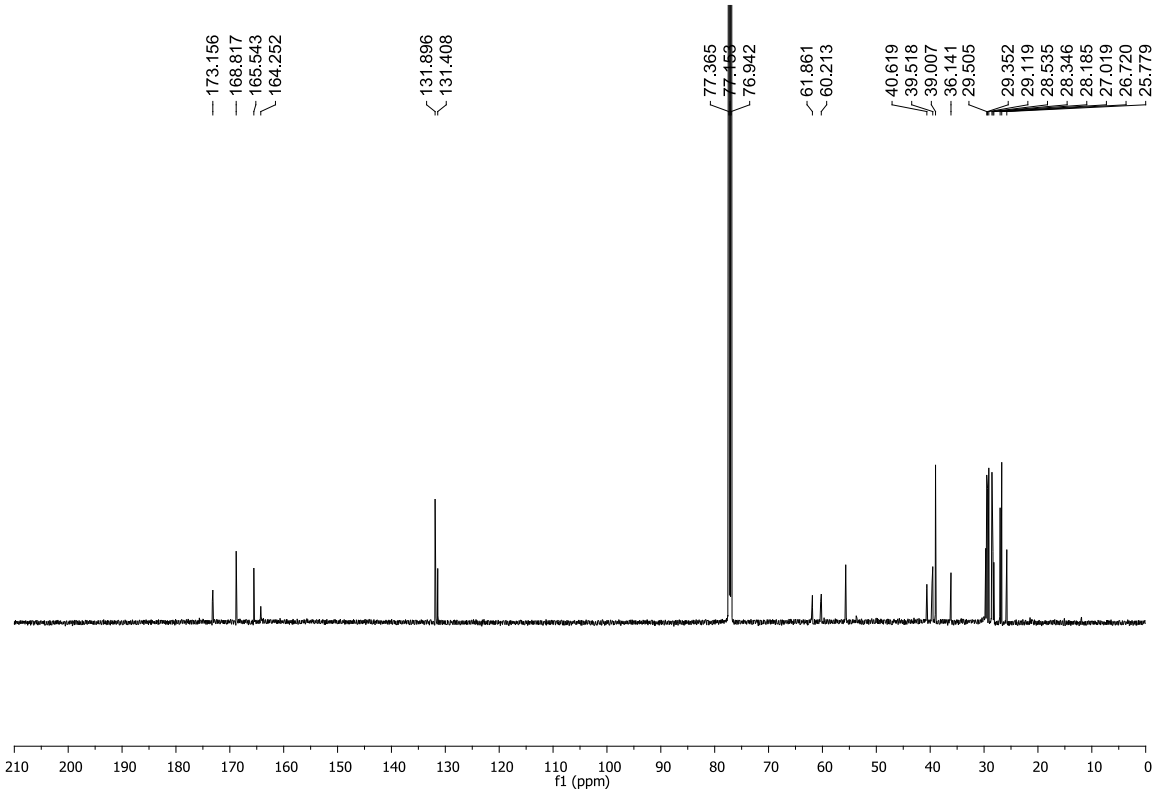
_N_-(10-(3-Bromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)decyl)-5-((4S)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide 6



TFA (5 mL) was added to tert-butyl (10-(5-((4*S*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)decyl)carbamate (125 mg, 0.25 mmol) and the reaction mixture stirred at 21 °C for 15 h. After this time, toluene was added (5 mL) and the solvent removed *in vacuo* to give crude 10-(5-((4S)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)decan-1-amonium 2,2,2-trifluoroacetate. 3-Bromo-maleic anhydride (45.0 mg, 0.25 mmol) was added in one portion to a solution of crude 10-(5-((4S)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)decan-1-amonium 2,2,2-trifluoroacetate in AcOH (10 mL) and the reaction was heated at reflux for 3 h. After cooling the reaction mixture to room temperature toluene was added (20 mL), the solvents were removed *in vacuo* (x2) and the crude residue was purified by column chromatography (2% to 10% MeOH/CH_2Cl_2) to afford *N*-(10-(3-bromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)decyl)-5-((4*S*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide **6** as a white solid (70 mg, 0.13 mmol, 68% yield): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.86 (s, 1H), 6.37-6.34 (br m, 1H), 6.04-5.98 (br m, 1H), 5.74-5.68 (br m, 1H), 4.51-4.49 (m, 1H), 4.31-4.29 (m, 1H), 3.53 (t, J = 7.0 Hz, 2H), 3.21-3.12 (m, 3H), 2.89 (dd, J = 4.5, 13.0 Hz, 1H), 2.73 (d, J = 13.0 Hz, 1H), 2.18 (t, J = 7.5 Hz, 2H), 1.74-1.54 (m, 6H), 1.47-1.39 (m, 4H), 1.28-1.22 (m, 12H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.2 (C), 168.8 (C), 165.5 (C), 164.3 (C), 131.9 (CH), 131.4 (C), 61.9 (CH), 60.2 (CH), 55.7 (CH), 40.6 (CH_2), 39.5 (CH_2), 39.0 (CH_2), 36.1 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 28.5 (CH_2), 28.4 (CH_2), 28.2 (CH_2), 27.0 (CH_2), 26.7 (CH_2), 25.8 (CH_2); IR (neat) 3352, 2975, 1732, 1584

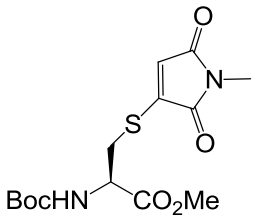
cm^{-1}; LRMS (ES^+) 581 (100, $[M^{79}Br+Na]^+$), 579 (100, $[M^{79}Br+Na]^+$); HRMS (CI) calcd for $C_{24}H_{38}N_4O_4SBr$ $[M^{79}Br+H]^+$ 577.1797, observed 577.1769.



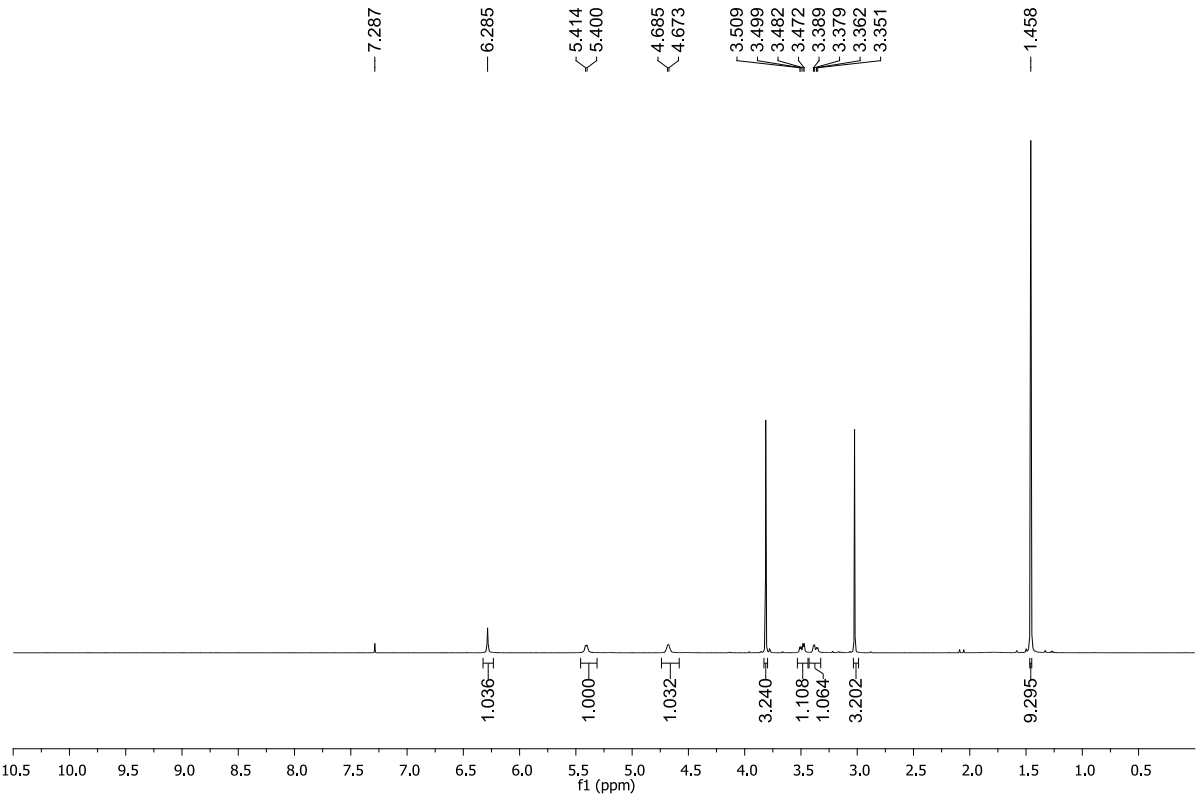


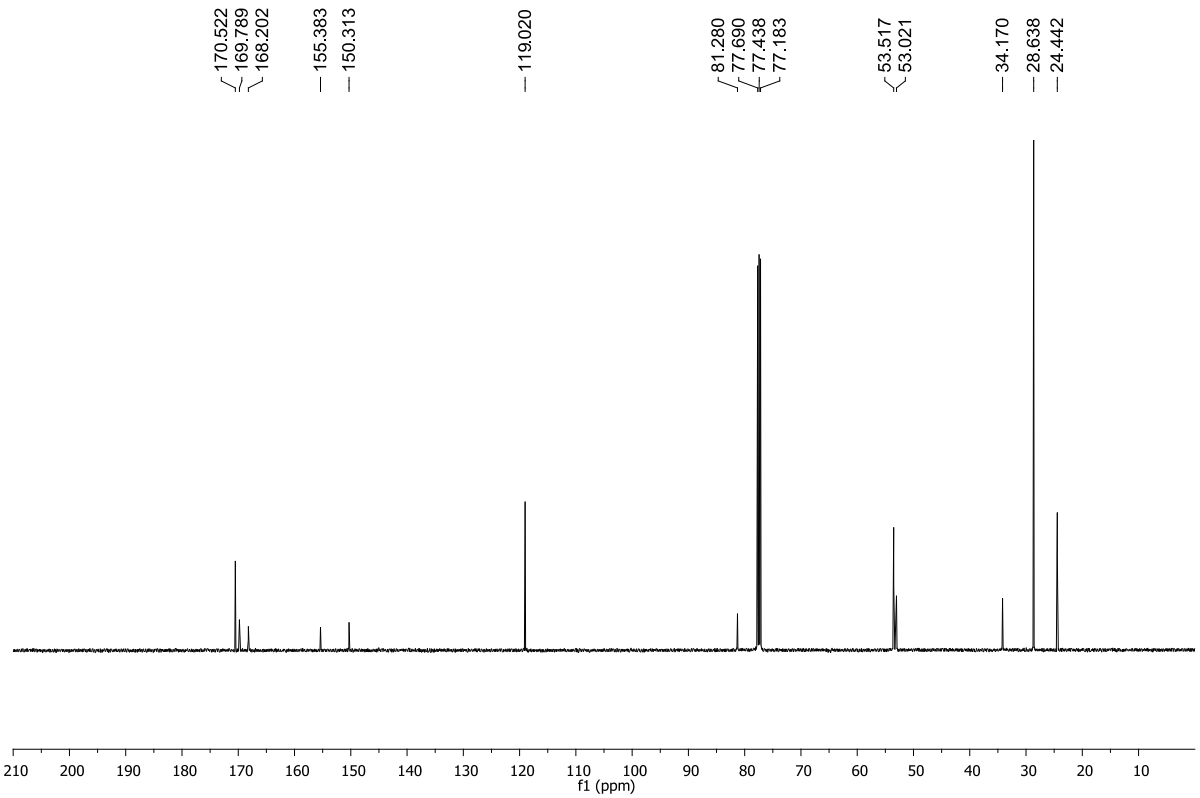
Small molecule studies

(*R*)-Methyl 2-((*tert*-butoxycarbonyl)amino)-3-((1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)thio)propanoate 10

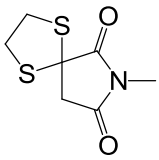


To a stirring solution of *N*-Boc-Cys-OMe (320 mg, 1.36 mmol) and NaOAc (820 mg, 4.08 mmol) in MeOH (10 mL) was added a solution of N-methyl bromomaleimide (258 mg, 1.36 mmol) in MeOH (10 mL) over a period of 10 min. After this time, the solvents were removed *in vacuo* and the crude residue was purified by column chromatography (10% to 30% EtOAc/Petrol) to afford (*R*)-methyl 2-((tert-butoxycarbonyl)amino)-3-((1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)thio)propanoate **10** as a pale white powder (393 mg, 1.14 mmol, 84%): m.p. 101-103 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.29 (s, 1H), 5.41 (d, 1H, J = 6.5 Hz), 4.69-4.67 (m, 1H), 3.81 (s, 3H), 3.49 (dd, 1H, J = 13.5 and 5.0 Hz), 3.37 (dd, 1H, J = 13.5 and 5.0 Hz), 3.02 (s, 3H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.5 (C), 169.8 (C), 168.0 (C), 155.3 (C), 150.3 (C), 118.7 (CH), 81.2 (C), 53.5 (CH_3), 53.0 (CH), 34.1 (CH_2), 28.6 (CH_3), 24.4 (CH_3); IR (solid) 3368, 2977, 1695 cm^{-1}; LRMS (ES$^+$) 367 (46, $[M+Na]^+$), 344 (100, $[M]^+$); HRMS (ES$^+$) calcd for $C_{14}H_{20}N_2O_6NaS$ $[M+Na]^+$ 367.0940, observed 367.0931; $^{20}\alpha_D$ = -18.6° (c 1.0, MeOH).

