Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

Aryloxymaleimides for cysteine modification, disulfide bridging and the dual functionalization of disulfide bonds

Cristina Marculescu,[a] Hanno Kossen,[a] Rachel E. Morgan,[a] Patrick Mayer,[a] Sally A. Fletcher,[a] Berend Tolner,[b] Kerry Chester,[b] Lyn H. Jones,[c] James R. Baker*[a]

[a] Department of Chemistry, University College London, 20 Gordon St, London, UK; Tel: (+44) 2076792653; E-mail: j.r.baker@ucl.ac.uk.
[b] UCL Cancer Institute, 72 Huntley Street, London,WC1E 6BT, UK,
[c] Pfizer, Chemical Biology Group, BioTherapeutics Chemistry, WorldWide Medicinal Chemistry, 610 Main Street,Cambridge MA 02139, USA

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I. Materials

All commercially available chemicals were used as received without further purification. Lyophilised somatostatin was purchased from Sigma Aldrich. The anti-CEA ds-scFv shMFE23 antibody fragment was prepared according to a literature protocol[1] and the Grb2 (L111C) SH2 adaptor protein was prepared according to a literature protocol[2].

All buffer solutions were prepared with double-deionised water and filter-sterilised.

II. General methods

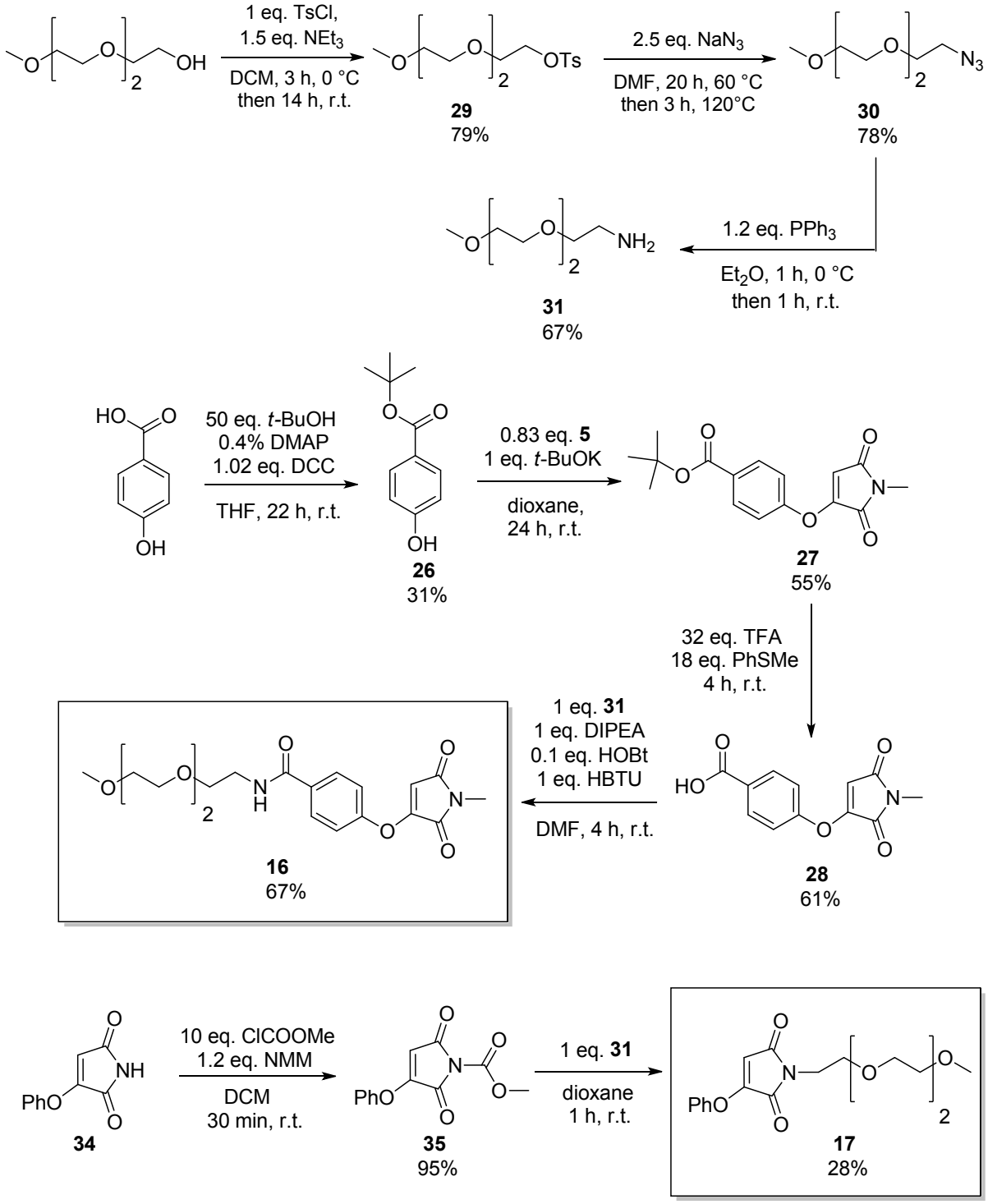
All reactions were carried out at room temperature, under argon unless otherwise stated. **^{1}H-NMR** spectra were recorded on Bruker AMX600 (600 MHz) instruments at room temperature. The chemical shifts are expressed in parts per million (ppm) referenced to the residual solvent peaks. Data are reported as follows: δ, chemical shift; integration, multiplicity (recorded as br, broad; s, singlet; d, doublet; t, triplet; q, quadruplet; qn, quintet, and m, multiplet), coupling constants (J in Hertz, Hz). **^{13}C-NMR** spectra were recorded on the same instruments at 150 MHz. The chemical shifts are expressed in parts per million (ppm), referenced to the residual solvent peaks. Assignments were obtained using DEPT experiments. **Infrared spectra** were obtained on a Perkin Elmer Spectrum 100 FTIR Spectrometer operating in ATR mode. Data is reported as follows: wavenumber (cm^{-1}), intensity (recorded as br, broad; s, strong; m, medium; w, weak). **Melting points** were measured with a Gallenkamp and are uncorrected. High and low resolution **mass spectrometry** was performed using a VG70 SE operating in modes ES, EI, FAB or CI depending on the sample. Normal phase silica gel 60 (0.04-0.063 mm, 230-400 mesh) (BDH) and sand (VWR) were used for **flash chromatography**. All reactions were monitored by thin layer chromatography (TLC), using TLC plates pre-coated with silica gel 60 F¬254 on aluminium (Merck KGaA) and $KMnO_4$ as chemical stain.

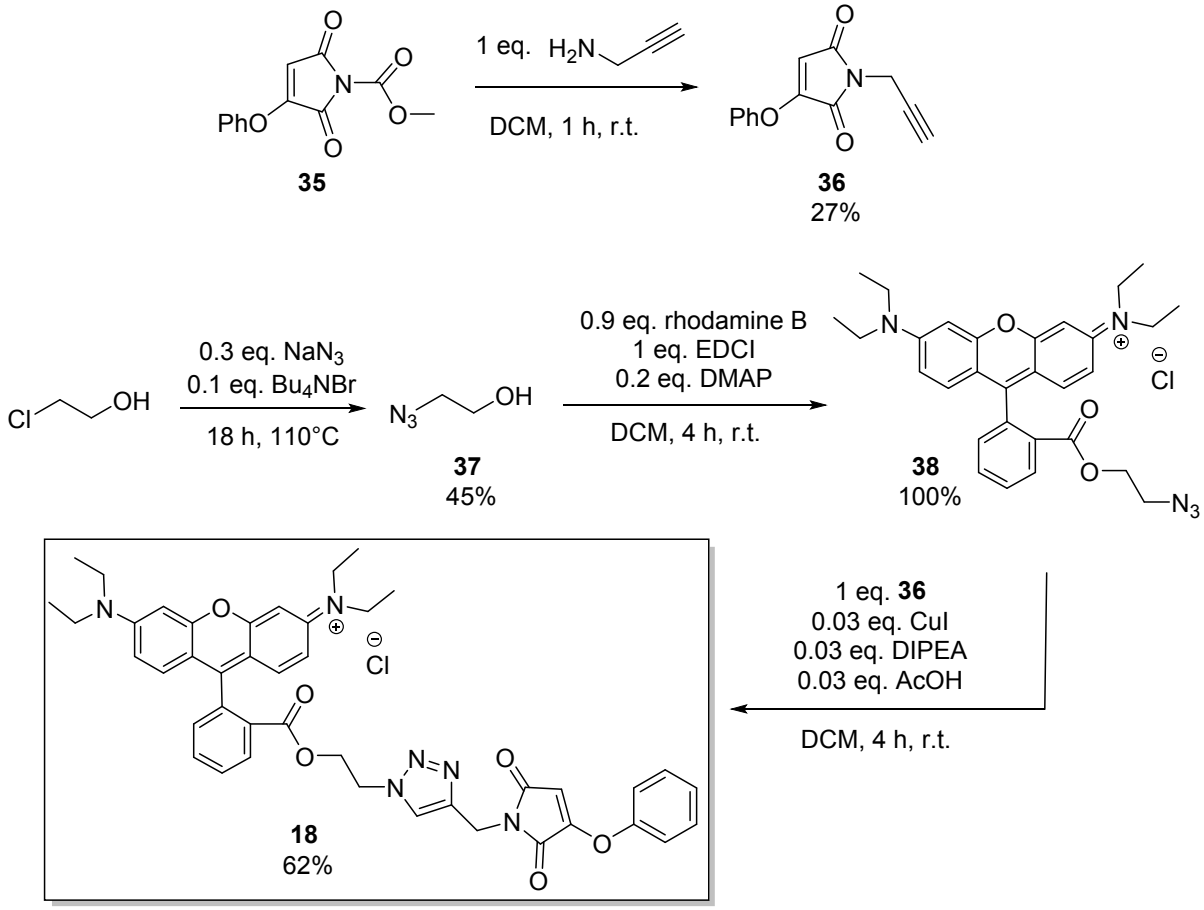
Somatostatin, anti-CEA ds-scfv antibody fragment and their conjugates were analysed by **LC-MS** using a Waters Acquity UPLC connected to Waters Acquity Single Quad Detector [column: Acquity UPLC BEH C18 1.7 µm 2.1 × 50 mm; wavelength 254 nm; mobile phase 95 : 5 water (0.1% formic acid) : acetonitrile (0.1% formic acid), gradient over 4 min to 5 : 95 water (0.1% formic acid) : acetonitrile (0.1% formic acid); flow rate 0.6 mL/ min; MS mode ES+/-; scan range (*m/z*) = 95 - 2090 Da; scan time 0.25 s]. Data was obtained in continuum mode. Sample volumes were between 10 - 30 µL. The electron spray source of the MS was operated with a capillary voltage of 3.5 kV and a cone voltage of 20 - 200 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 the MassLynx software.

Protein concentrations and UV-absorbances were obtained on a Carry BIO 100 **UV-Vis** spectrophotometer (Varian) equipped with a temperature controlled 12× sample holder in quartz cuvettes (1 cm path length, volume 75 µL) at room temperature, unless otherwise stated. Samples were baseline corrected.

III. Supporting schemes and figures

III.1 Synthetic scheme for the synthesis of aryloxymaleimides 16-18





Scheme S1. Synthesis of aryloxymaleimides **16-18**

III.2 Stability of succinimide bridged somatostatin 14 and maleimide bridged somatostatin 47 under cytoplasm mimicking conditions

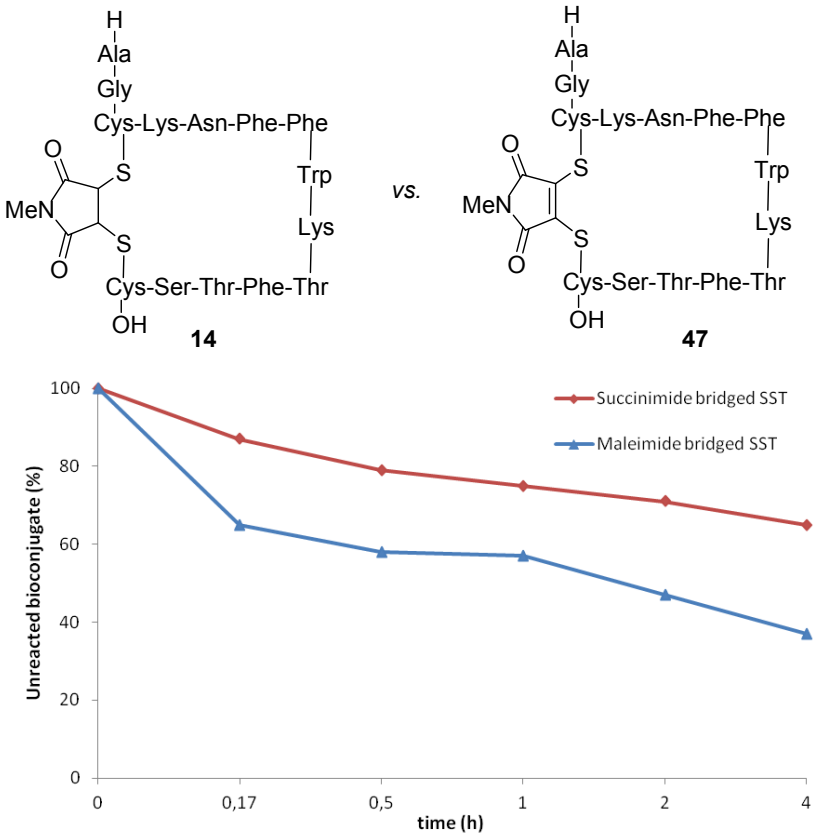
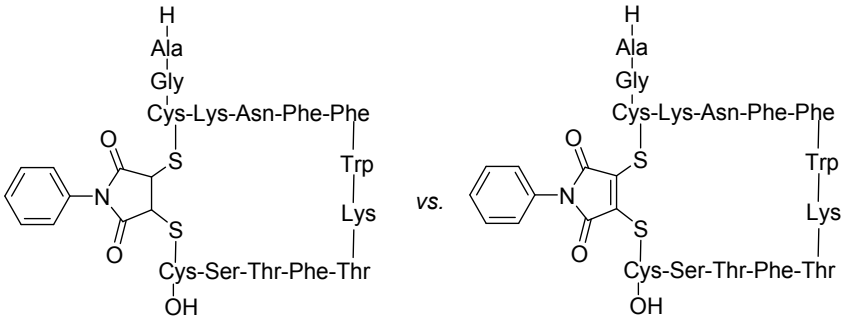


Fig. S1. Stability of succinimide bridged somatostatin **14** (brown) and maleimide bridged somatostatin **47** (blue) under cytoplasm mimicking conditions between 0 - 4 h

III.3 Hydrolytic stability of succinimide bridged somatostatin 45 and maleimide bridged somatostatin 48

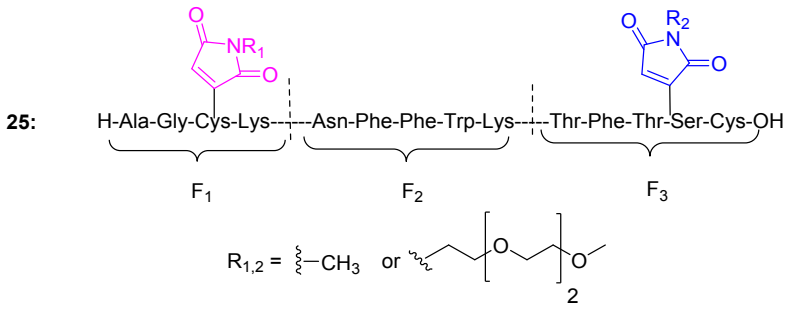


45 **48**

Conjugate	% Hydrolysed	
	after dialysis in pH 8 buffer	after 1 h @ 37 °C, pH 8
45	75	91
48	92	95

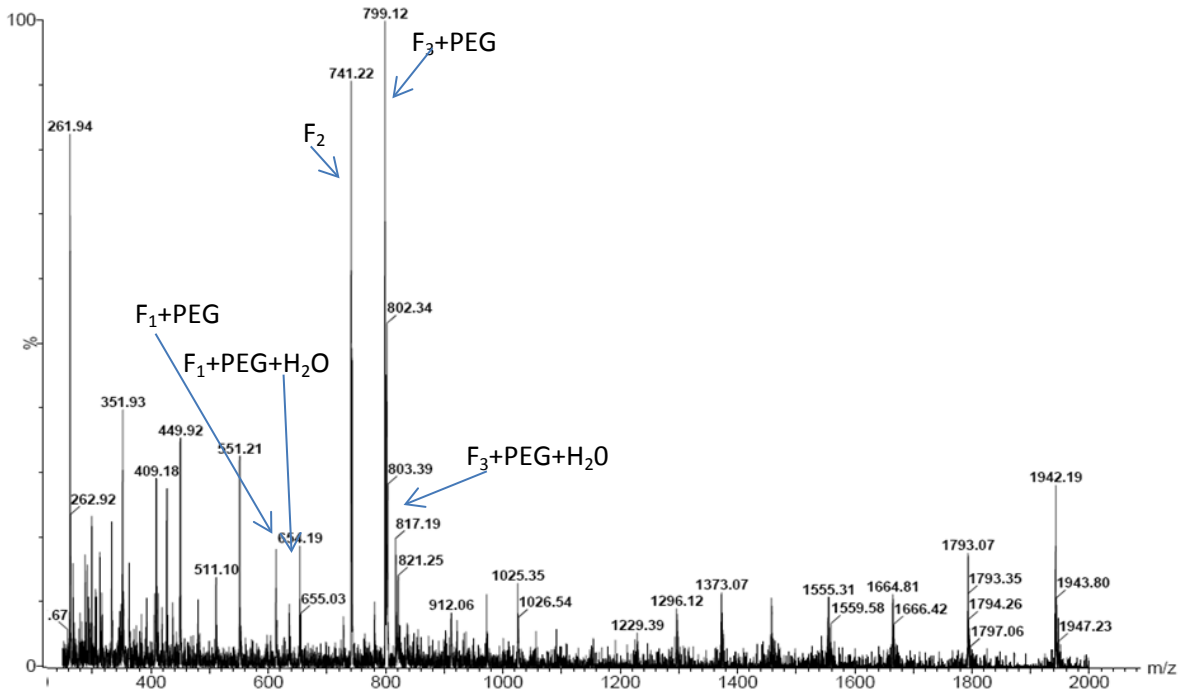
Fig. S2. Comparison between the hydrolytic stability of the succinimide bridged somatostatin **45** and maleimide bridged somatostatin **48**

III.4 Trypsin digest of dual labelled somatostatin conjugate 25



R_1	Ring hydrolysed	Mass F_1	R_2	Ring hydrolysed	Mass F_3	Mass F_2
PEG	No	**618**	PEG	No	**799**	**740**
PEG	Yes	**637**	PEG	Yes	**817**	
CH_3	No	486	CH_3	No	666	
CH_3	Yes	504	CH_3	Yes	684	

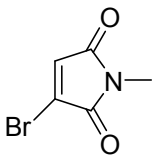
Possible fragments after the trypsin digest of **25**



SI Fig 3. Mass trace of the digest mixture after 15 h

IV. Synthesised compounds

3-Bromo-1-methyl-pyrrole-2,5-dione[3] (5)

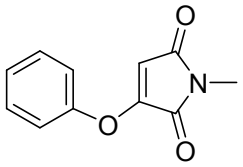


To *N*-methylmaleimide (2.87 g, 25.0 mmol) in $CHCl_3$ (125 mL), bromine (2.60 mL, 55.5 mmol) was added dropwise and the resulting mixture was refluxed for 2 h. The solvent was removed *in vacuo* and the resulting solid was dissolved in ethyl acetate (30 mL) and washed with 15% aq. $Na_2S_2O_3$ (20 mL) and then with brine (10 mL). The product was dried ($MgSO_4$), filtered and the solvent was evaporated *in vacuo* to afford 6.39 g of 2,3-dibromosuccinimide as yellow crystals. The succinimide product was dissolved in acetic acid (150 mL), then sodium acetate (5.94 g, 71.6 mmol) was added and the reaction mixture was refluxed for 2.5 h. The reaction mixture was left to cool down to room temperature and then the solvent was evaporated *in vacuo*. The crude mixture was dissolved in ethyl acetate (50 mL) and washed with sat. aq. Na_2CO_3 (3 × 25 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo*. Purification by

flash chromatography (petroleum ether : ethyl acetate, gradient elution from 80 : 20 to 60 : 40) afforded the title compound **5** as white crystals (2.86 g, 15.1 mmol) in 65% yield.

δ_H (600 MHz, $CDCl_3$) 6.88 (1H, s), 3.07 (3H, s); δ_C (150 MHz, $CDCl_3$) 168.7 (C), 165.5 (C), 132.0 (CH), 131.5 (C), 24.7 (CH_3); IR 3105 (w), 2947 (w), 1776 (m), 1704 (s), 1588 (s), 1493 (s), 1388 (m), 1231 (m), 969 (s), 706 (s); MS (CI+) *m/z* (relative intensity): 192 ($[^{81}M+H]^+$, 100), 190 ($[^{79}M+H]^+$, 100). Exact mass calcd. for $[C_5H_4NO_2Br]^+$+H: 189.9504. Measured: 189.9501 (CI+); m.p.: 100 °C (lit. m.p.[1]: 88 - 89 °C).

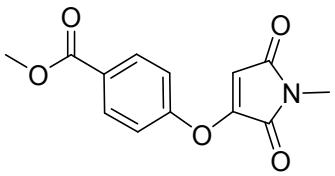
1-Methyl-3-phenoxy-pyrrole-2,5-dione (6)



To a solution of phenol (207 mg, 2.20 mmol) in dry dioxane (1 mL) potassium *tert*-butoxide (264 mg, 2.31 mmol) in dry dioxane (3 mL) was added dropwise and the solution was left stirring for 15 min. The mixture was then added dropwise to a solution of bromomaleimide **5** (400 mg, 2.10 mmol) in dry dioxane (1 mL). The mixture was stirred at room temperature for 30 min and then concentrated *in vacuo*. Water (10 mL) was added and the crude product was extracted with ethyl acetate (3 × 30 mL), dried ($MgSO_4$) and filtered. The organic solvent was evaporated *in vacuo* to give a beige solid. Purification by flash chromatography (petroleum ether : ethyl acetate, gradient elution from 90 : 10 to 80 : 20) afforded the title compound **6** as a white solid (320.9 mg, 1.58 mmol) in 75% yield.

δ_H (600 MHz, $CDCl_3$) 7.46-7.43 (2H, m), 7.31 (1H, t, *J* = 7.4 Hz), 7.18 (2H, d, *J* = 7.7 Hz), 5.27 (1H, s), 3.05 (3H, s); δ_C (150 MHz, $CDCl_3$) 170.1 (C), 165.8 (C), 159.6 (C), 153.9 (C), 130.4 (2 × CH), 126.9 (CH), 119.9 (2 × CH), 99.4 (CH), 23.7 (CH_3); IR 1714 (s), 1637 (m), 1313 (m), 1220 (w); MS (EI+) *m/z*, (relative intensity): 203 ($[M]^+$, 100), 94 (21). Exact mass calcd. for $[C_{11}H_9NO_3]^+$: 203.0577. Measured: 203.0580 (EI+); m.p.: 63 °C.

Methyl 4-(1-methyl-pyrrole-2,5-dione-3-yloxy)benzoate (7)

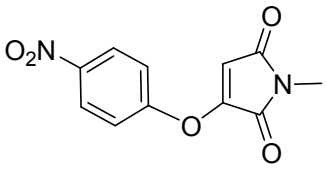


To a solution of methyl 4-hydroxybenzoate (170 mg, 1.10 mmol) in dry dioxane (1 mL) a mixture of potassium *tert*-butoxide (132 mg, 1.16 mmol) in dry dioxane (4 mL) and 18-crown-6 ether (306 mg,

1.16 mmol) was added dropwise and the resulting solution was stirred for 15 min at room temperature. The mixture was added dropwise to a solution of bromomaleimide **5** (200 mg, 1.05 mmol) in dry dioxane (1 mL) and stirred at room temperature for 19 h. After this time, the solvent was evaporated *in vacuo*, washed with 1 M aq. NaOH, extracted with ethyl acetate (3 × 30 mL), dried ($MgSO_4$), filtered and the solvent was evaporated *in vacuo*. Purification by flash chromatography (petroleum ether : ethyl acetate, gradient elution from 90 : 10 to 70 : 30) afforded title compound **7** as yellow solid (196 mg, 0.75 mmol) in 72% yield.

δ_H (600 MHz, $CDCl_3$) 8.15-8.13 (2H, m), 7.27-7.25 (2H, m), 5.37 (1H, s), 3.94 (3H, s), 3.06 (3H, s); δ_C (150 MHz, $CDCl_3$) 169.7 (C), 165.9 (C), 165.4 (C), 158.5 (C), 157.2 (C), 132.2 (2 × CH), 128.9 (C), 119.9 (2 × CH), 100.3 (CH), 52.6 (CH_3), 23.9 (CH_3); IR 1731 (s), 1713 (s), 1638 (m), 1600 (m), 1440 (m), 1285 (m), 1105 (m); MS (EI+) *m/z*, (relative intensity): 261 ($[M]^+$, 100), 86 (20). Exact mass calcd. for $[C_{13}H_{11}NO_5]^+$: 261.0632. Measured: 261.0636 (EI+); m.p.: 149 °C.

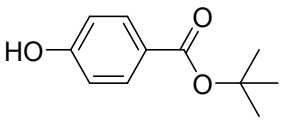
1-Methyl-3-(4-nitrophenoxy)-pyrrole-2,5-dione (8)



To a solution of 4-nitrophenol (155 mg, 1.10 mmol) in dry dioxane (1 mL) a mixture of potassium *tert*-butoxide (132 mg, 1.16 mmol) in dry dioxane (4 mL) and 18-crown ether (306 mg, 1.16 mmol) was added dropwise and the solution was stirred for 15 min. The resulting mixture was then added dropwise to a solution of bromomaleimide **5** (200 mg, 1.05 mmol) in dry dioxane (1 mL) and the stirring continued for another 20 h. After this time, the solvent was evaporated *in vacuo*. The resulting crude was redissolved in ethyl acetate (10 mL), washed with 1 M aq. NaOH (5 mL), extracted with ethyl acetate (3 × 10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography (petroleum ether : ethyl acetate, gradient elution from 90 : 10 to 70 : 30) afforded the title compound **8** as a white solid (114 mg, 0.46 mmol) in 44% yield.

δ_H (600 MHz, $CDCl_3$) 8.37-8.35 (2H, m), 7.39-7.37 (2H, m), 5.49 (1H, s), 3.07 (3H, s); δ_C (150 MHz, $CDCl_3$) 169.1 (C), 165.0 (C), 158.2 (C), 157.6 (C), 145.9 (C), 126.4 (2 × CH), 120.7 (2 × CH), 101.5 (CH), 23.9 (CH_3); IR 1713 (s), 1635 (m), 1588 (m), 1522 (m), 1350 (m), 1231 (w); MS (EI+) *m/z*, (relative intensity): 248 ($[M]^+$, 100), 150 (16). Exact mass calcd for $[C_{11}H_8N_2O_5]^+$: 248.0428. Measured: 248.0435 (EI+); m.p.: 123 - 124 °C.

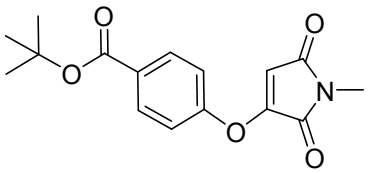
***tert*-Butyl 4-hydroxybenzoate[4] (26)**



To a solution of 4-hydroxybenzoic acid (2.50 g, 18.1 mmol), DMAP (0.08 g, 0.07 mmol) and *tert*-butanol (50 ml) in dry tetrahydrofuran (75 ml), a solution of DCC (3.82 g, 18.5 mmol) in dry tetrahydrofuran (25 ml) was added dropwise at room temperature for 30 min. The reaction mixture was stirred at room temperature for a further 22 h. The white solid was filtered and the filtrate was concentrated *in vacuo*. The crude filtate was dissolved in diethyl ether (40 mL) and washed with 0.3 M aq. Na_2CO_3 (3 × 20 mL), dried ($MgSO_4$), filtered and then concentrated *in vacuo*. Purification by flash chromatography (petroleum ether : ethyl acetate, 90 : 10) afforded the title compound **26** as a white solid (1.10 g, 5.67 mmol) in 31% yield.

δ_H (600 MHz, $CDCl_3$) 7.90 (2H, d, *J* = 8.4 Hz), 6.83 (2H, d, *J* = 8.4 Hz), 5.40 (1H, s), 1.58 (9H, s); δ_C (150 MHz, $CDCl_3$) 165.7 (C), 159.4 (C), 131.8 (2 × CH), 124.8 (C), 115.1 (2 × CH), 81.8 (C), 28.4 (3 × CH_3); IR 3338 (br), 1674 (s), 1607 (s), 1368 (s), 1154 (s); IR 3338 (br), 1673 (s), 1607 (s), 1368 (s), 1154 (s); MS (CI+) *m/z,* (relative intensity): 195 ($[M+H]^+$, 100). Exact mass calcd. for $[C_{11}H_{14}O_3]^+$+H: 195.1016. Measured: 195.1013 (CI+); m.p.: 119 °C (lit. m.p.[4]: 118 - 120 °C).

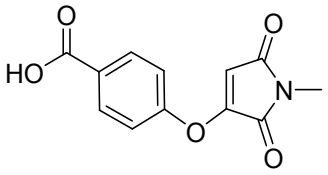
***tert*-Butyl 4-(1-methyl-pyrrole-2,5-dione-3-yloxy)benzoate (27)**



A solution of protected phenol **26** (300 mg, 1.55 mmol) in dry dioxane (1.5 mL) was added dropwise to a solution of potassium *tert*-butoxide (173 mg, 1.55 mol) in dry dioxane (4.5 mL) and stirred at room temperature for 15 min. The mixture was added dropwise to a solution of bromomaleimide **5** (255 mg, 1.29 mmol) in dry dioxane (1 mL) and the resulting solution was stirred at room temperature for 24 h. After this time, the solvent was concentrated *in vacuo* and the resulting crude dissolved in ethyl acetate (20 mL), washed with 1 M aq. NaOH (20 mL), water (20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was evaporated *in vacuo*. Purification by flash chromatography (dichloromethane : diethyl ether, 95 : 5) afforded the title compound **27** as a white solid (260 mg, 0.86 mmol) in 55% yield.

δ_H (600 MHz, $CDCl_3$) 8.05-8.04 (2H, m), 7.21-7.19 (2H, d), 5.32 (1H, s), 3.01 (3H, s) 1.56 (9H, s); δ_C (150 MHz, $CDCl_3$) 169.7 (C), 169.7 (C), 164.5 (C), 158.6 (C), 156.8 (C), 131.9 (2 × CH), 130.7 (C), 119.7 (2 × CH), 100.1 (CH), 81.8 (C), 28.2 (3 × CH_3), 23.8 (CH_3); IR 1712 (s), 1638 (m), 1291 (m); MS (CI+) *m/z,* (relative intensity): 304 ($[M+H]^+$, 30), 248 (100). Exact mass calcd. for $[C_{16}H_{17}NO_5]^+$+H: 304.1179. Measured: 304.1174 (CI+); m.p.: 139 °C.

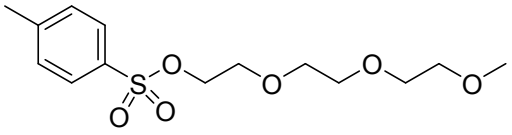
4-(1-Methyl-pyrrole-2,5-dione-3-yloxy)benzoic acid (28)



A solution of aryloxymaleimide **27** (250 mg, 0.82 mmol) and thioanisole (1.76 mL, 15 mmol) in dichloromethane (2 mL) was cooled to 0 °C and added dropwise over 10 min to TFA (2 mL, 26.3 mmol). The reaction mixture was stirred at room temperature for 4 h and then toluene (4 mL) was added to aid evaporation of TFA. Purification by flash chromatography (dichloromethane : methanol, gradient elution from 95 : 5 to 80 : 20) afforded the title compound **28** as white solid (122.9 mg, 0.50 mmol) in 61% yield.

δ_H (600 MHz, MeOD) 8.15 (2H, d, *J* = 9.0 Hz), 7.38 (2H, d, *J* = 9.0 Hz), 5.32 (1H, s), 2.99 (3H, s); δ_C (150 MHz, MeOD) 171.4 (C), 168.7 (C), 166.9 (C), 159.9 (C), 158.8 (C), 133.2 (2 × CH), 130.6 (C), 120.7 (2 × CH), 101.5 (CH), 23.6 (CH_3); IR 1726 (s), 1678 (m), 1643 (m), 1603 (m), 1294 (m); MS (CI+) *m/z,* (relative intensity): 248 ($[M+H]^+$, 100). Exact mass calcd. for $[C_{12}H_9NO_5]^+$+H: 248.0554. Measured: 248.0550 (CI+); m.p.: 237 °C.

2-(2-(2-Methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate[5] **(29)**

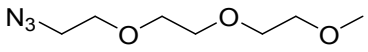


Triethylene glycol monomethyl ether (2.92 mL, 18.3 mmol) and triethylamine (3.80 mL, 27.4 mmol) were dissolved in dry dichloromethane (15 mL) and the solution was cooled to 0 °C. A solution of tosyl chloride (3.48 g, 18.3 mmol) in dry dichloromethane (5 mL) was then added dropwise. The mixture was stirred for 3 h at 0 °C and then allowed to warm to room temperature for 14 h. After that, diethyl ether was added (20 mL) and the resulting precipitate was filtered. The filtrate was

concentrated *in vacuo*. Purification by flash column chromatography (diethyl ether) afforded **29** as a colourless oil (4.57 g, 14.3 mmol) in 79% yield.

δ_H (600 MHz, $CDCl_3$) 7.80 (2H, d, *J* = 12.0 Hz), 7.34 (2H, d, *J* = 12.0 Hz), 4.16 (2H, t, *J* = 6.0 Hz), 3.69 (2H, t, *J* = 6.0 Hz), 3.61-3.59 (6H, m), 3.54-3.52 (2H, m), 3.37 (3H, s), 2.45 (3H, s); δ_C (150 MHz, $CDCl_3$) 144.9 (C), 133.1 (C), 129.9 (2 × CH), 128.1 (2 × CH), 72.0 (CH_2), 70.9 (CH_2), 70.7 (CH_2), 70.6 (CH_2), 69.3 (CH_2), 68.8 (CH_2), 59.2 (CH_3), 21.8 (CH_3); IR 2881 (br), 1355 (m), 1189 (m), 1170 (s), 1099 (m); MS (CI+) *m/z,* (relative intensity): 319 ($[M+H]^+$, 80), 199 (65), 147 (65), 103 (100). Exact mass calcd. for $[C_{14}H_{22}O_6S]^+$+H: 319.1210. Measured: 319.1210 (CI+).

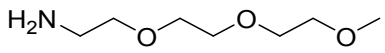
1-Azido-2-(2-(2-methoxyethoxy)ethoxy)ethane[5] (30)



To a solution tosylated PEG **29** (1.90 g, 5.98 mol) in dimethylformamide (40 mL) was added sodium azide (0.96 g, 14.9 mmol). The reaction mixture was stirred for 20 h at 60 °C and then for 3 h at 120 °C. The reaction mixture was allowed to cool to room temperature and then diethyl ether (50 mL) was added. The organic phase was washed with sat. aq. LiCl (5 × 20 mL), brine (20 mL), dried (Na_2SO_4), filtered and the solvent was removed *in vacuo* to afford the title compound **30** as a brown oil (887 mg, 4.69 mmol) in 78% yield.

δ_H (600 MHz, $CDCl_3$) 3.68-3.65 (8H, m), 3.55 (2H, t, *J* = 4.8 Hz), 3.40-3.38 (5H, m); δ_C (150 MHz, $CDCl_3$) 72.0 (CH_2), 70.81 (CH_2), 70.74 (CH_2), 70.74 (CH_2), 70.2 (CH_2), 59.2 (CH_3), 50.8 (CH_2); IR 2874 (br), 2098 (s), 1288 (br), 1104 (s); MS (CI+) *m/z,* (relative intensity): 190 ($[M+H]^+$, 20), 162 (65), 103 (100).

2-(2-(2-Methoxyethoxy)ethoxy)ethanamine[5] (31)

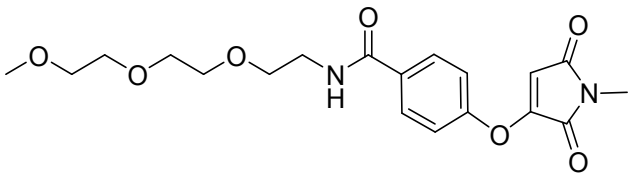


The PEG azide **30** (880 mg, 4.65 mmol) was dissolved in diethyl ether (50 mL) and the solution cooled at 0 °C. Triphenylphosphine (1.46 g, 5.58 mmol) was added and the mixture was stirred for 1 h at 0 °C and another 1 h at room temperature. The reaction was quenched with water (20 mL) and the mixture stirred vigorously for 4 h. Toluene (15 mL) was added and the mixture was stirred overnight. The layers were separated and the aq. layer was extracted once with toluene. *In vacuo* concentration of the aq. layer afforded the title compound **31** as a brown oil (506 mg, 3.10 mmol) in 67% yield.

δ_H (600 MHz, $CDCl_3$) 3.63-3.60 (6H, m), 3.53-3.52 (2H, m), 3.49 (2H, t, *J* = 4.8 Hz), 3.35 (3H, s), 2.85 (2H, t, *J* = 6.0 Hz); δ_C (150 MHz, $CDCl_3$) 73.0 (CH_2), 71.9 (CH_2), 70.7 (CH_2), 70.6 (CH_2), 70.3 (CH_2), 59.1

(CH_3), 41.7 (CH_2); IR 3379 (br), 2876 (br), 1570 (br), 1462 (br), 1304 (s), 1097 (s); MS (CI+) *m/z,* (relative intensity): 164 ([M+H]$^+$, 100), 88 (25). Exact mass calcd. for $[C_7H_{17}NO_3]^+$+H: 164.1287. Measured: 164.1289 (CI+).

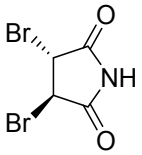
***N*-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)-4-(1-methyl-pyrrole-2,5-dione-3-yloxy)benzamide (16)**



To a solution of aryloxymaleimide **28** (50.0 mg, 0.20 mmol), HOBt (2.93 mg, 0.02 mmol) and HBTU (75.8 mg, 0.20 mmol) in dry dimethylformamide (14 mL), DIPEA (34 µL, 0.20 mmol) was added dropwise and the resulting reaction mixture was stirred at room temperature for 20 min. Then a solution of PEG amine **31** (32.2 mg, 0.20 mmol) in dry dimethylformamide (2 mL) was added dropwise and the stirring was continued for a further 4 h. After this time, the solvent was evaporated in *vacuo* and the resulting crude dissolved in dichloromethane (25 mL), washed with sat. aq. LiCl (2 × 20 mL), 15% aq. citric acid (10 mL), water (10 mL) and brine (10 mL). The combined organic layers were dried ($MgSO_4$), filtered and then the solvent evaporated *in vacuo*. Purification by flash chromatography (ethyl acetate : methanol, gradient elution from 100 : 0 to 80 : 20) afforded the title compound **16** as a yellow oil (52.6 mg, 0.13 mmol) in 67% yield.

