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Identification of abundant alkyl ether glycerophospholipids in the human lens by tandem mass spectrometry techniques

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RESEARCH ONLINE

Identification of abundant alkyl ether glycerophospholipids in the human lens by tandem mass spectrometry techniques

Abstract

Previous studies have shown that the human lens contains glycerophospholipids with ether linkages. These lipids differ from conventional glycerophospholipids in that the sn-1 substituent is attached to the glycerol backbone via an 1-O-alkyl or an 1-O-alk-1'-enyl ether rather than an ester bond. The present investigation employed a combination of collision-induced dissociation (CID) and ozone-induced dissociation (OzID) to unambiguously distinguish such 1-O-alkyl and 1-O-alk-1'-enyl ethers. Using these methodologies the human lens was found to contain several abundant 1-O-alkyl glycerophos-phoethanolamines, including GPEtn(16:0e/9Z-18:1), GPEtn(11Z-18:1e/9Z-18:1), and GPEtn(18:0e/9Z-18:1), as well as a related series of unusual 1-O-alkyl glycerophosphoserines, including GPSer(16:0e/9Z-18:1), GPSer(11Z-18:1e/9Z-18:1), GPSer(18:0e/9Z-18:1) that to our knowledge have not previously been observed in human tissue. Isomeric 1-O-alk-1'-enyl ethers were absent or in low abundance. Examination of the double bond position within the phospholipids using OzID revealed that several positional isomers were present, including sites of unsaturation at the n-9, n-7, and even n-5 positions. Tandem CID/OzID experiments revealed a preference for double bonds in the n-7 position of 1-O-ether linked chains, while n-9 double bonds predominated in the ester-linked fatty acids [e.g., GPEtn(11Z-18:1e/9Z-18:1) and GPSer(11Z-18:1e/9Z-18:1)]. Different combinations of these double bond positional isomers within chains at the sn-1 and sn-2 positions point to a remarkable molecular diversity of ether-lipids within the human lens.

Keywords

identification, abundant, alkyl, ether, glycerophospholipids, human, lens, spectrometry, tandem, techniques, mass

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Article

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Identification of Abundant Alkyl Ether Glycerophospholipids in the Human Lens by Tandem Mass Spectrometry Techniques

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Previous studies have shown that the human lens contains glycerophospholipids with ether linkages. These lipids differ from conventional glycerophospholipids in that the *sn*-1 substituent is attached to the glycerol backbone via an 1-*O*-alkyl or an 1-*O*-alk-1′-enyl ether rather than an ester bond. The present investigation employed a combination of collision-induced dissociation (CID) and ozone-induced dissociation (OzID) to unambiguously distinguish such 1-*O*-alkyl and 1-*O*-alk-1′-enyl ethers. Using these methodologies the human lens was found to contain several abundant 1-*O*-alkyl glycerophosphoethanolamines, including GPEtn(16:0e/9*Z*–18:1), GPEtn(11*Z*–18:1e/9*Z*–18:1), and GPEtn(18:0e/9*Z*–18:1), as well as a related series of unusual 1-*O*-alkyl glycerophosphoserines, including GPSer(16:0e/9*Z*–18:1), GPSer(11*Z*–18:1e/9*Z*–18:1), GPSer(18:0e/9*Z*–18:1) that to our knowledge have not previously been observed in human tissue. Isomeric 1-*O*-alk-1′-enyl ethers were absent or in low abundance. Examination of the double bond position within the phospholipids using OzID revealed that several positional isomers were present, including sites of unsaturation at the *n*-9, *n*-7, and even *n*-5 positions. Tandem CID/OzID experiments revealed a preference for double bonds in the *n*-7 position of 1-*O*-ether linked chains, while *n*-9 double bonds predominated in the ester-linked fatty acids [e.g., GPEtn(11*Z*–18:1e/9*Z*–18:1) and GPSer(11*Z*–18:1e/9*Z*–18:1)]. Different combinations of these double bond positional isomers within chains at the *sn*-1 and *sn*-2 positions point to a remarkable molecular diversity of ether-lipids within the human lens.

The main body of the human lens consists of concentric layers of slender, crescent shaped fiber cells, creating a tightly packed arrangement of cell membranes. Recent work from our laboratory has shown that the membrane lipid composition of the human lens is different from that of other animals in having high levels of dihydrosphingomyelins and ether glycerophospholipids.[1] Ether glycerophospholipids have one substituent attached via an 1-*O*-alkyl or an 1-*O*-alk-1′-enyl ether bond to the *sn*-1 position of the glycerol backbone.[2] The reactivity of the 1-*O*-alk-1′-enyl ether bond toward a range of reactive oxygen species has led to the theory that these lipids (commonly known as plasmalogens) may act as *in vitro* antioxidants.[3,4]

Previous ^{13}P NMR and matrix-assisted laser desorption ionization-time of flight (MALDI-ToF) analyses of the human lens lipids have reported that 1-*O*-alk-1′-alkenyl ethers represent 70% of glycerophosphoethanolamine (GPEtns) (14.6% of total phospholipids) in younger lenses,[5] and 14% of total cortical phospholipids in 70 year-old lenses.[6] The most recent ^{31}P NMR study into human lens phospholipids has cautiously reported two resonances as "PE-related I" and "PE-related II" that could not be positively assigned to 1-*O*-alkyl or 1-*O*-alk-1′-enyl ethers.[7] Interestingly, our recent electrospray ionization mass spectrometry (ESI-MS) survey of older human lenses (about 60 years-old) indicated that although 1-*O*-alk-1′-enyl ethers were present, the major components present in the GPEtn and GPSer classes in the human lens were more likely 1-*O*-alkyl ethers.[1] In the current study, we applied a comprehensive suite of established and novel ESI-MS techniques to structurally characterize these phospholipids.

"Shotgun lipidomics" is a contemporary method whereby a crude lipid extract is infused into an ESI mass spectrometer and each lipid present forms an ion that is detected by its mass-to-charge ratio.[8] Identification of the lipid is then conducted by analysis of a collision-induced dissociation (CID) mass spectrum that, in the case of glycerophospholipids, provides characteristic fragment ions for the headgroup class and the fatty acids esterified

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to the glycerol backbone.[9] Furthermore, when compared with an internal standard(s) of the same lipid class the abundance of the molecular ion can be used to quantify the lipid in the extract.[10] Despite its success, the shotgun lipidomic approach has limitations, most significantly its inability to distinguish isomeric lipids that differ only in the position of unsaturation, the site of fatty acid attachment to the lipid backbone (i.e., *sn*-positional isomers) and/or stereochemistry (e.g., *cis* or *trans* isomers). While *sn*-positional isomerism is not an impediment to the analysis of ether glycerophospholipids, owing to the tight regulation of the ether linkage to the *sn*-1 position,[11] the position of the double bond is the key difference between an unsaturated 1-*O*-alkyl ether and an 1-*O*-alk-1′-enyl ether. The recent development of ozone-induced dissociation (OzID) provides an important tool for the determination of double bond position in mass-selected lipid ions[12] and is thus ideally suited to the structural characterization of ether lipids. This approach is particularly valuable for lipids that are found in low amounts in biological tissues. In the present study we have employed OzID, along with CID and recently devised MS^3 approaches,[13] to characterize the molecular structure of ether-containing glycerophospholipids found in the human lens without the need for prior fractionation or derivatization.

METHODS

Materials. All organic solvents used were HPLC grade and purchased from Crown Scientific (Moorebank, Australia). Analytical grade butylated hydroxytoluene (BHT) was purchased from Sigma Aldrich (Castle Hill, Australia). Phospholipid standards were synthesized by Avanti Polar Lipids (Alabaster, U.S.A.) and purchased from Auspep (Parkville, Australia). Industrial grade compressed oxygen (purity 99.5%) for ozone production was obtained from BOC gases (Cringila, Australia).

Nomenclature. Where possible this paper adopts the nomenclature and abbreviation conventions of Fahy et al.[14] 1-*O*-alkyl ether and 1-*O*-alk-1′-enyl ether phospholipids are described by the (n:jk/s:t) nomenclature employed by Zemski Berry and Murphy, where *n* is the number of carbon atoms in the *sn*-1 substituent, *j* is the number of double bonds in the *sn*-1 hydrocarbon chain, *k* identifies the nature of *sn*-1 linkage to the glycerol backbone and is either an *e* for 1-*O*-alkyl- linkages or a *p* for 1-*O*-alk-1′-enyl linkages, *s* is the number of carbons, and *t* is the number of double bonds in the *sn*-2 substituent.[19] The position of the double bond in any carbon chain is indicated by the number of bonds from the glycerol end of the chain and immediately precedes the *E* or *Z* which defines the stereochemistry about the alkene [e.g., 1-*O*-hexadecyl-2-(9*Z*-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine is described as GPEtn(16:0e/9*Z*-18:1)]. It should be noted, that the stereochemistry about double bonds has not been explicitly determined in this study and is assumed to be *Z* in all cases.

Lenses. Human lenses were collected from eyes donated to the NSW Lions Eye Bank at the Sydney Eye Hospital, Sydney, and were stored at −80 °C until required. All work was approved by the human research ethics committees at the University of Sydney (#7292) and the University of Wollongong (HE 99/001).

