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Antagonists of *myo*-Inositol 3,4,5,6-Tetrakisphosphate Allow Repeated Epithelial Chloride Secretion

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Abstract—Cystic fibrosis (CF) patients suffer from a defect in hydration of mucosal membranes due to mutations in the cystic fibrosis transmembrane regulator (CFTR), an apical chloride channel in mucosal epithelia. Disease expression in CF knockout mice is organ specific, varying with the level of expression of calcium activated Cl^- channels (CLCA). Therefore, restoring transepithelial Cl^- secretion by augmenting alternate Cl^- channels, such as CLCA, could be beneficial. However, CLCA-mediated Cl^- secretion is transient, due in part to the inhibitory effects of *myo*-inositol 3,4,5,6-tetrakisphosphate [Ins(3,4,5,6)P_4]. This suggests that antagonists of Ins(3,4,5,6)P_4 could be useful in treatment of CF. We have, therefore, synthesized a series of membrane-permeant Ins(3,4,5,6)P_4 derivatives, carrying alkyl substituents on the hydroxyl groups and screened them for effects on Cl^- secretion in a human colonic epithelial cell line, T_{84}. While membrane-permeant Ins(3,4,5,6)P_4 derivatives had no direct effects on carbachol-stimulated Cl^- secretion, Ins(3,4,5,6)P_4 derivatives, but not enantiomeric Ins(1,4,5,6)P_4 derivatives, reversed the inhibitory effect of Ins(3,4,5,6)P_4 on subsequent thapsigargin activation of Cl^- secretion. The extent of the antagonistic effect of the Ins(3,4,5,6)P_4 derivatives varied with the position of the alkyl substituents. Derivatives with a cyclohexylidene ketal or a butyl-chain at the 1-position reversed the Ins(3,4,5,6)P_4-mediated inhibition of Cl^- secretion by up to 96 and 85%, respectively, whereas butylation of the 1- and 2-position generated a reversal effect of only 65%. Derivatives carrying the butyl chain only at the 2-position showed no antagonistic effect. These data: (1) Support the hypothesis that Ins(3,4,5,6)P_4 stereospecifically inhibits Ca^{2+} activated Cl^- secretion and that Ins(3,4,5,6)P_4 mediates most, if not all of the cholinergic-mediated inhibition of chloride secretion in T_{84} cells; (2) Demonstrate Ins(3,4,5,6)P_4-mediated inhibition can be completely reversed with rationally designed membrane-permeant Ins(3,4,5,6)P_4 antagonists; (3) Demonstrate that a SAR for membrane-permeant Ins(3,4,5,6) P_4 antagonists can be generated and screened in a physiologically relevant cell-based assay; (4) Indicate that Ins(3,4,5,6)P_4 derivatives could serve as a starting point for the development of therapeutics to treat cystic fibrosis.

Introduction

Transepithelial chloride flux by polarized epithelia controls a variety of physiological processes, such as intestinal and pancreatic secretion and renal functions.[1] For example, Cl^- secretion regulates the fluid balance of the intestinal lumen,[2] and is a driving force for the hydration of airway epithelia.[3] Given this key role in physiological functions, defects in Cl^- transport can result in life-threatening diseases, such as diarrhea and cystic fibrosis (CF).[4]

Transepithelial Cl^- secretion is gated through multiple apical Cl^- channels. One of these channels, the cystic fibrosis transmembrane regulator (CFTR) can be triggered by elevating cAMP levels, and consequently PKA-dependent phosphorylation.[5] The vast majority of CF patients suffer from a defect in hydration of mucosal membranes due to mutations in the gene coding for the CFTR. Mucosal epithelia also express Cl^- channels other than the CFTR such as the outwardly rectifying Cl^- channel (ORCC), calcium-activated Cl^- channels (CLCA) and volume-regulated Cl^- channels (ClC). While the ORCC appears to be controlled by the CFTR and therefore is dysfunctional in CF,[6–9] active CLCA are reportedly more abundant in CF tissue.[10,11] A number of studies indicate that phenotypes with increased activity of alternate Cl^- channels such as the CLCA correlate to milder clinical manifestations.[9,12–15] Therefore, in CF, cAMP-mediated Cl^- transport is defective,[16,17] leaving Cl^- secretion via the Ca^{2+}

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dependent pathway intact and even enhanced. Thus, it has been hypothesized that alternate ion channels may compensate for defects in CFTR function and could be therapeutically useful. There are compelling arguments for pursuing artificial activation of alternate Cl^- channels to counteract CF pathophysiology. This has lead to tests with Ca^{2+}-elevating agents such as purinergic agonists in the treatment of CF.[18] Currently, two compounds are in clinical development that elevate intracellular calcium [$(Ca^{2+})_i$] and thereby modulate Cl^- secretion; INS365, a PY2Y receptor agonist and duramycin, an antibiotic that triggers an increase in intracellular Ca^{2+} levels. However, an increase in [$(Ca^{2+})_i$] does not always lead to Cl^- secretion. Activation of phospholipase C (PLC) can lead to a transient activation of Cl^- secretion through the *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]/[Ca^{2+}]$_i$ pathway, but also promotes a long-term inhibitory feedback, preventing Cl^- secretion from being sustained.[19] We have demonstrated that the intracellular signaling molecule, Ins(3,4,5,6)P_4, becomes elevated after prolonged PLC activation and 'uncouples' Cl^- secretion from the rise in intracellular Ca^{2+} in mucosal epithelia.[20] This regulatory role for Ins(3,4,5,6)P_4 has been confirmed by several investigators.[21,22]

The observations that cholinergic but not histaminergic stimulation uncoupled the Ca^{2+}-mediated Cl^- secretion (CaMCS), and the inhibitory action of membrane-permeant derivative of Ins(3,4,5,6)P_4, have led to the conclusion that Ins(3,4,5,6)P_4 is the endogenous negative regulator of this conductance.[20,23] Studies by Xie et al. employing whole cell patch clamp technique with intracellular perfusion of inositol tetrakisphosphates indicated that Ins(3,4,5,6)P_4 modulated an apically located Ca^{2+}/calmodulin kinase regulated Cl^- channel in T_{84} cells.[22,24] Furthermore, Ca^{2+}-dependent Cl^- channels reconstituted in planar lipid bilayers could also be regulated by low levels of Ins(3,4,5,6)P_4 in the absence of phosphatase activities.[25] Electrophysiological analysis of the effects of Ins(3,4,5,6)P_4 in the CFPAC-1 cell line, originally derived from a CF patient suffering from the ΔF508 defect,[26] provided further evidence for an important and widespread intracellular function of Ins(3,4,5,6)P_4 as a negative regulator of Cl^- secretion.[21,27,28]

The pharmaceutical development of activators of Ca^{2+}-mediated Cl^- secretion in CF epithelia could be limited by the inhibitory action of Ins(3,4,5,6)P_4 on Cl^- transport. Therefore, there is an urgent need to understand the mechanism by which Ins(3,4,5,6)P_4 inhibits CaMCS, specifically identifying the downstream targets of Ins(3,4,5,6)P_4. Accordingly, we have synthesized a set of membrane-permeant Ins(3,4,5,6)P_4 derivatives to study the interaction of Ins(3,4,5,6)P_4 with its targets, and—more importantly—to find antagonists of the inhibitory action of Ins(3,4,5,6)P_4 (Fig. 1). Prodrug approaches, in which the highly polar phosphate groups are masked, are commonly used to help deliver biologically active molecules to the cytosol.[29] In fact, membrane-permeant derivatives of inositol polyphosphates have been successfully employed in the past,[20,30–36] and have been demonstrated to elevate, for instance, intracellular Ins(3,4,5,6)P_4 levels.[20] Preferred masking groups were acyloxymethyl groups. Here we demonstrate that some of these Ins(3,4,5,6)P_4 derivatives are potent antagonists of this inhibition following carbachol stimulation. Furthermore, the selection of modified Ins(3,4,5,6)P_4 derivatives enables us to elucidate some of the structural requirements for the putative Ins(3,4,5,6)P_4 interactions to its targets.

Results

Design and synthesis

We have previously shown that modifications of the hydroxyl groups of Ins(3,4,5,6)P_4 selectively influence the ability of membrane-permeant Ins(3,4,5,6)P_4 derivatives to inhibit carbachol-induced Cl^--secretion. In that study we demonstrated that a 2-deoxy derivative was a partial agonist for Ins(3,4,5,6)P_4, inhibiting Cl^- secretion, while the corresponding 1-deoxy derivative showed no effect on Cl^--secretion.[36] Therefore, the complete deletion of a hydroxyl group eliminated both the hydrogen bonding donor and acceptor potential for interaction with targets. In the present study, we sought derivatives that would provide the acceptor potential but are void of the hydrogen donor properties. Apart from the compounds mentioned above, some deoxyfluoro-Ins(3,4,5,6)P_4 derivatives were previously prepared.[37] Some of these compounds are suspected to have effects on protein phosphatases.[22]

We prepared a series of membrane-permeant *myo*-inositol 3,4,5,6-tetrakisphosphate derivatives alkylated at the hydroxyl groups, namely 1-*O*-butyl-2-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester [1-*O*-Bu-2-*O*-Bt-Ins(3,4,5,6)P_4/AM] (**1**), 2-*O*-butyl-1-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester [2-*O*-Bu-1-*O*-Bt-Ins(3,4,5,6)P_4/AM] (**2**), 1,2-di-*O*-butyl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester [1,2-di-*O*-Bu-Ins(3,4,5,6)P_4/AM] (**3**), and the corresponding enantiomeric compounds 3-*O*-Bu-2-*O*-Bt-Ins(1,4,5,6)P_4/AM (*ent*-**1**), 2-*O*-Bu-3-*O*-Bt-Ins(1,4,5,6)P_4/AM (*ent*-**2**) and 2,3-*O*-Bu-Ins(1,4,5,6)P_4/AM (*ent*-**3**), respectively[38] (Fig. 1).

Synthesis of the Ins(3,4,5,6)P_4 derivative *rac*-1,2-*O*-cyclohexylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*rac*-**4**) started from the common precursor *rac*-1,2-*O*-cyclohexylidene-*myo*-inositol (*rac*-**5**) (Scheme 1). The phosphorylation of *rac*-**5** proceeded smoothly using the method of Yu and Frazer-Reid[39] to give the fully protected tetrakisphosphate *rac*-**6** after purification by preparative HPLC. Deprotection of the phosphoric acid triester was achieved by catalytic hydrogenolysis with Pd/C in ethanol. Equal amounts of ethyl-*N*,*N*-di*iso*propylamine (DIEA) with respect to the number of deprotected phosphates produced during the reaction prevented the otherwise common hydrolysis of the acid labile cyclohexylidene group. After filtration and drying *rac*-**7** was obtained as its DIEA salt. *rac*-**7** was

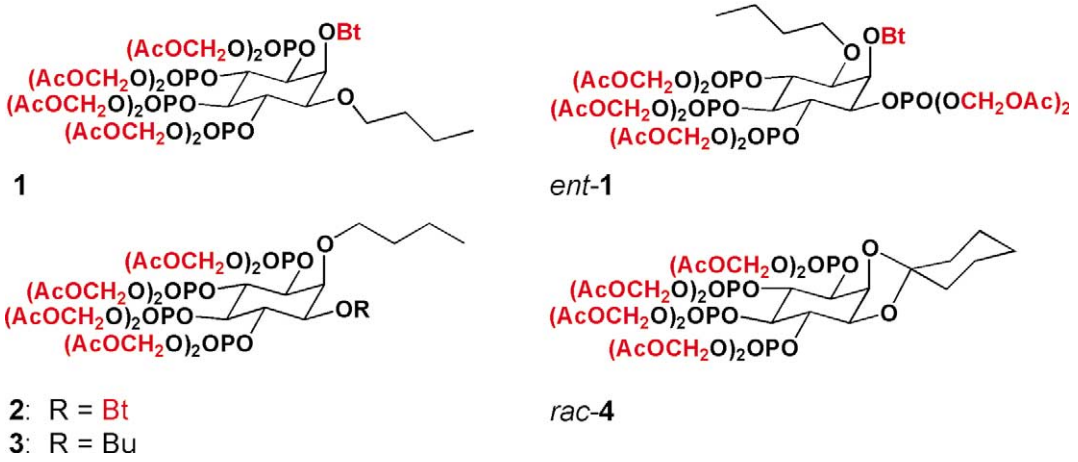
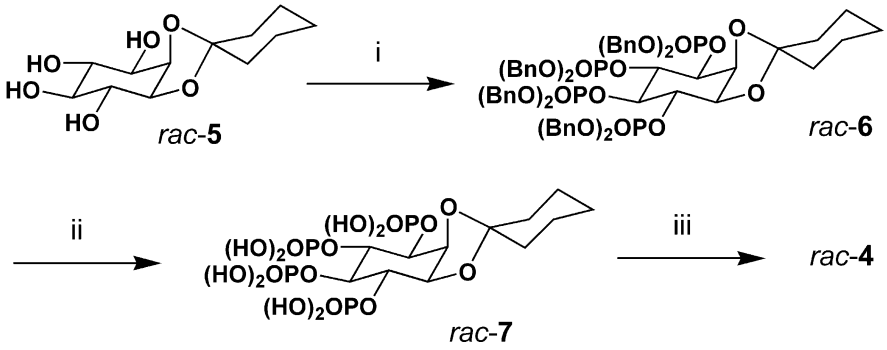


Figure 1. Structures of *myo*-inositol 3,4,5,6-tetrakisphosphate derivatives. Bioactivatable protecting groups are shown in red: 1-*O*-butyl-2-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (**1**) and the enantiomer 3-*O*-butyl-2-*O*-butyryl-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*ent*-**1**), as well as 2-*O*-butyl-1-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (**2**), 1,2-di-*O*-butyl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (**3**), and the racemic *rac*-1,2-*O*-cyclohexylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*rac*-**4**).



