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Introduction of a Fixed-Charge, Photolabile Derivative for Enhanced Structural Elucidation of Fatty Acids

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Abstract

Fatty acids are a structurally diverse category of lipids with a myriad of biochemical functions, which includes their role as building blocks of more complex lipids (e.g., glycerophospholipids and triacylglycerols). Increasingly, the analysis of fatty acids is undertaken using liquid chromatography-mass spectrometry (LC-MS), due to its versatility in the detection of lipids across a wide range of concentrations and diversity of molecular structures and masses. Previous work has shown that fixed-charge pyridinium derivatives are effective in enhancing the detection of fatty acids in LC-MS workflows. Herein, we describe the development of two novel pyridinium fixed-charged derivatization reagents that incorporate a photolabile aryl iodide that is selectively activated by laser irradiation inside the mass spectrometer. Photodissociation mass spectra of fatty acids conjugated to 1-(3-(aminomethyl)-4-iodophenyl)pyridin-1-ium (4-I-AMPP+) and 1-(4-(aminomethyl)-3-iodophenyl)pyridin-1-ium (3-I-AMPP+) derivatives reveal structurally diagnostic product ions. These spectra feature radical-directed dissociation of the carbon-carbon bonds within the fatty acyl chain, enabling structural assignments of fatty acids and discrimination of isomers that differ in site(s) of unsaturation, methyl branching or cyclopropanation. These derivatives are shown to be suitable for hyphenated LC-MS methods, and their predictable photodissociation behavior allows de novo identification of unusual fatty acids within a biological context.

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Introduction of a fixed-charge, photolabile derivative for enhanced structural elucidation of fatty acids

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ABSTRACT: Fatty acids are a structurally diverse category of lipids with a myriad of biochemical functions, which includes their role as building blocks of more complex lipids (*e.g.*, glycerophospholipids and triacylglycerols). Increasingly, the analysis of fatty acids is undertaken using liquid chromatography-mass spectrometry (LC-MS), due to its versatility in the detection of lipids across a wide range of concentrations and diversity of molecular structures and masses. Previous work has shown that fixed-charge pyridinium derivatives are effective in enhancing the detection of fatty acids in LC-MS workflows. Herein, we describe the development of two novel pyridinium fixed-charged derivatization reagents that incorporate a photolabile aryl iodide that is selectively activated by laser irradiation inside the mass spectrometer. Photodissociation mass spectra of fatty acids conjugated to 1-(3-(aminomethyl)-4-iodophenyl)pyridin-1-ium (4-I-AMPP$^+$) and 1-(4-(aminomethyl)-3-iodophenyl)pyridin-1-ium (3-I-AMPP$^+$) derivatives reveal structurally diagnostic product ions. These spectra feature radical-directed dissociation of the carbon-carbon bonds within the fatty acyl chain, enabling structural assignments of fatty acids and discrimination of isomers that differ in site(s) of unsaturation, methyl branching or cyclopropanation. These derivatives are shown to be suitable for hyphenated LC-MS methods and their predictable photodissociation behavior allows *de novo* identification of unusual fatty acids within a biological context.

INTRODUCTION

Fatty acids (FAs) are critical building blocks of complex cellular lipids (*e.g.*, triacylglycerols and glycerophospholipids) and also serve as metabolic precursors of a range of important lipid mediators. FAs are thus central to an abundance of functions in cellular biology, acting as energy sources for metabolism; structural components of the membrane bilayer; and – in conjunction with other biomolecules – participants in signal transduction.[1-3] Such functional diversity is reflected in the structural diversity of FAs, which can arise from differences in hydrocarbon chain length (*i.e.*, total number of carbon atoms); degree of unsaturation; position(s) and stereochemistry of carbon-carbon double bonds; degree and location of chain branching and carbocyclic motifs; and heteroatomic functionalization (*e.g.*, hydroxylation, halogenation and nitration).[2-4] This diversity results in an enormous number of possible structural permutations with the LIPID MAPS database currently listing more than seven thousand curated entries within the fatty acyl lipid category.[5] Due to this complexity, the confident identification of FAs in biological extracts presents an analytical challenge that is further confounded by the common occurrence of isomeric FAs that share an identical molecular mass and, oftentimes, similar physical properties.[6]

Traditionally, gas chromatography-mass spectrometry (GC-MS) has been deployed for identifying and quantifying FAs in biological samples due to the high peak capacity and reproducibility of the gas chromatograph.[7-8] In general, GC-MS analysis of FAs requires wet-chemical modification prior to injection in order to increase the volatility of the analytes and thus improve chromatographic performance. The most widely used of these derivatization strategies is conversion to fatty acid methyl esters (FAMEs).[9] FAME derivatives have a higher vapor pressure than their parent acids and thus, in combination with optimized column chemistries, efficient analytical separations can be achieved for a wide range of FAs. For some isomeric FAs, however, analytical separations are insufficient for robust identification and/or quantification of the individual components. Furthermore, the conventional (70 eV) electron ionization (EI) mass spectra of isomeric FAMEs are typically indistinguishable. This can mask the presence of structurally distinct FAs in both chromatographic and mass spectrometric dimensions.[10-12] The problem is further compounded for less common FAs where a lack of reference standards limits the use of retention time alignment or spectral matching for identification.[13] To address this challenge, alternative GC-MS derivatization methods have been developed to generate more structurally diagnostic mass spectra upon EI. Two of the most successful and widely adopted examples are 4,4-dimethyloxazoline (DMOX) and 3-picolinyl ester derivatives of FAs. Both derivatives produce EI mass spectra with fragmentation patterns that can identify double bond positions, methyl branch points and carbocycles within the acyl chain.[11, 14-17] A key design feature of these reagents is the incorporation of the nitrogen heteroatom that serves to localize the positive charge and direct radical-induced chain cleavage in a structurally sensitive manner.[11] Translating this

successful strategy to an LC-MS workflow is a central focus of the current work.

Advances in electrospray ionization mass spectrometry have underpinned the emergence of LC-MS as the pre-eminent technology for lipid analysis.[18-19] By sampling lipids directly from an extract solution, LC-MS can be deployed across a diversity of lipid structures spanning a wide range of molecular masses (*e.g.*, long and ultra-long chain FAs).[20-21] Non-esterified FAs (sometimes called free FAs) or FAs liberated from complex extracts by saponification can be detected by LC-MS in negative ion mode, however sensitivity can be compromised by the use of typical acidic mobile phases. Mass analysis of the deprotonated $[FA\text{-}H]^-$ anions can be used to assign the sum composition of the lipid (*i.e.*, total numbers of carbons, degree of unsaturation and functionalization) but cannot be used to assign structure. Tandem mass spectrometry (LC-MS/MS) of $[FA\text{-}H]^-$ anions by conventional, low energy (<100 eV) collision-induced dissociation (CID) typically results in decarboxylation ($-CO_2$), dehydration ($-H_2O$) and charge loss.[22] CID produces very little fragmentation from the carbon-carbon bonds in the acyl chain, thus restricting the utility of negative ion LC-MS/MS for *de novo* structural assignment. To surmount this impediment, charge-inversion of FAs has been explored using a number of approaches that can broadly be categorized as non-covalent and covalent.

Non-covalent charge inversion strategies involve the addition of selected salts to the electrospray solution yielding positively charged metal adduct ions. Adducts of the alkali metals ($[FA\text{-}H+2X]^+$, where X = Li^+ or Na^+), alkali earth metals ($[FA\text{-}H+Y]^+$, where Y = Mg^{2+}, Ca^{2+}, Ba^{2+}) and even some transition metals have been explored.[23-25] The tight binding of divalent metals, in particular, to the carboxylate moiety of the FA in $[FA\text{-}H+Y]^+$ can facilitate charge-remote fragmentation along the fatty acyl chain when activated by CID.[26] These even-electron dissociation processes reveal key structural details of FAs, but the requisite spray chemistry is not typically compatible with typical HPLC eluents. The recent demonstration of gas phase charge-inversion of $[FA\text{-}H]^-$ anions to metal-adducted cations may enable better integration of this approach with LC-MS methods.[27]

