Efficient Asymmetric Synthesis of Phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate

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Phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate, a candidate of the second messenger in cellular signal transduction, was efficiently synthesized in a homochiral form.

Key words phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate; phosphatidyl-D-*myo*-inositol 3-kinase; cellular signal transduction; second messenger

Polyphosphoinositides play an extremely important role in cellular signal transduction. Recently, it has been revealed that phosphatidyl-D-*myo*-inositol 3-kinase (PI 3-kinase) catalyzes the phosphorylation of phosphatidyl-D-*myo*-inositol 4,5-bisphosphate (PI 4,5-P_2) to phosphatidyl-D-*myo*-inositol 3,4,5-trisphosphate (PI 3,4,5-P_3: **1**).[1] Stephens *et al.* reported that PIP_3 5-phosphatase dephosphorylates **1** to phosphatidyl-D-*myo*-inositol 3,4-bisphosphate (PI 3,4-P_2).[2] Interestingly, **1** is resistant to known phospholipase Cs, and no other enzyme that metabolizes **1** has so far been found. Although the role and function of **1** remain unknown, it is a promising candidate for a second messenger in cellular signal transduction.

Many biological studies of PI 3-kinase have been reported, but there are only a few reports on the function of its product **1**.[3,4] Since the availability of **1** is severely limited, an efficient chemical synthesis is required. Recently, we reported the synthesis of 1-*O*-alkyl and 1-*O*-acyl-D-*myo*-inositol 3,4,5-trisphosphates,[5] which might serve as alternatives to **1** in studies of **1** and PI 3-kinase. Here, we present an efficient asymmetric synthesis of **1b**[6-9] with saturated lipid side chains from the chiral *myo*-inositol intermediate **3** developed by Prestwich and co-workers.[10]

Results and Discussion

The synthetic scheme of **1b** is depicted in Chart 2. The appropriately protected homochiral key intermediate **3** was synthesized according to Prestwich's method[10] from methyl α-D-glucopyranoside **2** through 9 steps in 26% overall yield. The amidite **7** with two stearoyl groups was obtained from 1,2-stearoyl-*sn*-glycerol and benzyl *N,N,N',N'*-tetraisopropylphosphoramidite, which were prepared from (*S*)-(+)-2,2-dimethyldioxolane-4-methanol and phosphorus trichloride, respectively.[9,11] The coupling of the inositol derivative **3** with the amidite **7** followed by *m*-chloroperbenzoic acids (*m*-CPBA) oxidation in one pot gave an epimeric mixture of **4**.[12] Removal of the *p*-methoxybenzyl (PMB) groups with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[13] in wet CH_2Cl_2 gave the triol **5**. Phosphitylation of the resulting triol with dibenzyl *N,N*-diethylphosphoramidite, followed by oxidation with *m*-CPBA in one pot gave fully protected PI 3,4,5-P_3 (**6**). Deprotection of all benzyl (Bn) and benzyloxymethyl (BOM) groups of **6** was carried out cleanly by hydrogenolysis over Pd black in *tert*-BuOH in the presence of $NaHCO_3$.[9] During the hydrogenolysis, partially or fully deprotected sodium salts precipitated. To obtain complete deprotection to **1b**, a small amount of water was added after 2 h to dissolve insoluble salts. The overall yield of **1b** from **2** was 21% through 13 steps.

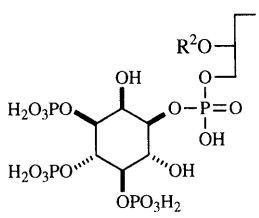
The synthesis described here should provide easy access to a variety of derivatives of **1b**. Clarification of the function of **1** in cellular signal transduction is in progress and the results will be reported elsewhere.

Experimental

^{1}H- and ^{31}P-NMR spectra were recorded on a JEOL JNM-A500 spectrometer with tetramethylsilane (^{1}H) and H_3PO_4 (^{31}P) as internal standards, respectively. The chemical shift values are expressed as δ (ppm) values. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet), br (broad). FAB-MS or HR FAB-MS were measured on a JEOL JMS-HX110 spectrometer.

1,2-Di-*O*-stearoyl-*sn*-glycerol Benzyl *N,N*-Diisopropylphosphoramidite (7) According to the procedure described in ref. 9, coupling of 1,2-di-*O*-stearoyl-*sn*-glycerol (300 mg, 0.48 mmol) with benzyl *N,N,N',N'*-tetraisopropylphosphoramidite (320 mg, 0.96 mmol) in the presence of 1*H*-tetrazole (68 mg, 0.97 mmol) gave **7** (409 mg, 0.48 mmol, 99%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, 7.0 Hz), 1.17 (12H, m), 1.24—1.30 (56H, br m), 1.60 (4H, m), 2.28 (2H, t, 6.0 Hz), 2.29 (2H, t, 6.0 Hz), 3.58—3.82 (4H, m), 4.17 (1H, m), 4.34 (1H, m), 4.63—4.76 (2H, m), 5.19 (1H, m), 7.23—7.39 (5H, m).

