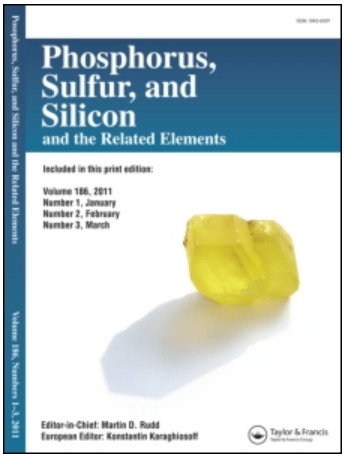

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Design, Synthesis and Biochemical Applications of Analogs of Phosphatidylinositol

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DESIGN, SYNTHESIS AND BIOCHEMICAL APPLICATIONS OF ANALOGS OF PHOSPHATIDYLINOSITOL

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Abstract A general methods for synthesis of diverse precursors of phosphoinositides have been elaborated and applied towards synthesis of a variety of inositol phosphates, phospholipids, and their phosphorothioate, oxygen-labeled and stereoisomeric analogs. The obtained compounds have been used to study mechanism of phosphatidylinositol phospholipase C. The acquired mechanistic information was then applied for design and synthesis of inhibitors of this enzyme.

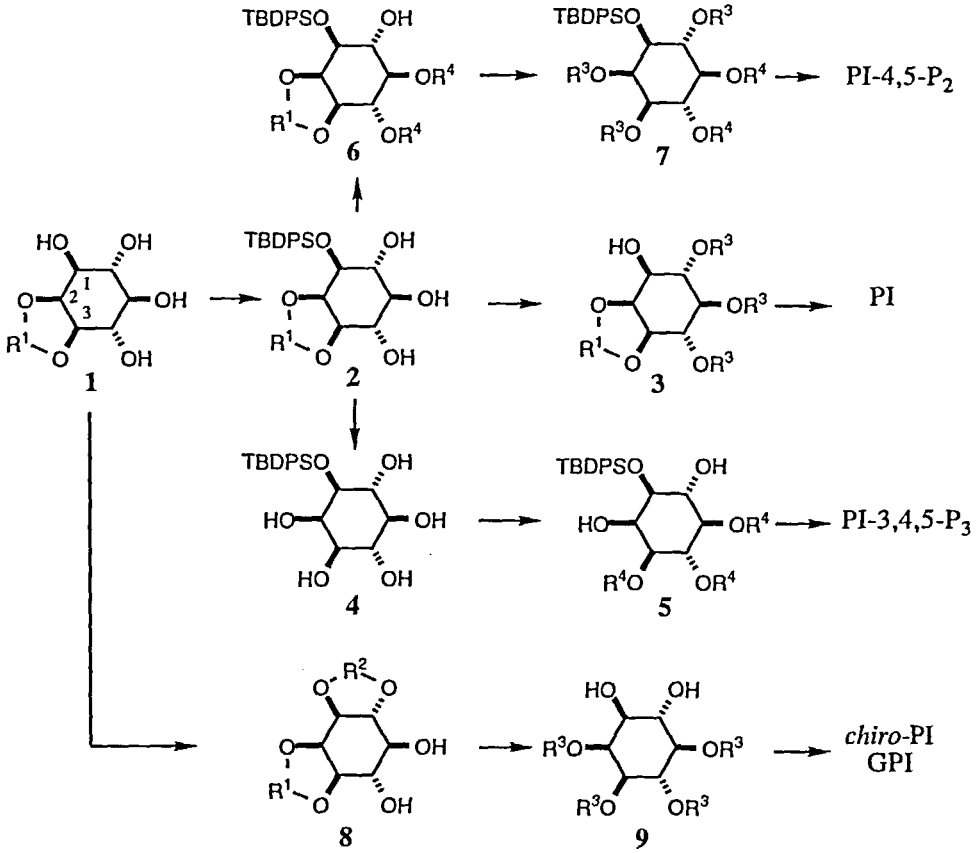
INTRODUCTION

Receptor-mediated cleavage of inositol phospholipids by phosphatidylinositol-specific phospholipase C (PI-PLC) is a key step in signal transduction of many hormones, neurotransmitters and growth factors [1]. Growth factors such as PDGF, EGF, FGF are known to increase levels of inositol trisphosphate second messenger by activation of PI-PLC-γ_1 [2]. On the other hand, insulin and TGF stimulate production of glycosylinositol phosphates (GIP), another family of putative second messengers known as insulin mediators, by activation of glycosylphosphatidylinositol-specific phospholipase C (GPI-PLC) [3]. One type of such insulin mediators have been found to contain the rare *chiro*-inositol instead of the typical *myo*-isomer [3]. Due to the central role of phospholipase C in signal transduction pathways the major thrust of our research is aimed at determining the mechanism of this enzyme [4,5], and design and synthesis of mechanism-based inhibitors as research tools for studying inositol-metabolism and as possible pharmacological agents against cancer, inflammation and hypertension. Furthermore, we work towards understanding the mechanism of transduction of insulin signal by investigating substrate properties of model *chiro*-phosphoinositides [6], and isolation and structure determination of *chiro*-inositol-containing phospholipids, precursors of the putative insulin mediators.

SYNTHESIS OF INOSITOL PHOSPHATES AND PHOSPHOLIPIDS

In view of our long-term interest in phosphoinositide metabolism it was essential to develop general methods enabling efficient synthesis of all naturally occurring inositol phosphates and phospholipids, and of some of their analogs. Our strategy has been to introduce highly versatile inositol intermediates [7] applicable towards synthesis of a variety of protected inositol precursors and further to synthesis of phosphomonoesters (inositol