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Synthesis and biological activities of (4,6-di-*O*-phosphonato-β-D-mannopyranosyl)-methylphosphonate as an analogue of 1L-*myo*-inositol 1,4,5-trisphosphate

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Abstract

The synthesis of the α and β anomers of the title compound (**1**) was accomplished from D-mannose. In the key step, the phosphonate analogues of the mannopyranosyl phosphates were prepared by a direct Wadsworth–Emmons condensation of a protected mannose derivative (**8**) with tetraethyl methylenebisphosphonate under two-phase conditions. In vitro bioassays have shown that the β anomer (**1a**) is a potent inhibitor of Ins(1,4,5)P_3 3-kinase and inhibits other enzymes.

1. Introduction

Since the discovery that 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] plays a pivotal role as a second messenger in transmembrane signalling, thus mobilizing calcium ions from the intracellular storage, intensive research efforts have been expended from the standpoint of both a fundamental interest and potential pharmacological intervention for therapeutic use. In order to understand which structural features in Ins(1,4,5)P_3 are essential for the activity, a number of analogues have been synthesized and their bioactivities studied [1]. It has been suggested from these studies that the vicinal 4- and 5-phosphates are essential for Ca^{2+} release, and that the 1-phosphate may be important for binding to the receptor. It is known that metabolism of Ins(1,4,5)P_3 occurs by phosphorylation to Ins(1,3,4,5)P_4 or by dephosphorylation to Ins(1,4)P_2. In its interactions with a

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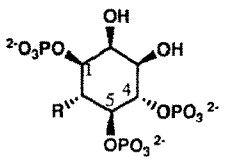
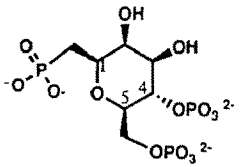
substrate, the 3-kinase enzyme exhibits high stereo- and positional selectivity, whereas the 5-phosphatase appears to be relatively nonspecific [2]. The unnatural 1L-*myo*-inositol 1,4,5-trisphosphate was synthesized and reported to have a poor ability to release Ca^{2+} ions, although it binds to the receptor and to the metabolic enzymes such as Ins(1,4,5)P_3 3-kinase and Ins(1,4,5)P_3/Ins(1,3,4,5)P_4 5-phosphatase at μM concentrations [3]. As part of our efforts to understand the molecular recognition aspect of the inositol phosphate-dependent signal transduction pathway, including phospholipase C, the inositol 1,4,5-trisphosphate receptor, and the metabolic enzymes, we have been studying the synthesis and bioactivities of various natural and unnatural inositol analogues [4]. There are certain structural similarities between *myo*-inositol and several hexopyranoses. We therefore constructed their 3D structures on the basis of MM2 calculations, and then compared their geometries by computer graphics. It has been found that the 1L isomer of *myo*-inositol 1,4,5-trisphosphate has a molecular size and shape very similar to D-mannose 1,4,6-trisphosphate, whereas the natural 1D isomer matches well with D-galactose 1,2,6-trisphosphate. The superimposition root mean squares in both pairs turned out to be ca. 0.055–0.063 Å, and their volume differences less than 2%. Therefore, we undertook syntheses of these D-mannose- and D-galactose-based structural analogues of Ins(1,4,5)P_3. Although a synthesis of D-mannose 1,4,6-trisphosphate has recently been completed, the anomeric phosphate is subject to chemical and stereochemical lability [4b]. We have therefore synthesized the corresponding phosphonate as a stable isostere, and now report the synthesis and biological activities of the title compound (**1**) [5].



