2-Deoxy Derivative Is a Partial Agonist of the Intracellular Messenger Inositol 3,4,5,6-Tetrakisphosphate in the Epithelial Cell Line T_{84}

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We have synthesized the first deoxy analogues of *myo*-inositol 3,4,5,6-tetrakisphosphate (**1**) [Ins(3,4,5,6)P_4], *rac*-2-deoxy-*myo*-inositol 3,4,5,6-tetrakisphosphate (*rac*-**2**), 2-deoxy-*myo*-inositol 1,4,5,6-tetrakisphosphate (*ent*-**2**), and *rac*-1-deoxy-*myo*-inositol 3,4,5,6-tetrakisphosphate (*rac*-**3**). In order to evaluate the binding properties of the three derivatives to the yet unidentified intracellular binding sites for Ins(3,4,5,6)P_4, the analogues were converted to membrane-permeant derivatives. Starting with common inositol precursors, various forms of Barton−McCombie deoxygenation and classical protection/deprotection procedures yielded the desired precursors *rac*-1-*O*-butyryl-2-deoxy-*myo*-inositol (*rac*-**12**), *ent*-3-*O*-butyryl-2-deoxy-*myo*-inositol (*ent*-**12**), and *rac*-2-*O*-butyryl-1-deoxy-*myo*-inositol (*rac*-**19**), respectively. Phosphorylation and subsequent deprotection yielded *rac*-**2**, *ent*-**2**, and *rac*-**3**. Alternatively, phosphorylation followed by alkylation with acetoxymethyl bromide gave the membrane-permeant derivatives 1-*O*-butyryl-2-deoxy-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*rac*-**5**), 3-*O*-butyryl-2-deoxy-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*ent*-**5**), and 2-*O*-butyryl-1-deoxy-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*rac*-**6**), respectively. We examined the potency of the membrane-permeant deoxy derivatives in inhibition of calcium-mediated chloride secretion (CaMCS) in intact T_{84} cells. Compared to the 1,2-di-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (**4**), the membrane-permeant derivative of Ins(3,4,5,6)P_4 (**1**), the 2-deoxy derivative (*rac*-**5**) exhibited a slightly weaker inhibitory effect, while the enantiomerically pure 2-deoxy-Ins(1,4,5,6)P_4 (*ent*-**5**) and the 1-deoxy derivative (*rac*-**6**) were inactive. As expected, the effect was stereoselective. Thus, the 1-hydroxyl group is apparently essential for binding and the inhibitory effect of Ins(3,4,5,6)P_4 on chloride secretion, whereas the 2-hydroxyl group plays a less important role.

Introduction

General recognition of the fundamental cellular role of several inositol-containing compounds[1,2] in signal transduction[3,4] has led to proliferation of biological[5,6] and chemical[7−9] studies aimed at unraveling the details of the complex actions of inositol polyphosphates. The ubiquitous second messenger *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] couples agonist stimulation of a variety of cell surface receptors to intracellular calcium mobilization.[10] Recently, particular attention has been focused on the more highly phosphorylated inositol polyphosphates.[11−14] While early studies were largely confined to describing their complex metabolism,[14] recent investigations have concentrated on elucidating their physiological functions.[12] Inositol tetrakisphosphates derived from InsP_5 have been a focal point,[15−17] fueled by the discovery that Ins(3,4,5,6)P_4 is responsible for the inhibition of calcium-mediated chloride secretion (CaMCS) in the intestinal epithelial cell line T_{84}.[18,19] The identification of this negative regulator was of special interest because chloride secretion [20,21] and resulting water flux across mucosal epithelia[22,23] are pathologically altered in cystic fibrosis and secretory diarrhea.

Although intracellular Ins(3,4,5,6)P_4 levels were shown to be increased following receptor stimulation,[18,19] probably due to an inhibitory effect of Ins(1,3,4)P_3 on the Ins(1,3,4,5,6)P_5 1-phosphatase/Ins(3,4,5,6)P_4 1-kinase equilibrium,[17,24] the membrane-permeant, bioactivatable Ins(3,4,5,6)P_4 derivative 1,2-*O*-Bt_2-Ins(3,4,5,6)P_4/AM (**4**) manifested the intracellular messenger function of Ins(3,4,5,6)P_4.[19] The treatment of T_{84} cells with **4** inhibited CaMCS without altering intracellular calcium levels.[19] Hence, the elevation of Ins(3,4,5,6)P_4 levels whether it was through muscarinic stimulation or addition of **4** resulted in an uncoupling of Cl^- secretion from intracellular calcium levels. At this point, we can only speculate as to the mechanism by which Ins(3,4,5,6)P_4 inhibits CaMCS. However, recent studies using the whole cell patch clamp technique with intracellular perfusion of inositol tetrakisphosphates indicated that Ins(3,4,5,6)P_4 directly modulated an apically located Ca^{2+}/calmodulin kinase-regulated Cl^- channel in T_{84} cells[25] and a Ca^{2+}-stimulated Cl^- current of CFPAC-1 cells, the latter being a cell line carrying a defect in the cystic fibrosis conductance regulator (CFTR).[26] Furthermore, Ca^{2+}-dependent Cl^- channels reconstituted in planar lipid bilayers were also regu-

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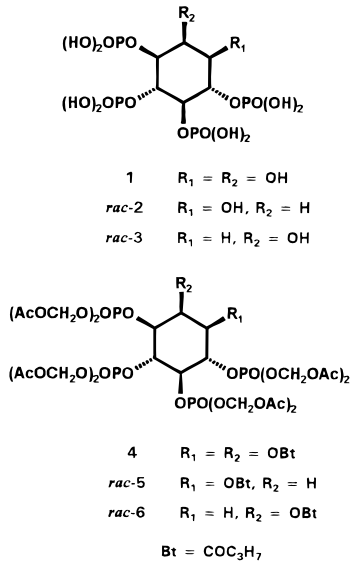


Figure 1. Structures of *myo*-inositol 3,4,5,6-tetrakisphosphate (**1**), 2-deoxy-Ins(3,4,5,6)P_4 (*rac*-**2**), 1-deoxy-Ins(3,4,5,6)P_4 (*rac*-**3**), and the membrane-permeant Ins(3,4,5,6)P_4 acetoxymethyl ester derivatives 1,2-*O*-Bt_2-Ins(3,4,5,6)P_4/AM (**4**), 1-*O*-Bt-2-deoxy-Ins(3,4,5,6)P_4/AM (*rac*-**5**), and 2-*O*-Bt-1-deoxy-Ins(3,4,5,6)-P_4/AM (*rac*-**6**). Numbering of *myo*-inositol derivatives refers to the D-configuration throughout this paper, exclusively. In case of racemic compounds only D-enantiomers are shown.

lated by low levels of Ins(3,4,5,6)P_4, suggesting that the binding site is closely associated with the chloride channel.[27]

Extending the use of membrane-permeant inositol phosphate derivatives, we are now aiming to use this methodology to characterize the unidentified Ins(3,4,5,6)-P_4-binding site(s). Deoxy derivatives proved to be useful analogues to study ligand/protein interaction in vitro in the past.[28−33] Therefore, we synthesized three membrane-permeant deoxy derivatives of Ins(3,4,5,6)P_4 (**1**): 1-*O*-butyryl-2-deoxy-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*rac*-**5**) [1-*O*-Bt-2-deoxy-Ins(3,4,5,6)P_4/AM], as a negative control, the enantiomerically pure Ins(1,4,5,6)P_4 derivative 3-*O*-butyryl-2-deoxy-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*ent*-**5**) [3-*O*-Bt-2-deoxy-Ins-(1,4,5,6)P_4/AM], and 2-*O*-butyryl-1-deoxy-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*rac*-**6**) [2-*O*-Bt-1-deoxy-Ins(3,4,5,6)P_4/AM] (Figure 1). Here we present the chemical synthesis and the effects of the compounds on CaMCS.

Results

Synthesis of 2-Deoxy-Ins(3,4,5,6)P_4 Derivatives. The Ins(3,4,5,6)P_4 analogue *rac*-2-deoxy-*myo*-inositol 3,4,5,6-tetrakisphosphate (*rac*-**2**) and its membrane-permeant derivative *rac*-1-*O*-butyryl-2-deoxy-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*rac*-**5**) were prepared from the common precursor *rac*-3,4,5,6-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol (*rac*-**7**).[34] Following the method of Garegg et al., the free hydroxyl group at the 2 position of the starting material was easily substituted by an iodo group with inversion at the reaction center (Scheme 1).[35] Free-radical dehalogenation[36] of *rac*-**8** at C-2 with AIBN/*n*-Bu_3SnH gave compound *rac*-**9**. Evidence of the dehalogenation was provided by the appearance of two complex signals for H-2_{ax} and H-2_{eq} in the 1.6−2.4 ppm region (Experimental Section). Deprotection of *rac*-**9** with DDQ[34] gave the alcohol *rac*-**10**. Esterification of the free hydroxyl group with butyric anhydride yielded *rac*-**11**, and subsequent removal of the benzyl groups by catalytic hydrogenolysis gave the desired 1-*O*-butyryl-2-deoxy-*myo*-inositol (*rac*-**12**) in 34% yield from *rac*-**7**.

