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Electronic Supporting Information (ESI)

Photoactivatable *bis*(thiosemicarbazone) derivatives for copper-64 radiotracer synthesis

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Synthesis and characterisation data

Compound 1

To a cooled (0 °C) solution of ethylenediamine (4.59 mL; 68.5 mmol; 0.25 M) in $CHCl_3$ (275 mL) was added dropwise a solution of di-*tert*-butyl dicarbonate (Boc_2O; 2.99 g; 13.7 mmol, 0.5 M) in $CHCl_3$ (27.5 mL) over period of 2 h. The reaction mixture was allowed to warm to rt and stirred for a further 3 h. After this time, the reaction mixture was filtered and concentrated under reduced pressure. The crude residue was dissolved in EtOAc (400 mL), washed with brine (3 × 150 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2; DCM:MeOH; 9:1 (v:v) + 1% NH_3OH) to afford compound **1** (1.95 g; 89% yield) as a colourless oil. $R_f = 0.31$ (DCM:MeOH; 9:1); **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K): δ (ppm) 4.86 (1H, br s, $N\underline{H}$), 3.17 (2H, m, $C\underline{H}_2$), 2.80 (2H, t, $^3J_{HH} = 6.0$ Hz, $C\underline{H}_2$) 1.44 (9H, s, $C(C\underline{H}_3)_3$), 1.36 (2H, s, $N\underline{H}_2$).

Compound 2

To a stirred solution of compound **1** (1.43 g; 8.9 mmol; 1 equiv) and triethylamine (1.25 mL; 8.9 mmol; 1 equiv) in EtOH (30 mL) was added CS_2 (0.54 mL; 8.9 mmol; 1 equiv) dropwise, whilst maintaining the reaction at rt. After 2 h, methyl iodide (0.56 mL, 8.9 mmol; 1 equiv) was added and the resulting mixture was stirred for a further 2 h. After this time, the solvent was removed under reduced pressure and the resulting residue was suspended in EtOAc (30 mL), washed with 1 M aq HCl (30 mL), sat aq $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo* to furnish compound **2** (2.03 g; 91% yield) as an off-white solid. **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K): δ (ppm) 8.32 (1H, br s, $N\underline{H}C{=}S$), 4.98 (1H, br s, $C\underline{H}_2NHC{=}S$), 3.79 (2H, q, $^3J_{HH} = 4.9$ Hz, $N\underline{H}C{=}O$), 3.42 (2h, t, $J = 5.4$ Hz, $C\underline{H}_2N\underline{H}C{=}O$), 2.59 (3H, s, SCH_3), 1.45 (9H, s, $C(CH_3)_3$). **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$, 298 K): δ (ppm) 199.3 (C=S), 157.8 (C=O), 80.7 ($\underline{C}(CH_3)_3$), 49.7 ($C\underline{H}_2NHC{=}S$), 39.2 ($C\underline{H}_2NHC{=}O$), 28.5 ($C(\underline{C}H_3)_3$), 18.1 ($S\underline{C}H_3$) ppm. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_9H_{18}N_2O_2S_2$ $[M+H]^+$ 251.08825, found 251.08808, $[M+Na]^+$ 273.07019, found 273.06992.

Compound 3

To a solution of compound **2** (2.03 g; 8.11 mmol; 1 equiv) in EtOH (30 mL) was added hydrazine hydrate (0.57 mL; 11.4 mmol; 1.4 equiv) and the reaction stirred under reflux for 3 h. After this time, the solvent was removed under reduced pressure and the resulting white residue was dissolved in $CHCl_3$ (20 mL). This solution was loaded on a plug of silica, washed with $CHCl_3$ (20 mL) and then eluted with MeOH (30 mL). The MeOH fraction was then concentrated *in vacuo* to afford compound **3** (1.79 g; 94% yield) as a colourless oil, which solidified over a few days. **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K): δ (ppm) 7.80 (1H, br s, $N\underline{H}NH_2$), 7.63 (1H, br s, $CH_2N\underline{H}C{=}S$), 5.03 (1H, br s, $N\underline{H}CO_2C(CH_3)_3$), 3.82 (2H, br s, $NHN\underline{H}_2$), 3.75 (2H, q, $^3J_{HH}$ = 5.8 Hz, $C\underline{H}_2NHC{=}S$), 3.37 (2H, q, $^3J_{HH}$ = 6.0 Hz, $C\underline{H}_2NHCO_2C(CH_3)_3$), 1.43 (9H, s, $C(C\underline{H}_3)_3$). **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$, 298 K): δ (ppm) 183.0 (C=S), 153.8 (C=O), 79.8 ($\underline{C}(CH_3)_3$), 44.7 ($C\underline{H}_2NHC{=}S$), 40.3 ($C\underline{H}_2NHC{=}O$), 28.5 ($C(\underline{C}H_3)_3$). **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_9H_{18}N_4O_2S$ $[M+H]^+$ 235.12239, found 235.12232.

Compound 4

A cooled (0 °C) solution of 4-methylthiosemicarbazide (2.00 g; 19 mmol; 1 equiv) in H_2O (100 mL) was acidified with conc aq HCl (~700 μL; 12.5 M), to which diacetyl (2,3-butanedione; 10.8 mL; 124 mmol; 6.5 equiv) was added rapidly. A suspension was formed instantly and stirring was continued for 1 h. After this time, the reaction mixture was filtered and the resulting solid was washed with H_2O (5 × 20 mL) and dried under high-vacuum to furnish compound **4** (2.50 g; 76% yield) as a white solid. **^{1}H NMR** (300 MHz, DMSO-d_6, 298 K): δ (ppm) 10.64 (1H, br s, $C{=}SN\underline{H}N$), 8.62 (1H, m, $CH_3N\underline{H}C{=}S$), 3.05 (3H, d, $^3J_{HH}$ = 4.5 Hz, $C\underline{H}_3NHC{=}S$), 2.42 (3H, s, $C\underline{H}_3C{=}O$), 1.96 (3H, s, $C\underline{H}_3C{=}N$). **^{13}C{^{1}H} NMR** (126 MHz, DMSO-d_6, 298 K): δ (ppm) 198.0 (C=O), 179.5 (C=S), 146.0 (C=N), 31.9 ($\underline{C}H_3NHC{=}S$), 25.2 ($\underline{C}H_3C{=}O$), 10.5 ($\underline{C}H_3C{=}N$). **HR-ESI-MS** (ESI–): *m/z* calcd. for $C_7H_{13}N_3OS$ $[M-H]^-$ 172.05501, found 172.05499.

Compound 5, H_2ATSM/en-Boc

To a stirred suspension of compound **4** (183 mg; 0.8 mmol; 1 equiv) in EtOH (33 mL) at 50 °C was added compound **3** (135 mg; 0.8 mmol; 1 equiv) portion-wise over 30 min. After this time, the reaction mixture was acidified with conc aq HCl (~100 μL; 12.5 M) and the reaction was stirred under reflux for 5 h. After this time, the reaction mixture was allowed to cool to rt and was poured into cold water (20 mL) to produce a white precipitate. The precipitate was then collected by vacuum filtration, washed with H_2O (2 × 10 mL), Et_2O (5 mL) and dried under reduced pressure to afford H_2ATSM/en-Boc, compound **5** (254 mg; 83% yield) as an off-white solid. **^{1}H NMR** (400 MHz, DMSO-d_6, 298 K): δ (ppm) 10.28 (2H, br s, 2 x $N\underline{H}N{=}C$), 8.45

(1H, t,$^3J_{HH}$= 5.0 Hz, CH$_2$N$\underline{H}$C=S), 8.38 (1H, q, $^3J_{HH}$ = 4.5 Hz, CH$_3$N$\underline{H}$C=S), 7.03 (1H, t, $^3J_{HH}$ = 5.0 Hz, N$\underline{H}$CO$_2$C(CH$_3$)$_3$), 3.59 (2H, td, $^3J_{HH}$ = 5.5, 5.0 Hz, C$\underline{H}_2$NHC=S), 3.18 (2H, td, $^3J_{HH}$ = 5.5, 5.0 Hz, C$\underline{H}_2$NHCO$_2$C(CH$_3$)$_3$), 3.02 (3H, d, $^3J_{HH}$ = 4.5 Hz, C$\underline{H}_3$NHC=S), 2.23 (3H, s, C$\underline{H}_3$C=N), 2.21 (3H, s, C$\underline{H}_3$C=N), 1.36 (9H, s, C(CH$_3$)$_3$). **^{13}C{^{1}H} NMR** (101 MHz, DMSO-d$_6$, 298 K): δ (ppm) 178.5 (C=S), 178.0 (C=S), 156.2 (C=O), 148.3 (CH$_3\underline{C}$=N), 148.0 (CH$_3\underline{C}$=N), 77.9 ($\underline{C}$(CH$_3$)$_3$), 44.4 ($\underline{C}$H$_2$), [39.1 ($\underline{C}$H$_2$) from DEPT-135 spectrum – see inset], 31.2 (S$\underline{C}$H$_3$), 28.2 (C($\underline{C}$H$_3$)$_3$), 11.7 ($\underline{C}$H$_3$C=N), 11.7 ($\underline{C}$H$_3$C=N). **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{14}H_{27}N_7O_2S_2$ [M+H]$^+$ 390.17404, found 390.17412, [M+Na]$^+$ 412.15599, found 412.15611.

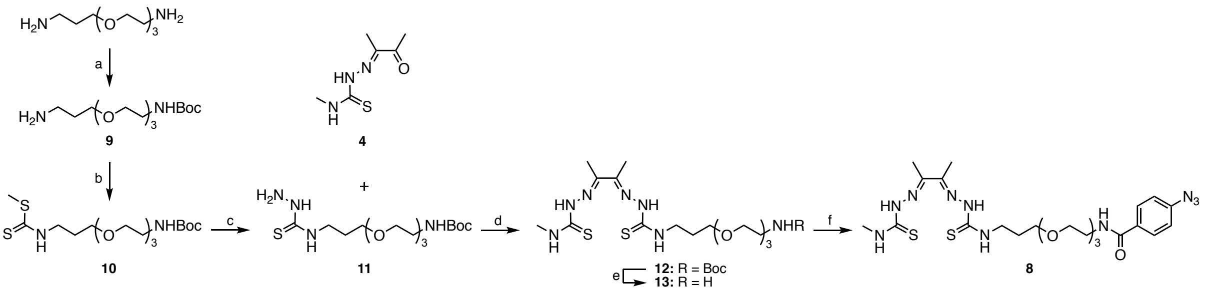
Compound 6, H$_2$ATSM/en

H$_2$ATSM/en-Boc, compound **5** (222 mg; 0.6 mmol; 1 equiv) was treated with TFA (3 mL; 22 mmol; 36 equiv) and the resulting colourless solution was stirred at rt for 2 h. After this time, the solvent was removed under reduced pressure and sat. aq NaHCO$_3$ (3 mL) was added slowly to produce a white suspension. After 20 min of stirring at rt the solid was collected by vacuum filtration and washed with H$_2$O (3 × 10 mL), Et$_2$O (2 × 10 mL) and dried under reduced pressure to furnish the TFA salt of H$_2$ATSM/en, compound **6** (126 mg; 76% yield) as an off-white solid. **^{1}H NMR** (400 MHz, DMSO-d$_6$, 298 K): δ (ppm) 10.19 (1H, br s, N$\underline{H}$N=C), 8.44 (1H, br s CH$_3$N$\underline{H}$C=S), 8.35 (1H, br s, CH$_2$N$\underline{H}$C=S), 7.78 (3H, br s, N$\underline{H}_3^+$), 3.38 (2H, br s, C$\underline{H}_2$NHC=S), 3.01 (5H, C$\underline{H}_3$NHC=S and C$\underline{H}_2$NH$_3^+$), 2.22 (6H, br s, 2 x C$\underline{H}_3$C=N). **^{13}C{^{1}H} NMR** (101 MHz, DMSO-d$_6$, 298 K): δ (ppm) 178.5 (C=S), 177.7 (C=S), 148.0 (CH$_3\underline{C}$=N), 147.7 (CH$_3\underline{C}$=N), 46.2 ($\underline{C}$H$_2$), 40.3 ($\underline{C}$H$_2$), 31.2 ($\underline{C}$H$_3$NHC=S), 11.7 ($\underline{C}$H$_3$C=N), 11.7 ($\underline{C}$H$_3$C=N). **Reverse-phase HPLC**: R_t 7.28 min. Method: 0.9 mL/min (MeCN/H$_2$O with 0.1%TFA): start 30% MeCN, hold until 1 min, gradient to 95% MeCN at 15 min, hold until 17 min. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_9H_{19}N_7S_2$ [M+H]$^+$ 290.12161, found 290.12136.

Compound 7, H$_2$ATSM/en-ArN$_3$

4-Azidobenzoic acid (16 mg; 0.1 mmol; 1.3 equiv) and BOP (41 mg; 0.1 mmol; 1.3 equiv) were added to a solution of H$_2$ATSM/en, compound **6** (21.3 mg; 0.074 mmol) in anh DMF (3 mL), followed by the addition of DIPEA (20 µL; 0.14 mmol; 2 equiv) in the dark and the reaction mixture was stirred at rt for 12 h. After this time, H$_2$O (10 mL) was added and the resulting precipitate was filtered, washed with H$_2$O (3 × 5 mL), EtOH (3 mL) and Et$_2$O (2 × 5mL), and dried under high-vacuum to furnish H$_2$ATSM/en-ArN$_3$, compound **7** (23 mg;

56% yield) as an off-white solid. **^{1}H NMR** (500 MHz, DMSO-d$_6$, 298 K): δ (ppm) 10.30 (1H, br s, $N\underline{H}C=N$), 10.22 (1H, br s, $N\underline{H}C=N$), 8.71 (1H, t, $^3J_{HH}$= 5.0 Hz, $CH_2N\underline{H}C=S$), 8.59 (1H, t, $^3J_{HH}$ = 5.0 Hz, $N\underline{H}C=O$), 8.38 (1H, q, $^3J_{HH}$ = 4.5 Hz, $CH_3N\underline{H}C=S$), 7.90 (2H, d, 8.5 Hz, Ar-CH) 7.20 (2H, d, 8.5 Hz, Ar-CH), 3.75 (2H, q, $^3J_{HH}$ = 6.5 Hz, $C\underline{H}_2NHC=S$) 3.52 (2H, q, $^3J_{HH}$ = 6.5 Hz, $C\underline{H}_2NHC=S$), 3.02 (3H, d, $^3J_{HH}$ = 4.5 Hz, $C\underline{H}_3NHC=S$), 2.22 (3H, s, $C\underline{H}_3C=N$), 2.21 (3H, s, $C\underline{H}_3C=N$). **^{13}C{^{1}H} NMR** (126 MHz, DMSO-d$_6$, 298 K): δ (ppm) 178.5 (C=S), 178.1 (C=S), 166.0 (C=O), 148.4 (C=N), 148.0 (C=N), 142.3 (Ar-C$_q$), 131.2 (Ar-CH), 130.7 (Ar-C$_q$), 129.2 (Ar-CH), 119.2 (Ar-CH), 118.9 (Ar-CH), 44.3 ($\underline{C}H_2$), 38.7 ($\underline{C}H_2$), 31.2 ($\underline{C}H_3NH$), 11.8 ($\underline{C}H_3C=N$), 11.6 ($\underline{C}H_3C=N$). **Reverse-phase HPLC**: R_t 9.62 min. Method: 0.9 mL/min (MeCN/H_2O with 0.1%TFA): start 30% MeCN, hold until 1 min, gradient to 95% MeCN at 15 min, hold until 17 min. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{16}H_{22}N_{10}OS_2$ $[M+H]^+$ 457.13090, found 457.13103.



Scheme S1. Synthesis of ATSM-PEG$_3$-ArN$_3$, compound **8**. *Reagents and conditions:* a) Boc$_2$O, CH_2Cl_2, rt, 16 h (70% yield); b) CS_2, Et_3N, methyl iodide, EtOH, rt, 4 h (84% yield); c) $H_2NNH_2{\cdot}H_2O$, EtOH, reflux, 3 h (96% yield); d) 3 M aq HCl, EtOH, reflux, 5 h (32% yield); f) TFA, CH_2Cl_2, rt, 2 h (80% yield); g) 4-azidobenzoic acid, HATU, DIPEA, DMF, rt, 16 h (20% yield).

Compound 9

4,7,10-Trioxa-1,13-tridecanediamine (6.06 g; 26.7 mmol; 1.6 equiv) was dissolved in CH_2Cl_2 (240 mL) to which a prepared solution of di-*tert*-butyl dicarbonate (3.80 g; 16.9 mmol; 1 equiv) in CH_2Cl_2 (60 mL) was added dropwise over 3 h. The reaction mixture was then stirred at rt for 16 h. After this time, the reaction mixture was washed with sat aq $NaHCO_3$ (3 × 100 mL) and concentrated under reduced pressure to afford compound **9** (3.78 g; 70% yield) as a colourless oil. **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K): δ (ppm) 3.50 – 3.67 (9H, m, $C\underline{H}_2$), 3.22 (2H, q, $^3J_{HH}$ = 6.4 Hz, $C\underline{H}_2$), 2.83 (1H, t, $^3J_{HH}$ = 6.6 Hz, $C\underline{H}_2$), 1.94 (1H, s, $C\underline{H}_2$), 1.75 (3H, p, $^3J_{HH}$ = 6.3 Hz, $C\underline{H}_2$), 1.43 (9H, s, $C(C\underline{H}_3)_3$). **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$, 298 K): δ (ppm) 156.2 (C=O), 79.1 ($\underline{C}(CH_3)_3$) 70.7 ($\underline{C}H_2$), 70.7 ($\underline{C}H_2$), 70.7 ($\underline{C}H_2$), 70.4 ($\underline{C}H_2$), 70.3 ($\underline{C}H_2$), 70.3 ($\underline{C}H_2$), 69.7 ($\underline{C}H_2$), 69.6 ($\underline{C}H_2$), 39.8 ($\underline{C}H_2$), 29.8 ($\underline{C}H_2$), 28.6 ($C(\underline{C}H_3)_3$). **HR-ESI-MS** (ESI+):

m/z calcd. for $C_{15}H_{32}N_2O_5$ $[M+H]^+$ 321.2384, found 321.2386.

