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Regioselective phosphorylation of vicinal 3,4-hydroxy *myo*-inositol derivative promoted practical synthesis of D-PtdIns(4,5)P2 and D-Ins(1,4,5)P3

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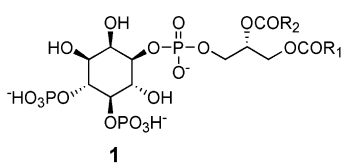
Abstract—The reactivity of 3 and 4-OH in 3,4-diol *myo*-inositol derivatives were observed through the phosphorylation, acylation and silylation. The results indicated that 3-OH is much more reactive than 4-OH, giving regiospecifically 3-mono-functionalized products. This investigation provided a concise methodology for the synthesis of natural D-form of PtdIns(4,5)P2 and D-Ins(1,4,5)P3 from L-1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyl disiloxane-1,3-diyl)-*myo*-inositol.

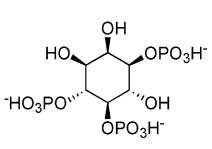
1. Introduction

Since the discovery of the biological role of *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P3] as a second messenger in intracellular signal transduction system in 1983,[1] the biological importance of inositol phosphates [Ins(P)ns] and then phosphatidylinositol phosphates [PtdIns(P)ns] have been well recognized in the past two decades. In the family of PtdIns(P)ns, PtdIns(4,5)P2 (**1**) is the key member due to its role as the precursor of at least three second-messenger molecules,[2] Ins(1,4,5)P3, PtdIns(3,4,5)P3 and diacylglycerol. In addition, PtdIns(4,5)P2 itself may also be involved in several other cellular processes such as exocytosis, cytoskeletal regulation, and intracellular trafficking of vesicles.[3] Recent studies established PtdIns(4,5)P2 itself is also a second-messenger.[4] As for inositol phosphates, Ins(1,4,5)P3 (**2**), that mediates the release of calcium ions from intracellular stores,[5] is the most important inositol phosphate biologically.



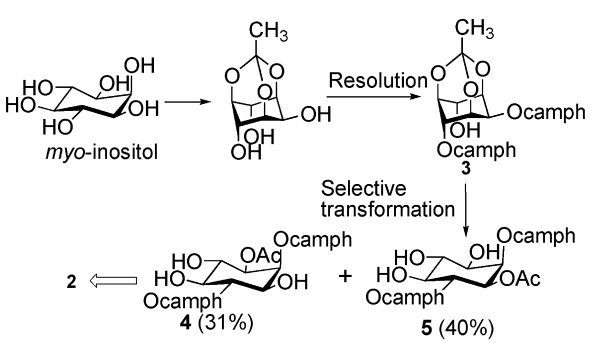
1

natural product: R_1CO=stearoyl, R_2CO=arachidonoyl
dipalmitoyl PtdIns(4,5)P2: $R_1=R_2=C_{15}H_{31}$



2

These events have been stimulating vehemently chemists to pursue a practical way to access these molecules. A number of reports have been appeared for the synthesis of the analogues of **1** from diverse starting materials, such as D-glucose,[6] *myo*-inositol,[7] and L-(−)-quebrachitol.[8] In spite of these, many of the old synthetic procedures were not entirely satisfactory, where long routes were required and the overall yield was low. For **2**, numerous routes hitherto have been reported,[9] among which two methods developed by Potter[10] and Ozaki[11] are very rapid, involving only about 5 steps. However, a serious problem involved in these methods is the formation of undesired regioisomers in almost equal amount to the required ones during selective protection–deprotection of hydroxyls, resulting in a low yield for the target **2**, although those undesired regioisomers can provide starting materials for alternative targets. In Potter's approach (Scheme 1), for instance, selective transformation of intermediate **3** yielded only about 31% of the desired **4**, accompanied by 40% of undesired



Scheme 1.

Keywords: phosphorylation; synthesis; PtdIns(4,5)P2; Ins(1,4,5)P3.
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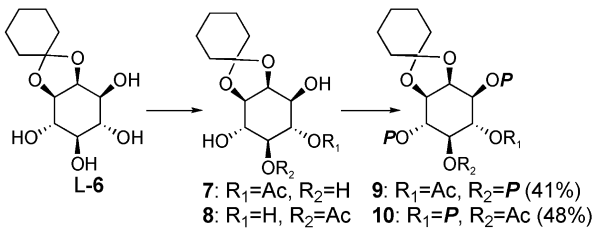
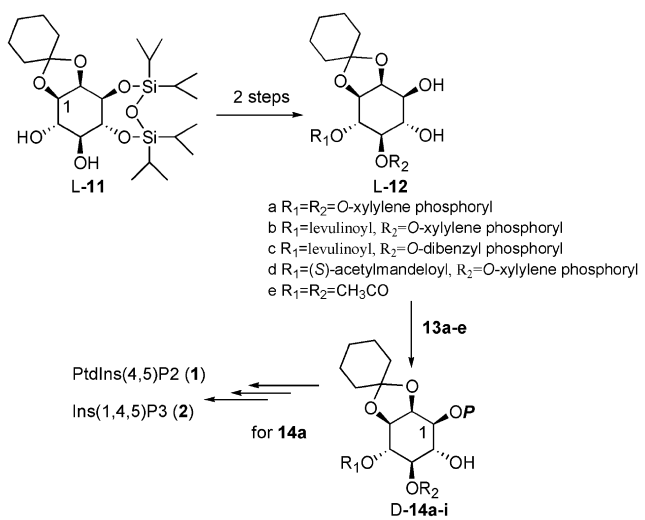


Scheme 2.

regioisomer **5**. Similarly, 41% of desired **9** and 48% of undesired **10** were formed, respectively, in Ozaki's method (Scheme 2), risen from the poor selectivity of acetylation of tetrol **6** to give inseparable mixture of diol **7** and **8**. Therefore, developing more practical methods for the syntheses of PtdIns(4,5) and Ins(1,4,5)P3 are still significant.

On the other hand, we have demonstrated that 1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol (**11**) may be a pivotal intermediate for the synthesis of diverse inositol phosphates such as Ins(4)P,[12a] Ins(5)P,[12a] Ins(1,3,4)P3,[12a] and phosphatidylinositol phosphates such as PI(5)P2,[12b] PI(3,4)P2,[13] PI(3,4,5)P3,[14] and PIM2.[15] In these cases, D-**11** was derived into the target molecules with the natural configuration. The opposite L-**11** has been the 'waste' one until now, whereas both enantiomers can be readily derived from the selective disiloxanylation with 1,3-dichlorotetraisopropyl-disiloxane[14b] of the corresponding D and L-1,2-*O*-cyclohexylidene-*myo*-inositol obtained by the enzyme-aided optical resolution[11b] with excellent yields (higher than 42% from *myo*-inositol, respectively). The utilization of L-**11** (Scheme 3) and the practical synthesis of **1** and **2** are of particular interest to us. These challenges have been accomplished in the synthesis of dipalmitoyl PtdIns(4,5)P2 (**1**) from L-**11**,[16] where regioselective phosphorylation of 1,2-*O*-cyclohexylidene-5,6-dis-*O*-(*o*-xylylene phosphoryl)-*myo*-inositol L-**12a** at 3-position, as a key reaction, provided a very rapid route to get **1**. We are interested in the extremely selective phosphorylation, and investigated furthermore the selectivity in the phosphorylation of various 3,4-diol derivatives of *myo*-inositol with different



Scheme 3.

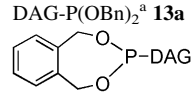
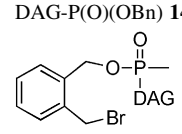
phosphites. Other electrophilic reactions such as benzoylation and silylation were also examined. In addition, to expand the application of the practical methodology, we studied the synthesis of D-Ins(1,4,5)P3 from L-**11**. The synthetic procedure of dipalmitoyl PtdIns(4,5)P2 **1** is also detailed.