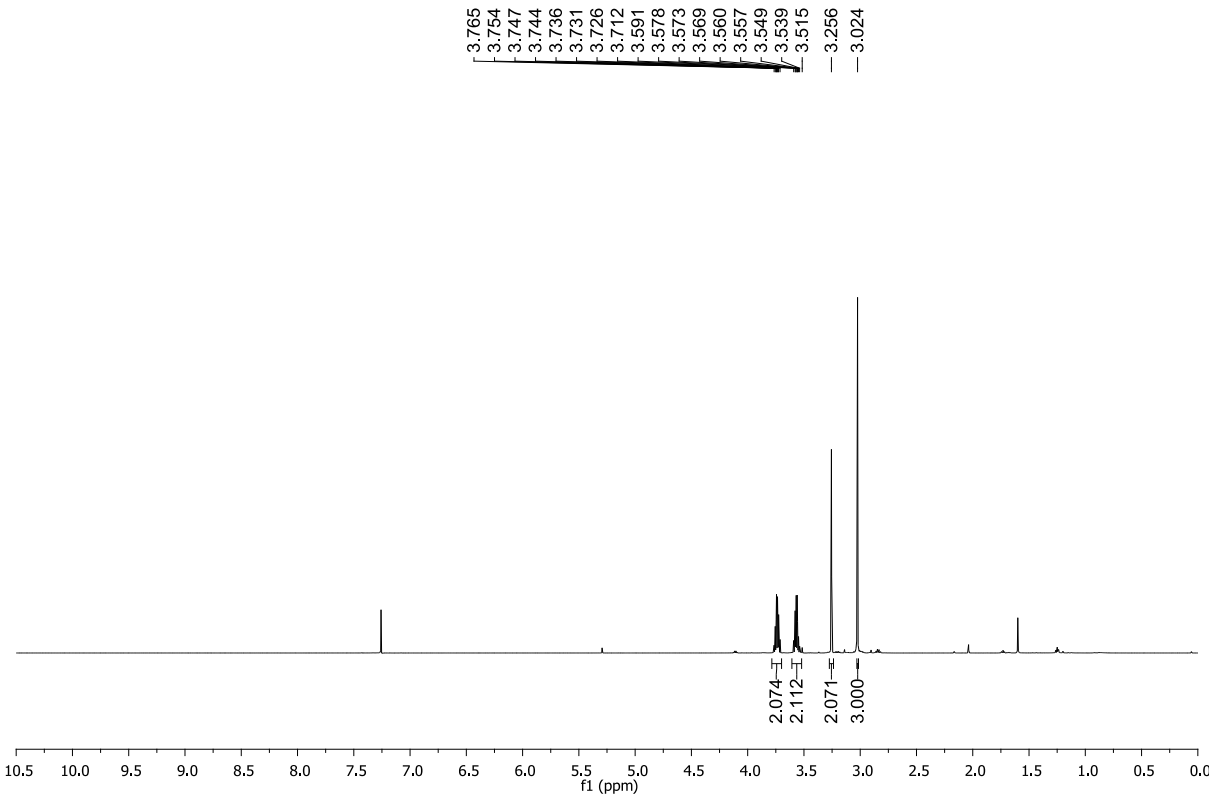


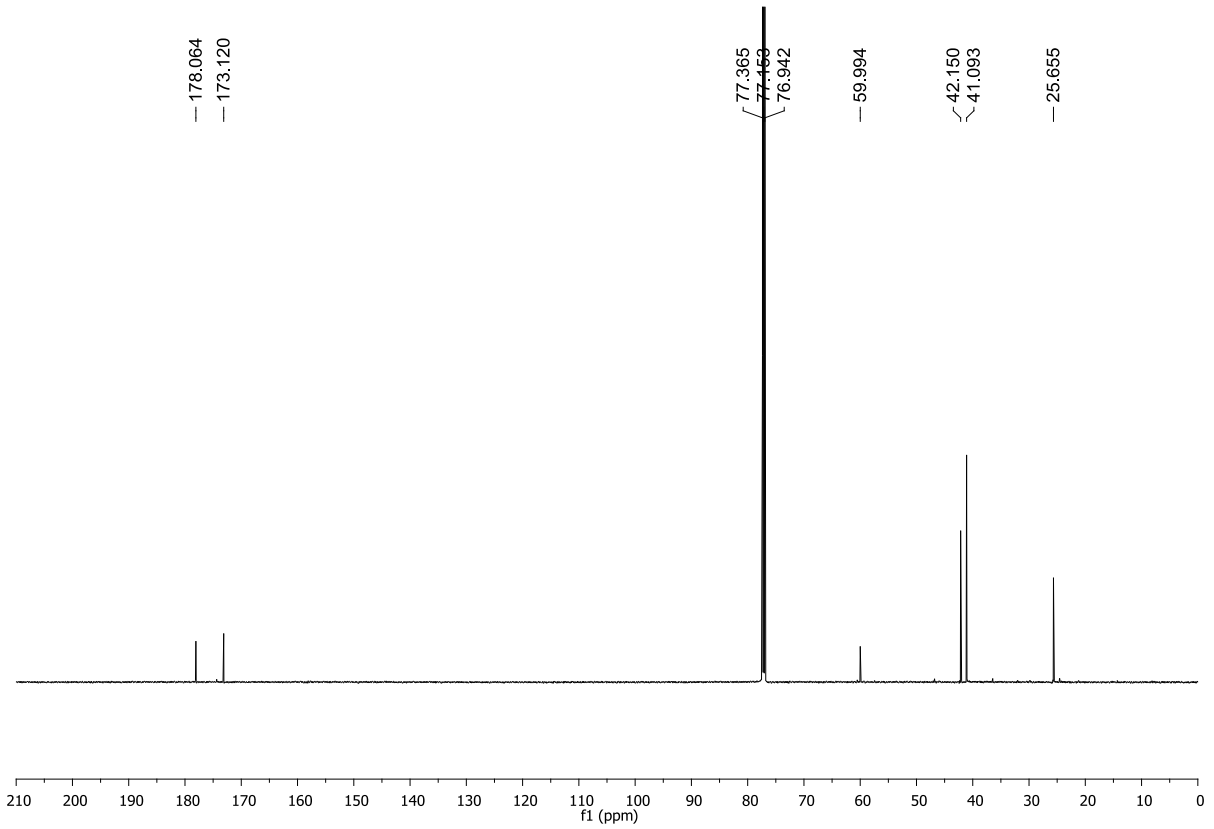


7-Methyl-1,4-dithia-7-azaspiro[4.4]nonane-6,8-dione 11

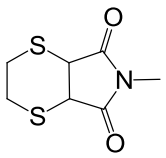


To a solution of (*R*)-Methyl 2-((tert-butoxycarbonyl)amino)-3-((1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)thio)propanoate **10** (40 mg, 0.12 mmol) in a mixture of DMF (1.5 mL) and sodium phosphate buffer (100 mM, pH 8.0, 13 mL) was added 1,2-ethanedithiol (11 mg, 0.12 mmol) in DMF (1.5 mL). The reaction mixture was heated at 37 °C for 24 h. The aqueous reaction mixture was extracted with EtOAc (2 x 25 mL). The organics were combined and washed with saturated aqueous LiCl (3 x 25 mL), saturated aqueous NaCl (25 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude residue purified by column chromatography (10% to 40% EtOAc/Petrol) to afford 7-methyl-1,4-dithia-7-azaspiro[4.4]nonane-6,8-dione **11** as a white solid (22 mg, 0.11 mmol, 95%): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 3.77-3.71 (m, 2H), 3.59-3.52 (m, 2H), 3.26 (s, 2H), 3.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 178.1 (C), 173.1 (C), 60.0 (C), 42.2 (CH_2), 41.1 (CH_2), 25.7 (CH_3); IR (solid) 2935, 1736, 1434 cm^{-1}; LRMS (ES$^+$) 203 (100, [M]$^+$); HRMS (ES$^+$) calcd for $C_7H_9NO_2S_2$ [M]$^+$ 203.0069, observed 203.0067.