Covalent derivatization of FAs has employed ester or amide linkages to append basic (and thus ionizable) or fixed-charge moieties to the carboxylic acid functional group. A wide range of these derivatization reagents have been reported (*e.g.*, di- and tri-methylaminoethyl esters, picolinylesters *etc.*) and all serve to enhance the sensitivity and selectivity of FAs in positive mode.[28-33] Within this class of reagents, Gelb and co-workers introduced the custom-designed *N*-(4-(aminomethyl)phenyl)pyridinium ($AMPP^+$).[34-35] Linked to the FA by a mild amide coupling procedure, the resulting pyridinium fixed-charge derivatives displayed excellent detection sensitivity in positive ion mode and significantly enhanced charge-remote fragmentation of the acyl chain upon collisional activation. CID spectra of mass selected FA-$AMPP^+$ derivatives displayed diagnostic product ions that could be used to assign site(s) of unsaturation and chain functionalization, thus providing a way of discriminating between isomeric FAs. Several groups have since employed $AMPP^+$ derivatization for the identification and quantification of non-esterified FAs in extracts from mouse plasma, human plasma and serum, as well as identification of unusual esterified FAs in extracts from bacteria.[34-40] Compared to the radical-directed fragmentation observed upon EI of picolinyl esters and DMOX derivatives however, the abundance of charge-remote fragments in even-electron $AMPP^+$ derivatives is relatively low as a proportion of the ion signal. Moreover, the product ion abundance declines for fragmentation toward the methyl end of the FA chain (*e.g.*, Supporting Information Figure S 16). These two factors conspire to make structural assignments of some FAs difficult, particularly for branched-chain FAs where structural variations are mostly located near the methyl terminus (*e.g.*, *iso*- and *anteiso*-FAs, Figures S17 and S18). It would be desirable therefore, to access FA derivatives that are compatible with LC-MS/MS workflows and capable of generating odd-electron radical cations.

Inspired by the work of Julian and co-workers on protein structure determination, we (and others) have previously demonstrated that radical ions of lipids can be produced by photodissociation (PD) of electrospray-generated, even-electron precursors.[41] These strategies utilized derivatives incorporating an aryl-iodide motif that can be appended to lipids using non-covalent or covalent strategies and activated selectively by laser irradiation at 266 nm inside the mass spectrometer.[42-45] In both instances, the resulting radical cations could be further activated by CID to affect dissociation of the fatty acyl chain and reveal a suite of FA structural motifs, including chain branching. Combining these learnings with findings from fixed-charge derivatives, we conceived FA derivatization reagents that incorporate both fixed-charge and photolabile motifs. These multi-functional derivatives would enhance detection efficiency and structure elucidation using both even-electron (*via* CID) and odd-electron (*via* PD) dissociation modalities. Herein we describe the design and synthesis of two such reagents, 4-I-$AMPP^+$ and 3-I-$AMPP^+$. PD mass spectra of FAs modified with these reagents are shown to yield extensive radical-directed dissociation of carbon-carbon bonds that can be used for structure elucidation and, specifically, discrimination of FA isomers. The compatibility of the reagents and the photodissociation workflow with LC-MS methods is demonstrated using a lipid extract derived from the complex human secretion *vernix caseosa*.

EXPERIMENTAL

Materials

Where possible, FAs are annotated using the recommended shorthand notation.[46] *cis*-Vaccenic acid (FA 18:1(11*Z*)), heptadecanoic acid (FA 17:0) and *cis*-nonadec-10-enoic acid (FA 19:1(10*Z*)) were obtained from Sigma-Aldrich (Castle Hill NSW, Australia); oleic acid (FA 18:1(9*Z*)) and petroselinic acid (FA 18:1(6*Z*)) were obtained from Tokyo Chemical Industry (Tokyo, Japan); 15-methylhexadecanoic acid (FA 16:0(15Me)), 14-methylhexadecanoic acid (FA 16:0(14Me)) and *cis*-octadec-8-enoic acid (FA 18:1(8*Z*)) were obtained from Larodan (Solna, Sweden); phytomonic acid (CFA 19:0(11,12cp)) from Sapphire Bioscience (Redfern NSW, Australia); and dihydrosterculic acid (CFA 19:0(9,10cp)) from Adelab Scientific (Thebarton SA, Australia). Food Industry FAME mix that includes 37 fatty acids as methyl esters was purchased from RESTEK (Bellefonte, PA, USA). All solvents, reagents and additives for LC-MS were Optima grade from Thermo Fisher Scientific (North Ryde NSW, Australia). The synthesis of derivatization reagents 4-I-$AMPP^+$ and 3-I-$AMPP^+$ is detailed in the Supporting Information, along with comprehensive characterization of the target reagents and synthetic intermediates. A triacylglycerol fraction of *vernix caseosa* was provided by Prof. Josef Cvačka (Charles University, Prague).[47-49] FA standards and lipid extracts were derivatized using a slight modification to a previously reported procedure as summarized in Figure 1 (for full details see Supporting Information).[34]

Liquid chromatography-mass spectrometry

All ESI mass spectra were acquired on a modified linear ion trap mass spectrometer (LTQ XL, Thermo Scientific, San Jose, CA, USA). For direct infusion experiments, a methanolic solution of FAs derivatized with I-AMPP$^+$ (~3 μM) was introduced into the ESI source via a syringe pump delivering 5 μL min^{-1}. To provide spray stability, the sample flow was supplemented through tee-infusion of a 50 μL min^{-1} flow of MeOH. Typical source parameters were: spray voltage +4.0 kV, capillary temperature 300 °C, tube lens voltage 50 V and capillary voltage 11 V. Nitrogen served as the sheath (arbitrary flow rate units between 5 and 10), auxiliary and sweep gases (between 0 and 5 arbitrary flow rate units). For collision-induced dissociation, ions were mass-selected with a window of 1–2 Da and a normalized collision energy (NCE) of 7–15 (arbitrary units) was applied over an activation time of 30 ms. The linear ion trap was modified to enable PD using 266 nm radiation from a Nd:YAG laser (Minilite II, Continuum, Santa Clara CA, USA) introduced at the posterior port of the ion trap mass spectrometer. Only a single laser pulse (pulse width approximately 5 ns) irradiated the selected ions per mass spectral cycle. HPLC experiments were carried out using a Dionex Ultimate 3000 RSLC (Thermo Scientific) system controlled by Chromeleon™ 7.2 Chromatography Data System software. Separations were performed using a C30 reverse-phase column (Acclaim™, 150 mm × 2.1 mm, 3.0 μm, Thermo Scientific) maintained at 45 °C with mobile phases and gradients provided as Supporting Information.

RESULTS AND DISCUSSION

Preparation of FA derivatives for photodissociation

Photodissociation mass spectrometry is finding increasing application in biomolecular structure elucidation. In general, these strategies use short wavelength ultraviolet radiation (most commonly 193 nm) to excite and fragment the analyte.[50-53] An alternative approach has been to introduce a photolabile functional group that is activated in a spectral region where the absorption cross-section of the analyte itself is weak (*e.g.*, 266 nm).[41] This has the advantage of initiating selective photochemistry from a defined location within the target ion and hence drive predictable fragmentation for structure elucidation. Aligned with the latter approach, we designed and synthesized two derivatization reagents, 4-I-AMPP$^+$ and 3-I-AMPP$^+$, incorporating both a fixed-positive charge to enhance sensitivity and a photolabile aryl iodide group as a target for photoactivation at 266 nm (Figure 1). Detailed synthesis and structural characterization details are provided in the Supporting Information. To evaluate the effectiveness of these reagents for FA structure elucidation, the two I-AMPP$^+$ reagents were conjugated to commercially available FA standards under mild amide coupling conditions (Figure 1).

Structure elucidation of unsaturated FAs

When infused into the mass spectrometer as a methanolic solution, oleic acid (FA 18:1(9*Z*)) derivatized with 4-I-AMPP$^+$ gave rise to an abundant even-electron molecular ion at *m/z* 575 (data not shown). Irradiation of the mass-selected precursor ion with a single 266 nm laser pulse yielded the [M-I]$^{\bullet+}$ radical cation at *m/z* 448, as shown in the photodissociation (PD) mass spectrum in Figure 2A. In addition to the loss of atomic iodine, secondary product ions corresponding to both acyl chain fragmentation and liberation of the AMPP$^+$ moiety are also observed at lower abundance. Product ions corresponding to loss of iodine followed by radical-induced scission of the acyl chain carbon-carbon bonds are observed in a sequence of 14 Da spacings ranging from *m/z* 433 ([M-I-CH_3]$^+$) to *m/z* 349 ([M-I-C_7H_{15}]$^+$). This trend is interrupted between *m/z* 349 and *m/z* 295, where only low abundant product ions are observed corresponding to the Δ^9-location of the carbon-carbon double bond. The two ions at *m/z* 349 and *m/z* 295 (Figure 2A) are the most abundant acyl chain products, consistent with radical-driven homolysis at the two activated allylic positions. The PD mass spectrum in Figure 2A also reveals abundant product ions at *m/z* 183 and *m/z* 169 arising from the AMPP$^+$ tag and are assigned as products from dissociation at the amide and benzyl positions, respectively, following initial loss of iodine.

