In-Depth Lipidome Annotation Through an Operatively Simple Method Combining Cross-Metathesis Reaction and Tandem Mass Spectrometry

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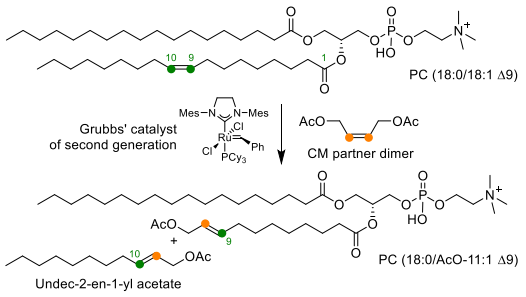
Abstract: The in-depth knowledge of lipid biological functions calls for a comprehensive lipid structure annotation that implies implementing a method to locate fatty acids unsaturations, which remains a thorny problem. To address this challenge we have associated Grubbs' cross-metathesis reaction and liquid chromatography hyphenated to tandem mass spectrometry. Thus the pretreatment of lipids-containing samples by Grubbs' catalyst and an appropriate alkene generates substituted lipids through cross-metathesis reaction under mild, chemoselective, and highly reproducible conditions. A systematic LC-MS/MS analysis of the reaction mixture allows locating unambiguously the double bonds in fatty acid side chains. This method has been successfully applied at a nanomole scale to commercial standard mixtures consisting of 10 lipid subclasses as well as in lipid extracts from an *in vitro* model of corneal toxicity.

Lipids are an essential class of metabolites playing a pleiotropic role in cells depending on their structures, the latter being actually very variable.[1,2] According to the international LipidMaps consortium, molecules arising from isoprene subunits condensation define the sterols or prenol lipid class, while lipids resulting from ketoacyl subunits condensation belong to the classes of fatty acids (FA), glycerolipids (GL), glycerophospholipids (PL) and sphingolipids (SL).[3,4] Within these latter classes, the structural diversity mainly originates from the nature of the fatty acids *i.e.* the chain length and the number and location of unsaturations. The location of double bonds in the FA chain is of prime relevance as in most cases it determines the biological activity of a given lipid.[5,6] For example, ω-3 polyunsaturated FAs (PUFA) display anti-inflammatory properties, while in contrast, ω-6 ones mainly act as pro-inflammatory metabolites. In ocular surface diseases, ω-3 PUFA has been shown to increase corneal wound healing and nerve regeneration through docosanoids biosynthesis.[7,8] Indeed, the use of ω-3-based therapy has been purposed specifically in dry eye disease, both topically and *per os*. Regarding mono-unsaturated fatty acid (MUFA) species, a shift from Δ9 to Δ11 C=C isomers in phosphatidylcholines (PC) and phosphatidylethanolamines (PE) containing oleic acid (18:1), has been reported in the plasma of type 2 diabetic patients.[9] Consequently, to clarify the role of lipids in physiological processes, it is indispensable to determine unambiguously the location of the double bond in FA chains of lipids.

For two decades, mass spectrometry (MS) has been increasingly used to study lipids.[10] Lipidomic analysis mainly uses liquid chromatography hyphenated to electrospray high resolution tandem mass spectrometry (LC-MS/MS).[11] Indeed, LC-MS/MS analysis is highly suitable to identify polar head groups, and therefore lipid subclasses but also FA chains.[11,12] However, LC-MS/MS alone can't determine position isomers when unsaturated FAs are involved,[13] unless using sophisticated analytical approaches implementing ion activation technics, such as ultraviolet-photodissociation (UVPD) or ozone-induced dissociation (OzID).[14,15] An online derivatization based on Paterno-Buchï (PB) photochemical reaction prior to MS analysis has also been successfully applied.[9,16] Nevertheless, these online sample preparation methods need technical adaptations of the mass spectrometer to be routinely used: UVPD, an hybrid MS^3 CID/UV mass spectrometer, OzID, the introduction of ozone within the collision cell and PB, the use of an UV lamp inside the ion source. Regarding offline sample preparation, a derivatization step based on an epoxidation reaction has been proposed to identify double bond lipid isomers.[17] Albeit very suitable for mono-unsaturated fatty acid (MUFA) species, this approach did not allow identifying double bond isomers in PUFA species readily. Furthermore, the above-mentioned methods implement harsh conditions that impede their chemoselectivity.

