

# A Versatile Approach to PI(3,4)P<sub>2</sub>, PI(4,5)P<sub>2</sub>, and PI(3,4,5)P<sub>3</sub> from L-(–)-Quebrachitol

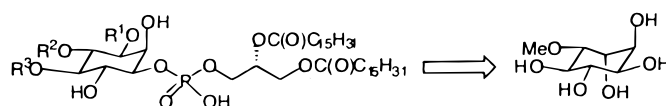
Lixin Qiao,<sup>†</sup> Youhong Hu,<sup>†</sup> Fajun Nan,<sup>†</sup> Garth Powis,<sup>‡</sup> and Alan P. Kozikowski<sup>\*,†</sup>

Drug Discovery Program, Institute for Cognitive and Computational Science,  
Georgetown University Medical Center, 3900 Reservoir Road NW,  
Washington, D.C. 20007, and Arizona Cancer Center, 1515 North Campbell Avenue,  
Tucson, Arizona 85724

kozikowa@giccs.georgetown.edu

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## ABSTRACT



PI(3,4)P<sub>2</sub> R<sup>1</sup> = R<sup>2</sup> = PO<sub>3</sub>H<sub>2</sub>, R<sup>3</sup> = H ;

PI(4,5)P<sub>2</sub> R<sup>1</sup> = H, R<sup>2</sup> = R<sup>3</sup> = PO<sub>3</sub>H<sub>2</sub> ;

PI(3,4,5)P<sub>3</sub> R<sup>1</sup> = R<sup>2</sup> = R<sup>3</sup> = PO<sub>3</sub>H<sub>2</sub> ;

L-(–)-quebrachitol

A versatile synthesis of PI(3,4)P<sub>2</sub>, PI(4,5)P<sub>2</sub>, and PI(3,4,5)P<sub>3</sub> is disclosed, starting from L-(–)-quebrachitol, a byproduct of latex production. The crystalline nature of most intermediates and the utilization of inexpensive protecting groups facilitate this synthetic route and its scale-up.

Phosphatidyl-*myo*-inositol (PI) occupies a unique position in that it can undergo reversible phosphorylation at multiple sites to generate five different phosphoinositides,<sup>1</sup> while its metabolites regulate two pathways important for cell proliferation, the inositol phosphate/diacylglycerol signaling pathway<sup>2,3</sup> and the phosphatidylinositol 3-phosphate (PI-3-kinase) pathway (depicted in Figure 1).<sup>4,5</sup> In the first pathway, PI-specific phospholipase C (PI-PLC) hydrolyzes a minor membrane phospholipid, PI(4,5)P<sub>2</sub>, to give the water-soluble Ins(1,4,5)P<sub>3</sub> and a lipophilic diacylglycerol (DAG). Ins(1,4,5)P<sub>3</sub> has been found more than two decades ago to be a second messenger<sup>6</sup> which transduces cellular signals through interacting specifically with membrane receptors to

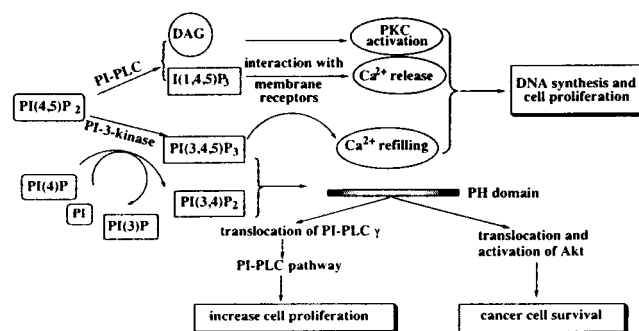


Figure 1. PI-PLC and PI-3-K pathway.

release Ca<sup>2+</sup> from stores in the endoplasmic reticulum, while DAG is an endogenous activator of protein kinase C (PKC).<sup>7</sup>

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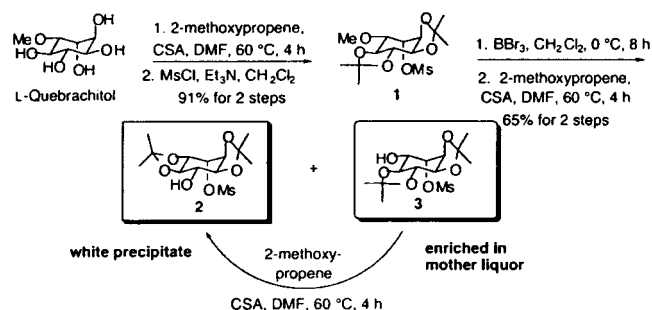
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Together, the increase in  $[Ca^{2+}]$  and the increased activity of PKC lead to a sequence of events that culminate in DNA synthesis and cell proliferation. In the second pathway, PI-3-kinase has been found associated with almost every growth factor receptor or oncogene transformation.<sup>8</sup> PI-3-kinase phosphorylates PI and PIP's with a free 3-OH group to give a class of compounds that are poor substrates for hydrolysis by PI-PLC. The PI-3-phosphates are present in the cell in varying amount; PI(3,4)P<sub>2</sub> and PI(3,4,5)P<sub>3</sub> predominate, while PI(3)P is present in smaller amounts. PI(3,4)P<sub>2</sub> and PI(3,4,5)P<sub>3</sub> are responsible for the effects of PI-3-kinase on tumor growth and apoptosis through activation of the pleckstrin homology (PH) domain of certain proteins.<sup>9</sup> The most extensively studied examples of PH domain-regulated signaling are the PH domain-dependent activation by PI(3,4)P<sub>2</sub> and PI(3,4,5)P<sub>3</sub> of PI-PLC $\gamma$ <sup>10</sup> and of Akt,<sup>11,12</sup> a proto-oncogene. Inhibiting Akt activation induces cancer cell apoptosis,<sup>13</sup> while activation of PI-PLC $\gamma$  will result in cell proliferation through the PI-PLC pathway. Because of the importance of the PIP<sub>n</sub>'s in studies of cell signaling, and due to the difficulty of isolating these materials from natural sources, efficient synthetic routes to these molecules are required.<sup>14</sup>

