SUPPLEMENTARY DATA

Mouse brain plasmalogens are targets for hypochlorous acid-mediated modification *in vitro* and *in vivo*

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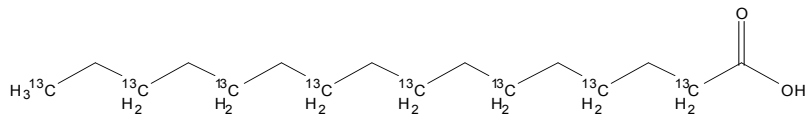
Fig. I: Summary of 2-Cl[$^{13}C_8$]HDA synthesis as outlined in Materials and methods

Fig. II: FT-ICR-MS analyses of phosphatidylinositol (A) and phosphatidylserine (B) subspecies composition

C57BL/6 mice were killed by cervical dislocation, brains were removed, homogenized in liquid N_2, lipids were extracted (twice) using a modified Folch extraction, dried under N_2, redissolved in $CHCl_3$/MeOH (1:1, v/v), and analyzed by a hybrid linear ion trap FT-ICR-MS in positive ESI mode as described in Materials and Methods. The corresponding m/z values and detailed percentage composition are given in Table I of the Supplement.

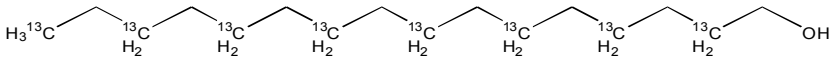
Fig. III: NICI-GC-MS analysis of 2-ClHDA and 2-Cl[$^{13}C_8$]HDA

Total ion current traces (left panel) and the corresponding full scan NICI spectra (right panel) of 2-ClHDA (**A**) and 2-Cl[$^{13}C_8$]HDA (**B**) PFB-oxime derivatives (0.5 nmoles injected). Potential fragmentation patterns and isotope distribution of the molecular ions are shown in the insets. Peaks eluting at 14.78 and 14.94 min correspond to the syn- and anti-isomers of 2-ClHDA PFB-oxime derivatives. Mass assignment is indicated in the insets.



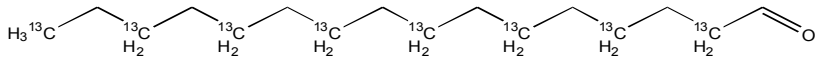
hexadecanoic-2,4,6,8,10,12,14,16-13C8-acid

1 **Reduction** 50°C/Et_2O/$LiAlH_4$/refluxing/EtOAc/H_20



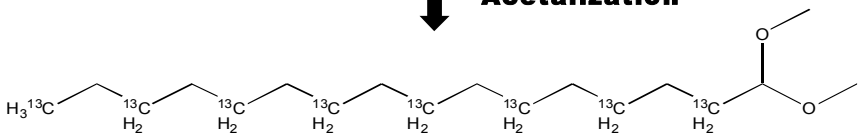
hexadecan-2,4,6,8,10,12,14,16-13C8-ol

2 **Swern-Oxidation** -70°C/CH_2Cl_2/N_2-atmosphere/DMSO /oxalylchloride/triethylamine



hexadecan-2,4,6,8,10,12,14,16-13C8-al

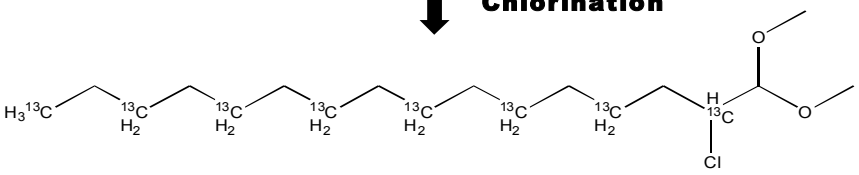
3 **Acetalization** 40°C/MeOH/trimethylorthoformate/ NH_4NO_3



hexadecan-2,4,6,8,10,12,14,16-13C8-al dimethylacetal

4 **Purification** Silica 60 column/hexane:Et_2O (97.5:2.5, v/v)