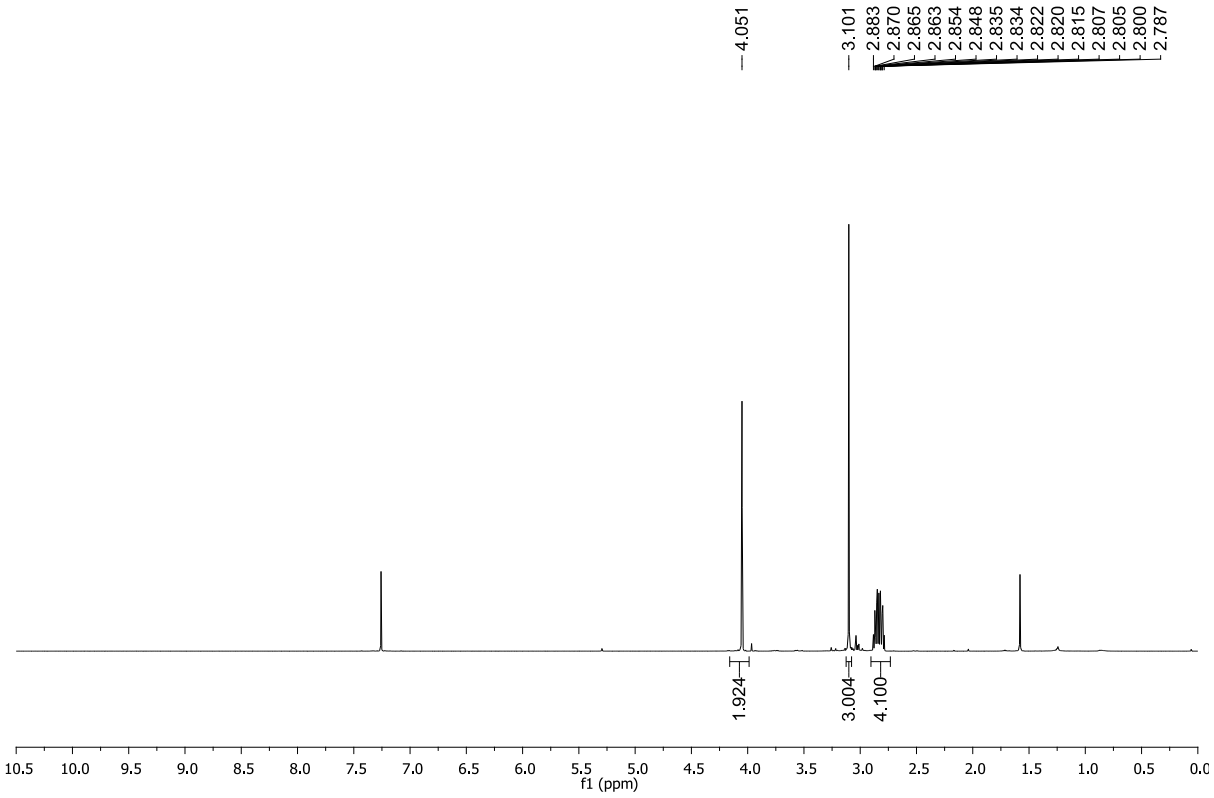


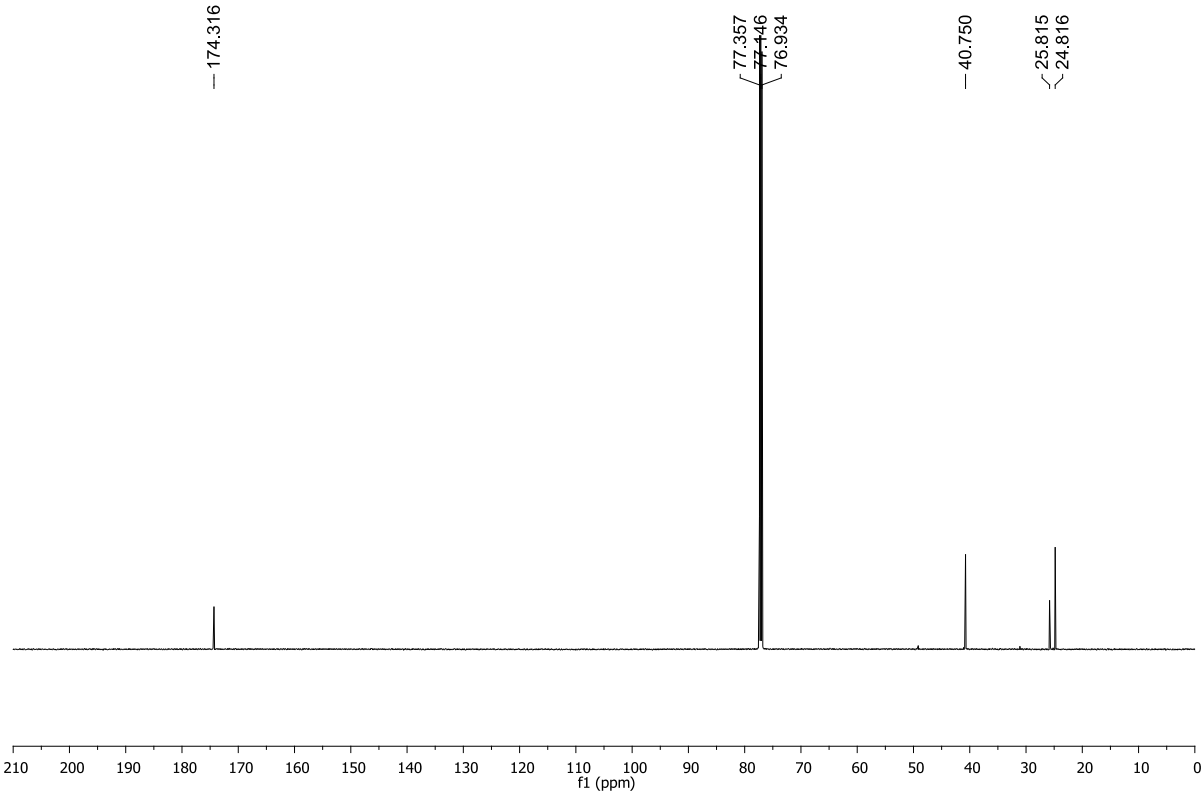


6-Methyldihydro-4a*H*-[1,4]dithiino[2,3-*c*]pyrrole-5,7(6*H*,7a*H*)-dione 12

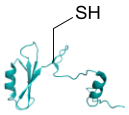


To a solution of *N*-methyl bromomaleimide (40 mg, 0.21 mmol) in a mixture of DMF (1.5 mL) and sodium phosphate buffer (100 mM, pH 8.0, 13 mL) was added 1,2-ethanedithiol (20 mg, 0.21 mmol) in DMF (1.5 mL). The reaction mixture was heated at 37 °C for 90 min. The aqueous reaction mixture was extracted with EtOAc (2 x 25 mL). The organics were combined and washed with saturated aqueous LiCl (3 x 25 mL), saturated aqueous NaCl (25 mL), dried ($MgSO_4$), the solvent removed *in vacuo* and the crude residue purified by column chromatography (10% to 30% EtOAc/Petrol) to afford 6-methyldihydro-4a*H*-[1,4]dithiino[2,3-*c*]pyrrole-5,7(6*H*,7a*H*)-dione **12** as a white solid (15 mg, 0.07 mmol, 35%): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.05 (s, 2H), 3.10 (s, 3H), 2.88-2.79 (m, 4H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.3 (C), 40.8 (CH_3), 25.8 (CH), 24.8 (CH_2); IR (solid) 2985, 1737, 1447, 1373 cm^{-1}; LRMS (ES^+) 203 (100, $[M]^+$); HRMS (ES^+) calcd for $C_7H_9NO_2S_2$ $[M]^+$ 203.0069, observed 203.0070.

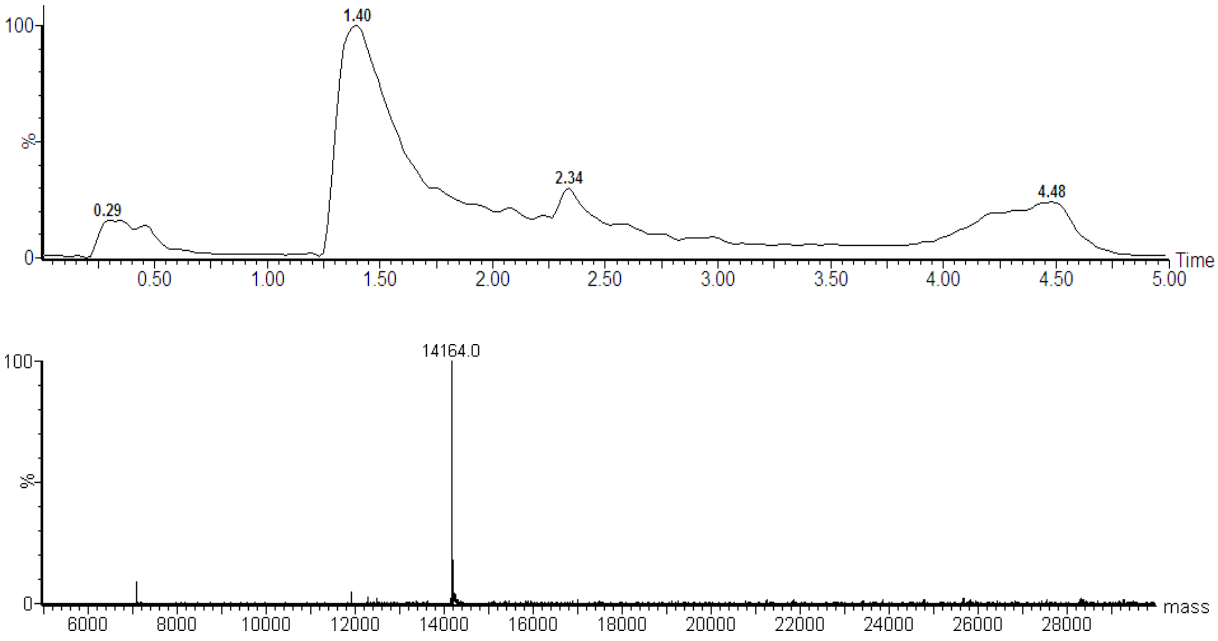


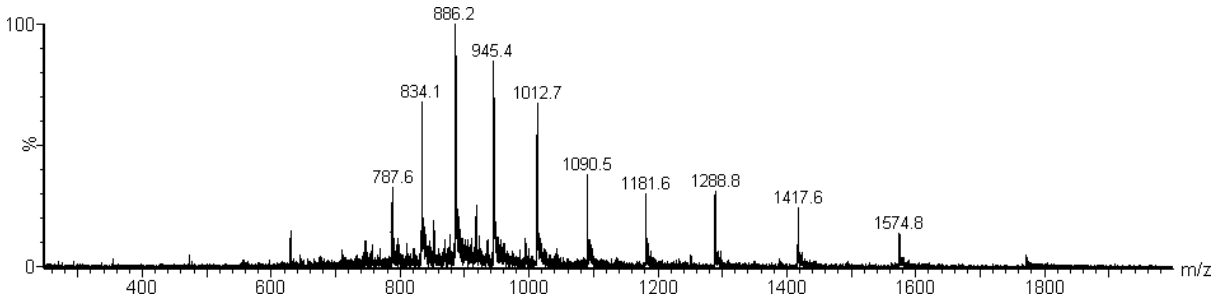


Cloning and expression of Grb2 SH2 (L111C) 5

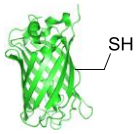


The cloning and expression of this protein was carried out by the method described by Caddick.[2] The mass of the monomeric protein **5** (mass 14164) was obtained using LC-MS:

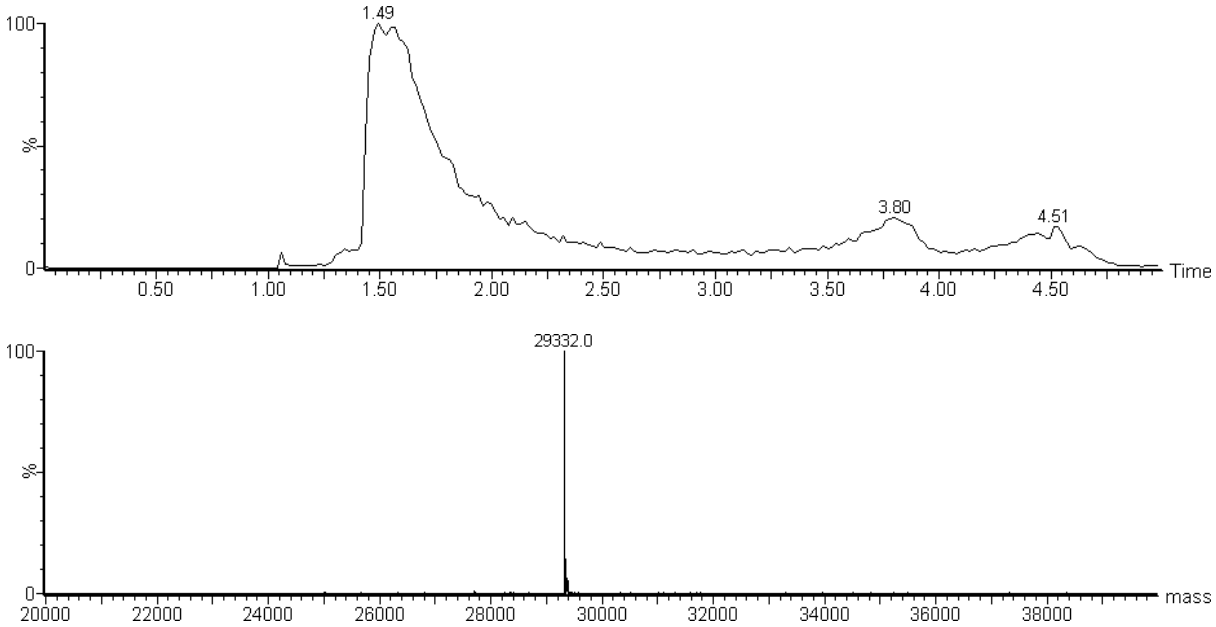


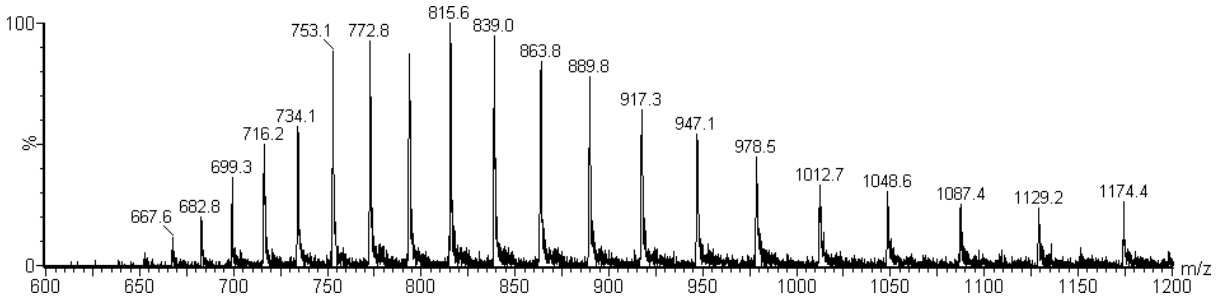


Cloning and expression of GFP (S147C) 7



The cloning and expression of this protein was carried out by the method described by Caddick.[3] The mass of the monomeric protein **7** (mass 29332) was obtained using LC-MS:

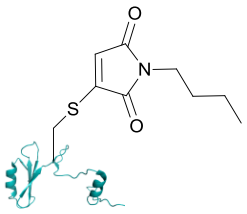




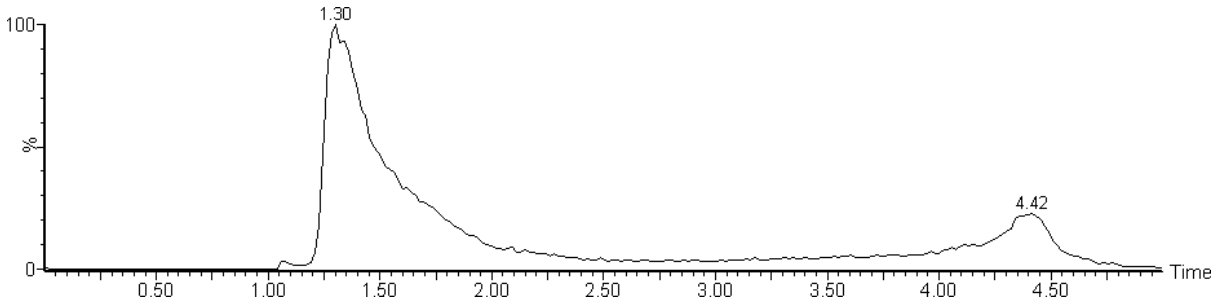
General Procedure for protein modification with bromomaleimide reagents

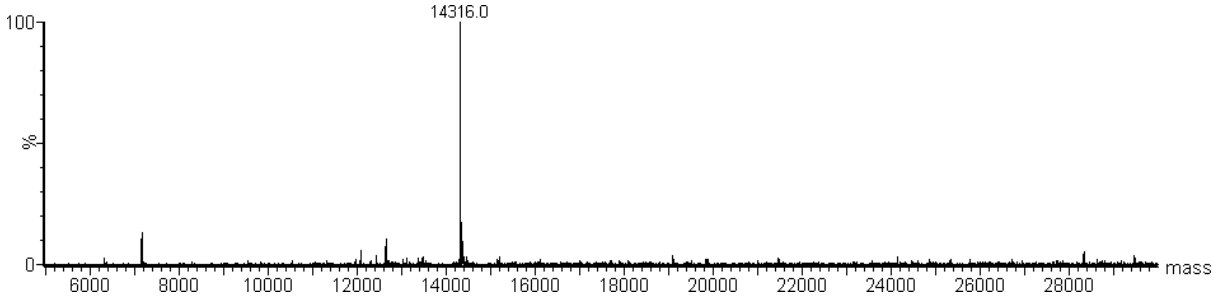
To a solution of protein (100 µL, [protein] 1.0 mg/mL, 100 mM sodium phosphate, 150 mM NaCl, pH 8.0) at 0 °C was added bromomaleimide (5 µL solution in DMF, 1 equivalent). The mixture was maintained at 0 °C for 1 h and analysed by LCMS.

