Regioselective hydrolysis of *myo*-inositol 1,3,5-orthobenzoate *via* a 1,2-bridged 2′-phenyl-1′,3′-dioxolan-2′-ylium ion provides a rapid route to the anticancer agent Ins(1,3,4,5,6)P_5

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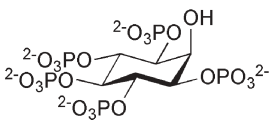
Acid hydrolysis of *myo*-inositol 1,3,5-orthobenzoate leads regioselectively to 2-*O*-benzoyl-*myo*-inositol *via* a 1,2-bridged 2′-phenyl-1′,3′-dioxolan-2′-ylium ion observed by ^{1}H and ^{13}C NMR spectroscopy, providing the precursor for a highly efficient route to the anticancer agent *myo*-inositol 1,3,4,5,6-pentakisphosphate.

The phosphatidylinositol 3-kinase (PI 3-K)/Akt signalling pathway plays a vital role in a wide array of biological and pathophysiological responses, including tumorigenesis, invasion and metastasis.[1] Therefore, specific inositol polyphosphates that have the potential to antagonise the activation of this pathway by competing with the binding of the lipid phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] to pleckstrin homology (PH) domains may prove useful in cancer therapy. *myo*-Inositol 1,3,4,5,6-pentakisphosphate [**1**, Ins(1,3,4,5,6)P_5], a water-soluble, non-toxic natural compound was recently identified as an excellent inhibitor of the (PI 3-K)/Akt pathway, with pro-apoptotic and antiangiogenic properties *in vitro* and *in vivo*, and thus has the potential to be developed as a novel anticancer therapeutic.[2] Ins(1,3,4,5,6)P_5 specifically inhibits Akt phosphorylation and kinase activity, blocks Akt phosphorylation *in vivo* and inhibits the growth of SKOV-3 xenografts as effectively as cisplatin.[2] Most surprisingly, this highly polar molecule is able to enter certain cell types to mediate the above effects, although the mechanism for this is as yet unclear. Consequently, it is essential to develop a rapid and scalable synthetic route to this potentially important new anticancer agent, to serve the needs of future *in vivo* investigations.

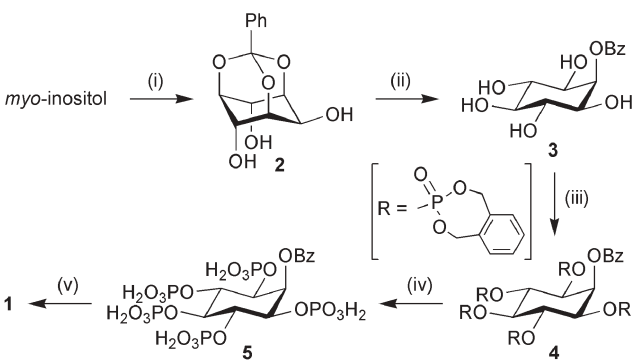


Ins(1,3,4,5,6)P_5 (**1**)

Ins(1,3,4,5,6)P_5 (**1**), which can be isolated from avian erythrocytes, has long been known to modulate the oxygen affinity of haemoglobin in several amphibians and the majority of birds, as does 2,3-bisphosphoglycerate in mammals.[3] Here we describe a rapid and high-yielding synthetic route to Ins(1,3,4,5,6)P_5 from *myo*-inositol, using *myo*-inositol 1,3,5-orthobenzoate (**2**, Scheme 1) as a new precursor. The route is faster and higher yielding than previously reported syntheses,[4] and requires only a single flash chromatographic purification step, allowing easy access to multigram quantities of **1**. During the course of this synthesis, our attention also focused on the mechanism of an unusual regioselective formation of 2-*O*-benzoyl-*myo*-inositol (**3**) *via* acid hydrolysis of **2**.

Myo-inositol orthobenzoate (**2**) was synthesised by transesterification of *myo*-inositol with trimethyl orthobenzoate and a catalytic amount of camphorsulfonic acid in DMSO at 80 °C, (Scheme 1).[5] Alternatively, the reaction can be carried out in DMF,[5,6] although higher temperatures (>140 °C) and larger amounts of acid catalyst and trimethyl orthobenzoate are needed. Use of DMSO gives a cleaner reaction compared to DMF, making purification easier. After work-up, **2** could be obtained in 90% yield. Although other *myo*-inositol 1,3,5-orthoesters have been utilised as synthetic precursors for inositol phosphates and lipids,[7] **2** had not previously been proposed as a useful synthetic intermediate until our recent report.[5] The main advantage of **2** is that it gives a stable benzoate ester upon hydrolysis, which can be directed to different positions on the ring by choosing suitable conditions and protection of other hydroxyl groups.

