

Electronic Supplementary Information

Discovery and Validation of SIRT2 Inhibitors Based on Tenovin-6: Use of a ^1H -NMR Method to Assess Deacetylase Activity

Figure S1. H1299 cells were treated with tenovin-43 at the indicated concentrations for 16 h together with 40 nM trichostatin A to reduce background due to non-sirtuin HDAC activity. K40-acetylated α -tubulin and total α -tubulin were analysed.

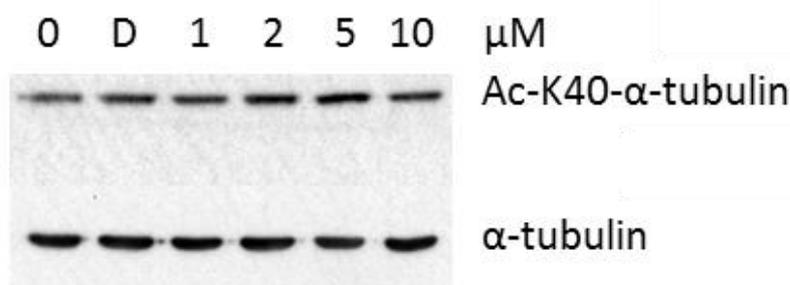


Figure S2. Band-selective ^1H , ^{13}C -HMBC of GLGKGGAK(Ac)RHR peptide at room temperature shows a crosspeak for the singlet at 1.86 ppm with the carbonyl carbon at 173.8 ppm which supports the assignment to the acetyl CH_3 . Furthermore, the spectrum enabled the identification of the terminal methylene group of the lysine residue (triplet at 3.03 ppm) which also shows a crosspeak with the carbonyl carbon at 173.8 ppm.

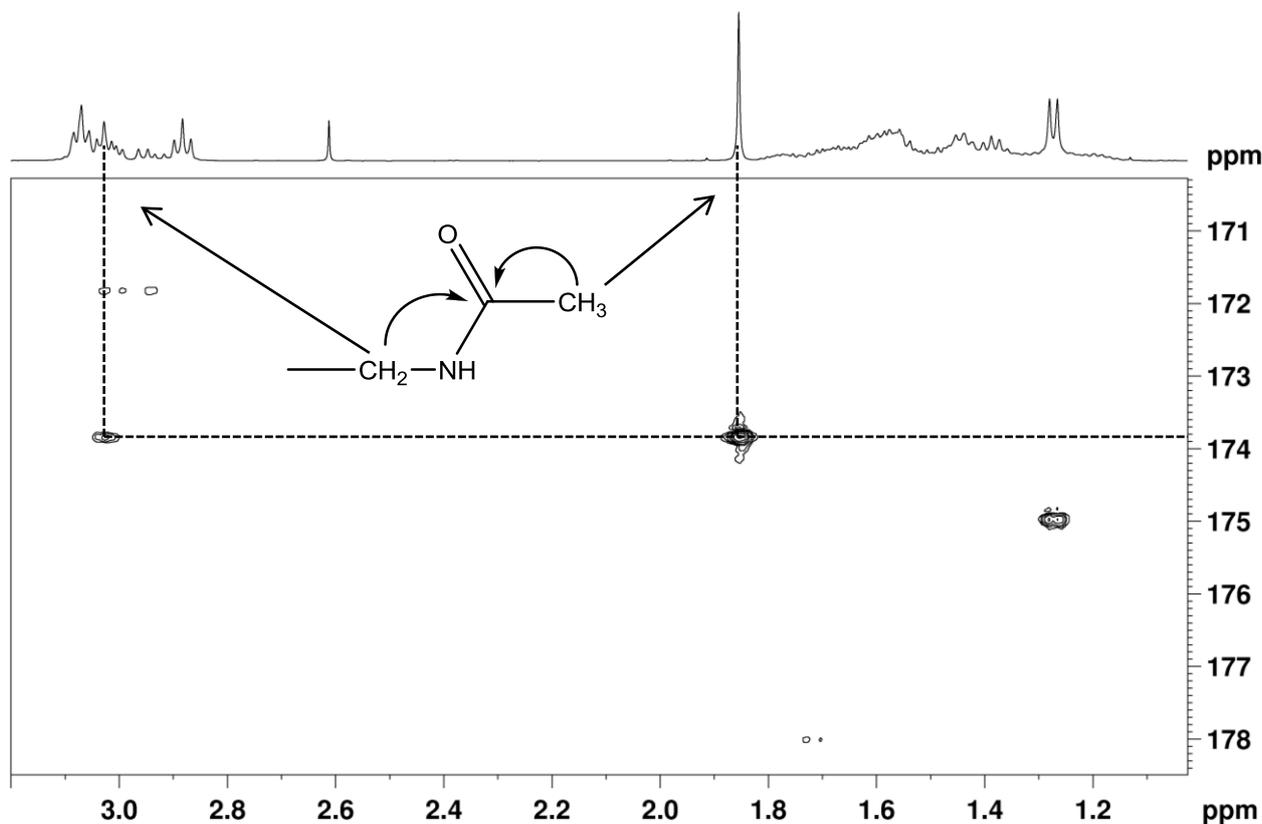


Figure S3. (a) 1D gs-TOCSY of GLGKGGAK(Ac)RHR peptide at r.t. The α -proton (4.12 ppm) was selectively excited and used as the starting point for coherence transfer to reveal all resonances of the N-acetylated lysine residue (1.61 (β), 1.20 (γ), 1.39 (δ) and 3.03 (ϵ) ppm). $^1\text{H-NMR}$ spectrum of: (b) the assay mixture before adding SIRT2 at r.t.; (c) the assay mixture after addition of SIRT2, incubation at 37 °C for 15 min. The dashed lines highlight the lysine resonances which were affected during the reaction.

