Synthesis of Phospholipids using an Inverse Phosphite Triester Approach

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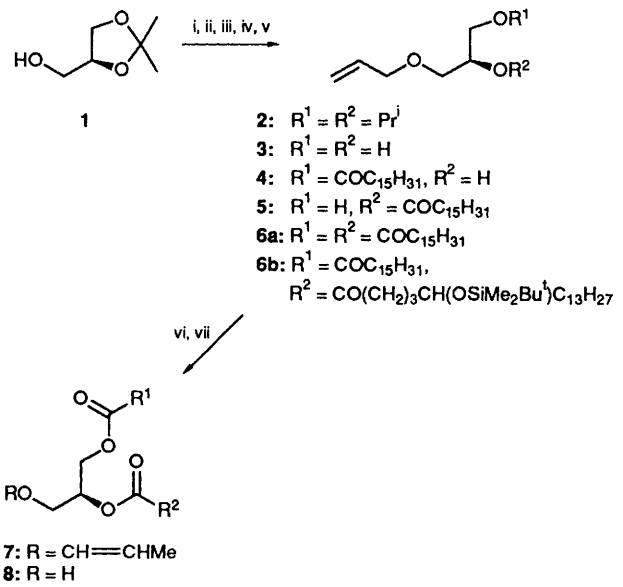
1,2-Diacylglycerols, prepared from allyl protected precursors, were transformed into glycerophosphatidylcholines through an acid catalysed coupling with a dialkyl phosphoramidite, followed by a one step deprotection–substitution reaction.

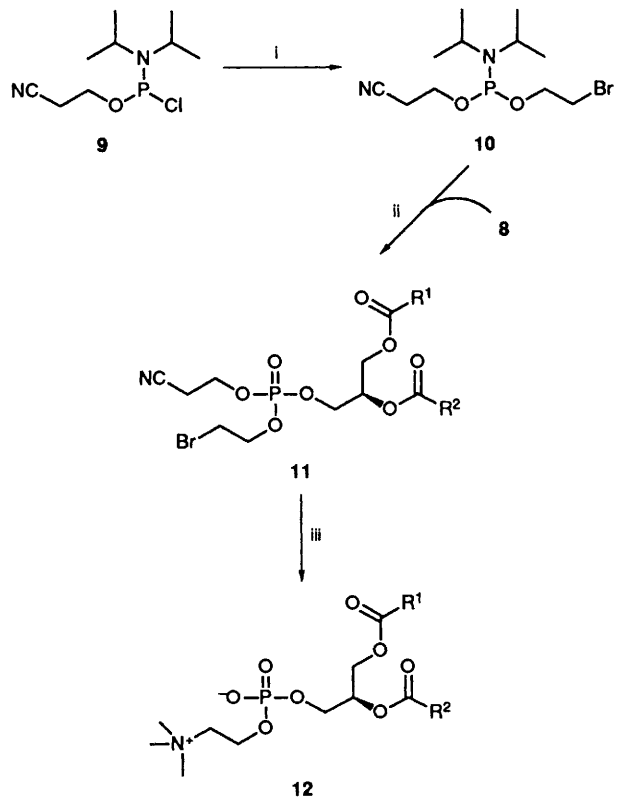
Amphiphilic molecules such as phospholipids have the property of forming aggregated structures such as vesicles and bilayers in water, or monolayers at air–water interfaces.[1] As part of our studies of such systems, we required a variety of glycerophosphatidylcholines with functional groups on the acyl chain at the sn-2 position. Current synthetic methods fall into two categories: in hemisynthesis, an inexpensive phospholipid is deacylated with phospholipase A_2 and the resulting lysophospholipid is reacylated with the carboxylic acid of interest.[2] Total synthesis methods involve the assembly of a 1,2-diacylglycerol followed by the introduction of the phosphocholine moiety.[3] Both of these strategies have inherent disadvantages, and the purification of the products is difficult. In this communication we describe the use of 1,2-diacyl-3-*O*-allyl-sn-glycerols as protected phospholipid precursors. We also describe an efficient, mild, acid catalysed phosphorylation strategy based on phosphoramidite coupling.[4] This procedure avoids the risk of base catalysed acyl migration, permits easy purification of the non-ionic intermediates, and reveals the zwitterionic phosphocholine moiety in the final deprotection–substitution step.

