

Molecules with O-acetyl group protect protein glycation by acetylating lysine residues

Garikapati Vannuruswamy^{#1}, Mashanipalya G. Jagadeeshaprasad^{#1,2}, K. Kashinath^{#2,3}, Suresh K Kesavan¹, Shweta Bhat¹, Arvind M. Korwar¹, Ashok D. Chougale¹, Ramanamurthy Boppana⁴, D. Srinivasa Reddy^{*2,3}, and Mahesh J. Kulkarni^{*1,2}.

¹ Mass-spectrometry and Proteomics Facility, Division of Biochemical Sciences, CSIR-National Chemical Laboratory, Pune-411008, India.

²Academy of Scientific and Innovative Research (AcSIR), New Delhi, India.

³Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune-411008, India.

⁴National Center for Cell Science, Pune-411007, India.

Supplementary table 1: List of Amadori modified peptides of *in-vitro* glycated BSA in presence or absence of acetyl group containing molecules

S.No	Modified site	Peptide start-end	modified peptide sequence	Peptide MH+ Da	Mono isotopic m/z Da (mmu/ppm)	Z	RT	XC	MC	Type of modification
Glycated Bovine Serum Albumin (GBSA) modifications:										
1	580	569-597	TVMENFVAFVDK* CCA ADDKEACFAVEGPK	3470.52	868.38 Da (-0.99 mmu/-1.14 ppm)	4	26.52	5.87	2	AML
2	318	310-340	SHCIAEVEK*DAIPENLPPLTADFAEDKDVK	3673.72	919.18 Da (-0.11 mmu/-0.12 ppm)	4	22.98	5.07	2	AML
3	346	341-359	NYQEAK*DAFLGSFLYEYSR	2463.13	821.71 Da (-0.68 mmu/-0.83 ppm)	3	26.40	4.33	1	AML
4	437	437-451	K *VPQVSTPTLVEVSR	1801.99	601.33 Da (+0.02 mmu/+0.03 ppm)	3	18.05	3.47	1	AML
5	88	76-100	TCVADESHAG CEK *SLHTLFGDELCK	3026.32	1009.44 Da (+1.16 mmu/+1.14 ppm)	3	19.18	4.50	1	AML
6	568	562-580	ATEEQLK*TVMENFVAFVDK	2361.15	1181.07 Da (-0.74 mmu/-0.63 ppm)	2	31.76	4.33	1	AML
7	548	548-557	K *QTALVELLK	1304.76	435.59 Da (+0.22 mmu/+0.51 ppm)	3	20.20	2.77	1	AML
8	548	548-557	K *QTALVELLK	1286.75	429.59 Da (+0.41 mmu/+0.95 ppm)	3	20.06	1.75	1	FL-1 _{H2O}
9	151	139-156	LKPDPNTLCDEFK*ADEKK	2310.11	578.28 Da (-0.43 mmu/-0.74 ppm)	4	17.13	1.83	2	AML
10	36	35-44	FK*DLGEEHFK	1411.67	471.22 Da (-0.44 mmu/-0.93 ppm)	3	13.49	1.98	1	AML
11	266	264-285	VHK* EC CHGDLLE C ADDRADLAK	2774.21	925.40 Da (-1.17 mmu/-1.27 ppm)	3	14.77	3.83	2	AML
12	299	298-309	LK* ECCD KPLLEK	1694.83	424.46 Da (-0.58 mmu/-1.37 ppm)	4	11.75	2.17	1	AML
13	285	281-297	ADLAK*YICDNQDTISSK	2103.97	701.99 Da (-0.96 mmu/-1.36 ppm)	3	17.93	3.44	1	AML
14	100	89-105	SLHTLFGDEL CK *VASLR	2108.06	527.77 Da (+0.1 mmu/+0.19 ppm)	4	25.01	3.22	1	AML
15	285	267-297	EC CHGDLLE C ADDRADLAK*YICDNQDTISSK	3834.61	959.41 Da (-0.22 mmu/-0.22 ppm)	4	21.50	5.24	2	AML
16	256	249-263	AEFVEVTK*LVTDLTK	1854.99	927.99 Da (-1.43 mmu/-1.55 ppm)	2	28.17	3.30	1	AML
17	597	581-607	CCA ADDKEACFAVEGPK*LVVSTQTALA	3073.41	1025.14 Da (-0.59 mmu/-0.58 ppm)	3	22.76	5.30	2	AML
18	528	524-544	AFDEK*LFTFHADICTLPDTEK	2660.24	887.42 Da (+1.16 mmu/+1.31 ppm)	3	24.25	3.80	1	AML
19	183	168-197	RHPYFYAPELLYYANK*YNGVFQ EC CAEDK	3935.74	787.95 Da (-4.39 mmu/-5.58 ppm)	4	25.07	6.42	2	AML
20	547&548	545-557	QIK* K *QTALVELLK	1836.05	612.68 Da (-0.8 mmu/-1.3 ppm)	3	18.11	3.13	2	AML
21	412	402-420	HLVDEPQNLIK*QNC DQ FEK	2517.19	839.73 Da ((+1.07 mmu/+1.27 ppm)	3	17.47	4.28	1	AML
GBSA treated with Aspirin										
1	285	267-297	EC CHGDLLE C ADDRADLAK*YICDNQDTISSK	3834.62	959.41 Da (+0.15 mmu/+0.16 ppm)	4	21.35	6.22	2	AML
2	580	569-597	TVMENFVAFVDK* CCA ADDKEACFAVEGPK	3470.52	868.38 Da (+0.35 mmu/+0.41 ppm)	4	26.80	5.85	2	AML
3	597	581-607	CCA ADDKEACFAVEGPK*LVVSTQTALA	3073.41	1025.14 Da (+0.02 mmu/+0.02 ppm)	3	22.74	5.19	2	AML
4	568	562-580	ATEEQLK*TVMENFVAFVDK	2361.15	1181.08 Da (+1.09 mmu/+0.93 ppm)	2	31.91	4.52	1	AML
5	346	341-359	NYQEAK*DAFLGSFLYEYSR	2463.13	821.71 Da (-0.19 mmu/-0.23 ppm)	3	26.42	4.43	1	AML
6	412	402-420	HLVDEPQNLIK*QNC DQ FEK	2517.20	839.73 Da (+3.09 mmu/+3.67 ppm)	3	17.84	4.13	1	AML
7	528	524-544	AFDEK*LFTFHADICTLPDTEK	2660.24	887.42 Da (+1.29 mmu/+1.45 ppm)	3	24.04	3.95	1	AML

