Design and synthesis of biotinylated inositol phosphates relevant to the biotin–avidin techniques†

Kensaku Anraku,[a] Teruhiko Inoue,[b] Kenji Sugimoto,[c] Takashi Morii,[c] Yasuo Mori,[d] Yoshinari Okamoto[e] and Masami Otsuka*[e]

Received 2nd January 2008, Accepted 7th March 2008
First published as an Advance Article on the web 1st April 2008
DOI: 10.1039/b719938d

Six bifunctional molecules containing biotin and various inositol phosphates were synthesized. These compounds were designed on the basis of X-ray structures of the complexes of D-*myo*-inositol 1,4,5-triphosphates (IP_3) and phospholipase C δ pleckstrin homology domain (PLCδ PH) considering the application to the biotin–avidin techniques. The building blocks of the inositol moiety were synthesized starting with optically resolved *myo*-inositol derivatives and assembled to the biotin linker through a phosphate linkage.

Introduction

D-*myo*-Inositol 1,4,5-triphosphate (IP_3) plays a key role in the signaling cascade that links extracellular messengers to intracellular Ca^{2+} mobilization.[1] Upon stimulation of a certain receptor, the associated G protein or tyrosine kinase activates a membrane-bound phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5-P_2) into two second messengers, IP_3 and diacylglycerol (DAG), bifurcating the signaling pathway. The hydrophilic IP_3 diffuses into the cytosol and activates the receptor of a Ca^{2+} channel on the endoplasmic reticulum, resulting in the release of Ca^{2+} from an internal store. Pleckstrin homology (PH) domains are structural modules of around 120 amino acids with sequence similarity to two regions in pleckstrin, the major protein kinase C substrate in platelets.[2–4] These domains are found in more than 100 different proteins, and appear to be important for membrane association of proteins involved in intracellular signaling and the cytoskeleton,[5–8] in some cases by binding specific phosphoinositides and their head groups.[9–11] As an example, PLCδ PH domain is an IP_3-binding region which sterospecifically recognizes and binds to PtdIns 4,5-P_2 with high affinity (the dissociation constant K_d value is 1.7 μM).[11] However, there have been a few reports on binding analysis of the inositol phosphate head groups and PH domains. Thus in order to study the relative affinity and specificity in the binding of inositol phosphates and diverse PH domains, we intend to prepare biotinylated inositol phosphates. The biotin–avidin techniques[12] would address the binding analysis of inositol phosphates and PH domains and the development of inositol phosphate mimetics that control PH domains, and search for novel PH domains without resort to radiolabels. We report here the synthesis of biotin derivatives of six different inositol phosphates, *i.e.*, D-1,4,5-IP_3, L-1,4,5-IP_3, D-2,4,5-IP_3, D-1-IP_1, L-1-IP_1, and 2-IP_1 (Fig. 1).

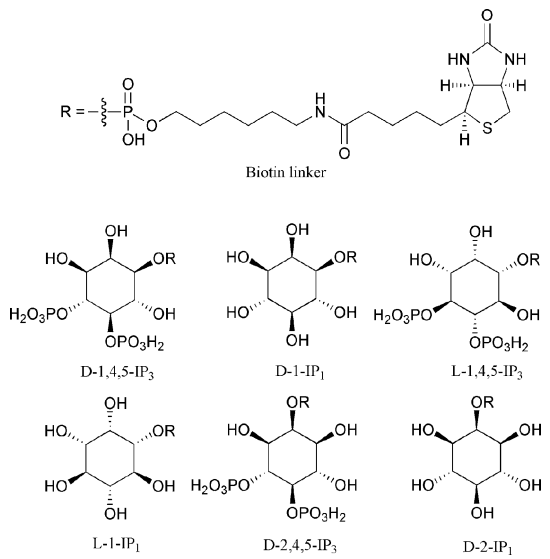


Fig. 1 Biotin derivatives of six different inositol phosphates.

Results and discussion

Fig. 2 shows the design and synthetic strategy of biotinylated inositol phosphates. The X-ray crystal structure of the IP_3–PLCδ PH domain complex[13] revealed that the inositol and 4,5-phosphate groups of IP_3 are accommodated in the binding pocket, therefore we thought that a biotin linker could be introduced at the 1-phosphate or 2-phosphate (the latter is an artificially-created derivative) of inositol without affecting the PH domain binding. Our synthetic strategy is to differentiate the six hydroxyl groups of D-*myo*-inositol through the optical resolution of diacetal intermediates[14] to obtain a suitably protected intermediate that could be coupled with the biotin linker by a bifunctional

[a]*Institute of Health Sciences, Kumamoto Health Science University, 325 Izumi-machi, Kumamoto 861-5598, Japan*
[b]*Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan*
[c]*Institute of Advanced Energy, Kyoto University, Uji, Kyoto 611-0011, Japan*
[d]*Laboratory of Molecular Biology, Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 615-8510, Japan*
[e]*Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan. E-mail: motsuka@gpo.Kumamoto-u.ac.jp; Fax: +81-96-371-4620; Tel: +81-96-371-4620*
† Electronic supplementary information (ESI) available: Further experimental and characterisation details. See DOI: 10.1039/b719938d

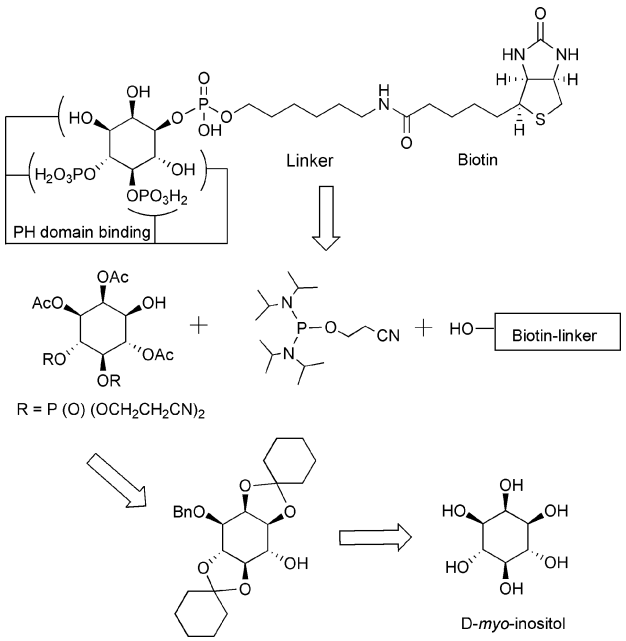
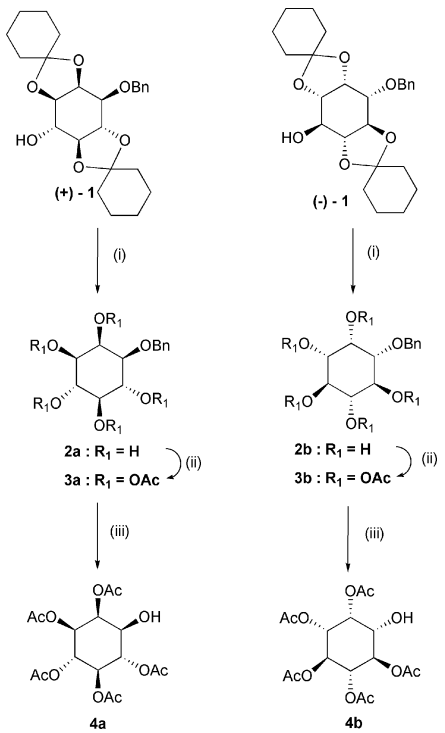


Fig. 2 Design and synthetic strategy of biotinylated D-*myo*-inositol 1,4,5-triphosphate.

phosphorylating agent.[15] As the target molecules have a sulfur in the biotin structure, our strategy does not end up with any benzyl or related protecting groups that require hydrogenolysis to remove them.

