Chinese Chemical Letters 35 (2024) 108775

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Visible-light-activated aziridination reaction enables simultaneous resolving of C=C bond location and the *sn*-position isomers in lipids

Qiongqiong Wan[1], Yanan Xiao[1], Guifang Feng, Xin Dong, Wenjing Nie, Ming Gao, Qingtao Meng*, Suming Chen*

The Institute for Advanced Studies, Renmin Hospital of Wuhan University, Wuhan University, Wuhan 430072, China

A R T I C L E I N F O

Article history:
Received 4 March 2023
Revised 13 June 2023
Accepted 5 July 2023
Available online 6 July 2023

Keywords:
Aziridination
Lipid isomers
C=C bond location
sn-Position
Mass spectrometry

A B S T R A C T

There is a close relationship between the biological functions of lipids and their structures, and various isomers greatly increases the complexity of lipid structures. The C=C bond location and *sn*-position are two of the essential attributes that determine the structures of unsaturated lipids. However, simultaneous identification of both attributes remains challenging. Here, we develop a visible-light-activated aziridination reaction system, which enables the dual-resolving of the C=C bond location and *sn*-position isomerism of in lipids when combines with liquid chromatography-mass spectrometry (LC-MS). Based on the derivatization of C=C bonds with PhI=NTs, their location in lipids could be easily identified by tandem MS. Especially, the *sn*-position isomers of unsaturated phosphatidylcholine (PC) can be separated and quantified by LC-MS after the derivatization. By using the proposed method, the significant changes of the *sn*-position isomers ratios of PC in mouse brain ischemia were revealed. This study offers a powerful tool for deep lipid structural biology.

Lipids is a kind of organic molecule that are very important to living organisms [1]. They are not only a component of cell membrane, but also plays an important role in signal transduction, coordination of metabolism and energy storage [1,2]. More and more studies have indicated the alteration of lipid metabolism is closely related to various diseases, such as tumor, diabetes, and cardiovascular diseases [2–6]. Unsaturated lipids are those with single or multiple C=C bonds on the fatty acyl chains, and they have different kinds of isomers that endows lipids with different structures and functions in living organisms [7–9]. For example, the position of the C=C bonds on fatty acyl chains gives rise to numerous isomers of unsaturated lipids [10–13]. In addition, a large number of *sn*-position isomers were also generated due to the different order of fatty acyl chains attached to the glycerol backbone in glycerides [14,15]. The biosynthesis and metabolism of lipid isomers are associated with many important biological processes and, as such, changes in these isomers have been found to be closely associated with many diseases [15,16]. Thus, the resolving of C=C location and *sn*-position isomers of unsaturated lipids could provide multidimensional information for in-depth understanding of the relationship between their structures and biological roles [15].

Mass spectrometry (MS) has become an effective method for lipid isomer analysis because of its high sensitivity and structural resolution [5,17–19]. For the analysis of positional isomers of double bonds, accurate identification of the double bond positions on the lipid chain can be achieved by subjecting the double bonds to chemical activation followed by tandem MS analysis [12,13,20,21]. However, the identification of *sn*-position isomers is more difficult and usually requires special fragmentation methods with MS^3 pathways [14,15]. For example, ozone-induced dissociation [13], ultraviolet photodissociation [14], and specific fragmentation induced by phospholipid-ion complexes [15]. Although these studies have enriched the library of methods for the analysis of lipid structure, the simultaneous identification of the C=C location and *sn*-position in unsaturated lipids with simple workflow remain challenging.

In this work, we aim to develop a new strategy that allows the simultaneous resolving of the C=C bond location and *sn*-position isomers of unsaturated lipids (Fig. 1). We envision that the chemical derivatization for C=C bond position identification may increase the chromatographic retention differences between *sn*-position isomers if the structure and property of the introduced group differ significantly from those of the aliphatic chains, which would allow for the separation and analysis of the *sn*-position isomers. To achieve this goal, we developed a visible-light-activated

---

* Corresponding authors.
E-mail addresses: qtmeng@whu.edu.cn (Q. Meng), sm.chen@whu.edu.cn (S. Chen).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cclet.2023.108775

