Supporting Information

Identification of Proteins Adducted by Lipid Peroxidation Products in Plasma and Modifications of Apolipoprotein A1 with a Novel Biotinylated Probe

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Table S1 and S2

Figure S1 and S2

Table S1. ω-end electrophile adducts found in ApoA1 from plasma supplemented with PLPBSO

sequences	**positions**	**mass shifts**			
		126	112	98	84
VK*DLATVYVDVLK	K12	x			x
DLEEVK*AK	K94	x	x		x
AK*VQPYLDDFQK	K96	x		x	x
K*WQEEMELYR	K107	x		x	x
QK*VEPLR	K118		x		
QK*LH*ELQEK	K133 or /H135	x			
LH*ELQEK	H135	x			
AH*VDALR	H155		x	x	x
TH*LAPYSDELR	H162		x	x	x
LAEYH*AK	H193	x		x	
AK*PALEDLR	K208	x		x	x

Mass shifts: 126: nonanal, 112: octanal, 98: heptanal, 84: hexanal
x: found

Table S2. Complete list of proteins identified from plasma following supplementation and oxidation in the presence of PLPBSO, affinity purification and base hydrolysis.

Protein name	Accession #	Average % Coverage
163 kDa protein	IPI00465313.2	19.60%
ALB protein	IPI00216773.4	58.60%
Alpha-1-antitrypsin precursor	IPI00553177.1	26.90%
Apolipoprotein A-I precursor	IPI000218541.1	68.30%
Apolipoprotein A-II precursor	IPI00021854.1	49.00%
Apolipoprotein A-IV precursor	IPI00304273.1	34.40%
Apolipoprotein B-100 precursor	IPI00022229.1	5.00%
Apolipoprotein D precursor	IPI00006662.1	18.50%
Apolipoprotein E precursor	IPI00021842.1	30.60%
Apolipprotein C-III precursor	IPI00021857.1	19.20%
Beta-globin gene from a thalassemia patient	IPI00382950.1	15.50%
Complement C3 precursor	IPI00164623.4	24.80%
Complement C4-A precursor	IPI00032258.4	8.60%
Factor VII active site mutant immunoconjugate	IPI00382606.1	5.30%
Fibrinogen beta chain precursor	IPI00298497.3	18.00%
Ig gamma-2 chain C region	IPI00399007	6.30%
Ig mu heavy chain disease protein	IPI00385264.1	13.50%
Inter-alpha-trypsin inhibitor heavy chain H1 precursor	IPI00292530.1	9.80%
Inter-alpha-trypsin inhibitor heavy chain H2 precursor	IPI00305461	10.20%
Serum amyloid A-4 protein precurosr	IPI00019399.1	24.60%
Serum paraoxonase/arylesterase 1	IPI00218732.2	17.20%

Figure S1. Total ion current LC-MS trace of peptides from a tryptic digest of oxidized plasma supplemented with PLPBSO (A) and oxidized, unsupplemented plasma (B). Plasma was supplemented with PLPBSO and then incubated with the free radical initiator AIPH as described under Experimental Procedures. After oxidation, biotinylated (PLPBSO-adducted) plasma proteins were captured on streptavidin-agarose, digested and analyzed by LC-MS. Very little non-specific binding was detected in the control sample (B).

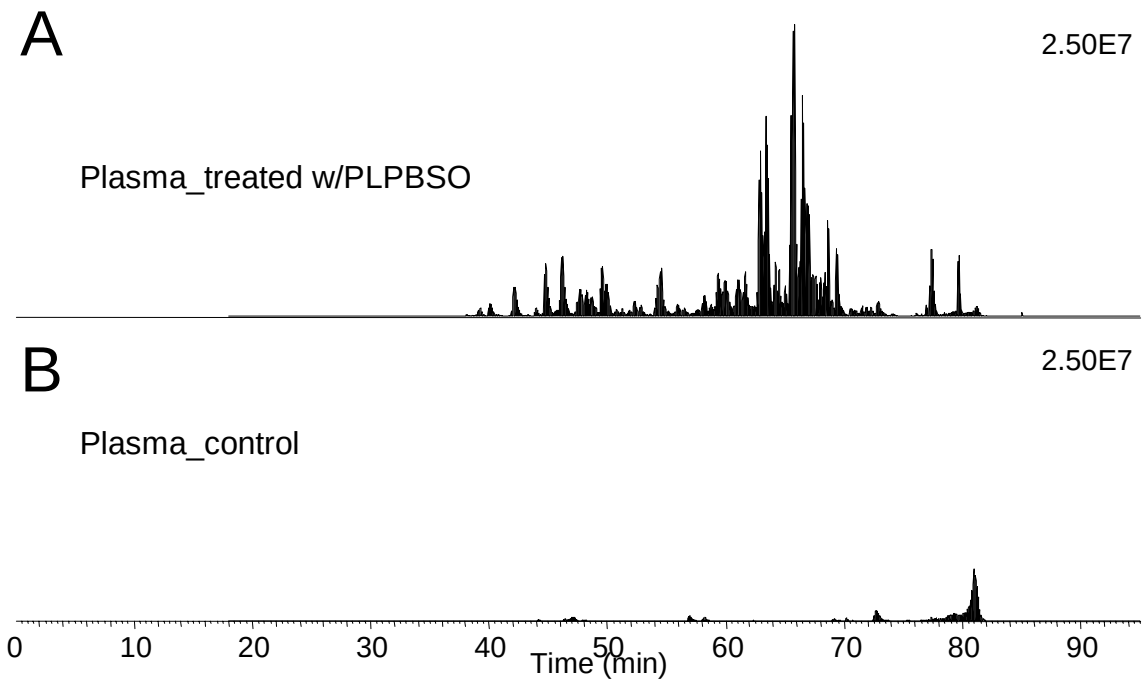
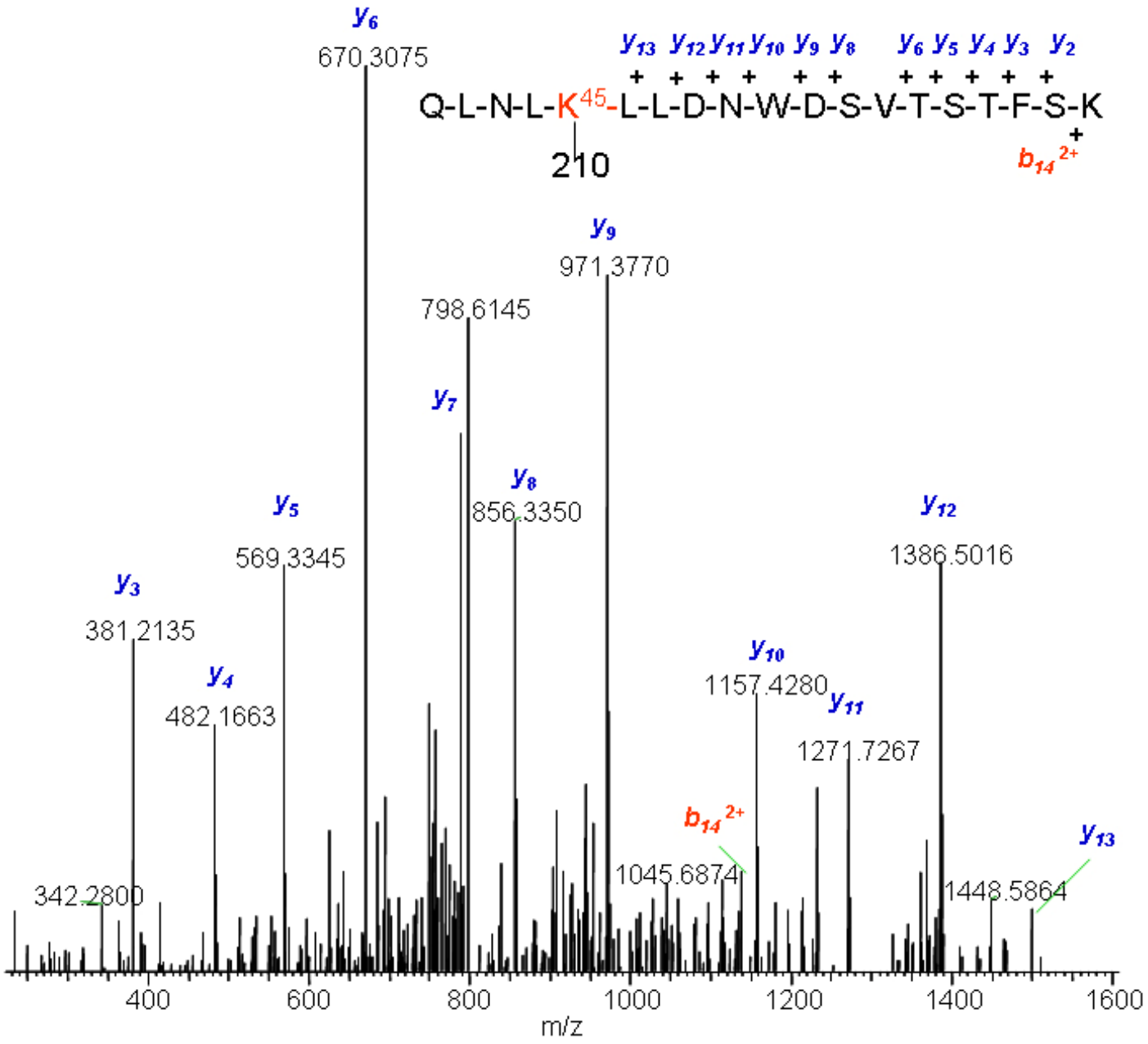


