# Synthesis and Biological Activity of D- and L-*chiro*-Inositol 2,3,4,5-Tetrakisphosphate: Design of a Novel and Potent Inhibitor of Ins(3,4,5,6)$P_4$ 1-Kinase/Ins(1,3,4)$P_3$ 5/6-Kinase

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The synthesis of a novel and potent Ins(3,4,5,6)$P_4$ 1-kinase/Ins(1,3,4)$P_3$ 5/6 kinase inhibitor and its enantiomer is described. D-*chiro*-Inositol 2,3,4,5-tetrakisphosphate [D-*chiro*-Ins(2,3,4,5)-$P_4$, **3**, Figure 1] and L-*chiro*-inositol 2,3,4,5-tetrakisphosphate [L-*chiro*-Ins(2,3,4,5)$P_4$, ***ent*-3**] were synthesized from D-1,6-di-*O*-benzyl-*chiro*-inositol and L-1,6-di-*O*-benzyl-*chiro*-inositol, respectively. We examined inhibition of the multifunctional Ins(3,4,5,6)$P_4$ 1-kinase/Ins(1,3,4)$P_3$ 5/6-kinase from bovine aorta by **3** and ***ent*-3**. Compound **3** was a potent inhibitor with an $IC_{50}$ of 1.5 $\mu$M, and ***ent*-3** was more than 20-fold less active. The results are compared to those for other inhibitory inositol polyphosphates with structure−activity relationship discussion. Compound **3** is a useful lead for development of further inhibitors of this important enzyme, and ***ent*-3** should find applications in the newly emerging Ins(1,4,5,6)$P_4$ signaling pathway.

## Introduction

There is considerable interest in research into the roles that inositol phosphates play in signal transduction. Among this family of polyphosphates, D-*myo*-inositol 3,4,5,6-tetrakisphosphate [Ins(3,4,5,6)$P_4$, **1**, Figure 1] is a well characterized intracellular signal, which inhibits the conductance of $Ca^{2+}$-activated $Cl^-$ channels in the plasma membrane,[1−3] thereby making an important contribution to the complex homeostatic control of salt and fluid secretion from epithelial cells.[4,5] Other important physiological activities regulated by $Ca^{2+}$-activated $Cl^-$ channels include smooth muscle contraction[6] and neurotransmission.[7] Recently, it has been shown that inositol 1,3,4-trisphosphate [Ins(1,3,4)$P_4$, **2**] acts in vivo as a specific regulator of cellular signaling by Ins(3,4,5,6)$P_4$; Ins(1,3,4)$P_3$ achieves this by competing with Ins(3,4,5,6)$P_4$ for phosphorylation by the same multifunctional kinase.[8] In this way, cellular levels of Ins(3,4,5,6)$P_4$ are elevated in response to receptor-dependent activation of PLC. The enantiomer of Ins-(3,4,5,6)$P_4$, namely D-*myo*-inositol 1,4,5,6-tetrakisphosphate [Ins(1,4,5,6)$P_4$, ***ent*-1**], is also thought to be of biological importance. Ins(1,4,5,6)$P_4$ produced in human intestinal epithelial cells in response to *Salmonella* invasion inhibits phosphoinositide 3-kinase signaling pathways,[9] and recently York et al. reported[10,11] that the enzymes that phosphorylate Ins(1,4,5)$P_3$ to Ins-(1,4,5,6)$P_4$, and then to Ins$P_6$, may play roles in regulating gene expression.

Ins(3,4,5,6)$P_4$ and its analogues have been synthesized by several groups.[12−17] Our aim in the present work was to design a synthesis of an analogue of this molecule and its enantiomer using the naturally occurring chiral inositol derivatives pinitol and quebrachitol, respectively, to avoid the tedious resolution of intermediates and to guarantee the optical purity of synthetic analogues. A focus upon designing new ligands based on the structure of Ins(3,4,5,6)$P_4$ is of 2-fold advantage. First, it may be possible to elucidate the functions of higher inositol phosphates by targeting their synthesis from Ins(3,4,5,6)$P_4$ using inhibitors; second, ligands active at the putative Ins(3,4,5,6)$P_4$ receptor could be directed at diseases that might be treated by either up-regulating or down-regulating $Ca^{2+}$-activated $Cl^-$ secretion.[18,19] A major challenge to pharmacological intervention at the effector site for Ins(3,4,5,6)$P_4$ comes from

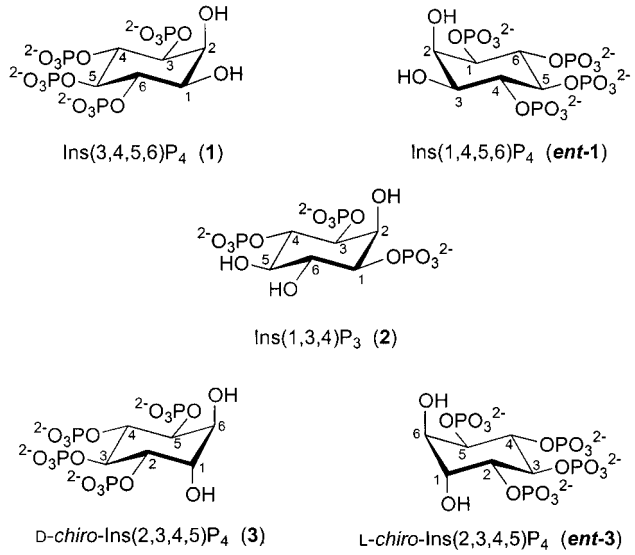


**Figure 1.** Structures of D-*myo*-inositol 3,4,5,6-tetrakisphosphate (**1**), D-*myo*-inositol 1,4,5,6-tetrakisphosphate (***ent*-1**), D-*myo*-inositol 1,3,4-trisphosphate (**2**), and synthetic analogues D- and L-*chiro*-inositol 2,3,4,5-tetrakisphosphates (**3** and ***ent*-3**).

