Chemical Resolution of 1,2-*O*-Cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol and Synthesis of Phosphatidyl-D-*myo*-inositol 3,5-Bisphosphate from Both L- and D-Enantiomers

Fushe Han,[a] Minoru Hayashi,[b] and Yutaka Watanabe*[b]

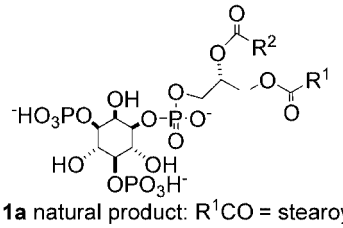
Keywords: Enantiomers / Resolution / *myo*-Inositol derivatives / Synthesis design / Biological activity

Chemical resolution of a versatile starting material, 1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol, which is used to access naturally occurring inositol phosphates and phosphatidylinositol phosphates, is described. Starting from both D- and L-enantiomers of the material, the synthesis of phosphatidyl-D-*myo*-inositol 3,5-bisphosphate [PtdIns(3,5)P2] has been conveniently accomplished via convergent routes. One of the key reactions in the synthetic procedure was the regioselective phosphorylation of suitably protected 1,2,4-triol derivatives of inositol. Phosphorylation of the triol attempted in a 1:12 (v/v) pyridine/CH_2Cl_2 mixture did not proceed at all, whereas in an optimized solvent system, pyridine/CH_2Cl_2 (1.1:1, v/v), the reaction afforded 68% of the desired 1-*O*-phosphate as a single product. Further investigation by ^1H NMR spectroscopy indicated that the reactivity of the three OHs on 1,2,4-triol derivatives is governed by intermolecular hydrogen bonding, which may be disrupted by an increase in the proportion of pyridine in the reaction solvent.

1. Introduction

The biological functions of phosphatidylinositol phosphates PtdInsPns, such as PtdIns(3)P, PtdIns(5)P, PtdIns(3,4)P2, PtdIns(4,5)P2 and PtdIns(3,4,5)P3, in intracellular-signal-transduction, exocytosis and the regulation of membrane-trafficking have been well recognized, and have attracted considerable interest in cell biology.[1] Recently, a previously unknown phosphatidylinositol 3,5-bisphosphate PtdIns(3,5)P2 (**1**) was found to occur in mammalian cell-lines.[2] It has been suggested that this molecule could be a new potential member of the PI cascade.[3] Although the biological role of PtdIns(3,5)P2 has not yet been well recognized, several investigations have shown that PtdIns(3,5)P2 is biologically important; for instance, in sorting membrane proteins into the lumen of the yeast vacuole and maintaining the vacuolar size.[4]

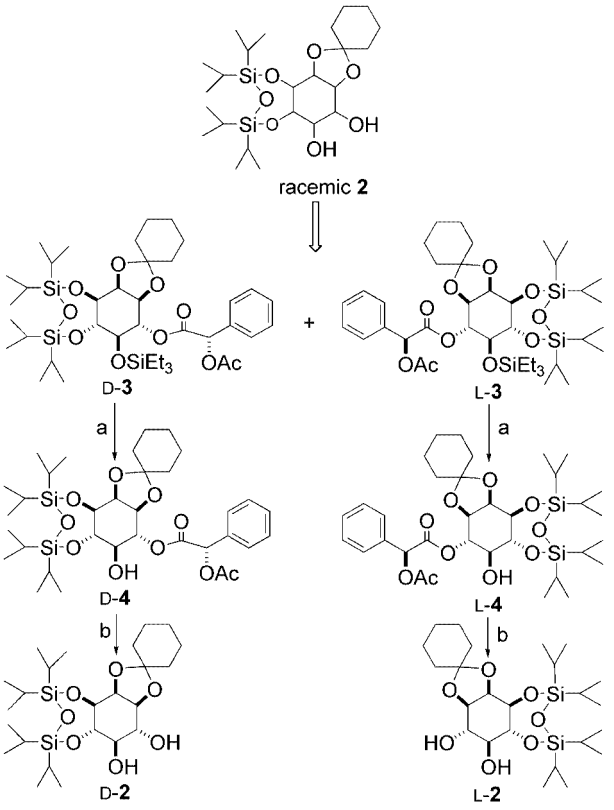
Due to its biological potential, PtdIns(3,5)P2 (**1**) is currently of keen interest to biochemists. This makes the chemical synthesis of natural **1a** and its analogues an important and attractive goal, as biological screening requires much larger quantities of material than can be obtained from natural sources. To date, six reports on the chemical synthesis of PtdIns(3,5)P2 analogues have appeared, in which glucose,[5] *myo*-inositol orthoacetate,[6] orthoformate,[7] and camphor ketal were used.[8] The inositol orthoester methods have advantages, both in terms of number of steps and of yield. We recently communicated a convenient method for synthesizing dipalmitoyl PtdIns(3,5)P2 **1b** from 1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol (**2**)[9] (Scheme 1). The highlights of our method include that it involves a convergent synthetic plan, which enables both D-and L-**2** to access the same target product with natural D-configuration; this being in contrast to previous methods. This drawback of the previous methods is apparent not only in the synthesis of PtdIns(3,5)P2 (**1**), but in the whole field of synthetic inositol chemistry where *myo*-inositol is used as the starting material. In addition, the convergent method developed in our laboratory is general insofar as it can be applied to the synthesis of other PtdInsPns and/or InsPn molecules. In particular, by



1a natural product: R^1CO = stearoyl, R^2CO = arachidonoyl
1b dipalmitoyl analogue: $R^1 = R^2 = C_{15}H_{31}$

[a] Venture Business Laboratory, Ehime University, Matsuyama 790-8577, Japan
[b] Department of Applied Chemistry, Faculty of Engineering, Ehime University, Matsuyama 790-8577, Japan
Fax: (internat.) + 81-89-927-9944
E-mail: wyutaka@dpc.ehime-u.ac.jp

  DOI: 10.1002/ejoc.200300517



Scheme 1. Reagents and conditions: (a) 1% HF (6 equiv.), MeCN/$CHCl_3$ (7:1 v/v), room temp., 36 h, 95%; (b) $NH_2NH_2{\cdot}H_2O$ (3.0 equiv.), CH_2Cl_2, room temp., 1.5 h, quantitative

employing the strategy of regiospecific functionalization at the 3-position of a vicinal 3,4-diol *myo*-inositol derivative.[9,10] Finally, one of the key reactions, regioselective phosphorylation of the triol derivatives, is unprecedented, and this markedly limits the number of steps.

During our synthetic studies, we found that the reactivity of the OHs on the triol derivatives was dramatically affected by the proportion of pyridine in the reaction solvent. Phosphorylation attempted in 1:12 (v/v) of pyridine/CH_2Cl_2 did not proceed at all, whereas that performed in a 1.1:1 pyridine/CH_2Cl_2 co-solvent system afforded the desired product in 68% yield. It is important to clarify the origins of this finding, since it may provide a new and rapid route for the synthesis of PtdInsPns and/or InsPns through the selective phosphorylation of a polyolic *myo*-inositol derivative, and this would markedly reduce the laborious protection-deprotection work. The results of our investigation into this phenomenon, along with an account of the chemical resolution of **2** and the experimental procedures for the synthesis of dipalmitoyl PtdIns(3,5)P2 (**1b**) are detailed in this report.

2. Results and Discussion

2. a. Chemical Resolution of 2

All of the naturally occurring PtdInsPns and InsPns and/or their analogues might be synthesized from D- and/or L-**2**. Hence, an effective preparation of optically pure **2** is required. Although D- and L-1,2-*O*-cyclohexylidene-*myo*-inositol, the precursor of optically active **2**, can be obtained rapidly using enzyme-aided resolution,[11] these compounds need to be converted into the corresponding optically active **2** separately. Moreover, the efficiency of this resolution was found to vary depending on the batch of enzyme. Owing to these shortcomings, we sought to develop an efficient and reliable method for obtaining both of the optically pure enantiomers of **2**. Earlier investigations in this laboratory showed that racemic **2** could be easily transformed into the fully protected **3** as column-separable diastereoisomers.[12] Therefore, selective desilylation of the triethylsilyl (TES) group on D- and L-**3** was attempted under various conditions (Table 1). Neither the tetraisopropyldisiloxane-1,3-diyl (TIPDS) nor the TES groups were removed by treatment with AcOH (Entries 1 and 2). After several experiments, hydrogen fluoride (HF) was found to be effective at selectively cleaving the TES group. **3** was then treated with HF at different concentrations to determine the most effective conditions (Entries 3−6). Treatment with six equivalents of aqueous 1% HF with 7:1 (v/v) MeCN/$CHCl_3$ as solvent for 36 h at room temperature produced the best result, affording 95% of the desired alcohol **4** (Entry 5). Removal of the acetylmandeloyl group from **4** proceeded smoothly by treatment with hydrazine monohydrate to give optically pure D- and L-**2** in quantitative yield in both cases. In addition to D- and L-**2**, the intermediates shown in the Scheme are also useful compounds for synthesis. D-**3** and D-**4** could be rapidly transformed into PtdIns(3,4,5)P3[13] and PtdIns(5)P,[14] respectively. Their opposite L-enantiomers may also serve as starting materials for alternative targets. For instance, L-**4** might be converted into D-PtdIns(5)P via the following sequence: (1) phosphorylation of L-**4** at the 5-position; (2) desilylation of TIPDS group to afford the vicinal 3,4-diol; (3) selective phosphorylation of the 3,4-diol[10b] to complete the precursor of D-PtdIns(5)P. This reaction sequence has, however, not yet been carried out.

