# Supporting Information

## Subcellular Delivery of Hydrogen Sulfide Using Small Molecule Donors Impacts Organelle Stress

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**1.** Fluorescence imaging of **MitoTCM** compared to NaSH

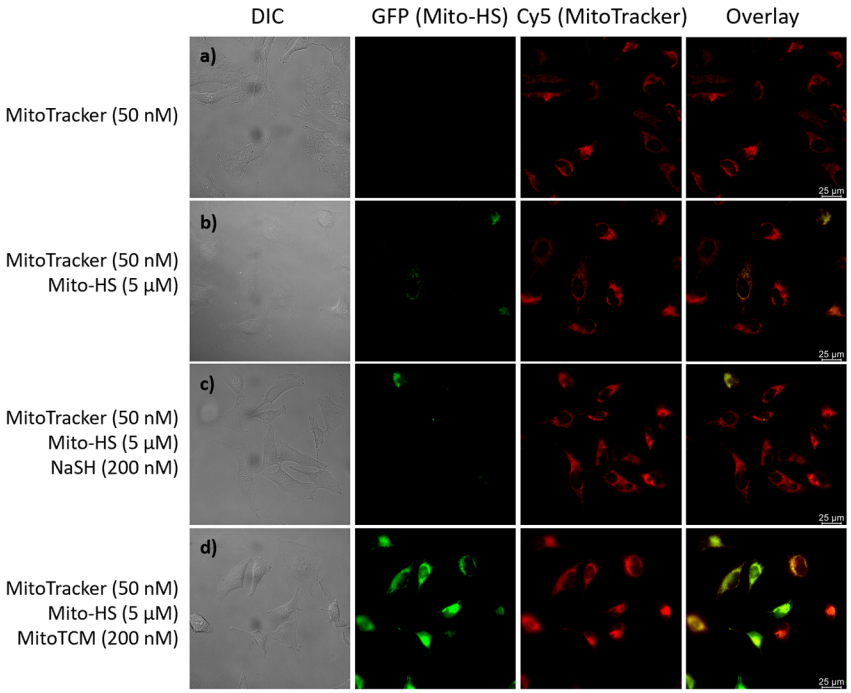



**Figure S1.** Fluorescence imaging of $H_2S$ produced by **MitoTCM** compared to NaSH in HeLa cells. Cells were incubated with MitoTracker (50 nM) and (a) vehicle (0.5% DMSO), (b) Mito-HS (5 µM), (c) Mito-HS (5 µM) and NaSH (200 nM), (d) Mito-HS (5 µM) and **MitoTCM** (200 nM). Cells were incubated with MitoTracker and vehicle or Mito-HS for 30 minutes, and then either the vehicle, NaSH or **MitoTCM** for 60 min followed by imaging. Bar scale: 25 µm.

**2.** Fluorescence imaging of **MitoTCM** compared to **TCM alkyne**

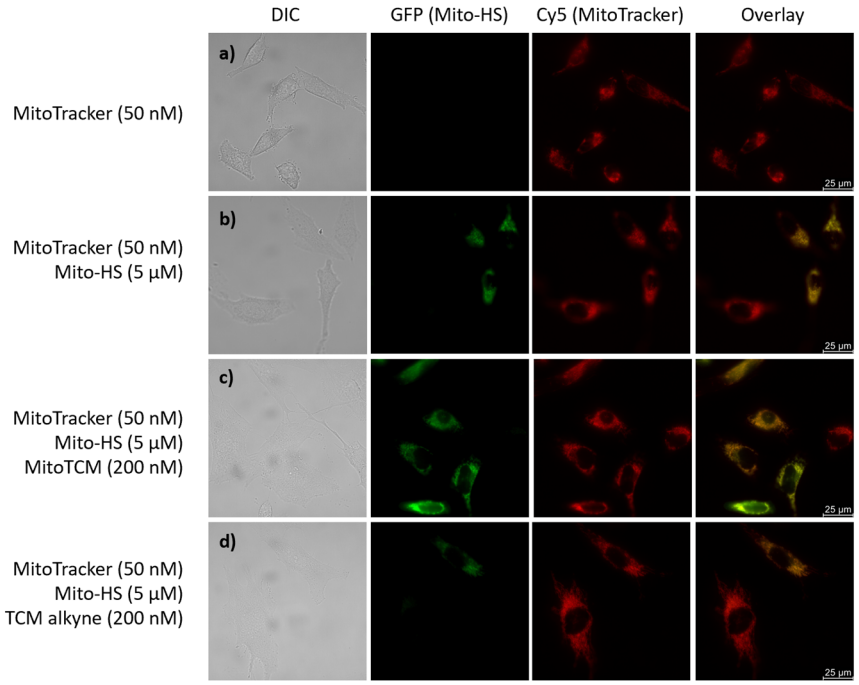



**Figure S2.** Fluorescence imaging of $H_2S$ produced by **MitoTCM** compared to **TCM alkyne** in HeLa cells. Cells were incubated with MitoTracker (50 nM) and (a) vehicle (0.5% DMSO), (b) Mito-HS (5 µM), (c) Mito-HS (5 µM) and **MitoTCM** (200 nM), (d) Mito-HS (5 µM) and **TCM alkyne** (200 nM). Cells were incubated with MitoTracker and vehicle or Mito-HS for 30 minutes, and then either the vehicle, **MitoTCM** or **TCM alkyne** for 60 min followed by imaging. Bar scale: 25 µm.

**3.** Fluorescence imaging of **LysoTCM** compared to **TCM alkyne**

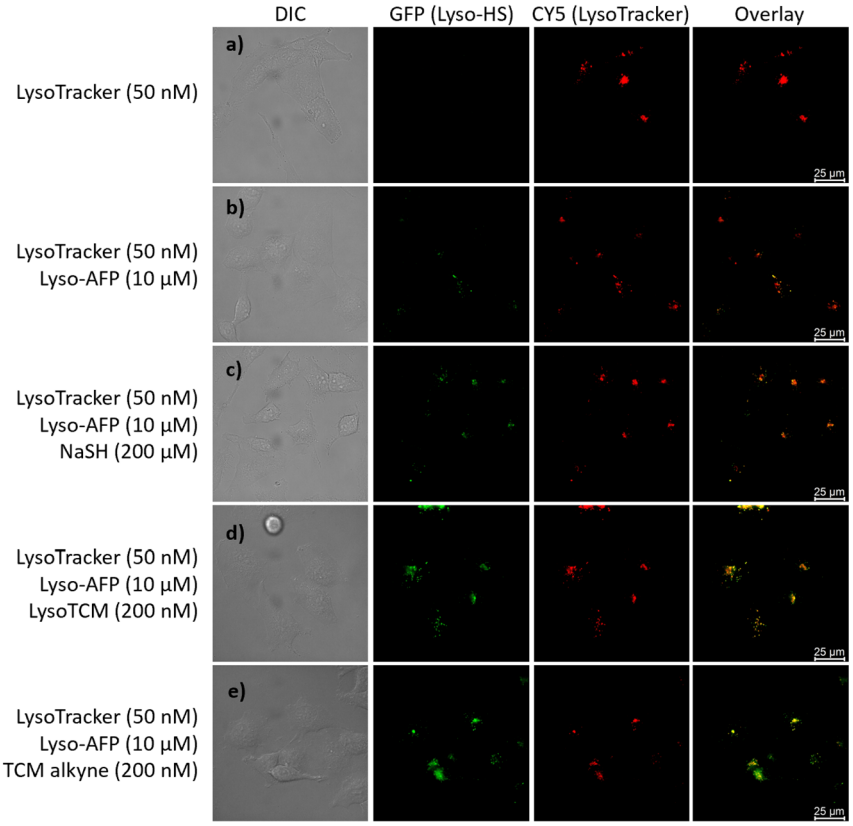



**Figure S3.** Fluorescence imaging of $H_2S$ produced by **LysoTCM** compared to **TCM alkyne** in HeLa cells. Cells were incubated with LysoTracker (50 nM) and (a) vehicle (0.5% DMSO), (b) Lyso-AFP (10 µM), (c) Lyso-AFP (10 µM) and NaSH (200 µM, positive control), (d) Lyso-AFP (10 µM) and **LysoTCM**, (e) Lyso-AFP (10 µM) and **TCM alkyne** (200 nM). Cells were incubated with LysoTracker and vehicle or Lyso-AFP for 30 minutes, and then either the vehicle, NaSH, **LysoTCM**, or **TCM alkyne** for 60 min followed by imaging. Bar scale: 25 µm.

**4.** Fluorescence imaging of **ERTCM** compared to **TCM alkyne**

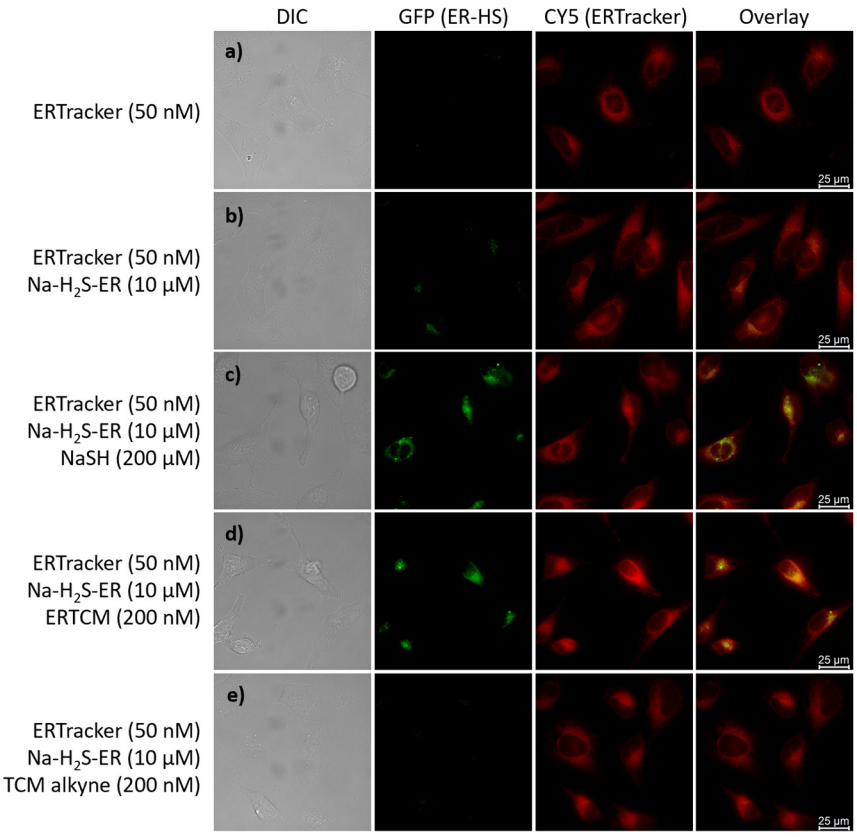



**Figure S4.** Fluorescence imaging of $H_2S$ produced by **ERTCM** compared to **TCM alkyne** in HeLa cells. Cells were incubated with ERTracker (50 nM) and (a) vehicle (0.5% DMSO), (b) Na-$H_2S$-ER (10 µM), (c) Na-$H_2S$-ER (10 µM) and NaSH (200 µM, positive control), (d) Na-$H_2S$-ER (10 µM) and **ERTCM**, (e) Na-$H_2S$-ER (10 µM) and **TCM alkyne** (200 nM). Cells were incubated with ERTracker and vehicle or Na-$H_2S$-ER for 30 minutes, and then either the vehicle, NaSH, **ERTCM**, or **TCM alkyne** for 60 min followed by imaging. Bar scale: 25 µm.