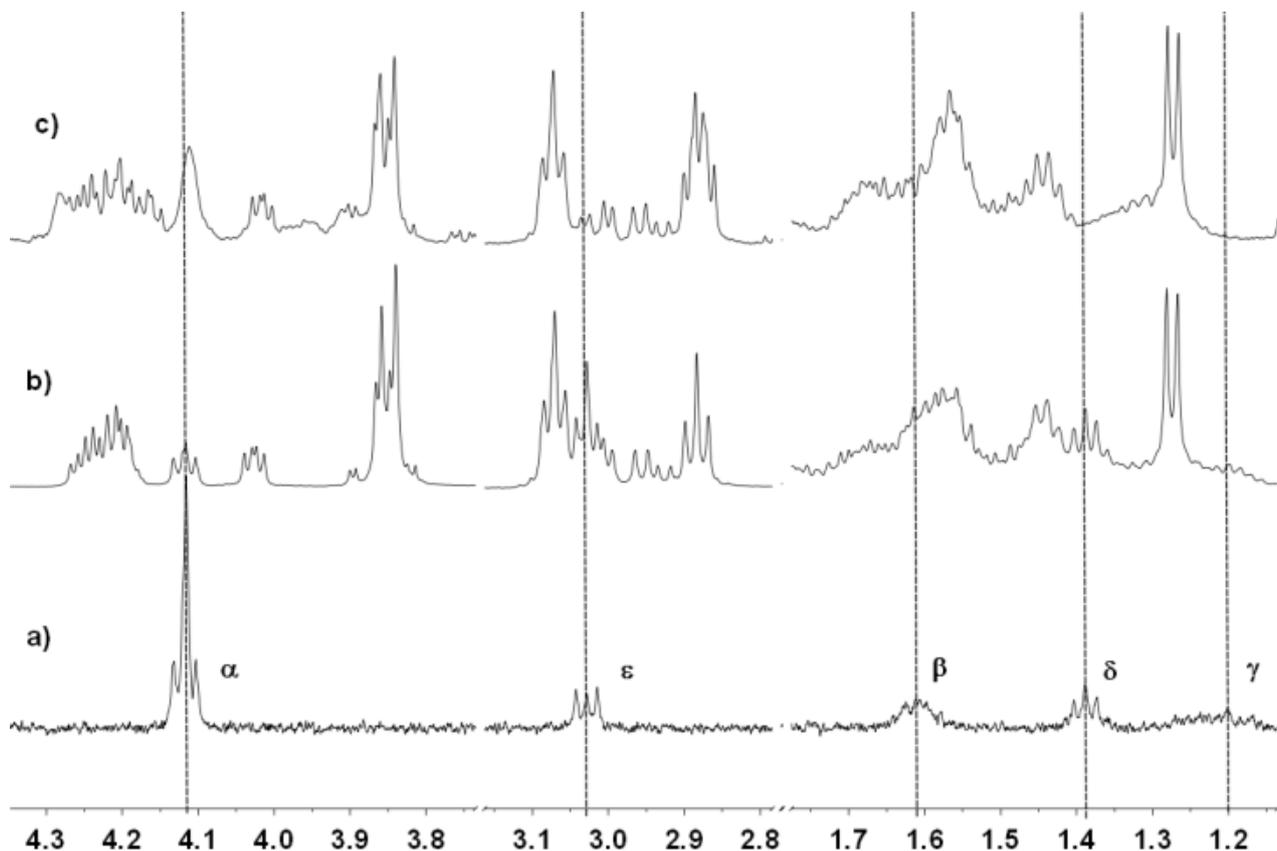
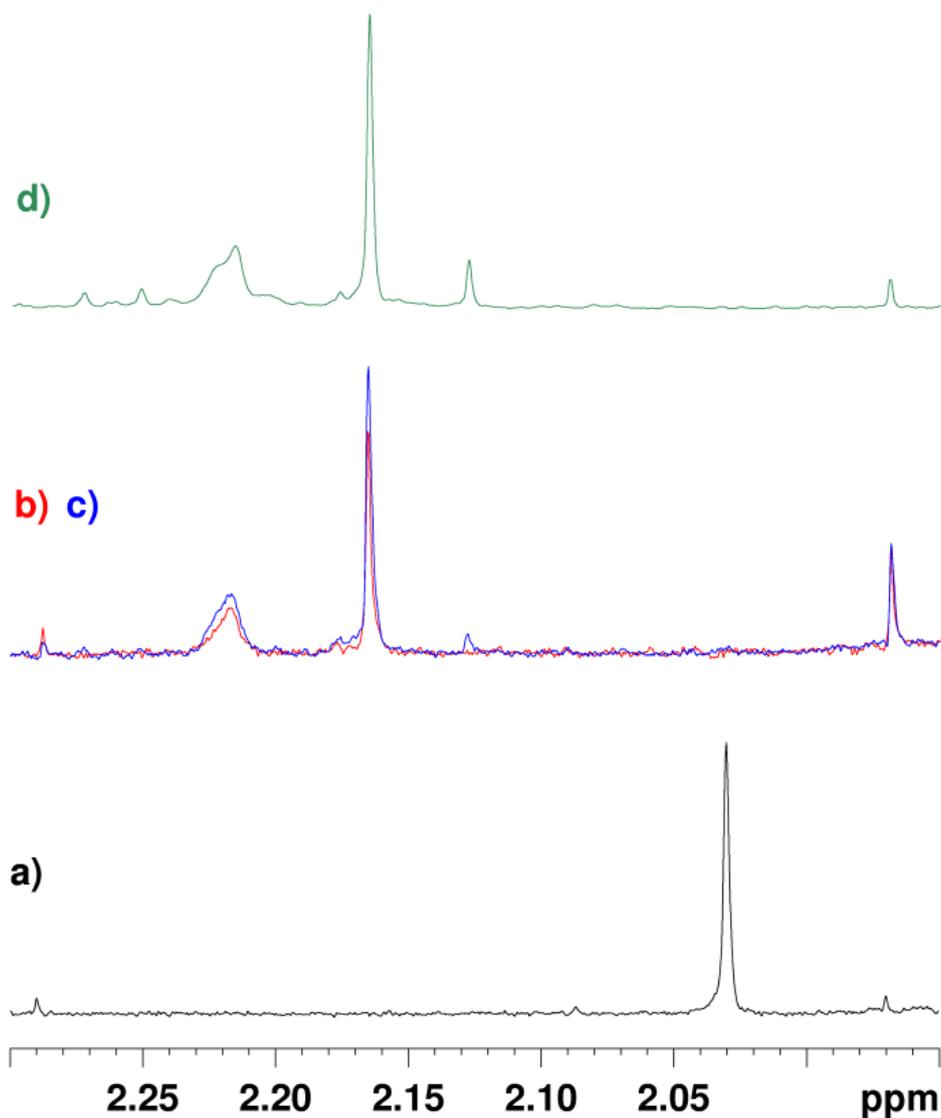


Figure S4. 1D ^1H -NMR with double solvent suppression (for both H_2O and Tris buffer) recorded at 37 °C. Sample contained 1 mM NAD^+ , 200 μM peptide, 10 μM SIRT2 in buffer (pH 8). (a) Before addition of enzyme. (b,c) After addition of enzyme and 15 mins. Incubation then addition of authentic *O*-acetyl ADP riboses (as prepared according to Perni *et al.* (2010)).(d) ^1H -NMR of authentic sample of *O*-acetyl-ADP riboses in buffer (pH 8).



It was also interesting to note the appearance of another acyl peak in this region over time at 1.97 ppm. It has been previously reported¹ that the esters hydrolyse readily in D_2O solution and this was observed in buffer at pH 8.

Figure S5. 1D ^1H -NMR with double solvent suppression (for both H_2O and Tris buffer) recorded at 37 °C. Sample contained 1 mM NAD^+ , 200 μM peptide, 10 μM SIRT2 in buffer (pH 8). (a) After 20-min Incubation with enzyme. A mixture of 2'-*O*-acetyl ADP ribose and 3'-*O*-acetyl ADP ribose is observed (b) After 24 h at room temperature (red) (c) After addition of sodium acetate (blue). The peaks directly overlap which implies that this peak is in fact acetate resulting from ester hydrolysis of the reaction products.

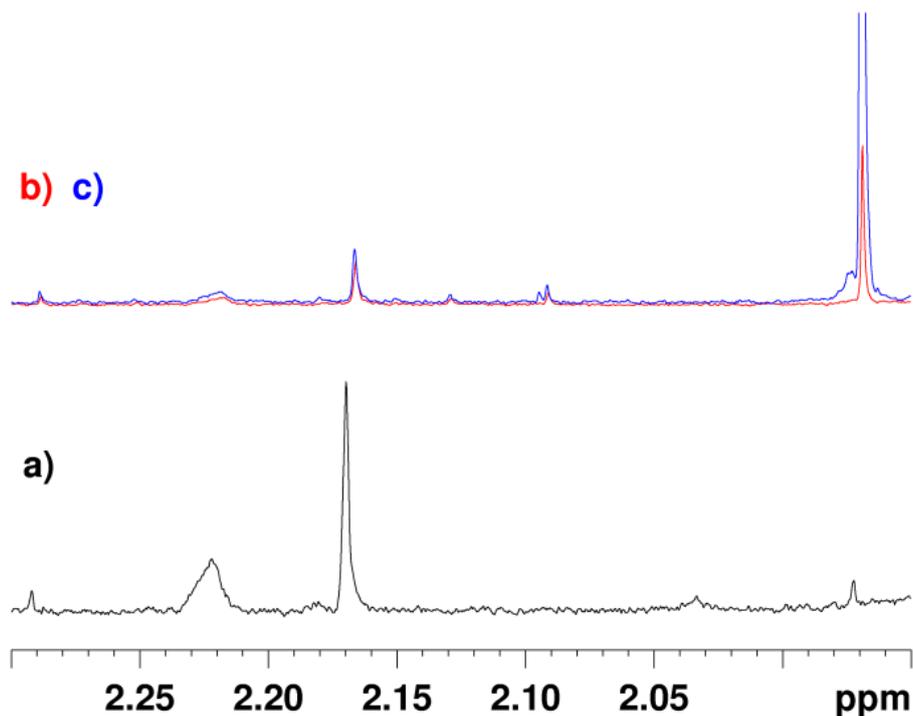


Figure S6. 1D ^1H -NMR with double solvent suppression (for both H_2O and Tris buffer) recorded at 37 °C. Sample contained 1 mM NAD^+ , 200 μM peptide, 10 μM SIRT2 in buffer (pH 8). (a) After 20-min Incubation with enzyme (shown in blue). The deacetylated peptide is observed. Doping with authentic material (shown in red). The peaks directly overlap which implies that this peaks are in fact from the deacetylated peptide product.