Lipid Extraction. Phospholipids were extracted from human lenses as previously described.[1] In brief, lenses were frozen under liquid nitrogen, ground using a mortar and pestle and chloroform/methanol (2:1 v/v) containing 0.01% BHT added at a ratio of 20:1 solvent to tissue (v/w). A methanolic internal standard solution was prepared comprising dinonadecanoyl phosphatidylcholine [GPCho(19:0/19:0); 95 μM], lauroyl dihydrosphingomyelin [SM(d18:0/12:0); 84 μM], diheptadecanoyl phosphatidylserine [GPSer(17:0/17:0); 34 μM], diheptadecanoyl phosphatidylethanolamine [GPEtn(17:0/17:0); 39 μM], diheptadecanoyl phosphatidylglycerol [GPGro(17:0/17:0); 25 μM], and diheptadecanoyl phosphatidic acid [GPA(17:0/17:0); 25 μM]. These concentrations were optimized to reflect the different concentrations of lipid classes within lens extracts. The internal standard solution was prepared by appropriate dilution of individual stock solutions where the concentration of each had been independently established by phosphorus assay.[15] The internal standard mixture was added to the homogenates at 1.4 mL g(tissue)$^{-1}$ for later quantification of phospholipids. The homogenates and solvents (including internal standards) were rotated overnight in an RSM7 rotary suspension mixer (Ratek Instruments, Boronia, Australia), and the lipids extracted as previously described[16] with the exception that aqueous sodium chloride was substituted with aqueous ammonium acetate (0.15 M). Samples were stored at −80 °C until analyzed. This protocol has been demonstrated to preserve alkenyl-ether bonds, which are known to be acid-labile.[1]

Mass Spectrometry. Precursor ion and neutral loss scans of human lens lipids were obtained as previously described.[1] In brief, a Waters QuattroMicro (Manchester, U.K.) equipped with a z-spray electrospray ion source was used. The capillary voltage was set to 3000 V, source temperature 80 °C, and desolvation temperature 120 °C. The cone voltage was set to 50 and 35 V in negative and positive ion modes, respectively. Nitrogen was used as the drying gas at a flow rate of 320 L h^{-1}. Phospholipid extracts were diluted to a final concentration of about 20 μM with the addition of methanol/chloroform (2:1 v/v). Samples were infused into the electrospray ion source at a flow rate of 10 μL min^{-1} using the instrument's on-board syringe pump. Neutral loss scans for GPEtn (141 Da) and GPSer (185 Da) were performed in positive ion mode using argon as the collision gas at a pressure of 3 mTorr and collision energies of 25 and 22 eV, respectively. Precursors of dehydrated glycerophosphate (m/z 153) were detected in negative ion mode using argon as the collision gas at 3 mTorr and a collision energy of 50 eV.

MS/MS and MS^3 spectra were obtained using a ThermoFinnigan LTQ quadrupole linear ion trap mass spectrometer fitted with an IonMax electrospray ionization source (now ThermoFisher, San Jose, U.S.A.). This instrument has been

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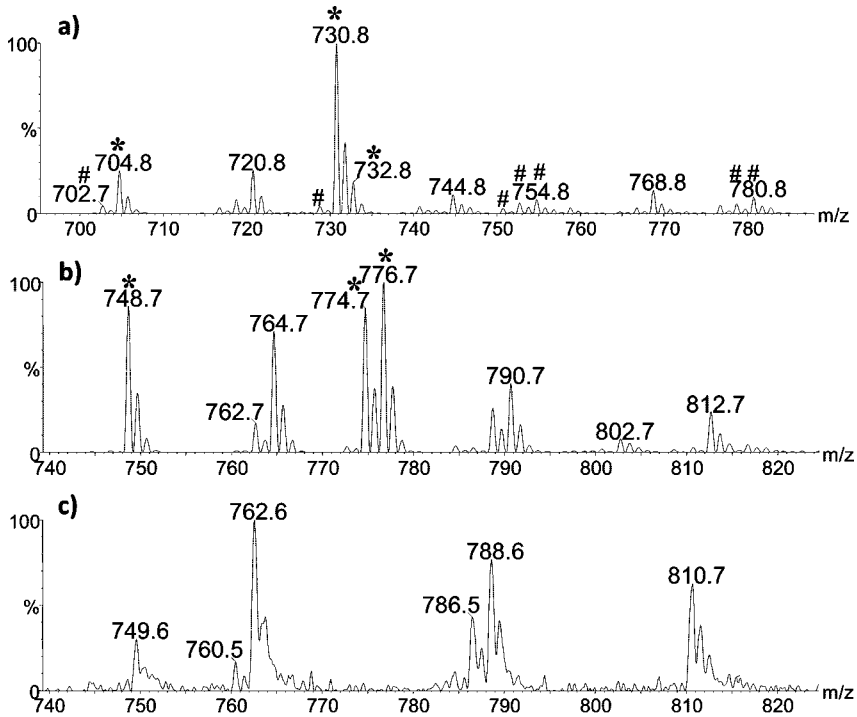

Figure 1. Tandem mass spectra obtained by infusing a total lipid extract from a 60 year-old human lens into an electrospray ionization triple quadrupole mass spectrometer set to scan for (a) a neutral loss of 141 Da (phosphoethanolamine) in positive ion mode, (b) a neutral loss of 185 Da (phosphoserine) in positive ion mode, and (c) precursors of *m/z* 153 (dehydrated glycerophosphate) in negative ion mode. Ions marked with a "#" were identified previously as 1-*O*-alk-1′-enyl-GPEtns.[1] Ions marked with a "*" are suspected 1-*O*-alkyl ether-phospholipids are the subject of the present investigation.

modified in-house, as previously described,[17] to allow the introduction of neutral reagent gases mixed with the helium buffer gas. In this study, crude lipid extracts were diluted to about 40 μM and directly infused at 5 μL min^{-1}, with the electrospray capillary voltage set between −3 and −6 kV in negative ion mode, and nitrogen was employed as the nebulizing gas. CID spectra were obtained by mass-selecting precursor ions with an isolation width of 1−3 Th and subjecting them to excitation with normalized collision energies of 25−45 arbitrary units. Ozone-induced dissociation (OzID) and CID/OzID spectra were obtained as previously described.[12] In brief, precursor ions were mass-selected with an isolation width of 2−3 Th, and trapped in the presence of ozone vapor for reaction times of 10 s prior to mass-dependent ejection and detection. Ozone was generated off-line at concentrations of 12% v/v in oxygen using an HC-30 ozone generator (Ozone Solutions, Sioux Center, IA) and was collected in a disposable, ozone-resistant plastic syringe that was then connected to the modified helium buffer gas supply line via a PEEKsil tubing restrictor (100 mm L × 1/16 in. o.d. × 0.025 mm i.d, SGE Analytical Science, Ringwood, Australia). A backing pressure (25 μL min^{-1}) was applied to the syringe using a syringe pump. At least 50 scans were acquired and averaged to achieve a sufficient signal-to-noise ratio. Relative abundances of OzID ions were found to be reproducible upon repeated acquisition.

Warning! *Ozone is a toxic gas and was produced in a fume cupboard. Excess ozone was destroyed by bubbling through an aqueous solution of sodium thiosulfate, sodium iodide, and Vitex indicator. Only ozone compatible materials were used.*

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RESULTS AND DISCUSSION

Phosphatidylethanolamines. Tandem mass spectra of [GPEtn+H]$^+$ ions, formed via electrospray ionization, contain prominent product ions resulting from the 141 Da neutral loss of the phosphoethanolamine headgroup. This pathway is observed regardless of whether the *sn*-1 substituent is an acyl, *O*-alkyl, or *O*-alk-1′-enyl group,[18,19] although in the latter case the fragmentation efficiency for this process is only about 30% of diacyl-GPEtn.[20] As such, neutral loss scans on a triple quadrupole mass spectrometer using an off-set of 141 Da are an effective means to survey GPEtn lipids in an extract[21] with internal standards providing for quantification when suitable correction factors are applied.[20] Figure 1a shows such a spectrum obtained from a human lens lipid extract on an electrospray ionization triple quadrupole mass spectrometer and thus represents a profile of GPEtn in this tissue. We have previously assigned many of the lipids in this spectrum, including several with masses consistent with GPEtn-ethers.[1] The identity of some of these ions as 1-*O*-alk-1′-enyl-GPEtn (# in Figure 1a) was confirmed by the presence of fragment ions distinctive of the *sn*-1 radyl[19] in the CID spectra of the respective [M+H]$^+$ molecular ions. Interestingly, however, three ions with masses consistent with GPEtn-ethers, namely *m/z* 704, 732, and the base-peak at *m/z* 730 (* in Figure

