Synthesis of 1D-3-Deoxy-, 1D-2,3-Dideoxy-, and 1D-2,3,6-Trideoxy-*myo*-inositol 1,4,5-Trisphosphate from Quebrachitol, Their Binding Affinities, and Calcium Release Activity

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Abstract: Syntheses of three optically pure, deoxygenated *myo*-inositol 1,4,5-trisphosphate analogues from quebrachitol are reported together with their binding affinities and $^{45}Ca^{2+}$ release activity. The ligand-binding affinities of the analogues were determined using membrane preparations from bovine adrenal cortex. The $^{45}Ca^{2+}$ release activities were compared to that of Ins(1,4,5)P_3, using the calcium mobilizing receptor of saponin permeabilized SH-SY5Y human neuroblastoma cells. 1D-3-deoxy-Ins(1,4,5)P_3 (**26**) and 1D-2,3-dideoxy-Ins(1,4,5)P_3 (**22**) exhibited very potent ligand and agonist properties, while 1D-2,3,6-trideoxy-Ins(1,4,5)P_3 (**19**) was much less potent. These data provide solid evidence of the critical role which HO-6 of Ins(1,4,5)P_3 plays in its binding affinity and its Ca^{2+} mobilizing activity.

Introduction

The importance of inositol phosphates for intracellular signaling is now well documented.[1] As a result of the stimulation of cell surface receptors by a variety of ligands, the membrane-located phosphatidylinositol 4,5-bisphosphate is hydrolyzed by phospholipase C to 1D-*myo*-inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) and diacylglycerol. Ins(1,4,5)P_3 binds to specific recognition sites on the endoplasmic reticulum resulting in the release of Ca^{2+} from intracellular stores, which constitutes a key event in signal transduction processes.[2] Recently, the Ins(1,4,5)P_3 receptor has been isolated,[3] cloned and sequenced,[4] and reconstituted.[5] The further metabolism of Ins(1,4,5)P_3 is quite complex and involves the action of various kinases and phosphatases with subsequent formation of a plethora of inositol phosphates whose biochemical role is in many cases still unclear and the subject of active investigation.[6] For example, an important initial transformation is the phosphorylation of Ins(1,4,5)P_3 through the action of an ATP-dependent 3-kinase to give 1D-*myo*-inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4). While the role of this metabolite is still under study, the suggestion has been made that it may play a role in refilling the intracellular Ca^{2+} stores with extracellular Ca^{2+}.[1a,7]

Elucidation of the molecular interactions of Ins(1,4,5)P_3 with its intracellular receptor and the metabolic enzymes Ins(1,4,5)P_3 3-kinase and 5-phosphatase could lead to the design of novel therapeutic agents capable of exhibiting Ins(1,4,5)P_3-like agonist or antagonist effects on Ca^{2+} release without being subject to metabolism by the 3-kinase or 5-phosphatase pathways. Although the critical importance of the vicinal 4,5-phosphate groups and the enhancing effect of the 1-phosphate group of Ins(1,4,5)P_3 in receptor binding is now well appreciated,[8] the individual role of the three hydroxyl groups is less clear. It is assumed that the binding affinity of Ins(1,4,5)P_3 is due to interaction of the phosphate groups with positively charged regions on the receptor, while the enhancing effect of the hydroxyl groups is a result of the formation of intermolecular hydrogen bonds with the receptor. In addition, intramolecular hydrogen bonds of the hydroxyl groups with the neighboring phosphate groups could fix the conformation of Ins(1,4,5)P_3 in solution.[9] The synthesis and activity of several deoxygenated analogues of Ins(1,4,5)P_3 have been reported,[10] including DL-2,3,6-trideoxy,[11] DL-2-deoxy-,[12] and 1D-6-deoxy-Ins(1,4,5)P_3.[13] For example, Ca^{2+} release studies have shown that DL-2,3,6-trideoxy-Ins(1,4,5)P_3 has 60–100-fold decreased potency,[11] DL-2-deoxy-Ins(1,4,5)P_3 was virtually equipotent to Ins(1,4,5)P_3,[12] and the 1D-6-deoxy-Ins(1,4,5)P_3 was 70-fold less potent[13] than Ins(1,4,5)P_3. Since the biological findings reported for some of these previously synthesized deoxygenated analogues may be complicated by the use of racemic materials, more detailed

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studies with optically pure derivatives were deemed valuable to probing the specific role of the hydroxyl groups of Ins(1,4,5)P_3. In this context, in a preliminary communication[14] we reported the preparation of 1D-3-deoxy-Ins(1,4,5)P_3, which was found to exhibit the same agonist effect as Ins(1,4,5)P_3 on Ca^{2+} release. Herein we describe in full syntheses of 1D-3-deoxy-Ins(1,4,5)P_3 (**26**) and the novel dideoxy and trideoxy compounds, namely 1D-2,3-dideoxy-Ins(1,4,5)P_3 (**22**) and 1D-2,3,6-trideoxy-Ins(1,4,5)-P_3 (**19**), together with their binding affinities and Ca^{2+} mobilizing activities.

Chemistry: Synthesis of 1D-Deoxy-*myo*-inositol 1,4,5-Trisphosphates 19, 22, and 26 from L-Quebrachitol

L-Quebrachitol [1L-2-*O*-methyl-*chiro*-inositol (**1**), Scheme I], isolated from waste rubber solids by extraction with EtOH, was converted to the diacetonide **2** by treatment with 2-methoxypropene in the presence of camphorsulfonic acid in analogy to a known procedure.[15] The remaining unprotected hydroxyl group in **2** was removed by application of the Barton deoxygenation procedure.[16] Thus, treatment of the alkoxide of **2** with CS_2 and MeI furnished the corresponding *S*-methyl xanthate (*S*-methyl dithiocarbonate) as an intermediate, which was further deoxygenated homolytically to **3** with *n*-Bu_3SnH. Next, removal of the protecting groups in **3** by BBr_3 provided 1D-3-deoxy-*myo*-inositol ((−)-viburnitol, **4**). This important chiral intermediate, possessing hydroxyl groups with the necessary *myo*-configuration for the construction of the target compounds **19**, **22**, and **26**, was converted to a mixture of regioisomeric bisacetonides **5**. Benzylation of **5** to **6**, followed by chemoselective cleavage of the transacetonide of **6** with a catalytic amount of AcCl in MeOH afforded the mixture of diols **7**. Next, benzoylation of **7** provided an isomeric mixture comprised of **8** and **9**. These products were separated readily by silica gel chromatography, and their structures were established by comparison of the ^1H NMR coupling constants of the protons adjacent to the carbon atoms bearing the benzoyloxy substituents, which in both cases resonate at lower field strengths (δ = 5.39 to 5.61). For **8** two doublet of doublets were observed with large couplings of J = 10.0, 8.0 Hz, and J = 10.0, 8.3 Hz, respectively, indicating a set of consecutive trans diaxial couplings in the H-4, H-5, H-6, and H-1 portion of the molecule. Similarly, the resonance for H-5 of isomer **9** appeared as a triplet with a large coupling constant of J = 9.0 Hz indicating trans diaxial geometry, while that for H-4 displayed a doublet of doublet of doublets with J = 10.9, 9.5, 4.7 Hz reflecting the coupling with the vicinal methylene group and H-5. Since isomer **8** could be transformed to the precursor **4** by deprotection, **9** could be prepared in good yield by recycling the undesired isomer **8**. Differentiation of the two hydroxyl groups of **10**, obtained by acetonide cleavage of **9**, was accomplished by selective benzoylation of the equatorial hydroxyl furnishing **11**.