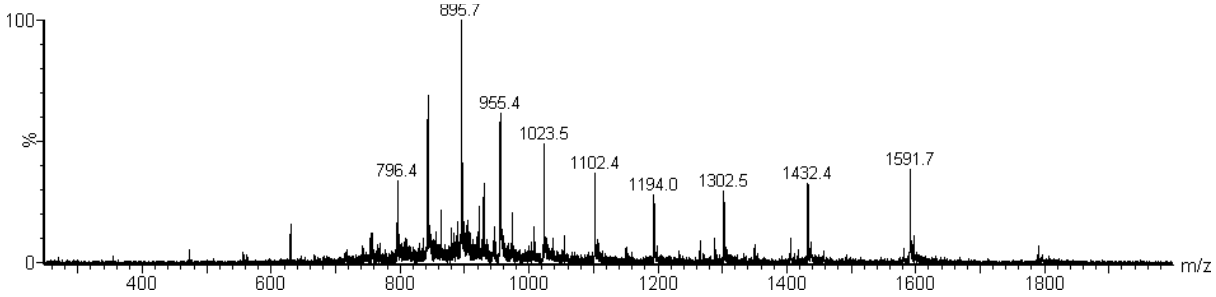
Grb2 SH2 (L111C) 5 conjugate with bromomaleimide 1



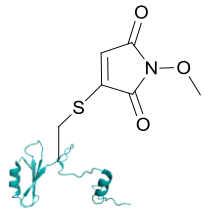
Expected Mass: 14316; Observed Mass: 14316



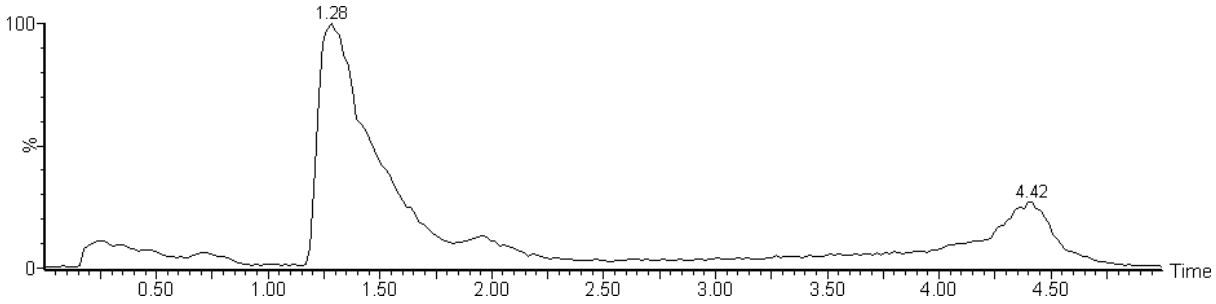


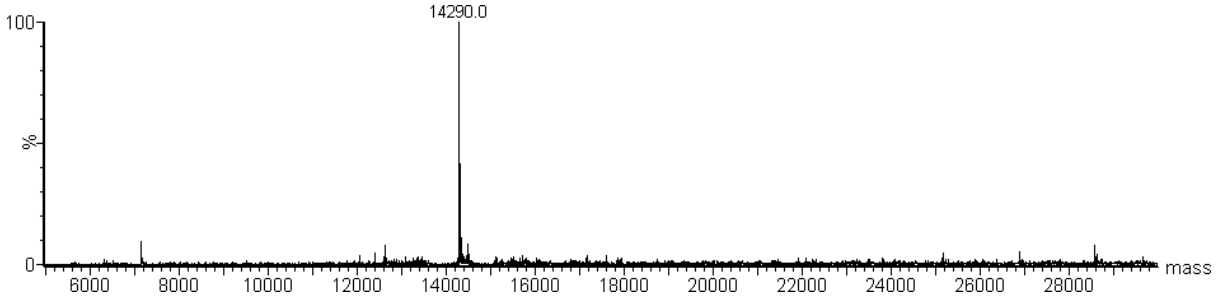


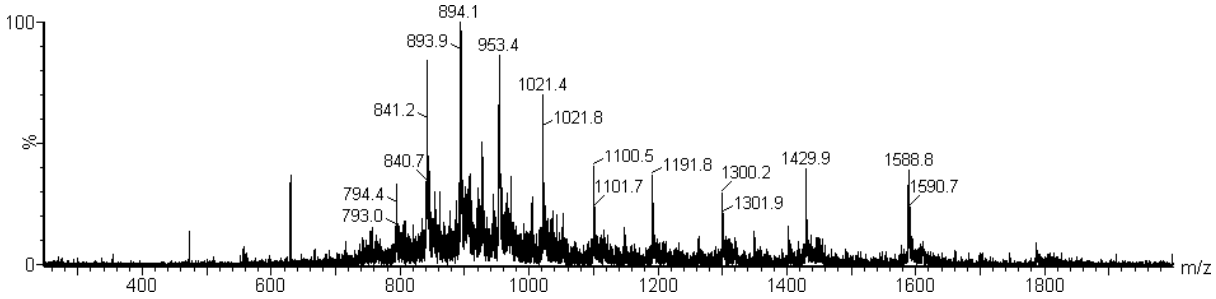
Grb2 SH2 (L111C) 5 conjugate with bromomaleimide 2



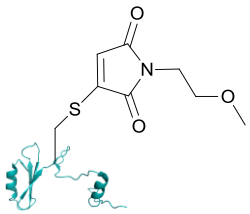
Expected Mass: 14290; Observed Mass: 14290