The syntheses of the D-1-IP_1 and L-1-IP_1 moieties were carried out as shown in Scheme 1. The optically resolved alcohol D-1-*O*-benzyl-2,3:5,6-di-*O*-cyclohexylidene-*myo*-inositol (+)-**1** was



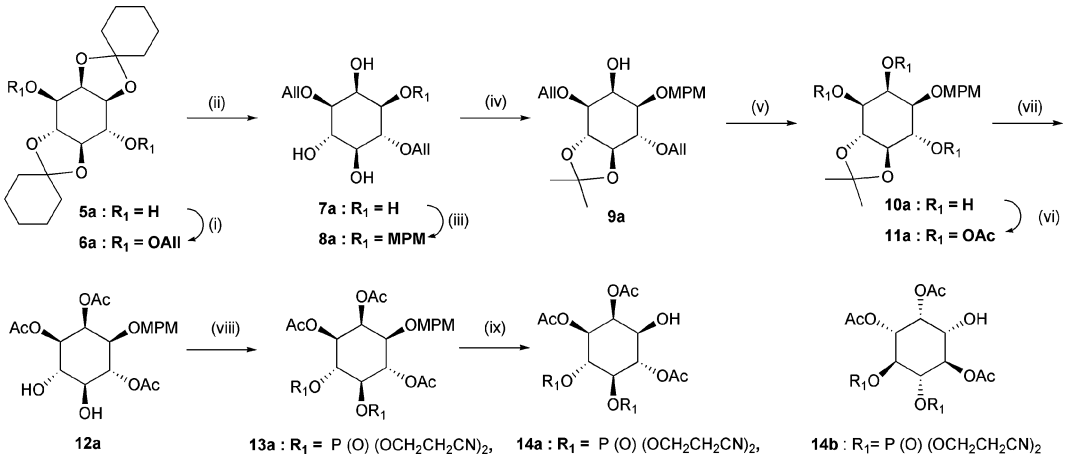
Scheme 1 *Reagents and conditions*: (i) TFA, MeOH, rt; (ii) Ac_2O, DMAP, Py, rt; iii) H_2/10% Pd-C, CH_3COOH, rt.

prepared as the starting material of the D-1-IP_1 moiety by the method of Billington *et al.*[14] Hydrolysis of the cyclohexylidene groups of (+)-**1** with acid provided **2a**, which was acetylated to give fully protected **3a**. Finally treatment with H_2/10% palladium-carbon gave the debenzylated D-1-IP_1 moiety **4a** in 73% yield (for 3 steps). The enantiomeric isomer L-1-*O*-benzyl-2,3:5,6-di-*O*-cyclohexylidene-*myo*-inositol (−)-**1** gave the L-1-IP_1 moiety **4b** by the same procedure.

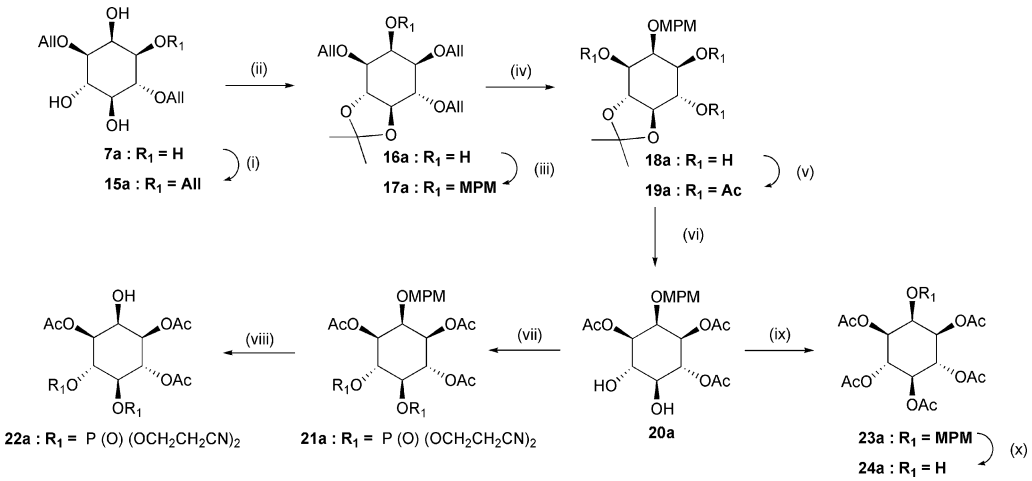
The syntheses of the D-1,4,5-IP_3 and L-1,4,5-IP_3 moieties were carried out as shown in Scheme 2. The starting material D-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol **5a** was prepared by hydrogenolysis of the benzyl group of (−)-**1**. Allylation of the resulting alcohol **5a** provided **6a**, which was further treated with *p*-toluenesulfonic acid and H_2O to give deprotected **7a** in 92% yield (for 2 steps). The *cis*-1,2-diol of **7a** was regioselectively *p*-methoxybenzylated by means of the dibutyltin oxide procedure.[16,17] Thus the tin complex of the 1,2-diol was regioselectively reacted with *p*-methoxybenzyl chloride in the presence of caesium fluoride to give **8a** exclusively in 97% yield. The introduction of isopropylidene acetal to the 4,5-vicinal alcohol under the usual conditions gave **9a** in 85% yield. Isomerization of the allyl groups of **9a** followed by the treatment with HgO, $HgCl_2$ gave **10a** in 67% yield.[18] Acetylation of the resulting alcohol provided **11a**, which was treated with *p*-toluenesulfonic acid and ethylene glycol to give the intermediate **12a** in 88% yield (for 2 steps), which is ready for the next phosphorylation. Bis(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite, considered one of the most effective phosphorylating agents, was prepared by the method of Uhlmann and Engels.[19] The 4,5-dihydroxy compound **12a** was converted to the 4,5-bisphosphonate with phosphoramidite and 1*H*-tetrazole and subsequent oxidation with MCPBA to afford **13a** in 93% yield. Oxidative cleavage of the *p*-methoxybenzyl group with CAN[20] gave the D-1,4,5-IP_3 moiety **14a** in 71% yield. The enantiomeric isomer D-2,3:5,6-di-*O*-cyclohexylidene-*myo*-inositol gave the L-1,4,5-IP_3 moiety **14b** by the same procedure.

D-2,4,5-IP_3 moiety was prepared from the intermediate **7a** as shown in Scheme 3. The regioselective allylation[16,17] at the 1-hydroxyl group of **7a** by the dibutyltin oxide method gave **15a** in 59% yield. Introduction of isopropylidene acetal to the 4,5-position of **15a** provided **16a**, which was further protected with a *p*-methoxybenzyl group at the 2-hydroxyl position to give fully protected **17a** in 66% yield (for 2 steps). Deprotection of the allyl groups[18] provided **18a**, which was acetylated to give **19a**. Finally the isopropylidene acetal was removed to afford **20a** in 42% yield (for 3 steps). Phosphorylation[19] of the 4,5-hydroxyl groups gave **21a**, which was treated with CAN[20] to give the D-2,4,5-IP_3 moiety **22a** in 59% yield (for 2 steps). The 2-hydroxy pentaacetate **24a** was prepared by exhaustive acetylation of **20a**, and removal of the *p*-methoxybenzyl group in 64% yield (for 2 steps).

The connection of inositol and biotin moieties prepared by the method of Pon[21] was carried out as shown in Scheme 4. The biotin-linker moiety was reacted with bifunctional phosphorylating agent (β-cyanoethyl)-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite[15] and 1*H*-tetrazole to give a rather labile phosphoramidite which was immediately condensed with inositol moiety **4a**, **14a**, **4b**, **14b**, **22a**, or **24a**. Subsequent oxidation of the condensation products with *tert*-BuOOH gave the fully protected biotinylated inositol phosphates **25a** (93% yield), **26a** (78% yield), **25b** (83% yield), **26b** (85% yield), **27** (99% yield), and **28** (52% yield). The ^{1}H NMR



Scheme 2 *Reagents and conditions*: (i) allyl-Br, NaH, DMF, rt; (ii) TsOH, THF–MeOH, reflux; (iii) (a) Bu_2SnO, toluene, reflux, 3 h; (b) CsF, MPM-Cl, DMF, −40 °C then rt; (iv) 2-methoxypropene, TsOH, DMF, rt; (v) (a) $(Ph_3P)_3RhCl$, DABCO, EtOH–benzene–H_2O, reflux, 5 h; (b) HgO, $HgCl_2$, acetone–H_2O, rt, 5 min; (vi) Ac_2O, DMAP, Py, rt; (vii) TsOH, ethylene glycol, CH_2Cl_2, rt, 10 min; (viii) (a) bis(β-cyanoethyl)-*N*,*N*-diisopropyl-phosphoramidite, 1*H*-tetrazole, CH_2Cl_2, rt, 1.5 h; (b) MCPBA, CH_2Cl_2, rt, 5 min; (ix) CAN, CH_3CN–H_2O, rt, 1 h.



