Synthesis of Phospholipid Headgroups *via* Nucleophilic Ring Opening of 1,3,2-Dioxaphospholanes

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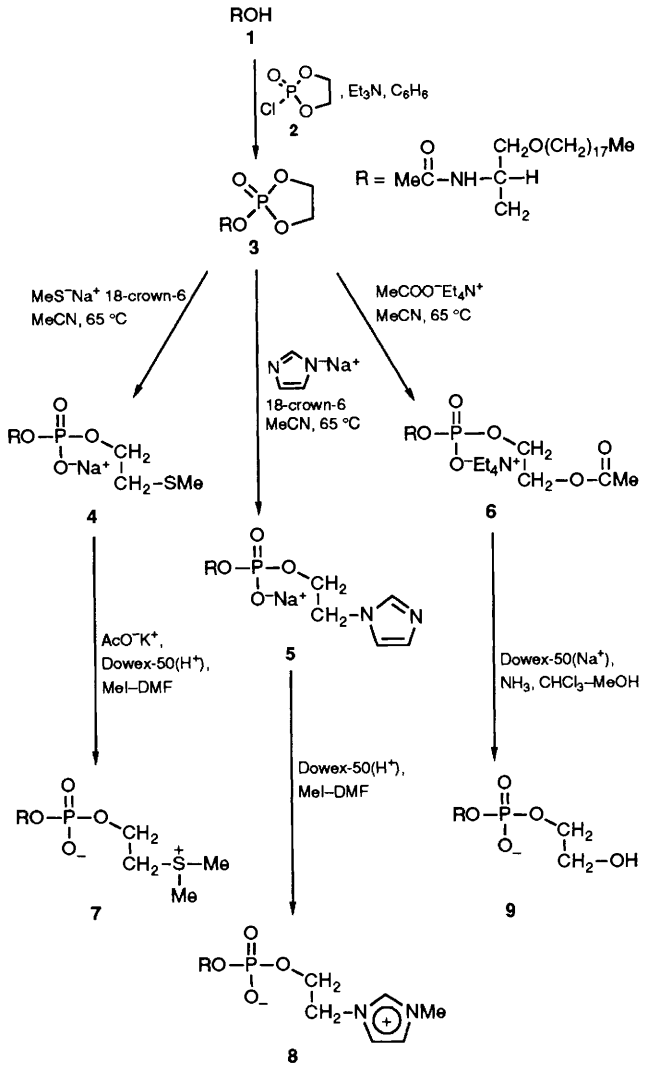
A new method for introduction of variable phospholipid headgroups *via* nucleophilic ring opening of 1,3,2-dioxaphospholanes is reported and applied to the synthesis of ether phospholipids.

Development of new methods for the preparation of structurally variable phospholipid derivatives involves the realization of two main synthetic objectives: (*i*) introduction of specific target functions into the glycerol skeleton, and (*ii*) elaboration of the phosphodiester headgroup in a manner that allows incorporation of various different polar phosphoester substituents. In contrast to the growing number of structurally modified glycerol derivatives that have been prepared and converted to biologically active phospholipid compounds,[1–5] relatively few general methods are currently available for the introduction of variable headgroup functions.[6] As evidenced, both naturally occurring and synthetic phospholipids show significant changes in thermotropic properties,[7] enzyme-substrate behaviour[8] and inhibitory potency,[9] as well as antihypertensive[10] and antitumour activity[11] in response to variation of the polar phosphoester substituent. Therefore, development of facile and efficient synthetic sequences leading to phospholipids with variable headgroups is necessary for assessing the functional role of the hydrophilic phosphoester component in determining the physicochemical, enzymological and cell-biological properties of the compounds.