8	437	437-451	K*VPQVSTPTLVEVSR	1801.99	601.33 Da (+0.02 mmu/+0.03 ppm)	3	18.07	3.41	1	AML
9	285	267-297	ADLAK*YICDNQDTISSK	2103.97	701.99 Da (+0.14 mmu/+0.2 ppm)	3	17.94	3.30	1	AML
10	256	249-263	AEFVEVTK*LVTDLTK	1854.99	928.00 Da (-0.27 mmu/-0.29 ppm)	2	27.91	3.17	1	AML
11	547&548	545-557	QIK*K*QTALVELLK	1836.05	612.69 Da (-0.06 mmu/-0.1 ppm)	3	18.67	2.88	2	AML
12	548	548-557	K*QTALVELLK	1304.76	435.59 Da (-0.08 mmu/-0.19 ppm)	2	20.46	2.74	1	AML
GBSA treated with Bisacodyl										
1	580	569-597	TVMENFVAFVDK*CCAADDKEACFAVEGPK	3470.52	868.38 Da (-0.32 mmu/-0.37 ppm)	4	26.52	5.85	2	AML
2	346	341-359	NYQEAK*DAFLGSFLYEYSR	2463.13	821.71 Da (+0.42 mmu/+0.51 ppm)	3	26.42	4.56	1	AML
3	568	562-580	ATEEQLK*TVMENFVAFVDK	2361.15	1181.08 Da (+0.48 mmu/+0.41 ppm)	2	31.65	4.54	1	AML
4	597	581-607	CCAADDKEACFAVEGPK*LVVSTQTALA	3073.41	1025.14 Da (-0.22 mmu/-0.22 ppm)	3	22.75	5.03	2	AML
5	528	524-544	AFDEK*LFTFHADICTLPDTEK	2660.25	887.42 Da (+2.63 mmu/+2.96 ppm)	3	24.24	4.16	1	AML
6	266	264-285	VHK*ECCHGDLLECADDRADLAK	2774.21	925.41 Da (-0.44 mmu/-0.48 ppm)	3	14.62	3.71	2	AML
7	437	437-451	K*VPQVSTPTLVEVSR	1801.99	601.33 Da (+0.44 mmu/+0.74 ppm)	3	18.07	3.51	1	AML
8	285	267-297	ADLAK*YICDNQDTISSK	2103.97	701.99 Da (-0.1 mmu/-0.14 ppm)	3	17.98	3.11	1	AML
9	548	548-557	K*QTALVELLK	1304.76	435.59 Da (+0.5 mmu/+1.15 ppm)	3	20.28	2.72	1	AML
GBSA treated with NDS-458										
1	580	569-597	TVMENFVAFVDK*CCAADDKEACFAVEGPK	3470. 52	868.38 Da (-0.93 mmu/-1.07 ppm)	4	26.529	5.88	2	AML
2	318	310-340	SHCIAEVEK*DAIPENLPPLTADFAEDKDVCCK	3673.72	919.18 Da (+0.56 mmu/+0.61 ppm)	4	23.16	4.66	2	AML
3	346	341-359	NYQEAK*DAFLGSFLYEYSR	2463.13	821.71 Da (+0.3 mmu/+0.36 ppm)	3	26.48	4.63	1	AML
4	568	562-580	ATEEQLK*TVMENFVAFVDK	2361.15	1181.08 Da (+1.58 mmu/+1.34 ppm)	2	31.73	4.48	1	AML
5	528	524-544	AFDEK*LFTFHADICTLPDTEK	2660. 24	887.42 Da (+2.08 mmu/+2.34 ppm)	3	24.26	3.86	1	AML
6	547&548	545-557	QIK*K*QTALVELLK	1716.01	858.51 Da (+0.32 mmu/+0.37 ppm)	2	21.63	3.76	2	Acety+AML
7	437	437-451	K*VPQVSTPTLVEVSR	1801.98	601.33 Da (-0.35 mmu/-0.58 ppm)	3	18.38	3.61	1	AML
8	285	267-297	ADLAK*YICDNQDTISSK	2103.97	701.99 Da (+0.02 mmu/+0.03 ppm)	3	17.94	3.39	1	AML
9	266	264-285	VHK*ECCHGDLLECADDRADLAK	2774.21	925.41 Da (-0.13 mmu/-0.15 ppm)	3	14.62	3.41	2	AML
10	100	89-105	SLHTLFGDELCK*VASLR	2108.07	527.77 Da (+0.65 mmu/+1.23 ppm)	4	25.33	3.04	1	AML
11	548	548-557	K*QTALVELLK	1304.76	435.59 Da (+0.19 mmu/+0.44 ppm),	3	20.57	2.62	1	AML
GBSA treated with Paracetamol										
1	580	569-597	TVMENFVAFVDK*CCAADDKEACFAVEGPK	3470.52	868.38 Da (-0.01 mmu/-0.02 ppm)	4	26.52	6.05	2	AML
2	88	76-100	TCVADESHAGCEK*SLHTLFGDELCK	3026.31	1009.44 Da (+0.67 mmu/+0.66 ppm)	3	19.48	5.04	1	AML
3	568	562-580	ATEEQLK*TVMENFVAFVDK	2361.15	1181.08 Da (+0.73 mmu/+0.62 ppm)	2	31.57	4.54	1	AML
4	285	267-297	ADLAK*YICDNQDTISSK	2103.97	701.99 Da (-0.35 mmu/-0.49 ppm)	3	17.87	3.47	1	AML
5	437	437-451	K*VPQVSTPTLVEVSR	1801.98	601.33 Da (-0.29 mmu/-0.48 ppm)	3	18.34	3.50	1	AML

6	548	548-557	K*QTALVELLK	1304.76	435.59 Da (+0.13 mmu/+0.3 ppm)	3	19.87	2.79	1	AML
7	346	341-359	NYQEAK*DAFLGSFLYEYSR	2463.13	821.71 Da (+0.24 mmu/+0.29 ppm)	3	26.34	4.60	1	AML
8	285	267-297	ECCHGDLLECADDRADLAK*YICDNQDTISSK	3834.62	959.41 Da (+1.98 mmu/+2.07 ppm)	4	21.49	5.38	2	AML
9	597	581-607	CCAADDKEACFAVEGPK*LVVSTQTALA	3073.41	1025.14 Da (+0.39 mmu/+0.38 ppm)	3	22.63	5.34	2	AML
10	528	524-544	AFDEK*LFTFHADICTLPDTEK	2660.24	1330.62 Da (+1.45 mmu/+1.09 ppm)	2	24.15	4.28	1	AML
11	412	402-420	HLVDEPQNLIK*QNCDQFEK	2517.19	839.73 Da (+1.5 mmu/+1.78 ppm)	3	17.95	4.24	1	AML
12	266	264-285	VHK*ECCHGDLLECADDRADLAK	2774.21	925.41 Da (-0.2 mmu/-0.21 ppm)	3	14.49	3.96	2	AML
13	100	89-105	SLHTLFGDELCK*VASLR	2108.07	527.77 Da (+0.77 mmu/+1.47 ppm)	4	25.22	3.05	1	AML
14	547&548	545-557	QIK*K*QTALVELLK	1836.05	612.69 Da (+0.43 mmu/+0.69 ppm)	3	19.10	2.96	2	AML
15	318	310-340	SHCIAEVEK*DAIPENLPPLTADFAEDKDVCK	3673.72	919.18 Da (-0.91 mmu/-0.99 ppm)	4	22.83	4.96	2	AML
GBSA treated with Acetazolamide										
1	580	569-597	TVMENFVAFVDK*CCAADDKEACFAVEGPK	3470.52	868.38 Da (-0.08 mmu/-0.09 ppm)	4	26.62	5.89	2	AML
2	318	310-340	SHCIAEVEK*DAIPENLPPLTADFAEDKDVCK	3673.72	919.18 Da (+0.62 mmu/+0.67 ppm)	4	22.91	4.99	2	AML
3	346	341-359	NYQEAK*DAFLGSFLYEYSR	2463.13	821.71 Da (-0.31 mmu/-0.38 ppm)	3	26.49	4.58	1	AML
4	568	562-580	ATEEQLK*TVMENFVAFVDK	2361.16	787.72 Da (+2.9 mmu/+3.69 ppm)	3	32.39	4.26	1	AML
5	412	402-420	HLVDEPQNLIK*QNCDQFEK	2517.19	839.73 Da (+0.95 mmu/+1.13 ppm)	3	17.73	4.14	1	AML
6	285	267-297	ADLAK*YICDNQDTISSK	2103.97	701.99 Da (-0.77 mmu/-1.1 ppm)	3	17.73	3.40	1	AML
7	437	437-451	K*VPQVSTPTLVEVSR	1801.98	601.33 Da (-0.35 mmu/-0.58 ppm)	3	18.41	3.54	1	AML
8	548	548-557	K*QTALVELLK	1304.76	435.59 Da (-0.02 mmu/-0.05 ppm)	3	20.25	2.67	1	AML
9	547&548	545-557	QIK*K*QTALVELLK	1836.05	459.76 Da (+0.06 mmu/+0.12 ppm)	4	18.87	2.58	2	AML
10	285	267-297	ECCHGDLLECADDRADLAK*YICDNQDTISSK	3834.61	959.41 Da (-0.03 mmu/-0.03 ppm)	4	21.28	5.11	2	AML
11	528	524-544	AFDEK*LFTFHADICTLPDTEK	2660.24	887.42 Da (+1.29 mmu/+1.45 ppm)	3	24.11	4.0	1	AML
12	266	264-285	VHK*ECCHGDLLECADDRADLAK	2774.21	925.40 Da (-0.93 mmu/-1 ppm)	3	14.58	4.13	2	AML
13	256	249-263	AEFVEVTK*LVTDLTK	1854.99	928.00 Da (-0.52 mmu/-0.56 ppm)	2	27.78	3.29	1	AML
14	100	89-105	SLHTLFGDELCK*VASLR	2108.07	527.77 Da (+0.22 mmu/+0.42 ppm)	4	25.52	3.25	1	AML
15	88	76-100	TCVADESHAGCEK*SLHTLFGDELCK	3026.31	1009.44 Da (-1.1 mmu/-1.09 ppm)	3	19.39	4.73	1	AML
GBSA treated with Acetohexamide										
1	580	569-597	TVMENFVAFVDK*CCAADDKEACFAVEGPK	3470.52	868.38 Da (+0.05 mmu/+0.05 ppm)	4	26.81	5.91	2	AML
2	568	562-580	ATEEQLK*TVMENFVAFVDK	2361.15	787.72 Da (+0.58 mmu/+0.74 ppm)	3	31.53	4.47	1	AML
3	346	341-359	NYQEAK*DAFLGSFLYEYSR	2463.13	821.71 Da (-0.31 mmu/-0.38 ppm)	3	26.49	4.59	1	AML
4	437	437-451	K*VPQVSTPTLVEVSR	1801.98	601.33 Da (-0.29 mmu/-0.48 ppm)	3	18.21	3.61	1	AML
5	100	89-105	SLHTLFGDELCK*VASLR	2108.06	527.77 Da (+0.04 mmu/+0.08 ppm)	4	25.44	2.92	1	AML
6	548	548-557	K*QTALVELLK	1304.76	435.59 Da (+0.04 mmu/+0.09 ppm)	3	20.27	2.75	1	AML