Scheme 3 *Reagents and conditions*: (i) (a) Bu_2SnO, toluene, reflux, 3 h; (b) CsF, allyl-Br, DMF, −40 °C then rt; (ii) 2-methoxypropene, TsOH, DMF, rt; (iii) MPM-Cl, NaH, DMF, rt; (iv) (a) $(Ph_3P)_3RhCl$, DABCO, EtOH–benzene–H_2O, reflux, 5 h; (b) HgO, $HgCl_2$, acetone–H_2O, rt, 5 min; (v) Ac_2O, DMAP, Py, rt; (vi) TsOH, ethylene glycol, CH_2Cl_2, rt, 10 min; (vii) (a) bis(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite, 1*H*-tetrazole, CH_2Cl_2, rt, 1.5 h; (b) MCPBA, CH_2Cl_2, rt, 5 min; (viii) CAN, CH_3CN–H_2O, rt, 1 h; (ix) Ac_2O, DMAP, Py, rt; (x) H_2/10% Pd-C, CH_3COOH, rt.

and FABMS data of these compounds showed no oxidation of the biotin sulfide.

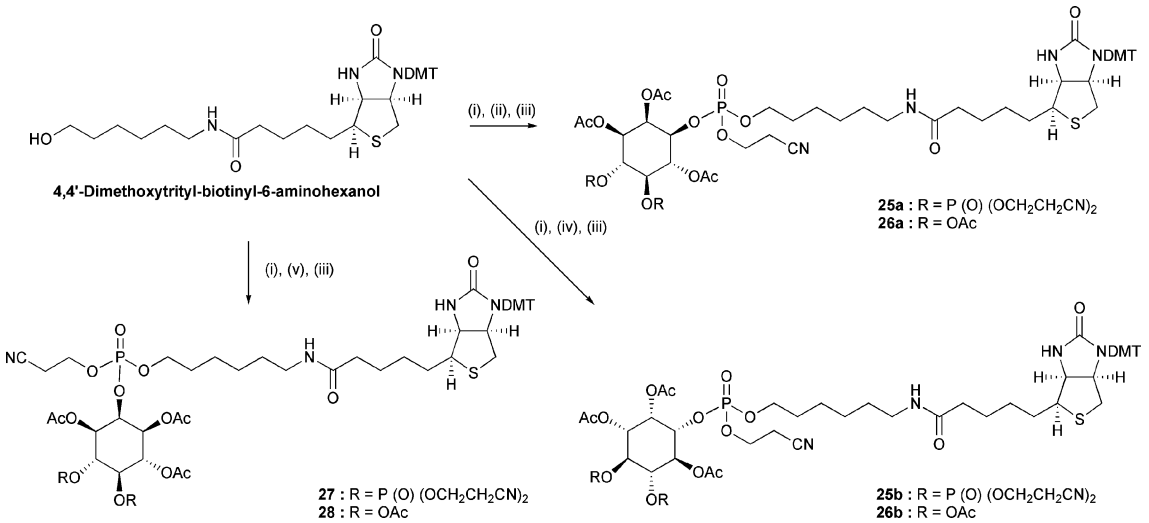
The final stage of the synthesis was carried out as shown in Scheme 5. After removal of trityl group of biotin with acid treatment, all protecting groups were removed in one step by reaction with NH_3 to give water-soluble biotinylated inositol phosphates. The biotinylated mono or polyphosphates were efficiently purified by anion-exchange chromatography with gradients of ammonium formate as eluent to give biotinylated D-1,4,5-IP_3 (32% yield), D-1-IP_1 (30% yield), L-1,4,5-IP_3 (35% yield), L-1-IP_1 (46% yield), D-2,4,5-IP_3 (32% yield), and D-2-IP_3 (66% yield) (for 2 steps).

Biological study of the synthetic biotinylated inositol phosphates using streptavidin beads was carried out. The original PLCδ PH domain bound efficiently to the prebound beads of biotinylated D-1,4,5-IP_3. The dissociation constant K_d of biotinylated D-1,4,5-IP_3 binding of the PLCδ PH domain was 250 ± 20 nM, which was comparable to that of non-tethered D-1,4,5-IP_3.[11] The detailed binding assay of the synthetic biotinylated inositol phosphates against the PLCδ PH domain or other PH domains will be reported elsewhere.

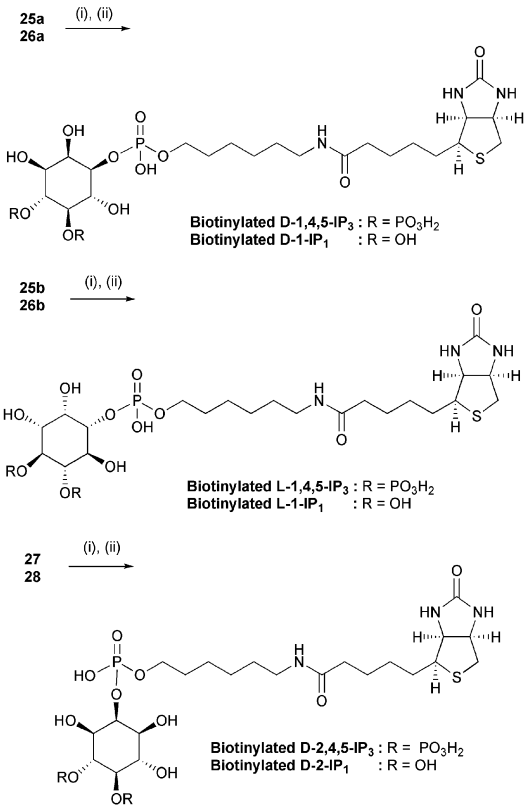
Exprimental

Materials and methods

Chemicals were purchased from Aldrich, Fluka, Kanto Chemical, Nacalai tesque, and Wako. Dehydrated CH_2Cl_2 was distilled from calcium hydride. Thin layer chromatography (TLC) was performed on precoated plates (Merck TLC sheets silica 60 F_{254}): products were visualized by spraying phosphomolybdic acid in EtOH, or basic potassium permanganate and heating at high temperature. Chromatography was carried out on Silica Gel 60 N (40–100 mesh). Anion exchange chromatography was performed using Dowex 1 × 8 (Cl^-, 50–100 mesh), eluting with ammonium formate containing formic acid. Column fractions containing biotinylated inositol polyphosphates were assayed for phosphate by



Scheme 4 *Reagents and conditions*: (i) (a) (β-cyanoethyl)-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite, 1*H*-tetrazole, CH_2Cl_2, rt, 1.5 h; (ii) **4a**, **14a**, 1*H*-tetrazole, CH_2Cl_2, rt, 2 h; (iii) *tert*-BuOOH, CH_2Cl_2, rt, 5 min; (iv) **4b** or **14b**, 1*H*-tetrazole, CH_2Cl_2, rt, 2 h; (v) **22a** or **24a**, 1*H*-tetrazole, CH_2Cl_2, rt, 2 h.



Scheme 5 *Reagents and conditions*: (i) TCA, CH_2Cl_2, rt, 1 h; (ii) aq. NH_3, MeOH, 55 °C, 10 h.

the Briggs test.[22] The optically resolved D-1-*O*-benzyl-2,3:5,6-di-*O*-cyclohexylidene-*myo*-inositol (+)-**1** and L-1-*O*-benzyl-2,3:5,6-di-*O*-cyclohexylidene-*myo*-inositol (−)-**1** were prepared by the method of Billington *et al.*[14] Treatment of the racemic **1** with (*R*)-camphanic acid chloride gave a mixture of the diastereomeric camphanate esters. Separation of the diastereomeric esters by recrystallization and chromatography, and subsequent hydrolysis of each ester gave the corresponding enatiomeric forms of the alcohol **1** in quantitative yield. $[\alpha]_D^{25} = +23.32$ and -23.16 (*c* 0.1, $CHCl_3$). (Diisopropylamino)dichlorophosphine was prepared by the method of Uhlmann and Engels.[19] by adding two equivalents of diisopropylamine to a solution of phosphorus trichloride in dry ethyl ether at −78 °C. The crude product was purified by distillation under reduced pressure and could be stored as a crystalline solid at −20 °C. Two equivalents of β-cyanoethanol in the presence of diisopropylethylamine were reacted with the purified product in dehydrated CH_2Cl_2, to afford bis(β-cyanoethyl)-*N*,*N*-diisopropylaminophosphoramidite. The biotin-linker moiety was prepared by the method of Pon[21] *N*-Hydroxysuccinimide-biotin was reacted with 6-amino-1-hexanol to give biotinyl-6-amino-1-hexanol, which was further converted into the dimethoxytrityl derivative by silylation of the OH group, N^1-protection with a dimethoxytrityl group, and removal of the *O*-silyl group. Specific rotations of enantiopure compounds were recorded by JASCO Dip-1000. NMR spectra (JEOL JNM-AL300MHz) were referenced to $SiMe_4$, or HDO. Infra-red spectra were recorded on a JASCO FT/IR-410. The samples were prepared as KBr discs, or thin films between sodium chloride discs. Microanalysis was carried out by Yanaco MT-5S. Mass spectra (EI) and high resolution mass spectra (HRMS) were recorded by a JEOL JMS-DX303HF. HRMS were recorded by using positive and negative fast atom bombardment (FAB) with 3-nitrobenzyl alcohol (NBA) (containing HMPA or not) as the matrix.

