Synthesis of Inositol Phosphodiesters by Phospholipase C-Catalyzed Transesterification

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Abstract: Transesterification of primary alcohol with inositol 1,2-cyclic phosphate (ICP) in the presence of phosphatidylinositol-specific phospholipase C (PI-PLC) resulted in the formation of O-alkyl inositol 1-phosphates. The starting ICP was obtained in a single step by PI-PLC catalyzed cleavage of phosphatidylinositol from the soybean phospholipid. The transesterification reaction was performed with a series of 20 structurally diverse hydroxyl compounds, ranging in the structural complexity from methanol to the series containing Ser-Tyr-Ser-Met tetrapeptides, to give the corresponding phosphodiester with 20–80% yields, depending mainly on the solubility of alcohol in water. The rates of transesterifications, and of the competing hydrolysis of ICP to inositol 1-phosphate (IP), were relatively insensitive to the alcohol structure. With polyhydroxyl compounds such as glycerol and hexitols, the enzyme displayed complete preference toward formation of the inositol phosphate derivatives of the primary hydroxyl groups. On the other hand, PI-PLC did not discriminate between primary hydroxyl groups in different environments and showed low selectivity for racemic alcohols featuring a chiral center at the β-position. The O-alkyl inositol phosphates formed were readily separable from the hydrolytic product, IP, by the anion-exchange chromatography, and were fully characterized by means of 'H and 31P NMR and electrospray MS. Our results provide a new, simple, and efficient two-step synthetic route to substituted O-alkyl inositol phosphates from inexpensive starting materials. The reported reaction was successfully applied to synthesis of complex inositol phosphate derivatives, as illustrated by inositol phosphoesters of mono- and oligosaccharides, nucleosides and peptides. The synthetic usefulness of this reaction, however, is not limited to the examples shown. Because transesterification activity of phospholipase C has not been reported before, its mechanism is discussed in a broad context of mechanisms of phosphoesterases.

Introduction

Development of new synthetic methods leading to inositol phosphates is of considerable interest in view of their role as second messengers in the transduction of cellular signals.1,2 The current state of art allows synthesis of all known naturally occurring inositol phosphates and phospholipids, and many of their analogs with almost any desired pattern of the phosphodiester or phosphomonoester substitution of inositol.3–5 Despite significant advances, however, most of the described synthetic methods still take more than 10 steps from inositol or any other precursor. The biggest effort in these syntheses is expended on elaborate protection–deprotection schemes, necessary to achieve the desired regio- and stereoselective phosphorylation pattern of inositol framework. Creating the molecular diversity needed for studies of structure–activity relationship of inositol-related enzymes and receptors,6–10 while feasible, is extremely labor intensive. In the past we have embarked on devising chemical syntheses of phosphoinositides applicable toward a goal of a broader application.8–10 In this paper we report on the first enzymatic synthesis of O-alkyl inositol phosphates starting from the corresponding alcohols, and the inexpensive and readily available soybean phospholipid, using phosphatidylinositol-specific phospholipase C (PI-PLC).

The action of PI-PLC on phosphatidylinositol (PI, 1), or its phosphorylated derivatives, produces the mixture of the corres-ponding 1,2-cyclic phosphate (ICP, 2) and inositol 1-phosphate (IP, 3) (Scheme 1) or their 4- and 5-phosphorylated con-geners,11–12 The bacterial species of PI-PLC cleave the non-phosphorylated phosphatidylinositol (PI) and glycosyl-PI to afford initially the cyclic phosphate13,14 as a sole product. This enzyme also catalyzes the subsequent slow hydrolysis of ICP into inositol 1-phosphate.14 In our earlier report8 we have

References:

1. Abbreviations: ESI MS, electrospray mass spectrometry; glycerol-3-phosphate (1-myo-inositol), G, IP, RP-HPLC, high performance liquid chromatography; ICP, inositol 1,2-cyclic phosphate; IP, inositol 1-phosphate; MOPS, morpholinepropanesulfonic acid; P, phosphate; phosphatidyl; PI-PLC, phosphatidylinositol-specific phospholipase C; SDC, sodium dodecyl sulfate; Trit, tris-hydroxy-aminomethane.

