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Synthesis of 4-*C*-Alkyl Inositol 1,4,5-Trisphosphates and 1,3,4,5-Tetrakisphosphates

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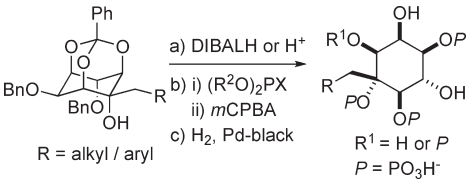

The preparation of 2,3,6-*O*-tribenzyl- and 2,6-*O*-dibenzyl-*myo*-inositols with β-primary, secondary, and tertiary 4-*C*-alkyl or aryl groups is reported. Five of these novel polyols are elaborated to 4-*C*-alkyl Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 analogues. Regio- and stereoselective introduction of 4-*C*-alkyl or aryl substituents proceeded via a 4-*exo*-methylene oxide. Subsequent regioselective reduction of an orthobenzoate provided a divergent method to access both $InsP_3$ and $InsP_4$ precursors. Previously unreported phosphorylation of the tertiary hydroxyl and global deprotection afforded novel analogues that retain their full complement of polar and charged binding features.

Introduction

The phosphatidylinositol phosphates (PtdInsP_n) and inositol phosphates ($InsP_n$) play diverse roles in cellular signaling. Ins(1,4,5)P_3 (**1a**, Figure 1), the cytosolic second messenger generated by the action of phospholipase C on PtdIns(4,5)P_2, provided the missing link between extracellular stimuli and internal Ca^{2+} release.[1] There are now over 30 $InsP_n$ species known to be generated from Ins(1,4,5)P_3 by a battery of kinases and phosphatases. $InsP_n$ have been implicated in many cell processes including nuclear function, ion chelation, and ATP-independent protein phosphorylation.[2] The seven PtdInsP_n found in nature, all derived from phosphatidylinositol (PtdIns), bind to many classes of protein to initiate a large number of downstream cellular responses.[3] In particular PtdIns(3,4,5)P_3 (**1b**, Figure 1), resulting from phosphoinositide-3-kinase mediated phosphorylation of PtdIns(4,5)P_2,[4] has been shown to influence a wide range of cellular pathways including growth, DNA synthesis, and apoptosis.[5] Perturbations of these signaling systems have been implicated in several disease states including cancer[6] and diabetes,[7] making them attractive targets for pharmacological intervention.

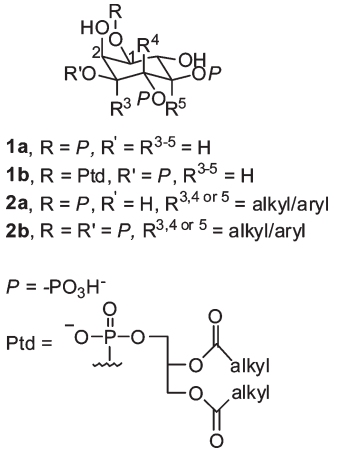

FIGURE 1. Ins(1,4,5)P_3 (**1a**), PtdIns(3,4,5)P_3 (**1b**), and novel *C*-alkyl Ins(1,4,5)P_3 (**2a**) and Ins(1,3,4,5)P_4 (**2b**) analogues.

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A number of different protein domains have been shown to bind the PtdInsP_n and InsP_n, and these domains are expressed on a multitude of proteins.[8] A few PtdInsP_n−protein interactions are reported to be high affinity with dissociation constants in the nanomolar range.[9] However, the majority (over 80%) appear to be somewhat indiscriminate in their PtdInsP_n binding partners and have low affinities in the micromolar range.[9] Study of these signaling systems is further complicated by some PtdInsP_n binding proteins acting as scaffolds and others that express more than one phosphoinositide binding domain.[10] Thus the same protein is often involved in more than one signaling pathway, leading to cross-talk between different processes. Consequently, altering the level of one PtdInsP_n or InsP_n ligand can simultaneously affect a range of downstream responses. Genetic approaches to the study of these systems exhibit related problems, for instance, suffering from signal rerouting during induction of protein expression, leading to difficulty in assigning a phenotype to an individual interaction.[11] This complexity and interdependence of the phosphoinositide signaling network has made isolation of individual PtdInsP_n−protein interactions difficult, hampering understanding of this system and the identification of potential therapeutic targets. It is for this reason that there has been much interest in the design of small molecule probes, including natural product analogues, to modulate these pathways selectively.

Following the chemical synthesis of the natural InsP_n and PtdInsP_n,[12−14] a number of modified analogues have been prepared to probe this system (for reviews see refs 12 and 15). Nearly all such modifications have employed simultaneous deletion or masking of a hydroxyl group, e.g., 3-methoxy-,[16] 3-amino-,[17] 3-chloro-, and 3-bromo-3-deoxy-Ins(1,4,5)P_3,[16] 6-methoxy-Ins(1,4,5)P_3, and 6-methyl-6-deoxy-Ins(1,4,5)P_3.[18] The 2-, 3-, and 6-deoxy analogues of Ins(1,4,5)P_3 were used to explore the structure−activity relationship between receptor binding and Ca^{2+} release.[18−20] Fluorinated analogues have demonstrated the significance of the 6-OH in binding to the Ins(1,4,5)P_3 receptor, and 3-F- and 2,2-F_2-Ins(1,4,5)P_3 were shown to be potent agonists.[21,22] Modification of InsP_n phosphate groups to generate phosphatase-resistant phosphorothioate analogues has also provided a convenient method to introduce ^{35}S radiolabels.[23] Other phosphate analogues including hydrogen phosphonates, methylphosphonates, and difluoromethylphosphonates have also been prepared.[12] A cyclic analogue, in which the 4-phosphate was esterified to a 3-*C*-hydroxymethyl substituent was prepared to study the interaction of conformationally restricted Ins(1,4,5)P_3 with its receptor.[24] Other reported modifications designed to address the difficulty of identifying the downstream effects of InsP_n binding have led to the preparation of caged analogues that are released upon irradiation, and photoaffinity analogues to identify and locate InsP_n receptors.[25]

The more complicated synthetic requirement of differentiating the 1-*O*-phosphatidate from the remaining sites of phosphorylation and the relative youth of the lipid signaling field mean that PtdInsP_n analogue synthesis has been less widely investigated. Even so a small number of deoxy- and fluoro-PtdInsP_n have been described, as well as phosphatase-resistant 3- and 5-methylenephosphonate and phosphorothioate analogues.[12,26] Other useful variations of the inositol phospholipids have left the headgroup unaffected while modifying the fatty acid esters or temporarily masking the phosphate charges: photoaffinity and fluorescent PtdInsP_n analogues have been reported;[27] immobilization of PtdInsP_n led to the identification of a number of binding proteins;[28] cell-permeant short-chain lipid precursors are enzymatically unblocked within the cell and address some of the problems of intracellular delivery of PtdInsP_n.[29]

The main contributions to PtdInsP_n−protein binding and specificity result from polar and Coulombic interactions between the receptor pocket and the lipid headgroup, as well as hydrophobic membrane penetration, electrostatic surface interactions, and shape complementarity.[30] When natural PtdInsP_n or InsP_n associate with a binding site, their unique 3D geometric arrangement of phosphate and hydroxyl groups corresponds to a constellation of precisely complementary polar and charged groups in the protein binding pocket. The majority of the preceding PtdInsP_n or InsP_n analogues involve deletions or substitutions of oxygen atoms compared to the natural ligand and must therefore have reduced polar or charged-based interactions with the protein. Therefore we proposed that novel analogues that did not modify *any* of the polar or charged features of the natural inositol headgroup would have tighter binding and/or greater protein discrimination than most previously reported classes of analogue. Consequently, we designed InsP_n and PtdInsP_n derivatives with an alkyl or aryl group appended to the inositol cyclohexane backbone. Apart from our own

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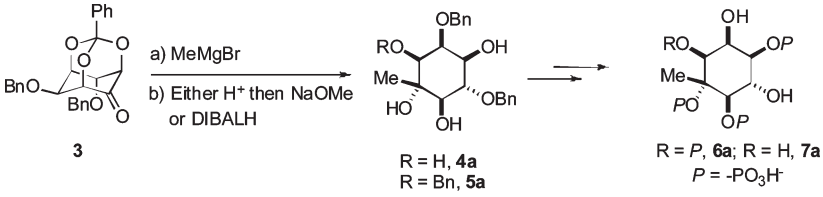
SCHEME 1. Preparation of 4-*C*-Methyl Ins(1,4,5)P_3 and 4-*C*-Methyl Ins(1,3,4,5)P_4



recent report of the first two members of this series of compounds, racemic 4-*C*-Me-Ins(1,4,5)P_3 and -Ins(1,3,4,5)-P_4,[31] the only other example of such an InsP_n analogue of which we are aware is the preparation of 3-*C*-CF_3-Ins(1,4,5)-P_3.[32] Notably, this analogue bound to Ins(1,4,5)P_3 receptors with virtually the same potency as natural Ins(1,4,5)P_3 (**1a**),[33] and although 4-*C*-Me-Ins(1,4,5)P_3 also binds to Ins(1,4,5)P_3 receptor Ca^{2+}-channels, it is a weaker agonist, perhaps reflecting the opposite directions of protrusion from the ring of the substituents on the two analogues. These initial results suggest that when small groups protrude from the inositol ring, such analogues will be accommodated in binding sites; we hope that such a conservative modification will generate new antagonists in receptors where binding triggers conformation change in the protein. By contrast, with larger protruding groups steric congestion in the binding site is likely to prevent these analogues from binding to the natural protein. However, these derivatives may find use in receptor−ligand engineering of phosphoinositide pathways, in a similar manner to the elegant steric complementation method of Shokat et al.,[34] who used N^6-alkylated ATP to dissect tyrosine kinase signaling.[35] As a result of the large number of binding proteins but small range of tightly controlled ligands, the PtdInsP_n and InsP_n signaling pathways may be amenable to investigation using this technique with suitable ligand analogues.