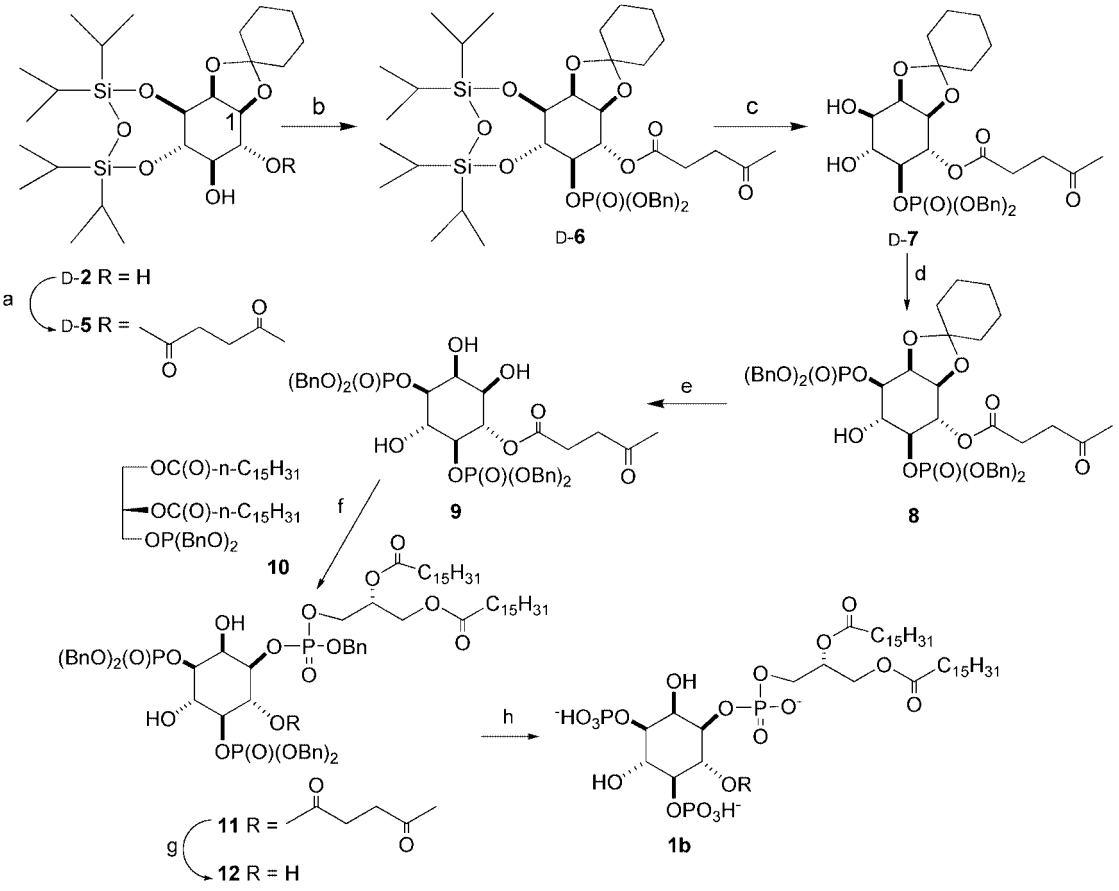
Table 1. Selective cleavage of TES group in D- and L-**3**

Entry	Acid (equiv.)	Solvent (v/v)	Time (h)	Yield of **2** (%)
1	AcOH (4−8)	THF	10	NR[a]
2	AcOH (8)	THF/H_2O (8:1)	10	NR
3	2% HF (6)	MeCN/$CHCl_3$ (7:1)	overnight	65
4	1% HF (6)	MeCN/$CHCl_3$ (7:1)	20	60[b]
5	1% HF (6)	MeCN/$CHCl_3$ (7:1)	36	95
6	1% HF (10)	MeCN/$CHCl_3$ (8:1)	28	91

[a] No reaction. [b] 33% starting material was recovered.

2.b. Synthesis of Dipalmitoyl PtdIns(3,5)P2 from both D- and L-2

As outlined in Scheme 2, diol D-**2** was easily converted into the fully protected D-**6**,[14] which was then transformed into diol D-**7**, without the migration of the phosphate functionality, by careful treatment with TBAF and AcOH at



Scheme 2. Conditions and reagents: (a) $CH_3CO(CH_2)_2COOH$, DCC, DMAP, CH_2Cl_2, room temp., 89%; (b) $(BnO)_2PN(iPr)_2$, 1*H*-tetrazole, CH_2Cl_2, room temp. then *m*CPBA, −78 °C to room temp., 96%; (c) TBAF·$3H_2O$, AcOH, THF, −15 to −10 °C, 92%; (d) $(BnO)_3P$, pyridinium tribromide, 2,6-lutidine, CH_2Cl_2, −42 to 0 °C, 91%; (e) $Py(HF)_x$, ethylene glycol, CH_2Cl_2, 0 °C to room temp., 84%; (f) **10**, pyridinium tribromide, 2,6-lutidine, pyridine/CH_2Cl_2 (v/v 1.1:1), −22 °C to room temp., 68%; (g) hydrazine monohydrate, pyridine/AcOH (v/v 4:1), 0 °C to room temp., 89%; (h) 5% Pd/C, H_2, EtOAc/MeOH (v/v 1:1), room temp., quant.

low temperature. As one of the key reactions, installation of a 3-phosphate group onto D-**7** to give the 3,5-diphosphate **8** was carried out in high yield (91%) using the protocol for selective phosphorylation of 3,4-diol derivatives.[10] Because of the overlap of some of the signals for the inositol protons in the ^{1}H NMR spectrum of **8**, the determination of the exact phosphorylation site of D-**7** was achieved by transforming **8** into its chloroacetate. The resonances for the six inositol methine protons of the chloroacetate derivative were clearly separated, and InsH-4 was shifted downfield from δ = 4.20 to 5.66 ppm as a triplet.

The synthesis of a 1-phosphatidyl derivative starting from **8** might be accomplished generally via the following procedure: (a) protection of the 4-OH in **8**; (b) removal of the cyclohexylidene moiety, affording a 1,2-diol intermediate; (c) selective 1-*O*-phosphorylation of the vicinal 1,2-diol derivative. However, we reasoned that such a circuitous procedure might be simplified through the direct selective phosphorylation of the triol **9**, since regioselective 1-*O*-phosphorylation of vicinal 1,2-diol derivatives of *myo*-inositol has been well documented by our own group[13] and others.[7a] On the other hand, selective functionalization (such as phosphorylation or acylation) at the 3-position of 3,4-diol derivatives has also been exploited very recently.[10b] These reported results clearly suggest that 1-OH would be the most reactive of the three hydroxy groups at the 1, 2, and 4 positions of the triol **9**. Therefore, the selective phosphorylation of **9** is expected to occur at 1-OH. Accordingly, 3,5-diphosphate derivative **8** was transformed into the triol **9** smoothly by the cleavage of the cyclohexylidene ketal with pyridinium poly(hydrogen fluoride) [$Py(HF)_n$], which has been used to cleave the isopropylidene group without the migration of an adjacent phosphate functionality.[15] Phosphorylation of **9** with dipalmitoylglycerol phosphite **10** was first attempted in a 1:12 (v/v) mixed solvent system of pyridine and CH_2Cl_2, and did not proceed at all (Table 2, Entry 1). Thus, the triol **9** showed unusually low reactivity compared to the 1,2- and 3,4-diol derivatives we had examined up to that point. After some trials, we found that the reaction was dramatically affected by the ratio of pyridine to CH_2Cl_2. A trace amount of the desired 1-*O*-phosphatidylinositol **11** was formed (as shown by TLC) in 1:5 pyridine/CH_2Cl_2 as solvent (Entries 2 and 3), and 36% of **11** was isolated when the reaction was carried out in a pyridine/CH_2Cl_2 mixture with the ratio 1:1.8 (Entry 4).