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the exquisite specificity with which it blocks $Cl^-$ channel conductance; Ins(1,3,4)$P_3$, Ins(1,3,4,5)$P_4$, Ins(1,3,4,6)$P_4$, Ins(1,4,5,6)$P_4$, and Ins(1,3,4,5,6)$P_5$ are all ineffective.[1−3] Moreover, at least one, and possibly both of the OH groups on Ins(3,4,5,6)$P_4$, are also critical determinants of ligand specificity.[18,20] We previously examined the impact on the 1-kinase activity of some analogues of Ins(1,3,4)$P_3$ that possess a phosphate group at the 2-position.[8a] Related to this work, and to further our understanding of the role of the hydroxyl group at the 1-position in structure−activity relationship for proteins that bind Ins(3,4,5,6)$P_4$, we synthesized D-*chiro*-inositol 2,3,4,5-tetrakisphosphate [D-*chiro*-Ins(2,3,4,5)$P_4$, **3**, Figure 1] and L-*chiro*-inositol-2,3,4,5-tetrakisphosphate [L-*chiro*-Ins(2,3,4,5)$P_4$, ***ent*-3**] for comparison to **1** and ***ent*-1**, respectively. Such compounds address not only the Ins(3,4,5,6)$P_4$ 1-kinase/Ins(1,3,4)$P_3$ 5/6-kinase but may also provide ligands for future studies of the Ins(3,4,5,6)$P_4$ receptor and for proteins that bind Ins(1,4,5,6)$P_4$. Like Ins(3,4,5,6)$P_4$ and Ins(1,4,5,6)$P_4$, D-*chiro*-Ins(2,3,4,5)$P_4$ and L-*chiro*-Ins(2,3,4,5)$P_4$ also possess four adjacent phosphates and one axial hydroxyl group at the 2-position [C-6 in D- or L-*chiro*-Ins(2,3,4,5)$P_4$]. The key difference lies in the orientation of the substituents at the 1-position. Both D- and L-*chiro*-Ins(2,3,4,5)$P_4$ possess an axial 1-hydroxyl group as a surrogate for the equatorial 1-hydroxyl of Ins(3,4,5,6)$P_4$ and the 3-hydroxyl of Ins(1,4,5,6)$P_4$, respectively. A molecule possessing this relatively conservative replacement, we hypothesized, might still be recognized by proteins that bind Ins(3,4,5,6)$P_4$. In particular for D-*chiro*-Ins(2,3,4,5)-$P_4$, we reasoned that the substitution of an axial hydroxyl group for the equatorial 1-hydroxyl in Ins(3,4,5,6)$P_4$ might provide an effective inhibitor of phosphorylation by the 1-kinase.

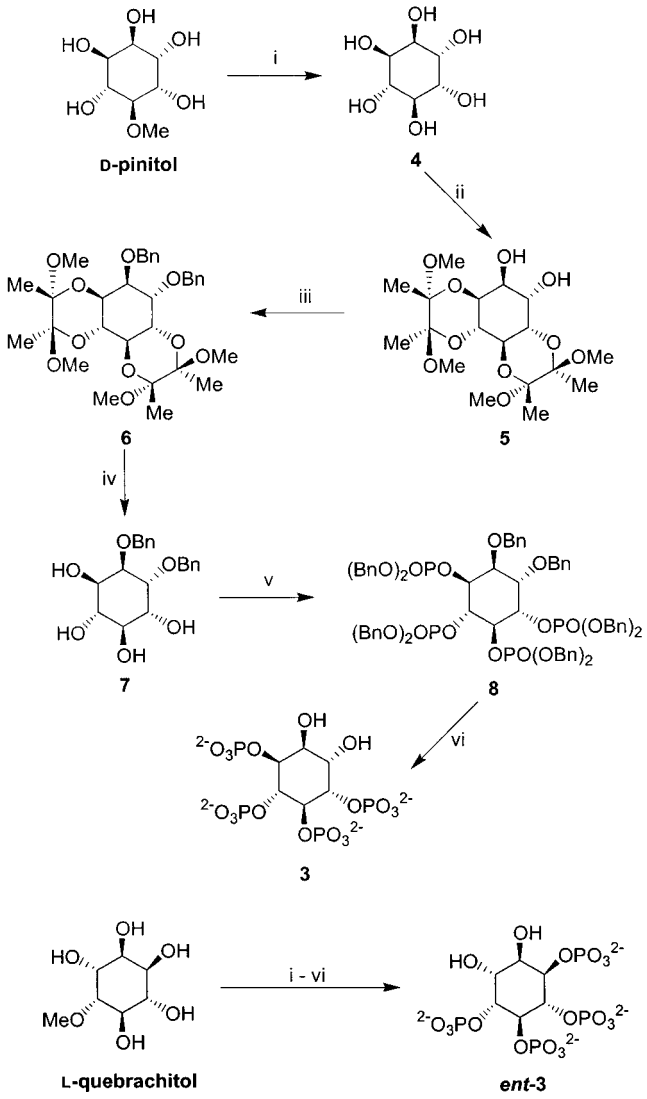
We therefore report here an efficient synthesis of the novel inositol polyphosphate D-*chiro*-Ins(2,3,4,5)$P_4$, **3**, and its enantiomer L-*chiro*-Ins(2,3,4,5)$P_4$, ***ent*-3**, together with a study of their interaction with the Ins(3,4,5,6)$P_4$ 1-kinase/Ins(1,3,4)$P_3$ 5/6-kinase.

## Results

The major problem in the synthesis of inositol polyphosphates is to obtain an intermediate suitable for phosphorylation. This requires multiple regiospecific protection of the hydroxyl groups in inositol with various permanent and temporary protecting groups.[21] Rapid and simplified routes are therefore of great advantage. The butane-2,3-diacetal (BDA) protecting group has been shown to be a useful tool for the selective protection of *trans*-1,2-diols in various monosaccharides.[22,23] Applying this method to *myo*-inositol as starting material, we previously synthesized *neo*-inositol.[24] *chiro*-Inositol has axial hydroxyl groups at positions 1 and 6, with the remaining four being equatorial (two *trans*-1,2-diols). These four hydroxyl groups can therefore be temporarily protected in one step using BDA groups, leaving the two axial hydroxyl groups available for further manipulation.

Thus, D-*chiro*-inositol (**4**, Scheme 1), obtained by O-demethylation[25,26] of naturally occurring D-pinitol with hydriodic acid, was treated with butanedione, trimethyl orthoformate, and $Et_2O{\cdot}BF_3$ in methanol for 30 h to afford D-2,3;4,5-bis-*O*-(2,3-dimethoxybutane-2,3-diyl)-*chiro*-inositol (**5**) in 97% yield. The $^1H$ NMR spectrum of **5** clearly showed that the molecule contained two chemically equivalent BDA groups, and the presence of a symmetry element in the NMR spectra of **5** and its derivatives confirmed that the desired pattern of protection had been achieved. Benzylation of diol **5** furnished the fully protected di-*O*-benzyl ether **6**. The BDA protecting groups were removed by treatment with trifluoroacetic acid in water to give crystalline D-1,6-di-*O*-benzyl-*chiro*-inositol (**7**). Phosphitylation of **7** with bis(benzyloxy)-(diisopropylamino)phosphine activated by 1*H*-tetrazole, followed by oxidation of the product with *m*-chloroperoxybenzoic acid (*m*CPBA), furnished the fully protected tetrakisphosphate **8**. Catalytic hydrogenolysis of **8** over palladium on carbon at atmospheric pressure gave the target tetrakisphosphate **3**, which was further purified by ion exchange chromatography on Q-Sepharose Fast Flow resin, eluting with a gradient of triethylammonium hydrogen carbonate buffer to give