An alkyl substituent could be introduced at any of the six cyclohexane ring positions, in place of the ring proton, without affecting the polar hydroxyl or charged phosphate groups. However, in PtdInsP_n the 1-*O* phosphatidate is the point of membrane attachment and must therefore be directed toward the membrane surface. Hence we considered it unlikely that a 1-*C* substituent, or indeed one at the adjacent 2- or 6-*C* would interact closely with a protein binding partner. In contrast, the signaling lipids are phosphorylated on the 3-, 4-, and 5-*O*, which must therefore contact the surfaces of their cognate binding proteins. Thus, we postulated that steric protrusions on the 3-, 4-, or 5-*C*, and particularly the 4-*C* at the center of this cluster of features, would be most likely to generate modified ligands with useful biological properties (**2**, Figure 1).

Preparation of such analogues required synthetic introduction of a steric protrusion followed by elaboration of these intermediates to the fully phosphorylated and deprotected PtdInsP_n or InsP_n analogues. With the exception of our recently reported phosphorylation of 4-*C*-methyl substituted inositols,[31] the phosphorylation of tertiary centers has not previously been explored. We anticipated that the increased steric hindrance of the tertiary hydroxyl by larger *C*-alkyl or aryl substituents would further obstruct and complicate polyphosphorylation of adjacent hydroxyl groups, which are already prone to competing cyclization reactions.[36] With an efficient route to 4-*C*-methylation validated, we identified the synthesis of a wider range of racemic 4-*C*-alkyl InsP_n head-groups as our first goal. Once established, the synthesis should be transferable to other *C*-alkyl isomers of the inositol ring and to the preparation of enantiomerically pure *C*-alkyl InsP_n and PtdInsP_n analogues.

Inositol orthoesters may be generated in high yield from *myo*-inositol,[37] and the protection of the remaining three hydroxyls has been extensively reported.[38] This provided an expedient route to isolate the 4-OH in 2,6-*O*-dibenzyl-*myo*-inositol 1,3,5-*O*-orthobenzoate[39] leading to the synthesis of 4-*C*-methyl Ins(1,4,5)P_3 and 4-*C*-methyl Ins(1,3,4,5)P_4 (Scheme 1).[31] Furthermore, chiral desymmetrization of inositol 1,3,5-*O*-orthobenzoates using (1*S*)-(−)-camphanic chloride has been reported previously.[39] The methyl group was stereo- and regioselectively introduced at the 4-*C* by oxidation of the 4-OH to inos-4-ose **3**, followed by addition of methyl magnesium bromide. Reduction with DIBALH, or acidic solvolysis, then selectively furnished the required 4-*C*-methyl triol or tetrol (**4a** and **5a**, Scheme 1). Subsequent phosphorylation and global deprotection generated the final analogues **6a** and **7a**. The extension of this chemistry to larger 4-*C*-alkyl derivatives was not straightforward, and the difficulties and solutions to the problems encountered in this endeavor are reported in this paper.

Results and Discussion

Attempted introduction of alkyl groups using Grignard reagents, as previously reported for the methyl group, was unsuccessful. The reaction of larger alkyl Grignard reagents with ketone **3** resulted in complete reduction of the inosose by β-hydride transfer, without any detectable alkyl addition. It was even noted that a small degree of ketone reduction (15%) occurred at rt with MeMgBr, which has no β-hydride, presumably a result of a one-electron transfer mechanism.[40]

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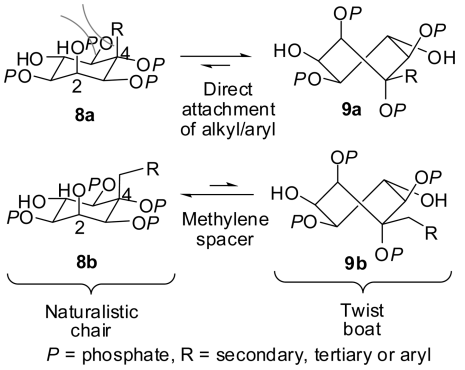

FIGURE 2. Introduction of a methylene spacer between bulky groups and inositol reduces undesirable 1,3-diaxial interactions.

Addition of an alkyl group to the inosose in this manner would attach it directly to the cyclohexane backbone of the inositol ring. Since the alkyl substituent will occupy an axial position in the final $InsP_n$ analogues, it was postulated that larger secondary and tertiary alkyl or aryl groups would experience significant 1,3-diaxial interactions, distorting the natural chair conformation of the inositol ring. Hence, incorporation of a methylene spacer between the alkyl group and cyclohexane ring should reduce undesirable diaxial interactions by allowing larger groups to point out radially (Figure 2).

A general route to analogues including a methylene spacer was envisaged via ring opening of an epoxide. This has the advantage of fixing the stereochemistry at the 4-*C* prior to introduction of the alkyl substituent. Therefore, dimethyl sulfoxonium methylide[41] was employed to stereoselectively add a methylene group across inos-4-ose **3**, generating *exo*-methylene oxide **10** in high yield, Scheme 2. Reduction of epoxide **10** using lithium aluminum hydride generated alcohol **11a** identical to that previously prepared,[31] confirming that epoxide **10** possessed 4-*C myo*-stereochemistry.

Upon addition of a range of alkyl and aryl mixed higher order cuprates,[42] epoxide **10** underwent nucleophilic ring opening to give the expected products **11b**−**g**. Initial reactions carried out in THF were low-yielding (e.g., maximum yield of **11c**, 48%); this may be due to solvent coordination destabilizing the alkyl lithium cuprate complex.[43] However, the addition of primary alkyl cuprates in ether to **10** was high-yielding (**11c**, 90%). The more sterically demanding *tert*-butyl lithium cuprate suffered from competing β-hydride reduction, which was not observed at all for the less sterically hindered groups, leading to production of both **11d** (36%) and **11a** (30%). The addition of BF_3 as a Lewis acid[44] to promote opening of epoxide **10** improved the proportion of alkylation (the ratio of **11d**:**11a** was 2:1 with BF_3, cf. 2:3 without BF_3, as judged by ^{1}H NMR of the crude material), but competing β-hydride reduction was still observed and the reaction could not be driven to completion. Addition of aryl lithium cuprates (phenyl and 2-naphthyl) to generate **11e** and **11f** was high-yielding, presumably due to the absence of β-hydrogens. Upon addition of isopropyl lithium cuprate to epoxide **10** two products were generated: the desired tertiary alcohol **11g** (26%) and, unexpectedly, conduritol **12a** (34%). No evidence of β-hydride reduction was observed. The structure of conduritol derivative **12a** was confirmed by X-ray crystallography. In order to establish whether this side reaction was independent of epoxide opening, 4-*C*-methyl orthobenzoate **11a** was treated with isopropyl lithium cuprate, in the same manner as epoxide **10**. The corresponding methyl conduritol **12b** was generated in 30% yield (with the remainder recovered as starting material). During initial attempts to effect nucleophilic opening of the epoxide, it was found that addition of methyl magnesium bromide to epoxide **10** generated bromomethyl inositol **13**, with none of desired 4-*C*-ethyl 11b. Addition of CuI[45] did not promote alkyl addition and a mixture of the bromo- and iodomethyl derivatives was obtained. However, direct addition of alkyl lithium reagents to epoxide **10** gave low yields, which became even lower with increasing basicity of the alkyl lithium reagent (Ph, 66%; *n*-butyl, 18%; *tert*-butyl, 0%). Analysis of the crude product mixture by mass spectrometry after treatment of epoxide **10** with *tert*-butyl lithium indicated the formation of dimers and tetramers due to intermolecular polymerization. This evidence of the alkyl lithium reagent acting as a base suggested a possible explanation for the generation of conduritols **12a** and **12b**, Scheme 3.

Crandall and Lin[46] proposed a mechanism to explain the olefinic products they isolated from treating epoxides with *tert*-butyl lithium involving proton abstraction, followed by generation of a carbene. In a similar manner, it is conceivable that isopropyl lithium has the correct combination of steric hindrance, lithium chelation, and basicity to remove the required ring proton (**14**), which equilibrates with scission of the orthobenzoate C−O bond to form carbene **15**, Scheme 3. Subsequent addition of isopropyl lithium and elimination of benzyl alcohol (**16**) would then lead to the observed conduritol framework.

With the 4-*C*-alkyl substituents introduced, acidic solvolysis of the orthobenzoate followed by removal of the resultant benzoate acyl ester[47] was expected to generate the 4-*C*-alkyl tetrols required for Ins(1,3,4,5)P_4 analogue synthesis, Scheme 2. The conditions for orthobenzoate solvolysis in the presence of the 4-*C*-alkyl groups were harsher than those previously reported,[47] requiring concentrated HCl−propan-1-ol (2:1 v/v) at reflux. The benzoate acyl esters generated from incomplete acidic solvolysis of the orthobenzoate may be separated[31,48] and the 1-*O*-benzoate used in the preparation of $PtdInsP_3$ analogues.[48] However, for the preparation of $InsP_4$ analogues, the mixed benzoate esters were cleaved to generate the 4-*C*-alkyl 2,6-*O*-dibenzyl tetrols, **4**. After transesterification of the benzoate esters with sodium methoxide in methanol, and purification of the resulting material by short column chromatography, the tetrols **4a**−**e** and **g** were