L-Ins(1,4,5)P3

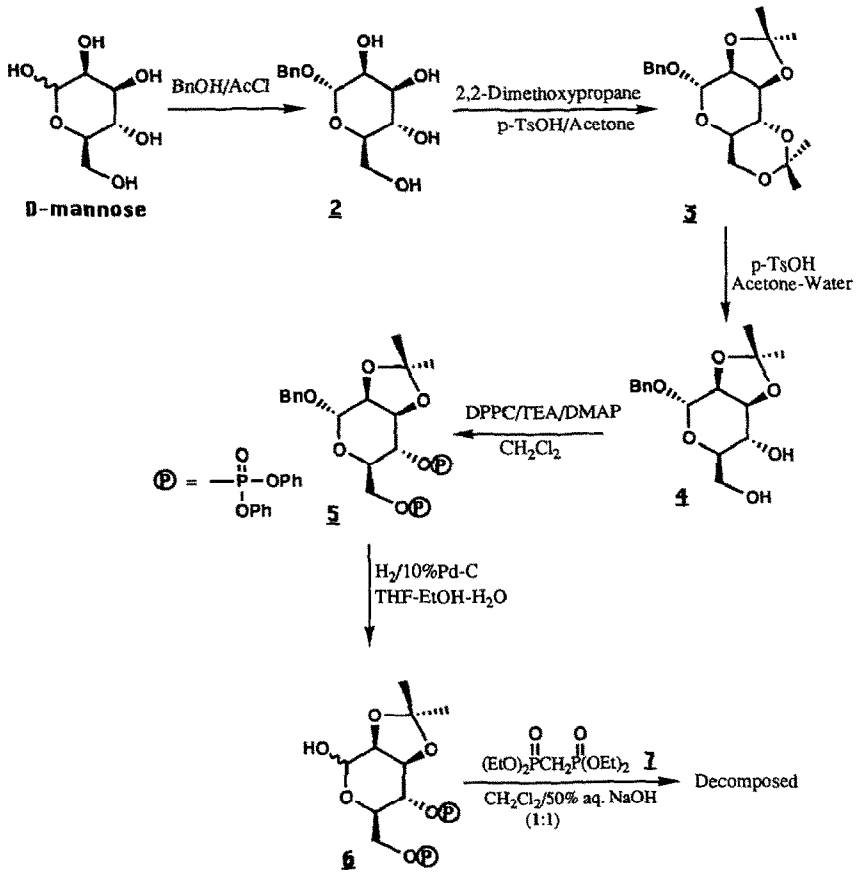


1a β-anomer
1b α-anomer

2. Results and discussion

Phosphonate analogues of hexopyranosyl phosphates have been synthesized on only a few occasions and the reported syntheses invariably involved multistep processes including Arbuzov reaction of halogenated *C*-glycosyl intermediates as their key step [6]. In contrast, phosphonate analogues from D-ribofuranose and D-arabinofuranose have been directly prepared by Wittig or Wittig-like condensations of suitably protected furanoses [7]. Our synthesis of the title compound was accomplished via the Wadsworth–Emmons reaction.

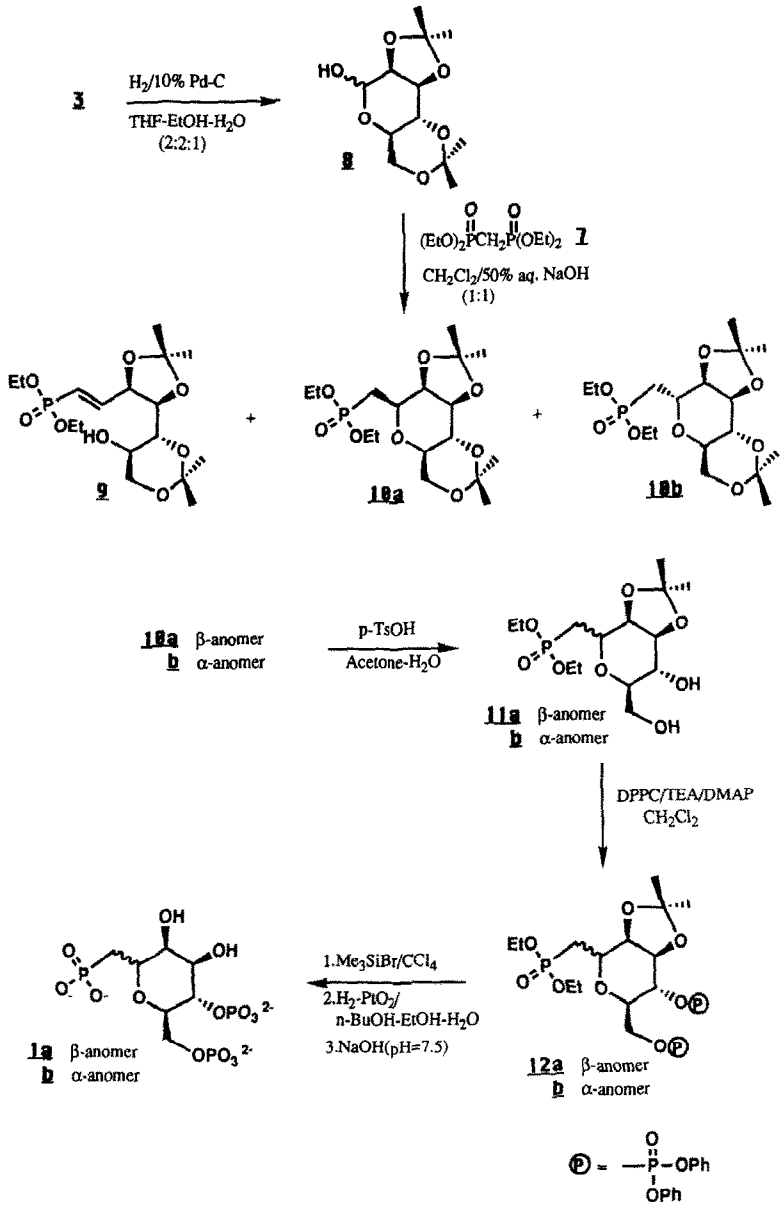
D-Mannose was converted into benzyl α-D-mannopyranoside (**2**) in 90% yield essentially according to the literature procedure [8]. Treatment of **2** with 2,2-dimethoxypropane in acetone in the presence of a catalytic amount of *p*-toluene-



Scheme 1.

sulfonic acid gave benzyl 2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside (**3**). When subjected to controlled hydrolysis conditions as described by Evans and Parrish [9], the diacetonide **3** was converted into benzyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**4**) in good yield. The 4- and 6-hydroxyl groups in **4** were readily phosphorylated by treatment with diphenyl phosphorochloridate in the presence of 4-dimethylaminopyridine and triethylamine, to provide **5**. Although formation of a cyclic phosphate is known to occur sometimes for diols and polyols under these conditions [10], it was not a problem in the present case. The benzyl protecting group in **5** was efficiently removed by hydrogenolysis to give 2,3-*O*-isopropylidene-4,6-di-*O*-diphenoxyphosphoryl-α-D-mannopyranose (**6**). However, all attempts to introduce the desired phosphonate functionality at the anomeric position failed. Apparently, the starting material did not survive the alkaline reaction conditions [11] (Scheme 1).