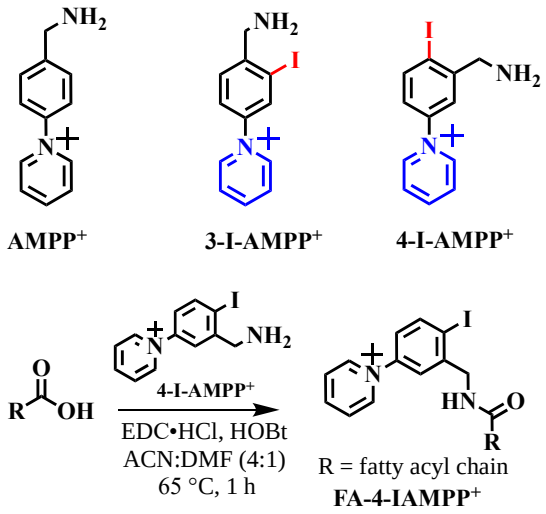

Figure 1: Structures of derivatization reagents AMPP$^+$, 3-I-AMPP$^+$ and 4-I-AMPP$^+$ and a summary of the derivatization protocol for fatty acids (RCO_2H) with 4-I-AMPP$^+$ under mild amide coupling conditions

Mass-selection and subsequent collisional activation of the PD-derived [M-I]$^{\bullet+}$ radical cation at *m/z* 448 in an MS^3 experiment gave rise to the PD/CID mass spectrum shown in Figure 2B. This spectrum displays a similar pattern of radical-driven dissociation along the acyl chain to the corresponding PD spectrum (*cf.* Figure 2A). Under collisional activation however, radical-directed and charge-remote chemistries appeared more competitive with both even- and odd-electron product ions observed for carbon-carbon bond cleavages along the chain (*e.g.*, *m/z* 349 and 350 were both observed). While still interpretable, the greater competition between radical-directed and charge-remote fragmentation modalities in the PD/CID spectrum could make the assignment of structure in complex mixtures more difficult than from the corresponding PD mass spectrum. With the latter providing the more easily interpretable spectra at a similar overall signal intensity, PD mass spectra were analyzed for all subsequent experiments with I-AMPP$^+$ derivatives.

The regioisomeric derivatization reagent 3-I-AMPP$^+$ was synthesized and conjugated to FA 18:1(9*Z*) for comparison. The PD mass spectrum of this derivative, compared to the equivalent PD spectrum of the 4-I-AMPP$^+$ congener is shown in Figure S1. These spectra are almost identical, with both derivatives showing a regular pattern of radical-driven dissociation along the acyl chain identifying the location of the carbon-carbon double bond. Careful analysis of the PD experiments with the two isomers, however, revealed that ~28% of precursor 4-I-AMPP$^+$ ions were converted to photoproduct ions under these conditions, compared to ~40% for the 3-I-AMPP$^+$ derivative

(Figure S2). The differing photo-fragmentation yields of the two derivatives arise from differences in (either or both) their absorption cross-sections or photodissociation efficiencies at 266 nm. Solution phase absorption measurements at 266 nm using equimolar solutions of 3-I-AMPP$^+$ and 4-I-AMPP$^+$ derivatives of FA 18:1(9*Z*) in 85% CH_3CN suggest a larger absorption cross-section for the latter (Figure S3B). Therefore, the greater PD product yield of the 3-I-AMPP$^+$ derivative is likely to result from a higher PD efficiency associated with the relative positioning of the charge, linker and iodine groups around the chromophore

The PD/CID mass spectra of FA 18:1(9*Z*) derivatized with the two reagents (Figure S4) also reveal differences in product ion abundances. Significantly, the 3-I-AMPP$^+$ derivative shows a greater abundance of product ions associated with the charge tag itself (*i.e.*, *m/z* 169 and 183), while the 4-I-AMPP$^+$ derivative shows systematically greater product yields distributed across the acyl chain fragments (Figure S5). Given the potential advantage of the latter for FA structure determination, along with its much lower cost of production, the 4-I-AMPP$^+$ isomer was selected as the derivatization reagent for all subsequent investigations of FA structure determination.

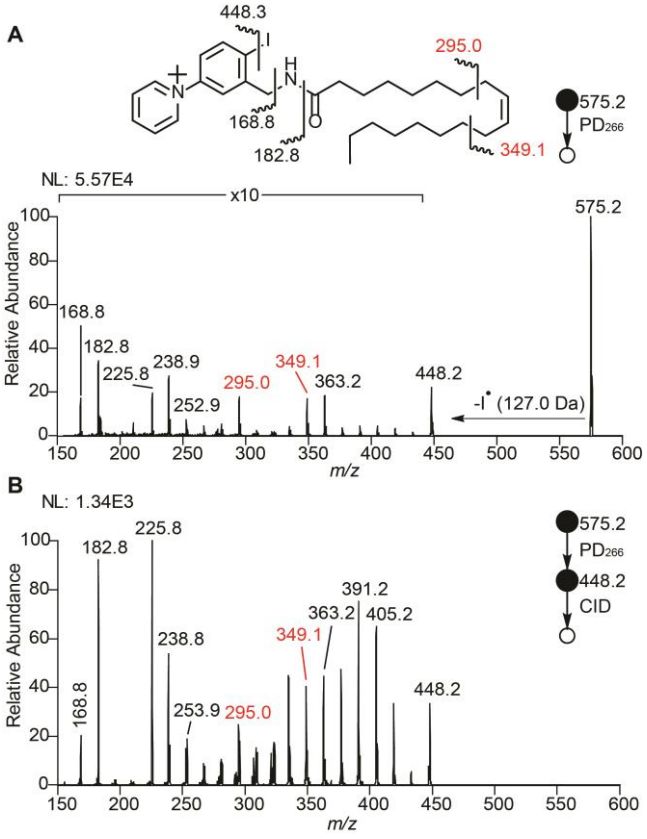

Figure 2: PD (MS^2) and PD/CID (MS^3) mass spectra of FA 18:1(9*Z*) derivatized with 4-I-AMPP$^+$. (A) PD spectrum of precursor ion *m/z* 575 and (B) PD/CID spectrum of the radical cation *m/z* 448 using a normalized collision energy of 7. Spectra represent an average of 80 individual scans with normalization limit (NL) indicative of the total ion count for each spectrum.

To establish the utility of this strategy for locating the carbon-carbon double bond in unsaturated lipids, 4-I-AMPP$^+$ derivatives of the isomeric fatty acids FA 18:1(11*Z*), FA 18:1(8*Z*) and FA 18:1(6*Z*) were prepared and subjected to PD. The PD mass spectra (Figure 3) show pairs of more abundant acyl-chain product ions at *m/z* 377 and *m/z* 323, *m/z* 335 and *m/z* 281, and *m/z* 307 and *m/z* 253, consistent with the Δ^{11}, Δ^8 and Δ^6 positioning of the double bonds, respectively. The characteristic 54 Da gap in the pattern of acyl chain fragment ions apparent in each case suggests this could serve as a characteristic marker of the double bond site in monounsaturated FAs, even when present in complex lipid mixtures.

