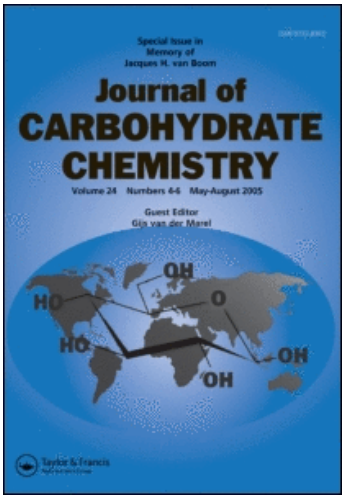

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Syntheses of All Regioisomers of *Myo*-Inositol Bisphosphate

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SYNTHESES OF ALL REGIOISOMERS OF *MYO*-INOSITOL BISPHOSPHATE

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ABSTRACT

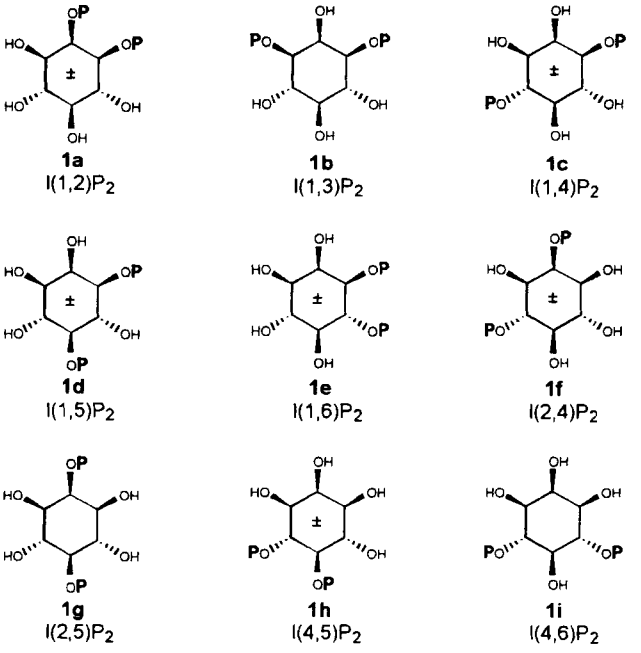
All possible nine regioisomers of *myo*-inositol bisphosphate were synthesized using various isopropylidene and benzoyl inositol derivatives as intermediates. These intermediates were successfully phosphorylated with diethyl chlorophosphite followed by oxidation. Selective diphosphorylations on several IBz_3 intermediates were also examined as possible routes to IP_2s. All of the protecting groups were easily removed in a one-pot procedure to give the sodium salts of the title compounds, IP_2 regioisomers.

INTRODUCTION

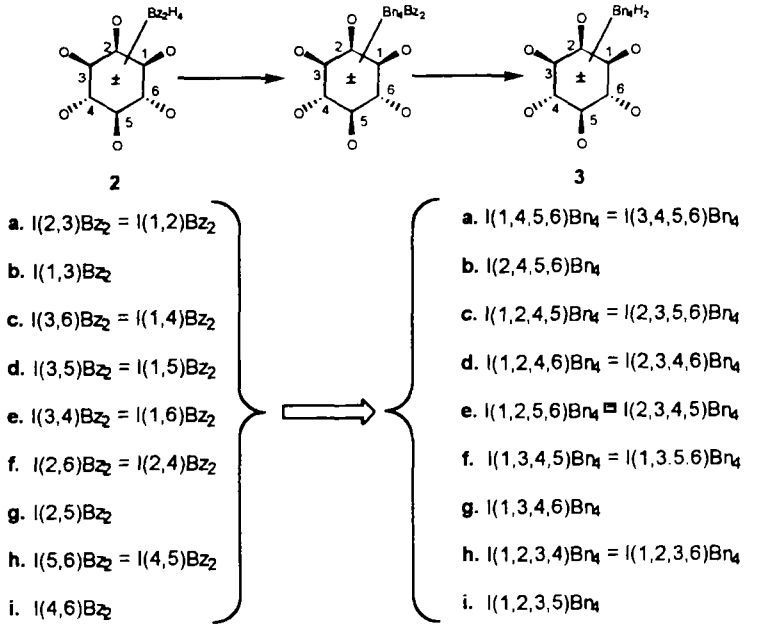
D-*myo*-Inositol 1,4,5-trisphosphate [D-I(1,4,5)P_3], released into the cytosol of cells by the phospholipase C (PLC)-catalyzed cleavage of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2], is known to mobilize the intracellular calcium.[1] One of the major metabolic pathways of IP_3 is removal of the 5-phosphate group by a specific 5-phosphatase located in plasma membrane as well as in the cytosol of stimulated cells to give I(1,4)P_2. Other phosphatases are responsible for the further degradation of the IP_2 formed in this hydrolysis, *via* I(1)P or I(4)P to give finally free inositol, which is recycled in the brain to provide more PIP_2.

Although the conversion of $I(1,4,5)P_3$ into $I(1,4)P_2$ had been regarded as merely a terminating step of Ca^{2+} mobilization signal, the novel activities of $I(1,4)P_2$ reported as DNA polymerase α activator[2] and cytoskeletal actin inducer[3] suggested that IP_2 might be another interesting signal molecule. Several other IP_2s were also detected in nature, but the biological functions of IP_2s have not been thoroughly studied yet.

Until now 5 out of all possible 9 (enantiomerically 15) IP_2 regioisomers (**1a-i**), $I(1,2)P_2$,[4] $I(1,3)P_2$,[5] $I(1,4)P_2$,[6] $I(1,5)P_2$[7] and $I(4,5)P_2$[8] have been synthesized by independent pathways, but systematic research on the structure and biological function has been hampered by the limited availability of IP_2 isomers. We previously reported divergent syntheses of all possible IP_1,[9] IP_3,[10] IP_4[11] and IP_5[12] regioisomers using the acyl migration methods. Here we describe the synthesis of the complete set of IP_2 regioisomers via application of the divergent method and each individual syntheses using various intermediates, thus completing the syntheses of all 38 regioisomers of IP_n (n= 1-5).



