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Comprehensive and Uniform Synthesis of All Naturally Occurring Phosphorylated Phosphatidylinositols

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Studies of cellular signal transduction mechanisms involving receptor-mediated generation of inositol phosphates and phosphorylated phosphatidylinositols require easy access to these naturally occurring products. Although numerous synthetic methods have been developed during the past decade, most of these methods suffer from excessive length and lack of generality. In this work we describe the comprehensive and uniform synthesis of all naturally occurring phosphatidylinositols such as phosphatidylinositol, phosphatidylinositol 3-phosphate, 4-phosphate, 5-phosphate, 3,4-bisphosphate, 3,5-bisphosphate, 4,5-bisphosphate, and 3,4,5-trisphosphate, featuring both saturated and unsaturated fatty acid chains.

Investigation of cellular signal transduction pathways involving inositol phospholipids (PIPn) and phosphates that involved major discoveries in the 1980s[1] continues to be an important field of research in cellular physiology. Inositol phospholipids are now known to be involved in the transduction of the vast array of cellular signals ranging from neurotransmission to vesicular trafficking.[1,2] Due to the broad scope of these signaling events several thousand research publications related to inositol phospholipids are published every year.[3] Much of this activity is enabled by the availability of phosphoinositides owing to success in developing synthetic methodologies leading to these compounds during the last 15 years.[4,5] Overall, eight major inositol phospholipids **1**−**8** (Figure 1) have been identified so far in cellular signaling pathways, and more are likely to be discovered. In principle, only three members of this family, including phosphatidylinositol (**1**), phosphatidylinositol 4-phosphate (**3**), and 4,5-bisphosphate (**7**), are available in sufficient quantities by isolation from natural sources.[6] Other phosphoinositides, while playing important roles in cell physiology, either are formed transiently or are present in only very low concentrations making preparative isolation of these compounds from natural sources unfeasible. To date, all known naturally occurring phosphoinositides, with the exception of certain glycosylated phosphatidylinositols[7] and the putative prostaglandyl inositol phosphate,[8] have been synthesized,[9−17] with some of the early methodology published from this Laboratory.[9a,18] In general, strategies of phosphoinositide syn-

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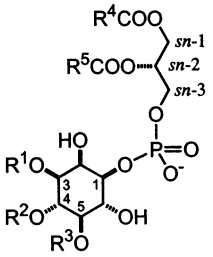

$R^1 = R^2 = R^3 = H$, PI (**1**)
$R^1 = PO_3^{2-}$; $R^2 = R^3 = H$, PI-3-P (**2**)
$R^2 = PO_3^{2-}$; $R^1 = R^3 = H$, PI-4-P (**3**)
$R^3 = PO_3^{2-}$; $R^1 = R^2 = H$, PI-5-P (**4**)
$R^1 = R^2 = PO_3^{2-}$; $R^3 = H$, PI-3,4-P_2 (**5**)
$R^1 = R^3 = PO_3^{2-}$; $R^2 = H$, PI-3,5-P_2 (**6**)
$R^1 = H$; $R^2 = R^3 = PO_3^{2-}$; PI-4,5-P_2 (**7**)
$R^1 = R^2 = R^3 = PO_3^{2-}$; PI-3,4,5-$P_3$ (**8**)
$R^4 = R^5 = C_{15}H_{31}$ (**8**, DP) or C_7H_{15} (**8a**, DO)
$R^4 = C_{17}H_{31}$, $R^5 = C_{19}H_{31}$ (**8b**, SA)

FIGURE 1. Structures of phosphatidylinositol phosphates (PIPn) **1**−**8**. Most naturally occurring PIPn presumed to participate in signal transduction pathways carry stearoyl and arachidonoyl residue at the glycerol *sn*-1- and *sn*-2-positions, respectively. All synthesized PIPn are 1,2-dipalmitoyl (DP) glycerides except compound **7**, which was also synthesized as the 1,2-dioctanoyl (DO) derivative (**7a**), and compound **8**, which was also synthesized as the dioctanoyl (**8a**) and 1-stearoyl-2-arachidonoyl (SA, **8b**) derivatives.

thesis are dominated by the necessity to solve three issues: (i) availability of *myo*-inositol derivatives with high enantiomeric purity, (ii) differential regioselective protection of inositol hydroxyl groups to be later converted into either the phosphodiester or phosphomonoester functions, and (iii) the final mild deprotection of the phosphate and inositol hydroxyl groups, compatible with the presence of carboxylic ester functions and double bonds in the diacylglycerol residue, and with the propensity of the neutral phosphate group toward migration between vicinal hydroxyl functions. While most of the earlier work focused mainly on reproducing the exact structure of the phosphatidylinositols polar headgroups, the more recent efforts have been devoted to replicating the precise structure of the diglyceride, including the presence of arachidonic acid at the 2-position of glycerol.

The first problem has been solved by various research groups using different approaches including (i) the use of enantiopure natural precursors such as D-glucose,[9c,11a,12b,14c,15g] L-*chiro*-inositol derivatives,[12g] and quinic acid,[12d,e,15a,f] (ii) kinetic resolution or desymmetrization via enantioselective enzymatic acylations of protected *myo*-inositol derivatives,[9b,15b,17] and (iii) separation of diastereomeric derivatives of *myo*-inositol with chiral auxiliaries.[4a−d,13b,18] In our early work[18] we have used the last approach, where the resolution of the inositol enantiomers is achieved by crystallization of diastereomeric ketals of *myo*-inositol with D- or L-camphor. This method, which combines in one step regioselective protection of the *cis*-diol function of inositol with diastereomer separation, is both short and efficient. Hence, following our initial reports, similar procedures have been adopted by others in their design of PIPn synthesis.[9e,12f,15k] The second problem has been solved by applying regioselective protection−deprotection sequences, typically to obtain inositol derivatives protected with benzyl or related groups. The use of benzylic groups is, however, completely unsuited in syntheses of inositol phospholipids containing the arachidonic acid moiety in the diacylglycerol residue. Synthesis of arachidonoyl PIPn, the true naturally occurring signaling molecules, required therefore a complete redesign[16a−c,19] of the previously developed synthetic strategies which inevitably lengthened the already long synthetic schemes. In addition, although arachidonoyl PI-3,4,5-P_3 has been synthesized,[16] it is unclear how the developed methodology could be used for the synthesis of other PIPn.