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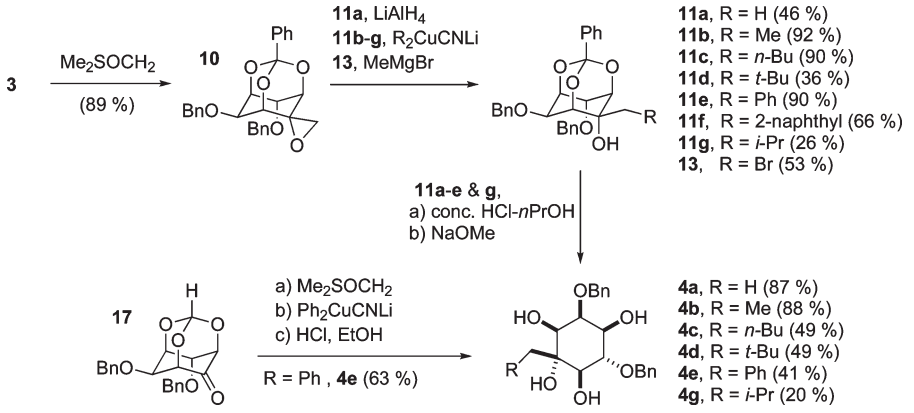
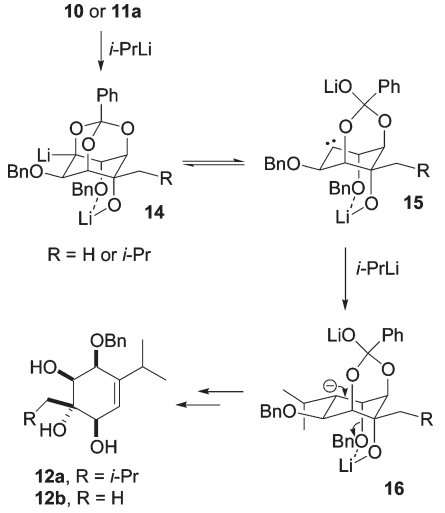
SCHEME 2. Introduction of 4-*C*-Alkyl Substituents and Preparation of 4-*C*-Alkyl-2,6-*O*-dibenzyl Tetrols



SCHEME 3. Proposed Mechanism Leading to Generation of Conduritol Derivates 12a and 12b



recovered in decreasing yields as the size of the 4-*C*-alkyl substituent increased.

Attempted crystallization of crude **4e** produced an off-white powder that was identified by HRMS and ^{1}H NMR as a mixture of the 4-*C*-benzyl-2-*O*-benzyl and -6-*O*-benzyl pentols resulting from benzyl ether solvolysis. As the inositol 1,3,5-*O*-orthoformates are reportedly more labile under acidic conditions than orthobenzoates,[49] 2,6-*O*-dibenzyl inos-4-ose-1,3,5-*O*-orthoformate **17** was prepared according to published procedures,[50] and the same manipulations as described above were applied to generate 4-*C*-benzyl tetrol **4e** from this intermediate, Scheme 2. As expected, the rate of acidic solvolysis of the orthoformate was faster than that of the orthobenzoate, requiring only 4 M HCl−ethanol (1:2 v/v) at 50 °C, instead of concd HCl−propan-1-ol at reflux, to initiate solvolysis. Desired tetrol **4e** (63%) was separated from unreacted starting material, and there was no sign of significant benzyl ether solvolysis. The conditions required for orthoformate solvolysis were still considerably harsher than those reported for their counterparts lacking a *C*-alkyl substituent.[51] It is likely that the smaller bridgehead group experiences a less severe steric clash with the 4-*C*-alkyl group during acidic solvolysis and the orthoformate is the recommended precursor for further preparations of *C*-alkyl tetrols by this method.

The ^{1}H NMR spectra of the 4-*C*-alkyl tetrols **4** were broad for R groups larger than H, and small coupling constants were observed for the inositol 6-H, although the 2-H resonances were similar to R = H. This indicated a local inversion of conformation for the 6-H [all R > H; 2-H (t, $J \approx$ 2.9 Hz), 6-H (t, $J \approx$ 3.4 Hz), cf. R = H; 2-H (t, J 3.1 Hz), 6-H (t, J 10 Hz)] as these values are close to the ^3J values observed for the 6-H of orthoesters **11** with purely gauche couplings. This demonstrates that in tetrols **4** where R > H the 4-*C*-steric protrusion is significantly distorting the inositol framework from the natural chair.

From our previously reported observation that DIBALH reduction of **11a** regioselectively generated only the 3-*O*-benzyl ether **5a**,[31] it was expected that treatment of **11b**−**f** would provide the corresponding 1,4,5-triols required for 4-*C*-alkyl Ins(1,4,5)P_3 analogue synthesis. However, treatment of **11b**−**f** with DIBALH gave two products, the desired 4-*C*-alkyl 1,4,5-triols **5b**−**f** and benzylidene acetals **18b**−**f** (Scheme 4 and Table 1).

The ^{1}H NMR spectra of tribenzyl derivatives **5** were broader than the spectra of the corresponding dibenzyl compounds **4**, with no measurable ^3J around the inositol ring except the 2-H. This indicates that the additional 3-OBn ether causes further ring distortion of the chair conformation compared to the 2,6-dibenzyl tetrols **4**. The regioselectivity of DIBALH reduction to the 1,4,5-triol was confirmed by acetylation of **11c** and **11e**. Locking the compounds into a single chair conformation, the ^{1}H NMR spectra of the acetates were sharp. The clear downfield shift of the 1- and 5-H proton signals from the remaining proton resonances confirmed regioselective formation of a 3-*O*-benzyl ether. However, mass spectrometry and the absence of a third set of ^{1}H or ^{13}C NMR acetate resonances revealed that in neither case had acetylation occurred at the more hindered tertiary 4-OH. This may be contrasted with acetylation of tetrol **4a**,[31] having only a 4-*C*-methyl alkyl substituent, which proceeded to completion under the same conditions. This demonstrates

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SCHEME 4. Reduction of Orthobenzoates 11a−f with DIBALH Generates Both Benzylidene Acetals 18 and 4-*C*-Alkyl Triols 5

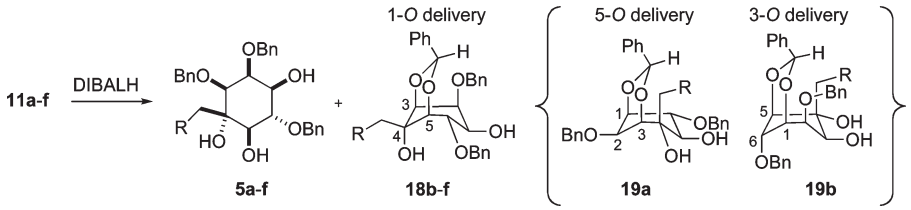

TABLE 1. Isolated Yields of Benzylidene Acetal 18 and 2,3,6-*O*-Tribenzyl Ether 5 after Treatment of 11 with 3.5 equiv of DIBALH (12 h, DCM, rt)

	4-*C*-alkyl	**18** (%)	**5** (%)
11a	methyl	0	55
11b	ethyl	19 (0[a])	32 (61[a])
11c	pentyl	13	54
11d	neopentyl	58	12
11e	benzyl	28	39
11f	(2-naphthyl)methyl	42	29

[a]Reduction of **11b** can be forced to completion over 48 h.

the greater hindrance of the 4-OH by the 4-*C*-CH_2R group when R > H.

Of the three possible benzylidene acetals, only 3,5-*O*-benzylidene acetal **18** was formed in each case. A key piece of evidence corroborating this assignment is a quartet in the ^{1}H NMR spectrum (d_6-DMSO), which exchanged to a triplet upon addition of D_2O. Such a resonance could not appear in the spectra of the other two possible isomers (**19**) in which the new C**H**(OH) only has one possible three bond coupling, apart from that to the -OH, Scheme 4. Other spectral details, including an observed NOE between the 6-H and acetal C-H, were consistent with this interpretation.

As reported by Gilbert and Holmes for inositol orthoformates,[52] such acetals are believed to occupy an approximate boat conformation. Generation of the 3,5-*O*-benzylidene acetal shows that the initial attack of DIBALH does not necessarily occur at the 5-O as reported for inositol orthoesters with only a 4-C hydrogen substituent.[52] This suggests that the steric influence of the 4-*C*-alkyl group is at least comparable to that of the equatorial 2-*O*-benzyl ether. Although addition of further DIBALH and longer reaction times forced complete conversion of 4-*C*-ethyl benzylidene acetal **18b** into **5b**, 4-*C*-benzyl benzylidene acetal **18e** was inert to further DIBALH treatment (24 h, 50 °C, 10 equiv of DIBALH). It is therefore postulated that **5** arises from transiently formed **19a**, as opposed to **18**. Furthermore, the absence of other isomeric reduction products demonstrates that **19b**, which would require the first equivalent of DIBALH to have reacted at the 3-O between the 2-OBn and 4-*C*-CH_2R equatorial substituents, cannot have formed. As the 4-*C*-alkyl substituent increases in size, benzylidene **18** becomes increasingly resistant to further DIBALH reduction and initial DIBALH delivery is favored more to the 1-O than the 5-O. Solvolysis of benzylidene acetals **18d−f** under mildly acidic conditions now conveniently generated corresponding 4-*C*-alkyl tetrols in improved yields (**4d**, 56%; **4e**, 75%; **4f**, 43%). Thus, although the orthobenzoates had proved poor substrates for acidic solvolysis to **4**, DIBALH reduction provided a divergent approach to building blocks for both 4-*C*-alkyl $InsP_3$ and $InsP_4$ analogue preparation.

The β-primary 4-*C*-alkyl triols (**5a−c**) and tetrols (**4a−c**) were phosphorylated by treatment with *N*,*N*-diisopropyldibenzyl phosphoramidite **20** and 1-*H*-tetrazole as activator, followed by oxidation of the intermediate phosphites with *m*CPBA.[53] Dibenzyl *H*-phosphonate reagent debris was removed by mildly basic oxidation with I_2 and water, followed by purification of the crude product by column chromatography to prepare fully protected $InsP_3$ (**21a−c**) and $InsP_4$ (**22a−c**) analogue precursors, Scheme 5.

All six fully protected species were globally deprotected using palladium black under an atmosphere of H_2 and buffered by $NaHCO_3$ to neutralize the acidic phosphomonoesters generated during deprotection.[54] A concentrated aqueous solution of the crude material was passed through a short column of DOWEX 50WX8-200 (H^+ form) to remove complexed Pd^{2+}. Acidic fractions were combined and neutralized with dilute ammonia before lyophilizing to provide the 4-*C*-alkyl Ins(1,3,4,5)P_4 and Ins(1,4,5)P_3 analogues **6a−c** and **7a−c** in high yield, Scheme 5.