7	528	524-544	AFDEK*LFTFHADICTLPDTEK	2660.25	887.42 Da (+2.57 mmu/+2.89 ppm)	3	24.23	4.22	1	AML
8	412	402-420	HLVDEPQNLIK*QNCDQFEK	2517.19	839.73 Da (-0.58 mmu/-0.69 ppm)	3	18.26	4.12	1	AML
9	285	267-297	ADLAK*YICDNQDTISSK	2103.97	701.99 Da (-0.77 mmu/-1.1 ppm)	3	17.93	3.33	1	AML
10	256	249-263	AEFVEVTK*LVTDLTK	1854.99	928.00 Da (-0.58 mmu/-0.62 ppm)	2	27.98	3.30	1	AML
11	548	548-548	K*QTALVELLK	1286.75	429.59 Da (+0.34 mmu/+0.8 ppm)	3	20.23	2.08	1	FL-1 _{H20}
12	285	267-297	ECCHGDLLECADDRADLAK*YICDNQDTISSK	3834.62	959.41 Da (+0.03 mmu/+0.03 ppm)	4	21.23	5.57	2	AML
13	318	310-340	SHCIAEVEK*DAIPENLPPLTADFAEDKDVK	3673.72	919.18 Da (-0.54 mmu/-0.59 ppm)	4	23.16	4.83	2	AML
GBSA treated with N-Acetyl Cysteine										
1	580	569-597	TVMENFVAFVDK*CCAADDKEACFAVEGPK	3470.52	868.38 Da (-1.36 mmu/-1.56 ppm)	4	26.55	5.93	2	AML
2	568	562-580	ATEEQLK*TVMENFVAFVDK	2361.15	1181.07 Da (-0.25 mmu/-0.21 ppm)	2	31.68	4.59	1	AML
3	346	341-359	NYQEAQ*DAFLGSLFYEYSR	2463.13	821.71 Da (-0.5 mmu/-0.6 ppm)	3	26.47	4.42	1	AML
4	285	267-297	ADLAK*YICDNQDTISSK	2103.97	701.99 Da (-0.53 mmu/-0.75 ppm)	3	17.99	3.47	1	AML
5	437	437-451	K*VPQVSTPTLVEYSR	1801.98	601.33 Da (-0.96 mmu/-1.6 ppm)	3	18.48	3.25	1	AML
6	547&548	545-557	QIK*K*QTALVELLK	1836.05	612.69 Da (-0.19 mmu/-0.3 ppm)	3	18.79	3.19	2	AML
7	548	548-557	K*QTALVELLK	1304.76	435.59 Da (-0.17 mmu/-0.4 ppm)	3	20.51	2.75	1	AML
8	548	548-557	K*QTALVELLK	1286.75	429.59 Da (-0.17 mmu/-0.41 ppm)	3	20.14	2.08	1	FL-1 _{H20}
9	318	310-340	SHCIAEVEK*DAIPENLPPLTADFAEDKDVK	3673.72	919.18 Da (-0.18 mmu/-0.19 ppm)	4	23.22	4.93	2	AML
10	412	402-420	HLVDEPQNLIK*QNCDQFEK	2517.19	839.73 Da (+1.31 mmu/+1.56 ppm)	3	17.82	4.17	1	AML
11	528	524-544	AFDEK*LFTFHADICTLPDTEK	2660.24	887.42 Da (+0.8 mmu/+0.9 ppm)	3	24.29	3.76	1	AML
12	256	249-263	AEFVEVTK*LVTDLTK	1854.99	928.00 Da (-0.82 mmu/-0.89 ppm)	2	27.81	3.28	1	AML
13	100	89-105	SLHTLFGDELCK*VASLR	2108.06	527.77 Da (-0.14 mmu/-0.27 ppm)	4	25.58	3.0	1	AML
14	285	267-297	ECCHGDLLECADDRADLAK*YICDNQDTISSK	3834.61	959.40 Da (-0.83 mmu/-0.86 ppm)	4	21.36	5.24	2	AML

Static modification: Carbamidomethyl (57.02 Da), Z-Charge state, RT-Retention time, MC-Missed cleavage, XC-Xcorr Value

\$: Acetylation, *: AML, AML: Amadori modified lysine, FL-1_{H20}: Fructosyl Lysine-1_{H20}

Supplementary table 2: List of acetylation modified peptides of BSA induced by O-acetyl group containing compounds (Aspirin, Bisacodyl, NDS-458)

S.No	Modified site	Peptide start-end	modified peptide sequence	Peptide MH+ Da	Mono isotopic m/z Da (mmu/ppm)	Z	RT	XC	M C
Aspirin induced acetylation modifications									
1	183	168-197	RHPYFYAPELLYYANK*YNGVFQECQAEDK	3815.73	954.68 Da (+1.26 mmu/+1.32 ppm)	4	28.22	7.65	2
2	44	35-65	FKDLGEEHFK*GLVLIAFSQYLQQCPFDEHVK	3764.90	753.78 Da (+5.64 mmu/+7.48 ppm)	5	33.61	5.56	2
3	336	310-340	SHCIAEVEKDAIPENLPPLTADFAEDK*DVCK	3553.68	889.17 Da (+1.79 mmu/+2.01 ppm)	4	24.31	5.08	2
4	420	413-433	QNCDQFEK*LGEYGFQNALIVR	2571.23	857.74 Da (+1.05 mmu/+1.22 ppm)	4	27.12	4.81	1
5	412	402-420	HLVDEPQNLIK*QNCDQFEK	2397.14	799.72 Da (-0.78 mmu/-0.97 ppm)	3	19.42	4.59	1
6	401	400-412	LK*HLVDEPQNLIK	1588.90	794.95 Da (-0.48 mmu/-0.6 ppm)	2	19.42	4.57	1
7	568	562-580	ATEEQLK*TMENFVAFVDK	2241.12	747.71 Da (+4.66 mmu/+6.24 ppm)	3	38.83	4.28	1
8	137	136-152	LK*PDPNTLCDEFKADEK	2061.9	687.99 Da (-0.03 mmu/-0.04 ppm)	3	19.80	4.18	1
9	44	37-65	DLGEEHFK*GLVLIAFSQYLQQCPFDEHVK	3489.73	873.18 Da (+5.77 mmu/+6.6 ppm)	4	35.03	4.32	1
10	374	372-386	LAK*EYEATLEECCA	1856.84	619.61 Da (+0.47 mmu/+0.75 ppm)	3	17.10	3.37	1
11	559	558-568	HK*PKATEEQLK	1350.73	450.91 Da (-0.19 mmu/-0.43 ppm)	3	8.52	3.33	1
12	204	184-209	YNGVFQECQAEDKGACLLPK*IETMR	3159.44	1053.81 Da (+1.74 mmu/+1.65 ppm)	3	24.70	3.29	2
13	204	198-209	GACLLPK*IETMR	1430.74	715.87 Da (-0.21 mmu/-0.3 ppm)	2	23.26	3.23	1
14	523	508-528	RPCFSALTPDETYVPK*AFDEK	2513.20	838.40 Da (+0.31 mmu/+0.37 ppm)	3	22.87	3.21	1
15	544	529-547	LFTFHADICTLPDTEK*QIK	2319.16	773.72 Da (+0.16 mmu/+0.21 ppm)	3	24.16	3.16	1
16	544	529-548	LFTFHADICTLPDTEK*QIKK	2447.26	612.57 Da (+0.56 mmu/+0.92 ppm)	4	22.62	1.66	2
17	266	264-285	VHK*ECCHGDLLEACADRADLAK	2654.17	664.29 Da (-1.11 mmu/-1.67 ppm)	4	15.96	3.34	2
18	65	45-75	GLVLIAFSQYLQQCPFDEHVK*LVNELTEFAK	3678.90	920.48 Da (+3.31 mmu/+3.6 ppm)	4	35.35	2.93	1
19	498	496-507	VTK*CCTESLVNR	1508.72	754.86 Da (+0.56 mmu/+0.74 ppm)	2	14.05	3.05	1
20	140	136-151	LK*PDPNTLCDEFK	1618.77	809.89 Da (+0.57 mmu/+0.7 ppm)	2	20.26	3.02	0
21	228	223-232	CASIQK*FGER	1237.59	619.30 Da (+0.23 mmu/+0.37 ppm)	2	15.54	3.02	1
22	548	548-557	K*QTALVELLK	1184.72	592.86 Da (+0.34 mmu/+0.57 ppm)	2	23.93	3.03	1
23	28	25-34	DTHK*SEIAHR	1235.61	412.54 Da (-0.39 mmu/-0.94 ppm)	3	8.07	3.02	1
24	489	483-496	LCVLHEK*TPVSEK	1581.82	527.94 Da (-0.5 mmu/-0.95 ppm)	3	15.79	2.85	1
25	304	298-309	LKECCDK*PLLEK	1574.79	787.89 Da (+0.14 mmu/+0.18 ppm)	2	15.33	2.84	1
26	463	460-468	CCTK*PESER	1208.50	604.75 Da (+0.05 mmu/+0.09 ppm)	2	8.26	2.74	0
27	248	246-256	FPK*AEFVEVTK	1336.71	668.86 Da (+0.11 mmu/+0.16 ppm)	2	21.11	3.09	1
28	100	89-105	SLHTLFGDELCK*VASLR	1988.02	663.34 Da (-0.1 mmu/-0.15 ppm)	3	28.14	3.20	1

