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Supporting Information

Discovery of a Potent Proteolysis Targeting Chimera Enables Targeting the Scaffolding Functions of FK506-Binding Protein 51 (FKBP51)

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Supplementary information

Contents

Supplementary figures and tables

alkyne	n =	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	target / E3-lig.	K_D(FKBP51) / nM					K_D(FKBP12) / nM					K_D(FKBP52) / nM				
1	a	1.2	11	8	4.1	3.5	0.05	0.1	0.1	0.09	0.1	2.2	16	12	6.9	6.4
	b	26	21	n.d.	33	7.7	0.5	0.5	n.d.	0.7	0.2	26	23	n.d.	30	15
	c	38	70	19	18	8	0.8	0.8	0.1	0.3	0.3	45	92	30	26	16
2	a	5.6	16	18	11	9.6	0.2	0.3	0.3	0.3	0.1	6.7	15	20	13	9.1
	b	204	20	9.8	4.8	6.2	114	0.2	1.4	0.1	0.1	n.d.	25	36	9.1	11
	c	38	2	4	6	6.5	0.4	0.2	0.06	0.2	0.07	47	26	6.4	9.3	7.6
3	a	64	303	279	211	178	1.4	6.9	6.6	3	7.4	45	277	218	169	184
	b	549	645	1000	1000	850	22	22	42	59	36	155	451	1000	1000	1000
	c	264	1035	1023	681	314	3.5	11	13	14	13	199	737	603	632	278
4	a	128	105	95	85	68	3.6	3.6	2.1	1.7	1.6	152	153	122	104	90
	b	226	266	95	106	134	12	16	11	4.7	2.8	256	236	111	144	172
	c	107	268	63	102	118	4.8	9.4	4	3.9	3.3	173	297	102	168	158
5	a	80	8.8	20	31	18	6.0	0.2	1.1	1.6	3.3	115	11	26	55	29
	b	115	157	122	144	384	8.5	12	6.5	4.2	227	319	441	280	154	1000
	c	8	25	17	28	12	0.2	2.1	2.4	1.5	0.7	1.9	43	16	30	60
6	a	5.3	0.9	1.2	1.1	1.9	0.6	0.06	0.2	0.1	0.2	6.3	0.23	2.9	2.2	1.7
	b	6.1	7	27	35	32	0.1	0.1	25	0.1	1.4	7.3	14	64	31	103
	c	2.3	4.4	3.2	4.2	2.1	0.2	0.4	0.5	0.5	0.4	0.7	5.7	5	8.4	4.9
7	a	676	745	355	317	357	95	131	95	67	54	1619	1726	1513	772	609
	c	506	387	590	357	537	84	62	108	91	158	1333	539	1685	817	783
8	a	7.7	6.4	2.9	10	1.9	43	19	28	50	24	n.d.	n.d.	n.d.	n.d.	n.d.
	b	457	314	269	191	176	408	1000	901	174	1000	n.d.	n.d.	n.d.	n.d.	n.d.
	c	1.8	8.4	1.1	0.9	1.8	109	180	59	54	62	n.d.	n.d.	n.d.	n.d.	n.d.
9	a	7.2	5.3	5.9	4.1	7.5	26	20	20	13	23	n.d.	n.d.	n.d.	n.d.	n.d.
	b	157	115	15	84	106	474	941	387	363	384	n.d.	n.d.	n.d.	n.d.	n.d.
	c	8.3	5.9	3.5	2	3.5	56	46	19	15	15	1000	1000	730	467	165
10	a	25	24	23	24	12	408	677	436	532	814	>3200	>3200	>3200	>3200	>3200
	b	82	110	93	104	118	25	377	108	88	136	n.d.	n.d.	n.d.	n.d.	n.d.
	c	19	16	22	18	5	306	256	206	256	226	>3200	>3200	>3200	>3200	>3200
11	a	100	59	54	50	36	1345	640	601	642	506	>3200	>3200	>3200	>3200	>3200
	b	61	94	110	84	83	35	160	177	80	228	n.d.	n.d.	n.d.	n.d.	n.d.
	c	26	26	34	40	30	1535	542	430	287	368	>3200	>3200	>3200	>3200	>3200
12	a	47	60	86	59	34	409	584	410	368	455	>3200	>3200	>3200	>3200	>3200
	b	70	75	104	87	98	85	96	78	110	129	n.d.	n.d.	n.d.	n.d.	n.d.
	c	22	21	18	24	25	111	177	111	81	162	>3200	>3200	>3200	>3200	>3200
13	a	84	142	101	68	59	30	108	24	28	18	n.d.	n.d.	n.d.	n.d.	n.d.
	b	32	45	47	50	83	20	100	97	42	228	n.d.	n.d.	n.d.	n.d.	n.d.
	c	54	28	6.7	28	35	84	70	15	35	48	n.d.	n.d.	n.d.	n.d.	n.d.
14	a	62	40	45	60	59	127	66	111	184	290	n.d.	n.d.	n.d.	n.d.	n.d.
	b	68	108	117	172	144	65	62	129	105	107	n.d.	n.d.	n.d.	n.d.	n.d.
	c	40	44	18	20	31	124	90	52	140	130	n.d.	n.d.	n.d.	n.d.	n.d.
15	a	52	26	14	23	67	1001	56	215	438	62	n.d.	n.d.	n.d.	n.d.	n.d.
	b	66	154	289	346	438	71	434	1000	1000	1000	n.d.	n.d.	n.d.	n.d.	n.d.
	c	272	309	401	503	1000	1000	1000	1000	1000	1000	n.d.	n.d.	n.d.	n.d.	n.d.

Figure S1.1 Binding affinities to FKBP51, FKBP12 and FKBP52 derived from competitive FP assays. Values represent the mean of at least two replicates.

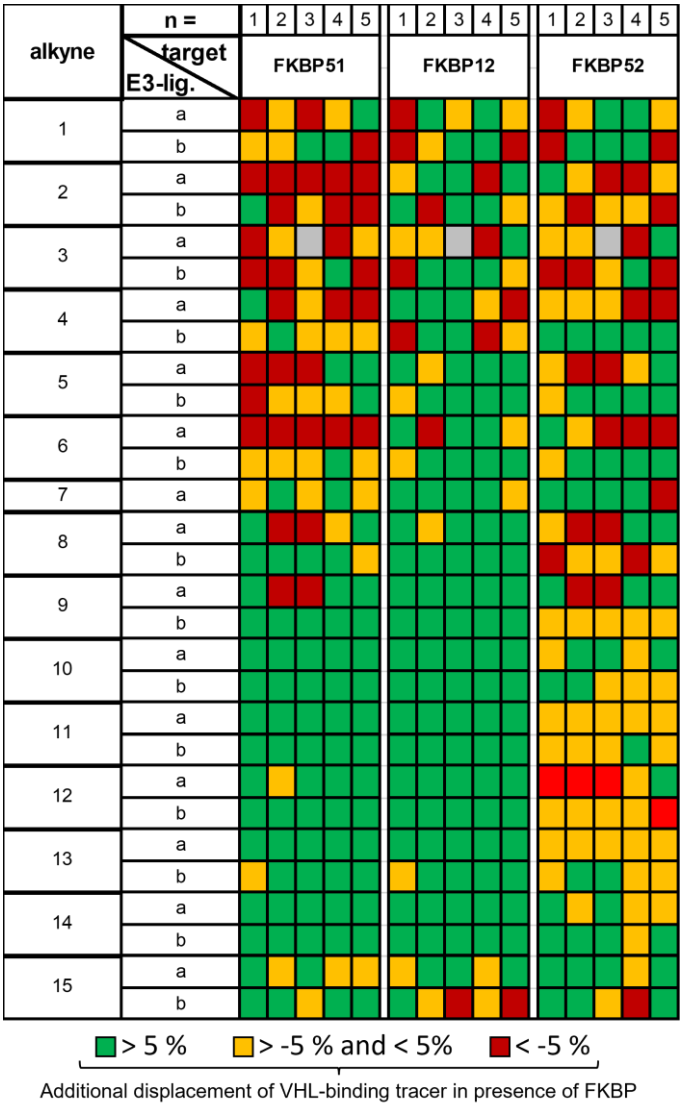


Figure S1.2 Systematic single point cooperativity screening assessed by fluorescence polarization-based VHL binding assay in the absence or presence of saturating FKBP concentrations. Constant or differential VHL binding in presence of FKBP is indicative of positive (green >5 %), no (yellow >-5 % and <5 %) or negative (red <-5 %) cooperativity. Grey: indicates not tested PROTACs

	c(PROTAC)	10 µM					0.5 µM					10 µM					0.5 µM				
	n =	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
alkyne	target / E3-lig.	FKBP51										FKBP12									
1	a	1.03	1.00	1.04	1.04	1.06	1.04	1.11	1.04	1.03	1.04	0.96	0.97	0.95	0.85	0.83	0.69	0.57	0.53	0.28	0.25
	b	0.97	1.00	0.95	0.95	1.08	1.04	1.03	0.91	0.93	1.04	0.93	0.98	0.70	0.64	0.62	0.66	0.89	0.41	0.25	0.27
	c	1.13	1.04	1.04	1.03	1.00	1.05	1.08	1.07	1.03	1.02	0.82	0.70	0.71	0.55	0.40	0.67	0.60	0.58	0.46	0.38
2	a	0.99	1.00	1.00	0.97	0.96	1.05	1.07	1.08	1.03	1.01	0.09	0.39	0.39	0.38	0.60	0.07	0.16	0.27	0.21	0.40
	b	0.95	1.05	1.04	1.02	0.94	1.11	1.15	1.03	1.06	1.04	0.82	0.96	0.75	0.68	0.57	0.72	0.79	0.36	0.30	0.38
	c	0.97	0.98	1.04	1.03	1.31	1.03	1.05	1.12	1.24	1.14	0.68	0.46	0.41	0.22	0.23	0.47	0.44	0.41	0.25	0.25
3	a	1.01	1.01	1.12	1.11	1.12	1.04	1.03	1.14	1.08	1.16	0.87	1.04	0.90	0.51	0.48	0.39	0.89	0.45	0.09	0.08
	b	0.98	0.99	1.04	0.99	0.99	1.11	1.03	1.01	1.03	1.02	0.90	0.94	0.60	0.17	0.34	0.85	0.90	0.45	0.25	0.19
	c	1.06	1.00	1.02	0.95	1.01	1.06	1.09	1.09	1.07	1.06	0.50	0.28	1.02	0.35	0.56	0.56	0.32	1.01	0.43	0.53
4	a	1.02	1.06	1.07	1.04	1.04	1.04	1.04	1.05	1.10	1.11	1.04	0.99	1.06	1.07	1.09	0.95	0.55	0.59	0.77	0.75
	b	1.08	1.00	0.97	0.99	0.99	1.07	1.10	1.04	1.06	1.05	0.95	0.68	0.68	0.81	0.85	0.94	0.50	0.79	0.94	0.73
	c	1.05	1.03	1.05	1.04	1.01	1.08	1.05	1.09	1.10	1.06	0.71	0.66	0.37	0.21	0.26	0.74	0.58	0.36	0.18	0.24
5	a	1.14	1.01	1.01	1.03	0.97	1.04	1.29	1.10	1.08	1.06	0.07	1.04	0.33	0.88	0.96	0.06	0.93	0.07	0.16	0.26
	b	0.88	0.93	0.87	0.95	0.96	1.12	1.14	1.12	1.07	0.96	0.66	0.22	0.14	0.19	0.25	1.02	0.20	0.15	0.12	0.11
	c	1.01	0.99	0.98	0.99	0.99	1.07	1.20	1.09	1.05	1.08	0.99	0.80	0.42	0.33	0.38	0.86	0.65	0.40	0.29	0.35
6	a	1.00	1.05	1.01	0.99	0.97	1.04	0.99	1.11	1.04	1.10	0.10	1.03	0.49	0.86	0.95	0.05	1.03	0.08	0.23	0.32
	b	0.90	0.99	0.85	0.84	0.92	1.07	1.11	1.05	0.76	0.79	0.80	0.33	0.25	0.11	0.10	1.06	0.21	0.13	0.08	0.07
	c	1.00	0.97	0.99	1.08	0.99	1.04	1.08	1.12	1.07	1.05	0.83	0.76	0.50	0.33	0.42	0.63	0.72	0.51	0.34	0.40
7	a	1.13	0.93	1.01	0.91	0.98	1.03	1.07	1.10	1.12	1.14	0.40	0.87	0.96	0.95	1.03	0.09	0.78	0.88	0.87	0.84
	c	1.00	1.00	0.99	1.01	0.99	1.18	1.12	1.24	1.16	1.13	0.82	0.91	0.95	0.88	0.94	0.93	0.96	0.97	0.97	0.96
8	a	0.80	0.89	0.96	0.88	0.87	1.03	1.02	1.04	0.99	1.00	0.46	0.77	0.88	0.88	0.89	0.92	0.97	0.97	0.98	0.98
	b	0.96	0.94	1.00	1.00	0.97	1.07	1.30	1.06	1.04	1.05	0.57	0.53	0.73	0.74	0.79	0.97	0.89	0.95	0.97	0.97
	c	0.93	0.98	0.99	0.98	0.95	1.01	1.02	1.04	1.01	1.22	0.89	0.74	0.89	0.93	0.94	0.97	0.98	0.98	0.99	0.99
9	a	0.69	1.11	0.88	0.78	0.84	0.82	0.91	0.97	0.89	0.86	0.17	0.46	0.47	0.41	0.52	0.16	0.13	0.25	0.38	0.32
	b	0.94	0.89	0.97	0.94	0.97	0.90	0.86	0.95	0.91	0.95	0.62	0.08	0.28	0.32	0.45	0.60	0.12	0.51	0.28	0.30
	c	0.98	0.99	0.97	0.96	0.92	1.00	0.99	1.02	1.03	1.01	0.31	0.19	0.24	0.29	0.23	0.39	0.22	0.32	0.46	0.34
10	a	0.80	0.73	0.72	0.72	0.76	0.87	0.79	0.58	0.58	0.48	0.19	0.23	0.44	0.22	0.42	0.29	0.30	0.77	0.20	0.42
	b	0.54	0.89	0.74	0.71	0.74	0.57	0.89	0.73	0.72	0.67	0.83	0.61	0.44	0.22	0.30	0.88	0.69	0.47	0.22	0.27
	c	1.01	1.02	0.97	0.98	0.99	1.02	1.02	1.10	1.00	1.03	0.88	0.36	0.61	0.36	0.35	0.89	0.52	0.70	0.47	0.45
11	a	0.93	0.89	0.80	0.82	0.84	0.97	0.93	1.04	0.88	0.92	0.61	0.43	0.52	0.52	0.72	0.71	0.41	0.60	0.58	0.68
	b	0.72	0.82	0.62	0.53	0.62	0.73	0.87	0.64	0.50	0.57	0.28	0.59	0.38	0.39	0.57	0.35	0.67	0.45	0.41	0.53
	c	0.88	0.78	0.91	0.92	0.88	0.93	0.86	0.94	0.93	0.93	0.25	0.26	0.22	0.20	0.18	0.29	0.32	0.31	0.29	0.29
12	a	0.94	0.81	0.84	0.84	0.85	0.98	0.95	0.88	0.94	0.90	0.68	0.45	0.32	0.22	0.34	0.82	0.53	0.37	0.28	0.27
	b	0.84	0.57	0.48	0.50	0.53	0.90	0.64	0.64	0.55	0.62	0.70	0.70	0.53	0.35	0.42	0.77	0.76	0.56	0.37	0.44
	c	0.83	0.77	0.89	0.87	0.89	0.89	0.83	0.91	0.95	1.02	0.07	0.15	0.22	0.22	0.23	0.12	0.21	0.30	0.30	0.32
13	a	0.95	0.92	0.90	0.99	1.11	0.86	0.74	0.85	0.91	0.96	0.55	0.58	0.56	0.60	0.50	0.42	0.44	0.42	0.41	0.45
	b	0.93	0.85	0.93	0.87	1.06	1.02	1.03	0.91	0.85	0.88	0.55	0.44	0.14	0.09	0.16	0.69	0.52	0.13	0.09	0.13
	c	1.04	1.03	1.06	1.18	1.11	1.01	1.04	1.04	1.03	1.04	0.72	0.56	0.56	0.50	0.41	0.76	0.59	0.43	0.36	0.33
14	a	0.87	0.81	0.90	1.06	0.95	0.78	0.92	0.93	0.95	0.81	0.43	0.63	0.40	0.33	0.52	0.46	0.59	0.32	0.20	0.24
	b	0.45	0.49	0.71	0.81	0.90	0.54	0.48	0.69	0.73	0.78	0.54	0.41	0.21	0.10	0.13	0.61	0.46	0.23	0.10	0.14
	c	1.05	1.04	1.04	1.02	1.01	1.06	1.02	1.03	1.07	1.05	0.66	0.49	0.47	0.59	0.22	0.84	0.56	0.73	0.62	0.43
15	a	1.04	1.00	1.01	1.03	1.08	1.07	1.08	1.24	1.04	1.07	0.91	1.02	1.01	0.99	1.01	0.96	1.02	1.01	1.01	1.03
	b	1.09	1.03	1.08	1.12	1.06	1.09	1.05	1.08	1.05	1.01	0.75	0.63	0.88	0.93	0.96	1.02	1.01	1.02	1.01	1.01
	c	1.01	0.99	1.17	1.01	1.09	1.03	1.02	1.05	1.06	1.08	1.02	1.01	1.01	1.00	1.00	1.01	1.01	1.02	1.01	1.01

Figure S1.3 Degradation activity profile of PROTAC candidates in FKBP51-eGFP and FKBP12-eGFP reporter assays. FKBP51-eGFP and FKBP12-eGFP level compared the DMSO control after 48 h treatment at 10 µM or 0.5 µM (green: >50 % reporter degradation; yellow: 50-25 % reporter degradation; red < 25 %). Values represent the mean of normalized (eGFP/mCherry) ratios on the DMSO control derived from biological duplicates and correspond to Fig. 1C.

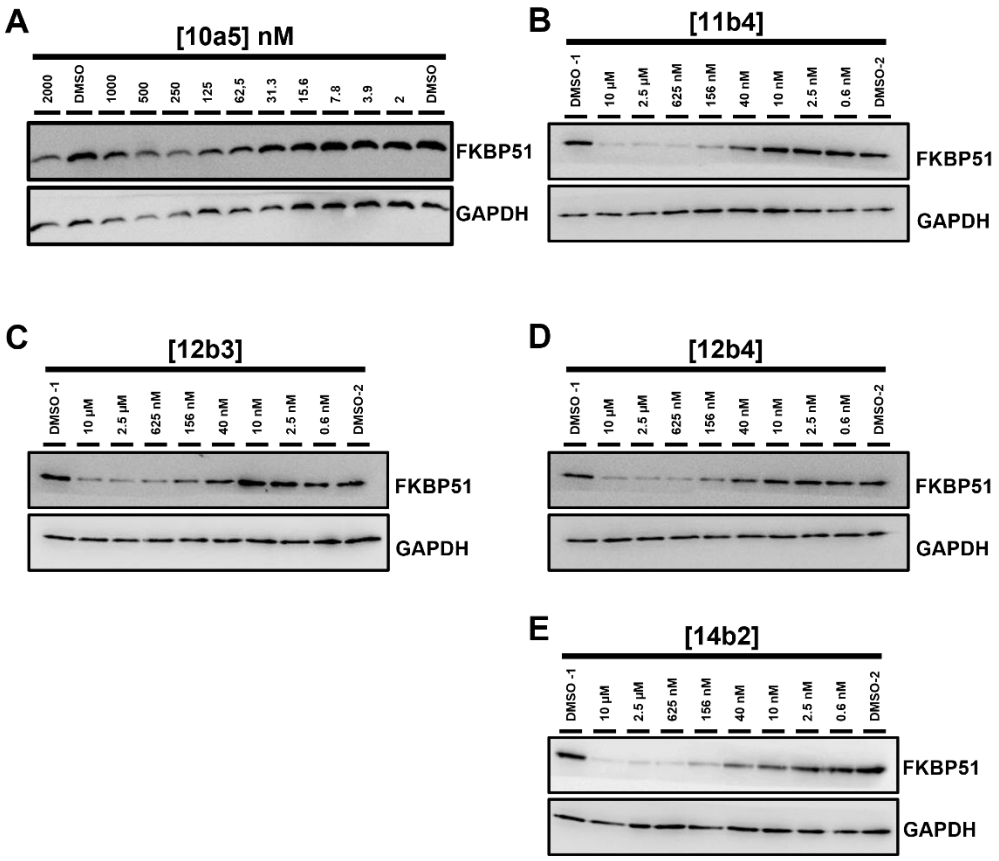


Figure S1.4 PROTAC-mediated FKBP51 degradation in HEK293 T cells after 24 h treatment. Uncropped Western blot images are depicted in Fig. S5.4.

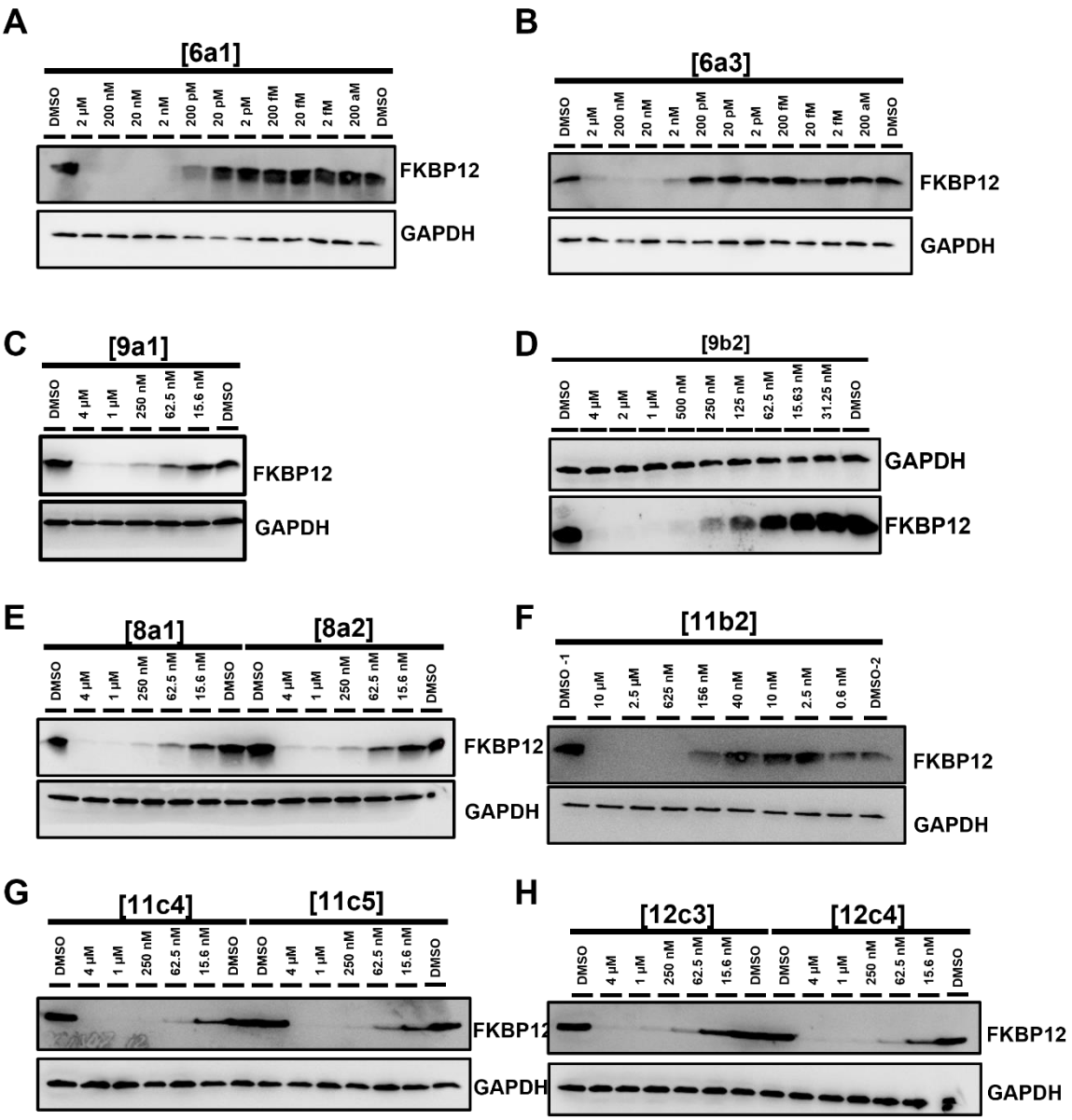
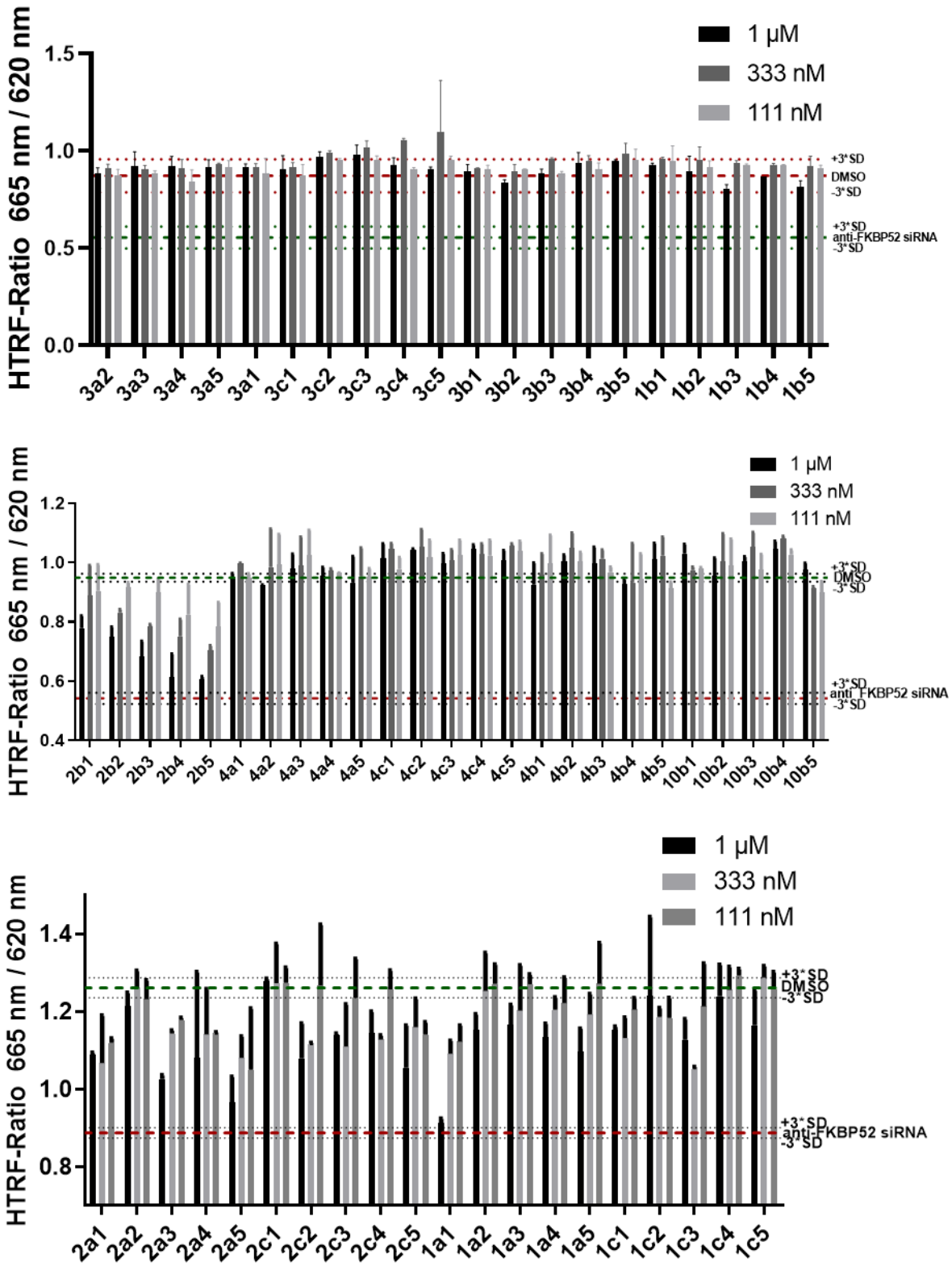


Figure S1.5 PROTAC mediated FKBP12 degradation in HEK293 T cells after 24 h treatment. Uncropped Western Blot images are depicted in Fig. S5.5.


HTRF-Ratio 665 nm / 620 nm
1 µM
333 nM
111 nM
+3*SD
DMSO
-3*SD
+3*SD
anti-FKBP52 siRNA
-3*SD
3a2 3a3 3a4 3a5 3a1 3c1 3c2 3c3 3c4 3c5 3b1 3b2 3b3 3b4 3b5 1b1 1b2 1b3 1b4 1b5
2b1 2b2 2b3 2b4 2b5 4a1 4a2 4a3 4a4 4a5 4c1 4c2 4c3 4c4 4c5 4b1 4b2 4b3 4b4 4b5 10b1 10b2 10b3 10b4 10b5
2a1 2a2 2a3 2a4 2a5 2c1 2c2 2c3 2c4 2c5 1a1 1a2 1a3 1a4 1a5 1c1 1c2 1c3 1c4 1c5

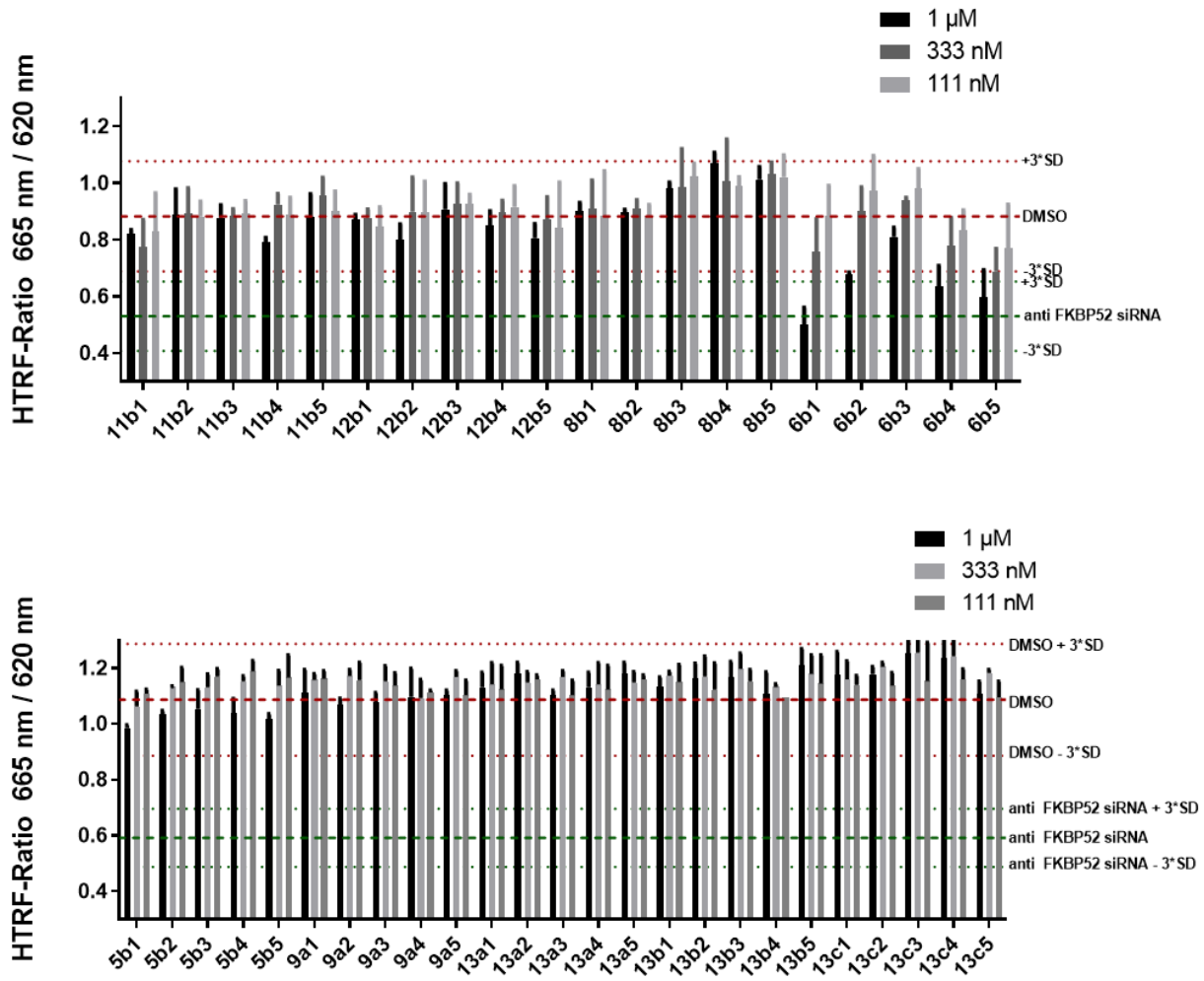
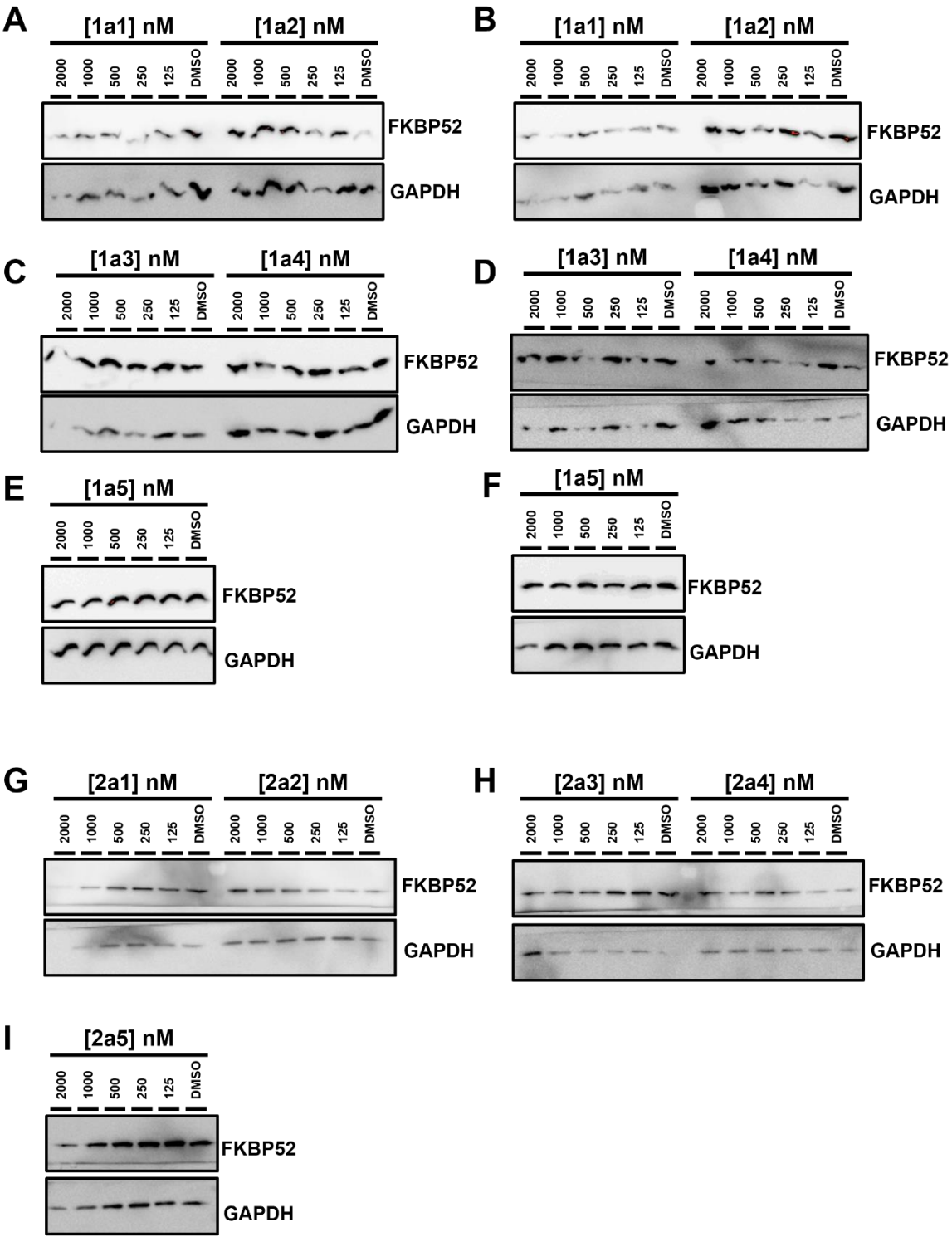


Figure S1.6 Relative HTRF-based quantification of endogenous FKBP52 levels after 24 h PROTAC treatment in HEK293T cell lysates. A homogeneous time-resolved FRET is observed between the fluorescent FKBP52-HTRF tracer (120 nM) Anti Rabbit IgG-Eu cryptate (1,2 nM) in combination with a primary anti FKBP52 antibody (1,25 nM) in the presence of FKBP52. HTRF-ratios in range of siRNA positive control indicate lower FKBP52 samples in the treated samples compared to the DMSO control and are indicative of active PROTACs. Bars and error bars represent mean and standard deviation of biological duplicates.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1000
500
250
125
DMSO
FKBP52
GAPDH
B
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GAPDH
C
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GAPDH
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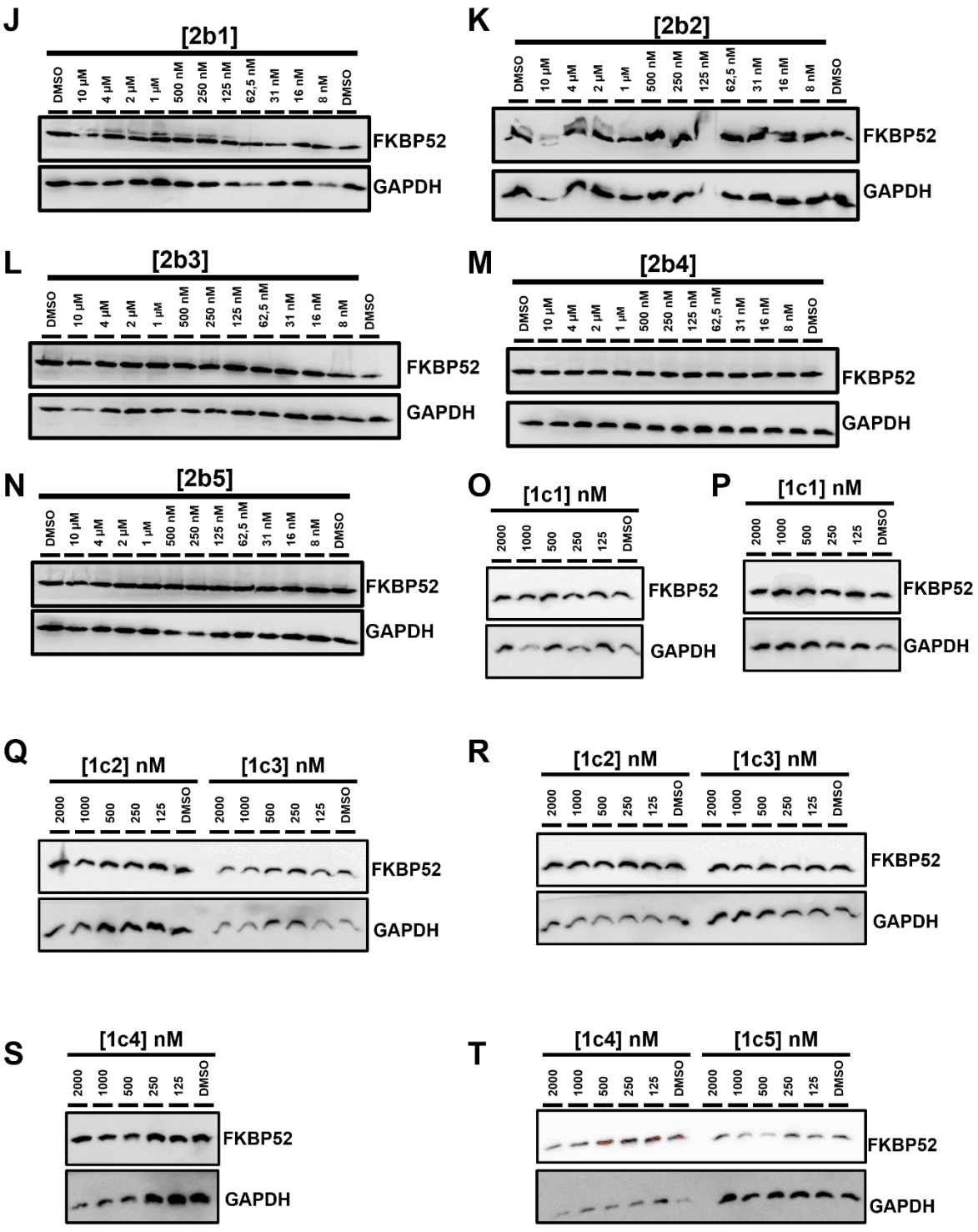
GAPDH