δ_H (600 MHz,$CDCl_3$) 7.94-7.92 (2H, m), 7.25-7.22 (2H, m), 5.33 (1H, s), 3.66-3.62 (10H, m), 3.53-3.51 (2H, m), 3.31 (3H, s), 3.04 (3H,s); δ_C (150 MHz, $CDCl_3$) 169.7 (C), 166.4 (C), 165.5 (C), 158.8 (C), 155.9 (C), 133.2 (C), 129.8 (CH), 119.9 (2 × CH), 100.1 (2 × CH), 71.8 (CH_2), 70.5 (CH_2), 70.4 (CH_2), 70.2 (CH_2), 70.0 (CH_2), 59.1 (CH_3), 40.0 (CH_2), 23.8 (CH_3); IR 1716 (s), 1639 (m), 1311 (w); MS (CI+) *m/z,* (relative intensity): 393 ([M+H]$^+$, 82), 273 (100). Exact mass calcd. for $[C_{19}H_{24}N_2O_7]^+$+H: 393.1656. Measured: 393.1661 (CI+).

(3r, 4r)-3,4-Dibromopyrrolidine-2,5-dione[3] (32)



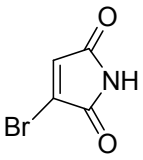
To maleimide (2.00 g, 20.0 mmol) in chloroform (15 mL) bromine (1.16 mL, 20.0 mmol) in chloroform (15 mL) was added dropwise. The reaction mixture was refluxed for 3 h, then allowed to cool down to room temperature over 1 h. The solid yellow precipitate was filtered and washed with

cold chloroform (2 × 35 mL) to afford the title compound **32** as white crystals (3.85 g, 14.9 mmol) in 75% yield.

δ_H (500 MHz, $CDCl_3$) 8.34 (1H, br s), 4.73 (2H, s); δ_C (125 MHz, $CDCl_3$) 169.8 (2 × C), 42.8 (2 × CH); IR 2945 (w), 1789 (m), 1708 (s), 1373 (s), 1170 (s); MS (ES+) *m/z* (relative intensity): 259 ($[^{81,81}M+H]^+$, 28), 257 ($[^{79,81}M+H]^+$, 29), 255 ($[^{79,79}M+H]^+$, 30); m.p.: 89 - 92 °C.

The stereochemistry of compound **32** was investigated. Based on molecular modelling (PC Model v 8.5) the calculated $^3J_{HH}$ for the *anti* isomer (corresponding to a torsional angle of 119°) is 2.2 Hz, exactly the same value that was measured when analysing the ^{13}C satellites in the ^1H spectrum. This demonstrates that the 2,3-dibromosuccinimide was obtained as the *anti* isomer.

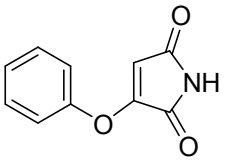
3-Bromo-pyrrole-2,5-dione (33)



Dibromosuccinimide **32** (965mg, 3.75 mmol) and sodium acetate trihydrate (1.54 g, 11.27 mmol) were dissolved in acetic acid (23 mL). The reaction mixture was refluxed for 1.5 h, then allowed to cool down to room temperature and stirred for a further 3 h before the solvent was evaporated *in vacuo*. The crude mixture was dissolved in ethyl acetate (50 mL) and washed with saturated aq. sodium bicarbonate solution (3 × 10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography (petroleum ether : ethyl acetate, gradient elution from 80 : 20 to 60 : 40) afforded the title compound **33** as white crystals (568 mg, 3.22 mmol) in 86% yield.

δ_H (500 MHz, $CDCl_3$) 7.70 (1H, br s), 6.89 (1H, d, J = 1.49 Hz); δ_C (150 MHz, $CDCl_3$) 168.0 (C), 164.9 (C), 132.9 (C), 132.3 (C); IR 3236 (m), 1782 (m), 1763 (m), 1716 (s), 1577 (m), 871 (m), 718 (m); MS (EI+) *m/z* (relative intensity): 177 ($^{81}[M]^+$, 95), 175 ($^{79}[M]^+$, 95), 134 (97), 132 (98), 106 (70), 104(70). Exact mass calcd. for $[C_4H_2NO_2{}^{79}Br]^+$: 174.9263. Measured: 174.9265 (EI+); m.p.: 149 °C (lit. m.p.[3]: 149 - 151 °C).

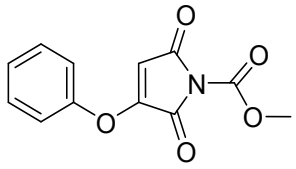
3-Phenoxy-pyrrole-2,5-dione (34)



To molten phenol (3.23 g, 34.3 mmol), potassium *tert*-butoxide (307 mg, 2.74 mmol) in dry dioxane (2 mL) was added dropwise and the solution was left stirring for 10 min at 40 °C. Then a solution of bromomaleimide **33** (400 mg, 2.28 mmol) in dry dioxane (2 mL) was added dropwise and the resulting mixture was stirred at 40 °C for 30 min. After this time, the solvent was evaporated *in vacuo*. Purification by flash chromatography (petroleum ether : ethyl acetate, gradient elution from 90 : 10 to 70 : 30) afforded the title compound **34** as a white solid (328 mg, 1.73 mmol) in 76% yield.

δ_H (600 MHz, $CDCl_3$) 8.44 (1H, br s), 7.43-7.40 (2H, m), 7.29-7.26 (1H, m), 7.17-7.15 (2H, m), 5.28 (1H, s); δ_C (150 MHz, $CDCl_3$) 170.4 (C), 166.1 (C), 159.6 (C), 153.9 (C), 130.5 (2 × CH), 127.0 (CH), 119.9 (2 × CH), 100.5 (CH); IR 3264 (br), 1732 (s), 1708 (s), 1627 (s), 1584 (s), 1488 (s), 1288 (s), 1215 (s); MS (EI+) *m/z,* (relative intensity): 189 ($[M]^+$, 85), 94 (100), 84 (95). Exact mass calcd. for $[C_{10}H_7NO_3]^+$: 189.0426. Measured: 189.0418 (EI+); m.p.: 85 °C.

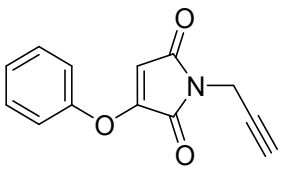
Methyl (3-phenoxy-pyrrole-2,5-dione)carboxylate (35)



To a solution of aryloxymaleimide **34** (360 mg, 1.41 mmol) in dichloromethane (6 mL) were added a solution of methyl chloroformate (1.09 mL, 14.1 mmol) in dichloromethane (1 mL) and a solution of *N*-methylmorpholine (186 µL, 1.69 mmol) in dichloromethane (1 mL). The reaction mixture was stirred at room temperature for 30 min. After this time, the solution was washed with water (3 × 5 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford the title compound **35** as a pale pink solid (329 mg, 1.33 mmol) in 95% yield.

δ_H (600 MHz, $CDCl_3$) 7.46-7.43 (2H, m), 7.33-7.30 (1H, m), 7.17-7.15 (2H, m), 5.42 (1H, s), 3.98 (3H, s); δ_C (150 MHz, $CDCl_3$) 164.6 (C), 161.0 (C), 159.3 (C), 153.6 (C), 148.0 (C), 130.6 (2 × CH), 127.4 (CH), 119.8 (2 × CH), 101.6 (CH), 54.4 (CH_3); IR 1769 (s), 1720 (s), 1641 (m), 1304 (s), 1259 (s), 1104 (m), 1075 (m); MS (CI+) *m/z,* (relative intensity): 248 ($[M+H]^+$, 100), 216 (75). Exact mass calcd. for $[C_{12}H_9NO_5]^+$+H: 248.0559. Measured: 248.0554 (CI+); m.p.: 70 - 72 °C.

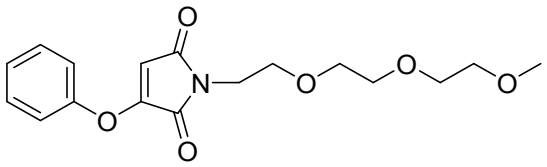
3-Phenoxy-1-(prop-2-ynyl)-pyrrole-2,5-dione (36)



A solution of propargylamine (4.90 µL, 0.08 mmol) in dichloromethane (0.2 mL) was added to a solution of aryloxymaleimide **35** (19.8 mg, 0.08 mmol) in dichloromethane (1 mL) and left to stir at room temperature for 1.5 h. After this time, the solvent was evaporated *in vacuo.* Purification by flash chromatography (ethyl acetate : ether petroleum, 70 : 30) afforded **36** as a transparent oil (5.00 mg, 0.02 mmol) in 27% yield.

δ_H (600 MHz, $CDCl_3$) 7.47-7.44 (2H, m), 7.34-7.31 (1H, m), 7.20-7.18 (2H, m), 5.33 (1H, s), 4.32 (2H, s), 2.24 (1H, s); δ_C (150 MHz, $CDCl_3$) 168.5 (C), 164.4 (C), 159.8 (C), 153.8 (C), 130.5 (CH), 127.1 (CH), 119.9 (CH), 99.8 (CH), 71.8 (CH), 26.9 (CH_2); IR 1715 (s), 1631 (s), 1585 (m), 1310 (s), 1220 (m); MS (CI+) *m/z,* (relative intensity): 228 ($[M+H]^+$, 100). Exact mass calcd. for $[C_{13}H_9NO_3]^+$+H: 228.0661. Measured: 228.0657 (CI+); m.p.: 55 °C.

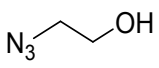
1-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)-3-phenoxy-pyrrole-2,5-dione (17)



A solution of amine **31** (32.8 mg, 0.20 mmol) in dichloromethane (0.5 mL) was added to a solution of aryloxymaleimide **35** (21.0 mg, 0.20 mmol) in dichloromethane (0.5 mL) and the resulting mixture was left to stir at room temperature for 15 h. After this time, the solvent was evaporated *in vacuo.* Purification by flash chromatography (dichloromethane : methanol, gradient elution from 97 : 3 to 95 : 5) afforded the title compound **17** as a transparent oil (7.60 mg, 0.02 mmol) in 28% yield.

δ_H (600 MHz, $CDCl_3$) 7.46-7.43 (2H, m), 7.32-7.30 (1H, t, *J* = 6.0 Hz), 7.20-7.18 (2H, d, *J* = 12 Hz), 5.27 (1H, s), 3.76-3.74 (2H, t, *J* = 6 Hz), 3.68-3.63 (8H, m), 3.55-3.54 (2H, m), 3.37 (3H, s); δ_C (150 MHz, $CDCl_3$) 169.9 (C), 165.6 (C), 159.5 (C), 153.9 (C), 130.4 (2 × CH), 126.9 (CH), 119.9 (2 × CH), 99.4 (CH), 72.0 (CH_2), 70.7 (2 × CH_2), 70.1 (CH_2), 68.1 (CH_2), 59.2 (CH_3), 37.1 (CH_2); IR 2917 (br), 1715 (s), 1633 (s), 1312 (m); MS (CI+) *m/z,* (relative intensity): 336 ($[M+H]^+$, 98), 216 (100). Exact mass calcd. for $[C_{17}H_{21}NO_6]^+$+H: 336.1447. Measured: 336.1445 (CI+).

2-Azidoethanol[7] **(37)**

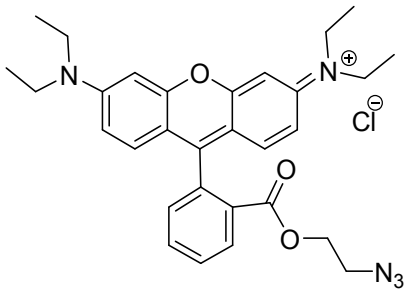


To a round-bottom flask containing sodium azide (2.42 g, 37.2 mmol) and tetrabutylammonium bromide (3.99 g, 12.4 mmol) was added 2-chloroethanol (1.00 g, 124 mmol) and the resulting mixture was stirred at 110 °C for 18 h. Purification by flash chromatography (petroleum ether : ethyl

acetate, gradient elution from 70 : 30 to 50 : 50) afforded the title compound **37** as a transparent oil (482 mg, 5.47 mmol) in 45% yield.

δ_H (600 MHz, $CDCl_3$) 3.78 (2H, t, *J* = 6.0 Hz), 3.45 (2H, t, *J* = 6.0 Hz); δ_C (150 MHz, $CDCl_3$) 61.6 (CH_2), 53.6 (CH_2); IR 3350 (br), 2937 (br), 2094 (s), 1288 (m); MS (CI+) *m/z,* (relative intensity): 116 ($[M+K]^+$, 53), 88 ($[M+H]^+$, 100). Exact mass calcd. for $[C_2H_5N_3O]^+$+H: 88.0511. Measured: 88.0513 (CI+).

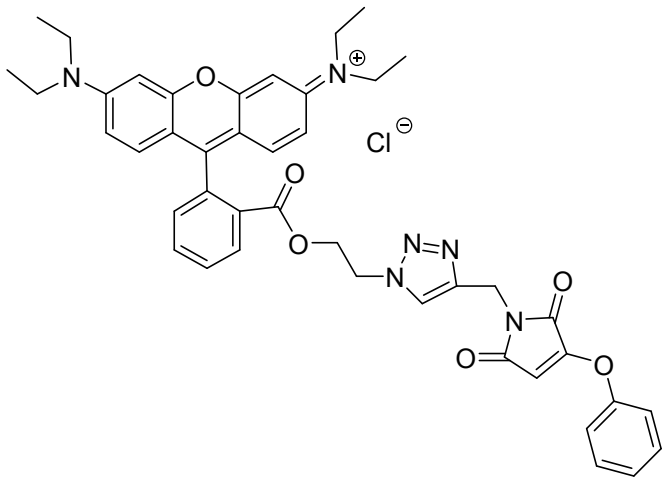
_N_-(9-(2-((2-Azidoethoxy)carbonyl)phenyl)-6-(diethylamino)-3H-xanthen-3-ylidene)-_N_-ethylethanaminium chloride (38)



A round bottom flask containing dichloromethane(1.5 mL) was wrapped in aluminium foil and rhodamine B (100 mg, 0.21 mmol), EDC·HCl (44.1 mg, 0.23 mmol), azide **37** (20 mg, 0.23 mmol) and DMAP (5.30 mg, 0.04 mmol) were added. The resulting reaction mixture was stirred at room temperature for 4 h. After this time, dichloromethane (5 mL) was added and the reaction mixture was washed with H_2O (10 mL). The aq. layer was extracted with dichloromethane (4 × 5 mL) and all organic layers were combined, washed with 0.1 M HCl (5 mL), brine (5 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by flash chromatography (chloroform : methanol, 98 : 2) afforded the title compound **38** as a purple solid (115 mg, 0.21 mmol) in 100% yield.

δ_H (600 MHz, $CDCl_3$) 8.24 (1H, d, *J* = 7.8 Hz), 7.77 (1H, t, *J* = 7.8 Hz), 7.69 (1H, t, *J* = 7.8 Hz), 7.25 (1H, d, *J* = 10.2 Hz), 7.01 (2H, d, *J*= 9.6 Hz), 6.86-6.84 (2H, d, *J* = 9.6 Hz), 6.73 (2H, d, *J* = 1.8 Hz), 4.11 (2H, t, *J* = 4.8 Hz), 3.59-3.56 (8H, m), 3.32 (2H, t, *J* = 4.8 Hz), 1.25 (12H, m); δ_C (150 MHz, $CDCl_3$) 166.8 (C), 158.4 (C), 157.8 (2 × C), 155.6 (2 × C), 133.7 (C), 133.5 (CH), 131.6 (CH), 131.3 (2 × CH), 130.6 (CH), 130.4 (CH), 129.4 (C), 114.4 (2 × CH), 113.5 (2 × C), 96.3 (2 × CH), 63.9 (CH_2), 49.6 (CH_2), 46.2 (4 × CH_2), 12.7 (4 × CH_3); IR 3384 (bs), 2971 (w), 2928 (w), 2102 (w), 1721 (m), 1586 (s), 1482 (m), 1413 (m), 1413 (s), 1338 (s), 1180 (s); Exact mass calcd. for $[C_{30}H_{34}N_5O_3]^+$: 512.2656. Measured: 512.2656 (NSI+).

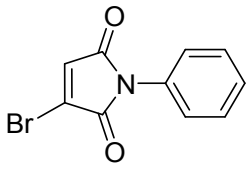
***N*-(6-(Diethylamino)-9-(2-((2-(4-((2,5-dioxo-3-phenoxy-pyrrole-2,5-dione-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)carbonyl)phenyl)-3H-xanthen-3-ylidene)-*N*-ethylethanaminium (18)**



To a mixture of copper iodide (0.19 mg, 0.001 mmol), *N*,*N*-diisopropylethylamine (DIPEA) (9.58 µL, 0.001 mmol) and acetic acid (9.00 µL, 0.001 mmol) in dichloromethane (1 mL) was added a mixture of azide **38** (15.5 mg, 0.03 mmol) and alkyne **36** (7.06 mg, 0.03 mmol) in dichloromethane (0.5 mL). The resulting reaction mixture was stirred at room temperature for 4 h. After this time the solvent was removed *in vacuo*. The crude was purified by flash chromatography (chloroform : methanol, gradient elution 98 : 2 to 90 : 10) to afford the title compound **18** as a purple solid (13.3 mg, 0.02 mmol) in 62% yield.

δ_H (600 MHz, $CDCl_3$) 8.21-8.20 (1H, m), 7.89 (1H, s), 7.77 (2H, t, *J* = 8.5 Hz), 7.44 (2H, t, J = 7.9 Hz), 7.31 (1H, t, *J* = 7.5 Hz), 7.28-7.27 (1H, m) 7.15 (2H, d, *J* = 7.9 Hz), 7.07 (2H, d, *J* = 9.4 Hz), 6.93 (2H, dd, *J* = 9.4 and 2.2 Hz), 6.79 (2H, d, *J* = 2.3 Hz), 5.29 (1H, s), 4.83 (2H, s), 4.65 (2H, t, *J* = 5.2 Hz), 4.49 (2H, t, *J* = 5.2 Hz), 3.67-3.59 (8H, m), 1.32 (12H, t, *J* = 7.1 Hz); δ_H (150 MHz,$CDCl_3$) 169.2 (C), 165.1 (C), 164.5 (C), 159.6 (C), 158.7 (C), 157.8 (2 × C), 155.7 (2 × C), 153.9 (C), 142.7 (C), 133.8 (C), 133.4 (CH), 131.8 (CH), 131.5 (CH), 130.9 (CH), 130.5 (2 × CH), 130.3 (CH), 129.3 (C), 127.0 (CH), 123.9 (CH), 119.9 (2 × CH), 114.6 (2 × CH), 113.6 (2 × C), 99.7 (CH), 96.4 (2 × CH), 63.6 (CH_2), 49.0 (CH_2), 46.3 (4 × CH_2), 32.9 (CH_2), 12.8 (4 × CH_3); IR 2970 (w), 1721 (s), 1588 (s), 1411 (m), 1342 (m), 1268 (s), 1247 (s); MS (ES+) *m/z,* (relative intensity): 739 ($[M]^+$, 100), 711 (50). Exact mass calcd. for $[C_{43}H_{43}N_6O_6]^+$:739.3239. Measured: 739.3221 (NSI+).

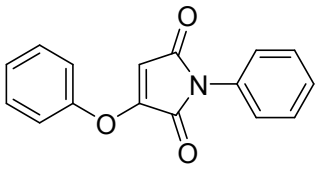
3-Bromo-1-phenyl-pyrrole-2,5-dione (39)



To a solution of *N*-phenyl maleimide (400 mg, 2.31 mmol) in (3 mL) was added dropwise a solution of bromine (237 µL, 4.62 mmol) in chloroform (1 mL) at room temperature. The reaction mixture was refluxed for 2 h and then allowed to cool to room temperature. The reaction mixture was diluted with chloroform (10 mL), washed with sat. aq. $Na_2S_2O_3$ (5 mL), water (5 mL) and brine (5 mL). The crude product was dried ($MgSO_4$), filtered and the solvent evaporated *in vacuo* to afford 636 mg of crude dibromosuccinimide as a yellow solid. Then, a part of the dibromosuccinimide (400 mg, 1.32 mmol) was dissolved in acetic acid (8 mL) and sodium acetate (325 mg, 3.96 mmol) was added. The reaction mixture was refluxed for 2 h and then the solvent was evaporated *in vacuo*. The crude residue was diluted with ethyl acetate (30 mL) and washed with water (20 mL), brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by flash chromatography (petroleum ether : ethyl acetate, gradient elution from 90 : 10 to 80 : 20) afforded the title compound **39** as a white solid (179.5 mg, 0.71mmol) in 54% yield.

δ_H (600 MHz, $CDCl_3$) 7.48 (2H, t, *J* = 7.8 Hz), 7.41-7.38 (1H, m), 7.34 (2H, d, *J* = 7.5 Hz), 7.03 (1H, s); δ_C (150 MHz, $CDCl_3$) 167.5 (C), 164.3 (C), 132.0 (CH), 131.9 (C), 131.1 (C), 129.4 (CH), 128.5 (CH), 126.2 (CH); IR 1711 (s), 1592 (m), 1504 (m), 1396 (s), 1148 (m); MS (EI+) *m/z,* (relative intensity): 253 ($[^{81}M]^+$, 100), 251 ($[^{79}M]^+$, 96), 86 (55), 84 (90). Exact mass calcd. for $[C_{10}H_6BrNO_2]^+$: 250.9582. Measured: 250.9582 (EI+); m.p.: 153 °C.

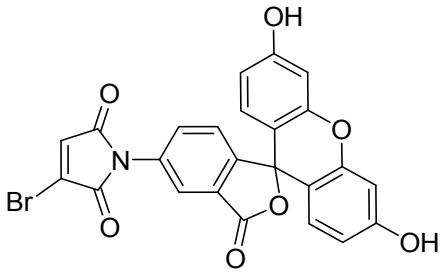
3-Phenoxy-1-phenyl-pyrrole-2,5-dione (40)



To a solution of phenol (22.3 mg, 0.24 mmol) in dry dioxane (0.5 mL) potassium *tert*-butoxide (26.9 mg, 0.24 mmol) in dry dioxane (2.5 mL) was added dropwise. The solution was left stirring for 15 min and then added dropwise to a solution of bromomaleimide **39** (50.0 mg, 0.20 mmol) in dry dioxane (1 mL). The mixture was stirred at room temperature for 2.5 h and then concentrated *in vacuo*. Purification by flash chromatography (petroleum ether : diethyl ether, gradient elution from 90 : 10 to 80 : 20) afforded the title compound **40** as a transparent oil (18.8 mg, 0.07 mmol) in 46% yield.

δ_H (600 MHz, $CDCl_3$) 7.61-7.51 (4H, m), 7.42-7.35 (4H, m), 7.28-7.26 (2H, m), 5.46 (1H, s); δ_C (150 MHz, $CDCl_3$) 168.9 (C), 164.5 (C), 159.4 (C), 153.9 (C), 131.1 (C), 130.5 (2 CH), 129.3 (2 CH), 128.1 (2 CH), 127.1 (2 CH), 126.2 (2 CH), 120.0 (CH), 99.5 (CH); IR 1715 (s), 1634 (m), 1401 (m), 1201 (m); MS (ES+) *m/z,* (relative intensity): 266 ($[M+H]^+$, 42), 180 (100). Exact mass calcd. for $[C_{16}H_{11}NO_3]^+$+H: 266.0817. Measured: 266.0827 (ES+); m.p.: 198 - 201 °C.

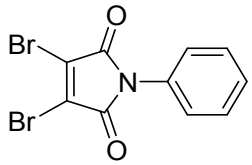
3-Bromo-1-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-5-yl)-pyrrole-2,5-dione (41)



To a solution of fluoresceinamine isomer 1 (104.2 mg, 0.3 mmol) in acetic acid (10 mL) was added monobromomaleic anhydride (28.7 µL, 0.3 mmol) and the resulting reaction mixture was stirred for 6 h at room temperature, then refluxed for 3 h. After this time the reaction mixture was allowed to cool to room temperature and the orange precipitate was filtered and dried *in vacuo* to afford the title compound **41** as an orange solid (96.8 mg, 0.19 mmol) in 64% yield.

δ_H (600 MHz, DMSO-d_6) 7.99 (1H, d, *J* = 1.3 Hz), 7.77 (1H, dd, *J* = 8.3 and 1.9 Hz), 7.73 (1H, s), 7.43 (1H, dd, *J* = 8.1 Hz), 6.69 (2H, d, *J* = 2.3 Hz), 6.62-6.57 (4H, m); δ_C (150 MHz, DMSO-d_6) 167.9 (C), 167.6 (C), 164.5 (C), 159.6 (C), 151.8 (C), 151.5 (C), 133.7 (CH),133.0 (C), 132.9 (CH), 131.2 (C), 129.1 (C), 126.7 (C), 124.8 (CH), 122.3 (CH), 112.8 (CH), 109.1 (C), 102.3 (CH), 83.4 (C); IR 3063 (br), 1724 (s), 1579 (s), 1536 (s), 1369 (s), 1208 (s); MS (ES+) *m/z* (relative intensity): MS (ES-) *m/z* (relative intensity): 506 ($[^{81}M\text{-}H^+]^-$, 100), 504 ($[^{79}M\text{-}H^+]^-$, 100). Exact mass calcd. for $[C_{24}H_{12}{}^{79}BrNO_7]^+$: 505.9875. Measured: 505.9897 (ES+).

3,4-dibromo-1-Phenyl-pyrrole-2,5-dione[8] (42)



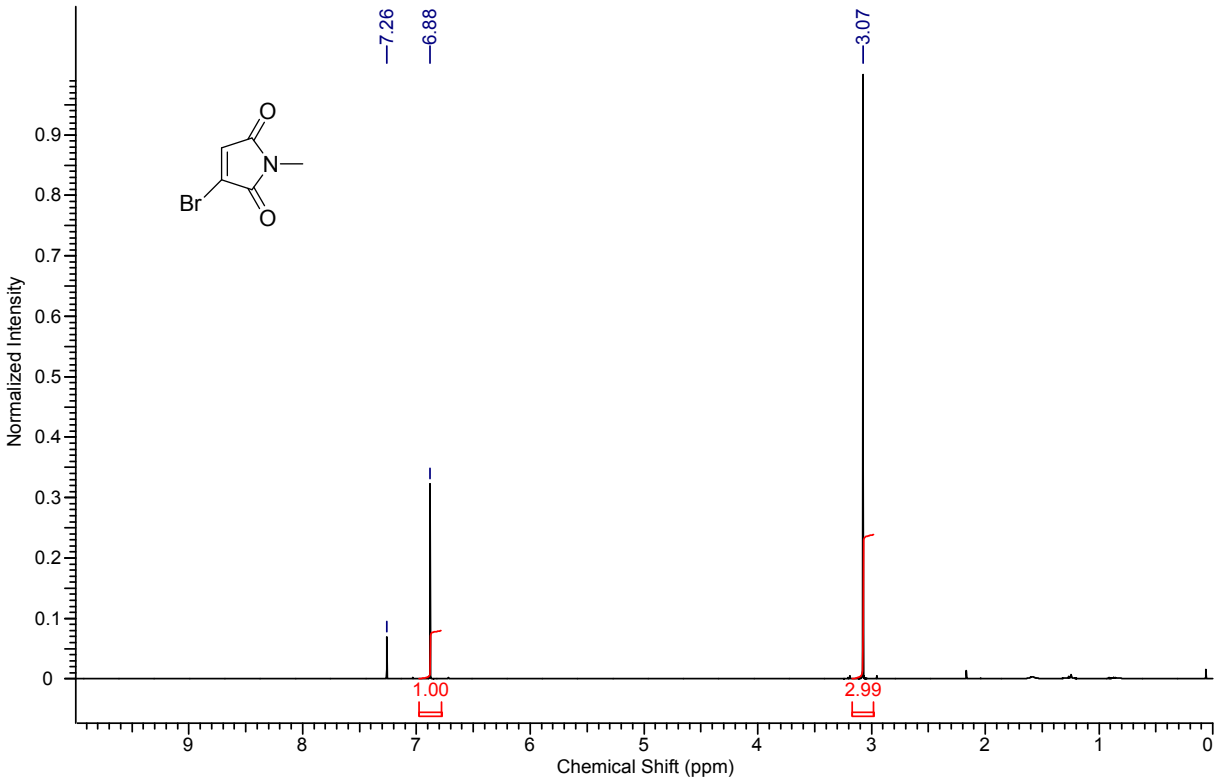
To a solution of dibromomaleic anhydride (300 mg, 1.17 mmol) in acetic acid (3.5 mL) was added aniline (118 µL, 1.29 mmol). The reaction mixture was refluxed for 3 h, stirred at room temperature for 15 h and then concentrated *in vacuo*. Purification by flash chromatography (petroleum ether : ethyl acetate, gradient elution from 90 : 10 to 80 : 20) afforded **42** as a yellow solid (91.5 mg, 0.28 mmol) in 24 % yield.

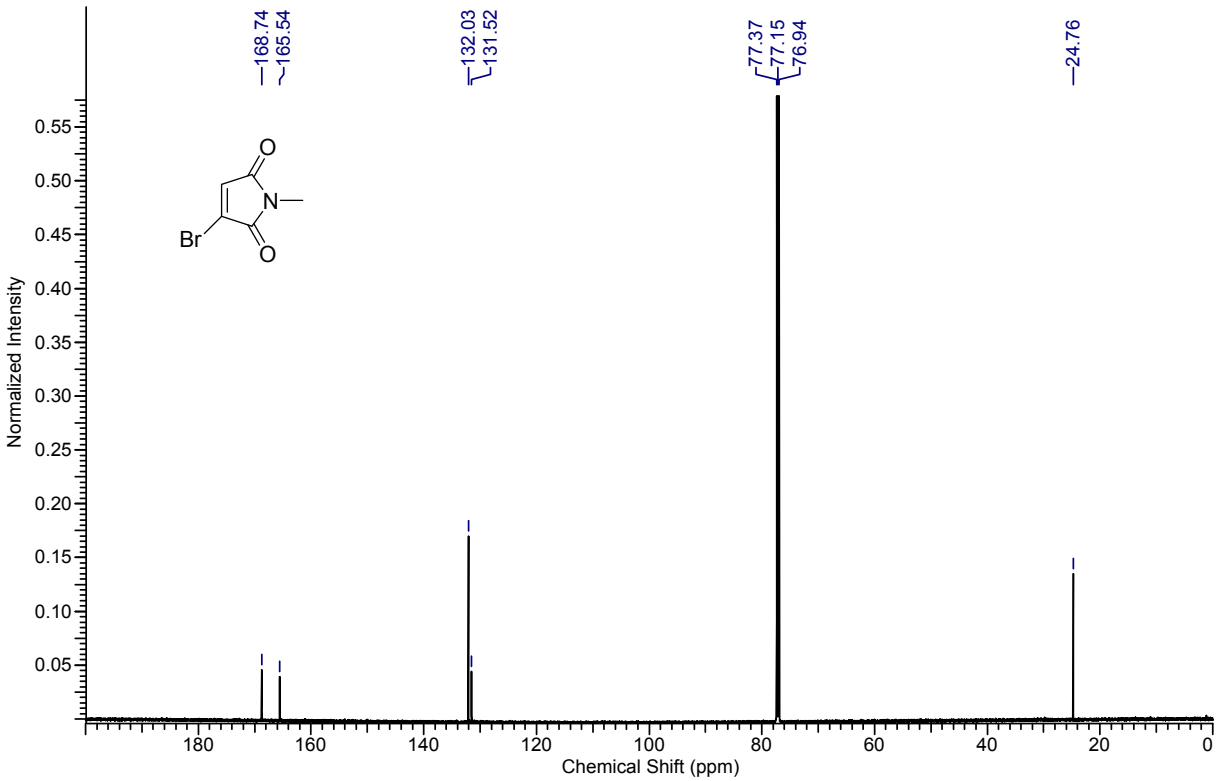
δ_H (600 MHz, $CDCl_3$) 7.50-7.47 (2H, m), 7.43-7.40 (1H, m), 7.34-7.33 (2H, m); δ_C (150 MHz, $CDCl_3$) 163.0 (C), 131.0 (C), 130.0 (C), 129.5 (CH), 128.8 (CH), 126.2 (CH); IR 1727 (s), 1717 (s), 1369 (w),

1229 (w); MS (CI+) *m/z,* (relative intensity): 334 ([^{81,81}M+H]$^+$, 45), 332 ([^{81,79}M+H]$^+$, 100), 330 ([^{79,79}M+H]$^+$, 45). Exact mass calcd. for [$C_{10}H_5{}^{79}BrNO_2$]$^+$+H: 329.9765. Measured: 329.8749 (CI+); m.p.: 169 °C.

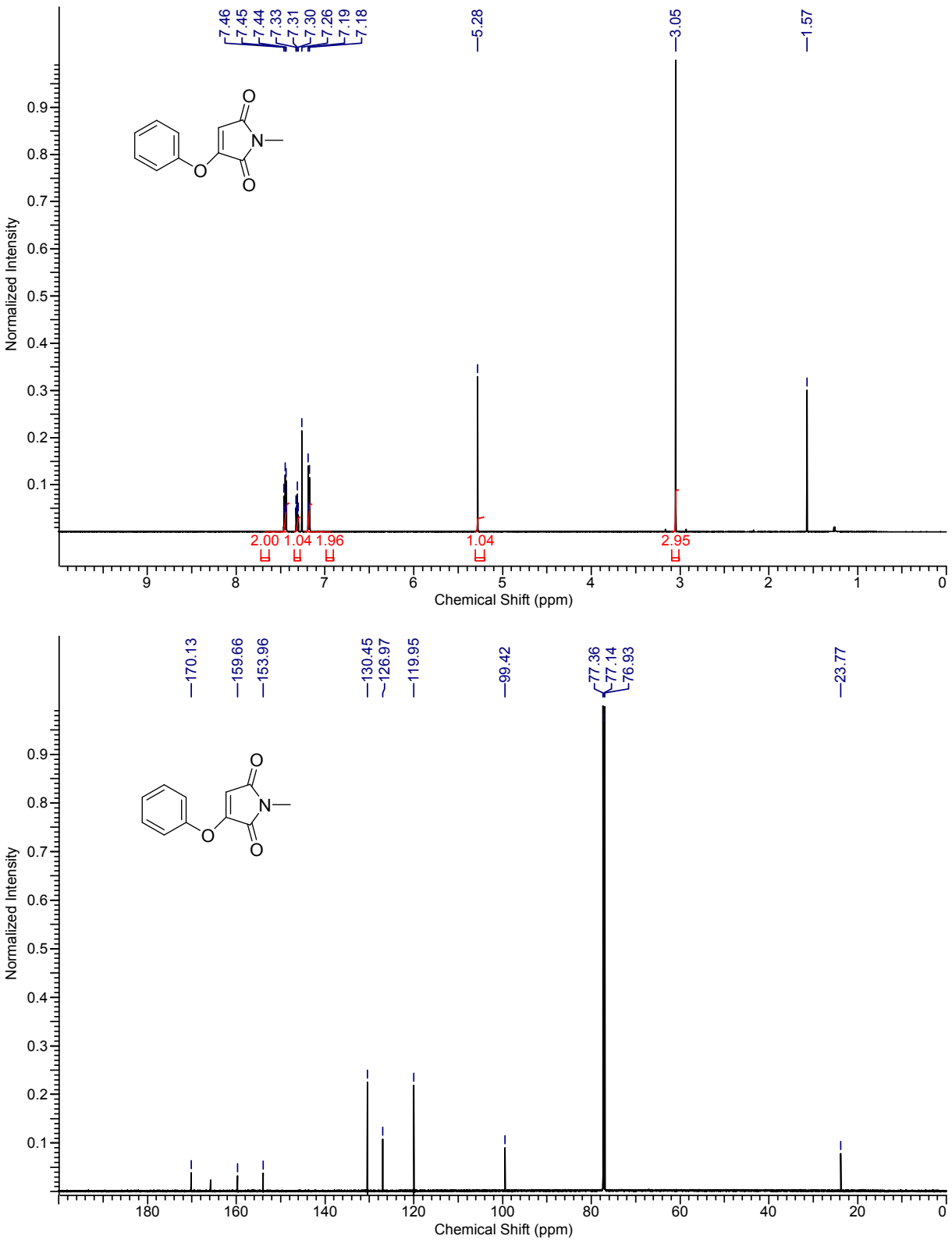
V. NMR Spectra

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

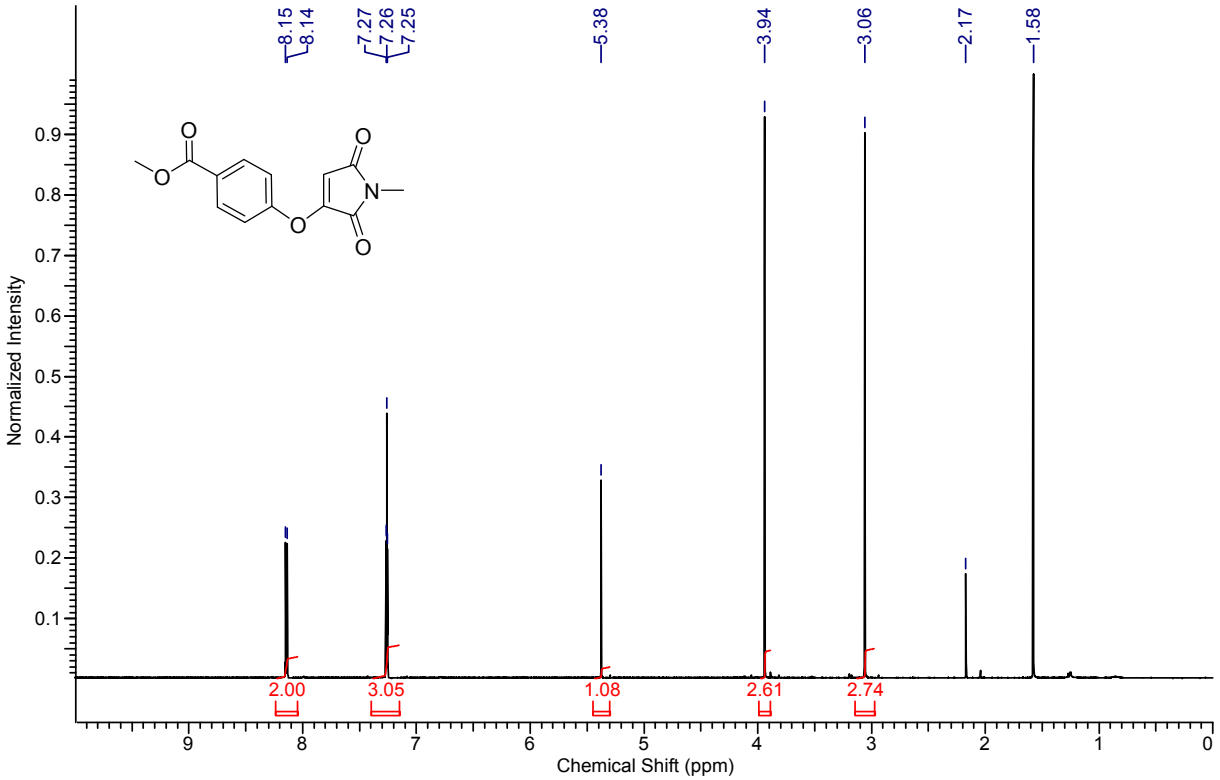


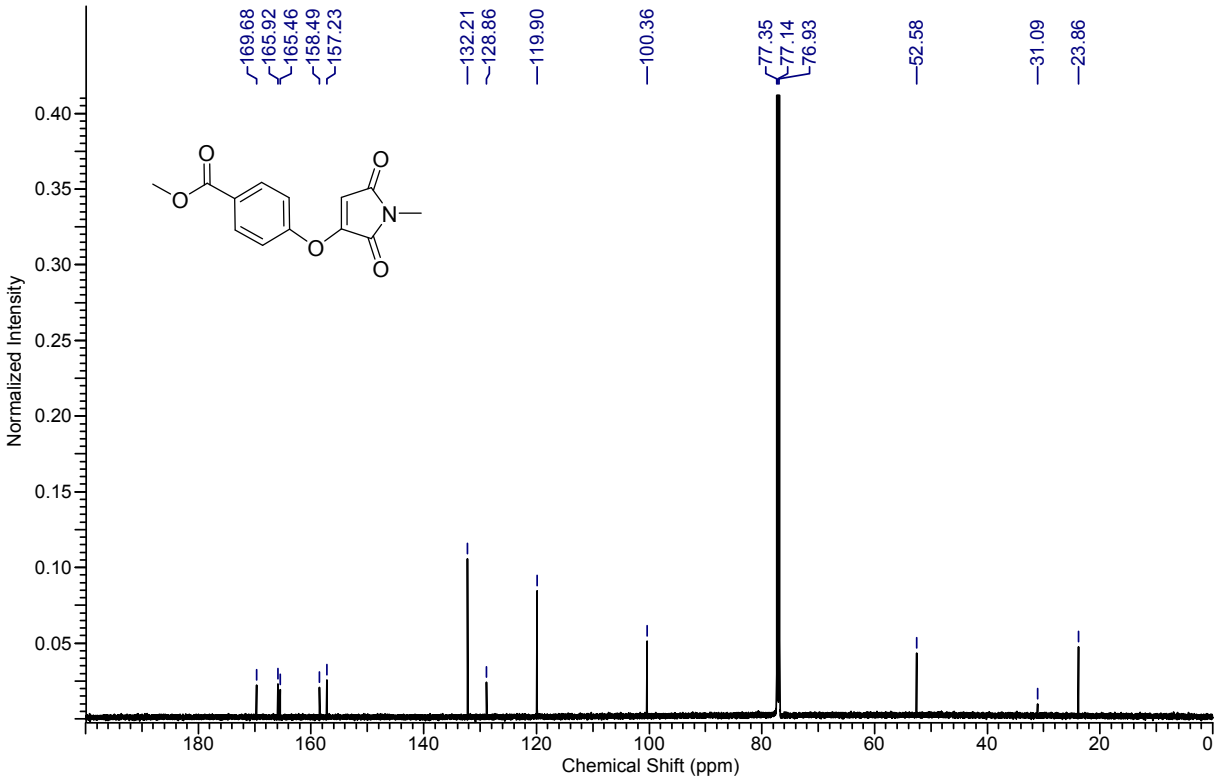


1-Methyl-3-phenoxy-pyrrole-2,5-dione (6)