Until now, we and others have only exploited inositol orthoesters to provide the correct regiochemistry of substitution[8] or, more interestingly, by ring opening to furnish substitution at the C-1/3 positions.[5] However, acid hydrolysis of **2** with 90% aqueous TFA gave 2-*O*-benzoyl-*myo*-inositol (**3**, Scheme 1) in less than 5 min with >99.5% selectivity as judged by ^{1}H NMR. When alcohols such as ethanol or methanol were used as solvent,



Scheme 1 *Reagents and conditions*: (i) $(MeO)_3CPh$, CSA, DMSO, 80 °C, 30–40 mbar, 3 h, 90%; (ii) TFA:H_2O, 10:1, RT, quantitative; (iii) *N,N*-diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amine, CH_2Cl_2, 5-phenyltetrazole, RT; *m*CPBA, 0 °C to RT, 96%; (iv) H_2, $Pd(OH)_2$/C, MeOH–H_2O, quantitative; (v) conc. aq. NH_3, 60 °C, 12 h, quantitative.

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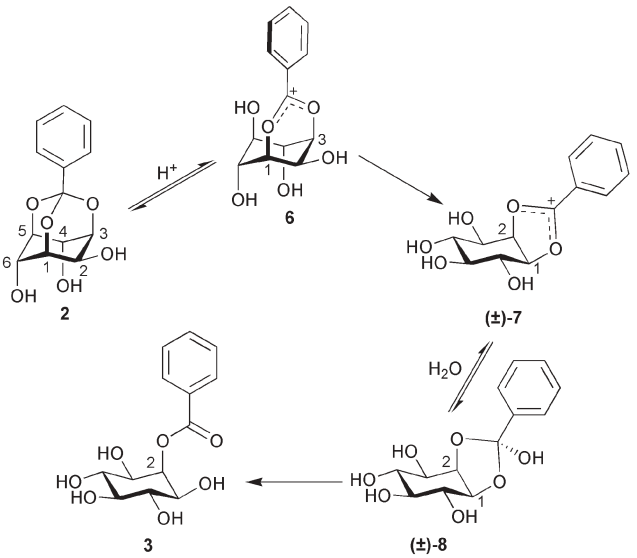
selectivity was reduced and (±)-1-*O*-benzoyl-*myo*-inositol was obtained as a minor product. Thus, treatment of **2** with TFA–methanol, 1 : 5 afforded a 13 : 1 mixture of **3** and (±)-1-*O*-benzoyl *myo*-inositol, respectively, while use of 1.0 M HCl–methanol, 1 : 2 gave a 10 : 1 mixture at room temperature and 1.0 M HCl–ethanol, 1 : 2 at 80 °C provided a 7 : 1 mixture. In all cases though, **3** was the major product.

Phosphitylation of **3**[4b] using *N*,*N*-diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amine in the presence of 5-phenyltetrazole,[9] followed by *in situ m*CPBA oxidation of the intermediate pentakisphosphite gave the symmetrical pentakisphosphate (**4**) in 96% yield. After purification of the product by flash column chromatography, the fully protected pentakisphosphate was subjected to hydrogenolysis over $Pd(OH)_2$ on carbon[10] to remove the benzyl protecting groups, and the benzoate ester was cleaved in concentrated aqueous ammonia. Benzamide was easily removed by aqueous work up with dichloromethane, giving Ins(1,3,4,5,6)P_5 (**1**) as the ammonium salt in 86% isolated overall yield from *myo*-inositol.

