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Supplementary Materials for

Class I histone deacetylases (HDAC1–3) are histone lysine delactylases

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This PDF file includes:

Supplementary Figures

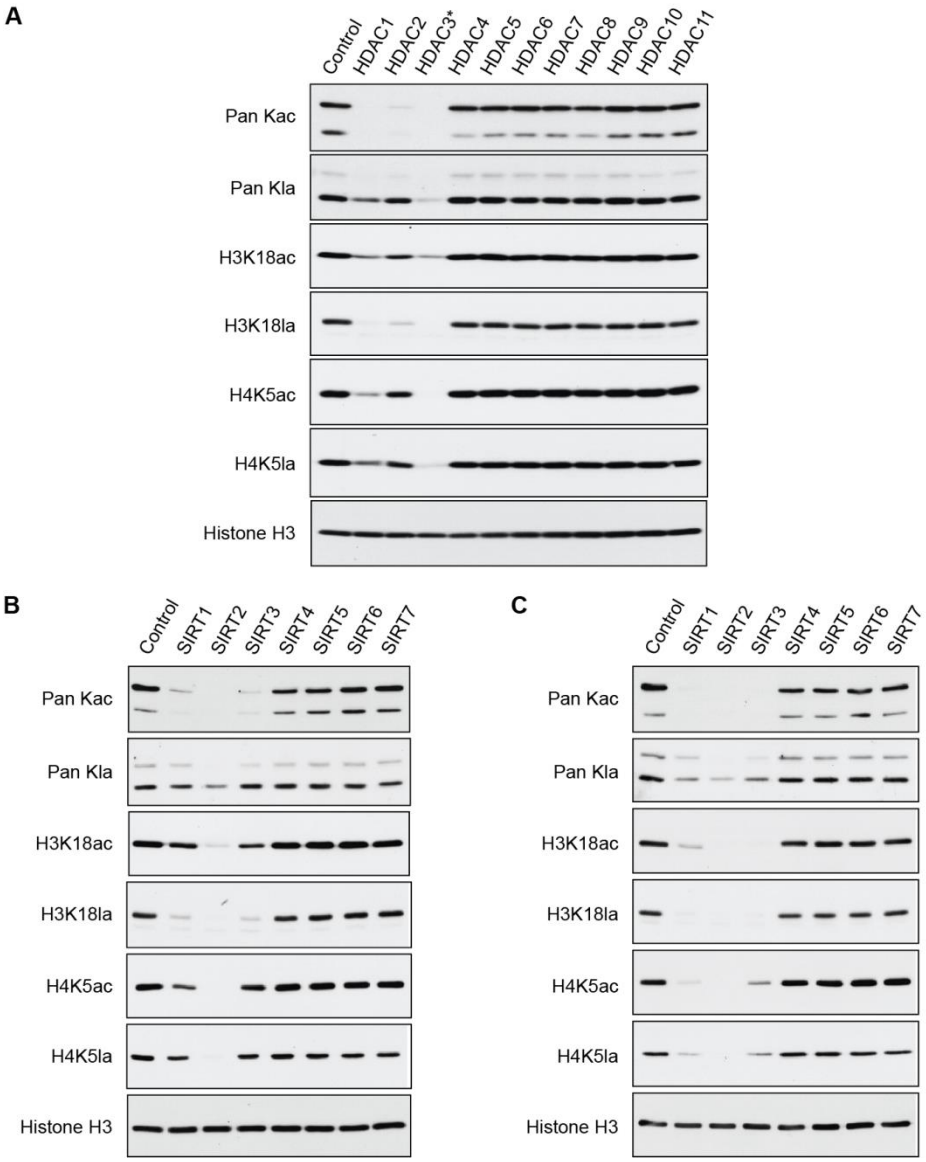


Fig. S1. Supplementary deacylase activity screening. (**A**) Deacylase activity screening using purified histones from HeLa cells and antibodies against Kac and Kla modifications, with histone H3 as loading control (1 h reaction). (**B**) Deacylase activity screening of recombinant sirtuin enzymes using purified histones from HeLa cells and antibodies against Kac and Kla modifications, with histone H3 as loading control (1 h reaction). (**C**) Deacylase activity screening of recombinant sirtuin enzymes using purified histones from HeLa cells (4 h reaction).

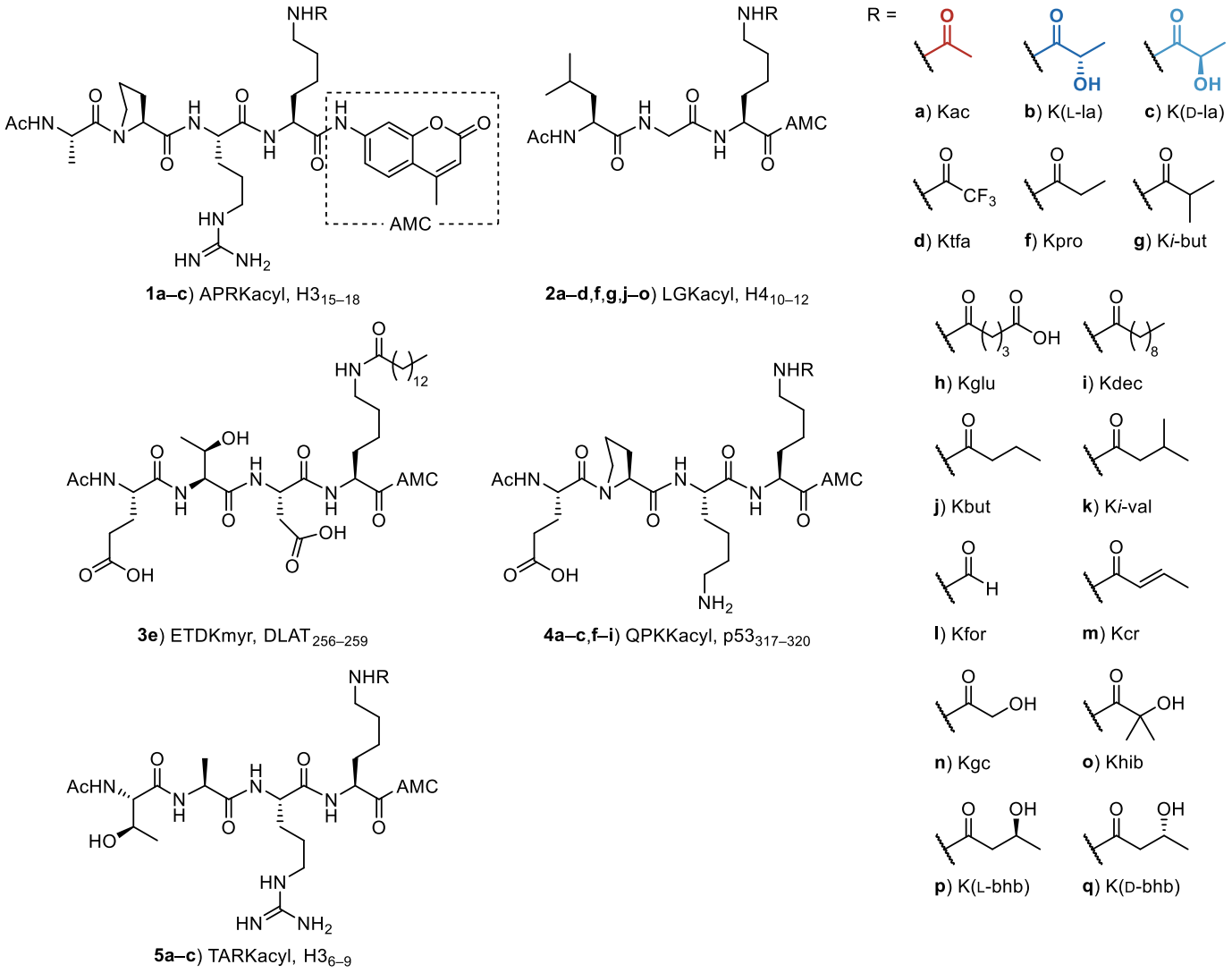


Fig. S2. Structure of fluorogenic substrates. AMC: 7-amino-4-methylcoumarin.

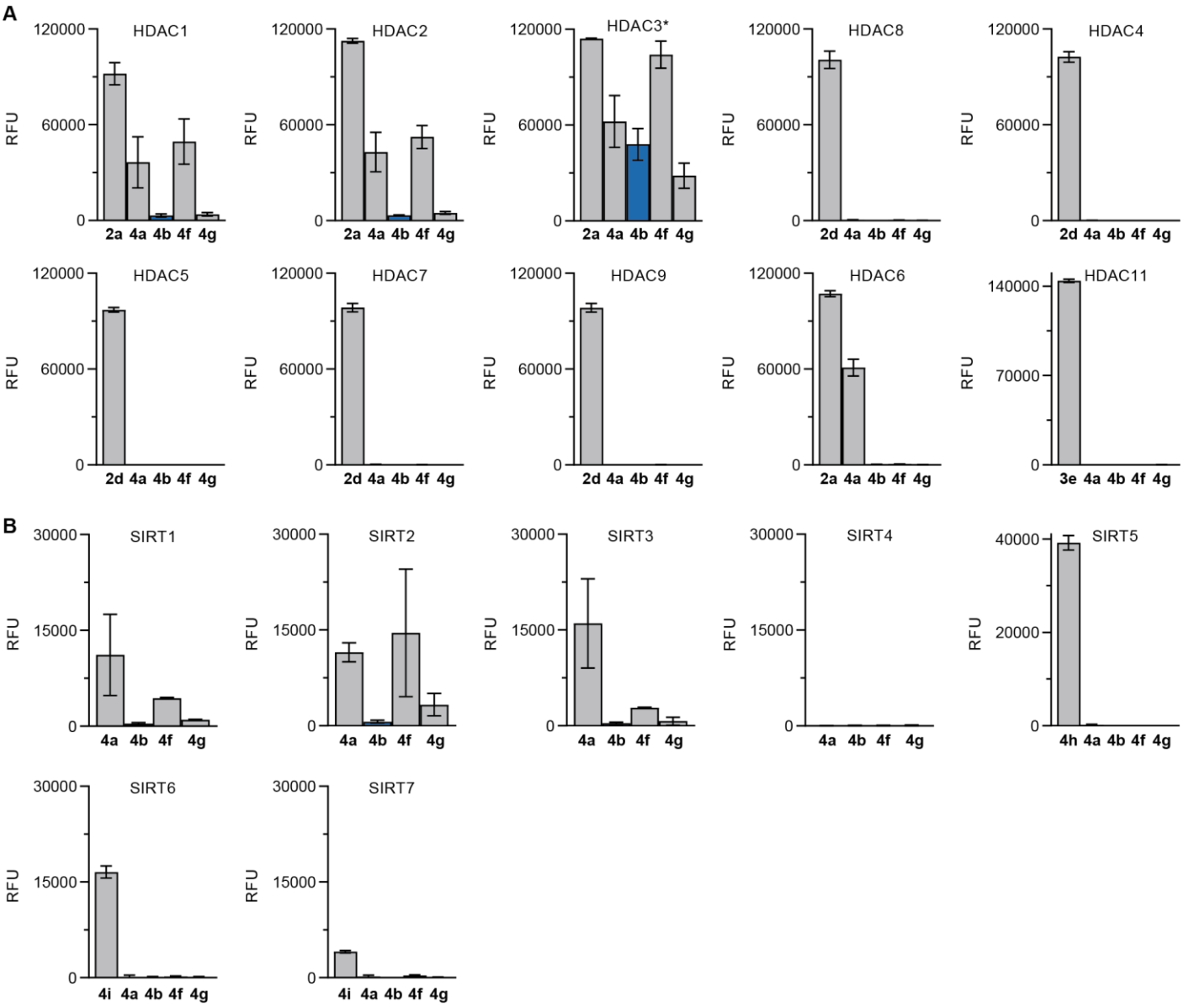
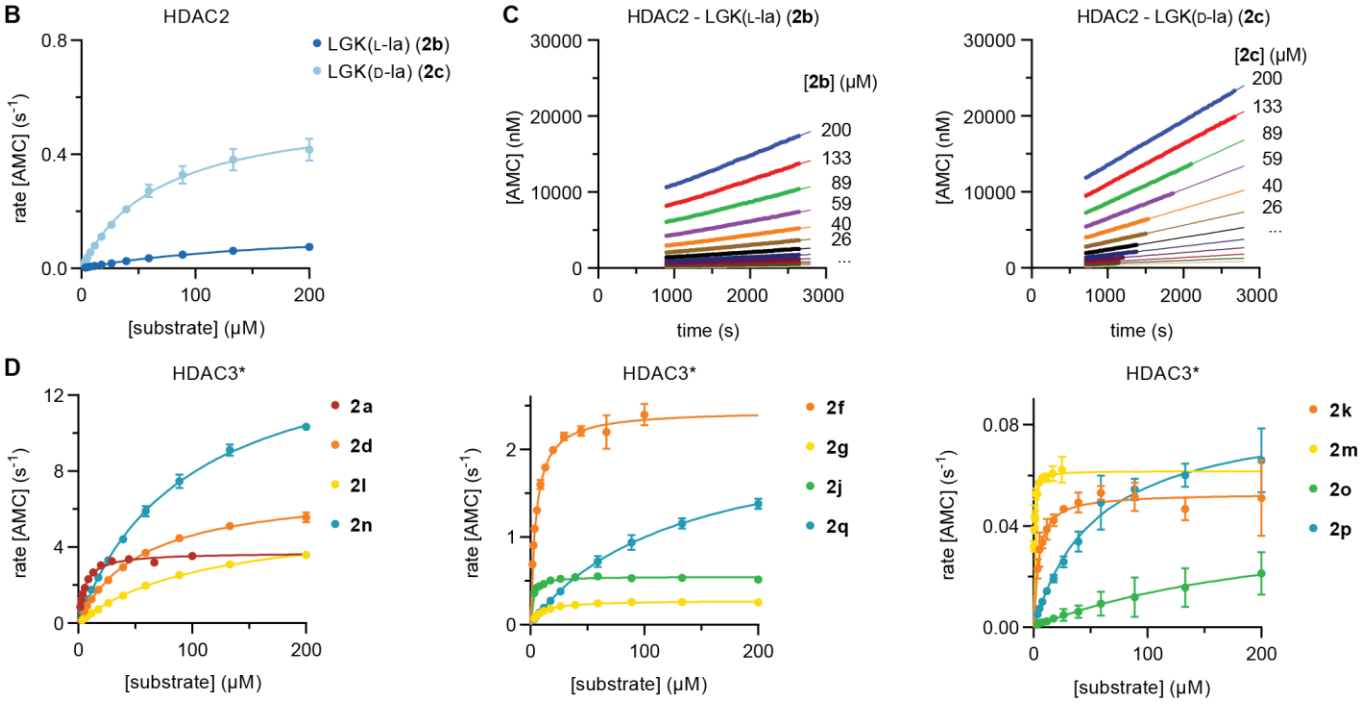


Fig. S3. Bar graphs corresponding to the heat map in Fig. 1D.

A

	HDAC1		HDAC2		HDAC3*	
	LGK(L-la) (**2b**)	LGK(D-la) (**2c**)	LGK(L-la) (**2b**)	LGK(D-la) (**2c**)	LGK(L-la) (**2b**)	LGK(D-la) (**2c**)
K_M (μM)	174 ± 5	68 ± 4	180 ± 10	69 ± 6	122 ± 7	67 ± 7
k_{cat} (s^{-1})	0.141 ± 0.002	0.35 ± 0.01	0.144 ± 0.006	0.57 ± 0.02	0.49 ± 0.02	0.92 ± 0.04
k_{cat}/K_M ($M^{-1}·s^{-1}$)	(0.81 ± 0.02)·10^3	(5.1 ± 0.3)·10^3	(0.80 ± 0.06)·10^3	(8.3 ± 0.7)·10^3	(4.0 ± 0.2)·10^3	(14 ± 1)·10^3



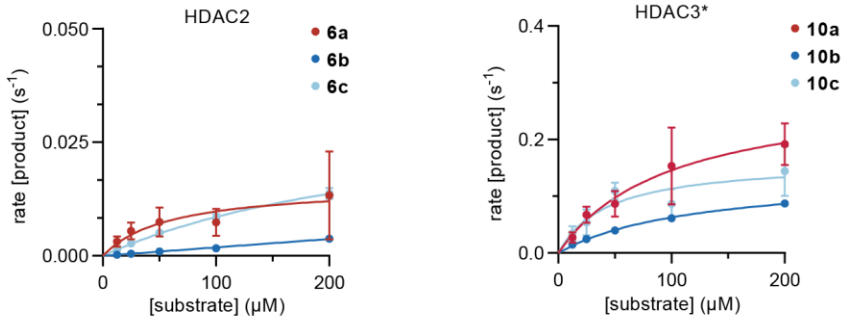
E

	HDAC3*		
	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}·s^{-1}$)
LGKac (**2a**)	5.4 ± 0.3	3.72 ± 0.06	(690 ± 40)·10^3
LGK(L-la) (**2b**)	122 ± 7	0.49 ± 0.02	(4.0 ± 0.2)·10^3
LGK(D-la) (**2c**)	67 ± 7	0.92 ± 0.04	(14 ± 1)·10^3
LGKtfa (**2d**)	56 ± 2	7.2 ± 0.1	(129 ± 5)·10^3
LGKpro (**2f**)	4.7 ± 0.2	2.45 ± 0.03	(520 ± 30)·10^3
LGK(*i*-but) (**2g**)	9.0 ± 0.6	0.28 ± 0.01	(31 ± 2)·10^3
LGKbut (**2j**)	1.7 ± 0.2	0.55 ± 0.01	(310 ± 40)·10^3
LGK(*i*-val) (**2k**)	4.1 ± 0.8	0.053 ± 0.002	(13 ± 2)·10^3

	HDAC3*		
	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}·s^{-1}$)
LGKfor (**2l**)	112 ± 3	5.7 ± 0.1	(50 ± 1)·10^3
LGKcr (**2m**)	0.43 ± 0.04	0.062 ± 0.001	(140 ± 10)·10^3
LGKgc (**2n**)	94 ± 4	15.3 ± 0.3	(163 ± 6)·10^3
LGKhib (**2o**)	240 ± 160	0.05 ± 0.02	(0.2 ± 0.1)·10^3
LGK(L-bhb) (**2p**)	56 ± 9	0.086 ± 0.006	(1.5 ± 0.2)·10^3
LGK(D-bhb) (**2q**)	125 ± 9	2.25 ± 0.09	(18 ± 1)·10^3

Fig. S4. Deacylase efficiencies of HDACs 1–3 against fluorogenic substrates. (**A**) Steady-state parameters of HDACs 1–3 against substrates **2b** and **2c** relative to curves presented in Figs. 2B and S4B. (**B**) Michaelis-Menten plots for HDAC2 against substrates **2b** and **2c**. Data represent mean ± SEM, *n* = 2. (**C**) Sample assay progression curves for HDAC2. Only data corresponding to the steady state is included in the analysis. (**D**) Michaelis-Menten plots for HDAC3/NCoR2 against substrates **2a**, **2d**, **2f**, **2g** and **2j–2q**. Data represent mean ± SEM, *n* = 2. (**E**) Steady-state parameters of HDAC3/NCoR2 against fluorogenic substrates relative to bar graphs in Fig. 4B. *HDAC3 incubated with the DAD of NCoR2.

A



B

	HDAC1			HDAC2		
	H3K9ac (**6a**)	H3K9(L-la) (**6b**)	H3K9(D-la) (**6c**)	H3K9ac (**6a**)	H3K9(L-la) (**6b**)	H3K9(D-la) (**6c**)
K_M (μM)	150 ± 50	900 ± 700	500 ± 200	60 ± 30	> 200	300 ± 40
k_{cat} (s^{-1})	0.07 ± 0.01	0.04 ± 0.03	0.09 ± 0.03	0.015 ± 0.03	> 0.02	0.034 ± 0.003
k_{cat}/K_M ($M^{-1}·s^{-1}$)	$(0.5 \pm 0.2)·10^3$	$(0.04 \pm 0.04)·10^3$	$(0.20 \pm 0.09)·10^3$	$(0.3 \pm 0.1)·10^3$	$< 0.04·10^3$	$(0.11 \pm 0.01)·10^3$

	HDAC3*					
	H3K9ac (**6a**)	H3K9(L-la) (**6b**)	H3K9(D-la) (**6c**)	H4K12ac (**10a**)	H4K12(L-la) (**10b**)	H4K12(D-la) (**10c**)
K_M (μM)	23 ± 8	90 ± 20	70 ± 20	100 ± 20	120 ± 10	40 ± 30
k_{cat} (s^{-1})	0.32 ± 0.03	0.24 ± 0.02	0.42 ± 0.04	0.29 ± 0.03	0.14 ± 0.01	0.16 ± 0.04
k_{cat}/K_M ($M^{-1}·s^{-1}$)	$(14 \pm 5)·10^3$	$(2.6 \pm 0.6)·10^3$	$(6 \pm 1)·10^3$	$(2.9 \pm 0.8)·10^3$	$(1.1 \pm 0.1)·10^3$	$(4 \pm 2)·10^3$

Fig. S5. Deacylase efficiencies of HDACs 1–3 against non-fluorogenic substrates. (**A**) Michaelis-Menten plots for HDAC2 against substrates **6a**–**c** and for HDAC3/NCoR2 against substrates **10a**–**c**. Data represent mean ± SEM, *n* = 2. (**B**) Steady-state parameters of HDACs 1–3 against non-fluorogenic histone substrates relative to bar graphs in Fig. 3F. *HDAC3 incubated with the DAD of NCoR2.

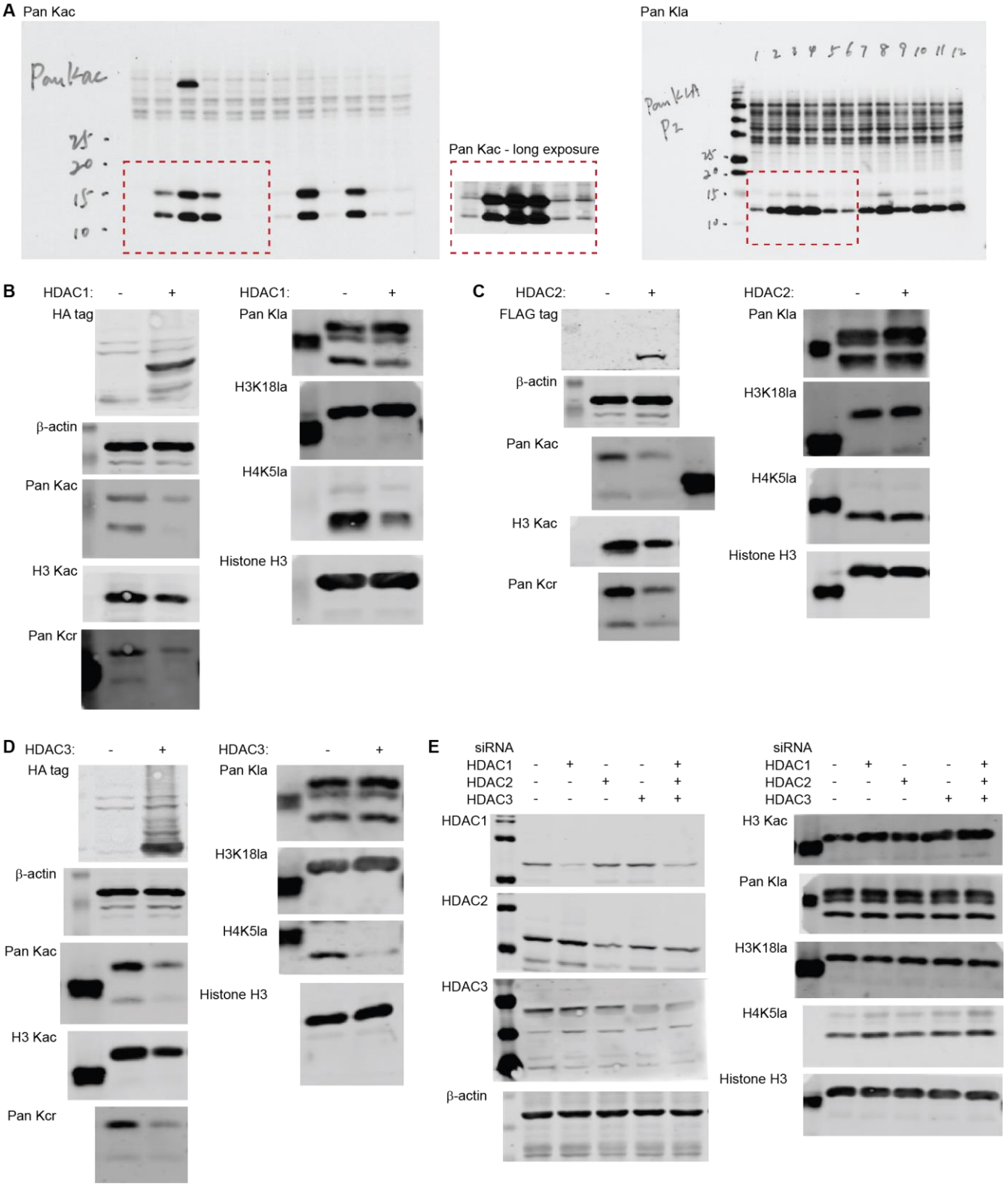


Fig. S6. Unmodified Western blots corresponding to Fig. 6. See legend in next page.

Fig. S6 (contd.) (**A**) Western blots depicted in Fig. 6A. (**B**) Western blots relative to samples transfected with HA-tagged HDAC1. (**C**) Western blots relative to samples transfected with FLAG-tagged HDAC2. (**D**) Western blots relative to samples transfected with HA-tagged HDAC3. (**E**) Western blots relative to samples with or without knockdown of HDAC1, 2, 3, or their combination.

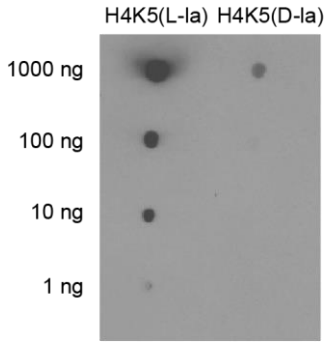


Fig. S7. Dot blot of H4K5la antibody against H4K5(L-la) and H4K5(D-la) peptides.

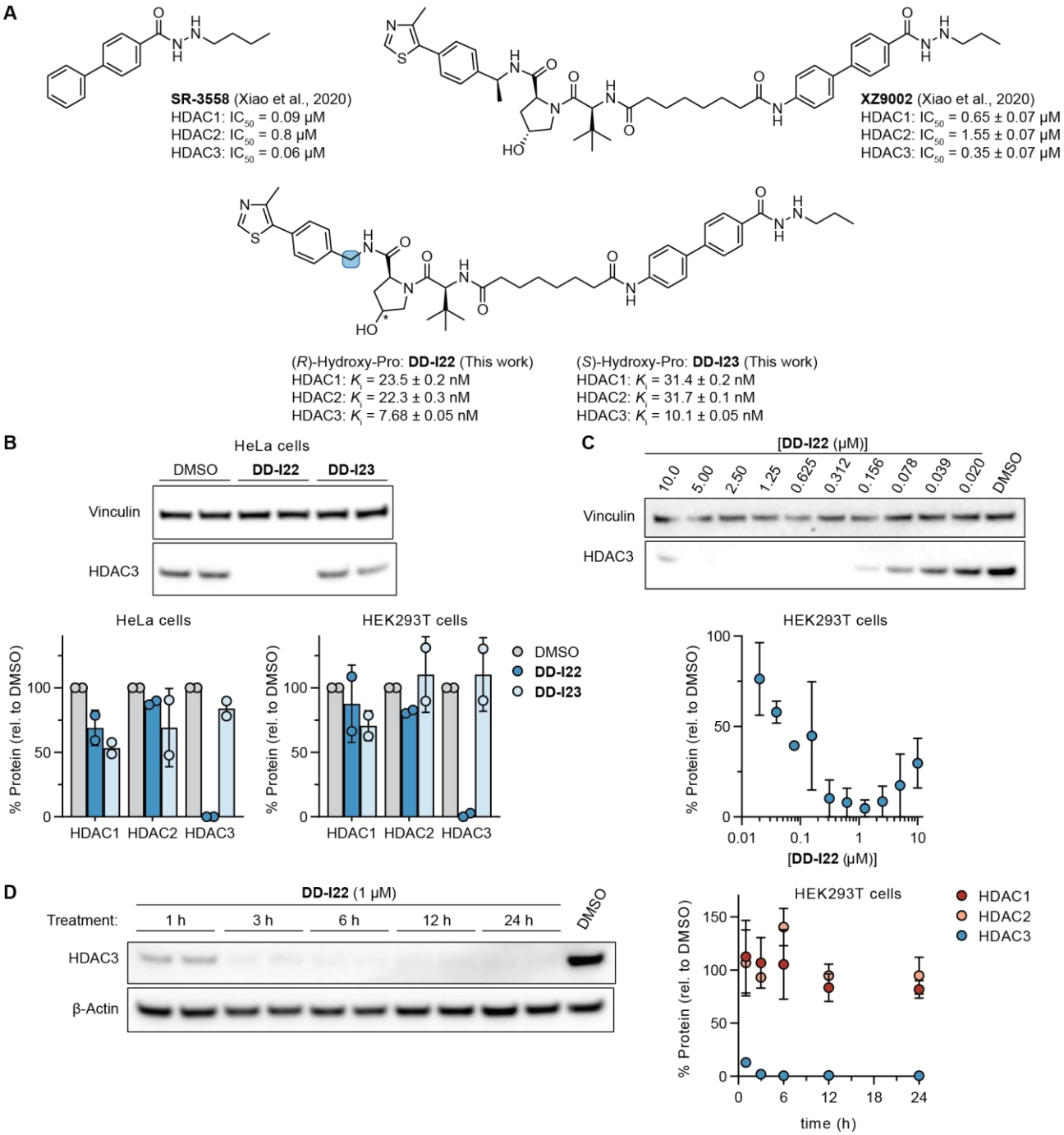


Fig. S8. Degradation of HDAC3 by PROTAC DD-I22. (**A**) Structures and HDAC inhibitory profiles of **SR-3558**, reported HDAC3-selective degrader **XZ9002** (*61*), and the HDAC3 PROTAC synthesized here (**DD-I22**) with corresponding **DD-I23** diastereomer unable to recruit the VHL E3 ligase. (**B**) Relative concentration of HDACs 1–3 upon 6 h treatment of HeLa or HEK293T cells with compounds **DD-I22** and **DD-I23** (1 μM), with sample HDAC3 blot. (**C**) Dose-response curve of compound **DD-I22** in HEK293T cells (6 h treatment), with sample blot. (**D**) Time-dependent concentration of HDACs 1–3 upon treatment with **DD-I22** at 1 μM concentration, with sample blot. All data represent mean ± SEM, *n* = 2.

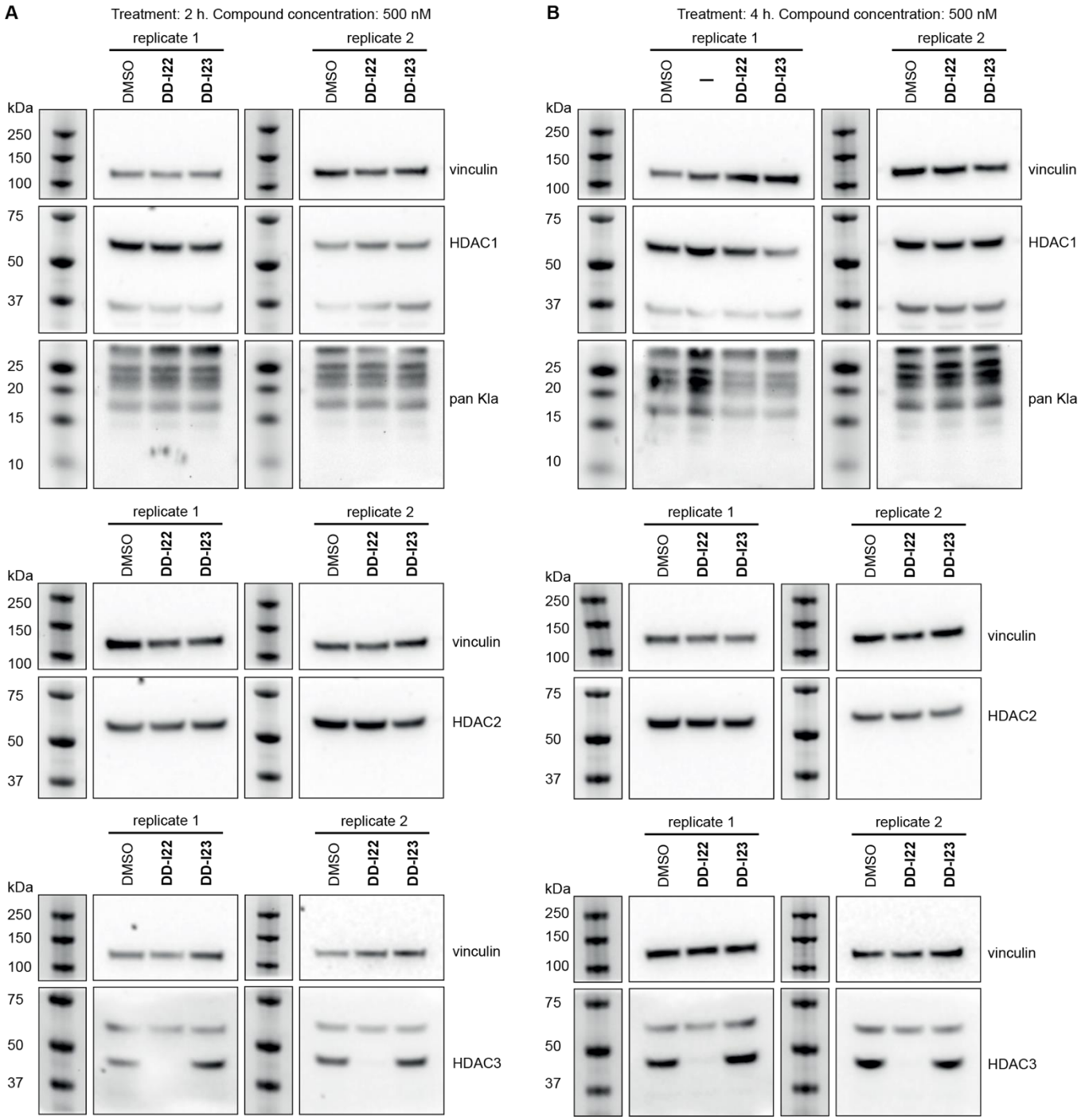


Fig. S9. Selective degradation of HDAC3 and overall Kla changes measured by Western blot. (**A**) Changes in concentration of HDAC1–3 and overall Kla modification after 2 h treatment with HDAC3-targeting PROTAC **DD-I22** or negative control **DD-I23** at 500 nM concentration. (**B**) Changes in concentration of HDAC1–3 and overall Kla modification after 4 h treatment with HDAC3-targeting PROTAC **DD-I22** or negative control **DD-I23** at 500 nM concentration. See Fig. S8 for compound structure and assay optimization.