In this work we describe a simple uniform methodology that is applicable to synthesis of all naturally occurring PIPn, including the unsaturated derivatives and phosphorothioate analogues[20] of PIPn.

Results and Discussion

The synthetic approach reported here is based on the following strategic consecutive steps (Schemes 1−3): (a) regioselective silylation of the 1-hydroxyl of inositol-camphor ketal; (b) removal of the ketal group; (c) regioselective benzoylation of the phosphomonoester positions; (d) exhaustive "capping" of all the remaining hydroxyl groups; (e) deacylation at the phosphomonoester posi-

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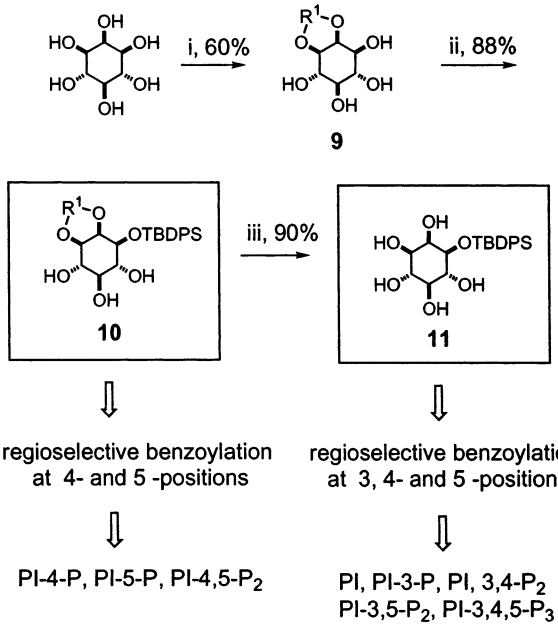
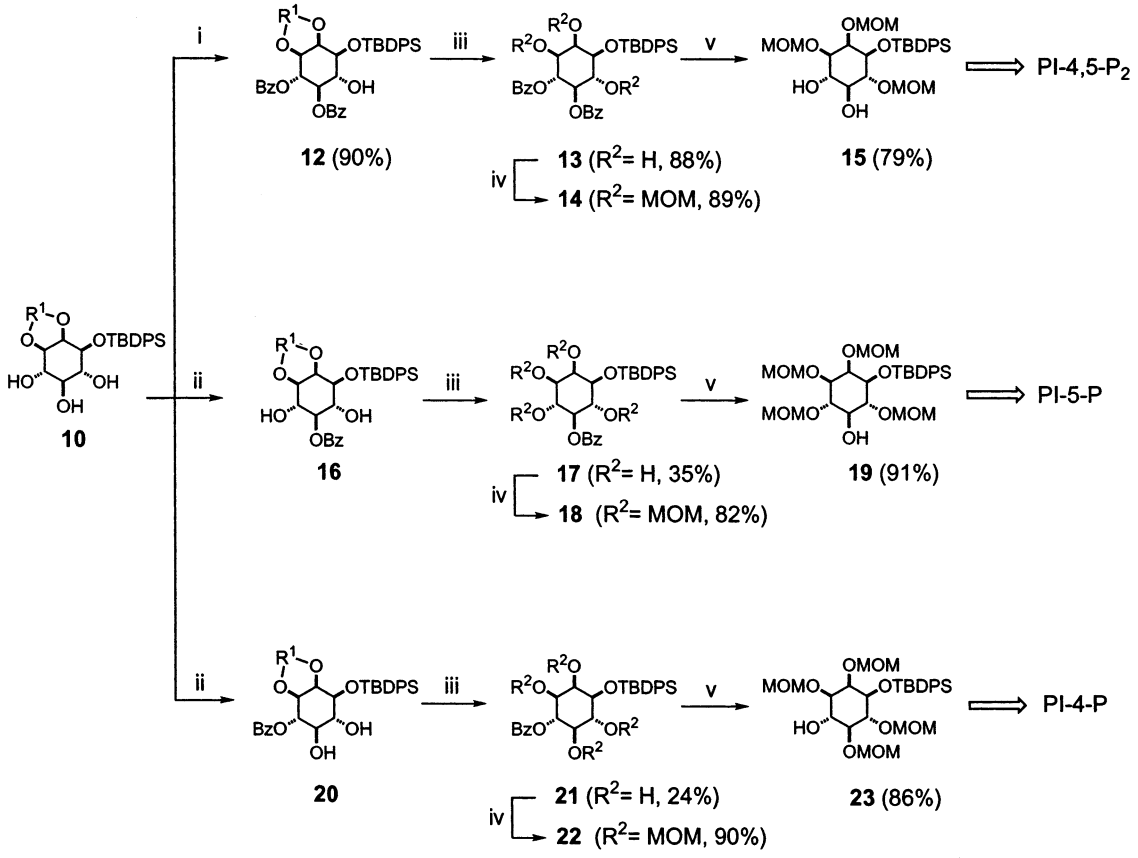
SCHEME 1. Generation of Key Intermediates 10 and 11 and Their Further Application to the Synthesis of PIPn