In contrary, cross-metathesis (CM) is a highly chemoselective, functional group tolerant reaction using mild conditions, so it appeared to us particularly suitable for lipidomic analysis. Indeed, biologically occurring MUFA and PUFA species contain one or several but-2-en-1,4-ylidene moieties, these alkenes therefore belong with category II in the Grubbs' CM classification.[18] They are thus expected to react readily with Ru carbene complexes such as the commercially available second-generation Grubbs' catalyst giving CM product easily. It can be anticipated that from the MS analysis of CM products the location of lipid double bonds would be easily and unequivocally determine (Scheme 1). In a previous study,

double bond location was determined in FA species using CM and mass spectrometry.[19] However, the method described in this study was designed for a use on pure FA samples at the mg scale, so it is sparsely adaptable to biological samples. The aim of our study was thus to explore the associating of CM and MS in order to devise a reproducible and routinely usable protocol allowing a universal identification of lipid isomers in biological samples, taking here, as example, a toxicological model of corneal damage.



Scheme 1. Cross-metathesis reaction applied to PC (18:0/18:1 Δ9).

We have first hypothesized that lipids could react with the Grubbs catalyst of second-generation under very mild conditions using (*Z*)-but-2-ene-1,4-diyl diacetate as CM partner. Indeed, this symmetrical alkene is highly reactive and allows preventing formation of ethylene and ethylene CM adducts. A first assay was performed at the mg scale with a commercial phospholipid. Briefly, 6.3 µmol (5.0 mg) of PC (18:0/18:1 Δ9) diluted in CH_2Cl_2 (0.5 ml) were treated at 40 °C for 2 hours with 5 equivalents of (*Z*)-but-2-ene-1,4-diyl diacetate in the presence of 5 mol% of Grubbs' catalyst. The reaction mixture was subsequently diluted (1/10,000) with MeOH and analyzed through direct infusion on a Synapt G2 (Q-ToF) high resolution mass spectrometer. The resulting mass spectrum displayed an intense peak at *m/z* 770.4953 corresponding to the $[M+Na]^+$ adduct PC (18:0/AcO-11:1 Δ9), the distal chain transformed into undec-2-en-1-yl acetate giving no signal (SI Figure S1). Therefore, the C10-C18 moiety of the unsaturated FA chain has been replaced through CM by a 2-acetoxyethyl-1-ylidene moiety.

Given that in a typical biological sample the overall content in phospholipids is of about tens of nmol for 10^6 cells, a scale down of our method was needed. However, at such a scale the concentration usually used in a CM reaction would require an amount of CH_2Cl_2 so small that it would anyway evaporate immediately at 40 °C. This led us implementing neat reaction conditions using *circa* one equivalent of the catalyst compared to the lipids and 50 equivalents of the CM partner. Using 2 nmol of the previous PC (18:0/18:1 Δ9), CM was performed as described here after. Briefly, Grubbs' catalyst (0.1 M) was pretreated with 50 equivalents of (*Z*)-but-2-ene-1,4-diyl diacetate in CH_2Cl_2 at 40 °C for 2 hours to afford a Ru carbene bearing the CM partner. This solution was diluted, then an appropriate volume was added to 2 nmol of PC (18:0/18:1 Δ9) and the solvent was subsequently removed under a flux of argon at 40 °C (SI Figure S2). The reaction mixture was then heated in a sealed tube at 40 °C for 2 hours, diluted in 100 µl of a $CHCl_3$/MeCN/H_2O/*i*-PrOH (20/30/10/30, v/v) mixture and 5 µL were injected in the LC-MS/MS system, the analysis being performed using previously described conditions.[20–22]