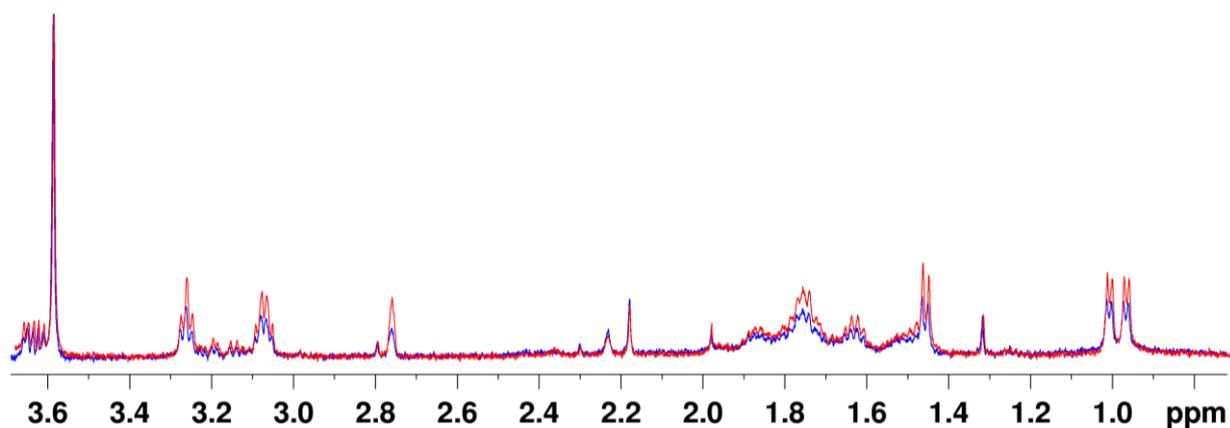


Figure S7. Fluorescence melting curves of SIRT2 at various added concentrations of tenovin-43. The midpoint of the melting temperature (T_m) is shifted towards higher temperature with increasing ligand concentration.

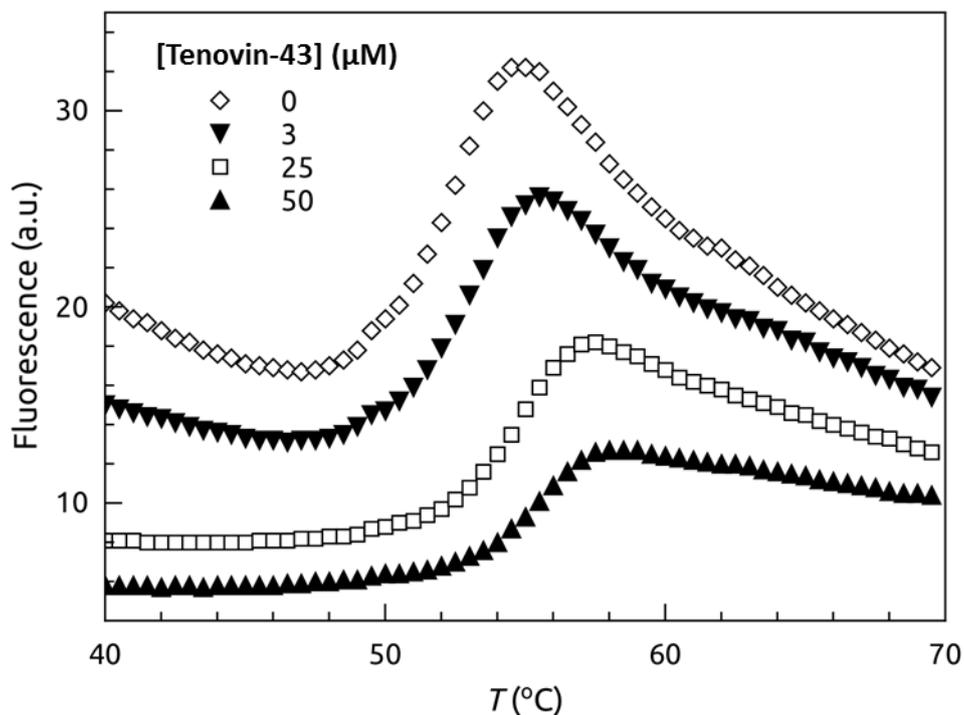


Figure S8. Fluorescence melting curves of SIRT2 at various added concentrations of AGK2. The midpoint of the melting temperature (T_m) remains unchanged.

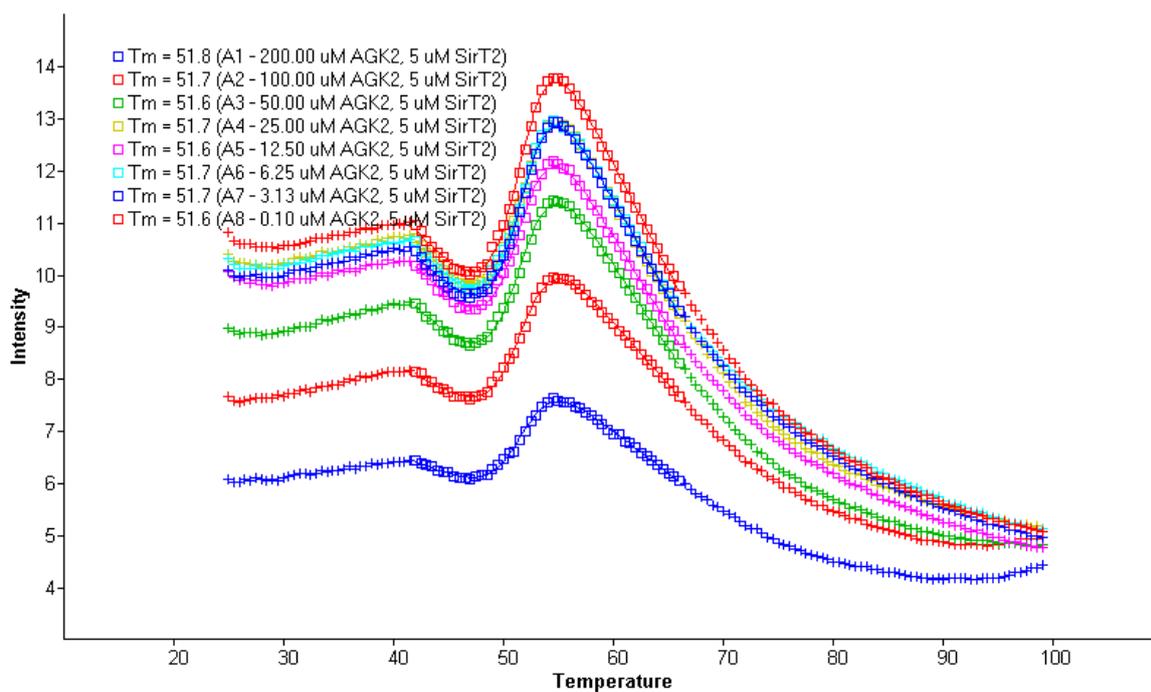


Figure S9. Ligand dosing curves showing the T_m shift dependence of SIRT2 on AGK2 concentration. Datapoints are experimental data obtained from curves as in Figure S6b while the lines are simulated according to the model as previously explained (Matulis *et al.*, 2005).

