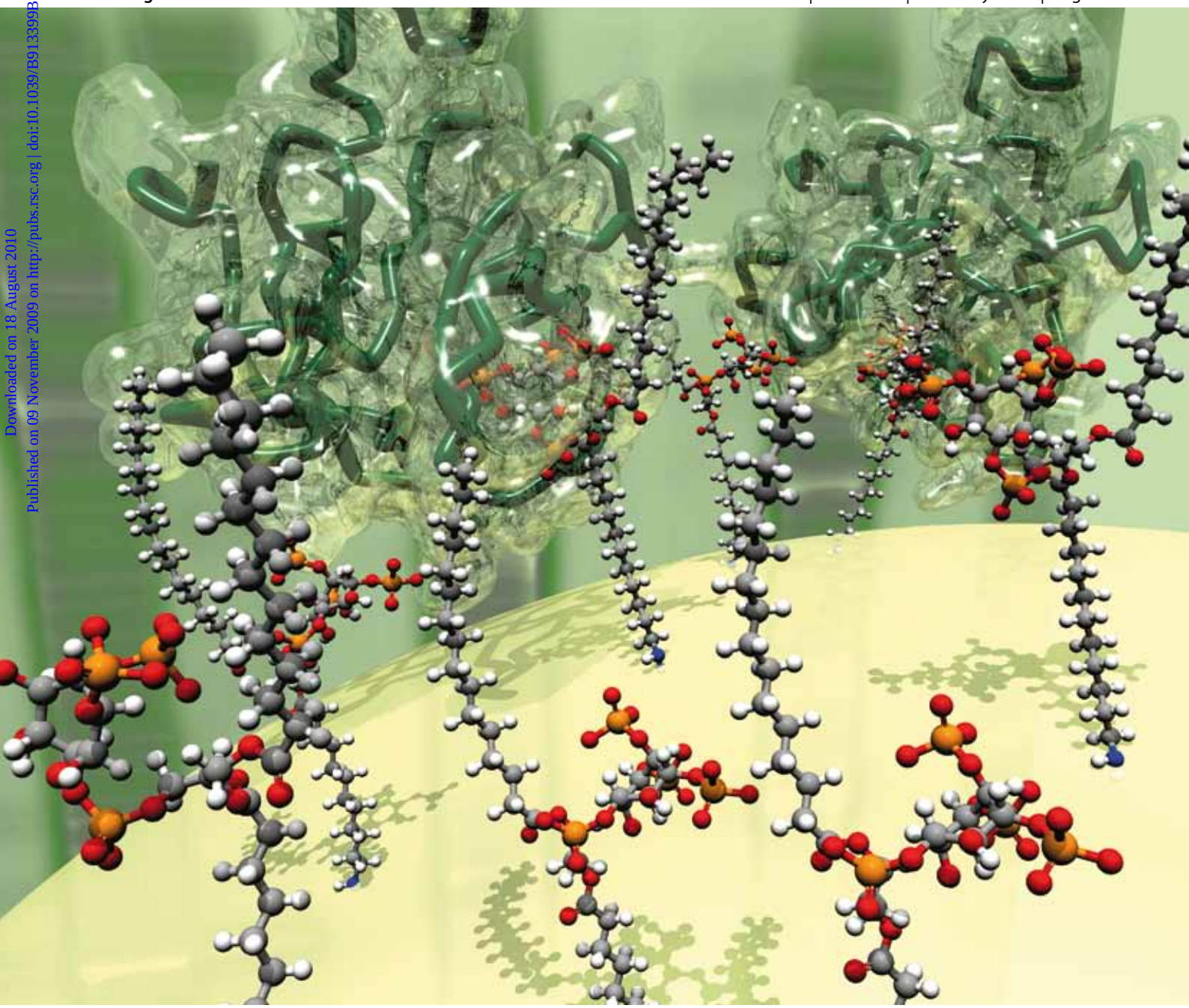
Organic & Biomolecular Chemistry

www.rsc.org/obc

Volume 8 | Number 1 | 7 January 2010 | Pages 1–284



ISSN 1477-0520

RSC Publishing

1477-0520(2010)8:1;1-J

Synthesis and biological evaluation of phosphatidylinositol phosphate affinity probes†

Stuart J. Conway,[a] James Gardiner,*[b] Simon J. A. Grove,[a] Melloney K. Johns,[a] Ze-Yi Lim,[a] Gavin F. Painter,[a] Diane E. J. E. Robinson,*[b] Christine Schieber,[b] Jan W. Thuring,[a] Leon S.-M. Wong,[b] Meng-Xin Yin,[b] Antony W. Burgess,[c] Bruno Catimel,[c] Phillip T. Hawkins,[d] Nicholas T. Ktistakis,[d] Leonard R. Stephens[d] and Andrew B. Holmes*[b]

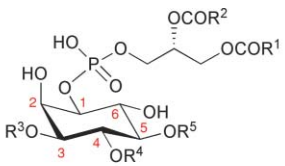
Received 6th July 2009, Accepted 29th September 2009
First published as an Advance Article on the web 9th November 2009
DOI: 10.1039/b913399b

The synthesis of the complete family of phosphatidylinositol phosphate analogues (PIPs) from five key core intermediates **A**–**E** is described. These core compounds were obtained from *myo*-inositol orthoformate **1** *via* regioselective DIBAL-H and trimethylaluminium-mediated cleavages and a resolution–protection process using camphor acetals **10**. Coupling of cores **A–E** with phosphoramidites **34** and **38**, derived from the requisite protected lipid side chains, afforded the fully-protected PIPs. Removal of the remaining protecting groups was achieved *via* hydrogenolysis using palladium black or palladium hydroxide on carbon in the presence of sodium bicarbonate to afford the complete family of dipalmitoyl- and amino-PIP analogues **42**, **45**, **50**, **51**, **58**, **59**, **67**, **68**, **76**, **77**, **82**, **83**, **92**, **93**, **99** and **100**. Investigations using affinity probes incorporating these compounds have identified novel proteins involved in the PI3K intracellular signalling network and have allowed a comprehensive proteomic analysis of phosphoinositide interacting proteins.

Introduction

Phosphatidylinositol phosphates (PtdInsPs or PIPs) are an important class of membrane phospholipids.[1,2] These lipids are involved in intracellular signalling mechanisms that are known to play a central role in fundamental cellular functions such as vesicle trafficking, apoptosis, cell proliferation and metabolism.[3] Furthermore, lack of regulation of these pathways is known to result in a range of disease conditions, such as cancer, diabetes, Alzheimer's disease and autoimmune disorders.[4]

PIPs are phosphorylated derivatives of *myo*-inositol. There are eight known naturally occurring classes of PIPs differing only in the degree of phosphorylation of the hydroxyl groups at the 3-, 4- and 5-positions of the inositol rings (Fig. 1). The natural materials carry a range of unsaturated fatty acid derivatives at the *sn*-1 and *sn*-2 positions. The nature and degree of saturation of the lipid chain is organism dependent. In this work we focus on saturated analogues and largely on palmitoyl derivatives, which, in our experience, have shown no marked influence on the binding properties of proteins to the synthetic PIPs. To understand the roles of each of these PIPs in their signalling pathways and their effect on downstream processes, it is necessary to identify proteins



naturally occurring: $R^1 = C_{17}H_{35}$, $R^2 = C_{19}H_{31}$
synthetic analogues: $R^1 = C_{15}H_{31}$ or $C_{11}H_{22}NH_2$, $R^2 = C_{15}H_{31}$

PI: $R^3 = R^4 = R^5 = H$
PI(3)P: $R^3 = PO(OH)_2$, $R^4 = R^5 = H$
PI(4)P: $R^3 = H$, $R^4 = PO(OH)_2$, $R^5 = H$
PI(5)P: $R^3 = R^4 = H$, $R^5 = PO(OH)_2$
PI(3,4)P2: $R^3 = R^4 = PO(OH)_2$, $R^5 = H$
PI(3,5)P2: $R^3 = R^5 = PO(OH)_2$, $R^4 = H$
PI(4,5)P2: $R^3 = H$, $R^4 = R^5 = PO(OH)_2$
PI(3,4,5)P3: $R^3 = R^4 = R^5 = PO(OH)_2$

Fig. 1 General structure of phosphatidylinositol phosphates.

that exhibit binding specificity. However, the inability to isolate practically useful amounts of these PIP compounds from cells has led to a need for efficient syntheses.[5-25] Furthermore, analogues of these compounds that can be used as affinity probes to investigate and isolate particular binding proteins, have become attractive targets[1,5,7,12,13,24,26–28] Our approach has been the incorporation of an ω-amino group on the *sn*-1 position of a saturated lipid side chain (abbreviated as NH_2-PIP) to facilitate immobilisation of the PIP by covalent linkage through an amide bond onto Affi-Gel® 10 beads.[12,28]

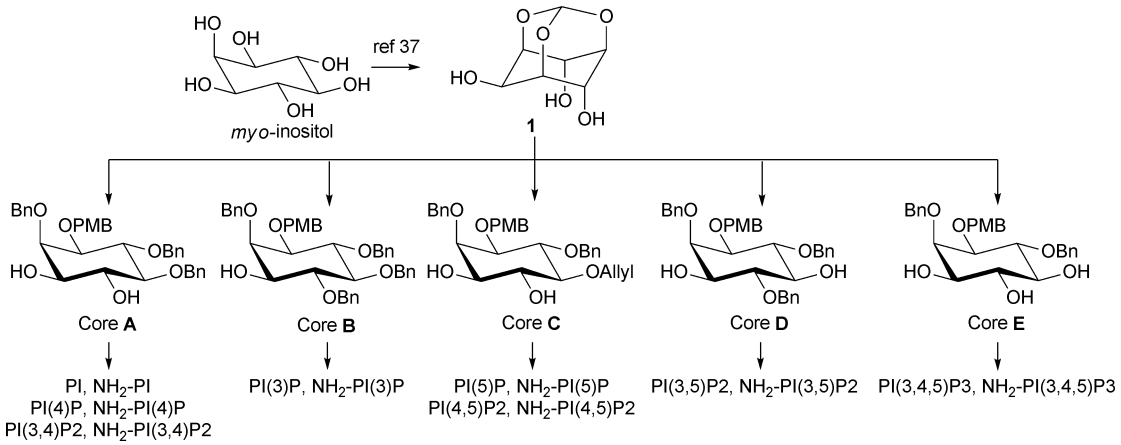
Herein, we describe our contribution over the last two decades to the synthesis of the complete family of PIP analogues, which we have used as probes for biological systems.[28-35] We have previously reported the synthesis of PI(3,4,5)P3 and PI(4,5)P2 affinity beads.[12,16]

Synthetic approach

Our synthetic approach is simple and divergent and involves the late-stage coupling of phosphoramidites, derived from the

[a]*Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK*
[b]*School of Chemistry, Bio21 Institute, University of Melbourne, Building 102, 30 Flemington Road, Parkville, Victoria 3010, Australia*
[c]*Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Royal Melbourne Hospital, Parkville, Victoria 3052, Australia*
[d]*Babraham Institute, Department of Signalling, Inositide Laboratory Cambridge, CB2 4AT, UK*

† Electronic supplementary information (ESI) available: Experimental procedures for **27–29**, **39–45**, **49**, **51–69**, **71**, **75**, **77**, **81**, **83–85**, **90**, **92**, **102**, **104–110**. See DOI: 10.1039/b913399b



Scheme 1 Core building blocks for the synthesis of all the PIPs.

requisite protected lipid side chains, to the suitably protected phosphorylated inositol cores followed by a global deprotection. All eight PIPs and their analogues can be synthesised from five inositol core compounds (**A–E**, Scheme 1).[36] Each of these intermediates can in turn be synthesised from the readily available *myo*-inositol orthoformate **1**[37] by judicial choice of protection, resolution and deprotection strategies. It should be noted that cores **D** and **E** can also be obtained from core **C** by suitable protecting group manipulation; furthermore, in principle all the PIPs can be obtained from core **C**, though this is not the most efficient route. This report focuses primarily on the most efficient routes to the target compounds.