2. Results and discussion

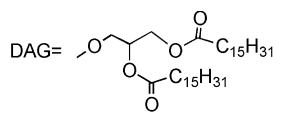
2.1. Phosphorylation of various vicinal 3,4-diols (12)

Although the phosphorylation of hydroxyls in 1,2-diol system of suitably protected *myo*-inositols have been well documented to give regiospecifically 1-*O*-phosphates with good yields,[7a,17] the selectivity of hydroxyls in 3,4 (1,6)-diol inositol derivatives is unpredictable based on these results because both 3 and 4-OH are equatorial hydroxyls to contrast with equatorial/axial 1,2-dihydroxyls, where 2-OH shows poor reactivity due to the sterically hindered axial orientation.[18] On the other hand, some reports[19] indicated that 3-OH on 1,2-ketal **6** and 1,2:5,6-diketal is more reactive than other OHs in their electrophilic reactions such as acylation, silylation and alkylation. Although some reports showed completely different results where the selectivity is poor,[20,21] even in some cases 4- and/or 5-OH are more reactive.[11,22] To facilitate the synthesis of dipalmityl PtdIns(4,5)P2 (**1**) and Ins(1,4,5)P3 (**2**), as well as to utilize the formerly 'wasted' L-**11**, we therefore studied the phosphorylating reaction of 3,4-diol **12** (racemic materials were used here), such reaction has rarely been investigated previously. Preliminary study on the phosphorylation of **12a** with 1,2-di-*O*-palmitoylglycerol phosphite **13a** in the presence of pyridinium tribromide regiospecifically occurred at the 3-OH position to give 3-monophosphate **14a** in excellent yield (Table 1, entry 1). To define the scope

Table 1. Phosphorylation of racemic diol **12** with various phosphites

Entry	Diol	Phosphite (**13**)	*P* in compound **14**	yield (%)
1	**12a**	DAG-P(OBn)$_2$[a] **13a**	DAG-P(O)(OBn) **14a**	86
2	**12a**	 **b**	 **b**	63
3	**12a**	DAG-P(OBn)$_2$ **13c**	DAG-P(O)(OBn) **14c**	88
4	**12a**	DAG-P(OMe)$_2$ **13d**	DAG-P(O)(OBn) **14d**	89
5	**12a**	P(OBn)$_3$ **13e**	P(O)(OBn)$_2$ **14e**	91
6[b]	**12b**	**13e**	P(O)(OBn)$_2$ **14f**	89
7	**12c**	**13e**	P(O)(OBn)$_2$ **14g**	91
8	**12d**	**13e**	P(O)(OBn)$_2$ **14h**	86
9	**12e**	**13e**	P(O)(OBn)$_2$ **14i**	86

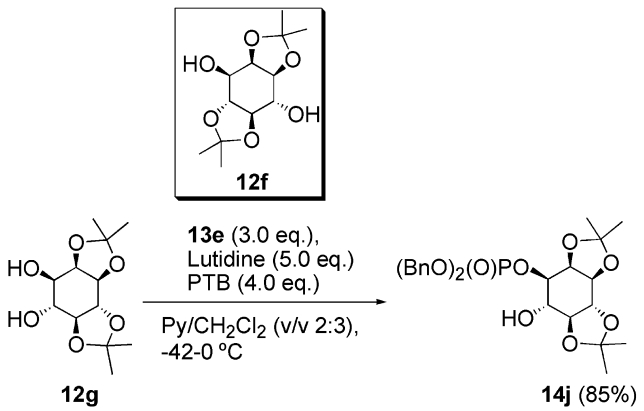
General reaction conditions: phosphite (3 equiv.), 2,6-lutidine (5 equiv.), pyridinium tribromide (PTB) (4 equiv.), −42°C, 10 min then 0°C, 1.5–2.0 h, solvent CH_2Cl_2 unless otherwise noted.

[a] DAG= 

[b] Solvent: CH_2Cl_2/Py (v/v 3:1).

of this reaction, we investigated the phosphorylation of **12a** with various phosphites **13b-e** using the phosphite-pyridinium tribromide approach. The reaction proceeded exclusively at the OH-3 position in all cases as listed in Table 1 (entries 2–5), without the formation of the another regioisomer and/or bisphosphorylated product as analyzed by TLC and NMR. The phosphorylation position was determined by transforming the phosphates **14** (except for **14e**) into their 4-*O*-chloroacetyl derivatives because signals corresponding to InsH-4 overlapped with those of α and γ-methylene protons in the glycerol moiety. The ^{1}H NMR spectra, combined with the ^{1}H–^{1}HCOSY, clearly showed that in each case the chemical shift of InsH-4 shifted downfield about 1.4 ppm. The results, summarized above, suggest that other vicinal 3,4-diol compounds might also show a good selectivity. To test this prediction, we have examined the phosphorylation reaction of various 3,4-diols bearing different substituents at 5- and 6-positions (**12b-e**) with tribenzyl phosphite **13e** (entries 6–9). As expected, in all cases, only 3-mono-phosphorylated products, **14f-i** were isolated in high yields, indicating the selectivity is less affected by substituents at 5- and 6-positions.

According to the literature,[20] such a good selectivity is probably due to the intramolecular hydrogen bonding between the 3-OH and oxygen at C-2, which results in the enhancement of the acidity of 3-OH. In addition, we thought that another plausible explanation for the high reactivity of 3-OH in vicinal 3,4-diol derivatives is due to their conformations. By comparison the vicinal coupling constants of inositol methine protons of 3,4-diol **12a-e** with those of the already reported chair-form 3,4-diketal **12f**[20] (Scheme 4 and Table 2), conformations of the all investigated 3,4-diol monoketal **12a-e** were apparently deviated from a normal chair-form. Conformation of **12a-e** deduced



Scheme 4.

Table 2. Coupling constants of some representative examples of diol **12** observed in $CDCl_3$

Compounds	Coupling constants (*J*, Hz)					
	H1–H2	H2–H3	H3–H4	H4–H5	H5–H6	H6–H1
12a	5.6	3.7	7.8	7.4	8.8	6.4
12e	5.6	4.0	8.8	8.8	9.5	7.2
12f[a]	4.8	4.8	9.4	9.4	10.5	6.4
12g[a]	6.2	4.2	5.3	9.1	10.2	8.1

[a] For the coupling constants, see also Ref. 20

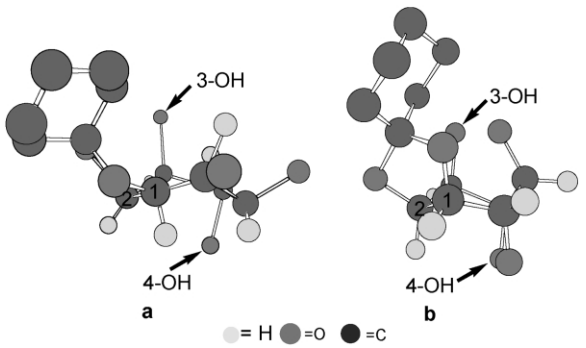
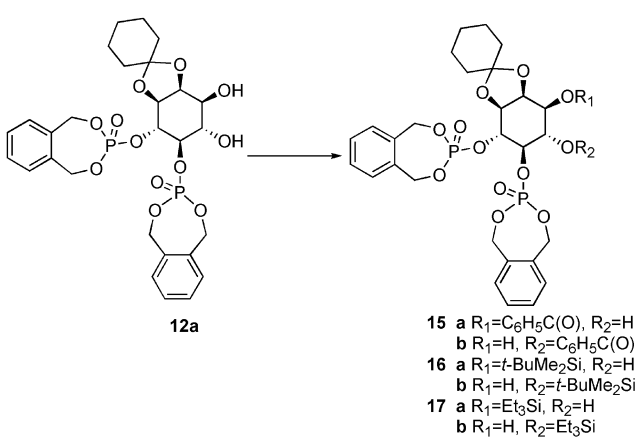


Figure 1. (a) Conformation of L-**12a** deduced from the coupling constants and MM-2 calculation as a represntative example of 3,4-diol monoketal derivatives **12a-e**; (b) chair-form conformation.

form the coupling constants and MM-2 calculation (Fig. 1(a)) suggests that it rather takes a boat-like one, furthermore, the 3-OH flipped to take an axial-like orientation. Such a conformation makes the 3-OH less crowded, but 4-OH sterically hindered to compare with a chair-form one (Fig. 1(b)), resulting in the higher reactivity of 3-OH than that of 4-OH. As a support experiment to examine our hypothesis, we conducted the phosphorylation of 1,2:5,6-diketal **12g**, which has been well defined to take a boat-form conformation because of the locked ring system.[20] Similarly, only 3-mono-phosphorylated product **14j** was obtained in high yield in the presence of 3 equiv. of phosphite **13e**. Whilst the selective functionalization of 1,2:4,5-diketal such as **12f** and dicyclohexylidene derivative was not always good,[20,23] di-functionalizd and/or 6-mono-functionalized by-product were isolated, in addition to the 3-mono-functionalized major product. Since 1,2:4,5-diketal can also form intramolecular hydrogen bond,[20] the selectivity differences between the boat-like 3,4-diol and chair-form 1,2:4,5-diketal may be caused by their different conformations. Thus, intramolecular hydrogen bonding as well as the favourable conformation may be the factors to influence the reactivity of 3- and 4-OH. Whilst the main reason is yet not clear.