In the literature,<sup>15</sup> *myo*-inositol has been widely used as a starting material for the synthesis of PIP<sub>n</sub>'s. To obtain the natural D enantiomers, optical resolution of *myo*-inositol intermediates is required.<sup>14a,15</sup> Although D-glucose<sup>16</sup> has also served as a chiral starting material, suitable protecting groups have to be introduced individually for different PIP<sub>n</sub>'s at the beginning to facilitate later incorporation of phosphate groups at selected positions. The naturally occurring cyclitol L-(–)-quebrachitol is a byproduct of rubber manufacture.<sup>17</sup> Although it has been widely used as a starting material for the synthesis of 3-modified phosphatidylinositols, to our knowledge, this is the first report demonstrating the use of L-(–)-quebrachitol in the preparation of phosphatidylinositol polyphosphates. Herein, we present a versatile approach to the synthesis of selected PIP<sub>n</sub>'s from L-quebrachitol.<sup>18</sup>

First, four of the five vicinal hydroxyls of L-(–)-quebrachitol were protected as their acetonides. After mesylation of the remaining 3-OH group, demethylation and concurrent removal of the acetonides with BBr<sub>3</sub> gave rise to a pentol bearing an intact mesylate group at position 3. Reprotection of the hydroxyl groups of this pentol with 2-methoxypropene resulted in the pair of regioisomers **2** and **3**. The differential solubilities of compounds **2** and **3** in hexanes–ethyl acetate (3/1 v/v) permit isolation of one key intermediate, compound **2**, by crystallization.<sup>19</sup> The other regioisomer, **3**, was found enriched in the mother liquor with a purity up to 96% (by <sup>1</sup>H NMR) (Scheme 1). The availability of intermediate **3**

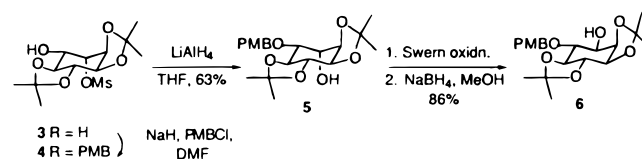
**Scheme 1.** Synthesis of Key Intermediates from L-Quebrachitol



allows the differential protection of 4-OH and gives ready access to PI(3,4)P<sub>2</sub> after inversion of the chiral center at position 3.

The synthesis of PI(3,4)P<sub>2</sub> from compound **3** is exemplified here to illustrate the synthetic strategy. The inversion of the stereochemistry at C-3 was readily accomplished through an oxidation/reduction sequence after LAH reduction of the mesylate (Scheme 2). Next, differential protection must be

**Scheme 2.** Inversion of the Chiral Center at C-3



introduced at O-5 and O-6. Generally, the synthesis of a given PIP<sub>n</sub> requires the preparation of an inositol intermediate with temporarily protected hydroxyl groups at the positions later to be phosphorylated. In our study, the *p*-methoxybenzyl group (PMB) was applied for this purpose. By procedures similar to those published before,<sup>20</sup> compound **6** was

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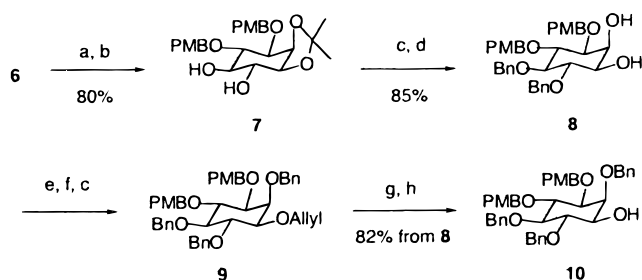
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transformed into diol **8**, bearing PMB groups at positions 3 and 4. At this stage, an allyl group was introduced for temporary protection at position 1. The mild conditions used for its removal have proven to be without effect on PMB groups.<sup>14c</sup> In contrast, attempts to protect position 1 by a MOM group was problematic, for the acidic conditions used for its removal also resulted in cleavage of the PMB groups. Thus, selective allylation at position 1 was achieved via a 1,2-*O*-stannylene intermediate.<sup>21</sup> The stoichiometric addition of CsF greatly improves the yield for this step. After benzylation at position 2, isomerization of the double bond in the allyl group with RhCl(PPh<sub>3</sub>)<sub>3</sub> under basic conditions and subsequent acidic hydrolysis in aqueous HCl–acetone furnished the desired compound **10** (Scheme 3).

