Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Utilising Fluorescent Reporters to Probe the Mode of Action of Norbornen-7-one CO Releasing Molecules

Supporting Information

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General experimental

Experiments requiring anhydrous conditions were performed under a dry nitrogen or argon atmosphere using apparatus heated and dried under vacuum, unless otherwise stated.

Anhydrous dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), toluene, acetonitrile (CH_3CN) and methanol (CH_3OH) were dried using the PURE SOLV MD-6 solvent purification system. All other reagents were purchased as analytical or reagent grade and used without further purification. Aqueous solutions of sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$) and ammonium chloride (NH_4Cl) were saturated. Reactions performed at room temperature (rt) were carried out at approximately 20°C and reaction temperatures from -78°C to0 °C were obtained using the following cooling bath mixtures: acetone/dry ice, -78°C; acetonitrile/dry ice, -40°C; NaCl/ice, -5°C; water/ice, 0°C.

Reactions were monitored by thin layer chromatography (TLC) carried out on 0.2 mm Kieselgel F254 (Merck) silica gel plates using UV light as a visualising agent and then stained and developed with heat using either vanillin in ethanolic sulfuric acid, ammonium heptamolybdate and cerium sulfate in aqueous sulfuric acid, or potassium permanganate and potassium carbonate in aqueous sodium hydroxide. Separation of mixtures was performed by flash chromatography using 0.063–0.1 mm silica gel with the indicated eluent.

Infrared spectra were recorded on a Bruker Optics Alpha FT-IR spectrometer with a diamond Attenuated Total Reflectance (ATR) top plate. No sample preparation was required. Absorption peaks are reported as wavenumbers (ν, cm^{-1}).

NMR spectra were recorded on a Varian 400-MR spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei at 25°C, or a Varian 500 MHz AR Premium Shielded Spectrometer operating at 500 MHz for ^{1}H nuclei and 125 MHz for ^{13}C nuclei at 25°C. ^{1}H NMR

chemical shifts are reported in parts per million (ppm) relative to the chloroform ($CDCl_3$, δ 7.26) or dimethylsulfoxide (DMSO-*d6*, δ 2.50) peak. ^1H NMR values are reported as chemical shifts δ, relative integral, multiplicity (s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet) and coupling constant (J, Hz). Coupling constants were taken directly from the spectra. ^{13}C NMR chemical shifts are reported in ppm relative to the chloroform ($CDCl_3$, δ 77.0) or dimethyl sulfoxide (DMSO-*d6*, δ 39.5) peak. ^{13}C NMR values are reported as chemical shifts δ. Assignments were made with the aid of DEPT, gCOSY, gHSQC, and gHMBC experiments.

Mass spectra were recorded on a Bruker micrOTOF-Q II mass spectrometer or a Shimadzu LC-MS-9030 Quadrupole Time-of-Flight Liquid Chromatograph Mass Spectrometer mass spectrometer by electrospray ionisation in positive and negative mode.

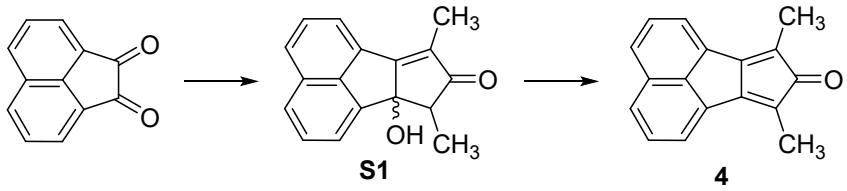
HPLC analysis

HPLC grade CH_3CN was purchased from Merck Chemicals. MilliQ grade water (H_2O) was obtained from a Millipore purification system. HPLC grade trifluoroacetic acid (TFA) was purchased from Scharlau. HPLC analyses were conducted using analytical RP-HPLC (Shimadzu LC–20AD equipped with an SPD-20A UV detector [210 and 254 nm] and a Shimadzu ELSD-LTII Low Temperature Evaporative Light Scattering Detector) using a Phenomenex Prodigy column (C–18, 5 µm, 3.00 × 250 mm) at 0.5 mL.min, heated to 40°C unless stated otherwise. The solvent system for all LC purposes was a mixture of A (0.05% TFA in H_2O) and B (0.05% TFA in CH_3CN) was unless otherwise stated.

LC-MS analysis

Mass spectrometry grade CH_3OH, CH_3CN and H_2O were purchased from Thermofisher Scientific. Mass spectrometry grade formic acid was purchased from Sigma-Aldrich. LC-MS analyses were conducted on an analytical RP-HPLC (Shimadzu LC–20AD equipped with a SPD-20A UV detector [210 and 254 nm] and a Shimadzu LC-MS-2020 Liquid Chromatograph Mass Spectrometer operating in positive ion or negative ion mode) using a Phenomenex Prodigy column (C–18, 5 µm, 3.00 × 250 mm) at 0.5 mL min^{-1} and heated to 40°C. The solvent system for LC purposes was a mixture of A (0.1% formic acid in H_2O) and B (0.1% formic acid in CH_3CN).

Acenaphthylene diene 4.



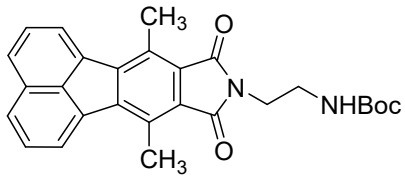
S1 **4**

The following procedure was adapted from Allen *et al.*[1] To a suspension of K_2CO_3 (7.59 g, 54.9 mmol) in CH_3OH (100 mL) was added pentan-3-one (5.8 mL, 54.9 mmol). Acenaphthenequinone (5 g, 27.4 mmol) was added portion-wise over 15 min and the reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated to a volume of 5 mL *in vacuo* and then suspended in H_2O (100 mL). The resulting precipitate was isolated by vacuum filtration and dried to afford the intermediate **S1** as a brown solid (5.25 g, 76%).

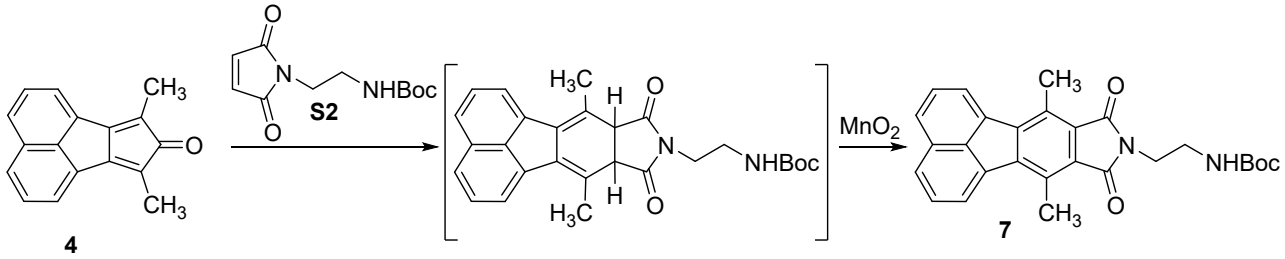
Without further purification a sample of intermediate **S1** (0.8 g, 3.20 mmol) suspended in Ac_2O (20 mL) and H_2SO_4 (20 µL, 0.38 µmol) was added. The resulting brown solution was stirred at rt for 2 h and then ice-cold water (40 mL) was slowly added. The mixture was stirred

for 1 h and the resulting precipitate was isolated by vacuum filtration to afford the title compound **4** as brown-yellow solid (636 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (3H, s), 2.26 (3H, s), 3.44–3.47 (2H, m), 7.17–7.47 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.9, 8.2, 8.5, 8.8, 9.0, 9.1, 15.7, 17.0, 53.4, 55.8, 62.7, 68.7, 120.5, 120.9, 121.1, 121.5, 122.0, 122.9, 123.3, 124.0, 124.3, 127.1, 127.5, 127.5, 127.7, 127.7, 127.8, 127.9, 128.0, 128.2, 130.4, 132.4, 132.6, 133.6, 134.4, 137.8, 141.4, 173.4, 206.6, 209.2.

Compound 7

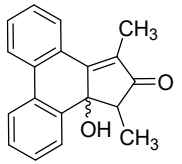


Method A: A stirred suspension of bromomaleimide **5**[2] (40 mg, 0.12 mmol) and acenaphthylene diene **4** (31 mg, 0.13 mmol) in toluene (2 mL) was heated to 60°C for 4 h. The reaction mixture was cooled to rt and then concentrated *in vacuo*. Purification by flash chromatography (0%, then 5%, then 10% EtOAc in CH_2Cl_2) gave the *title compound* **7** as a yellow solid (26 mg, 47%). M.p. 220 ºC (decomposition); R_f (10% EtOAc in CH_2Cl_2) 0.43; IR (ATR) ν_{max}/cm^{-1} 2927, 2845, 1749, 1686, 1510, 1426, 1163, 974; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (9H, s), 3.04 (6H, s), 3.44–3.47 (2H, m), 3.82 (2H, t, *J* = 4.0 Hz), 5.03 (1H, br s), 7.67 (2H, t, *J* = 7.6 Hz), 7.94 (2H, d, *J* = 7.6 Hz), 8.11 (2H, d, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5, 28.3, 37.6, 39.9, 79.5, 125.3, 128.0, 128.1, 128.2, 129.6, 132.1, 132.7, 135.6, 142.9, 156.0, 169.3; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{27}H_{26}N_2O_4Na^+$, 465.1785; found 465.1775.



Method B: Boc-protected acenaphthylene BP **4** can also be synthesised from the Boc-protected maleimide **S2**[3]. A stirred suspension of **S2** (50 mg, 0.21 mmol) and acenaphthylene diene **4** (47 mg, 0.20 mmol) in toluene (2 mL) was heated to 60°C for 4 h under an atmosphere of argon. The reaction mixture was cooled to rt and then MnO_2 (181 mg, 2.1 mmol) was added. The resulting brown suspension was heated to 120°C for 24 h, then cooled to rt and filtered through Celite. The Celite was washed with CH_2Cl_2 (50 mL) and the solvent removed *in vacuo* to obtain an orange oil. Purification by flash chromatography (0%, then 5%, then 10% EtOAc in CH_2Cl_2) gave the *title compound* **7** as a yellow solid (30 mg, 33%).

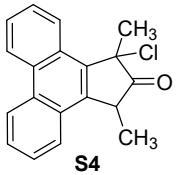
3,4-Dihydro-4-hydroxy-1,3-dimethyl-2*H*-cyclopenta[*l*]phenanthren-2-one (S3)



S3

The title compound **S3** was synthesised using the literature procedure reported by Jones.[4] K_2CO_3 (13.3 g, 96 mmol) and pentan-3-one (10.2 mL, 96 mmol) were suspended in CH_3OH (200 mL). To the above suspension was added phenanthrene-9,10-dione (10.0 g, 48 mmol) portion-wise over 15 min and the mixture was stirred at rt for 18 h. The reaction mixture was concentrated to ~20 mL *in vacuo* and suspended in H_2O (~100 mL). The insoluble solid was collected by vacuum filtration. The solid was then dissolved in DCM, washed with 1M HCl, H_2O and brine then dried over $MgSO_4$. The organic extracts were concentrated *in vacuo* to yield the title compound **S3** as a yellow-brown solid (11.2 g, 84%). IR (ATR) ν_{max}/cm^{-1} 3340, 1670; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (3H, d, *J* = 7.4 Hz), 2.08 (3H, s), 2.12 (1H, br s), 3.05 (1H, q, *J* = 7.5 Hz), 7.35–7.55 (5H, m), 7.70–7.74 (1H, m), 7.89–7.92 (1H, m), 7.96–7.98 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.8, 11.4, 48.0, 75.4, 124.5, 125.0, 126.5, 128.3, 128.5, 129.2, 129.4, 129.5, 130.9, 132.5, 133.4, 135.1, 139.4, 161.6, 208.8; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{19}H_{16}O_2Na^+$, 299.1043; found 299.1017.

1-Chloro-1,3-dihydro-1,3-dimethyl-2*H*-cyclopenta[*l*]phenanthren-2-one (S4)



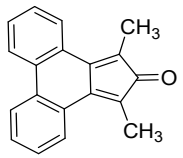
S4

The title compound **S4** was synthesised using the literature procedure reported by Jones.[4] 3,4-Dihydro-4-hydroxy-1,3-dimethyl-2*H*-cyclopenta[*l*]phenanthren-2-one (**S3**) (11.2 g, 40.6 mmol) was treated dropwise with acetyl chloride (38.2 mL, 487 mmol) and then the reaction mixture was stirred at 0–5°C for 1 h. The precipitate was filtered under vacuum and washed with petroleum ether to give the title compound **S4** as a pale pink solid (10.2 g, 85%) as a 2:1 mixture of diastereoisomers. IR (ATR) ν_{max}/cm^{-1} 1750; HRMS (ESI-TOF) *m/z*: [M - Cl]$^+$ calcd for $C_{19}H_{14}O^+$, 259.1117; found 259.1106.

Major diastereoisomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ *inter alia* 1.80 (3H, d, *J* = 7.5 Hz), 2.22 (3H, s), 4.06 (1H, q, *J* = 7.5 Hz), 7.68–7.81 (4H, m), 7.96–8.02 (1H, m), 8.46–8.55 (1H, m), 8.76–8.81 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ *inter alia* 19.2, 25.8, 44.4, 44.8, 68.6, 69.5, 123.8, 123.8, 123.8, 125.5, 125.6, 125.8, 125.84, 125.9, 127.0, 127.1, 127.12, 127.3, 127.4, 127.4, 127.4, 127.5, 127.6, 127.7, 127.9, 128.0, 128.1, 131.4, 131.7, 131.8, 134.9, 135.1, 136.9, 137.2, 213.1.

Minor diastereoisomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ *inter alia* 1.69 (3H, d, *J* = 7.6), 2.26 (3H, s), 4.20 (1H, q, *J* = 7.5 Hz), 7.68–7.81 (4H, m), 7.96–8.02 (1H, m), 8.46–8.55 (1H, m), 8.76–8.81 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ *inter alia* 18.9, 28.2, 44.4, 44.8, 68.6, 69.5, 123.8, 123.8, 123.8, 125.5, 125.6, 125.8, 125.8, 125.9, 127.0, 127.1, 127.1, 127.3, 127.4, 127.4, 127.4, 127.5, 127.6, 127.7, 127.9, 128.0, 128.1, 131.4, 131.7, 131.8, 134.9, 135.1, 136.9, 137.2, 211.9.

1,3-Dimethyl-2*H*-cyclopenta[*l*]phenanthren-2-one (9)

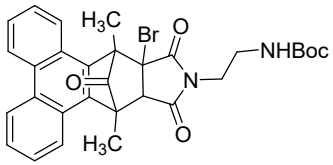


The title compound **9** was synthesised using the literature procedure reported by Jones.[4] 1-Chloro-1,3-dihydro-1,3-dimethyl-2*H*-cyclopenta[*l*]phenanthren-2-one (**S4**) (8.03 g, 27.2 mmol) was added over 5–10 min to a solution of NEt_3 (7.6 mL, 33.9 mmol) in refluxing toluene (50 mL). The reaction mixture was heated at reflux for 1 h and then allowed to cool to rt. CH_2Cl_2 was added and then the organic phase washed with H_2O and 2M H_2SO_4, then filtered and concentrated *in vacuo* to yield the title compound **9** as a pink solid (4.65 g, 66%) as a mixture of the monomer and the dimer. IR (ATR) ν_{max}/cm^{-1} 1760, 1680.

Dimer of **9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ *inter alia* 1.34 (3H, s), 1.40 (3H, s), 1.98 (3H, s), 2.10 (3H, s), 5.61 (1H, d, *J* = 8.1 Hz), 6.27 (1H, t, *J* = 7.7 Hz), 7.07 (1H, t, *J* = 7.6 Hz), 7.14–7.20 (2H, m), 7.47–7.53 (2H, m), 7.60 (1H, t, *J* = 7.6 Hz), 7.72–7.74 (3H, m), 7.92 (1H, d, *J* = 8.0 Hz), 8.04 (1H, d, *J* = 8.0 Hz), 8.46–8.48 (1H, m), 8.71 (1H, d, *J* = 8.5 Hz), 8.81–8.84 (1H, m); HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{38}H_{28}O_2Na^+$, 539.1982; found 539.1977.

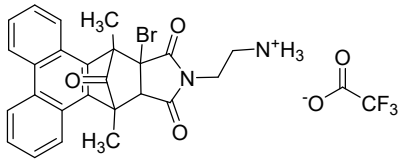
Monomer of **9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ *inter alia* 1.22 (3H, s), 1.88 (3H, s), 6.93 (1H, br s), 7.14–7.20 (1H, m), 7.48–7.59 (3H, m), 7.84 (1H, br s), 8.31–8.40 (2H, m); HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{19}H_{14}ONa^+$, 281.0937; found 281.0932.

Compound 10



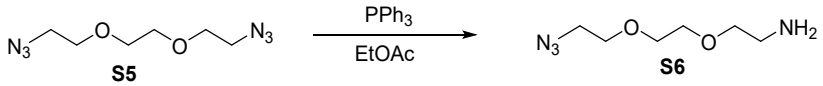
Diene dimer **9** (201 mg, 0.78 mmol) and *tert*-butyl 3-(3-bromo-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethylcarbamate (**5**)[2] (254 mg, 0.77 mmol) were stirred in toluene (50 mL) at 50 ºC for 18 h. The reaction mixture was concentrated *in vacuo* to give the crude product, which was triturated with Et_2O. The resulting precipitate was filtered under vacuum to give the *title compound* **10** as a white solid (350 mg, 78%, *endo* only). IR (ATR) ν_{max}/cm^{-1} 3410, 1790, 1710; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32 (9H, s), 1.86–1.89 (2H, m), 2.30 (3H, s), 2.33 (3H, s), 2.96–3.01 (2H, m), 3.05–3.10 (1H, m), 3.60 (1H, s), 7.65–7.72 (4H, m), 8.26–8.28 (1H, m), 8.32–8.34 (1H, m), 8.73 (2H, d, *J* = 8.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 14.9, 28.5, 39.2, 39.6, 55.0, 57.7, 58.3, 60.1, 79.4, 123.7, 125.1, 125.3, 126.5, 126.9, 127.8, 127.9, 128.2, 130.6, 130.9, 131.5, 134.9, 155.4, 171.9, 172.3, 198.2; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{30}H_{29}{}^{79}BrN_2O_5Na^+$, 599.1152; found 599.1135.

oCOm-62



Boc-protected-oCOm-62 (**10**) (202 mg, 0.349 mmol) was dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C. Trifluoroacetic acid (0.53 mL, 6.94 mmol) was added dropwise and the reaction mixture was allowed to warm to rt and stirred for 18 h. Removal of solvent *in vacuo* provided the *title compound* **oCOm-62** as a white solid (200 mg, 97%) and was used without further purification. IR (ATR) ν_{max}/cm^{-1} 2940, 1780, 1710; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.44–1.47 (2H, m), 2.18 (3H, s), 2.25 (3H, s), 2.78–2.85 (2H, m), 4.10 (1H, s), 7.42 (3H, br s), 7.69–7.79 (4H, m), 8.18 (1H, dd, J = 8.0, 1.6 Hz), 8.33 (1H, dd, J = 8.2, 1.5 Hz), 8.91–8.93 (2H, m); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.7, 14.4, 34.3, 35.2, 54.2, 57.5, 57.7, 58.4, 123.8, 123.9, 124.6, 124.6, 125.6, 126.2, 127.4, 127.5, 127.8, 130.1, 130.3, 130.5, 134.6, 170.9, 171.6, 198.2; HRMS (ESI-TOF) *m/z*: $[M - CF_3COO^-]^+$ calcd for $C_{25}H_{22}{}^{79}BrN_2O_3^+$, 477.0808; found 477.0811.

2-[2-(2-azidoethoxy)ethoxy}]ethanamine (S6)

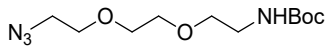


S5 **S6**

Triphenylphosphine (1.81 g, 6.90 mmol) in EtOAc (30 mL) was added dropwise at rt, to a stirred solution of diazide **S5**[5] (1.41 g, 7.04 mmol) in a mixture of EtOAc (30 mL) and 1M HCl (37 mL). The reaction mixture was vigorously stirred at rt for 7 h and then the EtOAc layer was removed. The aqueous phase was washed with EtOAc and then adjusted to pH 12 with 2M NaOH. The resulting aqueous solution was extracted with $CHCl_3$ and the combined organic extracts were dried over $MgSO_4$. The solvent was evaporated *in vacuo* to yield the title compound **S6** as a colourless oil (0.701 g, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87 (2H, t, J = 4.3 Hz), 3.39 (2H, t, J = 5.0 Hz), 3.52 (2H, t, J = 5.2 Hz), 3.65–3.70

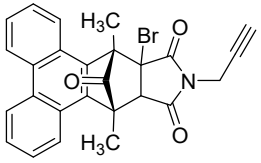
(6H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.9, 50.8, 70.2, 70.5, 70.8, 73.7. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_6H_{14}N_4O_2Na^+$, 197.1009; found 197.1004. The NMR data obtained were consistent with those from the literature.[5]

***Tert*-Butyl *N*-2-[2-[2-azidoethoxy)ethoxy]ethyl]carbamate (14)**



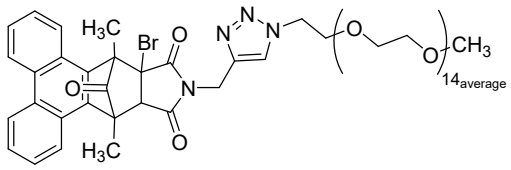
A solution of di-*tert*-butyl dicarbonate (1.75 g, 8.03 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a solution of **S6** (0.70 g, 4.00 mmol) in CH_2Cl_2 (40 mL) at 0 ºC. The reaction was stirred at rt for 18 h. Removal of the solvent *in vacuo* yielded the crude product which was then purified by flash chromatography (5%, then 40% EtOAc in petroleum ether) to yield the title compound **14** as a yellow oil (0.64 g, 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (9H, s), 3.28–3.34 (2H, m), 3.39 (2H, t, *J* = 5.1 Hz), 3.54 (2H, t, *J* = 5.2 Hz), 3.61–3.65 (4H, m), 3.67 (2H, t, *J* = 5.2 Hz), 5.00 (1H, br s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.5, 40.6, 50.8, 70.2, 70.4, 70.5, 70.7, 79.4, 156.1; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{11}H_{22}N_4O_4Na^+$, 297.1533; found 297.1527. The NMR data obtained were consistent with those from the literature.[6]

***Endo*-3a-bromo-2-(2-propyn-1-yl)-3a,4,7,7a-tetrahydro-4,7-dimethyl-5,6-phenanthro-1*H*-isoindole-1,3,8(2*H*)-trione (12)**



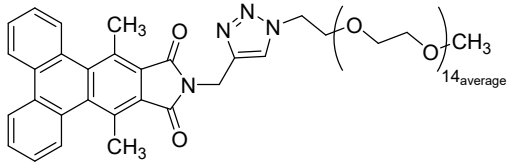
Exo-3a-bromo-2-(2-propn-1-yl)-3a,4,7,7a-tetrahydro-4,7-epoxy-*1H*-isoindole-1,3(*2H*)-dione (**11**)[7] (1.00 g, 3.55 mmol) was dissolved in toluene (60 mL) and heated at 124 ºC for 7 h in an open flask. The reaction mixture was then cooled to 50 ˚C and the diene dimer **9** (0.92 g, 3.55 mmol) was added. The reaction mixture was heated at 50 ºC for another 7 h and concentrated *in vacuo* to give the crude product, which was triturated from Et_2O and filtered under vacuum to yield the *title compound* **12** as a pale white solid (1.31 g, 78%, *endo* only). IR (ATR) ν_{max}/cm^{-1} 1780, 1710; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (1H, t, *J* = 2.6 Hz), 2.30 (3H, s), 2.33 (3H, s), 3.58–3.59 (2H, m), 3.64 (1H, s), 7.63–7.69 (4H, m), 8.24–8.26 (1H, m), 8.31 (1H, d, *J* = 9.4 Hz), 8.68–8.70 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 15.0, 28.1, 54.9, 57.8, 58.2, 60.3, 60.4, 70.6, 73.8, 123.3, 125.3, 125.5, 126.8, 127.2, 127.4, 127.4, 127.5, 127.8, 131.4, 131.6, 134.7, 170.5, 171.1, 197.9; HRMS (ESI-TOF) *m/z*: [M + Na]$^+$ calcd for $C_{26}H_{18}{}^{79}BrNO_3Na^+$, 494.0362; found 494.0356.

oCOm-57



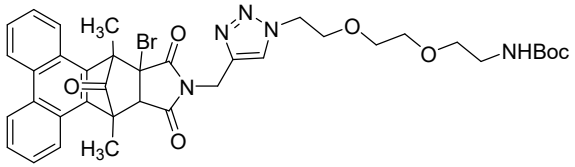
Cycloadduct **12** (0.30 g, 0.637 mmol) and CuI·P(OEt)$_3$ (0.028 g, 0.078 mmol) were dissolved in anhydrous THF (9 mL) under N_2, followed by the addition of a solution of mPEG-750-N_3 (**13**)[8] (0.43 g, 0.60 mmol) in anhydrous THF (3 mL). The reaction mixture was heated at 65 ºC for 5 days and then cooled to rt. Removal of the solvent *in vacuo* gave the crude product, which was purified by flash chromatography (0%, then 10% CH_3OH in $CHCl_3$) to yield the *title compound* **oCOm-57** as a yellow oil (0.75 g, 99%). R_f (10% CH_3OH in $CHCl_3$) 0.40; IR (ATR) ν_{max}/cm^{-1} 2870, 1790, 1710; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.25 (3H, s), 2.29 (3H, s), 3.38 (3H, s), 3.42–3.44 (2H, m), 3.49–3.51 (2H, m), 3.53–3.57 (6H, m), 3.60–3.66 (60H, m), 3.73–3.76 (2H, m), 4.01 (2H, t, *J* = 5.4 Hz), 4.17 (2H, s), 6.36 (1H, s), 7.62–7.71 (4H, m), 8.17–8.19 (1H, m), 8.23–8.25 (1H, m), 8.64 (2H, d, *J* = 8.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 14.9, 25.8, 34.2, 50.1, 54.9, 57.9, 58.2, 59.2, 60.1, 69.4, 70.5, 70.6, 70.7, 70.7, 70.8, 72.1, 123.3, 125.3, 125.5, 126.4, 126.7, 127.4, 127.4, 127.4, 127.7, 130.8, 131.0, 131.2, 134.6, 171.1, 171.6, 197.7; HRMS (ESI-TOF) *m/z*: [M + Na]$^+$ calcd for $C_{57}H_{81}{}^{79}BrN_4O_{18}Na^+$, 1211.4622; found 1211.4611; RP-HPLC[254 nm] (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100%B to 10%B over 1 min, then 10% B for 3 min), t_R = 12.1 min.

BP-57



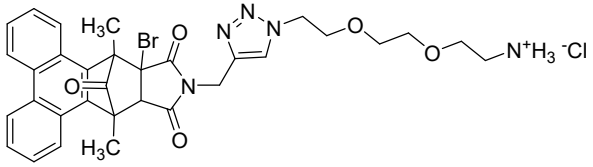
oCOm-57 (0.279 g, 0.235 mmol) and DBU (0.212 mL, 1.41 mmol) were dissolved in THF (15 mL) and stirred at rt for 18 h. The reaction mixture was then extracted into CH_2Cl_2, washed with H_2O and 1 M HCl, dried over $MgSO_4$ and concentrated *in vacuo* to give the crude product. Purification by flash chromatography (0%, then 4% CH_3OH in $CHCl_3$) yielded the *title compound* **BP-57** as a yellow oil (0.070 g, 27%). R_f (10% CH_3OH in $CHCl_3$) 0.56; Quantum yield Φ = 0.16 (1 mM in absolute EtOH, λ_{ex} = 400 nm, λ_{em} = 455 nm); IR (ATR) ν_{max}/cm^{-1} 2870, 1700; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.19 (6H, s), 3.37 (3H, s), 3.52–3.56 (2H, m), 3.59–3.67 (60H, m), 3.86 (2H, t, *J* = 5.1 Hz), 4.52 (2H, t, *J* = 5.1 Hz), 5.04 (2H, s), 7.55–7.58 (2H, m), 7.64-7.67 (2H, m), 7.82 (1H, br s), 8.32 (2H, d, *J* = 8.3 Hz), 8.52 (2H, d, *J* = 8.41 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.4, 29.8, 33.0, 50.4, 59.1, 69.6, 70.6, 72.1, 123.8, 124.1, 126.7, 126.8, 128.4, 129.0, 130.0, 131.7, 132.6, 138.0, 143.1, 168.5; HRMS (ESI-TOF) *m/z*: [M + Na]$^+$ calcd for $C_{56}H_{80}N_4O_{17}Na^+$, 1103.5411; found, 1103.5398.

Boc-protected oCOm-58 (15)



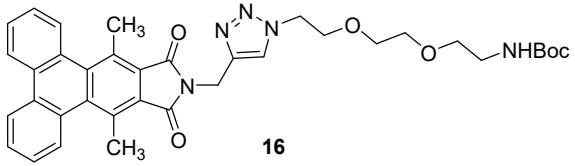
Cycloadduct **12** (0.39 g, 0.83 mmol) and CuI·P(OEt)$_3$ (0.0325 g, 0.0911 mmol) were dissolved in anhydrous THF (9 mL) under N_2, followed by the addition of a solution of *tert*-butyl *N*-2-[2-[2-azidoethoxy)ethoxy]ethyl]carbamate (**14**) (0.24 g, 0.86 mmol) in anhydrous THF (3 mL). The solution was heated at 65 ºC for 6 days and then cooled to rt. The reaction mixture was concentrated *in vacuo* to give the crude product. Purification by flash chromatography (80% EtOAc in petroleum ether, then 100% EtOAc) gave the *title compound* **15** as a pale yellow oil (0.37 g, 59%). R_f (100% EtOAc) 0.44; IR (ATR) ν_{max}/cm^{-1} 2970, 1790, 1710; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (9H, s), 2.25 (3H, s), 2.29 (3H, s), 3.26–3.30 (2H, m), 3.40–3.43 (2H, m), 3.45–3.48 (4H, m), 3.54–3.56 (2H, m), 3.64 (1H, s), 4.03 (2H, t, *J* = 5.3 Hz), 4.18 (2H, s), 4.95 (1H, br s), 6.41 (1H, s), 7.62–7.71 (4H, m), 8.19 (1H, dd, *J* = 8.1, 1.3 Hz), 8.25 (1H, dd, *J* = 8.2, 1.1 Hz), 8.64 (2H, d, *J* = 8.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 14.9, 28.6, 34.2, 40.5, 50.1, 54.9, 57.8, 58.2, 60.1, 60.2, 69.3, 70.2, 70.4, 70.5, 70.5, 123.3, 125.3, 125.5, 126.4, 126.7, 127.3, 127.4, 127.7, 130.8, 131.0, 131.2, 131.2, 134.6, 139.9, 156.1, 171.2, 171.7, 197.7; HRMS (ESI-TOF) *m/z*: [M + Na]$^+$ calcd for $C_{37}H_{40}{}^{79}BrN_5O_7Na^+$, 768.2003; found 768.1976.

oCOm-58



Adduct **15** (0.36 g, 0.48 mmol) was dissolved in anhydrous 1,4-dioxane (3 mL) and cooled to 0 ºC. A solution of 6M HCl in 1,4-dioxane (3 mL) was then added dropwise and the reaction mixture was warmed to rt and stirred for 18 h. The solvent was removed *in vacuo* to provide the amine hydrochloride salt which was then lyophilised to provide the crude product. The crude material was dissolved in 10% CH_3CN in H_2O containing 0.1% HCl (2 x 5 mL) and then loaded onto a C-18 solid phase extraction cartridge (pre-conditioning procedure: 100% CH_3CN [2 x 10 mL], then 90%, then 50% CH_3CN in H_2O [1 x 10mL each], 10% CH_3CN in H_2O [2 x 10 mL]). The compound was eluted from the C-18 cartridge (10% to 50% CH_3CN in H_2O containing 0.1% HCl [10% increments, 2 x 10 mL each], then 60% to 100% CH_3CN in H_2O [10% increments, 10 mL each]). The fractions containing the product were then lyophilised to yield the *title compound* **oCOm-58** as a white powder (197 mg, 60%, *endo* only). M.p 186 ºC; IR (ATR) ν_{max}/cm^{-1} 2870, 1780, 1710; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.14 (3H, s), 2.20 (3H, s), 2.87–2.92 (2H, m), 3.41–3.47 (6H, m), 3.55 (2H, t, J = 5.3 Hz), 3.98 (2H, s), 4.09 (2H, q, J = 5.1 Hz), 4.22 (1H, s), 6.51 (1H, s), 7.68–7.77 (7H, m), 8.11–8.13 (1H, m), 8.24–8.26 (1H, m), 8.87 (2H, dd, J = 7.9, 4.1 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.9, 14.5, 33.8, 38.5, 40.1, 49.3, 54.3, 57.5, 58.0, 58.2, 66.6, 68.4, 69.3, 69.4, 122.5, 123.8, 123.8, 124.7, 125.6, 126.1, 127.3, 127.3, 127.4, 127.7, 130.2, 130.4, 130.5, 134.5, 139.4, 170.8, 171.4, 197.9; HRMS (ESI-TOF) *m/z*: [M – Cl$^-$]$^+$ calcd for $C_{32}H_{33}{}^{79}BrN_5O_5^+$, 646.1660; found 646.1654; RP-HPLC[254 nm] (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100%B to 10%B over 1 min, then 10% B for 3 min), t_R = 9.52 min.

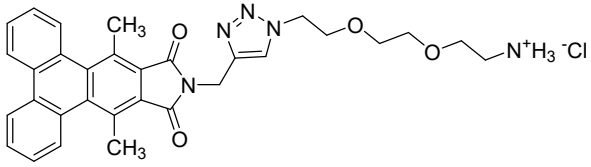
Boc-protected BP-58 (16)



16

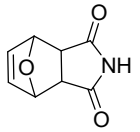
Boc-protected **oCOm-58** (**15**) (0.16 g, 0.21 mmol) was dissolved in anhydrous THF (10 mL) and cooled to 0 ºC. DBU (0.19 mL, 12.6 mmol) was added and the solution was warmed to rt and stirred for 1 h. CH_2Cl_2 was added and the organic phase was sequentially washed with saturated NH_4Cl, H_2O and then saturated NaCl. The organic phase was dried over $MgSO_4$ and the solvent removed *in vacuo* to give the crude compound, which was then filtered through a plug of silica (100% EtOAc) to give the *title compound* **16** as a pale yellow oil (98 mg, 74%). R_f (100% EtOAc) 0.40; IR (ATR) ν_{max}/cm^{-1} 1690; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (9H, s), 3.20 (6H, s), 3.33 (2H, br s), 3.53 (2H, t, *J* = 5.2 Hz), 3.58 (4H, s), 3.86 (2H, t, *J* = 5.1 Hz), 4.54 (2H, t, *J* = 5.1 Hz), 5.06 (2H, s), 5.14 (1H, br s), 7.55–7.59 (2H, m), 7.65–7.69 (2H, m), 7.85 (1H, s), 8.32–8.34 (2H, m), 8.52–8.54 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.3, 28.5, 32.9, 40.5, 50.3, 69.5, 70.2, 70.3, 70.6, 79.3, 123.7, 124.2, 126.5, 126.6, 128.4, 128.8, 129.8, 131.6, 132.5, 137.9, 143.1, 156.1, 168.4; HRMS-ESI (*m/z*): $[M + Na]^+$ calcd for $C_{36}H_{39}N_5O_6Na^+$, 660.2793; found 660.2778.

BP-58



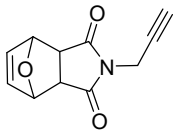
Boc-protected **BP-58** (**16**) (89 mg, 0.14 mmol) was dissolved in anhydrous 1,4-dioxane (3 mL) and cooled to 0 ºC. A solution of 6M HCl in 1,4-dioxane (3 mL) was added dropwise and the reaction mixture was warmed to rt and stirred for 18 h. The solvent was removed *in vacuo* to provide the amine hydrochloride salt, which was then lyophilised to provide the crude product. The crude material was dissolved in 10% CH_3CN in H_2O containing 0.1% HCl (2 x 5 mL) and then loaded onto a C-18 solid phase extraction cartridge (pre-conditioning procedure: 100% CH_3CN [2 x 10 mL], then 90%, then 50% CH_3CN in H_2O [1 x 10mL each], 10% CH_3CN in H_2O [2 x 10 mL]). The compound was eluted from the C-18 cartridge (10% to 50% CH_3CN in H_2O containing 0.1% HCl [10% increments, 2 x 10 mL each], then 60% to 100% CH_3CN in H_2O [10% increments, 10 mL each]). The fractions containing the product were then lyophilised to yield the *title compound* **BP-58** as a white powder (45 mg, 56%). Quantum yield Φ = 0.16 (1 mM in absolute EtOH, λ_{ex} = 400 nm, λ_{em} = 470 nm); IR (ATR) ν_{max}/cm^{-1} 1700; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.93–2.96 (2H, m), 3.16 (6H, s), 3.52–3.57 (6H, m), 3.83 (2H, t, *J* = 5.3 Hz), 4.52 (2H, t, *J* = 5.3 Hz), 4.90 (2H, s), 7.66 (2H, t, *J* = 7.7 Hz), 7.76 (2H, t, *J* = 7.6 Hz), 7.81 (3H, br s), 8.11 (1H, s), 8.44 (2H, d, *J* = 8.3 Hz), 8.73 (2H, d, *J* = 8.1 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.8, 32.8, 38.6, 49.4, 66.6, 68.6, 69.4, 69.5, 123.6, 123.9, 126.3, 126.9, 128.7, 128.8, 129.0, 131.0, 132.0, 137.0, 142.3, 167.7; HRMS (ESI-TOF) *m/z*: [M – Cl$^-$]$^+$ calcd for $C_{31}H_{32}N_5O_4^+$, 538.24488; found 538.24451; RP-HPLC[254 nm] (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100%B to 10%B over 1 min, then 10% B for 3 min), t_R = 9.74 min.

