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PHOSPHOLIPIDS CHIRAL AT PHOSPHORUS.SYNTHESIS,ABSOLUTE CONFIGURATIONS AND APPLICATIONS

KAROL S.BRUZIK, RU-TAI JIANG, and MING-DAW TSAI
Department of Chemistry, The Ohio State University,
Columbus, Ohio 43210

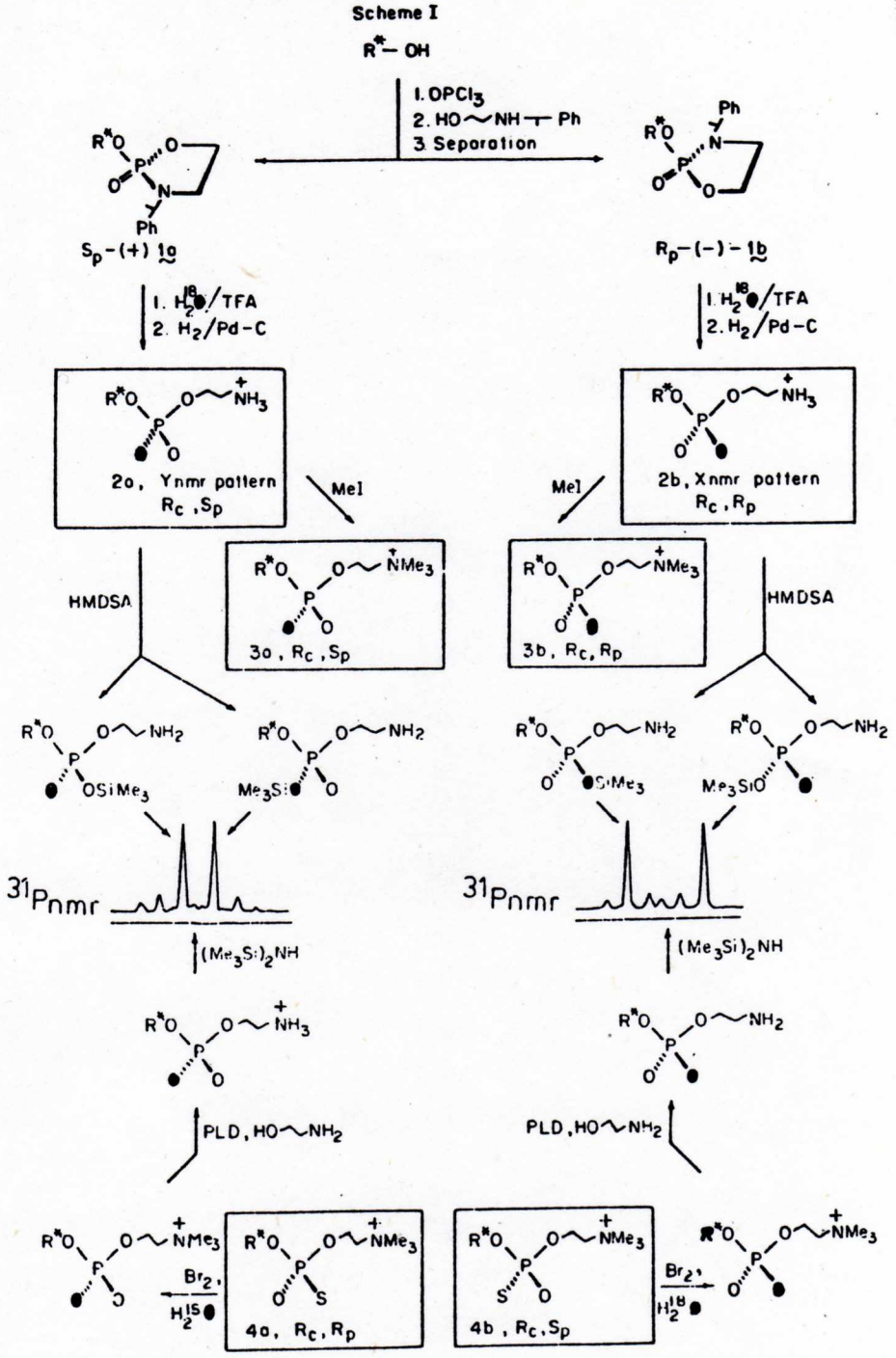
Abstract The synthesis of P-chiral phospholipids is described and their application in stereochemical studies is discussed.