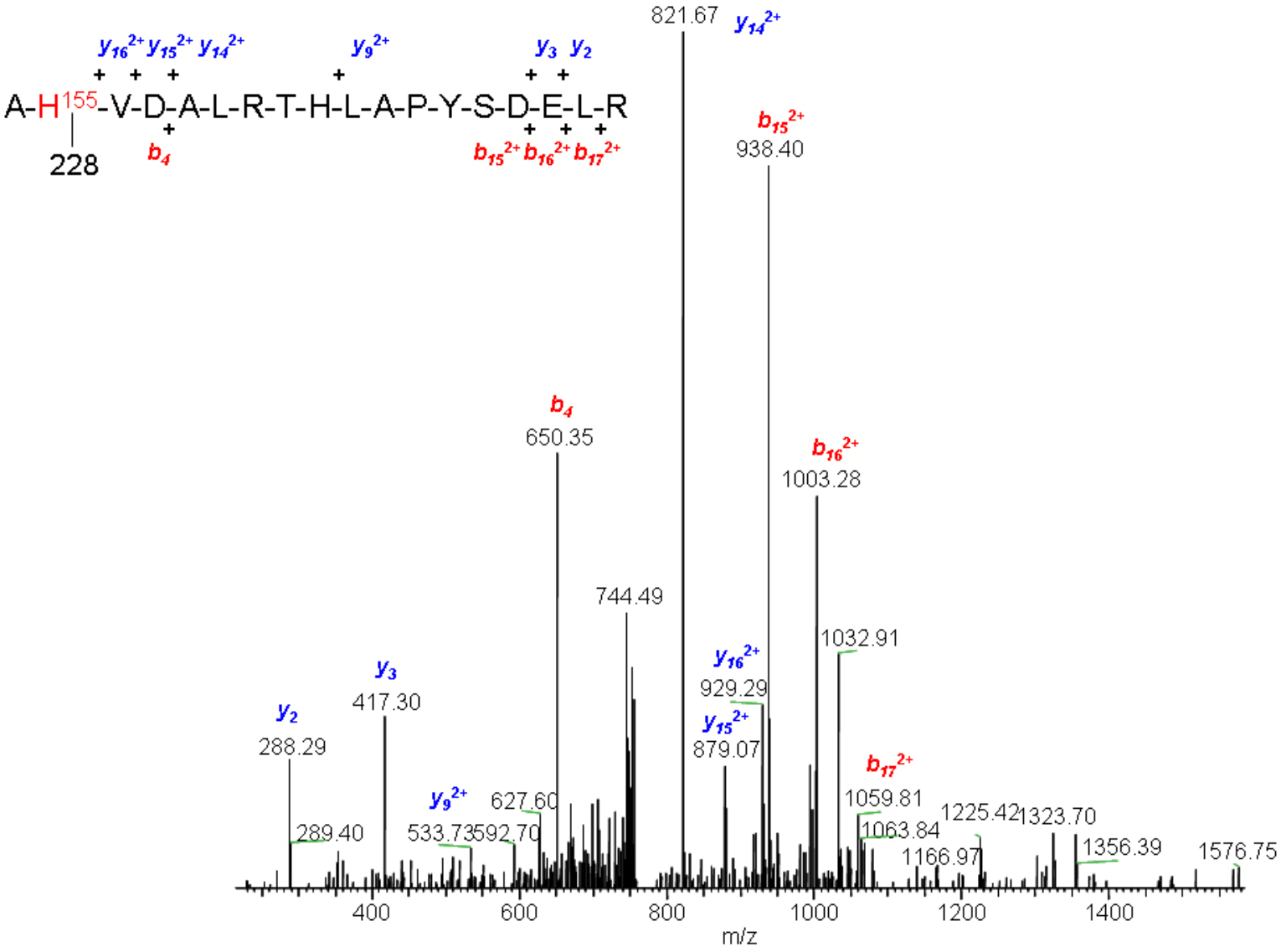
Figure S2. MS-MS spectra of ApoA1adducts formed with PLPBSO oxidation products or HNE.