The dissociation constants (K_d) of biotinylated D-1,4,5-IP_3 binding of the original PLCδ PH domain were estimated based on SDS–acrylamide gel electrophoretic analysis. 1 ml of streptavidin beads which has binding capacity up to 85 nmol free biotin was incubated with 5 nmol biotinylated D-1,4,5-IP_3 solution for 6 h at 4 °C in 30 mM HEPES, 50 mM NaCl, 0.005% Tween20, 3 mM EDTA (pH 7.4). The beads were washed with the same buffer and divided up into five different volumes (100, 50, 25, 10, 0 μl) in separate tubes, which volumes of beads were converted to biotinylated D-1,4,5-IP_3 concentrations (1.00, 0.50, 0.25, 0.10, 0 μM), respectively. The divided beads were incubated with 500 μl

of the PLCδ PH domain (0.20 μM) for 10 min at 4 °C in the same buffer. The beads were separated from the supernatant (S, nonbinding fraction) by centrifugation at 1000 g for 1 min at room temperature, the PLCδ PH domain was eluted from the beads with 50 μM IP_3 to give the binding fraction (B). The nonbinding fraction (S) and B were precipitated by addition of an equal volume of 20% trichloroacetic acid and centrifugation, and were quantified by analysis of 15% SDS–polyacrylamide gel electrophoresis followed by CBB staining. The quantification of each band was performed by analyzing the scaled gel data with NIH image (version 1.6) software to integrate the intensity of the dots of which each band was composed. The binding fraction $Q = [B]/([B] + [S])$ for the PLCδ PH domain to biotinylated D-1,4,5-IP_3 was plotted against biotinylated D-1,4,5-IP_3 concentration. The K_d was derived from the best fit for a theoretical dissociation equation; $Q_{fit} = [\text{biotinylated D-1,4,5-IP}_3]/(K_d + [\text{biotinylated D-1,4,5-IP}_3])$, where [biotinylated D-1,4,5-IP_3] is the concentration of biotinylated D-1,4,5-IP_3.

D-1-*O*-Benzyl-*myo*-inositol (2a)

To a solution of D-1-*O*-benzyl-2,3:5,6-di-*O*-cyclohexylidene-*myo*-inositol (+)-**1** (861 mg, 2.0 mmol) in MeOH (30 ml) was added TFA (30 ml), and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated under reduced pressure, and the residue was washed with AcOEt to give **2a** (443 mg, 82%) as a white solid. ^{1}H NMR (CD_3OD) δ 3.03–3.22 (m, 3H), 3.52 (t, $J = 9.5$ Hz, 1H), 3.67 (t, $J = 9.5$ Hz, 1H), 4.01 (s, 1H), 4.55 (d, $J = 11.7$ Hz, 1H), 7.15–7.35 (m, 5H). ^{13}C NMR (CD_3OD) δ 70.8, 73.0, 73.2, 73.7, 74.1, 76.5, 81.1, 128.6, 129.1, 129.3, 139.9. IR (KBr) 3350, 2850, 1730, 1360, 1200, 1060, 1030 cm^{-1}. MS (EI) *m/z* 271 $(M + H)^+$. Anal. calcd for $C_{13}H_{18}O_6$: C, 57.77; H, 6.71. Found: C, 57.80; H, 6.88.

D-1-*O*-Benzyl-2,3,4,5,6-penta-*O*-acetyl-*myo*-inositol (3a)

To a solution of **2a** (432 mg, 1.6 mmol) in pyridine (40 ml) was added acetic anhydride (1.51 ml, 16.0 mmol) followed by 4-dimethylaminopyridine (100 mg, 0.8 mmol), and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated under reduced pressure, and the crude product was purified by recrystallization (hexane–AcOEt) to afford **3a** (730 mg, 95%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.60 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.20 (s, 3H), 3.60 (dd, $J = 10.1$, 2.9 Hz, 1H), 4.39 (d, $J = 12.1$ Hz, 1H), 4.66 (d, $J = 12.1$ Hz, 1H), 4.94 (dd, $J = 10.6$, 2.7 Hz, 1H), 5.08 (t, $J = 9.9$ Hz, 1H), 5.39–5.52 (m, 2H), 5.76 (s, 1H), 7.22–7.34 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 20.5, 20.7, 20.8, 66.6, 69.1, 69.5, 70.9, 71.1, 71.8, 74.4, 127.8, 128.1, 128.5, 136.9, 169.6, 169.7, 169.8, 169.9, 170.0. IR (KBr) 2900, 1740, 1360, 1210, 1130 cm^{-1}. MS (EI) *m/z* 480 $(M + H)^+$. Anal. calcd for $C_{23}H_{28}O_{11}$: C, 57.50; H, 5.87. Found: C, 57.46; H, 5.94.

D-2,3,4,5,6-Penta-*O*-acetyl-*myo*-inositol (4a)

To a solution of **3a** (699 mg, 1.45 mmol) in acetic acid (30 ml) was added 10% palladium-carbon (50 mg), and the resulting mixture was stirred at room temperature under hydrogen for 10 h. The mixture was filtered through a pad of celite and concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2–MeOH = 14 : 1) to afford **4a** (525 mg, 93%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.09 (s, 3H), 2.21 (s, 3H), 3.90 (dd, $J = 10.1$, 2.9 Hz, 1H), 4.97 (dd, $J = 10.6$, 2.7 Hz, 1H), 5.15 (t, $J = 9.7$ Hz, 1H), 5.31 (t, $J = 9.9$ Hz, 1H), 5.45 (t, $J = 10.1$ Hz, 1H), 5.59 (t, $J = 2.9$ Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 20.5, 20.7, 20.8, 68.7, 69.2, 70.4, 70.8, 72.4, 169.7, 169.8, 169.9, 170.3, 171.0. IR (KBr) 3400, 1740, 1360, 1210, 1040 cm^{-1}. MS (EI) *m/z* 391 $(M + H)^+$. Anal. calcd for $C_{16}H_{22}O_{11}$: C, 49.23; H, 5.68. Found: C, 48.93; H, 5.81.

D-3,6-Di-*O*-allyl-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol (6a)

To a solution of (−)-**1** (1.94 g, 4.50 mmol) in MeOH (50 ml) was added 10% palladium-carbon (800 mg), and the resulting mixture was stirred at room temperature under hydrogen for 2 days. The residue was purified by recrystallization (hexane–AcOEt = 1 : 1) to afford **5a** (1.45 g, 95%) as a white solid. To a solution of **5a** (2.00 g, 5.9 mmol) in DMF (30 ml) was added NaH (576 mg, 24.0 mmol) followed by allyl bromide (1.04 ml, 12.0 mmol), and the resulting mixture was stirred at room temperature under argon for 24 h. The reaction was quenched with MeOH, and concentrated under reduced pressure, and the residue was diluted with AcOEt. The organic phase was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and then concentrated under reduced pressure. The crude product was purified by column chromatography (hexane–AcOEt = 5 : 1) to afford **6a** (2.65 g, >99%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.38–1.76 (m, 20H), 3.31 (t, $J = 9.9$ Hz, 1H), 3.63 (dd, $J = 10.6$, 6.4 Hz, 1H), 3.77 (dd, $J = 10.1$, 4.2 Hz, 1H), 3.97 (t, $J = 9.9$ Hz, 1H), 4.06 (t, $J = 4.9$ Hz, 1H), 4.22–4.37 (m, 4H), 4.45 (t, $J = 4.4$ Hz, 1H), 5.17–5.36 (m, 4H), 5.89–6.05 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 23.5, 23.7, 23.8, 23.9, 24.9, 25.0, 35.2, 36.3, 36.4, 37.6, 70.9, 71.2, 74.9, 76.0, 76.5, 78.4, 80.4, 80.8, 110.5, 112.6, 117.1, 117.8, 134.8, 134.9. IR (KBr) 2920, 2850, 1730, 1440, 1360, 1260, 1160 cm^{-1}. MS (EI) *m/z* 420 $(M + H)^+$. Anal. calcd for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.55; H, 8.77.