Compound 10

To a solution of compound **9** (3.6 g; 11.2 mmol;1 equiv) in EtOH (100 mL) was added CS_2 (0.8 mL;13 mmol; 1.2 equiv) dropwise, followed by Et_3N (1.9 mL; 13.4 mmol; 1.2 equiv) and the reaction stirred at rt. After 1.5 h, methyl iodide (1.9 mL, 13.4 mmol; 1.2 equiv) was added and stirred for a further 2 h. Then, the reaction mixture was concentrated under reduced pressure and the crude residue was suspended in EtOAc (100 mL) and washed with 1 M aq HCl (100 mL), sat aq $NaHCO_3$ solution (100 mL) and H_2O (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure to furnish compound **10** (3.80 g; 84% yield) as a pale-yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K): δ (ppm) 8.52 – 8.09 (1H, m, $CH_2N\underline{H}C{=}S$), 4.94 (1H, s, $N\underline{H}CO_2C(CH_3)_3$), 3.85 (1H, q, $^3J_{HH}$ = 5.6 Hz, $C\underline{H}_2$), 3.69 – 3.56 (7H, m, $C\underline{H}_2$), 3.53 (2H, t, $^3J_{HH}$ = 6.0 Hz, $C\underline{H}_2$), 3.21 (2H, t, $^3J_{HH}$ = 6.4 Hz, $C\underline{H}_2$), 2.59 (1H, s, $SC\underline{H}_3$), 1.92 (1H, p, $^3J_{HH}$ = 5.7 Hz, $C\underline{H}_2$-βN), 1.74 (2H, td, $^3J_{HH}$ = 6.3, 2.3 Hz, $C\underline{H}_2$-βN), 1.43 (9H, s, $C(C\underline{H}_3)_3$). **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$, 298 K): δ (ppm) 198.2 (C=S), 156.2 (C=O), 79.1 ($\underline{C}(CH_3)_3$), 70.7 ($\underline{C}H_2$), 70.7 ($\underline{C}H_2$), 70.5 ($\underline{C}H_2$), 70.5 ($\underline{C}H_2$), 70.4 ($\underline{C}H_2$), 69.7 ($\underline{C}H_2$), 46.8 ($\underline{C}H_2$-αNHC=S), 38.8 ($\underline{C}H_2$-αNHC=O), 29.8 ($\underline{C}H_2$-βN), 28.6 ($C(\underline{C}H_3)_3$), 27.7 ($\underline{C}H_2$-βN), 18.0 ($S\underline{C}H_3$). **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{17}H_{34}N_2O_5S_2$ $[M+H]^+$ 411.1982, found 411.1974; $[M+Na]^+$ 433.1801, found 433.1792.

Compound 11

To a solution of compound **10** (3.8 g; 9.25 mmol; 1 equiv) in EtOH (100 mL) was added hydrazine hydrate (1.90 mL; 13.9 mmol; 1.5 equiv) and the mixture was stirred under reflux for 3 h. After this time, the solvent was removed under reduced pressure and the crude residue was dissolved in $CHCl_3$ (20 mL). This solution was loaded on a plug of silica, washed with $CHCl_3$ (20 mL) and the product was eluted with MeOH (30 mL). The MeOH fraction was then concentrated *in vacuo* to afford compound **11** (3.5 g; 96% yield) as a pale-yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K): δ (ppm) 7.88 (1H, s, $CH_2N\underline{H}C{=}S$), 7.21 (1H, s, $NHC{=}SN\underline{H}NH_2$), 4.99 (1H, s, $N\underline{H}CO_2C(CH_3)_3$), 3.89 (1H, s, $NHC{=}SNHN\underline{H}_2$), 3.75 (1H, q, $^3J_{HH}$ = 5.9 Hz, $C\underline{H}_2$-αN), 3.62 (7H, ddt, $^3J_{HH}$ = 15.9, 10.3, 4.2 Hz, $C\underline{H}_2$), 3.53 (2H, q, $^3J_{HH}$ = 6.0 Hz, $C\underline{H}_2$), 3.21 (2H, p, $^3J_{HH}$ = 6.4 Hz, $C\underline{H}_2$), 1.89 (1H, p, $^3J_{HH}$ = 5.9 Hz, $C\underline{H}_2$-βN), 1.75 (2H, p, $^3J_{HH}$ = 6.5 Hz, $C\underline{H}_2$-βN), 1.43 (9H, s, $C(C\underline{H}_3)_3$). **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{16}H_{34}N_4O_5S$ $[M+H]^+$ 395.2323, found 395.2325.

Compound 12

To a suspension of compound **11** (1.70 g; 4.3 mmol; 1 equiv) was suspended in EtOH (50 mL) at 50 °C was added compound **4** (0.75 g; 4.3 mmol; 1 equiv) was added portions-wise over 1 h. After this time, the reaction mixture was acidified with 3 M aq HCl and stirred under reflux for 4 h. After this time, the reaction mixture was allowed to cool to rt and was poured into cold water (100 mL) to produce a white precipitate. The precipitate was then collected by vacuum filtration, washed with water (3 × 50 mL) and Et_2O (3 × 50 mL), and dried under high vacuum to furnish compound **12** (0.76 g; 32% yield) as a white solid. **^{1}H NMR** (400 MHz, DMSO-d_6, 298 K): δ = 10.18 (1H, s, $N\underline{H}N{=}C$), 8.44 – 8.34 (2H, m, $CH_2N\underline{H}C{=}S$, $CH_3N\underline{H}C{=}S$), 6.73 (1H, t, $^3J_{HH}$ = 5.6 Hz, $N\underline{H}CO_2C(CH_3)_3$), 3.62 (2H, q, $^3J_{HH}$ = 6.7 Hz, $C\underline{H}_2$-αN), 3.53 – 3.43 (10H, m, $C\underline{H}_2$), 3.39 – 3.31 (6H, m, $C\underline{H}_2$), 3.02 (3H, d, $^3J_{HH}$ = 4.6 Hz, $C\underline{H}_3NHC{=}S$), 2.95 (2H, q, $^3J_{HH}$ = 6.6 Hz, $C\underline{H}_2$), 2.20, 2.19 (6H, 2 x s, 2 x $C\underline{H}_3C{=}N$), 1.82 (2H, p, $^3J_{HH}$ = 6.5 Hz, $C\underline{H}_2$-βN), 1.58 (2H, p, $^3J_{HH}$ = 6.6 Hz, $C\underline{H}_2$-βN), 1.37 (9H, s, $C(C\underline{H}_3)_3$) ppm. **^{13}C{^{1}H} NMR** (101 MHz, DMSO-d_6, 298 K): δ = 178.47 (C=S), 177.7 (C=S), 155.6 (C=O), 148.0 ($CH_3\underline{C}{=}N$), 147.8 ($CH_3\underline{C}{=}N$), 77.4 ($\underline{C}(CH_3)_3$), 69.8 ($\underline{C}H_2$), 69.8 ($\underline{C}H_2$), 69.6 ($\underline{C}H_2$), 69.5 ($\underline{C}H_2$), 68.6 ($\underline{C}H_2$), 68.1 ($\underline{C}H_2$), 41.7 ($\underline{C}H_2$-αNHC=S), 37.2 ($\underline{C}H_2$-αNHC=O), 31.2 ($S\underline{C}H_3$), 29.7 ($\underline{C}H_2$-βN), 28.9 ($\underline{C}H_2$-βN), 28.3 ($C(\underline{C}H_3)_3$), 11.7 ($\underline{C}H_3C{=}N$), 11.6 ($\underline{C}H_3C{=}N$) ppm. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{22}H_{43}N_7O_5S_2$ $[M+H]^+$ 550.2840, found 550.2846.

Compound 13

To a solution of compound **12** in CH_2Cl_2 (20 mL) was added TFA (5 mL) and the reaction mixture was stirred at rt for 2 h. After this time, the solvent was removed under reduced pressure and the crude residue was co-evaporated several times with cyclohexane to afford the TFA salt of compound **13** (0.54 g; 80% yield) as a white solid. **^{1}H NMR** (400 MHz, DMSO-d_6, 298 K): δ (ppm) 8.38 (2H, s, $CH_2N\underline{H}C{=}S$, $CH_3N\underline{H}C{=}S$), 6.68 (3H, s, NH_2, $N\underline{H}N{=}C$), 3.66 – 3.57 (2H, m, $C\underline{H}_2$-αN), 3.53 – 3.38 (12H, m, $C\underline{H}_2$), 3.02 (3H, d, $^3J_{HH}$ = 4.0 Hz, $C\underline{H}_3NHC{=}S$), 2.56 (2H, t, $^3J_{HH}$ = 6.8 Hz, $C\underline{H}_2$), 2.20 (6H, d, $^3J_{HH}$ = 4.6 Hz, 2 x $C\underline{H}_3C{=}N$), 1.82 (2H, p, $^3J_{HH}$ = 6.6 Hz, $C\underline{H}_2$-βN), 1.55 (2H, p, $^3J_{HH}$ = 6.5 Hz, $C\underline{H}_2$-βN). **^{13}C{^{1}H} NMR** (101 MHz, DMSO-d_6, 298 K): δ (ppm) 178.5 (C=S), 177.7 (C=S), 148.0 ($CH_3\underline{C}{=}N$), 147.8 ($CH_3\underline{C}{=}N$), 69.8 ($\underline{C}H_2$), 69.7 ($\underline{C}H_2$), 69.6 ($\underline{C}H_2$), 69.5 ($\underline{C}H_2$), 68.6 ($\underline{C}H_2$), 68.4 ($\underline{C}H_2$), 41.7 ($\underline{C}H_2$-αNHC=S), 38.7 ($\underline{C}H_2$-αNHC=O), 33.2 ($\underline{C}H_2$-βN), 31.2 ($S\underline{C}H_3$), 30.7 ($\underline{C}H_2$-βN), 28.8 (impurity – C_q), 11.7

($\underline{C}H_3C$=N), 11.6 ($\underline{C}H_3C$=N) ppm. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{17}H_{35}N_7O_3S_2$ $[M+H]^+$ 450.2316, found 450.2321.

Compound 8, H_2ATSM-PEG$_3$-ArN$_3$

A solution of 4-azidobenzoic acid (54 mg; 0.33 mmol: 1.5 equiv) and HATU (127 mg; 0.33 mmol; 1.5 equiv) in anh DMF (4 mL) were stirred under an inert atmosphere at rt for 20 min. A solution of compound **13** (100 mg; 0.22 mmol; 1 equiv) in anh DMF (1 mL) was then added and the mixture was stirred for a further 10 min. Then, DIPEA (155 µL; 0.88 mmol; 4 equiv) was added and the reaction mixture stirred at rt for 16 h. After this time, the reaction mixture was concentrated under reduced pressure and the crude residue was washed with H_2O (3 × 25 mL), taken up in and concentrated under reduced pressure. The crude residue was then purified by reversed-phase flash chromatography (C18, 0 to 100% MeOH/H_2O with 0.1% TFA) to afford compound **8** as a yellow solid (26 mg; 20% yield). **^{1}H NMR** (400 MHz, MeOD, 298 K): δ = 7.84 (2H, d, $^3J_{HH}$ = 8.7 Hz, Ar-C$\underline{H}$), 7.13 (2H, d, $^3J_{HH}$ = 8.6 Hz, Ar-C$\underline{H}$), 3.76 (2H, t, $^3J_{HH}$ = 6.6 Hz, C$\underline{H}_2$-αNHC=S), 3.65 – 3.55 (12H, m, 6 x C$\underline{H}_2$), 3.46 (2H, t, $^3J_{HH}$ = 6.8 Hz, C$\underline{H}_2$-αNHC=O), 3.35 (s, MeOH), 3.15 (3H, s, SC$\underline{H}_3$), 2.19 (6H, d, $^3J_{HH}$ = 3.2 Hz, 2 x C$\underline{H}_3$C=N), 1.90 (4H, m, 2 x C$\underline{H}_2$-βN) ppm. **^{13}C{^{1}H} NMR** (101 MHz, MeOD, 298 K): δ = 169.0 (C=O), 148.8 (C=S), 148.6 (C=S), 144.8 (Ar-C$_q$), 132.3 (Ar-C$_q$), 130.2 (Ar-CH), 120.0 (Ar-CH), 71.6 ($\underline{C}H_2$), 71.5 ($\underline{C}H_2$), 71.3 ($\underline{C}H_2$), 71.3 ($\underline{C}H_2$), 70.5 ($\underline{C}H_2$), 70.3 ($\underline{C}H_2$), 49.9 (MeOH), 43.5 ($\underline{C}H_2$-αNHC=S), 38.8 ($\underline{C}H_2$-αNHC=O), 31.2 (S$\underline{C}H_3$), 30.4 ($\underline{C}H_2$-βN), 30.0 ($\underline{C}H_2$-βN), 11.1 ($\underline{C}H_3C$=N), 11.0 ($\underline{C}H_3C$=N) ppm. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{24}H_{39}N_{10}O_4S_2$ $[M+H]^+$ 595.25917, found 595.25933.

Metalation of compounds 6, 7 and 8

Zn-6

To a stirred suspension of ATSM/en, compound **6** (34.6 mg; 0.12 mmol; 1 equiv) in MeOH (3.5 mL) was added $Zn(AcO)_2 \cdot 2H_2O$ (31.6 mg; 0.144 mmol, 1.2 equiv) and the reaction mixture was stirred for 1 h, resulting in a yellow solution. The reaction mixture was diluted with H_2O, and Et_3N (~150 µL; 3 drops) was added producing a dark yellow precipitate. The suspension was filtered, washed with H_2O (3 × 5 mL), Et_2O (2 × 3 mL) and dried under high-vacuum to afford Zn-**6** (30.7 mg; 73% yield) as a yellow solid. **Reverse-phase HPLC**: *R*$_t$ 7.28 min. Method: 0.9 mL/min (MeCN/H_2O with 0.1% TFA): start 30% MeCN, hold until 1 min, gradient to 95% MeCN at 15 min, hold until 17 min. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_9H_{17}N_7S_2Zn$ $[M+H]^+$ 352.03511, found 352.03511.

Cu-6

To a stirred suspension of ATSM/en, compound **6** (16.6 mg; 0.057 mmol; 1 equiv) in MeOH (1.5 mL) was added $Cu(OAc)_2$ (11.3 mg; 0.062 mmol) and the reaction mixture was stirred for 1 h, resulting in a dark brown solution. The reaction mixture was diluted with H_2O, and Et_3N (~100 µL; 2 drops) was added producing a dark brown precipitate. The suspension was filtered, washed with H_2O (3 × 5 mL), Et_2O (2 × 3 mL) and dried under high-vacuum to furnish Cu-**6** (17.0 mg; 85% yield) as a dark brown solid. **Reverse-phase HPLC**: R_t 5.44 min. Method: 0.9 mL/min (MeCN/H_2O with 0.1%TFA): start 30% MeCN, hold until 1 min, gradient to 95% MeCN at 15 min, hold until 17 min. **HR-ESI-MS** (ESI+): *m/z* calc. for $C_9H_{17}N_7S_2Cu$ $[M+H]^+$ 351.03556, found 351.03526.

Zn-7

To a stirred suspension of H_2ATSM/en-ArN_3, compound **7** (26.7 mg; 0.0614 mmol; 1 equiv) in MeOH (6 mL) was added $Zn(OAc)_2{\cdot}2H_2O$ (33.7 mg; 0.154 mmol; 2.5 equiv) and the reaction mixture was stirred for 16 h under reflux, resulting in a yellow solution. The reaction mixture was diluted with H_2O (4.5 mL) and the organic solvent was removed under reduced pressure. The remaining aqueous solution was extracted with EtOAc (3 × 15 mL) and the combined organic fractions were dried over $MgSO_4$, concentrated under reduced pressure and dried under high-vacuum to furnish Zn-**7** (23.2 mg; 76% yield) as a yellow solid. **UV/vis**: λ_{max} = 430 nm. **Reverse-phase HPLC**: R_t 9.62 min. Method: 0.9 mL/min (MeCN/H_2O 0.1%TFA): start 30% MeCN, hold until 1 min, gradient to 95% MeCN at 15 min, hold until 17 min. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{16}H_{20}N_{10}OS_2Zn$ $[M+H]^+$ 497.06272, found 497.06261.

Cu-7

To a solution of H_2ATSM/en-ArN_3, compound **7** (3.2 mg; 7.40 µmol; 1 equiv) in DMF (0.2 mL) was added $Cu(AcO)_2$ (1.6 mg; 8.50 µmol; 1.2 equiv) and the mixture was stirred at rt for 1 h, resulting in a dark red/brown solution. The reaction mixture was diluted with H_2O (5 mL), affording a dark red precipitate. The solid was collected by vacuum filtration, washed with H_2O (3 × 5 mL), Et_2O (2 × 3 mL) and concentrated under reduced pressure to furnish Cu-**7** (2.75 mg; 75% yield) as a red/brown solid. **UV/vis**: λ_{max} = 476 nm. **Reverse-phase HPLC**: R_t 8.32 min. Method: 0.9 mL/min (MeCN/H_2O 0.1%TFA): start 30% MeCN, hold until 1 min, gradient to 95% MeCN at 15 min, hold until 17 min. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{16}H_{20}N_{10}OS_2Cu$ $[M+Na]^+$ 518.04512, found 518.04445.