a. KODA (M+210) adduct of ApoA1 peptide QLNL*K[45]LLDNWDSVTSTFSK

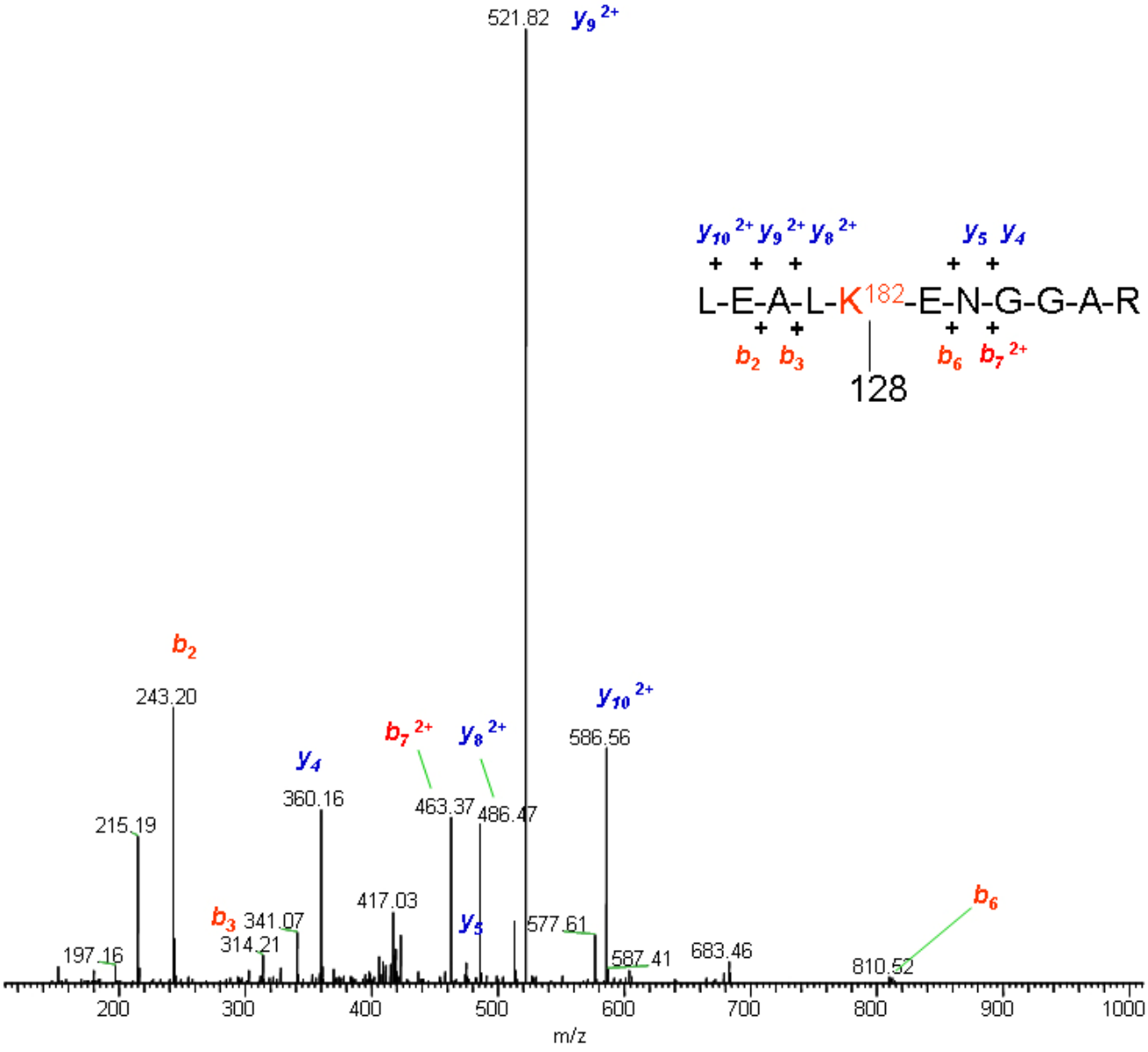


b. HODA (M+228) non-reduced Michael adduct of ApoA1 peptide

A*H^{155}VDALRTHLAPYSDELR

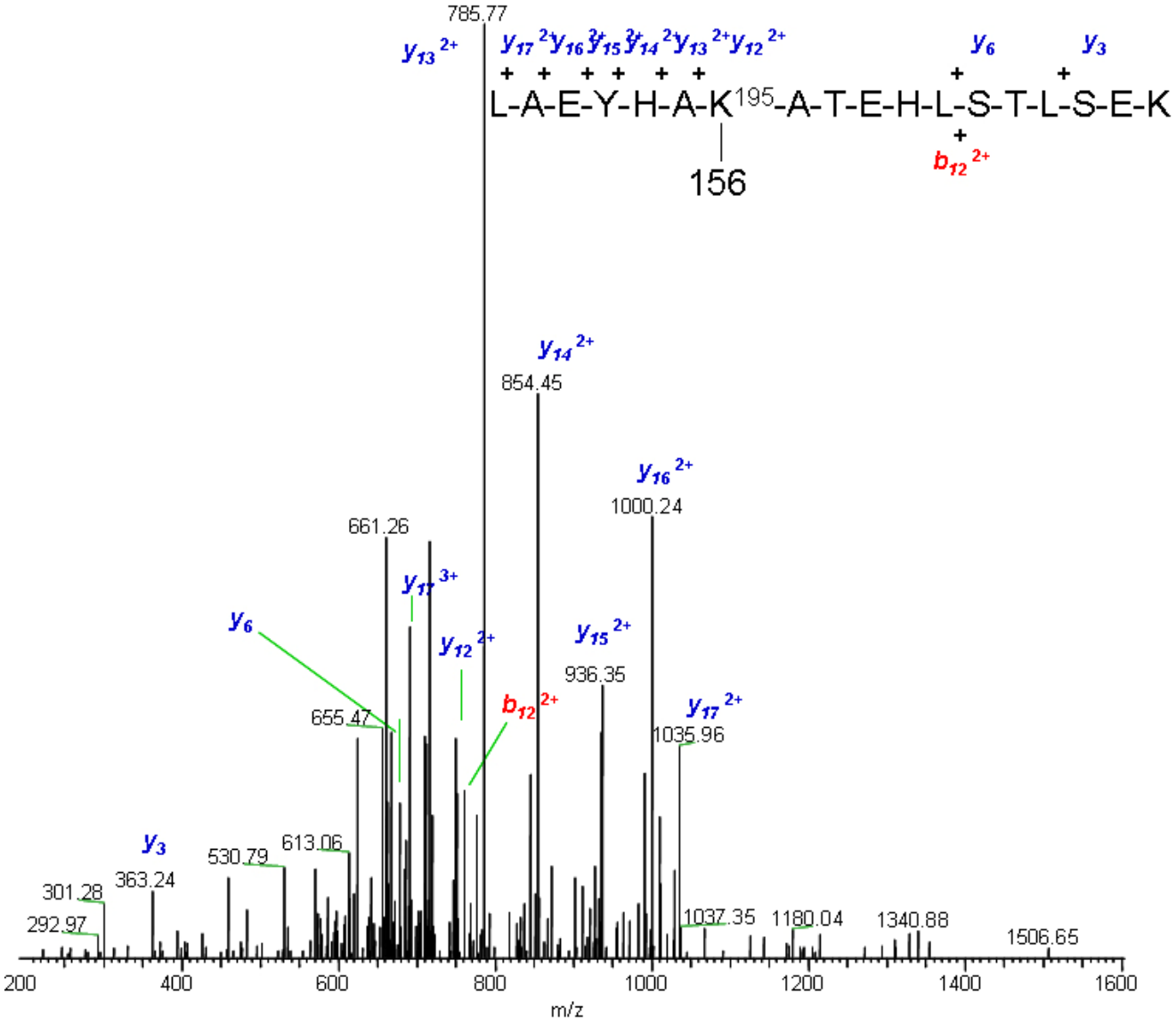