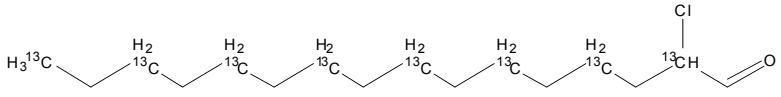
5 **Chlorination** 40°C/$MnCl_2$/MnO_2/trimethylchlorosilane /MeOH/ACN/NaOH



2-chlorohexadecan-2,4,6,8,10,12,14,16-13C8-al dimethylacetal

6 **Purification** Silica 60 column/hexane:Et_2O (90:10, v/v)

7 **Acetal-hydrolysis** 80°C/CH_2Cl_2/TFA/refluxing



2-chlorohexadecan-2,4,6,8,10,12,14,16-13C8-al

Fig. I

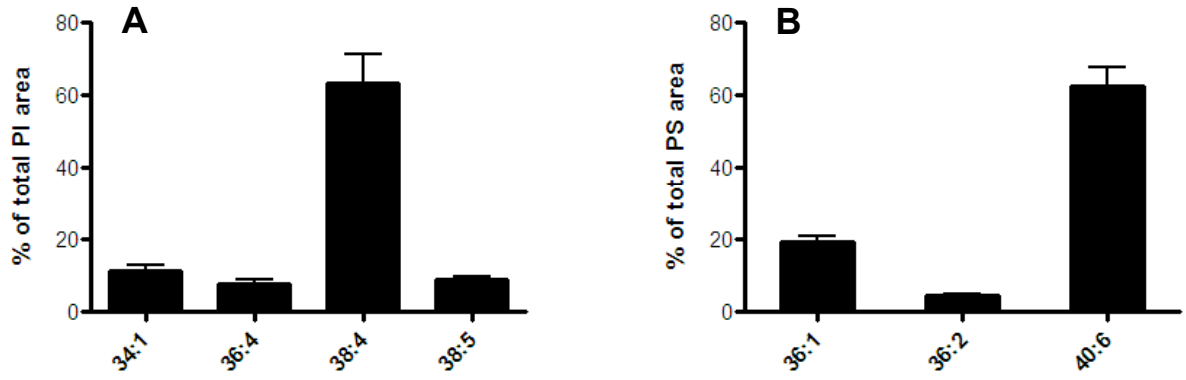

A
% of total PI area
80
60
40
20
0
34:1
36:4
38:4
38:5
B
% of total PS area
80
60
40
20
0
36:1
36:2
40:6

