Internally Quenched Fluorescent Peptides Provide Insights into Underexplored and Reversible Post-Translational Modifications

Jordi C. J. Hintzen[a], Kamiel D. Beckley[a], Emily L. Goldberg[b] and George M. Burslem[a,c*]

[a] Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.

[b] Department of Physiology, University of California San Francisco, San Francisco, CA 94158, USA.

[c] Department of Cancer Biology and Epigenetics Institute, Perelman School of Medicine, University of Pennsylvania; Philadelphia, PA, 19104

* Corresponding author: george.burslem@pennmedicine.upenn.edu

Contents

Materials

Solvents were removed under reduced pressure using an IKA rotary evaporator equipped with an IKA MVP10 basic vacuum pump. Dry solvents and reagents were purchased from commercial suppliers and used without further purification. Thinlayer chromatography was performed on Merck silica gel 60F254 plates. Flash column chromatography was conducted using a Teledyne ISCO CombiFlash NextGen 300+ purification system using 4g, 12g, or 24g RediSep Rf silica cartridges. Thermo Scientific silica gel (35-70 µm) was used for manual chromatography columns. Analytical LC-MS was performed using a system comprised of an Agilent 1260 Infinity II HPLC instrument equipped with an Agilent InfinityLab LS/MSD XT MS detector with electrospray ionisation. The system ran with a positive and negative switching mode and UV diode array detector using an Agilent ZORBAX SB-C18 RRHT (50 mm × 4.6 mm × 1.8 µm) column and gradient elution with two binary solvent systems: MeCN/H_2O or MeCN/H_2O plus 0.1% formic acid. Preparative HPLC was performed using a system comprising an Agilent 1260 Infinity II HPLC system equipped with an Agilent 1290 Prep Bin Pump, an Agilent Prep-C18 column (250 mm × 21.2 mm × 10 µm), and an Agilent prep autosampler and fraction collector. The system ran using a UV diode array detector and purification was performed using a gradient elution using a MeCN/H_2O binary solvent system. NMR analysis was conducted using a Bruker NEO400 spectrometer equipped with a 5mm BBFO IProbe (1H = 400 MHz and 13C = 100 MHz). Chemical shifts are quoted in parts per million (ppm) and coupling constants are given in Hz. Splitting patterns have been abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), td (triplet of doublets), t (triplet), q (quartet) and m (multiplet). 4-20% gradient SDS-PAGE gels were run at 180 V for 45 minutes and stained with Coomassie Blue dye unless used for Western blot. Plate reader assays were measured using a Varioskan Lux Microplate reader.

Table S1: Peptide data table, charge state for observed mass indicated in brackets

Peptide	Sequence	Formula	Observed Mass $[M+nH]^{n+}$	Δppm	Retention Time (min)
H3K9	AbzTARKSTGK(Dnp)	$C_{47}H_{74}N_{17}O_{16}$	1132.5504	0.490	8.65
H3K14	AbzSTGGKAPK(Dnp)	$C_{44}H_{65}N_{14}O_{15}$	1029.4731	-1.71	7.28
H3R14	AbzSTGGRAPK(Dnp)	$C_{44}H_{65}N_{16}O_{15}$	1058.4824	-1.21	8.32
H3R8	AbzQTARKSTK(Dnp)	$C_{50}H_{79}N_{18}O_{17}$	1203.5856	-0.78	6.58
H3Kc9	AbzTARKcSTGK(Dnp)	$C_{46}H_{72}N_{17}O_{16}S$	575.2143 (+2)	4.66	7.86
H3Kc14	AbzSTGGKcAPK(Dnp)	$C_{43}H_{63}N_{14}O_{15}S$	1147.4307	-0.57	7.47
H3Rc8	AbzQTARcKSTK(Dnp)	$C_{49}H_{75}N_{17}O_{17}S$	603.2785 (+2)	-3.04	8.28
H3K9ac	AbzTARKacSTGK(Dnp)	$C_{49}H_{76}N_{17}O_{17}$	1174.5566	-2.90	8.97
H3K9lac	AbzTARKlacSTGK(Dnp)	$C_{50}H_{78}N_{17}O_{18}$	1204.5727	-1.83	10.07
H3K9bhb	AbzTARKbhbSTGK(Dnp)	$C_{51}H_{80}N_{17}O_{18}$	1218.5862	0.01	10.27
H3Kc9ac	AbzTARKcacSTGK(Dnp)	$C_{48}H_{73}N_{17}O_{17}S$	597.3454 (+2)	-3.65	7.21
H3Kc9lac	AbzTARKclacSTGK(Dnp)	$C_{49}H_{75}N_{17}O_{18}S$	611.2765 (+2)	1.05	8.34
H3Kc9bhb	AbzTARKcbhbSTGK(Dnp)	$C_{50}H_{77}N_{17}O_{18}S$	618.1852 (+2)	2.77	10.15
H3K14ac	AbzSTGGKacAPK(Dnp)	$C_{46}H_{67}N_{14}O_{16}$	1071.4870	1.51	7.62
H3K14lac	AbzSTGGKlacAPK(Dnp)	$C_{47}H_{69}N_{14}O_{17}$	1101.4979	1.71	8.44
H3K14bhb	AbzSTGGKbhbAPK(Dnp)	$C_{48}H_{71}N_{14}O_{17}$	578.4339 (+2, +Na)	0.21	7.84
H3Kc14ac	AbzSTGGKcacAPK(Dnp)	$C_{45}H_{65}N_{14}O_{16}S$	1098.4467	-0.03	9.80
H3Kc14lac	AbzSTGGKclacAPK(Dnp)	$C_{46}H_{67}N_{14}O_{17}S$	558.2581 (+2)	1.40	5.80
H3Kc14bhb	AbzSTGGKcbhbAPK(Dnp)	$C_{47}H_{69}N_{14}O_{17}S$	567.2613 (+2)	2.68	5.78
H3K9me1	AbzTARKme1STGK(Dnp)	$C_{48}H_{76}N_{17}O_{16}$	1146.5666	1.32	7.47
H3K9me2	AbzTARKme2STGK(Dnp)	$C_{49}H_{78}N_{17}O_{16}$	1160.5822	1.30	7.27
H3K9me3	AbzTARKme3STGK(Dnp)	$C_{50}H_{81}N_{17}O_{16}$	1174.5978	1.23	7.21
H3Kc9me1	AbzTARKcme1STGK(Dnp)	$C_{47}H_{74}N_{17}O_{16}S$	1164.5218	0.26	7.31
H3Kc9me2	AbzTARKcme2STGK(Dnp)	$C_{48}H_{76}N_{17}O_{16}S$	1178.5387	1.34	7.51
H3Kc9me3	AbzTARKcme3STGK(Dnp)	$C_{49}H_{79}N_{17}O_{16}S$	1192.5535	0.60	7.41
H3K9meac	AbzTARKmeacSTGK(Dnp)	$C_{50}H_{78}N_{17}O_{17}$	1188.5793	3.08	10.02
H3Kc9meac	AbzTARKcmeacSTGK(Dnp)	$C_{49}H_{76}N_{17}O_{17}S$	1220.5374 (+Na)	1.67	8.92
H3K14meac	AbzSTGGKmeacAPK(Dnp)	$C_{47}H_{69}N_{14}O_{16}$	1085.5045	3.14	10.21
H3Kc14meac	AbzSTGGKcmeacAPK(Dnp)	$C_{46}H_{67}N_{14}O_{16}S$	557.2329 (+2)	0.21	9.31
H3K9S10ph	AbzTARKSphTGK(Dnp)	$C_{47}H_{74}N_{17}O_{19}P$	1212.5179	1.79	7.32
H3K9me2S10ph	AbzTARKme2SphTGK(Dnp)	$C_{49}H_{78}N_{17}O_{19}P$	1240.5489	1.47	7.29
H3K9me3S10ph	AbzTARKme3SphTGK(Dnp)	$C_{50}H_{81}N_{17}O_{19}P$	1254.5699	1.74	7.31
H3K9acS10ph	AbzTARKacSphTGK(Dnp)	$C_{49}H_{76}N_{17}O_{20}P$	1254.5294	2.46	8.03
αTK40	AbzPSDKTIGK(Dnp)	$C_{49}H_{72}N_{14}O_{17}$	1129.5300	-0.27	8.07
αTK40ac	AbzPSDKacTIGK(Dnp)	$C_{51}H_{74}N_{14}O_{18}$	1193.5220	-1.51	8.18
αTKc40ac	AbzPSDKcacTIGK(Dnp)	$C_{50}H_{72}N_{14}O_{18}S$	595.2479 (+2)	1.49	6.90