As an alternative (Scheme 2), when compound **8**, obtained from **3** via hydrogenolysis, was treated with tetraethyl methylenebisphosphonate and sodium



Scheme 2.

hydroxide under the two-phase conditions [12], a smooth reaction took place to give a mixture of unsaturated phosphonate **9** and the epimers of the cyclic sugar phosphonate **10**. Interestingly, in the case of 2,3,4,6-tetra-*O*-benzyl-α,β-D-manno-pyranose, we have observed an abnormal reaction of tetraethyl methylenebisphosphonate, leading solely to a diene. Preliminary studies have indicated that benzyl-

oxy groups are eliminated to give a diene and that elimination is related to sugar conformations [13]. The ratio of the cyclic products **10a** and **10b**, obtained in 44% yield, was found to be 1:2.7 in favor of the β anomer. The stereochemical assignments were made on the basis of the ^{1}H NMR resonance of H-5 which is expected to be sensitive to the steric environment of the anomeric position because of the 1,3-interactions. The H-5 peak in the α anomer appears at δ 3.15 as ddd, compared to δ 3.40 in the β anomer, clearly showing that H-5 in the α anomer experiences an upfield shift. In addition, the chemical shifts for the anomeric protons in the *C*-glycopyranosyl compounds were reported to occur at a lower field in the cis-1,2-substituted isomers than in the trans-1,2-substituted isomers. In the present case, the anomeric proton chemical shifts are observed at δ 4.28 in the cis-1,2-substituted β anomer (**10a**), and at δ 4.15 in the trans-1,2-substituted α anomer (**10b**). The fact that the ^{13}C NMR resonance of the anomeric carbon appears at δ 76.5 in the β anomer and at δ 75.5 in the α anomer is also consistent with the general observation that the C-1 chemical shift of the α anomer appears at a higher field than that of the β anomer [14].

The question whether or not the acyclic compound **9** might be an intermediate to the cyclic product **10** cannot be unequivocally answered at this time. When **9** was treated with sodium methoxide in methanol over an extended period, the formation of **10** was not observed. We have also examined the possible epimerization between the cyclic compounds **10a** and **10b** by ^{1}H NMR. Treatment of either anomer with sodium methoxide in methanol for 4 days resulted in no detectable epimerization [15].

Compound **10a** was hydrolyzed with *p*-toluenesulfonic acid in aqueous acetone to give **11a**, which was phosphorylated with diphenyl phosphorochloridate to yield the desired product **12a** in 87% yield. Compound **10b** was similarly converted into **12b** through **11b**. Compounds **12a** and **12b** have been thoroughly characterized, and all the spectral data are fully consistent with the assigned structures. In the final steps of the synthesis, **12a** and **12b** were each successively treated with an excess amount of bromotrimethylsilane [16], followed by hydrogenolysis over PtO_2 to yield (4,6-di-*O*-phosphonato-β-D-mannopyranosyl)methylphosphonate (**1a**) and its α anomer (**1b**).

Table 1
In vitro biochemical activity of Ins(1,4,5)P_3 analogues **1a** and **1b**

Test	**1a**	**1b**
Calcium release [relative to D-Ins(1,4,5)P_3]	≤1/1000	≤1/1000
Ins(1,4,5)P_3 receptor antagonist activity	None	None
3-Kinase, K_i (μM) [a]	26.8	None
1-Phosphatase (inhibition at 1.0 mM) [b]	ca. 30%	
5-Phosphatase (inhibition at 1.0 mM) [b]	ca. 17%	

[a] Determined by competitive binding of compounds with [^{3}H]-Ins(1,4,5)P_3 to Ins(1,4,5)P_3 3-kinase from rat.

[b] Mean value of assays performed in triplicate.

The in vitro bioactivities of the compounds have been examined and are summarized in Table 1. Compounds **1a** and **1b** did not significantly induce release of Ca^{2+}, and are not Ins(1,4,5)P_3 receptor antagonists. It was estimated that **1a** and **1b** are ca. 1000 times less potent than Ins(1,4,5)P_3 in their ability to release Ca^{2+} ion. Compound **1a** was found to mildly inhibit Ins(1,3,4)P_3/Ins(1,4)P_2 1-phosphatase from rat liver (ca. 30% inhibition at 1 mM) as well as the Ins(1,4,5)P_3 5-phosphatase preparation from bovine testis (ca. 17% inhibition at 1 mM). Compounds **1a** and **1b** were tested as inhibitors of the phosphorylation of [^{3}H]-Ins(1,4,5)P_3. The *E. coli*-expressed cDNA clone of Ins(1,4,5)P_3 3-kinase from rat brain was extensively inhibited by **1a** (K_i = 26.8 μM) but not by **1b**. Synthetic **1a** thus represents one of the most potent inhibitors reported of Ins(1,4,5)P_3 3-kinase *.

3. Experimental

General.—All reactions were performed in oven-dried glassware under a positive pressure of Ar. Liquids and solutions were transferred by syringes, and were introduced into reaction flasks through rubber septa. All solvents were carefully dried and distilled prior to use. Melting points were determined on a Thomas Hoover apparatus and are uncorrected. Analytical TLC was carried out on Merck 60 F_{254} silica gel plates (0.25-mm layer thickness) or Aldrich F_{254} cellulose plates (0.25-mm layer thickness). Visualization was done with UV light, and/or by spraying with a 5% solution of phosphomolybdic acid or with a *p*-anisaldehyde solution followed by charring with a heat gun. Column chromatography was performed on Merck 60 silica gel (70–230 mesh or 230–400 mesh) and eluted with a gradient mixture of hexane–EtOAc, unless indicated otherwise. Optical rotations were determined on a Jasco Model DIP-360 Digital Polarimeter. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker AM-300 spectrometer. Where anomer ratios are reported by ^{1}H NMR experiments, integrations were obtained under quantitative conditions. Chemical shifts are reported in δ ppm, and tetramethylsilane and phosphoric acid (85%) were used as internal and external standard for ^{1}H NMR and ^{31}P NMR, respectively. IR spectra were recorded with a BOMEM model FT-IR M100-C15 spectrometer for solutions in $CHCl_3$, using 0.2-mm path NaCl microcavity cells versus pure solvent reference, or for KBr pellets. Mass spectra (EI or FAB) were recorded on a KRATOS MS 25RFA system. Elemental analyses were performed by the Korea Basic Science Center, Seoul, Korea.