At this stage, **11** appeared as a crucial common precursor to the target compounds due to the proper protective group pattern, thus allowing differentiation and subsequent manipulation of the hydroxyl groups. Attempted preparation of the *S*-methyl xanthate of **11** under the conditions described for the Barton deoxygenation of **2** and **3** gave rise to a complex mixture of reaction products. Therefore the xanthate was replaced by the phenoxythiocarbonyl derivative **12**, which was prepared in good yield by treatment of **11** with phenyl chlorothionocarbonate[17] in the presence of DMAP. When **12** was treated with *n*-Bu_3SnH under the standard conditions, clean homolytic deoxygenation occurred to form product **13**. Debenzylation of **13** gave alcohol **14**, which when subjected to a similar deoxygenation procedure through the phenoxythiocarbonyl derivative **15** afforded the protected 2,3,6-trideoxy-*myo*-inositol **16**. Removal of the benzoate groups in **16** by basic hydrolysis with K_2CO_3 in MeOH gave triol **17**, which after phosphorylation with tetrabenzyl pyrophosphate[18] in the presence of NaH[19] furnished **18**. Next, hydrogenolysis of the benzyl groups of **18** over 10% Pd/C in EtOH, followed by titration of the free acid to pH = 10 with 1 N NaOH, gave the desired 1D-2,3,6-trideoxy-*myo*-inositol 1,4,5-tris(disodium phosphate) (**19**).

In a similar manner, removal of the benzoyl groups of the precursor **13** afforded triol **20**, which after phosphorylation to form **21** was debenzylated and titrated with 1 N NaOH to give the second target compound, 1D-2,3-dideoxy-*myo*-inositol 1,4,5-tris(disodium phosphate) (**22**). Finally, derivatization of the axial hydroxyl group of the intermediate **11** with ethyl vinyl ether in the presence of a catalytic amount of pyridinium *p*-toluenesulfonate produced the ethoxyethyl (EE) derivative **23**. Similar debenzoylation of **23** gave the triol **24**, which was phosphorylated to **25**, debenzylated, exposed to H_2O for cleavage of the EE protective group, and titrated with 1 N NaOH to give 1D-3-deoxy-*myo*-inositol 1,4,5-tris(disodium phosphate) (**26**).

Biology: Binding and Calcium Release Studies. Preparation of membranes from bovine adrenal cortices and the [^3H]Ins-(1,4,5)P_3 displacement studies to assess the binding affinity of each of the deoxygenated Ins(1,4,5)P_3 analogues were performed as described.[20] The Ca^{2+} releasing effects of the analogues were studied on saponin permeabilized SH-SY5Y human neuroblastoma cells preloaded with $^{45}Ca^{2+}$ as described previously.[21] In all cases four independent experiments were performed. The binding data have been corrected for nonspecific binding, and the calcium release data have been normalized to the ionomycin-sensitive stores. The results of the binding and calcium release studies of the deoxy analogues **26**, **22**, and **19** are presented in Table I. For comparison purposes, data are also provided both for the natural metabolite Ins(1,4,5)P_3 and for 1D-3-deoxy-3-fluoro-Ins(1,4,5)P_3, a compound synthesized previously by us[22] which is not subject to the action of 3-kinase.

Results and Discussion

1D-3-Deoxy-Ins(1,4,5)P_3 (**26**) and 1D-2,3-dideoxy-Ins(1,4,5)-P_3 (**22**) were bound by the Ins(1,4,5)P_3 receptor from bovine adrenal cortex with relatively high affinity (K_i = 23.4 and 39.6 nM, respectively) but with lower affinity than Ins(1,4,5)P_3 (K_d = 6.3 nM). Replacement of the 3-hydroxyl group of Ins(1,4,5)-P_3 by a fluorine substituent [i.e., 1D-3-deoxy-3-fluoro-Ins(1,4,5)-P_3] leads to a compound of slightly increased binding affinity and efficacy in comparison to analogue **26** in which this hydroxyl group is replaced by hydrogen. In contrast to these results, 1D-2,3,6-trideoxy-Ins(1,4,5)P_3 (**19**) exhibited substantially weaker binding affinity (see Table I) (K_i = 3948 nM) to bovine adrenal cortical membranes.

In agreement with the binding studies, analogues **26** and **22** as well as 1D-3-deoxy-3-fluoro-Ins(1,4,5)P_3 acted as full agonists in releasing Ca^{2+} from permeabilized SH-SY5Y cells (EC_{50} = 155.7, 185.7, and 120.2 nM, respectively) but were of lower potency than Ins(1,4,5)P_3 (EC_{50} = 52.1 nM). On the other hand, the trideoxy analogue **19** is a very poor effector molecule for Ca^{2+} release (EC_{50} > 10 000 nM) (Table I).

