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Supplementary Figures

Figure S1.

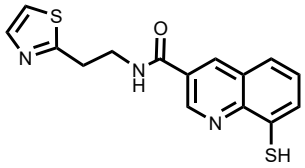


Figure S1: Structure of Capzimin, an Rpn11 inhibitor described by Perez *et al*.[1]

Fig.S2

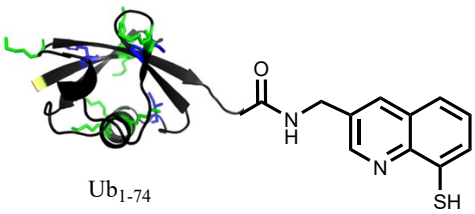


Figure S2: Schematic structure of **Ub_{74}-8MQ**, a non-covalent Rpn11 probe described by Hameed *et al*.[2]

Fig.S3

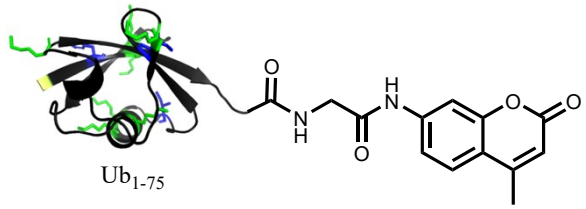


Figure S3: Schematic structure of **Ub-AMC**, a fluorogenic substrate for detection of deubiquitinase activity.[3]

Fig.S4

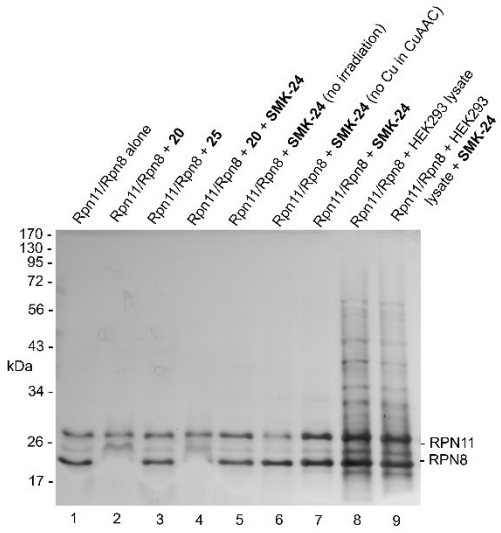


Figure S4: Silver stain of 40 µM diazirine probe **SMK-24** in photoaffinity labelling experiments with recombinant Rpn11 and HEK293 cell lysate.

Fig.S5

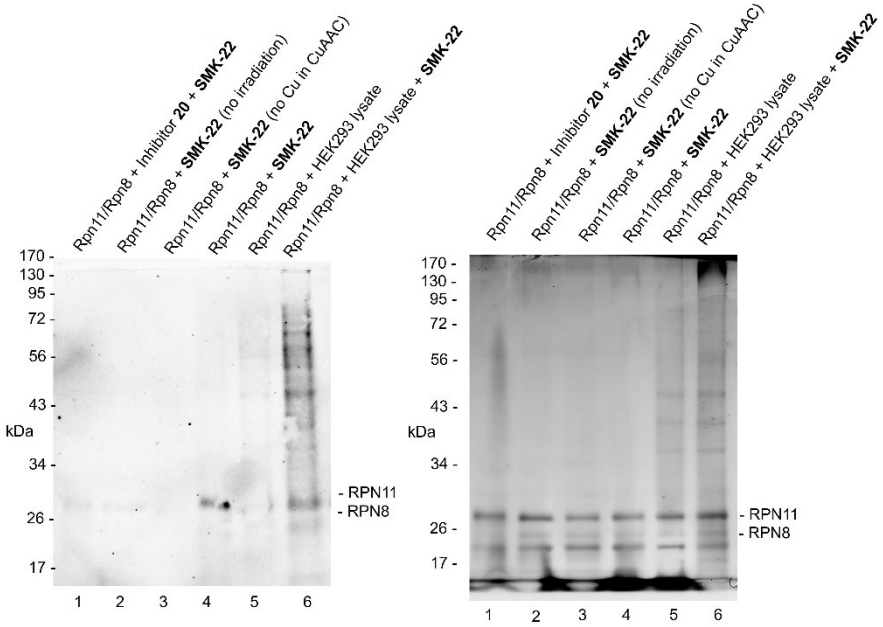


Figure S5: Western blot (left) and silver stain (right) of 40 µM benzophenone probe **SMK-22** in photoaffinity labelling experiments with recombinant RPN11 and HEK293 cell lysate.

Fig.S6

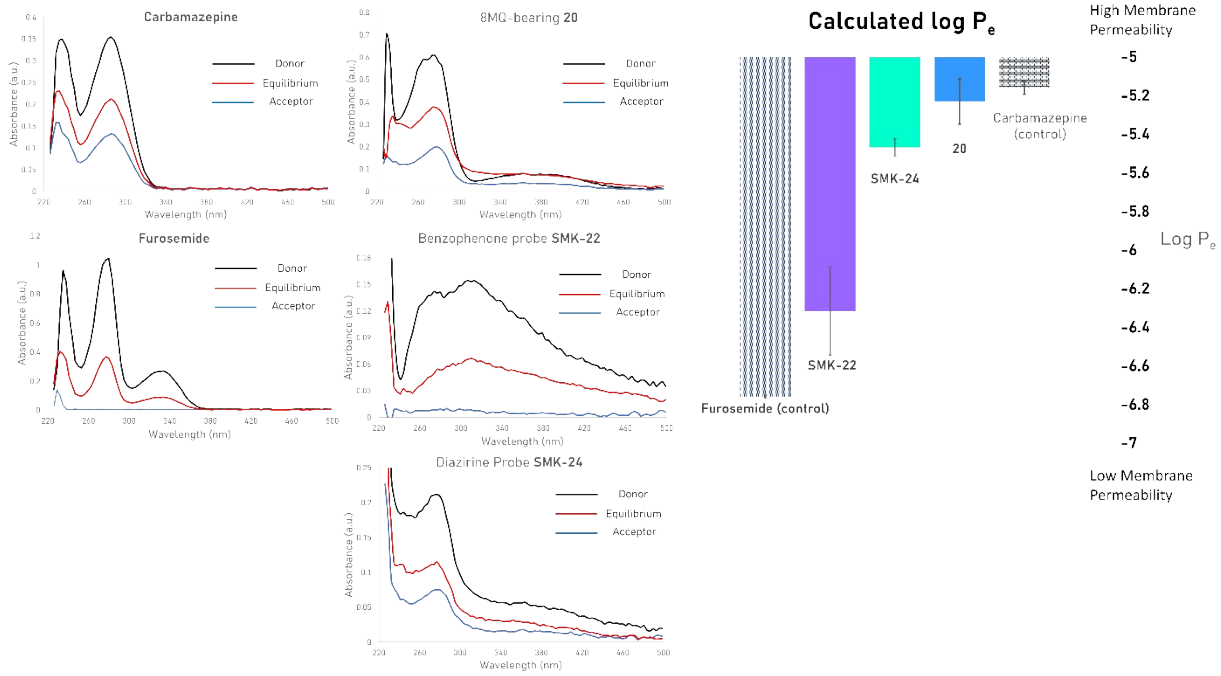
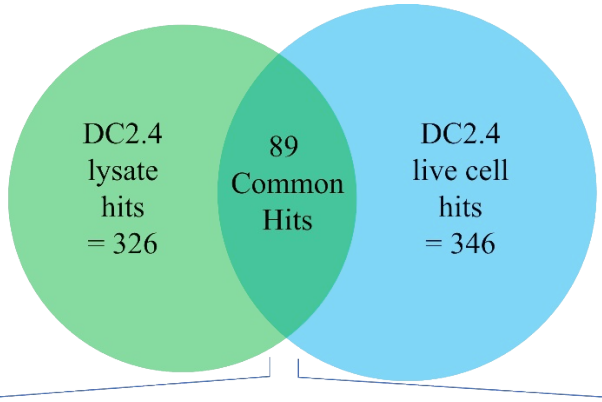


Figure S6: Parallel artificial membrane permeability assay (PAMPA) results for 8MQ-bearing **20**, benzophenone probe **SMK-22** and diazirine probe **SMK-24**. Carbamazepine and furosemide were used as high permeability and low permeability control compounds respectively. Carbamazepine (log P_e = -5.16) and furosemide (log P_e = -6.76) were in good agreement with reported values.[4] Samples were prepared and tested in triplicate. Mean average UV-Vis plots for respective test samples are given (left). Concentrations were calculated using calibration curves for each sample. log P_e values for probes was plotted (right).

Fig.S7



ACADL	ACP6	ADI1	ADO	ALDH1B1	ARHGDIB	ATP2A2	ATP5B	ATP6V0D1	ATP6V1B2
CALR	CANX	CD9	CLN3	CLUH	CREG1	CTSB	CTSD	CTSZ	ECH1
EMB	ESYT1	GLG1	GSTM1	HARS	HEATR3	HGSNAT	HNRNPAB	HSP90B1	HSPA9
KDELR2	LAMP2	MCM4	MCM6	MFGE8	MGEA5	MIF	MTAP	MTCO2	MYADM
NAP1L4	NENF	NQO2	NRD1	NUCB1	NUCB2	OSBPL8	P4HB	PARP1	PDCD6IP
PDIA4	PELP1	POR	PRCP	PRKCSH	PSAP	PSMB4	PSMB5	PTGR2	PTPRC
RAB14	RPN1	SCAMP3	SLC16A1	SLC16A3	SLC1A5	SLC29A1	SLC2A1	SLC38A2	SLC3A2
SLC7A1	SLC7A5	STEAP3	STOM	SYMPK	SYNCRIP	TARS2	TCIRG1	TFRC	TMEM176B
TPM3	TPR	TRIM28	TXN	TXNDC5	U2AF2	UGGT1	VAV3	VCP	

Figure S7: Venn diagram and table of total validated proteins hits from enrichment experiments in DC2.4 lysate using 10 µM **SMK-24** and enrichment experiments in DC2.4 live cells using 25 µM **SMK-24**. Listed are 89 hits enriched from both labelling mediums.

Fig.S8

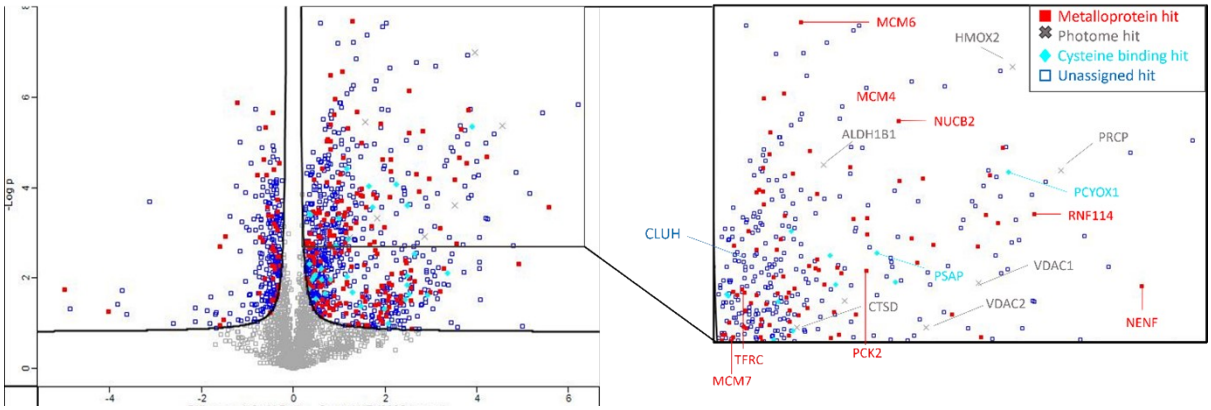


Figure S8: Identification of proteins enriched through treatment of 10 µM **SMK-24** in protein extract from lysed HEK293 cells. A selection of significantly enriched proteins are highlighted with nominal classification of binding mode.

Fig.S9

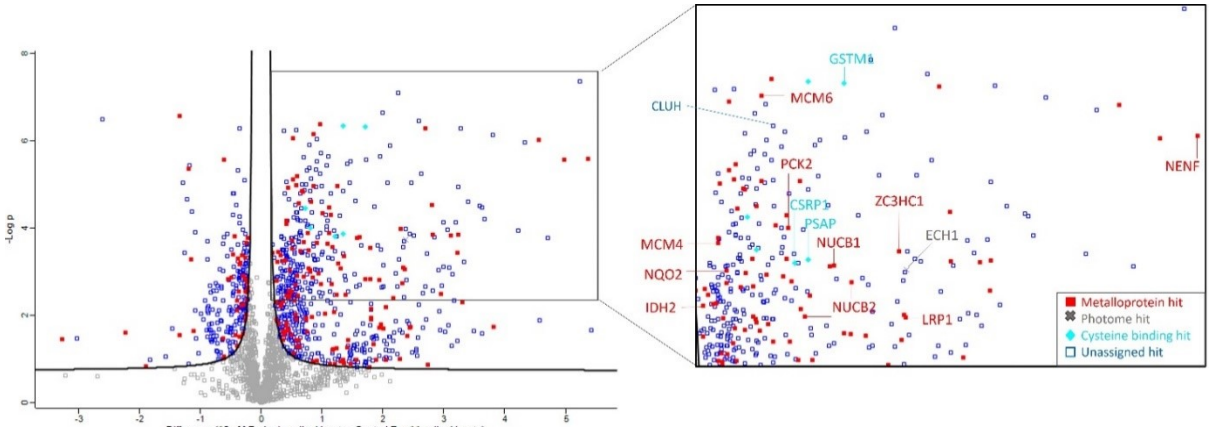


Figure S9: Identification of proteins enriched through treatment of 10 μM **SMK-24** in protein extract from lysed DC2.4 cells. A selection of significantly enriched proteins are highlighted with nominal classification of binding mode.

Fig.S10

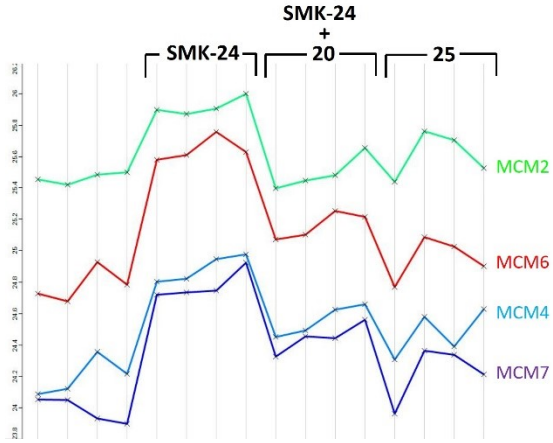


Figure S10: Label-free quantification (LFQ) profile plots for MCM2, MCM4, MCM6 and MCM7 in live DC2.4 cell labelling samples treated with vehicle (lanes 1-4), **SMK-24** (lanes 5-8), 8MQ-bearing **20** + **SMK-24** (lanes 9-12), and non-zinc-binding control diazirine **25** (lanes 13-16).

Fig.S11

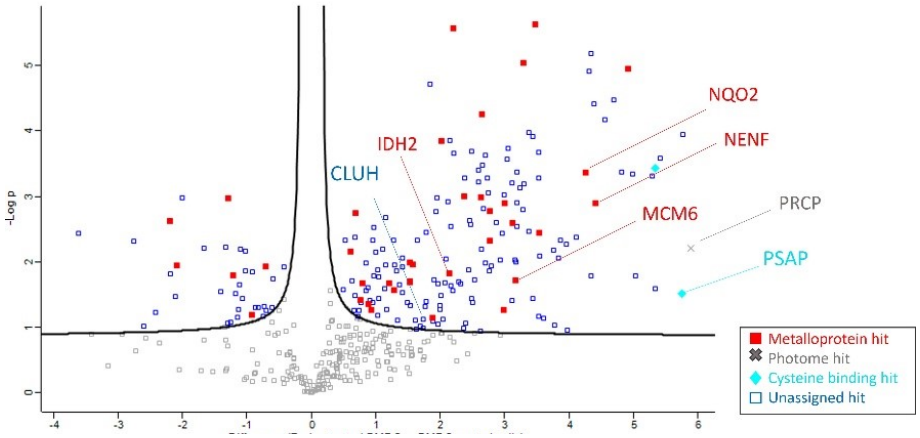
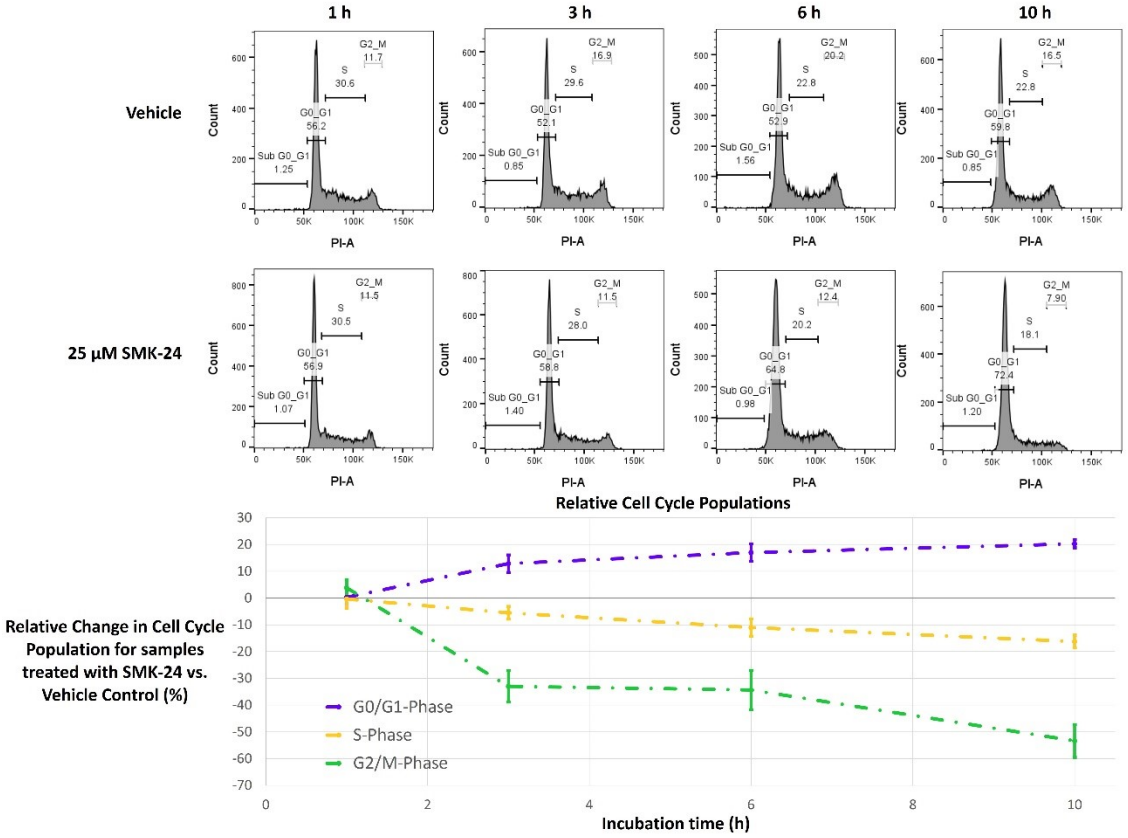
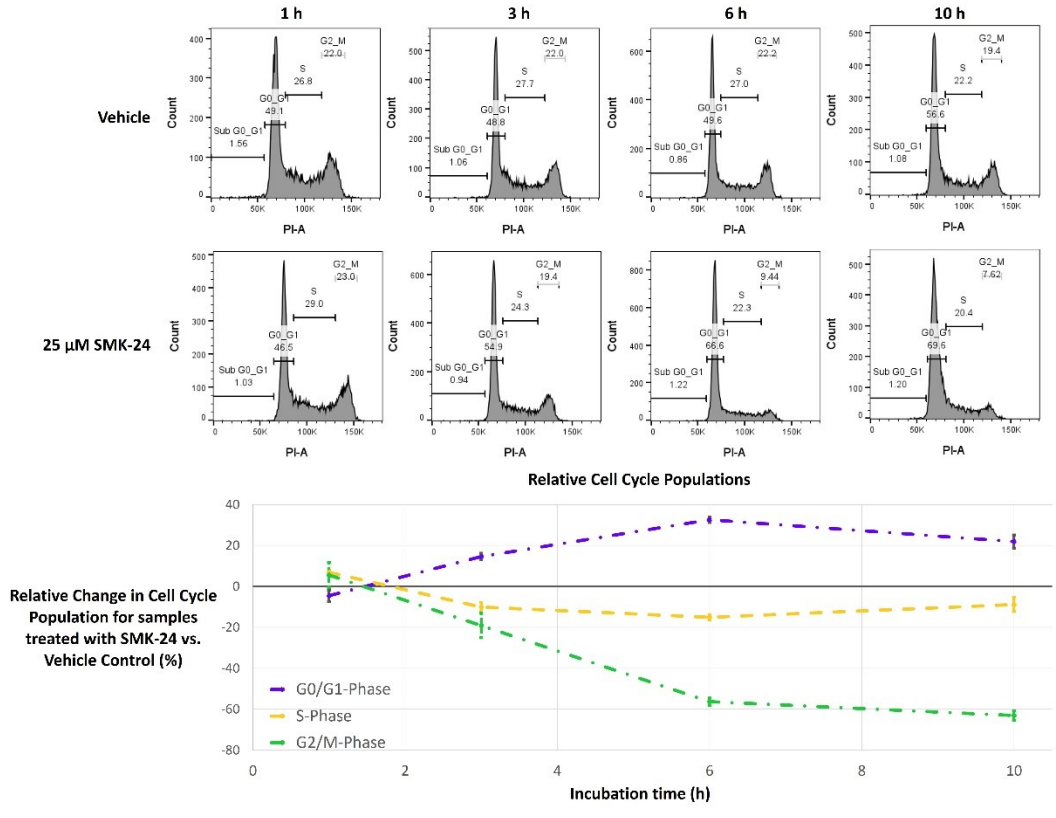


Figure S11: Identification of proteins enriched through treatment of 100 μM **SMK-24** in live bone marrow-derived dendritic cells. A selection of significantly enriched proteins are highlighted with nominal classification of binding mode.

