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Synthesis and biological activity of 3-hydroxy(phosphono)methyl-bearing phosphatidylinositol ether lipid analogues

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Abstract—Two 3-hydroxy(phosphono)methyl-bearing phosphatidylinositol ether lipid analogues were synthesized and shown to be inhibitors of Akt and PI3-K. These compounds were also shown to inhibit the growth of HT-29 human colon cancer cells and MCF-7 human breast cancer cells. © 2002 Elsevier Science Ltd. All rights reserved.

Phosphatidylinositol 3-kinase (PI3-K) phosphorylates the 3-position of phosphatidylinositol (PI), PI(4)P, and PI(4,5)P_2 to give rise to three signaling phospholipids: PI(3)P, PI(3,4)P_2, and PI(3,4,5)P_3, respectively.[1] These 3-phosphorylated PIs have the unique ability to bind to specific protein domains, the so-called pleckstrin homology (PH) domains, of a number of signaling proteins. One of the most extensively studied of the PH domain-regulated signaling proteins acting downstream of PI3-K is the proto-oncogenic serine/threonine kinase Akt. In particular, 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.[2] Akt is a proto-oncogene that inhibits apoptosis by phosphorylating a number of downstream targets, thus, the inhibition of Akt activation induces cancer cell apoptosis.[3] An important counterpart to PI3-K is the tumor suppressor PTEN, a protein that is able to bring about the dephosphorylation of PI(3,4,5)P_3, with specificity being shown for the phosphate at the D-3 position of the inositol ring. Mutations in the PTEN tumor suppressor gene appear to be a common occurrence in a number of human cancers.[4] Thus, PI3-K and Akt provide novel targets for drugs to inhibit the repression of apoptosis in cancer cells and thereby the opportunity to overcome the effects of the loss of the tumor suppressor PTEN.

In our previous work,[5] we have designed and synthesized some 3-modified PI ether lipid analogues[6] (Fig. 1), and found that all of these compounds exhibit some activity for the inhibition of Akt and PI3-K. Because of these favorable results, we deemed it valuable to investigate the activity of other 3-modified PI ether lipid analogues with the aim to further improve upon Akt inhibition and antiproliferative action. In this paper, we detail the synthesis and biology of analogues that bear a hydroxy(phosphono)methyl group at position 3. These compounds thus act as mimics of 3-phosphorylated PI.

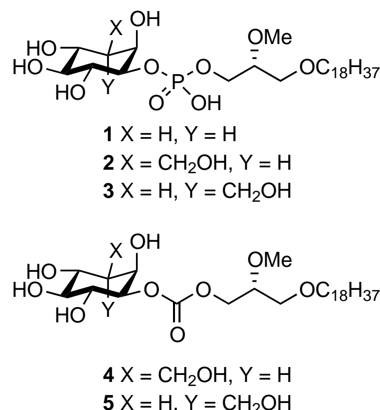

Figure 1.

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Compound **6** was synthesized according to our published method from L-quebrachitol.[6] After allyl protection of the 3-hydroxymethyl group, the *trans*-acetonide was selectively removed to give diol **7**. The two hydroxyl groups of **7** were protected by benzylation, and the second acetonide was removed to yield diol **8**. After selective *p*-methoxybenzylation of the 1-OH via a 1,2-*O*-stannylene intermediate and benzylation of the 2-OH, the allyl group was removed to give alcohol **9**. Dess–Martin oxidation of the hydroxymethyl group in **9** gave an aldehyde. Reaction of this aldehyde with lithium dibenzyl phosphite (prepared in situ from dibenzyl phosphite and butyllithium) yielded two phosphonates **10** and **11**, which can be separated by chromatography. Subsequently, the *p*-methoxybenzyl groups in **10** and **11** were removed by oxidation with CAN to give two key intermediates **12** and **13** (Scheme 1).