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provided evidence that in spite of the apparently different behavior of mammalian and bacterial enzymes, reflected by simultaneously varying the properties of the halides and acyclic products, respectively, both enzymes utilise analogous trans-esterification mechanisms, involving attack of the inositol 2-hydroxyl group on the phosphorus atom as a key step of the phospholipid cleavage.

Reverse reactions of esterases, proteases, and glucosidases are widely used in the synthesis of esters, peptides, and oligosaccharides, respectively. In contrast, examples of synthetic applications of phosphoesterases are limited to the transphosphatidyltransferacy activity of phospholipase A1 and trans-esterification activity of pancreatic ribonuclease A1,9,10 since venom phosphodiesterases,12,22 and alkaline phosphatase.23 This report is intended to fill this gap by exploring the synthetic potential of these phosphoesterases and synthesising the diversity of the series of inositol phosphodiesterase.

Results

Discovery of the Reverse Transphosphatidylation Catalyzed by PL-PLC. Hydrolysis of 1,2-diacyl-sn-glycero-3-phospho-(1-myos-inositol) (DPPH) in the presence of sodium deoxycholate (SDC) in 70 mM Tris-HCl buffer (pH 7.0) catalyzed by PL-PLC from both Bacillus thuringiensis or Bacillus cereus provided DPP (2, 15.61 ppm, Figure 1A). The monitoring of the increase of the percentage of IP catalyzed by PL-PLC from either source revealed formation of inositol 1-phosphate (IP), 3, 3.4 ppm as expected, and two unidentified products giving rise to signals at −0.3 and 0.5 ppm (Figure 1B). Since both enzyme preparations contained significant amounts of glycogen, the control reaction was performed using the dialyzed enzyme from B. thuringiensis in 70 mM MOPS-Na buffer at pH 7.0, which produced IP as an exclusive product (Figure 1C). In another experiment, the treatment of IP with PL-PLC from B. thuringiensis in MOPS buffer, and in the presence of 0.5 M glycogen, afforded IP and a product giving rise to the signal at −0.3 ppm (Figure 1D). These products were resolved by the anion-exchange chromatography on Sephadex QAE, and their identity was determined by NMR, MS, and high performance anion-exchange chromatography (HPAEC). The comparison of spectral and chromatographic data of this compound with those of the product of desylation of phosphatidylinositol, unequivocally established its structure as glycero-3-phospho-(1-myos-inositol) (DPP-IP). The treatment of IP with the dialyzed B. thuringiensis PL-PLC in the presence of 0.5 M Tris-HCl buffer at pH 7.0 resulted in the formation of a product giving rise to the signal at −0.5 ppm (Figure 1E), in addition to IP. Purification of this product and its analysis by 1H NMR and MS determined its structure as 1,2-diacyl-sn-glycero-3-phospho-(1-myos-inositol). Hence, both products 4 and 5 are formed as a result of the phosphophosphate C catalyzed transphosphatidylation of glycero or Trz, respectively, through the ring opening of the five-membered cyclic phosphate. In the blank experiments, incubation of IP with each of the alcoholase in the absence of enzyme over several days produced no reaction.

Driving Force for Transphosphatidylation. The hydrolysis of the cyclic five-membered phosphodiesterase is energetically more favorable in comparison with hydrolysis of the acyclic phosphodiesterase due to the presence of the C-O bond.

Table 1. H-H and F-F Coupling Constants for IP, IP, and Inositol

<table>
<thead>
<tr>
<th>Coupling (Hz)</th>
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<tbody>
<tr>
<td>H-H</td>
<td>Inositol</td>
<td>IP</td>
<td>DPP</td>
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<td>H-H</td>
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<tr>
<td>F-F</td>
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<td>F-F</td>
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In anhydro DMSO in the presence of boric trifluoride in the form of the mixture of the corresponding O-butyryl inositol 1- and 2-phosphates. The low yield (15%) of the acyclic diester and the low regioslectivity of this reaction indicated, however, that the utility of IP as a nonenzymatic phospho-esterase is doubtful (see also ref 25).