2.2. Benzoylation and silylation of 3,4-diol (Scheme 5)

The remarkably different reactivity between 3- and 4-hydroxyls in vicinal 3,4-diol derivatives in the phosphorylation is of interest, and therefore intrigued us to study



Scheme 5.

Table 3. Benzoylation and silylation of vicinal 3,4-diol **12a**

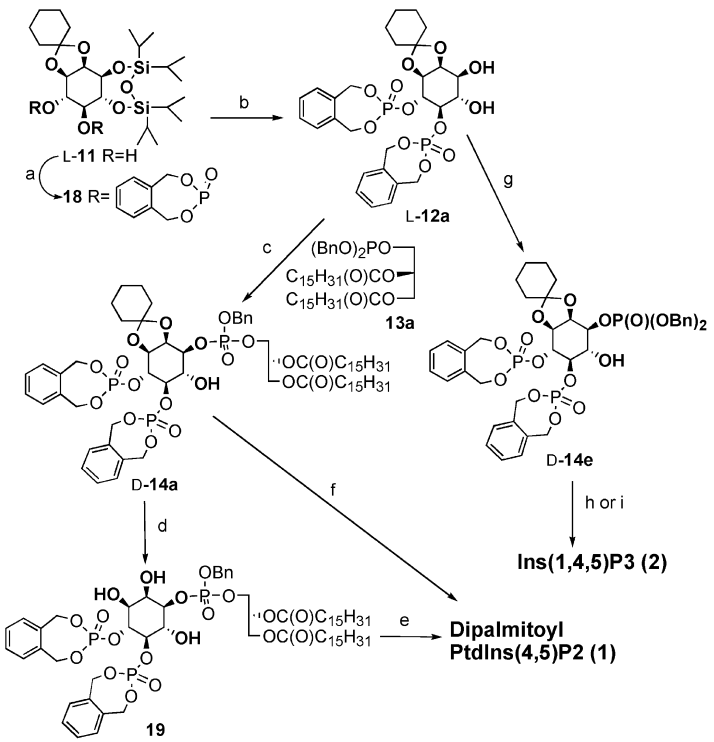
Entry	Reaction conditions	Yield (%) of the products[a]		S. M.[b] (%)
1	C_6H_5COCl (1.2 equiv.), Py (3.0 equiv.), DMAP[c] (cat.), 0°C, 5.3 h	**15a** (63)	**15b** (1)	ND[d]
2	C_6H_5COCl (2.0 equiv.), Py (3.0 equiv.), DMAP (cat.), 0°C, 4 h	**15a** (87)	**15b** (2)	
3	*t*-$BuMe_2SiCl$ (1.1 equiv.), imidzole (3.3 equiv.), DMF, rt, 16 h	**16a/16b**[e] (total yield 47%)		ND
4	*t*-$BuMe_2SiCl$ (1.1 equiv.), TBMP[f] (3.0 equiv.), DMAP[f] (cat.), CH_2Cl_2, rt, 16 h	**16a/16b** (total yield 51%)		ND
5	Et_3SiCl (1.05 equiv.), EDA[g] (3.0 equiv.), DMAP (cat.), CH_2Cl_2, rt, 14 h	**17a** (18.0)	**17b** (24)	28
6	Et_3SiCl (1.05 equiv.), EDA (3.0 equiv.), DMAP (cat.), CH_2Cl_2, 0°C, 4.5 h	**17a** (21)	**17b** (21)	33

[a] Isolated by column chromatography unless noted otherwise.
[b] Starting material recovered.
[c] DMAP: *N*,*N*-dimethylamino pyridine.
[d] Not determined.
[e] Inseparable regioisomer, the ratio of the regioisomer **16a/16b** was about 2:1 as determined by ^1H NMR analysis.
[f] TBMP: 2,6-*t*-butyl-4-methyl pyridine.
[g] EDA: ethyldiisopropylamine.

other electrophilic substitution reactions. Herein diol **12a** was chosen as a representative example of mono-ketal **12a-e** derivatives to study the functionalization such as acylation and silylation (Scheme 5). As outlined in Table 3, acylation of **12a** with benzoyl chloride carried out in different conditions has good selectivity (entries 1 and 2). Reaction carried out at 0°C occurred regiospecifically at 3-OH to give **15a** even if 2 equiv. of the chloride was used (entry 2). Silylation of **12a** with *t*-$BuMe_2SiCl$ under different conditions gave inseparable regioisomers **16a/16b** (entries 3 and 4). The ratio was about 2:1 as determined by the ^1H NMR spectra. Silylation with Et_3SiCl gave almost 1:1 separable regioisomers of **17a/17b** (entries 5 and 6). No disilylated product was detected in each case. The selectivity of silylation is poor, whilst that of acylation is quite good. The significant selectivity differences between the acylation and silyaltion led us to look into the reasons. Control experiment showed that migration of silyl groups in both pure **17a** or **17b** occurred to form about 1:1 mixture (monitored by TLC and NMR) of **17a** and **17b** in the presence of EDA and DMAP in CH_2Cl_2. Cat. amount of DMAP played the key role in promoting the migration. Such results suggested that the poor selectivity for the silylation of **12a** was indeed caused by the migration of silyl group in the real reaction system. This result may elucidate the previous experimental results that the selectivity for the silylation of 3- and 4-OH in diketal **12g** was also low.[20]

2.3. Synthesis of dipalmitoyl D-PtdIns(4,5)P2 (1) and D-Ins(1,4,5)P3 (2) (Scheme 6)

The outstanding regioselectivity of 3-OH in 3,4-diols **12a-e** provides a convenient route for the synthesis of PtdIns(4,5)P2[16] and Ins(1,4,5)P3. Thus, L-**11** was trans-



Scheme 6. *Reagents and conditions*: (a) *o*-xylylene *N*,*N*-diethyl phosphoramidite (XEPA), 1*H*-tetrazole, CH_2Cl_2, then *m*CPBA, 95%; (b) TBAF·$3H_2O$, HOAc, THF, −20°C, 81%; (c) $PyHBr_3$ (PTB), 2,6-lutidine, CH_2Cl_2, −42 to 0°C, 88%; (d) Py(HF)n, CH_2Cl_2, 0°C to rt, 1.5 h, 92%; (e) 10%-Pd/C-H_2 (25 wt%), EtOH/$CHCl_3$ (1:1), rt, overnight, quant.; (f) 10%−Pd/C−H_2, EtOAc, rt, 2.5 days, quant.; (g) $(BnO)_3P$, 2,6-lutidine, PTB, −42 to 0°C, 91%; (h) 10%−Pd/C−H_2 (25 wt%), EtOH; then H_2O/HOAc (2:1), 2 days, quant.; (i) 10%−Pd/C−H_2 (25 wt%), tributylamine (2 equiv.), MeOH/$CHCl_3$ (1:1), 3 days, quant.

formed into the diol L-**12a** through the phosphorylation with *o*-xylylene *N*,*N*-diethyl-phosphoramidite (XEPA)[24] to give bisphosphate **18**, followed by the desilylation. Phosphorylation of L-**12a** with 1,2-di-*O*-palmitoyl-*sn*-glycerol phosphite **13a** afforded D-**14a** as described above. With compound D-**14a** in hand, the final product, D-dipalmitoyl PtdIns(4,5)P2 **1** might be obtained by removal of the cyclohexylidene and benzylic groups. The cleavage of the cyclohexylidene ketal could be achieved smoothly by using pyridinium poly(hydrogen fluoride), which was used to decompose the isopropylidene group without the migration of the adjacent phosphate function.[25] The triol **19** thus obtained was hydrogenated to afford dipalmitoyl **1**. We eventually found that such a two-step strategy might be combined into a one-step procedure when the hydrogenolysis of D-**14a** was performed in a commercial grade EtOAc, resulting in the simultaneous removal of the cyclohexylidene ketal to give dipalmitoyl **1** in quantitative yield as its triethylammonium salt. No other undesired products were detected by TLC and spectral analyses. A comparison of EtOAc to MeOH suggested that an aprotic solvent was much more efficient than a protic one in promoting the spontaneous removal of the cyclohexylidene ketal, probably due to the protic solvent solvating the proton of the phosphoric acid, as a result, the catalytic ability of the phosphoric acid was decreased.