Zn-8

To a stirred suspension of H_2ATSM-PEG$_3$-ArN$_3$, compound **8** (3.0 mg; 0.005 mmol; 1 equiv) in MeOH (1 mL) was added $Zn(OAc)_2{\cdot}2H_2O$ (2.8 mg; 0.013 mmol; 2.5 equiv) and the reaction mixture was stirred for 16 h under reflux, resulting in a yellow solution. The reaction mixture was diluted with H_2O (1 mL) and the organic solvent was removed under reduced pressure. The remaining aqueous solution was extracted with EtOAc (3 × 5 mL) and the combined organic fractions were dried over $MgSO_4$, concentrated under reduced pressure and dried under high-vacuum to furnish Zn-**7** (3.2 mg; 97% yield) as a yellow solid. **UV/vis**: λ_{max} = 430 nm. **Reverse-phase HPLC**: R_t 11.89 min. Method: 0.9 mL/min (MeCN/H_2O with 0.1%TFA): start 30% MeCN, hold until 1 min, gradient to 95% MeCN at 15 min, hold until 17 min. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{24}H_{36}N_{10}O_4S_2Zn$ $[M+H]^+$ 657.17288, found 657.17266.

Cu-8

To a solution of ATSM-PEG$_3$-ArN$_3$, compound **8** (3.0 mg; 0.005 mmol; 1 equiv) in DMF (1 mL) was added $Cu(AcO)_2$ (1.1 mg; 0.006 mmol; 1.2 equiv) and the mixture was stirred at rt for 1 h, resulting in a dark red/brown solution. The reaction mixture was diluted with H_2O (5 mL), affording a dark red precipitate. The solid was collected by vacuum filtration, washed with H_2O (3 × 5 mL), Et_2O (2 × 3 mL) and concentrated under reduced pressure to furnish Cu-**7** (2.75 mg; 75% yield) as a red/brown solid. **UV/vis**: λ_{max} = 476 nm. **Reverse-phase HPLC**: R_t 10.84 min. Method: 0.9 mL/min (MeCN/H_2O with 0.1%TFA): start 30% MeCN, hold until 1 min, gradient to 95% MeCN at 15 min, hold until 17 min. **HR-ESI-MS** (ESI+): *m/z* calcd. for $C_{24}H_{36}N_{10}O_4S_2Cu$ $[M+H]^+$ 656.17340, found 656.17312.

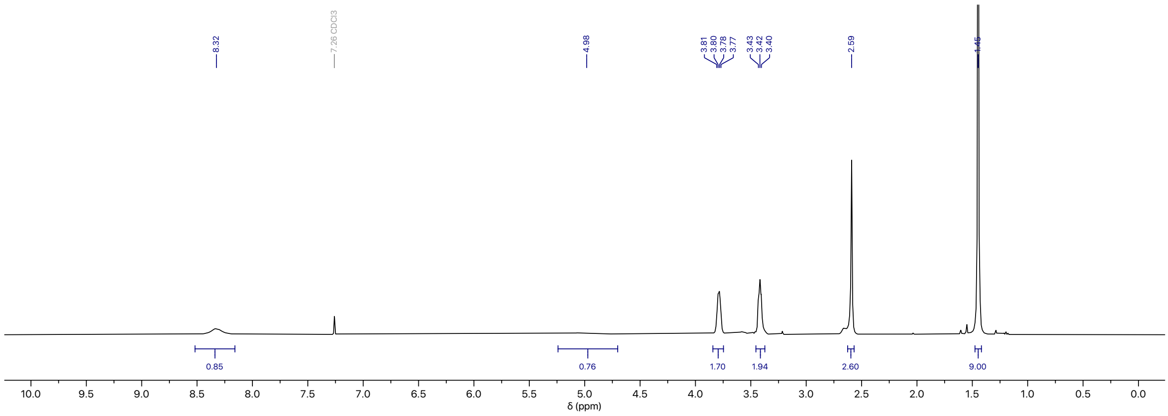


Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **2**

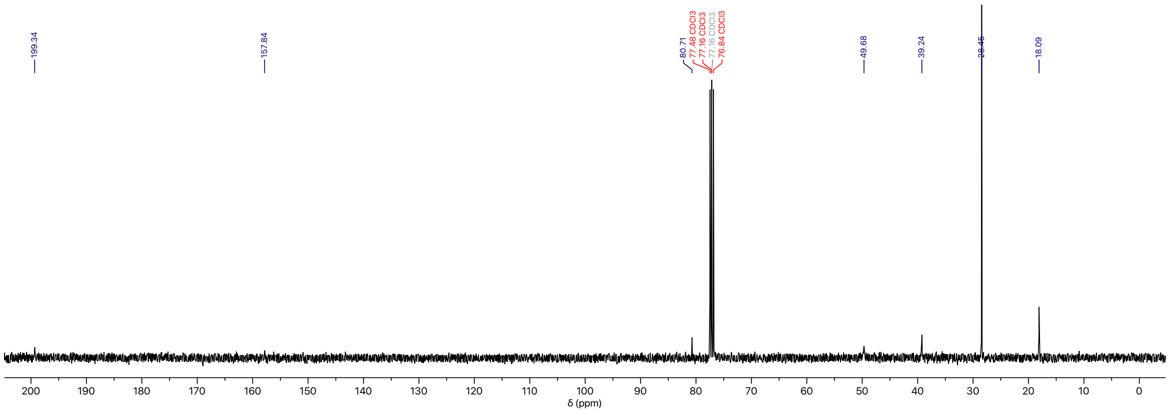


Figure S2. $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$, 298 K) spectrum of compound **2**

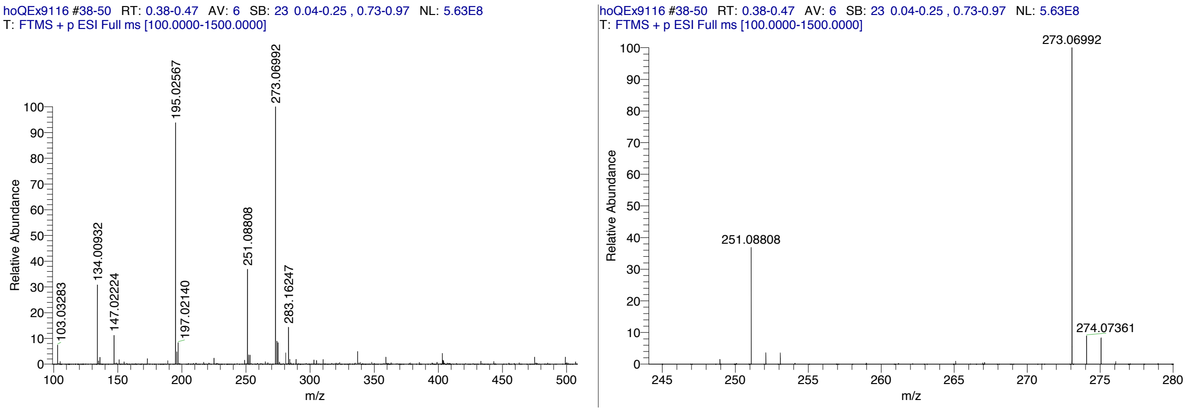


Figure S3. HR-ESI-MS spectrum of compound **2**

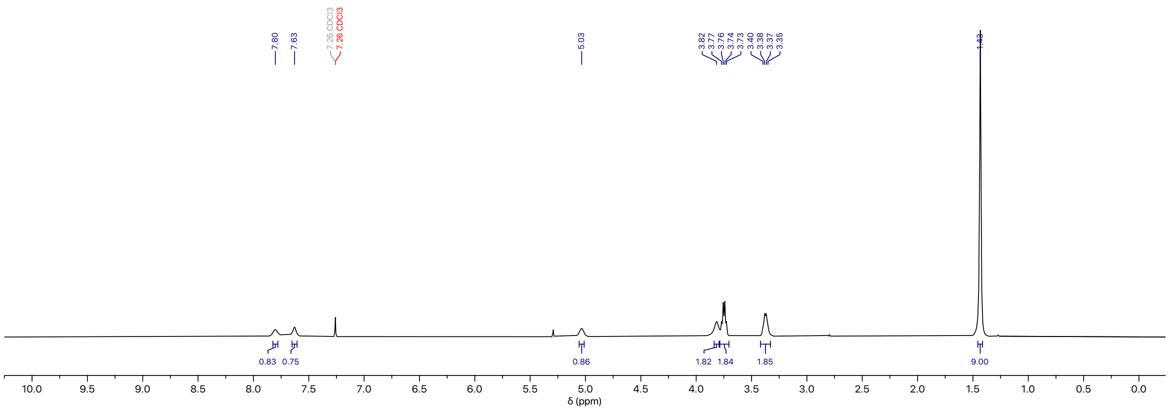


Figure S4. ^{1}H NMR (400 MHz, CDCl$_3$, 298 K) spectrum of compound **3**

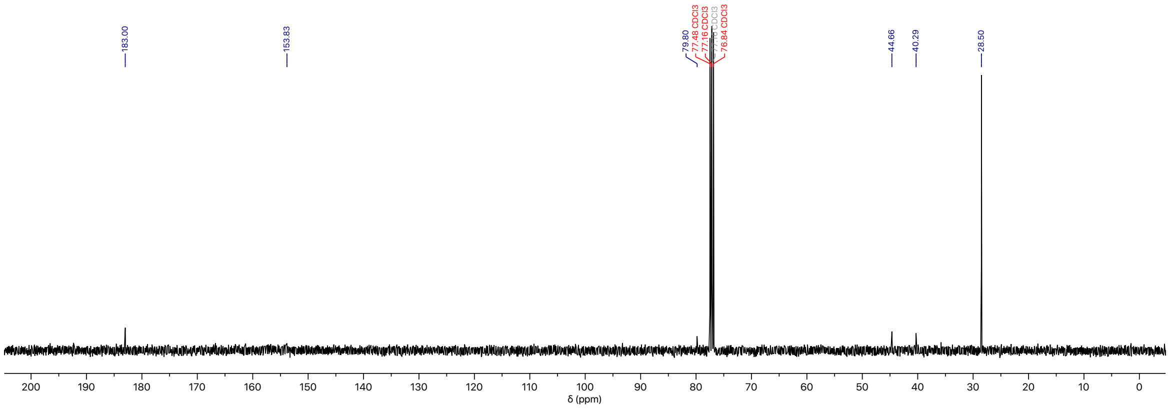


Figure S5. ^{13}C{^{1}H} NMR (101 MHz, CDCl$_3$, 298 K) spectrum of compound **3**

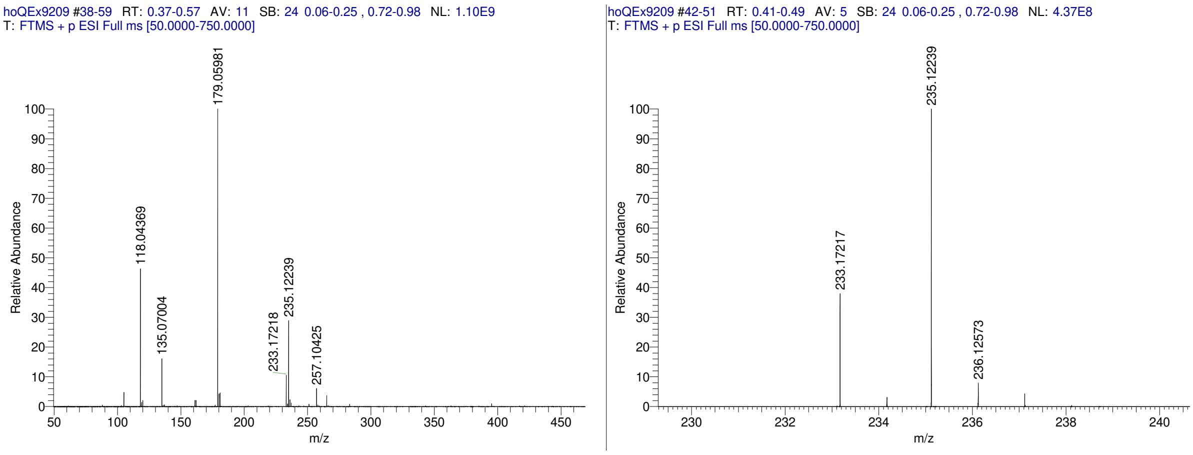


Figure S6. HR-ESI-MS spectrum of compound **3**

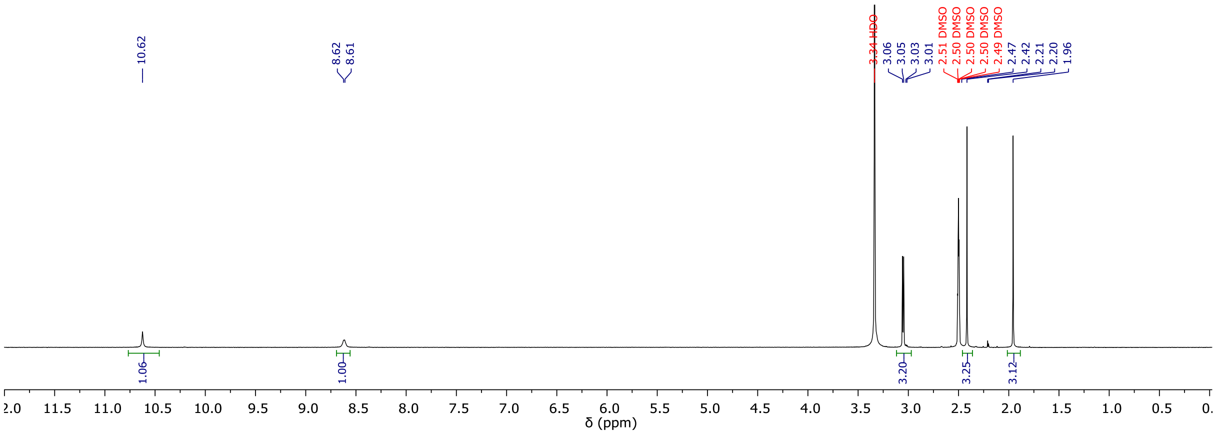


Figure S7. ^{1}H NMR (400 MHz, DMSO-d$_6$, 298 K) spectrum of compound **4**

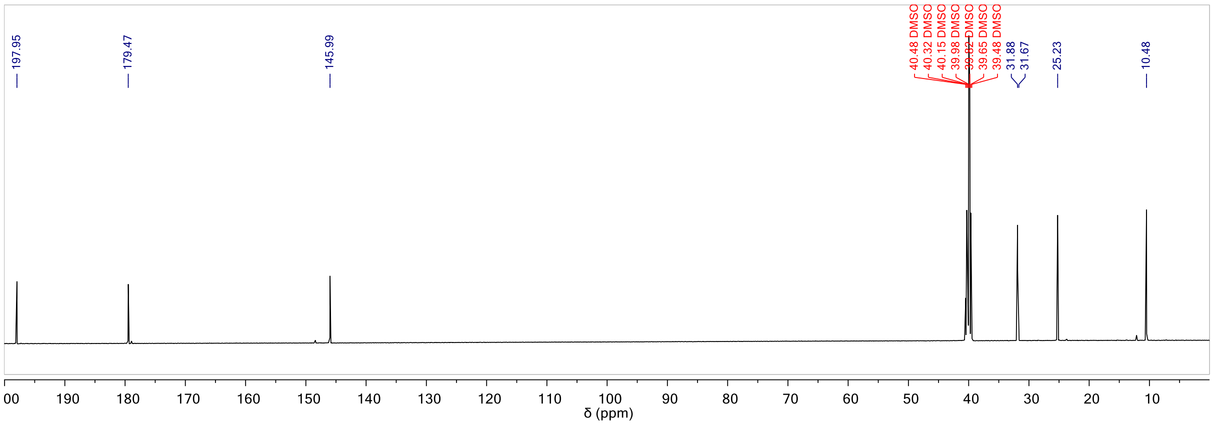


Figure S8. ^{13}C{^{1}H} NMR (101 MHz, DMSO-d$_6$, 298 K) spectrum of compound **4**