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, 2a,b),-3-|^{18}O|phosphocholine (|^{18}O|DPPC,3a,b) and -3-thiophosphocholine (DPPsC, 4a,b).

The synthesis of |^{18}O|DPPE is outlined in Scheme I. Condensation of 1,2-dipalmitoyl-sn-glycerol, $POCl_3$, and (R)-N-(1-methylbenzyl)-2-aminoethanol gave a diastereomeric mixture of oxazaphospholidines (1a + 1b), which was then separated by chromatography. Hydrolysis of 1a and 1b separately, followed by hydrogenolysis gave |^{18}O|DPPE (2a and 2b, respectively). Methylation of |^{18}O|DPPE (2a and 2b) gave |^{18}O|DPPC (3a and 3b, respectively). The diastereomeric mixture of DPPsC was synthesized by a known procedure[6] 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

Scheme I


R*–OH
1. OPCl3
2. HO~NH–Ph
3 Separation
Sp-(+) 1a
Rp-(-) 1b
1. H2 18O/TFA
2. H2/Pd-C
2a, Ynmr pattern Rc, Sp
2b, Xnmr pattern Rc, Rp
MeI
3a, Rc, Sp
3b, Rc, Rp
HMDSA
31Pnmr
(Me3Si)2NH
PLD, HO~NH2
Br2, H2 18O
4a, Rc, Rp
4b, Rc, Sp

isotope shifts, as shown in Scheme I.

The absolute configurations of all synthesized compounds were determined. Both isomers of $|^{17}O\ ^{18}O|$DPPE (5a and 5b, Scheme II), 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)-propane-1,2-diol of S_p configuration[7] 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 $Br_2/H_2\,^{18}O$ or $CNBr/H_2\,^{18}O$ (inversion)[8,9] followed by transphosphatidylation (retention),[1] 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.

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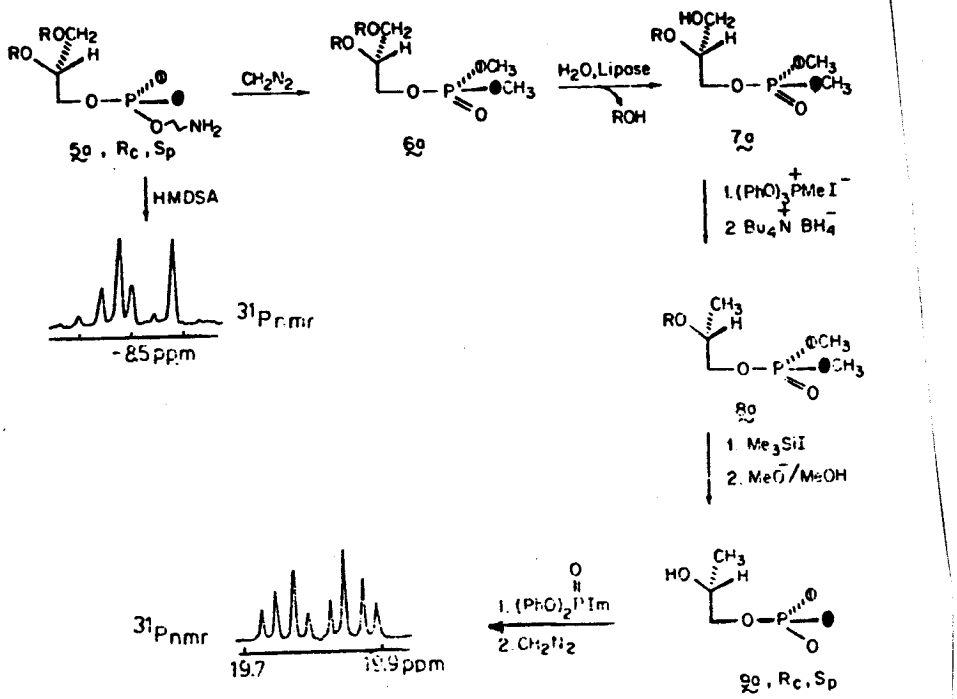
Scheme II



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