Synthesis of 2-Substituted *myo*-Inositol 1,3,4,5-Tetrakis(phosphate) and 1,3,4,5,6-Pentakis(phosphate) Analogs

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Some biologically interesting inositol poly(phosphate) derivatives, 2-*O*-(*p*-aminobenzoyl)-*myo*-inositol 1,3,4,5-tetrakis(phosphate), 2-*O*-(4-aminocyclohexylcarbonyl)-*myo*-inositol 1,3,4,5-tetrakis(phosphate), *myo*-inositol 1,3,4,5,6-pentakis(phosphate), and its 2-substituted analogs, were synthesized. Inositol tetrakis(phosphate) and pentakis(phosphate) affinity columns were also prepared. The inositol 1,3,4,5-tetrakis(phosphate) affinity column was found to be effetive in isolating the binding proteins from bovine cardiac membranes.

myo-Inositol 1,3,4,5-tetrakis(phosphate), a metabolite from the specific 3-kinase phosphorylation of the second messenger inositol 1,4,5-tris(phosphate),[1] was found to take part in a cellular signaling process, such as regulating Ca^{2+} homoeostasis[2] and activating the K^+ channel.[3] Biological interest in inositol 1,3,4,5-tetrakis(phosphate) has resulted in much attention being given to chemical synthesis.[4] In order to clarify what structural features of inositol phosphates are essential for activities, it is important to build up structure-activity profiles which describe the interaction of inositol phosphate with the receptor and metabolic enzymes by a chemical modification of the molecule.

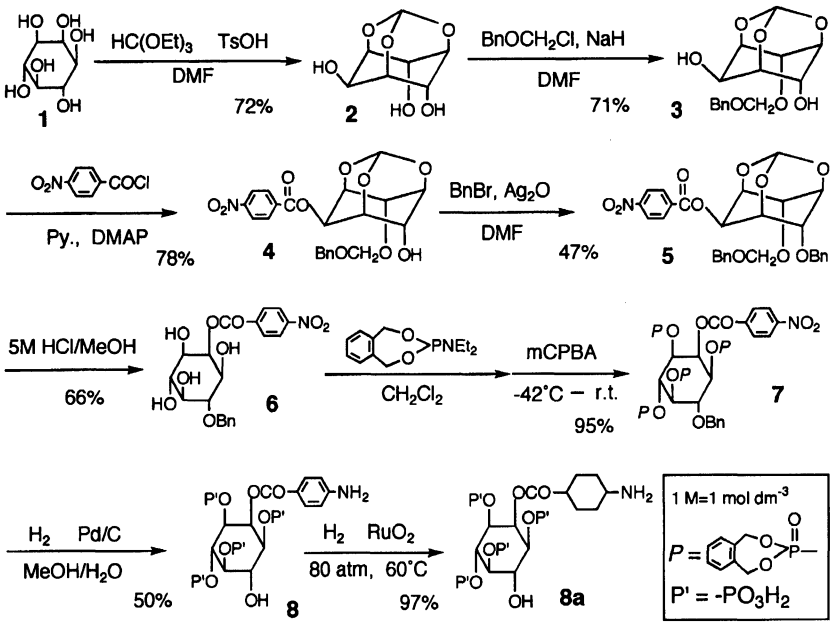
Along this line, we have synthesized 2-*O*-substituted inositol 1,3,4,5-tetrakis(phosphate) analogs, 2-*O*-(*p*-aminobenzoyl)inositol 1,3,4,5-tetrakis(phosphate) and 2-*O*-(4-aminocyclohexylcarbonyl)inositol 1,3,4,5-tetrakis(phosphate). In our previous papers, we reported on the biological behavior of these two analogs.[5] They had different effects on the binding, 5-phosphatase and 3-phosphatase activities from inositol 1,3,4,5-tetrakis(phosphate). The 2-*O*-(*p*-aminobenzoyl) analog was more potent than inositol 1,3,4,5-tetrakis(phosphate). These results provided important information concerning the binding of inositol 1,3,4,5-tetrakis(phosphate) and the structural features of the active sites of metabolic enzymes.[5] The specific recognition of the inositol phosphate derivative by certain enzymes can be used to either isolate or purify these enzymes, as shown in our preparation of an inositol 1,4,5-tris(phosphate) affinity column.[6] Thus, an inositol 1,3,4,5-tetrakis(phosphate) affinity column was also prepared by coupling of *p*-aminobenzoylated analog with a supporting matrix. Regarding another biologically interesting metabolite, inositol 1,3,4,5,6-pentakis(phosphate),[7] its 2-*O*-substituted analogs and affinity column were also synthesized. In this paper we describe all of the syntheses.

