Asymmetric Synthesis of Water-Soluble, Nonhydrolyzable Phosphonate Analogue of Phosphatidylinositol 4,5-Bisphosphate

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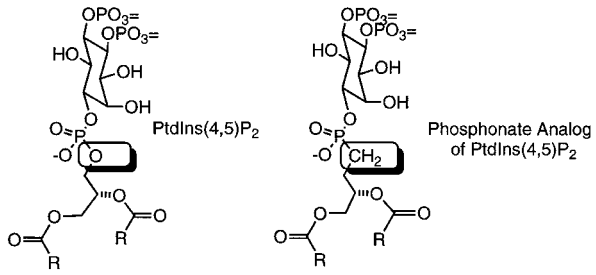
The hydrolysis of L-α-phosphatidyl-D-*myo*-inositol 4,5-bisphosphate (PtdIns(4,5)P_2) by phospholipase C (PLC) yields inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) and diacylglycerol, two key second messengers in cell signaling.[1] Four PLC isozymes (α, *β*, *γ*, and *δ*) are recognized on the basis of their modular structures and the mechanisms of their regulation.[2] The vertebrate *δ*1 isoform contains two binding sites for PtdIns(4,5)P_2, the catalytic domain and the pleckstrin homology (PH) domain, which are important for recruitment of PLC to the membrane[3] and for activation of the enzymatic activity.[4] Three-dimensional structures of this PH domain complexed to Ins(1,4,5)P_3 have been solved by NMR and X-ray crystallographic methods.[5−7] Moreover, X-ray structures of the combined C2, EF-hand, and catalytic domains have revealed many intimate details germane to the catalytic mechanism.[8−12] In biophysical studies, inhibition of PLC by basic peptides occurs by sequestration of substrates in two-dimensional domains.[13] Despite considerable effort, however, the precise mode of binding of the actual PtdIns-(4,5)P_2 substrate in the active site or in the PH domain remains elusive. The two principal difficulties are chemical in nature: (a) the natural substrate, with *sn*-1-*O*-stearoyl and *sn*-2-*O*-arachidonyl side diacylglyceryl chains, is micellar and cannot be readily employed in structural biological studies, and (b) the hydrolysis of the P−O bond occurs too rapidly for collection of NMR or crystallographic data.

Both problems also have chemical solutions. First, shorter chain analogues may be synthesized and employed for either NMR or cocrystallization experiments. The usefulness of short chain analogues as substrates for PLC isozymes is already recognized; that is, a micellar substrate is not required for PLC activity.[14] Second, analogues with slower hydrolytic rates, such as phosphorothioates,[15−17] or derivatives and analogues of the cyclic 1,2-phosphate intermediates[18] can be employed. Most recently, a number of phosphonate analogues of PtdIns have been prepared to study the structure and mechanism of bacterial PtdIns-specific PLC.[19,20] To date, however, no phosphonate analogues of PtdIns(4,5)P_2 have been reported as nonhydrolyzable substrates for the mammalian enzyme. The difficulty in synthesizing such analogues appeared to be the absence of an appropriate method to form the phosphonate C−P bond. We report herein an efficient route for the asymmetric synthesis of nonhydrolyzable phosphoinositide polyphosphates, and we show the application of this route for the preparation of a dibutyryl derivative of the PtdIns(4,5)P_2 phosphonate analogue.



Trivalent phosphorus chemistry is an essential component in the preparation of intermediates in the synthesis of inositol polyphosphates and polyphosphoinositides.[16,21,22] The tautomeric equilibrium between the trivalent hydrogen dialkyl phosphite and the pentavalent H-phosphonates has been employed to form new C−P bonds during the preparation of phosphonates. However, hydrogen dialkyl phosphites, often prepared by esterification of PCl_3 followed by hydrolysis, have not been extensively utilized in inositol chemistry. In the work described below, a hydrogen benzyl inosityl phosphite was prepared by addition of water to a phosphoramidite. This intermediate could be used to form the phosphonate analogue of the phosphodiester between a phosphorylated inositol headgroup and the *sn*-3-*O*-linked diacylglyceryl moiety.

