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Rapid and efficient routes to phosphatidylinositol 3,4,5-trisphosphates via *myo*-inositol orthobenzoate

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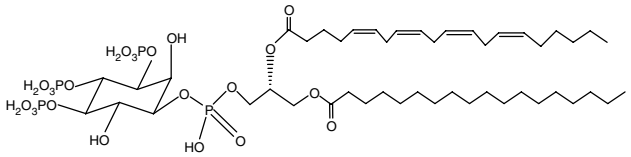
Abstract—Efficient routes to two phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] analogues with different acyl chains have been developed by using cheaply available *myo*-inositol as the starting material. The high yield of the orthobenzoate derivative, preferential formation of the required protected inositol diastereomer in its desymmetrization and ease of separation make the synthesis expedient, economical and high yielding. Due to the inherent problem of racemization of diacylglycerol (DAG), the synthesis of phosphatidylinositol phosphates [PIPns] with unambiguous stereochemical purity has always been difficult. Our methodology excludes the possibility of racemization in the DAG unit and thus provides access to PtdIns(3,4,5)P_3 of high optical purity. Since the acyl functionalities are introduced last, the methodology reported is amenable to the synthesis of PtdIns(3,4,5)P_3 with any acyl chain (or even a library of analogues).

The chemistry and biology of phosphorylated inositols has become an intense area of research during the last two decades due to their involvement in various cellular signalling processes.[1] Among the seven known phosphatidylinositol phosphates (PIPns), phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3, **1**], (Fig. 1) has attracted much attention due to its various biological roles[2] in Akt signalling, signal transduction[3] non-capacitative calcium influx,[4] cell regulation,[5] etc. The fact that PtdIns(3,4,5)P_3 interacts with many targets, namely, protein kinases, phospholipases, G-nucleotide exchange factors, etc, suggests that its role in cell signalling is particularly complex and there are many unsolved puzzles regarding the function of this lipid. Considering the central role of PtdIns(3,4,5)P_3 in these various phenomena and the fact that it occurs in nature in very small quantities, it is not surprising that there have been several attempts to synthesise this lipid.[6] As a part of our ongoing programme to synthesise various phosphoinositols, we were interested in developing an efficient synthetic protocol for PtdIns(3,4,5)P_3.



1.(2-*O*-arachidonyl-1-*O*-stearoyl-*sn*-glyceryl) Phosphatidylinositol 3,4,5-trisphosphate

Figure 1.

After the realization that D-*myo*-inositol 1,4,5-trisphosphate acts as a second messenger in cellular signal transduction, much attention has been focused on the development of efficient routes to various phosphoinositols.[1] Among various methods for the synthesis of phosphoinositols, those starting from the cheaply available *myo*-inositol (**2**) are advantageous in terms of cost, time and practicality. During the synthesis of phosphoinositols, various strategies for the selective protection and deprotection of inositol hydroxyl groups[7] have been developed. Initial protection of inositol hydroxyl groups as orthoesters for synthetic purposes has often been preferred over the more classical ketalisation methods in recent years. While the ketalisation of inositol gives a mixture of four ketals making the separation tedious and reducing the yield of the required ketal (less than 25%), the orthoesterification gives a single product, a *myo*-inositol 1,3,5-orthoester, in high yield (about 90%). Also, the known strategies for the selective protection of the remaining three free hydroxyl groups (2-OH,

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4-OH, or 6-OH) and the selective partial cleavage of the orthoester cage with reducing agents in protected orthoesters (to regenerate the 1(3)-OH or 5-OH) make *myo*-inositol 1,3,5-orthoesters versatile intermediates for synthetic purposes. For instance, orthoesters **3**–**5** (Fig. 2) have been synthesized and **3** and **4** have been used extensively for phosphoinositol synthesis.[1] In addition to the above mentioned reactions of orthoesters, acid hydrolysis of a 2-O-protected orthoester leaves the corresponding ester functionality at the 1(3)-O-position, except in the case of orthoformate as a formate ester is too labile to hydrolysis.[8] We envisioned that if a stable ester that can act as a temporary protecting group is introduced at the 1-O-position by this strategy, this reaction can be exploited for the efficient synthesis of phosphatidylinositol phosphates. We report herein efficient, rapid and high-yielding syntheses of two PtdIns(3,4,5)P_3 analogues, exploiting *myo*-inositol orthobenzoate, **8** (which will leave a stable benzoate ester at the 1-O-position on opening) as the synthetic precursor. Although the natural PtdIns(3,4,5)P_3 (and other PIPns) has an unsaturated arachidonyl chain at the *sn*2 position of the lipid, the unsaturated lipids often aggregate (due to their folding) making biological studies difficult in aqueous solutions. For these reasons, saturated and short chain lipids (more soluble in water) are preferred for biological studies.[6k,9] Thus, we have chosen two different saturated acyl chains in our synthesis of PtdIns(3,4,5)P_3 analogues.