D-3,6-Di-*O*-allyl-*myo*-inositol (7a)

To a solution of **6a** (2.65 g, 6.30 mmol) in a mixture of THF–H_2O (5 : 1, 60 ml) was added *p*-toluenesulfonic acid monohydrate (300 mg, 1.58 mmol), and the resulting mixture was refluxed for 3 h. The mixture was neutralized with Et_3N, and concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2 : MeOH = 7 : 1) to afford **7a** (1.80 g, >99%) as a white solid. ^{1}H NMR (CD_3OD) δ 3.02–3.42 (m, 4H), 3.61 (t, $J = 9.7$ Hz, 1H), 3.98–4.13 (m, 3H), 4.22–4.24 (m, 2H), 5.02–5.25 (m, 4H), 5.82–5.99 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 71.0, 72.1, 73.1, 73.6, 75.1, 76.3, 80.7, 82.5, 116.7, 117.4, 136.6, 137.2. IR (KBr) 3420, 2900, 1420, 1350, 1100, 1070, 930 cm^{-1}. MS (FAB) *m/z* 261 $(M + H)^+$. Anal. calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.21; H, 7.80.

D-3,6-Di-*O*-allyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (8a)

A mixture of **7a** (624 mg, 2.40 mmol) and dibutyltin oxide (750 mg, 3.00 mmol) in toluene (50 ml) was refluxed for 3 h in a Dean–Stark apparatus to remove water. The mixture was concentrated under reduced pressure. To the residue was added caesium fluoride (760 mg, 5.00 mmol), and the mixture was suspended in heated DMF (30 ml). To the resulting suspension was

added *p*-methoxybenzyl chloride (0.47 ml, 3.00 mmol) at −78 °C, and the mixture was stirred at room temperature under argon for 24 h. The reaction mixture was diluted with $CHCl_3$, filtered through a pad of celite, and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2 : MeOH = 12 : 1) to afford **8a** (800 mg, 88%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.53 (bs, 1H), 2.88–2.95 (m, 2H), 3.14 (dd, J = 9.4, 2.8 Hz, 1H), 3.31–3.40 (m, 2H), 3.63–3.93 (m, 5H), 4.07–4.44 (m, 5H), 4.63 (s, 2H), 5.16–5.33 (m, 4H), 5.87–6.04 (m, 2H), 6.88 (d, J = 8.6 Hz, 2H), 7.28 (d, J = 8.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 55.3, 66.9, 71.2, 72.2, 74.0, 78.8, 79.4, 80.0, 113.9, 116.9, 117.9, 129.5, 129.9, 134.4, 135.1, 159.4. IR (KBr) 3450, 2900, 1510, 1250, 1100, 1040 cm^{-1}. MS (FAB) m/z 381 (M + H)$^+$. Anal. calcd for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Found: C, 63.00; H, 7.44.

D-3,6-Di-*O*-allyl-1-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (9a)

To a solution of **8a** (1.43 g, 3.76 mmol) in DMF (30 ml) was added 2-methoxypropene (0.52 ml, 10.0 mmol) followed by dehydrated *p*-toluenesulfonic acid (95 mg, 0.50 mmol). The resulting mixture was stirred at room temperature under argon for 24 h. The mixture was neutralized with Et_3N and concentrated under reduced pressure, and the residue was diluted with AcOEt. The organic phase was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and then concentrated under reduced pressure. The crude product was purified by column chromatography (hexane–AcOEt = 3 : 2) to afford **9a** (1.23 g, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 3H), 1.39 (s, 3H), 2.73 (bs, 1H), 3.25–3.46 (m, 3H), 3.75–3.46 (m, 10H), 4.60 (d, J = 11.4 Hz, 1H), 4.67 (d, J = 11.4 Hz, 1H), 5.13–5.31 (m, 4H), 5.83–5.99 (m, 2H), 6.83 (d, J = 8.6 Hz, 2H), 7.25 (d, J = 8.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 26.6, 26.7, 55.0, 69.3, 70.4, 72.1, 72.7, 75.9, 76.6, 77.8, 78.8, 80.4, 111.3, 113.6, 116.2, 117.2, 129.3, 134.4, 134.9, 159.1. IR (KBr) 3500, 3080, 3000, 2900, 1610, 1510, 1240, 1080 cm^{-1}. MS (FAB) m/z 421 (M + H)$^+$. Anal. calcd for $C_{23}H_{32}O_7$: C, 65.70; H, 7.67. Found: C, 65.67; H, 7.76.

D-1-*O*-(*p*-Methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (10a)

To a solution of **9a** (552 mg, 1.31 mmol) in a mixture of EtOH–benzene–H_2O (7 : 3 : 1, 22 ml) was added diazabicyclo[2.2.2]octane (147 mg, 1.31 mmol) followed by triphenylphosphine rhodium(I) chloride (121 mg, 0.13 mmol), and the resulting mixture was refluxed for 5 h. The mixture was concentrated under reduced pressure, and the residue was diluted with AcOEt. The organic phase was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and then concentrated under reduced pressure. To a solution of the residue in a mixture of acetone–H_2O (10 : 1, 5 ml) was added mercury(II) oxide (284 mg, 1.31 mmol). To the resulting mixture was added dropwise a solution of mercury(II) chloride (355 mg, 1.31 mmol) in a mixture of acetone–H_2O (10 : 1, 5 ml). The resulting mixture was stirred at room temperature for 5 min. The mixture was neutralized with aqueous NaOH, filtered through a pad of celite, and concentrated under reduced pressure, and the residue was diluted with saturated aqueous NaCl. The aqueous phase was extracted with CH_2Cl_2, and the organic phase was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2 : MeOH = 12 : 1) to afford **10a** (200 mg, 45%) as a white solid. ^{1}H NMR (CD_3OD) δ 1.31 (s, 3H), 1.35 (s, 3H), 3.16–3.21 (m, 2H), 3.54–3.86 (m, 6H), 3.98 (s, 1H), 4.48 (d, J = 11.4 Hz, 1H), 4.55 (d, J = 11.4 Hz, 1H), 4.78 (bs, 3H), 6.77 (d, J = 8.6 Hz, 2H), 7.25 (d, J = 8.44 Hz, 2H). IR (KBr) 3350, 2900, 1520, 1250, 1070 cm^{-1}. MS (FAB) m/z 340 (M + H)$^+$. Anal. calcd for $C_{17}H_{23}O_7 - 1/2H_2O$: C, 58.61; H, 6.94. Found: C, 58.63; H, 7.06.

D-2,3,6-Tri-*O*-acetyl-1-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (11a)

To a solution of **10a** (390 mg, 1.15 mmol) in pyridine (10 ml) was added 4-dimethylaminopyridine (25 mg, 0.21 mmol) followed by acetic anhydride (0.47 ml, 5.00 mmol), and the resulting mixture was stirred at room temperature for 12 h. The mixture was diluted with toluene, the resulting azeotropic mixture was concentrated under reduced pressure, and then the residue was diluted with AcOEt. The organic phase was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and then concentrated under reduced pressure. The crude product was purified by column chromatography (hexane–AcOEt = 1 : 1) to afford **11a** (427 mg, >99%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H), 1.41 (s, 3H), 2.00 (s, 6H), 2.10 (s, 3H), 3.39–3.49 (m, 2H), 3.73 (s, 3H), 4.00 (t, J = 10.1 Hz, 1H), 4.24 (d, J = 11.7 Hz, 1H), 4.51 (d, J = 11.7 Hz, 1H), 4.93 (dd, J = 10.8, 2.7 Hz, 1H), 5.35 (t, J = 9.9 Hz, 1H), 5.74 (s, 1H), 6.80 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 20.8, 26.5, 26.6, 55.1, 67.7, 70.0, 70.7, 71.6, 74.6, 76.5, 77.2, 112.5, 113.7, 128.9, 129.4, 159.3, 169.5, 170.0. IR (KBr) 3000, 1750, 1510, 1370, 1230, 1060 cm^{-1}. MS (FAB) m/z 467 (M + H)$^+$. Anal. calcd for $C_{23}H_{30}O_{10} - 1/5H_2O$: C, 58.77; H, 6.52. Found: C, 58.88; H, 6.60.

