Electrophilic modification of PKM2 by 4-hydroxynonenal and 4-oxononenal results in protein cross-linking and kinase inhibition

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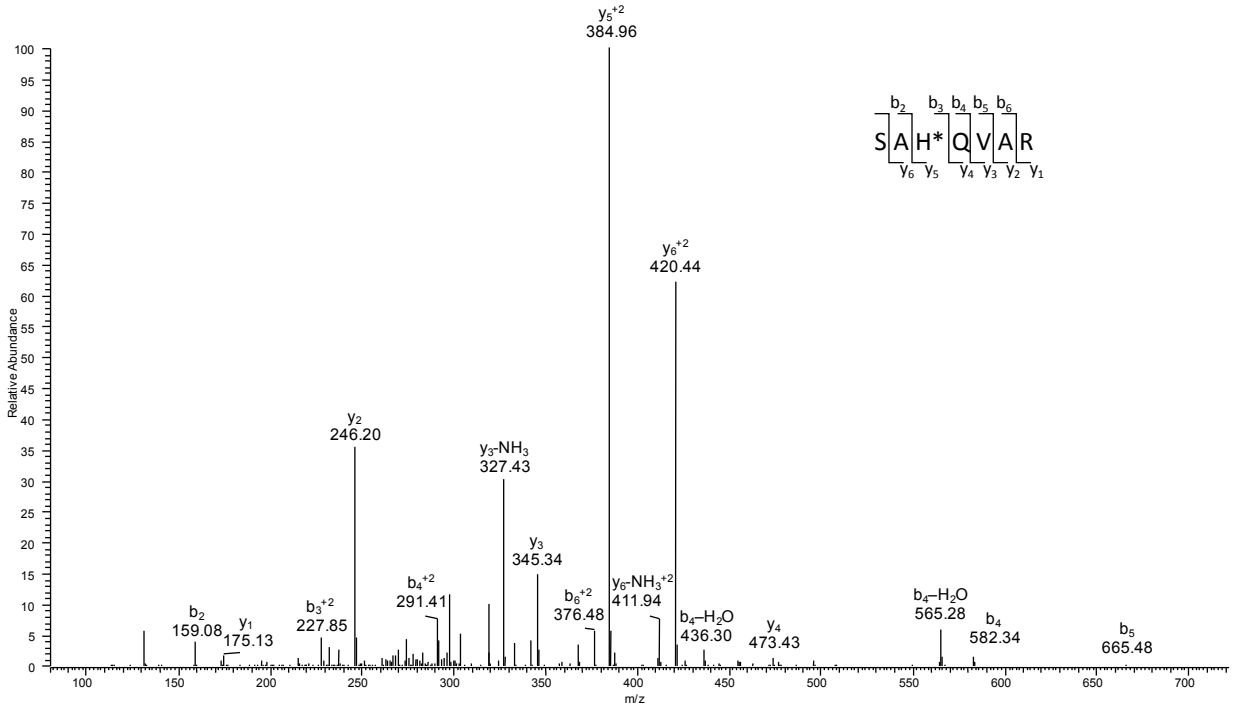
Supporting Information

Figure S1. Tandem mass spectra of peptides with Michael adducts from HNE or ONE on recombinant PKM2. Peptides include A) SAH*QVAR, B) CC*SGAIIVLTK, C) NTGIIC*TIGPASR, D) IENH*EGVR, E) NIK*IISK. An asterisk (*) indicates the site of modification.