***Exo*-3a,4,7,7a-tetrahydro-4,7-epoxy-*1H*-isoindole-1,3(*2H*)-dione (S7)**



Furan (9.4 mL, 129 mmol) was added to a suspension of maleimide (1.01 g, 10.4 mmol) in Et_2O (2 mL) and the resulting mixture was split evenly between 2 Kimax tubes. The tubes were sealed and heated at 80 ºC for 2 days. The suspension was diluted with CH_2Cl_2 and Et_2O and concentrated *in vacuo* to yield the crude compound, which was re-crystallised from CH_2Cl_2 and filtered under vacuum to yield the title compound **S7** as an off-white solid (1.71 g, 99%, *exo* only). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.89 (2H, s), 5.31 (2H, s), 6.52 (2H, s), 8.07 (1H, br s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 48.8, 81.1, 136.7, 176.1; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_8H_7NO_3Na^+$, 188.0318; found 188.0316. The NMR data obtained were consistent with those reported from the literature.[9]

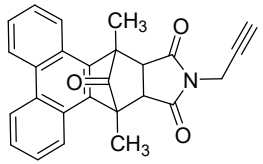
Exo-2-(2-propn-1-yl)-3a,4,7,7a-tetrahydro-4,7-epoxy-_1H_-isoindole-1,3(_2H_)-dione (S8)



To a mixture of furan adduct **S7** (1.71 g, 10.3 mmol) and K_2CO_3 (7.10 g, 51.4mmol) in anhydrous DMF (40 mL) was added propargyl bromide (1.42 mL, 18.9 mmol). The reaction mixture was heated at 50 ºC for 2 h, cooled to rt and diluted with EtOAc. The organic phase was washed with H_2O and the aqueous washing was back extracted with EtOAc. The combined organic extracts were washed with saturated NaCl, dried over $MgSO_4$ and the solvents removed *in vacuo* to yield the title compound **S8** as an off-white solid (1.33 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20 (1H, t, *J* = 2.6 Hz), 2.91 (2H, s), 4.24 (2H, d, *J* = 2.6 Hz), 5.30–5.31 (2H, m), 6.52–6.53 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.0, 47.7,

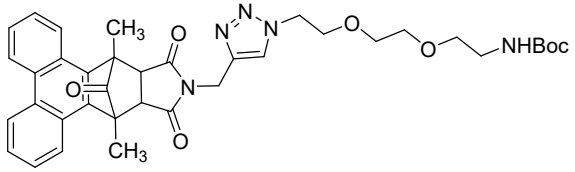
71.6, 77.4, 81.1, 136.7, 175.0; HRMS (ESI-TOF) *m/z*: [M +Na]$^+$ calcd for $C_{11}H_9NO_3Na^+$, 226.0475; found 226.0475. The NMR data obtained were consistent with those reported from the literature.[10]

Endo-2-(2-propyn-1-yl)-3a,4,7,7a-tetrahydro-4,7-dimethyl-5,6-phenanthro-1_H_-isoindole-1,3,8(_2H_)-trione (S9)



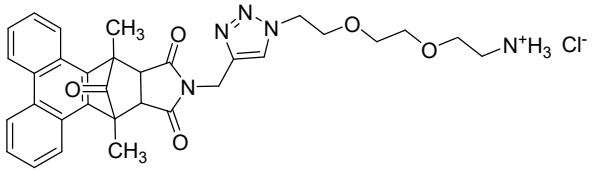
Furan adduct **S8** (1.33 g, 6.53 mmol) was dissolved in toluene (60 mL) and heated at 124 ºC for 9 h in an open flask. The reaction mixture was then cooled to 50 ºC and diene dimer **9** (1.66 g, 6.43 mmol) was added. The reaction mixture was heated at 50 ºC for 7 h and concentrated *in vacuo* to give the crude product, which was purified by flash chromatography (0%, then 5%, then 10% Et_2O in toluene) to yield the *title compound* **S9** as a light pink solid (1.33 g, 52%, *endo* only). R_f (10% Et_2O in toluene) 0.23; IR (ATR) ν_{max}/cm^{-1} 1780, 1710; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (1H, t, *J* = 2.5 Hz), 2.29 (6H, s), 3.39 (2H, s), 3.50 (2H, d, *J* = 2.5 Hz), 7.62–7.66 (4H, m), 8.33–8.36 (2H, m), 8.67–8.71 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.8, 27.4, 47.7, 54.9, 70.1, 74.4, 123.2, 125.3, 127.1, 127.2, 127.4, 131.1, 133.0, 173.7, 200.4; HRMS (ESI-TOF) *m/z*: [M + Na]$^+$ calcd for $C_{26}H_{19}NO_3Na^+$, 416.1257; found 416.1247.

Boc-protected DB-58 (S10)



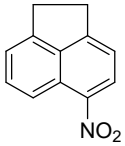
Cycloadduct **S9** (198 mg, 0.50 mmol) and CuI·P(OEt)$_3$ (18 mg, 0.050 mmol) was dissolved in anhydrous THF (9 mL) under N_2, followed by the addition of a solution of *tert*-butyl *N*-2-[2-[2-azidoethoxy)ethoxy]ethyl]carbamate (**14**) (139 mg, 0.51 mmol) in anhydrous THF (3 mL). The reaction mixture was heated at 65 ºC for 4 days and then cooled to rt. The solvent was removed *in vacuo* to give the crude product, which was purified by flash chromatography (0%, then 10% CH_3OH in $CHCl_3$) to yield the *title compound* **S10** as a yellow oil (185 mg, 55%). R_f (10% CH_3OH in $CHCl_3$) 0.35; IR (ATR) ν_{max}/cm^{-1} 1780, 1700; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (9H, s), 2.24 (6H, s), 3.27–3.28 (2H, m), 3.39 (2H, s), 3.40–3.42 (2H, m), 3.44–3.47 (4H, m), 3.54 (2H, t, *J* = 5.0 Hz), 4.03 (2H, t, *J* = 5.0 Hz), 4.10 (2H, s), 5.00 (1H, br s), 6.43 (1H, br s), 7.61–7.68 (4H, m), 8.29 (2H, dd, *J* = 7.9, 1.6 Hz), 8.65 (2H, dd, *J* = 8.1, 1.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.7, 28.6, 33.6, 40.5, 47.6, 50.0, 54.9, 69.3, 70.1, 70.4, 70.5, 79.5, 123.2, 125.3, 127.1, 127.1, 127.2, 130.6, 132.9, 156.1, 174.4, 200.2; HRMS (ESI-TOF) *m/z*: $[M + H]^+$ calcd for $C_{37}H_{42}N_5O_7^+$, 668.3079; found 668.3069.

DB-58



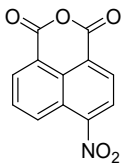
Adduct **S10** (192 mg, 0.29 mmol) was dissolved in 1,4-dioxane (3 mL) and cooled to 0 ºC. A solution of 6M HCl in 1,4-dioxane (1 mL) was added dropwise and the reaction mixture was warmed to rt and stirred for 18 h. Removal of the solvent *in vacuo* provided the amine hydrochloride salt, which was then lyophilised to provide the crude product. The crude material was dissolved in 10% CH_3CN in H_2O containing 0.1% HCl (2 x 5 mL) and then loaded onto a C-18 solid phase extraction cartridge (pre-conditioning procedure: 100% CH_3CN [2 x 10 mL], then 90%, then 50% CH_3CN in H_2O [1 x 10mL each], 10% CH_3CN in H_2O [2 x 10 mL]). The compound was eluted from the C-18 cartridge (10% to 50% CH_3CN in H_2O containing 0.1% HCl [10% increments, 2 x 10 mL each], then 60% to 100% CH_3CN in H_2O [10% increments, 10 mL each]). The fractions containing the product were lyophilised to yield the *title compound* **DB-58** as a white powder (117 mg, 67%). IR (ATR) ν_{max}/cm^{-1} 3390, 1770, 1700; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.13 (6H, s), 2.87–2.92 (2H, m), 3.40–3.47 (8H, m), 3.52 (2H, t, J = 5.0 Hz), 3.69 (2H, s), 3.89 (2H, s), 4.03 (2H, t, J = 5.1 Hz), 6.22 (1H, s), 7.67–7.63 (4H, m), 7.78 (3H, br s), 8.24 (2H, dd, J = 7.9, 1.8 Hz), 8.87–8.88 (2H, m); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.4, 33.2, 38.5, 47.1, 49.2, 50.7, 54.1, 58.2, 66.6, 68.4, 69.3, 69.4, 69.4, 122.0, 123.7, 124.7, 126.5, 127.0, 127.2, 129.9, 132.8, 140.4, 174.7, 200.1; HRMS (ESI-TOF) *m/z*: $[M]^+$ calcd for $C_{32}H_{34}N_5O_5^+$, 568.2555; found 568.2556; RP-HPLC[254 nm] (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100%B to 10%B over 1 min, then 10% B for 3 min), t_R = 8.77 min.

5-Nitro-1,2-dihydroacenaphthylene (S11)



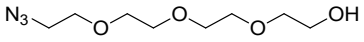
To a suspension of acenaphthene (7.7 g, 0.05 mol) in AcOH (60 mL), was added concentrated HNO_3 (70% aqueous, 5.5 mL) at rt. The reaction mixture was stirred for 4 hours at rt and then poured into ice-cold H_2O (200 mL). The resulting precipitate was isolated by Buchner filtration, washed with H_2O (200 mL) and then dried. The precipitate was then crystallised from hot AcOH (90 mL) heated to 90°C. The resulting crystals formed after cooling to rt were then recrystallised from hot EtOH (150 mL) heated 70°C to afford the title compound **S11** as yellow needles (3.86 g, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.44–3.47 (2H, m), 3.49–3.52 (2H, m), 7.34 (1H, dt, *J* = 7.7, 1.3 Hz), 7.47–7.44 (1H, m), 7.73 (1H, dd, *J* = 8.6, 7.0 Hz), 8.51 (1H, d, *J* = 7.7 Hz), 8.57 (1H, dd, *J* = 8.7, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.6, 30.6, 118.0, 120.2, 121.2, 124.4, 127.8, 132.0, 140.2, 146.6, 155.8. The NMR data were consistent with those obtained from the literature.[11]

6-Nitro-1*H*,3*H*-benzo[*de*]isochromene-1,3-dione (S12)



To a stirred solution of sodium dichromate dihydrate (3.74 g, 0.0125 mol) in AcOH (33 mL) was added a solution of **S11** (1.0 g, 0.005 mol) in AcOH (84 mL) at rt. The orange reaction mixture was heated at reflux for 5 hours and changed appearance to a dark green-black mixture. The reaction mixture was cooled to 0°C and ice-cold H_2O (50 mL) was slowly added. The resulting precipitate was isolated by Buchner filtration and washed with ice-cold H_2O (200 mL) until the filtrate was neutral as indicated by pH paper. The precipitate was then dried to give the title compound **S12** as a yellow powder (0.443 g, 36%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.12 (1H, dd, *J* = 8.7, 7.3 Hz), 8.57 (1H, d, *J* = 8.0 Hz), 8.64 (1H, d, *J* = 8.0 Hz), 8.66 (1H, dd, *J* = 7.3, 1.0 Hz), 8.76 (1H, dd, *J* = 8.7, 1.0 Hz); ^{13}C NMR (100 MHz, DMSO-*d6*) δ 120.1, 122.9, 124.1, 124.4, 129.9, 130.4, 130.7, 131.2, 133.3, 149.6, 159.5, 160.1; RP-HPLC-ELSD (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100% to 10% B over 1 min, 10% B for 3 min), t_R = 10.75 minutes. The NMR data were consistent with those obtained from the literature.[11]

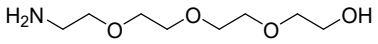
2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethanol (S13)



To a solution of tetraethylene glycol (50.9 g, 0.26 mmol) in THF (20 mL) at 0°C was added a solution of NaOH (1.7 g, 0.043 mmol) in H_2O (10 mL) and stirred for 0°C for 30 minutes. A solution of tosyl chloride (5.0 g, 0.026 mmol) in anhydrous THF (32 mL) was added dropwise over 30 minutes and the reaction mixture was then stirred at 0°C for 2 hours. The reaction mixture was poured onto ice-cold H_2O (100 mL) and the aqueous phase extracted with CH_2Cl_2 (3 x 120 mL). The combined organic extracts were washed with H_2O (2 x 100 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* to give the mono-tosylate as a pale oil (8.24 g) which was used immediately without purification.

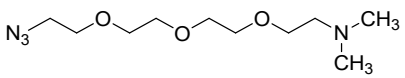
This was diluted in EtOH (133 mL) and NaN_3 (7.81 g, 0.12 mmol) was added. The resulting suspension was stirred at 70°C for 20 hours and then cooled to rt. H_2O (70 mL) was added and the EtOH removed *in vacuo*. The aqueous phase was then saturated with $NaCl_{(s)}$ and extracted with EtOAc (3 x 100 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to obtain a yellow oil. Purification by flash chromatography (CH_2Cl_2 load, 50% EtOAc in petroleum ether, then 100% EtOAc, then 10% CH_3OH in CH_2Cl_2) gave the title compound **S13** as a pale yellow oil (3.80 g, 71%). R_f (100% EtOAc) 0.25; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.39 (2H, t, *J* = 5.1 Hz), 3.60–3.62 (2H, m), 3.66–3.69 (10H, m), 3.72–3.74 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 50.7, 61.8, 70.0, 70.0, 70.4, 70.6, 70.7, 70.7, 72.4. The NMR data were consistent with those obtained from the literature.[12]

2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethan-1-ol (S14)



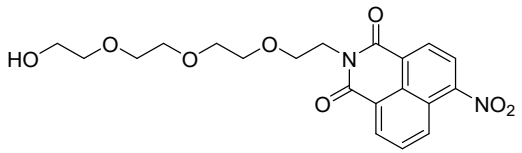
To a solution of azide **S13** (1.94 g, 8.85 mmol) in 10% H_2O in THF (50 mL) at rt was added triphenylphosphine (2.39 g, 9.11 mmol). The reaction was stirred at rt for 65 h and then diluted with H_2O (70 mL). The aqueous phase was extracted with toluene (3 x 100 mL) and the aqueous phase was concentrated *in vacuo* to get the crude product as a yellow oil. The crude product was azeotropically dried with toluene (3 x 50 mL) to afford the title compound **S14** as a yellow oil that was used without further purification (1.55 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.85 (2H, t, *J* = 5.0 Hz), 3.50–3.54 (2H, m), 3.58–3.60 (2H, m), 3.64–3.66 (8H, m), 3.70–3.72 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.4, 61.5, 70.1, 70.2, 70.5, 70.6, 73.0, 70.1. The NMR data was consistent with that obtained from the literature.[13]

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-*N,N*-dimethylethan-1-amine (S15)



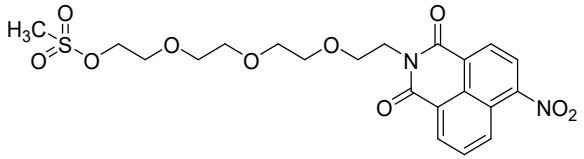
To a solution of **S13** (699 mg, 3.19 mmol) in THF at 0°C was added NEt_3 (1.3 mL, 9.33 mmol) dropwise and then mesyl chloride (0.49 mL, 6.38 mmol). The reaction mixture was stirred for 2 h and then allowed to warm to rt. Upon consumption of the starting material by TLC analysis, the reaction mixture was cooled to 0°C and then a solution of dimethylamine (5.7 mL, 3.17 mmol, 33% wt. in EtOH) was added dropwise. The reaction mixture was heated to 30°C for 4 days and then concentrated *in vacuo* to obtain an orange oil. The crude material was diluted in H_2O (2 x 5 mL) and then loaded onto a C-18 solid phase extraction cartridge (pre-conditioning procedure: 100% CH_3CN [2 x 10 mL], then 90%, then 50% CH_3CN in H_2O [1 x 10mL each], H_2O [2 x 10 mL]). The compound was eluted from the C-18 cartridge (0% to 40% CH_3CN in H_2O [10% increments, 2 x 10 mL each], then 50% to 100% CH_3CN in H_2O [10% increments, 10 mL each]). The fractions collected were analysed by RP-HPLC-ELSD and then concentrated *in vacuo* to afford the mesylate salt of **S15** as a pale yellow oil. This oil was diluted in EtOAc (50 mL) and the organic phase was washed with a 1:1 mixture of saturated $NaHCO_3$ and saturated NaCl (2 x 20 mL). The aqueous washings were back extracted with EtOAc (2 x 20 mL) and the combined organic extracts were dried over $MgSO_4$. The solvent was removed *in vacuo* to afford the title compound **S15** as a pale yellow oil (487 mg, 62 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (6H, s), 2.58 (2H, t, J = 5.8 Hz), 3.39 (2H, t, J = 5.8 Hz), 3.61–3.69 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 45.7, 50.7, 58.6, 69.0, 70.0, 70.4, 70.6, 70.6, 70.7. RP-HPLC-ELSD (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100% to 10% B over 1 min, 10% B for 3 min), t_R = 5.53 minutes. The NMR data were consistent with those obtained from the literature.[14]

2-(2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl)-6-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (S16)



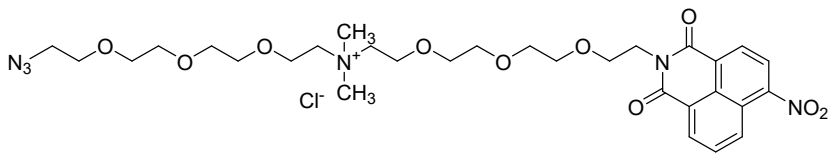
To a solution of **S14** (694 mg, 0.36 mmol) in anhydrous DMF (34 mL) was added nitro-1*H*,3*H*-naphthalic anhydride (**S12**) (822 mg, 0.034 mmol). The reaction mixture was heated to 100°C for 23 hours and then cooled to rt. The DMF was removed *in vacuo* at 55°C to give a dark orange oil that was purified by flash chromatography (CH_2Cl_2 load, then 0%, then 2%, then 5% CH_3OH in CH_2Cl_2) to yield the *title compound* **S16** as an orange oil (919 mg, 65%). R_f (5% CH_3OH in EtOAc) 0.20; IR (ATR) ν_{max}/cm^{-1} 3448, 3081, 2918, 2869, 1706, 1664, 1624, 1584, 1528, 1433, 1407, 1338, 1232, 1098, 839, 786, 760; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.62 (1H, br s), 3.52–3.54 (2H, m), 3.56–3.60 (4H, m), 3.61–3.65 (4H, m), 3.66–3.71 (2H, m), 3.84 (2H, t, *J* = 5.9 Hz), 4.45 (2H, t, *J* = 5.9 Hz), 7.98 (1H, dd, *J* = 8.7, 7.3 Hz), 8.39 (1H, d, *J* = 8.0 Hz), 8.68 (1H, d, *J* = 8.0 Hz), 8.73 (1H, dd, *J* = 7.3, 1.1 Hz), 8.83 (1H, dd, *J* = 8.7, 1.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.5, 61.7, 67.8, 70.1, 70.3, 70.5, 70.6, 72.4, 123.0, 123.6, 123.9, 126.9, 129.1, 129.3, 129.8, 129.9, 132.5, 149.6, 162.6, 163.4; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{20}H_{22}N_2NaO_8^+$ 441.1268; found 441.1258.

2-(2-(2-(2-(6-Nitro-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl methanesulfonate (S17)



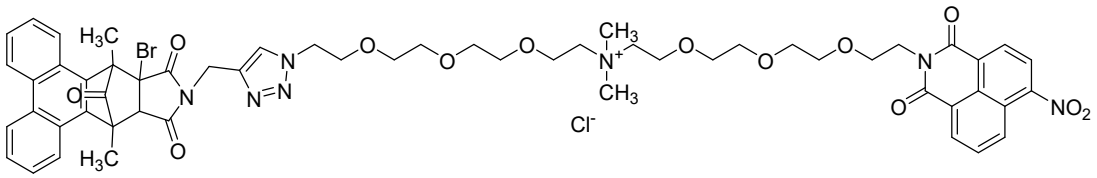
To a solution of alcohol **S16** (209 mg, 0.50 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0°C was added NEt_3 (209 µL, 1.5 mmol) and mesyl chloride (77 µL, 1.0 mmol). The reaction mixture was allowed to warm to rt and stirred for 20 h. H_2O (20 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo* to obtain an orange oil. Purification by flash chromatography (CH_2Cl_2 load, then 0%, then 2% CH_3OH in CH_2Cl_2) yielded the *title compound* **S17** as an orange oil (245 mg, 98%). R_f (5% CH_3OH in EtOAc) 0.38; IR (ATR) ν_{max}/cm^{-1} 2910, 2874, 1706, 1664, 1584, 1528, 1434, 1336, 1232, 1171, 1102, 972, 915, 786, 760, 729; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 (3H, s), 3.59–3.65 (6H, m), 3.67–3.69 (2H, m), 3.71–3.73 (2H, m), 3.83 2H, (t, *J* = 6.0 Hz), 4.34–4.36 (2H, m), 4.44 (2H, t, *J* = 6.0 Hz), 7.99 (1H, dd, *J* = 8.7, 7.3 Hz), 8.40 (1H, d, *J* = 8.0 Hz), 8.69 (1H, d, *J* = 8.0 Hz), 8.73 (1H, dd, *J* = 7.3, 1.1 Hz), 8.84 (1H, dd, *J* = 8.7, 1.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.7, 39.5, 67.8, 69.0, 69.2, 70.1, 70.5, 70.6, 122.9, 123.7, 123.9, 126.9, 129.1, 129.4, 129.9, 129.9, 132.5, 149.6, 162.5, 163.3; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{21}H_{24}N_2NaO_{10}S^+$ 519.1044; found 519.1035.

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-*N,N*-dimethyl-*N*-(2-(2-(2-(2-(6-nitro-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl)ethan-1-aminium chloride (17)



To a solution of amine **S15** (51 mg, 0.21 mmol) in anhydrous CH_3CN (1 mL) was added a solution of mesylate **S17** (104 mg, 0.21 mmol) in anhydrous CH_3CN (1 mL) at rt. The reaction mixture was stirred at 90°C for 21 h and then cooled to rt. The solvent was removed *in vacuo* and the crude material was then diluted in 10% CH_3CN in H_2O containing 0.1% HCl (2 x 5 mL) and then loaded onto a C-18 solid phase extraction cartridge (pre-conditioning procedure: 100% CH_3CN [2 x 10 mL], then 90%, then 50% CH_3CN in H_2O [1 x 10mL each], 10% CH_3CN in H_2O [2 x 10 mL]). The compound was eluted from the C-18 cartridge (10% to 50% CH_3CN in H_2O containing 0.1% HCl [10% increments, 2 x 10 mL each], then 60% to 100% CH_3CN in H_2O [10% increments, 10 mL each]). RP-HPLC analysis revealed the fractions which contained the product and these were pooled together. The solvent was removed *in vacuo* to afford the *title compound* **17** as an orange oil (123 mg, 81%). IR (ATR) ν_{max}/cm^{-1} 3369, 2963, 2928, 2871, 2104, 1707, 1666, 1584, 1530, 1342, 1233, 1114, 1060, 787; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.39 (2H, t, *J* = 4.9 Hz), 3.45 (6H, s), 3.57–3.69 (18H, m), 3.80 (2H, t, *J* = 6.1 Hz), 3.94–4.01 (8H, m), 4.42 (2H, t, *J* = 6.1 Hz), 8.01 (1H, dd, *J* = 8.7, 7.3 Hz), 8.43 (1H, d, *J* = 8.0 Hz), 8.70 (1H, d, *J* = 8.0 Hz), 8.74 (1H, dd, *J* = 7.3, 1.1 Hz), 8.85 (1H, dd, *J* = 8.7, 1.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.6, 50.6, 52.9, 59.0, 64.8, 64.9, 65.0, 65.1, 70.0, 70.1, 70.2, 70.2, 70.3, 70.3, 70.4, 70.4, 70.6, 122.8, 123.6, 124.0, 126.7, 129.1, 129.4, 130.0, 130.0, 132.5, 149.6, 162.5, 163.3 HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{30}H_{43}N_6O_{10}^+$ 647.3035; found 647.3024; RP-LC-MS[254 nm] (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100% to 10% B over 1 min, 10% B for 3 min), t_R = 7.23 minutes.

2-(2-(2-(2-(4-((9a-Bromo-9,13-dimethyl-10,12,14-trioxo-9,9a,10,12,12a,13-hexahydro-11H-9,13-methanophenanthro[9,10-*f*]isoindol-11-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-*N,N*-dimethyl-*N*-(2-(2-(2-(2-(6-nitro-1,3-dioxo-1H-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl)ethan-1-aminium chloride (oCOm-66)

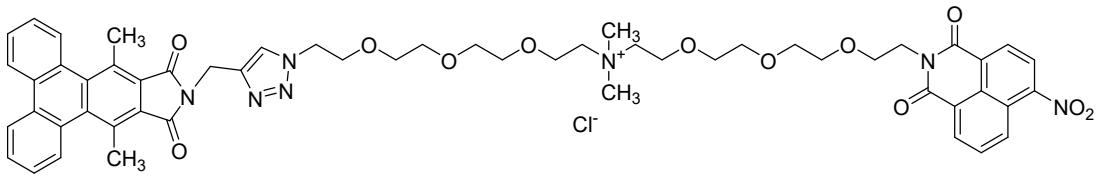


A solution of alkyne **12** (45 mg, 0.096 mmol) and azide **17** (45 mg, 0.091 mmol) in a 1:1 mixture of CH_3CN and toluene (2 mL) was purged with argon and then CuI▪$P(OEt)_3$ (5 mg, 0.014 mmol) was added at rt. The reaction mixture was heated to 70°C for 23 h and then more CuI▪$P(OEt)_3$ (5 mg, 0.014 mmol) was added. The reaction mixture was heated at 70°C for a further 24 h and then cooled to rt. Removal of the solvent *in vacuo* gave an orange oil. The crude material was diluted in 10% CH_3CN in H_2O containing 0.1% HCl (2 x 5 mL) and then loaded onto a C-18 solid phase extraction cartridge (pre-conditioning procedure: 100% CH_3CN [2 x 10 mL], then 90%, then 50% CH_3CN in H_2O [1 x 10mL each], 10% CH_3CN in H_2O [2 x 10 mL]). The compound was eluted from the C-18 cartridge (10% to 20% CH_3CN in H_2O containing 0.1% HCl [10% increments, 2 x 10 mL each], then 30% to 40% CH_3CN in H_2O containing 0.1% HCl [10% increments, 4 x 10 mL each], then 50% to 60% CH_3CN in H_2O containing 0.1% HCl [10% increments, 2 x 10 mL each], then 70% to 100% CH_3CN in H_2O containing 0.1% HCl [10% increments, 10 mL each]). RP-HPLC analysis revealed the fractions which contained the product and these were pooled together. The solvent was removed *in vacuo* to afford an orange oil (55 mg, 52%) consisting of the *title compound* **oCOm-66** and approximately 3% of **BP-66**.

Data for **oCOm-66**: Quantum yield Φ = 0.008 (1 mM in absolute EtOH, λ_{ex1} = 395 nm, λ_{em1} = 460 nm), Φ = 0.19 (1 mM in absolute EtOH, λ_{ex2} = 445 nm, λ_{em2} = 540 nm); IR (ATR) ν_{max}/cm^{-1} 3365, 2905, 2872, 1784, 1711, 1664, 1625, 1528, 1433, 1376, 1337, 1232, 1102, 1061, 908, 758; ^{1}H NMR (400 MHz, $CDCl_3$) δ *inter alia* 2.24 (3H, s), 2.28 (3H, s), 3.41–3.43 (8H, m), 3.48 (2H, br s), 3.56–3.67 (15H, m), 3.80 (2H, t, *J* = 6.0 Hz), 3.91–3.99 (8H, m), 4.07 (2H, br s), 4.18 (2H, s), 4.40 (2H, t, *J* = 6.0 Hz), 6.33 (1H, s), 7.61–7.68 (4H, m), 7.98 (1H, dd, *J* = 8.7, 7.3 Hz), 8.16 (1H, d, *J* = 7.9 Hz), 8.22 (1H, d, *J* = 8.1 Hz), 8.39 (1H, d, *J* = 7.9 Hz), 8.63–8.71 (4H, m), 8.81 (1H, dd, *J* = 8.7, 0.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 14.7, 34.1, 39.6, 50.4, 53.4, 54.6, 54.7, 57.7, 58.0, 60.0, 64.9, 65.1, 65.4, 67.7, 69.3, 70.2, 70.2, 70.3, 70.4, 70.4, 70.5, 122.7, 123.1, 123.3, 123.4, 123.6, 124.0, 125.0, 125.1, 126.1, 126.4, 126.6, 127.2, 127.3, 127.4, 127.7, 129.0, 129.4, 130.0, 130.1, 130.5, 130.7, 130.9, 132.5, 134.4, 139.9, 149.5, 162.5, 163.3, 171.0, 171.4, 197.4; HRMS (ESI-TOF) *m/z*: $[M - Cl]^+$ Calcd for $C_{56}H_{61}N_7O_{13}Br^+$ 1118.3505; found 1118.3493; RP-LC-MS[254 nm] (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100% to 10% B over 1 min, 10% B for 3 min), t_R = 9.00 minutes.

Data for **BP-66**: ^{1}H NMR (400 MHz, $CDCl_3$) δ *inter alia* 3.19 (6H, s); RP-LC-MS[254 nm] (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100% to 10% B over 1 min, 10% B for 3 min), t_R = 9.35 minutes.

2-(2-(2-(2-(4-((9,13-Dimethyl-10,12-dioxo-10,12-dihydro-11H-phenanthro[9,10-*f*]isoindol-11-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-*N,N*-dimethyl-*N*-(2-(2-(2-(2-(6-nitro-1,3-dioxo-1H-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl)ethan-1-aminium chloride (BP-66)



A solution of alkyne **12** (44 mg, 0.093 mmol) and azide **17** (32 mg, 0.047 mmol) in a 1:1 mixture of CH_3CN and toluene (2 mL) was purged with argon and then CuI▪$P(OEt)_3$ (2 mg, 0.006 mmol) was added at rt. The reaction mixture was heated to 80°C for 24 h and then more CuI▪$P(OEt)_3$ (2 mg, 0.006 mmol) was added. The reaction mixture was heated at 80 °C for a further 24 h and then cooled to rt. Removal of the solvent *in vacuo* gave an orange oil which was then diluted in CH_2Cl_2 (2 mL). DBU (50 µL, 0.33 mmol) was added at 0°C and the reaction mixture stirred for 1 h before the solvent was removed *in vacuo*. The crude material was dissolved in 10% CH_3CN in H_2O containing 0.1% HCl (2 x 5 mL) and then loaded onto a C-18 solid phase extraction cartridge (pre-conditioning procedure: 100% CH_3CN [2 x 10 mL], then 90%, then 50% CH_3CN in H_2O [1 x 10mL each], 10% CH_3CN in H_2O [2 x 10 mL]). The compound was eluted from the C-18 cartridge (10% to 50% CH_3CN in H_2O containing 0.1% HCl [10% increments, 2 x 10 mL each], then 60% to 100% CH_3CN in H_2O [10% increments, 10 mL each]). RP-HPLC analysis revealed the fractions which contained the product and these were pooled together. The solvent was removed *in vacuo* to afford the *title compound* **BP-66** as an orange oil (11 mg, 17%). Quantum yield Φ = 0.02 (1 mM in absolute EtOH, λ_{ex1} = 395 nm, λ_{em1} = 460 nm), Φ = 0.04 (1 mM in absolute EtOH, λ_{ex2} = 445 nm, λ_{em2} = 540 nm); IR (ATR) ν_{max}/cm^{-1} 3407, 2908, 2869, 1754, 1699, 1664, 1625, 1592, 1528, 1397, 1334, 1232, 1098, 1055, 785, 758; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.19 (6H, s), 3.38–3.45 (6H, m), 3.59–3.65 (16H, m), 3.78–3.80 (2H, m), 3.85–

3.92 (4H, m), 3.95–3.98 (2H, m), 4.03–4.06 (4H, m), 4.39 (2H, t, *J* = 5.9 Hz), 4.61–4.65 (2H, m), 5.10 (2H, s), 7.57 (2H, t, *J* = 7.4 Hz), 7.67 (2H, t, *J* = 7.6 Hz), 7.94 (1H, t, *J* = 8.0 Hz), 8.05 (1H, s), 8.31–8.37 (4H, m), 8.51 (2H, d, *J* = 7.9 Hz), 8.63 (1H, d, *J* = 7.7 Hz), 8.67 (1H, d, *J* = 8.0 Hz), 8.77 (1H, d, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 32.2, 39.5, 51.3, 52.9, 64.7, 64.9, 65.1, 67.7, 69.0, 70.1, 70.2, 70.2, 70.3, 70.4, 70.5, 122.7, 123.5, 123.6, 123.9, 125.3, 126.4, 126.5, 126.6, 128.4, 128.8, 128.9, 129.3, 129.6, 129.9, 129.9, 131.5, 132.4, 132.6, 137.9, 141.7, 149.5, 162.4, 163.2, 168.2; HRMS (ESI-TOF) *m/z*: $[M - Cl]^+$ Calcd for $C_{55}H_{60}N_7O_{12}^+$ 1010.4295; found 1010.4281; RP-LC-MS[254 nm] (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100% to 10% B over 1 min, 10% B for 3 min), t_R = 9.35 minutes.

Solubility studies

Solubility studies were conducted on **oCOm-57**, **oCOm-58**, **oCOm-66** and **BP-66** to determine their water solubilities at room temperature.

Water solubility studies for oCOm-57 and oCOm-58

A series of standard solutions of **oCOm-57** or **oCOm-58** in CH_3CN were prepared (five standards between the concentrations 0.07 and 0.46 mg mL^{-1}). The absorbance of the standards at 254 nm was analysed by HPLC in triplicate (UV detector = 254 nm, flow rate 0.5 mL min^{-1}). The average integrated peak areas obtained from the UV detector (254 nm) from each standard was used to generate the standard curves of **oCOm-57** (Figure S1) and **oCOm-58** (Figure S2) giving rise to equations (1) and (2) respectively.

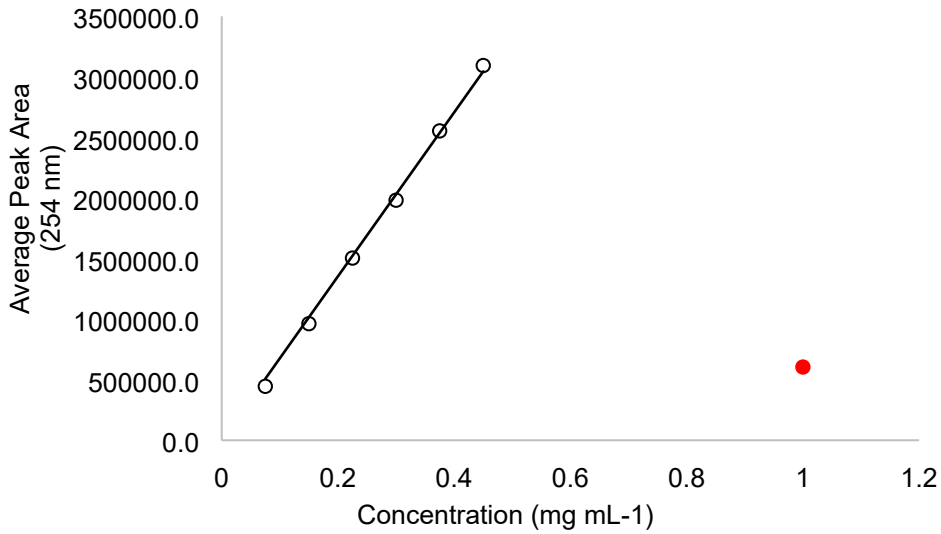


Figure S1: Standard curve for **oCOm-57** in CH_3CN (UV detector λ = 254 nm).

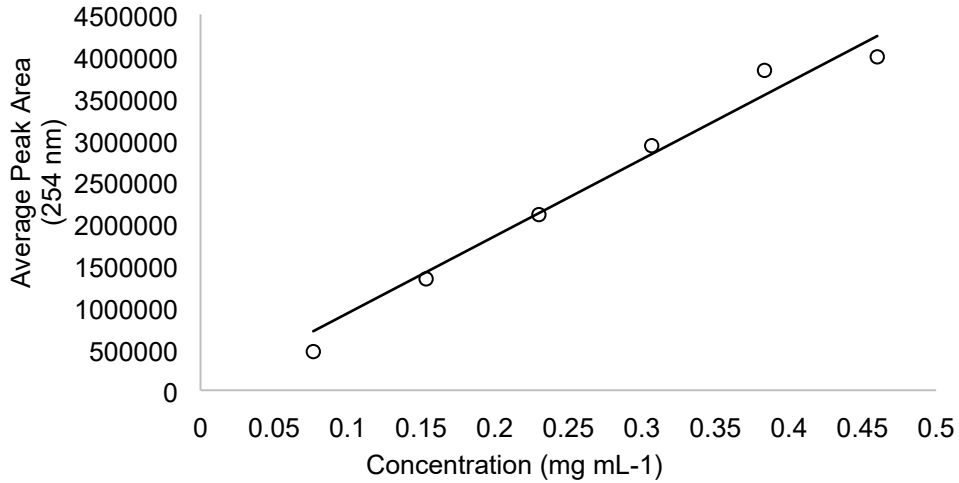


Figure S2: Standard curve for **oCOm-58** in CH_3CN (UV detector λ = 254 nm).

(1) y = 6760579.93 x where y = peak area integrated in UV detector (254 nm)
x = concentration of **oCOm-57** (mg mL^{-1})

(2) y = 9206378.67 x where y = peak area integrated in UV detector (254 nm)
x = concentration of **oCOm-58** (mg mL^{-1})

Tissue culture H_2O (1 mL, Invitrogen, DNAse and RNAse free) was added to a known amount of **oCOm-57** or **oCOm-58** at room temperature (where possible, more sample was added to make a saturated solution of the oCOm). This mixture was sonicated and gently heated to allow the maximum amount of sample to dissolve. The sample was then cooled to room temperature, filtered (syringe filter, nylon, 0.45 μm) and diluted with tissue culture H_2O to bring the concentration of the sample into the range of the standard curve. The diluted sample was analysed by HPLC in triplicate with the same flow rate and UV detector parameters as for the standards. The average peak area was used, in conjunction with the equation determined from the standard curve, to calculate the dissolved concentration of the original saturated solution of oCOm at room temperature.

A representative example is shown for **oCOm-57**. Equation (1) is rearranged to give equation (3) which provides an approximate concentration of **oCOm-57**.

(3) $$x = \frac{y}{6760579.93}$$

where y = 604440
dilution factor for **oCOm-57** = 200

x = 17.9 $mg.mL^{-1}$

The molecular weight of **oCOm-57** = 1190.189 g mol^{-1}, thus the concentration of **oCOm-57** dissolved in H_2O at 20°C = 0.0150 $mol.L^{-1}$ (15.0 mM)

Water solubility study for oCOm-66

HPLC analyses were conducted using analytical RP-HPLC (Shimadzu LC–20AD equipped with an SPD-20A UV detector [210 and 254 nm] and a Shimadzu ELSD-LTII Low Temperature Evaporative Light Scattering Detector) using a Phenomenex Prodigy column (C–18, 5 µm, 3.00 × 250 mm) at 0.5 mL.min, heated to 40°C. The solvent system was a mixture of A (0.05% TFA in H_2O) and B (0.05% TFA in CH_3CN) using a gradient of 10% B to 100% B over 12.5 minutes, 100% B for 2.5 minutes, then 100% B to 10% B over 1 minute, 10% B for 3 minutes.