29	160	157-167	FWGK*YLYEIAR	1487.77	744.38 Da (+0.99 mmu/+1.33 ppm)	2	27.48	2.6	1
30	455	452-458	SLGK*VGTR	859.50	430.25 Da (+0.45 mmu/+1.05 ppm)	2	11.40	2.46	1
31	36	35-44	FK*DLGEEHFK	1291.63	431.21 Da (-0.08 mmu/-0.2 ppm)	3	16.73	2.74	1
32	235	232-241	ALK*AWSVAR	1043.60	522.30 Da (+0.35 mmu/+0.66 ppm)	2	18.44	2.29	1
33	297	286-299	YICDNQDTISSK*LK	1726.83	863.91 Da (+0.2 mmu/+0.23 ppm)	2	17.84	2.67	1
34	160	156-167	KFWGK*YLYEIAR	1615.86	539.29 Da (+0.76 mmu/+1.41 ppm)	3	25.28	2.54	2
35	263	257-266	LVTDLTK*VHK	1195.70	399.23 Da (+0.2 mmu/+0.5 ppm)	3	15.13	2.48	1
36	437	437-451	K*VPQVSTPTLVEVSR	1681.95	561.32 Da (+0.8 mmu/+1.42 ppm)	3	20.18	2.02	1
37	495	490-498	TPVSEK*VTK	1030.57	515.79 Da (-0.07 mmu/-0.14 ppm)	2	11.42	2.34	1
38	304	300-309	ECCDK*PILLEK	1333.61	667.30 Da (-0.49 mmu/-0.73 ppm)	2	15.60	1.71	1
39	399	387-401	DDPHACYSTVFDK*LK	1837.84	613.28 Da (+0.66 mmu/+1.08 ppm)	3	22.08	1.46	1
40	528	524-544	AFDEK*LFTFHADICTLPDTEK	2540.20	847.40 Da (+0.72 mmu/+0.85 ppm)	3	26.59	4.06	1
41	374	372-399	LAK*EYEATLEECCA KDDPHACYSTVFDK	3392.48	848.87 Da (+1.9 mmu/+2.24 ppm)	4	19.91	3.28	2
42	266	264-280	VHK*ECCHGDLLECAADDR	2155.89	539.72 Da (+0.65 mmu/+1.21 ppm)	4	14.06	2.57	1
43	155	139-156	LKPDPNTLCDEFKADEK*K	2190.07	730.69 Da (+0.56 mmu/+0.77 ppm)	3	18.06	1.79	2
Bisacodyl induced acetylation modifications									
1	183	168-197	RHPYFYAPELLYYANK*YNGVFQEECCQAEDK	3815.73	954.68 Da (+1.26 mmu/+1.32 ppm)	4	28.16	7.43	2
2	44	35-65	FKDLGEEHFK*GLVLIAFSQYLQQCPFDEHVK	3764.89	941.97 Da (+4.83 mmu/+5.13 ppm)	4	33.55	6.73	2
3	318	310-340	SHCIAEVEK*DAIPENLPPLTADFAEDKDVK	3553.69	1185.23 Da (+4.03 mmu/+3.4 ppm)	3	24.92	5.49	2
4	44	37-65	DLGEEHFK*GLVLIAFSQYLQQCPFDEHVK	3489.74	873.19 Da (+8.21 mmu/+9.4 ppm)	4	35.07	5.27	1
5	420	413-433	QNCDQFEK*LGEYGFQNALIVR	2571.23	857.74 Da (+1.66 mmu/+1.93 ppm)	3	27.19	5.0	1
6	266	264-285	VHK*ECCHGDLLECAADDRADLAK	2654.18	885.39 Da (+1.75 mmu/+1.97 ppm)	3	15.93	4.53	2
7	401	400-412	LK*HLVDEPQNLIK	1588.90	794.95 Da (+0.62 mmu/+0.78 ppm)	2	19.32	4.72	1
8	140	139-155	LK*PDPNTLCDEFKADEK	2061.98	687.99 Da (+1.62 mmu/+2.36 ppm)	3	20.20	4.39	1
9	36&44	35-65	FK*DLGEEHFK*GLVLIAFSQYLQQCPFDEHVK	3806.90	952.48 Da (+4.94 mmu/+5.19 ppm)	4	35.18	4.13	2
10	489	483-496	LCVLHEK*TPVSEK	1581.83	791.42 Da (+1.12 mmu/+1.42 ppm)	2	15.82	4.03	1
11	568	562-580	ATEEQLK*TMENFVAFVDK	2241.12	798.49 Da (+1.74 mmu/+2.17 ppm)	3	35.77	3.90	1
12	100	89-105	SLHTLFGDELCK*VASLR	1988.02	663.34 Da (+0.64 mmu/+0.96 ppm)	3	28.28	3.82	1
13	374	372-386	LAK*EYEATLEECCA K	1856.84	928.92 Da (+1.93 mmu/+2.08 ppm)	2	17.31	3.87	1
14	547&548	545-557	QIK*K*QTALVELLK	1595.97	798.49 Da (+1.74 mmu/+2.17 ppm)	2	26.24	3.74	2
15	374	372-399	LAK*EYEATLEECCA KDDPHACYSTVFDK	3392.48	848.87 Da (+3.18 mmu/+3.75 ppm)	4	20.04	3.66	2
16	346	341-359	NYQEAK*DAFLGSFLYEYSR	2343.09	781.70 Da (+1.69 mmu/+2.16 ppm)	3	31.27	3.45	1
17	437	437-451	K*VPQVSTPTLVEVSR	1681.95	561.32 Da (+1.04 mmu/+1.86 ppm)	3	20.20	3.24	1
18	228	223-232	CASIQK*FGER	1237.60	619.30 Da (+0.78 mmu/+1.26 ppm)	2	15.64	3.20	1

