Supporting Information for

2-Aryl-5-carboxytetrazole as a New Photoaffinity Label for Drug Target Identification

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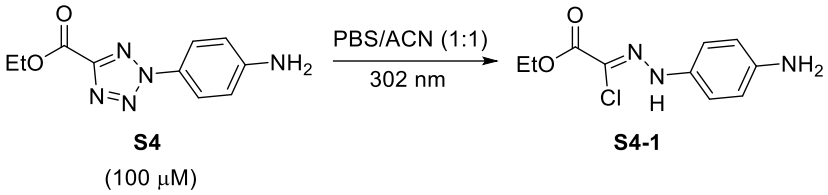
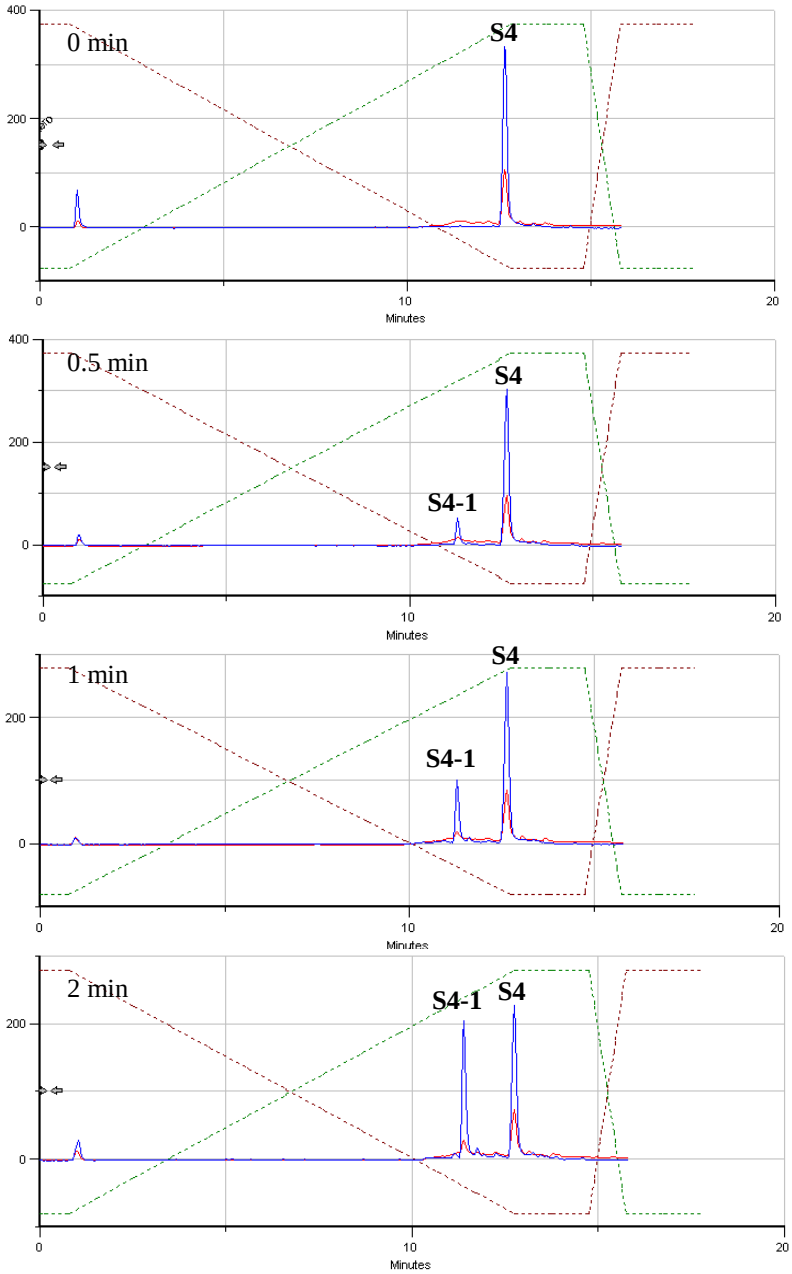
a)

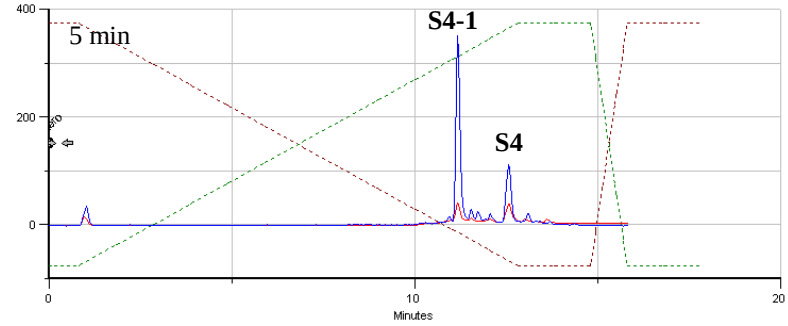


S4 (100 μM)

S4-1

b)





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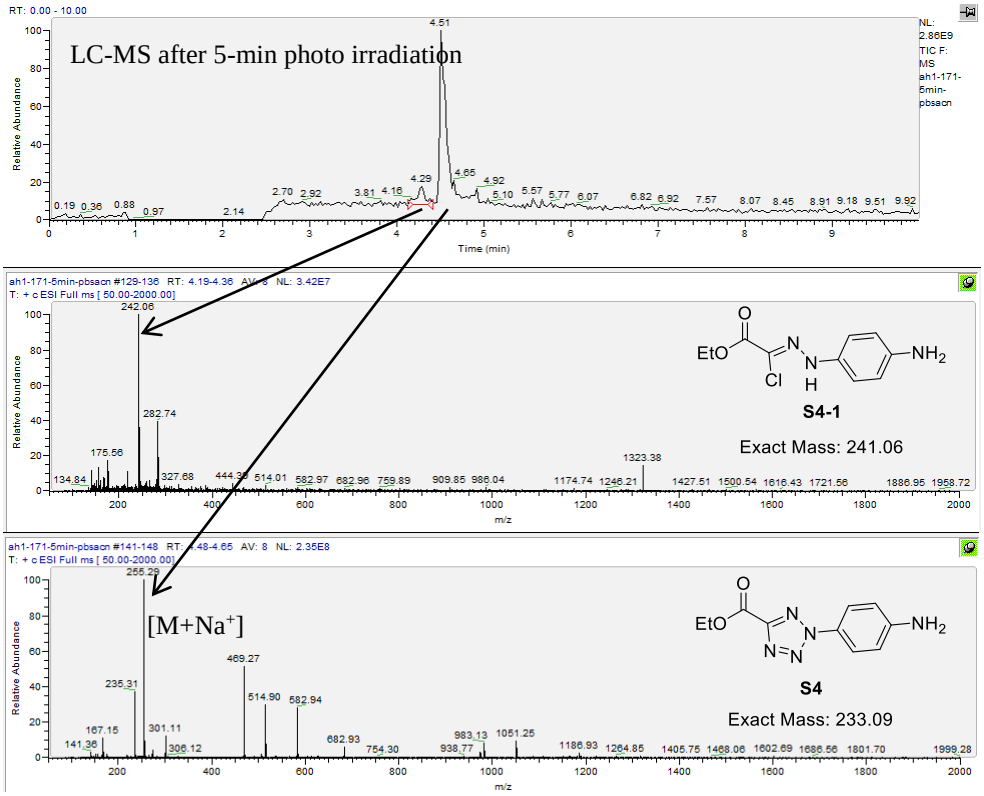


d)

Time (min)	% starting materials	% quenching product
0	100	0
0.5	88.4	11.6
1	77.2	22.8
2	57.0	43.0
5	28.5	71.5

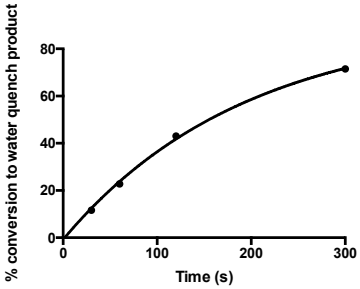


Figure S1. HPLC-based study of the medium-quenching of ACT **S4** (100 µM) in acetonitrile/PBS (1:1, 70 mM Cl^-, pH 7.5) under 302 nm photoirradiation. (a) Reaction scheme. (b) Time course of the quenching reaction after 0, 0.5, 1, 2, and 5-min photoirradiation monitored by reverse-phase HPLC. Red traces = absorbance at 254 nm; blue traces = absorbance at 365 nm. (c) Plot of percent conversion vs. photoirradiation time. The data were fitted to an exponential rise to maximum equation using: $y = (y_0 - a)e^{-k_{obs}t} + a$, to give k_{obs} = 0.005178 ± 0.0013 s^{-1}, with R^2= 0.9988.

Table S1. Kinome profiling of the Dasatinib probes.

kinase	**Das**	**1a**	**1b**	**2a**	**2b**	**3a**	**3b**
Abl	0.0004	0.0020	0.0007	0.0004	0.0004	0.0077	0.0036
ACVR1	0.122	4.6900	0.2580	4.3300	0.4400	4.6900	4.6900
Akt1	5.01	4.0700	4.0700	4.0700	4.0700	4.0700	4.0700
ALK	5.79	4.7700	4.7700	4.7700	4.7700	4.7700	4.7700
AUR1	2.81	5.5100	5.5100	5.5100	5.5100	5.5100	5.5100
AUR2	4.98	4.9800	2.9900	4.9800	4.9800	4.9800	4.9800
BRAF	0.022	0.4090	0.0909	0.1120	0.0473	2.5000	2.5000
BTK	0.0005	0.0067	0.0034	0.0014	0.0014	0.0613	0.2260
CAMK1D	5.48	5.4800	4.0200	5.4800	5.4800	5.4800	5.4800
CAMK2A	3.63	3.6300	3.6300	3.6300	3.6300	3.6300	3.6300
CAMKK2	4.96	4.9600	4.9600	4.9600	4.9600	4.9600	4.9600
CDK2	5.15	5.1500	5.1500	5.1500	5.1500	NV	NV
CDK7	5.05	5.0500	5.0500	5.0500	5.0500	NV	NV
CDK8	5	5.0000	5.0000	5.0000	5.0000	5.0000	5.0000
CDK9	1.91	3.5100	3.4600	4.8700	4.8700	NV	NV
Ck1 alpha1	4.28	6.2500	6.2500	6.2500	6.2500	6.2500	6.2500
CLK2	3.84	4.6400	4.6400	4.6400	4.6400	4.6400	4.6400
cMET	4.93	4.0200	2.6000	4.9300	4.9300	4.9300	4.9300
CSF1R	0.000486	0.0014	0.0004	0.0004	0.0005	0.0120	0.0033
DDR1	0.0149	0.0137	0.0044	0.0023	0.0015	0.1100	0.0116
Dyrk1A	2.08	5.1200	4.9800	5.1200	5.1200	5.1200	5.1200
DYRK1B	3.56	3.5600	1.7100	3.5600	3.5600	3.5600	3.5600
EGFR	3.31	4.4900	2.4400	4.4900	2.0400	4.4900	4.4900
Erk2	5.93	5.3300	5.3300	5.3300	5.3300	5.3300	NV
FAK	5.36	5.3600	5.3600	5.3600	5.3600	5.3600	5.3600
FGFR1	1.66	6.4400	2.5300	6.4400	6.4400	6.4400	6.4400
FLT1	2.21	5.3500	5.3500	5.3500	5.3500	5.3500	5.3500
Fyn	0.00049	0.0051	0.0013	0.0008	0.0009	0.0303	0.0166
GRK5	4.42	5.6000	4.0700	5.6000	5.6000	5.6000	NV
GSK3α	4.87	4.8700	4.8700	4.8700	4.8700	4.8700	4.8700
GSK3β	5.97	5.9700	5.9700	5.9700	5.9700	5.9700	5.9700
IGF1R	2.65	3.7200	3.7200	3.7200	3.7200	3.7200	3.7200
IKKE	4.73	4.7300	4.7300	4.7300	4.7300	4.7300	4.7300
InsR	3.78	7.0700	7.0700	7.0700	7.0700	7.0700	6.8400
JAK2	1.23	4.5900	3.8800	4.5900	2.8100	4.5900	4.5900
JAK3	2.63	4.9800	4.9800	4.9800	4.9800	4.9800	4.9800
JNK1	5.11	5.1100	5.1100	5.1100	5.1100	5.1100	5.1100
JNK2	6.68	6.6800	6.6800	6.6800	6.6800	6.6800	6.6800
Kdr	1.23	3.5400	3.5400	3.5400	3.5400	3.5400	3.5400
Lck	0.0004	0.0120	0.0042	0.0020	0.0014	0.0691	0.0588

LTK	2.77	4.2100	4.2800	4.2800	4.2800	4.2200	4.2800
MAP2K3	3.1	4.0400	3.3200	4.0400	4.0400	4.0400	NV
MAP3K10	1.4	5.5300	5.5300	5.5300	5.5300	5.5300	5.5300
MAP4K1	0.768	3.9900	3.6100	3.9900	3.9900	3.9900	NV
MAP4K2	1.07	2.3700	2.3700	2.3700	2.3700	2.3700	2.3700
MAP4K4	3.09	4.9400	4.9400	4.9400	4.9400	4.9400	4.9400
MEK1	0.696	4.8300	3.5200	4.8300	3.3500	4.8300	NV
MEK2	0.881	5.2800	5.2800	5.2800	4.8200	5.2800	5.2800
MST1	2.66	3.6500	3.6500	3.6500	3.6500	3.6500	3.6500
Nek2	1.88	5.1800	5.1800	5.1800	5.1800	5.1800	5.1800
p38 alpha	0.0624	0.0387	0.0932	0.1480	0.0292	4.9500	0.2860
PAK4KD	4.39	4.3900	4.3900	4.3900	3.6800	4.3900	4.3900
PDGFRA V561D	0.00273	0.0293	0.0067	0.0060	0.0052	0.2860	0.1550
PDGFRB	0.00195	0.0155	0.0063	0.0063	0.0025	0.3110	0.6370
Pim1	5.68	5.5900	5.5900	5.5900	5.5900	5.5900	5.5900
Pim2	4.19	5.0200	5.0200	5.0200	5.0200	5.0200	5.0200
PKA	4.89	4.8900	4.8900	4.8900	4.8900	4.8900	4.8900
PKC theta	3.86	1.5100	3.8600	3.8600	3.8600	3.8600	3.8600
PKC zeta	1.99	4.5800	4.5800	4.5800	4.5800	4.5800	NV
PKG1A	4	4.0000	4.0000	4.0000	4.0000	4.0000	4.0000
Plk3	1.55	3.7000	3.7000	3.7000	3.7000	3.7000	3.7000
Prkcn	3.73	2.7600	2.7600	2.7600	2.7600	2.7600	NV
RET	0.342	4.6000	1.2400	4.6000	1.4600	4.6000	4.6000
RIPK2	0.0028	0.0243	0.0109	0.0065	0.0037	0.3920	0.1070
Rock1	4.61	4.6100	4.6100	4.6100	4.6100	4.6100	4.6100
Rock2	5.28	5.2800	5.2800	NV	5.2800	4.8100	5.2800
Rsk2	4.64	4.6400	4.6400	4.6400	4.6400	4.6400	4.6400
SGK1	5.17	5.1700	5.1700	5.1700	5.1700	5.1700	5.1700
SIK1	0.000565	0.0042	0.0027	0.0010	0.0006	0.0431	0.0921
Src	0.000582	0.0051	0.0014	0.0009	0.0007	0.0316	0.0215
STK16	4.6	4.6000	4.6000	4.6000	4.6000	4.6000	4.6000
STK33	2.02	2.7000	2.7000	2.7000	2.7000	2.7000	2.7000
Syk CatDom	0.343	3.3000	2.2400	3.3000	3.3000	3.3000	3.3000
TAOK2	2.23	4.0000	2.0700	2.8400	0.5150	4.0000	4.0000
TBK1	3.27	4.0400	4.0400	4.0400	4.0400	4.0400	4.0400
TNK2	0.00192	0.0105	0.0047	0.0028	0.0033	0.1360	0.1800
TrkA	4.71	4.7100	4.7100	4.7100	4.7100	4.7100	4.7100
TrkB	5.3	5.3000	5.3000	5.3000	5.3000	5.3000	5.3000
TrkC	3.44	4.6500	4.6500	4.6500	4.6500	4.6500	4.6500
TYRO3	1.75	4.9800	3.3300	4.9800	4.6700	4.9800	4.9800
Wee1	0.0635	0.4650	0.2640	0.4710	0.0445	4.4000	4.4000
Zipk	5.41	5.7100	5.7100	5.7100	5.7100	5.7100	NV

K_i values are given in μM. Targets and off-targets are colored in blue. NV = no value.

Table S2. Binding affinity and cell proliferation data for the JQ-1 probes.

	Binding Assay K_i (μM)			Cell Proliferation Titer-Glo Assay IC_{50} (μM)		
	BRD2	BRD3	BRD4	Human MX-1	Human NCI-H1299	Human SKM-1
(+)-JQ-1	0.006	0.019	0.023	0.144	0.192	0.064
4a	0.002	0.004	0.004	1.380	7.140	0.031
4b	0.007	0.007	0.012	1.950	>10.000	0.110
5a	0.022	0.019	0.055	1.51	6.74	0.231
6a	0.006	0.018	0.022	2.030	0.911	0.067
6b	0.002	0.014	0.002	N/A	N/A	N/A

N/A = not available.

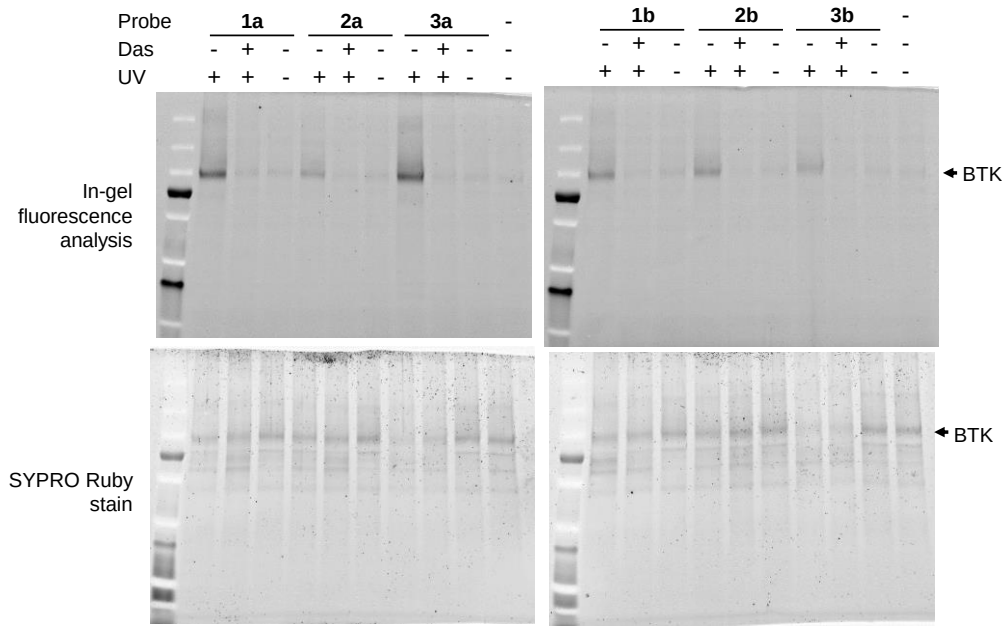


Figure S2. Full-size gel images of the labeling of recombinant BTK protein by the Dasatinib-derived photoaffinity probes.

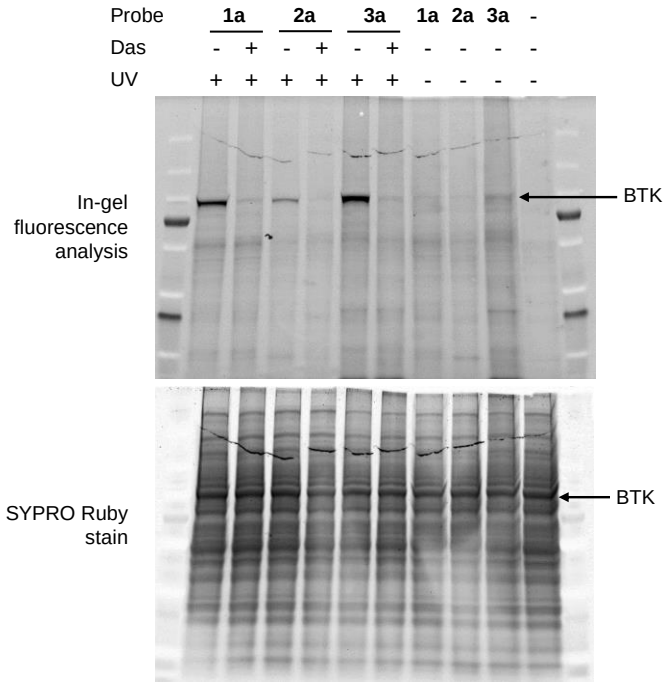


Figure S3. Selectively labeling of recombinant BTK protein by Dasatinib probes **1a**, **2a** and **3a** in the K562 cell lysate spiked with recombinant BTK protein. For the competition reactions, 0.2 μM of photoaffinity probe and 10 μM of Dasatinib were used.

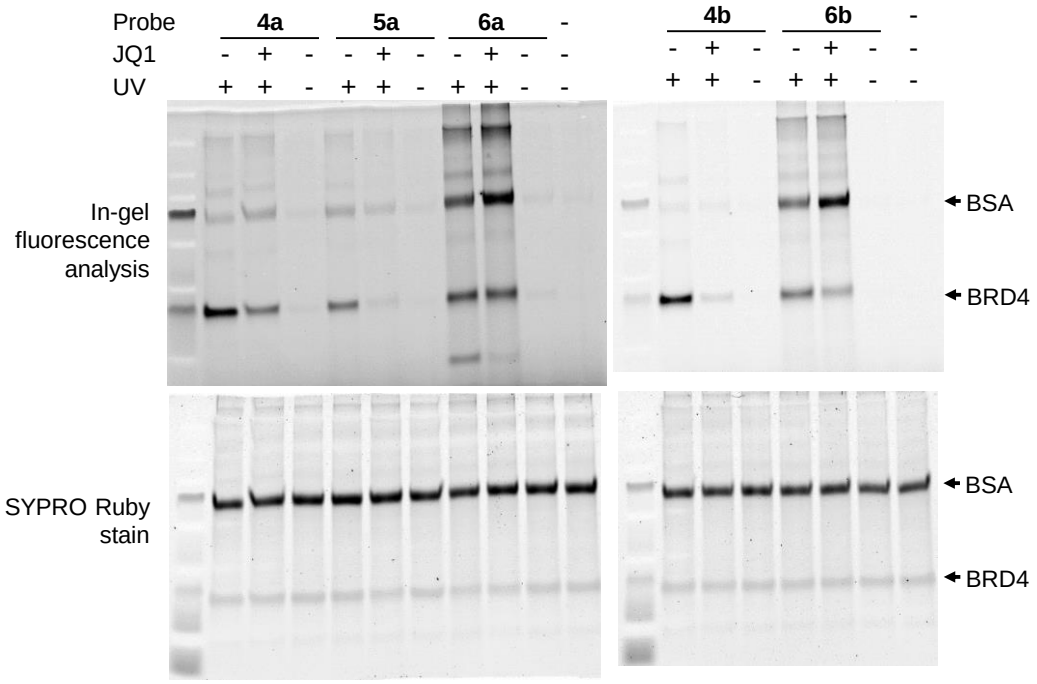


Figure S4. Full-size gel images of the labeling of recombinant BRD4 protein by the JQ-1-derived photoaffinity probes.

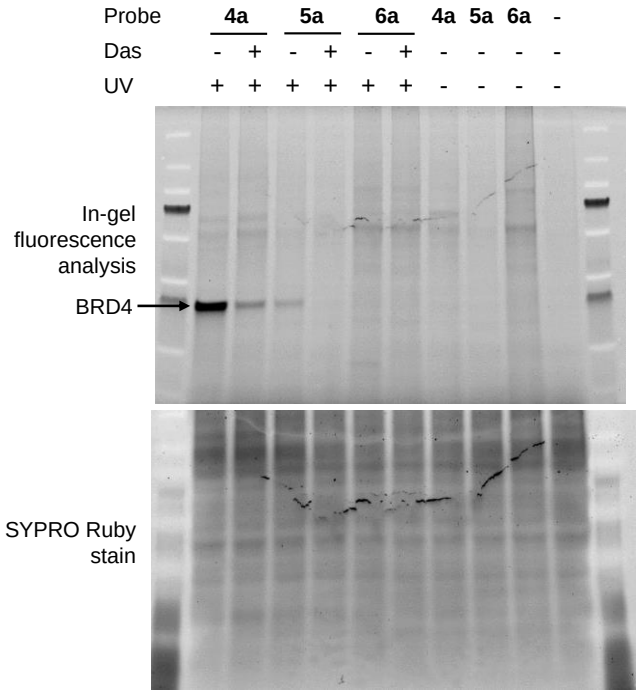
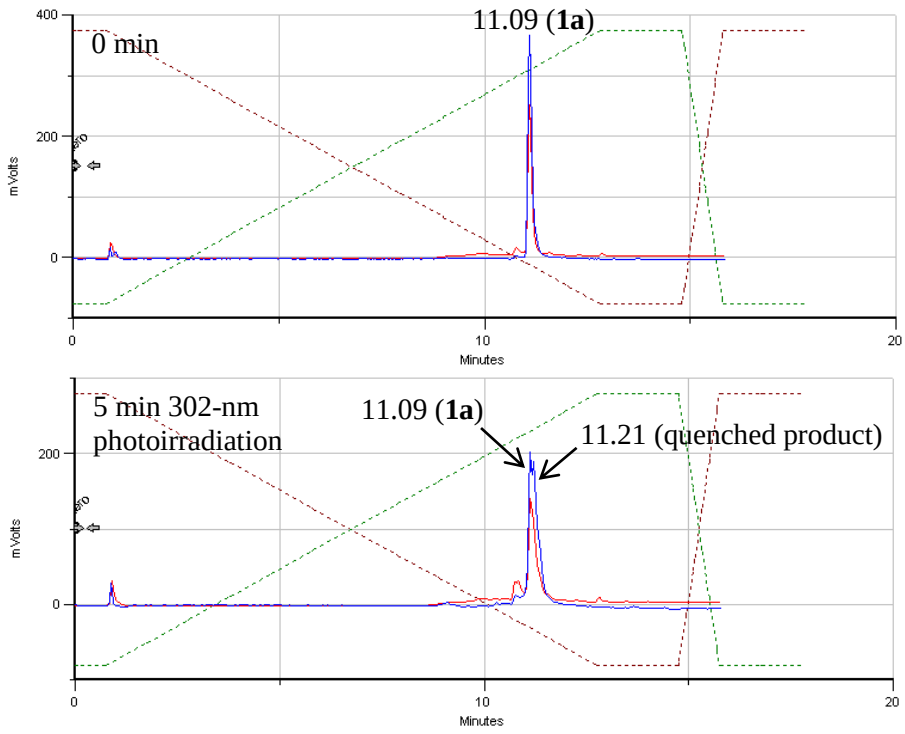


Figure S5. Labeling of recombinant BRD4 protein by JQ-1-derived photoaffinity probes **4a**, **5a** and **6a** in the. K562 cell lysate spiked with recombinant BRD4 protein.