In positive ion mode, the MS extracted ion chromatograms (EIC) at *m/z* 748.5123 and *m/z* 788.618 showed a first peak at t_R = 4.1 min and a second one at t_R = 7.1 min corresponding to the $[M+H]^+$ of PC (18:0/OAc-11:1 Δ9) and native PC (18:0/18:1 Δ9), respectively (Figure 1A). The MS/MS spectra displayed an intense peak at *m/z* 184.07 characteristic of the PC polar head group (Figure 1B and 1C). In negative ion mode, the EIC at *m/z* 732.479 showed an intense peak assigned to the $[M-CH_3]^-$ PC (18:0/OAc-11:1 Δ9) confirming information obtained under positive ion conditions. In addition, negative ion mode is required to identify the FA side chains. Indeed, the MS/MS spectrum displayed at *m/z* 283.26 a peak diagnostic of stearate in sn_1 position and at *m/z* 241.1453, a second peak corresponding to 11-acetoxyundec-9-enoate (FA (OAc-11:1 Δ9)) in sn_2 position (Figure 1E). These product ions are indicative of an oleate in sn_2 position and therefore allowed to unambiguously locate the double bond.

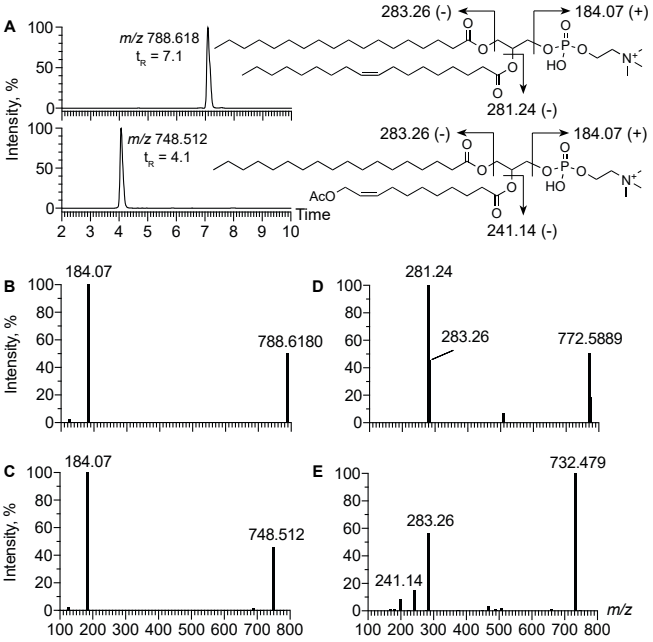


Figure 1. Analytical features of PC (18:0/18:1 Δ9) and its CM product PC (18:0/AcO-11:1 Δ9). (A) EIC at m/z 788.618 and m/z 748.5123 corresponding to [M+H]+ PC (18:0/18:1 Δ9) and PC (18:0/AcO-11:1 Δ9). PC (18:0/18:1 Δ9) and PC (18:0/AcO-11:1 Δ9) MS/MS spectra in (B, C) positive and (D, E) negative ion mode.

On one hand, it has been long established that the Grubb's catalysts are poisoned by basic amines.[23] On the other hand, among the phospholipid subclasses commonly encountered in biological samples, phosphatidylethanolamine (PE) and phosphatidylserine (PS), display basic primary amines functions. Accordingly, when a mixture of 20 commercially available standard phospholipids (SI Table S1) including PE and PS subclasses is submitted to our CM conditions, it gave a very poor yield of the expected CM products. Indeed, CM afford PC (18:0/OAc-11:1 Δ9) with approximatively 20% yield when it is performed on a pure PC (18:0/18:1 Δ9) solution and dramatically decreases to 5% when this PL is included in the standard lipid mixture (Figure 2B & 2C). To prevent the

deleterious effect of basic amines, the lipid mixture was pretreated with 1M HCl solution to give innocuous ammoniums.[23] CM performed under these conditions allowed to recover yields closed to those obtained with pure PC (18:0/18:1 Δ9) whatever the PL considered (Figure 2C). Thus, HCl pretreatment proves essential for CM reactions performed on lipid of biological lipid mixtures.