**Scheme 1.** Synthesis of D-*chiro*-Inositol 2,3,4,5-Tetrakisphosphate (**3**) and Its Enantiomer ***ent*-3**[a]




[a] Reagents and conditions: (i) 47% HI, reflux; (ii) butanedione, trimethyl orthoformate, $Et_2O{\cdot}BF_3$, methanol, 97%; (iii) benzyl bromide, NaH, DMF, 96%; (iv) trifluoroacetic acid, $H_2O$, 84%; (v) (a) $Pr^i_2NP(OBn)_2$, 1*H*-tetrazole, $CH_2Cl_2$, (b) *m*-chloroperoxybenzoic acid, 84%; (vi) Pd/C, $H_2$, methanol, $H_2O$, 81%.

**Table 1.** Potencies of Inositol Phosphates as Inhibitors of Ins(3,4,5,6)$P_4$ 1-Kinase Activity[a]

| inositol phosphate | $IC_{50}$ ($\mu$M) | inositol phosphate | $IC_{50}$ ($\mu$M) | inositol phosphate | $IC_{50}$ ($\mu$M) |
|---|---|---|---|---|---|
| Ins(1,3,4)$P_3$ (**2**) | 0.17 ± 0.05 | Ins(1,3,4)$P_3$ (**2**) | 0.2 ± 0.01 | Ins(1,3,4)$P_3$ (**2**) | 0.28[b] |
| DL-Ins(1,2,4)$P_3$ (**10**) | 0.7 ± 2 | Ins(1,3,4,5)$P_4$ (**14**) | 9 ± 1 | D-*chiro*-Ins(2,3,4,5)$P_4$ (**3**) | 1.5 ± 0.7 |
| Ins(1,2,3)$P_3$ (**11**) | 4.2 ± 0.9 | Ins(1,3,4,5,6)$P_5$ (**15**) | 15 ± 0.3 | L-*chiro*-Ins(2,3,4,5)$P_4$ (***ent*-3**) | >30 |
| DL-Ins(1,2,3,4)$P_4$ (**12**) | 1.6 ± 0.6 | Ins(1,3,4,6)$P_4$ (**16**) | 14.5 ± 0.2 | | |
| DL-Ins(1,2,4,6)$P_4$ (**13**) | 16.7 ± 4.7 | Ins(1,4,5)$P_3$ | 48 ± 1 | | |
| Ins(1,3,6)$P_3$ | 17 ± 7 | | | | |
| Ins(1,4,6)$P_3$ | >30 | | | | |
| Ins(1,4,5,6)$P_4$ (***ent*-1**) | >30 | | | | |

[a] Data in the first column from Yang et al.[8a] Data in the second column from Tan et al.[28] [b] This value is from $n = 1$, only performed to ensure consistency of the present assays with past data.

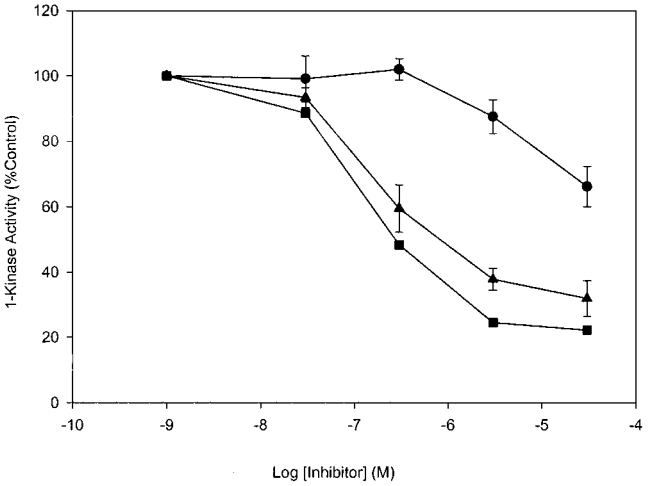



**Figure 2.** Inhibition of Ins(3,4,5,6)$P_4$ 1-kinase activity from bovine aorta by analogues of Ins(3,4,5,6)$P_4$. 1-Kinase activity was assayed as described in the Experimental Section: (■) Ins(1,3,4)$P_3$ (**2**), (▲) D-*chiro*-Ins(2,3,4,5)$P_4$ (**3**), (●) L-*chiro*-Ins(2,3,4,5)$P_4$ (***ent*-3**). Enzyme activity in each case is presented as a percentage of activity in the absence of any inhibitor.

the pure triethylammonium salt of **3**, quantified by a modification of the Briggs phosphate assay.[27]

The other enantiomer, L-*chiro*-inositol 2,3,4,5-tetrakisphosphate (***ent*-3**), was obtained in a fashion identical to that described for **3**, but starting with the naturally occurring L-quebrachitol (Scheme 1). Compounds were used as their triethylammonium salts for biological and physicochemical evaluation. Evaluation of the Ins(3,4,5,6)$P_4$ 1-kinase inhibitory activity of **3** and ***ent*-3** showed that **3** behaved as a potent inhibitor with an $IC_{50}$ of 1.5 ± 0.7 $\mu$M (Figure 2 and Table 1). As expected, the enantiomer ***ent*-3** was much less active ($IC_{50}$ > 30 $\mu$M).

## Discussion

The Ins(3,4,5,6)$P_4$ 1-kinase activity of the multifunctional enzyme shows considerable promiscuity in its interaction with inhibitors that bear some structural relationship to Ins(3,4,5,6)$P_4$[8a,28] (see data in Table 1). There has, however, been little structure−activity relationship discussion of such previous work. As Ins(1,3,4)$P_3$ and Ins(3,4,5,6)$P_4$ are co-substrates for the enzyme,[8] it is useful to consider which structural features are shared by the two molecules. In Figure 3, Ins(3,4,5,6)$P_4$ (**1**) and Ins(1,3,4)$P_3$ (**2**) are drawn so that their 1-hydroxyl and 6-hydroxyl groups, the sites of phosphorylation by the 1-kinase and 6-kinase activities of the enzyme, respectively, are made to coincide (this model ignores the weaker 5-kinase activity). Motifs common to the two molecules can then be identified, giving the schematic pattern **9**, which we propose as a preliminary pharmacophore for high affinity recognition of inositol phosphates by the multifunctional kinase. This pharmacophore consists of an all-equatorial 1-hydroxyl-3,4,6-trisphosphate pattern with absolute configuration as shown.