A solution of **oCOm-66** (4.0 $mg.mL^{-1}$) was prepared using CH_3CN. This solution was used to prepare the other standard solutions using CH_3CN (2.0, 1.0, 0.5, 0.25, 0.125 $mg\ mL^{-1}$). Each standard solution was filtered (0.45 µm nylon syringe filter) and then 3 µL of each solution was injected in duplicate. The integrated peak areas obtained from the UV detector (254 nm) was used to generate the standard curve (Figure S3) that is described by equation (4).

(4) $y = 8369160.49\ x$ where y = peak area integrated in UV detector (254 nm)
x = concentration of **oCOm-66** ($mg\ mL^{-1}$)

Equation (4) is rearranged to give equation (5) which provides an approximate concentration of **oCOm 66**.

(5) $$x = \frac{y}{8369160.49}$$

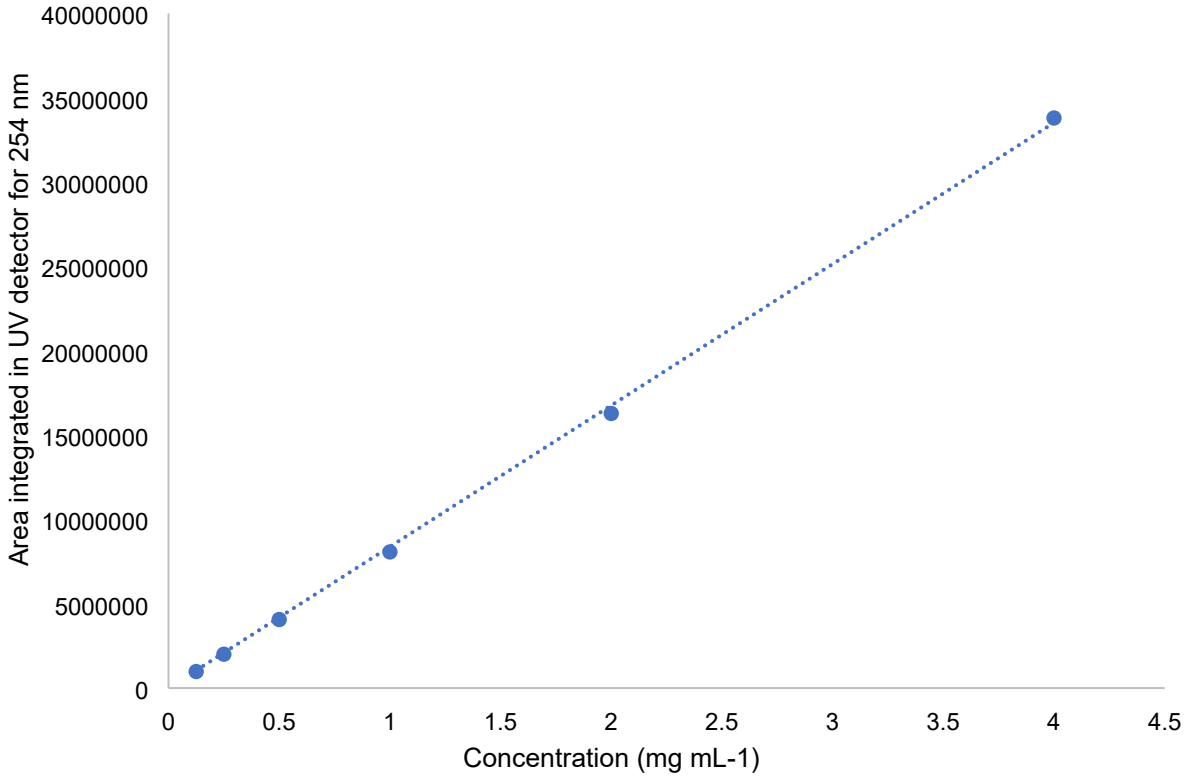


Figure S3: Standard curve for **oCOm-66** in CH_3CN (UV detector λ = 254 nm)

To determine the solubility of **oCOm-66** in H_2O, a sample of **oCOm-66** was dissolved in UltraPure Distilled H_2O (400 µL, Invitrogen, DNAse and RNAse free) and then filtered (0.45 µm nylon syringe filter) at 20˚C. An aliquot of the filtered solution (20 µL) was diluted with UltraPure Distilled H_2O (580 µL, 1/30 x dilution) and then 3 µL of the diluted sample was injected in duplicate. The integrated peak areas obtained from the UV detector (254 nm) was used in equation (2) to calculate the concentration of oCOm dissolved in H_2O.

$$x = \left(\frac{y}{8369160.49}\right) x\ dilution\ factor$$

where y = 10490053
dilution factor = 30

x = 37.6 mg mL^{-1}

The molecular weight of **oCOm-66** = 1155.494 g mol^{-1}, thus the concentration of **oCOm-66** dissolved in H_2O at 20˚C = 0.0325 mol L^{-1} (33 mM)

Water solubility study for BP-66

The water solubility study for **BP-66** was performed in a similar manner as **oCOm-66**. A solution of **BP-66** (3.1 mg mL^{-1}) was prepared using CH_3CN. This solution was used to prepare the other standard solutions using CH_3CN (1.55, 0.76, 0.39, 0.19, 0.097 mg mL^{-1}). Each standard solution was filtered (0.45 µm nylon syringe filter) and then 3 µL of each solution was injected in duplicate. The integrated peak areas obtained from the UV detector (254 nm) was used to generate the standard curve (Figure S4) that is described by equation (6).

(6) $y = 5054582.46\ x$ where y = peak area integrated in UV detector (254 nm)
x = concentration of **BP-66** (mg mL^{-1})

Equation (6) is rearranged to give equation (7) which provides an approximate concentration of **BP-66**.

(7) $$x = \frac{y}{5054582.46}$$

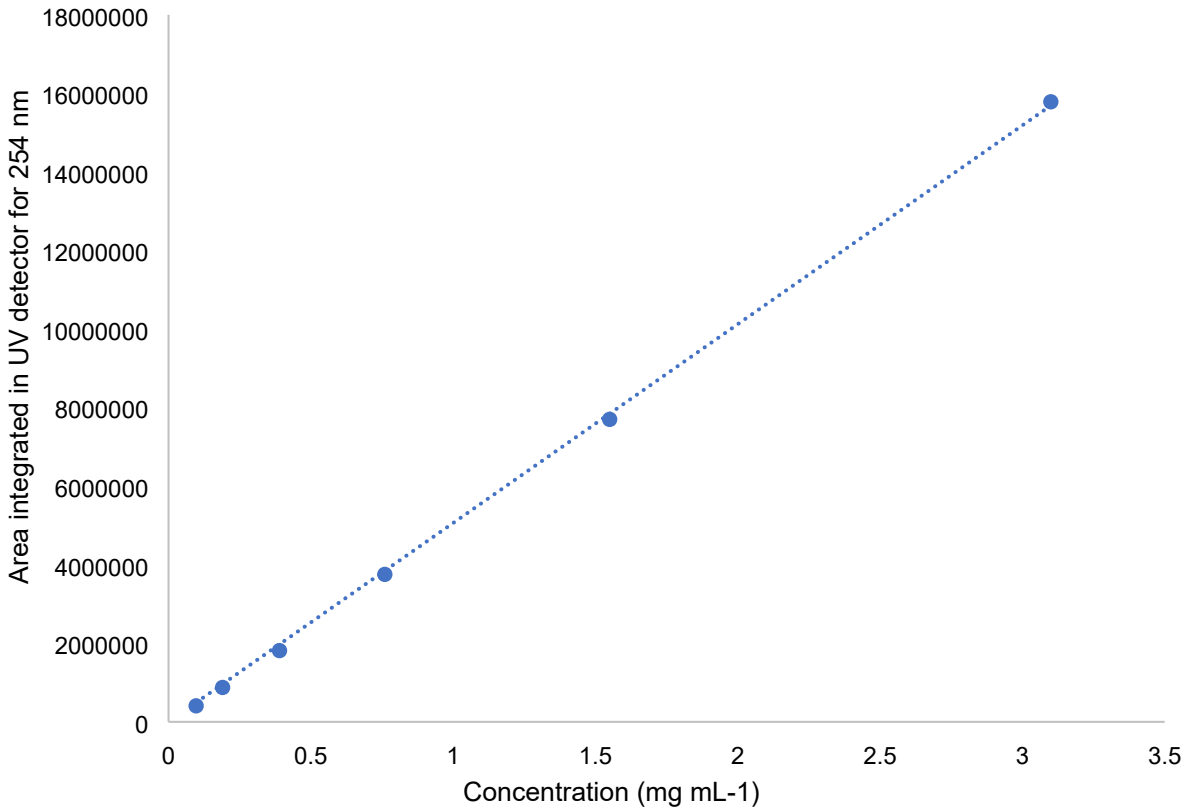


Figure S4: Standard curve for **BP-66** in CH_3CN (UV detector λ = 254 nm)

To determine the solubility of **BP-66** in H_2O, a sample of **BP-66** was dissolved in UltraPure Distilled H_2O (400 µL, Invitrogen, DNAse and RNAse free) and then filtered (0.45 µm nylon syringe filter) at 20˚C. An aliquot of the filtered solution (20 µL) was diluted with UltraPure

Distilled H_2O (580 µL, 1/30 x dilution) and then 3 µL of the diluted sample was injected in duplicate. The integrated peak areas obtained from the UV detector (254 nm) was used in equation (7) to calculate the concentration of oCOm dissolved in H_2O.

$$x = \left(\frac{y}{5054582.46}\right) x\ dilution\ factor$$

where y = 5195611
dilution factor = 30

x = 30.8 mg.mL^{-1}

The molecular weight of **BP-66** = 1046.572 g mol^{-1}, thus the concentration of **BP-66** dissolved in H_2O at 20°C = 0.0294 mol L^{-1} (29 mM)

Table S1: Water solubility of oCOm-57, oCOm-58, oCOm-66 and BP-66 at 20°C.

Compound	Average area integrated (UV = 254 nm)	Dilution factor	Solubility in H_2O (mg mL^{-1})	Solubility in H_2O (mM)
oCOm-57	604440	200	17.9	15.0
oCOm-58	6065839	200	131.8	192.9
oCOm-66	10490053	30	37.6	33
BP-66	5195611	30	30.8	29

UV-visible absorbance and fluorescence spectroscopy for oCOm-57, oCOm-58, BP-57 and BP-58.

Spectra were acquired using Greiner-96 F-Bottom well plates or Nunc-96 well plates on the BMG Labtech (Alphatech) CLARIOstar spectrophotometer, which was set to 37.8 ºC. UV-visible absorbance spectra were acquired with a wavelength step width of 2 nm. Fluorescence spectra were acquired using a gain of 1192 (set to 70% of the target value), a focal point of 6.9 mm and a step width of 1 nm (Figure S5 to Figure S12).

Stock solutions (5 mM) of **oCOm-57** and **oCOm-58** were prepared in tissue culture water (1 mL). These stock solutions were then serially diluted using tissue culture water to the following concentrations: 1 mM, 0.2 mM, 0.04 mM and 0.008 mM. Aliquots (100 µL) of the diluted solutions were then analysed by the spectrophotometer in triplicate. Concentrations in which the absorbance or fluorescence intensity were either too high or too low are not shown.

For **BP-57** and **BP-58**, stock solutions (5 mM) were prepared in tissue culture water (1 mL) or a mixture of 20% DMSO in 1x PBS buffer at pH 7.4. These stock solutions were then serially diluted using tissue culture water or 1x PBS buffer respectively to the following concentrations: 1 mM, 0.2 mM, 0.04 mM and 0.008 mM. Aliquots of each concentration was then analysed by the spectrophotometer in triplicate (100 µL each). Concentrations in which the absorbance or fluorescence intensity were either too high or too low are not shown.

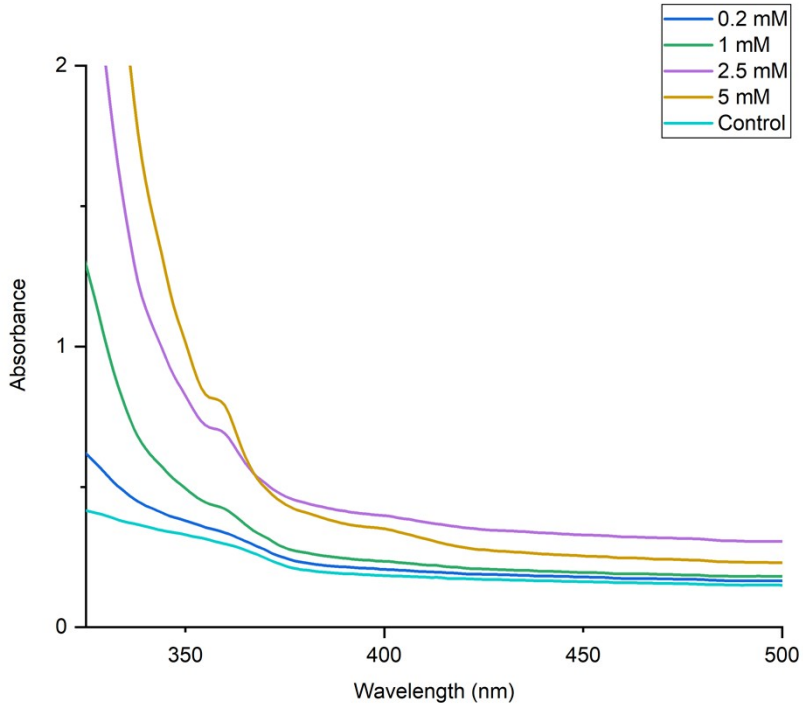


Figure S5: UV-visible spectrum of **oCOm-57** in tissue culture H_2O at 37.8°C.

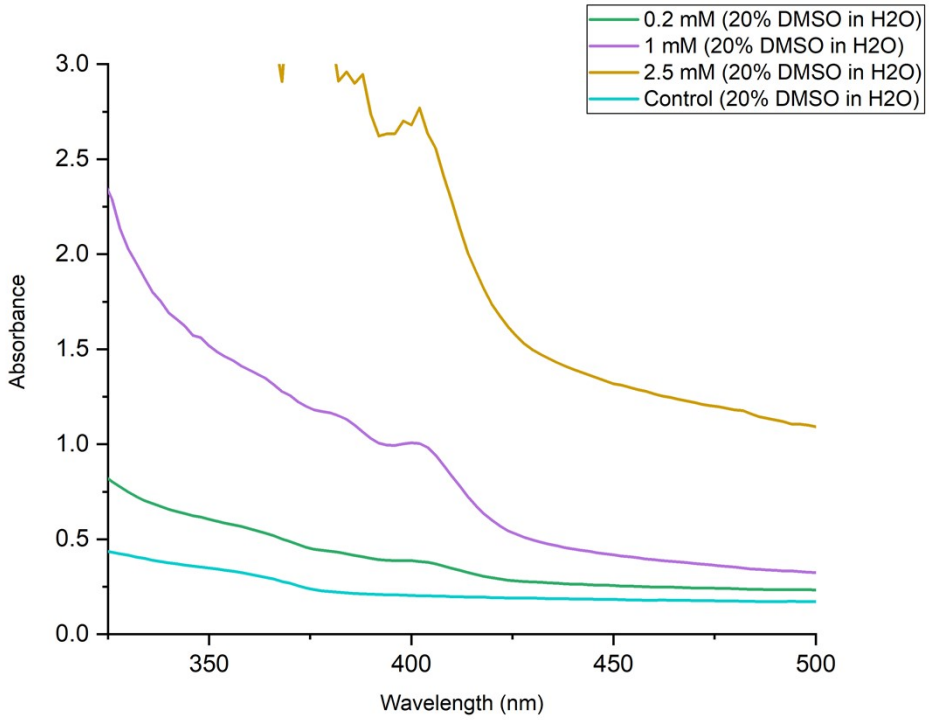


Figure S6: UV-visible spectrum of **BP-57** in 20% DMSO in tissue culture H_2O at 37.8°C.

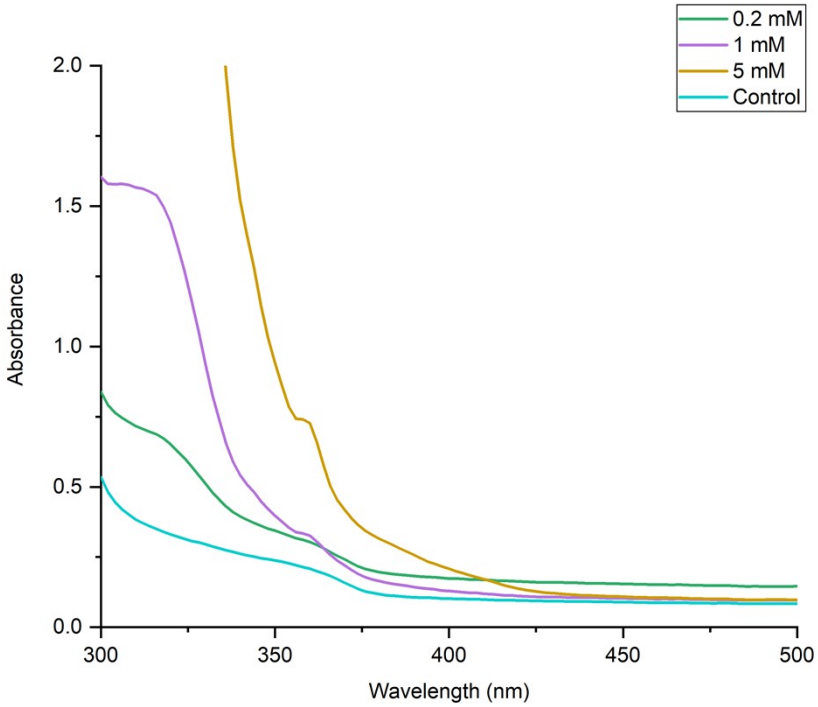


Figure S7: UV-visible spectra of **oCOm-58** in tissue culture H_2O at 37.8°C.

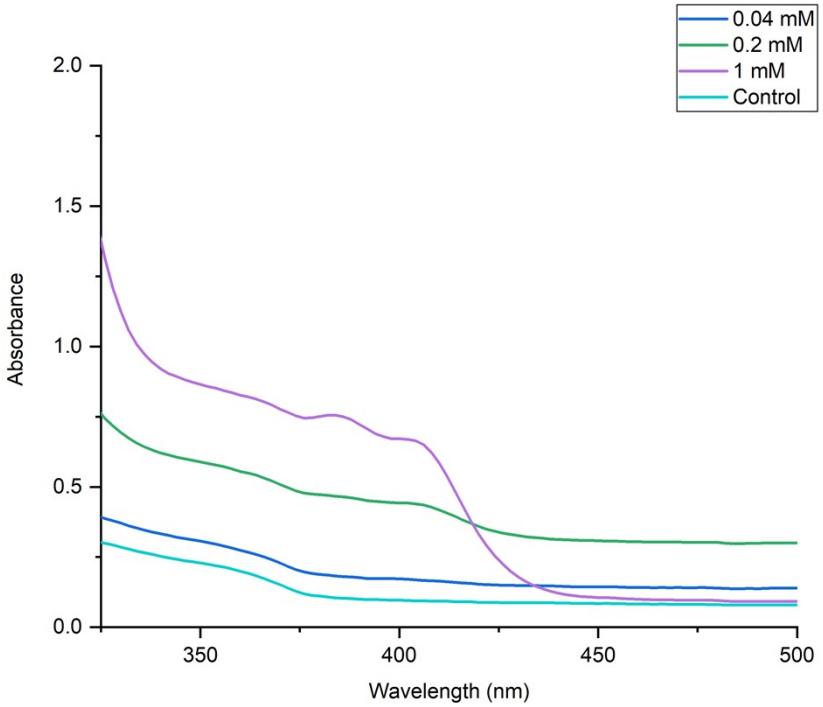


Figure S8: UV-visible spectrum of **BP-58** in tissue culture H_2O at 37.8°C.

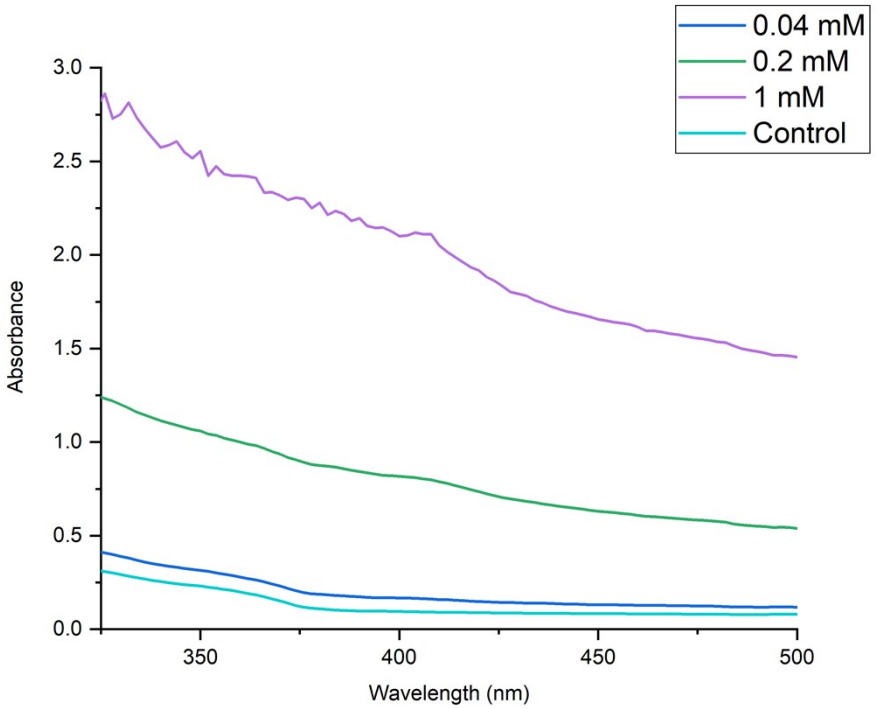


Figure S9: UV-visible spectrum of **BP-58** in a mixture of ≤ 20% DMSO in 1x PBS (pH 7.4) at 37.8°C.

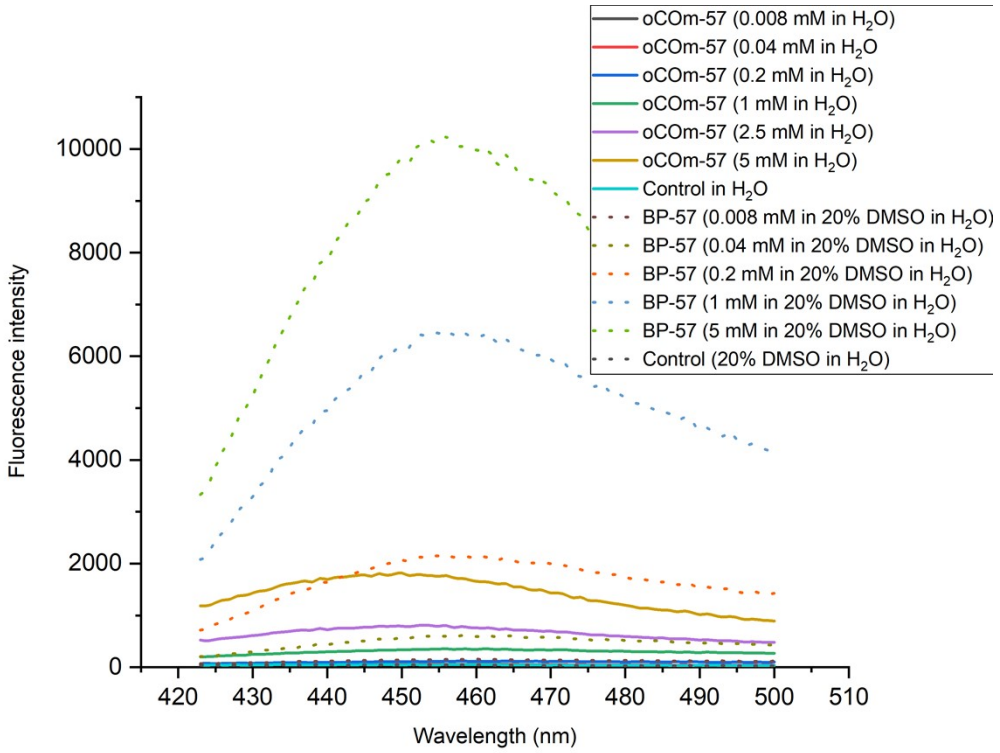


Figure S10: Fluorescence spectra for **oCOm-57** (solid lines) in tissue culture H_2O and **BP-57** (dotted lines) in < 20% DMSO in tissue culture H_2O (λ_{ex} = 400 nm at 37.8°C).

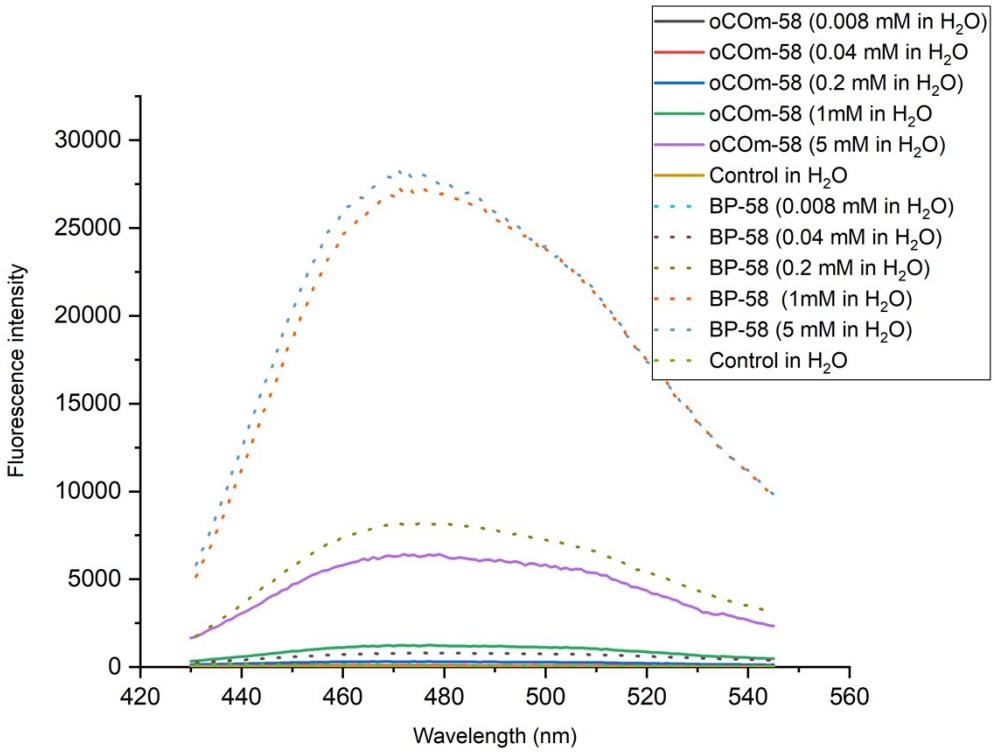


Figure S11: Fluorescence spectra for **oCOm-58** (solid lines) and **BP-58** (dotted lines) in tissue culture H_2O (λ_{ex} = 400 nm at 37.8°C).

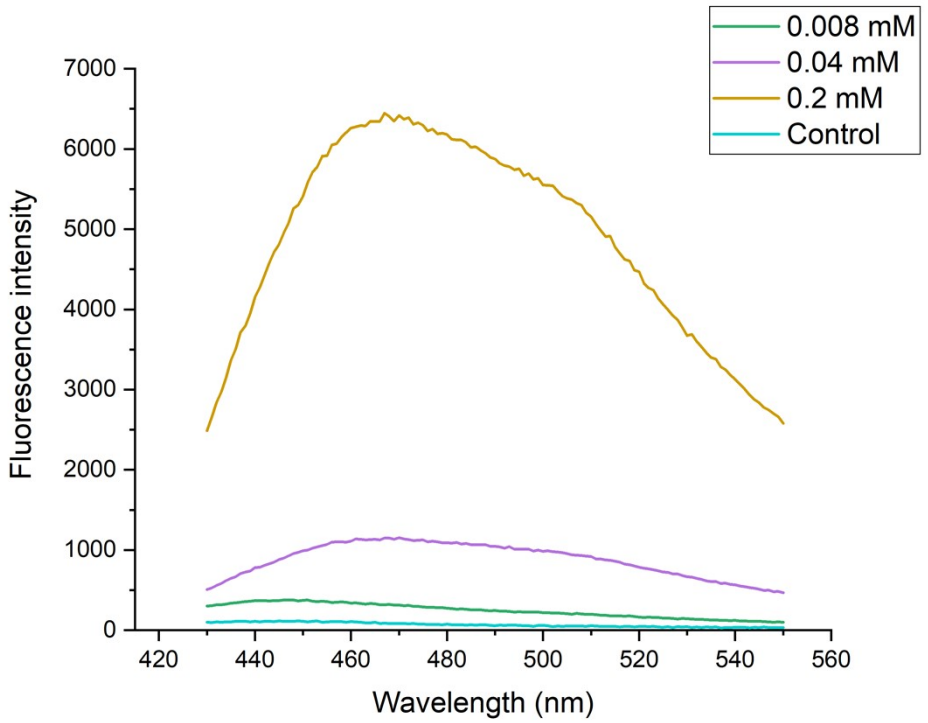


Figure S12: Fluorescence spectrum for **BP-58** in ≤ 20% DMSO in 1x PBS (pH 7.4) at 37.8°C.

UV-visible absorbance and fluorescence spectroscopy for oCOm-66 and BP-66.

The UV-visible and fluorescence spectra for the water soluble **oCOm-66**, **BP-66** and **DB-66** were acquired using Greiner-96 F-Bottom well plates or Nunc-96 well plates on the BMG Labtech (Alphatech) CLARIOstar spectrophotometer, which was set to 37.8 ºC. UV-visible

absorbance spectra were acquired with a wavelength step width of 2 nm. Fluorescence spectra were acquired using a gain of 1192 (set to 70% of the target value), a focal point of 6.9 mm and a stepwidth of 1 nm (Figure S13 to Figure S17).

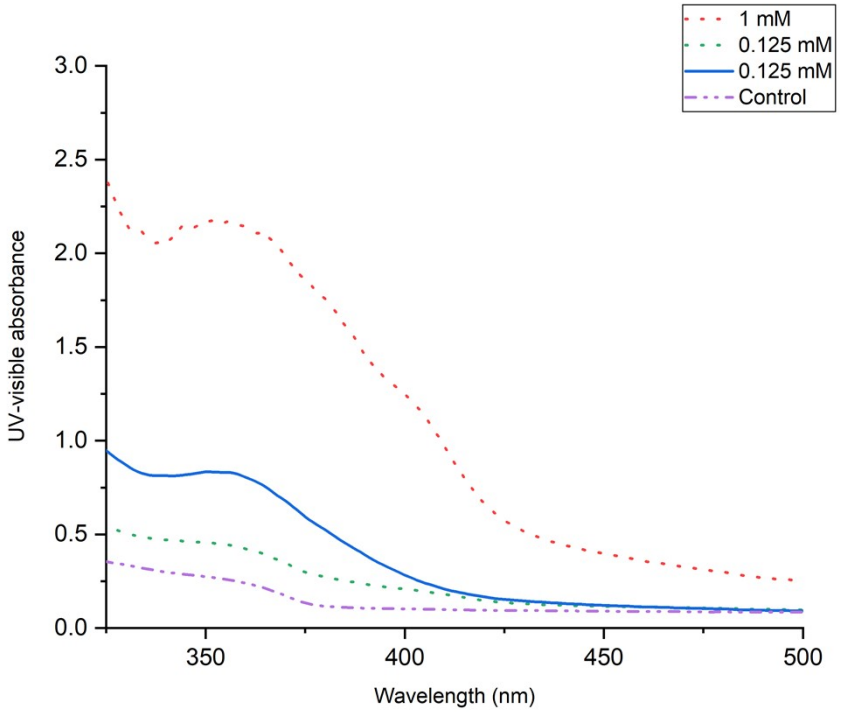


Figure S13: UV-visible spectrum of **oCOm-66** (solid line) and **BP-66** (dotted lines) at 37.8°C at various concentrations in H_2O in tissue culture H_2O.

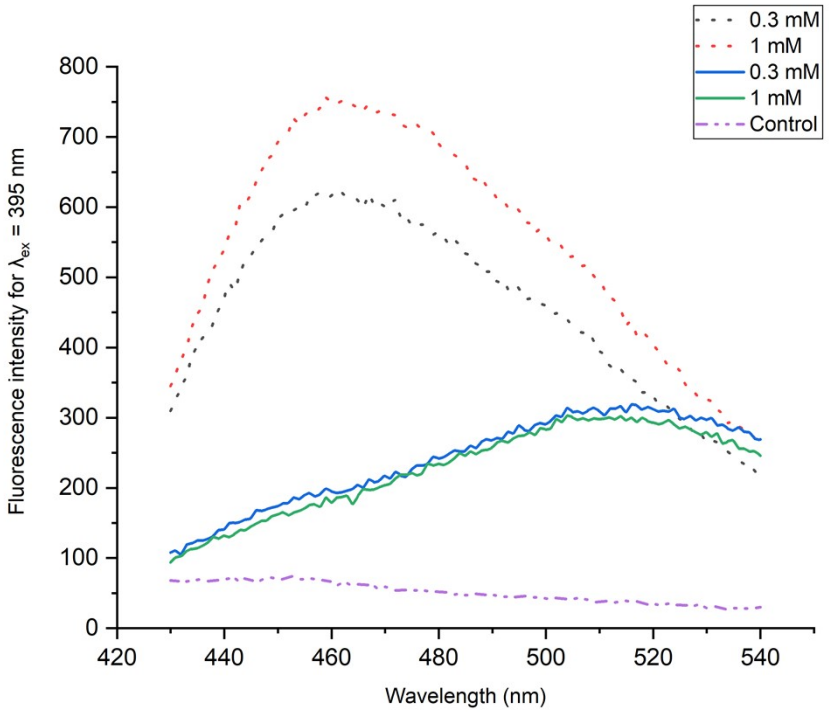


Figure S14: Fluorescence spectrum for **oCOm-66** (solid lines) and **BP-66** (dotted lines) at 37.8°C at various concentrations in H_2O (λ_{ex} = 395 nm).

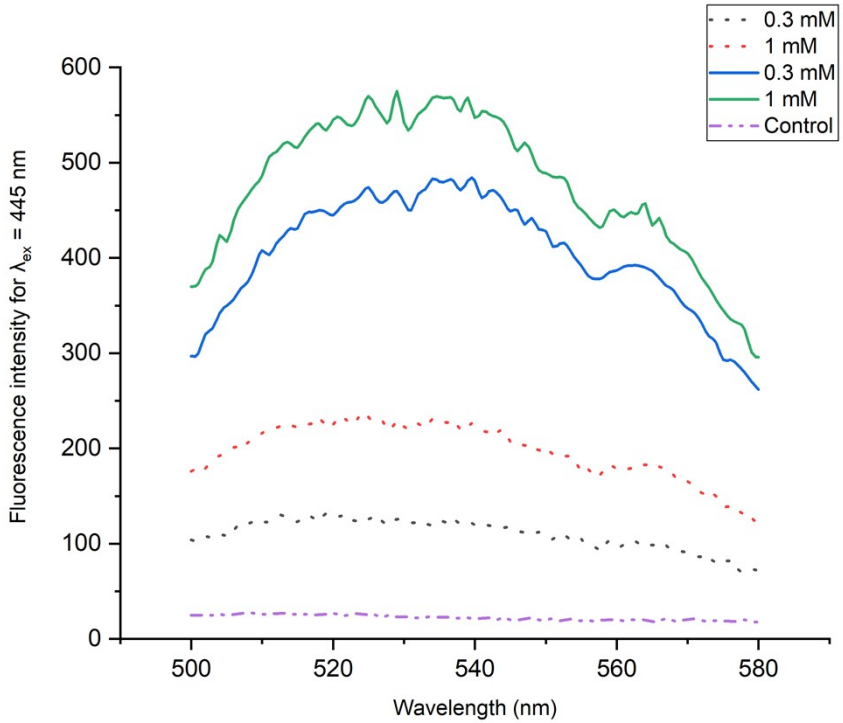


Figure S15: Fluorescence spectrum for **oCOm-66** (solid lines) and **BP-66** (dotted lines) at 37.8°C at various concentrations in H_2O (λ_{ex} = 445 nm).

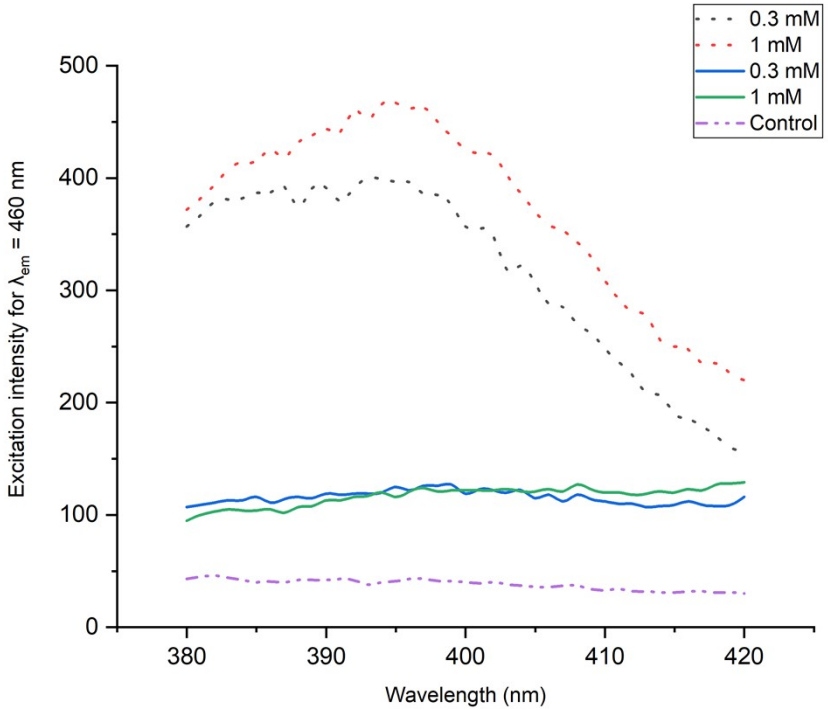


Figure S16: Excitation scan for **oCOm-66** (solid lines) and **BP-66** (dotted lines) at 37.8°C at various concentrations in H_2O when λ_{em} = 460 nm.