In order to investigate the reason for the low reactivity of triol **9**, we studied the relationship between the concentration and intra- vs. intermolecular interactions using ^{1}H

Table 2. Phosphorylation of triol **9** with phosphite **10** under different conditions

Entry	Base	Py/CH_2Cl_2 (v/v)	Temperature and time	Yield (%) of **11**
1	2,6-lutidine	1:12	−42 °C, 10 min; 0 °C, 2 h; then room temp., 2 h	nr[a]
2	2,6-lutidine	1:5	−42 °C, 10 min; 0 °C, 2 h; then room temp., 2 h	trace[b]
3	Et_3N	1:5	−22 °C, 10 min; 0 °C, 2 h; then room temp., 2 h	trace
4[c]	Et_3N	1:1.8	−22 °C, 10 min; 0 °C, 2 h; then room temp., 2 h	36
5	2,6-lutidine	1.1:1	−22 °C, 10 min; 0 °C, 1.5 h; then room temp., 1 h	68
6	−	1.1:1	−22 °C, 10 min; 0 °C, 1.5 h; then room temp., 1 h	61
7	2,6-lutidine	1.1:1	−22 °C, 10 min; 0 °C, 2 h;	47
8	2,6-lutidine	3:1	−22 °C, 10 min; 0 °C − room temp., 2 h	25[d]

[a] nr: no reaction. [b] Not isolated. [c] The molar ratio of phosphite to triol was 6:1 instead of 3:1 as in the other cases. [d] Some inseparable unknown products included.

NMR spectroscopy. We found that as the concentration of the solution increased in $CDCl_3$, the chemical shifts of the three OH protons shifted downfield, and finally merged into a single broad peak (Figure 1). The same phenomenon was observed in CD_2Cl_2 when spectra were recorded at various concentrations. These observations indicate the formation of intermolecular hydrogen bonds of the triol **9** at high concentrations. In contrast, in the case of a 1,2-diol, 3,6-di-*O*-benzyl-4,5-di-*O*-(dibenzylphosphoryl)-*myo*-inositol, intramolecular hydrogen bonding predominates throughout the range of concentrations tested for the triol (**9**) (Figure 2). It is interesting to note that the unprotected 4-OH in the triol **9** markedly changes the inherent intramolecular nature of the hydrogen bonding in the 1,2-diol system, facilitating intermolecular hydrogen bonding.

These findings directed us to increase the ratio of pyridine to CH_2Cl_2 in the phosphorylation reaction solvent in order to disrupt the intermolecular hydrogen-bonding network. As expected, the yield of the desired phosphorylation product **11** increased as the ratio of pyridine was increased, and finally 68% of **11** was obtained in an optimized 1.1:1 ratio of the mixed solvent system at room temperature (Entry 5). When the reaction was carried out in the absence of 2,6-lutidine or Et_3N in the same solvent system, 61% of **11** was obtained (Entry 6). These results suggested that the reaction was less affected by the type of base. In addition to the proportion of pyridine, temperature was also shown to be one of the factors to affect the reaction, since the yield decreased to 47% when the reaction was conducted at 0 °C (Entry 7). A further increase in the proportion of pyridine lowered the yield of **11** dramatically (Entry 8), presumably due to the decomposition of the reactive phosphorus intermediate by pyridine. In all cases, the desired compound **11** was formed as the single product, as shown by TLC and NMR spectroscopy. In order to determine the exact phosphorylation site, **11** was converted into the corresponding chloroacetate. Analysis of the ^1H NMR and ^1H-^1H COSY spectra of this compound clearly showed that phosphorylation had occurred at the 1-OH position. The methine protons at C-2 and C-4 were shifted downfield to δ = 5.88 and 5.52 ppm, respectively, from the region around δ = 4.20 ppm.

In a similar manner, the opposite enantiomer L-**2** was also transformed into the diol **15**, the key precursor of the final product is outlined in Scheme 3. L-**7**, in a similar man-

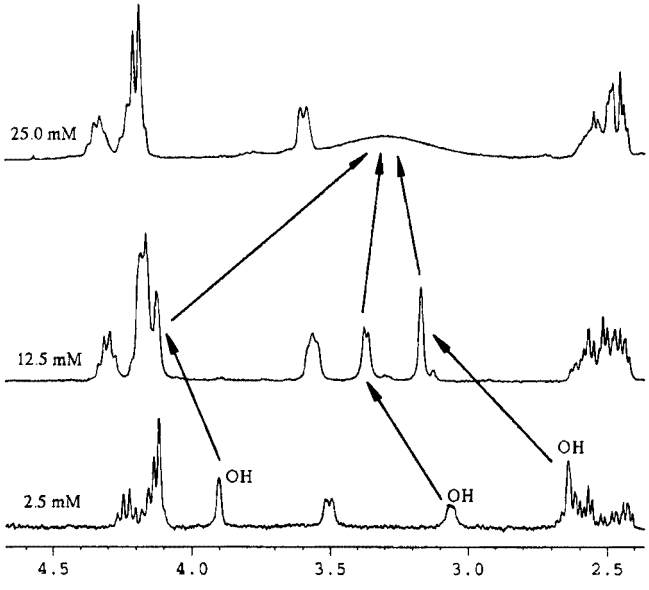


Figure 1. ^1H NMR spectra (400 MHz) of triol **9** in $CDCl_3$ at various concentrations

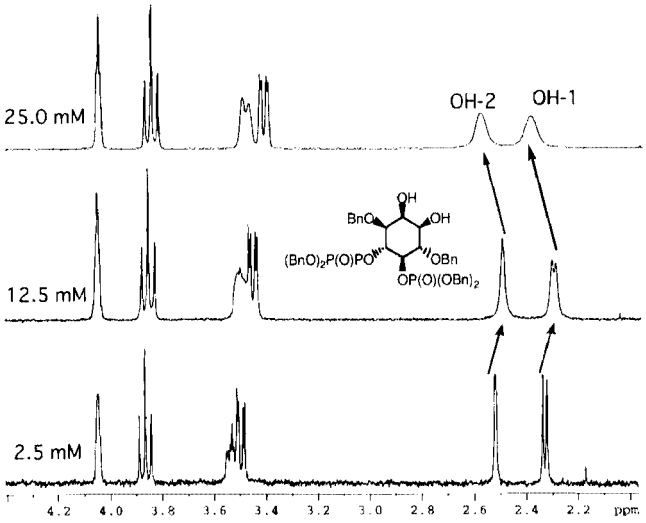
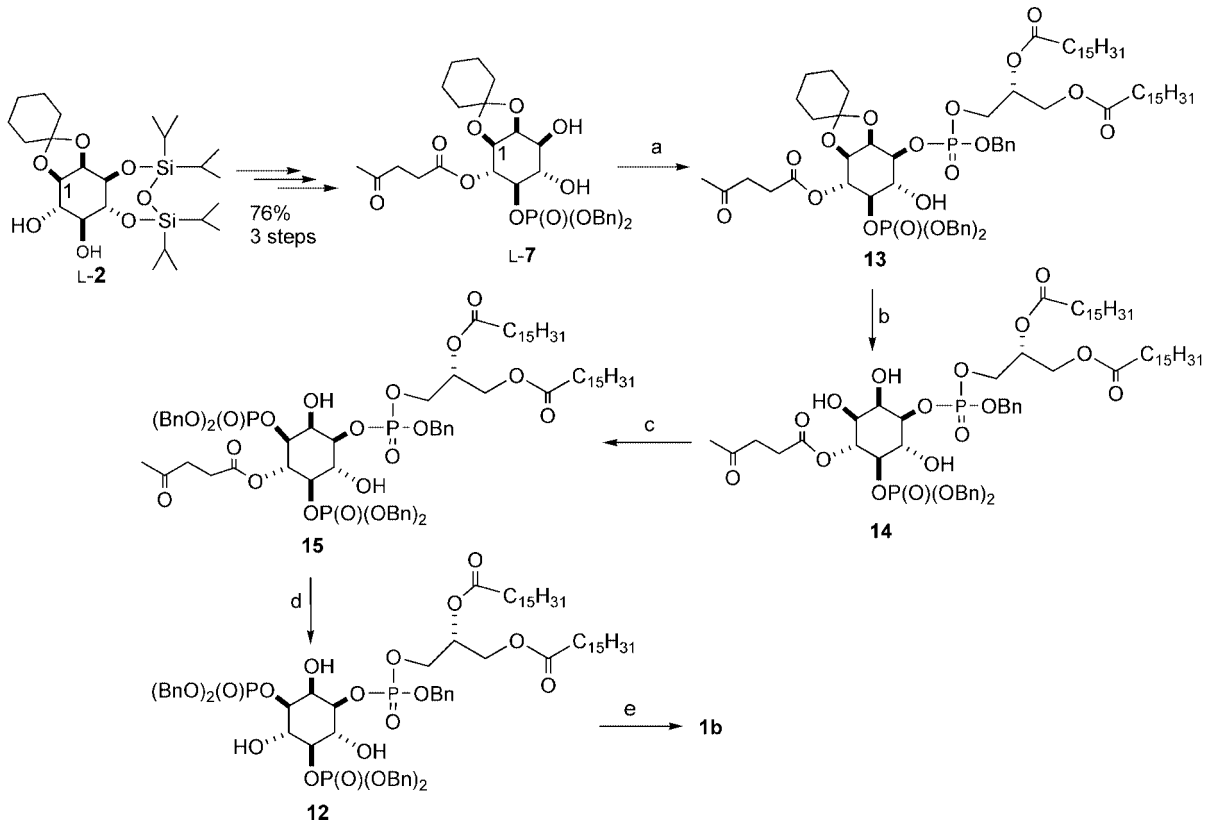