Scheme 1. (i) (a) $(BnO)_2PNiPr_2$, 1*H*-tetrazole, MeCN; (b) −40 °C, AcOOH; (ii) H_2, Pd/C (10%), ethanol, DIEA, 6d; (iii) AMBr, DIEA, MeCN.

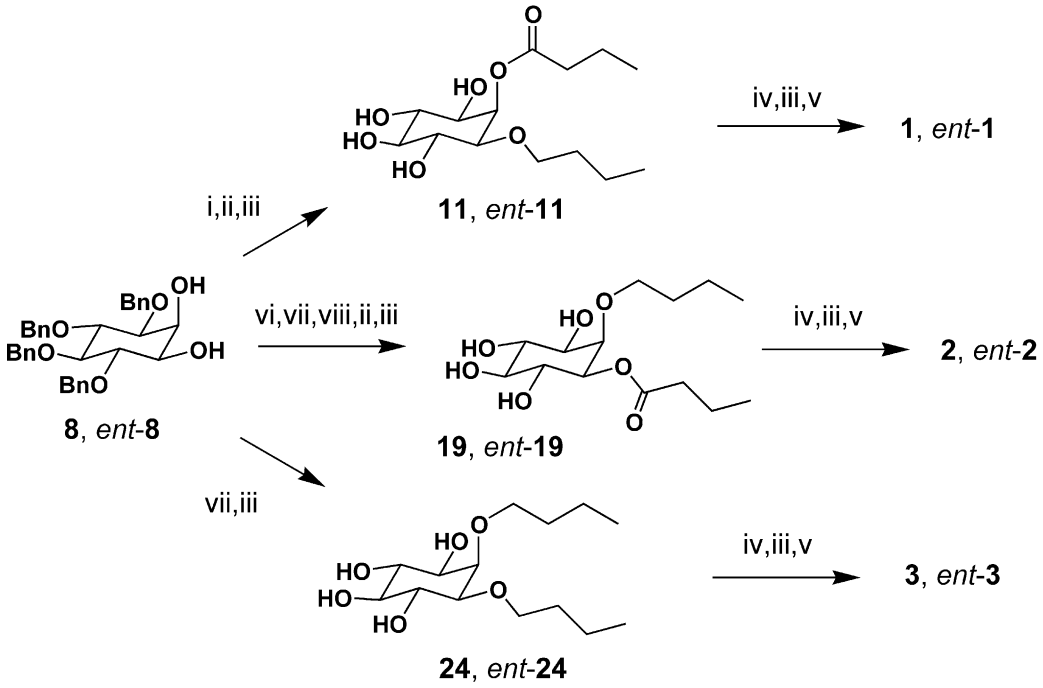
alkylated with acetoxymethyl bromide (AMBr) in the presence of DIEA to give the uncharged, bioactivatable octakis(acetoxymethyl) ester *rac*-**4**.

The synthesis strategy (Scheme 2) for mono- and dibutyl ether derivatives followed those previously reported for similar compounds.[40] In brief, enantiomerically pure 3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**8**) and 1,4,5,6-tetra-*O*-benzyl-*myo*-inositol (*ent*-**8**), respectively, were regioselectively alkylated with 1-butyl iodide at the 1/3-OH group via a cyclic dibutylstannyl intermediate. The remaining hydroxyl group was esterified with butyric anhydride. After removal of the benzyl groups, standard phosphorylation/deprotection procedures gave the tetrakisphosphates, which were alkylated with acetoxymethyl bromide, to yield the uncharged octakis(acetoxymethyl) esters **1** and *ent*-**1**, respectively. In case of 2-OH alkylated compounds, the initial alkylation of the 1/3-OH group was performed with allyl iodide, followed by the alkylation of the 2-OH group with 1-butyl iodide in the presence of sodium hydride. Selective removal of the allyl ether with tris(triphenyl)phosphin rhodium(I) chloride, followed by treatment with trifluoroacetic acid, gave alcohols **9** and (*ent*-**9**), respectively. Butyrylation and subsequent hydrogenolysis resulted in tetrols **11** and (*ent*-**11**), respectively. Phosphorylation and deprotection steps were performed as described above to finally give the octakis(acetoxymethyl) esters **2** and *ent*-**2**, respectively. The 1,2-di-*O*-butyl-*myo*-inositol (**24**) and 2,3-di-*O*-butyl-*myo*-inositol (*ent*-**24**) precursors were synthesized by alkylation of **8** and *ent*-**8**, respectively, with butyl iodide in the presence of NaH in DMF, followed by hydrogenolysis. The reaction sequence to the octakis(acetoxymethyl) esters **3** and *ent*-**3**, respectively, was performed as described above for the other tetrakisphosphates.

Finally, the monobutyrates **13**, *ent*-**13**, **19**, and *ent*-**19** were hydrolyzed with KOH to give the potassium salts of 1-*O*-butyl-*myo*-inositol 3,4,5,6-tetrakisphosphate (**14**) and 3-*O*-butyl-*myo*-inositol 1,4,5,6-tetrakisphosphate (*ent*-**14**) as well as 2-*O*-butyl-*myo*-inositol 3,4,5,6-tetrakisphosphate (**22**) and 2-*O*-butyl-*myo*-inositol 1,4,5,6-tetrakisphosphate (*ent*-**22**), respectively. Combined with the 1,2-di-*O*-butyl-*myo*-inositol 3,4,5,6-tetrakisphosphate (**26**) and its enantiomer *ent*-**26** a set of six orthogonally alkylated tetrakisphosphates is now available for in vitro studies.

Effect of inositol 3,4,5,6-tetrakisphosphate derivatives on calcium-mediated chloride secretion

The colonic epthelia cell line T_{84}, an established model for the study of Cl^- secretion, was grown to confluence



Scheme 2. (i) (a) Bu_2SnO, toluene, reflux, soxhlet with molecular sieves, 3 Å; (b) butyl iodide, CsF, DMF; (ii) Bt_2O, pyridine, DMAP; (iii) H_2, Pd/C (10%), AcOOH; (iv) (a) $(BnO)_2PNiPr_2$, tetrazole, MeCN; (b) −40 °C, AcOOH; (v) AMBr, DIEA, MeCN; (vi) (a) Bu_2SnO, toluene, reflux, soxhlet with molecular sieves, 3 Å; (b) allyl iodide, CsF, DMF; (vii) butyl iodide, NaH, DMF; (viii) (a) tris(triphenylphosphine)rhodium(I) chloride, EtOH/H_2O; (b) TFA. For details on intermediates, see text and Experimental. Only the enantiomers of the Ins(3,4,5,6)P_4 series are shown.

on polycarbonate membranes in Snapwells (Costar). The monolayers were mounted into modified Ussing chambers and Cl^- secretion was measured as short circuit current (I_{sc}) as described before.[36] We have previously reported that enantiomerically pure 1,2-di-*O*-butyryl-Ins(3,4,5,6)P_4/AM was capable of inhibiting charbachol-stimulated CaMCS by about 50% and that of thapsigargin by about 60%.[30] To compare the inhibitory effects of membrane-permeant derivatives of Ins(3,4,5,6)P_4 and Ins(1,4,5,6)P_4 on carbachol-stimulated CaMCS, cells were pre-incubated with 600 μM of the membrane-permeant derivatives **1**–**3**, respectively, for 30 min prior to mounting, or with 800 μM of the racemic compound *rac*-**4**. After an additional 10 min, 100 μM carbachol was added to the solution bathing the basolateral surface and the I_{sc} was monitored. We choose to stimulate CaMCS with carbachol and not with histamine due to the higher magnitude of the effect.[23] However, no significant change in I_{sc} was observed although some of the derivatives led to a slighty (10–15%) higher I_{sc} compared to control. These results indicate that the Ins(3,4,5,6)P_4-derivatives **1**–**3** and derivative *rac*-**4** did not inhibit CaMCS in T_{84} cells.

Effect of membrane-permeant Ins(3,4,5,6)P_4-derivatives on carbachol-induced inhibition of chloride secretion

The response of T_{84} cells to carbachol is followed by a prolonged period in which subsequent CaMCS is suppressed.[23] This inhibition of the Cl^- secretory response is due, at least in part, to carbachol-induced elevation in intracellular Ins(3,4,5,6)P_4 levels, uncoupling Cl^- secretion from $[Ca^{2+}]_i$. This effect could be simulated by using the membrane-permeant Ins(3,4,5,6)P_4 derivative, Bt_2Ins(3,4,5,6)P_4/AM, which generated intracellular Ins(3,4,5,6)P_4 levels of about 4 μM.[20] In this way, we tested whether the membrane-permeant derivatives **1**–**4** could reverse carbachol-induced inhibition of Cl^- secretion.

This protocol could potentially verify whether carbachol-mediated inhibition of Cl^- secretion is due in its entirety to the elevation of Ins(3,4,5,6)P_4 or whether other processes initiated through carbachol also contribute to the inhibition. To test this possibility, T_{84} monolayers were preincubated with 600 μM extracellular 1-*O*-Bu-2-*O*-Bt-Ins(3,4,5,6)P_4/AM (**1**) for 30 min prior to mounting. 25 min after stimulation with carbachol, 1 μM thapsigargin was added to both halves of the Ussing chamber in order to elevate $[Ca^{2+}]_i$ and subsequently stimulate Cl^- secretion (Fig. 2). As is shown in Figure 3, 1-*O*-Bu-2-*O*-Bt-Ins(3,4,5,6)P_4/AM (**1**) abolished the inhibitory effect of carbachol on thapsigargin-stimulated Cl^- secretion by about 88%, while the enantiomeric Ins(1,4,5,6)P_4/AM derivative (*ent*-**1**) was ineffective (Fig. 3). Lower concentrations (200 μM) showed no significant inhibition ($n = 4$, data not shown). To investigate whether the other butylated compounds were equally effective, cells were preincubated with similar amounts of 2-*O*-Bu-1-*O*-Bt-Ins(3,4,5,6)P_4/AM (**2**) (Fig. 2) or 1,2-di-*O*-Bu-Ins(3,4,5,6)P_4/AM (**3**) and I_{sc} was monitored under the same conditions. As shown in Figure 3, 1,2-di-*O*-Bu-Ins(3,4,5,6)P_4/AM (**3**) reversed the carbachol-mediated inhibition of CaMCS by about 65% compared to control, whereas 2-*O*-Bu-1-*O*-Bt-Ins(3,4,5,6)P_4/AM (**2**) was ineffective (Figs 2 and 3). The enantiomeric Ins(1,4,5,6)P_4 derivatives *ent*-**2** and *ent*-**3**

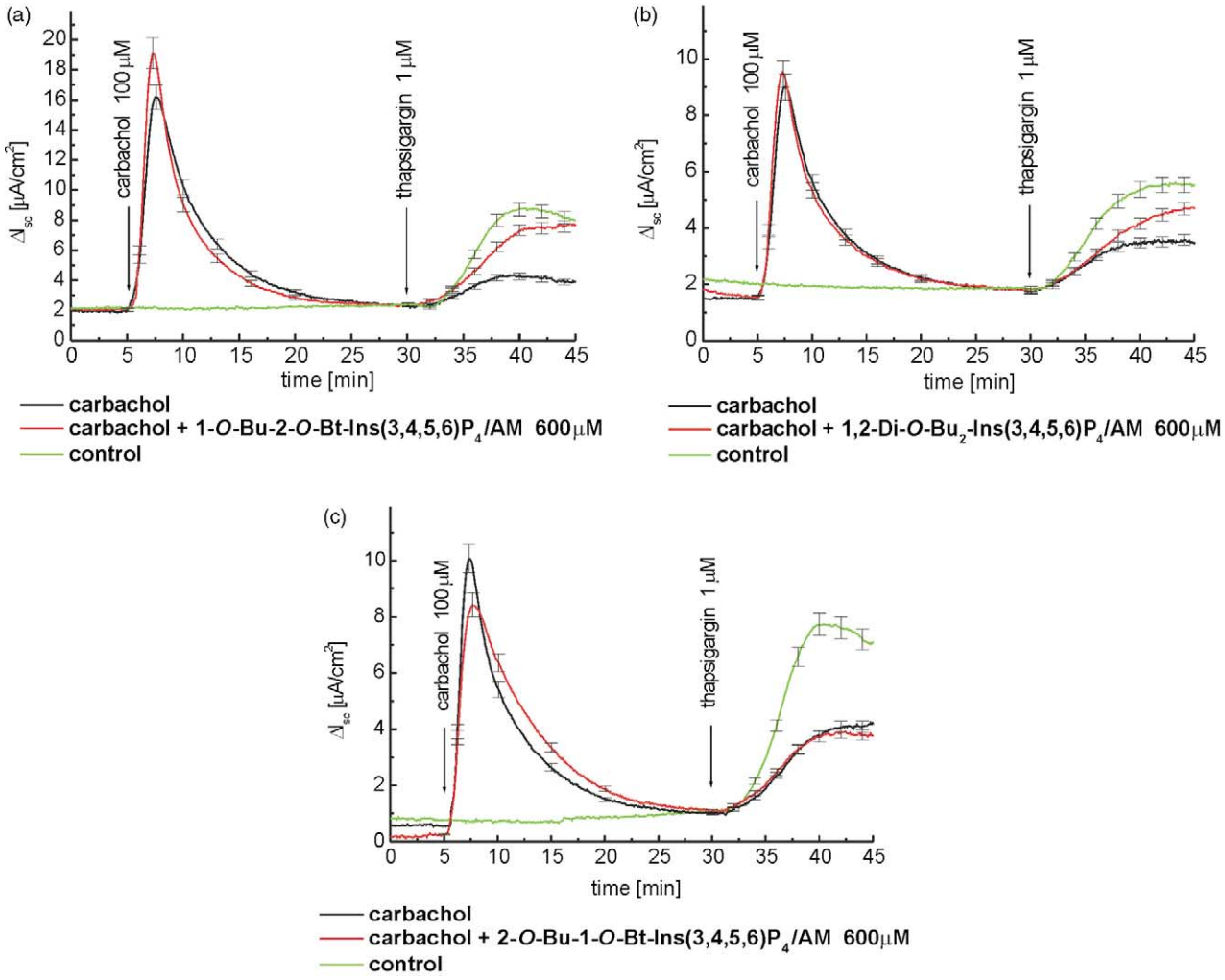


Figure 2. Time course of the reversed Ins(3,4,5,6)P_4 inhibition of thapsigargin-stimulated Cl^--secretion by membrane-permeant Ins(3,4,5,6)P_4 derivatives. T_{84} monolayers were incubated for 30 min with (A) **1**, (B) **2**, (C) **3** where indicated, or vehicle (DMSO/5% Pluronic) prior to mounting into Ussing chambers. Cl^- secretion was measured as short circuit current (ΔI_{sc}). Carbachol (10^{-4} M) was added to the basolateral side to induce elevated Ins(3,4,5,6)P_4 levels and a transient onset of Cl^--secretion.[20] After 25 min, thapsigargin (10^{-6} M) was added to the basolateral and the apical side to stimulate Cl^--secretion a second time. Data reflect monitoring every 4 s and are the average of six experiments.