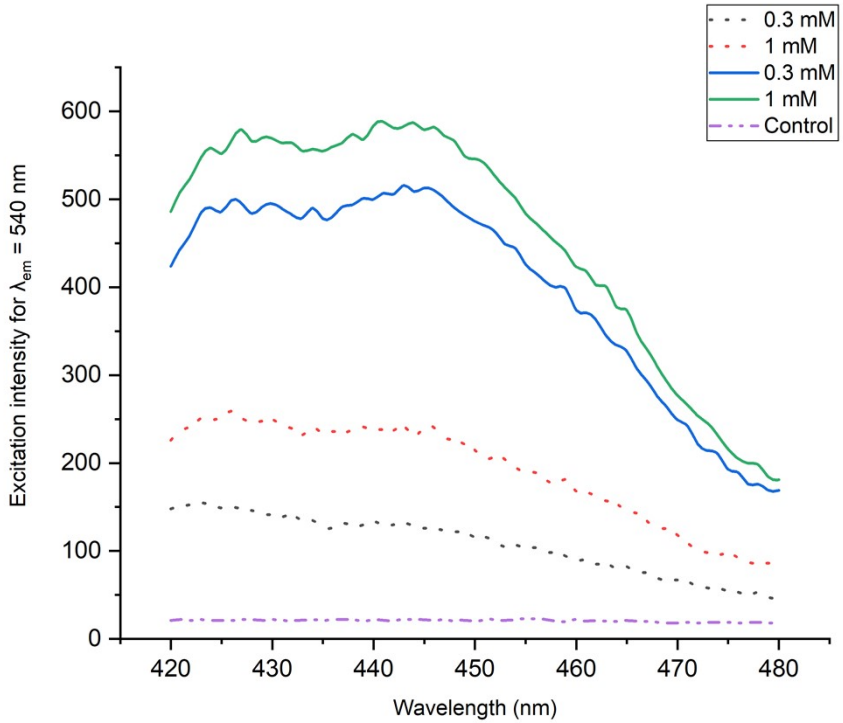


Figure S17: Excitation scan for **oCOm-66** (solid lines) and **BP-66** (dotted lines) at 37.8°C at various concentrations in H_2O when λ_{em} = 540 nm.

.

Table S2: Summary of excitation (λ_{ex}) and emission (λ_{ex}) wavelengths observed

Compound	λ_{ex1} (nm)[a]	λ_{em1} (nm)[a]	λ_{ex2} (nm)[b]	λ_{em2} (nm)[b]
oCOm-57	400	445[c]	–	–
BP-57[d]	400	455	–	–
oCOm-58	400	470[c]	–	–
BP-58	400	470	–	–
oCOm-66	395	460	445	540
BP-66	395	460	445	540

[a] λ_{ex1} and λ_{em1} are the excitation and emission wavelengths monitored for the conversion of the phenanthrene moiety to the triphenylene.
[b] λ_{ex2} and λ_{em2} are the excitation and emission wavelengths monitored for the naphthalimide.
[c] The fluorescence intensity for **oCOm-57** and **oCOm-58** at the specified wavelength was much weaker when compared to **BP-57** and **BP-58**.
[d] **BP-57** was not water soluble and the fluorescence spectrum was obtained in a solution of 20% DMSO in tissue culture H_2O.

CO release profiles

TRIS-sucrose buffer

The TRIS-sucrose buffer prepared from potassium chloride (1.49 g, 20 mmol), mannitol (2.73 g, 15 mmol), sucrose (1.71 g, 5 mmol) and TRIS (0.24 g, 1.98 mmol). MilliQ water (200 mL) was added to the above reagents to make the TRIS-sucrose buffer solution. If necessary, TRIS-HCl was added to the solution to adjust the pH to 7.4 at 37 ºC. The TRIS-sucrose buffer was stored at 4 ºC. Prior to each study an aliquot of buffer was removed and tested to confirm that the pH was 7.4 at 37 ºC.

CO release studies by HPLC

CO release studies were conducted to determine the rate of CO release of **oCOm-58** in aqueous buffer at 37 ºC at the specified pH. A sample of **oCOm-58** (approx. 2 mg) and buffer (1 mL) were allowed to equilibrate to 37 ºC for 1 hour. The buffer was then added to the sample and the timer started. Immediately after addition of the buffer, the sample was heated and/or briefly sonicated to facilitate dissolution, before being lightly sealed. Aliquots (30 µL) were removed from the vial at the specified times and then mixed with 0.5 M aqueous HCl (170 µL). Without delay, the quenched samples (3 µL injection) were analysed by RP-HPLC[254 nm] (Phenomenex Prodigy C-18 column, 10% to 100% B over 12.5 min, then 100% B for 2.5 min, then 100%B to 10%B over 1 min, then 10% B for 4 min) where the solvent system was a mixture of A (0.05% TFA in H_2O) and B (0.05% TFA in CH_3CN). The relative peak areas of **oCOm-58** and **BP-58** as detected by the UV detector (254 nm) were compared. The half-life was determined at the time point at which the peak areas of both the **oCOm-58** and **BP-58** were approximately 50% (Figure S18).

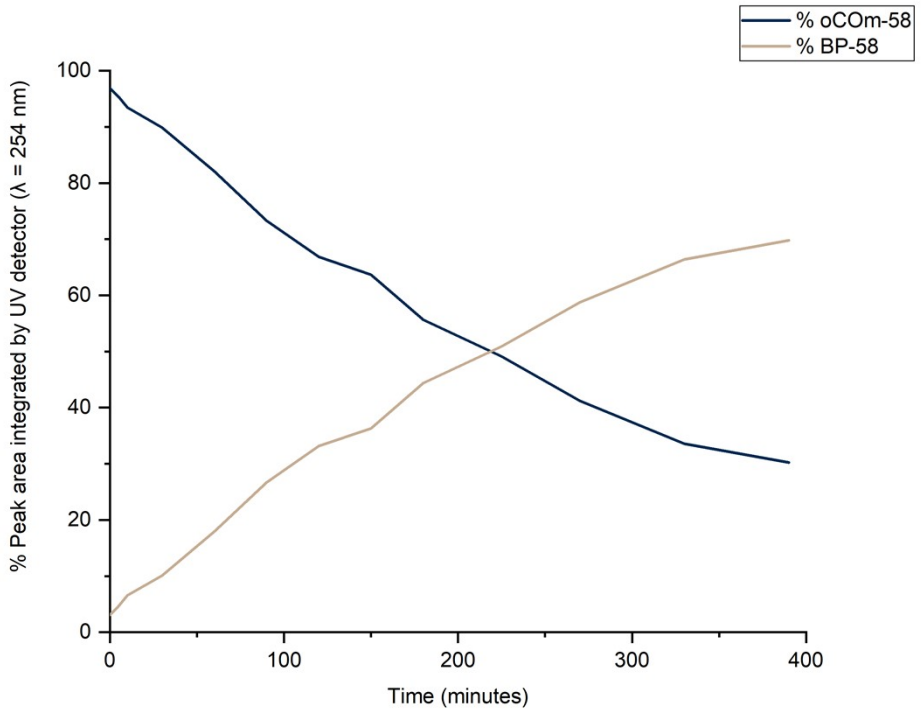


Figure S18: HPLC conversion study of **oCOm-58** to **BP-58** in TRIS-sucrose buffer pH 7.4 at 37˚C ($t_{1/2}$ = ~ 225 minutes).

Taking into consideration that the conversion of **oCOm-58** to **BP-58** is thought to occur through an $E1_{cB}$ type mechanism, the reaction was treated as a first order reaction in which the rate equation is described by equation (1):[ref]

$$rate = -\frac{\Delta\,[oCOm-58]}{\Delta\,[t]} = k[oCOm-58] \qquad (1)$$

The rate constant (k, s^{-1}) was determined by plotting *ln*[**oCOm-58**] against reaction time to give a linear relationship where:[ref]

$$ln[oCOm-58] = -k[t] + ln[oCOm-58]_0 \qquad (2)$$

As the reaction proceeds on a first order basis, the reaction half life ($t_{1/2}$) can be calculated using equation 3:[ref]

$$t_{1/2} = -\frac{ln\,(2)}{k} \qquad (3)$$

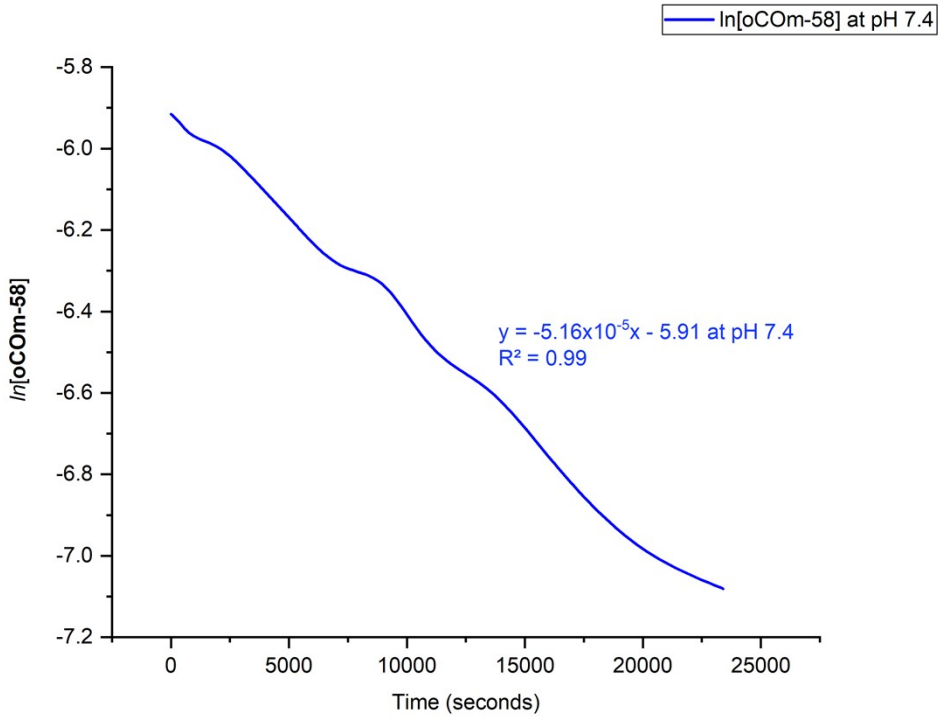


Figure S19: Plot of *ln*[**oCOm-58**] against time for **oCOm-58** (2.8 mM) at 37.8 ºC in 1 x PBS buffer at pH 7.4 (n = 1). Rate constant k = -5.16 x 10^{-5} s^{-1} and $t_{1/2}$ = 224 minutes at pH 7.4.

Briefly, the concentration of [**oCOm-58**] was calculated by multiplying the % of **oCOm-58** converted with the starting concentration of **oCOm-58** (2.8 mM). Figure S19 illustrates the relationship between *ln*[**oCOm-58**] against reaction time, in which the rate constant k = -5.16x10^{-5} s^{-1} and $t_{1/2}$ = 224 minutes at pH 7.4.

Conversion of oCOm-66 to BP-66 monitored by LC-MS analysis with Selective Ion Monitoring (SIM)

LC-MS analyses were conducted using analytical RP-HPLC (Shimadzu LC–20AD equipped with an SPD-20A UV detector [210 and 254 nm] and a Shimadzu LC-MS-2020 Liquid Chromatograph Mass Spectrometer operating in positive ion mode with Selective Ion Monitoring [SIM] for the *m/z* values of 1118, 1120, 560, 561, 1010 and 506) using a Phenomenex Prodigy C–18 column (5 µm, 3.00 × 250 mm) at 0.5 mL min^{-1}, heated to 40°C. The solvent system was a mixture of A (0.1% formic acid in H_2O) and B (0.1% formic acid in CH_3CN) using a gradient of 10% B to 100% B over 12.5 minutes, 100% B for 2.5 minutes, then 100% B to 10% B over 1 minute, 10% B for 3 minutes.

A 1 mM solution of **oCOm-66** in a mixture of 20% DMSO in 1x PBS buffer pH 7.4 was prepared (5 mL). The reaction mixture was heated and stirred at 37°C. Aliquots of the reaction mixture (100 µL) were taken periodically at the time intervals of 0, 60, 120, 180, 240, 300, 360 and 450 minutes (time after dissolution of **oCOm-66** in 1x PBS buffer). Each aliquot was immediately quenched by addition to a solution of 2M aqueous HCl (230 µL). Each of the quenched solutions were analysed by LC-MS using SIM (3 µL injection without pre-filtering as this affects the concentration of **BP-66**. The SIM values were used to identify peaks attributed to the **oCOm-66** and **BP-66**. The UV chromatogram (254 nm) was integrated at the retention times of 9.00 minutes for **oCOm-66** and 9.35 minutes for **BP-66** to give the % conversion (Figure S20 and Figure S21).

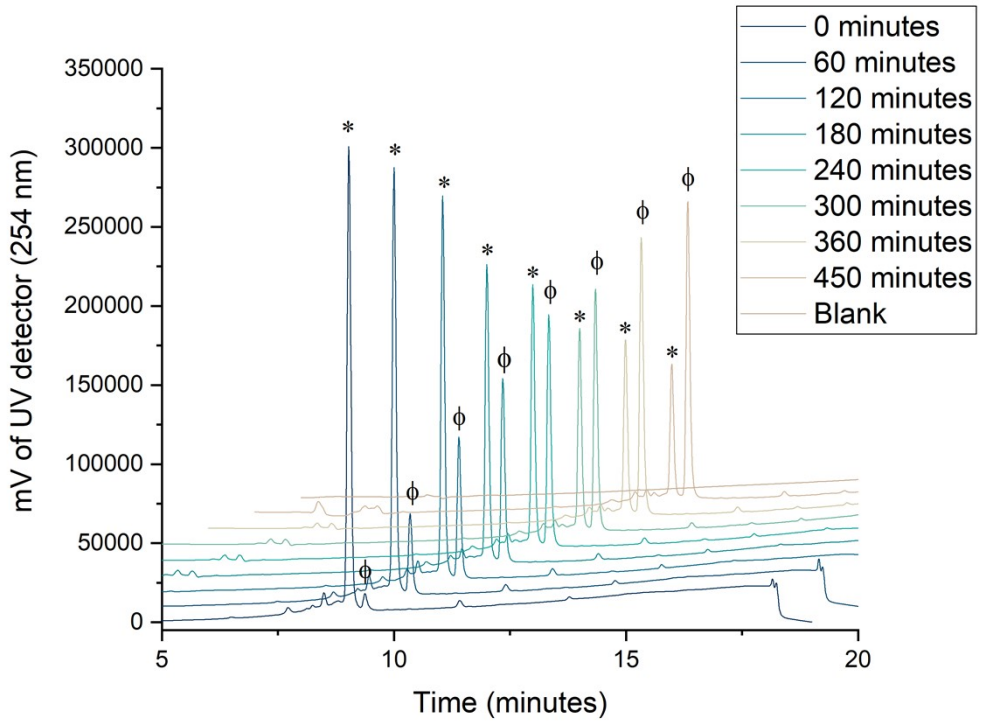


Figure S20: LC-MS chromatograms for the conversion of **oCOm-66** (*) to **BP-66** (ϕ) in 20% DMSO in 1 x PBS buffer at pH 7.4, 37˚C. Chromatograms were obtained from the UV detector (254 nm).

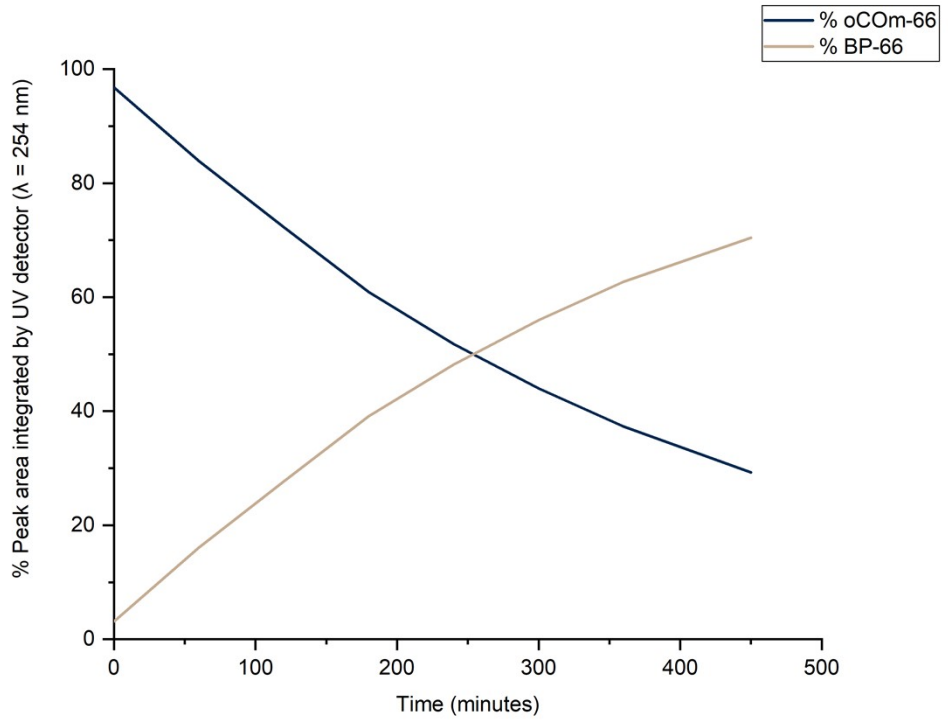


Figure S21: % conversion of the **oCOm-66** to **BP-66** in 20% DMSO in 1 x PBS buffer at 37°C at pH 7.4. % values were calculated from integrating the peak areas obtained from the chromatograms (UV detector = 254 nm, see Figure S20) at the retention times of 9.00 minutes for **oCOm-66** and 9.35 minutes for **BP-66**. Visual inspection of the graph indicates $t_{1/2}$ = ~250 minutes at pH 7.4, 37°C.

Taking into consideration that the conversion of **oCOm-66** to **BP-66** is thought to occur through an $E1_{cB}$ type mechanism, the reaction was treated as a first order reaction in which the rate equation is described by equation (1):[ref]

$$rate = -\frac{\Delta\,[oCOm-66]}{\Delta\,[t]} = k[oCOm-66] \qquad (1)$$

The rate constant (k, s^{-1}) was determined by plotting *ln*[**oCOm-66**] against reaction time to give a linear relationship where:[ref]

$$ln[oCOm-66] = -k[t] + ln[oCOm-66]_0 \qquad (2)$$

As the reaction proceeds on a first order basis, the reaction half life ($t_{1/2}$) can be calculated using equation 3:[ref]

$$t_{1/2} = -\frac{ln\,(2)}{k} \qquad (3)$$

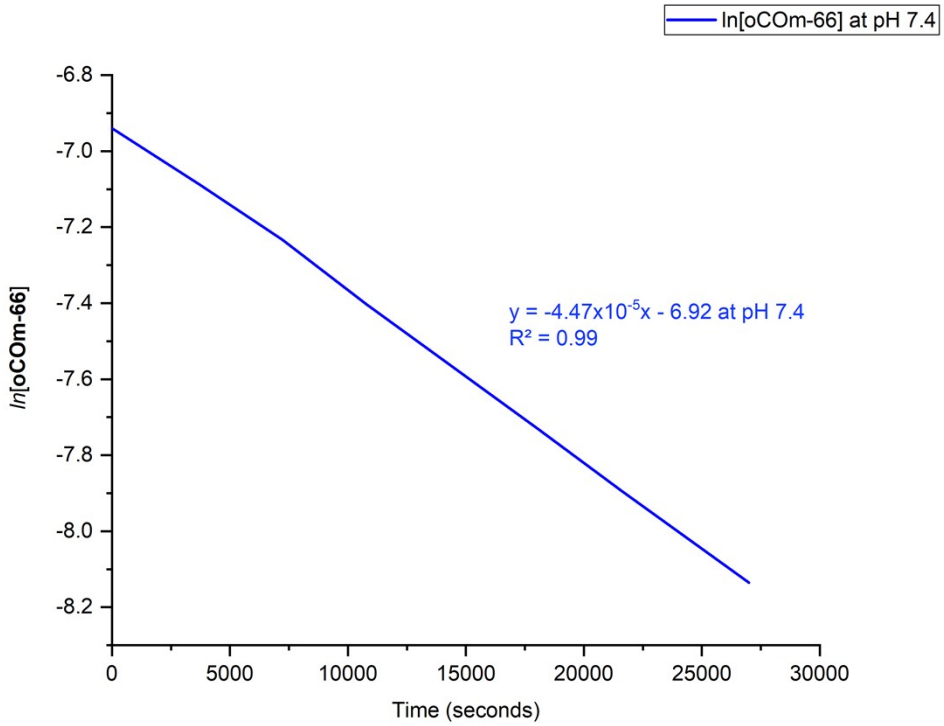


Figure S22: Plot of *ln*[**oCOm-66**] against time for **oCOm-66** (1 mM) at 37.8 ºC in 1 x PBS buffer at pH 7.4 (n = 1). Rate constant k = -4.47x10^{-5} s^{-1} and $t_{1/2}$ = 258 minutes at pH 7.4.

Briefly, the concentration of [**oCOm-66**] was calculated by multiplying the % of **oCOm-66** converted with the starting concentration of **oCOm-66** (1 mM). Figure S22 **i**llustrates the relationship between *ln*[**oCOm-66**] against reaction time, in which the rate constant k = -4.47x10^{-5} s^{-1} and $t_{1/2}$ = 258 minutes at pH 7.4.

Table S3: Calculation of the reaction constants' (k) and release half-lives for **oCOm-58** (2.8 mM) and **oCOm-66** (1 mM) at 37.8 ºC in 1 x PBS buffer at pH 7.4 by HPLC.

pH value	k (s^{-1})	$t_{1/2}$ (minutes)
oCOm-58		
7.4	-5.16×10^{-5}	224
oCOm-66		
7.4	-4.47×10^{-5}	258

CO release studies by fluorescence - monitoring the conversion of oCOm to BP

Spectra were acquired using Greiner-96 F-Bottom well plates or Nunc-96 well plates on the BMG Labtech (Alphatech) CLARIOstar spectrophotometer, which was set to 37.8 ºC, using a gain of 1192 (set to 70% of the target value), a focal point of 6.9 mm and a step width of 1 nm. The fluorescence spectrum was obtained every 600 seconds (10 min) using the excitation (λ_{ex}) and emission (λ_{em}) wavelengths specified in Figure S23 or , Figure S24 or Figure S25.

The sample of oCOm (1 mM) in either 1 x PBS buffer at pH 4.5 (3 mL) for **oCOm-57**, or a mixture of 20% DMSO in 1x PBS buffer at pH 4.5 (3 mL) for **oCOm-58**, was prepared and dispensed into the 96 well plate (100 µL in triplicate). *The next few steps were done quickly and without delay.* The pH of the oCOm solution was adjusted to 7.4 with 0.5 M aqueous NaOH and aliquots were dispensed into the 96 well plate (100 µL in triplicate). This process was repeated again at pH 8.1 for **oCOm-58** or pH 8.5 for **oCOm-5**7. The plate wells were then sealed using

EZ-Pierce™ Sealing film (EZPS-25, Excel Scientific) and immediately inserted into the pre-equilibrated (37.8°C) plate reader for the kinetic analysis. The kinetic analysis was left to acquire data for 14.5 h (**oCOm-58**) or 24 h (**oCOm-57**).

Visual analysis of Figure S23 indicated that the conversion of **oCOm-57** occurred faster at pH 8.1 than at pH 7.4. No conversion of **oCOm-57** to **BP-57** was observed at pH 4.6.

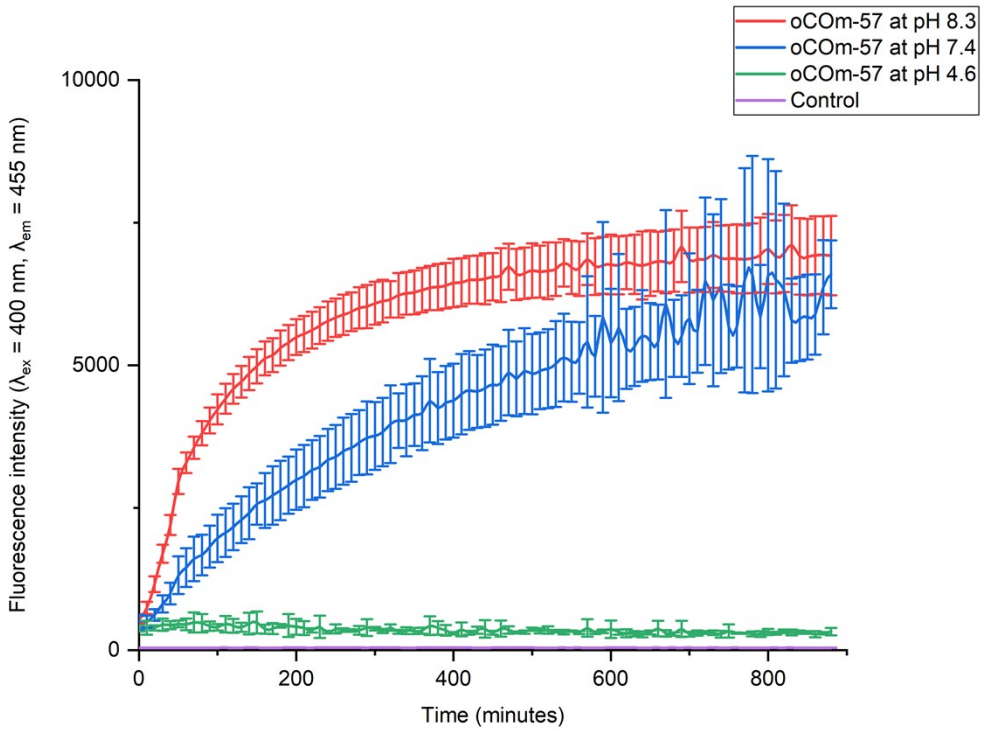


Figure S23: The conversion of **oCOm-57** (1 mM) to **BP-57** in 1x PBS buffer at the specified pH values at 37.8°C (λ_{ex} = 400 nm, λ_{em} = 455 nm) (n=3).

Taking into consideration that the conversion of **oCOm-57** to **BP-57** is thought to occur through an $E1_{cB}$ type mechanism, the reaction was treated as a first order reaction in which the rate equation is described by equation (1):[ref]

$$rate = -\frac{\Delta\,[oCOm-57]}{\Delta\,[t]} = k[oCOm-57] \qquad (1)$$

The rate constant (k, s^{-1}) was determined by plotting *ln*[**oCOm-57**] against reaction time to give a linear relationship where:[ref]

$$ln[oCOm-57] = -k[t] + ln[oCOm-57]_0 \qquad (2)$$

As the reaction proceeds on a first order basis, the reaction half life ($t_{1/2}$) can be calculated using equation 3:[ref]

$$t_{1/2} = -\frac{ln\,(2)}{k} \qquad (3)$$

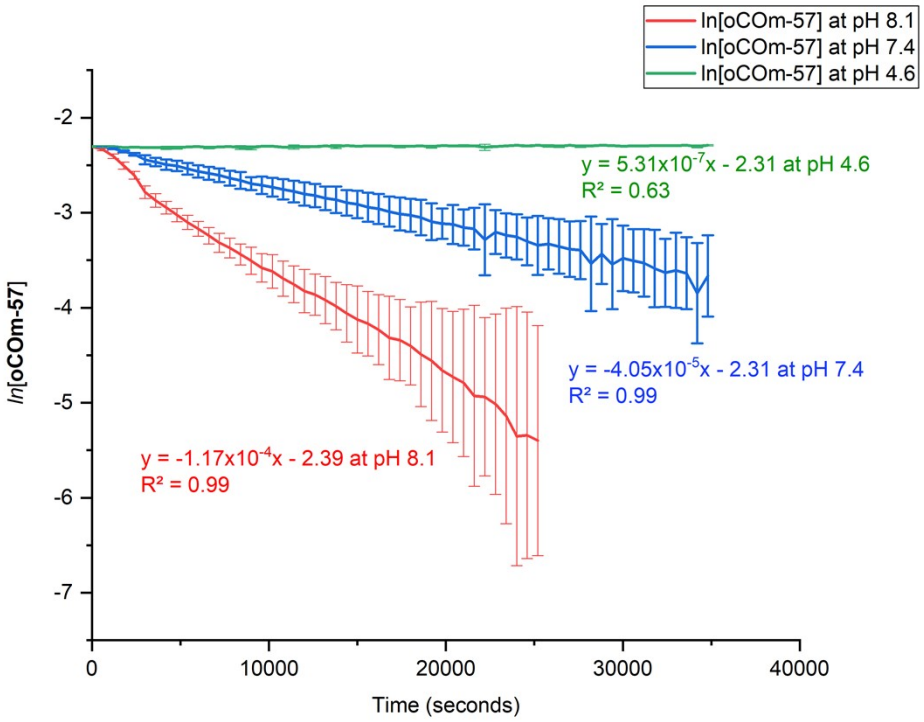


Figure S24: Plot of *ln*[**oCOm-57**] against time for **oCOm-57** (1 mM) at 37.8 ºC in 1 x PBS buffer at the specified pH values (n = 3).

Briefly, the concentration of [**oCOm-57**] was calculated by multiplying the % of **oCOm-57** converted (based on the change in fluorescence intensity) with the starting concentration of **oCOm-57** (1 mM). Figure S24 illustrates the relationship between *ln*[**oCOm-57**] against reaction time. Table S4 lists the rate constants and the release half-lives ($t_{1/2}$) calculated by equation 3.

A similar analysis was performed for **oCOm-58.** Visual inspection of Figure S25 indicated that **oCOm-58** converted to **BP-58** at a faster rate at pH 8.1 than at pH 7.4. The decrease in fluorescence intensity observed at pH 8.1 (n = 1) was attributed to the fast conversion of **oCOm-58** to **BP-58** resulting in the precipitation of **BP-58** and could result in the $t_{1/2}$ value being underestimated. No conversion of **oCOm-58** to **BP-58** was observed at pH 4.5. Figure S26 illustrates the relationship between *ln*[**oCOm-58**] against reaction time. Table S4 lists the rate constants calculated from Figure S26 and the release half-lives ($t_{1/2}$) calculated by equation 3.

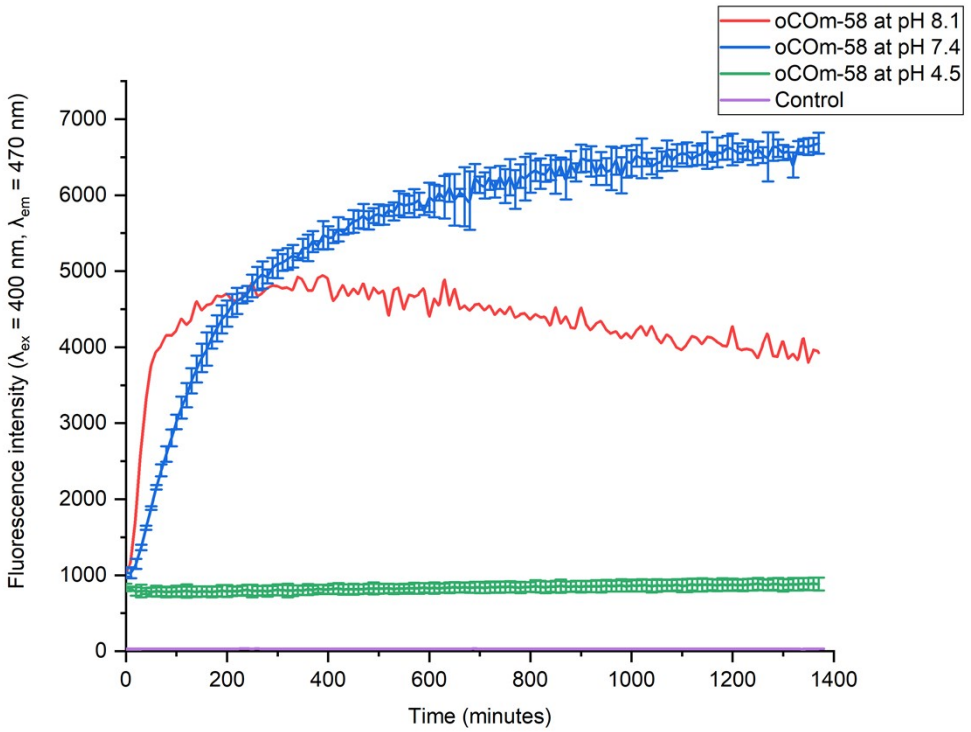


Figure S25: The conversion of **oCOm-58** (1mM) to **BP-58** in a mixture of 20% DMSO in 1x PBS buffer at the specified pH values at 37.8°C (λ_{ex} = 400 nm, λ_{em} = 470 nm) (n = 3, except for pH 8.1 n = 1). A large amount of precipitate formed when the conversion study of **oCOm-58** was conducted at pH 8.1. This was attributed to the formation of the water insoluble **BP-58**.

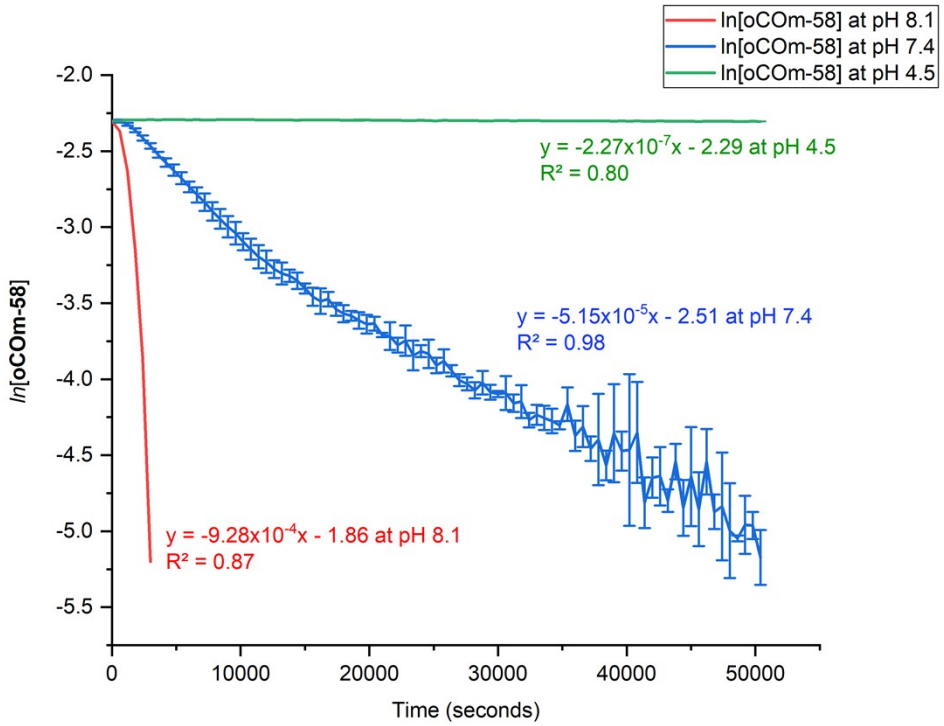


Figure S26: Plot of *ln*[**oCOm-58**] against time for **oCOm-58** (1 mM) at 37.8 ºC in 1 x PBS buffer at the specified pH values (n = 3, except for pH 8.1 where n = 1).

Table S4: Calculation of the reaction constants' (k) and release half-lives for **oCOm-57** and **oCOm-58** (1 mM) at 37.8 ºC in 1 x PBS buffer at the specified pH values by fluorescence.

pH value	k (s^{-1})	$t_{1/2}$ (minutes)
oCOm-57		
4.5	5.31×10^{-7}	–[a]
7.4	-4.05×10^{-5}	285
8.1	-1.17×10^{-4}	98
oCOm-58		
4.5	2.27×10^{-7}	–[a]
7.4	-5.15×10^{-5}	224
8.1	-9.28×10^{-4}	12

[a] No conversion of the oCOm to its' respective BP was observed in the time period for which the conversion study was monitored

An analogous procedure was used to monitor the conversion of **oCOm-66** to **BP-66** using the excitation (λ_{ex}) and emission (λ_{em}) wavelengths specified in Figure S27. A decrease in fluorescence intensity of the nitro-naphthalimide fluorophore was observed at pH 7.4 and pH 8.1 which is consistent with the conversion of **oCOm-66** to **BP-66**. As anticipated no conversion of **oCOm-66** to **BP-66** was observed at pH 4.5. Unfortunately, **BP-66** proved highly insoluble in a solution of 20% DMSO in 1x PBS buffer and no attempt was made to calculated the $t_{1/2}$ values as these would have been underestimated.

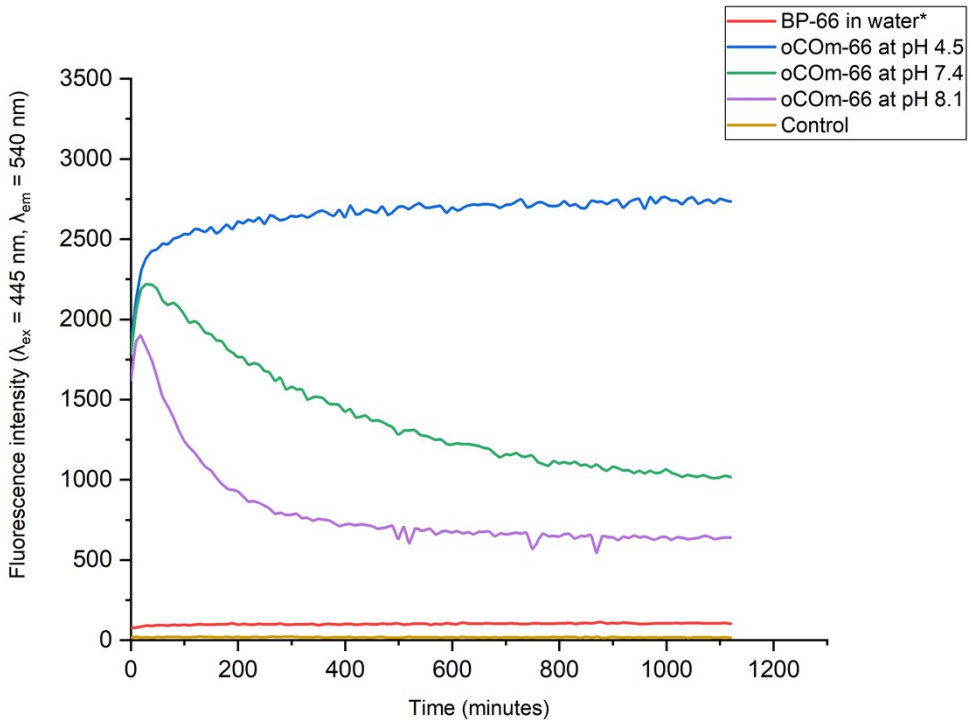


Figure S27: The conversion of **oCOm-66** (1 mM) to **BP-66** in a solution of 20% DMSO in 1x PBS buffer at the specified pH values at 37.8°C (λ_{ex} = 445 nm, λ_{em} = 540 nm) (n=1). Control samples of **DB-66** (1 mM, 20% DMSO in 1x PBS buffer pH 7.4) and **BP-66** (1 mM, H_2O) were also analysed concurrently. ***BP-66** is sparingly soluble in a solution of 20% DMSO in 1 x PBS buffer.