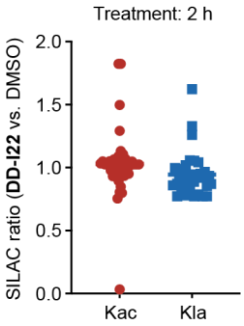


Fig. S10. MS quantification of Kac and Kla sites upon PROTAC treatment for 2 h. Data represent $n = 1$.

Supplementary Tables

Table S1. List of quantified acetylated and lactylated peptides, relative to Fig. 5. Symbols represent Kac (K#) and Kla (K*).

ipi	description	symbol	sequence	mass	charge	Ratio_Forward labeling	Ratio_Reverse labeling
P68431	HIST1H3A Histone H3.1	HIST1H3A	R.K#QLATK*AAR.K	1099,635	2	0,07	15
P04908	HIST1H2AB Histone H2A type 1-B/E	HIST1H2AB	R.GK#QGGK*AR.A	914,4934	2	0,07	11,99
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	K.SAPAPK*K#GSK.K	1083,592	2	0,07	14,82
P62805	HIST1H4A Histone H4	HIST1H4A	R.GKGGK*GLGK.G	872,508	2	0,07	14,9
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	K.K#GSK#K*AVTK.A	1101,639	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	K.GGK*GLGK#GGAK.R	1042,577	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	K.GGK*GLGK.G	687,3915	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	K.GLGK*GGAK#R.H	956,5403	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	R.GK#GGK*GLGK#GGAK#R.H	1467,816	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	R.GK#GGK*GLGK#GGAK.R	1269,704	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	R.GK#GGK*GLGK.G	914,5185	2	0,07	15
Q5QNW6-2	HIST2H2BF Isoform 2 of Histone H2B type 2-F	HIST2H2BF	K.K#GSK#K*AVTK.V	1101,639	2	0,07	15
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	-.PEPAK#SAPAPK*K.G	1333,724	2	0,07	NA
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	K.AVTK*AQK.K	816,4705	2	0,07	NA
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	K.KAVTK*AQK.K	944,5655	2	0,07	NA
P62805	HIST1H4A Histone H4	HIST1H4A	K.GGK#GLGK*GGAK#R.H	1240,689	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	K.GGK#GLGK*GGAK.R	1042,577	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	K.GGK*GLGK#GGAK#R.H	1240,689	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	R.GK*GGK#GLGK#GGAK#R.H	1467,816	3	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	R.GKGGK#GLGK*GGAK#R.H	1425,805	2	0,07	15
P68431	HIST1H3A Histone H3.1	HIST1H3A	R.K#STGGK*APR.K	1014,546	2	0,07	NA
P68431	HIST1H3A Histone H3.1	HIST1H3A	R.KQLATK*AAR.K	1057,624	2	0,07	15
P68431	HIST1H3A Histone H3.1	HIST1H3A	R.KQLATK*AAR.K	1057,624	3	0,07	15
Q5QNW6-2	HIST2H2BF Isoform 2 of Histone H2B type 2-F	HIST2H2BF	K.K#AVTK*VQK.K	1014,607	2	0,07	NA
P68431	HIST1H3A Histone H3.1	HIST1H3A	R.K*STGGK#APR.K	1014,546	2	0,07	15
P62805	HIST1H4A Histone H4	HIST1H4A	R.GK#GGKGLGK*GGAK#R.H	1425,805	3	0,08	15
Q99880	HIST1H2BL Histone H2B type 1-L	HIST1H2BL	-.PELAK*SAPAPK.K	1179,65	2	0,09	2,5
Q5QNW6-2	HIST2H2BF Isoform 2 of Histone H2B type 2-F	HIST2H2BF	K.AVTK*VQK.K	844,5018	2	0,09	4,42
P0C0S5	H2AFZ Histone H2A.Z	H2AFZ	-.AGGK#AGK#DSGK*AK.T	1329,689	2	0,1	4,74
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	K.SAPAPK*K.G	769,4334	2	0,14	2,02
Q5QNW6-2	HIST2H2BF Isoform 2 of Histone H2B type 2-F	HIST2H2BF	K.KAVTK*VQK.K	972,5968	2	0,14	2,15
Q99879	HIST1H2BM Histone H2B type 1-M	HIST1H2BM	-.PEPVK*SAPVPK.K	1219,681	2	0,21	2,11
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	-.PEPAK*SAPAPK.K	1163,619	3	0,25	NA
P23527	HIST1H2BO Histone H2B type 1-O	HIST1H2BO	-.PDPAK*SAPAPK.K	1149,603	2	0,25	NA
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	-.PEPAK*SAPAPK.K	1163,619	2	0,26	1,53
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	K.K#GSK*K#AVTK#AQK.K	1470,841	3	0,28	NA
P68431	HIST1H3A Histone H3.1	HIST1H3A	K.QLATK*AAR.K	929,5294	2	NA	15
O60814	HIST1H2BK Histone H2B type 1-K	HIST1H2BK	K.K#AVTK*AQK.K	986,576	2	NA	15
P58876	HIST1H2BD Histone H2B type 1-D	HIST1H2BD	-.PEPTK*SAPAPK.K	1193,629	2	NA	2,2

Supplementary Methods

Supplementary Methods relative to Fig. S8 and Fig. S9

*In vitro inhibition of HDACs 1–3 by **DD-I22** and **DD-I23***

End-point inhibition assays were performed in HEPES buffer containing 0.5 mg/mL BSA in a final volume of 25 µL per well, where substrate (**2a**, LGKac, 20 µM) was incubated with the inhibitor (3-fold dilution series) and HDAC1, HDAC2, or HDAC3/NCoR2 (1–4 nM) for 30 min at 37 °C. Then, a solution of trypsin (25 µL, 0.4 mg/mL; final concentration of 0.2 mg/mL) was added, and the assay was developed for 15 min at r.t. before fluorescence analysis. Background was subtracted from all data, and the residual enzyme activity was calculated relative to control wells without inhibitor. IC_{50} values were obtained by fitting the resulting data to the concentration–response equation with variable Hill slope (**Eq. 1**), and K_i values were calculated from the IC_{50} using the Cheng-Prusoff equation (**Eq. 2**), with K_M values as reported (HDAC1: 6 µM, HDAC2: 3 µM, HDAC3/NCoR2: 6 µM) (*35*).

$$\nu_{\mathrm{i}} = \nu_{\mathrm{bottom}} + \frac{\nu_{\mathrm{top}} - \nu_{\mathrm{bottom}}}{1+10^{(log\mathrm{IC}_{50} - \log[\mathrm{I}]\mathrm{h})}} \qquad \textbf{Eq. 1}$$

$$K_{\mathrm{i}} = \frac{\mathrm{IC}_{50}}{1+\frac{[\mathrm{S}]}{K_{\mathrm{M}}}} \qquad \textbf{Eq. 2}$$

*Cell culture, treatment with **DD-I22** or **DD-I23** and lysis*

Human embryonic kidney HEK293T cells were cultured in T175 flasks in Dulbecco's modified eagle medium (DMEM, ThermoFisher), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2 atmosphere. HeLa cells were cultured in T175 flasks in Minimum Essential Medium Eagle (MEM, Sigma-Aldrich). Cells were grown to 70–80% confluency as determined visually by a light microscope, washed twice with phosphate buffered saline (PBS) to remove dead and floating cells. Cells were treated with 2 mL trypsin-EDTA solution (Sigma-Aldrich) at 37 °C for 2–5 minutes. After complete detachment of cells, the cell suspension was diluted with medium and cells were were seeded at 1,000,000 cells per well in 2 mL medium on sterile 6-well plates and grown at 37 °C and 5% CO_2 atmosphere overnight. Compounds were dissolved in cell culture medium as 11-fold stocks and 200 µL per well were added to the cells. Unless otherwise noted, cells were treated for 6 h at 37 °C and 5% CO_2 atmosphere. Cells were washed with 2×1 mL PBS and 100 µL of lysis buffer (1% Triton X-100, 0.2% SDS in PBS) was added to the cells, which were then cooled to -20 °C for 20 min. Cell lysates were harvested by scraping, transferred to 1.5 mL Eppendorf tubes and sonicated on ice for 1 min (2 sec on/off pulses, ca 1.5 kJ/sample). Samples were centrifuged at 14 000 rcf for 30 min at 4 °C and the supernatant was collected. The total protein concentration was determined by a commercial BCA assay (ThermoFisher), and the sample concentrations were adjusted. All degradation experiments were performed in at least two independent biological replicates (duplicates).

SDS-PAGE and Western blot

SDS-PAGE was performed on an XCell SureLock mini-cell electrophoresis system (ThermoFisher). Samples were prepared from an appropriate volume of cell lysates to contain 20–40 µg total protein, diluted with MilliQ water to 13 µL and supplemented with 5 µL 4X NuPAGE LDS sample buffer (ThermoFisher) and 2 µL 10X NuPAGE sample reducing agent (ThermoFisher). Samples were heated to 85 °C for 10 min and spun down. 20 µL of the sample were loaded on NuPAGE 4–12% Bis-Tris gels (ThermoFisher) together with Precision Plus All Blue Prestained Protein Standard (Bio-Rad) as ladder.

Gels were run for 40 min at 200 V or for 90 min at 120 V in NuPAGE MES SDS running buffer (ThermoFisher). iBlot2® Dry Blotting system was used to transfer proteins from the gels onto polyvinylidene difluoride (PVDF) membranes which was utilized according to the manufactures instructions, using iBlot2® PVDF Regular Stacks. Transfer was performed at 30 V for 10 min at room temperature. After completion, the membrane was rinsed in TBST, cut, and blocked in 5% skim milk powder (w/v, Sigma-Aldrich) in TBST for 1 hour. The membrane was washed with TBST for 3×5 min and subsequently incubated with primary antibody (1:1000, or 1:250 for pan-Kla) in 5% BSA (w/v) at 4 °C overnight. The next day, the membrane was washed with TBST for 3×5 min and subsequently incubated with secondary antibody (1:10 000) in 2% skim milk (w/v) at room temperature for 1 h. The membrane was washed with TBST for 2×5 min and once with TBS. Blots were developed using ECL Western Blotting Substrate set (ThermoFisher) mixing the two solutions 1:1. Images were recorded on a Syngene PXi imaging station. Protein levels were quantified after western blotting using the software ImageJ (US National Institutes of Health). The following primary antibodies were used: beta-actin (Cell Signaling Technology, 13E5, rabbit mAb), HDAC1 (Cell Signaling Technology, 10E2, mouse mAb), HDAC2 (Cell Signaling Technology, 3F3, mouse pAb), HDAC3 (Cell Signaling Technology, 7G6C5, mouse mAb), histone H3 (Cell Signaling Technology, 96C10, mouse mAb), pan-K(L-la) (PTM Biolabs, PTM-1401, rabbit pAb), vinculin (Cell Signaling Technology, E1E9V, rabbit mAb). The following secondary antibodies were used for HRP detection: anti mouse-IgG HRP-conjugate (Cell Signaling Technology, 7076S), anti-rabbit-IgG HRP-conjugate (Cell Signaling, CST-7074S).

Chemical synthesis

General methods

All commercial reagents and solvents were of analytical grade and used without further purification. Anhydrous solvents were obtained from a PureSolv system. Reactions were conducted under an atmosphere of nitrogen whenever anhydrous solvents were used. Reactions were monitored by thin-layer chromatography (TLC) using silica gel-coated plates (analytical SiO_2-60, F-254) and by HPLC-MS analysis. TLC plates were visualized under UV light and/or by staining with (a) a solution of potassium permanganate (10 g/L), potassium carbonate (67 g/L) and sodium hydroxide (0.83 g/L) in water, (b) a solution of ninhydrin (3 g/L) in 3% acetic acid in water (v/v), or (c) a solution of molybdate-phosphoric acid (12.5 g/L) and cerium(IV)sulfate (5 g/L) in 3% conc. sulfuric acid in water (v/v). Evaporation of solvents was carried out under reduced pressure at a temperature below 40 °C. HPLC−MS analyses were performed on a Phenomenex Kinetex column (1.7 µm, 50×2.10 mm) using a Waters Acquity ultra high-performance liquid chromatography (UPLC) system. Gradient A with eluent I (0.1% HCOOH in H_2O) and eluent II (0.1% HCOOH in MeCN) rising linearly from 0% to 95% of II during t = 0.00-5.20 min was applied at a flow rate of 0.6 mL/min. Preparative reversed-phase HPLC purification was performed on a C18 Phenomenex® Luna column (5 µm, 250×20 mm) or a C8(2) Phenomenex® Luna column (5 µm, 250×21.2 mm) using an Agilent 1260 LC system equipped with a diode array UV detector and an evaporative light scattering detector (ELSD). Gradient B with eluent III (H_2O/MeCN/TFA, 95:5:0.1, v:v) and eluent IV (0.1% TFA in MeCN) rising linearly from 0-30% to 95% of IV during t = 5−45 min was applied at a flow rate of 20 mL/min. Analytical HPLC was performed on a C18 Infinity Poroshell 120 column (2.7 µm, 100×3.0 mm) using an Agilent 1260 Infinity II series system equipped with a diode array UV detector using eluent III and eluent IV, rising linearly from 0% to 50% or 95% of IV during t = 1−11 was applied at a flow rate of 1.2 mL/min at 40 °C. Nuclear magnetic resonance (NMR) spectra

were recorded on a Bruker Avance III HD equipped with a cryogenically cooled probe (^{1}H NMR and ^{13}C NMR recorded at 600 and 151 MHz, respectively). All spectra were recorded at 298 K. Chemical shifts are reported in ppm relative to deuterated solvent as internal standard (δ_H DMSO-d_6 2.50 ppm; δ_C DMSO-d_6 39.52 ppm). Assignments of NMR spectra are based on 2D correlation spectroscopy (COSY, HSQC, TOCSY and HMBC spectra). High-resolution mass spectrometry (HRMS) was recorded either on a QExactive Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a SMALDI5 ion source (TransMIT GmbH, Giessen, Germany), or on a Bruker Solarix WR by either matrix assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI). General methods adapted from Rajabi *et al.*, 2020 (*72*).

Synthesis of AMC-coupled fluorogenic substrates

Multiple fluorogenic substrate building blocks were synthesized following procedures reported in the literature (Fig. S11), and the Ac-Ala-Pro-Arg(Pbf)-Lys-AMC·TFA building block (**S5**) was prepared following similar procedures (Fig. S12). Then, substrates were obtained by acylation in solution (Figs. S13–S16). AMC=7-amino-4-methylcoumarin.

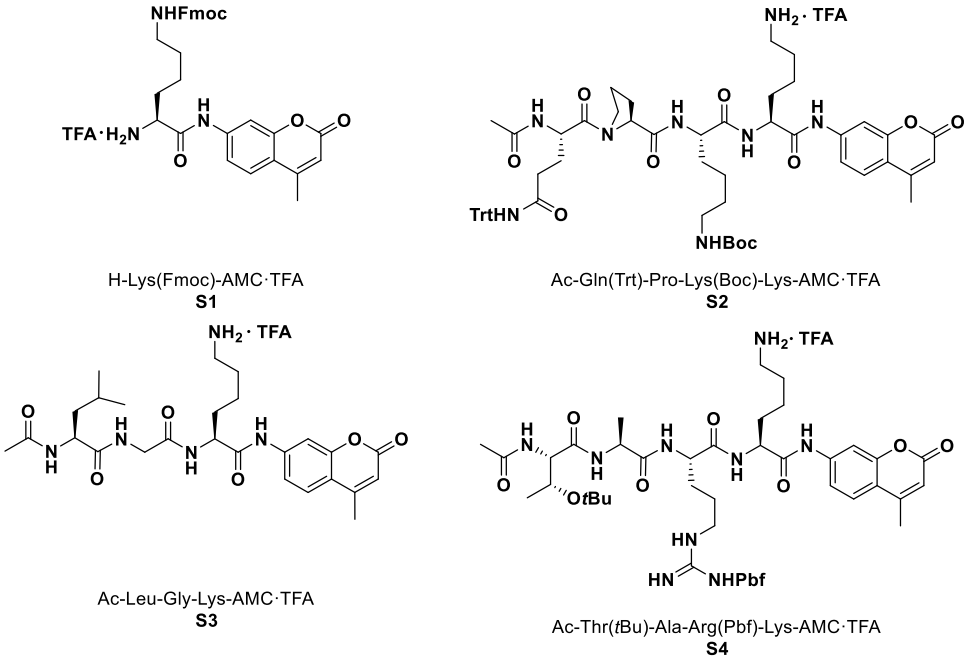


Fig. S11. Building blocks used for the synthesis of AMC-coupled substrates. Compounds **S1** (*39*), **S2** (*40*), **S3** (*31*), **S4** (*25*) were synthesized as published.

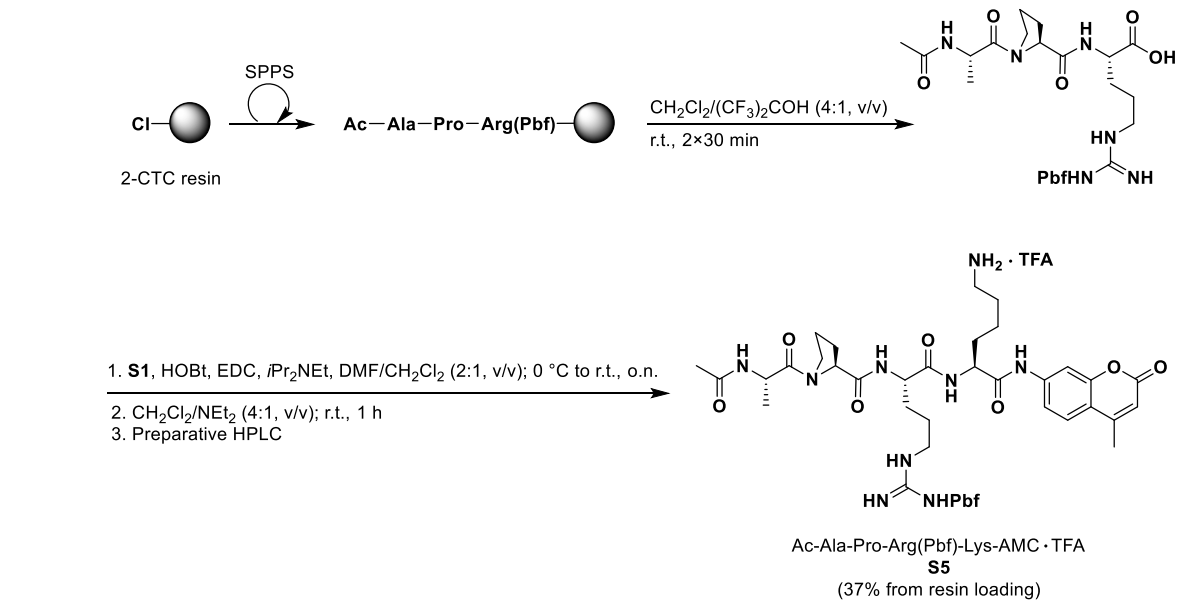


Fig. S12. Synthesis of building block S5.

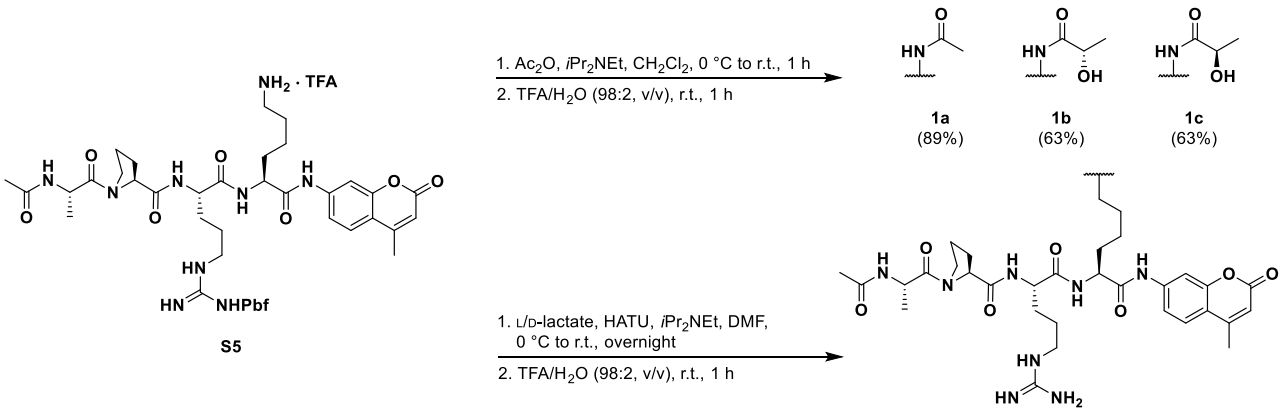


Fig. S13. Synthesis of substrates 1a–1c.

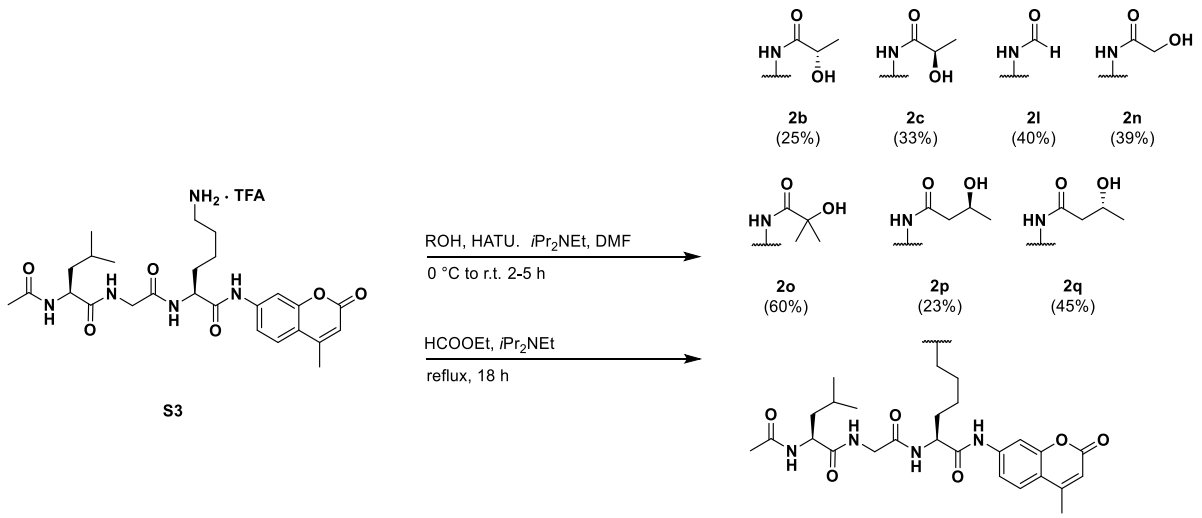


Fig. S14. Synthesis of substrates 2b, 2c, 2l, 2n, 2o, 2p and 2q.

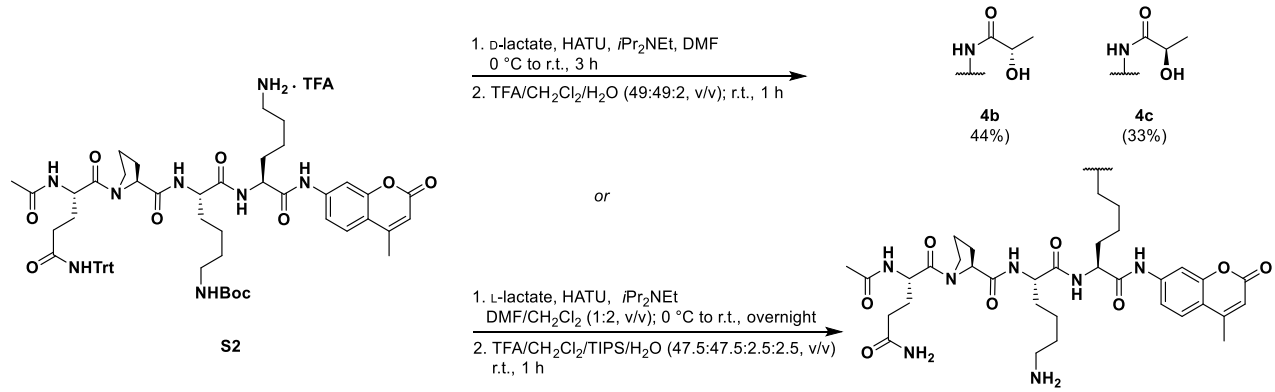
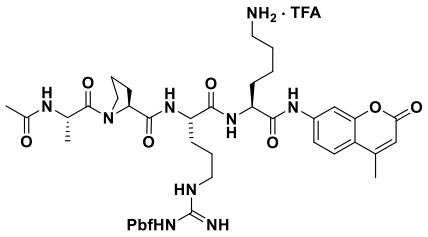
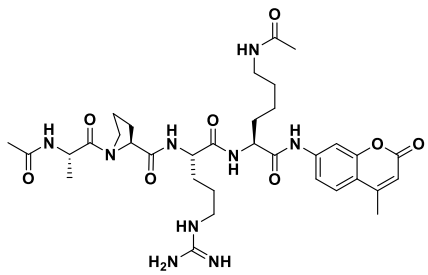


Fig. S15. Synthesis of substrates 4b and 4c.

Fig. S16. Synthesis of substrates 5b and 5c.

Ac-Ala-Pro-Arg(Pbf)-Lys-AMC (S5). 

Ac-Ala-Pro-Arg(Pbf)-resin (232 µmol resin loading) was synthesized on 2-chlorotrityl chloride (2-CTC) resin by using standard SPPS procedures as previously described.[1] The peptide was cleaved off the resin with CH_2Cl_2/hexafluoroisopropanol (4:1, v/v, 2×4 mL, 2×30 min) and concentrated under reduced pressure. Excess hexafluoroisopropanol was removed by co-evaporation with CH_2Cl_2:toluene (1:1, v/v, 15 mL) affording the crude 3-mer tentatively assigned as Ac-Ala-Pro-Arg(Pbf)-OH (HPLC-MS t_R 1.38 min, *m*/*z* 637.3; $[M+H]^+$, $C_{29}H_{45}N_6O_8S^+$, Calcd 637.3) as a white solid (166 mg, quant.), which was used without further purification. The crude was redissolved in anh. CH_2Cl_2/DMF (2:1, v/v, 6 mL) and cooled to 0 °C. Compound **S1** (144 mg, 0.23 mmol), HOBt (38 mg, 0.28 mmol), *i*Pr_2NEt (121 µL, 0.70 mmol) and then EDC (53 mg, 0.28 mmol) was added to the reaction mixture, which was stirred for 10 min at 0 °C and then overnight going towards ambient temperature. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with brine (20 mL), 5% $KHSO_4$ (3×20 mL), saturated aq. $NaHCO_3$ (2×20 mL), and brine (20 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure, affording a crude intermediate tentatively assigned as Ac-Ala-Pro-Arg(Pbf)-Lys(Fmoc)-AMC (HPLC-MS t_R 2.10 min, *m*/*z* 1144.6; $[M+H]^+$, $C_{60}H_{74}N_9O_{12}S^+$, Calcd 1144.5) as a white solid (297 mg), which was used without further purification. The crude was redissolved in CH_2Cl_2 (4.0 mL) and diethyl amine (1.0 mL) was added dropwise to the reaction mixture, which was stirred for 1 h at ambient temperature. Solvent was removed under reduced pressure, and preparative reversed-phase HPLC purification afforded the desired amine **S5** (87 mg, 37% based on resin loading), as a white fluffy TFA-salt after lyophilization. HPLC-MS t_R 1.31 min, *m*/*z* 922.5 ($[M+H]^+$, $C_{45}H_{64}N_9O_{10}S^+$, Calcd 922.4).

Ac-Ala-Pro-Arg-Lys(Ac)-AMC (1a). 
Compound **S5** (13 mg, 0.013 mmol) was dissolved in anh. CH_2Cl_2 (2.0 mL) and cooled to 0 °C. *i*Pr_2NEt (7 µL, 0.038 mmol) and acetic anhydride (0.019 mmol) was added to the reaction mixture, which was stirred for 1 h going towards ambient temperature. Solvent was removed under reduced pressure to afford the acetylated intermediate, tentatively assigned as Ac-Ala-Pro-Arg(Pbf)-Lys(Ac)-AMC (HPLC-MS t_R 1.64 min, *m*/*z* 964.5 ($[M+H]^+$, $C_{47}H_{65}N_9O_{11}S^+$, Calcd 964.5), which was used without further purification. TFA:H_2O (98:2, v/v, 1.0 mL) was added to the intermediate and stirred for 1 h at ambient temperature. Solvent was removed under a stream of nitrogen, and preparative reversed-phase HPLC purification afforded the title compound (8 mg, 89% from **S5**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.32 (s, 1H, NH_{AMC}), 8.10 (d, *J* = 7.3 Hz, 1H, NH_{Ala}), 8.04 (d, *J* = 7.4 Hz, 1H, $NH_{\alpha,Lys}$), 8.01 (d, *J* = 7.5 Hz, 1H, $NH_{\alpha,Arg}$), 7.86–7.78 (m, 2H, $NH_{\varepsilon,Lys}$, $H8_{AMC}$), 7.72 (d, *J* = 8.7 Hz, 1H, $H5_{AMC}$), 7.55 (t, *J* = 5.9 Hz, 1H, $NH_{\delta,Arg}$), 7.50 (dd, J = 8.7, 2.1 Hz, 1H, $H6_{AMC}$), 6.27 (d, *J* = 1.3 Hz, 1H, $H3_{AMC}$), 4.52 (p, *J* = 7.1 Hz, 1H, $H_{\alpha,Ala}$), 4.41–4.29 (m, 2H, $H_{\alpha,Pro}$, $H_{\alpha,Lys}$), 4.29–4.23 (m, 1H, $H_{\alpha,Arg}$), 3.71–3.52 (m, 2H, $H_{\delta,Pro}$), 3.11 (q, *J* = 6.4 Hz, 2H, $H_{\delta,Arg}$), 3.07–2.95 (m, 2H, $H_{\varepsilon,Lys}$), 2.40 (s, 3H, $CH_{3,AMC}$), 2.10–2.00 (m, 1H, $H_{\beta,Pro,A}$), 1.95–1.20 (m, 19H, $H_{\beta,Pro,A}$, $H_{\gamma,Pro}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $H_{\beta,Arg}$, $H_{\gamma,Arg}$, $COCH_{3,acetyl}$, $COCH_{3,Lys}$), 1.17 (d, *J* = 6.8 Hz, 3H, $H_{\beta,Ala}$). ^{13}C NMR (151 MHz, DMSO) δ 171.9 ($CO_{\alpha,Pro}$), 171.5 (CO_{Arg}), 171.3 (CO_{Ala}), 171.2 ($CO_{\alpha,Lys}$), 169.0 ($\underline{C}ONH_{\varepsilon,Lys}$), 168.8

($\underline{C}OCH_{3,acetyl}$), 160.0 ($C2_{AMC}$), 158.4 (q, J = 34.6 Hz, residual CO_{TFA}), 156.7 ($NHC(=NH)NH_2$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 126.0 ($C5_{AMC}$), 116.5 (q, J = 294.6 Hz, residual $CF_{3,TFA}$), 115.2 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.4 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 59.6 ($C_{\alpha,Pro}$), 53.7 ($C_{\alpha,Lys}$), 52.2 ($C_{\alpha,Arg}$), 46.8 ($C_{\delta,Pro}$), 46.2 ($C_{\alpha,Ala}$), 40.4 ($C_{\delta,Arg}$), 38.3 ($C_{\varepsilon,Lys}$), 31.3 ($C_{\beta,Lys}$), 29.0 ($C_{\beta,Pro}$), 28.8 ($C_{\beta,Arg}$, $C_{\delta,Lys}$), 25.0 ($C_{\gamma,Arg}$), 24.5 ($C_{\gamma,Pro}$), 22.9 ($C_{\gamma,Lys}$), 22.6 ($CO\underline{C}H_{3,Lys}$), 22.2 ($CO\underline{C}H_{3,acetyl}$), 18.0 ($CH_{3,AMC}$), 16.9 ($C_{\beta,Ala}$). Two sets of signals (approximately 4:1) were detectable due to rotamers. Only peaks for the major rotamer is given. Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.20 min (>98%, UV_{230}). HRMS calcd for $C_{34}H_{50}N_9O_8^+$ $[M+H]^+$, 712.3777; found 712.3776.