From these biological data we can conclude that the hydroxyl groups at positions 2 and 3 of Ins(1,4,5)P_3 play a relatively minor role in the recognition of this molecule by its receptor or in inducing

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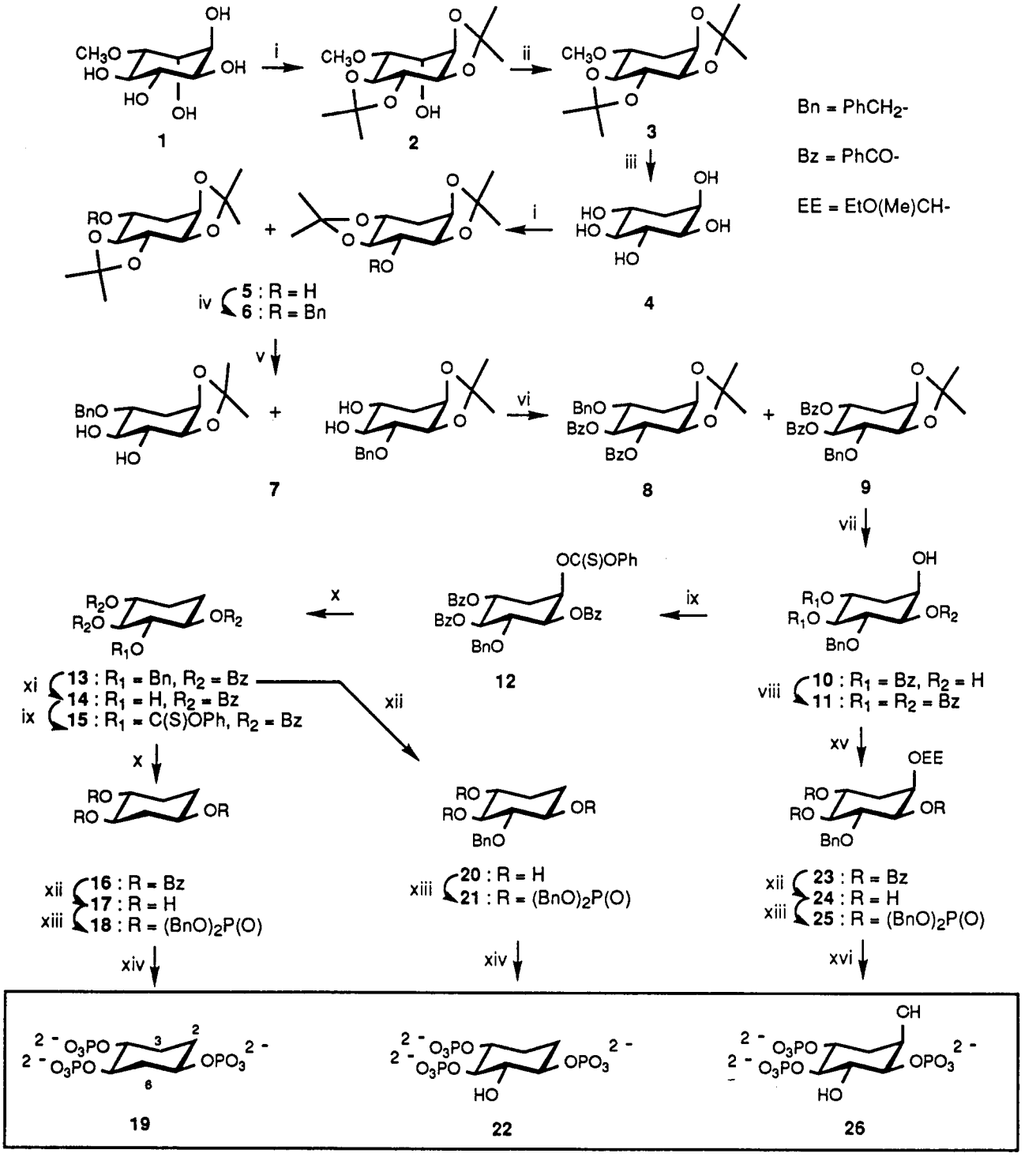
Scheme I.[a] Synthesis of 1D-3-Deoxy- (**26**), 1D-2,3-Dideoxy- (**22**), and 1D-2,3,6-Trideoxy-*myo*-inositol 1,4,5-Tris(disodium phosphate) (**19**)



[a] Reagents and conditions: (i) $H_2C{=}C(OMe)CH_3$, camphorsulfonic acid (CSA), DMF; (ii) (a) NaH, CS_2, THF, then MeI; (b) *n*-Bu_3SnH, AIBN, toluene; (iii) BBr_3, CH_2Cl_2; (iv) NaH, $PhCH_2Br$; (v) AcCl (catalyst), $MeOH/CH_2Cl_2$(1:2); (vi) PhCOCl, pyr, DMAP (catalyst), separate by silica gel chromatography; (vii) concentrated HCl (catalyst), MeOH/THF(4:1); (viii) PhCOCl, pyr, DMAP (catalyst); (ix) PhOC(S)Cl, DMAP, CH_2Cl_2; (x) *n*-Bu_3SnH, AIBN, toluene; (xi) H_2 (1 atm), 10% Pd/C, EtOH/THF (3:1); (xii) K_2CO_3, MeOH; (xiii) NaH, tetrabenzylpyrophosphate, DMF; (xiv) (a) H_2(50 psi), 10% Pd/C, EtOH; (b) 1 N NaOH; (xv) $H_2C{=}CHOEt$, pyridinium *p*-toluenesulfonate (catalyst), CH_2Cl_2; (xvi) (a) H_2 (50 psi), 10% Pd/C, EtOH; (b) H_2O; (c) 1 N NaOH.

the conformational changes required for mobilizing the Ins(1,4,5)-P_3 receptor associated Ca^{2+} pools.[12] On the other hand, the observed weak binding affinity and Ca^{2+} mobilizing activity of the 2,3,6-trideoxy analogue (**19**) are consistent with the reported[10,13] low affinity and activity of 1D-6-deoxy-Ins(1,4,5)P_3, thus confirming the critical nature of the hydroxyl group at the 6-position of Ins(1,4,5)P_3. The 6-hydroxyl group of Ins(1,4,5)P_3 may thus engage in essential hydrogen bonding interactions with appropriate amino acid residues of its receptor. Alternatively, intramolecular hydrogen bonding to the neighboring 1- and 5-phosphate groups may be important in defining a "receptor-recognizing" conformation of the Ins(1,4,5)P_3 molecule.[9] Furthermore, the reported poor Ca^{2+} mobilizing activity of the 6-*O*-methyl derivative of Ins(1,4,5)P_3 may, in the absence of competing steric factors, also point to the role of the 6-hydroxyl group as a hydrogen bond donor rather than an acceptor group.[11]

Table I. Binding and Calcium Release Data for Ins(1,4,5)P_3, the 3-Deoxy-3-fluoro Analogue, and the Deoxy Analogues **19**, **22**, and **26**

analogue	binding to bovine adrenal cortices (K_i)[a] (nM)	Ca^{2+} release from SH-SY5Y (EC_{50})[a] (nM)
1D-Ins(1,4,5)P_3	(K_d) 6.3 ± 0.5	52.1 ± 2.3
1D-3-deoxy-3-fluoro-Ins(1,4,5)P_3	13.3 ± 0.6	120.2 ± 10.9
1D-3-deoxy-Ins(1,4,5)P_3 (**26**)	23.4 ± 1.5	155.7 ± 20.1
1D-2,3-dideoxy-Ins(1,4,5)P_3 (**22**)	39.6 ± 1.0	185.7 ± 6.9
1D-2,3,6-trideoxy-Ins(1,4,5)P_3 (**19**)	3,948 ± 376	>10000

[a] Results represent the average of at least four experiments.