**5.** Fluorescence imaging of **GolgiTCM** compared to **TCM alkyne**

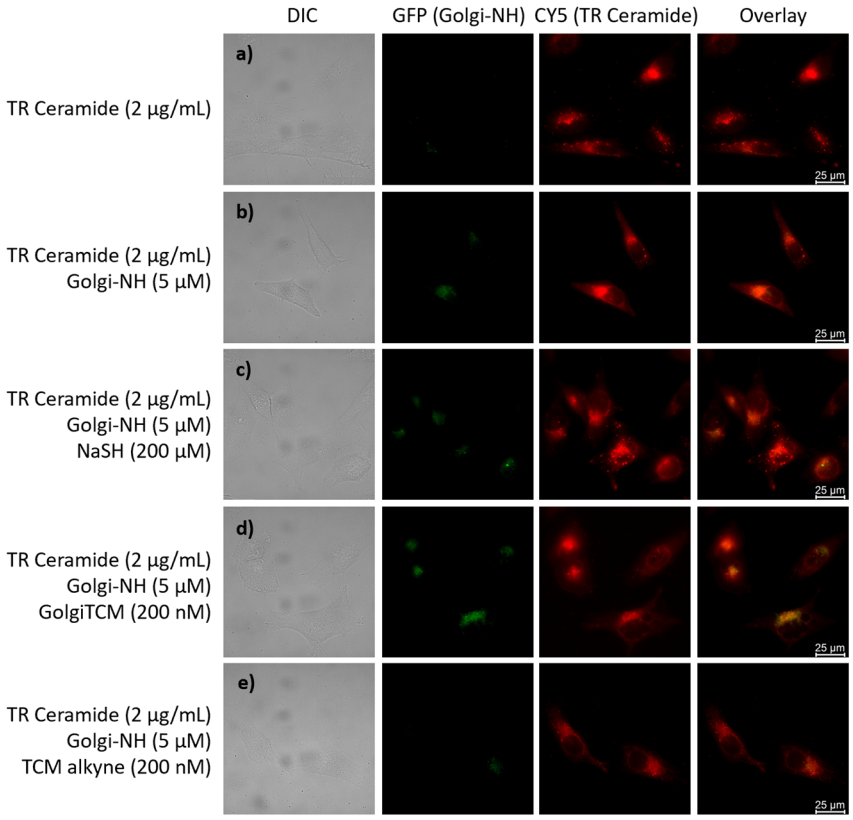



**Figure S5.** Fluorescence imaging of $H_2S$ produced by **GolgiTCM** compared to **TCM alkyne** in HeLa cells. Cells were incubated with BODIPY$^{TM}$ TR Ceramide (2 µg/mL) and (a) vehicle (0.5% DMSO), (b) Golgi-NH (5 µM), (c) Golgi-NH (5 µM) and NaSH (200 µM, positive control), (d) Golgi-NH (5 µM) and **GolgiTCM**, (e) Golgi-NH (5 µM) and **TCM alkyne** (200 nM). Cells were incubated with BODIPY$^{TM}$ TR Ceramide and vehicle or Golgi-NH for 30 minutes, and then either the vehicle, NaSH, **GolgiTCM**, or **TCM alkyne** for 60 min followed by imaging. Bar scale: 25 µm.

**6.** Selectivity experiment of localized delivery

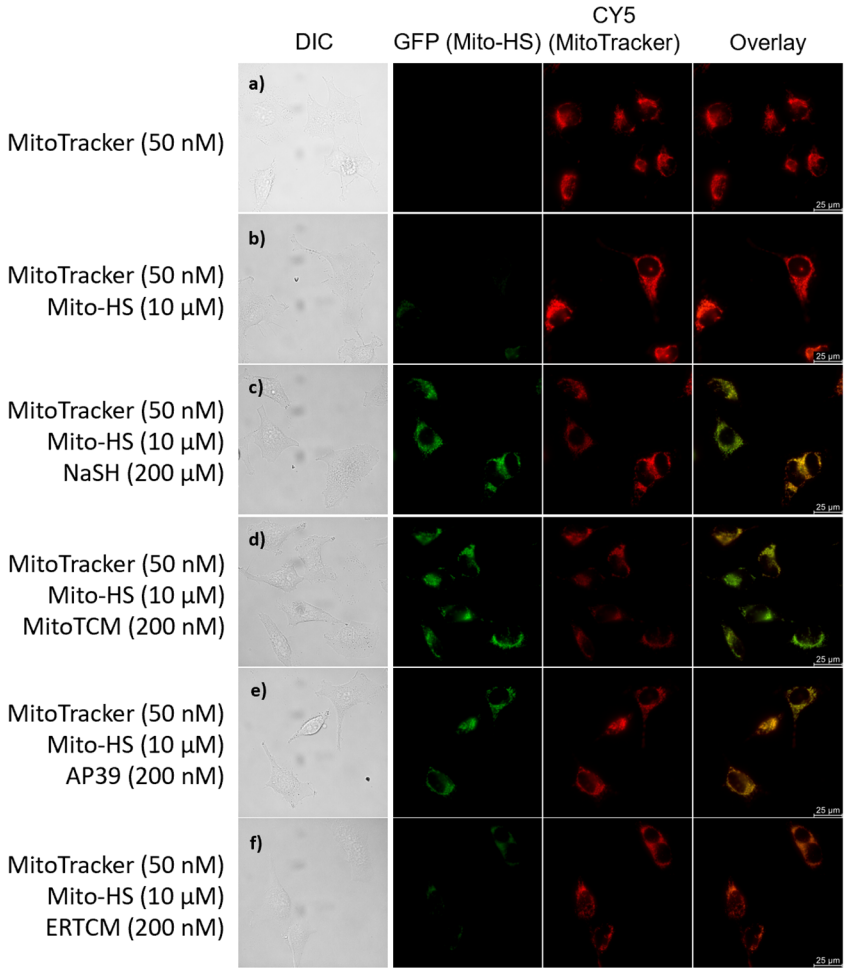



**Figure S6.** Fluorescence imaging of $H_2S$ produced by **MitoTCM**, AP39, and **ERTCM** in the presence of Mito-HS in HeLa cells. Cells were incubated with MitoTracker (50 nM) and (a) vehicle (0.5% DMSO), (b) Mito-HS (10 μM), (c) Mito-HS (10 μM) and NaSH (200 μM, positive control), (d) Mito-HS (10 μM) and **MitoTCM** (200 nM), (e) Mito-HS (10 μM) and AP39 (200 nM), (f) Mito-HS (10 μM) and **ERTCM** (200 nM). Cells were incubated with MitoTracker and vehicle or Mito-HS for 30 minutes, and then either the vehicle, **MitoTCM**, AP39, or **ERTCM** for 60 min followed by imaging. Bar scale: 25 μm.

**7.** Colocalization negative control experiment

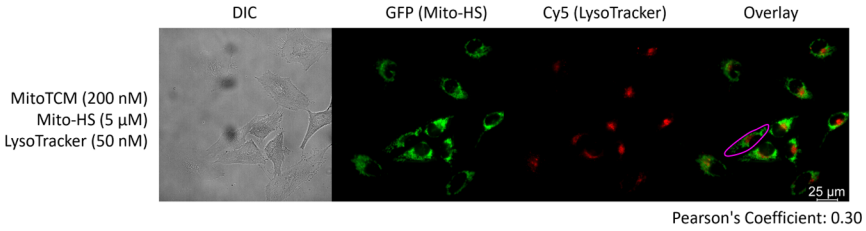



**Figure S7.** Fluorescence imaging of $H_2S$ produced by **MitoTCM** using Mito-HS in the presence of Lysotracker in HeLa cells. Cells were incubated with LysoTracker (50 nM) and Mito-HS (5 μM) for 30 minutes, then incubated with **MitoTCM** (200 nM) for 60 min followed by imaging. Pearson's coefficient = 0.30 suggesting minimal colocalization of $H_2S$ produced by MitoTCM and imaged with Mito-HS with LysoTracker. Bar scale: 25 μm.

**8.** Monensin Cell Viability Experiment

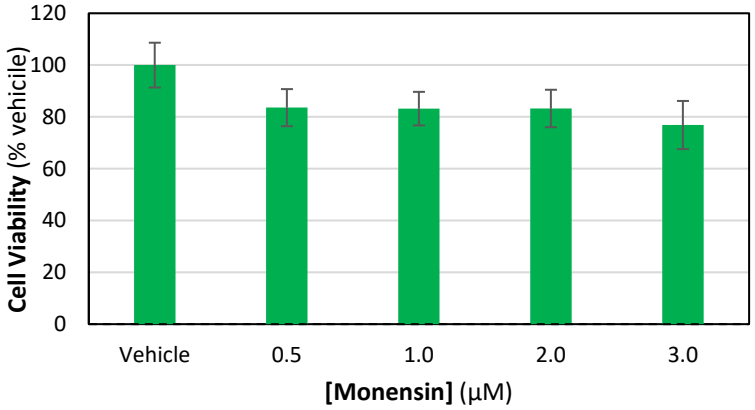
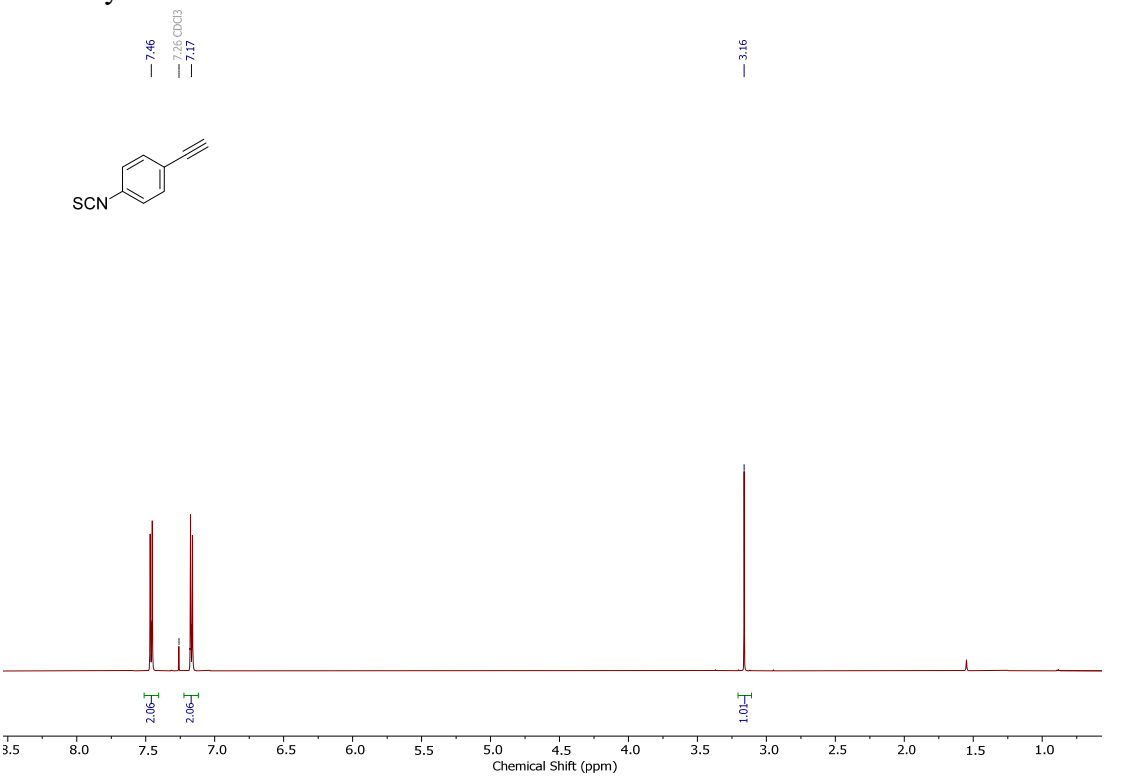
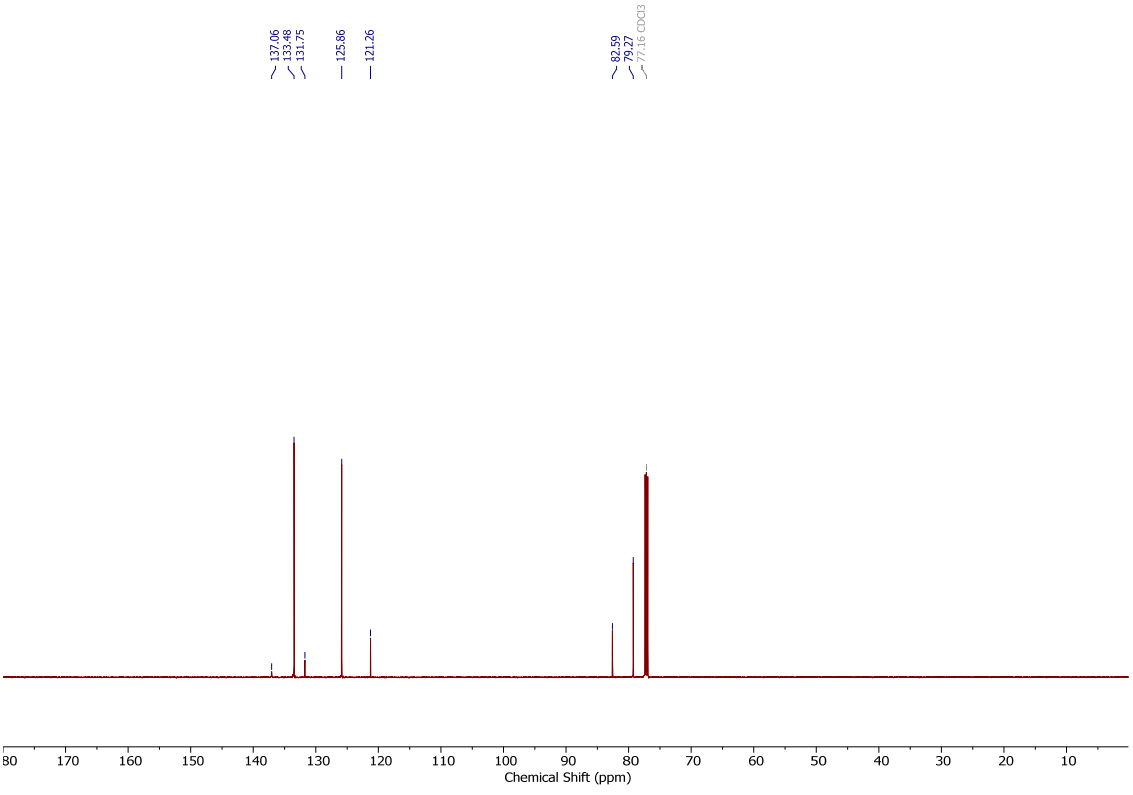



**Figure S8.** Cell viability of H9C2 cells in the presence of Monensin. H9C2 cells were treated with either DMSO (0.5%, vehicle) or Monensin (0.5, 1.0, 2.0, or 3 μM) for 19 h. Cell viability was assessed using a CCK-8 kit. Results are expressed as mean ± SD ($n$ = 12).