D-2,3,6-Tri-*O*-acetyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (12a)

To a solution of **11a** (360 mg, 0.77 mmol) in CH_2Cl_2 (10 ml) was added ethylene glycol (89 µl, 1.5 mmol) followed by *p*-toluenesulfonic acid monohydrate (10 mg, 0.051 mmol). The resulting mixture was stirred at room temperature for 10 min. The mixture was neutralized with Et_3N (0.1 ml, 0.72 mmol), and concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2 : MeOH = 12 : 1) to afford **12a** (168 mg, 77%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3H), 2.04 (s, 3H), 2.12 (s, 3H), 3.39–3.56 (m, 2H), 3.78 (s, 3H), 3.88 (s, 1H), 4.05 (bs, 2H), 4.29 (t, J = 11.7 Hz, 1H), 4.55 (d, J = 11.7 Hz, 1H), 4.79 (d, J = 13.0 Hz, 1H), 5.16 (t, J = 9.9 Hz, 1H), 5.69 (s, 1H), 6.85 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 20.6, 20.8, 55.0, 67.2, 70.8, 70.9, 71.0, 72.9, 73.0, 74.0, 113.6, 129.1, 129.2, 159.2, 170.1, 170.5, 170.9. IR (KBr) 3430, 2900, 1740, 1370, 1230, 1030 cm^{-1}. MS (FAB) m/z 427 (M + H)$^+$. Anal. calcd for $C_{20}H_{26}O_{10} - 1/2H_2O$: C, 55.17; H, 6.25. Found: C, 55.22; H, 6.01.

D-2,3,6-Tri-*O*-acetyl-1-*O*-(*p*-methoxybenzyl)-4,5-di-*O*-[bis(β-cyanoethyl)phosphoryl]-*myo*-inositol (13a)

To a solution of **12a** (250 mg, 0.60 mmol) in CH_2Cl_2 (10 ml) was added bis(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (0.77 ml, 3.00 mmol) followed by 1*H*-tetrazole (210 mg, 3.00 mmol). The resulting mixture was stirred at room temperature

for 1.5 h. To the mixture was added *m*-chloroperbenzoic acid (863 mg, 5.0 mmol) in small portions, and the resulting mixture was stirred for 5 min. The mixture was diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2 : MeOH = 13 : 1) to afford **13a** (296 mg, 68%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H), 2.12 (s, 3H), 2.19 (s, 3H), 2.78–2.85 (m, 8H), 3.60 (dd, J = 10.2, 2.4 Hz, 1H), 3.79 (s, 3H), 4.25–4.47 (m, 9H), 4.50–4.58 (m, 2H), 4.80 (dd, J = 18.7, 9.3 Hz, 1H), 5.06 (dd, J = 10.1, 2.6 Hz, 1H), 5.38 (t, J = 4.9 Hz, 1H), 5.68 (s, 1H), 6.80 (d, J = 8.2 Hz, 2H), 7.15 (d, J = 8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 19.2, 19.3, 19.4, 19.4, 20.6, 20.9, 55.1, 62.6, 62.7, 62.8, 62.9, 66.3, 68.5, 70.4, 71.4, 73.2, 76.5, 77.3, 113.7, 116.6, 116.7, 116.9, 117.0, 128.6, 129.3, 159.3, 169.7, 169.8, 170.0. IR (film) 2970, 2250, 1740, 1510, 1510, 1410, 1370, 1240, 1030, 940 cm^{-1}. MS (FAB) m/z 799 (M + H)$^+$.

D-2,3,6-Tri-*O*-acetyl-4,5-di-*O*-[bis(β-cyanoethyl)phosphoryl]-*myo*-inositol (14a)

To a solution of **13a** (296 mg, 0.37 mmol) in a mixture of CH_3CN–H_2O (9 : 1, 10 ml) was added diammonium cerium(IV) nitrate, and the resulting mixture was stirred at room temperature for 1.5 h. The mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (CH_2Cl_2 : MeOH = 10 : 1) to afford **14a** (68 mg, 27%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3H), 2.18 (s, 3H), 2.19 (s, 3H), 2.90–2.97 (m, 8H), 3.97 (dd, J = 10.1, 2.9 Hz, 1H), 4.30–4.41 (m, 8H), 4.68–4.86 (m, 2H), 5.21 (dd, J = 9.9, 2.9 Hz, 1H), 5.35 (t, J = 9.9 Hz, 1H), 5.50 (dd, J = 5.3, 2.9 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 20.1, 20.2, 20.2, 20.3, 20.8, 21.0, 21.5, 64.7, 64.8, 64.9, 68.1, 70.7, 71.7, 73.3, 78.4, 78.9, 118.6, 118.7, 118.8, 118.9, 171.5, 172.0, 172.2. IR (KBr) 3420, 2960, 1740, 1370, 1220, 1030 cm^{-1}.

D-1,3,6-Tri-*O*-allyl-*myo*-inositol (15a)

7a (521 mg, 2.00 mmol) was allowed to react under the same conditions as described for the preparation of **8a** to give **15a** (356 mg, 59%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.53 (s, 1H), 2.88–2.95 (m, 2H), 3.18 (dd, J = 4.8, 2.8 Hz, 1H), 3.27 (dd, J = 9.3, 2.8 Hz, 1H), 3.37 (t, J = 9.3 Hz, 1H), 3.65 (t, J = 9.3 Hz, 1H), 3.91 (t, J = 9.3 Hz, 1H), 4.11–4.42 (m, 7H), 5.15–5.34 (m, 6H), 5.87–6.02 (m, 3H). IR (KBr) 3450, 2900, 1110, 1030, 990, 910 cm^{-1}. Anal. calcd for $C_{15}H_{24}O_6$: C, 59.98; H, 8.05. Found: C, 59.96; H, 8.09.

D-1,3,6-Tri-*O*-allyl-4,5-*O*-isopropylidene-*myo*-inositol (16a)

15a (330 mg, 1.10 mmol) was allowed to react under the same conditions as described for the preparation of **9a** to give **16a** (340 mg, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 3H), 1.43 (s, 3H), 2.62 (s, 1H), 3.29–3.36 (m, 2H), 3.53 (dd, J = 9.9, 3.2 Hz, 1H), 3.82 (dd, J = 9.9, 8.6 Hz, 1H), 4.00 (t, J = 9.5 Hz, 1H), 4.13–4.38 (m, 7H), 5.14–5.34 (m, 6H), 5.87–6.02 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 27.3, 27.4, 69.9, 71.1, 72.7, 72.8, 76.5, 77.2, 78.3, 79.3, 81.5, 112.0, 116.9, 117.9, 118.0, 134.9, 135.0, 135.5. IR (KBr) 3475, 3080, 3000, 2900, 1640, 1460, 1420, 1370, 1230, 1080, 1000, 920 cm^{-1}. MS (FAB) m/z 341 (M + H)$^+$. Anal. calcd for $C_{18}H_{28}O_6$: C, 63.51; H, 8.29. Found: C, 63.27; H, 8.47.