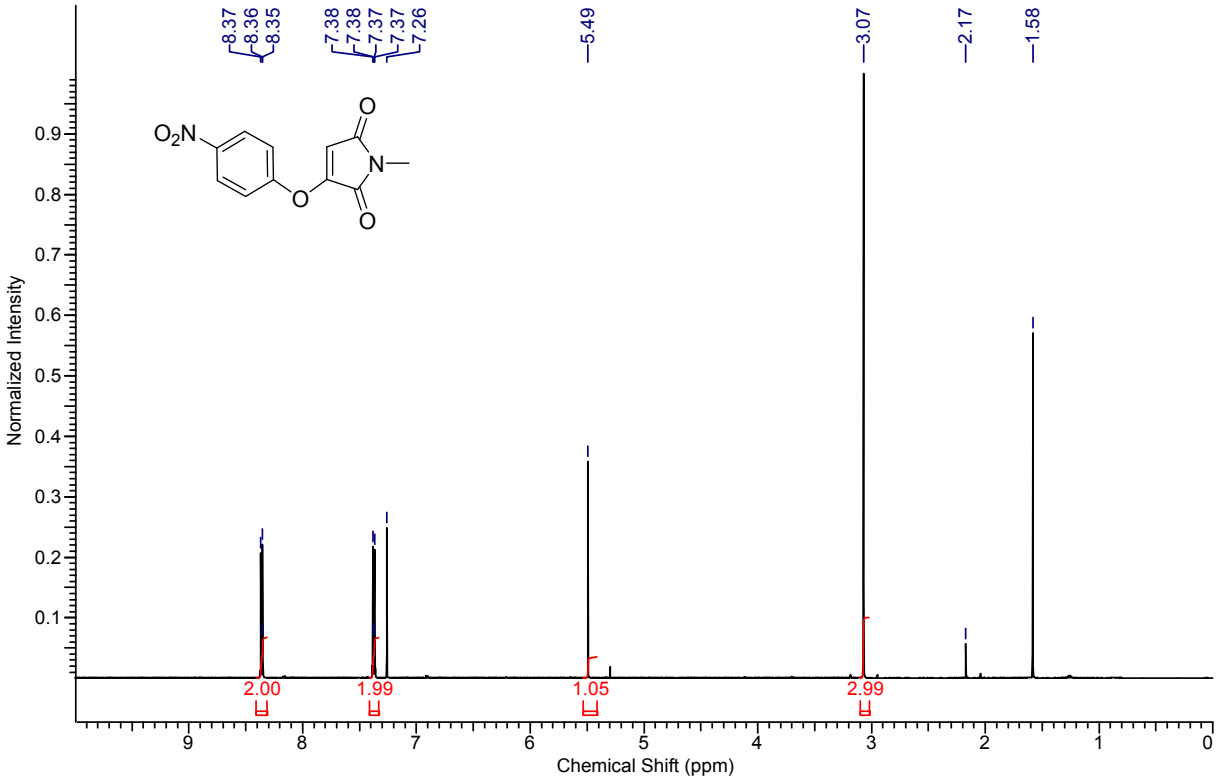


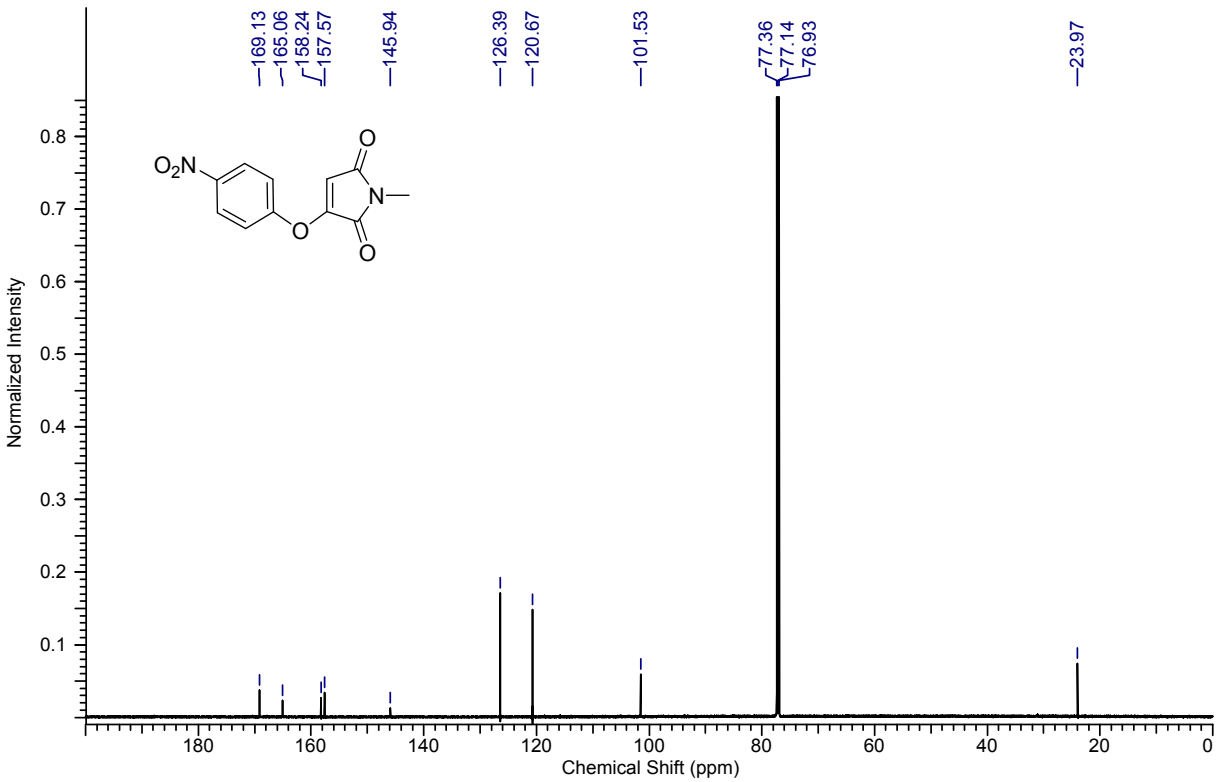
Methyl 4-(1-methyl-pyrrole-2,5-dione-3-yloxy)benzoate (7)



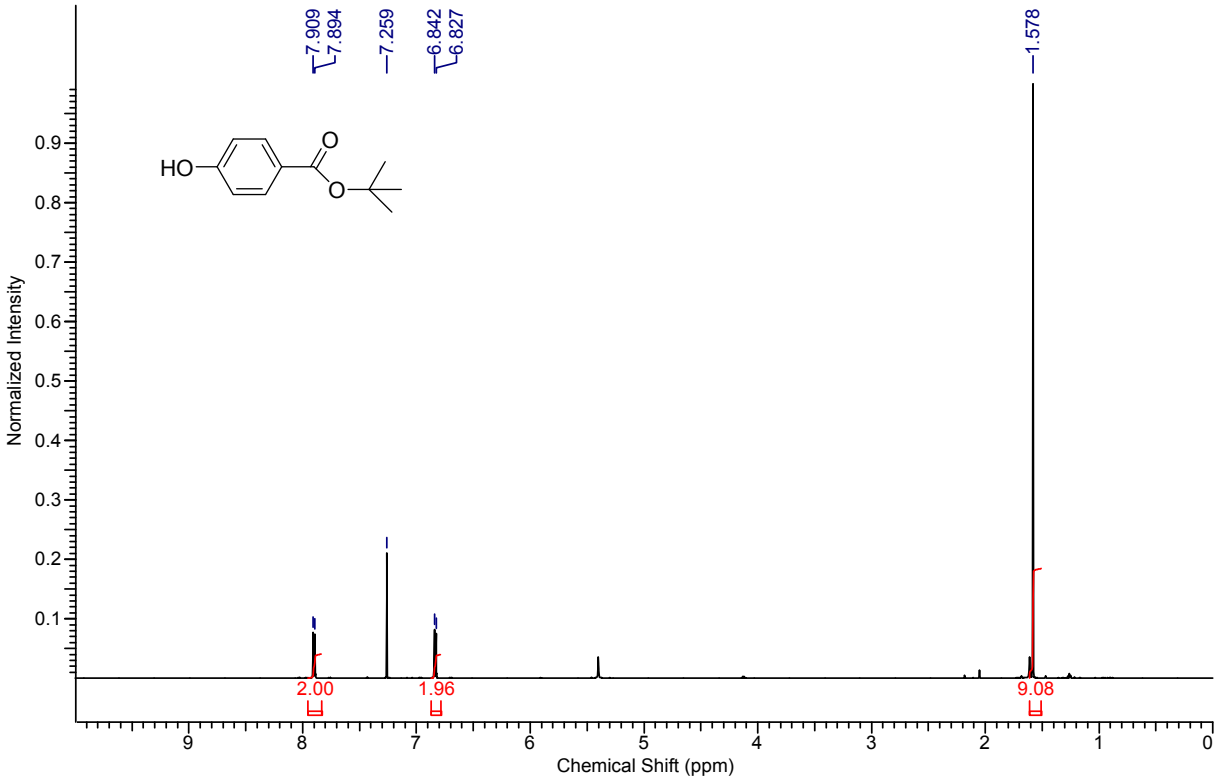


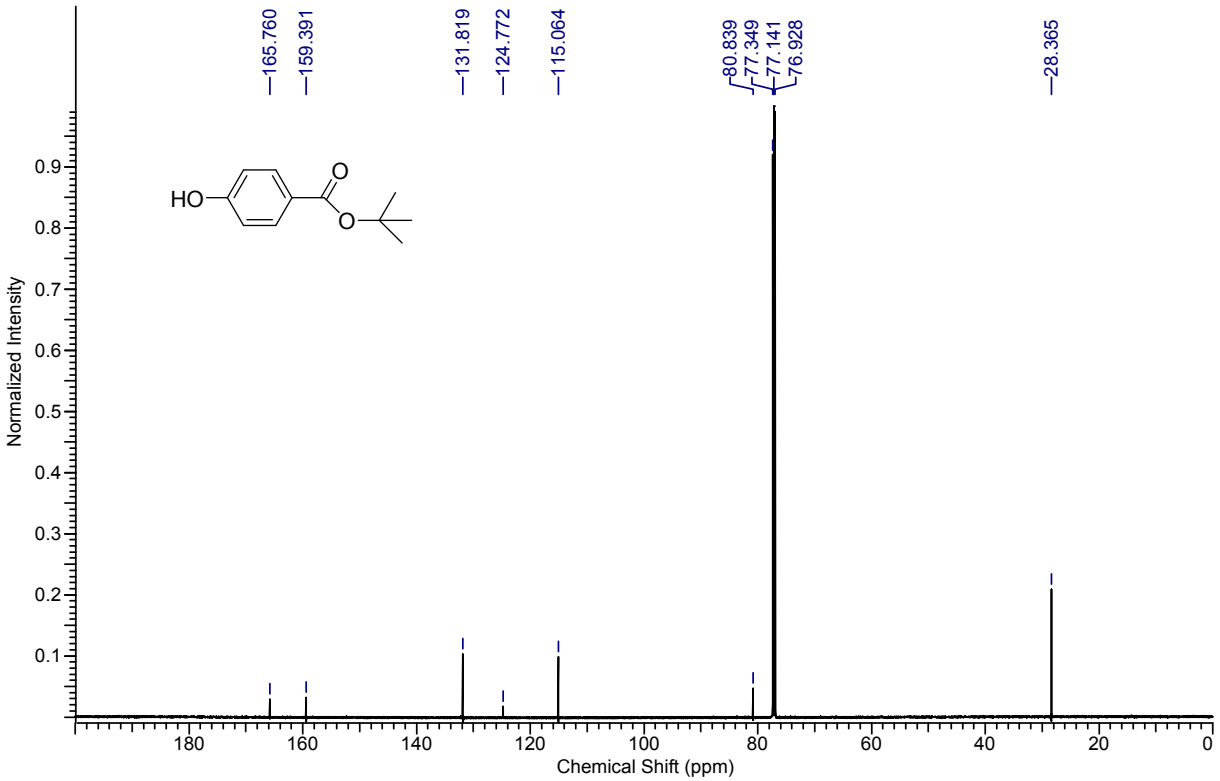
1-Methyl-3-(4-nitrophenoxy)-pyrrole-2,5-dione (8)



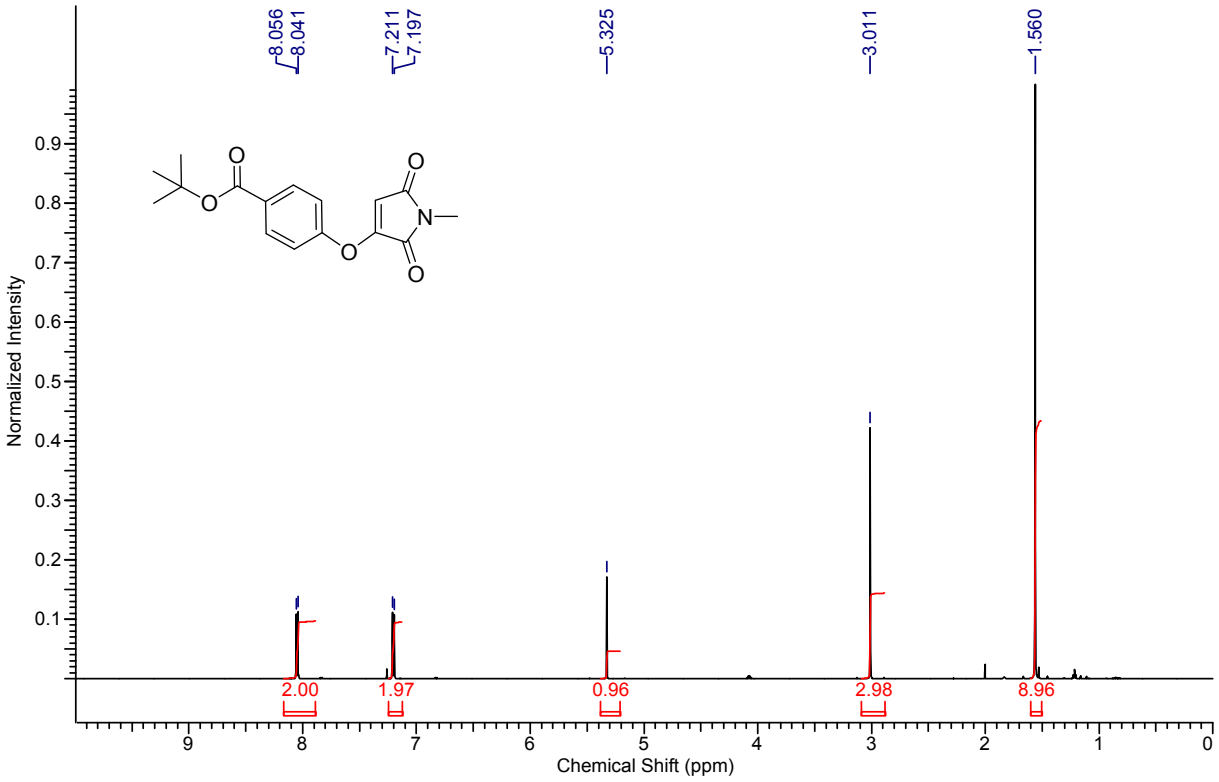


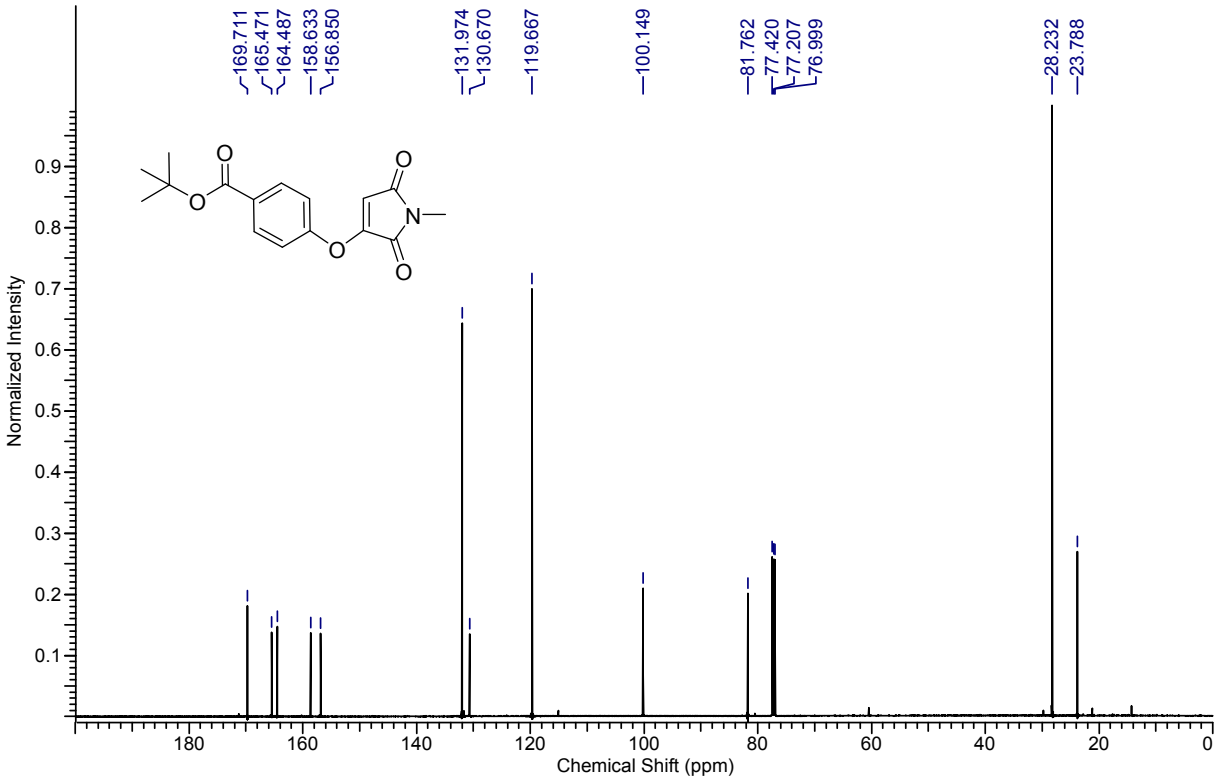
tert-Butyl 4-hydroxybenzoate (26)



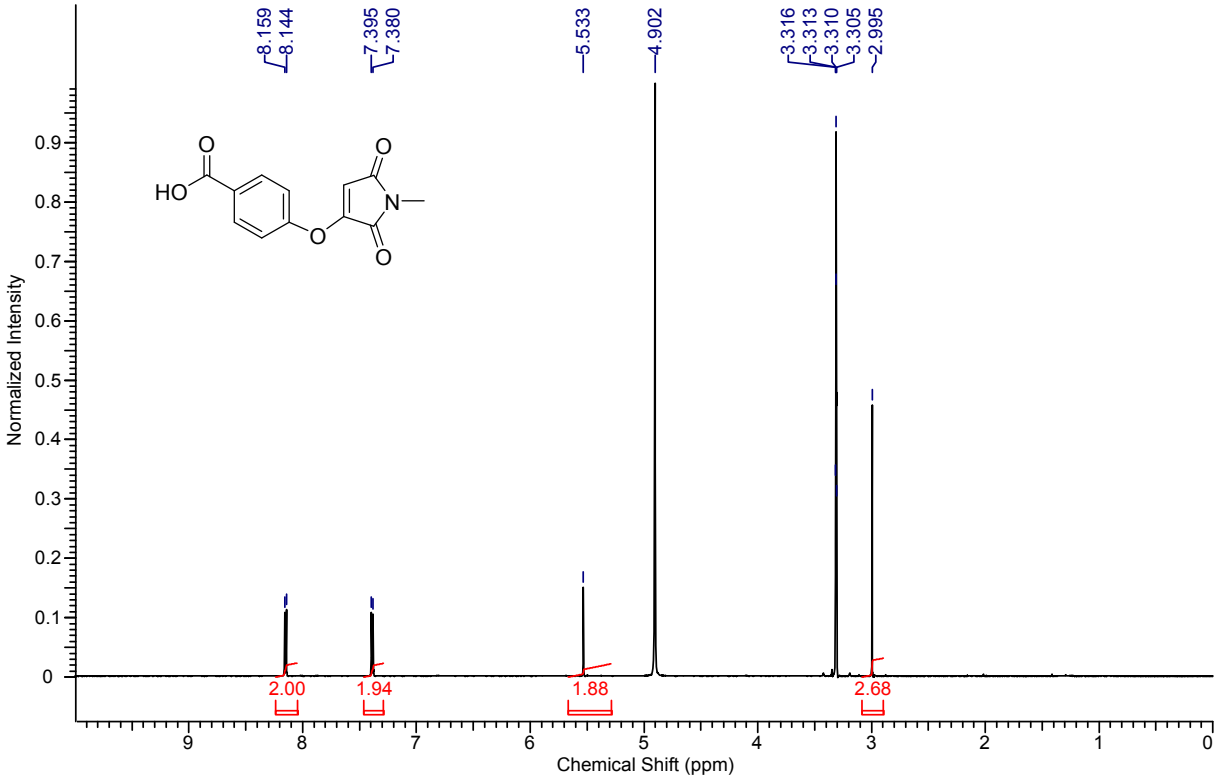


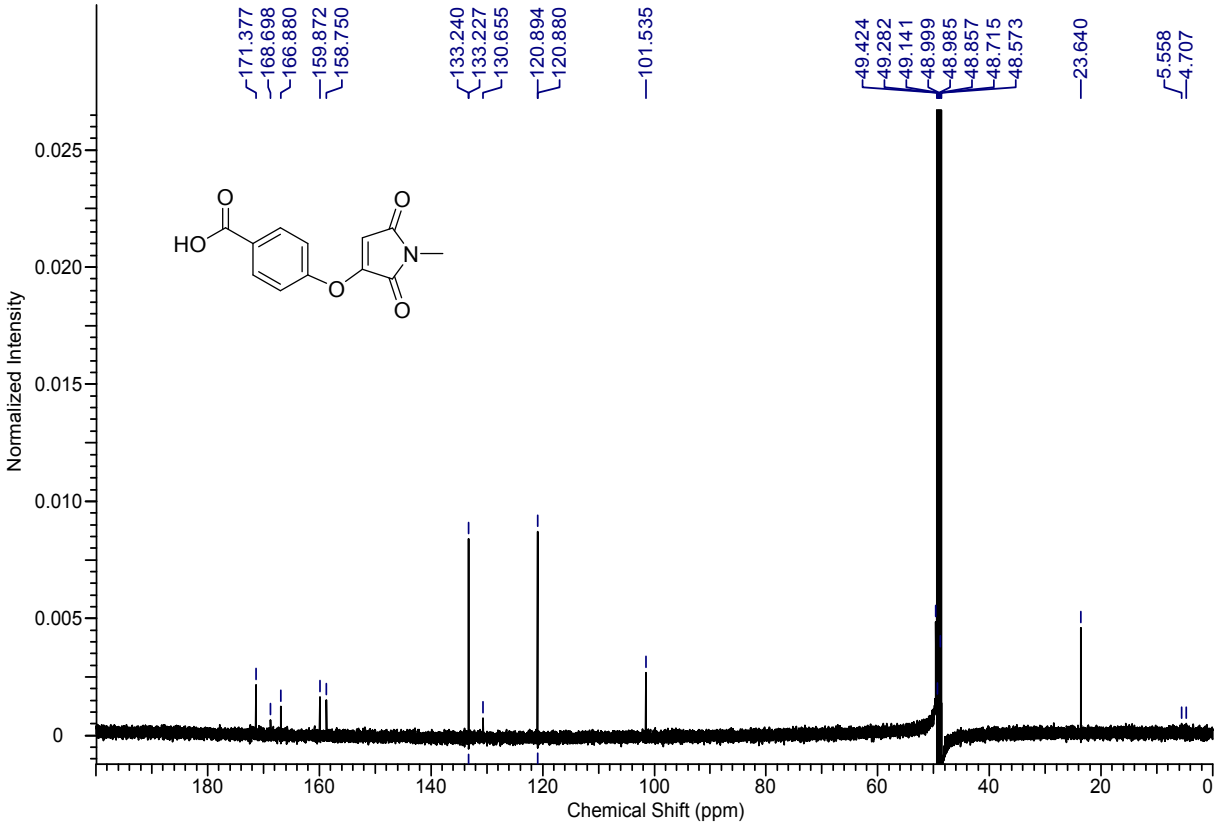
***tert*-Butyl 4-(1-methyl-pyrrole-2,5-dione-3-yloxy)benzoate (27)**



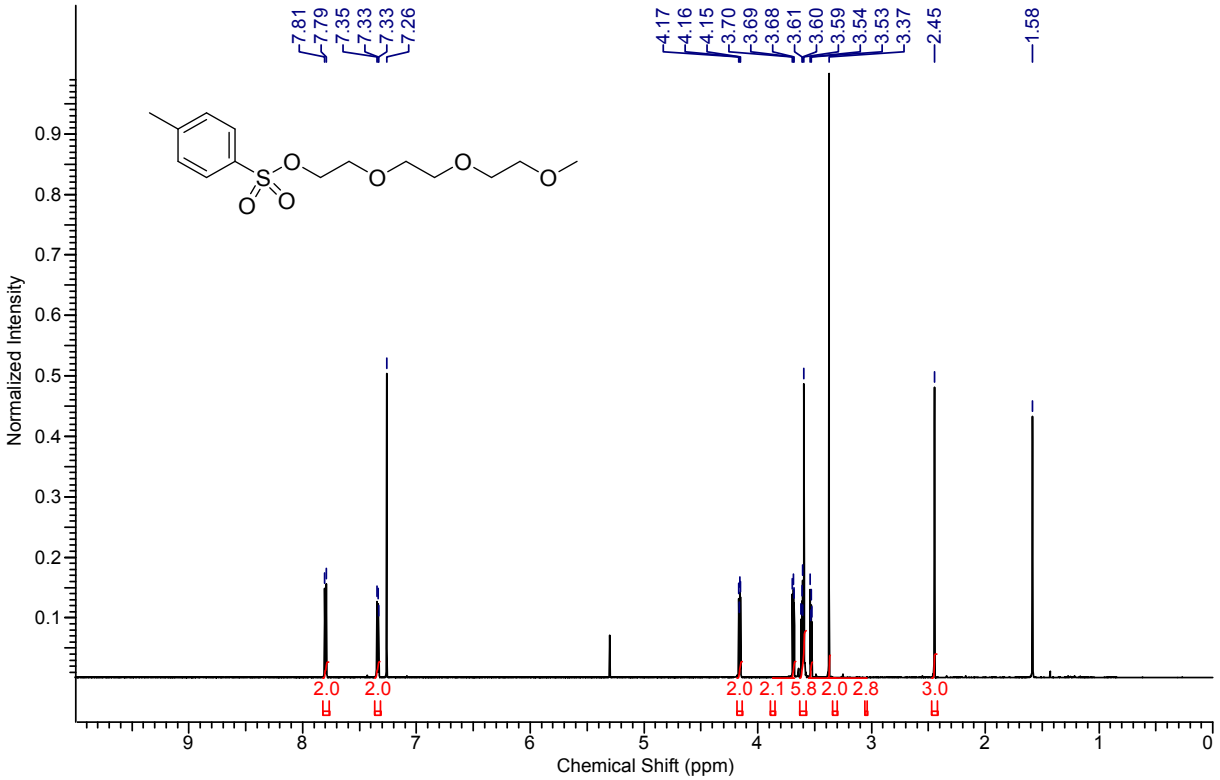


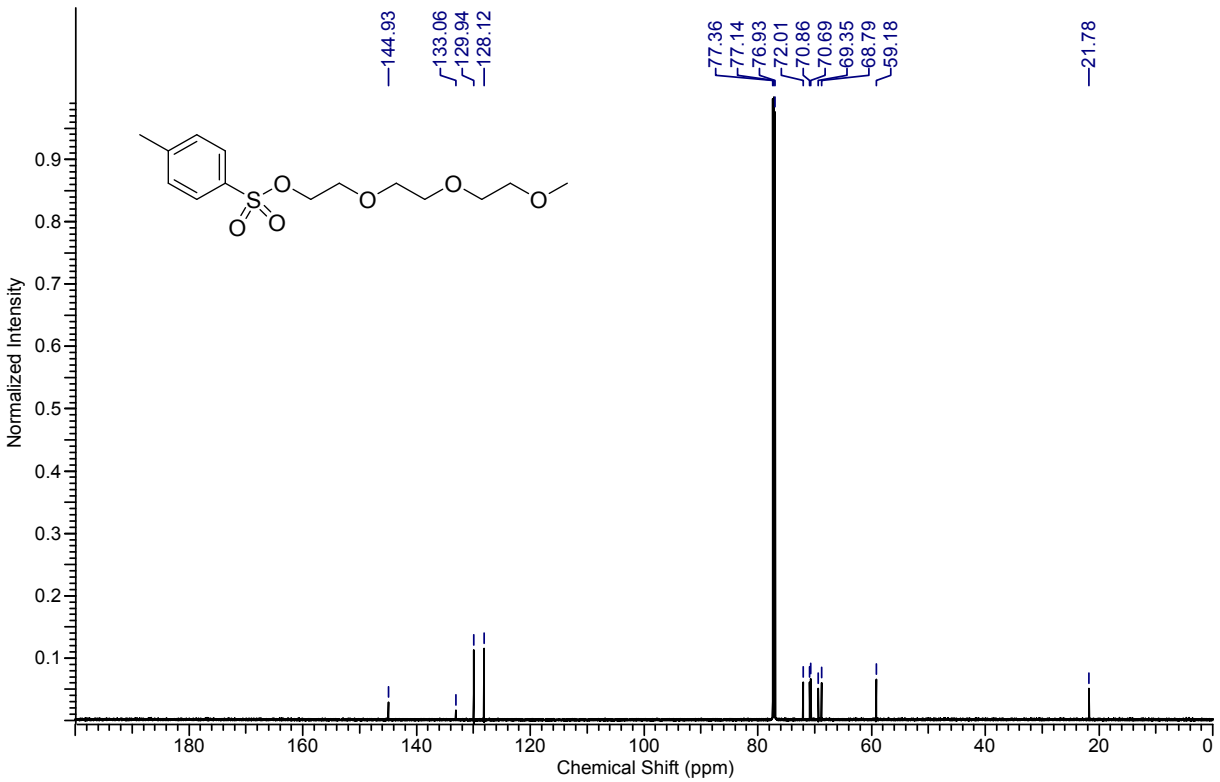
4-(1-Methyl-pyrrole-2,5-dione-3-yloxy)benzoic acid (28)



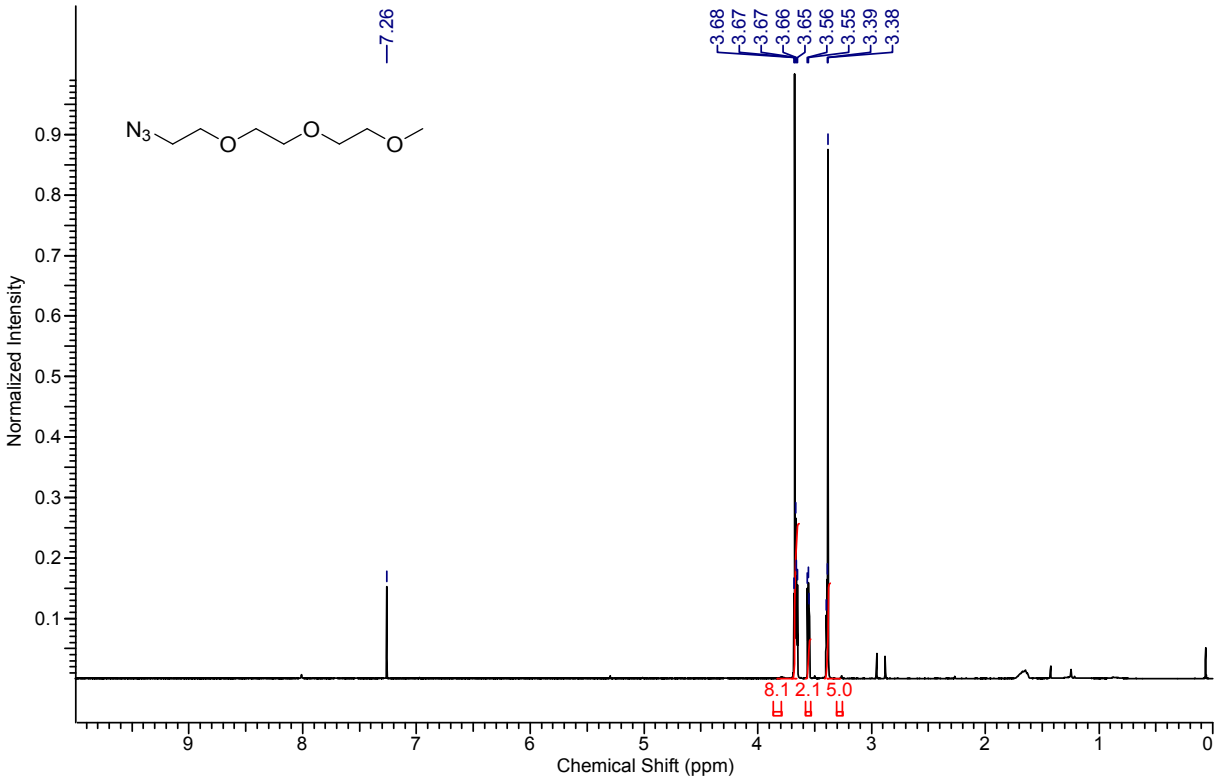


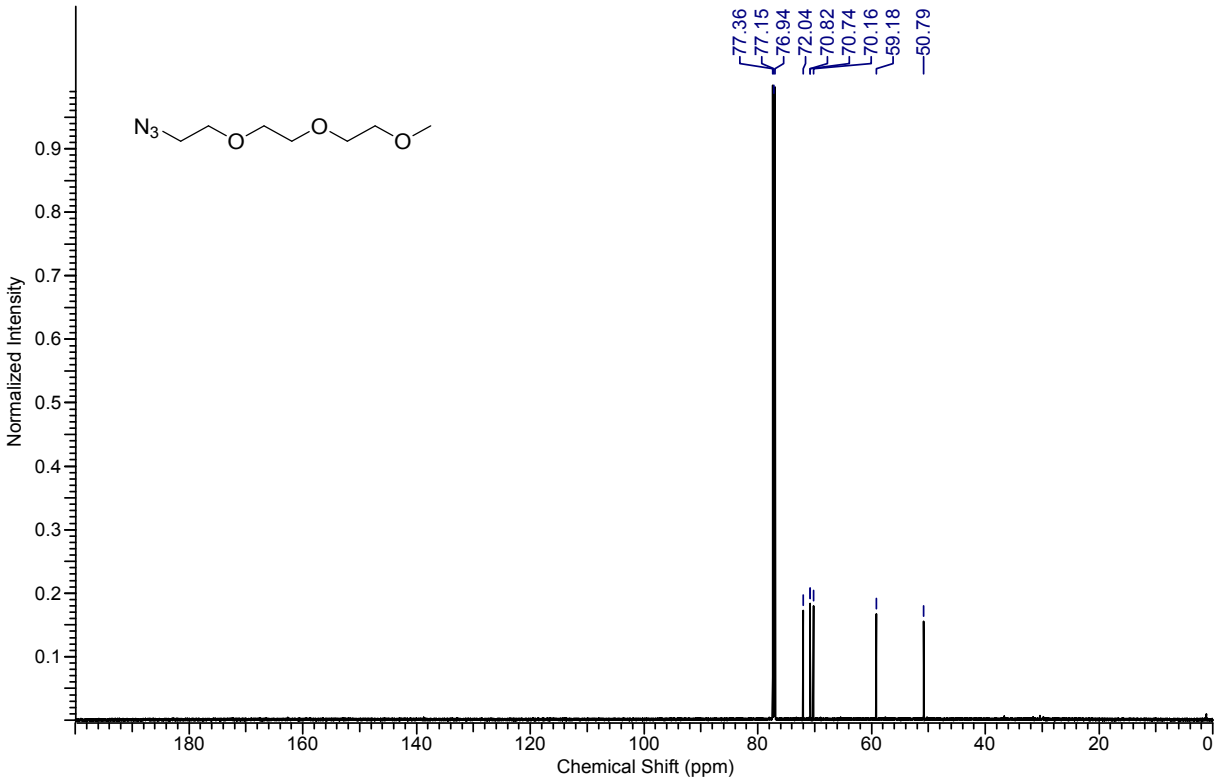
2-(2-(2-Methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (29)