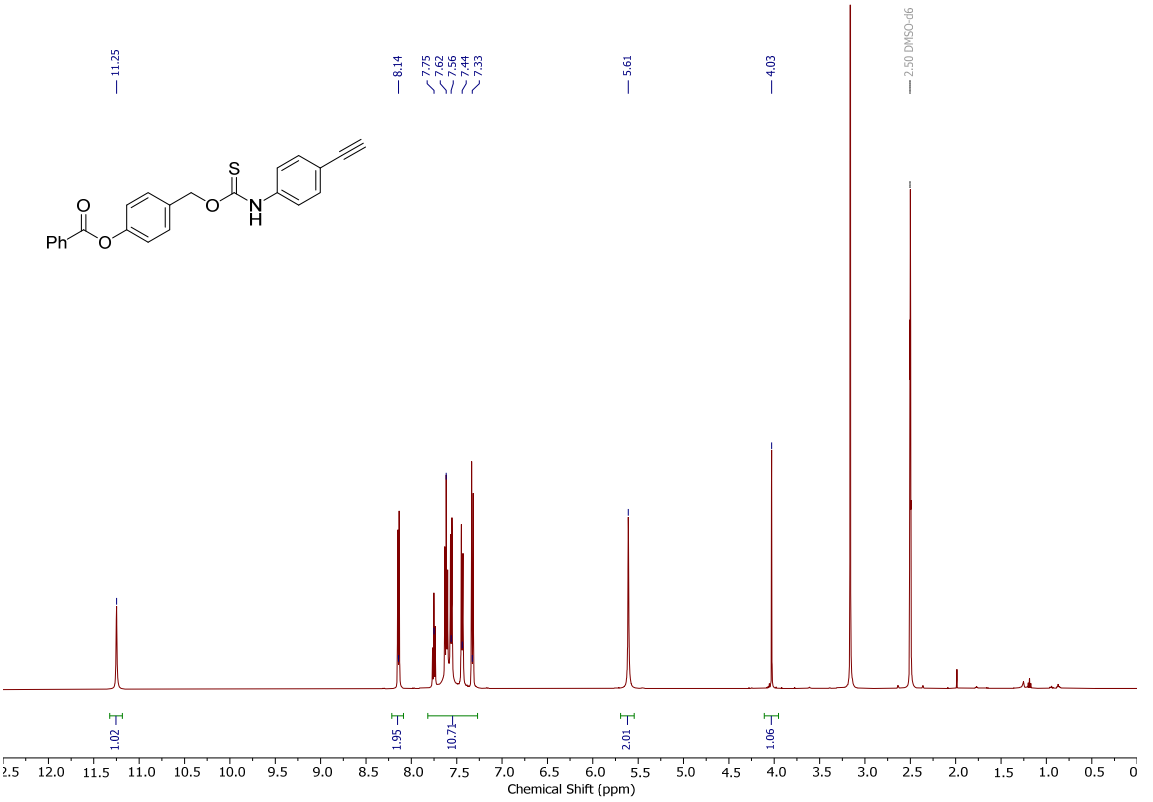
## 9. NMR Spectra

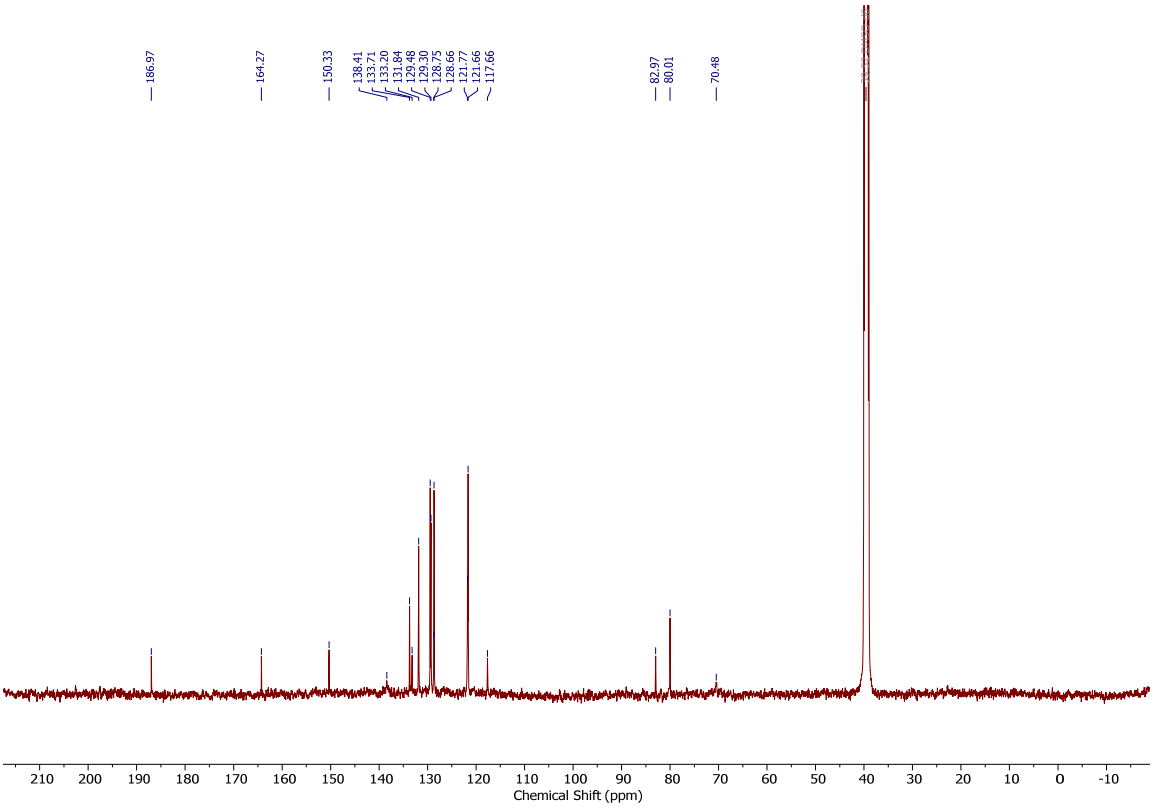
$^{1}H$ NMR (600 MHz, $CDCl_3$) and $^{13}C\{^{1}H\}$ NMR (151 MHz, $CDCl_3$) spectra of 1-ethynyl-4-isothiocyanatobenzene.



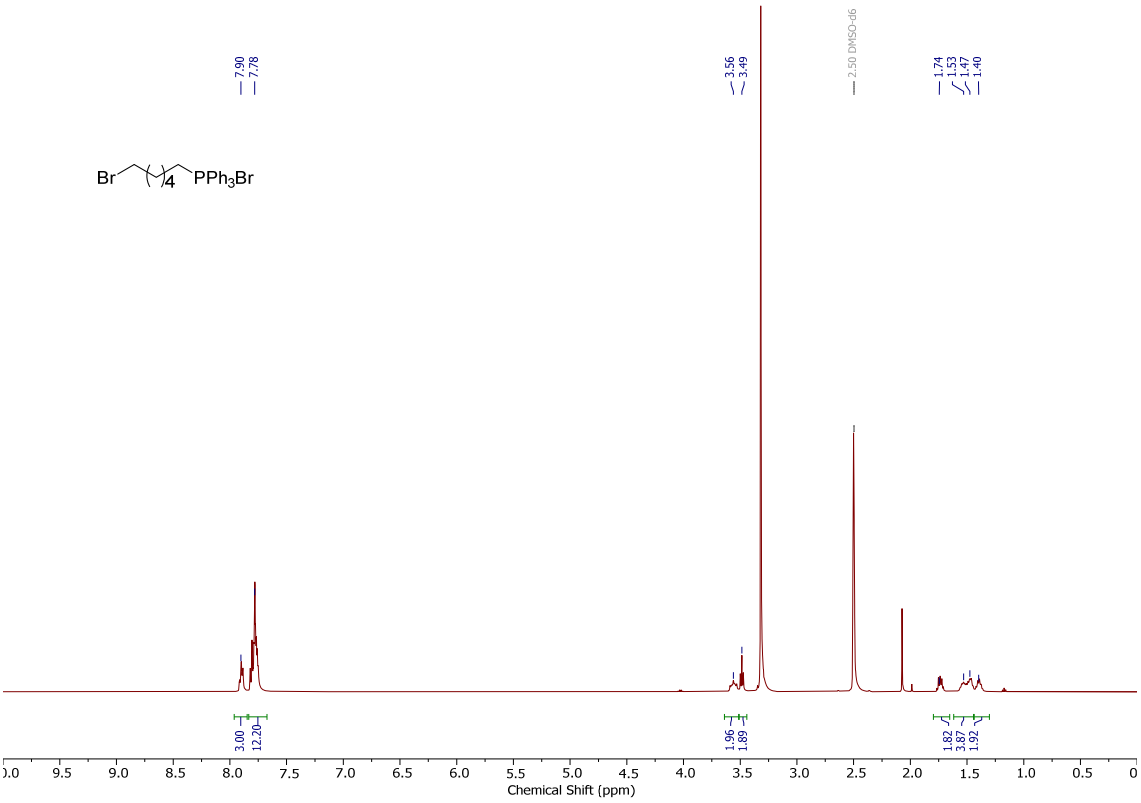



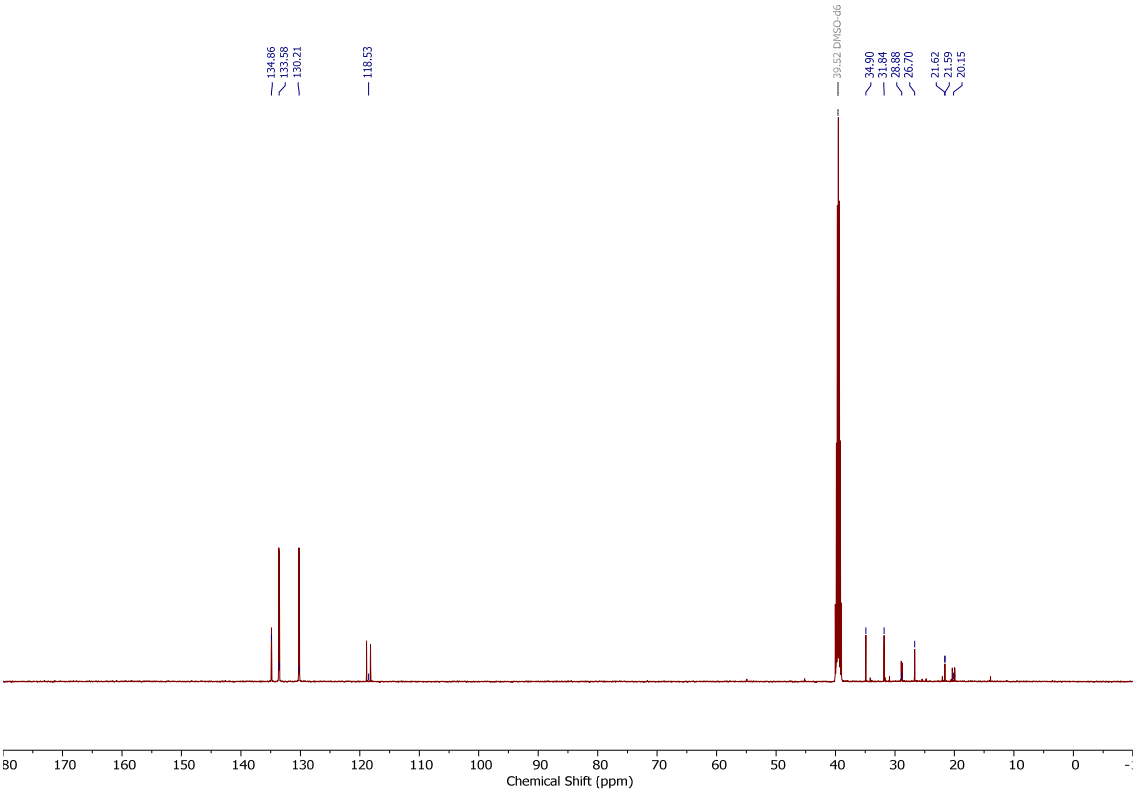
$^{1}$H NMR (500 MHz, DMSO-*d*$_6$, 60 °C) and $^{13}$C{$^{1}$H} NMR (126 MHz, DMSO-*d*$_6$, 60 °C) spectra of TCM alkyne



