Synthesis of unsaturated phosphatidylinositol 4,5-bisphosphate and analogues†

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Received 14th April 2009, Accepted 7th August 2009
First published as an Advance Article on the web 23rd September 2009
DOI: 10.1039/b907551h

A new approach for the synthesis of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] is described, compatible with unsaturated fatty acid esters, as well as phosphorothioate and acetylenic analogues. This strategy depends on masking the phosphate charges with base-labile cyanoethyl esters, and the hydroxyls of the target with mild acid-labile protecting groups. A two-step basic then acidic global unblocking of orthogonal protecting groups provides the target lipid. A xanthenylidene acetal was used for key temporary protection of the 4,5-diol, and the 6-*O* was protected with a 1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl (Cpep) acetal.

Introduction

Phosphoinositides continue to be a major field of investigation in cell biology due to the widespread involvement of these lipids in intracellular signalling.[1] They first came to prominence when phosphatidylinositol 4,5-bisphosphate [**1a**, PtdIns(4,5)P_2] was identified as the precursor of the second messengers *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **2**] and *sn*-1,2-diacylglycerol (**3a**, DAG), generated by the action of phospholipase C (Scheme 1). Ins(1,4,5)P_3 triggers the release of Ca^{2+} from intracellular stores, whilst DAG is well known as a co-activator of protein kinase C.[2] It later became apparent that PtdIns(4,5)P_2 (**1a**) is also the substrate for a second ubiquitous signalling enzyme, phosphoinositide 3-kinase, that converts it to PtdIns(3,4,5)P_3 (**4**).[3] This lipid serves as a membrane recognition motif for many acute signalling proteins bearing any one of several phosphoinositide binding domains.[4] Intriguingly, in mammalian phosphoinositides the *sn*-2-fatty acid ester of the glyceride is usually highly unsaturated, favouring arachidonoyl residues. Although this was not at first considered significant, there is now growing evidence that the unsaturated phosphoinositides are more active than their saturated analogues, and in some systems the saturated versions appear not to be recognised by receptors.[5]

Phosphoinositides are very difficult to isolate from natural sources, mainly due to their physical properties, with the active signalling lipids presenting an even greater challenge since they only occur in very small amounts and are rapidly degraded. Consequently there has been much interest in the chemical synthesis of these lipids for use in biological research.[6] From a synthetic point of view phosphoinositides share many features with oligo ribonucleotides: both are phosphorylated polyols with some free hydroxyls, and the phospho-diester linkage is a prominent motif. While the nucleobases of RNA bear little resemblance to phosphoinositides, thymine in particular is sensitive to reduction, as are the unsaturated fatty acid esters of phosphoinositides. On the basis of this comparison, it might be expected that the characteristic problems of RNA chemical synthesis[7] would also be relevant to the design of any phosphoinositide synthesis. In the case of RNA, apart from related issues of regiocontrol during protection of poly-hydroxylated systems, mono-phosphorylation of the 2′,3′-diol provides the ideal geometry for the remaining free hydroxyl to attack a neighbouring phospho-di- or triester. However, reports of

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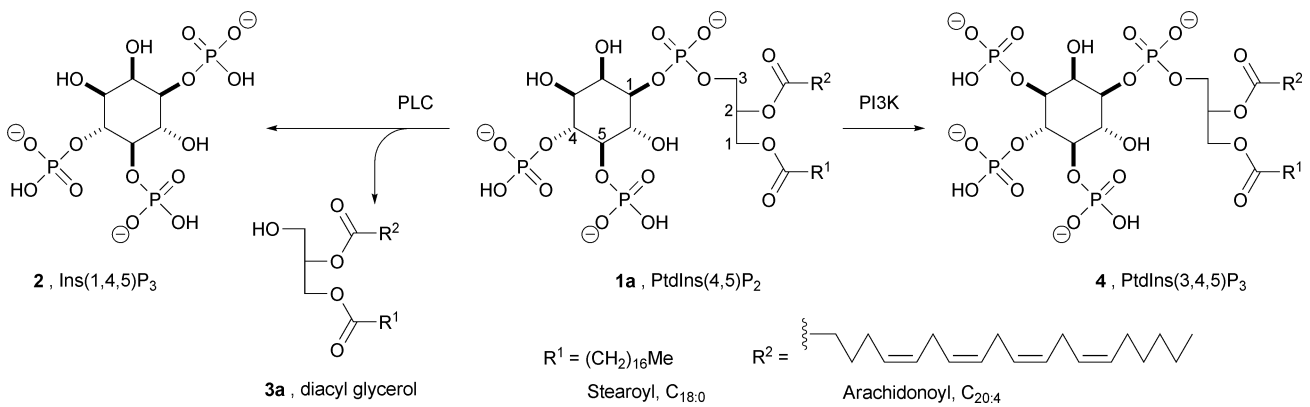
† Electronic supplementary information (ESI) available: Synthetic procedures and analytical data for compounds **8**, **9**, and **18–23**; facsimiles of the ^{1}H-, ^{13}C- and ^{31}P-NMR spectra for final products **1b**, **2**, **21** and **23**. See DOI: 10.1039/b907551h



Scheme 1 Signalling molecules derived from PtdIns(4,5)P_2; PLC = phospholipase C, PI3K = phosphoinositide 3-kinase.

the related attack of a free hydroxyl on a neighbouring phospho-mono- or diester in phosphoinositides are scant. A study of the acidolytic ring-opening of cyclic phosphoranes derived from cyclopentane and cyclohexane *cis* 1,2-diols – species that model the intermediates for acidic migration and hydrolysis of phosphoryl groups in RNA and phosphoinositides, respectively – demonstrated that the latter was less energetically favourable.[8] Reflecting this insight, Bruzik and Kubiak found that butyl and glyceryl inositol 1-phospho-diesters were stable under conditions that hydrolyse RNA.[9] However, the corresponding *para*-nitrophenyl phospho-diester underwent internal nucleophilic attack by both the 2-OH and 6-OH to give mixed inositol 1,2- and 1,6-cyclic phosphates. This suggests that acidification of phosphoinositides may lead to mechanistically similar degradation, although not as easily as for RNA. We are aware of only one case where unintended migration has been reported: when a cell-permeant derivative of Ins(1,3,5)P_3, with protected phosphates but free hydroxyls, was applied to cells it stimulated a similar response to Ins(1,4,5)P_3 (**2**), although at a significantly higher concentration, even though Ins(1,3,5)P_3 has no known biological effects. The authors accounted for this in terms of phospho-triester migration onto the free hydroxyls.[10] Thus, generating a phospho-triester adjacent to a free hydroxyl risks contamination with unwanted isomers.

Reviews of the chemical synthesis of phosphoinositides[11] have tended to emphasise techniques to manage regiocontrol and to obtain chiral intermediates, particularly by the separation of diastereoisomeric derivatives with chiral auxiliaries, or by starting from the chiral pool. However, we contend that the primary issue when designing a synthetic strategy for poly-unsaturated phosphoinositides and their analogues should be the choice of protective groups, and the corresponding conditions required for their global deprotection. Final purification of the target is effectively performed on the fully protected lipid precursor because it is very difficult to fractionate the deprotected lipid from any related contaminants. Consequently it is essential that the global deprotection causes no degradation of the final lipid, and that protective group and reagent debris are easily separated from the product. During global deprotection the most significant potential side-reaction that must be avoided is phosphoryl migration *via* the attack of neighbouring hydroxyls on electrophilic phospho-triesters, as demonstrated by migration in cell-permeant Ins(1,3,5)P_3 precursors.[10] For this reason, as is the case with RNA, the phosphates should all be unblocked before hydroxyl deprotection commences. During subsequent hydroxyl unblocking and any additional purification, extremes of pH that might initiate acid or base catalysed migration and hydrolysis should also be avoided, although the only decomposition so far reported during such treatments has been deacylation of the glyceride moiety.[12]

In the majority of earlier reports global deprotection was achieved by hydrogenolysis of perbenzylated lipid precursors; this is consistent with the above requirements as benzyl phosphate esters unblock far more rapidly than benzyl ethers.[6] Although these near neutral conditions do not cause phosphate migration, no reagent system has yet been reported that can deprotect the benzyl ethers of phosphoinositide precursors without also degrading the poly-unsaturated fatty acid esters of nature-identical glyceride moieties. Furthermore, it is not yet possible to deprotect benzyl ethers in the presence of both glyceride acyl esters and phosphatase resistant phosphorothioate groups (InsO-PSO_2^{2-}).