Of the four phosphate groups and two hydroxyl groups in Ins(3,4,5,6)$P_4$ (**1**, Figure 3), the presence of the vicinal 3,4-bisphosphate motif of Ins(3,4,5,6)$P_4$ is proposed to be essential for the inhibition of the multifunctional kinase [the compounds in Figure 3 have all been drawn in their putative binding modes relative to Ins(3,4,5,6)$P_4$]. For the racemic compounds DL-Ins(1,2,4)$P_3$, DL-Ins(1,2,3,4)$P_4$, and DL-Ins(1,2,4,6)$P_4$, only the D-enantiomers (shown as **10**, **12**, and **13**, respectively) are assumed to be active. Ins(1,4,5)$P_3$, Ins(1,4,6)$P_3$, and Ins(1,3,6)$P_3$ (not shown) are only weakly recognized (see Table 1) because their vicinal bisphosphate motifs have the opposite absolute stereochemistry to the 3,4-bisphosphate in pattern **9**. Surprisingly, however, ligands having an axial phosphate group [at C-2 in Ins(1,2,4)$P_3$ (**10**) or Ins(1,2,3)$P_3$ (**11**)], equivalent to C-4 in **9**, retain significant inhibitory activity,[8a] showing that the kinase is tolerant to stereochemical change at this position. All the results to date show that the phosphate group at C-5 of Ins(3,4,5,6)$P_4$ plays a relatively insignificant role, compared with the 3-, 4-, and 6-phosphate groups. Thus, ligands having an axial hydroxyl group at this position [at C-2 in Ins(1,3,4)$P_3$ (**2**)], an equatorial hydroxyl group [at C-3 in Ins(1,2,4)$P_3$ (**10**)], or an axial phosphate group [at C-2 in Ins(1,2,3,4)$P_4$ (**12**)] all have significant kinase inhibitory activity.[8a] In contrast, the 6-phosphate group in Ins(3,4,5,6)$P_4$ appears to be an important, although not essential, feature. Thus Ins(1,2,3)$P_3$ (**11**), which possesses an equatorial hydroxyl group at this relative position rather than an equatorial phosphate group, is a weak inhibitor.[8a]

The orientation of the 2-hydroxyl group in Ins(3,4,5,6)$P_4$ is not very important. Ins(1,3,4)$P_3$ (**2**), Ins(1,2,4)$P_3$ (**10**), and Ins(1,2,3,4)$P_4$ (**12**) which, in their putative binding orientations, all possess equatorial hydroxyl groups at the position corresponding to C-2 in Ins(3,4,5,6)$P_4$, are potent inhibitors.[8a] However, the weak activity of Ins(1,2,4,6)$P_4$ (**13**) compared to Ins(1,2,4)$P_3$ suggests that an equatorial phosphate group placed at the position corresponding to C-2 in pattern **9** interferes with recognition by the kinase. Thus, Ins(1,3,4,5)$P_4$ (**14**), the product of phosphorylation of Ins(1,3,4)$P_3$ by the 5-kinase activity of the enzyme, is only a weak inhibitor even though it contains pattern **9**, because its 5-phosphate group will hinder recognition

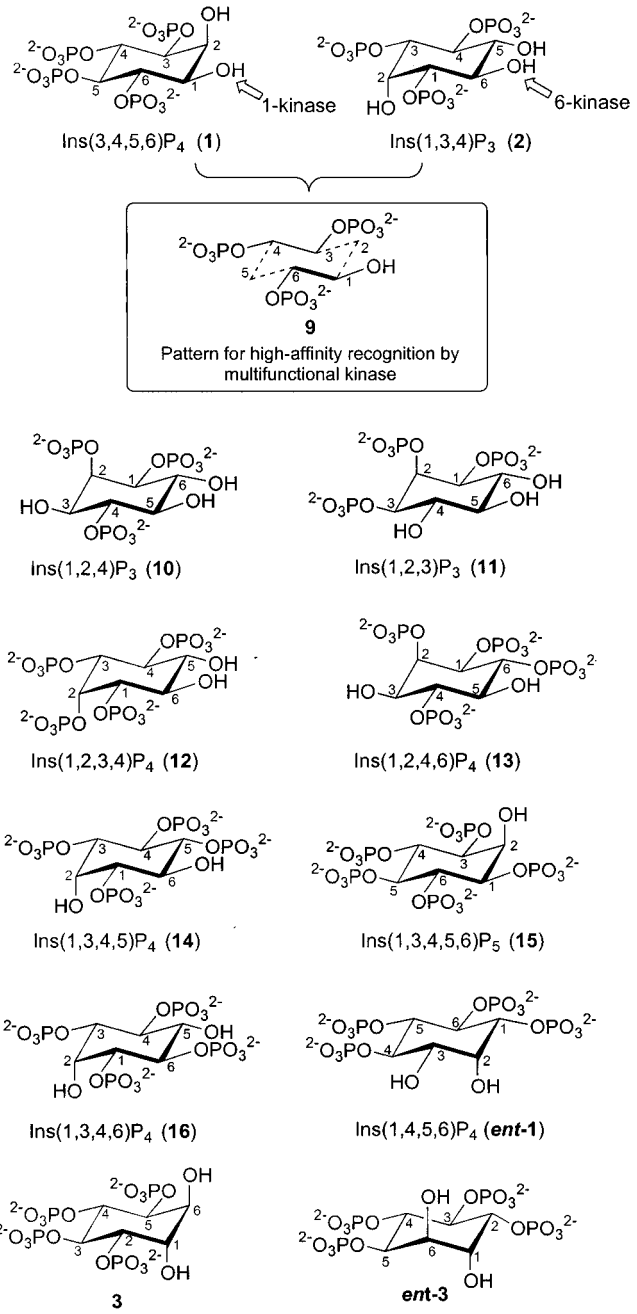



**Figure 3.** Comparison of the structures of the natural cosubstrates for the multifunctional kinase, Ins(3,4,5,6)$P_4$ (**1**) and Ins(1,3,4)$P_3$ (**2**), allows the identification of **9**, a pattern of three phosphate groups and one hydroxyl group that occurs in both molecules. The relative potencies of the weaker inhibitors ***ent*-1**, **3**, ***ent*-3** and analogues **10**−**16** can then be interpreted (see text) by reference to this pattern. All structures are shown in their putative binding modes relative to Ins(3,4,5,6)$P_4$ (**1**).

in this binding orientation. Finally, Ins(1,4,5,6)$P_4$ [***ent*-1**, the enantiomer of Ins(3,4,5,6)$P_4$] is also a very weak inhibitor of the kinase, a finding that may be explained by noting that, although ***ent*-1** does possess a bisphosphate of the correct stereochemistry, the inevitable placement of a phosphate group at the equatorial C-2 position in pattern **9** will interfere with recognition, as for Ins(1,3,4,5)$P_4$. Additionally, in the case of ***ent*-1**, no equivalent to the important 6-phosphate of Ins(3,4,5,6)-$P_4$ is present in potential binding orientations such as the one shown in Figure 3.