The sodium salt of 1,2-*O*-isopropylideneglycerol **1** was treated with allyl bromide and a catalytic amount of tetra-n-butylammonium iodide (TBAI) in tetrahydrofuran (THF) (Scheme 1). The isopropylidene group was removed by ethanolysis[5] catalysed by a cation exchange resin (Amberlite IR-120 H^+), giving **3** in 93% yield. When the diol was treated with one equivalent of palmitic acid in the presence of dicyclohexylcarbodiimide[6] (DCC) and dimethylaminopyridine (DMAP) in methylene chloride at 0 °C, a 5 : 1 mixture of regioisomers **4** and **5**, and a small amount of diacylated product **6a** were obtained in 85% overall yield. Pure **4** was isolated by chromatography, and **5** was recycled by isomerization with triethylamine in refluxing toluene. A variety of carboxylic acids could be esterified to the free sn-2 hydroxy of glycerol **4** with DCC and DMAP in methylene chloride at room temperature. Thus, a 95% yield of diacylglycerol **6b** was obtained as the sole product when using 5-*O*-(t-butyldimethylsilyl)octadecanoic acid.

The removal of the allyl ether from protected derivatives **6** was accomplished by isomerization to enol ether derivative **7** using tris(triphenylphosphine)rhodium chloride[7] [$(Ph_3P)_3$RhCl] in refluxing ethanol in the presence of diazabicyclooctane (DABCO). The reproducibility of this reaction was ensured by refluxing the catalyst with two equivalents of triphenylphosphine[8] prior to the addition of the allyl ether **6**. The hydrolysis of the enol ether was problematic at first because of the propensity for acyl chains at the sn-2 position to migrate to give the thermodynamically more stable 1,3-diacyl derivatives under standard conditions using mercury oxide



Scheme 1 *Reagents and conditions*: i, NaH, TBAI, $BrCH_2CH{=}CH_2$, THF, room temp., 12 h; ii, Amberlite IR-120 H^+, EtOH, room temp., 18 h; iii, $HO_2CC_{15}H_{31}$, DCC, DMAP, CH_2Cl_2, 0 °C, 3 h; iv, Et_3N, $PhCH_3$, reflux, 12 h; v, R^2CO_2H, DCC, DMAP, CH_2Cl_2, room temp., 1 h; vi, $(Ph_3P)_3$RhCl, DABCO, EtOH, reflux, 1 h; vii, NBS, H_2O, THF, room temp., 5 min



Scheme 2 *Reagents and conditions*: i, $BrCH_2CH_2OH$, Et_3N, CH_2Cl_2, room temp., 1 h; ii, **8**, tetrazole, MeCN, room temp., 1 h, then pyridine, I_2, H_2O, THF, room temp., 15 min; iii, Me_3N, MeCN, 65 °C, 48 h

and mercury chloride,[9] or with acid catalysts. The problem was eventually solved in the following way: hydrolysis of the enol ether was accomplished in neutral conditions by the use of *N*-bromosuccinimide (NBS) and water in THF. In the absence of any acid or base the alcohol **8** could be isolated in quantitative yield and used directly in the phosphorylation step.

We then examined the standard conditions used to introduce the phosphorylcholine moiety, but found that all used base catalysis in the coupling step,[3] leading to mixtures of isomers which were difficult to separate. A few applications of the phosphite triester method in the synthesis of phospholipids have been described,[10] but these examples suffer from essentially the same disadvantages as the previous methods. We decided to invert the coupling sequence, and to form the critical glycerol phosphite ester in the acid catalysed step. Therefore,[4] 2-cyanoethyl(*N*,*N*-diisopropylamino)chlorophosphinite **9** was condensed with bromoethanol in methylene chloride in the presence of triethylamine to give dialkyl phosphoramidite **10** in quantitative yield (Scheme 2). The phosphate triester **11** was obtained in 70% yield by the following sequence of reactions: an excess of tetrazole was added to a solution of diacylglycerol **8** and phosphoramidite **10** in dry acetonitrile. The intermediate phosphite triester was oxidized *in situ* by the addition of pyridine and a solution of iodine in aqueous THF. The neutral phosphate **11** could easily be purified at this stage using flash chromatography with mixtures of ethyl acetate and hexanes. No trace of isomeric 1,3-phospholipids could be detected by NMR spectroscopy (300 MHz). In the final step simultaneous removal of the cyanoethyl group and displacement of the bromide were accomplished by heating **11** in a 1:1 mixture of trimethylamine and acetonitrile in a pressure bottle at 65 °C for 48 h, producing the zwitterionic phosphatidylcholine **12** in 92% yield. Half-gram quantities of **12** could be obtained in 65% overall yield based on compound **6**. The advantage of this method is that purification of the intermediates and manipulation of the cationic head groups are simplified, and isomerization of the diacylglycerols is eliminated. We are currently working to extend this methodology to the synthesis of phosphatidylethanolamines and phosphatidylserines, and to introduce other functional groups on the sn-2 acyl chain.

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