Fig.S12


1 h
3 h
6 h
10 h
Vehicle
25 µM SMK-24
Count
PI-A
G2_M
S
G0_G1
Sub G0_G1
Relative Cell Cycle Populations
Relative Change in Cell Cycle Population for samples treated with SMK-24 vs. Vehicle Control (%)
G0/G1-Phase
S-Phase
G2/M-Phase
Incubation time (h)


1 h
3 h
6 h
10 h
Vehicle
25 µM SMK-24
Count
PI-A
G2_M
S
G0_G1
Sub G0_G1
Relative Cell Cycle Populations
Relative Change in Cell Cycle Population for samples treated with SMK-24 vs. Vehicle Control (%)
G0/G1-Phase
S-Phase
G2/M-Phase
Incubation time (h)

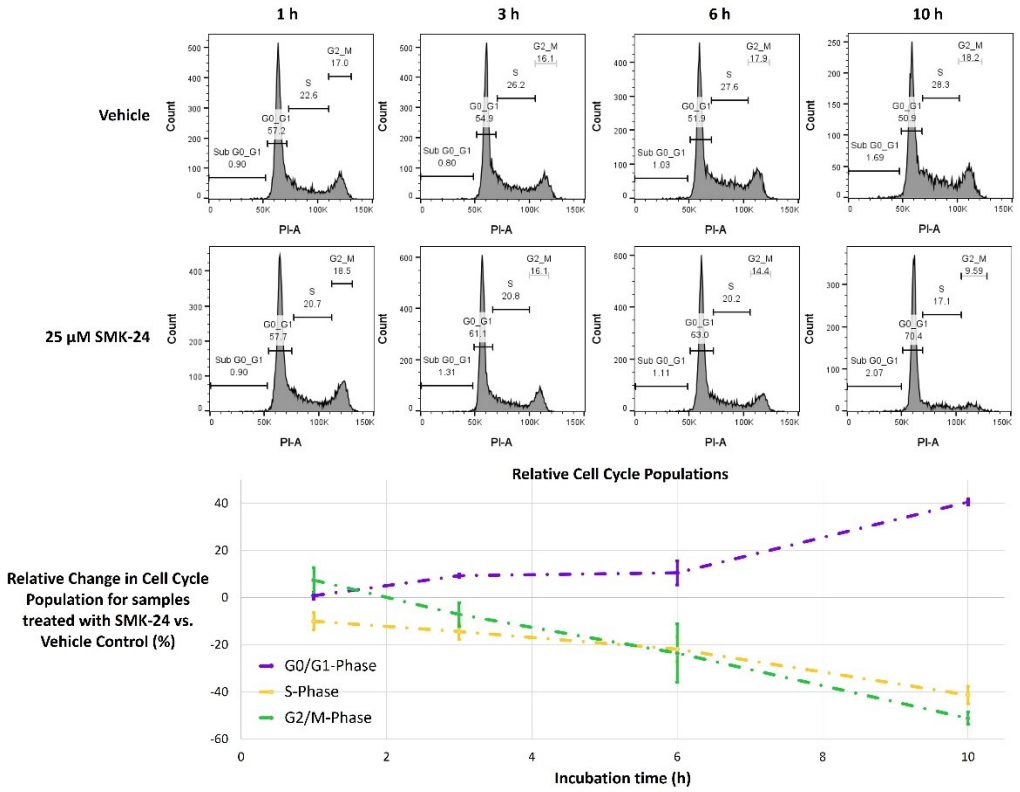


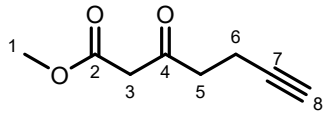
Figure S12: Effects of diazirine probe **SMK-24** on the cell cycle of HEK293 cells. Cells were treated with vehicle (0.1% DMSO, 150 µM DTT) or 25 µM **SMK-24** in DMEM growth medium for 1-10 h and thereafter fixed, stained with propidium iodide and analysed by flow cytometry. Plotted values represent the mean and standard deviation for three separate experiments. Samples prepared in triplicate per condition. Representative plots shown for each timepoint.

Chemical Methods

Chemical Synthesis

^{1}H and ^{13}C spectra were recorded on Bruker Avance III 400 MHz or Bruker Avance II 600 MHz system spectrometers in $CDCl_3$ or DMSO-d_6 relative to residual $CHCl_3$ (δ_H = 7.26 ppm, δ_C = 77.0 ppm) or DMSO (δ_H = 2.50 ppm, δ_C= 39.7 ppm). Chemical shifts are recorded in ppm and coupling constants are reported in Hertz (Hz) accurate to 0.2 Hz. ^{13}C NMR spectra are proton-decoupled. Spectra were assigned using HSQC, HMBC, ^{1}H selective TOCSY, DEPT and ^{15}N HSQC experiments. Mass spectrometry was recorded on a Bruker APCI or ESI HRMS system. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrophotometer. Melting point ranges were recorded using a Griffin melting point apparatus. Silica column chromatography was performed using Supelco high-purity grade silica, pore size 60 Å, particle size 40-63 µm. TLC analysis was carried out using precoated aluminium backed $60F_{254}$ slides and visualised by UV irradiation or anisaldehyde stain (9.2 mL p-methoxybenzaldehyde, 3.8 mL AcOH, 338 mL EtOH, 12.5 mL concentrated sulfuric acid). All reagents and solvents were obtained from commercial sources and used as received unless otherwise stated. Anhydrous DCM, THF, MeCN were obtained from a PureSolv solvent purification system. PE refers to the fraction of petroleum that boils at 40-60˚C.

Methyl 3-oxohept-6-ynoate (**1**)



In a flame-dried two-necked flask, magnesium chloride (1.88 g, 19.7 mmol, 1.04 equiv.) and potassium 3 methoxy-3-oxopropanoate (4.44 g, 28.4 mmol, 1.50 equiv.) were slurried in anhydrous THF (30 mL). The mixture was heated to 50 °C under an atmosphere of nitrogen and stirred for 4 h. In a separate flame-dried round-bottomed flask, 4-pentynoic acid (1.86 g, 19.0 mmol, 1.0 equiv.) in anhydrous THF (40 mL) was cooled to 0 °C and treated with 1,1-carbonyldiimidazole (3.69 g, 22.8 mmol, 1.2 equiv.) portion-wise such that flask temperature remained < 5 °C. The reaction was stirred at 0 °C for 10 min and then at ambient temperature for 1 h. The two-necked flask was cooled to ambient temperature and dropwise addition of the solution of activated 4-pentynoic acid was performed. The reaction mixture was stirred under an atmosphere of nitrogen overnight at ambient temperature. After this time, TLC analysis (DCM-MeOH-AcOH, 379:20:1) showed total consumption of starting oxoproponate (R_f = 0.2) and the formation of a new product (R_f = 0.9). The reaction was concentrated *in vacuo* and the crude residue was slurried in a biphasic mixture of EtOAc (100 mL) and 1 M K_2CO_3 solution (60 mL). The crude mixture was filtered through celite and the phases were separated. The organic extract was washed with saturated brine solution (50 mL), dried over anhydrous $MgSO_4$ and purified by silica column chromatography (Hexane-EtOAc, 4:1) to yield the desired product (R_f = 0.2), methyl-3-oxohept-6-ynoate, **1** (2.15 g, 13.9 mmol, 74%) as a colourless oil.

ν_{max}/cm^{-1} (neat) 3286 (C-H alkyne), 2956 (C-H), 2161 (C≡C), 1742 (C=O), 1715 (C=O), 1437 (C-H), 1407, 1365, 1320, 1256 (C-O), 1197, 1134, 1084, 1021, 991, 841.

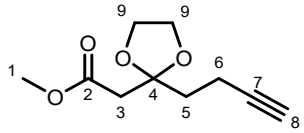
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.95 (t, $J_{H6,H8}$ = 2.6 Hz, 1H, H-8), 2.46 (td, $J_{H5,H6}$ = 7.2 Hz, $J_{H6,H8}$ = 2.6 Hz, 2H, H-6), 2.80 (t, $J_{H5,H6}$ = 7.2 Hz, 2H, H-5), 3.47 (s, 2H, H-3), 3.72 (s, 3H, H-1) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 12.8 (C-6), 41.6 (C-5), 48.9 (C-3), 52.4 (C-1), 69.0 (C-8), 82.4 (C-7), 167.3 (C-2), 200.4 (C-4) ppm.

HRMS (ESI^+): *m/z* found: 177.0519 $[M+Na]^+$, calculated $(C_8H_{10}NaO_3)^+$: 177.0522

The spectroscopic data are in agreement with those reported in the literature.[5]

Methyl 2-(2-(but-3-yn-1-yl)-1,3-dioxolan-2-yl)acetate (**2**)



A solution of ethylene glycol (3.07 mL, 54.9 mmol, 4.0 equiv.) and triethyl orthoformate (4.56 mL, 27.4 mmol, 2.0 equiv.) was treated with ketone **1** (2.12 g, 13.7 mmol, 1.0 equiv.). *p*-TsOH (236 mg, 1.37 mmol, 0.1 equiv.) was added and the reaction mixture was stirred at ambient temperature for 16 h under an atmosphere of nitrogen. After this time, TLC analysis (Hexane-EtOAc, 4:1) showed total consumption of ketone **1** (R_f = 0.2) and the formation of a new product (R_f = 0.3). The reaction was diluted with Et_2O (150 mL) and washed with saturated aq. sodium bicarbonate solution (100 mL). The organic extract was washed with saturated brine

solution (80 mL), dried over anhydrous $MgSO_4$ and purified by silica column chromatography (Hexane-EtOAc, 4:1) to yield the desired product methyl-2-(2-(but-3-yn-1-yl)-1,3-dioxolan-2-yl)acetate, **2** (959 mg, 4.84 mmol, 75%) as a colourless oil.

ν_{max}/cm^{-1} (neat) 3288 (C-H alkyne), 2954 (C-H), 2893 (C-H), 2160 (C≡C), 1734 (C=O), 1438 (C-H), 1330, 1209 (C-O), 1105, 1043, 949, 847, 770.

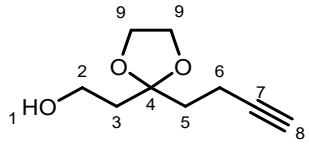
^1H NMR (400 MHz, $CDCl_3$): δ 1.92 (t, $J_{H6,H8}$ = 2.6 Hz, 1H, H-8), 2.10 (t, $J_{H5,H6}$ = 7.8 Hz, 2H, H-5), 2.29 (td, $J_{H5,H6}$ = 7.8 Hz, $J_{H6,H8}$ = 2.6 Hz, 2H, H-6), 2.66 (s, 2H, H-3), 3.68 (s, 3H, H-1), 3.95-4.03 (m, 4H, H-9).

^{13}C NMR (100 MHz, CDCl3): δ 12.7 (C-6), 36.3 (C-5), 42.5 (C-3), 51.8 (C-1), 65.2 (C-9), 68.1 (C-8), 83.9 (C-7), 108.2 (C-4), 169.6 (C-2).

HRMS (ESI^+): *m/z* found: 221.0789 $[M+Na]^+$, calculated $(C_{10}H_{14}NaO_4)^+$: 221.0784

The spectroscopic data are in agreement with those reported in the literature.[6]

2-(2-(but-3-yn-1-yl)-1,3-dioxolan-2-yl)ethan-1-ol (**3**)



A solution of ester **2** (6.00 g, 28.3 mmol, 1.0 equiv.) in THF (280 mL) was cooled to 0 °C under an atmosphere of nitrogen and treated with $LiAlH_4$ (1.61 g, 42.4 mmol, 1.5 equiv.) portion-wise. The reaction mixture was stirred at 0 °C for 2 h under nitrogen, after which the reaction was warmed to ambient temperature and stirred for a further 16 h. After this time, TLC analysis (Hexane-EtOAc, 1:1) showed total consumption of ester **2** (R_f = 0.5) and the formation of a new product (R_f = 0.2). The reaction was quenched by gradual addition to ice/dH_2O (150 mL) and volatiles were removed *in vacuo*. The crude was diluted with EtOAc (200 mL) and dH_2O (80 mL) and filtered through celite. The filtrate was separated and the organic extract was washed with saturated brine solution (150 mL) and dried over anhydrous $MgSO_4$. Volatiles were removed *in vacuo* to give methyl 2-(2-(but-3-yn-1-yl)-1,3-dioxolan-2-yl)acetate, **3** (4.17 g, 24.5 mmol, 87%) as a yellow oil.

ν_{max}/cm^{-1} (neat) 3370 (O-H), 3292 (C-H alkyne), 2959 (C-H), 2888 (C-H), 2117 (C≡C), 1490 (C-H), 1338, 1232 (C-O), 1125, 1044 (C-O), 948, 891, 826.

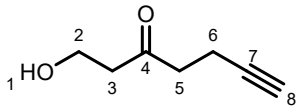
^1H NMR (400 MHz, $CDCl_3$): 1.90-1.96 (m, 5H, H-3 & H-5 & H-8), 2.26 (td, $J_{H5,H6}$ = 7.9 Hz, $J_{H6,H8}$ = 2.5 Hz, 2H, H-6), 2.49 (brs, 1H, H-1), 3.75 (t, $J_{H2,H3}$ = 5.5 Hz, 2H, H-2) 3.96-4.04 (m, 4H, H-9) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.1 (C-6), 35.9 (C-5), 38.2 (C-3), 58.7 (C-2), 64.9 (C-9), 68.2 (C-8), 83.9 (C-7), 111.0 (C-4) ppm.

HRMS (ESI^+): *m/z* found 171.1018 $[M+H]^+$, calculated $(C_9H_{15}O_3)^+$: 171.1016

The spectroscopic data are in agreement with those reported in the literature.[6]

1-Hydroxyhept-6-yn-3-one (**4**)



A solution of ketal **3** (5.90 g, 34.7 mmol, 1.0 equiv.) in THF (150 mL) was treated with 2 M HCl (77.0 mL, 154 mmol, 4.4 equiv.) and the resultant solution was stirred at ambient temperature for 60 h. After this time, TLC analysis (Hexane-EtOAc, 1:1) showed no resolution between ketal **3** (R_f = 0.2) and the new product (R_f = 0.2), however analysis of the crude product by IR confirmed the presence of an absorption band at ν=1708 cm^{-1} which was absent from the starting material. Volatiles were removed *in vacuo* and the crude mixture was diluted with EtOAc (200 mL) and washed with dH_2O (100 mL). The organic extract was washed with saturated brine solution (100 mL), dried over anhydrous $MgSO_4$ and purified by silica column chromatography (Hexane-EtOAc 1:1) to yield the desired product, 1-hydroxyhept-6-yn-3-one, **4** (3.06 g, 24.2 mmol, 70%) as a pale yellow oil.

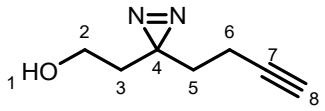
ν_{max}/cm^{-1} (neat) 3408 (O-H), 3288 (C-H alkyne), 2896 (C-H), 2118 (C≡C), 1708 (C=O), 1370, 1041 (C-OH), 943, 856, 637.

^1H NMR (400 MHz, $CDCl_3$) δ 1.95 (t, $J_{H6,H8}$ = 2.6 Hz, 1H, H-8), 2.26 (s, 1H, OH-1), 2.46 (td, $J_{H5,H6}$ = 7.1, $J_{H6,H8}$ = 2.6 Hz, 2H, H-6), 2.65-2.76 (m, 4H, H-3, H-5), 3.86 (t, $J_{H2,H3}$ = 5.4 Hz, 2H, H-2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7 (C-6), 41.7 (C-5), 44.5 (C-3), 57.7 (C-2), 68.9 (C-8), 82.7 (C-7), 209.0 (C-4) ppm.

The spectroscopic data are in agreement with those reported in the literature.[6]

2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-ol (**5**)



Ketone **4** (1 g, 7.93 mmol, 1.0 equiv.) was treated with anhydrous ammonia (20 mL, 787 mmol, 99.0 equiv.) at -78 °C. The solution was warmed to -30 °C and stirred for 5 h, before being cooled to -78 °C once again. A solution of hydroxylamine-*O*-sulfonic acid (1.17 g, 10.3 mmol, 1.30 equiv.) in anhydrous MeOH (25 mL) was added dropwise and the resultant mixture was warmed gradually to ambient temperature with stirring overnight in a sealed system. Following this, the system purged under a stream of nitrogen for 5 min. The resultant reaction mixture was filtered, cooled to 0 °C and treated with Et_3N (8.18 mL, 58.7 mmol, 7.4 equiv.) and then was treated with dropwise addition of a solution of iodine (2.62 g, 10.3 mmol, 1.3 equiv.) in anhydrous MeOH (22 mL) in darkness. The reaction was stirred 0 °C for 3 h. After this time, TLC analysis (Hexane-EtOAc, 1:1) showed total consumption of ketone **4** (R_f = 0.2) and the formation of a new product (R_f = 0.4). The reaction was concentrated *in vacuo* and the crude residue was diluted with Et_2O (80 mL) and washed with saturated brine solution (50 mL). The organic extract was dried over anhydrous $MgSO_4$ and purified by silica column chromatography (Hexane-EtOAc 1:1) to yield the desired product, 2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-ol, **5** (398 mg, 2.88 mmol, 36%) as a yellow oil.

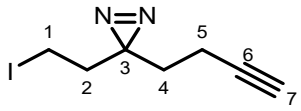
ν_{max}/cm^{-1} (neat) 3402 (O-H), 3296 (C-H alkyne), 2921 (C-H), 2119 (C≡C), 1586 (N=N), 1432 (C-H), 1333, 1260, 1154, 1048 (C-O), 866, 793.

^{1}H NMR (600 MHz, $CDCl_3$): δ 1.58 (brs, 1H, H-1), 1.68 (t, $J_{H5,H6}$ = 7.4 Hz, 2H, H-5), 1.70 (t, $J_{H2,H3}$ = 6.2 Hz, 2H, H-3), 2.00 (t, $J_{H6,H8}$ = 2.7 Hz, 1H, H-8), 2.04 (td, $J_{H5,H6}$ = 7.4 Hz, $J_{H6,H8}$ = 2.7 Hz, 2H, H-6), 3.48 (t, $J_{H2,H3}$ = 6.2 Hz, H-2) ppm.

^{13}C NMR (150 MHz, $CDCl_3$): δ 13.2 (C-6), 26.6 (C-4), 32.6 (C-5), 35.5 (C-3), 57.5 (C-2), 69.2 (C-8), 82.9 (C-7) ppm.

The spectroscopic data are in agreement with those reported in the literature.[7]

3-(but-3-yn-1-yl)-3-(2-iodoethyl)-3H-diazirine (**6**)



A solution of imidazole (584 mg, 8.58 mmol, 3.0 equiv.), triphenylphosphine (825 mg, 3.14 mmol, 1.1 equiv.) and iodine (871 mg, 3.43 mmol, 1.2 equiv.) in DCM (30 mL) was cooled to 0 °C. The mixture was treated with a solution of alcohol **5** (395 mg, 2.86 mmol, 1.0 equiv.) in DCM (10 mL) and stirred in darkness at 0 °C for 1 h and at ambient temperature for a further 13 h. After this time, TLC analysis (Hexane-EtOAc, 1:1) showed total consumption of alcohol **5** (R_f = 0.4) and the formation of a new product (R_f = 0.7). The reaction mixture was concentrated *in vacuo* and purified by silica column chromatography (Hexane-EtOAc 4:1) to yield the desired product, 3-(but-3-yn-1-yl)-3-(2-iodoethyl)-3H-diazirine, **6** (589 mg, 2.37 mmol, 83%) as a yellow oil.

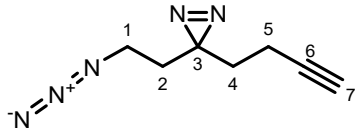
ν_{max}/cm^{-1} (neat) 3295 (C-H alkyne), 2922 (C-H), 2160 (C≡C), 1589 (N=N), 1429 (C-H), 1331, 1258, 1177, 636 (C-I).

^{1}H NMR (600 MHz, $CDCl_3$): δ 1.68 (t, $J_{H4,H5}$ = 7.4 Hz, 2H, H-4), 2.01 (t, $J_{H5,H7}$ = 2.6 Hz, 1H, H-7), 2.03 (td, $J_{H4,H5}$ = 7.4 Hz, $J_{H5,H7}$ = 2.6 Hz, 2H, H-5), 2.12 (t, $J_{H1,H2}$ = 7.6 Hz, 2H, H-2), 2.89 (t, $J_{H1,H2}$ = 7.6 Hz, 2H, H-1) ppm.

^{13}C NMR (150 MHz, $CDCl_3$): δ -4.0 (C-1), 13.3 (C-5), 28.6 (C-3), 31.8 (C-4), 37.5 (C-2), 69.4 (C-7), 82.4 (C-6) ppm.

The spectroscopic data are in agreement with those reported in the literature.[8]

3-(2-azidoethyl)-3-(but-3-yn-1-yl)-3H-diazirine (**7**)



A solution of alkyl iodide **6** (575 mg, 2.32 mmol, 1.0 equiv.) in DMF (10 mL) was treated with sodium azide (166 mg, 2.55 mmol, 1.1 equiv.) in darkness. The solution was heated to 70 °C under an atmosphere of nitrogen for 12 h. After this time, TLC analysis (Hexane-EtOAc, 9:1) showed total consumption of starting material **6** (R_f = 0.3) and the formation of a new product (R_f = 0.2). The reaction was cooled to ambient temperature and separated between EtOAc (150 mL) and dH_2O (100 mL). The organic extract was washed with saturated brine solution (100 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield the desired product 3-(2-azidoethyl)-3-(but-3-yn-1-yl)-3H-diazirine, **7** (377.1 mg, 2.31 mmol, 99%) as an orange oil.

ν_{max}/cm^{-1} (neat) 3300 (C-H alkyne), 2925 (C-H), 2094 (N=N=N), 1587 (N=N), 1433 (C-H), 1265, 1027, 914, 834, 637, 556.

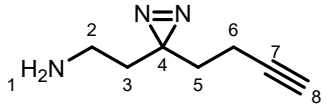
^{1}H NMR (600 MHz, $CDCl_3$): δ 1.68 (t, $J_{H4,H5}$ = 7.3 Hz, 2H, H-4), 1.70 (t, $J_{H1,H2}$ = 6.9 Hz, 2H, H-2), 2.01 (t, $J_{H5,H7}$ = 2.6 Hz, 1H, H-7), 2.03 (td, $J_{H4,H5}$ = 7.3 Hz, $J_{H5,H7}$ = 2.6 Hz, 2H, H-5), 3.16 (t, $J_{H1,H2}$ = 6.9 Hz, 2H, H-1) ppm.