Figure 2. ^1H NMR spectra (400 MHz) of 3,6-di-*O*-benzyl-4,5-di-*O*-(dibenzylphosphoryl)-*myo*-inositol in $CDCl_3$ at various concentrations



Scheme 3. (a) **10**, pyridinium tribromide, 2,6-lutidine, CH_2Cl_2, −42 to 0 °C, 87%; (b) $Py(HF)_x$, ethylene glycol, CH_2Cl_2, 0 °C to room temp., 89%; (c) $(BnO)_3P$, pyridinium tribromide, 2,6-lutidine, pyridine/CH_2Cl_2 (v/v 1:1), −22 to 0 °C, 81%; (d) hydrazine monohydrate, pyridine/AcOH (v/v 4:1), 0 °C to room temp., 91%; (e) 5% Pd/C, H_2, EtOAc/MeOH (v/v 1:1), room temp., quant.

ner to D-**7**, the phosphatidyl group was introduced prior to the 3-phosphate group, affording alcohol **13**. Removal of the cyclohexylidene ketal in **13** afforded the triol **14**, which was then selectively phosphorylated to give **15**. Finally, removal of the levulinoyl groups from **11** (Scheme 2) and **15** (Scheme 3) by treatment with hydrazine monohydrate in a mixture of pyridine and acetic acid [13,16] gave the triol **12**, and subsequent debenzylation by hydrogenolysis over 5% palladium on carbon afforded the dipalmitoyl PtdIns(3,5)P2 (**1b**) as its free acid. The structure of the final product was confirmed by its NMR and MS spectra. Further purification of the crude final product was not done, because no impurities were detected in its NMR spectra. No migration or decomposition of **1b** in its free acid form was seen when a sample was left standing in an open flask for four months at room temperature.

3. Conclusions

We have developed a convergent strategy to access PtdIns(3,5)P2 with the natural D-configuration from both enantiomers of **2**. The regioselective phosphorylation of the 3,4-diol, 5-dibenzyl 1,2-*O*-cyclohexylidene-6-*O*-levulinoyl-*myo*-inositol phosphate (**7**), played a key role in the convergent transformation of both enantiomers into the same target molecule. For the selective phosphorylation of triols **9** and **14**, a modified solvent system improved their reactivity, resulting in smooth regiospecific phosphorylation, to give the key intermediates (**11** and **15**, respectively) in the synthesis of the target molecule D-PtdIns(3,5)P2 (**1b**), in moderate to good yields. Regioselective phosphorylation reactions of diol and triol compounds markedly reduced the number of protection- and deprotection-steps, thus improving the synthetic route. In addition to the practical application to the synthesis of PtdIns(3,5)P2 (**1b**), the convergent synthetic strategy and the selective phosphorylation method of a polyol intermediate presented in this report might be applicable to the synthesis of other PtdInsPns and InsPns.

4. Experimental Section

General Methods: All solvents were purified according to standard procedures. Reagents were reagent grade, and were purified where further purification was required. Pyridinium tribromide (PTB) was recrystallized from AcOH and dried at 60 °C under reduced pressure. 2,6-Lutidine and 1,3-dichlorotetraisopropyldisiloxane were purified by distillation under reduced pressure. Compounds D- and L-**5** were prepared according to the reported method.[13] NMR spectra were recorded with a Bruker Avance 400 spectrometer unless otherwise noted. Optical rotations were measured using a JASCO P-1010 polarimeter with a 1-cm cell. Elemental analyses were performed with a Perkin−Elmer 240C machine. Melting points are uncorrected and were measured in open capillaries using a Yamato Melting Point Apparatus Model MP-21. Silica gel (Fuji Silysia Chemical Ltd., 200−400 mesh) was used for

flash chromatography. Stereospecifically numbered is abbreviated to "*sn*". "Ins" appearing in the text is the abbreviation of inositol.

Preparation of D- and L-6-*O*-Acetylmandeloyl-1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-5-*O*-triethylsilyl-*myo*-inositol (3): DMF (0.1 mL) was added dropwise to a CH_2Cl_2 solution (5 mL) of oxalyl chloride (1.70 g, 13.5 mmol) at 0 °C and the mixture was stirred for 5 min. Then, (*S*)-(+)-*O*-acetylmandelic acid (2.00 g, 10.3 mmol) in 10 mL CH_2Cl_2 was added dropwise. The mixture was stirred at ambient temperature for 3 h. Volatile materials were evaporated under reduced pressure and trace amounts of H_2O in the residue were removed by azeotropic distillation with benzene. The crude acetylmandeloyl chloride thus obtained was dissolved in 10 mL CH_2Cl_2 and added to a 30 mL CH_2Cl_2 solution of racemic **2** (3.75 g, 7.5 mmol) at 0 °C. Pyridine (2.13 g, 27.0 mmol) was added and the mixture was stirred at room temperature for 16 h. After being diluted with EtOAc, the organic layer was washed with aqueous $KHSO_4$, aqueous $NaHCO_3$ and brine, dried, concentrated and purified by chromatography to give (±)-6-*O*-acetylmandeloyl1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol (4.71 g, 89%) as a colorless oil. R_f = 0.52 (EtOAc/hexane, 1:10). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37−7.48 (m, 10 H, aromatic), 6.10 & 6.05 (s × 2, 2 H, methenyl), 5.15 & 5.23 (dd × 2, *J* = 7.6, 10.4 Hz, 2 H, InsH-6), 4.21 & 4.27 (t × 2, *J* = 4.2 Hz, 2 H, InsH-2), 4.07 (dd, *J* = 4.2, 7.6 Hz, 1 H, InsH-1), 3.93 & 4.02 (t × 2, *J* = 9.0 Hz, 2 H, InsH-4), 3.80−3.88 (m, 3 H, InsH-3, InsH-1), 3.25 & 3.44 (t × 2, *J* = 10.4 Hz, 2 H, InsH-5), 2.18 (s, 3 H, CH_3), 2.16 (s, 3 H, CH_3), 1.78 (br., 4 H, cyclohexylidene), 1.51−1.57 (m, 12 H, cyclohexylidene), 1.35 (br., 4 H, cyclohexylidene), 1.03 (br., 56 H, isopropyl) ppm. The compound thus obtained (1.73 g, 2.5 mmol), ethyldiisopropylamine (EDA, 2.13 mL, 12.3 mmol) and a catalytic amount of DMAP were dissolved in CH_2Cl_2 (18 mL) at 0 °C, and chlorotriethylsilane (TESCl, 1.64 mL, 9.8 mmol) was added dropwise. The ice-bath was removed, and the solution was stirred at room temperature for 20 h. The mixture was diluted with EtOAc and washed with H_2O, aqueous $KHSO_4$, aqueous $NaHCO_3$ and brine, then the organic phase was dried, concentrated and purified by chromatography (EtOAc/hexane, 1:50) to afford L-**3** (0.71 g, 38%), D-**3** (0.74 g, 39%) and 0.19 g mixture of D- and L-**3**. L-**3** R_f = 0.23 (EtOAc/hexane, 1:15). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36−7.47 (m, 5 H, aromatic), 6.15 (s, 1 H, methenyl), 5.07 (dd, *J* = 7.2, 8.4 Hz, 1 H, InsH-6), 4.12 (dd, *J* = 4.0, 4.8 Hz, 1 H, InsH-2), 3.96 (t, *J* = 9.2 Hz, 1 H, InsH-4), 3.75 (dd, *J* = 4.0, 9.2 Hz, 1 H, InsH-3), 3.59 (dd, *J* = 4.8, 7.2 Hz, 1 H, InsH-1), 3.45 (dd, *J* = 8.4, 9.2 Hz, 1 H, InsH-5), 2.18 (s, 3 H, CH_3), 1.76 (br., 2 H, cyclohexylidene), 1.28−1.56 (br., 8 H, cyclohexylidene), 1.05 (br., 28 H, isopropyl), 0.94 (t, *J* = 7.8 Hz, 9 H, $SiCH_2CH_3$), 0.63 (q, *J* = 7.8 Hz, 6 H, $SiCH_2CH_3$) ppm. **D-3:** R_f = 0.15 (EtOAc/hexane, 1:15). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36−7.47 (m, 5 H, aromatic), 6.10 (s, 1 H, methenyl), 5.06 (t, *J* = 5.4 Hz, 1 H, InsH-6), 4.37 (dd, *J* = 3.6, 5.4 Hz, 1 H, InsH-2), 4.18 (t, *J* = 5.4 Hz, 1 H, InsH-1), 4.04 (dd, *J* = 6.4, 9.6 Hz, 1 H, InsH-4), 3.79 (dd, *J* = 3.6, 9.6 Hz, 1 H, InsH-3), 3.43 (dd, *J* = 5.4, 6.4 Hz, 1 H, InsH-5), 2.18 (s, 3 H, CH_3), 1.69 (br., 2 H, cyclohexylidene), 1.28−1.55 (br., 8 H, cyclohexylidene), 1.03 (br., 28 H, isopropyl), 0.84 (t, *J* = 7.8 Hz, 9 H, $SiCH_2CH_3$), 0.45 (q, *J* = 7.8 Hz, 6 H, $SiCH_2CH_3$) ppm. $C_{40}H_{68}O_{10}Si_3$ (793.2).