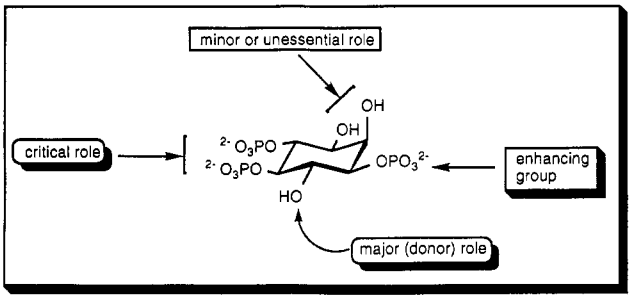


Figure 1. Relative inportance of the functional groups of Ins(1,4,5)P_3 to its binding and calcium mobilizing properties.

1D-3-Deoxy-3-fluoro-Ins(1,4,5)P_3 has previously been shown to be a useful pharmacological tool for studying inositol phosphate based cell signaling events because of its stability to the 3-kinase route of metabolism.[10,14,21,22] In view of the good binding affinity and calcium mobilizing activity of the deoxy analogues **22** and **26**, these compounds would also appear to be useful candidates for further biological study, since they too will be stable to the 3-kinase route of metabolism. Studies currently underway will elucidate the specific interactions of all three deoxy analogues with the metabolic enzymes.

In summary, the current work extends and consolidates previous findings regarding the importance of the individual hydroxyl groups of Ins(1,4,5)P_3 to receptor recognition and calcium mobilization, thus defining a minor role for the 2- and 3-hydroxyl groups and a critical role for the 6-hydroxyl group (Figure 1).

Experimental Section

General Methods. ^1H, ^{31}P, and ^{13}C NMR spectra were recorded on a Bruker WH-300 instrument (1 H frequency 300 MHz), in the solvent(s) noted. ^1H chemical shifts (δ) were reported with Me_4Si (δ = 0.00 ppm) or $CHCl_3$ (δ = 7.26 ppm) as internal standards. ^{31}P chemical shifts (δ) were reported relative to that for external aqueous 85% H_3PO_4. ^{13}C chemical shifts (δ) were reported with $CHCl_3$ (central peak, δ = 77.00 ppm) as internal standard. The following abbreviations are used: br = broad, d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet. Silica gel 60 (Merck, 230–400 mesh for flash chromatography) was used for column chromatography. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F-254 (0.25 mm, precoated on glass). Visualization of compounds on TLC was accomplished by UV illumination or by staining with a solution prepared from 25 g of ammonium molybdate and 1 g of ceric sulfate in 500 mL of 10% sulfuric acid, followed by heating.

1L-3,4:5,6-Di-*O*-isopropylidene-2-*O*-methyl-*chiro*-inositol (2). To a solution of (–)-quebrachitol (**1**) (25.5 g, 0.131 mol) and camphorsulfonic acid (0.61 g, 2.6 mmol) in anhydrous DMF (100 mL) was added 2-methoxypropene (44 mL, 0.46 mol) with stirring at room temperature under Ar. The exothermic reaction ceased after 15 min. The mixture was stirred at 60 °C for 4 h, cooled, diluted with CH_2Cl_2, washed with 10% aqueous $NaHCO_3$ and brine, and dried (Na_2SO_4). Concentration and column chromatography (20% EtOAc in hexanes) gave **2** (29.7 g, 82.5%) as a syrup, which can be crystallized from hexanes: mp 72–73 °C; $[\alpha]^{25}_D$ –7.8 (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.40–4.30 (m, 3 H), 3.79–3.58 (m, 3 H), 3.56 (s, 3 H), 2.81 (d, *J* = 3.1 Hz, OH), 1.52, 1.45, 1.44, and 1.37 (4 s, each 3 H). Anal. Calcd for $C_{13}H_{22}O_6$ (274.31): C, 56.92; H, 8.08. Found: C, 57.03; 8.01. These data were consistent with those previously reported[15] for **2**.

1D-3-Deoxy-1,2:5,6-di-*O*-isopropylidene-4-*O*-methyl-*myo*-inositol (3). To a solution of **2** (27.8 g, 0.101 mol) in THF (400 mL) was added 60% NaH in mineral oil (6.06 g, 0.152 mol) at 0 °C, and the mixture was stirred for 1 h at room temperature under Ar. After cooling to 0 °C, CS_2 (7.28 mL, 0.121 mol) was added dropwise. The mixture was stirred for 0.5 h at room temperature, cooled to 0 °C followed by dropwise addition of MeI (9.47 mL, 0.152 mol), and further stirred at room temperature for 0.5 h. The solvent was evaporated under reduced pressure, and then the residue was mixed with Et_2O, washed with H_2O, and dried (Na_2SO_4). Evaporation of the solvent gave the crude oily xanthate, which was dissolved in anhydrous toluene (500 mL). 2,2′-Azobis(2-methylpropionitrile) (AIBN) (1.92 g, 0.01 mol) and *n*-Bu_3SnH (48.9 mL, 0.182 mol) were added, and the mixture was refluxed for 1 h under Ar. Evaporation of the solvent and column chromatography (20% EtOAc in hexanes) gave **3** (23 g, 89%) as an oil: $[\alpha]^{25}_D$ –44.0 (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.42 (dt, *J* = 4.4, 3.8 Hz, 1 H), 4.19 (dd, *J* = 8.5, 5.7 Hz, 1 H), 3.66 (dt, *J* = 8.5, 5.7 Hz, 1 H), 3.56 (dd, *J* = 10.2, 8.5 Hz, 1 H), 3.44 (s, 3 H), 3.34 (dd, *J* = 10.2, 8.9 Hz, 1 H), 2.46 (ddd, *J* = 15.2, 5.7, 3.7 Hz, 1 H), 1.69 (ddd, *J* = 15.1, 8.1, 5.2 Hz, 1 H), 1.49 (s, 3 H), 1.43 (s, 3 H), 1.41 (s, 3 H), 1.33 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 111.19, 109.16, 79.37, 79.23, 76.99, 73.88, 57.00, 31.90, 28.02, 26.68, 25.41.

