

# Supporting Information

for

## Quantification of *N*-acetylcysteamine activated methylmalonate incorporation into polyketide biosynthesis

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**NMR spectra of synthesized compounds and ESI–MS spectra  
of D<sub>3</sub>-propionate incorporation**

## NMR spectra of the compounds

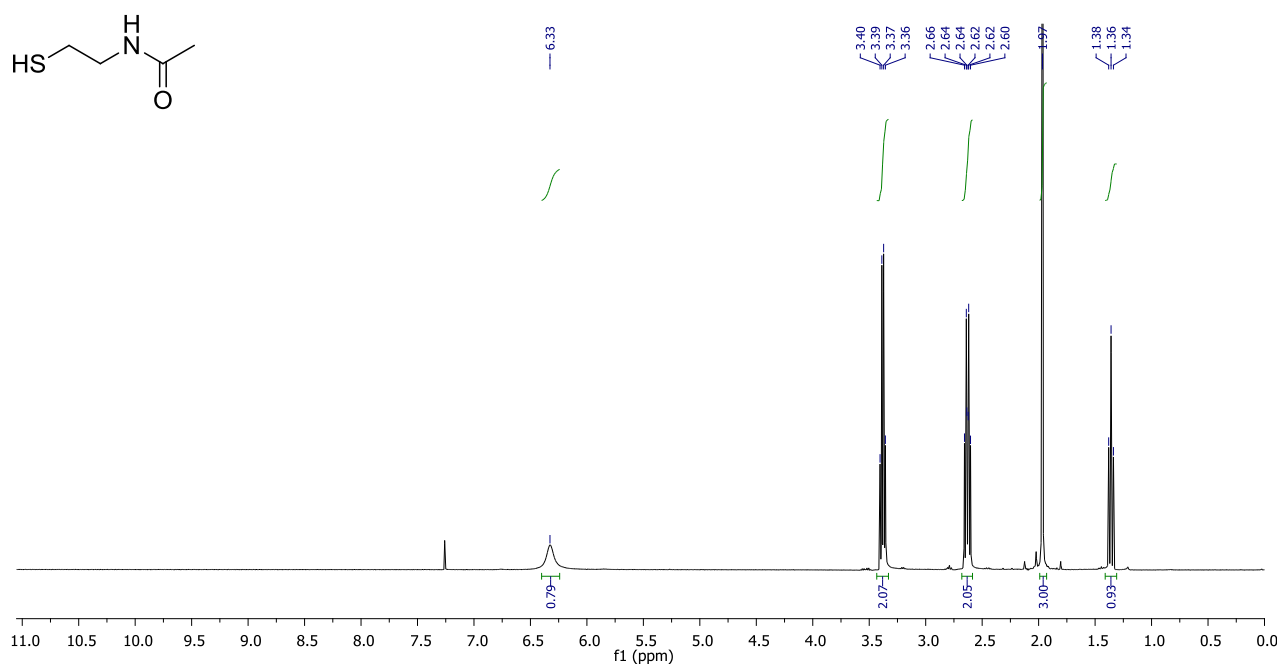


Figure S1:  $^1\text{H}$  NMR spectra of SNAC in  $\text{CDCl}_3$ .