In the present communication we report the synthesis of a new series of phospholipid derivatives containing a number of different types of headgroups. Our approach is based on the use of 2-chloro-2-oxo-1,3,2-dioxaphospholane **2** as the phosphorylating agent to obtain the substituted glycerophosphoryl intermediate **3** whose reactive five-membered phosphodiester ring can subsequently be cleaved by various nucleophiles for elaboration of the target phosphoester function. While ethylene chlorophosphate **2** and its alkyl ester derivatives have long been used as models for mechanistic studies toward elucidation of cyclic phosphate hydrolyses implicated in enzymatic degradation of nucleic acids,[12] synthetic application of the compounds has been rather limited.[1b,6]† Specifically, we now present evidence that nucleophilic ring opening of 2-alkoxy-2-oxo-1,3,2-dioxaphospholanes in nonprotic media *via* C–O bond cleavage readily affords *C*-substituted open chain phosphodiesters that can be exploited for the synthesis of phospholipids to introduce new polar headgroup functions into the molecule. The C–O *vs.* P–O bond cleavage turns out to be strongly dependent on the experimental conditions, particularly on the nature of the solvent. To achieve substitution at carbon, anhydrous acetonitrile appears to be particularly suitable when sulfur, nitrogen and oxygen nucleophiles are employed. Using highly purified dry MeCN, the ring opening reaction readily proceeds at 60–65 °C, and the

† Most of these focused on reactions at phosphorus leading to P–O cleavage; displacement at carbon has mainly been used for elaboration of the phosphocholine function by reaction of the five-membered phosphodiester ring with trimethylamine.



Scheme 1

resulting *C*-substituted phosphodiester products usually crystallize from the reaction mixture on cooling.‡

We have selected (1′-octadecyl-2′-acetamido-2′-deoxy)glyceryl-2-oxo-1,3,2-dioxaphospholane **3** as a model to study the ring opening reaction, since (*i*) the parent phosphocholine is a highly potent antitumour active ether phospholipid,[11] and (*ii*) these types of functionalized glycerol derivatives (with nucleophilic β-amido carbonyl substituents) have been the most difficult substrates to phosphorylate.[2,5] Significantly, while previous attempts aimed at electrophilic activation of 1,3,2-dioxaphospholanes toward ring opening *via* silylation[6] turned out to have rather limited applicability,§ the approach here presented should be suitable for preparation of a wide range of substituted glycerophospholipids.

For the synthesis of sulfur containing phospholipid derivatives compound **3**, prepared form alcohol **1** in quantitative yield *in situ*,[2b] was allowed to react with 1 equiv. 18-crown-6 solvated sodium methanethiolate in anhydrous acetonitrile at 65 °C for 48 h (Scheme 1). The resulting product **4** was first treated with KOAc to remove the crown ether, then purified by silica gel chromatography to afford the thioether compound **4** in 56% isolated yield.¶ The sodium salt of **4** was converted to the free acid *via* ion exchange chromatography (Dowex 50W-X8, $CHCl_3$–MeOH–H_2O 4:5:1) dried, and alkylated with methyl iodide in anhydrous dimethylformamide (DMF) at room temp. for 4 days. The corresponding sulfonium derivative **7**, isolated as a zwitterionic inner salt, was purified by gel filtration (Sephadex LH-20, $CHCl_3$–MeOH 1:1) yielding an analytically pure, white crystalline solid (50%). The yields realized in this synthesis greatly exceed the 4 to 7% recently reported by Kates[13] for preparation of structurally related sulfocholine analogues of platelet activating factor using conventional phosphorylation methods.

The imidazole substituted phospholipids were prepared by nucleophilic ring opening of cyclic phosphate **3** with crown-ether complexed sodium imidazolide, followed by methylation of the phosphoethyl imidazole intermediate with methyl iodide. Thus, treatment of compound **3** with stoichiometric amounts of Na-imidazole/18-crown-6 in anhydrous MeCN at 65 °C for 48 h yielded phospholipid **5** in its sodium-salt form. This product **5** was passed through a Dowex 50W-X8 (H^+) column using $CHCl_3$–MeOH–H_2O (5:4:1) as eluent to obtain the inner salt of **5**, which was purified on silica gel with $CHCl_3$–MeOH–H_2O (65:25:4) followed by $CHCl_3$–EtOH–aq. NH_3 (50:50:10) to afford an analytically pure sample of **5** in 50%. A sample of this phosphodiester **5** in dry DMF with an excess of methyl iodide and diisopropylethylamine was kept at room temp. for 24 h to produce the *N*-methylimidazolium derivative **8** isolated by gel filtration on Sephadex LH-20 with $CHCl_3$–MeOH (1:1) in quantitative yield, as an analytically pure phospholipid.