Nine regioisomers of IP_2 (P = PO_3^{2-})

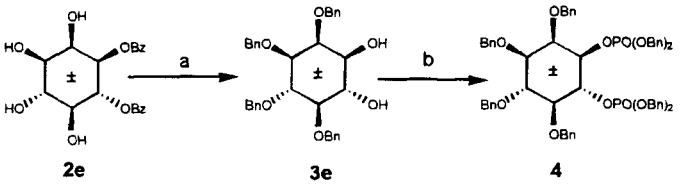


Scheme 1. Conversion scheme of IBz_2 into IBn_4

RESULTS AND DISCUSSION

One of the key problems in IP_n synthesis is the preparation of suitable intermediates, IR_{6-n}, which can be easily phosphorylated and deprotected under mild conditions to give a salt form of IP_n. As IBz_{6-n}s were proved to be convenient intermediates for IP_n syntheses in the previous works,[9-12] preparation of the 9 isomers of IBz_4 was expected to be the key to the synthesis of IP_2 regioisomers. We anticipated that IBz_2s, used as the precursors for IP_4s,[11] could also be utilized as intermediates in the synthesis of IP_2s; benzylation of IBz_2 at the free hydroxyl groups, followed by hydroyses of the benzoyl groups would provide corresponding IBn_4s, which could then be phosphorylated to give IP_2 (Scheme 1).

Thus, $I(1,6)Bz_2$, **2e**[10] was benzylated with BnBr and AgOTf, and the benzoyl groups were removed by basic methanolysis to give **3e**. $I(2,3,4,5)Bn_4$ (**3e**) was phosphorylated with the phosphoramidite reagent and a weak acid, 1H-tetrazole to give **4** (Scheme 2), which showed two phosphorus resonances at δ 0.68 and 1.28 ppm. This example clearly demonstrated the feasibility that all possible IP_2 isomers could be



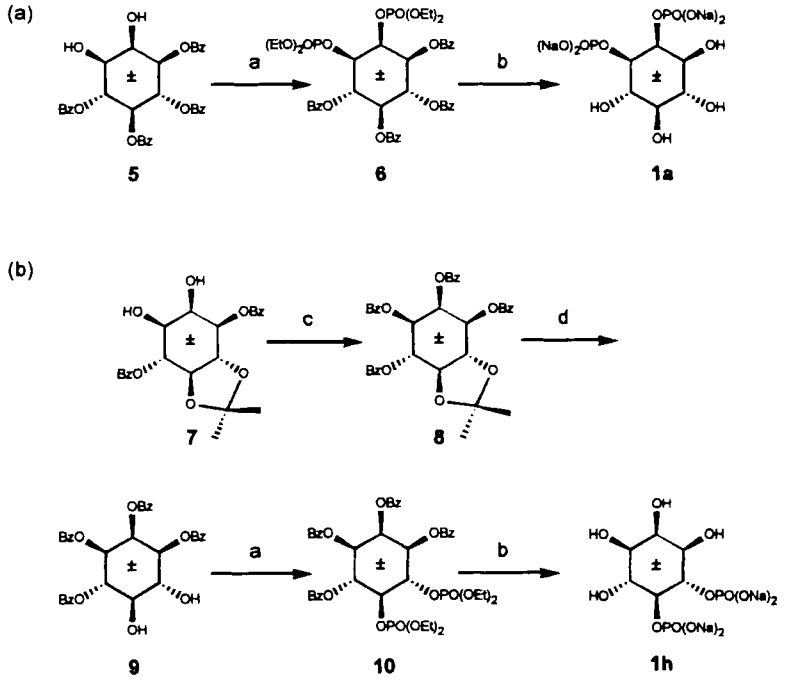
2e 3e 4

Scheme 2. A representative example of IP_2 synthesis from IBz_2.
a.(i) BnBr, AgOTf, 2,6-di-*t*-butyl-pyridine. (ii) NaOH, MeOH, reflux.
b.(i) $iPr_2NP(OBn)_2$, tetrazole, (ii) 30% H_2O_2

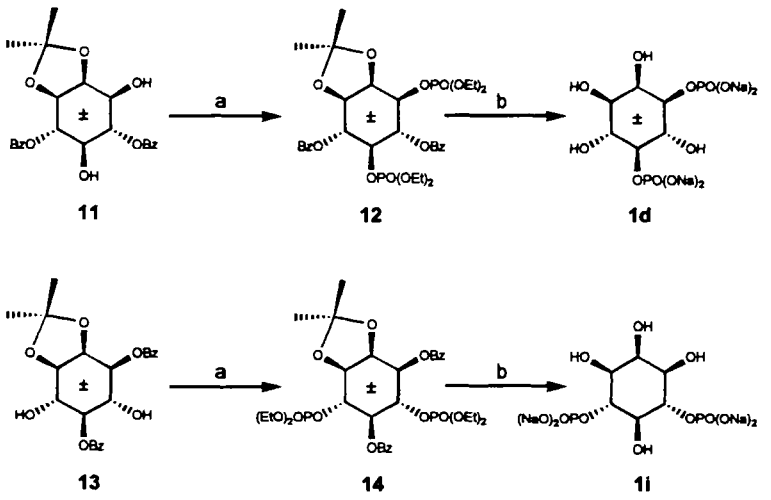
similarly synthesized from IBz_2. Nevertheless, various other intermediates and synthetic routes were examined in order to find the most efficient synthetic procedures.

I(1,4,5,6)Bz_4 (**5**), obtained by two step reactions from *myo*-inositol,[9] was phosphorylated by successive treatments with diethyl chlorophosphite and *N,N*-diisopropylethylamine in DMF, and then 30% hydrogen peroxide to yield **6**. In the final steps, the protecting groups of **6** were removed by successive reactions with TMSBr and then LiOH. The target compound I(1,2)P_2 (**1a**) was obtained after chromatography on Dowex 50 x 8-100 (H^+ form) resin, pH adjustment to 10 with NaOH, and then lyophilization (Scheme 3a). I(1,2,3,4)Bz_4 (**9**) which was derived by benzoylation and subsequent acid hydrolysis of **7**,[11] was similarly phosphorylated by the same procedure employed for **6** to afford **1h** via **10** (Scheme 3b).