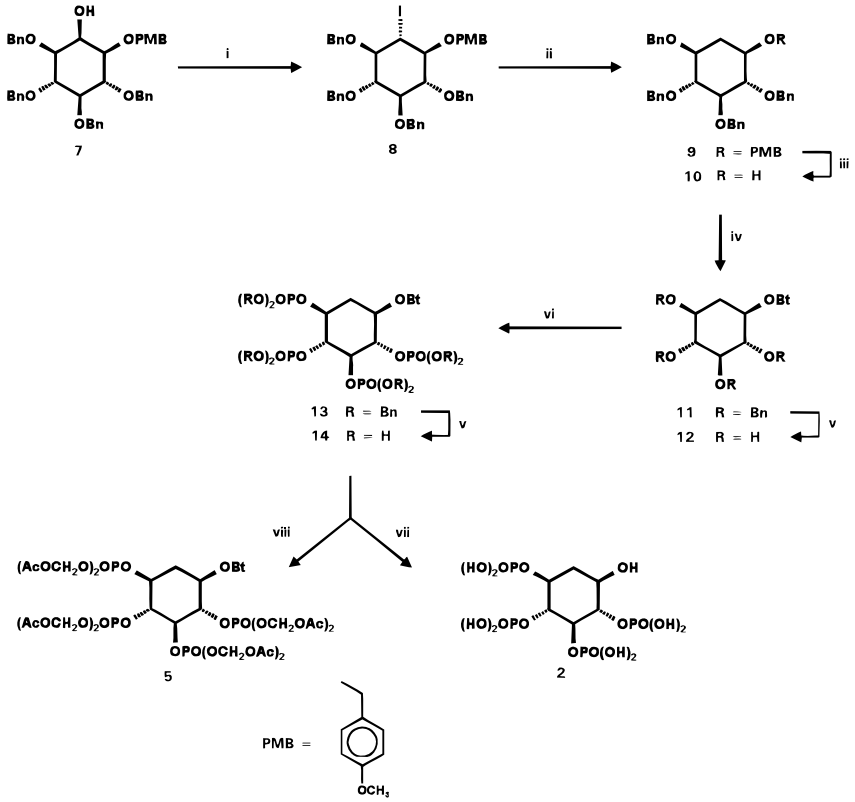
Phosphorylation was accomplished using dibenzyl *N*,*N*-diisopropylphosphoramidite,[37] followed by oxidation with peracetic acid to give the fully protected tetrakisphosphate derivative *rac*-**13** in 71% yield after purification by preparative HPLC (50 × 250 mm, RP-18, 10 μm) with 92% methanol as the eluent. Deprotection by catalytic hydrogenolysis yielded *rac*-1-*O*-butyryl-2-deoxy-Ins(3,4,5,6)P_4 (*rac*-**14**) as the free acid. The butyrate was removed by treatment with 1 M KOH (pH 12.8) to yield *rac*-2-deoxy-Ins(3,4,5,6)P_4 (*rac*-**2**). Alternatively, acid *rac*-**14** was alkylated with acetoxymethyl bromide (AMBr) in the presence of diisopropylethylamine (DIEA) to give the uncharged, bioactivatable octakis(acetoxymethyl) ester *rac*-**5**. The same synthetic sequence was repeated, starting from 1,4,5,6-tetra-*O*-benzyl-3-*O*-(4-methoxybenzyl)-*myo*-inositol (*ent*-**7**) which was prepared from enantiomerically pure 1,4,5,6-tetra-*O*-benzyl-*myo*-inositol.[34] The absolute configuration was confirmed by comparing the optical rotation value of the intermediate 1,4,5,6-tetra-*O*-benzyl-2-deoxy-*myo*-inositol (*ent*-**10**) to the published data.[38]

Synthesis of 1-Deoxy-Ins(3,4,5,6)P_4 Derivatives. The synthesis of 1-deoxy compounds required a slightly different approach since dehalogenation of an axial iodo group in the 1 position failed. Therefore, the 1-hydroxyl group of *rac*-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (*rac*-**15**) was regioselectively esterified with equimolar amounts of *O*-phenyl chlorothionoformate in dry pyridine in the presence of DMAP to give *rac*-**16** (Scheme 2). Higher amounts of *O*-phenyl chlorothionoformate or longer reaction times resulted in the formation of a 1,2-cyclic thiocarbonate. The structure of the latter was verified through the reaction of *rac*-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (*rac*-**15**) with thiophosgen[39] in pyridine/DMAP which gave the identical compound.

The remaining 2-OH group of *rac*-**16** was esterified with butyric anhydride, and subsequently the reduction of the fully protected phenoxythiocarbonyl compound *rac*-**17** with tributyltin hydride utilizing Barton's methodology[36] yielded the 1-deoxy derivative *rac*-**18**. The benzyl groups were removed by catalytic hydrogenolysis to form the tetrol *rac*-**19**. Phosphorylation to the fully protected tetrakisphosphate *rac*-**20** proceeded as described above. Hydrogenolysis afforded compound *rac*-**21**, which was treated with KOH (pH 12.8), followed by ion-exchange chromatography to give *rac*-2-*O*-butyryl-1-deoxy-Ins(3,4,5,6)P_4 (*rac*-**3**) as the free acid. Compound *rac*-**21** was alkylated with AMBr, as described above, to give the octakis(acetoxymethyl) ester *rac*-**6**.

Biochemistry. We have previously shown that exposure of T_{84} cells to the racemic mixture of 400 μM 1,2-di-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester [1,2-*O*-Bt_2-Ins(3,4,5,6,)P_4/AM] extracellularly corresponded to an intracellular concentration of 4 μM Ins(3,4,5,6)P_4 which reduced the thapsigargin-induced Cl^- secretion by approximately

Scheme 1[a]



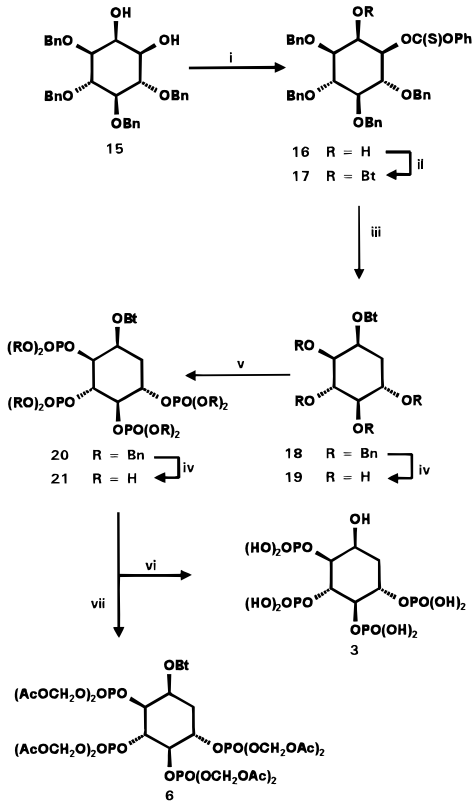
[a] Reagents and conditions: (i) I_2, imidazole, $(Ph)_3P$, toluene; (ii) *n*-Bu_3SnH, AIBN, toluene; (iii) DDQ, $CH_2Cl_2-H_2O$; (iv) Bt_2O, DMAP, pyridine; (v) H_2, Pd/C (10%), AcOH; (vi) a. $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, MeCN, b. − 40 °C, AcOOH; (vii) 1 M KOH, pH 12.8; (viii) AMBr, DIEA, MeCN.

40%. In contrast, 200 μM enantiomerically pure 2,3-*O*-Bt_2-Ins(1,4,5,6)P_4/AM was without effect.[19]

Here we demonstrate for the first time the ability of Ins(3,4,5,6)P_4 to inhibit carbachol-induced CaMCS by incubation with enantiomerically pure 1,2-*O*-Bt_2-Ins-(3,4,5,6,)P_4/AM (**4**).[34] Cells were grown to confluence on Snap-wells and mounted in modified Ussing chambers as described before.[40] For these experiments, T_{84} cells were pretreated with 200 μM 1,2-*O*-Bt_2-Ins(3,4,5,6)P_4/AM (**4**) for 30 min at 37 °C. CaMCS was stimulated by carbachol, a muscarinic receptor agonist which mobilizes intracellular calcium through a phospholipase C-dependent pathway. Preincubation of the monolayers with 1,2-*O*-Bt_2-Ins(3,4,5,6)P_4/AM (**4**) inhibited CaMCS by up to 50% (Figures 2 and 3).

To investigate the properties of the two deoxy Ins-(3,4,5,6)P_4 derivatives, *rac*-1-*O*-Bt-2-deoxy-Ins(3,4,5,6)-P_4/AM (*rac*-**5**) and *rac*-2-*O*-Bt-1-deoxy-Ins(3,4,5,6)P_4/AM (*rac*-**6**), on carbachol-mediated chloride secretion in T_{84} cells, short circuit current (I_{sc}) was measured after preincubation with these derivatives. As shown in Figure 2, only pretreatment with *rac*-1-*O*-Bt-2-deoxy-Ins(3,4,5,6)P_4/AM (*rac*-**5**) inhibited CaMCS, resulting in a 28% reduction of I_{sc} in response to carbachol, whereas pretreatment with *rac*-2-*O*-Bt-1-deoxy-Ins(3,4,5,6)P_4/AM (*rac*-**6**) was without effect. Inhibition was maximal at a concentration of 400 μM *rac*-**5**, while 200 μM was ineffective ($n = 3$, data not shown) and 800 μM ($n = 3$, data not shown) showed no increased potency. Because the 2-deoxy-Ins(3,4,5,6)P_4 derivative (*rac*-**5**) consists of a racemic mixture of Ins(3,4,5,6)P_4/Ins(1,4,5,6)P_4 derivatives and to confirm that the inhibition was enantiose-

Scheme 2[a]



[a] Reagents and conditions: (i) PhOC(S)Cl, DMAP, pyridine; (ii) Bt_2O, DMAP, pyridine; (iii) *n*-Bu_3SnH, AIBN, toluene; (iv) H_2, Pd/C (10%), AcOH; (v) a. $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, MeCN, b. −40 °C, AcOOH; (vi) 1 M KOH, pH 12.8; (vii) AMBr, DIEA, MeCN.