In order to ascertain the stereochemistry of **12** and **13**, we transformed **6** into phosphonate **14** by Dess–Martin oxidation and subsequent phosphite addition (Scheme 2). It is interesting that in this case only one isomer was obtained. Compound **14** proved to be crystalline, and we established its structure by X-ray analysis (Fig. 2).

Compound **14** was totally deprotected by hydrogenation and subsequent acidification to give **15**. By comparing the ^{1}H and ^{13}C NMR spectra of **15** with those of the deprotection products of **12** and **13**, we found that the hydrogenation product of **13** is identical to **15** (Scheme 2).

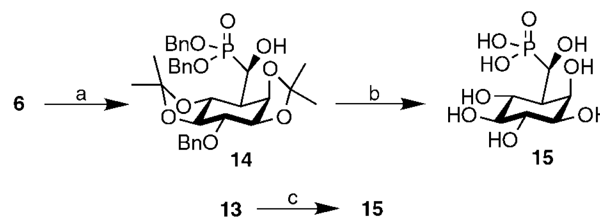
After solving the problem of stereochemistry, **12** and **13** were phosphorylated by reaction with the ether lipid phosphoramidite **17** catalyzed by 1*H*-tetrazole and subsequent oxidation of the phosphite intermediates with *tert*-butyl hydroperoxide. Finally the resulting phosphates **18** and **19** were completely deprotected by catalytic hydrogenation to give the desired PI analogues **20** and **21** (Scheme 3).[7,8]

Biological activity

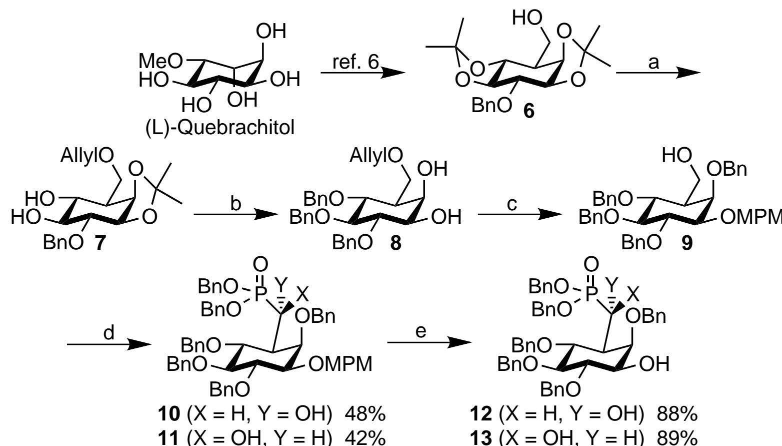
Data for the two new PI analogues from the PI3-K and Akt activity studies are presented in Table 1 along with comparison data obtained previously for compounds **2** and **3**.

The two new analogues exhibit comparable activity for the inhibition of Akt and PI3-K, while **21** is more selective for Akt than **20**. The compounds are more potent inhibitors of Akt and PI3-K than the hydroxymethyl-bearing compounds **2** and **3**, which suggests that the phosphonate moiety may increase their binding affinity for these two signaling proteins.

The growth inhibition of different cancer cell lines by compounds **20** and **21** was also tested (Table 2). We found that these PI analogues have similar cell growth inhibition effects on HT-29 human colon cancer and MCF-7 human breast cancer cell lines, but that they are somewhat less active than compounds **2** and **3**. They also exhibit some activity against the NIH3T3 mouse embryo derived cell line. The cell growth inhibitory effects of these PI analogues are probably due to a combination of their PI3-K and Akt inhibitory activities along with effects on other cell signaling proteins.[6]



Scheme 2. *Reagents and conditions*: (a) (i) Dess–Martin periodinane, CH_2Cl_2, rt, (ii) dibenzyl phosphite, BuLi, THF, −78°C, 85% for two steps; (b) (i) H_2, 20% $Pd(OH)_2$–C, *t*-BuOH, 1 atm, rt, (ii) 3N HCl, rt, 82%; (c) H_2, 20% $Pd(OH)_2$–C, *t*-BuOH, 1 atm, rt, 96%.