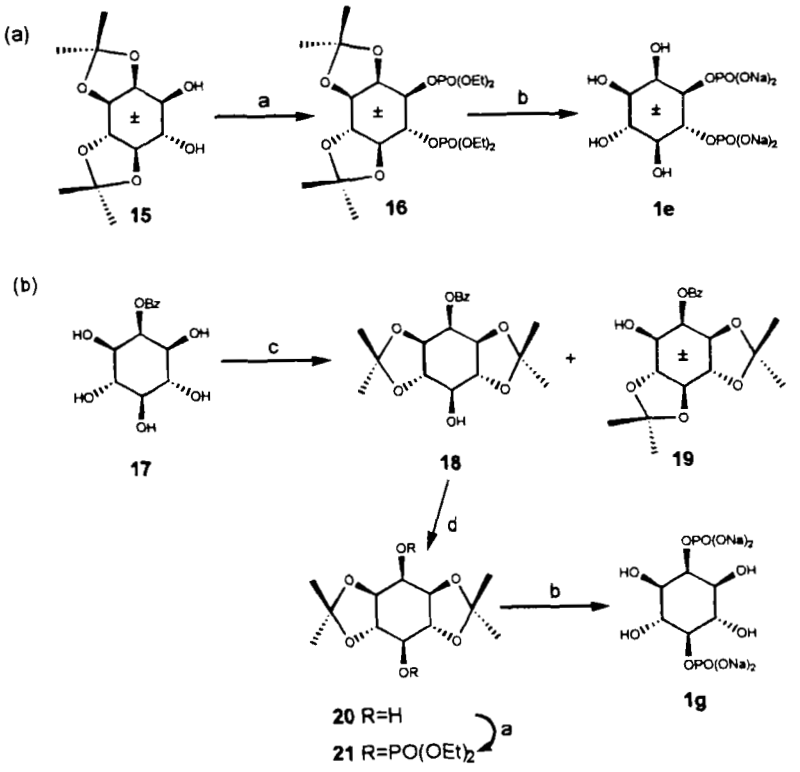
The IBz_2 isomers with the isopropylidene protecting group, **11** and **13**, which were used as intermediates in the synthesis of IP_4,[11] were similarly phosphorylated to provide the protected IP_2s, **12** and **14**, respectively. In addition to the base-labile Bz groups, the acid-labile isopropylidene protecting groups were to be removed in the deprotection step. The protecting groups were removed by the unusual protocols, i.e., successive reactions with TMSBr and then LiOH, followed by chromatography on Dowex 50 x 8-100 (H^+ form) resin. The isopropylidene group was eliminated simply by keeping the acid effluent for 1 day at rt. Lyophilization of the solvent gave the free acid form of IP_2, which was redissolved in a small amount of water, and adjusted to pH 10 with NaOH to give I(1,5)P_2 (**1d**) and I(4,6)P_2 (**1i**, Scheme 4).



Scheme 3. a.(i) $(EtO)_2PCl$, diisopropylethylamine, (ii) 30% H_2O_2. b.(i) TMSBr, (ii) 1N LiOH, (iii)H^+ ion exchange, (iv) pH 10 (NaOH). c. BzCl, pyridine. d. AcOH-EtOH.



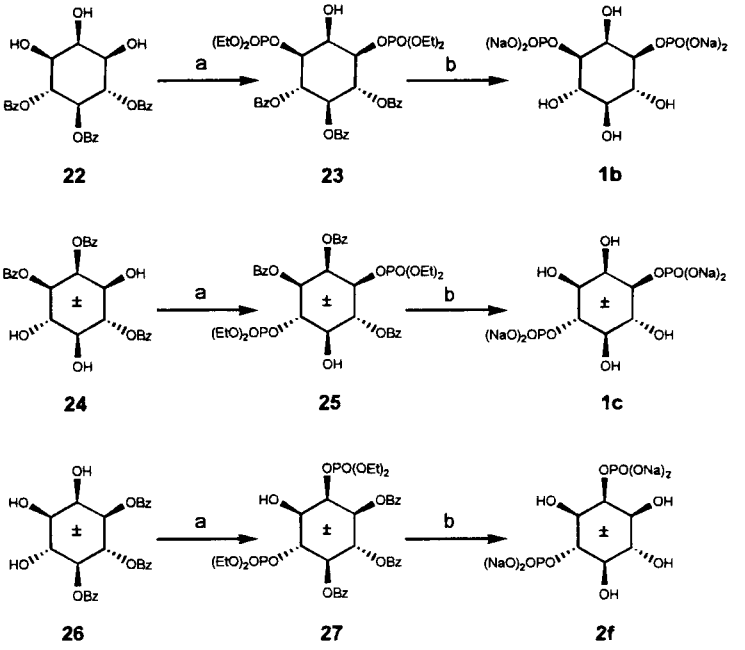
Scheme 4. a.(i) $(EtO)_2PCl$, diisopropylethylamine, (ii) 30% H_2O_2. b.(i) TMSBr, (ii) 1N LiOH, (iii) H^+ ion exchange, (iv) pH 10 (NaOH).



Scheme 5. a.(i) $(EtO)_2PCl$, diisopropylethylamine, (ii) 30% H_2O_2. b.(i) TMSBr, (ii) H^+ ion exchange, (iv) pH 10 (NaOH). c. 2,2-methoxypropane, TSA. d. NaOMe, MeOH.

myo-Inositol derivatives with two free hydroxyl groups, **15** and **20** were utilized as IP_2 precursors. Compound **15**[13] was phosphorylated by the same procedure as described for **6** to give **16**. Treatment with TMSBr and the subsequent acid-catalyzed hydrolysis of **16** removed all the protecting groups, and the pH adjustment of the product solution with NaOH gave the Na salt, I(1,6)P_2 (**1e**, Scheme 5a).

Acetonation of **17**[12] with 2,2-dimethoxypropane or 2-methoxypropene under the standard reaction conditions gave a mixture of **18** and **19** in almost equal amounts, regardless of the reagent used. Compound **18** was separated by column chromatography from **19**, then debenzoylated to give **20**. The 2,5-diol, **20** was similary converted to **21**, which was transformed to I(2,5)P_2 (**1g**, Scheme 5b).



Scheme 6. a.(i) $(EtO)_2PCl$ (3 eq.), diisopropylethylamine, (ii) 30% H_2O_2. b.(i) TMSBr, (ii) 1N LiOH, (iii) H^+ ion exchange, (iv) pH 10 (NaOH).