a)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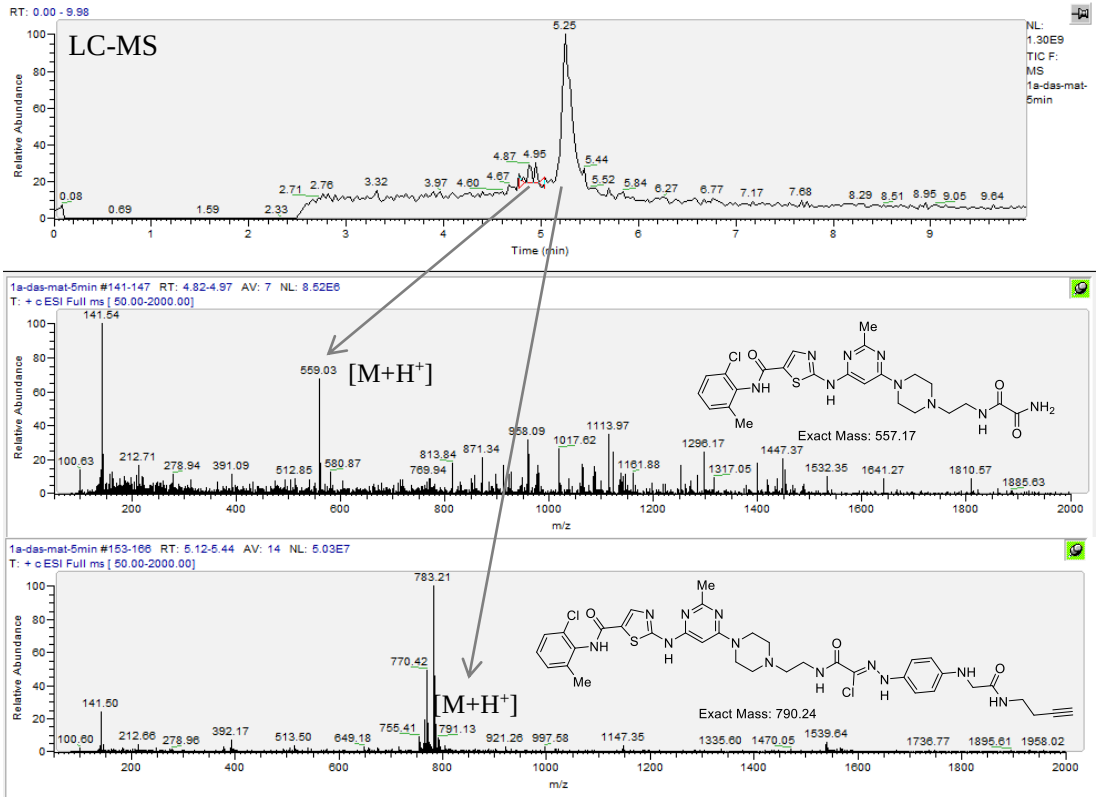
5 min 302-nm photoirradiation
11.09 (1a)
11.21 (quenched product)
mVolts
Minutes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Relative Abundance
Time (min)
[M+H+]
Exact Mass: 557.17
[M+H+]
Exact Mass: 790.24
m/z

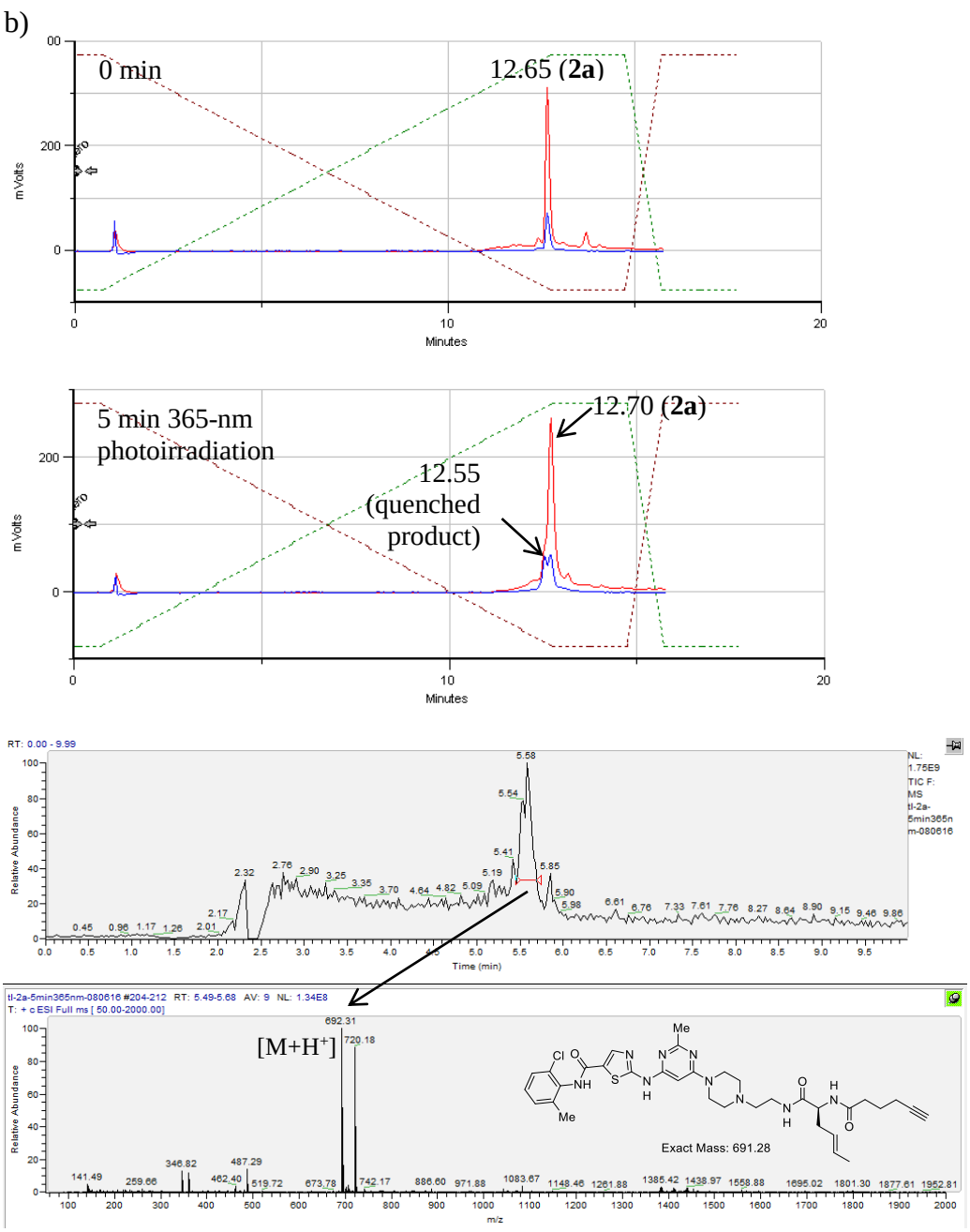


Figure S6. HPLC and LC-MS analyses of the photoactivation of (a) **1a** and (b) **2a** (100 μM) in PBS. Red trace = absorbance at 254 nm; blue trace = absorbance a 365 nm. The percent conversion was calculated to be 38% for **1a** and 44% for **2a** based on absorbance at 365 nm.

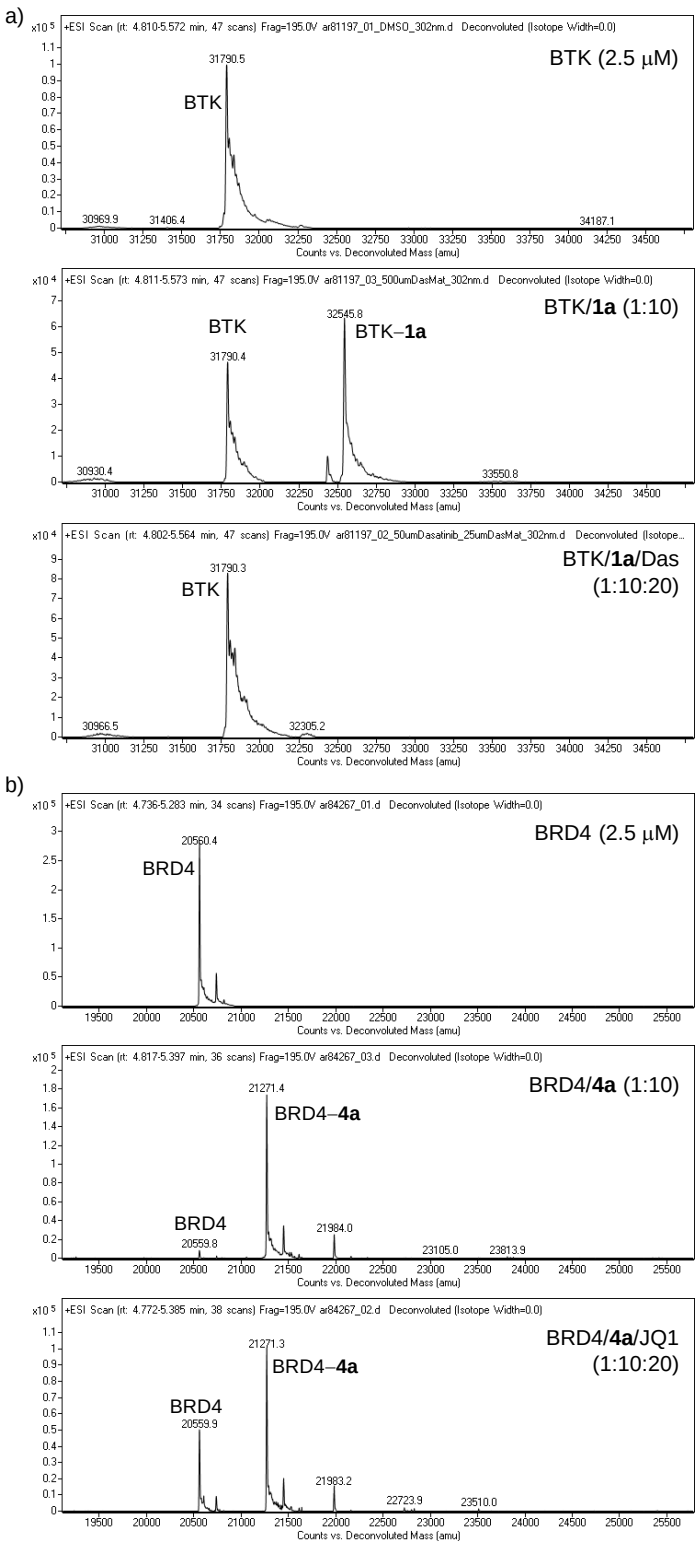


Figure S7. Inhibition of the ligand-dependent target cross-linking by the ACT-based probes as monitored by LC-MS. (a) Addition of Dasatinib (50 μM) completely abolished the labeling of BTK (2.5 μM) by probe **1a** (25 μM). (b) Addition of JQ-1 (50 μM) significantly reduced the labeling of BRD4 (2.5 μM) by probe **4a** (25 μM).

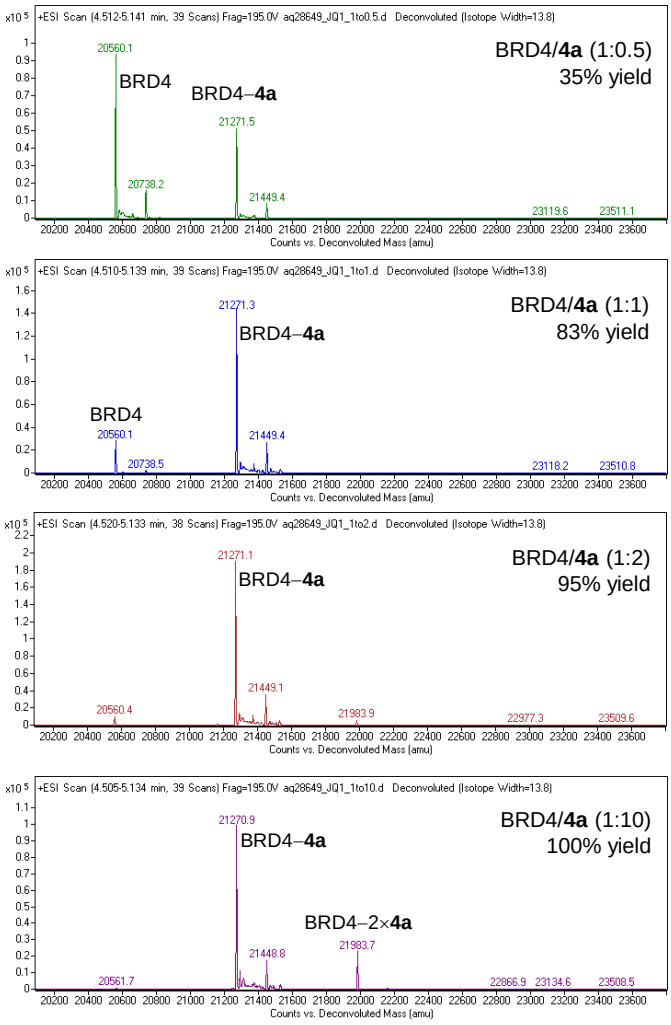
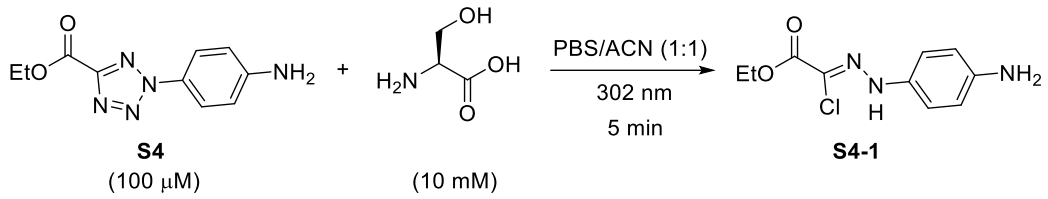


Figure S8. Concentration-dependent cross-linking of recombinant BRD4 protein by probe **4a** as monitored by LC-MS. The concentration of BRD4 used in the reactions was 2.5 μM.

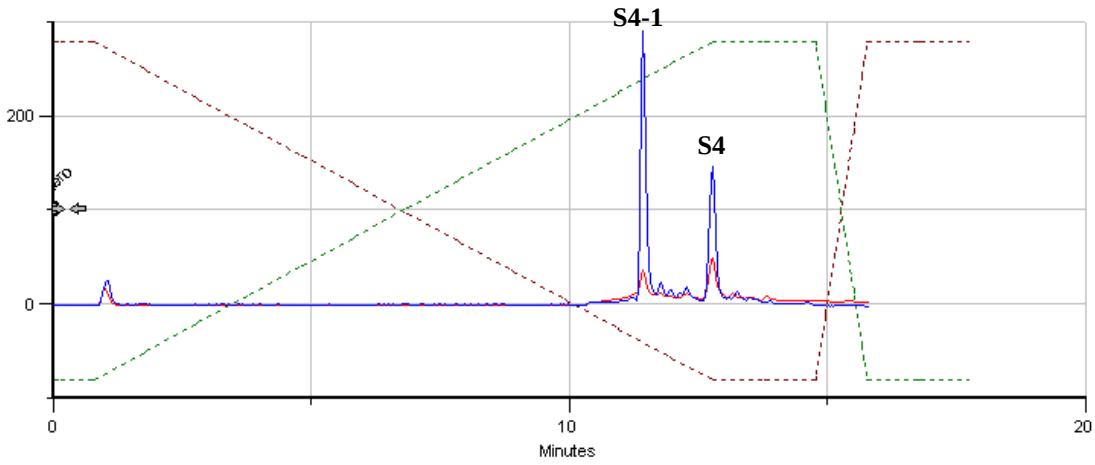
a)

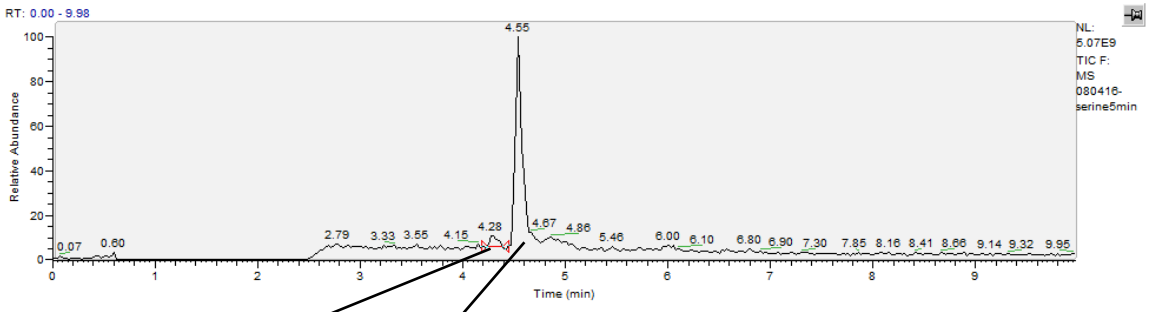


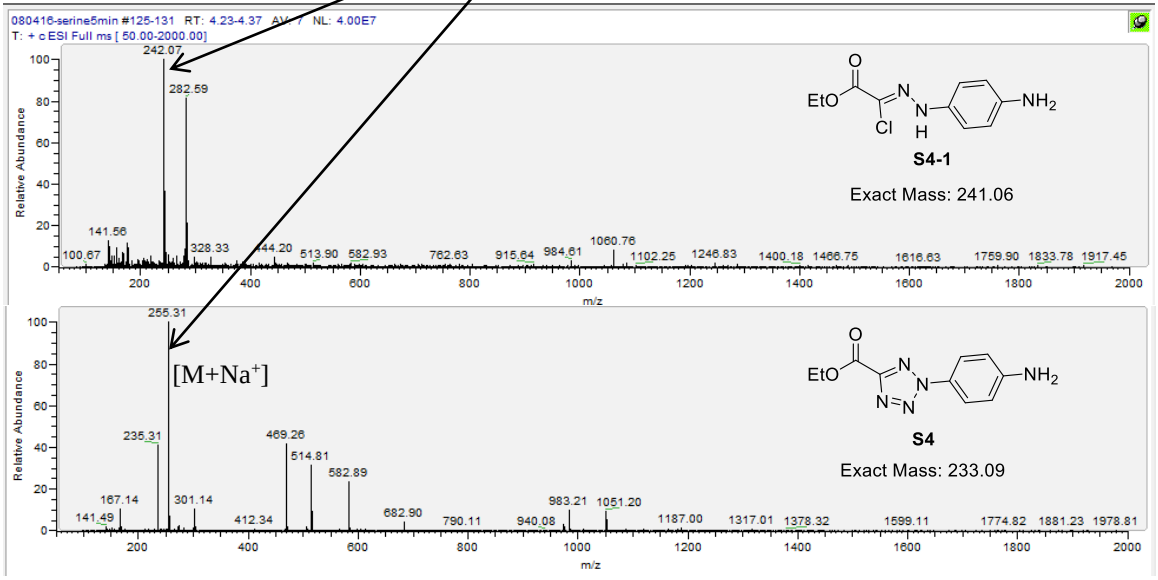
S4
(100 μM)

(10 mM)

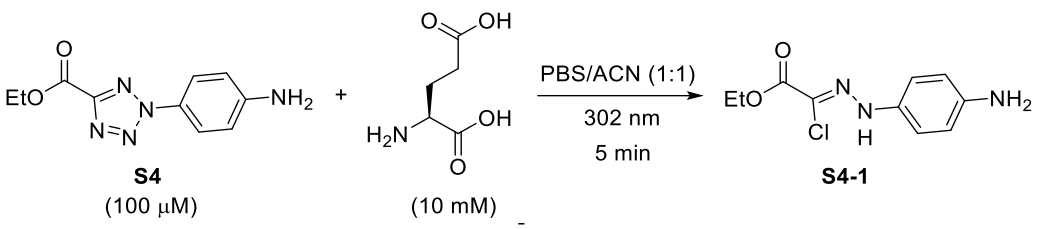
S4-1







b)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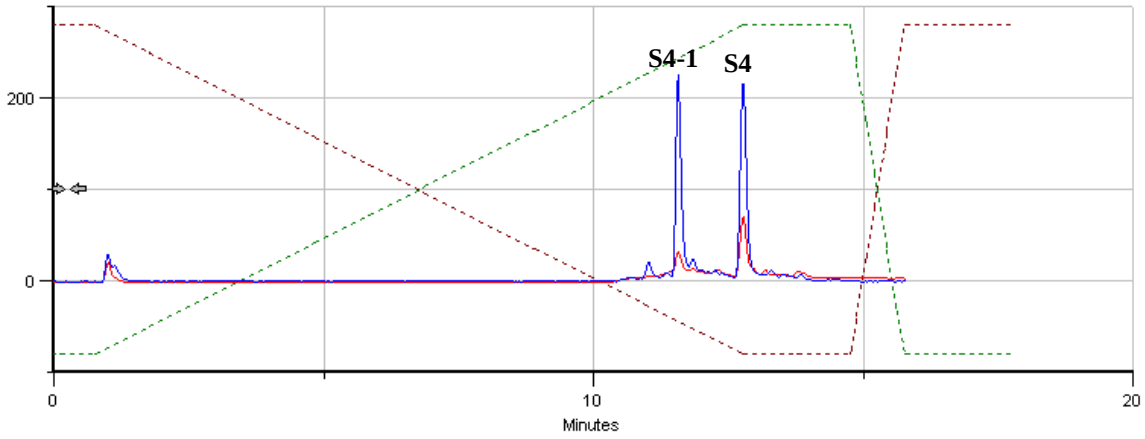
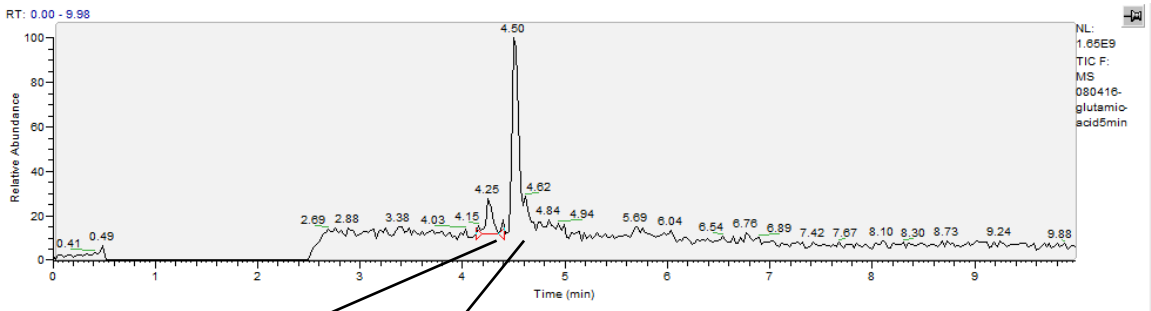
(100 μM)
H2N
OH
(10 mM)
PBS/ACN (1:1)
302 nm
5 min
Cl
H
S4-1


S4-1
S4
200
0
0
10
20
Minutes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Relative Abundance
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4.62
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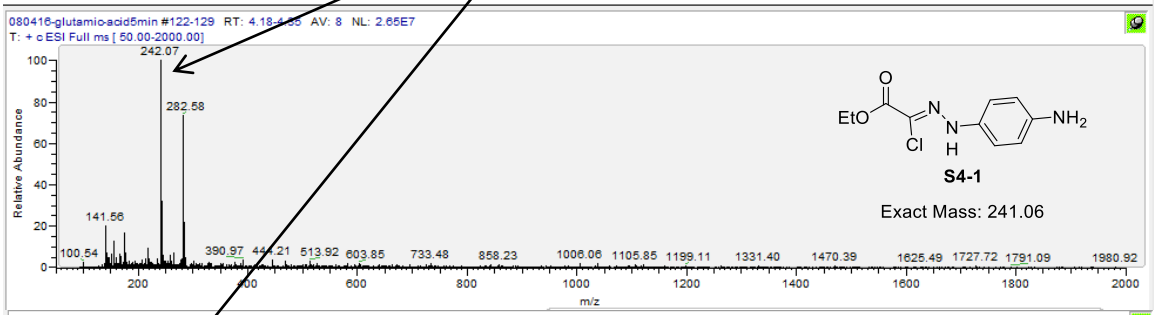
1.65E9
TIC F:
MS
080416-
glutamic-
acid5min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Relative Abundance
m/z
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NH2
Cl
H
S4-1
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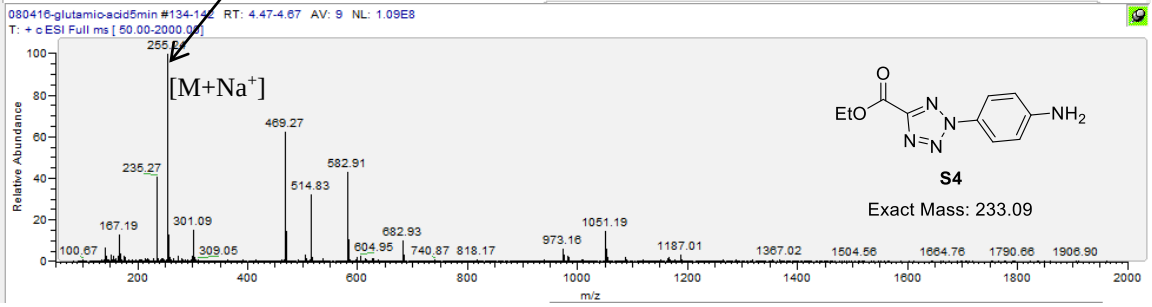
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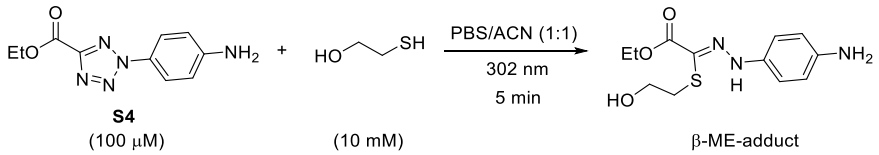
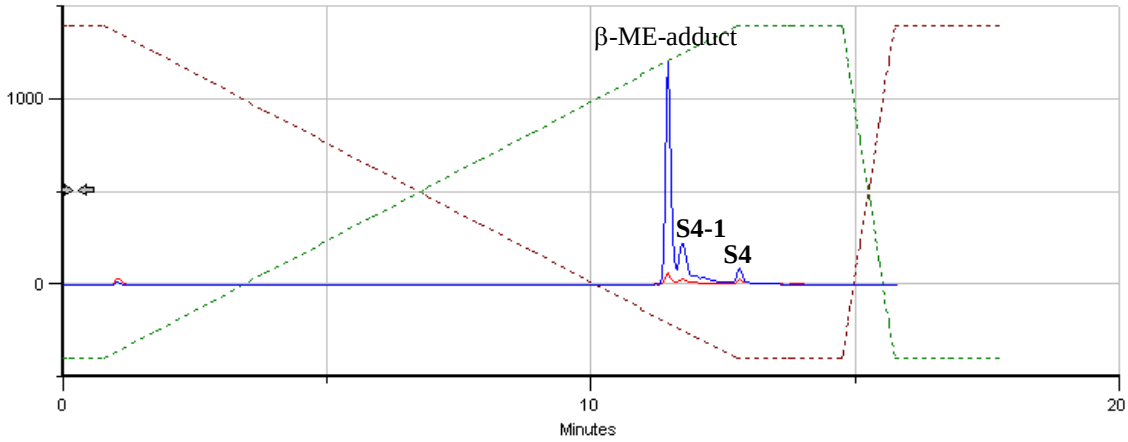
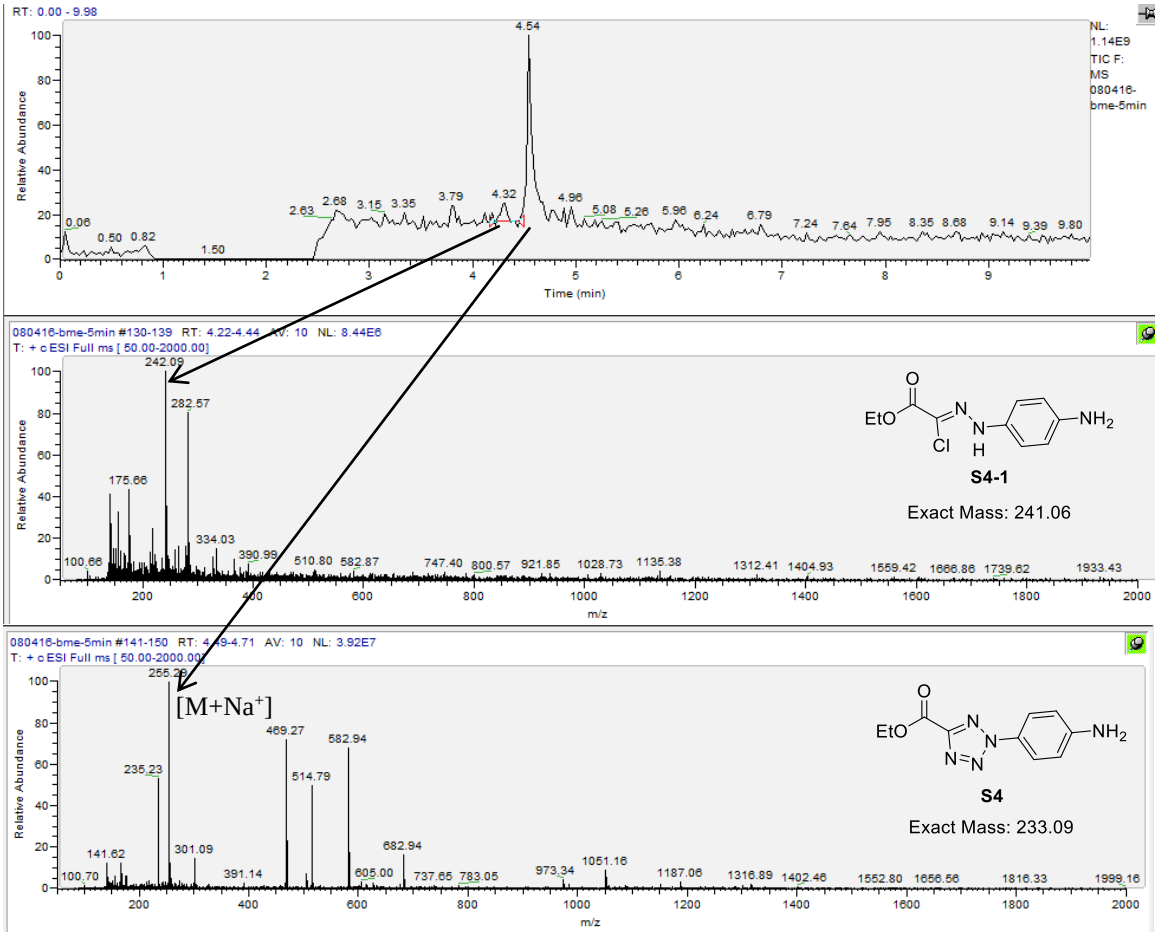
[M+Na+]
469.27
582.91
514.83
235.27
301.09
167.19
682.93
1051.19
973.16
Relative Abundance
m/z
EtO
NH2
S4
Exact Mass: 233.09