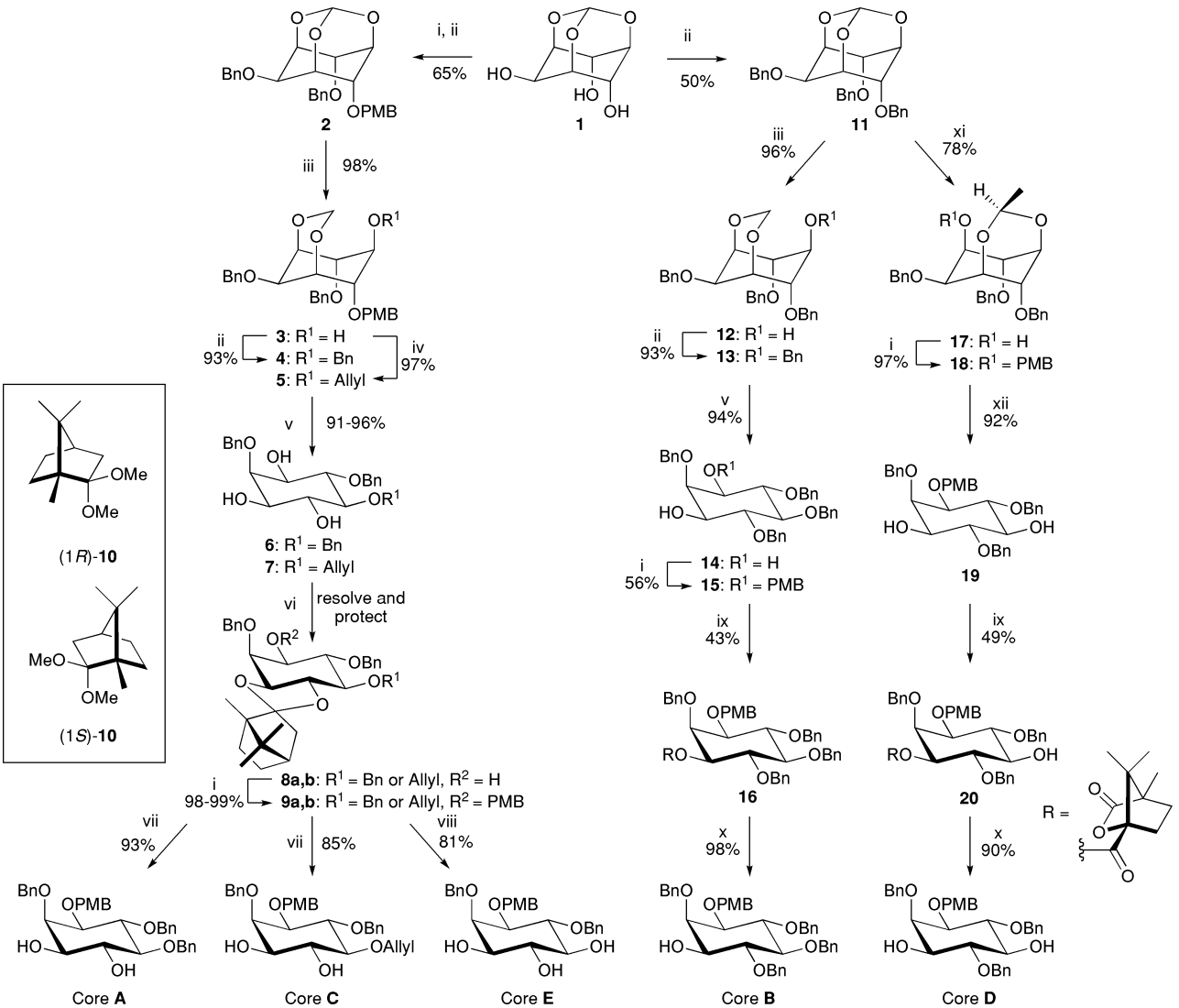
Synthesis of cores A–E

The syntheses of core molecules **A–E** are shown in Scheme 2 and have previously been reported.[12,16,38,39] To gain access to cores **A**, **C** and **E**, selective mono PMB-protection of **1** followed by benzylation of the remaining hydroxyl groups provides orthoformate **2**. Selective cleavage of the orthoformate by treatment with DIBAL-H affords 1,3-acetal **3**. Benzylation or allylation and subsequent removal of the acetal and PMB protecting groups give the racemic triols **6** and **7**. Resolution of the racemic triols **6** and **7** with simultaneous protection of the corresponding vicinal diols according to an elegant procedure previously reported by Bruzik[40,41] afforded an efficient approach to the unnatural (1L-series) and natural (1D-series) of PIPs. Thus, treatment of triol **7** with the camphor acetal (1*R*)-**10** afforded a mixture of four diastereomers of 3,4-acetal **8**, one of which could be separated by column chromatography from the mixture of the other three; deprotection of the acetal allows preparation of the unnatural enantiomer of the triol **7** corresponding to the 1L-series (for the use of this enantiomer see Scheme 12). The remaining mixture is therefore enriched in the desired enantiomer, which when protected with dimethylacetal (1*S*)-**10** provides diastereomer **8** with the correct absolute configuration corresponding to the natural 1D-series. It should be noted that direct resolution/protection of **6** or **7** with dimethylacetal (1*S*)-**10** also provides the required diastereomer of **8** in comparable yields. This route also allows separation of the two enantiomers of the inositol ring and provides access to the unnatural enantiomers of the PIPs.[21] Protection of the 1-position of the inositol ring of **8** as the *p*-methoxybenzyl ether, followed by deprotection of either the 3,4 and/or 5-positions afforded cores **A**, **C** and **E**.

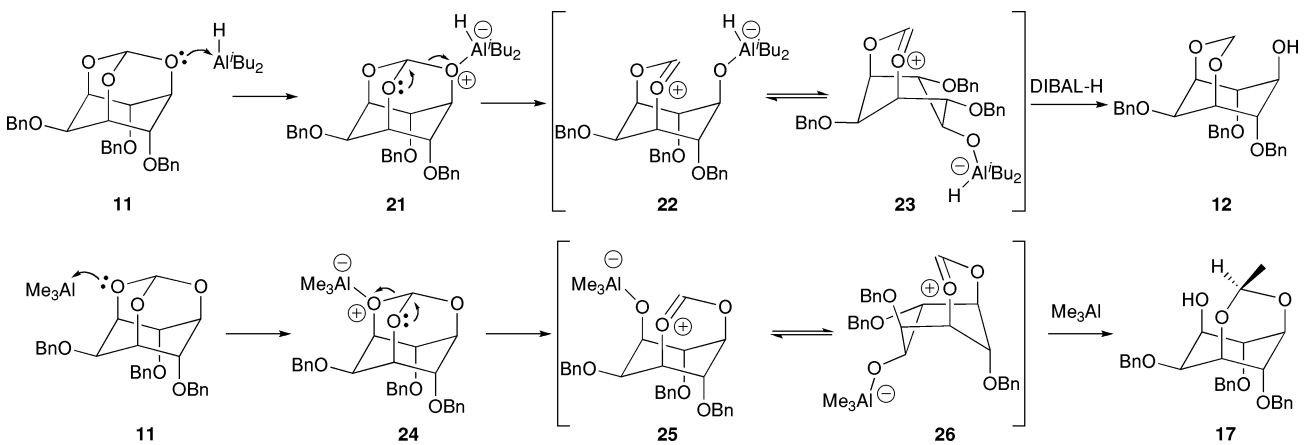
The synthesis of cores **B** and **D** begins with the complete benzylation of *myo*-inositol orthoformate **1** to form benzyl ether **11**. The differentially protected inositol acetals **12** and **17** can be obtained *via* selective Lewis acid-mediated reductive or alkylative cleavage of trisbenzyl orthoformate **11**.[39,42] As seen in the syntheses of cores **A**, **C** and **E**, the use of DIBAL-H selectively afforded the 1,3-acetal, **12**. In contrast, the use of trimethyl aluminium resulted in the 3,5-acetal, **17**. Protection of the free hydroxyl groups in **12** and **17** followed by removal of the acetal groups gave diols **14** and **19** respectively. Subsequent mono-protection of the 1-position of **14** gave racemic *p*-methoxybenzyl ether **15**. Racemic alcohols **15** and **19** were then resolved as their camphanate esters. Subsequent hydrolysis of esters **16** and **20** afforded enantiomerically pure cores **B** and **D** respectively.

The stereoselectivity of these Lewis acid-mediated reductive and alkylative cleavages of orthoformate **11** have been investigated and the mechanisms proposed (Scheme 3).[42] In the DIBAL-H reduction of **2** to **3**, and **11** to **12**, it was determined that at least 2 molar equivalents of DIBAL-H are required for the reaction to proceed to completion. It is proposed that the first equivalent of DIBAL-H acts as a Lewis acid, coordinating to the C-5 oxygen, presumably the most sterically accessible oxygen. Subsequent cleavage of the orthoformate affords the oxocarbenium ion **22**, the unfavourable 1,3-steric interactions in which can be accommodated by a ring flip to the boat conformation **23**. Reduction of this oxocarbenium ion by a second equivalent of DIBAL-H from the less hindered face produces the 1,3-acetal **12** exclusively.

