Regioselective Opening of *myo*-Inositol Orthoesters: Mechanism and Synthetic Utility

Himali Y. Godage, † Andrew M. Riley, † Timothy J. Woodman, † Mark P. Thomas, † Mary F. Mahon, ‡ and Barry V. L. Potter*,†

† Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, ‡ X-ray Crystallographic Suite, Department of Chemistry, University of Bath, Bath BA2 7AY, U.K.

Supporting Information

**ABSTRACT:** Acid hydrolysis of *myo*-inositol 1,3,5-orthoesters, apart from orthoformate, exclusively affords the corresponding 2-O-acyl *myo*-inositol products via a 1,2-bridged five-membered ring dioxolanylium ion intermediate observed by NMR spectroscopy. These C-2-substituted inositol derivatives provide valuable precursors for rapid and highly efficient routes to 2-O-acyl inositol 1,3,4,5,6-pentakisphosphates and *myo*-inositol 1,3,4,5,6-pentakisphosphate with biologically interesting and anticancer properties. Deuterium incorporation into the α-methylene groups of such alkyl ester products (2-O-C(O)CD₂R), when the analogous alkyl orthoester is treated with deuterated acid, is established utilizing the novel orthoester *myo*-inositol 1,3,5-orthobutyrate as an example. Such deuterated ester products provide intermediates for deuterium-labeled synthetic analogues. Investigation into this selective formation of 2-O-ester products and the deuterium incorporation is presented with proposed mechanisms from NMR experiments.

**INTRODUCTION**

Inositol phosphates are important intracellular messengers that play a vital role in many cellular functions including cell growth, migration, differentiation, apoptosis, and endocytosis. To facilitate biochemical investigations, it is vital that efficient routes to these naturally occurring inositol phosphates are established. As the demand for key synthetic intermediates increases, so does the need for differentially protected entities having free hydroxyl groups at specific positions. *myo*-Inositol 1,3,5-orthoesters have been extensively utilized as synthetic precursors for such inositol derivatives, since the O-1, O-3, and O-5 atoms can be protected in a single step leaving O-2, O-4, and O-6 for further manipulation (Scheme 1). Also, varied patterns of hydroxyl group protection can be obtained by the cleavage of orthoesters with reducing agents and in concert with chiral desymmetrization can lead to optically active targets.

In contrast, apart from orthoformate, acid hydrolysis of C-2 unprotected *myo*-inositol orthoesters affords the 2-O-acyl *myo*-inositol derivatives unexpectedly and demonstrated that this proceeds via the intermediacy of a 1,2-bridged 2′-phenyl-
1,3,5-dioxolan-2-ylium ion. We now report full details and extension of this work including mechanistic NMR and labeling experiments and show that the resulting protected inositol can be used to provide a highly efficient route to two emerging anticancer agents.23–25

■ RESULTS AND DISCUSSION

Regioselective Synthesis of myo-Inositol 2-O-Acetate and Mechanistic Investigations. Orthoacetate 2 was prepared by treating myo-inositol with triethyl orthoacetate and PTSA in DMF under reflux as previously reported.18 Treatment of 2 with aqueous TFA (10:1) gives 2-O-acetate 5 with complete selectivity in quantitative yield. This reaction is extremely fast and clean with no starting material observed by TLC even after 5 min of commencing the reaction.

Our finding that acid hydrolysis of 2 leads to the selective formation of ester 5 was unexpected. Previous studies on the hydrolysis of myo-inositol orthoesters have shown that the ester group is directed to the C-1 or C-3 position, although in these studies, the 2-OH group was protected. Interestingly, it was previously reported that acid hydrolysis of (±)-4-deoxy-myoinositol 1,3,5-orthopentanoate gave the 2-O-pentanoate, although the authors attributed this to acyl migration.26 We reasoned, however, that acyl migration could not explain the high regioselectivity in the formation of 5 from 2 and a series of NMR experiments was therefore undertaken in an attempt to observe intermediates in this hydrolysis reaction.

A 1H NMR spectrum taken immediately after treatment of 2 with deuterated TFA and CDCl3 showed only the starting material and some 2-O-acetate product 5, the latter presumably arising from adventitious water. A small amount of CDCl3 was used due to difficulty in attaining a lock signal in neat CF3CO2D. Although we were unable to observe any intermediates at this initial stage, a small change in the methyl peak of 5 was seen. Over time, the disappearance of the methyl singlet at 1.52 ppm and the appearance of new peaks next to it and their disappearance was observed by 1H NMR (Supporting Information, Figure 1), leading us to believe that the methyl group was being deuterated giving a mixture of −CH3−CH2D, and −CHD2 peaks. The reaction was therefore repeated, and further 1H and 13C NMR spectra were taken at different times. Deuterium NMR spectra showed an increase in the deuterated methyl peaks (−CH3D, −CH2D, and −CD3) at 1.52 ppm over time, complementary to the 1H NMR decrease in the methyl singlet (−CH3) proving that methyl group protons were exchanging (Supporting Information, Figure 2). This was further proven by 13C NMR spectra taken on a different time scale. Splitting patterns were seen progressively in the 13C NMR spectrum with initially a singlet (−CH3) at δ 23 ppm, a mixture of the singlet and a triplet (indicating −CH2D), a mixture along with a quintet showing some −CHD2, and a septet showing −CD3.

When acidic hydrolysis of orthoacetate 2 was carried out in other solvents such as ethanol, methanol, or acetonitrile, or with use of mineral acids such as HCl, the observed selectivity for 5 was reduced and longer reaction times were also needed. Furthermore, under the selective reaction conditions using 10:1 TFA/water (50%) but in the presence of another solvent such as methanol (50%), small amounts of 1/3-O-acetate were also obtained.

In order to further investigate this high regioselectivity and also to further understand the observed deuterium incorporation, both the C-2 hydroxyl-free and protected orthoacetates 4,6-di-O-benzyl orthoacetate (12) and 2,4,6-tri-O-benzyl orthoacetate (13) were synthesized (Scheme 3).

Scheme 3. Orthoacetates in Deuterated TFA

Metal alkoxide chelation-controlled regioselective protection of C-4 and C-6 hydroxyl groups was achieved in excellent yield when orthoacetate triol 2 was treated with lithium hydride and benzyl bromide in dry DMF. A higher yield was obtained when lithium hydride was used in comparison to that using n-butyl lithium.27 Observation of 12 in deuterated TFA and CDCl3 by NMR spectroscopy (1H, 2H, and 13C) again showed the disappearance of the signal corresponding to the methyl group of the orthoacetate at 1.56 ppm (1H NMR) and an increase in the height of the deuterated methyl peaks over time by 1H NMR spectra, once more confirming that the protons of the methyl group were exchanging with solvent deuterium. Although the major peaks seen were of the starting material which was being deuterated, there was a very small amount of hydrolyzed product, myo-inositol 4,6-di-O-benzyl 2-O-acetate, as well as a minute amount of some other broad peaks, perhaps corresponding to an intermediate species. This other species showed downfield broad signals at 5.91 and 5.72 ppm corresponding to one proton each and a multiplet around 4.21–4.27 ppm corresponding to four protons, along with a broad singlet at 2.67 ppm corresponding to three protons, perhaps representing a methyl group. However, since this species was observed only in trace amounts by 1H NMR and not by 13C NMR, identification of this potential intermediate was impossible.

Tribenzyl orthoacetate 13, synthesized by conventional benzylolation of 228 was treated with deuterated TFA, and after 15 min, only starting material was seen by NMR
spectroscopy, and no intermediates or changes to the methyl singlet were observed. Additionally, $^1$H NMR spectra taken up to 5 days showed no peaks due to deuterium incorporation into the methyl group of starting material 13.

**Regioselective Synthesis of myo-Inositol 2-O-Benzozate (6).** Orthobenzoate 3 was synthesized by transesterification of myo-inositol using DMSO as the solvent. Distilling off the formed methanol (for example, by carrying out the reaction in a rotary evaporator) shortened the reaction time. Initial attempts to carry out the reaction in DMF at the usual temperatures for orthoformate 1 and orthoacetate 2 synthesis (100 °C) gave very low yields, and higher temperatures (>140 °C) were required. When DMF was used a large excess of both the acid catalyst (p-toluenesulfonic acid) and trimethyl orthobenzoate was needed, perhaps due to thermal decomposition. Use of DMSO as the reaction solvent also gives a much cleaner reaction, making the purification easier.