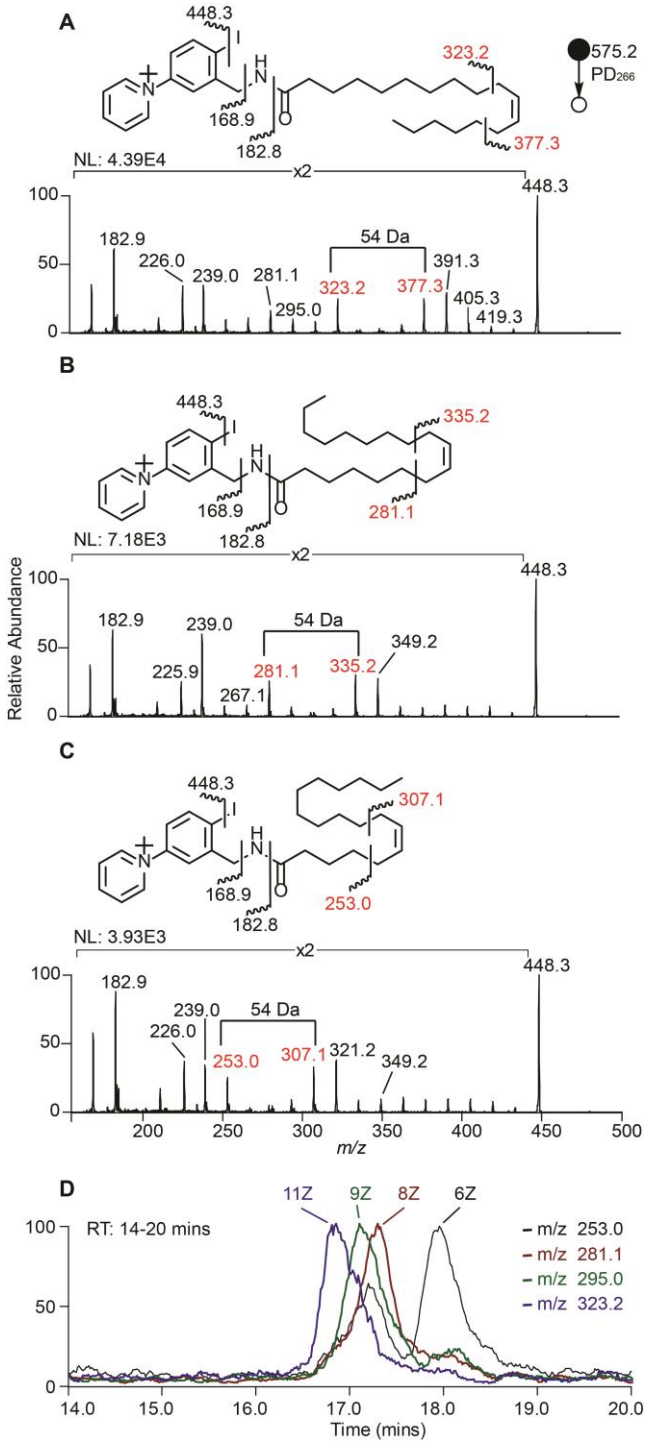

Figure 3: PD mass spectra of 4-I-AMPP$^+$ derivatives of the fatty acids: (A) FA 18:1(11*Z*), (B) FA 18:1(8*Z*) and (C) FA 18:1(6*Z*). (D) Extracted ion chromatograms (LC Method 1, see Supporting Information) of selected product ions of 4-I-AMPP$^+$ derivatives: FA 18:1(6*Z*) (*m/z* 253, black trace), FA 18:1(8*Z*) (*m/z* 281, red trace), FA 18:1(9*Z*) (*m/z* 295, green trace) and FA 18:1(11*Z*) (*m/z*

323, blue trace). Spectra shown in A-C were obtained by direct infusion of the derivatized FA. Each spectrum is an average of 80 scans.

To evaluate the compatibility of the PD approach with hyphenated LC-MS workflows, the individual standards and a mixture of all four isomeric 4-I-AMPP$^+$ FA 18:1 derivatives were injected sequentially onto a C30-reversed phase column and the eluent analyzed by PD in the ion-trap mass spectrometer. Extracted ion chromatograms (XICs) of the $[M\text{-}I]^{\bullet+}$ ion (*m/z* 448) reveal interesting trends in the relative retention times of the four isomers, with FA 18:1(11*Z*) eluting first, followed by FA 18:1(9*Z*), FA 18:1(8*Z*) and FA 18:1(6*Z*), suggesting a correlation between double bond position and retention on the C30 column (Figure S6). While overall the chromatographic resolution of the isomers is lower than can be achieved by GC, the isomer-specific fragmentation arising from PD was sufficient to discriminate between the isomers. For example, extracted ion chromatograms for specific product ions (*m/z* 253, 281, 295, and 323 for (6*Z*), (8*Z*), (9*Z*), and (11*Z*) respectively, Figure 3D) clearly identified each of the FA components in the isomeric mixture. The lower limit of detection for the I-AMPP$^+$ derivatized FAs was calculated to be 72 fmol on column (41 pg) based on the LC-PD response for serial dilutions of FA 18:1(9*Z*) (*i.e.*, peak area of the $[M\text{-}I]^{\bullet+}$ product ion, see Figure S15).

To assess the performance of the strategy across a wide range of saturated and unsaturated FAs a commercially available standard mixture of 37 FAMEs was hydrolyzed and derivatized with I-AMPP$^+$ prior to LC-MS analysis with ion activation by PD. Representative mass spectra are provided as Supporting Information and show that the same diagnostic fragmentation patterns identified for the FA 18:1 regioisomers (*i.e.*, 54 Da spacing at the site of unsaturation) can be identified in the PD mass spectra of mono-unsaturated FAs ranging in carbon-chain lengths from FA 14:1 to FA 24:1 (Figure S21). The same spectral patterns were observed in polyunsaturated FAs with 2-6 sites of unsaturation as indicated by the fragmentation diagrams provided in Supporting Information (Figure S21). Importantly, these experiments demonstrate that, as for the mono-unsaturated FAs, polyunsaturated FA isomers (*e.g.*, 18:3(6*Z*,9*Z*,12*Z*) and 18:3(9*Z*,12*Z*,15*Z*); FA 20:3(8*Z*,11*Z*,14*Z*) and 20:3(11*Z*,14*Z*,17*Z*)) can be distinguished by their PD mass spectra. The differences are sufficiently stark as to enable extracted ion chromatograms to visualize the elution profiles of each isomer despite incomplete chromatographic resolution. These results indicate that in both instances the *n*-3 PUFAs elute slightly earlier than their respective *n*-6 isomers (Figure S19 and S20). Future work is required to fully explore the discriminating power of this approach for even more complex polyunsaturated FAs (*e.g.*, conjugated polyenes).

Structure elucidation of cyclopropane FA

Some bacteria produce FAs incorporating cyclopropane rings within the acyl chain. Such cyclopropyl FAs (CFA) confer similar physicochemical properties to unsaturated FAs without the potential for chemical modification of the double bond, particularly under hostile conditions.[54] CFAs present an analytical challenge in that they are isomers of monounsaturated FAs. I-AMPP$^+$ derivatives of the two cyclopropane FAs CFA 19:0(9,10cp) and CFA 19:0(11,12cp) were prepared along with the isomeric monounsaturated fatty acid FA 19:1(10*Z*). The PD mass spectra of the three isomers obtained by direct infusion are presented in Figure 4. The PD mass spectrum of derivatized FA 19:1(10*Z*) (Figure 4A) is similar to those obtained from the other monounsaturated FAs (*vide supra*), where a pair of abundant and diagnostic product ions at *m/z* 363 and 309 (Δm = 54 Da) are observed from cleavage of the two allylic bonds adjacent to the Δ^{10} double-bond position. Radical-directed dissociation of the CFA isomers results in chain cleavage on either side of the cyclopropane moiety, yielding a distinctive 40 Da spacing between *m/z* 349 and 309 for CFA 19:0(9,10cp) (Figure 4B) and between *m/z* 363 and 323 for CFA 19:0(11,12cp) (Figure 4C). This distinctive fragmentation pattern provides a way of distinguishing the CFA isomers from each other and, importantly, a point of distinction between CFAs and isomeric monounsaturated FAs. Intriguingly, in addition to fragmentation adjacent to the cyclopropane motif, both CFAs show product ions consistent with cleavage across the carbocycle itself; a process that necessarily requires unimolecular rearrangement to facilitate cleavage of two carbon-carbon bonds. For example, the product ion at *m/z* 335 observed from PD of the I-AMPP$^+$ derivative of CFA 19:0(9,10cp) in Figure 4B can only be rationalized by cleavage of two of the endocyclic carbon-carbon bonds with retention of a bridging methylene on the charge-bearing product ion. Ring-opening of the strained cyclopropyl motif provides a driving force for this radical-driven unimolecular rearrangement and the characteristic product ion provides an additional point of spectral difference (Figure S9). LC-MS/MS analysis (LC Method 1, see Supporting Information) of the same series of derivatized FAs yielded high quality PD spectra across each chromatographic peak, which are represented as extracted ion chromatograms of diagnostic product ions (Figure S7).