J
[2b1]
DMSO
10 µM
4 µM
2 µM
1 µM
500 nM
250 nM
125 nM
62,5 nM
31 nM
16 nM
8 nM
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FKBP52
GAPDH
K
[2b2]
L
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M
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N
[2b5]
O
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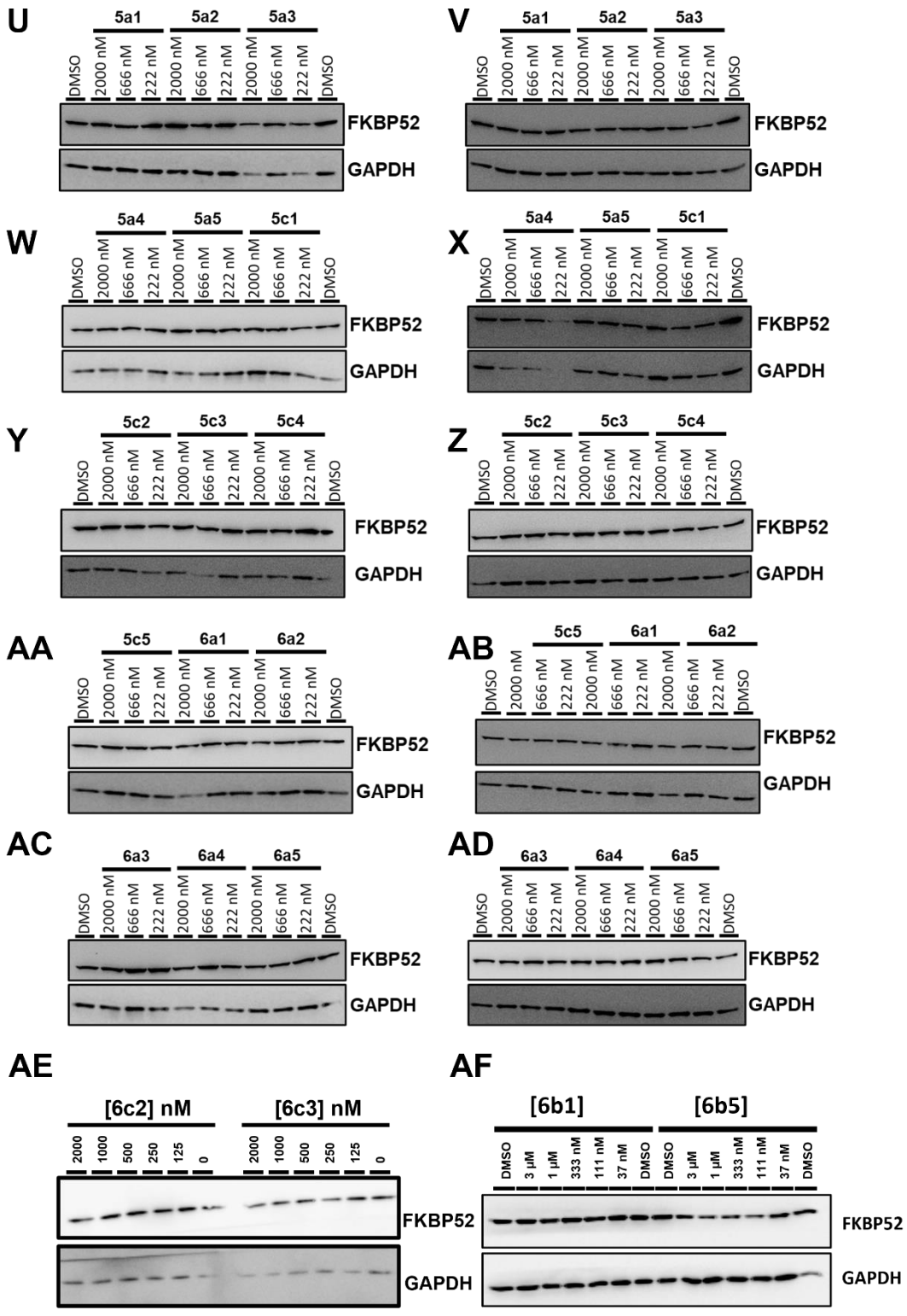
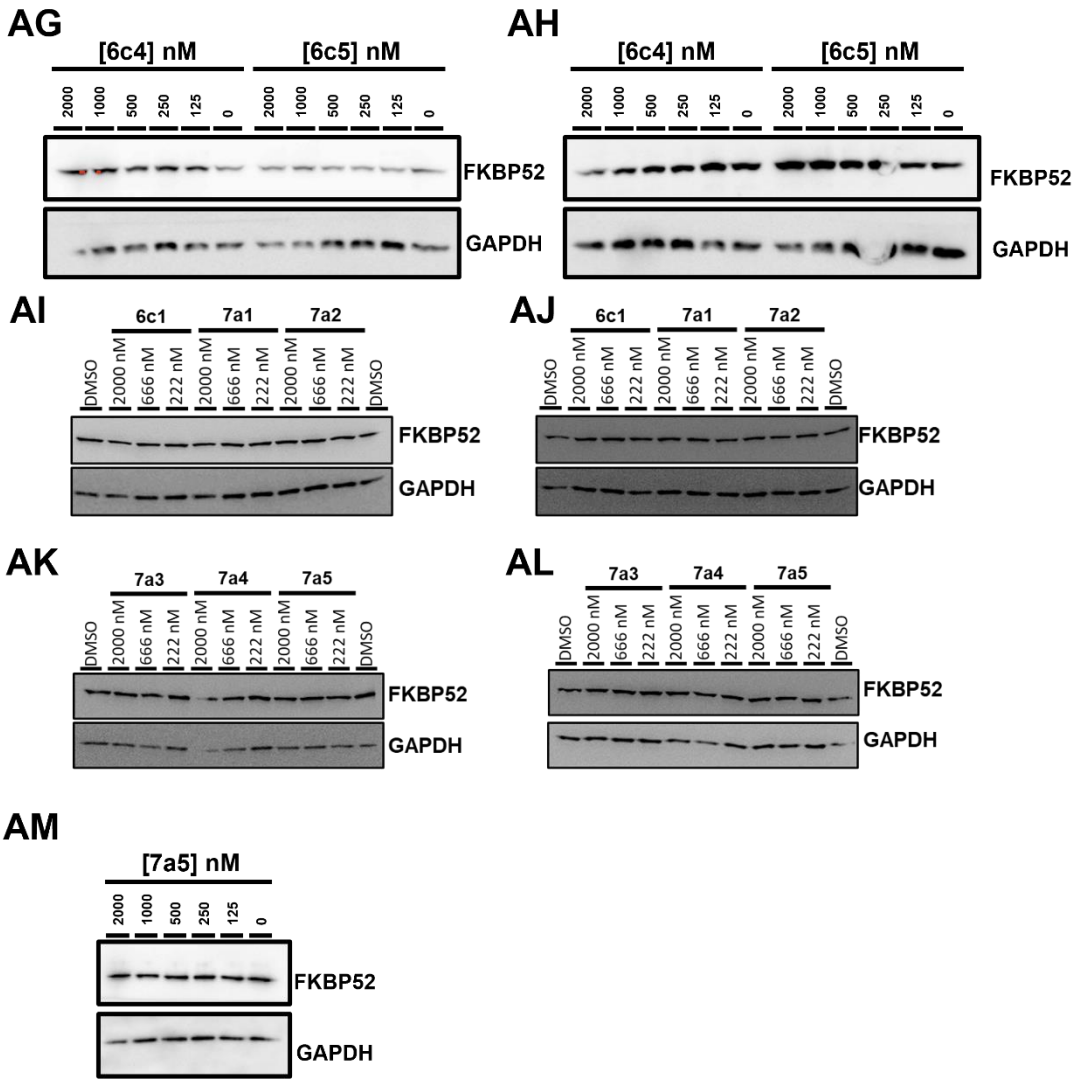
2000
1000
500
250
125
DMSO
P
Q
[1c2] nM
[1c3] nM
R
S
[1c4] nM
T
[1c5] nM


U
5a1
5a2
5a3
DMSO
2000 nM
666 nM
222 nM
FKBP52
GAPDH
V
W
5a4
5a5
5c1
X
Y
5c2
5c3
5c4
Z
AA
5c5
6a1
6a2
AB
AC
6a3
6a4
6a5
AD
AE
[6c2] nM
[6c3] nM
2000
1000
500
250
125
0
AF
[6b1]
[6b5]
3 µM
1 µM
333 nM
111 nM
37 nM


AG
[6c4] nM
[6c5] nM
2000
1000
500
250
125
0
FKBP52
GAPDH
AH
AI
6c1
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2000 nM
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AJ
AK
7a3
7a4
7a5
AL
AM
[7a5] nM

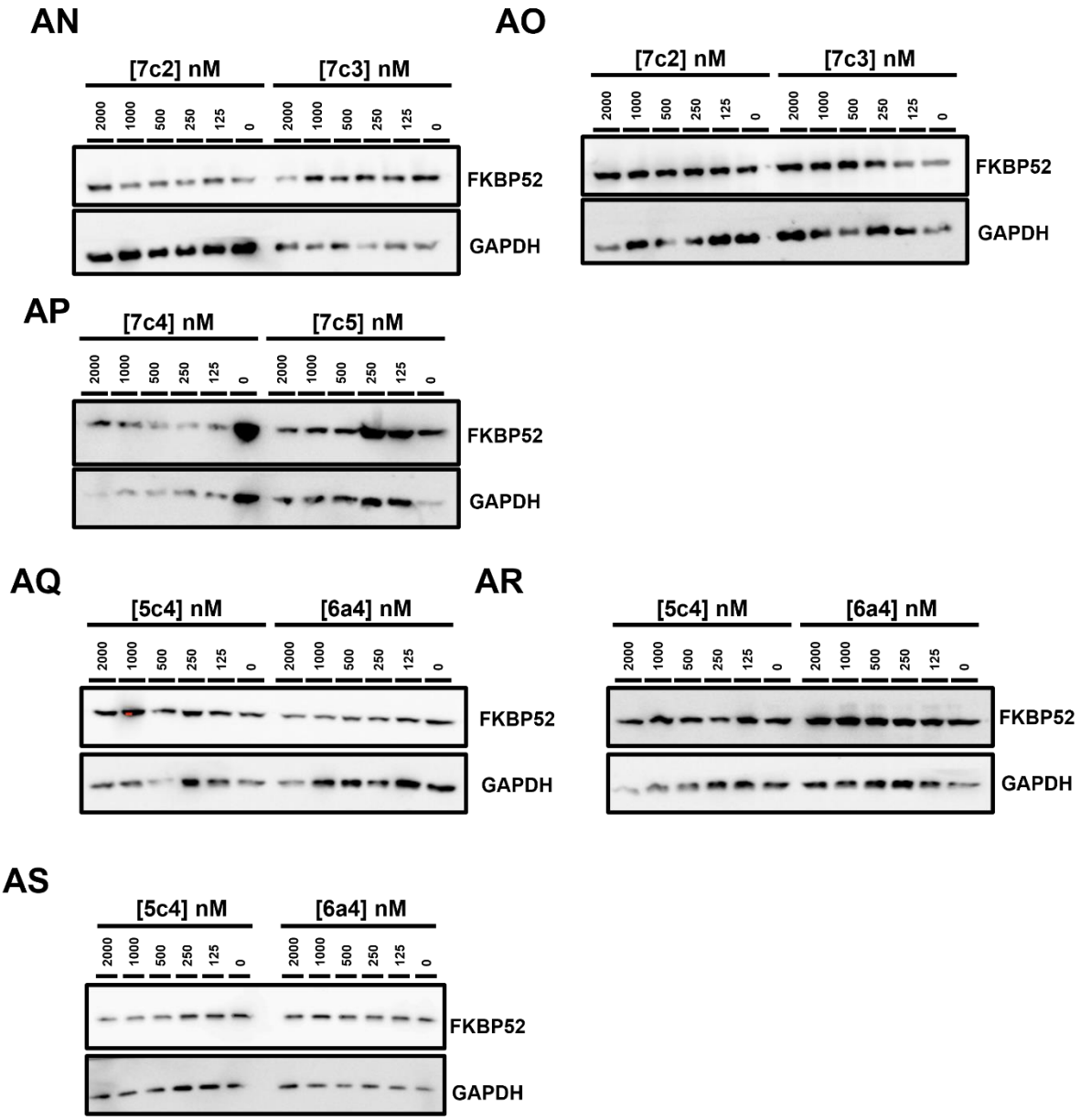


Figure S1.7 PROTAC mediated FKBP52 degradation in HEK293 T cells after 24 h treatment. Uncropped Western Blot images are depicted in Fig. S*5.6*.

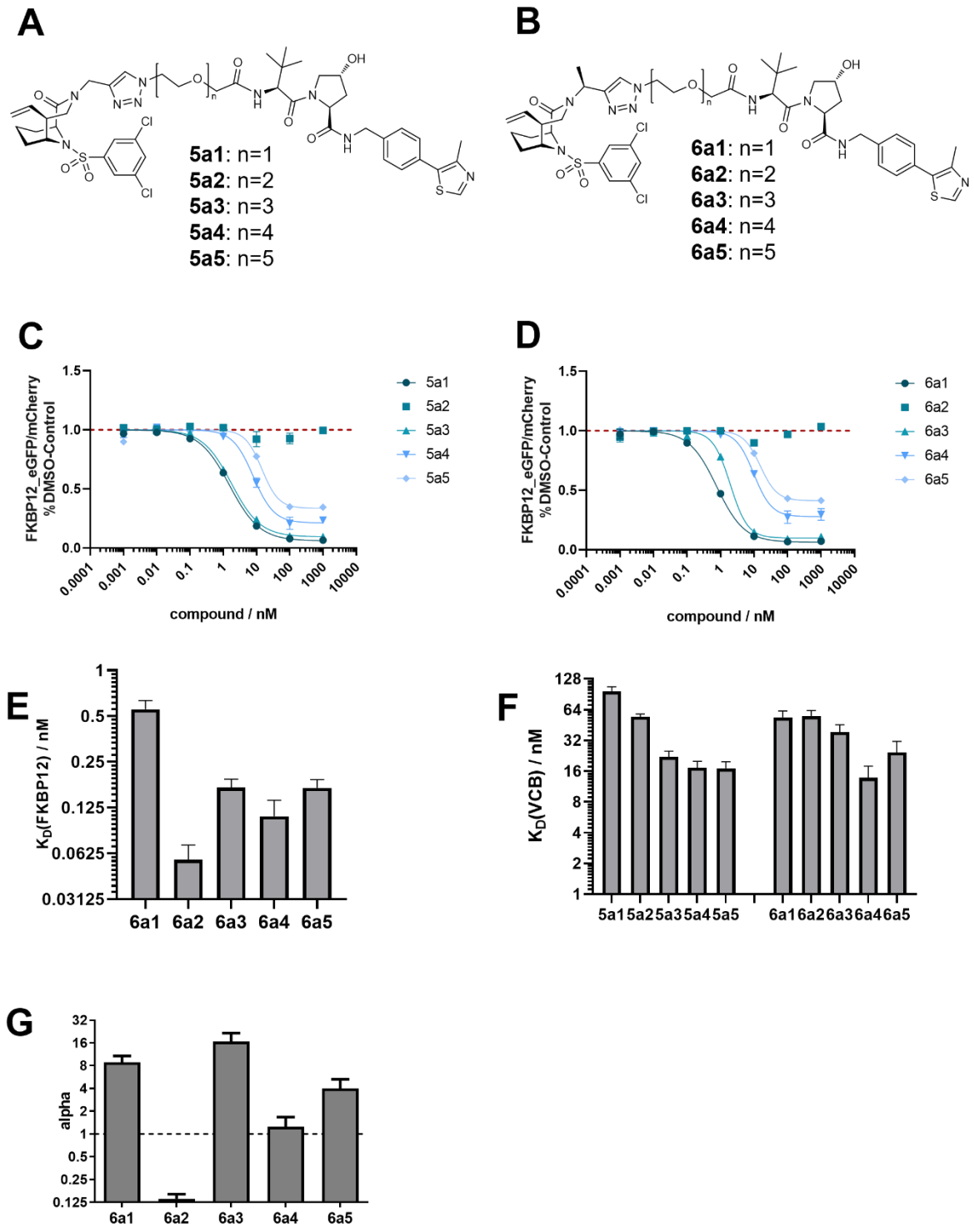


Figure S2.1 Degradation and affinity profile of 5a1-5a5 and 6a1-6a5. Chemical structures of A) 5a1-5a5 and B) 6a1-6a5. FKBP12_eGFP reporter degradation mediated by C) 5a1-5a5 and D) 6a1-6a5 after 48 hours treatment. Symbols and error bars represent mean and standard deviation of biological duplicates. E) FKBP12 binding affinities of 6a1-6a5 determined by competitive FP assays using a FKBP-FP tracer. Bars and error bars represent mean and standard deviation of duplicates. F) VCB binding affinities of 5a1-5a5 and 6a1-6a5 determined by competitive FP assays using a VHL-FP tracer. Bars and error bars represent mean and standard deviation of triplicates. G) Cooperativity (alpha) of 6a1-6a5 for binding to VCB.

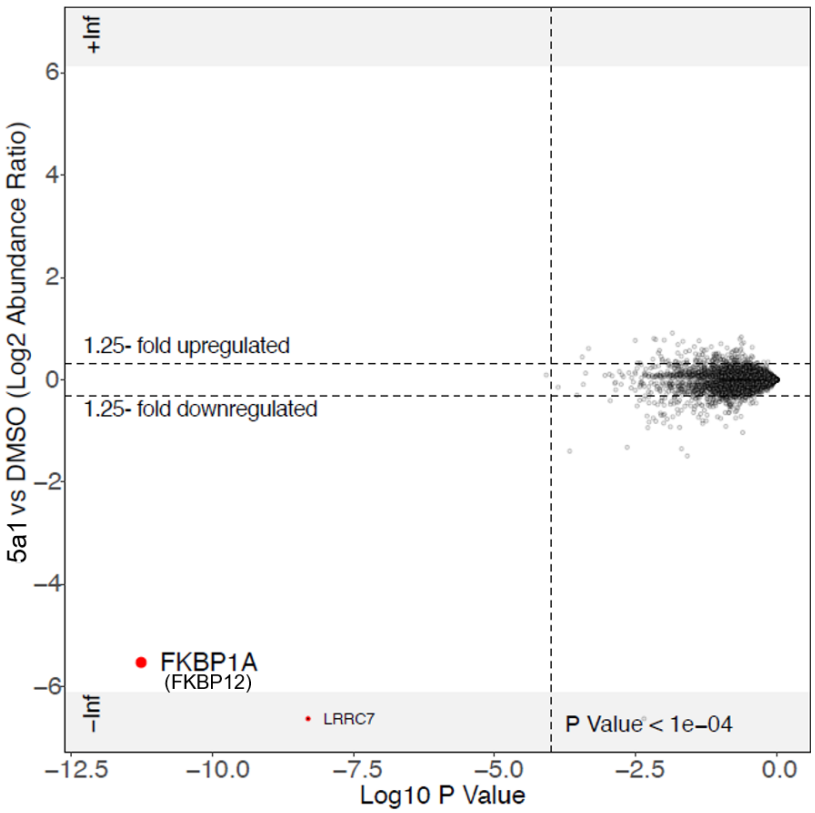


Figure S2.2 Label free quantitative proteomics of MOLT-4 cell lysates after treatment (5 h) with 5a1 (1 μM). FKBP12 (FKBP1A) is selectively degraded. +/- inf box (grey) contains proteins that were below detection level in all replicates of a specific treatment group.

Table S1 Binding affinities and cooperativities of PROTAC serias 5a1 and 6a binding to VCB. Binding constants in nM ± standard deviation from three replicates. The binding affinities for the PROTACs were determined in a competitive FP assay using a VCB-FP tracer either with the PROTAC alone or in presence of an excess (3 μM) of FKBP12.

PROTAC	K_D(PROTAC) / nM	K_D(PROTAC:FKBP12) / nM	α
5a1	97±10	2.0±0.3	47±9
5a2	55±3	436±31	0.1±0.01
5a3	22±3	2.4±0.5	9±2
5a4	17±3	7±1	2.5±0.6
5a5	17±3	8.2±0.9	2.1±0.4
6a1	54±9	6.0±0.9	9±2
6a2	55±8	397±27	0.1±0.02
6a3	39±7	2.3±0.6	17±5
6a4	14±4	11±2.0	1.2±0.4
6a5	24±7	6±1	4±1

Table S2 Binding affinities and cooperativities of selected PROTACs binding to FKBP51FK1. Binding constants in nM ± standard deviation from three replicates. The binding affinities for selected PROTACs were determined in a competitive HTRF assay using a FKBP-HTRF tracer either with the PROTAC alone or in presence of an excess (5 μM) of VCB.

PROTAC	K_D(PROTAC) / nM	K_D(PROTAC:VCB) / nM	α
10a4	56±6	0.54±0.08	104
10b1	23±2	1.6±0.1	14
11b4	31±4	2.7±0.2	11
11b5	23±3	2.2±0.2	10
12b3	8.7±0.8	7.5±0.7	1
12b4	23±3	5.0±0.5	5
12b5	21±2	5.2±0.4	4
14b1	26±3	0.81±0.06	32
14b2	40±5	0.66±0.08	61
SelDeg51	18±2	0.78±0.07	23
alkyne 14	22±2	-	-

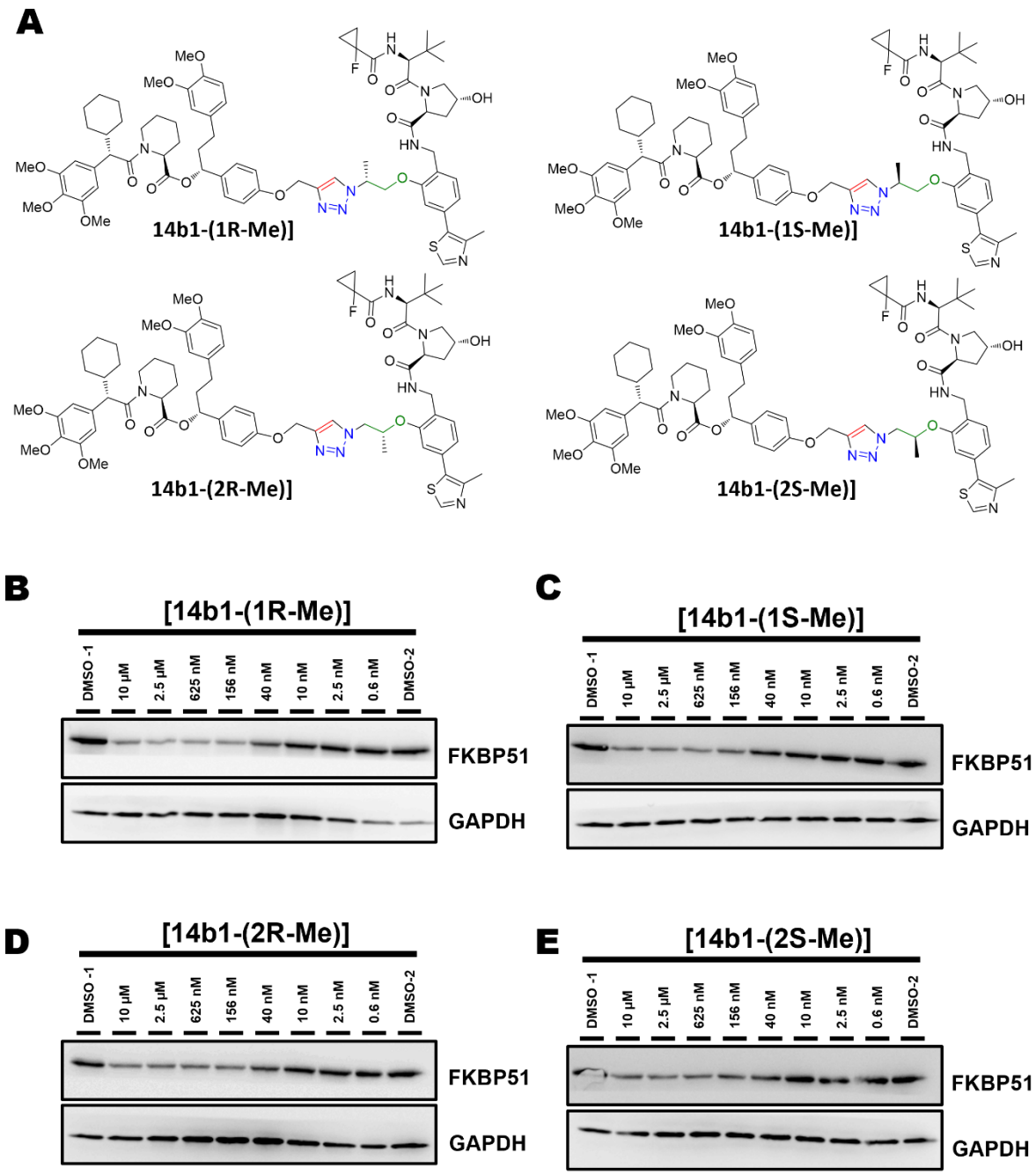


Figure S3.1 A) Chemical structures of linker-branched 14b1 analogues and B) western blot analysis of FKBP51 degradation after 24 h treatment in HEK293T cells. Uncropped Western Blot images are depicted in Fig. S5.7.

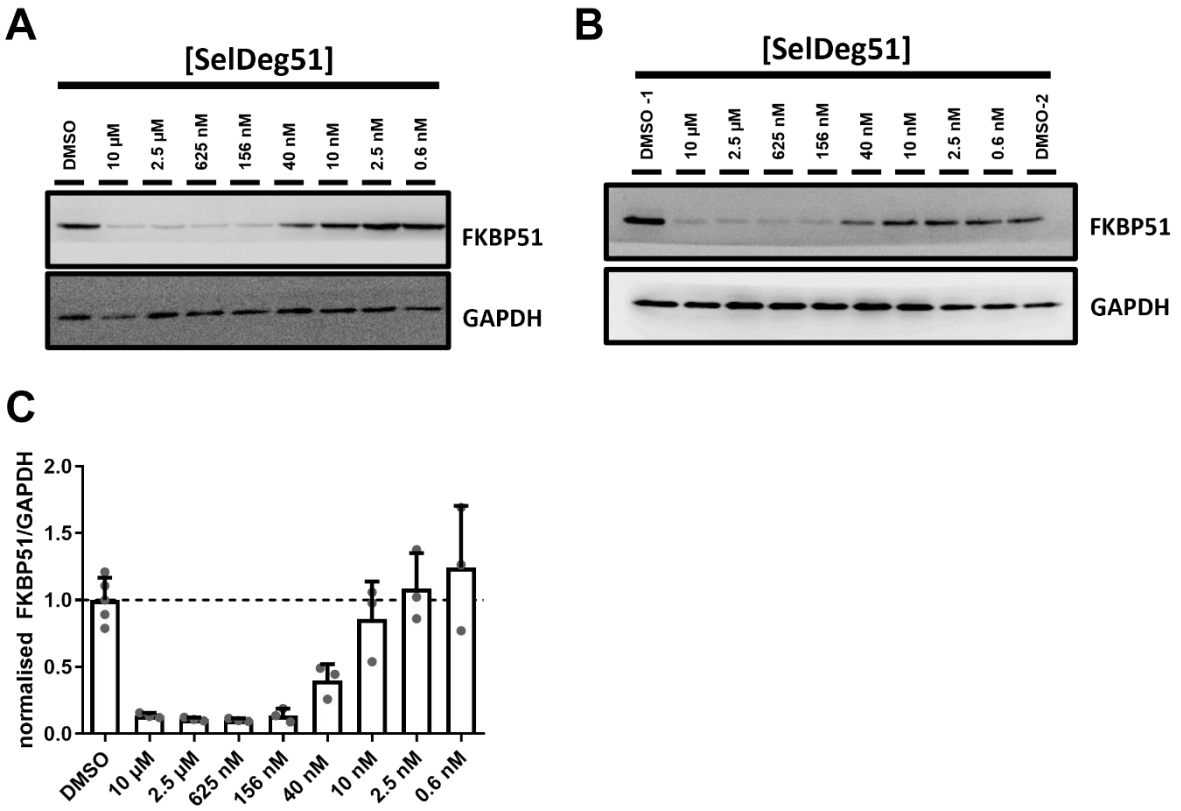
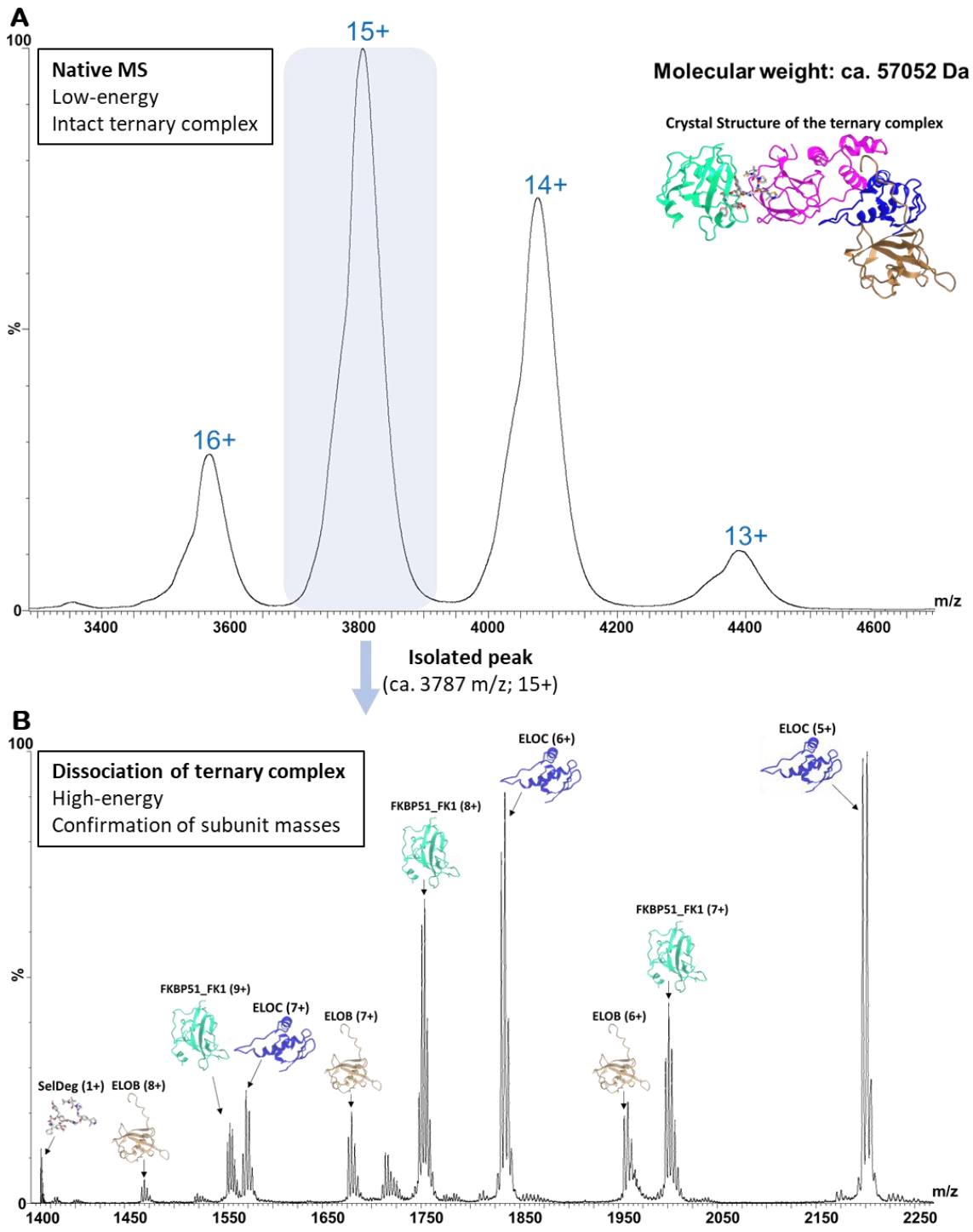


Figure S3.2 A), B) SelDeg51-mediated FKBP51 degradation after 24 h treatment. C) Quantitative analysis of the relative FKBP51 and GAPDH levels (Fig. 3B, Fig S3.2 A), B)), which are presented as ratios of FKBP51/GAPDH normalized to the DMSO-treated samples in the respective replicates. Bars and error bars represent mean and standard deviation of at least three replicates. Uncropped Western Blot images are depicted in Fig. S5.2.


A
100
Native MS
Low-energy
Intact ternary complex
15+
14+
16+
13+
Molecular weight: ca. 57052 Da
Crystal Structure of the ternary complex
%
0
3400
3600
3800
4000
4200
4400
4600
m/z
Isolated peak
(ca. 3787 m/z; 15+)
B
100
Dissociation of ternary complex
High-energy
Confirmation of subunit masses
ELOC (6+)
ELOC (5+)
FKBP51_FK1 (8+)
FKBP51_FK1 (7+)
FKBP51_FK1 (9+)
ELOC (7+)
ELOB (7+)
ELOB (6+)
SelDeg (1+)
ELOB (8+)
%
0
1400
1450
1550
1650
1750
1850
1950
2050
2150
2250
m/z

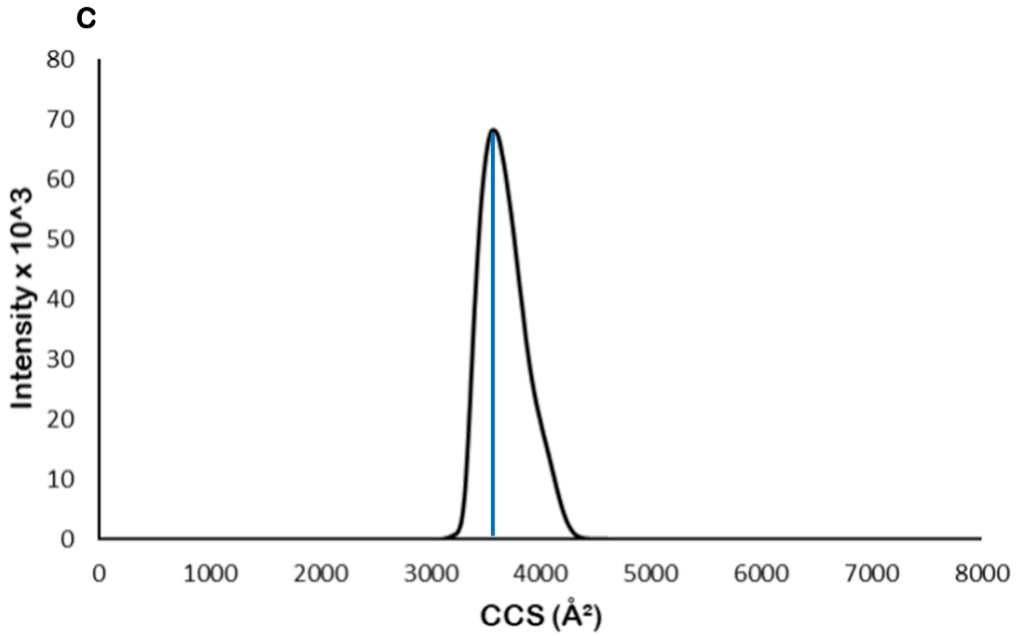


Figure S3.3 Native MS analysis of the FKBP51FK1:SelDeg51:VCB complex suggesting the presence in solution of a ternary complex similar to the obtained crystal structure. A) Native MS spectrum of the PROTAC-induced FKBP51:SelDeg51:VCB complex obtained under soft conditions to preserve the intact structure. The peaks represent the four most intense charge states of the intact ternary complex with a MW of ca. 57052 Da. Soft conditions were applied: Trap cell collision energy 10 V and Transfer cell collision energy 4 V. B) Tandem MS after isolating the most intense charge state of the FKBP51:SelDeg51:VCB complex (isolated peak highlighted in blue). Under harsh conditions (Trap cell collision energy 45 V; Transfer cell collision energy 8 V), the intact complex structure was disrupted causing ejection of the subunits. This experiment confirms that the isolated complex contained FKBP51, SelDeg51, and subunits of the VCB complex. C) Collision cross section plot generated from native MS-IM experimental data. The highest point corresponds to a CCS value of 3480 Å2. This experimentally-obtained CCS value is very close to the one derived from the crystal structure, further reinforcing the presence of the FKBP51:SelDeg51:VCB complex.

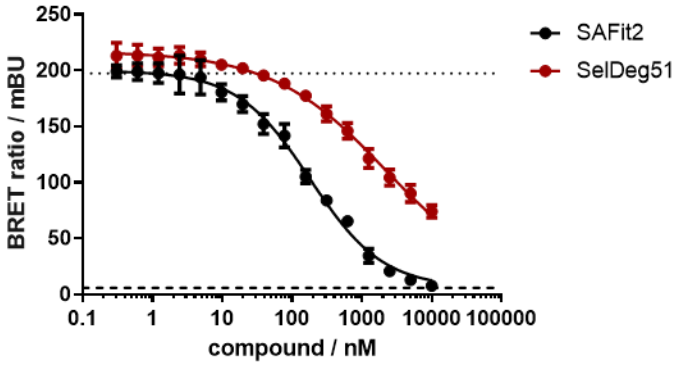


Figure S4.1 NanoBRET FKBP51 engagement assay. SelDeg51 engages FKBP51 in living HEK293 cells stably overexpressing FKBP51FK1-Nluc, although approx. ten-fold less efficient than its precursor SAFit2. Symbols and error bars represent mean and standard deviation of triplicates.

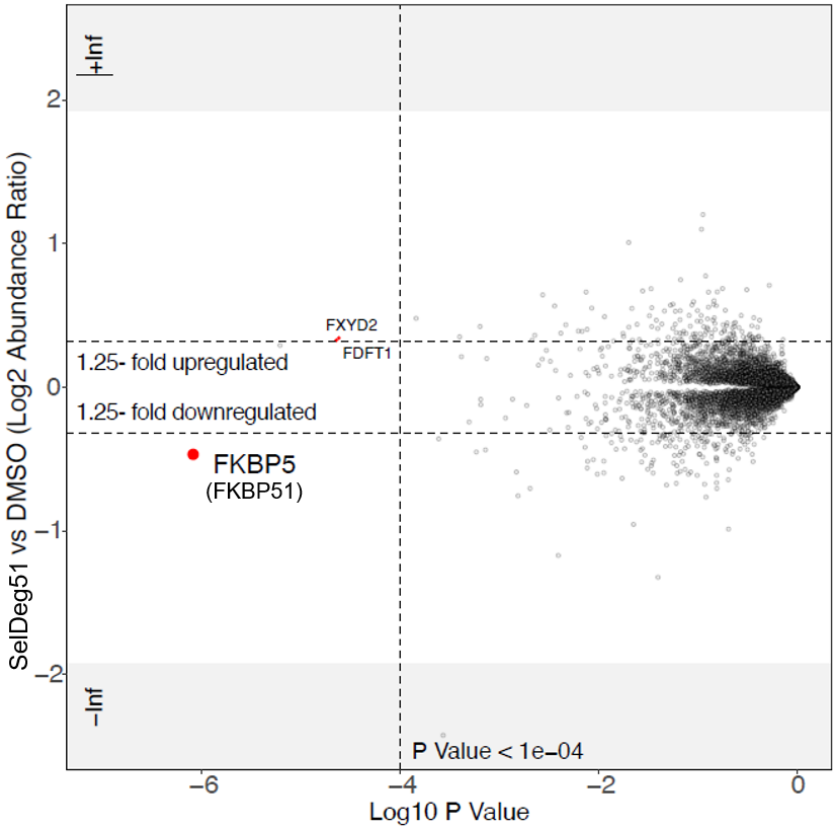


Figure S4.2 Label free quantitative proteomics of MOLT-4 cell lysates after treatment (5 h) SelDeg51 (1 µM). FKBP51 (FKBP5) is selectively degraded. +/- inf box (grey) contains proteins that were below detection level in all replicates of a specific treatment group.

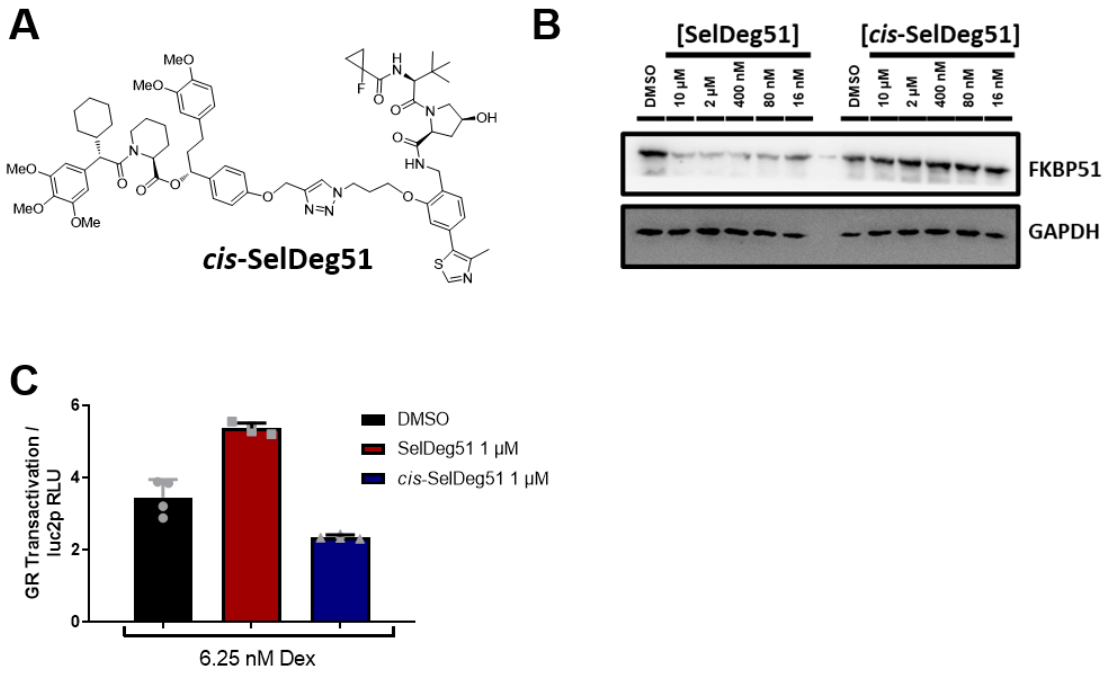


Figure S4.3 Degradation-inactive cis-SelDeg51 does not target FKBP51's scaffolding function. A) Chemical structure of cis-SelDeg51; B) SelDeg51 but not cis-SelDeg51 degrades FKBP51 in HEK293T cells, and C) reactivates GR-signalling in GR-reporter gene assays. Bars and error bars represent mean and standard deviation of biological quadruplicates. Uncropped Western Blot images are depicted in Fig. S5.8.

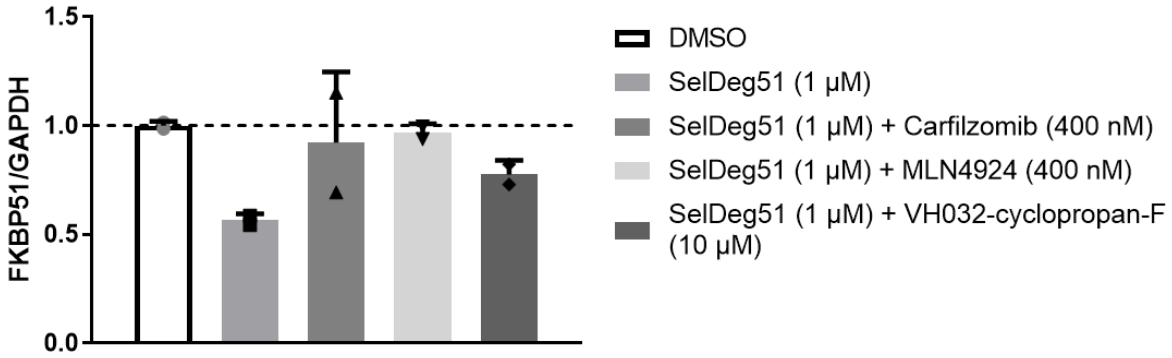


Figure S4.4 Quantitative analysis of the relative FKBP51 and GAPDH levels (Fig. 4D) which are presented as ratios of FKBP51/GAPDH normalized to the DMSO-treated samples in the respective replicates. Bars and error bars represent mean and standard deviation of two replicates.

Table S3 Refinement statistics of VCB:SelDeg51:FKBP51FK1 and FKBP12:6a2 crystal structures.

PDB entry	**8PC2 VCB:SelDeg51:FKBP51 FK1**	**8PDF FKBP12:6a2**
Data collection		
Beamline	BESSY II (BL14.1)	BESSY II (BL14.2)
Wavelength	λ = 0.9184 Å	λ = 0.9184 Å
Space group	I 1 2 1	C 1 2 1
Cell dimensions		
a, b, c (Å)	138.65, 68.42, 159.26	65.99, 36.51, 44.22
α, β, γ (°)	90, 114.45, 90	90, 102.19, 90
Resolution (Å)	48.69-2.80 (2.94-2.80)	43.22-1.20 (1.22-1.20)
R_{merge}	0.088 (0.523)	0.055 (1.318)
R_{pim}	0.070 (0.409)	0.036 (0.892)
$I/\sigma(I)$	12.0 (3.0)	11.7 (1.2)
CC1/2	0.997 (0.925)	0.999 (0.625)
Completeness (%)	99.1 (92.5)	99.9 (100)
Redundancy	4.6 (4.7)	6.3 (6.0)
Refinement		
Resolution (Å)	48.69-2.80	31.79-1.20
No. of reflections	33477	32324
R_{work}/R_{free} (%)	21.0/26.1	15.2/17.6
Total number of atoms	14339	1956
Average B, all atoms (Å^2)	79.0	21.0
R.m.s. deviations		
Bond lengths (Å)	0.0092	0.0174
Bond angles (°)	1.581	1.969
Ramachandran plot		
Favoured	862 (96%)	102 (95%)
Allowed	34 (4%)	5 (5%)
Outlier	2 (0%)	0

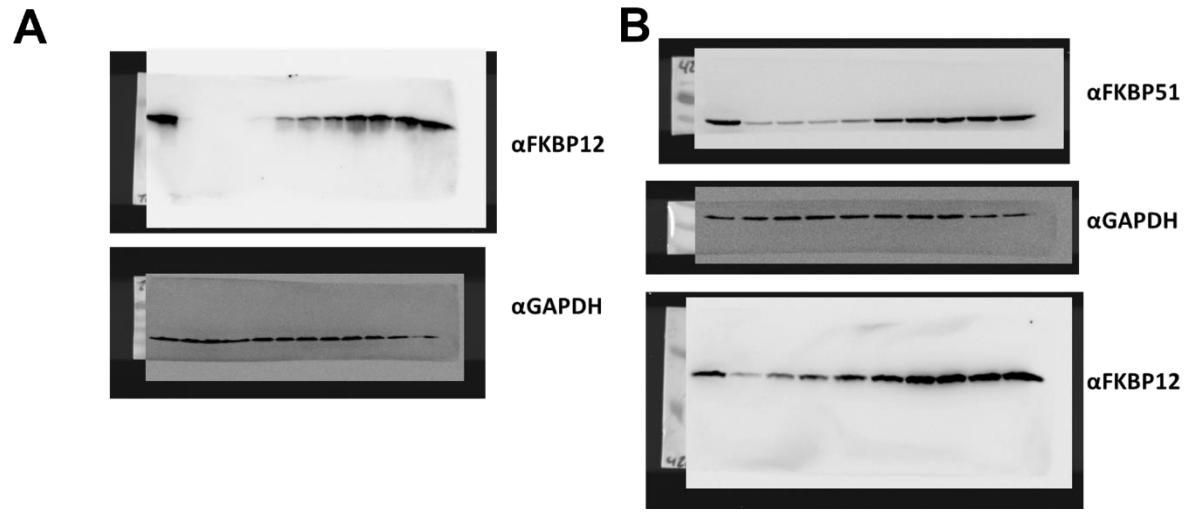


Figure S5.1 Uncropped images of Western Blots in **A** Figure 1D, **B** Figure 1E

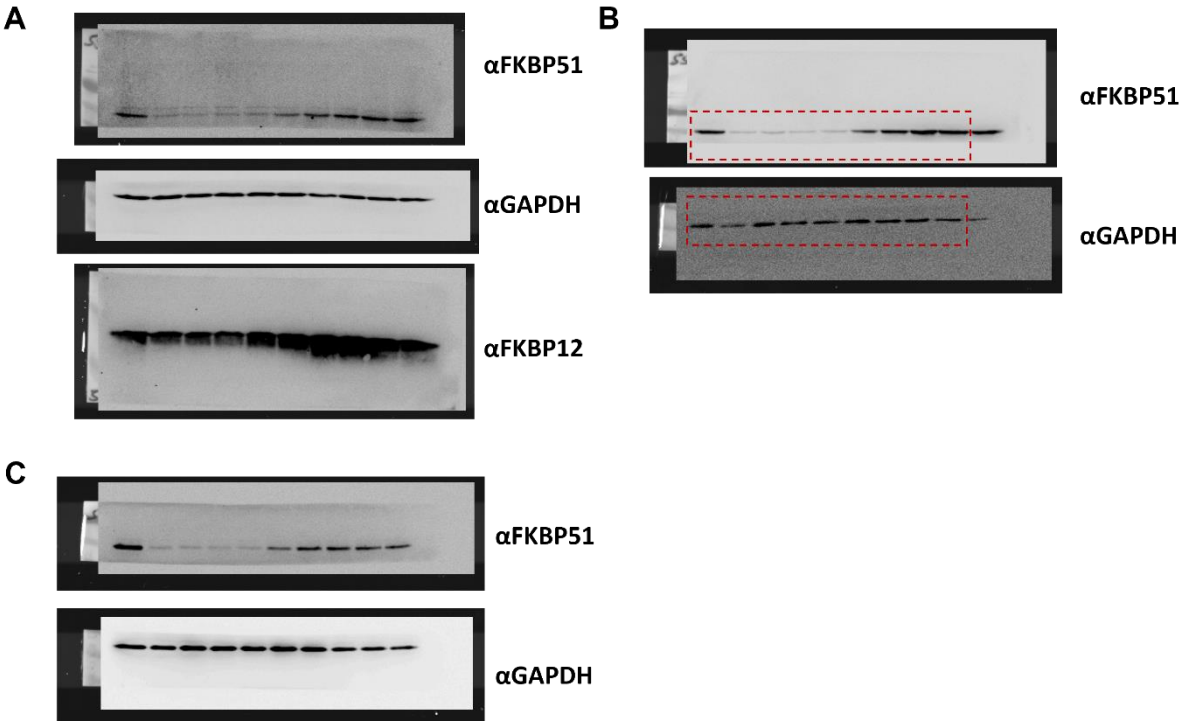


Figure S5.2 Uncropped images of Western Blots of SelDeg51-mediated FKBP51 and FKBP12 degradation after 24 h treatment. A) corresponds to Fig 3B, B) corresponds to Fig S3.2A, C) corresponds to Fig S3.2B.