Acid hydrolysis of 3 with aqueous TFA gave 2-O-benzoate 6, once more with complete selectivity and in quantitative yield. Again, this reaction was extremely fast. However, when alcohols such as ethanol or methanol were used as the solvent, along with longer reaction times, selectivity was reduced and 1- or 3-O-benzoyl-myoinositol (±6) was also obtained as a minor product (Scheme 4).

**Scheme 4. Acid Hydrolysis of 3 under Various Conditions**

Investigation into the Possibility of Acyl Migration.

While we believed that acyl migration could not be a result of the high regioselectivity in the formation of 2-O-ester products, further studies were undertaken to establish that 5 and 6 are not products of acyl migration of their 1- or 3-O-acetyl myoinositol regioisomers. We therefore tested the migratory abilities of the benzyl group in both 6 and (±)-14 under a variety of reaction conditions.

Both the (±)-14 and 6 regioisomers were tested individually for the migratory ability of their benzoyl group under the same reaction conditions used for the acid hydrolysis of orthoesters 2 and 3 (i.e., TFA/H$_2$O 10:1). However, no products due to migration were obtained even after extended reaction times, proving that 6 is not a migratory product from (±)-14. Partial migration was observed only at elevated temperatures and with much longer reaction times. For example, when (±)-14 was treated in 1 M HCl and ethanol at 80 °C for 10 h, a 6:1 mixture of (±)-14 and 6 was obtained, while treatment of 6 under the same reaction conditions gave a 4:1 mixture of 6 and (±)-14, respectively. Therefore, since more of the ester 6 is converted to (±)-14, the equatorial benzoyl ester (±)-14 may be the thermodynamically more stable product while the axial regioisomer 6 is the kinetic product.

**Role of a 1,2-bridged Dioxolanylium Ion Intermediate in Orthoester Opening.** After establishing that 2-O-benzoate 6 is not a migratory product of acid hydrolysis of orthobenzoate 3, further NMR experiments were undertaken in an attempt to observe the intermediates involved in the hydrolysis reaction. To our surprise, the $^1$H NMR spectrum taken immediately after the treatment of 3 with deuterated TFA showed a new species along with product 6, the latter presumably arising from adventitious water. (See Figure 3 in the Supporting Information for a full spectrum. Expansions are shown in Figure 1.)

No residual starting material was observed, indicating complete conversion to this novel intermediate, later identified as the 1,2-bridged 2'-phenyl-1,3'-dioxolan-2'-ylium ion (dioxolenium ion) (±)-15 (Scheme 5) possessing characteristic downfield signals at 6.12 and 6.29 ppm, corresponding to H-1 and H-2 of the inositol ring protons. H-1 and H-2 are strongly deshielded relative to the other inositol ring protons, and the coupling constant between them is unusually large ($J_{1,2}$ 9.0 Hz, while $J_{1,2}$ 1.9 Hz for orthobenzoate 3) owing 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 are clearly distinguishable from those in the starting material or product, being more deshielded ($\delta$H 8.40–8.37 (2H/Ar-ortho), 8.19–8.15 (1H/Ar-para) and 7.82–7.78 (2H/Ar-meta) compared to those in 3 ($\delta$H 7.68–7.66 (2H) and 7.40–7.38 (3H)) and product 6 ($\delta$H 8.03–8.01 (2H/Ar-ortho), 7.74–7.70 (1H/Ar-para) and 7.54–7.50 (2H/Ar-meta)). In addition, fully in line with the values reported for similar dioxolanylium ions and α,ω-di-alkoxybenzyl cations, the $^{13}$C NMR spectrum of (±)-15 also showed a signal attributable to C-2’ of the dioxolanylium ion at 183.3 ppm (Supporting Information, Figure 4).

It is likely that the dioxolanylium ion intermediate (±)-15 formed from myo-inositol orthobenzoate (3) is more stable than the corresponding intermediate for opening of myo-inositol orthoacetate (2). This would account for our finding that (±)-15 can readily be observed by $^1$H and $^{13}$C NMR. Therefore, we went on to synthesize the 4,6-di-O-methyl (16) and 4,6-di-O-benzyl (19) derivatives of 3, keeping the C-2 hydroxyl group free to allow formation of 1,2-bridged intermediate for further studies.

Thus, protection of the C-2 hydroxyl group of 3 as its TBDMS ether followed by C-4 and C-6 methylation afforded the compound 23 in good yield (Scheme 6). Finally, removal of the TBDMS group gave the required 4,6-di-O-Me orthobenzoate 16.

Treatment of 16 with deuterated TFA showed a similar dioxolanylium ion intermediate by $^1$H NMR for a second time with immediate consumption of starting material to give the 1,2-bridged dioxolanylium ion 17 (Scheme 5) comprising downfield signals at 5.92 and 6.16 ppm, representing strongly deshielded inositol ring protons H-1 and H-2 with a large $J_{1,2}$ value of 8.9 Hz (Supporting Information, Figure 5). $^{13}$C NMR spectra further confirmed the structure of the intermediate ion (±)-17 with a signal at 182.9 ppm corresponding to the carbocation (Supporting Information, Figure 6). A trace amount of 4,6-di-O-methyl 2-O-benzoate product 18 was also observed along with the dioxolanylium ion 17.

Chelation-controlled regioselective protection of C-4 and C-6 hydroxyl groups of 3 was achieved using benzyl bromide to afford 4,6-di-O-benzyl orthobenzoate 19 in high yield. When 19 was treated with deuterated TFA, along with hydrolyzed product di-O-benzyl 2-O-benzoate 21, a 1,2-bridged dioxola-
Dioxolanylium ion intermediate 20 was also observed. Dioxolanylium ion 20 exhibited spectroscopic data analogous to 15 and 17 with \(^1\)H NMR signals at 6.02 and 6.25 ppm and \(^{13}\)C NMR signal at 182.3 ppm. The aromatic protons of the dioxolanylium ion were also clearly noticeable since they were more deshielded in comparison to those in the starting material or product. Vicinal proton proton coupling constants (\(^3J_{\text{H,H}}\)) are compatible with slightly twisted boat conformation (see the SI for a computational molecular dynamics study of dioxolanylium ion 20).

Although it was not possible to observe the initial acid-catalyzed opening of the orthobenzoate cage owing to the rapidity of the reaction, it is most likely that the more stable 1,2-bridged dioxolanylium ion is produced by rearrangement of a 1,3-bridged dioxanylium (dioxenium) ion intermediate, when the 2-hydroxyl group in the starting material is free for instant rearrangement. Once the 2-hydroxyl group is protected, and there is therefore no possibility of rearrangement, only 1- or 3-\(\text{O}\)-substituted product is obtained,\(^{21}\) providing further evidence of the involvement of a 1,3-bridged dioxanylium intermediate in the initial stages of the acid hydrolysis. Therefore, 2,4,6-tri-\(\text{O}\)-methylated, 2,4,6-tri-\(\text{O}\)-benzylated, and 2,6-di-\(\text{O}\)-benzylated orthobenzoates were synthesized to facilitate NMR experiments, with the anticipation that the initial opening of the orthobenzoate cage might be observed due to the absence of a 2-hydroxyl, thus preventing the formation of any 1,2-bridged intermediates or even a potential transient 1,2,3-cage structure.

Figure 1. Proton NMR of the 1,2-bridged intermediate (±)-15 (labeled as I) and product 6 (labeled as P): (A) expansion of 3.90–6.45 ppm; (B) expansion of 7.40–8.50 ppm.