were also unable to reverse the inhibition of Cl^- secretion (Fig. 3). The racemic compound 1,2-*O*-cyclohexylidene-Ins(3,4,5,6)P_4/AM (*rac*-**4**) reversed the carbachol-induced inhibition of CaMCS by about 95% compared to control, at an extracellular concentration of 800 µM (Figs 2 and 3), while lower doses (400 µM) were not effective ($n=4$, data not shown). These data suggest that the Ins(3,4,5,6)P_4 derivatives **1**, **3**, and *rac*-**4**, but not **2**, are able to bind to the putative Ins(3,4,5,6)P_4-binding site, preventing Ins(3,4,5,6)P_4 from inhibiting CaMCS.

Effect of membrane-permeant Ins(3,4,5,6)P_4 derivatives on intracellular calcium levels

We have previously demonstrated that Ins(3,4,5,6)P_4 inhibits Ca^{2+}-dependent Cl^- secretion without altering intracellular Ca^{2+} mobilization.[20] Therefore, it was unlikely that the reversal of the inhibition could be due to an effect on intracellular calcium levels ($[Ca^{2+}]_i$). As expected, ratio imaging experiments with Fura-2-loaded T_{84} cells showed unchanged $[Ca^{2+}]_i$ levels after treatment with 600 µM extracellular for the compounds **1**–**3** or 800 µM for the derivative *rac*-**4**, respectively ($n=2$, data not shown). Furthermore, the rise in $[Ca^{2+}]_i$ in response to thapsigargin was not altered by any of the derivatives ($n=4$, data not shown).

Discussion

The recent discovery of Ins(3,4,5,6)P_4 as an endogenous negative regulator of CaMCS in T_{84} cells may assist in the design of therapeutic agents which modulate fluid secretion in diseased epithelia of patients suffering from hypersecretory or hyposecretory disorders such as secretory diarrhea or CF.

However, the downstream effector in the Ins(3,4,5,6)P_4-signaling pathway, the Ins(3,4,5,6)P_4 target(s), has not yet been identified. Photoaffinity labeled derivatives or affinity columns may help to characterize these proteins in the future. Knowledge of the canonical ligand/protein interaction properties of Ins(3,4,5,6)P_4 with its putative

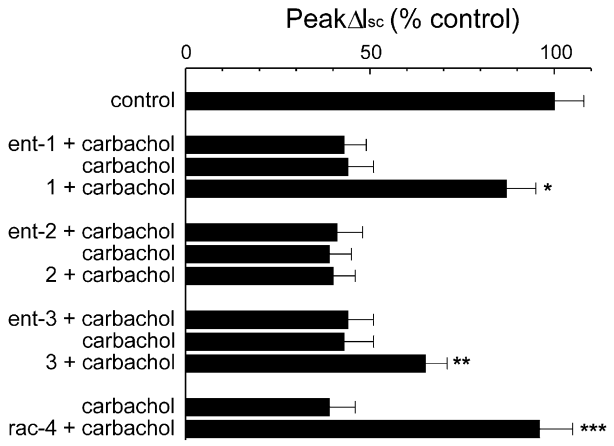


Figure 3. Ins(3,4,5,6)P_4/AM derivatives, but not Ins(1,4,5,6)P_4/AM derivatives reversed Ins(3,4,5,6)P_4 inhibition of thapsigargin-stimulated Cl^--secretion in T_{84} cells. Monolayers were preincubated with the indicated membrane-permeant tetrakisphosphate acetoxymethyl ester for 30 min prior to mounting into the Ussing chamber. CaMCS was first stimulated with carbachol (10^{-4} M) and after 25 min for a second time with thapsigargin (10^{-6} M). Data are mean peak $\Delta I_{sc} \pm$ SEM expressed as% control for six experiments. Control values represent the response to thapsigargin in co-incubated monolayers. Significant differences are denoted by probability values derived by Students' two-tailed *t*-test (* $p < 0.03$; ** $p < 0.04$; *** $p < 0.0009$). Concentration of the $InsP_4$ derivatives were 1-*O*-Bu-2-*O*-Bt-Ins(3,4,5,6)P_4/AM (**1**, 600 μM); 2-*O*-Bu-1-*O*-Bt-Ins(3,4,5,6)P_4/AM (**2**, 600 μM); 1,2-di-*O*-Bu-Ins(3,4,5,6)P_4/AM (**3**, 600 μM); *rac*-1,2-cyclo-Ins(3,4,5,6)P_4/AM (*rac*-**4**, 800 μM), 3-*O*-Bu-2-*O*-Bt-Ins(1,4,5,6)P_4/AM (*ent*-**1**, 600 μM); 2-*O*-Bu-3-*O*-Bt-Ins(1,4,5,6)P_4/AM (*ent*-**2**, 600 μM); 2,3-di-*O*-Bu-Ins(1,4,5,6)P_4/AM (*ent*-**3**, 600 μM).

binding sites will enable us to identify more potent ligands. The modified membrane-permeant Ins(3,4,5,6)P_4-derivatives presented here now enable us to map the unidentified binding-sites in the natural environment of the living cell with transepithelial Cl^--secretion as a read-out.

Derivatives for mapping the Ins(3,4,5,6)P_4-binding sites require the systematic modification of each of the functional groups, namely the hydroxyl and phosphate moieties to alter particular sites of interaction with the target. In this study, we modified the interaction potential of the hydroxyl groups by simple alkylation since previous studies have shown that the proper orientation of the phosphate moieties is essential to exhibit physiological function.[20,24] Alkylation of the respective hydroxyl group yielded Ins(3,4,5,6)P_4 derivatives, which exhibit the same stereochemistry, and an unchanged acceptor potential for hydrogen bonding. All alkylated Ins(3,4,5,6)P_4-derivatives (**1**–**4**) were unable to inhibit CaMCS. Therefore, it appears that the hydrogen bonding donor potential of the hydroxyl groups is essential for the ability to inhibit CaMCS. Furthermore, we previously reported that a 1-deoxy-derivative, where the donor and acceptor potential is missing, was incapable of inhibiting Cl^- secretion, indicating that the hydrogen bonding acceptor potential of the 1-hydoxyl group is critical. In contrast, the 2-hydroxyl group was shown to play a relatively minor role (Fig. 4), because the 2-deoxy Ins(3,4,5,6)P_4-derivative was partially active. We can therefore conclude that the lack of an effect of the 1-*O*-Bt-2-*O*-Bu-Ins(3,4,5,6)P_4-derivative (**2**) on Cl^- secretion resulted from steric hindrance of the butyl group at the 2-position rather than from loss of hydrogen bonding donor potential.

Of course, the lack of effect on Cl^- secretion does not necessarily reflect the binding properties to protein(s). We investigated the competition of our derivatives with naturally produced Ins(3,4,5,6)P_4 after carbachol stimulation. Since carbachol-mediated inhibition of thapsigargin-induced Cl^- secretion was essentially abolished by pretreatment with 1-*O*-Bu-2-*O*-Bt-Ins(3,4,5,6)P_4/AM (**1**) as well as *rac*-1,2-*O*-cyclohexylidene-Ins(3,4,5,6)P_4/AM (*rac*-**4**), compounds modified at the 1-hydroxyl position seemed to retain the ability to bind to the Ins(3,4,5,6)P_4 target protein thereby antagonizing the inhibitory effect of Ins(3,4,5,6)P_4. 1,2-Di-*O*-Bu-Ins(3,4,5,6)P_4/AM (**3**) had a slightly weaker effect and 2-*O*-Bu-1-*O*-Bt-Ins(3,4,5,6)P_4/AM (**2**) was inactive. Thus, a flexible bulky substituent at the 2-position appeared to abolish the binding properties of the Ins(3,4,5,6)P_4-derivative probably due to steric hindrance (Fig. 4). On the other hand, the more rigid positioning of the cyclohexylidene ketal in *rac*-**4** did not restrict binding, suggesting steric discrimination factors of the Ins(3,4,5,6)P_4-binding site. The binding ability of the 1-*O*-butyl compound (**1**) to the Ins(3,4,5,6)P_4-binding site points to the role of the 1-hydroxyl group as a hydrogen bonding acceptor rather than a donor group (Fig. 4). In addition, the lipophilicity of the butyl group (or the cyclohexylidene ketal) seems to promote binding as the sterically hindered 1,2-di-*O*-Bu-Ins(3,4,5,6)P_4 derivative (**3**) is also an antagonist, albeit with lower potency (Fig. 3). All enantiomeric Ins(1,4,5,6)P_4/AM-derivatives (*ent*-**1** through *ent*-**3**) did not prevent Ins(3,4,5,6)P_4 from inhibiting Cl^- secretion (Fig. 3), again indicating the importance of the proper orientation of the phosphates. In addition to the above findings, the complete reversal of the carbachol/Ins(3,4,5,6)P_4-mediated inhibition of the thapsigargin-induced Cl^- secretion suggests that carbachol inhibits subsequent CaMCS exclusively via Ins(3,4,5,6)P_4, with negligible contribution from other negative regulators.

For the development of future tools such as tethered compounds, the 1-hydroxyl group appears to be the most obvious choice to link a (hydrophobic) spacer to Ins(3,4,5,6)P_4. Furthermore, membrane-permeant deri-

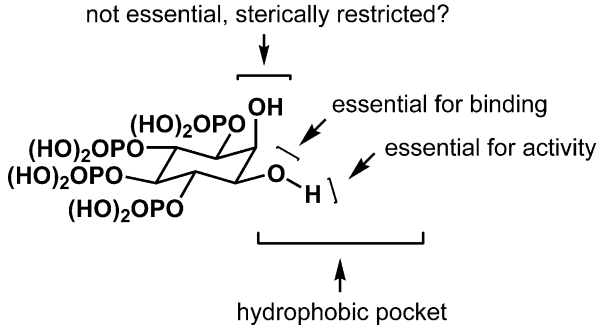


Figure 4. Relative importance of the hydroxyl groups of Ins(3,4,5,6)P_4 with respect to binding and inhibitory properties.

vatives of fluorescently labeled Ins(3,4,5,6)P_4-derivatives may help to locate the Ins(3,4,5,6)P_4 targets in living cells.

The antagonists identified in this study can serve as lead compounds for the development of pharmaceuticals, which can augment CaMCS by inactivating a feedback inhibitory mechanism. Such agents may overcome the chronically reduced electrolyte and water flux of epithelia from CF-patients. An important step for future optimization and drug development would be the identification of the putative Ins(3,4,5,6)P_4-binding proteins.

In summary, we have demonstrated that modified membrane-permeant derivatives are useful tools for probing inositol phosphate/protein interaction in intact living cells. This methodology enabled us to identify the critical nature of the hydrogen bonding acceptor potential of the 1-hydroxyl group for binding to the Ins(3,4,5,6)P_4 binding site. Furthermore, we were able to identify specific antagonists of Ins(3,4,5,6)P_4 prior to the identification of the Ins(3,4,5,6)P_4 targets. The preparation of additional derivatives in their membrane-permeant form might both increase our knowledge of the important messenger Ins(3,4,5,6)P_4 and open new possibilities for developing treatments for CF.

Experimental

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). Ethyl-*N*,*N*-di*iso*propylamine (DIEA, from Merck) was dried over sodium wire. Palladium on charcoal (10%) was from Acros Organics. Dibenzyl *N*,*N*-di*iso*propylphosphoramidite, tetrazole, peracetic acid (32%), dibutyltin oxide, and acetoxymethyl bromide were from Aldrich. 4-(Dimethylamino)pyridine (DMAP) and cesium fluoride (CsF) were from Fluka. Thapsigargin was purchased from Alexis Biochemicals, La Jolla, CA, USA. Carbachol was obtained from ICN, Irvine, CA, USA. Fura-2 acetoxymethyl ester (fura-2/AM) was purchased from Calbiochem, La Jolla, CA, USA. Cell culture membrane inserts (Snapwell, 0.4 μm pore size polycarbonate) were obtained from Corning Costar Corporation (Cambridge, MA, USA). All other reagents were at least reagent grade and were obtained commercially.

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 × 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 × 50 mm) filled with RP 18 material (Merck, LiChrospher 100, 10 μm). The eluents were methanol–water mixtures; compositions are given in % methanol (MeOH).