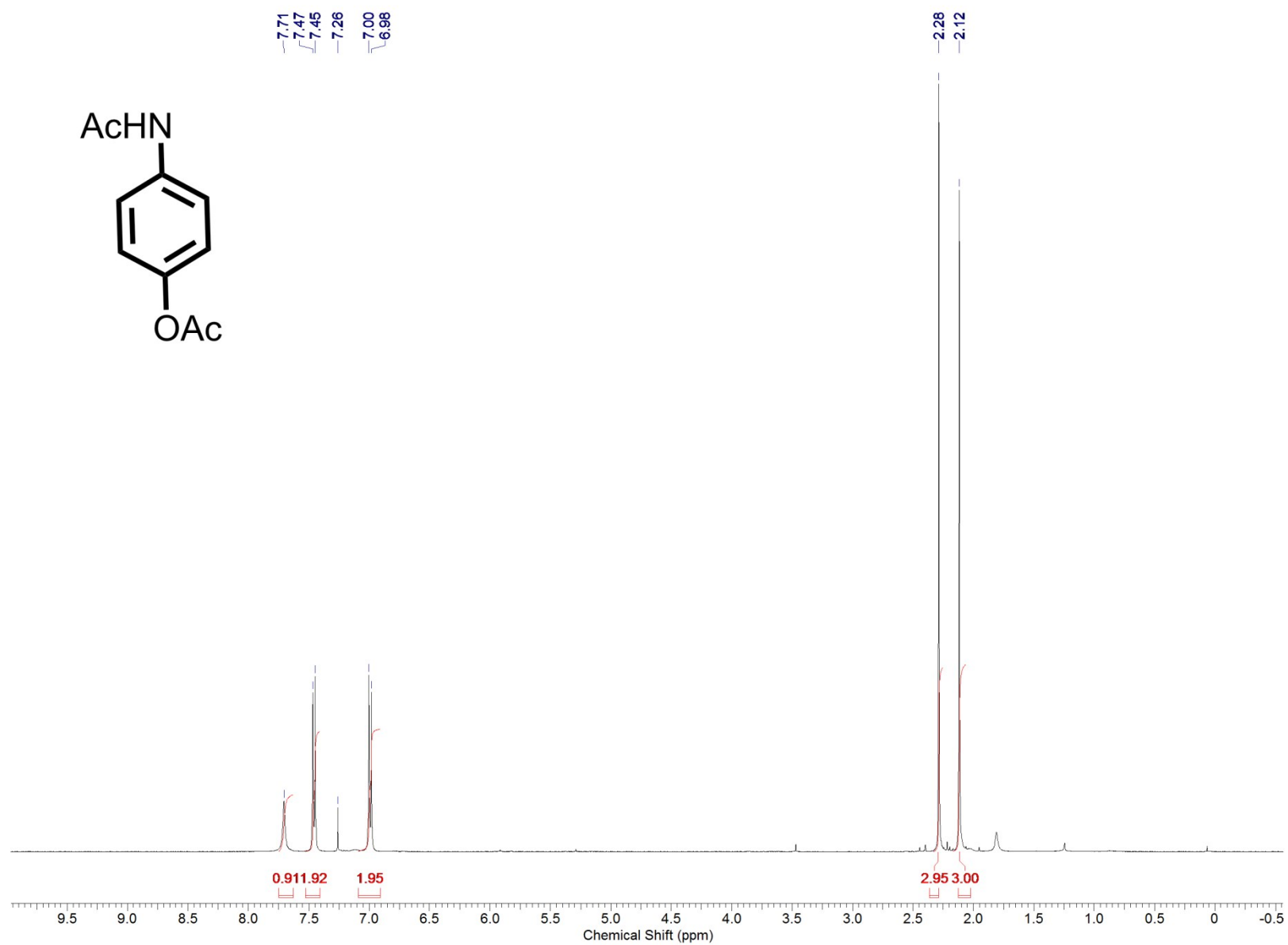
19	548	548-557	K*QTALVELLK	1184.72	592.86 Da (+0.83 mmu/+1.4 ppm)	2	23.63	3.31	1
20	266	264-280	VHK*ECCHGDLLECADDR	2155.90	539.73 Da (+1.33 mmu/+2.46 ppm)	4	14.25	3.01	1
21	160	157-167	FWGK*YLIEIAR	1487.77	744.38 Da (+1.11 mmu/+1.5 ppm)	2	27.52	2.87	1
22	256	249-263	AEFVEVTK*LVTDLTK	1734.95	867.97 Da (-0.08 mmu/-0.09 ppm)	2	31.20	2.91	1
23	160	156-167	KFWGK*YLIEIAR	1615.86	539.29 Da (+1.13 mmu/+2.09 ppm)	3	25.23	2.86	2
24	463	460-468	CCTK*PESER	1208.50	604.75 Da (+1.09 mmu/+1.8 ppm)	2	8.39	2.82	1
25	36	35-44	FK*DLGEEHFK	1291.63	431.21 Da (+0.53 mmu/+1.22 ppm)	3	16.45	2.73	1
26	523	508-528	RPCFSALTPDETYVPK*AFDEK	2513.20	838.40 Da (+2.32 mmu/+2.77 ppm)	3	22.85	2.73	1
27	204	198-209	GACLLPK*IETMR	1430.74	715.87 Da (-0.83 mmu/-1.15 ppm)	2	23.34	2.62	1
28	28	25-34	DTHK*SEIAHR	1235.61	412.54 Da (-0.08 mmu/-0.2 ppm)	3	8.28	2.61	1
29	401&412	400-420	LK*HLVDEPQNLIK*QNCDQFEK	2680.34	894.11 Da (+0.27 mmu/+0.31 ppm)	3	21.16	2.57	2
30	455	452-458	SLGK*VGTR	859.50	430.25 Da (+0.97 mmu/+2.25 ppm)	2	11.35	2.55	1
31	235	232-241	ALK*AWSVAR	1043.60	522.30 Da (+0.96 mmu/+1.83 ppm)	2	19.20	2.51	1
32	544	529-547	LFTFHADICTLPDTEK*QIK	2319.16	773.72 Da (+0.16 mmu/+0.21 ppm)	3	24.02	2.49	1
33	248	246-256	FPK*AEFVEVTK	1336.71	668.86 Da (+0.96 mmu/+1.44 ppm)	2	21.24	2.47	1
34	297	286-299	YICDNQDTISSK*LK	1726.83	863.92 Da (+1.6 mmu/+1.86 ppm)	2	17.86	2.28	1
35	304	298-309	LKECCDK*PLLEK	1574.79	525.60 Da (+0.68 mmu/+1.3 ppm)	3	15.46	2.01	1
36	263	257-266	LVTDLTK*VHK	1195.70	399.24 Da (+0.35 mmu/+0.88 ppm)	3	15.32	2.40	1
37	245	242-248	LSQK*FPK	889.51	445.26 Da (+0.48 mmu/+1.09 ppm)	2	14.06	1.69	1
38	495	490-498	TPVSEK*VTK	1030.57	515.79 Da (+0.72 mmu/+1.4 ppm)	2	11.32	1.53	1
39	399	287-401	DDPHACYSTVFDK*LK	1837.84	613.28 Da (+1.82 mmu/+2.97 ppm)	3	22.20	1.38	1
40	528	528-544	AFDEK*LFTFHADICTLPDTEK	2540.20	847.40 Da (+1.64 mmu/+1.93 ppm)	3	26.82	4.25	1
41	412	402-420	HLVDEPQNLIK*QNCDQFEK	2397.15	799.72 Da (-0.04 mmu/-0.05 ppm)	3	19.62	3.78	1
42	498	496-507	VTK*CCTESLVNR	1508.72	754.86 Da (+0.68 mmu/+0.9 ppm)	2	14.24	2.99	1
43	65	45-75	GLVLIAFSQYLQQCPFDEHVK*LVNELTEFAK	3678.90	920.48 Da (+3.25 mmu/+3.53 ppm)	4	35.37	2.46	1
NDS-458 induced acetylation modifications									
1	183	168-197	RHPYFYAPELLYYANK*YNGVVFQECQAEDK	3815.73	1272.58 Da (+3.25 mmu/+2.55 ppm)	3	27.88	8.17	2
2	528	524-544	AFDEK*LFTFHADICTLPDTEK	2540.20	1270.60 Da (+2.56 mmu/+2.01 ppm),	2	26.72	7.14	1
3	183	169-197	HPYFYAPELLYYANK*YNGVVFQECQAEDK	3659.63	1220.55 Da (+4 mmu/+3.28 ppm)	3	29.43	6.60	1
4	36&44	35-65	FK*DLGEEHFK*GLVLIAFSQYLQQCPFDEHVK	3806.90	952.48 Da (+2.91 mmu/+3.05 ppm)	4	35.12	6.60	1
5	580	569-597	TVMENFVAFVDK*CCAADDKEACFAVEGPK	3350.49	1117.50 Da (+2.38 mmu/+2.13 ppm)	3	28.75	6.57	2
6	266	264-285	VHK*ECCHGDLLECADDRADLAK	2654.18	885.39 Da (+2.54 mmu/+2.87 ppm)	3	15.89	5.91	2
7	336	310-340	SHCIAEVEKDAIPENLPPLTADFAEDK*DVCK	3553.69	889.17 Da (+2.78 mmu/+3.12 ppm)	4	24.32	5.71	2
8	420	413-433	QNCDQFEK*LGEYGFQNALIVR	2571.23	1286.12 Da (+3.07 mmu/+2.39 ppm)	2	27.18	5.54	1

9	401	400-420	LK*HLVDEPNLIK*QNCDQFEK	2680.34	894.12 Da (+2.41 mmu/+2.7 ppm)	3	21.25	5.46	2
10	44	35-65	FKDLGEEHFK*GLVLIAFSQYLQQCPFDEHVK	3764.90	753.78 Da (+5.58 mmu/+7.4 ppm)	5	33.49	5.55	2
11	399&401	287-412	DDPHACYSTVFDK*LK*HLVDEPNLIK	3166.57	792.40 Da (+7 mmu/+8.84 ppm)	4	33.37	5.22	2
12	568	562-580	ATEEQLK*TMENFVAFVDK	2241.11	1121.06 Da (+3.55 mmu/+3.16 ppm)	2	35.39	4.81	1
13	346	341-359	NYQEAK*DAFLGSFLYEYSR	2343.09	1172.05 Da (+3.15 mmu/+2.69 ppm)	2	31.18	5.05	1
14	547&548	545-557	QIK*K*Q TALVELLK	1595.98	798.49 Da (+3.51 mmu/+4.39 ppm)	2	26.38	4.80	2
15	401	400-412	LK*HLVDEPNLIK	1588.91	530.30 Da (+2.67 mmu/+5.04 ppm)	3	19.23	5.02	1
16	587	581-597	CCAADDK*EACFAVEGPK	1969.81	985.41 Da (+2.16 mmu/+2.19 ppm)	2	17.14	4.46	1
17	75	66-88	LVNELTEFAK*TCVADESHAGCEK	2650.22	884.07 Da (+3.55 mmu/+4.02 ppm)	3	24.10	4.66	1
18	285	267-297	ECCHGDLLECADDRADLAK*YICDNQDTISSK	3714.58	929.40 Da (+2.29 mmu/+2.47 ppm)	4	22.39	4.81	2
19	100	89-105	SLHTLFGDELCK*VASLR	1988.02	663.34 Da (+0.82 mmu/+1.23 ppm)	3	28.23	4.40	1
20	266	264-280	VHK*ECCHGDLLECADDR	2155.90	719.30 Da (+3.12 mmu/+4.33 ppm)	3	14.23	4.16	1
21	140	139-155	LK*PDPNTLCDEKADEK	2061.98	688.00 Da (+2.97 mmu/+4.31 ppm)	3	20.08	4.25	1
22	489	483-496	LCVLHEK*TPVSEK	1581.83	791.42 Da (+2.28 mmu/+2.88 ppm)	2	15.71	4.31	1
23	44	37-65	DLGEEHFK*GLVLIAFSQYLQQCPFDEHVK	3489.72	873.18 Da (+2.22 mmu/+2.55 ppm)	4	35.14	4.0	1
24	256	249-263	AEFVEVTK*LVTDLTK	1734.95	867.98 Da (+2.06 mmu/+2.37 ppm)	2	31.24	4.02	1
25	412	402-420	HLVDEPNLIK*QNCDQFEK	2397.15	799.72 Da (+1 mmu/+1.25 ppm)	3	19.50	4.10	1
26	160	156-167	KFWGK*YLYEIAR	1615.86	808.43 Da (+1.66 mmu/+2.06 ppm)	2	25.12	3.79	2
27	248	246-256	FPK*AEFVEVTK	1336.71	668.86 Da (+1.51 mmu/+2.26 ppm)	2	21.08	3.74	1
28	523	508-528	RPCFSALTPDETYVPK*AFDEK	2513.20	838.40 Da (+1.47 mmu/+1.75 ppm)	3	22.98	3.91	1
29	437	437-451	K*VPQVSTPTLVEVSR	1681.95	561.32 Da (+1.84 mmu/+3.27 ppm)	3	20.20	3.56	1
30	28	25-34	DTHK*SEIAHR	1235.61	412.54 Da (+0.53 mmu/+1.28 ppm)	3	8.31	3.53	1
31	544&547	529-548	LFTFHADICTLPDETEK*QIK*K	2489.28	830.43 Da (+2.42 mmu/+2.92 ppm)	3	23.96	3.53	2
32	297	286-299	YICDNQDTISSK*LK	1726.83	863.92 Da (+2.52 mmu/+2.92 ppm)	2	18.02	3.47	1
33	204	198-209	GACLLPK*IETMR	1430.75	715.87 Da (+0.7 mmu/+0.98 ppm)	2	23.23	3.40	1
34	374	372-386	LAK*EYEATLEECCAK	1856.84	928.92 Da (+1.93 mmu/+2.08 ppm)	2	16.99	3.57	1
35	299	298-309	LK*ECCDKPLLEK	1574.79	525.60 Da (+1.6 mmu/+3.04 ppm)	3	14.27	3.31	1
36	304	298-309	LKECCDK*PLLEK	1574.79	787.90 Da (+1.24 mmu/+1.58 ppm)	2	15.31	3.30	1
37	228	223-232	CASIQK*FGER	1237.60	619.30 Da (+1.15 mmu/+1.85 ppm)	2	15.51	3.27	1
38	495	490-498	TPVSEK*VTK	1030.58	515.79 Da (+1.46 mmu/+2.83 ppm)	2	11.25	3.12	1
39	160	157-167	FWGK*YLYEIAR	1487.77	744.38 Da (+1.85 mmu/+2.48 ppm)	2	27.47	3.12	1
40	36	35-44	FK*DLGEEHFK	1291.63	646.32 Da (+1.39 mmu/+2.15 ppm)	2	16.33	2.87	1
41	548	548-557	K*Q TALVELLK	1184.72	592.86 Da (+1.74 mmu/+2.94 ppm)	2	24.09	3.06	1
42	463	460-468	CCTK*PESER	1208.50	604.75 Da (+1.4 mmu/+2.31 ppm)	2	8.34	3.05	1