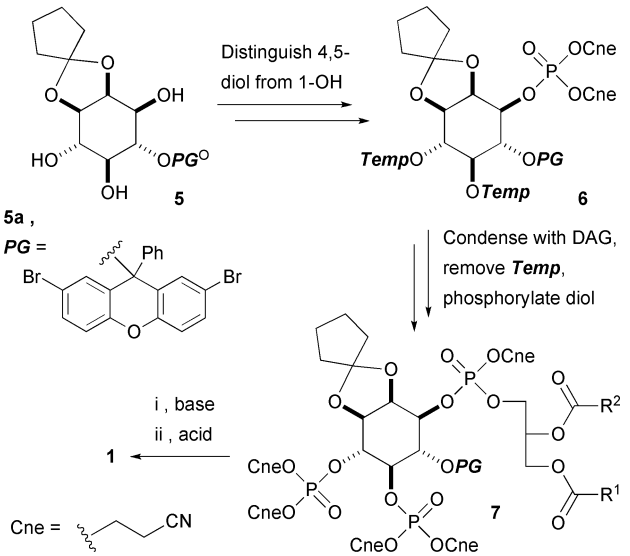
More recently explicitly two-step global deprotections have been designed for compatibility with a wider range of functionalities. Gaffney and Reese were the first to prepare nature-identical *sn*-1-stearoyl-2-arachidonoyl PtdIns(3,4,5)P_3 (**4a**) using global deprotection of base- then acid-labile protecting groups, on the phosphates then hydroxyls respectively.[13] In their preparation of poly-unsaturated PtdIns(3,4,5)P_3 (**4a**) the Watanabe group also selected base labile protecting groups on the phosphates, but used sensitive esters on the hydroxyls.[14,15] In the Bruzik group's effectively two-step global deprotection, benzyl phosphate esters and methoxy methyl ethers were exchanged for Tms-groups with neat TmsBr before solvolysis.[16] Later, Prestwich *et al.* essentially adopted Bruzik's methods to prepare a 3-*O* phosphorothioate analogue of PtdIns(3,4,5)P_3, although benzyl phosphates were replaced with methyl esters to avoid a potentially troublesome trans-esterification to sulfur.[17]

Defining PtdIns(4,5)P_2 head-group regiochemistry

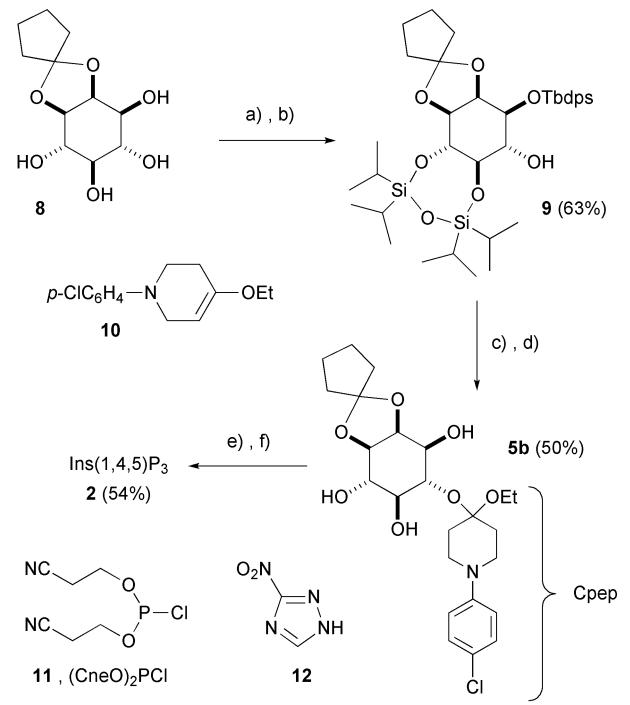
In this paper we report a novel route to unsaturated PtdIns(4,5)P_2. Having identified global deprotection as the overriding consideration in any phosphoinositide synthesis, we elected to adopt the strategy developed by Gaffney and Reese[13] because it was considered less prone to side-reactions and compatible with a wider range of targets than the other published methods, particularly hydrogenolysis. We rejected Bruzik's more flexible phosphate ester exchange with TmsBr because, even though it was noted that $(TmsO)_3P{=}O$ from Arbuzov attack on the inositol ring was virtually undetectable,[16a] it is not clear whether the less hindered TmsBr driven displacement of the phosphoryl group from the primary glyceryl *sn*-3-C was significant or not. More generally, many functionalities that may provide desirable analogues are weak nucleophiles to which powerfully Lewis acidic TmsBr could coordinate to initiate unwanted side-reactions. Also, Watanabe's chloroacetyl esters are unsuitable for preparing valuable sulfur-containing analogues due to *S*-alkylation.

We defined an ideal fully protected PtdIns(4,5)P_2 precursor **7** with acid-labile protecting groups on the hydroxyls of the target and base-labile cyanoethyl phosphate esters (Scheme 2). This became the primary goal of our synthesis with other issues, such as resolution of enantiomers, of secondary importance. In Reese and Ward's ground-breaking synthesis of Ins(1,4,5)P_3 (**2**)[18] a potential building block (**5a**) that might be elaborated to the PtdIns(4,5)P_2 precursor **7** was reported. Although their 2,3-cyclopentylidene acetal is consistent with Scheme 2, the route to install the 6-*O*-(2,7-dibromo-9-phenylxanthen-9-yl) (Dbxn) ether gave only a moderate yield after careful chromatography. Furthermore, when Gaffney and Reese condensed DAG **3a** to a 1-phospho-diester adjacent to a bulky Dbxn-like 6-*O* protecting group they only achieved a moderate yield.[13] Once a suitable building block (**5**) has been created, the key remaining problem will then be how to exercise regiocontrol over the triol to distinguish the 1-OH from the 4,5-diol.

The immediate aim was therefore to improve the efficiency of installing a smaller acid-labile 6-*O*-protecting group on readily prepared 2,3-*O*-cyclopentylidene *myo*-inositol (**8**,[18] Scheme 3) over the preparation of Dbxn-ether **5a**; notably, Reese and Ward[18]



Scheme 2 Summary of proposed route to poly-unsaturated PtdIns(4,5)P_2.



Scheme 3 Preparing a PtdIns(4,5)P_2 head-group building block.

successfully prepared a single enantiomer of building block **8** *via* resolution of 1,4,5,6-tetrabenzyl inositol. Tetrol **8** was selectively mono-silylated with 1.1 eq. TbdpsCl in good yield on a large scale (19 g, 70%), in a similar manner to that previously reported for inositol 2,3-*O*-cyclohexylidene and -camphanylidene acetals.[16] By contrast, comparable selectivity could not be obtained with a small excess of TbdmsCl. With the 1-*O* blocked by a large protective group, the 6-*O* was then expected to be the most hindered and reaction with the bi-functional Markiewicz dichlorodisiloxane gave exclusively 4,5-*O*-protected isomer **9**.[19] This compound, obtained in three steps from *myo*-inositol, already possesses all the regio-differentiation required for PtdIns(4,5)P_2 **1**, but the silyl temporary protecting groups are unsuitable for the later stages of the synthesis.

Next the isolated 6-OH had to be blocked with a permanent protecting group, *i.e.* one that could be carried through to total deprotection. Since modified phenylxanthen-9-yl ethers are very large and may hinder later coupling to DAG, we turned to less bulky linear acetals. As Mom-ethers are very robust, we first reacted the 6-OH with 4-methoxy-5,6-dihydro-2*H*-pyran (MDHP)[20] to generate an Mthp-acetal, but these proved unsuitable (see below). Instead the hydroxyl was blocked by addition of tetrahydropyridine enol ether **10**, under strictly anhydrous acidic conditions, to give an acyclic 1-chlorophenyl-4-ethoxypiperidinyl (Cpep) acetal,[21] followed by removal of the temporary silyl regio-directing groups with fluoride.