D- and L-6-*O*-Acetylmandeloyl-1,2-*O*-cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol (4). General Procedure: Aqueous HF (6.0 equiv.) was added dropwise to a $CH_3CN/CHCl_3$ (v/v 7:1) solution of **3** at 0 °C. The solution was stirred at room temperature for 36 h. After being diluted with EtOAc, the solution was washed with aqueous $NaHCO_3$ and brine. The organic phase was dried, concentrated, and purified by chromatography to give **4** (95%) as a colorless oil. R_f = 0.52 (EtOAc/hexane, 1:5); **L-4:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49 (dd, *J* = 3.9, 7.4 Hz, 2 H), 7.38 (m, 3 H), 6.05 (s, 1 H, methenyl), 5.21 (dd, *J* = 8.0, 10.8 Hz, 1 H, InsH-6), 4.21 (t, *J* = 4.4 Hz, 1 H, InsH-2), 3.99 (t, *J* = 9.2 Hz, 1 H, InsH-4), 3.82−3.88 (m, 2 H, InsH-3, InsH-1), 3.44 (dd, *J* = 9.2, 10.8 Hz, 1 H, InsH-5), 2.18 (s, 3 H, CH_3), 1.76 (br., 2 H, cyclohexylidene), 1.52 (br., 6 H, cyclohexylidene), 1.35 (br., 2 H, cyclohexylidene), 1.04 (m, 28 H, isopropyl) ppm. **D-4:** ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (dd, *J* = 3.9, 7.4 Hz, 2 H), 7.38 (m, 3 H), 6.10 (s, 1 H, methenyl), 5.14 (dd, *J* = 8.0, 10.8 Hz, 1 H, InsH-6), 4.28 (t, *J* = 4.4 Hz, 1 H, InsH-2), 4.06 (dd, *J* = 4.4, 8.0 Hz, 1 H, InsH-1), 3.92 (t, *J* = 9.2 Hz, 1 H, InsH-4), 3.83 (dd, *J* = 4.4, 9.2 Hz, 1 H, InsH-3), 3.24 (dd, *J* = 9.2, 10.8 Hz, 1 H, InsH-5), 2.16 (s, 3 H, CH_3), 1.74 (br., 2 H, cyclohexylidene), 1.54 (br., 6 H, cyclohexylidene), 1.37 (br., 2 H, cyclohexylidene), 1.03 (m, 28 H, isopropyl) ppm. $C_{34}H_{54}O_{10}Si_2$ (679.0).

D- and L-1,2-*O*-Cyclohexylidene-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol (2). General Procedure: Hydrazine monohydrate (20 equiv.) was added dropwise to a DMF solution of **4** at 0 °C, and the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with EtOAc and washed with aqueous $KHSO_4$, aqueous $NaHCO_3$ and brine. The organic phase was dried, concentrated, and purified by chromatography to give optically pure **2** (100%) as a white solid. L-**2**, $[\alpha]_D^{26}$ = +13.2 (*c* = 1.0, CH_2Cl_2); D-2, $[\alpha]_D^{26}$ = −13.6 (*c* = 1.0, CH_2Cl_2). For spectroscopic data for D- and L-**2**, see ref.[13].

5-Dibenzyl 1,2-*O*-Cyclohexylidene-6-*O*-levulinoyl-3,4-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*myo*-inositol Phosphate (6). D-6: Dibenzyl *N*,*N*-diisopropylphosphoramidite (379.5 mg, 1.11 mmol) and 1*H*-tetrazole (115.5 mg, 1.65 mmol) were added to a CH_2Cl_2 (10 mL) solution of D-**5** (330.0 mg, 0.55 mmol). The solution was stirred at room temperature for 1 h, then treated with 10 μL H_2O over 15 min. After being cooled to −78 °C, *m*CPBA (236.5 mg, 1.38 mmol) was added, and the mixture was stirred at ambient temperature for 1 h. The solution was diluted with EtOAc, and washed with aqueous Na_2SO_3, aqueous $NaHCO_3$, and brine. The organic phase was dried, concentrated, and purified by chromatography (hexane/EtOAc, 5:1) to afford D-**6** (454.2 mg, 96%) as a colorless viscous oil: $[\alpha]_D^{24}$ = −17.6 (*c* = 1.0, $CHCl_3$). **L-6:** Yield, 95%. $[\alpha]_D^{24}$ = +17.2 (*c* = 1.0, $CHCl_3$); R_f = 0.30 (hexane/EtOAc, 2:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31−7.36 (br., 10 H, aromatic), 5.26 (dd, *J* = 7.6, 10.0 Hz, 1 H, InsH-6), 5.00−5.05 (m, 2 H, $C_6H_5CH_2$), 4.86−4.95 (m, 2 H, $C_6H_5CH_2$), 4.28 (dd, *J* = 3.6, 4.8 Hz, 1 H, InsH-2), 4.25 (dd, *J* = 9.0, 10.0 Hz, 1 H, InsH-5), 4.15 (dd, *J* = 8.8, 9.0 Hz, 1 H, InsH-4), 4.02 (dd, *J* = 4.8, 7.6 Hz, 1 H, InsH-1), 3.91 (dd, *J* = 3.6, 8.8 Hz, 1 H, InsH-3), 2.52−2.70 (m, 4 H, CH_2CH_2), 2.05 (s, CH_3), 1.70−1.90 (m, 2 H, cyclohexylidene), 1.45−1.66 (m, 6 H, cyclohexylidene), 1.22−1.38 (m, 2 H, cyclohexylidene), 0.96−1.08 (m, 28 H, isopropyl) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = −0.29 ppm. $C_{43}H_{65}O_{12}PSi_2$ (861.1) (for D-**6**): calcd. C 59.97, H 7.61; found C 59.75, H 7.53.

