Syntheses of Phospholipids via Oxazaphospholanes

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Dedicated to Prof. Ivar Ugi on the occasion of his 60th birthday

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A method for the synthesis of the head group of phospholipids is described. It is formed by ring opening of oxazaphospholanes, either by mild tetratoze mediated hydrolysis of \( \lambda^3 \)-oxazaphospholanes or by methyaling ring cleavage of \( \lambda^1 \)-oxazaphospholanes. Oxidation can be performed by means of an oxaziridine.

Introduction

The availability of well defined phospholipids is a prerequisite for the study of membranes and their properties. Despite the existence of established methods their synthesis remains a challenge. The search for new synthetic methods continues.

One new method was adopted from oligonucleotide synthesis [1, 2]. It is based on a phosphite amide triester strategy. Reactive phosphorous ester amide chlorides are used for phosphorylative coupling. This phosphodiester approach needs the introduction of protecting groups for phosphorus and for the aminofunction and their cleavage at the end of the synthesis. A cyclic phosphite amide, like 1 could circumvent this necessity. During phosphite amide triester strategy an amine is the leaving group of the second phosphorylation step. Oxazaphospholanes, synthesized from a suitable aminoalkohol could on hydrolysis yield the fundamental part of the hydrophilic head group of the phospholipids and thus circumvent the use of protecting groups for phosphorus and amino group.

In this way, Eibl used \( \lambda^3 \)-oxazaphospholanes [3]. They were prepared in situ without the possibility to purify intermediates. Because of the known stability of phosphoric acid amides the cleavage of the phosphorus-nitrogen bond needed relatively drastic acidic conditions.

McGuigan [4] used 2-chloro-3-methyl-1,3,2-\( \lambda^3 \)-oxazaphospholane for phosphorylation. Oxidation by dinitrogen tetroxide was followed by mild tetratoze mediated hydrolysis.

Here we describe a three step method which is suitable for the synthesis of phospholipids of the choline- or ethanolamine type. Phosphorylation by 2-chloro-3-methyl-1,3,2-\( \lambda^3 \)-oxazaphospholane is followed by mild tetratoze mediated hydrolysis. Oxidation by tert-butylhydroperoxide yielded N-methyl ethanolalmino phospholipids and ether-lipids. The use of an alternative group of oxidizing agents, the oxaziridines, for the oxidation of intermediate phosphites is presented. It has not been used in phospholipid synthesis before. Finally an example is presented, where N-alkylating opening of an oxazaphospholane by dimethyl sulfate yielded an choline phospholipid.

Results and Discussion

The phosphorylating agent, namely 2-chloro-3-methyl-1,3,2-\( \lambda^3 \)-oxazaphospholane (1) was prepared from phosphorous trichloride and N-methyl ethanolamine at 0 °C [7, 8], According to \( ^{31} \)P, \( ^{1} \)H and \( ^{13} \)C NMR spectra it consists of two isomers (probably cis- and trans-).

1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine (2a) [9] and rac-glycero-1-octadecylether-2-methyl ether (2b) [10] were prepared as reported in the literature.

The reaction of 1 with primary alcohols, both short and long chain, has been reported before [4]. The analogous reaction with substituted glycerols
2a or 2b in the presence of triethylamine proceeded smoothly. The anticipated structures of the glycerol-1,3,2-\(\lambda^3\)-oxazaphospholanes (3) were in accord with the spectroscopic data.

The 1,2-dimyristoyl-sn-glycero-3-oxazaphospholane (3a) was oxidized by means of 3-(4-nitrophenyl)-2-tosyl-oxaziridine [11]. This oxidation can easily be followed by \(^{31}\) P NMR spectroscopy: the chemical shift of the \(\lambda^3\)-oxazaphospholane (\(\delta = 139.46\) ppm) changes to \(\delta = 21.43\) ppm, typical of \(\lambda^5\)-oxazaphospholanes [12]. Side reactions are not observed. The resulting imine precipitates and can be removed by filtration. To our knowledge, this kind of oxidation has not been used in phospholipid synthesis before.

The hydrolytic cleavage of \(\lambda^5\)-oxazaphospholanes requires drastically acidic conditions [3]. We found, that reaction with dimethylsulfate/water opened the ring under mild conditions and yielded the quarternary choline derivative directly. Again the anticipated structure was verified by spectroscopical means. The yield was 54\% after recrystallization from ether.