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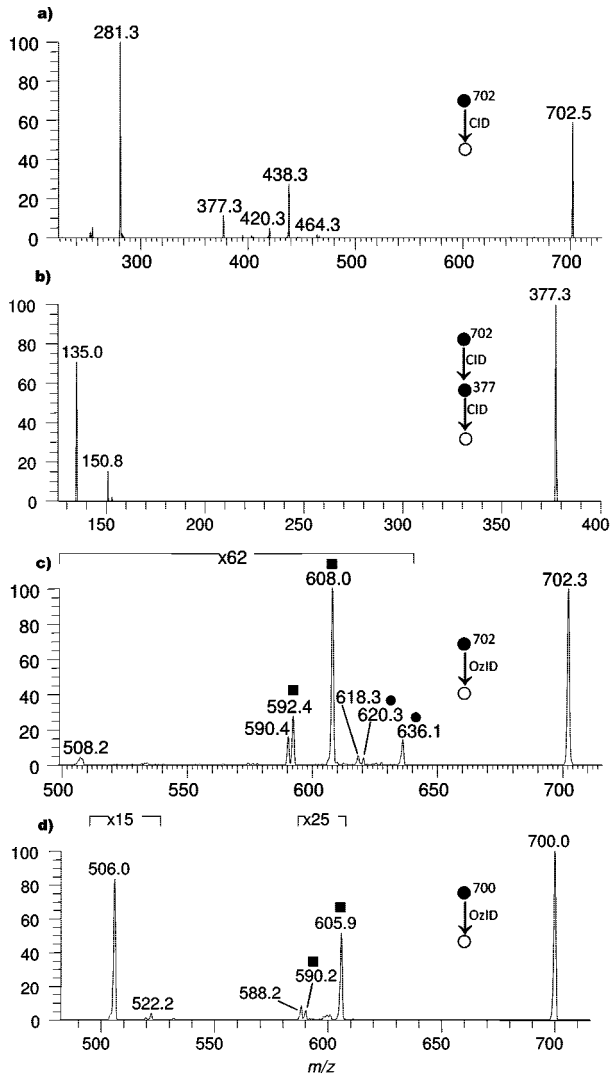

Figure 2. Negative ion electrospray ionization mass spectra obtained from (a−c) a total lipid extract from a 60 year-old human lens and (d) a commercial phospholipid standard GPEtn(16:0p/18:1). All spectra were obtained using a linear quadrupole ion-trap mass spectrometer. (a) CID of the GPEtn at *m/z* 702, (b) MS^3 of the *m/z* 377 fragment ion produced in (a) (i.e., 702 → 377 → CID), and (c) OzID of *m/z* 702. (d) OzID of a commercial GPEtn(16:0p/18:1) standard. It should be noted that the peculiar mass-defect and narrow peak-width of the unexpected ion at *m/z* 150.8 in (b) suggest this to be an artifact. ■ = OzID ions produced from oxidative cleavage of *n*-9 double bonds. ● = OzID ions produced from oxidative cleavage of *n*-7 double bonds.

1a), produced no such characteristic ions upon CID.[1] The structural elucidation of these lipids and a series of related GPSer-ethers is outlined below.

When subjected to negative ion electrospray ionization mass spectrometry, an $[M-H]^-$ molecular anion was identified at *m/z* 702 corresponding to the same lipid observed at *m/z* 704 in positive ion mode (Figure 1a). The CID mass spectrum of the *m/z* 702 precursor anion is shown in Figure 2a and was recorded on a quadrupole linear ion trap mass spectrometer. This spectrum reveals a single major fragment ion in the fatty acid carboxylate region of the spectrum at *m/z* 281. The fatty acid neutral loss portion of the spectrum is also instructive,[2] with an abundant fragment ion observed at *m/z* 438 corresponding to loss of an 18:1 fatty acid as a ketene (−264 Da) and a much less abundant fragment ion at *m/z* 420 corresponding to neutral loss of the entire 18:1 fatty acid (−282 Da). The greater relative abundance of the ion corresponding to the ketene loss (-R′CHCO, *m/z* 438) over the intact fatty acid loss (-RCO_2H, *m/z* 420) is typical of the fragmentation behavior of GPEtns[22] and can be attributed, at least in part, to the greater basicity of the phosphoethanolamine anion compared to other deprotonated headgroups.[23] The observation of fragment ions associated with only a single fatty acid, namely 18:1, suggests that the molecule either (i) contains two ester linked 18:1 fatty acids or (ii) the structure of the molecule permits the formation of only one carboxylate anion upon CID. The first possibility can be excluded in this instance as the $[M-H]^-$ molecular anion of GPEtn(18:1/18:1) would appear at *m/z* 742. The second explanation is often considered sufficient evidence for the presence of an ether-linked radyl in the lipid. This is because $R'CH_2O$-CH_2X ether bond (where X represents the remainder of the phospholipid) is less prone to heterolytic dissociation than the relatively more labile R′C(O)O-CH_2X ester-linkage. On the basis of the absence of fragments from a second fatty acid radyl and the molecular mass, a tentative assignment of this lipid as the 1-*O*-alkyl ether GPEtn(16:0e/18:1) could be made.

Hsu and Turk demonstrated that multiple-stage fragmentation of the $[M-H]^-$ molecular anions of ether-bearing GPEtn yield abundant product ions at *m/z* 135 that are not observed for the analogous diacyl GPEtn anions.[13] This diagnostic fragment ion is formed via consecutive neutral losses of (i) the ester-linked *sn*-2 radyl as a ketene, (ii) the ethanolamine headgroup (−61 Da), and (iii) the ether-linked *sn*-1 radyl as an alcohol. A suggested mechanism for this stepwise dissociation is outlined in Scheme 1, and step (iii) illustrates the possible involvement of an ion−molecule complex involving the alkoxide anion formed from heterolytic dissociation of the $R'CH_2O$-CH_2X ether bond and a neutral glycerylphosphate triester. The subsequent dissociation of this complex may proceed via (a) proton transfer and formation of the diagnostic phosphate fragment ion *m/z* 135, (b) a hydride transfer from the alkoxide to the phosphate followed by proton abstraction from the nascent aldehyde to form an enolate anion with a resultant neutral loss of 138 Da, or (c) direct dissociation to the alkoxide ion with a neutral loss of 136 Da. While the *m/z* 135 fragment ion is formed from both 1-*O*-alkenyl and 1-*O*-alky-1′-enyl etherss (Scheme 1a) the abundance of this fragment and the participation of the remaining two dissociation channels is dependent on the nature of the ether-linkage. Where the parent phospholipid is an 1-*O*-alk-1′-enyl ether, the nascent alkoxide anion is a resonance stabilized enolate anion (i.e., $R^{1\prime}CH{=}CHO^-$) and is consequently less basic. In such instances, formation of the alkoxide fragment anion (i.e., the −136 Da fragment ion, Scheme 1c) dominates over proton abstraction (i.e., *m/z* 135 Scheme 1a) and hydride transfer reactions cannot occur (i.e., the −138 Da fragment ion shown in Scheme 1b is not observed). Conversely, for an 1-*O*-alkyl ether, the nascent alkoxide (i.e., $R^{1\prime}CH_2CH_2O^-$) is more basic (e.g.,

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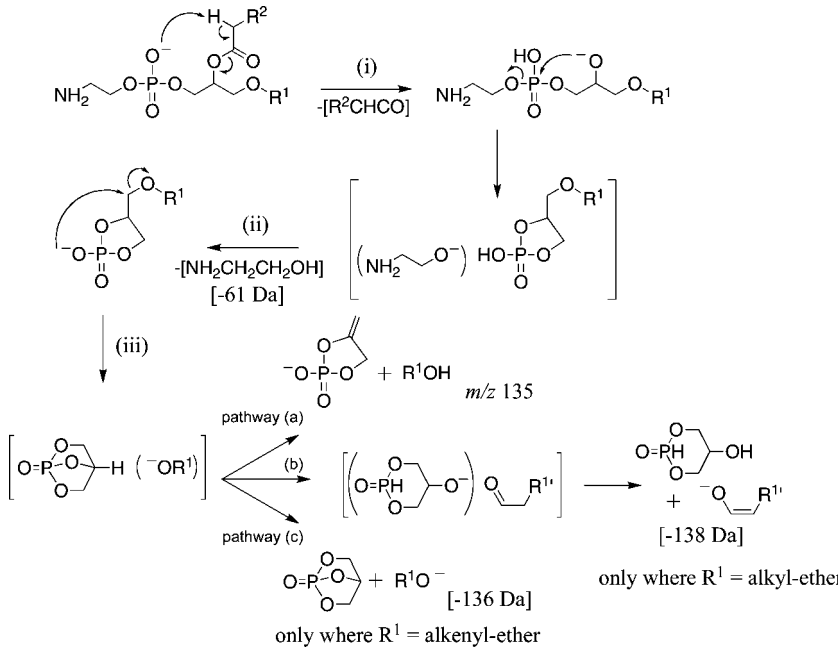
Scheme 1. Proposed Mechanism for the Sequential Fragmentation of 1-*O*-alkyl and 1-*O*-alk-1′-enyl GPEtn Based on the Observations of Hsu and Turk[13]