In a similar manner, Ins(1,4,5)P3, **2** was synthesized as follows. Phosphorylation of L-**12a** using tribenzyl phosphite **13e** afforded D-**14e** in 91% yield without formation of the other isomers. Spontaneous removal of cyclohexylidene ketal and benzylic groups in D-**14e** was first tried in EtOAc, however, it was not effective because the intermediates partially or completely hydrogenated would precipitate out from the solvent. Bruzik's[26] protocol employing MeOH as solvent was also not satisfactory. Several solvent system such as MeOH/EtOAc/triethylamine, MeOH/$CHCl_3$/triethylamine, MeOH/EtOAc/tributylamine and MeOH/$CHCl_3$/tributylamine were scanned. We finally found that when the hydrogenolysis was performed in a mixture of $CHCl_3$ and MeOH (v/v=1:1) in the presence of 2 equiv. of tributylamine, the cyclohexylidene ketal could be cleaved simultaneously to afford **2** completely. In this case, the amine and MeOH were necessary to dissolve the intermediates partially or completely hydrogenated in the medium. The ammonium salt of **2** obtained from our one-step procedure was transformed into its sodium salt by passing through the sodium form of ion-exchange resin. Alternatively, removal of benzylic groups followed by the cleavage of cyclohexylidene ketal using Vacca's protocol[27] was also applicable once the cleavage of cyclohexylidene ketal was performed at a 1:2 (v/v) H_2O/HOAc system, although the essential condition was proved to be difficult to cleave the ketal completely.

In summary, the finding as of the remarkable selective functionalization of 3-OH in vicinal 3,4-diol system, as well as the one-step deprotection of ketal and benzylic groups in the final stage, provided a practical methodology for the synthesis of dipalmitoyl PtdIns(4,5)P2 (**1**) and Ins(1,4,5)P3 (**2**) from L-**11**. It is the shortest and more practical method for the synthesis of **1** compared with the reported methods. For Ins(1,4,5)P3 (**2**), although this method presented here is not the shortest one, it refrains from the formation of the undesired regioisomers as appeared in the old methods, and thus makes this method advantageous.

3. Experimental

3.1. General methods

All solvents were purified according to the standard procedures. Reagents were reagent grade, and purified where further purification being required. Pyridinium tribromide (PTB) was purified by recrystallization from AcOH. 2,6-Lutidine, 1,3-dichlorotetraisopropyldisiloxane, benzoyl chloride and Et_3SiCl were purified by distillation under reduced pressure. NMR spectra were recorded on Bruker Avance 400 unless otherwise noted. Optical rotation was measured using JASCO P-1010 with 1 cm cell. Elemental analyses were performed on Perkin–Elmer 240C. Melting points were uncorrected and tested in open capillaries by using Yamato Melting Point Apparatus Model MP-21. Silica gel (Fuji Silysia Chemical Ltd., 200–400 mesh) was used for flash chromatography.

3.2. A general procedure for the phosphorylation of a diol 12 with a phosphite

To a CH_2Cl_2 solution of a diol **12** and phosphite **13** (3 equiv.) were added dropwise 2,6-lutidine (5 equiv.) and pyridinium tribromide (PTB, 4 equiv.) at −42°C. The solution was stirred vigorously at the same temperature for 10 min, and then at 0°C (an ice/H_2O including a small amount of NaCl bath) for about 2 h. After being diluted with EtOAc, the solution was washed with aq. $KHSO_4$, aq. $NaHCO_3$ and brine. The organic layer was dried, concentrated, and chromatographed (hexane/EtOAc=1:2) to afford **14**.

3.2.1. Compound 14a. The title compound (86%) as colorless gum. R_f=0.65 (hexane/EtOAc=1:3); ^{1}H NMR (400 MHz) δ: 7.31–7.39 (m, 13H), 5.54–5.65 (complex, 2H, ArCH_2O), 5.30 (t, 1H, J=13.6 Hz, ArCH_2O), 5.04–5.23 (complex, 8H in ArCH_2O, glyceryl 2-*H*), 4.85 (br 2×q, 1H, J=9.0 Hz, InsH-6), 4.64–4.71 (complex, 1.5H, InsH-3 and H-2), 4.60 (br 2×t, 0.5H, J=4.0 Hz, InsH-2), 4.39 (br 2×dd, 1H, J=4.4, 9.0 Hz, InsH-5), 4.11–4.35 (complex, 6H, InsH-1, H-4, glyceryl H-1, H-3), 2.28 (m, 4H, Pal H-2), 1.82 (m, 2H, cyclohexylidene H), 1.53–1.67 (br, 12H, Pal 3-H, cyclohexylidene H), 1.25 (br, 48H, Pal H4-15), 0.88 (t, 6H, J=6.2 Hz Pal CH_3); ^{13}C NMR (100.6 MHz) δ: 173.73 (2C), 173.59 (2C), 173.46, 173.33, 173.22, 173.17 (Pal CO), 128.30–135.89 (complex, aromatic C), 112.38 and 112.37 (spiral C), 79.57–80.13 (complex, InsC-5 C-6), 75.90–76.70 (m, InsC-3), 74.57 and 74.49 (m each, InsC-2), 68.63–71.80 (complex, InsC-4, glyceryl 2-C and ArCH_2O), 65.90–66.34 (m, glyceryl 3-C), 61.80–62.30 (m, glyceryl 1-C), 37.90 and 37.86 (cyclohexylidene C), 35.44, 34.40–34.55 (complex, Pal C-2), 32.31 (complex, Pal C-14), 29.49–30.10 (complex, Pal C4-13), 25.22 (complex, Pal C-3, cyclohexylidene C), 24.06 and 24.21 (s each, cyclohexylidene C), 23.08 (s, Pal C-15), 14.51 (s, Pal C-16), signals for InsC-1 was overlapped with those of $CDCl_3$; ^{31}P NMR (162 MHz) δ: −0.21, −0.23, −0.27 (s

each, 1P), −0.31, −0.44, −0.47 (s each, 1P), −0.50, −0.56, −0.61 (s each, 1P); Anal. Calcd. for $C_{70}H_{107}O_{19}P_3$: C, 62.48; H, 8.02; found: C, 62.10; H, 8.02.

3.2.2. Compound 14b. The title compound (63%) as colorless gum. R_f=0.66 (hexane/EtOAc=1:3); ^{1}H NMR (400 MHz) δ: 7.29–7.39 (m, 13H), 5.53–5.64 (complex, 2H, ArCH_2O), 5.29 (t, 1H, J=13.2 Hz, ArCH_2O), 5.08–5.28 (complex, 8H, ArCH_2O, glyceryl 2-H), 4.85 (br 2×q, 1H, J=9.0 Hz, InsH-6), 4.64–4.73 (complex, 1.5H, InsH-3, H-2), 4.54–4.63 (complex, 2.5H, InsH-2, CH_2Br), 4.40 (dd×2, 1H, J=4.0, 9.0 Hz, InsH-5), 4.01–4.36 (complex, 6H, InsH-1, H-4, glyceryl 1-H, 3-H), 2.28 (complex, 4H, Pal H-2), 1.82 (br, 2H, cyclohexylidene H), 1.38–1.64 (br, 12H, Pal H-3, cycohexylidene H), 1.25 (br, 48H, Pal H4-15), 0.89 (t, 6H, J=6.8 Hz, Pal CH_3); ^{31}P NMR (162 MHz) δ: −0.20, −0.22, −0.27 (s each, 1P), −0.32, −0.45, −0.48 (s each, 1P), −0.52, −0.59, −0.63 (s each, 1P).