Three IBz_3 isomers, which were previously used as precursors in the synthesis of IP_3s,[10] were selectively phosphorylated with limited amounts of the phosphorylating agent. When **22**, **24** and **26** were subjected to phosphorylation with 3 eq. $(EtO)_2PCl$, then oxidized with H_2O_2, selective bisphosphorylations were observed to our pleasant surprise. In these reactions compounds **23**, **25** and **27** were isolated as the major products in about 50% yields. While the conversion of $I(4,5,6)Bz_3$ (**22**) to **23** may be understood in terms of the steric availability of the 1- and 3-OH groups in comparison to the sterically more demanding 2-OH, the selective formations of **25** and **27** are fairly novel. It is generally known that electrophiles usually favor the equatorial hydroxyls over the axial (Scheme 6). The present observations may possibly be due to unfavorable steric effects between the vicinal phosphates, which may be more pronounced than that between equatorial/axial hydroxyl groups. Each protected IP_2 regioisomer was deprotected by the same standard method described for **1a** and **1h** to afford the IP_2 isomers, $I(1,3)P_2$ (**1b**), $I(1,4)P_2$ (**1c**) and $I(2,4)P_2$ (**1f**).

Thus, we successfully synthesized all 9 regioisomers of IP_2 (**1a-1i**) using various intermediates, i.e., IBz_4s, di-*O*-isopropylidene *myo*-inositols, *O*-isopropylidene IBz_2s, and IBz_3s, and the target compounds were fully characterized by ^1H, ^{13}C and ^{31}P NMR. Biological studies in which the synthetic set of the IP_2 isomers are subjected to the inhibition of metabolic enzymes, affinity measurement to specific IP_n receptors, and induction of cytoskeletal actin are currently in progress.

EXPERIMENTAL

DL-3,4,5,6-Tetra-*O*-benzoyl-*myo*-inositol 1,2-bis(diethyl phosphate) (6). To a solution of compound **5**[9] (120 mg, 0.20 mmol) in DMF (5 mL) at -42 °C were added dropwise diisopropylethylamine (1 mL, 5.7 mmol) and then diethyl chlorophosphite (0.2 mL, 1.4 mmol) with vigorous stirring. After 20 min, the reaction mixture was allowed to slowly warm up to rt, and stirred for additional 5 h. The mixture was cooled in an ice bath and sodium phosphate buffer (1 M, pH 7, 5 mL) and excess 30% H_2O_2 (5 mL) were added. After standing overnight at rt, the mixture was diluted with water, and thoroughly extracted with EtOAc. The organic layer was extensively washed with water, dried over $MgSO_4$, and concentrated *in vacuo* overnight to give the phosphorylated product **6** (146 mg, 84% yield). mp 186-188 °C; ^1H NMR ($CDCl_3$) δ 0.89-1.39 (m, 12H, $4CH_2CH_3$), 3.73-4.27 (m, 8H, $4CH_2CH_3$), 5.03 (tt, J = 0.6, 10.0 Hz, 1H, H-1), 5.45-5.49 (m, 2H, H-2 & H-3), 5.87 (t, J = 10.0 Hz, 1H, H-5), 6.11 (t, J = 10.0 Hz, 1H, H-6), 6.21 (t, J = 10.0 Hz, 1H, H-4), 7.25-8.01 (m, 20H, 4Ph), the assignment of each proton for **6** was made on the basis of the ^1H NMR double resonance experiments. ^{13}C NMR ($CDCl_3$) δ 16.11-16.70 ($4CH_2CH_3$), 64.92-65.22 ($4CH_2CH_3$), 70.24, 70.91, 71.05, 71.36, 74.46, 76.04 (inositol ring carbon), 128.95-134.13 (4Ph), 166.11 (2C), 166.14, 166.26, 4PhCO; ^{31}P NMR ($CDCl_3$) δ -1.39, -0.54 (2P); MS (FAB) *m/z* = 891 (M^++Na), 869 (M^++1).

Sodium Salt of DL-*myo*-Inositol 1,2-Bisphosphate (1a). To a solution of compound **6** (30 mg, 0.035 mmol) in chloroform (0.5 mL) at rt was added excess TMS-Br (0.1 mL), and the solution was stirred overnight. The solvent and excess reagent were evaporated and the reaction mixture was redissolved in MeOH (3 mL), and then treated with drops of water at 0 °C. After standing at rt for 10 min, the reaction mixture was

concentrated to dryness, treated with 1M LiOH (3 ml), and stirred at 80 °C for 3h. The basic solution was cooled and loaded on Dowex 50 x 8-100 (H+ form) and eluted with water. The acidic effluent was collected and lyophilized to dryness. The residue was redissolved in a small amount of water (1 mL), the pH adjusted to 10 with NaOH, and the solution lyophilized again to give the sodium salt of I(1,2)P_2 (**1a**) in quantitative yield. ^{1}H NMR (D_2O, pH 10) δ 3.22 (t, J = 9.2 Hz, 1H, H-5), 3.35 (br d, J = 9,8 Hz, 1H, H-3), 3.68 (t, J = 9.7 Hz, 1H, H-4), 3.78 (t, J = 9.2 Hz, 1H, H-6), 3.88 (br t, J = 9.4 Hz, 1H, H-1), 4.46 (br d, J = 7.1 Hz, 1H, H-2); ^{31}P NMR (D_2O, pH 10) δ 7.15, 7.49.

DL-1,2,3,4-Tetra-*O*-benzoyl-5,6-*O*-isopropylidene-*myo*-inositol (8). After compound **7**[11] (2 g) was treated with a large excess of BzCl in pyridine, the usual extractive work-up procedure gave a crystalline solid **8** (2.5 g, 85% yield). R_f 0.7 (EtOAc-hexane = 1 : 3, 3 times); mp 246-248 °C (from MeOH); ^{1}H NMR ($CDCl_3$) δ 1.50, 1.56 (2s, 6H, C**Me**$_2$), 4.02 (dd, J = 9.4, 10.0 Hz, 1H, H-5), 4.49 (dd, J = 9.4, 10.6 Hz, 1H, H-6), 5.67 (dd, J = 3.1, 10.6 Hz, 1H, H-1), 5.69 (dd, J = 3.1, 9.3 Hz, 1H, H-3), 6.11 (app t, J = 10.0 Hz, 1H, H-4), 6.23 (t, J = 3.1 Hz, 1H, H-2), 7.20-8.09 (m, 20H, 4Ph); ^{13}C NMR ($CDCl_3$) δ 27.44, 27.54 (C**Me**$_2$), 70.66, 71.05, 71.36, 72.35, 76.37, 77.43 (inositol ring carbon), 113.99 (**C**Me2), 128.95-134.25 (4Ph), 165.92, 166.02 (2C), 166.26 (4PhCO); MS (FAB) *m/z* = 637 (M^++1).