Results and Discussion

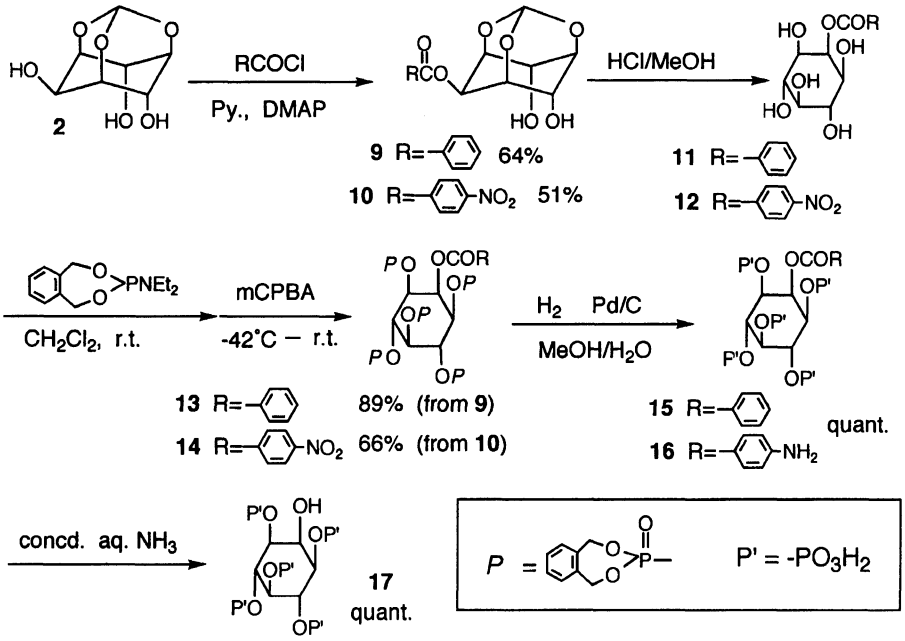
The syntheses of inositol 1,3,4,5-tetrakis(phosphate) analogs are shown in Scheme 1. *myo*-Inositol 1,3,5-orthoformate (**2**), which is easily available by one step from inositol,[8] was selectively protected at the C-4 position when it was treated with benzyloxymethyl chloride in the presence of NaH.[9] Then, the hydroxyl group at the C-2 position was benzoylated when **3** was subjected to benzoylation with *p*-nitrobenzoyl chloride. The treatment of **4** with benzyl bromide in the presence of silver(I) oxide gave fully protected inositol **5**. The hydrolysis of **5** under acidic conditions afforded the key intermediate **6**. Phosphorylation of **6** and deprotection were accomplished using a method developed in our laboratory[10] to give 2-*O*-(*p*-amino)benzoylinositol 1,3,4,5-tetrakis(phosphate) (**8**). The benzene ring of **8** was reduced by ruthenium(IV) oxide-catalyzed hydrogenation in an autoclave (80 atm) to give another analog, 2-*O*-(4-amino-cyclohexylcarbonyl)inositol 1,3,4,5-tetrakis(phosphate) (**8a**).

For the synthesis of inositol 1,3,4,5,6-pentakis(phosphate) and its analogs, **2** was used as the starting material (Scheme 2). The hydroxyl group at the C-2 position was benzoylated or *p*-nitrobenzoylated preferentially when **2** reacted with benzoyl or *p*-nitrobenzoyl chloride. The hydrolysis of orthoformate of **9** proceeded smoothly in 4 mol dm^{-3} HCl/MeOH. However, in the case of **10**, when it was treated in the same HCl solution, the *p*-nitrobenzoyl group migrated because of the effect of a strong electron-withdrawing group ($-NO_2$). A good result was obtained when 1 mol dm^{-3} HCl/MeOH was used. Intermediates **11** and **12** were subjected to phosphorylation and deprotection in the same way as in Scheme 1 without purification to give 2-*O*-substituted inositol 1,3,4,5,6-pentakis(phosphate) analogs **15** and **16**. Finally, the benzoyl group of **15** was hydrolyzed in concentrated aqueous ammonia to afford inositol 1,3,4,5,6-pentakis(phosphate) (**17**). Because being activated by poly(phosphate) groups, the benzoates at the C-2 position of **15** and **16** are sensitive to alkaline hydrolysis. When they were subjected to cellulose chromatography with an eluent containing 40% of concentrated aqueous ammonia (*n*-PrOH/aq $NH_3/H_2O=5/4/1$), a mixture containing 25% hydrolyzed product **17** was obtained. An eluent containing less than 3% of concentrated aqueous ammonia is recommended.

Activated CH-Sepharose 4B was selected as the supporting matrix for the preparation of an inositol phos-



Scheme 1.



Scheme 2.