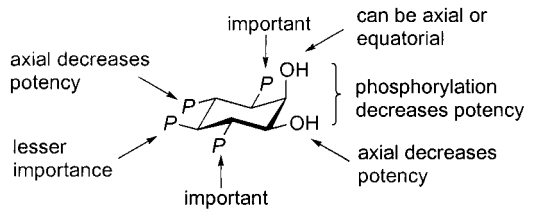



**Figure 4.** Schematic summary of structure−activity relationships for inhibition of the multifunctional kinase, based upon the structure of Ins(3,4,5,6)$P_4$. For clarity, phosphate groups are represented by the symbol *P*.

The biological data from evaluation of D- and L-*chiro*-inositol 2,3,4,5-tetrakisphosphate (**3** and ***ent*-3**) can be used to further develop this structure−activity analysis and improve our understanding of the catalytic site of the multifunctional kinase, which normally binds the equatorial hydroxyl group at C-1 of Ins(3,4,5,6)$P_4$ or C-6 of Ins(1,3,4)$P_3$ for phosphorylation. When a phosphate group is introduced at this position by the kinase [to give Ins(1,3,4,5,6)$P_5$ (**15**) and Ins(1,3,4,6)$P_4$ (**16**), respectively], the data show that the inhibitory activity is reduced significantly. However, without an equatorial 1-hydroxyl, but with an axial one at this position, D-*chiro*-Ins(2,3,4,5)$P_4$ (**3**) was relatively well recognized, with an affinity only about 5-fold less than that of the endogenous inhibitor Ins(1,3,4)$P_3$. Thus the enzyme shows some tolerance for an axial hydroxyl group at this position. It is interesting to note that, because **3** is $C_2$-symmetric, its likely orientation of binding to the active site of the kinase is particularly well defined. Furthermore, the fact that **3** has only axial hydroxyl groups clearly demonstrates that the enzyme does not absolutely require an equatorial hydroxyl group in high affinity ligands. The enantiomer, L-*chiro*-Ins(2,3,4,5)$P_4$ (***ent*-3**), was at least 20-fold less active than **3**, a result which can easily be explained using the same arguments outlined for Ins(1,4,5,6)$P_4$ above. These structure−activity arguments are summarized in Figure 4.

Apart from the naturally occurring Ins(3,4,5,6)$P_4$ and Ins(1,3,4)$P_3$, the most potent ligand that had previously been described is DL-Ins(1,2,4)$P_3$ with an apparent $IC_{50}$ of 0.7 $\mu$M. If we make the reasonable assumption that inhibition is largely due to only one enantiomer [Ins-(1,2,4)$P_3$, **10**] in the racemic mixture, the actual $IC_{50}$ is ca. 0.35 $\mu$M. D-*chiro*-Ins(2,3,4,5)$P_4$ (**3**) is only ca. 4-fold less active than the active isomer of Ins(1,2,4)$P_3$ but, as we demonstrate here, is simply available in chiral form without any optical resolution. L-*chiro*-Ins(2,3,4,5)-$P_4$ (***ent*-3**) is clearly unable to bind to the enzyme effectively.

Thus, we have synthesized the first potent chiral structurally modified inhibitor of Ins(3,4,5,6)$P_4$ 1-kinase/Ins(1,3,4)$P_3$ 5/6-kinase not based upon *myo*-inositol, and this compound should be useful for the further development of inhibitors. Moreover, the enantiomer ***ent*-3** should find applications elucidating more exact roles for Ins(1,4,5,6)$P_4$ and the enzymes that act upon it in what appears to be a new Ins(1,4,5,6)$P_4$-mediated signaling pathway.[10,11]

## Experimental Section

**Chemistry.** Quebrachitol, extracted from rubber byproduct, was purchased from the Malaysian Rubber Company, Kuala Lumpur. Pinitol was purchased from New Zealand Pharma-

ceuticals Ltd. TLC was performed on precoated plates (Merck TLC aluminum sheets silica $60F_{254}$). Spots were visualized by spraying of plates with phosphomolybdic acid in methanol followed by heating. Flash chromatography was carried out using Sorbsil C60 silica gel. NMR spectra were recorded with a JEOL GX-270 or EX-400 spectrometer. $^1$H chemical shifts were measured in ppm relative to tetramethylsilane (TMS), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad).$^{31}$P chemical shifts were measured in ppm relative to external 85% $H_3PO_4$ and are positive when downfield from this reference. Melting points (uncorrected) were determined using a Reichert-Jung Thermo Galen Kofler Block. Microanalysis was carried out by the University of Bath microanalysis service. Optical rotations were measured using an Optical Activity Ltd. AA-10 polarimeter, and $[\alpha]_D$ values are given in $10^{-1}$ deg cm$^2$ g$^{-1}$. Mass spectra were recorded at the Mass Spectrometry Service of the University of Bath. Ion-exchange chromatography was performed on a LKB-Pharmacia medium-pressure ion-exchange chromatograph using Q Sepharose Fast Flow with gradients of triethylammonium hydrogen carbonate (TEAB) as eluent. Column fractions containing inositol polyphosphate analogues were assayed for total phosphate content by a modification of the Briggs test.[27]

**Biology.** The activity of highly purified Ins(3,4,5,6)$P_4$ 1-kinase from bovine aorta was assayed as described previously.[8] Aliquots of the purified enzyme (0.7 $\mu$g) were incubated at 37 °C, with approximately 6000 dpm of 0.3 $\mu$M [$^3$H]Ins(3,4,5,6)$P_4$, plus the concentration of D- or L-*chiro*-Ins(2,3,4,5)$P_4$ indicated in the figure, in a final volume of 100 $\mu$L buffer containing 20 mM HEPES (pH 7.2), 6 mM $MgSO_4$, 0.4 mg/mL saponin, 100 mM KCl, 0.3 mg/mL bovine serum albumin, 5 mM ATP, 10 mM phosphocreatine, and 2.5 Sigma units of phosphocreatine kinase. The reaction was stopped after 30 min by adding 1 mL of ice-cold quenching buffer, which contained 1 mg/mL Ins$P_6$, 0.2 M ammonium formate, and 0.1 M formic acid. The amount of Ins(1,3,4,5,6)$P_5$ formed was analyzed by chromatography on gravity-fed anion exchange columns. The experiments were repeated three times for the analogues (**3** and ***ent*-3**); data for a single inhibition curve for Ins(1,3,4)$P_3$ for comparison with previous data only are presented.