Quantum Yields for BP-57, BP-58, oCOm-66 and BP-66

Quantum yields were obtained on an Edinburgh FS5 equipped with a SC-30 Integrating Sphere at approximately 20°C. The solvent reference (3 mL, EtOH) and sample (3 mL, 1 mM in EtOH) were separately dispensed into a matched pair of fluorescence quartz cuvettes (3 mL, 10 mm pathlength, 23/Q/10 Starna Scientific). Quantum yields were calculated using the Fluoracle software QY wizard.

The following parameters were used to calculate the quantum yields for **BP-57** and **BP-58**: bandwidth 0.85 nm, dwell time, 0.5 seconds, scan step 2 nm, for $\lambda_{ex\ max}$ = 400 nm the scatter range was set to λ_{ex} = 396–404 nm, for $\lambda_{em\ max}$ = 455 or 470 nm the emission range was set to λ_{em} = 408–534 nm.

The following parameters were used to calculate the quantum yields for **oCOm-66** and **BP-66**: Dwell time, 0.5 seconds, scan step 2 nm. For the phenanthrene or triphenylene fluorophore: bandwidth 0.9 nm, for $\lambda_{ex1\ max}$ = 395 nm the scatter range was set to λ_{ex1} = 385–405 nm, for $\lambda_{em1\ max}$ = 460 nm the emission range was set to λ_{em1} = 420–500 nm. For the naphthalimide fluorophore: bandwidth 1.0 nm, for $\lambda_{ex2\ max}$ = 445 nm the scatter range was set to λ_{ex2} = 435–460 nm, for $\lambda_{em2\ max}$ = 540 nm the emission range was set to λ_{em2} = 500–640 nm.

Confocal Studies on oCOm-57, oCOm-58, and BP-57

Methods:

AC16 human cardiomyocytes were plated at $9x10^3$ cells per well in DMEM-F12 (Gibco) + 10% foetal calf serum (50 µl) for 24 hours in 96 well black masked tissue culture plates (Corning, Tewksbury, MA, USA). **oCOm-57**, **oCOm-58**, **DB-58**, **BP-57** and **oCOm-66** (10µM) was added

to triplicate wells at five different time points to give total exposure times of 30, 60, 120, 180 and 240 minutes. Twenty minutes before imaging, the media was removed and replaced with magnesium free DPBS (50 µl; Gibco), to which was added the active mitochondrial membrane potential-dependent probe MitoTracker Red CM-H_2XRos (Invitrogen (MitoTracker)) to a final concentration of 200 nM and the CO-selective fluorescent probe COP-1 (1 µM) synthesised according to the method of Michel 2012. Each well was imaged at its appropriate time point on a Nikon A1 + Inverted Confocal Laser Scanning Microscope with a 40x objective lens using standard laser filter combinations for Green (λ_{ex} = 488 nm; λ_{em} = 525 nm), Red (λ_{ex} = 561 nm; λ_{em} = 595 nm) and blue (λ_{ex} = 405 nm; λ_{em} = 450 nm) fluorescence with a z stack of 12 slices collected for each image at times 30, 60, 120, 180 and 240 minutes after the addition of oCOm. Microscope settings were kept to the same parameters for all compounds to enable quantitative comparisons. Control wells with each of the fluorescent molecules alone (oCOm, MitoTraker and no COP-1) were included on all plates. Fluorescence intensity was determined using Fiji ImageJ software (Version 2.0.0rc, NIH, USA)[15] conducted on individual channels and calculated as intensity/cell normalised to the area of the cells and reported as the corrected total cell fluorescence (CTCF). CTCF = Integrated Density – (Area of selected cell X Mean fluorescence of background). Measures were obtained by analysing at least 10 cells per sample in at least three independent experiments. As we observed CO (as the COP-1 BODIPY probe) but no **BP-57** after cells were exposed to **oCOm-57**, we hypothesised that it remained outside the cells and was washed away when the media was removed to add the COP-1 and MitoTracker. To establish if **oCOm-57** was localised extracellularly or intracellularly cells were imaged following **oCOm-57** exposure both before and after washing with DPS.

Figure S28

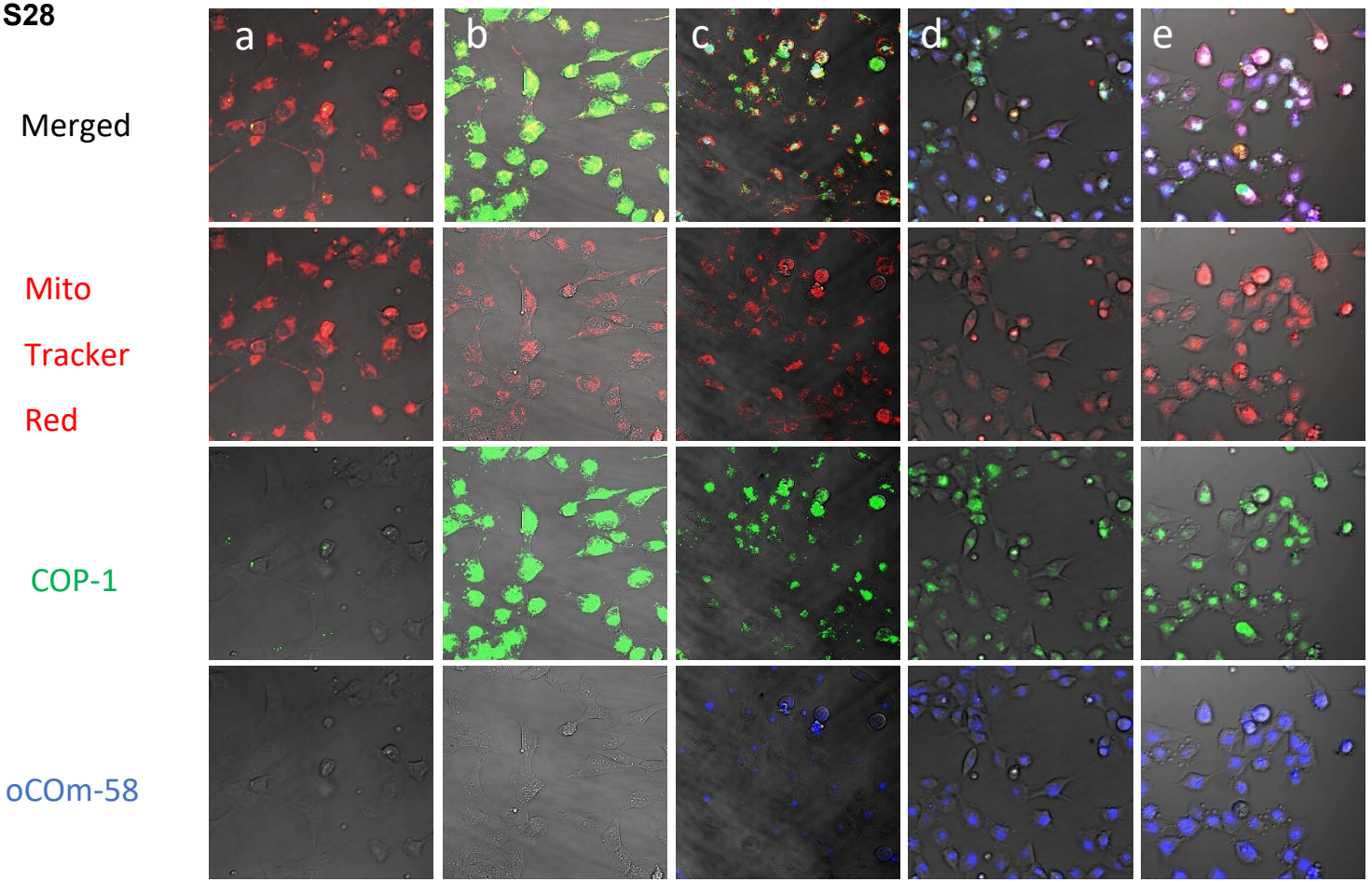


Figure S28 shows single channels of **Figure 6**: AC-16 cardiomyocytes treated with **oCOm-58** (10 μM), MitoTracker, and COP-1. Panels show merged and single channel fluorescence images of MitoTracker (red), CO activated COP-1 (green), and the spent breakdown-product **BP-58** (blue) in cells at sequential time points; a) 30 minutes – red indicates active mitochondria with no oCOm **BP-58** visible, b) 80 minutes – green shows CO release with some colocalisation (yellow) to the mitochondria is accompanied by decreased mitochondrial activity (red), c) 120 minutes – mitochondrial activity begins to return, d) 190 minutes – **BP-58** levels increase as CO is released, turquoise indicating colocalization of **BP-58** with CO whilst bright (white) patches indicate colocalization of **BP-58** with COP-1 and MitoTracker Red, e) 240 minutes – purple indicates that the spent by-product **BP-58** is co-localised with the active mitochondria, CO is still being released and colocalization of all three fluorescent signals are evident as bright white patches.

Figure S29

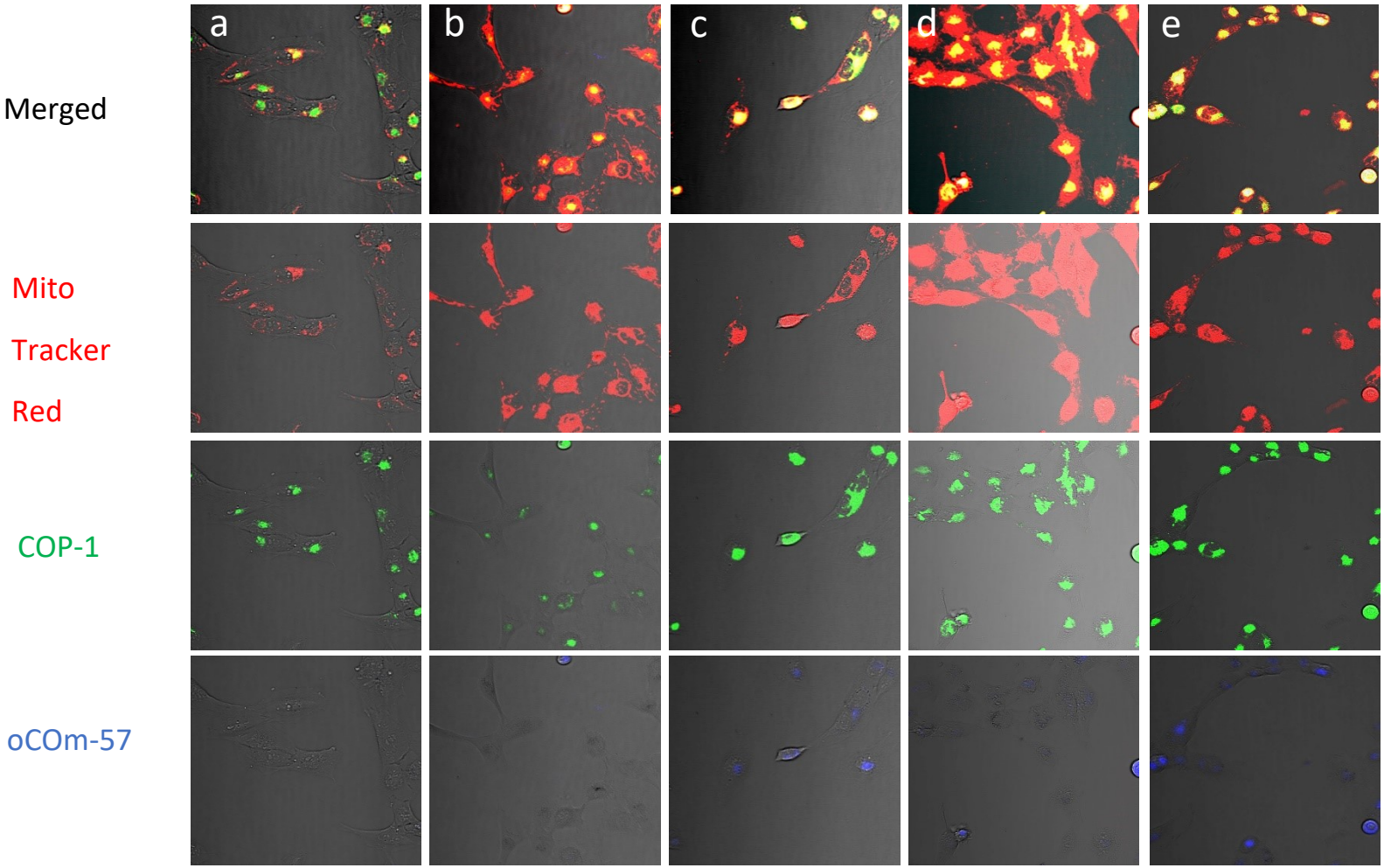


Figure S29 shows single channels of **Figure 8**: AC-16 cardiomyocytes treated with **oCOm-57** (10 μM), MitoTracker, and COP-1. Panels show merged and single channel fluorescence of MitoTracker (red), COP-1 due to CO release (green), and spent by-product **BP-5**7 (blue) in cells at sequential time points; a) 30 minutes & b) 60 mins - show CO release colocalised with active mitochondria (yellow), c) 120 minutes – shows intracellular CO release is accompanied by decreased mitochondrial activity and increased co-localisation of CO to the mitochondria (yellow), d) 180 minutes – mitochondrial activity increases, e) 240 minutes – CO continues to be co-localised to the mitochondria.

Figure S30

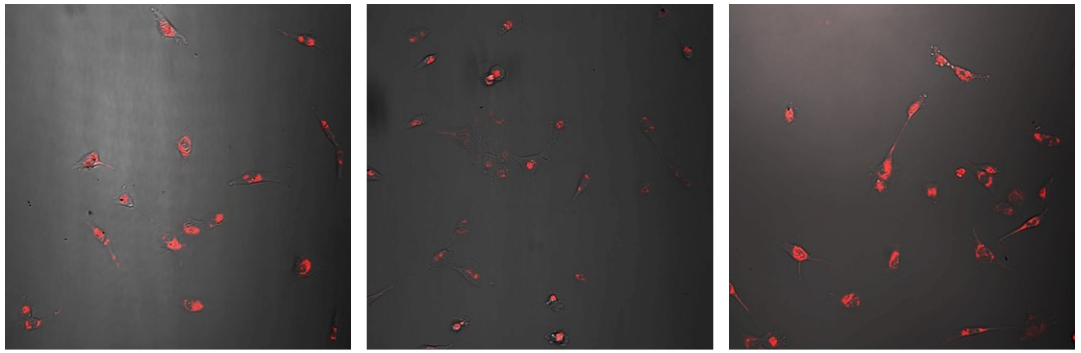
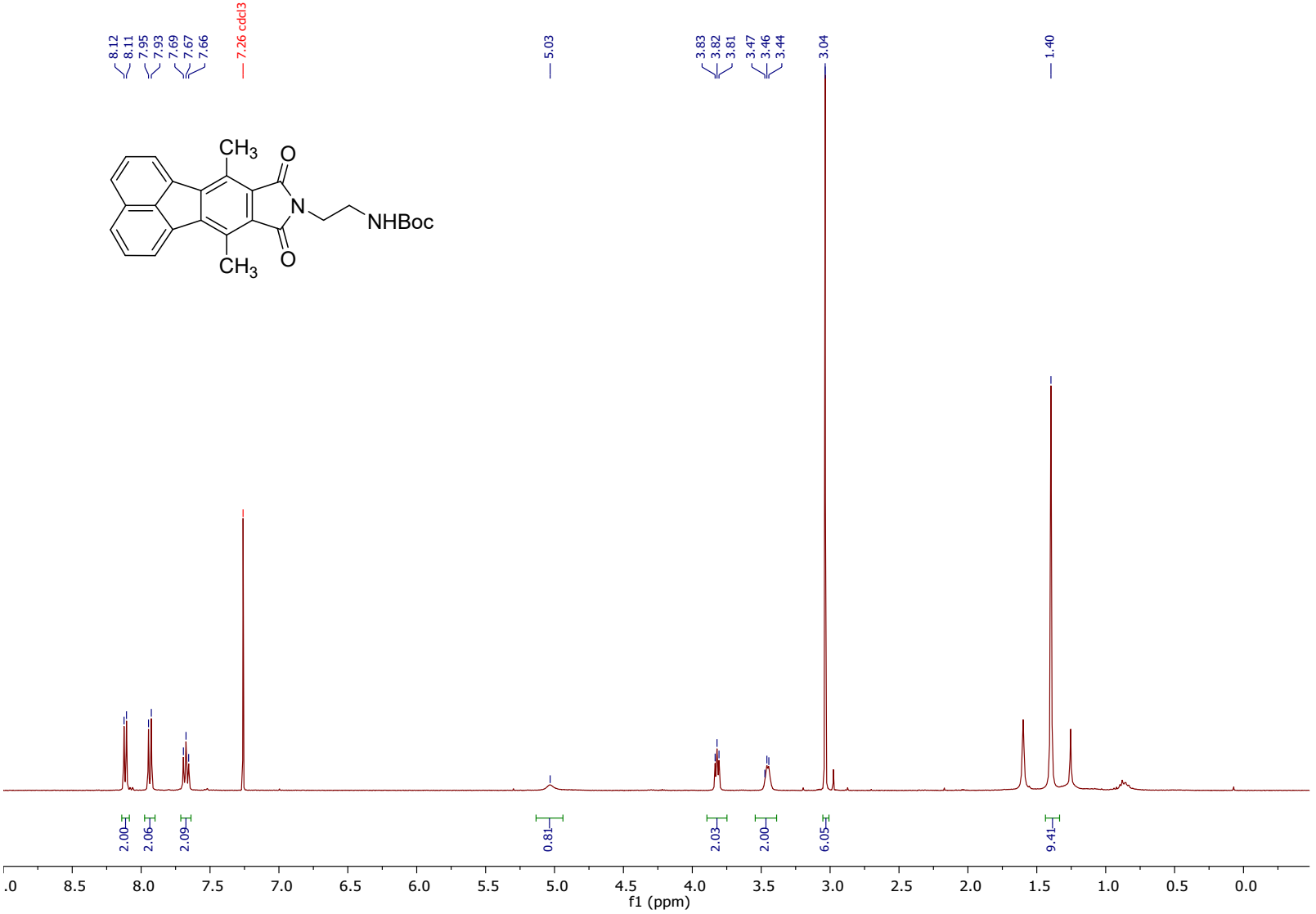


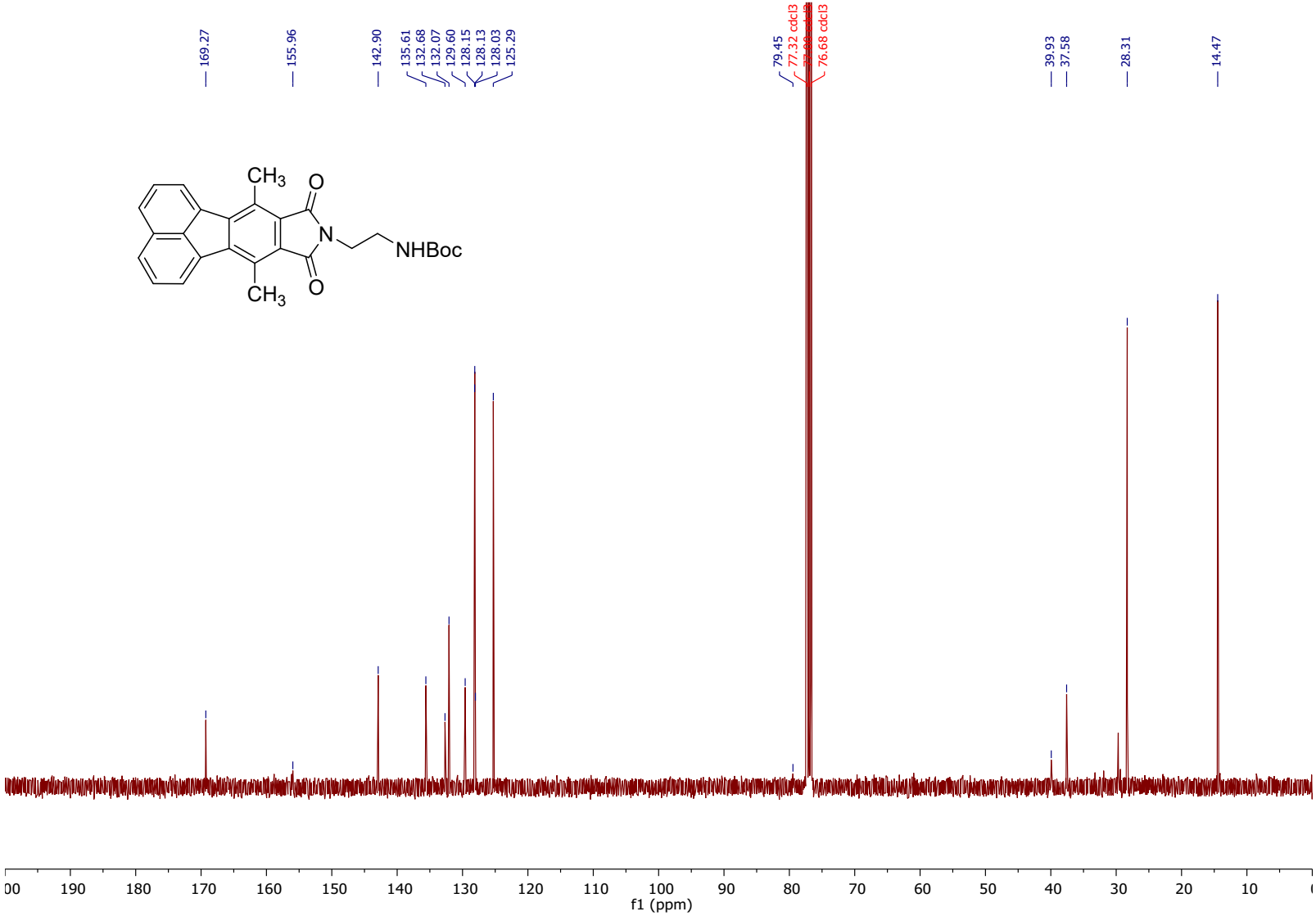
Figure S30: Pre-treatment with **oCOm-66** (10 μM) protects mitochondrial energetics for up to 4 h in AC16 cells maintained during live cell imaging as indicated by uptake of the fluorescent mitochondrial membrane potential dependent probe MitoTracker red: (a) Immediately after removal from incubator; (b & c) after 4 hours out of incubator. (b) without and (c) with prior exposure to **oCOm-66**.

Appendix:
^{1}H and ^{13}C NMR spectra of novel and unreported compounds

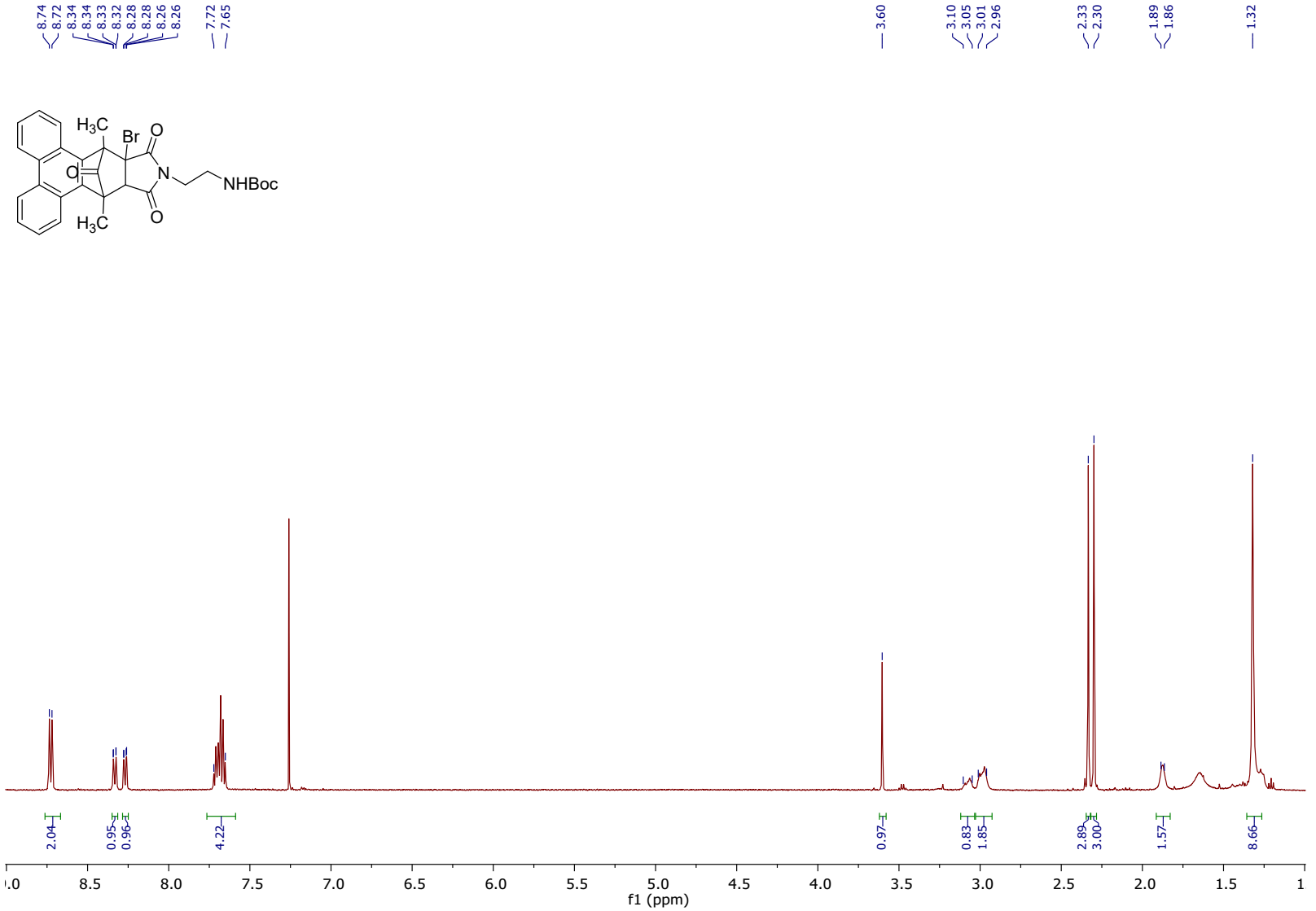
Compound **7** ^{1}H NMR spectrum (400 MHz, $CDCl_3$).



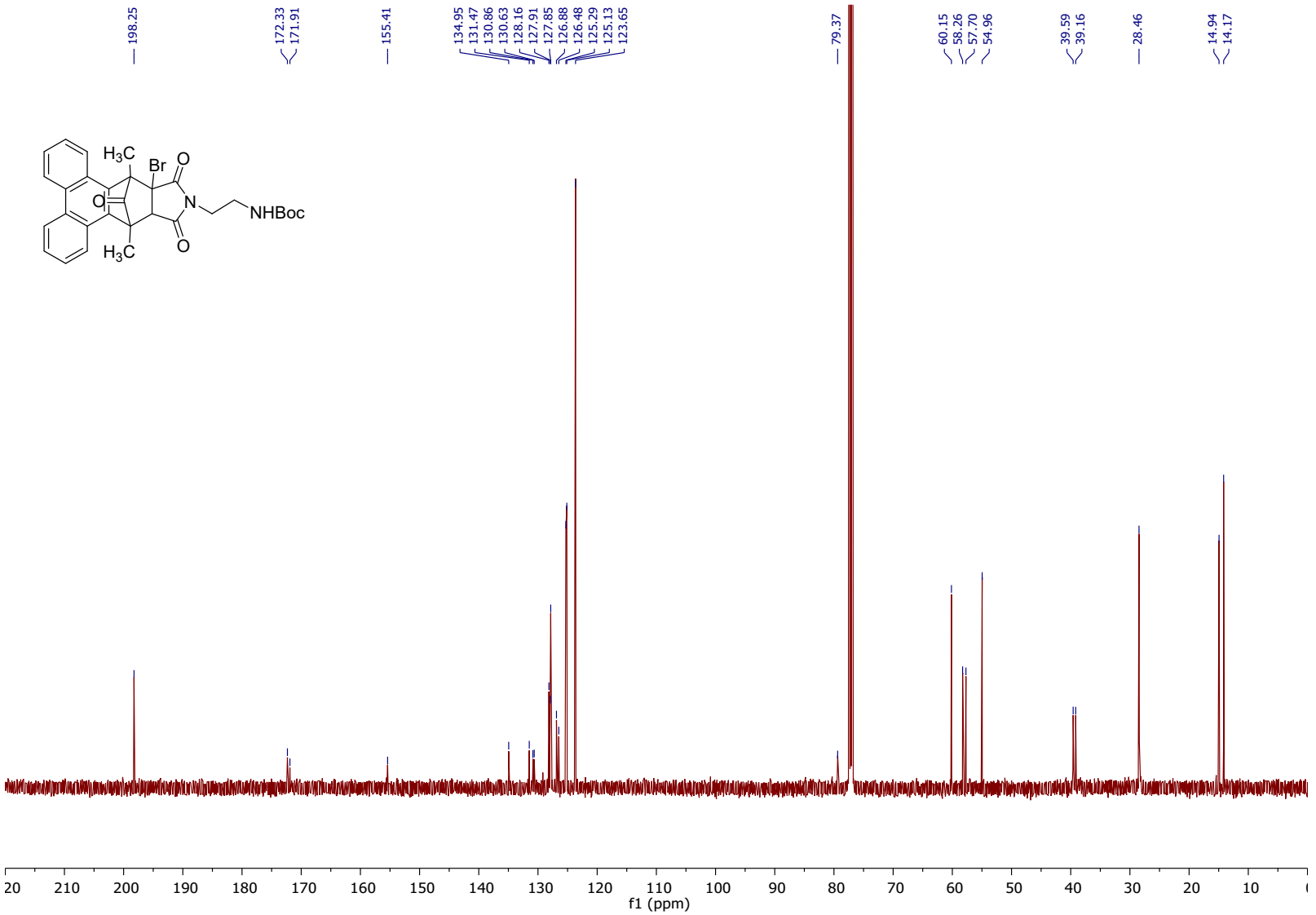
Compound **7**. ^{13}C NMR spectrum (100 MHz, $CDCl_3$).



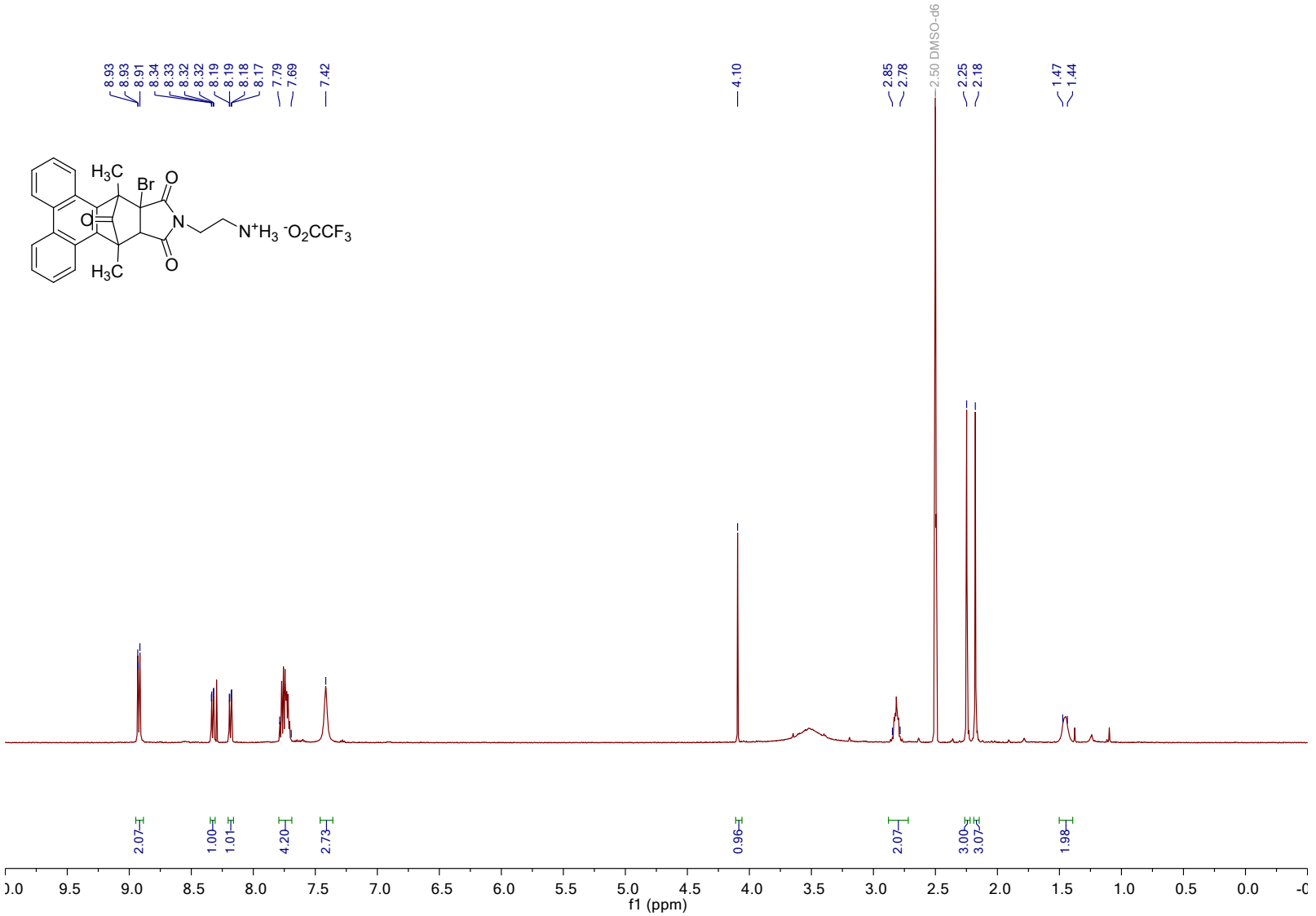
Compound **10**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



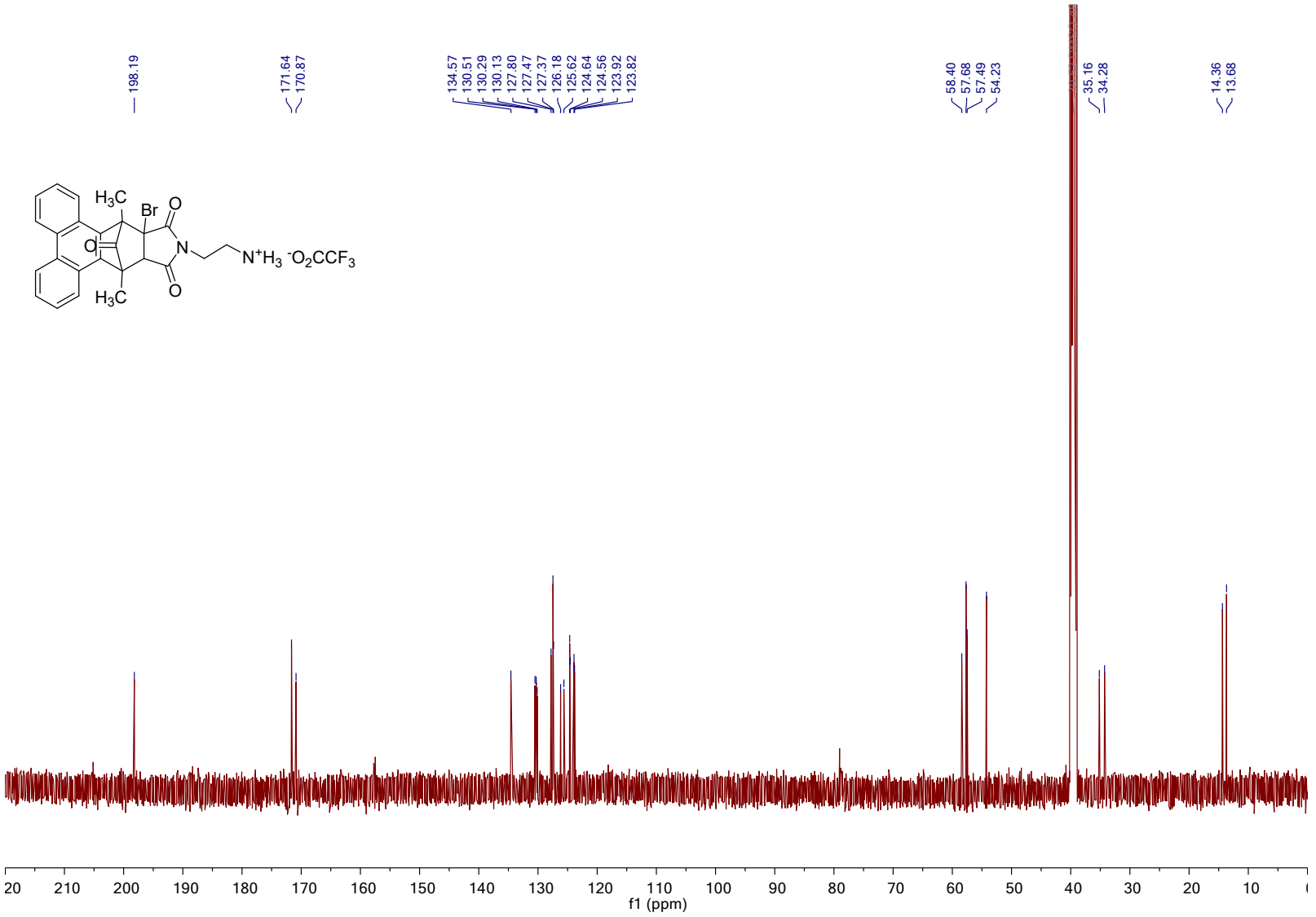
Compound **10**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



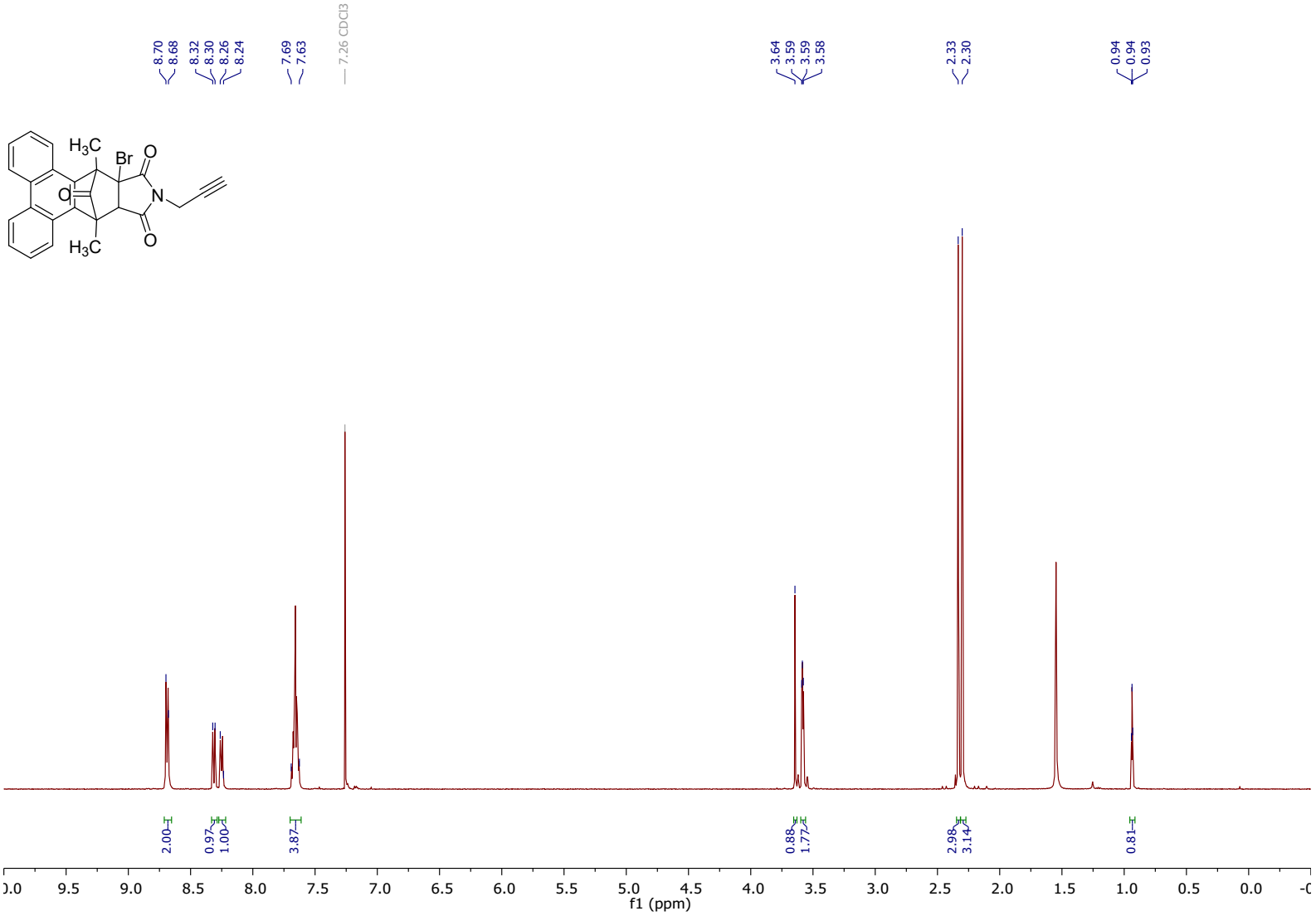
oCOm-62. ^{1}H NMR spectrum (500 MHz, DMSO-*d6*).



