Experimental procedures

Solid phase peptide synthesis (SPPS)

Solid phase peptide synthesis (SPPS) was performed using a microwave-assisted peptide synthesizer (CEM) or in a standard manual reaction vessel under argon. Rink-amide MBHA resin and Wang resin were purchased from Sigma-Aldrich. DMF, DMSO, NMP, DCM, MeOH, ACN and DIEA were dried and distilled using standard protocols. The peptides were purified on a Merck Hitachi HPLC using a reverse-phase C8 semi-preparative column (Vydac) with a gradient of 5% to 60% acetonitrile in water (both containing 0.001% (v/v) trifluoroacetic acid). The ^{1}H-NMR spectra were measured on 400 MHz or 600 MHz spectrometer (Bruker) and the ^{13}C-NMR spectra were measured at a 100 MHz frequency using $CDCl_3$ as solvent. The peptides identity was tested using MALDI-TOF Mass spectrometry on a PerSeptive Biosystems Voyager-DE PRO Biospectrometry workstation (Fig. S1). The peptides purity was confirmed using analytical HPLC (Fig. S2).

Fmoc deprotection: The peptidyl-resin was treated twice with 20% piperidine in NMP for 30 minutes using microwave irradiation. The product was washed 4 times using NMP.

HBTU/HOBt coupling of protected amino acids: Fmoc-protected amino acid (1.5 equiv), 1-hydroxybenztriazole hydrate (HOBt, 1.5 equiv), o-benzotriazole-1-yl-N,N,N,N-tetramethyluronium hexafluorophosphate (HBTU, 1.5 equiv) and diisopropyl-ethylamine (DIEA) (2 equiv) were dissolved in dry DMF. The solution was mixed with the resin-bound peptide and then irradiated in microwave for 5 minutes in the peptide synthesizer. The process was repeated twice and the resin was then washed with DMF, NMP and DCM.

HBTU coupling of malonic acid: Malonic acid (1 equiv) was activated by adding HBTU (1.2 equiv) and DIEA (1.5 equiv) in DMF or DMSO. The mixture was stirred for 10 minutes at 0 ^{o}C and then added into a vessel containing pre-swollen resin-bound peptide in DMF or DMSO. The reaction was then allowed to continue for another 1 hr. The completion of the reaction was monitored by the Kaiser-ninhydrin and chloranil tests. Negative response in these color tests indicated the completion of the reaction. The resin was then washed thoroughly with DMF, DCM, Ethanol and diethyl ether.

Cleavage of the peptide from the resin: A solution (10 ml) of trifluoroacetic acid (TFA)/TDW/triisopropylsilane (TIS) (92:4.5:3.5) was cooled to 0 ^{o}C and incubated with 200mg resin-bound peptide for 2h. The cleaved peptide was precipitated with ice cold diethyl ether, the solution centrifuged and the peptide washed twice more with ether. Then minimum volume of ACN/TDW (3:2) was used to dissolve the crude peptide. The solution was lyophilized before purification in HPLC.

Optimization of the acetylation reaction conditions

The activation of malonic acid (1 equiv) by HBTU (1.2 equiv) was optimized in presence of different bases (1.5 equiv) in different organic solvents as shown in Table S1. The mixture was stirred for 10 minutes at 0 ^{o}C and then added to pre-swollen resin-bound peptide 4 in the different solvents. The reaction was then allowed to continue for another 1 hr. The completion of the reaction was monitored by the Kaiser-ninhydrin and chloranil tests. The resin was then washed thoroughly with DMF, DCM, Ethanol and diethyl ether. A solution (10 ml) of trifluoroacetic acid (TFA)/TDW/triisopropylsilane (TIS) (92:4.5:3.5) was cooled to 0 ^{o}C and incubated with 200mg resin-bound peptide 4 for 2h. The cleaved peptide 30 was precipitated with ice cold diethyl ether, the solution centrifuged and the peptide washed twice more with ether. Then minimum volume of ACN/TDW (3:2) was used to dissolve the crude peptide and purified in HPLC. After optimization of bases and solvent system, we concluded that DIPEA/Et_3N in DMF/DMSO gave best yield in the shortest time (Table S1).