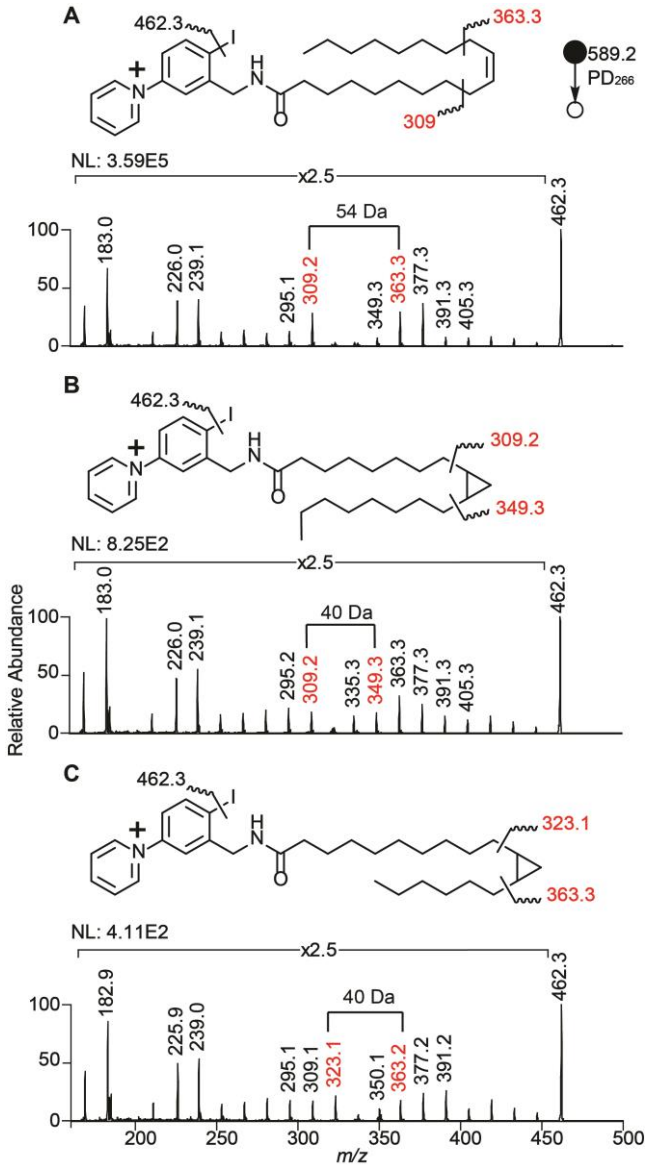

Figure 4: PD mass spectra of 4-I-AMPP$^+$ derivatives of (A) FA 19:1(10*Z*), (B) CFA 19:0(9,10cp), and (C) CFA 19:0(11,12cp). All spectra were derived from direct infusion and represent the average of 80 individual scans.

Structure elucidation of branched chain FAs

Branched chain FAs that incorporate one or more methyl groups along the acyl chain have been identified in some bacteria, in milk and in a range of human secretions, including *vernix caseosa* (covering newborn infants), sebum (secreted onto the skin) and meibum (produced in the eyelid and secreted into the tear film).[55-57] Differentiating methyl-branched FAs from straight chain isomers has not previously been demonstrated using conventional LC-MS ion activation modalities. To address this issue, straight chain FA 17:0 and two branched-chain variants, 15-methylhexadecanoic acid (FA 16:0(15Me)) and 14-methylhexadecanoic acid (FA 16:0(14Me)), were derivatized with 4-I-AMPP$^+$ and subjected to PD. The resulting PD mass spectra are displayed in Figure 5. The spectrum obtained from FA 17:0 displays an uninterrupted series of product ions between *m/z* 421 and 239 with 14 Da spacings (Figure 5A). In contrast, analysis of the analogous PD spectra of the branched-chain fatty acids FA 16:0(15Me) and FA 16:0(14Me) reveals a clear break from this sequence, with interruptions observed between *m/z* 421 and 393 (Figure 5B) and *m/z* 407 and 379 (Figure 5C), respectively. These 28 Da spacings are consistent with radical-directed cleavage of the acyl chain backbone on either side of the methyl-branch point, thus providing characteristic markers for the presence of methyl branches at the penultimate carbon (*iso*) and one carbon further away from the methyl terminus (*anteiso*).

Each of the isomeric derivatives were subjected to LC-MS individually and as a mixture. Extracted ion chromatograms of the common PD product ion (*m/z* 183) are presented in Supporting Information (Figure S10). Injection of each individual standard indicated that both branched-chain variants eluted earlier than the straight chain isomer FA 17:0 (14.26 mins, Figure S10C). Partial resolution of the two branched chain isomers was also observed, with the *anteiso* FA 16:0(14Me) (13.32 mins, Figure S10E) eluting slightly earlier than the *iso* FA 16:0(15Me) (13.60 mins, Figure S10D). Diagnostic PD product ions could be used to further enhance the resolution of the closely eluting branched chain isomers. Extracted ion chromatograms of *m/z* 407 and 393 reveal the contributions from *anteiso* and *iso* FAs, respectively (Figure 5D). This radical-driven fragmentation thus provides a means to not only identify common branched-chain isomers but also to undertake *de novo* structural identification and discovery of branched-chain lipids in complex biological extracts.

Identification of branched chain FAs in complex extracts

Vernix caseosa is a white waxy secretion covering the skin of infants *in utero*.[58] It is comprised of an exceedingly complex mixture of lipids that has challenged conventional analysis. Prior GC-MS analysis of FAMEs derived from *vernix caseosa* identified chromatographic features indicative of at least ten isomers of FA 17:0, but no definitive structural elucidation was possible based on the accompanying EI mass spectra.[47] Herein, the triacylglycerol fraction from a *vernix caseosa* extract was saponified, derivatized with 4-I-AMPP$^+$ and subjected to LC-MS incorporating the PD protocols described above. Figure 6A shows an MS^2 XIC (*m/z* 436) corresponding to the [M-I]$^{\bullet+}$ PD product of derivatized 17:0 fatty acids. Retention time alignment with derivatized FA 17:0 standards (Figure S10) indicated that the latest eluting feature (labeled as **1** in Figure 6A) corresponded to the straight chain FA 17:0 archetype. Moreover, the PD mass spectrum obtained for **1** showed an excellent match to the straight-chain standard (Figure S11). PD mass spectra obtained from the extract across the broad chromatographic feature between 12.4 and 13.7 mins provide evidence for a range of methyl-branched FA 17:0 isomers. For example, spectra obtained at 13.7 mins (**2**, Figure S12A) and 13.3 mins (**4**, Figure S13A) show a decrease in the peak intensity ratios I_{407}/I_{393} and I_{393}/I_{379}, respectively. Taken together with the alignment of these retention times with standards (*cf.* Figure 5D), this result indicates that both *iso* and *anteiso* variants of FA 17:0 are present in the extract, with the latter being more abundant.