DL-1,2,3,4-Tetra-*O*-benzoyl-*myo*-inositol (9). Compound **8** (1 g) in a solvent mixture of 80% aq. AcOH (25 mL) and EtOH (25 mL) was stirred at reflux for 1 h, and the solvents were evaporated to dryness to give **9** quantitatively. R_f 0.1 (EtOAc-hexane = 1 : 3); mp 206-207 °C (from EtOAc-Ether); ^{1}H NMR ($CDCl_3$-DMSO-d_6) δ 3.94 (dt, J = 5.0, 9.3 Hz, 1H, H-5), 4.27 (dt, J = 5.0, 9.3 Hz, 1H, H-6), 5.37 (d, J = 5.0, 1H, OH-1), 5.41 (dd, J = 3.1, 10.0 Hz, 1H, H-1), 5.52 (d, J = 5.0, 1H, OH-3), 5.59 (dd, J = 2.5, 10.6 Hz, 1H, H-3), 5.93 (dd, J = 10.0, 10.6 Hz, 1H, H-4), 6.08 (dd, J = 2.5, 3.1 Hz, 1H, H-2), 7.25-8.05 (m, 20H, 4Ph); ^{13}C NMR (DMSO-d_6) δ 69.66, 70.31, 70.74, 71.19, 72.05, 72.73 (inositol ring carbon), 128.48-134.01 (4Ph), 164.62, 165.02, 165.07, 165.37 (4PhCO); MS (FAB) *m/z* = 597 (M^++1).

DL-1,2,3,6-Tetra-*O*-benzoyl-*myo*-inositol 4,5-Bis(diethyl phosphate) (10). Compound **9** (41 mg, 0.069 mmol) was phosphorylated by the same procedure as described for **6** to give **10** (49 mg, 81% yield). R_f 0.25 (EtOAc-CH_2Cl_2 = 1 : 5); mp 136-

139 °C; ^{1}H NMR ($CDCl_3$) δ 0.80-1.31 (m, 12H, $4CH_2CH_3$), 3.56-4.23 (m, 8H, $4CH_2CH_3$), 5.03 (app q, J = 9.3 Hz, 1H, H-5), 5.30 (app q, J = 9.3 Hz, 1H, H-4), 5.60-5.67 (m, 2H, H-1 & H-3), 6.16-6.24 (m, 2H, H-2 & H-6), 7.26-8.07 (m, 20H, 4Ph); ^{13}C NMR ($CDCl_3$) δ 15.74-16.38 ($4CH_2CH_3$), 64.22-64.83 ($4CH_2CH_3$), [69.27, 69.78 (2C), 70.25, 70.51, 76.53, inositol ring carbon], 128.74-134.06 (4Ph), [165.49 (2C), 165.69, 165.87, 4PhCO]; ^{31}P NMR ($CDCl_3$) δ -1.43, -1.08 (2P); MS (FAB) *m/z* = 891 (M^++Na), 869 (M^++1).

Sodium Salt of DL-*myo*-Inositol 4,5-Bisphosphate (1h). Compound **10** (20 mg, 0.023 mmol) was deprotected by the same procedure as described for **1a** to give the sodium salt of **1h** in quantitative yield. ^{1}H NMR (D_2O, pH 10) δ 3.48 (dd, J = 2.9, 9.6 Hz, 1H, H-1), 3.58 (dd, J = 2.8, 9.7 Hz, 1H, H-3), 3.71-3.78 (m, 2H, H-5 & H-6), 3.90 (t, J = 2.8 Hz, 1H, H-2), 4.04 (q, J = 9.3 Hz, 1H, H-4); ^{31}P NMR (D_2O, pH 10) δ 7.23, 7.38.

DL-4,6-Bis-*O*-benzoyl-2,3-*O*-isopropylidene-*myo*-inositol 1,5-Bis(diethyl phosphate) (12). Compound **11**[11] (29 mg, 0.068 mmol) was phosphorylated by the same procedure as described for **6** to give **12** as an oil (40 mg, 85% yield). Rf 0.1 (EtOAc-CH_2Cl_2 = 1 : 5); ^{1}H NMR ($CDCl_3$) δ 0.74-1.74 (m, 18H, CMe_2 & $4CH_2CH_3$), 3.50-4.16 (m, 8H, $4CH_2CH_3$), 4.36 (dd, J = 4.8, 7.5 Hz, 1H, H-3), 4.70 (app t, J = 4.4 Hz, 1H, H-2), 4.79 (app q, J = 9.6 Hz, 1H, H-5), 4.91 (ddd, J = 4.0, 8.7, 10.1 Hz, 1H, H-1), 5.72 (dd, J = 7.5, 10.2 Hz, 1H, H-4), 5.96 (app t, J = 9.9 Hz, 1H, H-6), 7.43-8.19 (m, 10H, 2Ph); ^{13}C NMR ($CDCl_3$) δ 15.64-16.33 ($4CH_2CH_3$), 26.34, 28.03 (CMe_2), 64.18-64.73 ($4CH_2CH_3$), 70.92, 73.76, 73.92, 74.97, 75.69, 76.74 (inositol ring carbon), 111.74 (CMe2), 126.29-133.76 (2Ph), 165.68, 165.74 (2PhCO); ^{31}P NMR ($CDCl_3$) δ -1.33, -0.90; MS (FAB) *m/z* = 723 (M^++Na), 701 (M^++1).

Sodium Salt of DL-*myo*-Inositol 1,5-Bisphosphate (1d). To compound **12** (8 mg, 0.011 mmol) in chloroform (0.5 mL) at rt was added excess TMS-Br (0.1 mL), and the solution was stirred overnight. The solvent and excess reagent were evaporated and the reaction mixture was dissolved in MeOH (3 mL), and then treated with drops of water at 0 °C. After standing at rt for 10 min, the reaction mixture was concentrated to dryness and treated with 1M LiOH (3 mL) at 80 °C for 3h. The basic solution was cooled and loaded on a column of Dowex 50 x 8-100 (H^+ form) resin and eluted with water. The acidic effluent was collected, kept overnight at rt to remove the acetal protecting group.