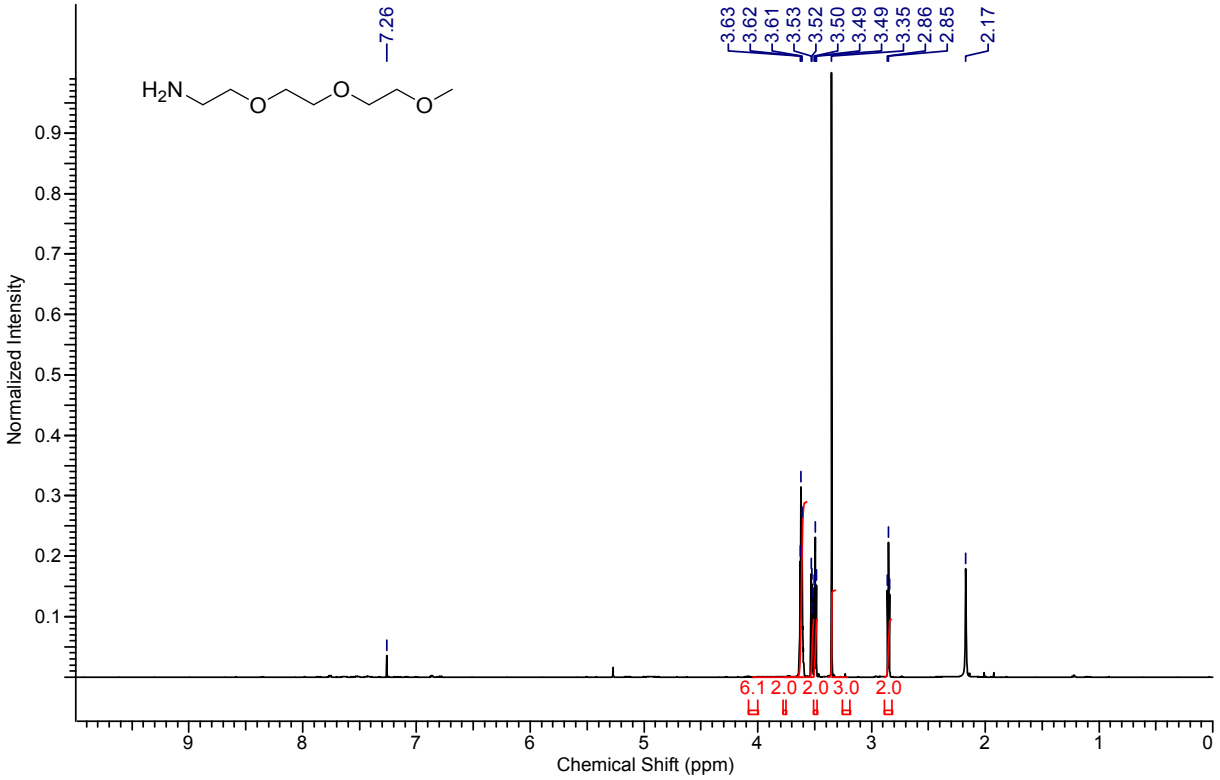


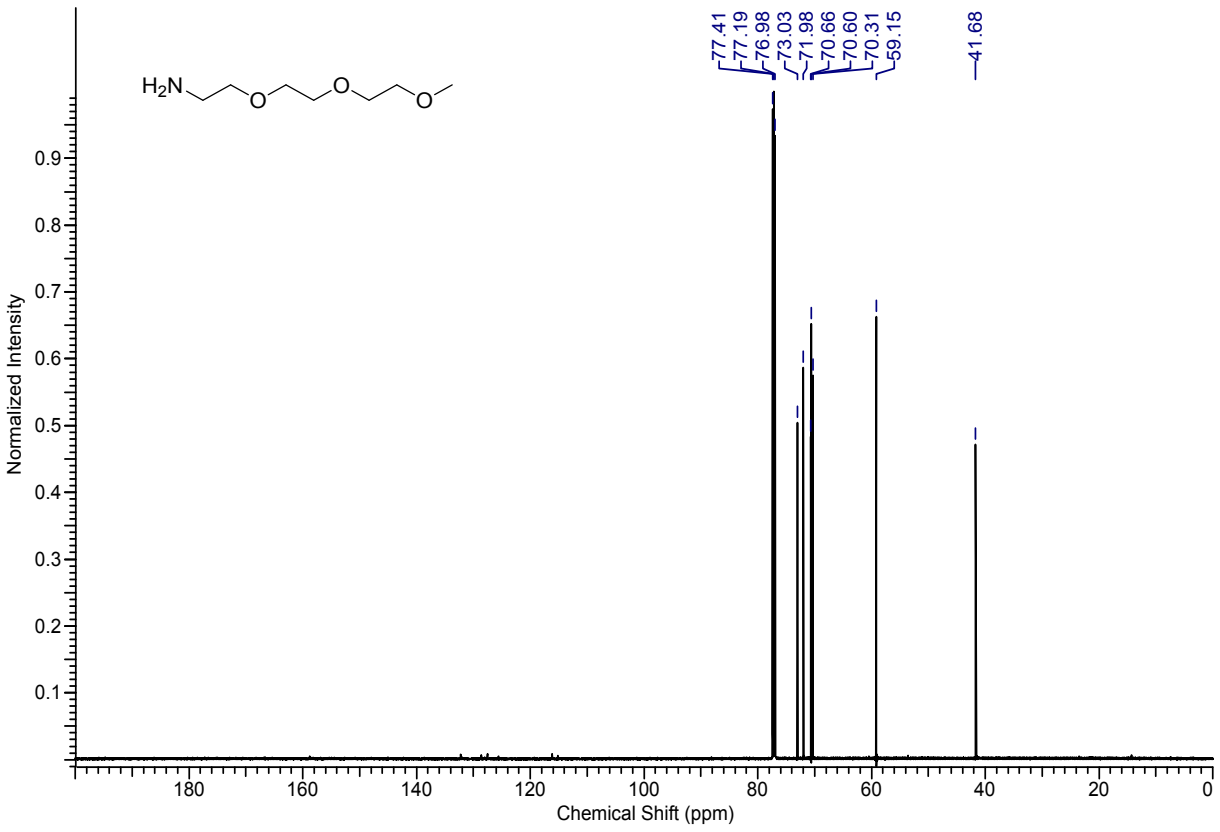
1-Azido-2-(2-(2-methoxyethoxy)ethoxy)ethane (30)



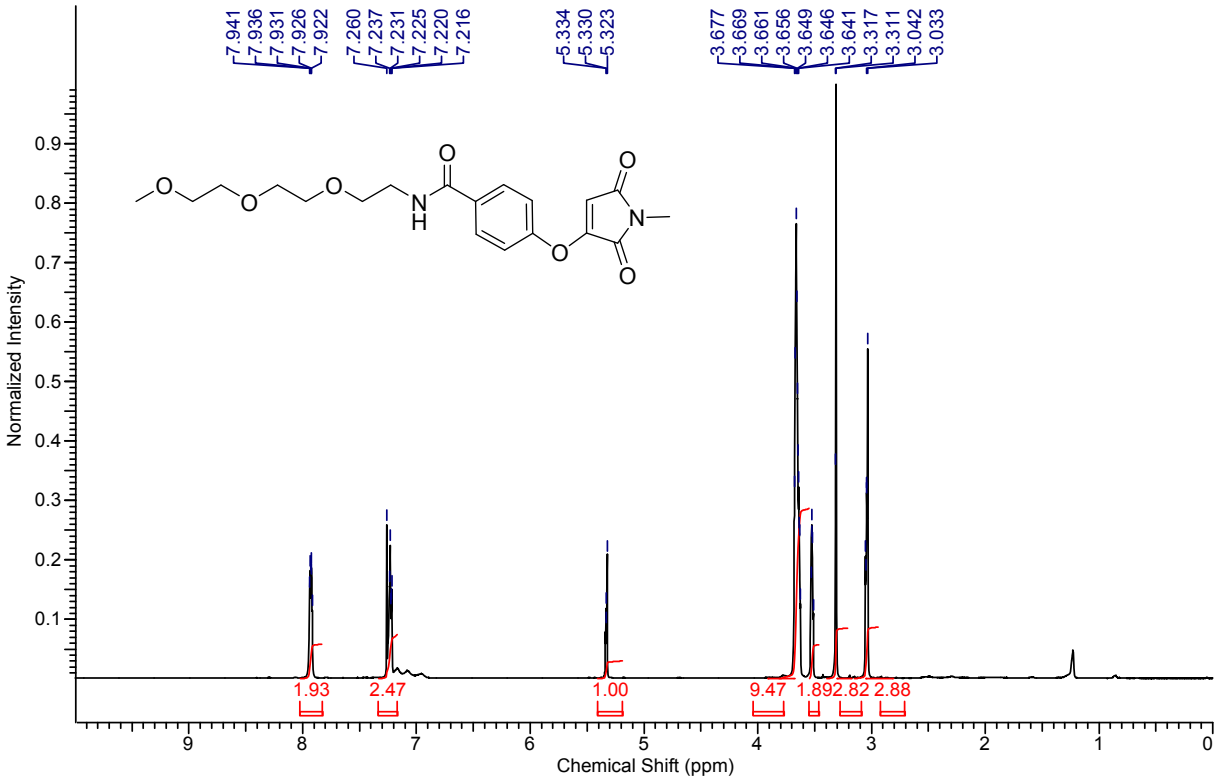


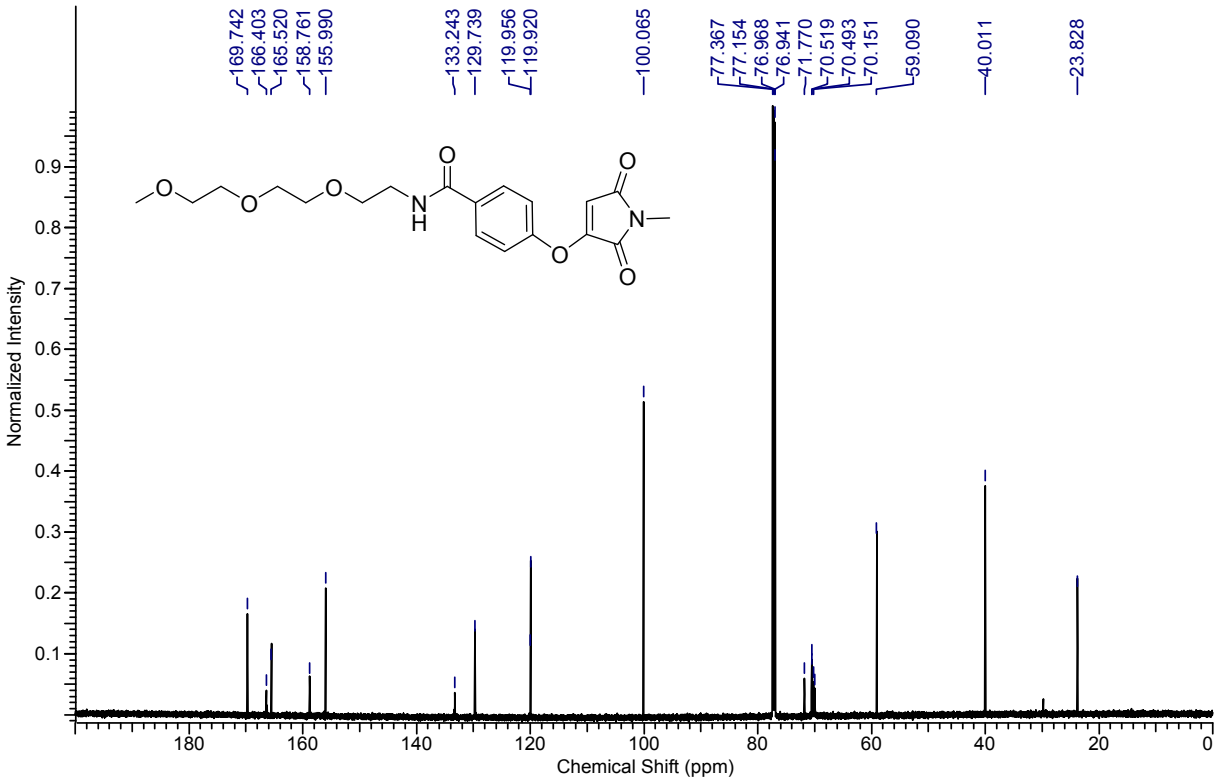
2-(2-(2-Methoxyethoxy)ethoxy)ethanamine (31)



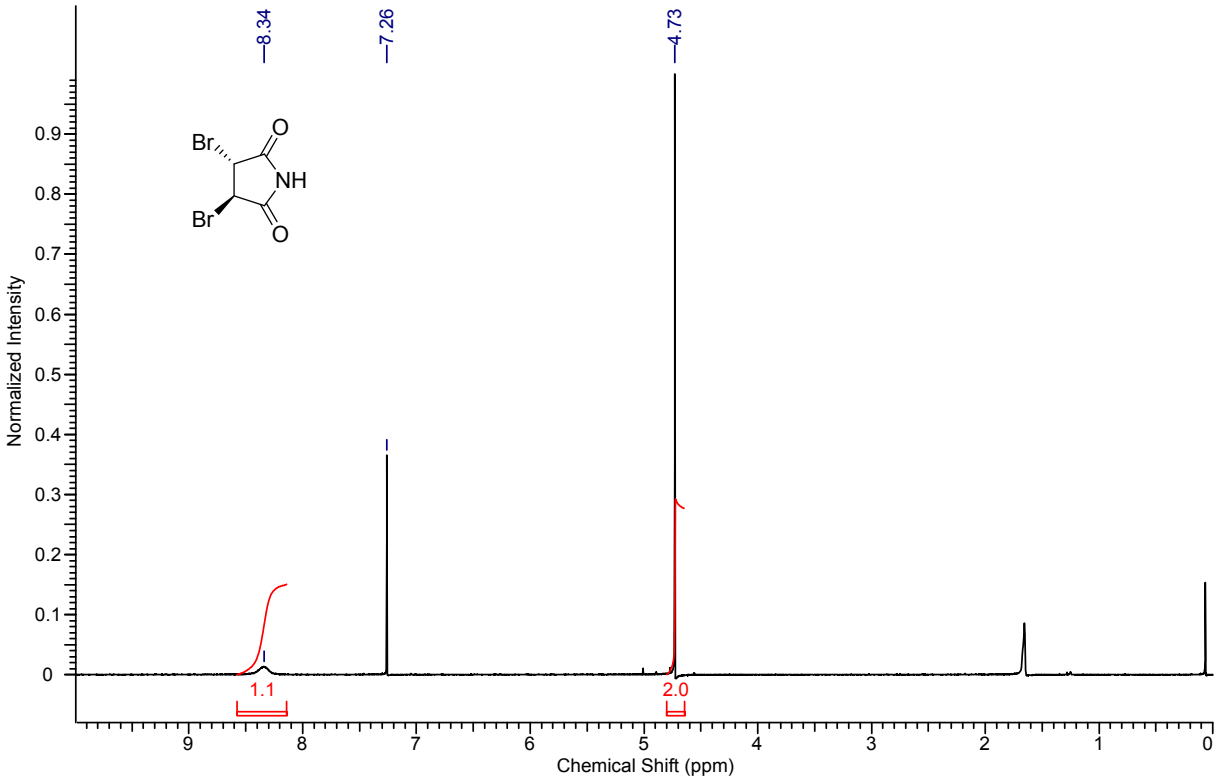


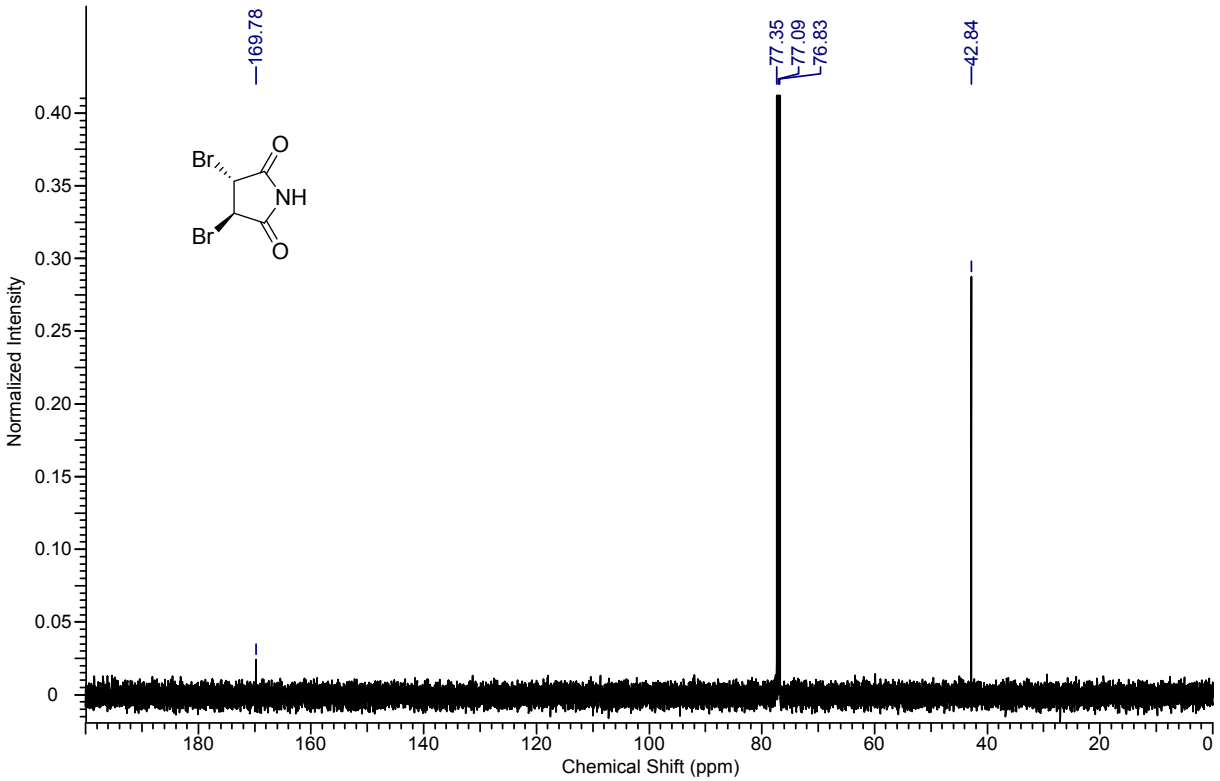
***N*-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)-4-(1-methyl-pyrrole-2,5-dione-3-yloxy)benzamide (16)**



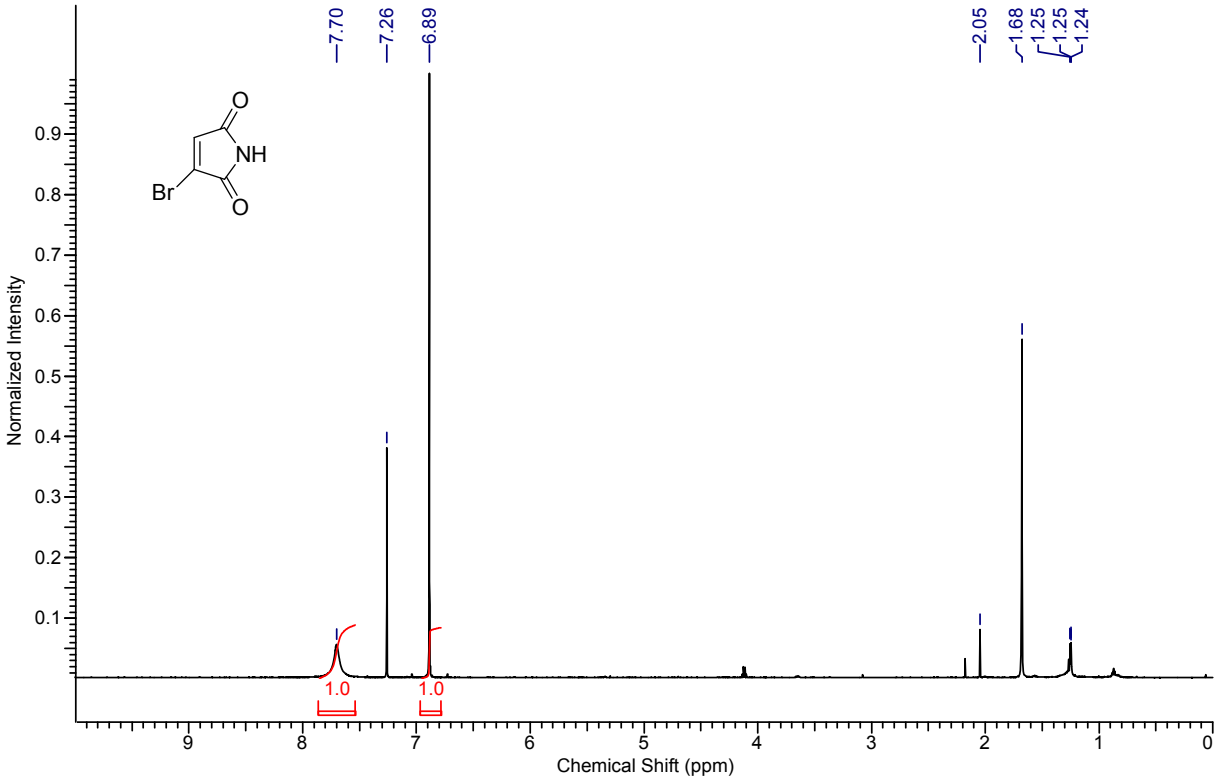


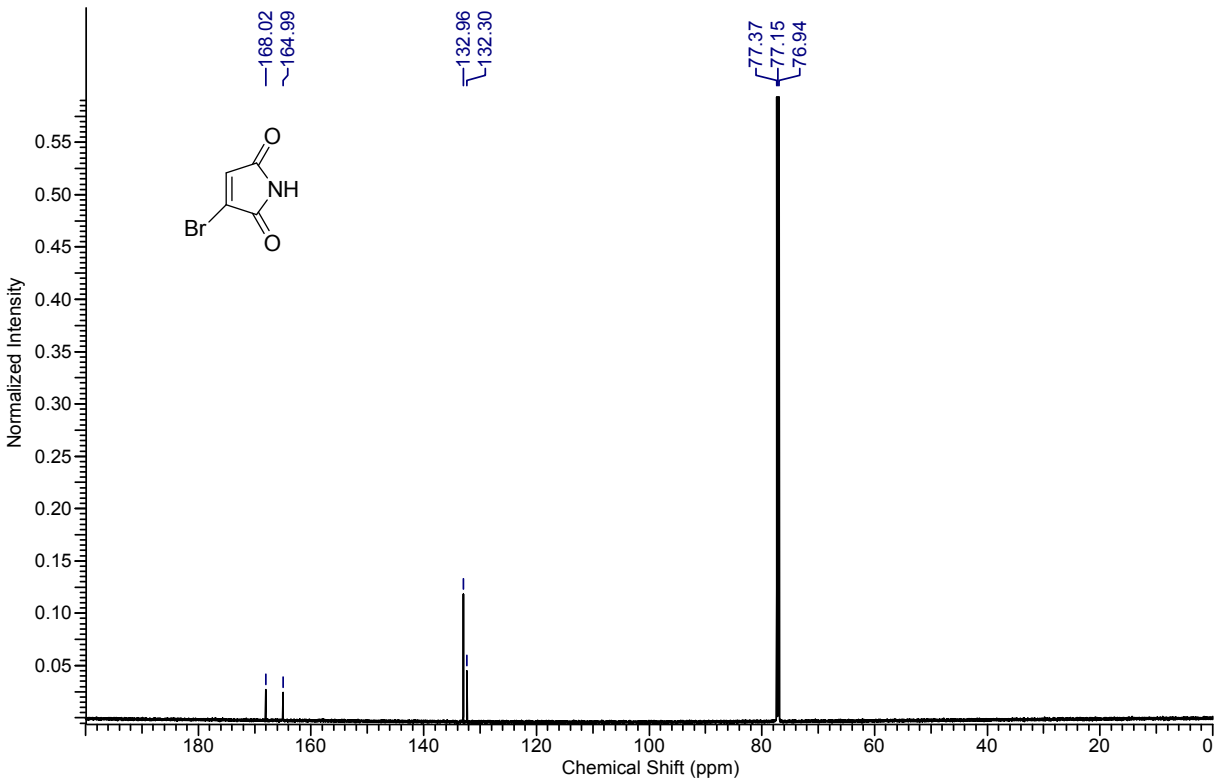
(3r, 4r)-3,4-Dibromopyrrolidine-2,5-dione (32)



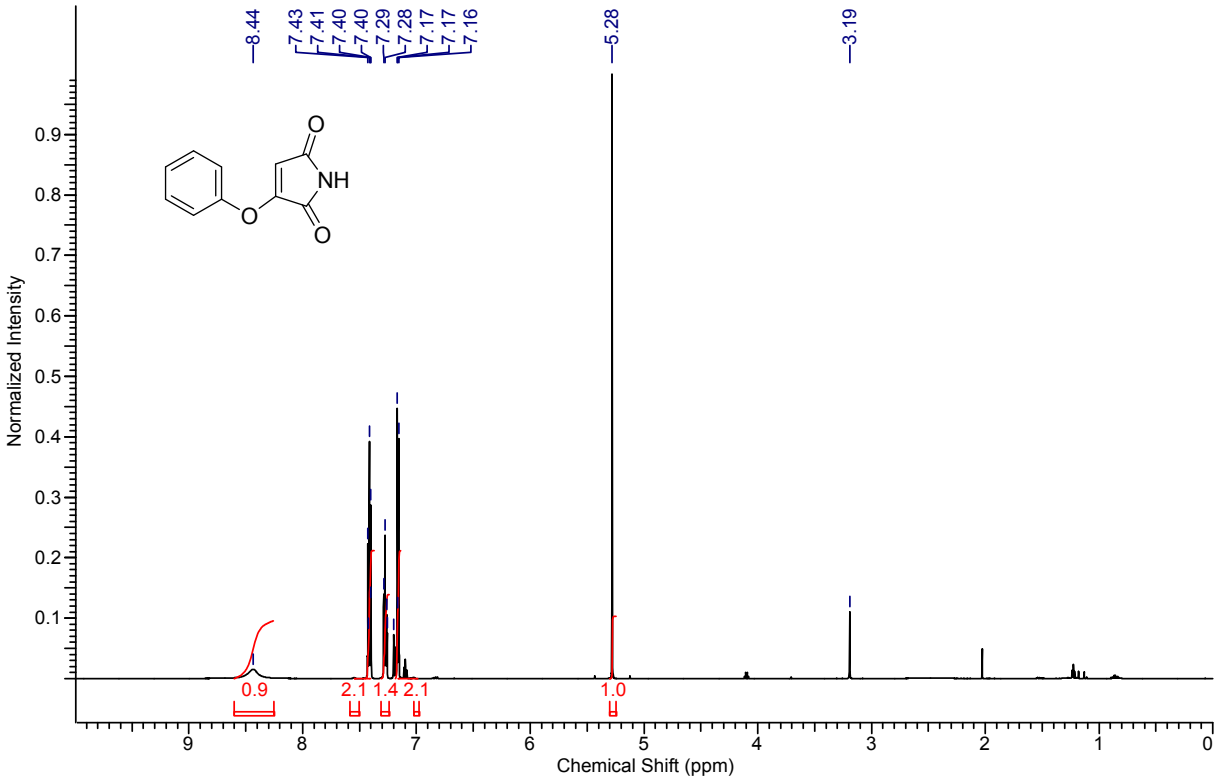


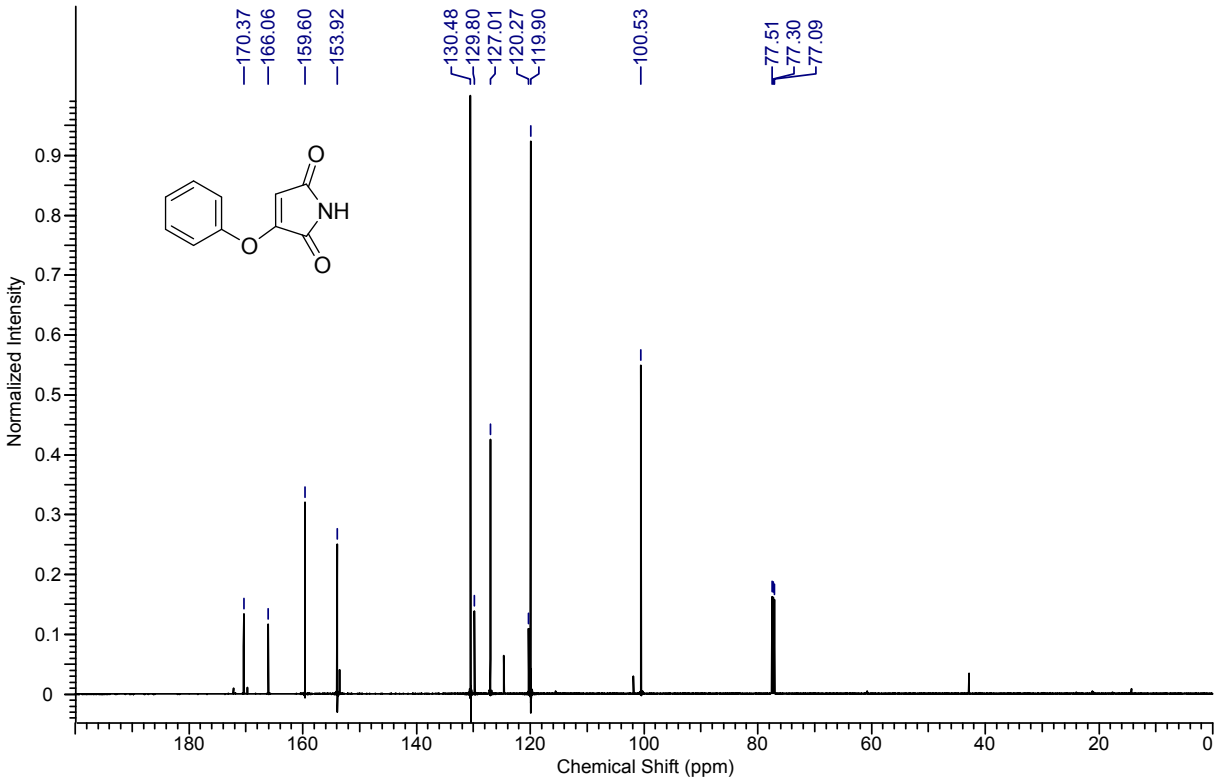
3-Bromo-pyrrole-2,5-dione (33)



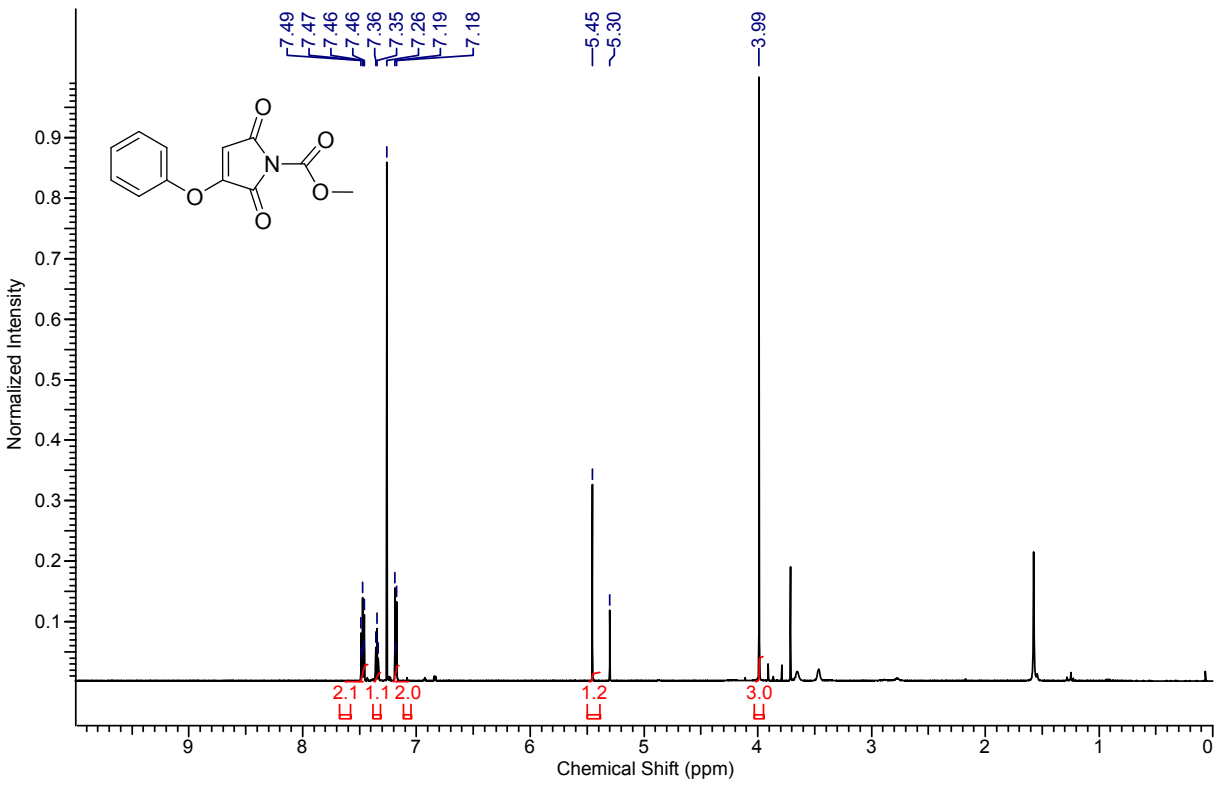


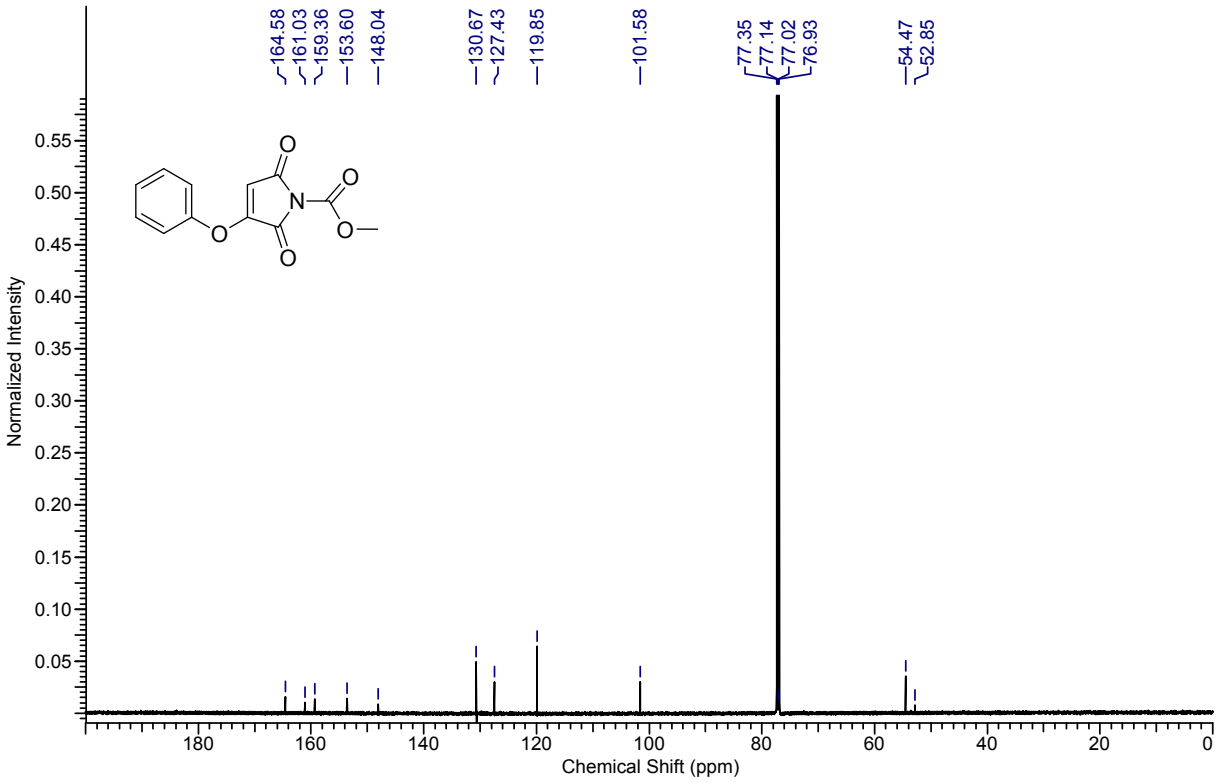
3-Phenoxy-pyrrole-2,5-dione (34)



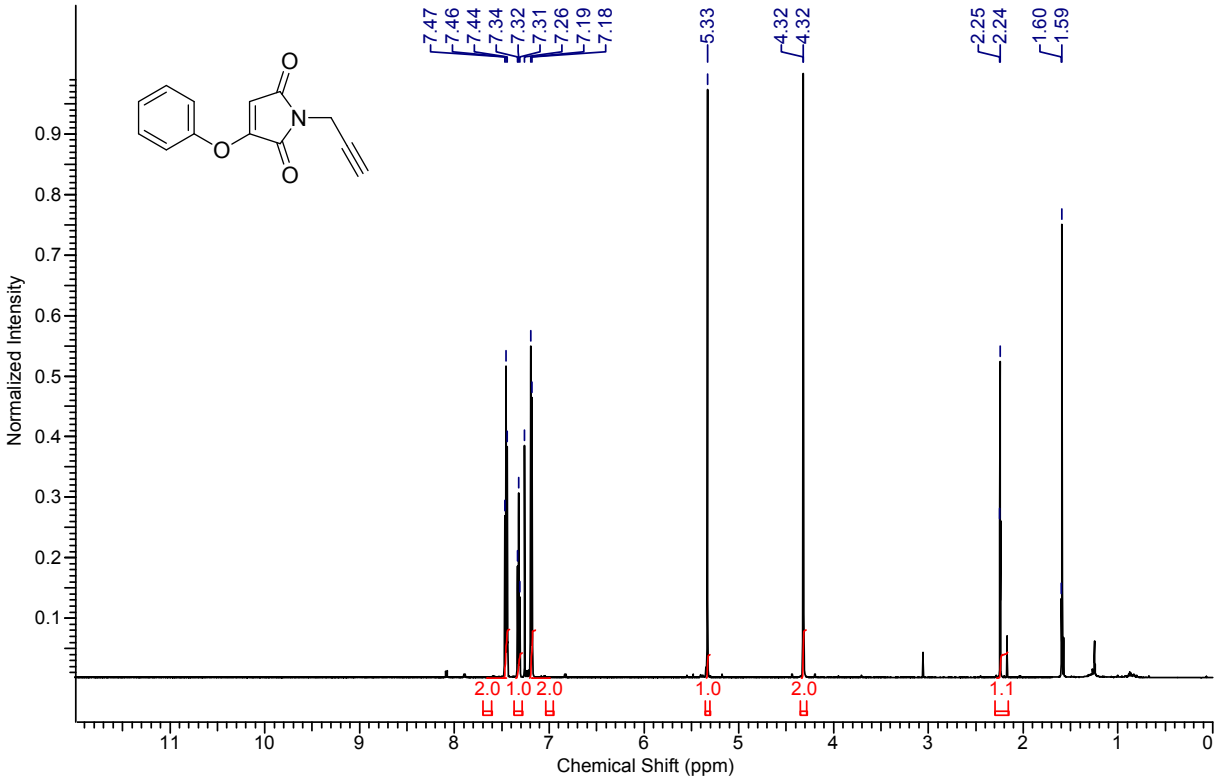


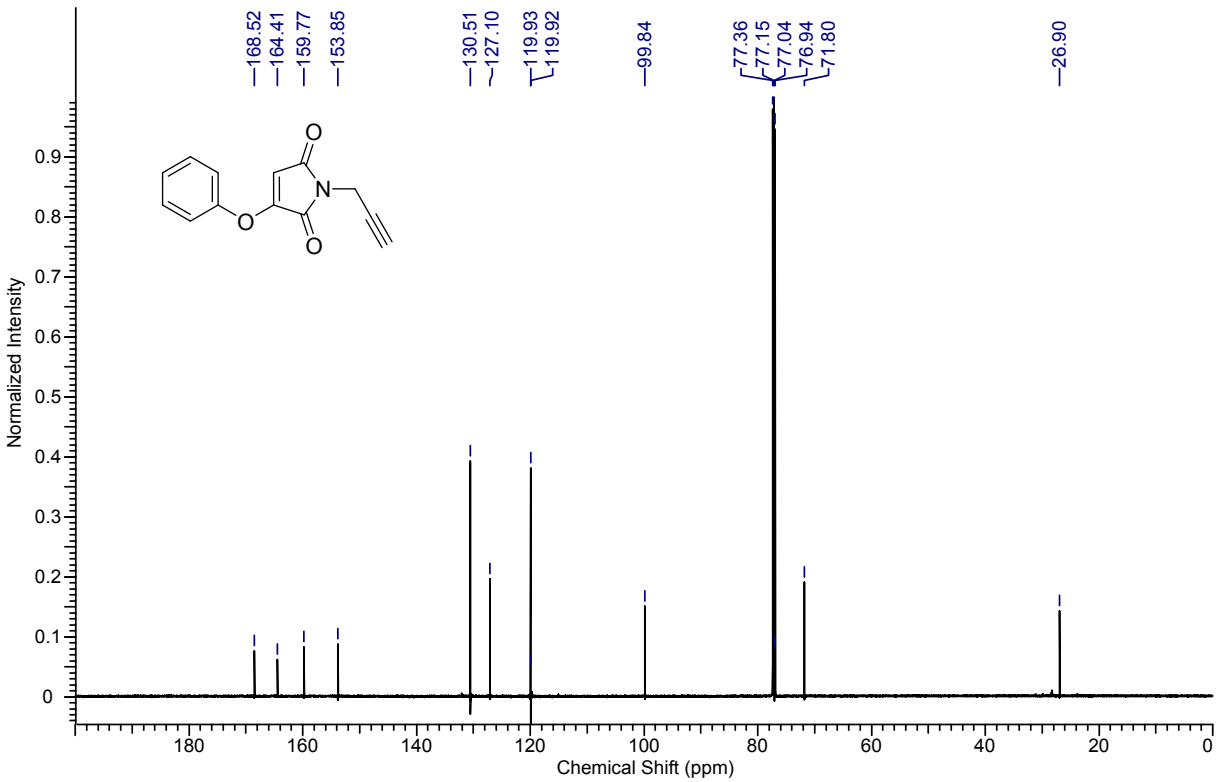
Methyl (3-phenoxy-pyrrole-2,5-dione)carboxylate (35)