Table S1: Optimization of the acetylation reaction conditions

	Reactants	Solvent	Base	Time (mins)	Yield (%)
1	KILNPEEIEKYVAEI - resin, **4** + Malonic acid, **55**	DMF	DIPEA	16	98
2		DMF	Et_3N	16	98
3		DMF	DBU	18	72
4		DMF	NMM	26	73
5		DMF	Pyridine	22	84
6		DMSO	DIPEA	17	96
7		NMP	DIPEA	25	75

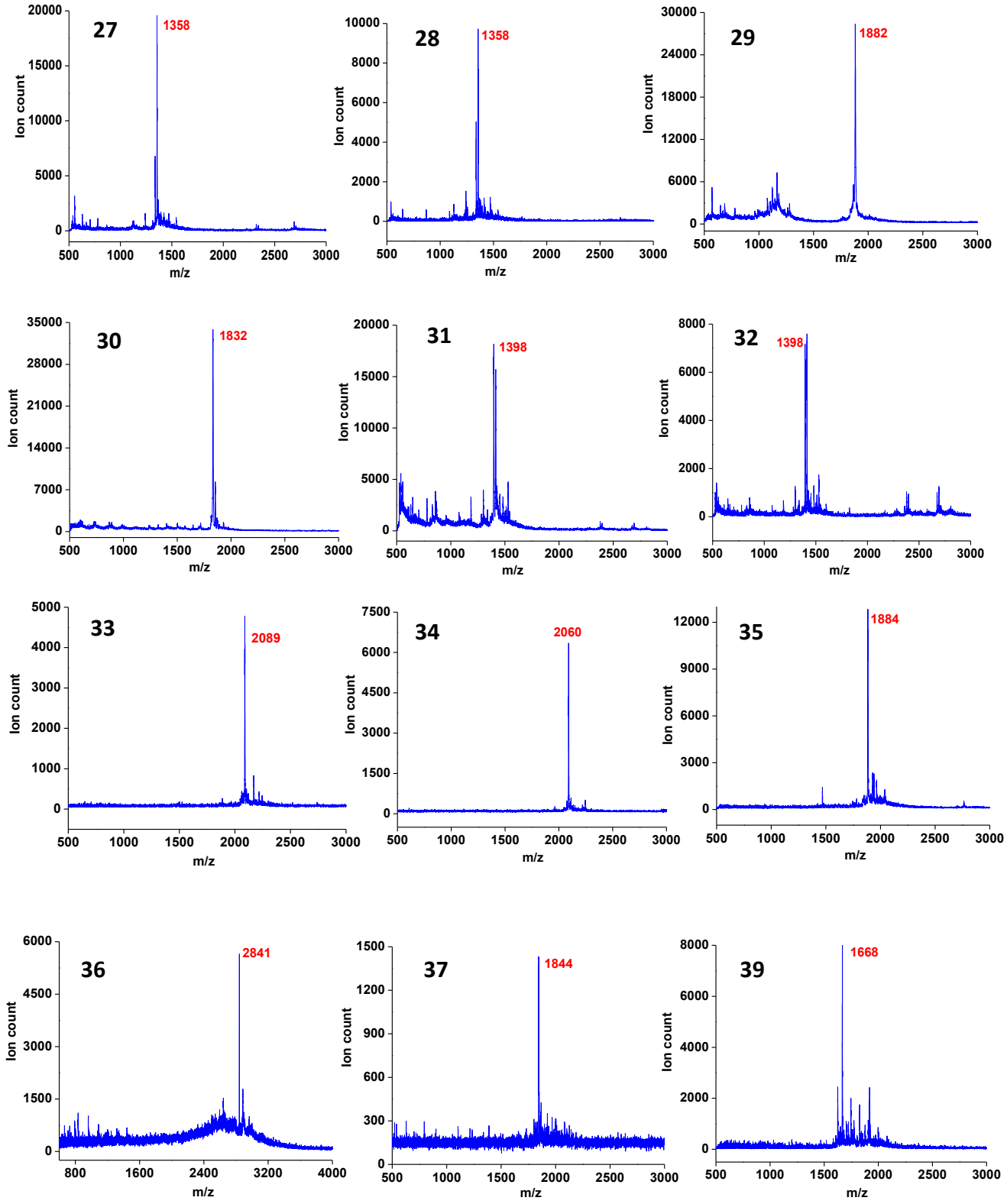

Figure S1: MALDI-TOF Mass of acetylated peptides as listed in table 1.