The D-*myo*-inositol intermediate **1** was synthesized from methyl α-D-glucopyranoside as previously described[23−26] via a Ferrier rearrangement route.[27] Coupling the inositol **1** with a phosphoramidite in the presence of *N*,*N*-diisopropylethylammonium 1*H*-tetrazole at rt gave the phosphoramidite intermediate **2** (Scheme 1). Because of the steric

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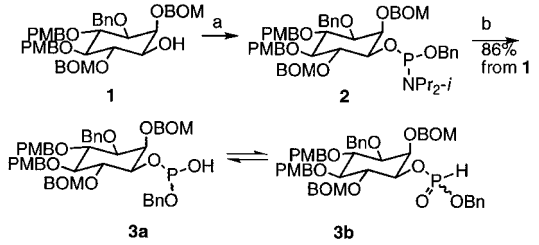
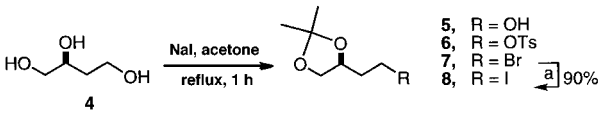
Scheme 1[a]



[a] Reaction conditions: (a) (BnO)P(NPr$_2$-*i*)$_2$, *N*,*N*-isopropylethylammonium 1*H*-tetrazole, CH_2Cl_2, rt, 2 d. (b) H_2O, 1*H*-tetrazole, rt, 1 h, CH_2Cl_2.

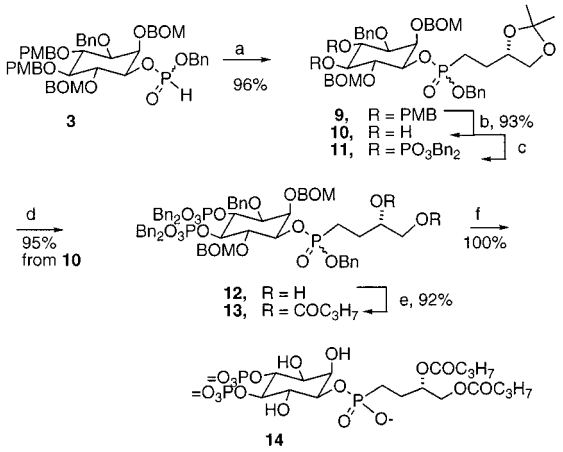
Scheme 2



hindrance of the secondary hydroxyl in precursor **1**, the sluggish reaction required more than 48 h for completion. Heating or using 1*H*-tetrazole accelerated the reaction but at the expense of increased byproduct formation. In the optimized reaction, 2 equiv of phosphoramidite and 1 equiv of *N*,*N*-diisopropylethylammonium 1*H*-tetrazole were added at $t = 0$ and again at $t = 24$ h; after 2 d, TLC showed >95% completion. At this point, 1*H*-tetrazole (2 equiv) was added followed by a small amount of water, and the reaction was stirred for an additional 1 h. The hydrogen phosphite **3** was obtained in 86% isolated yield (for two steps) based on the consumed starting inositol **1**. Two compounds, separable on SiO_2 column, corresponded to the diastereomers arising from the newly introduced chiral phosphorus. Moreover, the diastereomeric phosphites were in equilibrium with the H-phosphonate forms **3a** and **3b**, as recognized in the ^{31}P NMR spectrum of the mixture. The mixture was employed without separation, as the final product would not possess P-1 chirality.

The glyceryl synthon was prepared from (*S*)-(−)-1,2,4-butanetriol **4**. Thus, protection of the vicinal hydroxyls as an acetone acetal gave alcohol **5**, which was converted sequentially to the tosylate **6**, bromide[28] **7**, and finally to iodide **8** (Scheme 2). Reaction of the hydrogen phosphite **3** with sodium hydride in DMF generated an anion that was expected to react with tosylate **6** or with bromide **7**. In the event, the tosylate **6** and bromide **7** were not sufficiently reactive and debenzylation was observed as the predominant reaction. In contrast, iodide **8** reacted smoothly with the anion derived from **3** and gave the desired product **9** in 96% isolated yield (Scheme 3). Product **9** and compounds prepared from **9** were obtained as mixtures of two diastereomers (^{31}P, 34 and 35 ppm, in a 2:1 ratio) resulting from chirality of the tetrahedral phosphorus.[29] To avoid competing debenzylation, a reaction time of 30 min and only a 2-fold excess of sodium hydride is recommended. Oxidative removal of the PMB ethers from compound **9** with DDQ gave diol **10** in 93% yield, and reaction of diol **10** with dibenzyl *N*,*N*-diisopropylphosphoramidite followed by *m*-CPBA oxidation gave the protected bisphosphate **11**. The acetal in crude phosphonate **11** was then hydrolyzed; homogeneous **12** was obtained in 95% yield for two steps. The diol **12** could be acylated with any fatty acid; in this case, for the purpose of obtaining a water-soluble PtdIns(4,5)P_2, the dibutyrate was synthesized. Thus, carbodiimide-mediated esterification

