Lipid analogue for study of interactions between PIP_ns and proteins

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Lipid molecules not only make up the cell membrane that allow for the complex organization of life, but they are also crucial to the regulation of biological pathways. Phosphoinositides are the most important class of lipids that control cell functions. However, our understanding of the molecular basis for the interaction of lipids with the proteins they regulate is still ambiguous. Such an understanding is crucial both to conceiving the fundamental workings of living organisms and also for intervention in disease such as cancer and diabetes that result from mis-regulating of these pathways.

The Oakley lab has therefore developed a class of lipid analogues that allow us to display multiple copies of the polar head groups of the lipids on a spherical surface of polymers, PAMAM^{TM} dendrimers. To date, we have prepared two classes of dendrimers: those modified with enantiomerically pure $PI(4,5)P_2$ and those modified with racemic $PI(3,4,5)P_3$ headgroups. However, the preparation of these headgroup analogues was found to be inefficient. This report describes progress toward a more efficient synthesis of enantiomerically pure $PI(3,4,5)P_3$ headgroups.

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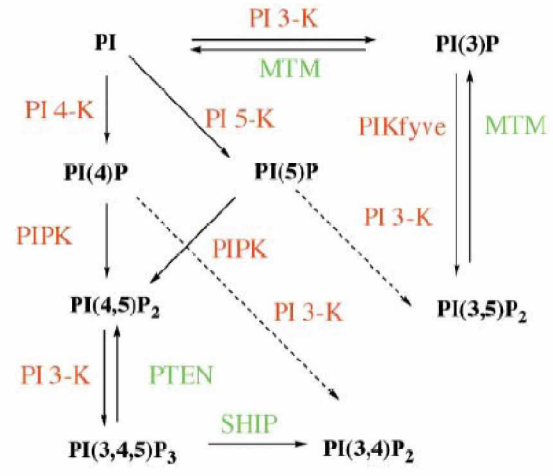
Introduction

Interactions between lipids and proteins modulate dynamically phosphoinositide (PIP_n) signaling transductions. PIP_ns are essential elements in tyrosine kinase-based signaling system and G protein receptor of signaling transductions (1 and 2). In addition, these lipid signaling plays important roles in membrane transport and endosome activities (3), including endocytosis, exocytosis, Golgi networks, and protein transport (4), in cell cycle (5 and 6), in remodeling of the actin cytoskeleton (7), and in mitogenesis and oncogenesis (8 and 9). As shown in Figure 1, PIP_ns are can be produced and transformed into relating analogues within cells by the intervention of various kinases (10) and phosphatases (11). PIP_n can bear various headgroups. Lipid



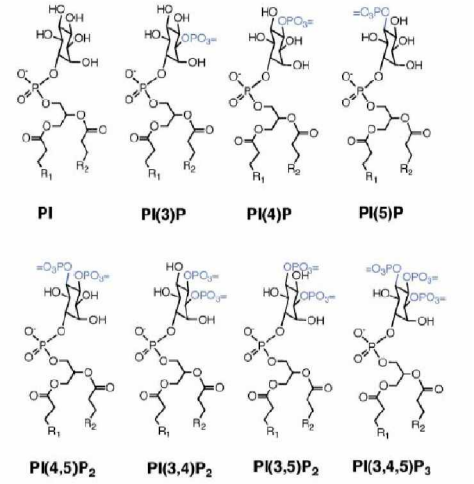


Fig 1. Interconversions of PIP_ns by lipid kinases (red) and lipid phosphatases (green). Figure taken from *Chem. & Biol*. **11** (2004) 619-637.

Fig 2. Structures of the phosphatidylinositol and its phosphates and synthetic analogs. Figure taken from polyphosphates and *Chem. & Biol*. **11** (2004) 619-637.

phosphatidylinositol (PI), the basic form, has no annexed phosphorylation on the inositol ring and the other seven PIP_ns consist of three monophosphates, three bisphosphates, and one trisphosphate (Figure 2).