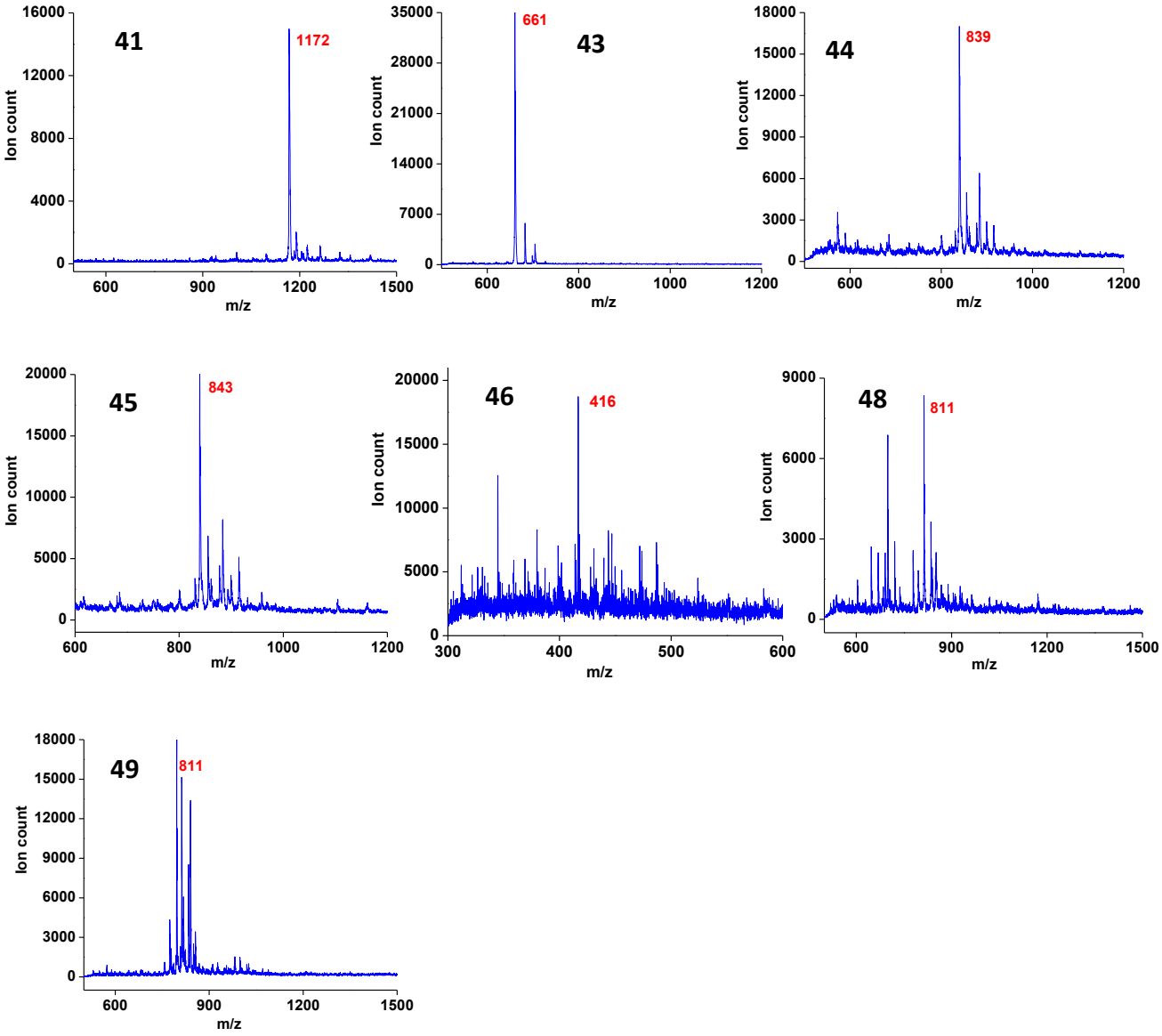

Figure S1 (continued): MALDI-TOF Mass of acetylated peptides as listed in table 1.