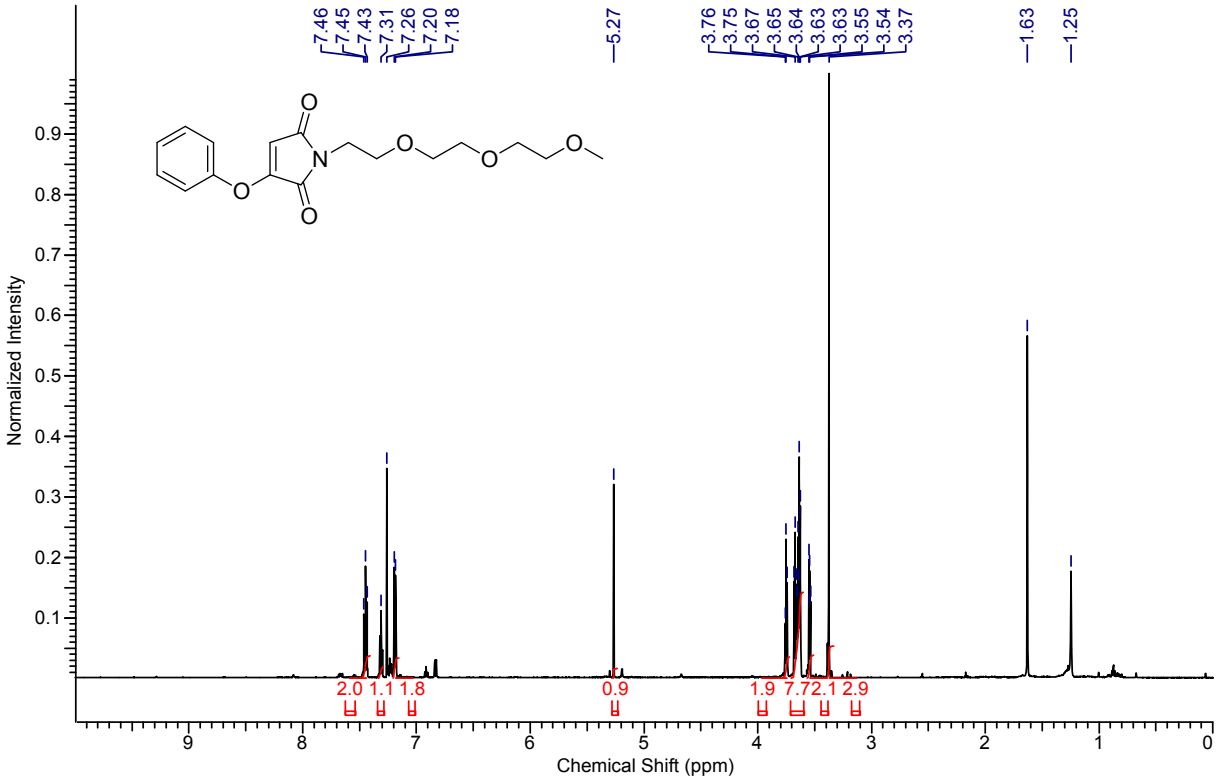


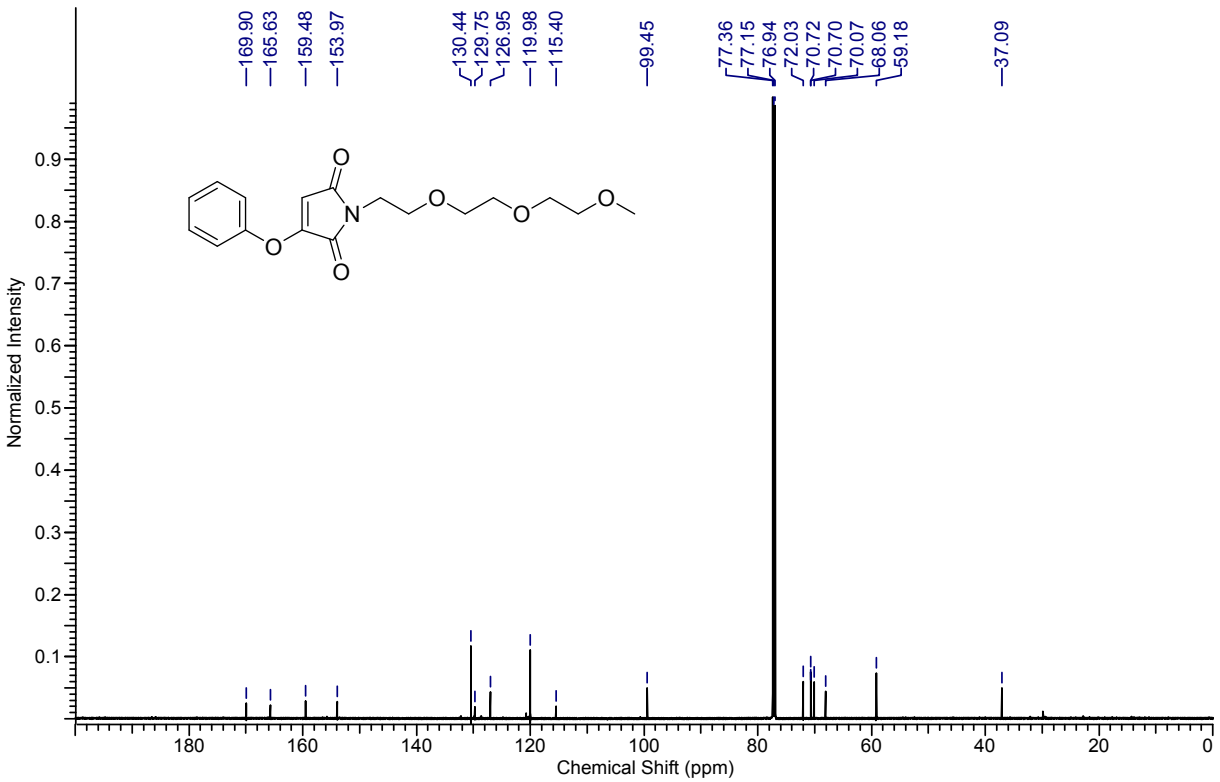
3-Phenoxy-1-(prop-2-ynyl)-pyrrole-2,5-dione (36)



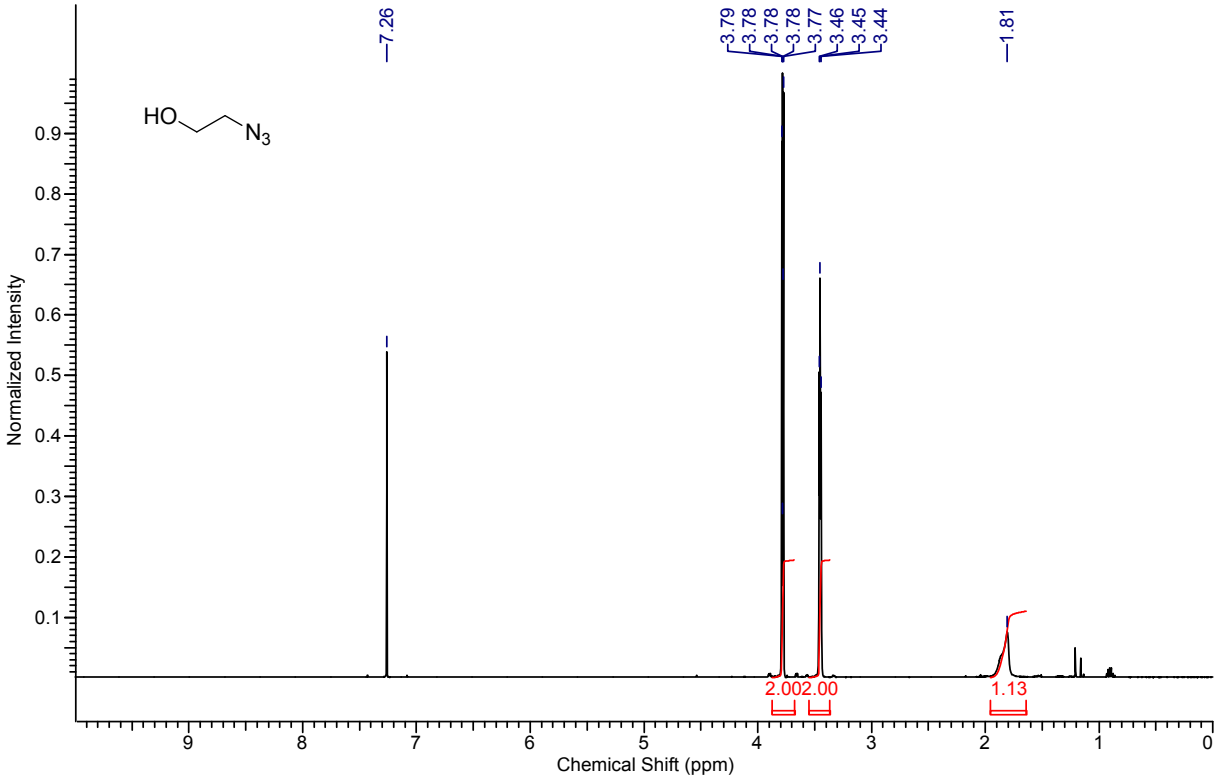


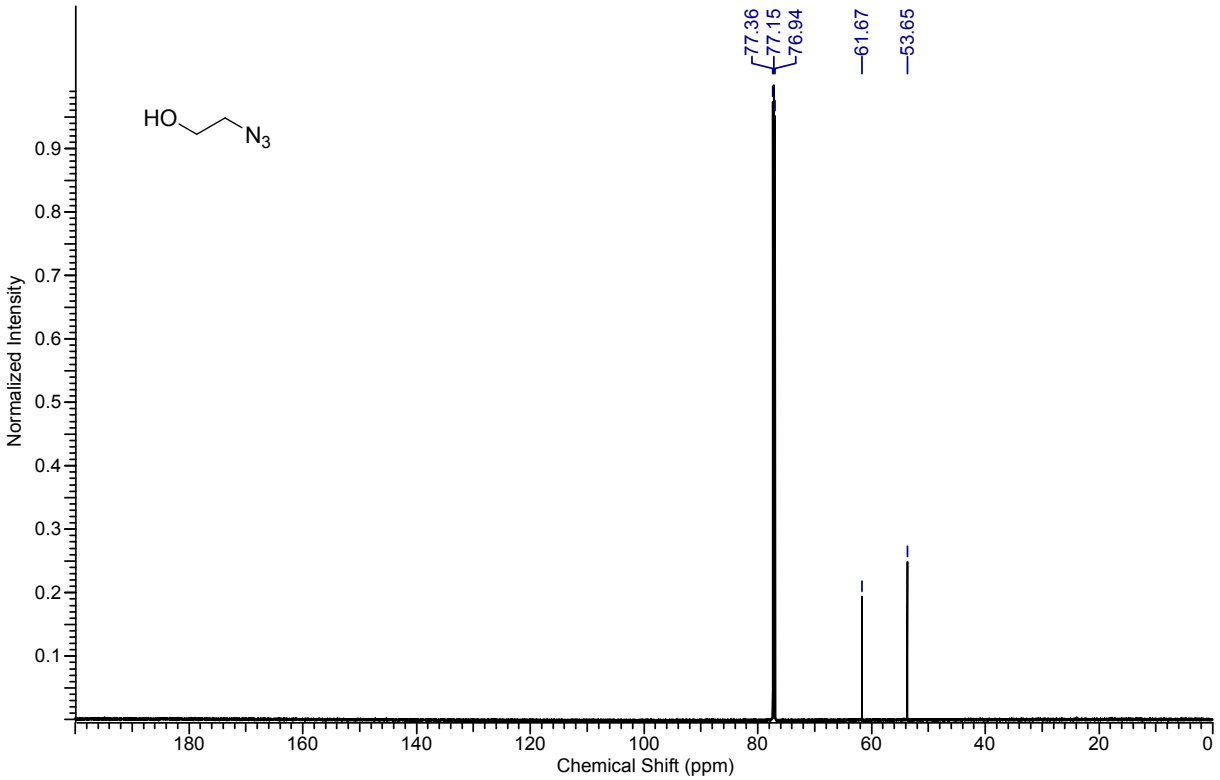
1-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)-3-phenoxy-1H-pyrrole-2,5-dione (17)



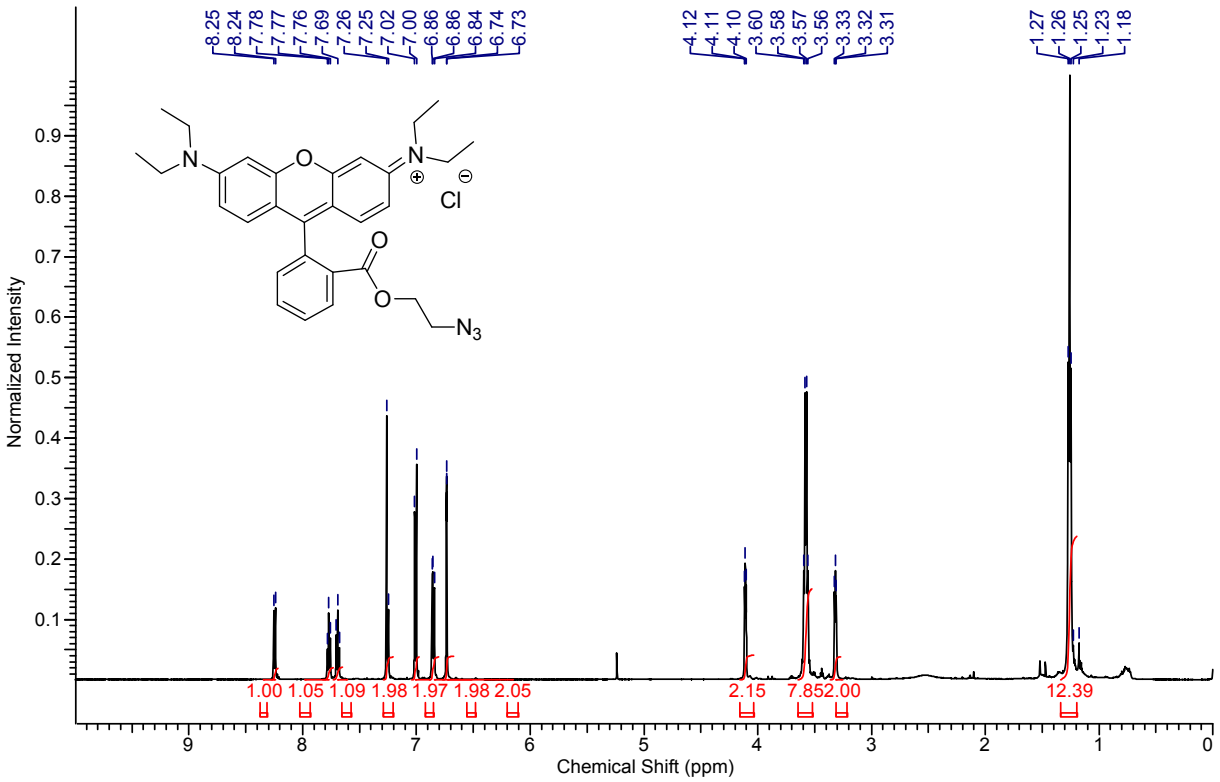


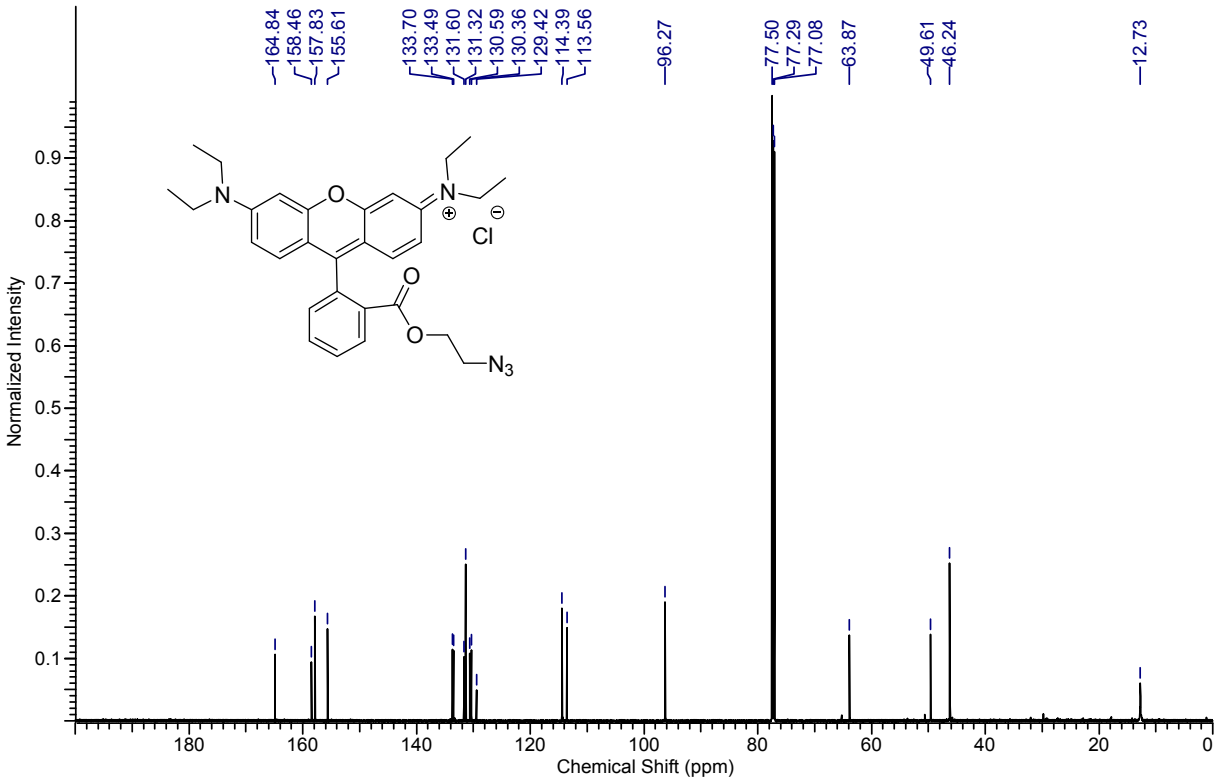
2-azidoethanol (37)



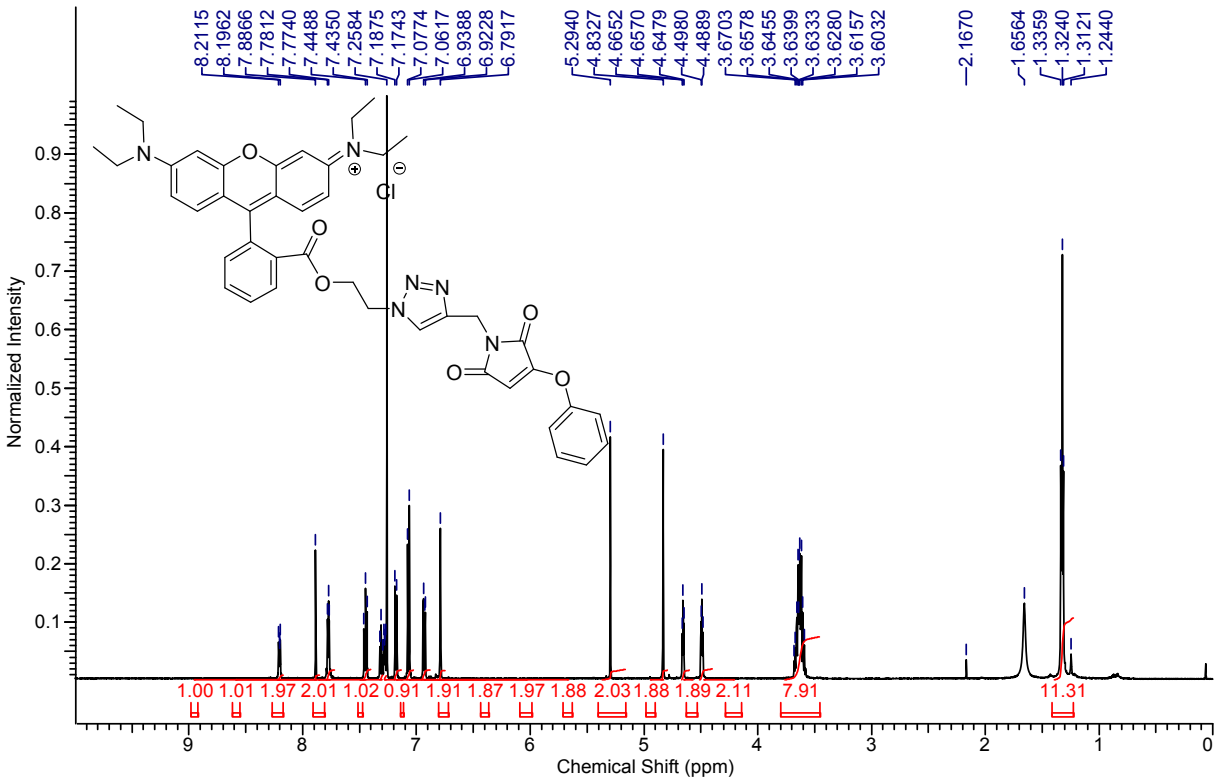


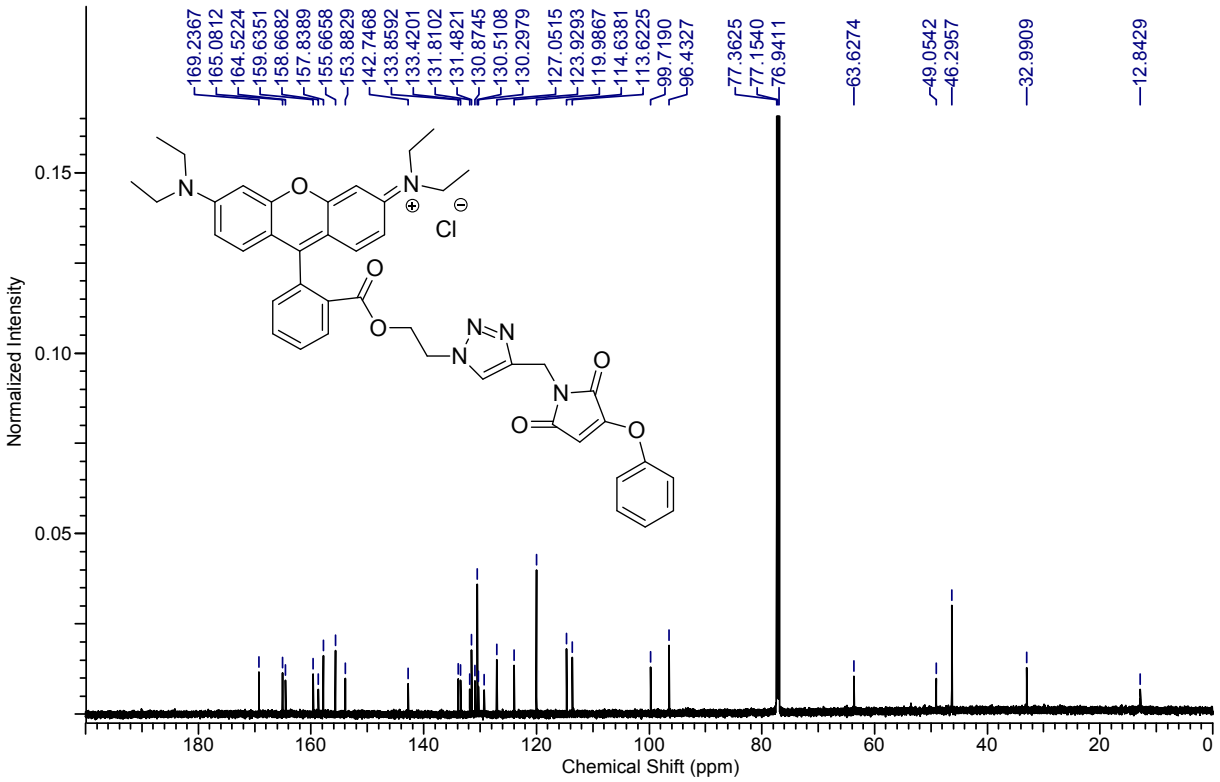
***N*-(9-(2-((2-Azidoethoxy)carbonyl)phenyl)-6-(diethylamino)-3H-xanthen-3-ylidene)-*N*-ethylethanaminium chloride (38)**



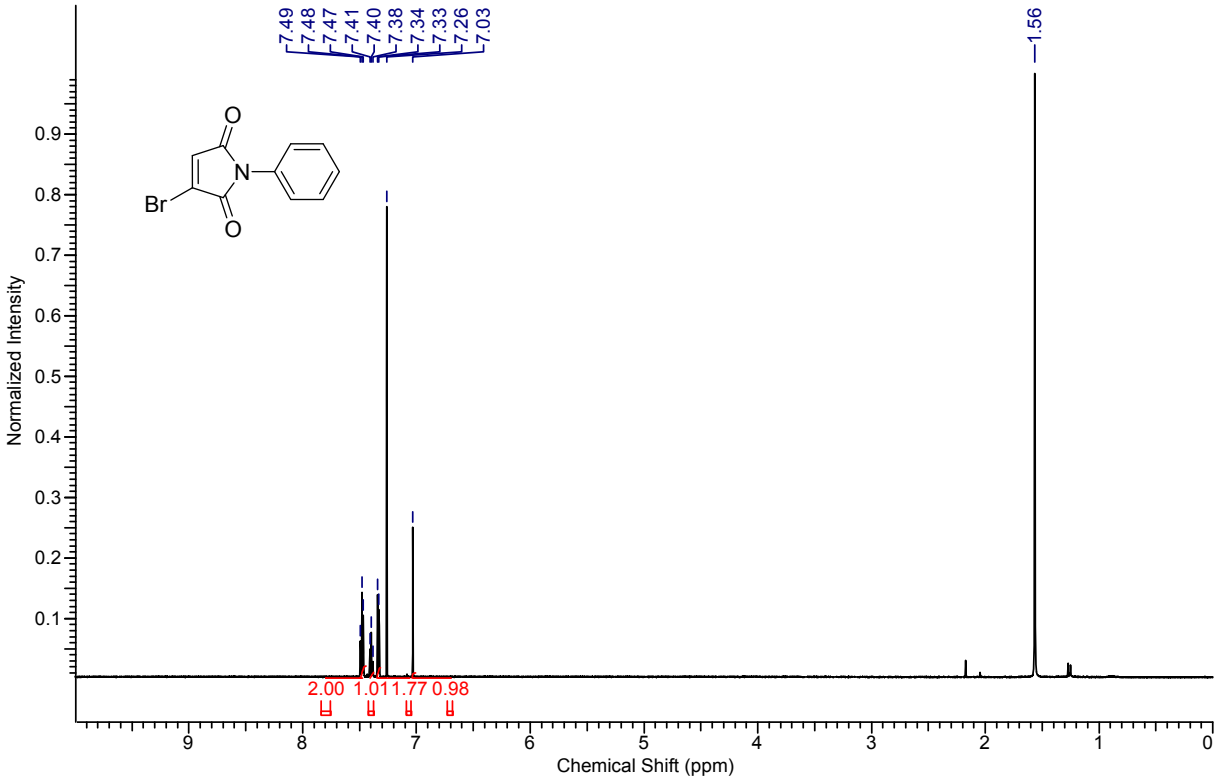


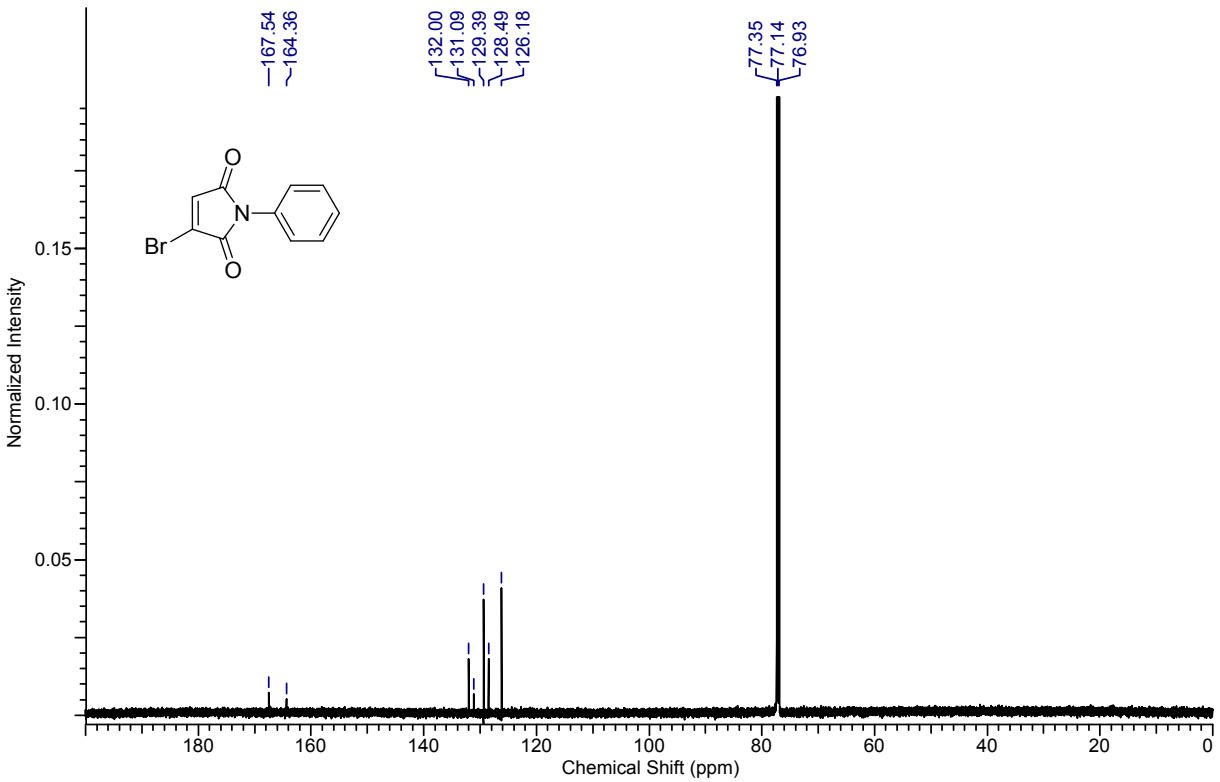
***N*-(6-(diethylamino)-9-(2-((2-(4-((2,5-dioxo-3-phenoxy-2,5-dihydro-1H-pyrrol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)carbonyl)phenyl)-3H-xanthen-3-ylidene)-*N*-ethylethanaminium (18)**



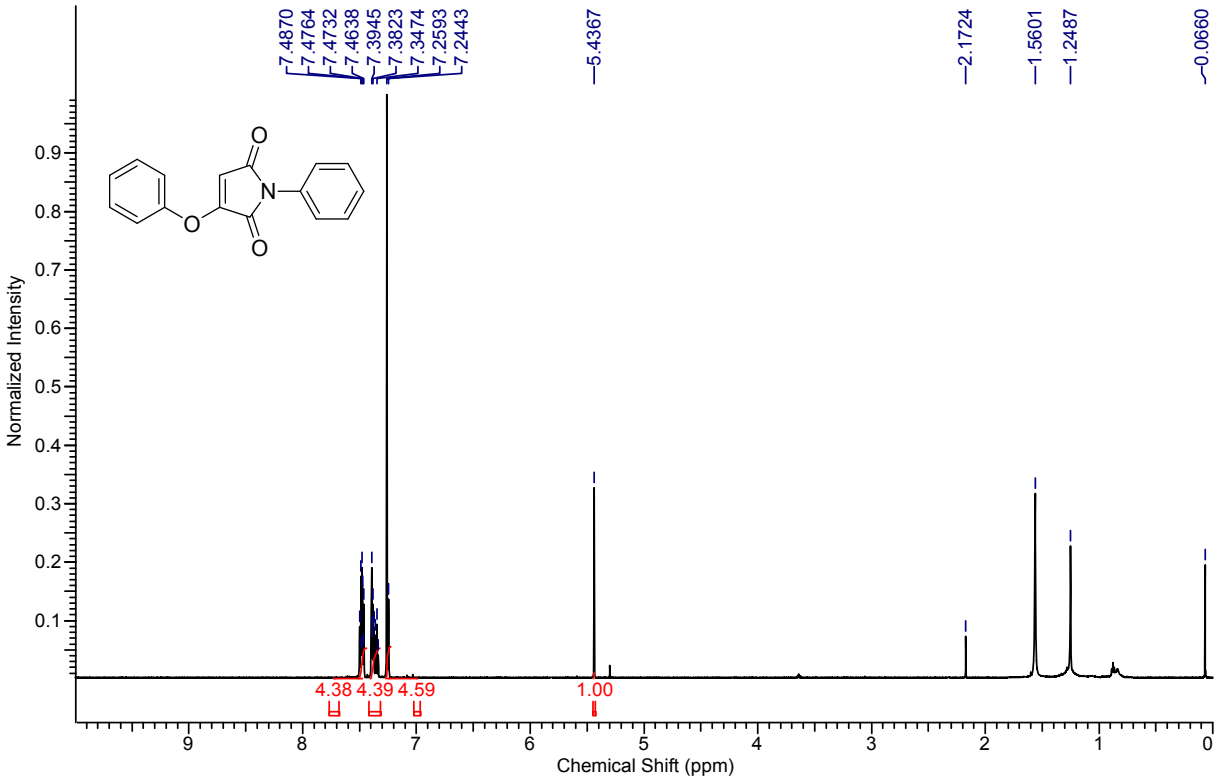


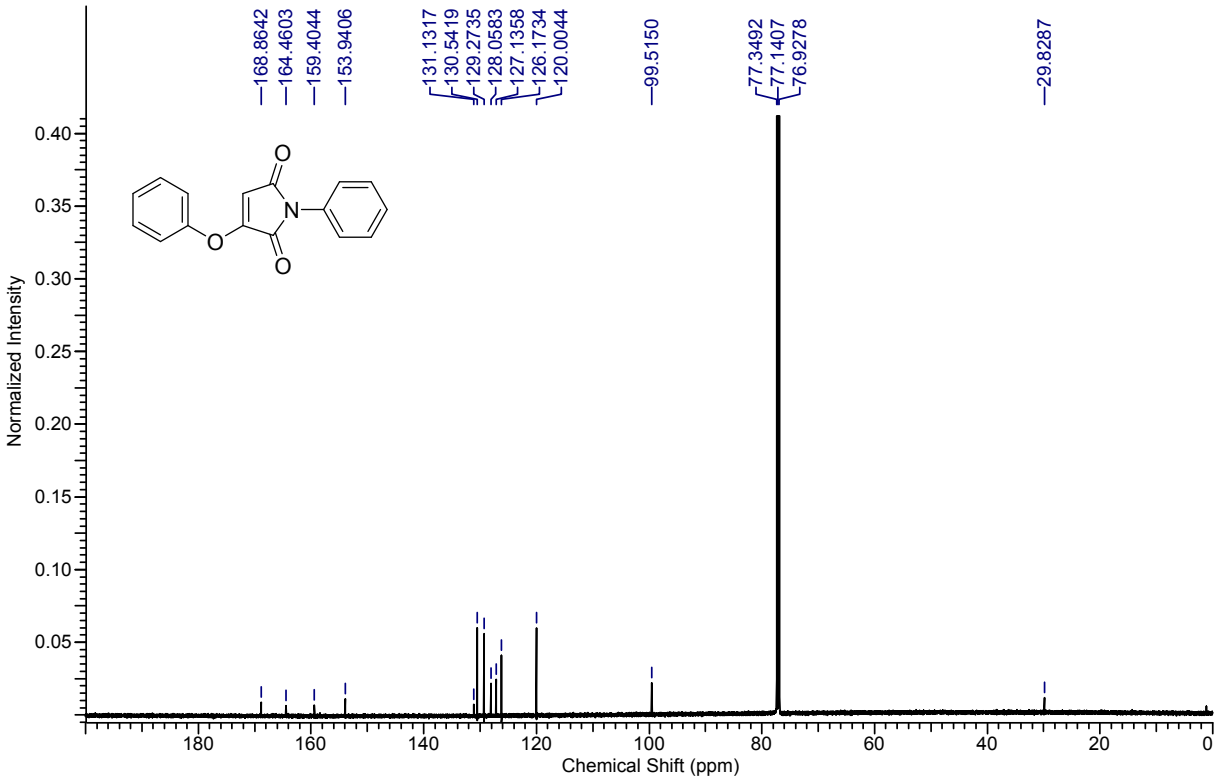
3-bromo-1-phenyl-pyrrole-2,5-dione (39)