c. 7-oxooctanoic acid imine adduct (M + 128) of ApoA1 peptide LEAL*K^{182}ENGGAR

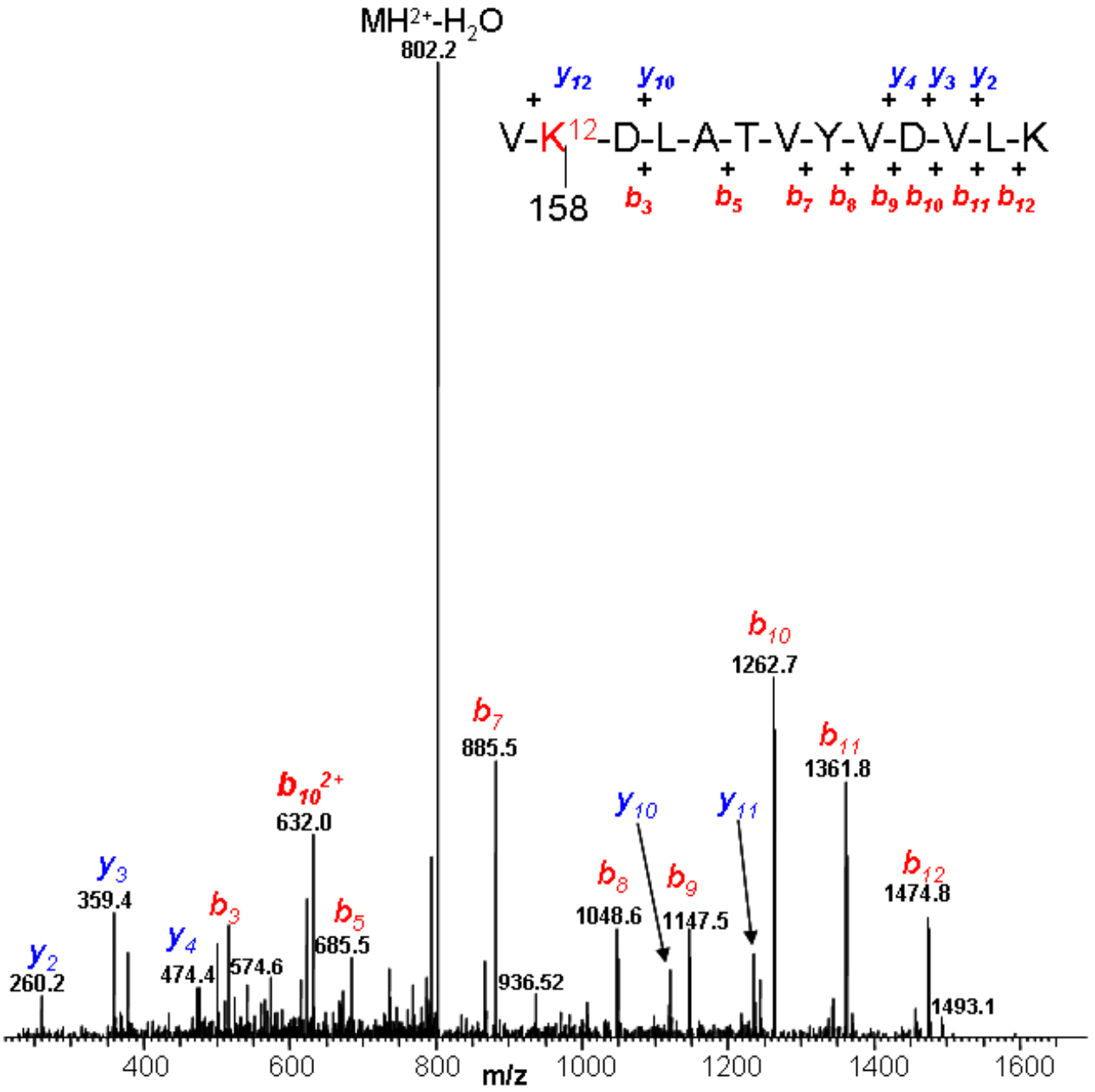


d. HODA (M+156) 9-oxononanoic acid imine adduct of ApoA1 peptide

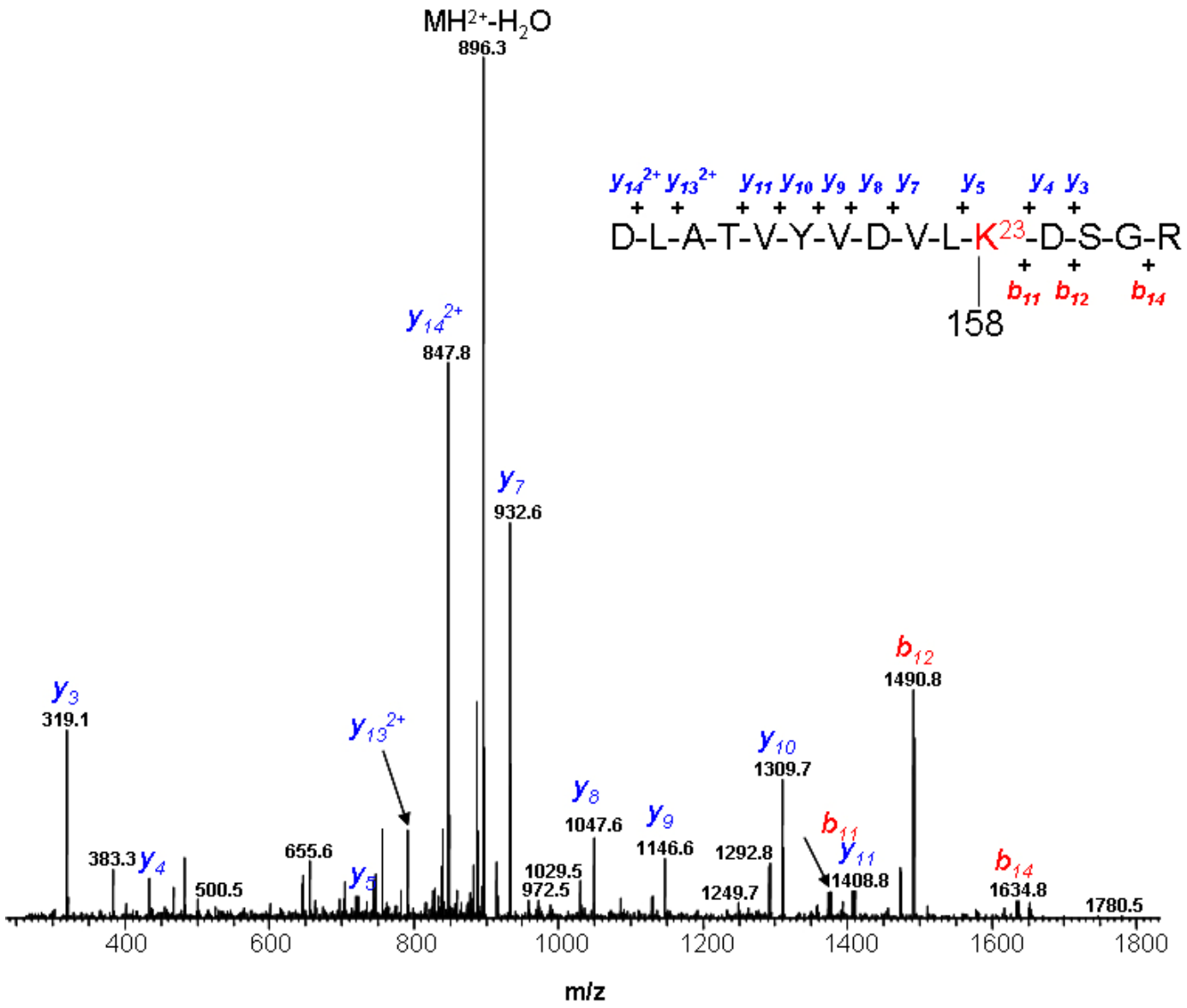
LAEYHA*K^{195}ATEHLSTLSEK