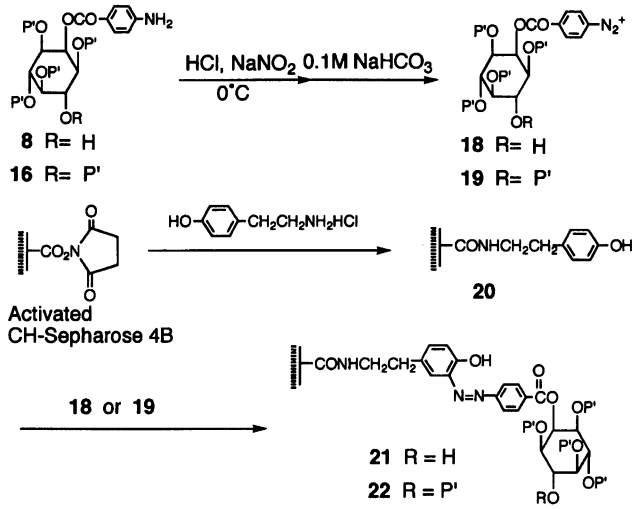
phate affinity column (Scheme 3).[6] It was pretreated with tyramine hydrochloride in a coupling medium (0.1 mol dm^{-3} $NaHCO_3$) to give **20**. In order to connect them with the supporting matrix **20**, 2-*O*-(*p*-aminobenzoyl)inositol tetrakis(phosphate) and pentakis(phosphate) analogs (**8** and **16**) were subjected to diazotization to give **18** and **19**. They were then used to couple with **20** in situ to give inositol tetrakis(phosphate) and pentakis(phosphate) affinity-column absorbents **21** and **22**.

As mentioned at the beginning of this article, the synthetic inositol 1,3,4,5-tetrakis(phosphate) analogs have created much biological interest; the results helped us to understand the structural features of metabolic enzymes. The application of an inositol tetrakisphosphate affinity column is promising.[5b,6] As shown in Fig. 1, the specific binding activity for [^{3}H]-inositol 1,3,4,5-tetrakis(phosphate) was retained on the affinity column and was eluted by increasing the salt concentration.

A biological study of inositol 1,3,4,5,6-pentakis(phosphate) and its analogs and the application of an affinity column are under investigation. The results will be reported elsewhere.

Experimental

All of the solvents and reagents used were of reagent



Scheme 3.

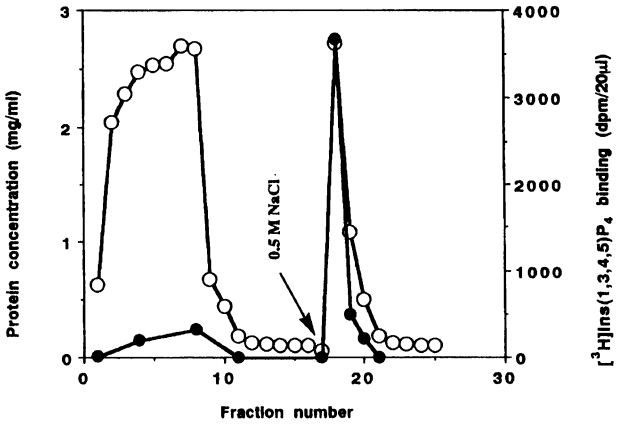


Fig. 1. Bovine cardiac membrane fraction was extracted with 1% Triton X-100 in a solution containing 50 mM NaCl, 10 mM Hepes buffer (pH 7.2) and 10 mM 2-mercaptoethanol. The extract was applied to a DEAE-anion exchange column equilibrated with the same buffer. High [^{3}H]inositol 1,3,4,5-tetrakis(phosphate) binding activities eluted from the column by a 0.2 M NaCl solution were then applied to a heparin affinity column after dialysis against the initial buffer as described. Fractions from a heparin column eluted by a 0.3 M NaCl solution were applied to an inositol 1,3,4,5-tetrakis(phosphate)-affinity column (1 M=1 mol dm^{-3}). At fraction 17, a 0.5 M NaCl solution was applied to the column to elute the adsorbed proteins. Each fraction was assayed for its protein concentration (○) or [^{3}H] inositol 1,3,4,5-tetrakis(phosphate) binding (●).

grade; in cases where further purification was required, the standard procedures were followed.[11] Thin-layer silica-gel chromatography (silica TLC) and cellulose chromatography (cellulose TLC) were performed on precoated silica-gel 60-F254 plates (E. Merck, Darmstadt) and cellulose powder plate Funaseru SF (Funakoshi), respectively. Silica gel (300—200 mesh, Wakogel C-300) was used for silica-gel chromatography, cellulose powder (Whatman CC-31) was used for cellulose chromatography, and the ratio of silica gel (cellulose powder) to the compound was in the range of 30:1—100:1. Organic solvents were removed on a rotary evaporator under the vacuum of a water aspirator with a bath temperature of 40 °C or lower. Elemental analyses were performed by the Advanced Center for the Chemical Analysis of Ehime University. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 270 MHz (JEOL GSX-270) with tetramethylsilane (δ=0 in $CDCl_3$) as an internal standard. IR spectra were recorded on a Hitachi EPI G-3 spectrometer. The melting points were recorded on a Yanaco melting-point apparatus and are uncorrected.