3.2.3. Compound 14c. The title compound (88%) as colorless gum. R_f=0.60 (hexane/EtOAc=1:3); ^{1}H NMR (400 MHz) δ: 7.30–7.39 (m, 13H), 5.55–5.65 (complex, 2H, ArCH_2O), 5.28 (t, 1H, J=13.2 Hz, ArCH_2O), 5.07–5.27 (complex, 8H, ArCH_2O, glyceryl 2-H), 4.85 (br 2×q, 1H, J=9.0 Hz, InsH-6), 4.64–4.73 (complex, 1.5H, InsH-3, H-2), 4.54 and 4.59 (2×t, 0.5H, J=4.0 Hz, InsH-2), 4.40 (2×dd, 1H, J=4.0, 9.0 Hz, InsH-5), 4.08–4.36 (complex, 6H, InsH-1 and H-4, glyceryl H-1, H-3), 2.28 (complex, 4H, Oct H-2), 1.82 (br, 2H, cyclohexylidene H), 1.38–1.64 (br, 12H, Oct H-3, cycohexylidene H), 1.25 (br, 16H, Oct H3-6), 0.87 (t, 6H, J=6.8 Hz, Oct CH_3); ^{31}P NMR (162 MHz) δ: −0.25, −0.27, −0.32 (s each, 1P), −0.33, −0.46 (0.5P) (s each, 1P), −0.58, −0.64 (s each, 1P).

3.2.4. Compound 14d. The title compound (89%) as colorless gum: R_f=0.59 (hexane/EtOAc=1:3); ^{1}H NMR (400 MHz) δ: 7.28–7.37 (m, 8H), 5.57–5.63 (complex, 2H, ArCH_2O), 5.30 (t, 1H, J=13.0 Hz, ArCH_2O), 5.03–5.25 (complex, 6H, ArCH_2O glyceryl 2-H), 4.85 (br 2×q, 1H, J=8.8 Hz, InsH-6), 4.52–4.68 (complex, 2H, InsH-3, H-2), 4.42 (2×dd, 1H, J=4.0, 8.8 Hz, InsH-5), 4.12–4.30 (complex, 6H, InsH-1 and H-4, glyceryl 1-H, 3-H), 3.79–3.87 (complex, 3H, OMe), 2.32 (complex, Oct H-2), 1.82 (br, 2H, cycohexylidene H), 1.40–1.65 (br, 12H, Oct H-3, cycohexylidene, H), 1.28 (br, 16H, Oct H4-6), 0.86 (t, 6H, J=7.2 Hz, Oct CH_3); ^{31}P NMR (162 MHz) δ: 0.59, 0.54, 0.52, 0.42 (s each, 1P), −0.23, −0.28, −0.31 (s each, 1P), −0.57, −0.58, −0.63 (s each, 1P).

3.2.5. Compound 14e. The title compound (91%) as white solid. R_f=0.41 (hexane/EtOAc=1:3); ^{1}H NMR (400 MHz) δ: 7.31–7.39 (m, 18H), 5.53–5.62 (complex, 2H, ArCH_2O), 5.30 (t, 1H, J=13.6 Hz, ArCH_2O), 5.01–5.22 (complex, 9H, ArCH_2O), 4.85 (dt, 1H, J=7.4, 9.2 Hz, InsH-6), 4.60 (dd, 1H, J=4.8, 9.0 Hz, InsH-3), 4.55 (t, 1H, J=4.8 Hz, InsH-2), 4.39 (dt, 1H, J=7.4, 9.2 Hz, InsH-5), 4.29 and 4.30 (2×t, 1H, J=7.4, 9.0 Hz, InsH-4), 4.22 (dd, 1H, J=4.8, 7.4 Hz, InsH-1), 1.82 (br, 2H, cyclohexylidene H), 1.51–1.64 (br, 6H, cyclohexylidene H), 1.25 (br, 2H, cyclohexylidene H); ^{13}C NMR (100.6 MHz) δ: 135.08–135.73 (m, aromatic C), 127.92–129.21 (m, aromatic C), 112.43 (s, spiral C), 79.65 (t, J=5.8 Hz, InsC-6), 79.23 (t, J=5.1 Hz, InsC-5), 76.30 (d, J=2.2 Hz, InsC-1), 75.81 (d, J=5.8 Hz, InsC-3), 74.09 (d, J=0.9 Hz, InsC-2), 70.79 (d, J=5.1 Hz, InsC-4), 69.85 (d, J=5.7 Hz), 69.52 (d, J=5.8 Hz), 69.31 and 69.12 (2×d, J=7.3 Hz), 68.90 (d, J=7.0 Hz), 68.68 (d, J=7.0 Hz) (ArCH_2O), 37.50 (s), 34.98 (s), 29.70 (s), 24.85 (s), 23.82 (s), 23.69 (s) (cyclohexylidene); ^{31}P NMR (162 MHz) δ: −0.23 (s), −0.27 (s), −0.46 (s). Anal. Calcd. for $C_{42}H_{47}O_{15}P_3$: C, 57.02; H, 5.35; found: C, 56.84; H, 5.47.

3.2.6. Compound 14f. The title compound (89%) as white solid. R_f=0.38 (hexane/EtOAc=1:3); ^{1}H NMR (400 MHz) δ: 7.34 (m, 14H, aromatic H), 5.28–5.37 (complex, 3H, InsH-6 and ArCH_2O), 5.06–5.19 (complex, 6H, ArCH_2O), 4.56 (dt, 1H, J=4.0, 9.2 Hz, InsH-3), 4.47 (dd, 1H, J=4.0, 4.8 Hz, InsH-2), 4.38 and 4.36 (t×2, 1H, J=9.2 Hz, InsH-5), 4.26 (t, 1H, J=9.2 Hz, InsH-4), 4.12 (dd, 1H, J=4.8, 6.8 Hz, InsH-1), 2.67–2.76 (complex, 4H, CH_2CH_2), 2.15 (s, 3H, CH_3), 1.81 (complex, 2H, cyclohexylidene), 1.63 (br, 2H, cyclohexylidene), 1.50 (br, 4H, cyclohexylidene), 1.26 (br, 2H, cyclohexylidene); ^{31}P NMR (162 MHz) δ: 0.29 (1P), 0.01 (1P).

3.2.7. Compound 14g. The title compound (91%) as white solid. R_f=0.35 (EtOAc/hexane=4:1); mp 155.5–157.0°C (sample was obtained by chromatography); ^{1}H NMR (400 MHz) δ: 7.35 (m, 20H, 4×C_6H_5), 5.26 (dd, 1H, J=7.4, 8.8 Hz, InsH-6), 4.50–5.19 (complex, 8H, 4×$C_6H_5CH_2$), 4.44–4.50 (complex, 2H, InsH-3, H-2), 4.13–4.23 (complex, 2H, InsH-4, H-5), 4.04 (dd, 1H, J=4.8, 7.4 Hz, InsH-1), 2.44–2.57 (complex, 4H, CH_2CH_2), 2.06 (s, 3H, CH_3), 1.60 (br, 2H, cyclohexylidene), 1.48 (br, 6H, cyclohexylidene), 1.33 (br, 2H, cyclohexylidene); ^{13}C NMR (100.6 MHz) δ: 206.52 (s), 172.17 (s), 136.16 (s), 136.08 (s), 136.01 (s), 135.94 (s), 128.26–129.50 (m), 112.01 (s), 79.04 (br), 76.50 (br), 75.93 (m), 74.56 (s), 73.91 (t, J=3.3 Hz), 70.28 (s), 70.23 (s, 2C), 70.17 (s), 69.97 (d, J=5.5 Hz), 38.04 (s), 37.69 (s), 35.39 (s), 30.10 (s), 28.29 (s), 25.34 (br), 24.15 (s), 23.98 (s); ^{31}P NMR (162 MHz) δ: 0.45 (1P), −0.05 (1P); calcd for $C_{45}H_{52}O_{14}P_2{\cdot}0.5H_2O$: C, 60.87; H, 6.02; found: C, 61.02; H, 6.24.