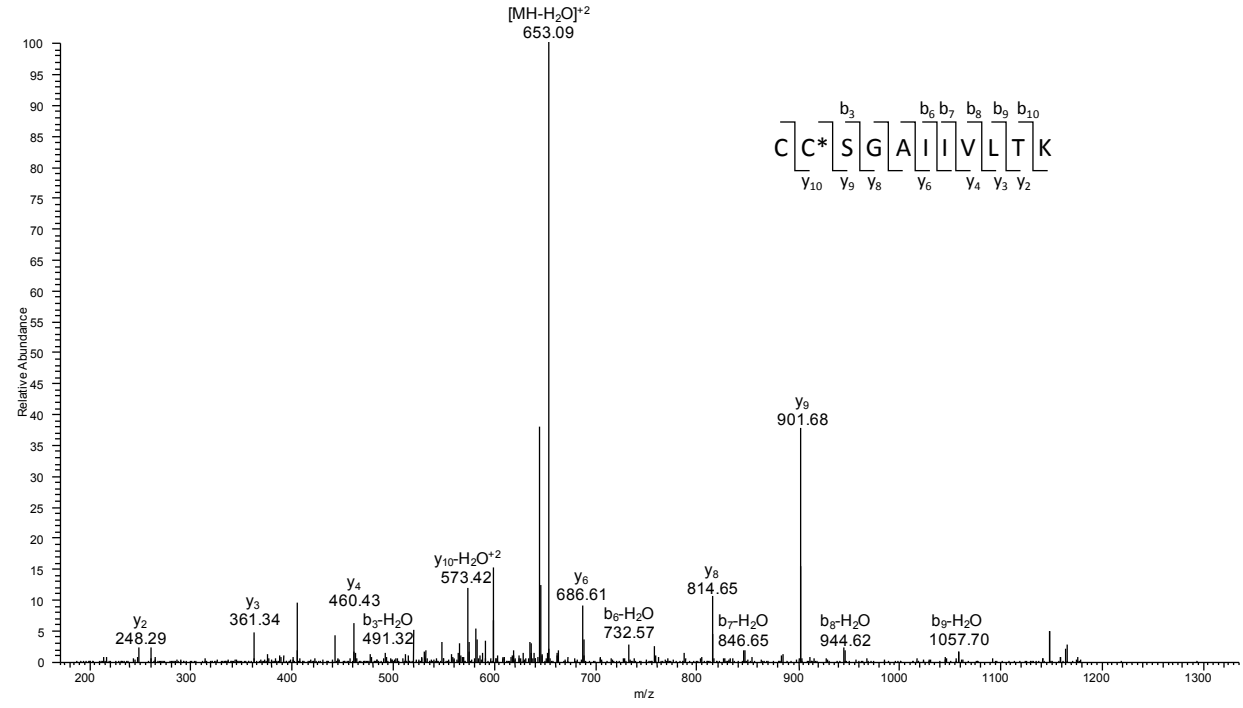
Figure S2. Tandem mass spectrum of peptides with a 4-ketoamide adducts from ONE on the peptide VLGEK*GK on recombinant PKM2.

Figure S1

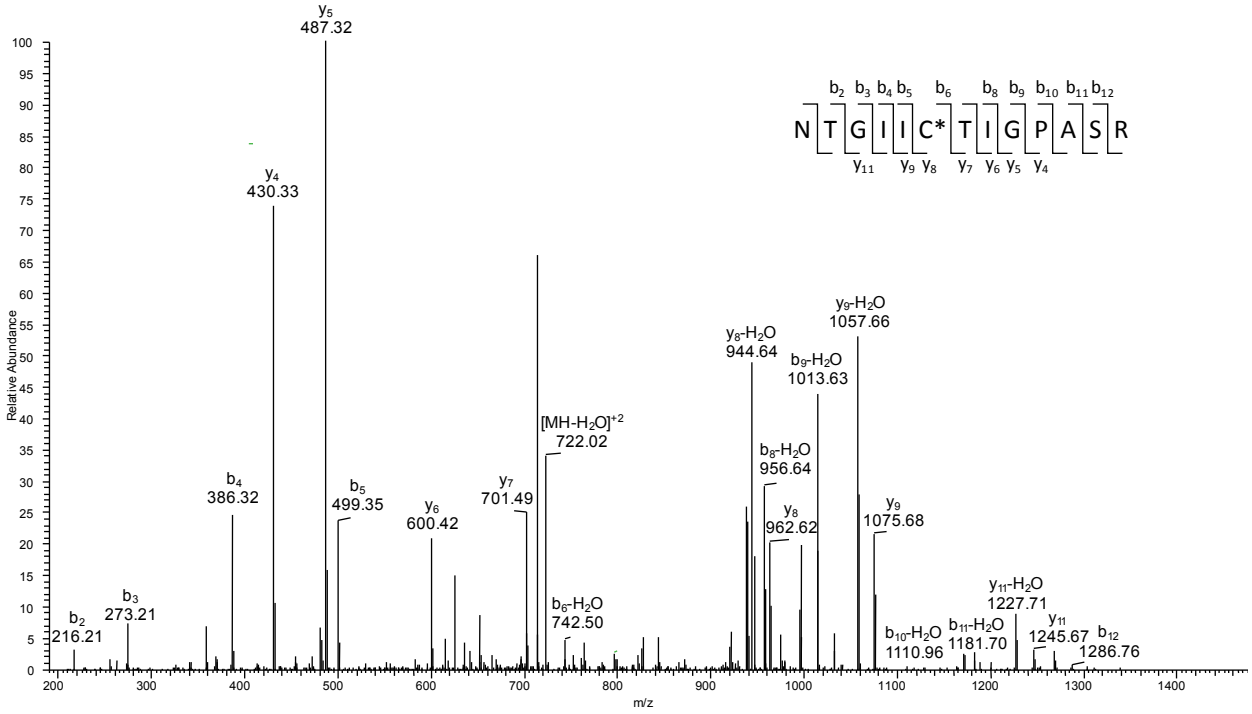
A)