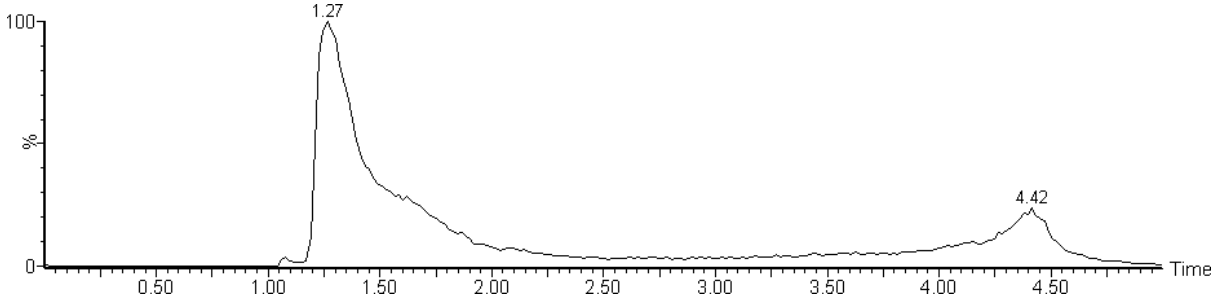


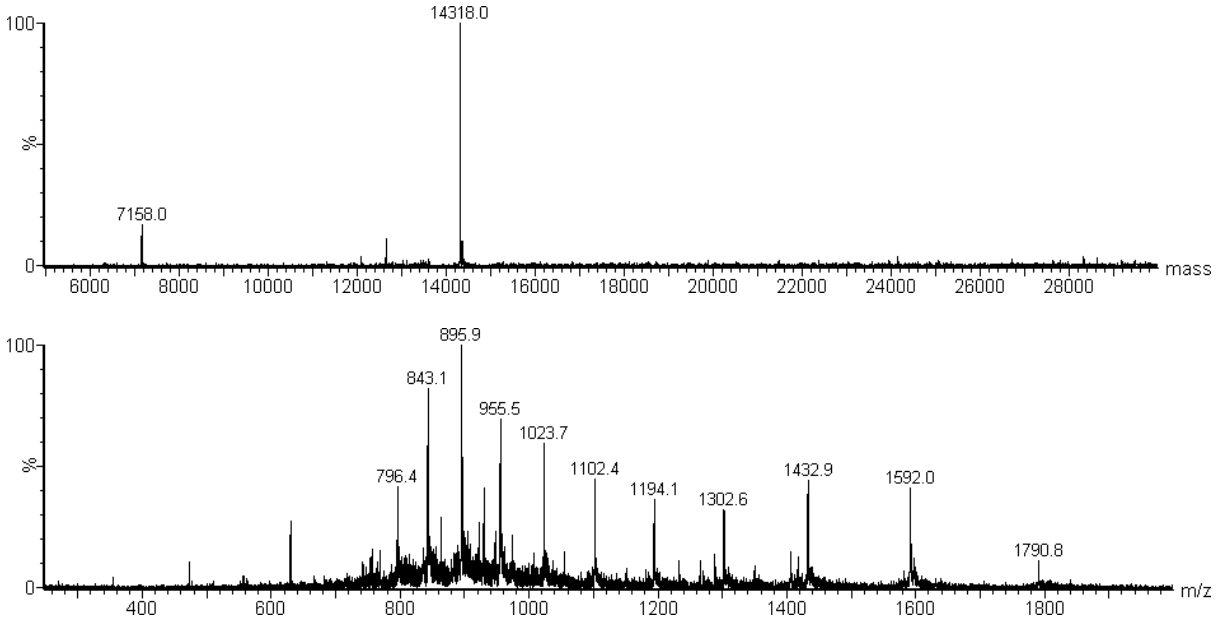


Grb2 SH2 (L111C) 5 conjugate with bromomaleimide 3

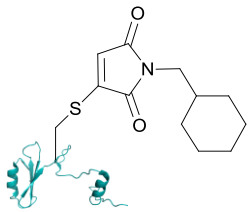


Expected Mass: 14318; Observed Mass: 14318

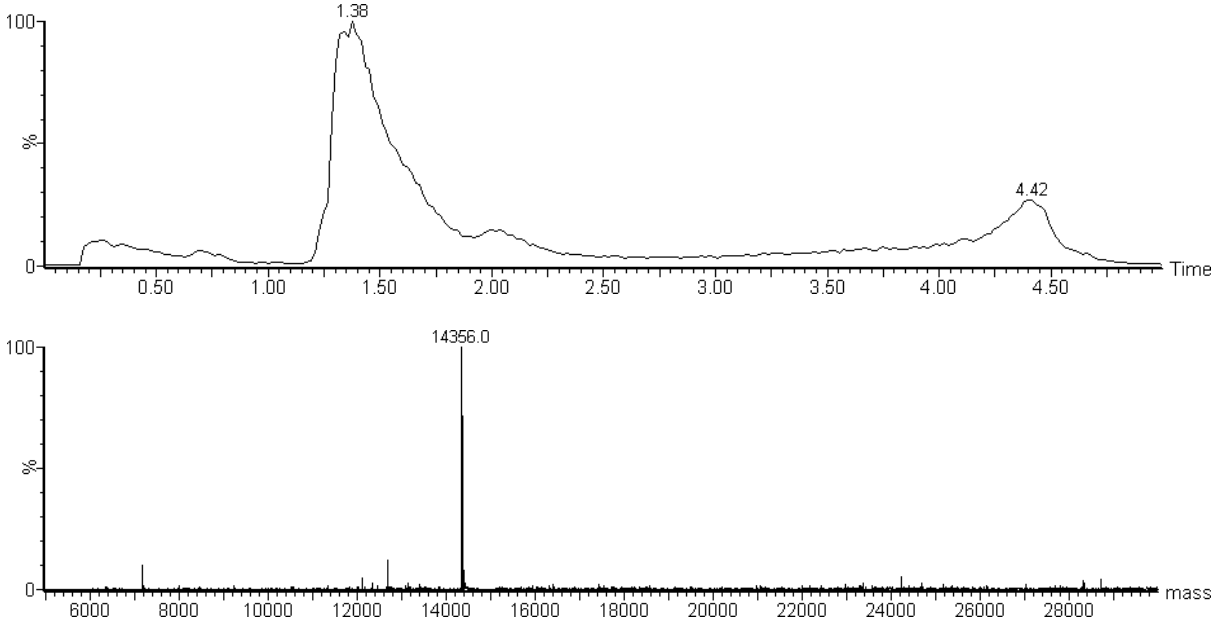


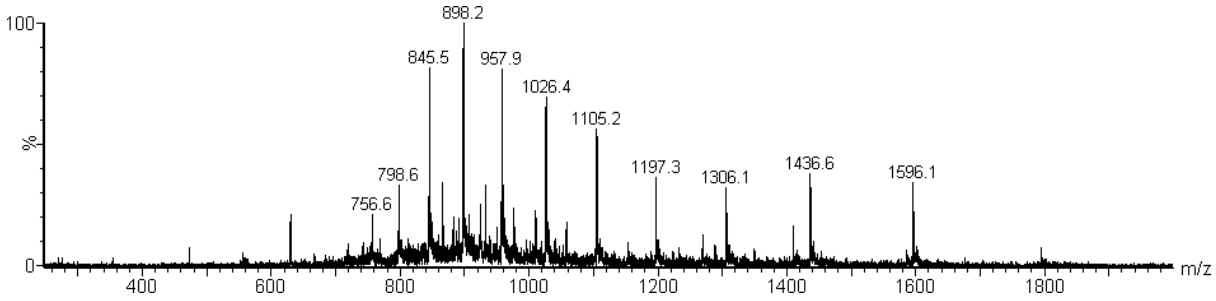


Grb2 SH2 (L111C) 5 conjugate with bromomaleimide 4

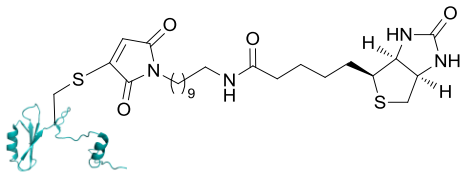


Expected Mass: 14356; Observed Mass: 14356

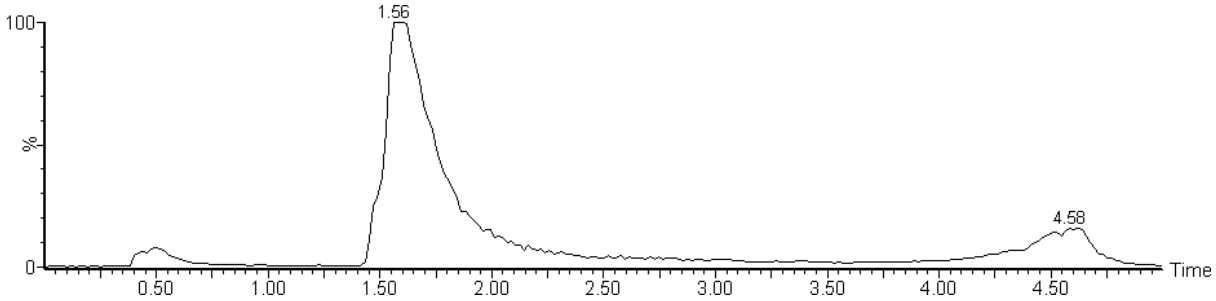


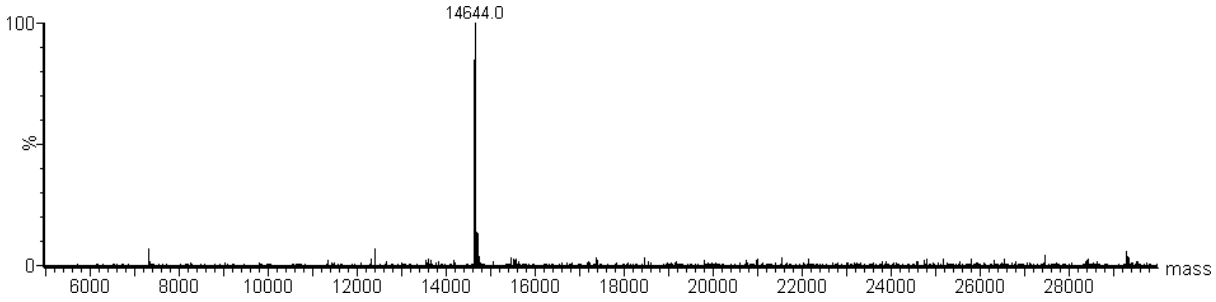


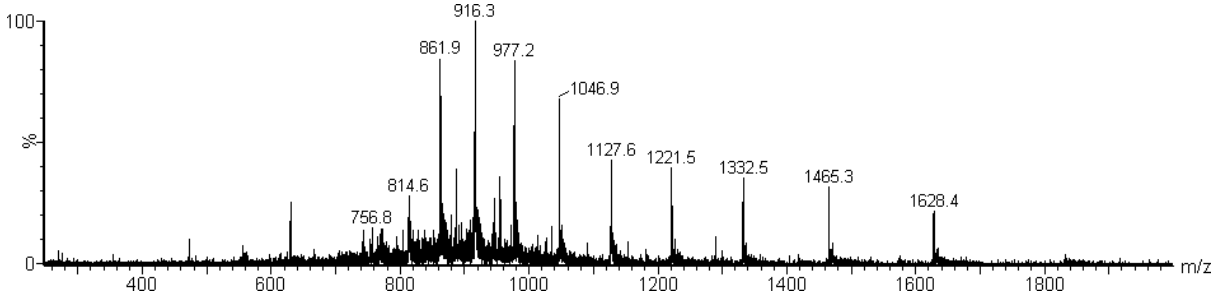
Grb2 SH2 (L111C) 5 conjugate with bromomaleimide 6



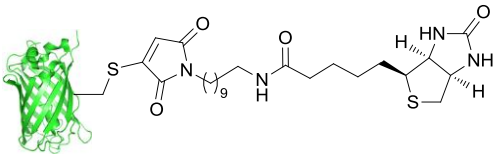
Expected Mass: 14641; Observed Mass: 14644



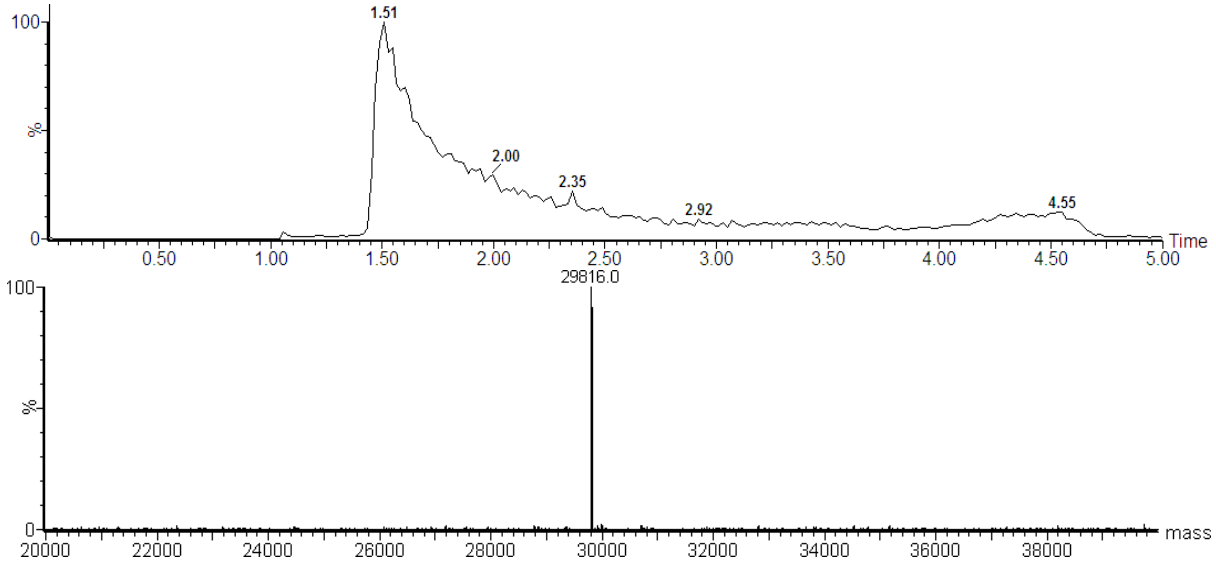


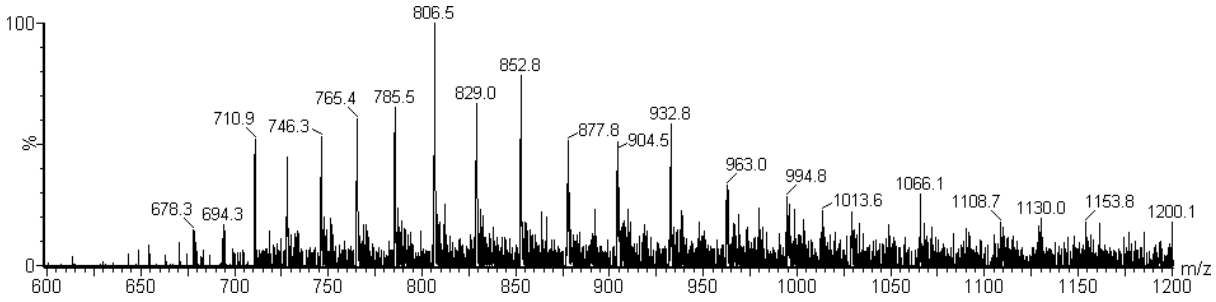


GFP (S147C) 7 conjugate with bromomaleimide 6



Expected Mass: 29809; Observed Mass: 29816

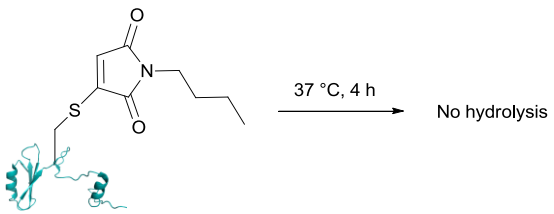


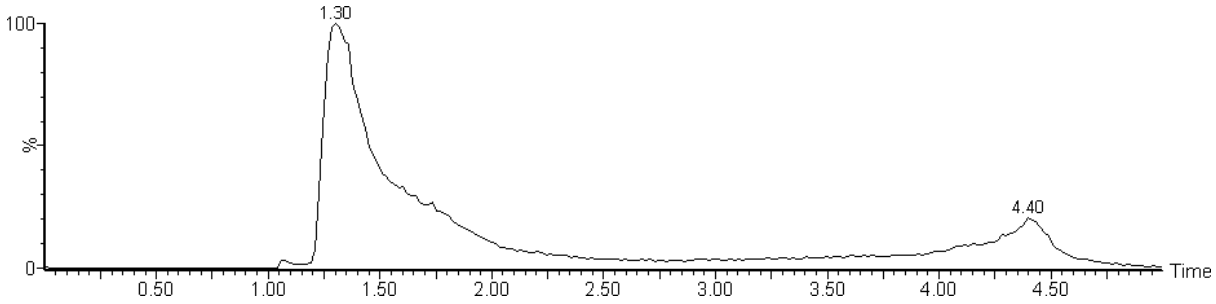


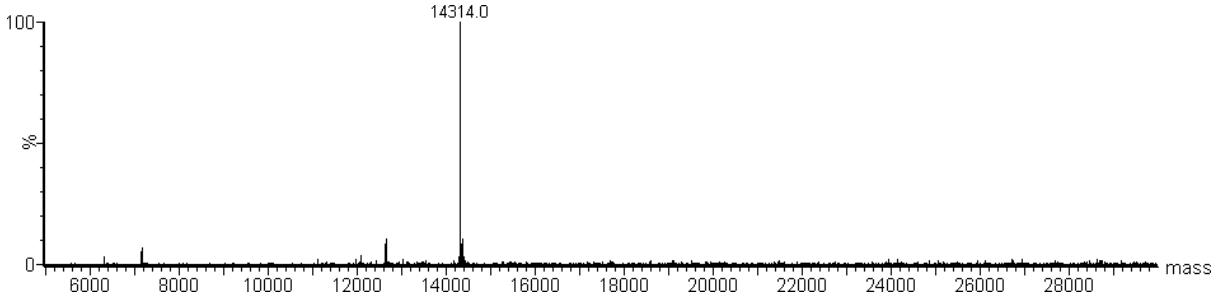
General Procedure for assessment of hydrolytic stability of protein conjugates

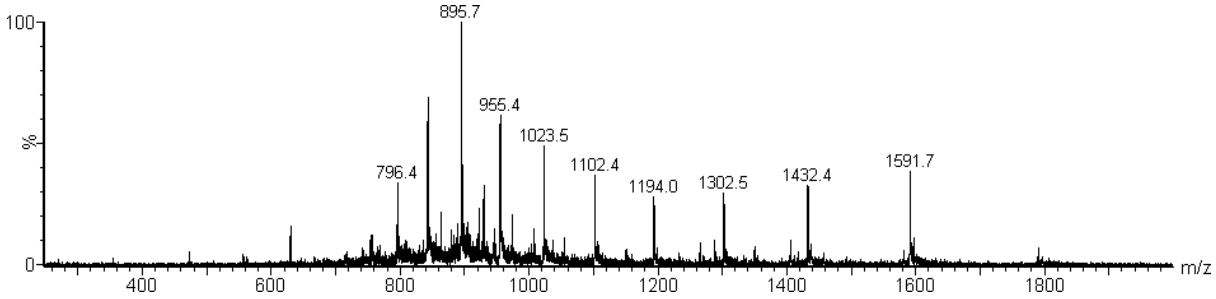
The protein conjugates (100 µL, [protein] 1.0 mg/mL, 100 mM sodium phosphate, 150 mM NaCl, pH 8.0) were heated at 37 °C for 4 h, and analysed by LCMS.