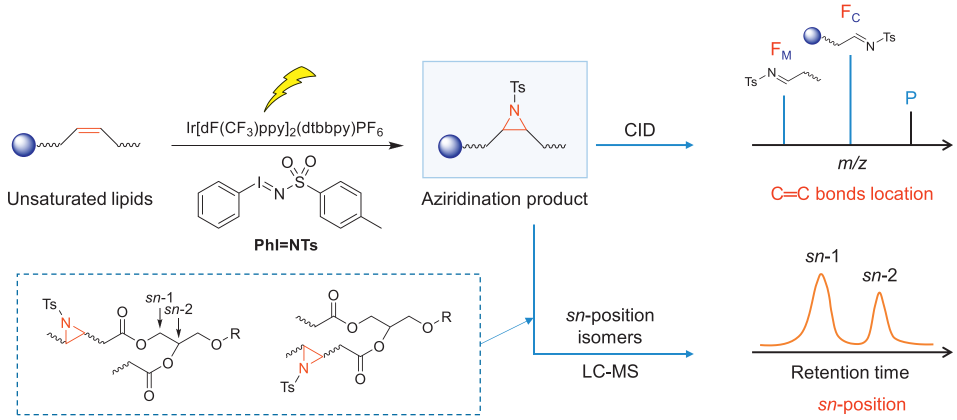

Fig. 1. Strategy for the simultaneous analysis the C=C bond location and *sn*-position isomers of unsaturated lipids with the aziridination derivatization. The derivative reagent PhI=NTs could react with unsaturated lipids under the photocatalysis of Ir[dF(CF$_3$)ppy]$_2$(dtbbpy)PF$_6$ to form the aziridination product. The product could be fragmented to the diagnostic ions to indicate the locations of C=C bonds *via* collision-induced dissociation in tandem MS. For the analysis of *sn*-position isomers, the derivatized products of isomers could be separated and analyzed by LC-MS.

aziridination reaction [22,23] that allows selective derivatization of the double bonds of unsaturated lipids, which subsequently allows fragmentation of the products using collision induced dissociation (CID) for C=C bond location identification. Meantime, the formed aziridine ring structure on one of the fatty acyl chains increases the separation resolution of the *sn*-position isomers by liquid chromatography (LC), allowing their qualitative and quantitative analysis. Using this method, we realized the identification of C=C bond locations of different types of unsaturated lipids, as well as the analysis of *sn*-position isomers of glycerophospholipids. Finally, the method was applied to the quantitative analysis of *sn*-isomers of phosphatidylcholine (PC) during cerebral ischemia in mice. We found that the process of cerebral ischemia was accompanied by significant changes in the proportion of PC *sn*-isomers.

The derivatization of C=C bonds in lipids typically form the O-containing ring structures [12,13,24]. However, the transferring of nitrenes to olefins to form N-containing three-membered ring by means of transition metal catalysts or photochemistry was also recognized well [22,23,25]. We reason that this aziridination reaction and the formed aziridine ring structure for unsaturated lipids could be adopted to identify the location of C=C bonds [26] and improve the separation of *sn*-position isomers when combined with LC-MS/MS. However, the previously reported reactions usually need the long reaction time or heating [22,25]. Therefore, we aim to develop a visible-light-activated rection system that could realize the aziridination of unsaturated lipids under accelerated and mild conditions.

We first synthesized the common nitrene precursor [(*N*-tosylimino)iodo]benzene (PhI=NTs) as derivatization reagents, and PC 16:0/18:1(9*Z*) as representative unsaturated lipids to screen the reaction systems under different photocatalysts. The blue light Kessil lamp (456 nm) was used to trigger the photoreaction. The aziridination product could be clearly observed in LC-MS analysis (Fig. S1 in Supporting information), which confirmed the visible-light-activated aziridination reaction indeed happened. After evaluating the efficiencies of different reaction systems, the photocatalyst Ir[dF(CF$_3$)ppy]$_2$(dtbbpy)PF$_6$ was chosen for the following experiments due to its highest efficiency (Fig. S2 in Supporting information).

To investigate the capability of the aziridination reaction for the identification of C=C bonds location, different kinds of lipid standards were used as examples. The lipid standards (100 µmol/L) first react with PhI=NTs under the photocatalysis for 3 min. Then, the reaction solution was subjected to the LC-MS/MS analysis (Fig. S3 in Supporting information). All the N-Ts aziridine products were observed that show a mass increase of 169 Da. Fig. 2 shows the MS/MS spectra of the formed aziridination products of oleic acid (FA 18:1(9*Z*)), linoleic acid (FA 18:2 (9*Z*)), DG 16:0/18:1(9*Z*), and PC 16:0/18:1(9*Z*). In a typical MS/MS spectrum, a pair of C=C diagnostic fragment ions generated by the cleavage of C–C bond and N–C bond in aziridine ring, denotated as F_M (containing Ts-N=C- unit and methyl end) and F_C (containing Ts-N=C- unit and carboxylic acid end), should be observed for each C=C (structures shown in Fig. 1).