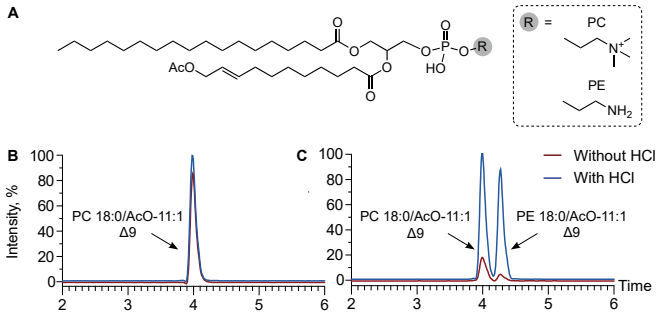


Figure 2. HCl pretreatment to prevent the inhibition of Grubbs' catalyst by amino groups. (A) CM product structures (B) EIC at m/z 748.5142 corresponding to the [M+H]+ ion of PC (18:0/AcO-11:1 Δ9) without and with HCl pretreatment in a reaction medium containing pure PC (18:0/18:1 Δ9) specie. (C) EIC at m/z 748.5142 and m/z 706.467 corresponding to the [M+H]+ ion of PC (18:0/AcO-11:1 Δ9) and PE (18:0/AcO-11:1 Δ9) without and with HCl pretreatment in a reaction medium containing a mixture of PC and PE species.

Our mixture of commercial lipid standards contains mono- and poly-unsaturated FA side chains representative of FAs commonly encountered in biological samples. While phospholipids such as PC (18:0/18:1 Δ9) lead to a single CM product, all C=C bonds present in phospholipids containing PUFA chain are expected to react leading to several CM products. Indeed, when CM was performed on PC (18:0/20:4 Δ5, Δ8, Δ11, Δ14) under the previous conditions, it led to 4 CM products: PC (18:0/OAc-16:4 Δ5, Δ8, Δ11, Δ14) (**C4**), PC (18:0/OAc-13:3 Δ5, Δ8, Δ11) (**C3**), PC (18:0/OAc-11:2 Δ5, Δ8) (**C2**) and PC (18:0/OAc-9:1 Δ5) (**C1**) (Figure 3A & 3B - SI Figure S3). Detecting simultaneously all possible CM products in the final reaction mixture is suitable as it allows locating all the double bonds and hence identifying phospholipids containing PUFA unambiguously. To ensure that the final reaction mixture contains all the CM products at concentrations letting optimal detection by mass spectrometry, a kinetic study was carried out by varying the temperatures with PC (18:0/20:4) and PC (16:0/20:4) (Figure 3D - 3G). Whatever the reaction time, chromatographic peaks corresponding to **C1** and **C4** products exhibited the highest and the lowest AUC compared to **C2** and **C3** (Figure 3D & 3E). As expected, in the course of the reaction, **C2**, **C3** and **C4** decreased in favor of **C1**. Moreover, at 4 hours of reaction at 40°C **C2**, **C3** and **C4** displayed suitable AUC and thus represents the best compromise to identify all the C=C bonds position (Figure 3D and 3E). In contrast, CM carried out at 60°C mainly led to **C1** products with a 4 times increased yield compared to 40°C, whatever the PC species (Figure 3F and 3G) while **C2**, **C3** and **C4** were sparsely detected in that case.