***myo*-Inositol 1,3,5-Orthoformate (2).** Compound **2** was synthesized by a modified procedure.[8a] To a solution of *myo*-inositol (22 g, 0.12 mol) in DMF (250 ml) were added triethyl orthoformate (36 ml, 0.22 mol) and *p*-toluenesulfonic acid monohydrate (6.0 g, 32 mmol). The mixture was heated to reflux. The formed ethanol was distilled off during heating, and heating was continued for 13 h. After being cooled to room temperature, a 10% $NaHCO_3$ solution (40 ml) was added and stirred for 30 min. The formed solid was removed by filtration. The solvent was distilled under reduced pressure by an aspirator to dryness. Methanol was added in order to extract the product, and the residual solid was removed by filtration once more. Finally, after methanol was evaporated the residual solid was recrystallized from MeOH–chloroform to give **2** as crystals (16.6 g, 72%). R_f 0.23 (acetone/hexane=1:1); mp 282—285 °C, (lit,[8b] mp 300—302 °C); ^{1}H NMR ($CD_3OD/CDCl_3$=1/9, v/v) δ=4.17 (3H, m), 4.22 (1H, m), 4.55 (2H, t, J=3.9 Hz), 5.5 (1H, d, J=1.2 Hz). Found: C, 43.93; H, 5.31%. Calcd for $C_7H_{10}O_6$: C, 44.21; H, 5.31%.

4-*O*-Benzyloxymethyl-*myo*-inositol 1,3,5-Orthoformate (3). To a solution of **2** (50 mg, 0.26 mmol) in DMF (2 ml) was added sodium hydride (12 mg, 0.29 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min; benzyloxymethyl chloride (40 μl, 0.29 mmol) was then added to reaction mixture at 0 °C, and stirring was continued at room temperature for 2 h. The reaction was quenched by the addition of water. An aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with brine. After the solvent was evaporated, the residue was chromatographed (SiO_2, AcOEt/Hexane=9/1) to give **3** (55 mg, 71%). Mp 96—97 °C, R_f 0.41 (AcOEt/Hexane=10/1), ^{1}H NMR ($CDCl_3$), δ=3.12 (1H, d, J=11.8 Hz, OH), 3.48 (1H, d, J=9.8 Hz, OH), 4.06 (1H, m), 4.23 (3H, m), 4.48 (1H, m), 4.62 (1H, m), 4.63 (2H, s), 4.87 (2H, s), 5.45 (1H, d, J=1.3 Hz), 7.35 (5H, m, aromatic); IR (Nujol) 3200, 1270, 1220, 1150, 1070, and 980 cm^{-1}. Found: C, 58.14; H, 5.86%. Calcd for $C_{15}H_{18}O_7$: C, 58.06; H, 5.85%.

2-*O*-(*p*-Nitrobenzoyl)-4-*O*-benzyloxymethyl-*myo*-inositol 1,3,5-Orthoformate (4). To a solution of **3** (652 mg, 2.20 mmol) in pyridine were added *p*-nitrobenzoyl chloride (120 mg, 2.24 mmol) and catalytic amount of 4-dimethylaminopyridine. The mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of water. An aqueous layer was extracted with ether. The combined organic layer was washed with a saturated $KHSO_4$, $NaHCO_3$ solution, and brine. After the solvent was evaporated, the residue was subjected to silica-gel chromatography (AcOEt/Hexane=1/2) to give **4** (764 mg, 78%). R_f 0.45 (AcOEt/Hexane=1/1), ^{1}H NMR ($CDCl_3$) δ=3.52 (1H, d, J=8.8 Hz, OH), 4.05—4.95 (9H, m), 5.45

(2H, m), 7.23 (5H, m, aromatic), 8.20 (4H, m, aromatic); IR (Nujol) 3420, 1700, 1590, 1510, 1255, 1150, 1080, 1035, and 990 cm^{-1}. Found: C, 57.72; H, 4.92; N, 2.69%. Calcd for $C_{22}H_{21}NO_{10}$: C, 57.54; H, 4.61; N, 3.05%.

