Electronic Supporting Information for

Simultaneous Imaging of Small Metabolites and Lipids in Rat Brain Tissues at Atmospheric Pressure by Laser Ablation Electrospray Ionization Mass Spectrometry

*Peter Nemes[1†], Amina S. Woods[2], and Akos Vertes[1]**

[1]Department of Chemistry, W. M. Keck Institute for Proteomics Technology and Applications, George Washington University, Washington, DC 20052;
[2]National Institute on Drug Abuse
National Institutes of Health, Baltimore, MD 21224

*To whom correspondence should be addressed. E-mail: vertes@gwu.edu. Phone: 202-994-2717. Fax: 202-994-5873.

[†]Current address: Department of Chemistry and the Institute for Genomic Biology, University of Illinois, Urbana-Champaign, Urbana, IL 61801.

METABOLITE ASSIGNMENTS

Assignments of the ions to particular metabolites and lipids in the brain samples were facilitated by accurate mass measurements, comparison of isotope distribution patterns and tandem mass spectrometry. The detected ions were assigned to particular metabolites according to the following protocol. Mass data were externally calibrated with reference ions of known mass to provide a mass accuracy of ~5 mDa or better between *m/z* 50 and 1000. Such a mass accuracy was found sufficient to search a human metabolome and a lipid database for a manageable set of hits for the observed ions.[1] Further narrowing of the results was achieved by tandem mass

spectrometry. For example, for the *m/z* 268.105 ion, the database search returned adenosine and its structural isomer deoxyguanosine (*m/z* 268.1046 theoretical) along with neuraminic acid (*m/z* 268.1032 theoretical) with 3 and 9 ppm mass accuracy, respectively. Fragmentation patterns induced by CAD for the related standards (see Figure S1) showed that only the behavior of the adenosine standard was consistent with the CAD spectrum recorded for the *m/z* 268.105 ion generated directly from the rat brain tissue. Formation of the *m/z* 136.062 fragment was not observed for the deoxyguanosine or neuraminic acid standards. The inset of Figure 1 in the text shows that CAD of the *m/z* 268.105 ion produced a fragment at *m/z* 136.062. The theoretical mass of the protonated adenine ($C_5H_5N_5$, *m/z* 136.0623) was also within 1 ppm agreement with this mass. Formation of this fragment was not observed for deoxyguanosine or neuraminic acid standards.

Limited assignment of the lipids started with comparing accurate masses to potential lipid structures generated by the Lipid Mass Spec. Prediction tool of the LIPID MAPS database.[2] Tandem mass spectrometry helped with the classification of the found lipids. The presence of the phosphocholine ion at *m/z* 184.074 in the tandem mass spectrum was an indication for a glycerophosphocholine (PC), whereas neutral loss of a 141.019 Da neutral group was interpreted as consistent with a glycerophosphoethanolamine (PE).

References

(1) Genome Alberta and Genome Canada, Human Metabolome Database, Version 2.0, http://www.hmdb.ca/, June 29, 2009.

(2) LIPID MAPS consortium - Nature Publishing Group, LIPID Metabolites And Pathways Strategy, http://www.lipidmaps.org/, June 29, 2009.

Figure S1. Small metabolite assignments were facilitated by CAD and tandem mass spectrometry. Fragmentation patterns induced by CAD for the LAESI-produced ions from standards **a)** adenosine ($[C_{10}H_{13}N_5O_4+H]^+$, *m/z* 268.1046 theoretical), **b)** 2'-deoxyguanosine ($[C_{10}H_{13}N_5O_4+H]^+$, *m/z* 268.1046 theoretical), and **c)** N-acetylneuraminic acid ($[C_{11}H_{19}NO_9+H]^+$, *m/z* 310.1148 theoretical) showed characteristic fragments at *m/z* 136.062 (adenine residue, *m/z* 136.0623 theoretical), *m/z* 152.059 (2'-deoxyguanine moiety, *m/z*), and the loss of one and two water molecules, respectively. Only the fragmentation pattern of the adenosine standard was consistent with the CAD spectrum recorded for *m/z* 268.105 directly from the rat brain tissue (see the inset in Figure 1).