^{13}C NMR (150 MHz, $CDCl_3$): δ 13.2 (C-5), 26.4 (C-3), 32.3 (C-4), 32.5 (C-2), 45.9 (C-1), 69.4 (C-7), 82.5 (C-6) ppm.

HRMS (ESI$^+$): *m/z* found: 164.0932 $[M+H]^+$, calculated $(C_7H_{10}N_5)^+$: 164.0931

The spectroscopic data are in agreement with those reported in the literature.[8]

2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-amine (**8**)



A solution of azide **7** (300 mg, 1.84 mmol, 1.0 equiv.) in THF (8 mL) and dH_2O (0.84 mL) was treated with triphenylphosphine (555 mg, 2.11 mmol, 1.15 equiv.) in darkness. The resultant solution was stirred at ambient temperature for 24 h. After this time, TLC analysis (Hexane-EtOAc, 4:1) showed total consumption of azide **7** (R_f = 0.5) and the formation of a new product (R_f = 0.0). The reaction was diluted with Et_2O (50 mL) and extracted with 1 M HCl (25 mL). The aqueous extract was adjusted to pH 14 by treatment with 1 M NaOH and extracted twice with Et_2O (50 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and purified by silica column chromatography (DCM-MeOH-NH_3, 57:3:1) to yield the desired product, 2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-amine, **8** (91.9 mg, 0.67 mmol, 37%) as a yellow oil.

ν_{max}/cm^{-1} (neat) 3411 (N-H), 3292 (C-H alkyne), 2919 (C-H), 2118 (C≡C), 1570 (N=N), 1433 (C-H), 1317, 1146, 1032, 910, 818, 697.

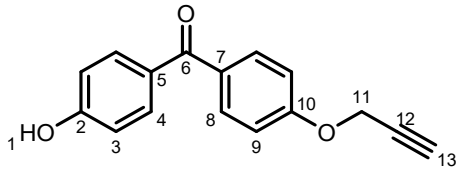
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.64 (t, $J_{H2,H3}$ = 6.9 Hz, 2H, H-3), 1.65 (t, $J_{H5,H6}$ = 7.4 Hz, 2H, H-5), 1.78 (brs, 2H, H-1), 1.99 (t, $J_{H6,H8}$ = 2.5 Hz, 1H, H-8), 2.02 (td, $J_{H5,H6}$ = 7.4 Hz, $J_{H6,H8}$ = 2.5 Hz, 2H, H-6), 2.53 (t, $J_{H13,H15}$ = 6.9 Hz, 2H, H-2) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.3 (C-6), 26.9 (C-4), 32.6 (C-5), 35.9 (C-3), 36.6 (C-2), 69.2 (C-8), 82.7 (C-7) ppm.

HRMS (ESI$^+$): *m/z* found: 138.1024 $[M+H]^+$, calculated $(C_7H_{12}N_3)^+$: 138.1026

The spectroscopic data are in agreement with those reported in the literature.[9]

(4-hydroxyphenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanone (**9**)



A solution of 4,4'-dihydroxybenzophone (1.0 g, 4.67 mmol, 2.0 equiv.) in acetone (10.0 mL) was cooled to 0 °C and treated with K_2CO_3 (326 mg, 2.36 mmol, 1.01 equiv.). Propargyl bromide (80% in toluene, 0.25 mL, 2.33 mmol, 1.0 equiv.) was added dropwise and the reaction was stirred at 50 °C for 3 h. After this time, TLC analysis (Hexane-EtOAc, 7:3) showed partial consumption of dihydroxybenzophone (R_f = 0.05) and the formation of two new products (R_f = 0.1) and (R_f = 0.3). Volatiles were removed *in vacuo* and the crude residue was redissolved in EtOAc (50 mL) and washed with dH_2O (40 mL). The organic extract was washed with saturated brine solution (40 mL) and dried over anhydrous $MgSO_4$. The crude product was purified by silica column chromatography (Hexane-EtOAc, 7:3) to yield the desired product, (4-hydroxyphenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanone, **9,** (471.9 mg, 1.87 mmol, 80%) as a pale orange solid.

mp 154-156 °C (Hexane-EtOAc)

ν_{max}/cm^{-1} (neat) 3276 (C-H alkyne) 3223 (O-H), 3068 (C-H ar), 2973 (C-H), 2113 (C≡C), 1588 (C=O), 1503 (C=C), 1429 (C-H), 1285 (C-O), 1166 (C-OH), 1014, 927, 850, 772, 685, 591.

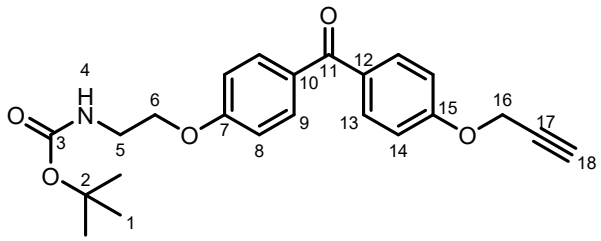
^{1}H NMR (400 MHz, DMSO-d_6): δ 3.64 (t, $J_{H11,H13}$ = 2.3 Hz, 1H, H-13), 4.91 (d, $J_{H11,H13}$ = 2.3 Hz, 2H, H-11), 6.89 (d, $J_{H3,H4}$ = 8.7 Hz, 2H, H-3), 7.12 (d, $J_{H8,H9}$ = 8.8 Hz, 2H, H-9), 7.63 (d, $J_{H3,H4}$ = 8.7 Hz, 2H, H-4), 7.69 (d, $J_{H8,H9}$ = 8.8 Hz, 2H, H-8), 10.34 (s, 1H, H-1) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ 55.6 (C-11), 78.7 (C-13), 78.8 (C-12), 114.5 (C-9), 115.1 (C-3), 128.3 (C-5), 131.0 (C-7), 131.5 (C-8), 132.2 (C-4), 160.1 (C-10), 161.5 (C-2), 193.0 (C-6) ppm.

HRMS (ESI$^+$): *m/z* found: 275.0681 $[M+Na]^+$, calculated $(C_{16}H_{12}NaO_3)^+$: 275.0679

The spectroscopic data are in agreement with those reported in the literature.[10]

Tert-butyl (2-(4-(4-(prop-2-yn-1-yloxy)benzoyl)phenoxy)ethyl)carbamate (**10**)



A solution of aryl alcohol **9** (150 mg, 0.60 mmol, 1.0 equiv.) in acetone (5 mL) was cooled to 0 °C and treated with K_2CO_3 (164 mg, 1.19 mmol, 2.0 equiv.). A solution of *tert*-butyl (2-bromoethyl)carbamate (160 mg, 0.71 mmol, 1.2 equiv.) in acetone (1 mL) was added dropwise and the mixture was stirred at 50 °C for 16 h. After this time, TLC analysis (Hexane-EtOAc, 3:1) showed partial consumption of **9** (R_f = 0.1) and the formation of a new product (R_f = 0.3). The reaction was cooled to ambient temperature, concentrated *in vacuo* and redissolved in EtOAc (50 mL). The organic phase was washed with dH_2O (30 mL) and saturated brine solution (20 mL), dried over anhydrous $MgSO_4$ and purified by silica column chromatography (Hexane-EtOAc, 3:1) to yield the desired product *tert*-Butyl(2-(4-(4-(prop-2-yn-1-yloxy)benzoyl)-phenoxy)ethyl)carbamate, **10** (69.2 mg, 0.16 mmol, 27%) as a colourless oil.

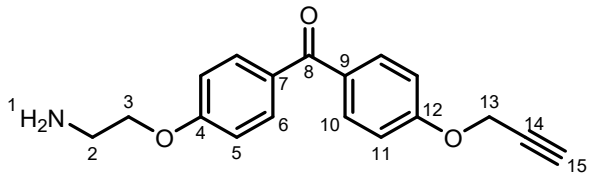
ν_{max}/cm^{-1} (neat) 3359 (N-H), 3292 (C-H alkyne), 3072 (C-H ar), 2977 (C-H), 2932 (C-H), 2128 (C≡C), 1703 (C=O), 1598 (C=O), 1505 (C=C), 1456 (C-H), 1285 (C-N), 1164 (C-O), 1020, 925, 849, 768, 682, 596.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (s, 9H, H-1), 2.56 (t, $J_{H16,H18}$ = 2.4 Hz, 2H, H-18), 3.57 (app. q, J = 5.0 Hz, 2H, H-5), 4.10 (t, $J_{H5,H6}$ = 5.0 Hz, 2H, H-6), 4.78 (d, $J_{H16,H18}$ = 2.4 Hz, 2H, H-16), 4.99 (app. brs, 1H, H-4), 6.96 (d, $J_{H8,H9}$ = 8.8 Hz, 2H, H-8), 7.05 (d, $J_{H13,H14}$ = 8.8 Hz, 2H, H-14), 7.78 (d, $J_{H8,H9}$ = 8.8 Hz, 2H, H-9), 7.79 (d, $J_{H13,H14}$ = 8.8 Hz, 2H, H-13) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.4 (C-1), 40.0 (C-5), 55.9 (C-16), 67.4 (C-6), 76.1 (C-18), 77.8 (C-17), 79.8 (C-2), 114.0 (C-8), 114.4 (C-14), 131.0 (C-10), 131.5 (C-12), 132.1 (C-9), 132.3 (C-13), 155.8 (C-3), 160.7 (C-15), 161.9 (C-7), 194.3 (C-11) ppm.

HRMS (APCI$^-$): *m/z* found: 394.1661 $[M-H]^-$, calculated $(C_2H_{24}NO_5)^-$: 394.1660

(4-(2-aminoethoxy)phenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanone (**11**)



A solution of Boc-protected **10** (60.0 mg, 0.15 mmol, 1.0 equiv.) in DCM (4 mL) was treated with TFA (0.06 mL, 0.76 mmol, 5.0 equiv.) and stirred at ambient temperature for 16 h under an atmosphere of nitrogen. After this time, TLC analysis (DCM-MeOH-Et_3N, 94:5:1) showed total consumption of Boc-protected **10** (R_f = 0.3) and the formation of a new product (R_f = 0.1). The reaction was diluted with DCM (15 mL) and washed with saturated aq. sodium bicarbonate solution (15 mL). The organic extract was washed with saturated brine solution (10 mL) and dried over anhydrous $MgSO_4$. Volatiles were removed *in vacuo* and the crude residue was recrystallised (Hexane-EtOAc, 1:1) to give (4-(2-aminoethoxy)phenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanone, **11** (17.8 mg, 0.06 mmol, 40%) as a white solid.

mp 92-94 °C (Hexane-EtOAc)

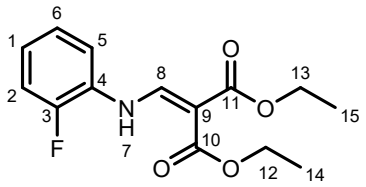
ν_{max}/cm^{-1} (neat) 3377 (N-H), 3294 (C-H alkyne), 3069 (C-H ar), 2936 (C-H), 2134 (C≡C), 1601 (C=O), 1504 (C=C), 1251, 1172 (C-O), 1151 (C-O), 1021, 927, 852, 763, 687, 631.

^{1}H NMR (600 MHz, DMSO-d_6): δ 2.91 (t, $J_{H2,H3}$ = 5.6 Hz, 2H, H-2), 3.64 (t, $J_{H13,H15}$ = 2.4 Hz, 1H, H-15), 4.02 (t, $J_{H13,H15}$ = 5.6 Hz, 2H, H-3), 4.92 (d, $J_{H13,H15}$ = 2.4 Hz, 2H, H-13), 7.08 (d, $J_{H5,H6}$ = 8.8 Hz, 2H, H-5), 7.13 (d, $J_{H10,H11}$ = 8.8 Hz, 2H, H-11), 7.70 (d, $J_{H5,H6}$ = 8.8 Hz, 2H, H-6), 7.72 (d, $J_{H10,H11}$ = 8.8 Hz, 2H, H-10) ppm.

^{13}C NMR (150 MHz, DMSO-d_6): δ 40.8 (C-2), 55.7 (C-13), 70.6 (C-3), 78.7 (C-15), 78.8 (C-14), 114.3 (C-5), 114.6 (C-11), 129.8 (C-7), 130.7 (C-9), 131.7 (C-10), 131.9 (C-6), 160.4 (C-12), 162.1 (C-4), 193.2 (C-8) ppm.

HRMS (ESI$^+$): *m/z* found: 296.1287 $[M+H]^+$, calculated $(C_{18}H_{18}NO_3)^+$: 296.1281

Diethyl 2-(((2-fluorophenyl)amino)methylene)malonate (**12**)



A solution of 2-fluoroaniline (1.00 g, 9.00 mmol. 1.0 equiv.) in EtOH (20 mL) was treated with diethyl ethoxymethylenemalonate (2.04 g, 9.45 mmol, 1.05 equiv.) and heated to reflux for 3 h under an atmosphere of nitrogen. After this time, TLC analysis (toluene-acetone, 19:1) showed partial consumption of starting aniline (R_f = 0.4) and the formation of a new product (R_f = 0.5). The reaction was cooled to ambient temperature and cooled to 0 °C for 1 h, whereupon white, needle-shaped crystals were observed to form. Solids were collected by filtration and dried *in vacuo* to yield the desired product diethyl 2-(((2-fluorophenyl)amino)methylene)malonate, **12** (1.25 g, 4.44 mmol, 49%) was collected as a flocculent white solid.

mp 74-77 °C (EtOH), (lit.[11] 80-81 °C)

ν_{max}/cm^{-1} (neat) 3158 (N-H), 3058 (C-H ar), 2984 (C-H), 2904 (C-H), 1680 (C=O), 1642 (C=O), 1609 (C=C), 1580 (C=C), 1511 (C=C), 1426 (C-H), 1389, 1345 (C-F), 1247, 1225 (C-O), 1095, 1028, 982, 916, 854, 800, 770, 747.

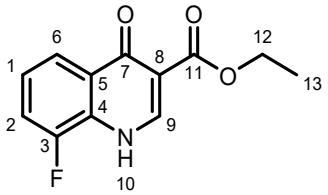
^{1}H NMR (600 MHz, $CDCl_3$): δ 1.33 (t, $J_{H13,H15}$ = 7.1 Hz, 3H, H-15), 1.38 (t, $J_{H12,H14}$ = 7.1 Hz, 3H, H-14), 4.26 (q, $J_{H13,H15}$ = 7.1 Hz, 2H, H-13), 4.33 (q, $J_{H12,H14}$ = 7.1 Hz, 2H, H-12), 7.06-7.11 (m, 1H, H-1), 7.13-7.19 (m, 2H, H-6 & H-2), 7.29 (app. t, J = 8.0 Hz, 1H, H-5), 8.51 (d, $J_{H7,H8}$ = 13.4 Hz, 1H, H-8), 11.06 (d, $J_{H7,H8}$ = 13.4 Hz, 1H, H-7) ppm.

^{13}C NMR (150 MHz, $CDCl_3$): δ 14.3 (C-14), 14.4 (C-15), 60.3 (C-13), 60.6 (C-12), 95.1 (C-9), 116.26 (d, $J_{C5,F3}$ = 1.1 Hz, C-5), 116.30 (d, $J_{C2,F3}$ = 18.7 Hz, C-2), 124.98 (d, $J_{C1,F3}$ = 3.8 Hz, C-1), 125.02 (C-6), 127.9 (d, $J_{C4,F3}$ = 10.6 Hz, C-4), 150.9 (C-8), 152.8 (d, $J_{C3,F3}$ = 244.9 Hz, C-3), 165.6 (C-11), 168.6 (C-10) ppm.

HRMS (ESI$^+$): *m/z* found: 304.0961 $[M+Na]^+$, calculated $(C_{14}H_{16}FNNaO_4)^+$: 304.0961

The spectroscopic data are in agreement with those reported in the literature.[12]

Ethyl 8-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**13**)



A solution of fluorophenyl **12** (1 g, 3.56 mmol, 1.0 equiv.) in diphenyl ether (10 mL) was heated to reflux at 250 °C for 1 h. The reaction was cooled to ambient temperature and TLC analysis (PE-EtOAc, 3:1) showed consumption of starting material **12** (R_f = 0.2) and the formation of a new product (R_f = 0.05). The reaction mixture was triturated in PE (20 mL) and stirred for 1 h. The resultant precipitate was collected and solids were washed with PE (15 mL) to yield the desired product ethyl 8-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate, **13** (0.53 g, 2.24 mmol, 63%) as a pale yellow powder.

mp 208-210 °C (PE-diphenyl ether), (lit.[13] 210-212 °C)

ν_{max}/cm^{-1} (neat) 3075 (C-H ar), 3030 (N-H), 2980 (C-H), 1711 (C=O), 1540 (C=C), 1450 (C-H), 1356 (C-F), 1250, 1129 (C-O), 1033, 976, 922, 779.

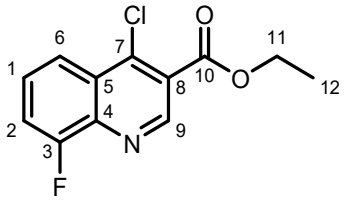
^{1}H NMR (400 MHz, DMSO-d_6): δ 1.28 (t, $J_{H12,H13}$ = 7.0 Hz, 3H, H-13), 4.22 (q, $J_{H12,H13}$ = 7.0 Hz, 2H, H-12), 7.40 (ddd, $J_{H1,H2}$ = 8.1 Hz, $J_{H1,H6}$ = 8.0 Hz, $J_{H1,F3}$ = 5.1 Hz, 1H, H-1), 7.65 (dd, $J_{H2,F3}$ = 11.0 Hz, $J_{H1,H2}$ = 8.1 Hz, 1H, H-2), 7.97 (d, $J_{H1,H6}$ = 8 Hz, 1H, H-6), 8.39 (s, 1H, H-9), 12.46 (s, 1H, H-10) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ 14.2 (C-13), 59.8 (C-12), 110.5 (C-8), 117.3 (d, $J_{C2,F3}$ = 16.7 Hz, C-2), 121.3 (d, $J_{C6,F3}$ = 3.3 Hz, C-6), 124.5 (d, $J_{C1,F3}$ = 6.9 Hz, C-1), 128.1 (d, $J_{C4,F3}$ = 9.9 Hz, C-4), 129.1 (C-5), 144.6 (C-9), 151.8 (d, $J_{C3,F3}$ = 247.3 Hz, C-3), 164.4 (C-11), 172.5 (C-7) ppm.

HRMS (ESI$^+$): *m/z* found: 236.0720 $[M+H]^+$, calculated $(C_{12}H_{11}FNO_3)^+$: 236.0717

The spectroscopic data are in agreement with those reported in the literature.[14]

Ethyl 4-chloro-8-fluoroquinoline-3-carboxylate (**14**)



Quinolone **13** (4.6 g, 19.6 mmol, 1.0 equiv.) was dissolved in $POCl_3$ (25.5 mL, 274 mmol, 14 equiv.) and heated to 100 °C for 3 h under an atmosphere of nitrogen. After this time, TLC analysis (PE-EtOAc, 3:1) showed partial consumption of starting material **13** (R_f = 0.05) and the formation of a new product (R_f = 0.4). The reaction was cooled to ambient temperature, concentrated *in vacuo*, diluted with DCM (200 mL) and washed with dH_2O (150 mL). The organic extract was washed with saturated aq. sodium bicarbonate solution (150 mL), saturated brine solution (100 mL) and dried over anhydrous $MgSO_4$. The organic extract was triturated by stirring in Et_2O (40 mL) and the precipitate was collected by filtration. Residual product in the filtrate was purified by silica column chromatography (PE-EtOAc 4:1) and products were combined to yield the desired product ethyl 4-chloro-8-fluoroquinoline-3-carboxylate (4.08 g, 16.1 mmol, 84%) as a cream solid.

mp 76-78 °C (PE-EtOAc)

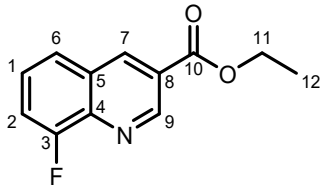
ν_{max}/cm^{-1} (neat) 3084 (C-H ar), 2993 (C-H), 1720 (C=O), 1581 (C=C), 1480 (C-H), 1355 (C-F), 1243 (C-O), 1162 (C-O), 1105, 1025, 968, 868, 775.

^{1}H NMR (600 MHz, $CDCl_3$): δ 1.47 (t, $J_{H11,H12}$ = 7.2 Hz, 3H, H-12), 4.51 (q, $J_{H11,H12}$ = 7.2 Hz, 2H, H-11), 7.56 (ddd, $J_{H2,F3}$ = 9.9 Hz, $J_{H1,H2}$ = 7.8 Hz, $J_{H2,H6}$ = 1.2 Hz, 1H, H-2), 7.66 (ddd, $J_{H1,H6}$ = 8.5, $J_{H1,H2}$ = 7.8, $J_{H1,F3}$ = 5.0 Hz, 1H, H-1), 8.21 (ddd, $J_{H1,H6}$ = 8.5 Hz, $J_{H2,H6}$ = 1.2 Hz, $J_{H6,F3}$ = 1.0 Hz, 1H, H-6), 9.23 (s, 1H, H-9) ppm.

^{13}C NMR (150 MHz, $CDCl_3$): δ 14.2 (C-12), 62.3 (C-11), 116.1 (d, $J_{C2,F3}$ = 18.8 Hz, C-2), 121.2 (d, $J_{C6,F3}$ = 4.8 Hz, C-6), 124.1 (C-8), 127.8 (d, $J_{C5,F3}$ = 1.4 Hz, C-5), 128.2 (d, $J_{C1,F3}$ = 8.1 Hz, C-1), 139.7 (d, $J_{C4,F3}$ = 12.3 Hz, C-4), 143.4 (C-7), 150.1 (C-9), 157.9 (d, $J_{C3,F3}$ = 256.9 Hz, C-3), 164.2 (C-10) ppm.

