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Synthesis of L-α-phosphatidyl-D-*myo*-inositol 3,5-bisphosphate from D-glucose

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Abstract—Efficient synthesis of L-α-phosphatidyl-D-*myo*-inositol 3,5-bisphosphate was achieved from 1,2,5,6-diisopropylidene-D-glucose by utilizing ring-closing metathesis and catalytic OsO_4 dihydroxylation.

L-α-Phosphatidyl-D-*myo*-inositol (PI) and its phosphates (PIPn) play important roles as second messengers in intracellular signal transduction.[1] We have already reported the synthesis of various lipid analogs of PIPns as artificial second messengers and biochemical probes.[2–4] Recently, L-α-phosphatidyl-D-*myo*-inositol 3,5-bisphosphate (PI 3,5-P2, **1a**) was identified as a novel phosphoinositide (Fig. 1).[5] In mouse cells, PI 3,5-P2 is synthesized by phosphorylation of PI 3-P at the D-5 position by Fab 1.[6] Several studies have shown that PI 3,5-P2 production is essential for sorting membrane proteins into the lumen of the yeast vacuole[7] and for the maintenance of vacuolar size.[8] Although PI 3,5-P2 may be important for membrane homeostasis, its biological role has not yet been defined clearly.

To supply suitably protected optically active *myo*-inositol derivatives with appropriate stereogenic centers, Ferrier rearrangement,[9,10] pinacol coupling[11] and ring-closing metathesis (RCM)[12] of chiral intermediates derived from precursors such as carbohydrates have been performed in addition to optical resolution of *myo*-inositol derivatives. In 1996, we reported the facile asymmetric synthesis of *myo*-inositol derivatives via a 1,7-diene intermediate, which was synthesized from tartaric acid with complete stereocontrol.[11d] Since then, several reports have appeared by other groups employing 1,7-dienes as key synthetic intermediates.[11e,12,13] In this communication, we describe an efficient asymmetric synthesis of PI 3,5P2 (**1b**), a novel lipid analog of **1a**,[14,†] by RCM of a properly protected 1,7-diene (**4**)

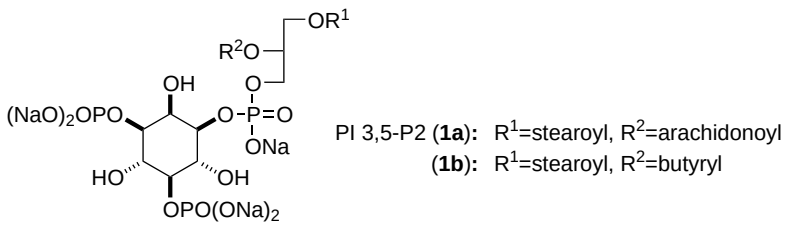

Figure 1.

Keywords: asymmetric synthesis; cyclitols; hydroxylation; inositols; metathesis; phospholipids; phosphoramidites.

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† According to our previous results, PI and PIP3 lipid analogs having a short saturated side chain at the *sn*-2 position were excellent substrates of PI 3-kinase and PIP3 5-phosphatase, respectively, while analogs with a long saturated side chain were not. Therefore, we employed butyrate as the *sn*-2 side chain of PI 3,5-P2, taking account of the potential biochemical application. See Ref. 3.