2,6-Di-*O*-benzyloxymethyl-3,4,5-tri-*O*-*p*-methoxybenzyl-D-*myo*-inositol 1-*O*-(1,2-Di-*O*-stearoyl-*sn*-glycerol Benzylphosphate) (4) Anhydrous CH_2Cl_2 (10 ml) was added to a mixture of anhydrous **7** (409 mg, 0.48 mmol, dried by azeotropic removal of water with benzene), 2,6-di-*O*-benzyloxymethyl-3,4,5-tri-*O*-*p*-methoxybenzyl-D-*myo*-inositol (**3**) (185 mg, 0.24 mmol) prepared by Prestwich's procedure[10] and 1*H*-tetrazole (120 mg, 1.70 mmol). After 30 min, a small amount of water was added. The mixture was further stirred for 30 min, then *m*-CPBA (330 mg, 1.34 mmol) was added at −40 °C, and the reaction mixture was allowed to warm to room temperature. It was extracted with AcOEt, and the organic solution was washed with aqueous 10% Na_2SO_3 and aqueous saturated $NaHCO_3$, then dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography with 5% AcOEt in hexane to give **4**. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, 7 Hz), 1.21—1.31 (56H, br), 1.50—1.59 (4H, m), 2.17—2.25 (4H, m), 3.38—3.43 (2H, m), 3.77 (9H, s), 3.91—4.25 (8H, m), 4.43—5.17 (17H, m), 6.78—6.82 (6H, m), 7.17—7.32 (21H, m).



1a R^1=stearoyl; R^2=arachidonoyl
1b R^1=R^2=stearoyl

phosphatidylinositol 3,4,5-trisphosphate

Chart 1

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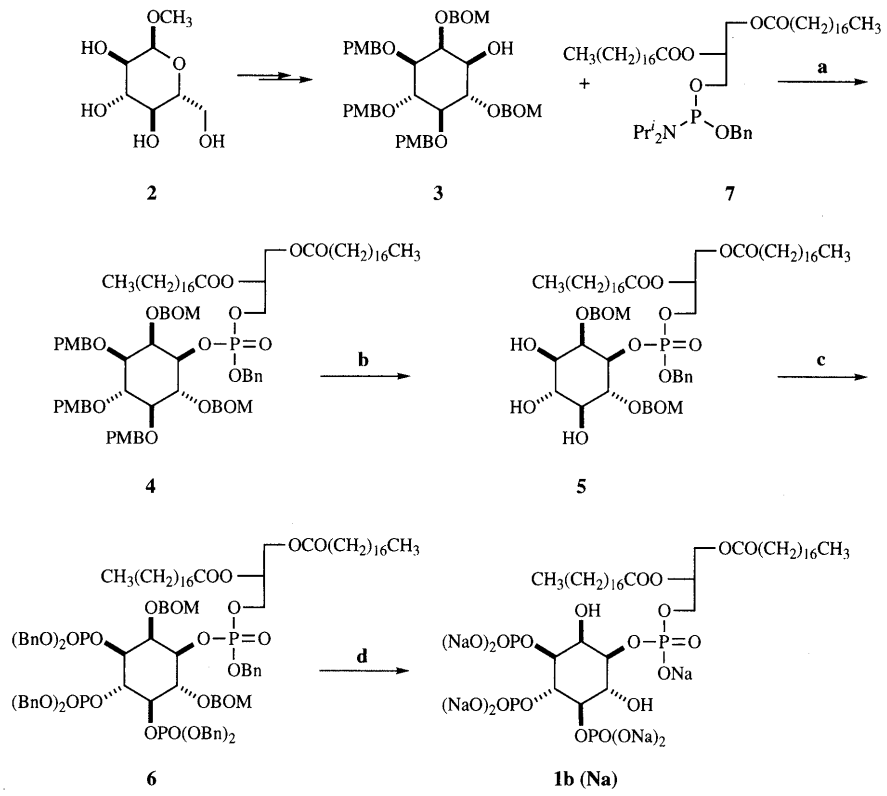

Reagents and conditions: **a.** 1*H*-tetrazole, CH_2Cl_2, r.t., 30 min, then *m*-CPBA, -40°C to r.t. **b.** DDQ, wet CH_2Cl_2, r.t, 1h. **c.** $(BnO)_2PNEt_2$, 1H-tetrazole, CH_2Cl_2, r.t., 30 min ; then *m*-CPBA, -40°C to r.t. **d.** H_2 (50 psi), Pd black, $NaHCO_3$, 85% t-BuOH/H_2O

Chart 2

IR ($CHCl_3$) cm^{-1}: 2900, 2870, 2830, 1740, 1610, 1590, 1510, 1460, 1360, 1300, 1240—1200, 1170, 1110, 1030, 920. FAB-MS (*m*-nitrobenzyl-alcohol (*m*NBA)) *m/z*: 1579, 1580 (M+Na). HR FAB-MS *m/z*: Calcd for $C_{92}H_{133}NaO_{18}P$, 1579.9127, Found 1579.9169.