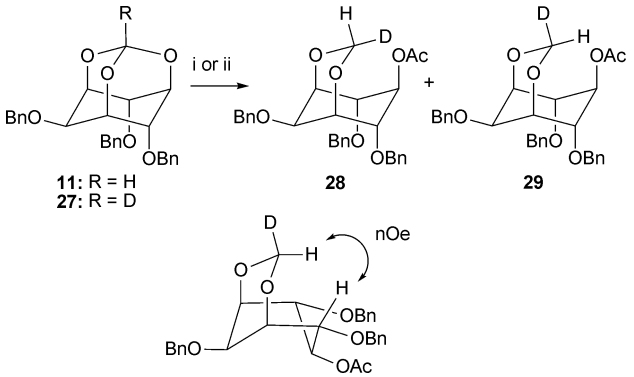
The stereoselectivity of the hydride delivery to the orthoformate was established through two deuterium-labelling experiments (Scheme 4). Firstly, reduction of orthoformate **11** using 2.5 molar equivalents of diisobutylaluminium deuteride[43] (DIBAL-D) resulted in acetals **28** and **29** in a 92 : 8 ratio. The reciprocal reaction in which deuterated orthoformate **27**[44] was treated with diisobutylaluminium hydride (DIBAL-H), afforded the two products with an inverted selectivity of 10 : 90 (**28** : **29**). The structures were confirmed by nOe experiments. This switch in selectivity between the two diastereomers **28** and **29**, indicates that the reductive cleavage is stereoselective in the delivery of the



Scheme 2 Synthesis of the core building blocks **A–E**. *Reagents and conditions*: i. NaH, PMBCl, DMF, 0 °C → rt; ii. NaH, BnBr, DMF, 0 °C → rt; iii. DIBAL-H (2.5 eq.), CH_2Cl_2/hexanes, 0 °C → rt; iv. NaH, allyl bromide, DMF, 0 °C → rt; v. HCl, MeOH, reflux; vi. (1*R*)-**10**, TsOH·H_2O (cat.), CH_2Cl_2, reflux, separate, AcCl, CH_2Cl_2–MeOH (2/1 v/v), then (1*S*)-**10**, TsOH·H_2O (cat.), CH_2Cl_2, reflux; vii. AcCl, CH_2Cl_2–MeOH (2/1 v/v), viii. From **9b**: $(Ph_3P)_3RhCl$, DABCO, EtOH–toluene/H_2O (7/3/1 v/v/v), reflux, then AcCl, CH_2Cl_2–MeOH (2/1 v/v); ix. (1*S*)-(–)-camphanic chloride, Et_3N or pyridine, CH_2Cl_2, rt; x. LiOH, THF–H_2O (10/1 v/v), rt; xi. Me_3Al (2.5 eq.), CH_2Cl_2/hexanes, 0 °C → rt; xii. HCl, CH_2Cl_2–MeOH (2/1 v/v), rt.



Scheme 3 Proposed mechanisms of the Lewis acid-mediated regioselective cleavage of orthoformate **11**[42]



Scheme 4 Deuterium labelling experiments for the cleavage of the orthoformates **11** and **27**. *Reagents and conditions*: i. From **11**: DIBAL-D (2.5 eq.), CH_2Cl_2, 0 °C → rt, then Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 83%, **28** : **29**: 92 : 8; ii. From **27**: DIBAL-H (2.5 eq.), CH_2Cl_2, 0 °C → rt, then Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 93%, **28** : **29**: 10 : 90.

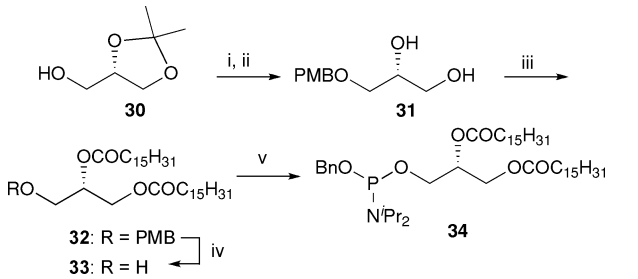
hydride (or deuteride) occurring preferentially on the *endo* face, *syn* to the broken C–O bond. This retention of stereochemistry discounts a mechanism pathway in which the orthoformate in **21** (Scheme 3) is cleaved *via* inter- or intramolecular delivery of the hydride. Whilst these experiments do not allow the distinction between intramolecular delivery of the hydride (or deuteride) in **22** and intermolecular delivery in **23**, the latter is a more feasible pathway when the unfavourable 1,3-steric interactions of **22** are considered.

In contrast, the trimethylaluminium-mediated cleavage of the orthoformate **11** exclusively affords the acetal **17**, which results from the cleavage of one of two identical alternative C–O bonds in the orthoformate **24**. This outcome indicates that trimethylaluminium is presumably sufficiently small to coordinate to one or both of the equivalent oxygens at the 1- or 3-positions (perhaps through intramolecular delivery by the equatorial benzyloxy group), allowing an alternative cleavage pathway resulting in the oxocarbenium ion **25**. Delivery of a methyl group to the *exo* face of the oxocarbenium **26** affords the 3,5-acetal **17**.

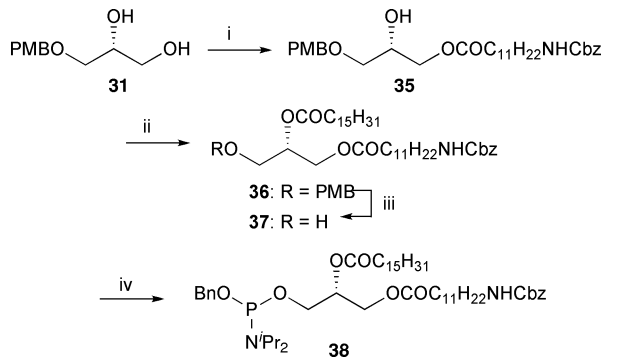
Synthesis of phosphoramidites

To install the lipid side chains, the phosphoramidites **34**[45] and **38**[12] were synthesised from (+)-1,2-*O*-isopropylidene-glycerol **30** (Scheme 5 and Scheme 6). The dipalmitoyl derivative **32** was obtained by esterification of the diol **31**, which resulted from PMB protection of the free alcohol in **30** followed by methanolysis of the acetal (Scheme 5). Subsequent removal of the PMB group followed by a 1*H*-tetrazole mediated coupling of the alcohol **33** with (benzyloxy)bis(*N*,*N*-diisopropylamino)phosphine[46] afforded the phosphoramidite **34**, which is used for the synthesis of the dipalmitoyl PIP analogues.

In order to prepare the PIP derivatives that can be attached to beads for use in protein affinity experiments, side chains containing an ω-amino group at the *sn*-1-position were also synthesised (Scheme 6). A DCC-mediated coupling of diol **31** allowed selective esterification of the primary alcohol to afford the alcohol **35** in good yield. Esterification of the free alcohol with palmitoyl chloride and removal of the PMB group provided the primary alcohol **37** which when coupled with (benzyloxy)bis(*N*,*N*-



Scheme 5 Synthesis of dipalmitoyl phosphoramidite **34**. *Reagents and conditions*: i. NaH, PMBCl, DMF, 0 °C → rt, quant.; ii. *p*-TsOH, MeOH, reflux, 82%; iii. $C_{15}H_{31}COCl$, pyr., DMAP, CH_2Cl_2, 0 °C → rt, 88%; iv. DDQ, CH_2Cl_2–H_2O (16/1, v/v), rt, 85%; v. $(BnO)P(N^iPr_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 92%.



Scheme 6 Synthesis of amino phosphoramidite **38**. *Reagents and conditions*: i. $CbzNHC_{11}H_{22}CO_2H$, DCC, DMAP, CH_2Cl_2, 0 °C → rt, 87%; ii. $C_{15}H_{31}COCl$, pyr., DMAP, CH_2Cl_2, 0 °C → rt, quant.; iii. DDQ, CH_2Cl_2–H_2O (16/1, v/v), rt, 80%; iv. $(BnO)P(N^iPr_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 92%.

diisopropylamino) phosphine gave phosphoramidite **38**, providing access to the NH_2-PIP analogues.

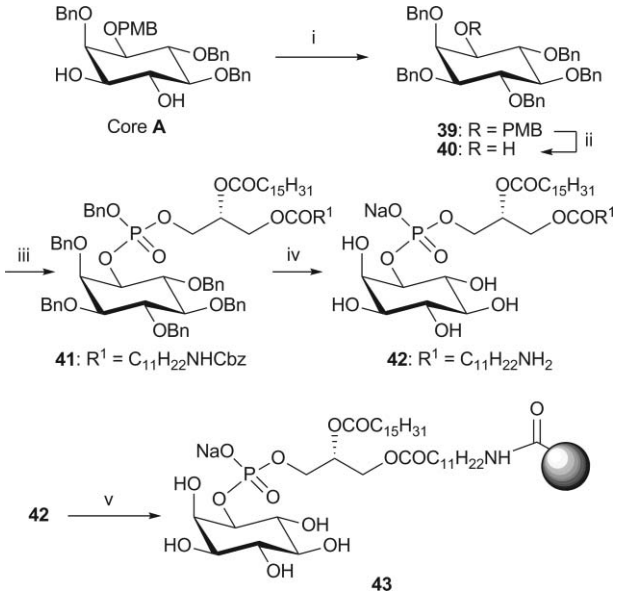
With all the necessary building blocks in hand, the whole family of the PIP analogues could be synthesised.

Synthesis of PI,[7,8,10] PI(3)P,[7,9,10,14–16 19,23] PI(4)P[10],[19] and PI(5)P[5,10,14,18] analogues

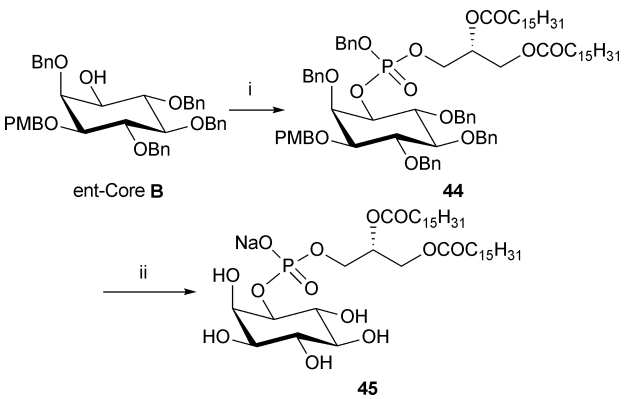
The simplest member of this family of compounds is phosphatidylinositol (PI), which contains the phospholipid side chain at the 1-position and none of the 3-, 4- or 5-positions are phosphorylated (Fig. 1).