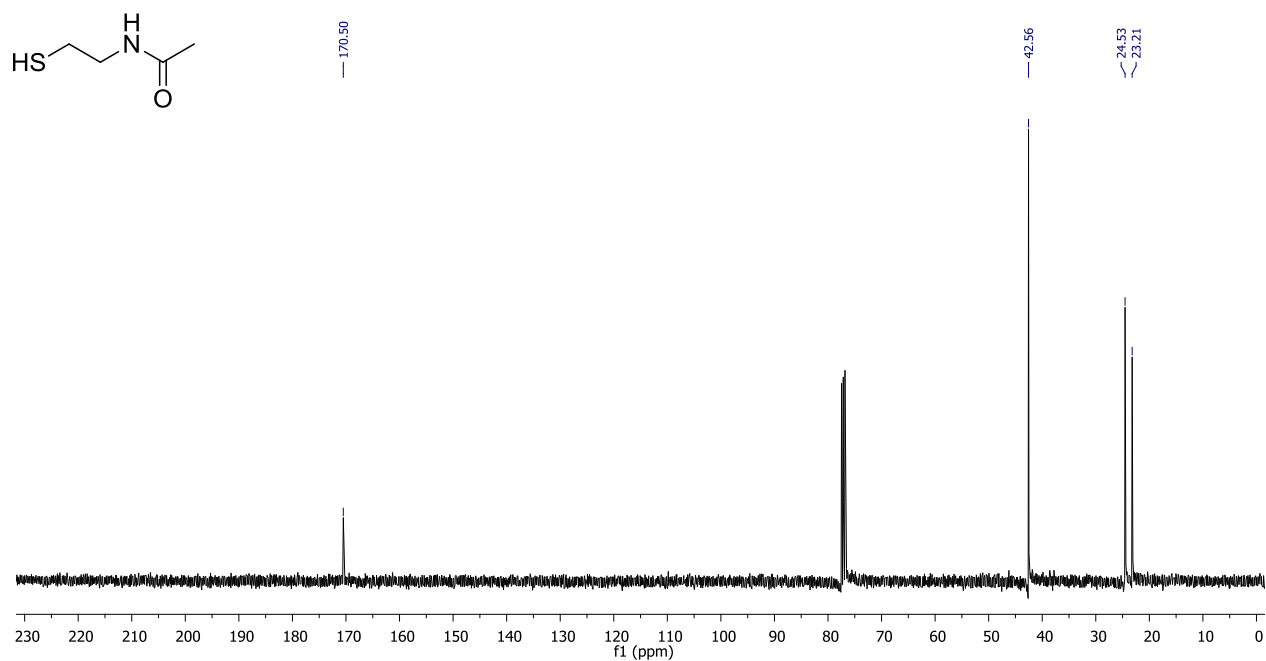
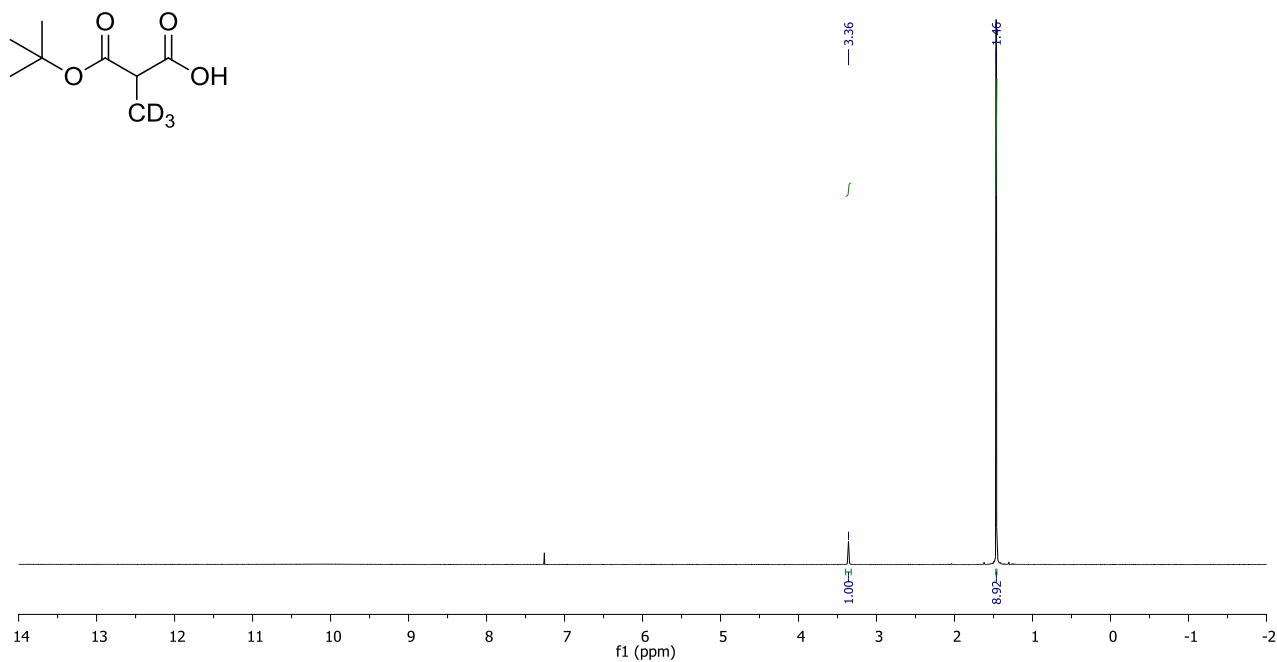
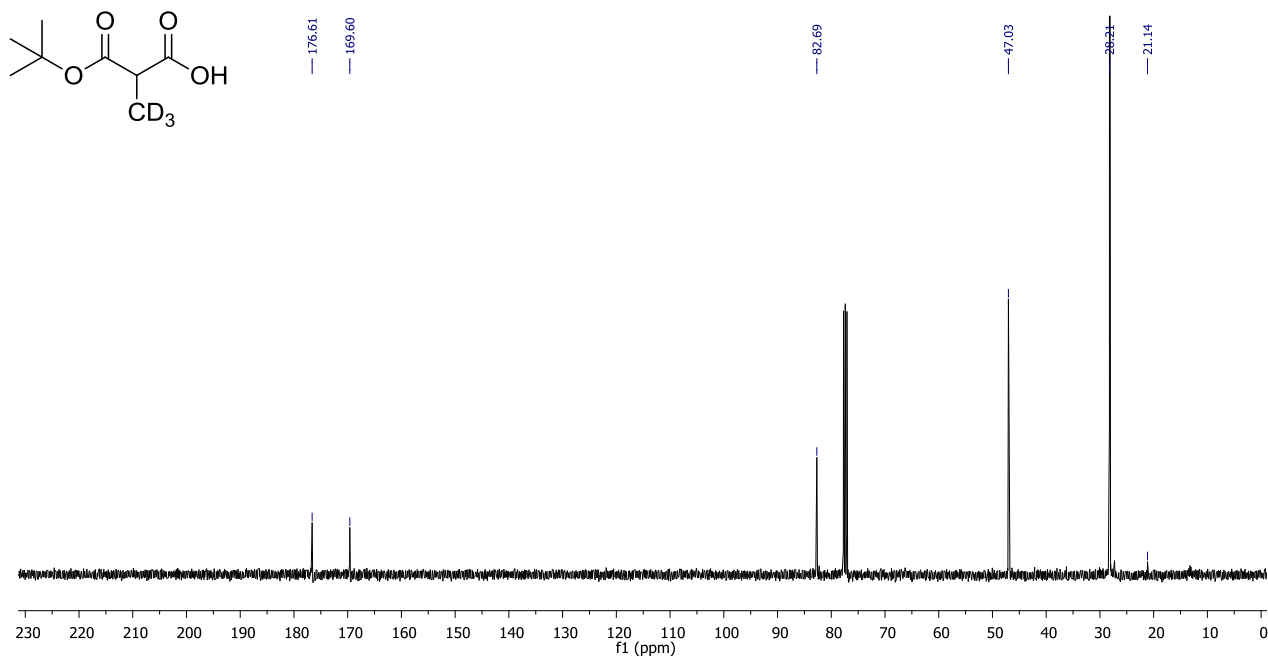