2-*O*-(*p*-Nitrobenzoyl)-4-*O*-benzyloxymethyl-6-*O*-benzyl-*myo*-inositol 1,3,5-Orthoformate (5). To a solution of **4** (854 mg, 1.92 mmol) in DMF were added Ag_2O (2.22 g 9.59 mmol) and benzyl bromide (2.28 ml, 19.2 mmol). The mixture was stirred at room temperature overnight. The reaction mixture was dissolved in ethyl acetate and washed with water, 1 mol dm^{-3} HCl, a saturated $NaHCO_3$ solution, and brine. After the solvent was evaporated, the residue was chromatographed (SiO_2, Et_2O/Hexane=1/4) to give **5** (480 mg, 47%). R_f 0.6 (AcOEt/Hexane=1/2), ^1H NMR ($CDCl_3$) δ=4.10—4.70 (10H, m), 5.35 (3H, m), 7.20 (10H, m, aromatic), 8.05 (4H, m, aromatic). Found: C, 63.27; H, 5.03; N, 2.48%. Calcd for $C_{29}H_{27}NO_{10}$: C, 63.37; H, 4.96; N, 2.55%.

2-*O*-(*p*-Nitrobenzoyl)-6-*O*-benzyl-*myo*-inositol (6). Compound **5** (278 mg, 0.52 mmol) was dissolved in a minimum amount of CH_2Cl_2. To the solution was added 5 mol dm^{-3} HCl/MeOH (25 ml). The mixture was stirred at room temperature overnight. After the solvent was evaporated, the residue was chromatographed (SiO_2, AcOEt/CH_2Cl_2/MeOH=10/5/1) to give **6** 139 mg, 66%. R_f 0.18 (MeOH/CH_2Cl_2=1/10). ^1H NMR ($CDCl_3$+CD_3OD), δ=3.60—3.80 (5H, m, inositol), 5.20 (2H, s), 5.52 (1H, t, J=2.7 Hz), 7.12 (5H, m). 8.02 (4H, m).

2-*O*-(*p*-Nitrobenzoyl)-6-*O*-benzyl 1,3,4,5-Tetrakis-*O*-[(*o*-xylene-α,α'-diyldioxy)phosphoryl]-*myo*-inositol (7). To a suspension of **6** (127 mg, 0.31 mmol) in CH_2Cl_2 were added 1*H*-tetrazole (264 mg, 3.77 mmol) and *o*-xylene-α,α'-diyl *N,N*-diethylphosphoramidite (451 mg, 1.89 mmol). The mixture was stirred at room temperature for 30 min. Several drops of water were added in order to decompose any excess of the reagent. Then, *m*-chloroperbenzoic acid (542 mg, 2.51 mmol) was added at −42 °C and stirred at room temperature for another 30 min. An aqueous layer was extracted with CH_2Cl_2 and the combined organic layer was washed successively with 10% Na_2SO_3, sat. $NaHCO_3$ solution, and brine. After the solvent had evaporated, the residue was chromatographed (SiO_2, MeOH/CH_2Cl_2=1/30) to give **7** (342 mg, 95%). R_f 0.43 (MeOH/CH_2Cl_2=1/10), ^1H NMR ($CDCl_3$), δ=4.30—5.50 (24H, m), 7.00 (21H, m, aromatic), 8.00 (4H, m, aromatic); IR (Nujol) 1725, 1550, 1250, and 1000 cm^{-1}. Found: C, 53.24; H, 4.73; N, 0.87%. Calcd for $C_{52}H_{49}NO_{21}P_4 \cdot H_2O$: C, 53.56; H, 4.41; N, 1.20%.

2-*O*-(*p*-Aminobenzoyl)-*myo*-inositol 1,3,4,5-Tetrakis(phosphate) (8). To a suspension of **7** (272 mg, 0.24 mmol) in a mixed solvent (MeOH/H_2O=4/1) was added 5% Pd/C (270 mg). The mixture was stirred at room temperature under a hydrogen atmosphere overnight. After the catalyst was filtered out, the filtrate was evaporated, and the residue was subjected to cellulose chromatography (Whatman CC-31, *n*-PrOH/aq NH_3/H_2O=5/4/1) to give **8** (80 mg, 50%). R_f 0.17 (*n*-PrOH/aq NH_3/H_2O=5/4/1), ^1H NMR (D_2O) δ=3.93 (1H, dd, $J_{6\text{-}5}=J_{6\text{-}1}$=9.8 Hz, H-6), 4.07 (1H, ddd, $J_{5\text{-}6}=J_{5\text{-}4}=J_{H\text{-}P}$=9.8 Hz, H-5), 4.10 (1H, ddd, $J_{1\text{-}6}=J_{H\text{-}P}$=9.8 Hz, $J_{1\text{-}2}$=2.4 Hz, H-1), 4.15 (1H, ddd, $J_{3\text{-}4}=J_{H\text{-}P}$=9.8 Hz, $J_{3\text{-}2}$=2.4 Hz, H-3), 4.39 (1H, ddd, $J_{4\text{-}5}=J_{4\text{-}3}=J_{H\text{-}P}$=9.8 Hz, H-4), 5.70 (1H, dd, $J_{2\text{-}1}=J_{2\text{-}3}$=2.4 Hz, H-2), 6.68 (2H, d, J=9.4 Hz, aromatic), 7.76 (2H, d, J=9.4 Hz, aromatic). Found: C, 21.45; H, 5.10; N, 9.46%. Calcd for $C_{13}H_{33}N_5O_{19}P_4 \cdot 2H_2O$: C, 21.58; H, 5.17; N, 9.68%.