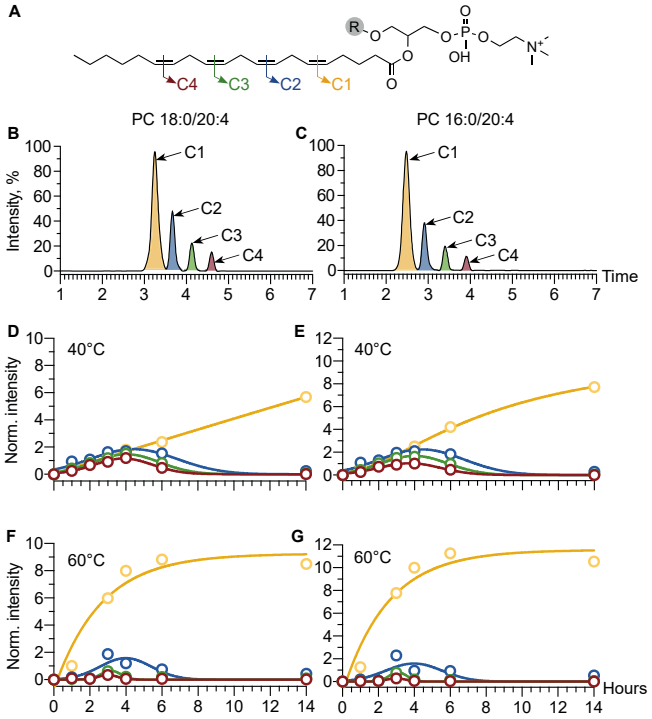


Figure 3. CM applied on polyunsaturated PC. (A) PC containing arachidonate chain. (B) EIC of CM products of PC (18:0/20:4 Δ5, Δ8, Δ11, Δ14) at m/z 812.5456, m/z 772.5141, m/z 732.4826 and m/z 692.4511 corresponding to [M+H]+ C4, C3, C2 and C1. (C) EIC of CM products of PC (16:0/20:4) at m/z 784.5142, m/z 744.4827, m/z 704.4512 and m/z 664.4197 corresponding to [M+H]+ ions of C4, C3, C2 and C1. CM kinetics on PC (18:0/20:4 Δ5, Δ8, Δ11, Δ14) and PC (16:0/20:4 Δ5, Δ8, Δ11, Δ14) at (D-E) 40°C and (F-G) 60°C.

Several phospholipids included in the standard lipid mixture contain two unsaturated FA side chains, and we expected CM to occur at both sn_1 and/or sn_2 position randomly (Figure 4B - 4D). This means that for instance PC (22:1/22:1 Δ13/Δ13) should give 3 CM products. Using this PC, we observed in positive ion mode that EIC at *m/z* 898.7252 and *m/z* 818.5181 displays two peaks at t_R = 8.1 min and t_R = 2.3 min corresponding to the $[M+H]^+$ ions of respectively native PC (22:1/22:1 Δ13/Δ13), and PC (OAc-15:1/OAc-15:1 Δ13/Δ13) issuing from a double CM process (Figure 4B). A third peak at *m/z* 858.6216 and t_R = 5.7 min is displayed; it corresponds to both PC (22:1/OAc-15:1 Δ13/Δ13) and its regio-isomer PC (OAc-15:1/22:1 Δ13/Δ13) which are almost co-eluted. However, a closer examination of the chromatographic peak located at t_R = 5.7 min reveals a doubling at t_R = 5.62 min and t_R = 5.72 min. Using the corresponding MS/MS spectra, it was possible to confirm the presence of the two regioisomers in both species, *i.e.* PC (22:1/OAc-15:1 Δ13/Δ13) and PC (OAc-15:1/22:1 Δ13/Δ13) respectively at t_R = 5.62 min at t_R = 5.72 min by calculating the ratio between peaks corresponding to the two fatty acid chains (SI Figure S4). On MS/MS spectra, an intense peak at *m/z* 184.07 corresponds to the PC polar head group (SI Figure S5). In negative ion mode, MS/MS spectrum exhibited peaks at *m/z* 337.31 and at *m/z* 297.21 due to respectively docos-13-enoate and 15-acetoxypentadec-13-enoate. While *m/z* 337.31 is due to native PC (22:1/22:1 Δ13/Δ13), and *m/z* 297.21 associated to CM modified PC which allows pinpointing the C=C bond (Figure 4E - 4G). Ring closure metathesis was also possible in that case, and interestingly PC

(22:1/22:1 Δ13/Δ13) gave a 30-membered ring product detected as a $[M+H]^+$ at *m/z* 646.45 at t_R = 3.4 min (Figure 4B).