Among these PIP_ns, PIP_2 (PtdIns(4,5,)P_2) and PIP_3 (PtdIns(3,4,5)P_3) play a variety of important roles in physiological processes. PIP_2 is a substrate for phospholipase C, and the receptor-activated action of PLC on PIP_2 generated the second messengers Ins(1,4,5)P_3 and 1,2-diacylglycerol (DAG) (12 and 13). PIP_2 has been recognized to be a crucial element in the recruitment of signaling proteins to membranes (14), as mediated by the pleckstrin homology (PH) domains (15). In addition, PIP_2 affects the organization of the cytoskeleton by sequestering profilin, thereby preventing the association of profilin with monomeric F-actin and thus permitting the polymerization of actins (16). PIP_2 can be converted by agonist-stimulated, receptor–mediated activation of phosphoinositide 3-kinase (PI 3-K) to PIP_3, the key element in a new intracellular signaling system (17).

Sarah Webb and Nichole Stewart in the Oakley lab have successfully prepared of the PAMAM^{TM} (Aldrich) dendrimer with multiple attached PIP_2 headgroups (Fig 3). These polymers will be used to mimic PIP_2 micelles as they are held in the phosphatidylcholine bilayer, and thus should help to gain a better understanding of the orientation in which molecule must be arranged in order to achieve binding with cytockeletal protein such as profilin. Lewis Belcher and Nichole Stewart in the

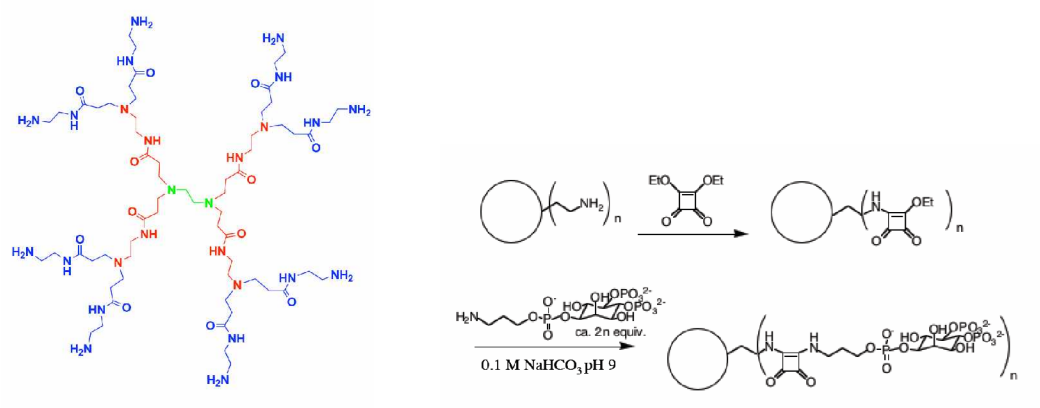
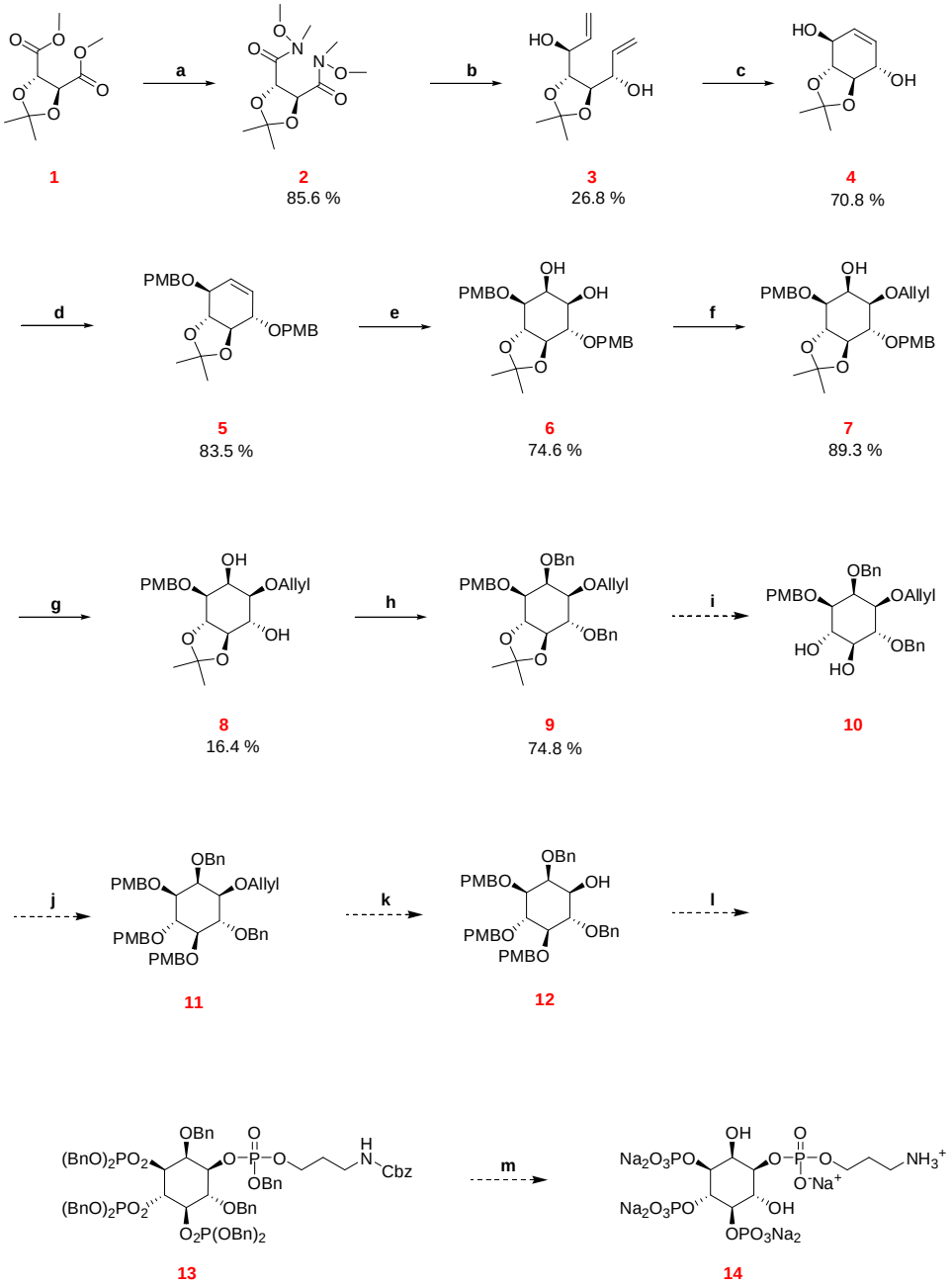