The inositol moiety of PI (**40**) was obtained from core **A** by benzylation of the 3- and 4-hydroxyl groups and removal of the PMB protecting group from the 1-position (Scheme 7). Coupling of the alcohol **40** with the amino-phosphoramidite **38** in the presence of an excess of 1*H*-tetrazole, followed by *m*CPBA oxidation of the intermediate phosphite (not isolated) provides the fully protected phosphate **41**. A global deprotection *via* hydrogenolysis in the presence of palladium black and sodium hydrogen carbonate[16,24] gave NH_2-PI sodium salt **42**,[47] which was coupled to NHS-activated Affi-Gel® 10 beads in the presence of sodium hydrogen carbonate to give affinity probe **43**.[48]

To demonstrate the versatility of our synthetic strategy the enantiomer of core **B** was treated with dipalmitoyl phosphoramidite **34** and 1*H*-tetrazole followed by oxidation with *m*CPBA to give the fully protected dipalmitoyl-PI **44** (Scheme 8). Global deprotection



Scheme 7 Synthesis of immobilised NH_2-PI **43**. *Reagents and conditions*: i. NaH (3 eq.), BnBr, (3 eq.), TBAI, DMF, 49%; ii. CAN, MeCN–H_2O (4/1, v/v), 85%; iii. phosphoramidite **38**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, 73%; iv. H_2 (4.1 bar), Pd black, $NaHCO_3$, ^{t}BuOH/H_2O (6/1), 76%; v. Affi-Gel® 10, $NaHCO_3$, $CHCl_3$–MeOH/H_2O (4/5/1, v/v/v), 0 °C → rt, 9% loading.

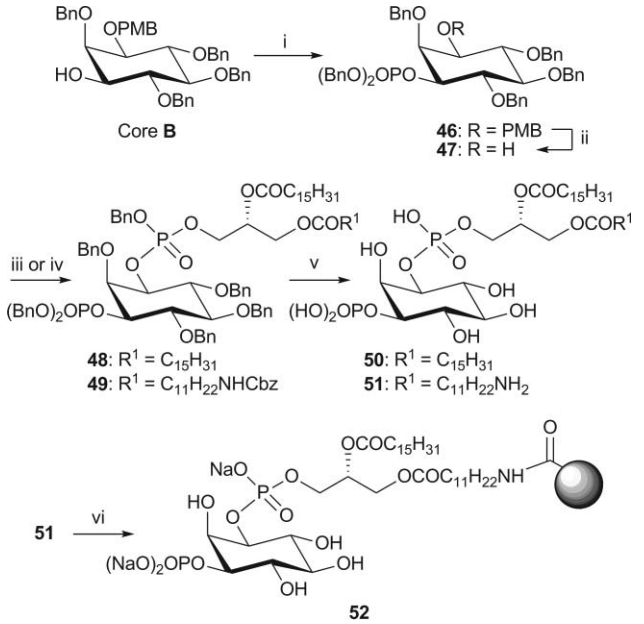


Scheme 8 Synthesis of dipalmitoyl-PI analogue **45**. *Reagents and conditions*: i. phosphoramidite **34**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*-CPBA, −78 °C → rt, 55% (ii) Pd black, $NaHCO_3$, ^{t}BuOH/H_2O (6 : 1), H_2 (3.1 bar), rt, 48 h, >99%.

via hydrogenolysis provides the dipalmitoyl-PI sodium salt **45** in a good yield.

The next group of phosphatidylinositols to consider is that where, in addition to the phospholipid side chain at the 1-position, one of either the 3-, 4- or 5-positions is phosphorylated.

For example, PI(3)P is obtained by first phosphorylating core **B** with bis(benzyloxy)(*N*,*N*-diisopropylamino)phosphine[49] followed by *in situ* oxidation with *m*CPBA, to install the phosphate group at the 3-position (Scheme 9). Removal of the PMB group in **46** and subsequent 1*H*-tetrazole-mediated coupling with the appropriate phosphoramidite afforded the fully protected dipalmitoyl-PI(3)P **48**[16] and NH_2-PI(3)P **49**. Standard global deprotection conditions afforded both analogues of PI(3)P (**50** and **51**). The affinity probe



Scheme 9 Synthesis of dipalmitoyl-PI(3)P **50** and immobilised NH_2-PI(3)P **52**. *Reagents and conditions*: i. $(BnO)_2P(N^iPr_2)$, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, 87%; ii. CAN, MeCN–H_2O (4/1, v/v), 83%; iii. phosphoramidite **34**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **48**: 90%; iv. phosphoramidite **38**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **49**: 84%; v. H_2 (4.1 or 3.8 bar), Pd black, ^{t}BuOH, **50**: 89% from **48**; **51**: 62% from **49**; vi. Affi-Gel® 10, $NaHCO_3$, $CHCl_3$–MeOH/H_2O (4/5/1, v/v/v), 0 °C → rt, 2% loading.

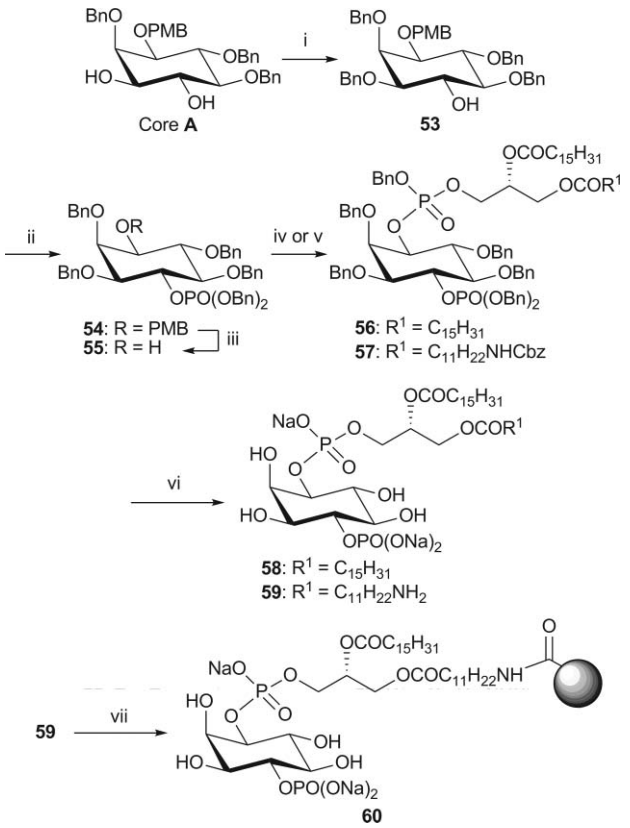
52 was obtained by coupling of **51** with NHS-activated Affi-Gel® 10 beads.

The analogues of PI(4)P were obtained from core **A** (Scheme 10). This synthesis required the selective protection of the hydroxyl group at the 3-position of the inositol ring, which was achieved using conditions developed by Gigg *et al.*[50] *In situ* generation of a stannane acetal in the presence of tetrabutylammonium bromide and benzyl bromide gave rise to the 3-benzylated derivative **53** and the corresponding 4-benzylated regioisomer in a 4 : 1 ratio (as judged by ^{1}H NMR analysis).

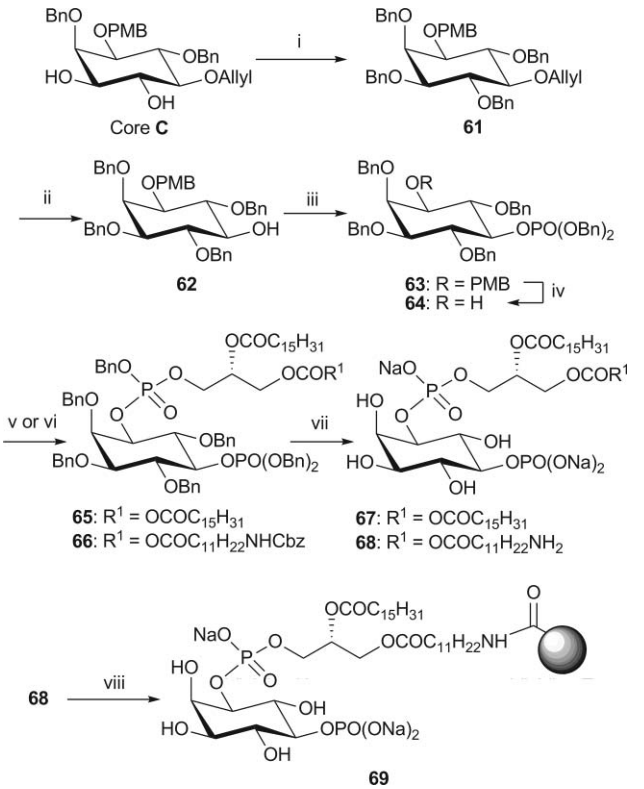
Subsequent phosphorylation of the remaining hydroxyl group at the 4-position of **53**, followed by removal of the PMB group, coupling to the requisite phosphoramidite, oxidation, and global deprotection smoothly provided both the dipalmitoyl analogue **58** and the amino analogue **59** of PI(4)P. The immobilised PI(4)P affinity probe **60** was synthesised in the standard manner.

The synthesis of PI(5)P analogues **67** and **68** from core **C** first required protection of the 3- and 4-hydroxyl groups (Scheme 11). The allyl group at the 5-position was then removed to allow installation of the 5-phosphate. This deprotection was achieved through isomerisation of the allyl ether **61** with Wilkinson's catalyst followed by acid catalysed methanolysis of the resulting enol ether. The standard phosphorylation, PMB removal, phosphoramidite coupling with *in situ* oxidation and global deprotection methods were then employed to obtain both analogues of PI(5)P. Amino-PI(5)P **68** was then immobilised onto Affi-Gel® 10 beads in the same manner as above.

Intermediate **61** in the synthesis of the PI(5)P analogues, can also be accessed from the unnatural L-enantiomer of triol **7** through facile protecting group manipulation (Scheme 12). This

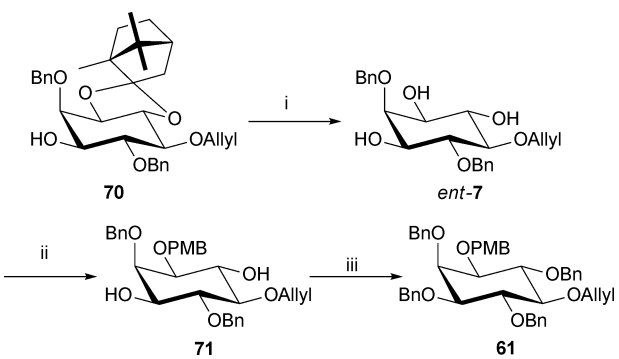


Scheme 10 Synthesis of dipalmitoyl-PI(4)P **58** and immobilised NH_2-PI(4)P **60**. *Reagents and conditions*: i. Bu_2SnO, BnBr, Bu_4NBr, MeCN, 3 Å MS, reflux, 76%; ii. $(BnO)_2P(N^iPr_2)$, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, 93% iii. CAN, MeCN–H_2O (4/1, v/v), 84%; iv. phosphoramidite **34**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*-CPBA, −78 °C → rt, **56**: 78%; v. phosphoramidite **38**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **57**: 77%; vi. H_2 (15 or 4.1 bar), Pd black, $NaHCO_3$, ^{t}BuOH/H_2O (6/1), **58**: 59% from **56**; **59**: 75% from **57**; vii. Affi-Gel® 10, $NaHCO_3$, $CHCl_3$–MeOH/H_2O (4/5/1, v/v/v), 0 °C → rt, 16% loading.