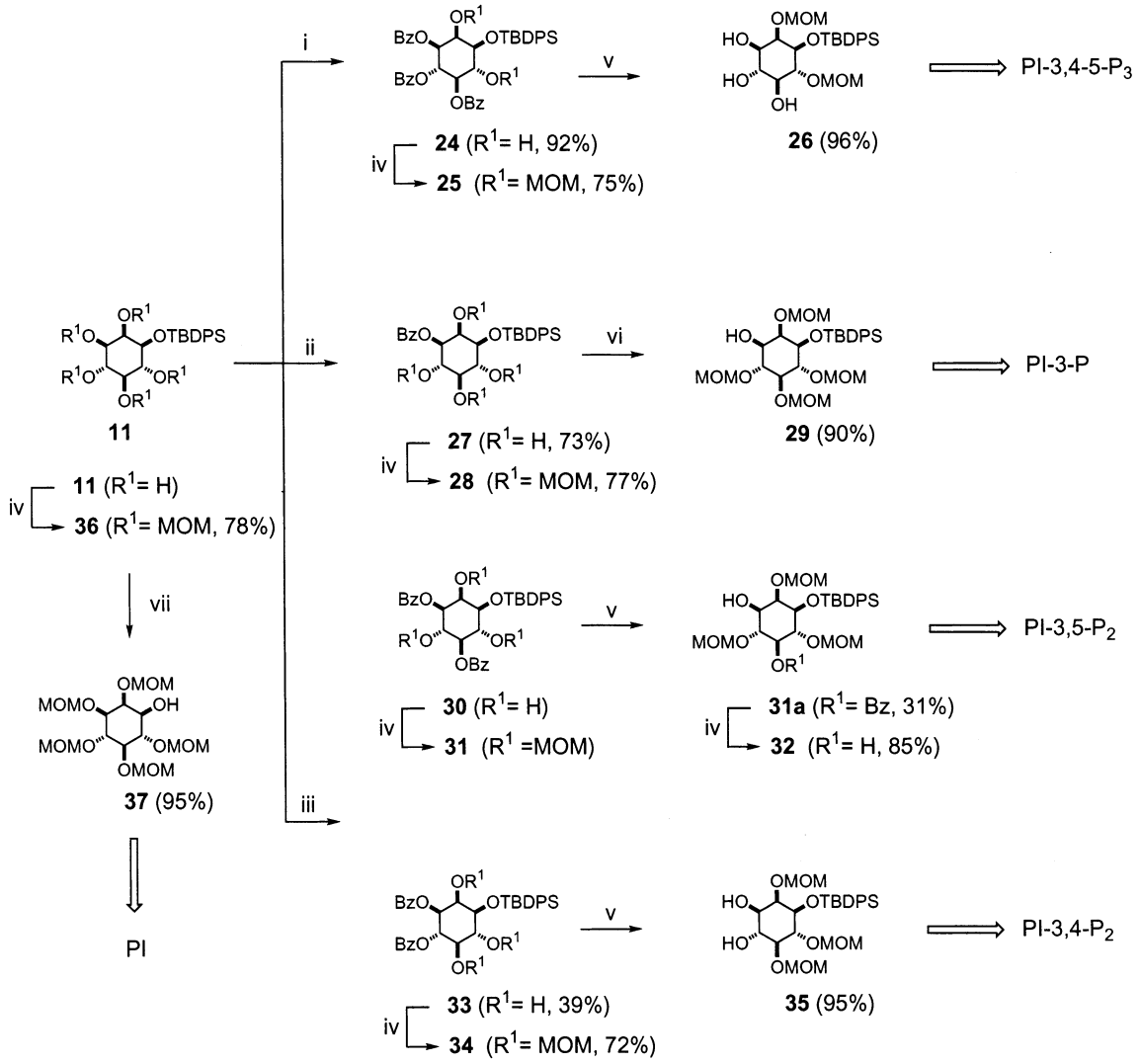
R^1 = bornanedi-2,2-yl; i: D-camphor dimethyl ketal, H_2SO_4; ii: $Ph_2tBuSiCl$, imidazole; iii: $HSCH_2CH_2OH$, BF_3-Et_2O

SCHEME 2. Synthesis of Precursors of PI-4,5-P_2, PI-5-P, and PI-4-P



R_1=bornanedi-2,2,-yl; i: 2 equiv. Bz-Cl/Py; ii: 1.3 equiv. Bz-Cl/Py; iii: TFA-$CHCl_3$; iv: MOM-Cl, v: MeONa/anh.MeOH

SCHEME 3. Synthesis of Precursors of PI-3,4,5-P_3, PI-3-p, PI-3,5-p_2, and PI-3,5-P_2



i: 3 equiv. BzCl/Py, ii: 1 equiv. BzCl/Py, iii: 2 equiv. BzCl/Py; iv: MOM-Cl/iPr_2EtN, v: $MeNH_2$, vi: MeONa/anh.MeOH; vii: $Bu_4N^+F^-$

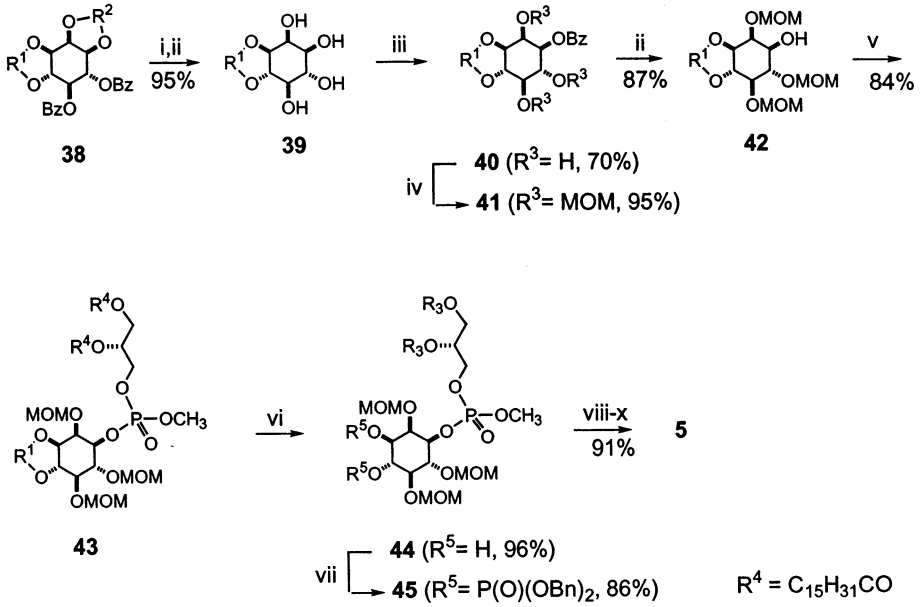
tions; (f) introduction of phosphate groups at the phosphomonoester positions; (g) removal of the silyl group at the phosphodiester position; (h) introduction of the phosphodiester residue; and (i) final deprotection of phosphate and hydroxyl groups. Depending on the exact structure of the target PIPn, steps b and c can be interchanged.