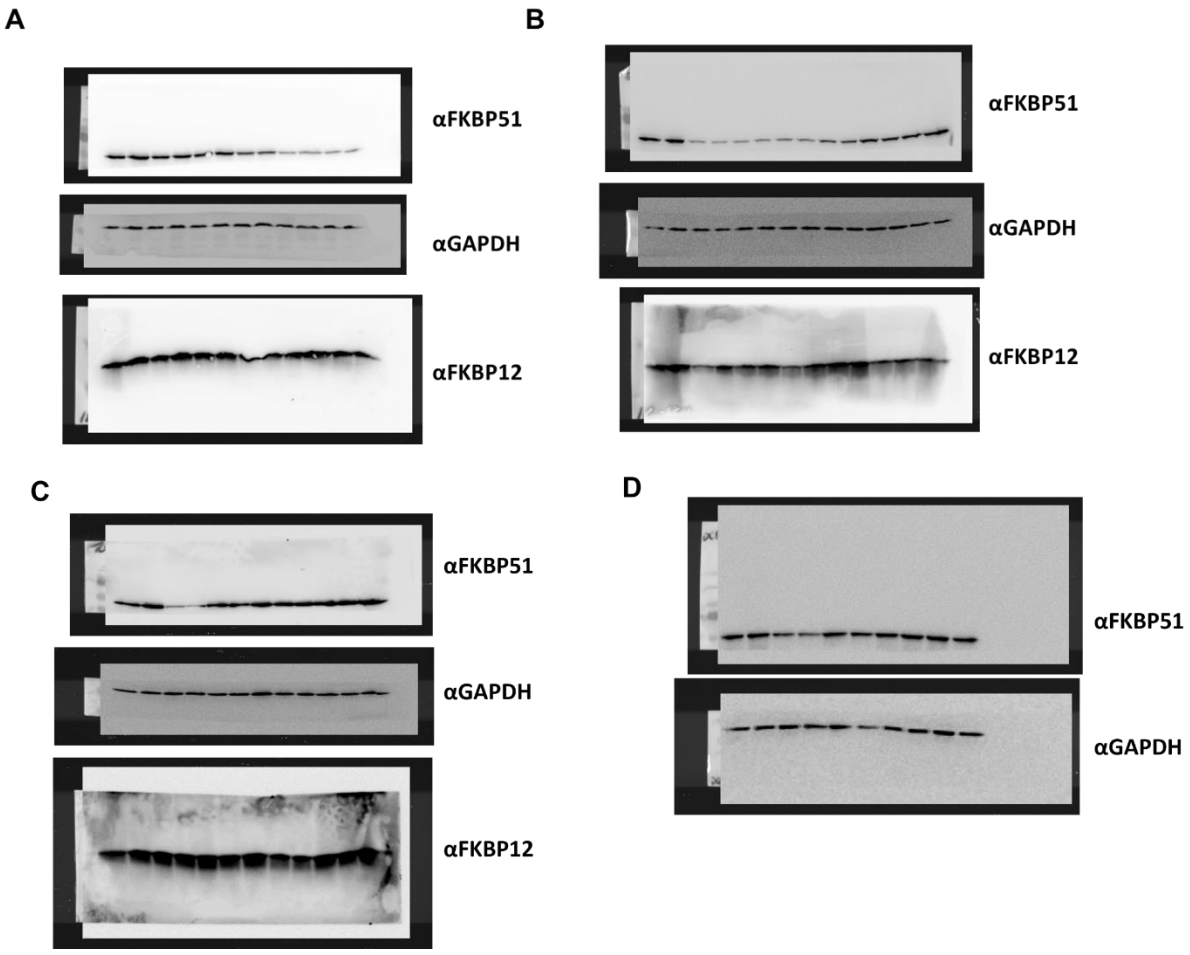


Figure S5.3 Uncropped images of Western Blots in **A** Figure 4A, **B** Figure 4B, **C** Figure 4C, and **D** Figure 4D

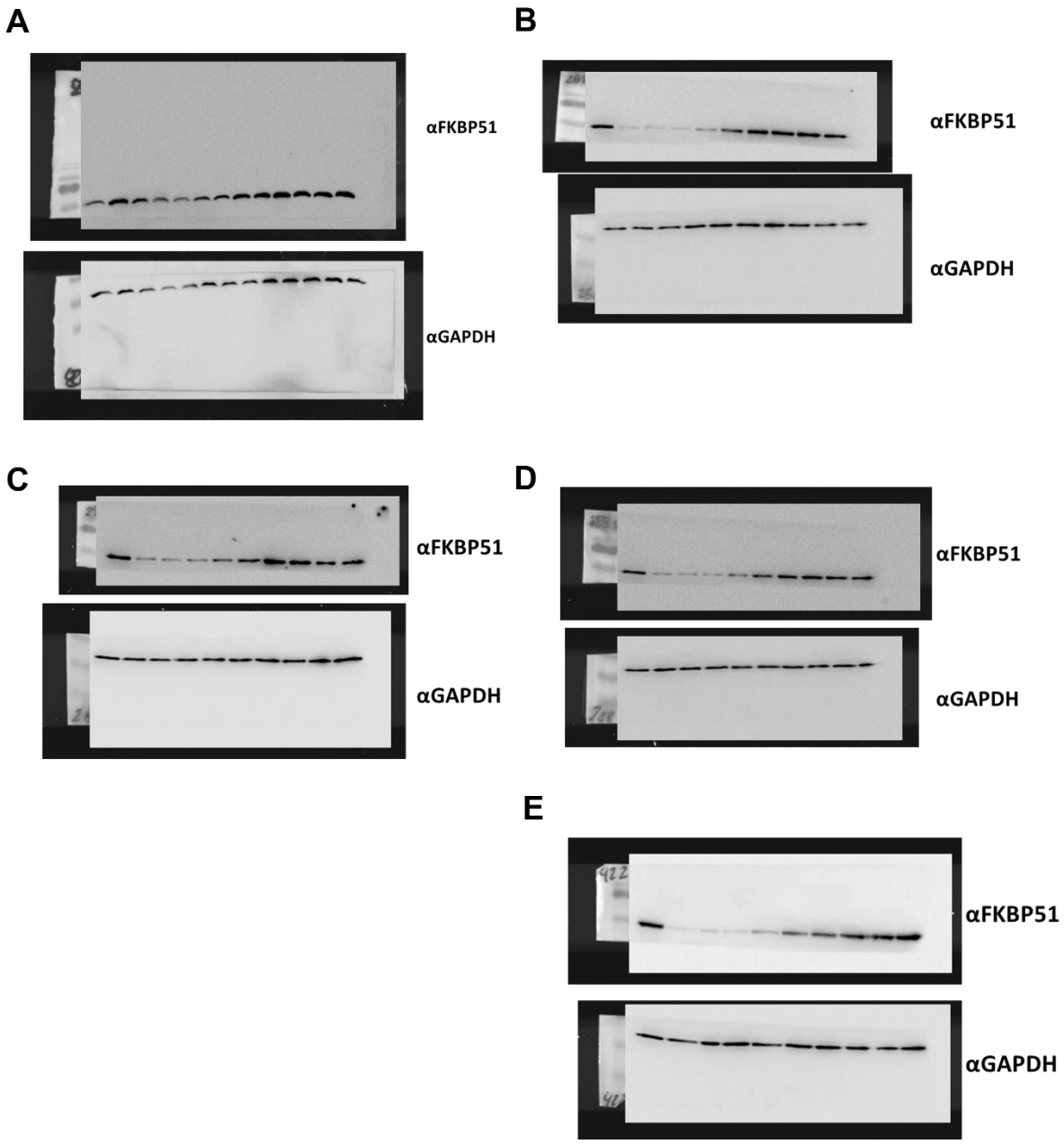


Figure S5.4 Uncropped images of Western Blots in Figure S1.4

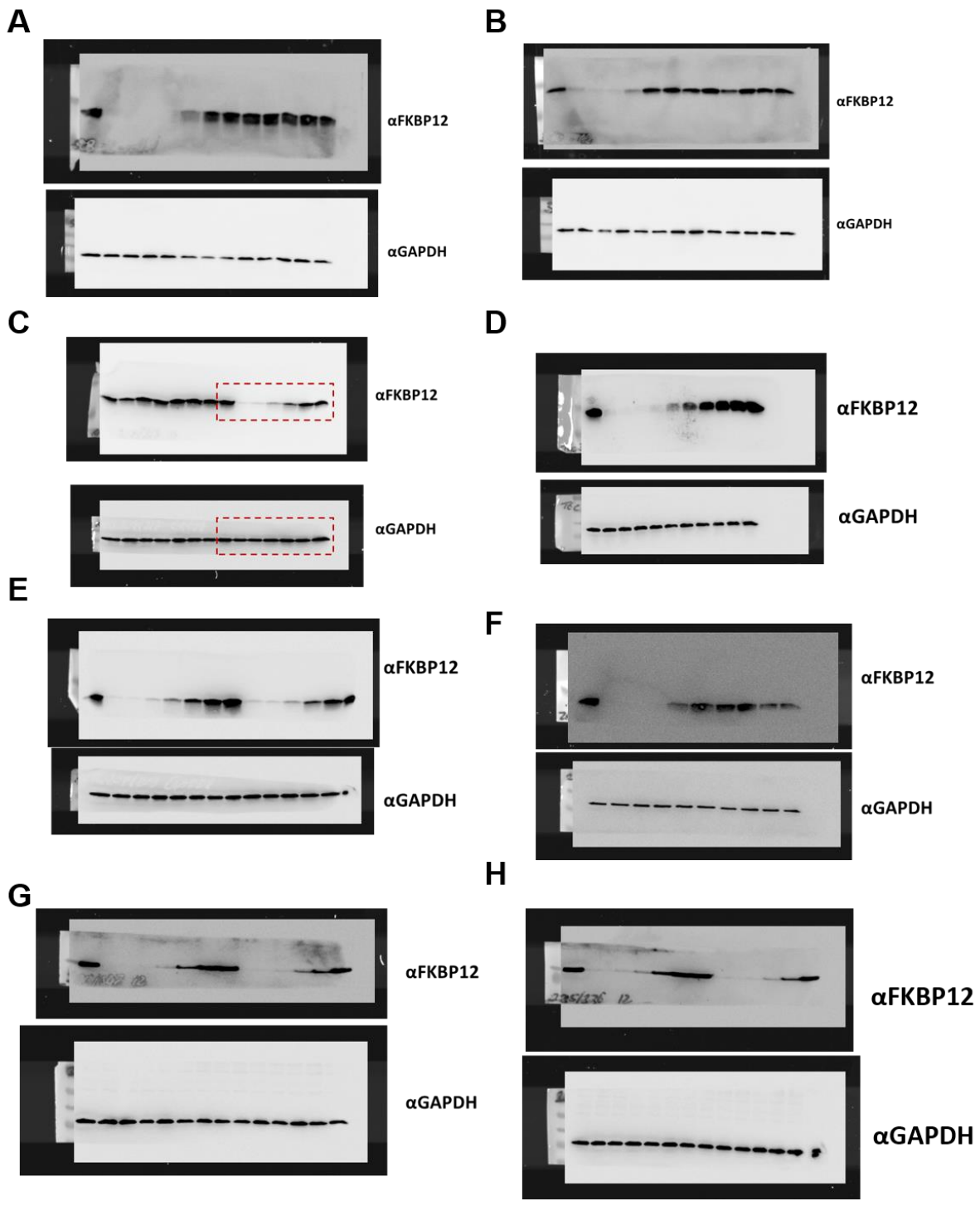
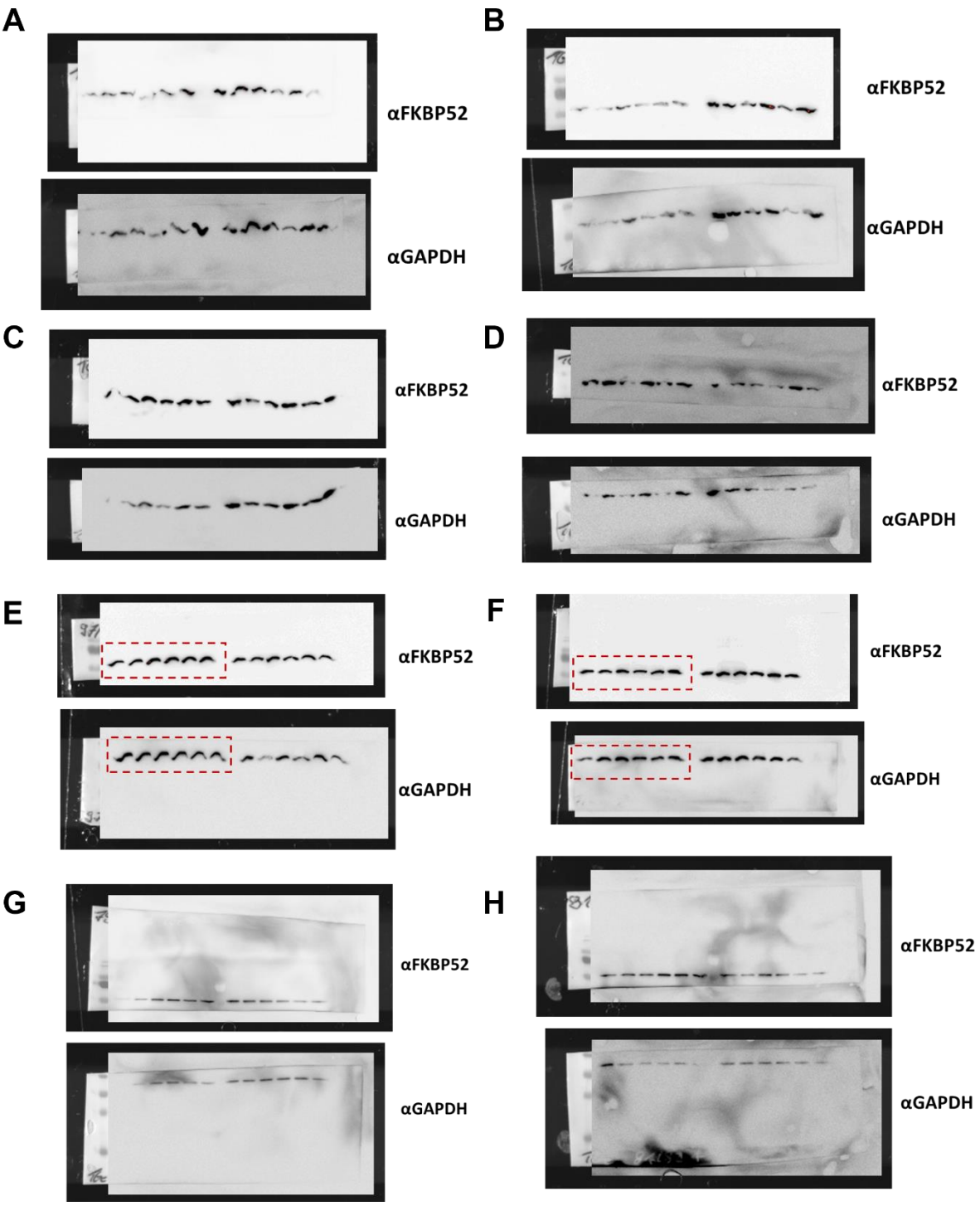
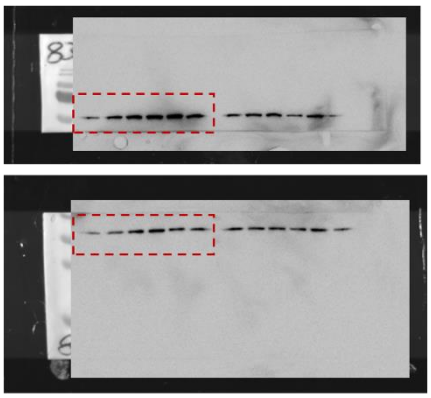


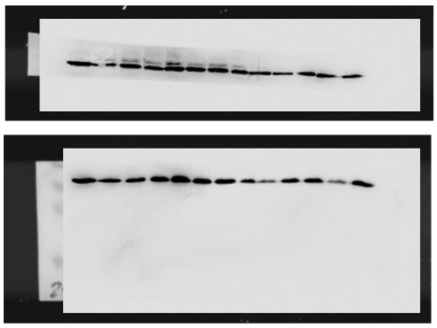
Figure S5.5 Uncropped images of Western Blots in Figure S1.5. The red dotted boxes indicate bands that are shown in Figure S1.5C.


A
αFKBP52
αGAPDH
B
αFKBP52
αGAPDH
C
αFKBP52
αGAPDH
D
αFKBP52
αGAPDH
E
αFKBP52
αGAPDH
F
αFKBP52
αGAPDH
G
αFKBP52
αGAPDH
H
αFKBP52
αGAPDH

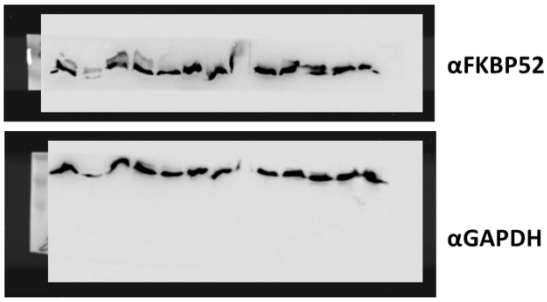
I



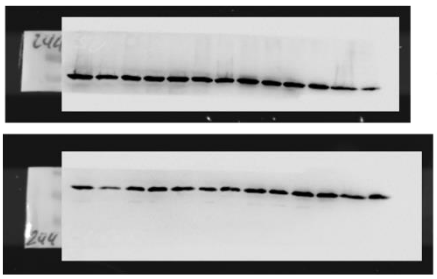
J


αFKBP52
αGAPDH

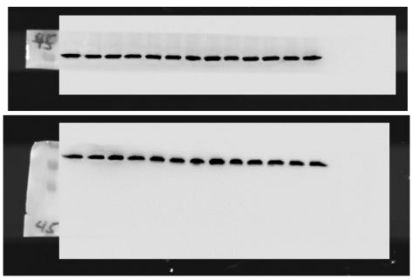
K


αFKBP52
αGAPDH

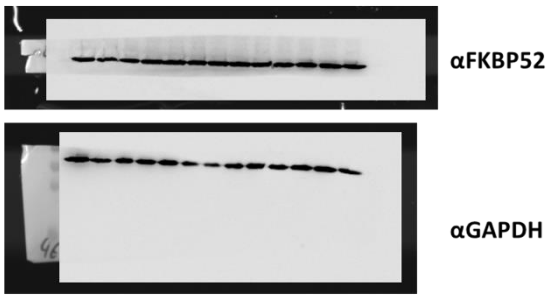
L


αFKBP52
αGAPDH

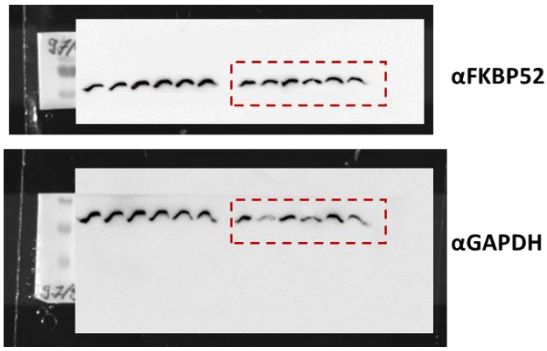
M


αFKBP52
αGAPDH

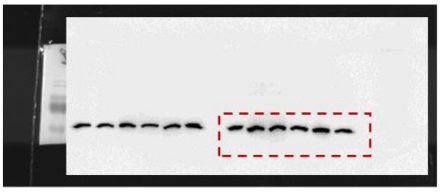
N


αFKBP52
αGAPDH

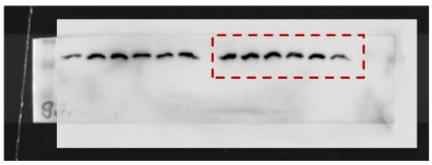
O


αFKBP52
αGAPDH

P

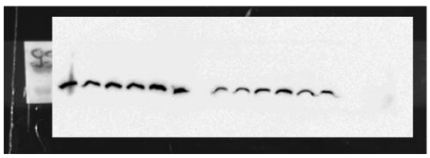


αFKBP52

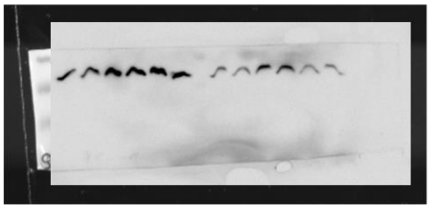


αGAPDH

Q

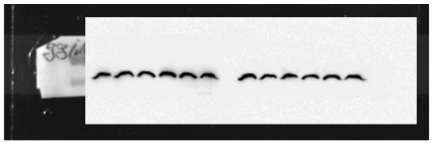


αFKBP52

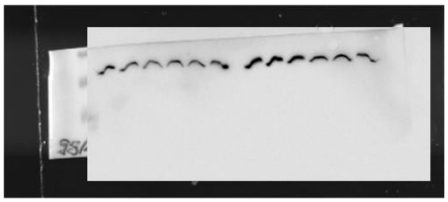


αGAPDH

R

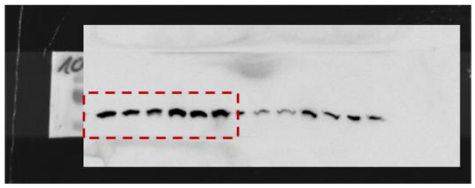


αFKBP52

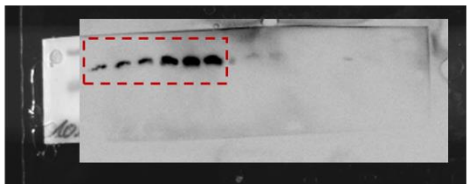


αGAPDH

S



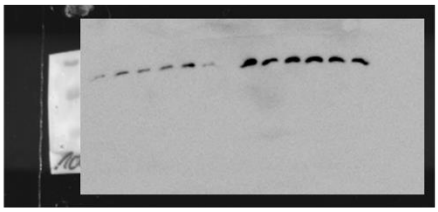
αFKBP52



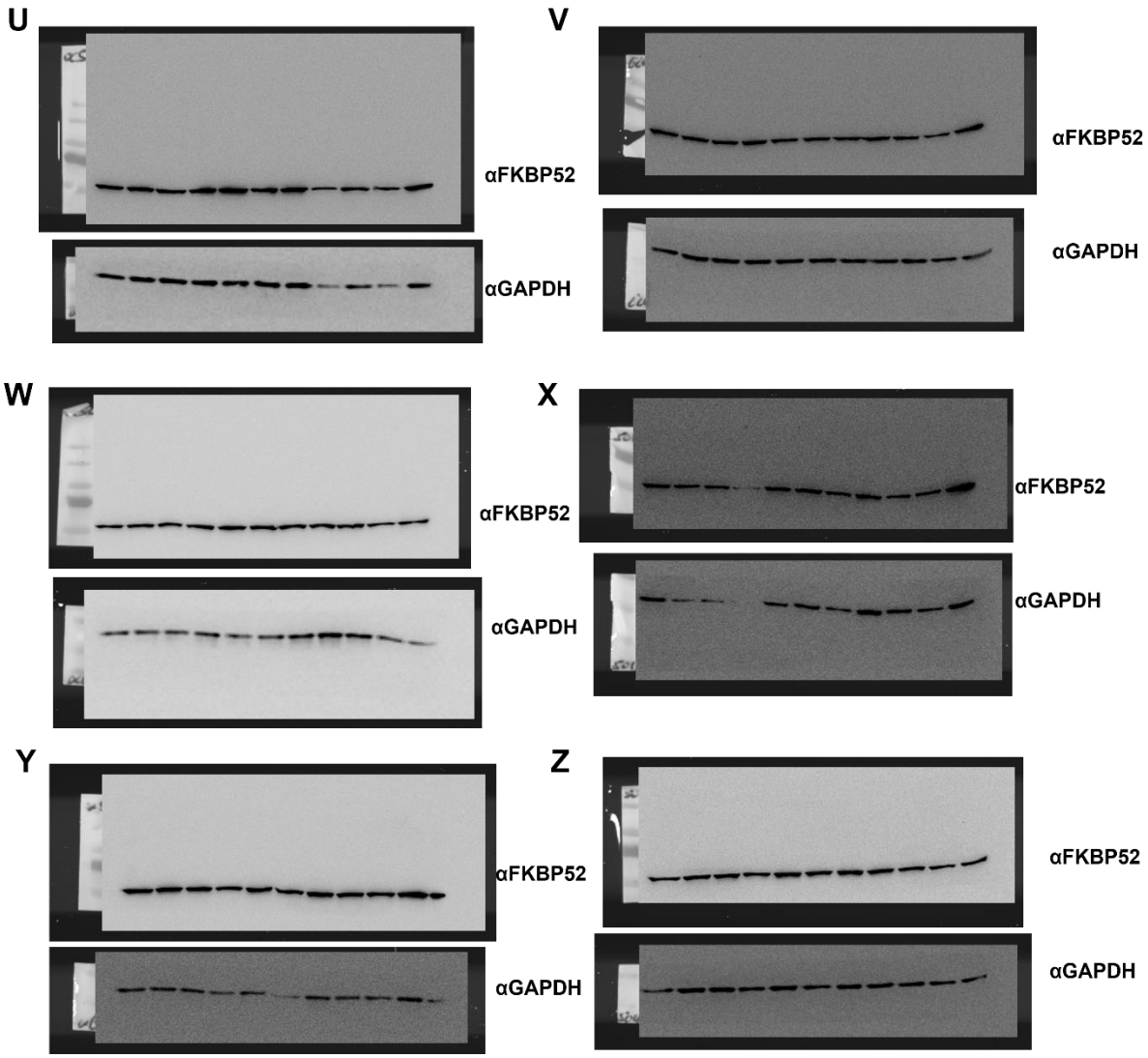
αGAPDH

T

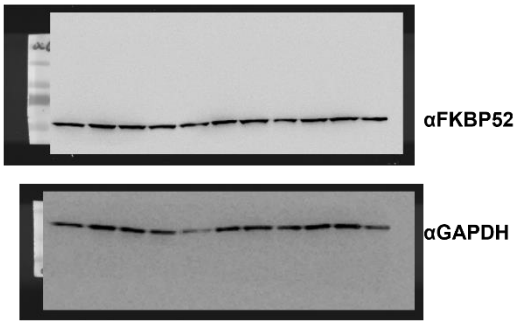
αFKBP52



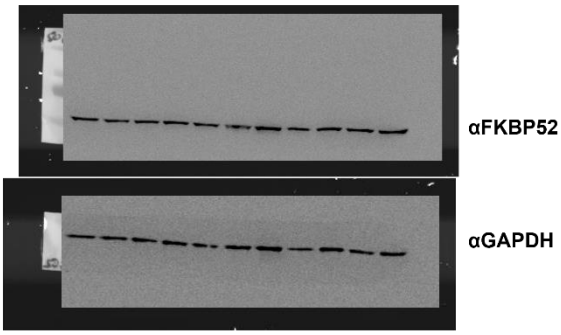
αGAPDH


U
αFKBP52
αGAPDH
V
αFKBP52
αGAPDH
W
αFKBP52
αGAPDH
X
αFKBP52
αGAPDH
Y
αFKBP52
αGAPDH
Z
αFKBP52
αGAPDH

AA



AB



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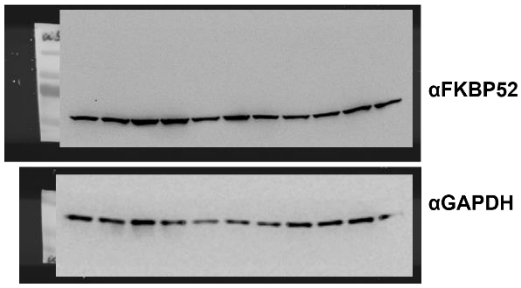

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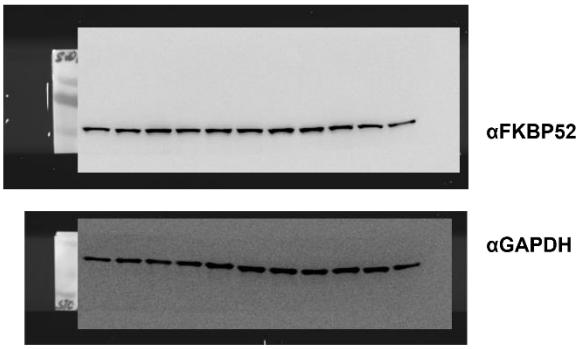

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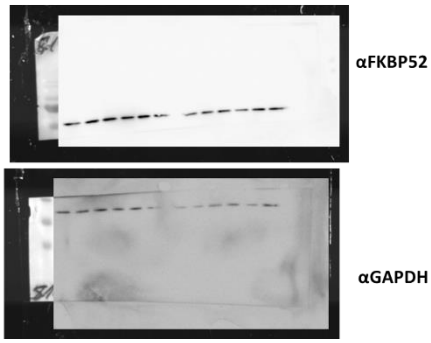
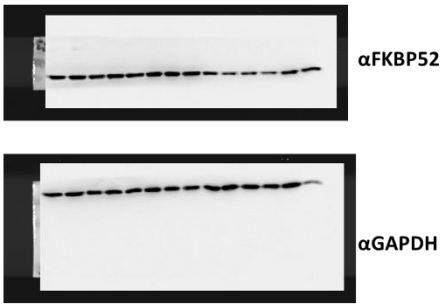
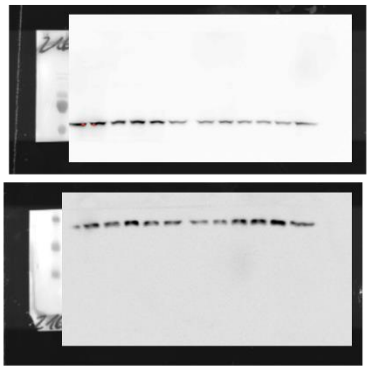


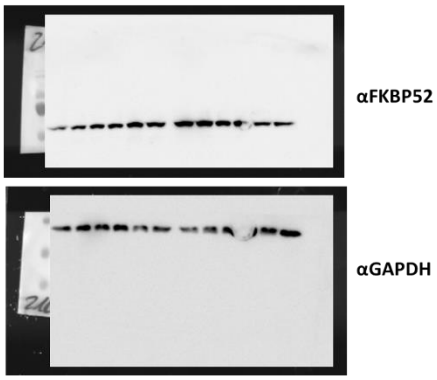
AF



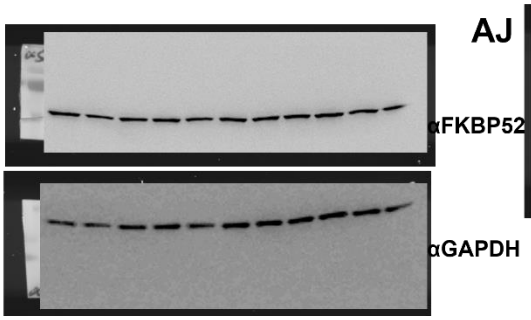
AG



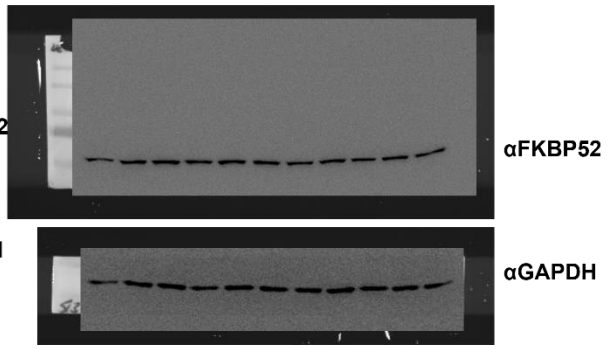
AH



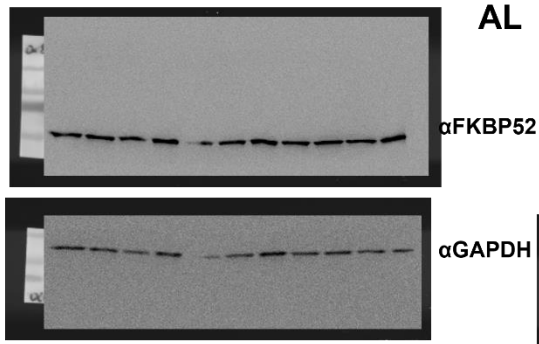
AI



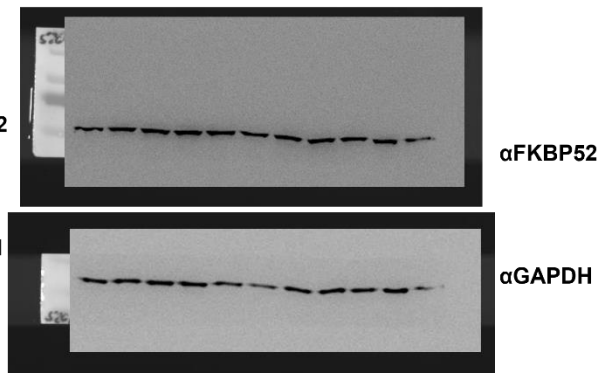
AJ



AK



AL



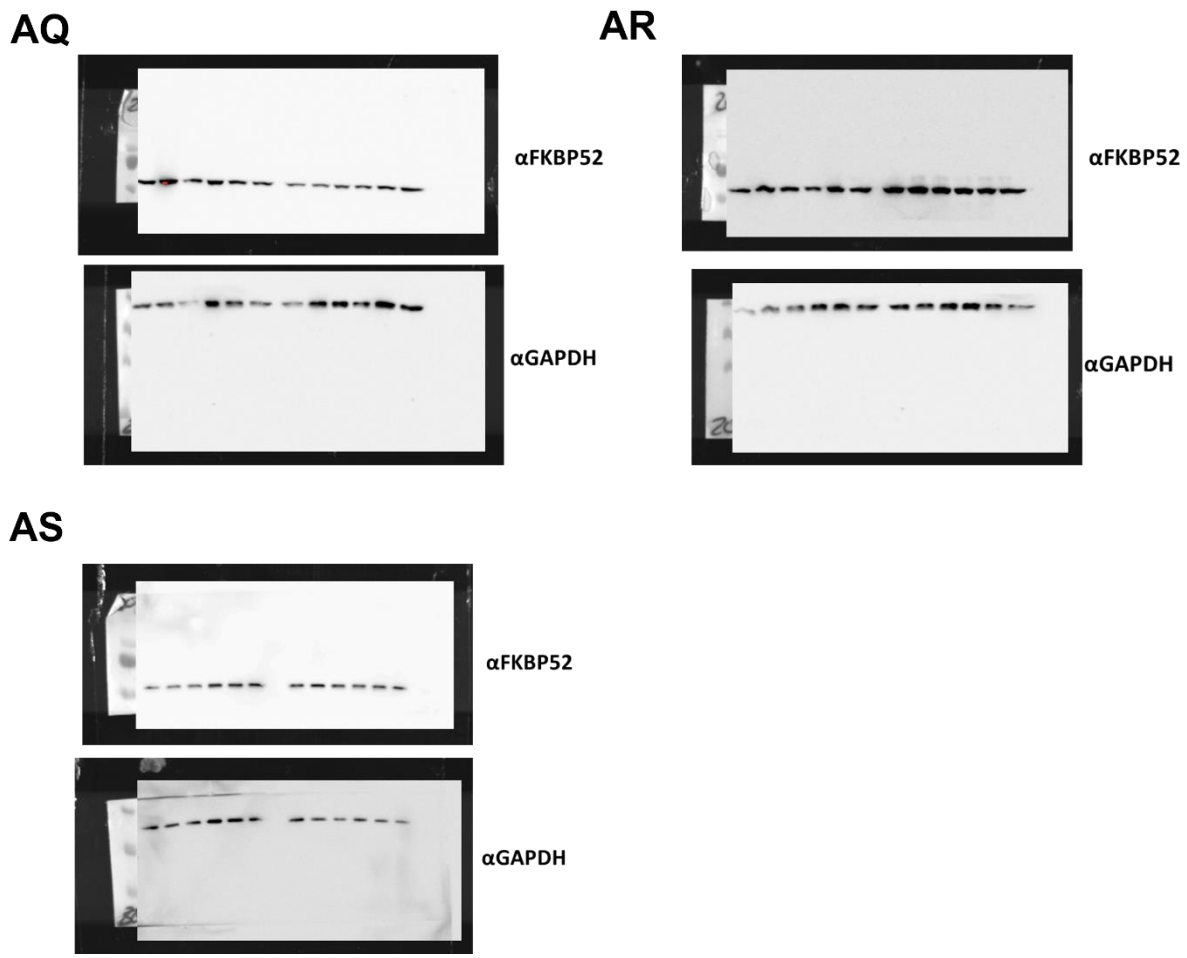


Figure S5.6 Uncropped images of Western Blots in Figure S1.5. The red dotted boxes indicate bands that are shown in the respective subfigures S1.7.

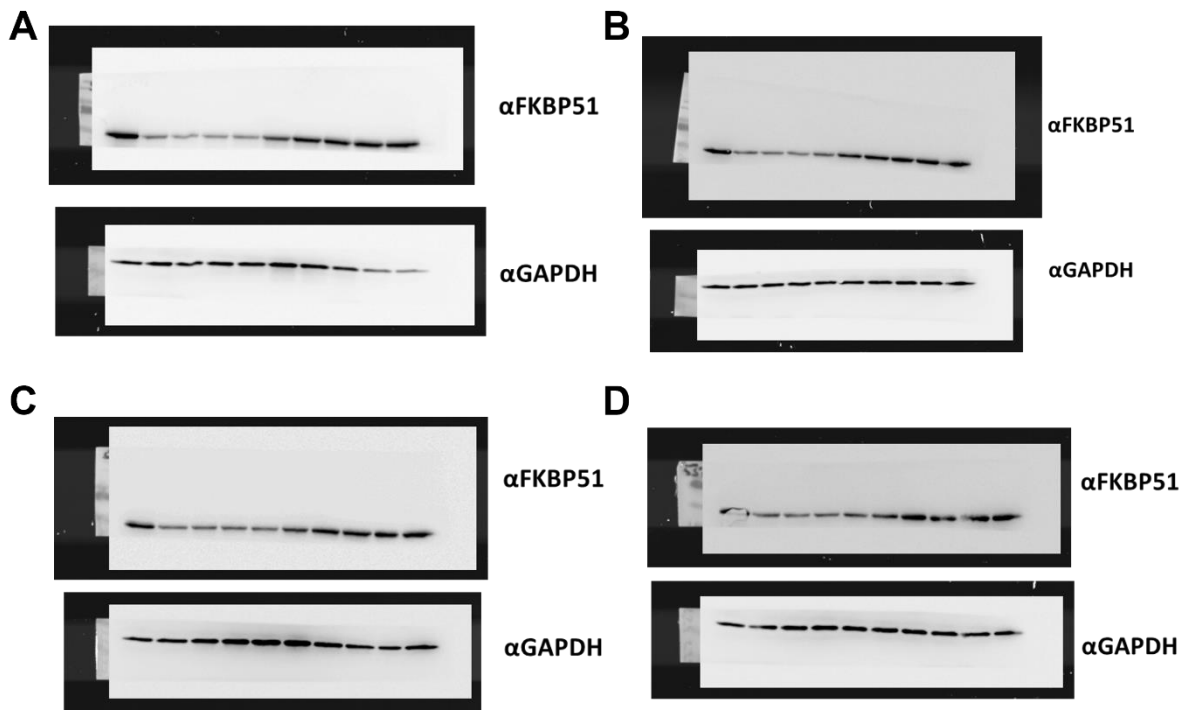


Figure S5.7 Uncropped images of Western Blots in **A** Figure S3.1B, **B** Figure S3.1C, **C** Figure S3.1D, and **D** Figure S3.1E.

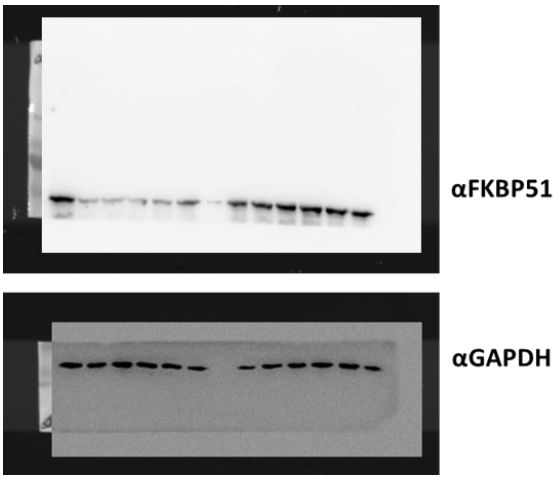
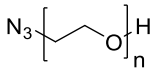


Figure S5.8 Uncropped images of Western Blots in Figure S4.3B

Chemistry methods

1. Synthesis of tosyl and azide derviatized linkers

1-3:



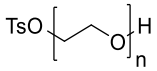
Cholonated ethylenglycoles of the length 1 to 3 (1.0 eq.), sodium azide (1.23 eq.), and sodium hydroxide (0.1 eq.) were stirred in water for 72 h at room temperature. Additional sodium azide (1.0 eq.) and sodium hydroxide (0.15 eq.) were added and stirred for 22 h. Sodium thiosulfate and brine were added and the solution was extracted with DCM. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure.

1:
Yield: 0.7 g (65 %, 8.0 mmol).
Appearance: slightly yellow oil.
1H-NMR: (300 MHz, Chloroform-*d*): δ = 2.25 (s, 1H), 3.38 – 3.47 (m, 2H), 3.72 – 3.80 (m, 2H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 53.6, 61.6.
TLC: Rf = 0.33 (DCM:MeOH = 50:1).