Hydrolytic stability of the conjugate of Grb2 SH2 (L111C) 5 with bromomaleimide 1

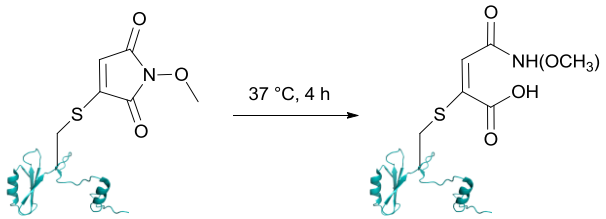




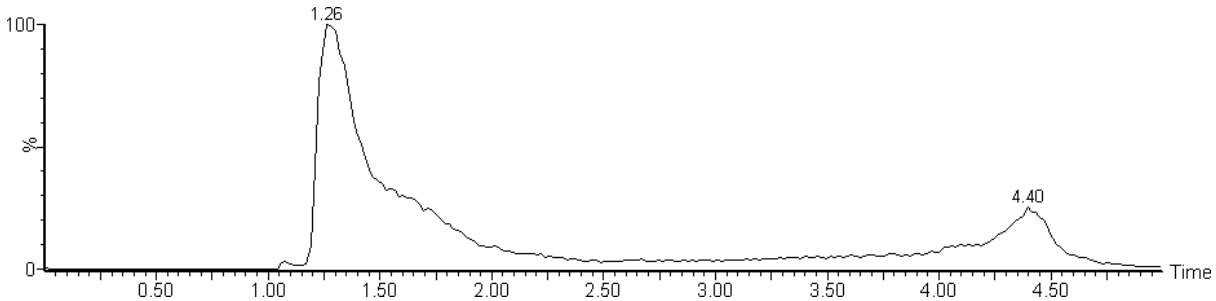


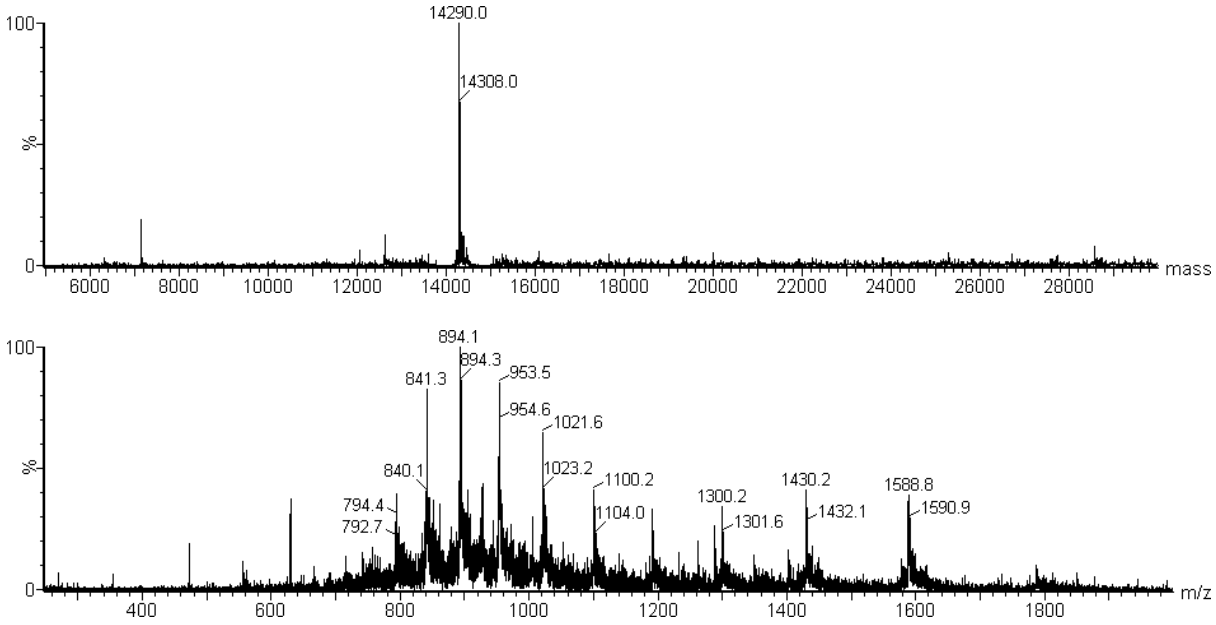


Hydrolytic stability of the conjugate of Grb2 SH2 (L111C) 5 with bromomaleimide 2

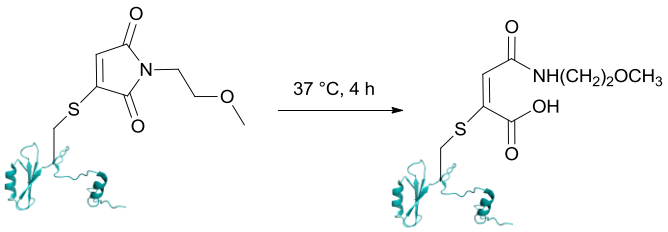


Expected Mass: 14308; Observed Mass: 14308

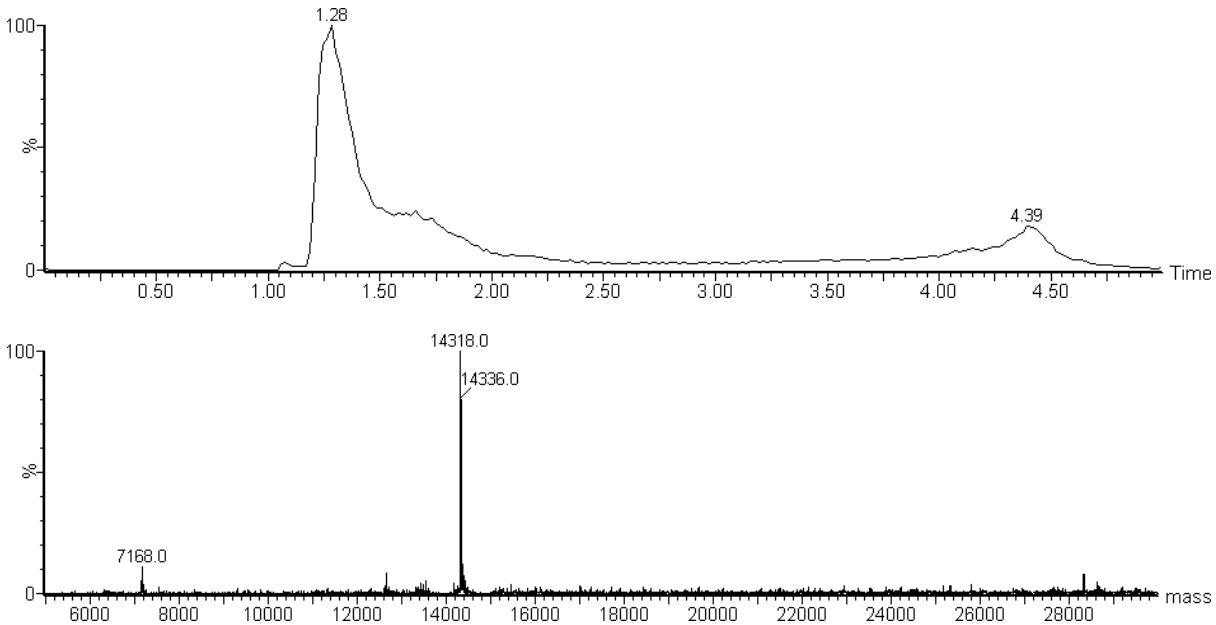


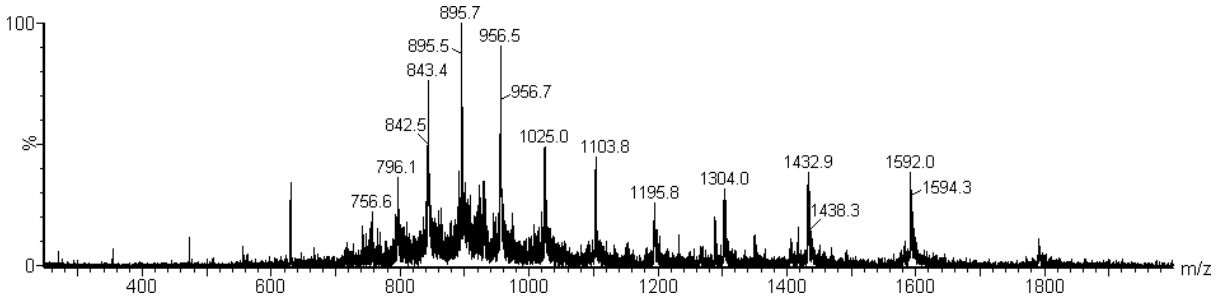


Hydrolytic stability of the conjugate of Grb2 SH2 (L111C) 5 with bromomaleimide 3

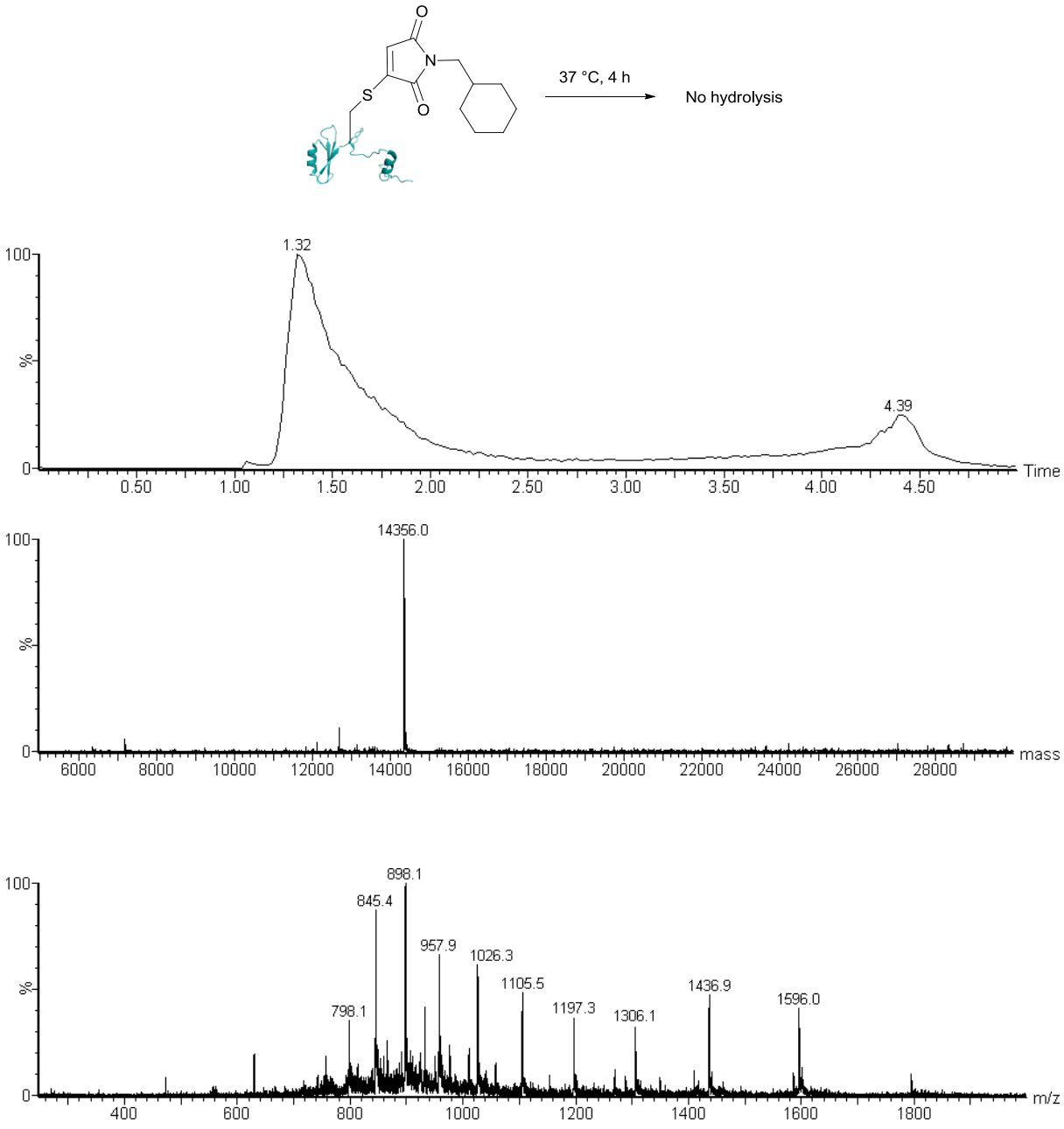


Expected Mass: 14336; Observed Mass: 14336

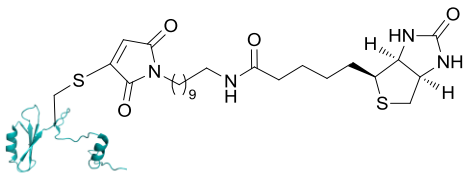


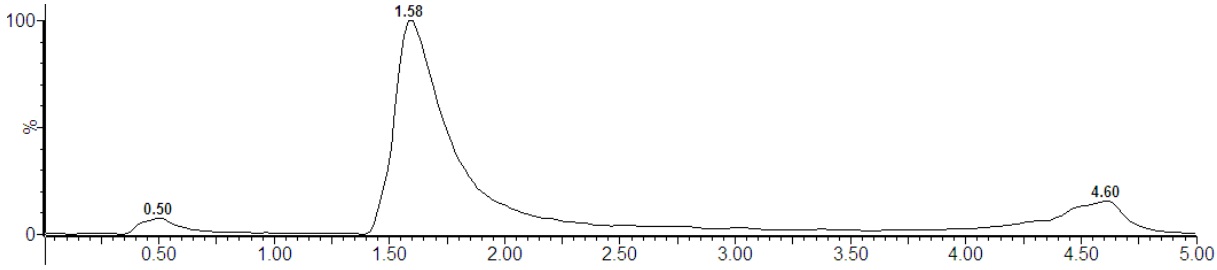


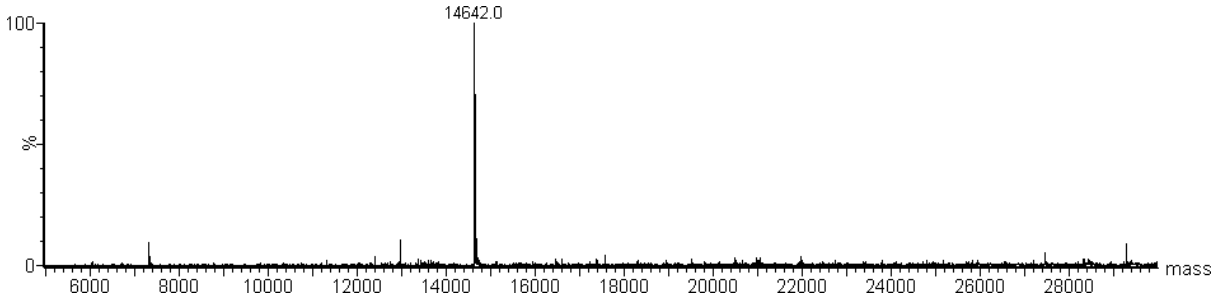
Hydrolytic stability of the conjugate of Grb2 SH2 (L111C) 5 with bromomaleimide 4