Scheme 5. Acid Hydrolysis of Orthobenzoate Derivatives Proceeds Regioselectively via a 1,2-Bridged Intermediate
Conventional methylation of 3 afforded the 2,4,6-tri-O-methyl orthobenzoate 24. However, when 24 was treated with deuterated TFA, signals in the $^1$H NMR spectrum were broadened suggesting a rapid equilibration. Inositol ring signals [$\delta_{H}$ 5.47 (2H), 4.55–4.52 (2H), 4.37–4.36 (2H)] were more deshielded in comparison to those in the starting material [$\delta_{H}$ 4.58–4.56 (3H), 4.28–4.26 (2H), 3.67 (1H)] in just CDCl$_3$ (Supporting Information, Figure 7). The signals corresponding to the phenyl ring protons [$\delta_{H}$ 8.09 (2H/Ar-ortho), 7.87 (1H/Ar-para) and 7.63–7.59 (2H/Ar-meta)] were also more deshielded, with broadened peaks for ortho and para protons from those in the starting material 24 [$\delta_{H}$ 7.65–7.63 (2H) and 7.34–7.31 (3H)]. Broadening of the signals in the $^{13}$C NMR spectrum was also significant, along with the disappearance of some signals corresponding to inositol ring carbons and aromatic ortho and para carbons as well as the orthoester carbon (O$_3$CPh), again consistent with a dynamic equilibrium (Supporting Information, Figure 8).

2,4,6-Tri-O-benzyl 1,3,5-orthobenzoate 25 was also obtained from 3, and treatment of 25 with deuterated TFA again showed broad signals in the $^1$H NMR spectrum with deshielded inositol and aromatic protons, especially H-1 and H-3 at 5.26 ppm instead of 4.52 ppm and ortho and para protons of orthobenzoate at 8.15 ppm and 8.00 ppm respectively instead of 7.23–7.69 ppm in the starting material 25, indicative of a rapid equilibration (Supporting Information, Figure 9). Significant broadening and disappearance/reduction of some signals corresponding to inositol ring carbons and the aromatic region of the orthobenzoate were also clearly distinguishable in the $^{13}$C NMR spectrum from those in 25, again demonstrating a dynamic equilibrium (Supporting Information, Figure 10).

Treatment of 26 with deuterated TFA yet again showed broad signals in the $^1$H NMR spectrum, with deshielded inositol and aromatic protons along with broadened or lost signals in the $^{13}$C NMR spectrum, once more suggesting a rapid equilibration. This may be due to reversible opening of the orthobenzoate cage 33 giving a rapidly interconverting mixture of starting material (24, 25, and 26) and the respective 1,3-dioxan-2-ylidium ion (27, 28, and 29) (Scheme 7). Nonetheless, addition of water to this equilibrium mixture did not give rise to the expected hydrolysis product, but instead resulted in ring closure to reform the starting material. This presumably results from destabilization of the dioxanylium intermediate due to dilution of the acid concentration, and demonstrates that hydrolysis is the rate limiting step of this reaction. Since the departing hydroxyl group remains in close proximity to the cationic center after ring-opening, the intramolecular ring closure will be much faster for a such 1,3-bridged dioxanylium ion intermediate than addition of water. Although in principal, the orthobenzoate cage could also be opened to give dioxanylium ions bridged between O-5 and O-1/3 of the inositol ring in the initial stage of acid hydrolysis, only 1/3-orthobenzoate ester products were obtained from acid hydrolysis of all 2,6-di and 2,4,6-tri-O-benzylated and 2,4,6-tri-O-methylated orthobenzoates 25, 26, and 24, respectively. However, on one occasion acid hydrolysis of 25 in TFA and DCM, gave a minor product 31 in 8% yield where a trifluoro acetyl group was substituted at the C-5 position along with the 1- or 3-O-benzoyl major product 30 (Scheme 8). This unexpected acylation at the C-5 position and not on the C-1 or C-3 positions also implies that the orthobenzoate cage may only be opened to give a bridge between O-1 and O-3, leaving the 5-hydroxyl group free for esterification by solvent. It could be that O-5 may perhaps be more easily protonated since O-1 and O-3 are more hindered due to the equatorial C-2 substituent.

This may, in addition, be due to the fact that symmetrical 1,3,5-bridged 2′-phenyl-1′,3′,5′-dioxan-2′-ylidium ions 27, 28, and 29 are thermodynamically more stable, since conformationally all four hydroxyl groups of the twisted boat could attain a less-hindered equatorial orientation in comparison to an alternative 1,5- or 3,5-bridged dioxanylium ion intermediate that would possess two equatorial and two axial hydroxyl groups.

Therefore, from the above observations we can postulate that the mechanism of acid hydrolysis of orthobenzoate 3 takes place via initial reversible ring-opening giving the 1,3-bridged 2′-phenyl-1′,3′,5′-dioxan-2′-ylidium ion 32 (indirectly observed as broad signals in the $^1$H and $^{13}$C NMR spectra of compounds with the 2-OH protected). This symmetrical, six-membered intermediate then rearranges immediately to the more stable 1,2-bridged 2′-phenyl-1′,3′-dioxolan-2′-ylidium ion (±)-15, which is then followed by the rate-determining attack by water, presumably from the less hindered face, to provide the hemiorthoester (±)-33 (Scheme 9). Subsequent decomposition of (±)-33, under stereoelectronic control, affords the product with an axial benzoate ester and equatorial hydroxyl groups (6). However, for substrates in which the 2-hydroxyl group is protected, rearrangement to the presumed five
membered dioxolanylium intermediate is not possible and thus slow hydrolysis gives product (±)-35 with the benzoate ester at O-1 or O-3, via a 1,3-bridged hemiorthoester intermediate 34.

**Acid Hydrolysis of myo-Inositol 1,3,5-Orthoformate (1).** Orthoformate 1 was also explored under the selective conditions used in opening orthoesters 2 and 3 onto the C-2 position. Orthoformate 1 was prepared using literature procedures. It should be noted that in the 1H NMR spectrum of the orthoformate, the signal that corresponds to the methylidyne proton is a small doublet of 1.1 Hz due to a 5 bond long-range spin coupling with C-2-H.

An 1H NMR spectrum taken immediately after treatment of 1 with deuterated TFA and CDCl3 showed only the starting material and no intermediate species or hydrolyzed products. When the same NMR sample was analyzed after being at room temperature for 15 h mainly the starting material was seen, along with very small amounts of products. Also, no deuterated starting material or products were seen even after 3 days. However, when the reactions were carried out in deuterated TFA and CDCl3 with a drop of D2O, the spectrum taken 5 min after the addition of D2O showed a 50:50 mixture of starting material and products, though no other intermediates were seen. Among the products, both the 2-O-formate and 1/3-O-formate products were present at a ratio of 2.5:1.

Acid hydrolysis of 1 was carried out using 10:1 mixture of TFA:water at room temperature for 15 min until all starting material had been consumed and the resulting mixture was evaluated by NMR after evaporation to establish product ratios. The 1H NMR spectrum showed the presence of inositol (arising due to hydrolysis of the formate ester group under the acidic conditions), 2-O-formate product and 1/3-O-formate product in a ratio of 1:1.7:1.2.

**Regioselective Synthesis of myo-Inositol 2-O-Butanoate 9 and Mechanistic Investigations.** Having studied orthoacetate 2, orthobenzoate 3, and orthoformate 1 opening under acid hydrolysis and seeing their similarities and differences, we synthesized another orthoester, this time with a longer alkyl chain to investigate further deuterium incorporation and intermediate cation formation. The novel orthoester, myo-inositol orthobutanoate (4), was prepared in high yield using analogous conditions for the synthesis of orthoacetate 1 by treatment of myo-inositol with trimethyl orthobutyrate and a catalytic amount of PTSA in DMF at 140 °C (this time due to shorter reaction time than for orthobenzoate synthesis under the same reaction conditions).

4 Was then treated with aqueous TFA to obtain myo-inositol 2-O-butanolate (7) in quantitative yield. Recrystallization of 7 in water and methanol afforded long thin crystals for which an X-ray crystal structure was obtained (Figures 2 and 3).

Therefore, from the above results, we can conclude that any 1,3,5-orthoester apart from orthoformate 1 should selectively open to give the axial 2-O-ester product under the above acidic hydrolysis conditions as already established for orthoacetate 2 and orthobenzoate 3. This ties in well with the rationalization offered by King and Allbutt\textsuperscript{36} based on the differences in steric strain among the possible transition states that fulfill the stereoelectronic requirements of dialkoxy-carbonium ion formation.
Mechanistic investigations of orthobutanoate 4 in deuterated TFA again showed deuterium incorporation at the α-position. However, unlike for orthobenzoate, only a minor species was seen by 1H NMR, perhaps attributed to a 1,2-bridged 2′-butyl-1′,3′-dioxolan-2′-ylium ion intermediate akin to (±)-15 which could not be fully identified due to the small amount present.