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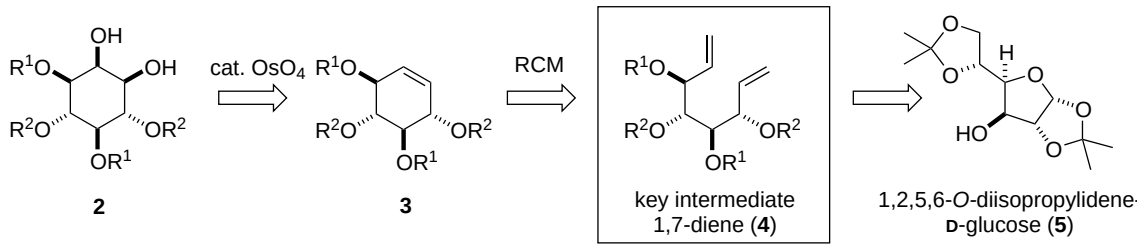
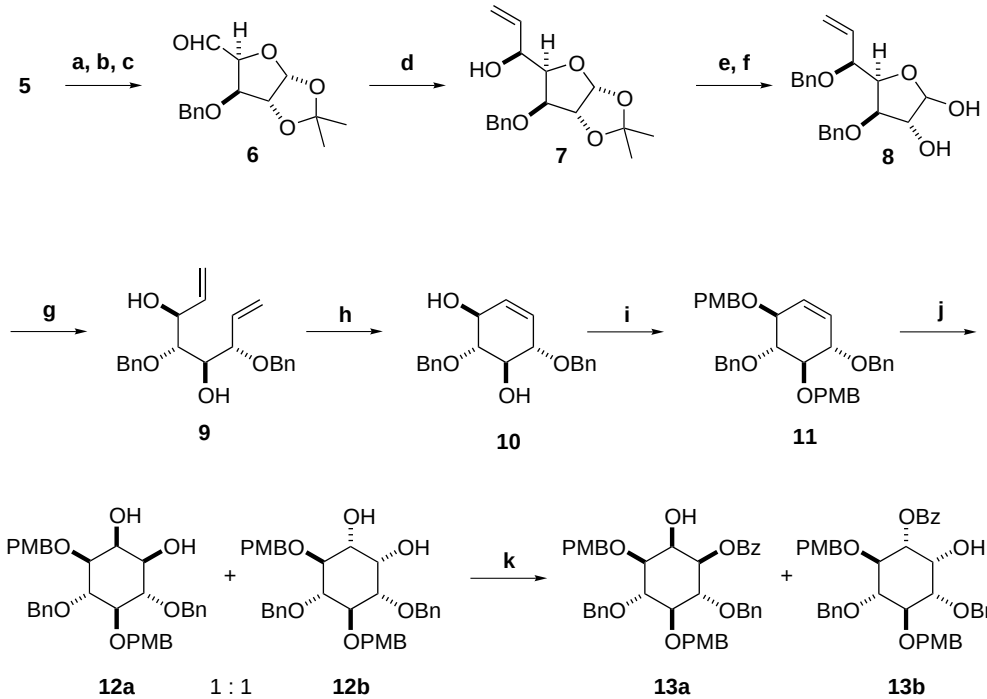


Scheme 1.



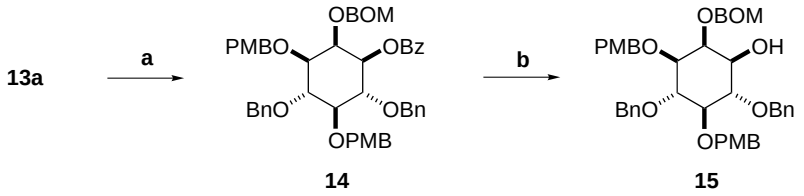
Scheme 2. (a) NaH, BnBr, DMF, 89%; (b) 60% aq. AcOH, rt, 95%; (c) $NaIO_4$, aq. $NaHCO_3$, CH_2Cl_2, rt, 2 h, 73%; (d) vinylmagnesium bromide (3.0 equiv.), CuBr–Me_2S (3.4 equiv.), THF–Me_2S, –40°C→rt, 2 h, 59%; (e) NaH, BnBr, DMF, 95%; (f) 80% AcOH, 70°C, 2 days, 92%; (g) $MePPh_3Br$, *n*-BuLi, ether, rt, overnight, 68%; (h) $Cl_2(PCy_3)_2Ru$=CHPh (10 mol%), CH_2Cl_2, rt, 1 h, 85%; (i) NaH, PMBCl, DMF, 92%; (j) OsO_4 (5 mol%), quinuclidine (5 mol%), NMO, CH_2Cl_2, rt, overnight, 98% (1:1 mixture of diastereoisomers); (k) BzCl (1.2 equiv.), pyridine, DMAP, 67% (+dibenzoates 27%).

derived from D-glucose, followed by catalytic OsO_4 dihydroxylation (Scheme 1).

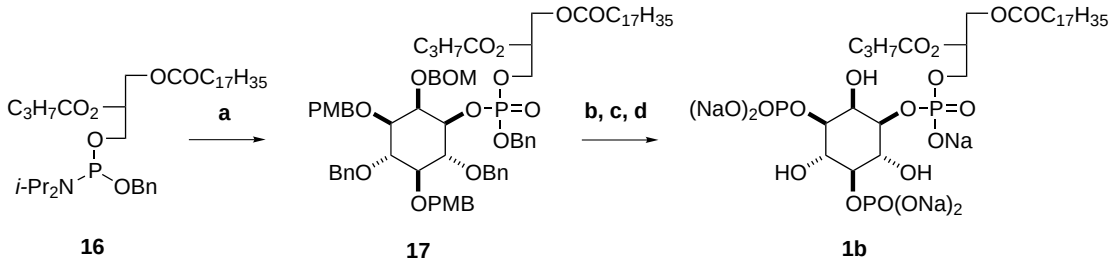
The 1,2-addition of an organocopper reagent prepared from vinylmagnesium bromide and CuBr–Me_2S to the known aldehyde (**6**) prepared from 1,2,5,6-diisopropylidene-D-glucose gave the desired allyl alcohol (**7**) as the sole product (Scheme 2).[15,16,‡] After protection of the C-2 hydroxy group as the benzyl ether, isopropylidene acetal was hydrolyzed with aq. AcOH to give the hemiacetal (**8**), which was subjected to Wittig methylenation to give the 1,7-diene (**9**). Ring-closing metathesis of **9** using Grubbs' catalyst $Cl_2(PCy_3)_2Ru$=CHPh[17] gave the cyclohexene (**10**) in 85% yield, and then two hydroxyl groups were protected as the PMB ether. The dihydroxylation of **11** utilizing a catalytic amount of osmium tetroxide with quinuclidine and NMO as co-oxidant gave the *cis*-diols (**12a**, **12b**) in 98% yield as an equimolar mixture of diastereoisomers by ^1H NMR analysis. Selective benzoylation of equatorially oriented hydroxyl groups of this mixture gave the benzoates (**13a**, **13b**), which were separated by silica gel column chromatography. Benzyloxymethylation of the desired **13a** followed by methanolysis of the benzoate afforded the appropriately protected *myo*-inositol (**15**) (Scheme 3).