Scheme 11 Synthesis of dipalmitoyl-PI(5)P **67** and immobilised NH_2-PI(5)P **69**. *Reagents and conditions*: i. NaH, BnBr, DMF, rt, 94%; ii. $RhCl(PPh_3)_3$, DIPEA, EtOH–toluene/H_2O (7 : 3 : 1 v : v : v) then AcCl, MeOH–CH_2Cl_2, 83%; iii. $(BnO)_2P(N^iPr_2)$, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, 91%; iv. CAN, MeCN–H_2O (4/1, v/v), 79%; v. phosphoramidite **34**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*-CPBA, −78 °C → rt, **65**: 62%; vi. phosphoramidite **38**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **66**: 64%; vii. H_2 (3.5 or 35 bar), Pd black, $NaHCO_3$, ^{t}BuOH/H_2O (6/1), **67**: 86% from **65**; **68**: 83% from **66**; viii. Affi-Gel® 10, $NaHCO_3$, $CHCl_3$–MeOH/H_2O (4/5/1, v/v/v), 0 °C → rt, 8% loading.

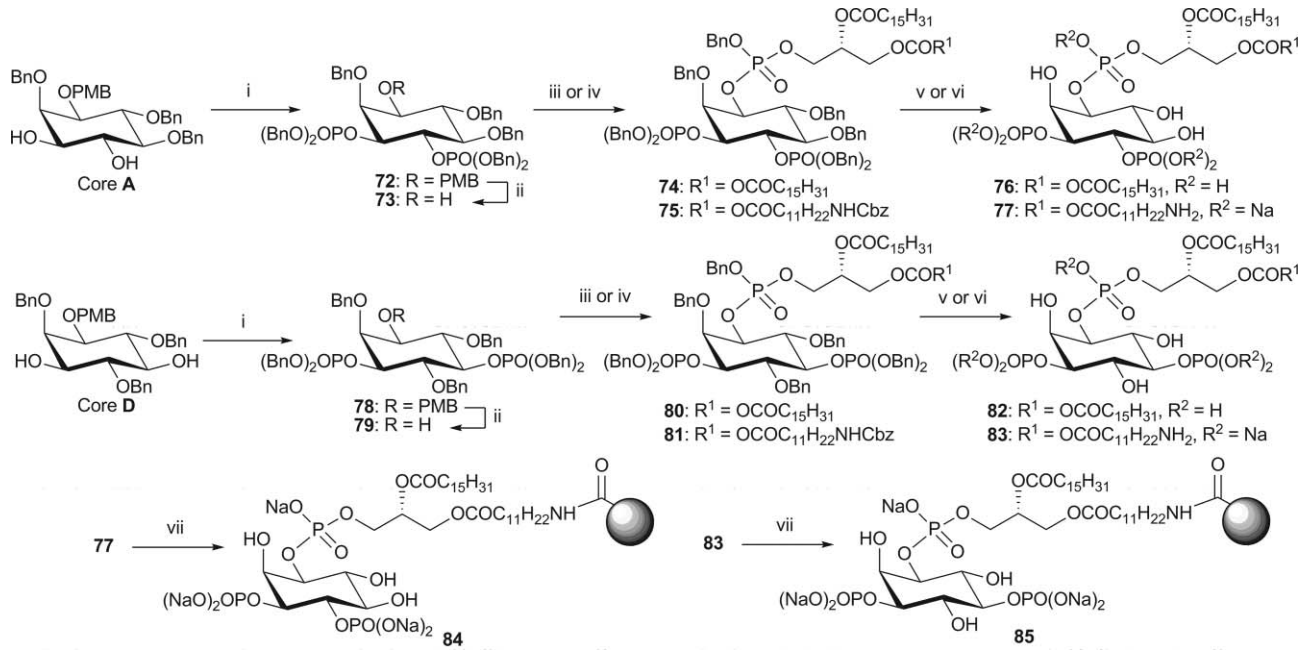
enantiomer was obtained from diastereomer **70**, resulting from the initial resolution step of racemic diol **7** with camphor (*R*)-**10** (Scheme 2), by acid catalysed methanolysis of the acetal **70**. Selective protection of the hydroxyl group at the 1-position as the PMB ether *via* the stannane acetal, followed by benzylation of the remaining hydroxyl groups afforded intermediate **61** in good yield with an optical rotation in agreement with an authentic sample (see Electronic Supporting Information for more details). Though this is not a direct route to PI(5)P, it makes good use of an otherwise unused by-product.



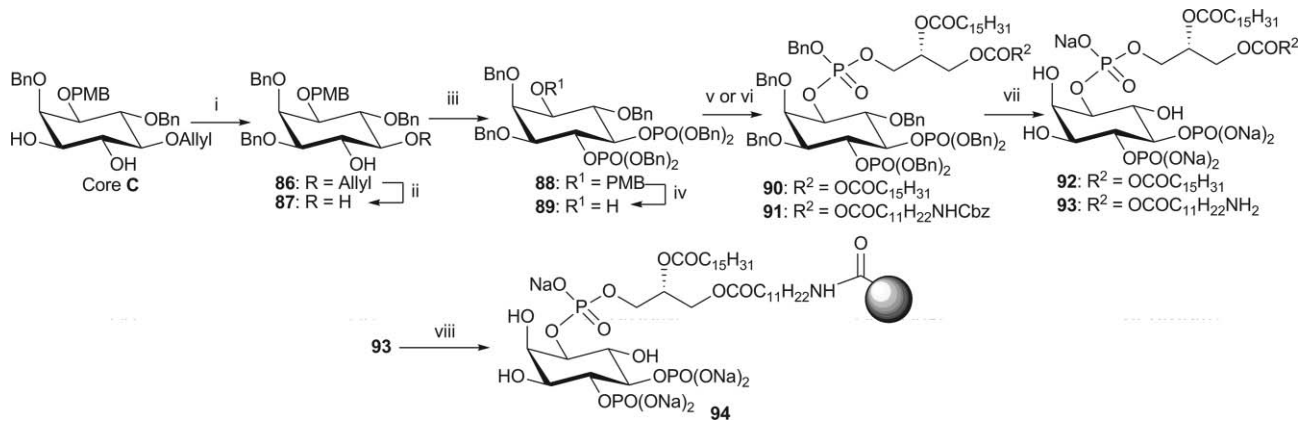
Scheme 12 Alternative synthesis of intermediate **61**. *Reagents and conditions*: i. AcCl, MeOH–CH_2Cl_2, (2/3 v/v), rt, 17 h, 97%; ii. Bu_2SnO, PMBCl, NaBr, Bu_4NBr, MeCN–toluene (2/1 v/v), 3 Å MS, reflux, 17 h, 72%; iii. NaH, BnBr, DMF, rt, 17 h, 66%.

Synthesis of analogues of PI(3,4)P2,[9,10,16,20,21,23,25] PI(3,5)P2[7,9,10,13–18] and PI(4,5)P2[10–12,22,25]

PI(X,Y)P2, the third subclass of phosphatidylinositols contains those in which two of the 3-, 4- or 5-positions are phosphorylated. There are three possible permutations for the bisphosphates: PI(3,4)P2, PI(3,5)P2 and PI(4,5)P2. PI(3,4)P2 and PI(3,5)P2 are easily obtained from cores **A** and **D** respectively (Scheme 13). This route follows the standard methods for phosphorylation and deprotection to afford the dipalmitoyl (**76**[16,21] and **82**[16]) and amino (**77** and **83**) analogues of PI(3,4)P2 and PI(3,5)P2 respectively. It should be noted that alternative hydrogenolysis conditions using palladium hydroxide on carbon in butanol were employed for the global deprotection of both the dipalmitoyl analogues **75** and **81**, affording **76** and **82** in comparable yields. Affinity probes **84** and **85** were then obtained in the manner as described above.



Scheme 13 Synthesis of dipalmitoyl-PI(3,4)P2, **76**, dipalmitoyl-PI(3,5)P2 **82** and immobilised NH_2-PI(3,4)P2 **84** and NH_2-PI(3,5)P2 **85**. *Reagents and conditions*: i. $(BnO)_2P(N^iPr_2)$, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **72**: 91%; **78**: 71%; ii. CAN, MeCN–H_2O (4/1, v/v), **73**: 90%; **79**: 72%; iii. phosphoramidite **34**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*-CPBA, −78 °C → rt, **74**: 91%; **80**: 93%; iv. phosphoramidite **38**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **75**: 83%; **81**: 88%; v. H_2 (3.5 or 4.1 bar), $Pd(OH)_2$-C, ^{t}BuOH, **76**: 97% from **74**; **82**: quant. from **80**; vi. H_2 (3.6 bar), Pd black, $NaHCO_3$, ^{t}BuOH/H_2O (6/1), **77**: 80% from **75**; **83**: 88% from **81**; vii. Affi-Gel® 10, H_2O, 4 °C, **84**: 3% loading; **85**: 3% loading.