1D-3-Deoxy-*myo*-inositol (1L-1-Deoxy-*chiro*-inositol or (–)-Viburnitol) (4). To a solution of **3** (22.9 g, 89 mmol) in anhydrous CH_2Cl_2 (600 mL) was added dropwise BBr_3 (33.6 mL, 0.355 mol) at 0 °C under Ar. The mixture was stirred at 0 °C for 1 h and then at 24 °C for 12 h, and the solvent was evaporated. The excess of BBr_3 in the residue was quenched by careful dropwise addition of MeOH at –20 °C, and the solvent was evaporated. After repeated addition of MeOH and evaporation of the solvent, the residue was dissolved in H_2O (200 mL) and extracted with CH_2Cl_2 (3 × 20 mL), and the aqueous phase was freeze dried to give **4** (12.7 g, 87.5%): mp 178–180 °C (EtOH); $[\alpha]^{25}_D$ –41.3 (*c* 0.4, H_2O); ^1H NMR (D_2O) δ 3.96–3.94 (m, 1 H), 3.65 (ddd, *J* = 12.0, 9.4, 4.8 Hz, 1 H), 3.48–3.35 (m, 2 H), 3.13 (t, *J* = 9.1 Hz, 1 H), 1.97 (dt, *J* = 14.0, 4.8 Hz, 1 H), 1.44 (ddd, *J* = 14.0, 11.9, 2.3 Hz, 1 H); ^{13}C NMR (D_2O) δ 77.68, 74.01, 72.99, 68.64 (2 C), 35.33. Anal. Calcd for $C_6H_{12}O_5$ (164.16): C, 43.90; H, 7.37. Found: C, 43.83; 7.34. These data were consistent with those previously reported[23] for **4**.

1D-3-Deoxy-1,2:5,6- and 1D-3-Deoxy-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (5). A mixture of **4** (12.5 g, 76 mmol), 2-methoxypropene (25.5 mL, 266 mmol), and camphorsulfonic acid (0.35 g, 0.02 mmol) in anhydrous DMF (50 mL) was stirred at 60 °C for 4 h. After cooling, the mixture was diluted with CH_2Cl_2 (300 mL), washed with saturated aqueous $NaHCO_3$ and brine, and dried (Na_2SO_4). Concentration and flash column chromatography (40% EtOAc in hexanes) gave the mixture of the bisacetonide regioisomers **5** (13.3 g, 72%) as a syrup.

1D-4-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene- and 1D-6-*O*-Benzyl-1,2:4,5-di-*O*-isopropylidene-3-deoxy-*myo*-inositol (6). A mixture of **5** (13.3 g, 54.5 mmol) and 60% NaH in mineral oil (4.36 g, 109 mmol) in anhydrous THF (150 mL) was stirred at 24 °C for 2 h, benzyl chloride (20 mL, 82 mmol) was added, and stirring was continued at the same temperature for 14 h. The excess of NaH was quenched with MeOH, the solvent was evaporated, and the residue was dissolved in Et_2O, washed with H_2O, and dried (Na_2SO_4). Concentration and column chromatography (20% EtOAc in hexanes) gave the mixture of the benzyl bisacetonide regioisomers **6** (16.8 g, 92.3%) as a syrup.

1D-4-*O*-Benzyl- and 1D-6-*O*-Benzyl-3-deoxy-1,2-*O*-isopropylidene-*myo*-inositol (7). To a solution of **6** (5.6 g, 16.8 mmol) in a mixture of CH_2Cl_2 (400 mL) and MeOH (200 mL) was added AcCl (8 drops). After stirring for 15 min at 24 °C, the reaction was quenched by addition of Et_3N (2 mL), the solvents were evaporated, and the residue was chromatographed (EtOAc) to give the mixture of the benzyl monoacetonide regioisomers **7** (4.44 g, 90%) as a syrup.

1D-5,6-Di-*O*-benzoyl-4-*O*-benzyl-3-deoxy-1,2-*O*-isopropylidene-*myo*-inositol (8) and 1D-4,5-Di-*O*-benzoyl-6-*O*-benzyl-3-deoxy-1,2-*O*-isopropylidene-*myo*-inositol (9). Benzoyl chloride (5.2 mL, 44.5 mmol) was added dropwise to a stirred solution of **7** (5.38 g, 18.3 mmol) and DMAP (0.25 g, 2 mmol) in anhydrous pyridine (25 mL) at 0 °C under Ar. Stirring was continued at 0 °C for 1 h and at 24 °C for 14 h, and the

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solvent was evaporated under vacuum. To the residue was added H_2O (100 mL), the mixture was extracted with Et_2O (3 × 50 mL), and the combined organic phases were washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine and dried (Na_2SO_4). Concentration and column chromatography (20% EtOAc in hexanes) gave the less polar **9** (3.58 g, 39%) followed by **8** (4.59 g, 50%). Data for **8**: mp 90–92 °C (C_6H_6/hexanes); $[\alpha]^{25}_D$ +5.5 (*c* 1.16, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.95–7.15 (m, 15 H), 5.61 (dd, *J* = 10.0, 8.0 Hz, 1 H), 5.39 (dd, *J* = 10.0, 8.3 Hz, 1 H), 4.60 (AB q, *J* = 12.0 Hz, 1 H), 4.50–4.46 (m, 1 H), 4.35 (dd, *J* = 7.8, 5.3 Hz, 1 H), 4.02 (ddd, *J* = 10.2, 8.3, 4.6 Hz, 1 H), 2.57 (ddd, *J* = 14.9, 4.4, 3.3 Hz, 1 H), 2.02 (ddd, *J* = 14.9, 10.2, 4.6 Hz, 1 H), 1.61 (s, 3 H), 1.36 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 165.91, 165.80, 137.87, 133.18, 133.04, 129.75, 128.68, 128.46, 128.35, 128.13, 79.65, 75.72, 75.34, 74.05, 70.20, 67.74, 32.56. Anal. Calcd for $C_{30}H_{30}O_7$ (502.56): C, 71.70; H, 6.02. Found: C, 71.84; 6.15. Data for **9**: mp 121–122 °C (C_6H_6/hexanes); $[\alpha]^{25}_D$ –95.4 (*c* 0.67, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.97–7.12 (m, 15 H), 5.56 (ddd, *J* = 10.9, 5.0, 4.7 Hz, 1 H), 5.46 (t, *J* = 9.0 Hz, 1 H), 4.78 (AB q, *J* = 12.0 Hz, 2 H), 4.47–4.43 (m, 1 H), 4.33 (t, *J* = 6.0 Hz, 1 H), 3.83 (dd, *J* = 8.5, 6.4 Hz, 1H), 2.6 (ddd, *J* = 7.1, 4.6, 2.5 Hz, 1 H), 2.03 (ddd, *J* = 14.8, 11.0, 4.0 Hz, 1 H), 1.53 (s, 3 H), 1.39 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 165.90, 165.61, 137.76, 133.02, 129.74, 129.66, 128.27, 128.14, 128.01, 127.56, 100.47, 79.61, 79.53, 74.00, 72.95, 72.90, 69.36, 29.80, 27.92, 25.76. Anal. Calcd for $C_{30}H_{30}O_7$ (502.56): C, 71.70; H, 6.02. Found: C, 71.59; H, 6.00.