Ac-Ala-Pro-Arg-Lys(L-La)-AMC (1b). By the method described for **1c**, the title compound was synthesized using **S5** (18 mg, 0.018 mmol), L-lactate (12 mg, 0.135 mmol), *i*Pr_2NEt (45 µL, 0.270 mmol), and HATU (13 mg, 0.034 mmol). Preparative reversed-phase HPLC purification afforded the title compound (12 mg, 89% from **S5**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.32 (s, 1H, NH_{AMC}), 8.10 (d, J = 7.5 Hz, 1H, NH_{Ala}), 8.05 (d, J = 7.3 Hz, 1H, $NH_{\alpha,Lys}$), 8.01 (d, J = 7.6 Hz, 1H, $NH_{\alpha,Arg}$), 7.80 (d, J = 2.0 Hz, 1H, $H8_{AMC}$), 7.72 (d, J = 8.7 Hz, 1H, $H5_{AMC}$), 7.66 (t, J = 5.5 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.55 (t, J = 5.9 Hz, 1H, $NH_{\delta,Arg}$), 7.50 (dd, J = 8.7, 2.1 Hz, 1H, $H6_{AMC}$), 6.27 (d, J = 1.3 Hz, 1H, $H3_{AMC}$), 4.52 (p, J = 7.0 Hz, 1H, $H_{\alpha,Ala}$), 4.40–4.29 (m, 2H, $H_{\alpha,Pro}$, $H_{\alpha,Lys}$), 4.26 (td, J = 7.9, 5.3 Hz, 1H, $H_{\alpha,Arg}$), 3.92 (qd, J = 6.8, 1.6 Hz, 1H, C(OH)$\underline{H}CH_3$), 3.67–3.58 (m, 1H, $H_{\delta,Pro,A}$), 3.59–3.51 (m, 1H, $H_{\delta,Pro,B}$), 3.11 (q, J = 6.7 Hz, 2H, $H_{\delta,Arg}$), 3.08–3.00 (m, 2H, $H_{\varepsilon,Lys}$), 2.40 (d, J = 1.3 Hz, 3H, $CH_{3,AMC}$), 2.10–2.00 (m, 1H, $H_{\beta,Pro,A}$), 1.95–1.21 (m, 16H, $H_{\beta,Pro,A}$, $H_{\gamma,Pro}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $H_{\beta,Arg}$, $H_{\gamma,Arg}$, $CH_{3,acetyl}$), 1.20–1.10 (m, 6H, C(OH)HC$\underline{H}_3$, $H_{\beta,Ala}$). ^{13}C NMR (151 MHz, DMSO) δ 174.4 ($\underline{C}ONH_{\varepsilon,Lys}$), 171.8 ($CO_{Pro}$), 171.5 ($CO_{Arg}$), 171.3 ($CO_{Ala}$), 171.2 ($CO_{\alpha,Lys}$), 168.8 ($\underline{C}OCH_{3,acetyl}$), 160.0 ($C2_{AMC}$), 158.4 (q, J = 35.1 Hz, residual CO_{TFA}), 156.7 ($NHC(=NH)NH_2$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 116.0 (q, J = 293.8 Hz, residual $CF_{3,TFA}$), 115.2 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.4 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 67.2 ($\underline{C}$(OH)HCH_3), 59.6 ($C_{\alpha,Pro}$), 53.7 ($C_{\alpha,Lys}$), 52.2 ($C_{\alpha,Arg}$), 46.8 ($C_{\delta,Pro}$), 46.2 ($C_{\alpha,Ala}$), 40.4 ($C_{\delta,Arg}$), 37.9 ($C_{\varepsilon,Lys}$), 31.3 ($C_{\beta,Lys}$), 29.0 ($C_{\beta,Pro}$), 28.93 ($C_{\delta,Lys}$), 28.85 ($C_{\beta,Arg}$), 25.0 ($C_{\gamma,Arg}$), 24.5 ($C_{\gamma,Pro}$), 22.8 ($C_{\gamma,Lys}$), 22.2 ($CO\underline{C}H_{3,acetyl}$), 21.1 (C(OH)H$\underline{C}H_3$), 18.0 ($CH_{3,AMC}$), 16.9 ($C_{\beta,Ala}$). Two sets of signals (approximately 6:1) were detectable due to rotamers. Only peaks for the major rotamer is given. Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.17 min (>98%, UV_{230}). HRMS calcd for $C_{35}H_{52}N_9O_9^+$ $[M+H]^+$, 742.3882; found 742.3884.

Ac-Ala-Pro-Arg-Lys(D-La)-AMC (1c). Compound **S5** (19 mg, 0.019 mmol) and sodium D-lactate (5 mg, 0.041 mmol) were dissolved in anh. DMF (1.0 mL) and cooled to 0 °C. *i*Pr_2NEt (14 µL, 0.082 mmol) and HATU (8 mg, 0.020 mmol) were added to the reaction mixture, which was stirred overnight going towards ambient temperature. Solvent was removed under reduced pressure to afford the crude intermediate tentatively assigned as Ac-Ala-Pro-Arg(Pbf)-Lys(D-la)-AMC (HPLC-MS t_R 1.61 min, *m*/*z* 994.5 ($[M+H]^+$, $C_{48}H_{68}N_9O_{12}S^+$, Calcd 994.5), which

was used without further purification. TFA/H_2O (98:2, v/v, 1.0 mL) was added to the intermediate, which was stirred for 1 h at ambient temperature. Solvent was removed under a stream of nitrogen, and preparative reversed-phase HPLC purification afforded the title compound (9 mg, 63% from **S5**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.32 (s, 1H, NH_{AMC}), 8.10 (d, J = 7.3 Hz, 1H, NH_{Ala}), 8.05 (d, J = 7.3 Hz, 1H, $NH_{\alpha,Lys}$), 8.00 (d, J = 7.5 Hz, 1H, $NH_{\alpha,Arg}$), 7.80 (d, J = 2.0 Hz, 1H, $H8_{AMC}$), 7.72 (d, J = 8.7 Hz, 1H, $H5_{AMC}$), 7.67 (t, J = 6.0 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.53 (t, J = 5.7 Hz, 1H, $NH_{\delta,Arg}$), 7.50 (dd, J = 8.7, 2.0 Hz, 1H, $H6_{AMC}$), 6.27 (d, J = 1.3 Hz, 1H, $H3_{AMC}$), 4.52 (p, J = 7.0 Hz, 1H, $H_{\alpha,Ala}$), 4.40–4.29 (m, 2H, $H_{\alpha,Pro}$, $H_{\alpha,Lys}$), 4.26 (td, J = 7.9, 5.4 Hz, 1H, $H_{\alpha,Arg}$), 3.92 (qd, J = 6.8, 2.0 Hz, 1H, C(OH)$\underline{H}CH_3$), 3.66–3.60 (m, 1H, $H_{\delta,Pro,A}$), 3.58–3.53 (m, 1H, $H_{\delta,Pro,B}$), 3.11 (q, J = 6.6 Hz, 2H, $H_{\delta,Arg}$), 3.08–2.99 (m, 2H, $H_{\varepsilon,Lys}$), 2.40 (d, J = 1.3 Hz, 3H, $CH_{3,AMC}$), 2.10–1.98 (m, 1H, $H_{\beta,Pro,A}$), 1.95–1.21 (m, 16H, $H_{\beta,Pro,A}$, $H_{\gamma,Pro}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $H_{\beta,Arg}$, $H_{\gamma,Arg}$, $CH_{3,acetyl}$), 1.19–1.14 (m, 6H, C(OH)HC$\underline{H}_3$, $H_{\beta,Ala}$). ^{13}C NMR (151 MHz, DMSO) δ 174.4 ($\underline{C}ONH_{\varepsilon,Lys}$), 171.8 ($CO_{\alpha,Pro}$), 171.5 ($CO_{Arg}$), 171.3 ($CO_{Ala}$), 171.2 ($CO_{\alpha,Lys}$), 168.8 ($\underline{C}OCH_{3,acetyl}$), 160.0 ($C2_{AMC}$), 158.2 (q, J = 33.3 Hz, residual CO_{TFA}), 156.7 ($NHC(=NH)NH_2$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.2 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.4 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 67.2 ($\underline{C}$(OH)HCH_3), 59.6 ($C_{\alpha,Pro}$), 53.7 ($C_{\alpha,Lys}$), 52.2 ($C_{\alpha,Arg}$), 46.8 ($C_{\delta,Pro}$), 46.2 ($C_{\alpha,Ala}$), 40.4 ($C_{\delta,Arg}$), 37.9 ($C_{\varepsilon,Lys}$), 31.3 ($C_{\beta,Lys}$), 29.0 ($C_{\beta,Pro}$), 28.91 ($C_{\delta,Lys}$), 28.85 ($C_{\beta,Arg}$), 24.9 ($C_{\gamma,Arg}$), 24.5 ($C_{\gamma,Pro}$), 22.8 ($C_{\gamma,Lys}$), 22.2 ($CO\underline{C}H_{3,acetyl}$), 21.1 (C(OH)H$\underline{C}H_3$), 18.0 ($CH_{3,AMC}$), 16.9 ($C_{\beta,Ala}$). Two sets of signals (approximately 6:1) were detectable due to rotamers. Only peaks for the major rotamer is given. Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.19 min (>98%, UV_{230}). HRMS calcd for $C_{35}H_{52}N_9O_9^+$ $[M+H]^+$, 742.3882; found 742.3878.

Ac-Leu-Gly-Lys(L-La)-AMC (2b). By the method described for **2c**, the title compound was synthesized using **S3** (27 mg, 0.032 mmol), L-lactate (11 mg, 0.127 mmol), iPr$_2$NEt (44 µL, 0.253 mmol), and HATU (45 mg, 0.118 mmol). Preparative reversed-phase HPLC purification afforded the title compound (6 mg, 25% from **S3**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.35 (s, 1H, NH_{AMC}), 8.30 (t, J = 5.8 Hz, 1H, NH_{Gly}), 8.08 (d, J = 7.4 Hz, 1H, NH_{Leu}), 8.01 (d, J = 7.6 Hz, 1H, $NH_{\alpha,Lys}$), 7.79 (d, J = 2.1 Hz, 1H, $H8_{AMC}$), 7.72 (d, J = 8.7 Hz, 1H, $H5_{AMC}$), 7.64 (t, J = 5.9 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.52 (dd, J = 8.7, 2.1 Hz, 1H, $H6_{AMC}$), 6.26 (d, J = 1.3 Hz, 1H, $H3_{AMC}$), 4.37 (ddd, J = 9.0, 7.6, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 4.22 (ddd, J = 9.1, 7.4, 6.0 Hz, 1H, $H_{\alpha,Leu}$), 3.92 (q, J = 6.7 Hz, 1H, C(OH)$\underline{H}CH_3$), 3.79–3.67 (m, 2H, $H_{\alpha,Gly}$), 3.05 (q, J = 6.8 Hz, 2H, $H_{\varepsilon,Lys}$), 2.40 (d, J = 1.2 Hz, 3H, $CH_{3,AMC}$), 1.85 (s, 3H, $CH_{3,acetyl}$), 1.78–1.70 (m, 1H, $H_{\beta,Lys,A}$), 1.70–1.55 (m, 2H, $H_{\beta,Lys,A}$, $H_{\gamma,Leu}$), 1.50–1.37 (m, 4H, $H_{\beta,Leu}$, $H_{\delta,Lys}$), 1.37–1.29 (m, 1H, $H_{\gamma,Lys,A}$), 1.29–1.20 (m, 1H, $H_{\gamma,Lys,B}$), 1.17 (d, J = 6.8 Hz, 3H, C(OH)HC$\underline{H}_3$), 0.88 (d, J = 6.6 Hz, 3H, $H_{\delta,Leu,1}$), 0.84 (d, J = 6.6 Hz, 3H, $H_{\delta,Leu,2}$). ^{13}C NMR (151 MHz, DMSO) δ 174.3 ($\underline{C}ONH_{\varepsilon,Lys}$), 172.9 ($CO_{Leu}$), 171.4 ($CO_{\alpha,Lys}$), 169.7 ($\underline{C}OCH_{3,acetyl}$), 169.0 ($CO_{Gly}$), 160.0 ($C2_{AMC}$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.3 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.3 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 67.2 ($\underline{C}(OH)HCH_3$), 53.6 ($C_{\alpha,Lys}$), 51.4 ($C_{\alpha,Leu}$), 42.0 ($C_{\alpha,Gly}$), 40.5 ($C_{\beta,Leu}$), 37.9 ($C_{\varepsilon,Lys}$), 31.4 ($C_{\beta,Lys}$), 28.9 ($C_{\delta,Lys}$), 24.2 ($C_{\gamma,Leu}$), 22.9 ($C_{\delta,Leu,1}$), 22.8 ($C_{\gamma,Lys}$), 22.5 ($CO\underline{C}H_{3,acetyl}$), 21.6 ($C_{\delta,Leu,2}$), 21.1 (C(OH)H$\underline{C}H_3$), 18.0 ($CH_{3,AMC}$). Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.97 min (>98%, UV_{230}). HRMS calcd for $C_{29}H_{41}N_5NaO_8^+$ $[M+Na]^+$, 610.2847; found 610.2842.

Ac-Leu-Gly-Lys(D-La)-AMC (2c). Compound **S3** (26 mg, 0.041 mmol) and D-lactate (11 mg, 0.124 mmol) were dissolved in anh. DMF (1.5 mL) and cooled to 0 °C. *i*Pr_2NEt (43 µL, 0.249 mmol) and HATU (44 mg, 0.116 mmol) were added to the reaction mixture, which was stirred for 3 h going towards ambient temperature. Solvent was removed under reduced pressure, diluted with CH_2Cl_2 (20 mL) and washed with aq. HCl (0.1 M, 4×10 mL), sat. $NaHCO_3$ (10 mL) and dried over $MgSO_4$. Solvent was removed under reduced pressure, and preparative reversed-phase HPLC purification afforded the title compound (8 mg, 33% from **S3**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.35 (s, 1H, NH_{AMC}), 8.31 (t, *J* = 5.9 Hz, 1H, NH_{Gly}), 8.08 (d, *J* = 7.3 Hz, 1H, NH_{Leu}), 8.01 (d, *J* = 7.6 Hz, 1H, $NH_{α,Lys}$), 7.79 (d, *J* = 2.0 Hz, 1H, $H8_{AMC}$), 7.72 (d, *J* = 8.7 Hz, 1H, $H5_{AMC}$), 7.64 (t, *J* = 5.9 Hz, 1H, $NH_{ε,Lys}$), 7.53 (dd, *J* = 8.7, 2.1 Hz, 1H, $H6_{AMC}$), 6.26 (d, *J* = 1.3 Hz, 1H, $H3_{AMC}$), 4.37 (ddd, *J* = 9.1, 7.6, 5.1 Hz, 1H, $H_{α,Lys}$), 4.22 (ddd, *J* = 9.0, 7.3, 6.0 Hz, 1H, $H_{α,Leu}$), 3.92 (q, *J* = 6.7 Hz, 1H, C(OH)HCH$_3$), 3.79–3.67 (m, 2H, $H_{α,Gly}$), 3.05 (q, *J* = 6.8 Hz, 2H, $H_{ε,Lys}$), 2.40 (d, *J* = 1.3 Hz, 3H, $CH_{3,AMC}$), 1.85 (s, 3H, $CH_{3,acetyl}$), 1.78–1.70 (m, 1H, $H_{β,Lys,A}$), 1.69–1.55 (m, 2H, $H_{β,Lys,A}$, $H_{γ,Leu}$), 1.50–1.38 (m, 4H, $H_{β,Leu}$, $H_{δ,Lys}$), 1.37–1.29 (m, 1H, $H_{γ,Lys,A}$), 1.29–1.20 (m, 1H, $H_{γ,Lys,B}$), 1.17 (d, *J* = 6.8 Hz, 3H, C(OH)HCH_3), 0.88 (d, *J* = 6.6 Hz, 3H, $H_{δ,Leu,1}$), 0.84 (d, *J* = 6.6 Hz, 3H, $H_{δ,Leu,2}$). ^{13}C NMR (151 MHz, DMSO) δ 174.3 (CONH$_{ε,Lys}$), 172.9 (CO_{Leu}), 171.4 ($CO_{α,Lys}$), 169.7 (COCH$_{3,acetyl}$), 169.0 (CO_{Gly}), 160.0 ($C2_{AMC}$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.3 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.3 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 67.2 (C(OH)HCH$_3$), 53.6 ($C_{α,Lys}$), 51.4 ($C_{α,Leu}$), 42.0 ($C_{α,Gly}$), 40.5 ($C_{β,Leu}$), 37.9 ($C_{ε,Lys}$), 31.4 ($C_{β,Lys}$), 28.9 ($C_{δ,Lys}$), 24.2 ($C_{γ,Leu}$), 22.9 ($C_{δ,Leu,1}$), 22.8 ($C_{γ,Lys}$), 22.5 (COCH$_{3,acetyl}$), 21.6 ($C_{δ,Leu,2}$), 21.1 (C(OH)HCH$_3$), 18.0 ($CH_{3,AMC}$). Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.98 min (>98%, UV_{230}). HRMS calcd for $C_{29}H_{41}N_5NaO_8^+$ $[M+Na]^+$, 610.2847; found 610.2842.

Ac-Leu-Gly-Lys(For)-AMC (2l). Compound **S3** (12 mg, 0.019 mmol) was dissolved in ethyl formate (0.77 mL, 9.5 mmol), and *i*Pr_2NEt (20 µL, 0.114 mmol) was added to the reaction mixture, which was stirred overnight at reflux. Solvent was removed under reduced pressure, and preparative reversed-phase HPLC purification afforded the title compound (4 mg, 40% from **S3**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.36 (s, 1H, NH_{AMC}), 8.32 (t, *J* = 5.9 Hz, 1H, NH_{Gly}), 8.09 (d, *J* = 7.3 Hz, 1H, NH_{Leu}), 8.00 (d, *J* = 7.7 Hz, 1H, $NH_{α,Lys}$), 7.98 (s, 1H, CHO), 7.98–7.94 (m, 1H, $NH_{ε,Lys}$), 7.79 (d, *J* = 2.1 Hz, 1H, $H8_{AMC}$), 7.72 (d, *J* = 8.6 Hz, 1H, $H5_{AMC}$), 7.52 (dd, *J* = 8.7, 2.1 Hz, 1H, $H6_{AMC}$), 6.26 (d, *J* = 1.3 Hz, 1H, $H3_{AMC}$), 4.38 (td, *J* = 8.1, 5.0 Hz, 1H, $H_{α,Lys}$), 4.22 (ddd, *J* = 9.1, 7.3, 6.0 Hz, 1H, $H_{α,Leu}$), 3.78–3.68 (m, 2H, $H_{α,Gly}$), 3.06 (q, *J* = 6.7 Hz, 2H, $H_{ε,Lys}$), 2.40 (d, *J* = 1.3 Hz, 3H, $CH_{3,AMC}$), 1.85 (s, 3H, $CH_{3,acetyl}$), 1.74 (ddt, *J* = 14.5, 10.4, 5.3 Hz, 1H, $H_{β,Lys,A}$), 1.69–1.56 (m, 2H, $H_{β,Lys,A}$, $H_{γ,Leu}$), 1.50–1.39 (m, 4H, $H_{β,Leu}$, $H_{δ,Lys}$), 1.39–1.31 (m, 1H, $H_{γ,Lys,A}$), 1.31–1.21 (m, 1H, $H_{γ,Lys,B}$), 0.88 (d, *J* = 6.6 Hz, 3H, $H_{δ,Leu,1}$), 0.84 (d, *J* = 6.5 Hz, 3H, $H_{δ,Leu,2}$). ^{13}C NMR (151 MHz, DMSO) δ 172.9 (CO_{Leu}), 171.3 ($CO_{α,Lys}$), 169.7 (COCH$_{3,acetyl}$), 169.0 (CO_{Gly}), 160.9 (CHO), 160.0 ($C2_{AMC}$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.3 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.4 ($C3_{AMC}$), 105.8 ($C8_{AMC}$), 53.5 ($C_{α,Lys}$), 51.5 ($C_{α,Leu}$), 42.1 ($C_{α,Gly}$), 40.5 ($C_{β,Leu}$), 36.9 ($C_{ε,Lys}$), 31.3 ($C_{β,Lys}$), 28.7 ($C_{δ,Lys}$), 24.2 ($C_{γ,Leu}$), 22.9 ($C_{δ,Leu,1}$), 22.8 ($C_{γ,Lys}$), 22.5 (COCH$_{3,acetyl}$), 21.6 ($C_{δ,Leu,2}$), 18.0 ($CH_{3,AMC}$). Analytical HPLC gradient 0–95% eluent II in eluent I

(11 min total runtime), t_R 4.96 min (>98%, UV_{215}). HRMS calcd for $C_{27}H_{37}N_5NaO_7^+$ $[M+Na]^+$, 566.2585; found 566.2580.

Ac-Leu-Gly-Lys(Gc)-AMC (2n). Compound **S3** (12 mg, 0.019 mmol) and glycolic acid (6 mg, 0.078 mmol) were dissolved in anh. DMF (1.0 mL) and cooled to 0 °C. *i*Pr_2NEt (20 µL, 0.114 mmol) and HATU (21 mg, 0.055 mmol) were added to the reaction mixture, which was stirred for 3.5 h going towards ambient temperature. Solvent was removed under reduced pressure, and preparative reversed-phase HPLC purification afforded the title compound (4 mg, 39% from **S3**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.35 (s, 1H, NH_{AMC}), 8.31 (t, J = 5.9 Hz, 1H, NH_{Gly}), 8.08 (d, J = 7.3 Hz, 1H, NH_{Leu}), 8.01 (d, J = 7.6 Hz, 1H, $NH_{\alpha,Lys}$), 7.79 (d, J = 2.0 Hz, 1H, $H8_{AMC}$), 7.72 (d, J = 8.7 Hz, 1H, $H5_{AMC}$), 7.69 (t, J = 6.0 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.52 (dd, J = 8.7, 2.0 Hz, 1H, $H6_{AMC}$), 6.26 (d, J = 1.3 Hz, 1H, $H3_{AMC}$), 4.37 (ddd, J = 9.1, 7.5, 5.0 Hz, 1H, $H_{\alpha,Lys}$), 4.22 (ddd, J = 8.9, 7.4, 6.0 Hz, 1H, $H_{\alpha,Leu}$), 3.77 (s, 2H, $C\underline{H}_2OH$), 3.76–3.68 (m, 2H, $H_{\alpha,Gly}$), 3.08 (q, J = 6.8 Hz, 2H, $H_{\varepsilon,Lys}$), 2.40 (d, J = 1.3 Hz, 3H, $CH_{3,AMC}$), 1.85 (s, 3H, $CH_{3,acetyl}$), 1.78–1.70 (m, 1H, $H_{\beta,Lys,A}$), 1.70–1.56 (m, 2H, $H_{\beta,Lys,A}$, $H_{\gamma,Leu}$), 1.50–1.39 (m, 4H, $H_{\beta,Leu}$, $H_{\delta,Lys}$), 1.38–1.30 (m, 1H, $H_{\gamma,Lys,A}$), 1.30–1.21 (m, 1H, $H_{\gamma,Lys,B}$), 0.88 (d, J = 6.6 Hz, 3H, $H_{\delta,Leu,1}$), 0.84 (d, J = 6.6 Hz, 3H, $H_{\delta,Leu,2}$). ^{13}C NMR (151 MHz, DMSO) δ 172.9 (CO_{Leu}), 171.6 ($\underline{C}ONH_{\varepsilon,Lys}$), 171.4 ($CO_{\alpha,Lys}$), 169.7 ($\underline{C}OCH_{3,acetyl}$), 169.0 ($CO_{Gly}$), 160.0 ($C2_{AMC}$), 158.2 (q, J = 36.8 Hz, residual CO_{TFA}), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.3 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.3 ($C3_{AMC}$), 105.8 ($C8_{AMC}$), 61.4 ($\underline{C}H_2OH$), 53.6 ($C_{\alpha,Lys}$), 51.5 ($C_{\alpha,Leu}$), 42.0 ($C_{\alpha,Gly}$), 40.5 ($C_{\beta,Leu}$), 37.8 ($C_{\varepsilon,Lys}$), 31.4 ($C_{\beta,Lys}$), 29.0 ($C_{\delta,Lys}$), 24.2 ($C_{\gamma,Leu}$), 22.9 ($C_{\delta,Leu,1}$), 22.8 ($C_{\gamma,Lys}$), 22.5 ($CO\underline{C}H_{3,acetyl}$), 21.6 ($C_{\delta,Leu,2}$), 18.0 ($CH_{3,AMC}$). Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.87 min (>99%, UV_{215}). HRMS calcd for $C_{28}H_{39}N_5NaO_8^+$ $[M+Na]^+$, 596.2690; found 596.2682.

Ac-Leu-Gly-Lys(Hib)-AMC (2o). By the method described for **2n**, the title compound was synthesized using **S3** (12 mg, 0.032 mmol), 2-hydroxyisobutyric acid (7 mg, 0.068 mmol), *i*Pr_2NEt (20 µL, 0.114 mmol), and HATU (20 mg, 0.053 mmol). Preparative reversed-phase HPLC purification afforded the title compound (7 mg, 60% from **S3**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.36 (s, 1H, NH_{AMC}), 8.29 (t, J = 5.9 Hz, 1H, NH_{Gly}), 8.07 (d, J = 7.4 Hz, 1H, NH_{Leu}), 8.02 (d, J = 7.5 Hz, 1H, $NH_{\alpha,Lys}$), 7.79 (d, J = 2.0 Hz, 1H, $H8_{AMC}$), 7.72 (d, J = 8.7 Hz, 1H, $H5_{AMC}$), 7.60 (t, J = 6.0 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.52 (dd, J = 8.7, 2.0 Hz, 1H, $H6_{AMC}$), 6.26 (d, J = 1.4 Hz, 1H, $H3_{AMC}$), 4.37 (td, J = 8.2, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 4.22 (ddd, J = 9.2, 7.4, 5.9 Hz, 1H, $H_{\alpha,Leu}$), 3.78–3.67 (m, 2H, $H_{\alpha,Gly}$), 3.04 (q, J = 6.8 Hz, 2H, $H_{\varepsilon,Lys}$), 2.40 (d, J = 1.4 Hz, 3H, $CH_{3,AMC}$), 1.85 (s, 3H, $CH_{3,acetyl}$), 1.74 (ddt, J = 15.3, 10.7, 5.4 Hz, 1H, $H_{\beta,Lys,A}$), 1.69–1.56 (m, 2H, $H_{\beta,Lys,A}$, $H_{\gamma,Leu}$), 1.49–1.39 (m, 4H, $H_{\beta,Leu}$, $H_{\delta,Lys}$), 1.36–1.29 (m, 1H, $H_{\gamma,Lys,A}$), 1.29–1.22 (m, 1H, $H_{\gamma,Lys,B}$), 1.21 (s, 3H, $CH_{3,Hib,A}$), 1.20 (s, 3H, $CH_{3,Hib,B}$), 0.88 (d, J = 6.6 Hz, 3H, $H_{\delta,Leu,1}$), 0.84 (d, J = 6.6 Hz, 3H, $H_{\delta,Leu,2}$). ^{13}C NMR (151 MHz, DMSO) δ 176.2 ($\underline{C}ONH_{\varepsilon,Lys}$), 172.9 ($CO_{Leu}$), 171.4 ($CO_{\alpha,Lys}$), 169.6 ($\underline{C}OCH_{3,acetyl}$), 169.0 ($CO_{Gly}$), 160.0 ($C2_{AMC}$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.3 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$),

112.3 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 71.8 (COH), 53.6 ($C_{\alpha,Lys}$), 51.4 ($C_{\alpha,Leu}$), 42.0 ($C_{\alpha,Gly}$), 40.5 ($C_{\beta,Leu}$), 38.0 ($C_{\varepsilon,Lys}$), 31.4 ($C_{\beta,Lys}$), 28.9 ($C_{\delta,Lys}$), 27.8 ($CH_{3,Hib,A}$, $CH_{3,Hib,B}$), 24.2 ($C_{\gamma,Leu}$), 22.9 ($C_{\delta,Leu,1}$), 22.7 ($C_{\gamma,Lys}$), 22.5 ($COCH_{3,acetyl}$), 21.6 ($C_{\delta,Leu,2}$), 18.0 ($CH_{3,AMC}$). Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 5.13 min (>97%, UV_{215}). HRMS calcd for $C_{30}H_{43}N_5NaO_8^+$ $[M+Na]^+$, 624.3004; found 624.2999.

Ac-Leu-Gly-Lys(L-Bhb)-AMC (2p). By the method described for **2n**, the title compound was synthesized using **S3** (12 mg, 0.032 mmol), L-β-hydroxybutyric acid (6 mg, 0.061 mmol), *i*Pr_2NEt (20 µL, 0.114 mmol), and HATU (20 mg, 0.053 mmol). Preparative reversed-phase HPLC purification afforded the title compound (3 mg, 23% from **S3**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.36 (s, 1H, NH_{AMC}), 8.31 (t, J = 6.0 Hz, 1H, NH_{Gly}), 8.08 (d, J = 7.2 Hz, 1H, NH_{Leu}), 8.00 (d, J = 7.7 Hz, 1H, $NH_{\alpha,Lys}$), 7.80 (d, J = 2.3 Hz, 1H, $H8_{AMC}$), 7.76 (t, J = 5.6 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.72 (d, J = 8.7 Hz, 1H, $H5_{AMC}$), 7.53 (dd, J = 8.8, 2.4 Hz, 1H, $H6_{AMC}$), 6.26 (s, 1H, $H3_{AMC}$), 4.41–4.33 (m, 1H, $H_{\alpha,Lys}$), 4.22 (dt, J = 7.3, 6.6 Hz, 1H, $H_{\alpha,Leu}$), 3.95–3.89 (m, 1H, CHOH), 3.78–3.66 (m, 2H, $H_{\alpha,Gly}$), 3.08–2.93 (m, 2H, $H_{\varepsilon,Lys}$), 2.40 (d, J = 2.6 Hz, 3H, $CH_{3,AMC}$), 2.17 (dd, J = 14.0, 7.2 Hz, 1H, $CH_{2,A}$CHOH), 2.06 (dd, J = 13.8, 6.0 Hz, 1H, $CH_{2,B}$CHOH), 1.85 (s, 3H, $CH_{3,acetyl}$), 1.78–1.70 (m, 1H, $H_{\beta,Lys,A}$), 1.69–1.54 (m, 2H, $H_{\beta,Lys,A}$, $H_{\gamma,Leu}$), 1.50–1.43 (m, 2H, $H_{\beta,Leu}$), 1.43–1.35 (m, 2H, $H_{\delta,Lys}$), 1.35–1.20 (m, 2H, $H_{\gamma,Lys}$), 1.02 (d, J = 6.2 Hz, 3H, CH(OH)CH_3), 0.88 (d, J = 6.6 Hz, 3H, $H_{\delta,Leu,1}$), 0.84 (d, J = 6.6 Hz, 3H, $H_{\delta,Leu,2}$). ^{13}C NMR (151 MHz, DMSO) δ 172.9 (CO_{Leu}), 171.4 ($CO_{\alpha,Lys}$), 170.5 ($CONH_{\varepsilon,Lys}$), 169.7 ($COCH_{3,acetyl}$), 169.0 (CO_{Gly}), 160.0 ($C2_{AMC}$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.3 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.3 ($C3_{AMC}$), 105.8 ($C8_{AMC}$), 63.8 (CHOH), 53.6 ($C_{\alpha,Lys}$), 51.5 ($C_{\alpha,Leu}$), 45.3 (CH_2CH), 42.0 ($C_{\alpha,Gly}$), 40.5 ($C_{\beta,Leu}$), 38.1 ($C_{\varepsilon,Lys}$), 31.4 ($C_{\beta,Lys}$), 28.8 ($C_{\delta,Lys}$), 24.2 ($C_{\gamma,Leu}$), 23.3 (CH(OH)CH_3), 22.9 ($C_{\delta,Leu,1}$), 22.8 ($C_{\gamma,Lys}$), 22.5 ($COCH_{3,acetyl}$), 21.6 ($C_{\delta,Leu,2}$), 18.0 ($CH_{3,AMC}$). Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.97 min (>95%, UV_{215}). HRMS calcd for $C_{30}H_{43}N_5NaO_8^+$ $[M+Na]^+$, 624.3004; found 624.2999.

Ac-Leu-Gly-Lys(D-Bhb)-AMC (2q). By the method described for **2n**, the title compound was synthesized using **S3** (12 mg, 0.032 mmol), D-β-hydroxybutyric acid (7 mg, 0.066 mmol), *i*Pr_2NEt (20 µL, 0.114 mmol), and HATU (20 mg, 0.053 mmol). Preparative reversed-phase HPLC purification afforded the title compound (5 mg, 45% from **S3**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.36 (s, 1H, NH_{AMC}), 8.32 (t, J = 5.9 Hz, 1H, NH_{Gly}), 8.08 (d, J = 7.3 Hz, 1H, NH_{Leu}), 7.99 (d, J = 7.7 Hz, 1H, $NH_{\alpha,Lys}$), 7.79 (d, J = 2.0 Hz, 1H, $H8_{AMC}$), 7.75 (t, J = 5.6 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.72 (d, J = 8.7 Hz, 1H, $H5_{AMC}$), 7.53 (dd, J = 8.7, 2.1 Hz, 1H, $H6_{AMC}$), 6.26 (d, J = 1.3 Hz, 1H, $H3_{AMC}$), 4.37 (td, J = 8.2, 5.1 Hz, 1H, $H_{\alpha,Lys}$), 4.24–4.19 (m, 1H, $H_{\alpha,Leu}$), 3.93 (dt, J = 7.1, 6.0 Hz, 1H, CHOH), 3.78–3.66 (m, 2H, $H_{\alpha,Gly}$), 3.08–2.95 (m, 2H, $H_{\varepsilon,Lys}$), 2.40 (d, J = 1.3 Hz, 3H, $CH_{3,AMC}$), 2.17 (dd, J = 13.8, 7.2 Hz, 1H, $CH_{2,A}$CHOH), 2.06 (dd, J = 13.8, 5.9 Hz, 1H, $CH_{2,B}$CHOH), 1.85 (s, 3H, $CH_{3,acetyl}$), 1.78–1.70 (m, 1H, $H_{\beta,Lys,A}$), 1.69–1.56 (m, 2H, $H_{\beta,Lys,A}$, $H_{\gamma,Leu}$), 1.49–1.43 (m, 2H, $H_{\beta,Leu}$), 1.43–1.36 (m, 2H, $H_{\delta,Lys}$), 1.36–1.29 (m, 1H, $H_{\gamma,Lys,A}$), 1.29–1.22 (m, 1H, $H_{\gamma,Lys,B}$), 1.03 (d, J = 6.2 Hz, 3H, CH(OH)CH_3), 0.88 (d, J = 6.6 Hz,

3H, $H_{\delta,Leu,1}$), 0.84 (d, J = 6.6 Hz, 3H, $H_{\delta,Leu,2}$). ^{13}C NMR (151 MHz, DMSO) δ 172.9 (CO_{Leu}), 171.4 ($CO_{\alpha,Lys}$), 170.5 ($\underline{C}ONH_{\varepsilon,Lys}$), 169.7 ($\underline{C}OCH_{3,acetyl}$), 169.0 ($CO_{Gly}$), 160.0 ($C2_{AMC}$), 158.2 (q, J = 36.5 Hz, residual CO_{TFA}), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.3 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.3 ($C3_{AMC}$), 105.8 ($C8_{AMC}$), 63.8 ($\underline{C}HOH$), 53.6 ($C_{\alpha,Lys}$), 51.5 ($C_{\alpha,Leu}$), 45.3 ($\underline{C}H_2CH$), 42.0 ($C_{\alpha,Gly}$), 40.5 ($C_{\beta,Leu}$), 38.1 ($C_{\varepsilon,Lys}$), 31.4 ($C_{\beta,Lys}$), 28.8 ($C_{\delta,Lys}$), 24.2 ($C_{\gamma,Leu}$), 23.3 ($CH(OH)\underline{C}H_3$), 22.9 ($C_{\delta,Leu,1}$), 22.8 ($C_{\gamma,Lys}$), 22.5 ($CO\underline{C}H_{3,acetyl}$), 21.6 ($C_{\delta,Leu,2}$), 18.0 ($CH_{3,AMC}$). Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.98 min (>97%, UV_{215}). HRMS calcd for $C_{30}H_{43}N_5NaO_8^+$ $[M+Na]^+$, 624.3004; found 624.3004.