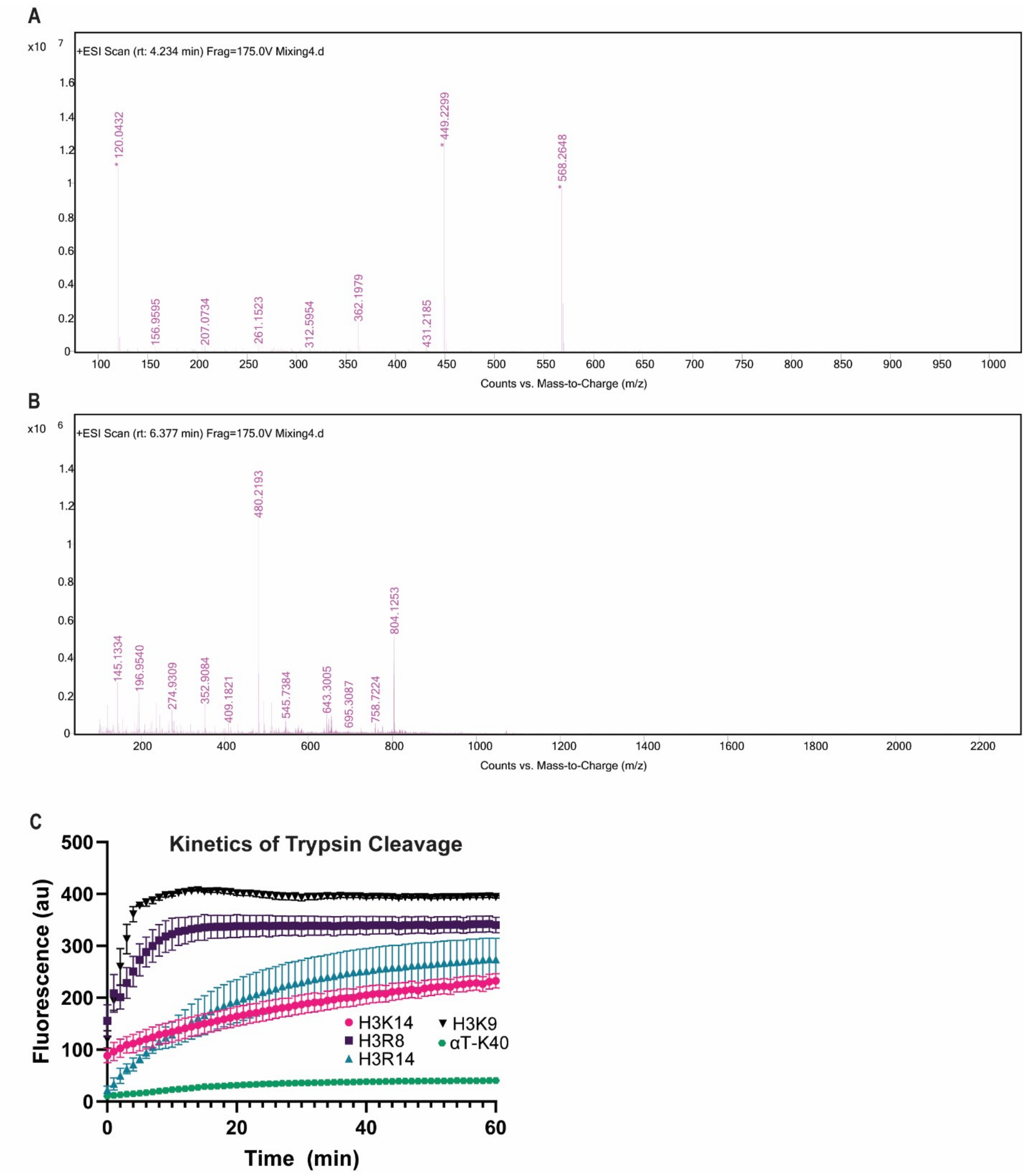


Fig. S1: System Validation. A) MS spectrum of the H3K14 Abz-containing fragment (exact mass 568.27), B) MS spectrum of the H3K14 Dnp-containing fragment (exact mass 480.21), C) Time-course of trypsin-catalyzed cleavage reactions of lysine and arginine peptides