2:
Yield: 1.6 g (98 %, 12.2 mmol).
Appearance: slightly yellow oil.
1H-NMR (500 MHz, Chloroform-*d*): δ = 2.59 (d, *J* = 3.7 Hz, 1H), 3.36 (t, *J* = 5.0 Hz, 2H), 3.56 (dd, *J* = 5.3, 3.8 Hz, 2H), 3.64 (t, *J* = 5.0 Hz, 2H), 3.67 – 3.74 (m, 2H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 50.7, 61.7, 70.0, 72.5.
TLC: Rf = 0.53 (CH:EA = 1:2).

3:
Yield: 2.1 g (97 %, 12.0 mmol).
Appearance: slightly yellow oil.
1H-NMR (500 MHz, Chloroform-*d*): δ = 2.48 – 2.67 (m, 1H), 3.29 – 3.42 (m, 2H), 3.51 – 3.75 (m, 10H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 50.7, 61.7, 70.0, 70.4, 70.7, 72.6.
TLC: Rf = 0.53 (CH:EA = 1:2).

4-5:

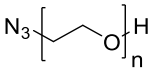


Ethylenglycoles of the length 4 to 5 (1.0 eq.), *p*-toluenesulfonyl chloride (1.0 eq.), silver oxide (1.5 eq.) and potassium iodide (0.2 eq.) were stirred in DCM for 90 min at 0 °C. The solution was allowed to warm to room temperature. The solution was filtered through celite, rinsed with DCM and concentrated under reduced pressure. The obtained product was purified by column chromatography

4:
Yield: 3.70 g (60 %, 10.8 mmol)
Appearance: slightly yellow oil.
1H-NMR (300 MHz, Chloroform-*d*): δ = 2.43 (s, 3H), 2.53 (s, 1H), 3.56 – 3.65 (m, 10H), 3.65 – 3.70 (m, 4H), 4.13 – 4.16 (m, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 7.78 (d, *J* = 8.1 Hz, 2H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 21.7, 61.8, 68.8, 69.3, 70.4, 70.6, 70.7, 70.8, 72.6, 128.0, 129.9, 133.1, 144.9.
TLC: Rf = 0.48 (DCM:MeOH = 20:1).
LC-MS: Mass (ESI), calculated = 349.4 [M+H]+, found = 349.4.

5:
Yield: 3.6 g (51 %, 9.2 mmol).
Appearance: slightly yellow oil.

1H-NMR (300 MHz, Chloroform-*d*): δ = 2.44 (s, 3H), 2.53 – 2.68 (m, 1H), 3.53 – 3.77 (m, 18H), 4.15 (t, *J* = 4.9 Hz, 2H), 7.33 (d, *J* = 7.9 Hz, 2H), 7.79 (d, *J* = 7.8 Hz, 2H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 21.8, 61.9, 68.8, 69.4, 70.5, 70.6, 70.7, 70.7, 70.9, 72.6, 128.1, 129.9, 133.2, 144.9.
TLC: Rf = 0.40 (DCM:MeOH = 20:1).
LC-MS: Mass (ESI), calculated = 393.5 [M+H]+, found = 393.4.

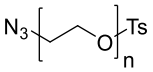


Tosylated ethylenglycoles of the length 4 to 5 (1.0 eq.) and sodium azide (2.0 eq.) were stirred in DMF for 40 h at room temperature. The solution was diluted with Brine and extracted with DCM. The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography.

4:
Yield: 2.2 g (94 %, 10.1 mmol).
Appearance: slightly yellow oil.
1H-NMR (500 MHz, Chloroform-*d*): δ = 2.74 (s, 1H), 3.33 (t, *J* = 5.1 Hz, 2H), 3.53 – 3.57 (m, 2H), 3.58 – 3.65 (m, 10H), 3.65 – 3.68 (m, 2H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 50.7, 61.7, 70.0, 70.4, 70.6, 70.7, 70.7, 72.5.
TLC: Rf = 0.30 (DCM:MeOH = 20:1).
LC-MS: Mass (ESI), calculated = 220.2 [M+H]+, found = 220.2.

5:
Yield: 2.3 g (97 %, 8.9 mmol).
Appearance: slightly yellow oil.
1H-NMR (500 MHz, Chloroform-*d*): δ = 2.75 (s, 1H), 3.30 – 3.42 (m, 2H), 3.53 – 3.76 (m, 18H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 50.7, 61.8, 70.1, 70.4, 70.6, 70.7, 70.7, 72.6.
TLC: Rf = 0.24 (DCM:MeOH = 20:1).
LC-MS: Mass (ESI), calculated = 264.2 [M+H]+, found = 264.2.
Mass (ESI), calculated = 281.2 [M+NH4]+, found = 281.1.

1-5:



Azide functionalized ethylenglycoles of the length 1 to 5 (1.0 eq.) and 4-toluenesulfonyl chloride (1.5 eq.) in DCM were cooled to 0 °C. Pyridine (2.0 eq.) was added and the mixture was stirred for 1 h at 0 °C, followed by 70 h at room temperature. The mixture was concentrated under reduced pressure. The obtained crude product was purified by column chromatography.

1:
Yield: 1.18 g (60%, 4.82 mmol).
Appearance: colourless liquid.
1H NMR (300 MHz, Chloroform-*d*) δ 2.47 (s, 3H), 3.49 (t, *J* = 5.1 Hz, 2H), 4.17 (t, *J* = 5.1 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.77 – 7.87 (m, 2H).
13C NMR (75 MHz, Chloroform-*d*) δ 21.6, 49.6, 68.1, 127.9, 130.0, 132.6, 145.3.
TLC: Rf = 0.30 (CH:EA = 5:1).
LC-MS: Mass (ESI), calculated = 242,3 [M+H+], found = 242.3.

2:
Yield: 2,54 g (82%, 9.43 mmol).
Appearance: colourless liquid.
1H NMR (300 MHz, Chloroform-*d*) δ 2.46 (s, 3H), 3.33 (t, *J* = 5.0 Hz, 2H), 3.54 – 3.70 (m, 2H), 3.66 – 3.76 (m, 2H), 4.13 – 4.23 (m, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.77 – 7.86 (m, 2H).
13C NMR (75 MHz, Chloroform-*d*) δ 21.6, 50.6, 68.7, 69.1, 70.1, 128.0, 129.8, 132.9, 144.9.
TLC: Rf = 0.75 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 286,3 [M+H+], found = 286.3.

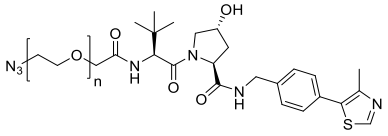
3:
Yield: 2,95 g (75%, 8.95 mmol).

Appearance: colourless liquid.
1H NMR (300 MHz, Chloroform-*d*) δ 2.45 (s, 1H), 3.37 (t, *J* = 5.0 Hz, 1H), 3.57 – 3.75 (m, 4H), 4.12 – 4.22 (m, 1H), 7.30 – 7.40 (m, 1H), 7.76 – 7.85 (m, 1H).
13C NMR (75 MHz, Chloroform-*d*) δ 21.6, 50.7, 68.8, 69.2, 70.1, 70.6, 70.8, 127.9, 129.8, 133.0, 144.8.
TLC: Rf = 0.68 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 330.4 [M+H+], found = 330.4.

4:
Yield: 2,3 g (61%, 6.1 mmol).
Appearance: colourless liquid.
1H NMR (300 MHz, Chloroform-*d*) δ 2.43 (s, 3H), 3.31 – 3.42 (m, 2H), 3.49 – 3.78 (m, 14H), 4.10 – 4.20 (m, 2H), 7.27 – 7.38 (m, 2H), 7.73 – 7.84 (m, 2H).
13C NMR (75 MHz, CDCl3) δ 21.6, 50.7, 63.0, 68.7, 68.8, 69.2, 70.0, 70.6, 70.6, 70.7, 127.9, 129.8, 133.1, 144.8.
TLC: Rf = 0.39 (Cyclohexane:Ethylacetate = 1:1).
LC-MS: Mass (ESI), calculated = 374.4 [M+H+], found = 374.4.

5:
Yield: 2,4 g (64%, 5.7 mmol).
Appearance: colourless liquid.
1H NMR (300 MHz, Chloroform-*d*) δ 2.43 (d, *J* = 0.7 Hz, 1H), 3.37 (dd, *J* = 5.6, 4.6 Hz, 1H), 3.54 – 3.75 (m, 8H), 4.10 – 4.22 (m, 1H), 7.31 – 7.38 (m, 1H), 7.73 – 7.84 (m, 1H).
13C NMR (75 MHz, CDCl3) δ 21.6, 50.7, 68.7, 69.2, 70.0, 70.5, 70.6, 70.7, 70.7, 76.6, 77.0, 77.5, 127.9, 129.8, 133.1, 144.7.
TLC: Rf = 0.20 (Cyclohexane:Ethylacetate = 1:1).
LC-MS: Mass (ESI), calculated = 418.2 [M+H+], found = 418.2.

2. Synthesis of a1-5



Azidoacetic acids of the length 1 to 5 (1.0 eq.) in DCM were added to VH032 (1.0 eq.) in DCM. Afterwards HATU (1.3 eq.) and DIPEA (5.0 eq.) were added and the mixture was stirred for 16 h at room temperature. DCM was added and the mixture was washed with brine. The organic phase was dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by chromatography.

a1:
Yield: 583 mg (53 %, 1.1 mmol).
Appearance: white foam.
1H-NMR (500 MHz, Chloroform-*d*): δ = 0.95 (s, 9H), 2.05 – 2.13 (m, 1H), 2.47 (s, 4H), 3.36 – 3.51 (m, 2H), 3.59 – 3.73 (m, 3H), 3.92 – 4.04 (m, 3H), 4.31 (dd, *J* = 15.1, 5.4 Hz, 1H), 4.46 – 4.56 (m, 3H), 4.71 (t, *J* = 7.9 Hz, 1H), 7.19 (d, *J* = 8.6 Hz, 1H), 7.30 – 7.36 (m, 4H), 7.42 (t, *J* = 6.0 Hz, 1H), 8.68 (s, 1H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 16.1, 26.5, 35.2, 36.2, 43.3, 50.8, 56.8, 57.4, 58.7, 70.2, 70.6, 128.2, 129.6, 130.9, 131.8, 138.3, 148.4, 150.5, 169.8, 171.0, 171.3.
TLC: Rf = 0.45 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 558.2 [M+H+], found = 558.3.
Mass (ESI), calculated = 1115.4 [2M+H+], found = 1114.8.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 10.4 min.

a2:
Yield: 881 mg (73 %, 1.5 mmol).
Appearance: white foam.
1H-NMR (500 MHz, Chloroform-*d*): δ = 0.92 – 0.99 (m, 9H), 2.03 – 2.14 (m, 1H), 2.36 – 2.51 (m, 4H), 3.36 (td, *J* = 9.4, 4.4 Hz, 2H), 3.57 – 3.72 (m, 8H), 3.90 – 4.03 (m, 3H), 4.32 (dt, *J* = 11.6, 4.4 Hz, 1H),

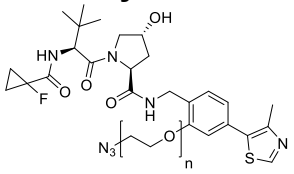
4.44 – 4.55 (m, 3H), 4.63 – 4.72 (m, 1H), 7.23 – 7.28 (m, 1H), 7.28 – 7.36 (m, 4H), 7.37 – 7.46 (m, 1H), 8.67 (s, 1H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 12.6, 16.0, 17.2, 18.6, 26.5, 35.4, 36.3, 43.2, 50.6, 55.5, 56.8, 57.1, 58.9, 70.1, 70.5, 71.2, 128.1, 129.5, 130.8, 131.8, 138.4, 148.3, 150.5, 170.3, 170.3, 171.1,
TLC: Rf = 0.45 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 602.3 [M+H+], found = 602.2.
Mass (ESI), calculated = 624.3 [M+Na+], found = 624.2.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 10.4 min.

a3:
Yield: 967 mg (75 %, 1.5 mmol).
Appearance: white foam.
1H-NMR (500 MHz, Chloroform-*d*): δ = 0.94 (s, 9H), 1.42 – 1.46 (m, 2H), 2.06 – 2.13 (m, 1H), 2.44 (ddd, *J* = 12.9, 7.8, 4.7 Hz, 1H), 2.49 (s, 3H), 3.31 – 3.37 (m, 2H), 3.60 – 3.67 (m, 12H), 3.94 – 4.03 (m, 3H), 4.33 (dd, *J* = 15.1, 5.5 Hz, 1H), 4.50 (tt, *J* = 6.5, 3.6 Hz, 3H), 4.69 (t, *J* = 7.9 Hz, 1H), 7.26 – 7.29 (m, 1H), 7.33 (d, *J* = 2.3 Hz, 4H), 7.40 (s, 1H), 8.70 (s, 1H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 17.2, 26.5, 35.2, 36.2, 43.3, 50.8, 56.8, 57.2, 58.8, 70.1, 70.2, 70.4, 70.6, 70.7, 71.2, 128.2, 129.5, 130.7, 131.9, 138.4, 148.2, 150.6, 170.5, 171.0, 171.3.
TLC: Rf = 0.45 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 646.3 [M+H+], found = 646.2.
Mass (ESI), calculated = 668.3 [M+Na+], found = 668.2.
HPLC: [20-80 % Solvent B, 20 min]: Rt = 8.5 min.

a4:
Yield: 1030 mg (75 %, 1.5 mmol).
Appearance: white foam.
1H-NMR (500 MHz, Chloroform-*d*): δ = 0.94 (s, 9H), 2.09 (ddt, *J* = 13.3, 8.1, 2.0 Hz, 1H), 2.44 (ddd, *J* = 12.9, 7.8, 4.7 Hz, 1H), 2.49 (s, 3H), 3.31 – 3.38 (m, 2H), 3.55 – 3.70 (m, 16H), 3.90 – 4.04 (m, 3H), 4.33 (dd, *J* = 15.1, 5.4 Hz, 1H), 4.45 – 4.53 (m, 3H), 4.69 (t, *J* = 7.9 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 7.30 – 7.35 (m, 4H), 7.42 (t, *J* = 6.0 Hz, 1H), 8.70 (s, 1H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 16.0, 26.5, 35.2, 36.3, 43.3, 50.8, 56.8, 57.2, 58.7, 70.1, 70.2, 70.4, 70.5, 70.7, 70.7, 71.2, 128.2, 129.5, 130.7, 131.9, 138.4, 148.2, 150.6, 170.5, 171.0, 171.3.
TLC: Rf = 0.47 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 690.3 [M+H+], found = 690.3.
Mass (ESI), calculated = 712.3 [M+Na+], found = 712.2.
HPLC: [20-80 % Solvent B, 20 min]: Rt = 8.4 min.

a5:
Yield: 330 mg (22 %, 0.5 mmol).
Appearance: yellow foam.
1H-NMR (300 MHz, Chloroform-*d*): δ = 0.94 (s, 9H), 2.09 (dd, *J* = 13.6, 8.2 Hz, 1H), 2.41 (td, *J* = 7.9, 3.8 Hz, 1H), 2.48 (s, 3H), 3.35 (t, *J* = 5.0 Hz, 2H), 3.54 – 3.72 (m, 20H), 3.88 – 4.05 (m, 3H), 4.32 (dd, *J* = 15.1, 5.5 Hz, 1H), 4.41 – 4.56 (m, 3H), 4.68 (t, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 1H), 7.32 (s, 4H), 7.41 (t, *J* = 6.0 Hz, 1H), 8.69 (s, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 16.0, 26.5, 35.2, 36.3, 43.2, 50.7, 56.8, 57.2, 58.8, 70.0, 70.1, 70.3, 70.4, 70.6, 70.6, 70.7, 71.2, 128.1, 129.5, 130.7, 131.9, 138.4, 148.2, 150.5, 170.5, 171.1, 171.2.
TLC: Rf = 0.49 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 734.4 [M+H+], found = 734.4.
Mass (ESI), calculated = 756.4 [M+H+], found = 756.3.
HPLC: [20-80 % Solvent B, 20 min]: Rt = 8.9 min.

3. Synthesis of b1-5



VH032-cyclopropane-F (0.70 g, 1,31 mmol, 1.0 eq.), tosyl and azide derviatized linkers of the length 1 to 5 (1.35 eq.) and potassiumcarbonate (2.7 eq.) were stirred in DMF for 24 h. The mixture was diluted

with water and was extracted with DCM. The organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by chromatography.

b1:
Yield: 0.68 g (86%, 1.13 mmol).
Appearance: colourless foam.
1H NMR (500 MHz, Chloroform-*d*) : δ = 0.97 (s, 8H), 1.25 – 1.42 (m, 4H), 2.10 (ddt, *J* = 13.1, 8.2, 2.1 Hz, 1H), 2.55 (s, 3H), 2.54 – 2.62 (m, 1H), 2.89 (d, *J* = 4.3 Hz, 1H), 2.98 (s, 0H), 3.65 (dd, *J* = 11.2, 3.9 Hz, 1H), 3.68 – 3.79 (m, 2H), 4.03 (dt, *J* = 11.3, 2.0 Hz, 1H), 4.18 – 4.29 (m, 2H), 4.46 (dd, *J* = 15.0, 5.4 Hz, 1H), 4.56 (dt, *J* = 14.4, 7.6 Hz, 3H), 4.75 (t, *J* = 7.7 Hz, 1H), 5.32 (s, 0H), 6.89 (d, *J* = 1.5 Hz, 1H), 7.03 (td, *J* = 8.5, 7.5, 2.6 Hz, 2H), 7.27 – 7.37 (m, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 8.71 (s, 1H).
13C-NMR (125 MHz, Chloroform-*d*): δ = 13.7, 13.8, 16.1, 26.3, 35.2, 35.8, 38.7, 50.4, 56.5, 57.5, 58.4, 67.2, 70.2, 76.8, 112.1, 122.4, 126.6, 129.9, 131.5, 132.4, 150.3, 170.2, 170.4, 171.0.
TLC: Rf = 0.53 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 602.3 [M+H+], found = 602.3.
HR-MS: Mass (ESI), calculated = 602.25556 [M+H+], found = 602.25554

b2:
Yield: 0.84 g (100%, 1.31 mmol).
Appearance: colourless foam.
1H NMR (500 MHz, Chloroform-*d*) : δ = 0.85 (s, 0H), 0.97 (s, 9H), 1.08 (s, 0H), 1.25 – 1.42 (m, 4H), 2.11 (ddt, J = 12.1, 7.8, 2.1 Hz, 1H), 2.53 (ddd, J = 12.2, 8.4, 5.6 Hz, 1H), 2.55 (s, 3H), 2.81 (d, J = 4.3 Hz, 1H), 2.91 (d, J = 0.6 Hz, 1H), 2.98 (s, 1H), 3.46 (ddd, J = 5.6, 4.3, 1.2 Hz, 2H), 3.66 (dd, J = 11.3, 3.9 Hz, 1H), 3.75 – 3.86 (m, 2H), 3.96 (hd, J = 6.1, 5.4, 2.8 Hz, 2H), 4.02 (dt, J = 11.3, 1.9 Hz, 1H), 4.18 – 4.29 (m, 2H), 4.48 (dd, J = 14.7, 5.6 Hz, 1H), 4.50 – 4.58 (m, 3H), 4.72 (t, J = 7.8 Hz, 1H), 5.32 (s, 0H), 6.92 (d, J = 1.6 Hz, 1H), 6.97 – 7.08 (m, 2H), 7.27 – 7.39 (m, 2H), 8.04 (s, 0H), 8.70 (d, J = 12.6 Hz, 0H), 8.70 (s, 1H).
13C-NMR (125 MHz, Chloroform-*d*): δ =13.7, 13.8, 16.1, 26.3, 35.2, 36.0, 38.9, 50.7, 56.5, 57.5, 58.4, 67.9, 69.7, 70.3, 112.6, 122.1, 126.7, 129.8, 131.7, 132.3, 148.5, 150.3, 156.6, 170.2, 170.4, 170.9.
TLC: Rf = 0.61 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 646.3 [M+H+], found = 646.3.
HR-MS: Mass (ESI), calculated = 646.28175 [M+H+], found = 646.28176

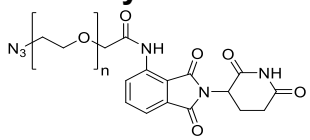
b3:
Yield: 0.78 g (87%, 1.14 mmol).
Appearance: colourless foam.
1H NMR (500 MHz, Chloroform-*d*) : δ = 0.97 (s, 8H), 1.06 (s, 0H), 1.23 – 1.41 (m, 3H), 1.69 (s, 0H), 2.03 – 2.15 (m, 1H), 2.44 (ddd, J = 13.5, 7.7, 4.7 Hz, 1H), 2.53 (d, J = 1.1 Hz, 3H), 2.85 (d, J = 3.9 Hz, 1H), 3.34 – 3.43 (m, 2H), 3.49 (s, 0H), 3.62 – 3.77 (m, 5H), 3.73 – 3.84 (m, 2H), 3.88 – 4.03 (m, 3H), 4.15 – 4.28 (m, 2H), 4.43 – 4.57 (m, 4H), 4.62 (s, 0H), 4.69 (t, J = 7.8 Hz, 1H), 6.91 (d, J = 1.6 Hz, 1H), 6.98 (dd, J = 7.6, 1.7 Hz, 1H), 7.04 (dd, J = 8.9, 3.5 Hz, 1H), 7.25 – 7.34 (m, 1H), 7.34 (d, J = 7.8 Hz, 1H), 8.69 (s, 1H).
13C-NMR (125 MHz, Chloroform-*d*): δ = 13.6, 13.7, 13.8, 16.1, 26.3, 35.3, 36.2, 39.0, 50.7, 56.6, 57.5, 58.5, 67.9, 69.8, 70.0, 70.3, 70.7, 70.9, 79.2, 112.8, 122.0, 126.8, 129.7, 131.7, 132.3, 148.5, 150.3, 156.8, 170.1, 170.2, 170.5, 170.7.
TLC: Rf = 0.50 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 690.3 [M+H+], found = 690.3.
HR-MS: Mass (ESI), calculated = 690.30829 [M+H+], found = 69030797

b4:
Yield: 0.81 g (84%, 0.95 mmol).
Appearance: colourless foam.
1H NMR (500 MHz, Chloroform-*d*) : δ = 0.76 – 0.84 (m, 0H), 0.81 (s, 0H), 0.88 (s, 7H), 0.90 (d, J = 1.4 Hz, 2H), 0.97 (s, 0H), 1.10 (d, J = 6.8 Hz, 0H), 1.14 – 1.32 (m, 4H), 1.99 – 2.10 (m, 1H), 2.07 – 2.13 (m, 0H), 2.16 – 2.24 (m, 0H), 2.29 – 2.38 (m, 1H), 2.45 (s, 3H), 2.46 (d, J = 7.8 Hz, 0H), 3.06 (s, 1H), 3.30 (t, J = 5.1 Hz, 2H), 3.58 (ddt, J = 5.8, 3.1, 2.3 Hz, 7H), 3.60 – 3.70 (m, 3H), 3.66 – 3.74 (m, 1H), 3.86 (tdd, J = 14.9, 6.4, 3.5 Hz, 3H), 4.13 (dddd, J = 24.2, 10.0, 5.7, 3.9 Hz, 2H), 4.33 – 4.45 (m, 2H), 4.42 – 4.57 (m, 2H), 4.60 (t, J = 7.8 Hz, 1H), 5.23 (d, J = 1.1 Hz, 0H), 6.83 (d, J = 1.7 Hz, 1H), 6.90 (dd, J = 7.6, 1.6 Hz, 1H), 6.98 (dd, J = 8.9, 3.7 Hz, 1H), 7.12 – 7.19 (m, 0H), 7.19 – 7.30 (m, 2H), 8.60 (s, 1H).
13C-NMR (125 MHz, Chloroform-*d*): δ = 13.6, 13.6, 13.7, 16.1, 26.2, 26.3, 35.5, 36.4, 39.0, 50.7, 56.6, 57.4, 58.6, 68.0, 69.6, 70.0, 70.2, 70.6, 70.7, 70.8, 76.8, 77.0, 77.3, 79.1, 112.8, 122.0, 126.8, 129.7, 131.7, 132.3, 148.5, 150.3, 156.8, 169.9, 170.6, 170.6.

TLC: Rf = 0.47 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 734.4 [M+H+], found = 734.4.
HR-MS: Mass (ESI), calculated = 734.33481 [M+H+], found = 734.33419

b5:
Yield: 0.67 g (66%, 0.86 mmol).
Appearance: colourless resin.
1H NMR (500 MHz, Chloroform-*d*) : δ = 0.98 (s, 8H), 1.09 (d, J = 18.7 Hz, 0H), 1.23 – 1.31 (m, 1H), 1.34 (dddd, J = 17.2, 14.4, 8.1, 3.4 Hz, 3H), 1.85 (s, 1H), 2.14 (ddt, J = 13.4, 8.0, 2.0 Hz, 1H), 2.42 (ddd, J = 12.8, 7.9, 4.7 Hz, 1H), 2.91 (d, J = 4.3 Hz, 1H), 3.36 – 3.42 (m, 2H), 3.53 (d, J = 5.1 Hz, 0H), 3.61 – 3.75 (m, 13H), 3.72 – 3.84 (m, 2H), 3.88 – 4.01 (m, 3H), 4.20 (ddd, J = 10.2, 6.3, 3.8 Hz, 1H), 4.25 (ddd, J = 10.2, 5.4, 3.6 Hz, 1H), 4.50 (d, J = 6.1 Hz, 2H), 4.51 – 4.59 (m, 2H), 4.69 (t, J = 7.9 Hz, 1H), 5.32 (s, 1H), 6.92 (d, J = 1.7 Hz, 1H), 6.99 (dd, J = 7.6, 1.6 Hz, 1H), 7.06 (dd, J = 8.9, 3.5 Hz, 1H), 7.14 (s, 0H), 7.27 – 7.38 (m, 3H), 8.70 (s, 1H).
13C-NMR (125 MHz, Chloroform-*d*): δ =13.6, 13.7, 13.7, 16.2, 26.3, 35.5, 36.4, 39.1, 50.7, 56.5, 57.5, 58.6, 68.0, 69.6, 70.0, 70.3, 70.5, 70.7, 70.8, 79.1, 112.8, 122.0, 126.8, 129.8, 131.7, 132.3, 148.5, 150.3, 156.8, 169.9, 170.1, 170.6, 170.7.
TLC: Rf = 0.51 (DCM:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 778.6 [M+H+], found = 778.6.
HR-MS: Mass (ESI), calculated = 778.36087 [M+H+], found = 778.36040

4. Synthesis of c1-5



Azidoacetic acids of the length 1 to 5 (1.0 eq.) were dissolved in DCM and DMF. The solution was cooled to 0 °C and oxalyl chloride (1.3 eq.) was added slowly. The solution was stirred for 30 min at 0 °C, allowed to warm to room temperature and stirred for additional 2 h. The solution was concentrated under reduced pressure. The intermediate was dissolved in THF (20 mL) and pomalidomide (1.0 eq.) was added. The solution was stirred for 3 h at 80 °C followed by 16 h at 70 °C. The solution was filtered and concentrated under reduced pressure. The obtained product was purified by chromatography.

c1:
Yield: 799 mg (99 %, 2.0 mmol).
Appearance: yellow solid.
1H-NMR (300 MHz, DMSO-*d6*): δ = 1.98 – 2.12 (m, 1H), 2.52 – 2.75 (m, 3H), 3.53 (t, *J* = 5.0 Hz, 2H), 3.77 (t, *J* = 5.0 Hz, 2H), 4.16 (s, 2H), 5.05 (dd, *J* = 13.1, 5.8 Hz, 1H), 7.54 (d, *J* = 6.7 Hz, 1H), 7.75 (d, *J* = 7.7 Hz, 1H), 8.68 (d, *J* = 8.3 Hz, 1H), 10.35 (s, 1H), 11.06 (s, 1H).
13C-NMR (75 MHz, DMSO-*d6*): δ = 22.3, 31.2, 49.3, 50.4, 70.2, 70.4, 116.2, 118.6, 124.7, 131.4, 136.2, 136.5, 166.8, 168.4, 169.0, 169.6, 172.8.
TLC: Rf = 0.27 (DCM:MTBE = 10:1).
LC-MS: Mass (ESI), calculated = 401.1 [M+H+], found = 401.1.
Mass (ESI), calculated = 418.1 [M+NH4+], found = 418.2.
Mass (ESI), calculated = 823.2 [2M+Na+], found = 822.8.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 10.6 min.

c2:
Yield: 660 mg (74 %, 1.5 mmol).
Appearance: yellow solid.
1H-NMR (300 MHz, DMSO-*d6*): δ = 2.05 – 2.18 (m, 1H), 2.56 – 2.72 (m, 2H), 2.86 – 3.02 (m, 1H), 3.37 – 3.47 (m, 2H), 3.66 – 3.71 (m, 2H), 3.73 – 3.78 (m, 2H), 3.79 – 3.85 (m, 2H), 4.25 (s, 2H), 5.19 (dd, *J* = 12.9, 5.4 Hz, 1H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.90 (dd, *J* = 8.4, 7.4 Hz, 1H), 8.76 (dd, *J* = 8.5, 0.7 Hz, 1H), 10.40 (s, 1H), 11.16 (s, 1H).
13C-NMR (75 MHz, DMSO-*d6*): δ = 21.9, 30.9, 48.9, 50.0, 69.3, 69.5, 70.2, 70.8, 116.0, 118.3, 124.3, 131.3, 135.9, 136.4, 166.6, 168.2, 169.2, 169.6, 172.6.
TLC: Rf = 0.13 (DCM:MeOH = 50:1).
LC-MS: Mass (ESI), calculated = 445.1 [M+H+], found = 445.3.
Mass (ESI), calculated = 462.1 [M+NH4+], found = 462.2.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 11.2 min.

c3:
Yield: 950 mg (97 %, 1.9 mmol).
Appearance: yellow solid.
1H-NMR (300 MHz, DMSO-*d6*): δ = 2.00 – 2.15 (m, 1H), 2.52 – 2.68 (m, 2H), 2.81 – 3.00 (m, 1H), 3.35 (dd, *J* = 5.6, 4.3 Hz, 2H), 3.56 (tt, *J* = 4.4, 2.0 Hz, 6H), 3.65 – 3.82 (m, 4H), 4.21 (s, 2H), 5.16 (dd, *J* = 12.9, 5.4 Hz, 1H), 7.63 (dd, *J* = 7.3, 0.7 Hz, 1H), 7.87 (dd, *J* = 8.5, 7.4 Hz, 1H), 8.73 (dd, *J* = 8.5, 0.8 Hz, 1H), 10.35 (s, 1H), 11.14 (s, 1H).
13C-NMR (75 MHz, DMSO-*d6*): δ = 21.9, 30.9, 49.0, 49.9, 69.2, 69.6, 69.7, 69.9, 70.2, 70.8, 116.0, 118.3, 124.4, 131.3, 135.9, 136.5, 166.6, 168.2, 169.3, 169.7, 172.7.
TLC: Rf = 0.27 (DCM:MeOH = 20:1).
LC-MS: Mass (ESI), calculated = 489.2 [M+H+], found = 489.4.
Mass (ESI), calculated = 506.2 [M+NH4+], found = 506.1.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 11.0 min.

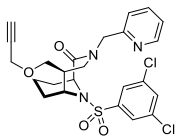
c4:
Yield: 503 mg (63 %, 0.9 mmol).
Appearance: yellow solid.
1H-NMR (300 MHz, DMSO-*d6*): δ = 2.08 (ddd, *J* = 9.5, 5.0, 2.7 Hz, 1H), 2.59 (dt, *J* = 9.6, 4.5 Hz, 1H), 2.71 – 2.97 (m, 2H), 3.47 – 3.62 (m, 10H), 3.65 – 3.71 (m, 2H), 3.73 – 3.80 (m, 2H), 4.21 (s, 2H), 5.16 (dd, *J* = 12.9, 5.4 Hz, 1H), 7.63 (dd, *J* = 7.3, 0.7 Hz, 1H), 7.87 (dd, *J* = 8.5, 7.4 Hz, 1H), 8.73 (dd, *J* = 8.5, 0.8 Hz, 1H), 10.36 (s, 1H), 11.13 (s, 1H).
13C-NMR (75 MHz, DMSO-*d6*): δ = 21.9, 30.9, 49.0, 50.0, 67.6, 69.2, 69.6, 69.7, 69.8, 69.8, 70.0, 70.2, 70.8, 116.1, 118.3, 124.4, 131.3, 135.9, 136.5, 166.6, 168.2, 169.4, 169.7, 172.7.
TLC: Rf = 0.19 (DCM:MeOH = 20:1).
LC-MS: Mass (ESI), calculated = 533.2 [M+H+], found = 533.6.
Mass (ESI), calculated = 550.2 [M+NH4+], found = 550.3.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 11.0 min.

c5:
Yield: 807 mg (70 %, 1.7 mmol).
Appearance: yellow solid.
1H-NMR (300 MHz, DMSO-*d6*): δ = 2.08 (ddt, *J* = 12.8, 5.4, 3.1 Hz, 1H), 2.52 – 2.74 (m, 2H), 2.90 (ddd, *J* = 17.3, 14.0, 5.4 Hz, 1H), 3.32 – 3.42 (m, 2H), 3.46 – 3.60 (m, 14H), 3.64 – 3.71 (m, 2H), 3.76 (dt, *J* = 4.8, 3.2 Hz, 2H), 4.20 (s, 2H), 5.16 (dd, *J* = 12.9, 5.4 Hz, 1H), 7.63 (d, *J* = 6.9 Hz, 1H), 7.81 – 7.93 (m, 1H), 8.72 (d, *J* = 8.1 Hz, 1H), 10.36 (s, 1H), 11.13 (s, 1H).
13C-NMR (75 MHz, DMSO-*d6*): δ = 21.9, 30.9, 48.9, 49.9, 69.2, 69.6, 69.7, 69.7, 69.8, 70.2, 70.7, 116.0, 118.3, 124.3, 131.3, 135.9, 136.5, 166.6, 168.2, 169.3, 169.6, 172.6.
TLC: Rf = 0.17 (DCM:MeOH = 20:1).
LC-MS: Mass (ESI), calculated = 577.2 [M+H+], found = 577.4.
Mass (ESI), calculated = 594.2 [M+NH4+], found = 594.2.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 11.0 min.

5. Synthesis of alkyne 1, 5, 6, 7, 10, 11, 12:

Synthesis of alkyne 1, 5, 6, 7, 10, 11 and 12 was previously described in [1].

6. Synthesis of alkyne 2:

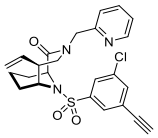


Starting from (1*S, 5S,* 6*R*)-10-((3,5-Dichlorophenyl)sulfonyl)-5-(hydroxymethyl)-3-(pyridin-2-ylmethyl)-3,10- diazabicyclo[4.3.1]decan-2-one; Synthesis previously described in [2].

(1*S,* 5*S,* 6*R*)-10-((3,5-Dichlorophenyl)sulfonyl)-5-(hydroxymethyl)-3-(pyridin-2-ylmethyl)-3,10-diazabicyclo[4.3.1]decan-2-one (48 mg, 99.1 µmol, 1.0 eq.) was dissolved in DMF (dry, 5 mL) at 0 °C. Sodium hydride (7.1 mg, 297 µmol, 3.0 eq.) was added and the mixture was stirred for 30 min at 0 °C. 3-Bromoprop-1-yne (47 mg, 396 µmol, 4.0 eq.) and tetrabutylammonium iodide (1.8 mg, 5.0 µmol, 0.05 eq.) were added and the mixture was was stirred for 18 h at 0 °C to room temperature. Ammonium chloride (sat., aq, 30 mL) was added and the mixture was extracted with EA (3 x 30 mL). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography (25 g SiO2, CH:EA = 1:1).

Yield: 29 mg (56 %, 54.9 µmol).
Appearance: white foam.
1H-NMR (500 MHz, Chloroform-*d*): δ = 1.30 (tdd, *J* = 13.3, 6.2, 4.1 Hz, 1H), 1.44 (ddt, *J* = 14.3, 9.5, 4.7 Hz, 71H), 1.50 – 1.66 (m, 3H), 2.33 (d, *J* = 15.0 Hz, 1H), 2.35 – 2.43 (m, 1H), 2.44 (t, *J* = 2.4 Hz, 1H), 3.24 (dd, *J* = 14.3, 1.9 Hz, 1H), 3.40 (d, *J* = 6.5 Hz, 2H), 3.76 (dd, *J* = 14.3, 10.7 Hz, 1H), 3.94 – 4.00 (m, 1H), 4.09 (d, *J* = 2.4 Hz, 2H), 4.79 (d, *J* = 8.5 Hz, 2H), 4.83 (d, *J* = 15.3 Hz, 1H), 7.20 (dd, *J* = 7.1, 5.2 Hz, 1H), 7.29 (d, *J* = 7.9 Hz, 1H), 7.56 (t, *J* = 1.8 Hz, 1H), 7.68 (td, *J* = 7.7, 1.7 Hz, 1H), 7.71 (d, *J* = 1.9 Hz, 2H), 8.53 (d, *J* = 4.9 Hz, 1H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 15.7, 28.2, 28.3, 44.5, 49.7, 52.9, 56.3, 57.2, 58.5, 70.5, 75.2, 79.3, 122.2, 122.6, 125.2, 132.8, 136.4, 137.3, 144.2, 149.2, 157.0, 170.5.
TLC: Rf = 0.53 (EA).
LC-MS: Mass (ESI), calculated = 522.1 [M+H]+, found = 522.6.

7. Synthesis of alkyne 3:



Starting from (1S,5R,6R)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one; Synthesis previously described in: Pomplun, et al. Chemogenomic Profiling of Human and Microbial FK506-Binding Proteins. J. Med. Chem. 2018, 61, 3660–3673.

3-Bromo-5-chlorobenzenesulfonyl chloride

3-Bromo-5-chloroaniline (1.79 g, 8.67 mmol, 1.0 eq.) was dissolved in acetonitrinle (150 mL) and cooled to 0 °C. Hydrocloric acid (37 %, aq, 3 mL), followed by sodium nitrite (0.72 g, 10.4 mmol, 1.2 eq.) and water (5 mL) were added and the mixture was stirred for 15 min at 0 °C. Thionyl chloride (56.5 mL, 780 mmol, 90 eq.) in water was added and the mixture was stirred for 20 min at 0 °C. Copper(II) chloride dihydrate (0.74 g, 4.33 mmol, 0.5 eq.) in water (5 mL) was added and the mixture was stirred for 3 h at 0 °C. The aqueous solution was extracted with wit EA (3 x 300 mL). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography (100 g SiO2, CH:EA = 9:1).

Yield: 1.57 g (63 %, 5.41 mmol).
Appearance: brown oil.
1H-NMR (300 MHz, Chloroform-*d*): δ = 7.87 (t, *J* = 1.8 Hz, 1H), 7.96 (t, *J* = 1.8 Hz, 1H), 8.06 (t, *J* = 1.7 Hz, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 124.1, 125.9, 128.2, 136.9, 138.1, 146.3.
TLC: Rf = 0.25 (CH).
HPLC: [0-100 % Solvent B, 20 min]: Rt = 16.7 min.
82 % purity (220 nm).

(1*S*, 5*S*, 6*R*)-10-((3-Bromo-5-chlorophenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one

(1*S*, 5*S*, 6*R*)-3-(Pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (750 mg, 2.76 mmol, 1.0 eq.), 3-bromo-5-chlorobenzenesulfonyl chloride (1041 mg, 3.59 mmol, 1.3 eq.) and DIPEA (0.94 mL, 5.53 mmol, 2.0 eq.) were dissolved in acetonitrile (dry, 75 mL) and stirred for 18 h at room temperature

under argon. Brine (100 mL) was added and the mixture was extracted with DCM (2 x 100 mL). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography (100 g SiO2, CH:EA = 1:1)

Yield: 710 mg (1.35 mmol, 49 %).
Appearance: white foam.
1H-NMR (300 MHz, Chloroform-*d*): δ = 1.14 – 1.38 (m, 4H), 1.44 – 1.69 (m, 3H), 2.23 – 2.35 (m, 1H), 2.69 (dd, *J* = 9.0, 2.2 Hz, 1H), 3.09 (dd, *J* = 14.2, 2.0 Hz, 1H), 3.94 – 4.04 (m, 2H), 4.66 – 4.76 (m, 2H), 4.85 (d, *J* = 15.1 Hz, 1H), 4.92 – 5.05 (m, 2H), 5.69 (ddd, *J* = 17.0, 10.2, 8.7 Hz, 1H), 7.17 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 7.27 – 7.31 (m, 1H), 7.65 (td, *J* = 7.7, 1.8 Hz, 1H), 7.69 (t, *J* = 1.8 Hz, 1H), 7.73 (t, *J* = 1.7 Hz, 1H), 7.83 (t, *J* = 1.6 Hz, 1H), 8.50 (ddd, *J* = 4.9, 1.8, 1.0 Hz, 1H).
TLC: Rf = 0.57 (EA).
LC-MS: Mass (ESI), calculated = 526.0 [M+H]+, found = 526.5.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 13.6 min.
> 99 % purity (220 nm).

(1*S*, 5*S*, 6*R*)-10-((3-Chloro-5-((trimethylsilyl)ethynyl)phenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one

(1*S*, 5*S*, 6*R*)-10-((3-Bromo-5-chlorophenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo [4.3.1]decan-2-one (710 mg, 1.35 mmol, 1.0 eq.), copper(I) iodide (129 mg, 0.68 mmol, 0.5 eq.) and **palladium-tetrakis(triphenylphosphine)** (786 mg, 0.68 mmol, 0.5 eq.) were dissolved in TMEDA (350 mL) under argon. Ethynyltrimethylsilane (1.88 mL, 13.5 mmol, 10.0 eq.) was added and the mixture was stirred for 3 h at 90 °C. Brine (2 L) was added and the mixture was extracted with DCM (4 x 1 L). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography (150 g SiO2, CH:EA = 2:1 → 1:1)

Yield: 510 mg (0.94 mmol, 70 %).
Appearance: white foam.
1H-NMR (500 MHz, Chloroform-*d*): δ = 0.25 (d, *J* = 2.0 Hz, 9H), 1.16 – 1.35 (m, 5H), 2.24 – 2.33 (m, 1H), 2.63 – 2.73 (m, 1H), 3.09 (dd, *J* = 14.2, 2.1 Hz, 1H), 3.97 – 4.04 (m, 2H), 4.70 – 4.77 (m, 2H), 4.84 (dd, *J* = 15.3, 1.7 Hz, 1H), 4.93 – 4.99 (m, 1H), 5.02 (dt, *J* = 9.9, 1.6 Hz, 1H), 5.63 – 5.76 (m, 1H), 7.17 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 1H), 7.30 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.56 – 7.61 (m, 1H), 7.66 (td, *J* = 7.7, 1.8 Hz, 1H), 7.67 – 7.73 (m, 1H), 7.73 – 7.78 (m, 1H), 8.51 (ddd, *J* = 4.9, 1.8, 1.0 Hz, 1H).
13C-NMR (126 MHz, Chloroform-*d*): δ = -0.2, 15.7, 26.5, 27.6, 49.2, 52.2, 54.9, 56.3, 57.0, 99.2, 101.4, 116.9, 122.1, 122.5, 126.2, 126.5, 128.0, 135.5, 135.6, 137.0, 137.4, 143.2, 149.3, 157.1, 170.6.
TLC: Rf = 0.50 (CH:EA = 1:2).
LC-MS: Mass (ESI), calculated = 542.2 [M+H]+, found = 542.9.
HPLC: [60-90 % Solvent B, 20 min]: Rt = 5.9 min.
94 % purity (220 nm).

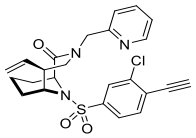
(*1S*, *5S*, *6R*)-10-((3-chloro-5-ethynylphenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one

(*1S*, *5S*, *6R*)-10-((3-Chloro-5-((trimethylsilyl)ethynyl)phenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (510 mg, 0.94 mmol, 1.0 eq.) and potassium carbonate (130 mg, 0.94 mmol, 1.0 eq) were dissolved in methanol (dry, 100 mL) and the mixture was stirred for 3 h at room temperature. The mixture was concentrated under reduced pressure and the obtained product was purified by column chromatography (40 g SiO2, CH:EA = 1:1).