For the analysis of oleic acid, both of the fragments containing methyl end (F_M, *m/z* 296.1684) and carboxylic acid end (F_C, *m/z* 326.1426) were observed in MS/MS spectrum of the product in positive ion mode, which clearly indicate the location of C=C bond at Δ9 position. In addition, the locations of C=C bonds in polyunsaturated linoleic acid FA 18:2 (9*Z*,12*Z*) could also be identified by this method. Interestingly, the products are mainly monosubstituted at each C=C bond that could simplify the identification. As shown in Fig. 2b, two pairs of the diagnostic fragment ions derived from the two products could be observed in the average MS/MS spectrum. The fragments at *m/z* 294.1530 and 326.1427 (F_M and F_C), and *m/z* 254.1212 and 366.1751 (F_M and F_C), unambiguously indicate the C=C bonds at Δ9 and Δ12, respectively. The 40 Da mass difference of fragments F_M (Δ9 *vs.* Δ12, *m/z* 294 and 254) and F_C (Δ9 *vs.* Δ12, *m/z* 366 and 326) also indicates the three-carbon shift (C_3H_4) of the C=C position.

The pinpointing of C=C bonds in glycerides and phosphoglyceride were also carried out. Figs. 2c and d show the MS/MS spectra of the aziridination products of PhI=NTs with DG 16:0/18:1(9*Z*) and PC 16:0/18:1 (9*Z*), respectively. Both of the diagnostic fragment ions (F_M at *m/z* 296.1682, F_C at *m/z* 620.3986) could be detected for DG, but the F_C fragment was observed with the loss of H_2O. For the analysis of PC 16:0/18:1(9*Z*), only the F_C ion at *m/z* 803.2641 could be observed after the CID, and the other F_M ion with the theoretical *m/z* of 296.1679 was not detected due to the limitation of ion trap for the low mass fragments. It is worth mentioning that the fragment ions derived from the loss of one of the intact fatty acyl chains were also observed. For example, *m/z* 691.3751 denotes the fragment ion that lost the 16:0 fatty acyl chain, while *m/z* 496.3399 denotes the fragment that lost the fatty acyl chain containing the N-Ts aziridine structure. This information is helpful to identify the length of fatty acyl chains and their degree of unsaturation in phospholipids.

To further investigate the generality of this aziridination reaction and demonstrate its capability in C=C bond position location, 17 more unsaturated lipids were taken for study (Table S1