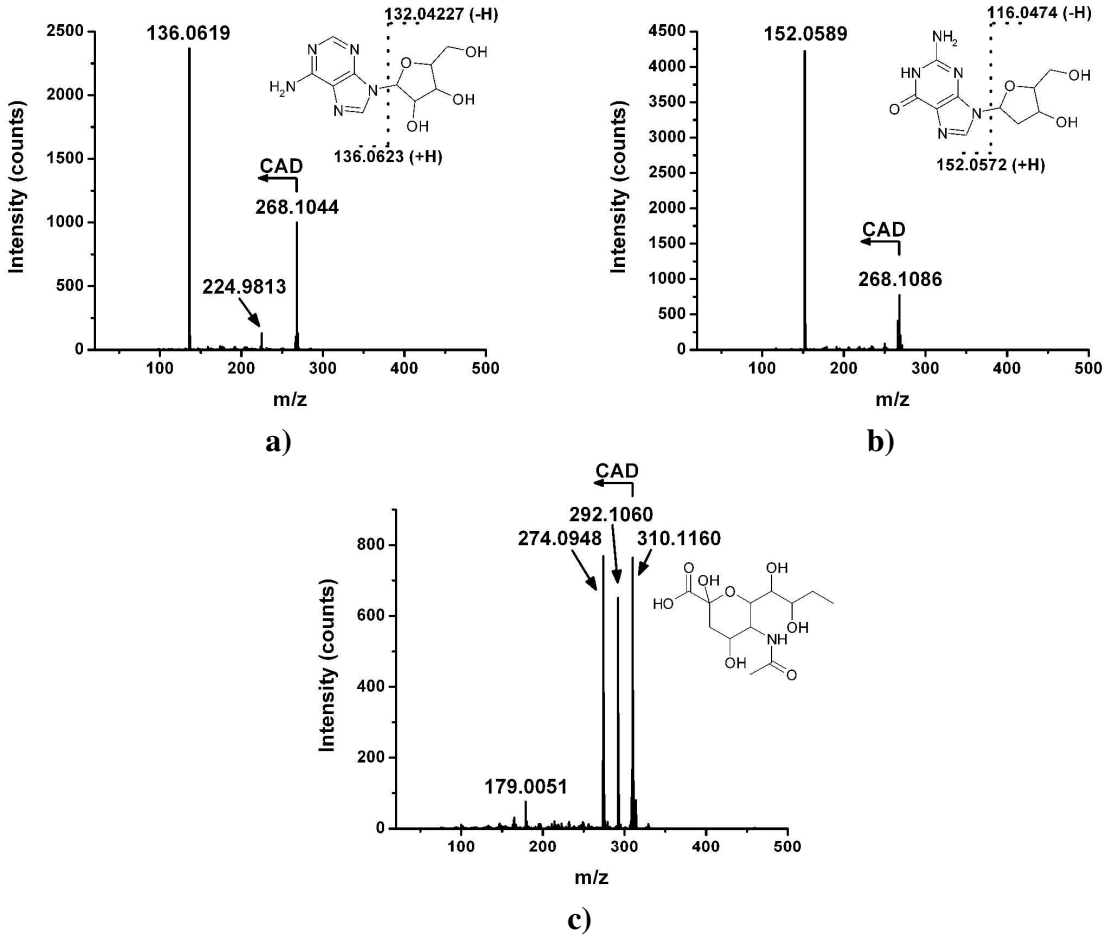
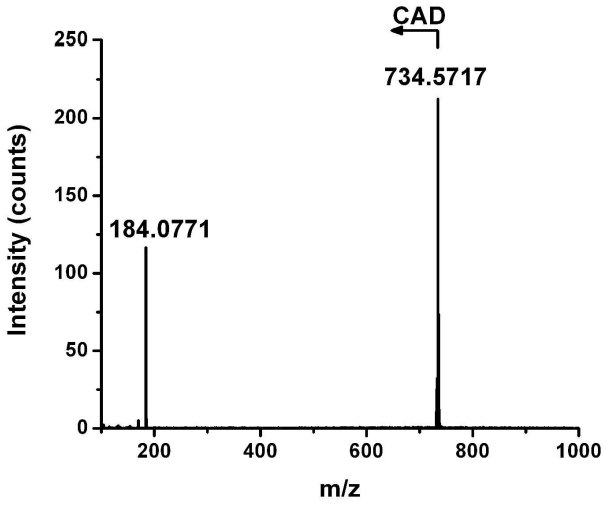
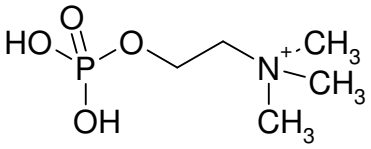