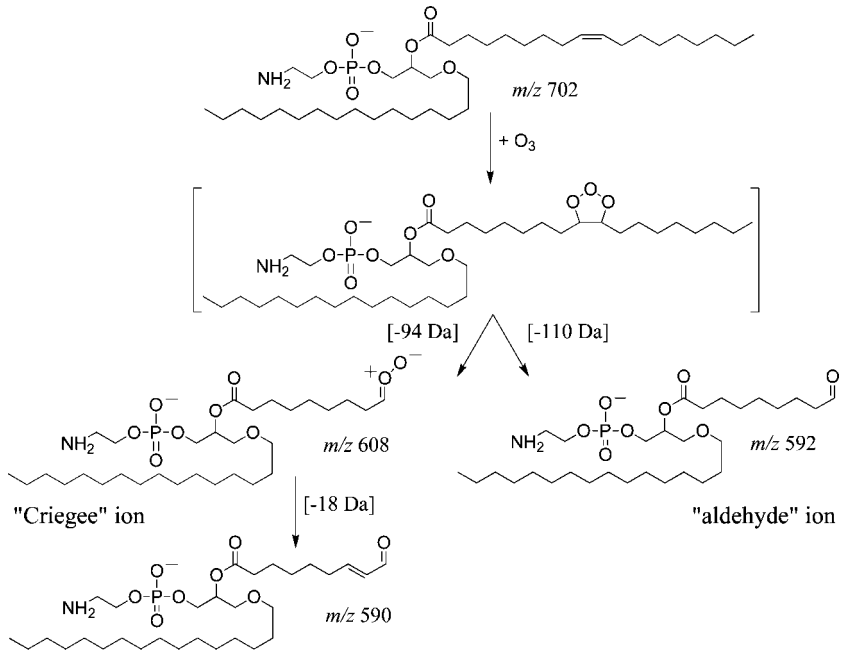
compare $\Delta_{298}H_{acid}[CH_3CH_2O-H] = 378 \pm 1$ kcal mol^{-1} with $\Delta_{298}H_{acid}[H-CH_2CHO] = 366 \pm 2$ kcal mol^{-1})[24] and favors the proton-transfer channel (Scheme 1a) forming *m/z* 135 as the major product ion. Furthermore, formation of the free alkoxide anion (Scheme 1c) is not observed for 1-*O*-alkyl ethers but rather the hydride transfer channel (Scheme 1b) participates producing a low abundance neutral loss of 138 Da. Such characteristic fragmentation in the MS^3 spectra thus identify the presence (or absence) of an ether-linked radyl at the *sn*-1 position via observation of the *m/z* 135 fragment (Scheme 1a) and can be further used to identify the nature of the bonding as predominantly 1-*O*-alk-1′-enyl or 1-*O*-alkyl ether, based on the relative abundance of this ion and/or via identification of ancillary 136 or 138 Da neutral losses.

In the present investigation, multistage mass spectrometry was performed on the *m/z* 702 molecular anion, using a quadrupole linear ion-trap mass spectrometer. As shown in Figure 2a, CID of the *m/z* 702 anion results in a product ion at *m/z* 438 via loss of the ester-linked 18:1 radyl as a neutral ketene. Spontaneous secondary fragmentation is also observed and proceeds via loss of ethanolamine, giving rise to the fragment ion at *m/z* 377 (cf. Scheme 1, steps i and ii). Instrumental conditions were optimized to maximize the ion abundance of *m/z* 377 in the MS^2 experiment such that mass-selection and subsequent fragmentation could be performed in an MS^3 experiment (Figure 2b, 702 → 377 → CID), obviating the need for further isolation-fragmentation steps (i.e., 702 → 438 → 377 → CID) with consequent losses in ion abundance. The MS^3 spectrum in Figure 2b shows an abundant *m/z* 135 product ion confirming the presence of an ether-linkage for the *sn*-1 radyl. Given that the only site of unsaturation in this lipid is associated with the ester-linked 18:1 radyl, this lipid can be assigned as the 1-*O*-alkyl ether, GPEtn(16:0e/18:1). In this instance, no ion corresponding to a 136 Da neutral loss (cf. Scheme 1c) is observed in the MS^3 spectrum (Figure 2b). It should be noted, however, that such fragments are known to be of very low abundance for 1-*O*-alkyl ethers (see above): a problem exacerbated in this case by the relatively low abundance of the precursor ion.

For the GPEtn(16:0e/18:1) ether, the negative ion MS^2 and MS^3 spectra (panels a and b of Figure 2) provide sufficient evidence to assign the only carbon−carbon double bond to the ester-linked 18:1 radyl. In the absence of further information, the position of the double bond would normally be assigned as *n*-9 (i.e., 9-bonds from the methyl end of the carbon chain) as this corresponds to the most naturally abundant 18:1 fatty acid, namely oleic acid (9*Z*−18:1). Recently, however, we have developed a novel mass spectrometric technique that allows the unequivocal determination of double bond position in intact phospholipids. In this approach the molecular ion of an unsaturated lipid is mass selected in an ion-trap and allowed to react with ozone vapor.[12] The resulting ion−molecule reaction produces two ozone induced dissociation (OzID) ions via oxidative cleavage of each double bond. The neutral loss information from OzID ions is characteristic of the position of the double bond(s) in the parent lipid. The OzID spectrum of the [GPEtn(16:0e/18:1)-H]$^-$ anion is shown in Figure 2c and reveals two pairs of chemically induced fragment ions that can be readily identified from the characteristic spacing of 16 Th. The OzID ions at *m/z* 592 and 608 correspond to the so-called aldehyde and Criegee ions formed from neutral losses of 110 and 94 Da and can be attributed to the oxidative cleavage of an *n*-9 double bond (Scheme 2). The greater abundance of the Criegee ion (*m/z* 608) compared with the aldehyde (*m/z* 592) is similar to that previously reported for diacyl-GPEtn.[12] The OzID data suggest that the lipid population giving rise to the [M−H]$^-$ ions at *m/z* 702 is predominantly GPEtn(16:0e/9*Z*−18:1), where the *sn*-2 radyl is assigned, as expected, to oleic acid, and only the stereochemistry about the double bond need be assumed. What is perhaps more interesting, however, is the second pair of OzID ions appearing at *m/z* 620 and 636. These ions are 28

(24) Linstrom, P. J.; Mallard, W. G., *NIST Chemistry WebBook, NIST Standard Reference Database*; National Institute of Standards and Technology: Gaithersburg, MD, 2003; http://webbook.nist.gov.

Scheme 2. Proposed Mechanism for the Ozone-Induced Dissociation of the *n*-9 Double Bond in the [GPEtn(16:0e/9*Z*−18:1)-H]⁻ Molecular Anion



Th higher than the *n*-9 fragment ions and have an abundance about 15% that of the *n*-9 pair. The higher mass fragments result from neutral losses of 82 and 66 Da and can be attributed to the oxidative cleavage of an *n*-7 double bond. Assuming similar efficiencies of ozone induced dissociation, these data suggest that approximately 15% of the *m/z* 702 ion population is due to the deprotonated *n*-7 lipid, GPEtn(16:0e/11*Z*−18:1).[25] Two further ions at *m/z* 590 and 618 in the OzID spectrum (Figure 2c) arise from water loss from the Criegee ions at *m/z* 608 and 636, respectively (Scheme 2).

The combination of CID and OzID spectra presented above demonstrate that two regioisomeric 1-*O*-alkyl ether phospholipids are present in the human lens, namely, GPEtn(16:0e/9*Z*−18:1) and GPEtn(16:0e/11*Z*−18:1) where the latter is found at about 15% of the concentration of the former. One might also expect the OzID spectrum (Figure 2c) to reveal additional fragment ions if any significant population of an isobaric 1-*O*-alkenyl ether [e.g., GPEtn(16:0p/18:0)] were present. For comparison, the OzID spectrum of a commercially available 1-*O*-alkenyl ether, GPEtn(16:0p/9*Z*−18:1) was obtained and is shown in Figure 2d. OzID ions are observed at *m/z* 590 and 606 corresponding to neutral losses of 110 and 94 Da resulting from oxidative cleavage of the *n*-9 double bond in the 18:1 radyl (cf. Scheme 2). Additionally, OzID ions are observed at *m/z* 506 and 522 corresponding to neutral losses of 194 and 178 Da. These fragments are consistent with a double bond at the *n*-15 position of the ether-linked radyl. It is also interesting to note that the abundance of these OzID ions is about 3 times greater than those resulting from ozonolysis of the 18:1 chain. This might be expected because of the enhanced reactivity of the more electron rich alkenyl-ether moiety over a conventional alkene. Indeed, for neutral alkenes the presence of a vinylic oxygen can enhance the rate of reaction with ozone by more than a factor of 12.[26] As such, the neutral losses, as well as the relative abundance of these ions in Figure 2d, clearly serve to identify the presence of an alkenyl-ether moiety within GPEtn-(16:0p/9*Z*−18:1). Given these observations, a re-examination of the OzID spectrum of *m/z* 702 (Figure 2c) reveals a broad peak of low abundance at *m/z* 508 (−194 Da) that might be attributable to a small amount of isobaric GPEtn(16:0p/18:0). This assignment is supported by the appearance of an *m/z* 283 ion, corresponding to the 18:0 carboxylate anion, at very low abundance in the CID spectrum in Figure 2a. In this case, OzID provides an alternative approach to unequivocal differentiation of isobaric 1-*O*-alkyl- and 1-*O*-alk-1′-enyl ether lipids in complex mixtures. It is possible to conclude in this instance that the lipids giving rise to $[M-H]^-$ anions at *m/z* 702 consist of a mixture of the alkyl ether lipids, GPEtn(16:0e/9*Z*−18:1) and GPEtn(16:0e/11*Z*−18:1), and that the isobaric alkenyl-ether GPEtn(16:0p/18:0) is present at very low levels.