2-*O*-(4-Aminocyclohexylcarbonyl)-*myo*-inositol 1,3,4,5-Tetrakis(phosphate) (8a). A mixture of **8** (15 mg, 0.02 mmol) and ruthenium oxide (22 mg, 0.16 mmol) in water (3.8 ml) and H_2 gas (80 atm) was charged in a 50 ml autoclave and heated at 60 °C for 2 h. The catalyst was filtered and the filtrate was evaporated. The residue was chromatographed on a cellulose column (*n*-PrOH/aq NH_3/H_2O=5/4/1) to give **8a** 14 mg, 97%, R_f 0.24 (*n*-PrOH/aq NH_3/H_2O=5/4/1). ^1H NMR (D_2O) δ=1.42—1.82 (8H, m), 2.76 (1H, m), 3.08 (1H, m), 3.72—4.32 (5H, m, inositol), 5.60 (1H, m). Found: C, 21.26; H, 6.02; N, 9.48%. Calcd for $C_{13}H_{39}N_5O_{19}P_4 \cdot 2H_2O$: C, 21.41; H, 5.94; N, 9.60%.

2-*O*-Benzoyl-*myo*-inositol 1,3,5-Orthoformate (9). To a solution of **2** (1.03 g, 5.40 mmol) in anhydrous pyridine (30 ml) were added benzoyl chloride (0.7 ml, 5.95 mmol) and a catalytic amount of 4-dimethylaminopyridine at 0 °C. The mixture was stirred at room temperature overnight. Ethyl acetate was added to the reaction system and washed with saturated $KHSO_4$, $NaHCO_3$ solution, and brine. After the solvent was removed, the residue was chromatographed (SiO_2, AcOEt/Hexane=1/2) to give product **9** (984 mg, 64%). R_f 0.13 (AcOEt/Hexane=1/2); mp 202—204 °C; ^1H NMR (CD_3OD/$CDCl_3$=1/9, v/v) δ=4.21 (3H, m), 4.30 (2H,m), 5.30 (2H, m), 7.21 (3H, m, aromatic), 7.58 (2H, m, aromatic). Found: C, 57.82; H, 4.93%. Calcd for $C_{14}H_{14}O_7$: C, 57.14; H, 4.81%.

2-*O*-(*p*-Nitrobenzoyl)-*myo*-inositol 1,3,5-Orthoformate (10). Compound **10** was obtained in the same way as **9** by using *p*-nitrobenzoyl chloride in a yield of 51%. R_f 0.36 (AcOEt/Hexane=1/1), mp 236—238 °C; ^1H NMR (DMSO−d_6/CD_3OD=1/9, v/v) δ=4.24 (1H, m), 4.39 (2H, m), 4.47 (2H, m), 5.57 (1H, s), 5.59 (1H, s), 8.36 (4H, m, aromatic). Found: C, 50.14; H, 4.07; N, 4.04%. Calcd for $C_{14}H_{13}NO_9$: C, 49.56; H, 3.87; N, 4.13%.

2-*O*-Benzoyl-*myo*-inositol (11). To a solution of **9** (120 mg, 0.41 mmol) in methanol (6 ml) was added concentrated hydrochloric acid (3 ml). The mixture was stirred at room temperature for 40 min. The solvent was evaporated to dryness under reduced pressure by an aspirator. The residue was dried under vacuum to give **11** quantitatively. R_f 0.5 ($CHCl_3$/MeOH=1/1), ^1H NMR (D_2O) δ=3.26 (1H, t, J=7.3 Hz, H-5), 3.66 (4H, m), 5.55 (1H, s, H-2), 7.41 (2H, t, J=7.6 Hz, aromatic), 7.56 (1H, t, J=7.6 Hz, aromatic), 7.91 (2H, d, J=7.6 Hz, aromatic).