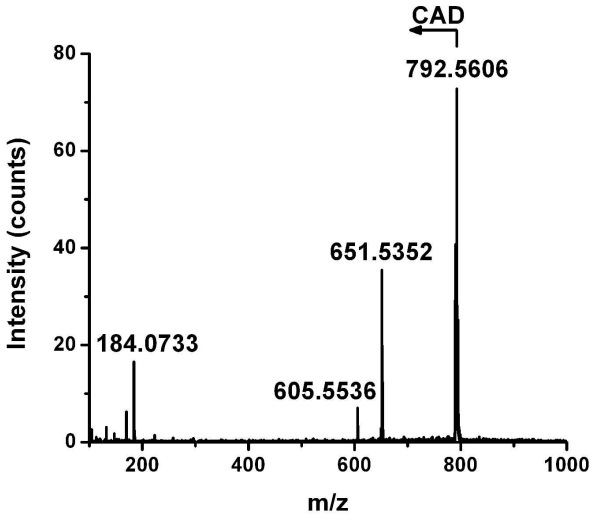
Figure S2. Narrowing the lipid assignments for the brain tissue samples was facilitated by CAD and tandem mass spectrometry. **a)** For example, the tandem mass spectrum obtained from the ion with *m/z* 734.572 showed the generation of *m/z* 184.077. The presence of the latter ion was consistent with the reported CAD behavior of glycerophosphocholines (PC) and enabled the PC(32:0) assignment in Table S2. **b)** Under CAD conditions, the ions registered at *m/z* 792.561 underwent neutral loss of 141.025 and also yielded ions with *m/z* 184.073. The former process was consistent with the loss of the phosphorylethanolamine head group reported for the glycerophosphoethanolamine (PE) class of lipids, whereas the production of the latter ion agreed with the fragmentation pattern of PCs. Such CAD spectra were indicative of the joint presence of PC and PE structural isomers, such as PC(37:6) and PE(40:6) in this case (see Table S2).

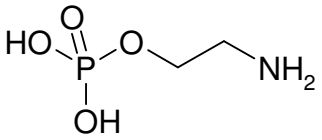




phosphocholine
m/z 184.074

a)





phosphorylethanolamine
$\Delta m/z$ = 141.019

b)

Figure S3. Optical image of a stained coronal rat brain section obtained at bregma ~0.7 mm delineates the major anatomical regions: cerebral cortex, corpus callosum, caudate putamen (striatum), intergeniculate leaf, anterior commissure and cingulum.