Scheme 1. *Reagents and conditions*: (a) (i) NaH, allyl bromide, DMF, 0°C to rt, (ii) AcCl (cat.), CH_2Cl_2–CH_3OH, rt, 72% for two steps; (b) (i) NaH, BnBr, DMF, 0°C to rt; (ii) AcCl (cat.), CH_3OH, rt, 90% for two steps; (c) (i) Bu_2SnO, toluene, reflux, then *p*-$MeOC_6H_4CH_2Cl$, CsF, DMF, rt, (ii) NaH, BnBr, DMF, 0°C to rt; (iii) $RhCl(PPh_3)_3$ (cat.), DABCO, EtOH, reflux, then 1N HCl, acetone, reflux, 85% for three steps; (d) (i) Dess–Martin periodinane, CH_2Cl_2, rt, (ii) dibenzyl phosphite, BuLi, THF, −78°C; (e) CAN, CH_3CN–H_2O, 4:1 (v/v), rt.

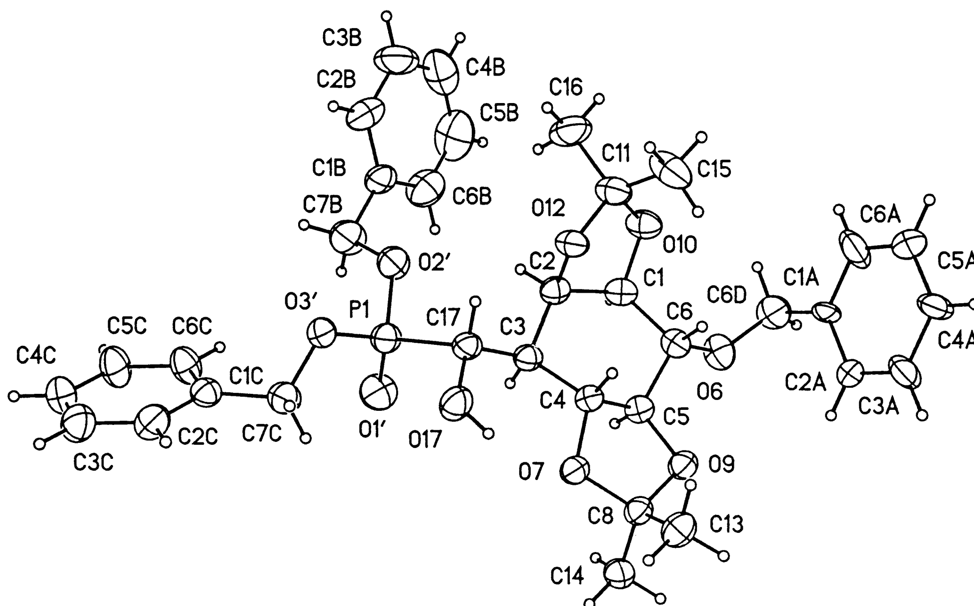
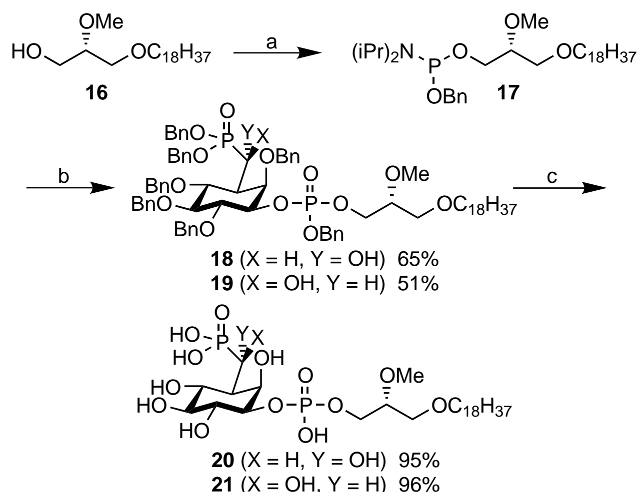


Figure 2. X-Ray structure of compound **14**.