2-*O*-(*p*-Nitrobenzoyl)-*myo*-inositol (12). A suspension of **10** (52 mg, 0.15 mmol) in 1 mol dm^{-3} HCl/MeOH was stirred at room temperature. The reaction was monitored by TLC. After the material had disappeared, the solvent was evaporated to dryness and the residue was dried under vacuum to give **12** quantitatively. R_f 0.43 ($CHCl_3$/MeOH=2/1), ^1H NMR (D_2O+DMSO-d_6) δ=3.60 (1H, m), 4.00 (4H, m), 5.95 (1H, s, H-2), 8.63 (2H, d, J=8.8 Hz, aromatic), 8.80 (2H, d, J=8.8 Hz, aromatic).

2-*O*-Benzoyl-1,3,4,5,6-pentakis-*O*-[(*o*-xylene-α,α'-diyldioxy)phosphoryl]-*myo*-inositol (13). To a suspension of **11** (50 mg, 0.18 mmol) and 1*H*-tetrazole (186 mg, 2.66 mmol) in CH_2Cl_2 (5 ml) was added *o*-xylene-α,α'-diyl *N,N*-diethyl phosphoramidite (345 mg, 1.44 mmol).

The mixture was stirred at room temperature for 5 h and then treated in the same way as **7** to give **13** (115 mg, 89%). R_f 0.5 (AcOEt/CH_2Cl_2/MeOH=20/10/1), ^{1}H NMR ($CD_3OD+CDCl_3$) δ=4.95—5.72 (25H, m), 6.39 (1H, m, H-2), 7.38 (20H, m, aromatic), 7.50 (2H, m, aromatic), 7.56 (1H, m, aromatic), 8.05 (2H, m, aromatic). Found: C, 52.61; H, 4.68%. Calcd for $C_{53}H_{51}O_{22}P_5$: C, 53.27; H, 4.31%.

2-*O*-(*p*-Nitrobenzoyl)-1,3,4,5,6-pentakis-*O*-[(*o*-xylene-α,α'-diyldioxy)phosphoryl]-*myo*-inositol (14). Compound **14** was obtained in the same way as **13** by using **12** (29 mg, 0.088 mmol) as material, 72 mg, 66%. R_f 0.33 ($CHCl_3$/acetone=6/4); mp 174—175 °C; ^{1}H NMR ($CDCl_3+CD_3OD$) δ=4.98—5.70 (25H, m), 6.41 (1H, t, J=2.4 Hz, H-2), 7.35 (20H, m,aromatic), 8.30 (4H, m, aromatic). Found; C, 49.56; H, 4.14; N, 0.92%. Calcd for $C_{53}H_{50}NO_{24}P_5\cdot 2H_2O$: C, 49.88; H, 4.27; N, 1.10%.

2-*O*-Benzoyl-*myo*-inositol 1,3,4,5,6-Pentakis(phosphate) (15). The same procedure as **8** was carried out. After the catalyst had been filtered out, the filtrate was evaporated to dryness. The residue was subjected to cellulose chromatography (Whatman CC-31, *n*-PrOH/aq NH_3/H_2O=11/0.5/8) to give **15** quantitatively. R_f 0.24 (*n*-PrOH/aq NH_3/H_2O=4.5/4.5/1) (cellulose TLC), ^{1}H NMR (D_2O) δ=4.11 (1H, ddd, $J_{5\text{-}4}=J_{5\text{-}6}=J_{H\text{-}P}$=9.5 Hz, H-5), 4.24 (2H, ddd, $J_{1\text{-}6}=J_{3\text{-}4}=J_{H\text{-}P}$=9.5 Hz, $J_{1\text{-}2}=J_{3\text{-}2}$=2.4 Hz, H-1, H-3), 4.45 (2H, ddd, $J_{4\text{-}3}=J_{4\text{-}5}=J_{6\text{-}5}=J_{6\text{-}1}=J_{H\text{-}P}$=9.5 Hz, H-4, H-6), 5.80 (1H, dd, $J_{2\text{-}1}=J_{2\text{-}3}$=2.4 Hz, H-2), 7.40 (2H, t, J=7.9 Hz, aromatic), 7.54 (1H, t, J=7.9 Hz, aromatic), 7.98 (2H, d, J=7.9 Hz, aromatic).