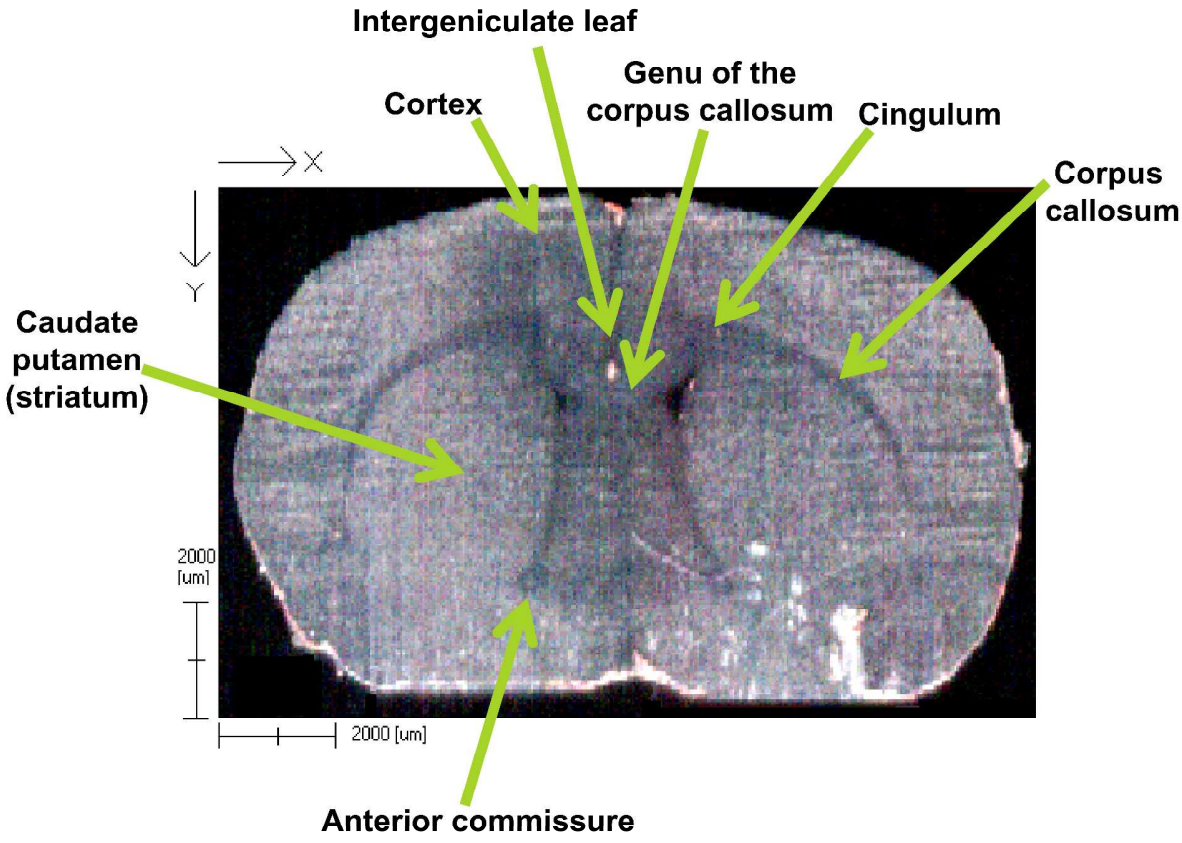


Figure S4. Distributions of species identified as a) hexose, b) adenosine, c) PC(36:1), d) the combination of PC(37:6) and PE(40:6), and spermidine corresponding to *m/z* 203.053, 268.105, 788.619 and 792.561, 146.166 ions, respectively.