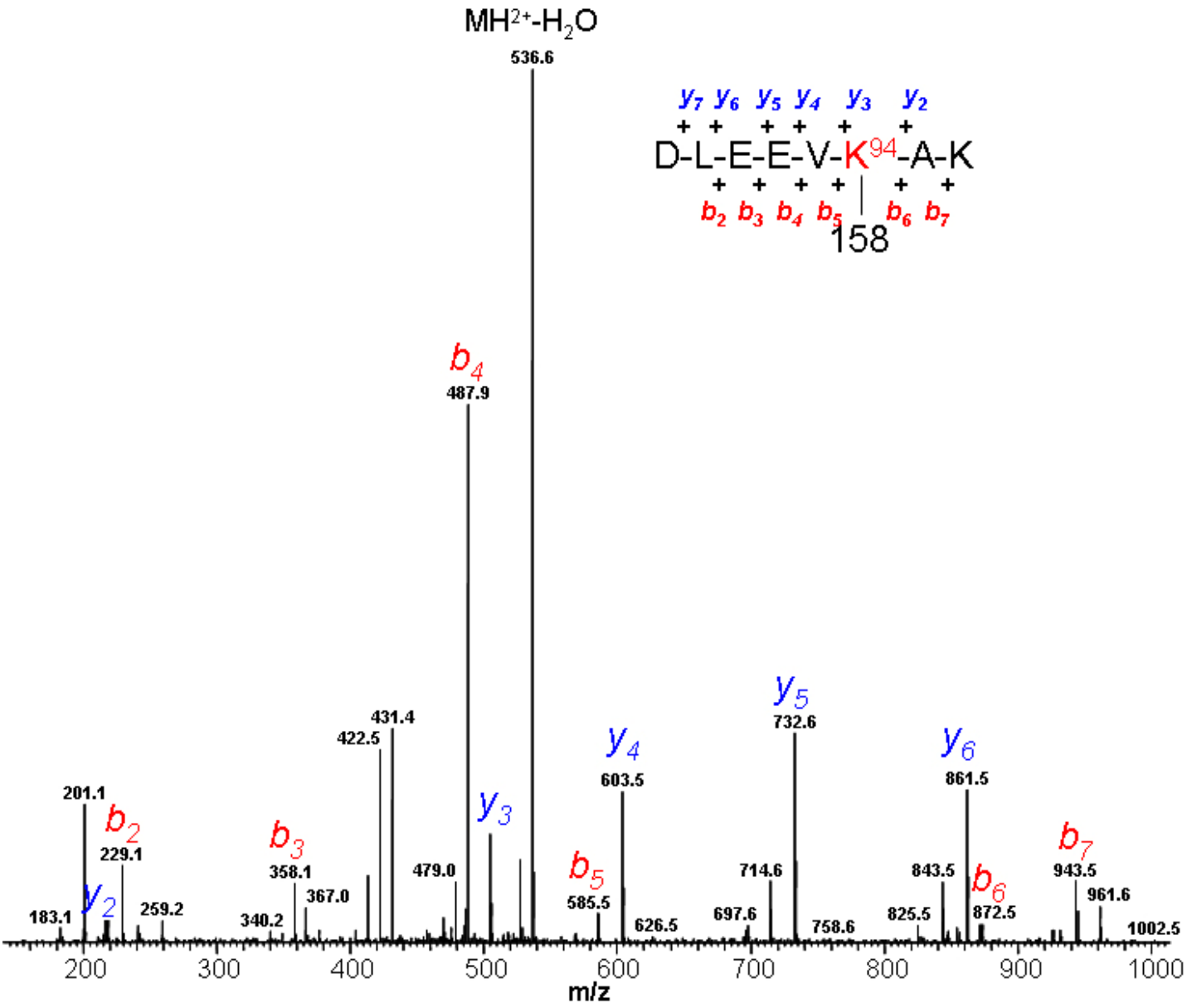
e. HNE (M+158) reduced Michael adduct of ApoA1 peptide V*K[12]DLATVYVDVLK



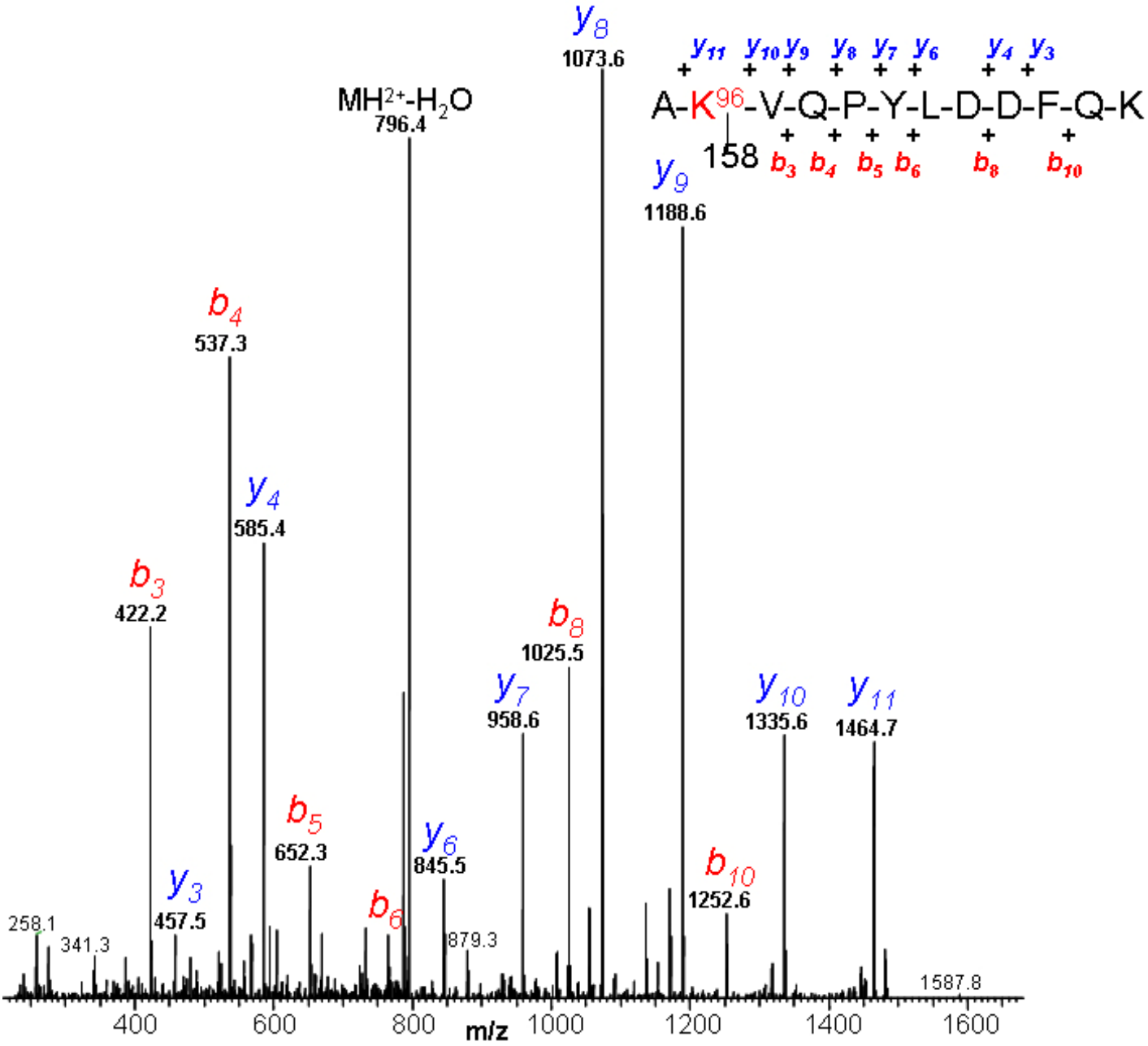
f. HNE (M+158) reduced Michael adduct of ApoA1 peptide DLATVYVDVL*K[23]DSGR