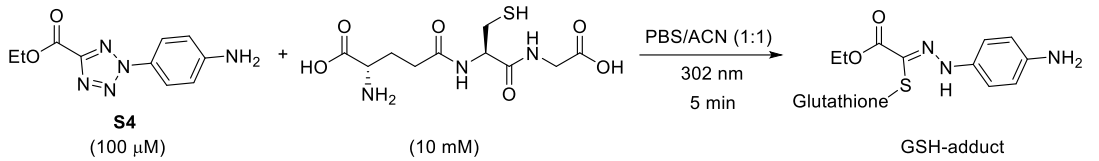
c)

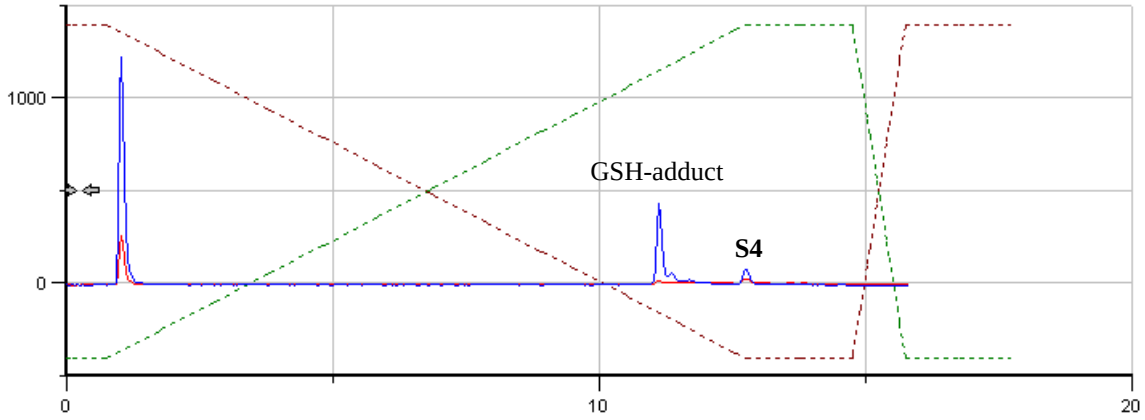

EtO
NH2
HO
SH
PBS/ACN (1:1)
302 nm
5 min
S4
(100 μM)
(10 mM)
β-ME-adduct

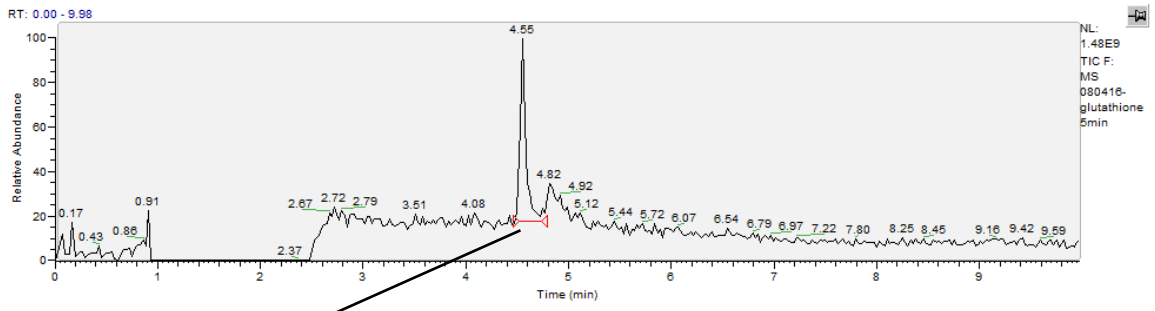

β-ME-adduct
S4-1
S4
1000
0
0
10
20
Minutes

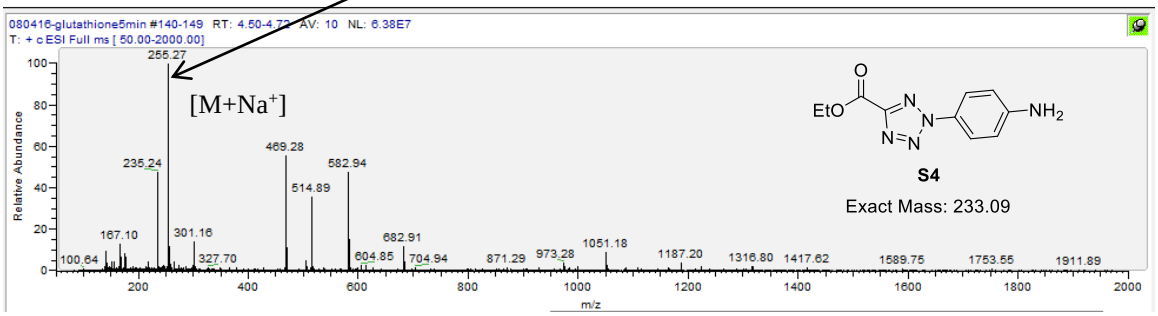

RT: 0.00 - 9.98
Relative Abundance
Time (min)
NL:
1.14E9
TIC F:
MS
080416-
bme-5min
080416-bme-5min #130-139 RT: 4.22-4.44 AV: 10 NL: 8.44E6
T: + c ESI Full ms [50.00-2000.00]
242.05
282.57
175.66
334.03
390.99
S4-1
Exact Mass: 241.06
m/z
080416-bme-5min #141-150 RT: 4.49-4.71 AV: 10 NL: 3.92E7
T: + c ESI Full ms [50.00-2000.00]
[M+Na+]
469.27
582.94
235.23
514.79
682.94
301.09
141.62
S4
Exact Mass: 233.09

d)

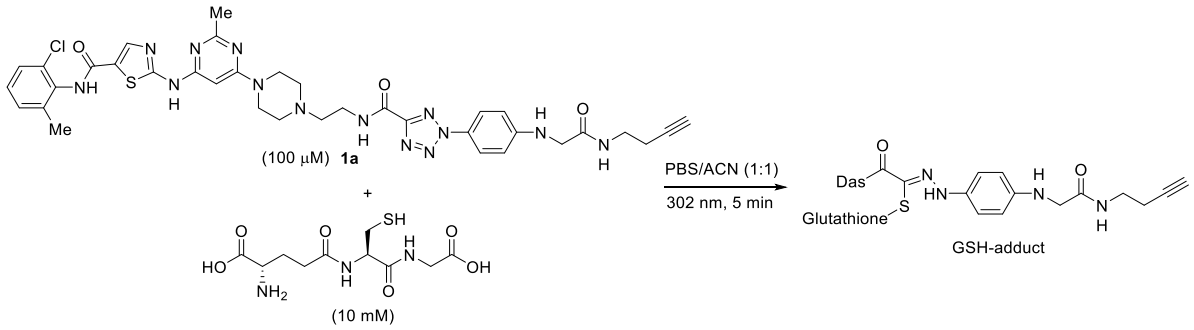

S4
(100 μM)
(10 mM)
PBS/ACN (1:1)
302 nm
5 min
Glutathione
GSH-adduct

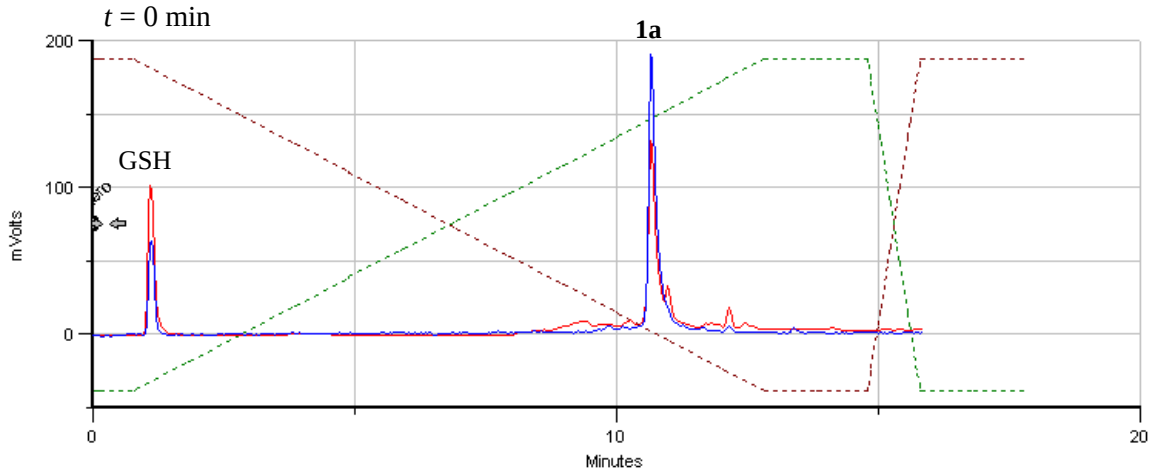

GSH-adduct
S4

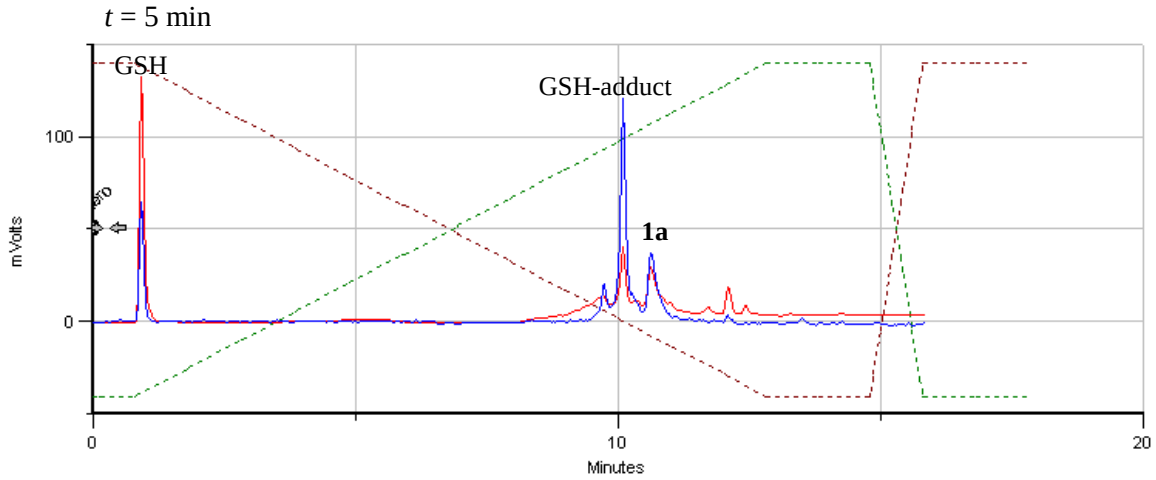

RT: 0.00 - 9.98
Relative Abundance
Time (min)
NL: 1.48E9 TIC F: MS 080416-glutathione 5min


080416-glutathione5min #140-149 RT: 4.50-4.72 AV: 10 NL: 6.38E7
T: + c ESI Full ms [50.00-2000.00]
[M+Na+]
Relative Abundance
m/z
S4
Exact Mass: 233.09

e)


(100 μM) 1a
(10 mM)
PBS/ACN (1:1)
302 nm, 5 min
Glutathione
GSH-adduct


t = 0 min
1a
GSH
mVolts
Minutes


t = 5 min
GSH
GSH-adduct
1a
mVolts
Minutes

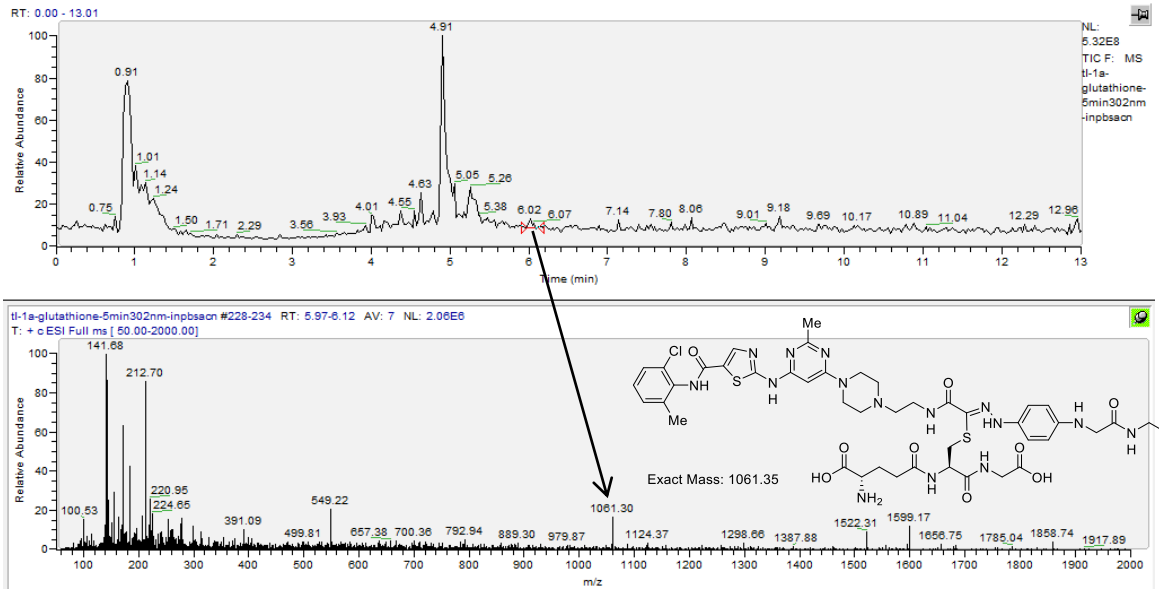
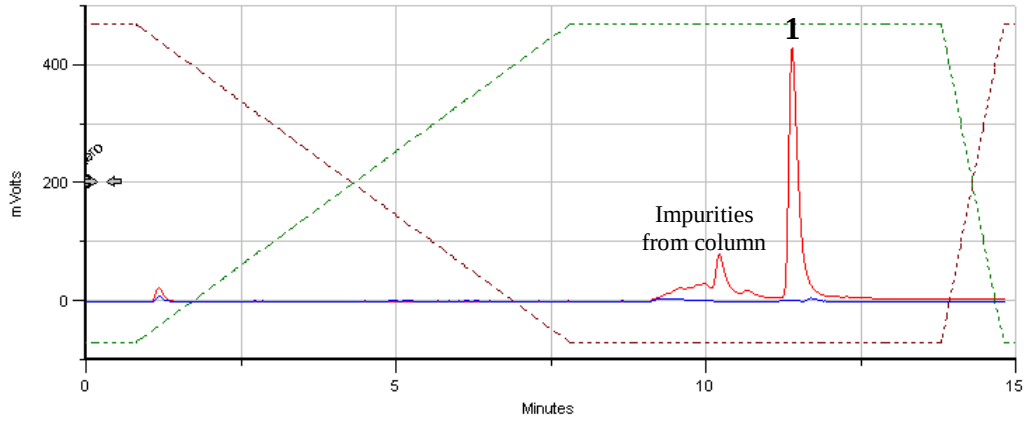
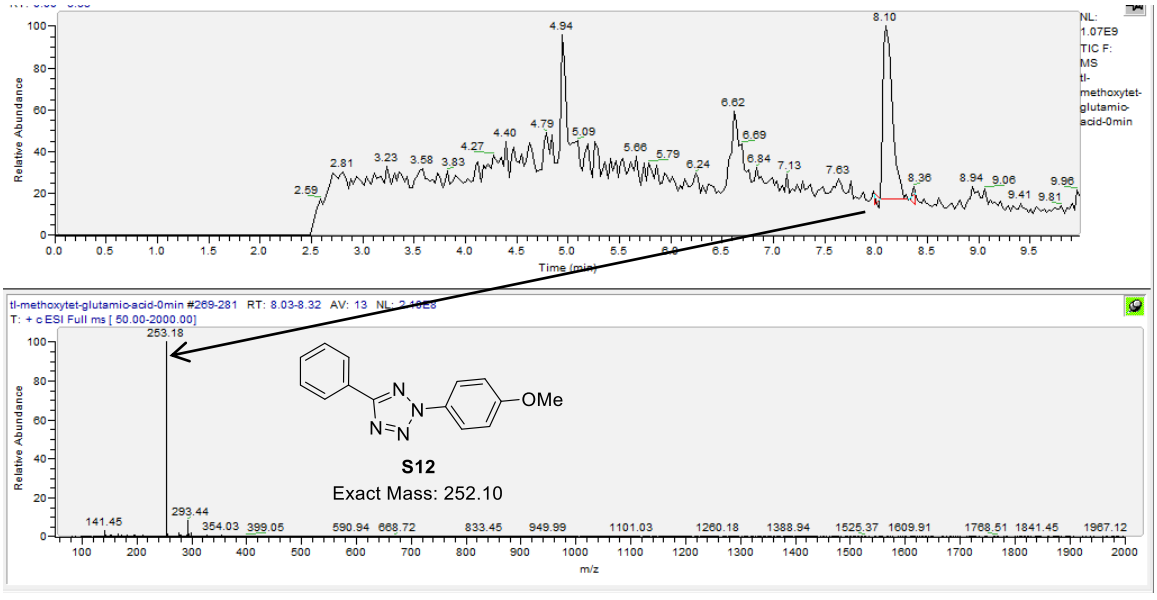

RT: 0.00 - 13.01
Relative Abundance
Time (min)
tl-1a-glutathione-5min302nm-inpbsacn #228-234 RT: 5.97-6.12 AV: 7 NL: 2.06E6
T: + c ESI Full ms [50.00-2000.00]
Exact Mass: 1061.35
m/z

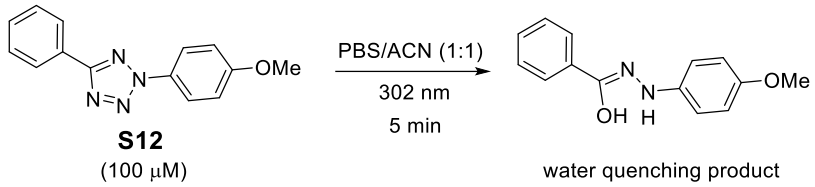
Figure S9. Model studies of the quenching reactions between 100 μM **S4** and 10 mM (a) L-serine, (b) L-glutamic acid, (c) β-mercaptoethanol, and (d) glutathione in acetonitrile/PBS (1:1, 70 mM Cl^-, pH 7.5) after 5-min 302 nm photoirradiation. (e) Quenching of probe **1a** (100 μM) by 10 mM glutathione in acetonitrile/PBS (1:1, 70 mM Cl^-, pH 7.5) after 5-min 302 nm photoirradiation. For HPLC chromatogram, red trace = absorbance at 254 nm; blue trace = absorbance at 365 nm. While L-serine and L-glutamic acid cannot compete effectively with the chloride quenching, β-mercaptoethanol and glutathione can form covalent adducts with the in situ generated carboxy-nitrile imine based on HPLC traces. However, the thiol-adduct masses were not detectable in (c) and (d), but detectable in (e), presumably due to differences in ionization potential of the adducts.

a) Diaryltetrazole **S12** (100 μM) without photoirradiation



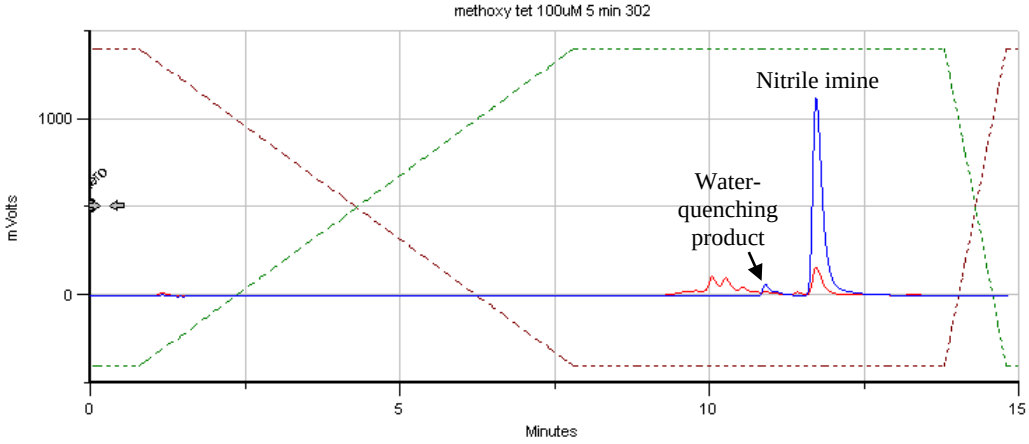


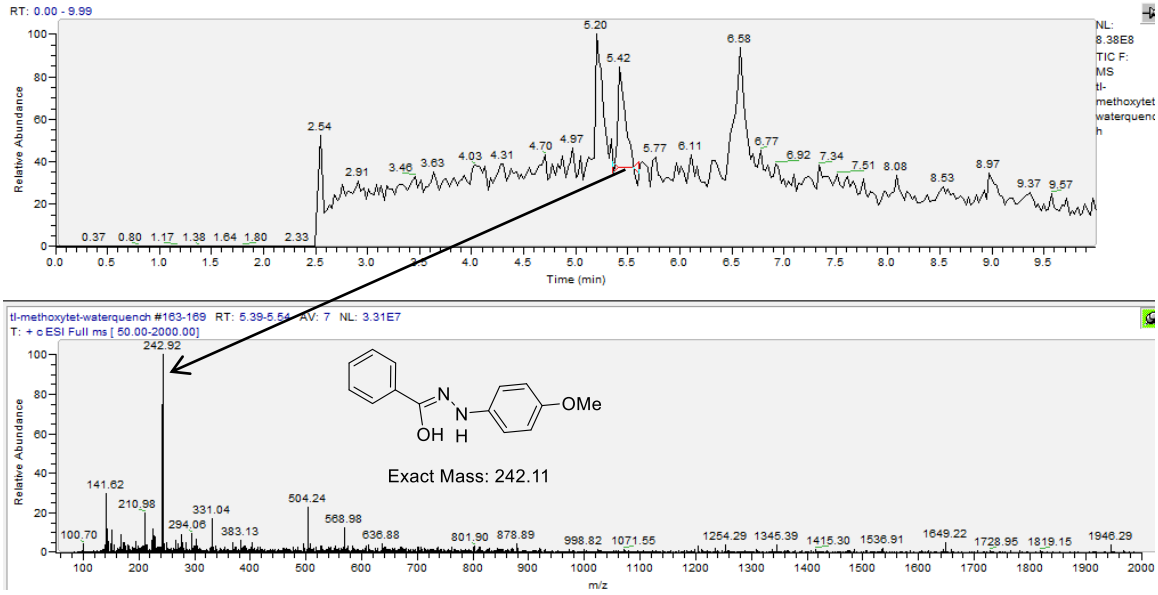
b) Diaryltetrazole **S12** only (100 μM) in PBS/ACN (1:1), irradiated at 302 nm for 5 min



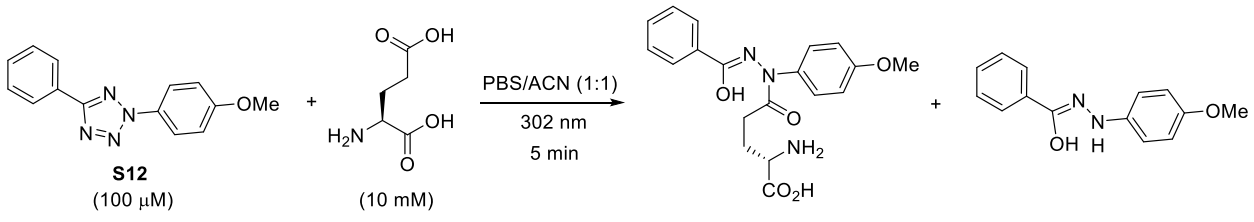
S12
(100 μM)

water quenching product





c) Diaryltetrazole **S12** (100 μM)+10 mM glutamic acid in PBS/ACN (1:1), irradiated at 302 nm for 5 min



S12
(100 μM)

(10 mM)

glutamate quenching product

water quenching product

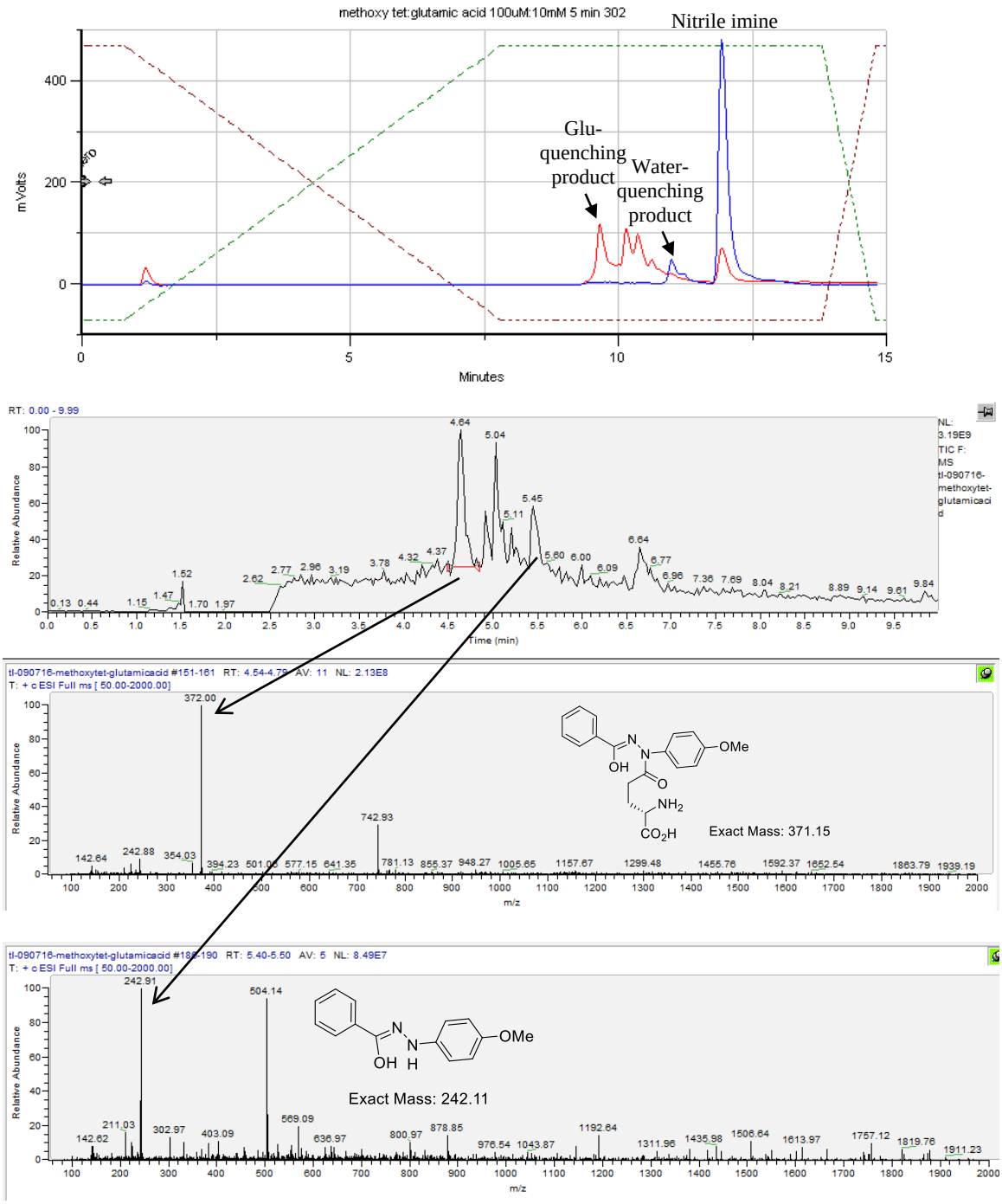


Figure S10. Model studies of the quenching reaction of **S12** by glutamic acid in PBS/acetonitrile (1:1).