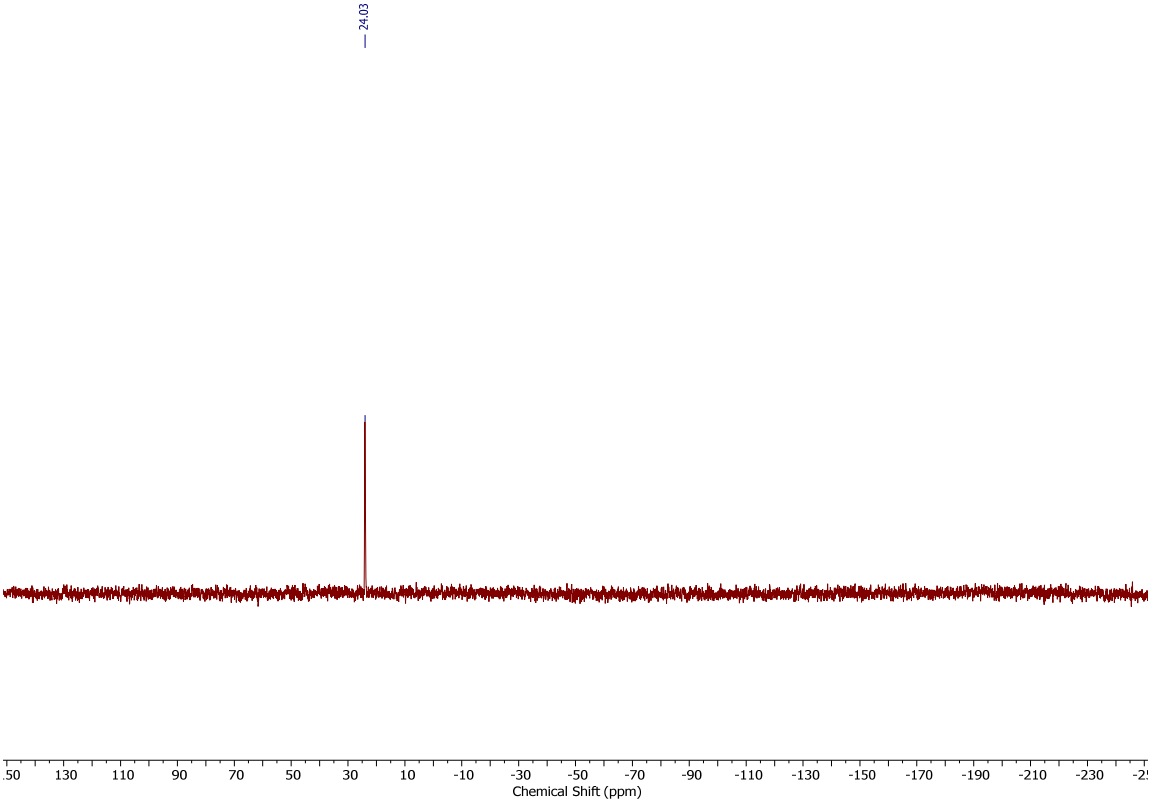



$^{1}$H NMR (500 MHz, DMSO-*d*$_6$) and $^{13}$C{$^{1}$H} NMR (126 MHz, DMSO-*d*$_6$) spectra of (6-bromohexyl)triphenylphosphonium bromide.

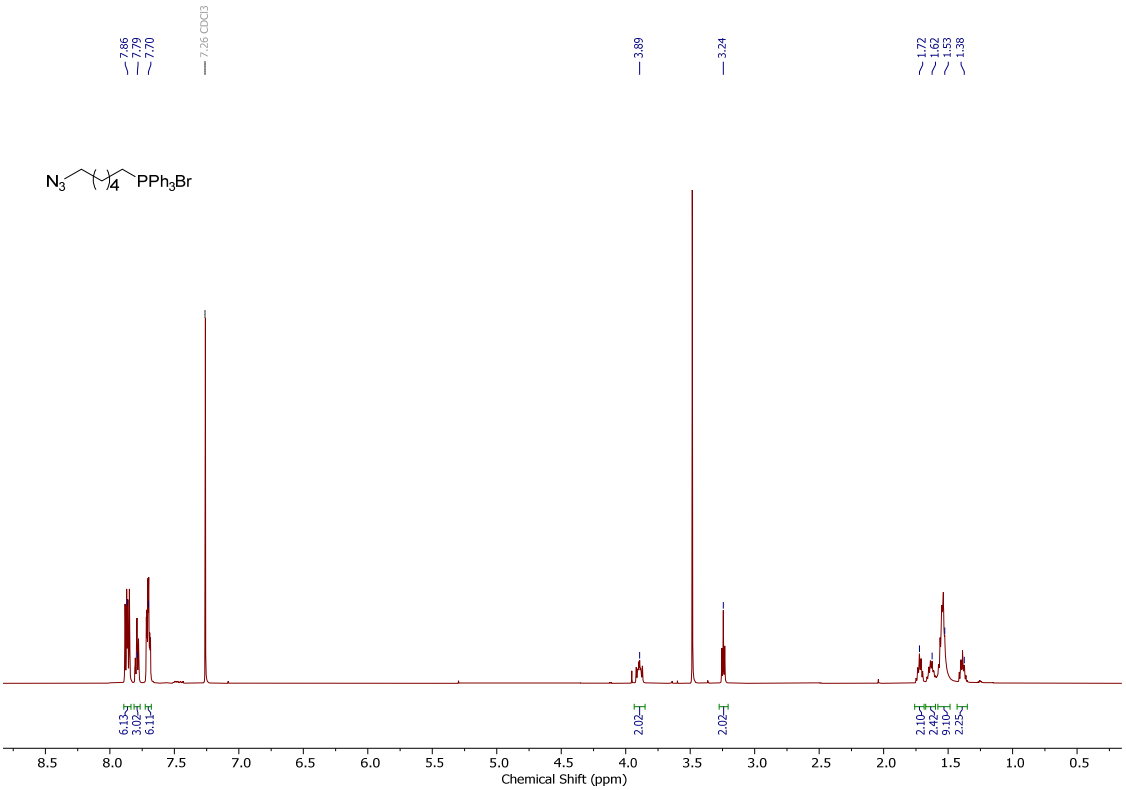


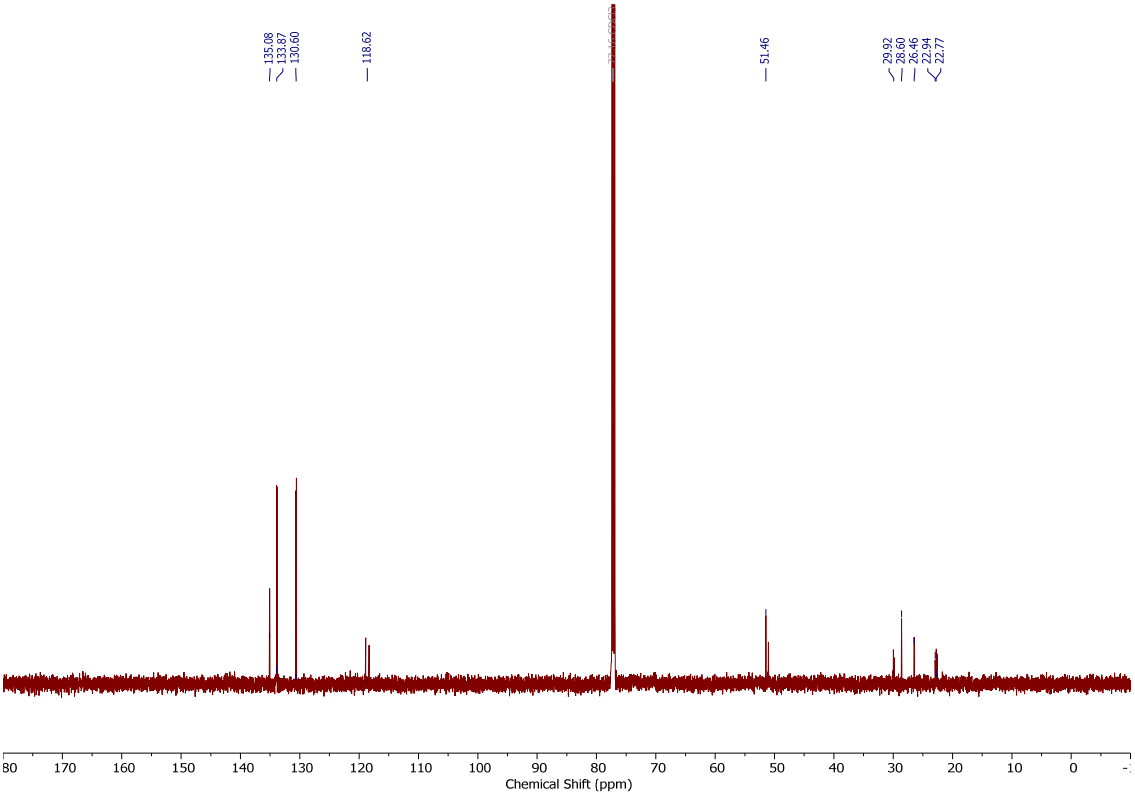



$^{31}P\{^{1}H\}$ NMR (202 MHz, DMSO-$d_6$) spectrum of (6-bromohexyl)triphenylphosphonium bromide.

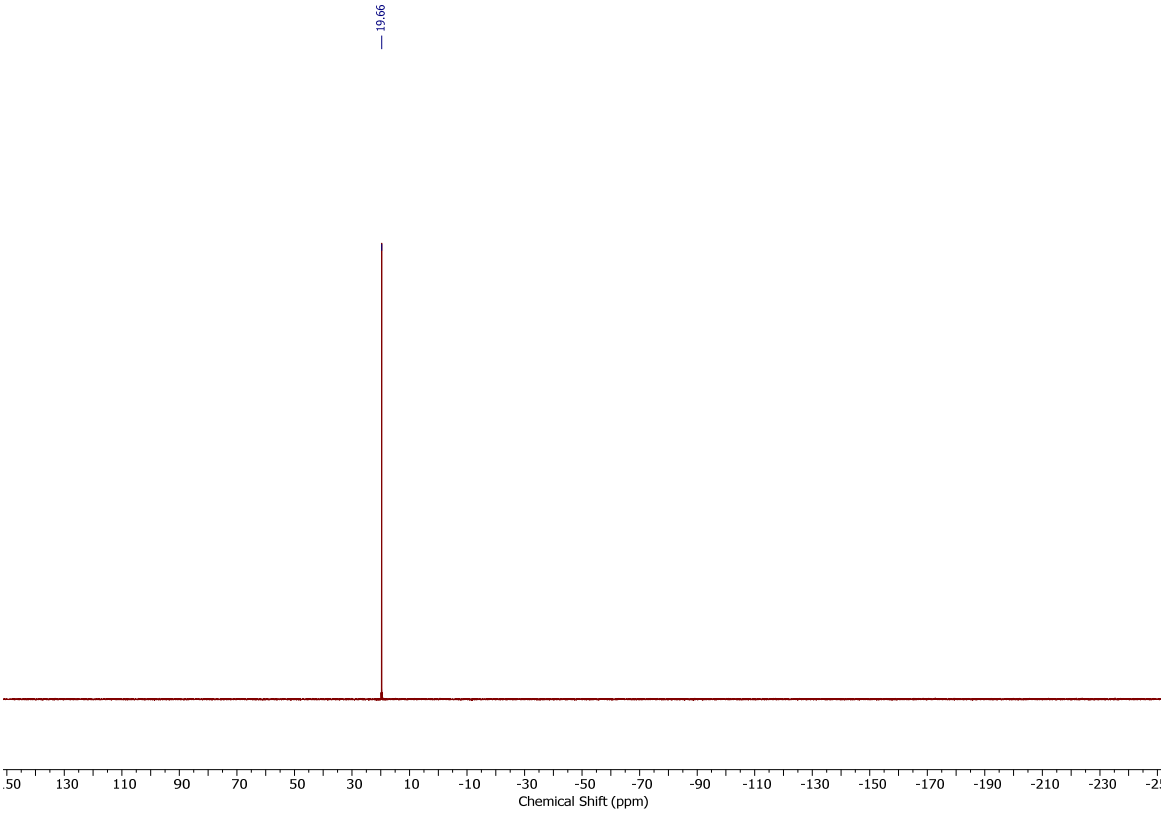


$^{1}$H NMR (500 MHz, DMSO-*d*$_6$) and $^{13}$C{$^{1}$H} NMR (151 MHz, CDCl$_3$) spectra of (6-azidohexyl)triphenylphosphonium bromide.