The coupling of **15** with phosphoramidite (**16**), in the presence of 1*H*-tetrazole, followed by *m*-CPBA oxida-

‡ The 1,2-addition of vinylmagnesium bromide to the aldehyde **6** took place with no selectivity (**7**:*epi*-**7**=1.25:1). Stereochemistry of these products was established by the comparison of the ^1H NMR spectrum of **11** derived from a known bisallyl alcohol. See Ref. 11d. ^1H NMR of **11** ($CDCl_3$, 500 MHz) δ 3.68 (1H, d, J=5.1 Hz), 3.69 (1H, d, J=5.1 Hz), 3.79 (6H, s), 4.17 (1H, d J=5.1 Hz), 4.18 (1H, d, J=5.1 Hz), 4.60 (1H, d, J=11.1 Hz), 4.64 (1H, d, J=11.1 Hz), 4.67 (1H, d, J=11.6 Hz), 4.71 (1H, d, J=11.6 Hz), 4.77 (1H, d, J=10.4 Hz), 4.84 (1H, d, J=10.4 Hz), 4.85 (1H, d, J=11.0 Hz), 4.92 (1H, d, J=11.0 Hz), 5.69 (1H, s), 5.70 (1H, s), 6.82 (2H, d, J=8.6 Hz), 6.84 (2H, d, J=8.6 Hz), 7.22–7.26 (4H, m), 7.28–7.37 (10H, m)



Scheme 3. (a) BOMCl, diisopropylethylamine, tetra-*n*-butylammonium bromide, CH_2Cl_2, 70°C, 2 days, 30% (recovered **13a** 68%); (b) K_2CO_3, MeOH, rt, 4 h, quant.



Scheme 4. (a) **15**, 1*H*-tetrazole (8.0 equiv.), CH_2Cl_2, rt, 2.5 h, then *m*-CPBA (4.2 equiv.), −78°C→rt, 45 min, quant.; (b) DDQ (3.0 equiv.), wet CH_2Cl_2, rt, 2.5 h, 52%; (c) $(BnO)_2PNEt_2$ (5.7 equiv.), 1*H*-tetrazole (6.0 equiv.), CH_2Cl_2, rt, 2.5 h, then *m*-CPBA (8.0 equiv.), −78°C→rt, 30 min, 82%; (d) H_2 (4.3 kgf/cm^2), Pd black, $NaHCO_3$, *tert*-BuOH/H_2O, quant.

tion gave the phosphodiester **17** (Scheme 4). PMB protecting groups of **17** were oxidatively removed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and the free hydroxyl groups were phosphorylated by the amidite method to give the fully protected PI 3,5-P2 precursor. Finally, hydrogenolysis with Pd black as a catalyst in the presence of $NaHCO_3$ gave PI 3,5-P2 (**1b**) as the sodium salt.[§]

In conclusion, we achieved efficient construction of the key 1,7-diene intermediate, which is adaptable for the synthesis of not only a series of inositol analogs, but also various six-membered cyclitols such as condritol B, and we also successfully completed the total synthesis of PI 3,5-P2 utilizing RCM and catalytic OsO_4 dihydroxylation. Clarification of the biochemical function of PI 3,5-P2 as a second messenger using this synthetic product as a biological tool is in progress.

Acknowledgements

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[§] Spectral data for **1b** (Na salt): ^{31}P NMR (D_2O, 202 MHz, 5% K_3PO_4 external reference): δ −2.87, −4.16, −5.46; FABMS *m*/*z* (*m*-NBA+Gly+NaCl): 875 $(M-H+2Na)^+$, 897 $(M-2H+3Na)^+$, 919 $(M-3H+4Na)^+$, 941 $(M-4H+5Na)^+$, 963 $(M-5H+6Na)^+$; HRMS (FAB) *m*/*z*: calcd for $C_{31}H_{58}O_{19}P_3Na_4$ $(M-3H+4Na)^+$, 919.2376, found: 919.2425.

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