The ^{1}H NMR spectra of tetrakisphosphates **6a−c** were well resolved, and the 6-H coupling constants were indicative of two antiperiplanar neighbors [**6a−c** 6-H (t, *J* 8.8−9.9 Hz), cf. natural $InsP_4$ 6-H (t, *J* 9.6 Hz)[31]], suggesting that these analogues occupied a naturalistic chair conformation. The ^{1}H NMR spectra of 4-*C*-ethyl and -pentyl $InsP_3$ derivatives **7b** and **7c** were considerably broader than those of the corresponding $InsP_4$ derivatives **6a−c** and 4-*C*-methyl $InsP_3$ (**7a**). Furthermore, the ^{13}C NMR was too broad to observe at room temperature, and ^{31}P coupling will have broadened the peaks further. This presumably arises from the presence of one less equatorial phosphate group in analogues **7a−c**. The spectra of both **7b** and **7c** exhibited a small amount of a second product. Re-examining the spectra of **7b** at 55 °C in an attempt to sharpen the observed peaks caused this second product to increase in intensity relative to **7b** until it was the only species remaining. This second product was identified as 4-*C*-ethyl Ins(1,5)P_2 **23b**, plus free phosphate. The decomposition of **7b** began during passage through DOWEX (H^+ form) and was exacerbated upon heating. As we suspected that weakly acidic NH_4^+ counterions could participate in formation of **23b**, the sodium salts of **7b** and **7c** were prepared by stirring freshly deprotected samples with DOWEX Na^+ form (rather than H^+ form) followed by HPLC. The sodium salts were not contaminated by dephosphorylation products and were stable at rt. However, during NMR experiments at 55 °C, the corresponding diphosphate again began to form,

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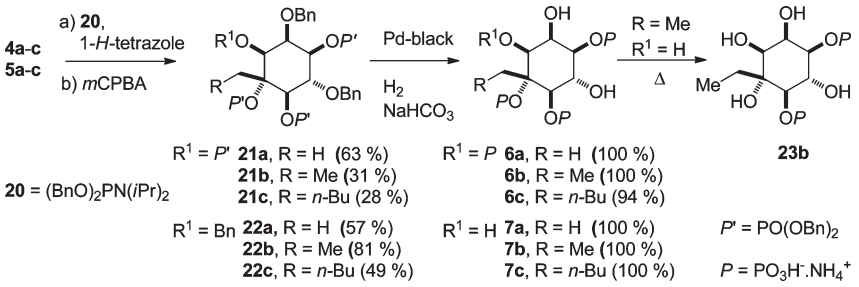
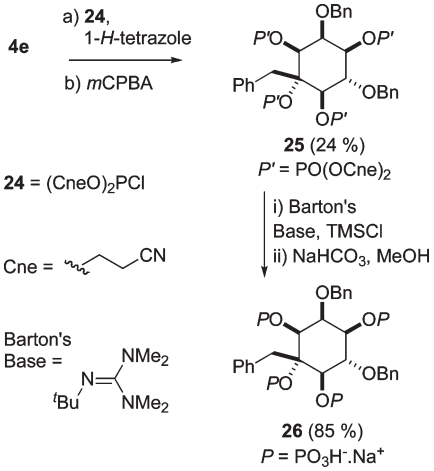
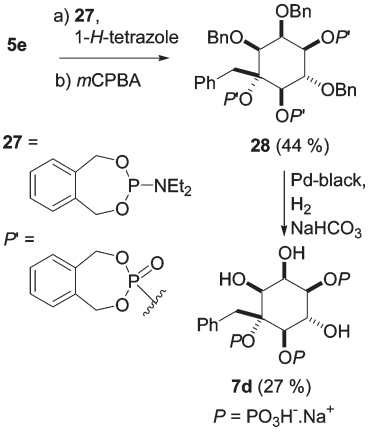
SCHEME 5. Phosphorylation and Deprotection of the Primary 4-*C*-Alkyl Tetrols and Triols



SCHEME 6. Phosphitylation of 4e with $(CneO)_2PCl$, followed by Oxidation and Deprotection



SCHEME 7. Preparation of 4-*C*-Benzyl Ins(1,4,5)P_3



although **7b** was fully characterized by ^{1}H NMR with <10% decomposition. At 55 °C the coupling constants of the inositol ring protons of 4-*C*-ethyl InsP_3 **7b** are slightly reduced compared to those observed in nonfluxional chair form **7a** [for **7b** 5-H (t, *J* 8.2 Hz), cf. **7a** 5-H (dd, *J* 9.5, 8.9 Hz)]. This suggests that the desired chair conformation is occupied to a greater extent than boat-like ones. We are uncertain if the observed fluxionality of trisphosphates **7b** and **7c** is related to their propensity for hydrolysis, but it is notable that this was not encountered with the less conformationally labile InsP_4 analogues **6a**−**c**.

Phosphorylation of tetrols **4d** and **4e**, having larger 4-*C*-alkyl substituents, using dibenzyl phosphoramidite **20** generated a mixture of the tris- and tetrakisphosphates as a result of incomplete phosphorylation at the tertiary hydroxyl. It was not possible to increase the reaction concentration further due to a thick precipitate, presumed to be diisopropyl ammonium tetrazolide. However, phosphitylation of 4-*C*-benzyl tetrol **4e** using di(2-cyanethyl)phosphorochloridite[55] **24**, followed by oxidation with *m*CPBA, effected complete phosphorylation of all four hydroxyls including the tertiary center to prepare tetrakisphosphate **25**, Scheme 6.

Fully protected tris(dicyanoethylphosphate) **25** required a two-step deprotection, first of the phosphate esters, followed by the 2- and 6-*O*-benzyl ethers. After exchange of the cyanoethyl esters for trimethyl silyl esters and separation of Barton's base salt [$(Me_2N)C{=}NtBu\cdot HCl$] by filtration,[36b] the trimethyl silyl phospho-diesters were removed by solvolysis in MeOH containing $NaHCO_3$. However, further deprotection of **26** by catalytic hydrogenolysis was incomplete and concomitant hydrogenolysis of the 4-*C*-benzyl substituent was observed.

Since dibenzyl phosphorochloridite is not known, we sought a more reactive phosphoramidite that would permit unblocking of all the protective groups on our analogue precursors in one step. Consequently, cyclic phosphoramidite **27** was prepared[56] as it is a less sterically hindered phosphitylating reagent with similar protective group properties to dibenzyl phosphoramidite **20** and it has performed well in reported polyphosphorylations.[57] Successful phosphorylation of **5e** was effected with phosphoramidite **27**, activated by 1-*H*-tetrazole, followed by oxidation, Scheme 7. Apart from lower steric demands, this success is also thought to be due to an increased reagent concentration compared to that of dibenzyl phosphoramidite **20** due to the absence of precipitation, presumably because diethyl ammonium tetrazolide is more soluble in this solvent system. Global deprotection[54] of **28** was followed by stirring with DOWEX (Na^+ form) to remove chelated Pd^{2+}, and then HPLC ($MeCN-H_2O$ 1:49 v/v) to remove the 4-*C*-cyclohexyl contaminant (~20% by ^{1}H NMR of the crude material), to afford 4-*C*-benzyl Ins(1,4,5)P_3, **7d**.

The ^{1}H NMR spectra of the 4-*C*-alkyl InsP_4 derivatives **6a**−**c** suggest a chairlike conformation. Occupation of the natural conformation, despite addition of the axial 4-*C*-alkyl

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group, suggests that these analogues will present the correct geometry of hydroxyl and phosphate groups during binding to their protein partners. It is not certain how the more fluxional nature of the 4-*C*-alkyl $InsP_3$ analogues will affect binding to their target proteins. However, the observed NMR spectra at elevated temperature suggest that they still occupy the natural conformation to a large extent.

Conclusion

Five new racemic analogues of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 have been synthesized in addition to the two previously reported.[31] It has been shown that the introduction of a wide range of 4-*C*-alkyl substituents is possible, with retention of the *myo*-stereochemistry and including a methylene spacer, via nucleophilic ring opening of a *myo*-inositol orthoester *exo*-methylene oxide. These 4-*C*-alkyl groups are stable to the conditions of orthobenzoate solvolysis and reduction to generate triols and tetrols for phosphorylation. In particular, treatment with DIBALH regioselectively partially reduced the 2,6-dibenzyl orthobenzoates to a hydrolytically sensitive benzylidene acetal, plus complete reduction to a 2,3,6-tribenzyl ether, generating both series of analogues from a single precursor. Although not all 4-*C*-alkyl triols and tetrols have been phosphorylated thus far and further work is needed to optimize the deprotection steps, the preparation of these compounds proves that the synthesis of phosphoinositide headgroup analogues that retain all of their original binding features but include an alkyl or aryl protrusion is possible. As such, these compounds are the first members of the new class of *C*-alkyl inositol phosphates. These analogues are expected to be valuable for biological studies. The susceptibility of these compounds to phosphoinositide phosphatases and the ability of the $InsP_3$ analogues to activate the endoplasmic $InsP_3$ receptor are being studied and will be reported in due course.

Experimental Section

General Method A. Acidic Solvolysis of Orthobenzoates 11 To Generate Tetrols 4a−e and g. 2,6-*O*-Dibenzyl-4-*C*-alkyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **11** (0.2 mmol) was taken up in concd HCl−propan-1-ol (1:2 v/v, 3 mL) and refluxed for 3 h. The solution was cooled and neutralized with satd $NaHCO_3$, and the products extracted into EtOAc. The combined organic layers were washed with H_2O, then brine, dried ($MgSO_4$), and all solvents were evaporated under reduced pressure. The crude mixture of benzoyl esters was evaporated from MeCN (3 × 1 mL) and taken up in MeOH (2 mL). After addition of NaOMe (25% w/v in MeOH, 0.15 mL, 0.1 mmol) the solution was refluxed for 3 h. The resulting solution was cooled and neutralized by dropwise addition of 4 M HCl, and all solvents were removed under reduced pressure. The crude material was taken up in dry MeOH, and the salts removed by filtration under gravity. The mother liquor was evaporated to dryness under reduced pressure.