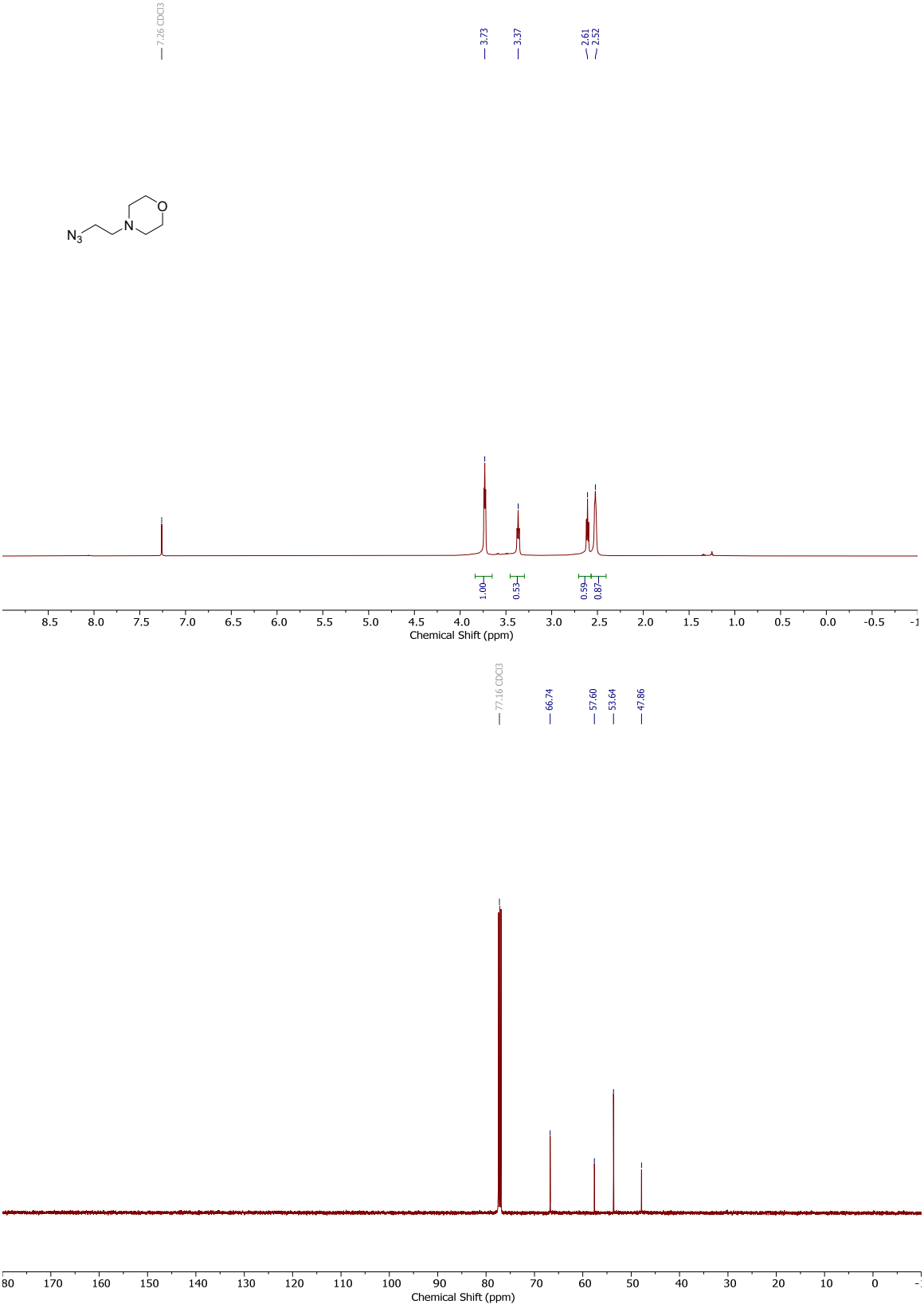


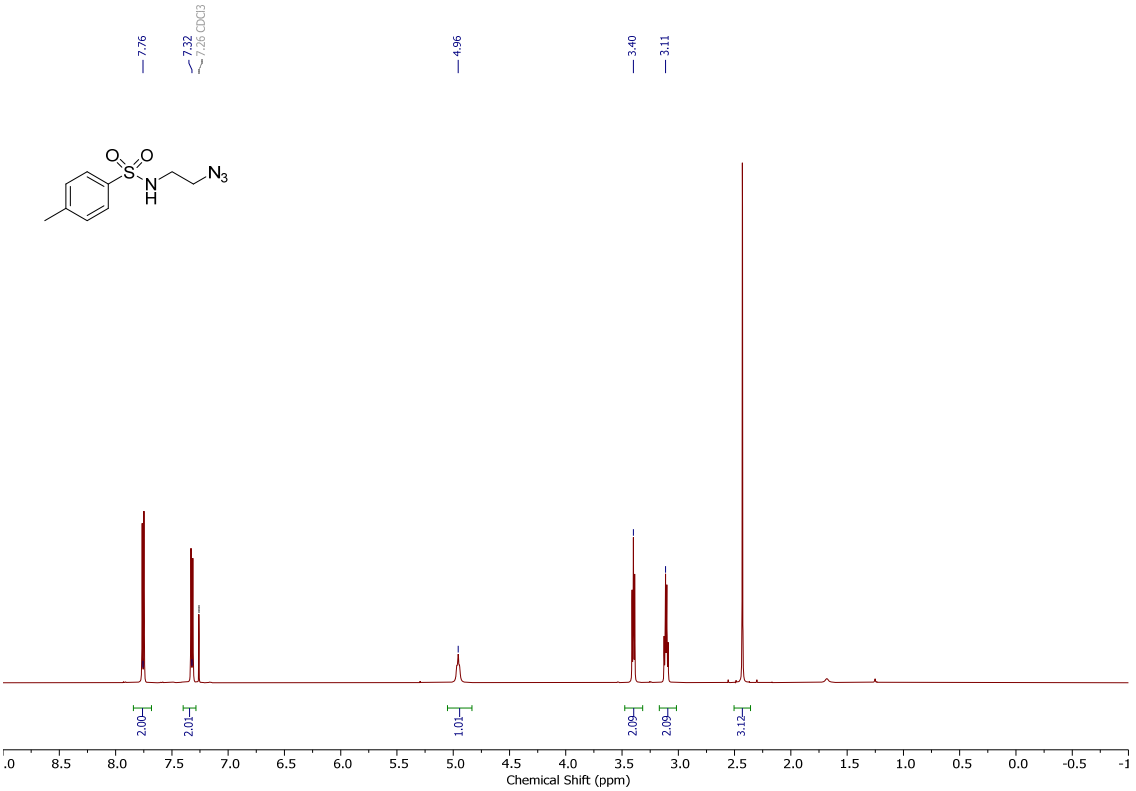
$^{31}P\{^{1}H\}$ NMR (202 MHz, $CDCl_3$) spectrum of (6-azidohexyl)triphenylphosphonium bromide.

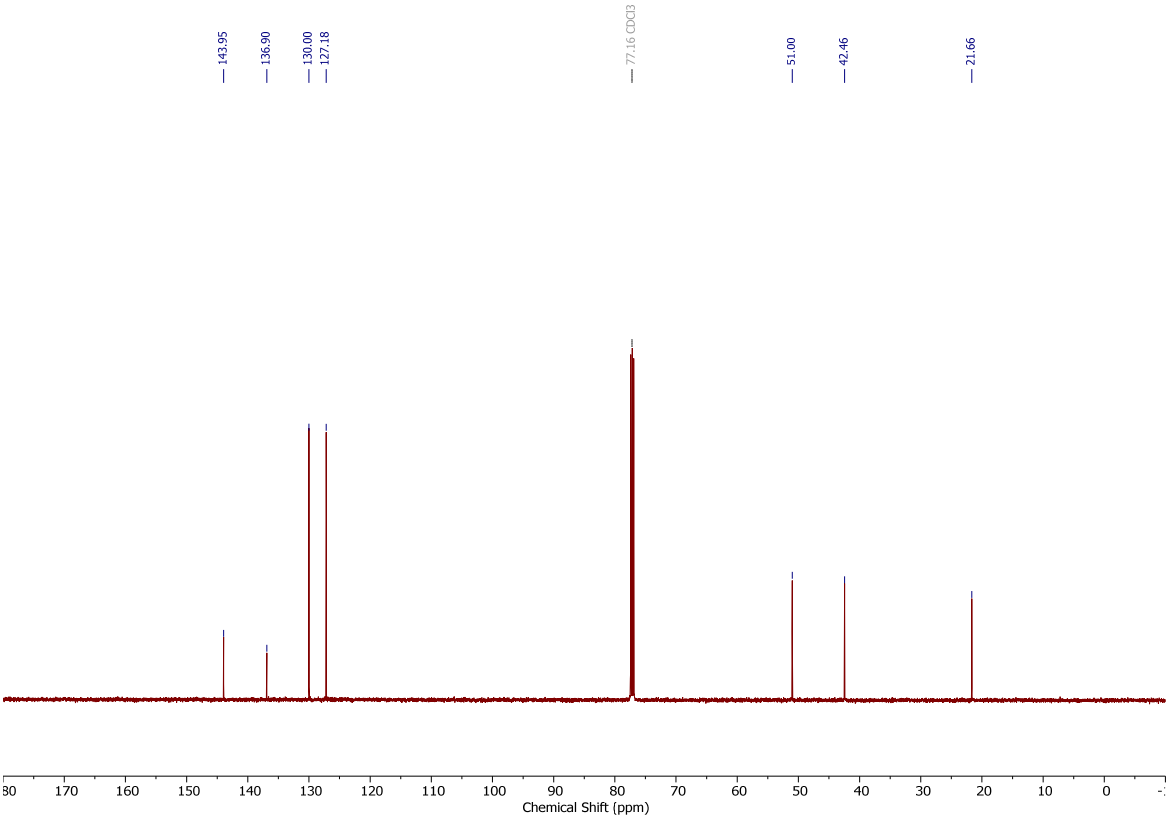


$^{1}$H NMR (500 MHz, $CDCl_3$) and $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$) spectra of 4-(2-azidoethyl)morpholine.

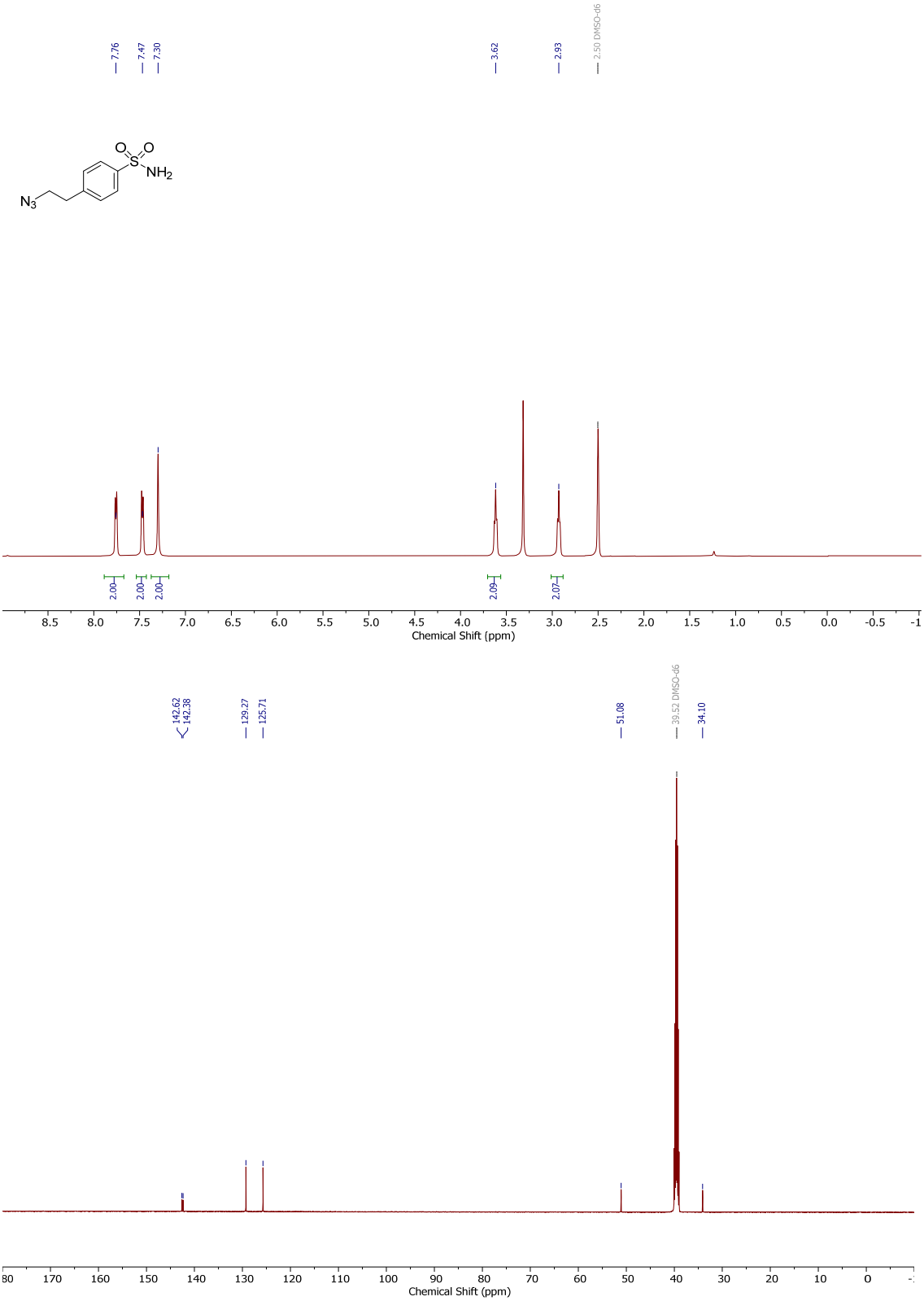


$^{1}$H NMR (500 MHz, $CDCl_3$) and $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$) spectra of *N*-(2-azidoethyl)-4-methylbenzenesulfonamide.