The unusual regioselectivity of the acid hydrolysis step was intriguing and merited further investigation. Although it has been reported that in the acid hydrolysis of (±)-4-deoxy-*myo*-inositol 1,3,5-orthopentanoate, the 2-*O*-pentanoate was obtained as a result of acyl migration,[11] we believed that acyl migration could not explain the highly regioselective formation of 2-*O*-benzoyl-*myo*-inositol from **2**. To confirm this, 1-*O*-benzoyl-*myo*-inositol was synthesised by acid hydrolysis of 2,6-di-*O*-benzyl-*myo*-inositol 1,3,5-orthobenzoate,[5] followed by chromatographic isolation of 1-*O*-benzoyl-2,6-di-*O*-benzyl-*myo*-inositol and subsequent removal of benzyl ethers by hydrogenolysis. Treatment of either 1-*O*-benzoyl-*myo*-inositol or 2-*O*-benzoyl-*myo*-inositol regioisomers under the same conditions used for the hydrolysis of **2** gave no products due to migration, and the starting materials remained unchanged even after extended reaction times. Partial migration was observed only at elevated temperatures and with longer reaction times. For example, treatment of 1-*O*-benzoyl-*myo*-inositol in 1.0 M HCl–ethanol, 1 : 2 at 80 °C for 10 h gave a 6.5 : 1 mixture of 1- and 2-*O*-benzoates, while treatment of 2-*O*-benzoate **3** under the same conditions gave a 1 : 4 mixture of 1- and 2-*O*-benzoates.

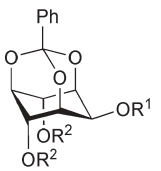
In order to investigate the mechanism of hydrolysis of orthobenzoate **2**, a series of NMR experiments was undertaken. A ^{1}H NMR spectrum taken immediately after treatment of **2** with deuterated TFA showed complete consumption of **2** to give a new species, along with traces of 2-*O*-benzoate **3**, the latter presumably arising from adventitious water. The new species was identified as the 1,2-bridged 2′-phenyl-1′,3′-dioxolan-2′-ylium ion (dioxolenium ion) (±)-**7** (Scheme 2). The ^{1}H NMR spectrum of (±)-**7** showed two characteristic downfield signals at 6.12 and 6.29 ppm, which were assigned to the H-1 and H-2 inositol ring protons, respectively. The coupling constant between H-1 and H-2 is unusually large (8.9 Hz) due to the incorporation of C-1 and C-2 into the five-membered dioxolanylium ring. The signals corresponding to the phenyl ring protons of the dioxolanylium ion were also clearly distinguishable and found to be more deshielded [δ_H 8.40–8.37 (2H/Ar-*ortho*), 8.19–8.15 (1H/Ar-*para*) and 7.82–7.78 (2H/Ar-*meta*)] in comparison to the phenyl ring of the product **3** δ_H [8.03–8.01 (2H/Ar-*ortho*), 7.74 to −7.70 (1H/Ar-*para*) and 7.54–7.50 (2H/Ar-*meta*)] and to the starting material **2**



Scheme 2 Proposed mechanism for the acid hydrolysis of **2**.

[δ_H 7.68–7.66 (2H) and 7.40–7.38 (3H)]. In addition, the ^{13}C NMR spectrum of (±)-**7** showed a signal at 183.3 ppm, attributed to C-2′ of the dioxolanylium ion, and fully in line with the values reported for similar dioxolanylium ions and α,α-dialkoxybenzyl cations.[12]

Similar dioxolanylium ion intermediates were also observed when 4,6-di-*O*-methyl orthobenzoate **9** and 4,6-di-*O*-benzyl orthobenzoate **10** were treated with deuterated TFA.† Again, immediate consumption of starting material to give the 1,2-bridged dioxolanylium ion was observed, followed by slower conversion of intermediate into the 2-*O*-benzoate product. Owing to the rapidity of the reaction, we were unable to observe the initial acid-catalysed opening of the orthobenzoate cage, but it seems likely that the formation of the dioxolanylium ion must in each case be preceded by a 1,3-bridged dioxanylium (dioxenium) ion intermediate akin to **6**, which rearranges immediately to the more stable 1,2-bridged species when the 2-hydroxyl group in the starting material is free.



9 (R^1 = H, R^2 = Me)
10 (R^1 = H, R^2 = Bn)
11 (R^1 = Me, R^2 = Me)
12 (R^1 = Bn, R^2 = Bn)

In order to observe the initial opening of the orthobenzoate cage, both 2,4,6-tri-*O*-methyl orthobenzoate **11** and 2,4,6-tri-*O*-benzyl orthobenzoate **12** were prepared, in which the 2-hydroxyl group of the inositol ring is protected, thus preventing the formation of any 1,2-bridged intermediates or even a transient 1,2,3-caged structure, during hydrolysis. However, treatment of orthobenzoates **11** and **12** with deuterated TFA resulted in broadening of the signals in the ^{1}H NMR spectrum, indicating a dynamic equilibrium. This may be due to reversible opening of the orthobenzoate cage[13] in each case, giving a rapidly interconverting mixture of substrate and 1,3-dioxan-2-ylium ions analogous to **6**. Considerable broadening was also observed in the ^{13}C NMR spectrum, thus eradicating the signals due to inositol ring carbons, and again suggesting a rapid equilibration. Addition of deuterated water to this equilibrium mixture resulted in ring closure to form