In Figure 1a, the base peak in the positive ion 141 Da neutral loss spectrum is observed at *m/z* 730. The corresponding $[M-H]^-$ negative ion was also identified at *m/z* 728, and the CID spectrum of this ion (Figure 3a) reveals a single carboxylate anion fragment at *m/z* 281 and fatty acid neutral loss ions at *m/z* 464 and 446 corresponding to exclusive loss of an 18:1 radyl. These data alone strongly suggest that this lipid has an ether at the *sn*-1 position, that is, it can be assigned as either GPEtn(18:1e/18:1) and/or GPEtn(18:0p/18:1). Further fragmentation of the *m/z* 403 ion observed, yields the MS^3 spectrum shown Figure 3b. This spectrum reveals an abundant *m/z* 135 fragment ion, confirming the assignment of the GPEtn as an ether-bearing lipid while the

(25) It should be emphasized that use of relative abundances of OzID ions to quantify the relative proportion of double bond regioisomers within a mixture assumes that all isomers react with ozone at the same rate (i.e., all such alkenes have similar rate constants with ozone). The percentages provided should thus be considered as a comparative guide and may contain systematic errors arising from small differences in reaction rate constants. While rate constants for these reactions are not currently available, preliminary data do indicate a correlation between the ratio of product ion abundances in OzID spectra upon systematic increase in the proportion of one of the regioisomers (see Supporting Information).

(26) Grosjean, E.; Grosjean, D. *Atmos. Environ.* **1998**, *32*, 3393–3402.

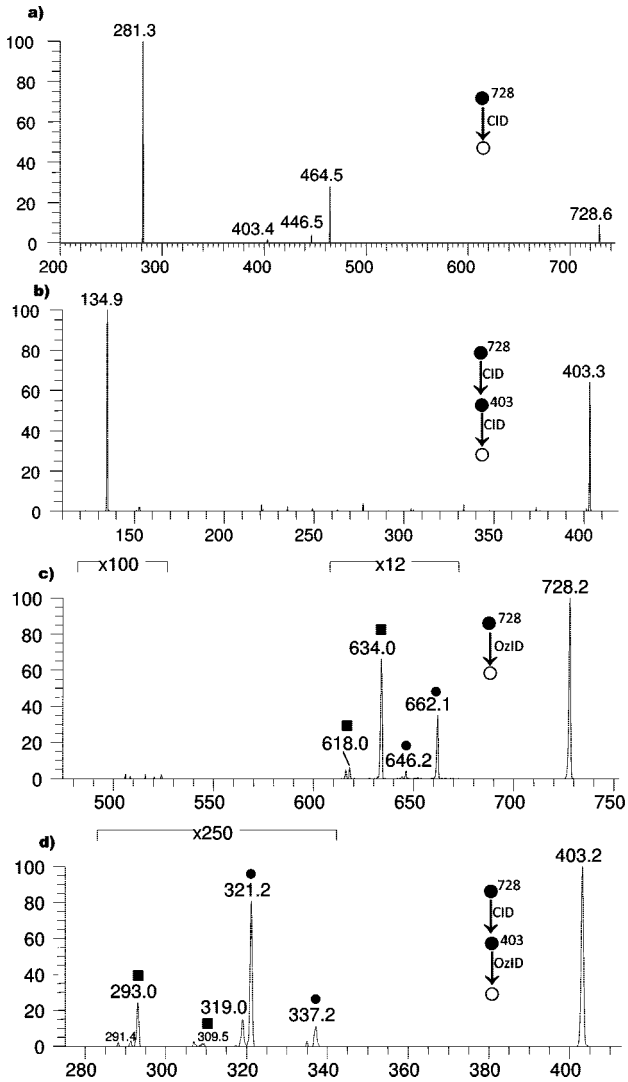
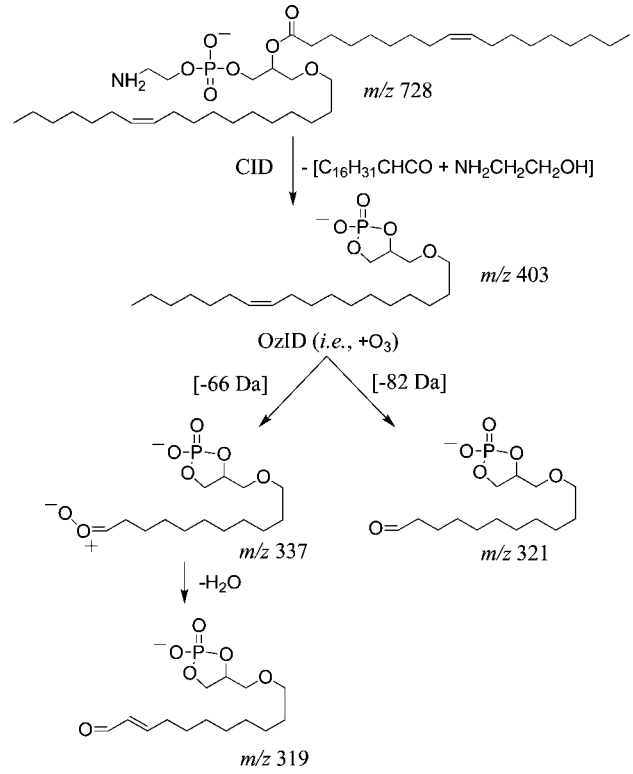


Figure 3. Negative ion electrospray ionization mass spectra obtained using a linear quadrupole ion-trap mass spectrometer: (a) CID of the GPEtn at *m/z* 728, (b) CID of the *m/z* 403 fragment ion produced in (a) (i.e., 728 → 403 → CID), (c) OzID of *m/z* 728, and (d) OzID on the *m/z* 403 fragment ion produced in (a) (i.e., 728 → 403 → OzID). ■ = OzID ions produced from oxidative cleavage of *n*-9 double bonds. ● = OzID ions produced from oxidative cleavage of *n*-7 double bonds.

absence of major fragment ion at *m/z* 267 (−136 Da) suggests that this is an 1-*O*-alkyl rather than an 1-*O*-alk-1′-enyl ether (cf. Scheme 1). Interestingly, the corresponding characteristic −138 Da fragment ion predicted at *m/z* 265 for an 18:1e radyl is not observed in this spectrum; however, such ions are known to be of low abundance for 1-*O*-alkyl ethers compared with the *m/z* 135 fragment ion.[13] Assignment of this lipid as the alkyl-ether GPEtn(18:1e/18:1) is confirmed, however, by the OzID spectrum (Figure 3c) that reveals none of the fragment ions expected for the *n*-17 alkenyl-ether GPEtn(18:0p/18:1) at *m/z* 506 and 522 (cf. Figure 2d). Rather in this spectrum two pairs of OzID ions are observed. The first at *m/z* 618 and 634 correspond to neutral losses of 110 and 94 Da and can be attributed to an *n*-9 double bond, while the second pair at *m/z* 646 and 662 correspond to neutral losses of 82 and 66 Da indicative of an *n*-7 double bonds. What cannot be determined from the OzID spectrum alone is whether this regiochemistry is associated with a particular

Scheme 3. Outline of the Pathway for Sequential CID/OzID Analysis of the [GPEtn(11*Z*−18:1e/9*Z*−18:1)-H]⁻ Molecular Anion to Reveal the *n*-7 Double Bond Position in the Ether-Linked Chain



substituent (i.e., *sn*-1 or *sn*-2) or whether this spectrum arises from a mixture of isomeric lipids as was the case for the GPEtn-(16:0e/18:1) described above. We have previously demonstrated that performing OzID on CID fragment ions, so-called CID/OzID, can be used to assign the double bond position on individual radyls. Indeed, the CID/OzID spectrum corresponding to the sequence *m/z* 728 → 464 → OzID from a human lens extract was presented as a proof of principle in our introductory paper on the OzID technique.[12] The complete structure elucidation of this ion was, however, beyond the scope of that study. Herein, we present an equivalent spectrum (Figure 3d) that was obtained by performing CID on the sequence *m/z* 728 → 403 → OzID, mass-selecting the *m/z* 403 product ion (discussed above), and allowing it to undergo reaction with ozone vapor (Scheme 3). Figure 3d reveals the most abundant pair of OzID ions at *m/z* 321 and 337, corresponding to neutral losses of 82 and 66 Da, along with an ion at *m/z* 319, which arises from dehydration of *m/z* 337. These data clearly indicate that the double bond on the *sn*-1 chain is predominantly at the *n*-7 position consistent with our previous report.[12] Interestingly, however, the improved signal-to-noise ratio in Figure 3d also reveals significant OzID ions at *m/z* 293 and 309 (−110 and 94 Da, respectively) indicative of an *n*-9 double bond. This indicates that isobaric lipids with isomeric ether chains are at the *sn*-1 position, namely, 11*Z*−18:1e and 9*Z*−18:1e. Given the robust nature of the ether-linkage it is not possible to remove the *sn*-1 radyl by CID while retaining the *sn*-2 radyl for subsequent OzID analysis. Conversely, OzID of the carboxylate fragment ion itself yields no characteristic ions. As such, it is not possible to assign the regiochemistry of the double bond on the 18:1 ester

