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Synthesis and Akt inhibitory properties of a 1D-3,4-dideoxyphosphatidylinositol ether lipid

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Abstract

1D-3,4-Dideoxyphosphatidylinositol ether lipid **2**, a PI analog, was synthesized through a sequence of protection/deprotection protocols and two Barton deoxygenation reactions, starting from L-(−)-quebrachitol. DDPIEL is 18-fold more potent than its monodeoxy counterpart DPIEL in the inhibition of PI3-K. © 2000 Published by Elsevier Science Ltd.

The proto-oncogenic serine/threonine kinase Akt (also known as RAC-PK or protein kinase B (PKB)) is one of the most extensively studied of the PH domain-regulated signaling proteins acting downstream of PI3-K. Binding of the PH domain of Akt to membrane PI(3)Ps causes the translocation of Akt to the plasma membrane bringing it into contact with membrane bound Akt kinase (phosphatidylinositol dependent kinase-1 and 2 (PDK1 and 2)), which phosphorylates and activates Akt.[1] Akt inhibits apoptosis by phosphorylating a number of downstream targets. Thus, the inhibition of Akt activation induces cancer cell apoptosis. Three mammalian isoforms of Akt have been identified, Akt1, Akt2, and Akt3. Akt1 has been found to be overexpressed in gastric adenocarcinomas, and Akt2 is overexpressed in breast, ovarian, and pancreatic cancer.[2] While the PH domain of Akt binds both PI(3,4)P_2 and PI(3,4,5)P_3 in vitro, only PI(3,4)P_2 activates Akt.[3] Thus, the design and synthesis of PI(3,4)P_2 antimetabolites capable of blocking Akt activation may offer a new approach to the design of anticancer agents that work by blocking Akt's ability to repress apoptosis in cancer cells.

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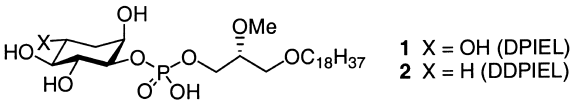
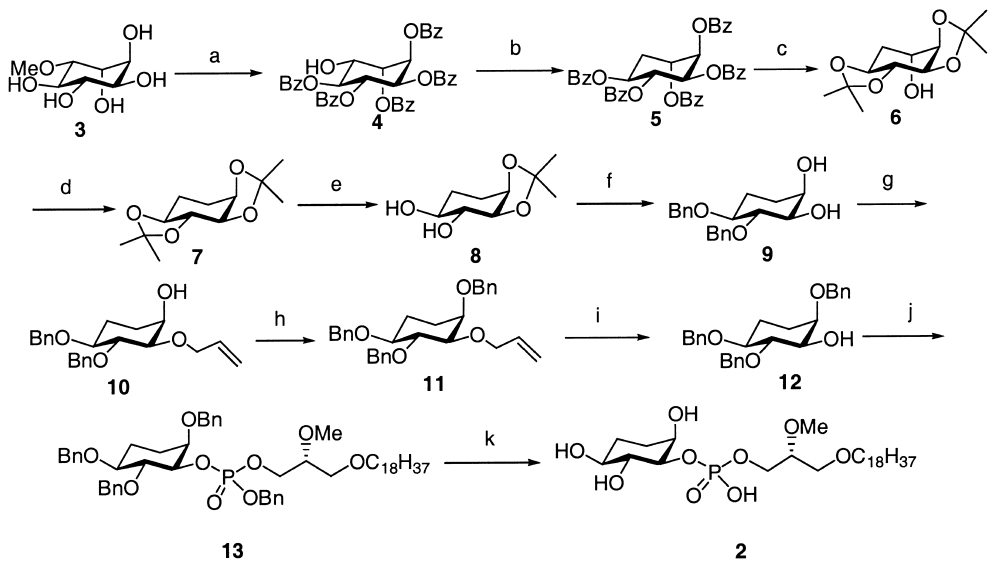


Figure 1.

In our previous work, 3-deoxy-PI ether lipid **1** (DPIEL, Fig. 1) was found to inhibit PI3-K ($IC_{50} = 14.8 \pm 5.6$ μM) and Akt ($IC_{50} = 1.5 \pm 0.3$ μM), and to block the growth of HT-29 colon cancer cells ($IC_{50} = 2.1$ μM) both in vitro and in tumor xenografts in *scid* mice. This phospholipid is currently under preclinical development through the RAID program of the National Cancer Institute (NCI).[4] Because of the promising activity exhibited by DPIEL, a PI analog that cannot be phosphorylated by PI3-K to produce PI(3)P-related metabolites (such as $PI(3,4)P_2$) due to the absence of the 3-OH group, it was deemed valuable to examine the activity of a PI analog lacking hydroxyl groups at both positions 3 and 4. The biological effect of the deletion of this additional hydroxyl group upon Akt and PI3-K activity, and cancer cell growth relative to DPIEL would provide important SAR data for further lead optimization. In the present paper we detail the synthesis of 1D-3,4-dideoxyphosphatidylinositol ether lipid **2** (DDPIEL) starting from L-quebrachitol and disclose preliminary information on its biological activity (Scheme 1).