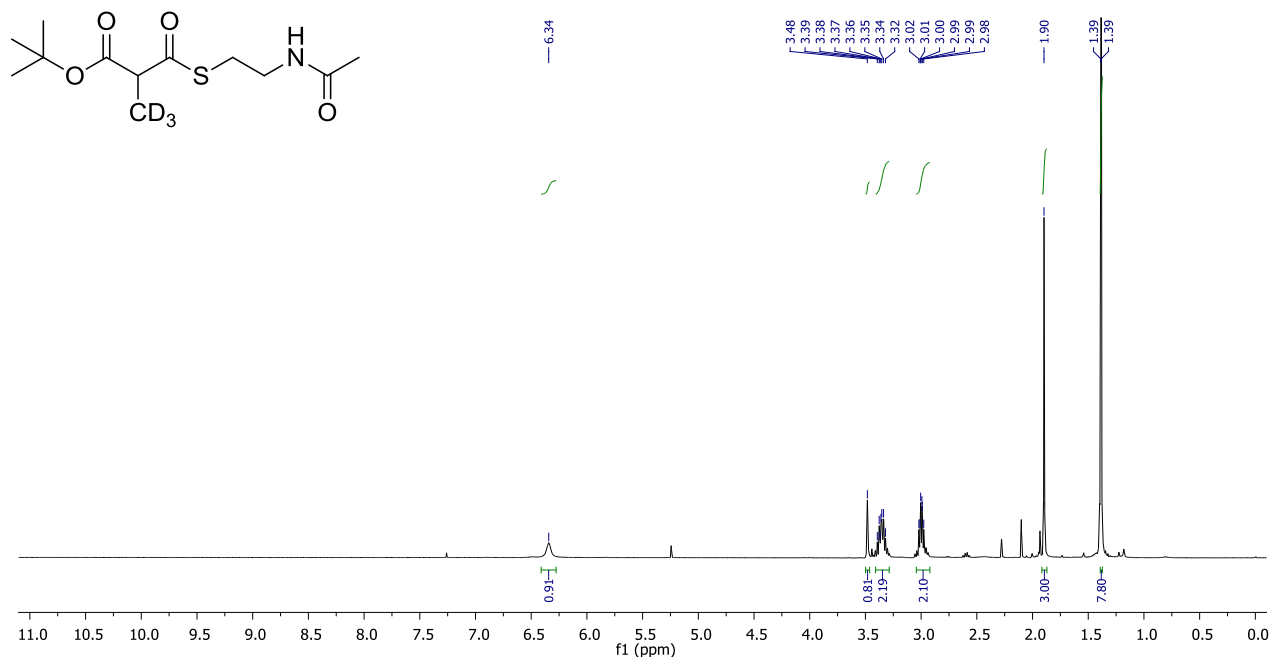
Figure S2:  $^{13}\text{C}$  NMR spectra of SNAC in  $\text{CDCl}_3$ .