phosphates, IP), phosphodiesters (phosphatidylinositol, PI) or both (phosphatidylinositol phosphates, PIP) [4-9]. Several of our synthetic procedures are summarized in Scheme 1. Acetalization of inositol with D-camphor dimethyl acetal produces the pure diastereomer of 2,3-acetal **1** (30%) in a single step, not requiring chromatographic separations [10,11]. Further regioselective protection of the acetal **1** with tert-butyldiphenylsilyl (TBDPS) group at the 1-position produces a pivotal intermediate **2**, which upon exhaustive protection gives rise to the precursor **3** of phosphatidylinositol.

Scheme 1



R^1: bornanedi-2,2-yl; R^2: tetraisopropyldisiloxanedi-1,3-yl;
R^3: methoxymethyl; R^4: benzoyl

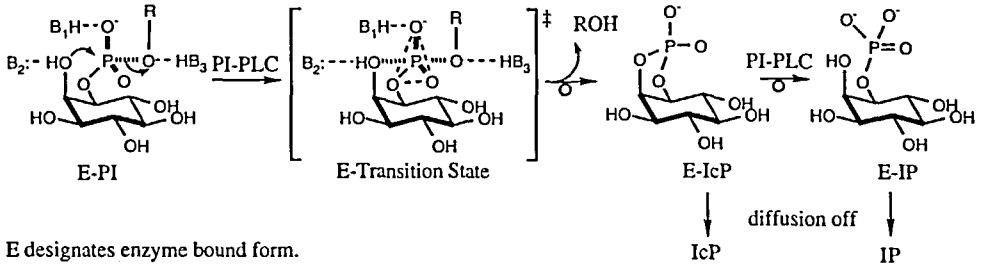
The deacetalization of the silyl derivative **2** affords the extremely versatile intermediate **4**, which upon selective tris-benzoylation gives rise to the precursor **5** of phosphatidylinositol 3,4,5-trisphosphate (PI-3,4,5-P_3). The respective monobenzoylation leads to a precursor of PI-3-P. On the other hand, benzoylation of the intermediate **2** produces selectively the 4,5-bisbenzoate **6**, which upon further deacetalization and protection gives a precursor **7** of PI-4,5-P_2. Silylation of the acetal **1** with 1,3-bissilyl dichloride occurs exclusively at the 1- and 6-positions to give the 4,5-diol **8** and a convenient access to the 1,6-diol **9** useful in syntheses of *chiro*-phosphatidylinositol and glycosylinositol phosphates. All the above shown intermediates and many other ones are generated in only 6-8 steps from inositol and have been used to produce almost every natural inositol phosphate and phospholipid [4-11]. An exemplary synthesis of an inositol phospholipid starting from the pentol **4** is described elsewhere in this volume in a related communication (see also ref. 9).

MECHANISM OF PHOSPHOLIPASE C

Despite significant sequence homology between mammalian and bacterial species of PI-PLC simultaneous vs. sequential formation of inositol 1,2-cyclic phosphate (IcP) and inositol 1-phosphate (IP) by these enzymes suggested different mechanisms. The origin of

this behavior was investigated by a stereochemical approach using P-chiral phosphorothioate and oxygen-isotope labeled analogs of PI as substrates [12]. PI-PLC from both mammalian and bacterial sources stereoselectively hydrolyzed the (Rp)-diastereomer of the phosphorothioate analog of PI to afford the 1,2-cyclic product with inversion of configuration at phosphorus. The bacterial enzyme further hydrolyzed IcP to IP with inversion of configuration [4]. Likewise, the mammalian enzymes produced IcP with inversion and IP with retention. These results can be best explained in terms of a unified mechanism presented in Scheme 2 [4].