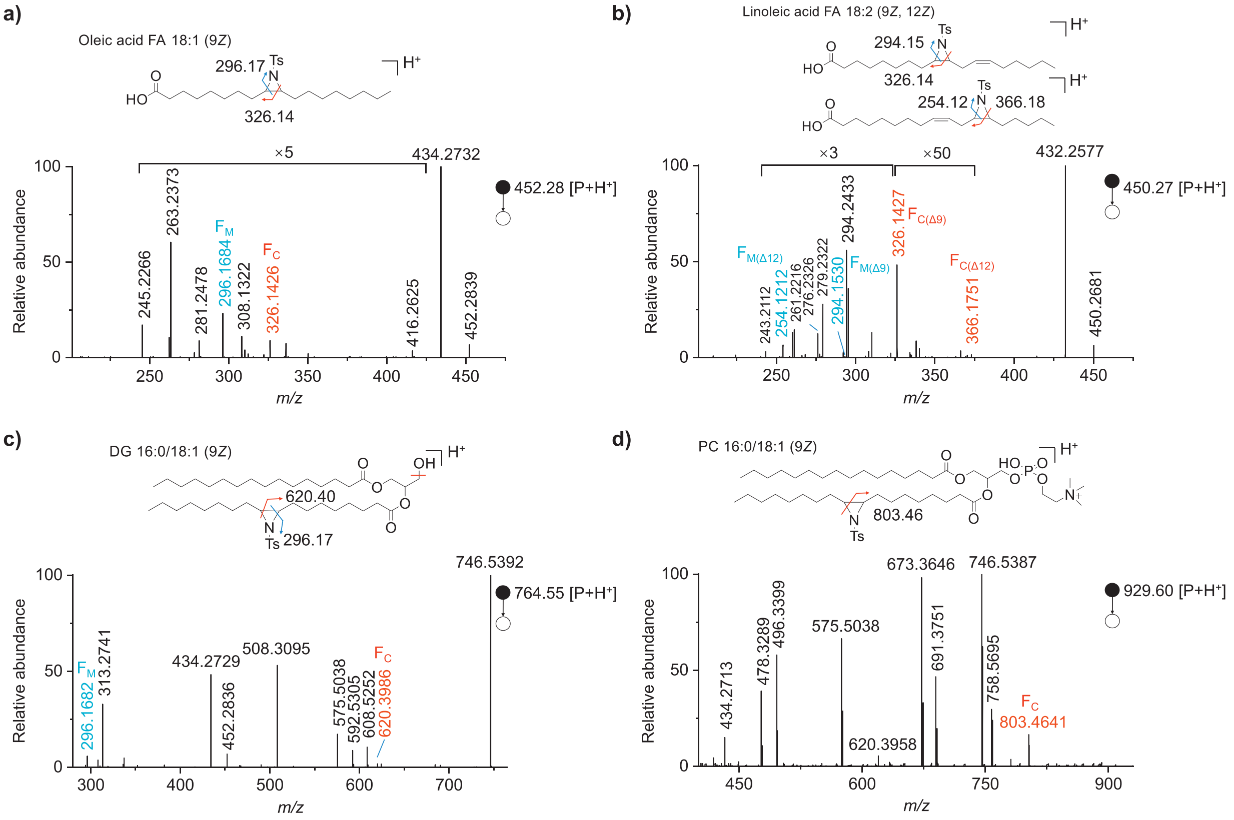

Fig. 2. MS/MS spectra of the derivatized products of PhI=NTs with different lipids: (a) Oleic acid (FA 18:1(9*Z*)), (b) linoleic acid (FA 18:2 (9*Z*,12*Z*)), (c) DG 16:0/18:1(9*Z*) and (d) PC 16:0/18:1(9*Z*). The chemical structures at the top of each MS/MS spectrum shows the positions of derivatized C=C bonds and the *m/z* values of the possible diagnostic fragment ions of reaction products *via* CID. The detailed dissociation pathways of these products are shown in Fig. S4 (Supporting information).

and Figs. S5-S21 in Supporting information), including monounsaturated and polyunsaturated fatty acids, PC, phosphatidic acid (PA), phosphatidylserine (PS), ceramide (Cer), triglyceride (TG), and lysophosphatidylethanolamine (LPE). The results show that all the locations of C=C bonds in these unsaturated lipids could be accurately identified, which indicates the wide applicability of the proposed method.

The above experiments have demonstrated the validity of this aziridination derivatization can help in the identification of double bond positions. However, for unsaturated glycerides, the quantification of the *sn*-position isomers is another challenge. We next want to examine whether the aziridination derivatization could benefit the analysis of *sn*-position isomers. The common pair of *sn*-position isomers PC 18:1(9*Z*)/16:0 and PC 16:0/18:1(9*Z*) was chosen as an example. The MS/MS spectra show that the two isomers have almost the same fragment ions, but the ratios between the neutral loss of the fatty acids as ketene $[M-RCH=C=O+H]^+$ and the neutral loss of free fatty acid $[M-RCH_2COOH+H]^+$ are different (Fig. S22 in Supporting information). In the case of the loss of the acyl group at *sn*-1, the abundances of $[M-RCH_2COOH+H]^+$ ions were higher than those of the corresponding $[M-RCH=C=O+H]^+$ ions, but, in the case of *sn*-2 substituents, the reverse was true. Although this information could be used to distinguish the *sn*-position isomers of PC, both of the isomers show almost the same retention time during chromatographic analysis (Fig. S23 in Supporting information). Thus, it is difficult to quantitatively analyze the isomer mixture directly. However, if the isomers were reacted with PhI=NTs, the resulting aziridination products can be well separated by LC (Fig. 3a). The possible reason is that the introduction of the N-Ts group greatly enhances the polarity of one of the fatty acyl chains, thus increasing the difference in affinity to the chromatographic stationary phase brought about by the difference in the *sn*-position of the fatty chains. The MS/MS spectra of the two products also follow the rules that the loss of fatty acid is more abundant than the loss of ketene in *sn*-1 position (Fig. 3b and Fig. S24 in Supporting information). More importantly, the location of C=C bond in the fatty acyl chain could be simultaneously identified. The F_C ion at *m/z* 803.4641 could be observed after the CID, which indicates the location of C=C bond at Δ9. The above results indicate that the combination of this aziridination derivatization and LC-MS/MS could provide an integrated solution for the identification of double bond positions and the analysis of *sn*-position isomers of unsaturated PCs.