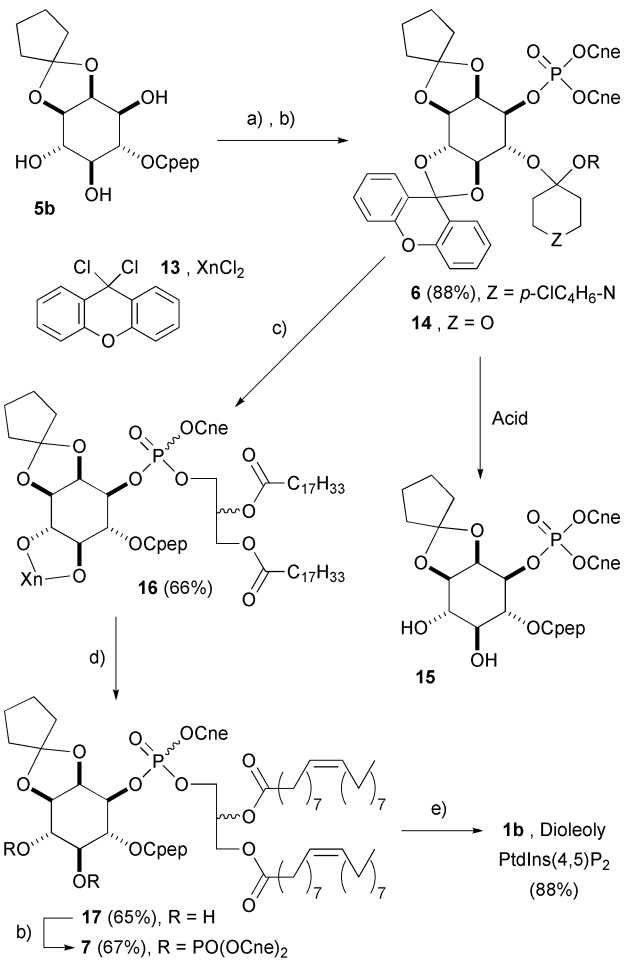
Before carrying key triol **5b** through to a more elaborate lipid, it was first phosphorylated and unblocked to give known Ins(1,4,5)P_3 (**2**) to ensure that the acid labile hydroxyl permanent protecting groups performed as expected. Phosphitylation of **5b** with dicyanoethyl phosphorochloridite [(CneO)$_2$PCl, **11**], in the presence of 1-*H*-3-nitro-1,2,4-triazole (**12**),[22] followed by oxidation of the intermediate trisphosphite with *tert*-butyl hydroperoxide gave the desired fully protected InsP_3 precursor.[13] Although water sensitive, chloridite **11** was preferred over the more commonly used (CneO)$_2$PNEt$_2$,[11,17b,18] because it gives reliable yields, and may be stored for long periods with refrigeration. Global deprotection was performed using Gaffney and Reese's method, first exchanging the Cne-phosphate esters to solvolytically sensitive silyl esters,[23] and then hydrolysing the acetals with aqueous acetic acid. One significant modification was made to the original procedure, replacing *N*,*N*,*N*′,*N*′-tetramethylguanidine with more volatile Barton's base [(Me$_2$N)$_2$C=NBu^t].[24] This ensured that almost all the base could be evaporated under high vacuum after exchanging the Cne- for Tms-phosphate esters. Consequently, the crude product contained only trace quantities of unnatural guanidinium cations, obviating the need for acidic ion exchange. After triturating out protecting group debris, *myo*-inositol 1,4,5-trisphosphate (**2**) was obtained in high purity without the need for further purification. This material had identical spectroscopic characteristics to Ins(1,4,5)P_3 produced in our own laboratory *via* an independent route,[25] and was consistent with published data from other laboratories.[18,26] Since the NMR spectra of the later diastereomeric intermediates in our new route to PtdIns(4,5)P_2 can be difficult to interpret fully, the successful preparation of racemic Ins(1,4,5)P_3 is also important because it verifies the distribution of phosphoryl groups around the inositol ring in all subsequent derivatives of triol **5b** which make up the remainder of this paper.

Temporary 4,5-*O*-protection and preparation of PtdIns(4,5)P_2

In Gaffney and Reese's preparation of PtdIns(3,4,5)P_3 (**4**)[13] the 3,4,5-triol was distinguished from the 1-OH by selective acylation of the relatively unhindered triol. However, because the 1-OH

is usually the most nucleophilic in a *myo*-inositol polyol due to a geometrically favourable hydrogen bond to the 2-*O*,[27] and is not so hindered in triol **5b**, this strategy was thought unlikely to work in this case. Furthermore, after elaborating **5b** to a phosphatidyl intermediate, the choice of conditions for removal of the 4,5-*O*-temporary protection is severely restricted by the range of functionality already present. Despite there being two acetals (cyclopentylidene and Cpep) already blocking hydroxyls in **5b**, we selected a xanthen-9,9-ylidene (Xn) acetal for this purpose.[28] A mildly acidic unblocking of the 4,5-diol would be compatible with the underlying poly-unsaturated PtdIns and, although the other acetals would be vulnerable, the *trans*-ring fusion of the 4,5-acetal is thermodynamically less favourable than the *cis*-fusion of the 2,3-cyclopentylidene. Furthermore, an Xn-acetal undergoes hydrolysis approximately 5-fold faster than a dialkyl acetal, and also provides a conveniently intense UV chromophore for following reactions by TLC.[28a]

Triol **5b** was treated with a slight excess of 9,9-dichloroxanthene (**13**, $XnCl_2$) and the 4,5-*O*-xanthenylidene triacetal was isolated in good yield as the only product (Scheme 4); in future studies, this derivative should be an ideal substrate for resolution of diastereomers by acylation with a chiral carboxylic acid, since basic deacylation to regenerate the mono-ol is orthogonal to the acid labile protective groups. However, phosphorylation of the remaining 1-OH (as for **5b**) only gave a disappointing yield of *ca.* 60%, and xanthone was observed to contaminate the crude product by TLC. This decomposition was ascribed to acidic hydrolysis during the aqueous work-up catalysed by nitrotriazole (**12**), the co-activator for phosphitylation. On this basis, replacement of **12** by *N*-methylimidazole provided a satisfactorily high yield of dicyanoethylphosphate **6**.



Reagents:
a) **13** , C_5H_5N ; b) **11** , *N*-Me-imidazole then Bu^tO_2H ; c) Et_3N then 1,2-dioleoyl glycerol (**3b**), 2,4,6-$Me_3C_6H_2SO_2Cl$, *N*-Me-imidazole ; d) TFA , pyrrole ; e) $(Me_2N)_2C{=}NBu^t$, TmsCl then AcOH.

Scheme 4 Converting key triol **5b** to dioleoyl PtdIns(4,5)P_2.

Before condensing the 1-*O*-phosphate with a glyceride, conditions were first sought to unblock selectively the 4,5-*O*-Xn acetal of **6**, as analysis of this reaction after coupling to a glyceride would be complicated by the presence of four diastereoisomers. It had originally been hoped that a simpler 4-methoxytetrahydropyran-4-yl (Mthp) linear acetal (**14**)[20] might be sufficient for 6-*O*-protection. However, when this was attempted no conditions could be found to unblock the temporary Xn-acetal without concurrent removal of the permanent 6-*O*-Mthp. Although Mthp-acetals are stabilised by the tetrahydropyran oxygen relative to acyclic dialkyl acetals, this was not sufficient to substantially exceed the entropic stabilisation of the cyclic 4,5-*O*-Xn acetal.

For this reason we turned to the Cpep-acetal which has a rate of acidolytic deprotection almost independent of hydrogen ion concentration over a wide range of pH, due to competing protonation of the piperidine nitrogen.[21] Thus, although treatment with a slight excess of dichloroacetic acid gave roughly equal rates of Xn- and Cpep-acetal hydrolysis, a short exposure to TFA gave almost quantitative conversion to diol **15**, as judged by ^{31}P- and ^{1}H-NMR of the crude reaction.

Having defined successful conditions for selective unblocking of the 4,5-*O*-Xn acetal, dicyanophosphate **6** was first treated with excess triethylamine, to remove one of the Cne-esters. The resultant phospho-diester salt was then condensed with two equivalents of 1,2-*O*-dioleoylglycerol (**3b**) to give fully protected PtdIns **16** as a mixture of diastereoisomers in the ratio 2:1:1:1. As with the preceding phosphitylation, using either nitrotriazole (**12**),[22] or even *para*-methoxypyridine *N*-oxide,[13,29] as condensation activators with mesitylene sulfonyl chloride led to concurrent partial unblocking of both Xn- and Cpep-acetals from fully protected PtdIns **16**. However, use of the more basic *N*-methyl imidazole suppressed these side-reactions.

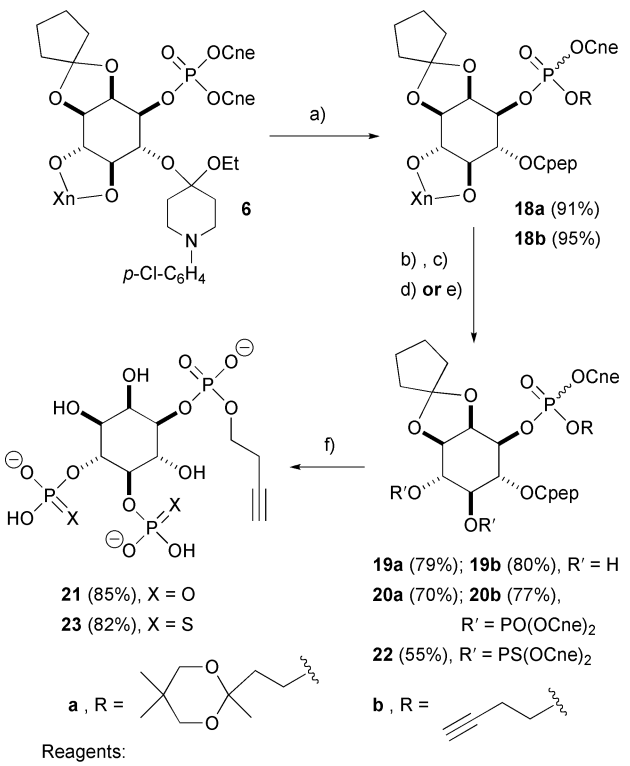
The 4,5-*O*-Xn acetal of **16** was unblocked by a brief treatment with TFA, and the resultant diol (**17**) phosphitylated with $(CneO)_2PCl$ and *N*-methylimidazole, followed by oxidation with *tert*-butyl hydroperoxide. Similarly to Gaffney and Reese's PtdIns(3,4,5)P_3 precursor, it was first necessary to fractionate crude fully protected PtdIns(4,5)P_2 **7** through a column of silanised silica to separate it from tricyanoethyl phosphate, before final purification by normal phase chromatography. As with the preceding preparation of Ins(1,4,5)P_3 (**2**), first basic then acidic global deprotection of **7** proceeded cleanly to give dioleoyl PtdIns(4,5)P_2 (**1b**) in good yield.