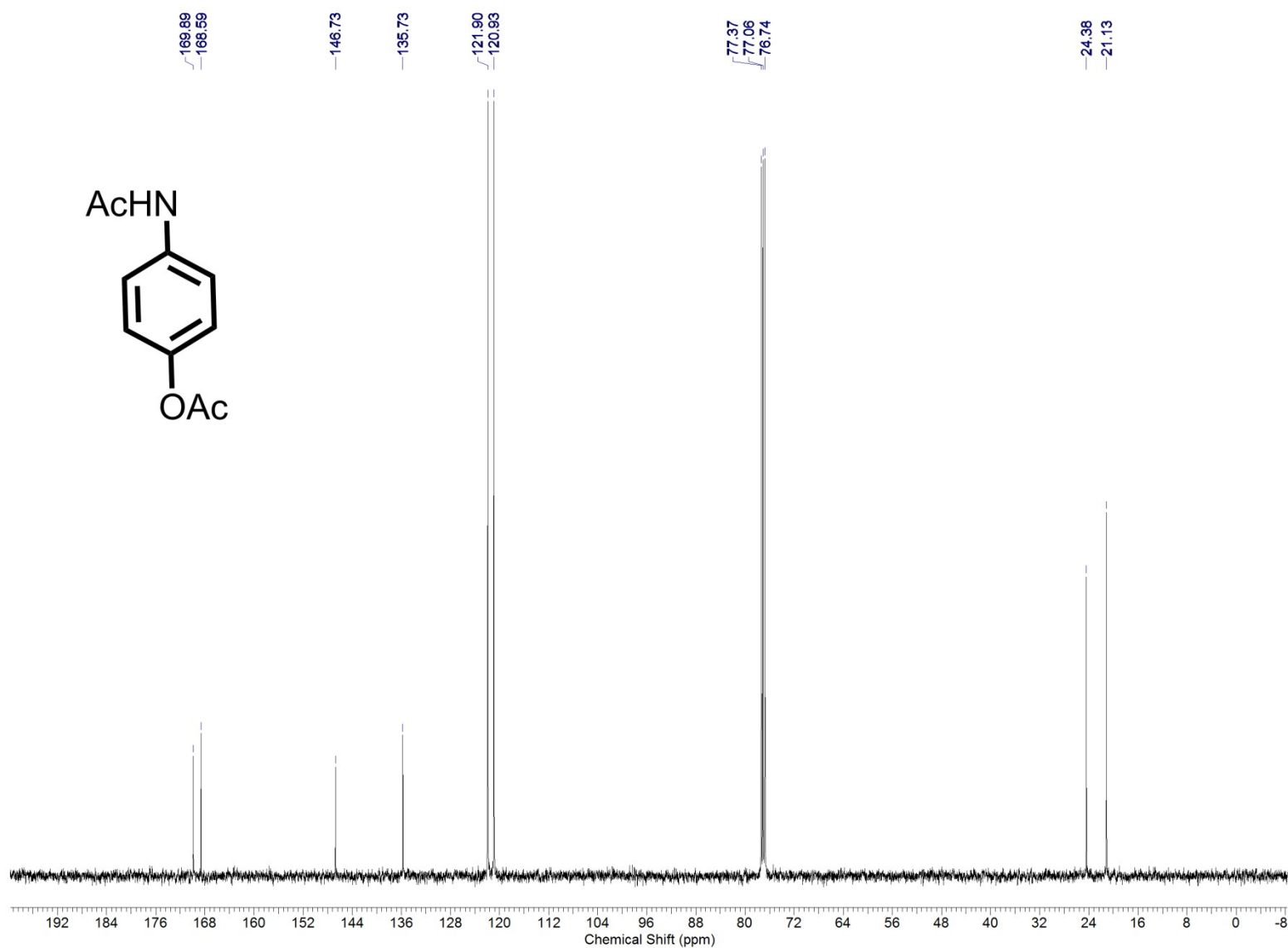
43	559	558-568	HK*PKATEEQLK	1350.74	450.91 Da (+0.81 mmu/+1.81 ppm)	3	8.59	3.46	2
44	140	139-156	LK*PDPNTLCDEFK*ADEKK	2232.08	744.70 Da (+1.38 mmu/+1.85 ppm)	3	20.43	2.73	2
45	235	232-241	ALK*AWSVAR	1043.60	522.30 Da (+1.81 mmu/+3.47 ppm)	2	18.90	2.65	1
46	263	257-266	LVTDLTK*VHK	1195.70	399.24 Da (+0.96 mmu/+2.41 ppm)	3	15.21	2.79	1
47	248	242-256	LSQKFPK*AEFVEVTK	1792.98	598.33 Da (+1.23 mmu/+2.06 ppm)	3	20.91	2.50	2
48	304	300-309	ECCDK*P LLEK	1333.61	667.31 Da (+0.98 mmu/+1.47 ppm)	2	15.53	1.95	1
49	65	45-75	GLVLIAFSQYLQQCPFDEHVK*LVNELTEFAK	3678.90	1226.97 Da (+4.12 mmu/+3.35 ppm)	3	35.22	5.20	1
50	498	496-507	VTK*CTESLVNR	1508.72	754.86 Da (+0.5 mmu/+0.66 ppm)	2	14.37	3.74	1
51	318&336	310-340	SHCIAEVEK*DAIPENLPPLTADFAEDK*DVCK	3595.69	899.67 Da (+0.91 mmu/+1.02 ppm)	4	26.09	3.16	2
52	544	529-547	LFTFHADICTLPDTEK*QIK	2319.17	773.72 Da (+1.02 mmu/+1.32 ppm)	3	23.90	2.89	1
53	156&160	156-167	K*FWGK*YLYEIAR	1657.87	829.44 Da (+2.61 mmu/+3.14 ppm)	2	28.84	2.86	2
54	285	281-297	ADLAK*YICDNQDTISSK	1983.93	992.47 Da (+3.01 mmu/+3.03 ppm)	2	20.15	2.59	1
55	455	452-459	SLGK*VGTR	859.50	430.25 Da (+0.94 mmu/+2.18 ppm)	2	11.50	2.52	1
56	528&544	524-547	AFDEK*LFTFHADICTLPDTEK*QIK	2951.45	984.49 Da (+3.02 mmu/+3.07 ppm)	3	27.38	2.05	2
57	140	139-155	LK*PDPNTLCDEFK*ADEK	2103.99	702.00 Da (+1.52 mmu/+2.16 ppm)	3	21.22	2.21	1

Static modification: Carbamidomethyl (57.02 Da), Z-Charge state, RT-Retention time, MC-Missed cleavage, XC-Xcorr Value

Supplementary Figure 1: ^1H NMR spectrum of compound NDS-458 in CDCl_3 (400 MHz)