*Benzyl α-*D*-mannopyranoside* (**2**).—Compound **2** was prepared from D-mannose in 89% yield according to the literature procedure [8]; mp 128–130°C (lit. [8] mp 130–132°C).

* Details of this study will be published elsewhere.

Benzyl 2,3:4,6-di-O-isopropylidene-α-D-mannopyranoside (**3**).—A solution of **2** (10 g, 37.0 mmol) and *p*-toluenesulfonic acid (1.75 g, 9.25 mmol) in acetone (20 mL) and 2,2-dimethoxypropane (20 mL) was stirred at room temperature for 1 h before addition of satd aq $NaHCO_3$ (10 mL). The solvent was evaporated, and the residue was treated with water (100 mL). The precipitate was filtered off, and recrystallized from EtOAc to give white crystals (12.05 g, 93.0%); mp 81–82°C; $[\alpha]_D^{25}$ +34.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.34, 1.43, 1.52, 1.55 (4 s, each 3 H, 2 Me_2C), 3.63 (m, 1 H), 3.68–3.87 (m, 3 H), 4.15–4.23 (m, 2 H), 4.49–4.72 (2 d, each 2 H, *J* 13.7 Hz, OCH_2Ph), 5.11 (s, 1 H, H-1), 7.26 (m, 5 H, Ar); ^{13}C NMR ($CDCl_3$): δ 18.8, 26.1, 28.2, and 29.0 (2 Me_2C), 61.5, 62.0, 69.4, 72.7, 74.9, and 76.1 (C-2,3,4,5,6 and CH_2Ph), 97.0, 99.7, and 109.4 (2 Me_2C and C-1), 128.0, 128.1, 128.5, and 136.8 (aromatic); EIMS *m/z* 350(M^+) and 335(M − Me). Anal. Calcd for $C_{19}H_{26}O_6$: C, 65.12; H, 7.48. Found: C, 65.04; H, 7.73.

Benzyl 2,3-O-isopropylidene-α-D-mannopyranoside (**4**).—Method 1. A solution of **2** (10.0 g, 37.0 mmol) and *p*-toluenesulfonic acid (1.75 g, 9.25 mmol) in dry acetone (50 mL) and 2,2-dimethoxypropane (50 mL) was stirred at room temperature for 4 h. Water (50 mL) was added, and stirring continued for 3 h. Saturated aq $NaHCO_3$ (10 mL) was added, and the solvents were evaporated. The residue was dried for 1 h at 90°C/30 torr, dissolved in water (50 mL), and extracted with hexane. Evaporation of the extract gave benzyl 2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside (**3**; 0.92 g, 7.1%). The aqueous layer was continuous extracted with $CHCl_3$ for 4 h. The extract was dried ($MgSO_4$) and evaporated to give **4** as a glassy product (10.21 g, 88.9%) which was sufficiently pure to be used in the next reaction.

Method 2. A solution of **3** (9.3 g, 26.3 mmol) and *p*-toluenesulfonic acid (0.72 g, 7.56 mmol) in acetone (30 mL) and water (30 mL) was stirred at room temperature for 4 h before addition of satd aq $NaHCO_3$ (5 mL). The solution was concentrated under reduced pressure, and an extractive work-up with $CHCl_3$ gave **4** (7.84 g, 96.0%) which was used in the next reaction without further purification; $[\alpha]_D^{25}$ +42.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.33, 1.50 (2 s, each 3 H, Me_2C), 1.68, 2.76 (2 bs, OH), 3.70 (m, 2 H), 3.82 (d, 2 H), 4.17 (m, 2 H), 4.49–4.73 (2 d, 2 H, *J* 11.7 Hz, OCH_2Ph), 5.09 (s, 1 H, H-1), 7.30 (m, 5 H, Ar); ^{13}C NMR ($CDCl_3$): δ 26.1, 27.7 (Me_2C), 62.5, 69.5, 69.7, 70.0, 75.6, and 76.2 (C-2,3,4,5,6 and CH_2Ph), 96.6 (C-1), 109.7 (Me_2C), 128.1, 128.2, 128.6, 136.8 (aromatic); IR (neat): 3409(br), 2986, 2924, 1454, 1376, 1073 cm^{-1}.

Benzyl 2,3-O-isopropylidene-4,6-di-O-diphenoxyphosphoryl-α-D-mannopyranoside (**5**).—A mixture of **4** (5.0 g, 16.13 mmol), triethylamine (7.4 mL, 52.5 mmol), and a catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2 (50 mL) was stirred until a clear solution was obtained. To this solution at 0°C was added diphenyl phosphorochloridate (11.0 mL, 52.5 mmol), and the resulting solution was stirred overnight at room temperature. The reaction was quenched with satd aq $NaHCO_3$ (10 mL) and the solution concentrated under reduced pressure. The mixture was partitioned between 0.1 M HCl and CH_2Cl_2. The organic layer was separated, washed with brine, dried ($MgSO_4$), and evaporated. The residue was flash chromatographed (gradient of EtOAc–hexane) to give **5** (11.5 g, 92%) as a white solid;