3.2.8. Compound 14h. The title compound (86% yield) as colorless gum. R_f=0.43 (hexane/AcOEt=1:2); ^{1}H NMR (400 MHz) δ: 7.28–7.47 (m, 28H, aromatic), 6.08 (s, 1H, methenyl), 6.05 (s, 1H, methenyl), 5.38 (dd, 2H, J=7.6, 9.2 Hz, InsH-6), 5.27–5.34 (complex, 2H, ArCH_2O), 4.71–5.18 (complex, 14H, ArCH_2O), 4.50–4.57 (complex, 3H, InsH-3 and InsH-2), 4.39 (dd, 1H, J=4.0, 4.8 Hz, InsH-2), 4.28–4.36 (complex, 4H, InsH-5 and InsH-4), 4.22 (dd, 1H, J=4.8, 7.6 Hz, InsH-1), 3.79 (dd, 1H, J=4.8, 7.6 Hz, InsH-1), 2.18 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 1.75 (br, 2H, cyclohexylidene), 1.48–1.61 (br, 6H, cyclohexylidene), 1.31 (br, 2H, cyclohexylidene); ^{31}P NMR (162 MHz) δ: 0.36 (1P), −0.19 (1P), −0.27 (2P).

3.2.9. Compound 14i. The title compound (86%) as white solid. ^{1}H NMR (400 MHz) δ: 7.29–7.37 (m, 10H, aromatic), 5.22 (dd, 1H, J=7.2, 9.6 Hz, InsH-6), 5.01–5.15 (complex, 4H, ArCH_2O), 4.83 (dd, 1H, J=9.2, 9.6 Hz, InsH-5), 4.51 (dt, 1H, J=4.0, 9.2 Hz, InsH-3), 4.44 (dd, 1H, J=4.0, 5.0 Hz, InsH-2), 4.14 (t, 1H, J=9.2 Hz, InsH-4), 4.10 (dd, 1H, J=5.0, 7.2 Hz, InsH-1), 2.07 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 1.79 (m, 2H, cyclohexylidene), 1.63 (br, 2H,

cyclohexylidene), 1.61 (br, 4H, cyclohexylidene), 1.32 (br, 2H, cyclohexylidene); ^{31}P NMR (162 MHz) δ: −0.33 (1P).

3.2.10. Phosphorylation of 1,2:5,6-di-*O*-isopropylidene *myo*-inositol (12g). To a pyridine (4 mL) and CH_2Cl_2 (6 mL) solution of **12g** (100 mg, 0.292 mmol) was added 2,6-lutidine (171.6 μL, 1.462 mmol), tribenzyl phosphite **13e** (308.8 mg, 0.877 mmol) and pyridinium tribromide (PTB, 374.3 mg, 1.170 mmol) at −42°C. The mixture was stirred at the same temperature for 10 min, then 0°C for 2 h. After being diluted with EtOAc, the solution was washed with $KHSO_4$ aq., $NaHCO_3$ aq., and brine, the organic layer was dried, concentrated and chromatographed (EtOAc/hexane=1:1) to afford **14j** (169 mg, 85%; gum solidifies on standing). R_f=0.33 (EtOAc/hexane=2:1); ^{1}H NMR (400 MHz) δ: 7.36 (m, 10H, 2×C_6H_5), 5.03–5.14 (complex, 4H, 2×$C_6H_5CH_2$), 4.48 (dd, 1H, J=4.4, 5.6 Hz, InsH-2), 4.21 (ddd, 1H, J=4.4, 6.4, 8.8 Hz, InsH-3), 4.24 (dd, 1H, J=5.6, 8.4 Hz, InsH-1), 4.16 (dd, 1H, J=6.4, 10.0 Hz, InsH-4), 3.79 (dd, 1H, J=8.4, 10.0 Hz, InsH-6), 3.33 (t, 1H, J=10.0 Hz, InsH-5), 1.47 (s, 3H, CH_3), 1.43 (s, 6H, 2×CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR (100.6 MHz) δ: 135.58, 135.54, 135.50, 135.47, 128.72, 128.68, 128.65, 128.63, 128.54, 128.44, 128.06, 128.03 (aromatic C), 112.58, 110.78 (spiral C), 79.89 (d, J=6.3 Hz), 78.32 (s), 77.32 (s), 76.07 (s), 75.31 (d, J=3.8 Hz), 71.80 (d, J=4.1 Hz) (InsC-1-C-6), 69.86 (d, J=5.6 Hz), 69.72 (d, J=5.6 Hz) (CH_2), 27.88 (s, CH_3), 26.88 (s, 2C, CH_3), 25.64 (s, CH_3); ^{31}P NMR (162 MHz) δ: 0.39. calcd for $C_{26}H_{33}O_9P\cdot0.5H_2O$: C, 58.97; H, 6.47; found: C, 58.86, H, 6.30.

3.2.11. Benzoylation of 1,2-*O*-cyclohexylidene-5,6-bis-*O*-(*o*-xylylene phosphoryl)-*myo*-inositol 12a. *Entry* 1. To a CH_2Cl_2 (2 mL) solution of **12a** (62 mg, 0.099 mmol), pyridine (24.0 μL, 0.298 mmol) and DMAP (cat.) was added benzoyl chloride (11.6 μL, 0.10 mmol) at 0°C for 5.3 h. After being diluted with EtOAc, the solution was washed with $KHSO_4$ aq., $NaHCO_3$ aq., and brine, the organic layer was dried, concentrated, and chromatographed (EtOAc/hexane=1:1) to afford **15a** (33.0 mg, 63%) as white solid. Physical and spectra data of **15a**: R_f=0.58 (EtOAc/hexane=3:1); ^{1}H NMR (400 MHz) δ: 8.10 (dd, 2H, J=1.2, 7.8 Hz, C_6H_5), 7.58 (t, 1H, J=7.8 Hz, C_6H_5), 7.46 (t, 2H, J=7.8 Hz, C_6H_5), 7.18–7.39 (complex, 8H, xylylene H), 5.57–5.67 (complex, 2H, ArCH_2O), 5.44 (dd, 1H, J=3.8, 7.8 Hz, InsH-3), 5.35 (t, 1H, J=13.4 Hz, ArCH_2O), 4.97–5.25 (complex, ArCH_2O, InsH-6), 4.65 (dd, 1H, J=3.8, 5.4 Hz, InsH-2), 4.56 (2×dd, 1H, J=7.0, 10.0 Hz, InsH-5), 4.38 (complex, 2H, InsH-1, H-4), 1.76 (complex, 2H, cyclohexylidene H), 1.58 (complex, 6H, cyclohexylidene H), 1.28 (br, 2H, cyclohexylidene H); ^{13}C NMR (100.6 MHz) δ: 165.94 (C=O), 135.50, 135.46, 135.26, 135.11, 133.25, 130.05 (2C), 129.64, 129.22 (2C), 129.19, 129.15, 129.13, 129.07, 128.88, 128.79, 128.41, 128.34 (aromatic C), 111.92 (spiral C), 80.7 (t, J=5.2 Hz), 79.77 (t, J=6.0 Hz), 77.24 (s), 73.17 (s), 71.44 (s), 70.97 (s) (InsC-1-C-6), 69.24 (t, 2C, J=7.7 Hz), 68.82 (d, J=6.8 Hz), 68.71 (d, J=6.8 Hz) (ArCH_2O), 37.25, 34.88, 24.92, 23.85, 23.71 (cyclohexylidene C); ^{31}P NMR (162 MHz) δ: −1.53, −1.88. calcd for $C_{35}H_{38}O_{13}P_2\cdot1.8H_2O$: C, 55.23; H, 5.51; found: C, 55.11; H, 5.39.

Entry 2. The reaction was carried out following the same procedure as entry 1 at 0°C for 4 h in the presence of 2.0 equiv. of benzoyl chloride. 86.5% yield for **15a**.

3.2.12. Silylation of 1,2-*O*-cyclohexylidene-5,6-bis-*O*-(*o*-xylylene phosphoryl)-*myo*-inositol 12a. *Entry* 3. To a DMF solution (1.5 mL) of **12a** (80 mg, 0.128 mmol) was added imidazole (28.7 mg, 0.422 mmol) and *t*-BuMe_2SiCl (21.2 mg, 0.141 mmol) at 0°C. The mixture was then stirred at rt for 16 h. After being diluted with EtOAc, the solution was washed with $KHSO_4$ aq., $NaHCO_3$ aq., and brine, the organic layer was dried, concentrated and chromatographed (EtOAc/hexane=1:1) to give the inseparable regioisomers of **16a** and **16b** (44.6 mg, 47%) as white solid. The ratio of the products was determined by their ^{1}H NMR spectra.

