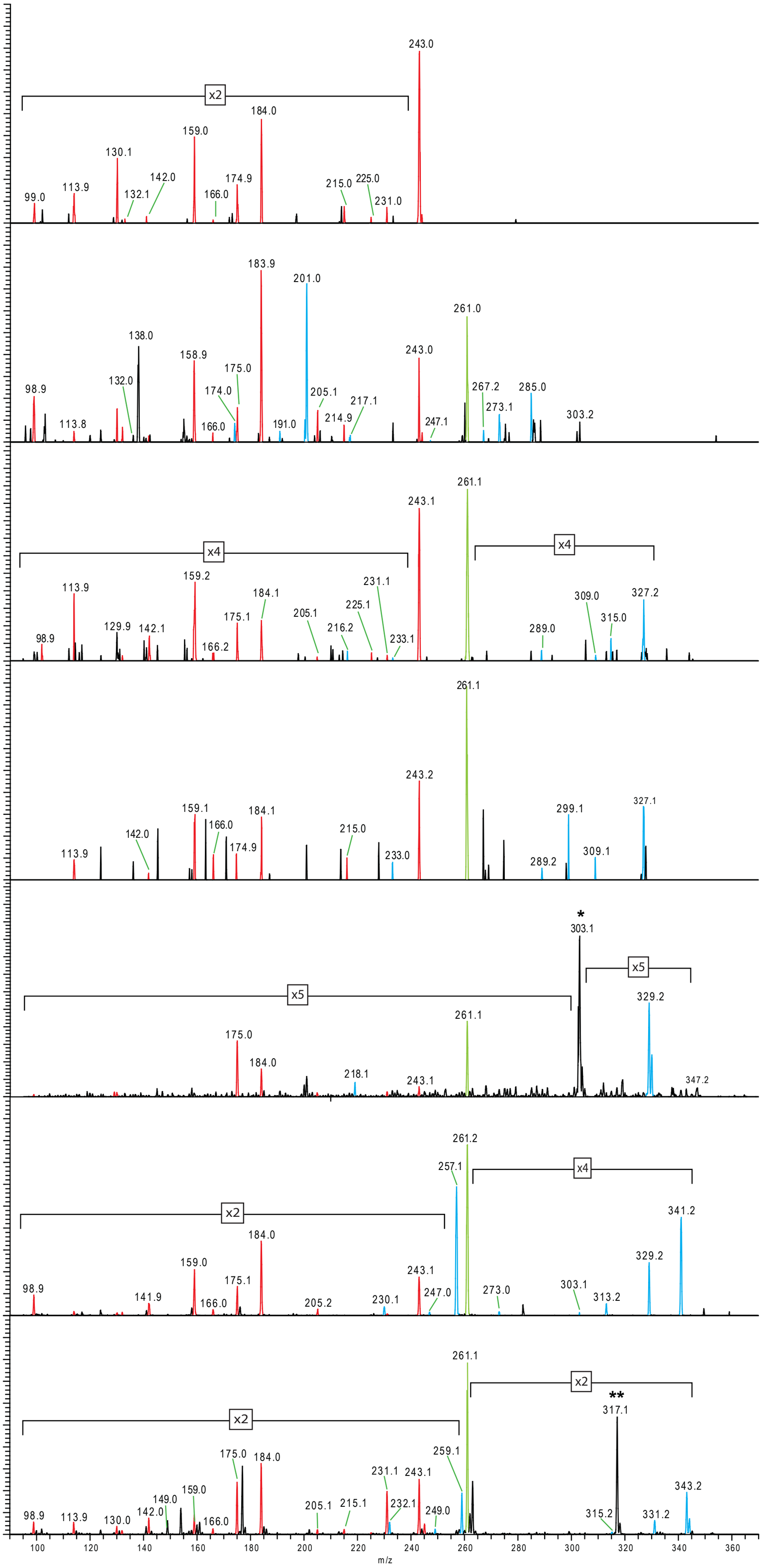
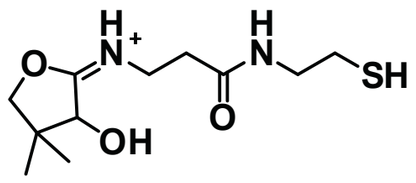
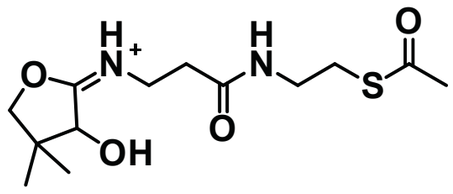