^{1}H NMR and ^{31}P NMR spectra were recorded on a Bruker DPX 200 or a Bruker AM 360 spectrometer. Chemical shifts were measured in ppm relative to tetramethylsilane for ^{1}H NMR spectra and external 85% H_3PO_4 for ^{31}P NMR spectra. Coupling constants (*J*) are given in Hz. Mass spectra were recorded using a Finnigan Mat 8222 mass spectrometer with fast atom bombardment (FAB) ionization. Peak intensities are given on a scale from 0 to 100. High resolution masses (HRMS) were determined relative to known compounds with a mass not differing more than 10%. Where indicated, high resolution masses were determined with direct chemical ionisation (DCI) and matched with peaks from perfluorinated kerosins (PFK) giving a maximum difference of 5 ppm. Meltings points (uncorrected) were determined using a Büchi B-540 apparatus. Optical rotations were measured at the sodium D-line in a 10-cm cell with a Perkin-Elmer 1231 polarimeter. Ultrafiltration of the palladium/carbon catalyst was performed with a Sartorius filtration apparatus SM 162 01 using filters from regenerated cellulose (Sartorius, SM 116 04). Element analyses were performed by Mikroanalytisches Labor Beller, Göttingen, Germany.

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 transports studies have been described previously.[41] In brief, T_{84} cells were grown in Dulbecco's modified Eagle's/F12 media (JRH, Lenexa, KS, USA) supplemented with 5% newborn calf serum (Hyclone, Logan, UT, USA) and 50 U/mL each of penicillin/streptomycin (Core Cell Culture Facility, UCSD). Medium was replaced every third day. 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, USA), 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, as described before.[36] The spontaneous potential difference across the monolayer was short-circuited with a voltage clamp (Model VCC MC6, Physiologic Instruments, San Diego, CA, USA). 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, USA). Increased I_{sc} stimulated through cholinergic pathways or through calcium-mobilizing agonists in T_{84} cells are fully reflective of Cl^- secretion.[42] The 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 and was adjusted to pH 7.4.

Preincubation of T_{84} cells with membrane-permeant $InsP_4$-derivatives

Confluent T_{84} cells on Snapwell inserts were rinsed with 1 mL of Ringer's solution on each side of the Snapwell. 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 the cells were washed with Ringer's solution (1 mL) and were ready for mounting. Cells for control experiments were incubated only with 100 μL of Ringer's solution with 2 μL of DMSO/5% Pluronic 127.

Synthesis

Enantiomerically pure 3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**8**) and 1,4,5,6-tetra-*O*-benzyl-*myo*-inositol (*ent*-**8**) were synthesized as described before.[40] *rac*-1,2-*O*-Cyclohexylidene-*myo*-inositol (*rac*-**5**) was synthesized by the method of Angyal and Tate.[43]

General procedure for phosphorylation

The selectively protected *myo*-inositol derivative and tetrazole were dissolved under argon in dry acetonitrile before dibenzyl *N,N*-di*iso*propylphosphoramidite was added. After stirring at room temperature for the indicated time, the reaction mixture was cooled to −40 °C and peracetic acid (32% v/w; 1 mol equiv for each mol equiv of phosphoramidite) was added. After the mixture reached room temperature the solvent was removed under reduced pressure and the residual oil was purified by preparative HPLC to give the desired inositol tetrakisphosphate derivative.

General procedure for removing the benzyl groups by hydrogenolysis

Either the fully protected *myo*-inositol tetrakisphosphate or the tetra-*O*-benzyl-*myo*-inositol derivative, respectively, was vigorously stirred with palladium on charcoal (10%; at least 0.1 mol palladium for each mol of benzyl groups) suspended in acetic acid under a hydrogen atmosphere in a self-built hydrogenation apparatus for the indicated time. The catalyst was removed by ultrafiltration and the filtrate was freeze-dried to give the respective product.

General procedure for the introduction of acetoxymethyl esters

The thoroughly dried inositol tetrakisphosphate derivative was suspended in dry acetonitrile under argon before dry DIEA (2.25 mol DIEA for each mol of hydroxy groups) and acetoxymethyl bromide (1 mol equiv for each mol equiv of DIEA) were added. After stirring the mixture at room temperature in the dark for 4 days, all volatile components were evaporated under reduced pressure and the crude residue was purified by preparative HPLC, with the solvent specified to give the inositol tetrakisphosphate octakis(acetoxymethyl) ester as a clear gum.

***rac*-1,2-*O*-Cyclohexylidene-*myo*-inositol 3,4,5,6-tetrakis (dibenzyl)phosphate (*rac*-6).** A solution of *rac*-1,2-*O*-cyclohexylidene-*myo*-inositol (*rac*-**5**) (130 mg, 500 μmol) and tetrazole (350 mg, 5.00 mmol) in acetonitrile (6 mL) was treated with dibenzyl *N,N*-diisopropylphosphoramidite[39] (1.68 mL, 5.00 mmol) for 26 h and subsequently oxidized with peracetic acid 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 (93% MeOH; 40 mL/min; $t_R = 22.35$ min) to give the fully protected compound *rac*-**6** (306 mg, 47%) as a colorless gum. ^{1}H NMR ($CDCl_3$, 360 MHz): δ 1.20–1.80 (10H, m, CH_2 [C_6H_{10}]), 4.26 (1H, dd, $J = 6.01$, 6.01 Hz, H-1), 4.66 (1H, dd, $J = 6.01$, 3.43 Hz, H-2), 4.76–4.81 (2H, m, H-3, H-5), 4.92–5.17 (18H, m, C*H*$_2$Ph, H-4, H-6), 7.20–7.40 (40H, m, CH_2*Ph*). ^{31}P NMR ($CDCl_3$, ^{1}H decoupled, 145.8 MHz): δ −1.69 (1 P, s), −1.59 (1 P, s), −1.52 (1 P, s), −1.00 (1 P, s). MS: *m*/*z* (−ve ion FAB) 1209 [(M−$C_7H_7^+$)$^-$, 1], 277 [OPO(OBn)$_2^-$, 100]. HRMS (DCI): *m*/*z* 1209.312 ± 10 ppm (M−$C_7H_7^+$)$^-$ (calcd for $C_{61}H_{65}O_{18}P_4$, 1209.312).

***rac*-1,2-*O*-Cyclohexylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate (*rac*-7).** *rac*-**6** (91 mg, 70 μmol) was dissolved in dry ethanol (4 mL) before dry ethyl-*N,N*-di*iso*propylamine (95 μL, 560 μmol) was added, followed by palladium (10%) on charcoal (84 mg, 800 μmol). After stirring the solution at room temperature for 6 days under a hydrogen atmosphere the catalyst was removed by filtration and the filtrate was dried to give the ethyl-*N,N*-di*iso*propylammonium salt of tetrakisphosphate *rac*-**7** (108 mg, 96%). ^{1}H NMR (D_2O, 360 MHz): δ 1.30–1.90 (10H, m, CH_2 [C_6H_{10}]), 4.01(1H, ddd, $J = 9.08$, 9.08, 8.23 Hz, H-5), 4.18–4.26 (2H, m, H-1, H-3), 4.32–4.40 (2H, m, H-4, H-6), 4.55 (1H, dd, $J = 4.26$, 3.69 Hz, H-2). ^{31}P NMR (D_2O, ^{1}H decoupled, 145.8 MHz): δ −0.24 (1 P, s), 0.10 (1 P, s), 0.85 (1 P, s), 0.90 (1 P, s). MS: *m*/*z* (+ve ion FAB) 581 [(M + H)$^+$, 80], 81 [PO(OH)$_2^+$, 100]. MS: *m*/*z* (−ve ion FAB) 579 [(M−H$^+$)$^-$, 100].

***rac*-1,2-*O*-Cyclohexylidene-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*rac*-4).** DIEA (205 μL, 1.20 mmol) and acetoxymethyl bromide (120 μL, 1.20 mmol) were added under argon to a suspension of compound *rac*-**7** (108 mg, 66 μmol) in acetonitrile (2 mL). After stirring the reaction mixture in the dark for 4 days all volatile compounds were evaporated off under reduced pressure and the crude residue was purified by preparative HPLC (68% MeOH, 40 mL/min, $t_R = 19.35$) to give compound *rac*-**4** (50 mg, 65%) as a syrup. ^{1}H NMR (toluene-d_8, 360 MHz): δ 1.20–1.75 (10H, m, CH_2 [C_6H_{10}]), 1.81–1.92 (24H, 8 s, 8 × OAc), 4.28 (1H, dd, $J = 5.51$, 5.51 Hz, H-1). 4.77 (1H, dd, $J = 5.51$, 3.54 Hz, H-2), 4.91–4.99 (2H, m, H-5, H-6), 5.07 (1H, ddd, $J = 8.66$, 8.27, 3.54 Hz, H-3), 5.17 (1H, ddd, $J = 9.05$, 8.66, 7.08 Hz, H-4), 5.65–5.94 (16H, m, C*H*$_2$OAc). ^{31}P NMR (toluene-d_8, ^{1}H decoupled, 145.8 MHz): δ −4.56 (1 P, s), −4.07 (1 P, s), −3.67 (1 P, s), −3.55 (1 P, s). MS: *m*/*z* (−ve ion FAB) 1083 [(M−CH_2OAc^+)$^-$, 35], 241 [OPO(OCH$_2$OAc)$_2^-$, 100]. HRMS: *m*/*z* 1157.168 [(M + H)$^+$] (calcd for $C_{36}H_{57}O_{34}P_4$, 1157.167).

D-3,4,5,6-Tetra-*O*-benzyl-1-*O*-butyl-*myo*-inositol (9). Dry **8** (250 mg, 463 μmol) and dry dibutyltin oxide (116 mg, 467 μmol) were heated under reflux in dry toluene (100 mL) in a Soxhlet apparatus with activated molecular sieves (3 Å) for 18 h. The reaction mixture was cooled to room temperature and evaporated to dryness under diminished pressure. CsF (140 mg, 926 μmol) 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 (10 mL) under argon and 1-butyl iodide (300 μL, 2.62 mmol) was added. After stirring the solution for 48 h, HPLC analysis (90% MeOH; 1.5 mL/min; $t_R = 7.43$ min) showed no further reaction. Excess of 1-butyl iodide and DMF were removed in high vacuum. The crude product was chromatographed by preparative HPLC (93% MeOH; 40 mL/min; $t_R = 22.30$ min) to give compound **9** (175 mg, 74%) as a colorless solid. Mp: 75.4–75.9 °C (from methanol). $[\alpha]_D^{24}$ +8.4° (*c* 0.98 in $CHCl_3$). ^1H NMR ($CDCl_3$, 360 MHz): δ 0.91 (3H, t, $J = 7.25$ Hz, CH_3), 1.34–1.44 (2H, m, CH_2), 1.57–1.65 (2H, m, CH_2), 2.46 (1H, s (br), OH), 3.23 (1H, dd, $J = 9.31$, 2.33 Hz, H-1), 3.43 (1H, dd, $J = 9.78$, 2.80 Hz, H-3), 3.45 (1H, dd, $J = 9.78$, 9.31 Hz, H-4), 3.57 (1H, dt, $J = 6.99$, 6.52 Hz, $OCH_2CH_2CH_2CH_3$), 3.57 (1H, dt, $J = 6.99$ Hz, $OCH_2CH_2CH_2CH_3$), 3.91 (1H, dd, $J = 9.31$, 9.31 Hz, H-5), 3.99 (1H, dd, $J = 9.78$, 9.31 Hz, H-6), 4.27 (1H, dd, $J = 2.80$, 2.33 Hz, H-2), 4.71–4.94 (8H, m, CH_2Ph), 7.25–7.39 (20H, m, CH_2Ph). MS: *m*/*z* (+ve ion FAB) 597 [$(M+H)^+$, 1], 91 [$C_7H_7^+$, 100]. Anal. ($C_{38}H_{44}O_6$) C: calcd 76.48; found 76.87; H: calcd 7.43, found 7.50.

D-1,4,5,6-Tetra-*O*-benzyl-3-*O*-butyl-*myo*-inositol (*ent*-9). A similar reaction with the diol *ent*-**8** gave compound *ent*-**9**. $[\alpha]_D^{24}$ −8.7° (*c* 1.01 in $CHCl_3$). Spectral data were in accordance with those obtained for enantiomer **9**.

D-3,4,5,6-Tetra-*O*-benzyl-1-*O*-butyl-2-*O*-butyryl-*myo*-inositol (10). A solution of **9** (178 mg, 298 μmol), butyric anhydride (210 μL, 596 μmol), and DMAP (38 mg, 30 μmol) in dry pyridine (3 mL) was stirred at room temperature for 18 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 (10 mL) and was washed once with phosphate buffer (10 mL), once with sodium hydrogen carbonate (10 mL), once with sodium hydrogen sulfate (10 mL), once again with phosphate buffer (10 mL) and then with brine (10 mL). The organic layer was dried over Na_2SO_4 and filtered. Evaporation of the solvent gave pure **10** (194 mg, 98%) as a colorless oil. $[\alpha]_D^{24}$ −10.4° (*c* 1.97 in $CHCl_3$). ^1H NMR ($CDCl_3$, 360 MHz): δ 0.91 (3H, t, $J = 7.25$ Hz, CH_3), 0.98 (3H, t, $J = 7.46$ Hz, CH_3), 1.32–1.42 (2H, m, CH_2), 1.50–1.60 (2H, m, CH_2), 1.69 (2H, tq, $J = 7.46$, 7.46 Hz, $O(O)CCH_2CH_2CH_3$), 2.39 (2H, t, $J = 7.46$ Hz, $O(O)CCH_2CH_2CH_3$), 3.31 (1H, dd, $J = 9.66$, 2.63 Hz, H-1), 3.44 (1H, dt, $J = 9.07$, 6.81 Hz, $OCH_2CH_2CH_2CH_3$), 3.48 (1H, dd, $J = 9.66$, 3.07 Hz, H-3), 3.49 (1H, dd, $J = 9.66$, 9.22 Hz, H-5), 3.67 (1H, dt, $J = 8.78$, 6.81 Hz, $OCH_2CH_2CH_2CH_3$), 3.81 (1H, dd, $J = 9.66$, 9.66 Hz, H-4), 3.86 (1H, dd, $J = 9.66$, 9.22 Hz, H-6), 4.51–4.93 (8H, m, CH_2Ph), 5.83 (1H, dd, $J = 3.07$, 2.63 Hz, H-2), 7.26–7.36 (20H, m, CH_2Ph). MS: *m*/*z* (+ve ion FAB) 667 [$(M+H)^+$, 21], 91 [$C_7H_7^+$, 100]. HRMS: *m*/*z* 667.364 [$(M+H)^+$] (calcd for $C_{42}H_{51}O_7$, 667.363).