Entry 4. To a CH_2Cl_2 solution (2.4 mL) of **12a** (48.6 mg, 0.078 mmol) was added *t*-BuMe_2SiCl (12.8 mg, 0.086 mmol) and 2,6-*t*-butyl-4-methylpyridine (TBMP 47.8 mg, 0.233 mmol) and at 0°C. The mixture was then stirred at rt for 16 h. Workup with the same procedure as entry 4 gave the mixture of **16a** and **16b** (39.3 mg, 51%) as white solid.

Entry 5. To a CH_2Cl_2 solution (3 mL) of **12a** (102 mg, 0.163 mmol) was added diisopropylethylamine (EDA) (63.2 mg, 0.490 mmol), DMAP (cat.) and Et_3SiCl (24.5 mg, 0.163 mmol) at 0°C. The mixture was then stirred at rt for 16 h. After being diluted with EtOAc, the solution was washed with $KHSO_4$ aq., $NaHCO_3$ aq., and brine, the organic layer was dried, concentrated and chromatographed (EtOAc/hexane=1:1) to give **17a** (21.3 mg, 18%), and **17b** (28.8 mg, 23.9%) as white solid.

Compound **17a**. R_f=0.39 ((EtOAc/hexane=2:1); ^{1}H NMR (400 MHz) δ: 7.24–7.36 (complex, 8H, aromatic H), 5.69 (dd, 1H, J=8.2, 13.6 Hz, ArCH_2O), 5.60 (dd, 1H, J=10.0, 13.6 Hz, ArCH_2O), 5.31–5.39 (complex, 2H, ArCH_2O), 4.93–5.18 (complex, 5H, ArCH_2O, InsH-6), 4.36–4.42 (complex, 2H, InsH-2, H-5), 4.26 (dd, 1H, J=5.8, 7.4 Hz, InsH-1), 4.11and 4.10 (2×t, 1H, J=6.9 Hz, InsH-4), 3.94 (dd, 1H, J=3.6, 6.9 Hz), 3.75 (s, OH-4), 1.80 (complex, 2H, cyclohexylidene H), 1.57–1.64 (complex, 6H, cyclohexylidene H), 1.25 (br, 2H, cyclohexylidene H), 1.00 (t, 9H, J=7.9 Hz, CH_3), 0.67 (q, 6H, J=7.9 Hz, CH_2); ^{13}C NMR (100.6 MHz) δ: 135.60, 138.58, 135.31, 135.29, 129.27, 129.18, 129.15, 129.11, 129.06, 128.87, 128.83 (aromatic C), 111.61 (spiral C), 80.45 (t, J=4.6 Hz), 80.14 (dd, J=5.6, 7.3 Hz), 79.62 (t, J=5.6 Hz), 76.58 (d, J=2.0 Hz), 75.34 (s), 71.34 (s) (InsC-1-C-6), 69.30 (d, J=7.2 Hz), 69.13 (d, J=7.2 Hz), 68.81 (d, J=6.9 Hz), 68.69 (d, J=6.9 Hz) (ArCH_2O), 37.00, 34.73, 25.02, 23.88, 23.72 (cyclohexylidene C), 6.76 (s, CH_3), 4.88 (s, CH_2); ^{31}P NMR (162 MHz) δ: −1.55, −2.22; calcd for $C_{34}H_{48}O_{12}P_2Si\cdot H_2O$: C, 53.96; H, 6.66; found: C, 53.94; H, 6.47.

Compound **17b**. R_f=0.28 (EtOAc/hexane=2:1); ^{1}H NMR (400 MHz) δ: 7.31–7.39 (complex, 8H, aromatic H), 5.53 (dd, 1H, J=10.0, 13.4 Hz, ArCH_2O), 5.40 (dd, 1H, J=10.0, 13.4 Hz, ArCH_2O), 5.28 (s, 2H, ArCH_2O), 5.24 (s, 2H, ArCH_2O), 5.09 (dd, 1H, J=13.4, 21.8 Hz, ArCH_2O), 4.99 (dd, 1H, J=13.6, 22.6 Hz, ArCH_2O), 4.76 (q, 1H, J=8.0 Hz,

InsH-6), 4.75 (s, 1H, OH-3), 4.60 and 4.58 (2×dd, J=5.5, 6.3 Hz, InsH-2), 4.40 (dd, 1H, J=3.7, 6.5 Hz, InsH-4), 4.29 (dd, 1H, J=6.5, 8.0 Hz, InsH-5), 4.26 (dd, 1H, J=3.7, 6.3 Hz, InsH-3), 3.93 and 3.92 (2×dd, 1H, J=5.5, 8.0 Hz, InsH-1), 1.73 (complex, 2H, cyclohexylidene H), 1.59 (complex, 6H, cyclhexylidene H), 1.27 (complex, 2H, cyclohexylidene H), 0.95 (t, 9H, J=7.9 Hz, CH_3), 0.62 (q, 6H, J=7.9 Hz, CH_2); ^{13}C NMR (100.6 MHz) δ: 135.60, 135.44, 135.42, 135.30, 129.43, 129.36, 129.32, 129.30, 129.25, 129.22, 129.14, 129.04 (aromatic C), 111.25 (spiral C), 82.79 (d, 1C, J=5.4 Hz, InsC-5), 81.58 (d, 1C, J=5.8 Hz, InsC-6), 75.84 (d, 1C, J=6.1 Hz, InsC-1), 74.68 (s, 1C, InsC-2), 73.52 (d, 1C, J=4.3 Hz, InsC-4), 69.25 (d, 1C, J=7.7 Hz), 69.18 (d, 1C, J=4.7 Hz) (ArCH_2O), 68.99 (d, 1C, J=6.9 Hz, InsC-3), 68.73 (t, 2C, J=6.8 Hz, ArCH_2O), 36.86, 34.43, 25.05, 23.96, 23.16 (cyclohexylidene C), 6.72 (CH_3), 4.22 (CH_2); ^{31}P NMR (162 MHz) δ: −1.09, −1.32. calcd for $C_{34}H_{48}O_{12}P_2Si\cdot0.5H_2O$: C, 53.61; H, 6.60; found: C, 53.52; H, 6.55.

Entry 6. The reaction was carried out by the same procedure as in entry 6 at 0°C for 4.5 h to afford **17a** (21.3%), and **17b** (20.5%).

3.2.13. *O*-Xylylene *N*,*N*-diethylphospharamidite L-1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-5,6-bis-*O*-(*o*-xylylene phosphoryl)-*myo*-inositol (18). To a CH_2Cl_2 solution (10 mL) of L-**11** (180 mg, 0.36 mmol) and 1-*H* tetrazole (151 mg, 2.15 mmol) was added dropwise *O*-xylylene-*N*,*N*-diethyl phospharamidite (XEPA) (274 mg, 1.15 mmol) at 0°C. The mixture was stirred at room temperature for 1.5 h, and additional 20 min after 12 μL H_2O was added. Then the solution was cooled to −78°C, and treated with *m*-CPBA (370 mg, 2.15 mmol) for 5 and 30 min at ambient temperature. After being diluted with EtOAc, the organic layer was washed with 10% aq. Na_2SO_3, aq. $NaHCO_3$, and NaCl, dried, and concentrated. The residue was chromatographed (EtOAc/hexane=1:5) to afford **18** (297 mg, 95%) as white solid. R_f=0.3 (EtOAC/hexane=1:2); $[\alpha]_D^{26}$=−3.9 (c=3, $CHCl_3$); mp: 82–83°C; ^{1}H NMR (400 MHz) δ: 7.22–7.36 (m, 8H), 5.46–5.57 (complex, 2H, ArCH_2O), 5.34 (t, 1H, J=12.8, ArCH_2O), 5.04–5.22 (complex, 5H, ArCH_2O), 4.81 (t×2, 1H, J=8.8 Hz, InsH-6), 4.57 (t×2, 1H, J=8.8 Hz, InsH-5), 4.34 (t, 1H, J=4.0 Hz, InsH-2), 4.10–4.20 (complex, 2H, InsH-1and H-4), 4.98 (dd, 1H, J=4.0, 8.8 Hz, InsH-3), 1.87 (br, 2H, cyclohexylidene), 1.62 (br, 6H, cyclohexylidene), 1.25 (br, 2H, cyclohexylidene), 1.05–1.12 (complex, 28H, isopropyl); ^{31}P NMR (162 MHz) δ: 0.88, −1.31.