Enantiomerically Pure Starting Materials. The synthesis of enantiomerically pure inositol derivatives developed in our laboratory is based on bornanediyl inositol ketals obtained from an inexpensive chiral auxiliary, D-camphor, and *myo*-inositol. Acetalization of inositol with D-camphor dimethyl acetal[18a] results in an equimolar mixture of four diastereoisomers of 1,2-bornanediyl-*myo*-inositol. Equilibration and crystallization of the mixture of four diastereomers in chloroform−methanol−water solution under mildly acidic conditions yields the least soluble isomer **9** (Scheme 1) in 60% yield.[18a−c] This isomer is the first key intermediate used in the synthesis of all PIPn reported here. In addition, the remaining three stereoisomers of ketal **9** can be separated and used to obtain various inositol phosphates.

Protection of the 1-Hydroxyl Group: Bigger Is Better. The second key element of our synthetic design is regioselective protection of the hydroxyl group at the inositol 1-position by the bulky silyl or acyl groups (Schemes 1−3). Only large groups such as *tert*-butyldiphenylsilyl and pivaloyl can be placed regioselectively at this position,[18b] whereas smaller groups (such as *tert*-butyldimethylsilyl and benzoyl) tend to give various isomers of both monoprotected and multiply protected ketals. The introduction of such bulky silyl or acyl groups sterically hinders the adjacent hydroxyl functions (the 6-hydroxyl in triol **10** and 2- and 6-hydroxyls in pentol **11**) and enables further highly regioselective protections of the remaining hydroxyls. An additional advantage of the large groups at the 1-position is their enhanced stability toward hydrolysis and migration under ketal-cleavage conditions applied later in the synthesis.

Protection at the Phosphomonoester Positions: Balance of Size and Reactivity. To achieve high regioselectivity in protecting single or multiple hydroxyl functions at 3-, 4-, and 5-positions, the use of bulkier

SCHEME 4. Synthesis of PI-3,4-P_2 Using Top-to-Bottom Assembly

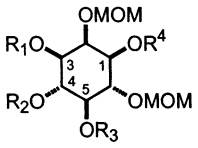


R^1 = -$(iPr)_2$Si-O-Si$(iPr)_2$-; R^2 = borrnanedi-2,2-yl; i: TFA/$CHCl_3$; ii: $MeNH_2$/MeOH; iii: BzCl/Py; iv: MOM-Cl/$EtiPr_2N$; v: (A) Cl-P(OMe)$NiPr_2$/$EtNiPr_2$, (B) DPG/tetrazol, (C) MCPBA; vi: $Bu_4N^+F^-$; vii: (A) $(BnO)_2PNiPr_2$, tetrazole, (B) MCPBA; viii: Me_3N; ix: H_2/Pd-C; x: EtSH/BF_3

protective groups for these positions would seem beneficial; however, we found that such groups later make it difficult to carry out exhaustive "capping" of the remaining hydroxyl functions.[18b] We found that application of benzoyl chloride in pyridine at −40 °C gives the best results, ensuring high regioselectivity particularly in simultaneous introduction of multiple groups, and at the same time still allows further exhaustive derivatization of the remaining hydroxyl groups. Thus, benzoylation of the triol **10** with 2 equiv of benzoyl chloride afforded exclusively the 4,5-bisbenzoate **12** (Scheme 2), whereas benzoylation of the pentol **11** with 3 equiv of this reagent produced only the tribenzoate **24** (Scheme 3), making the triol **10** and the pentol **11** strategically important intermediates applicable toward syntheses of PI-4,5-P_2 and PI-3,4,5-P_3, respectively.

In contrast, monobenzoylation of the triol **10** with slight excess of benzoyl chloride was completely nonregioselective and provided the mixture of three products: 5-benzoate **16**, 4-benzoate **20**, and 4,5-bisbenzoate **12** (Scheme 2). This mixture can be readily separated into the individual bisbenzoate **12** and the monobenzoate fraction; however, further separation of the monobenzoates proved difficult. To circumvent this problem, the obtained mixture of **12**, **16**, and **20** was subjected to deacetalization with TFA/water to form a mixture of the triol **13** and the tetrols **17** and **21**, which was then easily separable into individual compounds with excellent overall combined yield. Although the yields of individual products were lower due to a three-way split, the advantage of this method is the simultaneous generation of the precursors of PI-4-P, PI-5-P, and PI-4,5-P_2 in only a few steps and a minimal number of intermediate purifications. The precursors of these three PIPn must be separated at this stage, since their further processing results in derivatives having very similar chromatographic properties. Unlike in the case of the triol **10**, monobenzoylation of the pentol **11** also proceeded regioselectively to afford 3-benzoate **27** with good yield (Scheme 3). On the other hand, bisbenzoylation of the pentol **11** occurred nonregioselectively to afford the mixture of 3-benzoate **27**, 3,5-bisbenzoate **30**, 3,4-bisbenzoate **33**, and small amounts of the trisbenzoate **24** (Scheme 3). The formation of other monobenzoates and 4,5-bisbenzoate was not observed. The mixture was readily separated by chromatography into mono-, bis-, and trisbenzoate fractions; however, separation of the bisbenzoate fraction into individual 3,5- and 3,4-bisbenzoates (**30** and **33**, respectively) proved difficult and was deferred until later steps.