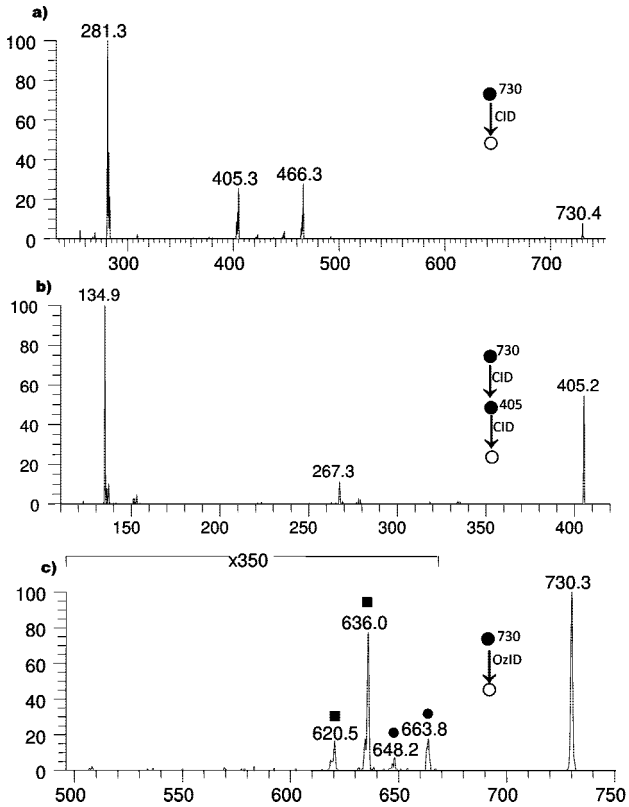


Figure 4. Negative ion electrospray ionization mass spectra obtained using a linear quadrupole ion-trap mass spectrometer: (a) CID of the GPEtn at *m/z* 730, (b) CID of the *m/z* 405 fragment ion produced in (a) (i.e., 730 → 405 → CID), and (c) OzID of *m/z* 730. ■ = OzID ions produced from oxidative cleavage of *n*-9 double bonds. ● = OzID ions produced from oxidative cleavage of *n*-7 double bonds.

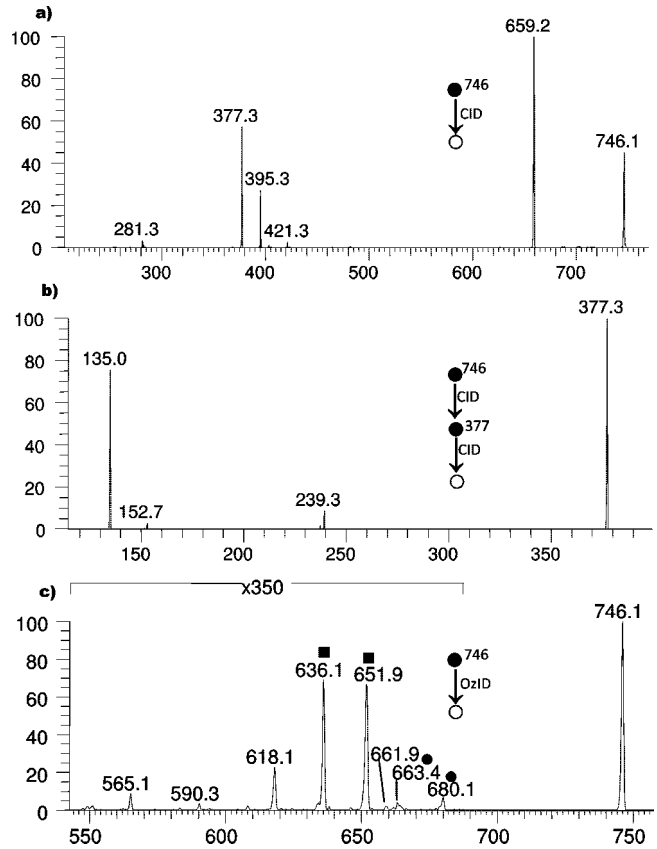


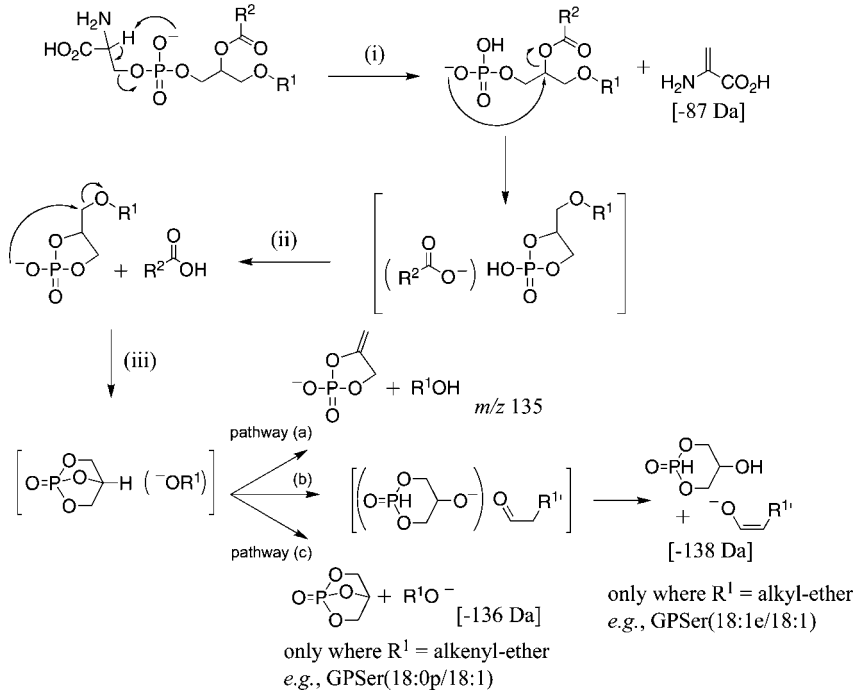
Figure 5. Negative ion electrospray ionization mass spectra obtained using a linear quadrupole ion-trap mass spectrometer: (a) CID of the GPSer at *m/z* 746, (b) CID of the *m/z* 377 fragment ion produced in (a) (i.e., 746 → 377 → CID), and (c) OzID of *m/z* 746. ■ = OzID ions produced from oxidative cleavage of *n*-9 double bonds. ● = OzID ions produced from oxidative cleavage of *n*-7 double bonds.

as exclusively *n*-9. The combined data from these experiments allows the characterization of this glycerophospholipid as predominately GPEtn(11*Z*−18:1e/9 *Z*−18:1) although some contribution from the isomers GPEtn(11*Z*−18:1e/11*Z*−18:1), GPEtn(9*Z*−18:1e/9*Z*−18:1), and GPEtn(9*Z*−18:1e/11*Z*−18:1) seems likely. Importantly, no evidence was found to suggest the presence of isomeric 1-*O*-alk-1′-enyl ethers GPEtn(18:0p/18:1) within the human lens.

The ion appearing at *m/z* 732 in the positive ion 141 Da neutral loss scan (Figure 1a) is significantly more abundant than would be expected from the M+2 isotope of the base peak at *m/z* 730 (i.e., M+2 abundance of *m/z* 730 is calculated to be 10% relative abundance while the observed abundance of *m/z* 732 is about 15%). A small fraction of this ion population was therefore tentatively assigned to a third ether-bearing GPEtn.[1] The CID and MS^3 mass spectra for the corresponding $[M-H]^-$ molecular anion at *m/z* 730 are shown in panels a and b of Figure 4, respectively. The fragment ions in these spectra show significant contributions from the ^{13}C-isotopologues of GPEtn(18:1e/18:1), but overall the fatty acid carboxylate ion at *m/z* 281 in the CID spectrum and the *m/z* 135 ion in the MS^3 are consistent with the presence of GPEtn(18:0e/18:1). In particular, the appearance of the *m/z* 267 (−138 Da) fragment ion in the MS^3 spectrum is characteristic of a saturated ether-linked 18:0 radyl at the *sn*-1 position[13] (cf. Scheme 1b). The presence of GPEtn-(18:0e/18:1) in this extract is further supported by the relative abundance of the *n*-9 (*m/z* 620 [−110 Da] and 636 [−94 Da]) and *n*−7 (*m/z* 648 [−82 Da] and 664 [−66 Da]) OzID ions in the spectrum shown in Figure 4c. For the *m/z* 730 molecular anion, the *n*-7 ions appear at about 20% of the abundance of the *n*-9 ions; this is significantly less than the 50% observed for *m/z* 728 and is closer to the 15% observed for the *m/z* 702.[25] These data thus suggest low concentrations of GPEtn(18:0e/9*Z*−18:1), and possibly an even smaller amount of the GPEtn(18:0e/11*Z*−18:1) isomer is present in the human lens.

Phosphatidylserine. A positive ion 185 Da neutral loss (-phosphoserine) spectrum obtained from a human lens total lipid extract is shown in Figure 1b. This spectrum identifies all glycerophosphoserine (GPSer) present in the lipid extract[21] and displays four dominant ions at *m/z* 748, 764, 774, and 776. The ion at *m/z* 764 is due to the protonated internal standard GPSer-(17:0/17:0), but the other three ions have previously been tentatively identified as GPSer ethers.[1] This assignment is supported by the negative ion *m/z* 153 precursor scan (dehydrated glycerophosphate) where the deprotonated ions of these GPSer lipids are not observed (Figure 1c). The *m/z* 153 ion is produced from diacyl-GPSer, glycerophosphatidic acid, glycerophosphoglycerol, and glycerophosphoinisitol.[21] The absence of these specific GPSer ions demonstrates that they are unable to form the *m/z* 153 ion as would be expected if they contained an ether-linked substitutent. To the authors' knowledge the only other mammalian tissue in which GPSer ethers have been identified is

Scheme 4. Proposed Mechanism for the Sequential Fragmentation of GPSer Phospholipids That Include 1-*O*-alkyl and 1-*O*-alk-1′-enyl Ether Moieties



the rat lung,[27] and accordingly, a more definitive structural characterization of these molecules was required. To achieve this, an analogous strategy to that used for the characterization of the GPEtn ethers was applied.