mp 86–88°C; $[\alpha]_D^{25}$ + 46.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.32 and 1.50 (2 s, each 3 H, Me_2C), 4.10 (m, 1 H), 4.18 (d, *J* 5.4 Hz, 1 H), 4.31–4.60 (m, 4 H), 4.38 and 4.63 (2 d, each 1 H, *J* 11.7 Hz, OCH_2Ph), 5.06 (s, 1 H, H-1), 7.23 (m, 25 H, aromatic); ^{13}C NMR ($CDCl_3$): δ 26.3 and 27.7 (Me_2C), 67.3, 69.2, 76.0, 76.3, 76.5, and 77.0 (C-2,3,4,5,6 and -OCH_2Ph), 95.8 (C-1), 110.3 (Me_2C), 120.1, 125.3, 128.2, 129.8, 150.5 (aromatic carbons); ^{31}P NMR ($CDCl_3$): δ − 13.7 and −14.3; FABMS: *m/z* 775 $(M + 1)^+$ and 667 $(M - OCH_2Ph)$. Anal. Calcd for $C_{40}H_{40}O_{12}P_2 \cdot 0.5H_2O$: C, 61.30; H, 5.27. Found: C, 61.37; H, 5.28.

2,3-O-Isopropylidene-4,6-di-O-diphenoxyphosphoryl-α-D-mannopyranose (**6**).—A suspension of **5** (4.20 g, 6.24 mmol) and 10% Pd–C (1.09) in 2:2:1 THF–EtOH–H_2O was hydrogenolyzed at 50 psi for 3 days. The mixture was filtered and washed with MeOH. The filtrate was evaporated to give **6** in quantitative yield; $[\alpha]_D^{25}$ −14.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.32 and 1.49 (2 s, each 3 H, Me_2C), 3.82–4.24 (m, 4 H), 4.39 (m, 2 H), 4.60 (m, 2 H), 5.28 (s, 1 H, H-1), 7.27 (m, 20 H, aromatic; ^{13}C NMR ($CDCl_3$): δ 26.3 and 27.7 (Me_2C), 66.6 (t, $J_{C,P}$ 6.75 Hz), 67.6 (d, $J_{C,P}$ 6.0 Hz), 76.3, 76.4, and 76.5 (C-2,3,4,5,6), 91.5 (C-1), 110.1 (Me_2C), 120.0–120.2, 125.1–125.4, 129.5–129.8, 150.4–150.6 (aromatic carbons); ^{31}P NMR ($CDCl_3$): δ − 11.4 and −12.1.

Tetraethyl methylenebisphosphonate (**7**).—To triethyl phosphite (51.3 mL, 0.3 mol) at 165°C was added CH_2Br_2 (7.02 mL, 0.1 mol) dropwise under Ar during 5 h. The reaction flask was equipped with a condenser and the condenser temperature was maintained at 54.6°C with a circulating water–ethylene glycol bath. In this way, unreacted starting materials were retained while ethyl bromide formed was removed. The mixture was heated for 24 h at 165°C and for 2 h at 185°C. Excess of triethyl phosphite was distillated off under reduced pressure and continued distillation gave **7**. Further purification by Kugelrohr distillation afforded **7** (18.27 g, 63.4%) as a colorless oil; bp 122–124°C/0.6 torr (lit. [17] bp 130°C/1.0 torr); ^1H NMR ($CDCl_3$): δ 1.35 (t, 12 H, *J* 7.1 Hz, 4 OCH_2CH_3), 2.45 (t, 2 H, $J_{H,P}$ 21.0 Hz, $-CH_2-$), 4.19 (quint, 8 H, *J* 7.1 Hz, 4 OCH_2CH_3); ^{31}P NMR ($CDCl_3$): δ 20.2 (lit. [17] δ 19.0). EIMS: *m/z* 288 (M^+).

2,3:4,6-Di-O-isopropylidene-α-D-mannopyranose (**8**).—A mixture of **3** (9.1 g, 25.9 mmol) and 10% Pd–C (2.0 g) in 2:2:1 THF–EtOH–H_2O was hydrogenolyzed at 50 psi for 3 days. The precipitate was filtered off and washed with MeOH. The filtrate was evaporated to give **8** (6.75 g, quantitative), which was recrystallized from 1:1 hexane–EtOAc; mp 139–140°C (lit. [18] mp 139–141°C); $[\alpha]_D^{25}$ −34° (*c* 1.0, $CHCl_3$) [lit. [18] $[\alpha]_D^{25}$ −39° (*c* 1.0, $CHCl_3$)].

Wadsworth–Emmons reaction of **8**.—A solution of **8** (260 mg, 1.0 mmol) in CH_2Cl_2 (3 mL) was mixed with tetraethyl methylenebisphosphonate (290 mg, 1.0 mmol) in aq 50% NaOH (3 mL) and the resulting two-phase mixture was vigorously stirred for 24 h [12]. The mixture was extracted with CH_2Cl_2 and the combined extracts were stored in a refrigerator, filtered, dried ($MgSO_4$), and evaporated. The residue was chromatographed (gradient of EtOAc and hexane) to give three products. The least polar product, diethyl (2,3:4,6-di-*O*-isopropylidene-β-D-mannopyranosyl)methylphosphonate (**10a**) was obtained as an oil (127 mg); $[\alpha]_D^{25}$ −6.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.32 (2t, 6 H, *J* 7.0 Hz,