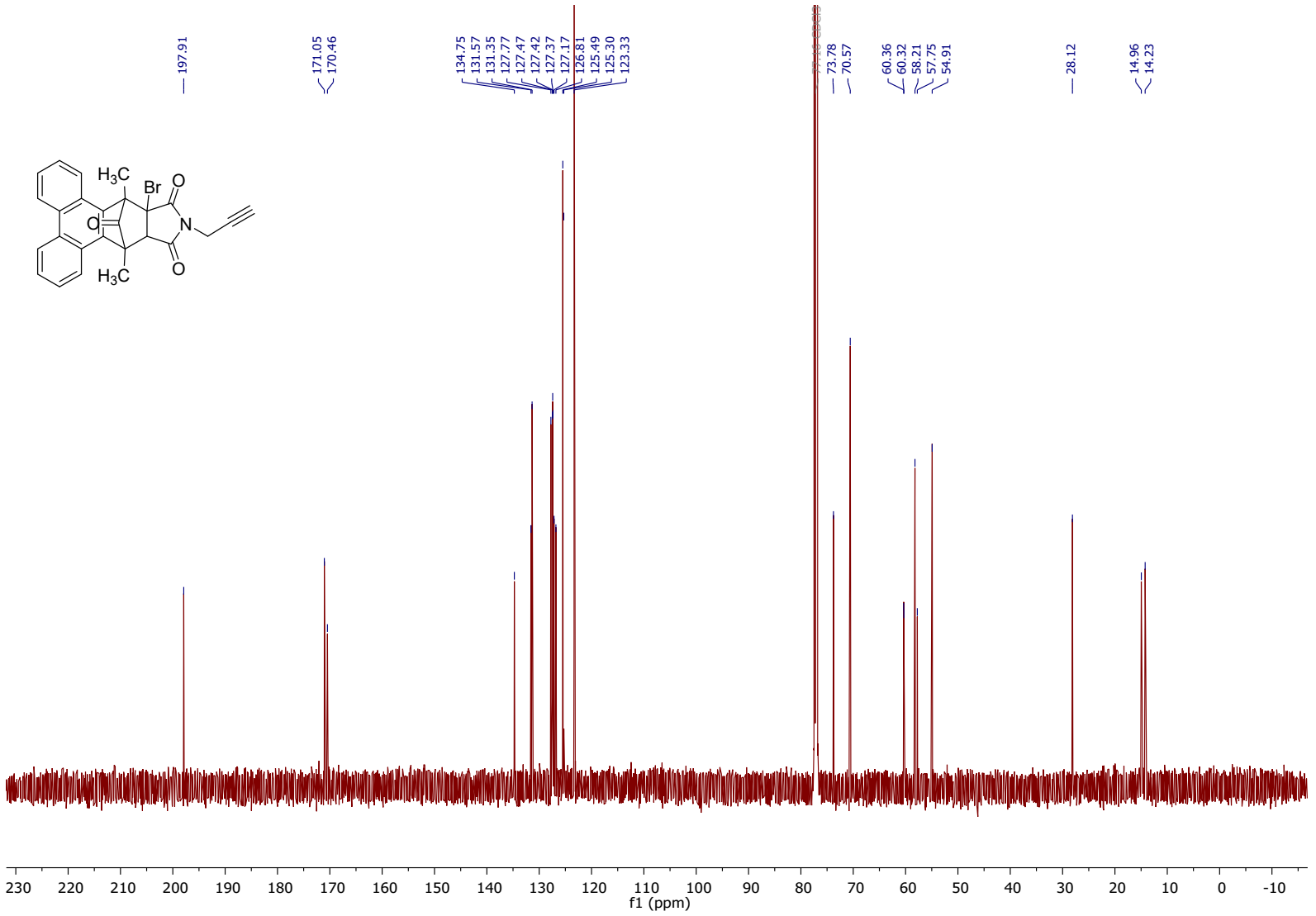
oCOm-62. ^{13}C NMR spectrum (125 MHz, DMSO-*d6*).



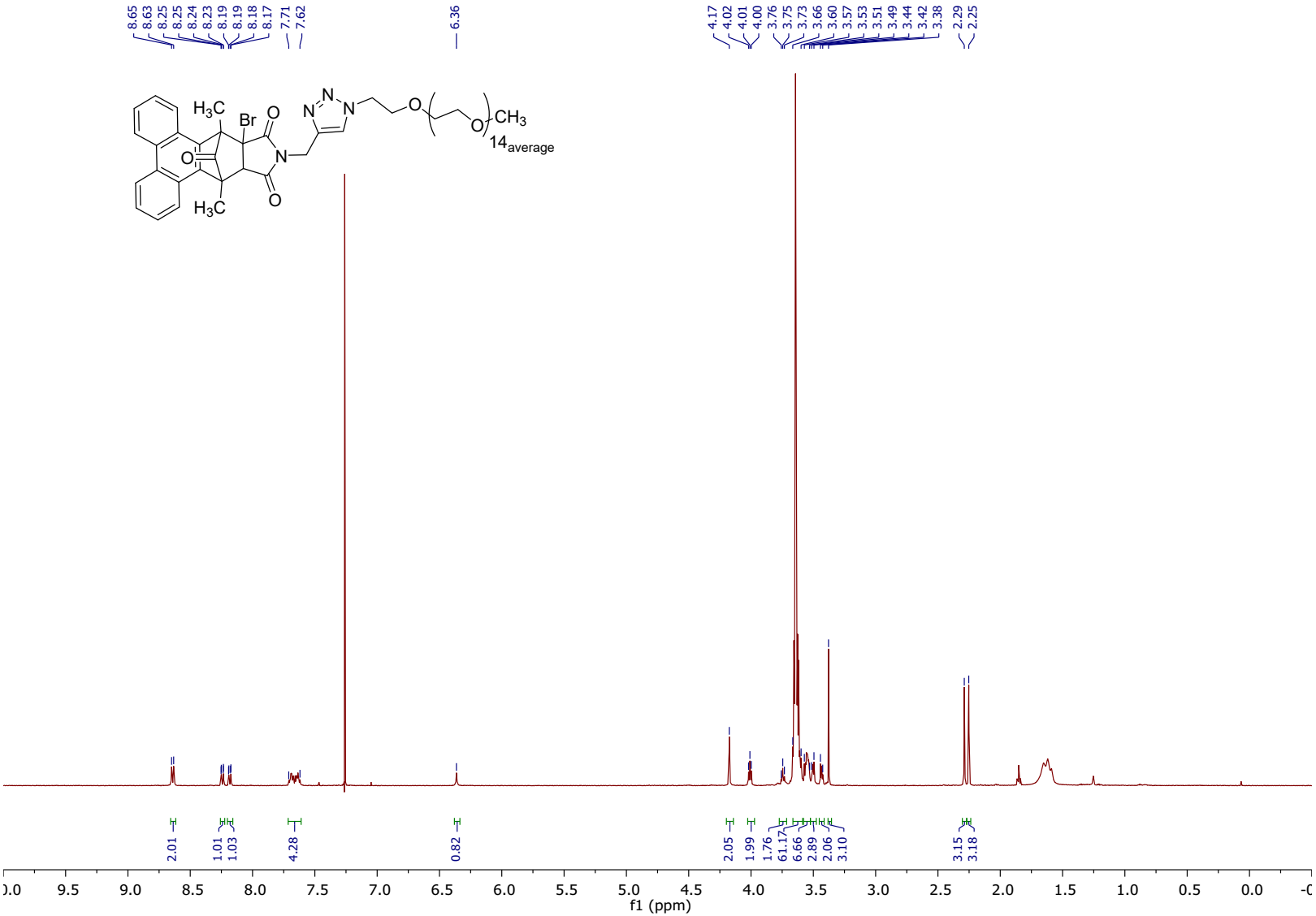
Endo-3a-bromo-2-(2-propyn-1-yl)-3a,4,7,7a-tetrahydro-4,7-dimethyl-5,6-phenanthro-1*H*-isoindole-1,3,8(2*H*)-trione (**12**). ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



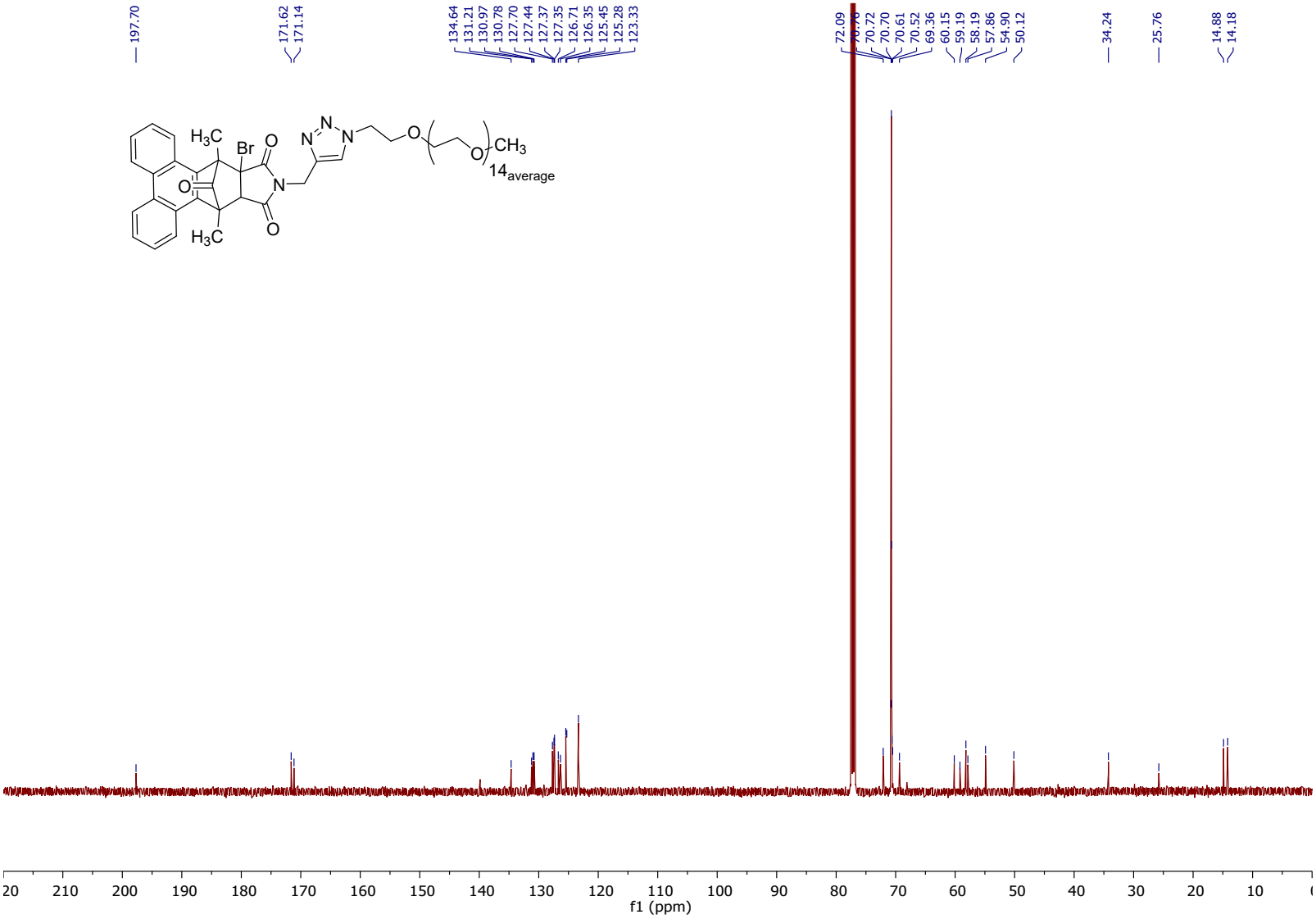
Endo-3a-bromo-2-(2-propyn-1-yl)-3a,4,7,7a-tetrahydro-4,7-dimethyl-5,6-phenanthro-1*H*-isoindole-1,3,8(2*H*)-trione (**12**). ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



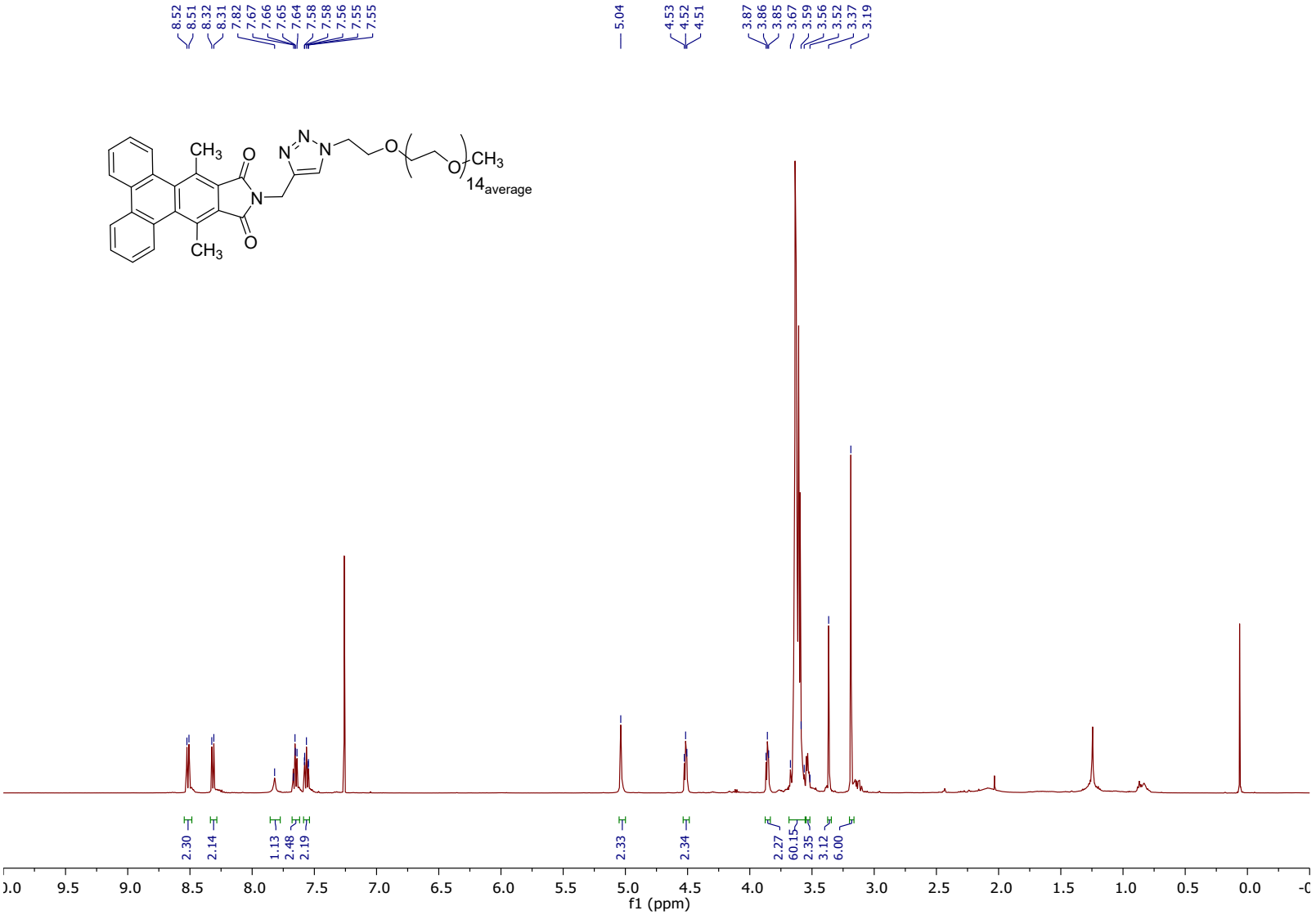
oCOm-57. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



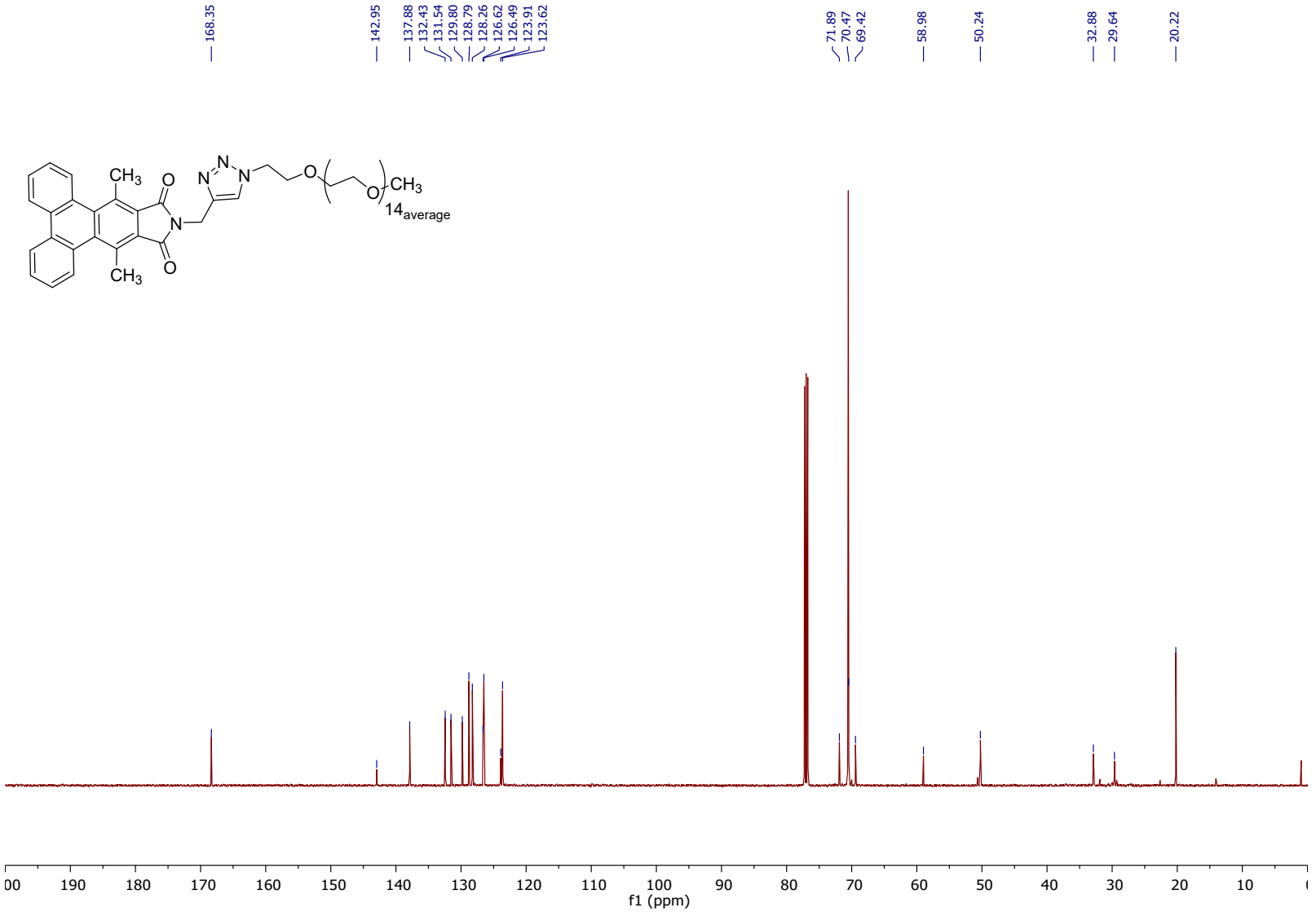
oCOm-57. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



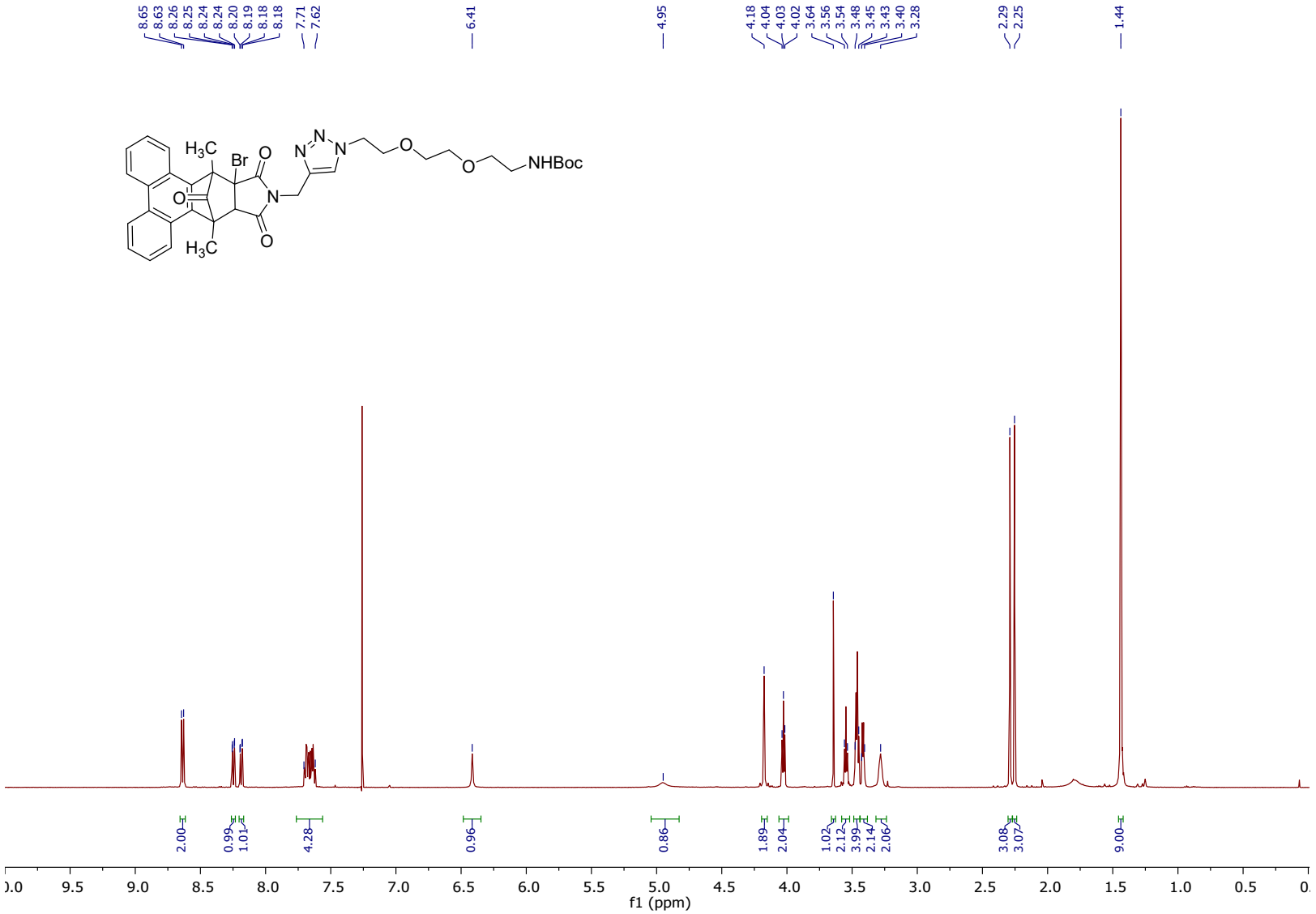
BP-57. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



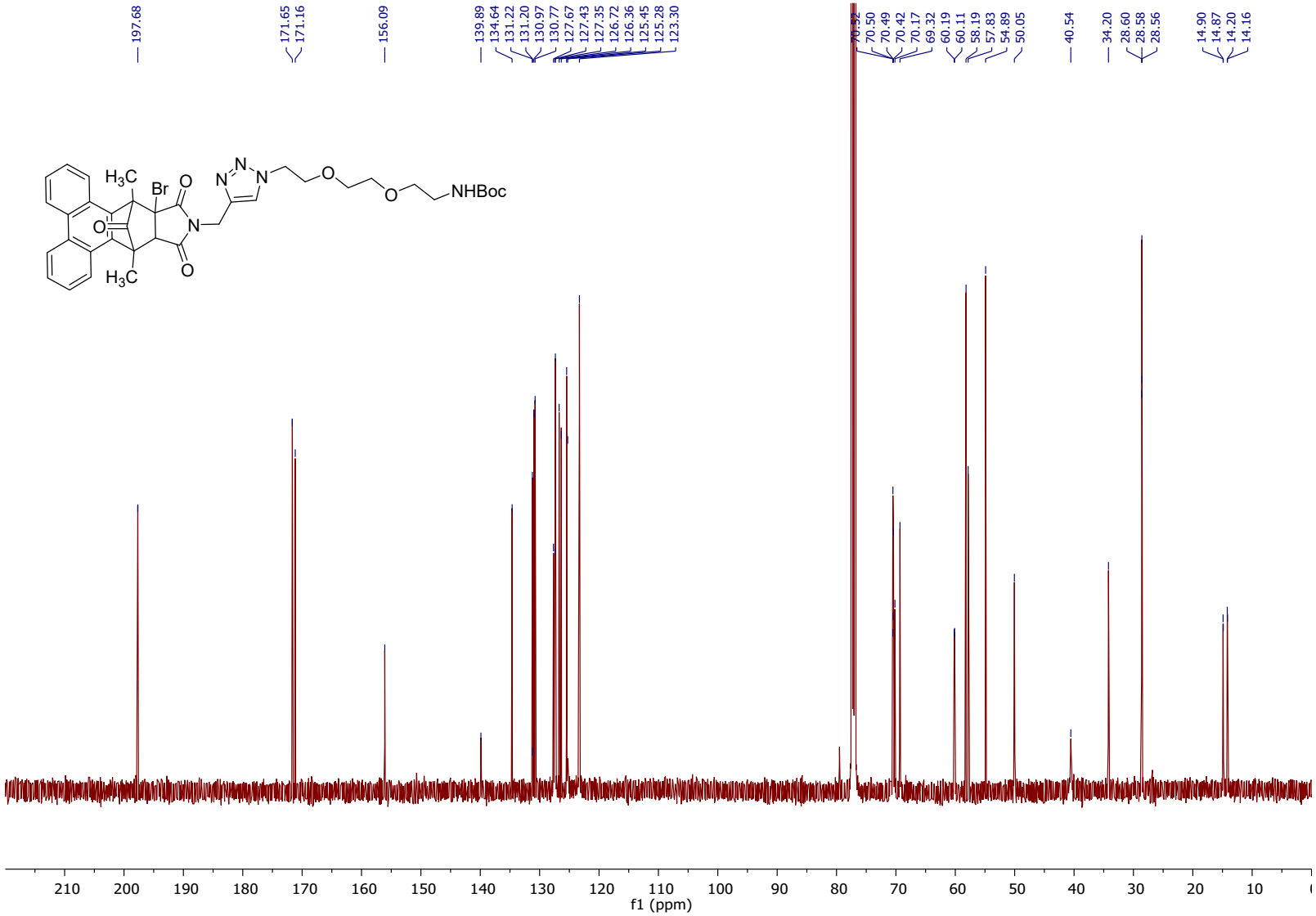
BP-57. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



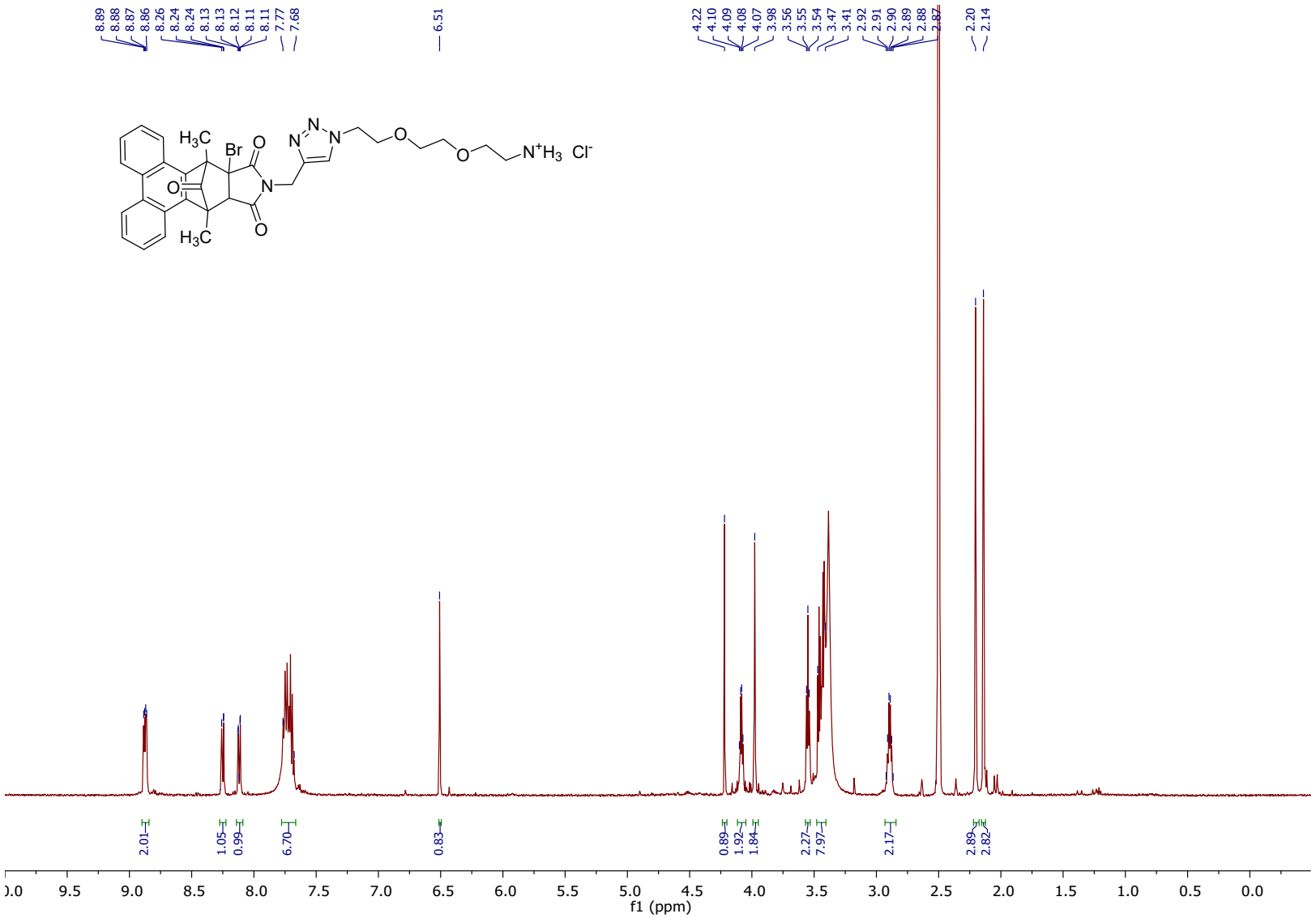
Compound **15**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



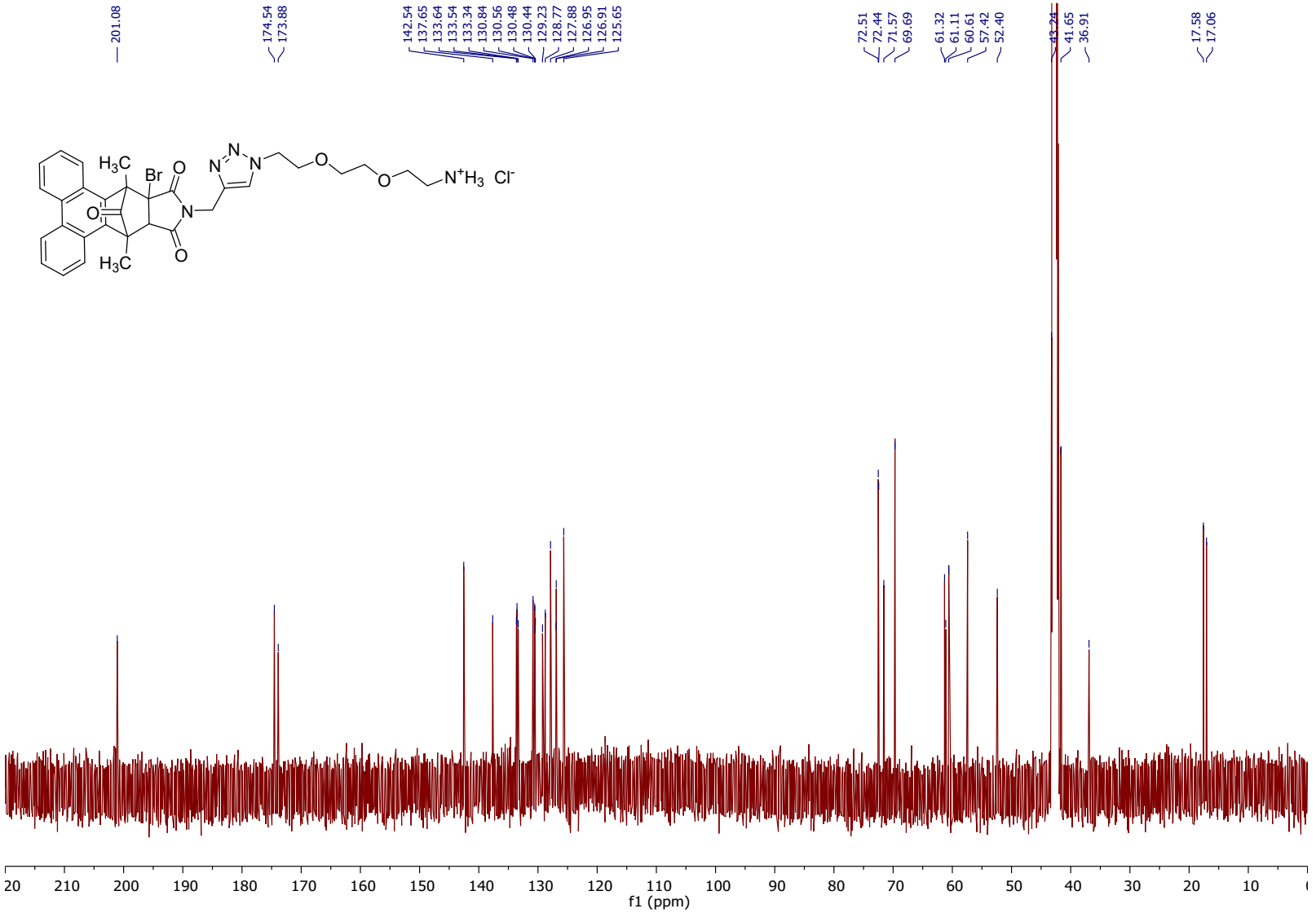
Compound **15**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



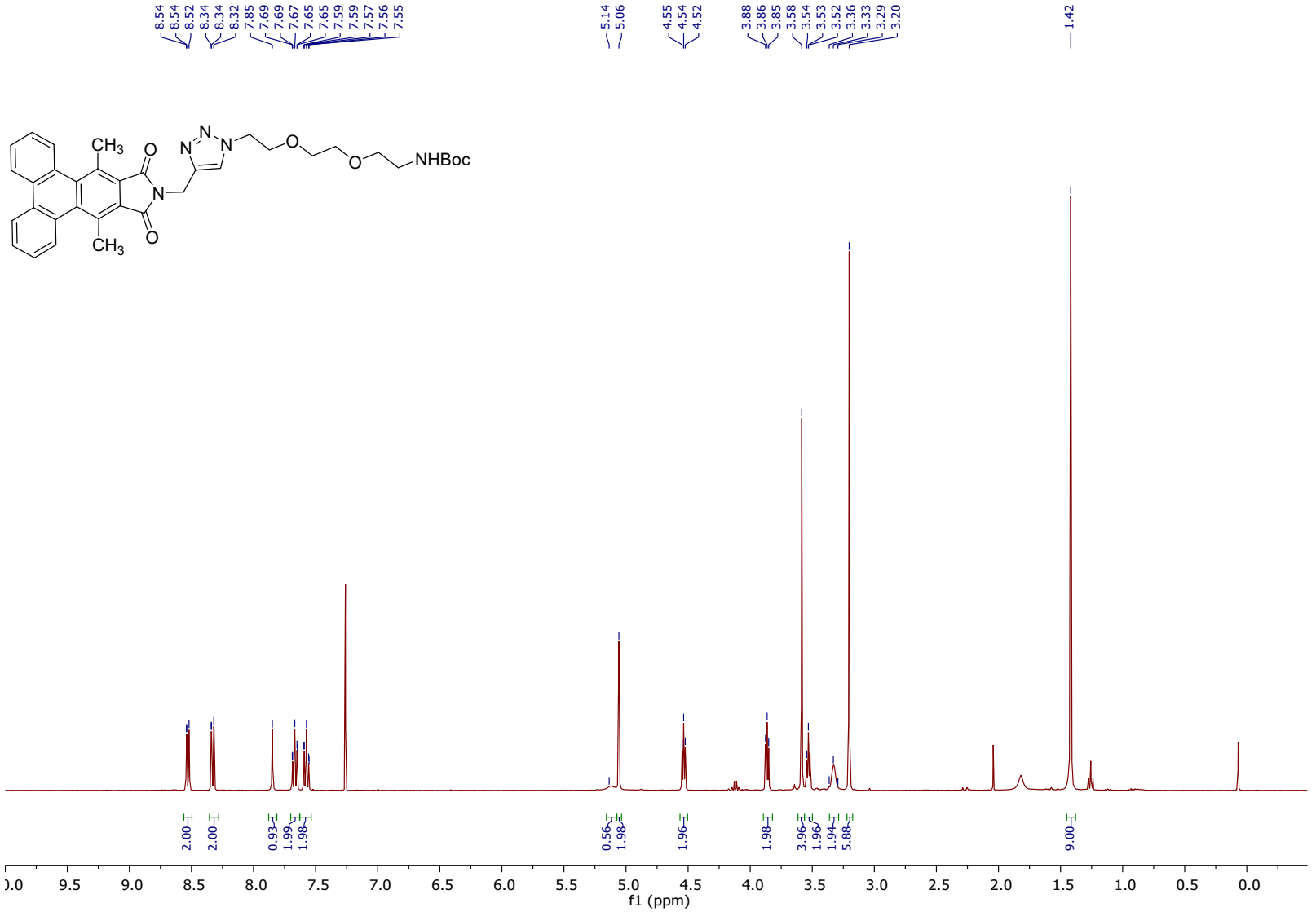
oCOm-58. ^{1}H NMR spectrum (500 MHz, DMSO-*d6*).