The resulting solution was lyophilized to dryness, and the residue redissolved in a small amount of water (1 mL). The pH of the solution was adjusted to 10 with NaOH, and the solution lyophilized again to give the sodium salt of *myo*-inositol bisphosphate (**1d**) in quantitative yield. ^{1}H NMR (D_2O, pH 10) δ 3.52 (dd, J = 2.6, 9.1 Hz, 1, H-3), 3.63-3.71 (m, 3H, H-4, H-5 & H-6), 3.82 (dt, J = 2.3, 7.5 Hz, 1H, H-1), 4.17 (t, J = 2.8 Hz, 1H, H-2); ^{31}P NMR (D_2O, pH 10) δ 6.25, 7.01.

DL-1,5-Bis-*O*-benzoyl-2,3-*O*-isopropylidene-*myo*-inositol 4,6-Bis(diethyl phosphate) (14). Compound **13**[11] (28 mg, 0.065 mmol) was phosphorylated by the same procedure as described for **12** to give **14** (36 mg, 79% yield). R_f 0.15 (EtOAc- CH_2Cl_2 = 1 : 5); mp 155-158 °C; ^{1}H NMR ($CDCl_3$) δ 0.77-1.66 (m, 18H, C**Me**$_2$ & 4CH_2C**H**$_3$), 3.56-4.19 (m, 8H, 4C**H**$_2$CH$_3$), 4.36 (dd, J = 5.1, 7.3 Hz, 1H, H-3), 4.69 (app t, J = 4.6 Hz, 1H, H-2), 4.90 (app q, J = 9.7 Hz, 1H, H-4), 5.26 (app q, J = 9.2 Hz, 1H, H-6), 5.46-5.57 (m, 2H, H-1 & H-5), 7.43-8.24 (m, 10H, 2Ph); ^{13}C NMR ($CDCl_3$) δ 15.70-16.41 (4CH_2**C**H$_3$), 26.29, 28.08 (C**Me**$_2$), 64.21-64.39 (4**C**H$_2$CH$_3$), 70.68, 71.90, 75.06, 75.09, 77.61, 78.31 (inositol ring carbon), 111.60 (**C**Me$_2$), 126.30-133.93 (2Ph), 165.94, 166.15 (2Ph**C**O); 31P NMR ($CDCl_3$) δ -0.83, -0.50; MS (FAB) *m/z* = 701 (M^++1).

Sodium Salt of *myo*-Inositol 4,6-Bisphosphate (1i). Deprotection of **14** (11 mg, 0.016 mmol) according to the procedures described for **1d** gave **1i** in quantitative yield. ^{1}H NMR (D_2O, pH 10) δ 3.38 (t, J = 9.2 Hz, 1H, H-5), 3.55 (dd, J = 2.6, 9.6 Hz, 1H, H-1 & H-3), 3.93 (br s, 1H, H-2), 4.08 (app q, J = 8.7 Hz, H-4 & H-6); ^{31}P NMR (D_2O, pH 10) δ 7.22.

DL-2,3:4,5-Di-*O*-isopropylidene-*myo*-inositol 1,6-Bis(diethyl phosphate) (16). Compound **15**[13] (45 mg, 0.17 mmol) was phosphorylated by the same procedure as described for **6** to give **16** as an oil (64 mg, 71% yield). R_f 0.3 (MeOH- CH_2Cl_2 = 1 : 20); ^{1}H NMR ($CDCl_3$) δ 1.28-1.55 (m, 24H, 2C**Me**$_2$ & 4CH_2C**H**$_3$), 3.53 (dd, J = 8.8, 10.5 Hz, 1H, H-5), 3.97 (dd, J = 8.1, 10.5 Hz, 1H, H-4), 4.13-4.27 (m, 8H, 4C**H**$_2$CH$_3$), 4.32 (dd, J = 6.1, 8.1 Hz, 1H, H-3), 4.60-4.66 (m, 2H, H-1 & H-2), 4.83 (app dt, 4.7, 8.9 Hz, 1H, H-6), H-H COSY spectra gave a definitive assignment of each proton for compound **16**. ^{13}C NMR ($CDCl_3$) δ 16.37-16.53 (4CH_2**C**H$_3$), [25.71, 27.28 (2C), 27.62, 2C**Me**$_2$], 64.43-64.64 (4**C**H$_2$CH$_3$), [75.13, 76.33, 77.33 (3C), 77.77, inositol ring carbon], 111.53,

113.07 (2CMe2); ^{31}P NMR ($CDCl_3$) δ 0.11, 0.49; MS (FAB) *m/z* = 555 (M^++Na), 533 (M^++1).

Sodium Salt of DL-*myo*-Inositol 1,6-Bisphosphate (1e). To compound **16** (22 mg, 0.041 mmol) in chloroform (0.5 mL) was added excess TMS-Br (0.1 mL) at rt, and the mixture was stirred overnight. The solvent and excess reagent were evaporated and the residue dissolved in MeOH (3 mL), and then the solution treated with drops of water at 0 °C. The solution was slowly warmed up to rt, held at rt for an additional 2 h, concentrated, and the pH adjusted to 10 with NaOH. Lyophilization of the aqueous solution gave the sodium salt of **1e** in quantitative yield. ^{1}H NMR (D_2O, pH 10) δ 3.39 (t, J = 9.0 Hz, 1H, H-5), 3.47 (dd, J = 2.8, 10.1 Hz, 1H, H-3), 3.56 (t, J = 9.3 Hz, 1H, H-4), 3.80 (dt, J = 2.4, 7.6 Hz, 1H, H-1), 4.04 (q, J = 9.2 Hz, 1H, H-6), 4.28 (br s, 1H, H-2); ^{31}P NMR (D_2O, pH 10) δ 5.95, 7.54.