Hence, having observed α-methylene protons exchange for deuterium in orthobutanoate 4, orthoacetate 2 and 12 using deuterated acid, we can postulate that the mechanism of exchange proceeds via a ketene acetal 38 or (±)-40 or both (Scheme 10). Although, having observed only a small amount of deuterium exchanged product and no such exchange in the starting material when tri-O-Bn orthoacetate 13 was treated with deuterated acid, this suggests that when the C-2 hydroxyl group is protected, the equilibrium may lie quite far on the side of the 1,3-dioxan-2-ylium ion 37 due to the rapid interconversion between the starting material 13 and the ion 37. Thus, when the C-2 hydroxyl group is free, the equilibrium being rapidly established may lie greatly toward the side of the ketene acetal (±)-40 due to the immediate rearrangement of 1,3-dioxan-2-ylium ion 37 to the 1,2-bridged ion (±)-39.

Treatment of 4 in deuterated TFA for 24 h followed by the addition of water in the subsequent hydrolysis gave the 2-O-C(O)CD₂CH₂CH₃ myo-inositol as the major product (over 92%) with trace amounts of 2-O-butanoate 7 and 2-O-C(O)CHDCH₂CH₃ myo-inositol, perhaps arising due to adventitious water. Therefore, any such alkyl orthoesters 36 could be treated in deuterated acid under anhydrous conditions for the deuterium exchange to take place before hydrolysis to yield 2-O-C(O)D₂R ester products 44 with deuterated methylene group at the α-position (Scheme 11). These deuterium incorporated products could provide valuable intermediates to many deuterium labeled synthetic analogues that could be used to study biologically important metabolic pathways or chemical reactions.

We have also proven that this deuterium incorporation only takes place before the hydrolysis step and thus before the formation of the hemiorthoester intermediate (±)-46 (Scheme 12). Therefore, once the products are formed deuterium incorporation at the α-methylene position is not possible in deuterated acid. To confirm this, 2-O-acetate and butanoate products 5 and 7 were treated in both deuterated TFA and CDCl₃ and deuterated TFA and D₂O for 9 days and monitored by NMR spectroscopy for deuterium exchange. No deuterium exchange was observed by 2H NMR spectroscopy over 9 days; only decomposition and migration products were seen by 1H NMR spectroscopy, further proving that deuterium incorporation only takes place via the ketene acetal (±)-40 and that no exchange takes place once products are formed through a mechanism similar to that occupy in migration of products involving a hemiorthoester intermediate (±)-46.

**Synthesis of 2-O-Benzoyl myo-Inositol 1,3,4,5,6-Pentakisphosphate 9 and myo-Inositol 1,3,4,5,6-Pentakisphosphate 11.** The efficient regioselective transformation observed also facilitates exploitation in the synthesis of inositol polyphosphates with 2-position substitutions that are of biological interest as well as providing a proficient route to the anticancer agent inositol pentakisphosphate 11. Thus, 2-O-benzoyl myo-inositol 6 was phosphitylated using N,N-diethyl-1,5-dihydro-2,4,3-benzodioxaphosphepin-3-amine in the presence of 5-phenyltetrazole. The commonly used 1H-tetrazole, which has potential explosive properties at room temperature, has recently become difficult to obtain due to shipping restrictions and we found that 5-phenyltetrazole is equally effective as an activator in such phosphitylation reactions. The intermediate pentakisphosphate was subse-

---

**Figure 2.** X-ray crystal structure of 7. Ellipsoids are represented at 30% probability.

**Figure 3.** Crystal packing diagram for compound 7 showing the extensive H-bonding network.
Subsequently oxidized in situ by \( m \)-CPBA to afford the symmetrical pentakisphosphate 47 in 96% yield (Scheme 13). 6 could also be phosphorylated with the phosphitylating agent bis-(benzyloxy)diisopropylaminophosphine in good yield (94%) using 5-phenyltetrazole followed by oxidation. After purification of the product, the phosphate protection was removed by hydrogenolysis to afford pure 2-\( O \)-benzoyl pentakisphosphate 9. Finally, the cleavage of benzoate ester in concentrated aqueous ammonia followed by simple removal of benzamide byproduct after aqueous work up with dichloromethane provided the \( \text{Ins}(1,3,4,5,6)\text{P}_5 \) 11 as the ammonium salt in 86% isolated overall yield from \( \text{myo} \)-inositol. Pentakisphosphate 11 could also be synthesized from 2-\( O \)-acetyl \( \text{myo} \)-inositol 5 or 2-\( O \)-butanoyl \( \text{myo} \)-inositol 7 in a similar method in high yield. This sequence greatly benefits from involving only a single chromatographic purification step thus, eliminating the need for the usual ion-exchange chromatography and also allows easy access to multigram scale quantities for in vivo studies. We
anticipate that this route can provide efficient synthetic access to a range of 2-substituted inositol phosphate derivatives of potential biological interest and such work is in progress.  

CONCLUSIONS

In summary, we show that acid hydrolysis of C-2 unprotected inositol-based orthoesters, apart from the orthoformate, can lead to exclusive formation of 2-O-acetyl myo-inositol products. This interesting regioselectivity is rationalized through the intermediacy of a 1,2-bridged '2-substituted-1,3'-dioxolan-2'-yl' ion that is observed by NMR spectroscopy and preceded by a 1,3-bridged dioxanylium ion intermediate. Deuterium incorporation into the α-methylene group at inositol C-2 of an alkyl ester (2-O-C(O)D)R is possible when the corresponding orthoester is treated in deuterated acid under anhydrous before hydrolysis. Furthermore, using these observations, we describe a most efficient route for gram-scale synthesis of Ins(1,3,4,5,6)-P₅, 11 and 2-O-Benzyl Ins(1,3,4,5,6)P₅ via myo-inositol 1,3,5-orthozenoate 3.

EXPERIMENTAL SECTION

All reagents and solvents either were of commercial quality or were synthesized and purified in the laboratory using standard procedures. Some solvents were redistilled and dried where necessary using standard procedures or purchased in anhydrous form. Petroleum ether 40–60 °C is abbreviated as pet. ether. ¹H NMR and ¹³C NMR chemical shifts are measured in ppm (δ) relative to internal tetramethylsilane (TMS), and ²⁹Si NMR chemical shifts are measured in ppm (δ) relative to phosphoric acid as an external standard. Signals are expressed and abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), and app (apparent). All ¹H NMR and ¹³C NMR assignments are based on gCOSY, gHMQC, gHMBC, and DEPT experiments. Coupling constants (J) are given in hertz. TLC was performed on precoated plates (aluminum sheets, silica gel 60 F₂₅₄) with detection by UV light or with phosphomolybdic acid in ethanol followed by heating. Flash chromatography was performed on silica gel (particle size 40–63 μm).