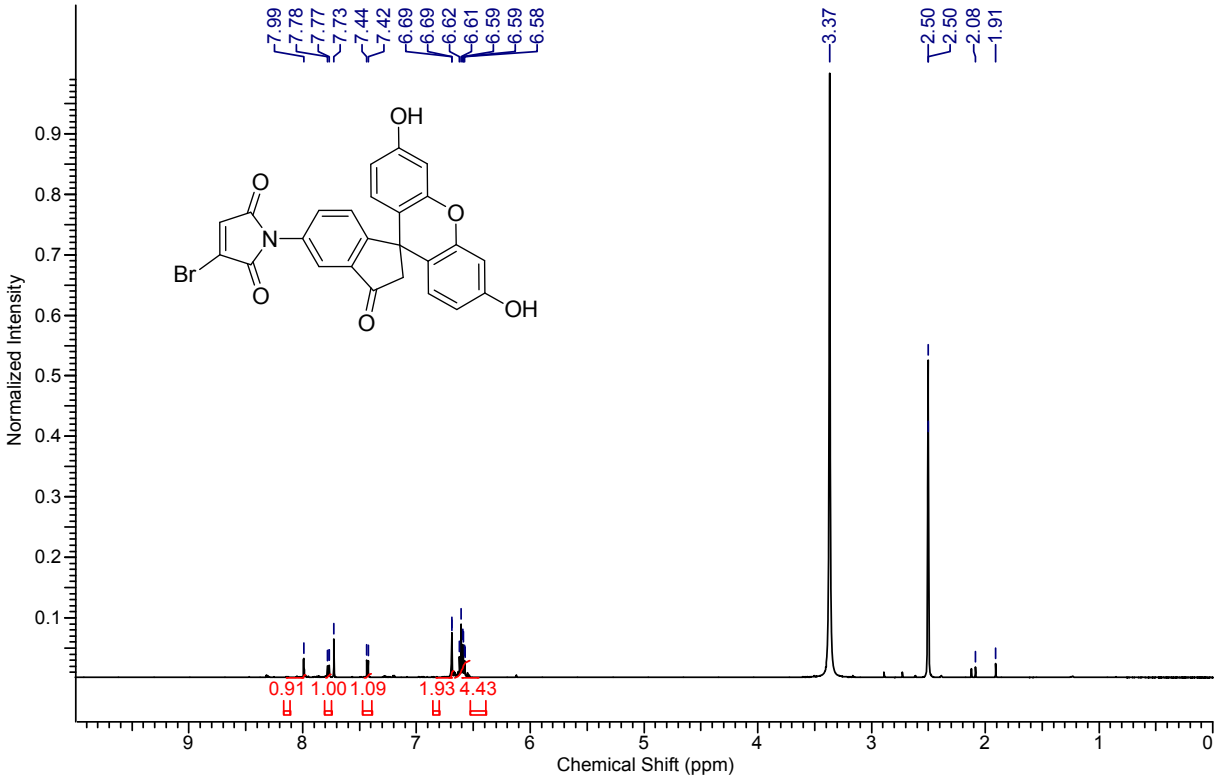


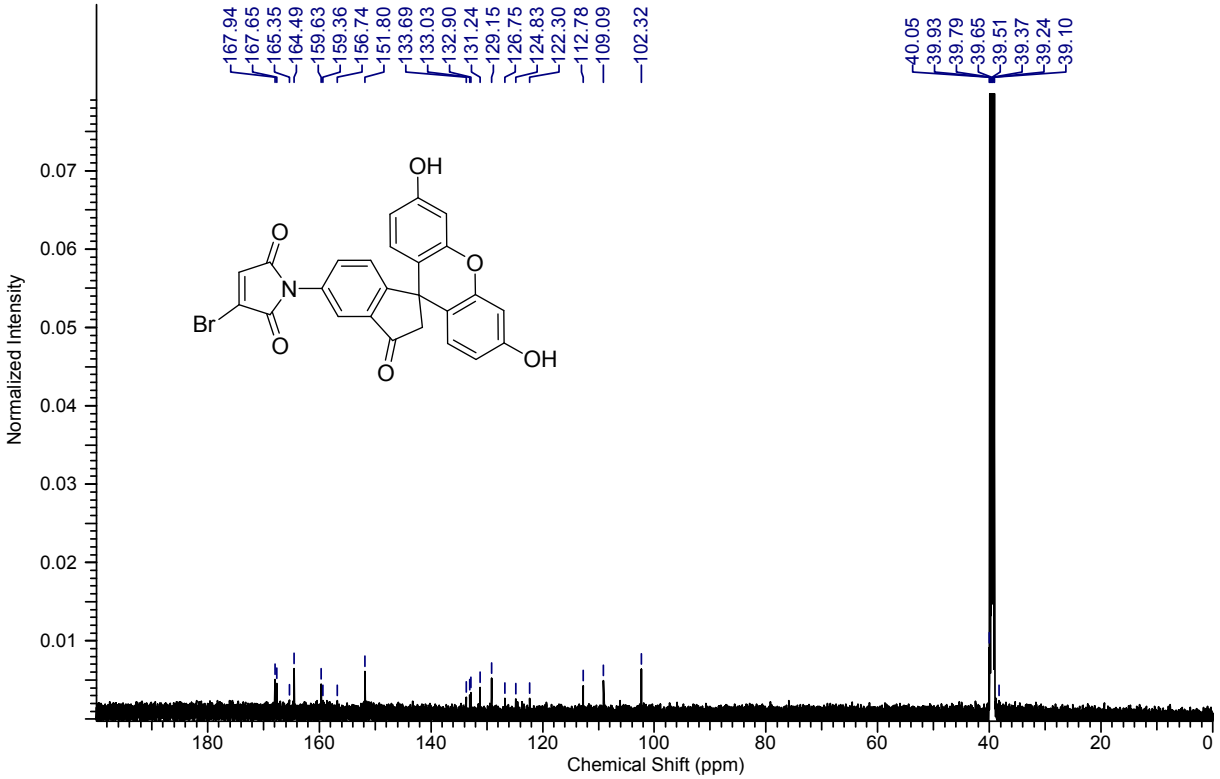
3-Phenoxy-1-phenyl-pyrrole-2,5-dione (40)



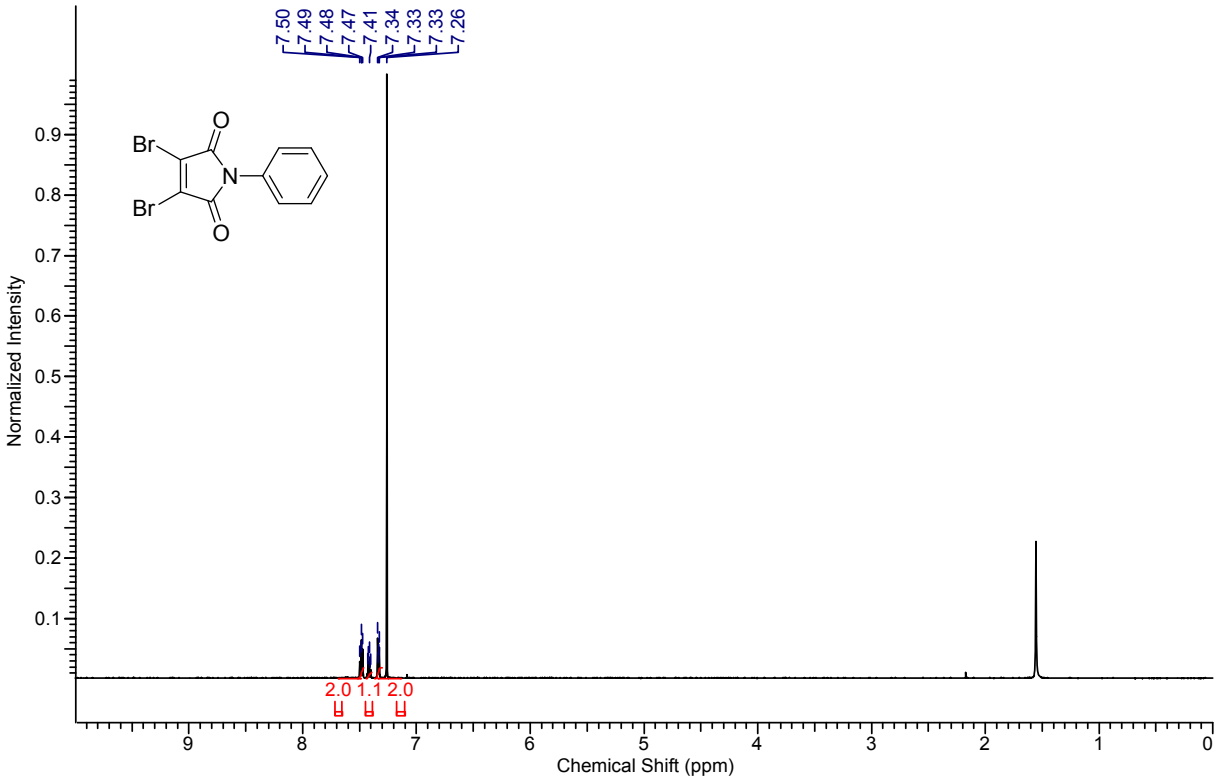


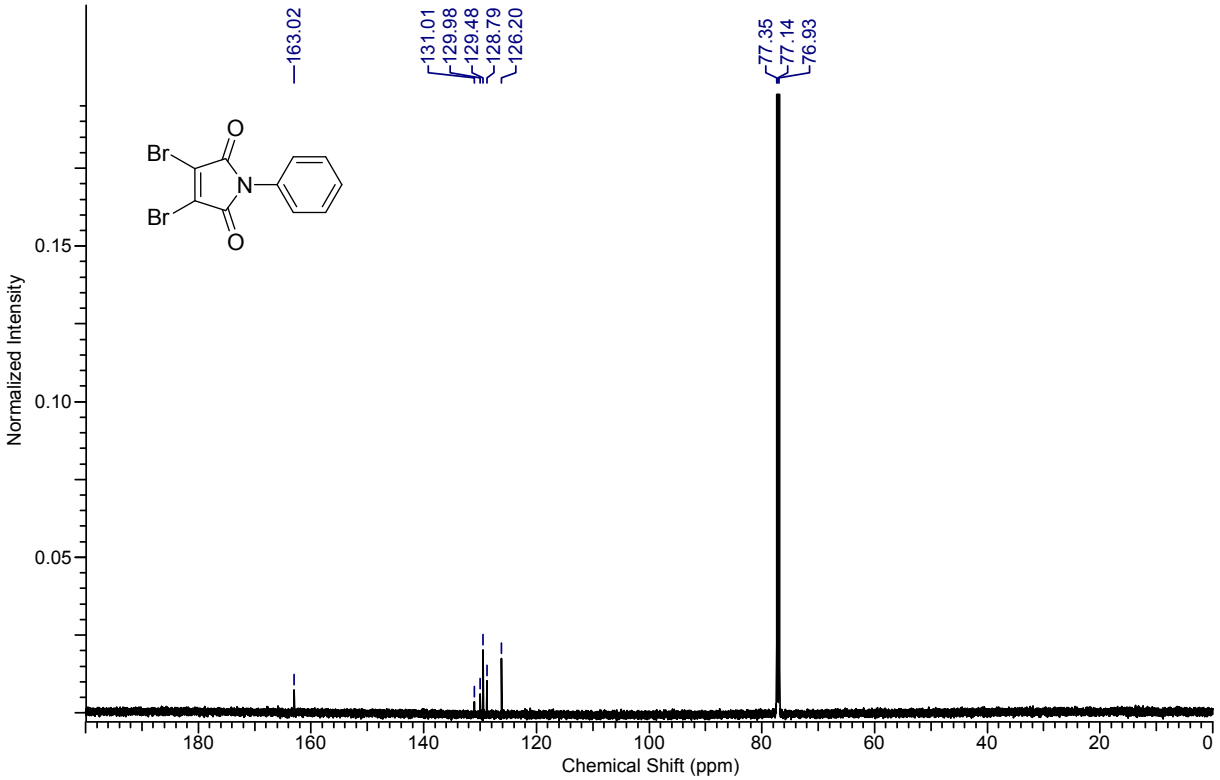
3-Bromo-1-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-5-yl)-pyrrole-2,5-dione (41)





3,4-Dibromo-1-phenyl-pyrrole-2,5-dione (42)





VI. Protein modification

VI.1 Modification of Grb2 (L111C) SH2

Reaction profile experiments of substituted maleimides 5 - 8 with Grb2 (L111C) SH2

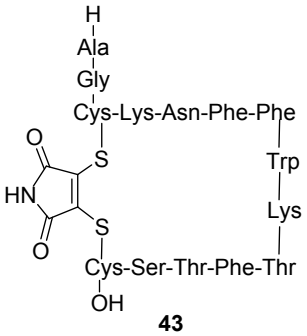
To a solution of Grb2 (L111C) SH2 **9** (100 µL, 70 µM, 100 mM sodium phosphate buffer, 150 mM NaCl, pH 8.0) at 0 °C was added the relevant substituted maleimide **5 - 8** (1 equiv., 5 µL from a 1.41 mM stock solution in dimethylformamide). The mixture was maintained on ice. Aliquots were taken at various time points and immediately analysed by LC-MS. The reaction progress was estimated based on the ratio of the MS peak heights corresponding to the native protein **9** and the protein conjugate **10**.

Modification of Grb2 (L111C) SH2 with excess of aryloxymaleimide 6

To a solution of Grb2 (L111C) SH2 **9** (100 µL, 70 µM, 100 mM sodium phosphate buffer, 150 mM NaCl, pH 8.0) at 0 °C was added aryloxymaleimide **6** (10 equiv., 5 µL from a 14.1 mM stock solution in dimethylformamide). The mixture was maintained on ice. After 10 min, an aliquot was taken and immediately analysed by LC-MS to show quantitative conversion to protein conjugate **10**.

VI.2 Modification of somatostatin

Reduction of somatostatin



43

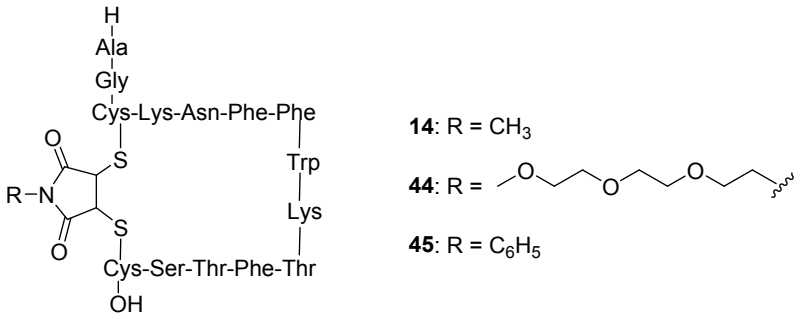
A solution of somatostatin **11*** (200 µM, 50 mM sodium phosphate buffer, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide) was reduced with TCEP (1.5 eq., 20 mM stock solution in the same buffer) for 1 h at room temperature. After this time, an aliquot (30 µL, 200 µM, 50 mM sodium phosphate buffer, pH 6.4) was taken and 2,3-dibromomaleimide (3 µL, 10 eq., from a 20 mM stock solution in DMF) was added. The reaction mixture was left to stand at room temperature for 1 min. Completion of the reduction was then checked by LC-MS, based on the disappearance of the MS peak corresponding to the native peptide **11** and appearance of the peak corresponding to the peptide conjugate **43**.

*Reactions were performed starting from 50 - 700 µL somatostatin solution.

Comparison between aryloxymaleimide 6 and bromomaleimide 5

A solution of somatostatin **11** (200 µL, 200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide) was reduced with TCEP (3 µL, 1.5 eq., from a 20 mM stock solution in the same buffer) for 1 h at room temperature. Completion of the reduction step was checked as described above. The reduced somatostatin solution was divided in two equal volumes and then treated with bromomaleimide **5** (1.5 eq., from a 20 mM stock solution in DMF) or aryloxymaleimide **6** (1.5 eq., from a 20 mM stock solution in DMF). The two reaction mixtures were left to stand at room temperature for 1 h and then analysed by LC-MS, based on the heights of the MS peaks corresponding to the peptide adducts. In the case of the reaction involving bromomaleimide **5**, a mixture of peptide conjugates **14** and **15** was obtained while in the case of the reaction involving aryloxymaleimide **6**, peptide conjugate **14** was obtained as a single product.

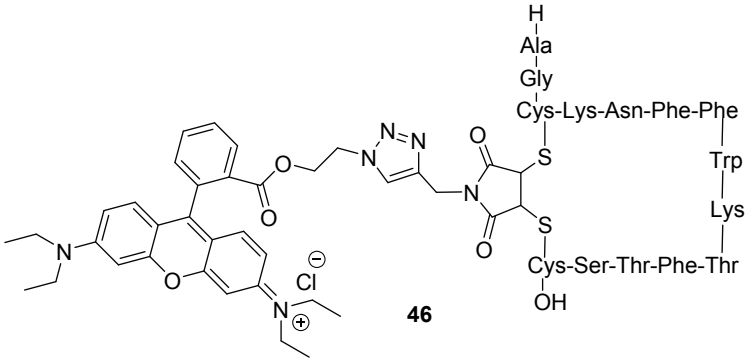
Stepwise bridging of somatostatin with aryloxymaleimides 6, 17 and 40



A solution of somatostatin **11*** (200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide) was reduced with TCEP (1.5 equiv., 20 mM stock solution in the same buffer) for 1 h at room temperature. The completion of the reduction step was checked as described above. The reduced somatostatin solution was then treated with aryloxymaleimides **6** or **17** or **40** (1.5 equiv., from a stock solution of 20 mM in dimethylformamide) and the reaction left to stand at room temperature for 1 h. After this time an aliquot was taken and analysed by LC-MS to show full conversion to the corresponding peptide conjugate**: 14** in the case of **6**, **44** in the case of **17** and **45** in the case of **40**.

*Reactions were performed starting from 50, 100, 300 and 700 µL of peptide solution.

Stepwise bridging of somatostatin with aryloxymaleimide 18



46

A solution of somatostatin **11** (100 µL, 200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide) was reduced with TCEP (1.2 equiv., 1.2 µL from a stock solution of 20 mM in the same buffer) and then aryloxymaleimide **18** (1.7 equiv., 1.7 µL from a 20 mM stock solution in dimethylformamide) was added. The reaction mixture was left at room temperature for 30 min and then another portion of TCEP (0.6 equiv., 0.6 µL from a 20 mM stock solution in the same buffer) was added. Quantitative conversion to the peptide conjugate **46** after another 30 min of incubation at room temperature was confirmed by LC-MS.

General procedure for the *in situ* bridging of somatostatin with aryloxymaleimides 6 and 17

A solution of somatostatin **11*** (200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide) was mixed with the relevant aryloxymaleimides **6** or **17** (1.5 equiv., from a 20 mM stock solution in dimethylformamide). To the resulting mixture was added TCEP (1.5 equiv., from a 20 mM stock solution in the same buffer) and the reaction mixture was left to stand at room temperature for 2 h. After this time an aliquot was taken and analysed by LC-MS to show quantitative conversion to the corresponding peptide conjugates: **14** in the case of **6** and **44** in the case of **17**.

*Reactions were performed starting from 50, 100 and 300 µL of somatostatin solution.

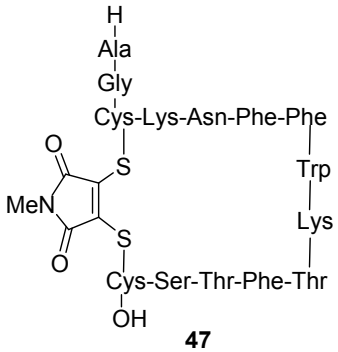
Organic solvent free procedure for the *in situ* bridging of somatostatin with aryloxymaleimide 16

A solution of somatostatin **11** (100 µL, 200 µM, 50 mM sodium phosphate, pH 6.4) was mixed with aryloxymaleimide **16** (1.5 equiv., 3 µL from a 10 mM stock solution in the same buffer). To the resulting mixture was added TCEP (1.5 eq, 1.5 µL from a 20 mM stock solution in the same buffer) and the reaction mixture was left to stand at room temperature. After 4 h, an aliquot was taken and analysed by LC-MS to show quantitative conversion to the corresponding peptide conjugate **14**.

Reaction of succinimide bridged somatostatin conjugate 14 with maleimide

Succinimide bridged somatostatin conjugate **14** (100 µL, 200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide), prepared as described above, was treated with maleimide (1. equiv., 1 µL from a 20 mM stock solution in dimethylformamide) and the reaction mixture was left to stand at room temperature. After 5 min an aliquot was taken and analysed by LC-MS to show that no reaction had occurred.

Stepwise bridging of somatostatin with *N*-methyl 2,3-dibromomaleimide



47

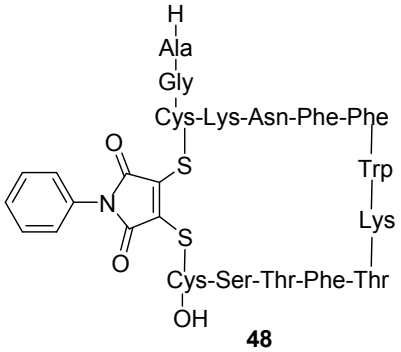
A solution of somatostatin **11** (200 µL, 200 µM, 50 mM sodium phosphate buffer, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide) was reduced with TCEP (1.5 equiv., 20 mM stock solution in the same buffer) for 1 h at room temperature. To confirm the completion of the reduction, a somatostatin solution aliquot (20 µL) was taken and mixed with 2,3-dibromomaleimide (10 equiv., 2 µL from a 20 mM stock solution in dimethylformamide). Quantitative insertion of the maleimide into the reduced disulfide bond to give the bridged adduct **43** was confirmed by LC-MS. The reduced somatostatin solution was then treated with the relevant *N*-methyl 2,3-dibromomaleimide (2 equiv., from a stock solution of 20 mM in dimethylformamide) and the reaction left to stand at room temperature. After 1 h an aliquot was taken and analysed by LC-MS to show quantitative conversion to peptide conjugate **47**.

Stability of somatostatin conjugates 14 and 47 under cytoplasm mimicking conditions

Somatostatin conjugates **14** and **47**, prepared as described above, were dialysed into 20 mM HEPES buffer (100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, pH 7.4). The concentration of the peptide conjugates was adjusted to 100 µM and the resulting reaction mixtures were incubated at 37 ^{o}C for 21 h in the presence of 1 mM reduced glutathione (from a 20 mM stock solution in the same buffer). Aliquots from the two reaction mixtures were taken at regular intervals and analysed by LC-MS (**SI Fig. 1**).

After 21 h, there was 0% maleimide bridged somatostatin **47** and 27% succinimide bridged somatostatin **14**.

Stepwise bridging of somatostatin with *N*-phenyl 2,3-dibromomaleimide 42



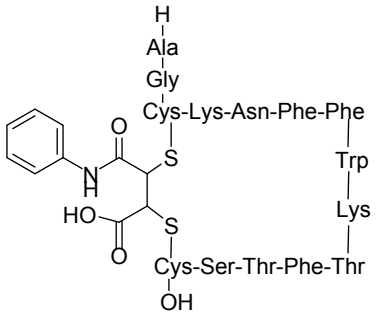
48

A solution of somatostatin (200 µL, 200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide) was reduced with TCEP (1.5 equiv., 20 mM stock solution in the same buffer) for 1 h at room temperature. To confirm the completion of the reduction, a somatostatin solution aliquot was taken and mixed with 2,3-dibromomaleimide (10 equiv., 20 mM stock solution in dimethylformamide). Quantitative insertion of the maleimide into the reduced disulfide bond to give the bridged adduct **43** was confirmed by LC-MS. The reduced somatostatin solution was then treated with *N*-phenyl 2,3-dibromomaleimides (2 equiv., 2 µL from a stock solution of 20 mM in dimethylformamide) and the reaction left to stand at room temperature. After 1 h, an aliquot was taken and analysed by LC-MS to show quantitative conversion to peptide conjugate **48**.

Hydrolytic stability of somatostatin conjugates 45 and 48

Somatostatin conjugates **45** and **48** (200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide), prepared as described above, were dialysed (Slide-A-Lyzer MINI Dialysis Devices, 2K MWCO) into pH 8 buffer (50 mM sodium phosphate) for 12 h at 0 ^{o}C. The concentration of the peptide conjugates was adjusted to 100 µM and the resulting reaction mixtures were incubated at 37 ^{o}C for 1 h. Aliquots were taken from the reaction mixtures immediately after dialysis and after 1 h of heating and were analysed by LC-MS to reveal the corresponding peptide adducts. The extent of the hydrolysis was estimated based on the ratio of the MS peak heights corresponding to the hydrolysed protein conjugate and the starting peptide conjugate (**SI Fig. 2**).

Thiol stability of hydrolysed somatostatin conjugate 49



49

Somatostatin conjugate **45** (200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide), prepared as described above, was dialysed (Slide-A-Lyzer MINI Dialysis Devices, 2K MWCO) into pH 8 buffer (50 mM sodium phosphate) for 12 h at 0 °C. The concentration of the peptide conjugate was adjusted to 100 µM and the resulting solution was incubated at 37 °C. After 16 h, an aliquot was taken and analysed by LC-MS to show complete hydrolysis to peptide conjugate **49**. The resulting reaction mixture was treated with 2-mercaptoethanol (100 equiv., 10 µL from a 100 mM stock solution in the pH 8 buffer and then incubated at 37 °C. After 21 h, an aliquot was taken and analysed by LC-MS to show that no reaction has occurred.

General procedure for the dual labelling of somatostatin

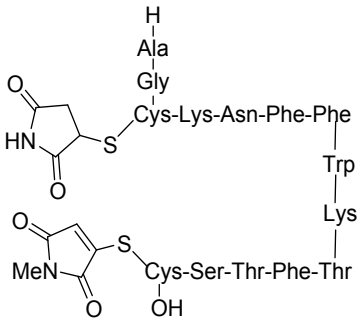
Succinimide bridged somatostatin conjugates **14**, **44** and **46** (200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide), prepared as described above, were treated with various amounts of the relevant bromomaleimide (**SI Table 1**) from 20 mM stock solution in dimethylformamide and heated at 37 °C. After 2 h an aliquot was taken and analysed by LC-MS to show quantitative conversion to the corresponding dual labelled conjugates.

*Reactions were performed starting from 100 and 250 µL of succinimide bridged somatostatin solution.

Starting material	Bromomaleimide (equiv.)	Product
14	**32** (1.5)	**22**
44	**41** (4)	**23**
46	**41** (4)	**24**
44	**5** (1.5)	**25**

SI Table 1. Amounts of reagents used for the dual labelling of somatostatin

Preparation of a dual labelled somatostatin conjugate with a cleavable and non-cleavable tag 50



50

Succinimide bridged somatostatin conjugate **14** (100 µL, 200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide), prepared as described above, was treated with maleimide (5 equiv., 5 µL from a 20 mM stock solution in dimethylformamide) and the resulting reaction mixture was left to stand at room temperature. After 12 h an aliquot was taken and analysed by LC-MS to show quantitative conversion to dual labelled conjugate **50**.

Thiol cleavage of dual labelled somatostatin conjugate 22

Dual labelled somatostatin conjugate **22** (100 µL, 200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide), prepared as described above, was treated with 2-mercaptoethanol (100 eq, 10 µL from a 200 mM stock solution in dimethylformamide) and the reaction mixture incubated at 37 ^{o}C. After 3 h an aliquot was taken and analysed by LC-MS to show quantitative conversion to reduced somatostatin **12**.

Digest of dual labelled somatostatin conjugate 25

A solution of dual labelled somatostatin conjugate **25** (200 µM, 50 mM sodium phosphate, pH 6.4, 40% acetonitrile, 2.5% dimethylformamide), prepared as described above, was dialysed (Slide-A-Lyzer MINI Dialysis Devices, 2K MWCO), 5000× dilution into pH 8.1 buffer (100 mM ammonium acetate, 1 mM calcium chloride). The concentration of the peptide conjugate was adjusted to 100 µM and to the resulting reaction mixture was added trypsin (0.1 equiv., from a 1 mM stock solution in the same buffer). The reaction mixture was incubated at 37 ^{o}C for 15 h and then the reaction mixture was analysed by LC-MS (**SI Fig. 3, 4**). Both F_1 and F_3 PEG adducts can be observed, indicating a mixture of the two possible regioisomers, together with the corresponding hydrolysed conjugates. This was sufficient evidence to support the lack of regioselectivity of the retro-Michael addition. Methylated F_1 and F_2 adducts were not observed but this is assumed to be correlated to the poor flying ability of these fragments.

VI.3 Modification of anti-CEA ds-scFv shMFE

In situ bridging of ds-scFv with aryloxymaleimide 6

To a solution of anti-CEA ds-scFv **19*** (70 µM, PBS pH 7.4) was added the aryloxymaleimide **6** (5 equiv., from a 70 mM stock solution in dimethylformamide) followed by benzeneselenol (25 equiv. from a 35 mM stock solution in dimethylformamide). The reaction mixture was left to stand at room temperature. After 30 min an aliquot was taken and analysed by LC-MS to show quantitative conversion to protein adduct **21**.

*Reactions were performed starting from 100 µL and 200 µL of protein solution.

In situ bridging of ds-scFv with pegylated aryloxymaleimide 17

To a solution of anti-CEA ds-scFv **19** (70 µM, in PBS, pH 7.4) was added the aryloxymaleimide **17** (15 equiv., from a 70 mM stock solution in dimethylformamide) followed by benzeneselenol (50 equiv., from a 35 mM stock solution in dimethylformamide). The reaction mixture was left to stand at room temperature. After 30 min an aliquot was taken and analysed by LC-MS to show quantitative conversion to protein adduct **20**.

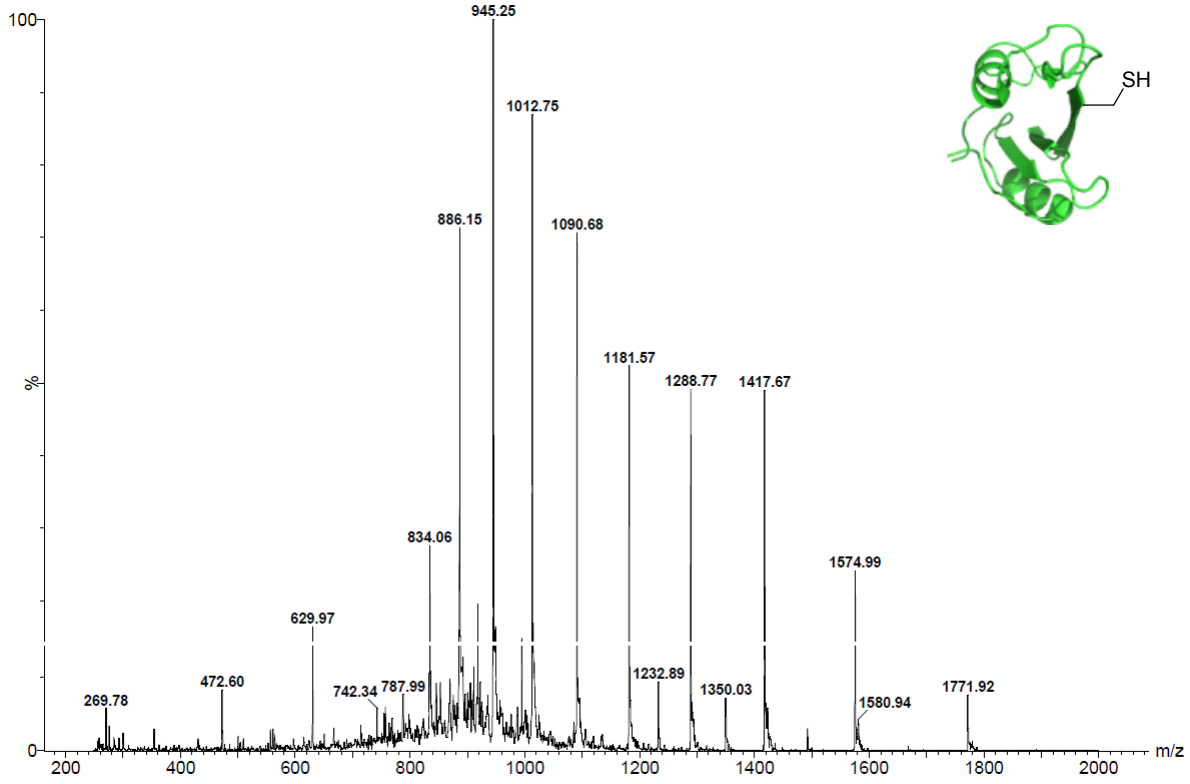
*Reactions were performed starting from 100 µL and 200 µL of protein solution.

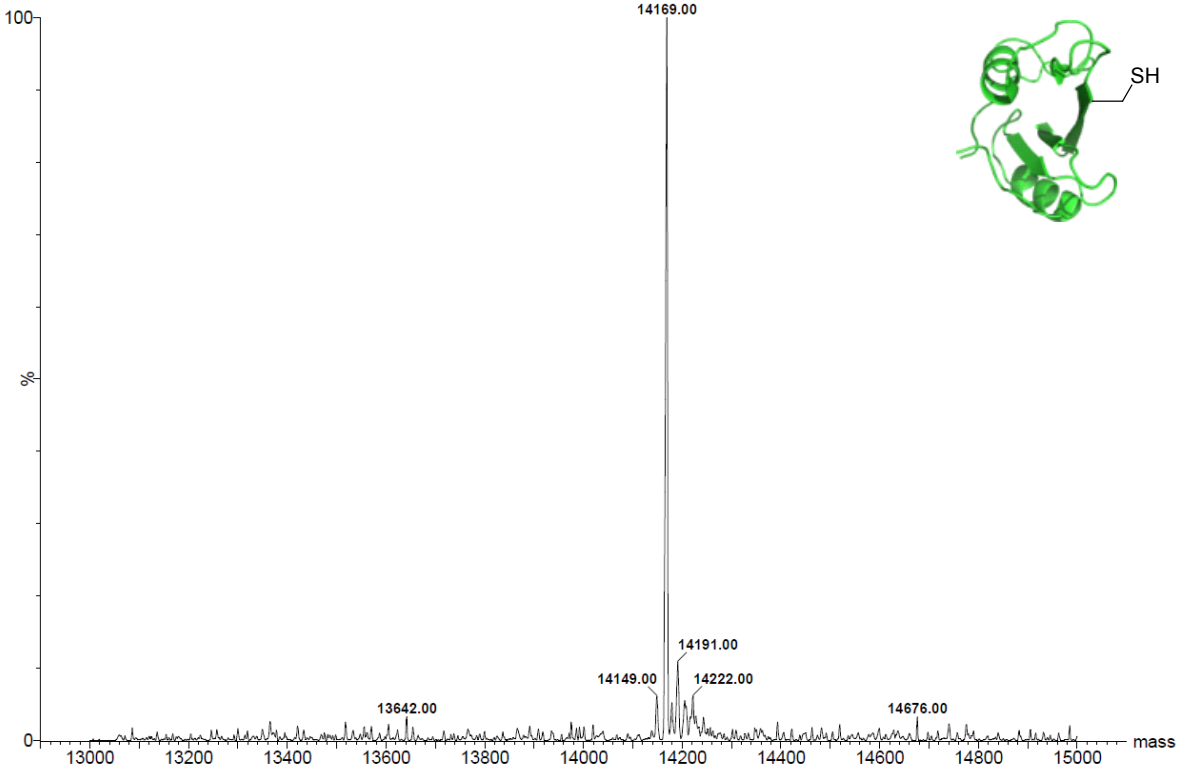
Thiol stability of succinimide bridged ds-scFv conjugate 21

Unpurified ds-scFv conjugate **21** (100 µL, 70 µM in PBS, pH 7.4), prepared as described above, was treated with 2-mercaptoethanol (100 equiv., 10 µL from a 70 mM stock solution in dimethylformamide). The same experiment was repeated using reduced glutathione (100 eq, 10 µL from a 70 mM stock solution in PBS, pH 7.4). The reaction mixtures were left to stand at room temperature. After 48 h, an aliquot was taken and analysed by LC-MS to show no degradation of the protein conjugate **21**.