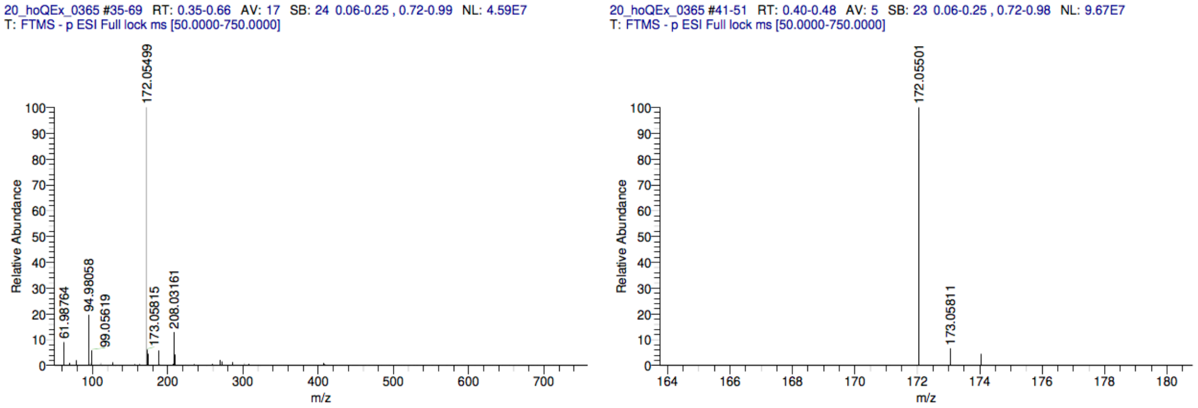


Figure S9. HR-ESI-MS spectrum of compound **4**

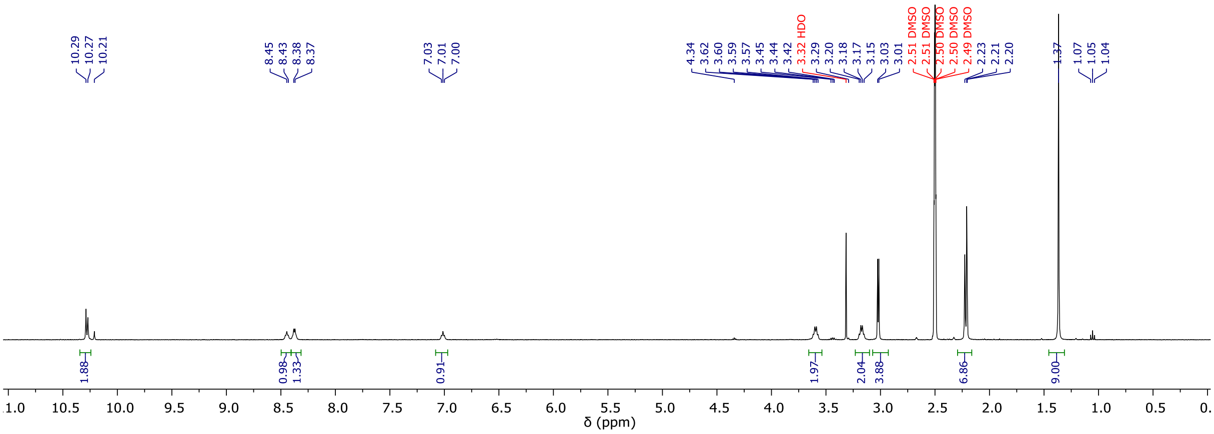


Figure S10. ^{1}H NMR (400 MHz, DMSO-d$_6$, 298 K) spectrum of compound **5**

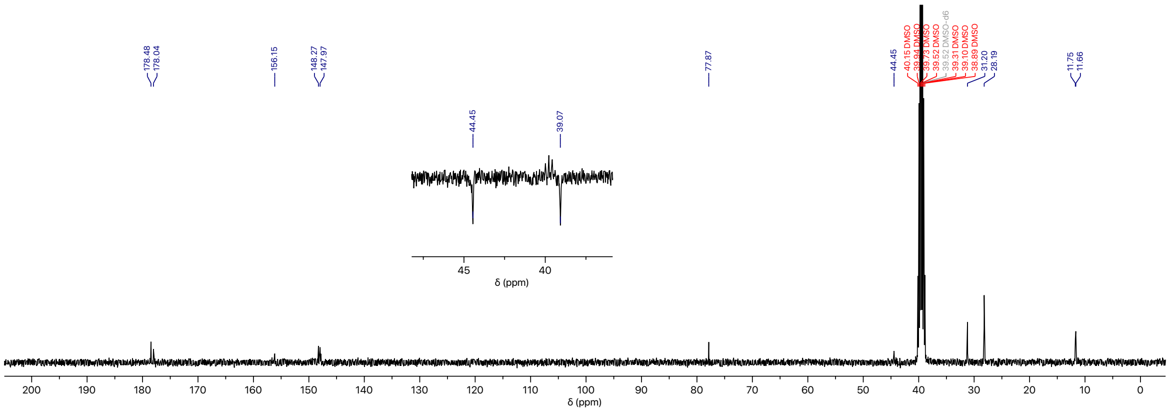


Figure S11. ^{13}C{^{1}H} NMR (101 MHz, DMSO-d$_6$, 298 K) spectrum of compound **5**

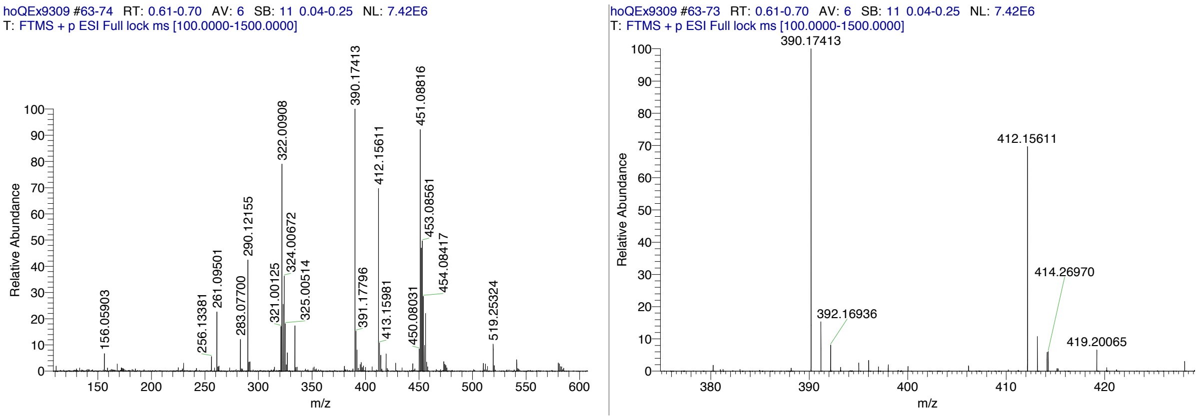


Figure S12. HR-ESI-MS spectrum of compound **5**

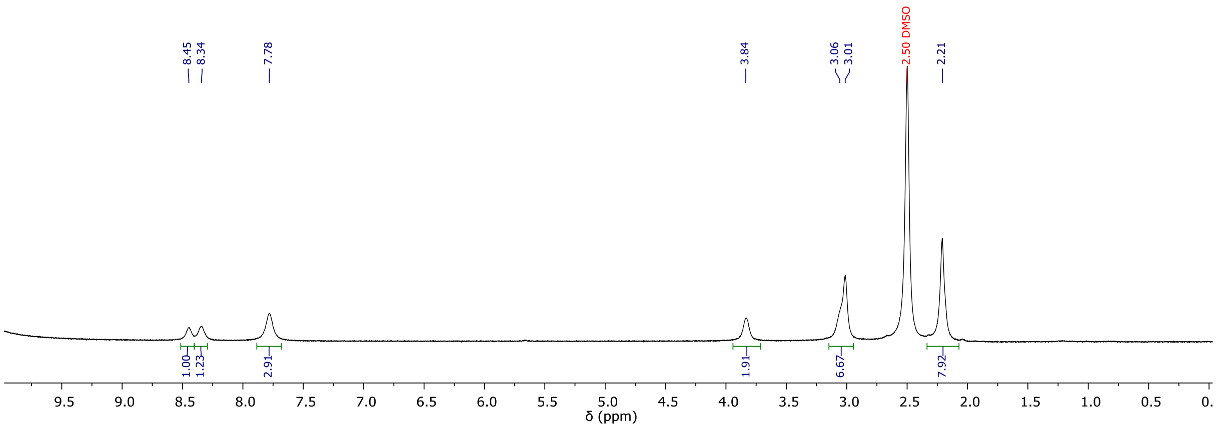


Figure S13. ^{1}H NMR (400 MHz, DMSO-d$_6$, 298 K) spectrum of compound **6**

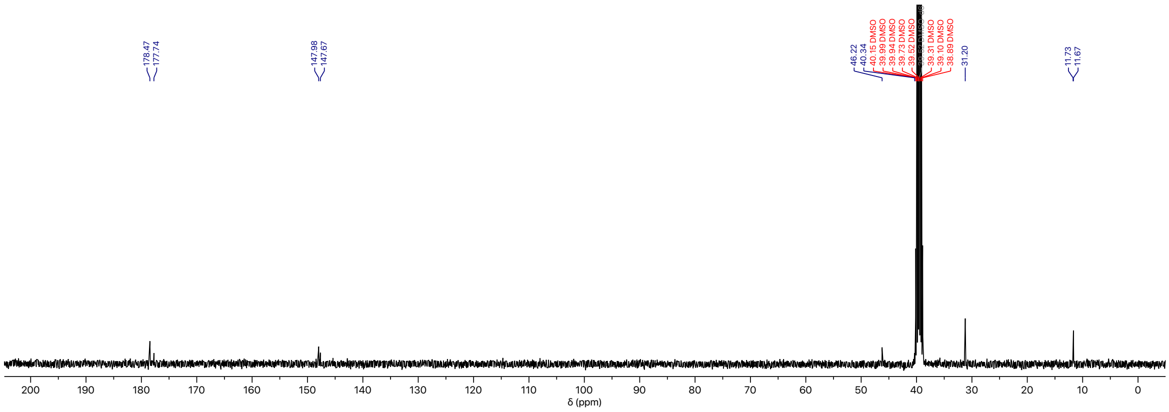


Figure S14. ^{13}C{^{1}H} NMR (101 MHz, DMSO-d$_6$, 298 K) spectrum of compound **6**

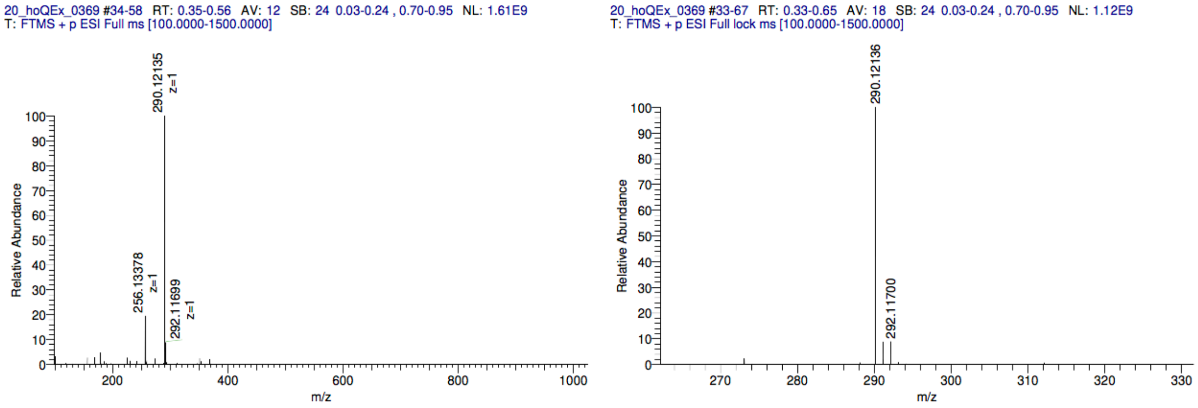


Figure S15. HR-ESI-MS spectrum of compound **6**

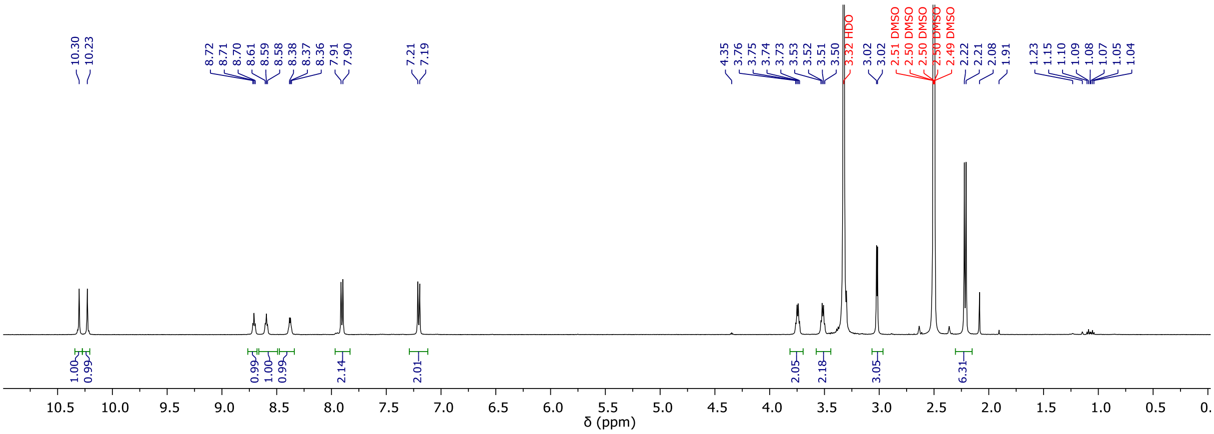


Figure S16. ^{1}H NMR (500 MHz, DMSO-d$_6$, 298 K) spectrum of compound **7**

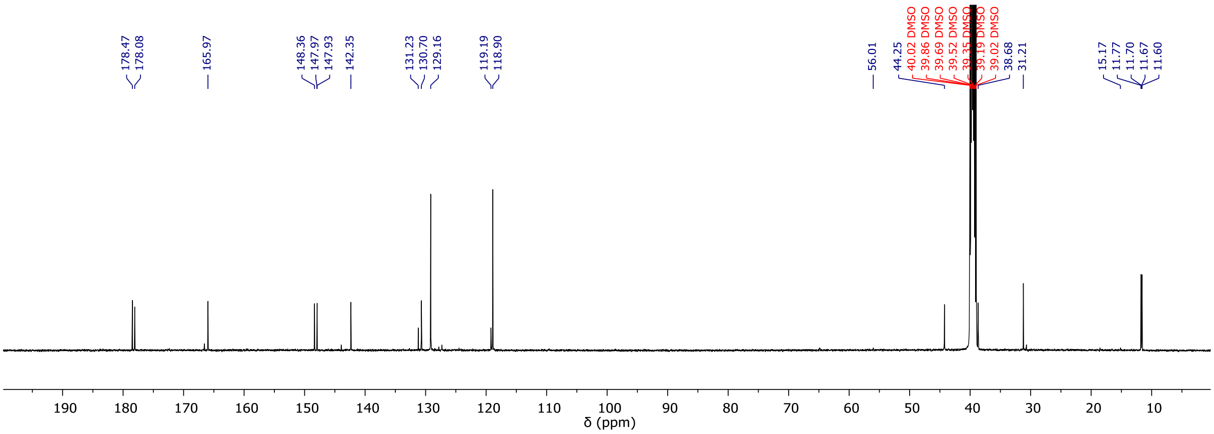


Figure S17. ^{13}C{^{1}H} NMR (126 MHz, DMSO-d$_6$, 298 K) spectrum of compound **7**

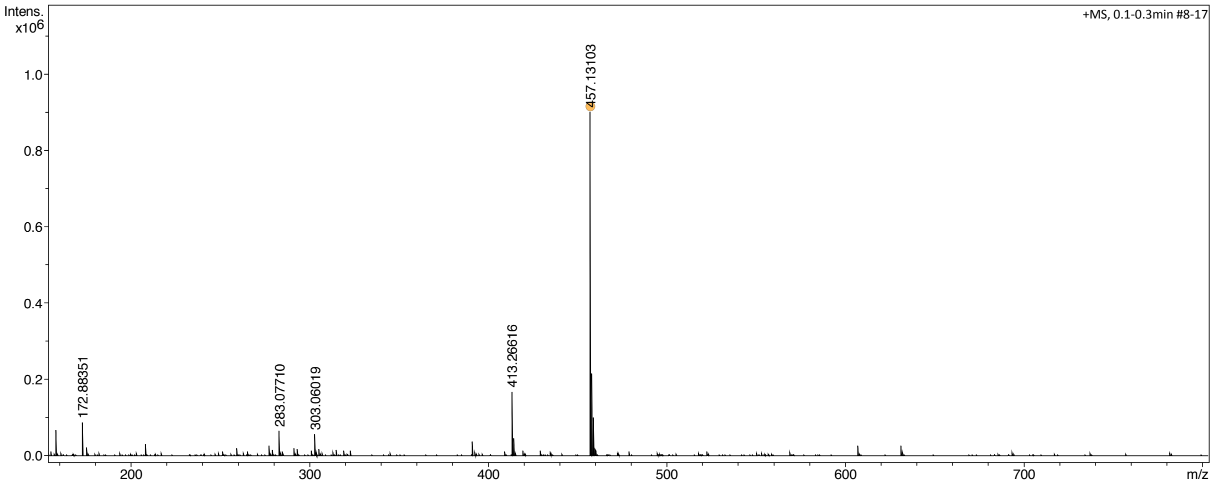


Figure S18. HR-ESI-MS spectrum of compound **7**

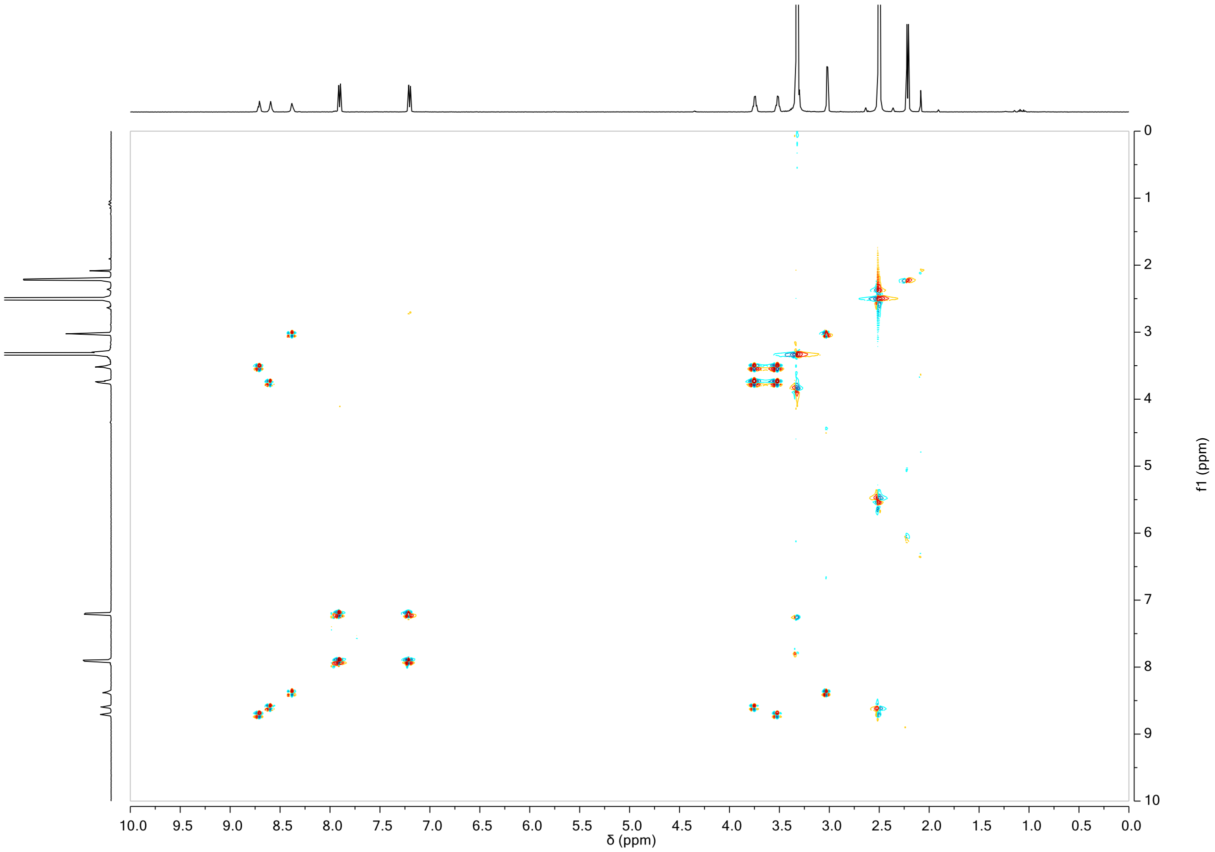


Figure S19. ^{1}H COSY NMR (400 MHz, DMSO-d$_6$, 298 K) spectrum of compound **7**