Finally, in the oxygen series, cleavage of the five-membered phosphodiester ring of **3** with tetraethylammonium acetate (0.2 mol dm^{-3}) in dry MeCN at 65 °C for 48 h gave the *sn*-3-phosphoethyl acetate **6**, which was converted to the sodium salt *via* ion exchange chromatography (Dowex 50W-X8, Na^+ form). Aminolysis of the product **6** with anhydrous NH_3 in $CHCl_3$–MeOH (1:2) at room temp. for 2 h led to the *sn*-3-phosphoethanol **9**, which was purified on silica gel chromatography ($CHCl_3$–EtOH–H_2O 50:50:4) to give an analytically pure white solid (**9**, 70%).

Throughout the series of ring-opening reactions activation of the nucleophile has turned out to be an important feature on the synthesis. Thus, cleavage of the dioxaphospholane ring can readily be accomplished using highly reactive anionic reagents rather than neutral nucleophiles. Specifically, in contrast to the rapid and effective cleavage of **3** by tetraethylammonium acetate, only traces of the open chain phosphodiester **9** are obtained with water, and cleavage by *N*-methylimidazole affords **8** in less than 10% yield. In line with these observations we have found that in the presence of 18-crown-6

‡ The crude phospholipids are usually obtained in high yield, however, silica gel chromatography of the final product frequently results in substantial losses on recovery and isolated yield of 50–70% are generally realized.

§ This method was only applicable to the synthesis of a very narrow range of substituted glycerophospholipids. Although addition of the catalyst (trimethylsilyl trifluoromethanesulfonate) allowed the reaction to take place at lower temperatures and at shorter reaction times, in the presence of an *sn*-2-acetoxy group the yield of the product dropped to 17%.[6]

¶ *Spectroscopic data* for **4**: ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.88 (br s, 3H, Me), 1.25 [br s, 32H, $(CH_2)_{16}$], 1.98 (s, 3H, MeCO), 2.15 (s, 3H, MeS), 2.65–2.82 (t, 2H, CH_2S), 3.24–3.58 [m, 5H, $(CH_2)_2O$, CH], 3.80–4.23 [m, 4H, $(CH_2O)_2P$]; $[\alpha]_D^{25}$ − 2.8 (*c* 0.99, $CHCl_3$–MeOH 4:1). **5**: ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.88 (br s, 3H, Me), 1.25 [br s, 32H, $(CH_2)_{16}$], 1.96 (s, 3H, MeCO), 3.10–3.58 [m, 5H, $(CH_2)_2O$, CH], 3.80–4.23 [m, 4H, $(CH_2O)_2P$], 7.30–7.67 (m, 3H, N–CH, CH=CH), 8.79 (s, 1H, NH); $[\alpha]_D^{25}$ −4.1 (*c* 1.25, $CHCl_3$–MeOH 4:1). **7**: ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.88 (br s, 3H, Me), 1.25 [br s, 32H, $(CH_2)_{16}$], 1.98 (s, 3H, MeCO), 2.95 (s, 6H, Me_2S), 3.30–4.47 [m, 11H, $(CH_2)O$, CH_2S, CH, $(CH_2O)_2P$]; $[\alpha]_D^{25}$ − 6.2 (*c*, 1.1, $CHCl_3$—MeOH 4:1). **9**: ^{1}H NMR ($CHCl_3$ + CD_3OD) δ 0.88 (br s, 3H, Me), 1.26 [br s, 32H, $(CH_2)_{16}$], 1.99 (s, 3H, MeCO), 3.22–4.31 [m, 11H, $(CH_2)_2$, $(CH_2O)_2P$, CH_2O, CH]; $[\alpha]_D^{25}$ −2.1 (*c* 0.89, $CHCl_3$–MeOH 2:1).

For all phospholipid compounds satisfactory elemental analyses were obtained.

the yield of thioether **4** increases from 20 to 56%, most probably due to a combined effect of raising the solubility of the reagent and generating a highly reactive 'desolvated' nucleophile (MeS^-) by chelating the positive counter ion.

In conclusion, the synthesis provides a rapid and efficient method for introduction of a wide range of polar phosphodiester substituents. It might also be pointed out that further activation of the hydroxy group in the phosphoethanol moiety of **9** may open the way for preparation of phospholipid derivatives with functional groups that would be unstable under the ring opening conditions.

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