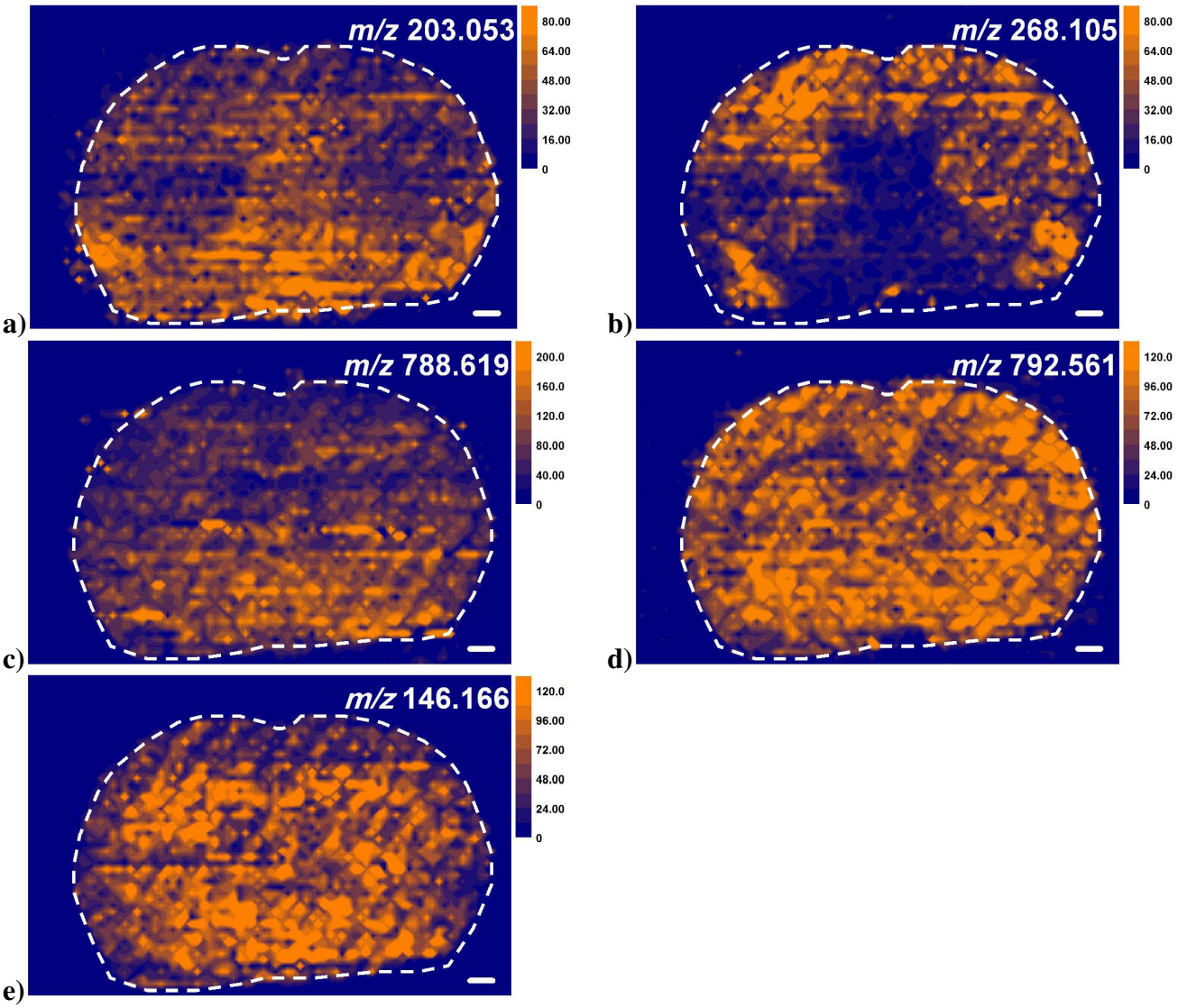


Table S1. Typical parameters of the imaging LAESI MS experiment.

Parameter	Value
Emitter	100 µm ID TaperTip (New Objective)
Spray voltage	3100 V
Solution supply rate	300 nL/min
Solvent composition	50 % (v/v) methanol, 50 % (v/v) water, 0.1 % (v/v) acetic acid
Laser wavelength, pulse width	2940 nm, 4 ns
Laser pulse repetition rate	20 Hz
Laser output energy	~100 µJ/pulse
Focusing element	ZnSe lens (75 mm focal length, antireflection coated for 3 µm wavelength)
MS data acquisition rate	2 spectra/second
MS orifice temperature	80 °C
MS acquisition mode, *m/z* interval	linear (+V) mode, *m/z* 50-1000
MS orifice-to-emitter tip distance	12 mm
MS orifice-to-laser beam distance	7 mm
MS orifice axis-to-sample surface distance	10 mm
Sample temperature	-5–0 °C
Ambient humidity in chamber	~10 rel.%
X and *Y* step size	200 µm and 200 µm
X and *Y* range during MS imaging	12.5 mm and 10.5 mm
Dwell time at each pixel	3.5 s

Table S2. Tentative assignment of small metabolites and lipids for positive species selected from more than 200 ions in the LAESI mass spectra of coronal rat brain sections.