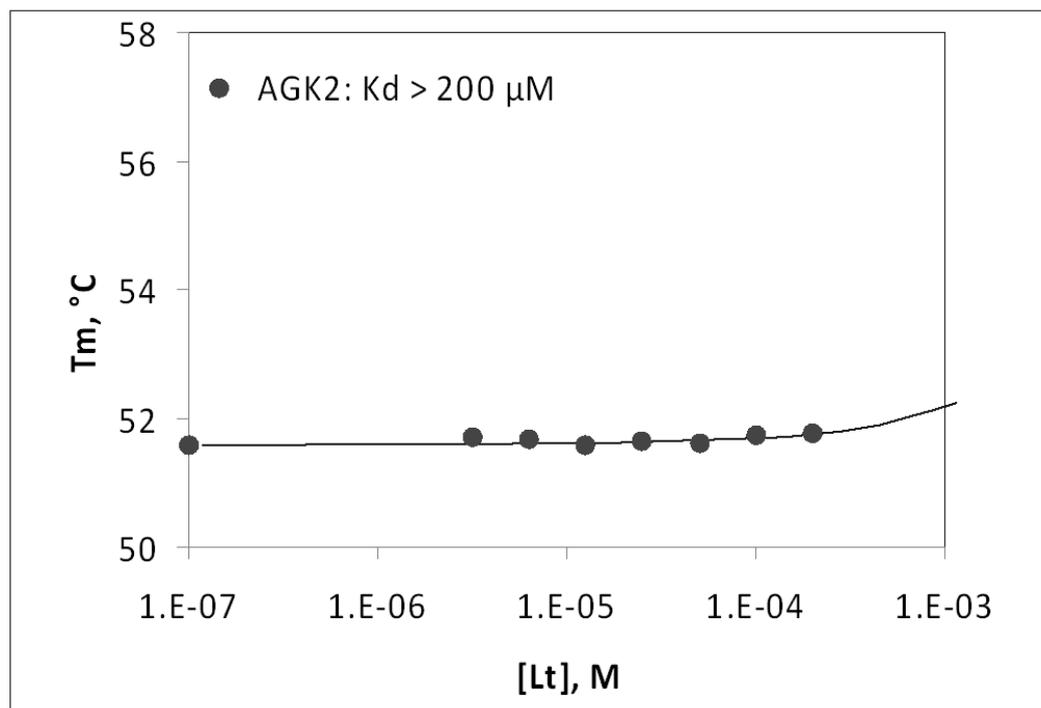
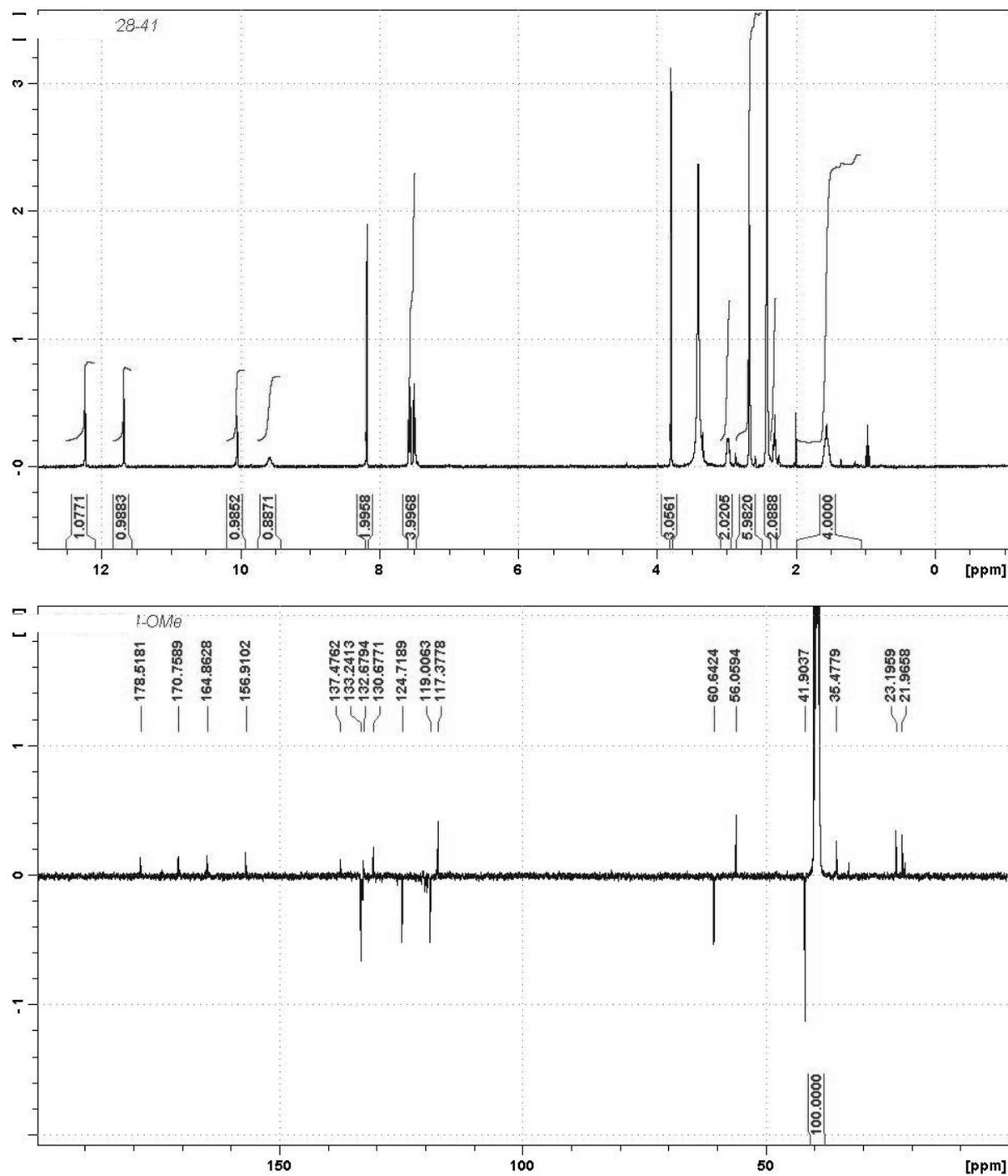


Table S1. Quantification of deacetylation reaction as observed by $^1\text{H-NMR}$.

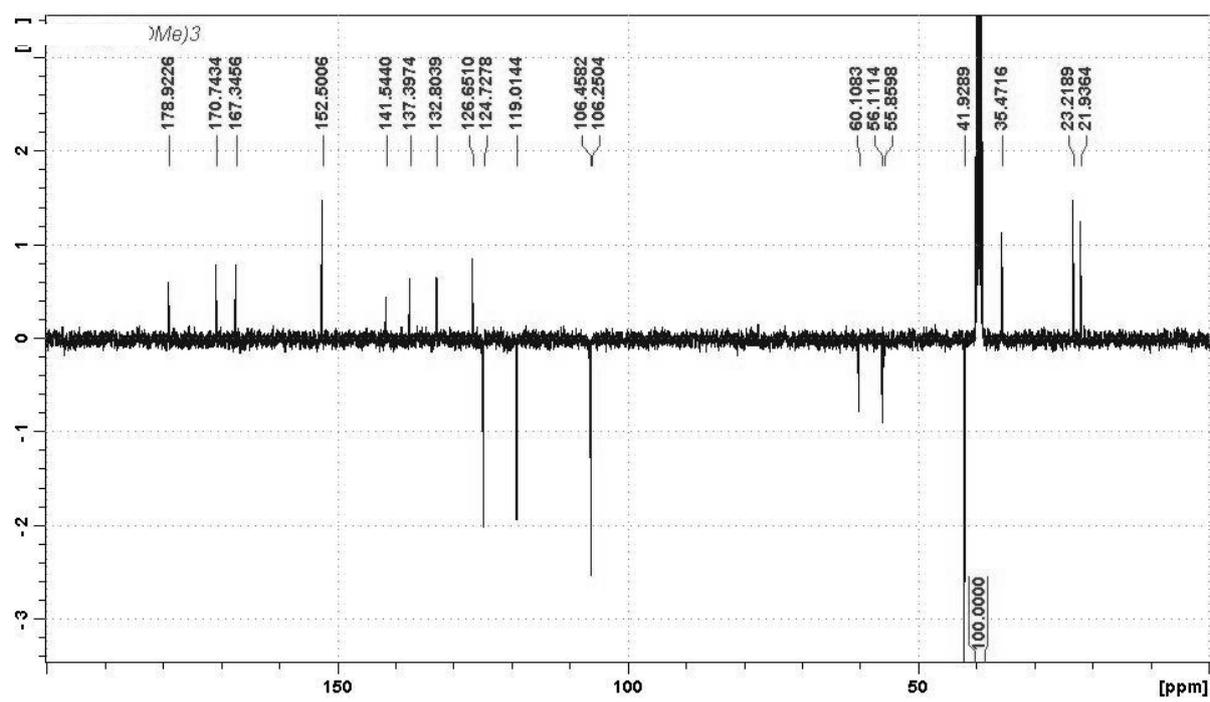
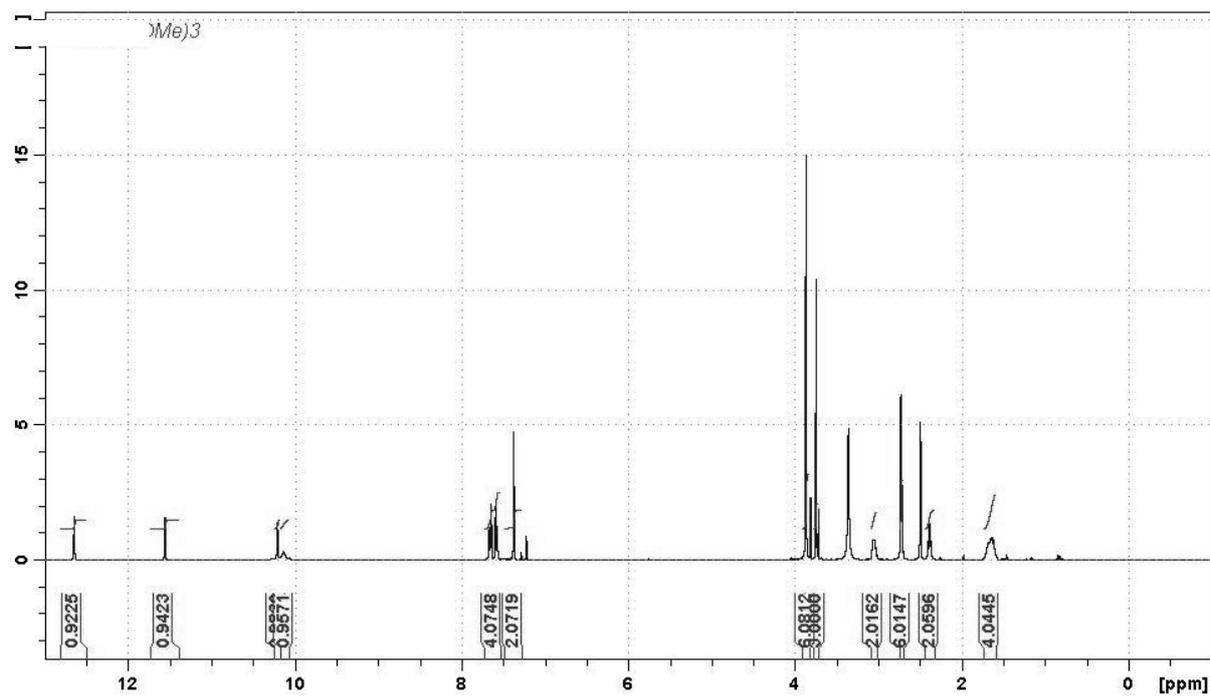
		Normalised Relative Integral Intensity of signal at 2.04 ppm	% deacetylation
(a)	Before adding enzyme	100	0
(b)	No inhibitor control	4	96
(c)	25 μM	11	89
(d)	50 μM	16	84
(e)	100 μM	34	66
(f)	200 μM	64	36
(g)	500 μM	89	10