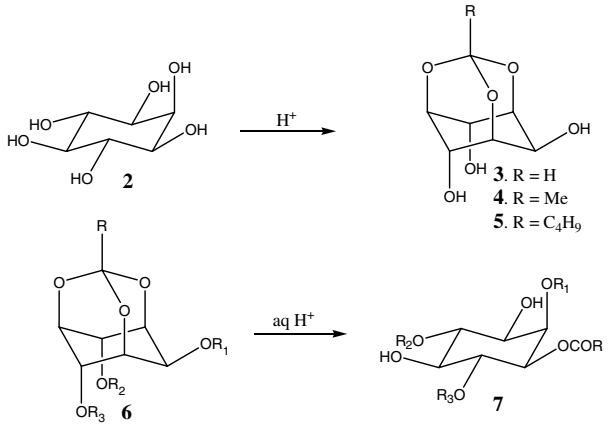
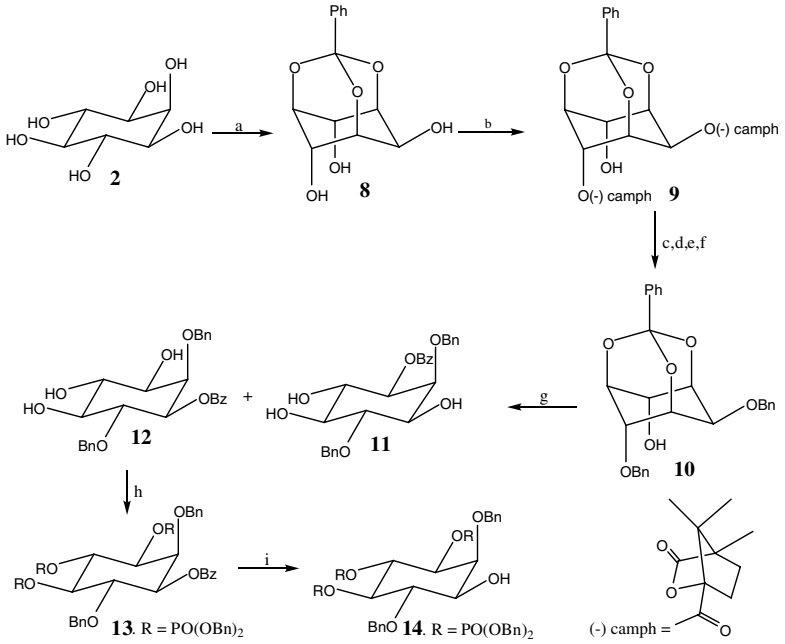


Figure 2.

We have recently developed[10] methodologies for the synthesis and resolution of *myo*-inositol 1,3,5-orthobenzoate (**8**) and derivatives that relate to earlier work on orthoester desymmetrization.[11] Thus, the orthoester triol **8** on desymmetrization by esterification with camphanoyl chloride provided diastereomer **9** (Scheme 1) preferentially, which was converted to dibenzyl ether **10** via protection of the 6-OH as the MIP acetal, deacylation, benzylation and deprotection of the MIP acetal. Thus, optically active dibenzyl ether **10** could be obtained in about 50% yield from *myo*-inositol. Acid hydrolysis of **10** provided two synthetically useful isomeric triols **11** (41%) and **12** (48%) that could be very easily separated. Triol **12** on phosphitylation with dibenzyl *N*,*N*-di-isopropyl phosphoramidite followed by in situ oxidation provided the protected trisphosphate **13**. Attempts to remove the benzoyl group by methanolysis (MeOH, Et_3N) resulted in a mixture of products presumably arising from phosphate migration under basic conditions. However, the treatment of **13** with the Grignard reagent EtMgCl provided alcohol **14**. The phosphate triesters were stable under these conditions.[12]