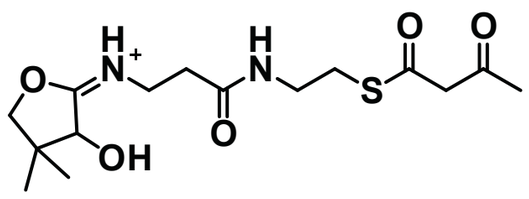
	Fragment m/z
a	**132.1**
b	**142.1**
c	149.1
d	**159.1**
e	**166.1**
f	**175.1**
g	**184.1**
h	205.1
i	**215.1**
j	**225.1**
k	**231.1**
l	**243.1**



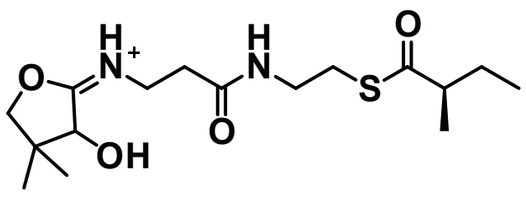
	Fragment m/z	+42 Da m/z
a	**132.1**	**174.1**
b	142.1	-
c	149.1	**191.1**
d	**159.1**	**201.1**
e	**166.1**	-
f	**175.1**	**217.1**
g	**184.1**	-
h	**205.1**	**247.1**
i	**215.1**	257.1
j	225.1	**267.1**
k	231.1	**273.1**
l	**243.1**	**285.1**



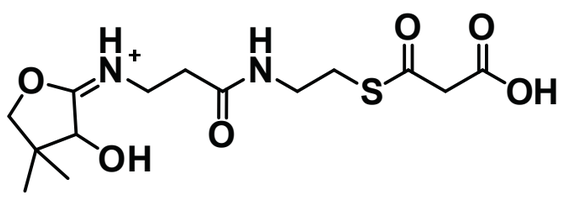
	Fragment m/z	+84 Da m/z
a	132.1	**216.1**
b	**142.1**	-
c	149.1	**233.1**
d	**159.1**	**243.1**
e	**166.1**	-
f	**175.1**	259.1
g	**184.1**	-
h	**205.1**	**289.1**
i	215.1	299.1
j	**225.1**	**309.1**
k	**231.1**	**315.1**
l	**243.1**	**327.1**



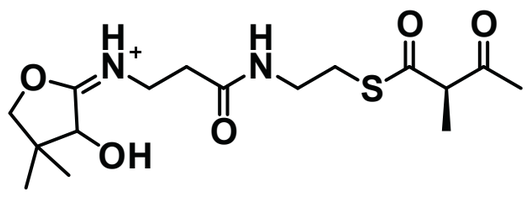
	Fragment m/z	+84 Da m/z
a	132.1	216.1
b	**142.1**	-
c	149.1	**233.1**
d	**159.1**	**243.1**
e	**166.1**	-
f	**175.1**	259.1
g	**184.1**	-
h	205.1	**289.1**
i	**215.1**	**299.1**
j	225.1	**309.1**
k	231.1	315.1
l	**243.1**	**327.1**



	Fragment m/z	+86 Da m/z
a	132.1	**218.1**
b	142.1	-
c	149.1	235.1
d	159.1	245.1
e	166.1	-
f	**175.1**	261.1
g	**184.1**	-
h	205.1	291.1
i	215.1	301.1
j	225.1	311.1
k	231.1	317.1
l	**243.1**	**329.1**