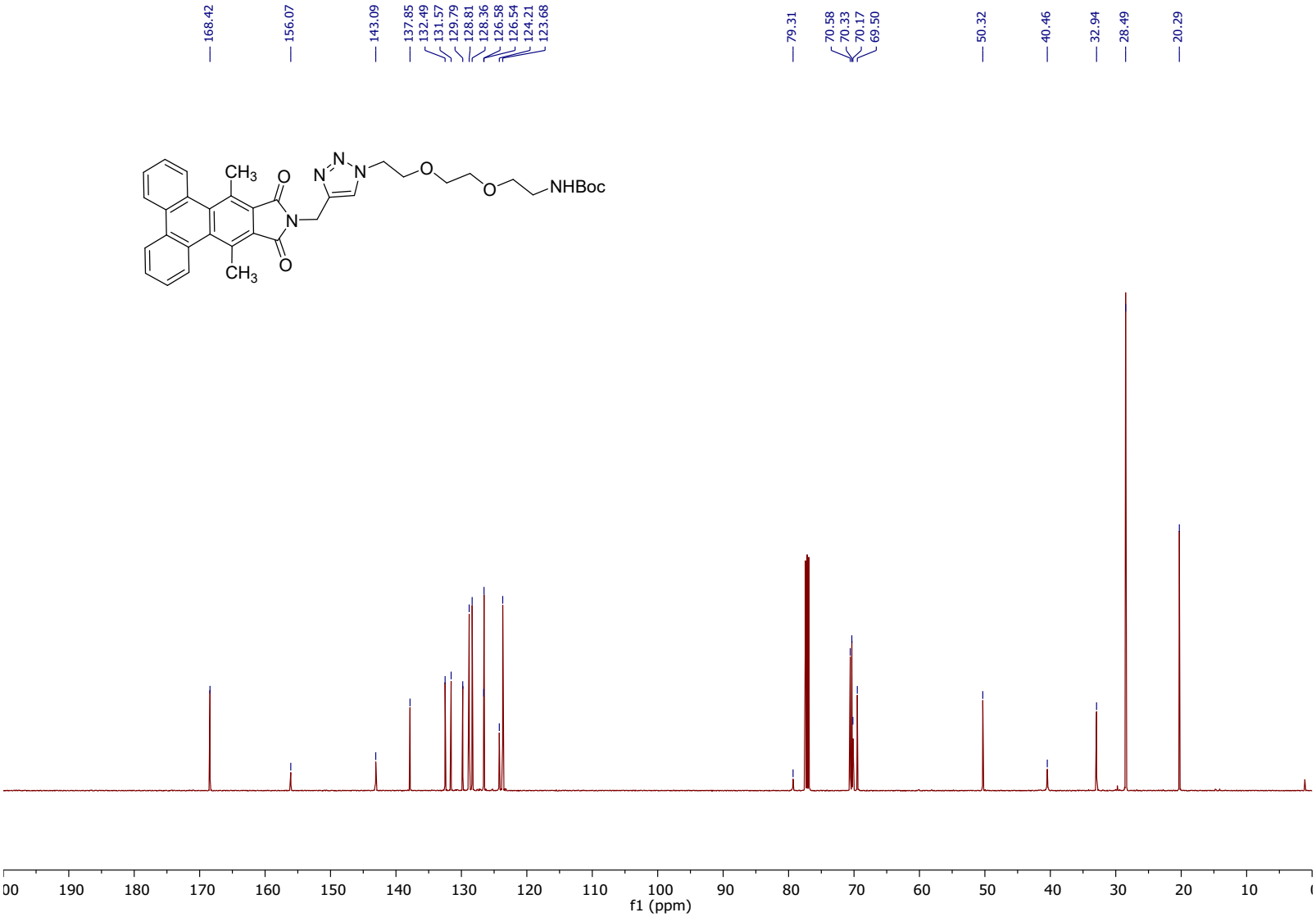
oCOm-58. ^{13}C NMR spectrum (125 MHz, DMSO-*d6*).



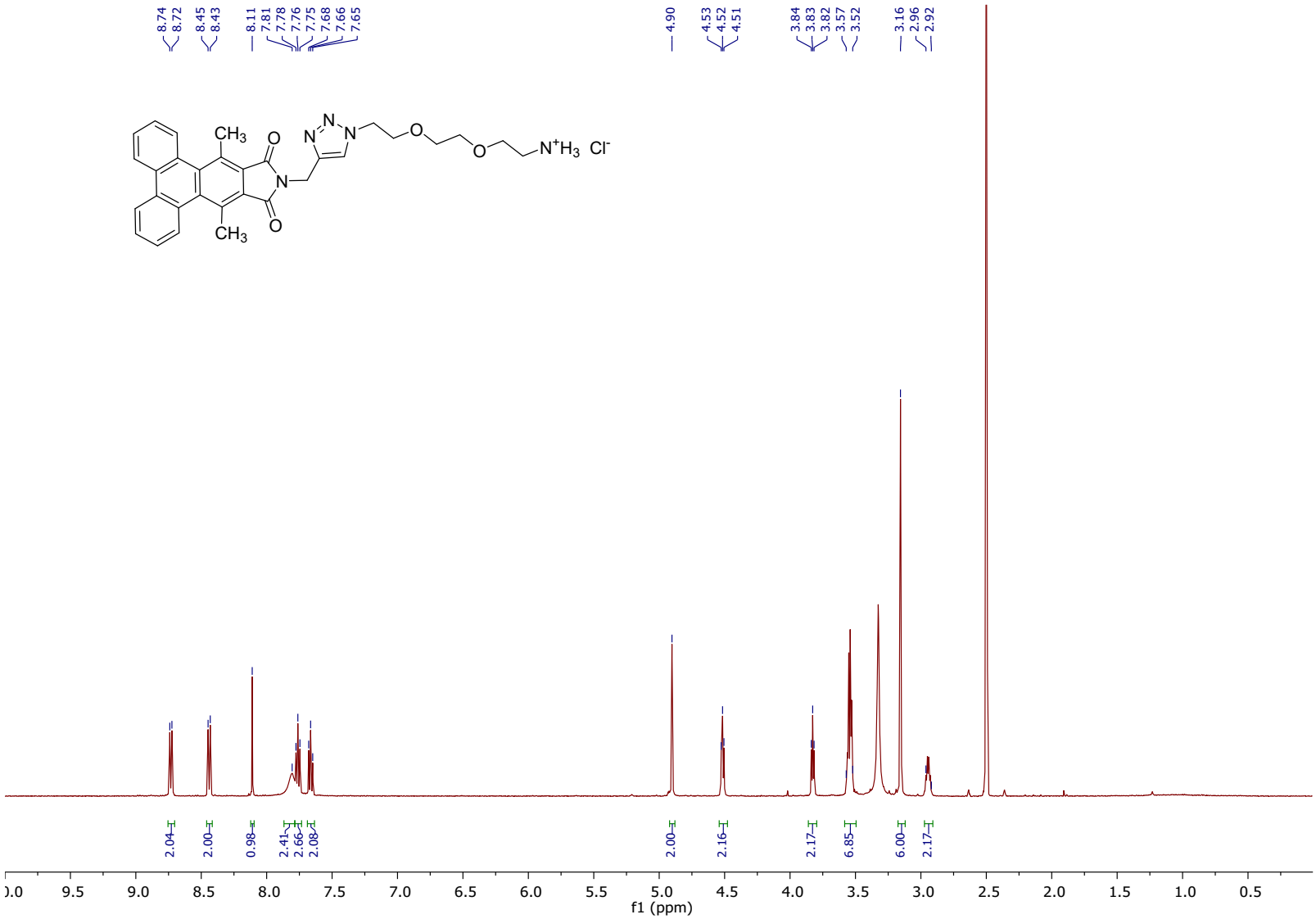
Compound **16**. ^{1}H NMR spectrum (400 MHz, $CDCl_3$).



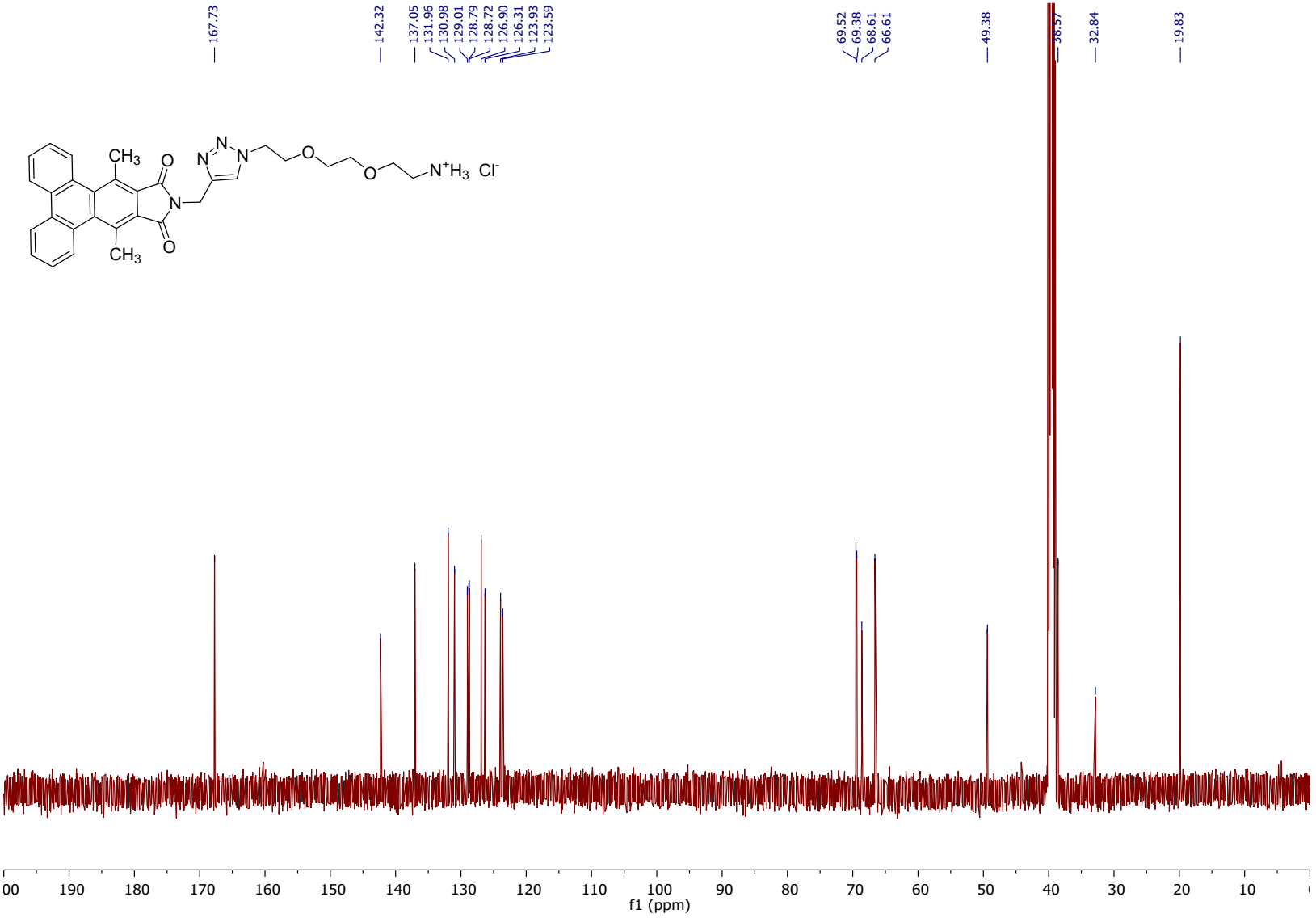
Compound **16**. ^{13}C NMR spectrum (100 MHz, $CDCl_3$).



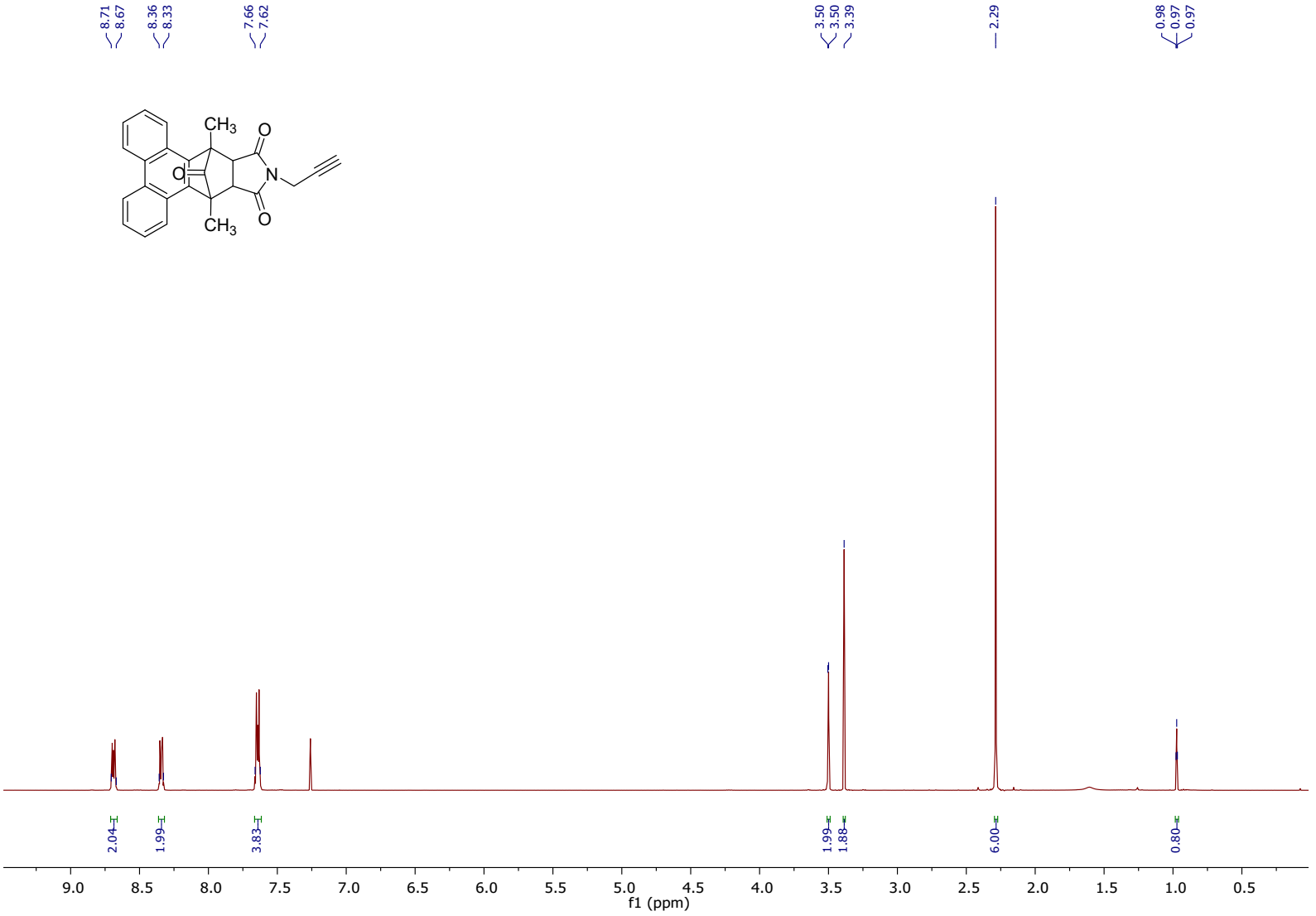
BP-58. ^{1}H NMR spectrum (500 MHz, DMSO-*d6*).



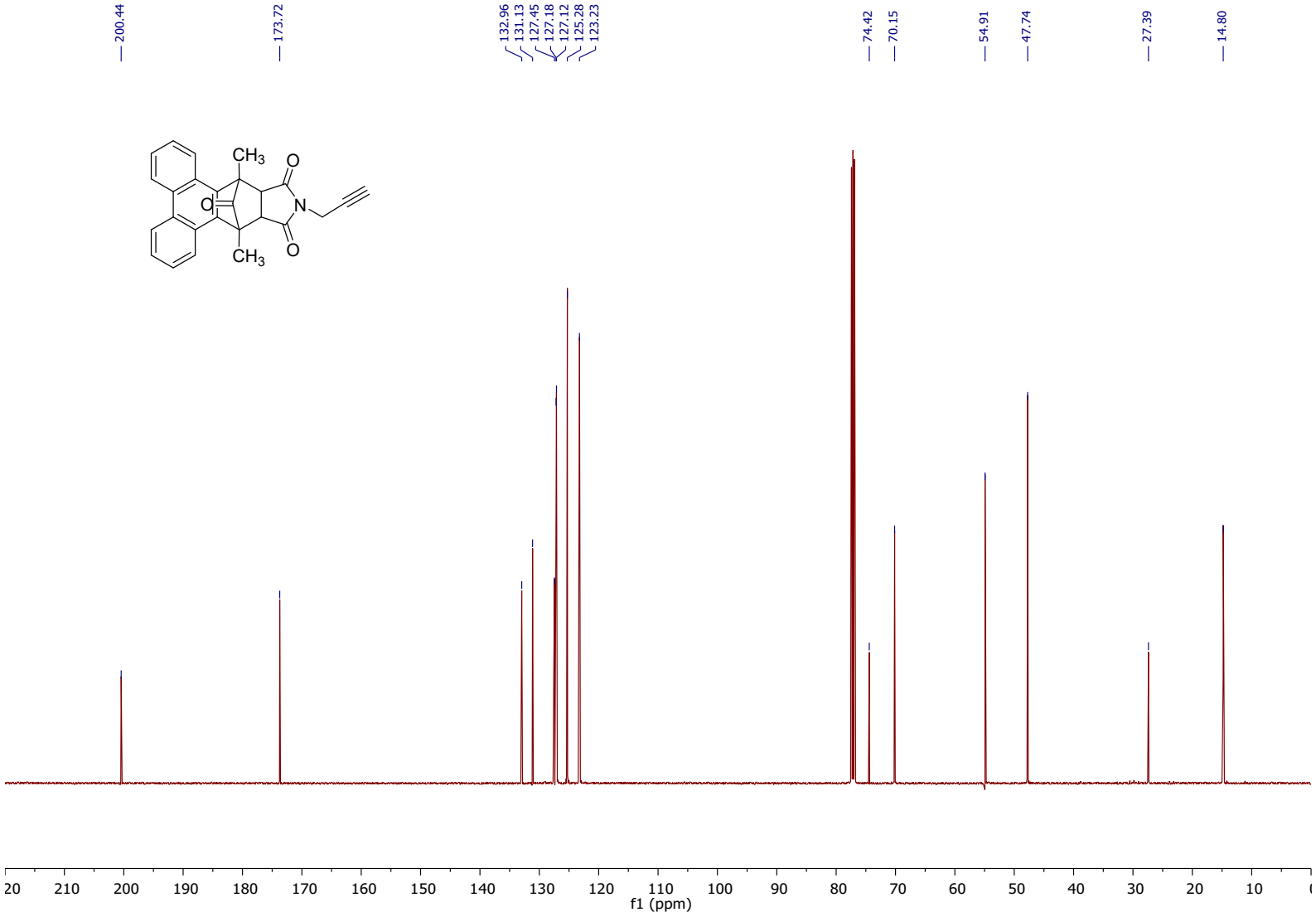
BP-58. ^{13}C NMR spectrum (125 MHz, DMSO-*d6*).



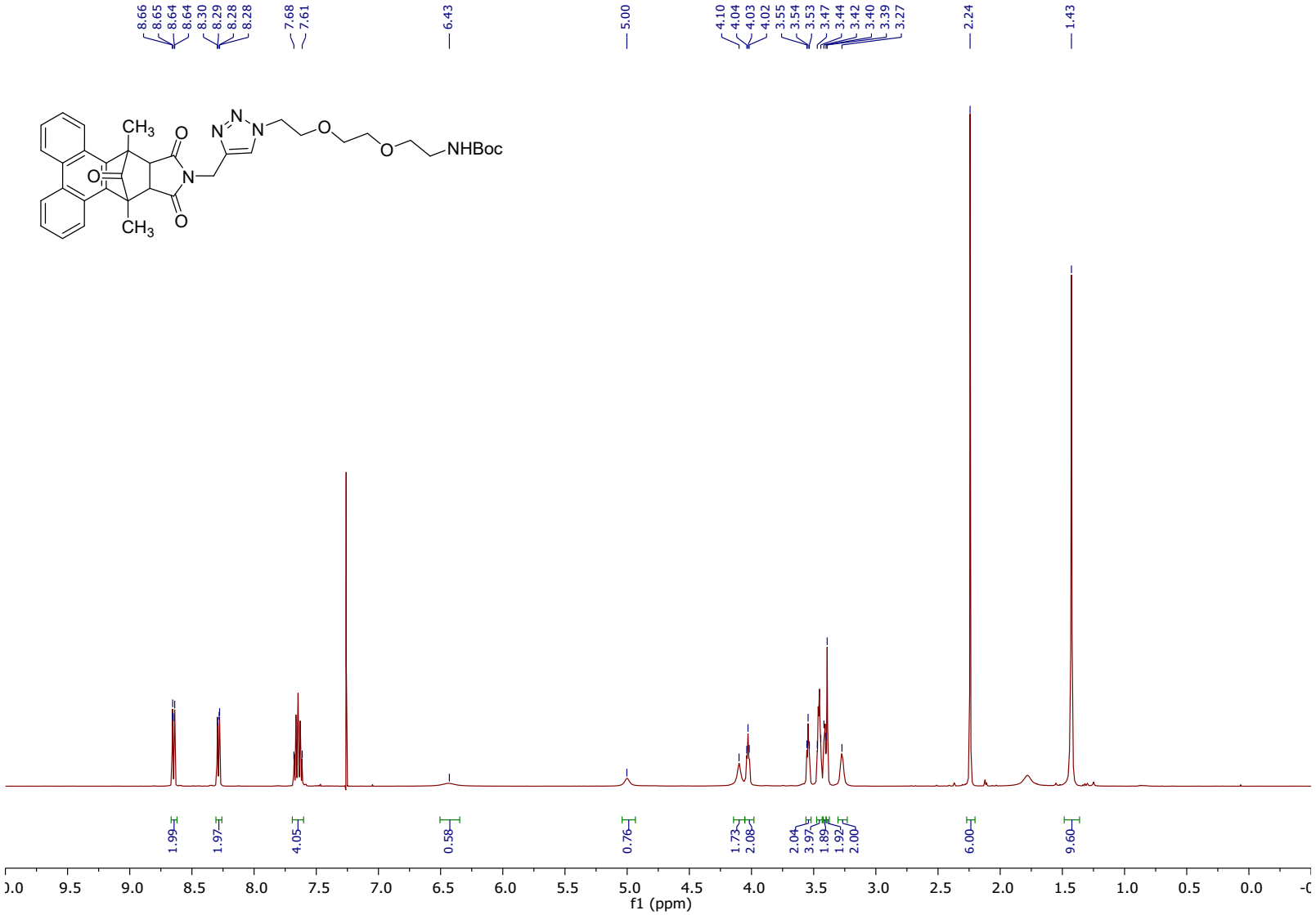
Endo-2-(2-propyn-1-yl)-3a,4,7,7a-tetrahydro-4,7-dimethyl-5,6-phenanthro-1*H*-isoindole-1,3,8(*2H*)-trione (**S9**). ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



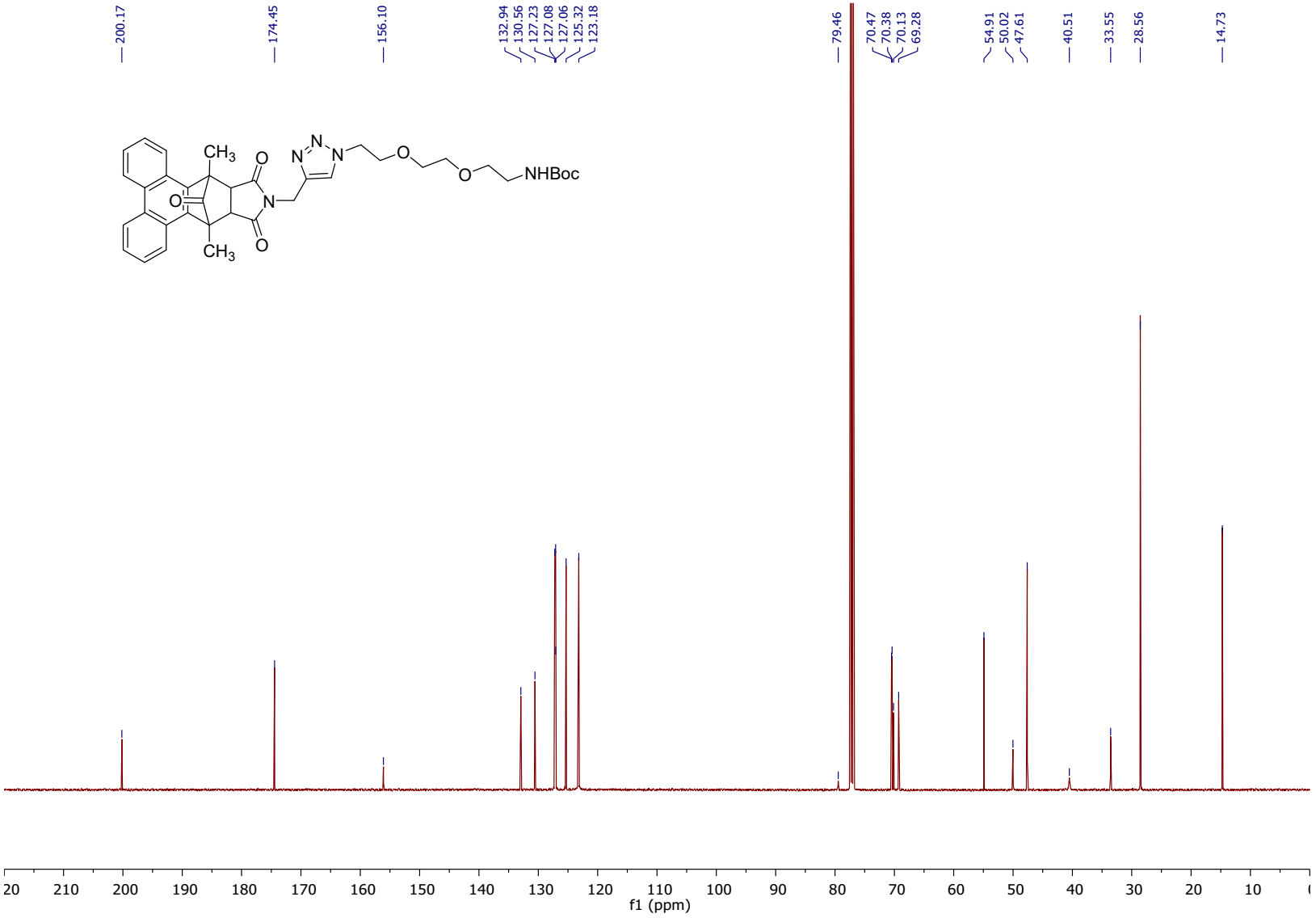
Endo-2-(2-propyn-1-yl)-3a,4,7,7a-tetrahydro-4,7-dimethyl-5,6-phenanthro-1*H*-isoindole-1,3,8(*2H*)-trione (**S9**). ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



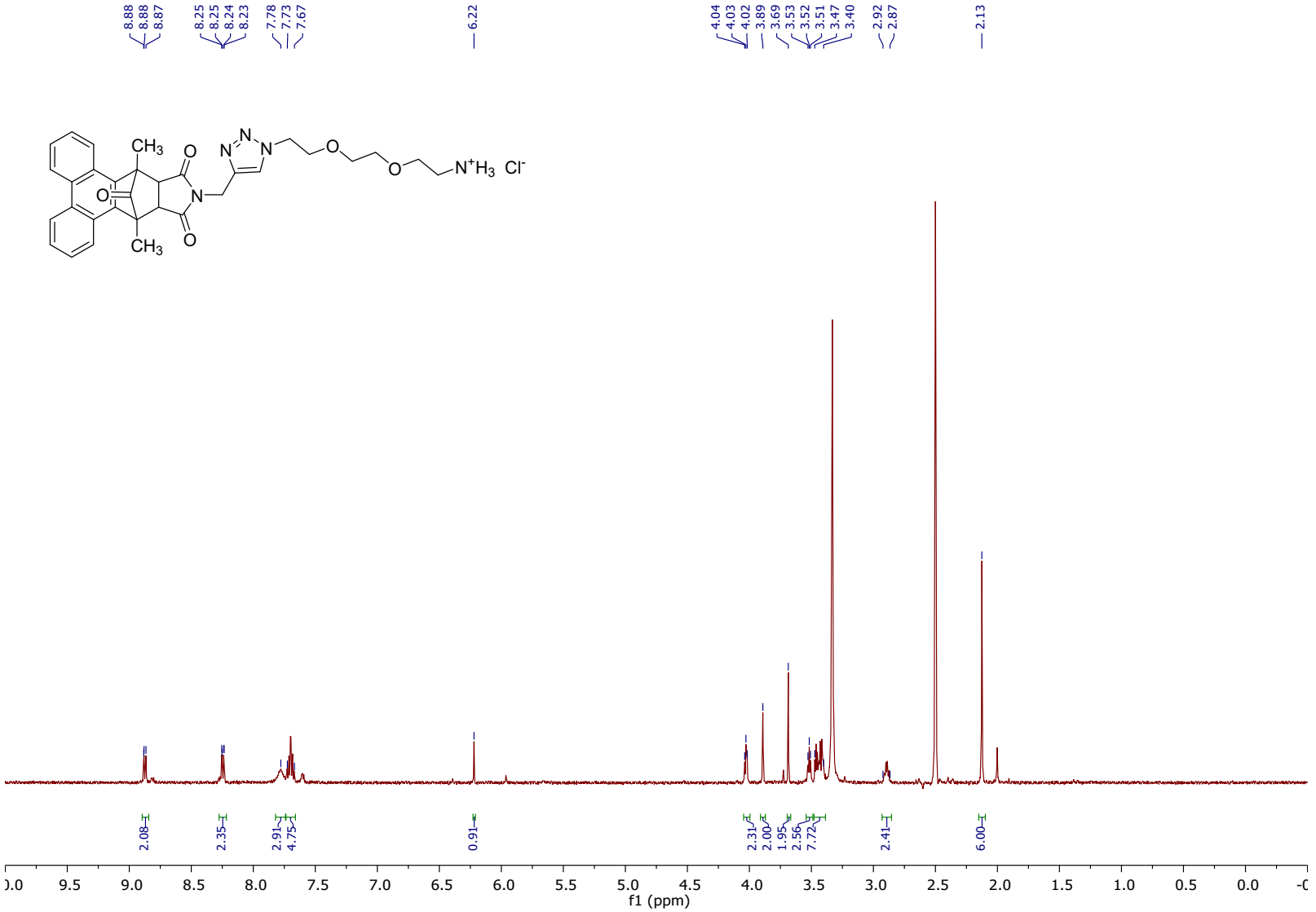
Compound **S10**. ^{1}H NMR spectrum (500 MHz, $CDCl_3$).



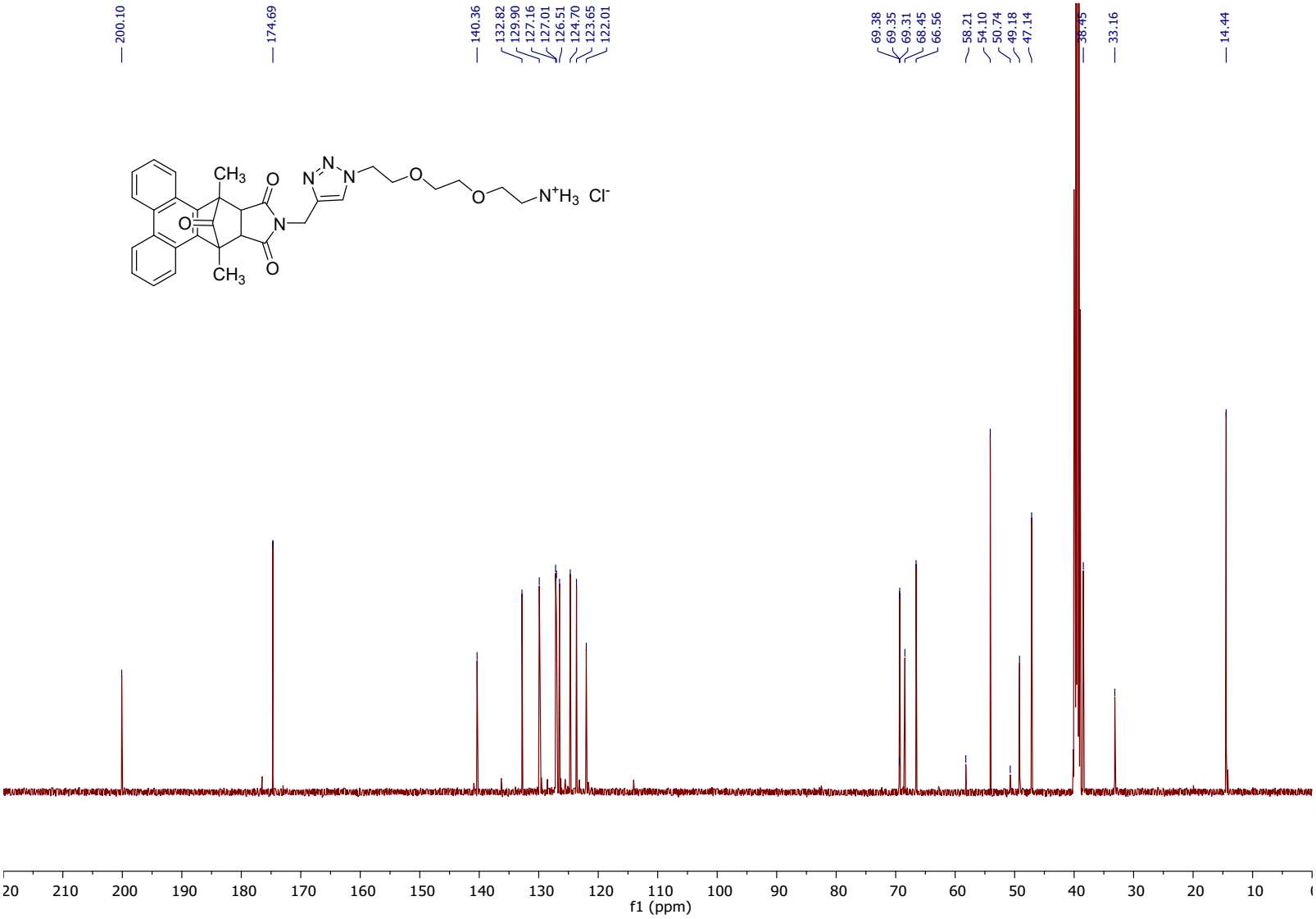
Compound **S10**. ^{13}C NMR spectrum (125 MHz, $CDCl_3$).