Fig. 3 (left): Structure of PAMAM^{TM} dendrimer : Ethylene diamine core (green), Generation 0 (G0) bearing 4 amine termini (red), and Generation 1 (G1) bearing 8 amine termini (blue), (right): General scheme for attachment of phospoinositide head group to the PAMAM^{TM} dendrimer scaffold. Figure taken from Webb's thesis (S.A. Webb, Synthesis and characterization of multivalent mimics of phosphatidylinositol-4,5-bisphosphate (PIP_2) micelles, 2004.)

Oakley lab also tried to prepare racemic PIP_3 by utilizing Prestwich's synthetic route (18). However, there are many troubles, including too low yield, tedious enantiomeric resolution steps, and problem of acyl group migration in this synthetic route. Thus, we have decided to adapt Bertozzi lab approach (19). – generates enantiomerically pure inositol derivatives from C2 symmetric conduritol.

Our ultimate objective is preparing a series of dendrimer analogues that vary in the phosphate substitution patterns on the inositol ring and the size of the dendrimers, from those containing 4 to 256 head groups and our short-term aim is to make PIP_3 headgroup and to establish a more efficient route for the preparation of this analogue. So, we will monitor binding of each protein to each of the dendrimer analogue.

Results & Discussion



Scheme 1. (a) $CH_3NHOCH_3{\cdot}HCl$, $AlMe_3$, CH_2Cl_2, -10 ºC; **(b)** i) vinyl magnesium bromide, THF, from -78 to -5 ºC; ii) $CeCl_3{\cdot}7H_2O$, $NaBH_4$, MeOH, -78 ºC; **(c)** $RuCl_2CHPhPCy_3IMesH_2$ (2 mol %), CH_2Cl_2, reflux; **(d)** PMBCl, NaH, DMF, rt; **(e)** K_2OsO_4, K_2CO_3, $K_3Fe(CN)_6$, methanesulfonamide, quinuclidine, *t*-BuOH, H_2O, rt; **(f)** i) Bu_2SnO, toluene, reflux; ii) allyl bromide, $Bu_4N^+I^-$, 60 ºC, toluene; **(g)** DDQ, CH_2Cl_2, 0 ºC; **(h)** BnBr, NaH, DMF, rt; **(i)** 1*M* HCl:MeOH 1:10, rt; **(j)** PMBCl, NaH, DMF, rt; **(k)** $PdCl_2$, MeOH, from 0 ºC to rt; **(l)** i) Phosphite linker, tetrazole, CH_2Cl_2; ii) *m*-CPBA, from -78 ºC to rt; iii) DDQ, wet CH_3CN; iv) $(BnO)_2PN{i}Pr_2$, tetrazole, CH_2Cl_2; v) *m*-CPBA,

from -78 ºC to rt; **(m)** i)H_2, Pd/C, ethanol; ii) Chelex column(Na^+ form), $Et_3NH^+HCO_3^-$ (TEAB) buffer, pH 8.3

Among the headgroups of PIP_ns, we first have been synthesizing an analogue of $PIP(3,4,5)_3$. A number of strategies for the synthesis of analogues of PIP_3 have been reported in the literature (20, 21 and 22). However, there are still the difficulties in synthesizing a selectively protected *myo*-inositol unit. Because *myo*-Inositol unit's chiral centers frequently require tedious resolution steps (23) and protections of their numerous hydroxyl groups make synthetic route longer and low yielding (24 and 25). As Ferrier rearrangement, pinacol coupling, and ring-closing metathesis methods (26, 27, 28, 29, 30, and 31) have been reported, the *myo*-inositol residue can be easily prepared from intermediates with the appropriate stereogenic centers.

In the work described here, we have adapted Bertozzi's synthetic route of differentially protected *myo*-inositol derivatives for the production of an enanthiomerically pure, tetherable PIP_3 analogue (19) (Scheme 1). Her synthetic route has a significant property, C2 symmetry. It gave us some merits such as no resolution step and no loss in yield due to the formation of diastereomers. We are directly following her only through compound **7.** After this, we will modify the PIP_3 analog with an aminopropyl tether at C1 position in order to serve as a bridge between PIP_3 analog and PAMAM^{TM} dendrimer.

To date, we have synthesized product **9**. Weinreb amide **2** was easily synthesized in high yield by conversion of commercially available dimethyl 2,3-*O*-isopropylidene-D-tartrate. After Grignard reaction via vinyl magnesium bromide, Luche reduction gave the 1,7-diene **3**. However, yield of this method of set of reactions was too low (< 10 %) in comparison to yield (73 %) of literature. We found a possible cause that work up with

aqueous NH_4Cl or dilute HCl solution can produce Michael adduct rather than diene. As shown in Fig 4, the liberated *N,O*-dimethylhydroxylamine added to the newly formed α,β-unsaturated ketone to afford Michael adduct (32). To remedy the