To further examine the quantitative capability of this method, a series of mixtures of PC 18:1(9*Z*)/16:0 and PC 16:0/18:1(9*Z*) was quantitatively investigated (Fig. S25 in Supporting information). The ratios of the chromatographic peak areas of the isomers were found to be directly proportional to their molar ratios, with a good linearity ($R^2 = 0.9964$). Notably, the slope of the curve (0.94) is close to 1, which means the molar ratio of the two isomers could be directly obtained from the ratio of their peak areas. This result demonstrated the capability of the proposed method for the relative quantification of *sn*-position isomers. In this case, the pair of derivatized isomers were not baseline-separated. Although the peak fitting can aid in accurate quantification, the optimization of chromatographic conditions to achieve baseline separation of the isomers is still recommended.

Having validated the method for the qualitative and quantitative analysis the isomers of unsaturated lipids, we next want to apply the method to the analysis of *sn*-position isomers of PC in biological samples. The *sn*-position of the fatty acyl chain is closely related to the biosynthesis and metabolic processes of glycerides,

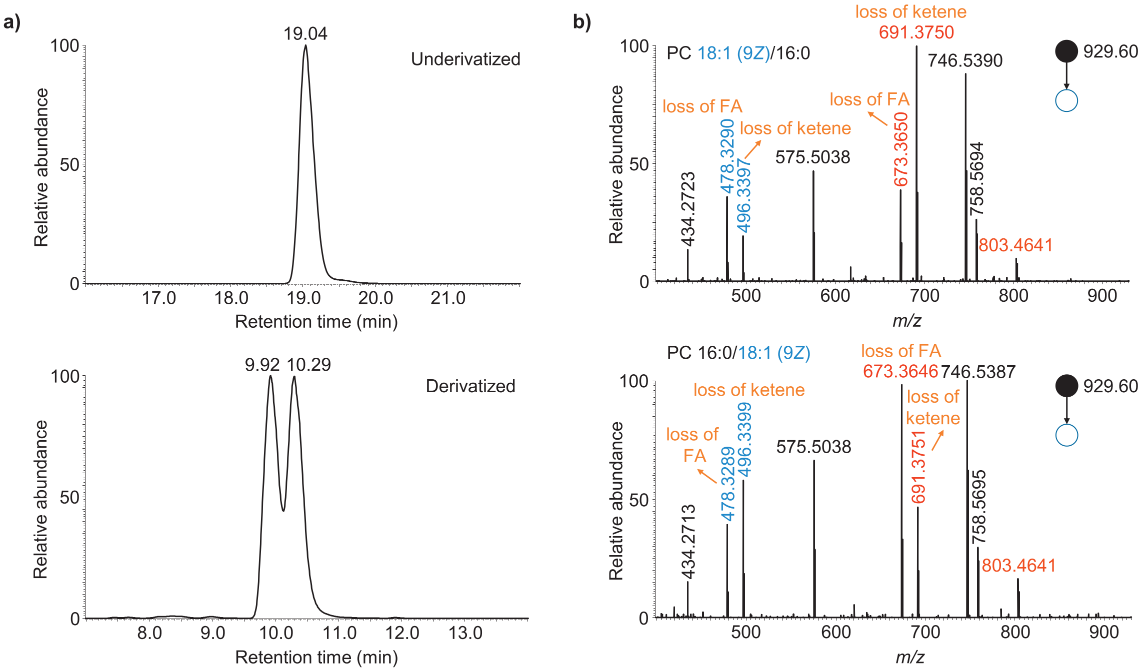

Fig. 3. Separation and analysis of PC *sn*-position isomers after derivatization by LC-MS/MS. (a) EICs of the mixture of PC 18:1(9*Z*)/16:0 and PC 16:0/18:1(9*Z*) before and after the PhI=NTs derivatization. (b) MS/MS spectra of the aziridination derivatization products of PC 18:1(9*Z*)/16:0 and PC 16:0/18:1(9*Z*) at *m/z* 929.60.