Yield: 170 mg (38 %, 0.36 mmol).
Appearance: white solid.
1H-NMR (500 MHz, Chloroform-*d*): δ = 1.18 – 1.32 (m, 2H), 1.47 – 1.54 (m, 2H), 1.59 (tt, *J* = 11.3, 3.0 Hz, 1H), 2.25 – 2.34 (m, 1H), 2.70 (td, *J* = 9.0, 6.8 Hz, 1H), 3.11 (dd, *J* = 14.2, 2.0 Hz, 1H), 3.25 (s, 1H), 3.97 – 4.06 (m, 2H), 4.71 – 4.79 (m, 2H), 4.86 (d, *J* = 15.2 Hz, 1H), 4.98 (dt, *J* = 17.0, 1.1 Hz, 1H), 5.04 (dd, *J* = 10.1, 1.3 Hz, 1H), 5.71 (ddd, *J* = 17.0, 10.1, 8.8 Hz, 1H), 7.16 – 7.22 (m, 1H), 7.31 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.64 (t, *J* = 1.7 Hz, 1H), 7.68 (td, *J* = 7.7, 1.8 Hz, 1H), 7.78 (t, *J* = 1.8 Hz, 1H), 7.81 (t, *J* = 1.5 Hz, 1H), 8.52 (ddd, *J* = 4.8, 1.8, 0.9 Hz, 1H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 15.7, 26.5, 27.7, 49.2, 52.2, 55.0, 56.4, 57.0, 80.5, 81.2, 116.9, 122.2, 122.6, 125.5, 126.8, 128.2, 135.7, 135.9, 137.0, 137.4, 143.4, 149.4, 157.1, 170.6.

TLC: Rf = 0.26 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 470.1 [M+H]+, found = 470.8.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 13.0 min.
[20-70 % Solvent B, 20 min]: Rt = 12.8 min.
> 99 % purity (220 nm).
HRMS (ESI) m/z: [M+H]+ calculated for C24H24ClN3O3S = 470.12997; found = 470.13016.

8. Synthesis of alkyne 4:



Starting from (1S,5R,6R)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one; Synthesis previously described in: Pomplun, et al. Chemogenomic Profiling of Human and Microbial FK506-Binding Proteins. J. Med. Chem. 2018, 61, 3660–3673.

(1*S*, 5*S*, 6*R*)-10-((4-Bromo-3-chlorophenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one

(1*S*, 5*S*, 6*R*)-3-(Pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (750 mg, 2.76 mmol, 1.0 eq.), 4-bromo-3-chlorobenzenesulfonyl chloride (1042 mg, 3.59 mmol, 1.3 eq.) and DIPEA (0.94 mL, 5.53 mmol, 2.0 eq.) were dissolved in acetonitrile (dry, 75 mL) and stirred for 18 h at room temperature under argon. Brine (100 mL) was added and the mixture was extracted with DCM (2 x 100 mL). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography (100 g SiO2, CH:EA = 1:1)

Yield: 904 mg (62 %, 1.72 mmol).
Appearance: white foam.
1H-NMR (300 MHz, Chloroform-*d*): δ = 1.15 – 1.39 (m, 2H), 1.44 – 1.69 (m, 3H), 2.29 (dq, *J* = 13.4, 2.7 Hz, 1H), 2.62 – 2.77 (m, 1H), 3.10 (dd, *J* = 14.2, 2.0 Hz, 1H), 3.95 – 4.07 (m, 2H), 4.71 – 4.78 (m, 2H), 4.84 (d, *J* = 15.2 Hz, 1H), 4.93 – 5.06 (m, 2H), 5.62 – 5.78 (m, 1H), 7.18 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 1H), 7.30 (dt, *J* = 7.9, 1.1 Hz, 1H), 7.55 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.67 (td, *J* = 7.7, 1.8 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 2.2 Hz, 1H), 8.52 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 15.5, 26.4, 27.5, 49.0, 52.1, 54.8, 56.2, 56.9, 116.8, 122.0, 122.4, 125.5, 127.8, 128.2, 134.7, 136.0, 136.9, 137.3, 141.8, 149.2, 157.0, 170.4.
TLC: Rf = 0.55 (EA).
LC-MS: Mass (ESI), calculated = 526.0 [M+H]+, found = 526.3.

(1*S*, 5*S*, 6*R*)-10-((3-Chloro-4-((trimethylsilyl)ethynyl)phenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one

(1*S*, 5*S*, 6*R*)-10-((4-Bromo-3-chlorophenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo [4.3.1]decan-2-one (702 mg, 1.34 mmol, 1.0 eq.), copper(I) iodide (128 mg, 0.67 mmol, 0.5 eq.) and **palladium-tetrakis(triphenylphosphine)** (774 mg, 0.67 mmol, 0.5 eq.) were dissolved in TMEDA (100mL) under argon. Ethynyltrimethylsilane (1.86 mL, 13.4 mmol, 10.0 eq.) was added and the mixture was stirred at 90 °C for 3 h. Brine (100 mL) was added and the mixture was extracted with DCM (2 x 100 mL). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography (150 g SiO2, CH:EA = 2:1 → 1:1)

Yield: 607 mg (1.12 mmol, 84 %).
Appearance: slightly brown foam.
1H-NMR (300 MHz, Chloroform-*d*): δ = 0.27 (s, 9H), 1.13 – 1.32 (m, 3H), 1.43 – 1.51 (m, 2H), 2.25 (d, *J* = 13.5 Hz, 1H), 2.59 – 2.74 (m, 1H), 3.07 (dd, *J* = 14.1, 2.0 Hz, 1H), 3.90 – 4.06 (m, 2H), 4.74 (d, *J* = 3.9 Hz, 2H), 4.80 (s, 1H), 4.91 – 5.07 (m, 2H), 5.59 – 5.77 (m, 1H), 7.17 (ddd, *J* = 7.6, 4.8, 1.1 Hz, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.54 – 7.71 (m, 3H), 7.84 (d, *J* = 1.6 Hz, 1H), 8.50 (dt, *J* = 4.9, 1.2 Hz, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = -0.2, 15.7, 26.4, 27.5, 49.1, 52.1, 54.8, 56.3, 57.0, 99.7, 105.1, 116.8, 122.1, 122.5, 124.3, 127.2, 127.5, 134.3, 137.0, 137.4, 137.4, 141.8, 149.3, 157.1, 170.6.
TLC: Rf = 0.50 (CH:EA = 1:2).
LC-MS: Mass (ESI), calculated = 542.2 [M+H]+, found = 542.8.

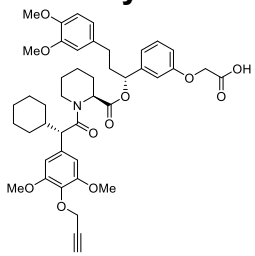
[5-100 % Solvent B, 20 min]: Rt = 15.1 min.
93 % purity (220 nm).

(1*S*, 5*S*, 6*R*)-10-((3-Chloro-4-ethynylphenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one

(1*S*, 5*S*, 6*R*)-10-((3-Chloro-4-((trimethylsilyl)ethynyl)phenyl)sulfonyl)-3-(pyridin-2-ylmethyl)-5-vinyl-3,10-diazabicyclo[4.3.1]decan-2-one (130 mg, 240 µmol, 1.0 eq.) and potassium carbonate (33 mg, 240 µmol, 1.0 eq) were dissolved in methanol (dry, 13 mL) and the mixture was stirred for 3 h at room temperature. Water (30 mL) was added and the mixture was extracted with EA (3 x 30 mL). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by preparative HPLC. The product was dried by lyophilisation.

Yield: 64 mg (57 %, 136 µmol).
Appearance: white solid.
1H-NMR (300 MHz, Chloroform-*d*): δ = 1.16 – 1.44 (m, 2H), 1.51 – 1.64 (m, 3H), 2.24 (d, *J* = 12.9 Hz, 1H), 2.77 (q, *J* = 8.3 Hz, 1H), 3.13 (d, *J* = 14.2 Hz, 1H), 3.59 (s, 1H), 4.07 (dd, *J* = 9.6, 3.8 Hz, 2H), 4.59 – 4.78 (m, 2H), 5.07 – 5.22 (m, 2H), 5.54 (d, *J* = 16.4 Hz, 1H), 5.75 (dt, *J* = 18.2, 9.4 Hz, 1H), 7.56 – 7.77 (m, 4H), 7.87 (s, 1H), 8.16 (t, *J* = 7.8 Hz, 1H), 8.78 (d, *J* = 5.4 Hz, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 15.5, 26.5, 27.4, 49.2, 53.1, 53.5, 54.9, 56.9, 78.9, 86.5, 117.7, 124.4, 124.5, 126.9, 127.3, 134.9, 136.5, 137.8, 142.1, 143.1, 144.2, 154.7, 171.7.
TLC: Rf = 0.40 (CH:EA = 1:2).
LC-MS: Mass (ESI), calculated = 470.1 [M+H]+, found = 470.1.
[50-100 % Solvent B, 2.7 min]: Rt = 1.0 min.
> 99 % purity (220 nm).
HRMS (ESI) m/z: [M+H]+ calculated for C24H24ClN3O3S = 470.12997; found = 470.12999.

9. Synthesis of alkyne 8:



Starting from (*R*)-1-(3-(2-(*tert*-Butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-piperidine-2-carboxylate; Synthesis previously described in: Gopalakrishnan R, Kozany C, Gaali S, Kress C, Hoogeland B, Bracher A, Hausch F: Evaluation of Synthetic FK506 Analogues as Ligands for the FK506-Binding Proteins 51 and 52. J. Med. Chem. 2012, 55:4114-4122.
And starting from (*S*)-2-(4-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethoxyphenyl)-2-cyclohexylacetic acid; Synthesis previously described [3].

(*R*)-1-(3-(2-(*tert*-Butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-((*S*)-2-(4-((*tert*-butyldiphenylsilyl)oxy)-3,5-dimethoxyphenyl)-2-cyclohexylacetyl)piperidine-2-carboxylate

(*S*)-2-(4-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethoxyphenyl)-2-cyclohexylacetic acid (362 mg, 0.68 mmol, 1.0 eq.) and HATU (176 mg, 0.75 mmol, 1.1 eq.) were dissolved in DCM (2 mL) and DMF (3mL). The mixture was cooled to 0 °C and DIPEA (356 µL, 2.04 mmol, 3.0 eq.) were added. The mixture was stirred for 15 min at 0 °C. (*R*)-1-(3-(2-(*tert*-Butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-piperidine-2-carboxylate (350 mg, 0.68 mmol, 1.0 eq.) in DCM (4 mL) was added and the mixture was stirred for 18 h at room temperature. Additional HATU (1.1 eq.) and DIPEA (3.0 eq.) were added and the mixture was stirred for 5 h at room temperature. The solution was concentrated under reduced pressure and the obtained product was purified by column chromatography.

Yield: 643 mg (92 %, 0.63 mmol).
Appearance: white foam.

1H-NMR (300 MHz, Chloroform-*d*): δ = 0.47 – 0.76 (m, 1H), 0.87 (t, *J* = 11.0 Hz, 1H), 1.06 – 1.15 (m, 14H), 1.19 – 1.35 (m, 3H), 1.48 (d, *J* = 1.9 Hz, 9H), 1.53 – 1.71 (m, 7H), 1.79 – 2.11 (m, 5H), 2.18 – 2.34 (m, 1H), 2.36 – 2.62 (m, 2H), 3.27 (s, 3H), 3.43 (d, *J* = 10.7 Hz, 3H), 3.79 – 3.89 (m, 6H), 4.50 (d, *J* = 1.8 Hz, 2H), 5.55 – 5.82 (m, 1H), 6.24 (d, *J* = 18.2 Hz, 2H), 6.47 – 7.01 (m, 6H), 7.08 – 7.45 (m, 10H), 7.60 – 7.73 (m, 5H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 20.2, 21.2, 25.6, 26.1, 26.3, 26.4, 26.4, 26.7, 27.1, 28.2, 30.6, 31.1, 33.0, 38.2, 41.4, 43.7, 55.3, 55.6, 55.8, 56.1, 65.8, 65.9, 75.7, 82.4, 105.7, 111.5, 111.8, 113.4, 113.9, 119.5, 120.4, 120.8, 127.0, 129.1, 129.2, 129.8, 130.5, 133.3, 133.8, 134.4, 135.4, 142.0, 147.4, 149.1, 151.0, 158.0, 168.1, 170.5, 172.6.
TLC: Rf = 0.78 (CH:EA = 1:1).
Rf = 0.35 (CH:EA = 3:1).
LC-MS: Mass (ESI), calculated = 1050.5 [M+H]+, found = 1050.6.
HPLC: [80-100 % Solvent B, 20 min]: Rt = 19.7 min.
75 % purity (220 nm).

(*R*)-1-(3-(2-(*tert*-Butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(4-hydroxy-3,5-dimethoxyphenyl)acetyl)piperidine-2-carboxylate

(*R*)-1-(3-(2-(*tert*-Butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-((*S*)-2-(4-((*tert*-butyldiphenylsilyl)oxy)-3,5-dimethoxyphenyl)-2-cyclohexylacetyl) piperidine-2-carboxylate (643 mg, 0.63 mmol, 1.0 eq.) was dissolved in THF (dry, 11 mL) and cooled to 0 °C under argon. TBAF (1 M in THF, 0.6 mL) was added and the mixture was stirred for 5 h at 0 °C to room temperature. The reaction was quenched with water (15 mL) and the mixture was extracted with DCM (3 x 40 mL). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography.

Yield: 420 mg (84 %, 0.53 mmol).
Appearance: white foam.
1H-NMR (300 MHz, Chloroform-*d*): δ = 1.47 (s, 9H), 1.56 – 1.70 (m, 10H), 1.76 – 1.98 (m, 4H), 2.03 (s, 2H), 2.25 – 2.31 (m, 1H), 2.32 – 2.48 (m, 2H), 2.49 – 2.63 (m, 1H), 2.75 (td, *J* = 13.4, 3.0 Hz, 1H), 3.31 – 3.37 (m, 1H), 3.67 (d, *J* = 9.2 Hz, 1H), 3.82 – 3.88 (m, 12H), 4.48 (s, 2H), 5.45 (d, *J* = 3.5 Hz, 2H), 5.58 (dd, *J* = 7.9, 5.7 Hz, 1H), 6.47 (s, 2H), 6.58 – 6.70 (m, 3H), 6.69 – 6.86 (m, 3H), 7.10 (t, *J* = 7.9 Hz, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 21.1, 25.7, 26.4, 26.7, 26.9, 28.2, 30.8, 31.1, 33.0, 38.1, 41.4, 43.7, 52.2, 55.0, 56.0, 56.4, 56.7, 60.5, 65.8, 75.7, 82.4, 105.7, 111.4, 112.0, 113.4, 113.7, 119.5, 120.4, 129.1, 129.7, 133.7, 133.8, 141.9, 147.1, 147.4, 149.0, 158.0, 168.1, 170.7, 172.6.
TLC: Rf = 0.42 (CH:EA = 2:1).
LC-MS: Mass (ESI), calculated = 790.4 [M+H]+, found = 790.4.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 19.8 min.
[70-100 % Solvent B, 20 min]: Rt = 7.1 min.
81 % purity (220 nm).

2-(3-((*R*)-1-(((*S*)-1-((*S*)-2-Cyclohexyl-2-(3,5-dimethoxy-4-(prop-2-yn-1-yloxy)phenyl) acetyl)piperidine-2-carbonyl)oxy)-3-(3,4-dimethoxyphenyl)propyl)phenoxy)acetic acid

(*R*)-1-(3-(2-(*tert*-Butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(4-hydroxy-3,5-dimethoxyphenyl)acetyl)piperidine-2-carboxylate (420 mg, 0.53 mmol, 1.0 eq.), 3-bromoprop-1-yne (76 mg, 0.64 mmol, 1.2 eq.) and potassium carbonate (732 mg, 5.30 mmol, 10.0 eq.) were stirred in acetone (5 mL) for 64 h at room temperature. The solution was filtered and concentrated under reduced pressure.

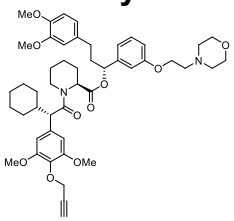
Appearance: colorless oil.
TLC: Rf = 0.23 (CH:EA = 3:1).
LC-MS: Mass (ESI), calculated = 828.4 [M+H]+, found = 828.5.

To crude (*R*)-1-(3-(2-(*tert*-butoxy)-2-oxoethoxy)phenyl)-3-(3,4-dimethoxyphenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,5-dimethoxy-4-(prop-2-yn-1-yloxy)phenyl)acetyl)piperidine-2-carboxylate in DCM (12 mL) TFA (5 mL) was added and the mixture was stirred for 1 h at room temperature. The solution was diluted with NH4Cl (sat., aq, 20 mL) and extracted with DCM (3 x 20 mL). The combined organic

phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography.

Yield: 220 mg (54 % o2s, 0.29 mmol).
Appearance: white solid.
1H-NMR (300 MHz, Chloroform-*d*): δ = 1.05 – 1.17 (m, 2H), 1.30 – 1.53 (m, 2H), 1.55 – 2.00 (m, 10H), 2.02 – 2.15 (m, 2H), 2.32 (d, *J* = 12.7 Hz, 1H), 2.44 – 2.66 (m, 2H), 2.86 (td, *J* = 13.4, 3.0 Hz, 1H), 3.35 (d, *J* = 9.3 Hz, 1H), 3.61 (s, 5H), 3.85 (d, *J* = 3.4 Hz, 9H), 4.03 – 4.17 (m, 1H), 4.63 (dd, *J* = 10.5, 3.6 Hz, 4H), 5.41 – 5.56 (m, 2H), 6.34 (s, 2H), 6.66 (dd, *J* = 9.2, 2.3 Hz, 4H), 6.74 – 6.84 (m, 2H), 7.16 (t, *J* = 7.8 Hz, 1H), 8.02 (s, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 21.0, 25.5, 26.3, 26.7, 27.3, 30.8, 31.5, 33.0, 38.3, 41.2, 43.7, 52.5, 55.4, 56.1, 56.2, 56.6, 60.1, 65.6, 74.9, 76.3, 79.7, 106.0, 110.2, 111.5, 111.9, 115.3, 119.7, 120.4, 129.7, 133.5, 133.6, 134.7, 142.5, 147.5, 149.1, 153.3, 158.0, 170.1, 171.4, 173.2.
TLC: Rf = 0.40 (CH:EA = 1:1, 1 % HCOOH).
HPLC: [0-100 % Solvent B, 20 min]: Rt = 18.1 min.
[50-100 % Solvent B, 20 min]: Rt = 10.7 min.
96 % purity (220 nm).
HRMS (ESI) m/z: [M+H]+ calculated for C44H53NO11 = 772.36914; found = 772.36891.

10. Synthesis of alkyne 9:



Starting from (*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl (*S*)-piperidine-2-carboxylate; Synthesis previously described in: Gopalakrishnan R, Kozany C, Gaali S, Kress C, Hoogeland B, Bracher A, Hausch F: Evaluation of Synthetic FK506 Analogues as Ligands for the FK506-Binding Proteins 51 and 52. J. Med. Chem. 2012, 55:4114-4122.
And starting from (*S*)-2-(4-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethoxyphenyl)-2-cyclohexylacetic acid; Synthesis previously described in [3].

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl (*S*)-1-((*S*)-2-(4-((*tert*-butyldiphenylsilyl)oxy)-3,5-dimethoxyphenyl)-2-cyclohexylacetyl)piperidine-2-carboxylate

(*S*)-2-(4-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethoxyphenyl)-2-cyclohexylacetic acid (MBA269, 218 mg, 0.41 mmol, 1.0 eq.) and HATU (106 mg, 0.45 mmol, 1.1 eq.) were dissolved in DCM (1 mL) and DMF (2mL). The mixture was cooled to 0 °C and DIPEA (215 µL, 1.23 mmol, 3.0 eq.) were added. The mixture was stirred for 15 min at 0 °C. (*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl (*S*)-piperidine-2-carboxylate (210 mg, 0.41 mmol, 1.0 eq.) in DCM (3 mL) was added and the mixture was stirred for 18 h at room temperature. Additional HATU (0.5 eq.) and DIPEA (1.5 eq.) were added and the mixture was stirred for 24 h at room temperature. The solution was concentrated under reduced pressure and the obtained product was purified by column chromatography.

Yield: 410 mg (97 %, 0.40 mmol).
Appearance: orange solid.
1H-NMR (300 MHz, Chloroform-*d*): δ = 0.79 – 1.07 (m, 1H), 1.13 – 1.28 (m, 1H), 1.36 (d, *J* = 11.6 Hz, 9H), 1.47 – 1.63 (m, 4H), 1.74 – 2.02 (m, 5H), 2.04 – 2.40 (m, 5H), 2.55 (d, *J* = 13.4 Hz, 1H), 2.71 (ddd, *J* = 22.7, 11.2, 6.0 Hz, 1H), 3.02 – 3.09 (m, 3H), 3.15 (s, 12H), 3.56 (d, *J* = 3.1 Hz, 2H), 3.58 – 3.70 (m, 2H), 3.71 (s, 1H), 4.01 – 4.19 (m, 8H), 4.24 – 4.53 (m, 3H), 5.58 – 5.91 (m, 1H), 6.50 – 6.79 (m, 2H), 6.84 – 7.32 (m, 5H), 7.40 – 7.66 (m, 6H), 7.82 – 8.06 (m, 4H), 8.24 (s, 2H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 20.5, 21.5, 26.8, 27.1, 30.9, 31.7, 33.3, 37.0, 38.5, 38.8, 41.9, 44.4, 53.0, 54.2, 55.6, 55.8, 56.3, 56.3, 57.9, 61.9, 65.0, 66.5, 76.6, 106.2, 112.4, 112.8, 113.4, 114.6, 119.7, 121.2, 127.5, 129.6, 129.7, 130.4, 131.0, 133.9, 134.5, 134.9, 135.8, 135.9, 142.5, 147.8, 149.4, 151.6, 158.9, 171.3, 174.0.
TLC: Rf = 0.15 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 1027.5 [M+H]+, found = 1027.6.
[5-100 % Solvent B, 2.6 min]: Rt = 1.9 min.
91 % purity (220 nm).

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(4-hydroxy-3,5-dimethoxyphenyl)acetyl)piperidine-2-carboxylate

((*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl (*S*)-1-((*S*)-2-(4-((*tert*-butyldiphenylsilyl)oxy)-3,5-dimethoxyphenyl)-2-cyclohexylacetyl)piperidine-2-carboxylate (365 mg, 355 µmol, 1.0 eq.) was dissolved in THF (dry, 7 mL) and cooled to 0 °C under argon. TBAF (1 M in THF, 355 µL) was added and the mixture was stirred for 5 h at 0 °C to room temperature. The reaction was quenched with water (10 mL) and the mixture was extracted with DCM (2 x 40 mL). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by column chromatography (25 g SiO2, EA:MeOH = 20:1).

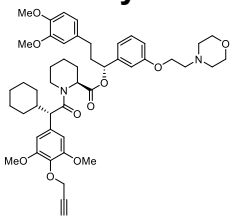
Yield: 197 mg (70 %, 250 µmol).
Appearance: white foam.
1H-NMR (300 MHz, Chloroform-*d*): δ = 0.53 – 0.95 (m, 2H), 1.29 (dd, *J* = 15.3, 5.6 Hz, 3H), 1.62 (t, *J* = 16.3 Hz, 5H), 1.80 – 2.12 (m, 4H), 2.29 (d, *J* = 13.6 Hz, 1H), 2.37 – 2.68 (m, 6H), 2.72 – 2.98 (m, 9H), 3.31 (d, *J* = 9.8 Hz, 1H), 3.73 (d, *J* = 20.9 Hz, 8H), 3.85 (d, *J* = 4.3 Hz, 7H), 4.07 (dt, *J* = 20.2, 6.0 Hz, 2H), 5.25 – 5.50 (m, 1H), 5.55 – 5.85 (m, 1H), 6.45 (d, *J* = 9.9 Hz, 2H), 6.52 (d, *J* = 5.0 Hz, 1H), 6.65 (d, *J* = 7.7 Hz, 2H), 6.69 – 7.00 (m, 2H), 7.10 (t, *J* = 8.0 Hz, 1H), 7.26 (s, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 21.1, 25.7, 26.4, 26.8, 26.9, 30.8, 31.3, 33.0, 38.0, 38.7, 41.4, 43.7, 52.2, 54.2, 55.2, 56.1, 56.4, 57.9, 65.1, 66.8, 75.8, 105.9, 111.5, 112.1, 113.1, 113.6, 119.3, 120.5, 128.8, 129.5, 129.9, 133.8, 134.2, 141.9, 147.5, 149.0, 158.8, 170.9, 172.5.
TLC: Rf = 0.18 (EA).
Rf = 0.42 (EA:MeOH = 10:1).
LC-MS: Mass (ESI), calculated = 789.4 [M+H]+, found = 789.7.
HPLC: [30-100 % Solvent B, 25 min]: Rt = 11.0 min.
96 % purity (220 nm).

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,5-dimethoxy-4-(prop-2-yn-1-yloxy)phenyl)acetyl)piperidine-2-carboxylate

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(4-hydroxy-3,5-dimethoxyphenyl)acetyl)piperidine-2-carboxylate (190 mg, 240 µmol, 1.0 eq.), 3-bromoprop-1-yne (34.5 mg, 290 µmol, 1.2 eq.) and potassium carbonate (332 mg, 2.40 mmol, 10.0 eq.) were stirred in acetone (3 mL) for 18 h at room temperature. Additional 3-bromoprop-1-yne (0.3 eq.) and potassium carbonate (1 eq.) were added and the mixture was stirred for 24 h at room temperature. The solution was filtered and concentrated under reduced pressure. The obtained product was purified by column chromatography.
Yield: 105 mg (53 %, 127 µmol).

Appearance: white foam.
1H-NMR (300 MHz, Chloroform-*d*): δ = 1.13 (ddd, *J* = 20.9, 10.3, 6.0 Hz, 2H), 1.19 – 1.36 (m, 3H), 1.51 – 1.71 (m, 5H), 1.96 – 2.13 (m, 4H), 2.25 – 2.30 (m, 1H), 2.57 (dq, *J* = 4.9, 2.4 Hz, 6H), 2.76 – 2.80 (m, 9H), 3.37 (d, *J* = 9.8 Hz, 1H), 3.72 (t, *J* = 4.7 Hz, 5H), 3.81 – 3.86 (m, 12H), 4.03 – 4.10 (m, 2H), 4.60 (d, *J* = 2.4 Hz, 2H), 5.42 – 5.49 (m, 1H), 5.54 (dd, *J* = 8.2, 5.5 Hz, 1H), 6.48 (s, 2H), 6.60 – 6.70 (m, 4H), 6.73 – 6.78 (m, 2H), 7.09 (t, *J* = 8.0 Hz, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 20.0, 24.5, 25.2, 25.6, 25.8, 29.6, 30.0, 31.8, 37.0, 37.6, 40.4, 42.6, 51.0, 53.1, 54.0, 54.9, 55.1, 56.6, 59.0, 64.7, 65.9, 73.7, 74.6, 78.6, 104.8, 110.3, 110.8, 112.0, 112.7, 117.5, 119.3, 128.5, 132.5, 133.3, 133.6, 140.8, 146.3, 147.8, 152.2, 157.6, 169.5, 171.3.
TLC: Rf = 0.38 (EA).
LC-MS: Mass (ESI), calculated = 827.4 [M+H]+, found = 827.7.
HPLC: [0-100 % Solvent B, 20 min]: Rt = 16.1 min.
[40-70 % Solvent B, 20 min]: Rt = 13.9 min.
96 % purity (220 nm).
HRMS (ESI) m/z: [M+H]+ calculated for C48H62N2O10 = 827.44772; found = 827.44770.

11. Synthesis of alkyne 13:



Starting from (*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-methoxyphenyl)propan-1-ol; Synthesis previously described in: Gopalakrishnan R, Kozany C, Gaali S, Kress C, Hoogeland B, Bracher A, Hausch F: Evaluation of Synthetic FK506 Analogues as Ligands for the FK506-Binding Proteins 51 and 52. J. Med. Chem. 2012, 55:4114-4122.
And starting from (*S*)-1-((*S*)-2-Cyclohexyl-2-(3-hydroxy-4,5-dimethoxyphenyl)acetyl)piperidine-2-carboxylic acid; Synthesis previously described in [3].

(*S*)-1-((*S*)-2-Cyclohexyl-2-(3,4-dimethoxy-5-(prop-2-yn-1-yloxy)phenyl)acetyl) piperidine-2-carboxylic acid

(*S*)-1-((*S*)-2-Cyclohexyl-2-(3-hydroxy-4,5-dimethoxyphenyl)acetyl)piperidine-2-carboxylic acid (214 mg, 0.53 mmol, 1.0 eq.), 3-bromoprop-1-yne (151 mg, 1.27 mmol, 2.4 eq.) and potassium carbonate (729 mg, 5.28 mmol, 10.0 eq.) were stirred in acetone (5 mL) for 18 h at room temperature. The solution was filtered and concentrated under reduced pressure.

Appearance: colorless oil.
TLC: Rf = 0.33 (CH:EA = 3:1).

To crude prop-2-yn-1-yl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,4-dimethoxy-5-(prop-2-yn-1-yloxy)phenyl)acetyl)piperidine-2-carboxylate (254 mg, 0.53 mmol, 1.0 eq.) in THF:water (1:1, 10 mL) lithium hydroxide (127 mg, 5.3 mmol, 10 eq.) was added and the mixture was stirred for 4 d at 70 °C. The solution was diluted with hydrochloric acid (1 M, aq, 50 mL) and extracted with DCM (3 x 30 mL).

The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by preparative HPLC. The product was dried by lyophilisation.

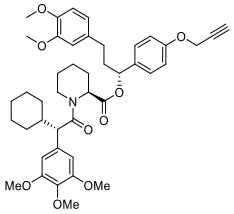
Yield: 180 mg (77 % o2s, 0.41 mmol).
Appearance: white solid.
1H-NMR (300 MHz, Chloroform-*d*): δ = 0.63 – 1.75 (m, 14H), 1.76 – 1.93 (m, 1H), 2.13 (dd, *J* = 61.4, 12.2 Hz, 2H), 2.45 (dt, *J* = 8.7, 2.4 Hz, 1H), 2.80 (dt, *J* = 84.2, 12.8 Hz, 1H), 3.25 (dd, *J* = 71.7, 9.7 Hz, 1H), 3.69 – 3.85 (m, 6H), 3.92 (d, *J* = 13.9 Hz, 1H), 4.71 (dd, *J* = 9.4, 2.6 Hz, 2H), 5.32 (q, *J* = 2.4 Hz, 1H), 6.42 – 6.60 (m, 2H), 10.69 (s, 2H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 20.9, 25.3, 26.1, 26.2, 26.2, 26.5, 26.6, 30.7, 32.8, 41.1, 43.9, 55.2, 56.1, 57.0, 61.0, 75.6, 78.7, 106.4, 108.8, 132.8, 137.8, 150.6, 153.3, 173.8, 176.0.
TLC: Rf = 0.30 (CH:EA = 1:1, 1 % HCOOH).
LC-MS: Mass (ESI), calculated = 444.2 [M+H]+, found = 444.2.
[5-100 % Solvent B, 2.6 min]: Rt = 2.0 min.
> 99 % purity (220 nm).

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-methoxyphenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,4-dimethoxy-5-(prop-2-yn-1-yloxy)phenyl)acetyl)piperidine-2-carboxylate

(*S*)-1-((*S*)-2-Cyclohexyl-2-(3,4-dimethoxy-5-(prop-2-yn-1-yloxy)phenyl)acetyl)piperidine-2-carboxylic acid (78 mg, 175 µmmol, 1.0 eq.) and 4-pyrolidinopyridine (104 mg, 700 µmol, 4.0 eq.) were weighted in a flask and flooded with argon. (*R*)-3-(3,4-Dimethoxyphenyl)-1-(3-methoxyphenyl)propan-1-ol (53 mg, 175 µmol, 1.0eq.) and toluene (dry, 15 mL) were added and the mixture was cooled to 0 °C. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (37 mg, 193 µmol, 1.1 eq.) was added and the mixture was stirred for 2 h at 0 °C to room temperature. The solution was diluted with hydrochloric acid (1 M, aq, 10 mL) and brine (40 mL) and extracted with DCM (2 x 40 mL). The combined organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by preparative HPLC. The product was dried by lyophilisation.

Yield: 66 mg (52 %, 91 µmol).
Appearance: white solid.
1H-NMR (500 MHz, Chloroform-*d*): δ = 0.50 – 0.80 (m, 1H), 0.82 – 1.02 (m, 1H), 1.04 – 1.74 (m, 8H), 1.76 – 2.19 (m, 4H), 2.24 – 2.50 (m, 3H), 2.59 (qdd, *J* = 20.3, 9.0, 5.8 Hz, 1H), 2.75 – 2.99 (m, 1H), 3.40 (d, *J* = 9.8 Hz, 1H), 3.65 – 3.88 (m, 15H), 3.95 – 4.03 (m, 1H), 4.63 – 4.78 (m, 2H), 5.40 – 5.49 (m, 1H), 5.53 – 5.59 (m, 1H), 6.39 – 6.53 (m, 1H), 6.55 – 6.71 (m, 4H), 6.73 – 6.81 (m, 2H), 6.84 – 6.97 (m, 1H), 7.22 (dt, *J* = 83.9, 7.9 Hz, 1H).
13C-NMR (126 MHz, Chloroform-*d*): δ = 20.9, 25.6, 26.1, 26.2, 26.6, 26.8, 30.7, 31.1, 32.8, 38.0, 41.3, 44.0, 52.5, 55.0, 55.3, 55.9, 56.1, 57.1, 61.0, 75.6, 76.0, 78.8, 106.4, 109.0, 111.5, 111.9, 112.5, 113.2, 118.5, 120.4, 129.8, 133.1, 133.5, 138.0, 141.7, 147.4, 148.9, 150.8, 153.6, 159.6, 170.3, 173.0.
TLC: Rf = 0.31 (CH:EA = 2:1).
LC-MS: [5-100 % Solvent B, 2.6 min]: Rt = 2.3 min.
> 99 % purity (220 nm).
HRMS (ESI) m/z: [M+H]+ calculated for C43H53NO9 = 728.37931; found = 728.37947.

12. Synthesis of alkyne 14:



Using (S)-2-Cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetic acid; Synthesis previously described in [4].

(E)-3-(3,4-Dimethoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

3,4-Dimethoxybenzaldehyde (6.65 g, 40.0 mmol, 1.0 eq.) and 1-(4-hydroxyphenyl)ethanone (5.45 g, 40.0 mmol, 1.0 eq.) were dissolved in EtOH (100 mL). The mixture was cooled to 0 °C and potassium hydroxide (8.98 g, 160 mmol, 4.0 eq.) in water (30 mL) was slowly added. The mixture was stirred for 18 h at 0 °C to room temperature. Additional potassium hydroxide (9.0 g 160 mmol, 4.0 eq.) was added and the mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure. Ice (100 mL) was added and a pH value of 5-6 was set by the addition of hydrochloric acid. The mixture was extracted with DCM (3 x 200 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by recrystallization in methanol.

Yield: 8.9 g (78 %, 31.3 mmol).
Appearance: yellow solid.
^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 3.74 (d, *J* = 1.5 Hz, 3H), 3.80 (d, *J* = 1.6 Hz, 3H), 6.82 – 6.89 (m, 2H), 6.93 (d, *J* = 8.3 Hz, 1H), 7.28 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.46 (d, *J* = 2.2 Hz, 1H), 7.59 (d, *J* = 15.4 Hz, 1H), 7.74 (d, *J* = 15.5 Hz, 1H), 8.00 – 8.05 (m, 2H), 10.31 (s, 1H).
^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 55.6, 55.7, 110.6, 111.5, 115.3, 119.7, 123.6, 127.8, 129.4, 131.1, 143.2, 149.0, 151.0, 162.0, 187.1.
TLC: R_f = 0.38 (CH:EA = 1:1)
LC-MS: Mass (ESI), calculated = 285.1 $[M+H]^+$, found = 285.2.
[5-100 % Solvent B, 3.0 min]: R_t = 1.7 min.
> 99 % purity (220 nm).

3-(3,4-Dimethoxyphenyl)-1-(4-hydroxyphenyl)propan-1-one

(*E*)-3-(3,4-Dimethoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (6.55 g, 23.0 mmol, 1.0 eq.) and ammonium chloride (123 g, 2.30 mol, 100 eq.) were dissolved in ethanol:water (2:1, 900 mL). Zn powder (4.5 g, 69.0 mmol, 3.0 eq) was slowly added over 1 h. After complete addition ethanol was removed under reduced pressure. The mixture was extracted with DCM (3 x 300 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 1.89 g (29 %, 6.60 mmol).
Appearance: white solid.
^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 2.87 (dd, *J* = 8.4, 6.8 Hz, 2H), 3.04 – 3.14 (m, 2H), 3.73 (d, *J* = 3.9 Hz, 6H), 6.66 (dd, *J* = 2.8, 1.7 Hz, 3H), 6.73 – 6.79 (m, 2H), 7.71 – 7.77 (m, 2H), 9.46 (s, 1H).
^{13}C-NMR (74 MHz, Chloroform-*d*): δ = 30.0, 40.0, 55.7, 55.9, 111.4, 111.9, 115.4, 120.2, 128.7, 130.4, 130.7, 134.1, 147.2, 148.8, 162.1, 197.9.
TLC: R_f = 0.28 (CH:EA = 2:1)
LC-MS: Mass (ESI), calculated = 287.1 $[M+H]^+$, found = 287.2.
[5-100 % Solvent B, 3.0 min]: R_t = 1.7 min.
96 % purity (220 nm).

(*R*)-4-(3-(3,4-dimethoxyphenyl)-1-hydroxypropyl)phenol

3-(3,4-Dimethoxyphenyl)-1-(4-hydroxyphenyl)propan-1-one (410 mg, 1.43 mmol, 1.0 eq.) was dissolved in *iso*-propanole (20 mL) and degassed by argon. $RuCl_2$[(S)-dm-segphos®][(*S*)-daipen] (36 mg, 0.03 mmol, 0.02 eq.) was added and the mixture was sparged with H_2. Potassium *tert*-butoxide (481 mg, 4.29 mmol, 3.0 eq.) was added and the mixture was stirred for 18 h at room temperature. The mixture was concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 266 mg (64 %, 0.92 mmol).
Appearance: white solid.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.93 – 2.19 (m, 2H), 2.55 – 2.71 (m, 2H), 3.83 – 3.91 (m, 6H), 4.63 (dd, *J* = 7.8, 5.5 Hz, 1H), 6.69 – 6.74 (m, 2H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.80 – 6.84 (m, 1H), 7.21 – 7.24 (m, 2H), 7.26 (d, *J* = 0.8 Hz, 1H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 136.9, 134.6, 127.6, 120.4, 115.5, 112.0, 111.5, 73.7, 56.1, 56.0, 40.6, 31.9.
TLC: R_f = 0.21 (CH:EA = 1:1).
R_f = 0.41 (DCM:MeOH = 20:1).

LC-MS: Mass (ESI), calculated = 289.1 $[M+H]^+$, found = 271.2.
[5-100 % Solvent B, 2.6 min]: R_t = 1.6 min.
88 % purity (220 nm).

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)propan-1-ol

(*R*)-4-(3-(3,4-Dimethoxyphenyl)-1-hydroxypropyl)phenol (1.38 g, 4.79 mmol, 1.0 eq.), 3-bromoprop-1-yne (684 mg, 5.75 mmol, 1.2 eq.) and potassium carbonate (6.62 g, 47.9 mmol, 10.0 eq.) were stirred in acetone (50 mL) for 18 h at room temperature. The solution was filtered and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 1.23 g (79 %, 3.89 mmol).
Appearance: white solid.
^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 1.91 – 2.19 (m, 3H), 2.52 (t, *J* = 2.4 Hz, 1H), 2.64 (tdt, *J* = 13.9, 9.1, 6.7 Hz, 2H), 3.84 (d, *J* = 1.3 Hz, 6H), 4.62 (dd, *J* = 7.7, 5.5 Hz, 1H), 4.67 (d, *J* = 2.4 Hz, 2H), 6.68 – 6.81 (m, 3H), 6.92 – 6.98 (m, 2H), 7.24 – 7.31 (m, 2H).
^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 31.7, 40.5, 55.8, 55.9, 55.9, 73.4, 75.6, 78.6, 111.4, 111.9, 114.9, 120.2, 127.2, 134.4, 137.7, 147.2, 148.9, 157.0.
TLC: R_f = 0.45 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 349.2 $[M+Na]^+$, found = 349.2.
[5-100 % Solvent B, 3.0 min]: R_t = 1.9 min.
[30-100 % Solvent B, 2.6 min]: R_t = 1.3 min.
> 99 % purity (220 nm).

1-((9H-Fluoren-9-yl)methyl) 2-((*R*)-3-(3,4-dimethoxyphenyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)propyl) (*S*)-piperidine-1,2-dicarboxylate

(*S*)-1-(((9H-Fluoren-9-yl)methoxy)carbonyl)piperidine-2-carboxylic acid (21 mg, 61 µmol, 1.0 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (14 mg, 74 µmol, 1.2 eq.) and 4-dimethylaminopyridine (2.2 mg, 18 µmol, 0.3 eq.) were cooled to 0 °C under argon. DCM (dry, 1 mL) was added. (*R*)-3-(3,4-Dimethoxyphenyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)propan-1-ol (20 mg, 61 µmol, 1.0 eq.) was added and the mixture was stirred for 15 min at 0 °C followed by 18 h at room temperature. The solution was concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 23 mg (58 %, 35 µmol).
Appearance: white foam.
TLC: R_f = 0.65 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 682.3 $[M+Na]^+$, found = 682.0.
[5-100 % Solvent B, 3.0 min]: R_t = 2.6 min.
99 % purity (220 nm).

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)propyl (*S*)-piperidine-2-carboxylate

1-((9H-Fluoren-9-yl)methyl) 2-((*R*)-3-(3,4-dimethoxyphenyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)propyl) (*S*)-piperidine-1,2-dicarboxylate (60 mg, 91 µmol, 1.0 eq.) and 4-methylpiperidine (44 µL, 373 µmol, 4.1 eq) were dissolved in DCM (400 µL) and the mixture was stirred for 3 h at room temperature. The obtained product was purified by by flash chromatography.

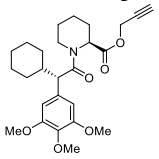
Yield: 30 mg (75 %, 69 µmol).
Appearance: yellow oil.
TLC: R_f = 0.26 (CH:EA = 1:1, 3 % TEA).
LC-MS: Mass (ESI), calculated = 438.2 $[M+H]^+$, found = 438.0.
[5-100 % Solvent B, 3.0 min]: R_t = 1.6 min.
99 % purity (220 nm).

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate

(*S*)-1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylic acid (32 mg, 105 µmol, 1.0 eq.) and HATU (27 mg, 116 µmol, 1.1 eq.) were dissolved in DCM (1.2 mL) and DMF (1.8mL). The mixture was cooled to 0 °C and DIPEA (55 µL, 315 µmol, 3.0 eq.) was added. The mixture was stirred for 15 min at 0 °C. (*R*)-3-(3,4-Dimethoxyphenyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)propyl (*S*)-piperidine-2-carboxylate (46 mg, 105 µmol, 1.0 eq.) in DCM (3 mL) was added and the mixture was stirred for 18 h at 0 °C to room temperature. The solution was concentrated under reduced pressure and the obtained product was purified by preparative HPLC. The product was dried by lyophilisation.

Yield: 68 mg (89 %, 93 µmol).
Appearance: white solid.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 0.70 – 0.81 (m, 1H), 0.89 (qd, *J* = 12.5, 3.3 Hz, 1H), 1.07 – 1.47 (m, 6H), 1.51 – 2.17 (m, 10H), 2.25 – 2.69 (m, 5H), 3.38 (d, *J* = 10.0 Hz, 1H), 3.72 – 3.88 (m, 17H), 3.96 (d, *J* = 14.7 Hz, 1H), 4.68 (dd, *J* = 18.2, 2.4 Hz, 2H), 5.48 (d, *J* = 5.4 Hz, 1H), 5.59 (dd, *J* = 7.7, 6.3 Hz, 1H), 6.47 (d, *J* = 62.9 Hz, 2H), 6.59 – 6.70 (m, 2H), 6.73 – 6.82 (m, 4H), 6.96 – 7.02 (m, 0H), 7.29 – 7.36 (m, 1H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 20.8, 25.6, 26.0, 26.1, 26.5, 30.6, 31.0, 32.7, 37.7, 41.2, 43.9, 52.2, 55.0, 55.7, 55.8, 55.9, 56.1, 56.3, 60.8, 75.5, 105.7, 111.3, 111.8, 114.6, 120.2, 127.8, 133.0, 133.4, 137.0, 147.3, 148.8, 153.2, 157.2, 170.2, 172.6.
TLC: R_f = 0.31 (CH:EA = 2:1).
LC-MS: [5-100 % Solvent B, 3.0 min]: R_t = 2.5 min.
[50-100 % Solvent B, 2.6 min]: R_t = 1.9 min.
99 % purity (220 nm).
HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{43}H_{53}NO_9$ = 728.37931; found = 728.38039.