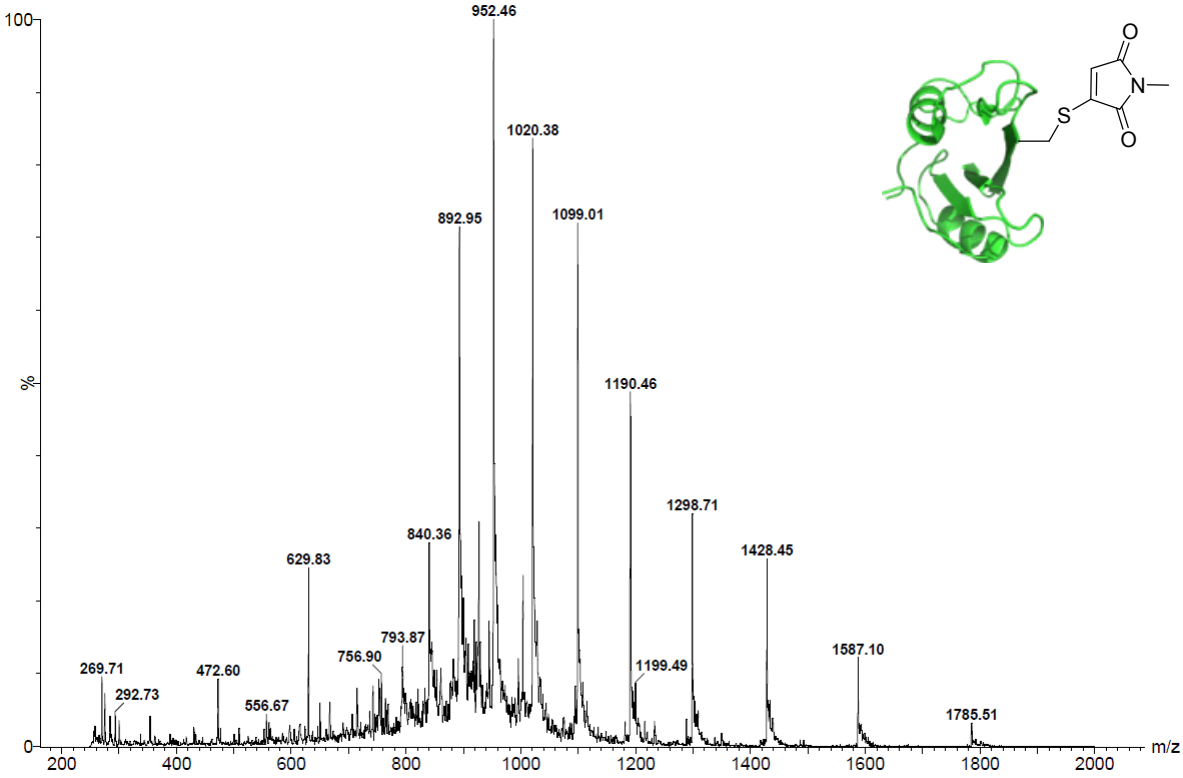
VII. LC-MS traces of protein conjugates

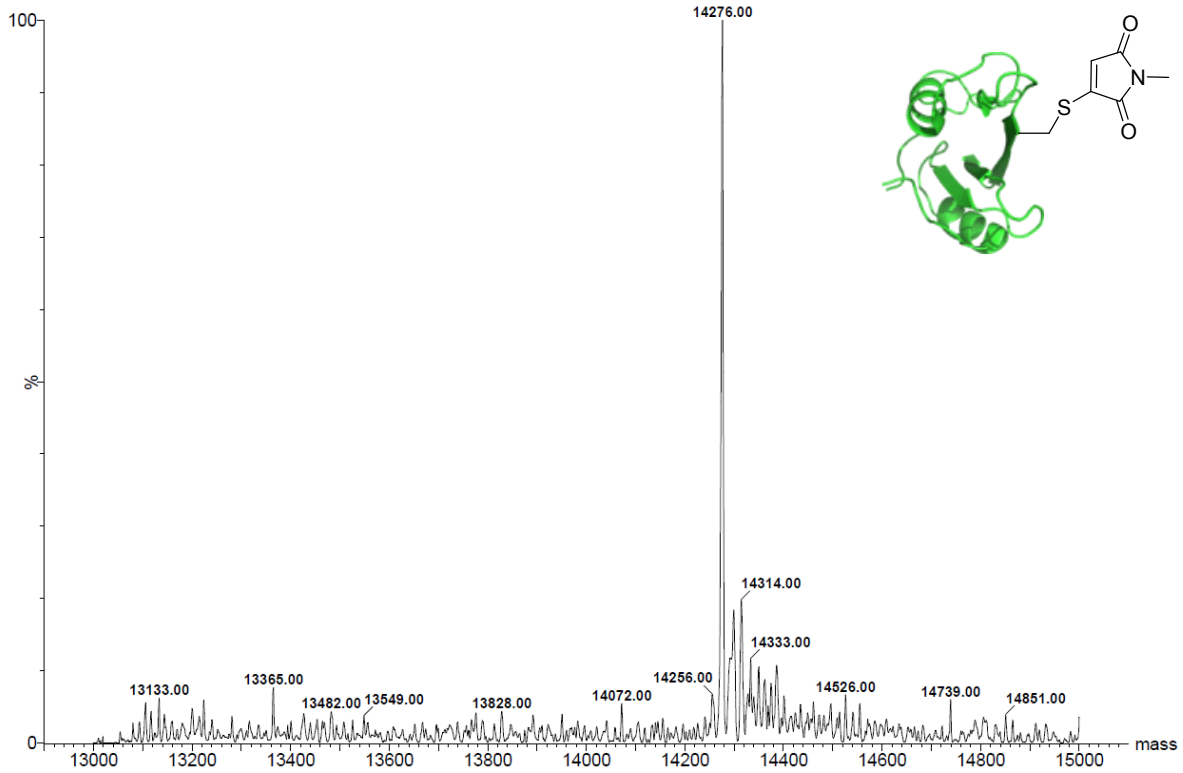
Unreacted Grb2 (L111C) SH2 **9** (calculated mass: 14171; observed mass: 14169)



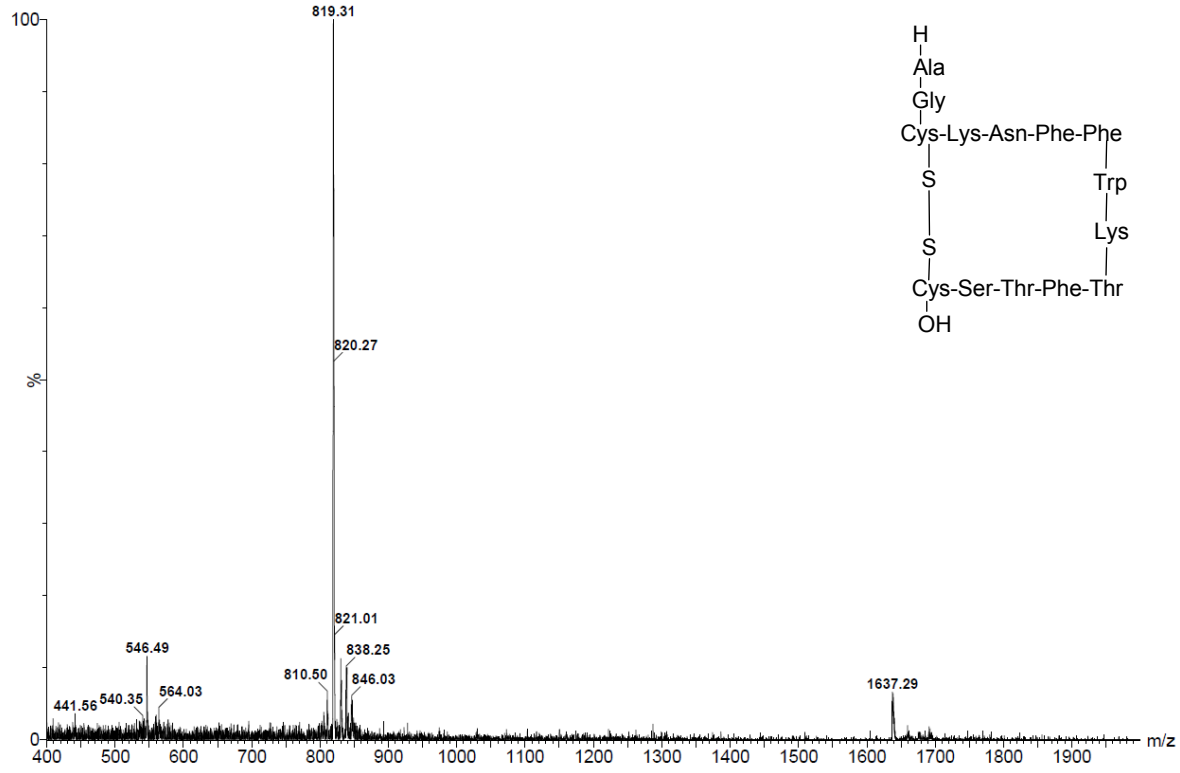


Grb2 (L111C) SH2 thiomaleimide adduct **10** (expected mass: 14278; observed mass: 14276)

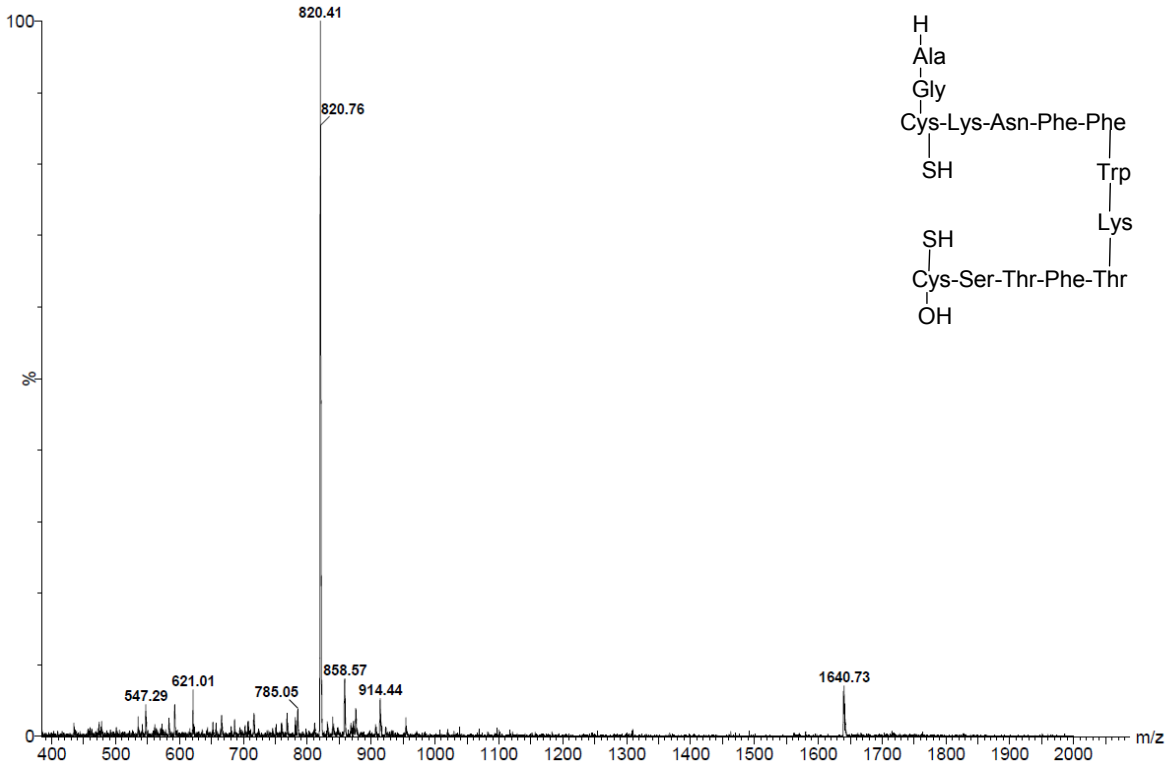




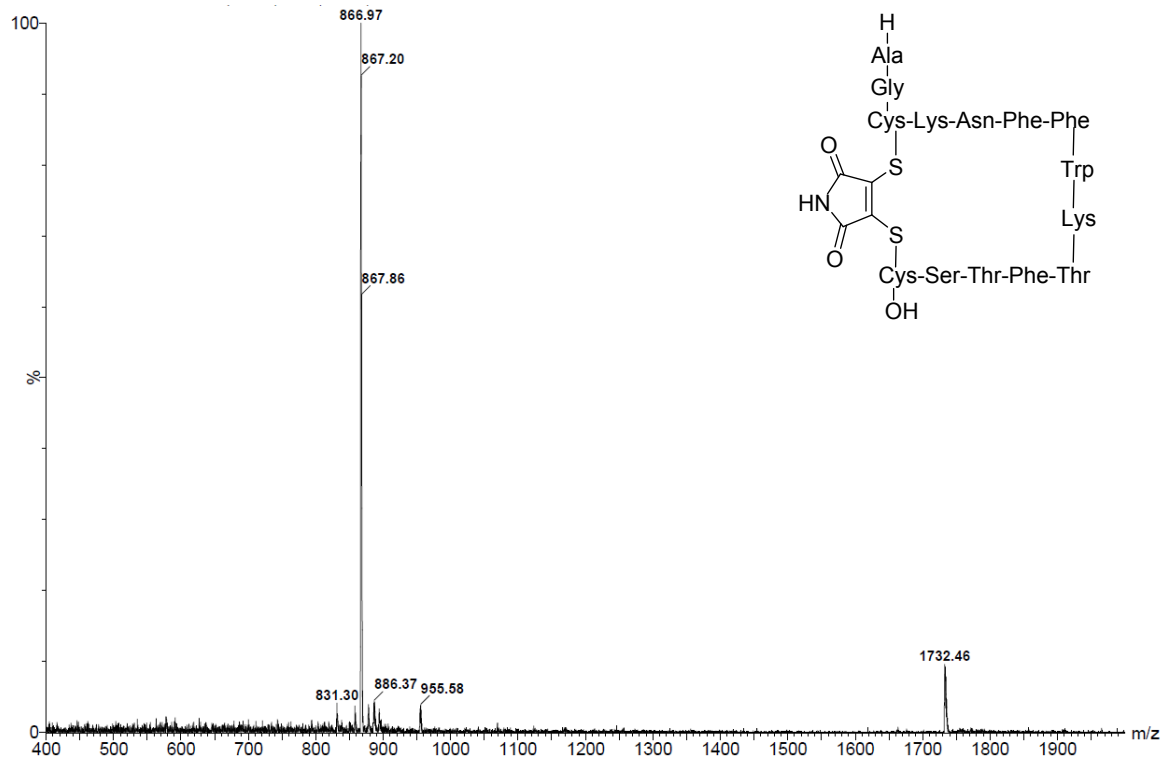
Unreacted somatostatin **11** (calculated mass: 1637; observed mass: 1637)



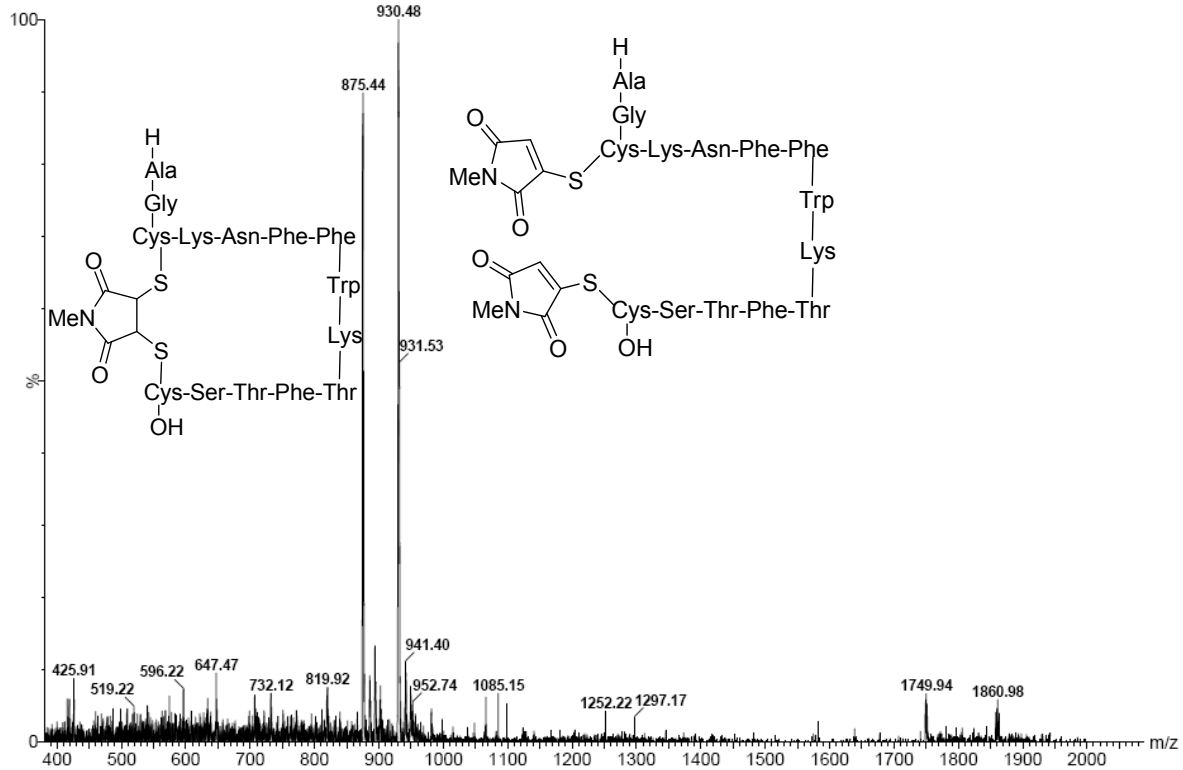
Reduced somatostatin **12** (calculated mass: 1639; observed mass: 1640)



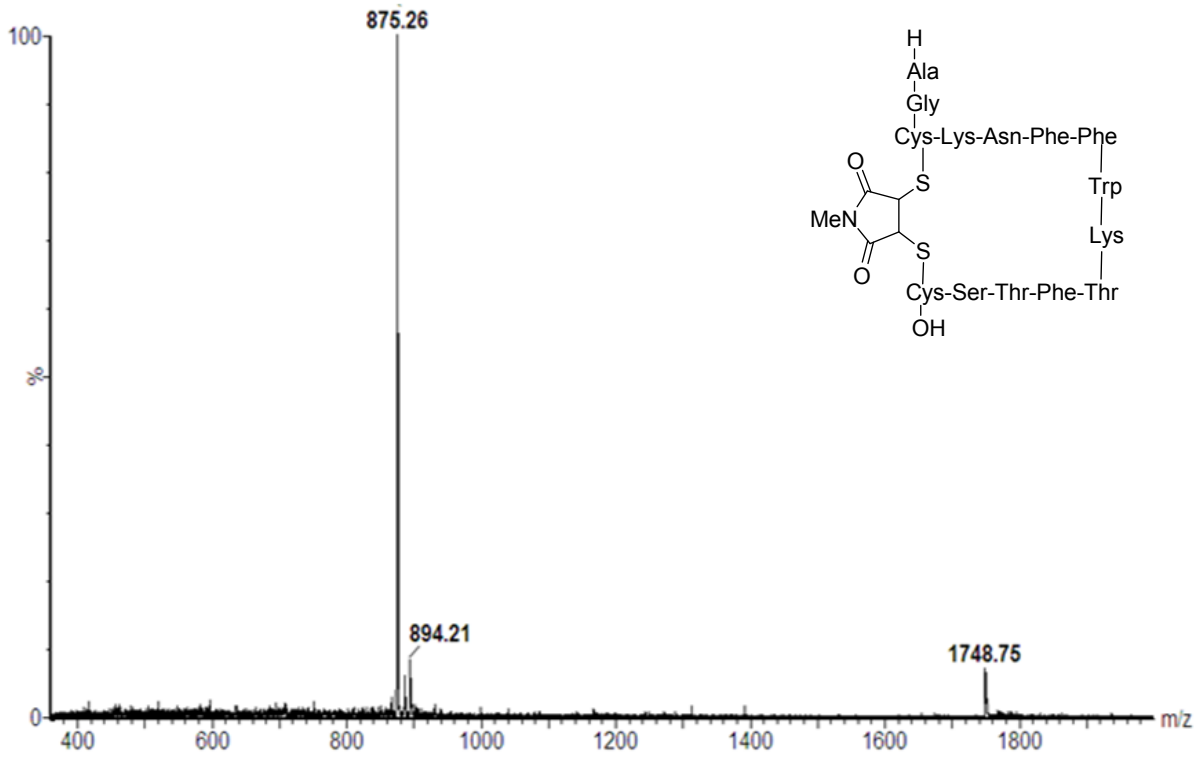
Maleimide bridged somatostatin **43** (expected mass: 1732; observed mass: 1732)



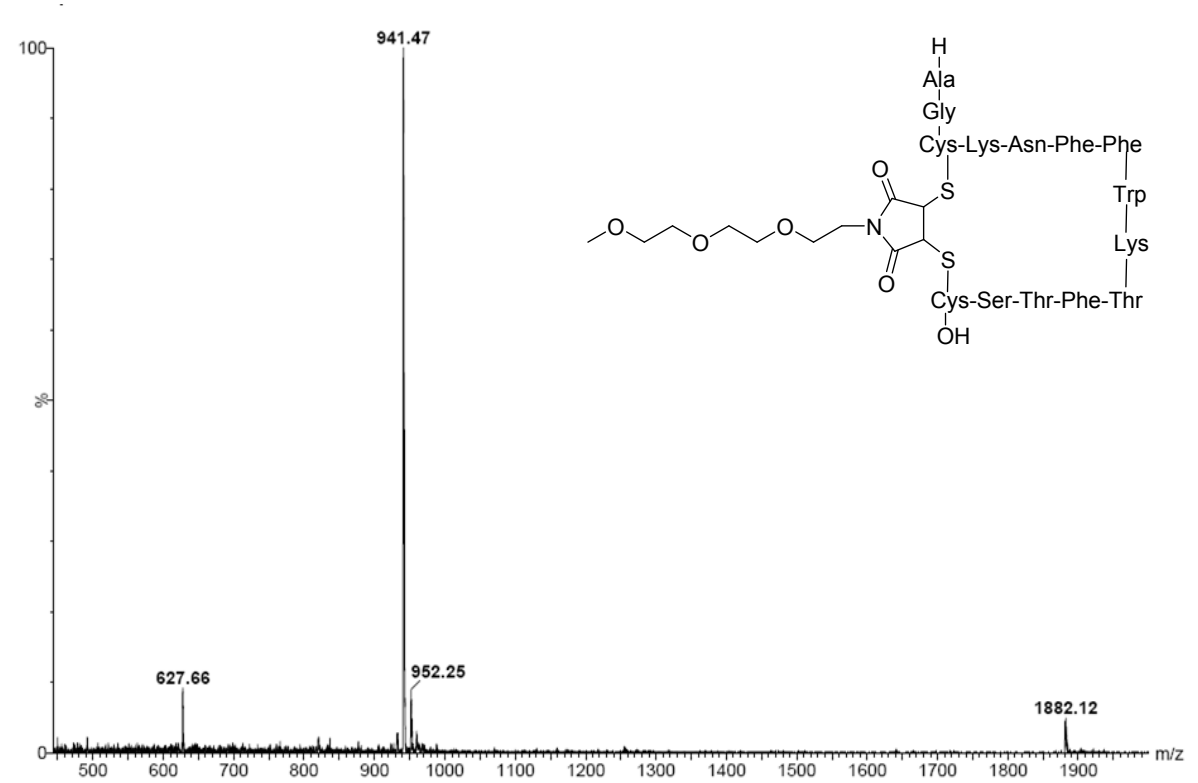
Mixture of *bis*-labelled somatostatin **15** and succinimide bridged somatostatin **14**



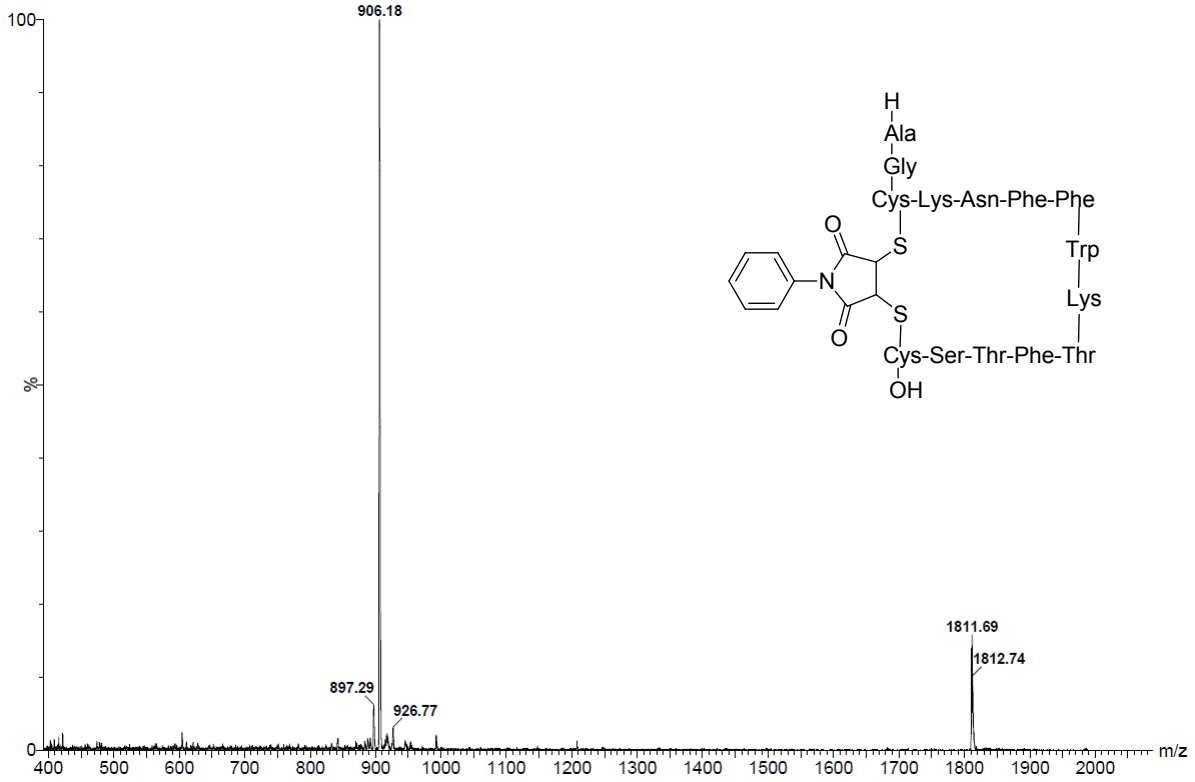
N-methyl succinimide bridged somatostatin **14** (expected mass: 1748; observed mass: 1749)



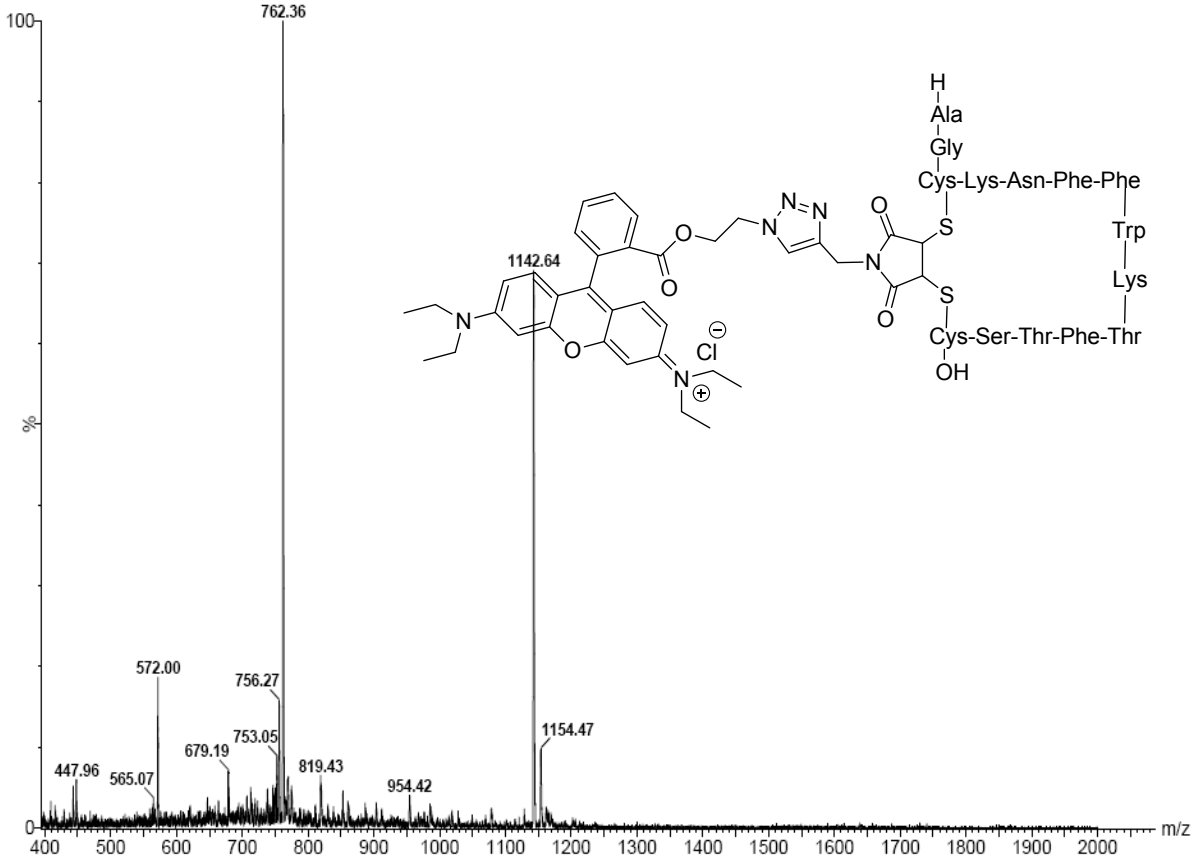
N-PEG succinimide bridged somatostatin **44** (expected mass: 1880; observed mass: 1882)

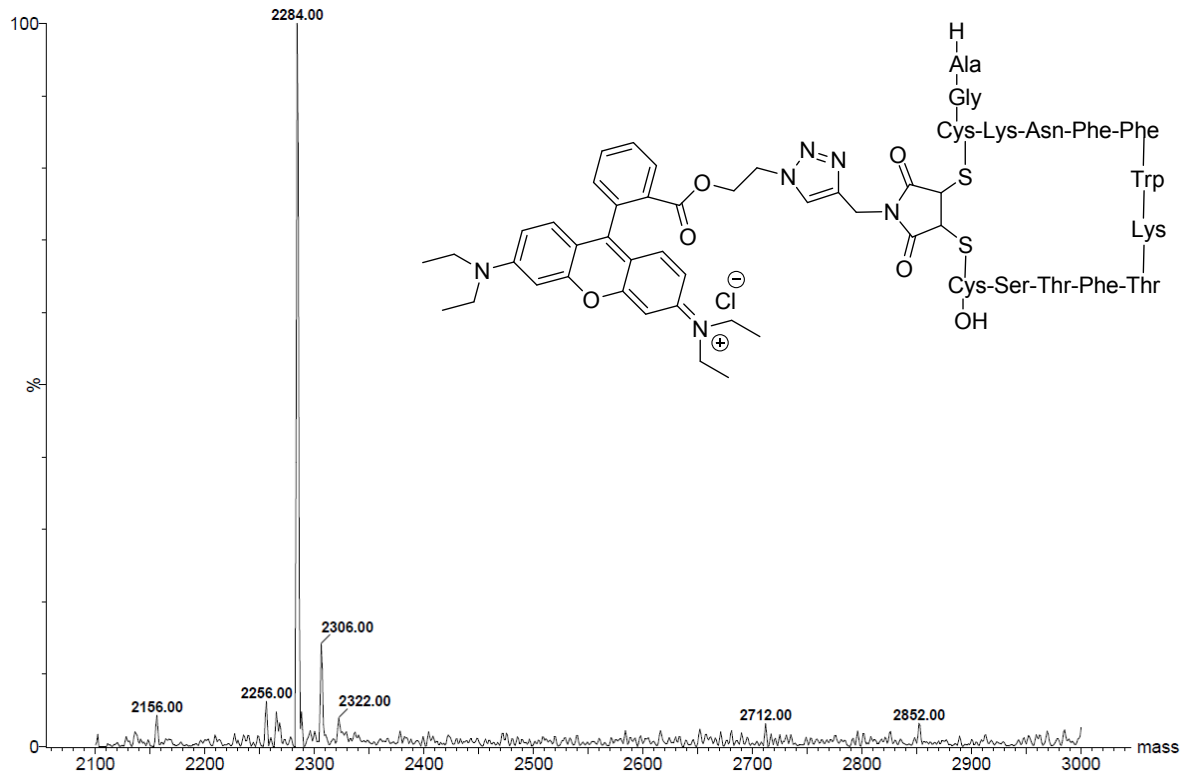


N-phenyl succinimide bridged somatostatin **45** (expected mass: 1810; observed mass: 1812)

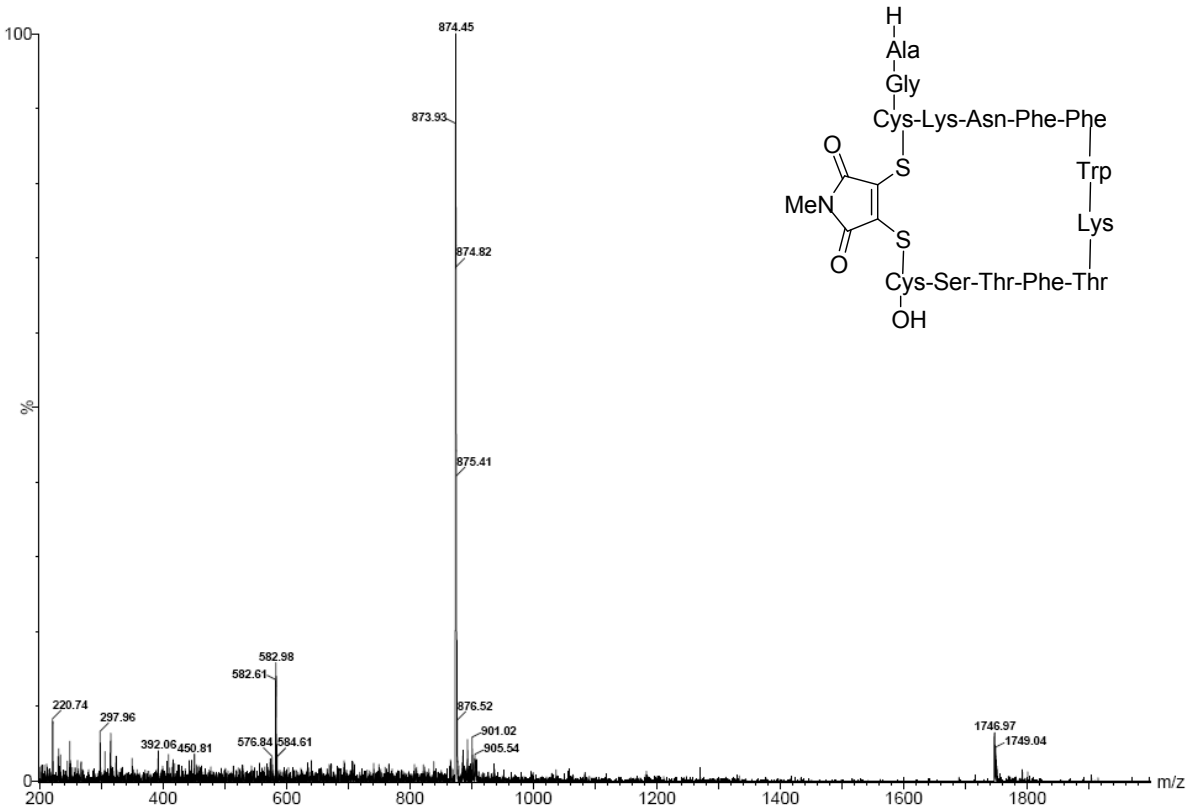


Rhodamine succinimide bridged somatostatin **46** (expected mass: 2283; observed mass: 2284)

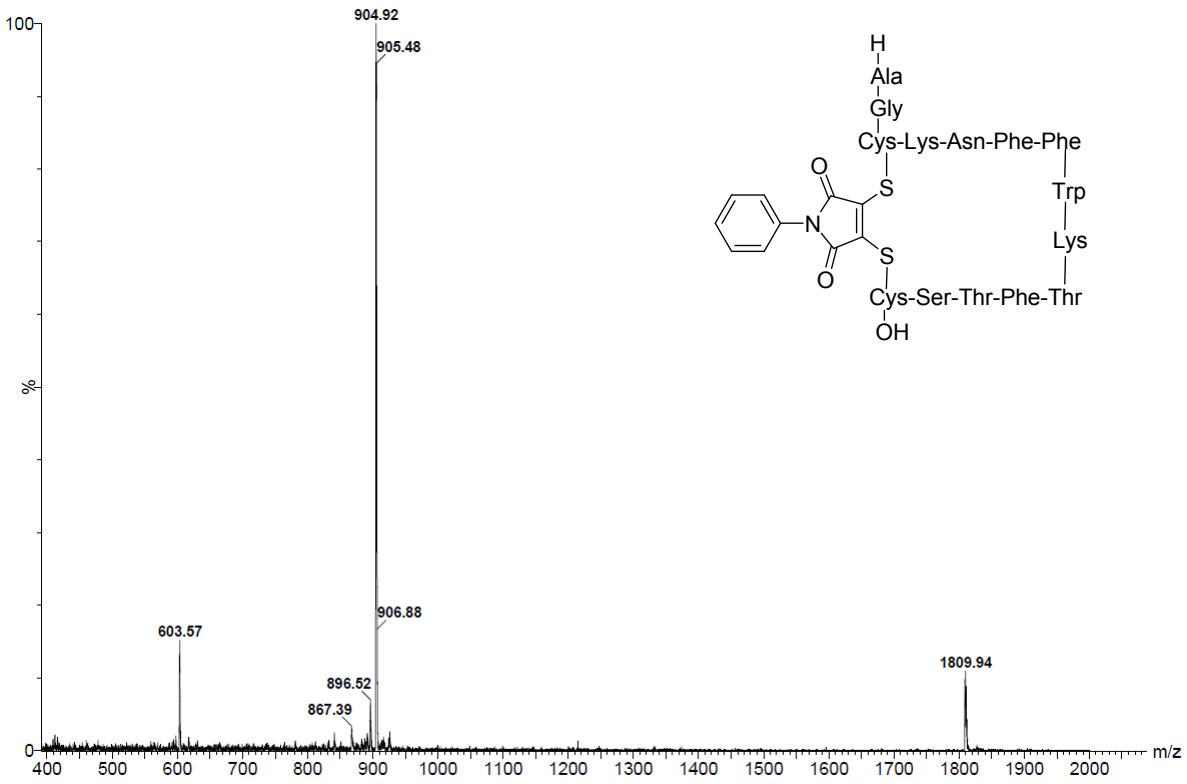




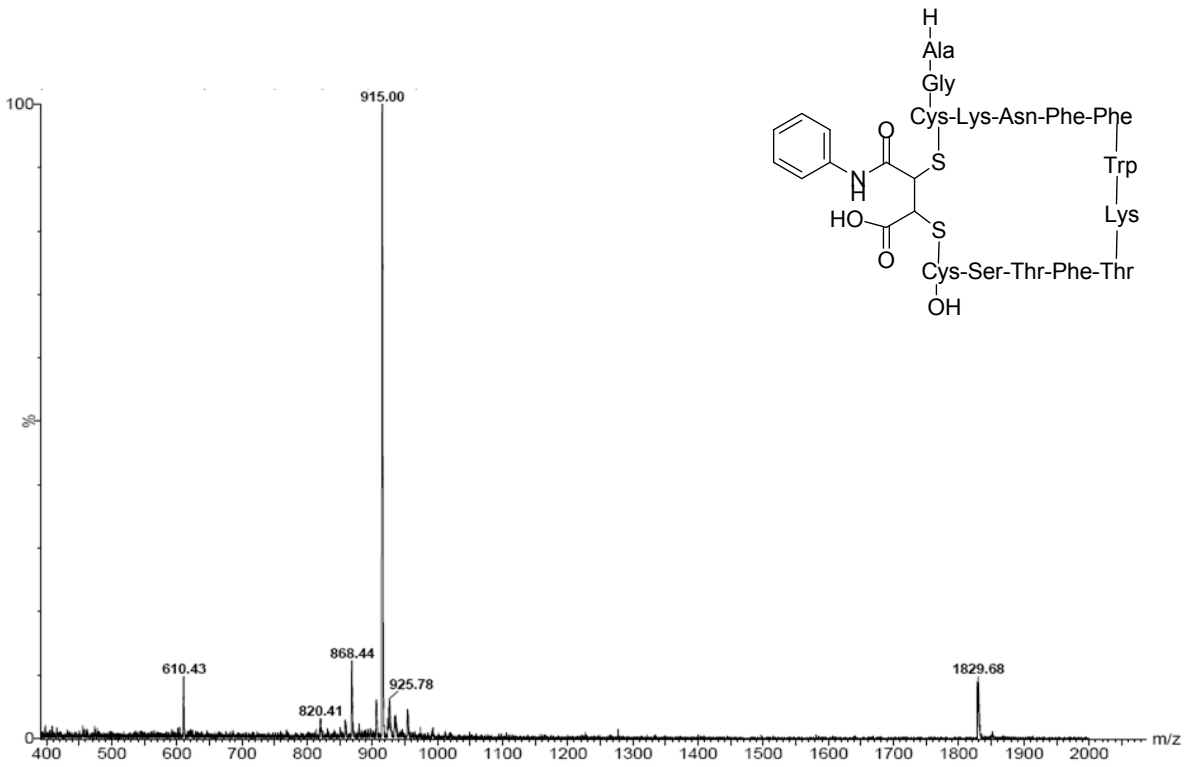
N-methyl maleimide bridged somatostatin **47** (expected mass: 1746; observed mass: 1747)



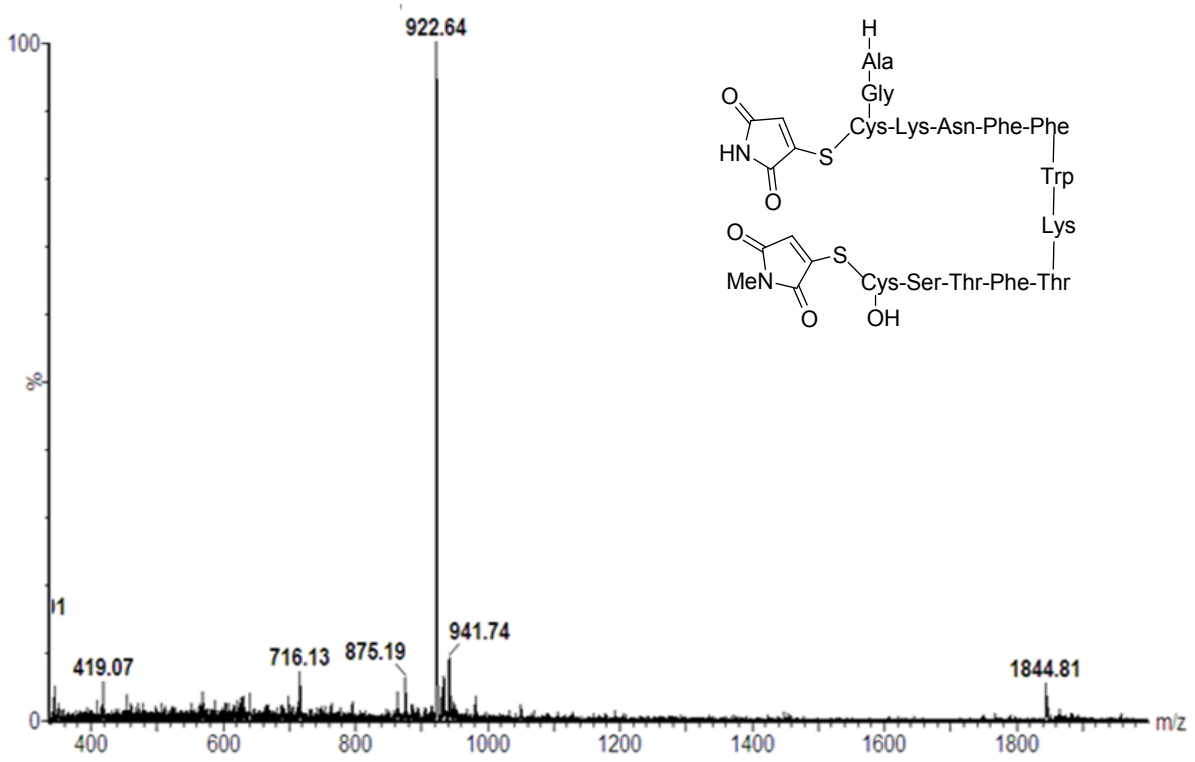
N-phenyl maleimide bridged somatostatin **48** (expected mass: 1808; observed mass: 1810)