2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol 4b. Compound **11b** (280 mg, 0.60 mmol) was refluxed in concd HCl−propan-1-ol according to general method A to recover a crude mixture of benzoyl esters (294 mg), HRMS (ESI^+) *m*/*z* (%) found [M + H] 493.2246 (32), $C_{29}H_{33}O_7$ requires 493.2226, $[M + Na]^+$ 515.2055 (100), $C_{29}H_{32}NaO_7$ requires 515.2046. The crude mixture was then treated with NaOMe in MeOH to afford **4b** (204 mg, 88% over 2 steps) as a pale yellow oil; R_f (EtOAc−hexane 7:3 v/v) 0.20; ^{1}H NMR (500 MHz), $CDCl_3$ δ 7.38−7.32 (m, 8H), 7.26−7.24 (m, 2H) (10 × Ar *H*), 4.72 (d, *J* = 11.5, 1H), 4.67 (d, *J* = 11.5, 1H), 4.65 (d, *J* = 11.6, 1H), 4.59 (d, *J* = 11.6, 1H) (4 × OCH*H*Ph), 4.28 (bs, 1H, Ins 1-*H*), 4.13 (t, *J* = 3.0, 1H, Ins 2-*H*), 4.04 (bs, 1H, Ins 5-*H*), 3.98 (t, *J* = 3.4, 1H, Ins 6-*H*), 3.80 (bs, 1H, Ins 4-O*H*), 3.73 (bd, *J* = 8.1, 1H, Ins 3-*H*), 3.32 (bd, *J* = 10.6, 1H, Ins 3-O*H*), 2.95 (bs, 1H, Ins 1-O*H*), 2.74 (bs, 1H, Ins 5-O*H*), 2.01 (dq, *J* = 14.4, 7.2, 1H), 1.85 (dq, *J* = 14.3, 7.0, 1H) (2 × CH*H*CH_3), 1.02 (t, *J* = 7.4, 3H, CH_2CH_3) ppm; ^{13}C NMR (125 MHz), $CDCl_3$ δ 137.4, 136.6 (2 × Ar *C*), 128.7 (2C), 128.7 (2C), 128.4, 128.3, 128.1 (2C), 127.9 (2C) (10 × Ar *C*H), 80.1 (Ins *C*H), 75.0 (Ins *C*), 74.1 (Ins *C*H), 73.4 (O*C*HPh), 73.3 (Ins *C*H), 71.3 (O*C*HPh), 70.9, 69.7 (2 × Ins *C*H), 27.7 (CH_2CH_3), 6.1 (CH_2CH_3) ppm; HRMS (ESI^+) *m*/*z* (%) found $[M + Na]^+$ 411.1799, $C_{22}H_{28}O_6Na$ requires 411.1784.

2,6-*O*-Dibenzyl-4-*C*-neopentyl-*myo*-inositol 4d. (a) Acidic solvolysis of the orthoester: **11d** (98 mg, 0.19 mmol) was refluxed with concd HCl−propan-1-ol according to general method A, to recover a crude mixture of benzoyl esters (90 mg); HRMS (ESI^+) *m*/*z* (%) found $[M + Na]^+$ 557.2520 (100), $C_{32}H_{38}O_7Na$ requires 557.2515. The crude mixture was treated with NaOMe in MeOH to afford **4d** (40 mg, 49%) as a clear oil. (b) Acidic solvolysis of the benzylidene acetal: **18d** (138 mg, 0.27 mmol) was taken up in CH_2Cl_2−EtOH−H_2O (5:1:1 v/v, 1.4 mL) and *p*-TsOH (10 mg, 0.05 mmol) added. After 24 h, all solvents were evaporated, and the crude material was purified by chromatography on flash silica. Elution with EtOAc−hexane (1:9 → 4:1 v/v) afforded **4d** (64 mg, 56%) as a clear glass; R_f (EtOAc−hexane 7:3 v/v) 0.23; ^{1}H NMR (400 MHz), $CDCl_3$ δ 7.41−7.35 (m, 8H), 7.29−7.26 (m, 2H) (10 × Ar *H*), 4.72 (d, *J* = 11.7, 1H), 4.69 (d, *J* = 11.7, 1H), 4.67 (d, *J* = 11.7, 1H), 4.60 (d, *J* = 11.7, 1H) (4 × OCH*H*Ph), 4.29 (bs, 1H, Ins 1-*H*), 4.16 (t, *J* = 2.9, 1H, Ins 2-*H*), 4.13 (bs, 1H, Ins 5-*H*), 4.01 (t, *J* = 3.4, 1H, Ins 6-*H*), 3.89 (bd, *J* = 5.4, 1H, Ins 3-*H*), 3.87 (bs, 1H, Ins 4-O*H*), 3.48 (bd, *J* = 6.8, 1H, Ins 3-O*H*), 3.07 (bs, 1H, Ins 1-O*H*), 2.85 (bs, 1H, Ins 5-O*H*), 1.98 (d, *J* = 14.7, 1H), 1.85 (d, *J* = 15.2, 1H) [2 × CH*H*$C(CH_3)_3$], 1.11 [s, 9H, $CH_2C(CH_3)_3$] ppm; ^{13}C NMR (125 MHz), $CDCl_3$ δ 137.5, 136.5 (2 × Ar *C*), 128.7 (2C), 128.6 (2C), 128.4, 128.2, 128.1 (2C), 127.9 (2C) (10 × Ar *C*H), 80.5 (Ins 2-*C*H), 76.6 (Ins 4-*C*), 75.7 (Ins 5-*C*H), 73.4 (OCH_2Ph), 73.0, 71.7 (Ins 6-*C*H + Ins 3-*C*H), 71.2 (OCH_2Ph), 69.2 (Ins 1-*C*H), 46.2 [$CH_2C(CH_3)_3$], 31.9 [$CH_2C(CH_3)_3$], 31.3 [$CH_2C(CH_3)_3$] ppm; HRMS (ESI^+) found $[M + Na]^+$ 453.2234, $C_{25}H_{34}O_6Na$ requires 453.2253.

General Method B. Reduction of the 4-*C*-Alkyl Orthobenzoates 11 Using Excess DIBALH. 2,6-*O*-Dibenzyl-4-*C*-alkyl-*myo*-inositol 1,3,5-*O*-orthobenzoate **11** (0.21 mmol) was evaporated from MeCN (3 × 1 mL), taken up in CH_2Cl_2 (1 mL), and cooled to −78 °C. DIBALH (1.0 M solution in hexanes, 0.84 mmol) was added dropwise at −78 °C. The solution was allowed to warm to rt over 2 h. The reaction was monitored by TLC (EtOAc−hexane, 3:7 v/v) and quenched by dropwise addition of H_2O (5 mL) when all starting material was consumed. The product was taken up in CH_2Cl_2 (10 mL) and separated from the aqueous layer, which was subsequently washed with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with satd $NaHCO_3$ and then brine. The organic layer was dried ($MgSO_4$), and all solvents were evaporated under reduced pressure.

2,6-*O*-Dibenzyl 4-*C*-ethyl-*myo*-inositol 3,5-*O*-Benzylidene Acetal 18b and 2,3,6-*O*-Tribenzyl-4-*C*-ethyl-*myo*-inositol 5b. (a) **11b** (220 mg, 0.46 mmol) was treated with DIBALH for 12 h, according to general method B. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc−hexane (0:1 → 1:1 v/v) afforded benzylidene acetal **18b** (42 mg, 19%) and tribenzyl ether **5b** (72 mg, 32%). (b) Treatment of **11b** (350 mg, 0.74 mmol) with DIBALH according to general method B for 48 h afforded only tribenzyl ether **5b** (216 mg, 61%) after chromatography on flash silica as detailed above. For **18b**: R_f (EtOAc−hexane, 3:7 v/v) 0.23; ^{1}H NMR (500 MHz), DMSO δ

7.40−7.25 (m, 15H, 15 × Ar *H*), 5.98 (s, 1H, PhC*H*O_2), 4.93 (s, 1H, ex, Ins 4-O*H*), 4.74 (d, *J* = 12.1, 1H, 2-OCH*H*Ph), 4.70 (s, 2H, 6-OCH_2Ph), 4.67 (d, *J* = 7.4, 1H, ex, Ins 1-O*H*), 4.64 (d, *J* = 12.1, 1H, 2-OCH*H*Ph), 4.46 (q, *J* = 7.7, 1H, ex → t, Ins 1-*H*), 4.20 (dd, *J* = 6.6, 1.9, 1H, Ins 3-*H*), 4.13 (dd, *J* = 8.2, 6.8, 1H, Ins 2-*H*), 3.94 (d, *J* = 6.3, 1H, Ins 6-*H*), 3.83 (d, *J* = 1.7, 1H, Ins 5-*H*), 2.01 (dq, *J* = 14.3, 6.9, 1H), 1.90 (dq, *J* = 14.3, 7.7, 1H) (2 × C*H*HCH$_3$), 0.90 (t, *J* = 7.4, 3H, CH_2CH_3) ppm; ^{13}C NMR (100 MHz), DMSO δ 139.20 (2C), 139.16 (3 × Ar *C*), 129.1, 128.60 (2C), 128.57 (2C), 128.5 (2C), 128.1 (2C), 128.0 (2C), 127.79, 127.78, 126.7 (2C) (15 × Ar *C*H), 92.6 (PhCHO$_2$), 85.0, 77.5, 72.9, 72.5 (4 × Ins *C*H), 72.0, 71.6 (2 × O*C*H$_2$Ph), 69.4 (Ins *C*H), 69.0 (Ins *C*), 28.8 (CH_2CH_3), 7.1 (CH_2CH_3) ppm; HRMS (ESI$^+$) *m*/*z* (%) found [M + Na]$^+$ 499.2093 (100), $C_{29}H_{32}O_6Na$ requires 499.2097. For **5b**: R_f (EtOAc−hexane, 3:7 v/v) 0.32; ^{1}H NMR (400 MHz), $CDCl_3$ δ 7.42−7.27 (m, 15H, 15 × Ar *H*), 4.91 (d, *J* = 10.8, 1H), 4.77 (d, *J* = 11.2, 1H), 4.70 (d, *J* = 11.7, 1H), 4.69 (d, *J* = 11.2, 1H), 4.67 (d, *J* = 10.8, 1H), 4.63 (d, *J* = 11.7, 1H) (6 × OCH*H*Ph), 4.37 (bs, 1H, Ins *H*), 4.16 (t, *J* = 3.2, 1H, Ins 2-*H*), 4.16−4.13 (m, 1H), 3.92 (bs, 1H), 3.74 (bs, 1H) (3 × Ins *H*), 2.11 (dq, *J* = 13.7, 6.8, 1H), 1.77 (dq, *J* = 13.7, 7.3, 1H) (2 × CH*H*CH$_3$), 1.03 (t, *J* = 7.3, 3H, CH_2CH_3) ppm; ^{13}C NMR (100 MHz), $CDCl_3$ δ 137.9, 137.7, 136.8 (3 × Ar *C*), 128.7 (2C), 128.6 (4C), 128.4, 128.0, 127.9 (5C), 127.7 (2C) (15 × Ar *C*H), 83.9, 80.5 (2 × Ins *C*H), 76.6 (O*C*H$_2$Ph), 75.9 (Ins *C*), 73.6 (Ins *C*H), 73.5, 71.1 (2 × O*C*H$_2$Ph), 70.7, 69.5 (2 × Ins *C*H), 27.8 (CH_2CH_3), 6.4 (CH_2CH_3) ppm; HRMS (ESI$^+$) *m*/*z* (%) found [M + Na]$^+$ 501.2251 (100), $C_{29}H_{34}O_6Na$ requires 501.2253.