1D-4,5-Di-*O*-benzoyl-6-*O*-benzyl-3-deoxy-*myo*-inositol (10). Concentrated HCl (4 drops) was added to a stirred solution of **9** (5.1 g, 10.85 mmol) in a mixture of MeOH (150 mL) and THF (50 mL) at 24 °C. Stirring was continued for 3 h at the same temperature, the solvents were evaporated, and the residue was chromatographed (80% EtOAc in hexanes) to give **10** (4.76 g, 95%) as a syrup: $[\alpha]^{25}_D$ –86.3 (*c* 0.54, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.02–7.20 (m, 15 H), 5.72–5.60 (m, 2 H), 4.72 (AB q, *J* = 11.2 Hz, 2 H), 4.22 (dd, *J* = 5.8, 2.8 Hz, 1 H) 4.07 (t, *J* = 9.0 Hz, 1 H), 3.79 (dd, *J* = 9.3, 3.0 Hz, 1 H), 2.94 (br s, 2 OH), 2.56 (dt, *J* = 13.6, 4.0 Hz, 1 H), 2.41 (ddd, *J* = 13.7, 11.1, 2.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 165.85, 165.73, 137.81, 133.11, 132.98, 129.68, 128.61, 128.40, 128.29, 128.06, 79.59, 75.65, 75.27, 73.99, 70.14, 67.68, 32.50.

1D-1,4,5-Tri-*O*-benzoyl-6-*O*-benzyl-3-deoxy-*myo*-inositol (11). Benzoyl chloride (1.39 mL, 12 mmol) was added dropwise to a stirred solution of **10** (4.62 g, 10 mmol) and DMAP (122 mg, 1 mmol) in anhydrous pyridine (50 mL) at 0 °C under Ar. Stirring was continued at 0 °C for 4 h, the mixture was left at 0 °C for 24 h, and the solvent was evaporated under vacuum. Workup as described for the preparation of **8** and **9**, followed by column chromatography (20% EtOAc in hexanes) gave **11** (5.15 g, 91%) as a syrup: $[\alpha]^{25}_D$–69.7 (*c* 0.33, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.08–7.01 (m, 20 H), 5.75–5.69 (m, 2 H), 5.36 (dd, *J* = 9.6, 2.8 Hz, 1 H), 4.68 (AB q, *J* = 11.2 Hz, 2 H), 4.50–4.42 (m, 2 H), 2.64–2.56 (m, 2 H), 1.93–1.89 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 165.85, 165.60, 137.52, 133.34, 133.04, 129.71, 129.60, 128.54, 128.31, 128.12, 127.85, 127.55, 77.19, 76.54, 75.15, 75.06, 69.87, 66.57, 32.68.

1D-1,4,5-Tri-*O*-benzoyl-6-*O*-benzyl-3-deoxy-2-*O*-(phenoxy(thiocarbonyl))-*myo*-inositol (12). Phenyl chlorothionoformate (0.63 mL, 4.57 mmol) was added dropwise to a solution of **11** (2.0 g, 3.53 mmol) and DMAP (0.6 g, 5 mmol) in anhydrous CH_2Cl_2 (20 mL), and the mixture was stirred at room temperature for 16 h under Ar. Workup as described for the preparation of **8** and **9**, followed by column chromatography (20% EtOAc in hexanes) gave **12** (2.25 g, 91%): mp 207–208 °C (C_6H_6/hexanes); $[\alpha]^{25}_D$ –38.2 (*c* 1.03 $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.07–7.05 (m, 25 H, 6.21 (s, 1 H), 5.78 (t, *J* = 9.0 Hz, 1 H), 5.66–5.56 (m, 2 H), 4.71 (AB q, *J* = 11.1 Hz, 2 H), 4.36 (t, *J* = 9.0 Hz, 1 H), 2.90 (dt, *J* = 14.4, 4.8 Hz, 1 H), 2.15 (t, *J* = 13.4 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 193.85, 165.79, 165.49, 153.45, 137.38, 133.51, 133.31, 133.23, 129.86, 129.80, 129.69, 129.60, 129.41, 128.57, 128.44, 128.24, 127.90, 127.73, 126.76, 121.87, 77.73, 75.31, 74.20, 73.50, 69.70, 30.01. Anal. Calcd for $C_{41}H_{34}O_9S$ (702.77): C, 70.07; H, 4.88. Found: C, 70.23; H, 5.04.

1D-1,4,5-Tri-*O*-benzoyl-6-*O*-benzyl-2,3-dideoxy-*myo*-inositol (13). A mixture of **12** (2.2 g, 3.13 mmol), *n*-Bu_3SnH (1.26 mL, 4.7 mmol), and AIBN (0.12 g, 0.63 mmol) in anhydrous toluene (50 mL) was refluxed for 1 h under Ar. Concentration followed by column chromatography (15% EtOAc in hexanes) gave **13** (1.7 g, 98.6%): $[\alpha]^{25}_D$ –69.6 (*c* 1.05, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.06–7.02 (m, 20 H), 5.68 (t, *J* = 9.5 Hz, 1 H), 5.35–5.23 (m, 2 H), 4.69 (AB q, *J* = 11.2 Hz, 2 H), 3.98 (t, *J* = 9.2 Hz, 1 H), 2.38–2.33 (m, 2 H), 1.86–1.69 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 166.05, 165.69, 165.63, 137.61, 133.19, 133.06, 130.13, 129.79, 129.74, 128.51, 128.39, 128.20, 127.96, 127.62, 80.77, 74.92, 74.52, 72.79, 25.74, 25.59.