PtdIns(4,5)P_2 analogues for "click" ligation

Having validated our synthetic route to unsaturated PtdIns(4,5)P_2 **1b**, this unique approach to phospholipids should also allow us to incorporate phosphorothioates into phosphoinositides, as

it does for RNA.[7a] In addition, other redox-sensitive groups valuable to chemical biology, such as azides, alkynes and many fluorophores, should also be compatible with this approach. We planned to screen PtdIns(4,5)P_2 head-group analogues against phosphoinositide phosphatases, and so needed to include phosphatase resistant phosphorothioates to avoid enzymatic digestion of the probe during screening. For screening purposes we also required a site for so-called "click" ligation of the analogue to a surface, or other species, under very mild conditions. For instance, *O*-alkyl oxyamines condense with ketones or aldehydes under mildly acidic conditions to give oxime *O*-ethers, and acetylenes and alkyl azides undergo Cu(I) catalysed [3+2] cycloaddition to give 1,2,3-triazoles.[30]

We therefore condensed the phospho-diester salt derived from fully protected inositol 1-phosphate **6** with 2-(2-hydroxyethyl)-2,5,5-trimethyl-1,3-dioxane[31] or homopropargyl alcohol, to provide protected Ins(1)P with tethers containing either a masked ketone (**18a**), or a terminal acetylene (**18b**), respectively (Scheme 5). In both cases a roughly 1:1 mixture of diastereoisomers was formed. The selective unblocking of the 4,5-*O*-Xn acetal with TFA then proceeded without difficulty, despite **18a** containing an additional acetal in the tether. Finally, diols **19a** and **19b** were phosphorylated as before to give the corresponding fully protected Ins(1,4,5)P_3 analogues **20a** and **20b** with either a masked 3-oxobutyl, or a but-3-ynyl tether, respectively.



Scheme 5 Acetylene conjugated Ins(1,4,5)P_3.

Unblocking of **20a** was first attempted using the same conditions as for dioleoyl PtdIns(4,5)P_2 **1b**. Exchange of the cyanoethyl- for Tms-phosphate esters proceeded as expected, as confirmed by ^{31}P-NMR of the pentakis(trimethylsilyl) intermediate,[32] where the resonance for a mono-silylated phospho-diester lies characteristically *ca*. 10 ppm downfield of the two disilyl phosphates. However, after treatment with 80% acetic acid for 3 hours, four peaks were observed in the ^{31}P-NMR,[33] two integrating to 1P each, but those at –0.07 and –0.53 ppm to only 0.5P. After 5 days in AcOH the ratio had changed to *ca*. 0.8P:0.2P in favour of the peak at –0.07 ppm, and both the ^{31}P- and ^{1}H-NMR spectra were nearly identical to those of Ins(1,4,5)P_3 **2**. In view of the long half-life in acetic acid it may be supposed that most of the hydrolysis of the side-chain occurred at higher pH, presumably by reverse-Michael reaction to give methyl vinyl ketone, when AcOH was evaporated and replaced by D_2O for analysis.

Since the 3-oxobutyl phosphate ester had proved unstable, we then unblocked fully protected but-3-ynyl Ins(1,4,5)P_3 **20b** using our standard conditions. At first it appeared that unmodified Ins(1,4,5)P_3 (**2**) had been produced because the inositol ring peaks in the ^{1}H-NMR spectrum were almost identical to those of **2**, and the ^{31}P-NMR spectra of the two compounds were completely superimposable. However, the mass spectrum of this derivative exhibited no molecular ion for Ins(1,4,5)P_3, or any of its fragments, only those corresponding to desired butynyl phospho-diester **21**. Furthermore, the ^{13}C-NMR spectrum contained a doublet for a methylene at 63.8 ppm corresponding to the 1-*C*H_2OP of the side-chain, clearly demonstrating that the inositol triphosphate head-group and butynyl chain were conjoined.

Confident that the acetylenic side-chain would withstand our total deprotection conditions, we again phosphitylated butynyl diol **19b** with (CneO)$_2$PCl (**11**), but this time the intermediate 4,5-*O*-bis(dicyanoethylphosphite) was thioylated with dibenzoyl tetrasulfide;[34] this crystalline but highly soluble reagent is much more reactive than elemental sulfur, easily prepared, and may be stored for years with refrigeration. The fully protected diphosphorothioate **22** (R = homopropargyl) was then unblocked under our standard conditions. This time the ^{1}H-NMR spectrum was very different to that of Ins(1,4,5)P_3, and the ^{31}P-NMR spectrum displayed a characteristic phosphorothioate resonance at 49.9 ppm integrating at twice the size of the phosphodiester signal at –0.62 ppm. Furthermore, the ^{13}C-NMR spectrum of butynyl diphosphorothioate **25** also exhibited a doublet for a methylene at 63.9 ppm, and the mass spectrum was consistent only with the desired product.

Conclusions

We developed a novel protective group strategy to access polyunsaturated PtdIns(4,5)P_2. This approach was chosen for its versatility, as the global deprotection conditions have already been demonstrated to be compatible with redox sensitive, nature-identical stearoyl arachidonoyl phosphoinositides. The interconversions of the key intermediates along this route relied upon the unusual properties of 1-chlorophenyl-4-ethoxypiperidinyl (Cpep) linear acetals adopted from oligonucleotide chemistry, and the selective unblocking of xanthen-9-ylidene cyclic acetals. We have also shown that this system can be extended to useful analogues containing acetylenic groups as a potential bioorthogonal handle, and phosphatase-resistant phosphorothioates.

Experimental

General experimental

^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on Brüker AC-270, AM-360, AV-400 and AV-500 spectrometers. Chemical shifts are referenced with respect to residual solvent signals, δ_H ($CHCl_3$) 7.25 ppm, δ_H (d_6-DMSO) 2.50 ppm, δ_H (HOD) 4.60 ppm, δ_C ($CHCl_3$) 77.50 ppm, δ_C (d_6-DMSO) 39.43 ppm, or an external reference, δ_P (H_3PO_4) 0.00 ppm. The splitting patterns for ^{1}H-NMR spectra are denoted as follows; s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), b (broad) and combinations thereof. Coupling constants (*J*) are in Hertz (Hz). ^{13}C-NMR assignments (C, CH, CH_2 and CH_3) and ^{1}H-NMR assignments, where given, were made with the aid of DEPT-90 and -135 and COSY experiments. Deuterated solvents were purchased from *Apollo Scientific Ltd.* (d_6-DMSO) or *Merck* (all others). 1-(4-Chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine was purchased from *Rasayan*, Inc., India. Other reagents were purchased from *Sigma-Aldrich Ltd.* or *Acros Organics* and used as supplied, except where specified. Mass spectra were recorded on a *VG* AutoSpec-Q (CI) or a *Micromass* LCT Premier (ESI) mass spectrometer. Reactions were carried out under anhydrous conditions under a nitrogen atmosphere. Dichloromethane, acetonitrile, toluene and triethylamine were distilled from calcium hydride; THF and diethyl ether were distilled from sodium metal and benzophenone; methanol was distilled from magnesium methoxide; all, except triethylamine, were stored over 4Å molecular sieves. Flash chromatography was carried out using silica from *British Drug Houses*, medium pressure liquid chromatography (MPLC) using *Merck* TLC grade silica, and reverse phase chromatography on *Merck* silanised silica. Thin layer chromatography was carried out using *Merck* silica gel 60 F_{254} glass-backed plates; compounds were visualised using UV light, *para*-anisaldehyde or $KMnO_4$ stains.