The spectroscopic data are in agreement with those reported in the literature.[12]

Ethyl 8-fluoroquinoline-3-carboxylate (**15**)



A solution of chloroquinoline **14** (2.92 g, 11.5 mmol, 1.0 equiv.) in MeCN (115 mL) was treated with triethylsilane (3.68 mL, 23.0 mmol, 2 equiv.) and purged with nitrogen for 5 min. The solution was treated with bis(triphenylphosphine)palladium(II) dichloride (404 mg, 0.58 mmol, 0.05 equiv.) and purged with nitrogen for a further 5 min, following which, the reaction mixture was heated to reflux for 16 h under an atmosphere of nitrogen. After this time, TLC analysis (PE-EtOAc, 4:1) showed partial consumption of chloroquinoline **14** (R_f = 0.3) and the formation of a new product (R_f = 0.2). The reaction was cooled to ambient temperature and concentrated *in vacuo*. The crude residue was dissolved in DCM (150 mL), washed with saturated aq. sodium bicarbonate solution (100 mL) and dried over anhydrous $MgSO_4$. The crude product was purified by silica column chromatography (PE-EtOAc, 4:1) to yield the desired product ethyl-8-fluoroquinoline-3-carboxylate (1.55 g, 7.08 mmol, 62%) as a pale yellow crystalline solid.

mp 64-66 °C (PE-EtOAc), (lit.[13] 51-53 °C)

ν_{max}/cm^{-1} (neat) 3052 (C-H ar), 2990 (C-H), 2909 (C-H), 1711 (C=O), 1608 (C=C), 1498 (C=C), 1474 (C-H), 1377 (C-F), 1271 (C-O), 1225 (C-O), 1190, 1114, 1023, 873, 832, 773, 710

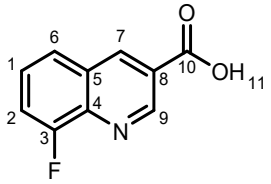
^{1}H NMR (600 MHz, $CDCl_3$): δ 1.46 (t, $J_{H11,H12}$ = 7.2 Hz, 3H, H-12), 4.49 (q, $J_{H11,H12}$ = 7.2 Hz, 2H, H-11), 7.53 (ddd, $J_{H2,F3}$ = 10.2 Hz, $J_{H1,H2}$ = 7.8 Hz, $J_{H2,H6}$ = 1.3 Hz, 1H, H-2), 7.57 (ddd, $J_{H1,H6}$ = 8.1 Hz, $J_{H1,H2}$ = 7.8 Hz, $J_{H1,F3}$ = 4.9 Hz, 1H, H-1), 7.75 (dd, $J_{H1,H6}$ = 8.1 Hz, $J_{H2,H6}$ = 1.3 Hz, 1H, H-6), 8.87 (d, $J_{H7,H9}$ = 2.0 Hz, 1H, H-7), 9.49 (d, $J_{H7,H9}$ = 2.0 Hz, 1H, H-9) ppm.

^{13}C NMR (150 MHz, $CDCl_3$): δ 14.3 (C-12), 61.8 (C-11), 115.9 (d, J = 18.8 Hz, C-2), 124.4 (C-8), 124.7 (d, $J_{C6,F3}$ = 4.8 Hz, C-6), 127.4 (d, $J_{C1,F3}$ = 7.8 Hz, C-1), 128.5 (d, $J_{C5,F3}$ = 2.1 Hz, C-5), 138.4 (C-7), 139.8 (d, $J_{C4,F3}$ = 11.7 Hz, C-4), 150.2 (C-9), 157.9 (d, $J_{C3,F3}$ = 256.6 Hz, C-3), 165.0 (C-10) ppm.

HRMS (APCI$^+$): *m/z* found: 220.0771 $[M+H]^+$, calculated $(C_{12}H_{11}FNO_2)^+$: 220.0768

The spectroscopic data are in agreement with those reported in the literature.[15]

8-fluoroquinoline-3-carboxylic acid (**16**)



A solution of ester **15** (1.00 g, 4.56 mmol, 1.0 equiv.) in THF (40 mL) and dH_2O (10 mL) was treated with LiOH (2.18 g, 91.2 mmol, 20 equiv.) and heated to reflux at 75 °C for 24 h under an atmosphere of nitrogen. After this time, TLC analysis (DCM-MeOH-AcOH, 379:20:1) showed total consumption of ester **15** (R_f = 0.9) and the formation of a new product (R_f = 0.1). The reaction was cooled to ambient temperature, diluted with DCM (160 mL) and quenched with 5% aq. citric acid solution (100 mL). The organic extract was washed with saturated brine solution (100 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield the desired product 8-fluoroquinoline-3-carboxylic acid, **16** (836 mg, 4.37 mmol, 96%) as a white solid.

mp 240-245 °C (DCM-THF), (lit.[13] 240 °C)

ν_{max}/cm^{-1} (neat) 3071 (C-H ar), 2453 (O-H), 1703 (C=O), 1608 (C=C), 1471 (C-H), 1378, 1230 (C-O), 1081, 983, 778, 722, 619

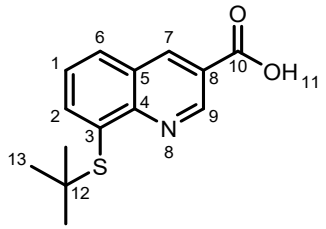
^{1}H NMR (400 MHz, DMSO-d_6): δ 7.64-7.75 (m, 2H, H-1 & H-2), 8.02 (d, $J_{H1,H6}$ = 7.5 Hz, 1H, H-6), 9.01 (s, 1H, H-7), 9.36 (s, 1H, H-9) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ 115.8 (d, $J_{C2,F3}$ = 18.3 Hz, C-2), 125.5 (d, $J_{C6,F3}$ = 4.5 Hz, C-6), 125.9 (C-8), 127.5 (d, $J_{C1,F3}$ = 8.0 Hz, C-1), 128.5 (d, $J_{C5,F3}$ = 2.0 Hz, C-5), 138.0 (C-7), 138.7 (d, $J_{C4,F3}$ = 11.4 Hz, C-4), 150.4 (C-9), 157.0 (d, $J_{C3,F3}$ = 253.4 Hz, C-3), 166.3 (C-10) ppm.

HRMS (APCI$^+$): *m/z* found: 192.0452 $[M+H]^+$, calculated $(C_{10}H_7FNO_2)^+$: 192.0455

The spectroscopic data are in agreement with those reported in the literature.[15]

8-(tert-butylthio)quinoline-3-carboxylic acid (**17**)



A solution of 8-fluoroquinoline **16** (920 mg, 4.81 mmol, 1.0 equiv.) in DMF (35 mL) was treated with 60% NaH dispersion in mineral oil (462 mg, 19.3 mmol, 4.0 equiv.) followed by 2-methyl-2-propanethiol (1.74 g, 19.3 mmol, 4.0 equiv). The reaction was heated to 140 °C and stirred for 34 h under an atmosphere of nitrogen. After this time, TLC analysis (DCM-MeOH-AcOH, 379:20:1) showed majority consumption of 8-fluoroaniline **16** (R_f = 0.1) and the formation of a new product (R_f = 0.3). The reaction was cooled to ambient temperature, concentrated *in vacuo* and the crude residue was diluted in DCM (100 mL). The organic extract was washed with 10% aq. citric acid solution (100 mL) and saturated brine solution (100 mL), dried over anhydrous $MgSO_4$ and purified by silica column chromatography (DCM-MeOH-AcOH, 379:20:1) to yield the desired product 8-(*tert*-butylthio)quinoline-3-carboxylic acid, **17** (964 mg, 3.69 mmol, 77%) as a yellow solid. Unreacted 8-fluoroquinoline **16** (117.9 mg, 0.62 mmol, 13%) was also recovered.

mp 204-207 °C (DCM-MeOH-AcOH)

ν_{max}/cm^{-1} (neat) 3064 (C-H ar), 2860 (C-H), 2435 (O-H), 1697 (C=O), 1617 (C=C), 1489 (C-H), 1362, 1230 (C-O), 996, 945 (O-H), 781 (C-H), 743 (C-S), 681 (C-S), 596.

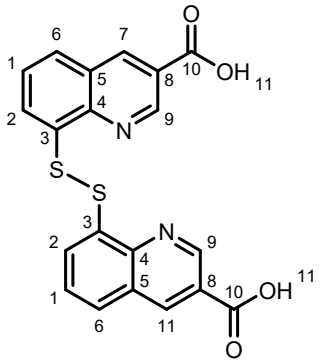
^{1}H NMR (600 MHz, DMSO-d_6): δ 1.31 (s, 9H, H-13), 7.69 (dd, $J_{H1,H6}$ = 8.1 Hz, $J_{H1,H2}$ = 7.2 Hz, 1H, H-1), 8.11 (dd, $J_{H1,H2}$ = 7.2 Hz, $J_{H2,H6}$ = 1.3 Hz, 1H, H-2), 8.19 (dd, $J_{H1,H6}$ = 8.1 Hz, $J_{H2,H6}$ = 1.3 Hz 1H, H-6), 8.98 (d, $J_{H7,H9}$ = 2.1 Hz, 1H, H-7), 9.35 (d, $J_{H7,H9}$ = 2.1 Hz, 1H, H-9) ppm.

^{13}C NMR (600 MHz, DMSO-d_6): δ 31.2 (C-13), 46.7 (C-12), 123.9 (C-8), 127.1 (C-1), 127.4 (C-5), 130.1 (C-6), 133.8 (C-3), 139.1 (C-7), 139.3 (C-2), 149.6 (C-9), 149.8 (C-4), 166.2 (C-10) ppm.

HRMS (APCI$^+$): *m/z* found: 262.0896 $[M+H]^+$, calculated $(C_{14}H_{16}NO_2S)^+$: 262.0896

The spectroscopic data are in agreement with those reported in the literature.[1]

8,8'-disulfanediylbis(quinoline-3-carboxylic acid) (**18**)



A slurry of thioether **17** (900 mg, 3.44 mmol, 1.0 equiv.) was dissolved in conc. HCl (65 mL) and heated to 100 °C for 20 h under an atmosphere of nitrogen. After this time, TLC analysis (DCM-MeOH-AcOH, 179:20:1) showed total consumption of starting material **17** (R_f = 0.4) and the formation of a new product (R_f = 0.1). The reaction was cooled to ambient temperature, pipetted dropwise on to ice and adjusted to pH 2 with addition of saturated aq. sodium bicarbonate solution. The resulting beige precipitate was collected and washed with dH_2O and dried *in vacuo*. The retained filtrate, a red solution, was stood in a flask at ambient temperature for 7 days with the flask vented, resulting in an additional portion of beige precipitate in a yellow solution. Solids were collected, washed with dH_2O and dried *in vacuo*. Solids were combined to yield the desired product, 8,8'-disulfanediylbis(quinoline-3-carboxylic acid), **18** (692 mg, 1.69 mmol, 98%) as a beige solid.

mp 302 °C (decomp.), (dH_2O)

ν_{max}/cm^{-1} (neat) 3064 (C-H ar), 2868 (C-H), 2571 (O-H), 2499 (O-H), 1694 (C=O), 1654, 1608 (C=C), 1463 (C-H), 1257 (C-O), 1185, 988, 926, 780, 741, 663 (C-S), 607 (S-S).

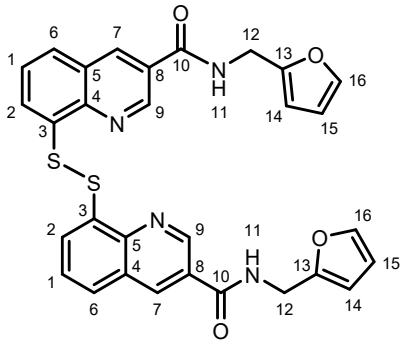
^{1}H NMR (400 MHz, DMSO-d_6): δ 7.63 (app. t, J = 7.8 Hz, 2H, H-1), 7.88 (dd, $J_{H1,H2}$ = 7.5 Hz, $J_{H2,H6}$ = 1.1 Hz, 2H, H-2), 8.06 (dd, $J_{H1,H6}$ = 8.3 Hz, $J_{H2,H6}$ = 1.1 Hz, 2H, H-6), 9.04 (d, $J_{H7,H9}$ = 2.1 Hz, 2H, H-7), 9.41 (d, $J_{H7,H9}$ = 2.1 Hz, 2H, H-9) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ 124.7 (C-8), 126.3 (C-2), 127.1 (C-5), 127.3 (C-6), 128.0 (C-1), 134.3 (C-3), 138.9 (C-7), 146.3 (C-4), 149.4 (C-9), 166.1 (C-10) ppm.

HRMS (APCI$^+$): *m/z* found: 409.0314 $[M+H]^+$, calculated $(C_{20}H_{13}N_2O_4S_2)^+$: 409.0311

The spectroscopic data are in agreement with those reported in the literature.[1]

8,8'-disulfanediylbis(N-(furan-2-ylmethyl)quinoline-3-carboxamide) (**19**)



A solution of carboxylic acid **18** (100 mg, 0.25 mmol, 1.0 equiv.) in DMF (4 mL) was treated with carbonyldiimidazole (118 mg, 0.73 mmol, 3.0 equiv.) and stirred at ambient temperature for 15 min. The mixture was then treated with a solution of furfurylamine (71.3 mg, 0.73 mmol, 3.0 equiv.) in DMF (1 mL) and the reaction mixture was stirred at ambient temperature for 48 h under an atmosphere of nitrogen. After this time, TLC analysis (DCM-MeOH-AcOH, 179:20:1) showed total consumption of starting material **18** (R_f = 0.1) and the formation of a new product (R_f = 0.4). The reaction was diluted with EtOAc (100 mL) and washed with saturated aq. sodium bicarbonate solution (75 mL) and saturated brine solution (75 mL). The organic extract was dried over anhydrous $MgSO_4$ and crude product was purified by silica column chromatography (Hexane-EtOAc, 1:1). The partially purified product was recrystallised from EtOAc to yield the desired product 8,8'-disulfanediylbis(*N*-(furan-2-ylmethyl)quinoline-3-carboxamide), **19** (24.6 mg, 0.04 mmol, 18%) as a white solid.

mp 244-247 °C (EtOAc)

ν_{max}/cm^{-1} (neat) 3276 (N-H), 3054 (C-H ar), 2925 (C-H), 1637 (C=O), 1543 (C=C), 1286 (C-O), 1147 (C-O), 1014, 916, 745, 663 (C-S), 598 (S-S).

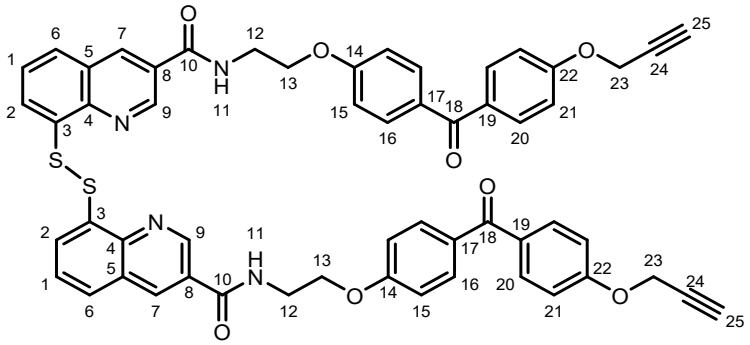
^{1}H NMR (600 MHz, DMSO-d$_6$): δ 4.58 (d, $J_{H11,H12}$ = 5.6 Hz, 4H, H-12), 6.38 (d, $J_{H14,H15}$ = 2.9 Hz, 2H, H-14), 6.44 (dd, $J_{H14,H15}$ = 2.9 Hz, $J_{H15,H16}$ = 1.9 Hz, 2H, H-15), 7.60-7.64 (m, 4H, H-1 & H-16), 7.85 (dd, $J_{H1,H2}$ = 7.5 Hz, $J_{H2,H6}$ = 1.1 Hz, 2H, H-2), 7.96 (dd, $J_{H1,H6}$ = 8.3 Hz, $J_{H2,H6}$ = 1.1 Hz, 2H, H-6), 8.92 (d, $J_{H7,H9}$ = 2.1 Hz, 2H, H-7), 9.38 (d, $J_{H7,H9}$ = 2.1 Hz, 2H, H-9), 9.40 (t, $J_{H11,H12}$ = 5.6 Hz, 2H, H-11) ppm.

^{13}C NMR (150 MHz, DMSO-d$_6$): δ 36.2 (C-12), 107.3 (C-14), 110.6 (C-15), 125.7 (C-2), 126.91 (C-6), 126.93 (C-5), 127.8 (C-8), 127.9 (C-1), 134.3 (C-3), 136.2 (C-9), 142.3 (C-16), 145.7 (C-4), 148.4 (C-7), 152.2 (C-13), 164.5 (C-10) ppm.

HRMS (ESI$^+$): *m/z* found: 567.1152 [M+H]$^+$, calculated ($C_{30}H_{23}N_4O_4S_2$)$^+$: 567.1155

The spectroscopic data are in agreement with those reported in the literature.[1]

8,8'-disulfanediylbis(N-(2-(4-(4-(prop-2-yn-1-yloxy)benzoyl)phenoxy)ethyl)quinoline-3-carboxamide) (**21**)



A solution of carboxylic acid **18** (25.0 mg, 0.06 mmol, 1.0 equiv.) in DMF (0.75 mL) was treated with carbonyldiimidazole (21.6 mg, 0.14 mmol, 2.2 equiv.) and stirred at ambient temperature for 15 min. The mixture was then treated with a solution of amine **11** (36.2 mg, 0.12 mmol, 2.0 equiv.) in DMF (0.5 mL) and the reaction mixture was stirred at ambient temperature for 48 h under an atmosphere of nitrogen. After this time, TLC analysis (DCM-MeOH-AcOH, 179:20:1) showed total consumption of starting material **18** (R_f = 0.1) and the formation of a new product (R_f = 0.6). The reaction was diluted with EtOAc (25 mL) and washed with saturated aq. sodium bicarbonate solution (20 mL) and saturated brine solution (20 mL). The organic extract was dried over anhydrous $MgSO_4$ and crude product was purified by silica column chromatography (Hexane-EtOAc, 1:1) to isolate the major product (R_f = 0.4). The product was recrystallised from EtOAc to yield the desired product 8,8' disulfanediylbis(*N*-(2-(4-(4-(prop-2-yn-1-yloxy)benzoyl)phenoxy)ethyl)quinoline-3-carboxamide), **21** (19.9 mg, 0.02 mmol, 34%) as a pale yellow solid.

mp 176-177 °C (EtOAc)

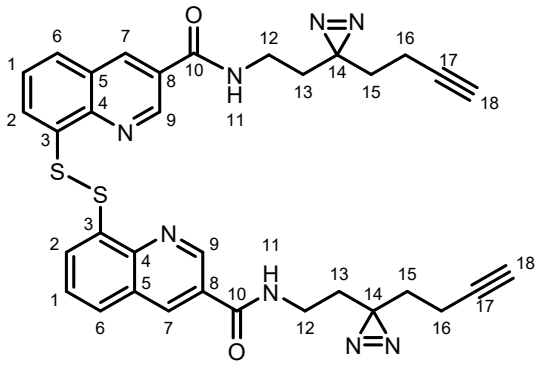
ν_{max}/cm^{-1} (neat) 3296 (N-H), 3068 (C-H ar), 2934 (C-H), 2163 (C≡C), 1634 (C=O), 1599 (C=C), 1541 (C=C), 1231 (C-O), 1171 (C-O), 1023, 928, 849, 764, 620 (C-S).

^{1}H NMR (600 MHz, DMSO-d$_6$): δ 3.64 (t, $J_{H23,H25}$ = 2.3 Hz, 2H, H-25), 3.79 (app. q, $J_{H12,H13}$ = 5.6 Hz, $J_{H11,H12}$ = 5.4 Hz, 4H, H-12), 4.33 (t, $J_{H12,H13}$ = 5.6 Hz, 4H, H-13), 4.91 (d, $J_{H23,H25}$ = 2.3 Hz, 4H, H-23), 7.12 (d, $J_{H15,H16}$ = 8.8 Hz, 4H, H-15), 7.16 (d, $J_{H20,H21}$ = 8.8 Hz, 4H, H-21), 7.61 (app. t, J = 7.6 Hz, 2H, H-1), 7.70 (d, $J_{H15,H16}$ = 8.8 Hz, 4H, H-16), 7.72 (d, $J_{H20,H21}$ = 8.8 Hz, 4H, H-20), 7.84 (dd, $J_{H1,H2}$ = 7.6 Hz, $J_{H2,H6}$ = 0.9 Hz, 2H, H-2), 7.96 (dd, $J_{H1,H6}$ = 7.5 Hz, $J_{H2,H6}$ = 0.9 Hz, 2H, H-6), 8.91 (d, $J_{H11,H12}$ = 2.2 Hz, 2H, H-7), 9.19 (t, $J_{H11,H12}$ = 5.4 Hz, 2H, H-11), 9.38 (d, $J_{H7,H9}$ = 2.2 Hz, 2H, H-9) ppm.

^{13}C NMR (150 MHz, DMSO-d$_6$): δ 40.1 (C-12), 55.7 (C-23), 66.4 (C-13), 78.7 (C-25), 78.8 (C-24), 114.4 (C-15), 114.6 (C-21), 125.6 (C-2), 126.8 (C-6), 126.9 (C-3), 127.8 (C-5), 127.9 (C-1), 130.0 (C-19), 130.7 (C-17), 131.6 (C-16), 131.9 (C-20), 134.3 (C-4), 136.1 (C-7), 145.8 (C-8), 148.4 (C-9), 160.4 (C-22), 161.8 (C-14), 165.0 (C-18), 193.1 (C-18) ppm.

HRMS (ESI$^+$): *m/z* found: 985.2338 [M+Na]$^+$, calculated ($C_{56}H_{42}N_4NaO_8S_2$)$^+$: 985.2336

8,8'-disulfanediylbis(N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)quinoline-3-carboxamide) (**23**)



A solution of carboxylic acid **18** (23.5 mg, 0.09 mmol, 1.0 equiv.) in DMF (1 mL) was treated with carbonyldiimidazole (30.2 mg, 0.19 mmol, 2.2 equiv.) and stirred at ambient temperature for 15 min. The mixture was treated with a solution of amine **8** (23.5 mg, 0.17 mmol, 2.0 equiv.) in DMF (1 mL) and the reaction mixture was stirred at ambient temperature for 48 h under an atmosphere of nitrogen. After this time, TLC analysis (DCM-MeOH-AcOH, 179:20:1) showed total consumption of starting material **18** (R_f = 0.1) and the formation of a new product (R_f = 0.3). The reaction was diluted with EtOAc (30 mL) and washed with saturated aq. sodium bicarbonate solution (25 mL) and saturated brine solution (25 mL). The organic extract was dried over anhydrous $MgSO_4$ and crude product was purified by silica column chromatography (Hexane-EtOAc, 1:1). The semi-pure product was further purified by silica column chromatography (DCM-MeOH, 99:1) to yield the desired product 8,8'-disulfanediylbis(*N*-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)quinoline-3-carboxamide), **23** (21.2 mg, 33 µmol, 38%) as a colourless oil.