Scheme 1. Reagents and conditions: (a) $PhC(OMe)_3$, CSA, DMSO, 80 °C, 5 h, 84%; (b) (1*S*)-(−)-camphanoyl chloride, Et_3N, DMAP, CH_2Cl_2, 0 °C–rt, 66%; (c) 2-methoxypropene, PTSA, THF, 0 °C–rt; (d) LiOH·H_2O, THF, MeOH, H_2O; (e) NaH, BnBr, DMF; (f) TFA, wet CH_2Cl_2 (85% for 4 steps); (g) 1 M HCl, EtOH (1:2), reflux, 5 h, **11** (41%), **12** (48%); (h) (1) $(BnO)_2PN(i\text{-}Pr)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 45 min, (2) *m*-CPBA, −78 °C to rt, 1 h, 94%; (i) EtMgCl, THF, −42 °C, 30 min, 96%.

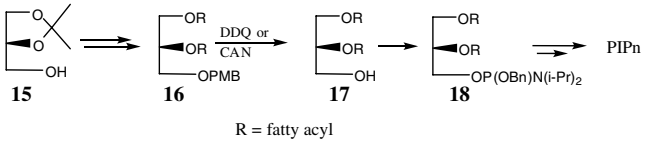
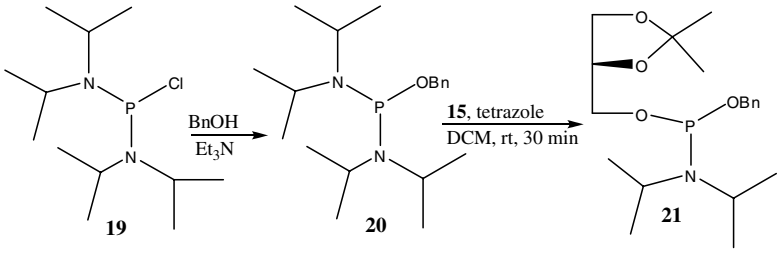


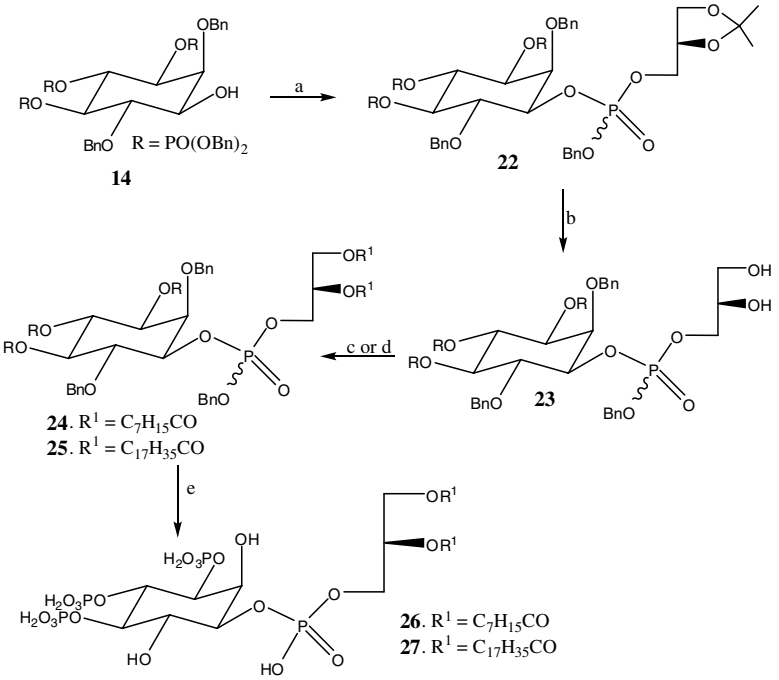
Figure 3.