Enzymatic Synthesis of IP. For transphosphatidylation reaction of alcohols with IP to be of any synthetic utility, the latter starting material has to be readily available. The earlier reported syntheses of the racemic IP27 and its phosphorhochrome analog28 from inositol have been ruled out as a source of this material, due to a greater difficulty in synthesis of the enantio- mERICally pure IP. Alternatively, we have considered naturally occurring phosphoinositides as a starting material. The crude phospholipid, containing P in as a major component, can be readily obtained in the multigram quantity from the inexpensive soybean phospholipid by the precipitation with methanol from its chloroform solution.29 The treatment of the sodium deoxycholate (SDC) dispersion of this phosphoinositide fraction with phosphatase C followed by the detergent removal via chloroform-methanol extraction at the pH 5.0, and further anion-exchange chromatography afforded IP contaminated with only small amounts of the glycosylated IP. In a typical procedure, treatment of 20 g soybean phospholipid with only 20 μg of PLC afforded 1.7 g of IP. This procedure can be further simplified by omitting the use of the detergent.

The time course of transphosphatidylation of Alcohols with IP. A representative time course of the enzymatic transphosphatidylation of an alcohol with IP catalyzed by PL-PLC, exemplified by the hydrolysis of IP with 1,6-butanediol (Figure 2). The formation of the O-1,6-di-hydrindoxyethyl-1-myos-inositol phosphate (6) was followed by HPAEC on Carboxap PA1 column giving rise to the 1,6-phosphodiester remaining in IP (Table 2). The concentration of IP increased mainly at the cost of the acyclic diester product, while the concentration of IP declined only very slowly. At the very long reaction times (several days) IP.

Figure 2. MM2 optimized conformations of IP and IP. The numerical values designates C1-C2-C1-C6 and C2-C1-C6-C5 torsional angles.
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was an exclusive product (not shown, but see Figure 1F for analogous reaction). Due to the breakdown of the acidic phosphodiester at the longer reaction times, their optimal yields in reactions of various alcohols were assured by monitoring the reactions by HPLC-PAD, reverse phase HPLC, or 1H NMR and stopping the reaction when the concentration of an acidic ester reached a highest point.

Alcohol Specificity of PI-Specific Phosphoplas C. In order to determine structural specificity of the enzyme we have examined a series of reactions of ICP with primary and secondary alcohols (Scheme 2). The results are summarized in Table 2 and in Figure 4A,B. The reactions of alcohols with ICP in the presence of PLC resulted in the formation of the corresponding acrylic acid phosphodiesters 4–22 with the yields generally exceeding 20%, but in some cases greater than 70%. The highest yields were obtained in cases of simple alcohols, where their high molecular concentrations were possible. The yields in some cases were limited by the poor solubility of the alcohols in water. The transesterification reaction was found to be of a general nature, with most alcohols participating at a comparable efficiency (Figure 4A). For example, the calculated alcohol efficiency parameter, defined as the ratio of the molar fraction of the alcohol in the product mixture in the molar fraction of alcohol in the starting alcohol/water solution, was as follows: methanol (6), 1-methylcyclohexanone (12), propanol (17), and pyridine (21), ethylene glycol (25), glycerol (28), methanol (29), and Triton X100. The rate of the formation of the acrylic diesters with such alcohols as ethylene glycol, glycerol, methanol, and isopropanol were very close indicating that this reaction is quite insensitive to the nature of substituents at the β-position such as hydroxyl, amino, tert-amylidammonium, carbonyl, and sulfonate group. The only exception among alcohols studied was Triton X100, which produced ca. 2-fold higher yield of the product. As shown in Figure 4B the presence of alcohols and their structural variation had little effect on the initial rates of the competing ICP hydrolysis, indicating that none of the alcohols studied was bound in the ICP site. Although the range of alcohol structures accepted by the enzyme was quite broad, we did note a few examples where we were unable to obtain products of transesterification. Reactions with secondary alcohols, isopropanol alcohol, 2-butanol, and cyclohexanol did not affect the corresponding phosphodiester, nor did the presence of such alcohols affect the rate of the hydrolysis of ICP (results not shown). Likewise, reactions with long chain primary alcohols such as dodecanol and hexadecanol also failed to afford the product. The attempt to