2-O-Acetyl myo-Inositol (5). A mixture of TFA (10 mL) and water (1 mL) was added to 2 (1.5 g, 7.3 mmol) prepared as described, and the solution was stirred for 5 min after which time TLC (ethyl acetate) indicated the complete conversion of starting material (R₀ = 0.0) to a product (R₀ = 0.4). The reaction mixture was then evaporated with water following by distillation in vacuo to obtain 5 (1.63 g, quantitative) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 2.12 (3H, s, CH₃), 3.25 (1H, t, J₁₂,J₁₃ = J₁₃,J₁₄ = 9.2 Hz, C-5, C-6), 3.57 (2H, t, J₁₂,J₁₃ = J₁₃,J₁₄ = 10.2 Hz, C-2, C-3, C-4), 3.65 (2H, t, J₁₂,J₁₃ = J₁₃,J₁₄ = 10.2 Hz, C-2, C-3, C-4, C-5), 3.74 (1H, t, J₁₂,J₁₃ = J₁₃,J₁₄ = 10.2 Hz, C-2, C-3, C-4, C-5), 4.36 (4H, AB, J₂₃,J₂₄ = 1.6 Hz, C₂H₂, CH₃), 7.29–7.33 (10H, m, Ar-H); ¹³C NMR (100.6 MHz, CDCl₃) δ 23.2 (q, CH₃), 67.6, 69.6, 72.1, 73.5 (2 × d, 2 × t, C-2, C-3, C-4, C-5), 73.8 (1H, t, C-2), 74.0, 74.4 (2 × C-1 and C-3, C-4 and C-6), 105.7 (s, C-2), 127.5, 128.2 (2 × d, Ar-C), 138.3 (s, Ar-C); HRMS (ESI-TOF) m/z [M + H⁺] calcd for C₂₉H₃₁O₆ 475.2121, found 475.2115.

myo-Inositol 1,3,5-Orthozenoate (3). Trimethyl orthobenzyl orthoacetate (10 mL, 55 mmol) was added to a suspension of oven-dried myo-inositol (9.0 g, 50 mmol) and camphorsulfonic acid (232 mg, 1.0 mmol) in anhydrous DMSO (30 mL). The resulting mixture was then heated at 60–80 °C under a pressure of 260–280 mbar for 3 h on a rotary evaporator, after which time TLC (ethyl acetate) indicated the complete consumption of starting material (R₀ = 0.0) and the formation of a major product (R₀ = 0.4). The resulting clear solution was allowed to cool, and the catalyst was neutralized by addition of triethylamine (1.0 mL). The reaction mixture was concentrated under reduced pressure, redissolved in hot ethyl acetate (500 mL), filtered through a pad of silica gel, and washed further with hot ethyl acetate (2 × 250 mL). The resulting filtrate was concentrated under reduced pressure to about a volume of approximately 100 mL, and the solution was kept in the refrigerator overnight. Concentration of the mother liquor and cooling gave a further crops of crystals to give a total yield of 12.2 g (92%) of colorless crystals of 3: mp 213–214 °C (ethyl acetate); ¹H NMR (400 MHz, CDCl₃) δ 4.11 (1H, dt, J₁₂,J₁₃ = J₁₃,J₁₄ = 1.9 Hz and J₁₄,J₁₅ = 6.3 Hz, C-2, C-3-H), 4.18–4.20 (2H, m, C-1-H and C-3-H), 4.23–4.25 (2H, m, C-1-H and C-3-H), 4.45–4.47 (2H, m, C-2-H and C-4-H), 5.38 (1H, d, C-2-OH), 5.57 (2H, d, J₁₆,J₁₇ = 6.2 Hz, C-4-OH and C-6-OH), 7.34–7.41 (3H, m, Ar-H), 7.56–7.61 (2H, m, Ar-H); ¹³C NMR (100.6 MHz, CDCl₃) δ 58.2 (d, C-2), 67.7 (d, C-4 and C-6), 70.6 (d, C-5), 76.3 (d, C-1 and C-3), 106.9 (s, O-C-Ar), 126.0 (d, Ar-C), 128.0 (d, Ar-C), 129.5 (d, Ar-C), 138.3 (s, CH₃); HRMS (ESI-TOF) m/z [M + H⁺] calcd for C₂₂H₂₂O₅ 372.1501, found 372.1500, and 372.1497; Anal. Calcd for C₂₂H₂₂O₅ 372.1501: C, 73.86; H, 5.86; found: C, 73.85; H, 5.88.

2-O-Benzyl myo-Inositol (6). A mixture of TFA (10 mL) and water (1 mL) was added to 3 (1.5 g, 5.6 mmol), and the solution was stirred for 3 min after which time TLC (ethyl acetate) indicated the complete conversion of starting material (R₀ = 0.0) to a product (R₀ = 0.4). The reaction mixture was then evaporated with water in vacuo to obtain 6 (1.6 g, quantitative) as a white solid: mp 240–242 °C (ethanol/water); ¹H NMR (400 MHz, CDCl₃) δ 3.26–3.30 (1H, m, C-1, C-3, C-4 and C-6), 3.64–3.71 (4H, m, C-1-H, C-3-H, C-4-H and C-6-H), 5.77 (1H, t, J₁₂,J₁₃ = J₁₃,J₁₄ = 2.7 Hz, C-2-H), 7.39–7.43 (3H, m, Ar-H), 7.54–7.68 (1H, Ar-H), 7.92 (2H, m, J₂₃,J₂₄ = 8.2 Hz, Ar-H), 12.18 (2H, Ar-H); ¹³C NMR (100.6 MHz, CDCl₃) δ 70.0, 73.0 (2 × d, C-1 and C-3, C-4 and C-6), 74.4 (d, C-5), 75.2 (2 × d, C-2, C-8), 128.9 (d, Ar-C), 128.9 (d, Ar-C), 134.2 (d, Ar-C), 168.1 (s, CO₂Ph); HRMS (ESI-TOF) m/z [M + H⁺] calcd for C₂₉H₂₄O₅, 468.1728, found 468.1724.
285.0974, found 285.0969. Anal. Calcd for \( \text{C}_4\text{H}_8\text{O}_2 \text{Si} \): C, 54.93; H, 5.67. Found: C, 54.90; H, 5.75.

Data for 1,2-bridged 2'-phenyl-1,3'-dioxolan-2'-ylion ion intermediate (±)-15 observed by \( ^{1}H \) NMR and \( ^{13}C \) NMR spectroscopy soon after the addition of deuterated trifluoroacetic acid (0.5 mL) into a NMR sample tube containing the dry myo-inositol, 1,3,5-orthobenzoate (30 mg, 0.19 mmol) and anhydrous deuterated chloroform (0.2 mL). A small amount of DCI was used due to the difficulty in attaining a lock signal in neat CF3CO2D. \( ^{1}H \) NMR (400 MHz, CF3CO2D and CDCl3) \( \delta \) 4.18 (1H, \( dd, J_{1,5} = 3.7 \text{ Hz}, \text{ C}-1 \text{-H} \)), 4.32 (2H, \( dd, J_{2,3} = 1.9 \text{ Hz}, \text{ C}-1 \text{-H} \)), 4.32 (1H, \( dd, J_{1,5} = 3.7 \text{ Hz}, \text{ C}-4 \text{-H} \)), 4.45 (1H, \( dd, J_{1,5} = 3.7 \text{ Hz}, \text{ C}-4 \text{-H} \)), 4.59 (1H, \( dd, J_{1,5} = 1.9 \text{ Hz}, \text{ C}-4 \text{-H} \)), 6.02 (1H, \( dd, J_{5,6} = 1.9 \text{ Hz}, \text{ C}-4 \text{-H} \)), 7.40 (1H, \( dd, J_{5,6} = 1.9 \text{ Hz}, \text{ C}-4 \text{-H} \)), 7.64—7.66 (2H, \( m, \text{ Ar-H} \)), 8.07—8.10 (2H, \( d, \text{ Ar-H} \)), 8.17 (1H, \( t, J_{4,5} = 12.1 \text{ Hz}, \text{ C} = \text{C} \)), 8.20 (1H, \( t, J_{4,5} = 12.1 \text{ Hz}, \text{ C} = \text{C} \)), 8.27 (2H, \( m, \text{ Ar-H} \)), 8.30 (2H, \( m, \text{ Ar-H} \)), 8.30 (2H, \( m, \text{ Ar-H} \)), 8.45 (2H, \( m, \text{ Ar-H} \)), 8.45 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)).

The resulting compound was purified by column chromatography (pet. ether/ethyl acetate, 1:5) to a solution of 4,6-di-O-benzyl myo-inositol 1,3,5-orthobenzoate (16). \( ^{1}H \) NMR (400 MHz, CF3CO2D) \( \delta \) 4.31 (3H, \( s, \text{ CH}_3 \)), 7.38 (2H, \( m, \text{ Ar-H} \)), 7.80 (2H, \( m, \text{ Ar-H} \)), 7.81 (1H, \( t, J_{4,5} = 12.1 \text{ Hz}, \text{ C} = \text{C} \)), 8.20 (2H, \( m, \text{ Ar-H} \)), 8.27 (2H, \( m, \text{ Ar-H} \)), 8.30 (2H, \( m, \text{ Ar-H} \)), 8.45 (2H, \( m, \text{ Ar-H} \)), 8.45 (2H, \( m, \text{ Ar-H} \)), 8.45 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)), 8.50 (2H, \( m, \text{ Ar-H} \)).