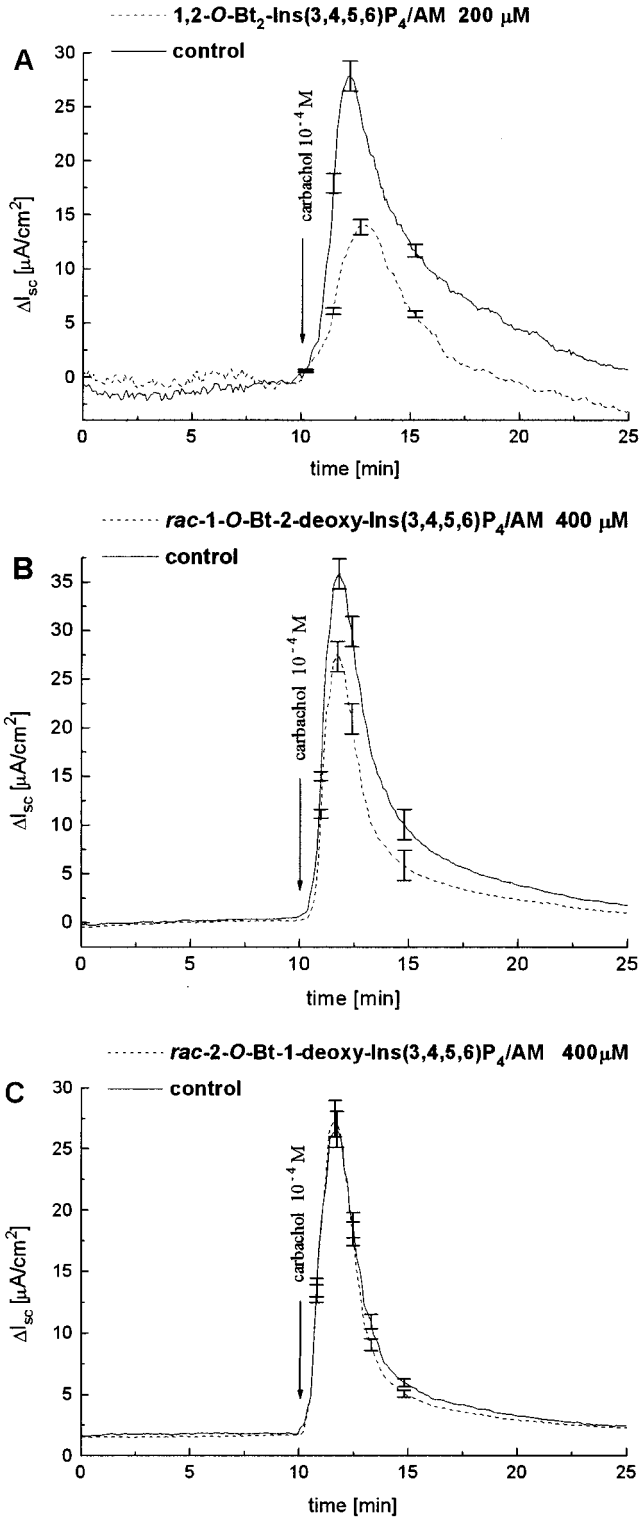


Figure 2. Time course of CaMCS inhibition by membrane-permeant Ins(3,4,5,6)P_4 derivatives. T_{84} monolayers were incubated for 30 min with (A) **4**, (B) *rac*-**5**, or (C) *rac*-**6** or vehicle (DMSO/5% Pluronic) prior to mounting into Ussing chambers. Cl^- secretion was measured as short circuit current (I_{sc}). Carbachol (10^{-4} M) was added to the basolateral side to induce CaMCS. Data reflect monitoring every 4 s and are the average of 6 experiments.

lective, we synthesized the enantiomerically pure derivative 3-*O*-Bt-2-deoxy-Ins(1,4,5,6)P_4/AM (*ent*-**5**). As expected, extracellular application of 200 μM *ent*-**5** did not inhibit CaMCS (Figure 3), showing that the effect of *rac*-**5** is due to the Ins(3,4,5,6)P_4 enantiomer.

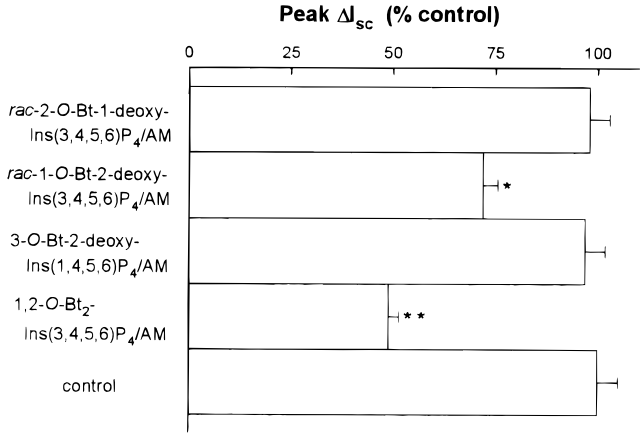


Figure 3. Inhibition of CaMCS by membrane-permeant deoxy $InsP_4$ derivatives compared to 1,2-*O*-Bt_2-Ins(3,4,5,6)P_4/AM (**4**). Monolayers were preincubated with the indicated membrane-permeant tetrakisphosphate acetoxymethyl ester for 30 min prior to mounting in the Ussing chamber. CaMCS was stimulated with carbachol (10^{-4} M). Data are mean peak I_{sc} SEM expressed as percent control for 4−6 experiments. Control values represent the response to carbachol in coincubated monolayers. Significant differences are denoted by probability values derived by Student's 2-tailed *t*-test (*p < 0.02; **p < 0.04). Concentrations of the $InsP_4$ derivatives were 1,2-*O*-Bt_2-Ins(3,4,5,6)P_4/AM (200 μM) (**4**), 1-*O*-Bt-2-deoxy-Ins-(3,4,5,6)P_4/AM (400 μM) (*rac*-**5**), 3-*O*-Bt-2-deoxy-Ins(1,4,5,6)-P_4/AM (200 μM) (*ent*-**5**), and 2-*O*-Bt-1-deoxy-Ins(3,4,5,6)P_4/AM (400 μM) (*rac*-**6**), respectively.

The lack of a biological effect of *rac*-2-*O*-Bt-1-deoxy-Ins(3,4,5,6)P_4/AM (*rac*-**6**) does not necessarily reflect the potential binding properties to the binding protein(s). Therefore we investigated whether *rac*-**6** would compete with naturally produced Ins(3,4,5,6)P_4 after carbachol stimulation. To analyze this potential antagonistic effect, *rac*-**6** was assayed for the reversal of the reported Ins(3,4,5,6)P_4-induced inhibition of Cl^- secretion by thapsigargin.[19] Cells were preincubated with *rac*-2-*O*-Bt-1-deoxy-Ins(3,4,5,6)P_4/AM (*rac*-**6**) for 30 min, mounted in Ussing chambers, and then treated with carbachol which induces transient Cl^- secretion followed by prolonged elevation of Ins(3,4,5,6)P_4 levels.[19] After 30 min thapsigargin was added to stimulate a second cycle of $[Ca^{2+}]_i$ elevation and subsequent chloride secretion. No significant differences in thapsigargin-induced I_{sc} were observed in *rac*-**6**-pretreated monolayers compared to controls (n = 4, data not shown) indicating that the 1-deoxy compound *rac*-**6** did not reverse the inhibition of CaMCS by physiologically mediated elevation of Ins-(3,4,5,6)P_4 levels. Therefore, *rac*-**6** does not inhibit CaMCS and thus is unable to bind to the Ins(3,4,5,6)-P_4-binding protein(s).

Finally, neither compound had any effect on $[Ca^{2+}]_i$ levels or on thapsigargin-induced elevation of Ca^{2+} levels in T_{84} cells, as was verified by continuous Fura-2 calcium ratio imaging for more than 30 min after incubation with the membrane-permeant inositol polyphosphate derivatives (data not shown).

Discussion

Recent confirmation that Ins(3,4,5,6)P_4 is an important intracellular negative regulator of CaMCS in epithelial cells[19,21,25−27] points out the need to characterize the intracellular target proteins. The latter should

be interesting targets for drug design, especially with respect to diseases where Cl^- secretion is pathological, e.g., in cystic fibrosis or secretory diarrhea. Agonists which mimic the Ins(3,4,5,6)P_4-mediated inhibition of CaMCS would result in a decrease in Cl^- secretion, while antagonists of Ins(3,4,5,6)P_4 would be expected to augment CaMCS. Unfortunately, no Ins(3,4,5,6)P_4-binding protein has yet been identified. However, using CaMCS as a readout, the availability of membrane-permeant derivatives of inositol polyphosphates now enables us to characterize the unidentified binding sites in their natural environment.