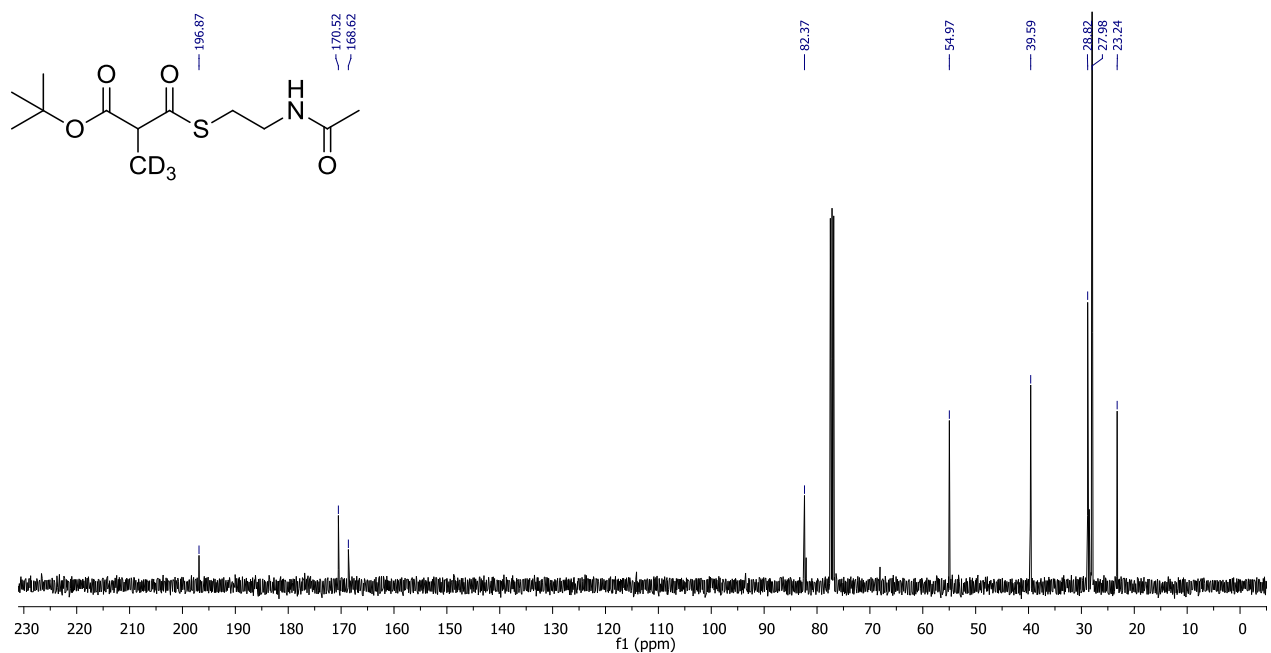
**Figure S3:**  $^1\text{H}$  NMR spectra of 3-(*tert*-butoxy)-2- $\text{D}_3$ -methyl-3-oxopropanoic acid (**2**) in  $\text{CDCl}_3$ .