Scheme 2

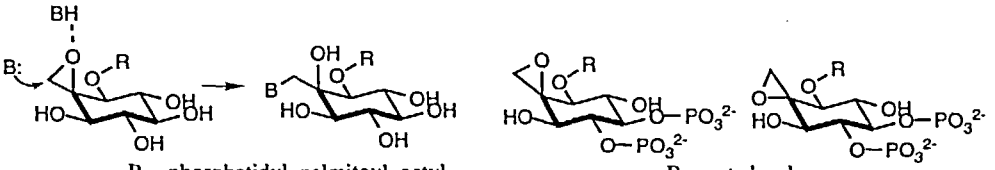


E designates enzyme bound form.

Formation of the pentacoordinated transition state with both the attacking 2-hydroxyl group and the diacyl glycerol oxygen leaving group occupying apical positions is preceded by deprotonation of the 2-hydroxyl group and protonation of the pro-*S* oxygen atom of the phosphate group in the case of all enzymes. The principal mechanistic difference between the enzymes is that the mammalian PI-PLC hydrolyzes the cyclic intermediate while it most likely still resides in the active site, while the bacterial enzyme releases the cyclic intermediate prior to the next hydrolytic step. Since the polar cyclic product is devoid of hydrophobic residue its active site binding upon return might not produce the catalytically competent enzyme conformation [13]. The presence of the hydrophobic side chain is important for efficient catalysis since the rate of hydrolysis is several times lower for short-chain PI [14], and several hundred times lower for short chain alkyl inositol phosphates, compared to natural PI. Furthermore, the mammalian PI-PLC binds the deacylated phosphatidylinositol 4-phosphate, but does not hydrolyze it [15].

Based upon the above postulated mechanism the replacement of the 2-hydroxyl group with the epoxide residue should confer inhibitory property to the analog of PI due to possible protonation of the axial oxygen of the epoxide and further epoxide opening by the enzymic base (Scheme 3). This amino acid residue would hence become alkylated causing irreversible inactivation of the enzyme. A series of compounds analogous to PI and PIP_2 have been synthesized, and their inhibitory properties towards PI-PLC are currently under investigation.

Scheme 3

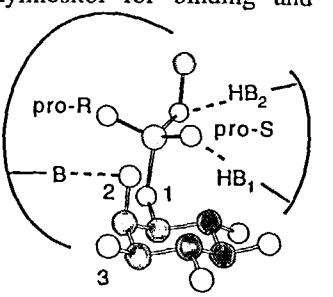


R = phosphatidyl, palmitoyl, octyl

R = octadecyl

MAPPING THE PHOSPHOLIPASE C ACTIVE SITE

In the absence of the three dimensional model of the active site the rational design of PI-PLC inhibitors can only rely on the mechanistic data and results of structure specificity studies. Significance of structural features of phosphatidylinositol for binding and catalysis can be summarized as follows [for a more detailed discussion see ref. 5]: (i) The presence of the charged phosphodiester group is not necessary for binding, since nonionic analogs bind with a similar affinity as PIP_2; [16] (ii) Protonation of the *pro*-S oxygen atom of the phosphoryl group is, however, essential for catalysis, since its replacement with sulfur causes a complete cessation of hydrolysis [12]; (iii) The presence of the 2-hydroxyl group is essential for catalysis, but not necessary for binding since the 2-deoxy analog of PI is an inhibitor of melanoma PI-PLC; (iv) The equatorial 3-hydroxyl group is essential for catalysis or binding, since its inversion causes 10^3-fold reduction of the cleavage rate [6]; likewise, PI 3-phosphates are completely resistant to PI-PLC; (v) The bridging oxygen of diacylglycerol moiety is essential for catalysis, since its replacement with sulfur causes 10-100 fold reduction in k_{cat} [17]; (vi) the exact structure of diacylglycerol and its configuration at the C-2 are insignificant, since both the single chain PI and the PI analog with a reversed configuration of the glycerol moiety are equally good substrates. In general, different main types of phospholipase C display various degrees of tolerance to substrate modifications at certain positions. Based on the findings above it should be possible to devise inhibitors with a narrow specificity towards these PI-PLC types.



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