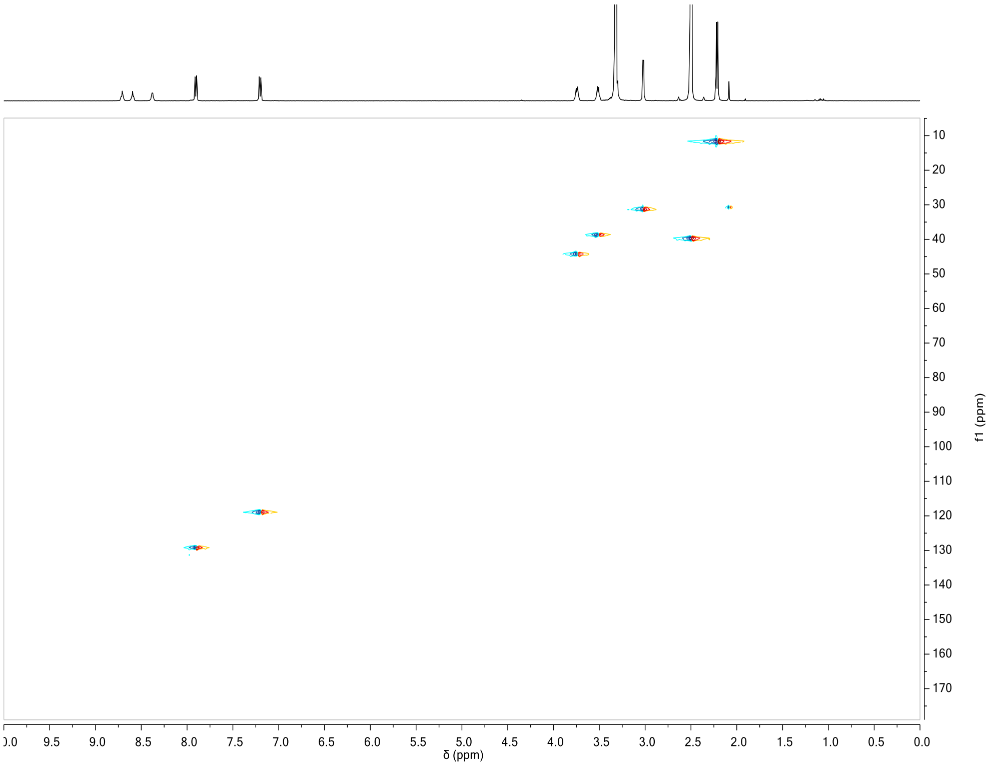


Figure S20. ^{1}H–^{13}C{^{1}H} HSQC NMR (400 MHz, DMSO-d$_6$, 298 K) spectrum of compound **7**

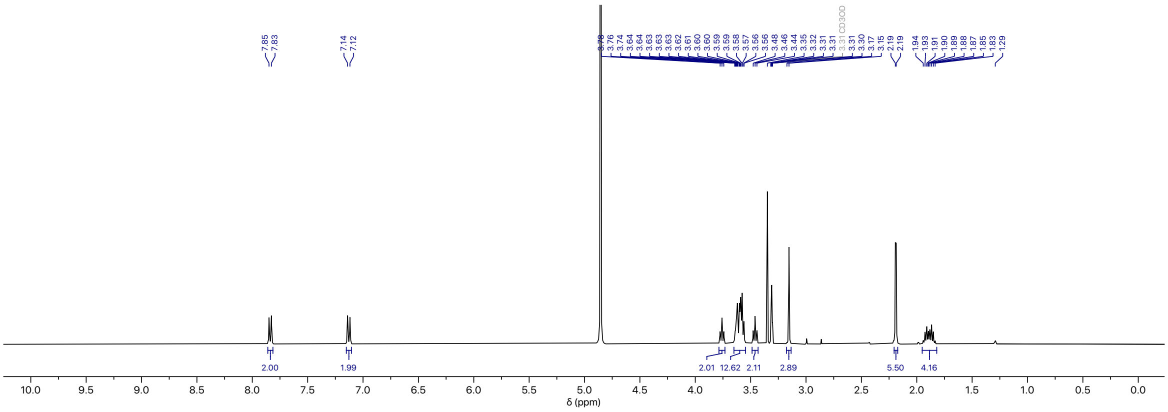


Figure S21 ^{1}H NMR (400 MHz, MeOD, 298 K) spectrum of compound **8**

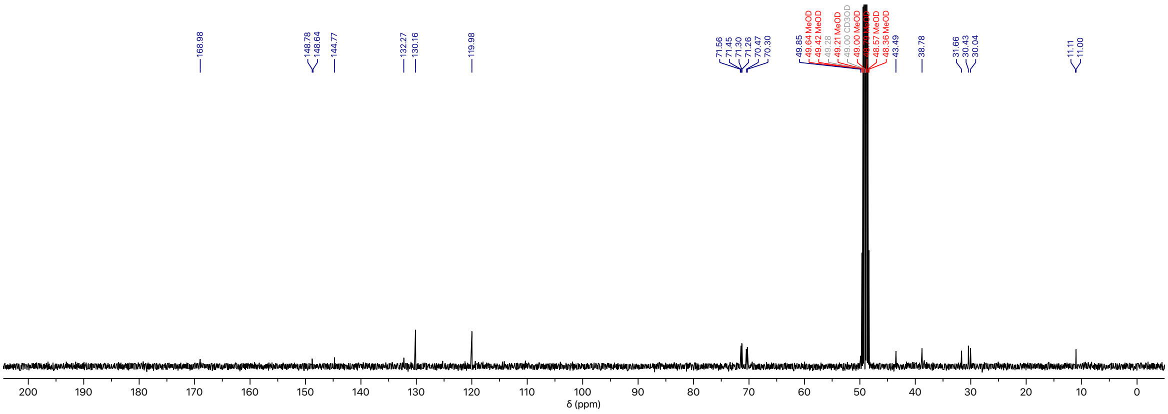


Figure S22. ^{13}C{^{1}H} NMR (101 MHz, MeOD, 298 K) spectrum of compound **8**

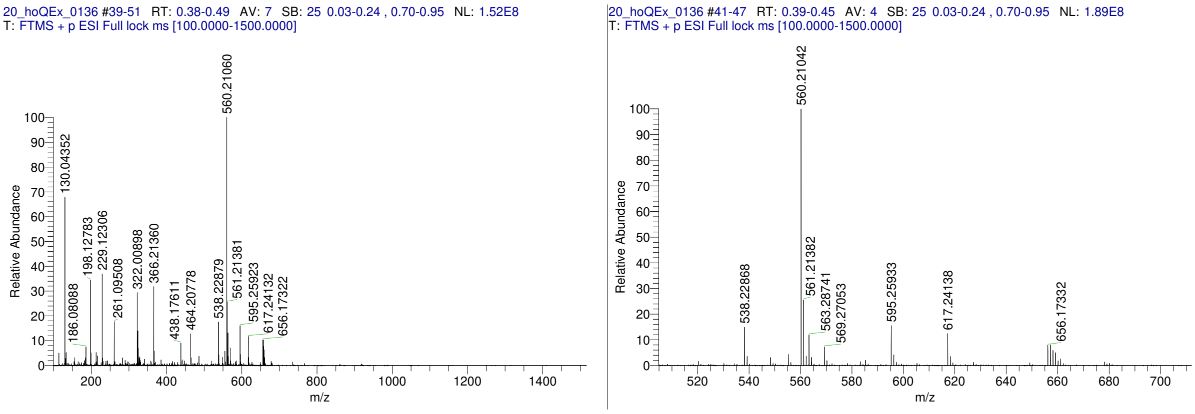


Figure S23. HR-ESI-MS spectrum of compound **8**

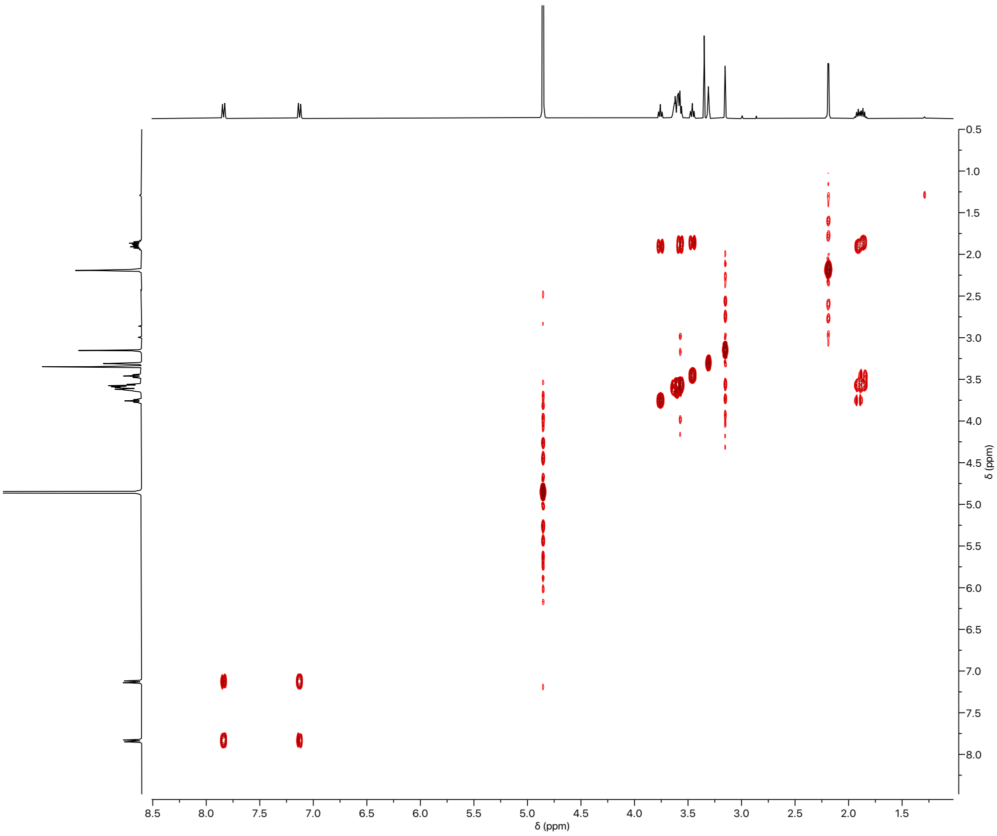


Figure S24.^{1}H COSY NMR (400 MHz, MeOD, 298 K) spectrum of compound **8**

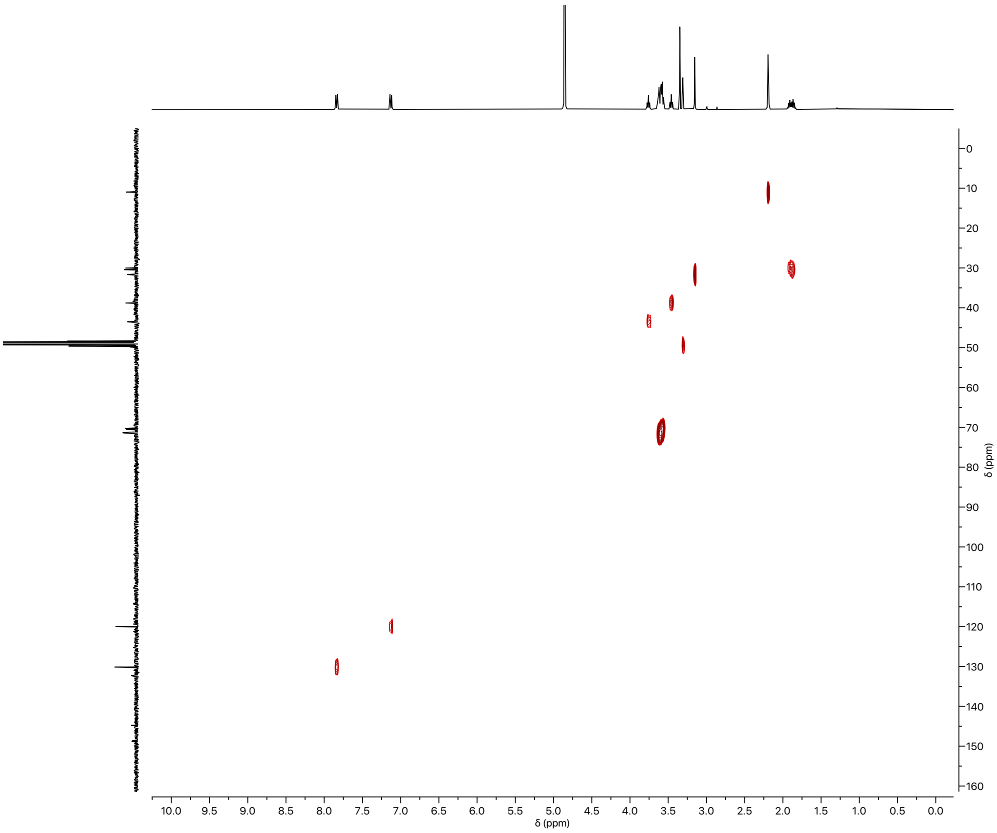


Figure S25. ^{1}H–^{13}C{^{1}H} HSQC NMR (400 MHz, MeOD, 298 K) spectrum of compound **8**

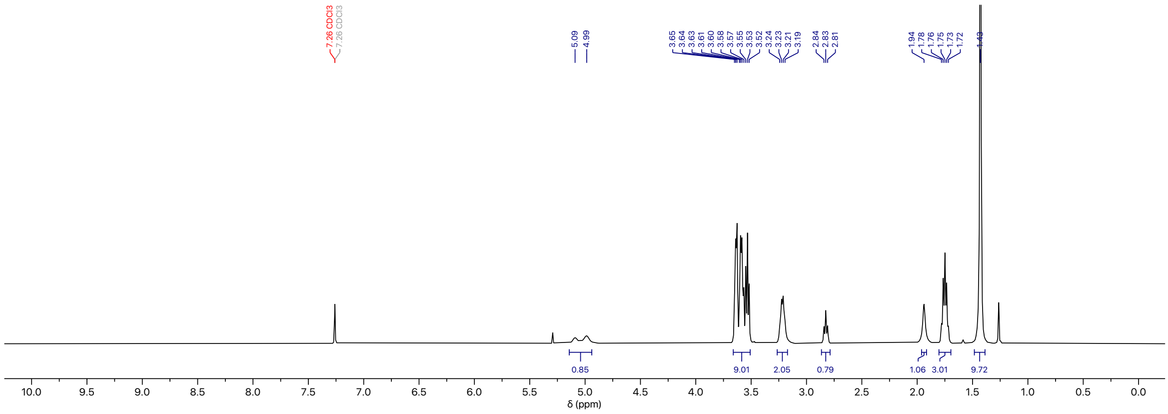


Figure S26. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **9**

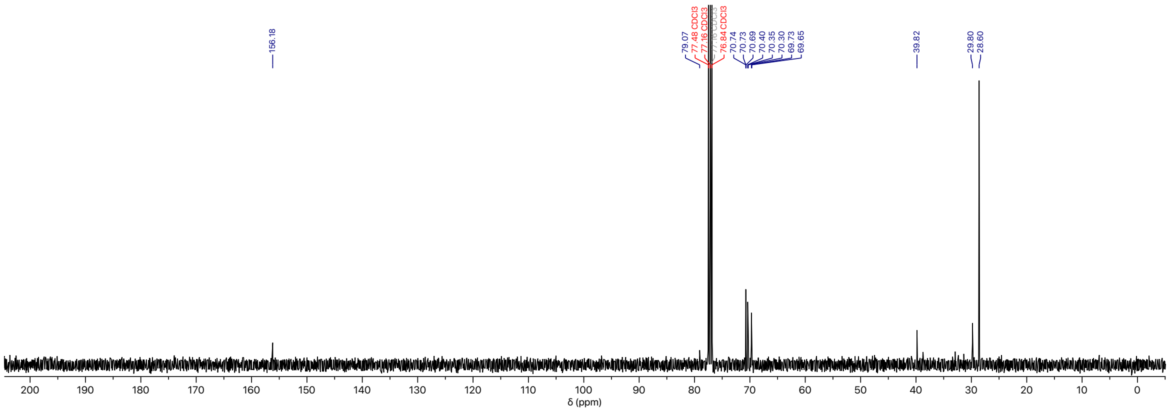


Figure S27. $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$, 298 K) spectrum of compound **9**

