Synthesis of a Thiophospho Analogue of Platelet Activating Factor (*RS*)- and (*S*)-1-Hexadecyl-2-acetylglycero-3-thiophosphocholine

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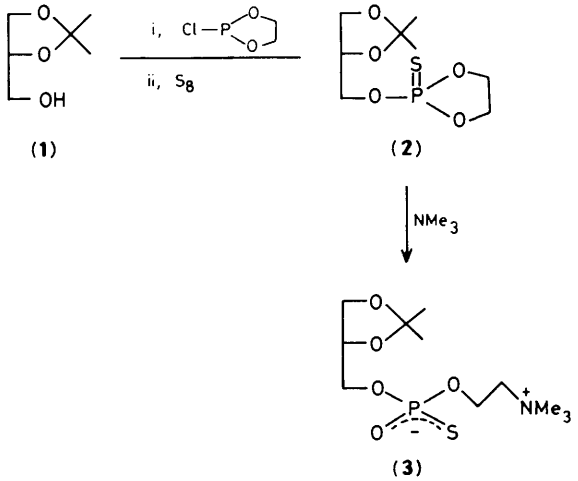
A sequence for synthesis of the title compound from chloro(di-isopropylamino)methoxyphosphine is reported.

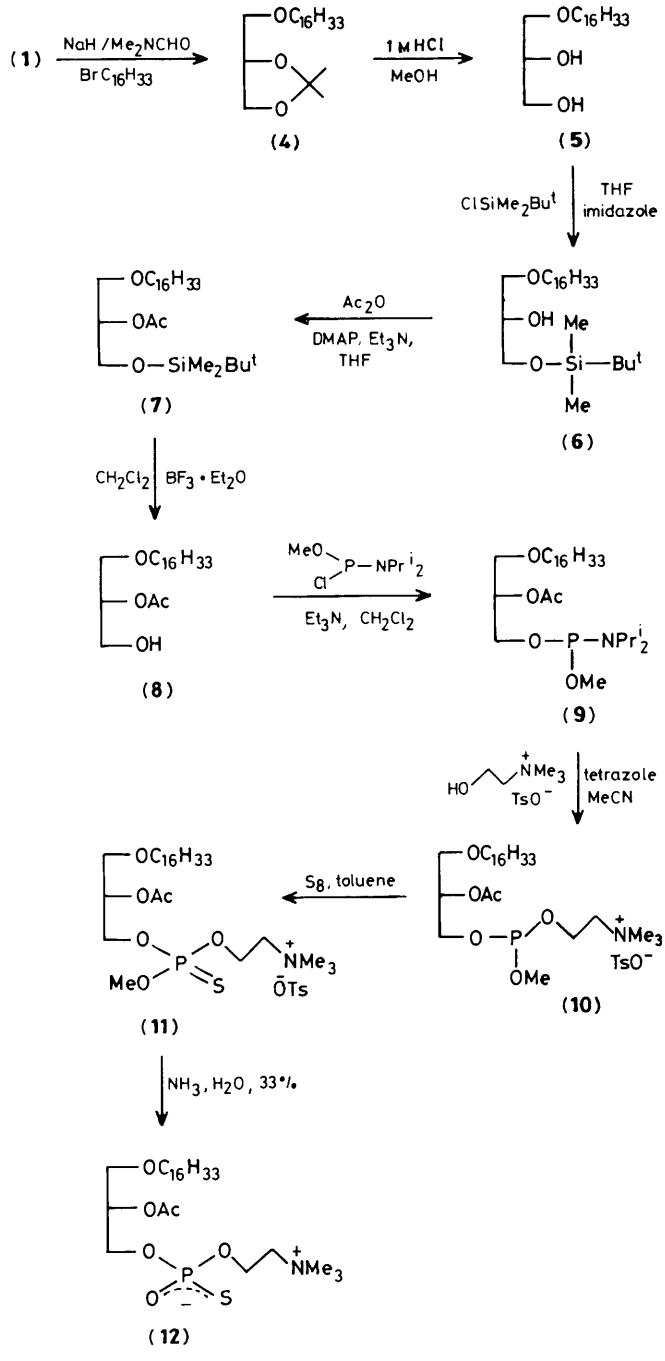
Initial developments in the synthesis of phospholipids as antagonists of platelet activating factor (acetylglycerophosphocholine)[1,2] have been extended to the design of glycerophospho derivatives chiral at phosphorus, used for stereochemical studies of enzymic reactions involving glycerides, such as phosphoglycerate kinase or phospholipase D.[3,4] Our interest in the search for inhibitors of the enzymes involved in the atypical glycolytic pathway in trypanosomes (inhibitors which may be proven active against sleeping sickness and Chagas disease[5]), coupled with the need for chiral thiophospholipids required for metabolism investigations,[6] encouraged us to develop the synthesis of the title compound; the presence of an acetyl group at position 2 makes this compound a pro-drug for the corresponding alcohol, and implied a specific approach to the synthesis, to minimize neighbouring group interference of the acetyl group with the phosphate moiety.[7] The interest of other groups[8] in similar compounds prompted us to disclose our results.