increase the alcohol solubility in water by adding the hydrophilic function, as in α-hydroxyhexanoic acid, produced a negative result, as well. On the other hand, the addition of 0.5 M isopropanol did not provide the acidic diester product, but it almost completely inhibited the hydrolysis of ICP, indicating isosteric binding in the ICP site as previously reported.88 Other unsuccessful attempts included alcohols with added electrophilic reactivity, such as in bromoethanol and glycidol.

In order to optimize transesterification conditions we have investigated the dependence of the yield of the phosphodiester upon the alcohol concentration (Figure 5). The yield of the diester 6 linearly increased with the concentration of hexane-1,6-diol and started leveling off only at concentrations above 2 M, indicating weak binding affinity of the alcohol to PI-PLC.

Regio- and Stereoselectivity of PI-Catalyzed Transesterification. With compounds featuring multiple hydroxyl groups such as glycerol and glycerol there is a possibility of two types of product isomerism due to phosphorylation of the primary vs secondary hydroxy group in mannositol and the pro-S vs the pro-R hydroxy group in glycerol. In all cases of polyols examined we have seen only phosphorylation of the primary hydroxy group, as evidenced by observation of either a quartet or a doublet of triplets (not shown) in 1H-coupled 13C NMR spectra. Phosphorylation of the hydroxyl group is also evident from the analysis of the 1H NMR spectra. The reaction of ICP with glycerol afforded two isomers giving rise to very closely spaced 1H NMR signals with 45:55 intensity ratio (Figure 6A). The addition to this product of a single isomer of GPlc, obtained by deacylation of PL, showed the increase of the down-field signal (Figure 6B). Both 1H NMR and 1H–1H COSY spectra were consistent with the presence of two stereoisomers having either the pro-R or pro-S hydroxy group phosphorylated. 1H NMR spectrum of the two stereoisomers is homotropic because of the C2 symmetry. Further experiments with racemic alcohols such as di-propyn-1,2-diol (12) and di-(17) also provided almost equimolar mixtures of diastereomers (not shown). In summary, while PI-PLC displays a strong preference for the primary hydroxy groups, it exhibits very low stereoselectivity with regard to configuration at the C-2 of an alcohol. This conclusion is consistent with our earlier results showing that synthetic manipulation of the PLC by PI-PLC is nonstereospecific with regard to configuration at the C-2 of the diacylglycerol moiety.

Application of PI-PLC-Catalyzed Transesterification to Synthesis of Complex Inositol Phosphodiester. The potential of the PI-PLC-catalyzed transesterification reaction as applied to derivatization of structurally complex compounds is illustrated by the syntheses discussed below: (A) 6-glucosylphospho-inositol (20), (B) 5′-uridylphospho-inositol (22), and (C) inositol-1-phosphate derivatives of Si-2'-deoxy-Ara-cytidine (23). (A) Monitoring of the transesterification of PI-PLC (the mixture of α- and β-isomers) with ICP by HPLC-PAD indicated formation of a new product giving rise to the peak at 15.4 min, in addition to those of ICP and IP (Figure 7A). Purification of this product by the preparative anion-exchange chromatography and analysis by 1H, 13C, and ESI-MS confirmed the identity of the product as 6-glucosylphosphoinositol. The product 20 constituted a mixture of both α- and β-isomers as indicated by the presence of two doublets at 5.20 ppm (3.8 Hz) and at 4.62 ppm (8.0 Hz), respectively, in 1H NMR spectrum. The two anisotonic products are not resolved by HPLC and 13C NMR. An analogous reaction of lactose with ICP produced a complex mixture of two α- and two β-isomers (21), due to the random phosphorylation of the 6-hydroxy groups in the galactose and glucose rings. The obtained mixture of lactose-phosphoinositol gave rise to a broad 13C NMR signal at 0.5 ppm, and two HPLC peaks at 13.6 min and 14.0 min. This
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Figure 7. (A) Formation of glucose inositol phosphate as observed by HPAE-PAD on CarboPak PA1. Identities of peaks are α, 2.8 min, glucose, α, 11.5 min, inositol, 11.5 min, IP; α, 15.5 min, inositol 1,4,5-trisphosphate. (B) HPAE of the purified 20. Chromatographic conditions are the same as in Figure 3.