Figure S10. ^1H and ^{13}C -NMR of Tenovin analogues.

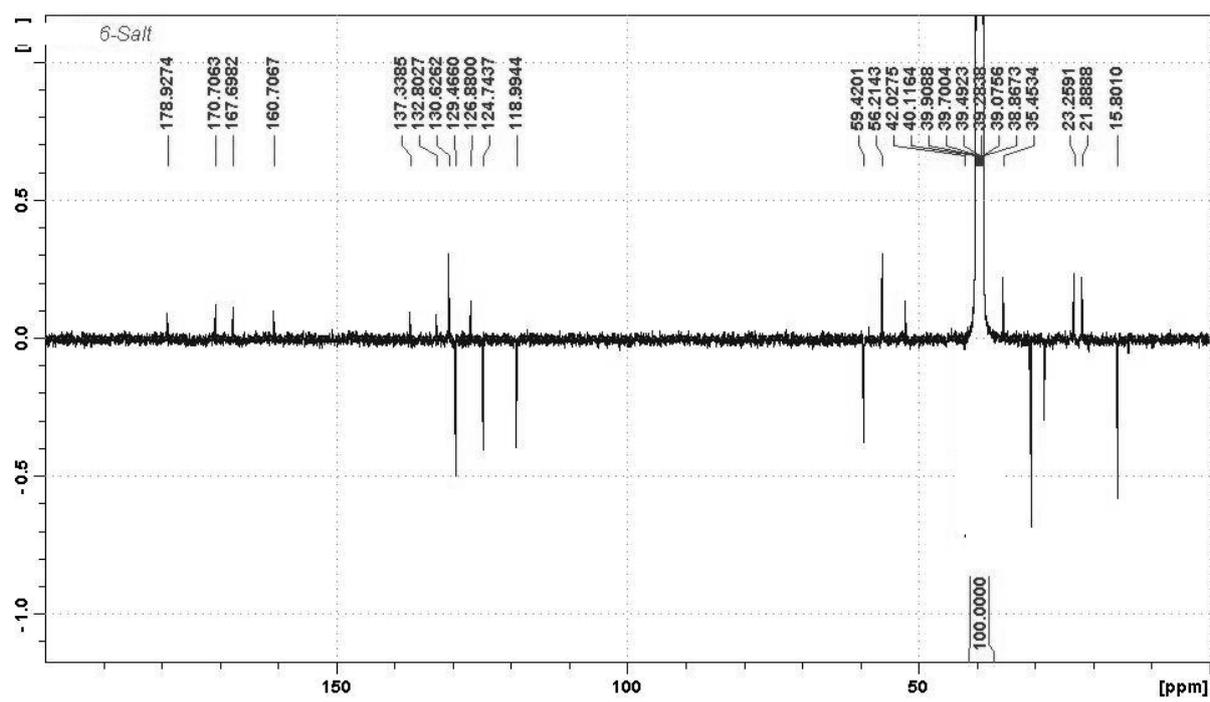
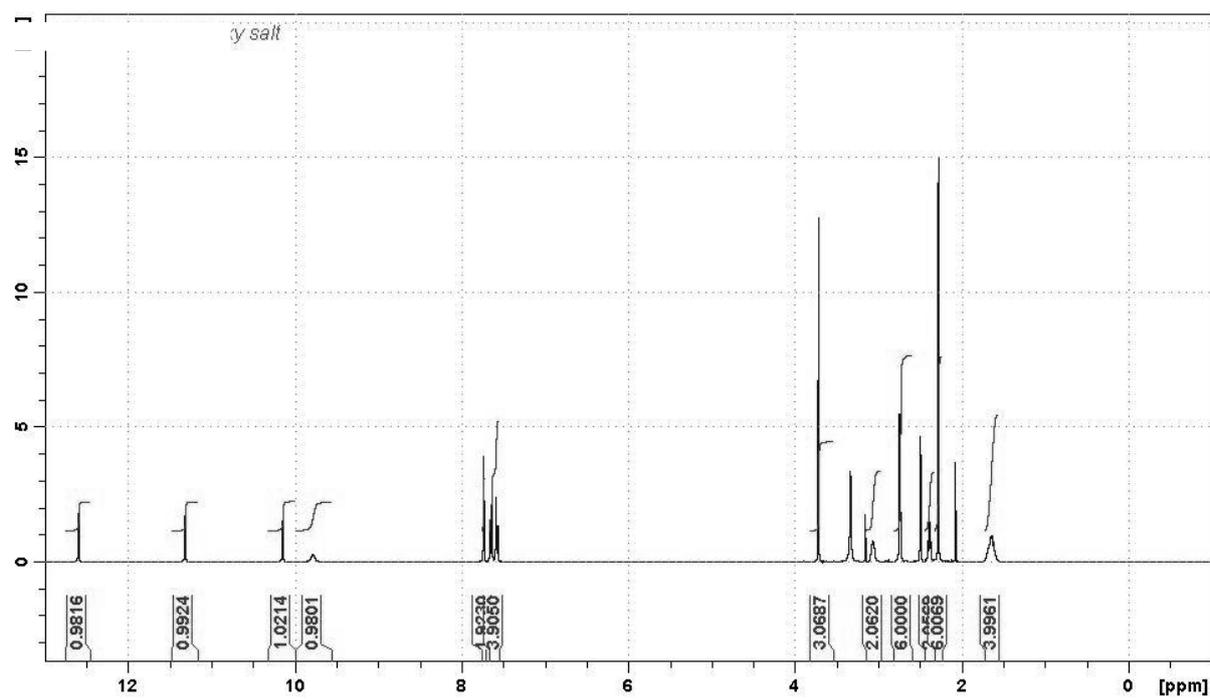
Tenovin-36