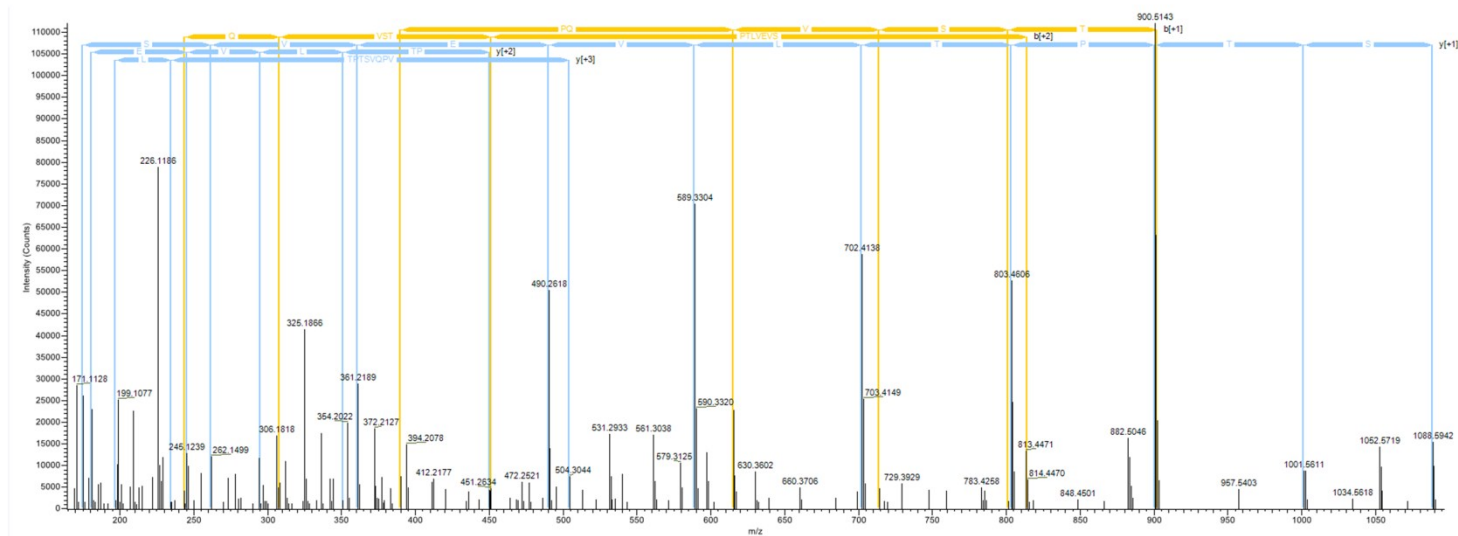


Supplementary Figure 2: ^{13}C NMR spectrum of compound NDS-458 in CDCl_3 (100 MHz)

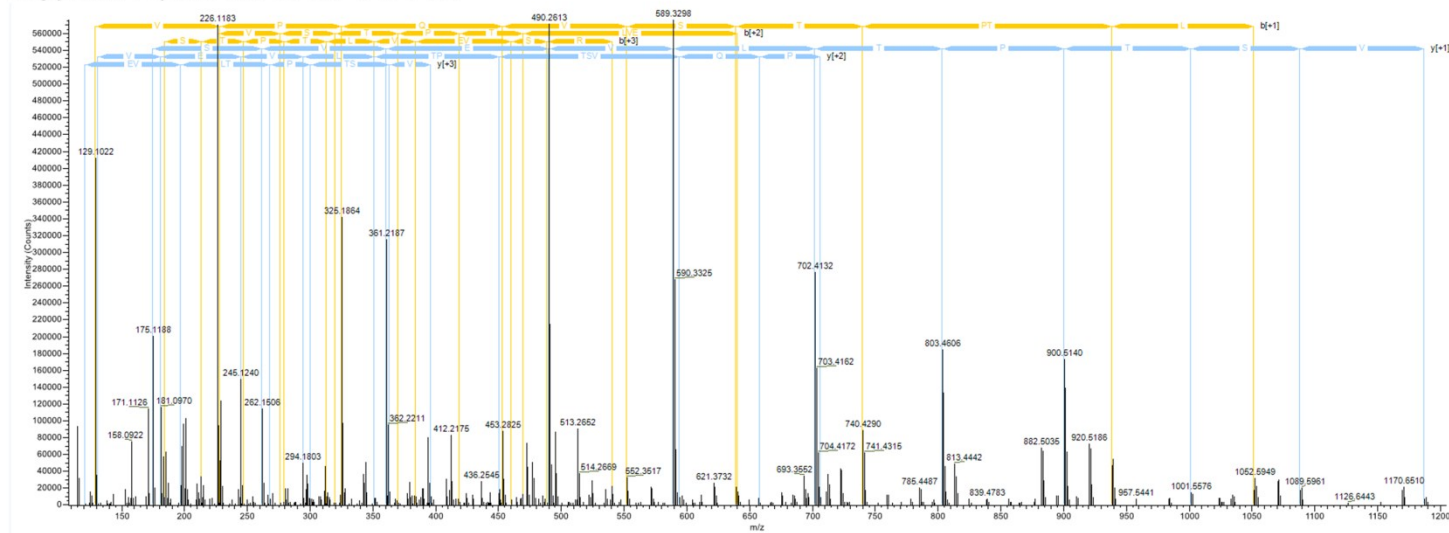


Supplementary Figure 3: A representative MS/MS annotation of Amadori modified peptide of BSA depicting diagnostic fragment ions (b ions) bearing 162.05 Da mass shift.