The usual protocol for the synthesis of PIPns is the phosphitylation of an appropriately functionalised inositol derivative with an alkyl 1,2-di-*O*-acyl glyceryl *N*,*N*-diisopropyl phosphoramidite (e.g., **18**) prepared from an optically pure diacylglycerol **17** (Fig. 3). However, diacylglycerol[13] is a synthon prone to racemization via acyl migration during its synthesis, purification, storage and reaction. Usually, diacylglycerol is prepared from optically pure 1,2-*O*-isopropylidene-*sn*-glycerol (**15**) through a series of operations via PMB ether **16**. However, under the (acidic) conditions for the deprotection of the PMB group (CAN or DDQ), the acyl groups are prone to migrate (racemization).[14] Moreover, Prestwich and co-worker observed this acyl migration during chromatographic purification of diacyl glycerol.[15] It is also known that 1,2-diacylglycerol isomerises readily to an equilibrium mixture of 1,2-, 1,3- and 2,3-diacylglycerols.[16] In addition, the formation of 1,3-diacyl glycerol during the coupling of 1,2-diacylglycerol with $BnOP(N\textit{i}\text{-}Pr_2)_2$ has been reported.[17]

Due to these problems, synthesis of PIPns with unambiguous stereochemical purity has always been difficult. Aneja et al. circumvented this problem by esterifying an appropriately protected inositol derivative with natural phosphatidic acid obtained by phospholipase D mediated lipolysis of phosphatidylcholines.[18] Although this offers a brilliant solution to the above problems, this method is limited to DAGs that occur in natural phosphatidylcholines. To exclude the problem of racemization and hence to provide access to PIPn of unambiguous optical purity, we introduced the acyl groups after the introduction of glycerophosphate. Furthermore, since the fatty acyl groups are introduced towards the end, a library of PIPns with different acyl chains can be prepared easily from the highly advanced common precursor.



Scheme 2.



Scheme 3. Reagents and conditions: (a) (1) **21**, 1*H*-tetrazole, DCM, rt, 1 h; (2) *m*-CPBA, −78 °C; (b) $CHCl_3$, TFA, MeOH (1:1:1, v/v/v), 0 °C, 20 min; (c) $C_7H_{15}COOH$, DCC, DMAP, DCM, rt, 12 h, 85%; (d) $C_{17}H_{35}COOH$, DCC, DMAP, DCM, rt, 12 h, 100%; (e) H_2 (60 psi), $Pd(OH)_2$ on carbon, *t*-BuOH, H_2O (5:1, v/v), 12 h, **26** (97%), **27** (88%).

Bifunctional phosphitylating reagent **20** was prepared from bis(*N*,*N*-diisopropylamino)chlorophosphine (Scheme 2).[19] Treatment of **20** with 1,2-*O*-isopropylidene-*sn*-glycerol (**15**) in the presence of tetrazole provided phosphoramidite **21** (95%).[20]

Trisphosphate **14** was phosphitylated with phosphoramidite **21** in the presence of tetrazole to afford **22** (99%) as a diastereomeric mixture (Scheme 3). The isopropylidene group was cleaved with TFA following Watanabe's protocol[21] to afford diol **23** (88%). Diol **23** is a highly advanced common precursor for the synthesis of various PtdIns(3,4,5)P_3 analogues with different acyl chains or affinity probes. DCC-promoted esterification of diol **23** with octanoic acid provided the fully protected lipid **24**. We have not observed any phosphate migration (cyclisation) under these conditions. Finally, the benzyl protecting groups were removed by hydrogenolysis to provide di-octanoyl (di-C_8)-PtdIns(3,4,5)P_3, **26** in good yield. Similarly distearoyl PtdIns(3,4,5)P_3, **27** was synthesized via stearoylation of diol **23** followed by hydrogenolysis. Thus, PtdIns(3,4,5)P_3 analogues with any acyl chain can be prepared from the diol **23**. The known method to deprotect the benzyl protecting groups without affecting the unsaturated fatty acyl chain,[22] extends the scope of our strategy for unsaturated lipid synthesis.

In conclusion, we have reported efficient routes for the syntheses of two PtdIns(3,4,5)P_3 analogues. Our strategy excludes the possibility of racemization in the lipid chain and thus provides access to PtdIns(3,4,5)P_3 analogues with an unambiguous optical homogeneity. Since the introduction of the acyl moiety is towards the end, a library of PtdIns(3,4,5)P_3 analogues with any acyl chain can be synthesized in a short period of time. Moreover, since the synthesis starts from a high yielding orthoesterification of *myo*-inositol, and the desymmetrization of the triol provides the required diastereomer preferentially, the total yield of the lipid is much better than previously reported methods.

Acknowledgement

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