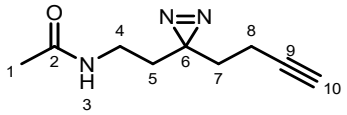
ν_{max}/cm^{-1} (neat) 3293 (C-H alkyne), 3063 (C-H ar), 2928 (C-H), 2161 (C≡C) 1637 (C=O) 1609 (C=C), 1544 (N=N), 1436 (C-H), 1369, 1289, 984, 926, 776, 663 (C-S).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (t, $J_{H15,H16}$ = 7.2 Hz, 4H, H-15), 1.94 (t, $J_{H12,H13}$ = 6.5 Hz, 4H, H-13), 2.02 (t, $J_{H16,H18}$ = 2.6 Hz, 2H, H-18), 2.07 (td, $J_{H15,H16}$ = 7.2 Hz, $J_{H16,H18}$ = 2.6 Hz, 4H, H-16), 3.46 (td, $J_{H12,H13}$ = 6.5 Hz, $J_{H11,H12}$ = 6.1 Hz, 4H, H-12), 6.87 (app. brs, 2H, H-11), 7.45 (app. t, J = 7.8 Hz, 2H, H-1), 7.67 (app. d, J = 7.6 Hz, 2H, H-2), 7.94 (dd, $J_{H1,H6}$ = 7.5 Hz, $J_{H2,H6}$ = 0.9 Hz, 2H, H-6), 8.63 (s, 2H, H-7), 9.38 (s, 2H, H-9) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.2 (C-16), 26.9 (C-14), 32.1 (C-15), 32.4 (C-13), 35.2 (C-12), 69.6 (C-18), 82.7 (C-17), 126.5 (C-2), 127.0 (C-5), 127.2 (C-6), 127.7 (C-7), 127.9 (C-1), 135.3 (C-3), 136.3 (C-9), 146.5 (C-4), 147.1 (C-8), 165.4 (C-10) ppm.

HRMS (APCI$^-$): *m/z* found: 645.1859 [M-H]$^-$, calculated ($C_{34}H_{29}N_8O_2S_2$)$^-$: 645.1860

N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)acetamide (**25**)



A solution of amine **8** (25.0 mg, 0.18 mmol, 1.0 equiv.) in DCM (6 mL) was treated with Et_3N (0.05 mL, 0.36 mmol, 2.0 equiv.) followed by with acetyl chloride (0.02 mL, 0.27 mmol, 2.0 equiv.). The resultant solution was stirred at ambient temperature for 16 h under an atmosphere of nitrogen. After this time, TLC analysis (DCM-MeOH-NH_3, 57:3:1) showed total consumption of starting amine **8** (R_f = 0.2) and the formation of two new products (R_f = 0.3 and R_f = 0.5). The reaction was diluted with DCM (10 mL) and washed with 1 M HCl (7 mL). The organic extract was dried over anhydrous $MgSO_4$, concentrated *in vacuo* and the crude material was redissolved in MeOH (5 mL). The solution was treated with solid sodium bicarbonate (30.8 mg, 0.36 mmol, 2.0 equiv.) and stirred at ambient temperature for 16 h under an atmosphere of nitrogen. After this time, TLC analysis (Hexane-EtOAc, 1:1) showed total consumption of higher running bis-acetylated product and presence of a single product (R_f = 0.1). The reaction was concentrated *in vacuo* and the crude residue was dissolved in DCM (10 mL) and washed with dH_2O (8 mL). The organic extract was washed with saturated brine solution (5 mL) and dried over anhydrous $MgSO_4$. Volatiles were removed *in vacuo* to yield the desired product *N*-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)acetamide, **25** (7.3 mg, 0.04 mmol, 22%) as a colourless oil.

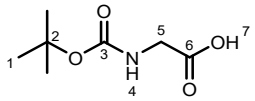
ν_{max}/cm^{-1} (neat) 3385 (N-H), 3238 (C-H alkyne) 2919 (C-H), 2114 (C≡C), 1649 (C=O), 1565 (N=N), 1463 (C-H), 1376, 1259, 1022, 798, 720.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.65 (t, $J_{H7,H8}$ = 7.2 Hz, 2H, H-7), 1.71 (t, $J_{H4,H5}$ = 6.6 Hz, 2H, H-5), 1.99 (s, 3H, H-1), 2.01 (t, $J_{H8,H10}$ = 2.6 Hz, 1H, H10), 2.02 (td, $J_{H7,H8}$ = 7.2 Hz, $J_{H8,H10}$ = 2.6 Hz, 2H, H8), 3.11 (app. q, $J_{H4,H5}$ = 6.6 Hz, $J_{H3,H4}$ = 6.3 Hz, 2H, H-4), 5.56 (brs, 1H, H-3) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 13.2 (C-8), 23.3 (C-1), 26.8 (C-6), 32.2 (C-7), 32.4 (C-5), 34.3 (C-4), 69.4 (C-10), 82.7 (C-9), 170.1 (C-2) ppm.

HRMS (ESI$^+$): *m/z* found: 202.0953 [M+Na]$^+$, calculated $(C_9H_{13}N_3NaO)^+$: 202.0951

Boc-glycine (**26**)



A solution of glycine hydrochloride (2.0 g, 17.9 mmol, 1.0 equiv.) in 1,4-dioxane (60 mL) and dH_2O (30 mL), was treated with sodium hydrogen carbonate (4.52 g, 53.8 mmol, 3.0 equiv.) and stirred at ambient temperature for 15 min. Boc anhydride (7.83g, 35.9 mmol, 2.0 equiv.) was added portion-wise and the mixture was stirred at ambient temperature for 60 h. After this time, TLC analysis (DCM-MeOH-AcOH, 379:20:1) showed total consumption of glycine (R_f = 0.2) and the formation of a new product (R_f = 0.6). Volatiles were removed *in vacuo* and the crude residue was diluted with dH_2O (50 mL) and adjusted to pH 3 with 1 M HCl. The solution was extracted with EtOAc (3 x 50 mL) and the combined organic extracts were washed with saturated brine solution (100 mL) and dried over anhydrous $MgSO_4$. Volatiles were removed *in vacuo* and the resultant white residue was dissolved in MeOH (50 mL) and washed with Hexane (2 x 50 mL). The MeOH extract was concentrated *in vacuo* to yield the desired product Boc-glycine **26**, (2.25 g, 12.8 mmol, 72%) as a white solid.

mp 48-50 °C (MeOH), (lit.[16] 78 °C)

ν_{max}/cm^{-1} (neat) 3405 (O-H), 3341 (N-H), 2977 (C-H), 1745 (C=O), 1686 (C=O), 1155 (C-O), 1054, 955, 856, 780, 672.

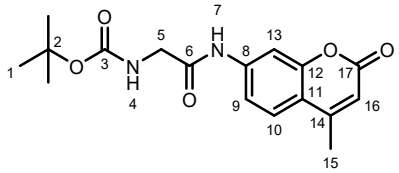
^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, H-1), 3.95 (d, $J_{H4,H5}$ = 5.0 Hz, 2H, H-5), 5.14 (app. brs, 1H, H-4), 10.49 (brs, 1H, H-7) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.3 (C-1), 42.2 (C-5), 80.4 (C-2), 156.3 (C-3), 174.8 (C-6) ppm.

HRMS (ESI$^+$): *m/z* found: 198.0739 [M+Na]$^+$, calculated $(C_7H_{13}NNaO_4)^+$: 198.0737

The spectroscopic data are in agreement with those reported in the literature.[17]

Tert-butyl (2-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-2-oxoethyl)carbamate (**27**)



A solution of Boc-glycine **26** (350 mg, 2.0 mmol, 1.0 equiv.) and 7-amino-4-methylcoumarin (350 mg, 2.0 mmol, 1.0 equiv.) in pyridine (6.0 mL) was cooled to -15 °C and treated with $POCl_3$ (0.21 mL, 2.20 mmol, 1.1 equiv.). The mixture was stirred at -15 °C for 15 min. After this time, TLC analysis (DCM-MeOH, 19:1) showed partial consumption of 7-amino-4-methylcoumarin (R_f = 0.5) and the formation of a new product (R_f = 0.3). The reaction was quenched with 5% aq. citric acid solution (20 mL) and extracted with EtOAc (40 mL). Upon standing for 30 min, a precipitate was observed to form in the organic extract. The precipitate was collected by filtration to yield the desired product *tert*-butyl (2-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-2-oxoethyl)carbamate, **27** (352.8 mg, 1.06 mmol, 53%) as an off-white solid.

mp 228-231 °C (EtOAc), (lit.[18] 219-222 °C)

ν_{max}/cm^{-1} (neat) 3358 (N-H), 3092 (C-H ar), 2981 (C-H), 1679 (C=O), 1619 (C=C), 1515 (C=C), 1394 (C-H), 1161 (C-O), 1070, 847, 753.

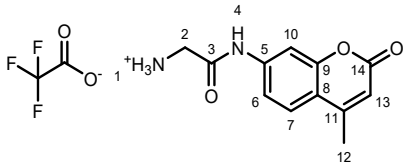
^{1}H NMR (600 MHz, DMSO-d_6): δ 1.39 (s, 9H, H-1), 2.39 (d, $J_{H15,H16}$ = 1.0 Hz, 3H, H-15), 3.79 (d, $J_{H4,H5}$ = 6.0 Hz, 2H, H-5), 6.26 (d, $J_{H15,H16}$ = 1.0 Hz, 1H, H-16), 7.11 (t, $J_{H4,H5}$ = 6.0 Hz, 1H, H-4), 7.52 (d, $J_{H9,H10}$ = 8.5 Hz, 1H, H-9), 7.71 (d, $J_{H9,H10}$ = 8.5 Hz, 1H, H-10), 7.77 (s, 1H, H-13), 10.57 (s, 1H, H-7) ppm.

^{13}C NMR (150 MHz, DMSO-d_6): δ 18.0 (C-15), 28.2 (C-1), 43.9 (C-5), 78.1 (C-2), 105.5 (C-13), 112.2 (C-16), 114.9 (C-14), 115.1 (C-9), 125.9 (C-10), 142.3 (C-8), 153.1 (C-11), 153.7 (C-12), 155.9 (C-3), 160.0 (C-17), 169.1 (C-6) ppm.

HRMS (ESI$^+$): *m/z* found: 355.1268 [M+Na]$^+$, calculated $(C_{17}H_{20}N_2NaO_5)^+$: 355.1264

The spectroscopic data are in agreement with those reported in the literature.[19]

2-((4-methyl-2-oxo-2H-chromen-7-yl)amino)-2-oxoethan-1-aminium 2,2,2-trifluoroacetate (**28**)



A solution of Boc-protected **27** (31.0 mg, 0.09 mmol, 1.0 equiv.) in DCM (3 mL) was treated with TFA (0.04 mL, 0.47 mmol, 5.0 equiv.) and stirred at ambient temperature for 5 h under an atmosphere of nitrogen. After this time, TLC analysis (DCM-MeOH-Et_3N, 94:5:1) showed total consumption of Boc-protected **27** (R_f = 0.5) and the formation of a new product (R_f = 0.1). Volatiles were removed *in vacuo* and 2-((4 methyl-2-oxo-2H-chromen-7-yl)amino)-2-oxoethan-1-aminium 2,2,2-trifluoroacetate, 28 (32.2 mg, 0.09 mmol, 99%) was collected as an off-white solid.

mp 228-231 °C (DCM)

ν_{max}/cm^{-1} (neat) 3361 (N-H), 3049 (C-H ar), 2924 (C-H), 1676 (C=O), 1614 (C=O), 1536 (C=C), 1391 (C-H), 1202, 1130 (C-O), 852, 727.

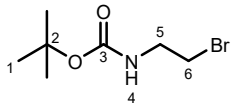
^{1}H NMR (400 MHz, DMSO-d_6): δ 2.41 (d, $J_{H12,H13}$ = 1.0 Hz, 3H, H-12), 3.87 (s, 2H, H-2), 6.29 (d, $J_{H12,H13}$ = 1.0 Hz, 1H, H-13), 7.54 (d, $J_{H6,H7}$ = 8.5 Hz, 1H, H-6), 7.76 (d, $J_{H6,H7}$ = 8.5 Hz, 1H, H-7), 7.80 (s, 1H, H-10), 8.33 (brs, 3H, H-1), 11.36 (s, 1H, H-4) ppm.

^{13}C NMR (150 MHz, DMSO-d_6): δ 18.0 (C-12), 41.3 (C-2), 105.7 (C-10), 112.5 (C-13), 113.9 (C-11), 115.1 (C-6), 126.2 (C-7), 142.4 (C-5), 153.1 (C-8), 153.6 (C-9), 160.0 (C-14), 173.0 (C-3) ppm.

HRMS (ESI$^+$): *m/z* found: 233.0926 $[M+H]^+$, calculated $(C_{12}H_{13}N_2O_3)^+$: 233.0921

The spectroscopic data are in agreement with those reported in the literature.[19]

Tert-butyl (2-bromoethyl)carbamate (**29**)



A solution of 2-bromoethylamine hydrobromide (1.50 g, 7.32 mmol, 1.0 equiv) in DCM (15 mL) was treated with Et_3N (1.02 mL, 7.32 mmol, 1.0 equiv.) at 0 °C. The mixture was treated with a solution of Boc-anhydride (1.60 g, 7.32 mmol, 1.0 equiv.) in DCM (4 mL) and stirred at 0 °C for 90 min. After this time, TLC analysis (DCM-MeOH-Et_3N, 94:5:1) showed total consumption of 2-bromoethylamine (R_f = 0.2) and the formation of a new product (R_f = 0.8). The reaction was diluted with DCM (50 mL) and washed with 5% aq. citric acid solution (40 mL), dH_2O (50 mL) and saturated brine solution (30 mL). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield the desired product *tert*-butyl-(2-bromoethyl)carbamate, **29** (1.59 g, 7.24 mmol, 97%) as a colourless oil.

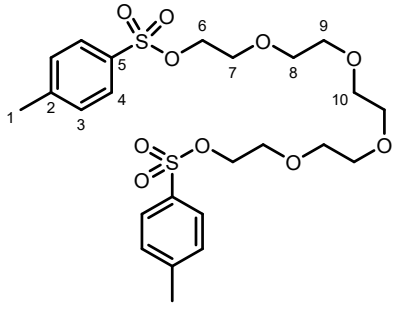
ν_{max}/cm^{-1} (neat) 3346 (N-H), 2979 (C-H), 1691 (C=O), 1366 (C-H), 1250, 1163 (C-O), 1117 (C-O), 1071, 950, 844, 778 (C-Br), 661.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H, H-1), 3.46 (t, $J_{H5,H6}$ = 5.7 Hz, 2H, H-6), 3.53 (app. q, J = 5.7 Hz, 2H, H-5), 4.94 (app. brs, 1H, H-4) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 28.3 (C-1), 32.8 (C-6) 42.4 (C-5), 79.8 (C-2), 155.6 (C-3) ppm.

The spectroscopic data are in agreement with those reported in the literature.[20]

3,6,9,12-tetraoxatetradecane-1,14-diyl bis(4-methylbenzenesulfonate) (**30**)



A solution of pentaethylene glycol (1.0 g, 4.20 mmol, 1.0 equiv.) in DCM (42 mL) was cooled to 0 °C and treated with *p*-TsCl (2.0 g, 10.5 mmol, 2.5 equiv.) portion-wise. DMAP (256 mg, 2.10 mmol, 0.5 equiv.) and Et_3N (1.46 mL, 10.5 mmol, 2.5 equiv.) were added and the mixture was stirred at 0 °C for 1 h, and then at ambient temperature for 4 h. After this time, TLC analysis (DCM-MeOH, 9:1) showed partial consumption of *p*-TsCl (R_f = 0.95) and the formation of a new product (R_f = 0.9). The reaction was diluted with DCM (100 mL) and washed with dH_2O (100 mL), 5% aq. citric acid solution (100 mL) and saturated brine solution (80 mL). The organic extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to obtain 3,6,9,12-tetraoxatetradecane-1,14-diyl bis(4-methylbenzenesulfonate), **30** (2.24 g, 4.10 mmol, 98%) as a pale yellow oil.

ν_{max}/cm^{-1} (neat) 3065 (C-H ar), 2872 (C-H), 1598 (C=C), 1452 (C-H), 1351 (S=O), 1174 (C-O), 1095 (C-O), 1011, 914, 772, 661.

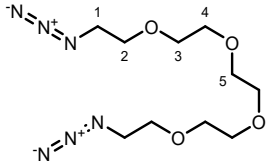
^1H NMR (400 MHz, $CDCl_3$): δ 2.44 (s, 6H, H-1), 3.57 (app. s, 8H, H-9 & H-10), 3.60 (app. s, 4H, H-8), 3.68 (t, $J_{H6,H7}$ = 4.8 Hz, 4H, H-7), 4.15 (t, $J_{H6,H7}$ = 4.8 Hz, 4H, H-6), 7.34 (d, $J_{H3,H4}$ = 8.1 Hz, 4H, H-3), 7.72 (d, $J_{H3,H4}$ = 8.1 Hz, 4H, H-4) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 21.6 (C-1), 68.6 (C-7), 69.2 (C-6), 70.5 (C-8), 70.6 (C-10), 70.7 (C-9), 127.9 (C-4), 129.8 (C-3), 133.0 (C-5), 144.8 (C-2) ppm.

HRMS (ESI^+): *m/z* found: 569.1486 $[M+Na]^+$, calculated $(C_{20}H_{34}NaO_{10}S_2)^+$: 569.1486

The spectroscopic data are in agreement with those reported in the literature.[21]

1,14-diazido-3,6,9,12-tetraoxatetradecane (**31**)



A solution of bis-tosyl **30** (2.20 g, 4.02 mmol, 1.0 equiv.) in DMF (16 mL) was treated with tetra-*N*-butylammonium iodide (74.3 mg, 0.2 mmol, 0.5 equiv.) and sodium carbonate (427 mg, 4.02 mmol, 1.0 equiv.). Sodium azide (785 mg, 12.1 mmol, 3.0 equiv.) was added portion-wise and the reaction was heated to 80 °C for 18 h. After this time, TLC analysis (DCM-MeOH, 99:1) showed total consumption of bis-tosyl **30** (R_f = 0.7) and the formation of a new product (R_f = 0.2). The reaction was cooled to 0 °C and diluted with saturated aq. sodium bicarbonate solution (100 mL) and extracted with EtOAc (3 x 100 mL). The organic extract was washed with saturated brine solution (100 mL), dried over anhydrous $MgSO_4$ and purified by silica column chromatography (DCM-MeOH, 99:1) to yield the desired product 1,14-diazido-3,6,9,12-tetraoxatetradecane, **31** (740 mg, 2.57 mmol, 64%) as a colourless oil.

ν_{max}/cm^{-1} (neat) 2888 (C-H), 2094 (N=N=N), 1443 (C-H), 1284, 1108 (C-O), 936, 851, 644, 556.

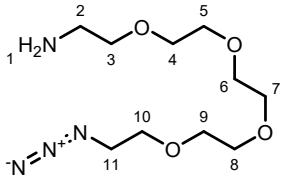
^1H NMR (400 MHz, $CDCl_3$): δ 3.37 (t, $J_{H1,H2}$ = 4.9 Hz, 4H, H-1), 3.63-3.68 (m, 16H, H-2 & H-3 & H-4 & H-5) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 50.6 (C-1), 70.0 (C-3), 70.5 (C-2), 70.6 (C-5), 70.7 (C-4) ppm.

HRMS (ESI^+): *m/z* found: 311.1444 $[M+Na]^+$, calculated $(C_{10}H_{20}N_6NaO_4)^+$: 311.1438

The spectroscopic data are in agreement with those reported in the literature.[22]

14-azido-3,6,9,12-tetraoxatetradecan-1-amine (**32**)



An ice-cold solution of bis-azide **31** (500 mg, 1.73 mmol, 1.0 equiv.) in Et_2O (3 mL) and THF (0.3 mL) was treated with 1M HCl (4.3 mL, 4.3 mmol, 2.5 equiv.). A solution of triphenylphosphine (455 mg, 1.73 mmol, 1.0 equiv.) in Et_2O (1.5 mL) was added dropwise and the reaction was stirred at 0 °C for 1 h. The reaction was stirred at ambient temperature for 60 h. After this time, TLC analysis (DCM-MeOH-Et_3N, 94:5:1) showed total consumption of bis-azide **31** (R_f = 0.9) and the formation of a new product (R_f = 0.2). The organic layer was removed and the aqueous was washed with portions of Et_2O (2 x 10 mL). The aqueous extract was adjusted to pH 14 through addition of 1M NaOH solution and then extracted with $CHCl_3$ (2 x 20 mL). The organic extract was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to yield the desired product 14-azido-3,6,9,12-tetraoxatetradecan-1-amine, **32** (407 mg, 1.55 mmol, 90%) as a colourless oil.

ν_{max}/cm^{-1} (neat) 3370 (N-H), 2869 (C-H), 2100 (N=N=N), 1573 (N-H), 1455 (C-H), 1300, 1095 (C-O), 940, 823, 640, 554.

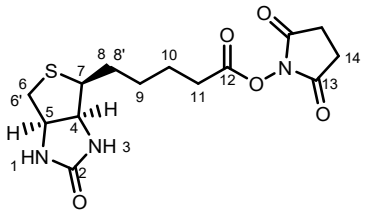
^{1}H NMR (600 MHz, DMSO-d_6): δ 1.30 (brs, 2H, H-1), 2.63 (t, $J_{H2,H3}$ = 5.8 Hz, 2H, H-2), 3.35 (t, $J_{H2,H3}$ = 5.8 Hz, 2H, H-3), 3.39 (t, $J_{H10,H11}$ = 5.0 Hz, 2H, H-11), 3.47-3.57 (m, 12H, H-4 & H-5 & H-6 & H-7 & H-8 & H-9), 3.60 (t, $J_{H10,H11}$ = 5.0 Hz, 2H, H-10) ppm.