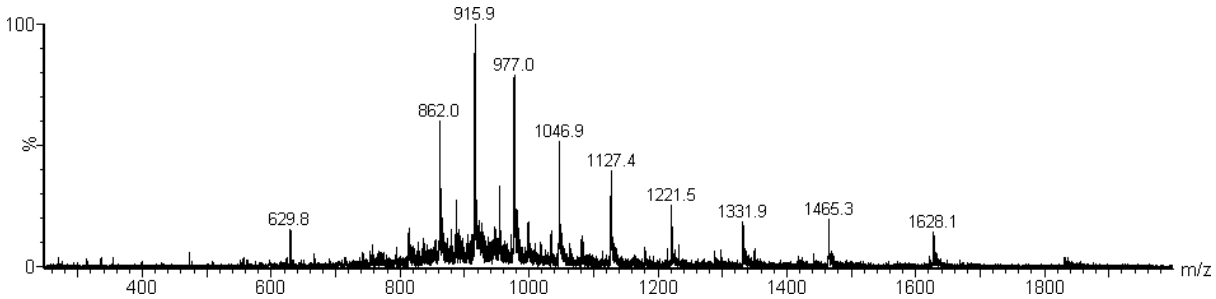


Hydrolytic stability of the conjugate of Grb2 SH2 (L111C) 5 with bromomaleimide 6

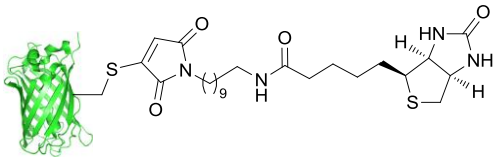


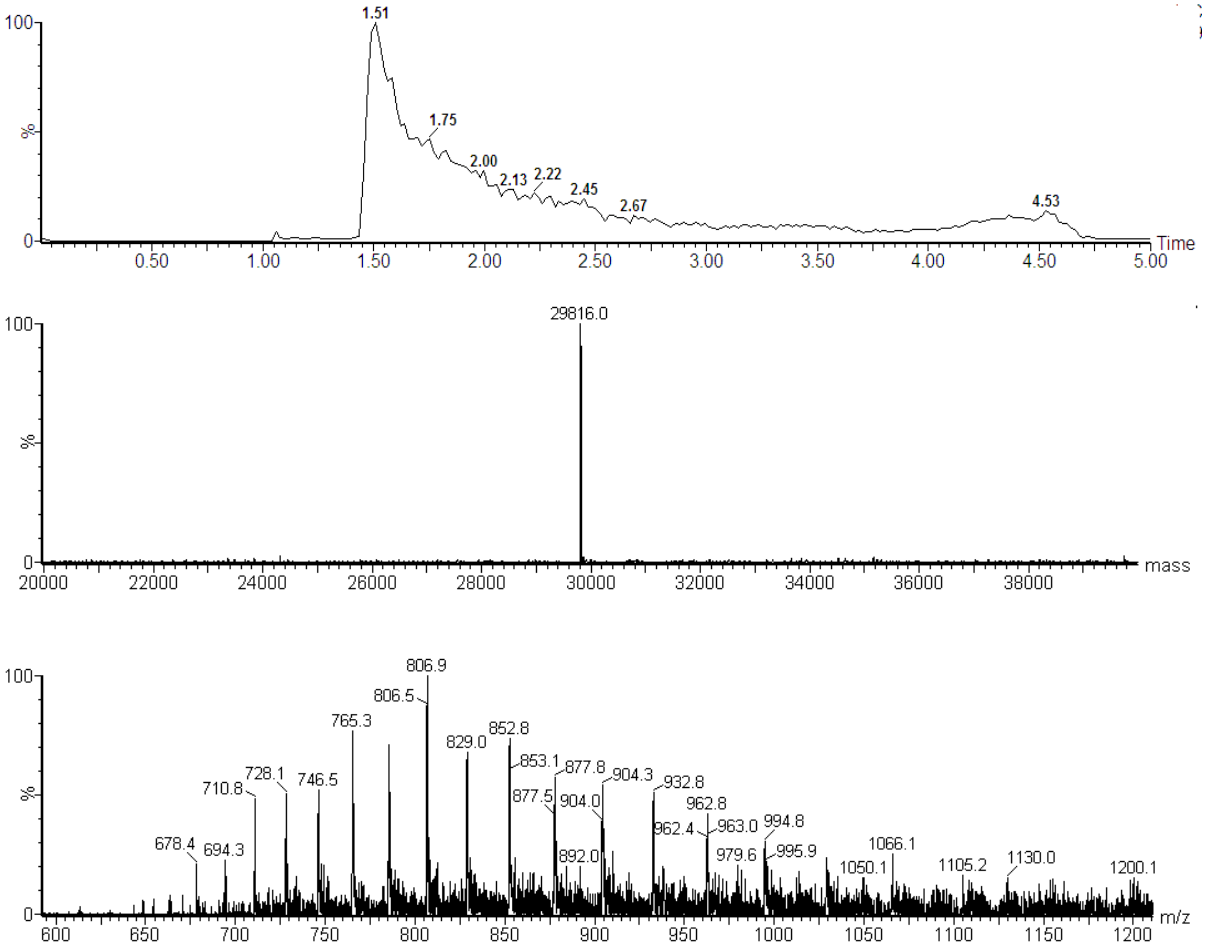






Hydrolytic stability of the conjugate of GFP (S147C) 7 with bromomaleimide 6

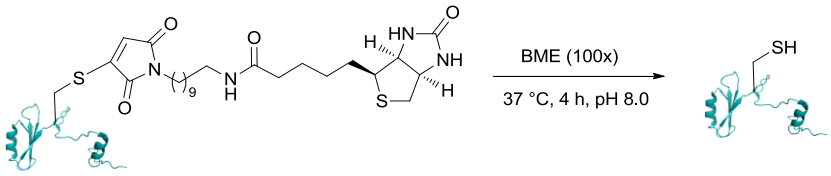




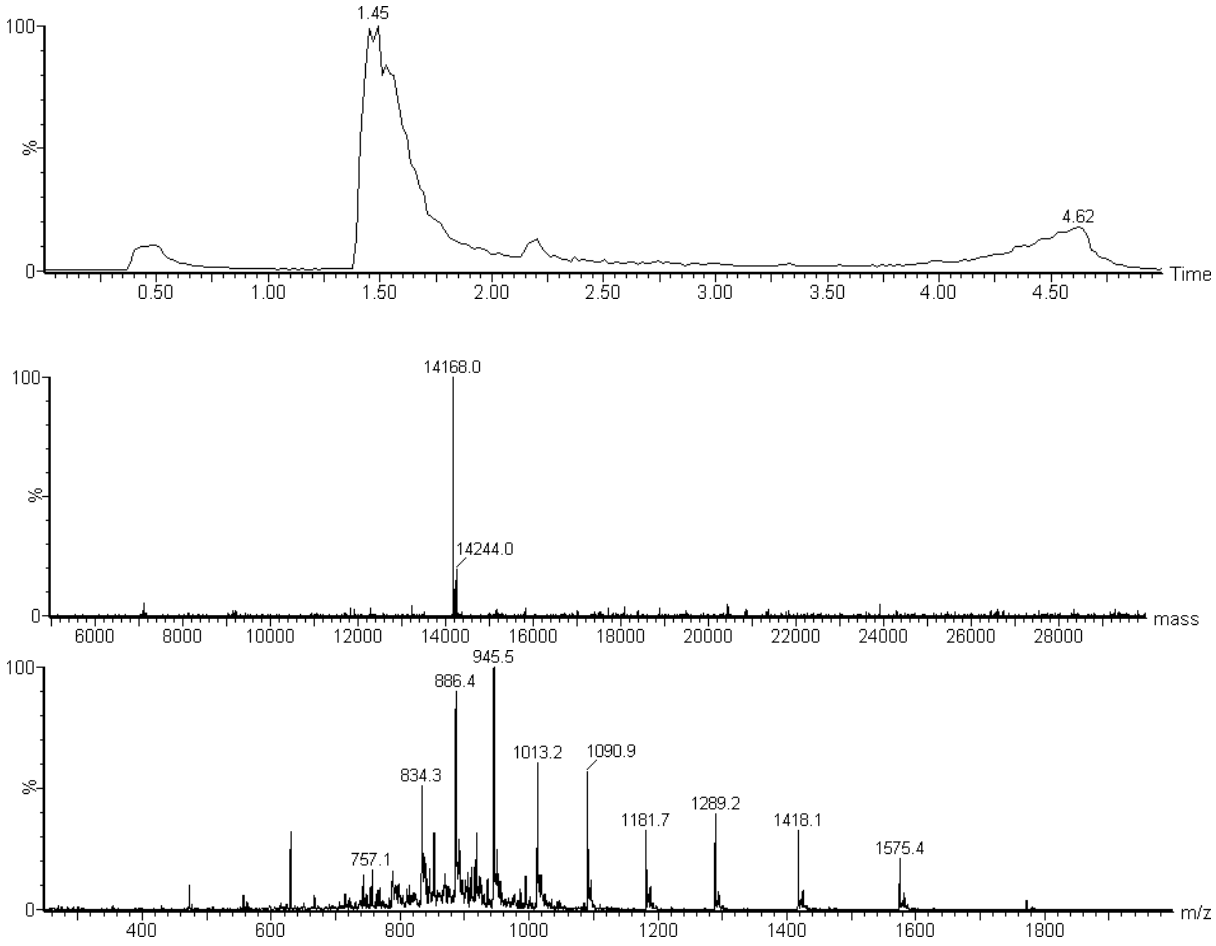
General Procedure for cleavage of protein bromomaleimide conjugates using *β*-mercaptoethanol

To a solution of protein-bromomaleimide conjugate (100 µL, [protein] 1.0 mg/mL, 100 mM sodium phosphate, 150 mM NaCl, pH 8.0) at 37 °C was added *β*-mercaptoethanol (5 µL solution in DMF, 100 equivalents). The mixture was maintained at 37 °C for 4 h and analysed by LCMS.

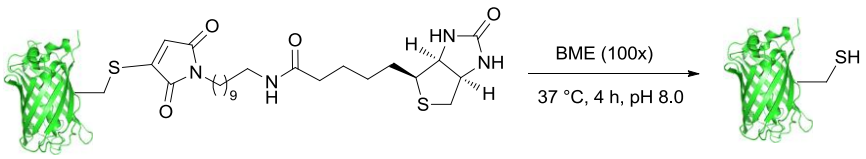
Cleavage of protein bromomaleimide conjugate 8 using *β*-mercaptoethanol



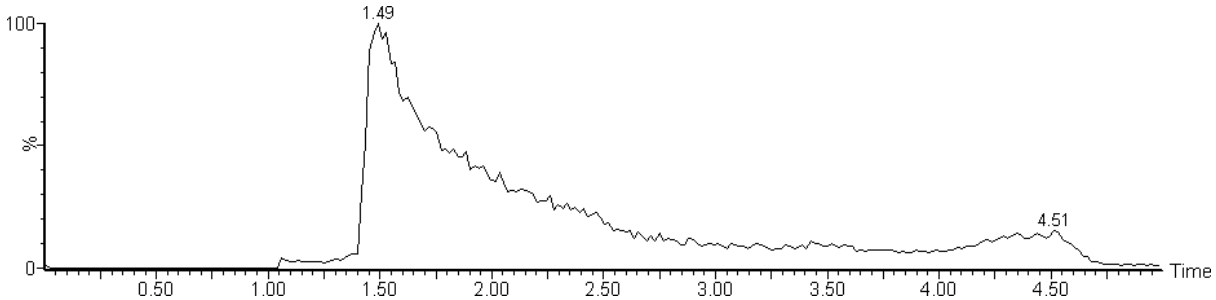
Expected Mass: 14164; Observed Mass: 14168