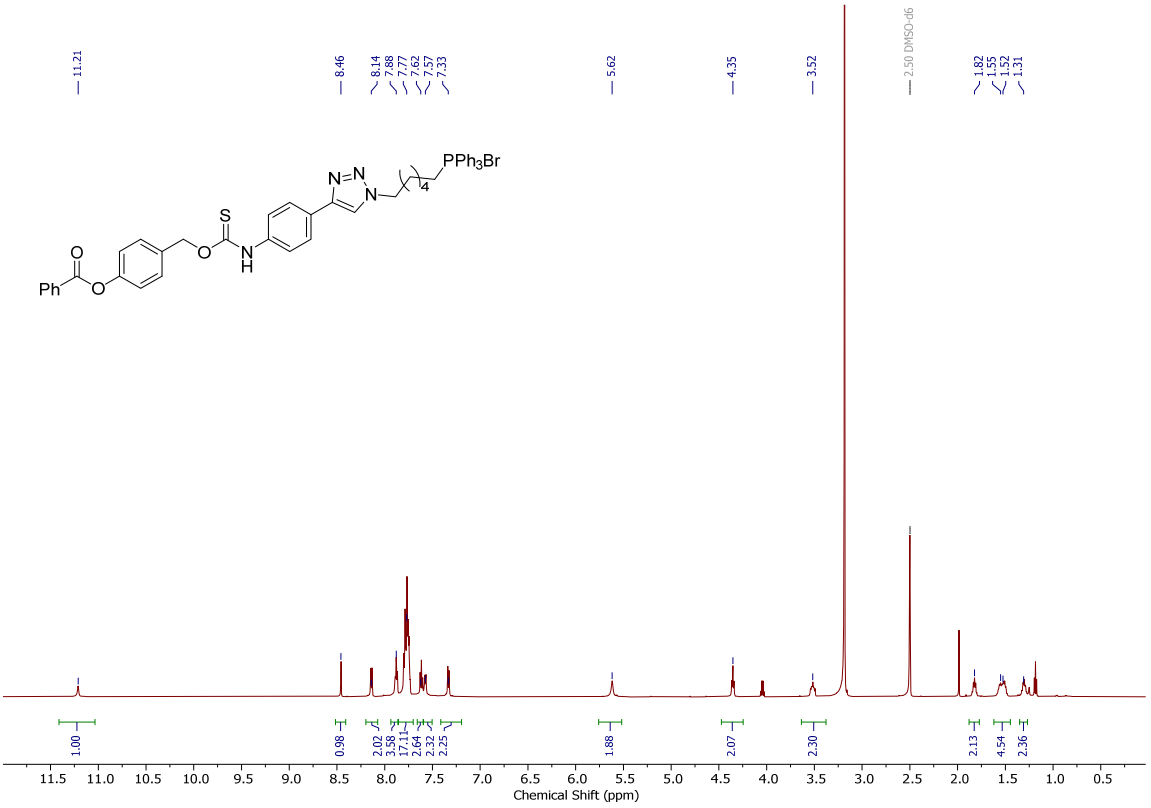


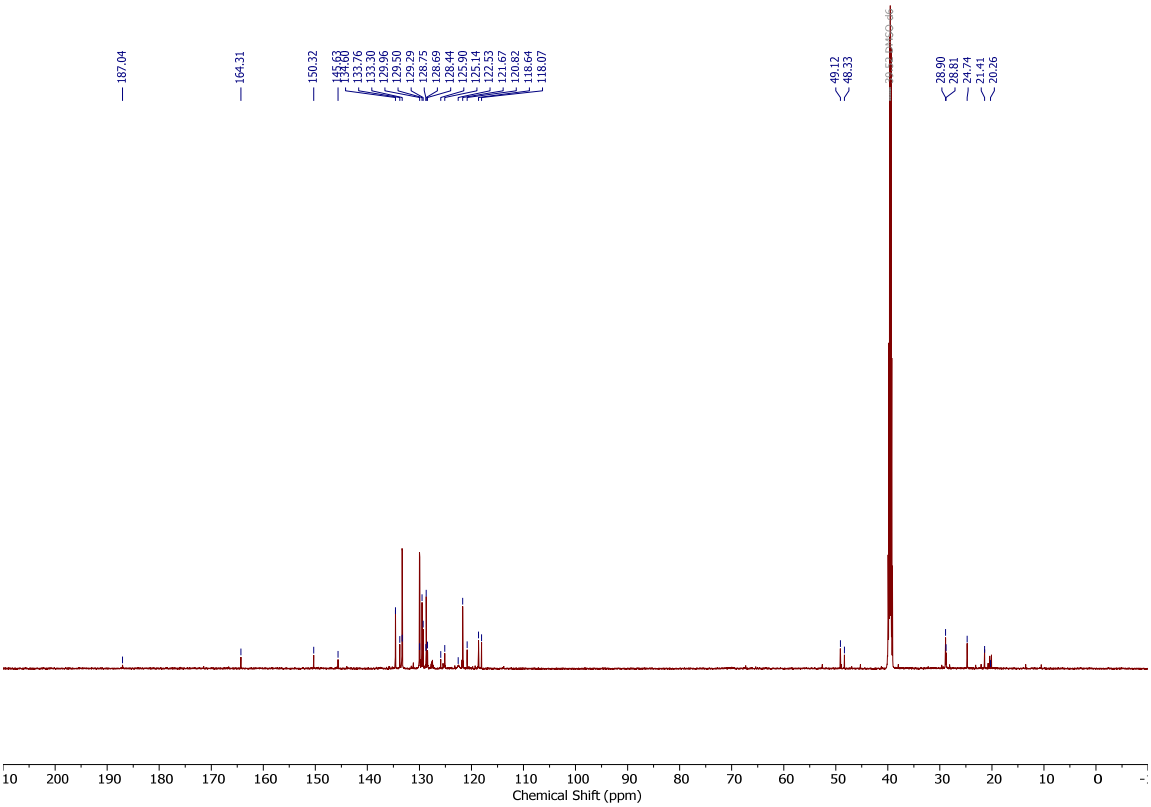



$^{1}H$ NMR (500 MHz, $CDCl_3$) and $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$) spectra of 4-(2-azidoethyl)-benzenesulfonamide.

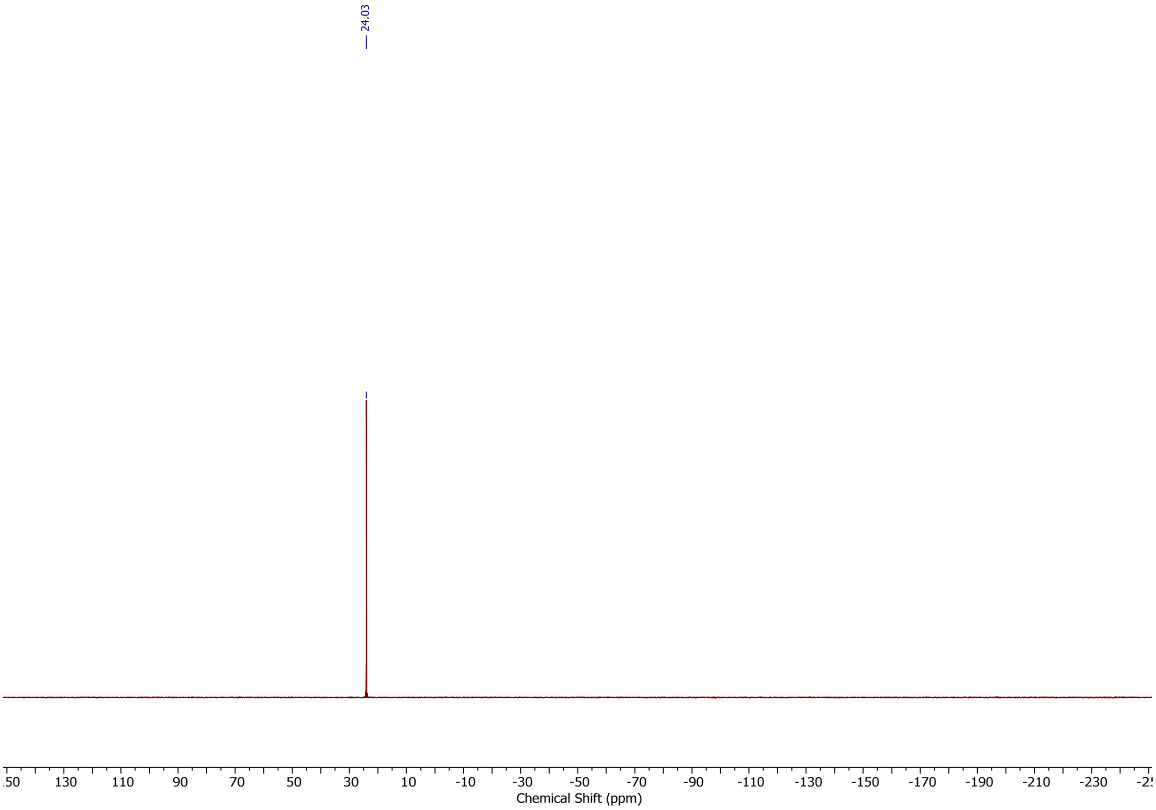


$^{1}$H NMR (600 MHz, DMSO-*d*$_6$, 60 °C) and $^{13}$C{$^{1}$H} NMR (126 MHz, DMSO-*d*$_6$, 60 °C) spectra of **MitoTCM**.