OCH_2CH_3), 1.35, 1.42, 1.51, and 1.52 (2 s, each 3 H, 2 Me_2C), 2.01 (ddd, 1 H, $J_{1'a,P} = J_{1'a,1'b} = 15.3$ $J_{1,1'a}$ 8.2 Hz, H-1'a), 2.16 (ddd, 1 H, ABMX system, $J_{1'b,P} = J_{1'b,1'a} = 15.3$, $J_{1'b,1}$ 5.0 Hz, H-1'b), 3.40 (ddd, 1 H, $J_{5,4}$ 10.0, $J_{5,6a}$ 10.8, $J_{5,6e}$ 5.5 Hz, H-5), 3.72 (dd, 1 H, $J_{6a,5} = J_{6a,6e} = 10.8$ Hz, H-6*a*), 3.86 (dd, 1 H, $J_{6e,6a}$ 10.8, $J_{6e,5}$ 5.5 Hz, H-6*e*), 3.97 (dd, 1 H, $J_{4,5}$ 10.0, $J_{3,4}$ 6.8 Hz, H-4), 4.10 and 4.11 (2 quint, each 2 H, *J* 7.0 Hz, OCH_2CH_3), 4.19–4.28 [overlapping, 3 H, H-1,2,3; $J_{1,2}$ 4.8 Hz (by *J*-resolved 2D), each 4.28, 4.21, 4.19 (by COSY)]; ^{13}C NMR ($CDCl_3$): δ 16.4 and 16.5 (2 OCH_2CH_3), 19.0, 25.4, 27.6, and 29.0 (2 Me_2C), 29.6 (d, $J_{C,P}$ 141.7 Hz, C-1'), 61.7 and 61.8 (2 d, 2 C, $J_{C,P}$ 6.2 Hz, 6.4, 2 OCH_2CH_3), 62.7 (C-6), 64.5, 69.7 (d, $J_{C,P}$ 4.2 Hz), 72.1 and 75.4 (C-2,3,4,5), 76.5 (d, $J_{C,P}$ 6.8 Hz, C-1), 99.6 and 110.0 (2 Me_2C); ^{31}P NMR ($CDCl_3$): δ 28.1; EIMS: *m/z* 395 (MH^+) and 379 (M − Me); FABMS: *m/z* 395 (M + 1) and 379 (M − Me).

The more polar product, diethyl (2,3 : 4,6-di-*O*-isopropylidene-α-D-mannopyranosyl)methylphosphonate (**10b**) was obtained as white crystals (47 mg); mp 87–90°C; $[\alpha]_D^{25}$ −39.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.32 (2t, 6 H, *J* 7.1 Hz, $-OCH_2CH_3$), 1.35, 1.42, 1.50, and 1.54 (4 s, 3 H, 2 Me_2C), 2.21 (ddd, 1 H, ABMX system, $J_{1'a,P}$ 18.4, $J_{1'a,1'b}$ 15.4, $J_{1'a,1}$ 6.7 Hz, H-1'a), 2.32 (ddd, 1 H, $J_{1'b,p}$ 18.4, $J_{1'b,1'a}$ 15.4, $J_{1'b,1}$ 6.7 Hz, H-1'b), 3.15 (ddd, 1 H, $J_{5,4} = J_{5,Ha} = 10.2$, $J_{5,6e}$ 5.6 Hz, H-5), 3.70 (dd, 1 H, $J_{4,5}$ 10.2, $J_{4,3}$ 7.0 Hz, H-4), 3.71 (dd, 1 H, $J_{6a,6e} = J_{6a,5} = 10.2$ Hz, H-6*a*), 3.88 (dd, 1 H, $J_{6e,6a}$ 10.2, $J_{6e,5}$ 5.6 Hz, H-6*e*), 4.10 and 4.11 (2 quin, 2 H, *J* 7.1 Hz, $-OCH_2CH_3$), 4.05–4.16 [overlapping, 2 H, H-1,3; each 4.15, 4.05 (by COSY)], 4.21 [dd, 1 H, $J_{1,2}$ 2.2, $J_{2,3}$ 5.1 Hz (by *J*-resolved 2D), H-2]; ^{13}C NMR ($CDCl_3$): δ 16.3 and 16.4 (each d, $J_{C,P}$ 5.3 and 5.6 Hz, 2 OCH_2CH_3), 18.8, 26.3, 28.4, and 29.0 (2 Me_2C), 28.5 (d, $J_{C,P}$ 141.2 Hz, C-1'), 61.5 and 62.0 (each d, $J_{C,P}$ 6.2 and 6.7 Hz, 2 OCH_2CH_3), 61.9 (C-6), 69.8, 72.1, 72.9, and 76.0 (C-2,3,4,5) 75.5 (d, $J_{C,P}$ 7.6 Hz, C-1), 99.6 and 109.5 (2 Me_2C); ^{31}P NMR ($CDCl_3$): δ 28.3; EIMS: *m/z* 394 (M^+) and 379 (M − Me). Anal. Calcd for $C_{17}H_{31}O_8P$: C, 51.76; H, 7.92. Found: C, 51.50; H, 7.88.