13. Synthesis of alkyne 15:



Starting from (S)-2-Cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetic acid; Synthesis previously described in [4].

Prop-2-yn-1-yl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate

(*S*)-1-((*S*)-2-Cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylic acid (250 g, 596 µmol, 1.0 eq.), 3-bromoprop-1-yne (213 mg, 1788 µmol, 3.0 eq.) and DIPEA (507 µL, 2980 µmol, 5.0 eq.) were stirred in acetonitrile (dry, 6 mL) for 18 h at room temperature. The solution was concentrated under reduced pressure. The obtained product was purified by preparative HPLC. The product was dried by lyophilisation.

Yield: 259 mg (95 %, 566 µmol).
Appearance: white solid.
TLC: R_f = 0.50 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 458.3 $[M+H]^+$, found = 458.2.
[5-100 % Solvent B, 2.6 min]: R_t = 2.1 min.
[30-100 % Solvent B, 2.6 min]: R_t = 1.8 min.
95 % purity (220 nm).

14. Synthesis of PROTACs:

Azides (1.0 eq.) and alkynes (1.0 eq.) were dissolved in *tert*-butanol, water and DMSO (1:1:10). The solution was degassed by argon. Copper(II) sulfate pentahydrate (1 M in water, 0.4 eq.) and (+)-sodium L-ascorbate (1 M in water, 0.4 eq.) were added. The solution was stirred for 18 h at room temperature. DCM was added and the mixture was washed with brine. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by chromatography and dried by lyophilisation.

Table 2.: Characterization of first generation PROTACs.

	linker length	1	2	3	4	5
alkyne	E3 ligand					
A1	a	**Yield**: 9.0 mg (58 %, 8.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.1 min. [20-80 % Solvent B, 20 min]: R_t = 10.2 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{48}H_{56}Cl_2N_{10}O_8S_2$ = 1035.31738; found = 1035.31674.	**Yield**: 6.5 mg (40 %, 6.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.3 min. [30-60 % Solvent B, 20 min]: R_t = 11.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{50}H_{60}Cl_2N_{10}O_9S_2$ = 1079.34360; found = 1079.34353.	**Yield**: 12.0 mg (71 %, 10.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.4 min. [30-100 % Solvent B, 20 min]: R_t = 7.8 min. 96 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{64}Cl_2N_{10}O_{10}S_2$ = 1123.36981; found = 1123.37016.	**Yield**: 12.0 mg (69 %, 10.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.4 min. [30-60 % Solvent B, 20 min]: R_t = 11.3 min. 96 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{68}Cl_2N_{10}O_{11}S_2$ = 1189.37797; found = 1189.37857.	**Yield**: 10.4 mg (57 %, 8.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.3 min. [20-80 % Solvent B, 20 min]: R_t = 11.4min. > 99 % purity. **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{72}Cl_2N_{10}O_{12}S_2$ = 1211.42224; found = 1211.42163.
	b	**Yield**: 10.2 mg (94 %, 9.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.7 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{50}H_{57}Cl_2FN_{10}O_8S_2$ = 1079.32361; found = 1079.32534.	**Yield**: 5.0 mg (44 %, 4.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.9 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{61}Cl_2FN_{10}O_9S_2$ = 1123.34983; found = 1123.35172.	**Yield**: 4.5 mg (39 %, 3.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.7 min]: R_t = 1.6 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{65}Cl_2FN_{10}O_{10}S_2$= 1167.37604; found = 1167.37653.	**Yield**: 4.3 mg (35 %, 3.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.1 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{69}Cl_2FN_{10}O_{11}S_2$ = 1211.40226; found = 1211.40402.	**Yield**: 7.2 mg (57 %, 5.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.7 min]: R_t = 1.6 min. [50-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{58}H_{73}Cl_2FN_{10}O_{12}S_2$= 1255.42847; found = 1255.43025.
	c	**Yield**: 10.0 mg (76 %, 11.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.28 (DCM:MeOH = 20:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.1 min. [30-60 % Solvent B, 20 min]: R_t = 9.9 & 10.1 min (diastereromers). > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{39}H_{37}Cl_2N_9O_9S$ = 878.18848; found = 878.18695.	**Yield**: 12.5 mg (91 %, 13.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.29 (DCM:MeOH = 20:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.4 min. [30-60 % Solvent B, 20 min]: R_t = 10.7 & 10.8 min (diastereomers). > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{41}H_{41}Cl_2N_9O_{10}S$ = 922.21469; found = 922.21361.	**Yield**: 13.0 mg (90 %, 13.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.25 (DCM:MeOH = 20:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.6 min. [30-60 % Solvent B, 20 min]: R_t = 11.1 min. 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{43}H_{45}Cl_2N_9O_{11}S$ = 966.24091; found = 966.23813.	**Yield**: 9.0 mg (59 %, 8.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.22 (DCM:MeOH = 20:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.6 min. [20-80 % Solvent B, 20 min]: R_t = 11.5 min. 96 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{45}H_{49}Cl_2N_9O_{12}S$ = 1010.26712; found = 1010.26580.	**Yield**: 15.0 mg (95 %, 14.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.21 (DCM:MeOH = 20:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.8 min. [20-80 % Solvent B, 20 min]: R_t = 11.7 min. 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{47}H_{53}Cl_2N_9O_{13}S$ = 1054.29334; found = 1054.29483.
A2	a	**Yield**: 12.8 mg (79 %, 11.9 µmol). **Appearance**: white solid.	**Yield**: 5.7 mg (34 %, 5.1 µmol). **Appearance**: white solid.	**Yield**: 11.5 mg (66 %, 9.8 µmol). **Appearance**: white solid.	**Yield**: 14.1 mg (77 %, 11.6 µmol). **Appearance**: white solid.	**Yield**: 10.6 mg (57 %, 8.4 µmol). **Appearance**: white solid.

		TLC: R_f = 0.29 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.2 min. [20-80 % Solvent B, 20 min]: R_t = 11.2 min. 100 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{50}H_{60}Cl_2N_{10}O_9S_2$ = 1079.3436; found = 1079.3426.	**TLC:** R_f = 0.33 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.3 min. [20-80 % Solvent B, 20 min]: R_t = 11.4 min. 95 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{64}Cl_2N_{10}O_{10}S_2$ = 1123.2398; found = 1123.3679.	**TLC:** R_f = 0.33 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.4 min. [20-80 % Solvent B, 20 min]: R_t = 11.6 min. 100 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{68}Cl_2N_{10}O_{11}S_2$ = 1167.3960; found = 1167.3971.	**TLC:** R_f = 0.31 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.4 min. [20-80 % Solvent B, 20 min]: R_t = 11.6 min. 100 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{72}Cl_2N_{10}O_{12}S_2$ = 1211.4222; found = 1211.4242.	**TLC:** R_f = 0.40 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.3 min. [20-80 % Solvent B, 20 min]: R_t = 11.6 min. 100 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{58}H_{76}Cl_2N_{10}O_{13}S_2$ = 1255.4485; found = 1255.4451.
	b	**Yield**: 5.0 mg (56 %, 4.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.7 min]: R_t = 1.5 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{61}Cl_2FN_{10}O_9S_2$ = 1123.34983; found = 1123.35134.	**Yield**: 5.0 mg (54 %, 4.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.7 min]: R_t = 1.5 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{65}Cl_2FN_{10}O_{10}S_2$ = 1167.37604; found = 1167.37735.	**Yield**: 5.3 mg (55 %, 4.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.07 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.7 min]: R_t = 1.6 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{69}Cl_2FN_{10}O_{11}S_2$= 1211.40226; found = 1211.40424.	**Yield**: 1.8 mg (18 %, 1.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.07 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.7 min]: R_t = 1.6 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{58}H_{73}Cl_2FN_{10}O_{12}S_2$= 1255.42847; found = 1255.43060.	**Yield**: 5.2 mg (50 %, 4.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.07 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.7 min]: R_t = 1.6 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{58}H_{73}Cl_2FN_{10}O_{12}S_2$= 1255.42847; found = 1255.43060.
	c	**Yield**: 13.3 mg (96 %, 14.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.75 (DCM:MeOH = 20:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.2 min. [20-80 % Solvent B, 20 min]: R_t = 11.0 min. 99 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{41}H_{41}Cl_2N_9O_{10}S$ = 922.2147; found = 922.2136.	**Yield**: 12.1 mg (83 %, 12.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.53 (DCM:MeOH = 20:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.6 min. [20-80 % Solvent B, 20 min]: R_t = 11.7 min. 96 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{43}H_{45}Cl_2N_9O_{11}S$ = 966.2409; found = 966.2404.	**Yield**: 10.4 mg (69 %, 10.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.56 (DCM:MeOH = 20:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.7 min. [20-80 % Solvent B, 20 min]: R_t = 11.8 min. 100 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{45}H_{49}Cl_2N_9O_{12}S$ = 1010.2671; found = 1010.2671.	**Yield**: 14.4 mg (91 %, 13.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.4.7 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.8 min. [20-80 % Solvent B, 20 min]: R_t = 11.9 min. 97 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{47}H_{53}Cl_2N_9O_{13}S$ = 1054.2933; found = 1054.2933.	**Yield**: 16.4 mg (99 %, 14.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.40 (DCM:MeOH = 10:1). **HPLC:** [0-100 % Solvent B, 20 min]: R_t = 12.8 min. [20-80 % Solvent B, 20 min]: R_t = 12.0 min. 97 % purity **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{49}H_{57}Cl_2N_9O_{14}S$ = 1098.3196; found = 1098.3194.
A3	a	**Yield**: 14.9 mg (97 %, 14.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.7 min 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{50}H_{59}ClN_{10}O_8S_2$= 514.18964; found = 514.19004.	**Yield**: 15.8 mg (98 %, 14.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.7 min 94 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{52}H_{63}ClN_{10}O_9S_2$ = 536.20275; found = 536.20290.	**Yield**: 12.5 mg (75 %, 11.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.7 min 93 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{54}H_{67}ClN_{10}O_{10}S_2$ = 558.21586; found = 558.21627.	**Yield**: 16.8 mg (97 %, 14.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.7 min 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{56}H_{71}ClN_{10}O_{11}S_2$ = 580.22896; found = 580.22869.	**Yield**: 15.1 mg (83 %, 12.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.7 min 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{58}H_{75}ClN_{10}O_{12}S_2$ = 602.24207; found = 602.24277.
	b	**Yield**: 6.5 mg (61 %, 6.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1).	**Yield**: 5.0 mg (45 %, 4.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1).	**Yield**: 3.8 mg (33 %, 3.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1).	**Yield**: 5.0 mg (42 %, 4.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.09 (DCM:MeOH = 10:1).	**Yield**: 6.1 mg (49 %, 4.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1).

		LC-MS: [50-100 % Solvent B, 2.7 min]: R_t = 1.2 min 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{60}ClFN_{10}O_8S_2$ = 1071.37823; found = 1071.37582.	**LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.0 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{64}ClFN_{10}O_9S_2$ = 1115.40445; found = 1115.40269.	**LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.1 min 95 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{68}ClFN_{10}O_{10}S_2$ = 1159.43066; found = 1159.43165.	**LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.1 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{58}H_{72}ClFN_{10}O_{11}S_2$ = 1203.45688; found = 1203.45299.	**LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.2 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{60}H_{76}ClFN_{10}O_{12}S_2$ = 1247.48309; found = 1247.47983.
	c	**Yield**: 8.8 mg (68 %, 10.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0.28 (DCM:MeOH = 20:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.6 min 96 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{41}H_{40}ClN_9O_9S$ = 870.24310; found = 870.24396.	**Yield**: 10.1 mg (74 %, 11.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.26 (DCM:MeOH = 20:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.6 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{43}H_{44}ClN_9O_{10}S$ =914.26931; found = 914.27007.	**Yield**: 10.7 mg (75 %, 11.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.25 (DCM:MeOH = 20:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.7 min 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{45}H_{48}ClN_9O_{11}S$ = 958.29553; found = 958.29647.	**Yield**: 9.0 mg (60 %, 9.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.24 (DCM:MeOH = 20:1). **LC-MS:** [30-100 % Solvent B, 2.2 min]: R_t = 1.5 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{47}H_{52}ClN_9O_{12}S$ = 1002.32174; found = 1002.32255.	**Yield**: 13.4 mg (85 %, 12.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.24 (DCM:MeOH = 20:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.8 min 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{50}H_{59}ClN_{10}O_8S_2$=1046.34796; found = 1046.34976.
A4	a	**Yield**: 6.4 mg (89 %, 6.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.8 min. 96 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{50}H_{59}ClN_{10}O_8S_2$ = 1027.37201; found = 1027.37209.	**Yield**: 6.8 mg (91 %, 6.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.8 min. 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{63}ClN_{10}O_9S_2$= 1071.39822; found = 1071.39822.	**Yield**: 2.2 mg (28 %, 2.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{67}ClN_{10}O_{10}S_2$ = 1115.42443; found = 1115.42399.	**Yield**: 4.7 mg (58 %, 4.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0.09 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{71}ClN_{10}O_{11}S_2$ = 1159.45065; found = 1159.44975.	**Yield**: 4.9 mg (58 %, 4.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0.09 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{58}H_{75}ClN_{10}O_{12}S_2$ = 1203.47686; found = 1203.47595.
	b	**Yield**: 2.2 mg (29 %, 2.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{60}ClFN_{10}O_8S_2$ = 1071.37823; found = 1071.37928.	**Yield**: 5.1 mg (65 %, 4.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{64}ClFN_{10}O_9S_2$ = 1115.40445; found = 1115.40662.	**Yield**: 5.3 mg (65 %, 4.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.09 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{68}ClFN_{10}O_{10}S_2$ = 1159.43066; found = 1159.43337.	**Yield**: 5.9 mg (70 %, 4.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.09 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. 96 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{58}H_{72}ClFN_{10}O_{11}S_2$ = 1203.45688; found = 1203.45942.	**Yield**: 6.9 mg (79 %, 5.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.09 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{60}H_{76}ClFN_{10}O_{12}S_2$ = 1247.48309; found = 1247.48621.
	c	**Yield**: 4.9 mg (80 %, 5.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.22 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.3 min.	**Yield**: 5.4 mg (84 %, 5.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.20 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.3 min.	**Yield**: 6.5 mg (97 %, 6.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.19 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.4 min.	**Yield**: 2.2 mg (31 %, 2.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.19 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.4 min.	**Yield**: 7.1 mg (97 %, 6.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.18 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.4 min.

		97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{41}H_{40}ClN_9O_9S$ = 870.24310; found = 870.24374.	> 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{43}H_{44}ClN_9O_{10}S$ = 914.26931; found = 914.26943.	95 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{45}H_{48}ClN_9O_{11}S$ = 958.29553; found = 958.29545.	99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{47}H_{52}ClN_9O_{12}S$ = 1002.32174; found = 1002.32214.	95 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{49}H_{56}ClN_9O_{13}S$ = 1046.34796; found = 1046.34806.
A5	a	TLC [DCM:MeOH 93:7]: Rf = 0.29. HPLC [0-100% Solvent B, 20 min]: Rt = 14.74 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 984.3058 [M+H]+, calculated 984.3058 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.33. HPLC [0-100% Solvent B, 20 min]: Rt = 14.81 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1028.3323 [M+H]+, calculated 1028.3327 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.26. HPLC [0-100% Solvent B, 20 min]: Rt = 14.79 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1072.3588 [M+H]+, calculated 1072.3589 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.26. HPLC [0-100% Solvent B, 20 min]: Rt = 14.79 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1116.3859 [M+H]+, calculated 1116.3851 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.21. HPLC [0-100% Solvent B, 20 min]: Rt = 14.78 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1160.4113 [M+H]+, calculated 1160.4113 [M+H]+
	b	**Yield**: 9.6 mg (93 %, 9.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.14 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{47}H_{56}Cl_2FN_9O_8S_2$= 1028.31271; found = 1028.30930.	**Yield**: 9.6 mg (90 %, 9.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.13 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.9 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{49}H_{60}Cl_2FN_9O_9S_2$= 1072.33893; found = 1072.33752.	**Yield**: 11.0 mg (98 %, 9.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.9 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{51}H_{64}Cl_2FN_9O_{10}S_2$= 1116.36514; found = 1116.36245.	**Yield**: 11.5 mg (99 %, 9.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.9 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{53}H_{68}Cl_2FN_9O_{11}S_2$= 1160.39136; found = 1160.38912.	**Yield**: 10.0 mg (82 %, 8.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.9 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{55}H_{72}Cl_2FN_9O_{12}S_2$= 1204.41757; found = 1204.41453.
	c	TLC [DCM:MeOH 97:3]: Rf = 0.36. HPLC [50-100% Solvent B, 20 min]: Rt = 6.49 min, purity (254 nm) = 96%. HRMS: m/z: found 827.17767 [M+H]+, calculated 827.17758 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.32. HPLC [0-100% Solvent B, 20 min]: Rt = 15.67 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 871.2043 [M+H]+, calculated 871.2038 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.30. HPLC [0-100% Solvent B, 20 min]: Rt = 15.57 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 915.22996 [M+H]+, calculated 915.23001 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.24. HPLC [0-100% Solvent B, 20 min]: Rt = 15.61 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 959.25696 [M+H]+, calculated 959.25622 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.26. HPLC [0-100% Solvent B, 20 min]: Rt = 15.51 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1003.28266 [M+H]+, calculated 1003.28244 [M+H]+
A6	a	TLC [DCM:MeOH 93:7]: Rf = 0.29. HPLC [0-100% Solvent B, 20 min]: Rt = 14.94 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 998.3224 [M+H]+, calculated 998.3221 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.30. HPLC [0-100% Solvent B, 20 min]: Rt = 15.05 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1042.3485 [M+H]+, calculated 1042.3483 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.23. HPLC [0-100% Solvent B, 20 min]: Rt = 15.12min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1086.3753 [M+H]+, calculated 1086.3746 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.17. HPLC [0-100% Solvent B, 20 min]: Rt = 15.07 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1130.4008 [M+H]+, calculated 1130.4008 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.21. HPLC [0-100% Solvent B, 20 min]: Rt = 15.08 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1174.4276 [M+H]+, calculated 1174.4270 [M+H]+
	b	**Yield**: 7.2 mg (69 %, 6.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.15 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.3 min. 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{48}H_{58}Cl_2FN_9O_8S_2$= 1042.3284; found = 1042.3305.	**Yield**: 7.0 mg (64 %, 6.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.14 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.3 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{50}H_{62}Cl_2FN_9O_9S_2$= 1086.3546; found = 1086.3564.	**Yield**: 7.0 mg (64 %, 6.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.14 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.4 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{66}Cl_2FN_9O_{10}S_2$= 1130.3808; found = 1130.3822.	**Yield**: 8.5 mg (72 %, 7.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.13 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.4 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{70}Cl_2FN_9O_{11}S_2$= 1174.4070; found = 1174.4089.	**Yield**: 7.6 mg (62 %, 6.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.13 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.4 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{74}Cl_2FN_9O_{12}S_2$= 1218.4332; found = 1218.4350.

	c	TLC [DCM:MeOH 97:3]: Rf = 0.36. HPLC [0-100% Solvent B, 20 min]: Rt = 15.56 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 841.19396 [M+H]+, calculated 841.19323 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.26. HPLC [0-100% Solvent B, 20 min]: Rt = 15.83 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 885.21874 [M+H]+, calculated 885.21944 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.30. HPLC [0-100% Solvent B, 20 min]: Rt = 15.81 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 929.24548 [M+H]+, calculated 929.24566 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.24. HPLC [0-100% Solvent B, 20 min]: Rt = 15.82 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 973.27224 [M+H]+, calculated 973.27187 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.24. HPLC [0-100% Solvent B, 20 min]: Rt = 15.87 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1017.29857 [M+H]+, calculated 1017.29809 [M+H]+
A7	a	TLC [DCM:MeOH 93:7]: Rf = 0.29. HPLC [0-100% Solvent B, 20 min]: Rt = 15.04 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 998.32293 [M+H]+, calculated 998.32213 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.33. HPLC [0-100% Solvent B, 20 min]: Rt = 15.11 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1042.34904 [M+H]+, calculated 1042.34835 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.29. HPLC [0-100% Solvent B, 20 min]: Rt = 15.12 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1086.37535 [M+H]+, calculated 1086.37456 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.21. HPLC [0-100% Solvent B, 20 min]: Rt = 15.12 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1130.39944 [M+H]+, calculated 1130.40078 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.24. HPLC [0-100% Solvent B, 20 min]: Rt = 15.10 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1174.42451 [M+H]+, calculated 1174.42699 [M+H]+
	c	TLC [DCM:MeOH 97:3]: Rf = 0.39. HPLC [0-100% Solvent B, 20 min]: Rt = 15.71 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 841.19364 [M+H]+, calculated 841.19323 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.32. HPLC [0-100% Solvent B, 20 min]: Rt = 15.93 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 885.21980 [M+H]+, calculated 885.21944 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.33. HPLC [0-100% Solvent B, 20 min]: Rt = 15.90 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 929.24586 [M+H]+, calculated 929.24566 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.27. HPLC [0-100% Solvent B, 20 min]: Rt = 15.92 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 973.27295 [M+H]+, calculated 973.27187 [M+H]+	TLC [DCM:MeOH 97:3]: Rf = 0.27. HPLC [0-100% Solvent B, 20 min]: Rt = 15.88 min, purity (254 nm) ≥ 99%. HRMS: m/z: found 1017.29876 [M+H]+, calculated 1017.29809 [M+H]+
A8	a	**Yield**: 14.5 mg (73 %, 10.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.9 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{70}H_{88}N_8O_{16}S$ =665.30923; found = 665.30995.	**Yield**: 14.0 mg (68 %, 10.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.9 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{72}H_{92}N_8O_{17}S$ =687.32233; found = 687.32312.	**Yield**: 11.7 mg (55 %, 8.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 2.0 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{74}H_{96}N_8O_{18}S$ = 709.33544; found = 709.33577.	**Yield**: 21.4 mg (98 %, 14.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 2.0 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{76}H_{100}N_8O_{19}S$ = 731.34855; found = 731.34905.	**Yield**: 18.3 mg (81 %, 12.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 2.0 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{78}H_{104}N_8O_{20}S$ =753.36166; found = 753.36246.
	b	**Yield**: 10.2 mg (74 %, 7.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.5 min. 96 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{72}H_{89}FN_8O_{16}S$ = 1373.61740; found = 1373.61759.	**Yield**: 11.9 mg (84 %, 8.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.5 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{74}H_{93}FN_8O_{17}S$ = 1417.64362; found = 1417.64346.	**Yield**: 12.2 mg (84 %, 8.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.5 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{76}H_{97}FN_8O_{18}S$ = 1461.66983; found = 1461.66976.	**Yield**: 10.9 mg (73 %, 7.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.5 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{78}H_{101}FN_8O_{19}S$ = 1505.69605; found = 1505.69640.	**Yield**: 14.8 mg (95 %, 9.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.10 (DCM:MeOH = 10:1, 1 % HCOOH). **LC-MS:** [50-100 % Solvent B, 2.2 min]: R_t = 1.5 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{80}H_{105}FN_8O_{20}S$ = 1549.72226; found = 1549.72245.
	c	**Yield**: 7.0 mg (60 %, 6.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.22 (DCM:MeOH = 20:1, 1 % HCOOH).	**Yield**: 1.4 mg (12 %, 1.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.20 (DCM:MeOH = 20:1, 1 % HCOOH).	**Yield**: 6.2 mg (49 %, 4.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.19 (DCM:MeOH = 20:1, 1 % HCOOH).	**Yield**: 7.1 mg (55 %, 5.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.18 (DCM:MeOH = 20:1, 1 % HCOOH).	**Yield**: 8.9 mg (66 %, 6.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.18 (DCM:MeOH = 20:1, 1 % HCOOH).

		LC-MS: [50-100 % Solvent B, 2.7 min]: R_t = 1.9 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{61}H_{69}N_7O_{17}$ = 1172.48227; found = 1172.48239.	**LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.9 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{63}H_{73}N_7O_{18}$ = 1216.50848; found = 1216.50858.	**LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 2.0 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{65}H_{77}N_7O_{19}$ = 1260.53470; found = 1260.53456.	**LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 2.0 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{67}H_{81}N_7O_{20}$ = 1304.56091; found = 1304.56107.	**LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 2.0 min > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{69}H_{85}N_7O_{21}$ = 1348.58713; found = 1348.58628.
A9	a	**Yield**: 4.8 mg (58 %, 3.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{74}H_{97}N_9O_{15}S$ = 692.84852; found = 692.84859.	**Yield**: 6.6 mg (77 %, 4.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.07 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{76}H_{101}N_9O_{16}S$ = 714.86163; found = 714.86206.	**Yield**: 5.8 mg (66 %, 3.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.07 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.0 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{78}H_{105}N_9O_{17}S$ = 736.87473; found = 736.87515.	**Yield**: 6.4 mg (70 %, 4.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.06 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.0 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{80}H_{109}N_9O_{18}S$ = 758.88784; found = 758.88805.	**Yield**: 6.2 mg (66 %, 4.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.06 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{82}H_{113}N_9O_{19}S$ = 780.90095; found = 780.90105.
	b	**Yield**: 11.3 mg (79 %, 7.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 1.7 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{76}H_{98}FN_9O_{15}S$ = 1428.69599; found = 1428.70331.	**Yield**: 9.5 mg (65 %, 6.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 1.7 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{78}H_{102}FN_9O_{16}S$ = 1472.72220; found = 1472.72922.	**Yield**: 8.0 mg (53 %, 5.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.08 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 1.7 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{80}H_{106}FN_9O_{17}S$ = 1516.74842; found = 1516.75723.	**Yield**: 7.8 mg (50 %, 5.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.07 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 1.7 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{82}H_{110}FN_9O_{18}S$ = 780.89095; found = 780.89167.	**Yield**: 10.1 mg (63 %, 6.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.07 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 1.7 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{84}H_{114}FN_9O_{19}S$ = 802.90406; found = 802.90462.
	c	**Yield**: 3.9 mg (53 %, 3.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.19 (DCM:MeOH = 20:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 0.9 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{80}H_{109}N_9O_{18}S$ = 1227.56085; found = 1227.55794.	**Yield**: 3.7 mg (49 %, 2.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.19 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{67}H_{82}N_8O_{17}$ = 1271.58707; found = 1271.58356.	**Yield**: 4.2 mg (53 %, 3.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.18 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{69}H_{86}N_8O_{18}$ = 1315.61328; found = 1315.61925.	**Yield**: 4.1 mg (50 %, 3.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.18 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.1 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{71}H_{90}N_8O_{19}$ = 1359.63950; found = 1359.63559.	**Yield**: 3.4 mg (61 %, 2.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.17 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.7 min]: R_t = 1.1 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{73}H_{94}N_8O_{20}$ = 1403.66571; found = 1403.67120.
A10	a	TLC [DCM:MeOH 95:5]: Rf = 0.25. HPLC [0-100% Solvent B, 20 min]: Rt = 17.4 min, purity (220 nm) = 99%. HRMS: m/z: found 1285.62133 [M+H]+, calculated 1285.62135 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.24. HPLC [0-100% Solvent B, 20 min]: Rt = 17.6 min, purity (220 nm) = 99%. HRMS: m/z: found 1329.64806 [M+H]+, calculated 1329.64756 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.24. HPLC [30-100% Solvent B, 20 min]: Rt = 17.4 min, purity (220 nm) = 99%. HRMS: m/z: found 1373.67337 [M+H]+, calculated 1373.67378 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.29. HPLC [0-100% Solvent B, 20 min]: Rt = 17.6 min, purity (220 nm) = 99%. HRMS: m/z: found 1417.69922 [M+H]+, calculated 1417.69999 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.23. HPLC [0-100% Solvent B, 20 min]: Rt = 17.5 min, purity (220 nm) = 98%. HRMS: m/z: found 1461.72591 [M+H]+, calculated 1461.72621 [M+H]+

	b	**Yield**: 6.7 mg (72 %, 5.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.18 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{71}H_{89}FN_8O_{14}S$ = 1329.62758; found = 1329.62725.	**Yield**: 7.2 mg (75 %, 5.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.18 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{73}H_{93}FN_8O_{15}S$ = 1373.65379; found = 1373.65357.	**Yield**: 6.6 mg (67 %, 4.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.17 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{75}H_{97}FN_8O_{16}S$ = 1417.68000; found = 1417.67861.	**Yield**: 7.2 mg (71 %, 4.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.16 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{77}H_{101}FN_8O_{17}S$ = 1461.70622; found = 1461.70535.	**Yield**: 8.0 mg (76 %, 5.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.16 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.7 min]: R_t = 1.5 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{79}H_{105}FN_8O_{15}S$ = 1505.73243; found = 1505.73167.
	c	TLC [DCM:MeOH 95:5]: Rf = 0.57. HPLC [0-100% Solvent B, 20 min]: Rt = 18.5 min, purity (220 nm) = 99%. HRMS: m/z: found 1128.49272 [M+H]+, calculated 1128.49244 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.52. HPLC [30-100% Solvent B, 20 min]: Rt = 15.8 min, purity (220 nm) ≥ 99%. HRMS: m/z: found 1172.51994 [M+H]+, calculated 1172.51866 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.50. HPLC [0-100% Solvent B, 20 min]: Rt = 18.6 min, purity (220 nm) = 98%. HRMS: m/z: found 1216.54470 [M+H]+, calculated 1216.54487 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.50. HPLC [0-100% Solvent B, 20 min]: Rt = 18.5 min, purity (220 nm) = 99%. HRMS: m/z: found 1260.57118 [M+H]+, calculated 1260.57109 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.31. HPLC [0-100% Solvent B, 20 min]: Rt = 18.5 min, purity (220 nm) = 99%. HRMS: m/z: found 1304.59712 [M+H]+, calculated 1304.59730 [M+H]+
A11	a	TLC [DCM:MeOH 93:7]: Rf = 0.38. HPLC [0-100% Solvent B, 20 min]: Rt = 17.73 min, purity (220 nm) = 99%. HRMS: m/z: found 1285.62041 [M+H]+, calculated 1285.62135 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.30. HPLC [0-100% Solvent B, 20 min]: Rt = 17.91 min, purity (220 nm) = 97%. HRMS: m/z: found 1329.64738 [M+H]+, calculated 1329.64756 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.30. HPLC [0-100% Solvent B, 20 min]: Rt = 17.96 min, purity (220 nm) = 97%. HRMS: m/z: found 1373.67431 [M+H]+, calculated 1373.67378 [M+H]+	TLC [DCM:MeOH 93:7]: Rf = 0.28. HPLC [0-100% Solvent B, 20 min]: Rt = 17.89 min, purity (220 nm) = 99%. HRMS: m/z: found 1417.69865 [M+H]+, calculated 1417.69999 [M+H]+	TLC [DCM:MeOH 90:10]: Rf = 0.56. HPLC [0-100% Solvent B, 20 min]: Rt = 17.88 min, purity (220 nm) = 95%. HRMS: m/z: found 1461.72644 [M+H]+, calculated 1461.72621 [M+H]+
	b	**Yield**: 11.3 mg (85 %, 8.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.13 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{71}H_{89}FN_8O_{14}S$ = 1329.62758; found = 1329.62524.	**Yield**: 12.4 mg (91 %, 9.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0. 12 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{73}H_{73}FN_8O_{15}S$ = 1373.65379; found = 1373.65473.	**Yield**: 13.0 mg (92 %, 9.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{75}H_{97}FN_8O_{16}S$ = 1417.68000; found = 1417.67842.	**Yield**: 13.7 mg (94 %, 9.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{77}H_{101}FN_8O_{17}S$ = 1461.70622; found = 1461.70402.	**Yield**: 13.3 mg (89 %, 8.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{79}H_{105}FN_8O_{18}S$ = 1505.73243; found = 1505.73207.
	c	TLC [DCM:MeOH 95:5]: Rf = 0.40. HPLC [0-100% Solvent B, 20 min]: Rt = 18.42 min, purity (220 nm) = 95%. HRMS: m/z: found 1128.49199 [M+H]+, calculated 1128.49244 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.42. HPLC [0-100% Solvent B, 20 min]: Rt = 18.55 min, purity (220 nm) = 98%. HRMS: m/z: found 1172.51801 [M+H]+, calculated 1172.51866 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.30. HPLC [0-100% Solvent B, 20 min]: Rt = 18.49 min, purity (220 nm) = 98%. HRMS: m/z: found 1216.54526 [M+H]+, calculated 1216.54487 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.27. HPLC [30-100% Solvent B, 20 min]: Rt = 15.86 min, purity (220 nm) ≥ 99%. HRMS: m/z: found 1260.57145 [M+H]+, calculated 1260.57109 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.41. HPLC [0-100% Solvent B, 20 min]: Rt = 18.47 min, purity (220 nm) = 98%. HRMS: m/z: found 1304.59619 [M+H]+, calculated 1304.59730 [M+H]+
A12	a	TLC [DCM:MeOH 90:10]: Rf = 0.49.	TLC [DCM:MeOH 90:10]: Rf = 0.57.	TLC [DCM:MeOH 90:10]: Rf = 0.57.	TLC [DCM:MeOH 90:10]: Rf = 0.61.	TLC [DCM:MeOH 90:10]: Rf = 0.58.

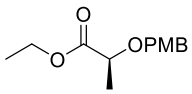
		HPLC [0-100% Solvent B, 20 min]: Rt = 17.73 min, purity (220 nm) = 97%. HRMS: m/z: found 1285.6204 [M+H]+, calculated 1285.62135 [M+H]+	HPLC [0-100% Solvent B, 20 min]: Rt = 17.85 min, purity (220 nm) = 99%. HRMS: m/z: found 1329.64624 [M+H]+, calculated 1329.64756 [M+H]+	HPLC [0-100% Solvent B, 20 min]: Rt = 17.91 min, purity (220 nm) = 96%. HRMS: m/z: found 1373.67136 [M+H]+, calculated 1373.67378 [M+H]+	HPLC [0-100% Solvent B, 20 min]: Rt = 17.84 min, purity (220 nm) ≥ 99%. HRMS: m/z: found 1417.70127 [M+H]+, calculated 1417.69999 [M+H]+	HPLC [0-100% Solvent B, 20 min]: Rt = 17.82 min, purity (220 nm) ≥ 99%. HRMS: m/z: found 1461.72733 [M+H]+, calculated 1461.72621 [M+H]+
	b	**Yield**: 10.6 mg (80 %, 8.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.13 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.7 min]: R_t = 0.9 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{71}H_{89}FN_8O_{14}S$ = 1329.6276; found = 1329.6291.	**Yield**: 11.3 mg (82 %, 8.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.7 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{73}H_{93}FN_8O_{15}S$ = 1373.65379; found = 1373.65102.	**Yield**: 10.0 mg (70 %, 7.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.2 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{75}H_{97}FN_8O_{16}S$ = 1417.68000; found = 1417.67846.	**Yield**: 12.2 mg (84 %, 8.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.2 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{77}H_{101}FN_8O_{17}S$ = 1461.7062; found = 1461.7084.	**Yield**: 10.1 mg (67 %, 6.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [60-100 % Solvent B, 2.2 min]: R_t = 0.9 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{79}H_{105}FN_8O_{18}S$ = 1505.7324; found = 1505.7366.
	c	TLC [DCM:MeOH 95:5]: Rf = 0.42. HPLC [0-100% Solvent B, 20 min]: Rt = 18.36 min, purity (220 nm) = 97%. HRMS: m/z: found 1128.49125 [M+H]+, calculated 1128.49244 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.31. HPLC [0-100% Solvent B, 20 min]: Rt = 18.48 min, purity (220 nm) ≥ 99%. HRMS: m/z: found 1172.51505 [M+H]+, calculated 1128.49244 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.36. HPLC [0-100% Solvent B, 20 min]: Rt = 18.44 min, purity (220 nm) = 98%. HRMS: m/z: found 1216.54480 [M+H]+, calculated 1216.54487 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.35. HPLC [0-100% Solvent B, 20 min]: Rt = 18.39 min, purity (220 nm) ≥ 99%. HRMS: m/z: found 1260.57131 [M+H]+, calculated 1260.57109 [M+H]+	TLC [DCM:MeOH 95:5]: Rf = 0.33. HPLC [0-100% Solvent B, 20 min]: Rt = 18.41 min, purity (220 nm) = 97%. HRMS: m/z: found 1304.5975 [M+H]+, calculated 1304.5973 [M+H]+
A13	a	**Yield**: 3.5 mg (69 %, 2.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.16 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.9 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{69}H_{88}N_8O_{14}S$ = 1285.62135; found = 1285.62040.	**Yield**: 5.0 mg (94 %, 3.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.16 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.9 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{71}H_{92}N_8O_{15}S$ = 1329.64756; found = 1329.64704.	**Yield**: 3.5 mg (64 %, 2.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.15 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.9 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{73}H_{96}N_8O_{16}S$ = 1373.67378; found = 1373.67526.	**Yield**: 4.1 mg (72 %, 2.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.14 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.9 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{75}H_{100}N_8O_{17}S$ = 1417.69999; found = 1417.70019.	**Yield**: 4.7 mg (81 %, 3.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.14 (DCM:MeOH = 10:1). **LC-MS:** [30-100 % Solvent B, 2.6 min]: R_t = 1.9 min. 95 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{77}H_{104}N_8O_{18}S$ = 1461.72621; found = 1461.72623.
	b	**Yield**: 4.9 mg (92 %, 3.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.13 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.6 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{71}H_{89}FN_8O_{14}S$ = 1329.62758; found = 1329.62804.	**Yield**: 4.7 mg (85 %, 3.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.6 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{73}H_{93}FN_8O_{15}S$ = 1373.65379; found = 1373.65448.	**Yield**: 5.0 mg (88 %, 3.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.6 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{75}H_{97}FN_8O_{16}S$ = 1417.68000; found = 1417.67944.	**Yield**: 5.1 mg (88 %, 3.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.6 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{77}H_{101}FN_8O_{17}S$ = 1461.70622; found = 1461.70634.	**Yield**: 4.8 mg (80 %, 3.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [50-100 % Solvent B, 2.6 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{79}H_{105}FN_8O_{18}S$ = 1505.73243; found = 1505.73201.
	c	**Yield**: 2.9 mg (64 %, 2.6 µmol). **Appearance**: white solid.	**Yield**: 4.6 mg (98 %, 3.9 µmol). **Appearance**: white solid.	**Yield**: 4.8 mg (98 %, 3.9 µmol). **Appearance**: white solid.	**Yield**: 4.1 mg (82 %, 3.3 µmol). **Appearance**: white solid.	**Yield**: 4.8 mg (92 %, 3.7 µmol). **Appearance**: white solid.

		TLC: R_f = 0.30 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.2 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.6 min. 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{60}H_{69}N_7O_{15}$= 1128.49244; found = 1128.49277.	**TLC:** R_f = 0.28 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.2 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.7 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{62}H_{73}N_7O_{16}$= 1172.51866; found = 1172.51918.	**TLC:** R_f = 0.27 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.2 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.6 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{64}H_{77}N_7O_{17}$= 1216.54487; found = 1216.54524.	**TLC:** R_f = 0.26 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.2 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.6 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{66}H_{81}N_7O_{18}$= 1260.57109; found = 1260.57124.	**TLC:** R_f = 0.26 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.2 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.6 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{68}H_{85}N_7O_{19}$= 1304.59730; found = 1304.59795.
A14	a	**Yield**: 9.0 mg (70 %, 7.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.16 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{69}H_{88}N_8O_{14}S$ = 1285.62135; found = 1285.62228.	**Yield**: 10.1 mg (76 %, 7.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.16 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{71}H_{92}N_8O_{15}S$ = 1329.64756; found = 1329.64684.	**Yield**: 9.7 mg (71 %, 7.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0.15 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 96 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{73}H_{96}N_8O_{16}S$ = 1373.67378; found = 1373.67298.	**Yield**: 10.0 mg (71 %, 7.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0.15 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{75}H_{100}N_8O_{17}S$ = 1417.69999; found = 1417.69950.	**Yield**: 9.8 mg (67 %, 6.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.14 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 94 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{77}H_{104}N_8O_{18}S$ = 1461.72621; found = 1461.72587.
	b	**Yield**: 11.1 mg (83 %, 8.3 µmol). **Appearance**: white solid. **TLC:** R_f = 0.13 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.6 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{71}H_{89}FN_8O_{14}S$ = 1329.6309; found = 1329.6296.	**Yield**: 11.7 mg (85 %, 8.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.6 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{73}H_{93}FN_8O_{15}S$ = 1373.6538; found = 1373.6532.	**Yield**: 13.0 mg (92 %, 9.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.12 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.6 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{75}H_{97}FN_8O_{16}S$ = 1417.6800; found = 1417.6798.	**Yield**: 12.8 mg (88 %, 8.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.6 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{77}H_{101}FN_8O_{17}S$ = 1461.7062; found = 1461.7065.	**Yield**: 13.3 mg (88 %, 8.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.11 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.6 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{79}H_{105}FN_8O_{18}S$ = 753.3699; found = 753.3701.
	c	**Yield**: 5.2 mg (46 %, 4.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.30 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for	**Yield**: 9.1 mg (78 %, 7.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.28 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for	**Yield**: 10.6 mg (87 %, 8.7 µmol). **Appearance**: white solid. **TLC:** R_f = 0.27 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 97 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for	**Yield**: 8.1 mg (64 %, 6.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.26 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for	**Yield**: 11.2 mg (86 %, 8.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.25 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.1 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.5 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for

		$C_{60}H_{69}N_7O_{15}$= 1128.49244; found = 1128.49311.	$C_{62}H_{73}N_7O_{16}$= 1172.51866; found = 1172.51949.	$C_{64}H_{77}N_7O_{17}$= 1216.54487; found = 1216.54567.	$C_{66}H_{81}N_7O_{18}$= 1260.57109; found = 1260.57244.	$C_{68}H_{85}N_7O_{19}$= 1304.59730; found = 1304.59794.
A15	a	**Yield**: 7.7 mg (75 %, 7.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.18 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.1 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{70}N_8O_{11}S$ = 1015.49575; found = 1015.49512.	**Yield**: 8.0 mg (75 %, 7.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.18 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.1 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{74}N_8O_{12}S$ = 1059.52197; found = 1059.52209.	**Yield**: 7.9 mg (72 %, 7.2 µmol). **Appearance**: white solid. **TLC:** R_f = 0.17 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.1 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{78}N_8O_{13}S$ = 1103.54818; found = 1103.54811.	**Yield**: 8.7 mg (76 %, 7.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.16 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.1 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{58}H_{82}N_8O_{14}S$ = 1147.57440; found = 1147.57469.	**Yield**: 7.7 mg (65 %, 6.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.16 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.2 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{60}H_{86}N_8O_{15}S$ = 1191.60061; found = 1191.60022.
	b	**Yield**: 9.1 mg (86 %, 8.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.16 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [30-100 % Solvent B, 2.6 min]: R_t = 1.8 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{54}H_{71}FN_8O_{11}S$ = 1059.50198; found = 1059.50206.	**Yield**: 9.4 mg (85 %, 8.5 µmol). **Appearance**: white solid. **TLC:** R_f = 0.15 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [30-100 % Solvent B, 2.6 min]: R_t = 1.8 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{56}H_{75}FN_8O_{12}S$ = 1103.52820; found = 1103.52878.	**Yield**: 10.3 mg (90 %, 9.0 µmol). **Appearance**: white solid. **TLC:** R_f = 0.15 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [30-100 % Solvent B, 2.6 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{58}H_{79}FN_8O_{13}S$ = 1147.55441; found = 1147.55490.	**Yield**: 10.5 mg (88 %, 8.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.14 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [30-100 % Solvent B, 2.6 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{60}H_{83}FN_8O_{14}S$ = 1191.58063; found = 1191.58131.	**Yield**: 11.9 mg (96 %, 9.6 µmol). **Appearance**: white solid. **TLC:** R_f = 0.14 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [30-100 % Solvent B, 2.6 min]: R_t = 1.8 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{62}H_{87}FN_8O_{15}S$ = 1235.60684; found = 1235.60729.
	c	**Yield**: 5.1 mg (59 %, 5.9 µmol). **Appearance**: white solid. **TLC:** R_f = 0.34 (DCM:MeOH = 20:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.0 min. 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{43}H_{51}N_7O_{12}$= 858.36685; found = 858.36666.	**Yield**: 5.2 mg (58 %, 5.8 µmol). **Appearance**: white solid. **TLC:** R_f = 0.32 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.0 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{45}H_{55}N_7O_{13}$= 902.39306; found = 902.39298.	**Yield**: 8.9 mg (94 %, 9.4 µmol). **Appearance**: white solid. **TLC:** R_f = 0.30 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.1 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{47}H_{59}N_7O_{14}$= 946.41928; found = 946.41926.	**Yield**: 5.0 mg (51 %, 5.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0.29 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.1 min. > 99 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{49}H_{63}N_7O_{15}$= 990.44549; found = 990.44627.	**Yield**: 9.4 mg (91 %, 9.1 µmol). **Appearance**: white solid. **TLC:** R_f = 0.28 (DCM:MeOH = 10:1). **LC-MS:** [5-100 % Solvent B, 2.6 min]: R_t = 2.0 min. [50-100 % Solvent B, 2.6 min]: R_t = 1.0 min. 98 % purity (220 nm). **HRMS** (ESI) m/z: $[M+H]^+$ calculated for $C_{49}H_{63}N_7O_{15}$= 1034.47171; found = 1034.47225.