Similar to the GPEtn ethers above, CID of the deprotonated *m/z* 746 ion (related to the $[M+H]^+$ ion observed at *m/z* 748 in Figure 1b) produced the spectrum shown in Figure 5a. The base peak in this spectrum is observed at *m/z* 659 corresponding to a neutral loss of 87 Da characteristic of the serine headgroup. The remaining fragment ions result from an ester-linked 18:1 fatty acid that is lost as (i) an intact neutral fatty acid (−282 Da at *m/z* 377), (ii) a neutral ketene (−264 Da at *m/z* 395), and (ii) a carboxylate anion at *m/z* 281. On the basis of these CID fragments and the molecular mass, a tentative assignment of this lipid as the monounsaturated alkyl-ether GPSer(16:0e/18:1) can be made. The *m/z* 377 fragment ion in Figure 5a is isobaric with that observed in the CID of the analogous GPEtn(16:0e/18:1) (Figure 2a). A plausible mechanism for formation of this ion from the $[GPSer(16:0e/18:1)\text{-}H]^-$ precursor ion is shown in Scheme 4 and predicts the structure of *m/z* 377 to be identical for both headgroups (cf. Scheme 1). This contention is supported by the MS^3 (746 → 377 → CID) spectrum shown in Figure 5b, which reveals an abundant *m/z* 135 fragment ion consistent with Figure 2b. The excellent signal-to-noise ratio in the GPSer spectrum also allows for the ready identification of the *m/z* 239 ion resulting from a neutral loss of 138 Da and characteristic of the 16:0e moiety. The OzID spectrum of *m/z* 746 (Figure 5c) contains two pairs of ions identifying an *n*-9 (*m/z* 636 and 652) and an *n*-7 (*m/z* 664 and 680) double bond, as well as an ion at *m/z* 618 arising from either the water loss from *m/z* 636 or the loss of hydrogen peroxide from *m/z* 652. This spectrum thus allows the identification of both GPSer(16:0e/9*Z*−18:1) and, to a lesser extent, GPSer(16:0e/11*Z*−18:1) in the human lens lipid extract. Assuming similar OzID efficiencies for these isobaric lipid ions, the *n*-7 abundance can be estimated to be 10% that of the *n*-9 isomer.[25] This is comparable to the ∼15% ratio for the analogous GPEtn(16:0e/18:1) phospholipids. Finally, the OzID spectrum in Figure 5c shows no significant ion abundance at *m/z* 552 that might indicate the presence of the isobaric 1-*O*-alk-1′-enyl ether, GPSer(16:0p/18:0).

The CID spectrum of an $[M-H]^-$ molecular anion of *m/z* 772 (related to the $[GPSer+H]^+$ cation at *m/z* 774 in Figure 1b) is shown in Figure 6a. As expected, the abundant −87 Da neutral loss confirms the presence of the serine headgroup while the fatty acid related fragments at *m/z* 281, 403, and 421 indicate the presence of a single ester-linked 18:1 radyl. The MS^3 spectrum obtained from the sequence 772 → 403 → CID is shown in Figure 6b where the abundance of the *m/z* 135 fragment ion indicates an ether-linkage at the *sn*-1 position. Less abundant *m/z* 265 (−138 Da) ions that might confirm this assignment are, however, difficult to unequivocally assign in this spectrum. The OzID spectrum of *m/z* 772 (Figure 6c) reveals no significant ions arising from oxidative cleavage of an *n*-17 double bond (*m/z* 550 or 566), as would be expected if the isobaric 1-*O*-alk-1′-enyl ether GPSer(18:0p/18:1) were also present in the extract. This spectrum does, however, contain ions clearly indicating the presence of double bonds at *n*-9 (*m/z* 662 [−110 Da] and 678 [−94 Da]), *n*-7 (*m/z* 690 [−82 Da] and 706 [−66 Da]), and even *n*-5 positions (*m/z* 718 [−54 Da] and 734 [−38 Da]). The CID/OzID spectrum obtained using the sequence 772 → 403 → OzID is shown in Figure 6d and is designed to examine the double bond position in the *sn*-1 chain having effectively removed the *sn*-2 radyl by CID (cf. Scheme 3).

Intriguingly, this spectrum also shows fragment ions arising from oxidative cleavage of double bonds at *n*-9 (*m/z* 293 [−110 Da] and 309 [−94 Da]), *n*-7 (*m/z* 321 [−82 Da] and 337 [−66

(27) Kaneshiro, E. S.; Guo, Z.; Sul, D.; Kallam, K. A.; Jayasimhulu, K.; Beach, D. H. *J. Lipid Res.* **1998**, *39*, 1907–1917.

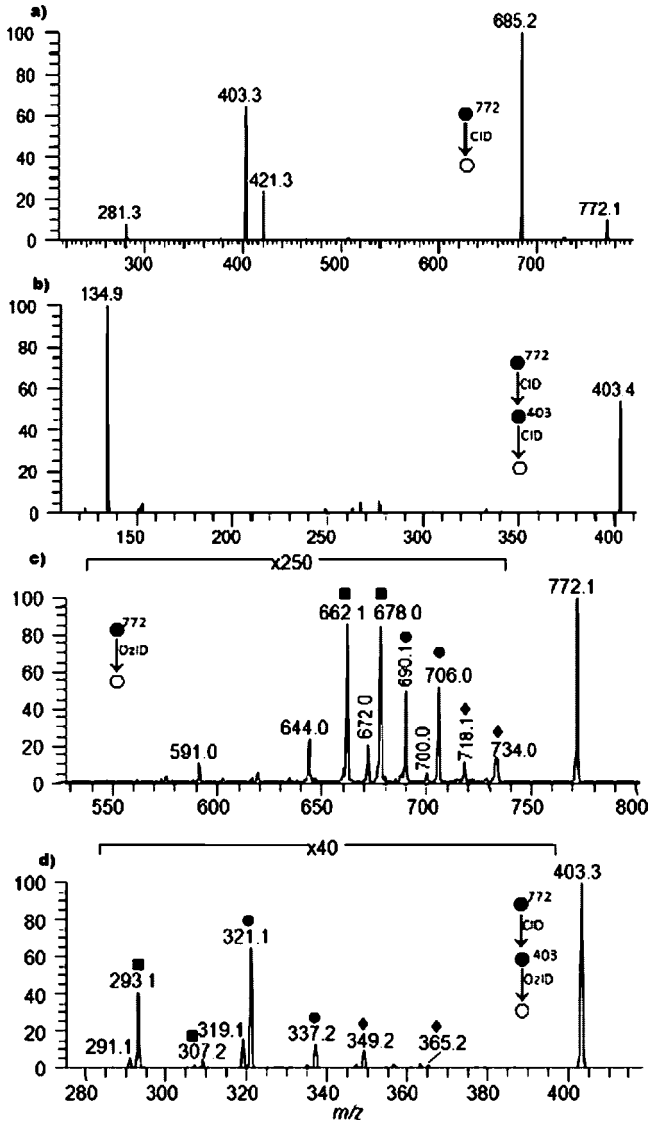


Figure 6. Negative ion electrospray ionization mass spectra obtained using a linear quadrupole ion-trap mass spectrometer: (a) CID of the GPSer at *m/z* 772, (b) CID of the *m/z* 403 fragment ion produced in (a) (i.e., 772 → 403 → CID), (c) OzID of *m/z* 772, and (d) OzID on the *m/z* 403 fragment ion produced in (a) (i.e., 772 → 403 → OzID). ■ = OzID ions produced from oxidative cleavage of *n*-9 double bonds. ● = OzID ions produced from oxidative cleavage of *n*-7 double bonds. ♦ = OzID ions produced from oxidative cleavage of *n*-5 double bonds.