2-*O*-Benzoyl-1,6:3,4-di-*O*-isopropylidene-*myo*-inositol (18) and **DL-2-*O*-Benzoyl-1,6:4,5-di-*O*-isopropylidene-*myo*-inositol (19).** Treatment of **17**[12] (500 mg, 1.8 mmol) with 2,2-dimethoxypropane (1 mL, 10 mmol) or 2-methoxypropene (1 mL, 8.1 mmol) and TSA (100 mg) in DMF (10 mL) at rt for 24 h, gave a mixture consisting of almost equal amounts of **18** and **19** (by TLC and ^{1}H NMR analysis). After the usual extractive work-up, column chromatography of the mixture on silica gel (EtOAc-hexane gradient) afforded the pure isomers. **18**: R_f 0.45 (EtOAc-hexane = 1 : 1); mp 181-183 °C (from ether-hexane); ^{1}H NMR ($CDCl_3$) δ 1.40, 1.48 (2s, 12H, 2C**Me**$_2$), 2.97 (d, J = 1.5 Hz, 1H, OH-5), 3.83 (dd, J = 2.1, 8.4 Hz, 2H, H-1 & H-3), 4.09-4.15 (m, 3H, H-4, H-5 & H-6), 6.06 (t, J = 2.1 Hz, 1H, H-2), 7.47-8.09 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ 27.00, 27.45 (2C**Me**$_2$), [65.16, 70.52, 77.39 (2C), 79.12 (2C), inositol ring carbon], 113.79 (2**C**Me2), 126.55-134.00 (Ph), 165.91 (Ph**C**O); MS (FAB) *m/z* = 365 (M^++1). **19**: R_f 0.50 (EtOAc-hexane = 1 : 1); mp 188-190 °C; ^{1}H NMR ($CDCl_3$) δ 1.39, 1.48, 1.51, 1.54 (4s, 12H, 2C**Me**2), 2.78 (d, J = 4.6 Hz, 1H, OH-3), 3.67 (dd, J = 2.0, 8.9 Hz, 1H, H-1), 3.74 (t, J = 9.3 Hz, 1H, H-5), 3.93 (t, J = 9.0 Hz, 1H, H-4), 4.24-4.30 (m, 2H, H-3 & H-6), 5.98 (t, J = 2.3 Hz, 1H, H-2), 7.46-8.07 (m, 5H, Ph), H-H COSY spectra gave a definitive assignment of each proton for compound **19**. ^{13}C NMR ($CDCl_3$) δ 26.95, 27.32, 27.36, 27.47 (2C**Me**$_2$), 70.28, 70.76, 75.36, 76.82, 78.87, 80.78 (inositol ring carbon),

113.63, 114.20 ($2\mathbf{C}Me_2$), 129.23-134.18 (Ph), 166.98 (PhCO); MS (FAB) *m/z* = 365 (M^++1).

1,6:3,4-Di-*O*-isopropylidene-*myo*-inositol (20). A mixture of **18** (100 mg) and NaOMe (20 mg) in MeOH (10 mL) was heated at reflux for 1h. After cooling, the solution was concentrated under reduced pressure. After addition of dichloromethane, the reaction mixture was filtered through silica gel. The filtrate was concentrated and washed with hexane to give a solid product **20** in quantitative yield. Rf 0.2 (EtOAc-hexane = 1 : 1); mp 212-214 °C; ^{1}H NMR ($CDCl_3$) δ 1.49, 1.51 (2s, 12H, $2C\mathbf{Me}_2$), 2.29, 2.46 (2s, 2H, OH-2 & OH-5), 3.62 (br d, J = 6.1 Hz, 2H, H-1 & H-3), 4.06-4.08 (m, 3H, H-4, H-5 & H-6), 4.61 (br s, 1H, H-2); ^{13}C NMR (DMSO-d6) δ 27.50, 27.85 ($2C\mathbf{Me}_2$), [63.12, 69.63, 78.72 (2C), 78.78 (2C), inositol ring carbon], 111.46 ($2\mathbf{C}Me_2$).

1,6:3,4-Di-*O*-isopropylidene-*myo*-inositol 2,5-Bis(diethyl phosphate) (21). Compound **20** (22 mg, 0.083 mmol) was phosphorylated by the same procedure as described for **16** to give **21** as an oil (36 mg, 82% yield). R_f 0.25 (EtOAc-CH_2Cl_2 = 1 : 5); ^{1}H NMR ($CDCl_3$) δ 1.23-1.44 (m, 24H, $2C\mathbf{Me}_2$ & $4CH_2C\mathbf{H}_3$), 3.66 (br d, J = 9.5 Hz, 2H, H-1 & H-3), 4.07 (app t, J = 9.5 Hz, 2H, H-4 & H-6), 4.13-4.24 (m, 8H, $4C\mathbf{H}_2CH_3$), 4.67 (app q, J = 8.9 Hz, 1H, H-5), 5.22 (br d, J = 8.3 Hz, 1H, H-2); ^{13}C NMR ($CDCl_3$) δ 16.28-16.49 ($4CH_2\mathbf{C}H_3$), 26.70, 27.22 ($2C\mathbf{Me}_2$), 64.28-64.35 ($4\mathbf{C}H_2CH_3$), [68.92, 74.93, 76.76 (2C), 77.12 (2C), inositol ring carbon], 111.36 ($2\mathbf{C}Me_2$); ^{31}P NMR ($CDCl_3$) δ 0.25, 0.92; MS (FAB) *m/z* = 555 (M^++Na), 533 (M^++1).

Sodium Salt of *myo*-Inositol 2,5-Bisphosphate (1g). Deprotection of **21** (23 mg, 0.042 mmol) according to the procedures described for **1e** gave **1g** in quantitative yield. ^{1}H NMR (D_2O, pH 10) δ 3.43 (br d, J = 9.6 Hz, 2H, H-1 & H-3), 3.68 (m, 3H, H-4, H-5 & H-6), 4.37 (app td, J = 2.4, 7.4 Hz, 1H, H-2); ^{31}P NMR (D_2O, pH 10) δ 7.04, 7.34.