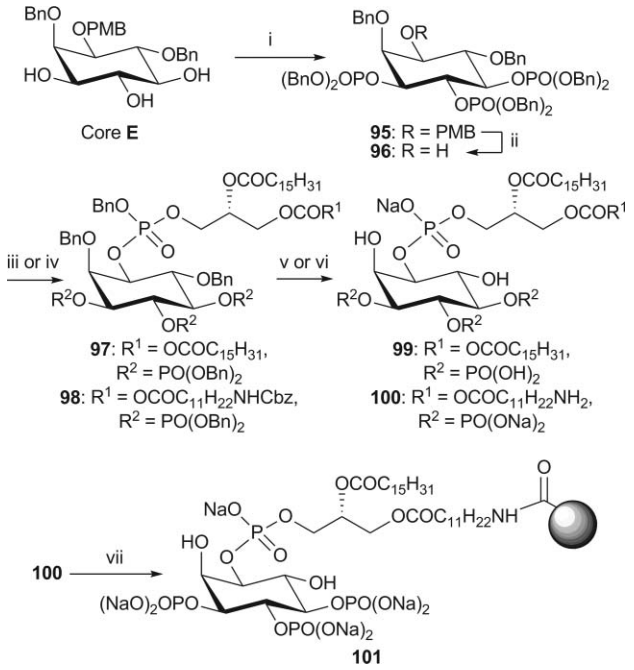
Scheme 14 Synthesis of dipalmitoyl-PI(4,5)P2 **92** and immobilised NH_2-PI(4,5)P2 **94**. *Reagents and conditions*: i. Bu_2SnO, BnBr, Bu_4NBr, CH_3CN, 3 Å MS, reflux, 75% ii, $Rh(PPh_3)_3Cl$, EtN^iPr_2, EtOH–toluene/H_2O (7/3/1), reflux, then AcCl, CH_2Cl_2–MeOH (2/1), 58%; iii. $(BnO)_2P(N^iPr_2)$, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, 75%; iv. CAN, MeCN–H_2O (4/1, v/v), 80%; v. phosphoramidite **34**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **90**: 83%; vi. phosphoramidite **38**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **91**: 82%; vii. H_2 (25 or 15 bar), Pd black, $NaHCO_3$, ^{t}BuOH/H_2O (6/1), **92**: 78% from **90**; **93**: 59% from **91**; viii. Affi-Gel® 10, $NaHCO_3$, H_2O, 0 °C, 3% loading.

The synthesis of the analogues of PI(4,5)P2, required some manipulation of the protecting groups to provide the correct substitution pattern (Scheme 14). Thus, the hydroxyl group in the 3-position of core **C** was selectively benzylated using the dibutyltin oxide strategy to afford alcohol **86**. Removal of the allyl group was achieved *via* isomerisation with Wilkinson's catalyst and methanolysis of the resulting enol ether, affording diol **87**. Subjection to the standard phosphorylation and deprotection conditions gave dipalmitoyl-PI(4,5)P2 **92** and NH_2-PI(4,5)P2 **93**.[12] Affinity probe **94**[12] was synthesised in the manner described previously.

Synthesis of PI(3,4,5)P3,[6,9,10,16,21–25,28] and related analogues

Lastly, analogues of PI(3,4,5)P3 were obtained from core **E** by a simple global phosphorylation (Scheme 15). The standard methods for the coupling with the side chain phosphoramidites, oxidation and global deprotection were used to gain access to both the analogues **99**[16,21] and **100**[28] of PI(3,4,5)P3.

To demonstrate the versatility of this approach to the synthesis of PIP analogues, shorter chain analogues of PI(3,4,5)P3 **108** and **109**, were synthesised using the corresponding hexanoyl[51] and octanoyl[24] phosphoramidites **102** and **103**, respectively (Fig. 2,



Scheme 15 Synthesis of dipalmitoyl-PI(3,4,5)P3 **99** and immobilised NH_2-PI(3,4,5)P3 **101**. *Reagents and conditions*: i. $(BnO)_2P(N^iPr_2)$, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, 78%; ii. CAN, MeCN–H_2O (4/1, v/v), 87%; iii. phosphoramidite **34**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*-CPBA, −78 °C → rt, **97**: 78%; iv. phosphoramidite **38**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **98**: 63%; v. H_2 (3.5 bar), $Pd(OH)_2$-C, ^{t}BuOH, **99**: 85% from **97**; vi. H_2 (4.1 or 3.5 bar), Pd black, $NaHCO_3$, ^{t}BuOH/H_2O (6/1), **100**: 92% from **98**; vii. Affi-Gel® 10, $NaHCO_3$, H_2O, 0 °C, 3% loading.

Scheme 16). An ω-amino analogue **110** with a hexanoyl chain at the secondary position of the glycerol side chain was also synthesised using the phosphoramidite **104**.[52] These shorter chain analogues are known to be more water-soluble than the longer chain PIPs.[19,27]

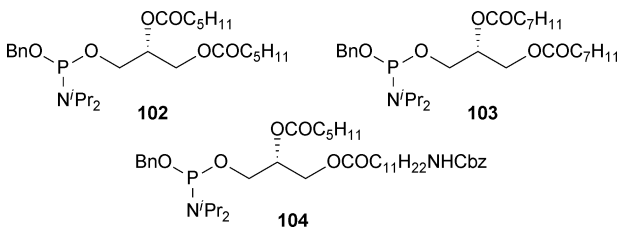
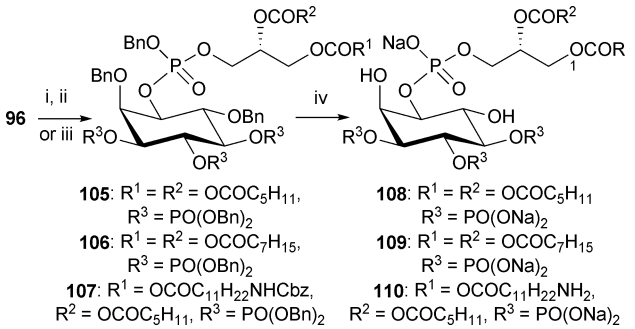


Fig. 2 Short chain phosphoramidites **102–104**.

Biological evaluation

During the course of our investigations we have used both the dipalmitoyl and amino analogues of the PIPs in the investigation of the PI3K pathway.[30-35] Our original experiments used cytosolic and membrane extracts from sheep brain or porcine neutrophils and leukocytes. Initial investigations using the dipalmitoyl analogue of PI(3,4,5)P3 revealed that Akt-1/PKB interacts directly with PI(3,4,5)P3 *via* the PH domain.[35] PI(3,4,5)P3-binding to Akt/PKB was shown to be necessary for upstream phosphorylation by directly activating upstream kinases. These investigations confirmed the role of Akt/PKB in PI3K-signalling.



Scheme 16 Synthesis of short chain analogues of PI(3,4,5)P3 **108–110**. *Reagents and conditions*: i. phosphoramidite **102**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **105**: 67%; ii. phosphoramidite **103**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*-CPBA, −78 °C → rt, **106**: 75%; iii. phosphoramidite **104**, 1*H*-tetrazole, CH_2Cl_2, rt, then *m*CPBA, −78 °C → rt, **107**: 79%; iv. H_2 (3.5, 3.1 or 25 bar), Pd black, $NaHCO_3$, ^{t}BuOH/H_2O (6/1), **108**: 96% from **105**; **109**: 80% from **106**; **110**: 78% from **107**.

Work using PI(3)P affinity probes in 2001, led to the discovery of the interactions of phosphatidylinositol phosphates with the PX domain and its role in the formation of reactive oxygen species (ROS) by neutrophils.[32] It was determined that PI(3)P specifically stimulates ROS formation by binding to the PX domain of $p40^{phox}$ which in turn can interact with $p67^{phox}$. Furthermore, $p40^{phox}$ was shown to target PI(3)P-containing membranes. In other work, two novel proteins FENS1 and DFCP1, containing one and two FYVE domains, respectively, were shown to bind PI(3)P specifically.[33] These proteins are involved in intracellular trafficking.[53]

In 2002, investigations with immobilised PI(3)P, PI(3,4)P2 and PI(3,5)P2 discovered five novel proteins and seven proteins previously uncharacterised as phosphoinositide-binding.[31] Interestingly, three of the proteins that were not known to bind PIPs, ATTP, MEG2 and Cdc42GAP, have different functions, but all proved to have an SEC14-like domain in common. This domain had not previously been known to bind to PIPs. One of the novel PI(3,4,5)P3 and PI(3,4)P2-binding proteins, ARAP3, is a PI3K-dependent, GTPase activating protein and was shown to be a genuine effector of PI3K signalling responsible for PI3K-dependent changes in the cell cytoskeleton and shape.

Investigations into the role of PI(3,5)P2 in vesicle recycling from vacuole/lysosomal compartments, using immobilised PI(3,5)P2 identified Svp1p as a specific PI(3,5)P2-binding protein.[30] This protein participates in the recycling of membrane proteins from the vacuole to the late endosome and at the time of discovery, represented a new family of phosphoinositide-binding proteins. It was shown that the binding of PI(3,5)P2 by Svp1p is necessary for normal function in vacuole membrane trafficking.

More recently we have used analogues of PI(3,5)P2, PI(4,5)P2 and PI(3,4,5)P3 to perform a comprehensive proteomic analysis of the phosphoinositide interactome in cell extracts from LIM1215 colorectal carcinoma cells.[29] The procedure for these experiments is outlined in Fig. 3. Phosphoinositide interacting protein complexes were pulled down using analogues of PI(3,5)P2, PI(4,5)P2 and PI(3,4,5)P3, either incorporated into liposomes or immobilised onto Affi-Gel® 10 beads in affinity-based assays. Proteins were

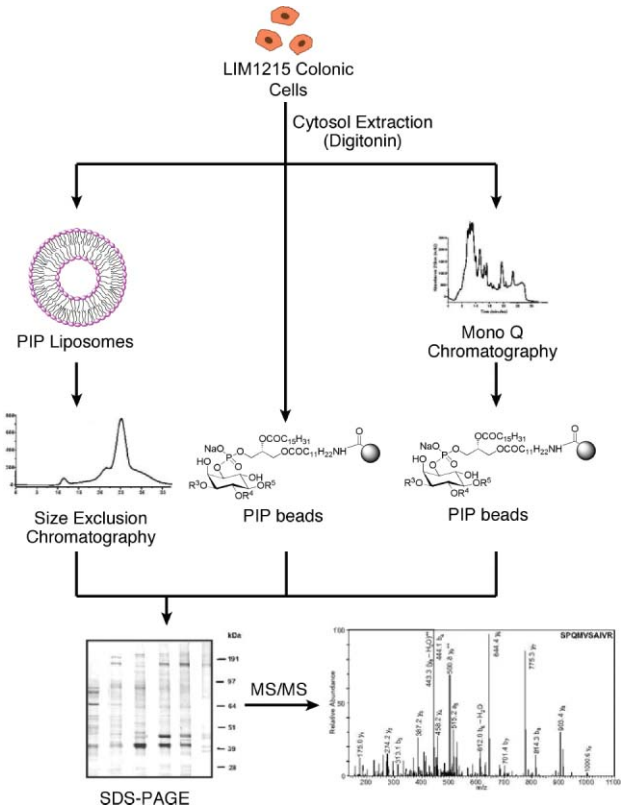


Fig. 3 Schematic of procedure for pull down experiments with liposomes incorporating PIPs and immobilised PIPs.[29]

then identified using nano-RP-HPLC ESI MS/MS analysis. The affinity probes were characterised using Biosensor technology. Thus, the phosphoinositide-containing liposomes were characterised using recombinant GST-tagged PH domains of General Receptor 1, Phospholipase C delta 1 and Dynamin, whilst the loading of the NH_2-PIP on the beads was assessed by the analysis of the supernatants from the immobilisation reactions and comparison with calibration curves generated by injecting various concentrations of each PIP over immobilised neomycin.[29] The NH_2-PIP analogues can also be immobilised onto a sensor chip allowing the characterisation of their interaction with their protein-binding partners.