3.2.14. L-1,2-*O*-Cyclohexylidene-5,6-di-*O*-(*o*-xylylene phosphoryl)-*myo*-inositol (L-12a). To a THF solution (3 mL) of **18** (298 mg, 0.34 mmol) were added HOAc (78.7 μL, 1.37 mmol) and TBAF·$3H_2O$ (325 mg, 1.03 mmol) at −20°C, and stirred for 12 h. Volatile materials were evaporated under reduced pressure at ambient temperature, the residue was chromatographed on silical gel (EtOAc/hexane=4:1) to afford L-**12a** (186 mg, 81%) as white solid. R_f=0.35 (EtOAc/MeOH 15:1); $[\alpha]_D^{25}$=+4.3 (c=1.56, $CHCl_3$); mp decomposed at above 170°C; ^{1}H NMR (400 MHz) δ: 7.32–7.39 (m, 8H), 5.62 (complex, 2H, ArCH_2O), 5.31 (t, 1H, J=13.6 Hz, ArCH_2O), 5.01–5.22 (complex, 5H, ArCH_2O), 4.91 (dd, 1H, J=6.4, 8.8 Hz, InsH-6), 4.74 (br, OH at C-4), 4.52 (dd, 1H, J=3.7, 5.6 Hz, InsH-2), 4.41 (dt, 1H, J=7.4, 8.8 Hz, InsH-5), 4.29 (dd, 1H, J=5.6, 6.4 Hz, InsH-1), 4.14 (dd, 1H, J=7.4, 7.8 Hz, InsH-4), 3.70 (dd, 1H, J=3.7, 7.8 Hz, InsH-3), 3.46 (br, OH at C-3), 1.79 (m, 2H, cyclohexylidene), 1.60 (br, 6H, cyclohexylidene), 1.41 (m, 2H, cyclohexylidene); ^{13}C NMR (100.9 MHz) δ: 135.54, 135.44, 135.22, 135.03, 129.32 (2C), 129.27 (2C), 129.18, 129.08, 128.88, 128.81 (aromatic), 111.88 (spiral C), 80.25 (InsC-5), 80.05 (InsC-6), 77.22 (InsC-1), 74.68 (InsC-2), 72.38 (InsC-4), 70.06 (InsC-3), 68.67–69.51 (m, ArCH_2O), 37.29, 34.86, 24.92, 23.83–23.66 (3C) (cyclohexylidene); ^{31}P NMR (162 MHz) δ: −0.31, −0.44; Anal. calcd for $C_{28}H_{34}O_{12}P_2\cdot2.5H_2O$: C, 50.23; H, 5.87; Found: C, 50.15; H, 5.78.

3.2.15. D-2,3-*O*-Cyclohexylidene-4,5-di-*O*-(*o*-xylylene phosphoryl)-*myo*-inositol 1-*O*-[(1,2-*O*-di-palmitoyl-*sn*-glceryl) benzyl] phosphate (D-14a). Phosphorylation of L-**12a** with 1,2-di-*O*-palmitoyl-*sn*-glycerol phosphite was proceeded with the general procedures. Physical and spectral data of D-**14a**: see Ref. 16.

3.2.16. D-1-*O*-(1,2-Di-*O*-palmitoyl-*sn*-glycerophospho)-*myo*-inositol 4,5-bis(dihydrogen phosphate) (1). *Procedure* (a). To a CH_2Cl_2 (2 mL) solution of D-**14a** (25.4 mg, 18.9 μmol) was added ethylene glycol (2.7 μL, 47 μmol) and pyridinium poly(hydrogen fluoride) (24 μL, 0.76 mmol) at 0°C. The mixture was stirred at the same temperature for 20 min, and additional 1.2 h at room temperature. Then saturated aq. $NaHCO_3$ (3 mL) was added slowly at 0°C and stirred for 15 min. After being diluted with EtOAc, the solution was washed with aq. $KHSO_4$, aq. $NaHCO_3$, and NaCl, organic layer was dried, concentrated, and chromatographed on silica gel (EtOAc/hexane=1:4) to afford **19** (21.8 mg, 92%). **19** (19.5 mg) thus obtained was hydrogenated overnight in EtOH/$CHCl_3$ (2 mL, v/v 1:1) in the presence of 10 mg Pd/C, then 100 μL Et_3N was added and stirred for 10 min at room temperature. After filtration to remove the catalyst, the filtrate was concentrated and dried in vacuum to give **1** as its triethylammonium salt (19.7 mg, quant.).

Procedure (b). 10%–Pd/C (5 mg) was suspended in a EtOAc (3 mL) solution of D-**14a** (20 mg, 15 μmol) and degassed. The solution was stirred under H_2 for 2.5 days at room temperature, then 100 μL Et_3N was added and stirred for 10 min. The catalyst was removed by filtration, washed with 5 mL $CHCl_3$, 5 mL MeOH. The combined organic layer was concentrated and dried in vacuum to give **1** as its triethylammonium salt (18.8 mg, quant.). R_f=0.3 ($CHCl_3$/acetone/MeOH/AcOH/H_2O 40/15/15/12/8); $[\alpha]_D^{26}$=+5.41, (c=0.61, $CHCl_3$); ^{1}H NMR (400 MHz) δ: 5.21 (br, 1H, glyceryl *sn*-2-H), 4.37–4.39 (br, 2H, InsH-2 and H-4), 4.25 (br, 1H, InsH-1), 3.10–4.20 (complex, 2H, glyceryl and InsH-5), 3.91–4.05 (complex, 4H, InsH-6, glyceryl *sn*-1 and 2), 3.60 (br, 1H, InsH-3), 3.11 (q, J=6.8 Hz, NCH_2CH_3), 2.26 (q, 4H, J=7.8 Hz, α-H in Pal), 1.57 (br, 4H, β-H in Pal), 1.38 (t, J=7.2 Hz, NCH_2CH_3), 1.25 (br, 48H, Pal), 0.88 (t, 6H, J=6.4 Hz, CH_3 in Pal); ^{31}P NMR (162 MHz) δ: 3.23, 2.57, 0.41; FAB-MS (triethanolamine, negative) m/z: 970 $[M-H]^-$, 992 $[M-2H+Na]^-$, 1014 $[M-3H+2Na]^-$.

3.2.17. D-2,3-*O*-Cyclohexylidene-1-*O*-(dibenzyl phophoryl)-5,6-di-*O*-(*o*-xylylene phosphoryl)-*myo*-inositol (D-14e). Phosphorylation of L-**12a** with tribenzyl phosphite was proceeded with the general procedures to afford D-**14e**. $[\alpha]_D^{26}=+2.33$, (c=1.4, $CHCl_3$); spectral data were consistent with racemic form **14e**.

3.2.18. D-*myo*-Inositol 1,4,5-trisphosphate (2). *Procedure* (a). 10%–Pd/C (10 mg) was suspended in a EtOH (2 mL) solution of D-**14e** (16.3 mg, 18.4 μmol) and degassed. The solution was stirred under H_2 for 4 h at room temperature. The catalyst was removed by filtration, washed with 10 mL EtOH. The combined organic layer was evaporated to dryness under reduced pressure. The residue was dissolved in a 1:2 (v/v) mixture of H_2O and HOAc, stirred for 44 h at room temperature. Volatile materials was evaporated under reduced pressure, then dried in vacuum for 5 h. The residue was passed through a Na^+-form of cation-resin using H_2O as mobile phase to afford trisodium salt of **1** (8.8 mg, quant.).

Procedure (b). To 8 mL mixed solvent of MeOH/$CHCl_3$ (v/v 1:1) was added D-**14e** (30 mg, 34 μmol), tributylamine (16.1 μL, 68 μmol) and 10 mg 10%-Pd/C, the solution was stirred under H_2 at room temperature for 3 days. The catalyst was removed by filtration, washed with 10 mixed solvent of MeOH/$CHCl_3$ (v/v 1:1), 10 mL MeOH. The combined organic layer was evaporated to dryness under reduced pressure, the residure was passed successively through cation-exchange column of H^+ and Na^+-form to give **1** as its trisodium salt quantitatively. $[\alpha]_D^{26}=-8.70$, (c=0.46, H_2O); spectral data were consistent with those of the specimen prepared formerly by us.[28]

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