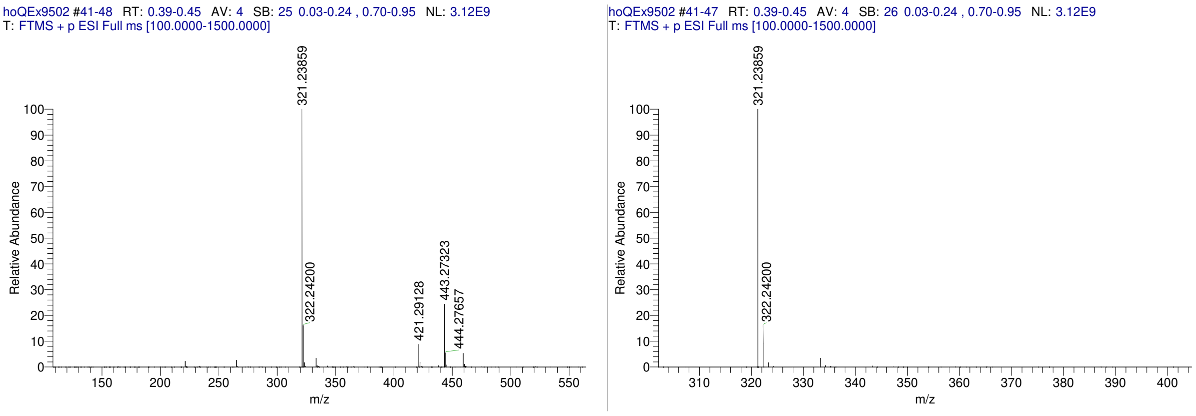


Figure S28. HR-ESI-MS spectrum of compound **9**

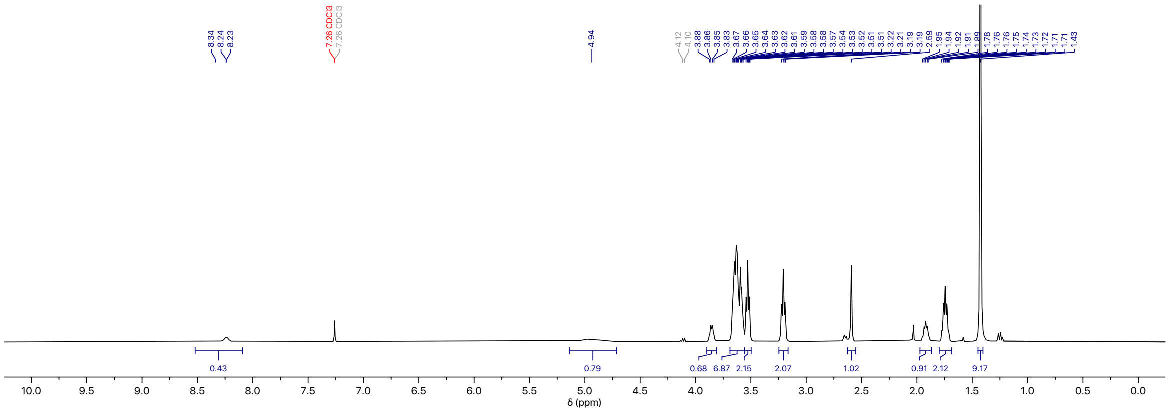


Figure S29. ^{1}H NMR (500 MHz, CDCl$_3$, 298 K) spectrum of compound **10**

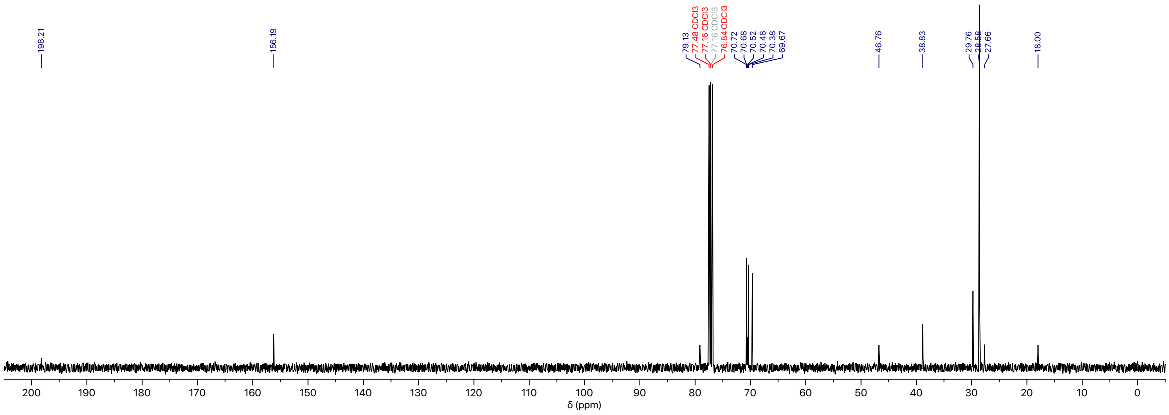


Figure S30. ^{13}C{^{1}H} NMR (126 MHz, CDCl$_3$, 298 K) spectrum of compound **10**

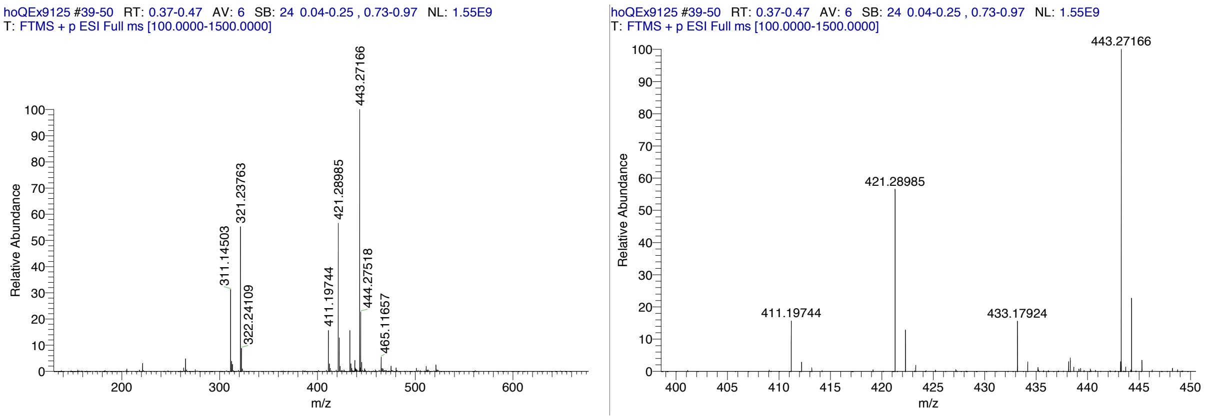


Figure S31. HR-ESI-MS spectrum of compound **10**

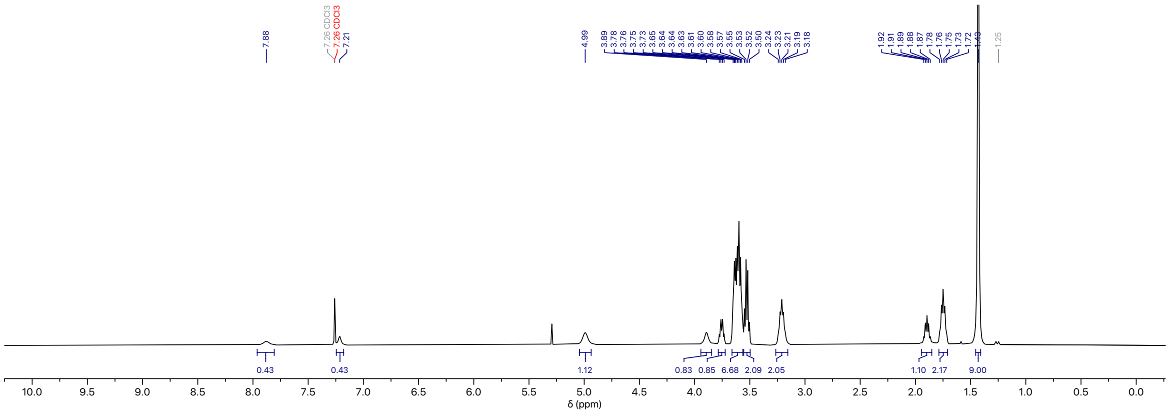


Figure S32. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) spectrum of compound **11**

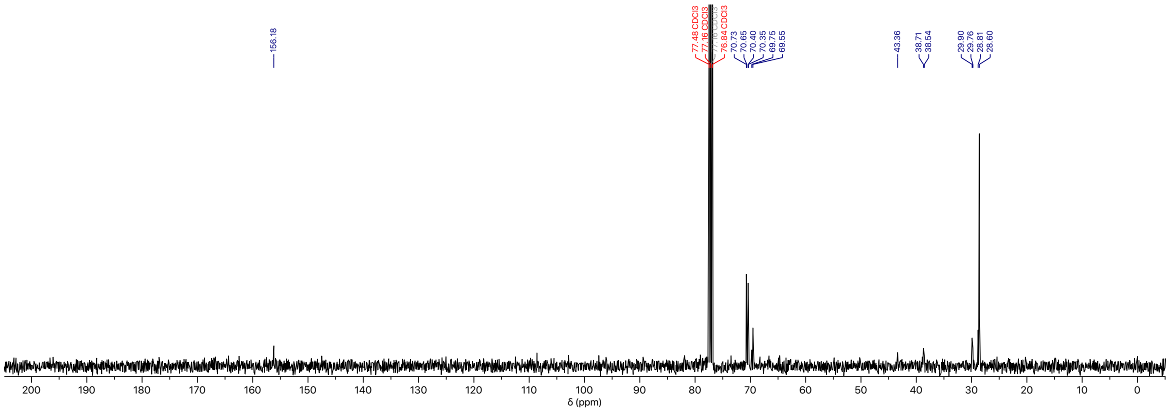


Figure S33. $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$, 298 K) spectrum of compound **11**

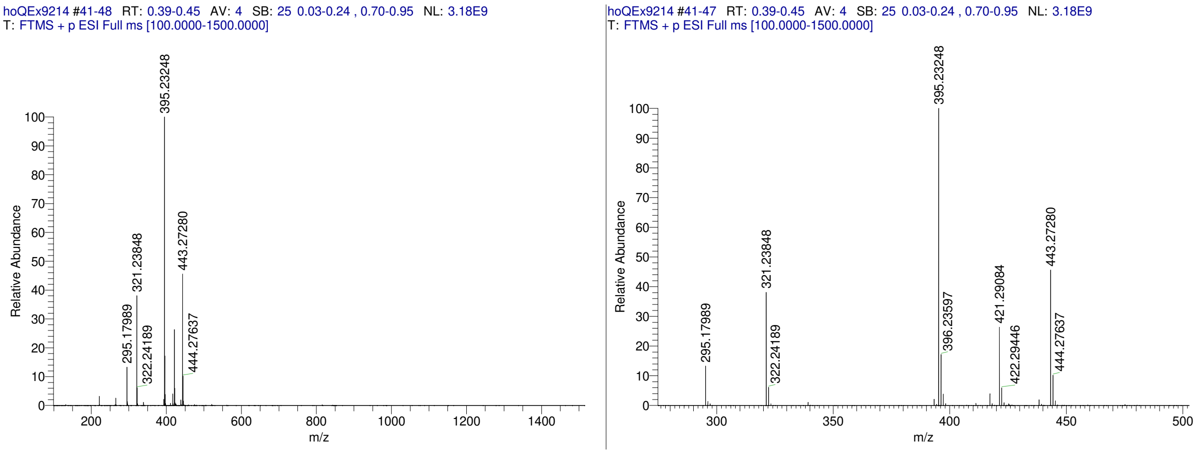


Figure S34. HR-ESI-MS spectrum of compound **11**

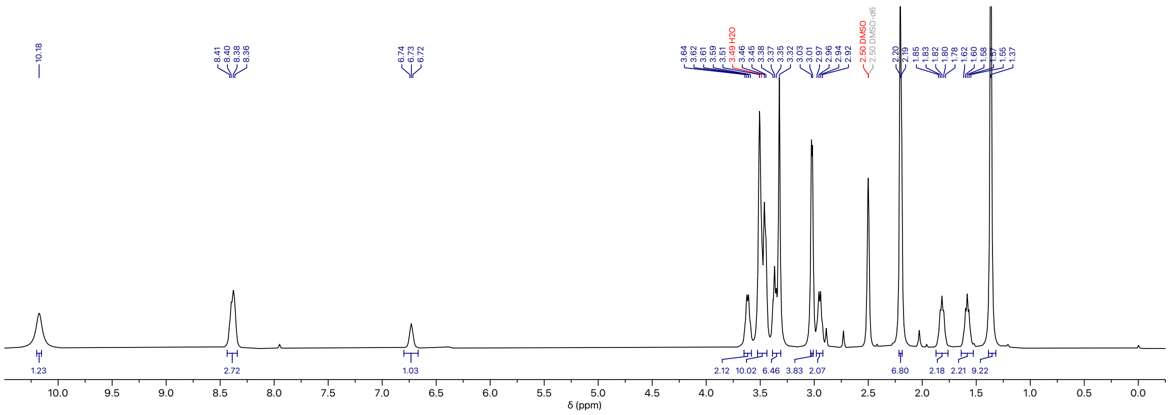


Figure S35. ^{1}H NMR (400 MHz, DMSO-d$_6$, 298 K) spectrum of compound **12**

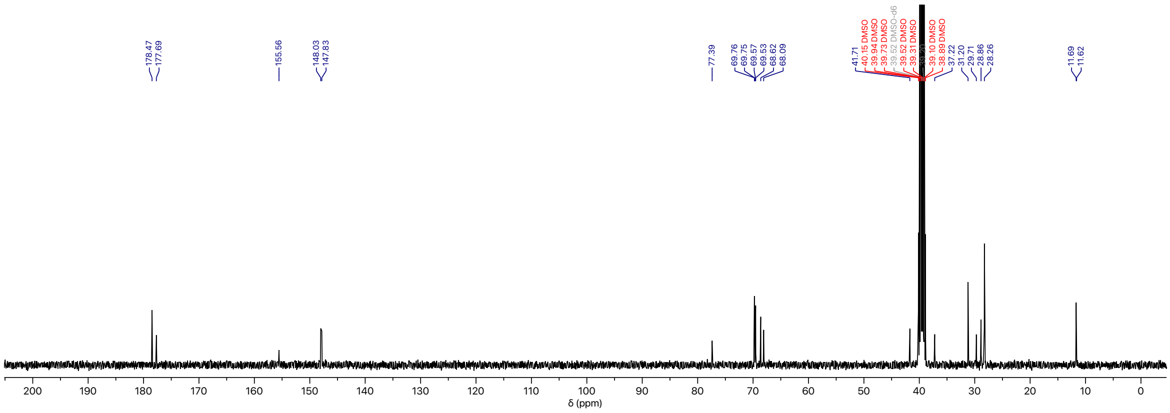


Figure S36. ^{13}C{^{1}H} NMR (101 MHz, DMSO-d$_6$, 298 K) spectrum of compound **12**