1-*O*-*tert*-Butyldiphenylsilyl-2,3-*O*-cyclopentylidene-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol. Freshly distilled TFA (1.9 mL, 25 mmol, 4.5 eq.) was added to a solution of 1-(4-chlorophenyl)-4-ethoxy-1,2,5,6-tetrahydropyridine (**10**, 3.90 g, 17 mmol, 3 eq.) in DCM (10 mL). Penta-protected inositol **9** (4.00 g, 5.5 mmol) in DCM (10 mL) was added over 1 h and the reaction was stirred overnight at rt. Excess triethylamine was added and the solution diluted with DCM. The organic layer was washed with sat. $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residual oil was fractionated by MPLC using a gradient of Et_2O-hexane (0:1 → 08:92 v/v) to afford the *title compound* as a colourless glass (3.45 g, 67%). HPTLC R_f (Et_2O-hexane, 1:9 v/v) 0.32; δ_H (400 MHz, $CDCl_3$) 7.88 (2H, dd, *J* 7.7, 1.1), 7.82 (2H, dd, *J* 7.6, 1.0), 7.45-7.36 (6H, m) (10 × Ph ***H***), 7.18 (2H, d, *J* 8.9), 6.76 (2H, d, *J* 9.0) (N-C_6***H***$_4$Cl), 4.71 (1H, t, *J* 8.6), 4.26-4.20 (2H, m), 4.05 (1H, t, *J* 8.1), 4.02 (1H, t, *J* 2.3), 3.78 (1H, dd, *J* 9.2, 1.7) (6 × Ins ***H***), 3.34-3.20 (2H, m, OC***H***$_2$Me), 3.16-3.08 (2H, m), 2.84-2.76 (2H, m) (C***H***$_2$NC***H***$_2$), 1.95 (2H, t, *J* 7.0), 1.77-1.64 (7H, m), 1.51 (1H, ddd, *J* 13.6, 10.0, 3.7) (5 × C***H***$_2$), 1.37-0.89 [39H, m, SiC***Me***$_3$ + (4 × Si***Pr***^{i}) + C***H***$_2$], 0.85 (3H, t, *J* 7.0, OCH_2***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 149.4 (Ar ***C***), 136.72 (2C), 136.63 (2C) (4 × Ph ***C***H), 134.0, 133.2 (2 × Ph ***C***), 129.58, 129.41 (2 × Ph ***C***H), 128.9 (2C), 127.6 (2C), 127.3 (2C) (6 × Ar ***C***H), 123.9 (Ar ***C***), 119.0 (acetal ***C***), 117.3 (2 × Ar ***C***H), 99.2 (acetal ***C***), 81.5, 78.2, 77.1, 76.6, 73.4, 71.1 (6 × Ins ***C***H), 55.6 (O***C***H_2Me), 46.6, 46.4 (***C***H_2N***C***H_2), 35.6, 35.2, 33.01, 32.91 (4 × ***C***H_2), 27.3 (SiC***Me***$_3$), 24.1, 23.3 (2 × ***C***H_2), 19.3 (Si***C***Me_3), 17.79, 17.72, 17.67,17.50, 17.45, 17.31, 17.26, 17.0 (4 × SiCH***Me***$_2$), 14.7 (OCH_2***Me***), 12.99, 12.95, 12.4, 12.1 (4 × Si***C***HMe_2) ppm; LRMS (ESI+) *m*/*z* found (%) $[M+H]^+$ 964.5 (33), $[M-OEt+H_2O]^+$ 936.5 (100), $[M-OEt]^+$ 918.5 (32), $[C_{13}H_{17}ClNO]^+$ 238 (46).

2,3-*O*-Cyclopentylidene-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol, 5b. 1M Tetrabutylammonium fluoride in THF (8.6 mL, 8.6 mmol, 3.6 eq.) was added to 1-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-cyclopentylidene-4,5-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol (2.30 g, 2.4 mmol) and the reaction was stirred at 75 °C overnight. The next day brine was added (30 mL) and the suspension was extracted with chloroform (× 3). The combined organic layers were dried ($MgSO_4$) and the solvent stripped off. The residue was fractionated by MPLC using a gradient of EtOAc-hexane (1:1 → 1:0 v/v) to afford the *title compound* as a colourless solid (864 mg, 75%). R_f (EtOAc) 0.54; δ_H (400 MHz, $CDCl_3$) 7.18 (2H, d, *J* 8.9), 6.84 (2H, d, *J* 9.0) (N-C_6***H***$_4$Cl), 4.30 (1H, dd, *J* 5.2, 4.1), 4.03-3.97 (2H, m) (3 × Ins ***H***), 3.95 (1H, bs, O***H***), 3.85 (1H, d, *J* 2.3), 3.81 (1H, dd, *J* 7.1, 3.7), 3.76 (1H, dd, *J* 10.1, 7.8) (3 × Ins ***H***), 3.66 (2H, q, *J* 7.0, OC***H***$_2$Me), 3.34-3.27 [4H, m, (2 × O***H***) + (2 × NCH***H***)], 3.17-3.11 (2H, m, 2 × NCH***H***), 2.16-2.07 (2H, m), 2.04-1.92 (4H, m), 1.82-1.77 (2H, m), 1.74-1.65 (4H, m) (6 × C***H***$_2$), 1.24 (3H, t, *J* 7.0, OCH_2***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 149.4 (Ar ***C***), 129.0 (2 × Ar ***C***H), 124.5 (Ar ***C***), 119.9 (acetal ***C***), 117.8 (2 × Ar ***C***H), 99.9 (acetal ***C***), 77.7, 75.50, 75.37, 74.1, 73.1, 70.0 (6 × Ins ***C***H), 56.6 (O***C***H_2Me), 46.78, 46.69 (***C***H_2N***C***H_2), 37.55, 37.47, 33.34, 33.25, 23.9, 23.5 (6 × ***C***H_2), 14.9 (***Me***) ppm; HRMS (EI+) *m*/*z* found $[M]^+$ 483.2037, $C_{24}H_{34}ClNO_7$ requires 483.2024.

1,4,5-*O*-Tris[di(cyanoethyloxy)phosphoryl]-2,3-*O*-cyclopentylidene-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol. Triol **5b** (130 mg, 0.27 mmol) and 1-*H*-3-nitro-1,2,4-triazole (**12**, 345 mg, 3.0 mmol, 11.3 eq.) were co-evaporated from pyridine-MeCN (1:1, 3 × 10 mL). The residue was re-dissolved in pyridine-MeCN (3:1 v/v, 4.0 mL). To this was added crude $(CneO)_2PCl$ (**11**, 948 mg, *ca.* 2.4 mmol, 9.0 eq.) in MeCN (4 mL) and the reaction was stirred for 1 h. The reaction was quenched with 3-hydroxypropionitrile (0.36 mL, 5.3 mmol, 12 eq.) and stirred for 15 min. The solvent was removed under high vacuum, the residue was re-dissolved in MeCN (3 mL) and cooled to 0 °C. *tert*-Butyl hydroperoxide (5M in hexane, 2.0 mL, 10.0 mmol) was added, the mixture allowed to warm to rt and it was stirred for 4 h. The solution was diluted with water until turbidity appeared and fractionated through a column of silanised silica, eluting with a gradient of water-MeCN (1:0 → 0:1 v/v). The appropriate fractions were combined and the MeCN evaporated under reduced pressure. The resulting aqueous suspension was saturated with NaCl and extracted with DCM (×3). The organic phase was dried (Na_2SO_4) and the solvent stripped off. The residual oil was fractionated by MPLC (silica pre-treated with 2% pyridine-DCM) using a gradient of MeOH-DCM (0:1 → 10:90 v/v) to afford the *title compound* as a colourless solid (172 mg, 62%). δ_H (400 MHz, $CDCl_3$) 7.17 (2H, d, *J* 8.9), 6.83 (2H, d, *J* 8.9) (N-C_6***H***$_4$Cl), 4.91

(1H, dt, *J* 8.9, 4.4), 4.84 (1H, q, *J* 8.4), 4.61-4.57 (2H, m), 4.52-4.48 (1H, m) (5 × Ins ***H***), 4.41-4.26 [13H, m, (6 × POC***H***$_2$) + Ins ***H***], 3.60-3.48 (2H, m, OC***H***$_2$Me), 3.37-3.28 (2H, m), 3.12-3.05 (2H, m) (C***H***$_2$NC***H***$_2$), 2.84-2.08 (12H, m, 6 × C***H***$_2$CN), 2.07-1.66 (12H, m) (6 × C***H***$_2$), 1.26 (3H, t, *J* 7.0, OCH$_2$***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 149.2 (Ar ***C***), 129.0 (2 × Ar ***C***H), 124.2 (Ar ***C***), 120.8 (acetal ***C***), 117.7 (2 × Ar ***C***H), 117.2, 117.01, 116.89 (2C), 116.70, 116.57 (6 × ***C***N), 101.49 (acetal ***C***), 81.55-81.43 (2C, m), 74.26, 72.69 (d, *J* 5.1), 72.16 (d, *J* 3.6), 70.51 (6 × Ins ***C***H), 63.24 (d, *J* 5.1), 62.93-62.76 (4C, m), 62.56 (d, *J* 5.0) (6 × PO***C***H$_2$), 57.04 (O***C***H$_2$Me), 46.84, 46.57, 36.01, 35.79, 33.44, 32.93, 23.94, 23.10, (8 × ***C***H$_2$), 19.71 (3C), 19.62 (3C) (6 × ***C***H$_2$CN), 15.1 (***Me***) ppm; δ_P (162 MHz, $CDCl_3$) −3.40, −3.64, −3.77 ppm; LRMS (FAB+) *m*/*z* (%) found $[M]^+$ 1041 (8), $[M–OEt]^+$ 996 (2).