The reaction mixture was then diluted with ethyl acetate (50 mL), washed with water (50 mL) and brine (50 mL), dried (MgSO4), and evaporated in vacuo. The resulting compound was purified by column chromatography (pet. ether/ethyl acetate, 1:5) to afford 16 (236 mg, 94% as a white solid: mp 121.0—122.5 °C (ethyl acetate)). \( ^{1}H \) NMR (400 MHz, CDCl3) \( \delta \) 3.13 (1H, \( CH(OH) \)), 12.0 (2H, C-2, C-3), 3.49 (6H, \( s, \text{ C} = \text{C} \)), 4.06 (1H, \( dt, J_{4,5} = 11.4 \text{ Hz}, \text{ C}-4 \text{-H} \)), 4.28 (2H, \( m, \text{ C}-4 \text{-H} \)), 4.28 (2H, \( m, \text{ C}-4 \text{-H} \)).

The reaction mixture was then diluted with ethyl acetate (50 mL), washed with water (50 mL) and brine (50 mL), dried (MgSO4), and evaporated in vacuo. The resulting compound was purified by column chromatography (pet. ether/ethyl acetate, 1:5) to afford 16 (236 mg, 94% as a white solid: mp 121.0—122.5 °C (ethyl acetate)). \( ^{1}H \) NMR (400 MHz, CDCl3) \( \delta \) 3.13 (1H, \( CH(OH) \)), 12.0 (2H, C-2, C-3), 3.49 (6H, \( s, \text{ C} = \text{C} \)), 4.06 (1H, \( dt, J_{4,5} = 11.4 \text{ Hz}, \text{ C}-4 \text{-H} \)), 4.28 (2H, \( m, \text{ C}-4 \text{-H} \)), 4.28 (2H, \( m, \text{ C}-4 \text{-H} \)).

The reaction mixture was then diluted with ethyl acetate (50 mL), washed with water (50 mL) and brine (50 mL), dried (MgSO4), and evaporated in vacuo. The resulting compound was purified by column chromatography (pet. ether/ethyl acetate, 1:5) to afford 16 (236 mg, 94% as a white solid: mp 121.0—122.5 °C (ethyl acetate)).
4.6-Di-o-benzyl 2-O-benzyl myo-Inositol (21): 1H NMR (400 MHz, CDCl3) δ 4.28 (1H, t, J = 6.7 Hz, Ar-C-H), 4.49 (2H, dd, J1 = 6.7 Hz, J2 = 10.1 Hz, C-1-H and C-3-H); 13C NMR (100.6 MHz, CDCl3) δ 70.3 (d, C-1 and C-3), 73.8 (d, C-2), 74.2 (d, C-5), 76.2 (2, CH2Ph), 80.0 (d, C-4 and C-6), 127.1 (s, Ar-C); HRMS (ESI-TOF) m/z [M + H]+ calcd for C24H27O8Na 403.1780, found 403.1777.

2,4,6-Tri-O-methyl 1,3,5-Orthoformato 24. Sodium hydride (451 mg, a 60% dispersion in oil, 11.3 mmol) was added portionwise to a solution of 3 (500 mg, 1.9 mmol) in dry THF (6 mL) at 0 °C. The resulting mixture was stirred for 10 min, and methyl iodide (0.70 mL, 11.5 mmol) was then added dropwise. Stirring was continued for a further 16 h, after which time TLC (1:1, pet. ether/ethyl acetate) showed the complete conversion of starting material (Rf 0.10) to a product (Rf 0.45). The reaction mixture was then diluted with ethyl acetate (20 mL), washed with water (50 mL) and brine (50 mL), dried (MgSO4), and evaporated in vacuo. The resulting compound was purified by column chromatography (petroleum ether/ethyl acetate, 2:1) to afford 24 (562 mg, 97%) as a white solid: mp 121.5–122.8 °C (ethyl acetate/hexane); 1H NMR (400 MHz, CDCl3) δ 3.48 (6H, s, C-4-OCH3 and C-6-OCH3), 3.53 (3H, s, C-2-OCH3), 3.67 (1H, br s, C-C2-H), 4.26–4.28 (2H, m, C-C4 and C-C6), 4.56–4.58 (3H, m, C-C1, C-3-C5 and C-3-H and C-5-H), 7.31–7.34 (3H, m, Ar-H), 7.63–7.65 (2H, m, Ar-H); 13C NMR (100.6 MHz, CDCl3) δ1 56.8 (C-2-OCH3), 57.8 (C-4-OCH3, C-5, C-2, and C-3), 68.3, 68.3 (2, C-2 and C-5), 70.6, 76.0 (2, C-1 and C-3, 1/3-C, and 3-C6), 107.8 (s, C-OPh), 125.3, 127.9 (2, C-2 and C-2-Ar-C, and C-2-Ar-C), 129.3 (d, C-3-Ar-C), 136.9 (s, Ar-C); HRMS (ESI-TOF) m/z [M + H]+ calcd for C17H22O7Na 309.1338, found 309.1333. Anal. Calc’d for C17H22O7Na (308.33) C, 62.33; H, 5.64. Found C, 62.30; H, 6.63. 1H NMR (400 MHz, CDCl3) δ 3.62 (6H, s, C-4-OCH3 and C-6-OCH3), 3.63 (3H, s, C-2-OCH3), 4.36 (2H, br s, Ins-H), 4.53 (1H, br d, Ins-H), 5.47 (2H, br s, Ins-H), 7.61 (2H, br t, Ar-H), 7.87 (1H, br s, Ar-H), 8.09 (2H, br s, Ar-H).
resulting solution was allowed to cool and the catalyst was neutralized by addition of triethylamine (0.22 mL). The reaction mixture was then concentrated under reduced pressure, and the resulting residue was purified by column chromatography (ethyl acetate) to afford 4 (1.12 g, 87%) as a white crystalline solid: mp 139-140 °C (ethyl acetate); 1H NMR (400 MHz, CDCl3) δ 0.72 (3H, t, J = 7.4 Hz, CO2CH2CH3), 1.24 (2H, t, J = 7.4 Hz, CH2OCH2CH3), 2.24 (2H, t, J = 7.4 Hz, (CH2)2CH3), 3.10 (1H, t, J = 4.9 Hz, CH2=CH2); 13C NMR (100.6 MHz, CDCl3) δ 14.4 (q, O3C(CH2)2), 17.0 (t, CO3(CH2)2CO2H), 60.3 (d, C-2/C-5), 69.0 (d, C-4 and C-6), 70.7 (d, C-2/C-5), 76.5 (d, C-1 and C-3), 110.2 (s, O3C(CH2)2), HRMS (ESI-TOF) m/z [M + Na]+ calcd for C10H18O7Na+ 273.0945, found 273.0942; Anal. Calcd for C10H18O7 (250.25): C, 48.00; H, 7.25.

To a solution of compound 4 (950 mg, quantitative) as a hygroscopic white foam: 31P NMR (109.4 MHz, CDCl3) δ 7.96, 13.95, 143.74 (1P, t, phosphite at C-5). The reaction mixture was cooled to −40 °C and 77% m-CPBA (7.49 g, 33.4 mmol) was added portionwise while stirring. The cooling bath was removed, and the mixture was allowed to reach room temperature. After 15 min, TLC (1:1, ethyl acetate/petroleum ether) showed complete oxidation of pentaphosphate to pentakis-(α,β-dihydroxyphosphoryl)-myo-inositol (5). The reaction mixture was then allowed to reach room temperature overnight under an atmosphere of hydrogen in a pressure vessel. The catalyst was filtered through a PTFE syringe filter, and the filtrate was evaporated under reduced pressure to give 5 (460 mg, quantitative) as a hygroscopic white foam: 31P NMR (109.4 MHz, H-decoupled, CDCl3) δ −0.02 (2P, s), 0.79 (2P, s), 1.09 (1P, s, phosphate at C-5); 1H NMR (400 MHz, CDCl3) δ 7.43-7.49 (2H, m, Bz-H), 7.51 (t, J = 7.8 Hz, Ar-C), 165.1 (s, Ar-CO2H), HRMS (ESI-TOF) m/z [M + H]+ calcd for C18H12O12P5 528.9952, found 528.9950; Anal. Calcd for C18H12O12P5 (528.9967): C, 48.27; H, 3.05; N, 17.98; P, 18.10; found: C, 48.17; H, 3.02; N, 17.98; P, 17.98.