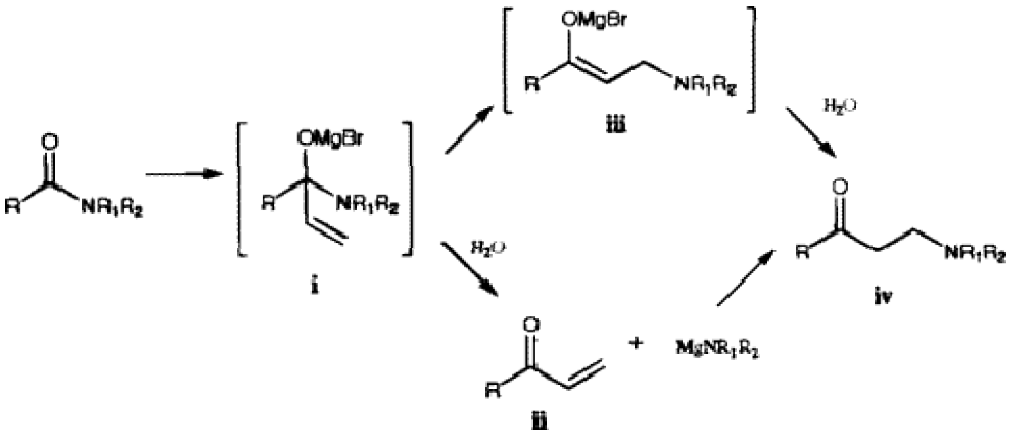


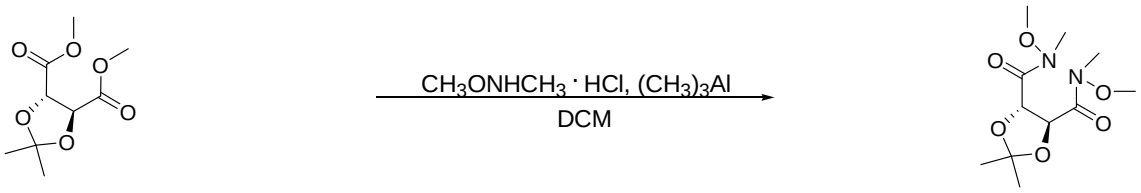
Fig 4. Mechanism of Michael reaction in Weinreb amide. Figure taken from A. Gomtsyan, *Org. Lett.* **2** (2002), 11-13.

unfavorable situation, we needed a solution to inactivate the expelled *N,O*-dimethylhydroxylamine. According to Miller's method (he had the same trouble we had with Grignard reaction) (33), quenching the reaction with acetic anhydride and methanol provided the desired diene **3** in better yield (26.8 %). This reaction route is still a challenging problem. Presumably, the other cause for low yield lies on variation of ratio of diastereomers depending on reduction conditions. Through Grubbs' second-generation catalyst, ring-closing metathesis afford to conduritol analog **4** in good yield. By utilizing benzyltrietylammonium chloride as phase transfer catalyst, simultaneous PMB protection of the C3 and C6 hydroxyl groups afforded compound **5** in low yield (10~50 %). Instead of this method in her paper, we synthesized product **5** in higher yield (83.5 %) by sodium

hydride as a base. Subsequent dihydroxylation (*syn*-addition) furnished the both oxygenated *myo*-inositol intermediate **6**. In this reaction, a period of reduction by Na_2SO_3 was essential to increase yield. Differentiation of the vicinal diol was achieved via selective C1 allylation to produce compound **7** with high regioselectivity (89.3 %). Selective elimination of the C6 *p*-methoxybenzyl ether was achieved by treatment with DDQ to afford compound **8.** However, this reaction proceeded in poor yield (16.4 %) in the presence of an unprotected C2 hydroxyl group. So we will try to C6 PMB deprotection in the situation of protected C2 hydroxyl group. Dibenzyl protection of the C2 and C6 hydroxyl groups afford compound **9** by the same method of PMB protection. High yield synthetic methods in compound **3** and **8** are needed in order to provide a more efficient route for the preparation of PIP_3 analogue.

Material & Method

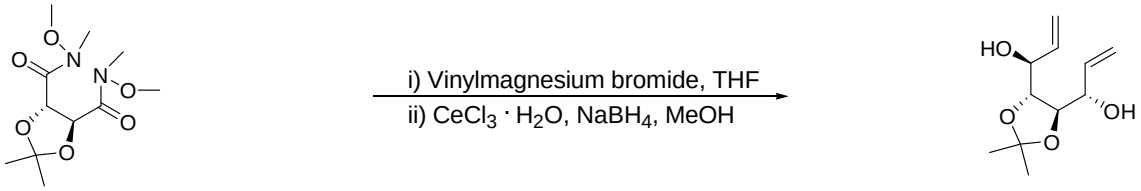
General Procedure. Unless otherwise noted, all reagents were obtained from commercial supplies and used without purification. Toluene and CH_2Cl_2 were distilled from CaH_2, and THF was distilled from sodium benzophenone ketyl, immediately prior to use. Chromatography was carried out using Merk 60 Å 230-400 mesh silica gel. Components were visualized either by ultraviolet light or ceric ammonium molybdate staining. Unless otherwise noted, all organic layers were dried over anhydrous $MgSO_4$, and all solvents were removed with a rotary evaporator under reduced pressure. NMR spectra were recorded on a 300 or 400 MHz spectrometer. Chemical shift are expressed in ppm downfield relative to tetramethylsilane. Coupling constants, *J*, are listed in Hertz.