D-1,4,5,6-Tetra-*O*-benzyl-3-*O*-butyl-2-*O*-butyryl-*myo*-inositol (*ent*-10). Compound *ent*-**9** was butyrylated as described above for the other enantiomer to give compound *ent*-**10**. $[\alpha]_D^{24}$ +10.7° (*c* 2.05 in $CHCl_3$). HRMS: *m*/*z* 667.367 [$(M+H)^+$] (calcd for $C_{42}H_{51}O_7$, 667.363). ^1H NMR and MS data were in accordance with those of enantiomer **10**.

D-1-*O*-Butyl-2-*O*-butyryl-*myo*-inositol (11). Compound **10** (178 mg, 267 μmol) was hydrogenated with palladium (10%) on charcoal under hydrogen as described in the general procedure to give tetrol **11** (81 mg, 99%) as a solid after freeze-drying. Mp 125–125.9 °C (from MeOH). $[\alpha]_D^{24}$ +26.5° (*c* 2.0 in MeOH). ^1H NMR (DMSO-d_6, 360 MHz): δ 0.84 (3H, t, $J = 7.38$ Hz, CH_3), 0.90 (3H, t, $J = 7.38$ Hz, CH_3), 1.21–1.31 (2H, m, CH_2), 1.36–1.44 (2H, m, CH_2), 1.54 (2H, tq, $J = 7.38$, 7.00 Hz, $O(O)CCH_2CH_2CH_3$), 2.24 (2H, t, $J = 7.00$ Hz, $O(O)CCH_2CH_2CH_3$), 2.97 (1H, dd, $J = 8.94$, 8.55 Hz, H-5), 3.10 (1H, dd, $J = 9.72$, 2.72 Hz, H-1), 3.25–3.35 (4H, m, H-3, H-4, H-6, $OCH_2CH_2CH_2CH_3$), 3.50 (1H, dt, $J = 8.94$, 6.60 Hz, $OCH_2CH_2CH_2CH_3$), 4.85 (4H, s (br), OH), 5.36 (1H, dd, $J = 2.72$, 2.33 Hz, H-2). MS: *m*/*z* (+ve ion FAB) 307 [$(M+H)^+$, 21], 71 [Bt^+, 100]; *m*/*z* (−ve ion FAB) 305 [$(M-H^+)^-$, 27], 87 [BtO^-, 100]. Anal. ($C_{14}H_{26}O_7$) C: calcd 54.89; found 54.45; H: calcd 8.55, found 8.56.

D-3-*O*-Butyl-2-*O*-butyryl-*myo*-inositol (*ent*-11). A similar reaction of the fully protected compound *ent*-**10** afforded tetrol *ent*-**11**. $[\alpha]_D^{24}$ −26.6° (*c* 0.76 in MeOH). ^1H NMR and MS data were in accordance with those obtained for enantiomer **11**.

D-1-*O*-Butyl-2-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakis(dibenzyl)phosphate (12). A solution of tetrol **11** (63 mg, 206 μmol) and tetrazole (174 mg, 2.47 mmol) in acetonitrile (2 mL) was treated with dibenzyl *N*,*N*-di*iso*propylphosphoramidite (834 μL, 2.47 mmol) for 18 h, oxidized with peracetic acid, and worked up as described above. Purification by preparative HPLC (93% MeOH; 40 mL/min; $t_R = 26.45$ min) gave compound **12** (165 mg, 60%) as a colorless gum. $[\alpha]_D^{24}$ −4.9° (*c* 1.08 in $CHCl_3$). ^1H NMR ($CDCl_3$, 360 MHz): δ 0.77 (3H, t, $J = 7.27$ Hz, CH_3), 0.93 (3H, t, $J = 7.27$ Hz, CH_3), 1.12–1.21 (2H, m, CH_2), 1.31–1.46 (2H, m, CH_2), 1.62 (2H, tq, $J = 7.26$, 7.26 Hz, $O(O)CCH_2CH_2CH_3$), 2.24 (2H, t, $J = 7.26$ Hz, $O(O)CCH_2CH_2CH_3$), 3.26 (1H, dt, $J = 8.23$, 5.81 Hz, $OCH_2CH_2CH_2CH_3$), 3.37 (1H, dd, $J = 9.20$, 2.90 Hz, H-1), 3.44 (1H, dt, $J = 8.23$, 7.26 Hz, $OCH_2CH_2CH_2CH_3$), 4.35 (1H, ddd, $J = 9.69$, 9.69, 2.42 Hz, H-3), 4.53 (1H, ddd, $J = 9.69$, 9.69, 9.69 Hz, H-5), 4.68 (1H, ddd, $J = 9.69$, 9.69, 9.20 Hz, H-6), 4.91 (1H, ddd, $J = 9.84$,9.69, 9.69 Hz, H-4), 4.92–5.02 (16H, m, CH_2Ph), 5.91 (1H, dd, $J = 2.90$, 2.42 Hz, H-2), 7.11–7.30 (40H, m, CH_2Ph). ^{31}P NMR ($CDCl_3$, ^1H decoupled, 145.8 MHz): δ −1.81 (1 P, s), −1.18 (1 P, s), −0.72 (1 P, s), −0.66 (1 P, s). MS: *m*/*z* (+ve ion FAB) 1347

$[(M+H)^+$, 8], 91 [$C_7H_7^+$, 100]. HRMS: *m/z* 1347.416 $[(M+H)^+]$ (calcd for $C_{70}H_{79}O_{19}P_4$, 1347.417).

D-3-*O*-Butyl-2-*O*-butyryl-*myo*-inositol 1,4,5,6-tetrakis (dibenzyl)phosphate (*ent*-12). Tetrol *ent*-**11** was phosphitylated and oxidized as described above for compound **12** to give the fully protected phosphate *ent*-**12**. $[\alpha]_D^{24}$ +4.8° (*c* 2.09 in $CHCl_3$). HRMS (DCI): *m/z* 1255.345 ± 10 ppm $[(M-C_7H_7^+)^-]$ (calcd for $C_{63}H_{79}O_{19}P_4$, 1255.345). ^{1}H NMR, ^{31}P NMR, and MS data were in accordance with those obtained for enantiomer **12**.

D-1-*O*-Butyl-2-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate (13). Compound **12** (160 mg, 118 μmol) was hydrogenated with palladium (10%) on carbon as described in the general procedure to give compound **13** (73 mg, 99%) as a solid after freeze-drying. $[\alpha]_D^{24}$ +4.1° (*c* 1.04 in H_2O, pH 1.6). ^{1}H NMR (D_2O, 360 MHz): δ 0.81 (3H, t, *J* = 7.30 Hz, CH_3), 0.91 (3H, t, *J* = 7.30 Hz, CH_3), 1.22–1.30 (2H, m, CH_2), 1.42–1.49 (2H, m, CH_2), 1.62 (2H, tq, *J* = 7.30, 7.30 Hz, O(O)CCH_2CH_2CH$_3$), 2.36–2.54 (2H, m, O(O)CCH_2CH$_2$CH$_3$), 3.56 (1H, dt, *J* = 9.49, 6.46 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 3.63 (1H, dt, *J* = 9.36, 6.74 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 3.70 (1H, dd, *J* = 9.74, 2.62 Hz, H-1), 4.27 (1H, ddd, *J* = 9.36, 9.36, 9.36 Hz, H-5), 4.31 (1H, ddd, *J* = 9.74, 9.74, 2.25 Hz, H-3), 4.39 (1H, ddd, *J* = 9.74, 9.36, 9.36 Hz, H-6), 4.49 (1H, ddd, *J* = 9.74, 9.36, 9.00 Hz, H-4), 5.75 (1H, dd, *J* = 2.62, 2.25 Hz, H-2), ^{31}P NMR (D_2O, ^{1}H decoupled, 145.8 MHz): δ −0.10 (2 P, s), 0.50 (1 P, s), 0.80 (1 P, s). MS: *m/z* (+ve ion FAB) 627 $[(M+H)^+$, 4], 71 [Bt^+, 100]. MS: *m/z* (−ve ion FAB) 625 $[(M-H^+)^-$, 100]. HRMS: *m/z* 625.025 $[(M-H^+)^-]$ (calcd for $C_{14}H_{29}O_{19}P_4$, 625.025).

D-3-*O*-Butyl-2-*O*-butyryl-*myo*-inositol 1,4,5,6-tetrakisphosphate (*ent*-13). A similar reaction with the fully protected compound *ent*-**12** afforded the free acid *ent*-**13** after freeze-drying. $[\alpha]_D^{24}$ −4.1° (*c* 0.78 in H_2O, pH 1.6). HRMS: *m/z* 625.023 $[(M-H^+)^-]$ (calcd for $C_{14}H_{29}O_{19}P_4$, 625.025). ^{1}H NMR and MS data were in accordance with those obtained for enantiomer **13**.

D-1-*O*-Butyl-*myo*-inositol 3,4,5,6-tetrakisphosphate (14). An aqueous solution of compound **13** (17 mg, 27 μmol) was treated with 1 M KOH (260 μL) to adjust the pH 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 **14** (14 mg, 94%). $[\alpha]_D^{24}$ +4.9° (*c* 0.53 in H_2O, pH 1.6). ^{1}H NMR (D_2O, 360 MHz): δ 0.78 (3H, t, *J* = 7.44 Hz, CH_3), 1.26 (2H, tq, *J* = 7.44, 7.44 Hz, CH_2), 1.43–1.52 (2H, m, CH_2), 3.42 (1H, dd, *J* = 9.60, 2.78 Hz, H-1), 3.52 (1H, dt, *J* = 9.71, 6.74 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 3.59 (1H, dt, *J* = 9.81, 6.74 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 4.16 (1H, ddd, *J* = 9.60, 9.60, 2.78 Hz, H-3), 4.21 (1H, ddd, *J* = 9.37, 9.37, 9.37 Hz, H-5), 4.35 (1H, dd, *J* = 2.78, 2.78 Hz, H-2), 4.39 (1H, ddd, *J* = 9.60, 9.60, 9.37 Hz, H-6), 4.46 (1H, ddd, *J* = 9.60, 9.60, 9.37 Hz, H-4). ^{31}P NMR (D_2O, ^{1}H decoupled, 145.8 MHz): δ 0.10 (2 P, s), 0.70 (1 P, s), 1.05 (1 P, s); *m/z* (−ve ion FAB) 555 $[(M-H^+)^-$, 100]. HRMS: *m/z* 554.979 $[(M-H^+)^-]$ (calcd for $C_{10}H_{23}O_{18}P_4$, 554.984).

D-3-*O*-Butyl-*myo*-inositol 1,4,5,6-tetrakisphosphate (*ent*-14). The butyryl group of *ent*-**13** was hydrolyzed by the same method described above to give the tetrakisphosphate *ent*-**14**. $[\alpha]_D^{24}$ −4.6° (*c* 0.35 in H_2O, pH 1.6). HRMS: *m/z* 554.982 $[(M-H^+)^-]$ (calcd for $C_{10}H_{23}O_{18}P_4$, 554.984). ^{1}H NMR, ^{31}P NMR, and MS data were in accordance with those obtained for enantiomer **14**.

D-1-*O*-Butyl-2-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (1). DIEA (131 μL, 768 μmol) and acetoxymethyl bromide (77 μL, 768 μmol) were added to a suspension of compound **13** (30 mg, 47 μmol) in acetonitrile (2 mL) as described in the general procedure. Purification by preparative HPLC (73% MeOH, 37.5 mL/min, t_R = 19.30) gave compound **1** (31 mg, 55%) as a colorless syrup. $[\alpha]_D^{24}$ −2.0° (*c* 1.00 in toluene). ^{1}H NMR (toluene-d_8, 360 MHz): δ 0.85 (3H, t, *J* = 7.48 Hz, CH_3), 0.97 (3H, t, *J* = 7.48 Hz, CH_3), 1.39 (2H, tq, *J* = 7.48, 7.28 Hz, CH_2), 1.51–1.67 (4H, m, 2 × CH_2), 1.75–1.75 (24H, 8 s, 8 × OAc), 2.08–2.13 (2H, m, CH_2), 3.07 (1H, dd, *J* = 9.45, 2.76 Hz, H-1), 3.46 (1H, dt, *J* = 7.68, 5.91 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 3.62 (1H, dt, *J* = 7.74, 7.68 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 4.62 (1H, ddd, *J* = 9.84, 9.84, 2.76 Hz, H-3), 4.67 (1H, ddd, *J* = 9.84, 9.84, 9.45 Hz, H-5), 4.80 (1H, ddd, *J* = 9.45, 9.45, 9.16 Hz, H-6), 5.04 (1H, ddd, *J* = 9.84, 9.84, 9.45 Hz, H-4), 5.63–5.96 (16H, m, CH_2OAc), 6.00 (1H, dd, *J* = 2.76, 2.76 Hz, H-2). ^{31}P NMR (toluene-d_8, ^{1}H decoupled, 145.8 MHz): δ −5.14 (1 P, s), −4.49 (1 P, s), −4.06 (1 P, s), −3.99 (1 P, s). MS: *m/z* (+ve ion FAB) 1131 $[(M-CH_2OAc^+ + 2H)^+$, 58], 987 $[(M-3\ CH_2OAc^+ + 4H)^+$, 100], *m/z* (−ve ion FAB) 1129 $[(M-CH_2OAc^+)^-$, 38], 241 $[OPO(OCH_2OAc)_2^-$, 100]. HRMS: *m/z* 1131.189 $[(M-CH_2OAc^+ + 2H)^+]$ (calcd for $C_{35}H_{59}O_{33}P_4$, 1131.189).