and changes in the ratio of *sn*-position isomers are likely to reflect abnormal physiological states in the disease process. Therefore, we first established the mouse middle cerebral artery occlusion (MCAO) model of focal ischemia, and identified the paired *sn*-position isomers of PC in mouse brain tissues by the PhI=NTs derivatization and LC-MS/MS (Fig. 4 and Fig. S26 in Supporting information). Then, analyzed the ratio changes of these isomers in normal and ischemic sides of the brain after different periods of reperfusion. We aim to examine the relationship between the relative changes of *sn*-position isomers and the ischemia. As shown in Fig. 4, two pairs of *sn*-position isomers of PC (PC 16:0/18:1 (Δ9) *vs.* PC 18:1 (Δ9)/16:0, and PC 16:0/18:1 (Δ11) *vs.* PC 18:1 (Δ11)/16:0) were identified in brain tissue, and the derivatized products could be separated and detected by LC-MS (Fig. 4a). Then, the peak area ratios of *sn*-position isomers in normal (left) and ischemic (right) brains after 1-day and 3-day reperfusion were compared. The results show that the ratios of PC 16:0/18:1 (Δ9) to PC 18:1 (Δ9)/16:0 were significantly higher in ischemic brain tissues ($P < 0.05$) than that in normal part after both 1-day and 3-day reperfusion. However, for the ratios of PC 16:0/18:1 (Δ11) to PC 18:1 (Δ11)/16:0, no significant change was observed between the ischemic and normal parts. We also found that isomers with double bonds at the *sn*-2 position (*e.g.*, PC 16:0/18:1 (Δ9)) were higher than those with double bonds at the *sn*-1 position (*e.g.*, PC 18:1 (Δ9)/16:0), which may indicate a preference for fatty acyl chain structures in the biosynthesis of phospholipids. These results suggest that the cerebral ischemia is accompanied by the changes in the ratio of *sn*-position isomers of PC, and that this change shows a preference for C=C bond positional isomers. The changes may be ascribed to the dysregulated biosynthesis or metabolism of the *sn*-position isomers of PC in brain ischemic process. These findings further validated the applicability of the proposed method to a deeper understanding of the relationship between the structural specificities and the biological functions of lipids.

In summary, we developed a visible-light-activated photocatalytic aziridination reaction system for the derivatization of unsaturated lipids. Based on this reaction system and LC-MS/MS, a

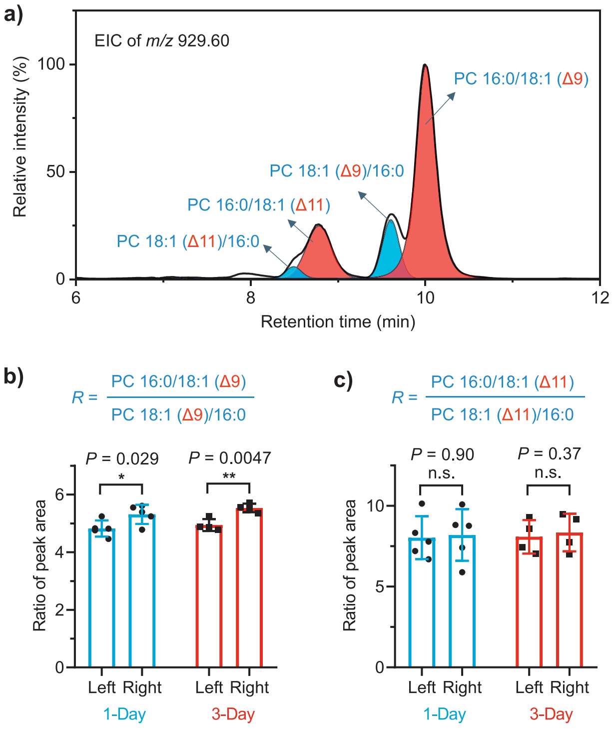

Fig. 4. Analysis of the *sn*-position isomer of PC by the PhI=NTs derivatization and LC-MS/MS. (a) EIC at *m/z* 929.60 showing the derivatized *sn*-position isomers of PC 34:1. The peak fitting was accomplished by using Origin software. (b, c) Comparisons of the ratio changes of *sn*-position isomers of PC 34:1 in normal (left) and ischemic (right) mouse brain tissues. The right brains were subjected to 1 h focal ischemia and then received reperfusion for 1 and 3 days. Two-sided Student's *t*-test was used without adjustment. * $P < 0.05$; ** $P < 0.01$; n.s., no significant difference. Data are presented as mean values ± SD, n=5 and n=4 biologically independent replicates for 1-day and 3-day reperfusion, respectively.