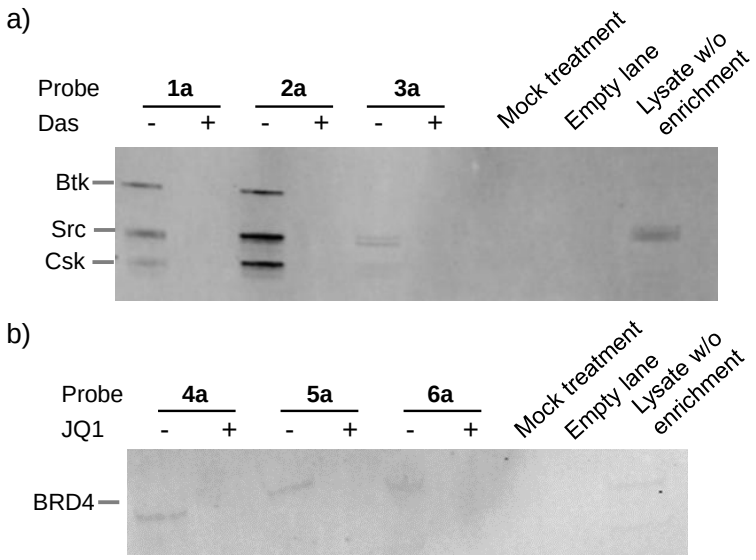


Figure S11. Western blot detection of the targets after the photoaffinity label-mediated cross-linking and subsequent pulldown. (a) Western blot of BTK, Src and Csk in the pull-downed samples using a mixture of anti-BTK, anti-Src, and anti-Csk antibodies followed by detection with an IR dye-conjugated secondary antibody. (b) Western blot of BRD4 in the pull-downed samples using an anti-BRD4 antibody followed by detection with an IR dye-conjugated secondary antibody.

Table S3. Kinase targets captured by probes **1a** and **2a** and identified by LC-MS/MS.

Accession	Gene ID	Description	# unique peptides	AUC: enriched by **1a**	AUC: inhibited by Dasatinib
Q06187	BTK	Tyrosine-protein kinase BTK	15	3.00E+06	N/D
P07948	LYN	Tyrosine-protein kinase Lyn	5	1.30E+06	N/D
P07947	YES1	Tyrosine-protein kinase Yes	4	1.30E+06	N/D
P41240	CSK	Tyrosine-protein kinase CSK	6	1.20E+06	N/D
Q16539	MAPK14	Mitogen-activated protein kinase 14	4	1.20E+06	N/D
Q53H12	AGK	Acylglycerol kinase, mitochondrial	3	4.40E+05	N/D

Accession	Gene ID	Description	# unique peptides	AUC: enriched by **2a**	AUC: inhibited by Dasatinib
P00519	ABL1	Tyrosine-protein kinase ABL1	3	3.80E+05	N/D
Q06187	BTK	Tyrosine-protein kinase BTK	15	2.30E+06	N/D
P41240	CSK	Tyrosine-protein kinase CSK	6	1.30E+06	N/D
P07948	LYN	Tyrosine-protein kinase Lyn	6	1.10E+06	N/D
Q16539	MAPK14	Mitogen-activated protein kinase 14	3	1.20E+06	N/D
P49137	MAPKAPK2	MAP kinase-activated protein kinase 2	2	4.70E+05	N/D
O43353	RIPK2	Receptor-interacting serine/threonine-protein kinase 2	3	8.30E+05	N/D
P07947	YES1	Tyrosine-protein kinase Yes	8	2.90E+06	N/D

N/D, not detected.

Table S4. Protein targets captured by probes **4a** and **5a** and identified by LC-MS/MS.

Accession	Gene ID	Description	# Unique peptides	AUC: enriched by **4a**	AUC: inhibited by JQ-1
P25440	BRD2	Bromodomain-containing protein 2	4	5.90E+05	N/D
Q15059	BRD3	Bromodomain-containing protein 3	3	3.50E+05	N/D
O60885	BRD4	Bromodomain-containing protein 4	3	6.00E+05	N/D
Q08554	DSC1	Desmocollin-1	2	2.40E+05	N/D

Accession	Gene ID	Description	# Unique peptides	AUC: enriched by **5a**	AUC: inhibited by JQ-1
P25440	BRD2	Bromodomain-containing protein 2	7	9.60E+05	N/D
Q15059	BRD3	Bromodomain-containing protein 3	3	3.10E+05	N/D
O60885	BRD4	Bromodomain-containing protein 4	4	8.10E+05	N/D
P04745	AMY1A	Alpha-amylase 1	2	8.20E+05	N/D
P19013	KRT4	Keratin, type II cytoskeletal 4	5	4.00E+05	N/D

N/D, not detected.

Table S5. Comparison of kinases identified from this study with those reported in the literature. The common kinase targets identified by probes **1a**/**2a** in the present study are colored in blue. N/D, not detected.

Gene ID	AUC: enriched by Das-ACT (**1a**)	AUC: enriched by Das-DA (**2a**)	JACS 2012 [a]		J. Proteomics 2011 [b]	PNAS 2007 [c]	Nat. Chem. Biol. 2010 [d]	Blood 2007 [e]	Nat. Biotech. 2007 [f]
			In vitro	In situ	In vitro	Immobilized Das	Immobilized Das	Immobilized Das	Immobilized, ligand
ABL1	N/D	3.80E+05				+	+	+	+ (BCR-ABL)
AGK	4.40E+05	N/D	+	+					
BTK	3.00E+06	2.30E+06	+	-		+		+	+
CSK	1.20E+06	1.30E+06	+	-	+	+	+	+	+
LYN	1.30E+06	1.10E+06	+	-		+	+	+	+
MAPK14	1.20E+06	1.20E+06					+	+	+
MAPKAPK2	N/D	4.70E+05	+	-					
RIPK2	N/D	8.30E+05					+	+	+
YES1	1.30E+06	2.90E+06	+	-		+	+	+	+

[a] "Cell-based proteome profiling of potential Dasatinib targets by use of affinity-based probes" Shi, H.; et al. *J. Am. Chem. Soc.* **2012**, *134*, 3001-3014. This paper described the in vitro and in situ photo-cross-linking enabled target capture and identification in HEPG2 and K562 cells. Only one kinase, AGK2, was identified in situ.

[b] "Dasatinib, Imatinib and staurosporine capture compounds—Complementary tools for the profiling of kinases by Capture Compound Mass Spectrometry (CCMS)" Fischer, J. J.; et al. *J. Proteomics* **2011**, *75*, 160-168. In vitro target capture from HEPG2 cell lysate using the Dasatinib/Imatinib-photo-cross-linker-biotin conjugates.

[c] "The Btk tyrosine kinase is a major target of the Bcr-Abl inhibitor Dasatinib" Hantschel, O.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104(33)*, 13283-13288. The immobilized Dasatinib was used in target capture and identification in K562 cell lysate.

[d] "A chemical and phosphoproteomic characterization of dasatinib action in lung cancer" Li, J.; et al. *Nat. Chem. Biol.* **2010**, *6*, 291-299. The immobilized Dasatinib was used in target capture in the H292, H441 and HCC827 cell lysates.

[e] "Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors" Bantscheff, M.; et al. *Nat. Biotechnol.* **2007**, *25*, 1035. An immobilized mixture of

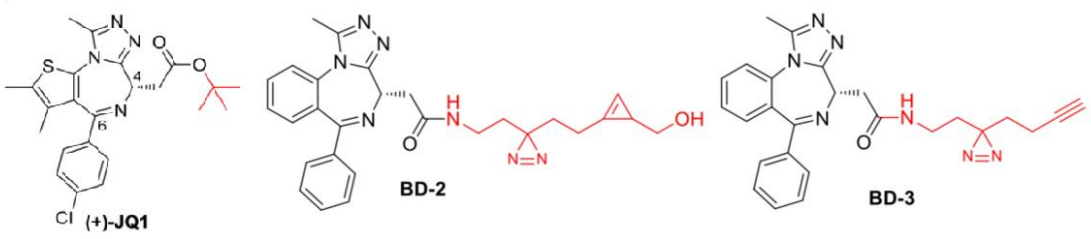
kinase inhibitors ("kinobeads") was used, together with pretreatment with a certain inhibitor (e.g. Das), in detecting the specific capture of kinase targets. The K562 and HeLa cell lysates were used in the in vitro capture.

[f] "Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and Dasatinib reveal novel kinase and nonkinase targets" Rix, U.; et al. Blood **2007**, *110*, 4055-4063. The immobilized Dasatinib was used in target capture and identification in K562 and primary CML cell lysates.

Table S6. Comparison of protein targets from this study with those reported in the literature. The common protein targets identified by probes **4a**/**5a** in the present study are colored in blue. N/D, not detected.

Gene ID	Protein Name	AUC: enriched by JQ-1-ACT (**4a**)	AUC: enriched by JQ-1-DA (**5a**)	JACS 2014 [a]
AMY1A	Alpha-amylase 1	N/D	8.20E+05	-
BRD2	Bromodomain-containing protein 2	5.90E+05	9.60E+05	-
BRD3	Bromodomain-containing protein 3	3.50E+05	3.10E+05	-
BRD4	Bromodomain-containing protein 4	6.00E+05	8.10E+05	-
DSC1	Desmocollin-1	2.40E+05	N/D	-
KRT4	Keratin, type II cytoskeletal 4	N/D	4.00E+05	-

[a] "Minimalist cyclopropene-containing photo-cross-linkers suitable for live-cell imaging and affinity-based protein labeling" Li, Z.; et al. *J. Am. Chem. Soc.* **2014**, *136*, 9990-9998. A related BRD4 inhibitor (structure shown below), not JQ-1, was used in this study. None of BRD proteins showed up in their high-confidence target list after *in situ* target capture in HEPG2 cells and subsequent LC-MS/MS analysis.

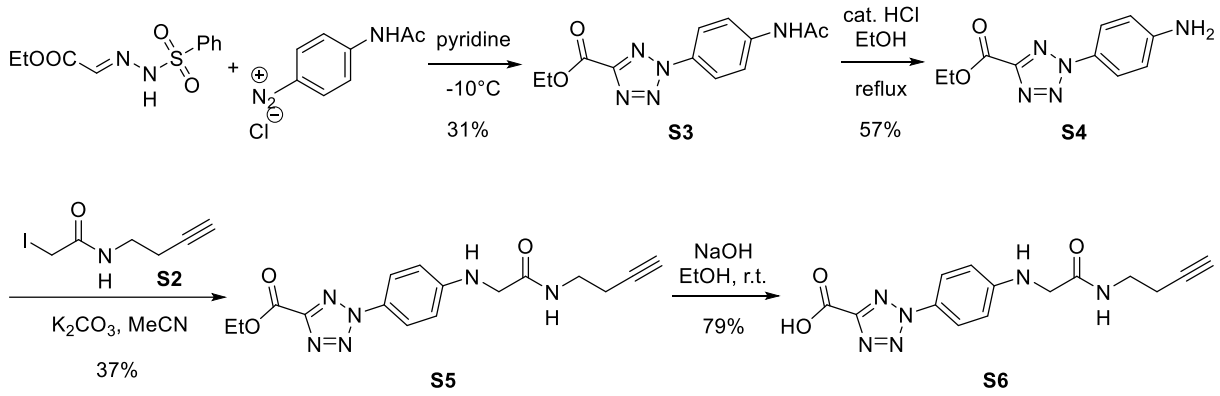


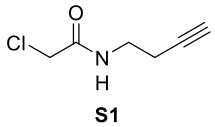
General Information

Recombinant human BTK protein used in the in-gel fluorescence experiments was purchased from Thermo Fisher (Cat. No. PR5442A). Recombinant human BTK (387-659) was used in the LC-MS based cross-linking experiments. Recombinant human BRD4 (44-168) protein used in the in-gel fluorescence and LC-MS studies was purchased from Active Motif (Cat. No. 31380). Solvents and chemicals were purchased from commercial sources and used directly without further purification. Flash chromatography was performed with SiliCycle P60 silica gel (40-63 μm, 60 Å). ^{1}H NMR spectra were recorded with Varian Mercury-300, Inova-400 or -500 MHz spectrometers at University at Buffalo and Agilent 400-MR, Varian Inova 500 MHz, Varian VNMRS 500 MHz, Bruker Avance III 500 MHz at Abbvie. Chemical shifts were reported in ppm using either TMS or deuterated solvents as internal standards (TMS, 0.00; $CDCl_3$, 7.26; CD_3OD, 3.31; DMSO-d_6, 2.50). Multiplicity was reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, brs = broad. ^{13}C NMR spectra were recorded at 75.4 or 126 MHz, and chemical shifts were reported in ppm using deuterated solvents as internal standards ($CDCl_3$, 77.0; DMSO-d_6, 39.5; CD_3OD, 49.05). High resolution mass spectrometry was performed on a Bruker solariX XR Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS). In-gel fluorescence images were acquired by scanning SDS-PAGE gels with a BioRad PharosFX imager. Invitrogen PVDF membranes were used for western blots and scanned using Licor. For photoirradiation with a hand-held UV lamp, if not specified, 5 min 302-nm photoirradiation was carried out for ACT probes, 10 min 365-nm photoirradiation was carried out for DA probes, and 10 min 302-nm photoirradiation was carried out for BP probes.

Experimental Procedures and Characterization Data

Scheme S1. Synthesis of the alkyne-functionalized ACT photo-affinity label

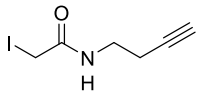




S1

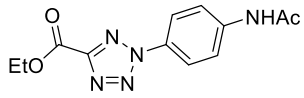
***N*-(But-3-yn-1-yl)-2-chloroacetamide (S1):** To a solution of but-3-yn-1-amine (250 mg, 3.62 mmol) and ethyldiisopropylamine (561 mg, 4.34 mmol) in 25 mL CH_2Cl_2 cooled in ice-water bath was added dropwise a solution of 2-chloroacetyl chloride (490 mg, 4.34 mmol) in 5 mL CH_2Cl_2. The ice-water bath was removed, and the mixture was stirred at r.t. for 3 h. Afterwards, additional 20 mL CH_2Cl_2 was added, and the organic layer was washed successively with 20 mL saturated $NaHCO_3$, 20 mL 2 N HCl and 20 mL brine, and dried over $MgSO_4$. The solvent was then removed under reduced pressure to give the desired product as a brownish oil (427 mg, 81% yield): R_f =

0.28 (hexanes/EtOAc = 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (s, 1H), 3.96 (s, 2H), 3.35 (q, J = 6.1 Hz, 2H), 2.34 (s, 2H), 1.98 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 80.9, 70.3, 42.5, 38.2, 19.0.



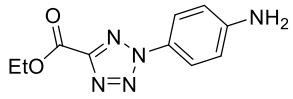
S2

***N*-(But-3-yn-1-yl)-2-iodoacetamide (S2):** To a solution of **S1** (415 mg, 2.85 mmol) in 15 mL acetone was added NaI (1.28 g, 8.55 mmol), and the mixture was refluxed for 4 h. After the solvent was evaporated, the residue was re-dissolved in 30 mL EtOAc and 30 mL water. The organic layer was separated, washed successively with 20 mL 10% $Na_2S_2O_3$, 20 mL H_2O and 20 mL brine. After drying the solution over $MgSO_4$, the solvent was removed with rotary evaporator to give the desired product as a light brown solid (471 mg, 70% yield), which was used directly without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.72 (s, 1H), 3.70 (s, 2H), 3.40 (q, J = 6.4 Hz, 2H), 2.41 (td, J = 6.5, 2.6 Hz, 2H), 2.04-2.01 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.4, 81.2, 70.4, 39.0, 19.1, -0.6.



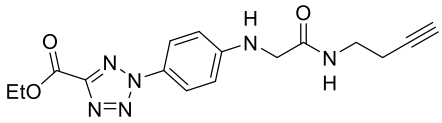
S3

Ethyl 2-(4-acetamidophenyl)-2*H*-tetrazole-5-carboxylate (S3): A solution of 50% ethyl 2-oxoacetate in toluene (3.042 g, 14.9 mmol) and benzenesulfonyl hydrazide (1.72 g, 11.5 mmol) in 60 mL EtOH was stirred at r.t. for 1 h. The solvent was removed under reduced pressure, and the residue was re-dissolved in 60 mL pyridine. Separately, to a suspension of *N*-(4-aminophenyl)acetamide (1.607 g, 10.7 mmol) and 4 mL concentrated HCl in 8 mL H_2O and 10 mL EtOH was added dropwise a solution of $NaNO_2$ (0.775 g, 5.2 mmol) in 3 mL H_2O at 0 °C. After 10-minute stirring, this solution was added dropwise to the previously prepared pyridine solution over 50 min at -10 °C. The mixture was warmed up to r.t. over 2 h with stirring before 100 mL H_2O was added. The solution was extracted with 70 mL CH_2Cl_2 three times, and the organic layers were collected, combined, washed successively with 3×100 mL 2 N HCl and 1×70 mL brine. After drying over $MgSO_4$, the solvent was removed on a rotary evaporator. The dark brown oily residue was applied to a silica gel flash chromatography using hexanes/EtOAc (1:1 → 1:4) as the eluent to give the crude product as a dark yellow oil (2.235 g). The crude product was recrystallized twice from EtOH to give the titled compound as a yellow powder (906 mg, 31% yield): R_f = 0.44 (hexanes/EtOAc = 1:4); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.07 (d, J = 9.1 Hz, 2H), 7.87 (d, J = 9.1 Hz, 2H), 4.46 (q, J = 7.1 Hz, 2H), 2.10 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.9, 157.2, 157.1, 141.5, 130.6, 121.1, 119.6, 62.3, 24.1, 14.0; HRMS (ESI) $[M+H]^+$ calcd for $C_{12}H_{14}N_5O_3$ 276.1091 $[M+H]^+$, found 276.1094.



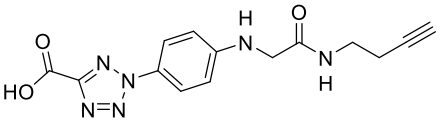
S4

Ethyl 2-(4-aminophenyl)-2*H*-tetrazole-5-carboxylate (S4): To a suspension acetamide derivative **S3** (201 mg, 0.73 mmol) in 5 mL EtOH was added 10 drops of concentrated HCl, and the mixture was refluxed until the starting material was consumed based on thin-layer chromatography. The mixture was cooled down to -20 °C, and the precipitate was collected with a glass filter, washed with cold EtOH, and dried under vacuum to give the titled compound as an off-white powder (97 mg, 57% yield): R_f = 0.44 (hexanes/EtOAc = 1:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.98 (s, 2.6H), 8.07 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 8.9 Hz, 2H), 4.45 (q, J = 7.1 Hz, 2H), 1.36 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.2, 157.1, 140.3, 131.5, 121.8, 121.3, 62.3, 14.0; HRMS (ESI) calcd for $C_{10}H_{12}N_5O_2$ 234.0986 $[M+H^+]$, found 234.0979.



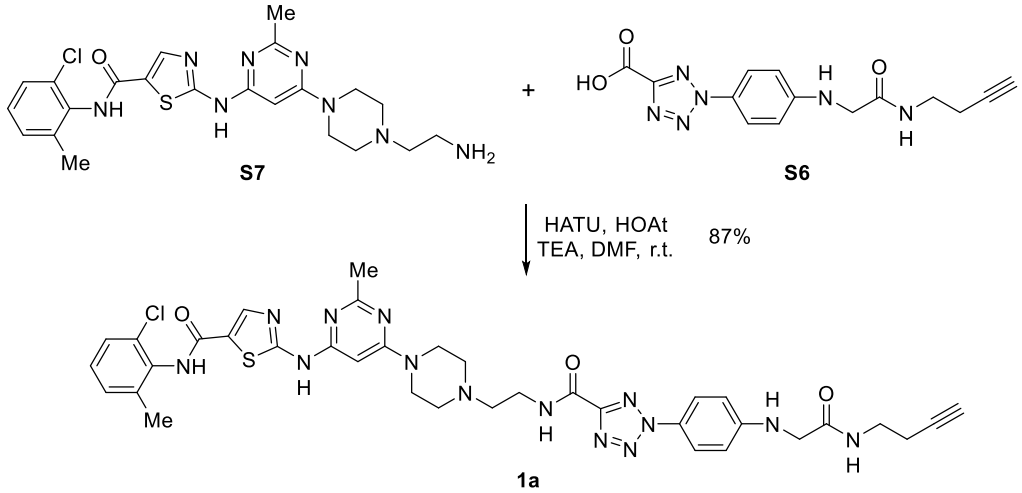
S5

Ethyl 2-(4-((2-(but-3-yn-1-ylamino)-2-oxoethyl)amino) phenyl)-2*H*-tetrazole-5-carboxylate (S5): A mixture of aniline **S4** (88 mg, 0.38 mmol), iodoacetamide **S2** (90 mg, 0.38 mmol) and K_2CO_3 (156 mg, 1.13 mmol) in 3 mL MeCN was refluxed for 2 days. The mixture was filtered through a layer of celite and the solvent was removed with a rotavapor. The residue was applied to a silica gel flash chromatography using hexanes/EtOAc (1:10) as the eluent to give the desired product as an off-white powder (48 mg, 37% yield): R_f = 0.25 (hexanes/EtOAc = 1:5); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, J = 9.0 Hz, 2H), 6.74 (d, J = 9.0 Hz, 2H), 6.71 (s, 1H), 4.77 (s, 1H), 4.56 (q, J = 7.1 Hz, 2H), 3.90 (d, J = 3.7 Hz, 2H), 3.47 (q, J = 6.3 Hz, 2H), 2.42 (td, J = 6.4, 2.6 Hz, 2H), 1.91 (t, J = 2.6 Hz, 1H), 1.48 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.5, 157.4, 156.7, 150.5, 125.4, 121.6, 112.4, 82.1, 72.2, 62.1, 46.3, 37.7, 18.7, 14.0; HRMS (ESI) calcd for $C_{16}H_{19}N_6O_3$ 343.1513 [M+H$^+$], found: 343.1514.



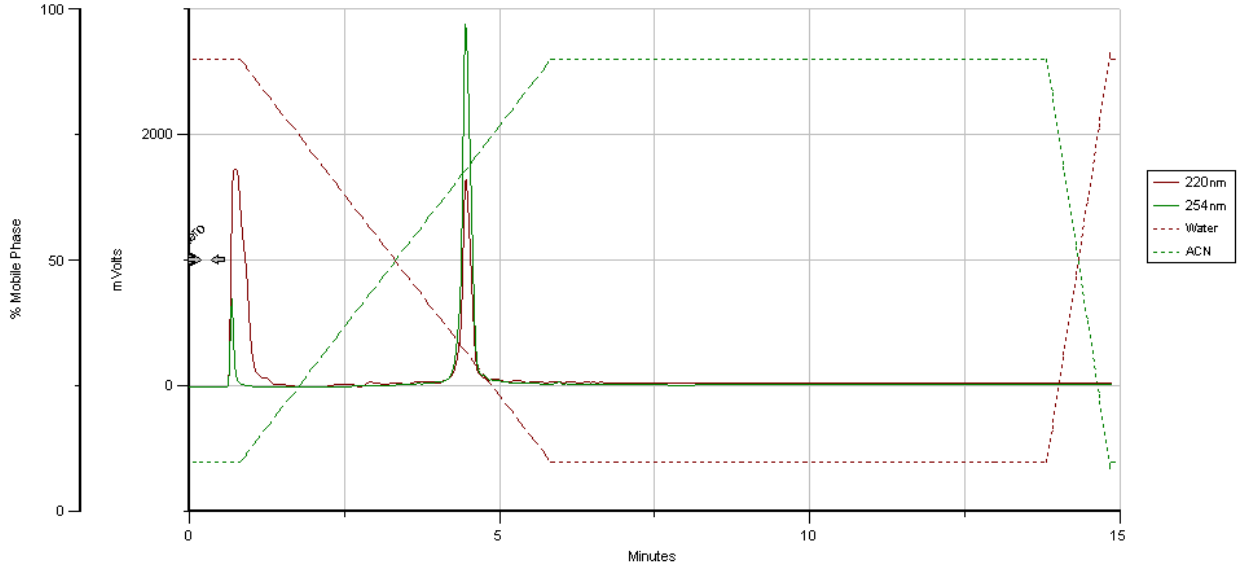
S6

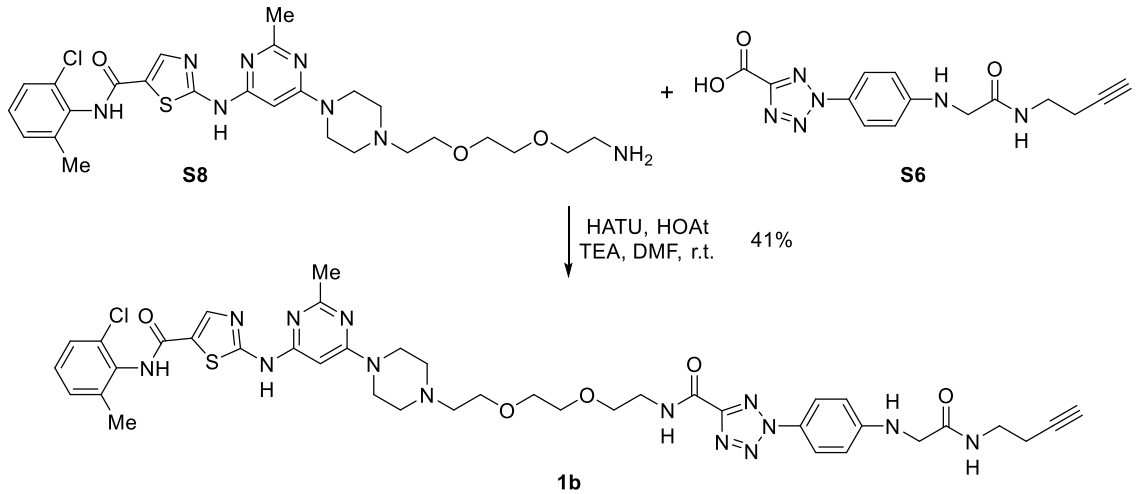
2-(4-((2-(But-3-yn-1-ylamino)-2-oxoethyl)amino) phenyl)-2*H*-tetrazole-5-carboxylic acid (S6): To a solution of tetrazole carboxylate **S5** (37 mg, 0.11 mmol) in 1 mL EtOH at r.t. was added 2 mL 2 N NaOH, and the mixture was stirred at r.t. until the starting material was completely consumed based on thin layer chromatography. The solution was added 5 mL EtOAc and then acidified. The precipitate that appeared was collected with a glass filter, washed with water, and dried under vacuum to give the titled compound as an off-white powder (27 mg, 79% yield): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16 (t, J = 5.8 Hz, 1H), 7.82 (d, J = 9.0 Hz, 2H), 6.74 (d, J = 9.1 Hz, 2H), 3.74 (s, 2H), 3.22 (q, J = 7.0 Hz, 2H), 2.84 (t, J = 2.6 Hz, 1H), 2.30 (td, J = 7.1, 2.7 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.6, 158.8, 157.6, 150.4, 125.6, 121.5, 112.4, 82.1, 72.1, 46.3, 37.7, 18.7; HRMS (ESI) calcd for $C_{14}H_{15}N_6O_3$ 315.1200 [M+H$^+$], found: 315.1202.