2-*O*-(*p*-Aminobenzoyl)-*myo*-inositol 1,3,4,5,6-Pentakis(phosphate) (16). Compound **16** was obtained in the same way as **15** from **14** (31 mg, 0.025 mmol) quantitatively. R_f 0.41 (*n*-PrOH/aq NH_3/H_2O=5.5/0.5/4) (cellulose TLC), ^{1}H NMR (D_2O) δ=4.09 (1H, ddd, $J_{5\text{-}4}=J_{5\text{-}6}=J_{H\text{-}P}$=9.5 Hz, H-5), 4.20 (2H, ddd, $J_{1\text{-}6}=J_{3\text{-}4}=J_{H\text{-}P}$=9.5 Hz, $J_{1\text{-}2}=J_{3\text{-}2}$=2.5 Hz, H-1, H-3), 4.43 (2H, ddd, $J_{4\text{-}3}=J_{4\text{-}5}=J_{6\text{-}5}=J_{6\text{-}1}=J_{H\text{-}P}$=9.5 Hz, H-4, H-6), 5.73 (1H, dd, $J_{2\text{-}1}=J_{2\text{-}3}$=2.5 Hz, H-2), 6.69 (2H, d, J=8.6 Hz, aromatic), 7.79 (2H, d, J=8.6 Hz, aromatic).

***myo*-Inositol 1,3,4,5,6-Pentakis(phosphate) (17).** To a suspension of **13** (72 mg, 0.061 mmol) in mixed solvent of methanol–water (4/1, v/v) was added 5% Pd/C (129 mg) at 0 °C. The mixture was stirred at room temperature under a hydrogen atmosphere overnight. After the catalyst had been filtered, the filtrate was evaporated to dryness. The residue was dissolved in concentrated aqueous ammonia and stirred at room temperature for 10 h. After the residual aqueous ammonia had been evaporated out, the residue was subjected to cellulose chromatography (Whatman CC-31, *n*-PrOH/aq NH_3/H_2O=5/4/1). The phosphate was finally transformed to potassium salt via a cation-exchange column to give **17** (42 mg, 90%). R_f 0.3 (*n*-PrOH/aq NH_3/H_2O=5/4/1) (cellulose TLC), ^{1}H NMR (D_2O) δ=4.00 (2H, ddd, $J_{1\text{-}6}=J_{3\text{-}4}=J_{H\text{-}P}$=9.5 Hz, $J_{1\text{-}2}=J_{3\text{-}2}$=2.7 Hz, H-1, H-3), 4.00 (1H, ddd, $J_{5\text{-}6}=J_{5\text{-}4}=J_{H\text{-}P}$=9.5 Hz, H-5), 4.23 (1H, dd, $J_{2\text{-}1}=J_{2\text{-}3}$=2.7 Hz, H-2), 4.30 (2H, ddd, $J_{4\text{-}3}=J_{4\text{-}5}=J_{6\text{-}5}=J_{6\text{-}1}=J_{H\text{-}P}$=9.5 Hz, H-4, H-6); ^{13}C NMR δ=71.3 (m, 1C), 74.9 (m, 2C), 76.8 (m, 2C), 78.2 (m, 1C). Found: C, 8.40; H, 2.24%. Calcd for $C_6H_{12}O_{21}P_5K_5\cdot 3H_2O$: C, 8.70; H, 2.18%.

Preparation of Affinity Column (22). Step 1. Pretreatment of Supporting Matrix. The supporting matrix (Activated CH-Sepharose 4B) (3.56 g) was swollen in 1 mmol dm^{-3} cool hydrochloric acid (0 °C) in 3G sintered glass filter for 15 min, and washed with the same hydrochloric acid (for 1 g of powder, 200 ml hydrochloric acid was used) under reduced pressure by an aspirator. Then, the swollen gel was washed with a coupling medium (0.1 mol dm^{-3} $NaHCO_3$, pH=8) twice and suspended in about 12 ml of the coupling medium.

To the suspension of swollen gel in the coupling medium was added tyramine (15 mg, 0.086 mmol); the mixture was then vibrated (vibrating mixer) at room temperature for 1 h. The gel was filtered and washed with the coupling medium 2—3 times. Then, the gel was suspended in the coupling medium once more for coupling (Step 3).

Step 2. Diazotization of 16. To a solution of **16** in small amount of water was added concentrated HCl (16 μl, 0.19 mmol) and $NaNO_2$ (4 mg, 0.06 mmol). The mixture was stirred at 0 °C for 30 min. The coupling medium was added to the reaction mixture so as to adjust the pH value to about 8. This solution was then used for coupling (Step 3).

Step 3. Coupling. To a suspension of the supporting matrix (Step 1) was added an inositol phosphate sample solution (Step 2); the mixture was vibrated at room temperature for 75 min. Gel was filtered and washed with a high-pH solution (0.05 mol dm^{-3} tris(hydroxymethyl)amino-methane, pH=8, 0.5 mol dm^{-3} NaCl) and a low-pH solution (0.05 mol dm^{-3} fomic acid, pH=4.0, 0.5 mol dm^{-3} NaCl) alternatively 4-5 times. The product (yellow gel) was kept in a refrigerator without drying.

An inositol 1,3,4,5-tetrakis(phosphate) affinity column (**21**) was prepared in the same way from **8**.

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