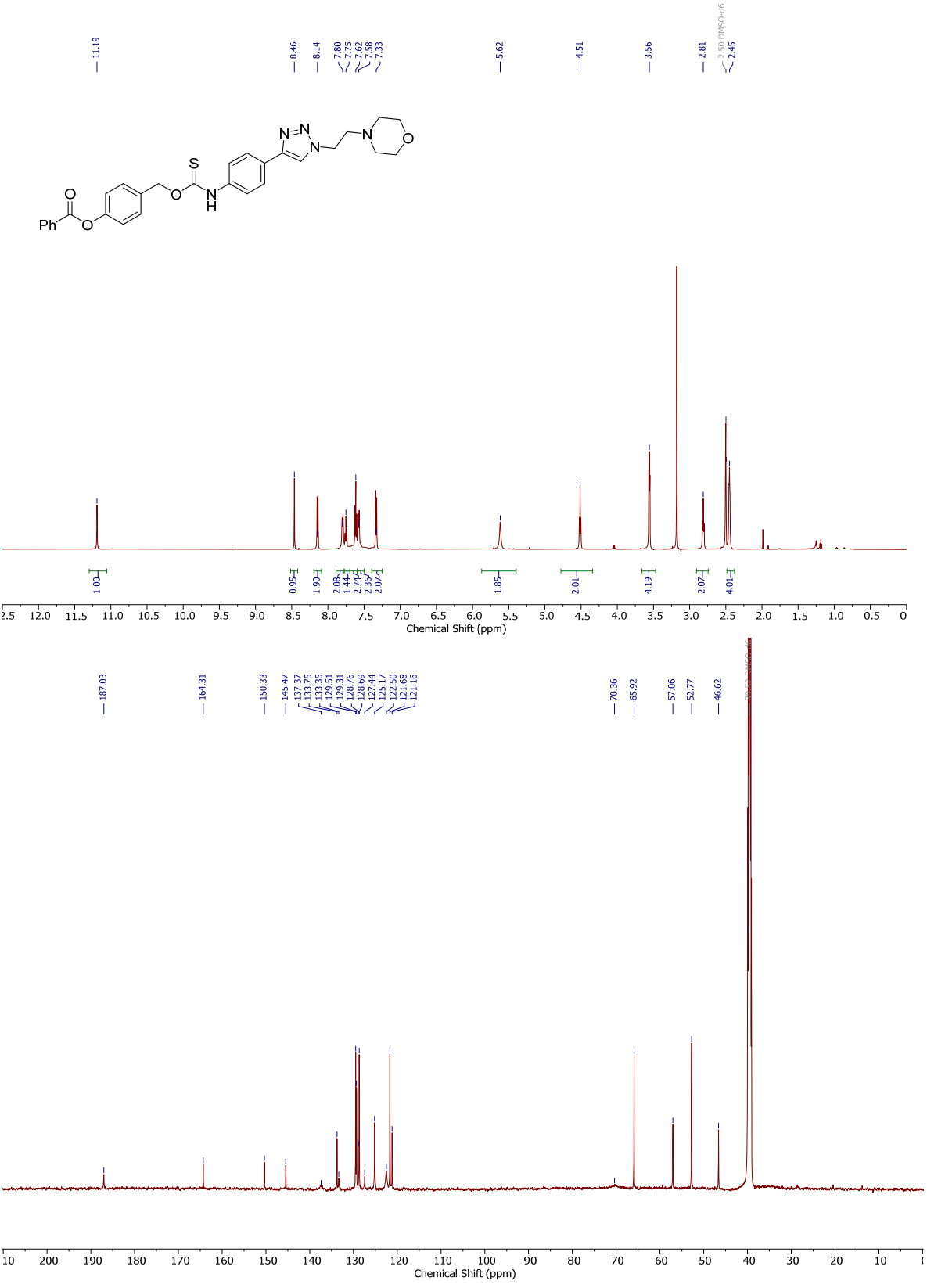


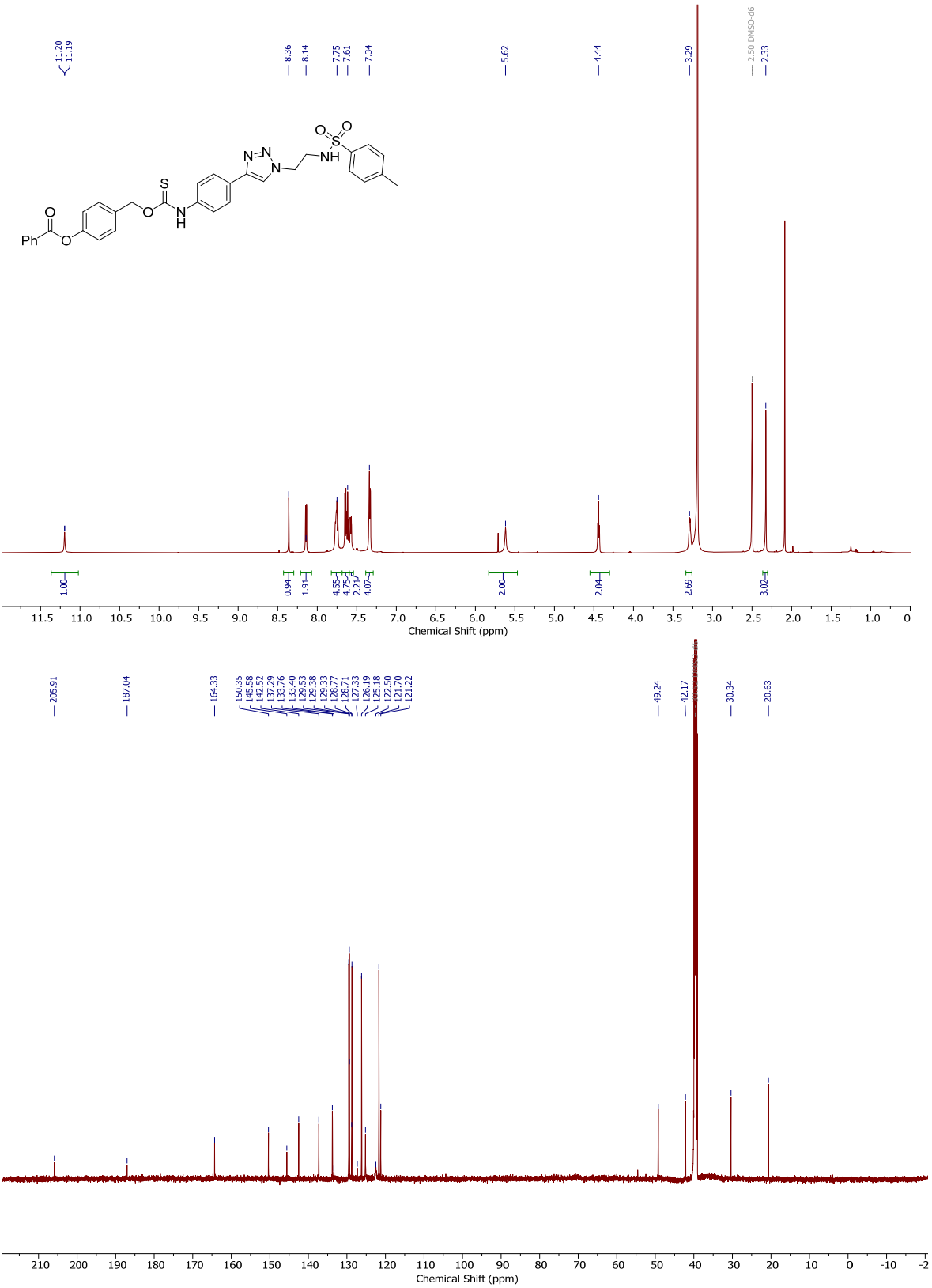
$^{31}P\{^{1}H\}$ NMR (202 MHz, DMSO-*d*$_6$, 60 °C) spectrum of **MitoTCM**.



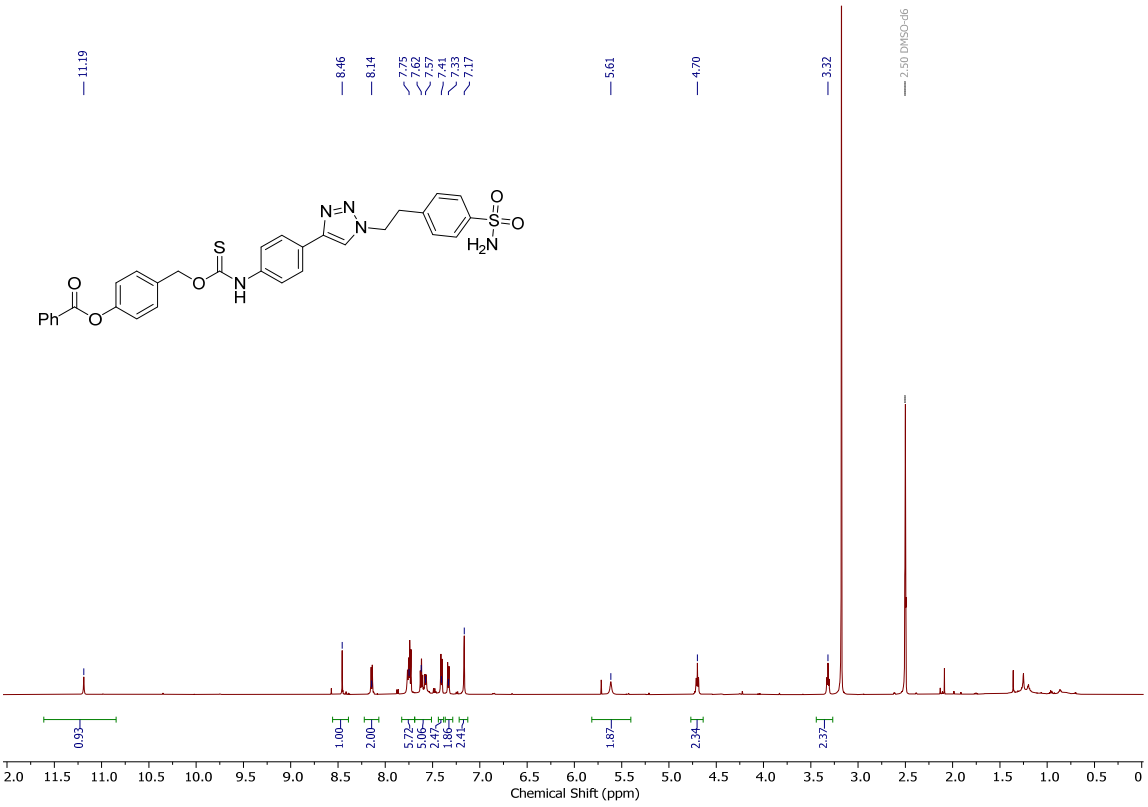
$^{1}$H NMR (600 MHz, DMSO-*d*$_6$, 60 °C) and $^{13}$C{$^{1}$H} NMR (151 MHz, DMSO-*d*$_6$, 60 °C) spectra of **LysoTCM**.

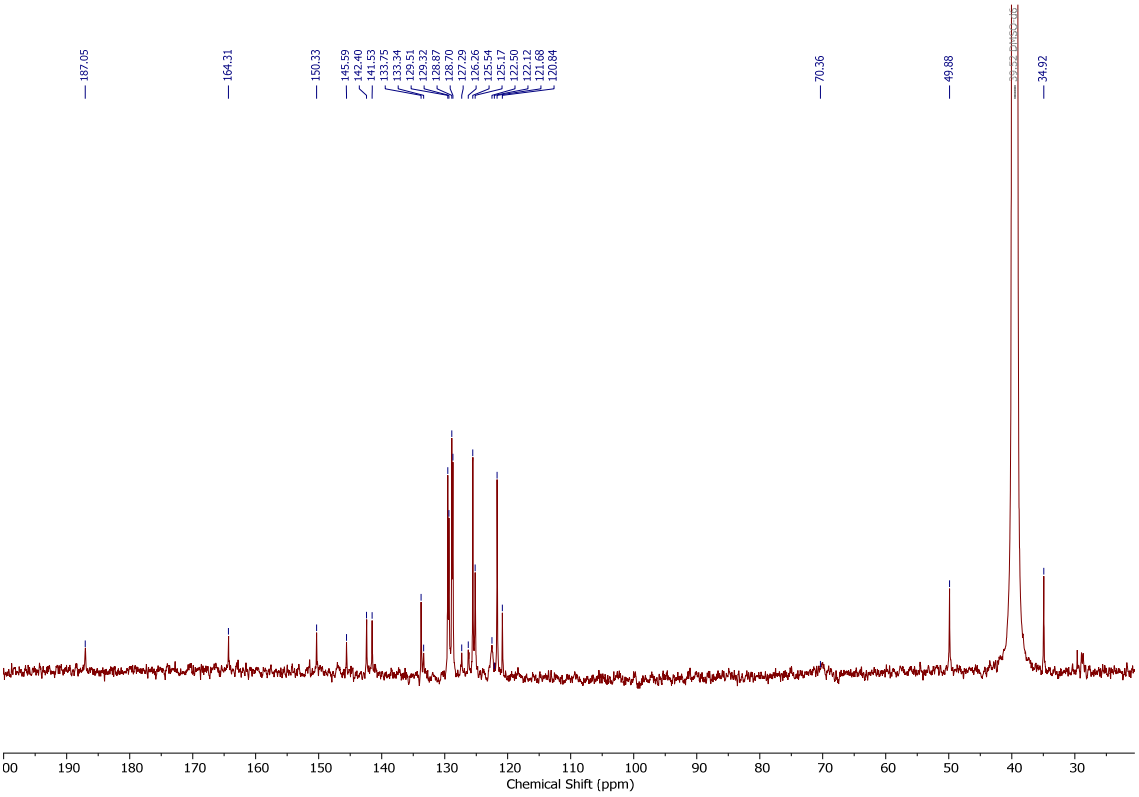


$^{1}$H NMR (600 MHz, DMSO-*d*$_6$, 60 °C) and $^{13}$C{$^{1}$H} NMR (151 MHz, DMSO-*d*$_6$, 60 °C) spectra of **ERTCM**.