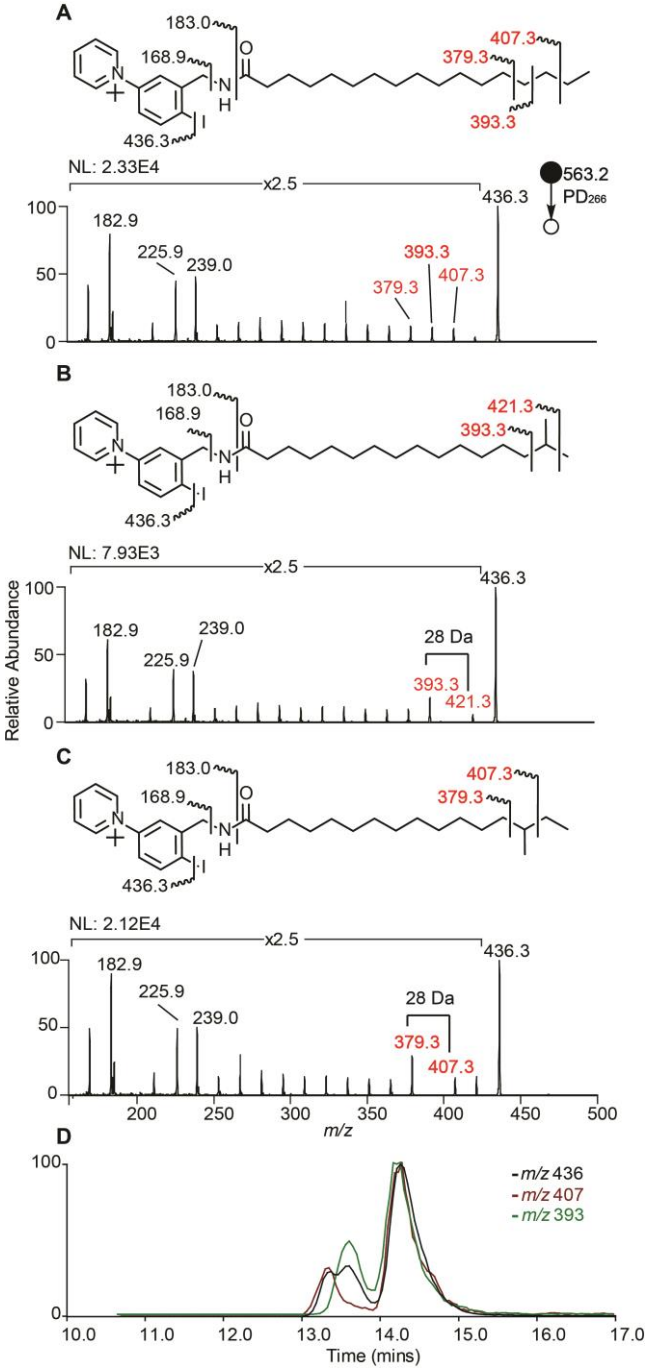

Figure 5: PD mass spectra of 4-I-AMPP$^+$ derivatives of (A) straight-chain FA 17:0, (B) *iso* branched-chain FA 16:0(15Me) and (C) *anteiso* branched-chain FA 16:0(14Me). (D) Extracted ion chromatograms (LC Method 2, see Supporting Information) of diagnostic PD product ions arising from methyl branching in a mixture of isomers; *m/z* 436 ([M-I]$^{\bullet+}$, black trace), *m/z* 407 (red trace), *m/z* 393 (green trace). Spectra A-C were obtained from direct infusion and represent the average of 80 individual scans.

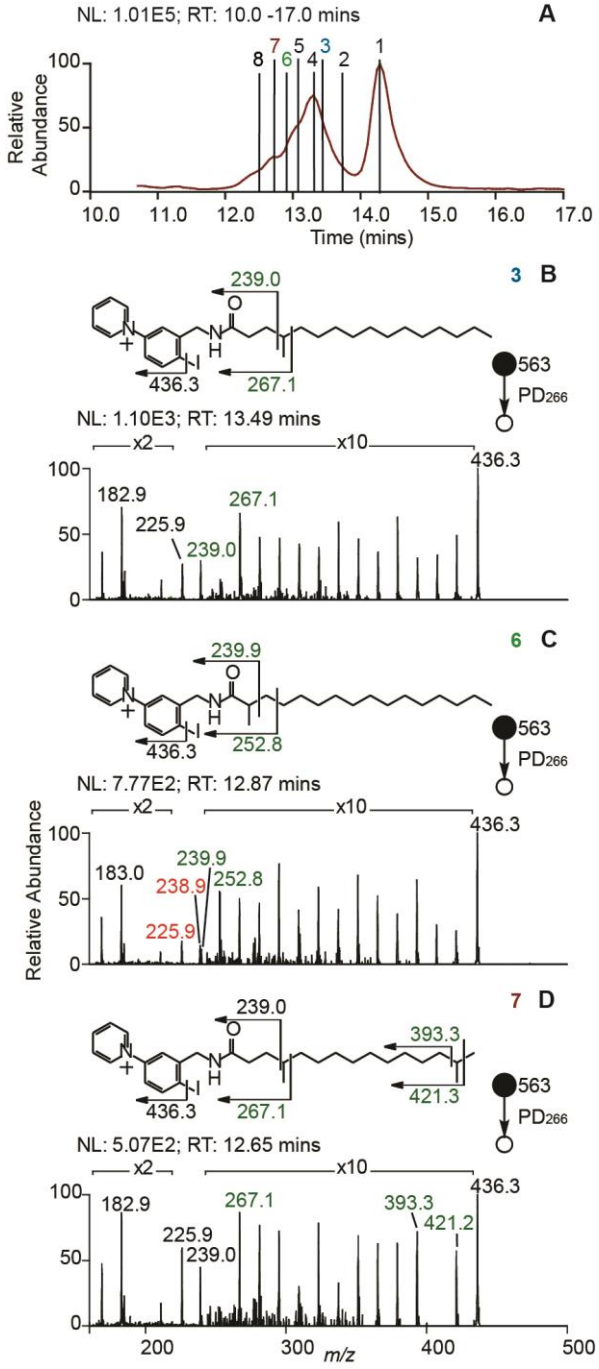

Figure 6: LC-MS analysis of 4-I-AMPP$^+$ derivatized *vernix caseosa*. (A) PD-based extracted ion chromatogram for [M-I]$^{\bullet+}$ for FA 17:0 (LC Method 2). PD mass spectra obtained at retention times of (B) 13.49 mins; (C) 12.87 mins; and (D) 12.65 mins are presented along with putative structural assignments. Mass spectra obtained at the remaining time points sequentially numbered in (A) are presented in Figure S11-14.

The PD mass spectra obtained at other time points across the chromatogram provide evidence for an even wider range of isomeric contributors. For example, spectra obtained at 13.49 mins (**3**, Figure 6B) and 12.87 mins (**6**, Figure 6C) exhibited distinctive product ion abundances at *m/z* 239, 240 and 253, consistent with the presence of FA 16:0(4Me) and FA 16:0(2Me), respectively. An analogous PD spectrum obtained from an ion eluting at 13.04 mins (**5**) is suggestive of FA 16:0(8Me) (Figure S14A).

Finally, PD mass spectra obtained at retention times consistent with apparent shoulders on the broad chromatographic feature (labelled **7** and **8** in Figure 6A) show fragmentation patterns consistent with dimethyl FAs. For example, the PD spectrum shown in Figure 6D reveals the complete absence of product ions at *m/z* 407 and 253, consistent with branch points at the 4- and 14-positions on a 15-carbon chain. These spectra, combined with shorter retention times than the mono-methylated isomers, provide evidence for the doubly-branched FA

15:0(4Me,14Me) and FA 15:0(8Me,14Me) within the extract (Figure S14B). Some of these branched chain isomers have previously been identified in *vernix caseosa* based on GC-MS analysis of methyl or picolinyl ester derivatives of fatty acids.[56, 59-61] These data highlight the structural diversity of fatty acids present within this wax secretion and that the methods developed here have broad application potential for FA analysis even where reference compounds are unavailable.

CONCLUSION

We have developed dual-function derivatization reagents for improving FA detection and structure elucidation. The I-AMPP$^+$ derivatives incorporate a fixed positive charge to enhance sensitivity in LC-MS workflows and a selectively photoactivated aryl-iodide moiety to facilitate the generation of structurally diagnostic ions *via* radical-directed dissociation. The photoproduct yield from PD of I-AMPP$^+$ derivatized FAs is 28 and 40% for 4- and 3-I-AMPP$^+$, respectively. Deploying the derivatives across a diverse set of FA structures yields PD mass spectra that show characteristic radical-driven fragmentation patterns, which are analogous to EI mass spectra of FAs modified as picolinyl esters and DMOX derivatives.[11, 14] These rich PD fragmentation patterns reveal modifications along the acyl chain, including unsaturation, chain branching and cyclopropanation. Moreover, these spectra distinguish regioisomers that differ only in the location of these structural features.

Characteristic fragmentation patterns are obtained for isomeric FAs on an LC-MS timescale, including in complex mixtures (*cf. vernix caseosa*). Future efforts could improve the yield of diagnostic PD product ions by synthetic modification of the I-AMPP$^+$ scaffold to maximize absorption overlap at 266 nm, or through use of shorter wavelengths (*e.g*., 213 nm) on commercially available PD-MS systems.

While LC-MS workflows cannot match the resolving power of GC, the I-AMPP$^+$ derivatives described here provide rich, structurally-sensitive fragmentation chemistry that identifies isomeric fatty acids in LC-MS analyses even when chromatographic resolution is incomplete. Moreover, the approach provides access to this structural information across a wide dynamic range, and is suitable for high molecular weight (non-volatile) compounds incompatible with GC-MS analysis. As an example, the I-AMPP$^+$ derivative was recently deployed in the structure elucidation of ultra-long chain *O*-acyl hydroxy fatty acids derived from human meibomian gland secretions.[21] In this case, PD mass spectra gave rise to radical-directed dissociation at all carbon-carbon bonds along both ester-linked chains. This near-complete "sequence coverage" was significantly greater than was achieved by CID of the conventional fixed charge AMPP$^+$ derivative for the same molecule. Thus, the PD strategy combined with I-AMPP$^+$ underpinned the first comprehensive structure assignment of these ultra-long chain lipids.