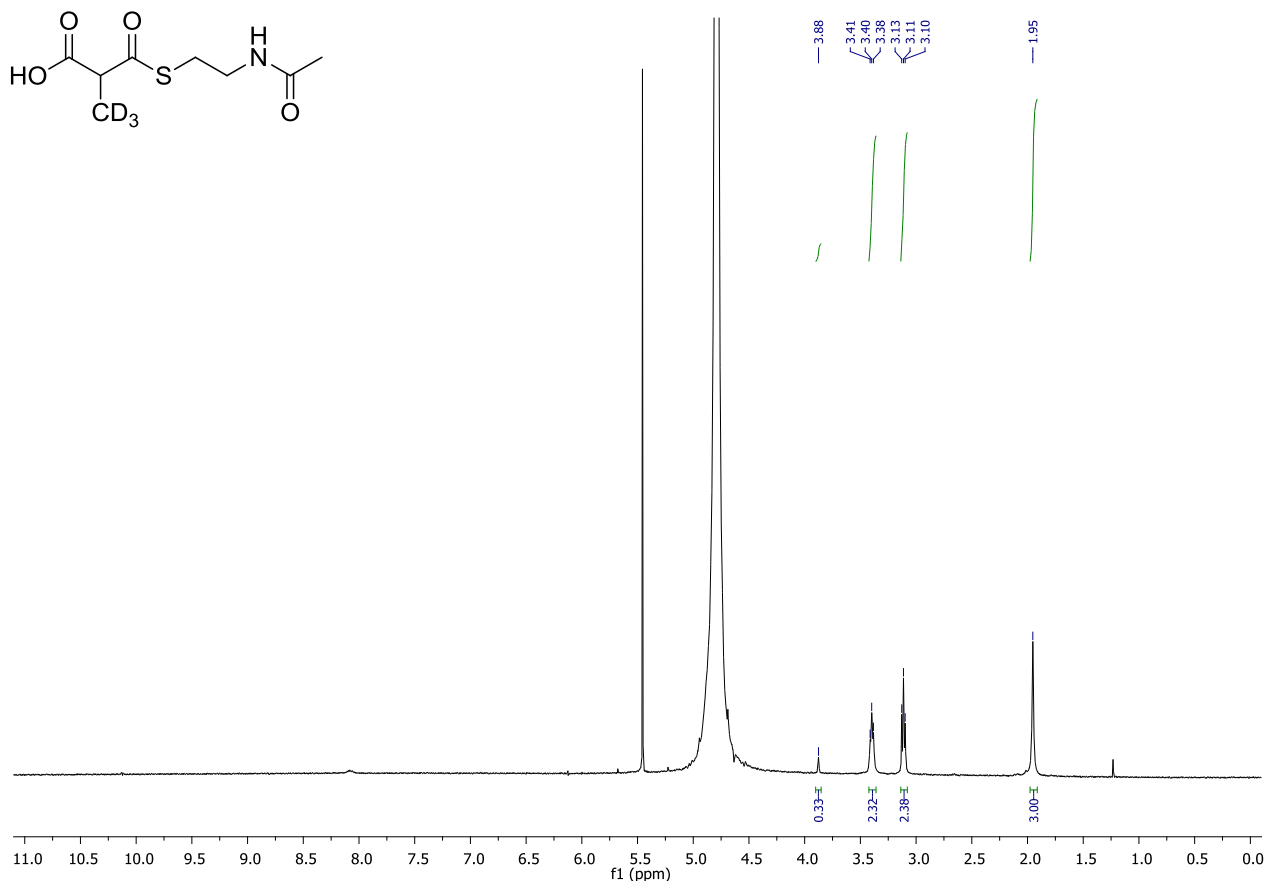
**Figure S4:**  $^{13}\text{C}$  NMR spectra of 3-(*tert*-butoxy)-2- $\text{D}_3$ -methyl-3-oxopropanoic acid (**2**) in  $\text{CDCl}_3$ .