Our initial approach was based on the opening of a dioxaphospholane ring by tertiary amines, as developed by Navech[9] and applied to thio-compounds by Chabrier[10] (Scheme 1). This route led to a mixture of products, resulting from reaction of trimethylamine not only at the endocyclic carbon atom but also at C-3 and at phosphorus, with loss of chemoselectivity. We also tested the reaction of (**1**) with $SPCl_3$ developed by Vasilenko *et al.*;[11] this method, used by Tsai *et al.*[12] for the synthesis of (*S*)-1,2-dipalmitoyl glycerothiophosphocholine, also led to a mixture of products owing to successive reactions with $SPCl_3$, despite appropriate stoicheiometry.

Our synthesis was eventually performed according to Scheme 2, which appears to be a general route. The starting material (**1**) was obtained by the classical reaction from glycerol for the racemate; the *S*-enantiomer was purchased from Fluka. The intermediate (**4**) was obtained in 98% yield and the diol (**5**) in 90% yield [ν_{OH} (KBr) 3300—3400 cm^{-1}; ^1H n.m.r. (80 MHz; $CDCl_3$) δ 0.9 (3H, s, CH_3), 1.22 (28H, s, $C_{14}H_{28}$), 2.8 (2H, s, OH), and 3.34—3.62 (7H, m, CH_2CHCH_2 and CH_2O)]. Protection of the primary hydroxy



Scheme 1



Scheme 2

group of (**5**) with t-butyldimethylsilyl chloride gave compound (**7**) after acetylation {ν_{CO} (film) 1740 cm^{-1}; ^1H n.m.r. (80 MHz; $CDCl_3$) δ 0.50 [6H, s, $Si(CH_3)_2$], 0.90 (3H, s, CH_3), 0.92 [9H, s, $C(CH_3)_3$], 1.30 (28H, s, $C_{14}H_{28}$), 2.1 [3H, s, $C(O)CH_3$], and 3.27—3.90 (7H, m, CH_2CHCH_2 and CH_2O)}. The yield of acetylation of (**6**) was improved from 60 to 90% by use of 4-*N*,*N*-dimethylaminopyridine (DMAP).[13] Deprotection was performed by the procedure described by Kelly[14] [excess of $BF_3{\cdot}Et_2O$ at 0 °C in dichloromethane, without isolation of (**8**)]. Phosphorylation of (**8**) [ν_{CO} (KBr) 1740, ν_{OH} 3400 cm^{-1}] was achieved with chloro(di-isopropylamino)methoxyphosphine in CH_2Cl_2 at 0 °C in the presence of an excess of triethylamine (yield 60%); the product (**9**) ($\delta^{31}P$ +149 p.p.m.) was transformed into (**10**) by reaction of choline tosylate (obtained from choline chloride and toluene-*p*-sulphonic acid exchange reaction, then dried as an oil over P_2O_5 under high vacuum) in the presence of tetrazole as catalyst;[15] the progress of the reaction was followed by ^{31}P n.m.r. [δ +138 p.p.m. for (**10**)]. The final step was achieved by heating (**10**) at 60 °C in toluene with an equivalent amount of sulphur for 24 h; these conditions produced partial hydrolysis of the methyl ester group of the intermediate thiophosphate (**11**) ($\delta^{31}P$ +68 p.p.m.); this hydrolysis was completed by adding ammonia (33% aqueous solution), and the final product (**12**) was obtained in an overall yield of 40% from (**9**). The low yield is due in part to the formation of two by-products, one from oxidation of the phosphoramidite group ($\delta^{31}P$ +14 p.p.m.; yield 20%) during the step (**8**) → (**9**), the other from partial oxidation of (**9**) during reaction with choline tosylate, isolated as a sulphurated compound ($\delta^{31}P$ +57.9 and +58.1 p.p.m.; yield 40%). Compound (**12**) was purified by t.l.c. on Chromatotron 7924 T (silica gel Merck 60 F_{254}; eluant $CHCl_3$–MeOH) {^1H n.m.r. (80 MHz; $CDCl_3$–CD_3OD) δ 0.96 (3H, s, CH_3), 1.26 (28H, s, $C_{14}H_{28}$), 2.23 [3H, s, $C(O)CH_3$], 3.4 [9H, s, $N(CH_3)_3$], and 3.6—4.0 (11H, m, CH_2CHCH_2, CH_2CH_2, and CH_2O); *m/z* 540(MH^+, 100%), 498 (17), 436 (21), and 394 (67); i.r.(film) ν_{CO} 1740, ν_{PO} 1240, ν_{P-OC} 1170, ν_{PO-C} 1050, ν_{P-O-} 1100 and 965, $\nu_{P=S,\ P-S-}$ 615—620 cm^{-1}}.

The same procedure was used for the racemic 2,3-*O*-isopropylideneglycerol and for the *S*-isomer. In the reaction with the racemate the two diastereoisomers were characterized by ^{31}P n.m.r. ($\delta^{31}P$ + 55.1 ± 0.3 and +55.6 ± 0.3 p.p.m., respectively); the same two diastereoisomers were obtained from (*S*)-(**1**) and were separated on Chromatotron. Both will be tested as enzymic substrates.

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