ASSOCIATED CONTENT

Supporting Information

Full experimental details including the synthesis and characterization of I-AMPP$^+$ reagents. Additional mass spectra and chromatograms. Supporting Information is available as a PDF file free of charge on the ACS Publications website. An archive of all raw data files associated with this manuscript can be accessed at https://doi.org/10.25912/5cf0779d91d75.

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Author Contributions

S.J.B., A.J.T. and T.W.M. conceived and designed the project. V.R.N., N.R.B., R.A. and M.J.K. designed and synthesized the reagents. V.R.N. D.L.M. and B.L.J.P. performed the LC-MS. V.R.N., S.J.B. and N.R.B. wrote the manuscript. All authors reviewed the manuscript and have approved the final version.

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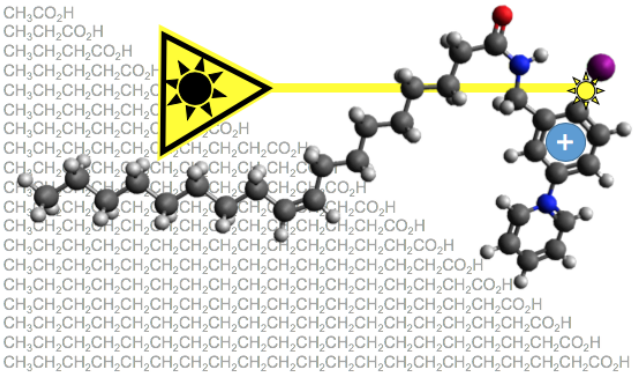
REFERENCES

1. Wenk, M. R., The emerging field of lipidomics. *Nat. Rev. Drug Discovery* **2005,** *4* (7), 594-610.
2. Fahy, E.; Subramaniam, S.; Brown, H. A.; Glass, C. K.; Merrill, A. H.; Murphy, R. C.; Raetz, C. R.; Russell, D. W.; Seyama, Y.; Shaw, W., A comprehensive classification system for lipids 1. *J. Lipid Res.* **2005,** *46* (5), 839-862.
3. Gurr, M. I.; Harwood, J. L.; Frayn, K. N., *Lipids: definition, isolation, separation and detection*. Blackwell Science: Oxford, 2002.
4. Shevchenko, A.; Simons, K., Lipidomics: coming to grips with lipid diversity. *Nat. Rev. Mol. Cell Biol.* **2010,** *11* (8), 593.
5. LIPID MAPS® Lipidomics Gateway. https://www.lipidmaps.org/ (accessed APR-2019).
6. Hancock, S. E.; Poad, B. L. J.; Batarseh, A.; Abbott, S. K.; Mitchell, T. W., Advances and unresolved challenges in the structural characterization of isomeric lipids. *Anal. Biochem.* **2017,** *524*, 45-55.
7. Eder, K., Gas chromatographic analysis of fatty acid methyl esters. *J. Chromatogr. B Biomed. Sci. Appl.* **1995,** *671* (1), 113-131.
8. Christie, W. W., *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids*. 4th ed.; The Oily Press: Oxford, 2010.
9. Aldai, N.; Murray, B. E.; Nájera, A. I.; Troy, D. J.; Osoro, K., Derivatization of fatty acids and its application for conjugated linoleic acid studies in ruminant meat lipids. *J. Sci. Food Agric.* **2005,** *85* (7), 1073-1083.
10. Hejazi, L.; Ebrahimi, D.; Guilhaus, M.; Hibbert, D. B., Discrimination among geometrical isomers of α-linolenic acid methyl ester using low energy electron ionization mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2009,** *20* (7), 1272-1280.
11. Dobson, G.; Christie, W. W., Structural analysis of fatty acids by mass spectrometry of picolinyl esters and dimethyloxazoline derivatives. *TrAC, Trends Anal. Chem.* **1996,** *15* (3), 130-137.
12. Destaillats, F.; Guitard, M.; Cruz-Hernandez, C., Identification of Δ6-monounsaturated fatty acids in human hair and nail samples by gas-chromatography–mass-spectrometry using ionic-liquid coated capillary column. *J. Chromatogr. A* **2011,** *1218* (52), 9384-9389.
13. Kramer, J. K. G.; Cruz-Hernandez, C.; Zhou, J., Conjugated linoleic acids and octadecenoic acids: Analysis by GC. *Eur. J. Lipid Sci. Technol.* **2001,** *103* (9), 600-609.
14. Harvey, D. J., Picolinyl derivatives for the characterization of cyclopropane fatty acids by mass spectrometry. *Biomed. Mass Spectrom.* **1984,** *11* (4), 187-192.
15. Fay, L.; Richli, U., Location of double bonds in polyunsaturated fatty acids by gas chromatography-mass spectrometry after 4, 4-dimethyloxazoline derivatization. *J. Chromatogr. A* **1991,** *541*, 89-98.
16. Yu, Q. T.; Liu, B. N.; Zhang, J. Y.; Huang, Z. H., Location of methyl branchings in fatty acids: fatty acids in uropygial secretion of Shanghai duck by GC-MS of 4, 4-dimethyloxazoline derivatives. *Lipids* **1988,** *23* (8), 804-810.
17. Harvey, D. J.; Tiffany, J. M., Identification of meibomian gland lipids by gas chromatography—mass spectrometry: application to the meibomian lipids of the mouse. *J. Chromatogr. A* **1984,** *301*, 173-187.