**Figure S5:** <sup>1</sup>H NMR spectra of *tert*-butyl 3-((2-acetamidoethyl)thio)-2-D<sub>3</sub>-methyl-3-oxopropanoate (**3**) in CDCl<sub>3</sub>.

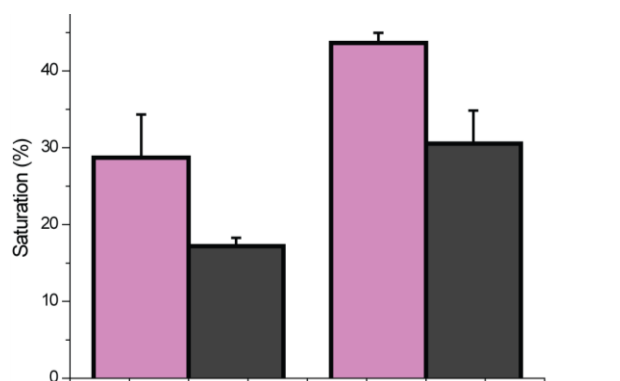


**Figure S6:** <sup>13</sup>C NMR spectra of *tert*-butyl 3-((2-acetamidoethyl)thio)-2-D<sub>3</sub>-methyl-3-oxopropanoate (**3**) in CDCl<sub>3</sub>.



**Figure S7:** <sup>1</sup>H NMR spectra of 3-((2-acetamidoethyl)thio)-2-D<sub>3</sub>-methyl-3-oxopropanoic acid (**4**) in D<sub>2</sub>O.

### Incorporation assay D<sub>3</sub>-methylmalonic acid versus D<sub>3</sub>-methylmalonic *N*-acetylcysteamine thioester (**4**)

