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A GENERAL PROTOCOL FOR THE PREPARATION OF PHOSPHOLIPIDS VIA PHOSPHITE COUPLING

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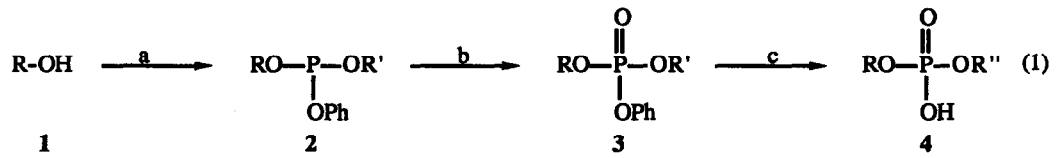
Abstract. A method for the facile preparation of a variety of phospholipids and their derivatives has been developed that utilizes a highly efficient phosphite coupling procedure for the synthesis of a phosphite triester, which may then be readily transformed into the corresponding phosphate diester by sequential oxidation and O-deprotection.

Phospholipids and related substances are presently the focus of considerable chemical and biological attention. Although the interest in phospholipids has been stimulated historically by their use as probes for studying membrane function and structure as well as lipid-protein interactions,[2] these compounds have been found more recently to exhibit a diversity of important biological activities.[3] Moreover, certain enzymes including protein kinase C and a flavin-containing monooxygenase appear to have a specific requirement for phospholipids.[4] Investigations of the lipolytic enzymes phospholipase A_2 and the phosphatidylinositol specific phospholipase C have intensified since arachadonic acid is released directly by the action of the former, and the secondary messengers inositol triphosphate and diacyl glycerides are produced by the latter.[5] Thus, it now seems evident that phospholipids and their analogs will play an increasingly crucial role in future studies of enzyme mechanism, and some such substances may ultimately emerge as useful inhibitors and regulators of enzymatic activity.[6]

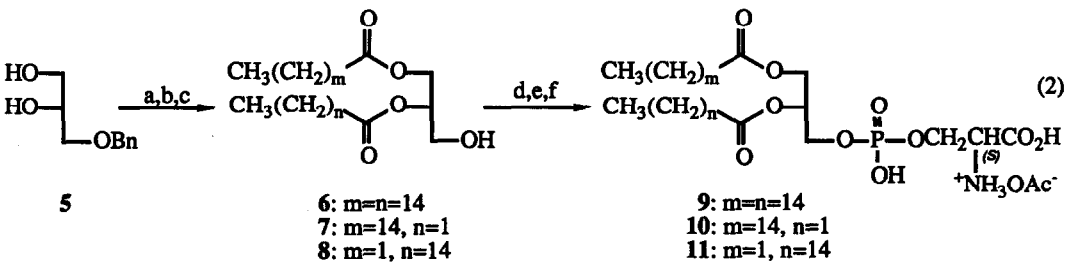
Traditional phosphate coupling procedures and modifications thereof have generally provided a suitable means for the preparation of phosphatidylethanolamine, phosphatidylcholine and derivatives thereof bearing different fatty acid side chains and/or polar head groups in generally good to excellent yields.[7] However, the application of these standard techniques to the efficient synthesis of structurally more complex and more highly functionalized phospholipids may be problematic. Although phosphite coupling reactions have been well established and exploited for the synthesis of oligonucleotides,[8] the utilization of these tactics for the construction of phospholipids and their derivatives has received only limited attention.[9] Consequently, we embarked upon an investigation to develop a method for the efficient synthesis of phospholipids and their analogues via a protocol involving a phosphite coupling as a key step (eq 1), and the results of these studies are recorded herein.



(a) (1) $PhOPCl_2$ (or CH_3OPCl_2), *i*-Pr_2EtN, THF, -78 °C; (2) R'OH, THF, -78 °C to 20 °C. (b) H_2O_2, CH_2Cl_2, 0 °C. (c) Pd black, PtO_2, AcOH, H_2.

In order to evaluate the viability of the strategy depicted in eq. 1, several exploratory experiments were conducted. Thus, 1-octadecanol [**1**, R = $CH_3(CH_2)_{17}$] was coupled with carbobenzyloxyethanolamine and benzyl carbobenzyloxyserine using phenyl dichlorophosphite (or methyl dichlorophosphite) in the presence of *N,N*-diisopropylethylamine, and subsequent oxidation of the intermediate phosphites **2** delivered the corresponding phosphate triesters **3** in excellent (89-92%) overall yields. The phenyl, benzyl and carbobenzyloxy protecting groups were then readily removed by hydrogenolysis under standard conditions[10] to provide the corresponding phosphatidylethanolamine and phosphatidylserine analogues **4** [R = $CH_3(CH_2)_{17}$; R" = CH_2CH_2+NH_3OAc^- and R" = (*S*)-CH_2CH(+NH_3OAc^-)CO_2H, respectively].

Having thus convincingly established the utility of phenyl and methyl dichlorophosphite as reagents for coupling simple alcohols with two of the common polar head groups present in natural phospholipids, attention was then focused upon exploring variations in the hydrophobic subunit. For example, the saturated derivative of phosphatidylserine **9** was prepared in six steps (45% overall yield) from **5**[7c] via a sequence that featured the phosphite coupling of 1,2-dipalmitoyl-*sn*-glyceride (**6**) with benzyl carbobenzyloxyserine (eq 2).