Scheme 1. Synthesis of DDPIEL from L-quebrachitol. *Reagents and conditions*: (a) (i) PhCOCl, DMAP (cat.), pyridine, rt; (ii) CrO_3, AcOH, rt; (iii) AcCl, MeOH, reflux; (b) (i) CS_2, DBU, MeI, DMF, rt; (ii) *n*-Bu_3SnH, AIBN, toluene, reflux; (c) (i) K_2CO_3, MeOH, rt; (ii) 2-methoxypropene, CSA (cat.), DMF, 0 to 60°C; (d) (i) NaH, CS_2, MeI, DMF, rt; (ii) *n*-Bu_3SnH, AIBN, toluene, reflux; (e) *p*-TsOH (cat.), MeOH–CH_2Cl_2 (1:5), rt; (f) (i) NaH, BnBr, DMF, rt; (ii) conc. HCl, MeOH, rt; (g) (i) Bu_2SnO, toluene, reflux; (ii) AllylBr, CsF, DMF, –50°C to rt; (h) NaH, BnBr, DMF; (i) (i) $RhCl(PPh)_3$, DABCO, EtOH, reflux; (ii) 1N HCl, acetone, reflux; (j) (i) $BnOP(N\text{-}iPr_2)_2$, diisopropylammonium tetrazolide, CH_2Cl_2, rt; (ii) 1-*O*-octadecyl-2-*O*-Me-*sn*-glycerol, tetrazole, CH_2Cl_2, rt; (iii) *t*-BuOOH, CH_2Cl_2, rt; (k) H_2, 20% $Pd(OH)_2/C$, *t*-BuOH, 65 psi

The five vicinal hydroxyl groups of L-(−)-quebrachitol **3** were first protected as their benzoates. Next, using Angyal's method,[5] the methyl group of the resulting pentabenzoate was converted into a formyl group by oxidation with chromium trioxide in acetic acid. The formyl group was then removed by treatment with acetyl chloride in methanol to provide **4** bearing a free hydroxyl at position 4 in 70% yield. Next, the hydroxyl group in **4** was removed by application of the Barton deoxygenation procedure.[6] Accordingly, treatment of **4** with CS_2 and MeI furnished the corresponding *S*-methyl xanthate intermediate, which was then deoxygenated homolytically to **5** with *n*-Bu_3SnH. Next, the benzoate groups of **5** were cleaved by the action of potassium carbonate, and the resulting free hydroxyl groups then reprotected with 2-methoxypropene to afford the bis-acetonide **6** possessing a free hydroxyl group at position 3 (31% yield). Removal of the hydroxyl group of **6** to give **7** was then effected in the same way as before by using the Barton reaction. While the treatment of **7** with a catalytic amount of AcCl in CH_2Cl_2–MeOH (5:1) for 5 minutes[7] gave rise to the corresponding tetraol, exposure of **7** to a catalytic amount of *p*-TsOH in CH_2Cl_2–MeOH (2:1) for 20 min at 0°C furnished the required diol **8** in 82% yield by selective cleavage of the *trans*-*O*-isopropylidene group.

Next, following protocols similar to those previously detailed by our group,[8] compound **8** was reprotected at positions 5, 6 by benzylation, and then the *cis*-acetonide was removed by acid hydrolysis to yield **9** in 98% yield. The diol **9** was selectively allylated at position 1 via a 1,2-*O*-stannylene intermediate to give compound **10** in 86% yield. After benzylation of **10** at position 2 to afford **11**, removal of the allyl group was accomplished in two steps that entailed: (a) isomerization of the double bond with $RhCl(PPh_3)_3$ and DABCO in ethanol, and (b) acidic hydrolysis of the resulting vinyl ether to afford compound **12** in 69% yield for the two steps. The intermediate **12** was transformed into the corresponding protected phosphatidylinositol analog **13** in accordance with established protocols in 30% yield.[4b,c] Finally, hydrogenolysis of the benzyl groups of compound **13** with 20% $Pd(OH)_2$/C in *t*-butanol gave the required 1D-3,4-dideoxyphosphatidylinositol ether lipid (DDPIEL) **2**[9] in 85% yield.

To evaluate the biological activity of DDPIEL, initial studies examined its ability to block the growth of NIH 3T3 cells (IC_{50} = 2.0 μM; for DPIEL, IC_{50} = 4.5 μM). DDPIEL is also a good inhibitor of Akt with an IC_{50} of 11.0±2.5 μM; it is about sevenfold less potent than DPIEL (1.5±0.3 μM). Interestingly, this dideoxy analog was found to be 18-fold more potent (IC_{50} = 0.8±0.1 μM) than the monodeoxy analog DPIEL (IC_{50} = 14.8±5.6 μM) in the inhibition of PI3-K. Further studies of the antiproliferative action of DDPIEL are underway, and these data will be reported separately.

In conclusion, the present work details the first synthesis of a novel PI analog, namely, 1D-3,4-dideoxyphosphatidylinositol ether lipid **2**, starting from L-(−)-quebrachitol as the chiral educt. The synthesis protocol entails a sequence of protection/deprotection protocols and two Barton deoxygenation reactions. DDPIEL is 18-fold more potent than its monodeoxy counterpart DPIEL in the inhibition of PI3-K.

Acknowledgements

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9. Compound **12**: $[\alpha]_D = -11.7°$ (*c* 0.12, $CHCl_3$/MeOH 1:1); ^1H NMR (300 MHz, $CDCl_3/CD_3OD$ 1:1, TMS) δ 4.14 (m, 3H), 4.00 (t, 1H, $J = 7.5$ Hz), 3.72 (t, 1H, $J = 9.0$ Hz), 3.62–3.41 (br m, 9H), 1.87 (dd, 1H, $J = 14.4$, 3.6 Hz), 1.76 (m, 2H), 1.58 (m, 3H), 1.27 (m, 30H), 0.88 (t, 3H, $J = 9.3$ Hz); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$ 1:1, TMS) δ 82.35, 79.54 ($J = 6.1$ Hz), 74.43, 73.45, 72.42, 69.92, 68.62, 66.54, 58.12, 32.47, 30.91, 30.20, 30.07, 30.03, 29.88, 27.29, 26.57, 26.48, 23.18, 14.29; ^{31}P NMR (121 MHz, $CDCl_3/CD_3OD$ 1:1, 85% H_3PO_4) δ −0.02.