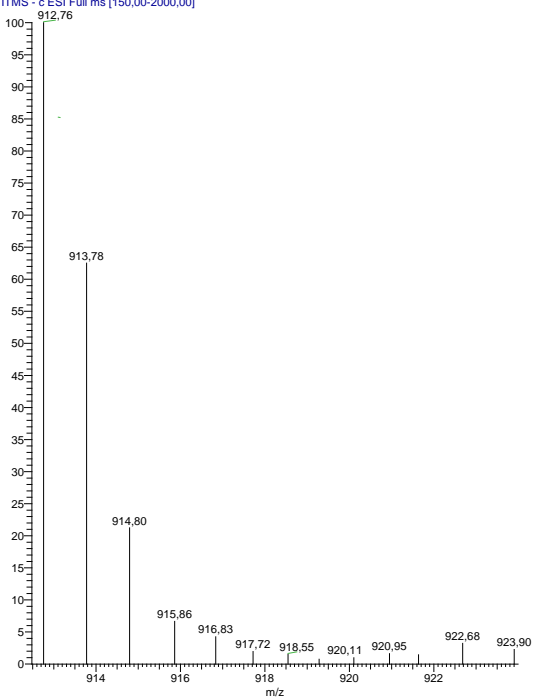


**Figure S8:** Relative incorporation of SNAC activated D<sub>3</sub>-methylmalonic acid (purple) and D<sub>3</sub>-methylmalonic acid (grey).

## ESI-MS spectra of D3-labeled compounds

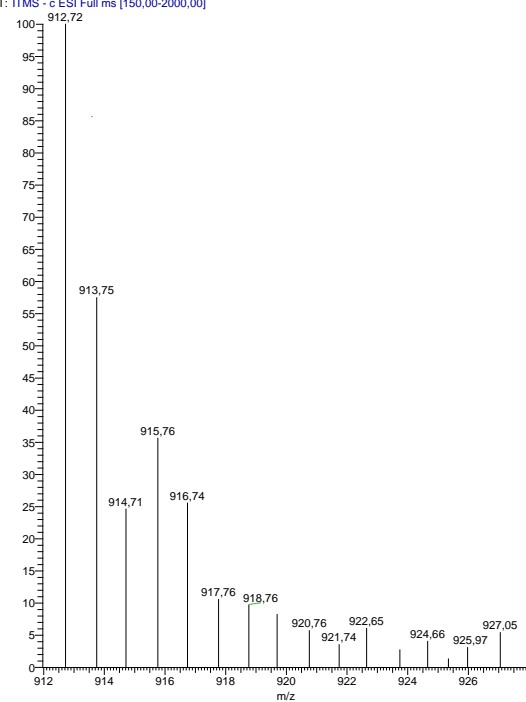
(A)

WT-0-2 #569 RT: 5.35 AV: 1 NL: 2.02E4  
T: ITMS - c ESI Full ms [150.00-2000.00]



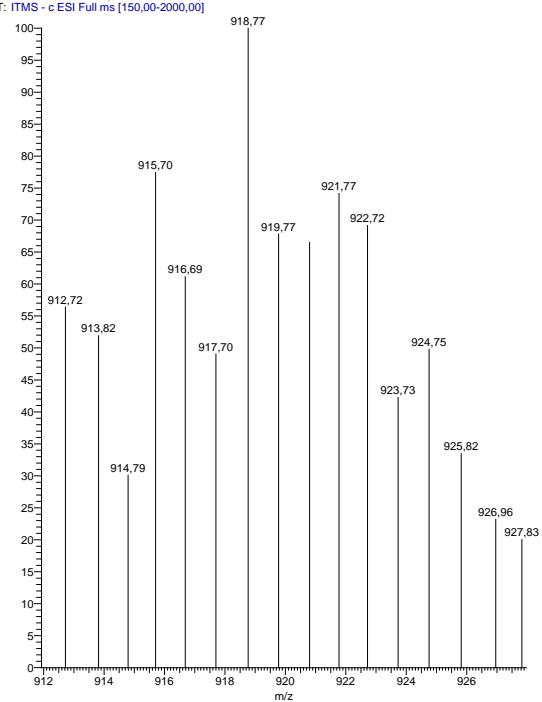
(B)

WT-2.5-3 #696 RT: 6.58 AV: 1 NL: 2.69E4  
T: ITMS - c ESI Full ms [150.00-2000.00]



(C)

WT-10-2 #717 RT: 6.81 AV: 1 NL: 4.72E3  
T: ITMS - c ESI Full ms [150.00-2000.00]



**Figure S9:** Showing the mass-to-charge ratio of rapamycin (HPLC/ESI-MS) after feeding the deuterated substrate at an increasing concentration. (A) 0 mM, (B) 2.5 mM and (C) 10 mM of **4**.