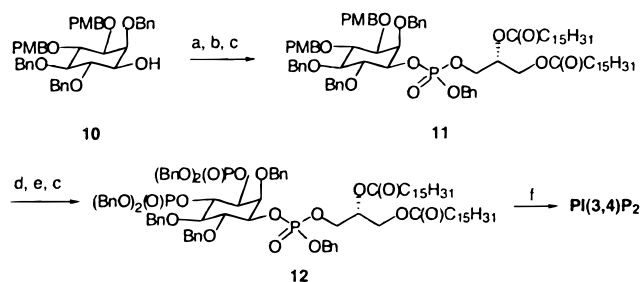
**Scheme 3.** Synthesis of Key Intermediate **8** from L-Quebrachitol<sup>a</sup>



<sup>a</sup> Legend: (a) NaH/PMBCl, DMF; (b) AcCl (cat.), MeOH–CH<sub>2</sub>Cl<sub>2</sub>; (c) BnBr, NaH, DMF; (d) concentrated HCl (cat.), MeOH; (e) Bu<sub>2</sub>SnO, toluene, reflux; (f) allyl bromide, CsF, DMF, 18 h, room temperature; (g) RhCl(PPh<sub>3</sub>)<sub>3</sub> (cat.), DABCO, EtOH, reflux; (h) acetone/1 N HCl (v/v 9/1), reflux.

Phosphitylation of compound **10** with *O*-benzyl *N,N,N',N'*-tetraisopropylphosphorodiamidite catalyzed by diisopropylammonium tetrazolide gave rise to the corresponding phosphoramidite, which was then coupled with 1,2-dipalmitoyl-*sn*-glycerol in the presence of tetrazole. Oxidation of the resulting phosphite with *tert*-butyl hydroperoxide provided the desired phosphate **11** in 60% overall yield for three steps (Scheme 4). Removal of the PMB groups with DDQ in wet chloroform provided the corresponding diol, which was purified by column chromatography on silica gel with CHCl<sub>3</sub>–MeOH (30/1 v/v) as eluent. The EtOAc–hexane solvent system failed to give a good separation of the diol from 2,3-dichloro-5,6-dicyanohydroquinone. Phosphorylation of this diol with dibenzyl diisopropylphosphoramidite and subsequent oxidation resulted in compound **12**. Eventually, debenzoylation of compound **12** with 20% Pd(OH)<sub>2</sub>/C in

**Scheme 4<sup>a</sup>**

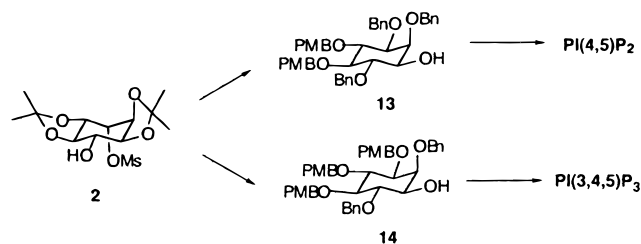


<sup>a</sup> Legend: (a) BnOP(NPr<sub>2</sub>)<sub>2</sub>, *i*-Pr<sub>2</sub>NH–tetrazole; (b) diacylglycerol, tetrazole; (c) *t*-BuOOH; (d) DDQ, CH<sub>2</sub>Cl<sub>2</sub>–H<sub>2</sub>O; (e) (BnO)<sub>2</sub>PNPr<sub>2</sub>, tetrazole; (f) 20% Pd(OH)<sub>2</sub>/C, *t*-BuOH.

*t*-BuOH under 70 psi of hydrogen delivered PI(3,4)P<sub>2</sub> in almost quantitative yield.

By steps similar to those shown in Scheme 3, compound **2** was transformed into the key intermediates **13** and **14**, which were further developed into PI(4,5)P<sub>2</sub> and PI(3,4,5)-P<sub>3</sub>, respectively (Scheme 5).

**Scheme 5**



In conclusion, we present an efficient approach to PI(3,4)-P<sub>2</sub>, PI(4,5)P<sub>2</sub>, and PI(3,4,5)P<sub>3</sub> starting from L-(–)-quebrachitol. The low cost of the starting material, the crystalline nature of most intermediates, the limited number of chromatographic separations, and the utilization of inexpensive protecting groups facilitate this synthetic route and its scale-up.

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**Supporting Information Available:** Experimental procedures for the synthesis of PI(3,4)P<sub>2</sub> from L-(–)-quebrachitol and spectral data for the final products and intermediates **2**–**14**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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