4,5,6-Tri-*O*-benzoyl-*myo*-inositol 1,3-Bis(diethyl phosphate) (23). To a solution of compound **22**[10] (110 mg, 0.22 mmol) in DMF (5 mL) at -42 °C were added dropwise diisopropylethylamine (1 mL, 5.74 mmol) and then diethyl chlorophosphite (0.1 mL, 0.69 mmol) with vigorous stirring. After 20 min, the reaction mixture was allowed to slowly warm to rt, and stirred for 1 h. The mixture was cooled in an ice bath and sodium phosphate buffer (1 M, pH 7, 5 mL) and excess 30% H_2O_2 (5 mL) were added. Upon standing overnight at rt, a solid was precipitated from the reaction mixture, which was

filtered using a vacuum apparatus, washed with water thoroughly, then dried to give pure product **23** (86 mg, 51% yield). ^{1}H NMR ($CDCl_3$) δ 0.98-1.27 (m, 12H, $4CH_2CH_3$), 3.46 (s, 1H, OH-2), 3.81-4.08 (m, 8H, $4CH_2CH_3$), 4.67-4.75 (m, 3H, H-1, H-2 & H-3), 5.68 (t, J = 10.6 Hz, 1H, H-5), 6.10 (t, J = 10.0 Hz, 2H, H-4 & H-6), 7.21-7.95 (m, 15H, 3Ph); ^{13}C NMR ($CDCl_3$) δ 16.23-16.59 ($4CH_2CH_3$), 64.94-65.13 ($4CH_2CH_3$), 70.62, 71.06 (2C), 71.35, 76.01 (inositol ring carbon), 128.88-133.83 (3Ph), [165.96 (2C), 166.17, 3PhCO]; ^{31}P NMR ($CDCl_3$) δ -1.56 (2P); MS (FAB) *m/z* = 787 (M^-+Na), 765 (M^-+1).

Sodium Salt of *myo*-Inositol 1,3-Bisphosphate (1b). Compound **23** (23 mg, 0.030 mmol) was deprotected by the same procedure as described for **1a** to give the sodium salt of **1b** in quantitative yield. ^{1}H NMR (D_2O, pH 10) δ 3.33 (t, J = 9.3 Hz, 1H, H-5), 3.70 (t, J = 9.4 Hz, 2H, H-4 & H-6), 3.88 (br t, J = 9.3 Hz, 2H, H-1 & H-3), 4.21 (br s, 1H, H-2); ^{31}P NMR (D_2O, pH 10) δ 6.83.

DL-2,3,6-Tri-*O*-benzoyl-*myo*-inositol 1,4-Bis(diethyl phosphate) (25). Compound **24**[10] was phosphorylated by the same procedure as described for **23** to give **25** in 54% yield: mp 156-158 °C; ^{1}H NMR ($CDCl_3$) δ 0.81-1.26 (m, 12H, $4CH_2CH_3$), 3.62-4.01 (m, 8H, $4CH_2CH_3$), 4.06 (dt, J = 1.9, 8.7 Hz, 1H, H-5), 4.24 (d, J = 1.9 Hz, 1H, OH-5), 4.93 (q, J = 10.0 Hz, 1H, H-4), 4.99 (dt, J = 2.5, 9.3 Hz, 1H, H-1), 5.46 (dd, J = 2.5, 10.0 Hz, 1H, H-3), 5.90 (t, J = 10.0 Hz, 1H, H-6), 6.15 (t, J = 2.5 Hz, 1H, H-2), 7.32-8.17 (m, 15H, 3Ph); ^{13}C NMR ($CDCl_3$) δ 15.28-15.83 ($4CH_2CH_3$), 64.15-64.70 ($4CH_2CH_3$), 69.83, 70.07, 72.31, 72.64, 73.06, 78.39 (inositol ring carbon), 128.34-133.63 (3Ph), 165.08, 165.17, 165.96 (3PhCO); ^{31}P NMR ($CDCl_3$) δ -0.87, 0.59; MS (FAB) *m/z* = 787 (M^++Na), 765 (M^++1).

Sodium Salt of DL-*myo*-Inositol 1,4-Bisphosphate (1c). Compound **25** (20 mg, 0.026 mmol) was deprotected by the same procedure for **1a** to give sodium salt of **1c** in quantitative yield. ^{1}H NMR (D_2O, pH 10) δ 3.47 (t, J = 9.3 Hz, 1H, H-5), 3.67 (dd, J = 2.7, 9.6 Hz, 1H, H-3), 3.80 (dd, J = 9.3, 9.6 Hz, 1H, H-6), 3.94 (ddd, J = 2.7, 8.7, 9.6 Hz, 1H, H-1), 4.15 (ddd, J = 8.0, 9.3, 9.6 Hz, 1H, H-4), 4.24 (t, J = 2.7 Hz, 1H, H-2); ^{13}C-NMR (D_2O, pH 10) δ 70.60, 71.05, 71.49, 73.55, 75.96, 76.94; ^{31}P NMR (D_2O, pH 10) δ 6.80, 7.29.

DL-1,5,6-Tri-*O*-benzoyl-*myo*-inositol 2,4-Bis(diethyl phosphate) (27). Compound **26**[10] was phosphorylated by the same procedure as described for **23** to give **27** in 46% yield: mp 187-189 °C; ^{1}H NMR ($CDCl_3$) δ 0.89-1.33 (m, 12H, $4CH_2CH_3$), 3.76-4.25 (m, 9H, $4CH_2CH_3$ & H-3), 4.72 (d, J = 5.0 Hz, 1H, OH-3), 4.95 (q, J = 9.3 Hz, 1H, H-4), 5.27 (td, J = 2.5, 8.7 Hz, 1H, H-2), 5.37 (td, J = 2.5, 10.6 Hz, 1H, H-1), 5.74 (t, J = 9.4 Hz, 1H, H-5), 6.11 (t, J = 10.0 Hz, 1H, H-6), 7.25-8.02 (m, 15H, 3Ph); ^{13}C NMR ($CDCl_3$) 16.13-16.17 ($4CH_2CH_3$), 64.88-65.55 ($4CH_2CH_3$), 70.21, 70.84, 71.35, 71.96, 76.96, 78.57 (inositol ring carbon), 129.03-134.12 (3Ph), [166.23 (2C), 166.27, 3PhCO]; ^{31}P NMR ($CDCl_3$) δ -0.03, 0.64; MS (FAB) *m/z* = 787 (M^++Na), 765 (M^++1).

Sodium Salt of DL-*myo*-Inositol 2,4-Bisphosphate (1f). Compound **27** (21 mg, 0.027 mmol) was deprotected by the same procedure as described for **1a** to give the sodium salt of **1f** in quantitative yield. ^{1}H NMR (D_2O, pH 10) δ 3.29-3.35 (m, 2H, H-1 & H-5), 3.48 (br d, J = 9.6 Hz, 1H, H-3), 3.68 (t, J = 9.7 Hz, 1H, H-6), 4.05 (q, J = 9.0 Hz, 1H, H-4), 4.38 (br d, J = 7.2 Hz, 1H, H-2); ^{31}P NMR (D_2O, pH 10) δ 7.22, 7.26.

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