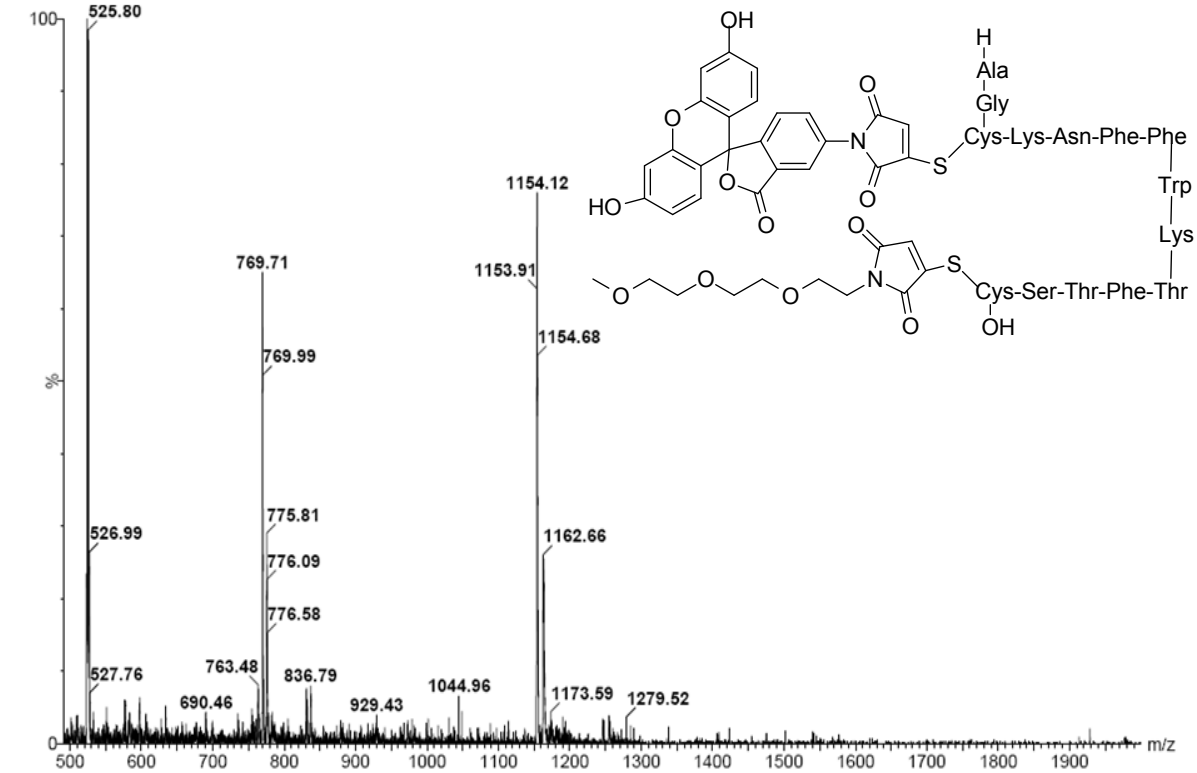
Hydrolysed *N*-phenyl succinimide bridged somatostatin **49** (expected mass: 1830; observed mass: 1830)

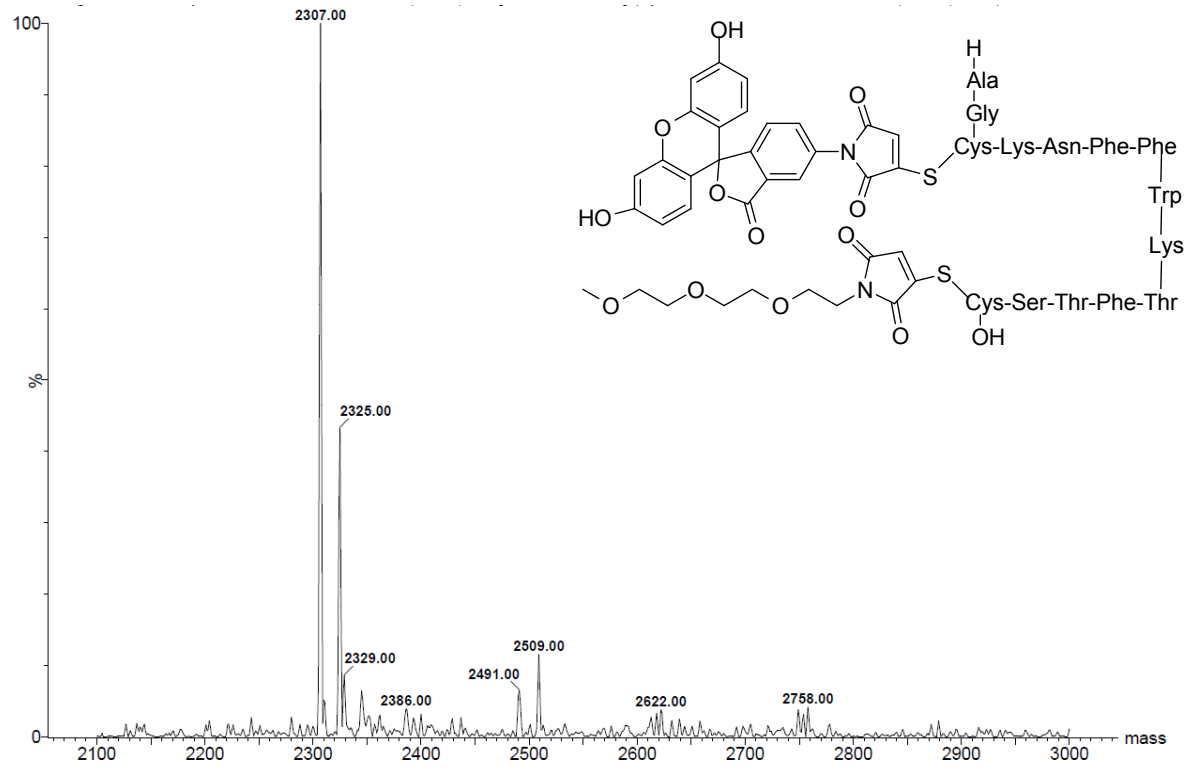


Hydrogen-methyl dual labelled somatostatin conjugate **22** (expected mass: 1845; observed mass: 1845)

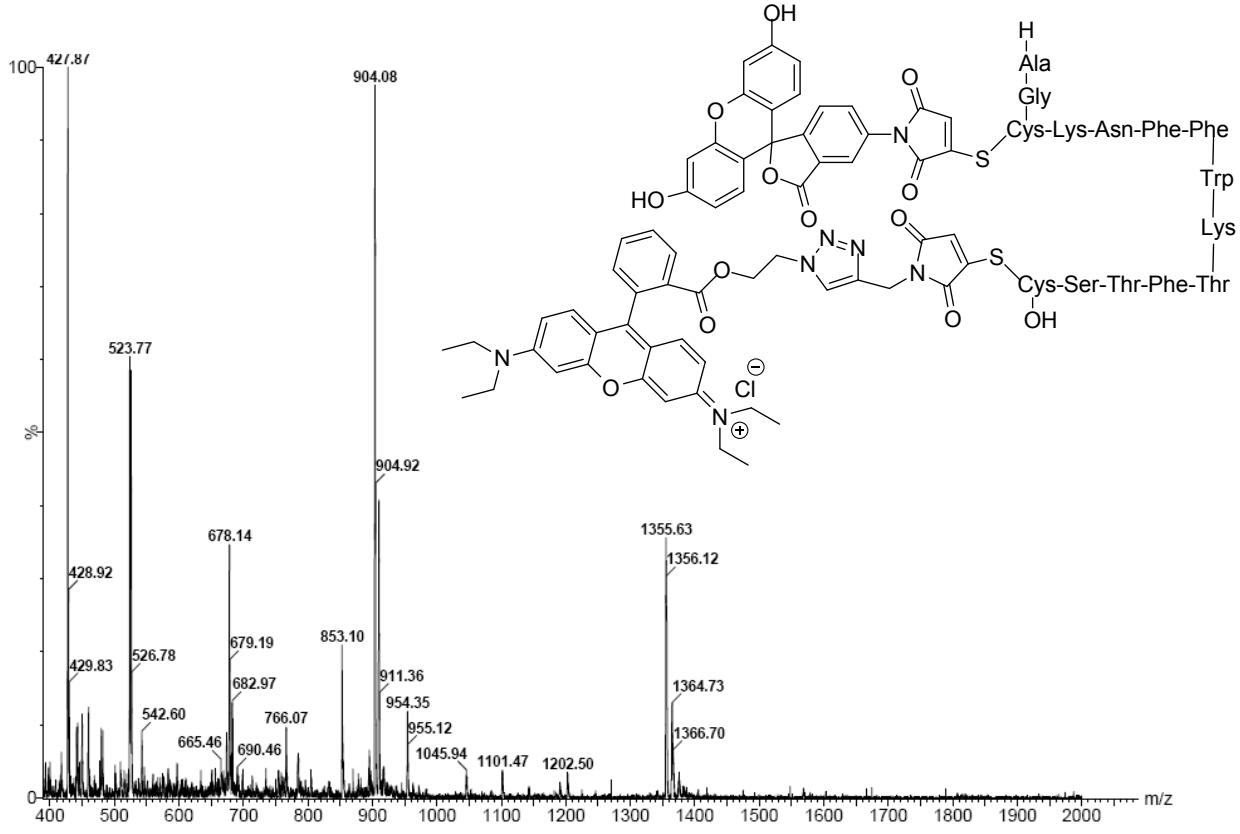


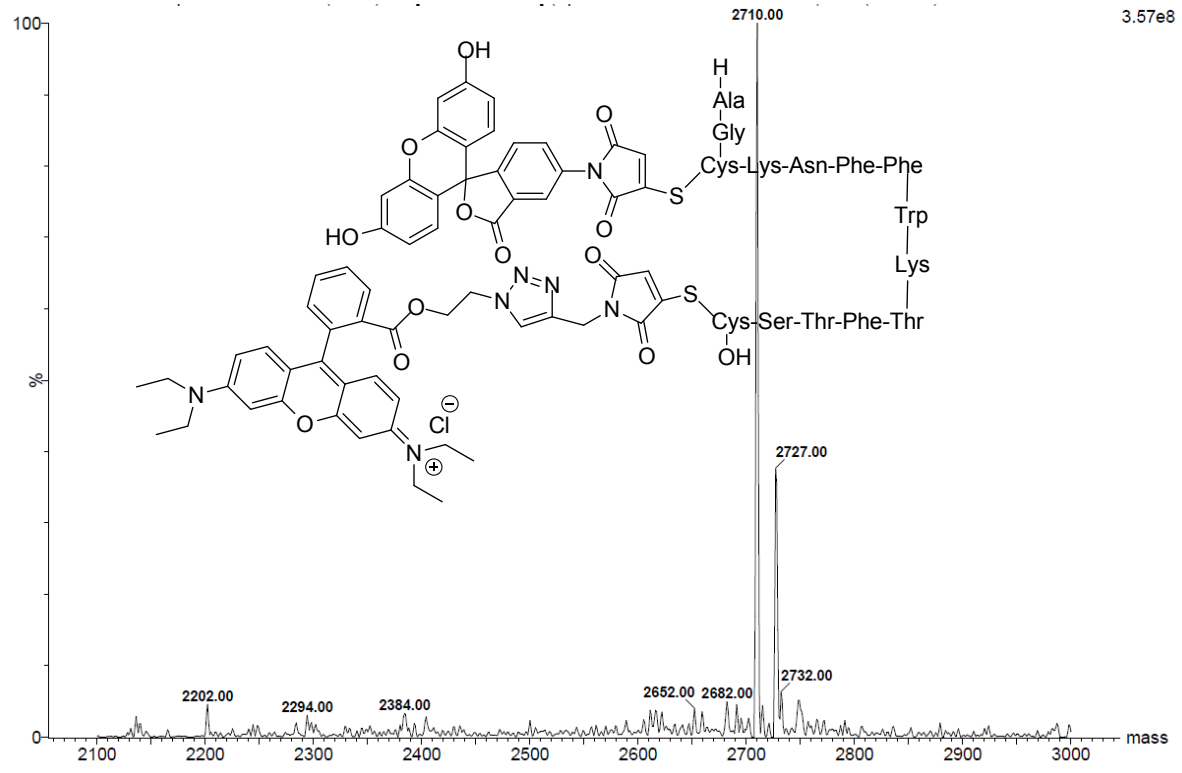
Fluoresceine-PEG dual labelled somatostatin conjugate **23** (expected mass: 2305; observed mass: 2307)



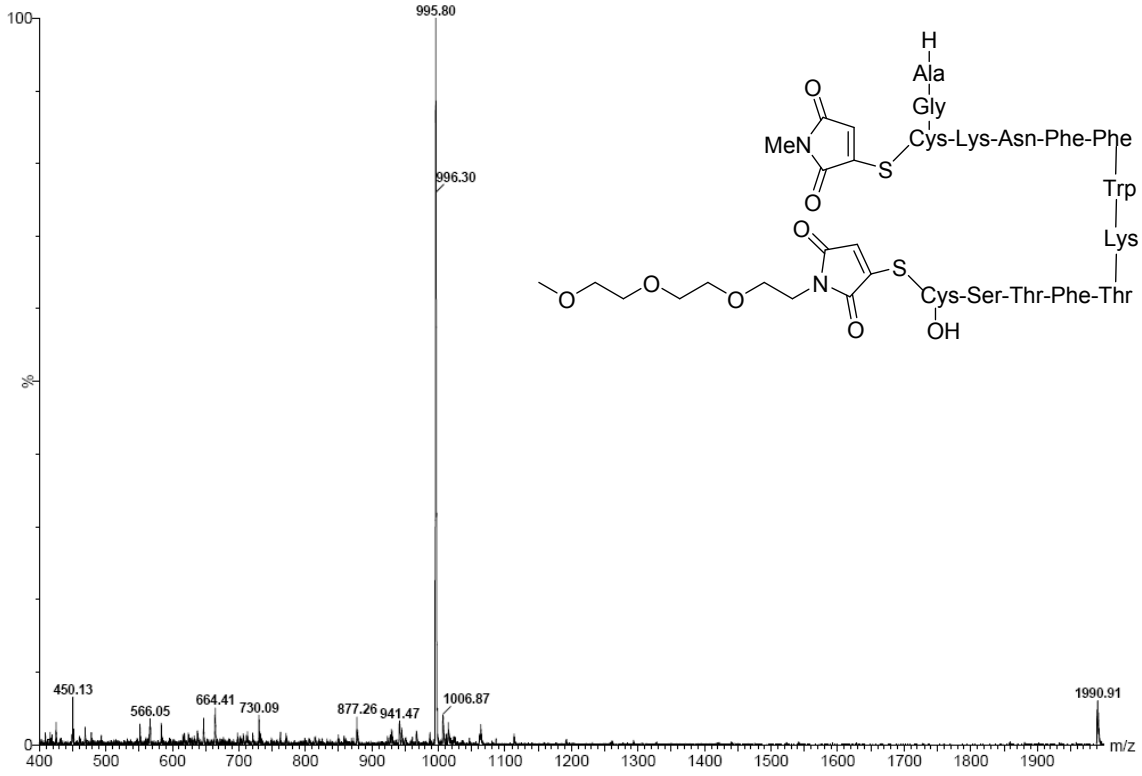


Rhodamine-Fluorescein dual labelled somatostatin conjugate **24** (expected mass: 2709; observed mass: 2710)

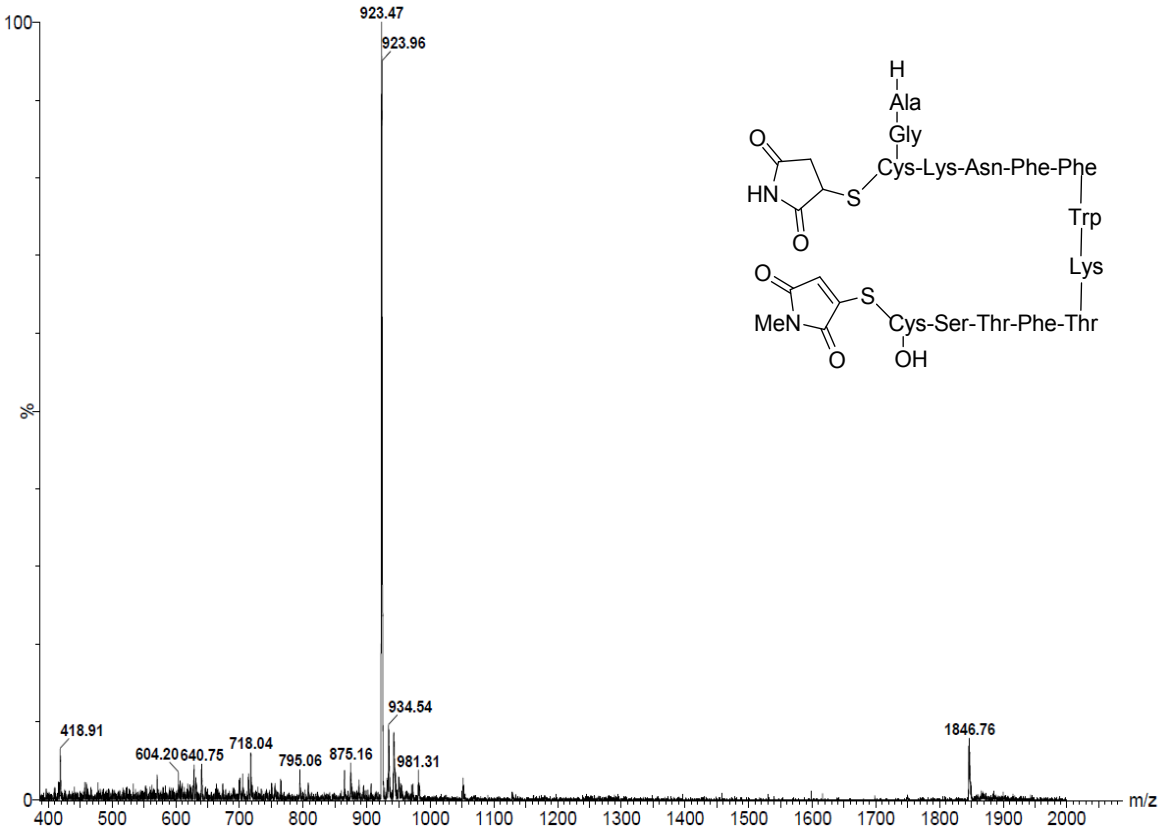




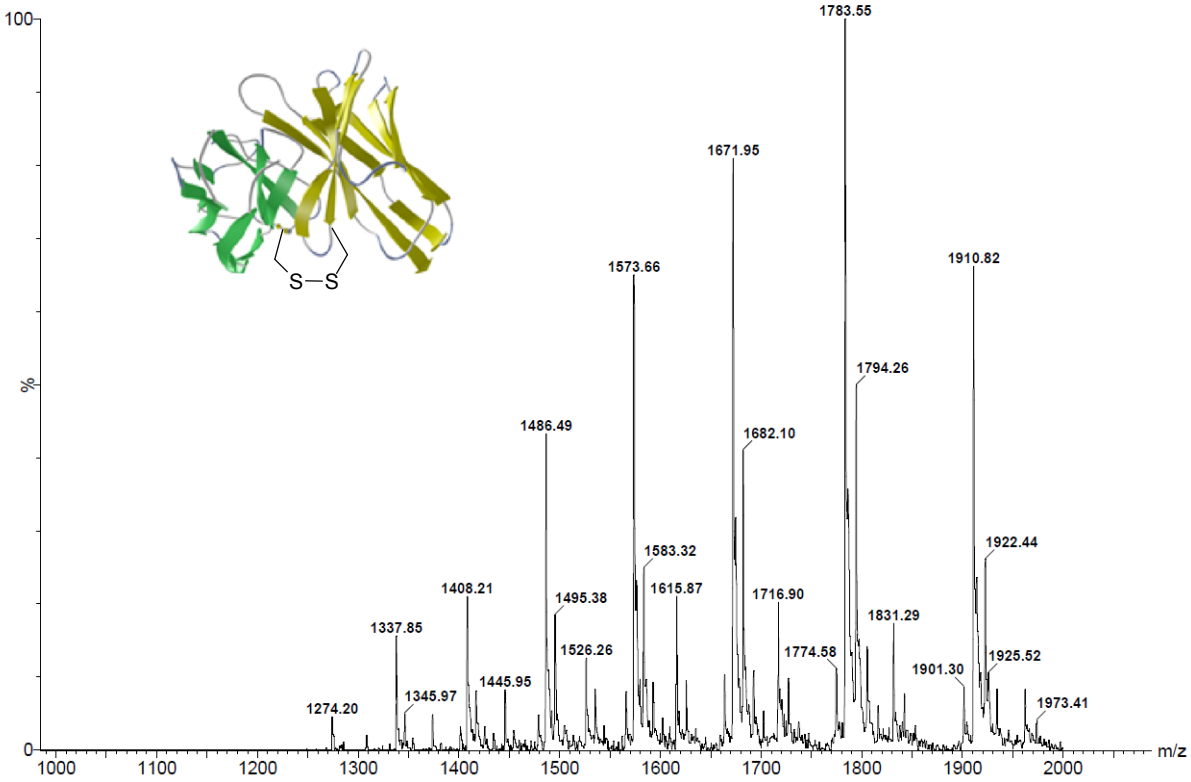
PEG-methyl dual labelled somatostatin conjugate **25** (expected mass: 1989; observed mass: 1991)

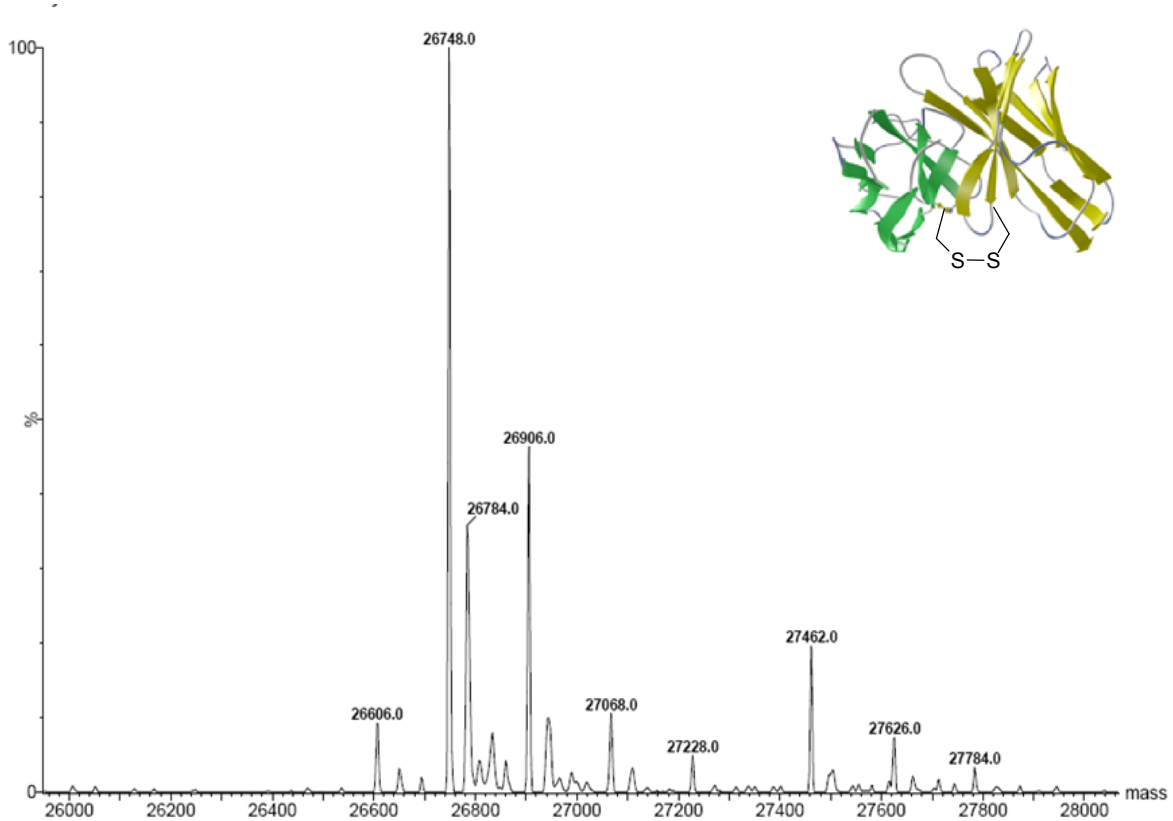


Dual labelled somatostatin conjugate with a cleavable and non-cleavable tag **50** (expected mass: 1845; observed mass: 1847)

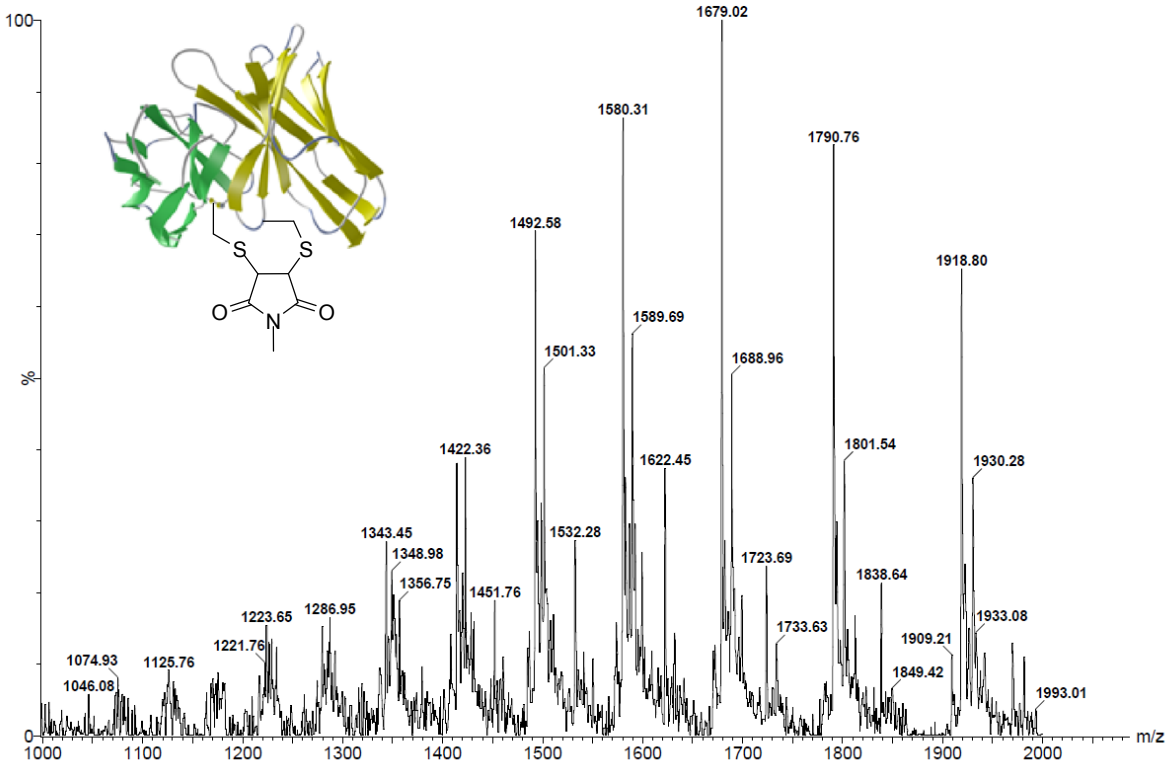


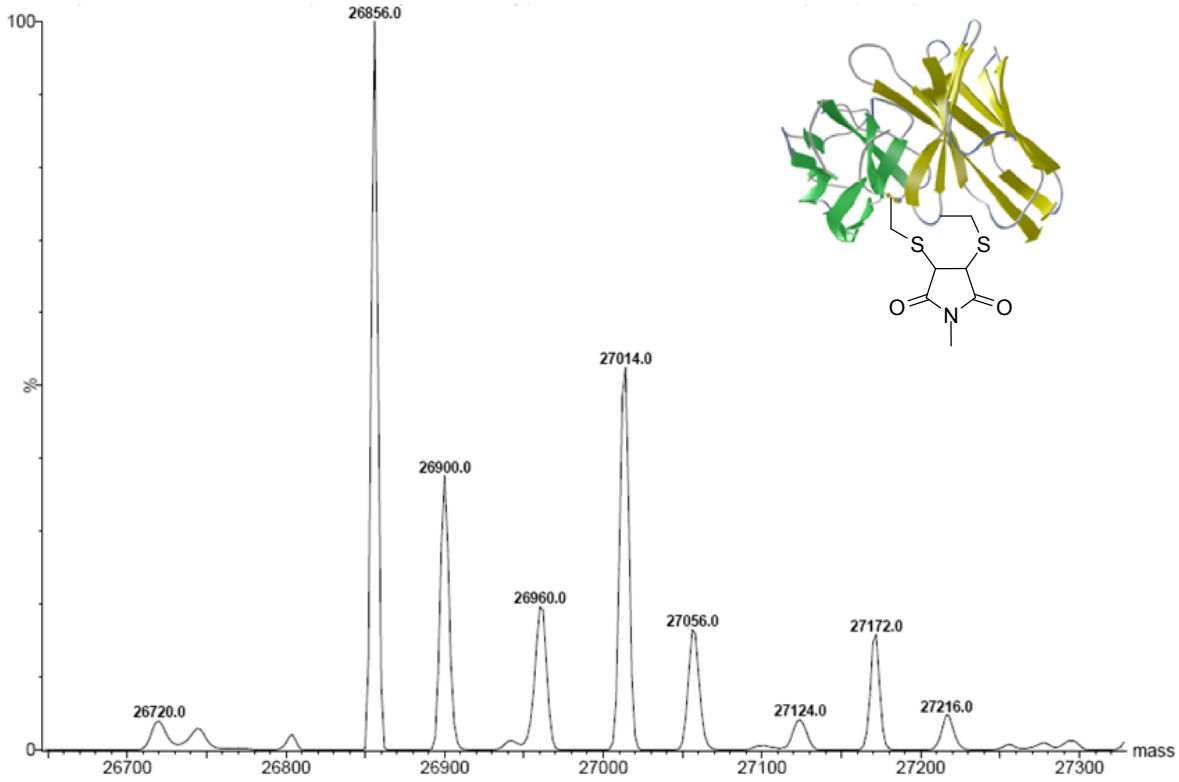
Unreacted anti-CEA ds-scFv **19** (observed mass: 26748)



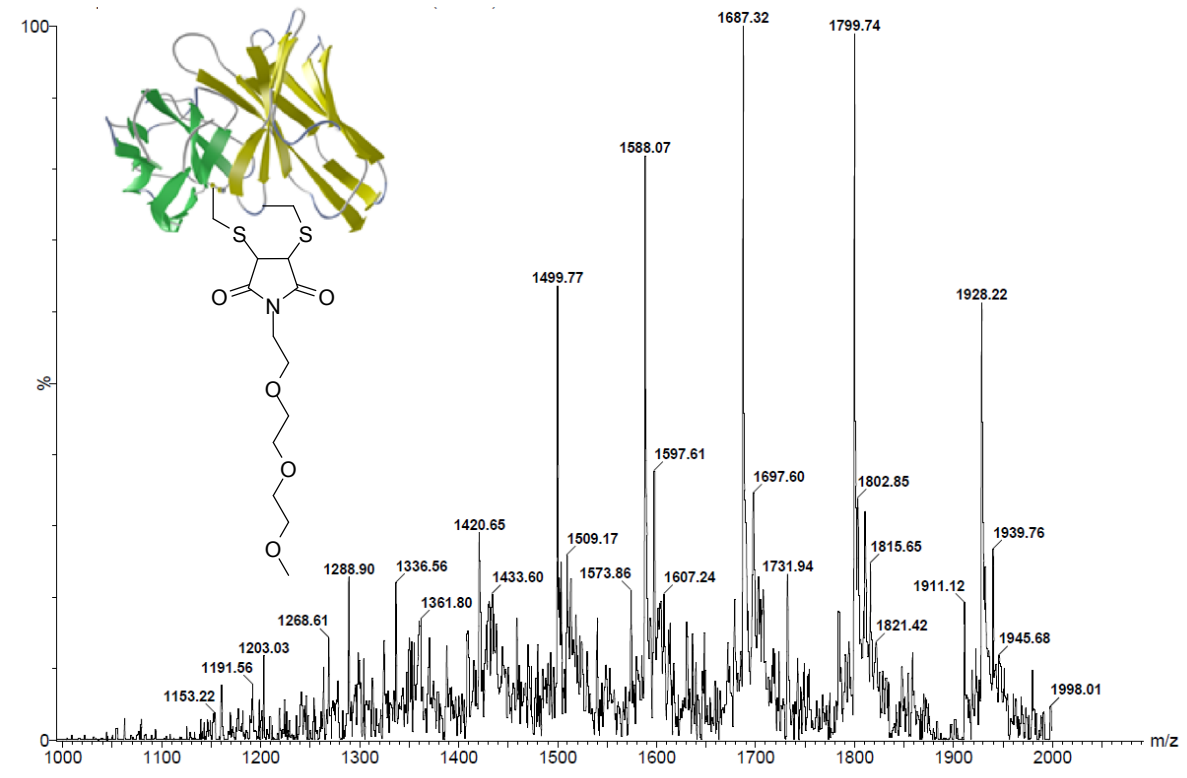


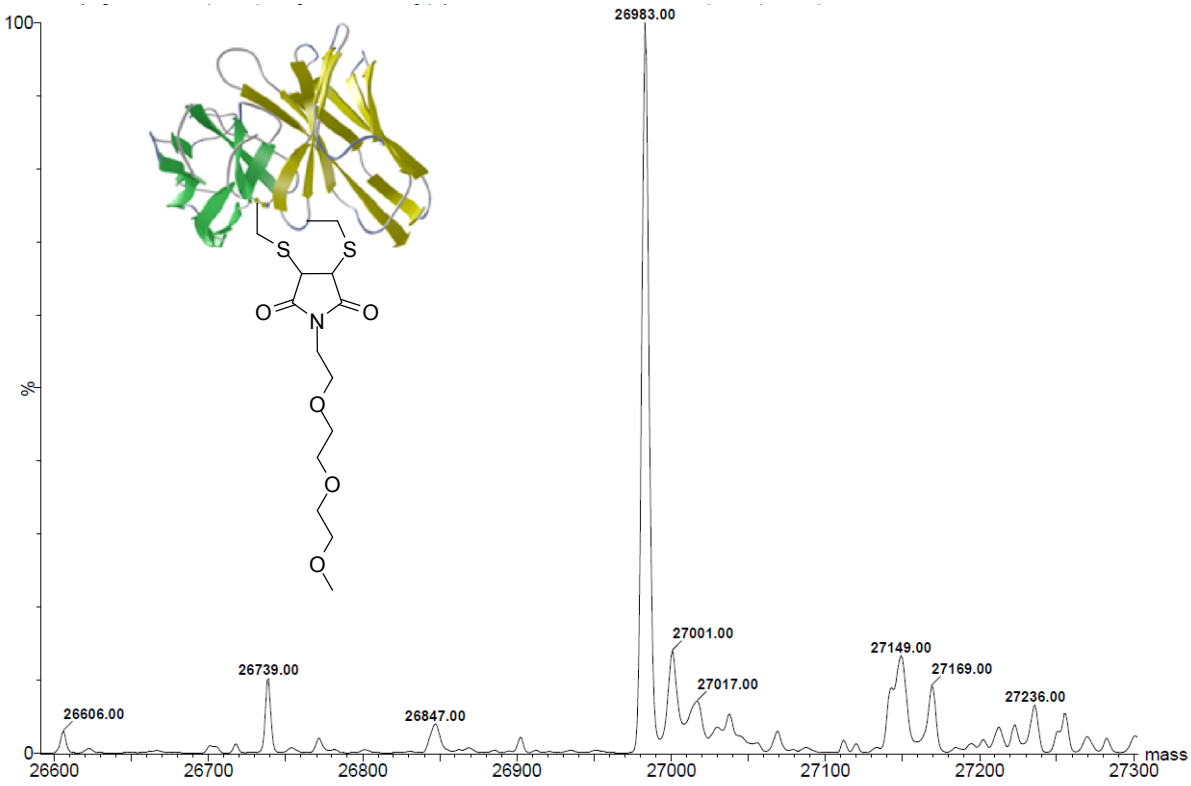
N-methyl succinimide bridged anti-CEA ds-scFv **21** (expected mass: 26859; observed mass: 26856)





N-PEG succinimide bridged anti-CEA ds-scFv **20** (expected mass: 26991; observed mass: 26983)





VIII. References

(1) Schumacher, F. F.; Sanchania, V. A.; Tolner, B.; Wright, Z. V. F.; Ryan, C. P.; Smith, M. E. B.; Ward, J. M.; Caddick, S.; Kay, C. W. M.; Aeppli, G.; Chester, K. A.; Baker, J. R. *Sci. Rep.*, **2013**, 3, 1525.

(2) Smith, M. E. B.; Schumacher, F. F.; Ryan, C. P.; Tedaldi, L. M.; Papaioannou, D.; Waksman, G.; Caddick, S.; Baker, J. R. *J. Am. Chem. Soc.,* **2010**, 132, 1960-5.

(3) Tedaldi, L. M.; Smith, M. E. B.; Nathani, R. I.; Baker, J. R. *Chem. Commun.* **2009**, 6583–6585.

(4) Iqbal, P.; Critchley, K.; Bowen, J.; Attwood, D.; Tunnicliffe, D.; Evans, S. D.; Preece, J. A. *J. Mater. Chem.* **2007**, *17*, 5097–5110.

(5) Clerc, J.; Schellenberg, B.; Groll, M.; Bachmann, A. S.; Huber, R.; Dudler, R.; Kaiser, M. *European J. Org. Chem.* **2010**, *2010*, 3991–4003.

(6) Sahoo, K.; Mhaske, S. B.; Argade, N. P. *Synthesis,* **2003**, 346–349.

(7) Norberg, O.; Deng, L.; Aastrup, T.; Yan, M.; Ramstrom, O.; *Analytical Chemistry* **2011**, 1000–1007.

(8) Dubernet, M.; Caubert, V.; Guillard, J.; Viaud-Massuard, M.-C. *Tetrahedron* **2005**, *61*, 4585–4593.