5-Dibenzyl 1,2-*O*-Cyclohexylidene-6-*O*-levulinoyl-*myo*-inositol Phosphate (7). D-7: AcOH (93.4 mg, 1.56 mmol) and TBAF·$3H_2O$ (367.9 mg, 1.17 mmol) were added to a THF (10 mL) solution of D-**6** (334.8 mg, 0.39 mmol) at −15 °C. The solution was stirred at −15 to −10 °C for 4.5 h, after which time, the solvent was removed under reduced pressure and the residue purified by chromatography (hexane/EtOAc, 1:3) to afford D-**7** (220.9 mg, 92%) as a white solid: R_f = 0.49 (EtOAc); m.p. 151.0−153.0 °C (sample was obtained by chromatography). **D-7:** $[\alpha]_D^{24}$ = −17.6 (*c* = 1.0, $CHCl_3$). **L-7:** Yield 88%. $[\alpha]_D^{24}$ = +17.4, (*c* = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz,

$CDCl_3$): δ = 7.35 (m, 10 H, 2 × C_6H_5), 5.28 (dd, *J* = 7.3, 9.2 Hz, 1 H, InsH-6), 5.00−5.10 (m, 4 H, 2 × $C_6H_5CH_2$), 4.44 (dd, *J* = 4.0, 5.1 Hz, 1 H, InsH-2), 4.09−4.16 (m, 2 H, InsH-5, InsH-1), 4.00 (dd, *J* = 8.8, 9.1 Hz, 1 H, H-4), 3.77 (dd, *J* = 4.0, 9.1 Hz, 1 H, InsH-3), 2.48−2.58 (m, 4 H, CH_2CH_2), 2.08 (s, 3 H, CH_3), 1.76 (m, 2 H, cyclohexylidene), 1.61 (m, 6 H, cyclohexylidene), 1.38 (m, 2 H, cyclohexylidene) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.81 (s), 172.12 (s), 135.99 (s), 135.93 (s), 135.92 (s), 135.85 (s), 128.25−129.11 (m), 111.93 (s), 79.84 (br.), 76.16 (d, *J* = 7.3 Hz), 75.24 (d, *J* = 6.0 Hz), 74.44 (d, *J* = 5.1 Hz), 72.28 (br.), 70.10−70.33 (br.), 70.30 (s), 70.25 (s), 38.09 (s), 37.64 (s), 35.42 (s), 30.14 (s), 28.28 (s), 25.27 (br.), 24.17 (s), 23.94 (s) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = −0.47 ppm. $C_{31}H_{39}O_{11}P$ (618.6) (for D-**7**): calcd. C 60.19, H 6.40; found C 60.14, H 6.61.

Phosphate 8: D-**7** (230.8 mg, 0.37 mmol), 2,6-lutidine (201.7 mg, 1.87 mmol) and tribenzyl phosphite (394.4 mg, 1.12 mmol) were dissolved in CH_2Cl_2 (16 mL) at −42 °C, and pyridinium tribromide (PTB) (478.0 mg, 1.49 mmol) was added. The solution was stirred at −42 °C for 10 min, and for a further 1.2 h at 0 °C, then diluted with EtOAc and washed with aqueous $KHSO_4$, aqueous $NaHCO_3$, and brine. The organic phase was dried, concentrated, and purified by chromatography (hexane/EtOAc, 1:2) to afford **8** (297.1 mg, 91%) as a white solid: R_f = 0.35 (EtOAc/hexane, 4:1); m.p. 155.5−157.0 °C (sample was obtained by chromatography). $[\alpha]_D^{25}$ = −2.9 (*c* = 2.7, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.35 (m, 20 H, 4 × C_6H_5), 5.26 (dd, *J* = 7.4, 8.8 Hz, 1 H, InsH-6), 4.50−5.19 (m, 8 H, 4 × $C_6H_5CH_2$), 4.44−4.50 (m, 2 H, InsH-3, H-2), 4.13−4.23 (m, 2 H, InsH-4, H-5), 4.04 (dd, *J* = 4.8, 7.4 Hz, 1 H, InsH-1), 2.44−2.57 (m, 4 H, CH_2CH_2), 2.06 (s, 3 H, CH_3), 1.60 (br., 2 H, cyclohexylidene), 1.48 (br., 6 H, cyclohexylidene), 1.33 (br., 2 H, cyclohexylidene) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 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, 2 C), 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) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 0.45 (1 P), −0.05 (1 P) ppm. $C_{45}H_{52}O_{14}P_2 \cdot 0.5H_2O$ (887.8): calcd. C 60.87, H 6.02; found C 61.02, H 6.24.

D-6-*O*-Levulinoyl-*myo*-inositol 3,5-Bis(dibenzyl Phosphate) (9): Ethylene glycol (33.9 mg, 0.55 mmol) and pyridinium poly(hydrogen fluoride) (624.8 μL, 21.86 mmol) were added to a CH_2Cl_2 (20 mL) solution of **8** (240.0 mg, 0.27 mmol) at 0 °C. The solution was stirred at 0 °C to room temperature for 3 h. After being re-cooled to 0 °C, an excess of saturated aqueous $NaHCO_3$ was added dropwise and stirred for 15 min. The mixture was washed with aqueous $NaHCO_3$, aqueous $KHSO_4$, and brine. The organic phase was dried, concentrated, and purified by chromatography (hexane/EtOAc, 1:5) to afford **9** (150.7 mg, 84%) as a colorless solid, along with recovered starting material **8** (35 mg, 14.6%): R_f = 0.37 (EtOAc); m.p. 179.0−181.0 °C. $[\alpha]_D^{24}$ = +3.3 (*c* = 1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3/D_2O$ one drop): δ = 7.33 (m, 20 H, 4 × C_6H_5), 5.35 (t, *J* = 9.8 Hz, 1 H, InsH-6), 4.93−5.12 (m, 8 H, 4 × $C_6H_5CH_2$), 4.23 (br. q, *J* = 9.8 Hz, 1 H, InsH-5), 4.12−4.17 (m, 3 H, InsH-2, InsH-3, InsH-4), 3.57 (br. dd, *J* = 4.6, 9.8 Hz, 1 H, InsH-1), 2.41−2.67 (m, 3 H, CH_2CH_2), 2.31 (t, *J* = 6.0 Hz, 0.5 H, CH_2CH_2), 2.27 (t, *J* = 6.0 Hz, 0.5 H, CH_2CH_2), 2.06 (s, 3 H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 207.91 (s), 173.17 (s), 136.25 (s), 136.17 (s), 136.06 (s), 135.98 (s), 128.29−128.95 (m), 79.58 (br.), 78.83 (br.), 73.34 (d, *J* = 3.9 Hz), 71.16−71.30 (m), 70.34 (d, *J* = 5.6 Hz), 70.18 (d, *J* = 5.8 Hz), 70.15 (s), 70.11 (s), 70.08 (s), 70.06 (s), 38.37 (s), 30.11 (s), 28.47 (s) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 0.11 (1 P), −0.06 (1 P) ppm. $C_{39}H_{44}O_{14}P_2 \cdot 0.5H_2O$ (807.7): calcd. C 57.99, H 5.62; found C 57.94, H 5.87.

Phosphate 11: A mixture of 1,2-di-*O*-palmitoyl-*sn*-glycerol (162.1 mg, 0.29 mmol), dibenzyl *N*,*N*-diisopropylphosphoramidite (114.8 mg, 0.33 mmol), and 1*H*-tetrazole (33.3 mg, 0.48 mmol) in CH_2Cl_2 (5 mL) was stirred at room temp. for 1 h. After being diluted with hexane, the solution was washed with brine and dried, and the solvents were evaporated. The benzene solution of crude **10** thus obtained was concentrated under reduced pressure to remove traces of H_2O. The residue, **10**, was mixed with **9** (75.9 mg, 0.10 mmol) in the solvent mixture benzene/CH_2Cl_2. The solvents were evaporated under reduced pressure, and dried in vacuo for 4 h. The resultant mixture of **9** and **10** was dissolved in pyridine (3.3 mL) and CH_2Cl_2 (3 mL). After being cooled to −22 °C, 2,6-lutidine (51.4 mg, 0.48 mmol) and PTB (121.7 mg, 0.38 mmol) were added. The solution was stirred vigorously at −22 °C for 10 min, and then at 0 °C (in an ice/H_2O bath containing a small amount of NaCl) for 1.5 h. The bath was removed, and the solution was stirred at ambient temperature for an additional 1 h. The solution was diluted with EtOAc, and washed with aqueous $KHSO_4$, aqueous $NaHCO_3$ and brine. The organic phase was dried, concentrated, and purified by chromatography (hexane/EtOAc, 1:2) to afford **11** (97.9 mg, 68%): R_f = 0.48 (EtOAc/hexane, 2:1). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.32 (m, 25 H, 5 × C_6H_5), 5.58 (dt, *J* = 6.4, 9.6 Hz, 1 H, InsH-6), 5.24 (m, 1 H, glyceryl *sn*-2 H), 4.91−5.10 (m, 5 × $C_6H_5CH_2$), 4.08−4.47 (m, 9 H, glyceryl *sn*-1 H, *sn*-3 H, InsH-1, -2, -3, -4 and -5), 2.24−2.61 (m, 4 H, CH_2 in levulinoyl), 2.26 (m, 4 H, α-CH_2 in palmitoyl), 2.01 (s, 3 H, CH_3 in levulinoyl), 1.55 (br., 4 H, β-CH_2 in palmitoyl), 1.25 (br., 48 H, CH_2 in palmitoyl), 0.88 (t, *J* = 6.4 Hz, 6 H, CH_3 in palmitoyl) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.51 (s), 206.45 (s), 173.84 (s), 173.71 (s), 173.61 (s), 173.46 (s), 172.43 (s), 172.30 (s), 135.70−136.20 (m), 128.25−129.01 (m), 79.38 (m), 78.94 (m), 76.57 (m), 75.12 (m), 70.23−70.70 (m), 69.83 (m), 66.40 (m), 62.21 (m), 37.68 (s), 37.54 (s), 34.55 (s), 34.40 (s), 32.34 (s), 29.55−30.12 (m), 28.23 (s), 25.24 (s), 23.11 (s), 14.55 (br.) ppm.^{31} P NMR (162 MHz, $CDCl_3$): δ = −0.02 (2 P), −0.18 (1 P), −0.28 (1 P), −1.08 (1 P), −1.14 (1 P) ppm, indicating that the phosphorylation product consisted of a 1:1 diastereomeric mixture based on the phosphorus center at the 1-position. $C_{81}H_{117}O_{21}P_3$ (1519.7): calcd. C 64.02, H 7.76; found C 64.32, H 7.69.