2-O-Benzoyl myo-inositol (10) (1.3 g, 2.52 mmol) was also carried out as above in methanol (110 mL) and water (10 mL) with 10% palladium hydroxide on activated charcoal (400 mg) to afford 9 (460 mg, quantitative) as a hygroscopic white foam: 31P NMR (109.4 MHz, H-decoupled, CDCl3) δ −0.02 (2P, s), 0.79 (2P, s), 1.09 (1P, s, phosphate at C-5); 1H NMR (270 MHz, CDCl3) δ 4.34 (1H, ap. quartet, dt, J = 5.4 Hz, J = 9.4 Hz, H-3, C-5-H), 1.32 (2H, d, J = 6.0 Hz, Bz-H), 2.74 (2H, t, J = 7.8 Hz, Ar-C), 13.8 (s, 1P, phosphate at C-5). The reaction mixture was cooled to −40 °C and m-CPBA (2.13 g, 77% m) was added portionwise while stirring. The cooling bath was removed, and the mixture was allowed to reach room temperature and diluted with dichloromethane (50 mL), washed with 10% sodium sulfite solution (2 × 100 mL), dried and solvent evaporated in vacuo. The residue was purified by column chromatography (chloroform/acetone, 4:1-1:1) to afford 7 (600 mg, 0.88 mmol) as a white crystalline solid: mp 193-200 °C (chloroform/methanol); 31P NMR (106.4 MHz, H-decoupled, CDCl3) δ 4.99 (m, 1P, phosphate at C-5), −3.37 (2P, s); 1H NMR (400 MHz, CDCl3) δ 8.92, 5.23, 5.30-3.57, 5.45-5.61, 6.00-6.10, 6.95 (5 × 10, CH3Ar), 7.04, 7.40 (2 × d, C-1 and C-3, C-4 and C-6), 7.14 (d, C-5), 7.23, 7.88, 8.49 (6 × 10, Ar-H), 7.15, 7.89-7.90, 8.00 (m, Bz-H, Bz-OH), 8.99-9.00 (m, Bz-H, Bz-OH), 13C NMR (100.6 MHz, CDCl3) δ 68.9, 69.0, 69.4, 69.5, 69.5 (5 × 10, CH3Ar), 70.4, 70.4 (2 × d, C-1 and C-3), 74.3 (d, C-5), 77.2 (d, C-2), 123.3, 128.9, 128.9, 129.1, 129.2, 129.3, 129.3, 129.9, 134.1, 135.0, 135.1, 135.6, 135.7 (14 × 10, 36 × Ar-C), 165.1 (s, CO2Ph), HRMS (ESI-TOF) m/z [M + H]+ calcd for C26H20OzP5 395.1526, found 395.1526.

2-O-Benzoyl 1,3,4,5,6-Pentakis-O-(α,bis(benzoyloxy)phosphoryl)-myo-inositol (11). Compound 10 (600 mg, 0.88 mmol) was dissolved in concentrated aqueous ammonia solution (30 mL) and heated at 60 °C overnight in a Pyrex pressure vessel.
tube. After evaporation of solution under vacuum, the residue was dissolved in water and the benzamide byproduct was removed by washing with dichloromethane to afford the pure ammonium salt of \( \text{C}_6\text{H}_16\text{O}_{21}\text{P}_5 \) (862.24) by: C. 10.30; H, 5.38; N, 12.40.

1. Via Symmetrical Conduritol B Derivatives.

Biochemistry

This material is available free of charge via the Internet at dx.doi.org/10.1021/jo3027774 J. Org. Chem. 2013, 78, 2275–2288.

REFERENCES

■ Corresponding Author

■ Supporting Information

Corresponding Author

*E-mail: b.v.l.potter@bath.ac.uk.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Wellcome Trust for financial support (Programme Grant No. 082837) to B.V.L.P. and A.M.R. and Dr. A. Nathubhai for proofreading this manuscript.