D-3-*O*-Butyl-2-*O*-butyryl-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*ent*-1). Alkylation of the phosphate *ent*-**21** as described above afforded the octakis(acetoxymethyl) ester *ent*-**1**. $[\alpha]_D^{24}$ +1.9° (*c* 1.46 in toluene). HRMS: *m/z* 1129.171 $[(M-CH_2OAc^+)^-]$ (calcd for $C_{35}H_{57}O_{33}P_4$, 1129.173). ^{1}H NMR, ^{31}P NMR, and MS data were in accordance with those obtained for enantiomer **1**.

D-1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (15). Dry **8** (690 mg, 1.28 mmol) and dry dibutyltin oxide (324 mg, 1.3 mmol) were heated to reflux in dry toluene (150 mL) in a Soxhlet apparatus with activated molecular sieves (3 Å) for 20 h. The reaction mixture was cooled to room temperature and evaporated to dryness under diminished pressure. CsF (388 mg, 2.56 mol) 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 (10 mL) under argon and 1-allyl iodide (329 μL, 3.58 mmol) was added. After stirring the solution for 20 h, HPLC analysis (95% MeOH; 1.5 mL/min; t_R = 3.20 min) showed no further reaction. Excess

of 1-allyl iodide and DMF were removed in high vacuum. The crude product was chromatographed by preparative HPLC (90% MeOH; 40 mL/min; $t_R = 26.15$ min) to give compound **15** (448 mg, 60%) as a solid. Mp 71–72 °C (from methanol). $[\alpha]_D^{24}$ +4.6° (*c* 0.98 in $CHCl_3$). ^1H NMR ($CDCl_3$, 360 MHz): δ 3.30 (1H, dd, *J* = 9.73, 3.10 Hz, H-1), 3.41 (1H, dd, *J* = 9.73, 2.65, H-3), 3.44 (1H, dd, *J* = 9.51, 9.51, H-5), 3.95 (1H, dd, *J* = 9.73, 9.51 Hz, H-6), 3.99 (1H, dd, *J* = 9.73, 2.51 Hz, H-4), 4.18 (1H, dd, *J* = 1.65, 1.33 Hz, OCH_2CHCH_2), 4.19 (1H, dd, *J* = 2.65, 1.33 Hz, OCH_2CHCH_2), 4.20 (1H, dd, *J* = 3.10, 2.65 Hz, H-2), 4.71–4.92 (8H, m, CH_2Ph), 5.19 (1H, ddt, *J* = 11.50, 1.33, 1.33 Hz, OCH_2CHCH_2), 5.29 (1H, ddt, *J* = 16.91, 2.65, 1.33 Hz, OCH_2CHCH_2), 5.94 (1H, dddd, *J* = 16.91, 11.50, 1.33, 1.33 Hz, OCH_2CHCH_2), 7.26–7.37 (20H, m, CH_2Ph). MS: *m*/*z* (+ve ion FAB) 581 $[(M+H)^+, 1]$, 91 $[C_7H_7^+, 100]$, *m*/*z* (−ve ion FAB) 580 $[(M-H^+)^-, 6]$, 489 $[(M-C_7H_7^+)^-, 100]$.

D-3-*O*-Allyl-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol (*ent*-15). A similar reaction and workup of the diol *ent*-**8** gave compound *ent*-**15**. $[\alpha]_D^{24}$ −3.9° (*c* 0.82 in $CHCl_3$). ^1H NMR and MS data were in accordance with those obtained for enantiomer **15**.

D-1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-2-*O*-butyl-*myo*-inositol (16). Sodium hydride (46 mg, 1.92 mmol) was added to a stirred solution of **15** (445 mg, 767 μmol) in dry DMF (5 mL) at room temperature in the dark. The mixture was stirred for 5 h, after which 1-butyl iodide (306 μL, 2.68 mmol) was added. The suspension was stirred for 18 h at 80 °C when HPLC (95% MeOH; 1.5 mL/min; $t_R = 7.35$) showed a complete reaction. Excess of 1-butyl iodide and DMF were evaporated off under reduced pressure. The mixture was then dissolved in *tert*-butyl methyl ether (40 mL) and washed once with phosphate buffer (20 mL), aq sodium dithionate (20 mL) and brine (20 mL), successively. The organic layer was dried over Na_2SO_4, filtered, and the ether was evaporated off to give an oil. The crude oil was purified by preparative HPLC (93% MeOH; 40 mL/min; $t_R = 37.10$ min) to give the title compound **16** (458 mg, 94%) as an oil. $[\alpha]_D^{24}$ +1.5° (*c* 2.29 in $CHCl_3$). ^1H NMR ($CDCl_3$, 360 MHz): δ 0.95 (3H, t, *J* = 7.27 Hz, CH_3), 1.39–1.49 (2H, m, CH_2), 1.58–1.66 (2H, m, CH_2), 3.24 (1H, dd, *J* = 9.69 2.42 Hz, H-1), 3.36 (1H, dd, *J* = 9.93, 2.42, H-3), 3.46 (1H, dd, *J* = 9.45, 9.45, H-5), 3.78 (2H, t, *J* = 6.54 Hz, $OCH_2CH_2CH_2CH_3$), 3.89 (1H, dd, *J* = 2.42, 2.42 Hz, H-2), 3.98 (1H, dd, *J* = 9.45, 9.45 Hz, H-6), 4.03 (1H, dd, *J* = 9.45, 9.45 Hz, H-4), 4.15 (1H, dd, *J* = 5.81, 1.00 Hz, OCH_2CHCH_2), 4.16 (1H, dd, *J* = 6.06, 1.00 Hz, OCH_2CHCH_2), 4.72–4.95 (8H, m, CH_2Ph), 5.19 (1H, ddt, *J* = 10.66, 1.45, 1.45 Hz, OCH_2CHCH_2), 5.32 (1H, ddt, *J* = 16.95, 1.45, 1.45 Hz, OCH_2CHCH_2), 5.95 (1H, dddd, *J* = 16.95, 10.66, 1.45, 1.45 Hz, OCH_2CHCH_2), 7.26–7.40 (20H, m, CH_2Ph). MS: *m*/*z* (+ve ion FAB) 637 $[(M+H)^+, 1]$, 91 $[C_7H_7^+, 100]$. HRMS: *m*/*z* 637.373 $[(M+H)^+]$ (calcd $C_{41}H_{49}O_6$, 637.353).

D-3-*O*-Allyl-1,4,5,6-tetra-*O*-benzyl-2-*O*-butyl-*myo*-inositol (*ent*-16). A similar reaction and workup of the compound *ent*-**15** gave compound *ent*-**16**. $[\alpha]_D^{24}$ −1.6° (*c* 1.87 in $CHCl_3$). HRMS: *m*/*z* 637.357 $[(M+H)^+]$ (calcd $C_{41}H_{49}O_6$, 637.353). ^1H NMR and MS data were in accordance with those obtained for enantiomer **16**.

D-3,4,5,6-Tetra-*O*-benzyl-2-*O*-butyl-*myo*-inositol (17). Tris(triphenylphosphine)-rhodium(I) chloride (140 mg, 150 μmol) and DIEA (25 μL, 140 μmol) were added to a suspension of **16** (458 mg, 720 μmol) in 50% ethanol (90 mL). The suspension was heated to reflux for 7 h. The mixture was cooled to room temperature before trifluoroacetic acid (7 mL) was added and the solution was stirred for additional 24 h, when HPLC analysis (90% MeOH; 1.5 mL/min; $t_R = 4.03$ min) showed no more starting material. After neutralization with aq NH_3 (2 N) the solvents were evaporated under reduced pressure to give a syrup. The syrup was dissolved in *tert*-butyl methyl ether (40 mL) and washed once with phosphate buffer (20 mL) and brine (20 mL), successively. The organic layer was dried over Na_2SO_4, filtered and the ether was evaporated off to give an oil. The crude oil was purified by preparative HPLC (92% MeOH; 40 mL/min; $t_R = 27.40$ min) to give **17** (275 mg, 74%) as a solid. Mp 112.3–113.1 °C (from MeOH). $[\alpha]_D^{24}$ +24.6° (*c* 0.97 in $CHCl_3$). ^1H NMR ($CDCl_3$, 360 MHz): δ 0.92 (3H, t, *J* = 7.36 Hz, CH_3), 1.32–1.43 (2H, m, CH_2), 1.53–1.61 (2H, m, CH_2), 3.41 (1H, dd, *J* = 9.96, 2.60 Hz, H-1), 3.45 (1H, dd, *J* = 9.52, 2.60 Hz, H-3), 3.46 (1H, dd, *J* = 9.31, 9.31 Hz, H-5), 3.61 (1H, dt, *J* = 9.09, 6.49 Hz, $OCH_2CH_2CH_2$), 3.74 (1H, dd, *J* = 9.52, 9.31 Hz, H-4), 3.86 (1H, dd, *J* = 2.60, 2.60 Hz, H-2), 3.95 (1H, dt, *J* = 8.95, 6.49 Hz, $OCH_2CH_2CH_2$), 3.98 (1H, dd, *J* = 9.96, 9.31 Hz, H-6), 4.79–4.94 (8H, m, CH_2Ph), 7.26–7.36 (20H, m, CH_2Ph). MS: *m*/*z* (+ve ion FAB) 597 $[(M+H)^+, 1]$, 91 $[C_7H_7^+, 100]$. MS: *m*/*z* (−ve ion FAB) 595 $[(M-H^+)^-, 100]$, $[(M-C_7H_7^+)^-, 20]$. Anal. ($C_{38}H_{44}O_6$) C: calcd 76.48; found 76.52; H: calcd 7.43, found 7.40.

D-1,4,5,6-Tetra-*O*-benzyl-2-*O*-butyl-myo-inositol (*ent*-17). A similar reaction of compound *ent*-**16** gave *ent*-**17**. $[\alpha]_D^{24}$ −24.9° (*c* 1.00 in $CHCl_3$). ^1H NMR and MS data were in accordance with those obtained for enantiomer **17**.

D-3,4,5,6-Tetra-*O*-benzyl-2-*O*-butyl-1-*O*-butyryl-*myo*-inositol (18). A solution of alcohol **17** (178 mg, 298 μmol) in dry pyridine (4 mL) was treated with butyric anhydride (158 μL, 447 μmol) and DMAP (38 mg, 29 μmol) and stirred at room temperature. After 18 h, HPLC analysis (90% MeOH; 1.5 mL/min, $t_R = 6.40$ min) showed no more starting material. The reaction mixture was evaporated under reduced pressure to give a crude oil. To remove residual pyridine the oil was dissolved in octane and evaporated three times. The residue was dissolved in *tert*-butyl methyl ether (20 mL) and was washed once with phosphate buffer (10 mL), once with sodium hydrogen carbonate (10 mL), once with sodium hydrogen sulfate (10 mL), once again with phosphate buffer (10 mL), and then with brine (10 mL). The organic layer was dried over Na_2SO_4 and filtered. Evaporation of the solvent gave pure **18** (176 mg, 89%) as a colorless oil. $[\alpha]_D^{24}$ −15.4° (*c* 1.00 in $CHCl_3$). ^1H NMR

($CHCl_3$, 360 MHz): δ 0.91 (3H, t, $J=7.21$ Hz, CH_3), 0.93 (3H, t, $J=7.21$ Hz, CH_3), 1.35–1.56 (4H, m, 2 × CH_2), 1.58–1.72 (2H, m, CH_2), 2.21–2.26 (2H, m, CH_2), 3.48 (1H, dd, $J=9.61$, 2.40 Hz, H-3), 3.51 (1H, dd, $J=9.66$, 9.37 Hz, H-5), 3.52 (2H, tq, $J=8.97$, 6.25 Hz, O(O)CCH_2CH$_2$CH$_3$), 3.76 (2H, t, $J=9.13$, 6.25 Hz, O(O)CCH_2CH2CH$_3$), 3.94 (1H, dd, $J=2.40$, 2.40 Hz, H-2), 4.00 (1H, dd, $J=9.61$, 9.61 Hz, H-4), 4.02 (1H, dd, $J=9.85$, 9.31 Hz, H-6), 4.73 (1H, dd, $J=9.85$, 2.40 Hz, H-1), 4.65–4.93 (8H, m, CH_2Ph), 7.25–7.34 (20H, m, CH$_2$*Ph*). MS: *m/z* (+ve ion FAB) 667 [$(M+H)^+$, 1], 91 [$C_7H_7^+$, 100]. HRMS: *m/z* 667.368 [$(M+H)^+$] (calcd for $C_{42}H_{51}O_7$, 667.363).

D-1,4,5,6-Tetra-*O*-benzyl-2-*O*-butyl-3-*O*-butyryl-*myo*-inositol (*ent*-18). Compound *ent*-**17** was butyrylated as described above for the other enantiomer to give compound *ent*-**18**. $[\alpha]_D^{24}$ +15.9° (*c* 1.10 in $CHCl_3$). HRMS: *m/z* 667.362 [$(M+H)^+$] (calcd for $C_{42}H_{51}O_7$, 667.363). ^{1}H NMR and MS data were in accordance with those of enantiomer **18**.