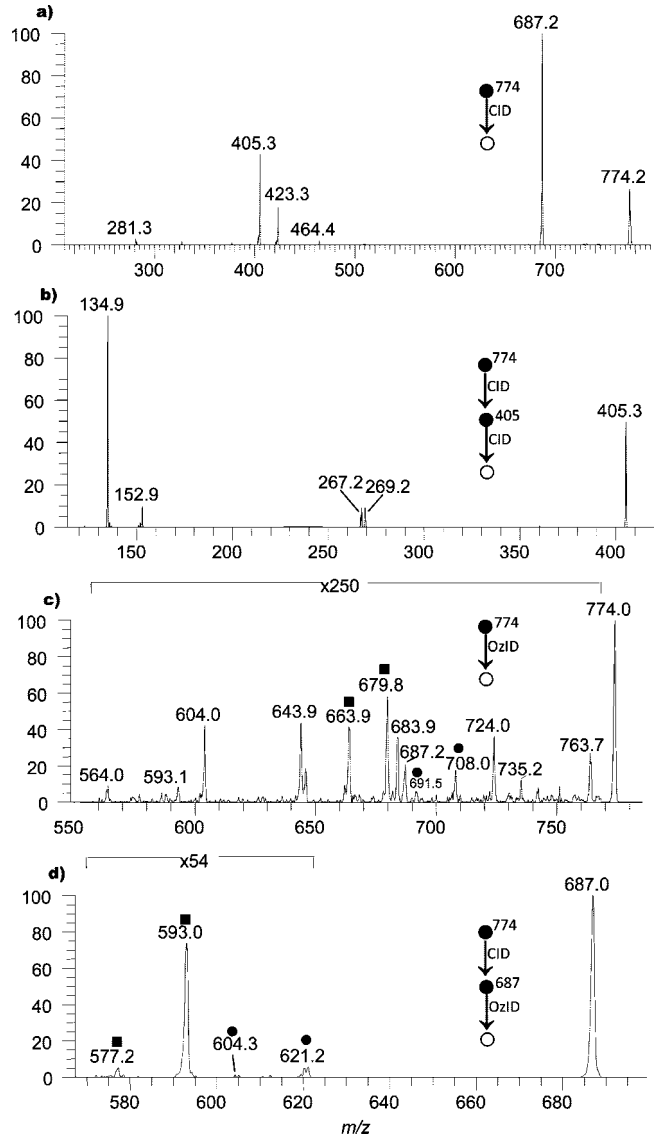


Figure 7. Negative ion electrospray ionization mass spectra obtained using a linear quadrupole ion-trap mass spectrometer: (a) CID of the GPSer at *m/z* 774, (b) CID of the *m/z* 405 fragment ion produced in (a) (i.e., 774 → 405 → CID), (c) OzID of *m/z* 774, and (d) OzID on the *m/z* 687 fragment ion produced in (a) (i.e., 774 → 687 → OzID). ■ = OzID ions produced from oxidative cleavage of *n*-9 double bonds. ● = OzID ions produced from oxidative cleavage of *n*-7 double bonds.

Da]), and *n*-5 (*m/z* 349 [−54 Da] and 365 [−38 Da]). This result indicates that all three regioisomeric radyls are present in the sample; however, the ratio of the peak heights in the CID/OzID spectrum differs significantly from that obtained from OzID of the molecular ion. Notably, the *n*-9 pair of ions are the most abundant in the OzID spectrum of the molecular ion (Figure 6c) while the *n*-7 ions are the most abundant in the CID/OzID spectrum (Figure 6d). On the basis of the most abundant ions and the analogy with GPEtn(18:1e/18:1), it seems likely that the major GPSer at this mass is GPSer(11*Z*−18:1e/9*Z*−18:1), while GPSer(9*Z*−18:1e/9*Z*−18:1) and GPSer(13*Z*−18:1e/9*Z*−18:1) are also contributing to the isobaric ion population. This in itself is a remarkable diversity of phospholipids, but it must be emphasized that contributions from a further 6 isomeric combinations cannot be rigorously excluded based on these data [i.e., GPSer(11Z−18:1e/11Z−18:1), GPSer(11Z−18:1e/13Z−18:1), GPSer(9*Z*−18:1e/11*Z*−18:1), GPSer(9*Z*−13:1e/13*Z*−18:1), GPSer (13*Z*−18:1e/11*Z*−18:1), and GPSer(13*Z*−18:1e/13*Z*−18:1)].

The CID spectrum of the $[M-H]^-$ ion at *m/z* 774 (the negative ion companion of the $[GPSer+H]^+$ cation identified at *m/z* 776 in Figure 1b) is shown in Figure 7a. The abundant −87 Da neutral loss in this spectrum clearly confirms the presence of a GPSer with the subsequent fragmentation indicative of an ester-linked 18:1 radyl (*m/z* 281, 405 and 423). In this spectrum an additional fatty acid carboxylate anion was observed at *m/z* 327, indicative of an ester-linked 22:6 fatty acid. Complementary neutral loss fragments for a 22:6 radyl are also observed at *m/z* 446 (−328 Da) and 464 (−310 Da) with the abundance ratio favoring the latter ion and suggesting that it may be a fragment

from an isobaric contributor such as GPEtn(18:1e/22:6) or GPEtn(18:0p/22:6). The MS^3 spectrum obtained using the sequence 774 → 405 → CID (Figure 7b) reveals an abundant ion at *m/z* 135, consistent with the assignment of the major contributing lipid to an alkyl-ether. The same spectrum shows an ion at *m/z* 267 (−138 Da) consistent with the presence of an 18:0e moiety, while the *m/z* 269 ion in this instance may result from a small amount of the 17:0 carboxylate anion perhaps arising from the isobaric impurity GPSer(17:0/18:1).

The presence of two isobaric ions at *m/z* 774 presents a challenge in assignment of double bond position based on the OzID spectrum (Figure 7c). Indeed, while this spectrum shows clear indications of *n*-7 (*m/z* 692 [−82 Da] and 708 [−66 Da]) and *n*-9 (*m/z* 664 [−110 Da] and 680 [−94 Da]) double bonds, presumably associated GPSer(18:0e/18:1), it also displays a large range of other product ions arising from sequential cleavage of the skip-conjugated double bonds in GPEtn(18:0p/22:6). To identify the unsaturation associated with the GPSer only, a CID/OzID spectrum was obtained using the sequence 774 → 687 → OzID (Figure 7d). This spectrum more closely resembles those of the other glycerophospholipids analyzed with two pairs of ions identifying the presence of *n*-7 (*m/z* 605 [−82 Da] and 621 [−66 Da]) and *n*-9 (*m/z* 577 [−110 Da] and 593 [−94 Da]) double bonds and the absence of ions that would identify the presence of a 1-*O*-alk-1′-enyl ether. Through the combination of CID and OzID experiments this GPSer was identified as predominantly GPSer(18:0e/9*Z*−18:1) with a lesser abundance of GPSer(18:0e/11*Z*−18:1) present at about 7% of the *n*-9 regioisomer.

CONCLUSION

This study illustrates the rapidly evolving capabilities of mass spectrometry for structure elucidation of lipids in complex mixtures with many of the lipids identified present at levels too low for conventional chromatographic approaches. The combination of CID (including MS^3) and OzID has provided near-complete elucidation of molecular structure where only the stereochemistry about the double bonds remains unassigned. For the characterization of ether-linked phospholipids in particular, these methods proved highly complementary. That is, in instances where characteristic CID fragment ions are of relatively low abundance and may not be detectable above the noise (e.g., the absence of characteristic 138 Da neutral loss ions in Figures 2b and 3b) it is reassuring to have structural confirmation of the nature of the ether-linkage by an alternative method.

A combination of mass spectrometric experiments has confirmed the presence of abundant ether-lipids in the human lens. Specifically, the ether-lipids identified in this study include GPEtn(16:0e/9*Z*−18:1), GPEtn(11*Z*−18:1e/9*Z*−18:1), GPEtn(18:0e/9*Z*−18:1)], GPSer(16:0e/9*Z*−18:1), GPSer(11*Z*−18:1e/9*Z*−18:1), and GPSer(18:0e/9*Z*−18:1). The combination of the structure elucidation presented here and previous quantitative estimates[1] suggest that 1-*O*-alkyl ethers account for more than two-thirds of all ether-bearing glycerophosphoethanolamines in older human lenses (about 60 years old). Indeed, the 1-*O*-alkyl ethers identified in both classes account for 52% and 66%, respectively, of the total GPEtn and GPSer content of the human lens.[1]

Evidence for lower levels of a range of other isomers including up to 9 isomers of GPSer(18:1e/18:1) differing only in the positions of double bonds on the two chains was also obtained. There is significant structural homology in the series of GPEtn and GPSer-ethers identified in this study that even extends to the relative distribution of double bond positional isomers. This observation may provide evidence for a precursor-product relationship in the lens and is in general agreement with the literature, which suggests that GPEtn can be formed through decarboxylation of GPSer and conversely GPSer can be formed from GPEtn via the action of phosphatidylserine synthase II.[11]

To our knowledge, this is the first report of 1-*O*-alkyl ether glycerophosphoserines in human tissue. The observation of double bonds at the *n*-5 position of unsaturated phospholipids is also unusual [e.g., GPSer(13*Z*−18:1e/9*Z*−18:1)] but is consistent with our previous report of the presence of low levels of *n*-5 dihydrosphingomyelins in human lens tissue.[12,28] The application of the novel CID/OzID technique provides evidence for a preference for *n*-7 double bonds on the ether chains suggesting some degree of specificity in the biosynthesis of these compounds.

The unusual molecular structures and the structural diversity identified in these lipids raises intriguing questions as to the role of these compounds within the human lens. It is interesting to note that mice deficient in dihydroxyacetonephosphate acyltransferase (DHAPAT), a peroxisomal enzyme that is required for the biosynthesis of ether lipids, develop early onset cataract among other pathologies.[3] Future work examining the change in this lipid population with age and the onset of disease may provide answers to these questions.

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SUPPORTING INFORMATION AVAILABLE

Additional data relating to ref 25. This material is available free of charge via the Internet at http://pubs.acs.org.

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