Fig. II

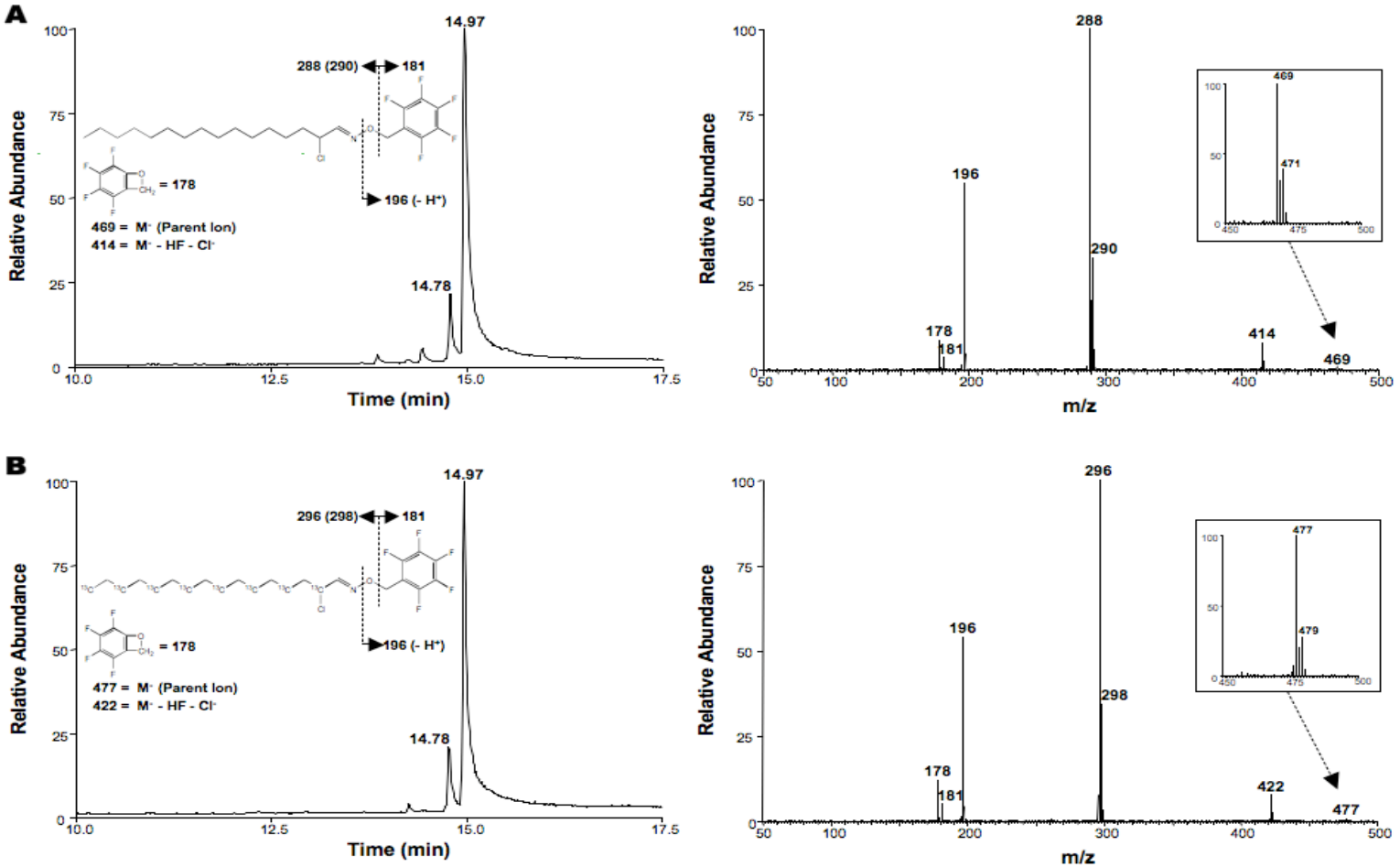

A
Relative Abundance
Time (min)
14.97
288 (290)
181
196 (- H+)
= 178
469 = M- (Parent Ion)
414 = M- - HF - Cl-
14.78
m/z
288
196
290
178
181
414
469
471
B
296 (298)
477 = M- (Parent Ion)
422 = M- - HF - Cl-
296
298
422
477
479

Fig. III

TABLE I: Percentage composition of C57BL/6 brain glycerophospholipids

Brain lipids were extracted and analyzed by FT-ICR-MS as described in Materials and Methods. Results shown are mean ± SD from three different brains.

Phosphatidylcholine

Species	m/z	m/z (+ H^+)	Mean	SD
30:0	705.53	706.54	0.28%	0.05%
32:0	733.56	734.57	17.04%	2.58%
32:1	731.55	732.55	1.23%	0.22%
34:0	761.59	762.60	4.63%	0.74%
34:1	759.58	760.59	37.56%	5.84%
34:2	757.56	758.57	0.81%	0.13%
34:3	755.55	756.55	0.16%	0.01%
36:0	789.62	790.63	0.09%	0.01%
36:1	787.61	788.62	13.56%	2.34%
36:2	785.59	786.60	2.92%	0.53%
36:3	783.58	784.59	0.09%	0.00%
36:4	781.56	782.57	5.07%	0.75%
38:1	815.64	816.65	0.68%	0.17%
38:2	813.62	814.63	0.50%	0.10%
38:4	809.59	810.60	4.83%	0.69%
38:5	807.58	808.59	1.64%	0.35%
38:6	805.56	806.57	4.29%	0.70%
38:7	803.55	804.55	0.10%	0.01%
40:1	843.67	844.68	0.16%	0.04%
40:2	841.66	842.66	0.16%	0.04%
40:4	837.62	838.63	0.35%	0.05%
40:6	833.59	834.60	2.69%	0.50%
40:7	831.58	832.59	0.88%	0.17%
42:1	871.70	872.71	0.16%	0.05%
42:2	869.69	870.69	0.10%	0.03%
Sum			100.00%	