2,6-*O*-Dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate 10. To a clear solution of trimethylsulfoxonium iodide (109 mg, 0.49 mmol) in DMSO (1 mL) was added NaH (60% dispersion in mineral oil, 21 mg, 0.54 mmol), and the mixture was stirred for 40 min. 2,6-*O*-Dibenzyl-inos-4-ose 1,3,5-*O*-orthobenzoate (**3**, 200 mg, 0.45 mmol) was evaporated from MeCN (1 mL × 3), taken up in THF (1 mL), and added to the reaction mixture, which was stirred at rt for 1 h. The reaction was quenched by dropwise addition of H_2O (2 mL), and the product was taken up in CH_2Cl_2. The organic layer was washed with satd $NaHCO_3$, H_2O, and then brine, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc−hexane (1:9 → 2:5 v/v) afforded **10** (184 mg, 89%) as a clear oil; R_f (EtOAc−hexane, 1:1 v/v) 0.70; ^{1}H NMR (400 MHz), $CDCl_3$ δ 7.68−7.26 (m, 15H, 15 × Ar *H*), 4.75 (d, *J* = 12.2, 1H), 4.70 (d, *J* = 12.1, 1H), 4.68 (d, *J* = 11.8, 1H) (3 × OCH*H*Ph), 4.55 (dq, *J* = 4.2, 2.0, 1H, Ins 1-*H*), 4.53 (d, *J* = 11.9, 1H, OCH*H*Ph), 4.42 (t, *J* = 3.8, 1H, Ins 6-*H*), 4.03 (t, *J* = 1.7, 1H, Ins 2-*H*), 3.95−3.94 (m, 2H, Ins 3-*H* + Ins 5-*H*), 2.95 (d, *J* = 4.7, 1H), 2.93 (d, *J* = 4.7, 1H) (2 × Ins 4-CCH*H*) ppm; ^{13}C NMR (100 MHz), $CDCl_3$ δ 137.8, 137.3, 136.7 (3 × Ar *C*), 129.6, 128.6 (2C), 128.5 (2C), 128.1, 128.0 (2C), 127.84 (3C), 127.79 (2C), 125.4 (2C) (15 × Ar *C*H), 108.1 (Ph*C*O_3), 74.9, 72.5, 72.3, 71.6 (4 × Ins *C*H), 71.5, 71.3 (2 × O*C*H$_2$Ph), 68.3 (Ins *C*H), 54.9 (Ins *C*), 51.0 (Ins 4-C*C*H$_2$) ppm; MS (ESI$^+$) *m*/*z* (%) found [M + H]$^+$ 459 (62), [M + Na]$^+$ 481 (100); HRMS (ESI$^+$) found [M + H]$^+$ 459.1800, $C_{28}H_{27}O_6$ requires 459.1808.

General Method C. Preparation of 2,6-*O*-Dibenzyl-4-*C*-alkyl-*myo*-inositol 1,3,5-*O*-Orthobenzoates 11 Using Alkyl Lithium Cuprates. CuCN (4.0 mmol) was evaporated from toluene (3 × 2 mL), taken up in ether (5 mL), and cooled to −78 °C. Alkyl lithium (8.0 mmol) was added dropwise to the stirred suspension, which turned pale yellow. In a separate vessel, 2,6-*O*-dibenzyl-4,4-*O*,*C*-methylidene-*myo*-inositol 1,3,5-*O*-orthobenzoate (**10**, 1.0 mmol) was evaporated from toluene (3 × 2 mL), taken up in ether (5 mL), and cooled to −78 °C. The solution of **10** was added slowly to the solution of alkyl lithium cuprate and stirred for 4 h at −78 °C, or until complete as judged by TLC. Excess reagent was quenched with satd NH_4Cl−NH_3 (9:1 v/v, 10 mL) and the two-phase mixture was stirred vigorously for a further 30 min before addition of brine (5 mL). After separating the organic layer, the aqueous phase was washed with ether (3 × 50 mL), and combined organic layers were dried ($MgSO_4$) and evaporated to dryness under reduced pressure.

2,6-*O*-Dibenzyl-4-*C*-ethyl-*myo*-inositol 1,3,5-*O*-Orthobenzoate 11b. Compound **10** (255 mg, 0.55 mmol) was treated with methyl lithium cuprate according to general method C. The crude material was fractionated using chromatography on flash silica. Elution with EtOAc−hexane (1:9 → 3:7 v/v) afforded **11b** (241 mg, 92%) as a pale yellow oil; R_f (EtOAc−hexane, 3:7 v/v) 0.57; ^{1}H NMR (400 MHz), $CDCl_3$ δ 7.69−7.67 (m, 2H), 7.48−7.34 (m, 11H), 7.25−7.23 (m, 2H) (15 × Ar *H*), 4.83 (d, *J* = 12.2, 1H), 4.71 (d, *J* = 12.2, 1H), 4.62 (d, *J* = 11.7, 1H), 4.57 (d, *J* = 11.7, 1H) (4 × OCH*H*Ph), 4.54 (t, *J* = 3.9, 1H, Ins 6-*H*), 4.49 (dq, *J* = 3.9, 2.0, 1H, Ins 1-*H*), 4.27 (q, *J* = 2.0, 1H, Ins 3-*H*), 4.23 (bs, 1H, Ins 4-O*H*), 4.10 (dt, *J* = 3.4, 1.5, 1H, Ins 5-*H*), 4.03 (t, *J* = 1.5, 1H, Ins 2-*H*), 2.15 (dq, *J* = 14.0, 7.3, 1H), 2.01 (dq, *J* = 14.1, 7.3, 1H) (2 × CH*H*CH$_3$), 1.03 (t, *J* = 7.3, 3H, CH_2CH_3) ppm; ^{13}C NMR (125 MHz), $CDCl_3$ δ 137.9, 137.0, 135.9 (3 × Ar *C*), 129.4, 128.8 (2C), 128.7, 128.5 (2C), 128.1 (2C), 128.0 (4C), 127.9, 125.4 (2C) (15 × Ar *C*H), 107.3 (Ph*C*O_3), 75.6 (Ins 3-*C*H), 74.7 (Ins 6-*C*H), 73.0, 71.1 (2 × O*C*H$_2$Ph), 71.0 (Ins 1-*C*H), 70.9 (Ins 4-*C*), 70.6 (Ins 5-*C*H), 66.4 (Ins 2-*C*H), 28.3 (CH_2CH_3), 6.4 (CH_2CH_3) ppm; MS (ESI$^+$) *m*/*z* (%) found [M + H]$^+$ 475 (100), [M + Na]$^+$ 497 (82), [2M + Na]$^+$ 971 (53); HRMS (ESI$^+$) found [M + H]$^+$ 475.2137, $C_{29}H_{31}O_6$ requires 475.2121.

General Method D. Phosphorylation of Triols 5 Using *N*,*N*-Diisopropyldibenzyl Phosphoramidite 20. 2,3,6-*O*-Tribenzyl-4-*C*-alkyl-*myo*-inositol (0.1 mmol) and 1-*H*-tetrazole (1.2 mmol) were evaporated from MeCN (3 × 2 mL) then taken up in MeCN (5 mL), and **20** (0.6 mmol) was added. After 2 h the solution was cooled to −40 °C, and *m*CPBA (0.7 mmol) added. Stirring was continued at 0 °C for 2 h. The solution was diluted with CH_2Cl_2, washed with 10% aq $Na_2S_2O_3$, satd $NaHCO_3$, H_2O, and brine. The organic layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure. The crude material was stirred with 1 M I_2 in pyridine−H_2O−THF (2:1:7 v/v, 3 mL) for 15 min before dilution with H_2O and $CHCl_3$. Saturated $Na_2S_2O_3$ was slowly added to the vigorously stirred solution until both layers were clear in color. The organic layer was washed with satd $NaHCO_3$ and then HCl (0.4 M) and dried ($MgSO_4$), and all solvents were evaporated under reduced pressure.