In principle, Ins(3,4,5,6)P_4 offers two target groups for systematic modification of its structure and therefore its molecular interaction potential: (i) the hydroxyl groups and (ii) the phosphates. Because previous experiments showed that proper stereochemistry and orientation of the phosphates were essential for the physiological function,[25−27] we modified the interaction potential of the hydroxyl groups by synthesizing deoxy derivatives. Usually hydroxyl groups serve as either hydrogen-bonding acceptors or donors or both. Replacing the hydroxyl groups with hydrogen atoms results in complete loss of the hydrogen bond interaction properties. From the biological data in this study, we can conclude that the hydroxyl group at the 2 position exhibits a relatively minor role in the recognition of Ins(3,4,5,6)P_4 by its unidentified target protein in T_{84} cells. On the other hand, the lack of an effect of the 1-deoxy compound *rac*-**6** indicates that the hydroxyl group at position 1 is critical. Thus, the 1-hydroxyl group of Ins(3,4,5,6)P_4 may be engaged in essential hydrogen-bonding interactions with the appropriate amino acid residues of its target, while the 2-hydroxyl group appears to be less involved. Nevertheless, the biological activity of *rac*-**5** was 50% of that of the membrane-permeant Ins(3,4,5,6)P_4 derivative **4**, and the effect could not be increased by raising the concentration. Therefore the 2-deoxy compound *rac*-**5** represents only a partial agonist, perhaps due to discrimination between the different binding sites postulated by Xie et al.[25]

The method described here for mapping proteins in living cells may be a promising approach for future investigations of inositol phosphate/protein interactions. Classical mapping approaches have been carried out in cell-free systems relying on highly purified proteins. This has been especially problematic when working with membrane-bound proteins or proteins which are modifiable by a number of solute cofactors present in cells. Although a higher number of membrane-permeant structurally modified Ins(3,4,5,6)P_4 derivatives would be desirable, the method used in the present study provides an alternative approach which enables us to elucidate the binding character of both identified and unidentified proteins in their natural environment. A prerequisite for this approach is the conversion of the membrane-impermeant inositol phosphates or one of its derivatives into the membrane-permeant, bioactivatable AM ester. These and previous studies demonstrated that the acyloxymethyl ester method is effective for delivering inositol phosphates to the cytosol of intact cells without perturbing the plasma membrane[19,41,42] and with minimal interference of other intracellular pathways.

In summary, the extracellular application of membrane-permeant derivatives of inositol 3,4,5,6-tetrakisphosphate provides a technique for in vivo mapping of Ins(3,4,5,6)P_4-binding proteins in T_{84} cells. Using this methodology we established that the role of the 2-hydroxyl group in ligand recognition is less important but that the role of the 1-hydroxyl group is essential. This information may aid in the search for drug candidates for the treatment of cystic fibrosis or secretory diarrhea.

Experimental Section

All chemical reagents were obtained in the highest purity available. Where necessary, solvents were dried and/or distilled before use. Acetonitrile was distilled from phosphorus(V) oxide and stored over molecular sieves (3 Å), as was dimethylformamide (DMF). Pyridine and toluene were stored over molecular sieves (4 Å). Diisopropylethylamine (DIEA) was dried over sodium wire. Palladium on charcoal (10%), iodine, and tri-*n*-butyltin hydride (*n*-Bu_3SnH) were from Acros Chemie. Dibenzyl *N*,*N*-diisopropylphosphoramidite, tetrazole, peracetic acid (32% v/w), dibutyltin oxide, α,α′-azoisobutyronitrile (AIBN), and acetoxymethyl bromide were from Aldrich. Butyric anhydride and DIEA were from Merck. 4-(Dimethylamino)pyridine (DMAP), cesium fluoride (CsF), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and *O*-phenyl chlorothionoformate were from Fluka. The ion-exchange resin Dowex 50 WX 8, H^+-form, was from Serva, Heidelberg.

Thapsigargin was purchased from Alexis Biochemicals, La Jolla, CA. Carbachol was obtained from ICN, Irvine, CA. Fura 2-acetoxymethyl ester (Fura-2/AM) was purchased from Calbiochem, La Jolla, CA. Pluronic 127 was a kind gift of BASF, Germany. Cell culture membrane inserts (Snapwell, 0.4 μm pore size polycarbonate) were obtained from Corning Costar Corp. (Cambridge, MA).

HPLC was performed on a LDC/Milton Roy Constametric III pump with a LDC/Milton Roy UV monitor D (254 nm) or a Knauer refractive index detector. The analytical column was a Merck Hibar steel tube (250 mm × 4 mm) filled with RP 18 material (Merck, LiChrosorb; 10 μm). Preparative HPLC was performed using a Shimadzu LC 8A pump with a preparative LDC UV III monitor (254 nm) or a Knauer refractive index detector and a Merck Prepbar steel column (250 mm × 50 mm) filled with RP 18 material (Merck, LiChrospher 100; 10 μm). The eluents were methanol−water mixtures; compositions are given in % methanol (MeOH).

^1H and ^{31}P NMR spectra were recorded on a Brucker AM 360 spectrometer. Chemical shifts were measured in ppm relative to tetramethylsilane for ^1H NMR spectra and external 85% H_3PO_4 for ^{31}P NMR spectra. *J* values are given in Hz. Mass spectra were recorded using a Finnigan Mat 8222 mass spectrometer with fast atom bombardment (FAB) ionization. High-resolution masses were determined relative to known compounds with a mass not differing more than 10%. Optical rotations were measured at the sodium D-line in a 10-cm cell with a Perkin-Elmer 1231 polarimeter. Meltings points (uncorrected) were determined using a Büchi B-540 apparatus. Ultrafiltration of the palladium/charcoal catalyst was performed with a Sartorius filtration apparatus SM 162 01 using filters from regenerated cellulose (Sartorius, SM 116 04). Elemental analyses were performed by Mikroanalytisches Labor Beller, Göttingen, Germany.

rac-3,4,5,6-Tetra-*O*-benzyl-*myo*-inositol (*rac*-**15**), *rac*-3,4,5,6-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol (*rac*-**7**), and enantiomerically pure 1,4,5,6-tetra-*O*-benzyl-*myo*-inositol (*ent*-**15**) were synthesized as described before.[13,34] *ent*-**15** had an optical rotation value of $[\alpha]^{24}_D$ +19 (c = 1.05 in $CHCl_3$) [lit.[43] $[\alpha]^{24}_D$ +23.4 (c = 4.5 in $CHCl_3$)]. The synthesis of enantiomerically pure 2-deoxy-*myo*-inositol 1,4,5,6-tetrakisphosphate (*ent*-**2**) and 3-*O*-butyryl-2-deoxy-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*ent*-**5**) followed the pathway for the racemic compounds. All analytical data were in accordance with those obtained for the racemic compounds.

Cell Culture. All studies were performed using monolayers of the T_{84} cell line and cells from passage numbers 20−29 only. Methods for the maintenance of T_{84} cells for use in transepthelial electrolyte transport studies have been described previously.[18] In brief, T_{84} cells were grown in Dulbecco's modified Eagle's/F12 media (JRH, Lenexa, KS) supplemented with 5% newborn calf serum (Hyclone, Logan, UT) and 50 U/mL each of penicillin/streptomycin (Core Cell Culture Facility, UCSD). Medium was replaced every third day. Cells were passaged by trypsinization. For the measurement of chloride secretion, 10^6 cells were seeded onto microporous filter inserts (see above) and maintained for 8−12 days prior to experiments in order to develop confluent monolayers with stable transepithelial resistance.

Chloride Secretion. Chloride secretion was measured as short circuit current (I_{sc}) across monolayers of T_{84} cells, mounted in modified Ussing chambers (Physiologic Instruments, San Diego, CA), bathed with Ringer solution warmed to 37 °C and gassed continuously with 95% O_2/5% CO_2 at a rate of 30−35 mL/min. The spontaneous potential difference across the monolayer was short-circuited with a voltage clamp (model VCC MC6, Physiologic Instruments, San Diego, CA). Short circuit current (I_{sc}) and conductance were recorded at 4-s intervals using Acquire and Analyze Software 1.2 (Physiologic Instruments, San Diego, CA). Increased I_{sc} values stimulated through cholinergic pathways or through calcium-mobilizing agonists in T_{84} cells are wholly reflective of Cl^- secretion.[40,44] Ringer's solution contained (in mM) 140 Na^+, 5.2 K^+, 1.2 Ca^{2+}, 0.8 Mg^{2+}, 119.8 Cl^-, 25 HCO_3^-, 2.4 $H_2PO_4^-$, 0.4 HPO_4^-, and 10 glucose.

Priming of T_{84} Cells with Membrane-Permeant $InsP_4$ Derivatives. Confluent T_{84} cells on Snap-well inserts were rinsed with 1 mL of Ringer's solution on each side of the Snap-well; 100 μL of Ringer's solution containing inositol tetrakisphosphate octakis(acetoxymethyl) ester and 2 μL of DMSO/5% Pluronic 127 was added to the basolateral reservoir. The cells were incubated at 37 °C for 30 min. The incubation mixture was discarded, and cells were washed with Ringer's solution (1 mL) and were ready for mounting. Cells for control experiments were incubated only with 2 μL of DMSO/5% Pluronic 127.

D-1,4,5,6-Tetra-*O*-benzyl-3-*O*-(4-methoxybenzyl)-*myo*-inositol (*ent*-7). Dry *ent*-**15** (2.7 g, 5 mmol) and dry dibutyl tin oxide (1.26 g, 5.06 mmol) were heated to reflux in dry toluene (150 mL) in a Soxhlet apparatus filled with activated molecular sieves (3 Å) for 4 h. The reaction mixture was cooled to room temperature and evaporated to dryness under diminished pressure. CsF (1.5 g, 9.9 mmol) was added to the residual oil, and the mixture was kept under high vacuum for 2 h. The residual syrup was dissolved in dry DMF (20 mL) under argon, and 4-methoxybenzyl chloride (6 mL, 40 mmol) was added. After the solution stirred for 5 h at 60 °C, HPLC analysis (90% MeOH; 1.5 mL/min; t_R = 5.30 min) showed no further reaction. Excess of 4-methoxybenzyl chloride and DMF were removed in high vacuum. The crude product was chromatographed by preparative HPLC (90% MeOH; 37.5 mL/min; t_R = 35.00 min) to give compound *ent*-**7** (2.6 g, 78%) as a solid. Mp: 127 °C (from methanol) (lit.[34] mp 128−129 °C). $[\alpha]^{24}_D$ +1.7 (c = 1.2 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 360 MHz): 3.37 (1 H, dd, J = 9.54, 2.73, H-1), 3.40 (1 H, dd, J = 9.50, 2.73 Hz, H-3), 3.48 (1 H, dd, J = 9.52, 9.52 Hz, H-5), 3.83 (3 H, s, OMe), 3.99 (1 H, dd, J = 9.54, 9.52 Hz, H-6), 4.03 (1 H, dd, J = 9.52, 9.50 Hz, H-4), 4.22 (1H, dd, J = 2.73, 2.73 Hz, H-2), 4.68 (2H, s, CH_2 in PMB), 4.77−4.98 (8 H, m, 4 × CH_2-Ph), 6.69 (2 H, d, PMB ArH), 7.28−7.43 (22 H, m, 4 × CH_2*Ph* and PMB ArH). MS: *m*/*z* (−ve ion FAB) 659 [(M − H^+)$^-$, 100], 569 [M − Bn^+, 15].