A

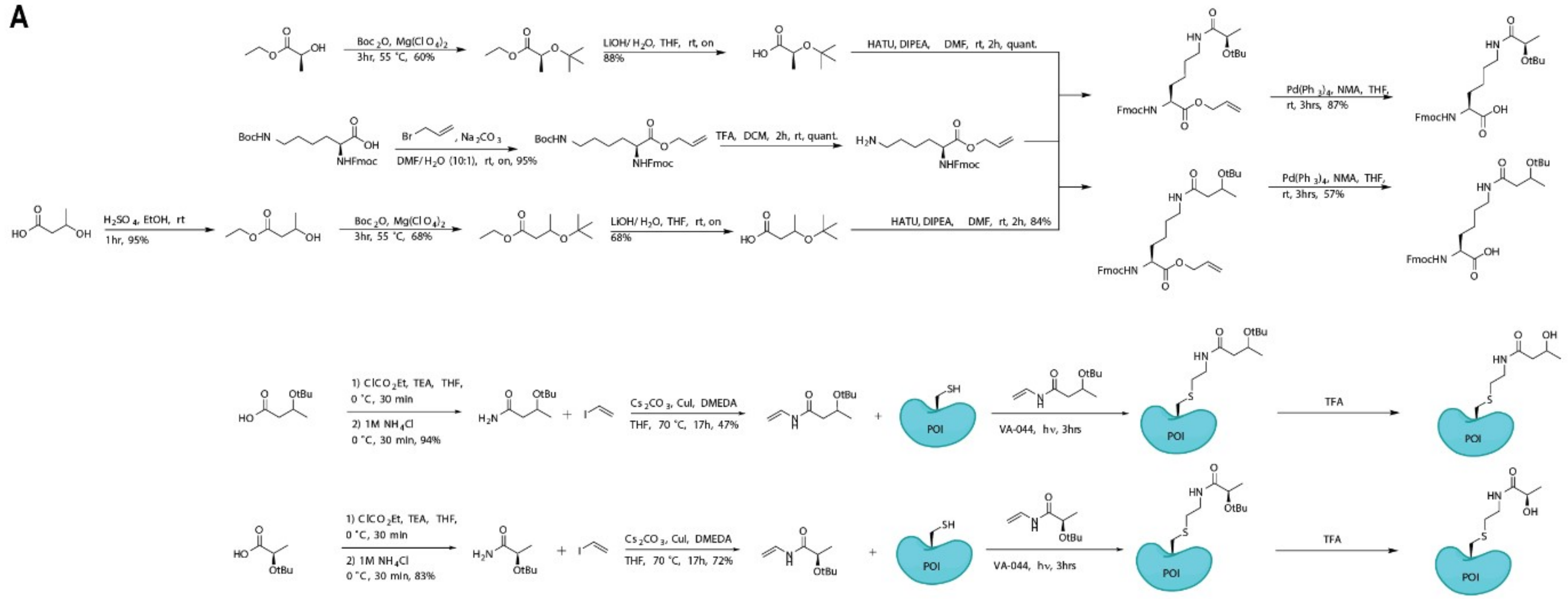


Fig. S2: Synthetic routes to obtain Fmoc-protected building blocks for Klac and Kbhb (top) and to install thialysine-derived mimics of Klac and Kbhb (bottom).

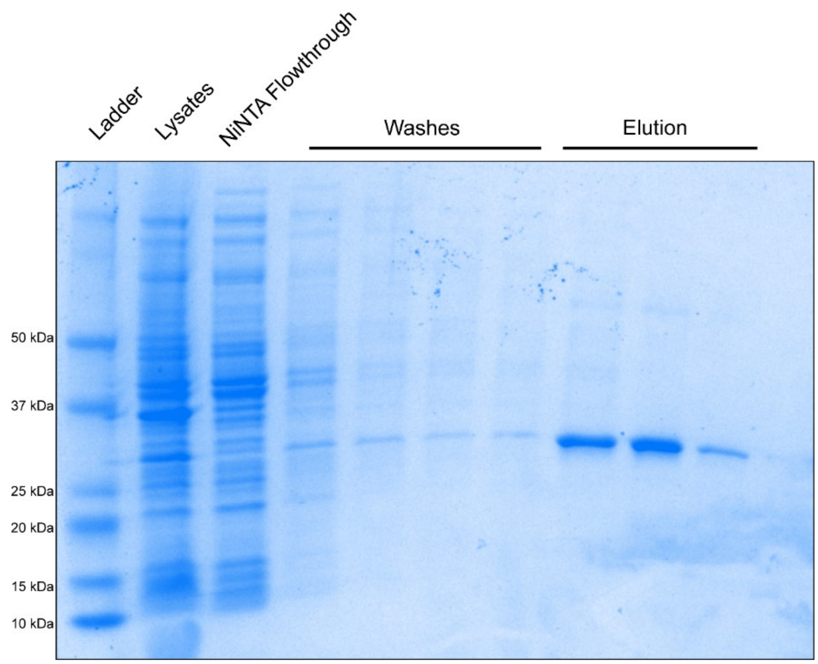


Fig. S3: SDS-PAGE gel for recombinantly expressed SIRT3 after Ni-NTA gel purification.

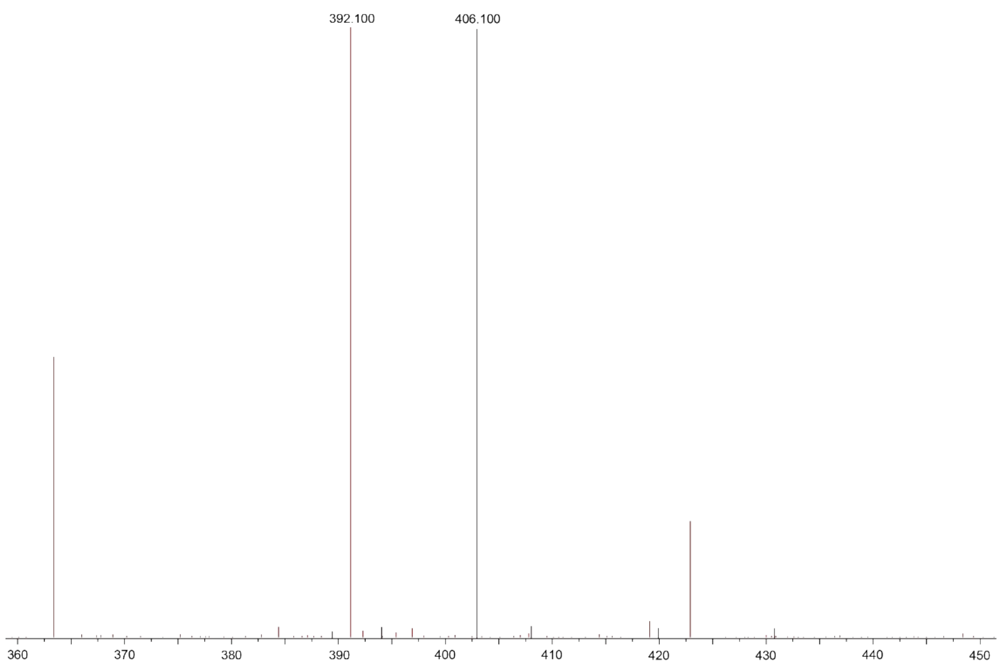


Fig. S4: Deacetylation of p53(378-386)K382ac by SIRT3. Under standard conditions after 1 hr incubation with 1 μM of SIRT3. Control reaction shown in black, SIRT3-catalyzed reaction shown in red. Peaks reported as $[M+3H]^{3+}$.

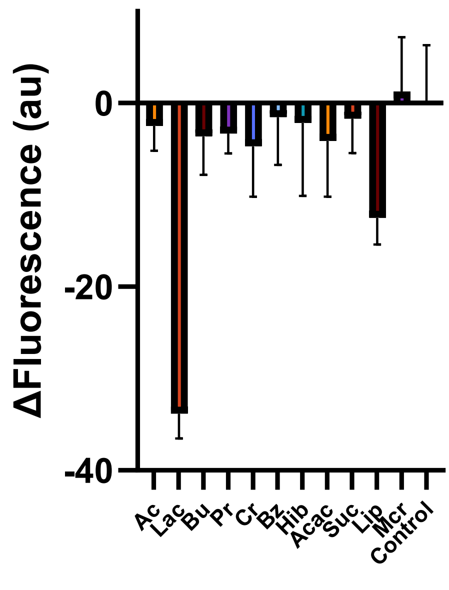


Fig. S5: Fluorescence differences for HDAC2-catalyzed acylation at the H3K9 site with a panel of acyl modifications at 10mM. Sample with solely the peptide and HDAC2 present taken as control.