Exhaustive Protection of the Remaining Hydroxyl Groups: Small Size and Stability toward Migration. Once the desired protection pattern had been established, coding the positions of the phosphodiester and monoester groups by the silyl and benzoate groups, respectively, exhaustive "capping" of the remaining hydroxyl functions was necessary. We have found that the methoxymethylene group (MOM) best met the criteria of mild introduction, high positional stability, and facile deprotection in the presence of phosphoester and acyl groups. The exhaustive introduction of the MOM group into compounds **13**, **17**, **21**, **24**, **27**, **30**, and **33** was carried out under identical conditions with use of MOM chloride in the presence of diisopropylethylamine in anhydrous DMF to provide the fully protected compounds **14**, **18**, **22**, **25**, **28**, **31**, and **34**, respectively, with good to excellent yields. The subsequent cleavage of the benzoates was carried out with either concentrated methylamine in methanol or with sodium methoxide in anhydrous methanol. The debenzoylation of the above fully protected inositols afforded final precursors for assembly of phospholipids such as 5-, 4-, and 3-alcohols (**19**, **23**, and **29**,



Intermediate → PIPn

37, $R^1 = R^2 = R^3$ = MOM, R^4 = OH, PI
29, R^1 = H, $R^2 = R^3$ = MOM, R^4 = *t*-$BuPh_2Si$, PI-3-P
23, R^2 = H, $R^1 = R^3$ = MOM, R^4 = *t*-$BuPh_2Si$, PI-4-P
19, R^3 = H, $R^1 = R^2$ = MOM, R^4 = *t*-$BuPh_2Si$, PI-5-P
35, $R^1 = R^2$ = H, R^3 = MOM, R^4 = *t*-$BuPh_2Si$, PI-3,4-P_2
32, $R^1 = R^3$ = H, R^2 = MOM, R^4 = *t*-$BuPh_2Si$, PI-3,5-P_2
15, $R^2 = R^3$ = H, R^1 = MOM, R^4 = *t*-$BuPh_2Si$, PI-4,5-P_2
26, $R^1 = R^2 = R^3$ = H, R^4 = *t*-$BuPh_2Si$, PI-3,4,5-P_3

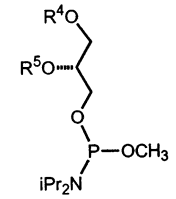
FIGURE 2. Structures of all final inositol intermediates used in this work prior to the phosphorylation steps. Except for the precursor of PI, **36**, which was obtained in 5 steps from inositol, all other intermediates have been obtained in 6 steps.

respectively), 4,5-, 3,5-, and 3,4-diols (**15**, **32**, and **35**, respectively), and the 3,4,5-triol (**26**). We have found that application of sodium hydroxide in methanol significantly lowers the yields due to competing desilylation of the 1-position.[21] All phosphorylation precursors discussed above have been subsequently used for a *bottom-to-top phospholipid assembly*, where introduction of the phosphomonoesters precedes that of phosphodiester.

Synthesis of the PI-3,4-P_2 Precursor for Top-to-Bottom Assembly. During preparation of the diastereomerically pure tetrol **9**, significant amounts of three other diastereomers are left behind. These can be utilized after separation into individual isomers, following silylation with 1,1,3,3-tetraisopropyldisiloxanediyl-1,3-dichloride and exhaustive benzoylation to give **38** (Scheme 4).[18b] The subsequent deacetalization and debenzoylation provided the cyclic 3,4-(1,1,3,3-tetraisopropyldisiloxanediyl)-*myo*-inositol **39**.[18b] This compound can be also more readily prepared in 3 steps from *myo*-inositol in 48% overall yield by employing transketalization of inositol with L- instead of D-camphor dimethyl ketal in the first step.[18b,c] Treatment of tetrol **39** with 1 equiv of benzoyl chloride occurred regioselectively to afford 1-benzoate **40** with 70% yield. The subsequent alkylation with MOM chloride gave the fully protected derivative **41** and the removal of the benzoate group with methylamine gave the 1-alcohol **42** with excellent overall yield.

Summary of Precursor Synthesis. For all phosphorylated phosphatidylinositols synthesized in this work we used a simple and uniform protection pattern (Figure 2). The inositol 1-position was silylated with the TBDPS group, the phosphomonoester positions (3-, 4-, and 5-hydroxyls) were esterified with the benzoate groups prior to their liberation, and all the remaining hydroxyl groups were protected as MOM-ethers. Using the same protective group strategy in synthesis of all PIPn offers numerous advantages such as the following: (i) only a small array of intermediates and reagents is needed to accomplish all PIPn syntheses; (ii) certain difficulties encountered in a particular step in one synthetic pathway are likely to be similar in other pathways, hence a short learning curve; and (iii) properties and reactivities of intermediates used in analogous steps along synthetic pathways are similar.

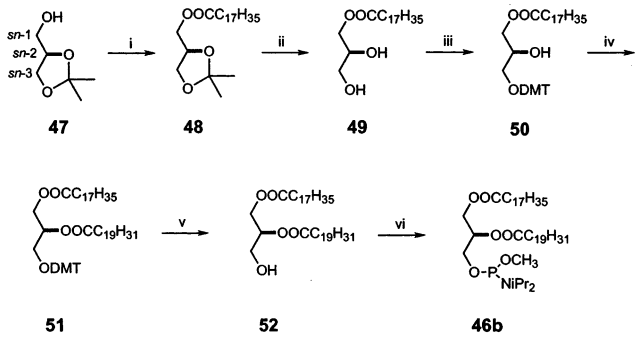
(21) Jiang et al. (ref 15k) have used cyanide for mild debenzoylation of a compound analogous to **25**. This reagent was completely unsuccessful in the removal of benzoates from other fully protected inositols described here.