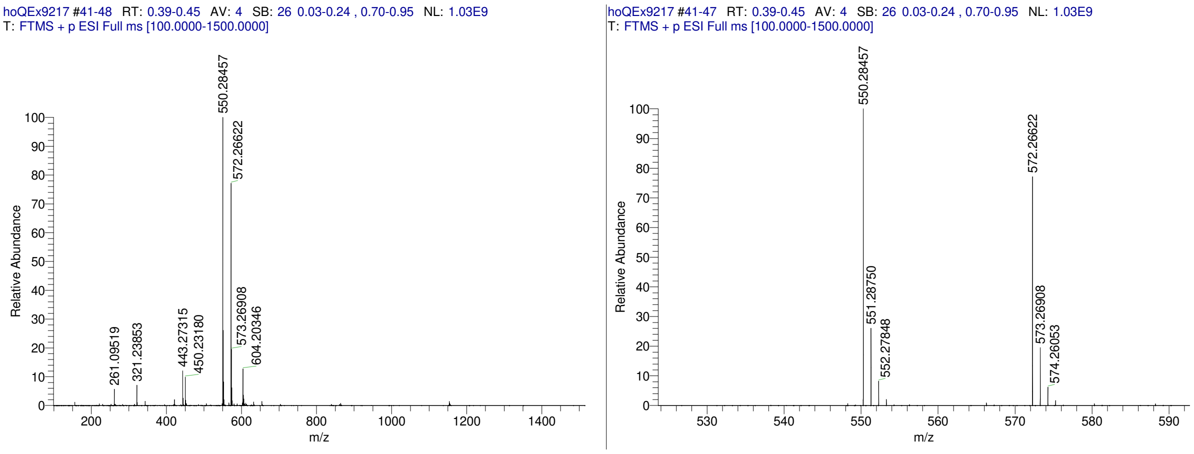


Figure S37. HR-ESI-MS spectrum of compound **12**

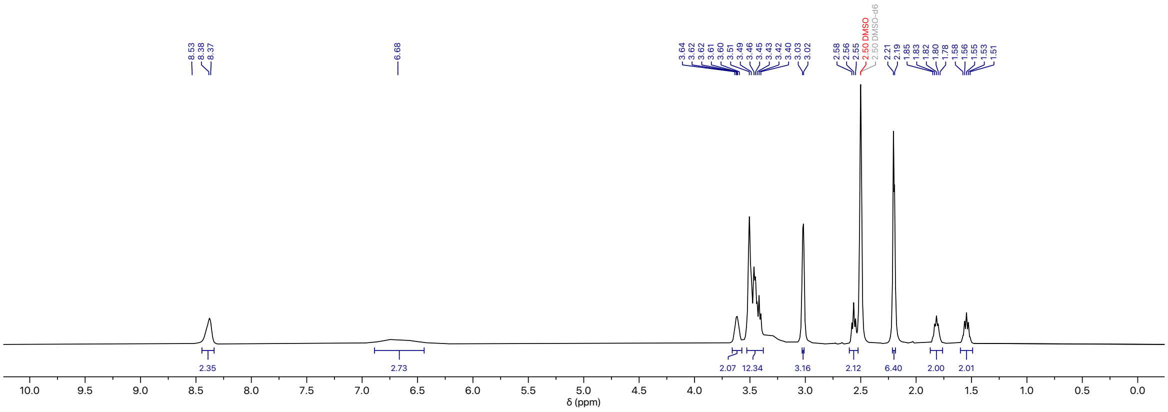


Figure S38. ^{1}H NMR (400 MHz, DMSO-d$_6$, 298 K) spectrum of compound **13**

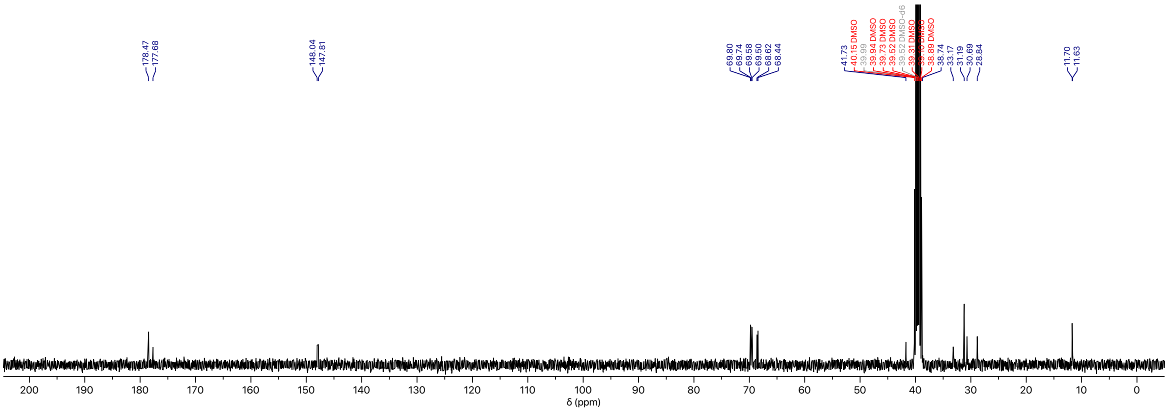


Figure S39. ^{13}C{^{1}H} NMR (101 MHz, DMSO-d$_6$, 298 K) spectrum of compound **13**

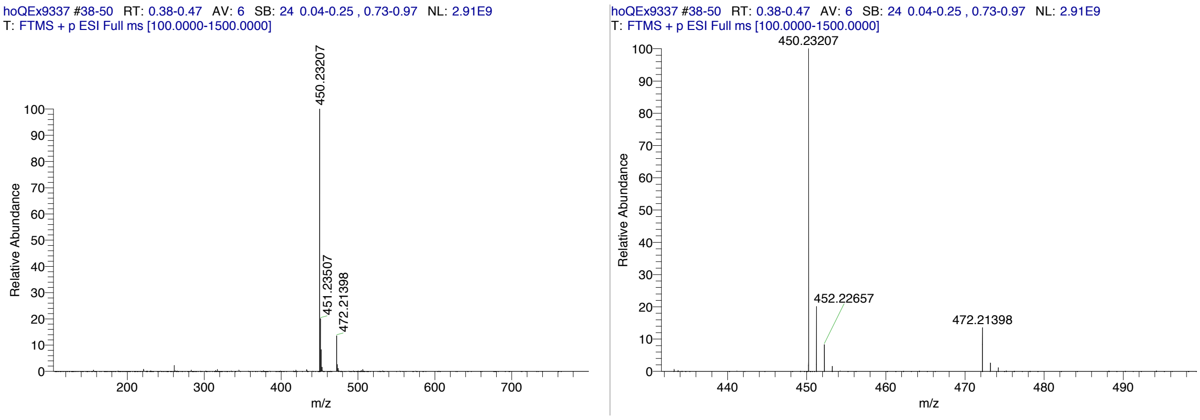


Figure S40. HR-ESI-MS spectrum of compound **13**

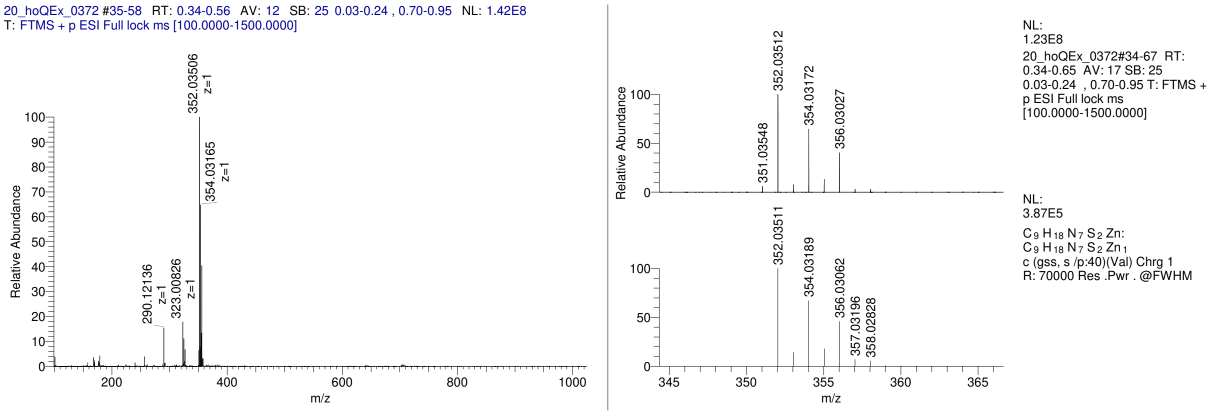


Figure S41. HR-ESI-MS spectrum of compound Zn-**6**

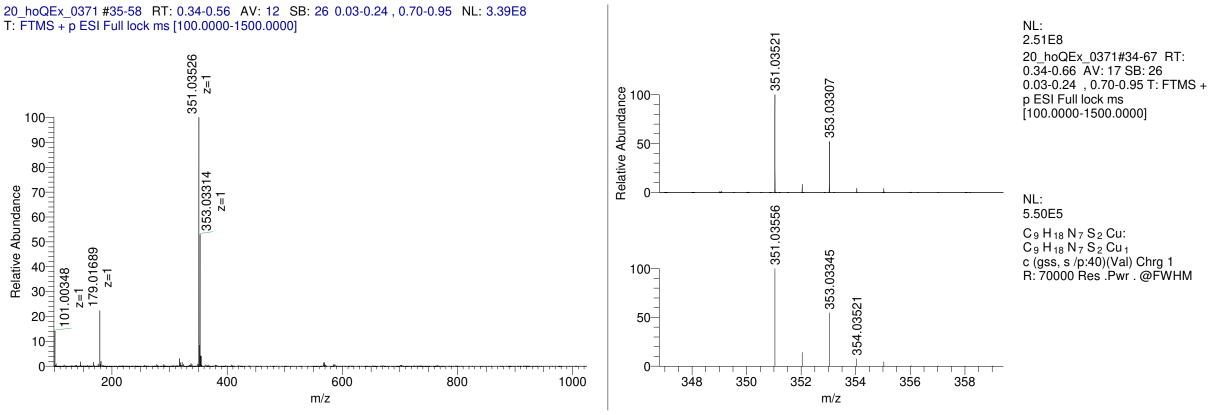


Figure S42. HR-ESI-MS spectrum of compound Cu-**6**

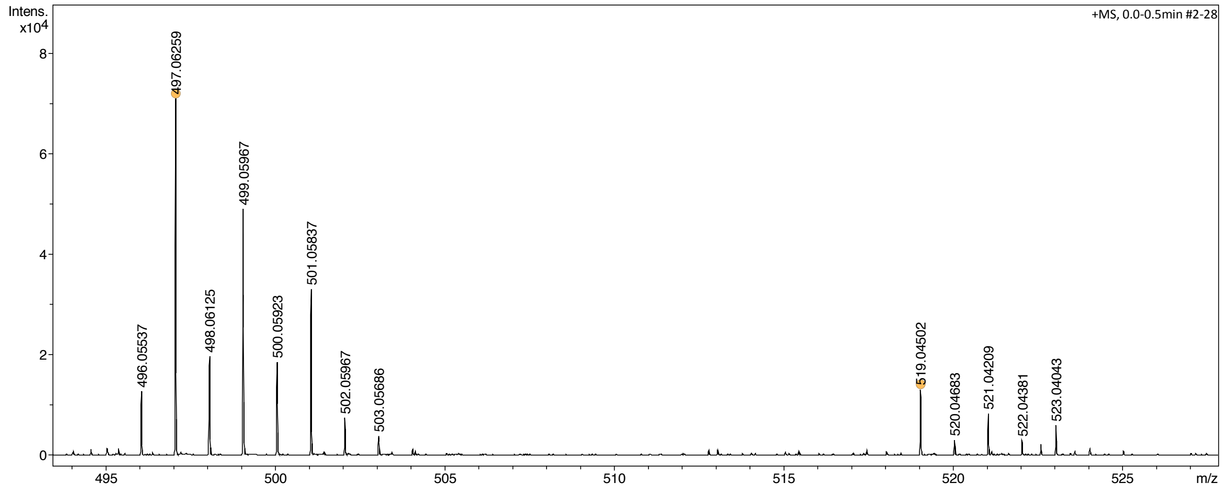


Figure S43. HR-ESI-MS spectrum of compound Zn-**7**

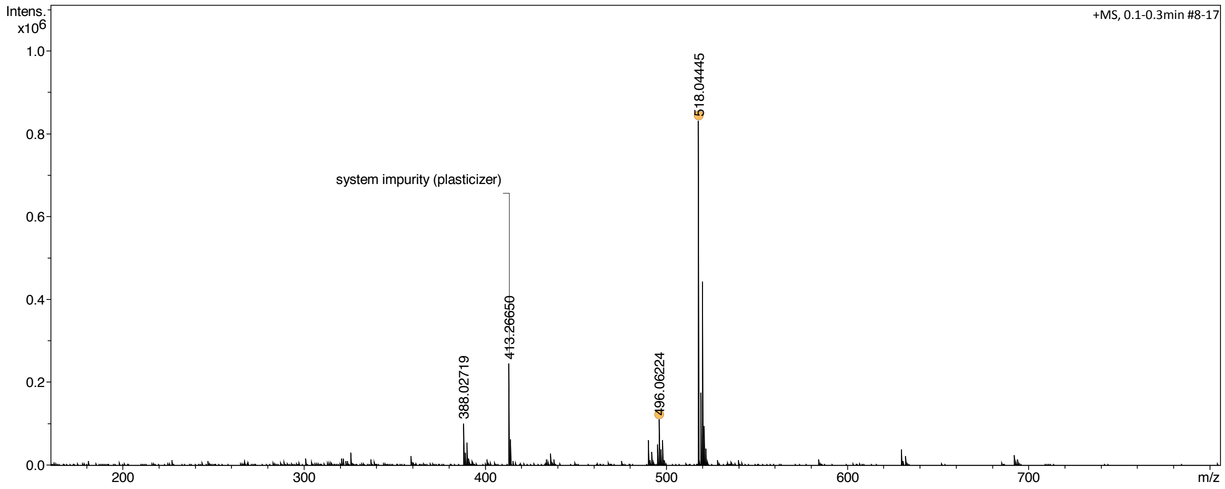


Figure S44. HR-ESI-MS spectrum of compound Cu-**7**

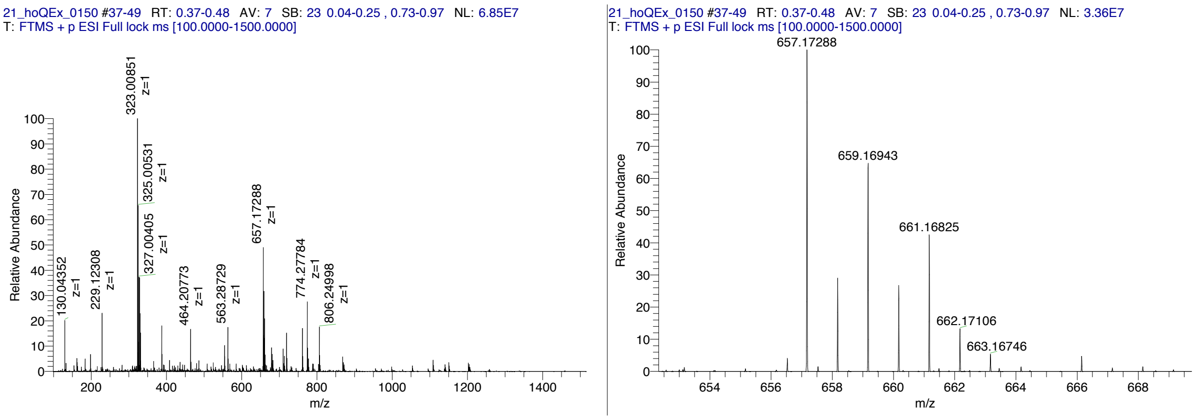


Figure S45. HR-ESI-MS spectrum of compound Zn-**8**

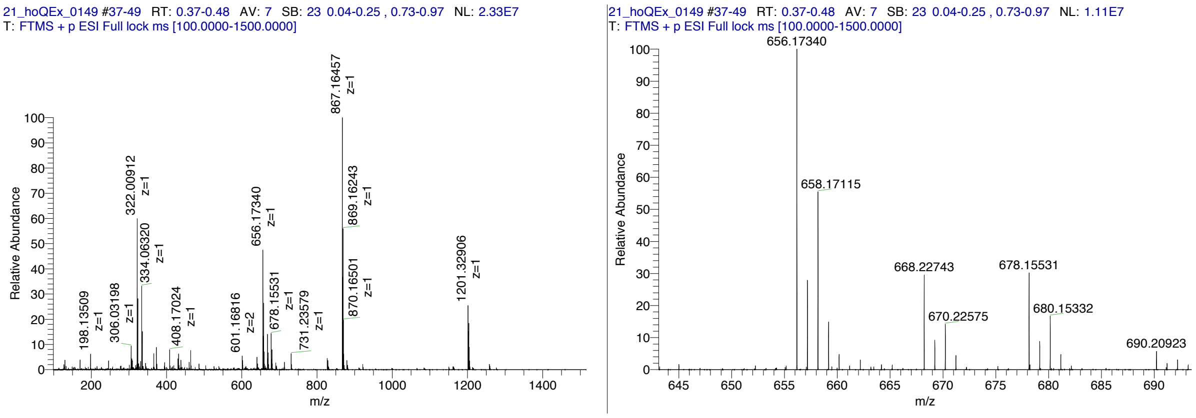


Figure S46. HR-ESI-MS spectrum of compound Cu-**8**

Photochemical activation

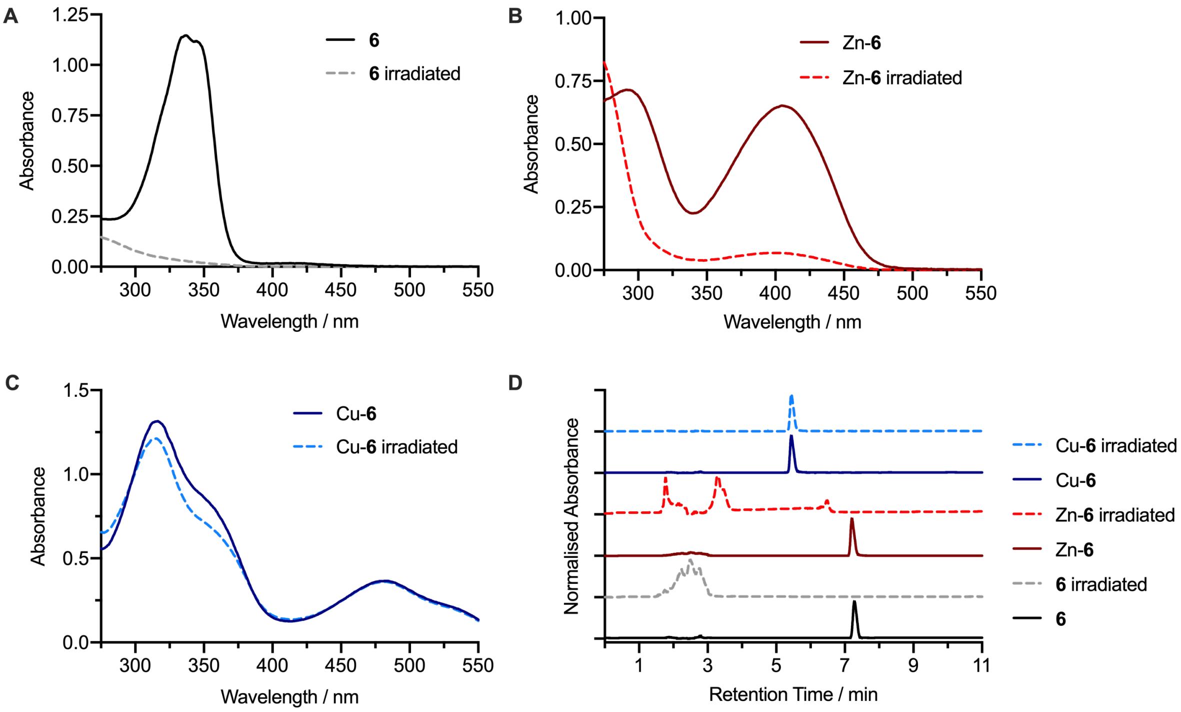


Figure S47. Irradiation stability for compound **6** (black: before; and grey: after irradiation) and corresponding metal complexes Zn-**6** (dark red: before; and red: after irradiation) and Cu-**6** (dark blue: before; and blue: after irradiation) in EtOH, before and after irradiation at 365 nm for 15 mins at pH 4.4. Electronic absorption spectra of (A) compound **6**, (B) Zn-**6**, (C) Cu-**6**, and (D) reverse-phase HPLC data (λ = 254 nm) for compounds **6**, Zn-**6**, and Cu-**6**.