15. Synthesis of linker analogues

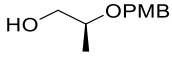
Ethyl (*S*)-2-((4-methoxybenzyl)oxy)propanoate



(-)-Ethyl L-lactate (11.8 g, 100 mmol, 1.0 eq.) and 1-(chloromethyl)-4-methoxybenzene (23.5 g, 150 mmol, 1.5 eq.), DIPEA (27.9 mL, 160 mmol, 1.6 eq.) and sodium iodide (1.5 g, 10 mmol, 0.1 eq.) were stirred at 150 °C for 2 h under argon. Water (100 mL) was added and the mixture was extracted with DCM (3 x 100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 16.6 g (70 %, 69.7 mmol).
Appearance: colorless oil.
^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 1.31 (t, *J* = 7.2 Hz, 3H), 1.43 (d, *J* = 6.9 Hz, 3H), 3.80 (s, 3H), 3.99 – 4.11 (m, 1H), 4.22 (qd, *J* = 7.1, 1.4 Hz, 2H), 4.40 (d, *J* = 11.3 Hz, 1H), 4.63 (d, *J* = 11.2 Hz, 1H), 6.84 – 6.94 (m, 2H), 7.27 – 7.35 (m, 2H).
^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 14.3, 18.7, 55.2, 60.8, 71.6, 73.7, 113.8, 129.6, 159.4, 173.3.
TLC: R_f = 0.18 (CH:EA = 9:1).
LC-MS: Mass (ESI), calculated = 261.1 $[M+Na]^+$, found = 261.2.
[5-100 % Solvent B, 3.0 min]: R_t = 2.0 min.
83 % purity (220 nm).

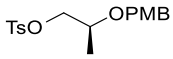
(*S*)-2-((4-Methoxybenzyl)oxy)propan-1-ol



Ethyl (*S*)-2-((4-methoxybenzyl)oxy)propanoate (5.0 g, 21.0 mmol, 1.0 eq.) was dissolved in THF (50 mL, dry) under argon. The mixture was cooled to 0 °C and LAH (1 M in THF, 23.1 mL, 23.1 mmol, 1.1 eq.) was added slowly. The mixture was stirred for 2 h at 0 °C to room temperature. Sodium hydroxide (aq, 1 M, 1 mL) and water (5 mL) were added. The solution was filtered and rinsed with DEE. The filtrate was washed with Brine (30 mL) and the organic phase was dried over $MgSO_4$ and concentrated under reduced pressure.

Yield: 4.1 g (quant., 21.0 mmol).
Appearance: colorless oil.
^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 1.19 (d, *J* = 6.1 Hz, 3H), 2.27 (s, 1H), 3.46 – 3.71 (m, 3H), 3.82 (s, 3H), 4.44 (d, *J* = 11.2 Hz, 1H), 4.61 (d, *J* = 11.3 Hz, 1H), 6.88 – 6.98 (m, 2H), 7.26 – 7.34 (m, 2H).
^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 15.9, 55.3, 66.3, 70.5, 75.3, 113.9, 129.4, 130.5, 159.3.
TLC: R_f = 0.18 (CH:EA = 2:1).
LC-MS: [30-100 % Solvent B, 3.2 min]: R_t = 1.5 min.
> 99 % purity (220 nm).

(*S*)-2-((4-methoxybenzyl)oxy)propyl 4-methylbenzenesulfonate

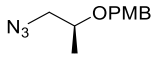


(*S*)-2-((4-Methoxybenzyl)oxy)propan-1-ol(1000 mg, 5.1 mmol, 1.0 eq.), triethylamine (3.0 eq.) and 4-dimethylaminopyridine (0.2 eq.) were dissolved in DCM and cooled to 0 °C. *p*-Toluenesulfonyl chloride (1.5 eq.) was added and the mixture stirred for 2 h at 0 °C to room temperature. Water was added and the mixture was extracted with DCM. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 1780 mg (99 %, 5.1 mmol).
Appearance: slightly yellow oil.

^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 1.17 (d, *J* = 6.4 Hz, 3H), 2.46 (s, 3H), 3.77 (td, *J* = 6.1, 4.5 Hz, 1H), 3.82 (s, 3H), 4.01 (dd, *J* = 5.3, 2.6 Hz, 2H), 4.46 (d, *J* = 3.9 Hz, 2H), 6.84 – 6.93 (m, 2H), 7.20 – 7.25 (m, 2H), 7.32 – 7.37 (m, 2H), 7.76 – 7.85 (m, 2H).
^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 16.8, 21.7, 55.3, 71.0, 72.0, 72.8, 113.8, 128.0, 129.3, 129.9, 130.1, 144.8, 159.2.
TLC: R_f = 0.35 (CH:EA = 3:1).
LC-MS: [5-100 % Solvent B, 3.0 min]: R_t = 2.2 min.
> 99 % purity (220 nm).

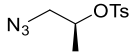
(*S*)-1-(((1-Azidopropan-2-yl)oxy)methyl)-4-methoxybenzene



(S)-2-((4-methoxybenzyl)oxy)propyl 4-methylbenzenesulfonate (3.10 g, 8.9 mmol, 1.0 eq.) and sodium azide (1.16 g, 17.8 mmol, 2.0 eq.) were dissolved in DMF (90 mL). The mixture was stirred for 18 h at 70 °C. The solvent was removed under reduced pressure. The crude product was dissolved in DCM and filtered through Celite. The solvent was removed under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 1.71 g (87 %, 7.7 mmol).
Appearance: slightly yellow oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.24 (d, *J* = 6.3 Hz, 3H), 3.22 (dd, *J* = 12.8, 3.9 Hz, 1H), 3.32 (dd, *J* = 12.8, 6.8 Hz, 1H), 3.74 (td, *J* = 6.4, 4.0 Hz, 1H), 3.83 (s, 3H), 4.52 (d, *J* = 11.3 Hz, 1H), 4.59 (d, *J* = 11.3 Hz, 1H), 6.89 – 6.95 (m, 2H), 7.30 – 7.35 (m, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 17.5, 55.3, 55.8, 70.7, 73.8, 113.9, 129.3, 130.3, 159.3.
TLC: R_f = 0.72 (CH:EA = 1:1).
LC-MS: [5-100 % Solvent B, 3.0 min]: R_t = 2.0 min.
87 % purity (220 nm).

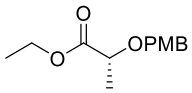
(*S*)-1-Azidopropan-2-yl 4-methylbenzenesulfonate



(*S*)-1-(((1-Azidopropan-2-yl)oxy)methyl)-4-methoxybenzene (1.65 g, 7.45 mmol, 1.0 eq.) was dissolved in DCM:TFA (9:1, 100 mL). The mixture was stirred for 30 min at room temperature. Water (50 mL) was added and the solution was extracted with DCM (3 x 50 mL). combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure.
TLC: R_f = 0.32 (CH:EA = 1:1)
Crude (*S*)-1-azidopropan-2-ol (0.75 g, 7.45 mmol, 1.0 eq.), triethylamine (3.1 mL, 22.4 mmol, 3.0 eq.) and 4-dimethylaminopyridine (182 mg, 1.49 mmol, 0.2 eq.) were dissolved in DCM (75 mL) and cooled to 0 °C. *p*-Toluenesulfonyl chloride (2.13 g, 11.2 mmol, 1.5 eq.) was added and the mixture was stirred for 18 h at 0 °C to room temperature. Water (80 mL) was added and the solution was extracted with DCM (3 x 50 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 274 mg (14 % o2s, 1.1 mmol).
Appearance: slightly yellow oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.34 (dd, *J* = 6.4, 1.0 Hz, 3H), 2.47 (s, 3H), 3.29 – 3.41 (m, 2H), 4.70 (pd, *J* = 6.3, 4.3 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.81 – 7.86 (m, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 18.4, 21.7, 55.1, 77.3, 127.8, 129.9, 133.8, 145.0.
TLC: R_f = 0.40 (CH:EA = 2:1).
LC-MS: Mass (ESI), calculated = 273.1 $[M+NH_4]^+$, found = 273.2.
[5-100 % Solvent B, 3.0 min]: R_t = 2.0 min.
99 % purity (220 nm).

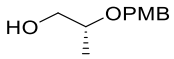
Ethyl (*R*)-2-((4-methoxybenzyl)oxy)propanoate



(-)-Ethyl D-lactate (12.5 g, 106 mmol, 1.0 eq.) and 1-(chloromethyl)-4-methoxybenzene (25.0 g, 160 mmol, 1.5 eq.), DIPEA (29.6 mL, 170 mmol, 1.6 eq.) and sodium iodide (1.6 g, 10.6 mmol, 0.1 eq.) were stirred for 2 h at 150 °C under argon. Water (100 mL) was added and the mixture was extracted with DCM (3 x 100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 8.9 g (35 %, 37.4 mmol).
Appearance: colorless oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.32 (t, *J* = 7.2 Hz, 3H), 1.43 (d, *J* = 7.1 Hz, 3H), 3.81 (d, *J* = 3.5 Hz, 3H), 4.05 (q, *J* = 6.9 Hz, 1H), 4.23 (qq, *J* = 6.8, 3.0 Hz, 2H), 4.41 (d, *J* = 11.3 Hz, 1H), 4.64 (d, *J* = 11.2 Hz, 1H), 6.90 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.3 Hz, 2H).
^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 14.3, 18.7, 55.3, 60.8, 71.6, 73.7, 113.8, 129.7, 159.4, 173.4.
TLC: R_f = 0.18 (CH:EA = 9:1).
LC-MS: Mass (ESI), calculated = 261.1 $[M+Na]^+$, found = 261.2.
[5-100 % Solvent B, 3.0 min]: R_t = 2.0 min.
85 % purity (220 nm).

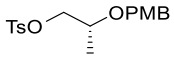
(*R*)-2-((4-Methoxybenzyl)oxy)propan-1-ol



Ethyl (*R*)-2-((4-methoxybenzyl)oxy)propanoate (8.80 g, 37.0 mmol, 1.0 eq.) was dissolved in THF (80 mL, dry) under argon. The mixture was cooled to 0 °C and LAH in THF (1 M, 41.0 mL, 41.0 mmol, 1.1 eq.) was added slowly. The mixture was allowed to warm to room temperature and was stirred for 2 h. Sodium hydroxide (aq, 1 M, 2 mL) and water (10 mL) were added. The solution was filtered and rinsed with DEE. The filtrate was washed with Brine (50 mL) and the organic phase was dried over $MgSO_4$ and concentrated under reduced pressure.

Yield: 7.30 g (quant., 37.0 mmol).
Appearance: slightly yellow oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.18 (d, *J* = 6.3 Hz, 3H), 2.27 – 2.47 (m, 1H), 3.50 (ddd, *J* = 11.0, 7.0, 2.8 Hz, 1H), 3.55 – 3.62 (m, 1H), 3.67 (ddq, *J* = 10.1, 7.0, 3.5 Hz, 1H), 3.81 (d, *J* = 3.0 Hz, 3H), 4.44 (d, *J* = 11.3 Hz, 1H), 4.59 (d, *J* = 11.2 Hz, 1H), 6.90 (d, *J* = 8.3 Hz, 2H), 7.26 – 7.33 (m, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 15.9, 55.3, 66.3, 70.5, 75.3, 113.9, 129.4, 130.6, 159.3.
TLC: R_f = 0.18 (CH:EA = 2:1).
LC-MS: Mass (ESI), calculated = 219.1 $[M+Na]^+$, found = 219.0.
[5-100 % Solvent B, 2.6 min]: R_t = 1.2 min.
85 % purity.

(*R*)-2-((4-methoxybenzyl)oxy)propyl 4-methylbenzenesulfonate

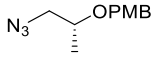


(*R*)-2-((4-Methoxybenzyl)oxy)propan-1-ol (7.3 g, 37.0 mmol, 1.0 eq.), triethylamine (3.0 eq.) and 4-dimethylaminopyridine (0.2 eq.) were dissolved in DCM and cooled to 0 °C. *p*-Toluenesulfonyl chloride (1.5 eq.) was added and the mixture stirred for 2 h at 0 °C to room temperature. Water was added and the mixture was extracted with DCM. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 9.5 g (73 %, 27.0 mmol).
Appearance: slightly yellow oil.

^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.17 (d, *J* = 6.4 Hz, 3H), 2.45 (s, 3H), 3.72 – 3.81 (m, 1H), 3.82 (d, *J* = 1.4 Hz, 3H), 3.96 – 4.06 (m, 2H), 4.41 – 4.51 (m, 2H), 6.87 (d, *J* = 8.1 Hz, 2H), 7.21 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 7.8 Hz, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 16.8, 21.7, 55.3, 71.0, 72.0, 72.8, 113.8, 128.0, 129.3, 129.9, 130.2, 133.0, 144.8, 159.3.
TLC: R_f = 0.35 (CH:EA = 3:1).
LC-MS: Mass (ESI), calculated = 373.1 $[M+Na]^+$, found = 373.0.
[5-100 % Solvent B, 3.0 min]: R_t = 2.2 min.
98 % purity (220 nm).

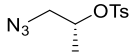
(*R*)-1-(((1-Azidopropan-2-yl)oxy)methyl)-4-methoxybenzene



(*R*)-2-((4-Methoxybenzyl)oxy)propyl 4-methylbenzenesulfonate (3.50 g, 10.0 mmol, 1.0 eq.) and sodium azide (1.30 g, 20.0 mmol, 2.0 eq.) were dissolved in DMF (50 mL). The mixture was stirred for 18 h at 70 °C. The solvent was removed under reduced pressure. The crude product was dissolved in DCM and filtered through Celite. The solvent was removed under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 1.82 g (82 %, 8.2 mmol).
Appearance: colorless oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.24 (d, *J* = 6.3 Hz, 3H), 3.22 (dd, *J* = 12.7, 3.4 Hz, 1H), 3.32 (dd, *J* = 12.7, 6.7 Hz, 1H), 3.71 – 3.77 (m, 1H), 3.83 (s, 3H), 4.52 (d, *J* = 11.3 Hz, 1H), 4.59 (d, *J* = 11.3 Hz, 1H), 6.92 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 17.5, 55.3, 55.8, 70.7, 73.8, 113.9, 129.3, 130.3, 159.3.
TLC: R_f = 0.72 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 244.1 $[M+Na]^+$, found = 244.0.
[5-100 % Solvent B, 3.0 min]: R_t = 2.0 min.
98 % purity (220 nm).

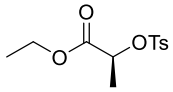
(*R*)-1-Azidopropan-2-yl 4-methylbenzenesulfonate



(*R*)-1-(((1-Azidopropan-2-yl)oxy)methyl)-4-methoxybenzene (1.82 g, 8.2 mmol, 1.0 eq.) was dissolved in DCM:TFA (9:1, 100 mL). The mixture was stirred for 30 min at room temperature. Water (50 mL) was added and the solution was extracted with DCM (3 x 50 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure.
TLC: R_f = 0.27 (CH:EA = 2:1).
Crude (*R*)-1-Azidopropan-2-ol (0.83 g, 8.2 mmol, 1.0 eq.), triethylamine (3.4 mL, 24.6 mmol, 3.0 eq.) and 4-dimethylaminopyridine (195 mg, 1.6 mmol, 0.2 eq.) were dissolved in DCM (75 mL) and cooled to 0 °C. *p*-Toluenesulfonyl chloride (2.35 g, 12.3 mmol, 1.5 eq.) was added and the mixture was stirred for 18 h at 0 °C to room temperature. Water (80 mL) was added and the solution was extracted with DCM (3 x 80 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by preparative HPLC. The product was dried by lyophilisation.

Yield: 711 mg (34 % o2s, 2.8 mmol).
Appearance: yellow oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.29 – 1.34 (m, 3H), 2.43 – 2.47 (m, 3H), 3.28 – 3.38 (m, 2H), 4.68 (pdd, *J* = 6.3, 4.2, 2.2 Hz, 1H), 7.34 – 7.38 (m, 2H), 7.82 (dq, *J* = 8.6, 2.3 Hz, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 18.3, 21.6, 55.0, 77.4, 127.8, 129.9, 133.7, 145.1.
TLC: R_f = 0.41 (CH:EA = 2:1).
LC-MS: Mass (ESI), calculated = 273.1 $[M+NH_4]^+$, found = 273.2.
[5-100 % Solvent B, 3.0 min]: R_t = 2.0 min.
98 % purity (220 nm).

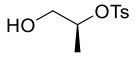
Ethyl (*S*)-2-(tosyloxy)propanoate



(*S*)-2-hydroxypropanoate (1.44 g, 12.2 mmol, 1.0 eq.), triethylamine (3.0 eq.) and 4-dimethylaminopyridine (0.2 eq.) were dissolved in DCM and cooled to 0 °C. *p*-Toluenesulfonyl chloride (1.5 eq.) was added and the mixture stirred for 2 h at 0 °C to room temperature. Water was added and the mixture was extracted with DCM. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 2.37 g (71 %, 8.7 mmol).
Appearance: colorless oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.21 (q, *J* = 6.9, 6.1 Hz, 3H), 1.51 (t, *J* = 5.6 Hz, 3H), 2.45 (d, *J* = 5.3 Hz, 3H), 4.12 (p, *J* = 6.7 Hz, 2H), 4.93 (t, *J* = 6.8 Hz, 1H), 7.35 (t, *J* = 6.9 Hz, 2H), 7.82 (t, *J* = 6.6 Hz, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 13.9, 18.4, 21.6, 61.8, 74.2, 128.0, 129.8, 133.4, 145.1, 169.0.
TLC: R_f = 0.41 (CH:EA = 3:1).
LC-MS: Mass (ESI), calculated = 273.1 $[M+H]^+$, found = 273.2.
[5-100 % Solvent B, 3.0 min]: R_t = 2.0 min.
93 % purity (220 nm).

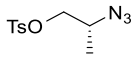
(*S*)-1-Hydroxypropan-2-yl 4-methylbenzenesulfonate



Ethyl (*S*)-2-(tosyloxy)propanoate (1.90 g, 7.0 mmol, 1.0 eq.) and lithium chloride (0.69 g, 16.3 mmol, 2.3 eq.) were dissolved in THF:EtOH (1:2, dry, 60 mL) under argon. The mixture was cooled to - 5 °C and sodium borohydride (0.62 g, 16.3 mmol, 2.3 eq.) was added slowly. The mixture was allowed to warm to room temperature and was stirred for 18 h. Chloroform (150 mL) and sodium sulfate (sat., aq, 150 mL) were added and the mixture was stirred for 1 h. The solution was filtered and rinsed with chloroform. The filtrate was washed with Brine (100 mL) and the organic phase was dried over $MgSO_4$ and concentrated under reduced pressure.

Yield: 1.09 g (68 %, 4.73 mmol).
Appearance: colorless oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.19 – 1.25 (m, 3H), 2.43 (d, *J* = 2.3 Hz, 3H), 3.58 – 3.64 (m, 2H), 4.66 (tdd, *J* = 9.0, 4.4, 2.8 Hz, 1H), 7.34 (dd, *J* = 8.1, 1.8 Hz, 2H), 7.80 (dd, *J* = 8.3, 1.8 Hz, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 16.9, 21.6, 65.4, 80.6, 127.8, 129.9, 133.8, 144.9.
TLC: R_f = 0.15 (CH:EA = 2:1).
R_f = 0.45 (CH:EA = 1:1).
LC-MS: [5-100 % Solvent B, 3.0 min]: R_t = 1.6 min.
> 99 % purity (220 nm).

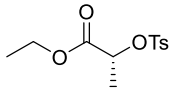
(*R*)-2-Azidopropyl 4-methylbenzenesulfonate



(*S*)-1-Hydroxypropan-2-yl 4-methylbenzenesulfonate (100 mg, 434 µmol, 1.0 eq.) and sodium azide (56 mg, 868 µmol, 2.0 eq.) were dissolved in DMF (5 mL). The mixture was stirred for 18 h at 70 °C. Brine (30 mL) was added and the solution was extracted with DCM (2 x 40 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure.
TLC: R_f = 0.29 (CH:EA = 2:1).
Crude (*R*)-2-azidopropan-1-ol (44 mg, 434 µmol, 1.0 eq.), triethylamine (181 µL, 1302 µmol, 3.0 eq.) and 4-dimethylaminopyridine (11 mg, 87 µmol, 0.2 eq.) were dissolved in DCM (5 mL) and cooled to 0 °C. *p*-Toluenesulfonyl chloride (124 mg, 651 µmol, 1.5 eq.) was added and the mixture stirred at 0 °C to room temperature for 3 h. Water (5 mL) was added and the solution was extracted with DCM (3 x 10 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 24 mg (22 % o2s, 94 µmol).
Appearance: colorless oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.01 – 1.06 (m, 3H), 2.24 – 2.30 (m, 3H), 3.54 (ddd, *J* = 11.0, 5.5, 3.3 Hz, 1H), 3.70 – 3.76 (m, 1H), 3.79 – 3.85 (m, 1H), 7.15 – 7.21 (m, 2H), 7.62 (td, *J* = 5.8, 2.7 Hz, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 15.7, 21.7, 55.6, 72.1, 127.9, 130.0, 132.5, 145.3.
TLC: R_f = 0.44 (CH:EA = 2:1).
LC-MS: Mass (ESI), calculated = 273.1 $[M+NH_4]^+$, found = 273.2.
[5-100 % Solvent B, 3.0 min]: R_t = 2.0 min.
99 % purity (220 nm).

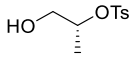
Ethyl (*R*)-2-(tosyloxy)propanoate



(*R*)-2-hydroxypropanoate (1.44 g, 12.2 mmol, 1.0 eq.), triethylamine (3.0 eq.) and 4-dimethylaminopyridine (0.2 eq.) were dissolved in DCM and cooled to 0 °C. *p*-Toluenesulfonyl chloride (1.5 eq.) was added and the mixture stirred for 2 h at 0 °C to room temperature. Water was added and the mixture was extracted with DCM. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 1.67 g (50 %, 6.1 mmol).
Appearance: colorless oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.17 (t, *J* = 7.2 Hz, 3H), 1.47 (d, *J* = 7.0 Hz, 3H), 2.41 (s, 3H), 4.05 – 4.11 (m, 3H), 4.89 (q, *J* = 6.9 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.78 (d, *J* = 8.2 Hz, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 13.9, 18.4, 21.6, 61.8, 74.2, 128.0, 129.8, 133.4, 145.1, 169.0.
TLC: R_f = 0.41 (CH:EA = 3:1).
LC-MS: Mass (ESI), calculated = 273.1 $[M+H]^+$, found = 273.2.
[5-100 % Solvent B, 3.0 min]: R_t = 2.0 min.
98 % purity (220 nm).

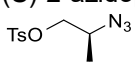
(*R*)-1-hydroxypropan-2-yl 4-methylbenzenesulfonate



Ethyl (R)-2-(tosyloxy)propanoate (1.67 g, 6.1 mmol, 1.0 eq.) and lithium chloride (0.78 g, 18.4 mmol, 3.0 eq.) were dissolved in THF:EtOH (1:2, dry, 60 mL) under argon. The mixture was cooled to - 5 °C and sodium borohydride (0.70 g, 18.4 mmol, 3.0 eq.) was added slowly. The mixture was allowed to warm to room temperature and was stirred for 18 h. Chloroform (150 mL) and sodium sulfate (sat., aq, 150 mL) were added and the mixture was stirred for 1 h. The solution was filtered and rinsed with chloroform. The filtrate was washed with Brine (100 mL) and the organic phase was dried over $MgSO_4$ and concentrated under reduced pressure.

Yield: 0.84 g (59 %, 3.6 mmol).
Appearance: colorless oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.24 (d, *J* = 6.5 Hz, 3H), 2.45 (s, 3H), 3.59 – 3.66 (m, 2H), 4.63 – 4.73 (m, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.82 (d, *J* = 8.3 Hz, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 16.9, 21.6, 65.5, 80.7, 127.8, 129.9, 133.9, 144.9.
TLC: R_f = 0.45 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 248.1 $[M+NH_4]^+$, found = 248.2.
[5-100 % Solvent B, 3.0 min]: R_t = 1.6 min.
> 99 % purity (220 nm).

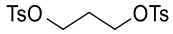
(*S*)-2-azidopropyl 4-methylbenzenesulfonate



(*R*)-1-hydroxypropan-2-yl 4-methylbenzenesulfonate (837 mg, 3.6 mmol, 1.0 eq.) and sodium azide (473 mg, 7.3 µmol, 2.0 eq.) were dissolved in DMF (30 mL). The mixture was stirred for 18 h at 70 °C. Brine (100 mL) was added and the solution was extracted with DCM (2 x 100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The solvent was removed under reduced pressure.
TLC: R_f = 0.29 (CH:EA = 2:1).
Crude (*S*)-2-azidopropan-1-ol (367 mg, 3.6 mmol, 1.0 eq.), triethylamine (1.5 mL, 10.9 mmol, 3.0 eq.) and 4-dimethylaminopyridine (89 mg, 0.7 mmol, 0.2 eq.) were dissolved in DCM (40 mL) and cooled to 0 °C. *p*-Toluenesulfonyl chloride (1039 mg, 5.5 mmol, 1.5 eq.) was added and the mixture stirred at 0 °C to room temperature for 18 h. Water (40 mL) was added and the solution was extracted with DCM (3 x 400 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 187 mg (20 % o2s, 0.7 mmol).
Appearance: colorless oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.25 (d, *J* = 6.7 Hz, 3H), 2.48 (s, 3H), 3.72 – 3.78 (m, 1H), 3.94 (dd, *J* = 10.3, 6.9 Hz, 1H), 4.02 (dd, *J* = 10.3, 4.4 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.83 (dd, *J* = 8.6, 1.9 Hz, 2H).
^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 15.9, 21.8, 55.7, 72.1, 128.1, 130.1, 132.7, 145.3.
TLC: R_f = 0.44 (CH:EA = 2:1).
LC-MS: Mass (ESI), calculated = 273.1 $[M+NH_4]^+$, found = 273.2.
[5-100 % Solvent B, 3.0 min]: R_t = 1.9 min.
99 % purity (220 nm).

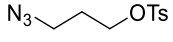
Propane-1,3-diyl bis(4-methylbenzenesulfonate)



Propane-1,3-diol (510 µL, 7.0 mmol, 1.0 eq.), triethylamine (5.9 mL, 42.0 mmol, 6.0 eq.) and 4-dimethylaminopyridine (340 mg, 2.8 mmol, 0.4 eq.) were dissolved in DCM (60 mL) and cooled to 0 °C. *p*-Toluenesulfonyl chloride (4.00 g, 21.0 mmol, 3.0 eq.) was added and the mixture stirred for 2 h at 0 °C to room temperature. Water (60 mL) was added and the solution was extracted with DCM (3 x 100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 1.84 g (68 %, 4.8 mmol).
Appearance: white solid.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 2.02 (p, *J* = 6.0 Hz, 2H), 2.47 (s, 6H), 4.08 (t, *J* = 6.0 Hz, 4H), 7.37 (d, *J* = 7.9 Hz, 4H), 7.76 (d, *J* = 8.0 Hz, 4H).
^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 21.8, 28.8, 66.0, 128.0, 130.1, 132.7, 145.2.
TLC: R_f = 0.71 (CH:EA = 1:1).
LC-MS: Mass (ESI), calculated = 385.1 $[M+H]^+$, found = 385.0.
[5-100 % Solvent B, 3.0 min]: R_t = 2.1 min.
> 99 % purity (220 nm).

3-azidopropyl 4-methylbenzenesulfonate



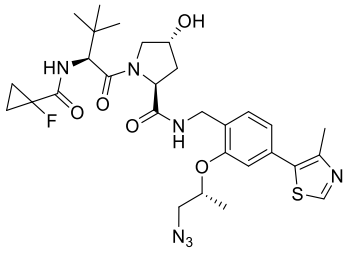
Propane-1,3-diyl bis(4-methylbenzenesulfonate) (1.84 g, 4.7 mmol, 1.0 eq.) and sodium azide (0.30 g, 4.7 mmol, 1.0 eq.) were dissolved in DMF (40 mL). The mixture was stirred for 18 h at 70 °C. The solvent was removed under reduced pressure. The crude product was dissolved in DCM and filtered through Celite. The solvent was removed under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 295 mg (25 %, 1.2 mmol).
Appearance: colorless oil.
^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 1.89 (p, *J* = 6.3 Hz, 2H), 2.46 (s, 3H), 3.38 (t, *J* = 6.5 Hz, 2H), 4.11 (t, *J* = 6.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 2H).

^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 21.6, 28.4, 47.3, 67.1, 127.9, 130.0, 132.7, 145.1.
TLC: R_f = 0.46 (CH:EA = 2:1).
LC-MS: Mass (ESI), calculated = 278.1 $[M+Na]^+$, found = 278.0.
[5-100 % Solvent B, 2.6 min]: R_t = 1.7 min.
72 % purity (220 nm).

16. Synthesis of b1 analogues

(2S,4R)-N-(2-(((R)-1-Azidopropan-2-yl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide



VH032-cyclopropane-F (53 mg, 100 µmol, 1.0 eq.), (*S*)-1-Azidopropan-2-yl 4-methylbenzenesulfonate (34 mg, 135 µmol, 1.35 eq) and potassium carbonate (2.7 eq.) were dissolved in DMF. The mixture was stirred for 18 h at 70 °C. The solvent was removed under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 48 mg (78 %, 78 µmol).

Appearance: white solid.

^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 0.95 (s, 9H), 1.20 – 1.36 (m, 5H), 1.38 (d, *J* = 6.2 Hz, 3H), 2.02 – 2.10 (m, 1H), 2.50 (s, 4H), 3.48 (dd, *J* = 12.9, 3.8 Hz, 1H), 3.56 (dd, *J* = 12.9, 6.3 Hz, 1H), 3.64 (dd, *J* = 11.2, 4.0 Hz, 1H), 3.67 – 3.71 (m, 1H), 3.94 (dt, *J* = 11.3, 1.9 Hz, 1H), 4.39 (dd, *J* = 15.1, 5.5 Hz, 1H), 4.51 (dd, *J* = 15.1, 6.5 Hz, 2H), 4.54 – 4.58 (m, 1H), 4.61 (tt, *J* = 6.3, 3.1 Hz, 1H), 4.69 (t, *J* = 7.7 Hz, 1H), 6.87 (d, *J* = 1.6 Hz, 1H), 6.96 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.07 (dd, *J* = 9.0, 3.6 Hz, 1H), 7.31 (t, *J* = 6.1 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 8.67 (s, 1H).

^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 13.7, 16.1, 17.2, 26.3, 35.6, 36.0, 38.8, 55.6, 56.6, 57.4, 58.6, 70.1, 73.2, 79.1, 113.3, 122.2, 127.3, 129.8, 131.7, 132.1, 148.5, 150.4, 154.9, 170.1, 170.2, 170.7.

TLC: R_f = 0.23 (EA).

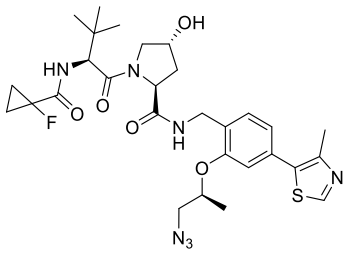
LC-MS: Mass (ESI), calculated = 616.3 $[M+H]^+$, found = 616.2.

[5-100 % Solvent B, 10.5 min]: R_t = 6.7 min.

[5-100 % Solvent B, 3.0 min]: R_t = 1.9 min.

98 % purity (220 nm).

(2S,4R)-N-(2-(((S)-1-Azidopropan-2-yl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide



VH032-cyclopropane-F (53 mg, 100 µmol, 1.0 eq.), (*R*)-1-Azidopropan-2-yl 4-methylbenzenesulfonate (34 mg, 135 µmol, 1.35 eq) and potassium carbonate (2.7 eq.) were dissolved in DMF. The mixture was stirred for 18 h at 70 °C. The solvent was removed under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 51 mg (82 %, 82 µmol).

Appearance: white solid.

^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 0.98 (s, 9H), 1.25 – 1.39 (m, 5H), 1.44 (d, *J* = 6.2 Hz, 3H), 2.12 (ddt, *J* = 13.5, 8.1, 2.1 Hz, 1H), 2.50 (ddd, *J* = 12.8, 7.7, 4.7 Hz, 1H), 2.55 (s, 3H), 3.33 (s, 1H), 3.49 (dd, *J* = 12.9, 3.8 Hz, 1H), 3.57 (dd, *J* = 13.0, 6.5 Hz, 1H), 3.66 (dd, *J* = 11.3, 3.8 Hz, 1H), 4.02 (dt, *J* = 11.5, 1.9 Hz, 1H), 4.48 – 4.59 (m, 4H), 4.64 (tt, *J* = 6.4, 3.2 Hz, 1H), 4.72 (t, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 1.6 Hz, 1H), 7.00 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.09 (dd, *J* = 9.0, 3.7 Hz, 1H), 7.24 (t, *J* = 6.1 Hz, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 8.70 (s, 1H).

^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 13.7, 13.8, 16.1, 17.4, 26.3, 35.4, 36.0, 38.7, 55.6, 56.6, 57.4, 58.6, 70.2, 73.4, 79.1, 113.3, 122.3, 127.3, 129.9, 131.6, 132.3, 148.5, 150.4, 155.0, 170.3, 170.6, 170.8.

TLC: R_f = 0.23 (EA).

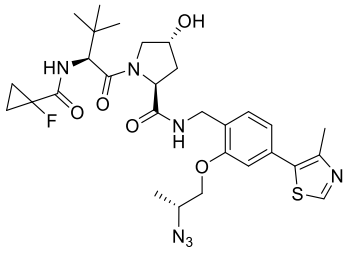
LC-MS: Mass (ESI), calculated = 616.3 $[M+H]^+$, found = 616.2.

[5-100 % Solvent B, 10.5 min]: R_t = 6.7 min.

[5-100 % Solvent B, 3.0 min]: R_t = 1.9 min.

98 % purity (220 nm).

(2S,4R)-N-(2-((R)-2-Azidopropoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide



VH032-cyclopropane-F (53 mg, 100 µmol, 1.0 eq.), (*R*)-2-azidopropyl 4-methylbenzenesulfonate (34 mg, 135 µmol, 1.35 eq) and potassium carbonate (2.7 eq.) were dissolved in DMF. The mixture was stirred for 18 h at 70 °C. The solvent was removed under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 41 mg (66 %, 66 µmol).

Appearance: white solid.

^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 0.96 (s, 9H), 1.28 (td, *J* = 9.9, 9.3, 2.8 Hz, 2H), 1.29 – 1.36 (m, 3H), 1.37 – 1.41 (m, 3H), 2.09 (ddt, *J* = 13.1, 8.2, 1.8 Hz, 1H), 2.52 (s, 4H), 3.66 (dd, *J* = 11.2, 4.0 Hz, 1H), 3.93 – 4.07 (m, 4H), 4.43 (dd, *J* = 15.2, 5.4 Hz, 1H), 4.51 – 4.61 (m, 3H), 4.72 (t, *J* = 7.7 Hz, 1H), 6.84 (d, *J* = 1.7 Hz, 1H), 6.99 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.09 (dd, *J* = 9.0, 3.6 Hz, 1H), 7.34 (t, *J* = 6.1 Hz, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 8.69 (s, 1H).

^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 13.7, 16.1, 16.2, 26.3, 35.6, 36.1, 38.6, 56.5, 56.6, 57.4, 58.7, 70.1, 71.7, 79.1, 112.1, 122.3, 126.6, 129.6, 131.6, 132.2, 148.6, 150.4, 155.9, 170.1, 170.7, 170.9.

TLC: R_f = 0.25 (EA).

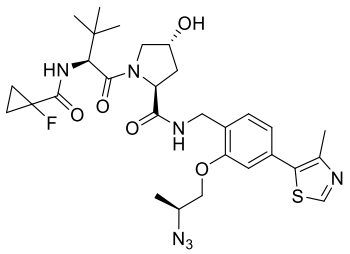
LC-MS: Mass (ESI), calculated = 616.3 $[M+H]^+$, found = 616.2.

[5-100 % Solvent B, 10.5 min]: R_t = 6.8 min.

[5-100 % Solvent B, 3.0 min]: R_t = 1.9 min.

> 99 % purity (220 nm).

(2S,4R)-N-(2-((S)-2-azidopropoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide



VH032-cyclopropane-F (53 mg, 100 µmol, 1.0 eq.), (*S*)-2-azidopropyl 4-methylbenzenesulfonate (34 mg, 135 µmol, 1.35 eq) and potassium carbonate (2.7 eq.) were dissolved in DMF. The mixture was stirred for 18 h at 70 °C. The solvent was removed under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 52 mg (83 %, 83 µmol).

Appearance: white solid.

^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 0.96 (s, 9H), 1.28 (td, *J* = 9.9, 9.3, 2.8 Hz, 2H), 1.29 – 1.36 (m, 3H), 1.37 – 1.41 (m, 3H), 2.09 (ddt, *J* = 13.1, 8.2, 1.8 Hz, 1H), 2.52 (s, 4H), 3.66 (dd, *J* = 11.2, 4.0 Hz,

1H), 3.93 – 4.07 (m, 4H), 4.43 (dd, *J* = 15.2, 5.4 Hz, 1H), 4.51 – 4.61 (m, 3H), 4.72 (t, *J* = 7.7 Hz, 1H), 6.84 (d, *J* = 1.7 Hz, 1H), 6.99 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.09 (dd, *J* = 9.0, 3.6 Hz, 1H), 7.34 (t, *J* = 6.1 Hz, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 8.69 (s, 1H).

^{13}C-NMR (75 MHz, Chloroform-*d*): δ = 13.7, 16.1, 16.2, 26.3, 35.6, 36.1, 38.6, 56.5, 56.6, 57.4, 58.7, 70.1, 71.7, 79.1, 112.1, 122.3, 126.6, 129.6, 131.6, 132.2, 148.6, 150.4, 155.9, 170.1, 170.7, 170.9.

TLC: R_f = 0.24 (EA).

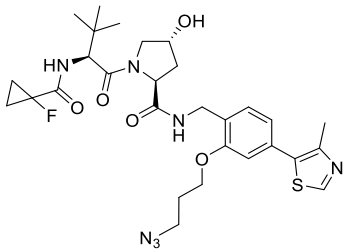
LC-MS: Mass (ESI), calculated = 616.3 $[M+H]^+$, found = 616.2.

[5-100 % Solvent B, 10.5 min]: R_t = 6.8 min.

[5-100 % Solvent B, 3.0 min]: R_t = 1.9 min.

> 99 % purity (220 nm).

(2S,4R)-N-(2-(3-Azidopropoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide



VH032-cyclopropane-F (53 mg, 100 µmol, 1.0 eq.), 3-azidopropyl 4-methylbenzenesulfonate (34 mg, 135 µmol, 1.35 eq) and potassium carbonate (2.7 eq.) were dissolved in DMF. The mixture was stirred for 18 h at 70 °C. The solvent was removed under reduced pressure. The obtained product was purified by flash chromatography.

Yield: 40 mg (65 %, 65 µmol).

Appearance: white solid.

^{1}H-NMR (500 MHz, Chloroform-*d*): δ = 0.91 (s, 9H), 1.23 – 1.35 (m, 4H), 2.02 – 2.08 (m, 1H), 2.09 – 2.15 (m, 2H), 2.51 (s, 4H), 3.52 – 3.64 (m, 4H), 3.94 (d, *J* = 11.3 Hz, 1H), 4.09 (td, *J* = 6.0, 4.2 Hz, 2H), 4.39 (dd, *J* = 14.9, 5.4 Hz, 1H), 4.46 – 4.56 (m, 3H), 4.71 (t, *J* = 7.7 Hz, 1H), 6.86 (d, *J* = 1.6 Hz, 1H), 6.95 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.07 (dd, *J* = 9.0, 3.6 Hz, 1H), 7.34 (dd, *J* = 12.6, 6.9 Hz, 2H), 8.67 (s, 1H).

^{13}C-NMR (126 MHz, Chloroform-*d*): δ = 13.8, 16.2, 26.4, 28.8, 35.6, 35.8, 38.8, 48.3, 56.6, 57.5, 58.6, 64.9, 70.2, 79.2, 112.1, 121.9, 126.3, 129.7, 131.8, 132.4, 148.6, 150.5, 156.5, 170.2, 170.5, 171.1.

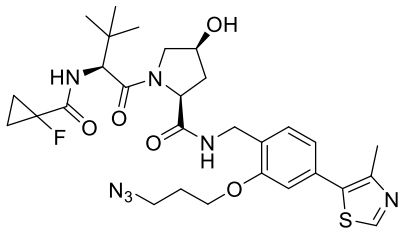
TLC: R_f = 0.24 (EA).

LC-MS: Mass (ESI), calculated = 616.3 $[M+H]^+$, found = 616.2.

[5-100 % Solvent B, 3.0 min]: R_t = 1.9 min.

93 % purity (220 nm).

(2S,4S)-N-(2-(3-azidopropoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide



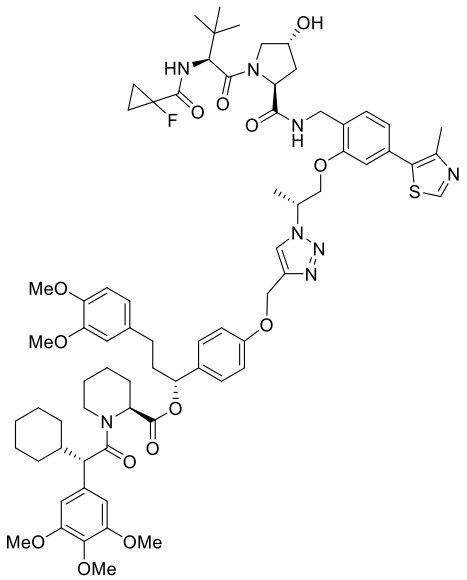
Cis-VH032-cyclopropane-F (24 mg, 44 μmol, 1.0 eq.) which was synthesized according to the procedures described for VH032-cyclopropane-F [5], 3-azidopropyl 4-methylbenzenesulfonate (1.35 eq.) and potassium carbonate (2.7 eq.) were stirred in DMF for 24 h. The mixture was diluted with water and was extracted with DCM. The organic phases were dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by chromatography.

Yield: 12 mg (44 %, 19 μmol).
Appearance: white solid.
1H-NMR (300 MHz, Chloroform-*d*): δ = 0.91 (s, 9H), 1.23 – 1.35 (m, 4H), 2.06 – 2.29 (m, 3H), 2.51 (s, 3H), 3.51 – 3.72 (m, 2H), 3.99 -3.74 (m, 3H), 4.08 – 4.19 (m, 2H), 4.34-4.48 (m, 2H), 4.50 – 4.64 (m, 2H), 4.74 (d, *J* = 8.8 Hz, 2H), 6.79 – 7.05 (m, 3H), 6.95 (dd, *J* = 7.7, 1.6 Hz, 1H), , 7.35 (d, *J* = 7.7 Hz, 1H), 7.56 (t, *J* = 6.2 Hz, 1H), 8.67 (s, 1H).
13C-NMR (75 MHz, Chloroform-*d*): δ = 13.8, 16.2, 26.4, 28.8, 35.6, 35.8, 38.8, 48.3, 56.6, 57.5, 58.6, 64.9, 70.2, 79.2, 112.1, 121.9, 126.3, 129.7, 131.8, 132.4, 148.6, 150.5, 156.5, 170.2, 170.5, 171.1.
TLC: R_f = 0.24 (EA).
HRMS: (ESI) m/z: [M+H]+ calculated for $C_{29}H_{39}FN_7O_5S$ =616.27119; found =616.27185.