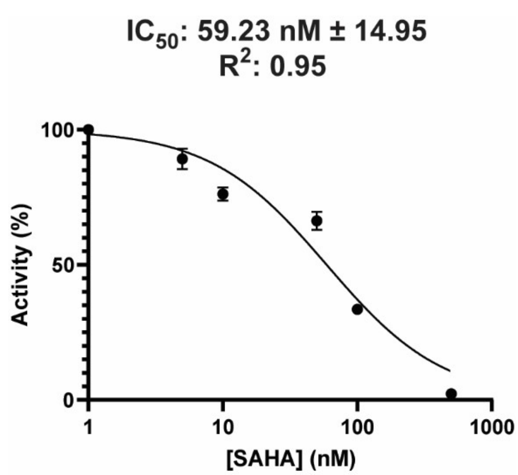


Fig. S6: Dose-response curve of HDAC2-catalyzed deacetylation of the H3K9 substrate in the presence of increasing concentrations of SAHA.

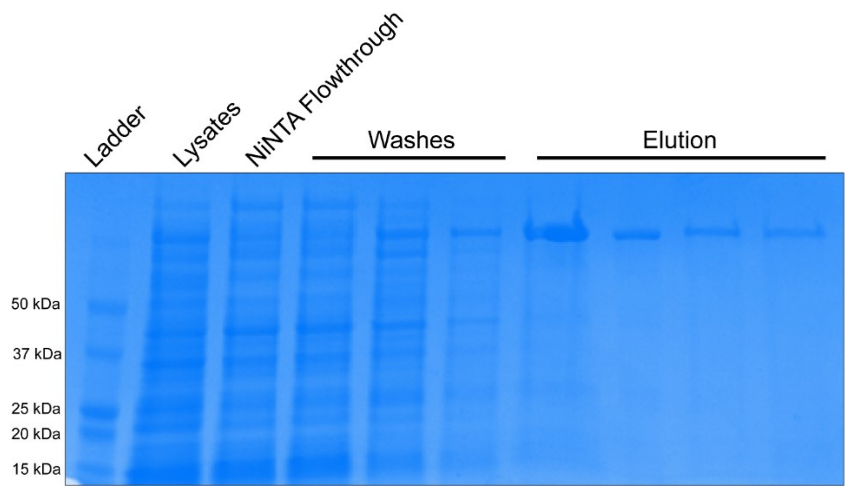


Fig. S7: SDS-PAGE gel for recombinantly expressed HDAC6 after Ni-NTA gel purification.

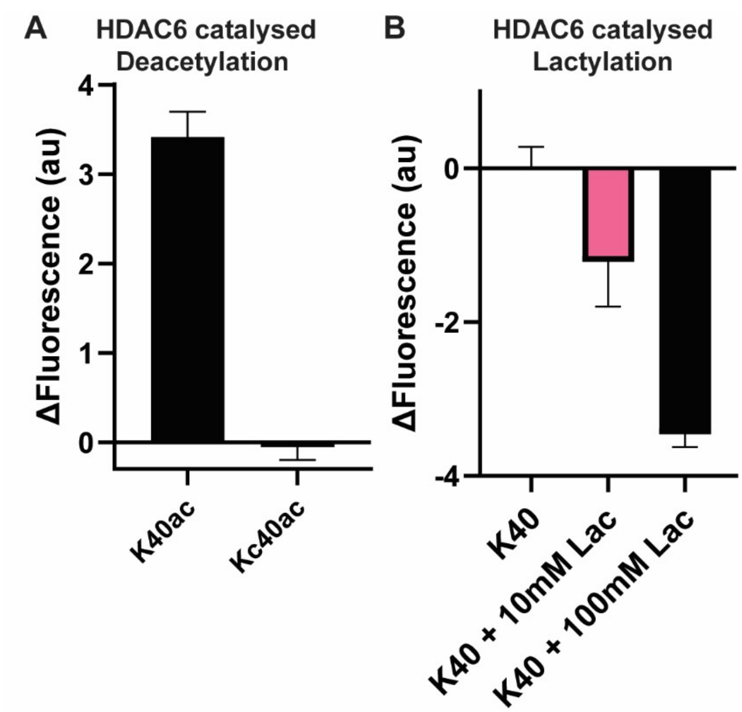


Fig. S8: Fluorescence differences for HDAC2-catalyzed deacetylation and lactylation reactions at the αT-K40, lactate was incubated at concentrations of 10 and 100mM.

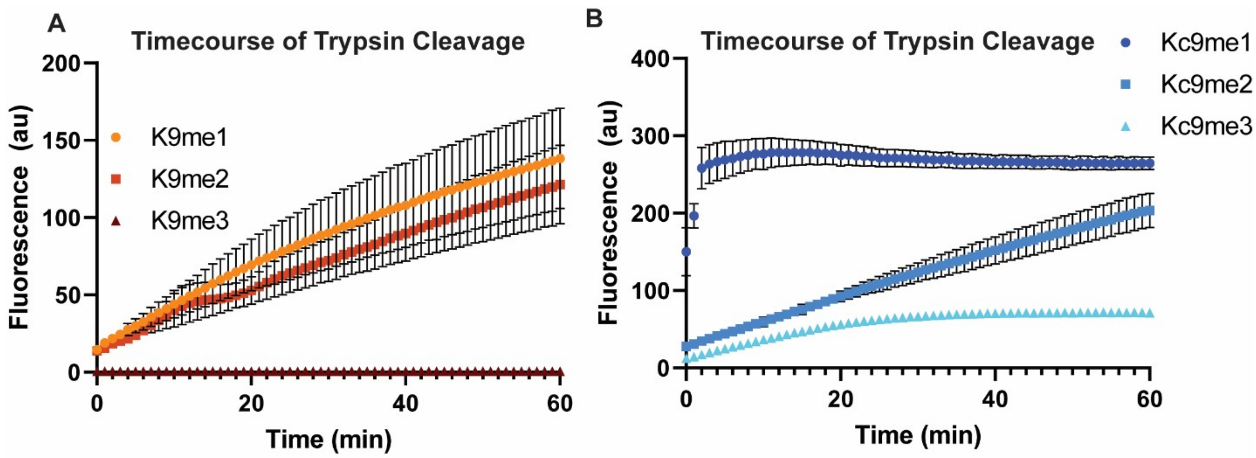


Fig. S9: Kinetics of trypsin cleavage of methylated peptides. A) Timecourse of fluorescence increase of methylated lysine peptides incubated with trypsin, B) Timecourse of fluorescence increased of methylated thialysine peptides incubated with trypsin.