Ac-Gln-Pro-Lys-Lys(L-La)-AMC (4b). L-lactate (5 mg, 0.053 mmol) was dissolved in anh. DMF/CH_2Cl_2 (1:2, v/v, 3.0 mL) and cooled to 0 °C. HATU (20 mg, 0.053 mmol), lutidine (12 µL, 0.106 mmol) and compound **S2** (50 mg, 0.048 mmol) were added to the reaction mixture, which was stirred overnight going towards ambient temperature. The solution was diluted with CH_2Cl_2 (25 mL), and washed with brine (30 mL), aq. HCl (0.5 M, 3×30 mL), sat. $NaHCO_3$ (3×30 mL), brine (30 mL) and dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude intermediate tentatively assigned as Ac-Gln(Trt)-Pro-Lys(Boc)-Lys(L-la)-AMC (HPLC-MS t_R 1.94 min, m/z 1111.9 ($[M-H]^-$, $C_{61}H_{75}N_8O_{12}^-$, Calcd 1111.6), which was used without further purification. TFA/CH_2Cl_2/H_2O/TIPS (47.5:47.5:2.5:2.5, v/v, 4.0 mL) was added to the intermediate and stirred for 1 h at ambient temperature. Solvent was removed under a stream of nitrogen, after which the crude was triturated in ice-cold ether followed by preparative reversed-phase HPLC purification to afford the title compound (19 mg, 44% from **S2**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ10.36 (s, 1H, NH_{AMC}), 8.11–8.02 (m, 3H, $NH_{\alpha,Gln}$, $NH_{\alpha,Lys}$ $NH_{\alpha,Lys(lactoyl)}$), 7.80 (d, J = 2.0 Hz, 1H, $H8_{AMC}$), 7.76–7.70 (m, 4H, $H5_{AMC}$, $NH_{\varepsilon,Lys}$), 7.67 (t, J = 6.0 Hz, 1H, $NH_{\varepsilon,Lys(lactoyl)}$), 7.48 (dd, J = 8.7, 2.0 Hz, 1H, $H6_{AMC}$), 7.34 (s, 1H, $CONH_{2,Gln,A}$), 6.82 (s, 1H, $CONH_{2,Gln,B}$), 6.27 (d, J = 1.2 Hz, 1H, $H3_{AMC}$), 4.46 (q, J = 8.0 Hz, 1H, $H_{\alpha,Gln}$), 4.38–4.30 (m, 2H, $H_{\alpha,Pro}$, $H_{\alpha,Lys(lactoyl)}$), 4.30–4.23 (m, 1H, $H_{\alpha,Lys}$), 3.92 (q, J = 6.8 Hz, 1H, $C(OH)\underline{H}CH_3$), 3.71–3.59 (m, 2H, $H_{\delta,Pro}$), 3.11–3.00 (m, 2H, $H_{\varepsilon,Lys(lactoyl)}$), 2.83–2.72 (m, 2H, $H_{\varepsilon,Lys}$), 2.40 (d, J = 1.1 Hz, 3H, $CH_{3,AMC}$), 2.19–2.09 (m, 2H, $H_{\delta,Gln}$), 2.09–2.00 (m, 1H, $H_{\beta,Pro,A}$), 1.94–1.21 (m, XH, $H_{\beta,Lys(lactoyl)}$, $H_{\gamma,Lys(lactoyl)}$, $H_{\delta,Lys(lactoyl)}$, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $H_{\beta,Pro,B}$, $H_{\gamma,Pro}$, $H_{\beta,Gln}$, $CH_{3,acetyl}$), 1.16 (d, J = 6.8 Hz, 3H, $C(OH)HC\underline{H}_3$). ^{13}C NMR (151 MHz, DMSO) δ 174.4 ($\underline{C}ONH_{\varepsilon,Lys(lactoyl)}$), 173.9 ($CO_{\delta,Gln}$), 171.9 ($CO_{\alpha,Lys}$), 171.7 ($CO_{Pro}$), 171.4 ($CO_{\alpha,Lys(lactoyl)}$), 170.5 ($CO_{\alpha,Gln}$), 169.2 ($\underline{C}OCH_{3,acetyl}$), 160.0 ($C2_{AMC}$), 158.3 (q, J = 34.4 Hz, residual CO_{TFA}), 153.7 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 126.0 ($C5_{AMC}$), 116.2 (q, J = 295.4 Hz, $CF_{3,TFA}$), 115.2 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.3 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 67.3 ($\underline{C}(OH)HCH_3$), 59.5 ($C_{\alpha,Pro}$), 53.7 ($C_{\alpha,Lys(lactoyl)}$), 52.3 ($C_{\alpha,Lys}$), 49.9 ($C_{\alpha,Gln}$), 46.9 ($C_{\delta,Pro}$), 38.8 ($C_{\varepsilon,Lys}$), 37.9 ($C_{\varepsilon,Lys(lactoyl)}$), 31.4 ($C_{\gamma,Gln}$), 31.1 ($C_{\beta,Lys}$), 31.0 ($C_{\beta,Lys(lactoyl)}$), 29.1 ($C_{\beta,Pro}$), 29.0 ($C_{\delta,Lys(lactoyl)}$), 27.2 ($C_{\beta,Gln}$), 26.5 ($C_{\delta,Lys}$), 24.5 ($C_{\gamma,Pro}$), 22.9 ($C_{\gamma,Lys(lactoyl)}$), 22.3 ($C_{\gamma,Lys}$), 22.1 ($CH_{3,acetyl}$), 21.1 ($C(OH)HCH_3$), 18.0 ($CH_{3,AMC}$). Two sets of signals (approximately 9:1) were detectable due to rotamers. Only peaks for the major rotamer is given. Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 3.93 min (>98%, UV_{230}). HRMS calcd for $C_{37}H_{55}N_8O_{10}^+$ $[M+H]^+$, 771.4036; found 771.4024.

Ac-Gln-Pro-Lys-Lys(D-La)-AMC (4c). Compound **S2** (35 mg, 0.031 mmol) and D-lactate (8 mg, 0.092 mmol) were dissolved in anh. DMF (1.0 mL) and cooled to 0 °C. *i*Pr_2NEt (32 µL, 0.184 mmol) and HATU (33 mg, 0.086 mmol) were added to the reaction mixture, which was stirred for 3 h going towards ambient temperature. Solvent was removed under reduced pressure, to afford the crude intermediate tentatively assigned as Ac-Gln(Trt)-Pro-Lys(Boc)-Lys(D-la)-AMC (HPLC-MS t_R 1.94 min, *m/z* 1113.6 ([M+H]$^+$, $C_{61}H_{77}N_8O_{12}^*$, Calcd 1113.6), which was used without further purification. TFA/CH_2Cl_2/H_2O (49:49:2, v/v, 1.6 mL) was added to the intermediate and stirred for 1 h at ambient temperature. Solvent was removed under a stream of nitrogen, and preparative reversed-phase HPLC purification afforded the title compound (8 mg, 33% from **S2**) as a white fluffy material. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.35 (s, 1H, NH_{AMC}), 8.11–8.04 (m, 3H, $NH_{\alpha,Gln}$, $NH_{\alpha,Lys}$ $NH_{\alpha,Lys(lactoyl)}$), 7.80 (d, *J* = 2.0 Hz, 1H, $H8_{AMC}$), 7.79–7.69 (m, 4H, $H5_{AMC}$, $NH_{\varepsilon,Lys}$), 7.67 (t, *J* = 6.0 Hz, 1H, $NH_{\varepsilon,Lys(lactoyl)}$), 7.48 (dd, *J* = 8.7, 2.1 Hz, 1H, $H6_{AMC}$), 7.33 (s, 1H, $CONH_{2,Gln,A}$), 6.81 (s, 1H, $CONH_{2,Gln,B}$), 6.27 (d, *J* = 1.4 Hz, 1H, $H3_{AMC}$), 4.47 (td, *J* = 8.2, 5.9 Hz, 1H, $H_{\alpha,Gln}$), 4.39–4.30 (m, 2H, $H_{\alpha,Pro}$, $H_{\alpha,Lys(lactoyl)}$), 4.29–4.22 (m, 1H, $H_{\alpha,Lys}$), 3.92 (q, *J* = 6.8 Hz, 1H, C(OH)$\underline{H}CH_3$), 3.72–3.59 (m, 2H, $H_{\delta,Pro}$), 3.11–3.02 (m, 2H, $H_{\varepsilon,Lys(lactoyl)}$), 2.82–2.73 (m, 2H, $H_{\varepsilon,Lys}$), 2.40 (d, *J* = 1.3 Hz, 3H, $CH_{3,AMC}$), 2.18–2.10 (m, 2H, $H_{\delta,Gln}$), 2.09–2.00 (m, 1H, $H_{\beta,Pro,A}$), 1.96–1.21 (m, 20H, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $H_{\beta,Lys(lactoyl)}$, $H_{\gamma,Lys(lactoyl)}$, $H_{\delta,Lys(lactoyl)}$, $H_{\beta,Pro,B}$, $H_{\gamma,Pro}$, $H_{\beta,Gln}$, $CH_{3,acetyl}$), 1.17 (d, *J* = 6.7 Hz, 3H, C(OH)HC$\underline{H}_3$). ^{13}C NMR (151 MHz, DMSO) δ 174.3 ($\underline{C}ONH_{\varepsilon,Lys(lactoyl)}$), 173.9 ($CO_{\delta,Gln}$), 171.9 ($CO_{\alpha,Lys}$), 171.7 ($CO_{Pro}$), 171.4 ($CO_{\alpha,Lys(lactoyl)}$), 170.5 ($CO_{\alpha,Gln}$), 169.2 ($\underline{C}OCH_{3,acetyl}$), 160.0 ($C2_{AMC}$), 158.1 (q, *J* = 32.4 Hz, residual CO_{TFA}), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.2 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.3 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 67.2 ($\underline{C}$(OH)HCH_3), 59.5 ($C_{\alpha,Pro}$), 53.7 ($C_{\alpha,Lys(lactoyl)}$), 52.3 ($C_{\alpha,Lys}$), 49.8 ($C_{\alpha,Gln}$), 46.9 ($C_{\delta,Pro}$), 38.8 ($C_{\varepsilon,Lys}$), 37.9 ($C_{\varepsilon,Lys(lactoyl)}$), 31.3 ($C_{\gamma,Gln}$), 31.1 ($C_{\beta,Lys}$), 31.0 ($C_{\beta,Lys(lactoyl)}$), 29.1 ($C_{\beta,Pro}$), 28.9 ($C_{\delta,Lys(lactoyl)}$), 27.1 ($C_{\beta,Gln}$), 26.5 ($C_{\delta,Lys}$), 24.5 ($C_{\gamma,Pro}$), 22.8 ($C_{\gamma,Lys(lactoyl)}$), 22.3 ($C_{\gamma,Lys}$), 22.1 ($CH_{3,acetyl}$), 21.1 (C(OH)$HCH_3$), 18.0 ($CH_{3,AMC}$). Two sets of signals (approximately 10:1) were detectable due to rotamers. Only peaks for the major rotamer is given. Analytical HPLC gradient 0–50% eluent II in eluent I (11 min total runtime), t_R 5.20 min (>98%, UV_{230}). HRMS calcd for $C_{37}H_{55}N_8O_{10}^+$ [M+H]$^+$, 771.4036; found 771.4027.

Ac-Thr-Ala-Arg-Lys(L-La)-AMC (5b). By the method described for **5c**, the title compound was synthesized using **S4** (8 mg, 0.008 mmol), L-lactate (5 mg, 0.054 mmol), *i*Pr_2NEt (19 µL, 0.109 mmol), and HATU (5 mg, 0.012 mmol). Preparative reversed-phase HPLC purification afforded the title compound (2 mg, 26% from **S4**) as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) 10.41 (s, 1H, NH_{AMC}), 8.09 (d, *J* = 7.12Hz, 1H, $NH_{\alpha,Lys}$), 8.02–7.93 (m, 2H, $NH_{\alpha,Arg}$, NH_{Ala}), 7.86–7.78 (m, 2H, NH_{Thr}, $H8_{AMC}$), 7.72 (d, *J* = 8.7 Hz, 1H, $H5_{AMC}$), 7.67 (t, *J* = 5.9 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.51–7.43 (m, 2H, $NH_{\delta,Arg}$, $H6_{AMC}$), 6.27 (s, 1H, $H3_{AMC}$), 4.37–4.25 (m, 3H, $H_{\alpha,Ala}$, $H_{\alpha,Arg}$, $H_{\alpha,Lys}$), 4.18 (dd, *J* = 8.3, 4.4 Hz, 1H, $H_{\alpha,Thr}$), 4.00–3.89 (m, 2H, $H_{\beta,Thr}$, C(OH)$\underline{H}CH_3$), 3.14–3.00 (m, 4H, $H_{\delta,Arg}$, $H_{\varepsilon,Lys}$), 2.40 (s, 3H, $CH_{3,AMC}$), 1.91 (s, 3H, $CH_{3,acetyl}$), 1.78–1.20 (m, 13H, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $H_{\beta,Arg}$, $H_{\gamma,Arg}$, $H_{\beta,Ala}$), 1.16 (d, *J* = 6.7 Hz,

3H, C(OH)HC<u>H</u>$_3$), 1.05 (d, J = 6.3 Hz, 3H, $H_{\gamma,Thr}$). ^{13}C NMR (151 MHz, DMSO) δ 174.3 (<u>C</u>ONH$_{\varepsilon,Lys}$), 172.3 (CO_{Ala}), 171.4 (CO_{Arg}), 171.3 ($CO_{\alpha,Lys}$), 170.1 (CO_{Thr}), 169.8 (<u>C</u>OCH$_{3,acetyl}$), 160.0 ($C2_{AMC}$), 156.6 (NHC(=NH)NH$_2$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.2 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.3 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 67.2 (<u>C</u>(OH)HCH$_3$), 66.5 ($C_{\beta,Thr}$), 58.4 ($C_{\alpha,Thr}$), 53.7 ($C_{\alpha,Lys}$), 52.1 ($C_{\alpha,Arg}$), 48.3 ($C_{\alpha,Ala}$), 40.4 ($C_{\delta,Arg}$), 37.9 ($C_{\varepsilon,Lys}$), 31.4 ($C_{\beta,Lys}$), 28.97 ($C_{\beta,Arg}$ / $C_{\delta,Lys}$), 28.95 ($C_{\beta,Arg}$ / $C_{\delta,Lys}$), 24.9 ($C_{\gamma,Arg}$), 22.8 ($C_{\gamma,Lys}$), 22.6 ($CH_{3,acetyl}$), 21.1 (C(OH)<u>C</u>H$_3$), 19.7 ($C_{\gamma,Thr}$), 18.0 ($CH_{3,AMC}$), 17.9 ($C_{\beta,Ala}$). Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.04 min (>98%, UV_{230}). HRMS calcd for $C_{34}H_{52}N_9O_{10}^+$ [M+H]$^+$, 746.3832; found 746.3826.

Ac-Thr-Ala-Arg-Lys(D-La)-AMC (5c). Compound **S4** (7 mg, 0.007 mmol) and sodium D-lactate (5 mg, 0.041 mmol) were dissolved in anh. DMF (1.0 mL) and cooled to 0 °C. iPr$_2$NEt (14 µL, 0.082 mmol) and HATU (4 mg, 0.010 mmol) were added to the reaction mixture, which was stirred overnight going towards ambient temperature. Additional HATU (4 mg, 0.010 mmol) was added, and the reaction mixture was stirred 1 h until all starting material was consumed. Solvent was removed under reduced pressure to afford the crude intermediate tentatively assigned as Ac-Thr(tBu)-Ala-Arg(Pbf)-Lys(D-la)-AMC (HPLC-MS t_R 1.77 min, m/z 1054.6 ([M+H]$^+$, $C_{51}H_{76}N_9O_{13}S^+$, Calcd 1054.5), which was used without further purification. TFA/TIPS/H$_2$O (95:2.5:2.5, v/v, 1.0 mL) was added to the intermediate, which was stirred for 1 h at ambient temperature. Solvent was removed under a stream of nitrogen, and preparative reversed-phase HPLC purification afforded the title compound (1 mg, 25% from **S4**), as a white fluffy material after lyophilization. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.41 (s, 1H, NH_{AMC}), 8.09 (d, J = 7.1 Hz, 1H, $NH_{\alpha,Lys}$), 8.04–7.94 (m, 2H, $NH_{\alpha,Arg}$, NH_{Ala}), 7.86–7.78 (m, 2H, NH_{Thr}, $H8_{AMC}$), 7.72 (d, J = 8.5 Hz, 1H, $H5_{AMC}$), 7.67 (t, J = 6.0 Hz, 1H, $NH_{\varepsilon,Lys}$), 7.52–7.44 (m, 2H, $NH_{\delta,Arg}$, $H6_{AMC}$), 6.30–6.25 (m, 1H, $H3_{AMC}$), 4.37–4.26 (m, 3H, $H_{\alpha,Ala}$, $H_{\alpha,Arg}$, $H_{\alpha,Lys}$), 4.18 (dd, J = 8.2, 4.3 Hz, 1H, $H_{\alpha,Thr}$), 4.00–3.89 (m, 2H, $H_{\beta,Thr}$, C(OH)<u>H</u>CH$_3$), 3.10 (q, J = 6.6 Hz, 2H, $H_{\delta,Arg}$), 3.06 (q, J = 6.4 Hz, 3H, $H_{\varepsilon,Lys}$), 2.40 (s, 3H, $CH_{3,AMC}$), 1.91 (s, 3H, $CH_{3,acetyl}$), 1.79–1.21 (m, 13H, $H_{\beta,Lys}$, $H_{\gamma,Lys}$, $H_{\delta,Lys}$, $H_{\beta,Arg}$, $H_{\gamma,Arg}$, $H_{\beta,Ala}$), 1.17 (d, J = 6.6 Hz, 3H, C(OH)HC<u>H</u>$_3$), 1.05 (d, J = 6.2 Hz, 3H, $H_{\gamma,Thr}$). ^{13}C NMR (151 MHz, DMSO) δ 174.3 (<u>C</u>ONH$_{\varepsilon,Lys}$), 172.3 (CO_{Ala}), 171.4 (CO_{Arg}), 171.3 ($CO_{\alpha,Lys}$), 170.1 (CO_{Thr}), 169.8 (<u>C</u>OCH$_{3,acetyl}$), 160.0 ($C2_{AMC}$), 156.6 (NHC(=NH)NH$_2$), 153.6 ($C8a_{AMC}$), 153.1 ($C4_{AMC}$), 142.1 ($C7_{AMC}$), 125.9 ($C5_{AMC}$), 115.2 ($C6_{AMC}$), 115.1 ($C4a_{AMC}$), 112.3 ($C3_{AMC}$), 105.7 ($C8_{AMC}$), 67.2 (<u>C</u>(OH)HCH$_3$), 66.5 ($C_{\beta,Thr}$), 58.4 ($C_{\alpha,Thr}$), 53.7 ($C_{\alpha,Lys}$), 52.1 ($C_{\alpha,Arg}$), 48.3 ($C_{\alpha,Ala}$), 40.4 ($C_{\delta,Arg}$), 37.9 ($C_{\varepsilon,Lys}$), 31.4 ($C_{\beta,Lys}$), 29.0 ($C_{\beta,Arg}$), 28.9 ($C_{\delta,Lys}$), 24.9 ($C_{\gamma,Arg}$), 22.8 ($C_{\gamma,Lys}$), 22.6 ($CH_{3,acetyl}$), 21.1 (C(OH)<u>C</u>H$_3$), 19.7 ($C_{\gamma,Thr}$), 18.0 ($CH_{3,AMC}$), 17.9 ($C_{\beta,Ala}$). Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 4.05 min (>98%, UV_{230}). HRMS calcd for $C_{34}H_{52}N_9O_{10}^+$ [M+H]$^+$, 746.3832; found 746.3826.

Additional substrate sources

Code	Sequence	Source
2a	Ac-Leu-Gly-Lys(Ac)-AMC	(*35*)
2d	Ac-Leu-Gly-Lys(Tfa)-AMC	(*35*)
2f	Ac-Leu-Gly-Lys(Pro)-AMC	(*46*)
2g	Ac-Leu-Gly-Lys(*i*-But)-AMC	(*29*)
2j	Ac-Leu-Gly-Lys(But)-AMC	(*46*)
2k	Ac-Leu-Gly-Lys(*i*-Val)-AMC	(*29*)
2m	Ac-Leu-Gly-Lys(Cr)-AMC	(*31*)
3e	Ac-Glu-Thr-Asp-Lys(Myr)-AMC	(*29*, *37*)
4a	Ac-Gln-Pro-Lys-Lys(Ac)-AMC	Enzo Life Sciences and (*40*)
4f	Ac-Gln-Pro-Lys-Lys(Pro)-AMC	(*40*)
4g	Ac-Gln-Pro-Lys-Lys(*i*-But)-AMC	(*40*)
4h	Ac-Gln-Pro-Lys-Lys(Glu)-AMC	(*38*)
4i	Ac-Gln-Pro-Lys-Lys(Dec)-AMC	(*37*)

Synthesis of non-fluorogenic histone peptide substrates and antibody specificity controls

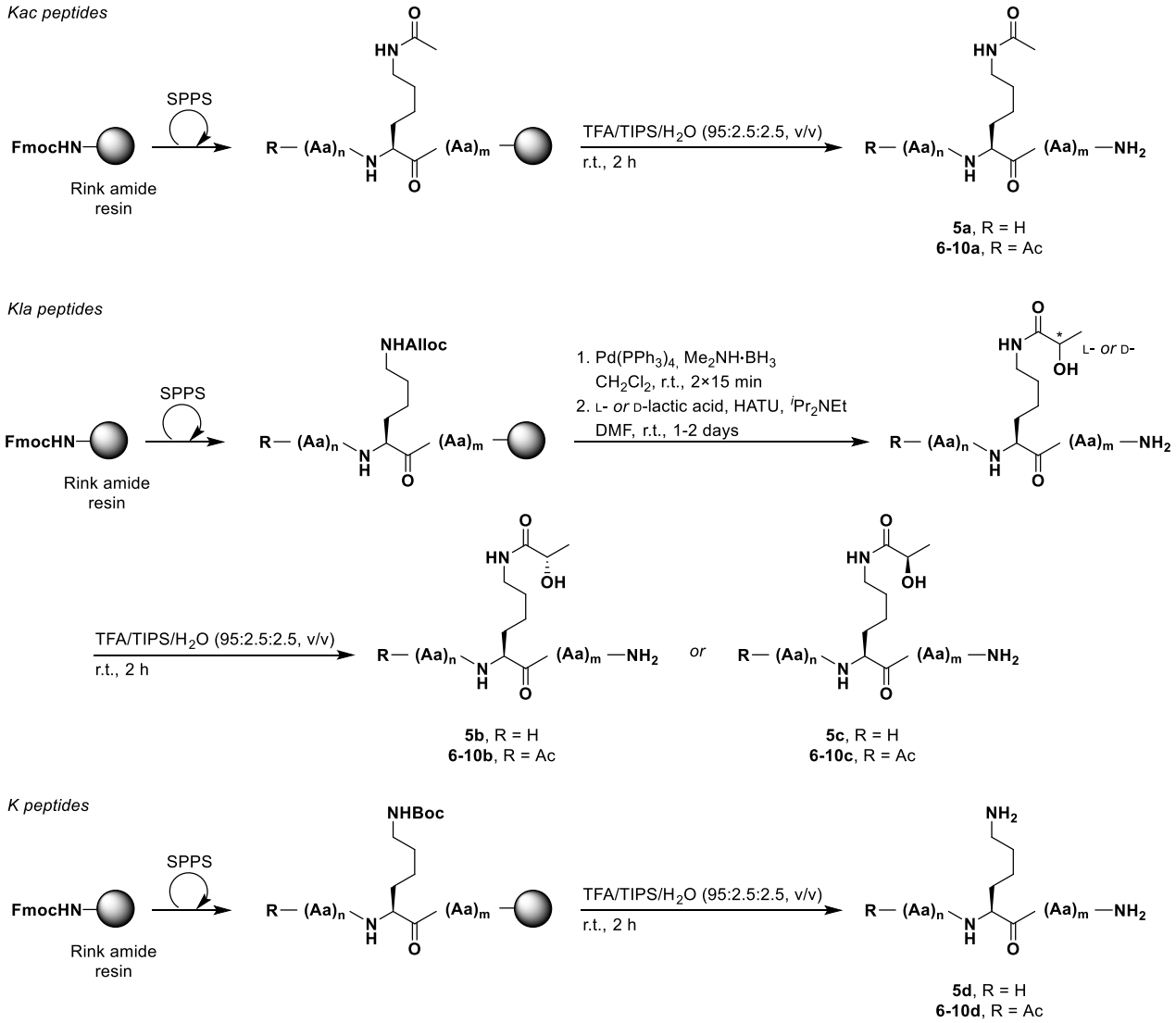


Fig. S17. Synthesis of peptides 5–10 as Kac (a), K(L-la) (b), K(D-la) (c) or K (d) versions.

Non-fluorogenic peptide substrates and controls for antibody dot-blot experiments were synthesized by automated solid phase peptide synthesis (SPPS) using standard Fmoc/*t*Bu chemistry on a Biotage Syro Wave synthesizer (Fig. S17). The following commercially available protected amino acids were used: Boc-Ser(*t*Bu)-OH, Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(*t*Bu)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Lys(Ac)-OH, Fmoc-Lys(Alloc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Boc-Pro-OH (final amino acid for sequence **5**), Fmoc-Pro-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH and Fmoc-Trp(Boc)-OH. SPPS was performed on 0.02–0.08 mmol scale using preloaded TentaGel® S RAM resin (0.24 mmol/g, Rapp Polymere; #S30023). Fmoc deprotection was performed twice: (1) piperidine in DMF (2:3, v/v) for 3 min and (2) piperidine in DMF (1:4, v/v) for 2×8 min. Deprotection was followed by washing with DMF (2×45 s), CH_2Cl_2 (45 s), and DMF (2×45 s). Coupling reactions were

performed as double couplings using Fmoc-Xaa-OH (5.0 equiv to the resin loading), HBTU (5 equiv) and *i*Pr_2NEt (10 equiv, 2.0 M in NMP) in DMF (final concentration = 0.2 M) for 2×40 min.
On-resin Alloc deprotection was performed by addition of borane dimethylamine complex (5 equiv.) and $Pd(PPh_3)_4$ (10 mol%) in anh. CH_2Cl_2 (2.5 mL) to the resin for 2×15 min, followed by washing with CH_2Cl_2 (3×4.0 mL), DMF (3×4.0 mL) and CH_2Cl_2 (3×4.0 mL). Then, on-resin lysine lactylation was performed using L- *or* D-lactic acid (5 equiv.), HATU (4.5 equiv.), and and *i*Pr_2NEt (10 equiv) in DMF (2.5 mL) for 1–2 days, followed by washing with DMF (3×4.0 mL) and CH_2Cl_2 (3×4.0 mL). Alloc deprotection and on-resin lactylation were repeated when test cleavages showed remaining starting material.
Cleavage and global deprotection of the peptides was performed with a mixture of TFA/TIPS/H_2O (95:2.5:2.5, v/v, 4 mL) for 2 h. TFA was removed under a stream of nitrogen, and the crude peptides were triturated in ice-cold diethylether and purified by preparative reversed-phase HPLC to afford the desired products as white fluffy powder after lyophilization (4–30% yield, Fig. S18).

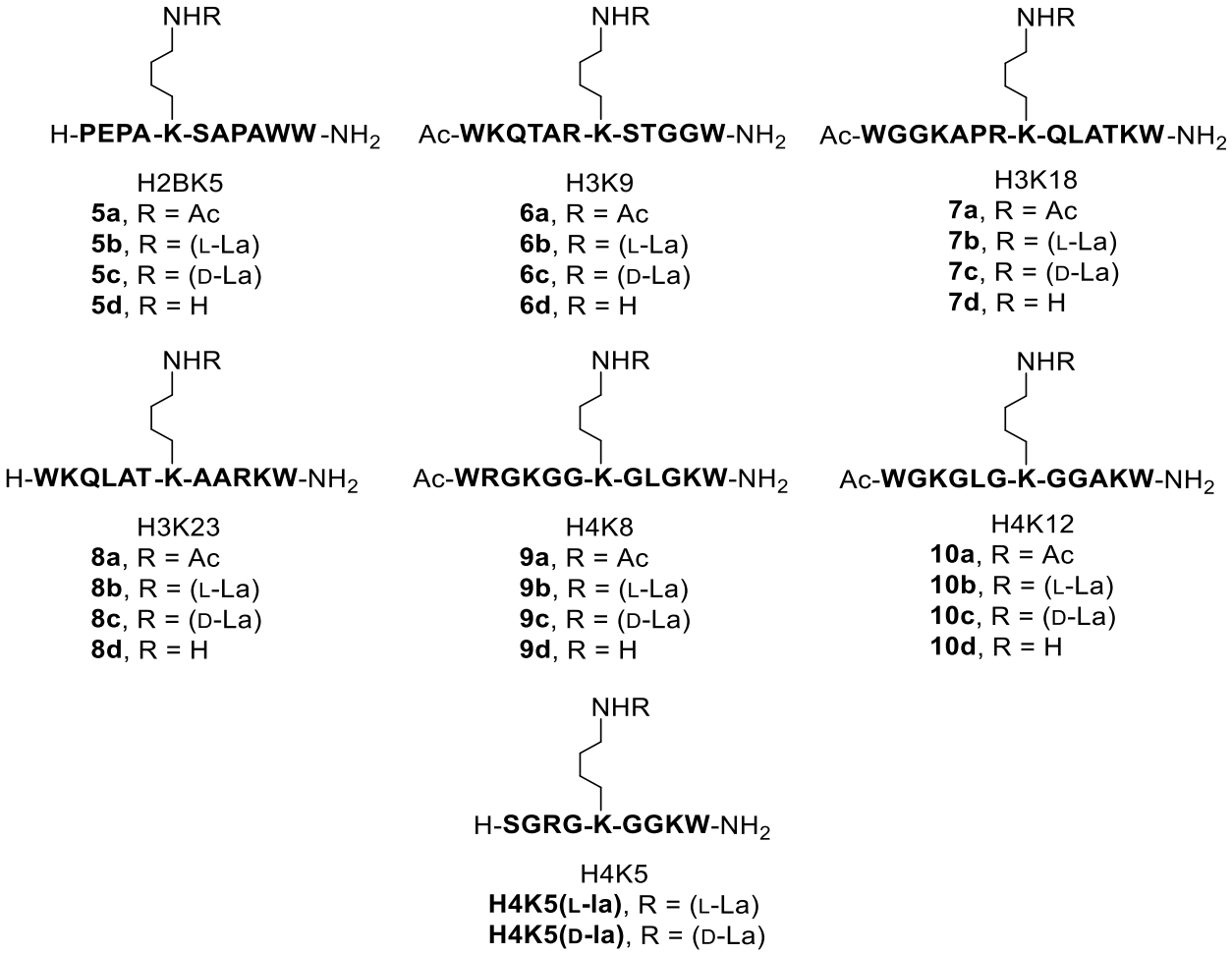


Fig. S18. Structure of non-fluorogenic peptide substrates and lysine controls, and of peptide controls for antibody dot-blot experiments.

