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A NEW EFFICIENT AND VERSATILE SYNTHESIS OF ALKYL PHOSPHORYLCHOLINES

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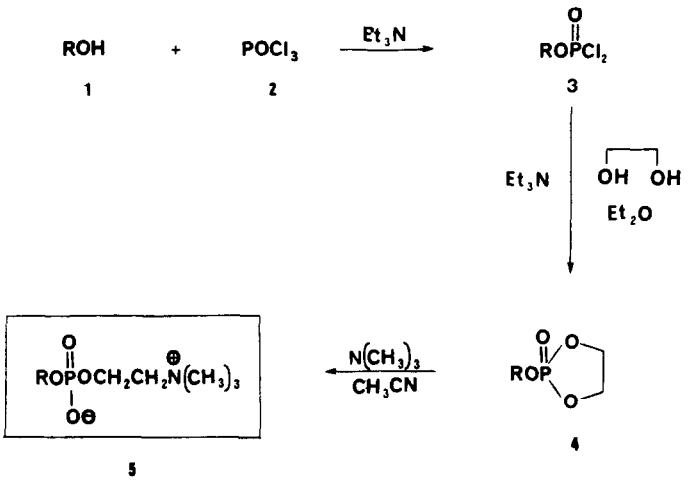
ABSTRACT: A short and general synthetic method is described for the preparation of new phosphorylcholines.

Phospholipids are a class of compounds that have recently been the focus of chemical and biochemical attention. Besides being natural-membrane components, phospholipids have been implicated in a variety of physiological processes.[1] Phospholipase A_2 (PLA_2), for example, is an esterase responsible for liberating arachidonic acid from membrane phospholipids.[2] Since arachidonic acid is a biosynthetic precursor of mediators of inflammation, PLA_2 inhibitors may represent a new class of anti-inflammatories.[3]

As interest in phospholipids develop, a general synthesis of various unnatural phosphorylcholines will be required to study these biochemical processes. A classical synthetic procedure to phosphatidyl cholines[4] requires stoichiometric amounts of expensive silver salts and an aqueous step. Another approach[5] employs 2-chloro-2-oxo-1,2,3-dioxaphospholane, a cyclic secondary chloro-phosphate which reacts slowly with hindered alcohols. A recent synthetic route reacts phosphatidic acid chloride with choline tosylate followed by hydrolysis.[6] While all three approaches are ideal for lecithins, they have severe limitations for the preparation of phosphorylcholines from unreactive alcohols. Aqueous solutions of unusual and short-chain (water-soluble) phospholipids are also difficult to extract with these methods.

We have discovered an inexpensive, anhydrous, and efficient (two-pot, three-step) process (Figure 1) for making alkyl phosphorylcholines. Treating the requisite alcohol **1** with stoichiometric amounts of phosphorous oxychloride (**2**) and triethylamine under nitrogen in anhydrous ether at 0°C generates in 0.5 h the dichlorophosphate **3** in quantitative yield. The reaction mixture is filtered to remove the precipitated triethylamine hydrochloride salt then diluted (0.08-0.1M), cooled (0°C) and exposed to triethylamine (2 equivalents) and ethylene glycol. After twelve hours at room temperature, the dichlorophosphate **3** is completely converted (TLC) into cyclic phosphate **4**, sufficiently pure to be used directly in the last step. Optional silica gel chromatography provides pure **4** using non-polar solvents (dichloromethane, ether). Since phosphorylation reactivity decreases with increasing phosphate order[7] (1°>2°>3°), this process takes advantage of facile primary to secondary phosphate reactivity followed by the intramolecular cyclization to the cyclic phosphate **4** in good yield (65-75%, purified).



Heating an acetonitrile solution of 4 and trimethylamine (3 equivalents) in a sealed tube for 30 h at 75°C affords, upon cooling, the precipitated n-alkyl phosphorylcholines 5 in good yield (65-70%). Generally, the reaction mixture can be filtered and recrystallized (acetonitrile, tetrahydrofuran, acetone) or subjected to silica-gel column chromatography ($CHCl_3$:CH_3OH:H_2O; 65:25:4) followed by lyophilization to provide pure phospholipids.[8] By this process, the synthesis of alkyl phosphorylcholines from the requisite alcohol is achieved in a modest (35-50%) overall yield.

Several n-alkyl- and n-alkylglycolether-phosphorylcholines[9] have been efficiently prepared in this manner (Table 1). Although most alcohols are rapidly phosphorylated with phosphorous oxychloride, the 3-(thioalkyl)propanols[10] resisted phosphorylation in ether under conditions where both alkyl- and glycolether-alcohols reacted quantitatively. The 3-(thioalkyl) propanols lack of reactivity suggested potential micelle formation, thereby shielding the reactive alcohols from the polar phosphorylating agent. Changing from ether with a low dielectric constant (4.2) to tetrahydrofuran (7.6) or acetonitrile (38.8) should disrupt micelle formation and promote phosphorylation. As shown in Table 1, combinations of tetrahydrofuran and acetonitrile resulted in quantitative phosphorylation. Uneventful cyclic phosphate formation (THF) followed by trimethylamine treatment completed the synthesis of 3-(thioalkyl)propyl-phosphorylcholines.

This approach offers several synthetic advantages. In an efficient two-pot process, substrates prone to form micelles are easily transformed into phospholipids without aqueous steps. Use of reactive and inexpensive reagents (phosphorous oxychloride, ethylene glycol) ensures rapid phosphorylation of unreactive alcohols and facile cyclic phosphate formation. Optional cyclic phosphate purification affords the synthetic flexibility to prepare more complicated phospholipids that can be purified with non-polar solvents prior to phosphorylcholine transformation.

These alkyl-phosphorylcholines were designed as phospholipase A_2 inhibitors and their biochemcial properties will be described elsewhere[11]

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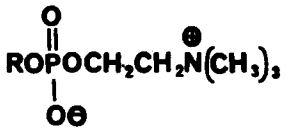
TABLE I'

Alkyl Phosphorylcholines



ENTRY	R	PS	MP(°C)[a]	OVERALL YIELD(%)[b]
Alkyl				
6	C_6H_3	Et_2O	200 - 201	46
7	C_8H_{17}	Et_2O	202 - 204	49
8	$C_{12}H_{25}$	Et_2O	251 - 252	47
9	$C_{18}H_{37}$	Et_2O	222 - 224	50
10	$9\Delta\text{-}C_{18}H_{37}$	Et_2O	168 - 170	48
S-Alkyl				
11	$C_{16}H_{33}S(CH_2)_3$	THF:CH_3CN (2:1)	229 - 233	41
12	$C_{18}H_{37}S(CH_2)_3$	THF:CH_3CN (2:1)	178 - 180	45
13	$9\Delta\text{-}C_{18}H_{35}S(CH_2)_3$	THF	215 - 220	35
Glycol				
14	$C_{16}H_{33}O(CH_2)_2$	Et_2O	207 - 210	44
15	$C_{18}H_{37}O(CH_2)_2$	Et_2O	198 - 201	45
16	$9\Delta\text{-}C_{18}H_{35}O(CH_2)_2$	Et_2O	170 - 174	45

PS = phosphorylation solvent; CH_3CN = acetonitrile; THF = tetrahydrofuran.

(a) = Melting points were determined on a Thomas-Hoover apparatus and are uncorrected.

(b) = Overall yield was based upon starting alcohol for phosphorylation.

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