***rac*-3,4,5,6-Tetra-*O*-benzyl-2-deoxy-2-iodo-1-*O*-(4-methoxybenzyl)-*scyllo*-inositol (*rac*-8).** A mixture of dry *rac*-**7** (1.32 g, 2 mmol), triphenylphosphine (2.15 g, 8.2 mmol), imidazole (549 mg, 8.2 mmol), and iodine (1.52 g, 6 mmol) was stirred under reflux in dry toluene (100 mL) for 41 h. The reaction mixture was cooled to room temperature, saturated aqueous sodium hydrogen carbonate (100 mL) was added, and the mixture was stirred for 10 min. Iodine was added in portions until the toluene phase remained iodine-colored and was stirred for an additional 15 min. Excess of iodine was removed by the addition of aqueous sodium dithionite solution. The organic and the aqueous phase were separated, and the organic phase was washed with brine twice, dried over Na_2SO_4, and filtered. Evaporation of the solvent and crystallization from methanol gave pure *rac*-**8** (1.01 g, 73%). Mp: 131.5−132.4 °C (from methanol). ^{1}H NMR ($CDCl_3$, 360 MHz): 3.49 (1 H, dd, J = 9.27, 9.27 Hz, H-4), 3.50 (1 H, dd, J = 9.27, 9.27 Hz, H-6), 3.61 (1H, dd, J = 9.50, 9.50 Hz, H-5), 3.64−3.67 (2 H, m, H-1, H-3), 3.80 (3 H, s, OMe), 4.06 (1 H, dd, J = 11.08, 11.08 Hz, H-2), 4.82−4.92 (10 H, m, 4 × CH_2Ph and CH_2 in PMB), 6.87 (2 H, d, PMB ArH), 7.23−7.37 (22 H, m, 4 × CH_2*Ph* and PMB ArH). MS: *m*/*z* (+ve ion FAB) 924 [(M + NBA + H)$^+$, 1], 121 [PMB^+, 100]. Anal. ($C_{42}H_{43}O_6I$) C: calcd, 65.45; found, 65.89. H: calcd, 5.62; found, 5.72.

D-1,4,5,6-Tetra-*O*-benzyl-2-deoxy-2-iodo-3-*O*-(4-methoxybenzyl)-*scyllo*-inositol (*ent*-8). Compound *ent*-**7** (1.18 g, 1.8 mmol) was halogenated in the same way as described for *rac*-**8** to give the title compound *ent*-**8** (949 mg, 68%) as a white solid. Mp: 132−133 °C (from methanol). $[\alpha]^{24}_D$ −14.7 (c = 0.92 in $CHCl_3$). ^{1}H NMR and MS data were in accordance with those obtained for *rac*-**8**.

***rac*-3,4,5,6-Tetra-*O*-benzyl-2-deoxy-1-*O*-(4-methoxybenzyl)-*myo*-inositol (*rac*-9).** Compound *rac*-**8** was dissolved in dry toluene (150 mL), and AIBN (63 mg, 355 mol) and *n*-Bu_3-SnH (1.63 mL, 6.3 mmol) were added. The solution was heated under an argon atmosphere for 2 h. The reaction mixture was cooled and then washed once with phosphate buffer (30 mL) and once with brine (30 mL). The organic layer was dried over Na_2SO_4 and filtered. Evaporation of the solvent and crystallization from methanol gave *rac*-**9** (692 mg, 76%). Mp: 76.2−76.8 °C (from methanol). ^{1}H NMR ($CDCl_3$, 360 MHz): 1.64 (1 H, ddd, J = 12.73, 12.73, 12.73 Hz, H-2 (ax)), 2.39 (1 H, ddd, J = 12.73, 4.39, 4.39 Hz, H-2 (eq)), 3.39−3.46 (2 H, m, H-1, H-3), 3.46 (1 H, dd, J = 9.22, 8.87 Hz, H-5), 3.55 (1 H, dd, J = 9.22, 9.22 Hz, H-4), 3.56 (1 H, dd, J = 9.22, 9.22 Hz, H-6), 3.81 (3 H, s, OMe), 4.60−4.94 (10 H, m, 4 × CH_2Ph and CH_2 in PMB), 6.86 (2 H, d, PMB ArH), 7.24−7.33 (22 H, m, 4 × CH_2*Ph* and PMB ArH). MS: *m*/*z* (−ve ion FAB) 643 [(M − H^+)$^-$, 1], 523 [(M − PMB^+)$^-$, 100]. Anal. ($C_{42}H_{44}O_6$) C: calcd, 78.23; found, 77.78. H: calcd, 6.88; found, 6.78.

D-1,4,5,6-Tetra-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-*myo*-inositol (*ent*-9). Compound *ent*-**8** (837 mg, 1.11 mmol) was dehalogenated as decribed for *rac*-**9** to give the title compound *ent*-**9** (571 mg, 80%) as a white solid. Mp: 75.8−76.3 °C (from methanol). $[\alpha]^{24}_D$ −3.5 (c = 0.88 in $CHCl_3$). ^{1}H NMR and MS data were in accordance with those obtained for *rac*-**9**.

***rac*-3,4,5,6-Tetra-*O*-benzyl-2-deoxy-*myo*-inositol (*rac*-10).** DDQ (367 mg, 1.62 mmol) was added to a solution of *rac*-**9** (600 mg, 931 μmol) in CH_2Cl_2 (10 mL) containing small amounts of water (5%). After the suspension was stirred at room temperature for 28 min, HPLC analysis (90% MeOH; 1.5 mL/min; t_R = 4.27 min) showed the reaction to be complete. The solvent was evaporated under reduced pressure, and the reaction mixture was purified by preparative HPLC (90% MeOH; 40 mL/min; t_R 20.00 min) to give *rac*-**10** (314 mg, 65%) as a solid. Mp: 126.7 °C (from methanol) [lit.[32] mp 121−122 °C]. ^{1}H NMR ($CDCl_3$, 360 MHz): 1.64 (1 H, ddd, J = 12.73, 12.73, 12.73 Hz, H-2 (ax)), 2.39 (1 H, ddd, J = 12.73, 4.39, 4.39 Hz, H-2 (eq)), 3.39−3.46 (2 H, m, H-1, H-3), 3.46 (1 H, dd, J = 9.22, 8.87 Hz, H-5), 3.55 (1 H, dd, J = 9.22, 9.22 Hz, H-4), 3.56 (1 H, dd, J = 9.22, 9.22 Hz, H-6), 3.81 (3 H, s, OMe), 4.60−4.94 (10 H, m, 4 × CH_2Ph and CH_2 in PMB), 6.86 (2 H, d, PMB ArH), 7.24−7.33 (22 H, m, 4 × CH_2*Ph* and PMB ArH). MS: *m*/*z* (+ve ion FAB) 525 [(M + H)$^+$, 1], 91 [Bn^+, 100].

D-1,4,5,6-Tetra-*O*-benzyl-2-deoxy-*myo*-inositol (*ent*-10). The 4-methoxybenzyl group of *ent*-**9** (540 mg, 873 μmol) was removed as described for compound *rac*-**10** to give *ent*-**10** (315 mg, 69%) as a solid. Mp: 126 °C (from methanol) [lit.[32] mp 121−122 °C]. $[\alpha]^{24}_D$ −20.1 (c = 0.68 in $CHCl_3$) [lit.[38] $[\alpha]^{24}_D$ −24

(c = 2 in $CHCl_3$)]. ^{1}H NMR and MS data were in accordance with those obtained for *rac*-**10**.

***rac*-3,4,5,6-Tetra-*O*-benzyl-1-*O*-butyryl-2-deoxy-*myo*-inositol (*rac*-11).** A solution of *rac*-**10** (186 mg, 355 μmol), butyric anhydride (70 μL, 426 mol), and DMAP (12 mg, 10 μmol) in dry pyridine (5 mL) was stirred at room temperature for 18 h. The solvent was evaporated under high vacuum to give an oil. Residual pyridine was removed by evaporating three times with octane. The residue was dissolved in *tert*-butyl methyl ether (30 mL) and was washed with phosphate buffer (20 mL), with sodium hydrogen carbonate (20 mL), with sodium hydrogen sulfate (20 mL), again with phosphate buffer (20 mL), and finally with brine (20 mL). The organic layer was dried over Na_2SO_4 and filtered. Evaporation of the solvent and crystallization from methanol gave pure *rac*-**11** (196 mg, 94%) as needles. Mp: 110.1−110.3 °C (from methanol). ^{1}H NMR ($CHCl_3$, 360 MHz): 0.93 (3 H, t, J = 7.37 Hz, CH_3), 1.45 (1 H, ddd, J = 12.25, 12.25, 12.25 Hz, H-2 (ax)), 1.56−1.67 (2 H, m, $-CH_2$), 2.12−2.28 (2 H, m, $-CH_2$), 2.42 (1 H, dd, J = 12.25, 4.35, 4.35 Hz, H-2 (eq)), 3.50−3.61 (4 H, m, H-3, H-4, H-5, H-6), 4.61−4.95 (9H, m, CH_2Ph, H-1), 7.23−7.37 (20 H, m, CH_2Ph). MS: m/z (+ve ion FAB) 595 [$(M + H)^+$, 2], 91 [Bn^+, 100]. Anal. ($C_{38}H_{42}O_6$) C: calcd, 76.74; found, 76.87. H: calcd, 7.12; found, 7.31.