Glycated Peptide: **K[Amadori]VPQVSTPTLVEVSR**

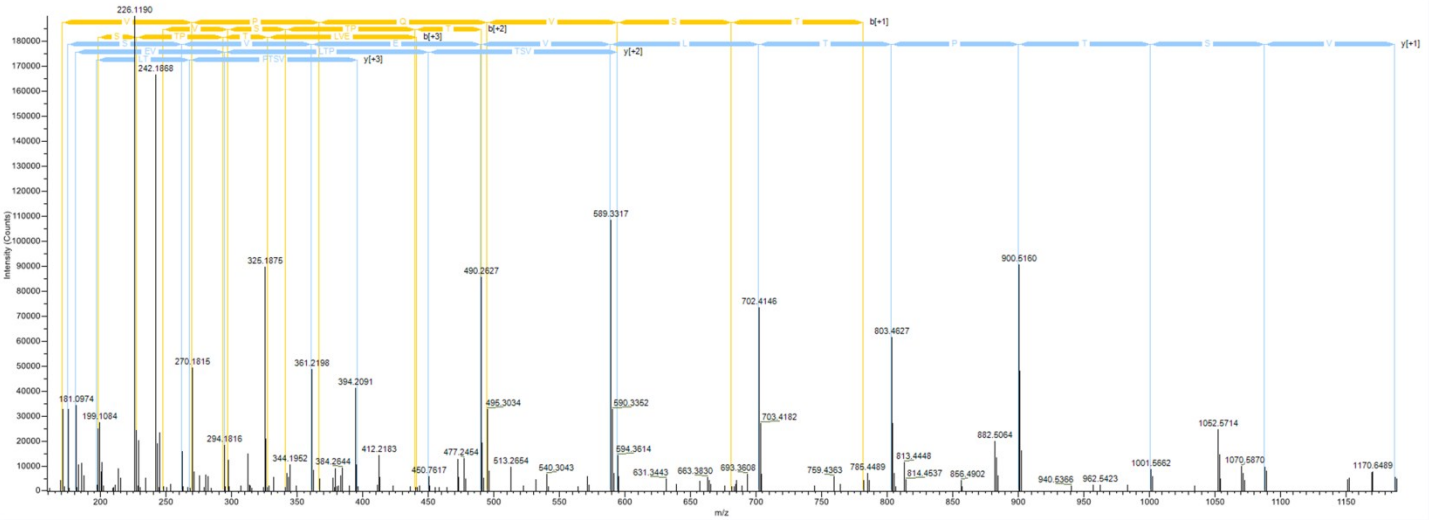


Unglycated Peptide: **KVPQVSTPTLVEVSR**

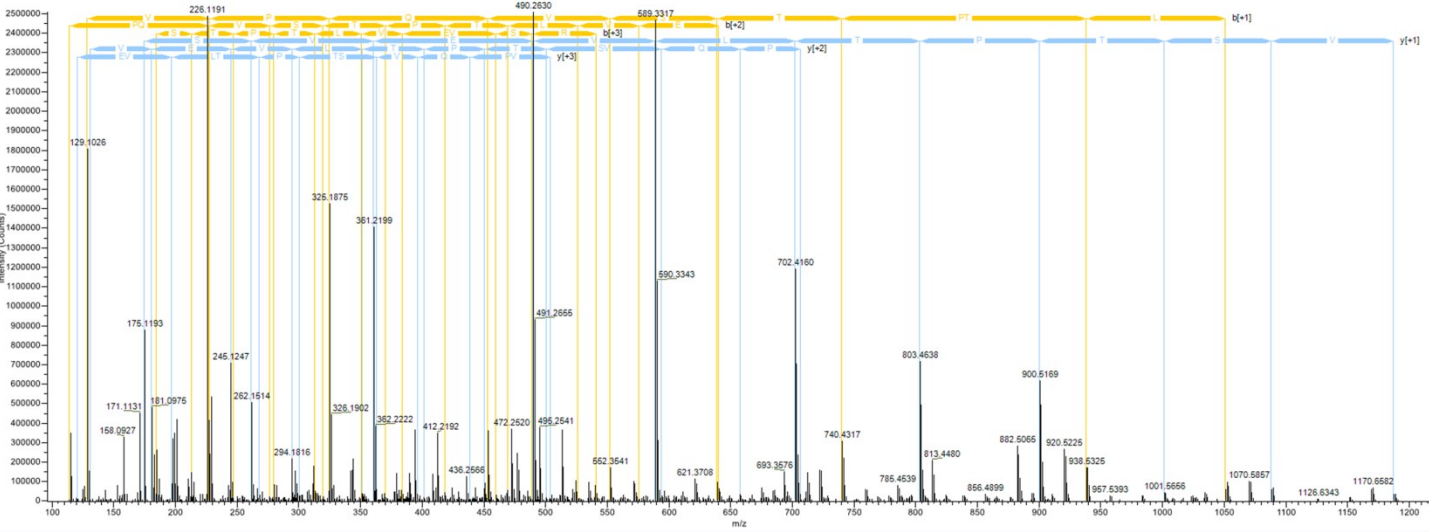


Supplementary Figure 4: A representative MS/MS annotation of Acetylated peptide of BSA depicting 42.02 Da mass shift.

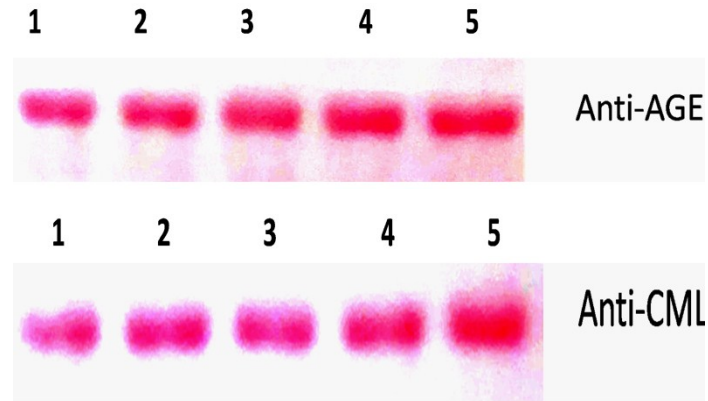
Acetylated Peptide: **K[Acetyl]VPQVSTPTLVEVSR**



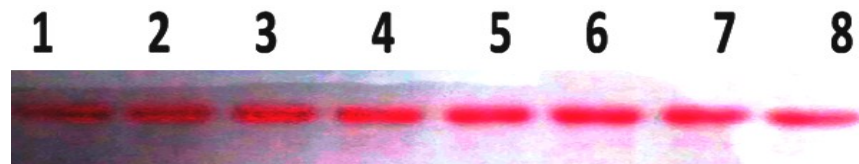
Unacetylated Peptide: **KVPQVSTPTLVEVSR**



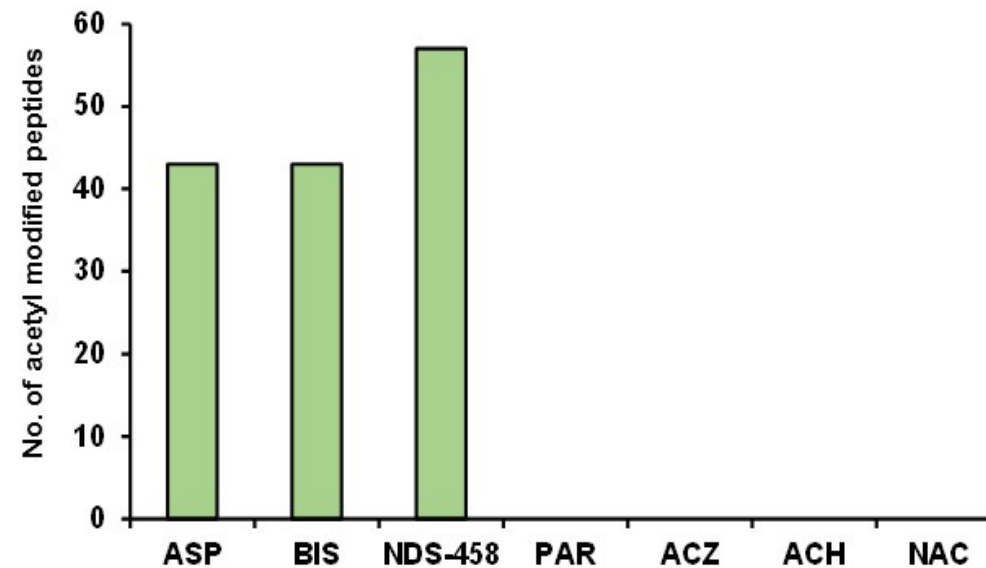
Supplementary Figure 5: Ponceau stain blot analysis utilizing anti-AGE and anti-CML antibodies of 1) Control BSA, 2) Glycated BSA, 3) Glycated BSA with ASP, 4) Glycated BSA with BIS and 5) Glycated BSA with NDS-458.



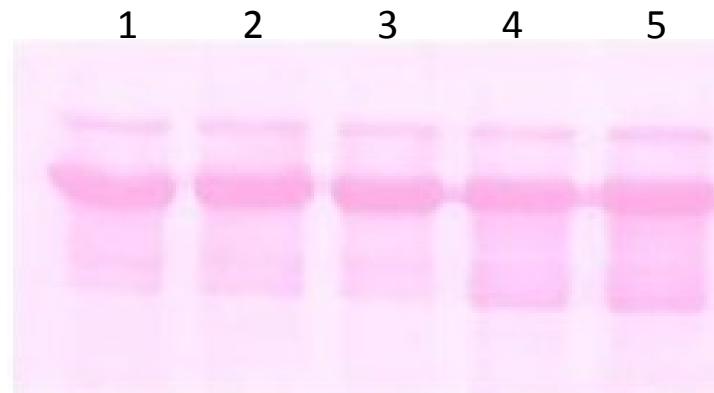
Supplementary Figure 6: Ponceau stain blot analysis utilizing anti-Acetyl lysine antibody of 1) BSA, 2) BSA with ASP, 3) BSA with BIS, 4) BSA with NDS-458, 5) BSA with Paracetamol, 6) BSA with Acetazolamide, 7) BSA with Acetohexamide and 8) BSA with N-Acetyl Cysteine.



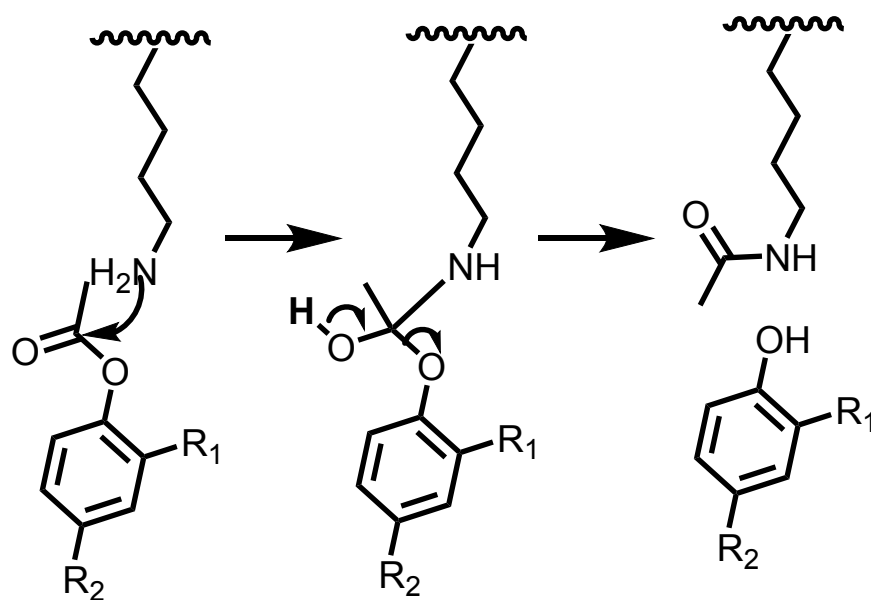
Supplementary Figure 7: Bar graph depicting the number of *in vitro* acetyl modified peptides of BSA, BSA in presence of various acetyl group containing molecules.



Supplementary Figure 8: Ponceau stain blot analysis utilizing anti-Acetyl lysine and anti-CML antibodies in in-vivo of plasma proteins of 1) Control mice, 2) Diabetic mice, 3) Aspirin treated control mice, 4) Diabetic mice treated with aspirin and 5) Pre-aspirin treated then STZ induced diabetic mice.



Supplementary Figure 9: Plausible mechanism of acetylation of amines by O-Acetyl group containing compounds.



$\text{R}_1 = \text{COOH}, \text{R}_2 = \text{H}$: Aspirin
 $\text{R}_1 = \text{H}, \text{R}_2 = \text{NHCOCH}_3$: NDS-458