**D-(2′*R*,3′*R*)-2,3;4,5-Bis-*O*-(2′,3′-dimethoxybutane-2′3′-diyl)-*chiro*-inositol (5).** To a stirred suspension of D-*chiro*-inositol (**4**) (5.00 g, 27.7 mmol) in dry methanol (90 mL) were added trimethyl orthoformate (24 mL, 220 mmol), butanedione (5.8 mL, 66 mmol), and $Et_2O{\cdot}BF_3$ (0.4 mL, 3 mmol). The mixture was stirred at room temperature for 30 h. The solvent was evaporated, and the residue was partitioned between $CH_2Cl_2$ (100 mL) and $H_2O$ (100 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness. Purification of the residue by flash chromatography on silica gel gave the bisacetal **5** (11.0 g, 97%); mp 103−120 °C (from diisopropyl ether); $[\alpha]_D = -151$ ($CH_2Cl_2$, $c = 1$); $\delta_H$ (400 MHz, DMSO $d_6$) 1.16, 1.20 (12 H, 2 s, 4 × $CH_3$), 3.12, 3.14 (12 H, 2 s, 4 × $OCH_3$), 3.64−3.75 (6 H, m, 6 × CH), 5.13 (2 H, d, $J = 3.4$ Hz, $D_2O$ exch., 2 × OH); $\delta_C$ (100 MHz, DMSO $d_6$) 17.63 (2 × $CH_3$), 17.68 (2 × $CH_3$), 47.09 (2 × $OCH_3$), 47.13 (2 × $OCH_3$), 66.33 (2 × CH), 67.55 (2 × CH), 70.15 (2 × CH), 97.99 (2 × $C_q$ of BDA), 99.21 (2 × $C_q$ of BDA); *m*/*z* (+ve ion FAB) 407 (M, 100%), 377 (40), 101 (100), 73 (28); Anal. ($C_{18}H_{32}O_{10}$) C, H, N.

**L-(2′*S*,3′*S*)-2,3;4,5-Bis-*O*-(2′,3′-dimethoxybutane-2′3′-diyl)-*chiro*-inositol** (***ent*-5**) was obtained in a fashion identical to that described for **5**; mp 105−120 °C (from diisopropyl ether); $[\alpha]_D = +145$ ($CH_2Cl_2$, $c = 0.8$); spectroscopic data were identical to those obtained for **5**; Acc. Mass ($C_{18}H_{32}O_{10}$).

**D-(2′*R*,3′*R*)-1,6-Di-*O*-benzyl-bis-*O*-2,3;4,5-(2′,3′-dimethoxybutane-2′,3′-diyl)-*chiro*-inositol (6).** A mixture of bisacetal (**5**) (0.82 g, 2.0 mmol), benzyl bromide (1.0 g, 5.8 mmol) and NaH (0.24 g of a 60% dispersion in oil, 6.0 mmol) in dry DMF was stirred at room temperature for 20 h, and the excess of NaH was destroyed by adding methanol. The solvents were evaporated to dryness in vacuo, and the residue was taken up in ethyl acetate and washed with saturated aqueous $NaHCO_3$, then $H_2O$. The organic phase was dried ($MgSO_4$) and concentrated, followed by flash chromatography to give the fully protected di-*O*-benzyl ether **6** (1.13 g, 96%); mp: 120−124 °C (from methanol/water); $[\alpha]_D = -160$ ($CHCl_3$, $c = 0.2$) $\delta_H$ (400 MHz, $CDCl_3$) 1.27, 1.28 (12 H, 2 s, 4 × $CH_3$), 3.18, 3.27 (12 H, 2 s, 4 × $OCH_3$), 3.66−3.67 (2 H, m, H-1 and H-6), 3.90−3.93 (2 H, m, 2 × CH), 4.02−4.05 (2 H, m, 2 × CH), 4.46 and 4.83 (4 H, AB, $J_{AB}$=12.2 Hz, 2 × $CH_2$), 7.21−7.51 (10 H, m, ArH); *m*/*z* (+ve ion FAB) 587 [($M^+ - H^\bullet$) 100%], 557 (7), 101.0 (40), 91.0 (100); Anal. ($C_{32}H_{44}O_{10}$) C, H.

**L-(2′*S*,3′*S*)-1,6-Di-*O*-benzyl-bis-*O*-2,3;4,5-(2′,3′-dimethoxybutane-2′3′-diyl)-*chiro*-inositol** (***ent*-6**) was obtained in a fashion identical to that described for **6**; mp 117−120 °C (from methanol/water); $[\alpha]_D = +152$ ($CHCl_3$, $c = 5$); spectroscopic data were identical to those obtained for **6**; Anal. ($C_{32}H_{44}O_{10}$) C, H.

**D-1,6-Di-*O*-benzyl-*chiro*-inositol (7).** To **6** (1.4 g, 2.4 mmol) was added a mixture of trifluoroacetic acid (20 mL) and water (20 mL). The mixture was stirred at room temperature for 20 min and then evaporated to dryness in vacuo to give a white solid, which was recrystallized from ethyl acetate to give tetraol **7** (0.73 g, 84%); mp: 174−175 °C (from ethyl acetate); $[\alpha]_D = -24$ (MeOH, $c = 0.4$); $\delta_H$ (400 MHz, DMSO $d_6$) 3.34−3.36 (2 H, m, 2 × CH), 3.53−3.56 (2 H, m, 2 × CH), 3.71−3.72 (2 H, m, H-1 and H-6), 4.56 and 4.69 (4 H, AB, $J_{AB} = 12.2$ Hz, 2 × $CH_2$), 4.55−4.65 (4 H, br, partially buried, $D_2O$ exch., 4 × OH), 7.25−7.35 (10 H, m, ArH); *m*/*z* (+ve ion FAB) 359 [($M^+ - H^\bullet$) 20% ], 721 (75), 383 (28), 181 (28), 91 (100); Acc. Mass ($C_{20}H_{23}O_6$).