D-1,4,5,6-Tetra-*O*-benzyl-3-*O*-butyryl-2-deoxy-*myo*-inositol (*ent*-11). Compound *ent*-**10** (169 mg, 322 μmol) was butyrylated as described for *rac*-**11** to give the fully protected inositol *ent*-**11** (183 mg, 96%) as a solid. Mp: 110.4−111.3 °C (from methanol). $[\alpha]^{24}_D$ −14.0 (c = 1.18 in $CHCl_3$). ^{1}H NMR and MS data were in accordance with those obtained for *rac*-**11**.

***rac*-1-*O*-Butyryl-2-deoxy-*myo*-inositol (*rac*-12).** Compound *rac*-**11** (177 mg, 297 mol) was suspended in acetic acid (4 mL) and hydrogenated with palladium (10%) on charcoal (178 mg, 1.78 mmol) under a hydrogen atmosphere in a self-built hydrogenation apparatus for 6 h. The catalyst was removed by ultrafiltration, and the filtrate was freeze-dried to give tetrol *rac*-**12** (68 mg, 98%) as a solid. Mp: 174.9−175.9 °C (from ethanol). ^{1}H NMR (DMSO-d_6, 360 MHz): 0.87 (3 H, t, J = 7.25 Hz, CH_3), 1.24 (1 H, ddd, J = 12.37, 12.37, 12.37 Hz, H-2 (ax)), 1.48−1.58 (2 H, m, $-CH_2$), 1.90 (1 H, ddd, J = 12.37, 4.21, 4.21 Hz, H-2 (eq)), 2.26 (1 H, t, J = 7.25, $-CH_2$), 2.93−3.02 (2 H, m, H-3, H-5), 3.17−3.30 (2 H, m, H-4, H-6), 4.52 (1 H, ddd, J = 12.37, 9.59, 4.21 Hz, H-1), 4.79 (1 H, d, J = 4.01 Hz, OH), 4.81 (1 H, d, J = 3.90 Hz, OH), 4.86 (1 H, d, J = 3.90 Hz, OH), 4.94 (1 H, d, J = 4.01 Hz, OH). MS: m/z (+ve ion FAB) 235 [$(M + H)^+$, 100]. MS: m/z (−ve ion FAB) 233 [$(M - H^+)^-$, 28], 87 [BtO^-, 100]. Anal. ($C_{10}H_{18}O_6$) C: calcd, 51.27; found, 51.09. H: calcd, 7.75; found, 7.57.

D-3-*O*-Butyryl-2-deoxy-*myo*-inositol (*ent*-12). Compound *ent*-**11** (170 mg, 286 μmol) was hydrogenated as described for *rac*-**12** to give tetrol *ent*-**12** (65 mg, 97%) as a solid. Mp: 174−174.8 °C (from ethanol). $[\alpha]^{24}_D$ +25.8 (c = 1.39 in MeOH). ^{1}H NMR and MS data were in accordance with those obtained for *rac*-**12**.

***rac*-1-*O*-Butyryl-2-deoxy-*myo*-inositol 3,4,5,6-Tetrakis-(dibenzyl)phosphate (*rac*-13).** A solution of tetrol *rac*-**12** (44 mg, 190 μmol) and tetrazole (160 mg, 2.28 mmol) in acetonitrile (2 mL) was treated with dibenzyl *N*,*N*-diisopropylphosphoramidite (767 μL, 2.28 mmol) for 36 h and subsequently oxidized with peracetic acid (153 μL, 2.28 mmol) at −40 °C. After the mixture warmed to room temperature, the solvent was removed under reduced pressure and the residual oil was purified by preparative HPLC (92% MeOH; 40 mL/min; t_R = 21.55 min) to give the fully protected compound *rac*-**13** (172 mg, 71%) as an oil. ^{1}H NMR ($CDCl_3$, 360 MHz): 0.79 (3 H, t, J = 7.57 Hz, CH_3), 1.45 (2 H, tq, J = 7.57, 7.57 Hz, $-CH_2$), 1.46 (1 H, ddd, J = 12.92, 11.14, 11.14 Hz, H-2 (ax)), 2.08 (2 H, t, J = 7.57 Hz, $-CH_2$), 2.58 (1 H, ddd, J = 12.92, 4.90, 4.90 Hz, H-2 (eq)), 4.28−4.37 (1 H, m, H-3), 4.44 (1 H, dd, J = 9.24, 9.24, 9.24 Hz, H-5), 4.53 (1 H, ddd, J = 9.24, 9.02, 9.02 Hz, H-4), 4.56 (1 H, ddd, J = 9.24, 9.24, 9.24 Hz, H-6), 4.83−5.08 (17 H, m, 8 × CH_2Ph, H-1), 7.12−7.32 (40 H, m, 8 × CH_2Ph). ^{31}P NMR ($CDCl_3$, ^{1}H decoupled, 145.8 MHz): −1.67 (1 P, s), −1.33 (1 P, s), −0.85 (1 P, s), −0.64 (1 P, s). MS: m/z (+ve ion FAB) 1275 [$(M + H)^+$, 4], 91 [Bn^+, 100]. MS: m/z (−ve ion FAB) 1183 [$(M - Bn^+)^-$, 16], 277 [$OPO(OBn)^-$, 100]. MS: m/z 1275.366 $(M + H)^+$ (calcd for $C_{66}H_{71}O_{18}P_4$, 1275.359).

D-3-*O*-Butyryl-2-deoxy-*myo*-inositol 1,4,5,6-Tetrakis-(dibenzyl)phosphate (*ent*-13). Tetrol *ent*-**12** (49 mg, 209 μmol) was phosphorylated as described for compound *rac*-**13** to give the fully protected phosphate *ent*-**13** (197 mg, 74%) as a clear oil. $[\alpha]^{24}_D$ +1.6 (c = 0.97 in $CHCl_3$). MS: m/z 1275, 350 $(M + H)^+$ (calcd for $C_{66}H_{71}O_{18}P_4$, 1275.359). ^{1}H and ^{31}P NMR data were in accordance with those obtained for *rac*-**13**.

***rac*-1-*O*-Butyryl-2-deoxy-*myo*-inositol 3,4,5,6-Tetrakisphosphate (*rac*-14).** Compound *rac*-**13** (165 mg, 130 μmol) was hydrogenated with palladium (10%) on charcoal as described for compound *rac*-**12** to give the title compound *rac*-**14** (65 mg, 90%) as a hygroscopic solid after freeze-drying. ^{1}H NMR (D_2O, 360 MHz): 0.67 (3 H, t, J = 7.41 Hz, CH_3), 1.33−1.43 (2 H, m, $-CH_2$), 1.62 (1 H, ddd, J = 11.72, 11.71, 11.38 Hz, H-2 (ax)), 2.17 (2 H, m, $-CH_2$), 2.25 (1 H, ddd, J = 11.38, 2.24, 2.24 Hz, H-2 (eq)), 4.07−4.23 (4 H, m, H-3, H-4, H-5, H-6), 4.85 (1 H, m, H-1). ^{31}P NMR (D_2O, ^{1}H decoupled, 145.8 MHz): −0.39 (2 P, s), −0.13 (1 P, s), 0.41 (1 P, s). MS: m/z (+ve ion FAB) 555 [$(M + H)^+$, 100], 485 [$(M - Bt^+ + 2\ H^+)^+$], 40]. MS: m/z (−ve ion FAB) 553 [$(M - H^+)^-$, 100]. MS: m/z 552.963 $(M - H^+)^-$ (calcd for $C_{10}H_{21}O_{18}P_4$, 552.967).

D-3-*O*-Butyryl-2-deoxy-*myo*-inositol 1,4,5,6-Tetrakisphosphate (*ent*-14). Compound *ent*-**13** (160 mg, 125 μmol) was hydrogenated as described for *rac*-**14** to give the free acid *ent*-**14** (66.4 mg, 96%) as a hygroscopic solid. $[\alpha]^{24}_D$ −2.4 (c = 1.15 in H_2O). MS: m/z 552.972 $(M - H^+)^-$ (calcd for $C_{10}H_{21}O_{18}P_4$, 552.967). ^{1}H and ^{31}P NMR data were in accordance with those obtained for *rac*-**14**.