y5+2
384.96
b2 b3 b4 b5 b6
SAH*QVAR
y6 y5 y4 y3 y2 y1
y6+2
420.44
y2
246.20
y3-NH3
327.43
y3
345.34
b4+2
291.41
b6+2
376.48
y6-NH3+2
411.94
b3+2
227.85
b2
159.08
y1
175.13
b4–H2O
436.30
y4
473.43
b4–H2O
565.28
b4
582.34
b5
665.48
Relative Abundance
m/z

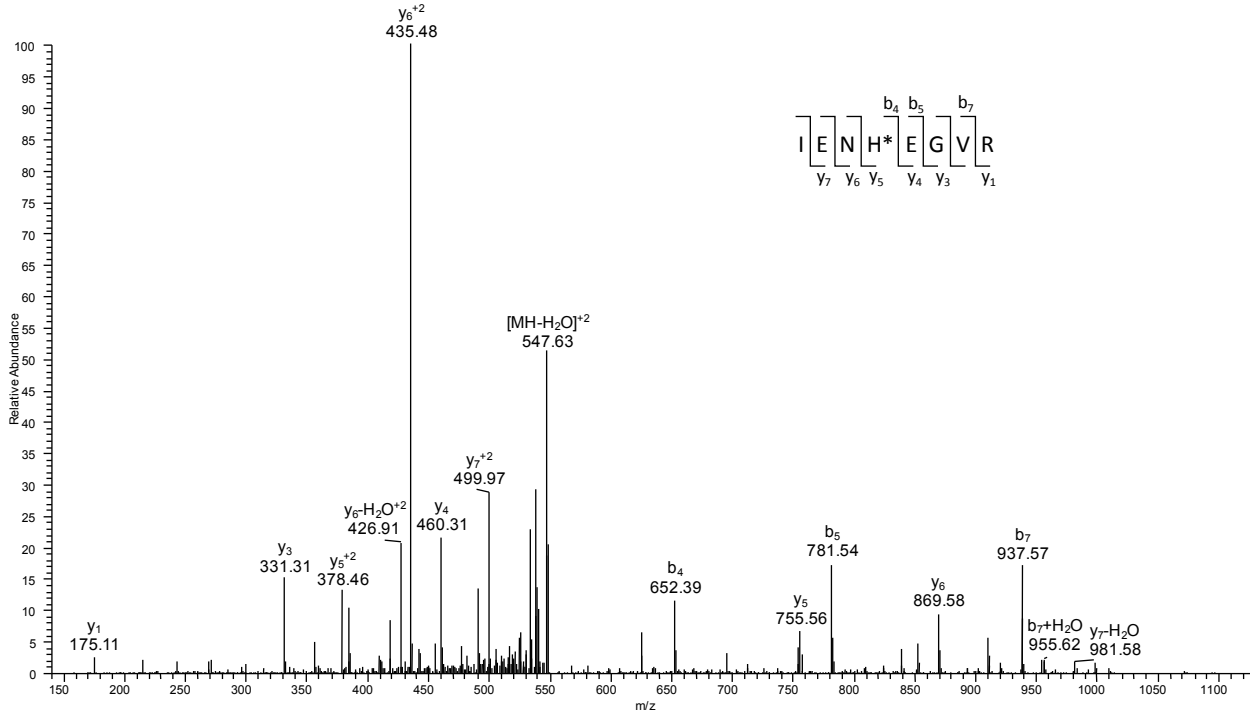
B)