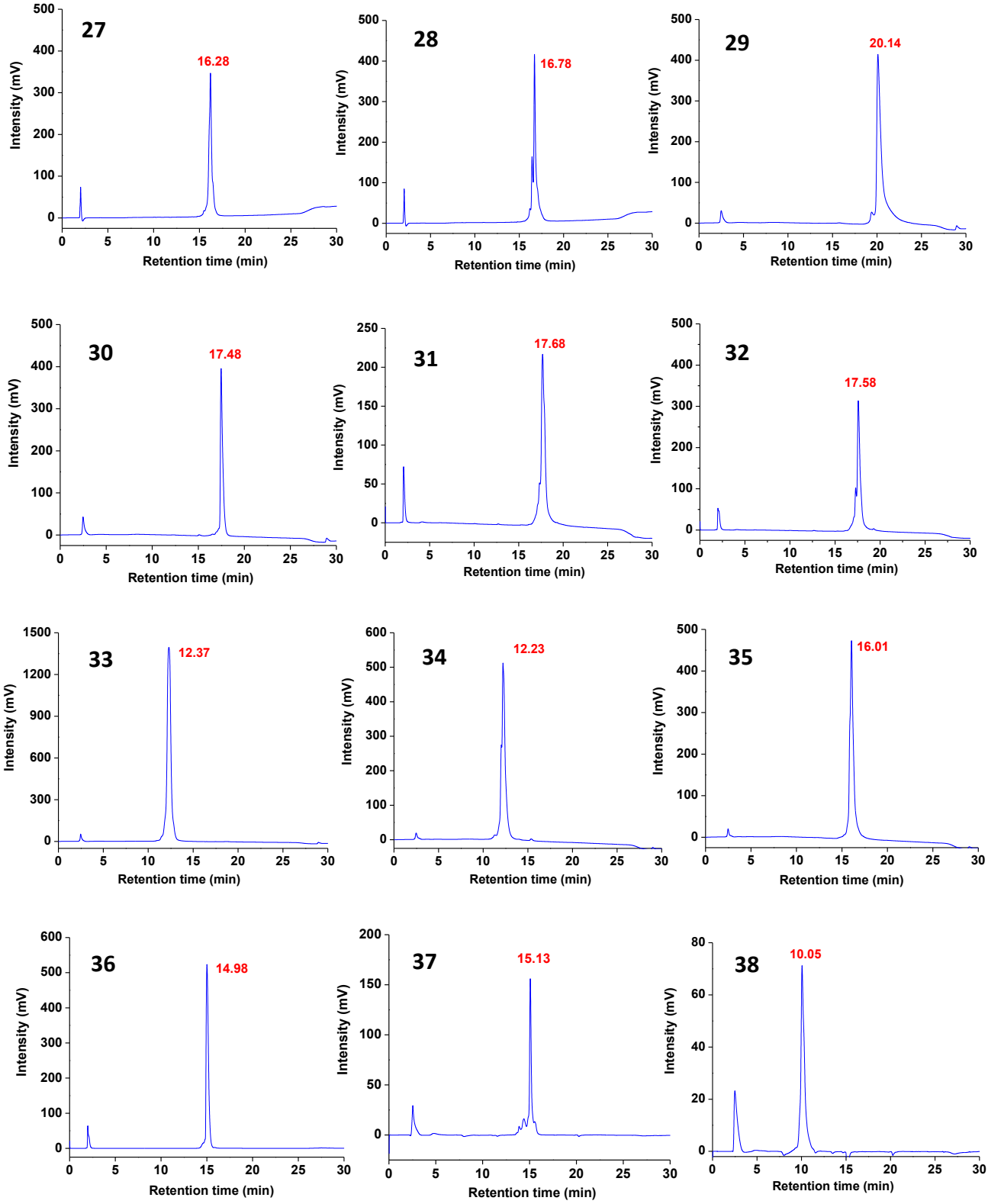

Figure S2: Reverse phase HPLC trace (in 5-60 Acetonitrile / Water) of acetylated peptides as listed in table 1.