Synthesis of 1a: A solution of dasatinib amine·3TFA salt **S7** (15.0 mg, 0.018 mmol), tetrazole carboxylic acid **S6** (6.8 mg, 0.022 mmol), HATU (8.3 mg, 0.022 mmol), 3-hydroxytriazolo[4,5-b]pyridine (HOAt; 3.0 mg, 0.022 mmol), and triethylamine (10.6 μL, 0.076 mmol) in 150 μL DMF was stirred at r.t. overnight. The reaction mixture was applied to a medium-pressure silica gel flash chromatography system equipped with an automatic fraction collector running the MeOH/CH_2Cl_2

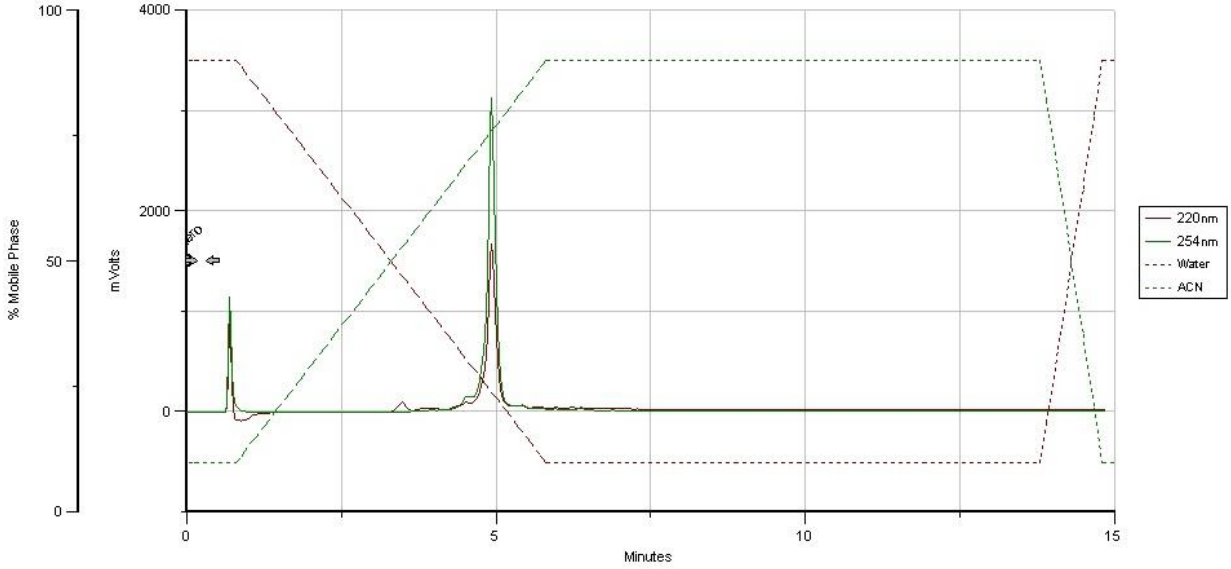
(2:98→20:80) eluent gradient. The suitable fractions were pooled and evaporated under reduced pressure. The residue was re-dissolved in 3 mL MeCN and 3 mL H_2O containing 0.5% TFA, and the solution was lyophilized to dryness to give the titled compound as a white powder (16.0 mg, 87% yield): R_f = 0.63 (MeOH/CH_2Cl_2 = 1:5); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 11.46 (s, 0.7H), 9.87 (s, 0.8H), 8.97 (t, J = 5.5 Hz, 0.6H), 8.22 (s, 1H), 8.15 (t, J = 5.6 Hz, 0.7H), 7.82 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 7.4 Hz, 1H), 7.32 - 7.22 (m, 2H), 6.78 – 6.71 (m, 3H), 6.06 (s, 1H), 3.74 (d, J = 5.4 Hz, 2H), 3.57 – 3.44 (m, 6H), 3.34 – 3.28 (m, 2H), 3.26 – 3.18 (m, 2H), 2.98 (brs, 1H), 2.83 (s, 1H), 2.57 (t, J = 6.6 Hz, 2H), 2.55 – 2.51 (m, under the DMSO signal), 2.41 (s, 3H), 2.30 (td, J = 6.8, 2.1 Hz, 2H), 2.24 (s, 3H); HRMS (ESI) calcd for $C_{36}H_{40}ClN_{14}O_3S$ 783.2812 [M+H$^+$], found 783.2817. The product purity was also verified by analytical HPLC as shown below.

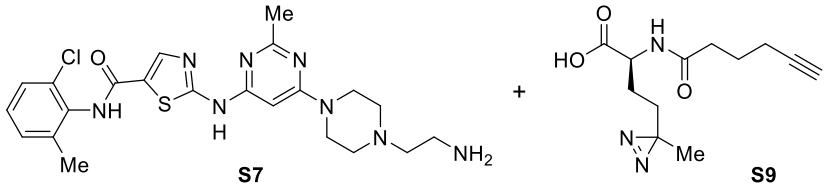




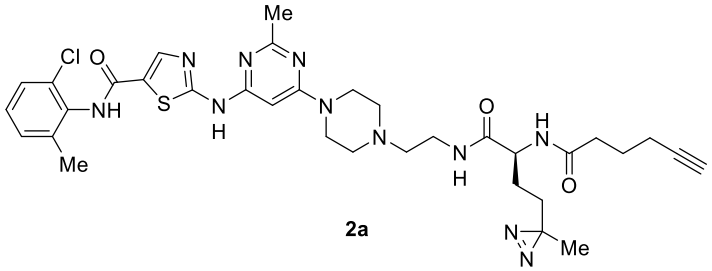
Synthesis of 1b: A solution of dasatinib amine·3TFA salt **S8** (15.0 mg, 0.016 mmol), tetrazole carboxylic acid **S6** (6.2 mg, 0.020 mmol), HATU (7.5 mg, 0.020 mmol), HOAt (2.7 mg, 0.020 mmol), and triethylamine (9.6 μL, 0.069 mmol) in 150 μL DMF was stirred at r.t. overnight. The reaction mixture was applied to a medium-pressure silica gel flash chromatography system equipped with an automatic fraction collector running the MeOH/ CH_2Cl_2 (2:98→20:80) eluent

gradient. The suitable fractions were pooled and evaporated under reduced pressure. The residue was re-dissolved in 3 mL MeCN and 3 mL H_2O containing 0.5% TFA, and the solution was lyophilized to dryness to give the titled compound as a white powder (7.4 mg, 41% yield): R_f = 0.61 (MeOH/CH_2Cl_2 = 1:5); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 11.45 (s, 0.8H), 9.87 (s, 1H), 8.98 (s, 1H), 8.22 (s, 1H), 8.15 (s, 1H), 7.82 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 7.2 Hz, 1H), 7.32 - 7.22 (m, 2H), 6.77 – 6.70 (m, 3H), 6.04 (s, 1H), 4.09 (d, J = 4.0 Hz, 1H), 3.73 (d, J = 5.2 Hz, 2H), 3.62 – 3.43 (m, 18H), 3.27 – 3.12 (m, 7H), 2.82 (s, 0.8H), 2.49 – 2.44 (m, overlapping with the DMSO signal), 2.40 (s, 3H), 2.30 (t, J = 5.5 Hz, 2H), 2.24 (s, 3H); HRMS (ESI) calcd for $C_{40}H_{48}ClN_{14}O_5S$ 871.3336 $[M+H]^+$, found 871.3358. The product purity was also verified by analytical HPLC as shown below.



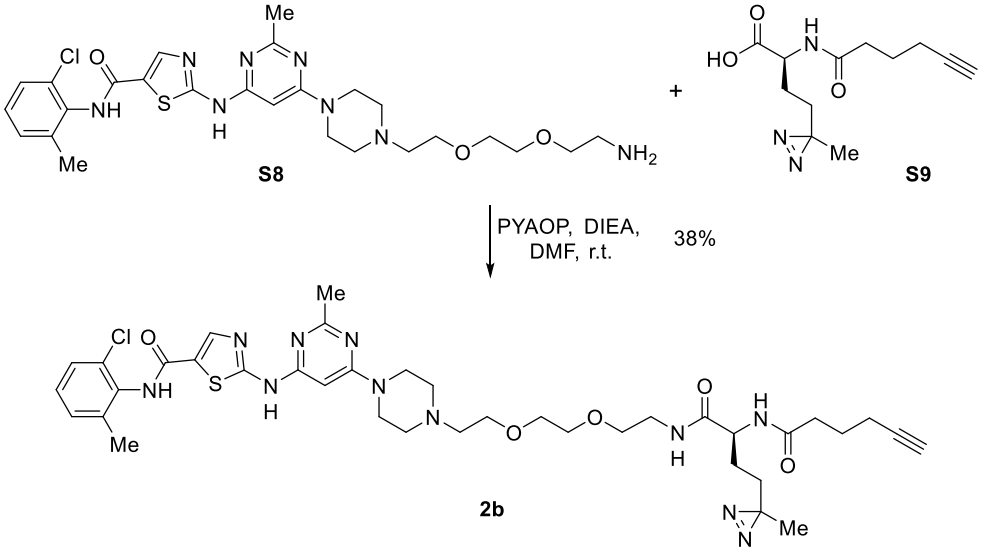


PYAOP, DIEA
DMF, r.t.
25%

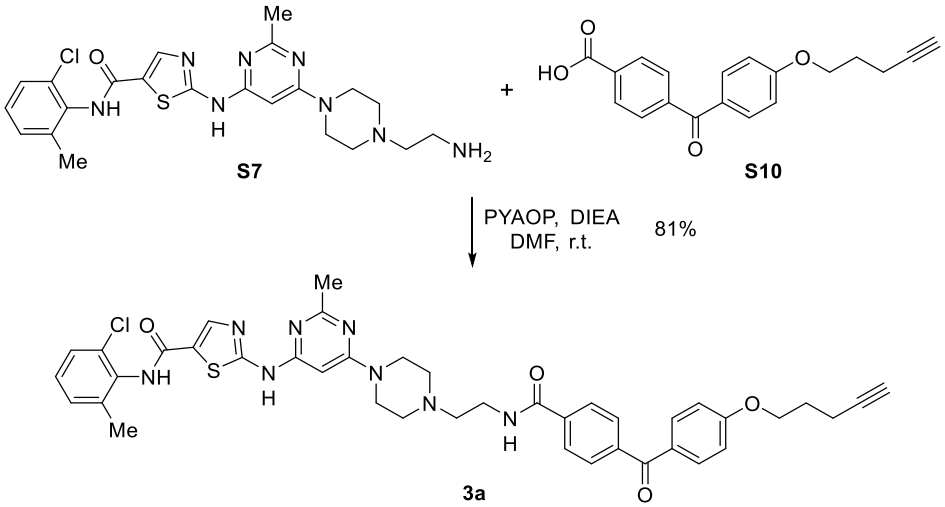


Synthesis of 2a: A solution of dasatinib amine·3TFA salt **S7** (27.1 mg, 0.033 mmol), diazirine acid **S9** (8.2 mg, 0.033 mmol), (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP; 17.01 mg, 0.033 mmol) and ethyldiisopropylamine (0.023 mL, 0.131 mmol) were combined in DMF (3 mL) and stirred for 2 h at r.t. The crude reaction mix was diluted

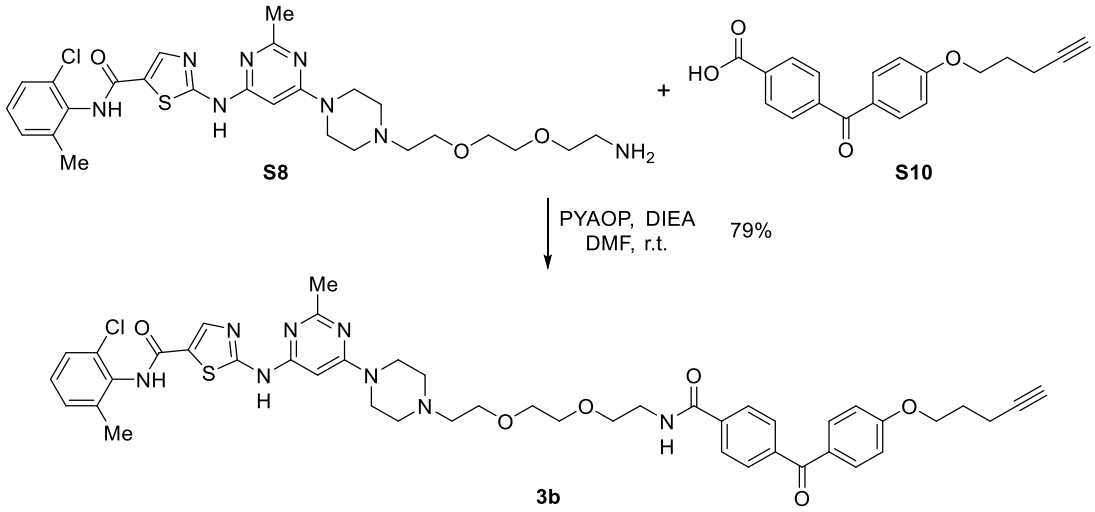
with 90% DMSO/water to 4 mL and purified on preparative HPLC. The eluents were A: 0.1% TFA-water; B: acetonitrile. 0-5 min isocratic followed by 1% increase in acetonitrile per min gradient. The appropriate fractions were collected and lyophilized to dryness to give the titled compound (7.7 mg, 25% yield): ^{1}H-NMR (500 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.91 (s, 1H), 9.67 (brs, 1H), 8.24 (s, 1H), 8.21 (t, J = 5.7 Hz, 1H), 8.10 (d, J = 7.3 Hz, 1H), 7.40 (dd, J = 7.7, 1.4 Hz, 1H), 7.32 - 7.23 (m, 2H), 6.15 (s, 1H), 4.36 (brs, 1.6H), 4.12 – 4.04 (m, 1H), 3.60 (m, under the water signal), 3.29 – 3.06 (m, under the water signal), 3.01 (td, J = 6.6, 3.9 Hz, 1H), 2.79 (t, J = 2.6 Hz, 1H), 2.45 (s, 3H), 2.26 – 2.20 (m, 5H), 2.15 (td, J = 7.2, 2.6 Hz, 2H), 1.76 – 1.71 (m, 1H), 1.69 – 1.62 (m, 2H), 1.54 – 1.22 (m, 4H), 0.99 (s, 3H); HRMS (ESI) calcd for $C_{34}H_{42}ClN_{11}O_3S$ 719.2960 [M+H$^+$], found 720.2950.



Synthesis of 2b: A solution of dasatinib amine·3TFA salt **S8** (10 mg, 10.90 µmol), diazirine acid **S9** (7.3 mg, 0.029 mmol), PyAOP (15.15 mg, 0.029 mmol) and ethyldiisopropylamine (0.020 mL, 0.116 mmol) were combined in DMF (3 mL) and stirred for 2 h at r.t. The crude reaction mix was diluted with 90% DMSO/water to 4 mL and purified preparative HPLC. The eluents were A: 0.1% TFA-water; B: acetonitrile. 0-5 min isocratic; 1%/min acetonitrile gradient. The appropriate fractions were collected and lyophilized to dryness to give the titled compound (8.8 mg, 38% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.91 (s, 1H), 9.79 (brs, 1H), 8.24 (s, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.92 (t, J = 5.7 Hz, 1H), 7.40 (dd, J = 7.7, 1.4 Hz, 1H), 7.32 – 7.24 (m, 2H), 6.15 (s, 1H), 4.35 (d, J = 12.5 Hz, 2H), 4.15 (td, J = 8.2, 5.2 Hz, 1H), 3.81 – 3.75 (m, 2H), 3.64 – 3.54 (m, under the water signal), 3.32 – 3.07 (m, under the water signal), 3.04 – 2.98 (m, 1H), 2.78 (t, J = 2.7 Hz, 1H), 2.45 (s, 3H), 2.24 (s, 3H), 2.20 (td, J = 7.2, 1.3 Hz, 2H), 2.13 (td, J = 7.2, 2.7 Hz, 2H), 1.76 – 1.71 (m, 1H), 1.69 - 1.60 (m, 2H), 1.52 – 1.21 (m, 5H), 0.97 (s, 3H); HRMS (ESI) calcd for $C_{38}H_{51}ClN_{11}O_5S$ 808.3485 [M+H$^+$], found 808.3477.

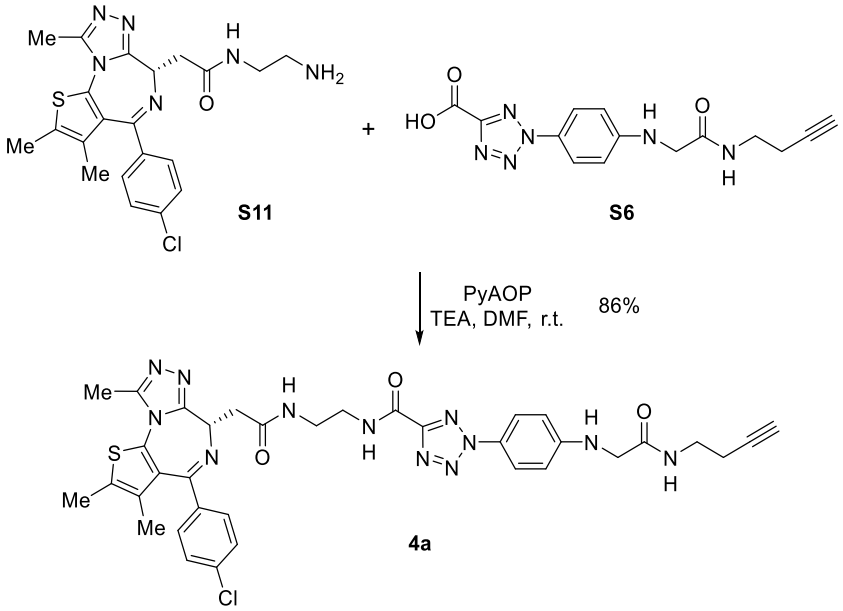


Synthesis of 3a: A solution of dasatinib amine·3TFA salt **S7** (30 mg, 0.036 mmol), benzoic acid **S10** (12.27 mg, 0.040 mmol), PyAOP (20.75 mg, 0.040 mmol) and ethyldiisopropylamine (0.025 mL, 0.145 mmol) were combined in DMF (3 mL) and stirred for 2 h at r.t. The crude reaction mix was diluted with 90% DMSO/water to 4 mL and purified preparative HPLC. The eluents were A: 0.1% TFA-water; B: acetonitrile. 0-5 min isocratic; 1% /min acetonitrile gradient after that point. The appropriate fractions were collected and lyophilized to dryness to give the titled compound (29.3 mg, 81% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.66 (brs, 1H), 9.92 (s, brs, 2H), 8.96 (t, J = 5.6 Hz, 1H), 8.24 (s, 1H), 8.03 (d, J = 8.5 Hz, 2H), 7.80 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.9 Hz, 2H), 7.40 (dd, J = 7.6, 1.1 Hz, 1H), 7.32 - 7.22 (m, 2H), 7.12 (d, J = 8.9 Hz, 2H), 6.18 (s, 1H), 4.39 (brs, 2H), 4.16 (t, J = 6.2 Hz, 2H), 3.77 – 3.66 (m, 4H), 3.44 – 3.35 (m, 2H), 3.34 - 3.20 (m, 2H), 3.17 (s, 2H), 2.84 (t, J = 2.6 Hz, 1H), 2.46 (s, 3H), 2.36 (td, J = 7.1, 2.6 Hz, 2H), 2.24 (s, 3H), 1.93 (p, J = 6.7 Hz, 2H); HRMS (ESI) calcd for $C_{41}H_{42}ClN_8O_4S$ 777.2739 [M+H$^+$], found 777.2736.

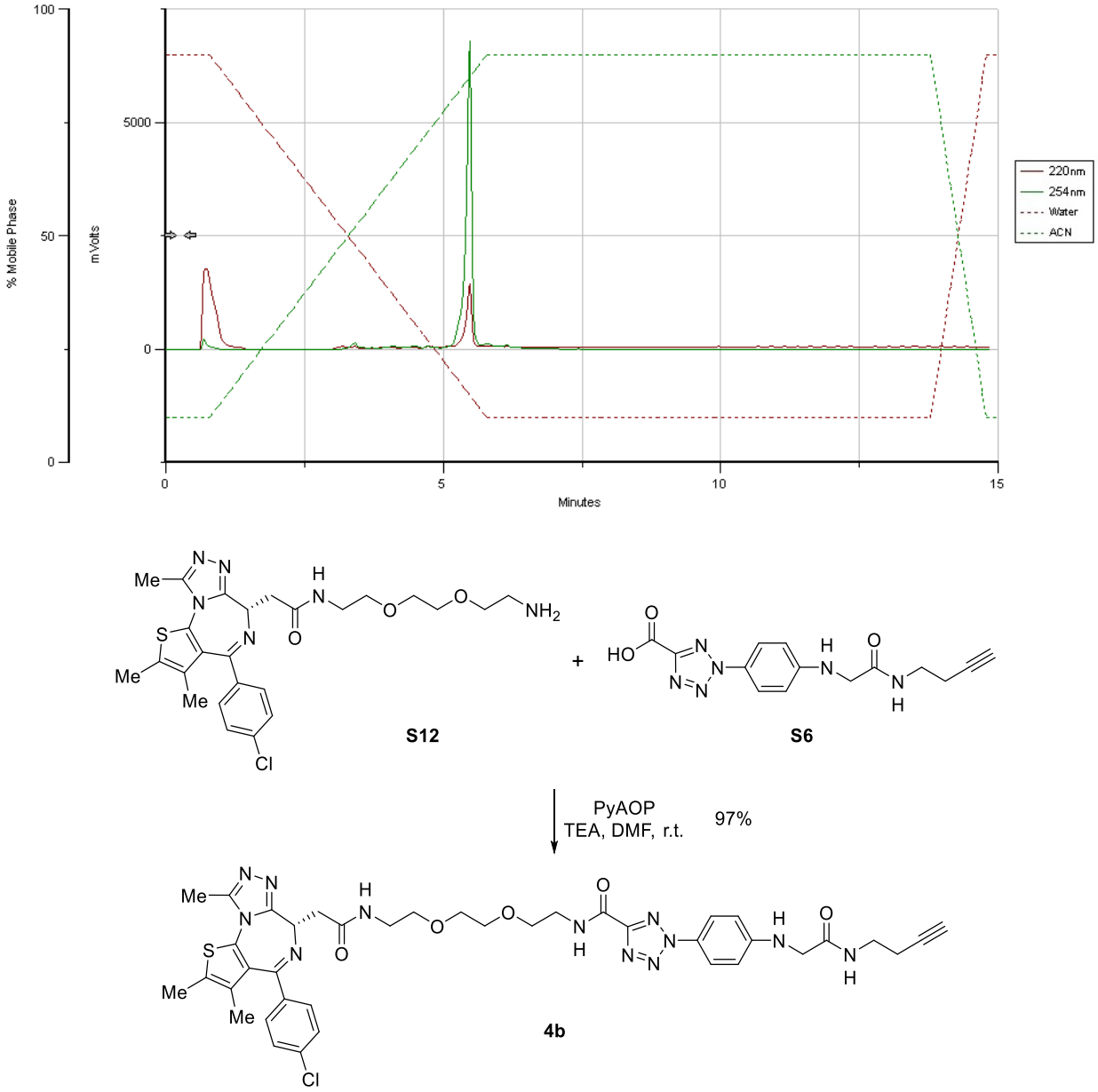


Synthesis of 3b: A solution of dasatinib amine·3TFA salt **S8** (30mg, 0.033 mmol), benzoic acid **S10** (11.09 mg, 0.036 mmol), PyAOP (18.76 mg, 0.036 mmol) and ethyldiisopropylamine (0.023 mL, 0.131 mmol) were combined in DMF (3 mL) and stirred for 2 hrs at r.t. The crude reaction

mix was diluted with 90% DMSO/water to 4 mL and purified on preparative HPLC. The eluents were A: 0.1% TFA-water; B: acetonitrile. 0-5 min isocratic; 1% /min acetonitrile gradient after that point. The appropriate fractions were collected and lyophilized to dryness to give the titled compound (28.4 mg, 79% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.65 (brs, 1H), 10.06 (brs, 1H), 9.92 (s, 1H), 8.74 (t, J = 5.5 Hz, 1H), 8.24 (s, 1H), 8.00 (d, J = 8.3 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H), 7.72 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 6.7 Hz, 1H), 7.31 - 7.19 (m, 2H), 7.08 (d, J = 8.8 Hz, 2H), 6.16 (s, 1H), 4.33 (brs, 2H), 4.13 (t, J = 6.2 Hz, 2H), 3.83 – 3.74 (m, 2H), 3.66 - 3.53 (m, 8H), 3.52 – 3.43 (m, 2H), 3.34 (s, 2H), 3.26 (brs, 2H), 3.17 (s, 2H), 3.10 (s, 2H), 2.82 (t, J = 2.6 Hz, 1H), 2.43 (s, 3H), 2.34 (td, J = 7.0, 2.6 Hz, 2H), 2.24 (s, 3H), 1.92 (p, J = 6.6 Hz, 2H); HRMS (ESI) calcd for $C_{45}H_{49}ClN_8O_6S$ 865.3263 [M+H$^+$], found 865.3278.