From the cytosolic extracts of LIM1215 colon cancer cells we identified 529 proteins/protein complexes that appeared to interact specifically with their phosphoinositide targets: 69 proteins interact specifically with PI(3,5)P2, 146 with PI(4,5)P2, 141 with PI(3,4,5)P3, 32 with both PI(3,5)P2 and PI(4,5)P2, 36 with both PI(3,5)P2 and PI(3,4,5)P3, 41 with both PI(4,5)P2 and PI(3,4,5)P3, and 64 with all 3 phosphoinositides (Fig. 4).

Our affinity method was validated by the purification of a large number of proteins that possess known phosphoinositide or phospholipid binding domains (for example: PH, PX, FERM, PTB/PID, Phosphatidylinositol 3- and 4-kinase, RANBP1, C1, C2, MARCKS, PDZ, PHD, Septin, Annexin, Clathrin adaptor, Calponin, Phosphatidylinositol transfer protein, HEAT, Inositol monophosphatase family, Cral-Trio, SPC2, START, SPFH). Furthermore, small GTPases of Ras, Rab, Arf and Rho families that are known to interact with phosphoinositide phosphates, were also purified. Thus at least 30% of the total number of proteins

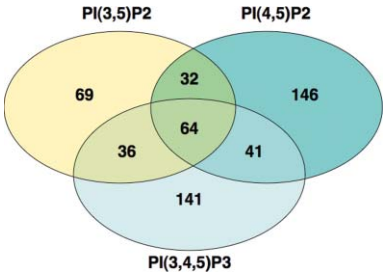


Fig. 4 Venn diagram analysis of the purified phosphoinositide interacting proteins showing the number of common and unique proteins pulled down by PI(3,5)P2, PI(4,5)P2 and PI(3,4,5)P3.

identified in our study were able to interact directly with their phosphoinositide targets, suggesting that the remaining proteins were presumably purified as part of interactome complexes.

Interestingly, the majority of purified proteins had molecular function and processes corresponding to previously well characterized cellular functions of phosphoinositide (*e.g.* molecular transport, protein trafficking, vesicle mediated transport, actin cytoskeletal regulation and GTPases regulated function, cell-to-cell signalling and interaction, cellular development, movement, assembly organization, growth and proliferation). A large number of purified proteins were also reported to have direct and indirect interactions with the PI3K/Akt signalling pathway.

Furthermore, proteins with different functions were found to bind to the liposomes and beads. Broadly speaking, those that require a membrane environment, such as GTPases and cell adhesion molecules, bound to the liposomes, whereas those involved in functions such as transport and trafficking were identified using beads. Phosphatases and kinases were identified in both pull-down experiments. This is depicted in Fig. 5 for PI(4,5)P2. Experiments are continuing in an effort to further our understanding of the PIP interactomes.

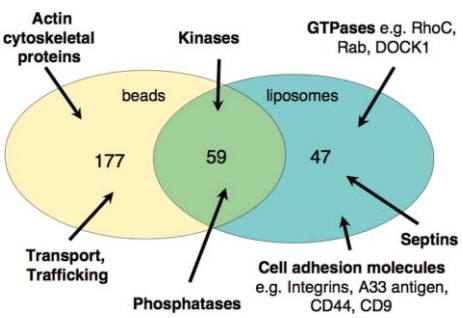


Fig. 5 Venn diagram analysis of the purified PI(4,5)P2 interacting proteins showing the number of common and unique proteins pulled down by immobilised-NH2-PI(4,5)P2 and PI(4,5)P2-liposomes.

Conclusions

The complete family of phosphatidylinositol phosphate analogues has been synthesised by a divergent strategy. Over the course of these investigations, affinity probes incorporating these analogues have been used to identify a range of important proteins in the PI3K signalling pathway, which were novel at the time of discovery.

A comprehensive proteomic analysis of phosphoinositide interacting proteins in cell extracts from LIM1215 colorectal carcinoma

cells has also been carried out. Our studies provide an initial detailed assessment of the phosphoinositide interactome which can be used in the study of the cellular responses mediated by phosphoinositides, to identify signalling pathways that are affected by a change of phosphoinositide cellular concentration (*e.g.* 3-phosphoinositide signalling cascade) and as a tool in drug discovery and biomarker development.

Acknowledgements

We thank the BBSRC and EPSRC for financial support and for provision of the Swansea Mass Spectrometry Service. This work was supported in part by the Australian Research Council, Discovery Project, Grant DP0770668 and an NHMRC Program Grant, 487922. General support was provided by CSIRO and VESKI (Victorian Endowment for Science, Knowledge and Innovation). We also thank Daiso Co., Ltd, Japan for the generous gift of (+)-1,2-*O*-isopropylidene-glycerol. SJC thanks Hughes Hall, Cambridge for a Research Fellowship. SJAG thanks GSK for a CASE studentship. Z-YL thanks Cambridge Commonwealth Trust, Universities UK (ORS award), New Hall, Cambridge and Tan Kar Kee Foundation, Singapore for generous financial support.