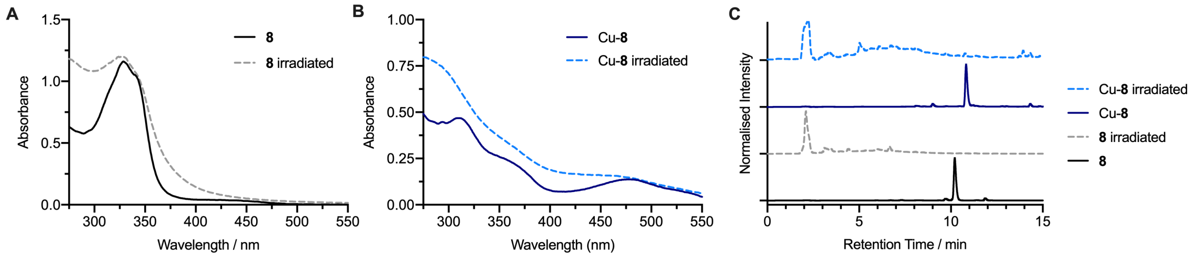


Figure S48. Photochemical degradation of compound **8** (black: before; and grey: after irradiation) and Cu-**8** (dark blue: before; and blue: after irradiation) in EtOH, before and after irradiation at 365 nm for 15 mins at pH 4.4. Electronic absorption spectra of (A) compound **8**, (B) Cu-**8**, and (C) reverse-phase HPLC data (λ = 254 nm) for compounds **8**, Zn-**8**, and Cu-**8**.

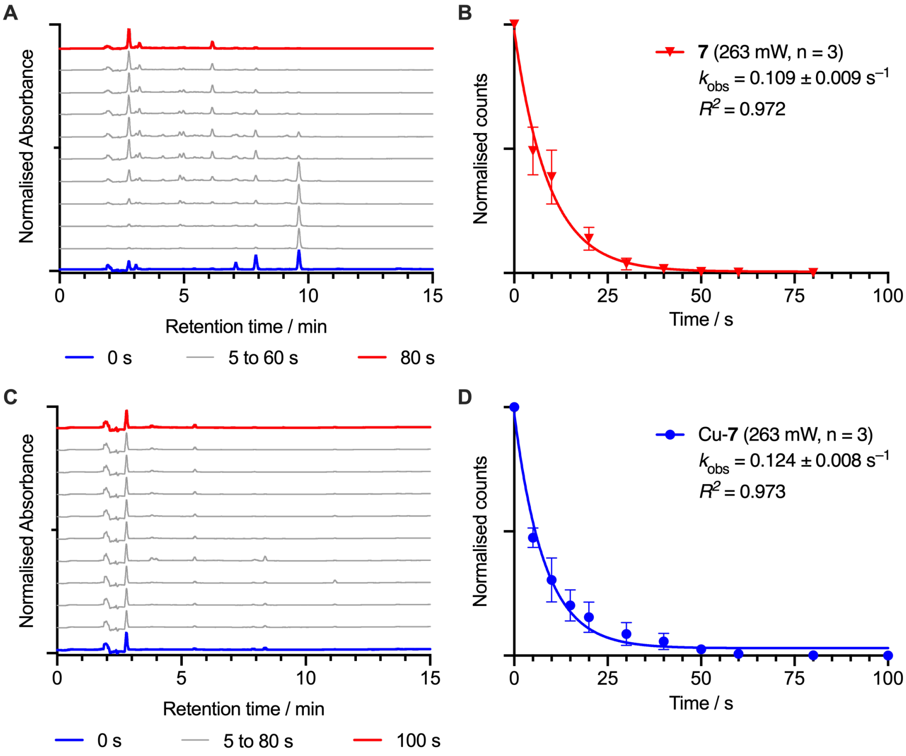


Figure S49. Photochemical activation kinetics measured by HPLC analysis during the photolysis of solutions of H_2ATSM/en-ArN_3 (**7**) and Cu-**7** in H_2O at 365 nm for up to 100 seconds at room temperature. (A) Stack plots showing the change in the reverse-phase HPLC chromatograms of (A) **7**, and (C) Cu-**7** *versus* irradiation time. The corresponding kinetic plots produced from integration and normalisation of the peak intensity associated with the starting materials are show for (B) **7**, and (D) Cu-**7**. All data points are the mean (with error bars representing 1 standard deviation) derived from independent measurements that were performed in triplicate. Data were fitted with a mono-exponential function to derive the experimentally observed first-order rate constants, k_{obs} / s^{-1}, for photoinduced degradation.

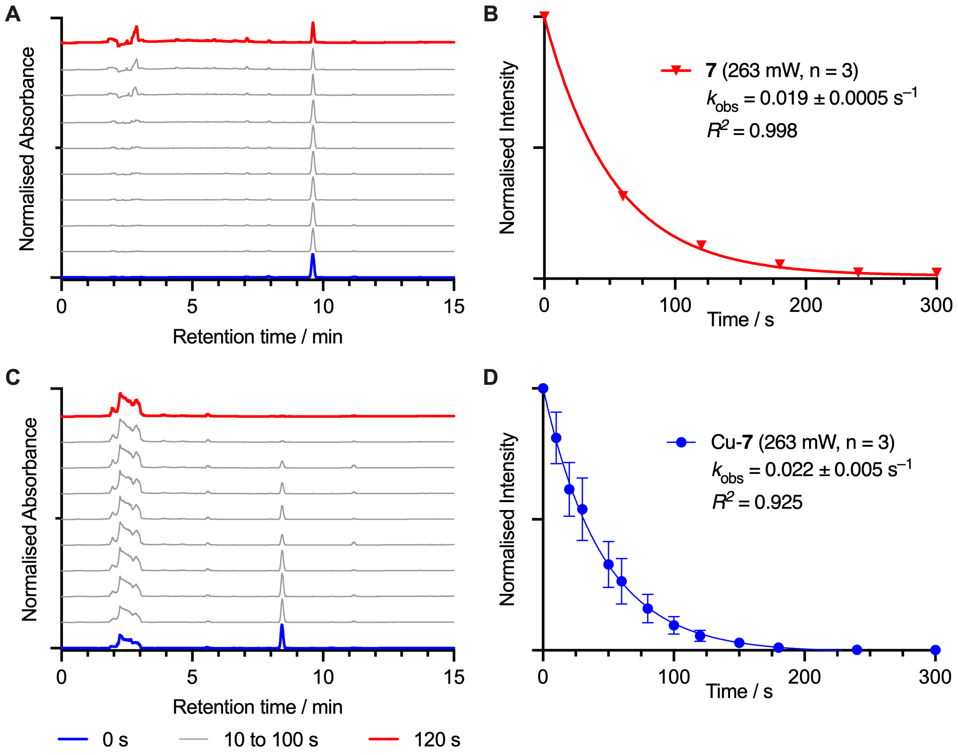


Figure S50. Photochemical activation kinetics measured by HPLC analysis during the photolysis of solutions of H_2ATSM/en-ArN_3 (**7**) and Cu-**7** in DMF at 365 nm for up to 120 seconds at room temperature. (A) Stack plots showing the change in the reverse-phase HPLC chromatograms of (A) **7**, and (C) Cu-**7** *versus* irradiation time. The corresponding kinetic plots produced from integration and normalisation of the peak intensity associated with the starting materials are show for (B) **7**, and (D) Cu-**7**. All data points are the mean (with error bars representing 1 standard deviation) derived from independent measurements that were performed in triplicate. Data were fitted with a mono-exponential function to derive the experimentally observed first-order rate constants, k_{obs} / s^{-1}, for photoinduced degradation.

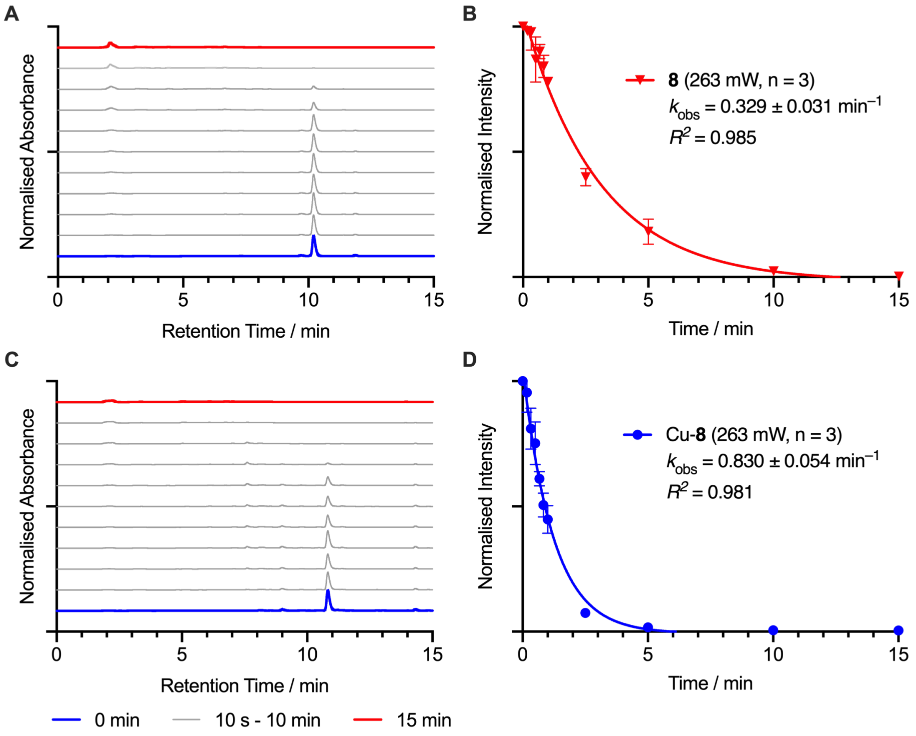


Figure S51. Photochemical activation kinetics measured by HPLC analysis during the photolysis of solutions of H_2ATSM/en-PEG$_3$-ArN$_3$ (**8**) and Cu-**8** in EtOH at 365 nm for up to 900 seconds at room temperature. (A) Stack plots showing the change in the reverse-phase HPLC chromatograms of (A) **8**, and (C) Cu-**8** versus irradiation time. The corresponding kinetic plots produced from integration and normalisation of the peak intensity associated with the starting materials are show for (B) **8**, and (D) Cu-**8**. All data points are the mean (with error bars representing 1 standard deviation) derived from independent measurements that were performed in triplicate. Data were fitted with a mono-exponential function to derive the experimentally observed first-order rate constants, k_{obs} / s^{-1}, for photoinduced degradation.

^{64}Cu-Radiolabelling of H$_2$ATSM/en-ArN$_3$ derivatives

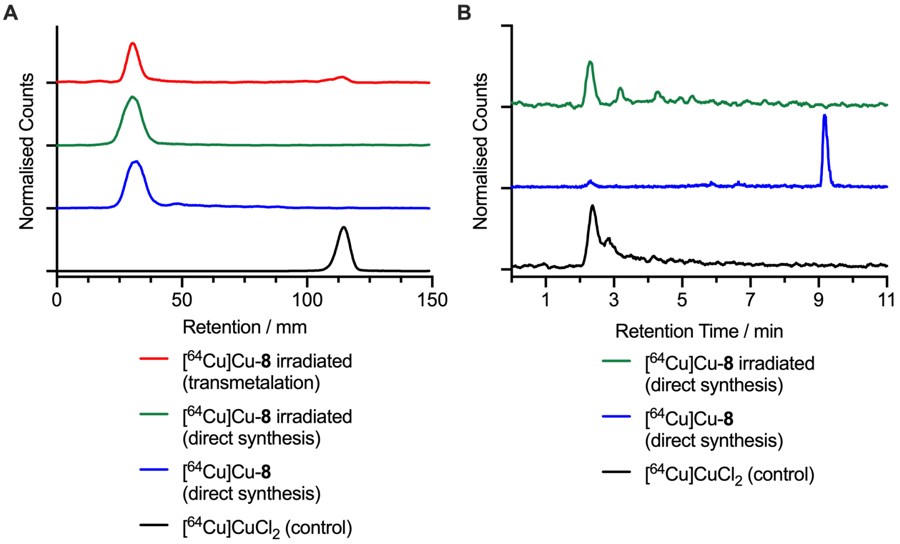


Figure S52. Radiochemical characterisation of [^{64}Cu]Cu-**8**. (A) Radio-iTLC chromatograms developed in 50 mM DTPA pH7.4 of [^{64}Cu]Cu-**8** produced by direct synthesis from **8** (blue trace), as well as the sample of the reaction mixture measured after irradiation of [^{64}Cu]Cu-**8** at 365 nm for 15 minutes from either direct synthesis (green trace) or transmetallation (red trace). The profile of [^{64}Cu]CuCl$_2$ as a control is shown in the black trace. (B) Radio-HPLC chromatograms of the reaction mixtures of [^{64}Cu]Cu-**8** produced by direct synthesis (blue radiotrace) and after irradiation (green radiotrace), as well as the elution profile of 'free' ^{64}Cu^{2+} ions (black radiotrace) shown as a control.

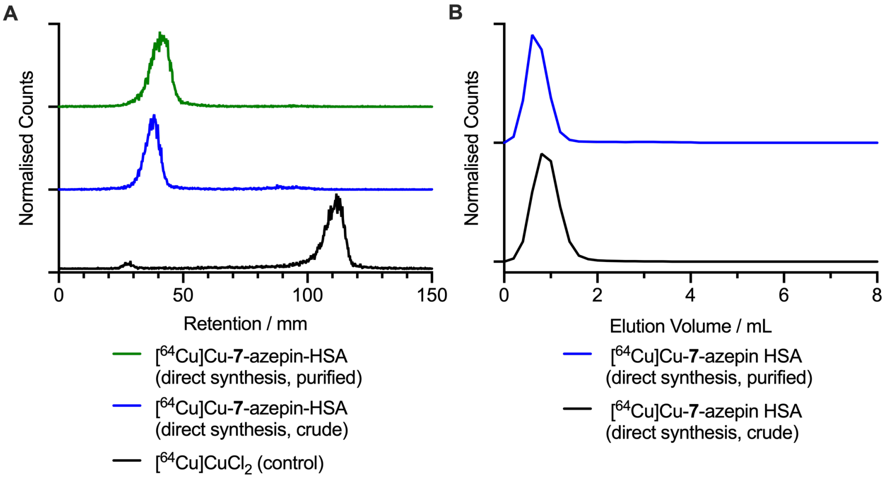


Figure S53. Radiochemical characterisation data of the [^{64}Cu]Cu-labelled protein conjugate [^{64}Cu]CuATSM/en-azepin-HSA *via* direct synthesis by (A) radio-iTLC (eluent: 50 mM DTPA pH7.4), and (B) radio-PD-10.

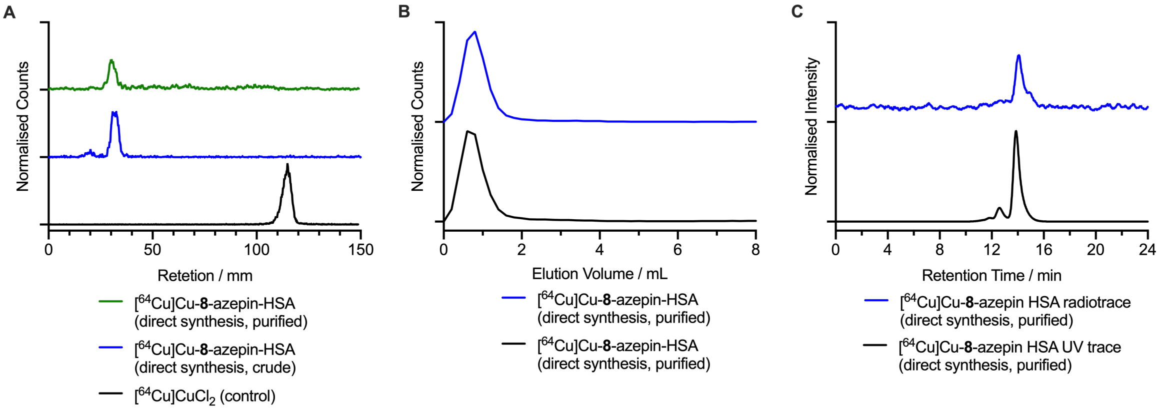


Figure S54. Radiochemical characterisation data of the [^{64}Cu]Cu-labelled protein conjugate [^{64}Cu]CuATSM/en-PEG$_3$-azepin-HSA *via* direct synthesis by (A) radio-iTLC and (B) radio-PD-10. Note: radio-iTLC chromatograms were developed by using a DTPA eluent (50 mM, pH7.4) where the [^{64}Cu]CuCl$_2$ complex forms the [^{64}Cu][Cu(DTPA)]$^{3-}$ complex *in situ*.