Figure 8. (A) A representative RP-HPLC of the reaction mixture of St-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 9. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with 22 and PL-PLC after 48 h at 30 °C. The UV absorbance of the phospholipid products 22, α, 15.4 min; 23a, α, 15.5 min; 23b, α, 15.8 min. Reaction conditions: tetrapeptide 0.04 M, PL-PLC 4 μg. Chromatographic conditions are described in Experimental Section. (B) An overlay of the UV spectra of the tetrapeptide, the product 23a and the product 23b.

Figure 10. (A) Deuterium-labeled HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 11. (A) A representative RP-HPLC of the reaction mixture of St-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 12. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 13. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 14. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 15. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 16. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 17. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 18. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 19. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 20. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 21. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 22. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 23. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.

Figure 24. (A) A representative RP-HPLC of the reaction mixture of Ser-Tyr-Ser-Met tetrapeptide with IP to form uridine inositol phosphate (UI). (B) HPLC of the purified 22. See Experimental Section for chromatographic conditions. (C) A subregion of the UV spectrum of the tetrapeptide, the product 23a and the product 23b.
phosphates (Scheme 3), and (II) those which use an internal hydrolyzable group as a substrate for a polyether enzyme. First, note the similarities between the transannexation reaction catalyzed by PIPLC to those of other phospholipases.

(A) Snake venom phospholipases A2 cleave the phospho-ester linkage of 7,5-diminoquinine and nucleoside triphosphates by a trans-annexation mechanism involving formation of the covalent phospho-enzyme intermediate. This enzyme also catalyzes transannexation of various primary amines, which nucleoside triphosphates through alcohol attack at the reactive phospho-enzyme intermediate. The rate of transannexation is slow as compared to ATP, and it is dependent on the independent structure of the alcohol. In general, the alcohol efficiency parameters for this enzyme are several-fold lower than those for PIPLC, although a similar pattern is observed in the phospho-enzyme intermediate. The recently reported ester group exchange in phosphatidylcholine by PLD in the presence of several hexosides and glucose is proposed to play a role in the diabetic metabolism, while transannexation of phosphatidylcholine by phospholipase C could be important in fetal alcohol syndrome.

(B) Ribonuclease A catalyzes transannexation of single primary alcohols with 2,3-cyclic nucleoside phosphates, at a much higher rate as compared to the alcohol. This enzyme is related to the basic PIPLC by an analogous catalytic mechanism, and by the fact that the cyclic phospho-ester is the physiologically end-product of the RNA cleavage. The latter is due to the lower phospho-ester that is formed, which results mostly due to the kcat value from the absence of a part of the substrate structure needed to bind to the phospho-enzyme intermediate. In contrast, the reaction rate is brought about by the removal of the hydrophobic chains from the substrate.

In summary, we have shown a potential for PIPLC in synthesis of active-site related to phospholipase C. One of the possible areas of application of the produced compounds in synthesis of structure-activity relationship of isolated-related enzymes such as PIPLC, or in phospholipase C. Both of these enzymes take part in lipidic recycling, and thus could contain targets of antigingival drug action.