Molecular species[a]	Formula[b]	*m/z* calculated	*m/z* measured	Δ*m/z* mDa	Δ*m/m* ppm
ethanolamine	C_2H_7NO (Na^+)	84.0425	84.0435	1.0	11.4
γ-butyrolactone	$C_4H_6O_2$ (H^+)	87.0446	87.0436	-1.0	-11.5
alanine, N-methylglycine	$C_3H_7NO_2$ (H^+)	90.0555	90.0557	0.2	2.2
γ-aminobutyric acid and/or 2-methylalanine	$C_4H_9NO_2$ (H^+)	104.0712	104.0705	-0.7	-6.7
choline	$C_5H_{14}NO$ (+)	104.1075	104.1079	0.4	3.8
spermidine	$C_7H_{19}N_3$ (H^+)	146.1657	146.1655	-0.2	-1.6
creatine	$C_4H_9N_3O_2$ (H^+)	132.0773	132.0768	-0.5	-3.8
hexose	$C_6H_{12}O_6$ (Na^+)	203.0532	203.0528	-0.4	-1.8
spermine	$C_{10}H_{26}N_4$ (H^+)	203.2236	203.2200	-3.6	-17.7
glycerophosphocholine	$C_8H_{20}NO_6P$ (H^+)	258.1106	258.1108	0.2	0.7
adenosine	$C_{10}H_{13}N_5O_4$ (H^+)	268.1046	268.1054	0.8	3.2
adenosine monophosphate	$C_{10}H_{14}N_5O_7P$ (H^+)	348.0709	348.0711	0.2	0.6
cholesterol (+ H^+ - H_2O)	$C_{27}H_{44}$ (H^+)	369.3521	369.3518	-0.3	-0.8
heme	$C_{34}H_{32}N_4O_4Fe$ (+)	616.1773	616.1760	-1.2	-2.0
PC(O-33:3) and/or PE(O-36:3)	$C_{41}H_{78}NO_7P$ (H^+)	728.5594	728.5588	-0.6	-0.8
PC(32:0)[c]	$C_{40}H_{80}NO_8P$ (H^+)	734.5700	734.5717	1.7	2.3
PC(34:1)[c]	$C_{42}H_{82}NO_8P$ (H^+)	760.5856	760.5852	-0.4	-0.5
PC(35:4) and PE(38:4)[d]	$C_{43}H_{78}NO_8P$ (H^+)	768.5543	768.5530	-1.3	-1.7
PC(36:4)[c]	$C_{44}H_{80}NO_8P$ (H^+)	782.5700	782.5715	1.6	2.0
PC(36:2)[c]	$C_{44}H_{84}NO_8P$ (H^+)	786.6012	786.5987	-2.5	-3.2
PC(36:1)[c]	$C_{44}H_{86}NO_8P$ (H^+)	788.6169	788.6188	1.9	2.4
PC(37:6) and PE(40:6)[d]	$C_{45}H_{78}NO_8P$ (H^+)	792.5543	792.5606	6.3	7.9
PC(38:6)[c]	$C_{46}H_{80}NO_8P$ (H^+)	806.5700	806.5651	-4.8	-6.0
PC(38:4)[c]	$C_{46}H_{84}NO_8P$ (H^+)	810.6012	810.6001	-1.1	-1.4
PC(40:6)[c]	$C_{48}H_{84}NO_8P$ (H^+)	834.6012	834.5974	-3.8	-4.6

[a]PC and PE indicate glycerophosphocholines and glycerophosphoethanolamines, respectively. The numbers in parentheses denote the combined acyl chain length and the number of double bonds on them. O- indicates a structure with one of the ester linkages replaced by an ether link in a glycerolipid, turning it into a plasmalogen. [b]Symbols in parentheses indicate protonated (H^+), sodiated (Na^+) species or preformed ions (+). [c]Fragmentation pattern confirms PC. [d]Fragmentation pattern is consistent with the presence of both PC and PE.