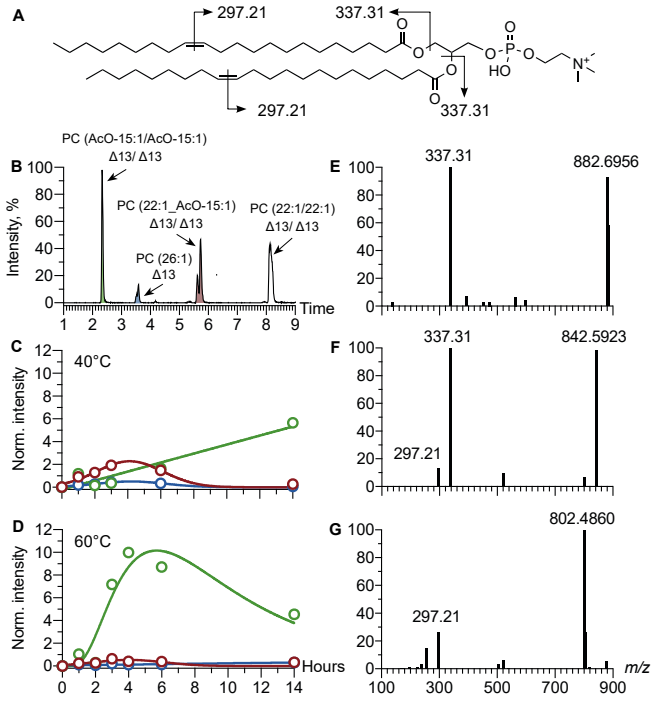


Figure 4. CM on phospholipids containing unsaturated FA chains both on sn_1 and sn_2 positions. (A) Structure of PC (22:1/22:1 13Z/13Z) and *m/z* value of product ions corresponding to FA chains of CM products in ESI- mode. (B) EIC at m/z 818.5180 (t_R = 2.3 min), m/z 646.4444 (t_R = 3.5 min), m/z 858.6216 (t_R = 5.7 min) and m/z 898.72 (t_R = 8.2 min) corresponding to PC (AcO-15:1/AcO-15:1 Δ13/Δ13), PC (26:1 13E), PC (22:1/ AcO-15:1 Δ13/Δ13) and native PC (22:1/22:1 Δ13/Δ13) respectively. Kinetic study of CM products of PC 22:1/22:1 Δ13/Δ13) at (C) 40°C and (D) 60°C. Negative ion mode MS/MS spectra of (E) PC (22:1/22:1 Δ13/Δ13), (F) PC (22:1/ AcO-15:1 Δ13/Δ13) and (G) PC (AcO-15:1/ AcO-15:1 Δ13/Δ13).

Within PC subclass, CM has thus been successfully applied to identify isomers of lipid species commonly encountered in biological samples *i.e.*, PL containing SFA/MUFA, SFA/PUFA and MUFA/MUFA side chains. The kinetic profiles we previously described for PC at 40 and 60°C were reproducibly observed for all lipid species whatever the phospholipid subclasses considered, *i.e.* PE, PS, PG and PI as well as with several sphingolipids and glycerolipids including Cer, SM, and DG, TG, respectively (Table S1 Figure S6-S25). Regarding the sensitivity of the method, CM was successfully applied on 10 pmol individual amount for species of the lipid standard mixture.

We previously reported that benzalkonium chloride (BAK), a quaternary ammonium, have a major impact on the lipidome of human corneal epithelial (HCE) cell line, however we could not determine double bound position of lipids at that time.[24,25] So this complex example appeared particularly relevant to assess the efficiency of our method. BAK is the more often encountered preservative in multidose eye drops which have long term indication for glaucoma and dry eye, two diseases deeply impacting quality of life.[26] HCE cells exposed to BAK is also a well-established toxicological model of corneal damage,[26–28] for which we previously demonstrated a striking decrease in PC species whatever the FA side chainsconsidered.[24,25] So we were delighted to see that our method succeeded at fully determining the structure of the 5 following PC species revealing a homeostasis change following BAK exposure. Indeed, in PC (18:1/18:1), FA side chains were identified as an oleyl (18:1 Δ9) and an octadec-11-enoyl (18:1 Δ11) (SI Figure S26). In PC (16:1/18:1) FA chains were identified as palmitoleyl (16:1 Δ9) and oleyl (18:1 Δ9) in sn_1 and sn_2, respectively. PC (16:0/18:1) and PC (16:0/16:1) contain oleyl (18:1 Δ9) and palmitoleyl (16:1 Δ9) in sn_2 position respectively. Finally, PC (18:0/20:3) contained an eicosatrienoyl (20:3 Δ5 Δ8 Δ11) in sn_2 position. Therefore, regarding PC subclass, our method has revealed BAK-induced changes mainly on lipid species containing ω-9 and ω-7 FA (Figure 5). It may thus be suggested that BAK induces ocular injury by targeting ω-9 and ω-7 FA. Indeed, stearoyl CoA desaturase (SCD), which is involved in the ω-9 FA metabolism, has been previously described as impacted by BAK exposure.[29] To confirm these results, the investigation of enzymes involved in FA elongation and desaturation metabolism remains to be carried out.