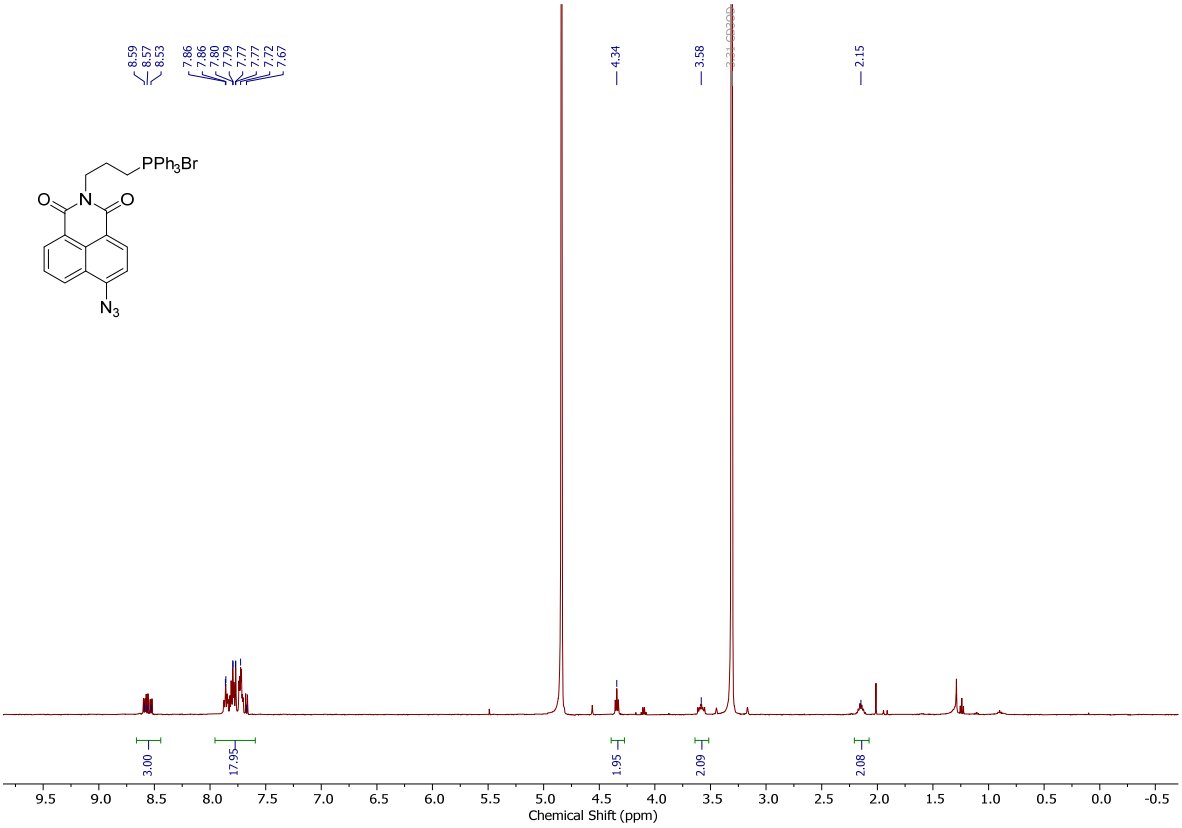


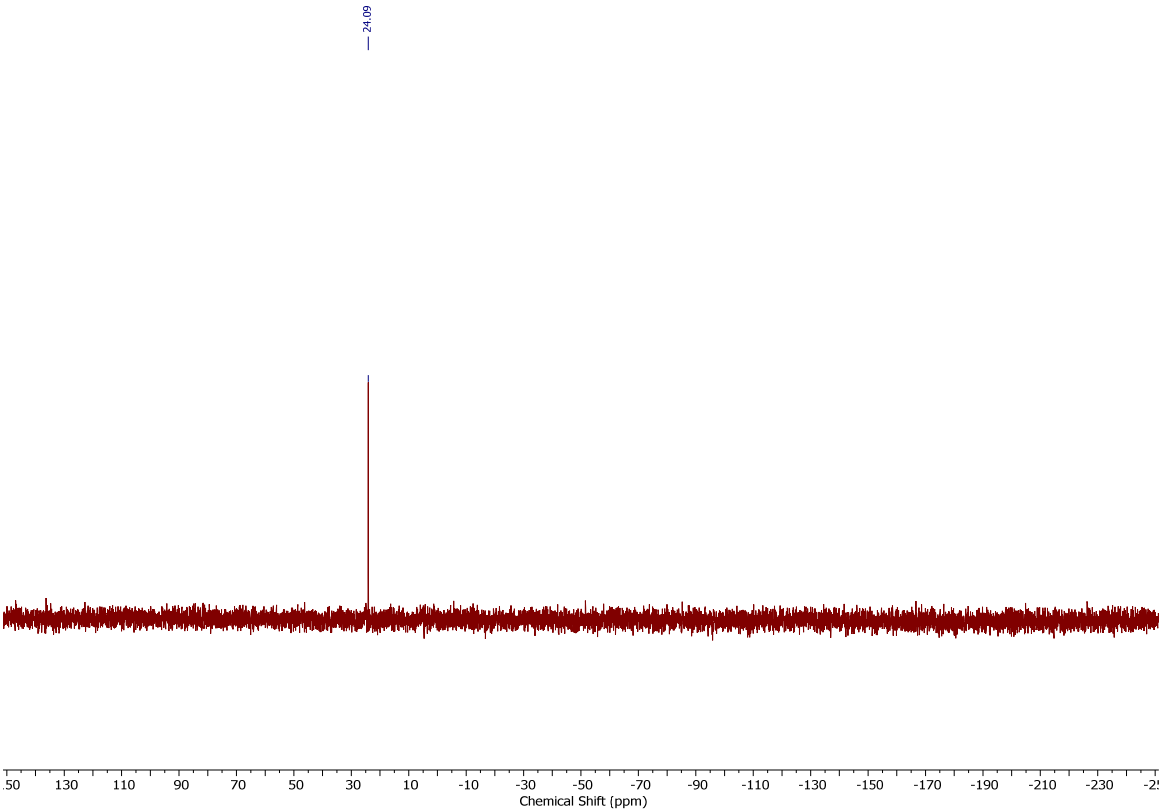
$^{1}$H NMR (600 MHz, DMSO-*d*$_6$, 60 °C) and $^{13}$C{$^{1}$H} NMR (151 MHz, DMSO-*d*$_6$, 60 °C) spectra of **GolgiTCM**.



$^{1}$H NMR (500 MHz, $CD_3OD$) and $^{31}P\{^{1}H\}$ NMR (202 MHz, $CD_3OD$) spectra of Mito-HS.



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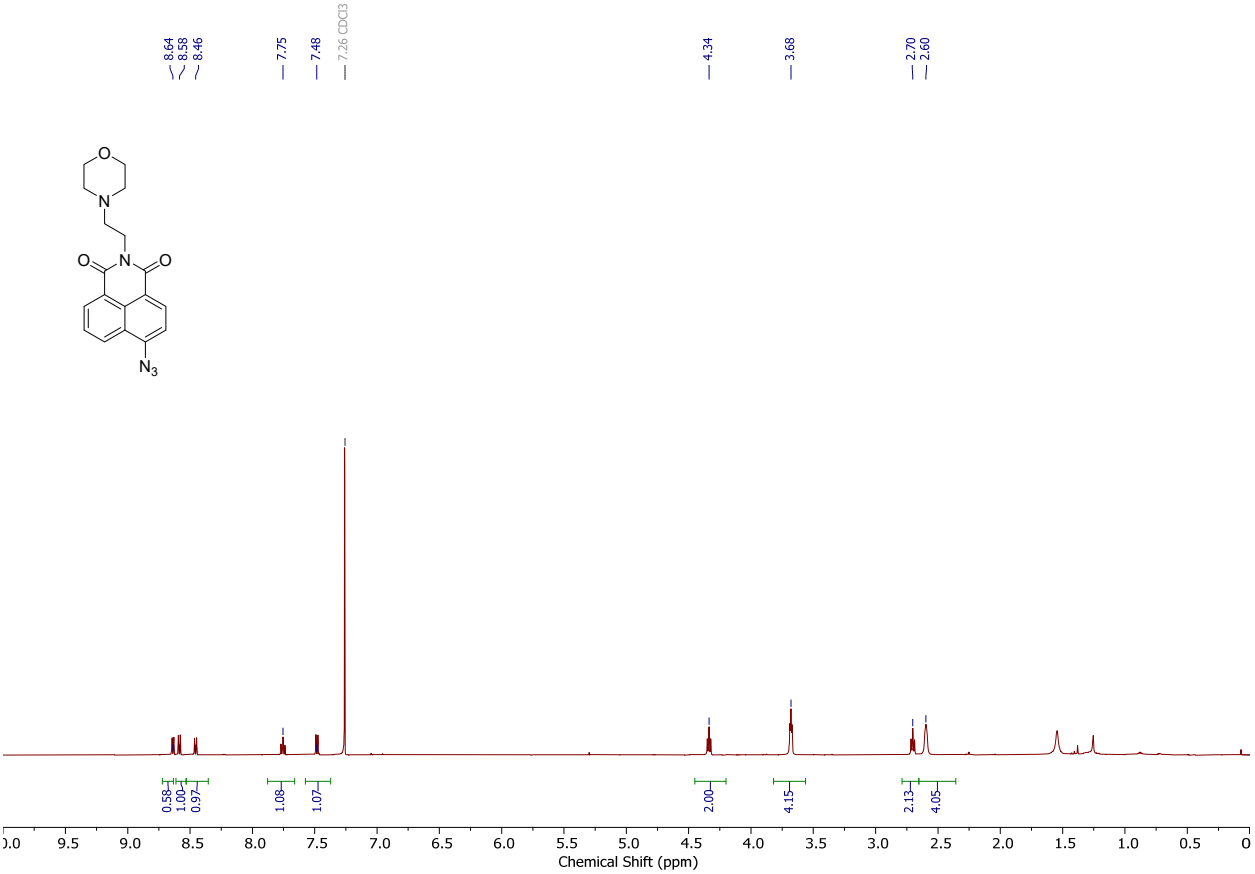

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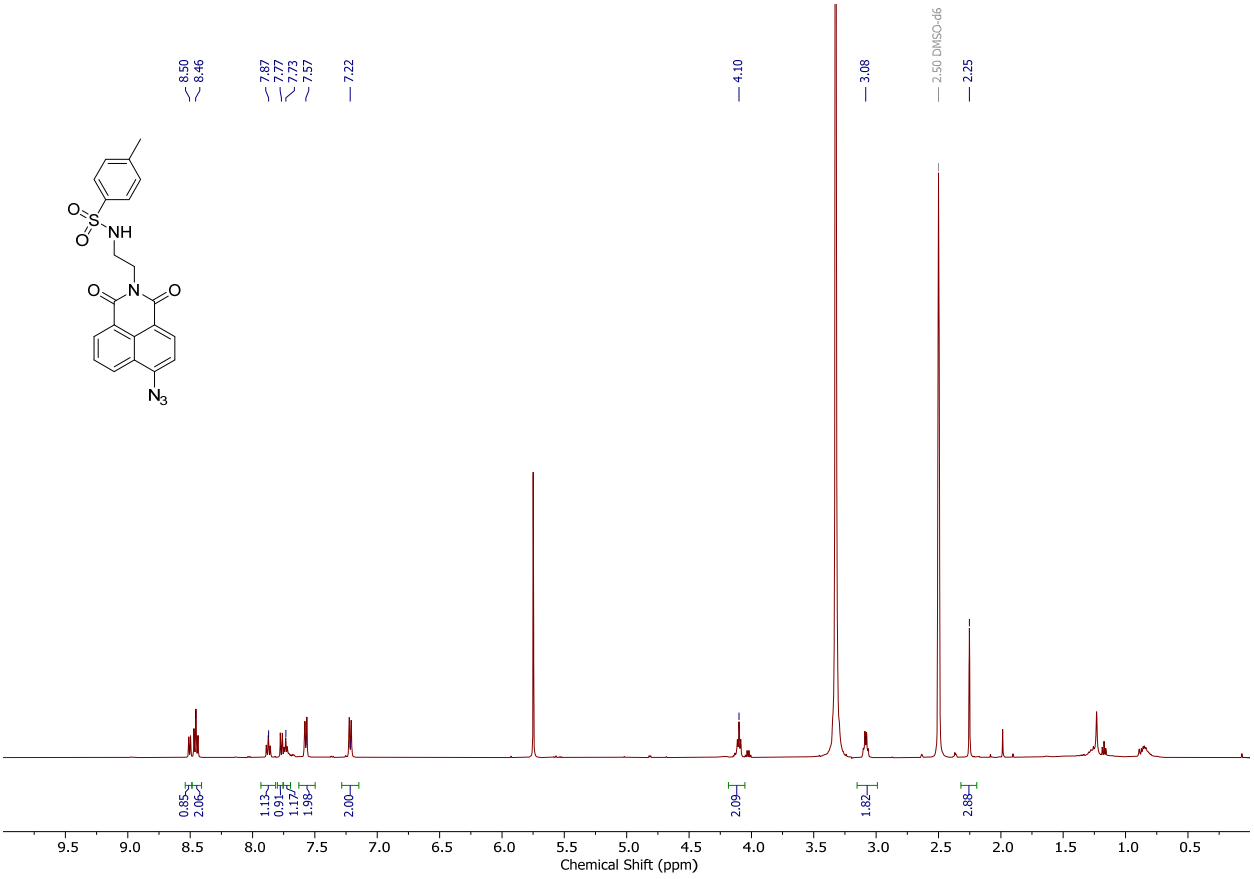

$^{1}$H NMR (500 MHz, DMSO-$d_6$) spectrum of Golgi-NH.