[MH-H2O]+2
653.09
b3 b6 b7 b8 b9 b10
CC*SGAIIVLTK
y10 y9 y8 y6 y4 y3 y2
y9
901.68
y10-H2O+2
573.42
y4
460.43
y3
361.34
y2
248.29
b3-H2O
491.32
y6
686.61
b6-H2O
732.57
y8
814.65
b7-H2O
846.65
b8-H2O
944.62
b9-H2O
1057.70
Relative Abundance
m/z

C)


y5 487.32
y4 430.33
b4 386.32
b5 499.35
y6 600.42
y7 701.49
[MH-H2O]+2 722.02
b6-H2O 742.50
b2 216.21
b3 273.21
y8-H2O 944.64
b8-H2O 956.64
y8 962.62
b9-H2O 1013.63
y9-H2O 1057.66
y9 1075.68
b10-H2O 1110.96
b11-H2O 1181.70
y11-H2O 1227.71
y11 1245.67
b12 1286.76
b2 b3 b4 b5 b6 b8 b9 b10 b11 b12
N T G I I C* T I G P A S R
y11 y9 y8 y7 y6 y5 y4
Relative Abundance
m/z

D)


y6+2 435.48
[MH-H2O]+2 547.63
y7+2 499.97
y6-H2O+2 426.91
y4 460.31
y3 331.31
y5+2 378.46
y1 175.11
b4 652.39
b5 781.54
y5 755.56
y6 869.58
b7 937.57
b7+H2O 955.62
y7-H2O 981.58
b4 b5 b7
I E N H* E G V R
y7 y6 y5 y4 y3 y1
Relative Abundance
m/z

E)