**L-1,6-Di-*O*-benzyl-*chiro*-inositol** (***ent*-7**) was obtained in a fashion identical to that described for **13**; mp 172−173 °C (from ethyl acetate); $[\alpha]_D = +26$ (MeOH, $c = 0.3$); spectroscopic data were identical to those obtained for **7**; Acc. Mass ($C_{20}H_{23}O_6$).

**D-1,6-Di-*O*-benzyl-*chiro*-inositol 2,3,4,5-tetrakis(*O*,*O*-dibenzyl phosphate) (8).** The tetraol **7** (0.18 g, 0.50 mmol) and 1*H*-tetrazole (0.35 g, 5.0 mmol) in dry dichloromethane (10 mL) were stirred at room temperature for 10 min, and then bis(benzyloxy)(diisopropylamino)phosphine (0.83 g, 2.4 mmol) was added. The mixture was stirred for 1 h and cooled to 0 °C, followed by addition of *m*-chloroperoxybenzoic acid (60%, 1.0 g, 3.5 mmol). After further stirring for 30 min, the mixture was diluted with ethyl acetate (50 mL) and washed with 10% sodium metabisulfite (50 mL), aqueous $NaHCO_3$, brine, and water (50 mL of each). The organic phase was dried over magnesium sulfate and evaporated to dryness. The residue was chromatographed on silica gel to give compound **8** (0.59 g, 84%) as an oil; $[\alpha]_D = +4.7$ ($CHCl_3$, $c = 0.6$); $\delta_H$ (400 MHz, $CDCl_3$) 4.13 (2 H, br s, H-1 and H-6), 4.35 and 4.44 (4 H, AB, $J_{AB} = 11.7$ Hz, 2 × $CH_2$), 4.55−4.65 (2 H, br m, 2 × CH), 4.86−5.01 (18 H, m, 8 × $CH_2$ and 2 × CH), 6.92−7.31 (50 H, m, ArH); $\delta_P$ (160 MHz, $CDCl_3$), $^1$H-decoupled) −2.23 (2 P, s), −1.36 (2 P, s); *m*/*z* (+ve ion FAB) 1401 [($M + H)^+$ 10%], 271 (8), 181 (12), 91 (100); Acc. Mass ($C_{76}H_{77}O_{18}P_4$).

**L-1,6-Di-*O*-benzyl-*chiro*-inositol 2,3,4,5-tetrakis(*O*,*O*-dibenzyl phosphate)** (***ent*-8**) was obtained in a fashion identical to that described for **8**; $[\alpha]_D = -3.7$ ($CHCl_3$, $c = 0.8$); spectroscopic data were identical to those obtained for **7**; Acc. Mass ($C_{76}H_{77}O_{18}P_4$).

**D-*chiro*-Inositol 2,3,4,5-tetrakisphosphate (3).** A solution of **8** (0.30 g, 0.21 mmol) in methanol (20 mL) and a few drops of water was stirred vigorously with 10% Pd/C (1.2 g) under atmosphere of $H_2$ at room temperature overnight. The catalyst was removed by filtration, and the solvents were evaporated in vacuo. The residue was purified by ion-exchange chromatography on Pharmacia Q Sepharose Fast Flow resin eluting with a gradient of TEAB buffer (0.1−1 M) gave the pure triethylammonium salt of compound **3** (0.17 mmol, 81%), which eluted at 750−850 mM buffer; $[\alpha]_D = +8.9$ (MeOH, $c = 1$); $\delta_H$ (400 MHz, $CD_3OD$) 4.10 (2 H, br s, H-1 and H-6), 4.49−4.53 (2 H, m, 2 × CH), 4.53−4.57 (2 H, m, 2 × CH); $\delta_P$ (160 MHz, $CDCl_3$), $^1$H-decoupled) −0.14 (2 P, s), 0.38 (2 P, s); Acc. Mass ($C_6H_{15}O_{18}P_4$).

**L-*chiro*-Inositol 2,3,4,5-tetrakisphosphate (*ent*-3)** was obtained from ***ent*-8** in a fashion identical to that described

for **3**; $[\alpha]_D = -10.1$ (MeOH, $c = 1$); spectroscopic data were identical to those obtained for **3**; Acc. Mass ($C_6H_{15}O_{18}P_4$).

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## References

(1) Ho, M. W.; Shears, S. B.; Bruzik, K. S.; Duszyk, M.; French, A. S. Inositol 3,4,5,6-tetrakisphosphate specifically inhibits a receptor-mediated $Ca^{2+}$-dependent $Cl^-$ current in CFPAC-1 cells. *Am. J. Physiol.* **1997**, *272*, 1160−1168.

(2) Xie, W.; Kaetzel, M. A.; Bruzik, K. S.; Dedman, J. R.; Shears, S. B.; Nelson, D. J. Inositol 3,4,5,6-tetrakisphosphate inhibits the calmodulin-dependent protein kinase II-activated chloride conductance in T84 colonic epithelial cells. *J. Biol. Chem.* **1996**, *271*, 14092−14097.

(3) Ismailov, I. I.; Fuller, C. M.; Berdiev, B. K.; Shlyonsky, V. G.; Benos, D. J.; Barrett, K. E. A biologic function for an "orphan" messenger: D-*myo*-inositol 3,4,5,6-tetrakisphosphate selectively blocks epithelial calcium-activated chloride current. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10505−10509.

(4) Vajanaphanich, M.; Schultz, C.; Rudolf, M. T.; Wasserman, M.; Enyedi, P.; Craxton, A.; Shears, S. B.; Tsien, R. Y.; Barrett, K. E.; Traynor-Kaplan, A. Long-term uncoupling of chloride secretion from intracellular calcium level by $Ins(3,4,5,6)P_4$. *Nature* **1994**, *371*, 711−714.

(5) Carew, M. A.; Yang, X.; Shears, S. B. $Ins(3,4,5,6)P_4$ inhibits an apical calcium-activated chloride conductance in polarized monolayers of a cystic fibrosis cell-line. *J. Biol. Chem.* **2000**, *275*, 26906−26913.

(6) Large, W. A.; Wang, Q. Characteristics and physiological role of the $Ca^{2+}$-activated $Cl^-$ conductance in smooth muscle. *Am. J. Physiol.* **1996**, *271*, C435−C454.

(7) Frings, S.; Reuter, D.; Kleene, S. J. Neuronal $Ca^{2+}$-activated $Cl^-$ channels − homing in on an elusive channel species. *Prog. Neurobiol.* **2000**, *60*, 247−289.