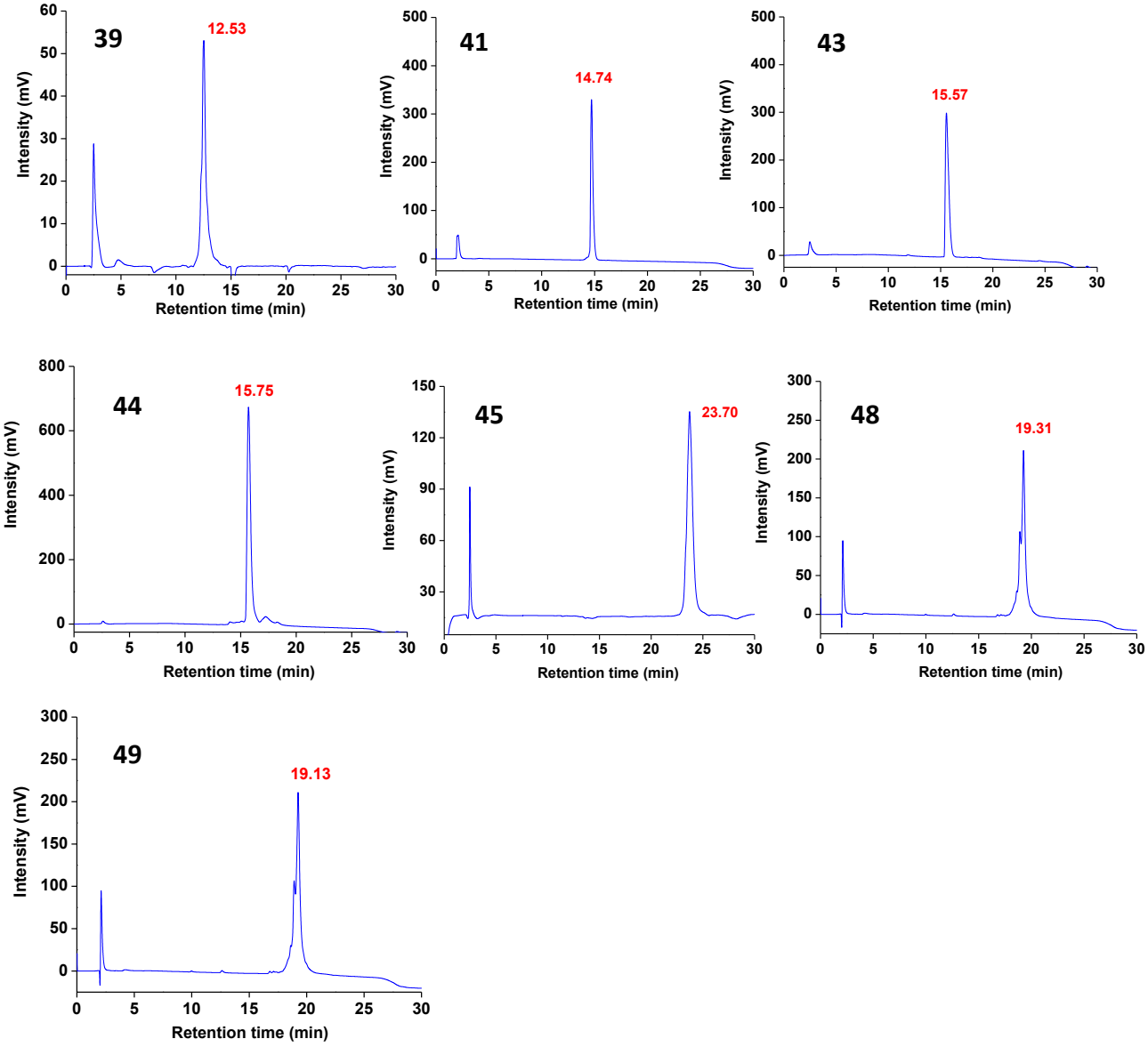

Figure S2 (continued): Reverse phase HPLC trace (in 5-60 Acetonitrile / Water) of acetylated peptides as listed in table 1.