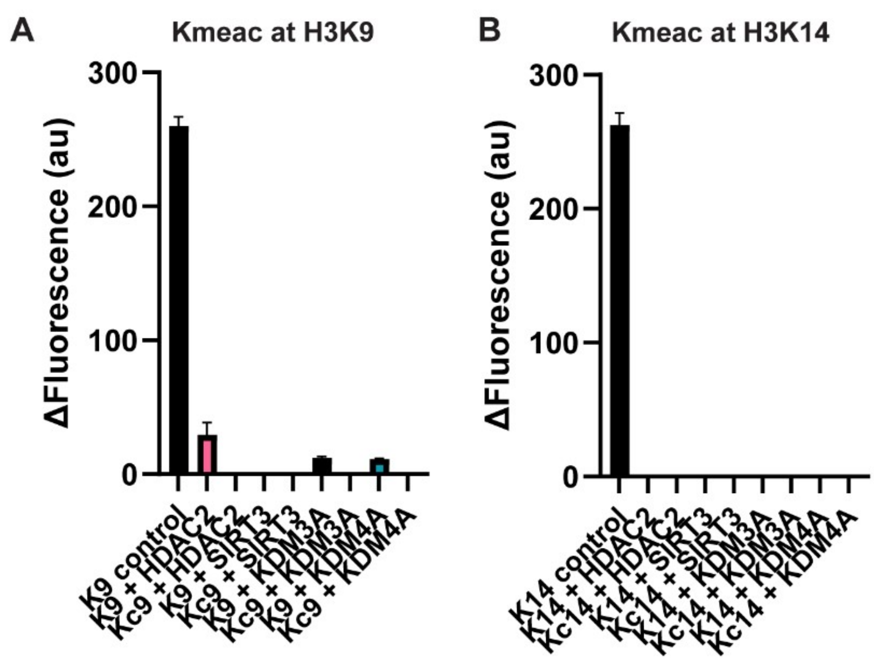


Fig. S10: Studies of enzymatic removal of the methylacetyl modification. Substrates detected by fluorescent turn-on by trypsin cleavage histone-derived Abz/Dnp substrates at the A) H3K9 site and B) H3K14 site.

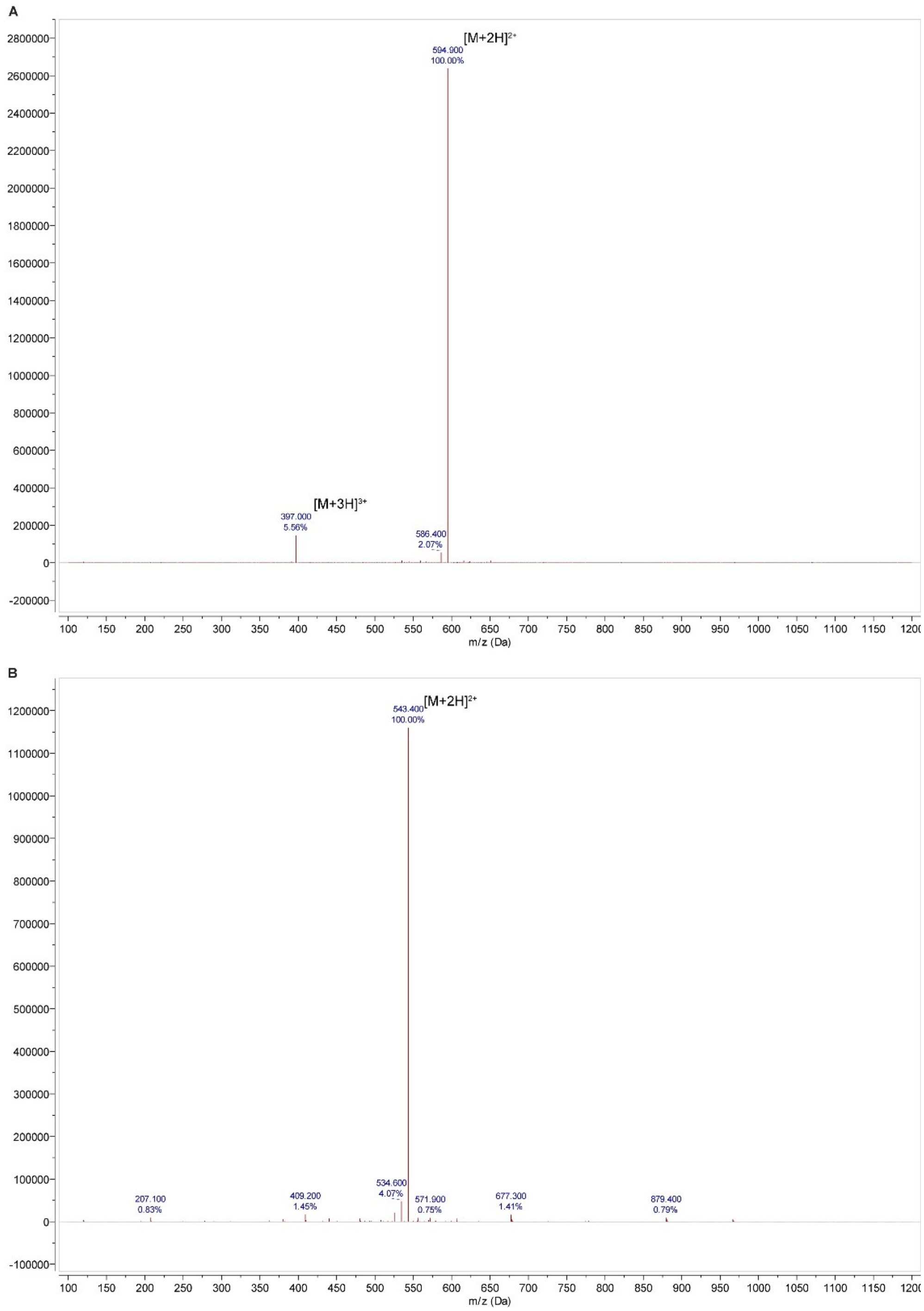
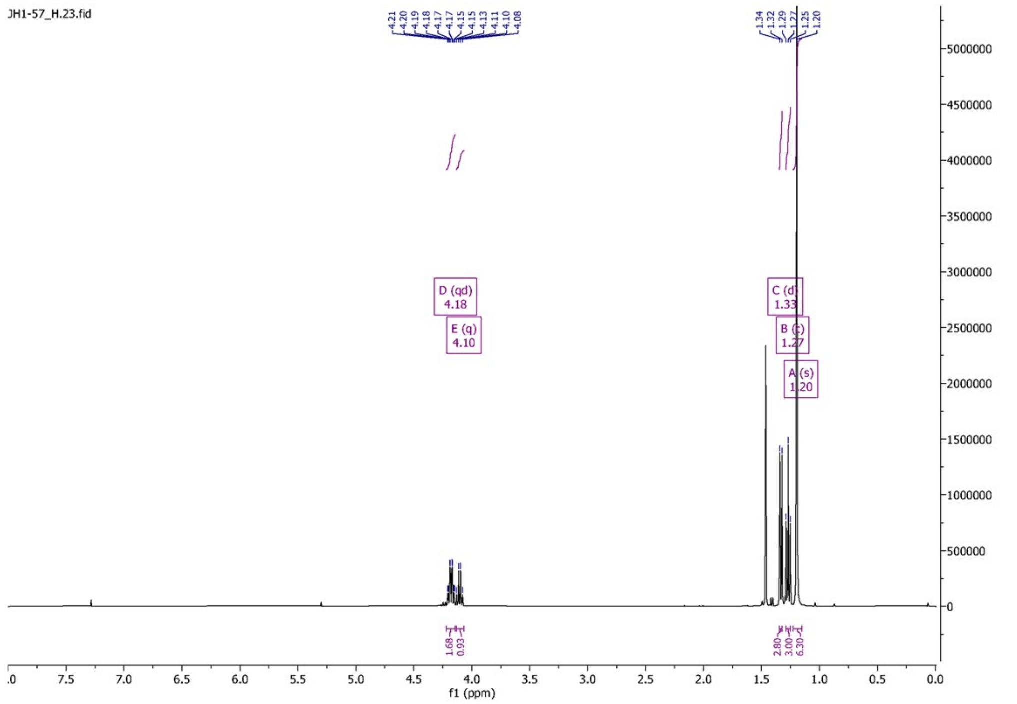