	Fragment m/z	+98 Da m/z
a	132.1	**230.1**
b	**142.1**	-
c	149.1	**247.1**
d	**159.1**	**257.1**
e	**166.1**	-
f	**175.1**	**273.1**
g	**184.1**	-
h	**205.1**	**303.1**
i	215.1	**313.1**
j	225.1	323.1
k	231.1	**329.1**
l	**243.1**	**341.1**



	Fragment m/z	+100 Da m/z
a	132.1	**232.1**
b	**142.1**	-
c	**149.1**	**249.1**
d	**159.1**	**259.1**
e	**166.1**	-
f	**175.1**	275.1
g	**184.1**	-
h	**205.1**	305.1
i	**215.1**	**315.1**
j	225.1	325.1
k	**231.1**	**331.1**
l	**243.1**	**343.1**

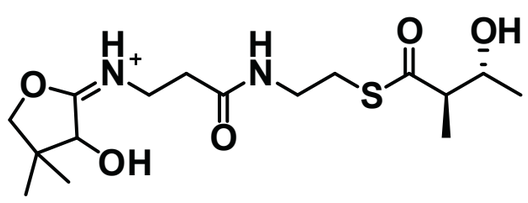


Figure SI-1. Linear trap MS3 confirmation of intermediate-loaded pantetheine ejection ions.

In addition to using FT-ICR-MS2 to verify the identity of all observed intermediate-loaded pantetheine ejection ions, ejection ions were subsequently trapped (during the same experiment) and subjected to additional rounds of tandem-MS. Pantetheine ejection ions yield a characteristic pattern of fragment ions, by which their identity can be confirmed (green colored peaks). The LovF intermediates remained bound to thiol-containing pantetheine fragments, resulting in mass shifts of these fragments (blue colored peaks). Each of the intermediate-loaded pantetheine ejection ions also yielded a prominent peak at 261.1 m/z (red colored peaks) as some of the ejection ions trapped in each experiment released their covalently bound intermediate during CID. Two unique pantetheine ejection ion fragmentation patterns occurred: The malonyl-loaded pantetheine ejection ions were observed to predominantly undergo a McLafferty type rearrangement resulting in acetyl-loaded, intact pantetheine ion (labeled *) at 303.1 *m/z*. This ion was trapped and fragmented again in the linear ion trap, giving the same fragmentation pattern that would be observed from MS^3 fragmentation of an actual acetyl-loaded pantetheine ejection ion . MS^3 of the β-hydroxy-α-methylbutyryl-loaded pantetheine ejection produced a moderate amount of the standard fragment ions, but also produced a prominent ion with a mass of 317.1 *m/z* (labeled **). This mass is consistent with that of a pantetheine ejection ion bearing a 56 Da species ion, and likely was the result of a β-elimination reaction upon MS^3. Subsequent MS^4 of the 317.1 *m/z* species resulted in the typical diagnostic pattern of pantetheine ejection ions in which the thiol containing fragments all showed a mass-addition of 56 Da.