46, $R^4 = R^5 = C_{15}H_{31}CO$
46a, $R^4 = R^5 = C_7H_{15}CO$
46b, $R^4 = C_{17}H_{35}CO$, $R^5 = C_{19}H_{31}CO$

FIGURE 3. Structures of Phosphoramidite Reagents Used

SCHEME 5. Synthesis of Arachidonic Acid-Containing Phosphoramidite Reagent 46c

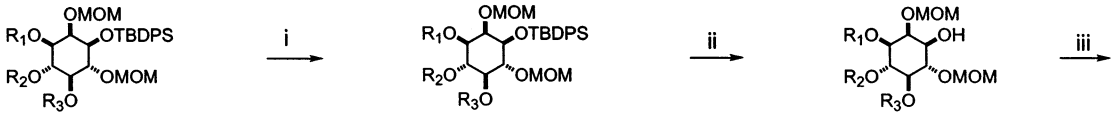


i: stearic acid/DCC/DMAP; ii: Amberlyst-H^+/MeOH; iii: DMT-Cl/Py; iv: arachidonic acid/DCC/DMAP; v: TFA/pyrrole; vi: Cl-P(OMe)($NiPr_2$), $EtNiPr_2$

Strategies in Phospholipid Molecule Assembly. An additional advantage of our syntheses is in that all PIPn precursors reported in this work, except alcohol **42**, require the *bottom-to-top* assembly of phospholipid molecules, whereby introduction of the phosphomonoester group (initially as a fully protected phosphate triester) precedes the liberation of the 1-hydroxyl, further followed by introduction of the phosphatidate group at that position. This strategy is preferred to the *top-to-bottom assembly*, with reverse order of phosphatidate and phosphomonoester introduction, because it allows generation of a greater diversity of phospholipid species and molecular probes in fewer synthetic steps. For example, the precursor **26** was used in this work to generate three different species of PI-3,4,5-P_3 (**8**, **8a**, and **8b**) using different phosphoramidite reagents (**46**, **46a**, and **46b**, respectively, Figure 3) late in the synthesis. The top-to-bottom assembly would require introduction of phosphoramidate reagents 2 steps earlier in the synthesis; an additional 4 steps would be required to complete syntheses of all three products.

Synthesis of 2-*O*-Arachidonoyl-1-*O*-stearoyl-*sn*-glycerol. With the exception of the unsaturated derivatives, the diacyl glycerol components needed for the assembly of the phosphatidate moiety are either com-

SCHEME 6. Assembly and Deprotection of PIPn



R^1 = H, R^2 = R^3 = MOM, **29**
R^2 = H, R^1 = R^3 = MOM, **23**
R^3 = H, R^1 = R^2 = MOM, **19**
R^1 = R^2 = H, R^3 = MOM, **35**
R^2 = R^3 = H, R^1 = MOM, **15**
R^1 = R^3 = H, R^2 = MOM, **32**
R^1 = R^2 = R^3 = H, **26**

R^1 = P(O)(OBn)$_2$, R^2 = R^3 = MOM, **53** (81%)
R^2 = P(O)(OBn)$_2$, R^1 = R^3 = MOM, **57**
R^3 = P(O)(OBn)$_2$, R^1 = R^2 = MOM, **60**
R^1 = R^2 = P(O)(OBn)$_2$, R^3 = MOM, **63** (58%)
R^2 = R^3 = P(O)(OBn)$_2$, R^1 = MOM, **66** (84%)
R^1 = R^3 = P(O)(OBn)$_2$, R^2 = MOM, **70**
R^1 = R^2 = R^3 = P(O)(OBn)$_2$, **73** (84%)

R^1 = R^2 = R^3 = MOM, **37**
R^1 = P(O)(OBn)$_2$, R^2 = R^3 = MOM, **54** (87%)
R^2 = P(O)(OBn)$_2$, R^1 = R^3 = MOM, **58** (80%, 2 steps)
R^3 = P(O)(OBn)$_2$, R^1 = R^2 = MOM, **61** (83%, 2 steps)
R^1 = R^2 = P(O)(OBn)$_2$, R^3 = MOM, **64** (97%)
R^2 = R^3 = P(O)(OBn)$_2$, R^1 = MOM, **67** (73%)
R^1 = R^3 = P(O)(OBn)$_2$, R^2 = MOM, **71** (87%, 2 steps)
R^1 = R^2 = R^3 = P(O)(OBn)$_2$, **74** (95%)

iv or v or vi
see Scheme 7

R^1 = R^2 = R^3 = MOM, **55** (87%)
R^1 = P(O)(OBn)$_2$, R^2 = R^3 = MOM, **56** (83%)
R^2 = P(O)(OBn)$_2$, R^1 = R^3 = MOM, **59** (95%)
R^3 = P(O)(OBn)$_2$, R^1 = R^2 = MOM, **62** (98%)
R^1 = R^2 = P(O)(OBn)$_2$, R^3 = MOM, **65** (89%)
R^2 = R^3 = P(O)(OBn)$_2$, R^1 = MOM, **68** (72%)
R^2 = R^3 = P(O)(OBn)$_2$, R^1 = MOM, R^4 = R^5 = C_7H_{15}, **92**
R^1 = R^3 = P(O)(OBn)$_2$, R^2 = MOM, **72** (91%)
R^1 = R^2 = R^3 = P(O)(OBn)$_2$, **75** (78%)
R^1 = R^2 = R^3 = P(O)(OBn)$_2$, R^4 = $C_{17}H_{35}CO$, R^5 = $C_{19}H_{31}CO$, **76**