1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-ethyl-*myo*-inositol 22b. Compound **5b** (67 mg, 0.14 mmol) was phosphitylated using **20** and then oxidized with *m*CPBA as described in general method D. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc−hexane (1:9 → 1:0 v/v) afforded **22b** (143 mg, 81%); R_f (EtOAc−hexane, 3:7 v/v) 0.11; ^{1}H NMR (400 MHz), $CDCl_3$ δ 7.41−7.00 (m, 45H, 45 × Ar *H*), 5.09−4.67 [m, 18H, (17 × OCH*H*Ph) + Ins 5-*H*], 4.61−4.52 (m, 3H, OCH*H*Ph + Ins 1-*H* + Ins 2-*H*), 4.43 (d, *J* = 2.3, 1H, Ins 3-*H*), 4.08−4.02 (m, 1H, Ins 6-*H*), 2.48 (dqd, *J* = 14.9, 7.4, 3.0, 1H, CH*H*CH$_3$), 2.18 (dq, *J* = 14.1, 7.4, 1H, CH*H*CH$_3$), 1.19 (t, *J* = 7.4, 3H, CH_2CH_3) ppm; ^{13}C NMR (100 MHz), $CDCl_3$ δ 138.33, 138.26, 137.9 (3 × Ar *C*), 136.3 (d, *J* = 8.0), 136.1 (d, *J* = 7.8), 136.0 (d, *J* = 8.1), 135.9 (d, *J* = 7.4), 135.7 (d, *J* = 5.8), 135.6 (d, *J* = 6.4) (6 × Ar *C*CH$_2$OP), 128.5 (2C), 128.5 (2C), 128.4, 128.34 (3C), 128.29 (3C), 128.25 (2C), 128.17 (2C), 128.13 (4C), 128.08 (2C), 127.98, 127.91 (3C), 127.83 (2C), 127.78 (3C), 127.59 (2C), 127.50 (4C), 127.45 (2C), 127.3 (2C), 127.23, 127.16, 126.9 (3C) (45 × Ar *C*H), 89.9−89.7 (m, Ins 4-*C*), 78.7, 77.6, 77.3 (2C) (4 × Ins *C*H), 74.4 (2 × O*C*H$_2$Ph), 73.8 (Ins *C*H), 69.5 (d, *J* = 5.2), 69.3−69.1 (5C, m), 68.8 (d, *J* = 5.9) [O*C*H$_2$Ph + (6 × PO*C*H$_2$Ph)], 25.8 (d, *J* = 4.3, *C*H$_2$CH$_3$), 9.4 (CH$_2$*C*H$_3$) ppm; ^{31}P NMR (162 MHz), $CDCl_3$ δ −1.82, −2.15, −7.07 ppm; MS (ESI$^+$) *m*/*z* (%) found [M + H]$^+$ 1259 (55), [M + Na]$^+$ 1281 (100).

General Method E. Global Deprotection of $InsP_3$ Precursors 22 Using H_2 and Pd-Black. 1,4,5-*O*-Tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-alkyl-*myo*-inositol (0.05 mmol) was taken up in ^{t}BuOH−H_2O (6:1 v/v, 6 mL) to which was added $NaHCO_3$ (0.41 mmol) and Pd-black (1.03 mmol). The solution was stirred under an atmosphere of H_2 (1 atm) for 36 h. The catalyst was filtered off and washed with H_2O (4×10 mL), and the mother liquor was concentrated under reduced pressure, before being taken up in H_2O, washed with CH_2Cl_2 (×2) and lyophilized.

4-*C*-Ethyl-*myo*-inositol 1,4,5-*O*-Trisphosphate 7b. (a) As the ammonium salt: 1,4,5-*O*-tris(dibenzyloxyphosphoryl)-2,3,6-*O*-tribenzyl-4-*C*-ethyl-*myo*-inositol (**22b**, 138 mg, 0.11 mmol) was hydrogenated according to general method E. The powdery solid was redissolved in the minimum volume of H_2O and passed through DOWEX 50WX8-200 H^+ resin. Acidic fractions of eluent were combined, neutralized with aq ammonia, and lyophilized to yield **7b** (49 mg, 100%) as a pale brown powdery salt. (b) As the sodium salt: crude 4-*C*-ethyl-*myo*-inositol 1,4,5-*O*-trisphosphate (45 mg, 0.09 mmol) was redissolved in the minimum volume of H_2O and stirred with sodiated DOWEX 50WX8-200 resin (prepared by stirring the H^+ form with NaOH for 30 min before washing with H_2O). The crude material was purified by preparative RP-HPLC. Elution over a gradient of H_2O−MeCN (flow rate 0.1 mL min^{-1}, 0−10 min 49:1 v/v, 10−17 min 49:1 → 1:1 v/v, retention time 6.7 min) afforded **7b** (47 mg, 100%); ^{1}H NMR (500 MHz), D_2O, 328 K δ 4.61 (t, J = 3.4, 1H, Ins 2-*H*), 4.48 (t, J = 8.2, 1H, Ins 5-*H*), 4.40−4.37 (m, 1H, Ins 3-*H*), 4.34−4.29 (m, 1H, Ins 1-*H*), 4.27−4.23 (m, 1H, Ins 6-*H*), 2.34 (dq, J = 14.8, 7.3, 1H), 2.22 (dq, J = 14.6, 7.2, 1H) (2 × CH*H*CH$_3$), 1.31 (t, J = 7.3, 3H, CH$_2$C*H$_3$*) ppm; ^{13}C NMR (125 MHz), D_2O, 328 K δ 83.9−83.7 (m, Ins 4-*C*), 78.6−78.1 (br m, Ins 5-*C*H), 75.5 (d, J = 4.4, Ins 1-*C*H), 73.1 (d, J = 5.5, Ins 3-*C*H), 72.2 (d, J = 5.5, Ins 6-*C*H), 69.3−69.1 (m, Ins 2-*C*H), 25.0 (*C*H$_2$CH$_3$), 9.1 (CH$_2$*C*H$_3$) ppm; ^{31}P NMR (162 MHz), D_2O, 328 K δ 4.45, 4.08, 0.52 ppm; HRMS (ESI$^-$) m/z (%) found [M − H]$^-$ 446.9842 (100), $C_8H_{18}O_{15}P_3$ requires 446.9859.

4-*C*-Ethyl-*myo*-inositol 1,5-*O*-Diphosphate (23b) Formed from Decomposition of ammonium salt of 4-*C*-ethyl-*myo*-inositol 1,4,5-*O*-Trisphosphate 7b. ^{1}H NMR (500 MHz), D_2O, 328 K δ 4.61 (t, J = 3.4, 1H, Ins 2-*H*), 4.53 (td, J = 8.1, 3.4, 1H, Ins 1-*H*), 4.46−4.41 (m, 1H, Ins 6-*H*), 4.31 (t, J = 8.7, 1H, Ins 5-*H*), 4.03 (d, J = 3.4, 1H, Ins 3-*H*), 2.28 (dq, J = 18.0, 6.0, 1H), 2.17 (dq, J = 17.6, 6.0, 1H) (2 × CH*H*CH$_3$), 1.37 (t, J = 6.2, 3H, CH$_2$C*H$_3$*) ppm; ^{13}C NMR (125 MHz), D_2O, 328 K δ 80.8−80.5 (m, Ins 5-*C*H), 77.2 (d, J = 3.3, Ins 4-*C*), 76.2 (d, J = 5.5, Ins 1-*C*H), 74.7 (Ins 3-*C*H), 70.7 (t, J = 3.3, Ins 6-*C*H), 69.7−69.4 (m, Ins 2-*C*H), 25.4 (*C*H$_2$CH$_3$), 8.0 (CH$_2$*C*H$_3$) ppm; ^{31}P NMR (162 MHz), D_2O, 328 K δ 1.09, 0.77 ppm; HRMS (ESI$^-$) m/z (%) found [M − H]$^-$ 367.0184 (100), $C_8H_{17}O_{12}P_2$ requires 367.0195.

1,3,4,5-*O*-Tetrakis(dicyanoethyloxyphosphoryl)-2,6-*O*-dibenzyl-4-*C*-benzyl-*myo*-inositol 25. Compound **4e** (200 mg, 0.44 mmol) and 3-nitro-1,2,4-1*H*-triazole (709 mg, 6.22 mmol) were evaporated from MeCN (3 × 2 mL) and taken up in pyridine (2 mL). Dicyanoethyl phosphorochloridite **24** (ca. 0.5 M solution in CH_2Cl_2, 6.22 mL, 3.11 mmol) was added, and the solution was stirred for 2 h. After this, 3-hydroxypropionitrile (88 μL, 1.33 mmol) was added, and the reaction was stirred for a further 1 h, before cooling to 0 °C. *m*CPBA (75%, 1.07 g, 6.22 mmol) was added portion-wise, and the resulting solution was allowed to warm to rt and stirred for a further 2 h. The reaction was quenched with $Na_2S_2O_3$ (10% solution in H_2O) and stirred for 30 min before extracting with CH_2Cl_2 (4 × 50 mL). The combined organic layers were washed with brine and dried ($MgSO_4$), and all solvents were evaporated under reduced pressure. The crude residue (1.60 g) was separated from phosphorylating reagent debris by chromatography on silanized silica. Elution with MeCN−H_2O (1:19 → 1:1 v/v) afforded the crude product, which was then fractionated by chromatography on flash silica. Elution with MeOH−CH_2Cl_2 (0:1 → 3:50 v/v) afforded **25** (127 mg, 24%) as a clear oil; R_f (MeOH−CH_2Cl_2, 1:25 v/v) 0.37; ^{1}H NMR (400 MHz), $CDCl_3$ δ 7.51−7.46 (m, 4H), 7.42−7.36 (m, 5H), 7.35−7.25 (m, 5H), 7.23−7.19 (m, 1H) (15 × Ar *H*), 5.37−5.34 (m, 1H, Ins *H*), 5.06−5.04 (m, 1H, Ins *H*), 5.04 (d, J = 12.7, 1H), 5.00 (d, J = 11.4, 1H), 4.97 (d, J = 11.0, 1H), 4.89 (d, J = 11.0, 1H) (4 × OCH*H*Ph), 4.78−4.72 (m, 2H, 2 × Ins *H*), 4.27−3.95 [m, 17H, (8 × OC*H$_2$*CH$_2$CN) + Ins *H*], 3.65 (dd, J = 15.0, 3.5, 1H), 3.47 (d, J = 15.4, 1H) (2 × CCH-*H*Ph), 2.76−2.54 (m, 16H, 8 × OCH$_2$C*H$_2$*CN) ppm; ^{13}C NMR (100 MHz), $CDCl_3$ δ 137.8, 137.6 (2 × Ar *C*)*, 131.8, 128.7 (3C), 128.6 (3C), 128.2 (2C), 128.0, 127.9 (2C), 127.8, 127.5, 126.6 (15 × Ar *C*H), 117.1, 117.0, 116.8, 116.7, 116.6, 116.5 (2C), 116.4 (8 × OCH$_2$CH$_2$*C*N), 88.1−87.9 (m, Ins 4-*C*), 77.21, 77.15, 77.1, 77.0, 76.9 (5 × Ins *C*H), 76.2, 74.9 (2 × O*C*H$_2$Ph), 63.1−62.9 (4C, m), 62.9 (d, J = 5.5), 62.8 (d, J = 4.9), 62.7 (d, J = 4.9), 62.7 (d, J = 4.9) (8 × OCH$_2$*C*H$_2$CN), 37.5 (C*C*H$_2$Ph), 19.6 (d, J = 7.6), 19.6 (d, J = 8.2), 19.6 (d, J = 6.5), 19.5 (d, J = 8.2, 2C), 19.5 (d, J = 7.6, 2C), 19.3 (d, J = 7.6) (8 × OCH$_2$*C*H$_2$CN) ppm; ^{31}P NMR (162 MHz), $CDCl_3$ δ −3.17, −3.99, −4.17, −8.61 ppm; MS (ESI$^+$) m/z (%) found 338 (100), [M + H]$^+$ 1195 (61), [M + Na]$^+$ 1217 (92). * Third Ar *C* peak not detected in ^{13}C NMR spectrum.