1 **2**

2,2-Dimethyl-[1,3]dioxolane-4,5-dicarboxylic acid bis-(methoxy-methyl-amide) (2)

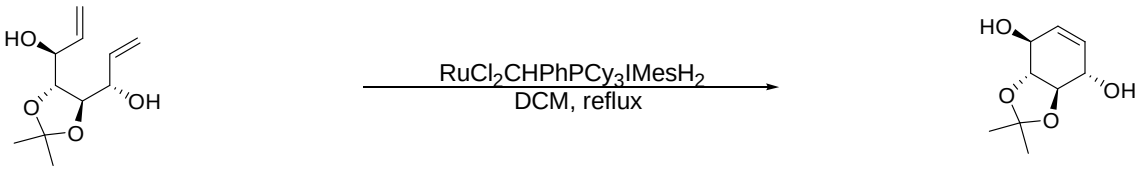
To a solution of *N,O*-dimethyl hydroxylamine hydrochloride (17.4g, 178.7 mmol) in CH_2Cl_2 (100 mL) was added trimethylaluminum (89.4 mL, 2M in n-hexane) dropwise at 0 °C under argon. The solution was allowed to warm to room temperature. After stirring for 30 min at room temperature, the solution was cooled to -10 °C. (-)-dimethyl 2,3-isopropylidne-D-tartrate (13.0 g, 59.6 mmol) in CH_2Cl_2 (50 mL) was added dropwise and then stirred for 3 hours at -10 °C under argon. The resulting solution was quenched with 1N HCl (100 mL) and then extracted with CH_2Cl_2 (3 X 80 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by flash chromatography (acetone : chloroform = 5 : 95) to afford compound **2** as a white solid (11.1 g, 85.6 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.15 (s, 2H), 3.67 (s, 6 H), 3.20 (s, 6 H), 1.49 (s, 6H). The ^{1}H NMR data are in accord with those previously reported.



2 **3**

1-[5-(1-Hydroxy-allyl)-2,2-dimethyl-[1,3]dioxolan-4-yl]-prop-2-en-1-ol (3)

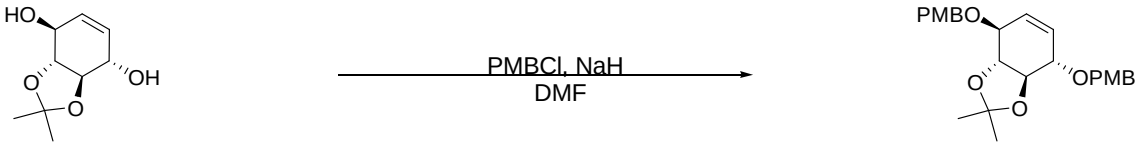
To a solution of bis-Weinreb amide **2** (5.0 g, 18.1 mmol) in 80 mL of THF at -78 °C was added vinyl magnesium bromide (54.3 mL, 1M in THF) under argon. After stirring for 30 minutes, the solution was warmed to 0 °C and then stirred for 1 hour at the same temperature. Acetic anhydride (30 mL) was added to the solution and then it was followed by methanol (30 mL) at 0 °C. The resulting solution was reduced to 1/4 of its original volume under reduced pressure and then diluted with diethyl ether. The mixture was washed with saturated NH_4Cl (1 X 100 mL) and dried over anhydrous $MgSO_4$. The mixture was filtered and concentrated under reduced pressure. To the previous mixture in MeOH (100 mL) was added cerium (III) chloride heptahydrate (20.2 g, 54.3 mmol). After stirring until cerium (III) chloride heptahydrate was dissolved, the solution was cooled to -78 °C. Sodium borohydride (2.1 g, 54.3 mmol) was added to the solution in small portion at -78 °C. After stirring for 1h, the solution was quenched with saturated NH_4Cl (50 mL), extracted with EtOAC (3 X 50 mL), and dried over $MgSO_4$. Flash chromatography (EtOAC : n-hexane = 1 : 2) furnished compound **3** as a pale yellow oil (1.04 g, 26.8 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.87 (ddd, *J* = 17.2, 10.4, 4.8 Hz, 2H), 5.36 (dt, *J* = 17.2, 1.3 Hz, 2H), 5.25 (dd, *J* = 10.4, 1.2 Hz, 2H), 4.14-4.10 (m, 2H), 3.99 (dd, *J* = 2.5, 1.3 Hz, 2H), 2.31 (d, *J* = 7.0 Hz, 2H), 1.43 (s, 6H). The ^{1}H NMR data are in accord with those previously reported.