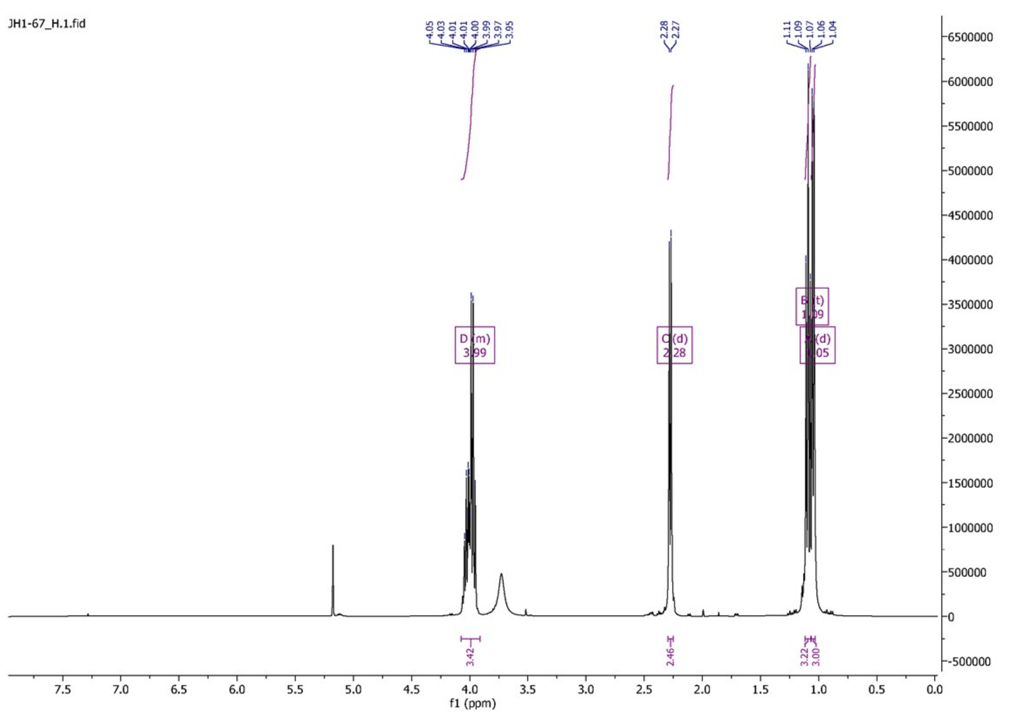
Figure S11: Exemplar LC/MS traces of Kmeac peptides incubated with HDAC2 for A) H3K9meac and B) H3K14meac.