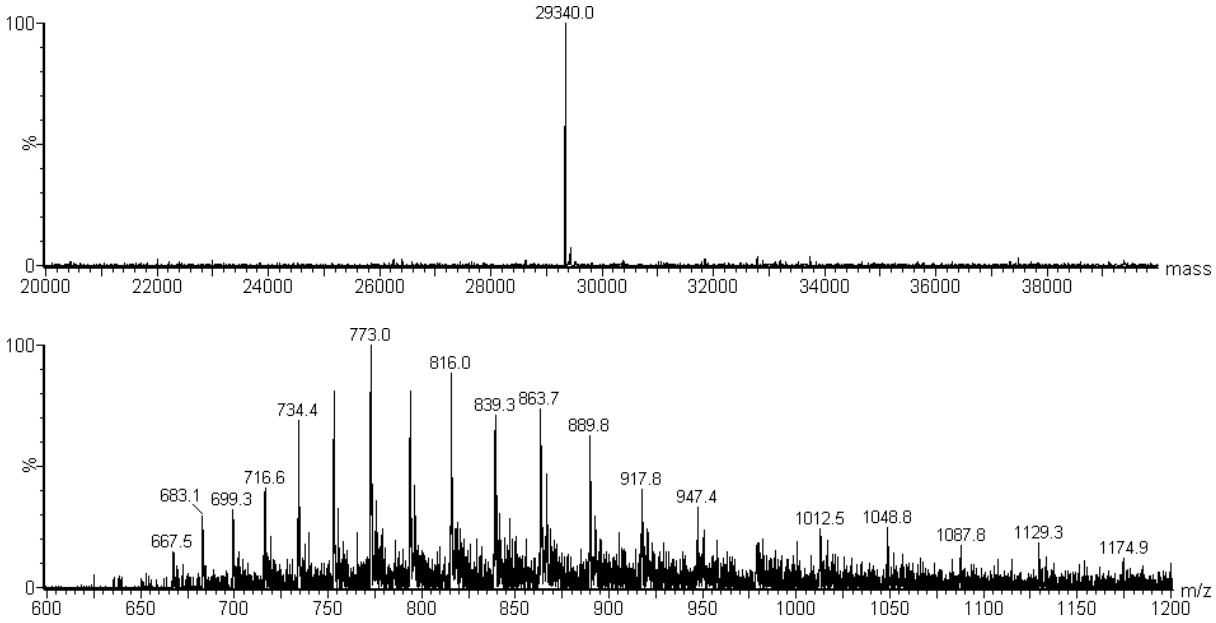


Cleavage of protein bromomaleimide conjugate 9 using *β*-mercaptoethanol



Expected Mass: 29332; Observed Mass: 29340

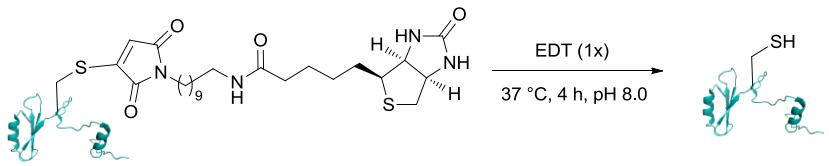




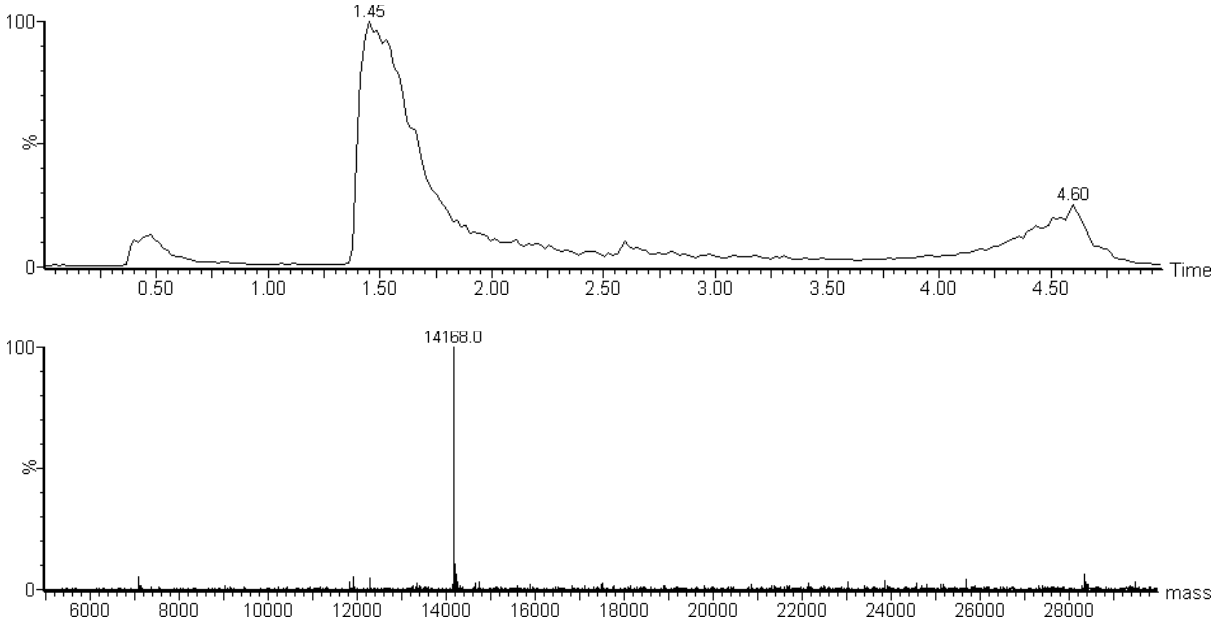
General Procedure for cleavage of protein bromomaleimide conjugates using EDT

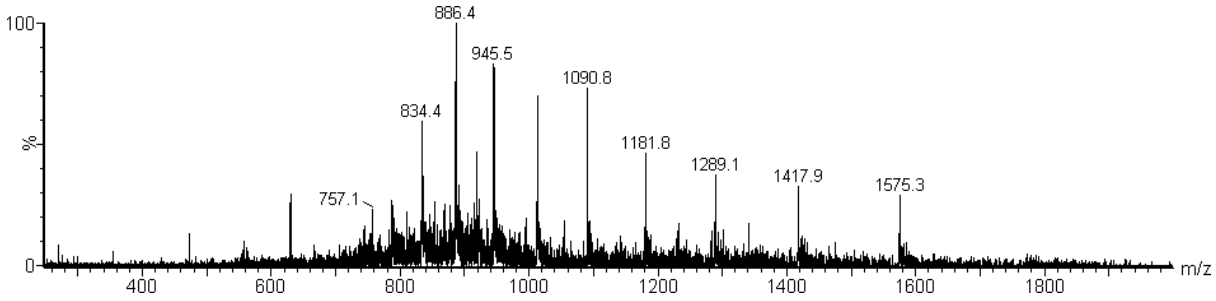
To a solution of protein-bromomaleimide conjugate (100 µL, [protein] 1.0 mg/mL, 100 mM sodium phosphate, 150 mM NaCl, pH 8.0) at 37 °C was added ethanedithiol (5 µL solution in DMF, 1 equivalent). The mixture was maintained at 37 °C for 4 h and analysed by LCMS.

Cleavage of protein bromomaleimide conjugate 8 using EDT

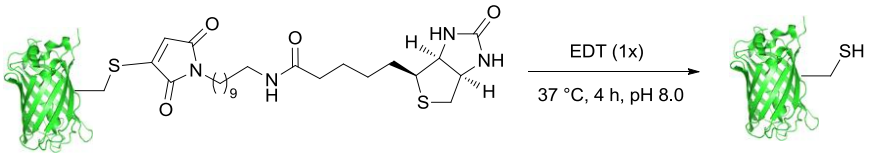


Expected Mass: 14164; Observed Mass: 14168

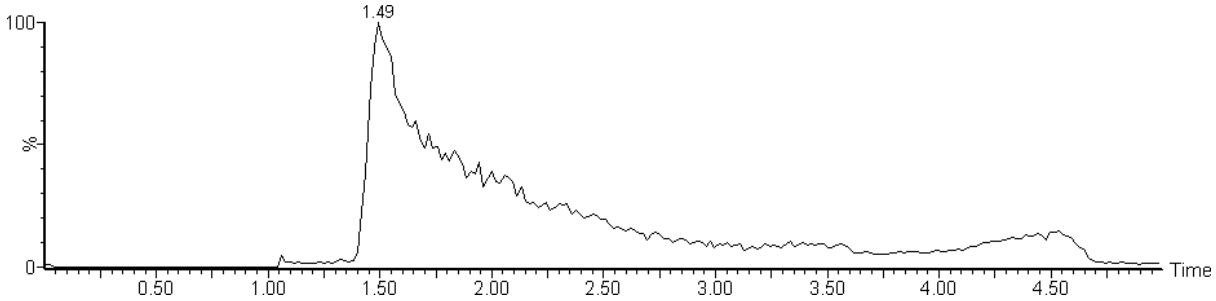


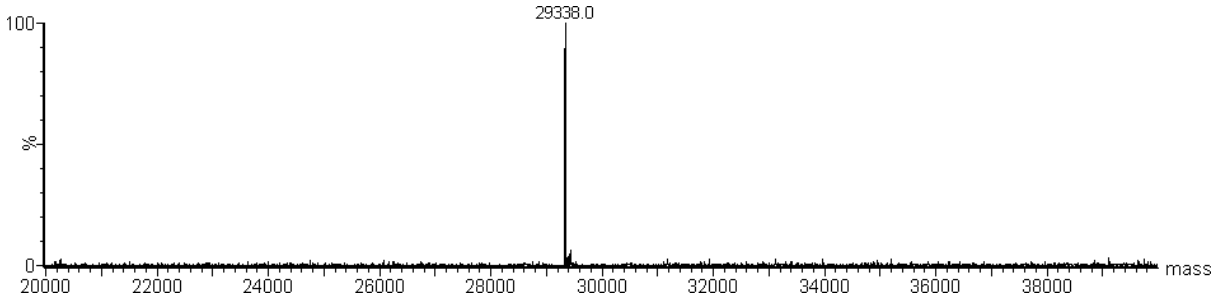


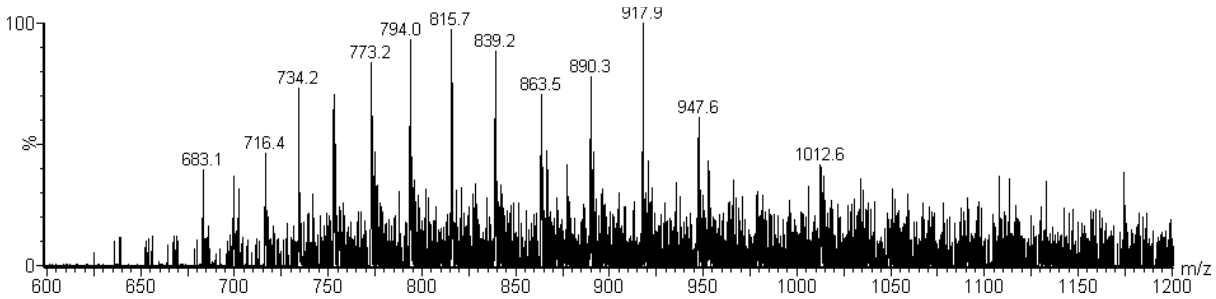
Cleavage of protein bromomaleimide conjugate 9 using EDT



Expected Mass: 29332; Observed Mass: 29338



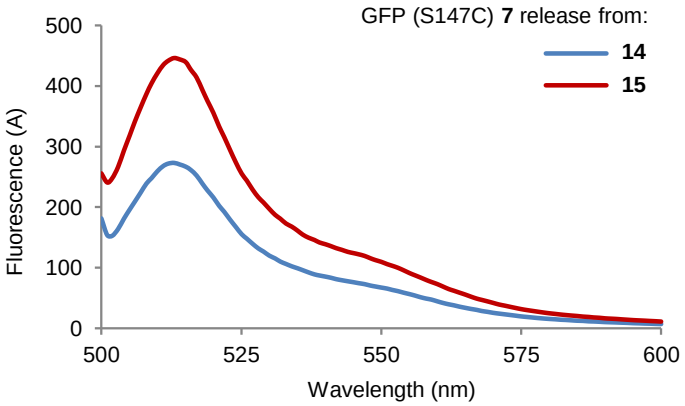




General procedure for pull down experiments with Streptavidin beads

20 µL PureProteome Streptavidin Magnetic Beads (pre-washed with 100 mM sodium phosphate, 150 mM NaCl, pH 8.0 (500 µL)) were incubated with derivatised GFP (S147C) **14** or **15** (100 µL, 1.0 mg/mL in 100 mM sodium phosphate, 150 mM NaCl, pH 8.0) with shaking (800 rpm) for 4 h at 37 °C. Following incubation, the magnetic beads were washed with 100 mM sodium phosphate, 150 mM NaCl, pH 8.0 (3 x 1 mL). 100 mM Sodium phosphate, 150 mM NaCl, pH 8.0 (100 µL) and

ethanedithiol (5 μL, 6 mM solution in DMF, 1 equivalent) were added to the beads and the mixture was shaken (800 rpm) for 4 h at 37 °C. The supernatant was removed and transferred into a Starna Scientific 26.100-F quartz fluorescence cuvette with a 10 mm path length. The fluorescence spectrum was obtained at room temperature using a Cary Eclipse Fluorescence Spectrophotometer. The sample was excited at 494 nm, and the emission intensity was scanned at 120 nm/min with an averaging time of 0.5 s and a data interval of 1 nm. The excitation and emission slit was set to 5 nm.



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2. V. Chudasama, M. E. B. Smith, F. F. Schumacher, D. Papaioannou, G. Waksman, J. R. Baker and S. Caddick, *Chem. Commun.*, 2011, 47, 8781-8783.

3. R. Nathani, P. Moody, M. E. B. Smith, R. J. Fitzmaurice and S. Caddick, *ChemBioChem*, 2012, 13, 1283-1285.