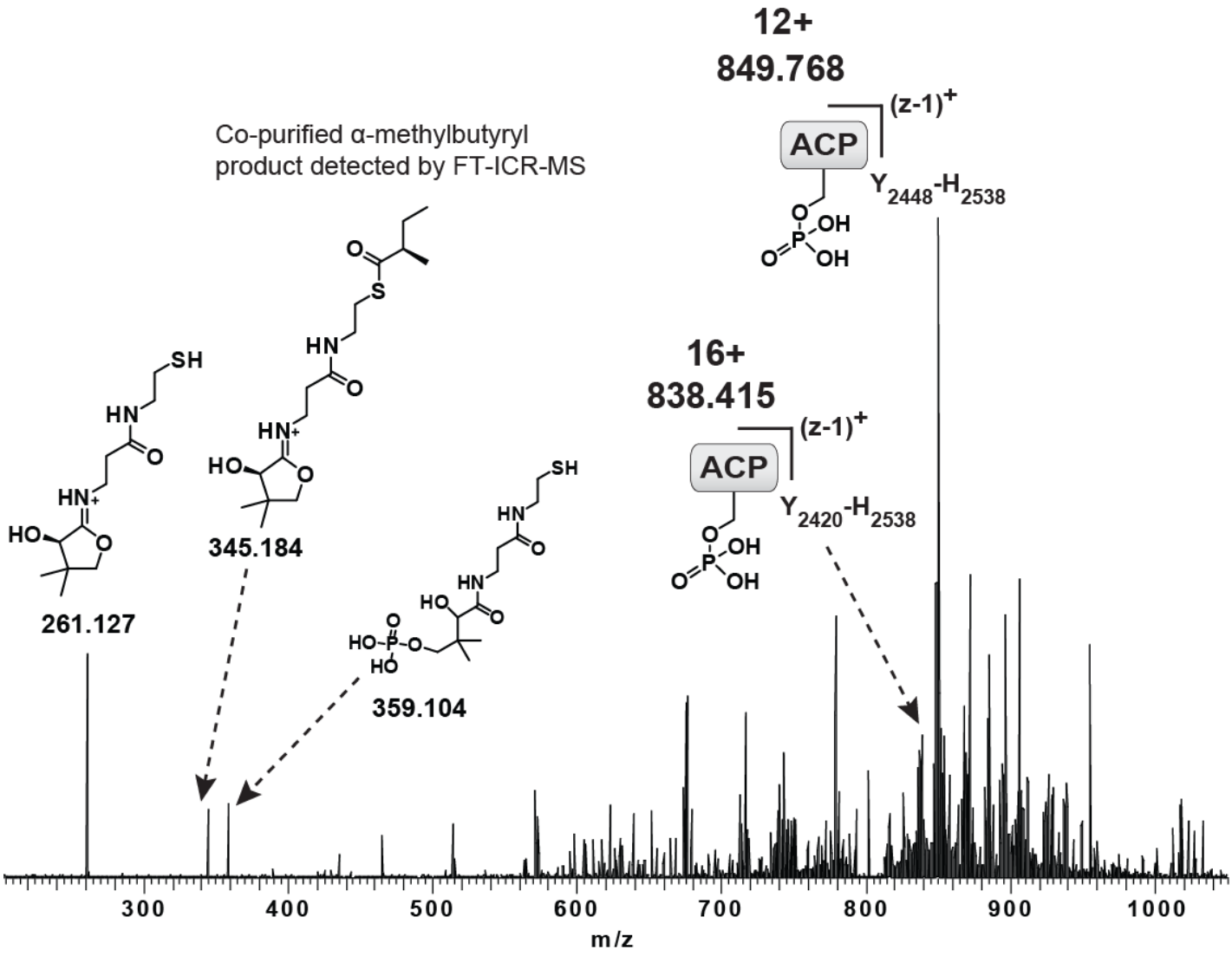


Figure SI-2. Detection of α-methylbutyrate by PPant ejection. FT-ICR-MS^2 of *holo*-LovF active site peptide following incubation with acetoacetyl-CoA, SAM, and NADPH. A low intensity α-methylbutyryl-loaded PPant ejection ion (345.184 *m/z*) was detected and could be distinguished from the adjacent acetoacetyl-loaded PPant ejection ion (345.148 *m/z*). An increase in the HPLC retention time of the ACP active site bearing covalently bound α-methylbutryate resulted overlap in elution times of the Y_{2420}-H_{2538} α-methylbutyryl-*S*-ACP peptide with the Y_{2448}-H_{2538} ACP peptide bearing other intermediates generated from the *in vitro* reaction. As a result, post-ejection parent phosphopeptides for both Y_{2420}-H_{2538} and the Y_{2448}-H_{2538} ACP peptides were detected.