1D-1,4,5-Tri-*O*-benzoyl-2,3-dideoxy-*myo*-inositol (14). Hydrogenation of the benzyl ether **13** (0.50 g, 0.91 mmol) over preequilibrated 10% Pd/C (0.1 g) in a mixture of EtOH (45 mL) and THF (15 mL) at room temperature and atmospheric pressure for 14 h gave after column chromatography (20% EtOAc in hexanes) **14** (0.39 g, 94%) as a syrup: $[\alpha]^{25}_D$ –60.1 (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.06–7.24 (m, 15 H), 5.53 (t, *J* = 9.7 Hz, 1 H), 5.30–5.13 (m, 2 H), 4.09–3.99 (m, 1 H), 2.98 (d, *J* = 4 Hz, OH), 2.33–2.25 (m, 2 H), 1.80–1.65 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 166.44, 166.30, 165.94, 133.17, 133.09, 129.81, 129.71, 129.63, 129.48, 128.34, 128.28, 76.12, 74.94, 74.22, 72.43, 25.77, 25.57.

1D-1,4,5-Tri-*O*-benzoyl-2,3-dideoxy-6-*O*-(phenoxy(thiocarbonyl))-*myo*-inositol (15). Under conditions similar to those for the preparation of **12**, **14** (357 mg, 0.776 mmol) gave after column chromatography (20% EtOAc in hexanes) **15** (0.396 g, 86%): ^1H NMR ($CDCl_3$) δ 8.08–6.48 (m, 20 H), 6.24 (t, *J* = 9.8 Hz, 1 H), 5.82 (t, *J* = 9.8 Hz, 1 H), 5.41–5.34 (m, 2 H), 2.50–2.43 (m, 2 H), 1.87–1.81 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 195.17, 165.75, 165.63, 165.48, 153.22, 133.42, 133.33, 133.25, 129.99, 129.79, 129.45, 129.37, 129.29, 129.15, 128.51, 128.41, 126.45, 121.48, 82.63, 73.20, 72.43, 25.60.

1D-1,4,5-Tri-*O*-benzoyl-2,3,6-trideoxy-*myo*-inositol (16). Under conditions similar to those for the preparation of **13**, **15** (396 mg, 0.664 mmol) gave after column chromatography (10% EtOAc in hexanes) **16** (0.24 g, 81.6%): $[\alpha]^{25}_D$ –60.6 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.03–7.31 (m, 15 H, 5.44–5.17 (m, 3 H), 2.67–2.60 (m, 1 H), 2.40–2.26 (m, 2 H), 2.02 (dt, *J* = 12.6, 10.2 Hz, 1 H), 1.83–1.70 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 165.97, 165.66, 133.04, 130.18, 129.83, 129.66, 128.35, 73.07, 71.20, 69.61, 34.71, 28.42, 25.47.

1D-2,3,6-Trideoxy-*myo*-inositol (17). To a solution of **16** (0.19 g, 0.43 mmol) in a mixture of MeOH (4 mL) and THF (2 mL) was added finely ground K_2CO_3 (70 mg), and the resulting mixture was stirred at room temperature for 4 h. Concentration and column chromatography (10% MeOH in EtOAc) gave **17** (47 mg, 84%) as a solid: $[\alpha]^{25}_D$ –28.9 (*c* 0.52, H_2O); ^1H NMR (DMSO-d_6) δ 4.53 (br s, 3 OH), 3.50–3.20 (m, 1 H, overlapping with the signal of H_2O), 3.13–3.01 (m, 2 H), 1.96–1.88 (m, 1 H), 1.72–1.62 (m, 2 H), 1.17–1.04 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 73.97, 72.15, 67.05, 42.29, 33.22, 29.32.

1D-2,3,6-Trideoxy-*myo*-inositol 1,4,5-Tris(dibenzylphosphate) (18). A mixture of **17** (45 mg, 0.341 mmol) and tetrabenzyl pyrophosphate (1.29 g, 2.39 mmol) in anhydrous DMF (3 mL) was stirred at room temperature for 15 min under Ar and cooled to 0 °C. NaH (60%) in mineral oil (0.15 g, 3.75 mmol) was added, the mixture was stirred for 14 h at 0 °C, and the solvent was evaporated under vacuum at room temperature. The residue was stirred with anhydrous CH_2Cl_2 (20 mL) for 15 min and filtered through Celite, and the filtrate was concentrated and chromatographed (70% EtOAc in hexanes) to give **18** (196 mg, 63%) as a solid: $[\alpha]^{25}_D$ –17.9 (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.41–7.22 (m, 30 H), 5.05–4.95 (m, 12 H), 4.25–4.17 (m, 3 H), 2.48 (d, *J* = 11.3 Hz, 1 H), 2.18–2.14 (m, 1 H), 1.98–1.94 (m, 1 H), 2.18–2.14 (m, 1H), 1.98–1.94 (m, 1 H), 1.68 (dt, *J* = 12.6, 10.5 Hz, 1 H), 1.50–1.28 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 135.85, 135.77, 128.67, 128.58, 128.07, 127.98, 75.57, 72.96, 72.88, 69.56, 69.48, 69.42, 37.43, 29.62, 26.15. ^{31}P NMR ($CDCl_3$) δ –1.23 (2 P), –1.41.

1D-2,3,6-Trideoxy-*myo*-inositol 1,4,5-Tris(disodium phosphate) (19). Hydrogenation of **18** (157 mg, 0.172 mmol) with 10% Pd/C (0.2 g) in EtOH (60 mL) at 50 psi and room temperature for 14 h, filtration through Celite, and evaporation of the solvent under vacuum gave the crude acid which was dissolved in distilled H_2O (50 mL). The aqueous solution was titrated to pH = 10 with 1 N NaOH and freeze dried. The residue was suspended in MeOH, filtered, and dried in a desiccator (P_2O_5) to give the hexasodium salt **19** (80 mg, 92%) as a hygroscopic white powder: $[\alpha]^{25}_D$ +43.3 (*c* 0.6, H_2O); ^1H NMR (D_2O) δ 3.86–3.76 (m, 1 H), 3.76–3.65 (m, 2 H), 2.44–2.39 (m, 1 H), 1.80–1.98 (m, 1 H), 1.35–1.10 (m, 3 H) including 1.29 (q, *J* = 11.5 Hz, 1 H); ^{13}C NMR (D_2O) δ 78.48, 76.68, 73.09, 42.51, 33.87, 30.56; ^{31}P NMR (D_2O) δ 6.65, 6.61, 6.52.