method features the simultaneous identification of the C=C bond location and *sn*-position in unsaturated lipid isomers was established. The fine structures and relative ratios of the unsaturated *sn*-position isomers of PC were identified and quantified in mouse brain tissue, which revealed the significant changes of *sn*-position isomers ratio in the cerebral ischemia for the first time. This study provides a promising approach for the in-depth structural lipidomics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Nos. 22074111, 22004093 and 22004092) and the National Key Research and Development Program of China (No. 2021YFC2700700). We also thank the support of the start-up funds of Wuhan University and the National Youth Talents Plan of China. The authors thank Dr. Liang Wu for the help in the design of the derivatization reagent.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.108775.

References

[1] M.P. Wymann, R. Schneiter, Nat. Rev. Mol. Cell Biol. 9 (2008) 162–176.
[2] X.L. Han, Nat. Rev. Endocrinol. 12 (2016) 668–679.
[3] Z. Wang, E. Klipfell, B.J. Bennett, et al., Nature 472 (2011) 57–63.
[4] M.R. Wenk, Cell 143 (2010) 888–895.
[5] P. Xie, J. Zhang, P. Wu, et al., Chin. Chem. Lett. 34 (2023) 107349.
[6] Y. Hao, Z. Zhang, G. Feng, et al., iScience 24 (2021) 102974.
[7] B. Antonny, S. Vanni, H. Shindou, et al., Trends Cell Biol. 25 (2015) 427–436.
[8] L.M. Bond, M. Miyazaki, L.M. O'Neill, F. Ding, J.M. Ntambi, Fatty acid desaturation and elongation in mammals, in: N.D. Ridgway, McLeod R.S. (Eds.), Biochemistry of Lipids, Lipoproteins and Membranes, Elsevier, Netherlands, 2015, pp. 185–208.
[9] G. Feng, M. Gao, L. Wang, et al., Nat. Commun. 13 (2022) 2652.
[10] G. Feng, Y. Hao, L. Wu, et al., Chem. Sci. 11 (2020) 7244–7251.
[11] C. Sun, C. Ma, L. Li, et al., Chin. Chem. Lett. 33 (2022) 2073–2076.
[12] X. Ma, Y. Xia, Angew. Chem. Int. Ed. 53 (2014) 2592–2596.
[13] M.C. Thomas, T.W. Mitchell, S.J. Blanksby, J. Am. Chem. Soc. 128 (2006) 58–59.
[14] P.E. Williams, D.R. Klein, S.M. Greer, et al., J. Am. Chem. Soc. 139 (2017) 15681–15690.
[15] W. Cao, S. Cheng, J. Yang, et al., Nat. Commun. 11 (2020) 375.
[16] N.S. Kelley, N.E. Hubbard, K.L. Erickson, J. Nutr. 137 (2007) 2599–2607.
[17] R. Bandu, H.J. Mok, K.P. Kim, Mass Spectrom. Rev. 37 (2018) 107–138.
[18] Q.Y. Cheng, J. Xiong, F. Wang, et al., Chin. Chem. Lett. 29 (2018) 115–118.
[19] X.M. Tang, T.T. Ye, X.J. You, et al., Chin. Chem. Lett. 34 (2023) 107531.
[20] T. Porta Siegel, K. Ekroos, S.R. Ellis, Angew. Chem. Int. Ed. 58 (2019) 6492–6501.
[21] S. Tang, X. Chen, Y. Ke, et al., Anal. Chem. 94 (2022) 12750–12756.
[22] P. Müller, C. Fruit, Chem. Rev. 103 (2003) 2905–2920.
[23] M. Nakanishi, A.F. Salit, C. Bolm, Adv. Syn. Catal. 350 (2008) 1835–1840.
[24] Y. Zhao, H. Zhao, X. Zhao, et al., Anal. Chem. 89 (2017) 10270–10278.
[25] S. Masakado, Y. Kobayashi, Y. Takemoto, Chem. Pharm. Bull. 66 (2018) 688–690.
[26] G. Feng, M. Gao, R. Fu, et al., Biorxiv (2022) https://doi.org/10.1101/2022.04.24.489320.