***myo*-Inositol 1,4,5-triphosphate, 2.** 1,4,5-*O*-Tris[di(cyanoethyloxy)phosphoryl]-2,3-*O*-cyclopentylidene-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol (240 mg, 0.23 mmol) was evaporated from MeCN (3 × 5 mL) and re-dissolved in MeCN (2.0 mL). TmsCl (0.7 mL, 5.52 mmol, 24 eq.) then Barton's base (0.81 mL, 6.91 mmol, 30 eq.) were added. The reaction was stirred at rt for 16 h then the evaporated under reduced pressure and the residue was evaporated from MeCN (3 × 5 mL). The residue was triturated with Et_2O under argon. The filtrate was concentrated and taken up in 1M methanolic ammonia (3 mL). This solution was evaporated under reduced pressure and the residue dissolved in 90% acetic acid (10 mL). After 16 h, the solution was evaporated under reduced pressure and co-evaporated from ethanol (3 × 10 mL). The solids were triturated with DCM, then with MeCN to give the *title compound* as a colourless amorphous solid (88 mg, 88%). δ_H (400 MHz, D_2O) 4.12 (1H, q, *J* 9.1), 4.11 (1H, bs), 3.89-3.83 (2H, m), 3.73 (1H, t, *J* 9.4), 3.56 (1H, dd, *J* 9.7, 1.8) ppm; δ_C (100 MHz, D_2O) 78.1 (b), 76.7 (b), 75.0 (d, *J* 5.1), 70.9 (d, *J* 4.5), 70.6, 70.26 ppm; δ_P (162 MHz, D_2O) 1.54, 0.97, –0.07 ppm; LRMS (ESI-) *m*/*z* (%) found $[M–H]^-$ 418.3 (100).

2,3-*O*-Cyclopentylidene-4,5-*O*-(xanthen-9-ylidene)-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol. Triol **5b** (1.00 g, 2.07 mmol) was evaporated from pyridine (3 × 10 mL), then taken up in pyridine-DCM (1:1 v/v, 20 mL). The solution was cooled to 0 °C and solid 9,9-dichloroxanthene (**13**, 697 mg, *ca.* 80%, 2.3 mmol, 1.1 eq.) was added under a stream of argon. After stirring for 15 min the reaction was allowed to warm to rt. After 2 h, Et_3N (2 mL) and water (0.5 mL) were added, then the solvent was evaporated *in vacuo*. The residue was taken up in DCM (20 mL) and washed with sat. $NaHCO_3$ (× 3), then dried over $MgSO_4$. The solvent was stripped off and the crude material fractionated by MPLC, using a gradient of EtOAc-hexane (0:1 → 1:1 v/v) containing 0.5% Et_3N, to afford the *title compound* as a colourless glass (1.26 g, 92%). R_f (EtOAc-hexane, 1:4 v/v) 0.22; δ_H (400 MHz, $CDCl_3$) 7.73 (1H, dd, *J* 7.8, 0.9), 7.67 (1H, dd, *J* 7.8, 1.1), 7.64-7.41 (2H, m), 7.32-7.21 (4H, m) (8 × Ar ***H***), 7.18 (2H, d, *J* 8.9), 6.80 (2H, d, *J* 9.0) (N-C$_6$***H***$_4$Cl), 4.79 (1H, dd, *J* 10.9, 7.4), 4.63 (1H, d, *J* 7.8), 4.54 (1H, dd, *J* 7.8, 3.3), 4.46 (1H, t, *J* 7.6), 4.14-4.09 (2H, m) (6 × Ins ***H***), 3.64 (1H, quin, *J* 7.4), 3.51 (1H, quin, *J* 7.4) (2 × OCH***H***Me), 3.30 (1H, ddd, *J* 12.2, 6.3, 4.2), 3.24-3.18 (2H, m), 3.03 (1H, dt, *J* 13.2, 5.6) (C***H***$_2$NC***H***$_2$), 2.76 (1H, s, ex, O***H***), 2.06-1.92 (6H, m), 1.81-1.68 (6H, m) (6 × C***H***$_2$), 1.28 (3H, t, *J* 7.0, OCH$_2$***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 151.91, 151.75, 149.6 (3 × Ar ***C***), 130.07, 130.02, 128.9 (2C), 126.2, 125.3 (6 × Ar ***C***H), 124.1 (Ar ***C***), 123.33, 123.23 (2 × Ar ***C***H), 122.99, 122.80 (2 × Ar ***C***), 120.7 (acetal ***C***), 117.6 (2C), 116.89, 116.74 (4 × Ar ***C***H), 103.4, 99.9 (2 × acetal ***C***), 81.2, 76.55, 76.48, 75.3, 74.8, 71.1 (6 × Ins ***C***H), 56.1 (O***C***H$_2$Me), 47.2, 46.6 (***C***H$_2$N***C***H$_2$), 35.34, 35.31, 34.1, 32.2, 24.0, 22.8 (6 × ***C***H$_2$), 15.0 (***Me***) ppm; HRMS (ESI+) *m*/*z* (%) found $[M+H]^+$ 662.2521 (100), $C_{37}H_{41}ClNO_8$ requires 662.2521, $[M–OEt]^+$ 616.2115 (45).

1-*O*-[Di(2-cyanoethyloxy)phosphoryl]-2,3-*O*-cyclopentylidene-4,5-*O*-(xanthen-9-ylidene)-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin4-yl]-*myo*-inositol, 6. 2,3-*O*-Cyclopentylidene-4,5-*O*-(xanthen-9-ylidene)-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol (1.50 g, 2.3 mmol) was evaporated from pyridine (3 × 10 mL). The residue was re-dissolved in pyridine-MeCN (1:3 v/v, 14 mL) to which was added *N*-methylimidazole (1.08 mL, 13.6 mmol, 6 eq.), followed by crude (CneO)$_2$PCl (**11**, 1.64 g, *ca.* 4.6 mmol, 2.0 eq.) in MeCN (6 mL). After 30 min, 3-hydroxypropionitrile (0.92 mL, 14 mmol, 6 eq.) was added and stirring continued for 15 min. The solvent was stripped off, the residue was re-dissolved in MeCN (15 mL) and the solution was cooled to 0 °C. 5M *tert*-Butyl hydroperoxide in hexane (3.6 mL, 18 mmol) was added and the mixture was allowed to warm to rt. After 2 h the solution was diluted with water until turbidity appeared and fractionated through a column of silanised silica, eluting with a gradient of water-MeCN (1:0 → 0:1 v/v). The appropriate fractions were combined and the MeCN evaporated under reduced pressure. The resulting aqueous suspension was saturated with NaCl, extracted with DCM (×3), the organic phase was dried over Na_2SO_4 and the solvent stripped off. The residual oil was fractionated by MPLC using a gradient of EtOAc-hexane (1:1 → 9:1 v/v) to afford the *title compound* as a colourless glass (1.84 g, 96%). R_f (EtOAc-hexane, 8:2 v/v) 0.27; δ_H (400 MHz, $CDCl_3$) 7.68 (1H, dd, *J* 7.7, 1.4), 7.64 (1H, dd, *J* 7.8, 1.5), 7.49-7.44 (2H, m), 7.32-7.28 (3H, m), 7.24 (1H, td, *J* 7.6, 1.0) (8 × Ar ***H***), 7.20 (2H, d, *J* 8.9), 6.82 (2H, d, *J* 9.1) (N-C$_6$***H***$_4$Cl), 4.77 (1H, dt, *J* 9.0, 2.4), 4.71 (1H, dd, *J* 7.0, 1.9), 4.61 (1H, dd, *J* 11.1, 7.7), 4.57 (1H, t, *J* 3.4), 4.51 (1H, t, *J* 7.4) (5 × Ins ***H***), 4.46-4.31 (4H, m, 2 × PO***C***H$_2$), 4.07 (1H, dd, *J* 10.9, 7.1, Ins ***H***), 3.60 (1H, quin, *J* 7.4), 3.51 (1H, quin, *J* 7.3) (2 × OCH***H***Me), 3.33 (1H, ddd, *J* 12.7, 6.0, 4.0), 3.24-3.17 (2H, m), 3.02 (1H, ddd, *J* 12.4, 8.3, 3.6) (C***H***$_2$NC***H***$_2$), 2.85 (4H, t, *J* 6.1, 2 × ***C***H$_2$CN), 2.11-1.91 (6H, m), 1.79-1.69 (6H, m) (6 × C***H***$_2$), 1.09 (3H, t, *J* 6.9, OCH$_2$***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 151.9 (2C), 149.4 (3 × Ar ***C***), 130.32, 130.27, 128.9 (2C), 125.62, 125.47 (6 × Ar ***C***H), 124.1 (Ar ***C***), 123.37, 123.34 (2 × Ar ***C***H), 122.58, 122.53 (2 × Ar ***C***), 121.2 (acetal ***C***), 117.6 (2C), 117.01, 116.98 (4 × Ar ***C***H), 116.4 (2 × ***C***N), 103.7, 100.4 (2 × acetal ***C***), 80.9, 80.2 (d, *J* 6.8), 76.5, 75.8, 73.8 (d, *J* 4.7), 69.8 (6 × Ins ***C***H), 62.69-62.60 (m, 2 × PO***C***H$_2$), 56.4 (O***C***H$_2$Me), 47.2, 46.5 (***C***H$_2$N***C***H$_2$), 35.9, 35.5, 34.0, 33.2, 23.8, 22.9 (6 × ***C***H$_2$), 19.72-19.60 (m, 2 × ***C***H$_2$CN), 15.0 (***Me***) ppm; δ_P (162 MHz, $CDCl_3$) −3.59 ppm; LRMS (ESI+) *m*/*z* (%) $[M+Na]^+$ 870.0 (31), $[M+H]^+$ 847.8 (41), $[M–OEt]^+$ 801.9 (100), $[M–C_{13}H_{16}ClNO]^+$ 610.8 (44).