Contrary to the drastic conditions needed to hydrolyze \(\lambda^5\)-oxazaphospholanes, their \(\lambda^3\)-analogs react under very mild acidic conditions, tetrazole is sufficient. The reaction of analogous open chain compounds is the basis of the phosphite-amide-approach in oligonucleotide synthesis [13]. 3b is opened within minutes by tetrazole/water. The reaction can be followed by the appearance of the phosphorous signal, characterized by the coupling constant (697 Hz) of the tautomeric phosphorous diester. It was oxidized by tert-butylhydroperoxide [1]. Purification and recrystallization yielded 67\% of the kephaline.

**Experimental**

All solvents were reagent grade. \(^{1}\)H NMR and \(^{13}\)C NMR spectra were obtained on a Bruker XP 360 spectrometer (360 MHz) in CDCl\(_3\) with tetramethylsilane as internal standard. \(^{31}\)P NMR spectra were recorded on a Jeol JNM FX 90 (36.4 MHz) in CDCl\(_3\) with H\(_3\)PO\(_4\) as external standard.

2-(1',2'-Dimyristoyl-sn-glycero)-3-methyl-1,3,2-\(\lambda^3\)-oxazaphospholane (3a)

2-Chloro-3-methyl-1,3,2-\(\lambda^3\)-oxazaphospholane (1) (1.37 g, 9.8 mmol) dissolved in 10 ml dichloromethane was added dropwise to a cooled solution of 5.02 g (9.8 mmol) 1,2-dimyristoyl-sn-glycerol (2a) and 2.73 ml (19.6 mmol) triethylamine in 10 ml dichloromethane. During the addition an inert nitrogen atmosphere and 0 °C were maintained. The mixture was stirred for 30 min at 0 °C and for further 30 min at room temperature. Di-
chloromethane was added and the solution was extracted twice with brine, dried (MgSO₄) and evaporated to obtain 3a in 89% yield.

³¹P NMR: δ = 138.8, 139.4 (diastereomers).

¹H NMR: δ = 5.12 (m, 1H; sn-2-H); 4.43–4.03 (2m, 4H; sn-1-H, P–O–CH₃); 3.87–3.74 (m, 2H; sn-3-H); 3.17–2.89 (2m, 2H; P–N–CH₃); 2.72 (dd, JₚH = 11.8 Hz, 3H; N–CH₃); 2.31 (m, 4H; OOC–CH₃); 1.61 (m, 4H; OOC–CH₂–C–OOC); 1.26 (s, 40H; CH₃). 

¹³C NMR: δ = 70.56 (C-sn-2, 2JCₚ = 6.8 Hz); 68.83 (P–O–CH₃, 2JCₚ = 9.7 Hz); 63.97 (C-sn-3, 2JCₚ = 11.8 Hz); 61.31 (C-sn-1); 49.18 (P–N–CH₃, 2JCₚ = 5.8 Hz); 31.41 (N–CH₃); fatty acids: 173.02, 172.65 (C-1*); 34.08, 33.90 (C-2*); 31.41 (C-12); 29.49–28.43 (C-11)–(C-4); 22.69, 22.51 (C-13*); 19.60 (C-14).

After stirring for further 30 min the mixture was poured into 20 ml of the same solvent. The temperature was maintained at 0 °C, the reaction was carried out under a blanket of dry nitrogen. Subsequently the reaction mixture was stirred for 1 h at room temperature. Now 0.42 g (6 mmol) tert-tetrazole and 0.2 ml (11 mmol) water were added, followed by 3 ml (22 mmol) triethylamine and 1 ml (7.8 mmol) tert-butyldihydroperoxide (70% solution in water) after 30 min. After further 30 min the mixture was poured into 20 ml water, 25 ml chloroform and 50 ml methanol. Addition of 25 ml chloroform and 25 ml water with shaking after each addition yielded two phases. The organic phase was evaporated, the residue dissolved in a little of dichloromethane and precipitated from ether and acetone repeatedly and dried to yield 0.66 g (67%) colourless powder. The NMR spectral data are consistent with the proposed structure.

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