R^1 = R^2 = R^3 = H, **1** (66%)
R^1 = PO_3H_2, R^2 = R^3 = H, **2** (98%)
R^2 = PO_3H_2, R^1 = R^3 = H, **3** (94%)
R^3 = PO_3H_2, R^1 = R^2 = H, **4** (95%)
R^1 = R^2 = PO_3H_2, R^3 = H, **5** (80%)
R^2 = R^3 = PO_3H_2, R^1 = H, **7** (86%)
R^2 = R^3 = P(O)(OBn)$_2$, R^1 = MOM, R^4 = R^5 = C_7H_{15}, **7a**
R^1 = R^3 = PO_3H_2, R^2 = H, **6** (77%)
R^1 = R^2 = R^3 = PO_3H_2, **8** (79%)
R^1 = R^2 = R^3 = PO_3H_2, R^4 = $C_{17}H_{35}CO$, R^5 = $C_{19}H_{31}CO$, **8a**

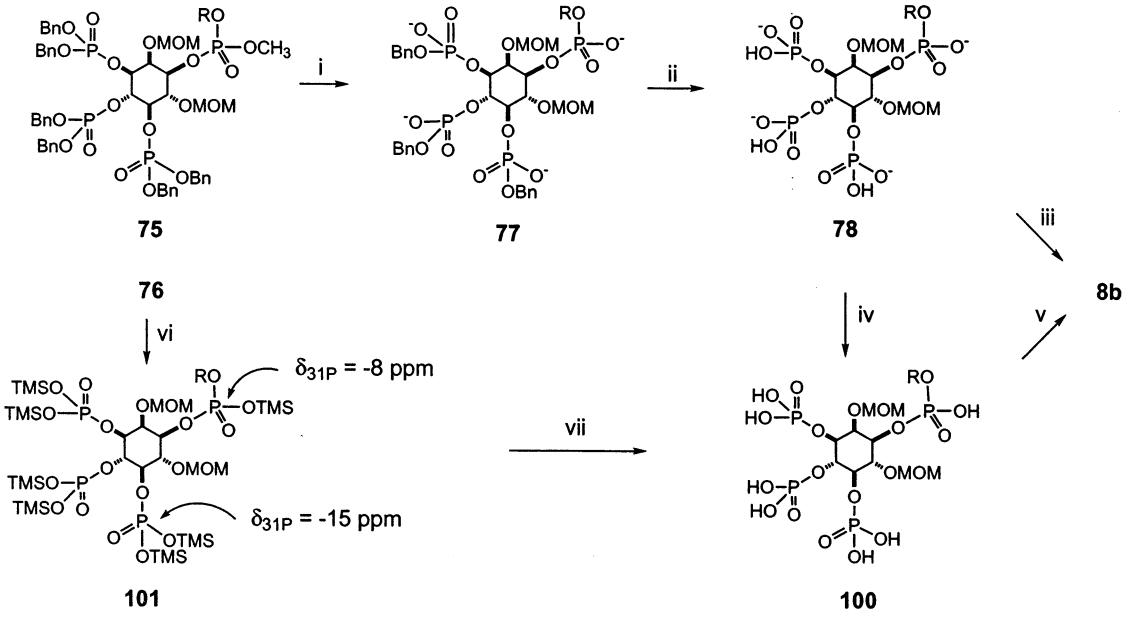
R^4 = R^5 = $C_{15}H_{31}CO$ unless otherwise indicated; i: (a) $(BnO)_2PNiPr_2$, tetrazole, (b) MCPBA, -30°C; ii: $Bu_4N^+F^-$; iii: (a) reagent **46-46b**, tetrazole, (b) MCPBA except compound **76**: Bu_4N^+, IO_4^-; (iv): (a) Me_3N, (b) H_2/Pd, (c) BF_3/EtSH; v: (a) Me_3N, (b) H_2/Pd, (c) Dowex-H^+, (d) EtSH; vi: (a) Me_3SiBr, (b) MeOH, (c) EtSH

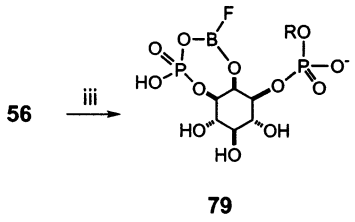
mercially available or can be easily obtained by using the well-known procedures.[22] Synthesis of 2-arachidonoyl-1-stearoyl-*sn*-glycerol **52** used in this work was patterned after the recently described procedure,[16b,23] with significant simplifying improvements as shown in Scheme 5. These include (i) application of the common DCC/DMAP condensing reagent for introduction of both the stearoyl and arachidonoyl groups, (ii) using milder conditions for cleaving the isopropylidene group (Amberlyst resin in MeOH), and (iii) using the common dimethoxytrityl group for the regioselective protection of the primary hydroxyl group, rather than the more esoteric pixyl group. The obtained yields and product purity were comparable to those reported by Gaffney and Reese.[16b,23]

Phospholipid Assembly and Deprotection Sequences. The hydroxyl derivatives **29**, **23**, **19**, **35**, **15**, **32**, and **26** have been converted into the corresponding phosphotriesters **53**, **57**, **60**, **63**, **66**, **70**, and **73** (Scheme 6), respectively, using *O*,*O*-dibenzyl-*N*,*N*-diisopropylphosphoramidite in the presence of tetrazole followed by oxidation with *m*-chloroperbenzoic acid. The silyl group in the 1-position was removed with TBAF, and the resulting 1-alcohols **54**, **58**, **61**, **64**, **67**, **71**, and **74** were treated with one of the reagents **46**−**46b** in the presence of tetrazole followed by oxidation with MCPBA (except compound **76**) to give the fully protected PIPn derivatives **56**, **59**, **62**, **65**, **68** (or **69**), **72**, and **75**, respectively. In the case of synthesis of arachidonoyl derivative **76**, oxidation

(22) Martin, S. F.; Josey, J. A.; Wong, Y.-L.; Dean, D. W. *J. Org. Chem.* **1994**, *59*, 4805−4820.
(23) Gaffney, P. R. J.; Reese, C. B. *Tetrahedron Lett.* **1997**, *38*, 2539−242.