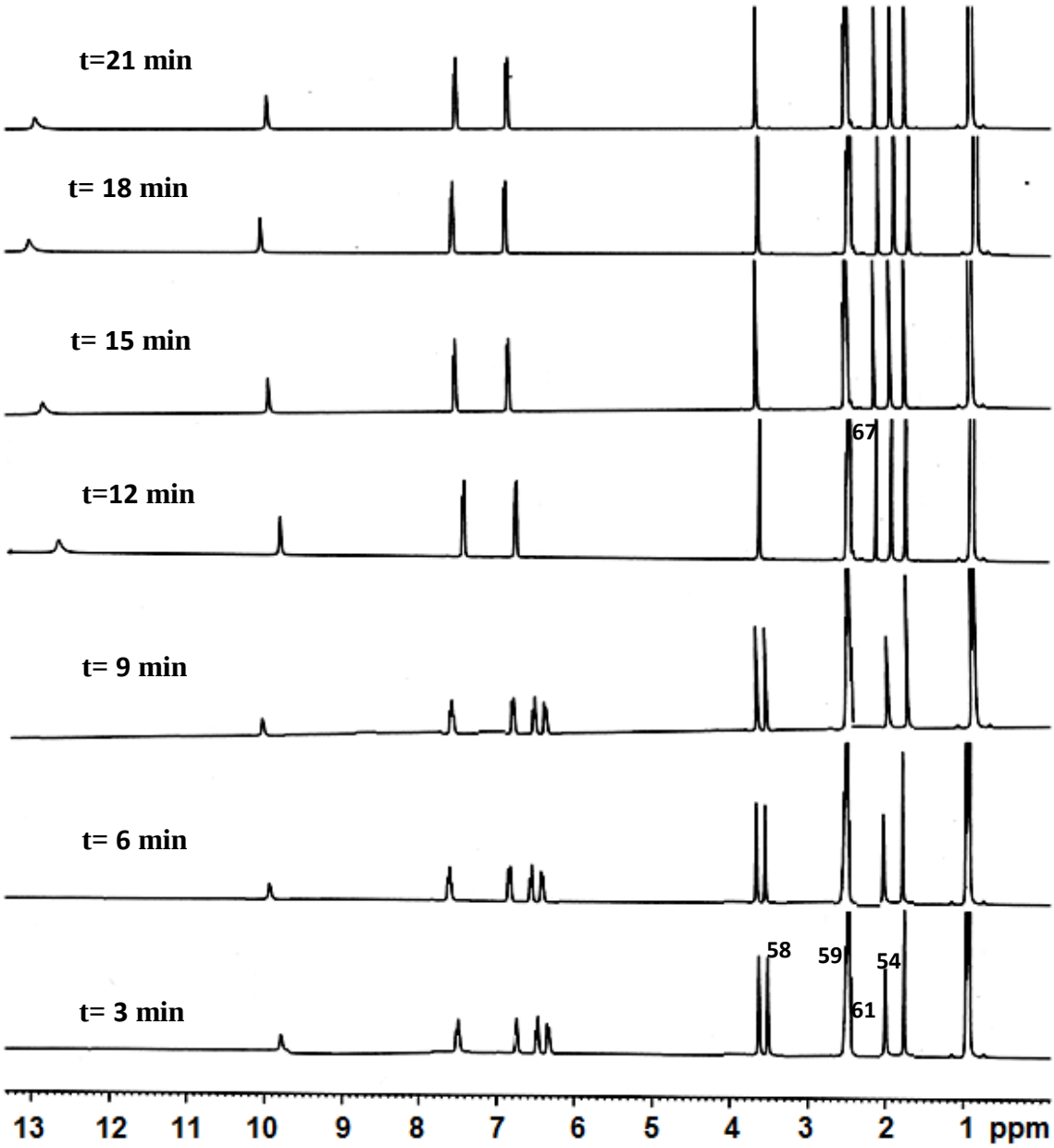

Figure S3: **^{1}H NMR kinetics of the acetylation of anisidine (53) in d_6-DMSO**

The plot of the integral of the peak at 1.86 ppm (acetylated anisidine, 54) relative to the peak at 2.7 ppm (tetramethyl urea, 59) versus the reaction time shows a sigmoidal nature. This indicates that the formation of the N-acetyl anisidine (54) from the ketene (61) in scheme 2 (main text) is irreversible. The process is fast and highly spontaneous since it took only 20 minutes to reach saturation.