The third product (**9**, 50 mg) was isolated as an oil; ^1H NMR ($CDCl_3$): δ 1.31 (t, 6 H, *J* 7.1 Hz, 2 OCH_2CH_3), 1.38 and 1.41 (2 s, 3 H, Me_2C), 1.46 (s, 6 H, Me_2C), 2.98 (bs, 1 H, OH), 3.60–394 (overlapping, 3 H, H-6,7a,7b), 3.84 (overlapping, 1 H, H-5), (overlapping, 1 H, H-4), 4.07 and 4.08 (2 quint, each 2 H, *J* 7.1 Hz, 2 OCH_2CH_3), 4.70 (m, 1 H, H-3), 6.02 (ddd, 1 H, $J_{1,P}$ 20.2, $J_{1,2}$ 17.1, $J_{1,3}$ 1.6 Hz, H-1), 6.76 (ddd, 1 H, $J_{2,P}$ 21.8, $J_{1,2}$ 17.1, $J_{2,3}$ 4.3 Hz, H-2); ^{31}P NMR ($CDCl_3$): δ 18.9; IR (neat): 3359(br), 2940, 1639, 1450, 1379, 1106, 762 cm^{-1}; EIMS: *m/z* 395 (MH^+) and 379 (M − Me). Anal. Calcd for $C_{17}H_{31}O_8P \cdot 2H_2O$: C, 47.43; H, 8.19. Found: C, 47.29; H, 7.94.

Diethyl (2,3-O-isopropylidene-β-D-mannopyranosyl)methylphosphonate (**11a**).—A solution of **10a** (540 mg, 1.37 mmol) and *p*-toluenesulfonic acid (56.0 mg, 0.29 mmol) in acetone (5 mL) and water (5 mL) was stirred at room temperature. The reaction was found to be complete in 4 h by TLC, and was quenched with satd aq $NaHCO_3$ (1 mL). The solution was evaporated under reduced pressure, the residue dissolved in water, and the solution extracted with EtOAc. The organic extract was dried with anhyd Na_2SO_4 and evaporated to give **11a** as a colorless glass (310 mg, 64%); ^1H NMR ($CDCl_3$): δ 1.33 and 1.34 (2 t, each 3 H, *J* 7.0 Hz, 2

OCH_2CH_3), 1.35 and 1.49 (2 s, each 3 H, Me_2C), 1.97 and 2.23 (m, 2 H, H-1'), 3.24 (bs, 1 H, OH), 3.54 (m, 1 H), 3.82 (bs, 2 H), 4.00–4.23 (m, 9 H); ^{13}C NMR ($CDCl_3$): δ 16.3 and 16.4 (2 OCH_2CH_3), 25.0 and 27.2 (Me_2C), 30.3 (d, $J_{C,P}$ 141.3 Hz, C-1'), 61.7 and 62.1 (2 d, $J_{C,P}$ 5.0 and 6.1 Hz, 2 OCH_2CH_3), 61.7 (C-6), 69.0 (d, $J_{C,P}$ 6.3 Hz, C-1), 68.1, 76.7, and 76.9 (C-3,4,5), 78.7 (d, $J_{C,P}$ 1.7 Hz, C-2), 110.1 (Me_2C); EIMS: m/z 355 (MH^+) and 339 (M − Me).

Diethyl (2,3-O-isopropylidene-α-D-mannopyranosyl)methylphosphonate (**11b**).—Compound **10b** (50 mg, 0.125 mmol) was converted into **11b** (23 mg, 52%), using the procedure described for the preparation of **11a**; ^1H NMR ($CDCl_3$): δ 1.31 and 1.32 (2 t, each 3 H, J 7.0 Hz, 2 OCH_2CH_3), 1.34 and 1.50 (2 s, each 3 H, Me_2C), 1.83 (bs, 1 H, OH), 2.23 (m, 2 H, H-1'), 2.80 (bs, 1 H, OH), 3.25 (m, 1 H), 3.65 (dd, 1 H, J_1 9.8, J_2 7.2 Hz), 3.74 (dd, 1 H, J_1 11.8, J_2 5.5 Hz), 3.83 (m, 2 H), 4.07 and 4.08 (2 quint, 4 H, J 7.0 Hz, 2 OCH_2CH_3), 4.01–4.16 (m, 2 H).

Diethyl (2,3-O-isopropylidene-4,6-di-O-diphenoxyphosphoryl-β-D-mannopyranosyl)methylphosphonate (**12a**).—A mixture of **11a** (230 mg, 0.65 mmol), Et_3N (0.74 mL, 5.25 mmol), and a catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2 (10 mL) was stirred until a clear solution was obtained. To this solution at 0°C was added diphenyl phosphorochloridate (1.10 mL, 5.25 mmol), and the resulting solution was allowed to warm to ambient temperature and stirred overnight. The reaction was quenched with satd aq $NaHCO_3$ (10 mL), the solvents were evaporated, and the product was partitioned between 0.1 M HCl and CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed (gradient of EtOAc–hexane) to give **12a** (464 mg, 87.3%) as a colorless glass; $[\alpha]_D^{25}$ −4.0° (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.27 and 1.28 (2 t, each 3 H, J 7.1 Hz, 2 OCH_2CH_3), 1.31 and 1.48 (2 s, each 3 H, 2 Me_2C), 2.08 (m, 2 H, H-1'a,1'b), 3.88 (m, 1 H, H-5), 4.11 (m, 4 H, J 7.1 Hz, 2 OCH_2CH_3), 4.30 (overlapping, 4 H, H-1,2,3,6), 4.48 [ddd, 1 H, $J_{5,6a}$ 3.2, $J_{6a,6b}$ 11.1 Hz, H-6a (by COSY)], 4.73 [ddd, 1 H, $J_{3,4}$ 6.2, $J_{4,5}$ 9.0 Hz, H-4 (by COSY)], 7.24 (m, 20 H, 4 OPh); ^{13}C NMR ($CDCl_3$): δ 16.3 and 16.4 (2 OCH_2CH_3), 26.0 and 27.6 (Me_2C), 29.1 (d, $J_{C,P}$ 140.6 Hz, C-1' by DEPT 135 degree), 62.0 and 62.1 (each d, $J_{C,P}$ 6.2 Hz, 2 OCH_2CH_3 by DEPT 135 degree), 66.8 (d, $J_{C,P}$ 6.0 Hz, C-6 by DEPT 135 degree), 69.0 (d, $J_{C,P}$ 2.0 Hz), 71.1 (t, $J_{C,P}$ 6.9 Hz), 75.3 (d, $J_{C,P}$ 6.4 Hz), 75.4, 75.8 (d, $J_{C,P}$ 9.6 Hz), 110.3 (Me_2C), 120.1, 125.4, 129.6, and 150.4 (4 OPh); ^{31}P NMR ($CDCl_3$): δ 27.0, −11.3, −12.1; FABMS: m/z 841 $(M + Na)^+$ and 819 $(M + 1)^+$.