Plasmenyl Phosphatidylcholine

Species	m/z	m/z (+ H^+)	Mean	SD
32:0	717.57	718.58	8.15%	0.82%
32:1	715.55	716.56	3.08%	0.88%
34:0	745.60	746.61	53.70%	6.07%
34:1	743.58	744.59	11.08%	3.68%
36:0	773.63	774.64	6.54%	0.65%
36:1	771.61	772.62	8.84%	2.16%
36:2	769.60	770.61	4.93%	2.03%
38:1	799.65	800.65	3.01%	0.75%
40:0	829.69	830.70	0.25%	0.11%
40:1	827.68	828.68	0.42%	0.07%
Sum			100.0%	

Phosphatidylethanolamine

Species	m/z	m/z (+ H^+)	Mean	SD
34:0	719.55	720.55	1.03%	0.18%
34:1	717.53	718.54	2.49%	0.25%
36:0	747.58	748.59	0.98%	0.11%
36:1	745.56	746.57	5.89%	0.53%
36:2	743.55	744.55	3.48%	0.23%
36:4	739.52	740.52	2.01%	0.18%
38:1	773.59	774.60	2.80%	0.53%
38:2	771.58	772.59	0.51%	0.11%
38:4	767.55	768.55	22.34%	2.27%
38:5	765.53	766.54	3.62%	0.23%
38:6	763.52	764.52	8.04%	0.89%
40:1	801.62	802.63	0.45%	0.01%
40:4	795.58	796.59	4.43%	0.60%
40:5	793.56	794.57	0.76%	0.11%
40:6	791.55	792.55	38.69%	3.71%
40:7	789.53	790.54	2.47%	0.20%
Sum			100.00%	

Plasmenyl Phosphatidylethanolamine

Species	m/z	m/z (+ H^+)	Mean	SD
32:0	675.52	676.53	0.04%	0.01%
34:0	703.55	704.56	0.29%	0.02%
34:1	701.54	702.54	5.23%	0.53%
36:1	729.57	730.58	11.28%	1.13%
36:2	727.55	728.56	11.52%	0.88%
36:4	723.52	724.53	1.69%	0.17%
38:0	759.61	760.62	1.73%	0.21%
38:1	757.60	758.61	5.48%	0.77%
38:2	755.58	756.59	6.44%	0.62%
38:4	751.55	752.56	9.83%	0.98%
38:5	749.54	750.54	6.66%	0.58%
38:6	747.52	748.53	8.90%	0.85%
40:1	785.63	786.64	0.31%	0.04%
40:2	783.61	784.62	0.78%	0.09%
40:4	779.58	780.59	5.59%	0.68%
40:6	775.55	776.56	19.81%	1.07%
40:7	773.54	774.54	4.41%	0.35%
Sum			100.0%	

Phosphatidylinositol

Species	m/z	m/z (- H^+)	Mean	SD
34:1	836.54	835.53	11.33%	1.46%
36:0	866.59	865.58	1.79%	0.55%
36:4	858.53	857.52	7.57%	1.32%
38:0	894.62	893.61	2.32%	1.71%
38:4	886.56	885.55	63.09%	8.28%
38:5	884.54	883.53	8.67%	1.06%
38:6	882.53	881.52	1.73%	0.64%
40:6	910.56	909.55	3.30%	0.81%
40:7	908.54	907.53	0.19%	0.02%
Sum			100.00%	

Phosphatidylserine

Species	m/z	m/z (+ H^+)	Mean	SD
34:1	761.52	762.53	0.97%	0.07%
36:1	789.55	790.56	19.36%	1.28%
36:2	787.54	788.54	4.31%	0.43%
38:1	817.58	818.59	2.87%	0.24%
38:4	811.54	812.54	3.20%	0.27%
40:1	845.61	846.62	1.13%	0.29%
40:4	839.57	840.57	3.46%	0.19%
40:5	837.55	838.56	0.97%	0.16%
40:6	835.54	836.54	62.60%	5.22%
40:7	833.52	834.53	1.13%	0.20%
Sum			100.00%	