^{13}C NMR (150 MHz, DMSO-d_6): δ 41.4 (C-2), 50.0 (C-11), 69.2 (C-10), 69.57 (C-9), 69.69 (C-4), 69.8 (C-5 & C-6 & C-7 & C-8), 73.1(C-3) ppm.

HRMS (ESI$^+$): *m/z* found: 263.1713 $[M+H]^+$, calculated $(C_{10}H_{23}N_4O_4)^+$: 263.1714

The spectroscopic data are in agreement with those reported in the literature.[23]

Biotin-NHS-ester (**33**)



D-Biotin (300 mg, 1.23 mmol, 1.0 equiv.) was suspended in DMF (10 mL) and stirred at 80 °C for 30 min. The mixture was cooled to ambient temperature and treated with *N*-hydroxy succinimide (198 mg, 1.72 mmol, 1.4 equiv.) followed by *N,N'*-dicyclohexyl carbodiimide (304 mg, 1.47 mmol, 1.2 equiv.) portion-wise and stirred for 24 h at ambient temperature. After this time, TLC analysis (acetone-Hexane-AcOH, 16:4:1) showed total consumption of D-Biotin (R_f = 0.1) and formation of a new product (R_f = 0.8). The reaction material was filtered and the filtrate was concentrated *in vacuo*. Et_2O (20 mL) was added to the filtrate residue and the resulting white precipitate was collected by filtration to yield the desired product Biotin-NHS-ester, **33** (312.0 mg, 0.91 mmol, 75%) as a white solid.

mp 198-202 °C (Et_2O)

ν_{max}/cm^{-1} (neat) 3223 (N-H), 2940 (C-H), 1819, 1788 (C=O), 1729 (C=O), 1700 (C=O), 1369 (C-H), 1211 (C-O), 1071, 992 (N-O), 861, 813, 740, 656 (C-S).

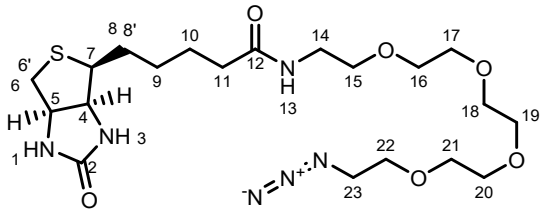
^{1}H NMR (400 MHz, DMSO-d_6): δ 1.35-1.56 (m, 3H, H8 & H-9), 1.58-1.70 (m, 3H, H-8' & H-10), 2.57 (d, $J_{H6,H6'}$ = 12.4 Hz, 1H, H-6), 2.67 (t, $J_{H10,H11}$ = 7.3 Hz, 2H, H-11), 2.81 (s, 4H, H-14), 2.84-2.86 (m, 1H, H-6'), 3.07 3.14 (m, 1H, H-7), 4.11-4.17 (m, 1H, H-4), 4.30 (dd, $J_{H5,H6'}$ = 7.6 Hz, $J_{H4,H5}$ = 5.2 Hz, 1H, H-5), 6.36 (s, 1H, H-3), 6.41 (s, 1H, H-1) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ 24.3 (C-9), 25.4 (C-14), 27.5 (C-8), 27.8 (C-10), 30.0 (C-11), 39.7 (C-6), 55.2 (C-7), 59.1 (C-5), 61.0 (C-4), 162.6 (C-2), 168.9 (C-12), 170.2 (C-13) ppm.

HRMS (ESI$^+$): *m/z* found: 364.0932 $[M+Na]^+$, calculated $(C_{14}H_{19}N_3NaO_5S)^+$: 364.0938

The spectroscopic data are in agreement with those reported in the literature.[24]

Biotin-PEG4-azide (**34**)



A solution of amine **32** (200 mg, 0.76 mmol, 1.0 equiv.) in DMF (2 mL) was treated with Et_3N (0.32 mL, 2.28 mmol, 3.0 equiv.) followed by Biotin-NHS-ester **33** (311 mg, 0.91 mmol, 1.2 equiv.). The mixture was stirred at ambient temperature for 16 h under an atmosphere of nitrogen. After this time, TLC analysis (acetone-Hexane-AcOH, 16:4:1) showed total consumption of Biotin-NHS-ester **33** (R_f = 0.8) and the formation of a new product (R_f = 0.4). The reaction was concentrated *in vacuo* and the resultant crude product was purified by silica column chromatography (acetone-Hexane-AcOH, 16:4:1) to yield the desired product Biotin-PEG4-azide, **34** (359.5 mg, 0.74 mmol, 96%) as an off white solid.

mp 97-99 °C (acetone-Hexane-AcOH)

ν_{max}/cm^{-1} (neat) 3282 (N-H), 2921 (C-H), 2867 (C-H), 2101 (N=N=N), 1696 (C=O), 1645 (C=O), 1418 (C-H), 1265, 1106 (C-O), 944, 869, 727, 697 (C-S).

^{1}H NMR (600 MHz, DMSO-d_6): δ 1.21-1.35 (m, 2H, H-10), 1.41-1.47 (m, 1H, H-8), 1.47-1.54 (m, 2H, H-9), 1.57-1.65 (m, 1H, H-8'), 2.06 (t, $J_{H10,H11}$ = 7.4 Hz, 2H, H-11), 2.57 (app. d, J = 12.4 Hz, 1H, H-6), 2.82 (dd, $J_{H6,H6'}$ = 12.4 Hz, $J_{H5,H6'}$ = 5.2 Hz, 1H, H-6'), 3.06-3.12 (m, 1H, H-7), 3.18 (dd, $J_{H13,H14}$ = 5.8 Hz, $J_{H14,H15}$ = 5.5 Hz, 2H, H-14), 3.36-3.42 (m, 4H, H-15 & H-23), 3.47-3.57 (m, 12H, H-16 & H17 & H-18 & H19 & H20 & H21), 3.60 (t, $J_{H22,H23}$ = 5.0 Hz, 2H,

H-22), 4.11-4.15 (m 1H, H-4), 4.28-4.33 (dd, $J_{H4,H5}$ = 7.4 Hz, $J_{H5,H6'}$ = 5.2 Hz, 1H, H-5), 6.36 (s, 1H, H-3), 6.42 (s, 1H, H-1), 7.83 (t, $J_{H13,H14}$ = 5.5 Hz, 1H, H-13) ppm.

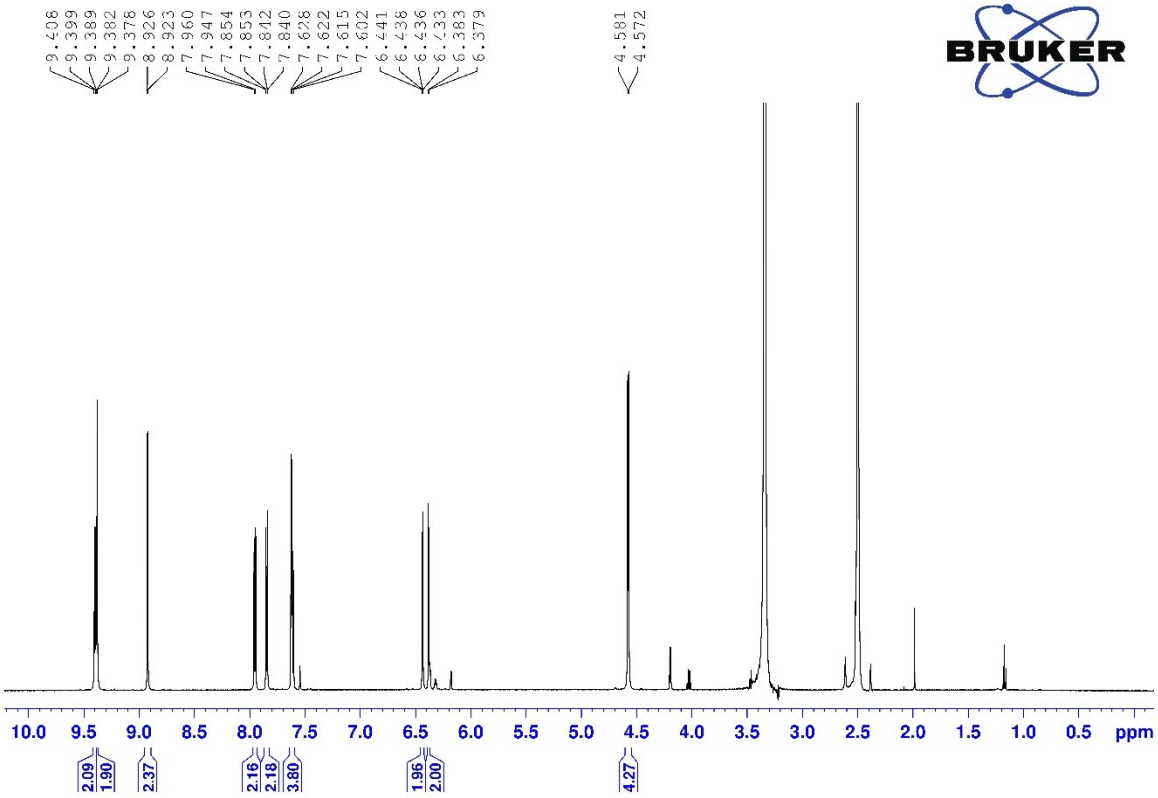
^{13}C NMR (150 MHz, DMSO-d_6): δ 25.3 (C-9), 28.0 (C-8), 28.2 (C-10), 35.1 (C-11), 38.4 (C-14), 39.8 (C-6), 50.0 (C-23), 55.4 (C-7), 59.2 (C-5), 61.0 (C-4), 69.2 (C-15), 69.3 (C-22), 69.6-69.8 (C-16 & C-17 & C-18 & C-19 & C-20 & C-21), 162.7 (C-2), 172.1 (C-12) ppm.

HRMS (ESI$^-$): *m/z* found: 487.2344 $[M-H]^-$, calculated $(C_{20}H_{35}N_6O_6S)^-$: 487.2344

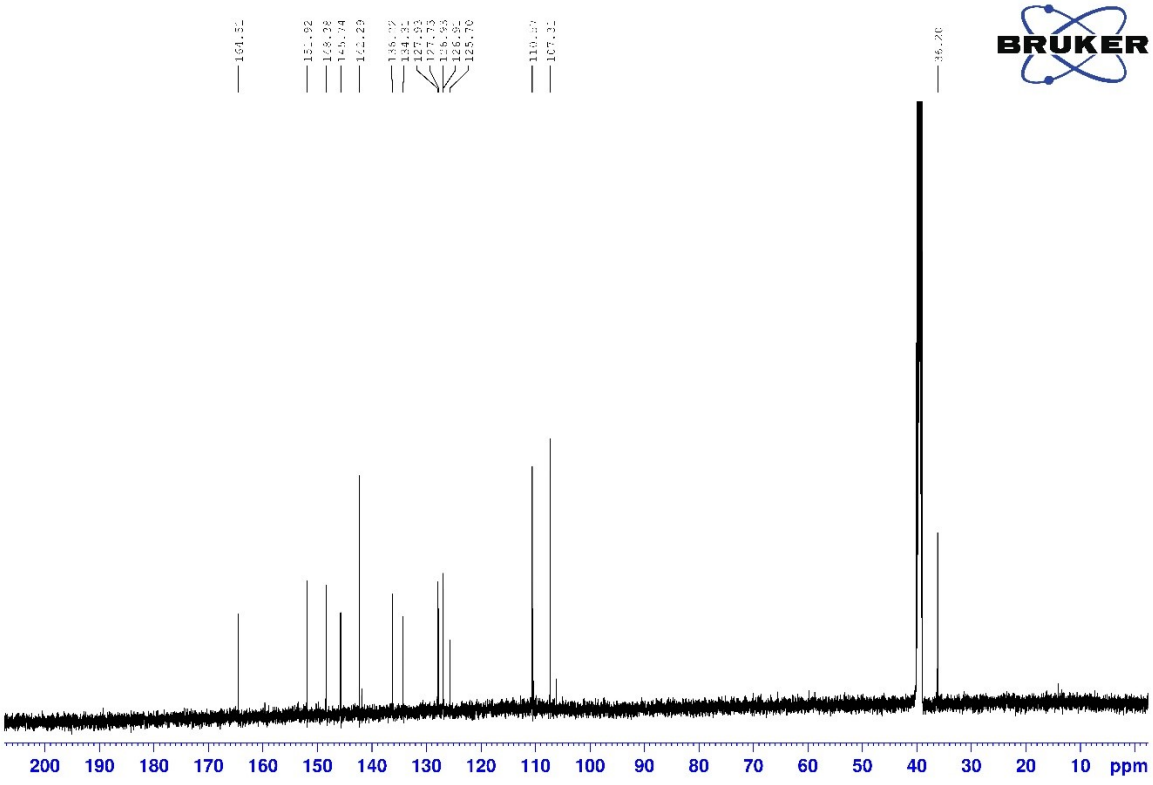
The spectroscopic data are in agreement with those reported in the literature.[25]

[1]H and [13]C NMR for test 8MQ probes as disulfide precursors **19, 21** and **23**, and diazirine control **25**.

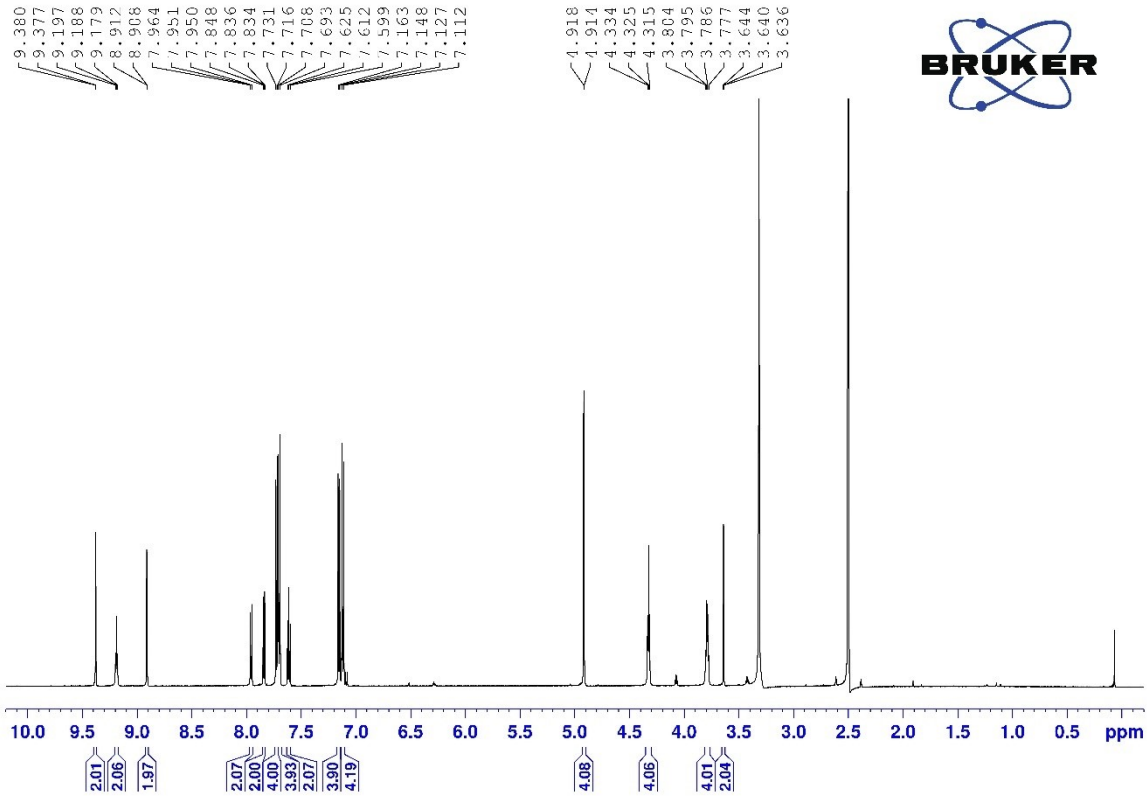
8,8'-disulfanediylbis(N-(furan-2-ylmethyl)quinoline-3-carboxamide) (**19**) [1]H NMR



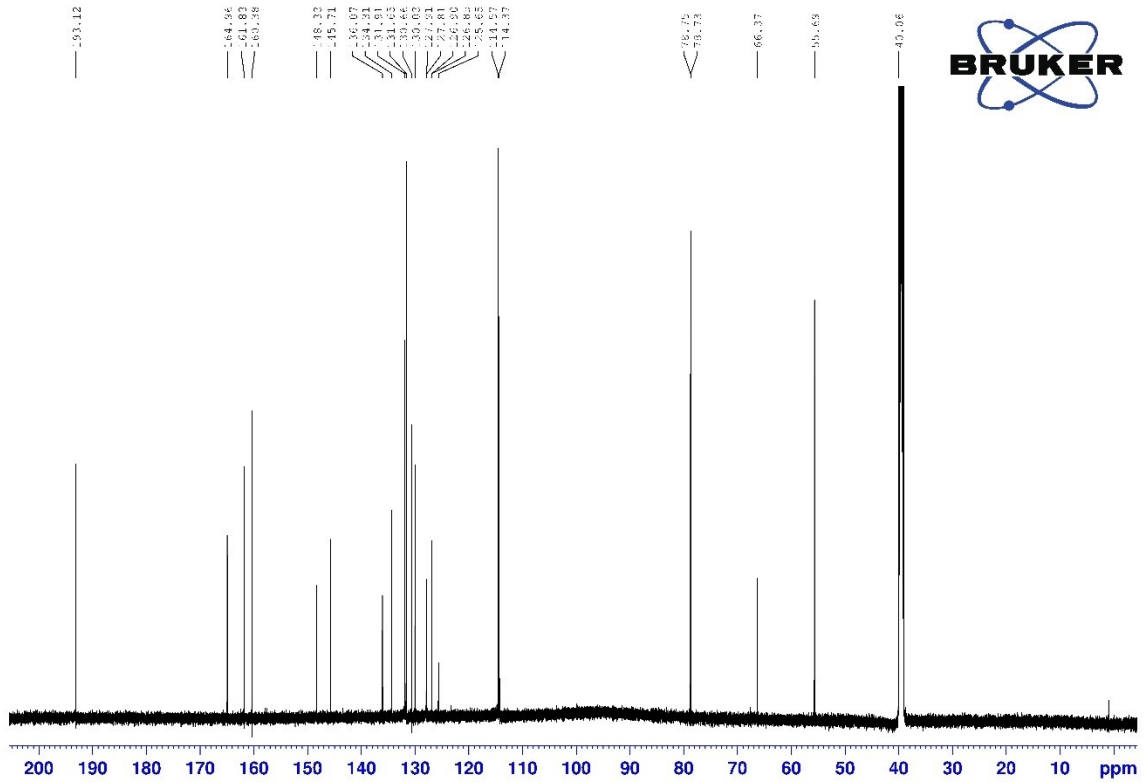
8,8'-disulfanediylbis(N-(furan-2-ylmethyl)quinoline-3-carboxamide) (**19**) [13]C NMR



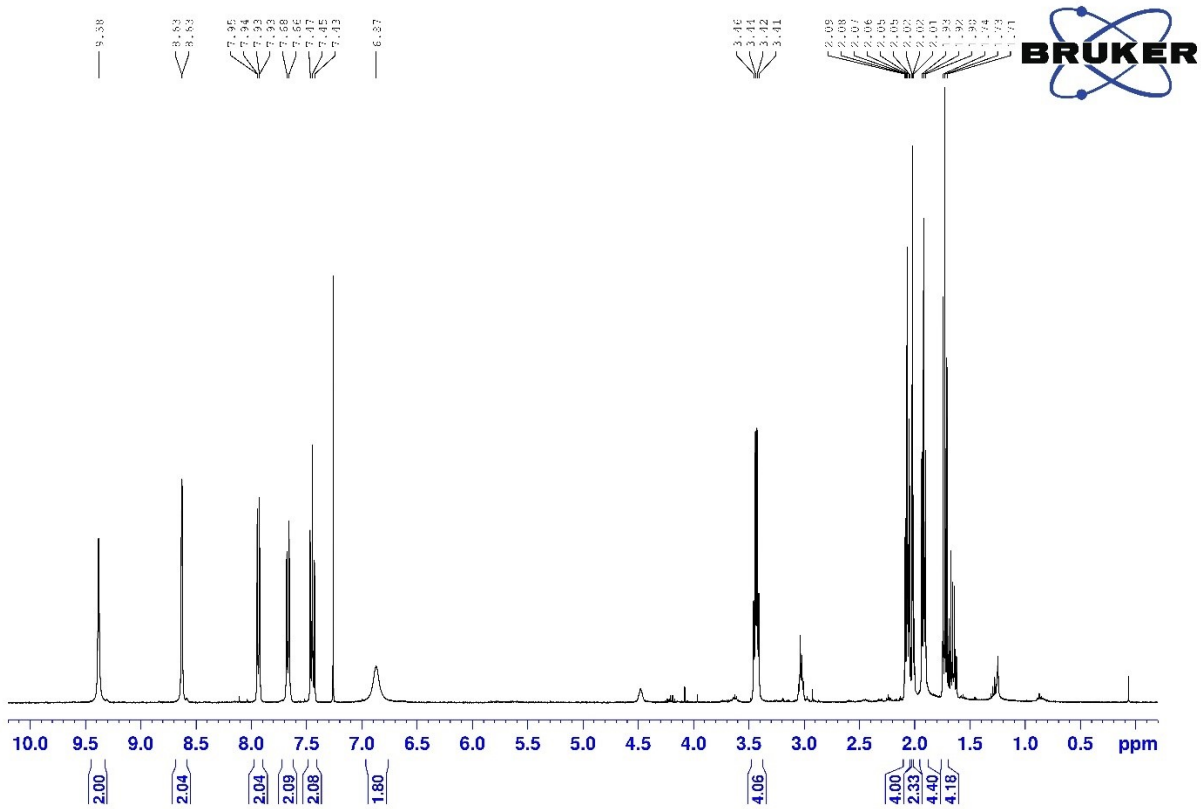
8,8'-disulfanediylbis(N-(2-(4-(4-(prop-2-yn-1-yloxy)benzoyl)phenoxy)ethyl)quinoline-3-carboxamide) (**21**)
^{1}H NMR