1-*O*-[(1,2-*O*-Dioleoylglycer-3-yloxy)(2-cyanoethyloxy)phosphoryl]-2,3-*O*-cyclopentylidene-4,5-*O*-(xanthen-9-ylidene)-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol, 16. Fully protected inositol 1-phosphate **6** (156 mg, 0.184 mmol) was taken up in MeCN-Et_3N (2:1 v/v, 2.4 mL) and stirred at rt for 16 h. The solvent was evaporated under reduced pressure and the residue was

re-evaporated from pyridine (3 × 3 mL). The residue was dissolved in MeCN (0.3 mL) and to this was added *N*-methylimidazole (0.15 mL, 1.8 mmol, 10 eq.), followed by dioleoylglycerol (**3b**, 228 mg, 0.37 mmol, 2 eq.). A solution of mesitylene sulfonyl chloride (201 mg, 0.92 mmol, 5 eq.) in pyridine (0.6 mL) was added drop-wise over 2 min to the reaction mixture. After 30 min water (0.1 mL) was added and the solution concentrated under reduced pressure. The residue was dissolved in EtOAc (80 mL) and washed with sat. $NaHCO_3$ (2 × 25 mL). The combined aqueous layers were back-extracted with EtOAc (2 × 10 mL), and the combined organic layers were dried ($MgSO_4$) then evaporated under reduced pressure. The residual oil was fractionated by MPLC (silica slurried with 1% pyridine-hexane) using a gradient of EtOAc-hexane (1:9 → 8:2 v/v) to afford the *title compound* as a colourless oil (170 mg, 66%). TLC R_f (EtOAc-hexane, 2:3 v/v) 0.40; δ_H (400 MHz, $CDCl_3$) 7.70-7.63 (2H, m), 7.45 (2H, bt, *J* 7.7), 7.30-7.26 (3H, m), 7.22 (1H, bt, *J* 7.7) (8 × Ar ***H***), 7.18 (2H, d, *J* 8.8), 6.82-6.78 (2H, m) (N-C_6***H***$_4$Cl), 5.41-5.31 [5H, m, (2 × ***H***C=C***H***) + glyc 2-***H***], 4.75-4.68 (2H, m), 4.62 (1H, dd, *J* 10.5, 8.0), 4.56-4.47 (2H, m), 4.42-4.17 (6H, m), 4.06 (1H, dd, *J* 11.0, 6.8) [(6 × Ins ***H***) + (2 × glyc OC***H***$_2$) + POC***H***$_2$], 3.64-3.45 (1H, m, OC***H***$_2$Me), 3.34-3.28 (1H, m), 3.23-3.14 (2H, m), 3.05-2.96 (1H, m) (C***H***$_2$NC***H***$_2$), 2.81 (2H, t, *J* 6.2, C***H***$_2$CN), 2.36-2.28 (4H, m, 2 × C***H***$_2$$CO_2$), 2.11-1.89 (14H, m), 1.79-1.67 (6H, m) 1.65-1.46 (4H, m) 1.39-1.23 (40H, m) (32 × C***H***$_2$), 1.09-1.03 (3H, m, OCH_2***Me***), 0.90 (6H, t, *J* 6.7, 2 × ***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 173.2, 172.85-172.75 (m) (2 × ***C***O_2), 151.9 (2C), 149.5 (3 × Ar ***C***), 130.20 (2C), 130.02 (2C), 129.7 (2C), 128.9 (2C), 125.70-125.65 (m), 125.46 [(6 × Ar ***C***H) + (2 × H***CC***H)], 124.2 (Ar ***C***), 123.3 (2 × Ar ***C***H), 122.6 (2 × Ar ***C***), 121.2 (acetal ***C***), 117.6 (2C), 117.0 (2C) (4 × Ar ***C***H), 116.1 (***C***N), 103.62-103.57 (m), 100.4 (2 × acetal ***C***), 81.1 (0.8C), 80.9 (0.2C), 79.5 (d, *J* 4.7), 76.49-76.45 (m), 75.8, 73.8 (b), 69.82 (0.2C), 69.73 (0.8C), 69.31-69.17 (m) [(6 × Ins ***C***H) + Glyc 2-***C***H], 66.3 (b), 62.40-62.31 (m), 61.53 (0.6C), 61.43 (0.4C) [(2 × Glyc ***C***H_2O) + PO***C***$H_2$$CH_2$CN], 56.4 (O***C***$H_2$Me), 47.2, 46.5 (***C***$H_2$N***C***$H_2$), 35.97 (0.2C), 35.90 (0.8C), 35.43 (0.6C), 35.35 (0.4C), 34.09-33.94 (3C, m), 33.2, 31.9 (2C), 29.77 (2C), 29.73 (2C), 29.5 (2C), 29.3 (4C), 29.22-29.10 (6C, m), 27.23 (2C), 27.20 (2C), 24.8 (2C), 23.8, 22.86, 22.69 (2C) (34 × ***C***H_2), 19.65-19.54 (m, ***C***H_2CN), 15.0 (OCH_2***Me***), 14.1 (2C, 2 × ***Me***) ppm; δ_P (162 MHz, $CDCl_3$) –2.82 (0.2P), –2.86 (0.4P), –2.93 (0.2P), –3.08 (0.2P) ppm; HRMS (ESI+) *m*/*z* (%) found $[M+H]^+$ 1397.7667 (49), $C_{79}H_{115}ClN_2O_{15}P$ requires 1397.7724.

1-*O*-[(1,2-*O*-Dioleoylglycer-3-yloxy)(2-cyanoethyloxy)phosphoryl]-2,3-*O*-cyclopentylidene-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol, 17. TFA-DCM (1:9 v/v, 0.53 mL, 3 eq.) was added to a solution of fully protected PtdIns **16** (330 mg, 0.24 mmol) in pyrrole-DCM (1:9 v/v, 1.5 mL, 9 eq.). After 50 s the reaction was quenched with sat. $NaHCO_3$ (60 mL) and extracted with $CHCl_3$ (3 × 15 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residual oil was fractionated by MPLC (silica slurried with 1% pyridine-hexane) using a gradient of hexane-EtOAc (4:1 → 0:1 v/v) to afford the *title compound* as a colourless oil (188 mg, 65%). TLC R_f (EtOAc) 0.24; δ_H (400 MHz, $CDCl_3$) 7.20 (2H, bd, *J* 8.1), 6.87 (2H, bd, *J* 8.1) (N-C_6***H***$_4$Cl), 5.41-5.32 (4H, m, 2 × ***H***C=C***H***), 5.27-5.20 (1H, m Glyc 2-***H***), 4.79 (1H, bs, O***H***), 4.61-4.53 (1H, m), 4.47-4.40 (1H, m), 4.36-4.12 (8H, m), 4.09-4.05 (1H, m) [(5 × Ins ***H***) + (2 × glyc OC***H***$_2$) + OC***H***$_2$$CH_2$CN], 3.84-3.77 (1H, m, Ins ***H***), 3.77-3.69 (1H, m), 3.66-3.57 (1H, m) (2 × OCH***H***Me), 3.42-3.29 (3H, m) [O***H*** + (2 × NCH***H***)], 3.24-3.05 (2H, m) (2 × NCH***H***), 2.76-2.64 (2H, m, C***H***$_2$CN), 2.36-2.27 (4H, m, 2 × C***H***$_2$$CO_2$), 2.13-1.91 (14H, m), 1.82-1.67 (6H, m), 1.67-1.55 (4H, m) (12 × C***H***$_2$), 1.39-1.19 (43H, m) [(20 × C***H***$_2$) + OCH_2***Me***], 0.92-0.87 (6H, m, 2 × ***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 173.3, 172.88 (0.2C), 172.78 (0.8C) (2 × ***C***O_2), 149.35 (0.4C), 149.28 (0.6C) (Ar ***C***), 130.0 (2C), 129.7 (2C), 129.0 (2C) [(2 × Ar ***C***H) + (2 × H***CC***H)], 124.35 (0.4C), 124.29 (0.6C) (Ar ***C***), 120.1 (acetal ***C***), 117.6 (2 × Ar ***C***H), 116.29 (0.6C), 116.14 (0.4C) (***C***N), 100.2 (acetal ***C***), 77.3, 76.24-76.15 (m), 74.09-73.08 (2C, m), 73.53-73.47 (2C, m), 69.25-69.17 (m) [(6 × Ins ***C***H) + Glyc 2-***C***H], 65.99-65.76 (m), 62.25-62.08 (m), 61.7 [(2 × Glyc ***C***H_2O) + PO***C***H_2], 56.7 (O***C***H_2Me), 47.66-46.33 (m, ***C***H_2N***C***H_2), 37.21-36.97 (2C, m), 34.1, 33.9, 33.4, 33.08 (0.2C), 32.95 (0.8C), 31.9 (2C), 29.76 (2C), 29.73 (2C), 29.5 (2C), 29.32 (4C), 29.22 (2C), 29.13 (4C), 27.22 (2C), 27.18 (2C), 24.8 (2C), 23.8, 23.4, 22.7 (2C) (34 × ***C***H_2), 19.53-19.40 (m, ***C***H_2CN), 14.9 (OCH_2***Me***), 14.1 (2C, 2 × ***Me***) ppm; δ_P (162 MHz, $CDCl_3$) –2.23 (0.2P), –2.30 (0.4P), –2.44 (0.4P) ppm; HRMS (EI+) *m*/*z* (%) found $[M+H]^+$ 1219.7261 (49), $C_{66}H_{109}ClN_2O_{14}P$ requires 1219.7305.