Peptide purity and high-resolution mass spectrometry (HRMS)

	R =	Purity*	t_R (min)	Formula	HRMS (*m/z*)	found	(calcd)
5a	Ac	99%	4.68	$C_{62}H_{85}N_{15}O_{15}$	$[M+H]^+$	1280.64216	(1280.64223)
5b	L-La	98%	4.68	$C_{63}H_{87}N_{15}O_{16}$	$[M+H]^+$	1310.65211	(1310.65280)
5c	D-La	98%	4.68	$C_{63}H_{87}N_{15}O_{16}$	$[M+H]^+$	1310.65224	(1310.65280)
5d	H	99%	4.49	$C_{60}H_{83}N_{15}O_{14}$	$[M+H]^+$	1238.63224	(1238.63167)
6a	Ac	95%	4.10	$C_{67}H_{101}N_{21}O_{18}$	$[M+H]^+$	1488.7724	(1488.7705)
6b	L-La	95%	4.13	$C_{68}H_{103}N_{21}O_{19}$	$[M+H]^+$	1518.7804	(1518.7810)
6c	D-La	95%	4.13	$C_{68}H_{103}N_{21}O_{19}$	$[M+H]^+$	1518.7847	(1518.7810)
6d	H	94%	3.94	$C_{65}H_{99}N_{21}O_{17}$	$[M+H]^+$	1446.7613	(1446.7599)
7a	Ac	96%	4.19	$C_{80}H_{124}N_{24}O_{18}$	$[M+H]^+$	1709.9610	(1709.9596)
7b	L-La	97%	4.40	$C_{81}H_{126}N_{24}O_{19}$	$[M+H]^+$	1739.9722	(1739.9702)
7c	D-La	99%	4.40	$C_{81}H_{126}N_{24}O_{19}$	$[M+H]^+$	1739.9720	(1739.9702)
7d	H	98%	4.18	$C_{78}H_{122}N_{24}O_{17}$	$[M+H]^+$	1667.9512	(1667.9491)
8a	Ac	98%	5.00	$C_{74}H_{116}N_{22}O_{16}$	$[M+2H]^{2+}$	785.45399	(785.45426)
8b	L-La	94%	5.02	$C_{75}H_{118}N_{22}O_{17}$	$[M+2H]^{2+}$	800.45976	(800.45954)
8c	D-La	96%	5.01	$C_{75}H_{118}N_{22}O_{17}$	$[M+2H]^{2+}$	800.45980	(800.45954)
8d	H	97%	4.65	$C_{72}H_{114}N_{22}O_{15}$	$[M+2H]^{2+}$	764.44908	(764.44898)
9a	Ac	97%	4.31	$C_{66}H_{101}N_{21}O_{14}$	$[M+2H]^{2+}$	706.89903	(706.89912)
9b	L-La	96%	4.31	$C_{67}H_{103}N_{21}O_{15}$	$[M+2H]^{2+}$	721.90423	(721.90440)
9c	D-La	97%	4.30	$C_{67}H_{103}N_{21}O_{15}$	$[M+H]^+$	1442.80226	(1442.69300)
9d	H	98%	4.11	$C_{64}H_{99}N_{21}O_{13}$	$[M+2H]^{2+}$	685.89396	(685.89384)
10a	Ac	98%	4.52	$C_{63}H_{94}N_{18}O_{14}$	$[M+H]^+$	1327.72851	(1327.72697)
10b	L-La	98%	4.53	$C_{64}H_{96}N_{18}O_{15}$	$[M+H]^+$	1357.73773	(1357.73753)
10c	D-La	97%	4.53	$C_{64}H_{96}N_{18}O_{15}$	$[M+H]^+$	1357.73711	(1357.73753)
10d	H	99%	4.31	$C_{61}H_{92}N_{18}O_{13}$	$[M+H]^+$	1285.71807	(1285.71640)
H4K5(L-la)	L-La	99%	3.86†	$C_{43}H_{70}N_{16}O_{12}$	$[M+H]^+$	1003.5427	(1003.5432)
H4K5(D-la)	D-La	98%	3.86†	$C_{43}H_{70}N_{16}O_{12}$	$[M+H]^+$	1003.5428	(1003.5432)

*Peptide purity measured by integration of HPLC chromatograms at 215 nm or 230 nm. Please find HPLC traces below. Retention times correspond to linear gradients of eluent III and eluent IV, rising linearly from] 0% to 95% of IV during t = 1−11, or 0% to 50% of IV during t = 1−11 in the case of H4K5 dot-blot controls (†).

*Synthesis of compounds **DD-I22** and **DD-I23***

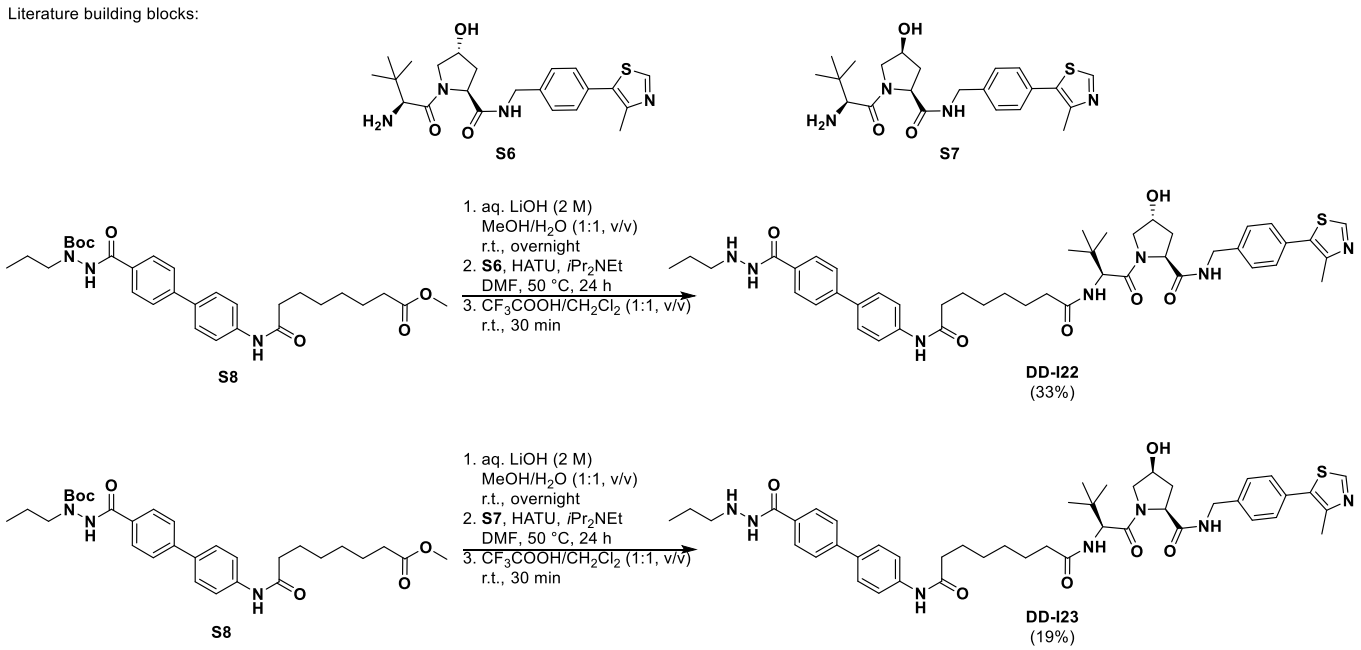


Fig. S19. Synthesis of compounds DD-I22 and DD-I23. Compound **S8** was synthesized following literature procedures (*61*).

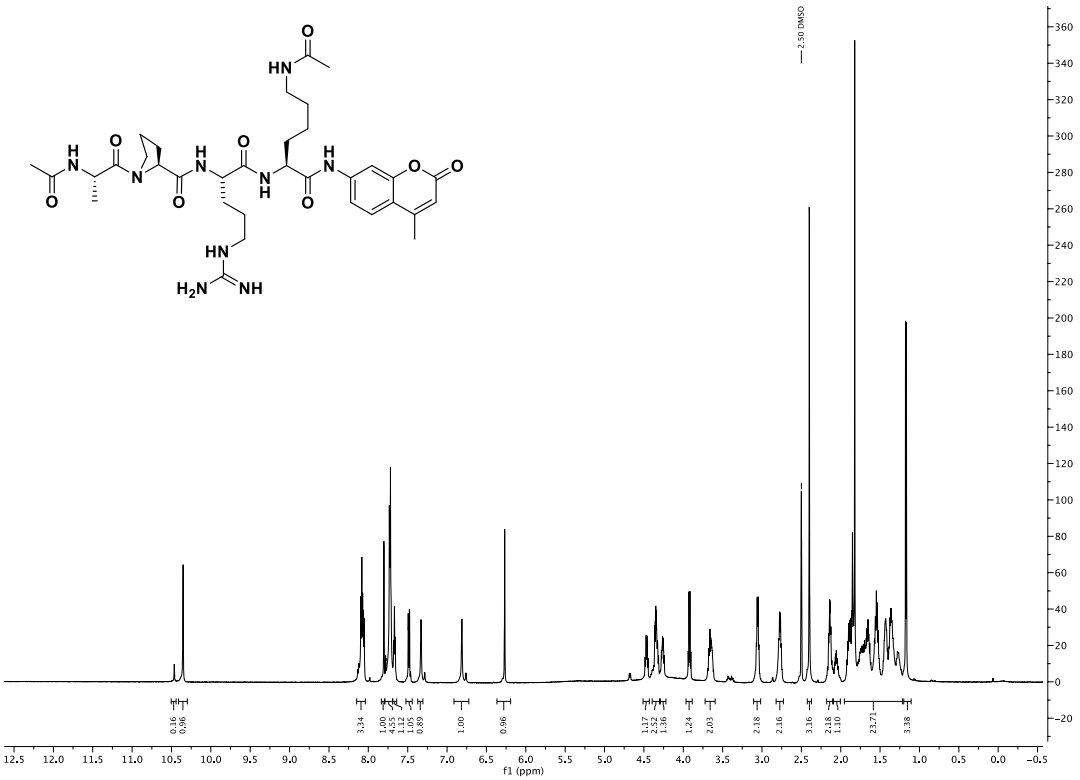
***N*^{1}-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-*N*^{8}-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)octanediamide (DD-I22).** Compound **S8** (35 mg, 0.065 mmol) was dissolved in a THF/MeOH mixture (2 mL), and aq. LiOH (250 µM, 1 mL, 0.260 mmol) was added and the reaction mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure, and water was removed by co-evaporation with toluene (3×3 mL) to afford white powder (HPLC-MS t_R 1.85 min, *m*/*z* 526.2; $[M+H]^+$, $C_{29}H_{40}N_3O_6$, Calcd 526.6) as a white solid, which was redissolved in anh. DMF (2.5 mL). Compound **S6** (35 mg, 0.065 mmol), HATU (26 mg, 0.068 mmol) and *i*Pr$_2$NEt (90 µL, 0.520 mmol) were added to the reaction mixture, which was stirred at 50 °C for 5 days. The reaction mixture was diluted with EtOAc (10 mL) and washed with water (8 mL). The aqueous phase was extracted with EtOAc (2×10 mL). The combined organic phases were washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated under reduced pressure affording crude intermediate (HPLC-MS t_R 2.02 min, *m*/*z* 938.4; $[M+H]^+$, $C_{51}H_{68}N_7O_8S^+$, Calcd 939.2). The crude was dissolved in a TFA/CH_2Cl_2 mixture (1:1, v/v) and stirred at room temperature for 1 h. Volatiles were removed under the stream of N_2, and preparative reversed-phase HPLC purification afforded the title compound (19 mg, 35%) as a white fluffy material after lyophilization. Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 5.76 min (>95%, UV_{230}). HRMS calcd for $C_{46}H_{59}N_7O_6^+$ $[M+H]^+$, 838.4320; found 838.4303. ^{1}H NMR (600 MHz, MeOD) δ 8.87 (s, 1H, $CH_{thiazole}$), 7.91 (d, J = 8.1 Hz, 2H, C3'$H_{biphenyl}$ and C5'$H_{biphenyl}$), 7.76 (d, J = 8.2 Hz, 2H, C1'$H_{biphenyl}$ and C6'$H_{biphenyl}$),

7.72 – 7.59 (m, 4H, C2H$_{biphenyl}$, C3H$_{biphenyl}$, C5H$_{biphenyl}$ and C6H$_{biphenyl}$,), 7.47 – 7.37 (m, 4H, CH$_{Ar,VHL}$), 4.64 (s, 1H, CH$_{\alpha,tBuG}$), 4.62 – 4.54 (m, 1H, CH$_{\alpha,HyP}$), 4.54 – 4.47 (m, 2H, CHOH, CH$_{2,a}$NH$_{VHL}$), 4.35 (d, J = 15.4 Hz, 1H, CH$_{2,b}$NH$_{VHL}$), 3.91 (d, J = 11.0 Hz, 1H, HOCHCH$_{2,a}$N), 3.80 (dd, J = 10.9, 3.9 Hz, 1H, HOCHCH$_{2,b}$N), 3.14 (t, J = 7.7 Hz, 2H, CH$_3$CH$_2$CH$_2$N), 2.46 (s, 3H, CH$_{3,thiazole}$), 2.40 (t, J = 7.5 Hz, 2H, OCCH$_2$CH$_2$CH$_{2,linker-biphenyl}$), 2.30 (hept, J = 7.1 Hz, 2H, OCCH$_2$CH$_2$CH$_{2,linker-VHL}$), 2.24 – 2.18 (m, 1H, HOCHCH$_{2,a}$C), 2.12 – 2.05 (m, 1H, HOCHCH$_{2,b}$C), 1.76 – 1.68 (m, 4H, OCCH$_2$CH$_2$CH$_{2,linker}$x2), 1.64 (p, J = 7.2 Hz, 2H, CH$_3$CH$_2$CH$_2$N), 1.45 – 1.35 (m, 5H, OCCH$_2$CH$_2$CH$_{2,linker}$x2), 1.04 (m, 13H, CH$_3$CH$_2$CH$_2$N, C(CH$_3$)$_{3,tBuG}$). ^{13}C NMR (151 MHz, MeOD) δ 176.0 (CO$_{linker-VHL}$), 174.8 (CO$_{linker-biphenyl}$), 174.5 (CO$_{HyP}$), 172.4 (CO$_{tBuG}$), 168.1 (CO$_{hydrazide}$), 152.9 (CH$_{thiazole}$), 149.0(CH$_3$C$_{thiazole}$), 146.2 (C1'$_{biphenyl}$), 140.4 (NHCH$_2$C$_{Ar,VHL}$), 136.5 (C4$_{biphenyl}$) 136.3 (C1$_{biphenyl}$), 133.5 (C$_{Ar}$CS), 131.5 (C$_{Ar}$CS), 130.5 (C$_{Ar}$CO$_{hydrazide}$), 130.4 (C3''H$_{Ar,VHL}$, C5''H$_{Ar,VHL}$), 129.2 (C3'H$_{biphenyl}$, C5'H$_{biphenyl}$), 129.0 (C2''H$_{Ar,VHL}$, C6''H$_{Ar,VHL}$), 128.5 (C2H$_{biphenyl}$, C6H$_{biphenyl}$), 127.9 (C2'H$_{biphenyl}$, C6'H$_{biphenyl}$), 121.5 (C3H$_{biphenyl}$, C5H$_{biphenyl}$), 71.1 (HOCH), 60.8 (CH$_{\alpha,HyP}$), 59.0 (CH$_{\alpha,tBuG}$),zz 58.0 (HOCHCH$_2$N), 54.0 (CH$_3$CH$_2$CH$_2$N), 43.7 (CH$_2$NH$_{VHL}$), 38.9 (HOCHCH$_2$C), 37.9 (OCH$_2$CH$_2$CH$_{2,linker-biphenyl}$), 36.5 (OCH$_2$CH$_2$CH$_{2,linker-VHL}$, C(CH$_3$)$_{3,tBuG}$), 29.9 (OCCH$_2$CH$_2$CH$_{2,linker}$ x2), 27.0 (C(H$_3$)$_{3,tBuG}$), 26.8 (OCCH$_2$CH$_2$CH$_{2,linker}$), 26.7 (OCCH$_2$CH$_2$CH$_{2,linker}$), 20.3 (CH$_3$CH$_2$CH$_2$N), 15.8 (CH$_{3,thiazole}$), 11.4 (CH$_3$CH$_2$CH$_2$N).

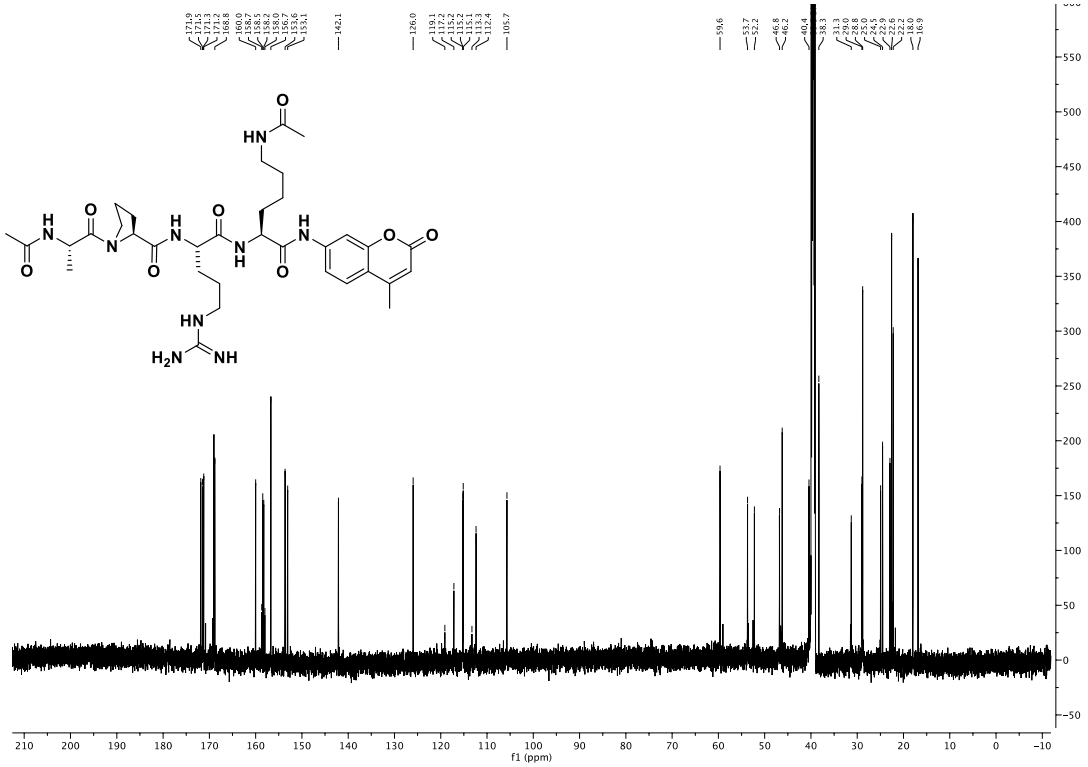
***N*^{1}-((*S*)-1-((2*S*,4*S*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-*N*^{8}-(4'-(2-propylhydrazine-1-carbonyl)-[1,1'-biphenyl]-4-yl)octanediamide (DD-I23).** Compound **S8** (35 mg, 0.065 mmol) was dissolved in THF/MeOH mixture (2 mL), and aq. LiOH (250 mM, 1 mL, 0.260 mmol) was added and the reaction mixture was stirred at 40 °C overnight. The reaction mixture was concentrated under reduced pressure, and water was removed by co-evaporation with toluene (3×2 mL) to afford white powder (HPLC-MS t_R 1.85 min, *m*/*z* 526.2; [M+H]$^+$, $C_{29}H_{40}N_3O_6$, Calcd 526.6) as a white solid, which was redissolved in anh. DMF (2.5 mL). Compound **S7** (35 mg, 0.065 mmol), HATU (26 mg, 0.068 mmol) and DIPEA (90 µL, 0.520 mmol) were added to the reaction mixture which was stirred at 50 °C for 2 days. The reaction mixture was diluted with EtOAc (15 mL) and washed with water (10 mL). The aqueous phase was extracted with EtOAc (2x15 mL). The combined organic phases were washed with water (20 mL) and brine (20 mL), dried over $MgSO_4$ and concentrated under reduced pressure affording crude intermediate (HPLC-MS t_R 2.06 min, *m*/*z* 938.4; [M+H]$^+$, $C_{51}H_{68}N_7O_8S^+$, Calcd 939.2). The crude was dissolved in a TFA/CH_2Cl_2 mixture (1:1, v/v) and stirred at room temperature for 1 h. Volatiles were removed under the stream of N_2, and preparative reversed-phase HPLC purification afforded the title compound (10 mg, 19%) as a white fluffy material after lyophilization. Analytical HPLC gradient 0–95% eluent II in eluent I (11 min total runtime), t_R 5.81 min (>97%, UV_{230}). HRMS calcd for $C_{46}H_{59}N_7O_6^+$ [M+H]$^+$, 838.4320; found 838.4305. ^{1}H NMR (600 MHz, MeOD) δ 9.91 (brs, 1H, NH$_{biphenyl}$), 8.89 (s, 1H, s, 1H, CH$_{thiazole}$), 8.59 (t, J = 6.0 Hz, 1H, NH$_{VHL-Ar}$), 7.95 – 7.91 (m, 2H, C3'H$_{biphenyl}$ and C5'H$_{biphenyl}$), 7.85 (d, J = 8.1 Hz, 1H, NH$_{tBuG}$), 7.80 – 7.75 (m, 2H, C1'H$_{biphenyl}$ and C6'H$_{biphenyl}$), 7.72 – 7.63 (m, 4H, C2H$_{biphenyl}$, C3H$_{biphenyl}$, C5H$_{biphenyl}$ and C6H$_{biphenyl}$,), 7.47 – 7.36 (m, 4H, CH$_{Ar,VHL}$), 4.55 – 4.49 (m, 3H, CH$_{\alpha,tBuG}$, CH$_{\alpha,HyP}$, CH$_{2,a}$NH$_{VHL}$), 4.42 – 4.35 (m, 2H, CH$_{2,b}$NH$_{VHL}$, CHOH), 4.05 (dd, J = 10.5, 5.1 Hz,

1H, HOCHC$\underline{H}_{2,a}$N), 3.70 (dd, J = 10.4, 3.8 Hz, 1H, HOCHC$\underline{H}_{2,b}$N), 3.20 (t, J = 5.9 Hz, 2H, $CH_3CH_2C\underline{H}_2N$), 2.49 – 2.38 (m, 6H, $CH_{3,thiazole}$, HOCHC$\underline{H}_{2,a}$C, OCC$\underline{H}_2CH_2CH_{2,linker\text{-}biphenyl}$), 2.35 – 2.23 (m, 2H, OCC$\underline{H}_2CH_2CH_{2,linker\text{-}VHL}$), 1.98 (dt, J = 13.3, 4.5 Hz, 1H, HOCHC$\underline{H}_{2,b}$C), 1.81 – 1.68 (m, 4H, $OCCH_2C\underline{H}_2CH_{2,linker}$, $CH_3C\underline{H}_2CH_2N$), 1.67 – 1.60 (m, 2H, $OCCH_2C\underline{H}_2CH_{2,linker}$), 1.46 – 1.36 (m, 5H, $OCCH_2CH_2C\underline{H}_{2,linker}$x2), 1.10 – 0.99 (m, 12H, $C\underline{H}_3CH_2CH_2N$, $C(C\underline{H}_3)_{3,tBuG}$). ^{13}C NMR (151 MHz, MeOD) δ 176.4 ($CO_{linker\text{-}VHL}$), 174.9 ($CO_{linker\text{-}biphenyl}$), 174.7 ($CO_{HyP}$), 172.6 ($CO_{tBuG}$), 167.9 ($CO_{hydrazide}$), 152.8 ($CH_{thiazole}$), 148.8 ($CH_3\underline{C}_{thiazole}$), 146.2 ($C1'_{biphenyl}$), 140.4 ($NHCH_2\underline{C}_{Ar,VHL}$), 140.0 ($C4_{biphenyl}$), 136.1 ($C1_{biphenyl}$), 133.4 ($C_{Ar}\underline{C}S$), 131.4 ($\underline{C}_{Ar}CS$), 130.3 ($C3''H_{Ar,VHL}$, $C5''H_{Ar,VHL}$), 129.2 ($C3'H_{biphenyl}$, $C5'H_{biphenyl}$), 129.0 ($C2''H_{Ar,VHL}$, $C6''H_{Ar,VHL}$), 128.4 ($C2H_{biphenyl}$, $C6H_{biphenyl}$), 127.8 ($C2'H_{biphenyl}$, $C6'H_{biphenyl}$), 121.5 ($C3H_{biphenyl}$, $C5H_{biphenyl}$), 71.5 (HOCH), 61.0 ($CH_{\alpha,HyP}$), 59.4 ($CH_{\alpha,tBuG}$), 57.5 (HOCH$\underline{C}H_2N$), 53.8 ($CH_3CH_2\underline{C}H_2N$), 43.9 ($\underline{C}H_2NH_{VHL}$), 37.8 ($O\underline{C}H_2CH_2CH_{2,linker\text{-}biphenyl}$), 36.3 ($OCH_2\underline{C}H_2CH_{2,linker\text{-}VHL}$, HOCH$\underline{C}H_2C$), 35.8 ($\underline{C}(CH_3)_{3,tBuG}$), 29.8 ($OCCH_2CH_2\underline{C}H_{2,linker}$ x2) 26.9 ($(CH_3)_{3,tBuG}$), 26.7 ($OCCH_2\underline{C}H_2CH_{2,linker}$), 26.6 ($OCCH_2\underline{C}H_2CH_{2,linker}$), 19.9 ($CH_3\underline{C}H_2CH_2N$), 15.7 ($CH_{3,thiazole}$), 11.3 ($\underline{C}H_3CH_2CH_2N$).

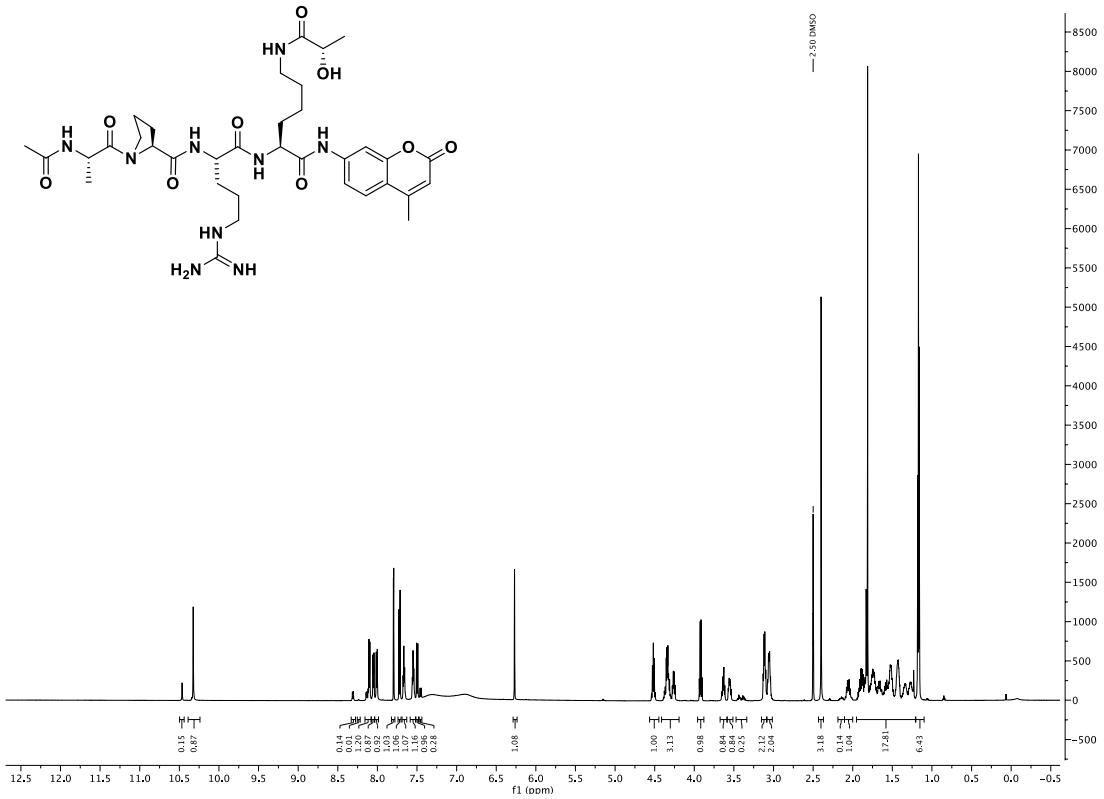
NMR spectra



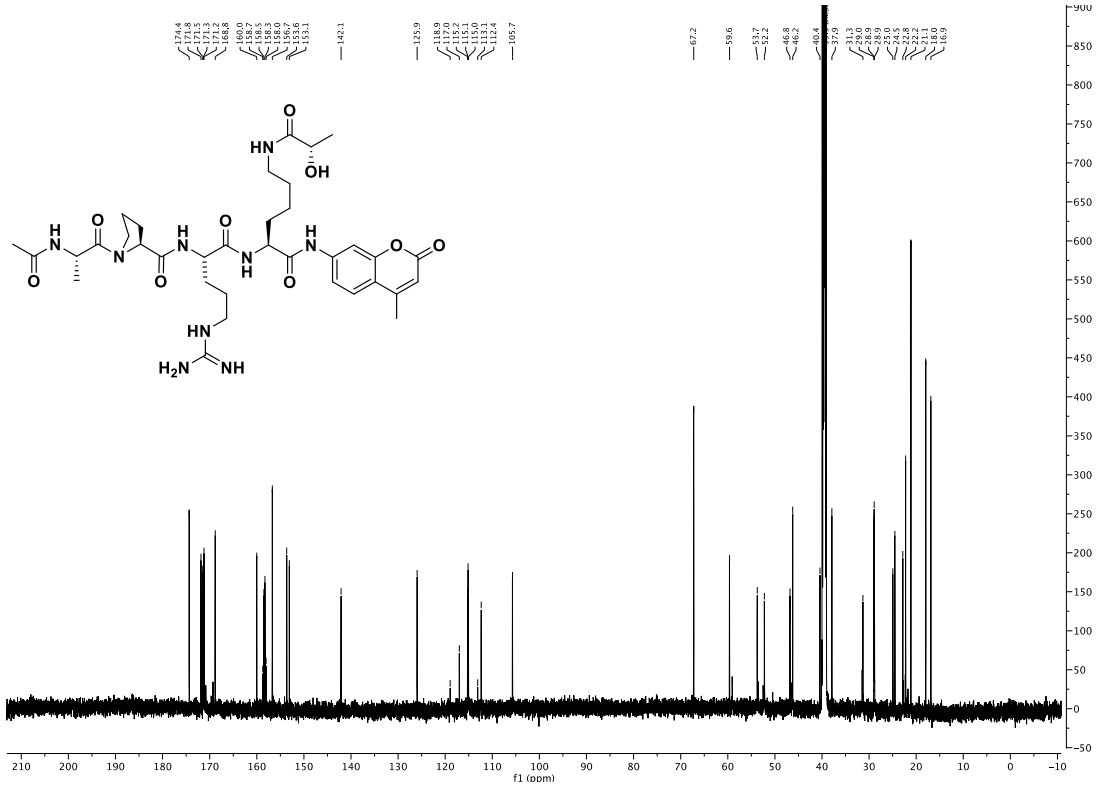
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **1a**



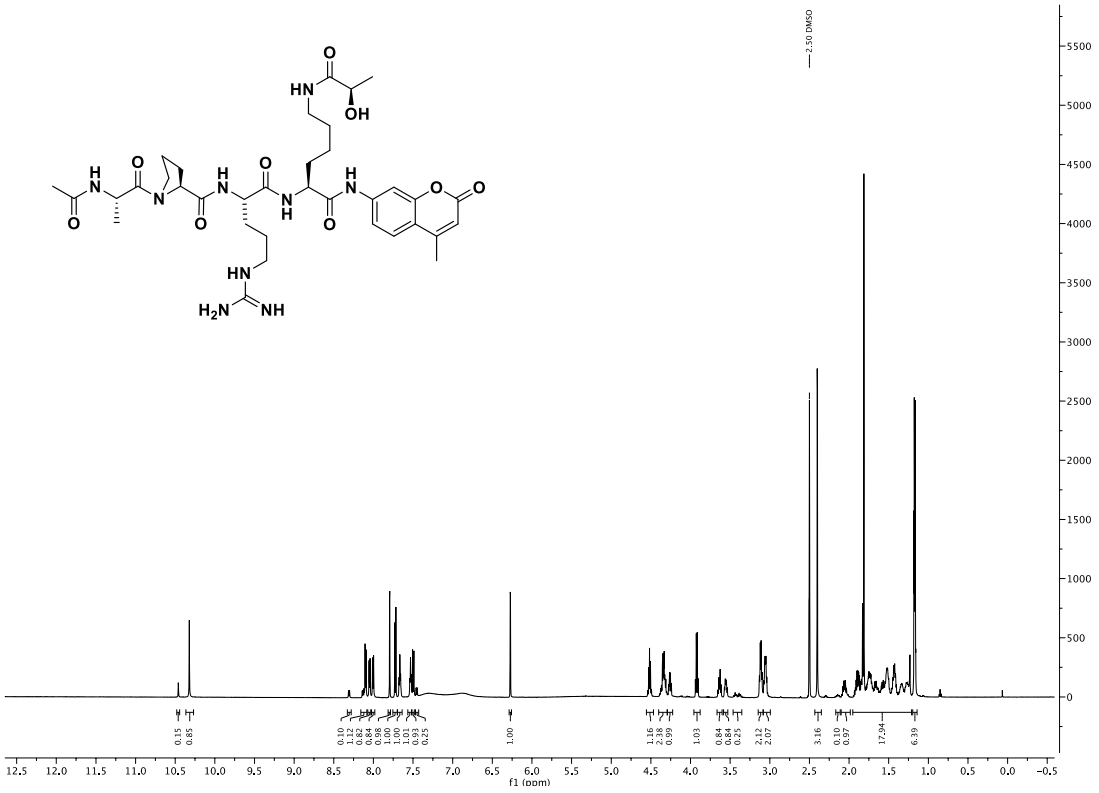
^{13}C NMR spectra (151 MHz, DMSO) of compound **1a**



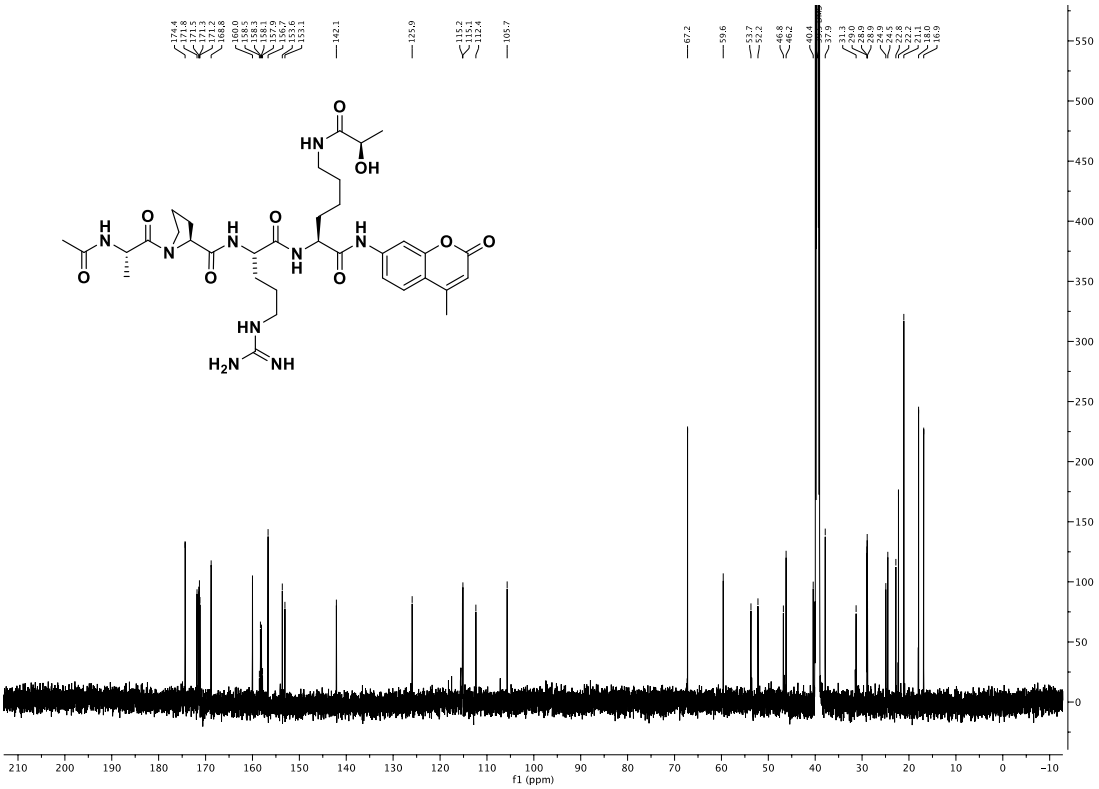
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **1b**