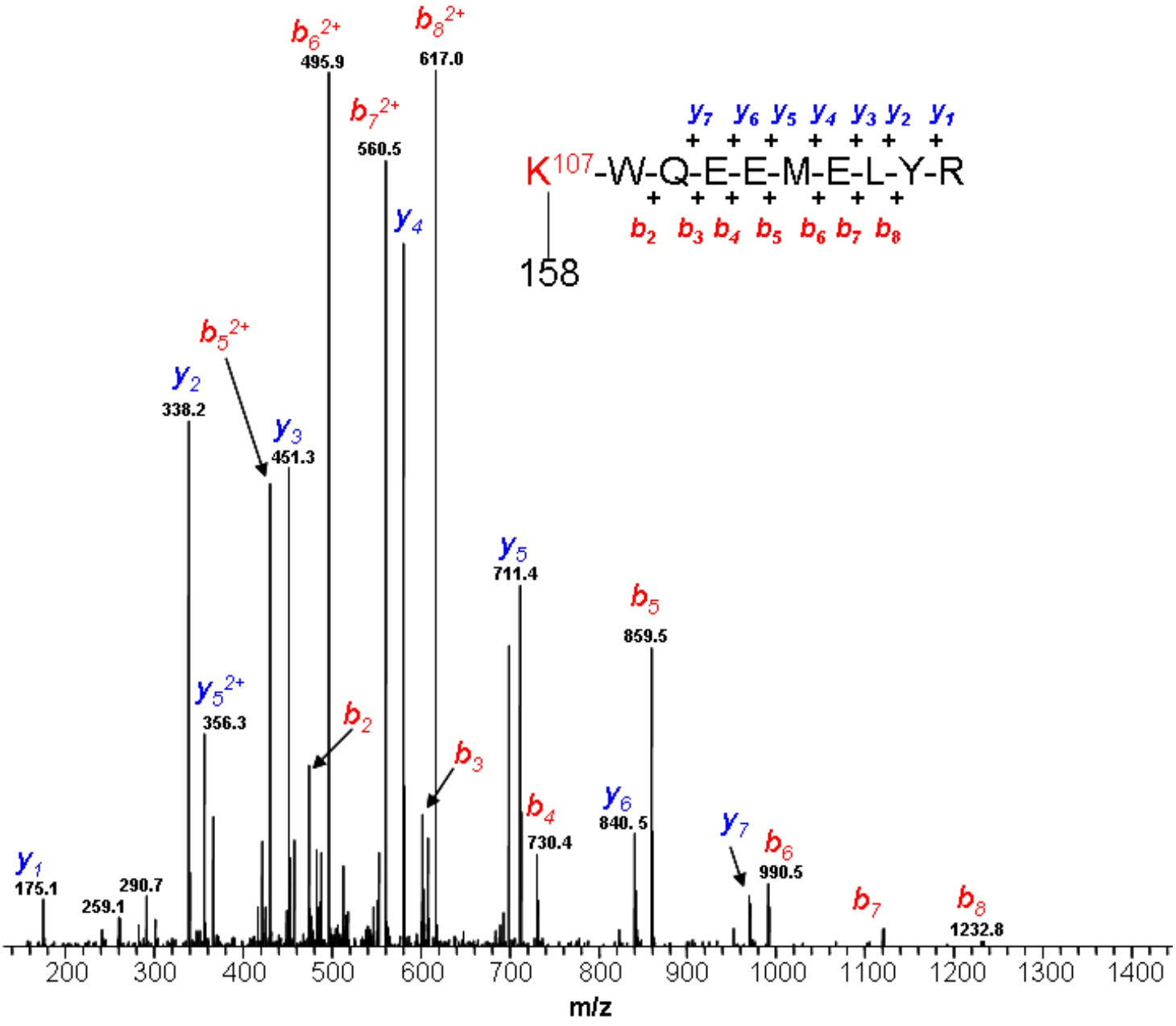
g. HNE (M+158) reduced Michael adduct of ApoA1 peptide DLEEV*K^{94}AK



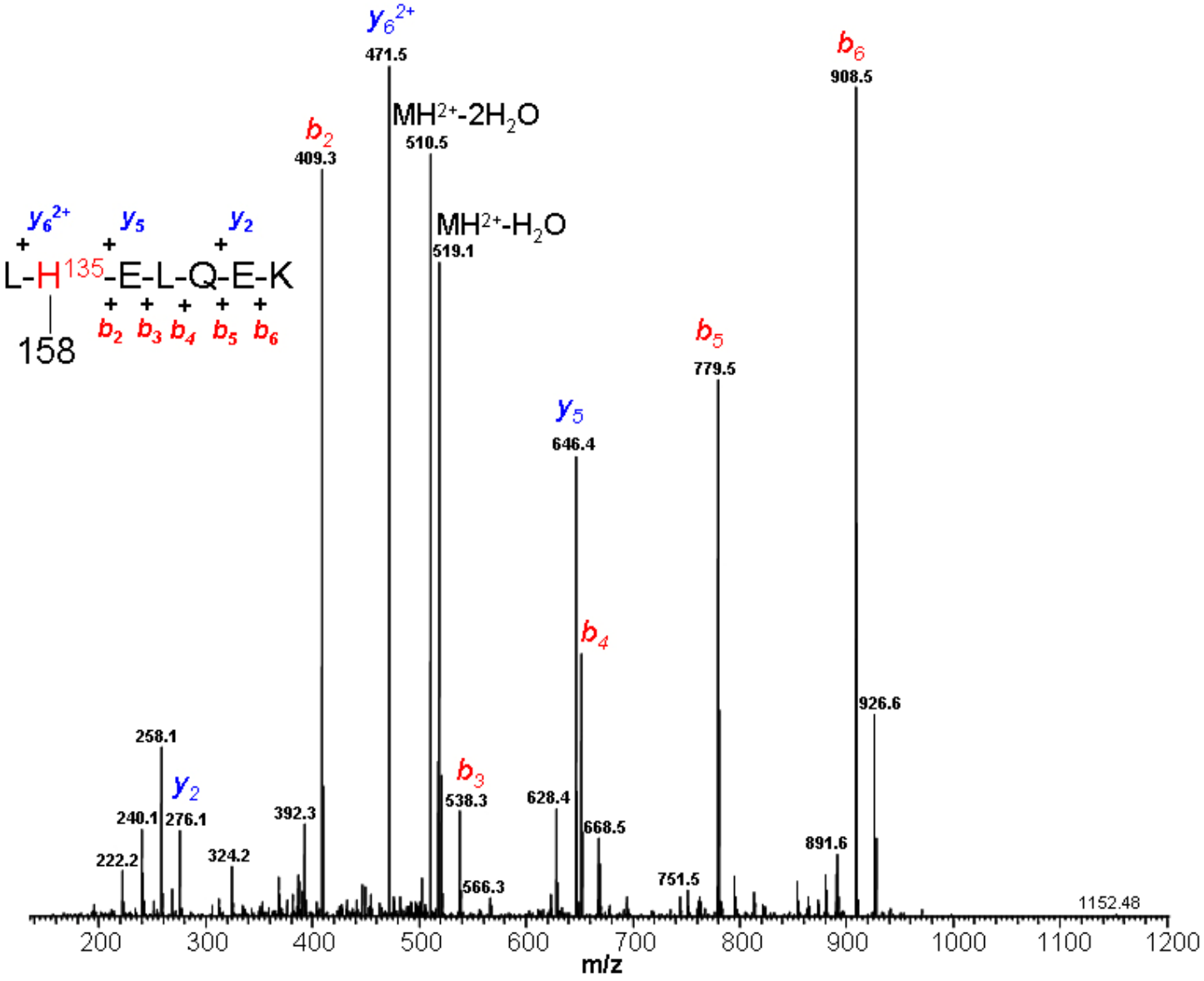
h. HNE (M+158) reduced Michael adduct of ApoA1 peptide A*K^{96}VQPYLDDFQK