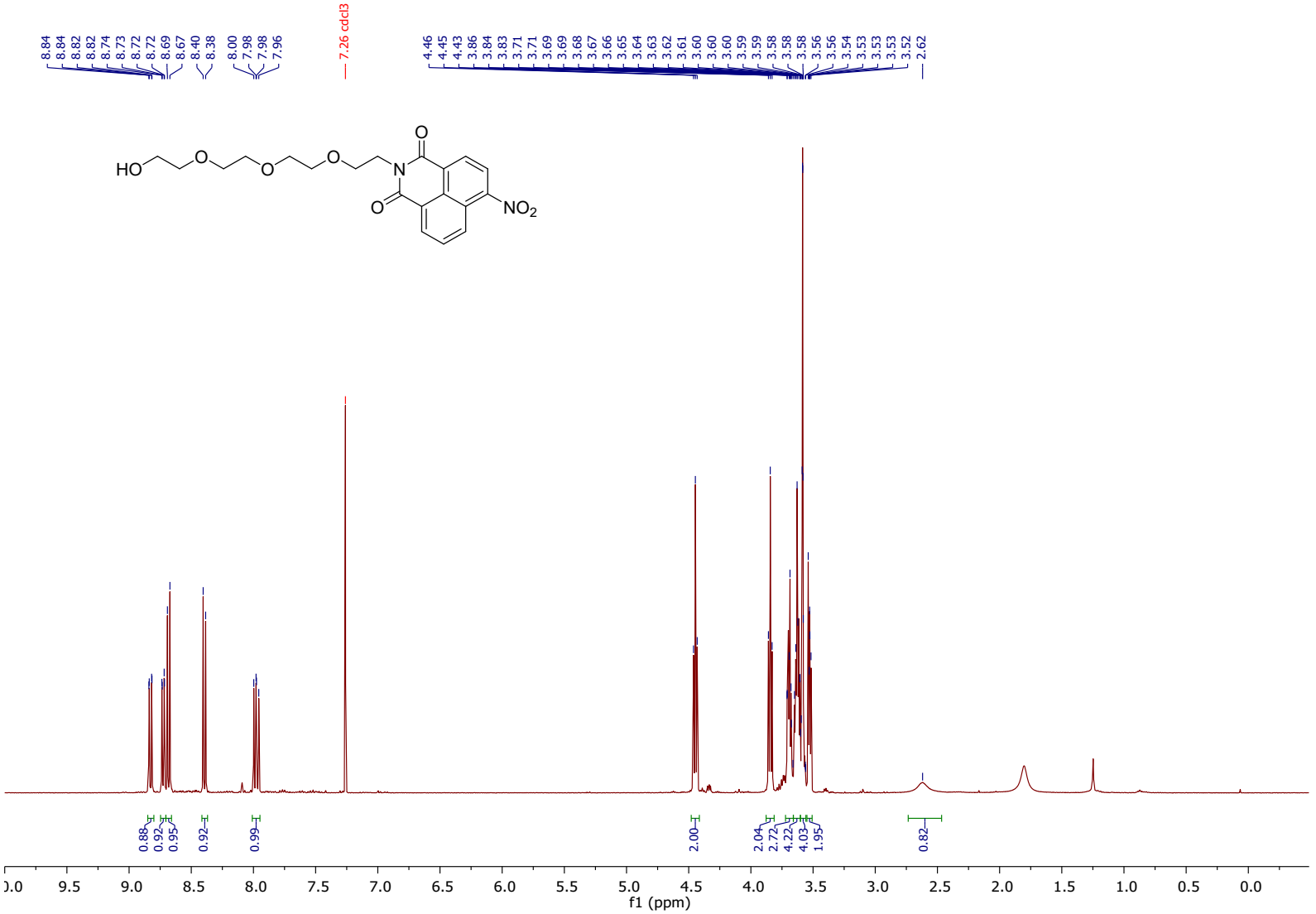
DB-58. ^{1}H NMR spectrum (500 MHz, DMSO-*d6*).



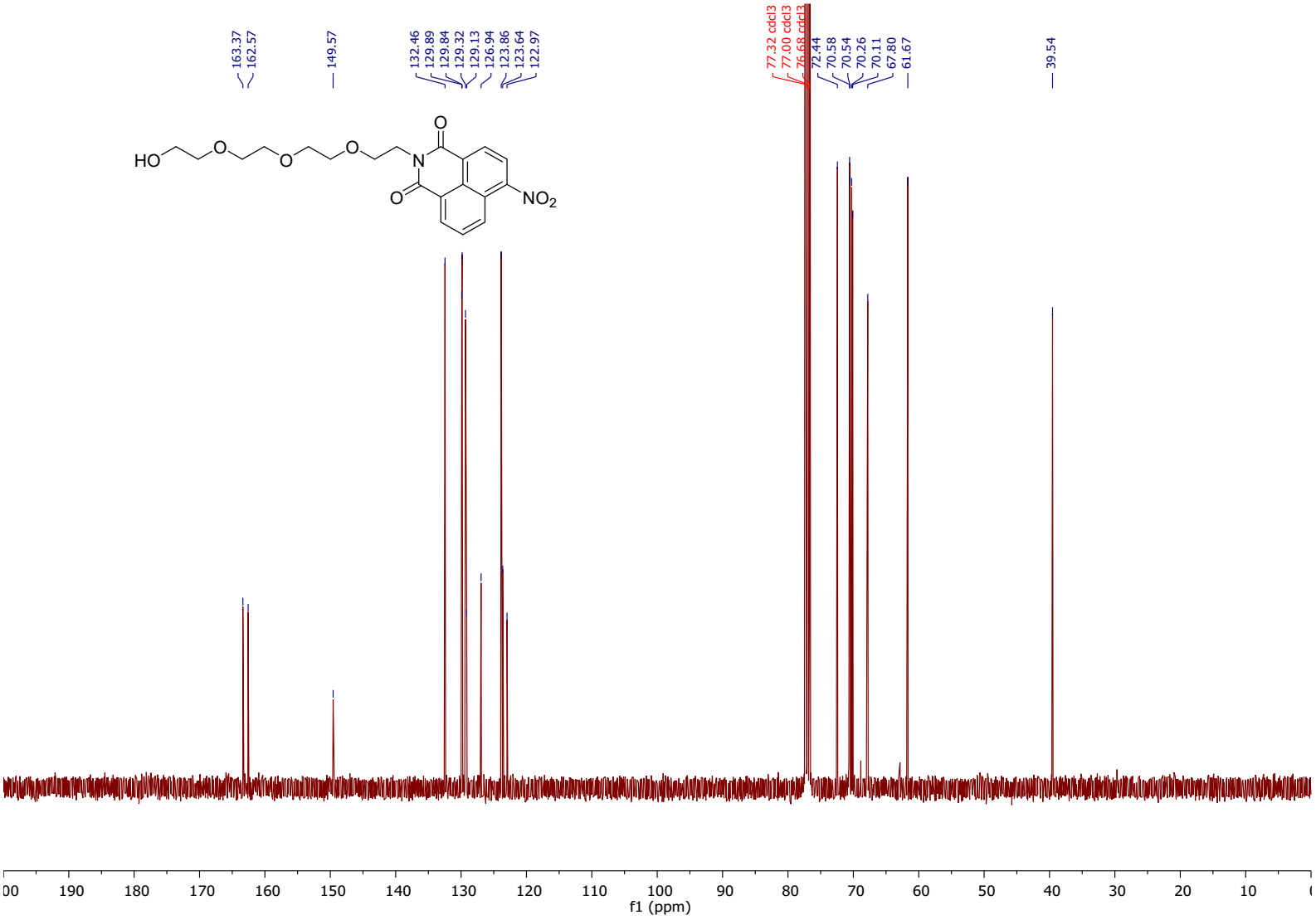
DB-58. ^{13}C NMR spectrum (125 MHz, DMSO-*d6*).



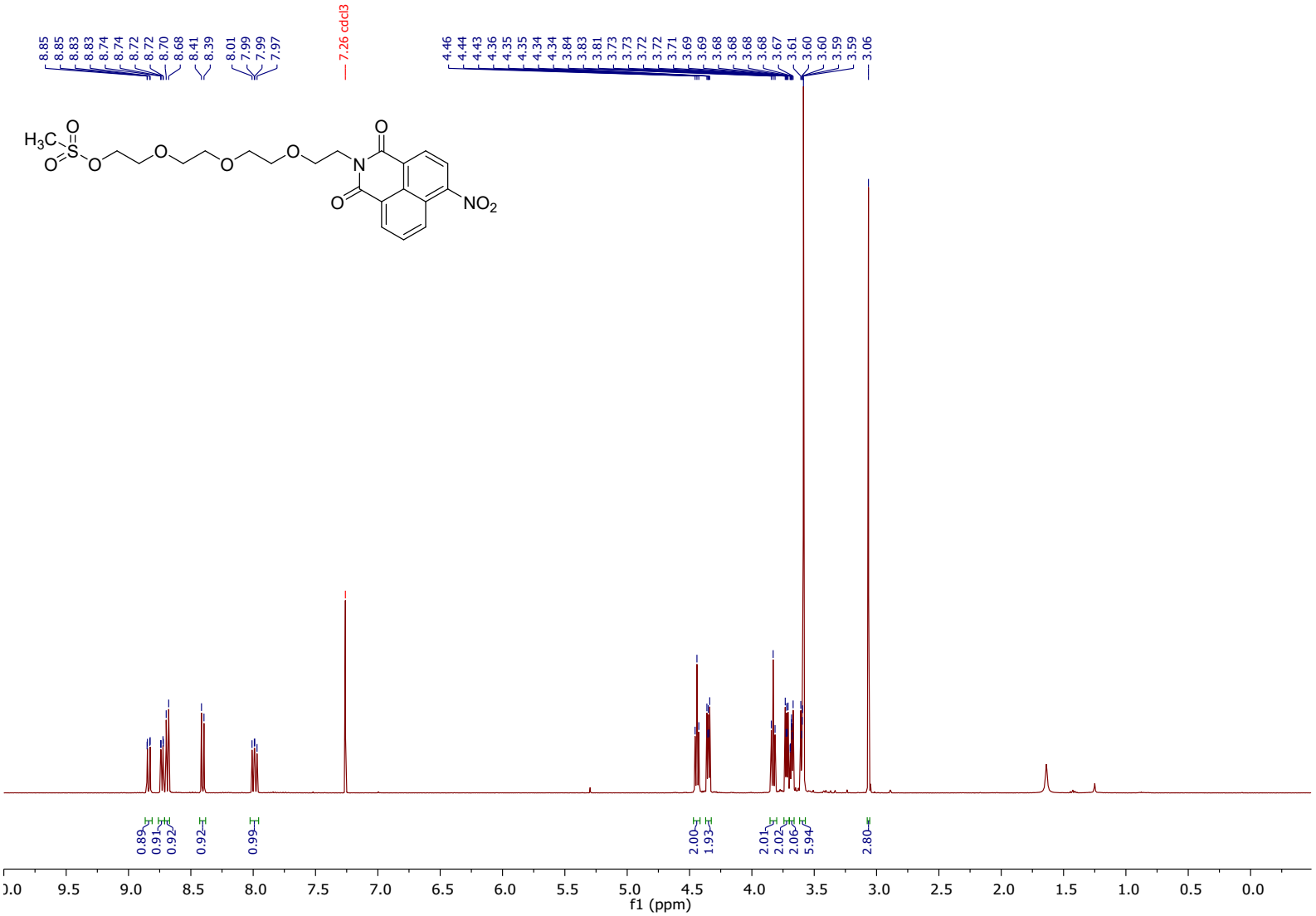
2-(2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl)-6-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**S16**).
^{1}H NMR spectrum (400 MHz, $CDCl_3$).



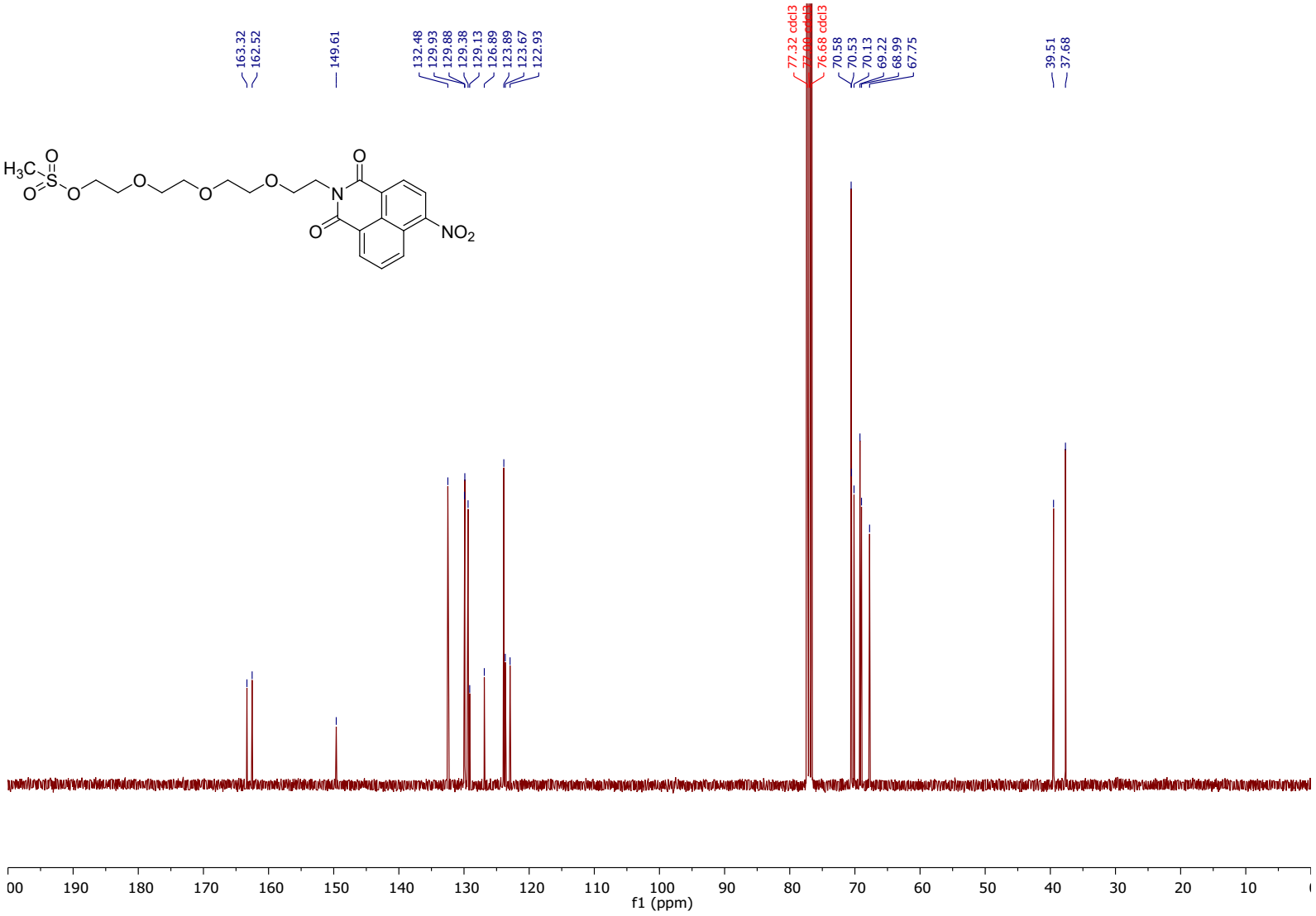
2-(2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl)-6-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**S16**).
^{13}C NMR spectrum (100 MHz, $CDCl_3$).



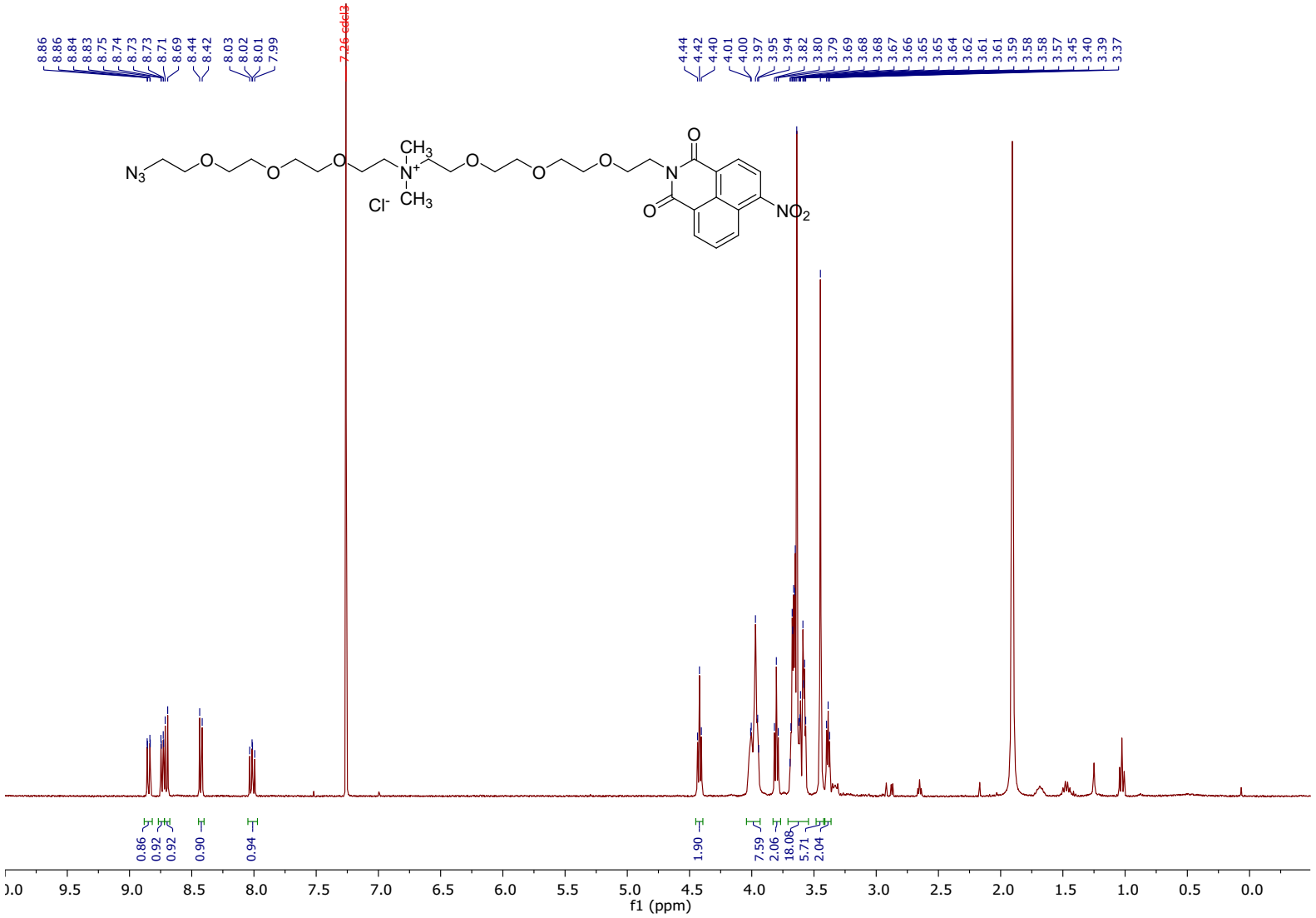
2-(2-(2-(2-(6-Nitro-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl methanesulfonate (**S17**).
^{1}H NMR spectrum (400 MHz, $CDCl_3$).



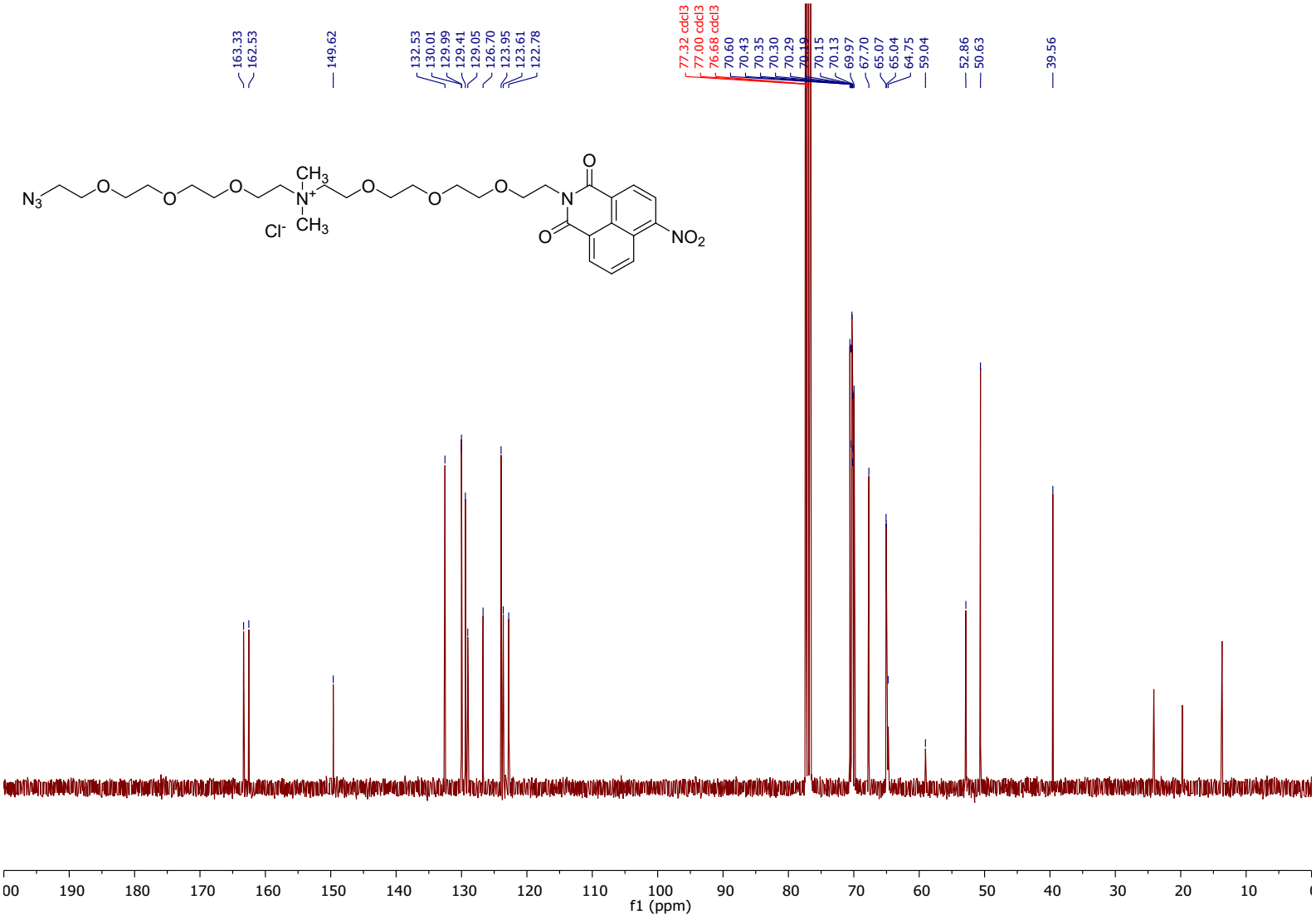
2-(2-(2-(2-(6-Nitro-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl methanesulfonate (**S17**).
^{13}C NMR spectrum (100 MHz, $CDCl_3$).



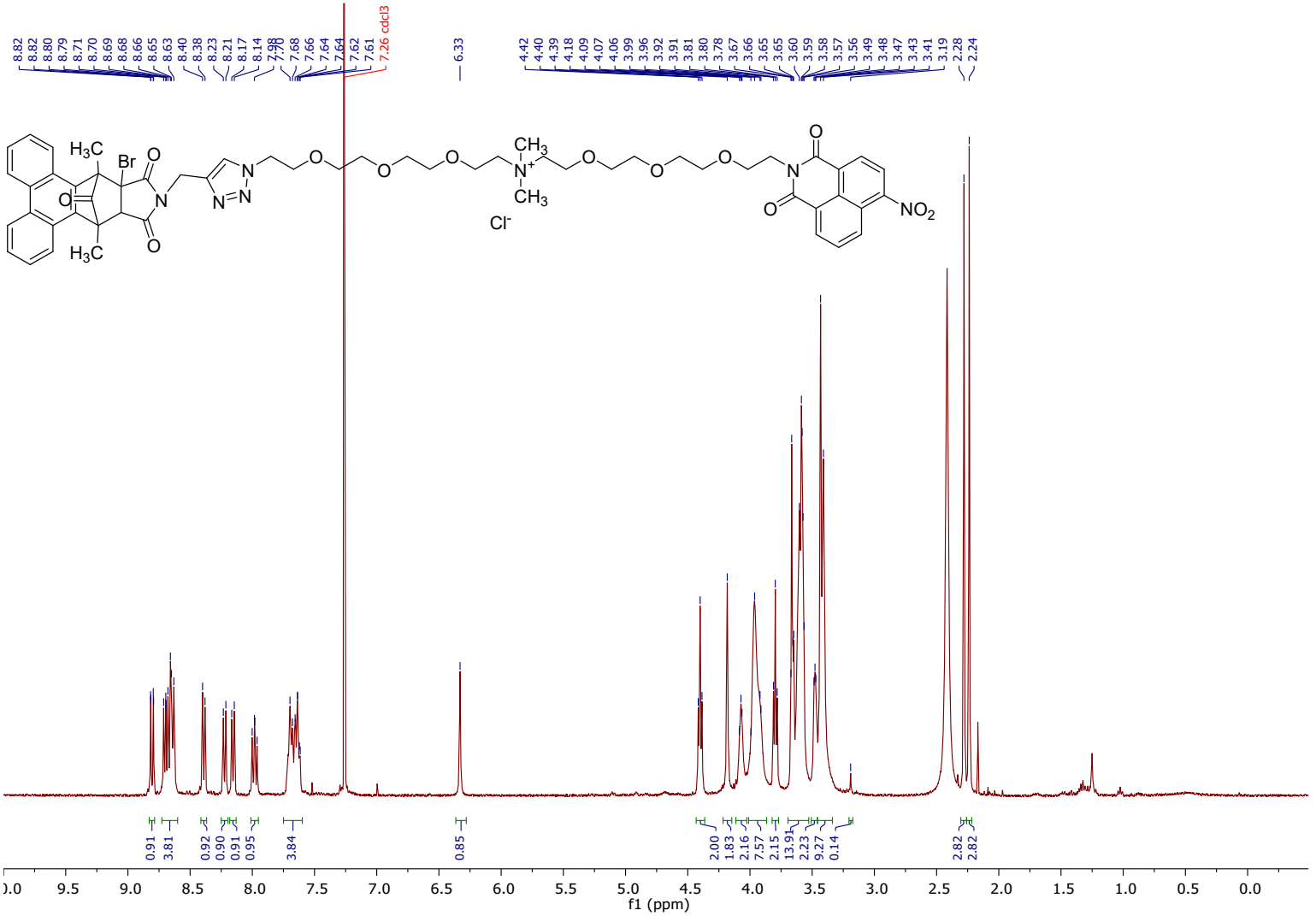
2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-*N,N*-dimethyl-*N*-(2-(2-(2-(2-(6-nitro-1,3-dioxo-1H-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl)ethan-1-aminium chloride (**17**). ^{1}H NMR spectrum (400 MHz, $CDCl_3$).



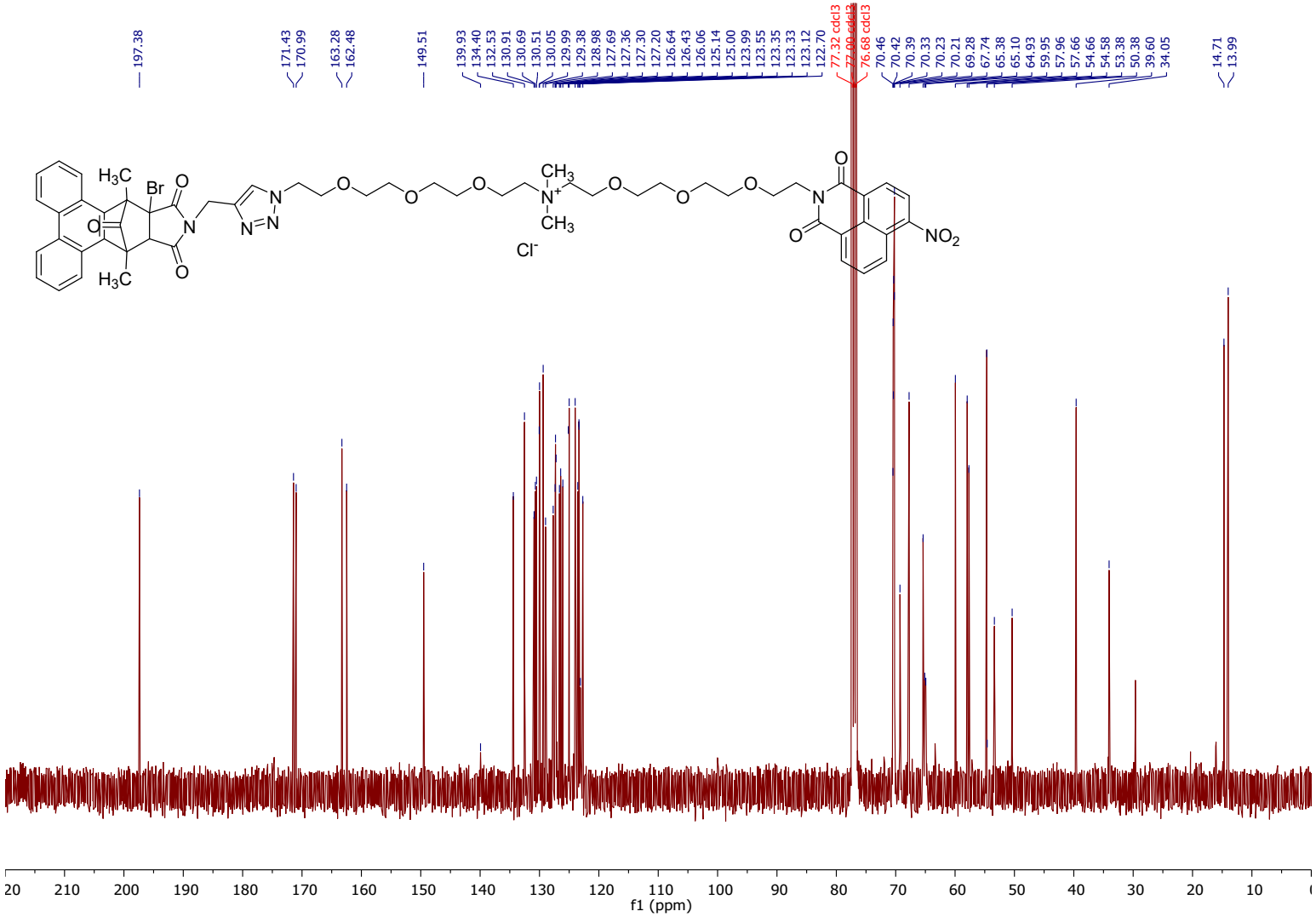
2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-*N,N*-dimethyl-*N*-(2-(2-(2-(2-(6-nitro-1,3-dioxo-1H-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl)ethan-1-aminium chloride (**17**). ^{13}C NMR spectrum (100 MHz, $CDCl_3$).



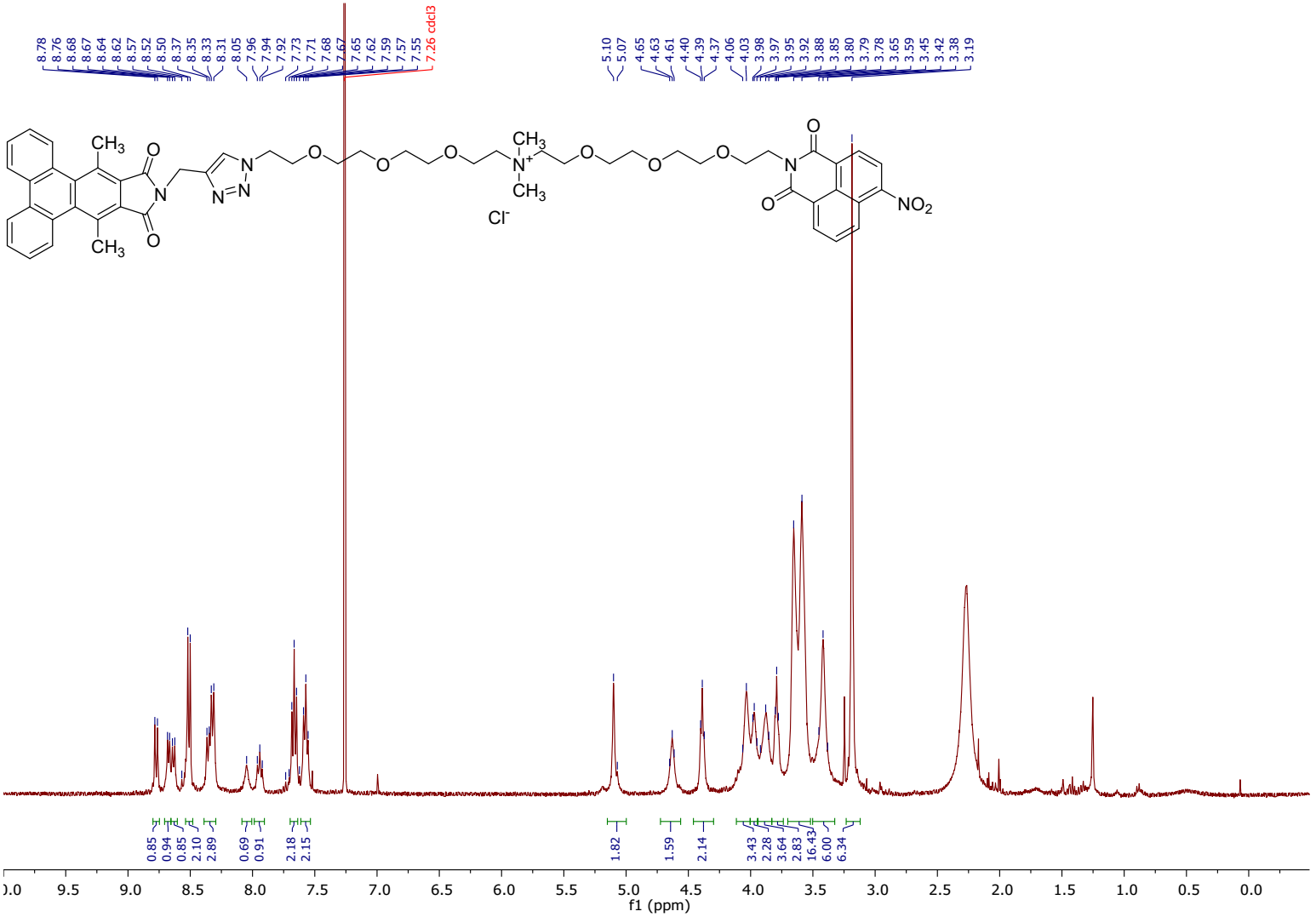
2-(2-(2-(2-(4-((9a-Bromo-9,13-dimethyl-10,12,14-trioxo-9,9a,10,12,12a,13-hexahydro-11H-9,13-methanophenanthro[9,10-*f*]isoindol-11-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-*N,N*-dimethyl-*N*-(2-(2-(2-(2-(6-nitro-1,3-dioxo-1H-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl)ethan-1-aminium chloride (**oCOm-66**). ^{1}H NMR spectrum (400 MHz, $CDCl_3$).



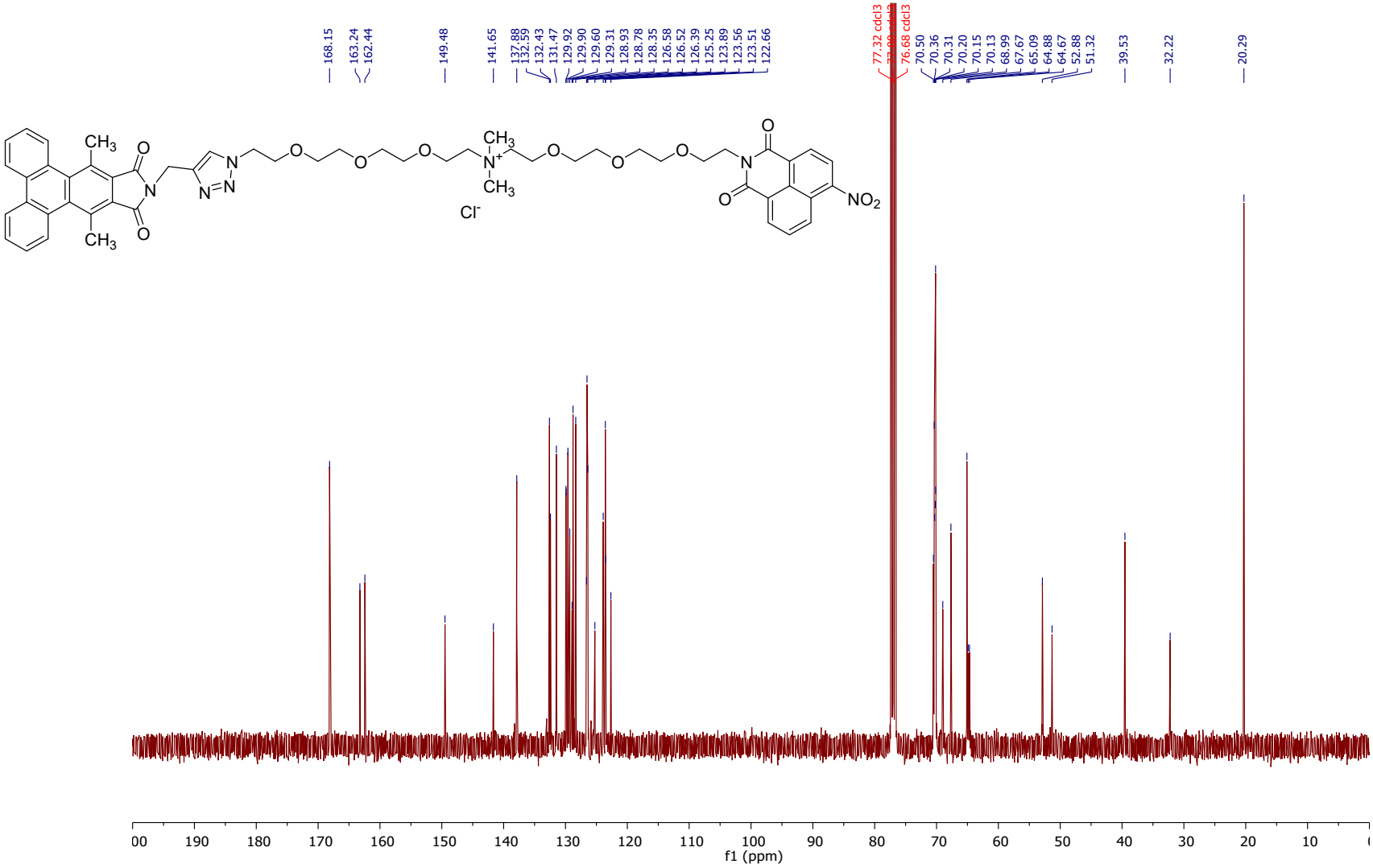
2-(2-(2-(2-(4-((9a-Bromo-9,13-dimethyl-10,12,14-trioxo-9,9a,10,12,12a,13-hexahydro-11H-9,13-methanophenanthro[9,10-*f*]isoindol-11-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-*N,N*-dimethyl-*N*-(2-(2-(2-(2-(6-nitro-1,3-dioxo-1H-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl)ethan-1-aminium chloride (**oCOm-66**). ^{13}C NMR spectrum (100 MHz, $CDCl_3$).



2-(2-(2-(2-(4-((9,13-Dimethyl-10,12-dioxo-10,12-dihydro-11H-phenanthro[9,10-*f*]isoindol-11-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-*N,N*-dimethyl-*N*-(2-(2-(2-(2-(6-nitro-1,3-dioxo-1H-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl)ethan-1-aminium chloride (**BP-66**). ^{1}H NMR spectrum (400 MHz, $CDCl_3$).



2-(2-(2-(2-(4-((9,13-Dimethyl-10,12-dioxo-10,12-dihydro-11H-phenanthro[9,10-*f*]isoindol-11-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-*N,N*-dimethyl-*N*-(2-(2-(2-(2-(6-nitro-1,3-dioxo-1H-benzo[*de*]isoquinolin-2(3*H*)-yl)ethoxy)ethoxy)ethoxy)ethyl)ethan-1-aminium chloride (**BP-66**). ^{13}C NMR spectrum (100 MHz, $CDCl_3$).



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