D-1,3,6-Tri-*O*-allyl-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (17a)

To a solution of **16a** (760 mg, 2.23 mmol) in DMF (30 ml) was added NaH (240 mg, 3.00 mmol) followed by *p*-methoxybenzyl chloride (0.44 ml, 3.00 mmol), and the resulting mixture was stirred at room temperature for 24 h. The reaction was quenched with MeOH, and concentrated under reduced pressure, and the residue was diluted with AcOEt. The organic phase was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and then concentrated under reduced pressure. The crude product was purified by the column chromatography (hexane–AcOEt = 3 : 1) to afford **17a** (748 mg, 73%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 3H), 1.43 (s, 3H), 3.23–3.33 (m, 2H), 3.46 (dd, J = 10.4, 2.8 Hz, 1H), 3.80 (s, 3H), 3.85–3.91 (m, 1H), 4.01–4.46 (m, 8H), 4.77 (s, 2H), 5.12–5.33 (m, 6H), 5.84–5.99 (m, 3H), 6.86 (d, J = 8.6 Hz, 2H), 7.36 (d, J = 8.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 26.9, 27.0, 55.2, 70.6, 72.3, 72.4, 74.7, 76.3, 77.1, 77.4, 78.0, 79.4, 82.0, 111.5, 113.6, 116.3, 116.6, 116.7, 129.5, 131.0, 134.8, 135.0, 135.3, 159.1. IR (film) 3030, 3000, 2900, 1610, 1460, 1370, 1220, 1100, 1030 cm^{-1}. MS (EI) m/z 461 (M + H)$^+$. Anal. calcd for $C_{26}H_{36}O_7$ − 1/2H_2O: C, 66.50; H, 7.94. Found: C, 66.80; H, 7.83.

D-2-*O*-(*p*-Methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (18a)

17a (290 mg, 0.63 mmol) was allowed to react under the same conditions as described for the preparation of **10a** to give **18a** (106 mg, 49%) as a colorless oil. ^{1}H NMR (CD_3OD) δ 1.41 (s, 6H), 3.26–3.30 (m, 2H), 3.65–3.67 (m, 1H), 3.78 (s, 3H), 3.83–3.96 (m, 2H), 4.09 (s, 1H), 4.58 (d, J = 11.4 Hz, 1H), 4.66 (d, J = 11.4 Hz, 1H), 6.88 (d, J = 8.6 Hz, 2H), 7.36 (d, J = 8.6 Hz, 2H). ^{13}C NMR (CD_3OD) δ 27.1, 27.2, 55.7, 71.2, 71.8, 72.1, 73.3, 78.8, 80.2, 83.0, 112.4, 114.7, 130.9, 131.7, 160.9. IR (KBr) 3350, 2900, 1510, 1250, 1070 cm^{-1}. Anal. calcd for $C_{17}H_{24}O_7$ − 1/3H_2O: C, 58.95; H, 7.18. Found: C, 58.79; H, 7.16.

D-1,3,6-Tri-*O*-acetyl-2-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (19a)

18a (103 mg, 0.30 mmol) was allowed to react under the same conditions as described for the preparation of **12a** to give **19a** (140 mg, >99%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H), 1.41 (s, 3H), 2.00 (s, 6H), 2.10 (s, 3H), 3.39–3.49 (m, 2H), 3.73 (s, 3H), 4.00 (t, J = 10.1 Hz, 1H), 4.24 (d, J = 11.7 Hz, 1H), 4.51 (d, J = 11.7 Hz, 1H), 4.93 (dd, J = 10.8, 2.7 Hz, 1H), 5.35 (t, J = 9.9 Hz, 1H), 5.74 (s, 1H), 6.80 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 20.3, 20.5, 20.6, 20.8, 26.5, 26.6, 55.1, 67.7, 70.0, 70.7, 71.6, 74.6, 76.5, 77.2, 112.5, 113.7, 128.9, 129.4, 159.3, 169.5, 170.0. IR (KBr) 3000, 1750, 1515, 1370, 1220, 1100, 1060, 1030 cm^{-1}. Anal. calcd for $C_{23}H_{30}O_{10}$: C, 59.22; H, 6.48. Found: C, 59.04; H, 6.57.

D-1,3,6-Tri-*O*-acetyl-(*p*-methoxybenzyl)-*myo*-inositol (20a)

19a (115 mg, 0.27 mmol) was allowed to react under the same conditions as described for the preparation of **13a** to give **20a** (140 mg, 94%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 3.49–3.56 (m, 1H), 3.67–3.75 (m, 2H), 3.79 (s, 3H), 4.00–4.06 (m, 2H), 4.53–4.63 (m, 2H), 4.78 (dd,

J = 10.3, 2.2 Hz, 1H), 4.92 (dd, J = 10.4, 2.6 Hz, 1H), 5.41 (t, J = 9.7 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 20.6, 20.7, 20.8, 55.1, 71.2, 71.3, 72.0, 72.9, 73.1, 74.6, 113.6, 129.5, 129.8, 159.2, 169.9, 170.6, 170.8. IR (KBr) 3450, 2900, 1740, 1510, 1360, 1230, 1030 cm^{-1}. Anal. calcd for $C_{20}H_{26}O_{10} - 1/2H_2O$: C, 55.17; H, 6.25. Found: C, 55.47; H, 6.15.

D-1,3,6-Tri-*O*-acetyl-2-*O*-(*p*-methoxybenzyl)-4,5-di-*O*-[bis(β-cyanoethyl)phosphoryl]-*myo*-inositol (21a)

20a (135 mg, 0.32 mmol) was allowed to react under the same conditions as described for the preparation of **14a** to give **21a** (256 mg, >99%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3H), 2.13 (s, 3H), 2.20 (s, 3H), 2.71–2.85 (m, 8H), 3.42–3.62 (m, 2H), 3.80 (s, 3H), 4.10–4.59 (m, 10H), 4.80 (dd, J = 18.7, 9.3 Hz, 1H), 5.05 (dd, J = 10.1, 2.6 Hz, 1H), 5.38 (t, J = 9.9 Hz, 1H), 5.68 (s, 1H), 6.86 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H). IR (film) 2960, 2250, 1740, 1610, 1510, 1410, 1360 cm^{-1}. MS (FAB) m/z 799 (M + H)$^+$.

D-1,3,6-Tri-*O*-acetyl-4,5-di-*O*-[bis(β-cyanoethyl)phosphoryl]-*myo*-inositol (22a)

21a (240 mg, 0.30 mmol) was allowed to react under the same conditions as described for the preparation of **14a** to give **22a** (120 mg, 59%) as a colorless oil. ^{1}H NMR (CD_3OD) δ 2.02 (s, 3H), 2.09 (s, 3H), 2.15 (s, 3H), 2.88–2.94 (m, 8H), 4.18–4.40 (m, 9H), 4.81–4.93 (m, 2H), 5.01 (dd, J = 10.4, 2.6 Hz, 1H), 5.14 (dd, J = 9.7, 2.6 Hz, 1H), 5.56 (t, J = 10.1 Hz, 1H). ^{13}C NMR (CD_3OD) δ 20.1, 20.2, 20.3, 20.7, 20.9, 21.2, 21.3, 64.7, 64.8, 64.9, 68.4, 70.8, 71.9, 72.1, 78.4, 78.9, 118.6, 118.8, 118.9, 119.0, 171.6, 171.8, 171.9. IR (KBr) 3400, 2950, 1740, 1360, 1220, 1030 cm^{-1}.

1,3,4,5,6-Penta-*O*-acetyl-2-*O*-(*p*-methoxybenzyl)-*myo*-inositol (23a)

To a solution of **20a** (115 mg, 0.27 mmol) in pyridine (15 ml) was added 4-dimethylaminopyridine (30 mg, 0.25 mmol) followed by acetic anhydride (0.15 ml, 1.60 mmol), and the resulting mixture was stirred at room temperature for 12 h. The mixture was diluted with toluene, and the resulting azeotropic mixture was concentrated, and then the residue was diluted with AcOEt. The organic phase was washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and then concentrated under reduced pressure. The crude product was purified by column chromatography (hexane–AcOEt = 1 : 1) to afford **23a** (118 mg, 86%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 15H), 3.80 (s, 3H), 4.12 (s, 1H), 4.62 (s, 2H), 4.97 (dd, J = 10.3, 1.8 Hz, 2H), 5.14 (t, J = 9.5 Hz, 1H), 5.61 (t, J = 9.9 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 20.3, 20.4, 20.4, 55.1, 70.0, 70.9, 71.1, 74.1, 74.6, 113.7, 129.4, 129.6, 159.3, 169.5, 170.0. IR (KBr) 2950, 1740, 1360, 1230, 1040 cm^{-1}. Anal. calcd for $C_{24}H_{30}O_{12}$: C, 56.47; H, 5.92. Found: C, 56.39; H, 5.93.