2,6-*O*-Dibenzyl-4-*C*-benzyl-*myo*-inositol 1,3,4,5-*O*-Tetrakisphosphate 26. Compound **25** (77 mg, 0.06 mmol) was evaporated from MeCN (3 × 1 mL) and then taken up in MeCN (1 mL). Barton's base (155 μL, 0.77 mmol) and TmsCl (82 μL, 0.65 mmol) were added, and the solution was stirred for 16 h. All solvents were evaporated under reduced pressure, and the residue was evaporated from toluene (2 × 2 mL). The residue was washed with ether−hexane (1:1 v/v) and carefully filtered under N_2 using a filter stick. The filtrate was evaporated to dryness and then stirred with $NaHCO_3$ (20 mg, 0.24 mmol) suspended in MeOH (2 mL). After 10 min, all solvents were evaporated, and the residue was evaporated from MeCN (2 × 5 mL), to afford **26** (47 mg, 85%) as a white residue; ^{1}H NMR* (500 MHz), MeOD δ 7.68 (d, J = 7.3, 2H), 7.53 (d, J = 7.3, 2H), 7.35−7.33 (m, 6H), 7.26 (t, J = 6.8, 2H), 7.09 (t, J = 6.8, 2H), 7.03 (t, J = 6.8, 1H) (15 × Ar *H*), 5.04 (bd, J = 8.3, 1H, Ins 3-*H*), 5.01 (s, 2H, OC*H$_2$*Ph), 4.98 (d, J = 10.5, 1H), 4.95 (d, J = 10.5, 1H) (2 × OCH*H*Ph), ~4.94 (under HOD, Ins 5-*H*), 4.72 (t, J = 3.4, 1H, Ins 2-*H*), 4.37 (bt, J = 8.3, 1H, Ins 1-*H*), 4.06 (t, J = 9.8, 1H, Ins 6-*H*), 3.55 (d, J = 14.2, 1H), 3.31 (d, J = 14.2, 1H) (2 × CCH*H*Ph) ppm; ^{13}C NMR** (100 MHz), MeOD δ 139.5, 138.4, 138.1 (3 × Ar *C*), 131.8 (2C), 129.5 (2C), 127.7 (4C), 127.6 (2C), 127.1, 126.8, 126.4 (2C), 124.6 (15 × Ar *C*H), 79.3 (b, Ins 5-*C*H), 78.7 (Ins 2-*C*H), 77.3 (b, Ins 6-*C*H), 76.1 (O*C*H$_2$Ph), 75.6 (b, Ins 1-*C*H), 75.3 (b, Ins 3-*C*H), 74.9 (O*C*H$_2$Ph), 36.8 (b, C*C*H$_2$Ph) ppm; ^{31}P NMR (162 MHz), MeOD δ 0.13, −0.17, −0.23, −2.99 ppm; HRMS (ESI$^-$) m/z (%) found [M − H]$^-$ 769.0649 (65), $C_{27}H_{33}O_{18}P_4$ requires 769.0617.; *Also includes nonstoichiometric Barton's Base counterion δ_H 2.98, 1.39 ppm, δ_C 161.2, 56.0, 39.4, 28.6 ppm. **Quatenary 4-*C* not detected in ^{13}C NMR spectrum.

1,4,5-*O*-Tris(5,6-benzo-2-oxo-1,3,2-dioxaphosphapan-2-yl)-2,3,6-*O*-tribenzyl-4-*C*-benzyl-*myo*-inositol 28. Compound **5e** (79 mg, 0.15 mmol) and 1-*H* tetrazole (102 mg, 1.46 mmol) were evaporated from MeCN (3 × 1 mL) and taken up in MeCN (1 mL). The resulting suspension was placed in a water bath at 25 °C, and *N*,*N*-diethylamino-5,6-benzo-1,3,2-dioxaphosphepane (158 μL, 1.02 mmol) was added, when all solids dissolved immediately. After 2 h the solution was cooled to 0 °C, and *m*CPBA (336 mg, 1.46 mmol) was added. The solution was then allowed to warm to rt and stirred for a further 90 min, before addition of $Na_2S_3O_3$ (10% aq solution). After 30 min the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic

layers were washed with brine and dried ($MgSO_4$), and all solvents were evaporated under reduced pressure. The crude material was fractionated by chromatography on flash silica. Elution with EtOAc−hexane (1:9 → 1:0 v/v) afforded **28** (69 mg, 44%) as a clear oil; R_f (EtOAc−hexane, 3:7 v/v) 0.19; ^{1}H NMR (400 MHz), $CDCl_3$ δ 7.52−7.47 (m, 4H), 7.35−6.95 (m, 28H) (32 × Ar *H*), 5.30−4.74 (m, 17H, 3 × Ins *H*, 3 × OC*H*$_2$Ph, 8 × OCH*H*Ar), 4.65−4.60 (m, 3H, Ins *H*, OCH_2Ar), 4.39 (dd, J = 13.7, 9.8, 1H, OCH*H*Ar), 4.27−4.21 (m, 1H, Ins *H*), 4.09−3.98 (m, 2H, OCH*H*Ar + CH*H*Ph), 3.61 (d, J = 11.7, 1H, CCH*H*Ph) ppm; ^{13}C NMR (100 MHz), $CDCl_3$ δ 138.2, 137.8, 137.7, 135.7, 135.6 (2C), 135.5, 135.2, 135.1, 132.6 (10 × Ar *C*), 129.2, 129.1 (2C), 129.02 (2C), 128.98 (2C), 128.8 (3C), 128.7 (2C), 128.6 (2C), 128.5 (2C), 128.4 (3C), 128.1 (4C), 127.74 (2C), 127.69, 127.5 (2C), 127.4 (2C), 127.29, 127.26 (32 × Ar *C*), 88.2−88.1 (m, Ins *C*), 82.8−82.7 (m), 78.9, 77.3, 76.5−76.4 (m) (4 × Ins *C*H), 76.3, 75.7 (2 × O*C*H_2Ph), 74.7−74.6 (m, Ins *C*H), 71.9 (O*C*H_2Ph), 68.9 (d, J = 6.4), 68.6−68.1 (m, 5C) (6 × O*C*H_2Ar), 36.5 (C*C*H_2Ph) ppm; ^{31}P NMR (162 MHz) $CDCL_3$ δ −0.48, −2.27, −9.11 ppm; MS (ESI$^+$) m/z (%) found $[M + H]^+$ 1087 (40), $[M + Na]^+$ 1109 (100).

4-*C*-Benzyl-*myo*-inositol 1,4,5-*O*-Trisphosphate 7d. Compound **28** (64 mg, 0.06 mmol) was hydrogenated according to general method E. The crude material (30 mg) was purified by preparative RP-HPLC. Elution over a gradient of H_2O−MeCN (flow rate 1.0 mL min^{-1}, 0−4 min 49:1 v/v, 4−17 min 49:1 → 1:1 v/v, retention time 2.8 min, detection at 210 and 254 nm) yielded **7d** (8 mg, 27%) as a pale brown powdery salt; ^{1}H NMR (500 MHz), D_2O δ 7.44 (d, J = 7.5, 2H), 7.22 (t, J = 7.4, 2H), 7.14 (t, J = 7.3, 1H) (5 × Ar *H*), 4.41−4.33 (m, 1H), 4.30−4.27 (m, 1H), 4.20−4.15 (m, 1H), 4.08−3.99 (m, 2H) (5 × Ins *H*), 3.21 (s, 2H, C*C*H_2Ph) ppm; ^{31}P NMR (162 MHz), D_2O δ 0.91, 0.11, −4.33 ppm; HRMS (ESI$^-$) m/z (%) found $[M - H]^-$ 508.9998 (100), $C_{13}H_{20}O_{15}P_3$ requires 509.0015.

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Supporting Information Available: Experimental procedures and NMR characterizations for **4c**, **4e**, **4f**, **4g**, **5c** and its diacetate, **5d**, **5e** and its diacetate, **5f**, **6b**, **6c**, **7c**, **11c**, **11d**, **11e**, **11f**, **11g**, **12b**, **13**, intermediates from **17** to **4e**, **20b**, **20c**, and **21c**; copies of the ^{1}H NMR and ^{13}C NMR spectra for all new compounds and crystallographic data for unexpected conduritol **12a**. This material is available free of charge via the Internet at http://pubs.acs.org.