1D-6-*O*-Benzyl-2,3-dideoxy-*myo*-inositol (20). Under conditions similar to those for the preparation of **17**, **13** (0.50 g, 0.91 mmol) gave after column chromatography (EtOAc) **20** (191 mg, 88.4%) as a solid: mp 126–127 °C (C_6H_6/hexanes); $[\alpha]^{25}_D$ –27.2 (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.44–7.27 (m, 5 H), 4.86 (AB q, *J* = 11.5 Hz, 2 H), 3.61–3.44 (m, 2 H), 3.35 (t, *J* = 9.0 Hz, 1 H), 2.96 (br s, 2 OH), 2.44 (br s, OH), 1.98–1.87 (m, 2 H), 1.43–1.24 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 138.61, 128.80, 128.13, 128.01, 86.11, 77.85, 75.11, 72.74, 72.35, 28.28, 28.05. Anal. Calcd for $C_{13}H_{18}O_4$ (238.28); C, 65.53; H, 7.61. Found: C, 65.41; H, 7.62.

1D-6-*O*-Benzyl-2,3-dideoxy-*myo*-inositol 1,4,5-Tris(dibenzylphosphate) (21). Under conditions similar to those for the preparation of **18**, **20** (119 mg, 0.5 mmol) gave after column chromatography (60% EtOAc in hexanes) **21** (446 mg, 87.6%) as a white solid: mp 93–95 °C (C_6H_6/hexanes); $[\alpha]^{25}_D$ −11.5 (*c* 1.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.38–7.02 (m, 35 H), 5.06–4.70 (m, 14 H), 4.47 (q, *J* = 9.0 Hz, 1 H), 4.33–4.28 (m, 2 H), 3.47 (t, *J* = 9.0 Hz, 1 H), 2.27–2.17 (m, 2 H), 1.52–1.30 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 137.95, 136.03, 135.92, 135.76, 135.68, 135.59, 128.47, 128.43, 128.37, 128.31, 128.26, 128.07, 127.95, 127.90, 127.75, 127.37, 127.24, 81.57, 80.38, 78.09, 74.50, 69.55, 69.47, 69.32, 69.22, 26.44, 25.99; ^{31}P NMR ($CDCl_3$) δ −0.83, −1.11, −1.30.

1D-2,3-Dideoxy-*myo*-inositol 1,4,5-Tris(disodium phosphate) (22). Under conditions similar to those for the preparation of **19**, **21** (324 mg, 0.318 mmol) gave after column chromatography (60% EtOAc in hexanes) **22** (148 mg, 90%) as a hygroscopic white powder: $[\alpha]^{25}_D$ −16.5 (*c* 1.0, H_2O); ^{1}H NMR (D_2O) δ 3.69–3.65 (m, 3 H), 3.33 (t, *J* = 8.6 Hz, 1 H), 2.17–1.95 (m, 2 H), 1.30–1.10 (m, 2 H); ^{13}C NMR (D_2O) δ 81.97, 79.64, 77.97, 76.61, 29.92, 29.68; ^{31}P NMR (D_2O) δ 7.71, 6.66, 6.19.

1D-1,4,5-Tri-*O*-benzoyl-6-*O*-benzyl-3-deoxy-2-*O*-(1-ethoxyethyl)-*myo*-inositol (23). A mixture of **11** (364 mg, 0.643 mmol), ethyl vinyl ether (0.067 mL, 0.71 mmol), and pyridinium *p*-toluenesulfonate (0.02 g) in anhydrous CH_2Cl_2 (10 mL) was stirred at 24 °C for 12 h under Ar. Concentration and column chromatography (20% EtOAc in hexanes) gave **23** (1.03 g, 95%) as a mixture of diastereoisomers.

1D-6-*O*-Benzyl-3-deoxy-2-*O*-(1-ethoxyethyl)-*myo*-inositol (24). Under conditions similar to those for the preparation of **17**, **23** (0.50 g, 0.78 mmol) gave after column chromatography (EtOAc) the diastereomeric mixture **24** (0.24 g, 94%) as a syrup.

1D-3-Deoxy-*myo*-inositol 1,4,5-Tris(disodium phosphate) (26). Under conditions similar to those for the preparation of **18**, **24** (68 mg, 0.21 mmol) gave after column chromatography (60% EtOAc in hexanes) the 1,4,5-tris(dibenzylphosphate) **25** (116 mg, 50%) as a diastereomeric mixture. Hydrogenation of this material under conditions similar to those for the preparation of **19** gave the crude acid which was dissolved in distilled H_2O (50 mL) and stirred for 4 h at room temperature. Titration with 1 N NaOH to pH = 10, followed by freeze drying afforded the hexasodium salt **26** (32 mg, 56%) as a hygroscopic white powder: $[\alpha]^{25}_D$ −28.9 (*c* 0.74, H_2O); ^{1}H NMR (D_2O) δ 4.21 (br s, 1 H), 4.04 (br s, 1 H), 3.60–3.85 (m, 3 H), 2.43–2.24 (m, 1 H), 1.56–1.36 (m, 1 H); ^{13}C NMR (D_2O) δ 80.20, 79.87, 75.58, 72.80, 69.78, 36.13; ^{31}P NMR (D_2O) δ 7.74, 6.63, 6.21.

Ins(1,4,5)P_3 Binding Assay. The preparation of bovine adrenal cortices and [^{3}H]Ins(1,4,5)P_3 binding and displacement assays were performed essentially as described.[20] Bound and free [^{3}H]Ins(1,4,5)P_3 were separated by rapid filtration through Whatman GF/B glass fiber filters. Specifically bound [^{3}H]Ins(1,4,5)P_3 (approximately 2500 dpm/assay) was displaced by Ins(1,4,5)P_3, 1D-3-deoxy-3-fluoro-Ins(1,4,5)P_3 or deoxy analogues **19**, **22**, and **26**. Nonspecific binding (approximately 150 dpm/assay) was defined by the addition of 10 μM Ins(1,4,5)P_3.

Cell Culture and Ca^{2+} Mobilization. Monolayers of SH-SY5Y human neuroblastoma cells (passage 80–90), initially a kind gift of Dr. J. L. Biedler (Sloane-Kettering Institute, New York, USA) were grown as described.[24] Prior to use, the cells were saponin permeabilized and the $^{45}Ca^{2+}$ loading and mobilization experiments were performed at pH 7.2 as described.[25]

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