***rac*-1-*O*-Butyryl-2-deoxy-*myo*-inositol 3,4,5,6-Tetrakisphosphate Octakis(acetoxymethyl) Ester (*rac*-5).** DIEA (201 μL, 1.19 mmol) and acetoxymethyl bromide (119 μL, 1.19 mmol) were added to a suspension of compound *rac*-**14** (37 mg, 66 μmol) in dry acetonitrile (2 mL) under an argon atmosphere. After the reaction mixture stirred in the dark for 4 days, all volatile compounds were removed under reduced pressure and the crude residue was purified by preparative HPLC (69% MeOH; 40.00 mL/min; t_R = 12.10) to give compound *rac*-**5** (31 mg, 50%) as a clear syrup. ^{1}H NMR (toluene-d_8, 360 MHz): 0.96 (3 H, t, J = 7.48 Hz, CH_3), 1.54 (1 H, ddd, J = 12.70, 12.70, 12.31 Hz, H-2 (ax)), 1.63−1.74 (2 H, m, $-CH_2$), 1.81−1.87 (24 H, 8 s, 8 × OAc), 2.30 (1 H, dt, J = 16.28, 7.68 Hz, $-CH_2$), 2.52 (1 H, dt, J = 16.28, 7.88 Hz, $-CH_2$), 2.64 (1 H, ddd, J = 12.31, 5.31, 5.31 Hz, H-2 (eq)), 4.40−4.59 (4 H, m, H-3, H-4, H-5, H-6), 5.03 (1 H, ddd, J = 12.20, 9.53, 5.31 Hz, H-1), 5.65−5.95 (16 H, m, 8 × CH_2OAc). ^{31}P NMR (toluene-d_8, ^{1}H decoupled, 145.8 MHz): −4.68 (1 P, s), −4.32 (1 P, s), −3.77 (1 P, s), −3.65 (1 P, s). MS: m/z (+ve ion FAB) 1131 [$(M + H)^+$, 12], 987 [$(M - 2\ CH_2OAc^+ + 3\ H^+)^+$, 100]. MS: m/z (−ve ion FAB) 1059 [$(M - Bt^+)^-$, 16], 241 [$OP(OCH_2OAc)_2^-$, 100]. MS: m/z 1059.130 $(M - CH_2OAc^+ + 2\ H)^+$ (calcd for $C_{31}H_{51}O_{32}P_4$, 1059.131).

D-3-*O*-Butyryl-2-deoxy-*myo*-inositol 1,4,5,6-Tetrakisphosphate Octakis(acetoxymethyl) Ester (*ent*-5). The free acid *ent*-**14** (40 mg, 72 μmol) was alkylated as described for compound *rac*-**5** to give the acetoxymethyl ester *ent*-**5** (45 mg, 56%) as a clear syrup. $[\alpha]^{24}_D$ −1.0 (c = 1 in toluene). MS: m/z 1059.138 $(M - CH_2OAc^+ + 2\ H)^+$ (calcd for $C_{31}H_{51}O_{32}P_4$, 1059.131). ^{1}H and ^{31}P NMR data were in accordance with those obtained for *rac*-**5**.

***rac*-2-Deoxy-*myo*-inositol 3,4,5,6-Tetrakisphosphate (*rac*-2).** Compound *rac*-**14** (17 mg, 30 μmol) was treated with 1 M KOH (260 μL) to adjust the pH value to 12.8. The solution was stirred at room temperature for 2 days. The reaction mixture was directly poured onto an ion-exchange column (Dowex 50 WX 8, H^+) for purification. Lyophilization gave compound *rac*-**2** (14 mg, 95%). ^{1}H NMR (D_2O, 360 MHz): 1.55 (1 H, ddd, J = 12.09, 12.09, 12.09 Hz, H-2 (ax)), 2.24 (1 H, ddd, J = 12.09, 4.31, 4.31 Hz, H-2 (eq)), 4.79 (1 H, ddd, J = 12.09, 8.77, 4.31 Hz, H-1), 5.14 (1 H, ddd, J = 9.25, 9.25, 8.83

Hz, H-5), 5.18−5.73 (3 H, m, H-3, H-4, H-6). ^{31}P NMR (D_2O, ^{1}H decoupled, 145.8 MHz): −0.08 (1 P, s), 0.28 (1 P, s), 0.34 (1 P, s), 0.90 (1 P, s). MS: *m/z* (+ve ion FAB) 485 [$(M + H)^+$, 100]. MS: *m/z* (−ve ion FAB) 483 [$(M - H^+)^-$, 100]. MS: *m/z* 482.928$(M - H)^+$ (calcd for $C_6H_{15}O_{17}P_4$, 482.926).

D-2-Deoxy-*myo*-inositol 1,4,5,6-Tetrakisphosphate (*ent*-2). The free acid *ent*-**14** (19 mg, 34 μmol) was saponified as described for compound *rac*-**2** to give the title compound *ent*-**2** (15 mg, 91%) as a solid. $[\alpha]^{24}_D$ +1.7 (c = 0.58 in H_2O). MS: *m/z* 482.927 $(M - H)^+$ (calcd for $C_6H_{15}O_{17}P_4$, 482.926). ^{1}H and ^{31}P NMR data were in accordance with those obtained for *rac*-**2**.

***rac*-3,4,5,6-Tetra-*O*-benzyl-1-*O*-phenoxy(thiocarbonyl)-*myo*-inositol (*rac*-16).** A solution of *rac*-**15** (1 g, 2 mmol), *O*-phenyl chlorothionoformate (270 μL, 2 mmol), and DMAP (122 mg, 1 mmol) in dry pyridine (6 mL) was stirred at room temperature for 20 min. The solvents were evaporated under high vacuum to give an oil. Residual pyridine was removed by evaporating three times with octane. The residue was dissolved in *tert*-butyl methyl ether (50 mL) and was washed twice with phosphate buffer (40 mL) and then with brine (40 mL). The organic layer was dried over Na_2SO_4 and filtered. Purification by preparative HPLC (91% MeOH; 37.5 mL/min; t_R = 40.00 min) yielded compound *rac*-**16** (843 mg, 63%). Mp: 122.7−123.1 °C (from methanol). ^{1}H NMR ($CDCl_3$, 360 MHz): 3.59 (1 H, dd, J = 9.58, 2.45 Hz, H-3), 3.60 (1 H, dd, J = 9.36, 9.36 Hz, H-5), 4.01 (1 H, dd, J = 9.58, 9.36 Hz, H-4), 4.28 (1 H, dd, J = 10.25, 9.36 Hz, H-6), 4.59 (1 H, dd, J = 2.45, 2.45 Hz, H-2), 4.72−4.91 (8 H, m, 4 × C*H*$_2$Ph), 5.35 (1 H, dd, J = 10.25, 2.45 Hz, H-1), 7.03−7.44 (25 H, m, 4 × CH$_2$*Ph* and C(S)OPh ArH). MS: *m/z* (+ve ion FAB) 677 [$(M + H)^+$, 1], 91 [Bn^+, 100]. Anal. ($C_{41}H_{40}O_7S$) C: calcd, 72.76; found, 72.43. H: calcd, 5.96; found, 5.93.

***rac*-3,4,5,6-Tetra-*O*-benzyl-2-*O*-butyryl-1-*O*-phenoxy-(thiocarbonyl)-*myo*-inositol (*rac*-17).** A solution of *rac*-**16** (532 mg, 787 mol), butyric anhydride (140 μL, 850 mol), and DMAP (19 mg, 15 mol) in dry pyridine (5 mL) was stirred at room temperature for 1 h. The solvents were evaporated under high vacuum to give an oil. Residual pyridine was removed by evaporating three times with octane. The residue was dissolved in *tert*-butyl methyl ether (30 mL) and was washed with phosphate buffer (20 mL), with sodium hydrogen carbonate (20 mL), with sodium hydrogen sulfate (20 mL), again with phosphate buffer (20 mL), and finally with brine (20 mL). The organic layer was dried over Na_2SO_4 and filtered. Evaporation of the solvent gave pure *rac*-**17** (476 mg, 82%) as an oil. ^{1}H NMR ($CDCl_3$, 360 MHz): 0.99 (3 H, t, J = 7.31 Hz, CH_3), 1.71 (2 H, m, $-CH_2$), 2.41 (2 H, m, $-CH_2$), 3.62 (1 H, dd, J = 9.45, 9.45 Hz, H-5), 3.63 (1 H, dd, J = 9.67, 2.58 Hz, H-3), 3.92 (1 H, dd, J = 9.67, 9.45 Hz, H-4), 4.11 (1 H, dd, J = 10.32, 9.45 Hz, H-6), 4.71−4.94 (8 H, m, 4 × C*H*$_2$Ph), 5.38 (1 H, dd, J = 10.32, 2.58 Hz, H-1), 6.08 (1 H, dd, J = 2.58, 2.58 Hz, H-2), 7.04−7.43 (25 H, m, 4 × CH$_2$*Ph* and C(S)O*Ph*). MS: *m/z* (+ve ion FAB) 747 [$(M + H)^+$, 1], 91 [Bn^+, 100]. MS: *m/z* 731.310 $(M + H)^+$ (calcd for $C_{45}H_{47}O_7S$, 731.304).

***rac*-3,4,5,6-Tetra-*O*-benzyl-2-*O*-butyryl-1-*O*-deoxy-*myo*-inositol (*rac*-18).** Compound *rac*-**17** (476 mg, 638 mol) was dissolved in dry toluene (100 mL), and AIBN (30 mg, 150 mol) and *n*-Bu_3SnH (305 μL, 1.15 mmol) were added. The solution was heated to reflux under an argon atmosphere for 2 h. The reaction mixture was cooled and then washed with phosphate buffer (30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and filtered. Evaporation of the solvent and purification by preparative HPLC (89% MeOH; 37.5 mL/min; t_R = 50.30 min) gave *rac*-**18** (134 mg, 35%) as an oil. ^{1}H NMR ($CDCl_3$, 360 MHz): 0.96 (3 H, t, J = 7.45 Hz, CH_3), 1.50 (1 H, ddd, J = 14.21, 11.89, 2.33 Hz, H-1 (ax)), 1.61−1.71 (2 H, m, $-CH_2$), 2.25 (1 H, ddd, J = 14.21, 4.31, 4.31 Hz, H-1 (eq)), 2.33 (2 H, m, $-CH_2$), 3.54 (1 H, dd, J = 10.01, 2.49 Hz, H-3), 3.56 (1 H, dd, J = 9.32, 9.32 Hz, H-5), 3.82 (1 H, ddd, J = 11.89, 9.32, 4.31 Hz, H-6), 3.84 (1 H, dd, J = 9.32, 9.32 Hz, H-4), 4.55−4.95 (8 H, m, 4 × C*H*$_2$Ph), 5.55 (1 H, ddd, J = 4.31, 2.49, 2.33 Hz, H-2), 7.26−7.39 (20 H, m, 4 × CH$_2$*Ph*). MS: *m/z* (+ve ion FAB) 595 [$(M + H)^+$, 1], 91 [Bn^+, 100]. MS: *m/z* 235.120 $(M + H)^+$ (calcd for $C_{10}H_{19}O_6$, 235.118).