Table S3: Pearson product–moment cross-correlation coefficients calculated between the lateral distributions of 25 *m/z*-selected ions revealed the co-localization of metabolites in the rat brain tissue section. Numbers in bold indicate correlations for distributions presented in Figure 2 or 3 in the text.

m/z	**84.04**	**87.04**	**104.07**	**104.11**	**132.08**	**146.17**	**203.05**	**203.22**	**258.11**	**268.10**	**369.35**	**616.18**	**702.57**	**728.56**	**734.57**	**760.59**	**760.84**	**768.55**	**776.58**	**782.57**	**788.62**	**792.55**	**806.57**	**810.60**	**834.59**
84.04	1.00	0.48	0.41	0.25	**0.71**	0.62	0.52	0.55	0.62	0.55	0.45	0.13	0.42	0.41	0.30	0.35	0.10	0.61	0.38	0.30	0.35	0.57	0.48	0.38	0.47
87.04		1.00	**0.81**[ab]	0.24	0.50	0.62	0.49	0.46	0.53	0.18	0.43	0.12	0.44	0.44	0.29	0.35	0.11	0.50	0.38	0.28	0.45	0.50	0.42	0.43	0.52
104.07			1.00	**0.11**[b]	0.49	0.69	0.49	0.53	0.54	0.23	0.60	0.08	0.49	0.51	0.30	0.38	0.06	0.61	0.45	0.26	0.47	0.62	0.47	0.45	0.56
104.11				1.00	0.34	0.25	0.34	0.41	0.34	0.17	0.03	0.09	0.25	0.20	0.27	0.31	0.11	0.26	0.25	0.44	0.36	0.24	0.42	0.44	0.46
132.08					1.00	0.68	0.67	0.74	0.78	0.62	0.38	0.18	0.46	0.42	0.42	0.47	0.16	0.65	0.41	0.42	0.48	0.61	0.58	0.53	0.64
146.17						1.00	0.57	0.71	0.61	0.52	0.70	0.13	0.65	0.69	0.41	0.49	0.11	0.75	0.49	0.43	0.57	0.69	0.59	0.55	0.62
203.05							1.00	0.62	0.69	0.42	0.34	0.21	0.45	0.43	0.42	0.47	0.20	0.56	0.36	0.46	0.53	0.50	0.53	0.54	0.58
203.22								1.00	0.65	0.66	0.39	0.09	0.45	0.41	0.52	0.55	0.07	0.75	0.60	0.56	0.55	0.75	0.71	0.59	0.71
258.11									1.00	0.53	0.36	0.17	0.46	0.42	0.36	0.39	0.14	0.57	0.39	0.37	0.44	0.52	0.50	0.44	0.55
268.10										1.00	0.36	0.06	0.37	0.35	0.33	0.35	0.03	0.53	0.37	0.30	0.31	0.56	0.48	0.28	0.39
369.35											1.00	0.03	0.67	**0.76**[a]	0.25	0.32	0.01	0.59	0.37	0.20	0.43	0.53	0.36	0.34	0.38
616.18												1.00	0.34	0.32	0.04	0.09	**0.98**[ab]	0.17	0.08	0.06	0.07	0.12	0.21	0.15	0.22
702.57													1.00	0.85	0.38	0.47	0.34	0.60	0.45	0.33	0.58	0.55	0.50	0.47	0.54
728.56														1.00	0.34	0.44	0.31	0.60	0.42	0.35	0.57	0.53	0.48	0.45	0.51
734.57															1.00	0.88	0.05	0.67	0.54	0.77	0.83	0.64	0.72	0.74	0.69
760.59																1.00	**0.11**[b]	0.75	0.56	0.78	0.89	0.73	0.80	0.82	0.76
760.84																	1.00	0.16	0.06	0.08	0.08	0.11	0.21	0.17	0.22
768.55																		1.00	0.65	0.68	0.74	0.87	0.82	0.77	0.80
776.58																			1.00	0.49	0.57	0.76	0.68	0.52	0.63
782.57																				1.00	0.75	0.61	0.78	0.82	0.73
788.62																					1.00	0.70	0.76	0.85	0.80
792.55																						1.00	0.86	0.70	0.81
806.57																							1.00	0.80	0.87
810.60																								1.00	0.86
834.59																									1.00

[a]See the corresponding molecular ion image in Figure 2 in the text.

[b]See the corresponding molecular ion image in Figure 3 in the text.