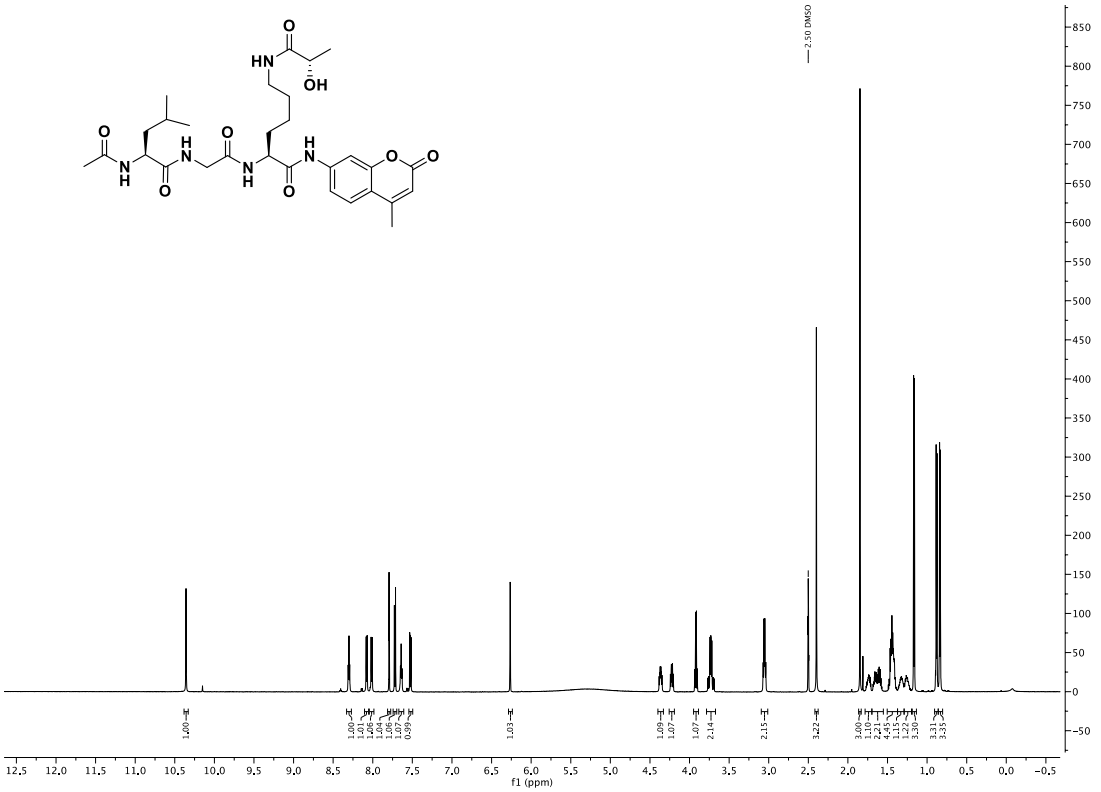
^{13}C NMR spectra (151 MHz, DMSO) of compound **1b**



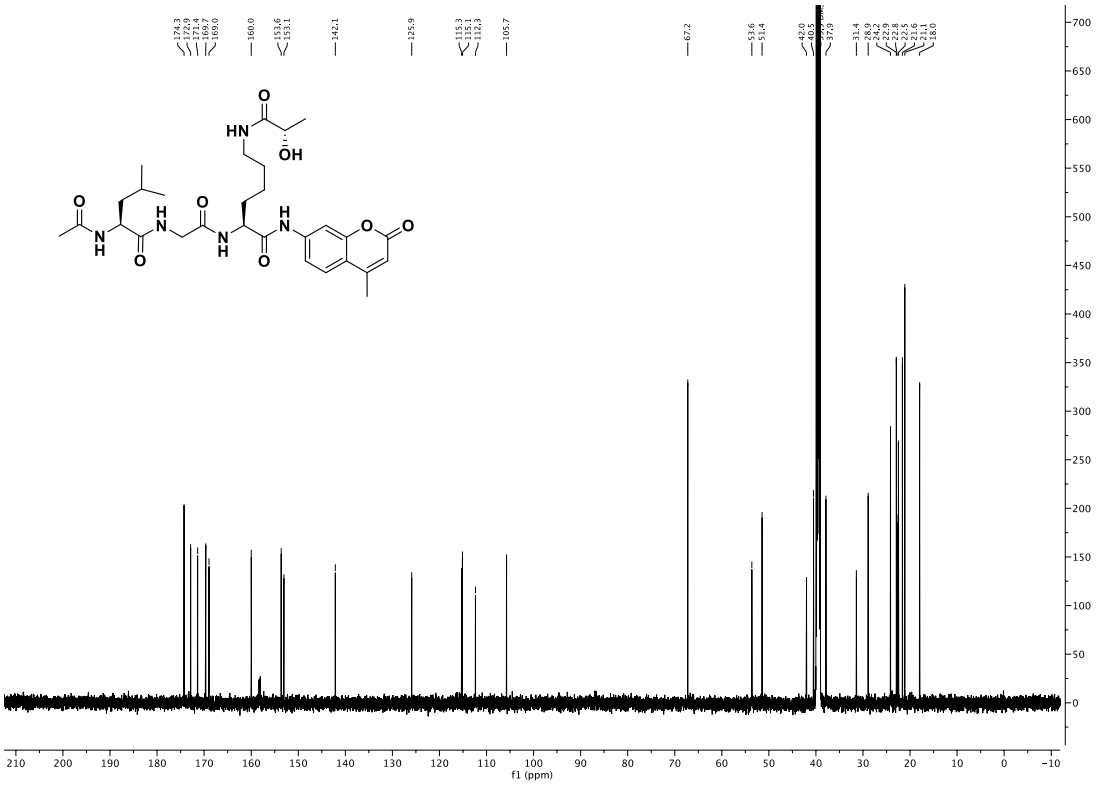
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **1c**



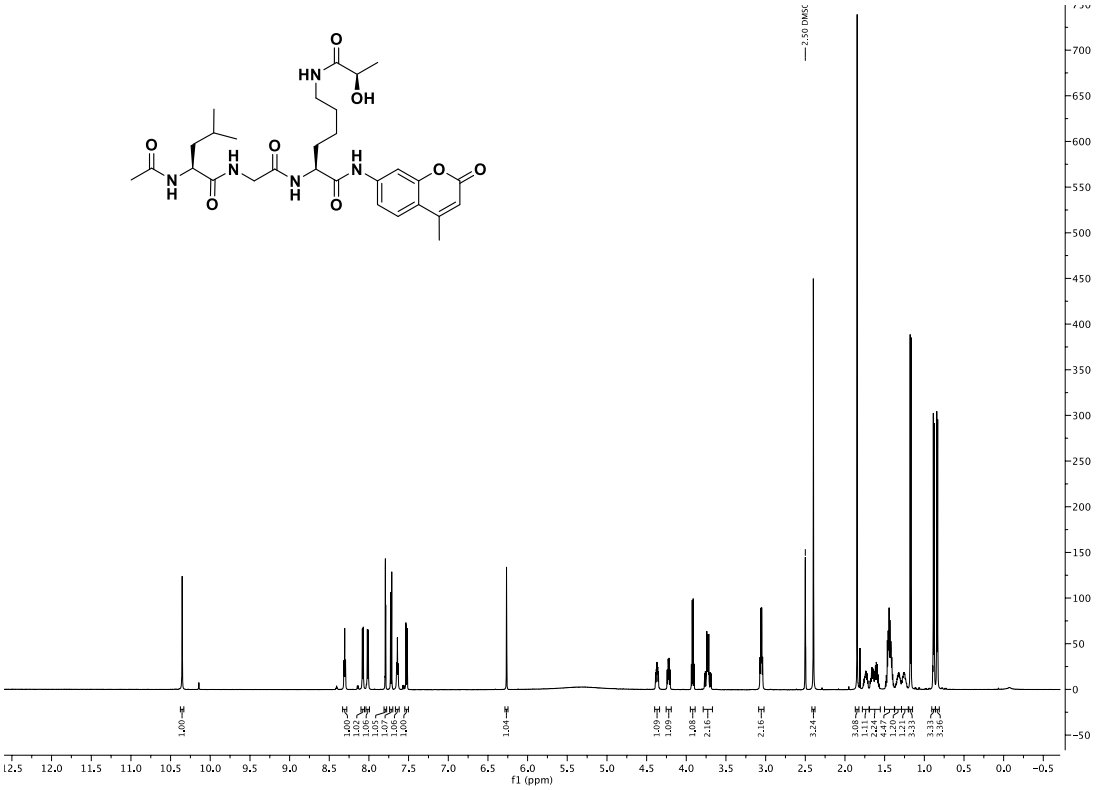
^{13}C NMR spectra (151 MHz, DMSO) of compound **1c**



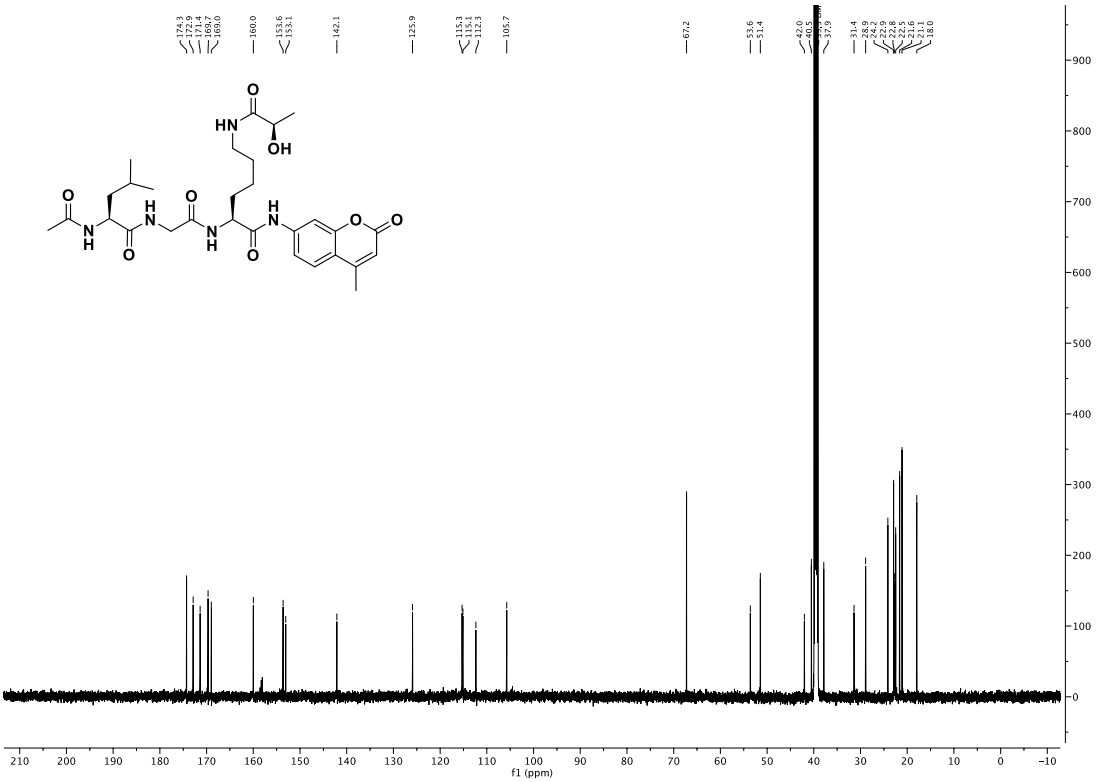
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **2b**



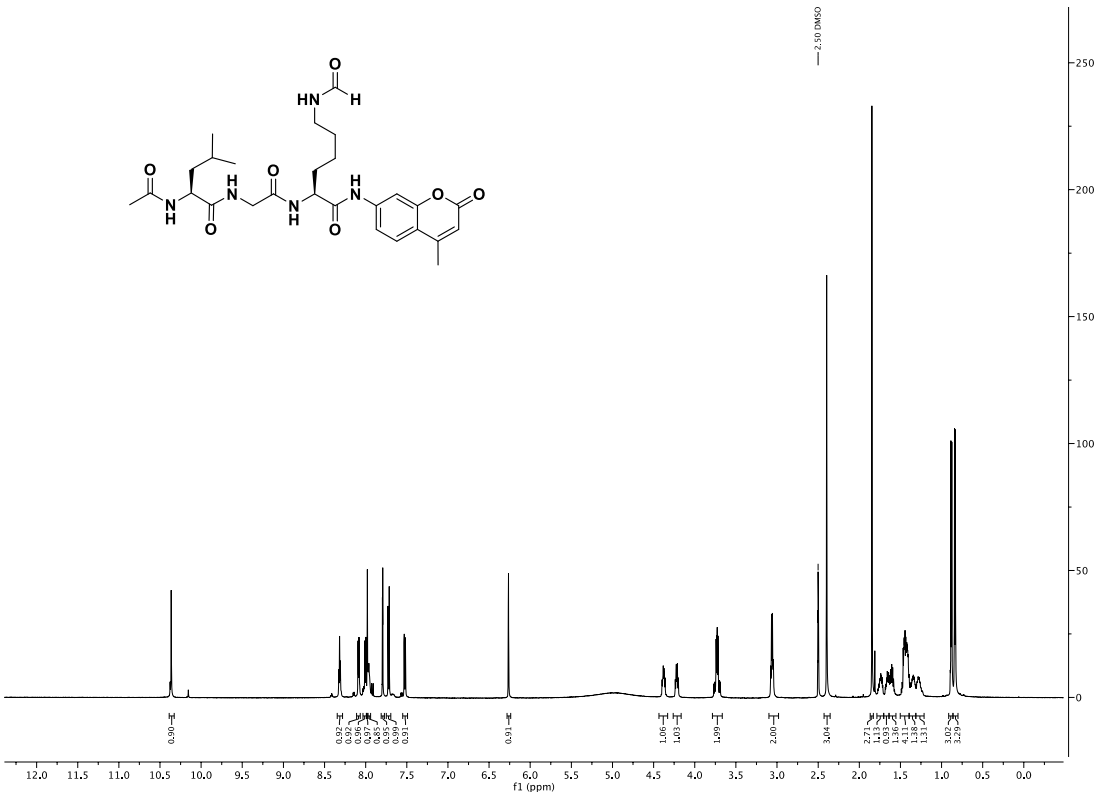
^{13}C NMR spectra (151 MHz, DMSO) of compound **2b**



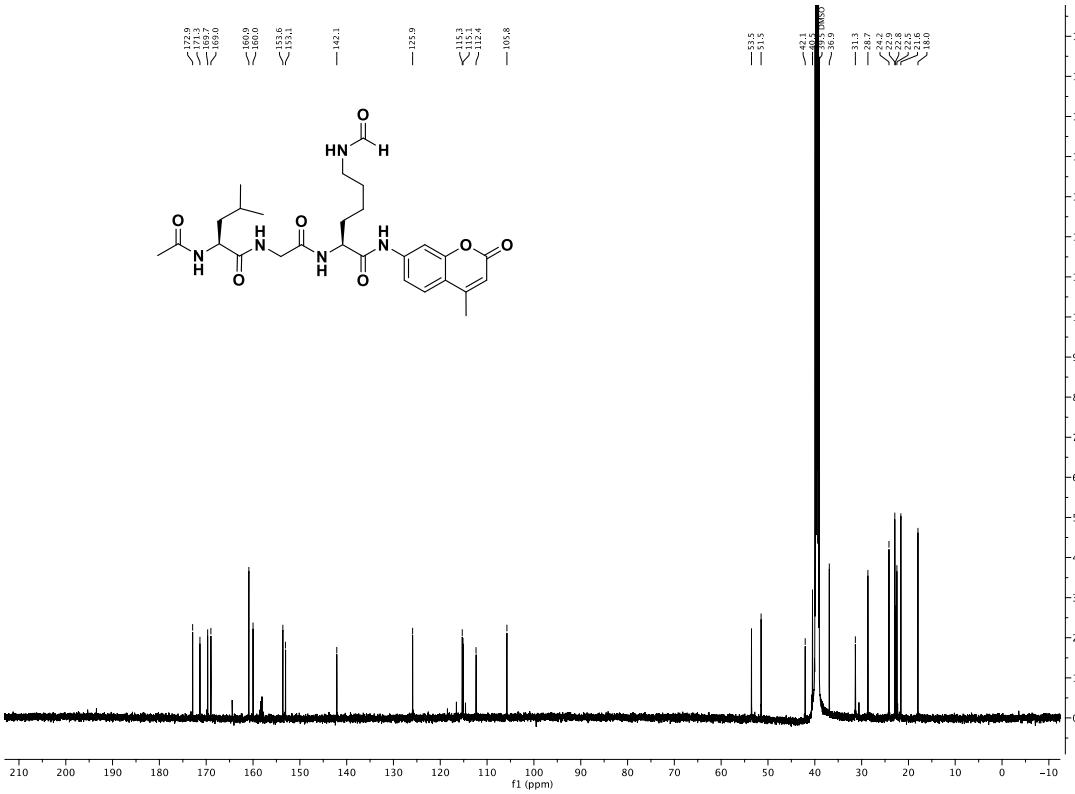
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **2c**



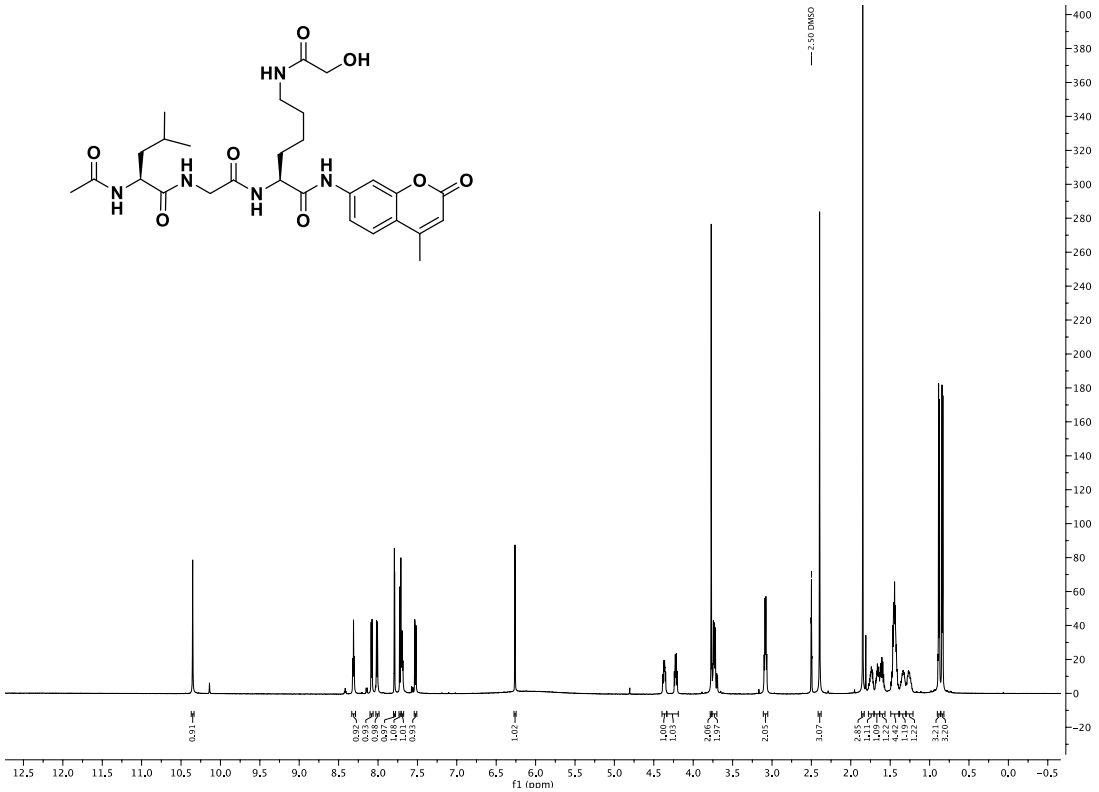
^{13}C NMR spectra (151 MHz, DMSO) of compound **2c**



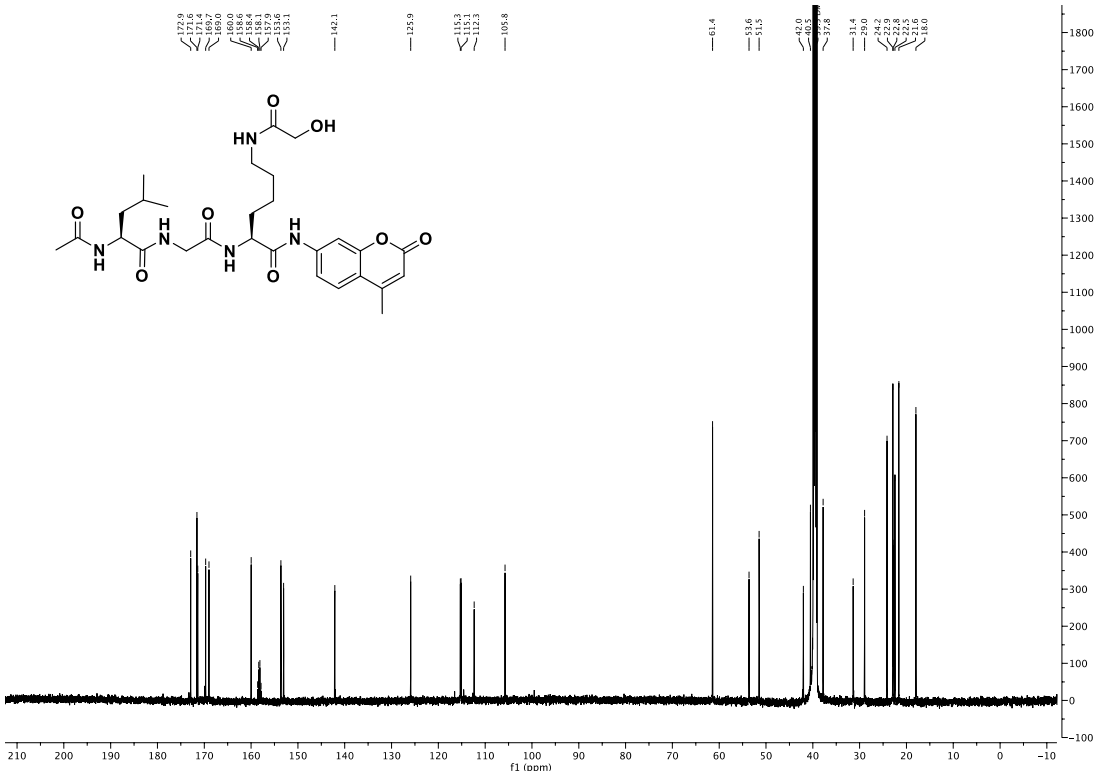
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **2l**



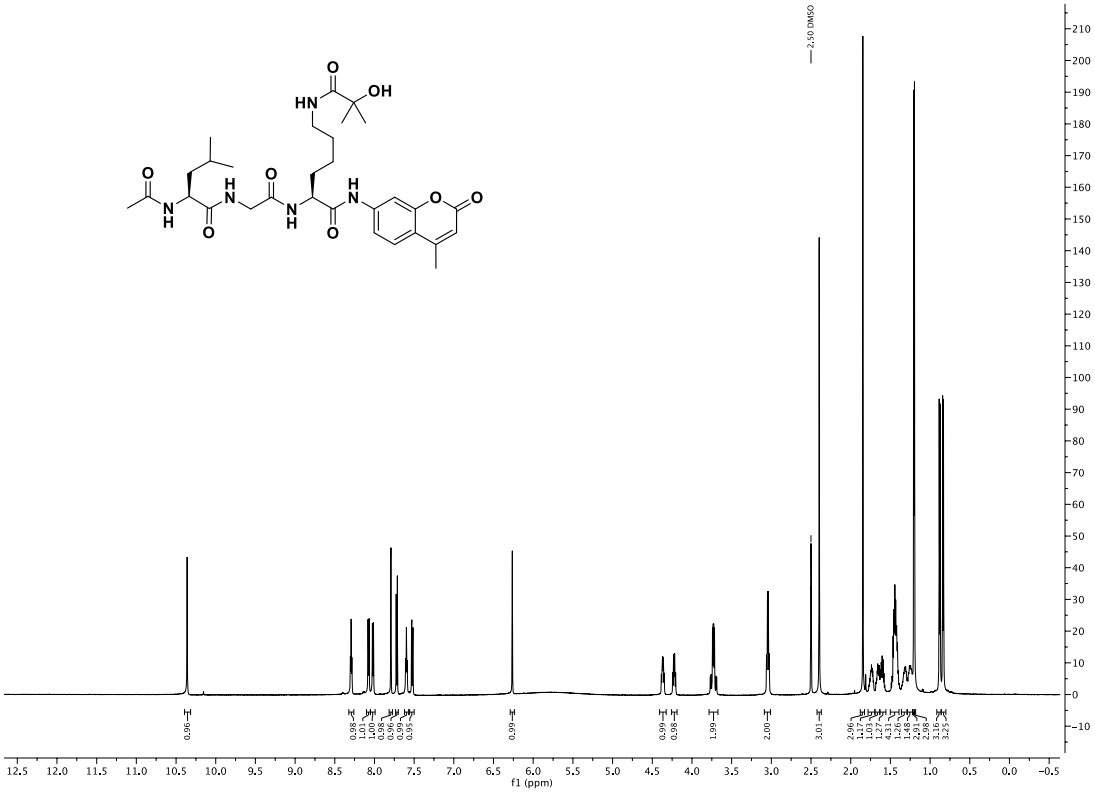
^{13}C NMR spectra (151 MHz, DMSO) of compound **2l**



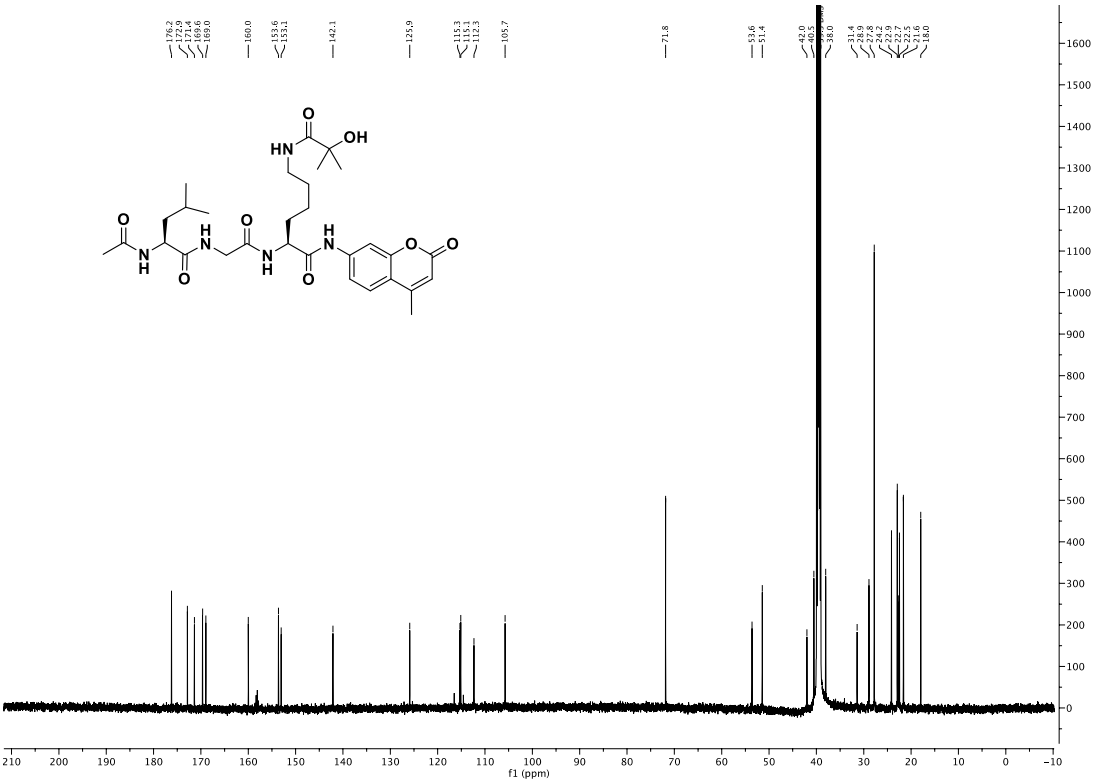
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **2n**



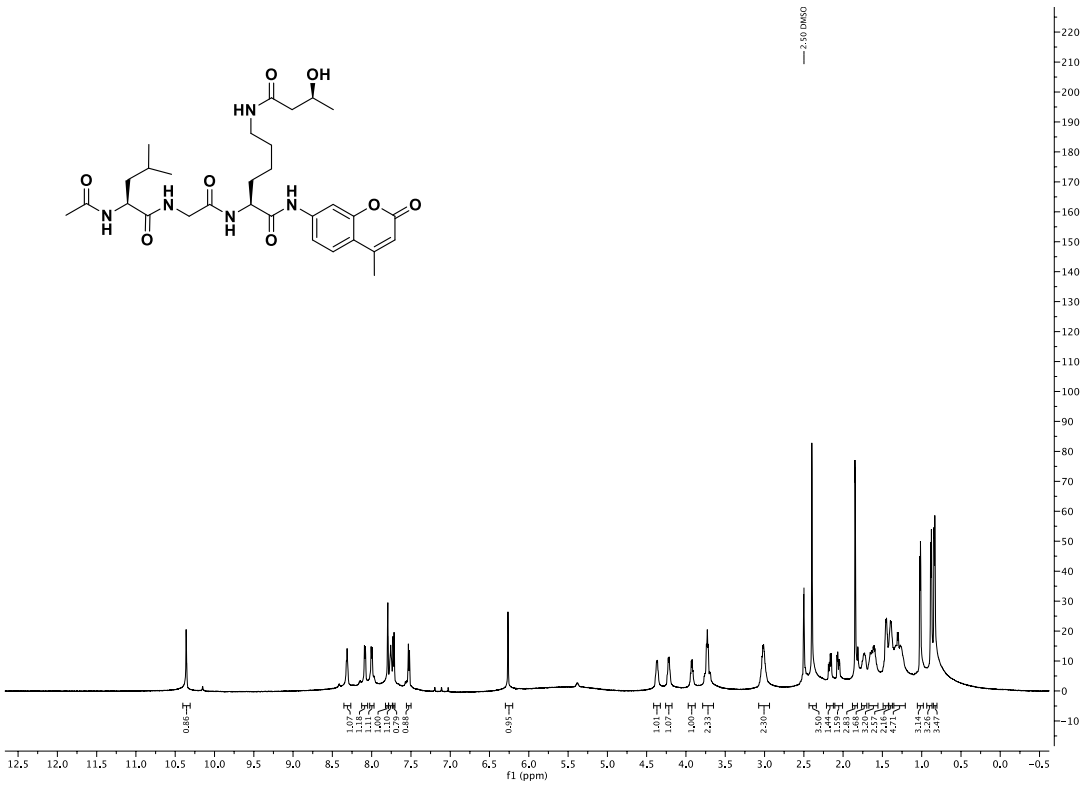
^{13}C NMR spectra (151 MHz, DMSO) of compound **2n**



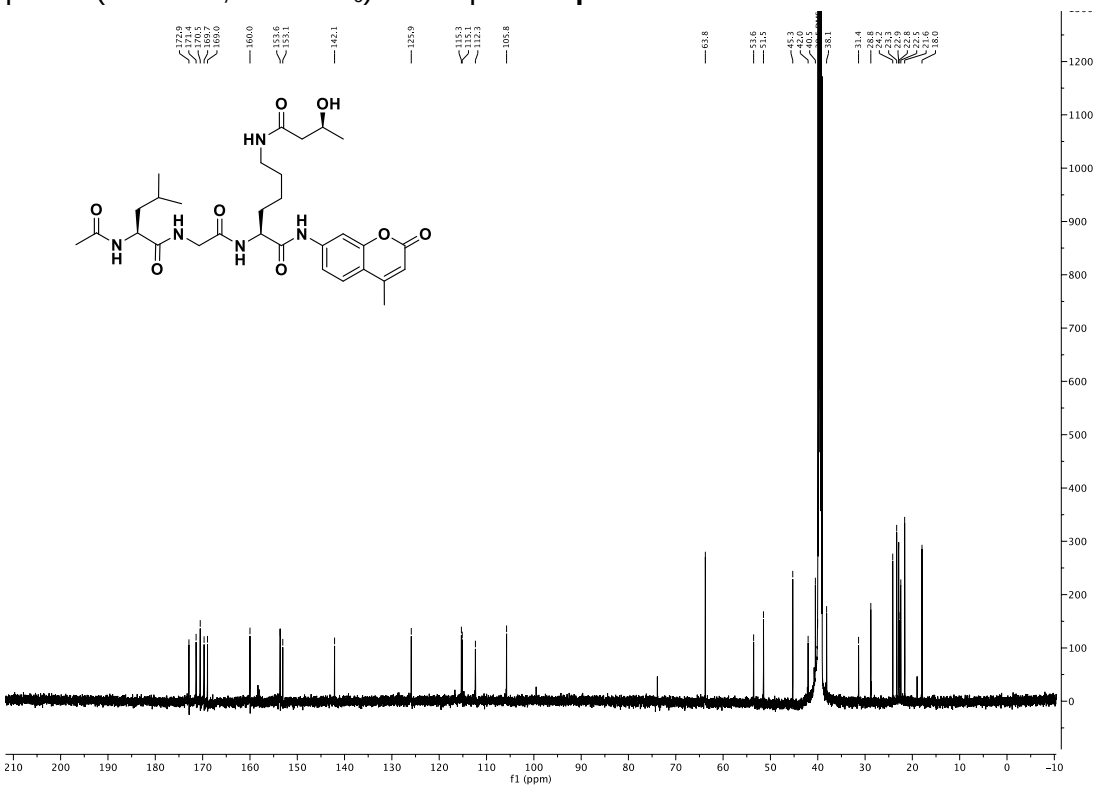
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **2o**