■ REFERENCES

(1) Irvine, R. F.; Schell, M. J. Back in the Water: The Return of the Inositol Phosphates. Nature Rev. Mol. Cell Biol. 2001, 2, 327–338. (2) Shi, Y.; Azab, A. M.; Thompson, M. N.; Greenberg, M. L. Inositol Phosphates and Phosphoinositides in Health and Disease. Biology of Inositols and Phosphoinositides. Subcell. Biochem. 2006, 39, 265–292. (3) Conway, S. J.; Miller, G. J. Biology-Enabling Inositol Phosphates, Phosphatidylinositol Phosphates and Derivatives. Nat. Prod. Rep. 2007, 24, 687–707. (4) Kliba, B.; Balc, M. Recent Advances in Inositol Chemistry: Synthesis and Applications. Tetrahe- dron 2011, 67, 2355–2389. (5) Potter, B. V. L.; Lampre, D. Chemistry of Inositol Lipid-Mediated Cellular Signaling. Angew. Chem., Int. Ed. Engl. 1995, 34, 1933–1972. (6) Lu, P. J.; Gou, D. M.; Shieh, W. R.; Chen, C. S. Molecular-Interactions of Endogenous \( \text{d}-\text{myo-Inositol} \) and \( \text{L}-\text{myo-Inositol} \) 1,4,5-Trisphosphate Recognition site. Biochemistry 1994, 33, 11586–11597. (7) Podeschwa, M. A. L.; Plettenburg, O.; Altenbach, H. J. Flexible Stereo- and Regioselective Synthesis of \( \text{myo-Inositol} \) Phosphates (Part 1): Via Symmetrical Con- duritol B Derivatives. Eur. J. Org. Chem. 2008, 3101–3115. (8) Shashidhar, M. S. Regioselective Protection of \( \text{myo-Inositol} \) Orthoesters - Recent Developments. ARKIVOC 2002, VII, 63–75. (9) Sureshan, K. M.; Shashidhar, M. S.; Praveen, T.; Das, T. Regioselective Protection and Deprotection of Inositol Hydrosul- phates. Chem. Rev. 2003, 103, 4477–4503. (10) Sureshan, K. M.; Shashidhar, M. S. Sulphonate Protecting Groups. Regioselective O-Sulfonylation of \( \text{myo-Inositol} \) Orthoesters. Tetrahedron Lett. 2001, 42, 3037–3039. (11) Gilbert, I. H.; Holmes, A. B.; Young, R. C. Synthesis of Protected \( \text{myo-Inositol} \). Tetrahedron Lett. 1990, 31, 2633–2634. (12) Gilbert, I. H.; Holmes, A. B.; Pechtcher, M. J.; Young, R. C. Lewis Acid Catalysed Rearrangements of \( \text{myo-Inositol} \) Orthophosphate Derivatives. Carbohydr. Res. 1992, 234, 117–130. (13) Conway, S. J.; Gardiner, J.; Gou, D. M.; Johns, M. K.; Lim, Z.; Baker, G. F.; Robinson, D. E. J.; Schieber, C.; Thuring, J. W.; Wang, L. S.-M.; Yin, M.-X.; Burgess, A. W.; Catmell, B.; Hawkins, P. T.; Kristakas, N. T.; Stephens, L. R.; Holmes, A. B. Synthesis and Biological Evaluation of Phosphatidylinositol Phosphate Affinity Probes. Org. Biomol. Chem. 2010, 8, 66–76. (14) Painter, G. F.; Grove, S. J.; Albert, I. H.; Holmes, A. B.; Raithby, P. R.; Hill, M. L.; Hawkins, P. T.; Stephens, L. R. General Synthesis of 3-Phosphorylated \( \text{myo-Inositol} \) Phospholipids and Their Derivatives. J. Chem. Soc., Perkin Trans. 1 1999, 923–936. (15) Yeh, S. M.; Lee, G. H.; Wang, Y.; Luh, T. Y. Chelation-Assisted C-O Bond Cleavage of Ortho Ester. A Convenient Synthesis of \( \text{myo-Inositol} \) Derivatives Having Free Hydroxy Group(s) at Specific Position(s). J. Org. Chem. 1997, 62, 8315–8318. (16) Riley, A. M.; Mahon, M. F.; Potter, B. V. L. Rapid Synthesis of the Enantiomers of \( \text{myo-Inositol} \)-1,3,4,5-Tetrasphosphate by Direct Chiral Desymmetrization of \( \text{myo-Inositol} \) Orthophosphate. Angew. Chem., Int. Ed. Engl. 1997, 36, 1472–1474. (17) Lee, H. W.; Kishi, Y. Synthesis of Mono and Unsymmetrical Bis Ortho-esters of \( \text{sclyl-Inositol} \). J. Org. Chem. 1985, 50, 4402–4404. (18) Garrett, S. W.; Liu, C. S.; Riley, A. M.; Potter, B. V. L. Rapid and Practical Synthesis of \( \text{myo-Inositol} \)-1,4,5-Trisphosphate. J. Chem. Soc., Perkin Trans. 1 1998, 1367–1368. (19) Praveen, T.; Shashidhar, M. S. Convenient Synthesis of 4,6-Di-O-benzyl-\( \text{myo-Inositol} \) and \( \text{myo-Inositol} \), 1,3,5-Orthoesters. Carbohydr. Res. 2001, 330, 409–411. (20) Sureshan, K. M.; Shashidhar, M. S. Sulphonate Protecting Groups. Regioselective O-Acylation of \( \text{myo-Inositol} \), 1,3,5-Orthoesters: The Role of Acyl Migration. Tetrahedron Lett. 2000, 41, 4185–4188. (21) Riley, A. M.; Godage, H. Y.; Mahon, M. F.; Potter, B. V. L. Chiral Desymmetrisation of \( \text{myo-Inositol} \), 1,3,5-Orthobenzoate Gives Rapid Access to Precursors for Second Messenger Analogues. Tetrahedron Asymn. 2006, 17, 171–174. (22) Godage, H. Y.; Riley, A. M.; Woodman, T. J.; Potter, B. V. L. Regioselective Hydrolysis of \( \text{myo-Inositol} \), 1,3,5-Orthobenzoate via a 1,2-Bridged \( 2-\text{Phenyl-1,3-dioxolan-2-ylum} \) Ion Provides a Rapid Route to the Anticancer Agent \( \text{Ins}(1,3,4,5,6)\)P5. Chem. Commun. 2006, 28, 2989–2991. (23) Falasch, M.; Chiozzotto, D.; Godage, H. Y.; Mazzoletti, M.; Riley, A. M.; Previdi, S.; Potter, B. V. L.; Broginni, M.; Maffucci, T. A Novel Inhibitor of the PISK/Akt Pathway Based on the Structure of \( \text{myo-Inositol} \), 1,3,4,5,6-Pentakisphosphate. Br. J. Cancer 2010, 102, 104–114. (24) Piccolo, E.; Vignati, S.; Maffucci, T.; Innominato, P. F.; Riley, A. M.; Potter, B. V. L.; Pandolli, P. B.; Broginni, M.; Iacabelli, S.; Innocenti, P.; Falasch, M. Inositol Pentakisphosphate Promotes Apoptosis through the PI3-K/Akt Pathway. Onco gene 2004, 23, 1754–1765. (25) Maffucci, T.; Piccolo, E.; Cumashi, A.; Iezzi, M.; Riley, A. M.; Saardi, A.; Godage, H. Y.; Rossi, C.; Broginni, M.; Iacobelli, S.; Potter, B. V. L.; Innocenti, P.; Falasch, M. Inhibition of the Phosphatidylinositol 3-Kinase/Akt Pathway by Inositol Pentakisphosphate Results in Antiangiogenic and Antitumor Effects. Cancer Res. 2005, 65, 8339–8349. (26) Bianomote, M. A.; Vasella, A. An Advantageous Synthesis of 1D- and 1L-1,2,3,5,4-Cyclohexanepentol. Helv. Chim. Acta 1998, 81, 688–694. (27) Devaraj, S.; Shashidhar, M. S.; Dixit, S. S. Chelation Controlled Regiospecific O-Substitution of \( \text{myo-Inositol} \) Orthoesters: Convenient Access to Orthogonally Protected \( \text{myo-Inositol} \) Derivatives. Tetrahe- dron 2005, 61, 529–536.
(28) Riley, A. M.; Potter, B. V. L. L. α-Phosphatidyld-\textit{myo}-Inositol 3,5-Bisphosphate: Total Synthesis of a New Inositol Phospholipid via \textit{myo}-Inositol Orthoacetate. \textit{Tetrahedron Lett.} \textbf{1998}, \textit{39}, 6769–6772.

(29) Bhosekar, G.; Murali, C.; Gonnade, R. G.; Shashidhar, M. S.; Bhadbhade, M. M. Identical Molecular Strings Woven Differently by Intermolecular Interactions in Dimorphs of \textit{myo}-Inositol 1,3,5-Orthobenzoate. \textit{Cryst. Growth Des.} \textbf{2005}, \textit{5}, 1977–1982.

(30) Pindur, U.; Müller, J.; Flo, C.; Witzel, H. Ortho Esters and Dialkoxycarbenium Ions—Reactivity, Stability, Structure, and New Synthetic Applications. \textit{Chem. Soc. Rev.} \textbf{1987}, \textit{16}, 75–87.

(31) Childs, R. F.; Frampton, C. S.; Kang, G. J.; Wark, T. A. Structures of Some Dialkoxyphenylmethylum Ions—Steric Inhibition of Resonance. \textit{J. Am. Chem. Soc.} \textbf{1994}, \textit{116}, 8499–8505.

(32) Crich, D.; Dai, Z. M.; Gastaldi, S. On the role of Neighboring Group Participation and Ortho Esters in Beta-Xylosylation: C-13 NMR Observation of a Bridging 2-Phenyl-1,3-dioxalenium Ion. \textit{J. Org. Chem.} \textbf{1999}, \textit{64}, 5224–5229.

(33) Lam, P. W. K.; McClelland, R. A. Direct Observation of the Reversible Ring-Opening of the Hydrolysis of 3-Phenyl-2,4,10-trioxadadamantane. \textit{J. Chem. Soc., Chem. Commun.} \textbf{1980}, \textit{883–884}.

(34) McClelland, R. A.; Lam, P. W. K. Hydrolysis of Trioxadadamantane Ortho Esters. 1. Dialkoxycarbocation—Ortho Ester Equilibrium and Acidity Function. \textit{Can. J. Chem.} \textbf{1984}, \textit{62}, 1068–1073.

(35) McClelland, R. A.; Lam, P. W. K. Hydrolysis of Trioxadadamantane Ortho Esters. 2. Kinetic Analysis and the Nature of the Rate-Determining Step. \textit{Can. J. Chem.} \textbf{1984}, \textit{62}, 1074–1080.

(36) King, J. F.; Allbutt, A. D. Remarkable Stereoselectivity in Hydrolysis of Dioxolenium Ions and Orthoesters Fused to Anchored 6-Membered Rings. \textit{Can. J. Chem.} \textbf{1970}, \textit{48}, 1754–1769.

(37) Li, S. G.; Dory, Y. L.; Deslongchamps, P. Hydrolysis of Cyclic Orthoesters: Experimental Observations and Theoretical Rationalization. \textit{Tetrahedron} \textbf{1996}, \textit{52}, 14841–14854.

(38) Deslongchamps, P. Stereoelectronic Effects in Organic Chemistry. In \textit{Organic Chemistry Series}, 1st ed.; Baldwin, J. E., Eds.; Pergamon Press: Oxford, 1983; Vol. 1, pp 82–85.

(39) Ozaki, S.; Koga, Y.; Ling, L.; Watanabe, Y.; Kimura, Y.; Hirata, M. Synthesis of 2-Substituted \textit{myo}-Inositol 1,3,4,5,6-Pentakis(phosphate) and 1,3,4,5,6-Pentakis(phosphate) Analogs. \textit{Bull. Chem. Soc. Jpn.} \textbf{1994}, \textit{67}, 1058–1063.