Phosphate 13: 2,6-Lutidine (100.5 mg, 0.93 mmol) was added to a CH_2Cl_2 (10 mL) solution of L-**7** (115.0 mg, 0.19 mmol) and **10** (3 equiv.). After being cooled to −42 °C, PTB (238.2 mg, 0.74 mmol) was added to the solution. The mixture was stirred at −42 °C for 10 min, and 0 °C for 2 h. The solution was diluted with EtOAc, washed with aqueous $KHSO_4$, aqueous $NaHCO_3$, and brine. The organic phase was dried, concentrated, and purified by chromatography (hexane/EtOAc, 1:2) to afford **13** (217.2 mg, 87%): R_f = 0.43 (hexane/EtOAc, 1:2). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.28 (br., 15 H, 3 × C_6H_5), 5.27 (br. dd, *J* = 6.4, 9.6 Hz, 1 H, InsH-4), 5.19 (m, 1 H, glyceryl *sn*-2 H), 5.00−5.15 (m, 6 H, 3 × $C_6H_5CH_2$), 4.49−4.54 (m, 2 H, InsH-2, InsH-5), 4.36 (t, *J* = 6.2 Hz, 0.5 H, InsH-3), 4.33 (t, *J* = 6.2 Hz, 0.5 H, InsH-3), 4.09−4.29 (m, 6 H, glyceryl *sn*-1 H, *sn*-3 H, InsH-1, InsH-6), 2.49−2.60 (m, 4 H, CH_2 in levulinoyl), 2.31 (br. q, *J* = 6.0 Hz, 4 H, α-CH_2 in palmitoyl), 2.09 (s, 3 H, CH_3 in levulinoyl), 1.78 (br., 2 H, cyclohexylidene), 1.60 (br., 10 H, β-CH_2 in palmitoyl, cyclohexylidene), 1.51 (br., 2 H, cyclohexylidene), 1.28 (br., 48 H, CH_2 in palmitoyl), 0.91 (t, *J* = 6.0 Hz, 6 H, CH_3 in palmitoyl) ppm. ^{31} P NMR (162 MHz, $CDCl_3$): δ = 0.56 (1 P), 0.43 (1 P), −0.29 (1 P), −0.43 (1 P) ppm.

$C_{73}H_{112}O_{18}P_2$ (1339.6): calcd. C 65.45, H 8.43; found C 65.66, H 8.52.

Phosphate 14: Ethylene glycol (8.8 mg, 0.14 mmol) and pyridinium poly(hydrogen fluoride) (80.7 μL, 2.82 mmol) were added to a CH_2Cl_2 (5 mL) solution of **13** (95.0 mg, 0.071 mmol) at 0 °C. The solution was stirred at 0 °C to room temperature for 4 h. After being re-cooled to 0 °C, an excess of saturated aqueous $NaHCO_3$ was added dropwise and the mixture was stirred for 15 min. The mixture was diluted with EtOAc, and washed with aqueous $NaHCO_3$, aqueous $KHSO_4$, and brine. The organic phase was dried, concentrated, and purified by chromatography (hexane/EtOAc, 1:3) to afford **14** (81.3 mg, 89%): R_f = 0.31 (EtOAc/hexane, 2:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32−7.38 (m, 15 H, 3 × C_6H_5), 5.38 (br. dd, J = 6.4, 9.2 Hz, 1 H, InsH-4), 5.23 (m, 1 H, glyceryl *sn*-2 H), 4.94−5.17 (m, 6 H, 3 × $C_6H_5CH_2$), 4.25−4.33 (m, 3 H, glyceryl *sn*-1 H, InsH-5), 4.10−4.22 (m, 5 H, glyceryl *sn*-3 H, InsH-1, InsH-2 and InsH-6), 3.59 (br., 1 H, InsH-3), 3.20 (br., 1 H, 3-OH), 2.46−2.59 (m, 4 H, CH_2 in levulinoyl), 2.27 (m, 4 H, α-CH_2 in palmitoyl), 2.07 (s, 3 H, CH_3 in levulinoyl), 1.57 (br., 4 H, β-CH_2 in palmitoyl), 1.25 (br., 48 H, CH_2 in palmitoyl), 0.89 (t, J = 6.6 Hz, 6 H, CH_3 in palmitoyl) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 207.84 (s), 173.89 (s), 173.77 (s), 173.62 (s), 173.58 (s), 173.12 (s), 173.08 (s), 135.85−136.04 (m), 128.47−129.05 (m), 78.04−79.60 (m), 73.27 (d, J = 3.6 Hz), 69.83−71.20 (m), 65.84 (m), 61.82 (m), 38.49 (s), 38.46 (s), 34.58 (s), 34.41 (s), 32.33 (s), 29.52−30.11 (m), 28.46 (s), 25.23 (s), 23.09 (s), 14.51 (s) ppm. ^{31} P NMR (162 MHz, $CDCl_3$): δ = 0.30 (1 P), 0.09 (1 P), −0.16 (1 P), −0.44 (1 P) ppm. $C_{67}H_{104}O_{18}P_2$ (1259.5): calcd. C 63.89, H 8.32; found C 63.64, H 8.16.

Phosphate 15: 14 (65.0 mg, 0.051 mmol**)** was evaporated with benzene and dried in vacuo for 3 h. Pyridinium tribromide (PTB) (65.7 mg, 0.205 mmol) was added to 6 mL of a pyridine/CH_2Cl_2 (1:1) solution of **14**, 2,6-lutidine (27.7 mg, 0.257 mmol), and tribenzyl phosphite (54.2 mg, 0.154 mmol) at −42 °C. The mixture was stirred at the same temperature for 15 min and for a further 2 h at 0 °C, then diluted with EtOAc, and washed with aqueous $KHSO_4$, aqueous $NaHCO_3$ and brine. The organic phase was dried, concentrated, and purified by chromatography (hexane/EtOAc, 1:2) to afford **15** (63.2 mg, 81%): R_f = 0.47 (EtOAc/hexane, 2:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (m, 25 H, 5 × C_6H_5), 5.58 (m, 1 H, InsH-4), 5.22 (m, 1 H, glyceryl *sn*-2 H), 4.98−5.16 (m, 10 H, 5 × $C_6H_5CH_2$), 4.12−4.42 (m, 9 H, glyceryl *sn*-1 H, *sn*-3 H, InsH-1, -2, -3, -5 and -6), 2.38 (m, 2 H, CH_2 in levulinoyl), 2.25−2.29 (m, 6 H, CH_2 in levulinoyl, α-CH_2 in palmitoyl), 1.94 (s, 3 H, CH_3 in levulinoyl), 1.57 (br., 4 H, β-CH_2 in palmitoyl), 1.25 (br., 48 H, CH_2 in palmitoyl), 0.89 (t, J = 6.4 Hz, 6 H, CH_3 in palmitoyl) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 205.89 (s), 173.41 (s), 173.31 (s), 173.10 (s, 2 C), 171.81 (s, 2 C), 135.49−135.74 (m), 127.92−129.07 (m), 79.16 (m), 78.93 (m), 77.76 (m), 75.08 (m), 69.33−70.20 (m), 65.96 (m), 61.78 (s), 37.31 (s), 34.14 (s), 34.00 (s), 31.92 (s), 28.84−29.68 (m), 27.81 (s), 24.81 (s), 22.69 (s), 14.11 (s) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 0.50 (1 P), 0.23 (1 P), −0.07 (1 P), −0.50 (1 P), −0.94 (1 P), −1.04 (1 P) ppm. $C_{81}H_{117}O_{21}P_3 \cdot 2.5H_2O$ (1564.7): calcd. C 62.17, H 7.85; found C 62.15, H 7.70.