2,6-Di-*O*-benzyloxymethyl-D-*myo*-inositol 1-*O*-(1,2-Di-*O*-stearoyl-*sn*-glycerol Benzylphosphate) (5) A solution of **4** in wet CH_2Cl_2 5 ml at room temperature was treated with 95% DDQ (440 mg, 1.84 mmol). After having been stirred for 1 h, the reaction mixture was poured into aqueous saturated $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with 60% AcOEt in hexane to give **5** (254 mg, 0.21 mmol, 90% from **3**). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, 7.0 Hz), 1.23—1.27 (56H, br m), 1.53—1.60 (4H, br), 1.86 (1H, br), 2.22—2.28 (4H, m), 3.01 (1H, br), 3.34—3.44 (2H, m), 3.50 (1H, br), 3.70 (1H, t, 9.0 Hz), 3.78—3.85 (1H, m), 4.04—4.31 (7H, m), 4.53—5.09 (9H, m), 5.15 (1H, m), 7.27—7.35 (15H, m). IR ($CHCl_3$) cm^{-1}: 2920, 2850, 2400, 1740, 1460, 1380, 1160, 1110, 1020, 920. FAB-MS (*m*NBA) *m/z*: 1219 (M+Na). HR FAB-MS *m/z*: Calcd for $C_{68}H_{109}NaO_{15}P$, 1219.7402, Found 1219.7417.

2,6-Di-*O*-benzyloxymethyl-3,4,5-tris-*O*-(dibenzylphosphoryl)-D-*myo*-inositol 1-*O*-(1,2-Di-*O*-stearoyl-*sn*-glycerol Benzyl Phosphate) (6) Dibenzyl *N*,*N*-diethylphosphoramidite (0.10 ml, 0.39 mmol) was added to a mixture of **5** (54 mg, 0.042 mmol) and 1*H*-tetrazole (50 mg, 0.71 mmol) in 3 ml of dry CH_2Cl_2 at room temperature. The mixture was stirred for 30 min, then a small amount of water was added. After additional stirring for 10 min, the mixture was treated with *m*-CPBA (100 mg, 0.41 mmol) at −40 °C, and the whole was allowed to warm to room temperature. It was extracted with AcOEt, washed with aqueous 10% Na_2SO_3, aqueous saturated $NaHCO_3$ and brine, and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography with 30% AcOEt in CH_2Cl_2 to give **6** (69 mg, 0.035 mmol, 84%). ^{1}H-NMR δ ($CDCl_3$): 0.80—0.90 (6H, br), 1.10—1.40 (56H, br), 1.50—1.70 (4H, br), 2.20 (4H, br), 2.90—3.10 (2H, br), 3.90—5.10 (31H, br m), 7.10—7.40 (45H, br m). IR ($CHCl_3$) cm^{-1}: 2940, 2860, 1740, 1600, 1500, 1460, 1380, 1270, 1080. FAB-MS (*m*NBA) *m/z*: 1999 and 2000 (M+Na), 1977 (M+H). HR FAB-MS *m/z*: Calcd for $C_{110}H_{148}NaO_{24}P_4$, 1999.9209, Found 1999.9206.

3,4,5-Tris-*O*-phosphoryl-D-*myo*-inositol 1-*O*-(1,2-Di-*O*-stearoyl-*sn*-glycerol Phosphate) Sodium Salt (1b (Na)) Pd black (28 mg) and $NaHCO_3$ (6.4 mg, 0.078 mmol) were added to a solution of **6** (22 mg, 0.011 mmol) in 8.3 ml of 85% *tert*-BuOH in water, and the mixture was shaken under H_2 (50 psi) for 3 h. Precipitated insoluble sodium salts were dissolved by the addition of water and the mixture was shaken again under H_2 (50 psi) for a further 3 h. The catalyst was removed by filtration and washed with EtOH and water. The filtrate was concentrated *in vacuo* and lyophilized to give **1b** (Na) as the heptasodium salt. (13.6 mg). ^{1}H-NMR (D_2O) δ: 0.70 (6H, br), 1.00—1.50 (60H, br), 2.10—2.40 (4H, br), 3.80—4.50 (9H, br m), 5.20 (1H, br). ^{31}P-NMR (D_2O) δ: −0.127, 1.984, 3.563, 4.419. Negative FAB-MS *m/z*: 1105 (M−H), 1127 (M+Na−2H), 1149 (M+2Na−3H), 1171 (M+3Na−4H).

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