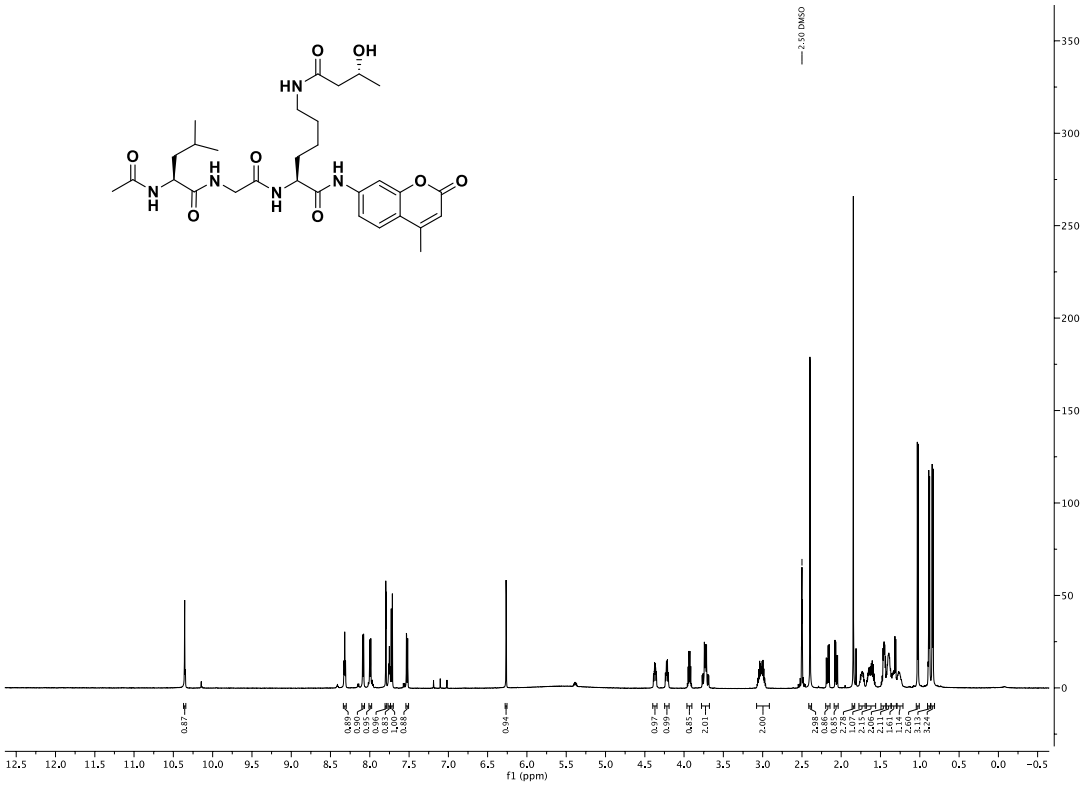
^{13}C NMR spectra (151 MHz, DMSO) of compound **2o**



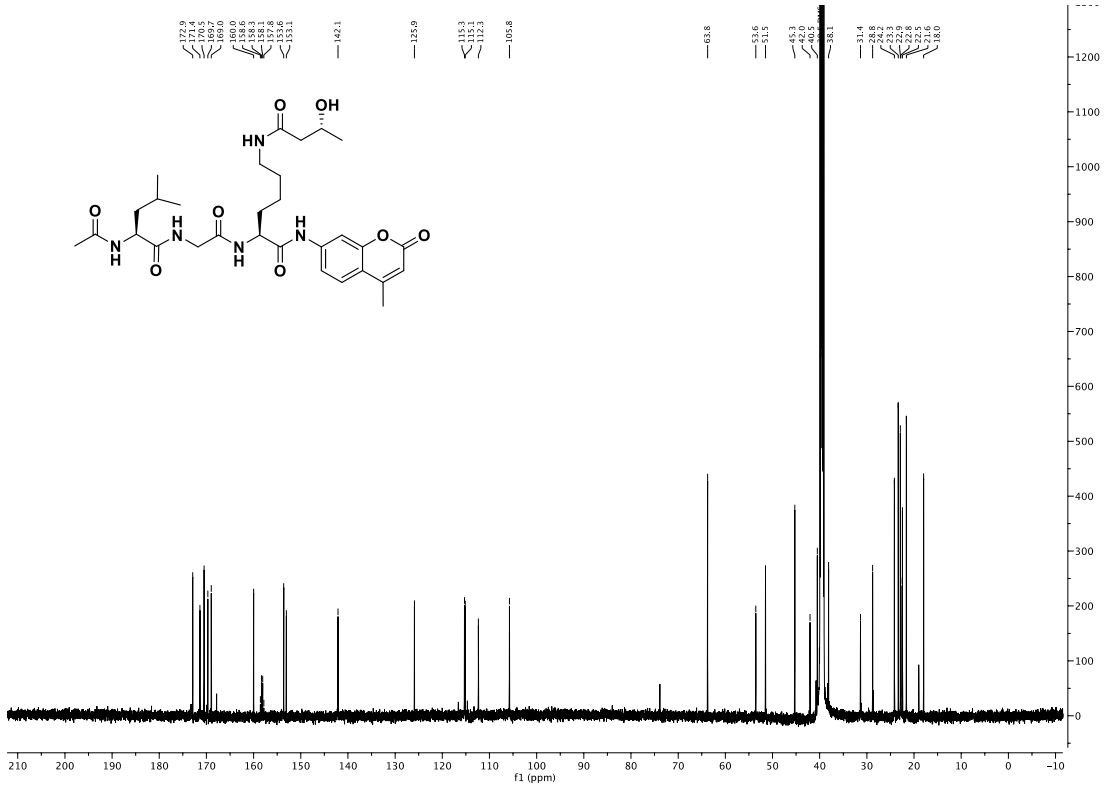
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **2p**



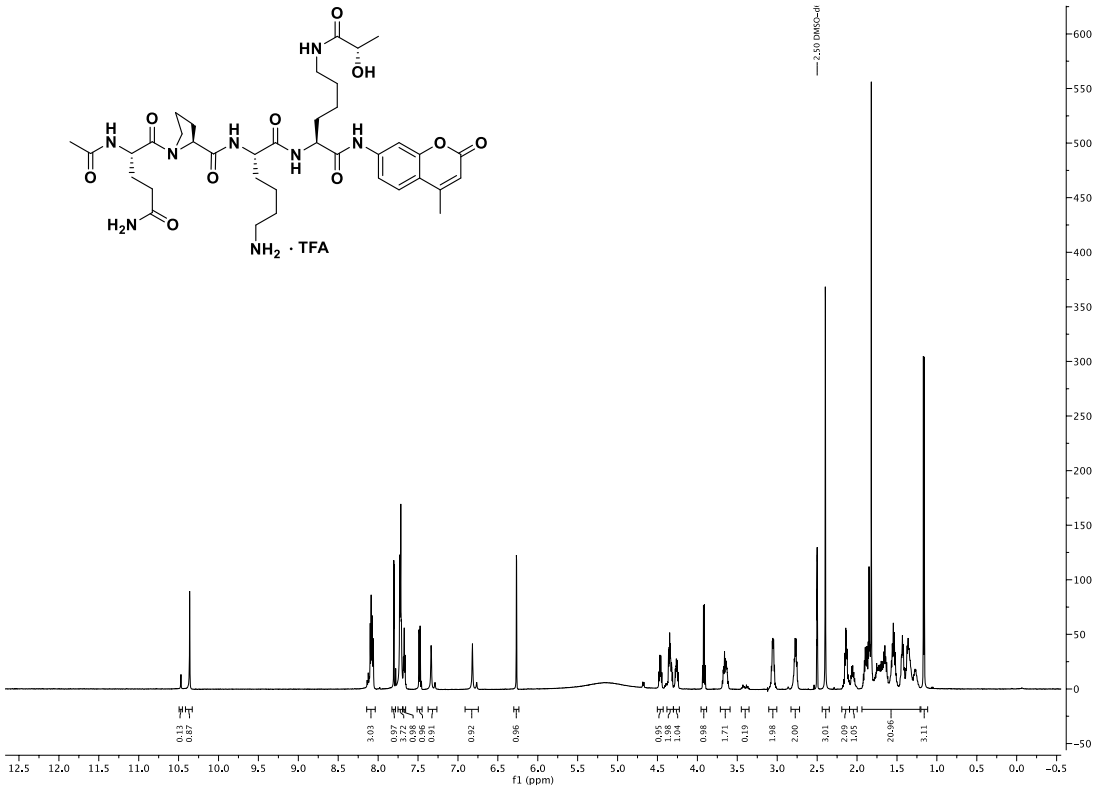
^{13}C NMR spectra (151 MHz, DMSO) of compound **2p**



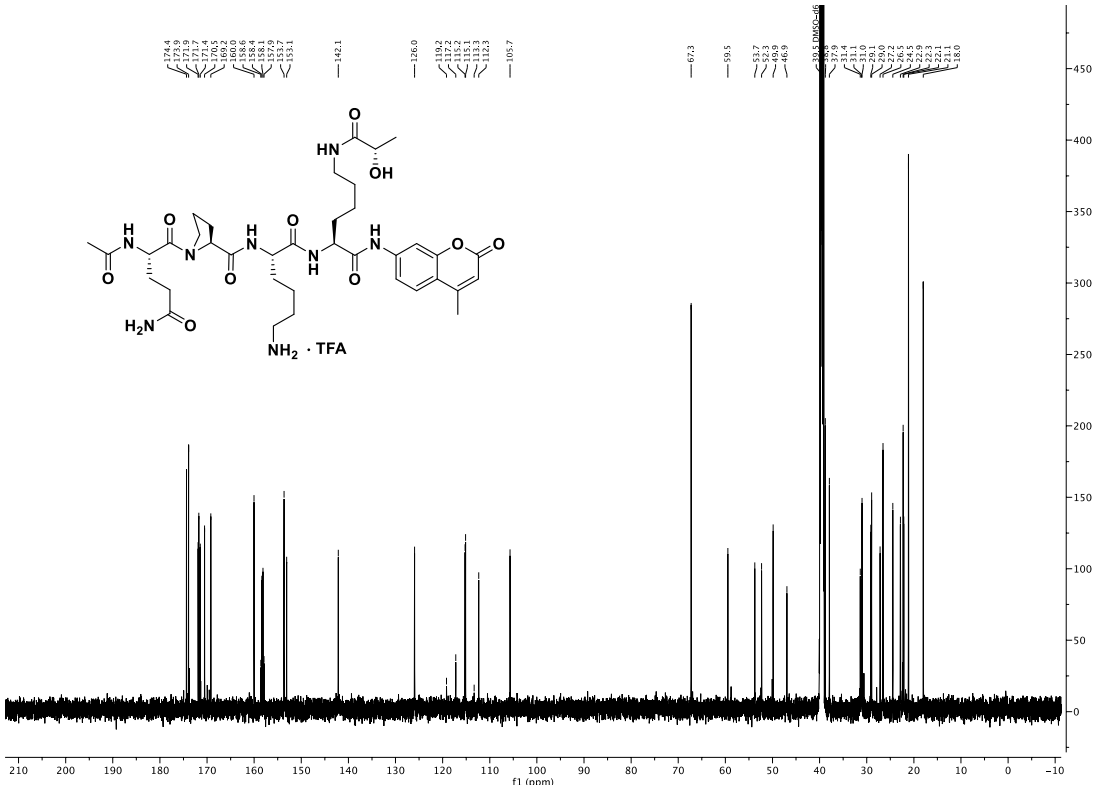
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **2q**



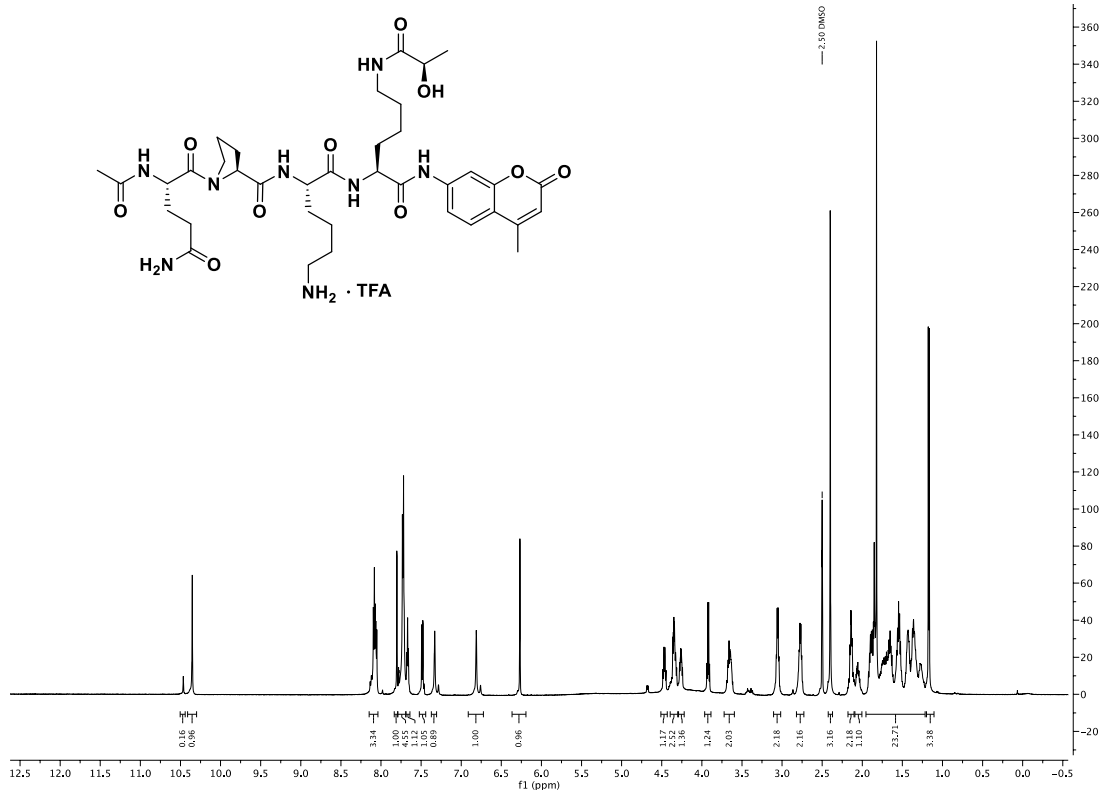
^{13}C NMR spectra (151 MHz, DMSO) of compound **2q**



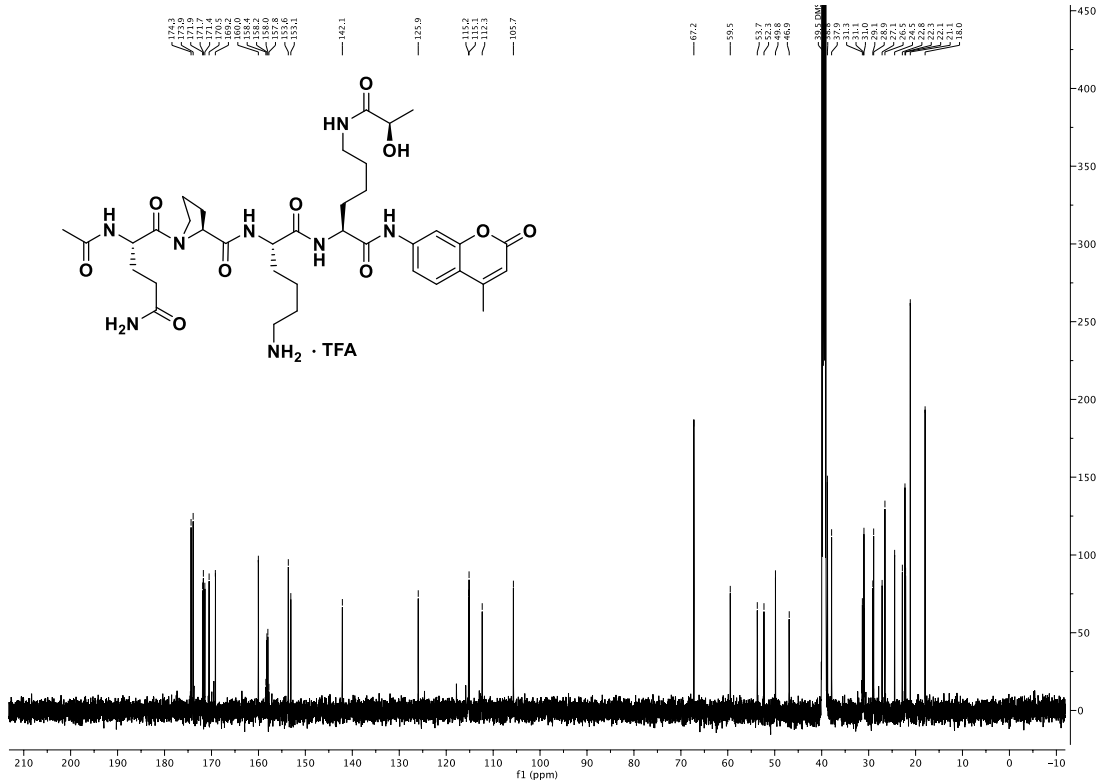
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **4b**



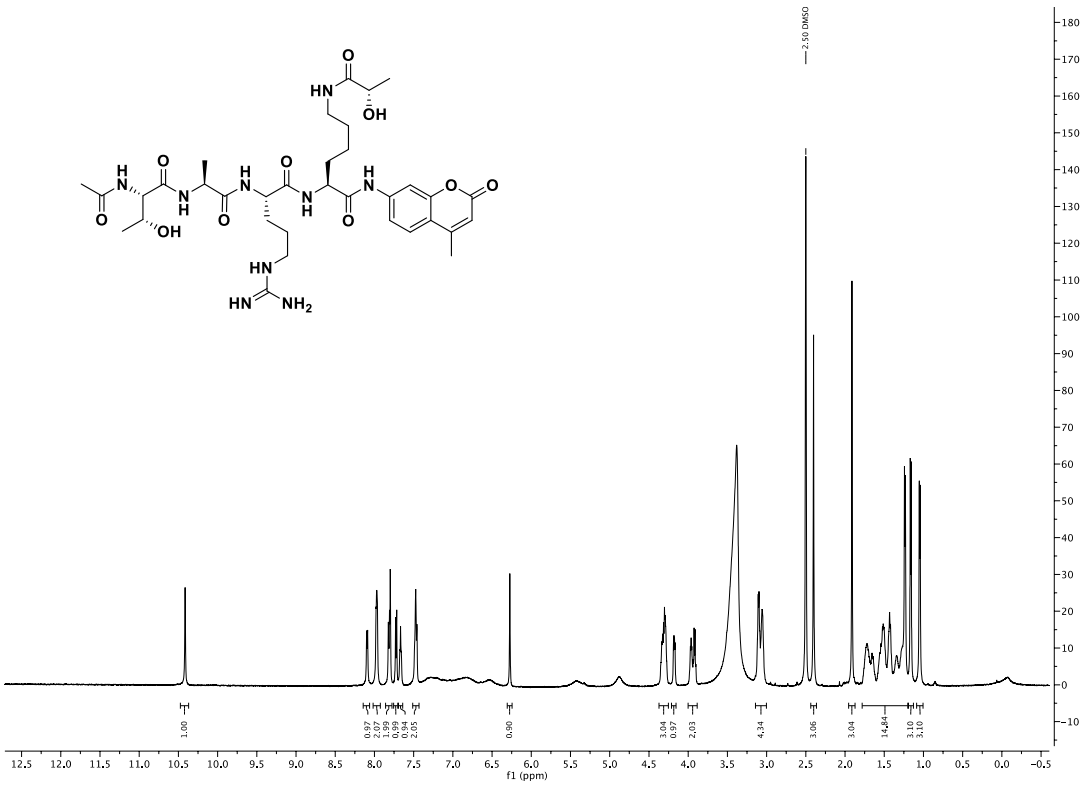
^{13}C NMR spectra (151 MHz, DMSO) of compound **4b**



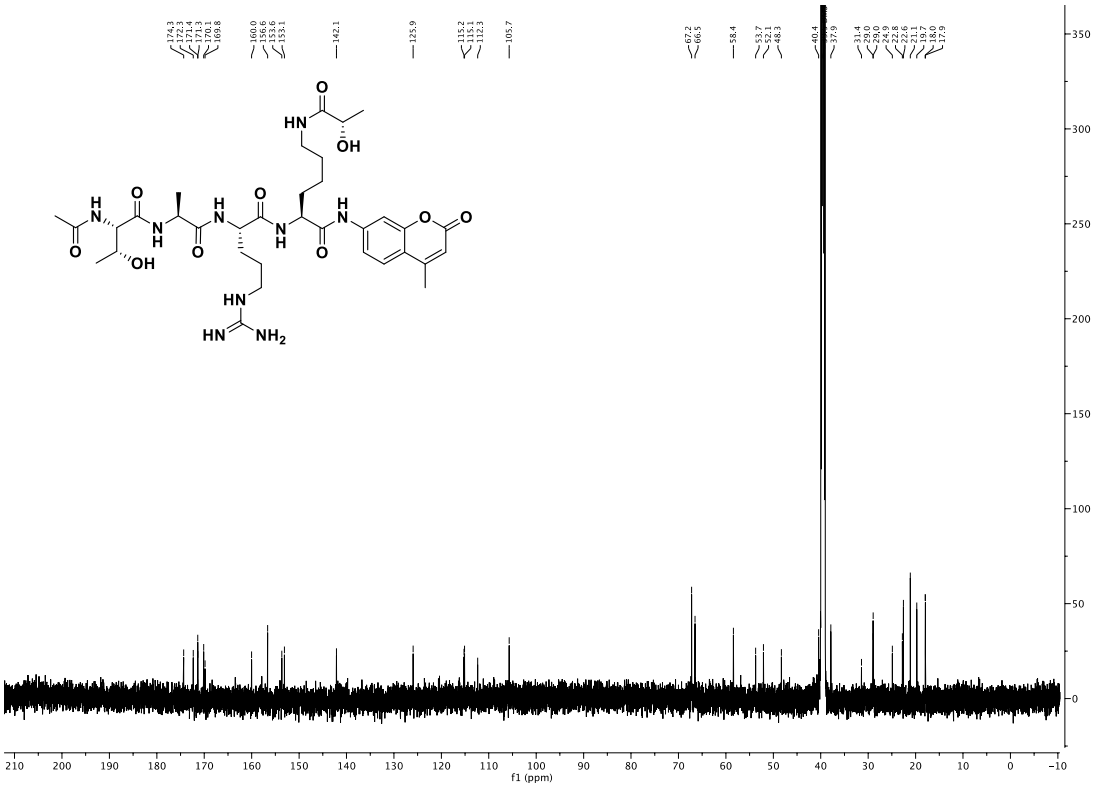
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **4c**



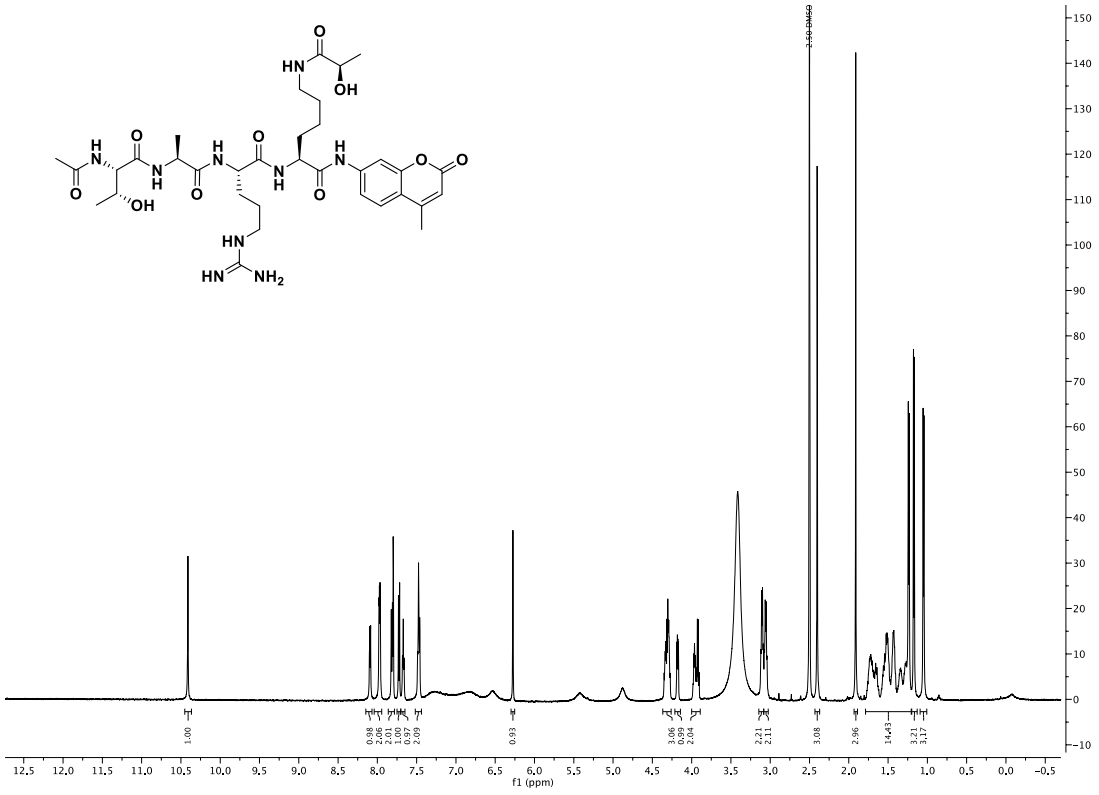
^{13}C NMR spectra (151 MHz, DMSO) of compound **4c**



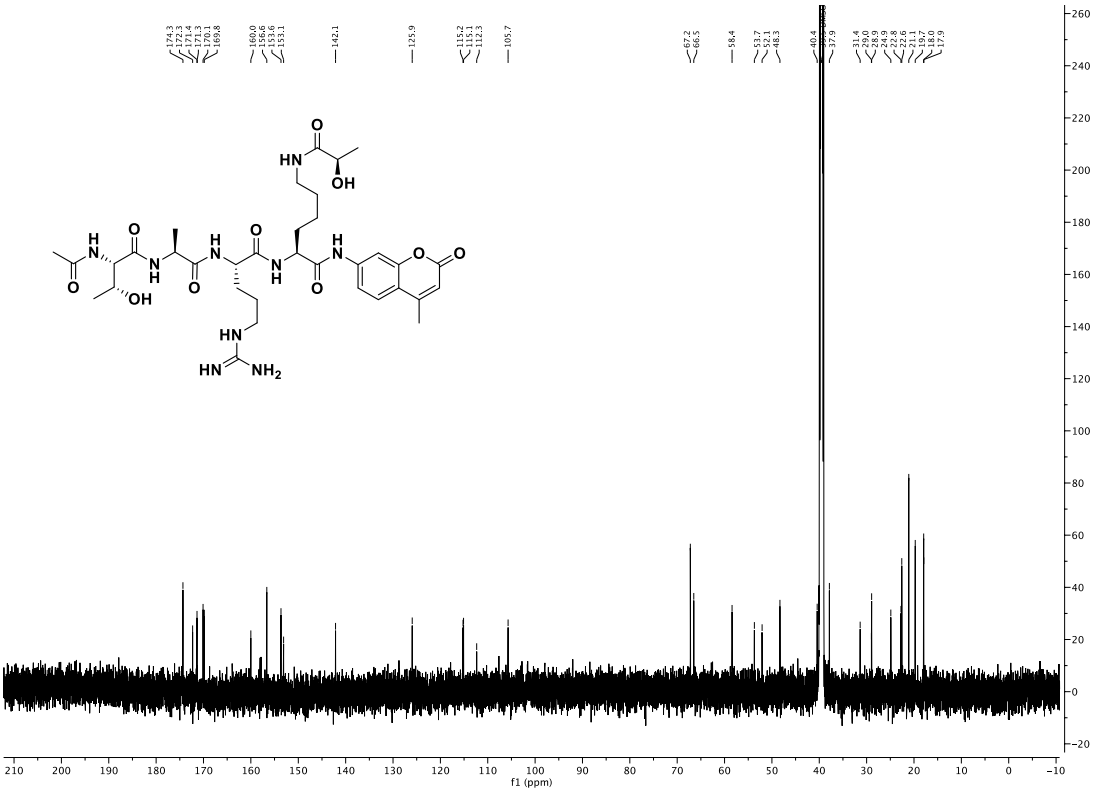
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **5b**



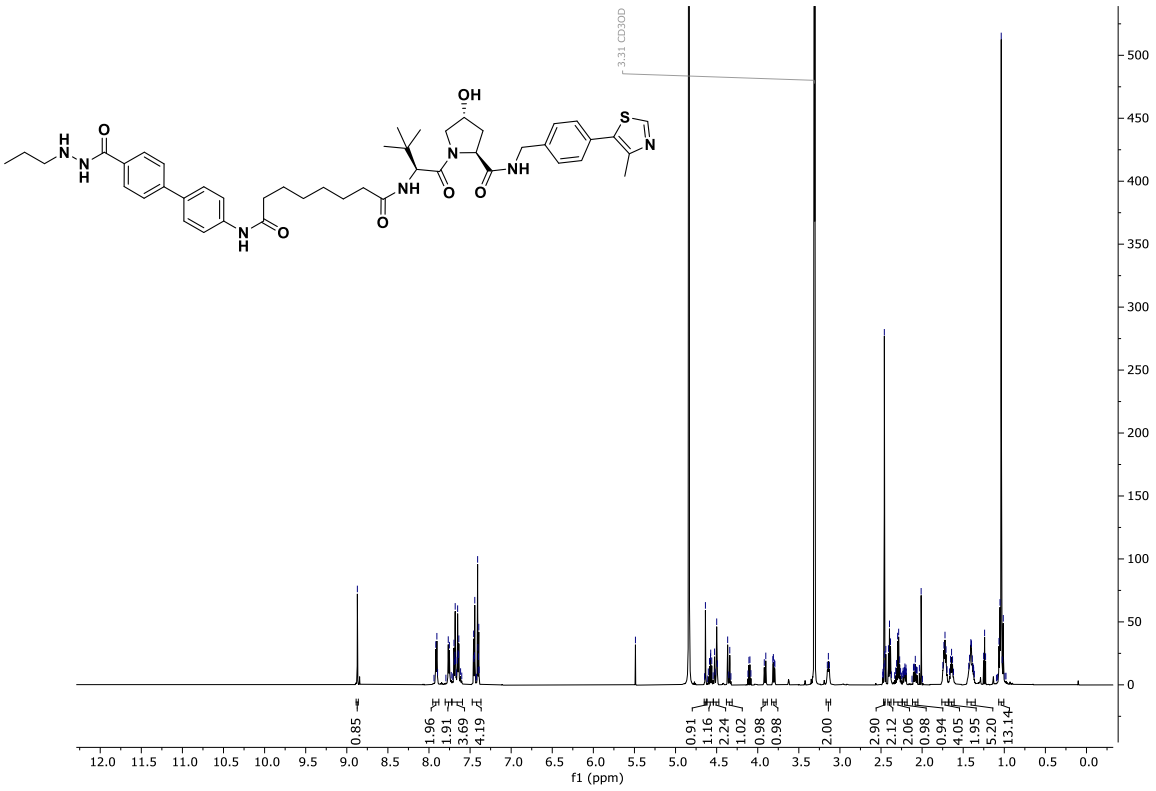
^{13}C NMR spectra (151 MHz, DMSO) of compound **5b**



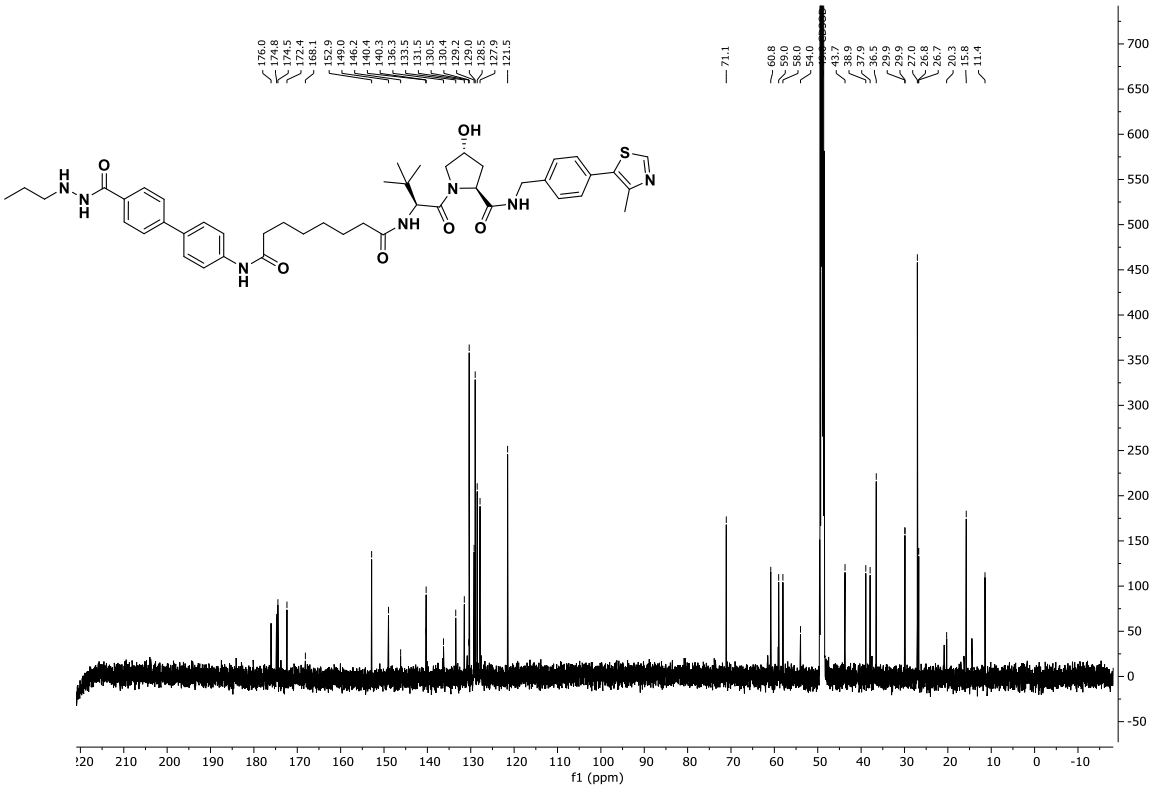
^{1}H NMR spectra (600 MHz, DMSO-d_6) of compound **5c**



