PHOSPHOLIPIDS CHIRAL AT PHOSPHORUS. SYNTHESIS, ABSOLUTE CONFIGURATIONS AND APPLICATIONS

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Abstract The synthesis of P-chiral phospholipids is described and their application in stereochemical studies is discussed.

Recently we have initiated the stereochemical study of phospholipids, aimed at probing the mechanism of phospholipase-catalyzed reactions and the role of the phosphate head group of phospholipids in protein-lipid interactions and in other membrane functions.\(^1\)–\(^5\) For the purpose of those studies we have developed methods for the synthesis of separate diastereomers of chiral phospholipids, 1,2-dipalmitoyl-sn-glycero-3-\(^{18}\)O-phosphoethanolamine (\(^{18}\)O-DPPE, \(2_{a,b}\)), 3-\(^{18}\)O-phosphocholine (\(^{18}\)O-DPPPC, \(3_{a,b}\)) and -3-thiophosphocholine (DPPSC, \(4_{a,b}\)).

The synthesis of \(^{18}\)O-DPPE is outlined in Scheme 1. Condensation of 1,2-dipalmitoyl-sn-glycerol, POCI\(_3\), and (R)-N-(1-methylbenzyl)-2-aminooethanol gave a diastereomeric mixture of oxazaphospholidines (\(1_{a} + 1_{b}\)), which was then separated by chromatography. Hydrolysis of \(1_{a}\) and \(1_{b}\) separately, followed by hydrogenolysis gave \(^{18}\)O-DPPE (\(2_{a}\) and \(2_{b}\), respectively). Methylation of \(^{18}\)O-DPPE (\(2_{a}\) and \(2_{b}\)) gave \(^{18}\)O-DPPPC (\(3_{a}\) and \(3_{b}\), respectively). The diastereomeric mixture of DPPSC was synthesized by a known procedure and separated by stereospecific hydrolysis catalyzed by phospholipase A\(_2\).

The relative configurations of \(^{18}\)O-DPPE were determined by \(^{31}\)P NMR of their O-trimethylsilyl derivatives on the basis of \(^{18}\)O
isotope shifts, as shown in Scheme I.

The absolute configuration of all synthesized compounds were determined. Both isomers of $^{17}O^{18}O$ DPPE (5a and 5b, Scheme I), synthesized analogously to 2a and 2b, were converted to 1-$^{16}O^{17}O$

$^{18}O$-phosphopropane-1,2-diols (9a and 9b) as shown in Scheme II. By using $^{17}O^{18}O$ DPPE (5a, same configuration as 2a) obtained from (+)-$^{17}O$-oxazaphospholidine, 1-$^{16}O^{17}O^{18}O$-phospho-(R)-propene-1,2-diol of $S_p$ configuration was obtained. Therefore the $^{17}O^{18}O$

DPPE with the $^{31}P$ NMR pattern of its trimethylsilyl derivative shown in Scheme II is of $S_p$ configuration. The configuration of DPPsC was related to that of $^{18}O$ DPPE by desulfurization with $\text{Br}_2/H_2O$ or $\text{CNBr/H}_2O$ (inversion) followed by transphospha-

tidylation (retention), as shown in Scheme I.

The synthesized P-chiral phospholipids were used to determine steric course of the hydrolysis and of the transphosphatidylation catalyzed by cabbage phospholipase D. The results showed that the reactions proceed with retention of configuration. Both isomers of DPPsC were used to study the stereospecificity of phospholipases (pl) A$_2$ and C from different sources. It was found that all four pl A$_2$ studied showed stereospecificity toward (R$_p$)-DPPsC.

(S$_p$)-DPPsC is the preferred isomer of pl C.

Separate isomers and mixture of DPPsC were applied to study interactions between polar head-groups in phospholipid bilayers. $^{31}P$ and $^{14}N$ NMR studies of phospholipid bilayers revealed that both $^{14}N$ nuclear quadrupolar splitting and $^{31}P$ chemical shift anisotropy decrease in the following order: (S$_p$)-DPPsC > (R$_p$)-DPPsC > (R$_p$) + (S$_p$)-DPPsC. It can be concluded therefore that the configuration of the phosphate group in phospholipid is important in the structure of the phospholipid bilayer.

REFERENCES