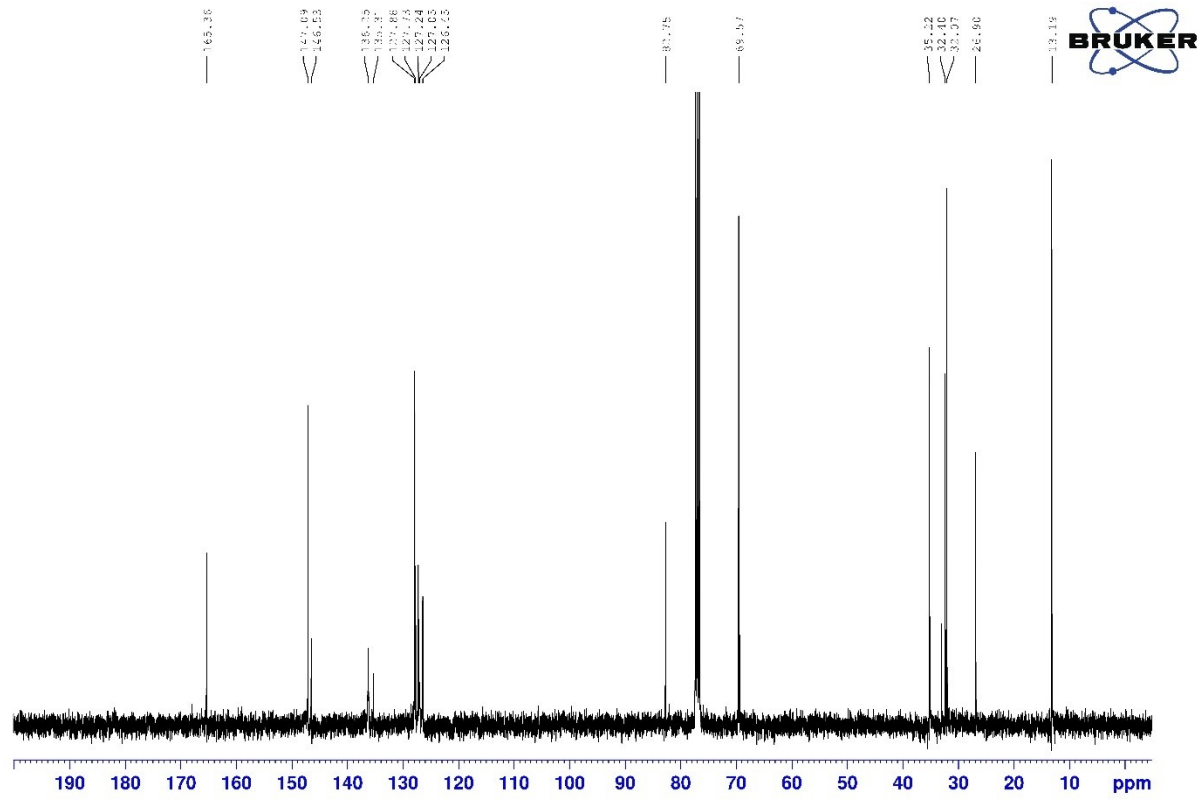
8,8'-disulfanediylbis(N-(2-(4-(4-(prop-2-yn-1-yloxy)benzoyl)phenoxy)ethyl)quinoline-3-carboxamide) (**21**)
^{13}C NMR



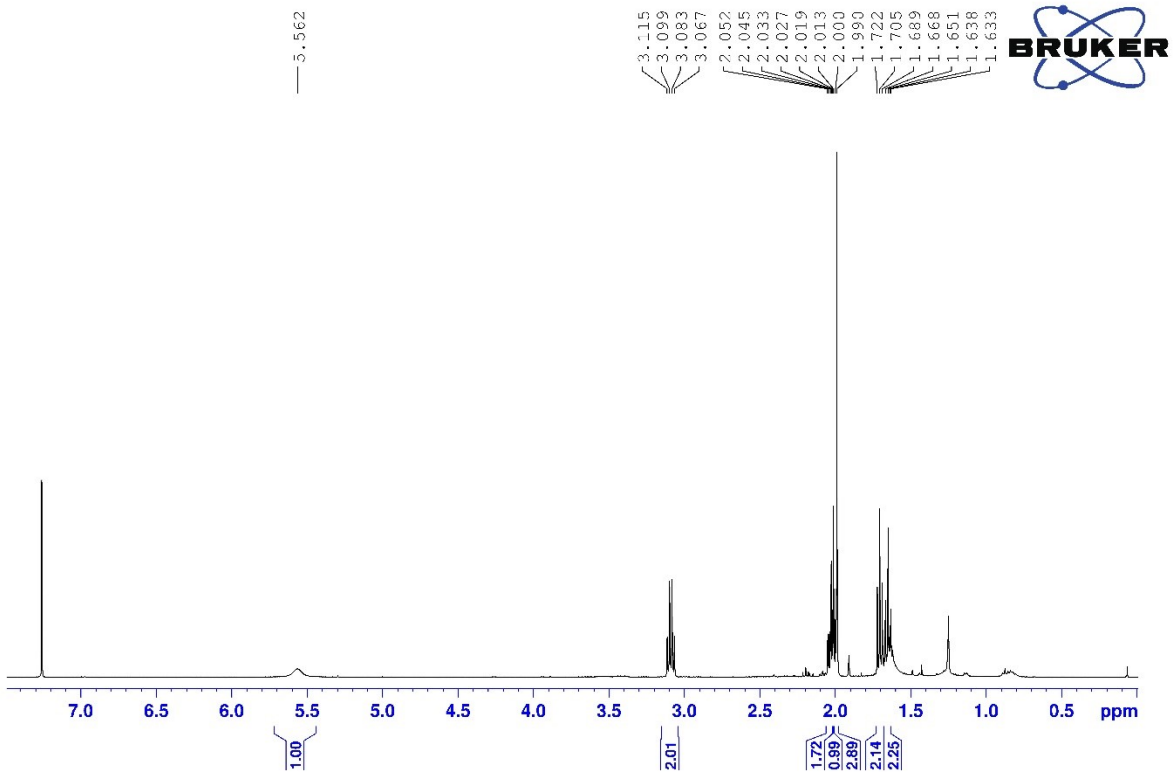
8,8'-disulfanediylbis(N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)quinoline-3-carboxamide) (**23**) ^{1}H NMR



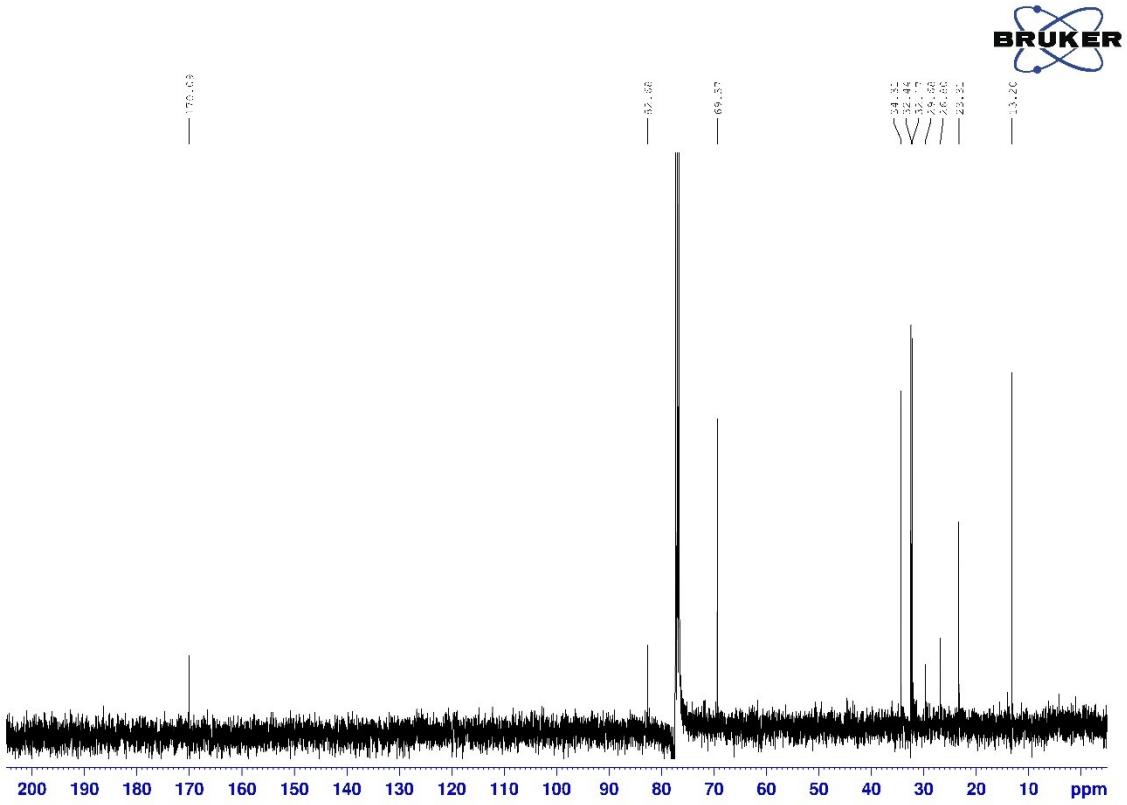
8,8'-disulfanediylbis(N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)quinoline-3-carboxamide) (**23**) ^{13}C NMR



N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)acetamide (**25**) ^{1}H NMR



N-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethyl)acetamide (**25**) ^{13}C NMR



Reduction of disulfides to generate 8MQ-bearing **20**, **SMK-22** and **SMK-24**

10 mM disulfide (**19**/**21**/**23**) solution in DMSO (10 µL) was treated with a solution of 50 mM DTT in homogenate buffer (50 mM Tris-Cl pH 7.4, 5 mM $MgCl_2$, 250 mM sucrose) (12 µL), vortexed for 30 s and incubated in darkness at 37 °C for 5 min. A visual cue for the successful reduction of the disulfide to liberate the corresponding 8MQ was seen in the development of a vibrant yellow colour in solution. The solution was diluted with 1 mM DTT in homogenate buffer (8 µL) and vortexed for 10 s. The solution was further treated with 1 mM DTT in homogenate buffer (10 µL) and vortexed for a further 10 s. The resulting solution corresponded with a 5 mM solution of 8MQ probes **20**/**SMK-22**/**SMK-24** and was diluted to the relevant concentration in homogenate buffer or growth medium.

UV-Vis absorption for detection of Zn^{2+} binding to **SMK-24**

UV-vis absorption spectra were recorded with a spectroscopic window of 600-200 nm on a Varian Cary 50 UV-vis spectrophotometer. A baseline correction of 1% DMSO in MeCN (spectroscopic grade) solvent was applied for all spectra. A solution of 50 µM **SMK-24** and 150 µM TCEP* in MeCN (spectroscopic grade) (2.7 mL) was added to a 1 cm quartz cuvette. The solution was titrated with aliquots of 1.5 mM $Zn(II)(ClO_4)_2$ in MeCN such that the total volume in the cuvette did not increase by >5%. Following addition of each aliquot, the capped cuvette was mixed by inversion 8-10 times and a UV-vis absorption spectra was measured. The data collected were fitted to metal-ligand binding models using nonlinear regression analysis through ReactLab Equilibria software (version 1.0, Jplus Consulting Pty Ltd).

* n.b. TCEP was used as the reducing agent to form **SMK-24** in this experiment to avoid DTT acting as a competing Zn^{2+} chelator.

Parallel Artificial Membrane Permeability Assay (PAMPA)

A 96-well Multiscreen filter plate (Merck) was combined with a 96-well Multiscreen Transport Receiver Plate (Merck) and filters were treated with 1% lecithin in dodecane (5 µL/well). A solution of 5% DMSO in PBS pH 7.4 (300 µL) was added to each receiver well. Solutions of Carbamazepine (500 µM), Furosemide (500 µM), 8MQ-bearing **20** (500 µM), **SMK-22** (500 µM) and **SMK-24** (500 µM) in 5% DMSO in PBS pH 7.4 were added to respective donor wells (150 µL). The combined plates were incubated at ambient temperature for 12 h. Concentrations of solutions in donor and acceptor wells were measured relative to equilibrium concentration using a Nanodrop 1000 Spectrophotometer and used to calculate log P_e. The effective permeability P_e was calculated using the following equation:

$$\log P_e = \log \{C \times \ln(1 - \frac{[Drug]_{acceptor}}{[Drug]_{equilibrium}})\}$$

$$\text{where } C = \frac{(V_D \times V_A)}{\{(V_D + V_A) \times Area \times time\}}$$

$V_D = 0.15\ cm^3$

$V_A = 0.45\ cm^3$

$Area = 0.3\ cm^2$

$time = 43{,}200\ s$

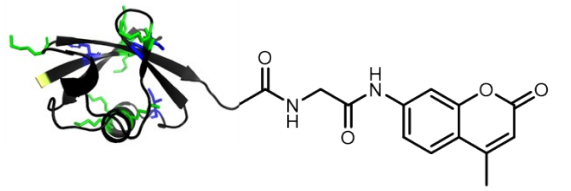
Samples were prepared in triplicate. Mean values were reported ± one standard deviation.

Biological Methods

Expression and Purification of HA-Ub_{1-75}-MESNa

HA-Ub_{1-75}-Int-CBD was expressed and purified from BL21 (DE3) competent cells (Thermo Fisher Scientific) transfected with a pTYB2 plasmid encoding for the HA-ubiquitin-(1-75)-Intein-CBD fusion protein as per literature method.[26,27] Plasmid bearing cells were obtained from a glycerol stock and grown in LB medium (8 mL) treated with ampicillin (100 μg/mL)(Fisher Scientific) for 16 h at 37 °C at 180 rpm. Cells were transferred to undergo a second grow phase in LB medium (300 mL) treated with ampicillin (100 μg/mL) and grown at 37 °C at 180 rpm until an OD_{600} of 0.6-0.9 for the growth was measured. Expression was induced by addition of IPTG to a final concentration of 0.4 mM, after which cells were incubated at 18 °C for 16 h at 180 rpm. After this time, the cultures were centrifuged for 15 min at 6,000 rpm and the supernatant was decanted. The cell pellet was re-suspended in column buffer (25 mL) and lysed using pulsed sonication via sonication (3 s pulse, 3 s rest, 5 min total, 40% amplitude). The resultant lysate was centrifuged for 45 min at 12,000 rpm and the supernatant was decanted onto chitin resin (2.5 mL) equilibrated with column buffer (25 mL). The column was washed with column buffer (25 mL) and then treated with a solution of 50 mM MESNa in column buffer (15 mL). The column was incubated at 37 °C for 18 h at 80 rpm. HA-Ub_{75}-MESNa was eluted with an additional portion of column buffer (5 mL) and the eluent was concentrated to <500 μL by spin filtration at 13,200 rpm using 5 kDa MW cut-off Vivaspin 500 centrifuge filters. HA-Ub_{75}-MESNa was desalted using a NAP-5 column and eluted in column buffer in accordance with manufacturer guidelines to give a final volume of ≤1000 μL. HA-Ub_{75}-MESNa concentration was measured on a Nanodrop 1000 Spectrophotometer (0.7 mg/mL, 1000 μL).[28]

Synthesis of fluorogenic deubiquitinase substrate, HA-Ub_{1-76}-AMC



A solution of HA-Ub_{1-75}-MESNa (500 μL, 0.70 mg/mL) was treated with Tris-Cl buffer pH 7.5 (10 μL, 100 mM) followed by *N*-hydroxysuccinimide (45 μL, 2 M). The mixture was incubated at 37 °C for 10 min with shaking at 180 rpm. Amine **28** (25 mg, 0.07 mmol) was suspended in MeCN-dH_2O-DMSO (4:2:1, 100 μL) and treated with aliquots of 1M NaOH to adjust pH to 9-10. The free-base Gly-AMC solution was combined with the NHS-ester activated HA-Ub_{1-75} and was incubated at 37 °C for 12 h with shaking at 180 rpm. The mixture was desalted using a NAP-5 column according to the manufacturer's instructions and eluted with column buffer. The eluent was concentrated by spin filtration using 5 kDa MW cut-off Vivaspin 500 centrifuge filters at 13,200 rpm. The probe concentration was measured on a Nanodrop 1000 Spectrophotometer (3.30 mg/mL, 100 μL).

Transfection, Expression and Purification of Rpn11-Rpn8 heterodimer from BL21 competent cells

BL21 (DE3) competent cells (50 μL) were incubated on ice with an Rpn11-Rpn8 bearing pETDuet-1 plasmid (2 μL) for 30 min as per manufacturers guidelines. The mixture was incubated in a water bath at 42 °C for 30 s, and incubated on ice for 2 min. Cells were supplemented with SOC microbial growth medium (250 μL) and incubated for 1 h at 37 °C with shaking at 220 rpm. Cells were streaked on 1.0% agar plates infused with ampicillin (100 μg/mL) and incubated at 37 °C for 20 h. Extracted colonies were transferred to individual centrifuge tubes containing LB growth medium (5 mL) treated with ampicillin (100 μg/mL) and were incubated at 37 °C for 16 h with shaking at 180 rpm. After this time, cells were pelleted by centrifugation for 5 min at 4,000 rpm. The supernatant was aspirated, and the cell pellet was suspended in 70% glycerol solution (1 mL). Glycerol stocks were flash frozen and stored at -80 °C.

His6-tagged Rpn11 and StrepII-tagged Rpn8 were co-expressed and purified as per literature method.[2,28] Plasmid bearing BL21 (DE3) cells were obtained from the glycerol stock and grown in 2xYT growth medium (8 mL) treated with ampicillin (100 μg/mL)(Fisher Scientific) for 16 h at 37 °C at 180 rpm. Cells were transferred to undergo a second grow phase in 2xYT growth medium (300 mL) treated with ampicillin (100 μg/mL) and grown at 37 °C at 180 rpm until an OD_{600} of 0.6-0.9 for the growth was measured. Expression was induced by addition of IPTG to a final concentration of 0.4 mM, after which BL21 (DE3) cells were incubated at 18 °C for 16 h at 180 rpm. Cells were centrifuged for 15 min at 6,000 rpm and supernatant was decanted to remove depleted LB medium. The cell pellet was re-suspended in ice-cold column buffer (25 mL) containing PMSF (20 μM) and lysed using pulsed sonication via a sonication tip (4 s pulse, 4 s rest, 5 min total, 30% amplitude). The resultant lysate was centrifuged for 45 min at 12,000 rpm and the supernatant was combined with pre-washed Ni-NTA agarose resin and rotated overnight at 4 °C. The slurry was centrifuged for 5 min at 2,200 rpm and the supernatant was aspirated. The resin was washed with 20 mM imidazole wash

buffer (0.7 mL, 50 mM sodium phosphate pH 8.0, 300 mM NaCl, 20 mM imidazole), centrifuged for 5 min at 2,200 rpm and the supernatant was aspirated. The wash-centrifuge-aspirate steps were repeated twelve times. The resin was treated a further two times with 50 mM imidazole wash buffer (0.7 mL, 50 mM sodium phosphate pH 8.0, 300 mM NaCl, 20 mM imidazole). Following this, the resin was treated with 150 mM imidazole elution buffer (0.7 mL, 50 mM sodium phosphate pH 8.0, 300 mM NaCl, 150 mM imidazole) and centrifuged for 5 min at 2,200 rpm. The supernatant was aspirated and retained. The elution steps were repeated six times. The combined elution washes were concentrated by spin filtration at 9,000 rpm using 10 kDa MW cut-off Vivaspin 500 centrifuge filters. The concentrate was washed with storage buffer (500 µL, 20 mM Tris-Cl, pH 8.0, 1 mM DTT, 10% glycerol, 50 mM NaCl) and concentrated once again to a volume of 425 µL. The concentrate was filtered through a NAP-5 column and eluted with storage buffer to yield the desired recombinant heterodimer Rpn11-Rpn8 (1.36 mg/mL, 900 µL).

Rpn11-Rpn8 Deubiquitinase Activity Detection by Fluorescence Assay

In a CELLSTAR black 384-well plate, Rpn11-Rpn8 (2.7 µL, 1.36 mg/mL) was combined with solutions of 8MQ probe in homogenate buffer. Wells were treated with HA-Ub_{1-76}-AMC (0.91 µL, 3.30 mg/mL) and incubated at 37 °C for 3 h. Fluorescence (ex. = 341 nm, em. = 440 nm) measurements were collected every 10 min on a SpectraMax Gemini EM Microplate Reader. Background fluorescence was subtracted from data points and data was normalised to the vehicle control. Samples were prepared in triplicate

Cell Culture

DC2.4 mouse dendritic cells were cultured in RPMI 1640 culture medium (Gibco) supplemented with 10% fetal calf serum (FCS)(Invitrogen), 1% GlutaMAX™ (Gibco), 1% non-essential amino acids (Thermo Fisher Scientific), sodium pyruvate (1 mM), penicillin (100 IU/mL), streptomycin (50 µg/mL), and 2-mercaptoethanol (50 µM, Thermo Fisher Scientific), and incubated at 37 °C, 5% CO_2. Cells were grown to 70-80% confluency and passaged every 2-3 days by trypsinisation. Experiments were performed using cultures between passage 15 and 36.

Bone marrow-derived dendritic cells (BMDC) were obtained as previously described.[30] Cells were plated in IMDM growth medium (Gibco) supplemented with 10% FCS (Invitrogen), 1% GlutaMAX™ (Gibco), penicillin (100 IU/mL), streptomycin (50 µg/mL), and 2-mercaptoethanol (50 µM, Thermo Fisher Scientific). Cells were supplemented with additional growth medium on day 2. On day 4, loosely adherent cells were split and plated in complete IMDM culture medium. On day 9 or 10, cells were harvested for lysate labelling or replated for live cell labelling experiments.

HEK293 cells were cultured in DMEM high glucose GlutaMAX culture medium (Gibco) supplemented with 10% FCS (Invitrogen), penicillin (100 IU/mL) and streptomycin (50 µg/mL) according to literature procedures.[30] Cells were incubated at 37 °C, 5% CO_2, grown to 70-80% confluency and passaged every 2-3 days by trypsinisation. Experiments were performed using cultures between passage 30 and 40.

Cell Lysis

HEK293/DC2.4/BMDC cell pellet (2 x 10^7 cells) were suspended in ice-cold homogenate buffer (200 µL) and combined with 100 µL glass beads. Suspension was vortexed for 20 seconds and returned to ice for 90 seconds for a total of 20 repetitions. The suspension was centrifuged for 5 min at 13,200 rpm. Lysate supernatant was aspirated, and concentration was measured by Bradford assay.