5

6: m=n=14
7: m=14, n=1
8: m=1, n=14

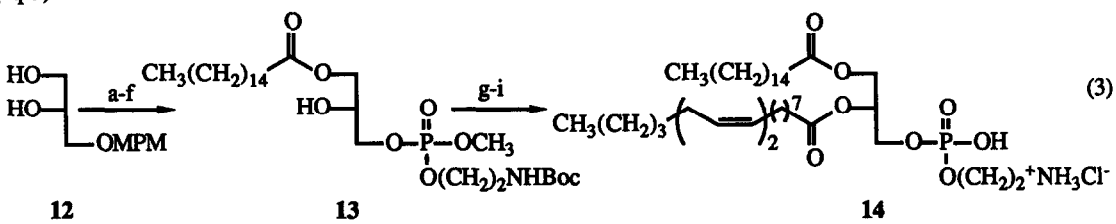
9: m=n=14
10: m=14, n=1
11: m=1, n=14

(a) $CH_3(CH_2)_mCO_2H$, DMAP, DCC, CH_2Cl_2, 0 °C. (b) $CH_3(CH_2)_nCO_2H$, DMAP, DCC, CH_2Cl_2, 20 °C. (c) Pd/C, EtOH, AcOH, H_2, 45 °C. (d) (1) (*S*)-$HOCH_2CH(NHCbz)CO_2Bn$, $PhOPCl_2$, *i*-Pr_2NEt, THF, -78 °C; (2) **6**, **7** or **8**, THF, -78 °C to 20 °C. (e) H_2O_2, CH_2Cl_2, 0 °C. (f) Pd black, PtO_2, AcOH, H_2.

Mixed diacyl phospholipids have been most commonly prepared utilizing phospholipase A_2 to cleave the secondary acyl group from a diacyl phospholipid, followed by the reacylation of the *sn*-2 hydroxyl of the lysophospholipid with a different fatty acid.[11] Although these substances have also been prepared by chemical synthesis via mixed 1,2-diacyl-*sn*-glycerides, the sequences are rather lengthy involving several protection and deprotection steps.[12] We have recently discovered that 3-*sn*-benzyl glycerol (**5**) may be acylated sequentially in a

straightforward fashion *without the occurrence of deleterious 1,2-acyl migration* to allow ready and efficient access to either of the mixed 1,2-diacyl-*sn*-glycerides **7** or **8** (65% over three steps) (eq 2). The subsequent conversion of **7** and **8** into the corresponding mixed 1,2-diacyl-*sn*-phosphatidylserine analogues **10** and **11** was then achieved through the agency of phosphite coupling followed by oxidation and deprotection via catalytic hydrogenolysis (65-70% overall).

Most of the naturally occurring and biologically active phospholipids contain unsaturated *sn*-2-acyl chains possessing from one to four double bonds. Unfortunately, entry to this class of compounds via known synthetic methods has been severely restricted due to the inherent limitations in the choice protecting groups for the polar head groups as well as the propensity for the more highly unsaturated acyl chains to suffer facile oxidation. One solution to this dilemma has been revealed in a recent report of the preparation semi-synthetic, mixed-chain phosphatidylethanolamines from myristoyl-*N*-Boc-lysophosphatidylethanolamine.[13] We have developed a more general approach to this problem that features a modification of the previous procedure (eqs 1, 2) for the efficient synthesis of the lysophospholipid **13** (ca. 50% from **12**) which can be smoothly converted into **14** in a straightforward fashion (75%) (eq 3).



(3)

12 **13** **14**

(a) $CH_3(CH_2)_{14}CO_2H$, DMAP, DCC, CH_2Cl_2, 0 °C. (b) $BnOCH_2Cl$, Proton Sponge, DME. (c) DDQ, CH_2Cl_2, H_2O. (d) $HOCH_2CH_2NHBoc$, CH_3OPCl_2, *i*-Pr_2NEt, THF, -78 °C to 20 °C. (e) H_2O_2, CH_2Cl_2, 0 °C. (f) Raney Ni (W-4), H_2, EtOH. (g) linoleic acid, DMAP, DCC, CH_2Cl_2. (h) NaI, MeCOEt, 85 °C. (i) TFA 50%, CH_2Cl_2, 0 °C.

Thus, the present methodology constitutes a facile and general means of preparing a variety of natural and unnatural phospholipids containing a diverse array of head groups and unsaturated acyl side chains in the *sn*-2 position. Extension of this work to the syntheses of ether lipids, phosphatidylinositols and enzymatic probes is currently under investigation and will be reported in due course.

General Experimental Procedure for Phosphite Coupling and Oxidation: To a vigorously stirred solution of phenyl dichlorophosphite (3.69 g, 19.0 mmol) and *N,N*-diisopropylethylamine (6.10 g, 47.4 mmol) in dry, oxygen free THF (25 mL) was added dropwise (-78 °C) a solution of ROH (15.8 mmol) in THF (minimum volume). After stirring an additional 10 min at -78 °C, a solution of R'OH (19.0 mmol) in THF (minimum volume) was added slowly. The suspension was then stirred for 2 h at -78 °C, the cooling bath was removed, and the suspension was stirred for an additional 1 h. The solvent was removed under reduced pressure and the residue suspended in EtOAc, and the solids were removed by suction filtration through Celite. Evaporation of the filtrate under reduced pressure yielded the crude phosphite triester **2**, which could be purified, if necessary, by flash chromatography (silica gel; ethyl acetate/hexane). The **2** thus obtained was dissolved in CH_2Cl_2 (100 mL), 30% H_2O_2 (1.2-1.5 equiv.) was added, and the mixture was stirred vigorously for 2 h at room temperature. Saturated aq. NaCl (50 mL) was added, and the layers

were separated. The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield the crude triphosphate **3**, which was purified by flash chromatography (silica gel; EtOAc/hexane) (85-92%).

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