***rac*-2-*O*-Butyryl-1-*O*-deoxy-*myo*-inositol (*rac*-19).** Compound *rac*-**18** (75 mg, 126 mol) was hydrogenated with palladium (10%) on charcoal under hydrogen as described for compound *rac*-**12** to give tetrol *rac*-**19** (28 mg, 99%) as a solid after freeze-drying. Mp: 138.7−139.5 °C (from ethanol). ^{1}H NMR (DMSO-d_6, 360 MHz): 0.89 (3 H, t, J = 7.53 Hz, CH_3), 1.41 (1 H, ddd, J = 14.14, 11.90, 2.34 Hz, H-1 (ax)), 1.50−1.59 (2 H, m, $-CH_2$), 1.89 (1 H, ddd, J = 14.14, 4.27, 4.27 Hz, H-1 (eq)), 2.26 (2 H, m, $-CH_2$), 2.96 (1 H, dd, J = 8.75, 5.90 Hz, H-3), 3.25−3.32 (2 H, m, H-4, H-5), 3.39 (1 H, m, H-6), 4.65 (1 H, d, J = 4.48 Hz, OH), 4.73 (1 H, s(br), OH), 4.77 (1 H, s(br), OH), 5.00 (1 H, ddd, J = 4.27, 2.34, 2.34 Hz, H-2). MS: *m/z* (+ve ion FAB) 235 [$(M + H^+)^-$, 18], 71 [$(Bt^+)^-$, 100]. MS: *m/z* (−ve ion FAB) 233 [$(M - H^+)^-$, 18], 87 [BtO^-, 100]. Anal. ($C_{10}H_{18}O_6$) C: calcd, 51.27; found, 51.09. H: calcd, 7.75; found, 7.57.

***rac*-2-*O*-Butyryl-1-deoxy-*myo*-inositol 3,4,5,6-Tetrakis-(dibenzyl)phosphate (*rac*-20).** A solution of tetrol *rac*-**19** (20 mg, 85 mol) and tetrazole (74 mg, 1.02 mmol) in acetonitrile (2 mL) was treated with dibenzyl *N*,*N*-diisopropylphosphoramidite (345 μL, 1.02 mmol) for 18 h, oxidized with peracetic acid at −40 °C, and worked up as described for compound *rac*-**13**. Purification by preparative HPLC (93% MeOH; 40 mL/min; t_R = 15.50 min) gave compound *rac*-**20** (80 mg, 73%) as an oil. ^{1}H NMR ($CDCl_3$, 360 MHz): 0.90 (3 H, t, J = 7.52 Hz, CH_3), 1.51−1.63 (2 H, m, $-CH_2$), 1.70 (1 H, ddd, J = 12.98, 10.48, 2.73 Hz, H-1 (ax)), 2.22 (2 H, t, J = 7.52 Hz, $-CH_2$), 2.50 (1 H, ddd, J = 12.98, 4.90, 4.90 Hz, H-1 (eq)), 4.47 (1 H, ddd, J = 9.87, 9.81, 2.73 Hz, H-3), 4.59−4.71 (2 H, m, H-4, H-5), 4.91−5.09 (17 H, m, 8 × C*H*$_2$Ph, H-6), 5.55 (1 H, ddd, J = 4.90, 2.73, 2.73 Hz, H-2), 7.15−7.30 (40 H, m, 8 × CH$_2$*Ph*). ^{31}P NMR ($CDCl_3$, ^{1}H decoupled, 145.8 MHz): −1.58 (1 P, s), −1.12 (1 P, s), −0.92 (2 P, s). MS: *m/z* (+ve ion FAB) 1275 [$(M + H)^+$, 2], 91 [Bn^+, 100]. MS: *m/z* (−ve ion FAB) 1183 [$(M - Bn^+)^-$, 14], 277 [$OPO(OBn)^-$, 100]. MS: *m/z* 1275.358 $(M + H)^+$ (calcd for $C_{66}H_{71}O_{18}P_4$, 1275.359).

***rac*-2-*O*-Butyryl-1-deoxy-*myo*-inositol 3,4,5,6-Tetrakisphosphate (*rac*-21).** Compound *rac*-**20** (165 mg, 130 mol) was hydrogenated with palladium (10%) on charcoal as described for compound *rac*-**12** to give title compound *rac*-**21** (65 mg, 90%) as a solid after freeze-drying. ^{1}H NMR (D_2O, 360 MHz): 0.85 (3 H, t, J = 7.39 Hz, CH_3), 1.51−1.61 (2 H, m, $-CH_2$), 1.82 (1 H, ddd, J = 13.52, 2.58, 2.21 Hz, H-1 (ax)), 2.31−2.37 (3 H, m, H-1(eq), $-CH_2$), 4.26 (1 H, ddd, J = 9.29, 9.18, 9.18 Hz, H-5), 4.27−4.37 (2 H, m, H-3, H-4), 4.48 (1 H, ddd, J = 9.36, 9.36, 9.12 Hz, H-6), 5.38 (1 H, ddd, J = 4.76, 2.21, 2.21 Hz, H-2). ^{31}P NMR (D_2O, ^{1}H decoupled, 145.8 MHz): −0.21 (1 P, s), −0.01 (1 P, s), 0.49 (1 P, s), 0.53 (1 P, s). MS: *m/z* (+ve ion FAB) 555 [$(M + H)^+$, 100], 485 [$(M - Bt^+ + 2 H^+)^+$, 30]. MS: *m/z* (−ve ion FAB) 553 [$(M - H^+)^-$, 100]. MS: *m/z* 552.960 $(M - H^+)^-$ (calcd for $C_{10}H_{21}O_{18}P_4$, 552.967).

***rac*-2-*O*-Butyryl-1-deoxy-*myo*-inositol 3,4,5,6-Tetrakisphosphate Octakis(acetoxymethyl) Ester (*rac*-6).** DIEA (139 μL, 816 mol) and acetoxymethyl bromide (82 μL, 820 mol) were added to a suspension of compound *rac*-**21** (18 mg, 32 mol) in acetonitrile (1 mL) as described for compound *rac*-**2**. Purification by preparative HPLC (69% MeOH; 40.00 mL/min; t_R = 11.50) gave compound *rac*-**6** (21 mg, 55%) as a clear syrup. ^{1}H NMR (toluene-d_8, 360 MHz): 0.85 (3 H, t, J = 7.48 Hz, CH_3), 1.51−1.68 (3 H, m, H-1 (ax), $-CH_2$), 1.78−1.86 (24 H, 8 s, 8 × OAc), 2.08−2.17 (2 H, m, $-CH_2$), 2.57 (1 H, ddd, J = 14.37, 4.53, 4.53 Hz, H-1 (eq)), 4.70 (1 H, ddd, J = 9.06, 9.06, 2.34 Hz, H-3), 4.73−4.84 (2 H, m, H-4, H-5), 5.02 (1 H, ddd, J = 9.06, 9.06, 9.06 Hz, H-6), 5.59−5.92 (17 H, m, 8 × C*H*$_2$OAc, H-2). ^{31}P NMR (toluene-d_8, ^{1}H decoupled, 145.8 MHz): −4.75 (1 P, s), −4.56 (1 P, s), −3.72 (1 P, s), −3.69 (1 P, s). MS: *m/z* (+ve ion FAB) 1131 [$(M + H)^+$, 2], 987 [$(M - 2 CH_2OAc^+ + 3 H)^+$, 100], MS: *m/z* (−ve ion FAB) 1059 [$(M - Bt^+)^-$, 6], 241 [$OP(OCH_2OAc)_2^-$, 100]. MS: *m/z* 1059.133 $(M - CH_2OAc^+ + 2 H)^+$ (calcd for $C_{31}H_{51}O_{32}P_4$, 1059.131).

***rac*-1-Deoxy-*myo*-inositol 3,4,5,6-Tetrakisphosphate (*rac*-3).** Compound *rac*-**21** (20 mg, 35 μmol) was treated with

1 M KOH (285 μL) to adjust the pH value to 12.8. The solution was stirred at room temperature for 2 days. The reaction mixture was directly poured onto an ion-exchange column (Dowex 50 WX 8, H^+) for purification. Lyophilization gave compound *rac*-**3** (18 mg, 94%). ^{1}H NMR (D_2O, 360 MHz): 1.63 (1 H, ddd, J = 13.72, 10.50, 2.31 Hz, H-1 (ax)), 2.27 (1 H, ddd, J = 13.72, 2.45, 2.31 Hz, H-1 (eq)), 4.62 (1 H, ddd, J = 9.45, 9.45, 9.45 Hz, H-5), 4.68 (1 H, ddd, J = 10.50, 2.31, 2.31 Hz, H-2), 4.95−5.05 (2 H, m, H-3, H-4), 5.18 (1 H, m, H-6). ^{31}P NMR (D_2O, ^{1}H decoupled, 145.8 MHz): −0.28 (1 P, s), −0.06 (1 P, s), 0.14 (1 P, s), 0.27 (1 P, s). MS: *m*/*z* (+ve ion FAB) 485 [$(M + H)^+$, 100]. MS: *m*/*z* (−ve ion FAB) 483 [$(M - H^+)^-$, 100]. MS: *m*/*z* 482.924 $(M - H)^+$ (calcd for $C_6H_{15}O_{17}P_4$, 482.926).

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