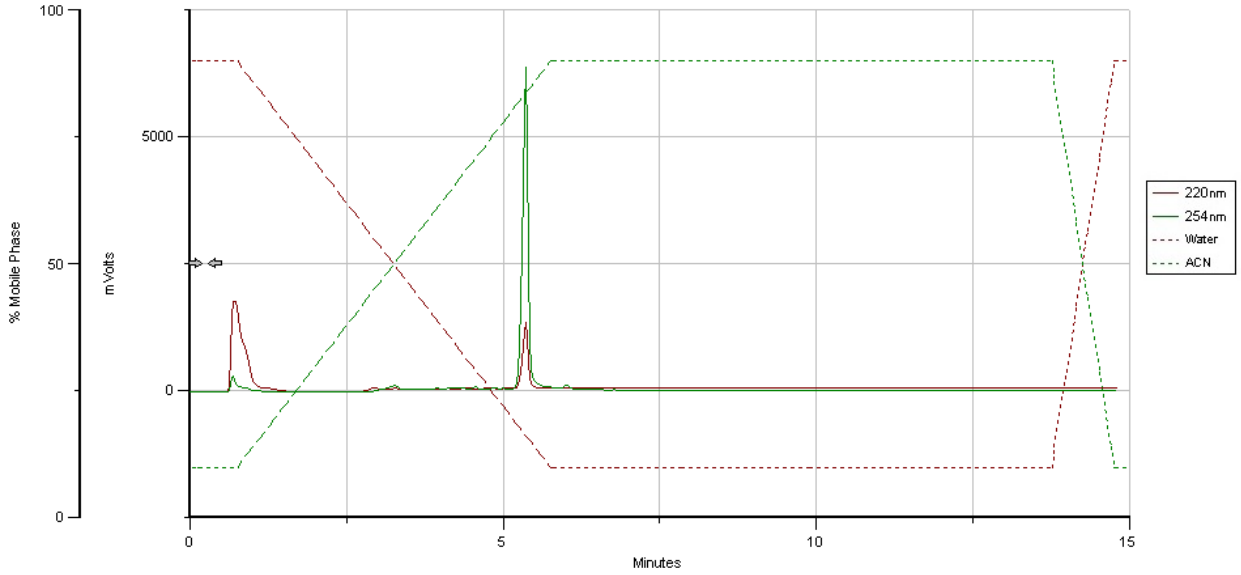


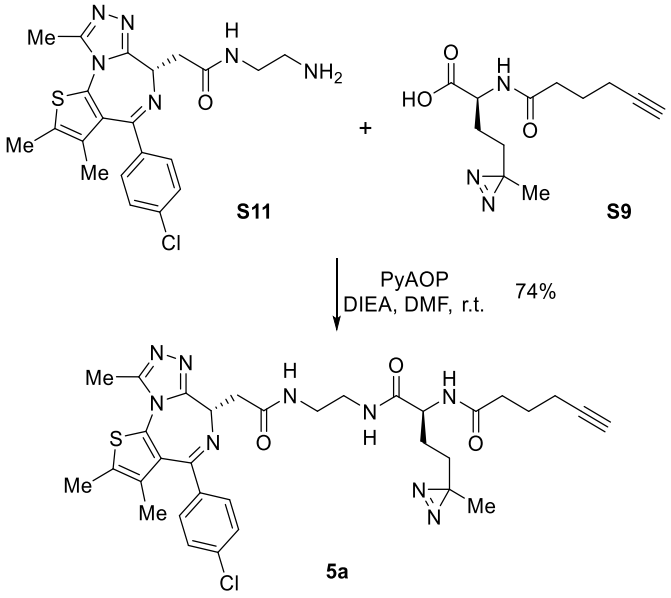
Synthesis of 4a: A solution of (+)-JQ-1 amine·TFA salt **S11** (10.0 mg, 0.018 mmol), tetrazole carboxylic acid **S6** (6.2 mg, 0.020 mmol), PyAOP (11.2 mg, 0.022 mmol), and triethylamine (6.3 μL, 0.045 mmol) in 150 μL DMF was stirred at r.t. for 2 h. The reaction mixture was applied to a medium-pressure silica gel flash chromatography system equipped with an automatic fraction collector running the MeOH/CH_2Cl_2 (1:99→10:90) eluent gradient. The suitable fractions were pooled and evaporated under reduced pressure. The residue was re-dissolved in 3 mL MeCN and 3 mL H_2O containing 0.5% TFA, and the solution was lyophilized to dryness to give the titled compound as a white powder (11.4 mg, 86% yield): R_f = 0.40 (MeOH/CH_2Cl_2 = 1:10): ^{1}H NMR (500 MHz, CD_3OD) δ 7.89 (d, J = 8.9 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 8.9 Hz, 2H), 4.65 (dd, J = 8.0, 6.2 Hz, 1H), 3.85 (s, 2H), 3.63 (t, J = 5.4 Hz, 2H), 3.56 (t, J = 5.4 Hz, 2H), 3.46 – 3.33 (m, 4H), 3.21 (q, J = 7.3 Hz, 1H), 2.66 (s, 3H), 2.40 (s 3H), 2.37 (td, J = 6.8, 2.3 Hz, 2H), 2.25 (s, 1H), 1.62 (s, 3H); HRMS (ESI) calcd for $C_{35}H_{36}ClN_{12}O_3S$ 739.2437 [M+H$^+$], found 739.2419. The product purity was verified by analytical HPLC.



Synthesis of 4b: A solution of (+)-JQ-1 amine·TFA salt **S12** (10.0 mg, 0.016 mmol), tetrazole carboxylic acid **S6** (5.4 mg, 0.017 mmol), PyAOP (9.7 mg, 0.019 mmol), and triethylamine (5.4 μL, 0.039 mmol) in 150 μL DMF was stirred at r.t. for 4 h. The reaction mixture was applied to a medium-pressure silica gel flash chromatography system equipped with an automatic fraction collector running the MeOH/CH_2Cl_2 (1:99→10:90) eluent gradient. The suitable fractions were pooled and evaporated under reduced pressure. The residue was re-dissolved in 3 mL MeCN and 3 mL H_2O containing 0.5% TFA, and the solution was lyophilized to dryness to give the titled compound as a white powder (12.8 mg, 97% yield): R_f = 0.36 (MeOH/CH_2Cl_2 = 1:10); ^{1}H NMR (500 MHz, CD_3OD) δ 7.86 (d, J = 8.9 Hz, 2H), 7.43 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 6.73 (d, J = 8.9 Hz, 2H), 4.64 (dd, J = 8.7, 5.4 Hz, 1H), 4.58 (s, 0.6H), 3.83 (s, 2H), 3.75 – 3.59 (m, 11H), 3.50 – 3.40 (m, 3H), 3.39 – 3.32 (m, under the solvent signal), 2.67 (s, 3H), 2.42 (s 3H), 2.37 (td, J = 6.8, 2.5 Hz, 2H), 2.25 (t, J = 2.4 Hz, 1H), 1.66 (s, 3H); HRMS (ESI) calcd for

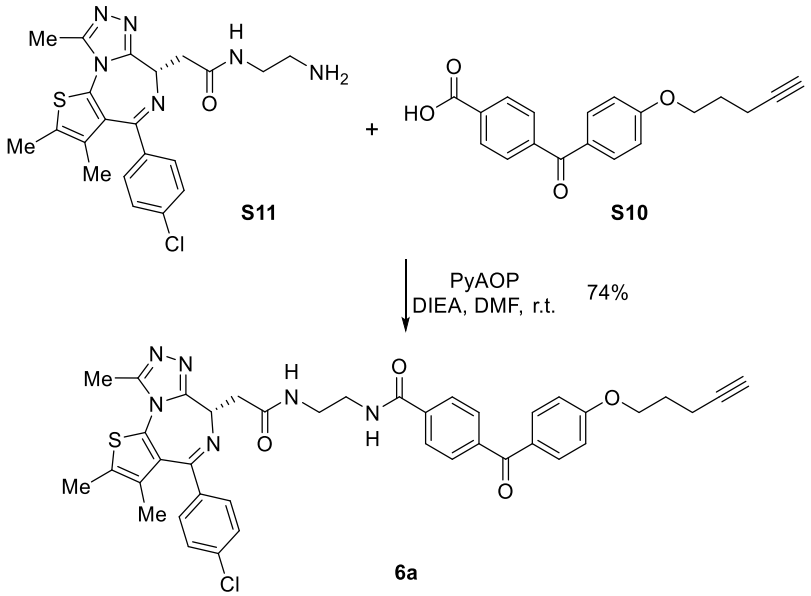
$C_{39}H_{44}ClN_{12}O_5S$ 827.2961 [M+H$^+$], found 827.2946. The product purity was also verified by analytical HPLC.



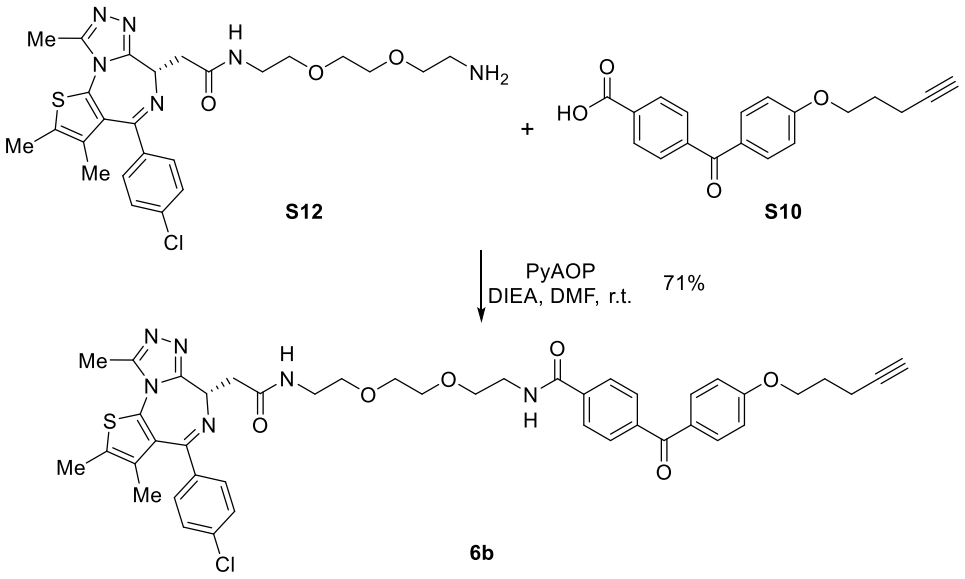


Synthesis of 5a: A solution of (+)-JQ-1 amine·TFA salt **S11** (20 mg, 0.036 mmol), diazirine acid **S9** (12.21 mg, 0.049 mmol), PyAOP (28.1 mg, 0.054 mmol) and ethyldiisopropylamine (0.025 mL, 0.144 mmol) were combined in DMF (2 mL) and stirred for 2 h at r.t. The crude reaction mix was diluted with 90% DMSO/water to 4 mL and purified on preparative HPLC. The eluents were A: 0.1% TFA-water; B: acetonitrile. 0-5 min isocratic; 1% /min acetonitrile gradient after that point. The appropriate fractions were collected and lyophilized to dryness to give the titled compound (18 mg, 74% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.90 (t, J = 5.0 Hz, 1H), 7.48 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 8.5 Hz, 2H), 4.52 (t, J = 7.1 Hz, 1H), 4.10 (td, J = 8.0, 5.2 Hz, 1H), 3.24 (d, J = 7.1 Hz, 2H), 3.20 – 3.08 (m, 4H), 2.73 (t,

J = 2.6 Hz, 1H), 2.60 (s, 3H), 2.40 (s, 3H), 2.20 (t, *J* = 7.5 Hz, 2H), 2.11 (td, *J* = 7.1, 2.6 Hz, 2H), 1.67 – 1.58 (m, 5H), 1.54-1.45 (m, 1H), 1.38 – 1.21 (m, 3H), 0.94 (s, 3H); HRMS (ESI) calcd for $C_{33}H_{39}ClN_9O_3SNa$ 698.2405 [M+Na$^+$], found 698.2422.

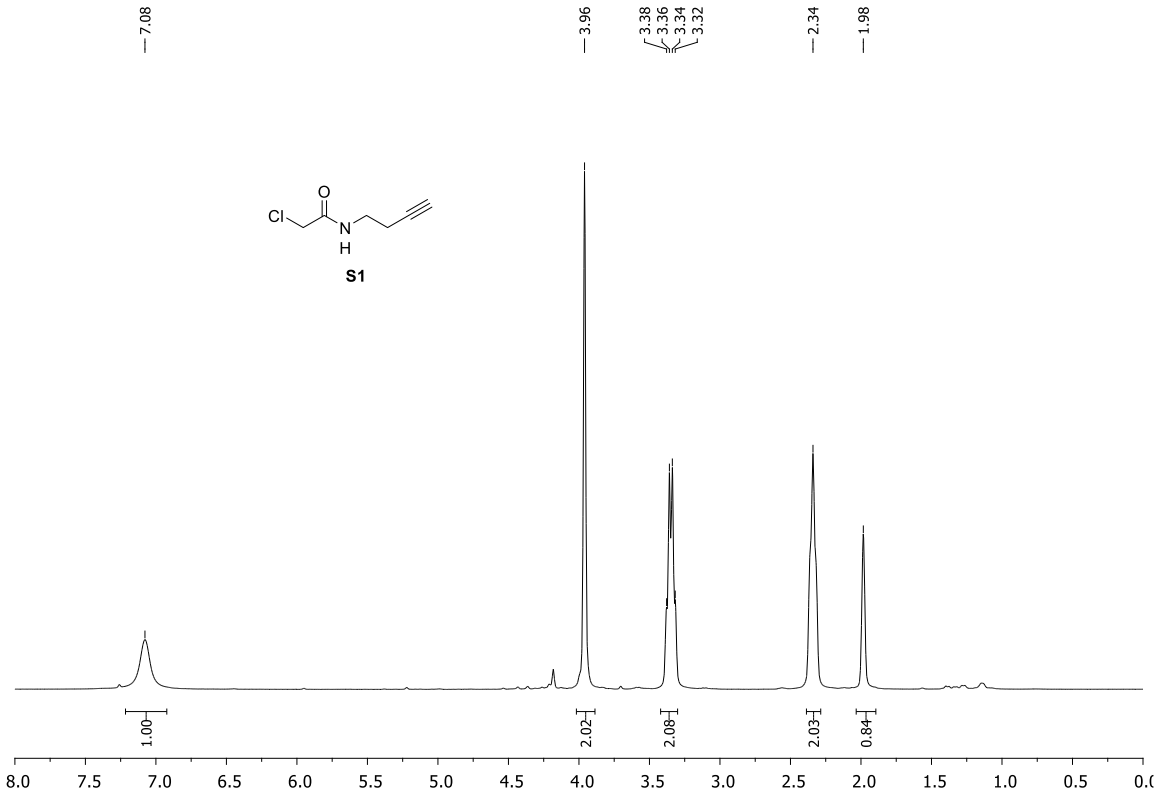


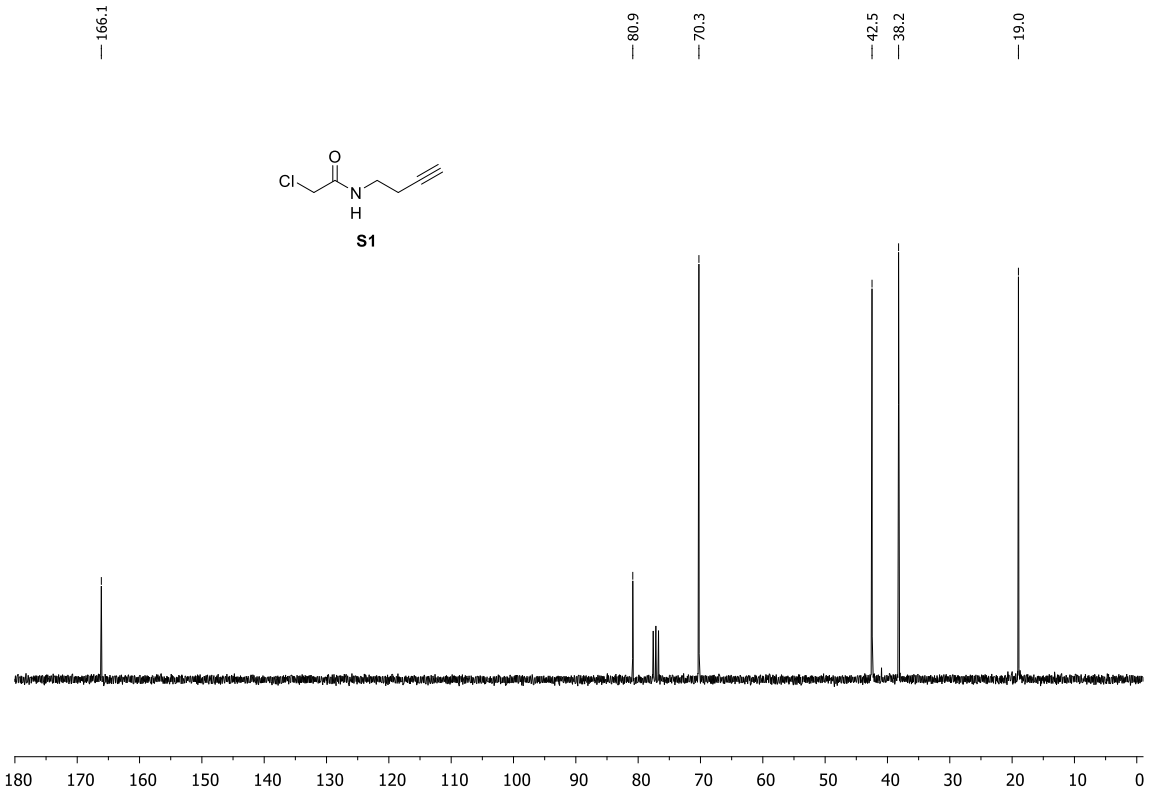
Synthesis of 6a: A solution of (+)-JQ-1 amine·TFA salt **S11** (25 mg, 0.045 mmol), benzoic acid **S10** (15.22 mg, 0.049 mmol), PyAOP (25.7 mg, 0.049 mmol) and ethyldiisopropylamine (0.031 mL, 0.180 mmol) were combined in DMF (3 mL) and stirred for 2 h at r.t. The crude reaction mix was diluted with 90% DMSO/water to 4 mL and purified on preparative HPLC. The eluents were A: 0.1% TFA-water; B: acetonitrile. 0-5 min isocratic; 1% /min acetonitrile gradient after that point. The appropriate fractions were collected and lyophilized to dryness to give the titled compound (24 mg, 74% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.66 (t, *J* = 5.2 Hz, 1H), 8.41 (t, *J* = 5.6 Hz, 1H), 7.98 (d, *J* = 8.3 Hz, 2H), 7.77 – 7.73 (m, 4H), 7.46 (d, *J* = 8.7 Hz, 2H), 7.42 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 8.8 Hz, 2H), 4.55 (t, *J* = 7.1 Hz, 1H), 4.17 (t, *J* = 6.2 Hz, 2H), 3.42 – 3.22 (m, under the water signal), 2.84 (t, *J* = 2.6 Hz, 1H), 2.59 (s, 3H), 2.41 (s, 3H), 2.37 (td, *J* = 7.1, 2.6 Hz, 2H), 1.95 (p, *J* = 6.5 Hz, 2H), 1.60 (s, 3H); HRMS (ESI) calcd for $C_{40}H_{37}ClN_6O_4S$ 733.2365 [M+H$^+$], found 733.2354.

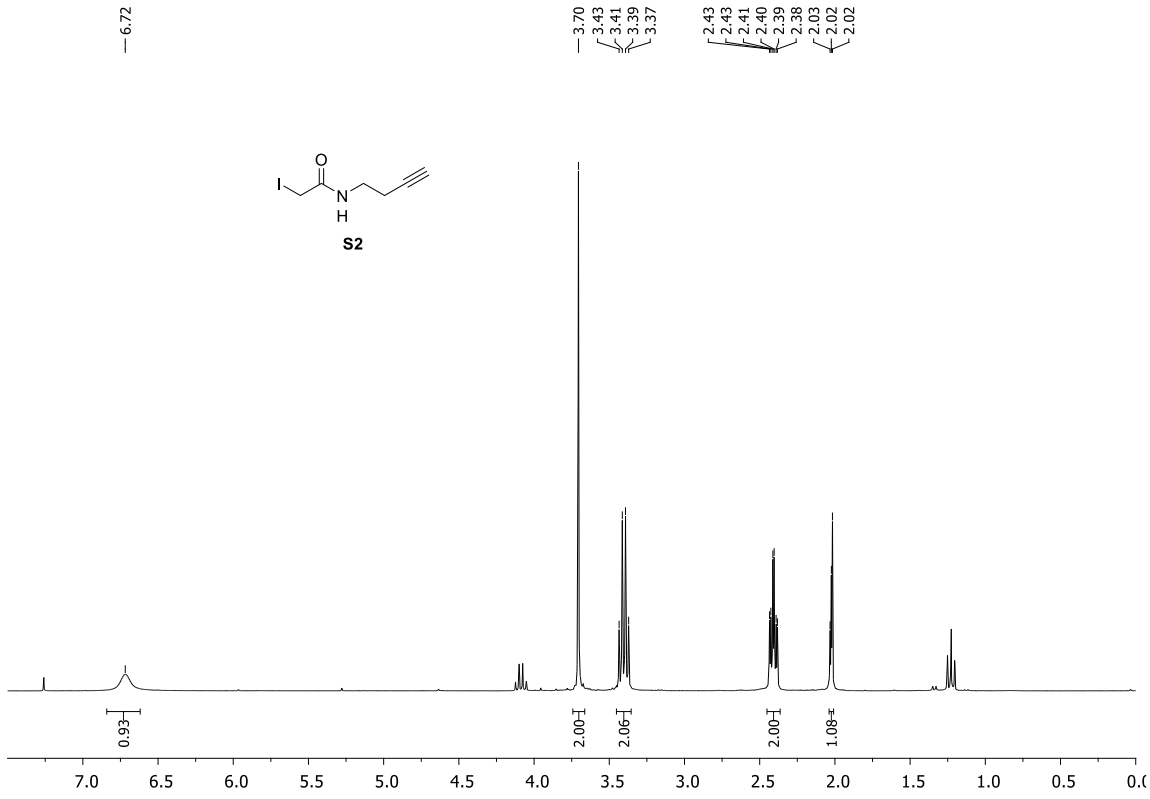


Synthesis of 6b: A solution of (+)-JQ-1 amine•TFA salt **S12** (25 mg, 0.033 mmol), benzoic acid **S10** (11.17 mg, 0.036 mmol), PyAOP (18.89 mg, 0.036 mmol) and ethyldiisopropylamine (0.023 mL, 0.132 mmol) were combined in DMF (3 mL) and stirred for 2 h at r.t. The crude reaction mix was diluted with 90% DMSO/water to 4 mL and purified on preparative HPLC. The eluents were A: 0.1% TFA-water; B: acetonitrile. 0-5 min isocratic; 1% /min acetonitrile gradient after that point. The appropriate fractions were collected and lyophilized to dryness to give the titled compound (19.1 mg, 71% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 (t, J = 5.2 Hz, 1H), 8.40 (t, J = 5.6 Hz, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.77 – 7.70 (m, 4H), 7.45 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 8.8 Hz, 2H), 4.53 (t, J = 7.1 Hz, 1H), 4.16 (t, J = 6.2 Hz, 2H), 3.40 – 3.22 (m, under the water signal), 2.82. (t, J = 2.6 Hz, 1H), 2.58 (s, 3H), 2.39 (s, 3H), 2.35 (td, J = 7.1, 2.6 Hz, 2H), 1.93 (p, J = 6.5 Hz, 2H), 1.58 (s, 3H); HRMS (ESI) calcd for $C_{44}H_{45}ClN_6O_6SNa$ 843.2708 [M+Na$^+$], found 843.2701.