18. Blanksby, S. J.; Mitchell, T. W., Advances in mass spectrometry for lipidomics. *Annu. Rev. Anal. Chem.* **2010,** *3*, 433-465.
19. Ryan, E.; Reid, G. E., Chemical derivatization and ultrahigh resolution and accurate mass spectrometry strategies for "shotgun" lipidome analysis. *Acc. Chem. Res.* **2016,** *49* (9), 1596-1604.
20. Hirabayashi, T.; Anjo, T.; Kaneko, A.; Senoo, Y.; Shibata, A.; Takama, H.; Yokoyama, K.; Nishito, Y.; Ono, T.; Taya, C., PNPLA1 has a crucial role in skin barrier function by directing acylceramide biosynthesis. *Nat. Commun.* **2017,** *8*, 14609.
21. Hancock, S. E.; Ailuri, R.; Marshall, D. L.; Brown, S. H. J.; Saville, J. T.; Narreddula, V. R.; Boase, N. R.; Poad, B. L. J.; Trevitt, A. J.; Willcox, M. D. P.; Kelso, M. J.; Mitchell, T. W.; Blanksby, S. J., Mass spectrometry-directed structure elucidation and total synthesis of ultra-long chain (O-acyl)-ω-hydroxy fatty acids. *J. Lipid Res.* **2018,** *59* (8), 1510-1518.
22. Murphy, R. C., *Tandem mass spectrometry of lipids: molecular analysis of complex lipids*. The Royal Society of Chemistry: Cambridge, 2014.
23. Afonso, C.; Riu, A.; Xu, Y.; Fournier, F.; Tabet, J. C., Structural characterization of fatty acids cationized with copper by electrospray ionization mass spectrometry under low-energy collision-induced dissociation. *J. Mass Spectrom.* **2005,** *40* (3), 342-349.
24. Hsu, F.-F.; Turk, J., Distinction among isomeric unsaturated fatty acids as lithiated adducts by electrospray ionization mass spectrometry using low energy collisionally activated dissociation on a triple stage quadrupole instrument. *J. Am. Soc. Mass Spectrom.* **1999,** *10* (7), 600-612.
25. Zehethofer, N.; Pinto, D. M.; Volmer, D. A., Plasma free fatty acid profiling in a fish oil human intervention study using ultra-performance liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2008,** *22* (13), 2125-2133.
26. Adams, J.; Gross, M. L., Tandem mass spectrometry for collisional activation of alkali metal-cationized fatty acids: a method for determining double bond location. *Anal. Chem.* **1987,** *59* (11), 1576-1582.
27. Randolph, C. E.; Foreman, D. J.; Betancourt, S. K.; Blanksby, S. J.; McLuckey, S. A., Gas-Phase Ion/Ion Reactions Involving Tris-Phenanthroline Alkaline Earth Metal Complexes as Charge Inversion Reagents for the Identification of Fatty Acids. *Anal. Chem.* **2018,** *90* (21), 12861-12869.
28. Li, X.; Franke, A. A., Improved LC− MS Method for the Determination of Fatty Acids in Red Blood Cells by LC− Orbitrap MS. *Anal. Chem.* **2011,** *83* (8), 3192-3198.
29. Johnson, D. W.; Trinh, M.-U.; Oe, T., Measurement of plasma pristanic, phytanic and very long chain fatty acids by liquid chromatography-electrospray tandem mass spectrometry for the diagnosis of peroxisomal disorders. *J. Chromatogr. B* **2003,** *798* (1), 159-162.
30. Johnson, D. W., Alkyldimethylaminoethyl ester iodides for improved analysis of fatty acids by electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2000,** *14* (21), 2019-2024.
31. Johnson, D. W., Dimethylaminoethyl esters for trace, rapid analysis of fatty acids by electrospray tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **1999,** *13* (23), 2388-2393.
32. Yang, W.-C.; Adamec, J.; Regnier, F. E., Enhancement of the LC/MS analysis of fatty acids through derivatization and stable isotope coding. *Anal. Chem.* **2007,** *79* (14), 5150-5157.
33. Higashi, T.; Ichikawa, T.; Inagaki, S.; Min, J. Z.; Fukushima, T.; Toyo'oka, T., Simple and practical derivatization procedure for enhanced detection of carboxylic acids in liquid chromatography–electrospray ionization-tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2010,** *52* (5), 809-818.
34. Bollinger, J. G.; Thompson, W.; Lai, Y.; Oslund, R. C.; Hallstrand, T. S.; Sadilek, M.; Turecek, F.; Gelb, M. H., Improved sensitivity mass spectrometric detection of eicosanoids by charge reversal derivatization. *Anal. Chem.* **2010,** *82* (16), 6790-6796.
35. Bollinger, J. G.; Rohan, G.; Sadilek, M.; Gelb, M. H., LC/ESI-MS/MS detection of FAs by charge reversal derivatization with more than four orders of magnitude improvement in sensitivity. *J. Lipid Res.* **2013,** *54* (12), 3523-3530.
36. Wang, M.; Han, R. H.; Han, X., Fatty acidomics: global analysis of lipid species containing a carboxyl group with a charge-remote fragmentation-assisted approach. *Anal. Chem.* **2013,** *85* (19), 9312-9320.
37. Yang, K.; Dilthey, B. G.; Gross, R. W., Identification and quantitation of fatty acid double bond positional isomers: a shotgun lipidomics approach using charge-switch derivatization. *Anal. Chem.* **2013,** *85* (20), 9742-9750.
38. Hsu, F.-F., Characterization of hydroxyphthioceranoic and phthioceranoic acids by charge-switch derivatization and CID tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2016,** *27* (4), 622-632.
39. Frankfater, C.; Jiang, X.; Hsu, F.-F., Characterization of Long-Chain Fatty Acid as N-(4-Aminomethylphenyl) Pyridinium Derivative by MALDI LIFT-TOF/TOF Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2018**, 1-12.
40. Tatituri, R. V.; Wolf, B. J.; Brenner, M. B.; Turk, J.; Hsu, F.-F., Characterization of polar lipids of Listeria monocytogenes by HCD and low-energy CAD linear ion-trap mass spectrometry with electrospray ionization. *Anal. Bioanal. Chem.* **2015,** *407* (9), 2519-2528.
41. Ly, T.; Julian, R. R., Residue-specific radical-directed dissociation of whole proteins in the gas phase. *J. Am. Chem. Soc.* **2008,** *130* (1), 351-358.
42. Pham, H. T.; Ly, T.; Trevitt, A. J.; Mitchell, T. W.; Blanksby, S. J., Differentiation of complex lipid isomers by radical-directed dissociation mass spectrometry. *Anal. Chem.* **2012,** *84* (17), 7525-7532.
43. Pham, H. T.; Trevitt, A. J.; Mitchell, T. W.; Blanksby, S. J., Rapid differentiation of isomeric lipids by photodissociation mass spectrometry of fatty acid derivatives. *Rapid Commun. Mass Spectrom.* **2013,** *27* (7), 805-815.
44. Pham, H. T.; Julian, R. R., Characterization of glycosphingolipid epimers by radical-directed dissociation mass spectrometry. *Analyst* **2016,** *141* (4), 1273-1278.
45. Pham, H. T.; Julian, R. R., Mass shifting and radical delivery with crown ether attachment for separation and analysis of phosphatidylethanolamine lipids. *Anal. Chem.* **2014,** *86* (6), 3020-3027.
46. Liebisch, G.; Vizcaíno, J. A.; Köfeler, H.; Trötzmüller, M.; Griffiths, W. J.; Schmitz, G.; Spener, F.; Wakelam, M. J., Shorthand notation for lipid structures derived from mass spectrometry. *J. Lipid Res.* **2013**, 1523-1530.
47. Míková, R.; Vrkoslav, V.; Hanus, R.; Háková, E.; Hábová, Z.; Doležal, A.; Plavka, R.; Coufal, P.; Cvačka, J., Newborn boys and girls differ in the lipid composition of vernix caseosa. *PloS One* **2014,** *9* (6), e99173.
48. Kalužíková, A.; Vrkoslav, V.; Harazim, E.; Hoskovec, M.; Plavka, R.; Buděšínský, M.; Bosáková, Z.; Cvačka, J., Cholesteryl esters of ω-(O-acyl)-hydroxy fatty acids in vernix caseosa. *J. Lipid Res.* **2017,** *58* (8), 1579-1590.
49. Harazim, E.; Vrkoslav, V.; Buděšínský, M.; Harazim, P.; Svoboda, M.; Plavka, R.; Bosakova, Z.; Cvačka, J., Nonhydroxylated 1-O-acylceramides in vernix caseosa. *J. Lipid Res.* **2018,** *59* (11), 2164-2173.
50. Ly, T.; Julian, R. R., Ultraviolet photodissociation: developments towards applications for mass-spectrometry-based proteomics. *Angew. Chem. Int. Ed.* **2009,** *48* (39), 7130-7137.
51. Julian, R. R., The mechanism behind top-down UVPD experiments: making sense of apparent contradictions. *J. Am. Soc. Mass Spectrom.* **2017,** *28* (9), 1823-1826.
52. Ryan, E.; Nguyen, C. Q. N.; Shiea, C.; Reid, G. E., Detailed structural characterization of sphingolipids via 193 nm ultraviolet photodissociation and ultra high resolution tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2017,** *28* (7), 1406-1419.
53. Williams, P. E.; Klein, D. R.; Greer, S. M.; Brodbelt, J. S., Pinpointing double bond and sn-positions in glycerophospholipids via hybrid 193 nm ultraviolet photodissociation (UVPD) mass spectrometry. *J. Am. Chem. Soc.* **2017,** *139* (44), 15681-15690.
54. Chang, Y. Y.; Cronan, J. E., Membrane cyclopropane fatty acid content is a major factor in acid resistance of Escherichia coli. *Mol. Microbiol.* **1999,** *33* (2), 249-259.

55. Kaneda, T., Iso-and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiol. Rev.* **1991,** *55* (2), 288-302.
56. Ran-Ressler, R. R.; Devapatla, S.; Lawrence, P.; Brenna, J. T., Branched chain fatty acids are constituents of the normal healthy newborn gastrointestinal tract. *Pediatr. Res.* **2008,** *64* (6), 605.
57. Chen, J.; Green-Church, K. B.; Nichols, K. K., Shotgun lipidomic analysis of human meibomian gland secretions with electrospray ionization tandem mass spectrometry. *Invest. Ophthalmol. Visual Sci.* **2010,** *51* (12), 6220-6231.
58. Visscher, M. O.; Narendran, V.; Pickens, W. L.; LaRuffa, A. A.; Meinzen-Derr, J.; Allen, K.; Hoath, S. B., Vernix caseosa in neonatal adaptation. *J. Perinatol.* **2005,** *25* (7), 440.
59. Hauff, S.; Vetter, W., Exploring the fatty acids of vernix caseosa in form of their methyl esters by off-line coupling of non-aqueous reversed phase high performance liquid chromatography and gas chromatography coupled to mass spectrometry. *J. Chromatogr. A* **2010,** *1217* (52), 8270-8278.
60. Nicolaides, N., The structures of the branched fatty acids in the wax esters of vernix caseosa. *Lipids* **1971,** *6* (12), 901-905.
61. Nicolaides, N.; Apon, J.; Wong, D. H., Further studies of the saturated methyl branched fatty acids of Vernix caseosa lipid. *Lipids* **1976,** *11* (11), 781-790.



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