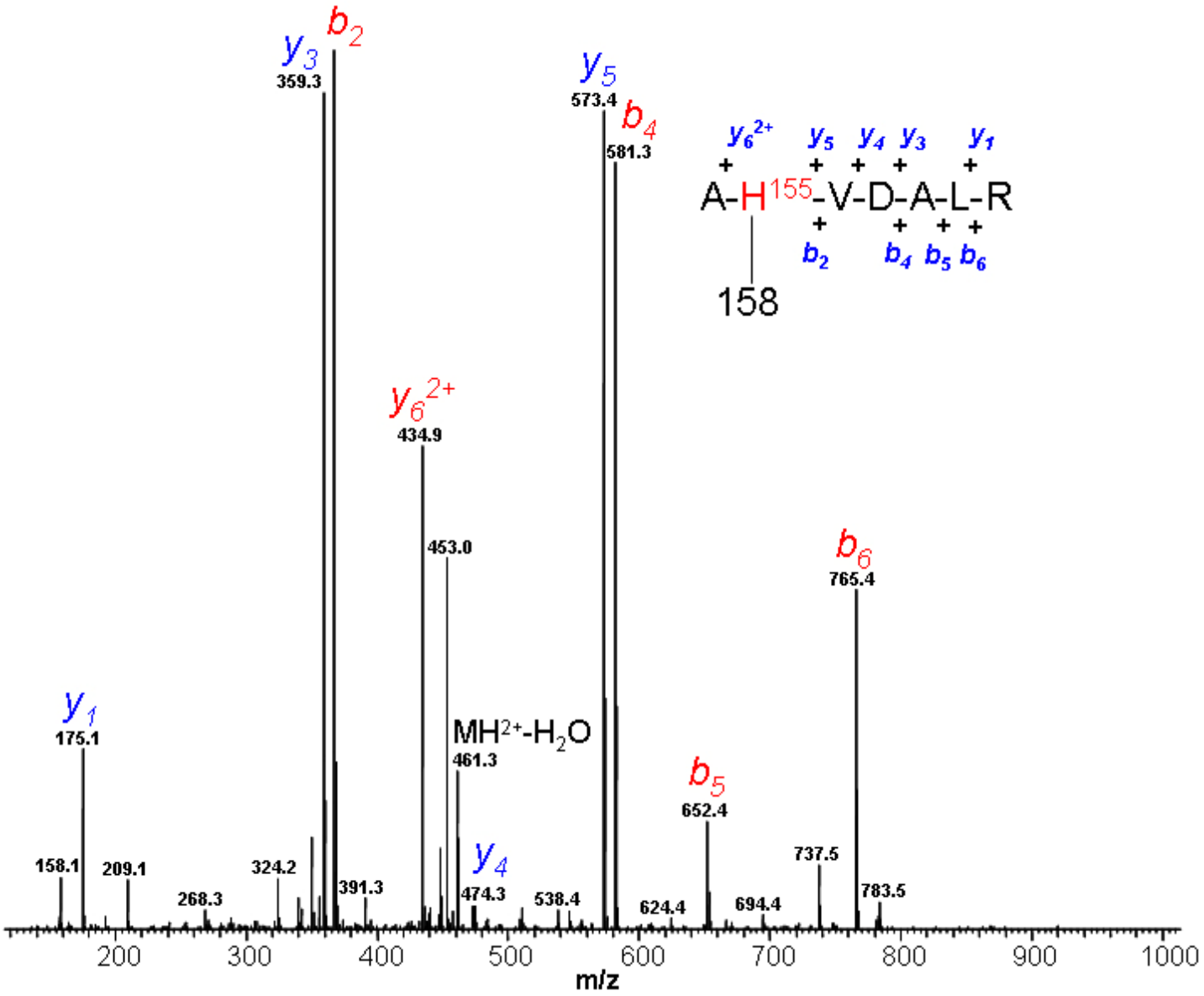
i. HNE (M+158) reduced Michael adduct of ApoA1 peptide *K^{107}WQEEMELYR



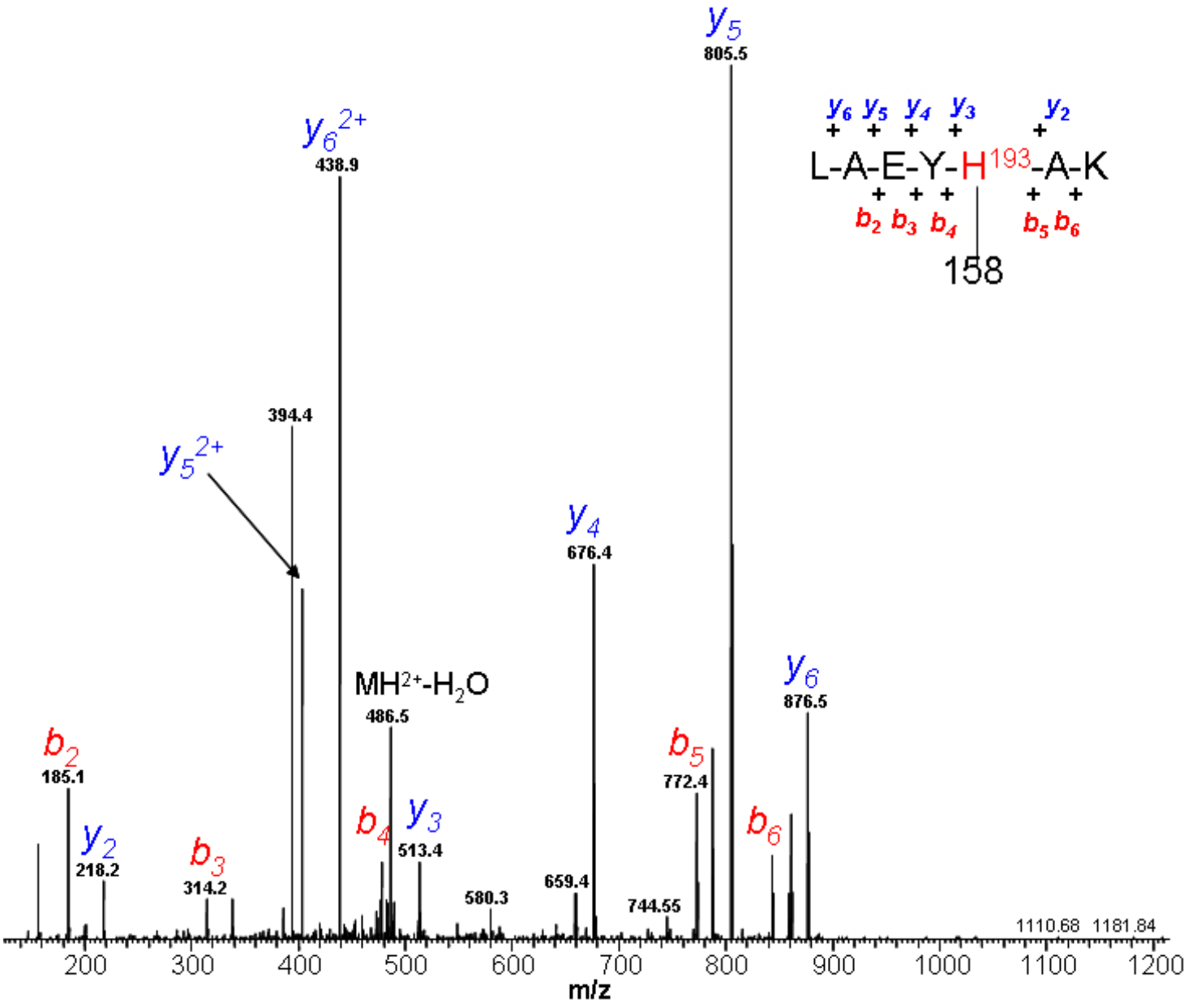
j. HNE (M+158) reduced Michael adduct of ApoA1 peptide L*H^{135}ELQEK