Scheme 3[a]



[a] Reaction conditions: (a) NaH, DMF, **8**, rt, 30 min; (b) DDQ, CH_2Cl_2−H_2O (100/1, v/v), rt, 2 h; (c) $(BnO)_2P(NPr_2\text{-}i)$, 1*H*-tetrazole, CH_2Cl_2, rt, 1 h, then *m*-CPBA, 30 min; (d) *p*-TsOH·H_2O, MeOH, rt, 1 h; (e) butyric acid, DCC, DMAP, CH_2Cl_2, rt, overnight; (f) Pd/C (10%), 95% EtOH, H_2, 50 psi; then ion exchange.

with butyric acid gave the fully protected diester **13** in 92% yield with minor contamination by the dicyclohexylurea (DCU) byproduct. The target phosphonate analogue **14** was obtained in essentially quantitative yield, free from DCU, by hydrogenolysis of all protecting groups at 50 psi H_2 followed by ion-exchange purification. Removal of the protecting groups converted the diastereomeric mixtures for compounds **9**−**13** into a single stereoisomer with the D-*myo* inosityl headgroup and the methylene analogue of the *sn*-1,2-*O*-diacyl-3-phosphoglyceryl moiety.[29]

In conclusion, an efficient route for preparation of the nonhydrolyzable phosphoinositide polyphosphates is described. Specifically, a phosphonate analogue of a short-chain analogue of PtdIns(4,5)P_2 was prepared in which the *sn*-3 oxygen of the diacylglyceryl moiety is substituted by CH_2, rendering the product resistant to hydrolysis by PLC. This phosphonate analogue was obtained in 67% yield for seven steps from a protected inositol precursor. This route offers a high-yield strategy for preparation of diacylglyceryl analogues (with any chain length) of phosphoinositide phosphonates (with any inositol phosphorylation pattern) from readily available homochiral inositol precursors.

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Supporting Information Available: Experimental procedures for the synthesis as well as spectral and analytical data of compounds **3** and **8**−**14** (5 pages).

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(29) Compound **9**: ^{31}P NMR (81 MHz, $CDCl_3$) δ 34.88, 33.91 (2s, 1: 2); HRMS (FAB) calcd for $C_{59}H_{70}O_{14}P$ (MH^+) 1033.4503, found 1033.4515. Compound **13**: ^{31}P NMR (81 MHz, $CDCl_3$) δ 34.41, 33.71 (2s, 1: 2, combined as 1P), −0.49, −0.58 (combined as 1P), −0.81, −0.87 (combined as 1P); HRMS (FAB) calcd for $C_{68}H_{76}O_{18}P_3$ (MH^+) 1273.4244, found 1273.4294. Compound **14**: ^{1}H NMR (500 MHz, D_2O) δ 5.10−5.00 (m, 1H, *sn*-2), 4.06 (dd, $J = 2.5$, 12 Hz, 1H), 4.15 (q, $J = 8.5$ Hz, 1H), 4.10−4.05 (m, 2H), 3.95−3.90 (m, 2H), 3.87 (t, $J = 9.0$ Hz, 1H), 3.78 (t, $J = 9.5$ Hz, 1H), 3.60 (dd, $J = 3$, 10 Hz, 1H), 2.35−2.25 (m, 4H, CH_2CO), 1.85−1.45 (m, 8H, $2CH_2$ + CH_2CH_2P), 0.82 (2t, $J = 7.2$ Hz, 6H, CH_3) ppm; ^{31}P NMR (^{1}H-*decoupled*, 81 MHz, D_2O) δ 30.22 (s, 1P), 6.45 (s, 1P); 5.35 (s, 1P). ^{31}P NMR (^{1}H-*coupled*, 81 MHz, D_2O) δ 30.30 (dt, $^2J_{HP} = 16.4$ Hz, $^3J_{HP} = 8.1$ Hz, 1P), 6.58 (d, $^3J_{HP} = 8.4$ Hz, 1P), 5.30 (d, $^3J_{HP} = 7.0$ Hz, 1P); ^{13}C NMR (50 MHz, D_2O) δ 178.85, 178.80, 80.28 (t, $J = 5.0$ Hz), 78.30 (q, $J = 2.9$ Hz), 76.65 (d, $J = 6.6$ Hz), 75.11, 74.72, 73.70, 73.45, 73.39, 79.09, 38.35, 37.93, 26.50 (t, $J = 3.1$ Hz), 20.28, 20.17, 15.07.