^{1}H and ^{13}C NMR spectra



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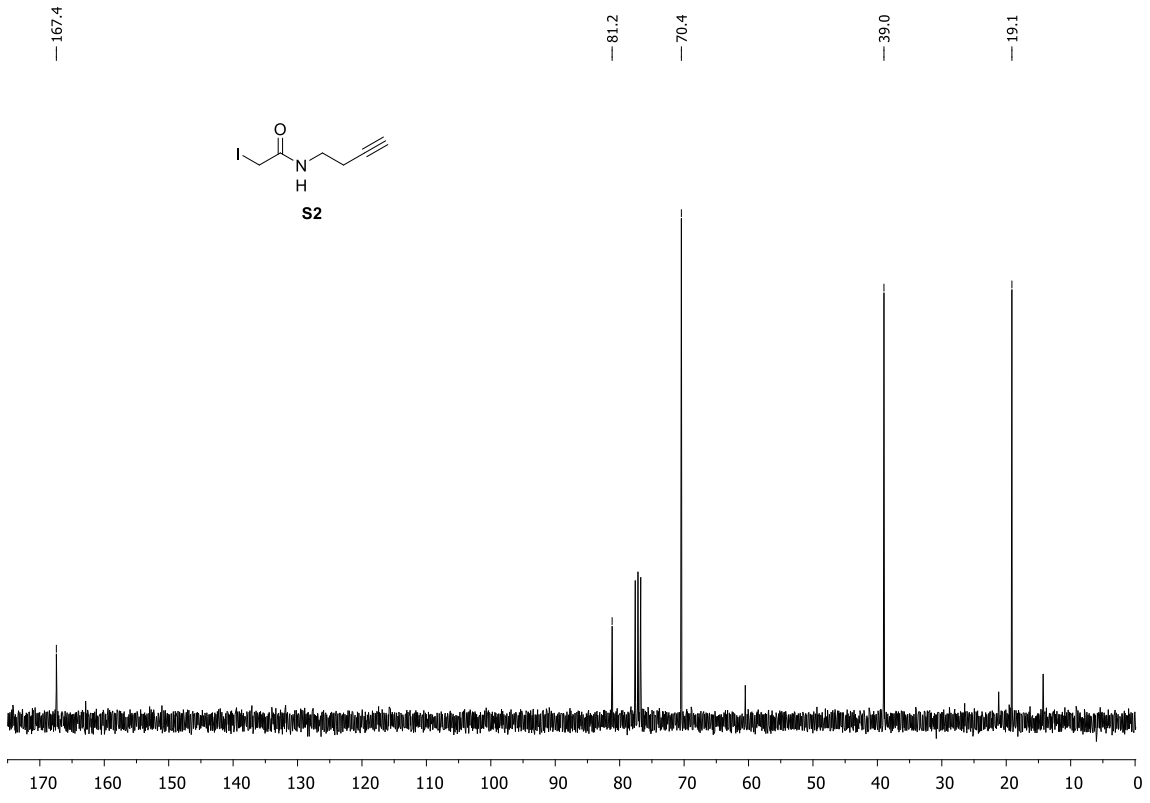
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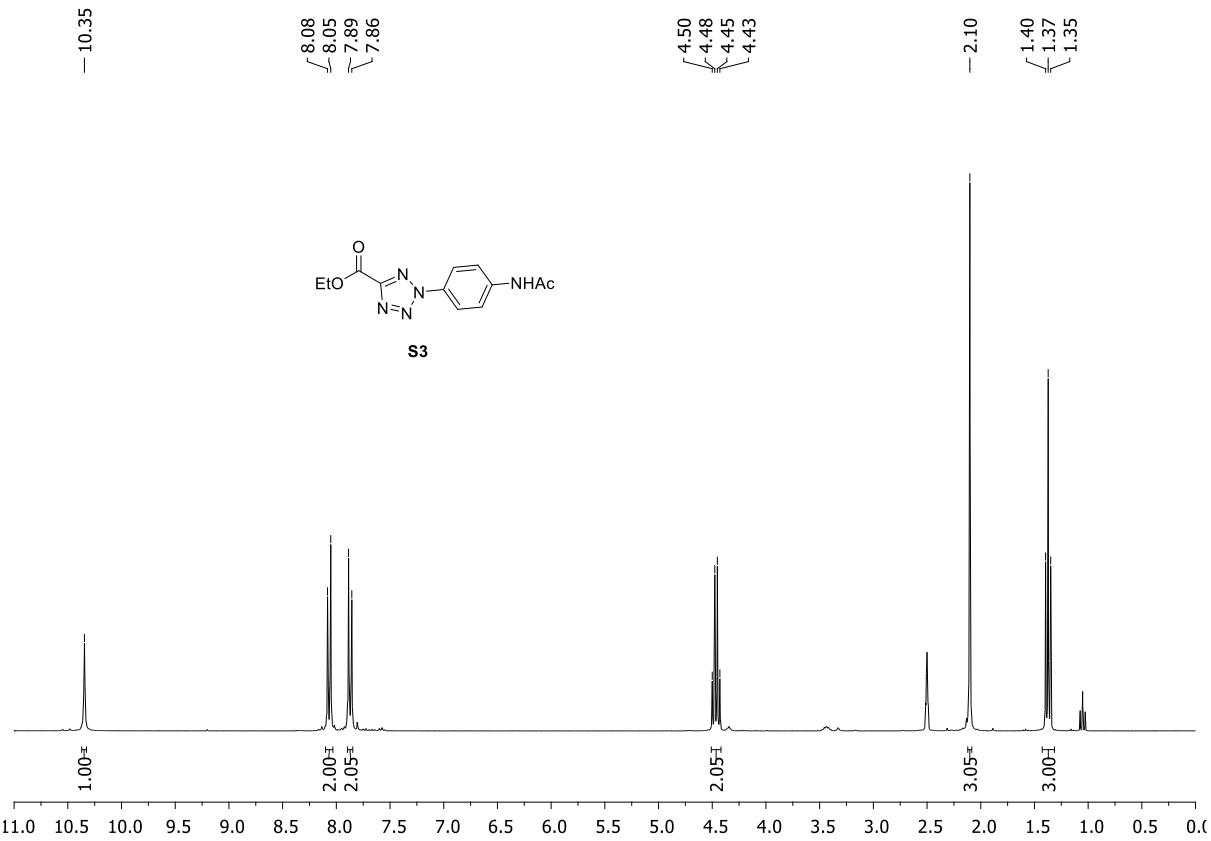
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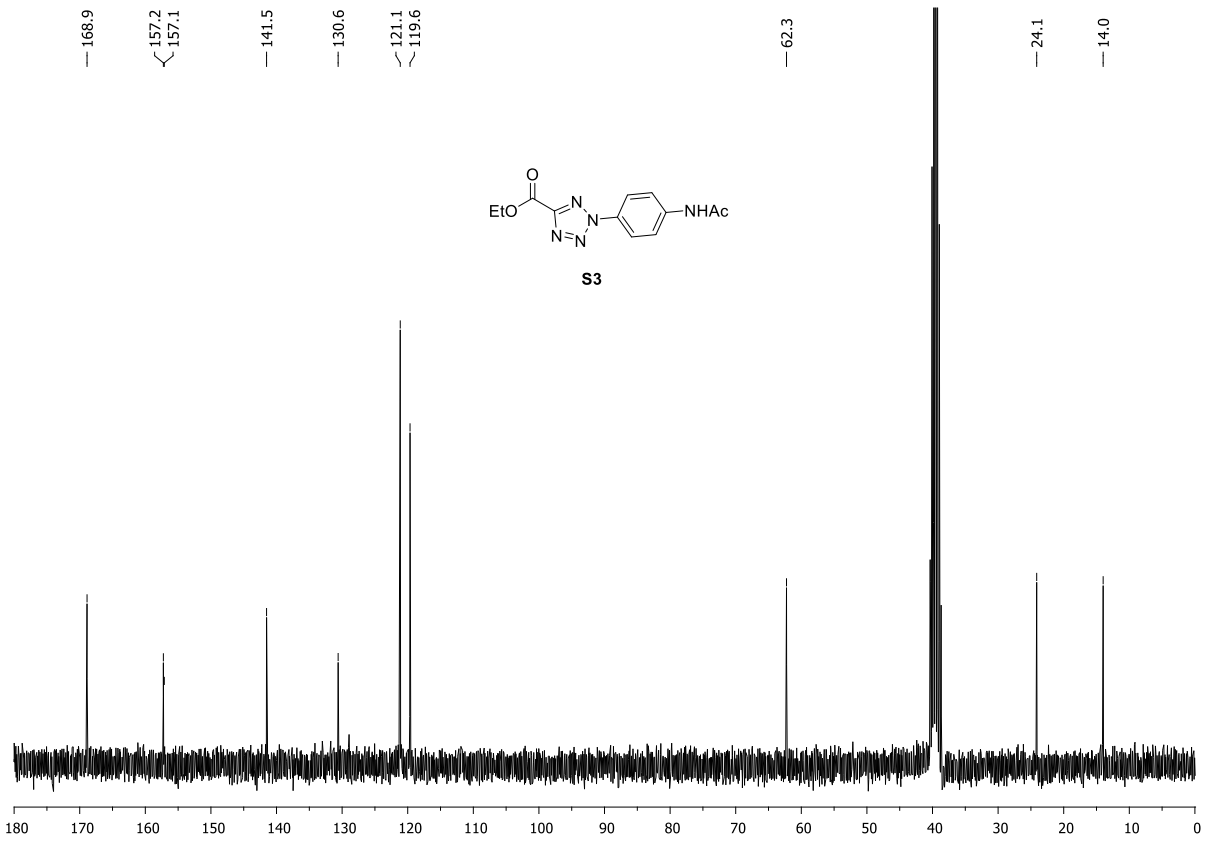
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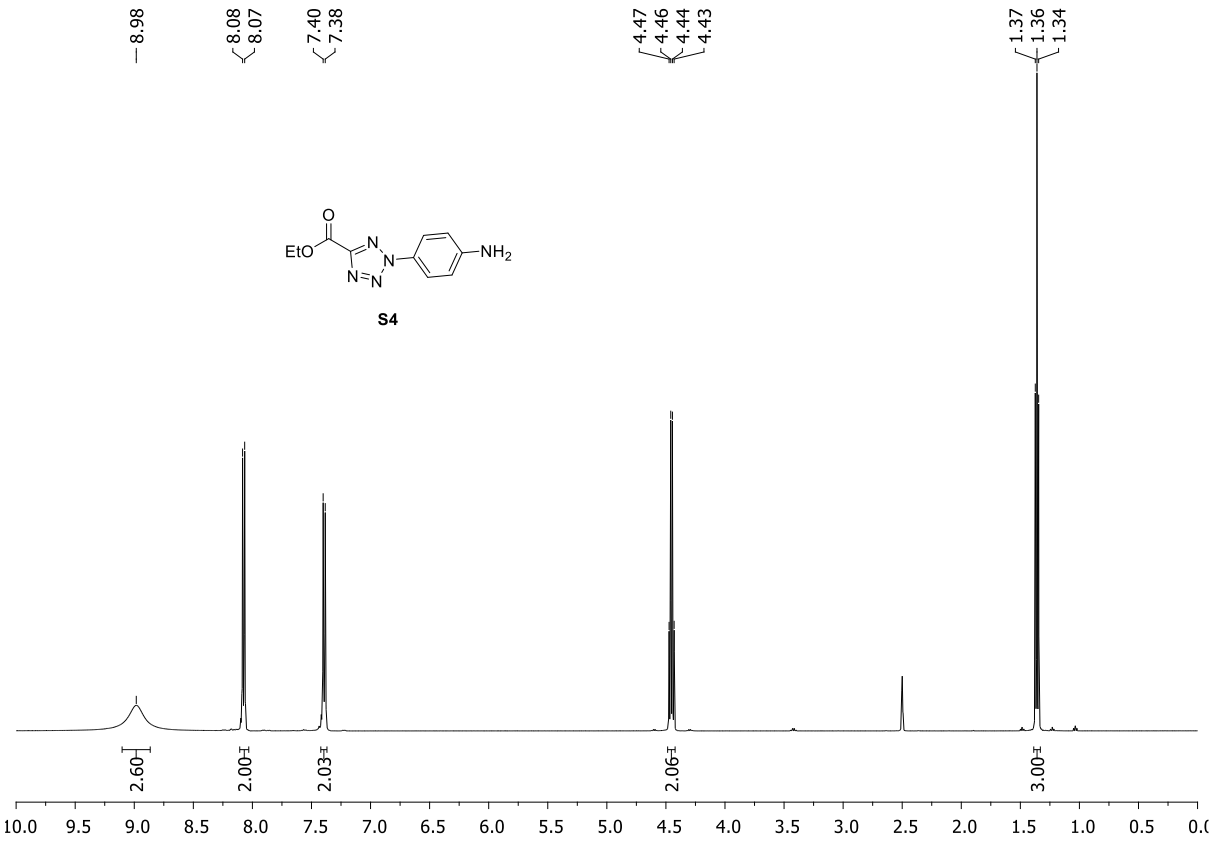
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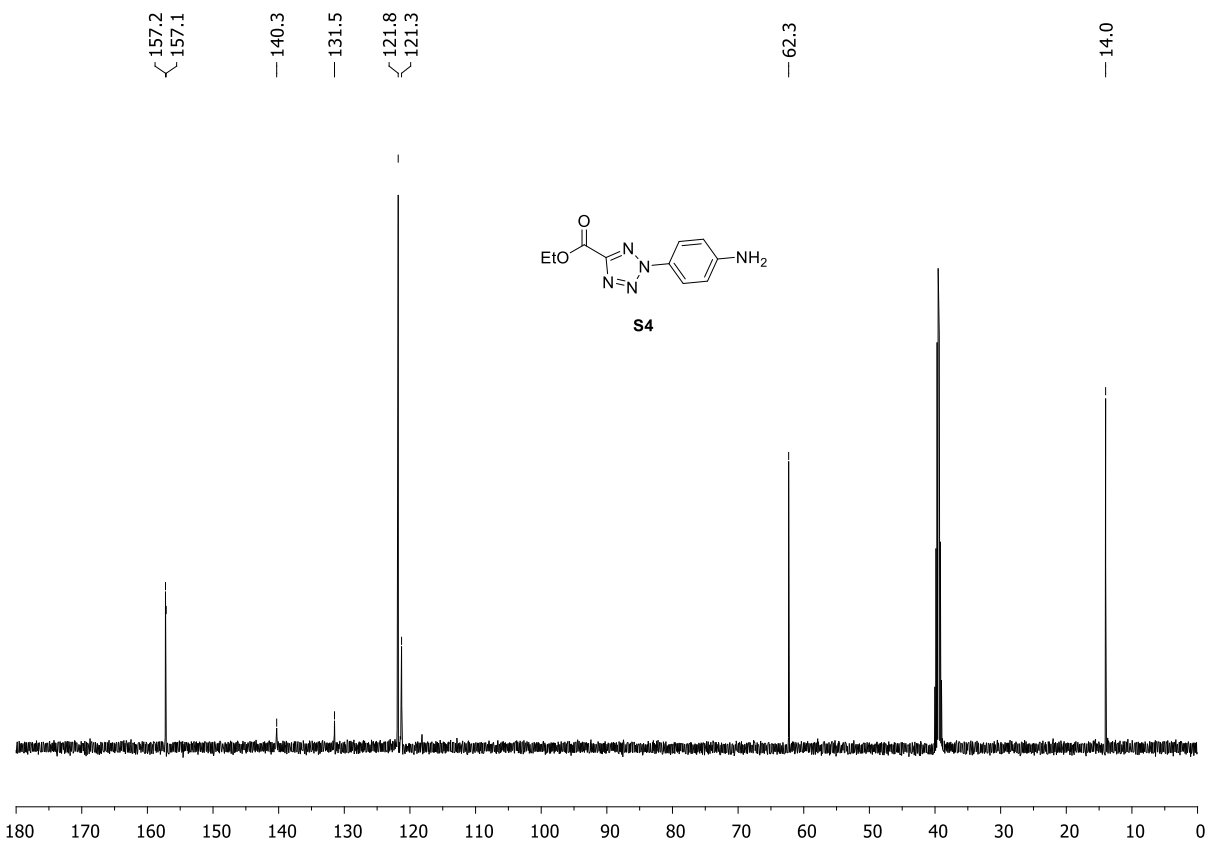
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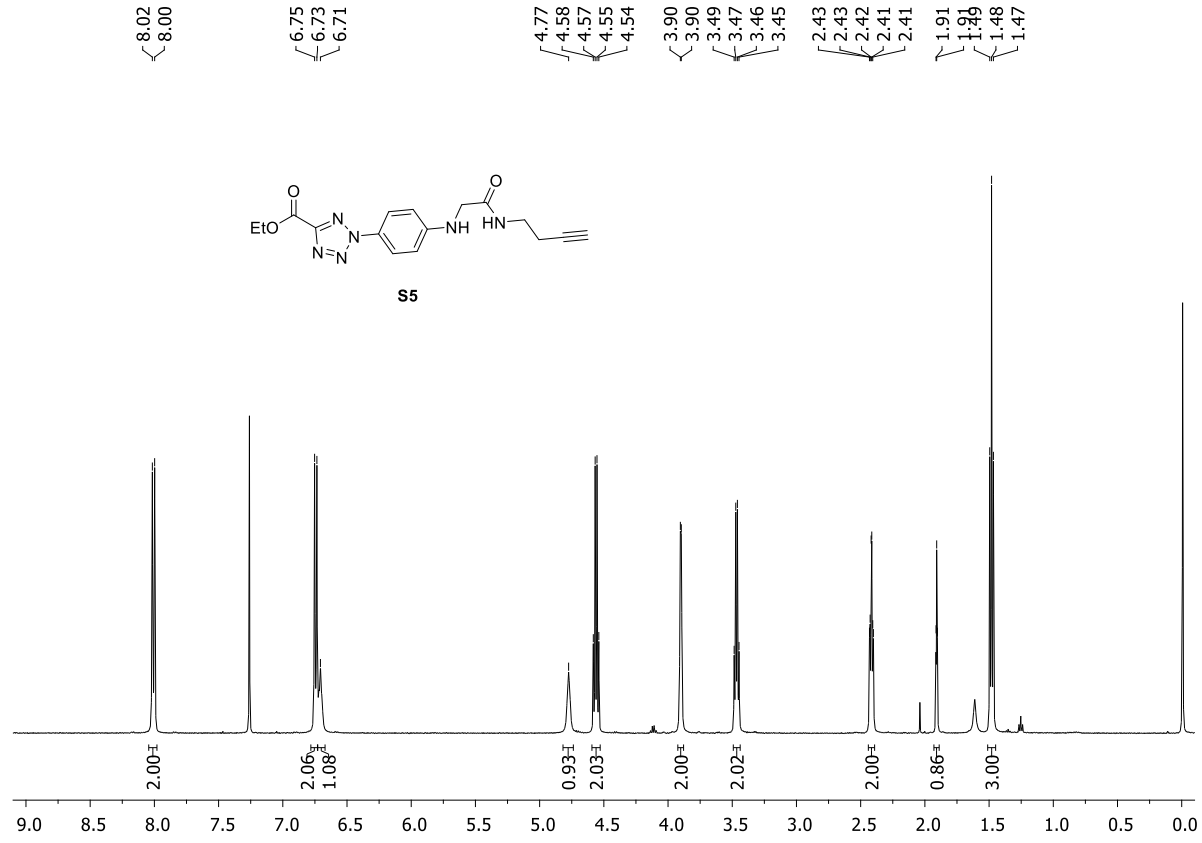
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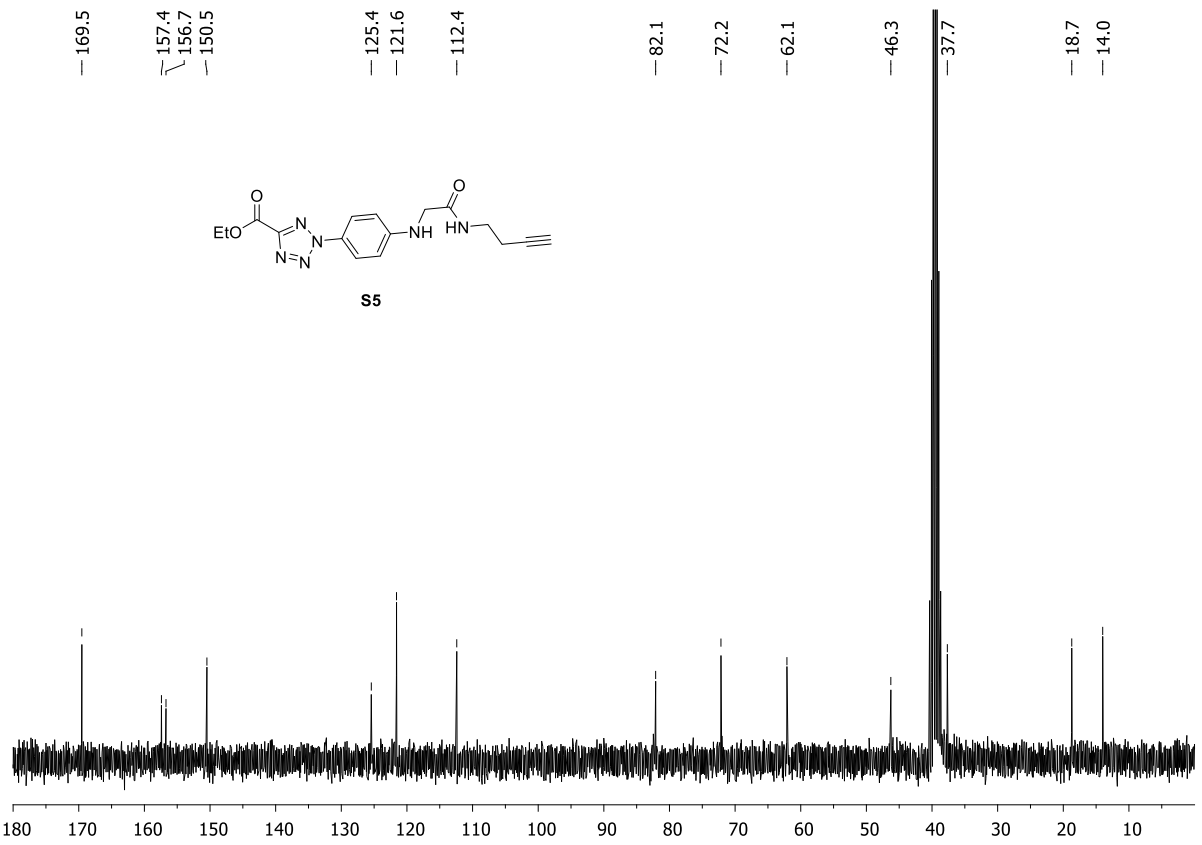
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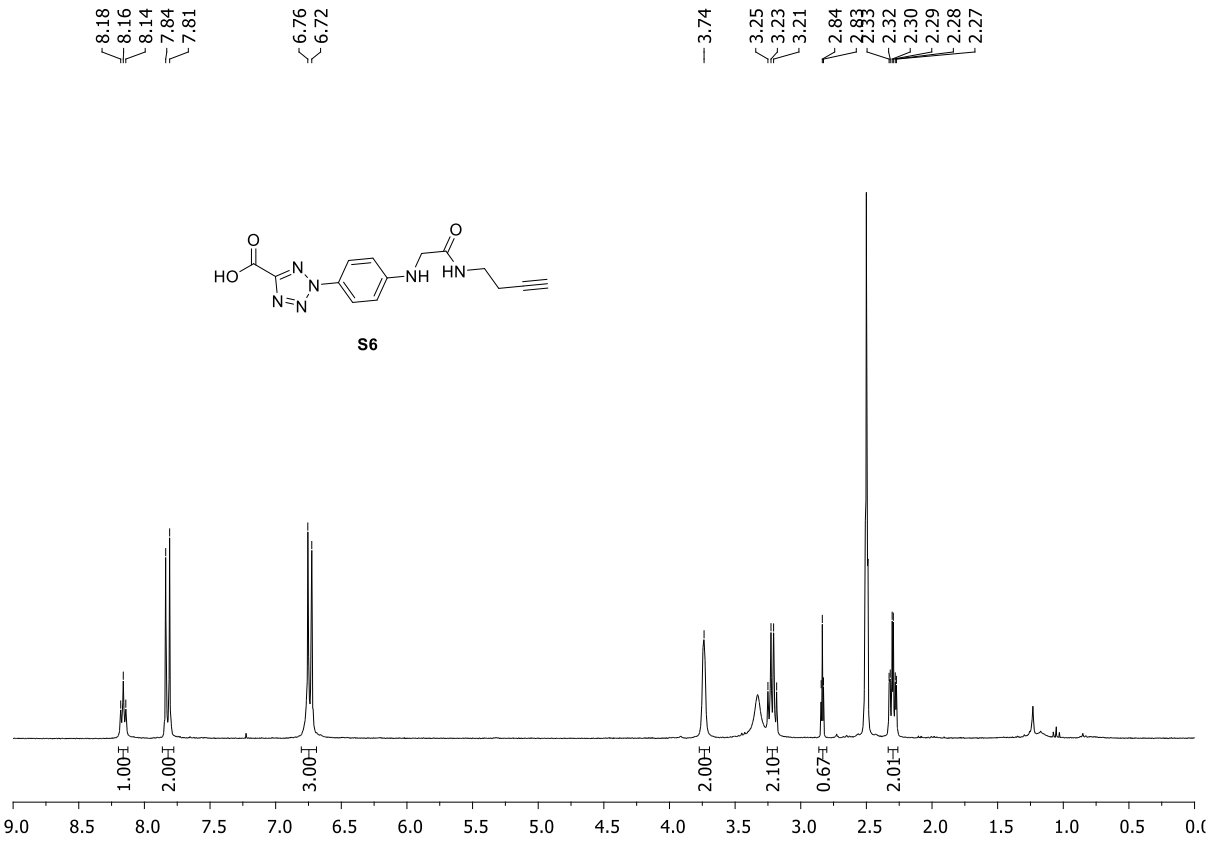
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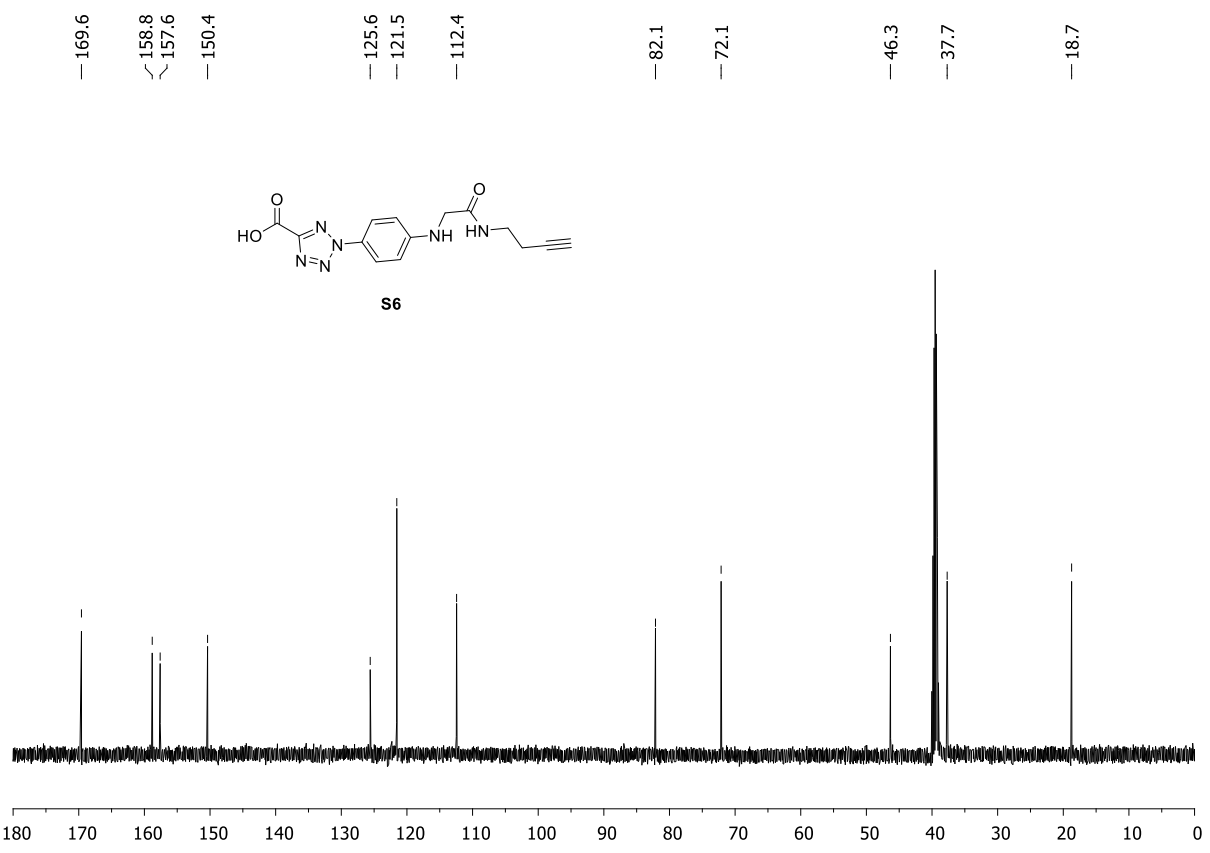
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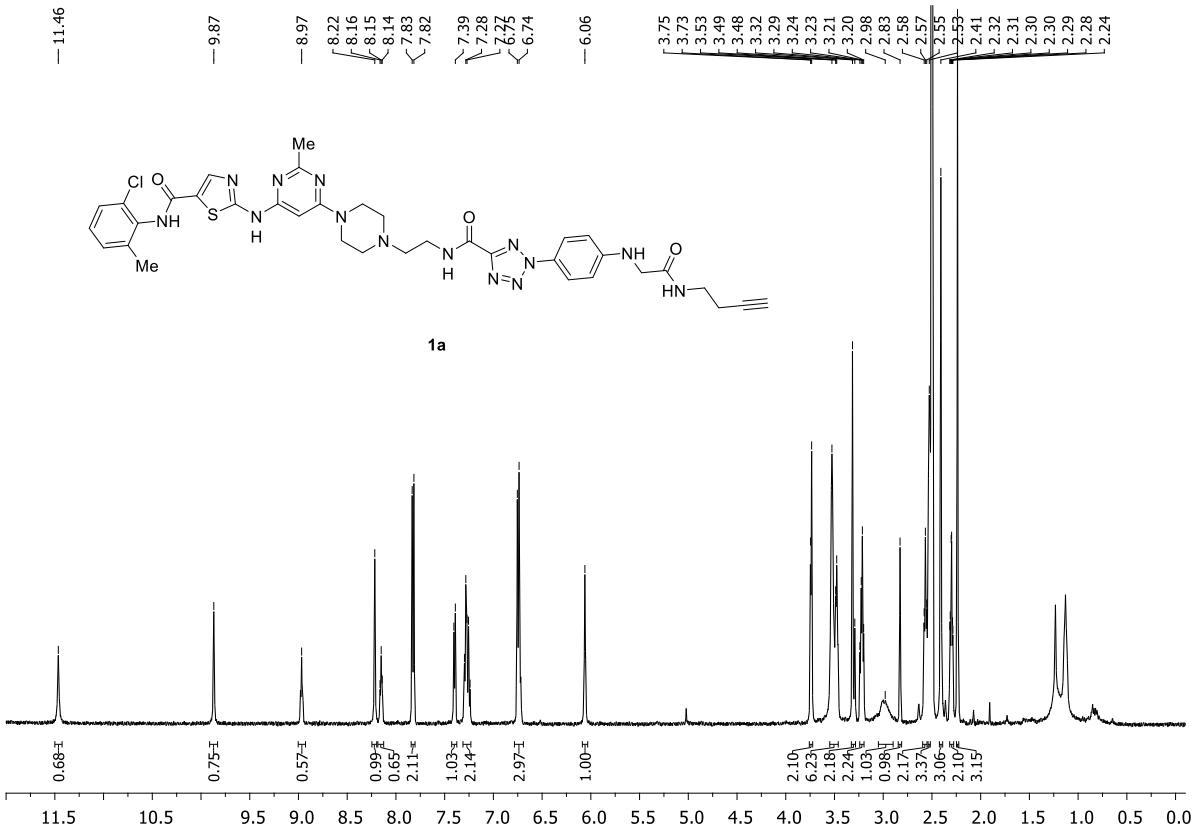