Experimental Section

[1] NMR spectra were recorded in D$_2$O with a Bruker AM-400, AC-250, or AC-300 spectrometer. The samples were dissolved in pH 7.0 buffer (in the case of diesters) and would be converted into a phospholipid, an energetically disadvantageous process due to a higher solvation energy of the products as compared to the diester.

The observed transannexation of alcohol by ICP in the presence of PI-PLC fails, thus, into a general property of group I and II phospholipases. We note the similarities between the transannexation reaction catalyzed by PIPLC to those of other phospholipases.

(B) Snake venom phospholipases A2 cleave the cleavage of 7,5-diminoquinine and nucleoside triphosphates by a transannexation mechanism involving formation of the covalent phospho-enzyme intermediate. This enzyme also catalyzes transannexation of various primary alcohols, which nucleoside triphosphates through alcohol attack at the reactive phospho-enzyme intermediate. The rate of transannexation is slow as compared to ATP, and it is dependent on the independent structure of the alcohol. In general, the alcohol efficiency parameters for this enzyme are several-fold lower than those for PIPLC, although a similar pattern is observed in the phospho-enzyme intermediate. The recently reported ester group exchange in phosphatidylcholine by PLD in the presence of several hexosides and glucose is proposed to play a role in the diabetic metabolism, while transannexation of phosphatidylcholine by phospholipase C could be important in fetal alcohol syndrome.

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Mannitol-1-phosphate (1-myo-inositol) (13): 1H NMR δ 4.07 (tr, H-2, J = 2.7 Hz, 1H), 4.0 (m, H-1', 1H), 3.89 (m, H-1', 1H), 3.78 (dtt, H-1, J = 10.8, 2.8 Hz, 1H), 2.6 (m, 3H), 3.58–3.54 (m, 3H), 3.51–3.41 (m, 3H), 3.35 (dd, H-3, J = 10, 2.7 Hz, 1H), 3.12 (tr, H-5, J = 9.3 Hz, 1H); 13C NMR δ 0.99 ppm; ESMs m/z 423.0.

Iditol-1-phosphate (1-myo-inositol) (14): 1H NMR δ 4.23 (tr, H-2, J = 2.6 Hz, 1H), 4.02–3.9 (m, H-1', 1H-2', 1H-4H), 3.84 (m, 3H, H-3), 3.78–3.6 (m, 6H), 3.53 (dd, H-3, J = 10.0 Hz, 1H), 3.31 (tr, H-5, J = 9.5 Hz, 1H); 13C NMR δ 0.63 ppm; ESMs m/z 423.0.

(1-Hydroxypropyl) 1-myo-inositol phosphate (15): 1H NMR δ 4.04 (tr, H-2, J = 2.4 Hz, 1H), 3.85 (trd, H-1', overlapped, 2H), 3.75 (ddd, H-2, J = 2.9, 9.7, 12.4 Hz, 1H), 3.55 (tr, H-6, J = 9.6 Hz, 1H), 3.46 (tr, H-4, J = 9.3 Hz, 1H), 3.35 (dd, H-3, J = 2.5, 10.1 Hz, 1H), 3.13 (tr, H-5, J = 9.2 Hz, 1H), 2.96 (tr, H-3, J = 7.3 Hz, 2H), 1.82 (p, H-2', J = 6.6 Hz, 2H); 13C NMR δ 0.68 ppm; ESMs m/z 317.0.

Cholinephospho-1-myo-inositol (16): 1H NMR δ 4.28 (m, H-1'A, 2H), 4.15 (tr, H-2, J = 2.8 Hz, 1H), 3.87 (ddd, H-1, J = 2.8, 8.4, 9.9 Hz, 1H), 3.67 (tr, H-6, J = 9.7 Hz, 1H), 3.58 (m, H-2', 2H), 3.56 (H-4, J = 9.3 Hz, 1H), 3.47 (dd, H-3, J = 2.7, 10 Hz, 1H), 3.24 (tr, H-5, J = 9.3 Hz, 1H), 3.13 (s, Me, 9H); 13C NMR δ 0.6. ESMs m/z 329.9.