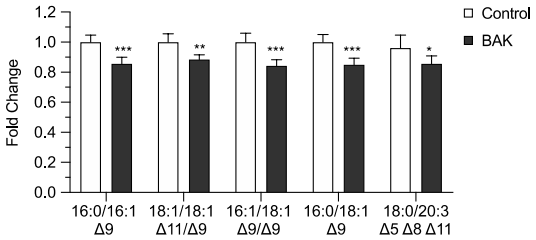


Figure 5. Effect of BAK on the cell content of PC species containing ω-9 and ω-7 FA. Results are expressed as mean fold change ± standard deviation compared to control (Multiple t-test with FDR rate at 0.01. n = 5. *p < 0.05; **p < 0.01; ***p < 0.001).

In summary, we have demonstrated that associating Grubbs catalyzed CM reaction and LC-MS/MS analysis allows an accurate identification of a broad scope of lipid isomers through an operatively simple and reproducible procedure. We have optimized conditions to obtain all the possible CM products enabling a full identification of MUFA and PUFA with a level of sensitivity allowing the analysis of biological samples. We are currently pursuing this study testing CM partners that could transfer an ionizable functional group allowing the identification of the distal moiety of FA chains. Furthermore, any sophisticated adaptation of the LC-MS system previously developed is needed to analyze complex biological lipid samples with our method. Regarding MS acquisition, both low- and high-resolution mass spectrometry are suitable and CM products exhibit same fragmentation patterns in MS/MS as native lipids facilitating identification. A remaining key challenge is the post-acquisition of the big amount of data in the case of biological sample. The data processing strategy is currently in progress and will be the subject of a dedicated study.

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[1] M. R. Wenk, *Cell* **2010**, *143*, 888–895.

[2] P. J. Meikle, S. A. Summers, *Nat. Rev. Endocrinol.* **2017**, *13*, 79–91.

[3] E. Fahy, S. Subramaniam, H. A. Brown, C. K. Glass, A. H. Merrill, R. C. Murphy, C. R. H. Raetz, D. W. Russell, Y. Seyama, W. Shaw, T. Shimizu, F. Spener, G. van Meer, M. S. VanNieuwenhze, S. H. White, J. L. Witztum, E. A. Dennis, *J. Lipid Res.* **2005**, *46*, 839–862.

[4] E. Fahy, S. Subramaniam, R. C. Murphy, M. Nishijima, C. R. H. Raetz, T. Shimizu, F. Spener, G. van Meer, M. J. O. Wakelam, E. A. Dennis, *J. Lipid Res.* **2009**, *50*, S9–S14.

[5] C. N. Serhan, J. Savill, *Nat. Immunol.* **2005**, *6*, 1191–1197.

[6] H. Martinez-Seara, T. Róg, M. Pasenkiewicz-Gierula, I. Vattulainen, M. Karttunen, R. Reigada, *Biophys. J.* **2008**, *95*, 3295–3305.

[7] W. C. Gordon, N. G. Bazan, *Curr. Eye Res.* **2013**, *38*, 995–1005.

[8] T. L. Pham, H. E. P. Bazan, *J. Lipid Res.* **2020**, jlr.TR120000954.