SCHEME 7. Various Deprotection Sequences of PIP-3,4,5-P_3





of the tervalent phosphorus intermediate was performed with tetra-*n*-butylammonium periodate to avoid double bond peroxidation. For comparison, Gaffney and Reese[16b] used the triester method for attachment of the phosphatidate group where the oxidation step is eliminated altogether, and Watanabe and Nakatomi[16c] employed oxidation with *tert*-butyl hydroperoxide.

The removal of protective groups from the phosphate and hydroxyl functions can be carried out in several ways. We have previously reported the procedure[9a] whereby the fully protected PIPn derivatives were first treated with trimethylamine to give the corresponding phosphodiesters (such as **77**) at all phosphorylated positions (Scheme 7). These phosphodiesters were then subjected to catalytic hydrogenolysis to cleave all the remaining benzyl groups to afford a tribasic acid **78** and finally treated with ethanethiol in the presence of catalytic BF_3 to cleave all MOM groups. This approach, while successful with most compounds, has had several problems. First, the removal of the MOM group from the 3-phosphate derivative **56** resulted in formation of the mixed cyclic fluoroboro-phosphate **79** as a side product.[24] While, **79** can be easily removed by chromatography in the case of the PI-3-monophosphate, chromatographic purification of more highly phosphorylated PIs after the final deprotection steps is not feasible due to the very high polarity of these products. We have first improved the above procedure by the replacement of BF_3 with intramolecular protic acid catalysis (sequences iv and v, Scheme 7) provided by neighboring phosphate groups in their dibasic acid forms. Thus, the tetraalkylammonium salt of MOM-protected PIPn resulting from hydrogenolysis (such as **78**, Scheme 7) was first converted into the acid form with a Dowex cation-exchange resin and rendered anhydrous by freeze-drying with anhydrous dioxane. The resulting hexabasic acid **80** was treated with ethanethiol to afford the final trisphosphate **8**. This procedure was also successfully applied to deprotection of PI-3,4-P_2 precursor **65**; however, its extension to PI-3-P precursor **56** resulted in an incomplete cleavage of MOM-ethers, even after extended periods of time in EtSH. It is possible that due to high aggregation of $(MOM)_3$−PI-3-P in aqueous media, the cation exchange to produce the tribasic acid form of PI-3-P does not occur completely. The additional obvious flaw of both above approaches was in that the hydrogenolytic removal of benzyl groups is incompatible with the presence of the arachidonic moiety in the diacyl glycerol residue. Our attempts at selective hydrogenolysis of benzyl esters in the presence of alkenes

(24) The structure of this byproduct is corroborated by ^{31}P NMR showing one sharp and one broad signal, ^{19}F NMR, and ESMS data. The broadening of the ^{31}P NMR signal of the phosphate at the 3-position is expected due to interactions with the quadrupolar boron-7 nucleus.

with modified palladium catalysts failed to give satisfactory results. An additional difficulty of the modified procedure is that the acid form of PIPn is fragile and can potentially undergo both deacylation and phosphate migration during handling of its aqueous solutions.

The final improvement of the synthesis was achieved by the replacement of both trimethylamine and hydrogenolytic steps with TMS bromide. Thus, the fully protected derivatives **59**, **62**, **72**, **75**, and **76** were dissolved in TMS-bromide as solvent under strictly anhydrous conditions (Scheme 7). The ^{31}P NMR spectrum of the reaction mixture, recorded immediately after warming up the mixture to room temperature, showed only two groups of resonances at −15 and −8 ppm at the 3:1 intensity ratio. The chemical shifts of these signals indicated the presence of the bis-silyl and mono-silyl phosphate esters, respectively, and proved that all phosphate alkyl esters (except inositol) have been replaced by the silyl groups. In the control experiment, the inositol−phosphate ester bond was found almost completely resistant to cleavage, yielding <1% of tris-TMS-phosphate (δ_{31P} −25 ppm) after 24 h at room temperature. The mixture was concentrated under vacuum to remove excess TMS bromide and methyl and benzyl bromide products. The subsequent ^{1}H NMR spectrum of the product indicated the complete removal of all aromatic residues. While it has been reported that TMS bromide also removes MOM groups,[25] we have found that even after 24 h at least one of the MOM groups remained present in the product. The TMS-esters were cleaved by being dissolved in methanol and further treated with ethanethiol, resulting in complete deprotection of MOM groups within 1−6 h to afford >98% pure final products **3**, **4**, **6**, **8a**, and **8b**. Although so far this simple deprotection procedure was successfully tried in the synthesis of PI-4-P, PI-5-P, PI-3,5-P_2, and both saturated and unsaturated PI-3,4,5-P_3, it seems reasonable to assume that it should have general applicability to all saturated and unsatured PIPn.

In summary, we have developed a comprehensive approach to the synthesis of all so far identified naturally occurring phosphatidylinositol phosphates. The overarching strategy is the use of two key synthetic intermediates **10** and **11** from which precursors of PIPn could be generated in only 4−5 steps (6−7 steps from inositol) and the use of analogous protective groups in all precursors. In addition, our method enables synthesis of both saturated analogues of PIPn, as well as the true natural products that contain arachidonic acid. Future work will be directed at further shortening of syntheses by direct regioselective introduction of phosphomonoester groups into the 3-, 4-, and 5-positions.

Acknowledgment. This work was financially supported by the National Institutes of Health (GM 57568).

Supporting Information Available: Experimental procedures and characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0206418

(25) Hanessian, S.; Delorme, D.; Dufresne, Y. *Tetrahedron Lett.* **1984**, *25*, 2515.