D-2-*O*-Butyl-1-*O*-butyryl-*myo*-inositol (19). Compound **18** (170 mg, 255 μmol) was hydrogenated with palladium (10%) on carbon under hydrogen as described in the general procedure to give tetrol **19** (75 mg, 97%) as a solid after freeze-drying. Mp 131.2–131.8 °C (from ethanol). $[\alpha]_D^{24}$ +41.9° (*c* 1.10 in MeOH). ^{1}H NMR (DMSO-d_6, 360 MHz): δ 0.87 (3H, t, $J=7.16$ Hz, CH_3), 0.89 (3H, t, $J=7.33$ Hz, CH_3), 1.28–1.48 (4H, m, 2 × CH_2), 1.52–1.62 (2H, m, CH_2), 2.24–2.36 (2H, m, CH_2), 2.96 (1H, dd, $J=9.21$, 9.21 Hz, H-5), 3.26 (1H, dd, $J=9.55$, 2.39 Hz, H-3), 3.35 (1H, dd, $J=9.55$, 9.21 Hz, H-4), 3.44 (1H, dt, $J=9.43$, 6.39 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 3.52 (1H, dd, $J=10.23$, 9.21 Hz, H-6), 3.57 (1H, dd, $J=2.39$, 2.39 Hz, H-2), 4.47 (1H, dt, $J=9.21$, 6.39 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 4.47 (1H, dd, $J=10.23$, 2.39 Hz, H-1), 4.71 (2H, s (br), OH), 4.82 (1H, s (br), OH), 4.86 (1H, s (br), OH), MS: *m/z* (+ve ion FAB) 307 [$(M+H)^+$, 100]. MS: *m/z* (−ve ion FAB) 305 [$(M-H^+)^-$, 34], 87 [BtO^-, 100]. Anal. ($C_{14}H_{44}O_6$) C: calcd 54.89; found 54.90; H: calcd 8.55, found 8.51.

D-2-*O*-Butyl-3-*O*-butyryl-*myo*-inositol (*ent*-19). A similar reaction of the fully protected compound *ent*-**18** afforded tetrol *ent*-**19**. $[\alpha]_D^{24}$ −40.5° (*c* 1.00 in MeOH). ^{1}H NMR and MS data were in accordance with those obtained for enantiomer **19**.

D-2-*O*-Butyl-1-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakis(dibenzyl)phosphate (20). A solution of compound **19** (55 mg, 178 μmol) and tetrazole (152 mg, 2.15 mmol) in acetonitrile (2 mL) was treated with dibenzyl *N*,*N*-di*iso*-propylphosphoramidite (726 μL, 2.15 mmol) for 22 h, then oxidized with peracetic acid, and worked up as described. Purification by preparative HPLC (92% MeOH; 40 mL/min; $t_R=29.00$ min) gave compound **20** (192 mg, 80%) as a colorless oil. $[\alpha]_D^{24}$ −3.4° (*c* 1.02 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 360 MHz): δ 0.79 (3H, t, $J=7.30$ Hz, CH_3), 0.86 (3H, t, $J=7.30$ Hz, CH_3), 1.23–1.38 (2H, m, CH_2), 1.41–1.57 (4H, m, 2 × CH_2), 2.01–2.17(2H, m, CH_2), 3.54 (1H, dt, $J=8.52$, 6.63 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 3.60 (1H, dt, $J=8.52$, 7.74 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 4.13 (1H, dd, $J=2.52$, 2.52 Hz, H-2), 4.22 (1H, ddd, $J=9.73$, 9.73, 2.52 Hz, H-3), 4.43 (1H, ddd, $J=9.51$, 9.51, 9.51 Hz, H-5), 4.44 (1H, ddd, $J=9.51$, 9.51, 9.28 Hz, H-6), 4.89 (1H, ddd, $J=9.73$,9.73, 9.51 Hz, H-4), 4.91–5.08 (17H, m, CH_2Ph, H-1), 7.13–7.27 (40H, m, CH$_2$*Ph*). ^{31}P NMR ($CDCl_3$, ^{1}H decoupled, 145.8 MHz): δ −1.64 (1 P, s), −1.42 (1 P, s), −0.76 (1 P, s), −0.53 (1 P, s). MS: *m/z* (+ve ion FAB) 1347 [$(M+H)^+$, 33], 71 [Bt^+, 100], *m/z* (−ve ion FAB) 1255 [$(M-C_7H_7^+)^-$, 16], 277 [$OPO(OBn)_2^-$, 100]. HRMS: *m/z* 1347.418 [$(M+H)^+$] (calcd for $C_{70}H_{79}O_{19}P_4$, 1347.417).

D-2-*O*-Butyl-3-*O*-butyryl-*myo*-inositol 1,4,5,6-tetrakis(dibenzyl)phosphate (*ent*-20). Compound *ent*-**19** was phosphitylated and oxidized as described above for compound **19** to give the fully protected phosphate *ent*-**20**. $[\alpha]_D^{24}$ +3.1° (*c* 1.10 in $CHCl_3$). HRMS (DCI): *m/z* 1255.354 ± 10 ppm [$(M-C_7H_7^+)^-$] (calcd for $C_{63}H_{71}O_{19}P_4$, 1255.354). ^{1}H NMR and MS data were in accordance with those obtained for enantiomer **20**.

D-2-*O*-Butyl-1-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate (21). Compound **20** (190 mg, 141 μmol) was hydrogenated with palladium (10%) on carbon as described in the general procedure to give title compound **21** (87 mg, 99%) as a solid after freeze-drying. $[\alpha]_D^{24}$ +9.9° (*c* 1.10 in H_2O, pH 1.6). ^{1}H NMR (D_2O, 360 MHz): δ 0.75 (3H, t, $J=7.15$ Hz, CH_3), 0.76 (3H, t, $J=7.31$ Hz, CH_3), 1.19–1.29 (2H, m, CH_2), 1.38–1.53 (4H, m, 2 × CH_2), 2.30 (2H, t, $J=7.47$, O(O)CCH_2CH$_2$CH$_3$), 3.58 (1H, dt, $J=9.64$, 6.44 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 3.67 (1H, dt, $J=9.64$, 6.36 Hz, OCH_2CH$_2$CH$_2$CH$_3$), 4.01 (1H, dd, $J=2.54$, 2.23 Hz, H-2), 4.22 (1H, ddd, $J=9.22$, 9.22, 8.90 Hz, H-5), 4.23 (1H, ddd, $J=9.54$, 9.54, 2.54 Hz, H-3), 4.43 (1H, ddd, $J=9.90$, 9.22, 9.22 Hz, H-6), 4.48 (1H, ddd, $J=9.54$, 9.54, 9.54 Hz, H-4), 4.94 (1H, dd, $J=9.90$, 2.23 Hz, H-1). ^{31}P NMR (D_2O, ^{1}H decoupled, 145.8 MHz): δ −0.20 (2 P, s), 0.40 (1 P, s), 0.50 (1 P, s). MS: *m/z* (+ve ion FAB) 627 [$(M+H)^+$, 8], 71 [Bt^+, 100], *m/z* (−ve ion FAB) 625 [$(M-H^+)^-$, 35], 79 [$OP(O)_2^-$, 100]. HRMS: *m/z* 625.030 [$(M-H^+)^-$] (calcd for $C_{14}H_{29}O_{19}P_4$, 625.025).

D-2-*O*-Butyl-3-*O*-butyryl-*myo*-inositol 1,4,5,6-tetrakisphosphate (*ent*-21). A similar reaction with the fully protected substrate *ent*-**20** afforded the free acid *ent*-**21** after freeze-drying. $[\alpha]_D^{24}$ −9.7° (*c* 1.00 in H_2O, pH 1.6). HRMS: *m/z* 625.027 [$(M-H^+)^-$] (calcd for $C_{14}H_{29}O_{19}P_4$, 625.025). ^{1}H NMR and MS data were in accordance with those obtained for enantiomer **21**.

D-2-*O*-Butyl-*myo*-inositol 3,4,5,6-tetrakisphosphate (22). Compound **21** (33 mg, 52 μmol) was treated with 1 M KOH (453 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 **22** (27 mg, 93%). $[\alpha]_D^{24}$ −1.1° (*c* 0.89 in H_2O, pH 1.6). ^{1}H NMR (D_2O, 360 MHz): δ 0.77 (3H, t, $J=7.37$ Hz, CH_3), 1.21–1.31 (2H, m, CH_2), 1.39–1.52 (2H, m, CH_2), 3.63 (1H, dt,

J = 9.30, 6.18 Hz, $OCH_2CH_2CH_2CH_3$), 3.67 (1H, dd, J = 10.00, 2.63 Hz, H-1), 3.75 (1H, dt, J = 9.30, 6.56 Hz, $OCH_2CH_2CH_2CH_3$), 3.94 (1H, dd, J = 2.63, 2.37 Hz, H-2), 4.13 (1H, ddd, J = 9.21, 9.21, 9.21 Hz, H-5), 4.17 (1H, ddd, J = 9.47, 9.47, 2.37 Hz, H-3), 4.30 (1H, ddd, J = 9.47, 9.47, 9.21 Hz, H-4), 4.43 (1H, ddd, J = 10.00, 9.73, 9.21 Hz, H-6). ^{31}P NMR (D_2O, ^{1}H decoupled, 145.8 MHz): δ −0.18 (1 P, s), 0.55 (2 P, s), 0.95 (1 P, s). MS: m/z (+ve ion FAB) 557 [$(M+H^+)^+$, 100], m/z (−ve ion FAB) 555 [$(M-H^+)^-$, 100]. HRMS: m/z 554.983 [$(M-H^+)^-$] (calcd for $C_{10}H_{23}O_{18}P_4$, 554.984).

D-2-*O*-Butyl-*myo*-inositol 1,4,5,6-tetrakisphosphate (*ent*-22). The butyryl group of substrate *ent*-**21** was hydrolyzed by the method described above to give the tetrakisphosphate *ent*-**22**. $[\alpha]_D^{24}$ +1.6° (*c* 0.60 in H_2O, pH 1.6). HRMS: m/z 554.985 [$(M-H^+)^-$] (calcd for $C_{10}H_{23}O_{18}P_4$, 554.984). ^{1}H NMR and MS data were in accordance with those obtained for enantiomer **22**.

D-2-*O*-Butyl-1-*O*-butyryl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (2). DIEA (182 μL, 1.06 mmol) and acetoxymethyl bromide (107 μL, 1.06 mmol) were added to a suspension of compound **21** (37 mg, 59 μmol) in acetonitrile (2 mL) as described in the general procedure. Purification by preparative HPLC (72% MeOH, 40 mL/min, t_R = 21.12 min) gave compound **2** (33 mg, 46%) as a syrup. $[\alpha]_D^{24}$ +1.1° (*c* 1.07 in toluene). ^{1}H NMR (toluene-d_8, 360 MHz): δ 0.93 (3H, t, J = 7.28 Hz, CH_3), 0.97 (3H, t, J = 7.48 Hz, CH_3), 1.39–1.49 (2H, m, CH_2), 1.51–1.60 (2H, m, CH_2), 1.77–1.86 (24H, 8 s, 8 × OAc), 2.39 (1H, dt, J = 16.67, 7.38 Hz, CH_2), 2.63 (1H, dt, J = 16.67, 7.68, CH_2), 3.78 (1H, dt, J = 9.45, 6.30 Hz, $OCH_2CH_2CH_2CH_3$), 3.87 (1H, dt, J = 9.06, 6.30 Hz, $OCH_2CH_2CH_2CH_3$), 4.40 (1H, dd, J = 2.36, 2.36 Hz, H-2), 4.68 (1H, ddd, J = 9.55, 9.55, 2.36 Hz, H-3), 4.79 (1H, ddd, J = 9.55, 9.45, 9.45 Hz, H-5), 5.03 (1H, ddd, J = 9.55, 9.55, 9.55 Hz, H-4), 5.09 (1H, ddd, J = 10.04, 9.55, 9.55 Hz, H-6), 5.21 (1H, dd, J = 10.04, 2.36 Hz, H-1), 5.58–5.93 (16H, m, CH_2OAc). ^{31}P NMR (toluene-d_8, ^{1}H decoupled, 145.8 MHz): δ −4.42 (1 P, s), −3.98 (1 P, s), −3.48 (2 P, s). MS: m/z (+ve ion FAB) 1131 [$(M-CH_2OAc^+ + 2H)^+$, 44], 987 [$(M-3\ CH_2OAc^+ + 4H)^+$, 100], m/z (−ve ion FAB) 1129 [$(M-CH_2OAc^+)^-$, 18], 241 [$OPO(OCH_2OAc)_2^-$, 100]. HRMS: m/z 1131.187 [$(M-CH_2OAc^+ + 2H)^+$] (calcd for $C_{35}H_{59}O_{33}P_4$, 1131.189).

D-2-*O*-Butyl-3-*O*-butyryl-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*ent*-2). Alkylation of the phosphate *ent*-**23** as described above afforded the octakis(acetoxymethyl) ester *ent*-**2**. $[\alpha]_D^{24}$ −1.3° (*c* 0.60 in toluene). HRMS: m/z 1129.177 [$(M-CH_2OAc^+)^-$] (calcd for $C_{35}H_{57}O_{33}P_4$, 1129.173). ^{1}H NMR and MS data were in accordance with those obtained for enantiomer **2**.