[9] W. Zhang, D. Zhang, Q. Chen, J. Wu, Z. Ouyang, Y. Xia, *Nat. Commun.* **2019**, *10*, 1–9.

[10] T. Züllig, M. Trötzmüller, H. C. Köfeler, *Anal. Bioanal. Chem.* **2019**, 2191–2209.

[11] J. Wang, C. Wang, X. Han, *Anal. Chim. Acta* **2019**, *1061*, 28–41.

[12] M. Holčapek, G. Liebisch, K. Ekroos, *Anal. Chem.* **2018**, *90*, 4249–4257.

[13] T. Porta Siegel, K. Ekroos, S. R. Ellis, *Angew. Chemie* **2019**, *131*, 6560–6569.

[14] D. R. Klein, J. S. Brodbelt, *Anal. Chem.* **2017**, *89*, 1516–1522.

[15] B. L. J. Poad, H. T. Pham, M. C. Thomas, J. R. Nealon, J. L. Campbell, T. W. Mitchell, S. J. Blanksby, *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1989–1999.

[16] X. Ma, Y. Xia, *Angew. Chemie - Int. Ed.* **2014**, *53*, 2592–2596.

[17] T. H. Kuo, H. H. Chung, H. Y. Chang, C. W. Lin, M. Y. Wang, T. L. Shen, C. C. Hsu, *Anal. Chem.* **2019**, *91*, 11905–11915.

[18] A. K. Chatterjee, T. L. Choi, D. P. Sanders, R. H. Grubbs, *J. Am. Chem. Soc.* **2003**, *125*, 11360–11370.

[19] Y. Kwon, S. Lee, D.-C. Oh, S. Kim, *Angew. Chemie* **2011**, *123*, 8425–8428.

[20] J. Lanzini, D. Dargère, A. Regazzetti, A. Tebani, O. Laprévote, N. Auzeil, *Biochimie* **2015**, *118*, 234–243.

[21] J. Petit, A. Wakx, S. Gil, T. Fournier, N. Auzeil, P. Rat, O. Laprévote, *Biochimie* **2018**, *149*, 1–8.

[22] R. Magny, A. Regazzetti, K. Kessal, G. Genta-Jouve, C. Baudouin, S. Mélik-Parsadaniantz, F. Brignole-Baudouin, O. Laprévote, N. Auzeil, *Metab. 2020, Vol. 10, Page 225* **2020**, *10*, 225.

[23] C. P. Woodward, N. D. Spiccia, W. R. Jackson, A. J. Robinson, *Chem. Commun.* **2011**, *47*, 779–781.

[24] R. Magny, K. Kessal, A. Regazzetti, A. Ben Yedder, C. Baudouin, S. M. Parsadaniantz, F. Brignole-Baudouin, O. Laprévote, N. Auzeil, *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2020**, 158728.

[25] R. Magny, N. Auzeil, E. Olivier, K. Kessal, A. Regazzetti, M. Dutot, S. Mélik-Parsadaniantz, P. Rat, C. Baudouin, O. Laprévote, F. Brignole-Baudouin, *Biochimie* **2020**, DOI 10.1016/j.biochi.2020.07.015.

[26] C. Baudouin, A. Labbé, H. Liang, A. Pauly, F. Brignole-Baudouin, *Prog. Retin. Eye Res.* **2010**, *29*, 312–334.

[27] F. Brignole-Baudouin, N. Desbenoit, G. Hamm, H. Liang, J. P. Both, A. Brunelle, I. Fournier, V. Guerineau, R. Legouffe, J. Stauber, D. Touboul, M. Wisztorski, M. Salzet, O. Laprevote, C. Baudouin, *PLoS One* **2012**, *7*, DOI 10.1371/journal.pone.0050180.

[28] M. De Saint Jean, C. Debbasch, F. Brignole, P. Rat, J. M. Warnet, C. Baudouin, *Curr. Eye Res.* **2000**, *20*, 85–94.

[29] M. Lee, K. M. Joo, S. Choi, S. H. Lee, S. Y. Kim, Y. J. Chun, D. Choi, K. M. Lim, *Toxicol. Vitr.* **2018**, *47*, 94–102.