Scheme 3. *Reagents and conditions*: (a) $BnOP(N(i\text{-}Pr)_2)_2$, diisopropylammonium tetrazolide, CH_2Cl_2, rt, 98%; (b) (i) **12** or **13**, 1*H*-tetrazole, CH_2Cl_2, rt, (ii) *tert*-butyl hydroperoxide, CH_2Cl_2, rt; (c) H_2, $Pd(OH)_2$–C, *t*-BuOH, 1 atm, rt.

Table 1. IC_{50} values for inhibition of Akt and PI3-K activity

Compound	IC_{50} (μM)	
	Akt	PI3-K
2	7.8±0.8	31.0±7.0
3	9.1±1.7	18.5±1.7
20	4.5±1.3	5.7±1.2
21	2.5	8.8±1.7

In conclusion, the present paper details the synthesis and biological activity of two new 3-hydroxy(phosphono)methyl-bearing PI analogues. These compounds are structurally related to PI-3-phosphate and act as reasonably good inhibitors of Akt and PI3-K.

Table 2. Effects of compounds **2**, **3**, **20** and **21** on the growth inhibition of cell lines in vitro (72 h exposure)

Compound	IC_{50} (μM)		
	HT-29	MCF-7	NIH3T3
2	4.5	5.0	–
3	7.5	2.0	–
20	7.8	9.0	19.8
21	7.8	8.0	19.8

Acknowledgements

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7. Compound **20**: $[\alpha]_D^{25}=-4.6$ (*c* 0.2, $CHCl_3$–CH_3OH, 1:1); ^1H NMR (300 MHz, CD_3OD–$CDCl_3$, 1:1, TMS): δ 4.75 (br s, 1H), 4.40 (br d, $J=6.9$ Hz, 1H), 4.22–4.02 (m, 2H), 4.02–3.88 (m, 2H), 3.83 (t, $J=9.3$ Hz, 1H), 3.70–3.40 (m, 9H), 1.80 (m, 1H), 1.55 (m, 2H), 1.25 (br s, 30H), 0.88 (t, $J=6.6$ Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD–$CDCl_3$, 1:1): δ 81.07, 79.53, 72.45, 71.80, 70.53, 69.92, 68.64, 67.94, 67.79, 67.76, 58.25, 42.74, 32.45, 30.21, 30.17, 30.05, 30.02, 29.88, 26.54, 23.18, 14.40; ^{31}P NMR (CD_3OD–$CDCl_3$, 1:1, 121 MHz, 85% H_3PO_4): δ 23.49, −0.42. Anal. calcd for $C_{29}H_{60}O_{14}P_2$: C, 50.14; H, 8.71; found: C, 50.14; H, 8.69%.
8. Compound **21**: $[\alpha]_D^{25}=5.1$ (*c* 0.43, $CHCl_3$–CH_3OH, 1:1); ^1H NMR (300 MHz, CD_3OD–$CDCl_3$, 1:1, TMS): δ 4.37 (br s, 1H), 4.35–4.25 (m, 1H), 4.23–3.98 (m, 4H), 3.69 (t, $J=9.3$ Hz, 1H), 3.53–3.40 (m, 9H), 2.10–1.93 (m, 1H), 1.62–1.50 (m, 2H), 1.26 (br s, 30H), 0.88 (t, $J=6.6$ Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD–$CDCl_3$, 1:1): δ 81.32, 79.72, 77.89, 72.58, 71.72, 71.28, 71.08, 70.10, 68.82, 66.88, 58.33, 44.76, 32.61, 30.36, 30.32, 30.23, 30.18, 30.04, 26.72, 23.34, 14.46; ^{31}P NMR (121 MHz, CD_3OD–$CDCl_3$, 1:1, 85% H_3PO_4): δ 23.49, −0.30. Anal. calcd for $C_{29}H_{60}O_{14}P_2\cdot1.5H_2O$: C, 48.28; H, 8.80; found: C, 48.31; H, 8.84%.