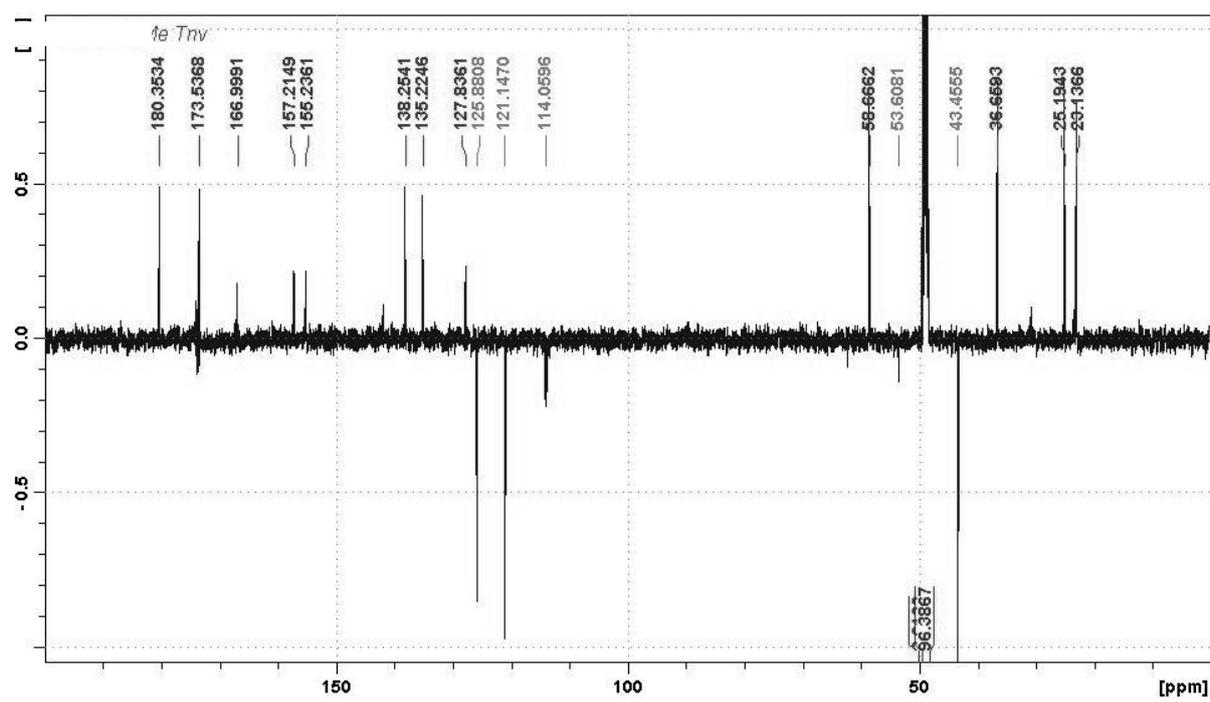
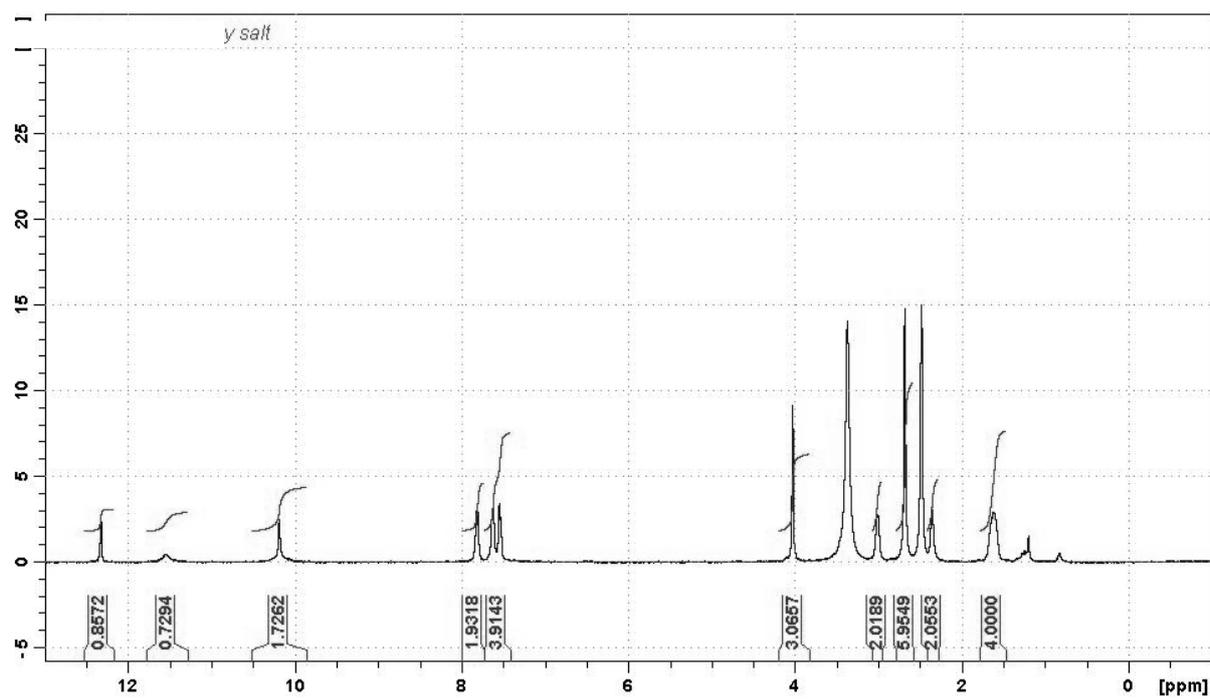
Tenovin-37