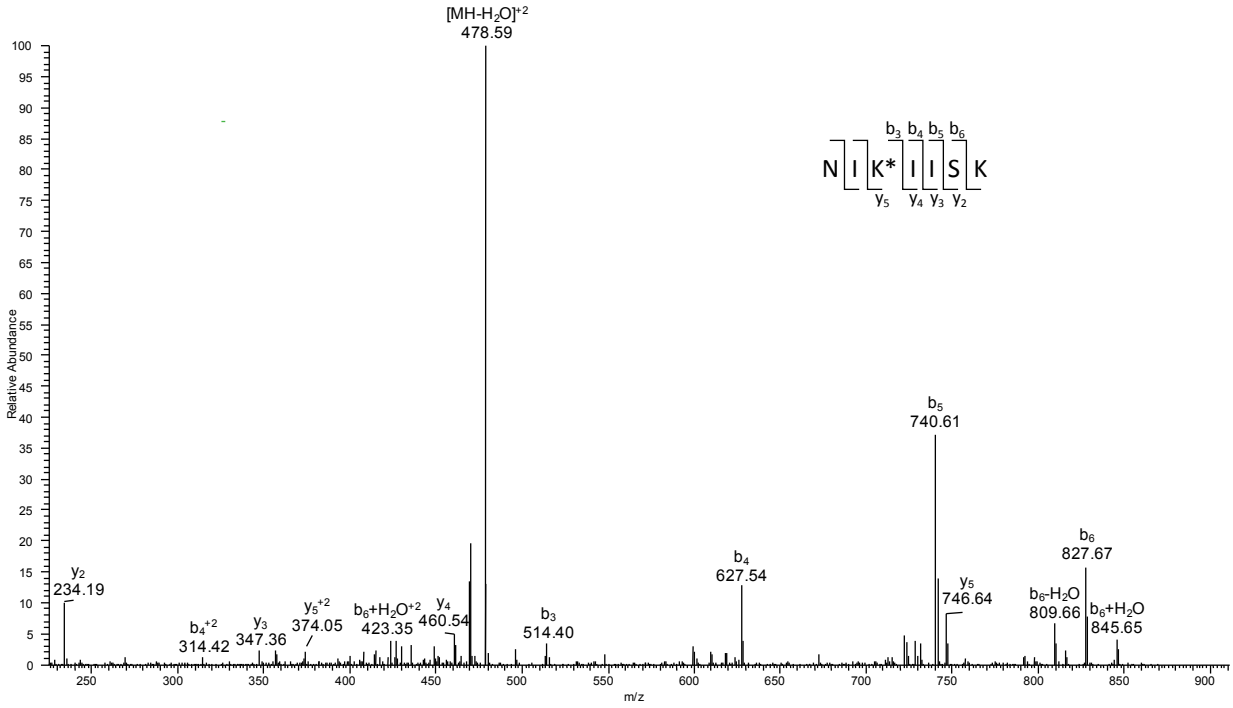
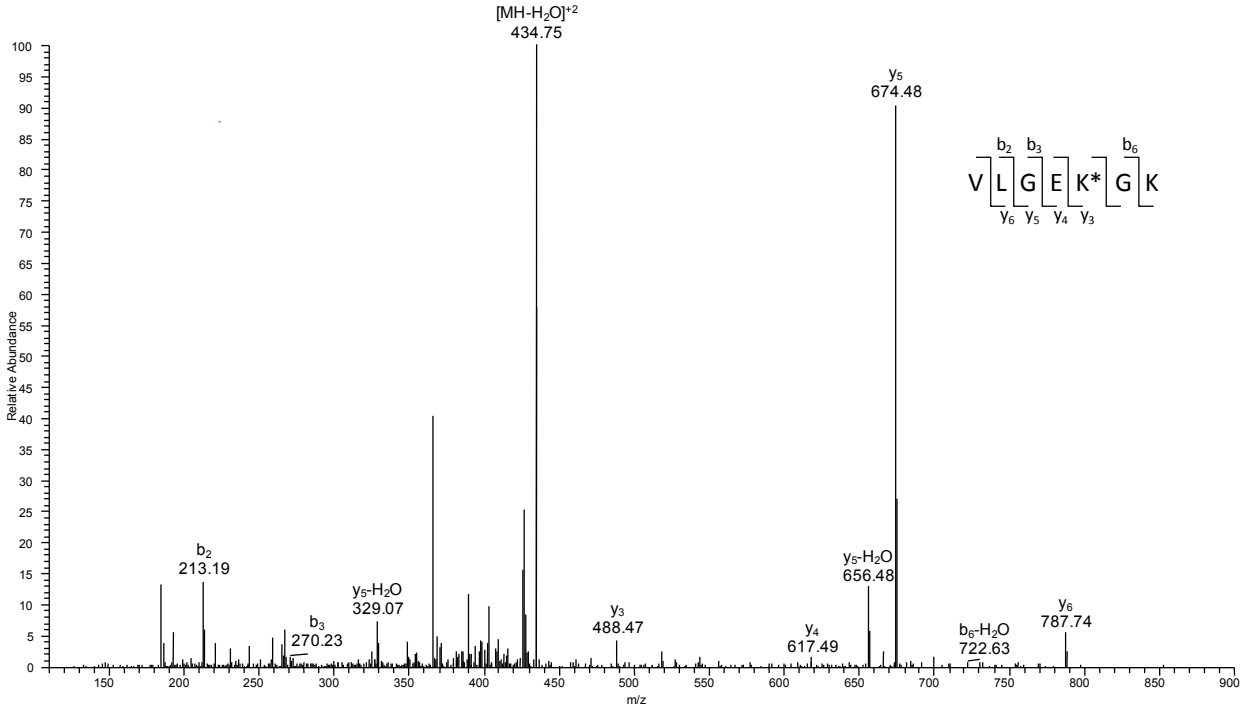

[MH-H2O]+2
478.59
b3 b4 b5 b6
N I K* I I S K
y5 y4 y3 y2
b5
740.61
b6
827.67
b4
627.54
y2
234.19
y5
746.64
b6-H2O
809.66
b6+H2O
845.65
b4+2
314.42
y3
347.36
y5+2
374.05
b6+H2O+2
423.35
y4
460.54
b3
514.40
Relative Abundance
m/z

Figure S2


[MH-H2O]+2
434.75
y5
674.48
b2 b3 b6
V L G E K* G K
y6 y5 y4 y3
b2
213.19
y5-H2O
329.07
b3
270.23
y3
488.47
y4
617.49
y5-H2O
656.48
b6-H2O
722.63
y6
787.74
Relative Abundance
m/z