D-3,4,5,6-Tetra-*O*-benzyl-1,2-di-*O*-butyl-*myo*-inositol (23). Sodium hydride (13 mg, 522 μmol) was added to a stirred solution of **8** (94 mg, 174 μmol) in dry DMF (3 mL) at room temperature in the dark. The mixture was stirred for 5 h, after which 1-butyl iodide (120 μL, 1.04 mmol) was added. The suspension was stirred for 36 h at 80 °C, until HPLC (95% MeOH; 1.5 mL/min; t_R = 7.26) showed predominantly one product. Excess of 1-butyl iodide and DMF were evaporated under reduced pressure. The mixture was then dissolved in *tert*-butyl methyl ether (30 mL) and washed once with phosphate buffer (10 mL), aq sodium dithionate (10 mL) and brine (10 mL), successively. The organic layer was dried over Na_2SO_4, filtered, and the ether was evaporated off to give an oil. The crude oil was purified by preparative HPLC (95% MeOH, 40 mL/min, t_R = 27.24 min) to give the title compound **23** (100 mg, 88%) as an oil. $[\alpha]_D^{24}$ +1.3° (*c* 1.00 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 360 MHz): δ 0.92 (6H, t, J = 7.52 Hz, CH_3), 1.36–1.47 (4H, m, 2 × CH_2), 1.55–1.63 (4H, m, 2 × CH_2), 3.14 (1H, dd, J = 9.57, 2.28 Hz, H-3), 3.34 (1H, dd, J = 10.02, 2.28, H-1), 3.43 (1H, dd, J = 9.34, 9.34, H-5), 3.52 (1H, dt, J = 9.11, 6.60 Hz, $OCCH_2CH_2CH_3$), 3.60 (1H, dt, J = 9.11, 6.60 Hz, $OCCH_2CH_2CH_3$), 3.75 (2H, t, J = 6.60 Hz, $OCH_2CH_2CH_2CH_3$), 3.89 (1H, dd, J = 2.28, 2.28 Hz, H-2), 3.92 (1H, dd, J = 9.57, 9.34 Hz, H-4), 4.00 (1H, dd, J = 10.02, 9.34 Hz, H-6), 4.71–4.93 (8H, m, CH_2Ph), 7.24–7.39 (20H, m, CH_2Ph). MS: m/z (+ve ion FAB) 653 [$(M+H)^+$, 1], 91 [$C_7H_7^+$, 100]. HRMS: m/z 653.383 $(M+H)^+$ (calcd for $C_{42}H_{53}O_6$ 653.384).

D-1,4,5,6-Tetra-*O*-benzyl-2,3-di-*O*-butyl-*myo*-inositol (*ent*-23). A similar reaction of the compound *ent*-**8** gave compound *ent*-**23**. $[\alpha]_D^{24}$ −1.2° (*c* 2.20 in $CHCl_3$). Spectral data were in accordance with those obtained for enantiomer **23**.

D-1,2-Di-*O*-butyl-*myo*-inositol (24). Compound **23** (100 mg, 153 μmol) was hydrogenated with palladium (10%) on carbon under hydrogen as described in the general procedure to give tetrol **19** (40 mg, 92%) as a solid after freeze-drying. Mp 143.7–144.5 °C (from ethanol). $[\alpha]_D^{24}$ +18.7° (*c* 0.80 in MeOH). ^{1}H NMR (DMSO-d_6, 360 MHz): δ 0.89 (3H, t, J = 7.52 Hz, CH_3), 0.91 (3H, t, J = 7.38 Hz, CH_3), 1.24–1.39 (4H, m, CH_2), 1.41–1.53 (4H, m, CH_2), 2.88 (1H, dd, J = 9.55, 2.63 Hz, H-3), 2.93 (1H, dd, J = 9.93, 2.63 Hz, H-1), 3.14 (1H, dt, J = 9.51, 6.42 Hz, $OCH_2CH_2CH_2CH_3$), 3.31 (1H, dd, J = 9.52, 9.52 Hz, H-5), 3.39–3.67 (6H, m, 3 × $OCH_2CH_2CH_2CH_3$, H-2, H-4, H-6), 4.43 (1H, s, OH), 4.52 (1H, s, OH), 4.59 (2H, s (br), OH), MS: m/z (+ve ion FAB) 293 [$(M+H)^+$, 100]. MS: m/z (−ve ion FAB) 291 [$(M-H^+)^-$, 100], Anal. ($C_{14}H_{28}O_6$) C: calcd 57.51; found 56.32; H: calcd 9.65, found 9.91.

D-2,3-Di-*O*-butyl-*myo*-inositol (*ent*-24). A similar reaction and workup of the fully protected compound *ent*-**23** afforded tetrol *ent*-**24**. $[\alpha]_D^{24}$ −18.9° (*c* 1.36 in MeOH). ^{1}H NMR and MS data were in accordance with those obtained for enantiomer **24**.

D-1,2-Di-*O*-butyl-*myo*-inositol 3,4,5,6-Tetrakis(dibenzyl)-phosphate (25). A solution of compound **24** (40 mg, 137 μmol) and tetrazole (115 mg, 1.64 mmol) in acetonitrile (2 mL) was treated with dibenzyl *N*,*N*-di*iso*propylphosphoramidite (552 μL, 1.64 mmol) for 18 h, oxidized with peracetic acid, and worked up as described in the general procedures. Purification by preparative HPLC

(93% MeOH; 40 mL/min; $t_R = 26.05$ min) gave compound **25** (133 mg, 73%) as an oil. $[\alpha]_D^{24}$ −2.3° (*c* 1.00 in $CHCl_3$). ^1H NMR ($CDCl_3$, 360 MHz): δ 0.79 (3H, t, $J = 7.31$ Hz, CH_3), 0.86 (3H, t, $J = 7.31$ Hz, CH_3), 1.13–1.38 (4H, m, 2 × CH_2), 1.41–1.56 (4H, m, 2 × CH_2), 3.24 (1H, dd, $J = 9.89$, 2.15 Hz, H-1), 3.36 (1H, dt, $J = 8.31$, 7.41 Hz, $OCH_2CH_2CH_2CH_3$), 3.41 (1H, dt, $J = 8.31$, 5.87 Hz, $OCH_2CH_2CH_2CH_3$), 3.60 (1H, dt, $J = 8.88$, 6.55 Hz, $OCH_2CH_2CH_2CH_3$), 3.71 (1H, dt, $J = 8.88$, 6.55 Hz, $OCH_2CH_2CH_2CH_3$), 4.16 (1H, ddd, $J = 9.89$, 9.89, 2.47 Hz, H-3), 4.21 (1H, dd, $J = 2.47$, 2.15 Hz, H-2), 4.48 (1H, ddd, $J = 9.67$, 9.67, 9.67 Hz, H-5), 4.79 (1H, ddd, $J = 9.89$, 9.67, 9.67 Hz, H-6), 4.95 (1H, ddd, $J = 9.89$, 9.67, 9.67 Hz, H-4), 4.96–5.09 (16H, m, CH_2Ph), 7.12–7.32 (40H, m, CH_2Ph). ^{31}P NMR ($CDCl_3$, ^1H decoupled, 145.8 MHz): δ −1.82 (1 P, s), −1.59 (1 P, s), −0.81 (1 P, s), −0.68 (1 P, s). MS: *m*/*z* (+ve ion FAB) 1333 $[(M+H)^+, 2]$, 91 $[C_7H_7^+, 100]$, *m*/*z* (−ve ion FAB) 1241 $[(M-C_7H_7^+)^-, 8]$, 277 $[OPO(OBn)_2^-, 100]$. HRMS: *m*/*z* 1333.416 $[(M+H^+)^+]$ (calcd for $C_{70}H_{81}O_{19}P_4$, 1333.417).

D-2,3-Di-*O*-butyl-*myo*-inositol 1,4,5,6-tetrakis(dibenzyl)-phosphate (*ent*-25). Compound *ent*-**24** was phosphitylated and oxidized as described above for compound **25** to give the fully protected phosphate *ent*-**25**. $[\alpha]_D^{24}$ +2.5° (*c* 1.15 in $CHCl_3$). HRMS (DCI): *m*/*z* 1241.3748 ± 10 ppm $[(M-C_7H_7^+)^-]$ (calcd for $C_{63}H_{73}O_{18}P_4$, 1241.3748). ^1H NMR and MS data were in accordance with those obtained for enantiomer **25**.

D-1,2-Di-*O*-butyl-*myo*-inositol 3,4,5,6-tetrakisphosphate (26). Compound **25** (134 mg, 100 μmol) was hydrogenated with palladium (10%) on carbon as described in the general procedure to give title compound **26** (52 mg, 87%) as a solid after freeze-drying. $[\alpha]_D^{24}$ +4.1° (*c* 1.10 in H_2O, pH 1.6). ^1H NMR (D_2O, 360 MHz): δ 0.70 (3H, t, $J = 7.37$ Hz, CH_3), 0.71 (3H, t, $J = 7.37$ Hz, CH_3), 1.13–1.24 (4H, m, 2 × CH_2), 1.32–1.46 (4H, m, 2 × CH_2), 3.39 (1H, dd, $J = 9.47$, 2.33 Hz, H-1), 3.45 (1H, dt, $J = 8.59$, 8.16 Hz, $OCH_2CH_2CH_2CH_3$), 3.51 (1H, dt, $J = 8.59$, 5.79 Hz, $OCH_2CH_2CH_2CH_3$), 3.56 (1H, dt, $J = 9.47$, 6.31 Hz, $OCH_2CH_2CH_2CH_3$), 3.65 (1H, dt, $J = 9.47$, 6.84 Hz, $OCH_2CH_2CH_2CH_3$), 4.02–4.08 (2H, m, H-2, H-3), 4.11 (1H, ddd, $J = 9.47$, 9.47, 9.47 Hz, H-5), 4.27 (1H, ddd, $J = 9.47$, 9.47, 9.47 Hz, H-4), 4.39 (1H, ddd, $J = 9.47$, 9.21, 9.21 Hz, H-6). ^{31}P NMR (D_2O, ^1H decoupled, 145.8 MHz): δ −0.69 (1 P, s), −0.41 (2 P, s), −0.12 (1 P, s). MS: *m*/*z* (+ve ion FAB) 613 $[(M+H)^+, 15]$, 81 $[PO(OH)_2^+, 100]$, *m*/*z* (−ve ion FAB) 611 $[(M-H^+)^-, 90]$. 79 $[OP(O)_2^-, 100]$. HRMS: *m*/*z* 611.043 $[(M-H^+)^-]$ (calcd for $C_{14}H_{31}O_{18}P_4$, 611.046).

D-2,3-Di-*O*-butyl-*myo*-inositol 1,4,5,6-tetrakisphosphate (*ent*-26). A similar reaction with the fully protected substrate *ent*-**25** afforded the free acid *ent*-**26** after freeze-drying. $[\alpha]_D^{24}$ −4.3° (*c* 1.00 in H_2O, pH 1.6). HRMS: *m*/*z* 611.047 $[(M-H^+)^-]$ (calcd for $C_{14}H_{31}O_{18}P_4$, 611.046). ^1H NMR and MS data were in accordance with those obtained for enantiomer **26**.

D-1,2-Di-*O*-butyl-*myo*-inositol 3,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (3). DIEA (187 μL, 1.00 mmol) and acetoxymethyl bromide (111 μL, 1.00 mmol) were added to a suspension of compound **26** (34 mg, 55 μmol) in acetonitrile (2 mL) as described in the general procedure. Purification by preparative HPLC (73% MeOH, 40 mL/min, $t_R = 20.40$ min) gave compound **3** (42 mg, 65%) as a syrup. $[\alpha]_D^{24}$ −2.3° (*c* 1.03 in toluene). ^1H NMR (toluene-d_8, 360 MHz): δ 0.92 (3H, t, $J = 7.28$ Hz, CH_3), 0.99 (3H, t, $J = 7.48$ Hz, CH_3), 1.33–1.47 (4H, m, 2 × CH_2), 1.48–1.54 (2H, m, CH_2), 1.61–1.69 (2H, m, CH_2), 1.77–1.87 (24H, 8 s, 8 × OAc), 3.11 (1H, dd, $J = 9.84$, 2.36 Hz, H-1). 3.46 (1H, dt, $J = 8.79$, 7.28 Hz, $OCH_2CH_2CH_2CH_3$), 3.56 (1H, dt, $J = 8.79$, 7.28 Hz, $OCH_2CH_2CH_2CH_3$), 3.84 (1H, t, $J = 6.50$ Hz, 2 × $OCH_2CH_2CH_2CH_3$), 4.34 (1H, dd, $J = 2.36$, 2.36 Hz, H-2), 4.49 (1H, ddd, $J = 9.45$, 9.45, 2.36 Hz, H-3), 4.68 (1H, ddd, $J = 9.65$, 9.65, 9.65 Hz, H-5), 4.90 (1H, ddd, $J = 9.65$, 9.45, 9.45 Hz, H-4), 5.06 (1H, ddd, $J = 9.84$, 9.65, 9.65 Hz, H-6), 5.68–5.96 (16H, m, CH_2OAc). ^{31}P NMR (toluene-d_8, ^1H decoupled, 145.8 MHz): δ −5.12 (1 P, s), −3.98 (1 P, s), −3.69 (1 P, s), −3.42 (1 P, s). MS: *m*/*z* (+ve ion FAB) 1189 $[(M+H)^+, 3]$, 1045 $[(M-2 \times CH_2OAc^+ + 3H)^+, 100]$, *m*/*z* (−ve ion FAB) 1115 $[(M-CH_2OAc^+)^-, 30]$, 241 $[OPO(OCH_2OAc)_2^-, 100]$. HRMS: *m*/*z* 1117.210 $[(M-CH_2OAc^+ + 2H)^+]$ (calcd for $C_{35}H_{61}O_{32}P_4$, 1117.210).

D-2,3-Di-*O*-butyl-*myo*-inositol 1,4,5,6-tetrakisphosphate octakis(acetoxymethyl) ester (*ent*-3). Alkylation of the phosphate *ent*-**26** as described above afforded the octakis(acetoxymethyl) ester *ent*-**3**. $[\alpha]_D^{24}$ +2.5° (*c* 1.10 in toluene). HRMS: *m*/*z* 1115.192 $[(M-CH_2OAc^+)^-]$ (calcd for $C_{35}H_{59}O_{32}P_4$, 1115.194). ^1H NMR and MS data were in accordance with those obtained for enantiomer **3**.

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