Phosphate 1b: 15 (80.0 mg, 0.053 mmol) was dissolved in a 1.25 mL mixture of pyridine and AcOH (v/v 4:1). The solution was cooled to 0 °C, then hydrazine monohydrate (6.5 mg, 0.132 mmol) was added, and the mixture was stirred at 0 °C to room temperature for 1.3 h. The reaction mixture was diluted with EtOAc and washed with aqueous $KHSO_4$, aqueous $NaHCO_3$, and brine. The organic layer was dried, concentrated, and purified by chromatography (hexane/EtOAc, 1:3) to afford **12** (68.2 mg, 89%): R_f = 0.47 (EtOAc/hexane, 2:1); compound **11** was converted into **12** by the same procedure in 91% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33 (br., 25 H, 5 × C_6H_5), 5.22 (m, 1 H, glyceryl *sn*-2 H), 5.03−5.15 (m, 10 H, 5 × $C_6H_5CH_2$), 4.12−4.32 (m, 10 H, glyceryl *sn*-1 H, *sn*-3 H, InsH-1 to -6), 2.26 (m, 4 H, α-CH_2 in palmitoyl), 1.56 (br., 4 H, β-CH_2 in palmitoyl), 1.25 (br., 48 H, CH_2 in palmitoyl), 0.88 (t, J = 6.4 Hz, 6 H, CH_3 in palmitoyl) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.85 (s), 173.77 (s), 173.54 (s, 2 C), 135.95−136.16 (m), 128.22−129.10 (m), 82.08 (m), 77.43−79.60 (m), 69.81−70.53 (m), 66.25 (m), 61.74 (m), 34.56 (s), 34.40 (s), 33.34 (s), 29.00−31.00 (m), 25.23 (s), 23.10 (s), 14.49 (m) ppm. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 1.10 (2 P), 0.15 (1 P), −0.05 (1 P), −0.07 (1 P), −0.54 (1 P) ppm. Compound **12** (20.0 mg) thus obtained was dissolved in a 8 mL mixture of EtOAc and MeOH (v/v 1:1). The solution was flushed with N_2, then 5% Pd/C (10 mg) was added. The suspension was hydrogenated at 1 atm for 16 h at room temperature. The catalyst was removed by filtration, and the residue was washed sequentially with MeOH and $CHCl_3$. The combined solutions were concentrated in vacuo to afford **1b** (13.4 mg, quant.) as its free acid from. $[\alpha]_D^{24}$ = −1.4 [c = 0.27, $CHCl_3$/MeOH, 1:1 (v/v)]. ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD/D_2O, 1:1:0.1): δ = 5.27 (br., 1 H, glyceryl *sn*-2 H), 4.41 (br. s, 1 H, InsH-2), 4.30 (br., 0.5 H, glyceryl *sn*-1 H), 4.20 (m, 0.5 H, glyceryl *sn*-1 H), 4.03−4.17 (m, 6 H, glyceryl *sn*-1 H, *sn*-3 H, InsH-1, H-3, H-5), 3.96 (br., 2 H, InsH-4, H-6), 2.34 (m, 4 H, α-CH_2 in palmitoyl), 1.60 (br., 4 H, β-CH_2 in palmitoyl), 1.28 (br., 48 H, CH_2 in palmitoyl), 0.89 (br., 6 H, CH_3 in palmitoyl) ppm. ^{31}P NMR (162 MHz,$CDCl_3$/CD_3OD/D_2O, 1:1:0.1): δ = 5.33 (1 P), 4.54 (1 P), 4.12 (1 P) ppm. Negative FABMS (triethylammonium salt): *m/z* (%) = 1008 (25) [M − 2H + K]$^-$, 992 (35) [(M − 2H + Na]$^-$, 970 (100) [M − H]$^-$, 648 (50) [$C_{15}H_{31}COOCH_2CH(OCOC_{15}H_{31})CH_2OPO_3H^-$], 255 (80) [$C_{15}H_{31}COO]^-$. HRMS (FAB$^-$, triethanolamine) calcd. for $C_{41}H_{80}O_{19}P_3^-$ (970.0): 969.4506; found 969.4523.

Acknowledgments

We are grateful to the Center for Cooperative Research and Development of Ehime University for MS analysis.

[1] [1a] N. Divecha, R. F. Irvine, *Cell* **1995**, *80*, 269−278. [1b] P. De Camilli, S. D. Emr, P. S. McPherson, P. Novick, *Science* **1996**, *271*, 1533−1539. [1c] K. Hinchliffe, R. Irvine, *Nature* **1997**, *390*, 123−124.

[2] [2a] C. C. Whiteford, C. A. Brearley, E. T. Ulug, *Biochem. J.* **1997**, *323*, 597−601. [2b] S. K. Dove, F. T. Cooke, M. R. Douglas, L. G. Sayer, P. J. Paker, R. H. Michell, *Nature* **1997**, *390*, 187−192.

[3] [3a] L. Rameth, K. Tolias, B. Duckworth, L. Cantley, *Nature* **1997**, *390*, 192−196. [3b] K. Tolias, L. Cantley, *Chemistry and Physics of Lipids* **1999**, *98*, 1−169.

[4] [4a] G. Odorizzi, M. Babst, S. D. Emr, *Cell* **1998**, *95*, 847−858. [4b] J. D. Gary, A. E. Wurmser, C. J. Bonangelino, L. S. Weisman, S. D. Eur, *J. Cell. Biol.* **1998**, *143*, 65−79. [4c] F. K. Cooke, S. K. Dove, R. K. McEwen, G. Painter, A. B. Holmes, M. N. Hall, R. H. Michell, P. Parker, *J. Curr. Biol.* **1998**, *8*, 1219−1222.

[5] [5a] A. Nishikawa, S. Saito, K. Hashimoto, K. Koga, R. Shirai, *Tetrahedron Lett.* **2001**, *42*, 9195−9198; [5b] J. Peng, G. D. Prestwich, *Tetrahedron Lett.* **1998**, *39*, 3965−3968.

[6] A. M. Riley, B. V. L. Potter, *Tetrahedron Lett.* **1998**, *39*, 6769−6772.

[7] [7a] J. R. Falck, U. M. Krishna, K. R. Katipally, J. H. Capdevila, E. T. Ulug, *Tetrahedron Lett.* **2000**, *41*, 4271−4275. [7b] J. R. Falck, U. M. Krishna, J. H. Capdevila, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1711−1713. [7c] G. F. Painter, S. J. A. Grove, I. H. Gilbert, A. B. Holmes, P. R. Raithby, M. L. Hill, P. T. Hawkins, L. R. Stephens, *J. Chem. Soc., Perkin Trans. 1* **1999**, 923−935.
[8] R. J. Kubiak, K. S. Bruzik, *J. Org. Chem.* **2003**, *68*, 960−968.
[9] F. S. Han, M. Hayashi, Y. Watanabe, *Chem. Lett.* **2003**, *32*, 724−725.
[10] [10a] F. S. Han, M. Hayashi, Y. Watanabe, *Chem. Lett.* **2003**, *32*, 46−47. [10b] F. S. Han, M. Hayashi, Y. Watanabe, *Tetrahedron* **2003**, *59*, 7703−7711.
[11] [11a] L. Ling, S. Ozaki, *Tetrahedron Lett.* **1993**, *34*, 2501−2504. [11b] L. Ling, S. Ozaki, *Carbohydr. Res.* **1994**, *256*, 49−58.
[12] Y. Watanabe, M. Nakatomi, *Tetrahedron Lett.* **1998**, *39*, 1583−1586.
[13] Y. Watanabe, M. Tomioka, S. Ozaki, *Tetrahedron* **1995**, *51*, 8969−8976.
[14] Y. Watanabe, H. Ishikawa, *Tetrahedron Lett.* **2000**, *41*, 8509−8512.
[15] Y. Watanabe, Y. Kiyosawa, A. Tatsukawa, M. Hayashi, *Tetrahedron Lett.* **2000**, *41*, 4641−4643.
[16] J. H. Van Boom, P. M. J. Burgers, *Tetrahedron Lett.* **1976**, 4875−4878.

Received August 14, 2003