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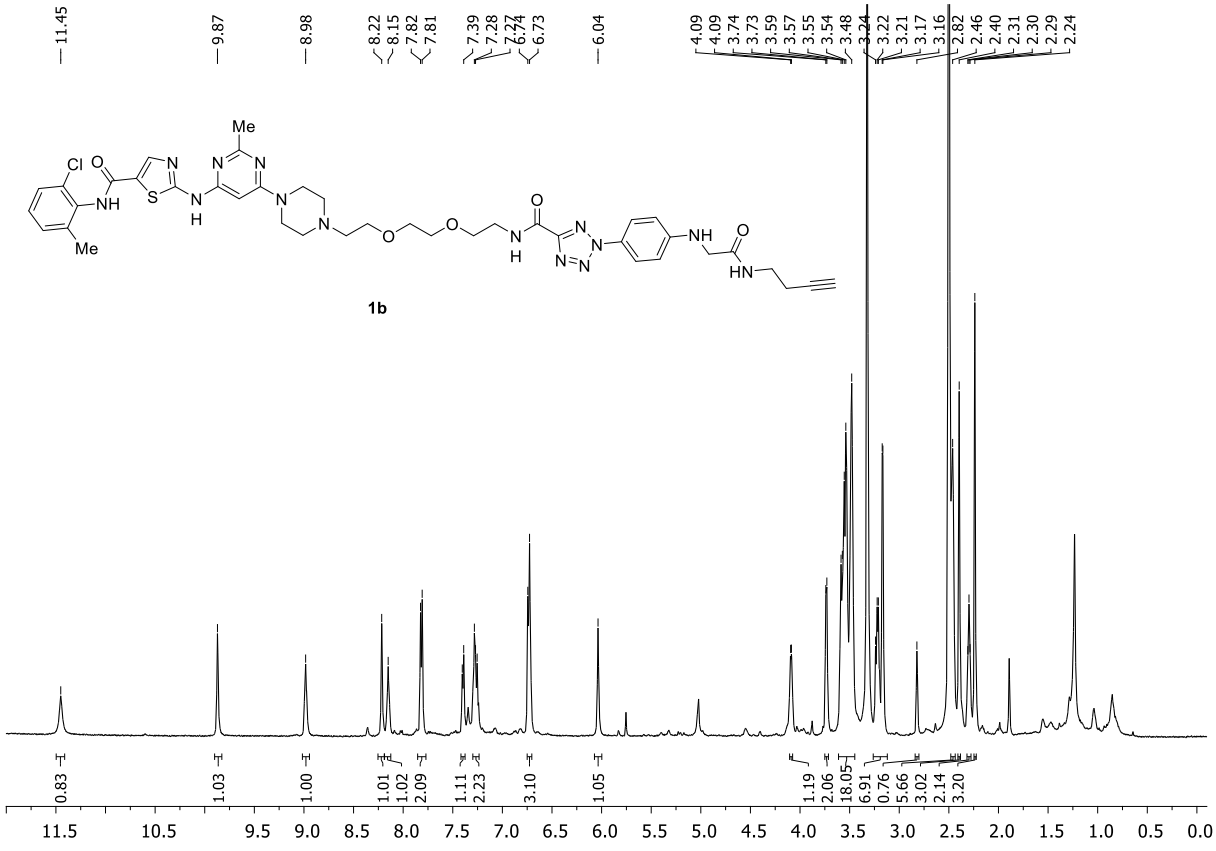
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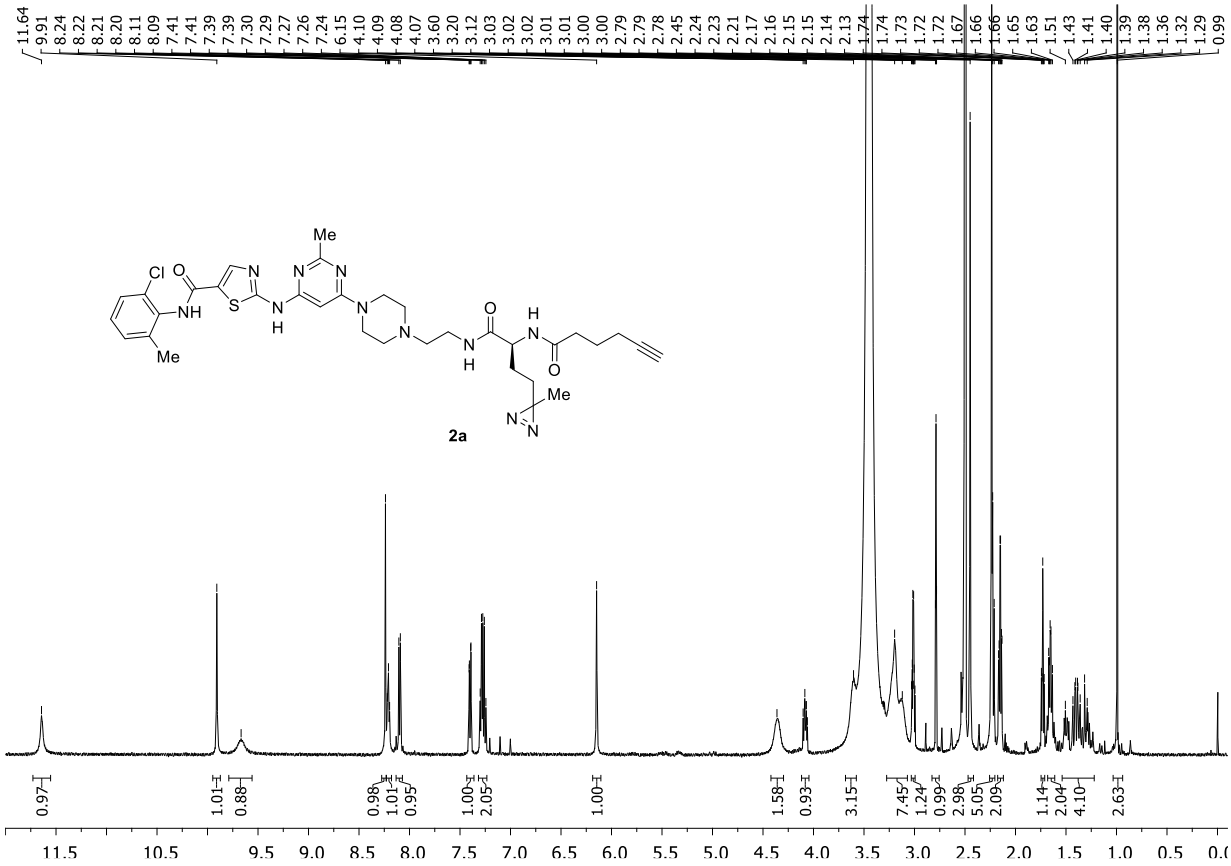
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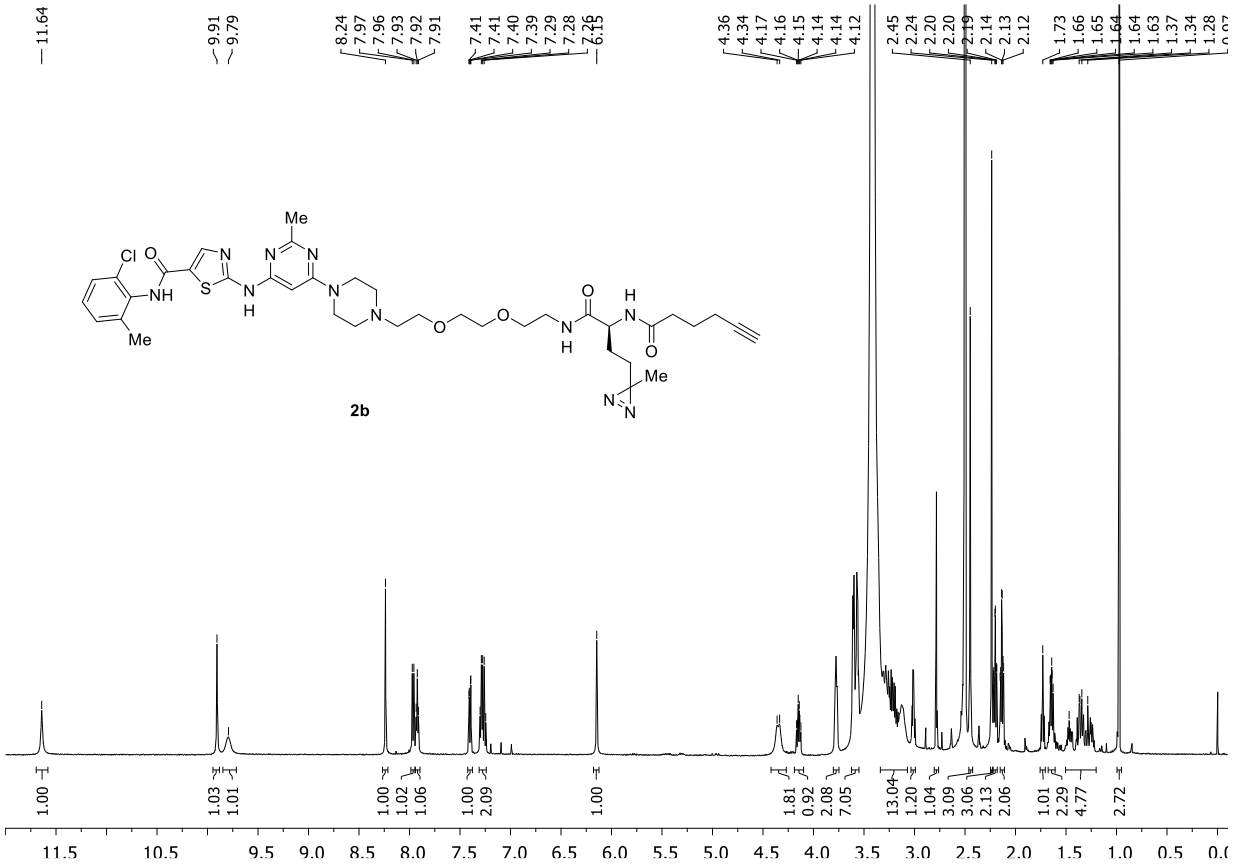
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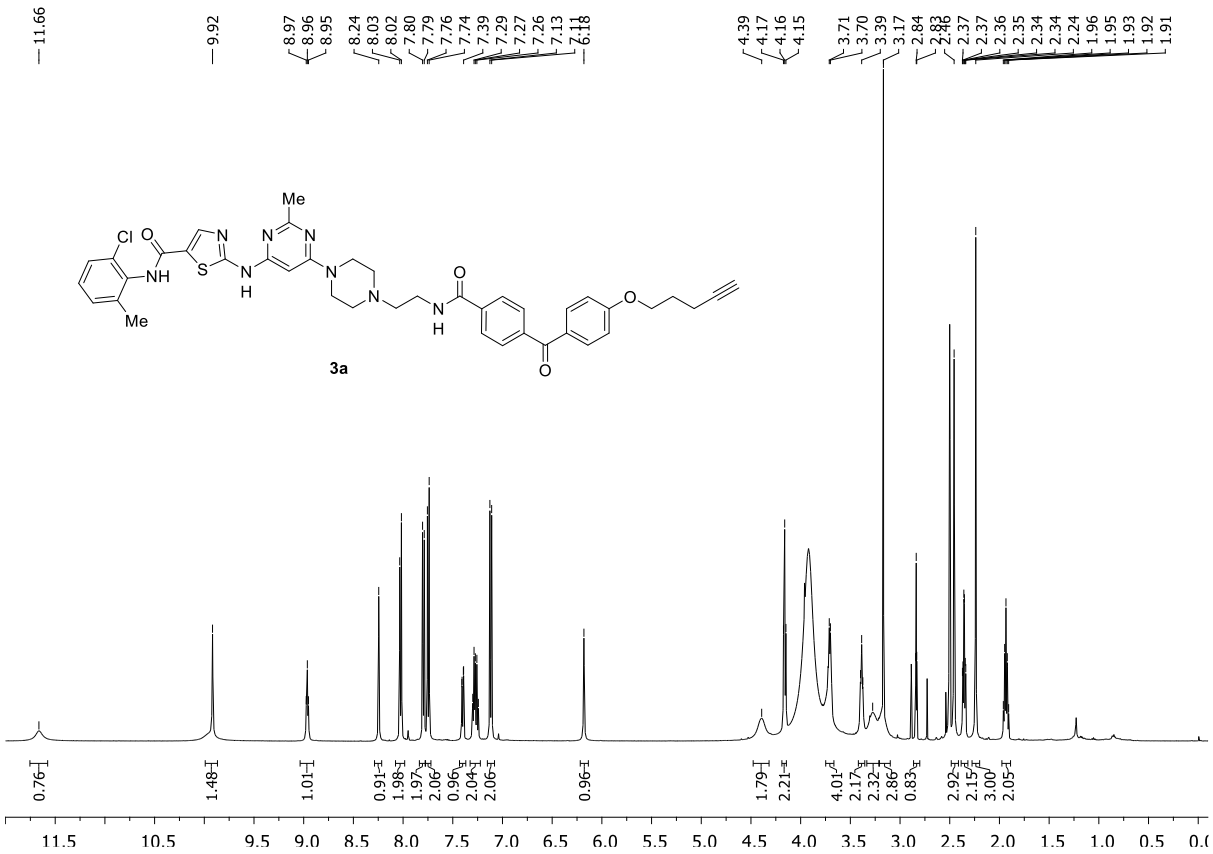
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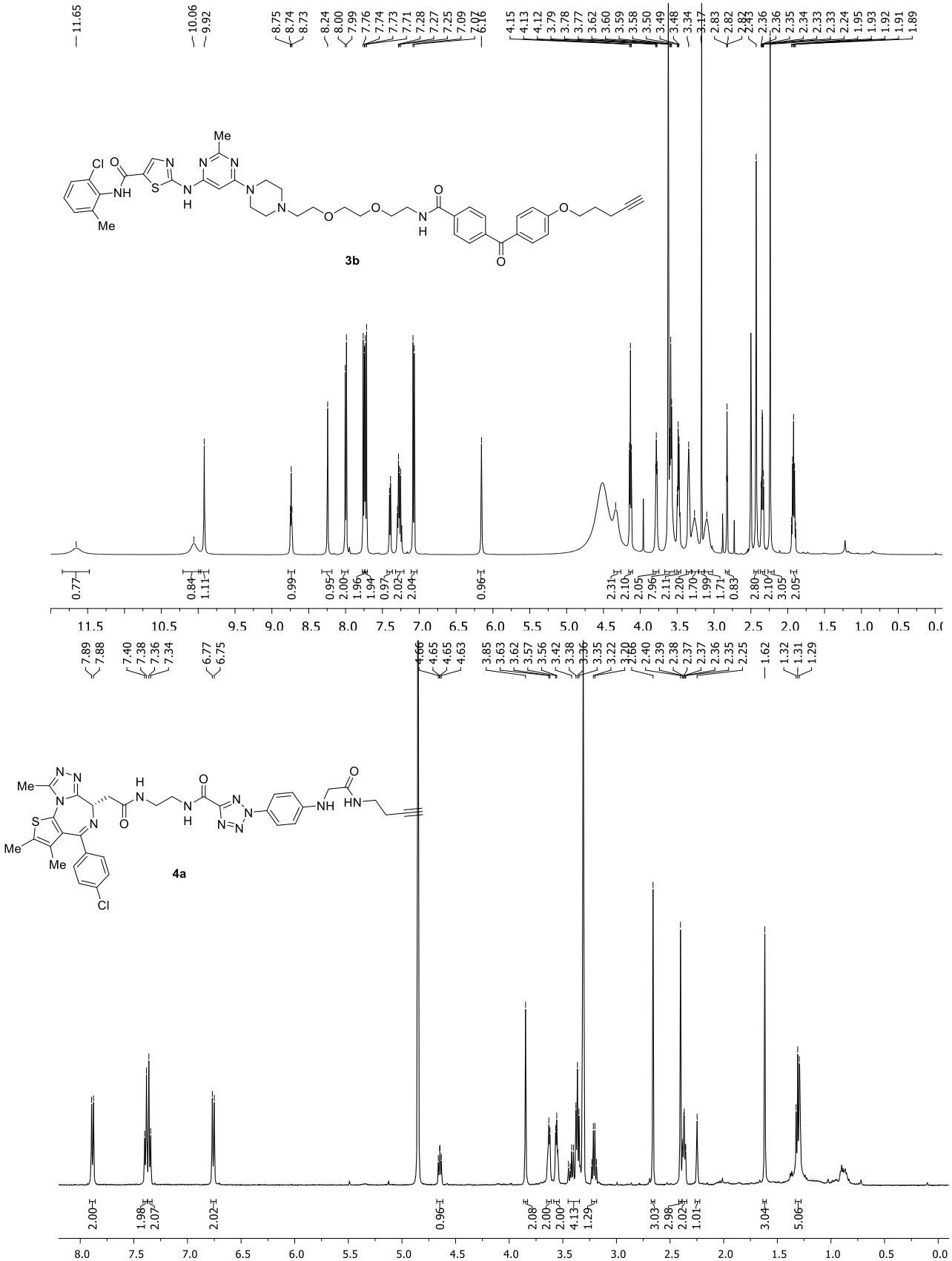
3a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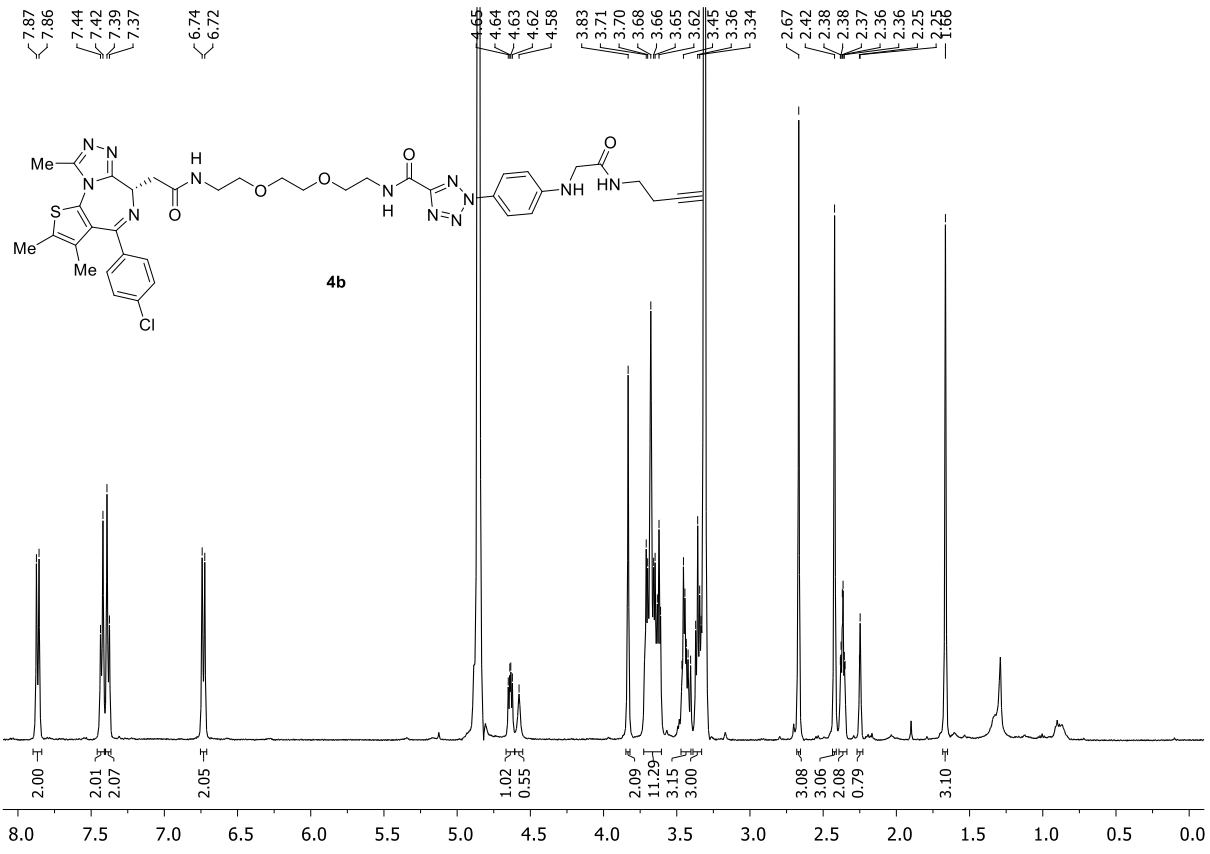
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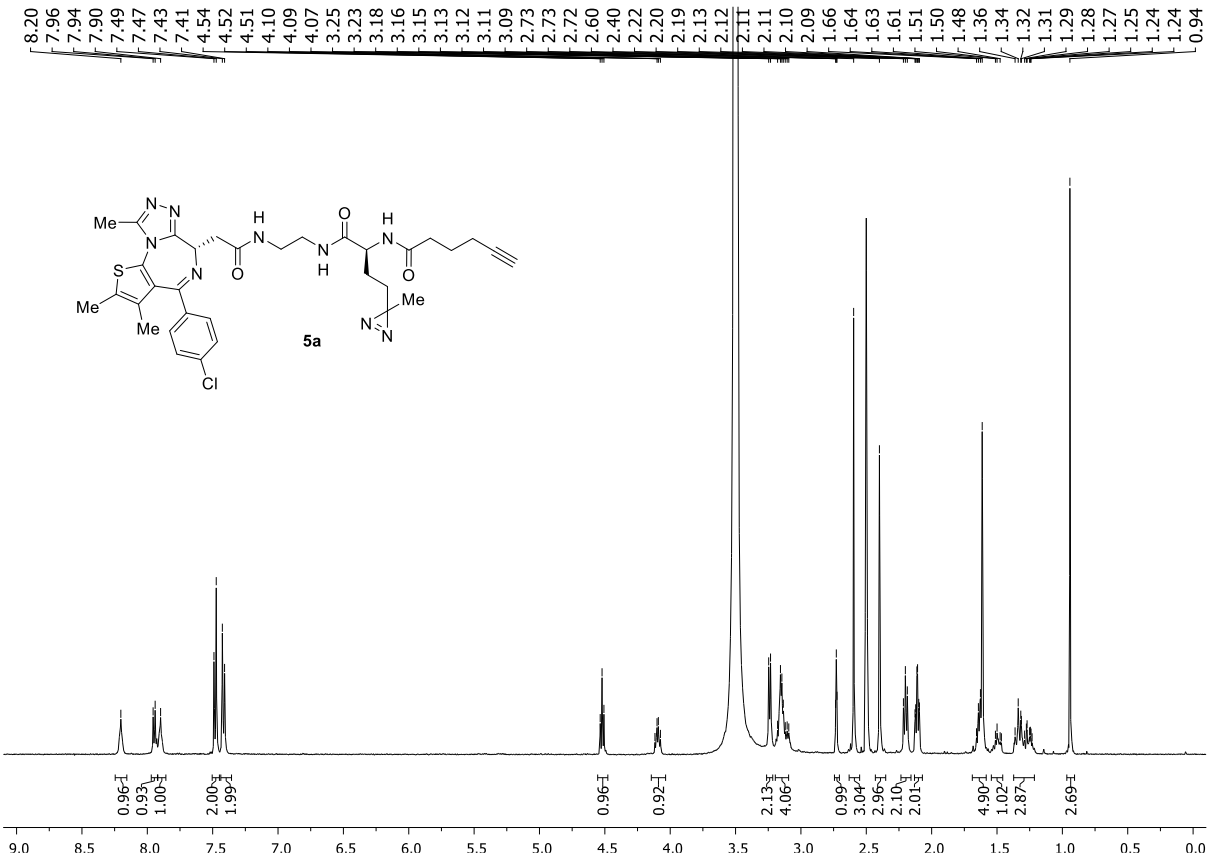
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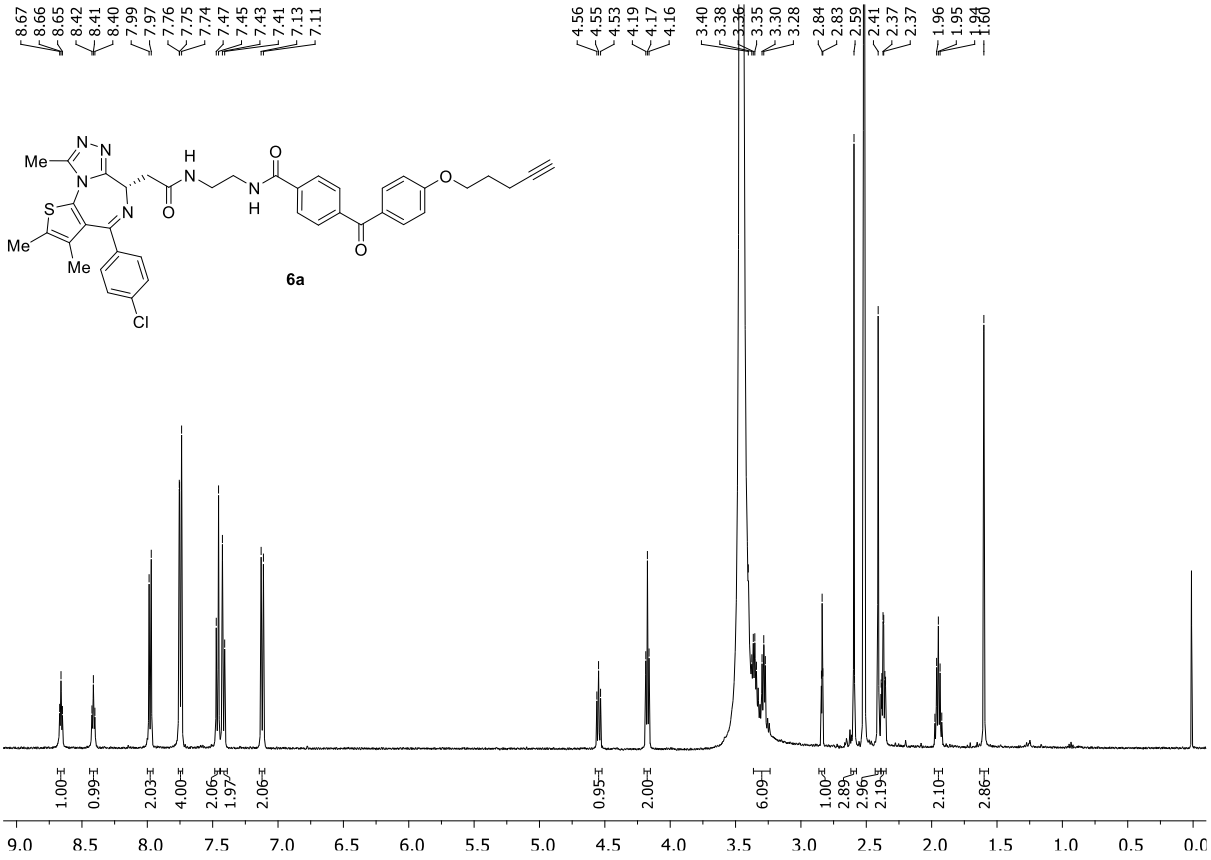
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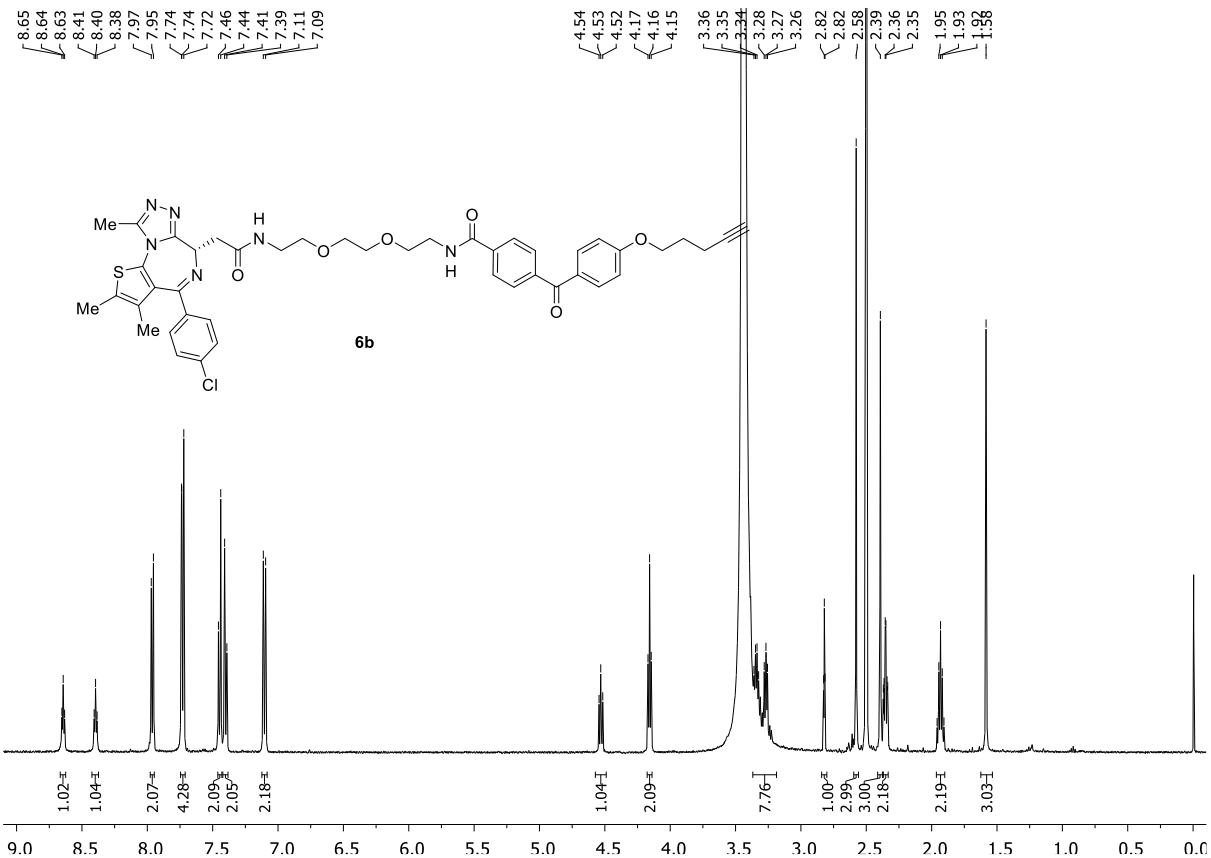
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