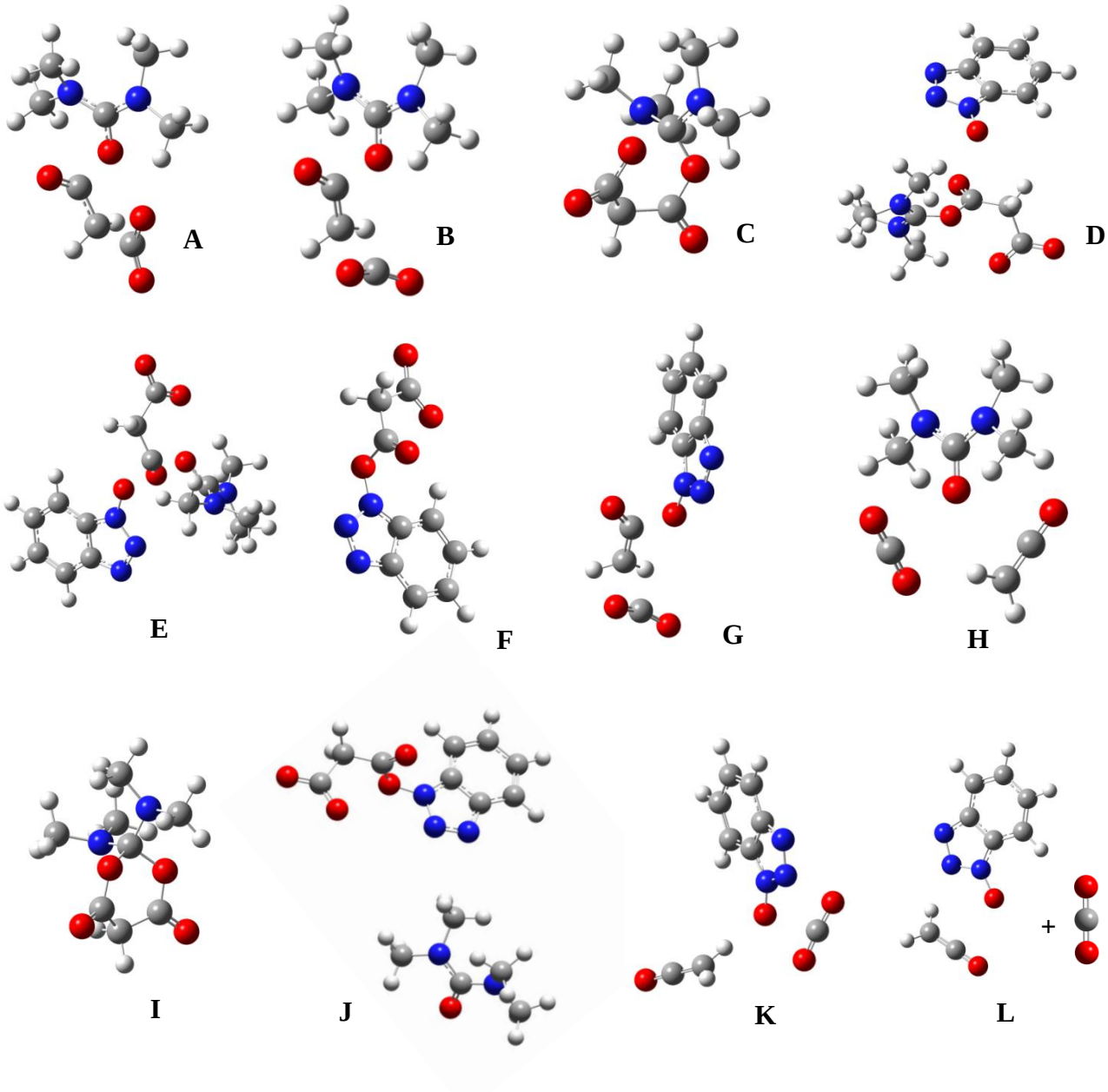

Figure S4: Optimized structures of compounds (58-64) for the three different possible pathways of acetylation as shown in figure 2 in the main text. A. Minimized Structure of the reactant (**58**) for path A. **B.** Optimized transition state structure of path A (**TS1**) for the self-dissociation process. **C.** Optimized transition state structure of path B (**TS2**). **D.** Minimized structure of reactant complex for the first step of path C (**59…62**) where the attack of 58 by HOBt anion takes place. **E.** Optimized transition state of first step of path C (**TS3a**). **F.** Minimized structure of the reactant complex for path C (**63**). **G.** Optimized transition state of path C (**TS3b**), which represents the self-dissociation. **H.** Minimized structure of the product for path A (**59--61**). **I.** Minimized intermediate product for path B (**64**) from where no self-dissociation is possible. **J.** Minimized structure of the product for the first step of path C (**63…59**) **K.** Minimized structure of the final product for path C (**60** + [**61…62**]). **L.** Minimized structure of the product ([**60….62**] + $\mathbf{CO_2}$) where it was considered that the CO_2 already left the reaction medium.