the starting material, not a hydrolysis product, showing that addition of water is the rate limiting step for the hydrolysis of **11** and **12**. For a 1,3-bridged dioxanylium ion, intramolecular ring closure will be much faster than addition of water, since the departing hydroxyl group remains in close proximity to the cationic centre.[14] However, when the 2-hydroxyl group is unprotected, as in **6**, an alternative pathway is available, namely a rearrangement to give a 1,2-bridged dioxolanylium ion such as (±)-**7**. We cannot exclude the participation of dioxanylium ions bridged between O-5 and O-1/3 of the inositol ring in the initial opening of the orthobenzoate cage, although it is notable that acid hydrolysis of **11**, **12** and of 2,6-di-*O*-benzyl-*myo*-inositol orthobenzoate[5] (1.0 M HCl–ethanol 1 : 2, reflux, 5 h) gives only 1/3-*O*-benzoate ester products in each case.

From these results, we postulate the mechanism for the acid hydrolysis of *myo*-inositol 1,3,5-orthobenzoate (**2**) to be as shown in Scheme 2. Acid catalysed reversible opening of **2** initially gives the symmetrical 1,3-bridged 2′-phenyl-1′,3′-dioxan-2′-ylium ion **6** (observed as broad signals in the ^{1}H NMR spectrum for **11** and **12**). This intermediate then immediately rearranges to the more stable 1,2-bridged 2′-phenyl-1′,3′-dioxolan-2′-ylium ion (±)-**7**. Attack by water from the less hindered face of (±)-**7** then gives hemi-orthoester (±)-**8**, which subsequently decomposes, under stereoelectronic control,[15] to form the product with an axial benzoate ester and equatorial hydroxyl group (**3**). For derivatives of **2** in which the 2-hydroxyl group is protected, however, the six-membered ring dioxanylium ion cannot rearrange, and is slowly hydrolysed to give products with the benzoate ester at O-1 or O-3, *via* a 1,3-bridged hemi-orthoester intermediate.

In summary, we have described a convenient route to the novel anticancer agent Ins(1,3,4,5,6)P_5 *via* *myo*-inositol 1,3,5-orthobenzoate (**2**). This is the first synthesis of any inositol polyphosphate using an inositol orthobenzoate precursor and has considerable advantages, including speed, high yield and the potential for large scale synthesis. The interesting regioselectivity of the acid-catalyzed orthobenzoate hydrolysis was rationalised by NMR spectroscopy through the intermediacy of a 1,2-bridged 2′-phenyl-1′,3′-dioxolan-2′-ylium ion.

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Notes and references

† Data for 1,2-bridged 2′-phenyl-1′,3′-dioxolan-2′-ylium ion from **9**: ^{1}H NMR (400 MHz, CF_3CO_2D) δ_H 3.61 (3H, s, CH_3), 3.78 (3H, s, CH_3), 3.95 (1H, br t, H-4), 4.15 (1H, dd, $J_{4,5}$ 3.6 Hz, $J_{5,6}$ 9.0 Hz, H-5), 4.53 (1H, dd, $J_{1,6}$ 6.1 Hz, H-6), 4.76 (1H, br t, H-3), 5.92 (1H, dd, $J_{1,2}$ 8.9 Hz, H-1), 6.16 (1H, dd, $J_{2,3}$ 2.7 Hz, H-2), 7.78 (2H, t, Ar–H_{meta}), 8.14 (1H, t, Ar–H_{para}), 8.34 (2H, d, Ar–H_{ortho}); ^{13}C NMR (400 MHz, CF_3CO_2D) δ_C 58.4, 59.7 (2 × q, 2 × CH_3), 66.7 (d, C-3), 72.3 (d, C-5), 79.1 (d, C-6), 84.0 (d, C-4), 87.0 (d, C-2), 89.6 (d, C-1), 115.6 (s, Ar–C_{ipso}), 131.0 (d, Ar–C_{meta}), 134.5 (d, Ar–C_{ortho}), 143.3 (d, Ar–C_{para}), 182.9 (s, C-2′).

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10 It was found that use of palladium on carbon in the hydrogenolysis led to contamination of **1** with traces of paramagnetic ions, as shown by severe broadening of signals in ^{31}P NMR spectra.

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