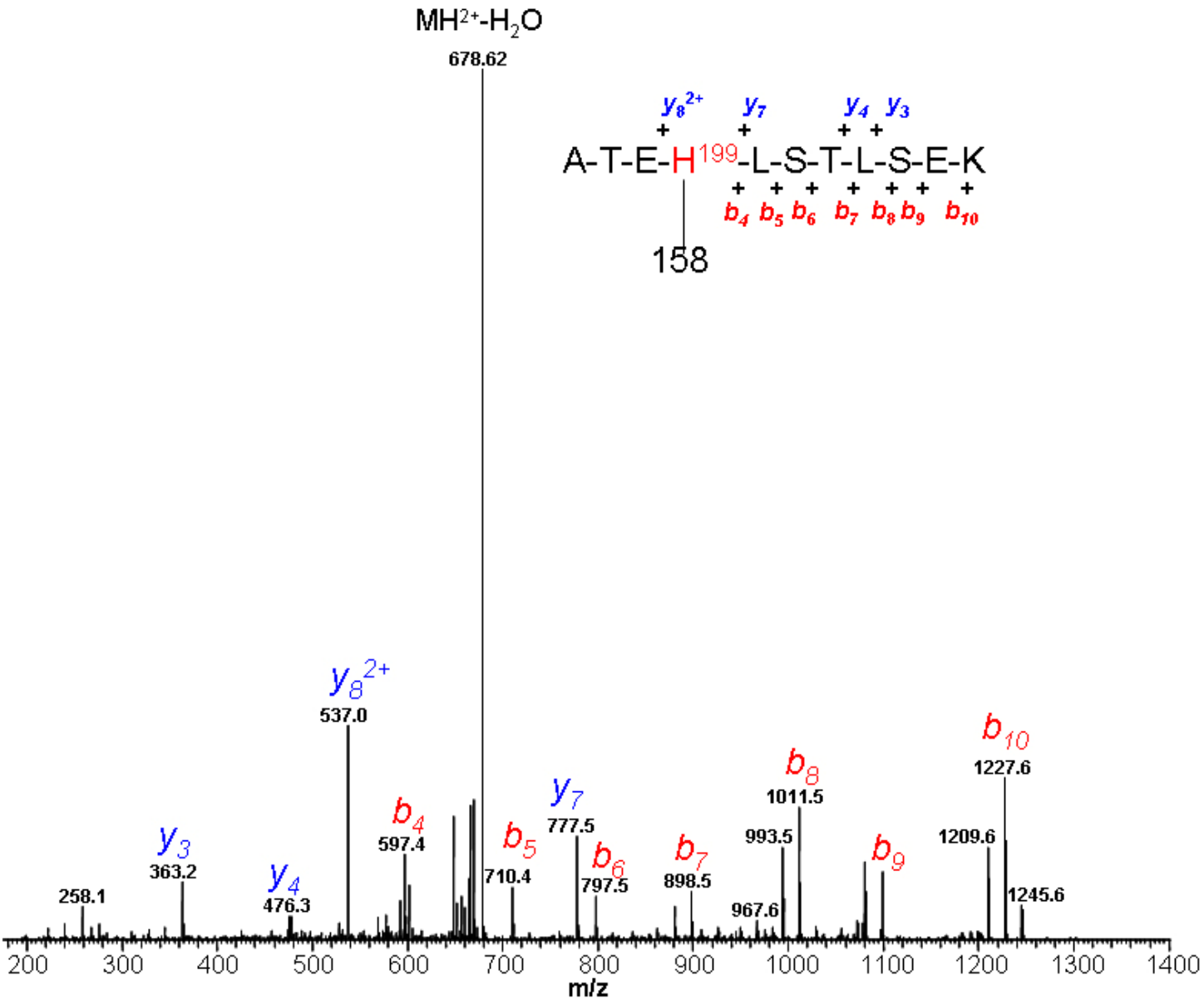
k. HNE (M+158) reduced Michael adduct of ApoA1 peptide A*H^{155}VDALR



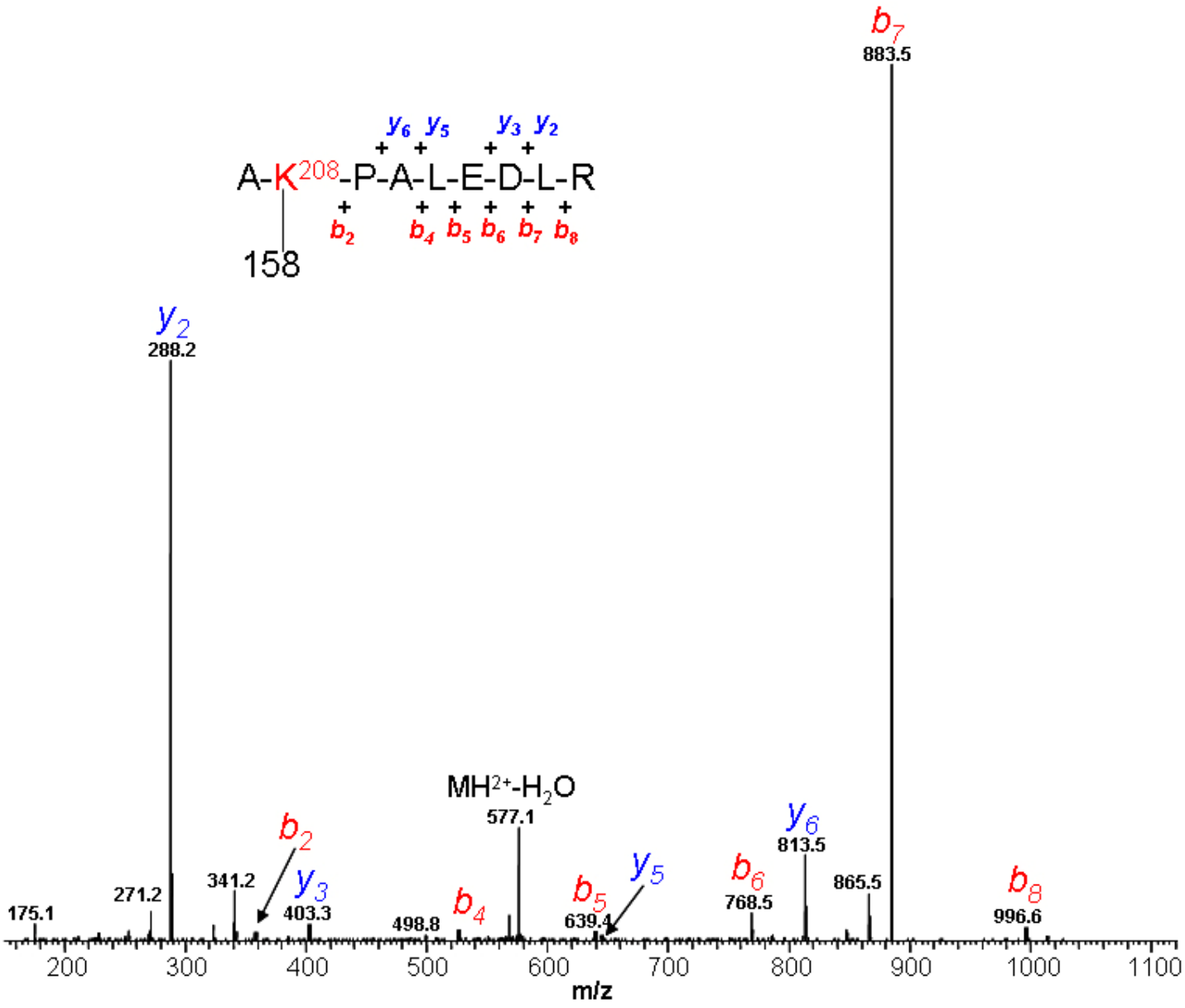
l. HNE (M+158) reduced Michael adduct of ApoA1 peptide LAEY*H[193]AK



m. HNE (M+158) reduced Michael adduct of ApoA1 peptide ATE*H[199]LSTLSEK



n. HNE (M+158) reduced Michael adduct of ApoA1 peptide A*K^{208}PALEDLR



o. HNE (M+158) reduced Michael adduct of ApoA1 peptide VSFLSALEEYT*K^{238}K