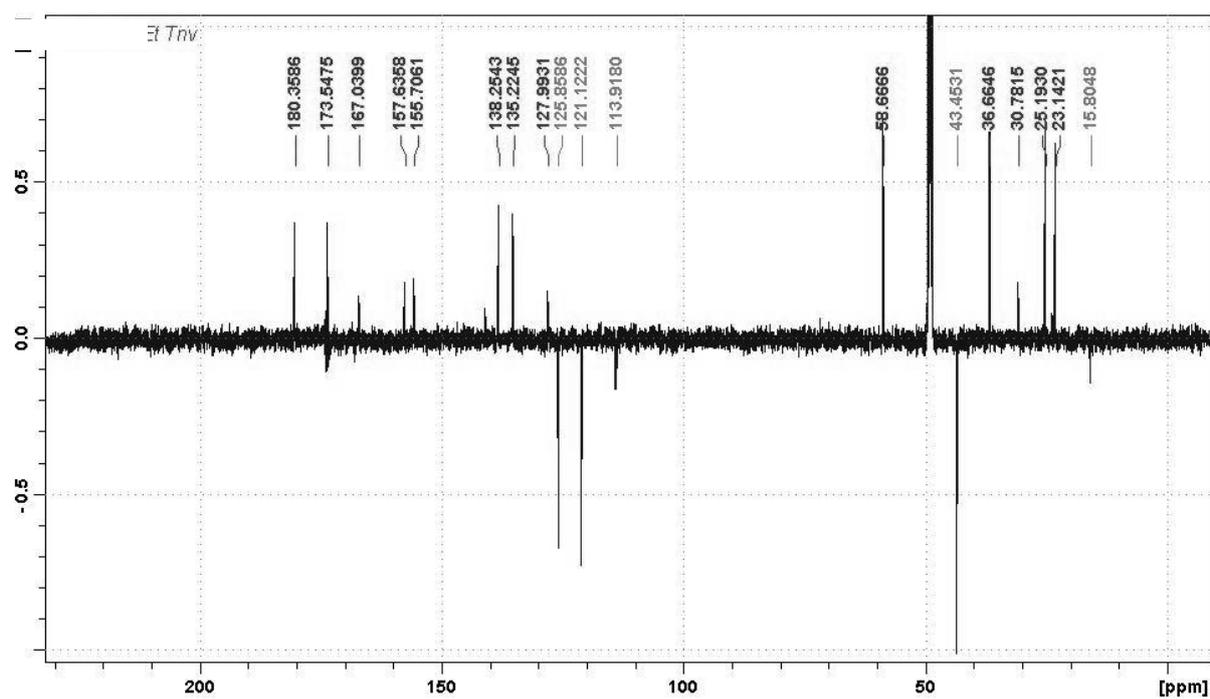
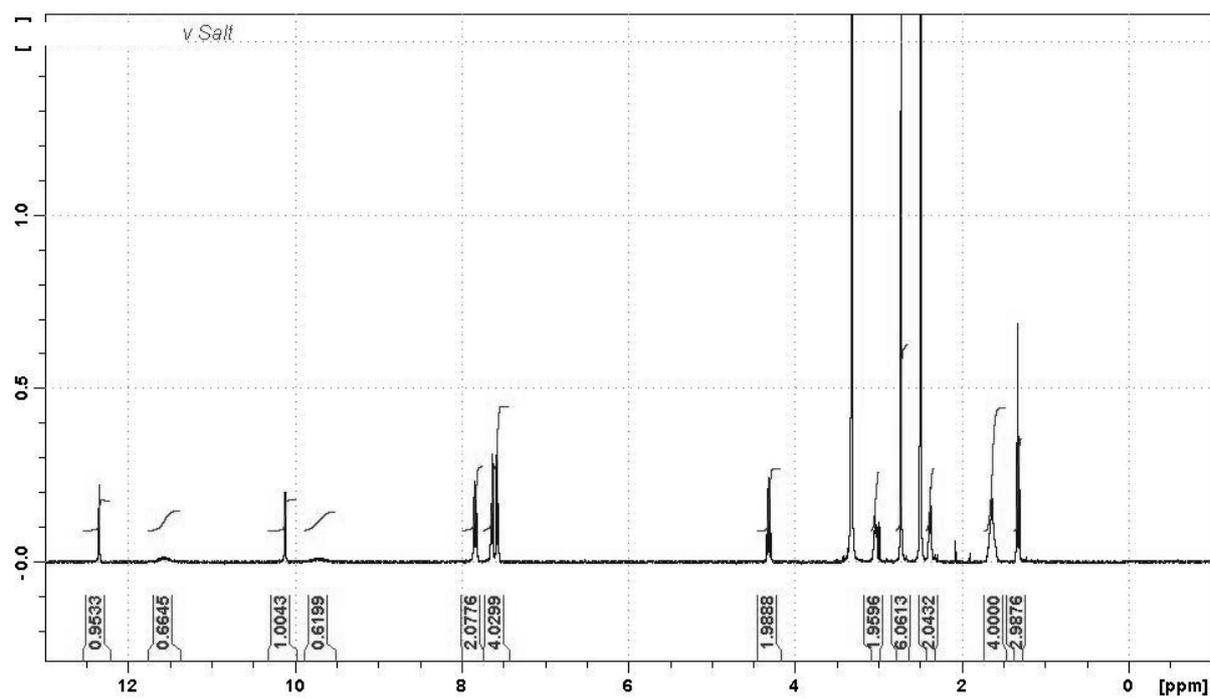
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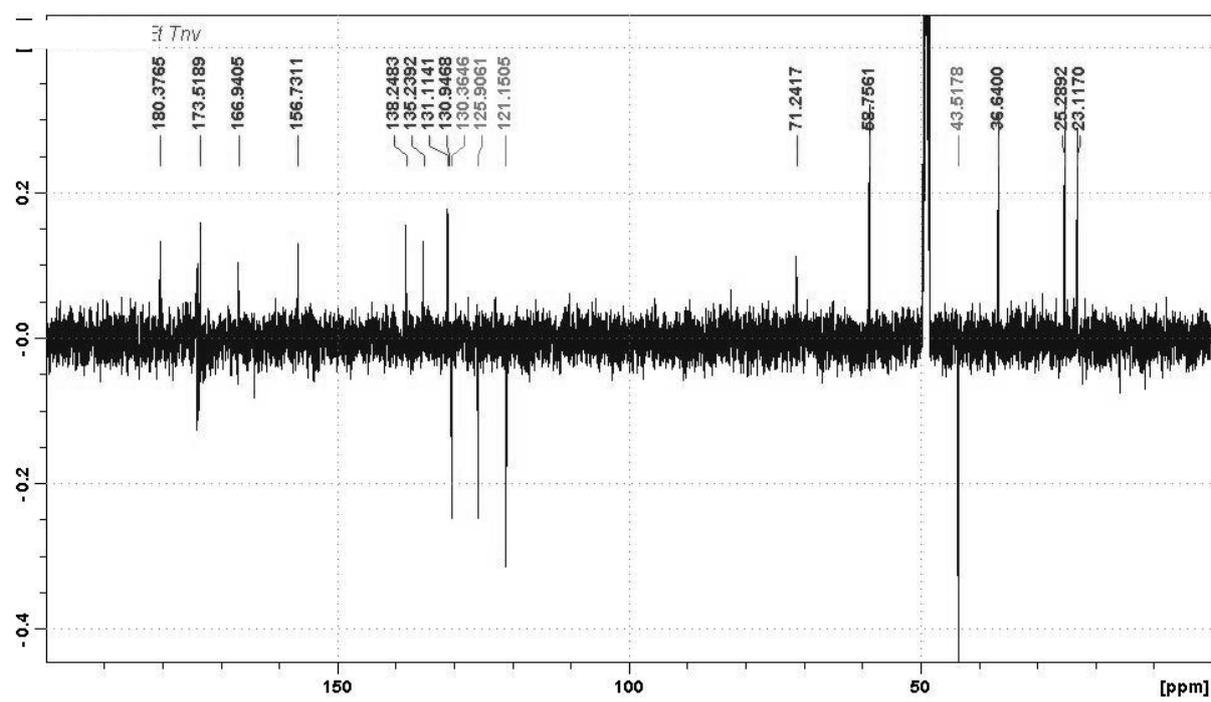
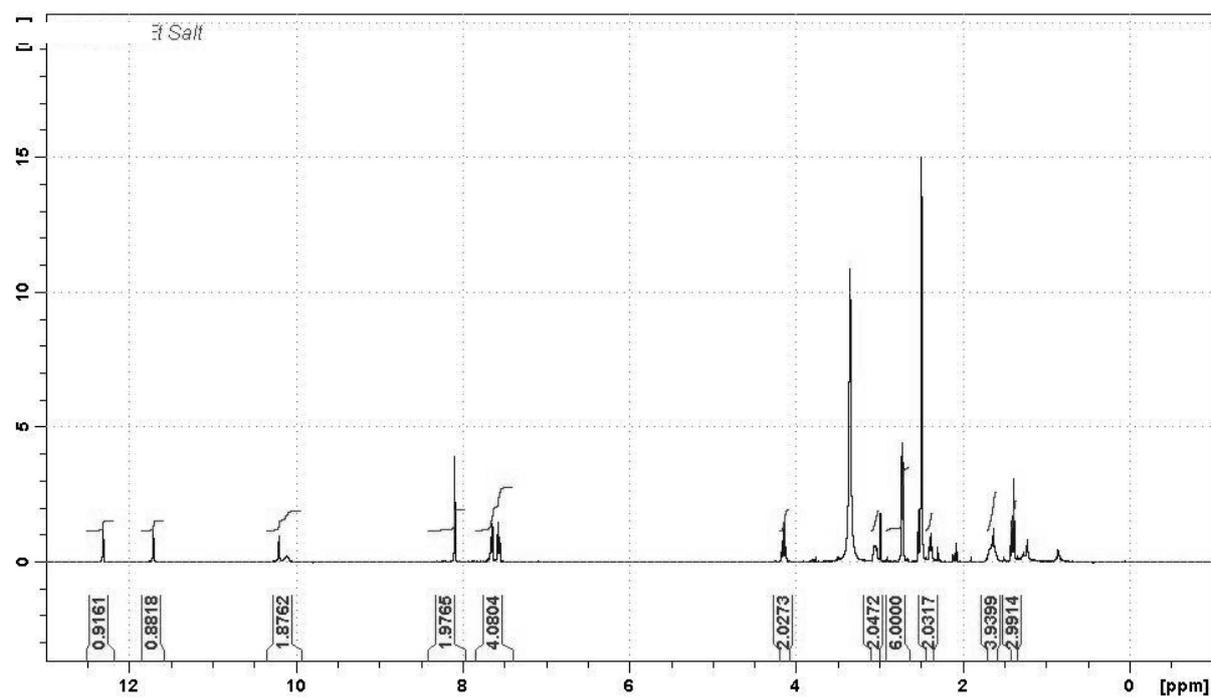
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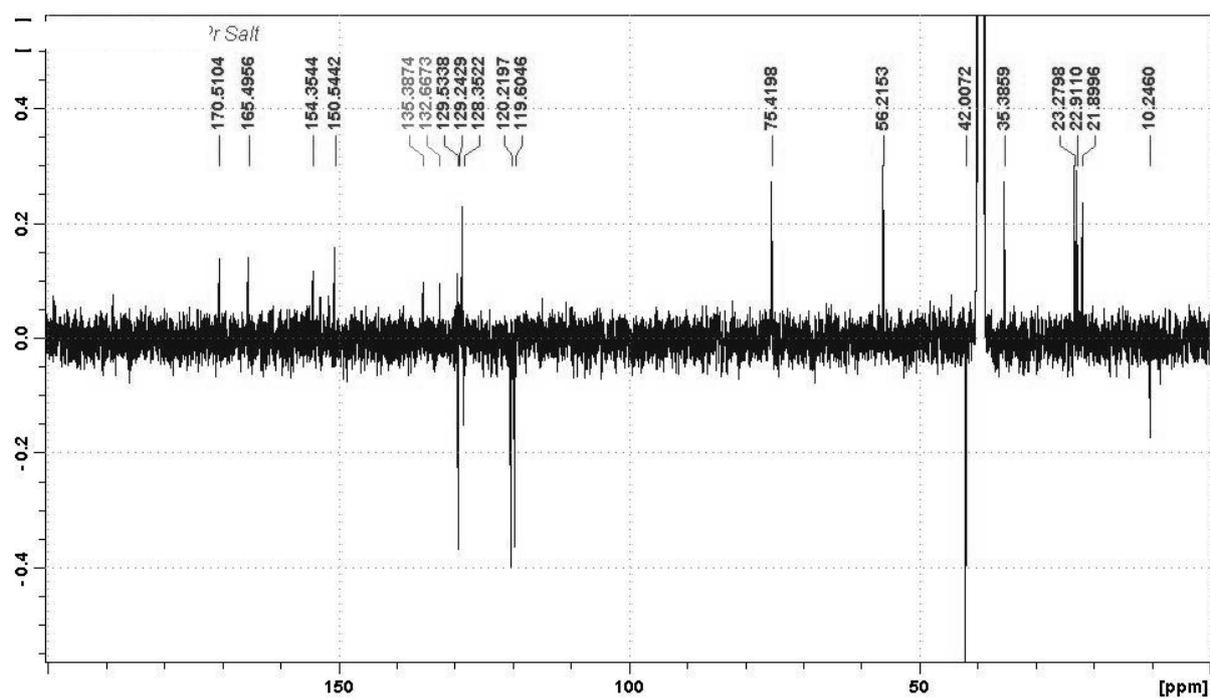
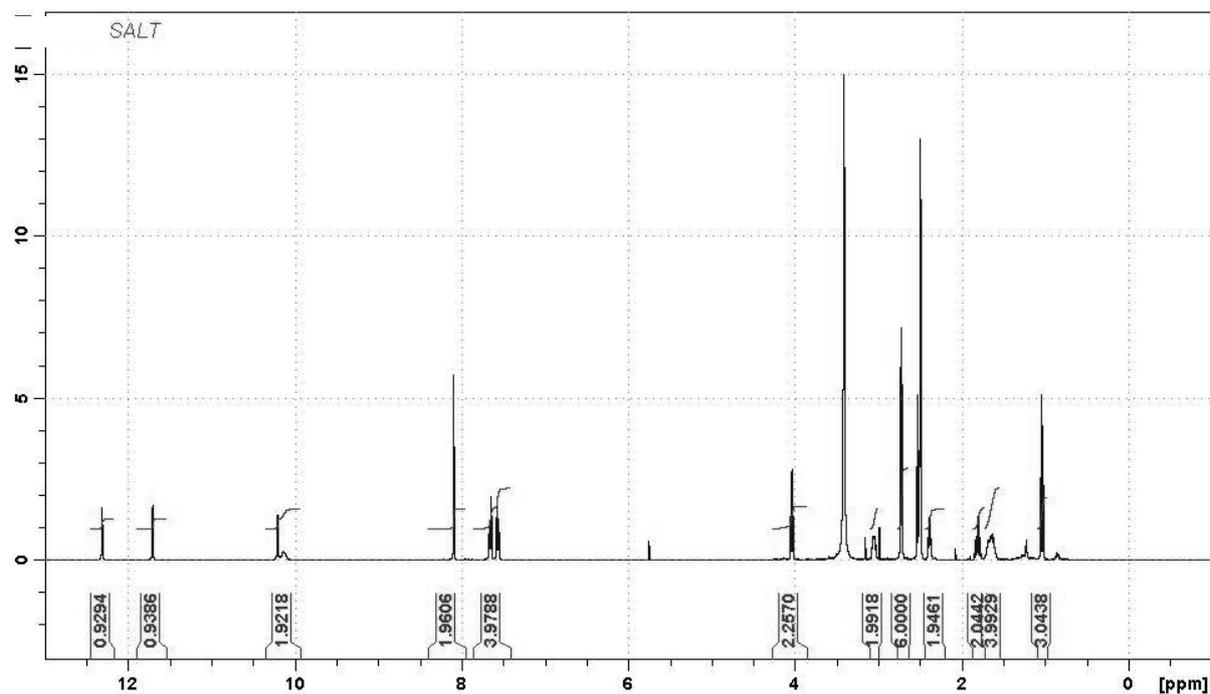
Tenovin-40