Diethyl (2,3-O-isopropylidene-4,6-di-O-diphenoxyphosphoryl-α-D-mannopyranosyl)methylphosphonate (**12b**).—The 4,6-diol **11b** (20 mg, 0.056 mmol) was converted into **12b** (30 mg, 65.4%) by using the procedure described for the preparation of **12a**; $[\alpha]_D^{25}$ −14.0° (c 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.31 (quin, 6 H, J 7.0 Hz, 2 OCH_2CH_3), 1.37 and 1.49 (2 s, each 3 H, Me_2C), 2.19 (m, 2 H, H-1'a,1'b), 3.65 [m, 1 H, H-5 (by COSY)], 4.08 [overlapping, 5 H, 2 OCH_2CH_3 and H-1 (by COSY)], 4.29 (overlapping, 3 H, H-2,3,6b), 4.45 [ddd, 1 H, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 11.3 Hz, H-6a (by COSY)], 4.60 (ddd, 1 H, $J_{3,4}$ 6.7, $J_{4,5}$ 11.0 Hz, H-4), 7.25 (m, 20 H, 4 OPh); ^{13}C NMR ($CDCl_3$): δ 16.3 and 16.4 (2 OCH_2CH_3), 26.2 and 27.7 (Me_2C), 27.9 (d, $J_{C,P}$ 140.4 Hz, C-1' by DEPT 135 degree), 61.7 and 62.1 (each d,

$J_{C,P}$ 6.2 Hz, 2 OCH_2CH_3), 65.3, 67.4 (d), 71.3, 75.1 (d, $J_{C,P}$ 6.0 Hz), 75.7 (d, $J_{C,P}$ 7.0 Hz), and 76.8 (C-1,2,3,4,5,6), 110.4 (Me_2C), 120.1, 125.4, 129.7, and 150.4 (4 OPh); ^{31}P NMR ($CDCl_3$): δ 27.7, −11.4, and −12.0; FABMS: m/z 841 $(M + Na)^+$ and 819 $(M + 1)^+$.

Hexasodium (4,6-di-O-phosphonato-β-D-mannopyranosyl)methylphosphonate (**1a**).—To a solution of **12a** (20 mg, 0.024 mmol) in CCl_4 (0.5 mL) was added bromotrimethylsilane (20 μL, 0.15 mmol). The ^{31}P NMR spectra taken after 20 min showed loss of the signals corresponding to the phosphonic ethyl esters. After an additional 30 min, water (1 mL) was added to the mixture and the solvents were removed under reduced pressure. The residue was dissolved in 1:1:2 n-BuOH–EtOH–H_2O and hydrogenated under H_2 (50 psi) and PtO_2 (80 mg) overnight. The product mixture was filtered and washed with water. The filtrate was concentrated and lyophilized. A solution of the resultant solid in water (0.5 mL) was treated with 0.5 M NaOH to pH 7.5 and lyophilized to give **1a** (9.0 mg, 68%); $[\alpha]_D^{25}$ +6.6° (*c* 1, H_2O); ^1H NMR (D_2O, pH 7.5): δ 1.81 (m, 2 H, H-1'a,1'b), 3.70–4.26 (m, 7 H); ^{31}P NMR (D_2O): δ 23.2, 5.2, and 4.9.

Hexasodium (4,6-di-O-phosphonato-α-D-mannopyranosyl)methylphosphonate (**1b**).—Compound **12b** (20 mg, 0.024 mmol) was similarly converted into **1b** (6.0 mg, 46.3%); $[\alpha]_D^{25}$ +3.2° (*c* 0.5, H_2O); ^1H NMR (D_2O, pH 8.5): δ 1.62 (m, 2 H, H-1'a,1'b), 3.80–4.25 (m, 7 H); ^{31}P NMR (D_2O): δ 17.2, 5.3, and 5.2.

Evaluation of calcium release activity with permeabilized hepatocytes [19].—The calcium release activity and Ins(1,4,5)P_3 receptor antagonism were measured by M.J. Berridge at the University of Cambridge, UK.

Evaluation of bioactivities for Ins(1,4,5)P_3 5-phosphatase and Ins(1,3,4)P_3/Ins(1,4)P_2 1-phosphatase.—The synthetic compounds were examined for their potential activity as inhibitor or activator of bovine testis Ins(1,4,5)P_3 5-phosphatase and rat liver Ins(1,3,4)P_3/Ins(1,4)P_2 1-phosphatase by R.H. Michell at the University of Birmingham, UK.

Evaluation of inhibitory activity with Ins(1,4,5)P_3 3-kinase [20].—The synthetic compounds were evaluated for their potential activity as inhibitors of Ins(1,4,5)P_3 3-kinase obtained from cDNA clone of rat brain by K.Y. Choi in the POSTECH, Korea.

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