Notes and references

1 G. D. Prestwich, *Chem. Biol.*, 2004, **11**, 619.
2 T. F. J. Martin, *Annu. Rev. Cell Dev. Biol.*, 1998, **14**, 231.
3 A. Toker, *Cell. Mol. Life Sci.*, 2002, **59**, 761; M. Overduin, M. L. Cheever and T. G. Kutateladze, *Mol. Interv.*, 2001, **1**, 150; L. V. Dekker and A. W. Segal, *Science*, 2000, **287**, 982; S. Corvera and M. P. Czech, *Trends Cell Biol.*, 1998, **8**, 442; A. Toker and L. C. Cantley, *Nature*, 1997, **387**, 673; M. J. Berridge, *Nature*, 1993, **365**, 388.
4 M. Vicinanza, G. D'Angelo, A. Di Campli and M. A. De Matteis, *Cell. Mol. Life Sci.*, 2008, **65**, 2833; T. L. Yuan and L. C. Cantley, *Oncogene*, 2008, **27**, 5497; M. P. Wymann and R. Scheiter, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**, 162; A. G. Bader, S. Y. Kang and P. K. Vogt, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 1475; D. W. Parsons, T. L. Wang, Y. Samuels, A. Bardelli, J. M. Cummins, L. DeLong, N. Silliman, J. Ptak, S. Szabo, J. K. V. Willson, S. Markowitz, K. Kinzler, B. Vogelstein, C. Lengauer and V. E. Velculescu, *Nature*, 2005, **436**, 792; B. T. Hennessy, D. L. Smith, P. T. Ram, Y. L. Lu and G. B. Mills, *Nat. Rev. Drug Discovery*, 2005, **4**, 988; C. Pendaries, H. Tronchere, M. Plantavid and B. Payrastre, *FEBS Lett.*, 2003, **546**, 25; L. Shayesteh, Y. L. Lu, W. L. Kuo, R. Baldocchi, T. Godfrey, C. Collins, D. Pinkel, B. Powell, G. B. Mills and J. W. Gray, *Nat. Genet.*, 1999, **21**, 99.
5 K. J. Kayser-Bricker, P. A. Jordan and S. J. Miller, *Tetrahedron*, 2008, **64**, 7015.
6 K. M. Sureshan, A. M. Riley and B. V. L. Potter, *Tetrahedron Lett.*, 2007, **48**, 1923; D. S. Wang, A. L. Hsu and C. S. Chen, *Bioorg. Med. Chem.*, 2001, **9**, 133; P. R. J. Gaffney and C. B. Reese, *J. Chem. Soc., Perkin Trans. 1*, 2001, 192; R. Shirai, K. Morita, A. Nishikawa, N. Nakatsu, Y. Fukui, N. Morisaki and Y. Hashimoto, *Tetrahedron Lett.*, 1998, **39**, 9485; S. G. Aneja, A. Parra, C. Stoenescu, W. Y. Xia and R. Aneja, *Tetrahedron Lett.*, 1997, **38**, 803.
7 Y. J. Xu, B. R. Sculimbrene and S. J. Miller, *J. Org. Chem.*, 2006, **71**, 4919.
8 Y. Watanabe, Y. Kiyosawa, S. Hyodo and M. Hayashi, *Tetrahedron Lett.*, 2005, **46**, 281; N. Morisaki, K. Morita, A. Nishikawa, N. Nakatsu, Y. Fukui, Y. Hashimoto and R. Shirai, *Tetrahedron*, 2000, **56**, 2603; R. Aneja and S. G. Aneja, *Tetrahedron Lett.*, 2000, **41**, 847; J. G. Ward and R. C. Young, *Tetrahedron Lett.*, 1988, **29**, 6013; P. A. Gent, R. Gigg and C. D. Warren, *Tetrahedron Lett.*, 1970, **11**, 2575.
9 B. R. Sculimbrene, Y. Xu and S. J. Miller, *J. Am. Chem. Soc.*, 2004, **126**, 13182; D. S. Wang and C. S. Chen, *J. Org. Chem.*, 1996, **61**, 5905; K. S. Bruzik and R. J. Kubiak, *Tetrahedron Lett.*, 1995, **36**, 2415.
10 R. J. Kubiak and K. S. Bruzik, *J. Org. Chem.*, 2003, **68**, 960.
11 F. H. Han, M. Hayashi and Y. Watanabe, *Chem. Lett.*, 2003, **32**, 46; F. Han, M. Hayashi and Y. Watanabe, *Tetrahedron*, 2003, **59**, 7703; J. R. Falck, U. M. Krishna and J. H. Capdevila, *Tetrahedron Lett.*, 1999, **40**, 8771; C. E. Dreef, C. J. J. Elie, P. Hoogerhout, G. A. van der Marel and J. H. van Boom, *Tetrahedron Lett.*, 1988, **29**, 6513.
12 Z.-Y. Lim, J. W. Thuring, A. B. Holmes, M. Manifava and N. T. Ktistakis, *J. Chem. Soc., Perkin Trans. 1*, 2002, 1067.
13 A. Nishikawa, S. Saito, Y. Hashimoto, K. Koga and R. Shirai, *Tetrahedron Lett.*, 2001, **42**, 9195.
14 J. R. Falck, U. M. Krishna, K. R. Katipally, J. H. Capdevila and E. T. Ulug, *Tetrahedron Lett.*, 2000, **41**, 4271.
15 J. R. Falck, U. M. Krishna and J. H. Capdevila, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1711.
16 G. F. Painter, S. J. A. Grove, I. H. Gilbert, A. B. Holmes, P. R. Raithby, M. L. Hill, P. T. Hawkins and L. R. Stephens, *J. Chem. Soc., Perkin Trans. 1*, 1999, 923.
17 A. M. Riley and B. V. L. Potter, *Tetrahedron Lett.*, 1998, **39**, 6769.
18 J. R. Peng and G. D. Prestwich, *Tetrahedron Lett.*, 1998, **39**, 3965.
19 J. Chen, L. Feng and G. D. Prestwich, *J. Org. Chem.*, 1998, **63**, 6511.
20 K. K. Reddy, J. H. Ye, J. R. Falck and J. H. Capdevila, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2115; K. K. Reddy, J. Rizo and J. R. Falck, *Tetrahedron Lett.*, 1997, **38**, 4729.
21 S. J. A. Grove, A. B. Holmes, G. F. Painter, P. T. Hawkins and L. R. Stephens, *Chem. Commun.*, 1997, 1635.
22 Q. M. Gu and G. D. Prestwich, *J. Org. Chem.*, 1996, **61**, 8642.
23 T. Desai, J. Gigg, R. Gigg and E. Martin-Zamora, *Carbohydr. Res.*, 1996, **296**, 97.
24 K. K. Reddy, M. Saady, J. R. Falck and G. Whited, *J. Org. Chem.*, 1995, **60**, 3385.
25 L. X. Qiao, Y. H. Hu, F. J. Nan, G. Powis and A. P. Kozikowski, *Org. Lett.*, 2000, **2**, 115.
26 H. Zhang, Y. Xu, N. Markadieu, R. Beauwens, C. Erneux and G. D. Prestwich, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 762; S. J. Conway and G. J. Miller, *Nat. Prod. Rep.*, 2007, **24**, 687; Y. Xu, S. A. Lee, T. G. Kutateladze, D. Sbrissa, A. Shisheva and G. D. Prestwich, *J. Am. Chem. Soc.*, 2006, **128**, 885; H. L. Zhang, Y. Xu, Z. Zhang, E. R. Liman and G. D. Prestwich, *J. Am. Chem. Soc.*, 2006, **128**, 5642; P. W. Rzepecki and G. D. Prestwich, *J. Org. Chem.*, 2002, **67**, 5454; G. D. Prestwich, *Acc. Chem. Res.*, 1996, **29**, 503; T. L. Andresen, D. M. Skytte and R. Madsen, *Org. Biomol. Chem.*, 2004, **2**, 2951; J. Chen, A. A. Profit and G. D. Prestwich, *J. Org. Chem.*, 1996, **61**, 6305; R. J. Kubiak and K. S. Bruzik, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1231; C. Mihai, J. Mataka, S. Riddle, M. D. Tsai and K. S. Bruzik, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1235; O. Thum, J. Chen and G. D. Prestwich, *Tetrahedron Lett.*, 1996, **37**, 9017; H. L. Zhang, N. Markadieu, R. Beauwens, C. Erneux and G. D. Prestwich, *J. Am. Chem. Soc.*, 2006, **128**, 16464; A. P. Kozikowski, L. X. Qiao, W. Tuckmantel and G. Powis, *Tetrahedron*, 1997, **53**, 14903.
27 J. Chen and G. D. Prestwich, *J. Org. Chem.*, 1998, **63**, 430.
28 G. F. Painter, J. W. Thuring, Z.-Y. Lim, A. B. Holmes, P. T. Hawkins and L. R. Stephens, *Chem. Commun.*, 2001, 645.
29 B. Catimel, M.-X. Yin, C. Schieber, M. Condron, H. Patsiouras, J. Catimel, D. E. J. E. Robinson, L. S.-M. Wong, E. C. Nice, A. B. Holmes and A. W. Burgess, *J. Proteome Res.*, 2009, **8**, 3712; B. Catimel, C. Schieber, M. Condron, H. Patsiouras, L. Connolly, J. Catimel, E. C. Nice, A. W. Burgess and A. B. Holmes, *J. Proteome Res.*, 2008, **7**, 5295.
30 S. K. Dove, R. C. Piper, R. K. McEwen, J. W. Yu, M. C. King, D. C. Hughes, J. Thuring, A. B. Holmes, F. T. Cooke, R. H. Michell, P. J. Parker and M. A. Lemmon, *EMBO J.*, 2004, **23**, 1922.
31 S. Krugmann, K. E. Anderson, S. H. Ridley, N. Risso, A. McGregor, J. Coadwell, K. Davidson, A. Eguinoa, C. D. Ellson, P. Lipp, M. Manifava, N. Ktistakis, G. Painter, J. W. Thuring, M. A. Cooper, Z. Y. Lim, A. B. Holmes, S. K. Dove, R. H. Michell, A. Grewal, A. Nazarian, H. Erdjument-Bromage, P. Tempst, L. R. Stephens and P. T. Hawkins, *Mol. Cell*, 2002, **9**, 95.
32 C. D. Ellson, S. Gobert-Gosse, K. E. Anderson, K. Davidson, H. Erdjument-Bromage, P. Tempst, J. W. Thuring, M. A. Cooper, Z. Y. Lim, A. B. Holmes, P. R. J. Gaffney, J. Coadwell, E. R. Chilvers, P. T. Hawkins and L. R. Stephens, *Nat. Cell Biol.*, 2001, **3**, 679.
33 S. H. Ridley, N. Ktistakis, K. Davidson, K. E. Anderson, M. Manifava, C. D. Ellson, P. Lipp, M. Bootman, J. Coadwell, A. Nazarian, H. Erdjument-Bromage, P. Tempst, M. A. Cooper, J. Thuring, Z. Y. Lim, A. B. Holmes, L. R. Stephens and P. T. Hawkins, *J. Cell Sci.*, 2001, **114**, 3991.
34 M. Manifava, J. W. J. F. Thuring, Z.-Y. Lim, L. Packman, A. B. Holmes and N. T. Ktistakis, *J. Biol. Chem.*, 2001, **276**, 8987; K. E. Anderson,

P. Lipp, M. Bootman, S. H. Ridley, J. Coadwell, L. Ronnstrand, J. Lennartsson, A. B. Holmes, G. F. Painter, J. Thuring, Z. Y. Lim, H. Erdjument-Bromage, A. Grewal, P. Tempst, L. R. Stephens and P. T. Hawkins, *Curr. Biol.*, 2000, **10**, 1403; R. K. McEwen, S. K. Dove, F. T. Cooke, G. F. Painter, A. B. Holmes, A. Shisheva, Y. Ohya, P. J. Parker and R. H. Michell, *J. Biol. Chem.*, 1999, **274**, 33905.

35 L. Stephens, K. Anderson, D. Stokoe, H. Erdjument-Bromage, G. F. Painter, A. B. Holmes, P. R. J. Gaffney, C. B. Reese, F. McCormick, P. Tempst, J. Coadwell and P. T. Hawkins, *Science*, 1998, **279**, 710; D. Stokoe, L. R. Stephens, T. Copeland, P. R. J. Gaffney, C. B. Reese, G. F. Painter, A. B. Holmes, F. McCormick and P. T. Hawkins, *Science*, 1997, **277**, 567; S. R. James, C. P. Downes, R. Gigg, S. J. A. Grove, A. B. Holmes and D. R. Alessi, *Biochem. J*, 1996, **315**, 709.

36 Stereochemical representation: *myo*-inositol (Scheme 1), structures **1**, **11–14** and **27–29** are *meso*-compounds. Structures **2–7**, **15** and **17–19** refer to racemic compounds. Structures in Fig. 1, cores **A**–**E**, structures **8–10**, **16**, **20** and **30–110** refer to single enantiomers.

37 D. C. Billington, R. Baker, J. J. Kulagowski, I. M. Mawer, J. P. Vacca, S. J. Desolms and J. R. Huff, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1423; H. W. Lee and Y. Kishi, *J. Org. Chem.*, 1985, **50**, 4402.

38 S. J. A. Grove, I. H. Gilbert, A. B. Holmes, G. F. Painter and M. L. Hill, *Chem. Commun.*, 1997, 1633.

39 I. H. Gilbert and A. B. Holmes, *Tetrahedron Lett.*, 1990, **31**, 2633.

40 K. S. Bruzik and M.-D. Tsai, *J. Am. Chem. Soc.*, 1992, **114**, 6361.

41 Bruzik subsequently used camphor dimethyl acetal for the early resolution of *myo*-inositol as a strategy for the synthesis of analogues of all eight naturally occurring PIPs. See reference 10 for more details.

42 I. H. Gilbert, A. B. Holmes, M. J. Pestchanker and R. C. Young, *Carbohydr. Res.*, 1992, **234**, 117.

43 *Organometallic Syntheses*, ed. J. J. Eisch, and R. B. King, Academic Press, New York, Vol. 2, p. 137; J. J. Eisch, and S. G. Rhee, *J. Am. Chem. Soc.*, 1974, 96, 7276.

44 Synthesised from *myo*-inositol and deuterated triethyl orthoformate, see Electronic Supporting Information for more details.

45 M. K. Johns, M.-X. Yin, S. J. Conway, D. E. J. E. Robinson, L. S.-M. Wong, R. Bamert, R. E. H. Wettenhall and A. B. Holmes, *Org. Biomol. Chem.*, 2009, **7**, 3691.

46 Synthesised from phosphorus trichloride by the method of W. Bannwarth and A. Trzeciak, *Helv. Chim. Acta*, 1987, **70**, 175.

47 These compounds are notoriously difficult to characterise because of problems with solubility and/or micelle formation (also see reference 19). Characterisation data is available in the Electronic Supporting Information.

48 The percentage loading was determined by either ^{1}H NMR analysis of the supernatant (reference 12) or Biocore analysis (reference 29).

49 J. W. Perich and R. B. Johns, *Tetrahedron Lett.*, 1987, **28**, 101.

50 T. Desai, J. Gigg, R. Gigg, E. Martin-Zamora and N. Schnetz, *Carbohydr. Res.*, 1994, **258**, 135.

51 Synthesised in a similar manner to dipalmitoyl phosphoramidite **34**, see Electronic Supporting Information.

52 Phosphoramidite **104** was synthesised from **35** following the procedure for the synthesis of **38**, see Electronic Supporting Information for more details.

53 E. L. Axe, S. A. Walker, M. Manifava, P. Chandra, H. L. Roderick, A. Habermann, G. Griffiths and N. T. Ktistakis, *J. Cell Biol.*, 2008, **182**, 685.