1-*O*-[(1,2-*O*-Dioleoylglycer-3-yloxy)(2-cyanoethyloxy)phosphoryl]-2,3-*O*-cyclopentylidene-4,5-*O*-bis[di(2-cyanoethyloxy)phosphoryl]-6-*O*-[1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl]-*myo*-inositol, 7. PtdIns diol **17** (160 mg, 0.34 mmol) was evaporated from pyridine (3 × 3 mL) and the residue was re-dissolved in pyridine (0.3 mL) and MeCN (1.0 mL). To this was added *N*-methylimidazole (0.13 mL, 1.57 mmol, 12 eq.), then crude $(CneO)_2PCl$ (**11**, 176 mg, *ca.* 0.52 mmol, 4 eq.) in MeCN (1 mL). After 30 min the reaction was quenched with 3-hydroxypropionitrile (0.11 mL, 1.57 mmol, 12 eq.) and stirred for 15 min. The solvent was stripped off, the residue re-dissolved in MeCN (1.2 mL), and the solution cooled to 0 °C. *tert*-Butyl hydroperoxide (5M in hexane, 0.4 mL, 2.1 mmol) was added, the mixture allowed to warm to rt and it was stirred for 165 min. The solution was diluted with water until turbidity appeared and fractionated through a column of silanised silica, eluting with a gradient of water-MeCN (1:0 → 0:1 v/v). The appropriate fractions were combined and the MeCN evaporated under reduced pressure. The resulting aqueous suspension was saturated with NaCl and extracted with $CHCl_3$ (×3). The organic phase was dried (Na_2SO_4) and the solvent stripped off. The residual oil was fractionated by MPLC using a gradient of MeOH-DCM (0:1 → 5:95 v/v) to afford the *title compound* as a near-colourless oil (140 mg, 67%). TLC R_f (MeOH-DCM, 8:92) 0.32; δ_H (400 MHz, $CDCl_3$) 7.20 (2H, d, *J* 9.0), 6.86 (2H, d, *J* 8.8) (N-C_6***H***$_4$Cl), 5.39-5.32 (4H, m, 2 × ***H***C=C***H***), 5.32-5.25 (1H, m + glyc 2-***H***), 4.93-4.84 (2H, m), 4.65-4.59 (2H, m), 4.53-4.50 (1H, m), 4.45-4.13 (15H, m) [(6 × Ins ***H***) + (2 × Glyc OC***H***$_2$) + (5 × POC***H***$_2$$CH_2$CN)], 3.63-3.51 (2H, m, OC***H***$_2$Me), 3.39-3.32 (2H, m), 3.16-3.04 (2H, m) (4 × NCH***H***), 2.88-2.80 (10H, m, 5 × C***H***$_2$CN), 2.37-2.30 (4H, m, 2 × C***H***$_2$$CO_2$), 2.11-1.84 (14H, m), 1.81-1.68 (6H, m), 1.66-1.57 (4H, m) (12 × C***H***$_2$), 1.38-1.23 [43H, m, (20 × C***H***$_2$) + OCH_2***Me***], 0.89 (6H, t, *J* 6.8, 2 × ***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 173.35-173.18 (m), 172.93-172.80 (m) (2 × ***C***O_2), 149.2 (Ar ***C***), 130.0 (2C), 129.7 (2C), 129.0 (2C), [(2 × Ar ***C***H) + (2 × H***CC***H)], 124.5 (Ar ***C***), 120.86 (0.8C), 120.81 (0.2C) (acetal ***C***), 117.7 (2 × Ar ***C***H), 117.07-116.37 (5 × ***C***N), 101.46 (acetal ***C***), 81.61-81.25 (2C, m), 74.48-74.34 (m),

72.77-72.56 (m), 72.2, 70.7, 69.35-69.16 (m) [(6 × Ins ***C***H) + Glyc 2-***C***H], 66.38-66.22 (m), 62.26-63.20 (m), 62.82-62.79 (2C, m), 62.61-62.50 (2C, m), 61.77-61.71 (m) [(2 × Glyc ***C***H_2O) + (5 × PO***C***H_2CH_2CN)], 57.0 (O***C***H_2Me), 46.9, 46.7 (***C***H_2N***C***H_2), 36.07 (0.6C), 36.04 (0.4C), 35.82 (0.4C), 35.75 (0.6C), 34.11, 33.96, 33.5, 33.0, 31.9 (2C), 29.7 (4C), 29.5 (2C), 29.31 (4C), 29.24, 29.22, 29.14 (2C), 29.11 (2C), 27.22 (2C), 27.18 (2C), 24.8 (2C), 23.9, 23.1, 22.7 (2C) (34 × ***C***H_2), 19.61-19.54 (m, 5 × ***C***H_2CN), 15.1 (OCH$_2$***Me***), 14.1 (2C, 2 × ***Me***) ppm; δ_P (162 MHz, $CDCl_3$) –2.90 (0.4P), –3.08 (0.2P), –3.17 (0.2P), –3.27 (0.2P), –3.50 (1P), –3.65 (1P) ppm; LRMS (ESI+) *m*/*z* (%) found $[M+H]^+$ 1591.7 (77), $[M+Na-C_{13}H_{16}ClNO]^+$ 1376.6 (100), $[M-C_{13}H_{16}ClNO]^+$ 1354.6 (97).

Dioleoyl phosphatidyl-*myo*-inositol 4,5-diphosphate, 1b. Fully protected dioleoyl PtdInsP_2 **7** (115 mg, 0.072 mmol) was taken up in MeCN (0.9 mL) to which was added TmsCl (0.18 mL, 1.4 mmol, 20 eq.) and Barton's base (0.21 mL, 1.8 mmol, 25 eq.). The reaction was stirred at rt for 16 h, then the solvent was evaporated under reduced pressure and the residue was evaporated from MeCN (3 × 5 mL). The resulting mixture was triturated with Et_2O, then the filtrate was evaporated to dryness and taken up in 1M methanolic ammonia (2 mL). After 25 min the solution was evaporated under reduced pressure and the residue was dissolved in 80% acetic acid (5 mL). After 3 h, the solvent was stripped off under reduced pressure and the residue re-evaporated from EtOH (3 × 10 mL). The solids were triturated with DCM and then with MeCN to give the *title compound* as a near-colourless solid (61 mg, 88%). δ_H (400 MHz, d_4-AcOH-D_2O-$CDCl_3$ 4:2:1 v/v) 5.44-5.37 (4H, m, 2 × ***H***C=C***H***), 5.35-5.30 (1H, m, Glyc 2-***H***), 4.50-4.43 (2H, m), 4.34 (1H, bs), 4.27-4.08 (5H, m) [(4 × Ins ***H***) + (2 × Glyc OC***H***$_2$)], 4.02 (1H, bt, *J* 9.5, Ins-***H***), 3.78 (1H, bd, *J* 9.6, Ins 3-***H***), 2.42 (2H, t, *J* 7.5), 2.35 (2H, t, *J* 7.7) (2 × C***H***$_2$$CO_2$) 2.11-2.04 (8H, m, 2 × C***H***$_2$CHCHC***H***$_2$), 1.69-1.59 (4H, m, 2 × C***H***$_2$$CH_2CO_2$), 1.43-1.29 (40H, m, 20 × C***H***$_2$), 0.93 (6H, t, *J* 6.8, 2 × ***Me***) ppm; δ_C (100 MHz, $CDCl_3$) 174.46, 174.27 (2 × ***C***O_2), 129.9 (2C), 129.6 (2C) (2 × H***CC***H), 78.56-78.38 (m), 77.31-77.19 (m), 75.8 (d, *J* 5.3), 70.9 (2C, b), 70.52-70.34 (m), 70.3 [(6 × Ins ***C***H) + Glyc 2-***C***H], 63.97-63.82 (m), 63.0 (2 × Glyc ***C***H_2O), 34.19, 34.07, 31.9 (2C), 29.80, 29.77, 29.72 (2C), 29.51 (2C), 29.37-29.08 (10C, m), 27.2 (4C), 24.96, 24.83, 22.6 (2C) (28 × C***H***$_2$), 13.9 (2C, 2 × ***Me***) ppm; δ_P (162 MHz, $CDCl_3$) 0.77, 0.33, –1.12 ppm; HRMS (ESI–) *m*/*z* (%) found $[M-H]^-$ 1021.4791 (58), $C_{45}H_{84}O_{19}P_3$ requires 1021.4820, $[M-2H+Na]^-$ 1043.4681 (44), $[M-2H]^{2-}$ 510.2282 (100).

Acknowledgements

Funding from the UK Medical Research Council (PRJG, grant no. G 120/709) and Biotechnology and Biological Sciences Research Council (NP, grant no. 28/B17388) are gratefully acknowledged.

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