1,3,4,5,6-Penta-*O*-acetyl-*myo*-inositol (24a)

To a solution of **23a** (110 mg, 0.21 mmol) in acetic acid (10 ml) at room temperature was added 10% palladium-carbon (50 mg), and the resulting mixture was stirred at room temperature under hydrogen for 10 h. The mixture was filtered through a pad of celite and concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2–MeOH = 14 : 1) to afford **24a** (61 mg, 74%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 9H), 2.10 (s, 6H), 3.12 (bs, 1H), 4.33 (s, 1H), 5.03 (dd, J = 10.3, 2.4 Hz, 2H), 5.20 (t, J = 9.7 Hz, 1H), 5.60 (t, J = 10.1 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 68.2, 69.5, 70.7, 70.8, 169.7, 169.8. IR (KBr) 3420, 2950, 1740, 1370, 1230, 1040 cm^{-1}. MS (FAB) m/z 391 (M + H)$^+$. Anal. calcd for $C_{16}H_{22}O_{11} - 1/3H_2O$: C, 48.49; H, 5.76. Found: C, 48.53; H, 5.68.

D-2,3,6-Tri-*O*-acetyl-4,5-di-*O*-[bis(β-cyanoethyl)phosphoryl]-*myo*-inositol 1-{[(4,4′-dimethoxytrityl)biotinyl-6-aminohexyl](β-cyanoethyl)phosphate} (25a)

To a solution of 1-*N*-(4,4-dimethoxytrityl)biotinyl-6-aminohexan-1-ol (97 mg, 0.15 mmol) in CH_2Cl_2 (5 ml) was added (β-cyanoethyl)-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite (48 μl, 0.15 mmol) followed by 1*H*-tetrazole (11 mg, 0.15 mmol), and the resulting mixture was stirred at room temperature under argon for 1.5 h. To the mixture was added **14a** (84 mg, 0.12 mmol) followed by 1*H*-tetrazole (11 mg, 0.15 mmol), and the resulting mixture was stirred at room temperature for further 2 h. To the mixture was added *tert*-butylhydroperoxide (0.10 ml, 0.78 mmol), and the resulting mixture was stirred at room temperature for a further 5 min, and concentrated under reduced pressure to half volume. The residue was purified by column chromatography (CH_2Cl_2 : MeOH = 13 : 1, 0.5%Et_3N) to afford **25a** (45 mg, 38%) as a colourless oil. ^{1}H NMR ($CDCl_3$) δ 1.36–1.67 (m, 14H), 2.03–2.29 (m, 11H), 2.43–2.45 (m, 1H), 2.70–2.80 (m, 10H), 3.08–3.20 (m, 3H), 3.79 (s, 6H), 4.02–4.29 (m, 15H), 4.67–4.81 (m, 3H), 5.22–5.26 (m, 1H), 5.49 (t, J = 9.7 Hz, 1H), 5.63–5.73 (m, 2H), 5.99 (bs, 1H), 6.80 (d, J = 8.4 Hz, 4H), 7.11–7.29 (m, 9H). IR (KBr) 3400, 2950, 1750, 1700, 1650, 1510, 1220, 1130 cm^{-1}. MS (FAB) m/z 1440 (M + H)$^+$.

D-1-*O*-[(Biotinyl-6-aminohexyl) hydrogen phosphoryl]-*myo*-inositol 4,5-bis(hydrogenphosphate):biotinylated D-*myo*-inositol 1,4,5-triphosphate

To a solution of **25a** (45 mg, 0.031 mmol) in CH_2Cl_2 (10 ml) was added trichloroacetic acid (30 mg), and the resulting mixture was stirred at room temperature for 30 min. The mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (CH_2Cl_2 : MeOH = 7 : 1) to afford dimethoxytrityl-free crude compound. To a solution of crude compound in MeOH (5 ml) was added 28% NH_4OH (5 ml), and the resulting mixture was stirred at 55 °C for 10 h. The mixture was concentrated under reduced pressure, and the residue was purified by anion-exchange chromatography to afford the ammomium salt of biotinylated D-*myo*-inositol 1,4,5-triphosphate (23 mg, 32%) as a white solid. ^{1}H NMR (D_2O) δ 1.22–1.58 (m, 14H), 2.09 (t, J = 7.1 Hz, 2H), 2.63 (d, J = 13.0 Hz, 1H), 2.85 (dd, J = 13.0, 4.9 Hz, 1H), 3.02 (d, J = 6.6 Hz, 2H), 3.15–3.21 (m, 1H), 3.58 (d, J = 8.1 Hz, 1H), 3.72–3.96 (m, 5H), 4.11–4.29 (m, 3H), 4.33–4.48 (m, 1H). ^{13}C NMR (D_2O) δ 27.3, 27.9, 28.4, 30.3, 30.5, 30.9, 32.4, 38.2, 41.9, 42.3, 58.0, 62.9, 64.7, 69.1, 72.8, 73.4, 78.2, 79.7, 81.1, 168.0, 179.3. IR (KBr) 3400, 3200, 2900, 1700, 1400, 1030 cm^{-1}. MS (FAB) m/z 746 (M + H)$^+$. FABHRMS calcd m/z

for $C_{22}H_{41}N_3O_{17}P_3S$ 744.1370. Found: 744.1358 ($M - H^+$). Anal. calcd for $C_{22}H_{42}N_6\ O_{17}P_3S - 5/2NH_3 - 5/2H_2O$: C, 31.71; H, 6.59; N, 9.25. Found: C, 31.51; H, 6.64; N, 9.29. $[\alpha]_D^{25} = -3.8$ (*c* 0.1, H_2O).

Acknowledgements

The present study was financially supported in part by a Grant-in-Aid for Scientific Research (NO.17390030 to MO), the Kumamoto Technology and Industry Foundation (to YO), and the Shorai Foundation for Science and Technology (to MO).

Notes and references

1 M. J. Berridge, *Nature*, 1993, **361**, 315–325.
2 M. Tyers, R. A. Rachubinski, M. I. Stewart, A. M. Varrichio, R. G. L. Shorr, R. J. Haslam and C. B. Harley, *Nature*, 1988, **333**, 470–473.
3 R. J. Haslam, H. B. Koide and B. A. Hemmings, *Nature*, 1993, **363**, 309–310.
4 B. J. Mayer, *Cell*, 1993, **73**, 629–630.
5 J. M. Kavran, D. E. Klein, A. Lee, M. Falasca, S. J. Isakoff, E. Y. Skolnik and M. A. Lemmon, *J. Biol. Chem.*, 1988, **273**, 30497–30508.
6 G. Shaw, *BioEssays*, 1996, **18**, 35–46.
7 A. Musacchio, T. Gibson, P. Rice, J. Thompson and M. Saraste, *Trends Biochem. Sci.*, 1993, **19**, 343–348.
8 T. Gibson, M. Hyvönen, A. Musacchio, M. Saraste and E. Birney, *Trends Biochem. Sci.*, 1994, **19**, 349–353.
9 J. E. Harlan, P. J. Hajduk, H. S. Yoon and S. W. Fesik, *Nature*, 1994, **371**, 168–170.
10 M. Hyvönen, M. J. Macias, M. Nilges, H. Oschkinat, M. Saraste and M. Wilmanns, *EMBO J.*, 1995, **14**, 4676–4685.
11 M. A. Lemmon, K. M. Ferguson, R. O'Brien, P. B. Sigler and J. Schlessinger, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 10472–10476.
12 M. Wilchek and E. A. Bayer, *Methods Enzymol.*, 1990, **184**, 123–138.
13 K. M. Ferguson, M. A. Lemmon, P. B. Sigler and J. Schlessinger, *Cell*, 1995, **83**, 1037–1046.
14 D. C. Billington, R. Baker, J. J. Kulagowski and I. M. Mawer, *J. Chem. Soc., Chem. Commun.*, 1987, **4**, 314–316.
15 W. Bannwarth and A. Trzeciak, *Helv. Chim. Acta*, 1987, **70**, 175–186.
16 N. Nagashima and M. Ohno, *Chem. Lett.*, 1987, 141–144.
17 C. Liu and B. L. Potter, *J. Org. Chem.*, 1997, **62**, 8335–8340.
18 E. J. Corey and W. Suggs, *J. Org. Chem.*, 1973, **38**, 3224.
19 E. Uhlmann and J. Engels, *Tetrahedron Lett.*, 1986, **27**, 1023–1026.
20 R. Johansson and B. Samuelsson, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2371–2374.
21 R. T. Pon, *Tetrahedron Lett.*, 1991, **32**, 1715–1718.
22 D. Lampe, C. Liu and B. V. L. Potter, *J. Med. Chem.*, 1994, **37**, 907–912.