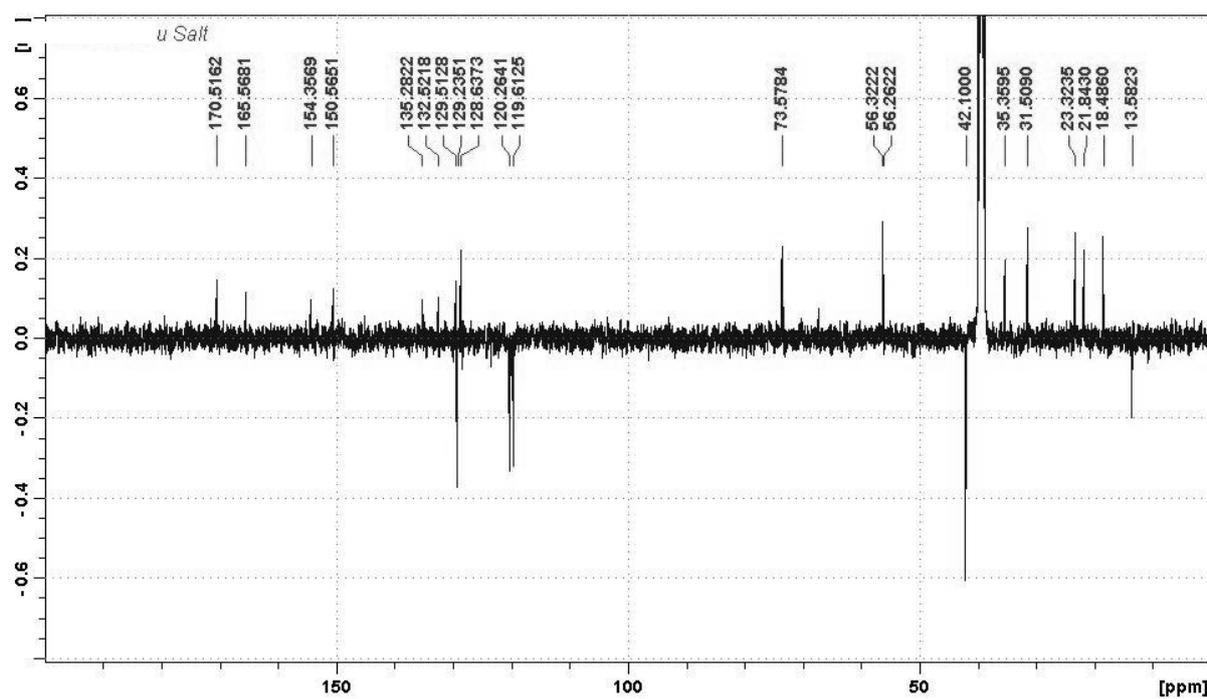
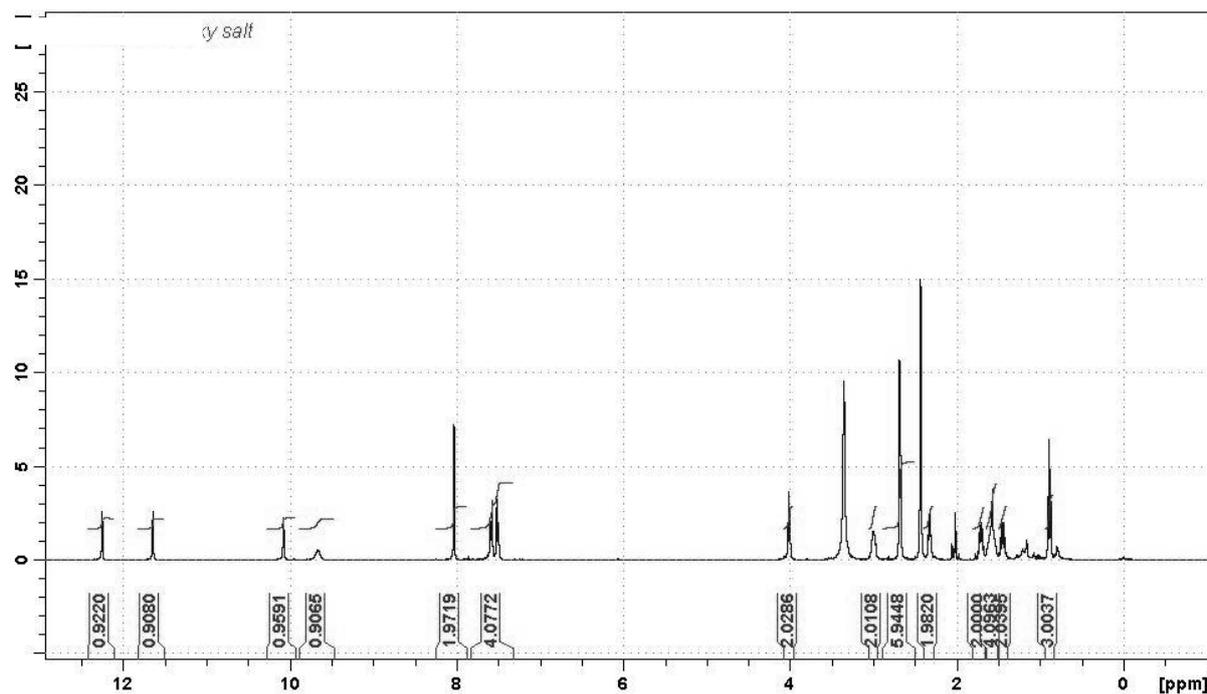
Tenovin-41



Tenovin-42



Tenovin-43



Tenovin-44