NMR spectra for newly synthesized compounds

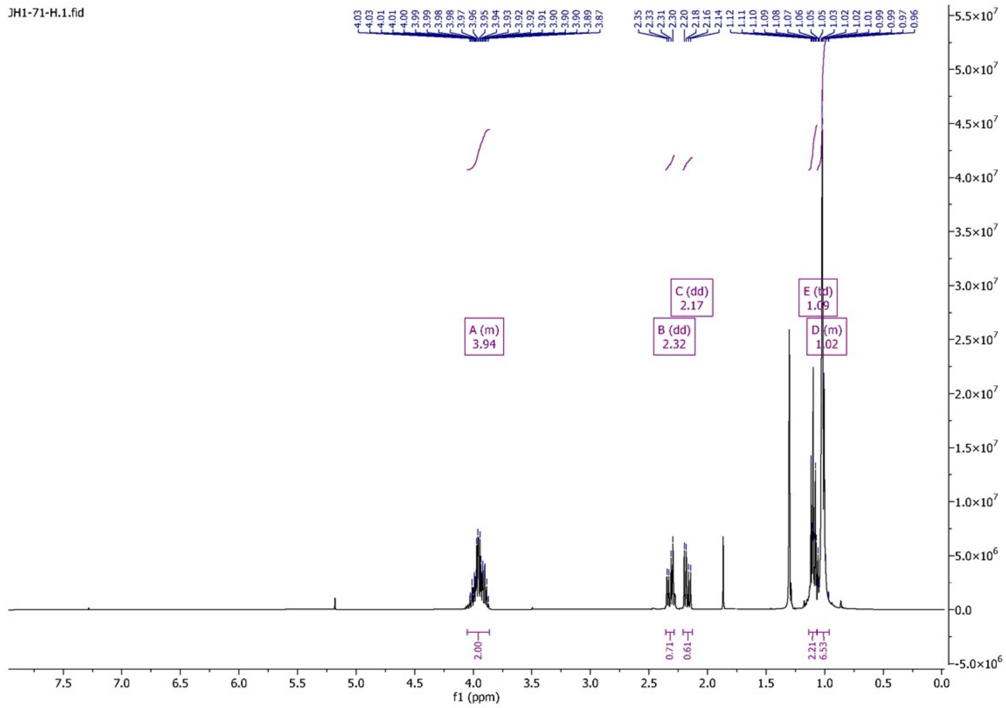
Ethyl (S)-2-(tert-butoxy)propanoate



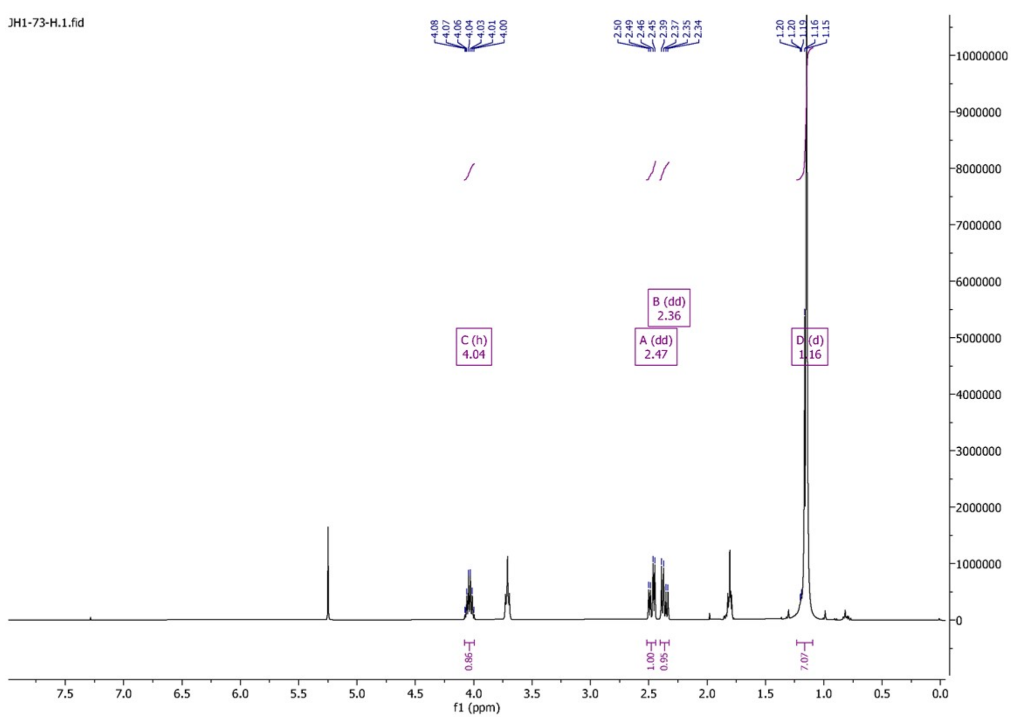
Ethyl 3-hydroxybutanoate

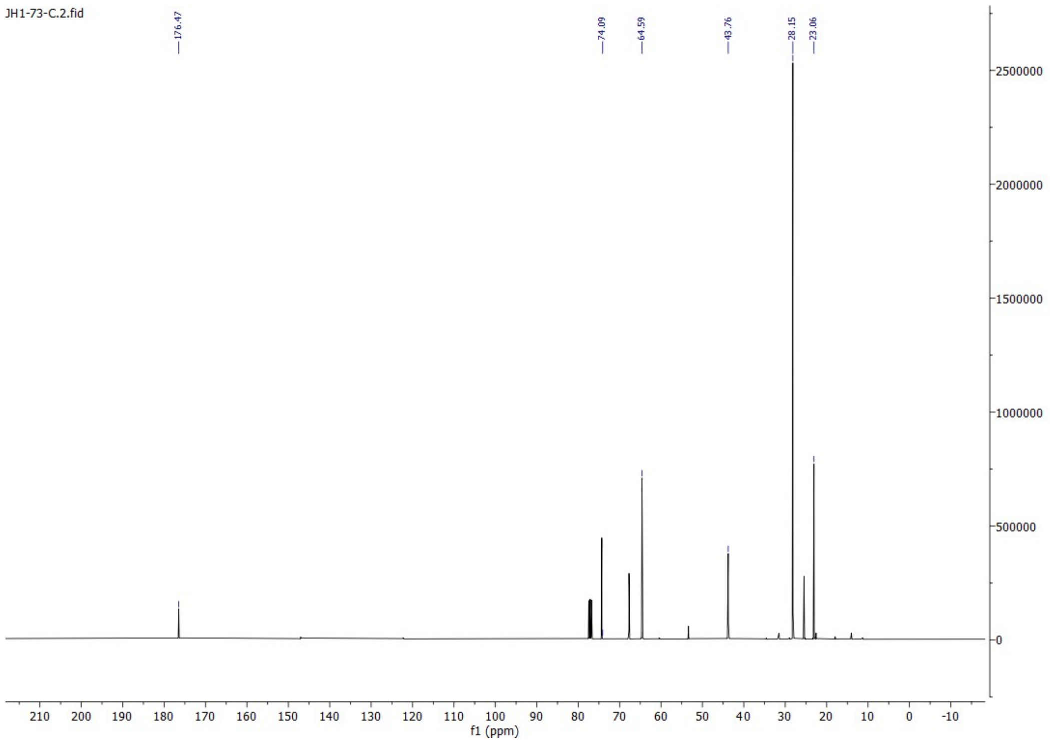


Ethyl 3-(tert-butoxy)butanoate

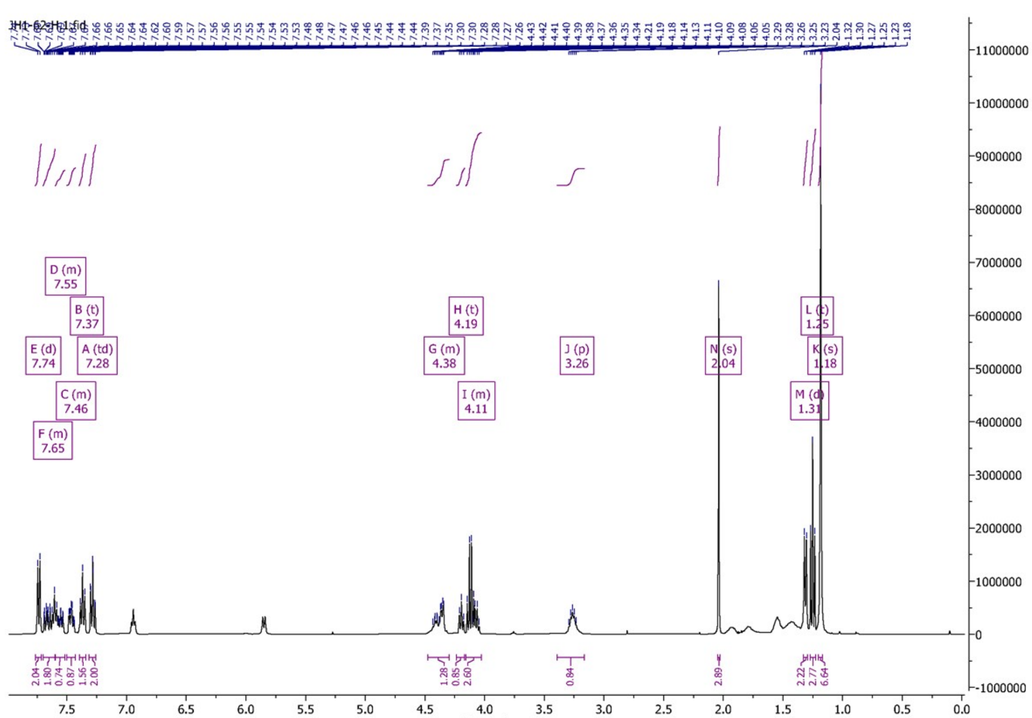