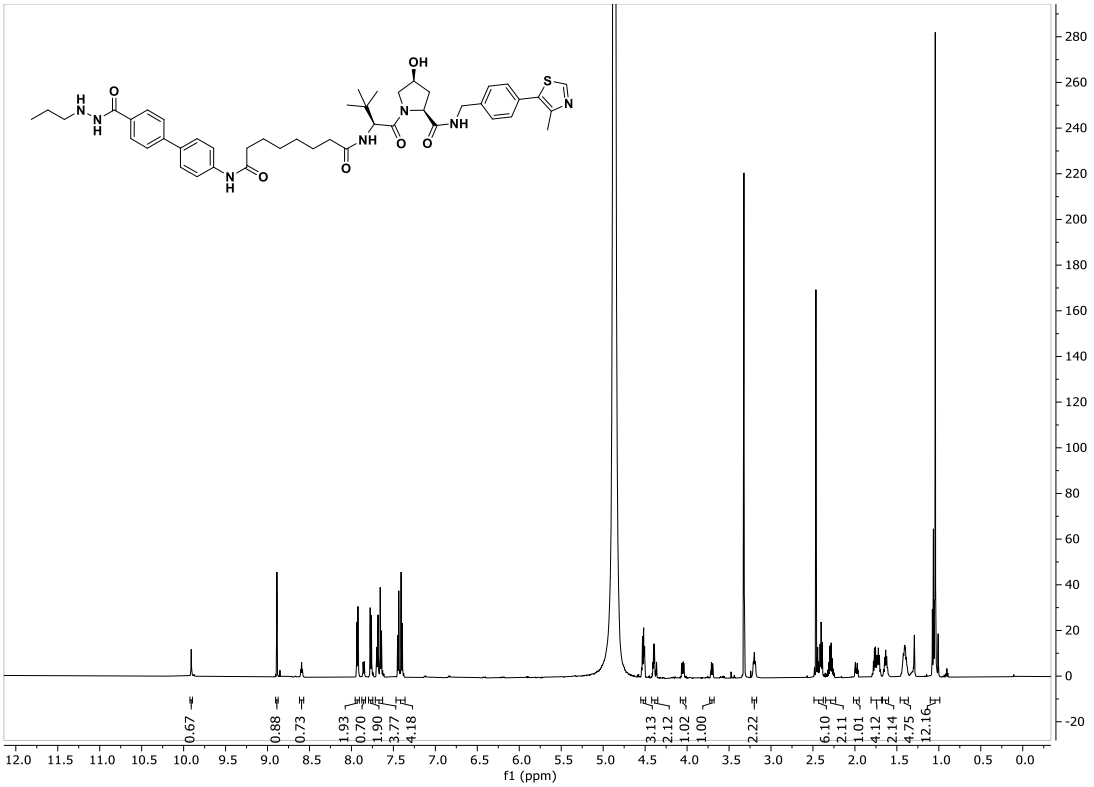
^{13}C NMR spectra (151 MHz, DMSO) of compound **5c**



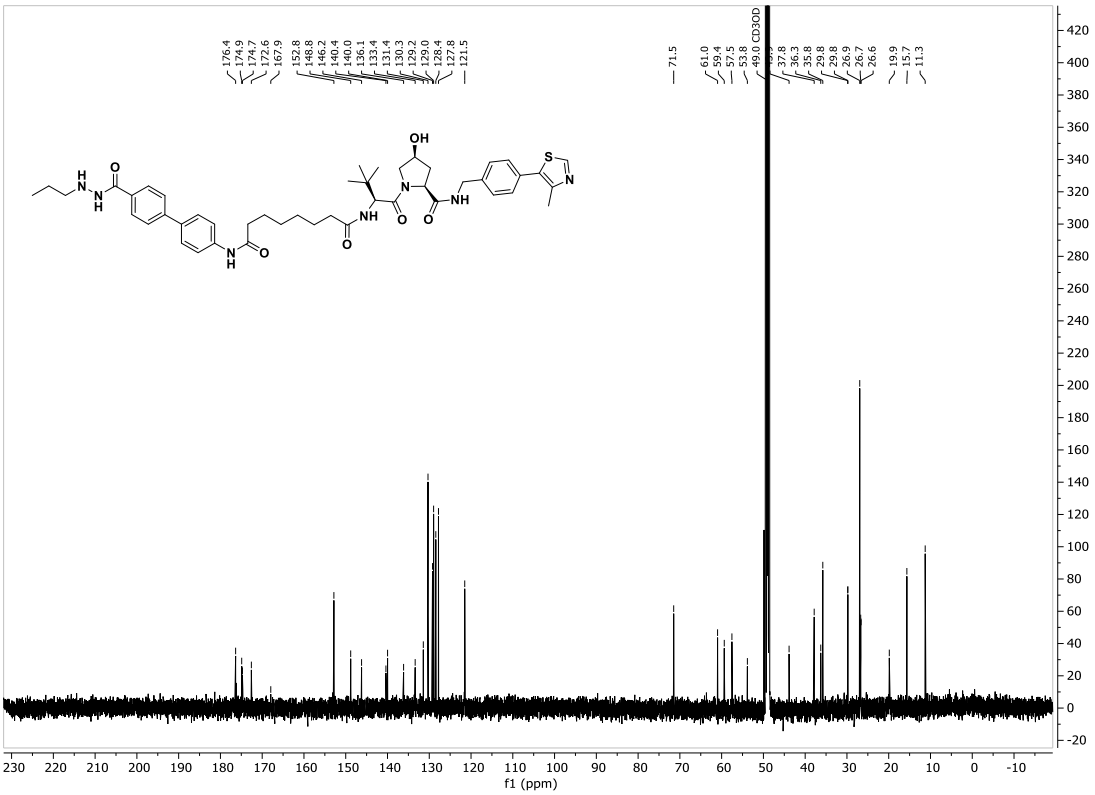
^{1}H NMR spectra (600 MHz, MeOD) of compound **DD-I22**



^{13}C NMR spectra (151 MHz, MeOD) of compound **DD-I22**

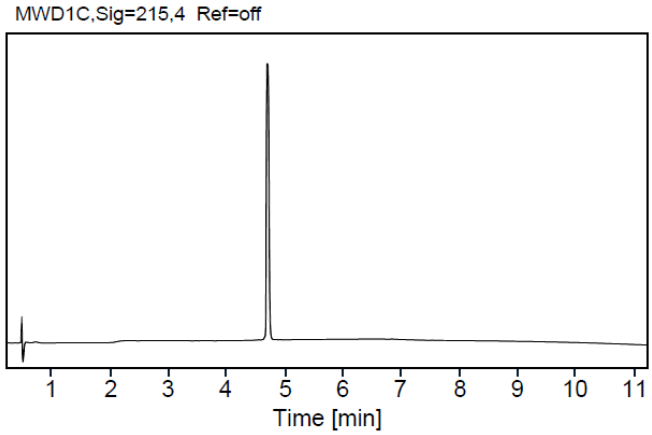


^{1}H NMR spectra (600 MHz, CD_3OD) of compound **DD-I23**

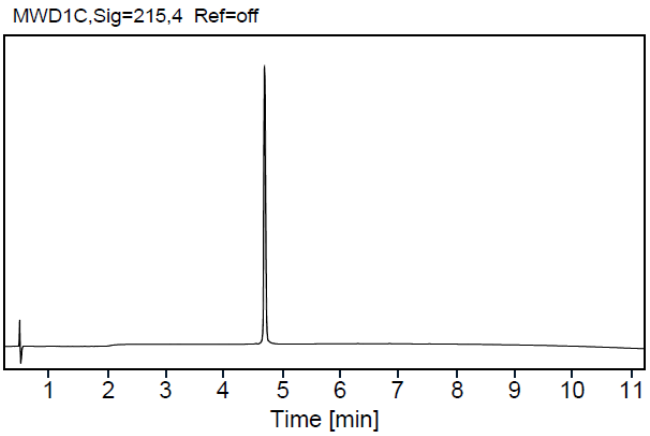


^{13}C NMR spectra (151 MHz, CD_3OD) of compound **DD-I23**

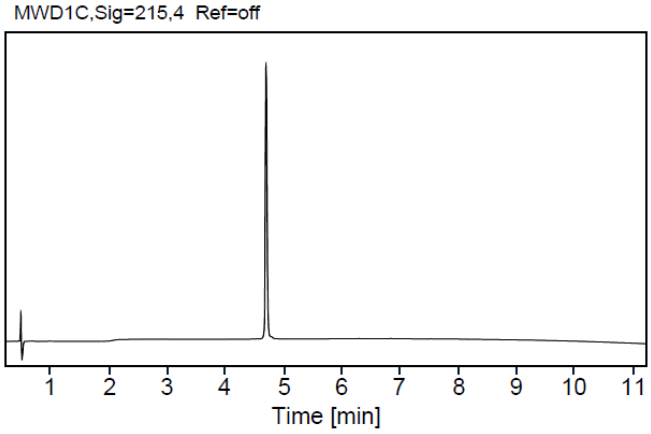
HPLC purity traces



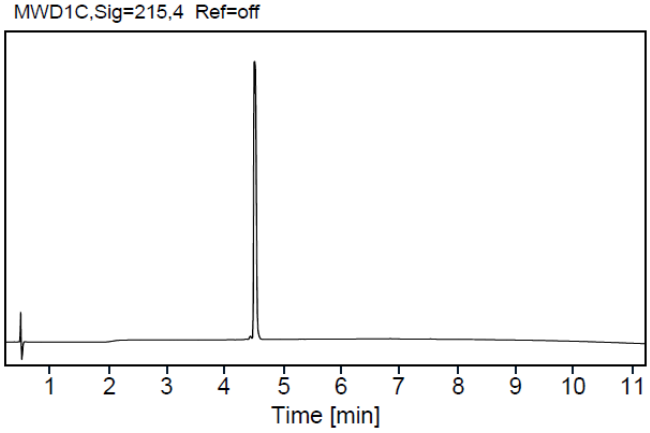
HPLC trace of peptide **5a**



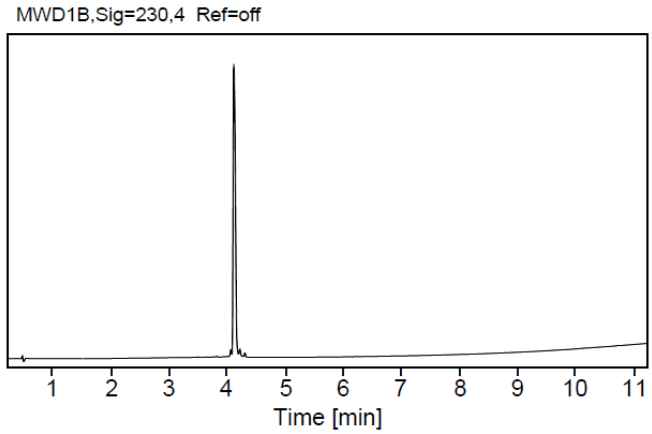
HPLC trace of peptide **5b**



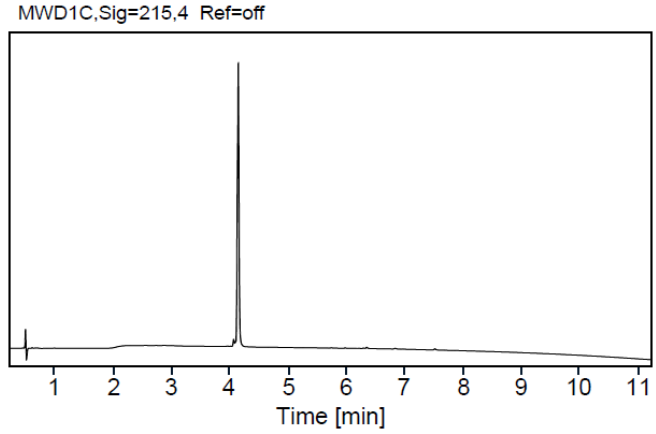
HPLC trace of peptide **5c**



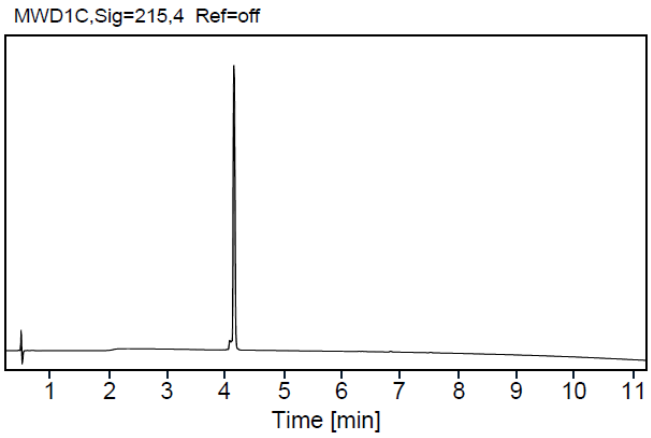
HPLC trace of peptide **5d**



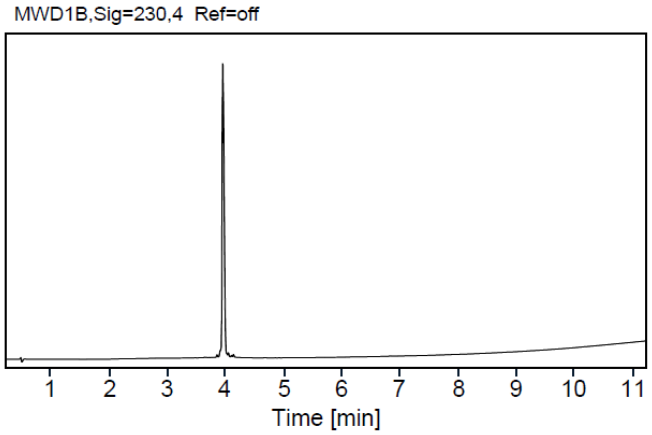
HPLC trace of peptide **6a**



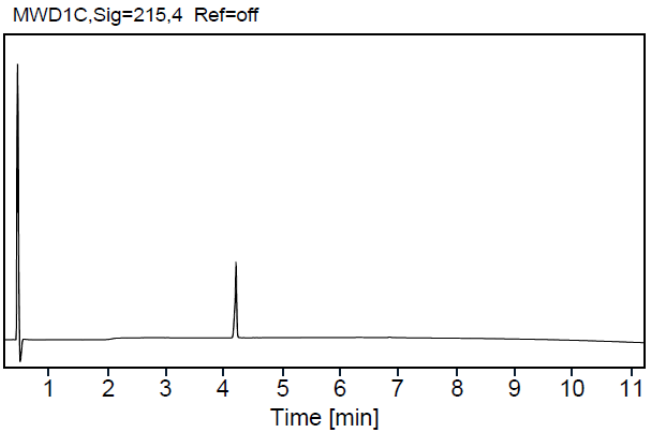
HPLC trace of peptide **6b**



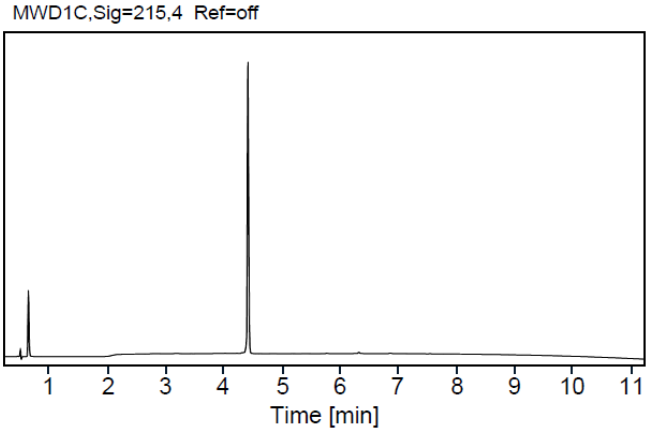
HPLC trace of peptide **6c**



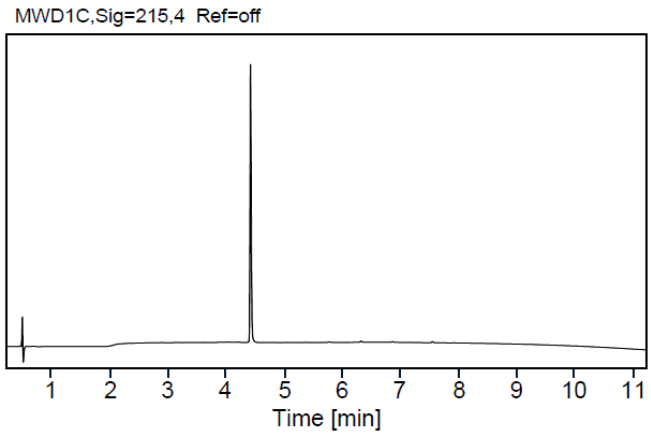
HPLC trace of peptide **6d**



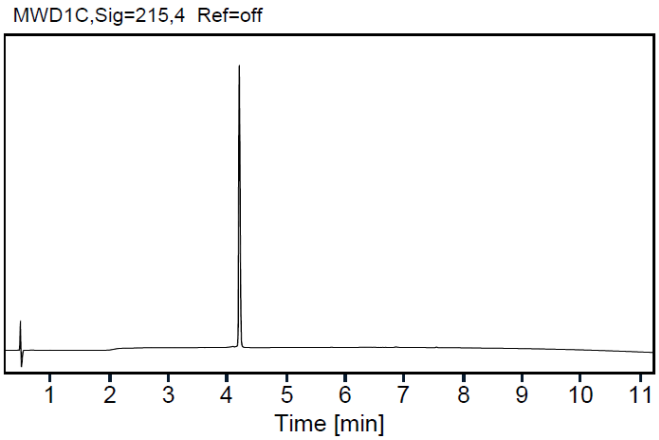
HPLC trace of peptide **7a**



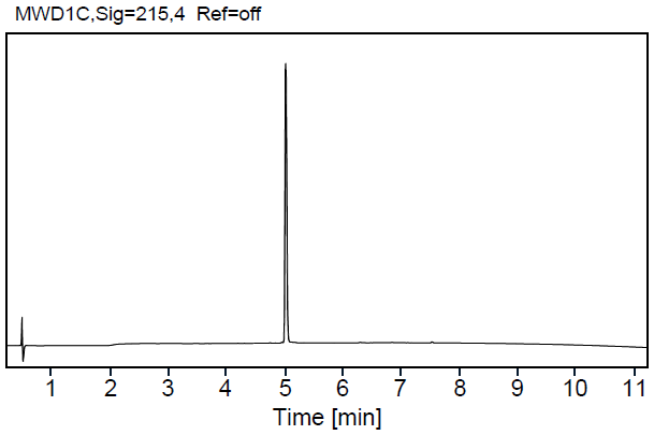
HPLC trace of peptide **7b**



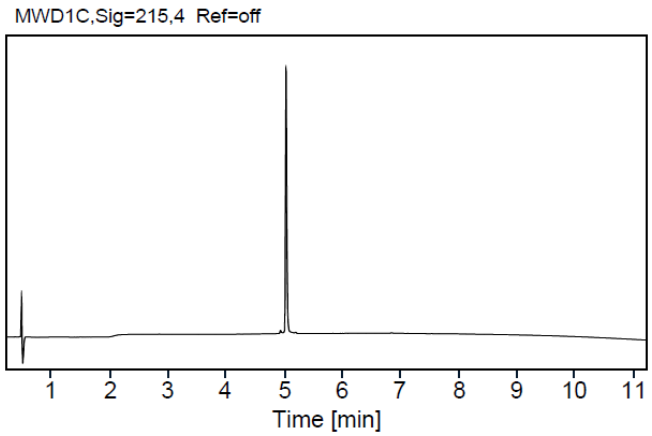
HPLC trace of peptide **7c**



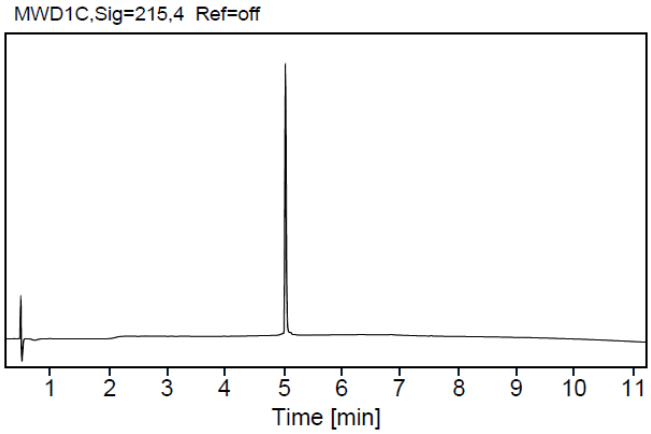
HPLC trace of peptide **7d**



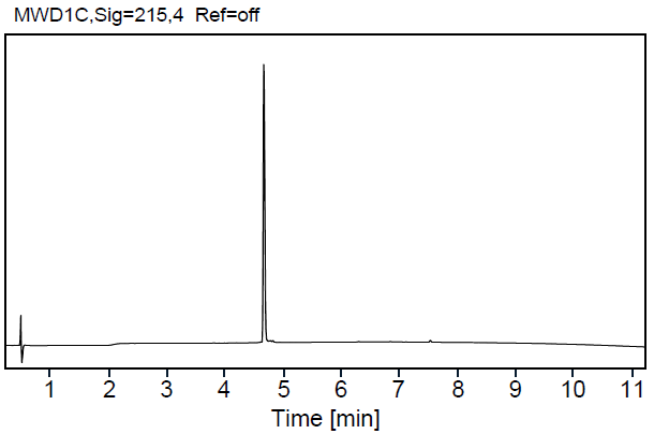
HPLC trace of peptide **8a**



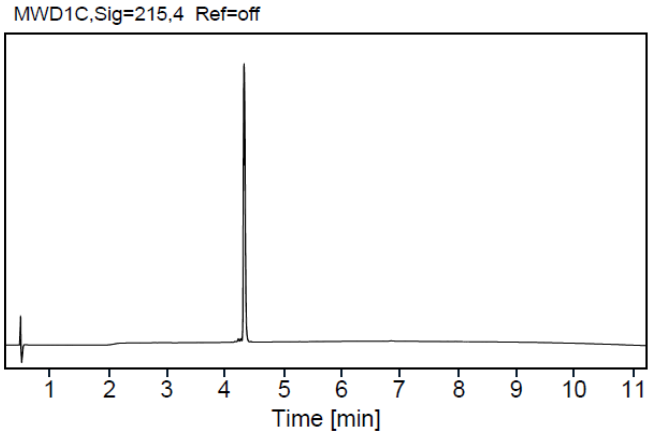
HPLC trace of peptide **8b**



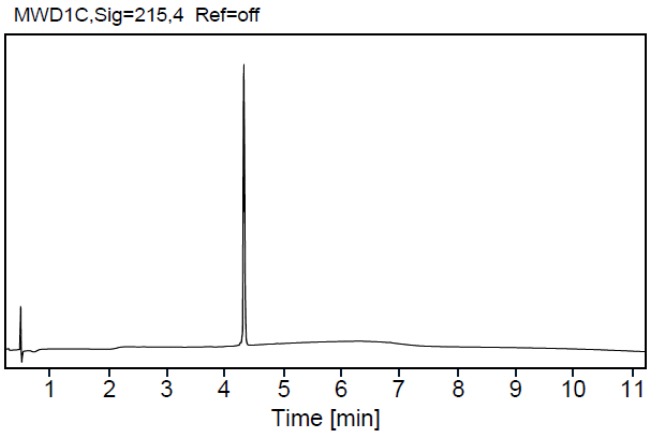
HPLC trace of peptide **8c**



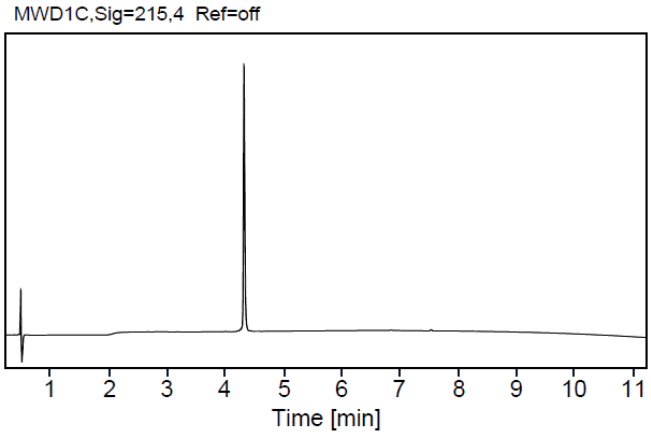
HPLC trace of peptide **8d**



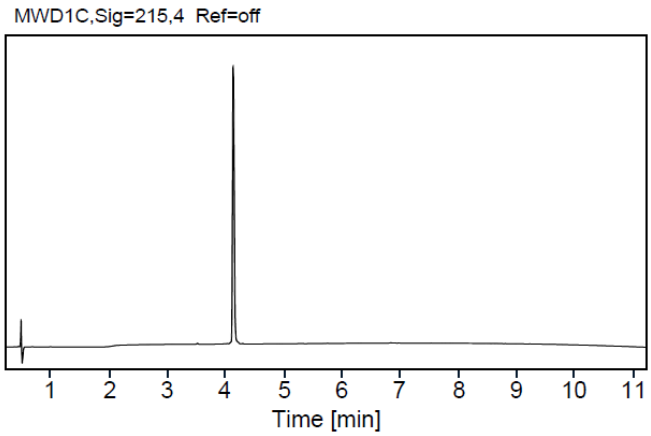
HPLC trace of peptide **9a**



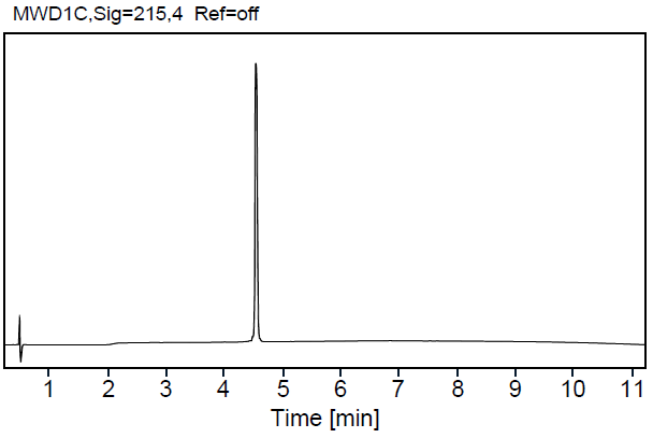
HPLC trace of peptide **9b**



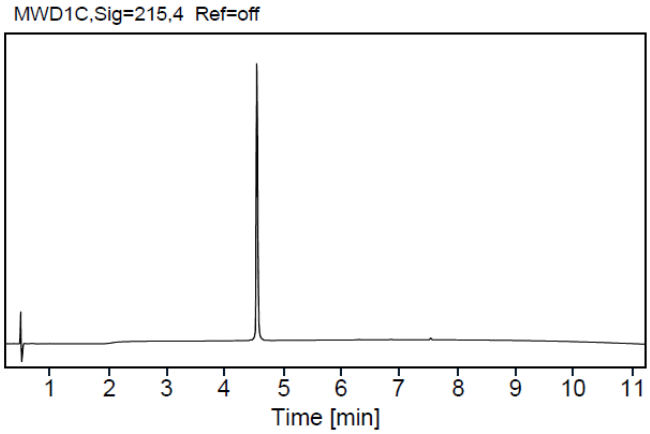
HPLC trace of peptide **9c**



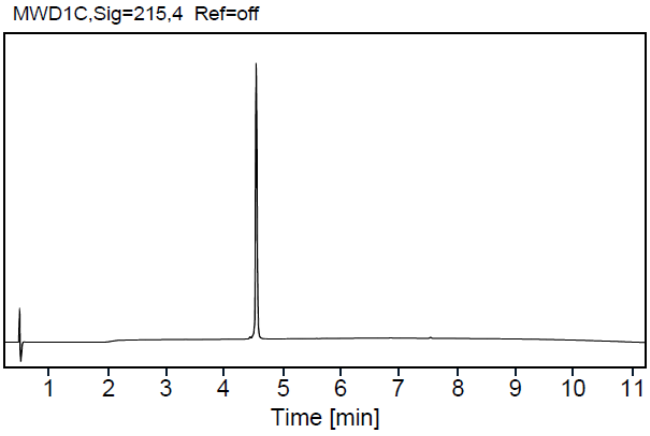
HPLC trace of peptide **9d**



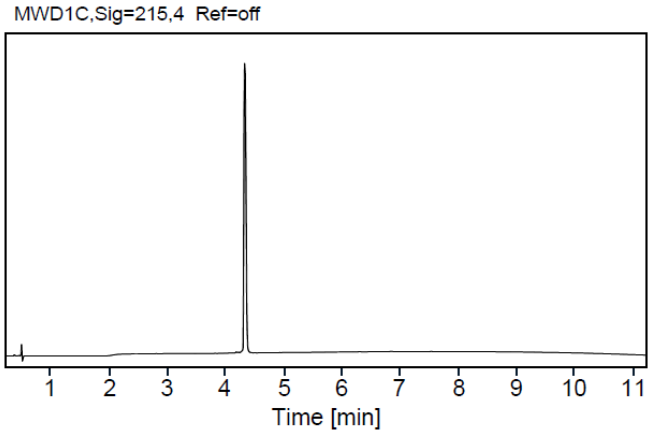
HPLC trace of peptide **10a**



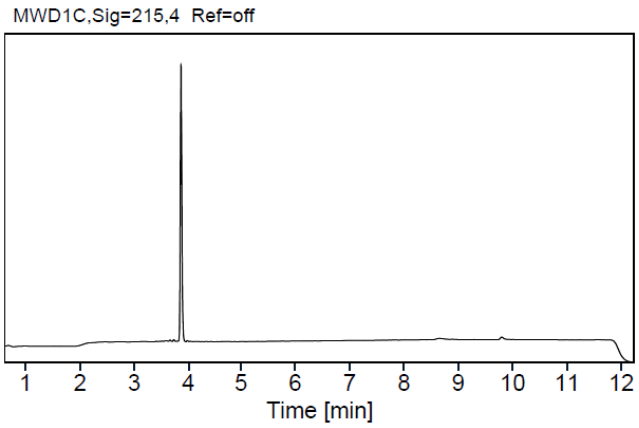
HPLC trace of peptide **10b**



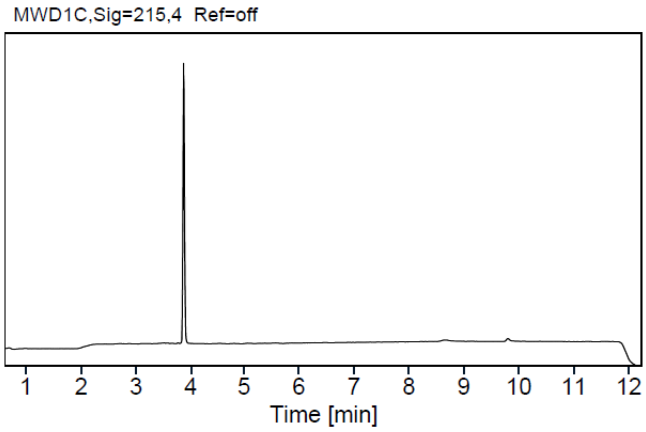
HPLC trace of peptide **10c**



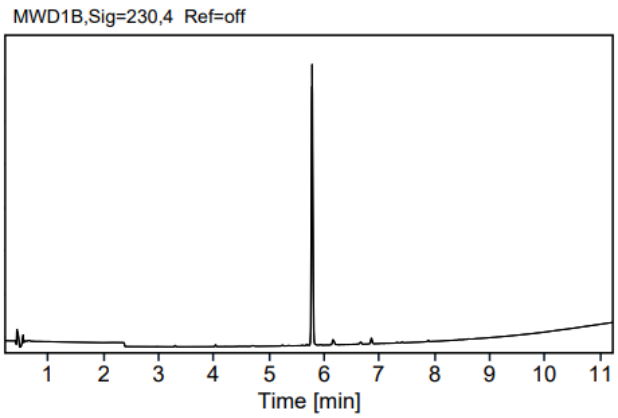
HPLC trace of peptide **10d**



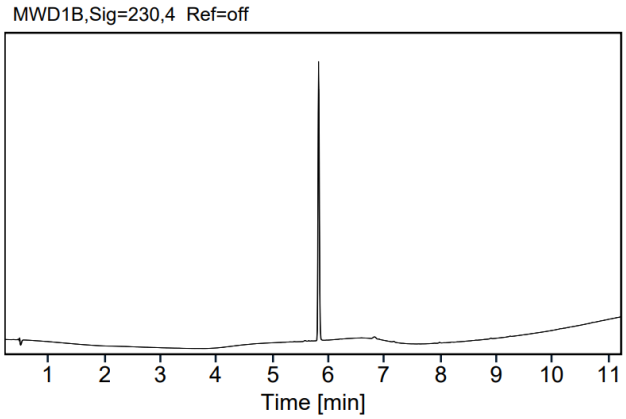
HPLC trace of peptide **H4K5(L-Ia)**



HPLC trace of peptide **H4K5(D-Ia)**



HPLC trace of compound **DD-I22**



HPLC trace of compound **DD-I23**