MTT cell viability assay

DC2.4 cells were seeded in a 96-well plate (Sarstedt) with 1x10^5 cells/well, and incubated at 37 °C, 5% CO_2 for 3 h to allow cells to attach. A solution of **SMK-24** was prepared in FCS-free RPMI 1640 culture medium (Gibco) supplemented with 1% GlutaMAX™ (Gibco), 1% non-essential amino acids (Thermo Fisher Scientific), sodium pyruvate (1 mM), penicillin (100 IU/mL), streptomycin (50 µg/mL), and 2-mercaptoethanol (50 µM, Thermo Fisher Scientific) to give a concentration range of 3.1-200 µM. Growth medium was aspirated from seeded wells and replaced with prepared solutions of **SMK-24** in triplicate. Controls of 1% DMSO in FCS-free growth medium and complete (FCS-enriched) growth medium were also prepared in triplicate. Cells were incubated at 37 °C, 5% CO_2 for 1-24 h and centrifuged (5 min, 1,500 rpm). Growth medium was aspirated from each sample and replaced with FCS-enriched growth medium containing 500 µg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and incubated at 37 °C, 5% CO_2 for 3 h. Growth medium was aspirated and residue was dissolved in DMSO (100 µL) and incubated at 37 °C for 30 min with 600 rpm shaking. Absorbance was measured on a CLARIOstar plate reader (BMG Labtech) at 570 nm. The data set was normalised using the 1% DMSO in FCS-free growth medium control. No difference was observed between cells incubated with 1% DMSO in FCS-free growth medium and FCS-enriched growth medium.

Protein Labelling

Recombinant Rpn11-Rpn8 labelling

Recombinant Rpn11-Rpn8 (1.47 µL, 1.36 µg/µL) was diluted in homogenate buffer and aliquoted in glass microvials (Supelco) and treated with probe solutions in homogenate buffer. Samples were incubated in darkness at 37 °C for 10 min with 180 rpm shaking and then irradiated at 365 nm for 5 min in a LZC photoreactor oven (Luzchem).

Cell Lysate Labelling

Method A

Protein extract of mammalian cell lysate (2.19 µL, 22.8 µg/µL) in homogenate buffer was aliquoted in a glass microvial (Supelco), treated with probe solution in homogenate buffer and vortexed for 10 s. Samples were incubated in darkness at 37 °C for 10 min with 180 rpm shaking and then irradiated at 365 nm for 5 min in a LZC photoreactor oven (Luzchem).

Method B

Protein extract of mammalian cell lysate (23.6 µL, 8.36 µg/µL) in homogenate buffer and aliquoted in a V-bottom 96-well plate, treated with probe solution in homogenate buffer and vortexed for 10 s. Samples were prepared in quadruplicate. The 96-well plate was incubated at 37 °C for 10 min with 600 rpm shaking. The plate was irradiated on a cooling block at 350 nm for 10 min in a CaproBox (Caprotec Bioanalytics).

Live Cell Labelling

DC2.4 cells were seeded in 6 cm^2 tissue culture dishes (Sarstedt) with $5x10^6$ cells/dish, and incubated at 37 °C, 5% CO_2 for 3 h to allow cells to attach. Solution of probe in FCS-free RPMI 1640 culture medium (Gibco) supplemented with 1% GlutaMAX™ (Gibco), 1% non-essential amino acids (Thermo Fisher Scientific), sodium pyruvate (1 mM), penicillin (100 IU/mL), streptomycin (50 µg/mL), and 2-mercaptoethanol (50 µM, Thermo Fisher Scientific) was prepared to desired concentration. Growth medium was aspirated from seeded plates and replaced with prepared solutions of **SMK-24**, controls 8MQ-bearing **20** or diazirine **25**, or FCS-free control growth medium. Plates were incubated at 37 °C, 5% CO_2 for 1 h, prior to irradiation on a cooling block at 350 nm for 10 min in a CaproBox (Caprotec Bioanalytics). Cells were harvested by scraping and the suspended cells were pelleted (4 min, 3,500 rpm). The supernatant was aspirated and cells were stored at -21 °C prior to lysis. Cells were lysed as per 'Cell Lysis' method. Samples were prepared in quadruplicate.

CuAAC conjugation to **34**

A solution of 50 mM $CuSO_4$ (20 µL) was treated with a solution of 100 mM THPTA in tBuOH-DMSO (4:1, 10 µL). To this was added a solution of 100 mM sodium ascorbate (15 µL) and the mixture was vortexed for 10 s. A solution of 50 mM Biotin-PEG4-azide, **34** in DMSO (30 µL) was added and the mixture was vortexed for 20 s. The freshly prepared CuAAC reaction mixture was added to labelling samples, such that the volume of addition corresponded to 40 equiv. of azide relative to 1 equiv. of alkyne bearing probe **SMK-22**/**SMK-24**. Samples were incubated at 37 °C for 3 h with 600 rpm shaking.

SDS-PAGE

Labelled protein samples (30 µL) were combined with Laemlli's buffer (0.2 M Tris pH 6.8, 30% glycerol 0.4% β-mercaptoethanol, 9% SDS, bromophenol blue) (30 µL) and heated at 95 °C for 5 min. Proteins were resolved by 12% SDS:PAGE (resolving gel: 1.5 M Tris pH 8.8 (1.3 mL), 40% acrylamide/Bis-acrylamide (29:1) (1.5 mL), dH_2O (2 mL), 10% SDS (50 µL), 10% ammonium persulfate (APS) (50 µL), Tetramethylethylenediamine (TEMED) (5 µL); stacking gel: 0.5 M Tris pH 6.8 (630 µL), acrylamide (0.3 mL), dH_2O (1.3 mL), 10% SDS (25 µL), 10% APS (25 µL), TEMED (2.5 µL). followed by heating at 95 °C for 5 min. The proteins were loaded along with Fisher EZ-Run™ Pre-Stained Rec Protein Ladder. Resolution was performed at 150 V for 1.5 h and visualised by Western blotting and silver staining.

Silver Staining

SDS-PAGE gels were treated with fixative solution (40% EtOH, 10% AcOH) for 1 h at ambient temperature. Gels were washed with 20% EtOH solution (2 x 10 min), then rehydrated in dH_2O (2 x 10 min). Gels were sensitised in 0.02% $Na_2S_2O_3$ solution for 50 s and immediately washed with dH_2O (2 x 1 min). Gels were incubated in a solution of 12 mM $AgNO_3$ (aq.) solution containing 0.02% formaldehyde at 4 °C for 1 h. Gels were washed with dH_2O (2 x 20 s) and treated with developer solution (3% K_2CO_3, 0.05% formaldehyde). Development was halted with 5% AcOH. Stained gels were visualised using a Typhoon TLA9500 laser scanner (GE Healthcare).

Western blotting

Method A:

Proteins resolved by SDS-PAGE were transferred to nitrocellulose Western blotting membrane in blotting transfer buffer (25 mM Tris, 190 mM Glycine, 20% MeOH) at 15 V, 4 °C for 16 h. The membrane was blocked with incubation in 5% BSA in PBST at 4 °C for 3 h. Fluorescent IRDye 800CW Streptavidin (LI-COR Biosciences) was diluted 1:5000 in 2% BSA in PBST, added to the membrane and incubated at ambient temperature for 1 h. The membrane was washed with PBST (4 x 5 min) and fluorescence was detected using an Odyssey Infrared Imaging System (LI-COR Biosciences).

Method B:

Proteins resolved by SDS-PAGE gels were transferred by Trans-Blot Turbo Transfer system (Bio-Rad) to Trans-Blot Turbo Mini 0.2 µm nitrocellulose membrane (Bio-Rad). The membrane was blocked with incubation in 5% BSA in PBST at 4 °C for 3 h. The fluorescent dye Streptavidin, Alexa Fluor 647 (Invitrogen) was diluted 1:5000 in 2% BSA in PBST and incubated at ambient temperature for 1 h. The membrane was washed with PBST (4 x 5 min) and fluorescence was detected on ChemiDoc MP Imaging System (Bio-Rad) in the Epi-red channel.

Proteomics

$CHCl_3$/MeOH Protein Precipitation

Protein samples were treated sequentially with MeOH (666 µL), $CHCl_3$ (166 µL) and H_2O (150 µL) and vortexed. The precipitated protein was pelleted by centrifugation (10 min, 3,500 rpm) and solvent was aspirated. The pellet was washed by resuspension in MeOH (600 µL), sonicated (10 s, 30% amplitude) and centrifuged (5 min, 14,000 rpm) and the remaining solvent was aspirated.[31]

Avidin Binding and Enrichment

The pelleted protein was dissolved in 6M urea buffer (250 µL), treated with 1M DTT (2.5 µL) and heated to 65 °C for 15 min. Following cooling, the sample was treated with freshly prepared 0.5 M iodoacetamide (20 µL) and incubated in darkness at ambient temperature for 30 min. The sample was treated with 10% SDS solution (70 µL) and incubated at 65 °C for 5 min. Samples were combined with a suspension of PBS (5 mL) containing pre-washed avidin agarose resin (50 µL) and incubated while rotating at ambient temperature for 6 h. Beads were pelleted by centrifugation (2 min, 4,000 rpm) and supernatant was aspirated. Beads were washed with 0.5% SDS in PBS (1 mL), PBS (3 x 1 mL), resuspended in PBS (0.5 mL), transferred to a low binding tube. Beads were pelleted by centrifugation (2 min, 4,000 rpm) and supernatant was aspirated.

On-Bead Digestion

Avidin beads bearing enriched proteins were suspended in a solution of digestion buffer (250 µL, 100 mM Tris pH 7.8, 100 mM NaCl, 1 mM $CaCl_2$, 2% MeCN) containing trypsin (3.6 µL, 0.25 mg/mL) (sequencing grade, Promega). Samples were incubated at 37 °C for 16 h with shaking at 950 rpm. Trypsin was inactivated through treatment with formic acid (12.5 µL) and samples were filtered through a Bio-Spin column (Bio-Rad) under centrifugation (2 min, 2,500 rpm) and filtrate was collected.

StageTip Peptide Purification

C18 StageTips were conditioned sequentially with MeOH (50 µL), 0.5% formic acid in 80% MeCN/MilliQ H_2O (50 µL) and 0.5% formic acid in MilliQ H_2O (50 µL). Samples were added to conditioned StageTips with centrifugation (2 min, 2,000 rpm) and peptides were washed with 0.5% formic acid in MilliQ H_2O (100 µL) and centrifuged (2 min, 2,000 rpm). Peptides were eluted into new low-binding tubes with 0.5% formic acid in 80% MeCN/MilliQ H_2O (100 µL) and the eluent was lyophilised using an Eppendorf Concentrator Plus 5301 speedvac (Eppendorf).

LC-MS/MS

Lyophilised peptide samples were reconstituted in a solution of 0.1% formic acid, 3% MeCN in MilliQ H_2O (50 µL) containing 20 fmol/µL yeast enolase peptide digest standard (Waters). Samples were measured as previously reported on an Ultimat 3000 RSLCnano combined with a Q Exactive High Frequency Orbitrap (Thermo Scientific).[33] Peptides were resolved using a nanoEase M/Z HSS C18 T3 100Å, 1.8 µm, 75 µm x 250 mm analytical column (Waters) at 40 °C with a non-linear gradient 1 to 90% MeCN in H_2O with 0.1% formic acid. Electro-spray ionization (ESI) of the eluent via a nanoESI source (Thermo Scientific) using stainless steel nano-bore emitters (40 mm, OD 1/32", ES542, Thermo Scientific). Mass analysis on the Q Exactive High Field Orbitrap was performed in the positive mode. A default charge of 2+ was applied to the data dependent

acquisition without the use of lock mass. External calibration with LTQ Velos ESI positive ion calibration solution (Pierce, Thermo) was performed every 5 days to <2 ppm.

The tune file for the survey scan was set to scan range of 350 – 1400 m/z, 60.000 resolution (m/z 200), 1 microscan, automatic gain control (AGC) of 1e6, max injection time of 50 ms, no sheath, aux or sweep gas, spray voltage ranging from 1.7 to 3.0 kV, capillary temp of 250°C and an S-lens value of 80. For the 10 data dependent MS/MS events the loop count was set to 10 and the general settings were resolution to 15,000, AGC target 1e5, max IT time 100 ms, isolation window of 1.6 m/z, no fixed first mass and normalized collision energy (NCE) of 28 eV. For individual peaks the data dependent settings were 5.00e4 for the minimum AGC target yielding an intensity threshold of 5.0e5 that needs to be reached prior of triggering an MS/MS event. No apex trigger was used, unassigned, +1 and charges >+8 were excluded with peptide match mode preferred, isotope exclusion on and dynamic exclusion of 20 sec. In between experiments, routine wash and control runs were done by injecting 5 µl 97.3.0.1 solution, 5 µl of 10 fmol/µl BSA or enolase digest and 1 µl of 10 fmol/µl angiotensin III (Fluka, Thermo)/oxytocin (Merck) to check the performance of the platform on each component (nano-LC, the mass spectrometer (mass calibration/quality of ion selection and fragmentation) and the search engine).

Data Processing

MaxQuant (version 2.2.0.0) was used for the identification and label-free quantification of peptides using a custom fasta file based upon the Mus musculus proteome from the Uniprot database (UPID: UP000000589, downloaded March 30, 2023) and Homo sapien proteome from the Uniprot database (UPID: UP000005640, downloaded November 14, 2022). Each proteome was supplemented with the inclusion of BETAS background (BSA P02769, yeast enolase P00924, trypsin pig P00761, avidin P02701 and streptavidin P22629).

The following adjustments to MaxQuant standard settings were applied: The digestion enzyme was set to Trypsin/P with a maximum of 2 missed cleavages. Label-free quantification was chosen with an LFQ min. ratio count of 1. "Match between runs" was enabled. The minimum number of peptides required for protein identification was set to 3. The peptide length was set to be between 8 and 25 with a maximum peptide mass of 4600 Da. Oxidation (M) and Acetyl (Protein N-term) were set as variable peptide modifications and Carbamidomethyl (C) was set as fixed peptide modification. Upon completion of analysis, the "peptides.txt" and "proteingroups.txt" files were used for further analysis. LFQ intensities of identified proteins were analysed in Perseus v2.0.7.0 for Windows. Values of four biological replicates were used per test condition. Data sets were filtered to remove contaminants and log2(x) and p-values were determined for remaining proteins. Standard volcano plot settings were applied with a false discovery rate of 0.05 using multiple t-tests to calculate the p-values. Log2(x) values show the ratio between the samples treated with **SMK-24** and control samples containing vehicle, 8MQ-bearing **20** or diazirine **25**.

Flow Cytometry

HEK293 cells were seeded in 6-well culture dishes (Sarstedt) $3x10^5$ cells/well, and incubated at 37 °C, 5% CO_2 for 12 h to allow cells to attach. Growth medium was aspirated from seeded plates and replaced with 25 µM **SMK-24** in DMEM growth medium enriched with 1% GlutaMAX™, 10% FCS and 200 µg/mL penicillin & streptomycin or vehicle control. Samples were prepared in triplicate and incubated at 37 °C, 5% CO_2. Cells were collected by trypsinisation, fixed with ice-cold 70% EtOH and stored at -20 °C for 12 h.

Fixed cells were briefly thawed and then pelleted by centrifugation (8 min, 2,500 rpm). The supernatant was aspirated and fixed cells were resuspended in propidium iodide staining solution (400 µL, PBS treated with 0.1% Triton X-100, 0.02 mg/mL propidium iodide, 0.2 mg/mL RNAase). Samples were incubated in darkness at 37 °C for 30 min and analysed by flow cytometry using a BD FACSCanto II (BD Biosciences, New Jersey USA). Cellular debris were excluded by gating forward/side scatter plots, and PI positive cells were gated based on forward scatter/PI fluorescence plots. 10,000 gated events were acquired, and results were analysed using FlowJo software (version 10.9.0). Experiments were performed on three independent occasions. Mean values and standard error were calculated. One-way ANOVA statistical analysis was performed followed by Dunnett's multiple comparison test; (*, $p < 0.10$; **, $p < 0.05$; ***, $p < 0.01$). Results with > 95% confidence were reported. Samples were prepared in triplicate.

References

1 C. Perez, J. Li, F. Parlati, M. Rouffet, M. Yuyong, A. L. Mackinnon, T. F. Chou, R. J. Deshaies and S. M. Cohen, *J. Med. Chem.*, 2017, **60**, 1343–1361.

2 D. S. Hameed, A. Sapmaz, L. Burggraaff, A. Amore, C. J. Slingerland, G. J. P. van Westen and H. Ovaa, *Angew. Chemie - Int. Ed.*, 2019, **58**, 14477–14482.

3 L. C. Dang, F. D. Melandri and R. L. Stein, *Biochemistry*, 1998, **37**, 1868–1879.

4 W. Doherty, E. M. Dürr, H. T. Baddock, S. Y. Lee, P. J. McHugh, T. Brown, M. O. Senge, E. M. Scanlan and J. F. McGouran, *Org. Biomol. Chem.*, 2019, **17**, 8094–8105.

5 L. Wirtz, D. Auerbach, G. Jung and U. Kazmaier, *Synthesis (Stuttg).*, 2012, **44**, 2005–2012.

6 M. Walko, E. Hewitt, S. E. Radford and A. J. Wilson, *RSC Adv.*, 2019, **9**, 7610–7614.

7 P. Kleiner, W. Heydenreuter, M. Stahl, V. S. Korotkov and S. A. Sieber, *Angew. Chemie - Int. Ed.*, 2017, **56**, 1396–1401.

8 Y. Shan, J. Wang, R. Si, Y. Ma, J. Li, Q. Zhang, W. Lu and J. Zhang, *Bioorg. Chem.*, 2021, **108**, 104551.

9 Z. Li, P. Hao, L. Li, C. Y. J. Tan, X. Cheng, G. Y. J. Chen, S. K. Sze, H.-M. Shen and S. Q. Yao, *Angew. Chemie*, 2013, **125**, 8713–8718.

10 B. Lee, W. Sun, H. Lee, H. Basavarajappa, R. S. Sulaiman, K. Sishtla, X. Fei, T. W. Corson and S. Y. Seo, *Bioorganic Med. Chem. Lett.*, 2016, **26**, 4277–4281.

11 E. Leyva, E. Monreal and A. Hernández, *J. Fluor. Chem.*, 1999, **94**, 7–10.

12 B. Hu, R. Bernotas, R. Unwalla, M. Collini, E. Quinet, I. Feingold, A. Goos-Nilsson, A. Wilhelmsson, P. Nambi, M. Evans and J. Wrobel, *Bioorganic Med. Chem. Lett.*, 2010, **20**, 689–693.

13 E. H. Erickson, C. F. Hainline, L. S. Lenon, C. J. Matson, T. K. Rice, K. F. Swingle and M. Van Winkle, *J. Med. Chem.*, 1979, **22**, 816–823.

14 S. Sarkar, P. Ghosh, A. Misra and S. Das, *Synth. Commun.*, 2015, **45**, 2386–2393.

15 *US Pat.*, US2009186918A1, 2007.

16 J. Voskuhl, M. Waller, S. Bandaru, B. A. Tkachenko, C. Fregonese, B. Wibbeling, P. R. Schreiner and B. J. Ravoo, *Org. Biomol. Chem.*, 2012, **10**, 4524–4530.

17 J. Naskar, M. G. B. Drew, I. Deb, S. Das and A. Banerjee, *Org. Lett.*, 2008, **10**, 2625–2628.

18 G. Laconde, M. Amblard and J. Martinez, *European J. Org. Chem.*, 2018, **2019**, 85–90.

19 J. Liang, G. Fang, X. Huang, Z. Mei, J. Li, C. Tian and L. Liu, *Sci. China Chem.*, 2013, **56**, 1301–1306.

20 C. Lang, X. Zhang, Q. Luo, Z. Dong, J. Xu and J. Liu, *European J. Org. Chem.*, 2015, **2015**, 6458–6465.

21 H. Mao, J. B. Thorne, J. S. Pharr and R. E. Gawley, *Can. J. Chem.*, 2006, **84**, 1273–1279.

22 A. Blanc, M. Todorovic and D. M. Perrin, *Chem. Commun.*, 2019, **55**, 385–388.

23 W. H. Wen, M. Lin, C. Y. Su, S. Y. Wang, Y. S. E. Cheng, J. M. Fang and C. H. Wong, *J. Med. Chem.*, 2009, **52**, 4903–4910.

24 X. Zheng, Z. Li, W. Gao, X. Meng, X. Li, L. Y. P. Luk, Y. Zhao, Y. H. Tsai and C. Wu, *J. Am. Chem. Soc.*, 2020, **142**, 5097–5103.

25 J. Hoogenboom, M. Fiers, R. Immink, H. Zuilhof and T. Wennekes, *Tetrahedron Lett.*, 2016, **57**, 2406–2409.

26 S. Chong, F. B. Mersha, D. G. Comb, M. E. Scott, D. Landry, L. M. Vence, F. B. Perler, J. Benner, R. B. Kucera, C. A. Hirvonen, J. J. Pelletier, H. Paulus and M. Q. Xu, *Gene*, 1997, **192**, 271–281.

27 A. Borodovsky, H. Ovaa, N. Kolli, T. Gan-Erdene, K. D. Wilkinson, H. L. Ploegh and B. M. Kessler, *Chem. Biol.*, 2002, **9**, 1149–1159.

28 N. C. Taylor, G. Hessman, H. B. Kramer and J. F. McGouran, *Chem. Sci.*, 2020, **11**, 2967–2972.

29 E. J. Worden, C. Padovani and A. Martin, *Nat. Struct. Mol. Biol.*, 2014, **21**, 220–227.

30 T. van Leeuwen, W. Doelman, R. W. R. van den Kieboom, B. I. Florea and S. I. van Kasteren, *ChemBioChem*, 2023, **24**, e202300082.

31 T. Geiger, A. Wehner, C. Schaab, J. Cox and M. Mann, *Mol. Cell. Proteomics*, 2012, **11**, M111.014050.

32 E. J. Van Rooden, B. I. Florea, H. Deng, M. P. Baggelaar, A. C. M. Van Esbroeck, J. Zhou, H. S. Overkleeft and M. Van Der Stelt, *Nat. Protoc.*, 2018, **13**, 752–767.

33 B. L. H. Beerkens, Ç. Koç, R. Liu, B. I. Florea, S. E. Le Dévédec, L. H. Heitman, A. P. Ijzerman and D. Van Der Es, *ACS Chem. Biol.*, 2022, **17**, 3131–3139.