3-(tert-butoxy)butanoic acid

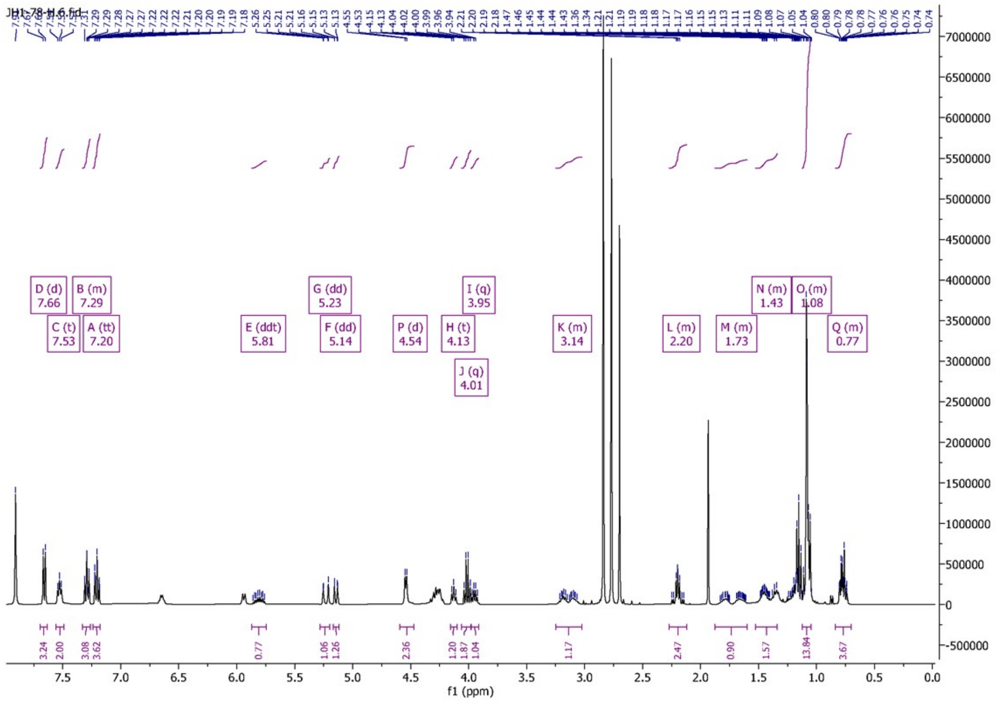


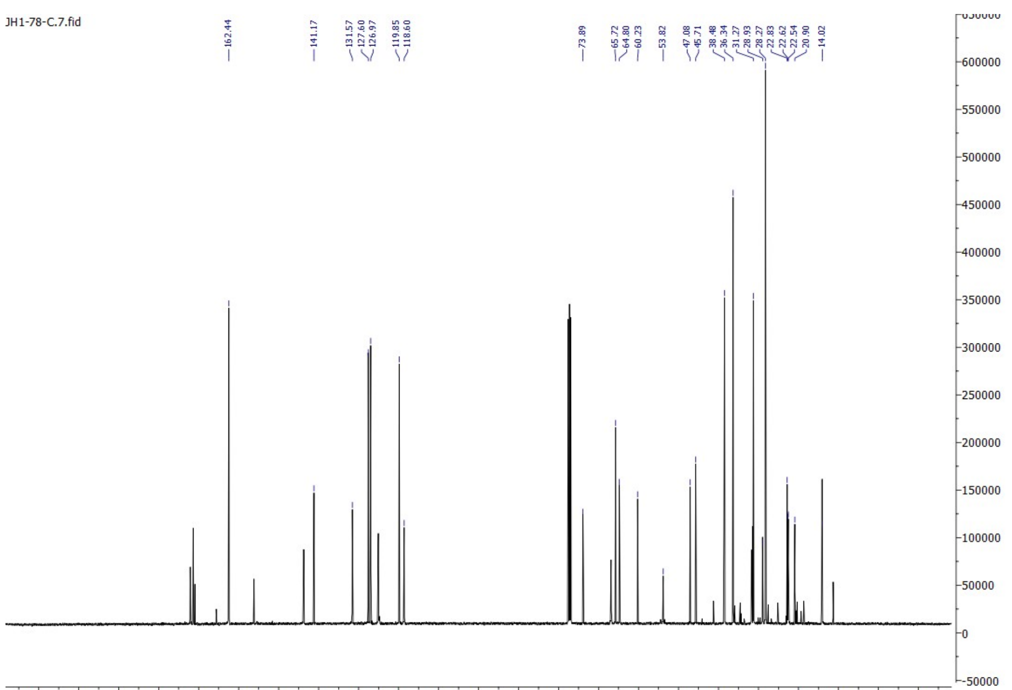


Fmoc-Lys(lac)-OH

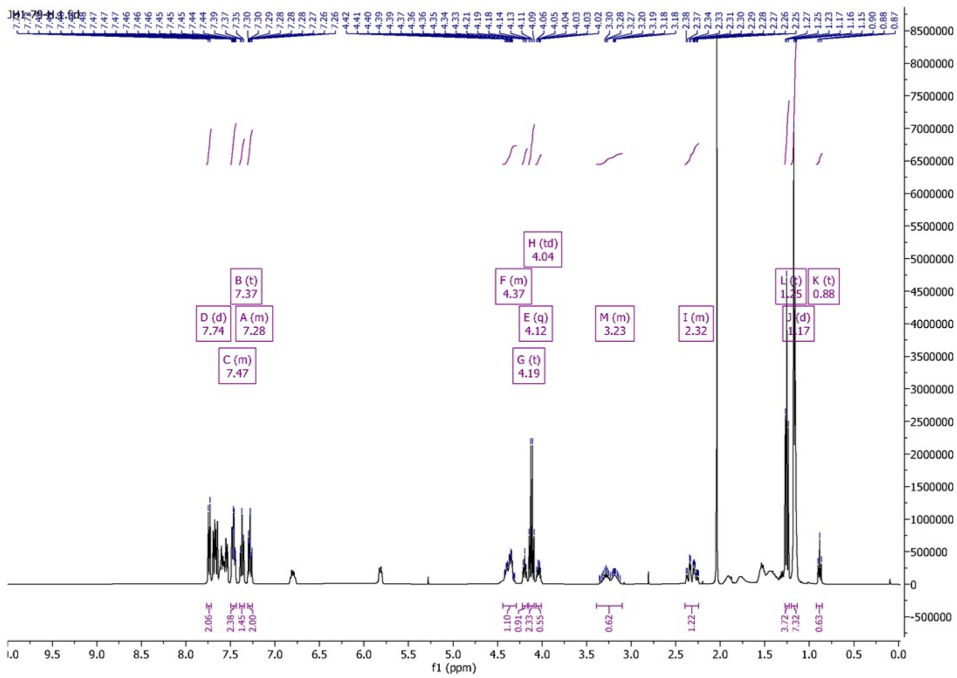


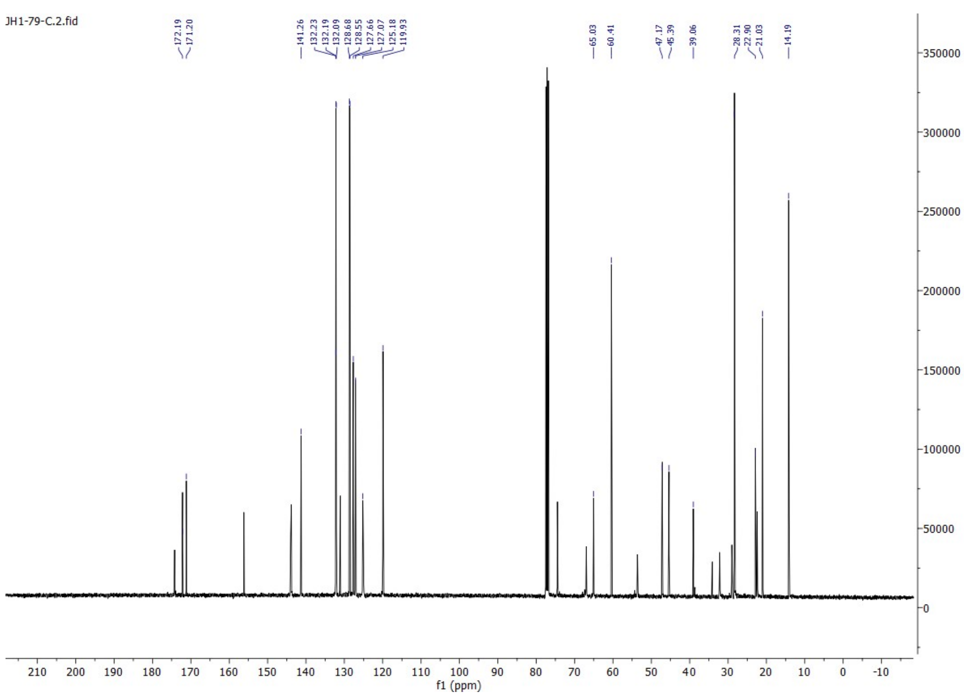
Fmoc-Lys(bhb)-OAllyl





Fmoc-Lys(bhb)-OH





3-(tert-butoxy)butanamide (mixture of rotamers)