Serine-phospho-1-myo-inositol (17): 1H NMR δ 4.09 (tr, H-1', J = 5.4 Hz, 2H), 4.04 (tr, H-2, J = 2.7 Hz, 1H), 3.81 (tr, H-2', J = 4.29 Hz, 1H), 3.75 (ddd, H-1, J = 12.0, 9.0, 3.0 Hz, 1H), 3.53 (tr, H-6, J = 9.8 Hz, 1H), 3.41 (dd, H-4, J = 18.6, 9.3 Hz, 1H), 3.35 (dd, H-3, J = 10.02, 1.8 Hz, 1H), 3.12 (tr, H-5, J = 9.3 Hz, 1H); 13C NMR δ 0.74 ppm; ESMs m/z 346.

Pentaethyleneglyco-phospho-1-myo-inositol (18): 1H NMR δ 4.24 (tr, H-2, J = 2.8 Hz, 1H), 4.05 (m, CH_2O, 2H), 3.94 (ddd, H-1, 1H), 3.68–3.77 (m, OCH_2CH_2O_16H), 3.63 (m, H-6, CH_2OH, 4H), 3.53 (dd, H-3, 1H), 3.31 (tr, H-5, 1H); 13C NMR δ 0.49 ppm; ESMs m/z 479.0 (M + H^+).

Biotinylpentaethyleneglyco-phospho-1-myo-inositol (19): 1H NMR δ 4.60 (dd, H-1, 4.42 (dd, H-1, 4.25 (m, CH_2O, 2H), 3.94 (ddd, H-1, 1H), 3.60–3.80 (m, OCH_2CH_2O_16H), 3.52 (dd, H-3, 1H), 3.78 (m, H-6, 2H), 2.96 (dd, H-1, 2.75 (d, H-1), 2.42 (tr, H-2, 1.32–1.80 (m, 6H); 13C NMR δ 0.44 ppm; ESMs m/z 706.

Glucose-6-phospho-1-myo-inositol (20): 5.20 (d, J = 4.8 Hz, 4.62 (d, J = 8.0 Hz, 4.23 (tr, J = 2.7 Hz, 2H), 4.16 (dd, J = 5.3, 9.6 Hz, 4.11 (tr, J = 4.9 Hz, 4.06 (q, J = 5.3 Hz, 3.93 (m, H-1, 1H), 3.71 (tr, J = 9.7 Hz, 1H), 3.61 (tr, J = 9.7 Hz, 1H), 3.53–3.43 (m, 3H), 3.29 (tr, J = 5.4 Hz, 1H), 3.25 (m); 13C NMR δ 0.65 ppm; ESMs m/z 421.4.

Uridine-5'-phospho-1-myo-inositol (22): 1H NMR δ 7.89 (d, J = 8.0 Hz, 1H), 5.92 (d, H-1', J = 4.7 Hz, 1H), 5.90 (d, J = 8.0 Hz, 1H), 4.31 (m, H-5', 2H), 4.2 (m, H-2' overlapped with H-4' and H-2', 3.09 (m, H-3', 1H), 3.92 (m, H-1', 1H), 3.71 (tr, H-6, J = 9.6 Hz, 1H), 3.61 (H-4, J = 9.8 Hz, 1H), 3.50 (dd, H-3, J = 2.8, 6.0 Hz, 1H), 3.27 (tr, H-5, J = 9.4 Hz, 1H); 13C NMR δ 0.47 ppm; ESMs m/z 485.

Transesterification of S-Tyr-Ser-Met with ICP. The solution (50 µL) of tetrapeptide (1 mg) in water was treated with PI-PLC solution (4 µL, 1 mg/mL). The progress of the reaction was monitored by RP-HPLC on C18 column using gradient elution as specified earlier. The reaction was stopped after 48 h and the mixture subjected to LC-ESIMS, using chromatographic conditions as described earlier.

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Supporting Information Available. 1H and 13C NMR spectra and ESM data for compounds 2–23 synthesized in this work (27 pages). See any current masthead page for ordering and Internet access instructions.

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