17. Synthesis of branched 14b1 PROTACs

14b1-(1R-Me)

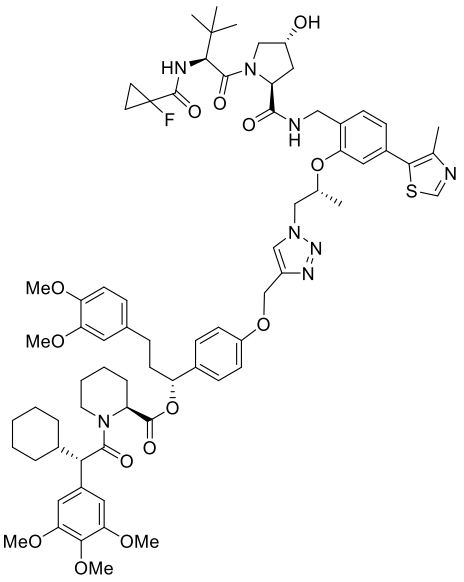
(*R*)-3-(3,4-Dimethoxyphenyl)-1-(4-((1-((*R*)-1-(2-(((2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)propan-2-yl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate



Yield: 11.8 mg (88 %, 8.8 µmol).
Appearance: white solid.
TLC: R_f = 0.15 (DCM:MeOH = 10:1).
LC-MS: [30-100 % Solvent B, 3.0 min]: R_t = 2.2 min.
> 99 % purity (220 nm).
HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{72}H_{91}FN_8O_{14}S$ = 1343.64323; found = 1343.64382.

14b1-(2R-Me)

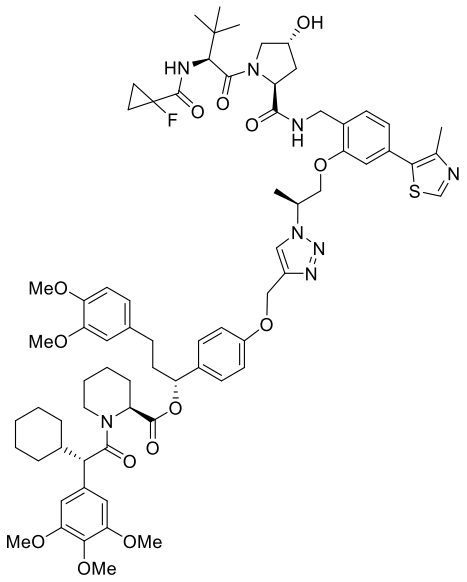
(*R*)-3-(3,4-Dimethoxyphenyl)-1-(4-((1-((*R*)-2-(2-(((2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate



Yield: 12.3 mg (92 %, 9.2 µmol).
Appearance: white solid.
TLC: R_f = 0.15 (DCM:MeOH = 10:1).
LC-MS: [30-100 % Solvent B, 3.0 min]: R_t = 2.2 min.
> 99 % purity (220 nm).
HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{72}H_{91}FN_8O_{14}S$ = 1343.64323; found = 1343.64367.

14b1-(1S-Me)

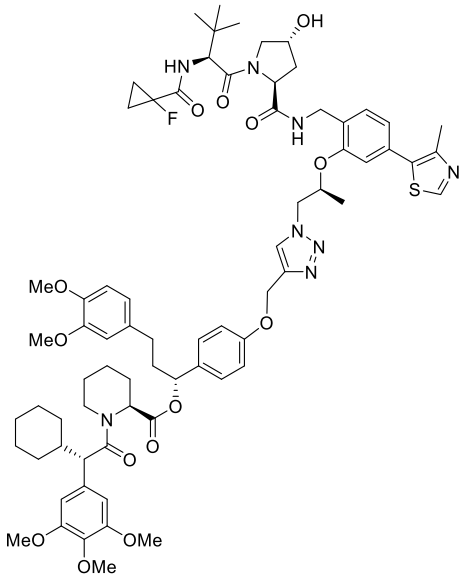
(*R*)-3-(3,4-dimethoxyphenyl)-1-(4-((1-((*S*)-1-(2-(((2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)propan-2-yl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate



Yield: 10.4 mg (78 %, 7.8 µmol).
Appearance: white solid.
TLC: R_f = 0.15 (DCM:MeOH = 10:1).
LC-MS: [30-100 % Solvent B, 3.0 min]: R_t = 2.2 min.
> 99 % purity (220 nm).
HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{72}H_{91}FN_8O_{14}S$ = 1343.64323; found = 1343.64370.

14b1-(2S-Me)

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(4-((1-((*S*)-2-(2-(((2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate

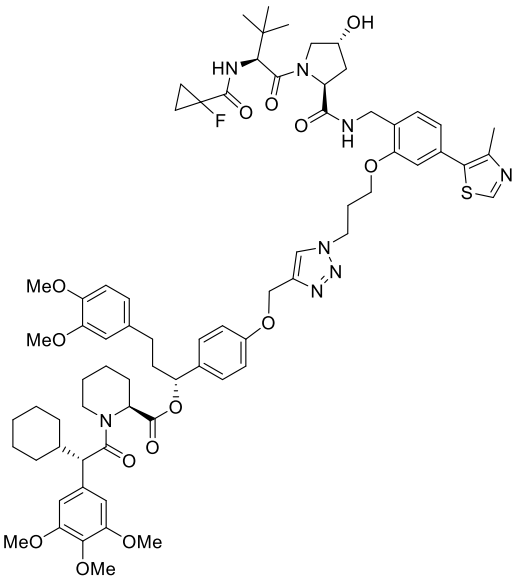


Yield: 9.0 mg (67 %, 6.7 µmol).
Appearance: white solid.
TLC: R_f = 0.15 (DCM:MeOH = 10:1).
LC-MS: [30-100 % Solvent B, 3.0 min]: R_t = 2.2 min.

> 99 % purity (220 nm).
HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{72}H_{91}FN_8O_{14}S$ = 1343.64323; found = 1343.64376.

18. Synthesis of SelDeg51

(*R*)-3-(3,4-Dimethoxyphenyl)-1-(4-((1-(3-(2-(((2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl (*S*)-1-((*S*)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate

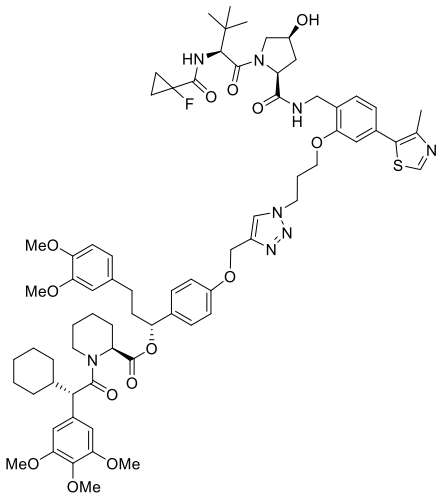


According to the PROTAC synthesis procedure, the SelDeg51 has been obtained after a click reaction.

Yield: 10.0 mg (75 %, 7.5 µmol).
Appearance: white solid.
TLC: R_f = 0.15 (DCM:MeOH = 10:1).
LC-MS: [30-100 % Solvent B, 3.0 min]: R_t = 2.2 min.
> 99 % purity (220 nm).
HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{72}H_{91}FN_8O_{14}S$ = 1343.64323; found = 1343.64340.

19. Synthesis of cis-SelDeg51

(R)-3-(3,4-dimethoxyphenyl)-1-(4-((1-(3-(2-(*((2S,4S)*-1-((S)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)propyl (S)-1-((S)-2-cyclohexyl-2-(3,4,5-trimethoxyphenyl)acetyl)piperidine-2-carboxylate



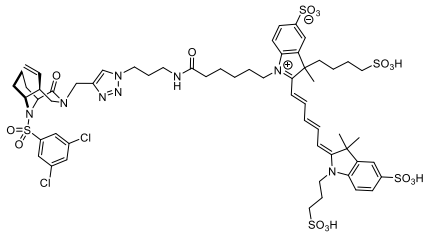
According to the PROTAC synthesis procedure, the *cis*-SelDeg51 has been obtained after a click reaction.

Yield: 22 mg (82 %, 16 µmol).
Appearance: white solid.
TLC: Rf = 0.20 (DCM:MeOH = 10:1).
HPLC: [30-100 % Solvent B, 25 min]: Rt = 19.19 min.
[30-100 % Solvent B, 3 min]: Rt = 2.2 min.
97 % purity (220 nm).
HRMS (ESI) m/z: [M+H]+ calculated for $C_{72}H_{92}FN_8O_{14}S$ = 1343.64323; found = 1343.64574.

20. Synthesis of tracers

FKBP52-HTRF tracer

1-(6-((3-(4-(((5*S*)-10-((3,5-Dichlorophenyl)sulfonyl)-2-oxo-5-vinyl-3,10-diazabicyclo[4.3.1]decan-3-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)amino)-6-oxohexyl)-2-((1E,3E)-5-((E)-3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-methyl-3-(4-sulfobutyl)-3H-indol-1-ium-5-sulfonate

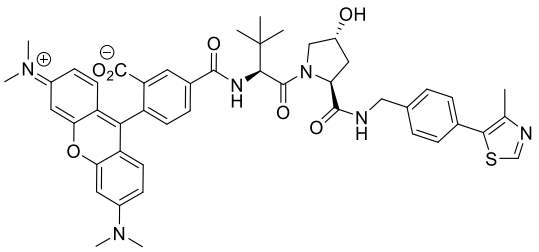


1-(6-((3-Azidopropyl)amino)-6-oxohexyl)-2-((1*E*,3*E*)-5-((*E*)-3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-methyl-3-(4-sulfobutyl)-3H-indol-1-ium-5-sulfonate (2.9 mg, 3.0 µmol, 1.0 eq.) in DCM was added to alkyne 5 (1.3 mg, 3.0 µmol, 1.0 eq.) in DCM. Afterwards HATU (1.3 eq.) and DIPEA (5.0 eq.) were added and the mixture was stirred for 16 h at room temperature. DCM was added and the mixture was washed with brine. The organic phase was dried over MgSO4 and concentrated under reduced pressure. The obtained product was purified by chromatography.
To determine the purity absorption of a 5 µM solution at 647 nm and 650 nm was detected and compared to its theoretical values. This showed a purtiy of over 99 %.

Yield: 2.0 mg (48 %, 1.4 µmol).
Appearance: blue solid.
TLC: R_f = 0.19 (DCM:MeOH = 2:1).
HRMS (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{59}H_{74}Cl_2N_8O_{16}S_5$ = 691.16745; found = 691.16817.

VHL-FP tracer

2-(6-(Dimethylamino)-3-(dimethyliminio)-3H-xanthen-9-yl)-5-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamoyl)benzoate



A mixture of (2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (VH032) (2.6 mg, 6.0 µmol, 1.0 eq.), TEA (8.4 µL, 60.0 µmol, 10.0 eq.) and TAMRA-NHS (3.2 mg, 6.0 µmol, 1.0 eq.) in DCM:DMF (1:1, 400 µL) was stirred for 90 h at room temperatue and concentrated under reduced pressure. The obtained product was purified by chromatography (2 g SiO2, DCM:MeOH = 15:1 → 5:1). The obtained product was purified by preparative HPLC. The product was dried by lyophilisation.

Yield: 4.2 mg (5.0 µmol, 83 %).

Appearance: pink solid.

^{1}H-NMR (300 MHz, Chloroform-*d*): δ = 1.09 (m, 3H), 1.11 (m, 3H), 1.17 (d, *J* = 3.7 Hz, 9H), 1.25 – 1.37 (m, 6H), 2.14 (ddd, *J* = 13.2, 9.1, 4.4 Hz, 1H), 2.23 – 2.31 (m, 1H), 2.48 (s, 3H), 3.74 – 3.84 (m, 1H), 3.91 (dd, *J* = 11.0, 3.9 Hz, 1H), 4.03 (d, *J* = 11.2 Hz, 1H), 4.36 (d, *J* = 15.5 Hz, 1H), 4.54 – 4.61 (m, 2H), 4.64 (dd, *J* = 9.1, 7.5 Hz, 1H), 5.00 (s, 1H), 6.96 (d, *J* = 2.4 Hz, 2H), 7.04 (dd, *J* = 9.4, 2.6 Hz, 2H), 7.20 (dd, *J* = 9.5, 1.5 Hz, 2H), 7.40 – 7.52 (m, 5H), 8.16 (dd, *J* = 7.9, 1.6 Hz, 1H), 8.67 (s, 1H), 8.87 (s, 1H).

TLC: R_f = 0.22 (DCM:MeOH = 10:1).

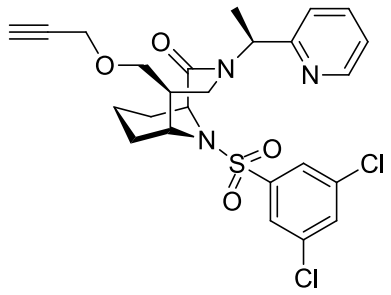
HPLC: [30-50 % Solvent B, 20 min]: R_t = 10.8 min.

95 % purity (220 nm).

HRMS (ESI) m/z: $[M+2H]^{2+}$ calculated for $C_{47}H_{50}N_6O_7S$ = 843.35345; found = 843.35360

FKBP-HTRF tracer

(1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-5-((prop-2-yn-1-yloxy)methyl)-3-((S)-1-(pyridin-2-yl)ethyl)-3,10-diazabicyclo[4.3.1]decan-2-one



18$^{(S\text{-}Me)}$ [6] (15 mg, 0.030 mmol, 1.0 eq.) was dissolved in 1.3 mL dry THF and cooled to 0°C under Ar atmosphere. NaH (60% in mineral oil, 8.33 mg, 0.211 mmol, 7.0 eq.) was added, followed by properglybromide (80wt% in toluene, 34 uL, 0.301 mmol, 10.0 eq.). The reaction was stirred at rt overnight. The reaction was quenched with brine and little water and the crude product was extracted with DCM (3x20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by prep. HPLC.

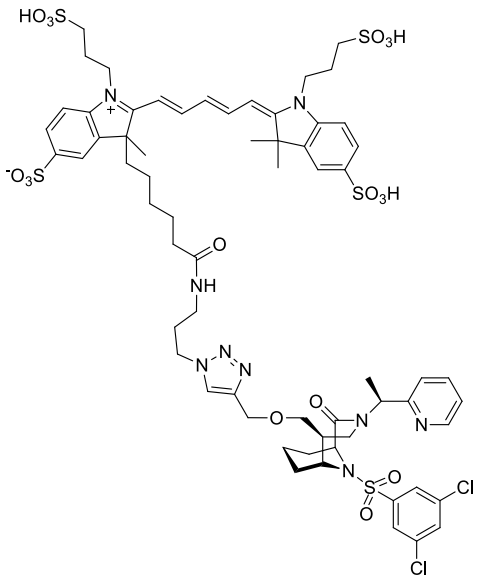
Yield: 14 mg (87%)

Appearance: colourless solid

NMR:

1H NMR (500 MHz, Chloroform-d) δ 8.83 (d, J = 5.3 Hz, 1H), 8.05 (td, J = 7.9, 1.6 Hz, 1H), 7.69 (d, J = 1.8 Hz, 2H), 7.60 – 7.55 (m, 2H), 7.54 – 7.50 (m, 1H), 5.97 (q, J = 7.0 Hz, 1H), 4.76 – 4.68 (m, 1H), 4.15 (s, 2H), 4.04 – 3.95 (m, 1H), 3.51 – 3.44 (m, 2H), 3.44 – 3.36 (m, 1H), 3.20 (dd, J = 14.5, 1.8 Hz, 1H), 2.44 (t, J = 2.4 Hz, 1H), 2.40 (dt, J = 12.8, 6.8 Hz, 1H), 2.28 – 2.19 (m, 1H), 1.70 (d, J = 7.0 Hz, 3H), 1.64 – 1.53 (m, 3H), 1.52 – 1.41 (m, 1H), 1.36 – 1.25 (m, 1H).
13C NMR (126 MHz, CDCl3) δ 170.80, 157.85, 145.42, 143.86, 141.58, 136.46, 132.93, 125.11, 124.20, 123.98, 79.20, 75.23, 70.25, 58.66, 57.19, 55.80, 52.78, 45.38, 45.29, 28.30, 28.14, 15.63, 15.26.
HR-MS: (ESI) m/z: $[M+H]^+$ calculated for $C_{25}H_{28}Cl_2N_3O_4S$ = 536.11721; found = 536.11740

3-(6-((3-(4-((((1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-2-oxo-3-((S)-1-(pyridin-2-yl)ethyl)-3,10-diazabicyclo[4.3.1]decan-5-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)amino)-6-oxohexyl)-2-((1E,3E)-5-((E)-3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-methyl-1-(3-sulfopropyl)-3H-indol-1-ium-5-sulfonate



(1S,5R,6R)-10-((3,5-dichlorophenyl)sulfonyl)-5-((prop-2-yn-1-yloxy)methyl)-3-((S)-1-(pyridin-2-yl)ethyl)-3,10-diazabicyclo[4.3.1]decan-2-one (1.14 mg, 2.13 umol, 1.0 eq.) and Alexa647-azide (Jenabioscience, 2.0 mg, 2.13 umol 1.0 eq.) was dissvoled in 1000 uL DMSO, 100 uL tert-butanol and 100 uL water under Ar atmosphere. $CuSO_4$ (0.1M aqueus solution, 21 uL, 2.1 umol 1.0 eq.) and Na-L-ascorbate (0.1M aqueous solution, 21 uL, 2.1 umol, 1.0 eq.) were added and the reaction was stirred at rt overnight. The crude product was directly purified by prep. HPLC.

Yield: 2.1 mg (68%)

Appearance: blue solid

HR-MS: (ESI) m/z: $[M+H]^+$ calculated for $C_{64}H_{80}Cl_2N_9O_{17}S_5$ = 1476.36473; found = 1476.36400

Biochemical methods

Mammalian cell culture

Human embryonic kidney 293T (HEK293T) cells, the HEK293T FKBP12-eGFP reporter cell line and Hela AZ-GR cells [7] were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (Gibco) + 1% Penicillin-Streptomycin (Gibco) and in case of the FKBP12-eGFP and Hela AZ-GR reporter cell lines with 200 µg/mL Hygromycine B (Roth) at 37 °C and 5% CO_2 unless indicated otherwise.

Selection process and generation of FKBP12-eGFP reporter cell line

10^6 HEK293T cells were seeded in a 10 cm plate and left to attach overnight. On the next day, the medium was aspirated and 9 mL fresh medium was added to the cells. 8 µg pcDNA3.1(H+)-FKBP12-eGFP-IRES2-mCherry plasmid was mixed with 22,5 µg PEI in 1 mL Opti-MEM (Gibco), incubated for 30 min at room temperature and added to the plate. Following overnight incubation, the medium was exchanged and after 48 hours the culture medium was replaced by selection medium (DMEM +10% FBS + 1% P/S + 900 µg/mL Hygromycin B) for 14 days. The medium was exchanged every 2-3 days. To obtain monoclonal populations the cells were washed, trypsinized and subcloned into 96 well plates at a density of 1 cell/well. After expansion, cells were screened for simultaneous expression of FKBP12-eGFP and mCherry.

FKBP12-eGFP Reporter Assay

HEK293T cells stably expressing FKBP12-eGFP and mCherry (pcDNA3.1(H+)-FKBP12-eGFP-IRES2-mCherry stably incorporated) were seeded at 10^4 cells/well in black PLL coated 96 well plates and left to attach overnight. The medium was aspirated and 50 µL fresh medium (without Hygromycin B) was added. PROTAC solutions at 2-fold concentration were prepared in medium (without Hygromycin B) and 50 µL were added to the respective wells. After 48 h, the cells were washed with DPBS (Gibco) and lysed in 50 µL NETN buffer (100 mM NaCl, 20 mM Tris-Cl, 0.5 mM EDTA, 0.5% (v/v) Nonidet P-40, pH 8.0) + proteasome inhibitor (Roche) on ice for 30 minutes. The eGFP and mCherry fluorescence was measured using a Tecan Spark (mCherry: Ex: 580 nm, Em:620 nm; eGFP: Ex: 485 nm Em: 525 nm). Following, eGFP to mCherry ratios were calculated and normalized to the eGFP/mCherry ratio of the DMSO control.

For dose-response experiments the data was analysed with GraphPad Prism 8 and fitted by a four parameter IC_{50} fit.

FKBP12-eGFP reporter sequence:

MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQ
MSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEEDPPVATMVSKGEELFTGVVPILVELDG
DVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSA
MPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADK
QKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFV
TAAGITLGMDELYK

FKBP51-eGFP Reporter Assay

2 x 10^6 HEK293T cells were seeded in a 10 cm plate and left to attach overnight. The medium was replaced with 9 mL fresh medium and the cells were transiently transfected by adding a previously incubated (30 min, room temperature) mixture of 5 µg pEGFP-N-FKBP51-IRES2-mCherry plasmid and 22,5 µg PEI in 1 mL Opti-MEM reduced serum medium (Gibco). Following overnight incubation, the cells were washed, trypsinized and 2 x 10^4 transiently transfected cells in 50 µl medium were added to each well of a black PLL coated 96 well plate. Additionally, PROTAC solutions were prepared at 2-fold concentration in medium and added to the respective wells. After 48 h, the cells were washed with DPBS (Gibco) and lysed in 50 µL NETN buffer (100 mM NaCl, 20 mM Tris-Cl, 0.5 mM EDTA, 0.5% (v/v) Nonidet P-40, pH 8.0) + proteosome inhibitor (Roche) on ice for 30 minutes. The eGFP and mCherry fluorescence was measured using a Tecan Spark (mCherry: Ex: 580 nm, Em: 620 nm; eGFP: Ex: 485 nm Em: 525 nm). Following, eGFP to mCherry ratios were calculated and normalized to the eGFP/mCherry ratio of the DMSO control.

FKBP51-eGFP reporter sequence:

MTTDEGAKNNEESPTATVAEQGEDITSKKDRGVLKIVKRVGNGEETPMIGDKVYVHYKGKLSNGKKFD
SSHDRNEPFVFSLGKGQVIKAWDIGVATMKKGEICHLLCKPEYAYGSAGSLPKIPSNATLFFEIELLDFK
GEDLFEDGGIIRRTKRKGEGYSNPNEGATVEIHLEGRCGGRMFDCRDVAFTVGEGEDHDIPIGIDKAL
EKMQREEQCILYLGPRYGFGEAGKPKFGIEPNAELIYEVTLKSFEKAKESWEMDTKEKLEQAAIVKEK
GTVYFKGGKYMQAVIQYGKIVSWLEMEYGLSEKESKASESFLLAAFLNLAMCYLKLREYTKAVECCDK
ALGLDSANEKGLYRRGEAQLLMNEFESAKGDFEKVLEVNPQNKAARLQISMCQKKAKEHNERDRRIY
ANMFKKFAEQDAKEEANKAMGKKTSEGVTNEKGTDSQAMEEEKPEGHVEDPPVATMVSKGEELFTG
VVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHM
KQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYN
SHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEK
RDHMVLLEFVTAAGITLGMDELYK

Western Blot analysis

2 x 10^5 HEK293 T cells per well were seeded in PLL coated 12 well plates and cultured over night. Cells were treated with indicated concentrations of PROTACs and/or compounds for 24 h unless indicated otherwise. After the incubation period, the PROTAC/compound containing medium was aspirated, the cells were washed with 500 µL 4°C DPBS and lysed on ice in 100 µL/well NETN buffer (100 mM NaCl, 20 mM Tris-Cl, 0.5 mM EDTA, 0.5% (v/v) Nonidet P-40, pH 8.0) + proteasome inhibitor (Roche). MLN4924 and Carfilzomib were obtained from Cell Signaling Technology.

Following lysis, lysates were transferred to 1.5 mL Eppendorf tubes, spun down for 15 minutes at 4°C and 15000 g. 60 µL supernatant was mixed with 20 µL 4 x Lämmli-buffer containing β-mercaptoethanol and heated to 95°C for 10 minutes. The proteins were separated on a SDS-page and transferred to Nitrocellulose membrane (Amersham) using Semi-Dry Rapid Blotting System (Bio-Rad). After blocking the membrane in 5% milk powder (Roth) in TBS buffer for 30 minutes, the membrane was cut at heights of 45 kDa and 20 kDa. Protein levels were probed overnight with the respective primary antibodies (FKBP51: A301-430 (Bethyl); FKBP52: A301-427A (Bethyl); FKBP12: ab24373 (Abcam); GAPDH: 14C10 (CST)). The membranes were washed 3 times for 5 minutes in TBS buffer and the FKBP51, FKBP52 and FKBP12 blots were probed with secondary antibody (A120-112P (Bethyl)) for 2 h. All blots were washed 3 times for 5 minutes in TBS buffer. Proteins levels were visualized using Immobilon Western Chemiluminescent HRP Substrate (Millipore) and LAS-3000 (Fujifilm) device.

Western blots were quantified using (Fiji) is just Image J software.

FKBP52-HTRF Quantification

3.5 x 10^4 HEK293T cells/well were seeded in PLL coated 24 well plates and left to attach overnight. Cells in two wells/plate were transiently transfected (Lipofectamin 2000) with 20 pmol/well anti-FKBP52 siRNA (Silencer Validated siRNA siRNA ID: s50, ThermoFisher). After overnight incubation, the medium of all wells was exchanged to medium containing PROTAC or DMSO for 24 hours. Then the cells were washed with 500 µL/well cold DBPS (Gibco) and lysed in lysis buffer (150 mM NaCl, 25 mM Tris-Cl, 200 mM KF, 0.5% Triton X-100, 0.5%sodium deoxycholate, 0.1% SDS, 5%(w/v) BSA, supplemented with protease inhibitor cocktail (Roche) and 1 mM PMSF, pH 8.0) on ice for 30 min. The lysates were transferred to 1.5 mL Eppendorf tubes and spun down for 20 min at 18000 g at 4 °C. 16 µL lysate were transferred to ProxiPlates (Revvity) and 4 µL (600 nM FKBP52-HTRF tracer, 6,25 nM primary FKBP52 antibody (A301-427A (Betyhl) and 6 nM secondary pAB Anti Rabbit IgG-Eu cryptate (61PARKLA (Revvity)) in lysis buffer were added. After overnight incubation at 4°C, the HTRF signal was measured with a Tecan Spark (Ex.: 320 nm, Em.: 620 nm and 665 nm, lag time: 150 µs, integration time: 500 µs).

GR activation reporter gene assay

2 x 10^4 Hela AZ-GR [7] cells per well were seeded in PLL coated 96 well plate. After overnight incubation, the medium was aspirated and replaced with 50 µl medium supplemented with 2-fold Dexamthesone and 50 µL 2-fold PROTAC in medium. In case of competition experiments, 50 µL 2-fold Dexamthesone, 25 µL 4-fold PROTAC and 25 µL 4-fold compound containing medium was added. After 48 hours incubation, the cells were washed with 50 µL 4°C DPBS and lysed on ice in 60 µL passive lysis buffer (Promega) for 30 minutes. 20 µL lysate were transferred to a white 96 well half area plate (Greiner) and 20 µL per well Bio-Glow Substrate (Promega) was added. After 5 minutes incubation at room temperature the luminescence was assessed using a Tecan Spark device. Fold-induction was calculated in reference to the unstimulated control and significance was tested by One-way Anova tests using GraphPad Prism 8.

GR activation qPCR assay

6*10^5 A549 cells per 6cm dish were seeded and immediately co-treated (64 h) with dexamethasone (25 nM) and with DMSO or compounds as indicated. Afterwards, the cells were washed with cold (4 °C) DPBS (Gibco) and the RNA was isolated using PureLink™ RNA Mini Kit from (Invitrogen, Thermos Fisher Scientific) according to the manufactures instructions. 1 µg RNA was transcribed in cDNA using standard reverse transcription (according to NEB standard first strand cDNA synthesis protocol using ProtoScript II RT (NEB #M0368), Oligo d(T)23VN (50 µM, NEB#S1327S), Murine RNase Inhibitor (NEB#M0314L) and dNTP mix (NEB#N0447S). FKBP5, GILZ and GAPDH were quantified using commercially available PowerUP™ SYBR™ Green Master Mix (Thermos Fisher Scientific), QuantStudio 5 qPCR machine (Thermos Fisher Scientific), and the following primers.

FKBP5: fw: 5′-AAATCCAAACGAAGGAGCAA-3′ [8], rev: 5′-GCCACATCTCTGCAGTCAAA-3′ [8]

GILZ: fw: 5′-ACCGAAATGTATCAGACCCCCA-3′ [8], rev: 5′-CGATCTTGTTGTCTATGGCCACC-3′ [8]

GAPDH: fw: 5′-AAGAAGGTGGTGAAGCAGGC-3′ [9], rev: 5′- ACCACCCTGTTGCTGTAGCCAA -3’ [10]

FKBP5 and *GILZ* levels were normalized to the corresponding *GAPDH* levels and afterwards *FKBP5* and *GILZ* induction was calculated in reference to the unstimulated (no Dex, no compounds) control.

FKBP51 target engagement NanoBRET

A FKBP ligand/ PROTAC dilution series was performed at a 100-fold concentration of the final sample in DMSO. Following, the ligand/PROTAC was diluted to a 2-fold concentration in Opti-MEM reduced serum medium (Gibco) and 20 µL were transferred to a white non-binding 384 well plate (Greiner). HEK293T cells stably expressing FKBP51FK1-Nluc fusion were adjusted to a concentration of 9.05 x 10^5 cells/mL. The fluorescent tracer 2c from [11] was diluted to 3.2 µM (8-fold) in Opti-MEM reduced serum medium. A 2-fold cell-tracer mixture was prepared by mixing 3 parts detached cells with one part of the 8-fold tracer dilution and 800 nM MLN4924 (CST). 20 µL/well of the 2-fold tracer-cell mixture were added on top of the PROTAC/compound solution and the plate was briefly spun down, sealed with aluminium foil and incubated for 2 h at 37°C. 20 µL/well 6.6 µM hydrolysed hikarazine (compound 26dl, [12]) and 7.5 µM extracellular Nluc inhibitor (compound 43, [13]) were added on top to the assay plate. For BRET detection, the donor (445-470 nm) and acceptor (610-700 nm) emissions were measured for 1 s using a Tecan Spark in well-wise measuring mode. The IC50 values were determined by a four parameter IC_{50} fit using GraphPad Prism 8.

Protein purification

VCB complex

The codon optimized gene for VHL amino acids 54-213 was cloned behind a SUMO tag and transformed in *E. coli* BL21 (DE3) cells bearing a plasmid for the co-expression of EloB and EloC. The plasmid encoding for EloB and EloC was a kind gift of Alessio Ciulli. A single colony was used to inoculate 50 ml LB medium supplemented with 50 µg/ml kanamycin and 33 µg/ml streptomycin and incubated at 37 °C overnight. For the main culture, 1 L LB medium supplemented with 50 µg/ml kanamycin and 33 µg/ml streptomycin was inoculated to an OD600 of 0.1 and incubated at 37 °C and 180 rpm until an OD600 of 0.6 was reached. The culture was cooled to 25 °C, induced by addition of 0.5 mM isopropyl 1-thio-D-galactopyranoside and further incubated for additional 16 hours.

Cells were harvested by centrifugation (13,000 x g, 15 min, 4 °C) and the cell pellet was solubilized in lysis buffer (20 mM HEPES, 500 mM NaCl, pH 8) supplemented with 1 mM PMSF, 1 mM TCEP, 2 mg/ml lysozyme, and 0.1 mg/ml DNase I. After incubation for 1 hour, the cells were lysed using sonication and cellular debris were removed by centrifugation (20,000 x g, 30 min, 4 °C). The supernatant was loaded on a Nickel-NTA (Machery Nagel) column equilibrated with lysis buffer. The column was washed with 10 column volumes of washing buffer (20 mM HEPES, 300 mM NaCl, 10 mM imidazole pH 8) and the protein was eluted with elution buffer (20 mM HEPES, 300 mM NaCl, 300 mM imidazole pH 8). Target protein containing fractions were dialyzed against 20 mM HEPES, 300 mM NaCl, 1 mM TCEP, pH 8 and the His-SUMO tag was cleaved by addition of recombinant Ulp1. The cleaved His-SUMO tag was removed by passing the protein mixture through a Nickel-NTA column. The VCB containing flow-through was finally purified by size exclusion chromatography using a HiLoad® 16/600 Superdex® 75 pg column (Cytiva) equilibrated with 20 mM HEPES, 150 mM NaCl, pH 7.5. The pure protein was concentrated to 17 mg/ml using an Amicon® Ultra 2 mL centrifugal filter, flash frozen in liquid nitrogen and stored at -80 °C.

Crystallography

For crystallization trials the ternary complex was formed by mixing 3614 µl 20 mM HEPES, 150 mM NaCl, pH 7.5, 50 µl of FKBP51 (16-140, A19T, C103A, C107I) at 31 mg/ml, 100 µl of SelDeg51 at 1 mM in DMSO and 236 µl of VCB at 17 mg/ml, yielding 4 ml complex at 1.3 mg/ml (23.6 µM). This solution was concentrated to 400 µl using an Amicon® Ultra 2 mL centrifugal filter and purified by size exclusion

chromatography using a 10/300 GL Superdex® Increase 75 column (Cytiva) equilibrated with 20 mM HEPES, 150 mM NaCl, pH 7.5. The fraction containing all components of the protein complex was concentrated to 10-13 mg/ml and used directly for crystallization trials. Crystallization was performed at 4 °C using the sitting drop vapour-diffusion method by equilibrating mixtures of 0.5 µl protein complex with 0.5 µl reservoir solution against 30 µl reservoir solution containing 15% PEG3350, 0.2 M tri-sodium citrate, 0.1 M HEPES-NaOH pH 7.5 and 10% glycol. Crystals were fished and flash frozen in liquid nitrogen.

The complex of FKBP12 and PROTAC 6a2 was prepared by mixing FKBP12 (C22V) at 25.6 mg/ml formulated in 20 mM HEPES pH 8.0 and 20 mM NaCl with a slight molar excess of ligand previously dissolved at 20 mM in DMSO. Crystallization was performed at room temperature using the hanging drop vapour-diffusion method, equilibrating mixtures of 1 µl protein complex and 1 µl reservoir against 500 µl reservoir solution. Crystals were obtained from reservoir solutions containing 1.36 Na/K tartrate, 0.2M ammonium citrate and 0.1M MES pH 6.5. Crystals were fished, cryoprotected with LV CryoOil™ (Jena Bioscience) and flash frozen in liquid nitrogen.

The crystallographic experiments were performed on the BL14.1 and BL14.2 beamlines at the Helmholtz-Zentrum BESSY II synchrotron, Berlin, Germany [14]. Diffraction data were integrated with XDS and further processed with the implemented programs of the CCP4i and CCP4i2 interface [15,16]. The data reduction was conducted with Aimless [16,17]. Crystal structures were solved by molecular replacement using Phaser [18]. Iterative model improvement and refinement were performed with Coot and Refmac5 [19]. The dictionaries for 6a2 and SelDeg51 were generated wit PRODRG implemented in CCP4i [20].

Burrow surface area calculation

Interface areas were calculated PISA [21].

Single point cooperativity screening

In order to quickly screen for positive cooperativity competitive fluorescence polarization assays were carried out. Therefore, 200 nM of the respective PROTAC in 20 mM HEPES pH 8.0, 150 mM NaCl and 0.002% Triton X-100 was placed in a 384-well assay plate and incubated with 8 nM VCB complex and 1 nM VHL tracer alone or in the presence of 1 µM of FKBP12, FKBP51FK1 or FKBP52FK1. After incubation for 30 min at room temperature, the fluorescence polarization was measured with a Tecan Spark (Ex.: 535 nm, Em.: 595 nm). The obtained results for each 4 replicates were normalized with respect to the maximal binding signal and the additional tracer displacement upon FKBP addition was determined.

Fluorescence polarization assay for FKBP binding

For the dertermination of the binding affinity of PROTACs to FKBP proteins competitive fluorescence polarization assays using a FKBP-FP tracer were carried out as described earlier [2,3].

Fluorescence polarization assay for VCB binding

The influence of FKBP12 binding on the binding of PROTACs to VCB was investigated using a competitive fluorescence polarization assay. A serial dilution of the respective PROTAC in 20 mM

HEPES pH 8.0, 150 mM NaCl and 0.002% Triton X-100 alone or supplemented 2 µM FKBP12 was placed in a 384-well assay plate and incubated with 8 nM VCB complex and 1 nM VHL-FP tracer. After incubation for 30 min at room temperature, the fluorescence polarization was measured with a Tecan Spark (Ex.: 535 nm, Em.: 595 nm). The obtained results for each 3 replicates were normalized with respect to the maximal binding signal and the data was fitted using a competitive binding model as described by Wang [22].

HTRF assay for FKBP51 binding

For the determination of the binding affinity of PROTACs alone or in complex with VCB to FKBP51 competitive HTRF assays were performed. Therefore, a serial dilution of the respective PROTAC in 20 mM HEPES pH 8.0, 150 mM NaCl, 5% PEG3350 and 0.002% Triton X-100 alone or supplemented 5 µM VCB was placed in a white 384-well PROXiPlate Plus (Revvity) and incubated with 2.5 nM His-tagged FKBP51FK1, 5 nM FKBP-HTRF tracer and MAb Anti-6HIS TB cryptate Gold as recommended by the supplier (Revvity). After incubation for 30 min at room temperature, the HTRF signal was measured with a Tecan Spark (Ex.: 320 nm, Em.: 620 nm and 665 nm, lag time: 150 µs, integration time: 500 µs). The obtained results for each 3 replicates were normalized with respect to the maximal binding signal and the data was fitted using a competitive binding model as described by Wang. [22]

Label free proteomics Proteomics

MOLT4 cells were treated with DMSO or 1 µM of 5a1 or SelDeg51 for 5 hr and cells were harvested by centrifugation at 4 ºC before snap freezing in liquid nitrogen. Cells were lysed by addition of lysis buffer (8 M Urea, 50 mM NaCl, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (EPPS) pH 8.5, Protease and Phosphatase inhibitors) and homogenization by bead beating (BioSpec) for three repeats of 30 seconds at 2400. Bradford assay was used to determine the final protein concentration in the clarified cell lysate. 50 µg of protein for each sample was reduced, alkylated and precipitated using methanol/chloroform as previously described [23] and the resulting washed precipitated protein was allowed to air dry. Precipitated protein was resuspended in 4 M Urea, 50 mM HEPES pH 7.4, followed by dilution to <1 M urea with the addition of 200 mM EPPS, pH 8. Proteins were digested with LysC (1:50; enzyme:protein) and trypsin (1:50; enzyme:protein) overnight at 37 °C. Sample digests were acidified with formic acid to a pH of 2-3 prior to desalting using C18 solid phase extraction plates (SOLA, Thermo Fisher Scientific). Desalted peptides were dried in a vacuum-centrifuged and reconstituted in 0.1% formic acid for LC-MS analysis.

Data were collected using a TimsTOF Pro2 (Bruker Daltonics, Bremen, Germany) coupled to a nanoElute LC pump (Bruker Daltonics, Bremen, Germany) via a CaptiveSpray nano-electrospray source. Peptides were separated on a reversed-phase C_{18} column (25 cm x 75 µm ID, 1.6 µM, IonOpticks, Australia) containing an integrated captive spray emitter. Peptides were separated using a 50 min gradient of 2 - 30% buffer B (acetonitrile in 0.1% formic acid) with a flow rate of 250 nL/min and column temperature maintained at 50 ºC.

DDA was performed in Parallel Accumulation-Serial Fragmentation (PASEF) mode to determine effective ion mobility windows for downstream diaPASEF data collection (Meier et al., 2020). The ddaPASEF parameters included: 100% duty cycle using accumulation and ramp times of 50 ms each, 1 TIMS-MS scan and 10 PASEF ramps per acquisition cycle. The TIMS-MS survey scan was acquired between 100 – 1700 *m/z* and 1/k0 of 0.7 - 1.3 V.s/cm^2. Precursors with 1 – 5 charges were selected and those that reached an intensity threshold of 20,000 arbitrary units were actively excluded for 0.4 min. The quadrupole isolation width was set to 2 *m/z* for *m/z* <700 and 3 *m/z* for *m/z* >800, with the *m/z* between 700-800 *m/z* being interpolated linearly. The TIMS elution voltages were calibrated linearly with three points (Agilent ESI-L Tuning Mix Ions; 622, 922, 1,222 *m/z*) to determine the reduced ion mobility

coefficients ($1/K_0$). To perform diaPASEF, the precursor distribution in the DDA *m/z*-ion mobility plane was used to design an acquisition scheme for DIA data collection which included two windows in each 50 ms diaPASEF scan. Data was acquired using sixteen of these 25 Da precursor double window scans (creating 32 windows) which covered the diagonal scan line for doubly and triply charged precursors, with singly charged precursors able to be excluded by their position in the m/z-ion mobility plane. These precursor isolation windows were defined between 400 - 1200 *m/z* and 1/k0 of 0.7 - 1.3 V.s/cm^2.

LC-MS data analysis

The diaPASEF raw file processing and controlling peptide and protein level false discovery rates, assembling proteins from peptides, and protein quantification from peptides was performed using library free analysis in DIA-NN 1.8 [24]. Library free mode performs an in silico digestion of a given protein sequence database alongside deep learning-based predictions to extract the DIA precursor data into a collection of MS2 spectra. The search results are then used to generate a spectral library which is then employed for the targeted analysis of the DIA data searched against a Swissprot human database (January 2021). Database search criteria largely followed the default settings for directDIA including: tryptic with two missed cleavages, carbomidomethylation of cysteine as a fixed modification, and oxidation of methionine as a variable modification and precursor Q-value (FDR) cut-off of 0.01. Precursor quantification strategy was set to Robust LC (high accuracy) with RT-dependent cross run normalization. Proteins with low summed abundance across the treatments (<16k) were excluded from further analysis and proteins with missing values were imputed by random selection from a gaussian distribution either with a mean of the non-missing values for that treatment group or with a mean equal to the median of the background (in cases when all values for a treatment group are missing). Protein abundances were scaled using in-house scripts in the R framework (R Development Core Team, 2014). The resulting data comparisons (treatment vs control groups) were filtered to include only proteins that had a minimum of 2 abundance counts per protein in at least 4 replicates followed by statistical analysis using the limma package within the R framework [25].

Native MS

Reagents and Standards

Analytical-grade reagents and solvents were all acquired from Merck (Darmstadt, DE). The PROTAC-induced complex sample was prepared at a concentration of 40 µM in 200 mM aqueous ammonium acetate (pH 6.8). Before starting the MS-based experiments, the solution was desalted with a 10k molecular weight cut-off Zeba spin column (Thermo Fisher Scientific, Waltham, MA, USA). This step is crucial to get rid of non-volatile salts that may interfere with the MS measurement.

Native mass spectrometry

Approximately 7 µL of sample solution was loaded into in-house pulled glass needles and then sprayed through direct infusion with a nanoESI source coupled to a Synapt XS ion mobility-mass spectrometer (Waters, Milford, MA, USA). The glass needles were pulled with a P97 micropipette puller (Sutter Instruments, Novato, CA, USA). A spray voltage of 1.3 kV was applied using a stainless steel wire inserted into the distal end of the glass needle. A sampling cone potential of 30 V and a source temperature of 30 °C were used. For the measurements under gentle conditions in S3.2A, we used Trap and Transfer collision energies of 10 and 4 V, respectively. For the harsh conditions in S3.2B, these values were changed to 45 V and 8 V, and a broad precursor window centered at 4000 *m/z* was selected in the quadrupole. Additional MS parameters were kept at default values. Data processing was

performed with MassLynx 4.2. For acquisition of the ion mobility data, three different calibrants were used: bovine serum albumin, myoglobin, and cytochrome c. IM-MS data were acquired with a wave height of 40 V and wave velocities of 800, 1000, and 1200 m/s. A logarithmic function was applied to calculate the collision-cross section (CCS).[26] To compare the obtained values, we calculated CCS values based on the crystal structure of the complex using two software packages: IMPACT [27] and DrifScope v3.0.

Supplementary references

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