(8) (a) Yang, X.; Rudolf, M.; Carew, M. A.; Yoshida, M.; Nerreter, V.; Riley, A. M.; Chung, S.-K.; Bruzik, K. S.; Potter, B. V. L.; Schultz C.; Shears, S. B. Inositol 1,3,4-trisphosphate acts in vivo as a specific regulator of cellular signaling by inositol 3,4,5,6-tetrakisphosphate. *J. Biol. Chem.*, **1999**, *274*, 18973-18980. (b) Yang, X.; Shears, S. B. Multitasking in signal transduction by a promiscuous human $Ins(3,4,5,6)P_4$ 1-kinase/$Ins(1,3,4)P_3$ 5/6-kinase. *Biochem. J.* **2000**, *352*, 343−351.

(9) Eckmann, L.; Rudolf, M. T.; Ptasznik, A.; Schultz, C.; Jiang, T.; Wolfon, N.; Tsien, R.; Fierer, J.; Shears, S. B.; Kagnoff, M. F.; Traynor-Kaplan, A. E. D-*myo*-Inositol 1,4,5,6-tetrakisphosphate produced in human intestinal epithelial calls in response to Salmonella invasion inhibits phosphoinositide 3-kinase signaling pathways. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14456−14460.

(10) York, J. D.; Odom, A. R.; Murphy, R.; Ives, E. B.; Wente, S. R. A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. *Science* **1999**, *285*, 96.

(11) Odom, A. R.; Stahlberg, A.; Wente, S. R.; York, J. D. A role for nuclear inositol 1,4,5-trisphosphate kinase in transcriptional control. *Science* **2000**, *287*, 2026−2029.

(12) Pietrusiewicz, K. M.; Salamonczyk, G. M.; Bruzik, K. S. The synthesis of homochiral inositol phosphates from *myo*-inositol. *Tetrahedron* **1992**, *48*, 5523−5542. Corrigendum: *Tetrahedron* **1994**, *50*, 573−574.

(13) Ozaki, S.; Ling, L.; Ogasawara, T.; Watanabe, Y.; Masato, H. A convenient chemoenzymatic synthesis of D- and L-*myo*-inositol 1,4,5,6-tetrakisphosphate. *Carbohydr. Res.* **1994**, *259*, 307−310.

(14) Roemer, S.; Rudolf, M. T.; Stadler, C.; Schultz, C. Synthesis of D-*myo*-inositol 3,4,5,6- and 1,4,5,6-tetrakisphosphate analogues and their membrane-permeant derivatives. *J. Chem. Soc., Chem. Commun.* **1995**, 411−412.

(15) Roemer, S.; Stadler, C.; Rudolf, M. T.; Schultz, C. Membrane-permeant analogues of the putative second-messenger *myo*-inositol 3,4,5,6-tetrakisphosphate. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1683−1694.

(16) Solomons, K. R. H.; Freeman, S.; Schwalbe, C. H.; Shears, S. B.; Nelson, D. J.; Xie, W.; Bruzik, K. S.; Kaetzel, M. A. Synthesis of D-1,2-dideoxy-1,2-difluoro-*myo*-inositol 3,4,5,6-tetrakisphosphate and its enantiomer as analogues of *myo*-inositol 3,4,5,6-tetrakisphosphate. *Carbohydr. Res.* **1998**, *309*, 337−343.

(17) Adelt, S.; Plettenburg, O.; Stricker, R.; Reiser, G.; Altenbach, H.-J.; Vogel, G. Enzyme-assisted total synthesis of the optical antipodes D-*myo*-inositol 3,4,5-trisphosphate and D-*myo*-inositol 1,5,6-trisphosphate: aspects of their structure−activity relationship to biologically active inositol phosphates. *J. Med. Chem.* **1999**, *42*, 1262−1273.

(18) Rudolf, M. T.; Li, W.; Wolfson, N.; Traynor-Kaplan, A. E.; Schultz, C. 2-Deoxy derivative is a partial agonist of the intracellular messenger inositol 3,4,5,6-tetrakisphosphate in the epithelial cell line T84. *J. Med. Chem.* **1998**, *41*, 3635−3644.

(19) Schultz, C.; Roemer, S.; Stadler, C.; Rudolf, M. T.; Wolfson, E.; Traynor-Kaplan, A. E. Antagonists of Ins(3,4,5,6)P4 reverse inhibition of chloride secretion in colonic epithelia. *Gastroenterology* **1997**, *112*, A401.

(20) Xie, W.; Solomons, K. G. H.; Freeman, S.; Kaetzel, M. A.; Bruzik, K. S.; Nelson, D. J.; Shears, S. B. Regulation of $Ca^{2+}$-dependent $Cl^-$ conductance in T84 cells: cross-talk between $Ins(3,4,5,6)P_4$ and protein phosphatases. *J. Physiol.* **1998**, *510*, 661−673.

(21) Potter, B. V. L.; Lampe, D. Chemistry of inositol lipid mediated cellular signaling. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933−1972.

(22) Montchamp, J.-L.; Tian, F.; Hart, M. E.; Frost, J. W. Butane 2,3-bisacetal protection of vicinal diequatorial diols. *J. Org. Chem.*, **1996**, *61*, 3897−3899.

(23) Hense, A.; Ley, S. V.; Osborn, H. M. I.; Owen, D. R.; Poisson, J.-F.; Warriner, S. L.; Wesson, K. E. Direct preparation of diacetals from 1,2-diketones and their use as 1,2-diol protection groups. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2023−2031.

(24) Riley, A. M.; Jenkins, D. J.; Potter, B. V. L. A concise synthesis of *neo*-inositol. *Carbohydr. Res.* **1998**, *314*, 277−281.

(25) Liu, C.; Nahorski, S. R.; Potter, B. V. L. Synthesis from quebrachitol of 1L-*chiro*-inositol 2,3,5-trisphosphate, an inhibitor of the enzymes of 1D-*myo*-inositol 1,4,5-trisphosphate metabolism. *Carbohydr. Res.* **1992**, *234*, 107−115.

(26) Tegge, W.; Ballou, C. E. Chiral synthesis of D-*myo*-inositol and L-*myo*-inositol 1,4,5-trisphosphate. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 94−98.

(27) Lampe, D.; Liu, C.; Potter, B. V. L. Synthesis of selective non-$Ca^{2+}$-mobilizing inhibitors of D-*myo*-inositol 1,4,5-trisphosphate 5-phosphatase. *J. Med. Chem.* **1994**, *43*, 3295−3303.

(28) Tan, Z.; Bruzik, K S.; Shears, S. B. Properties of the inositol 3,4,5,6-tetrakisphosphate 1-kinase purified from rat liver. *J. Biol. Chem.* **1997**, *272*, 2285−2290.

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