3 **4**

2,2-Dimethyl-3a,4,7,7a-tetrahydro-benzo[1,3]dioxole-4,7-diol (4)

To the compound **3** (822 mg, 3.84 mmol) in CH_2Cl_2 (50 mL) was added Grubb's second generation catalyst, $RuCl_2CHPhPCy_3IMesH_2$, (65.2 mg, 0.077 mmol). The resulting solution was heated at reflux for 4 h and dried under reduced pressure. The crude product was purified by flash chromatography (EtOAC : n-hexane = 2 : 1) to afford compound **4** as a colorless oil (506 mg, 70.8 %). ^{1}H NMR ($CDCl_3$, 300 MHz): 5.64 (s, 2H), 4.47 (s, 2H), 3.51 (dd, J = 5.6, 2.3 Hz, 2 H), 2.61 (s, 2H), 1.44 (s, 6H). The ^{1}H NMR data are in accord with those previously reported.

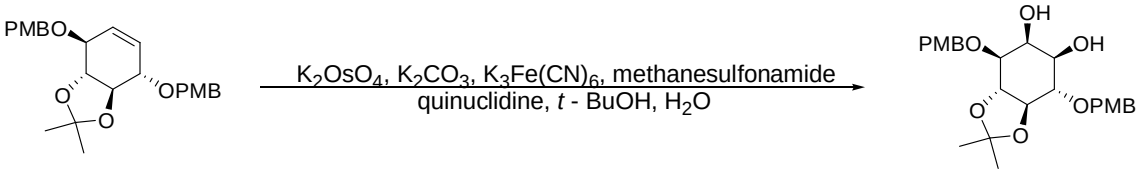


4 **5**

4,7-Bis-(4-methoxy-benzyloxy)-2,2-dimethyl-3a,4,7,7a-tetrahydro-benzo[1,3] dioxole (5)

To a solution of conduritol **5** (560 mg, 3.06 mmol) in DMF (30 mL) was added 60 % dispersion sodium hydride (144 mg, 6.1 mmol). After the solution was cooled to 0 °C, 4-methoxybenzyl chloride (1.05 mL, 7.64 mmol) was added. The solution was stirred for 20 h at room temperature under argon. The solution was quenched with water and extracted with CH_2Cl_2 (3 X 15 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by flash chromatography (EtOAC : n-hexane = 1 : 4) to give compound **5** as a yellow solid (1.09 g, 83.5 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28 (d, J = 7.1 Hz, 4 H), 6.86 (d, J = 7.1 Hz, 4 H), 5.64 (s, 2H), 4.74 (d, A of AB, J_{AB} = 11.4 Hz, 2H), 4.58 (d, B of AB, J_{AB} = 11.4 Hz,

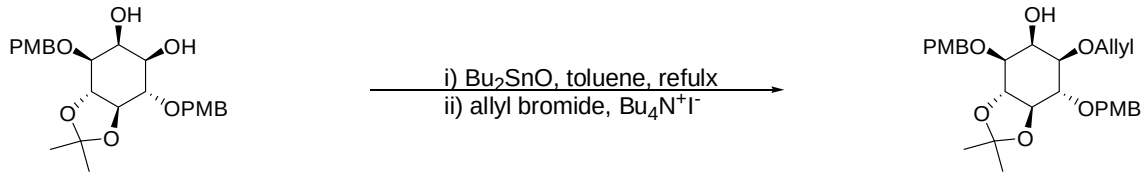
2H), 4.21(d, *J* = 5.9 Hz, 2 H), 3.78 (s, 6H), 3.61 (dd, *J* = 5.7, 1.8 Hz, 2H), 1.47 (s, 6H). The ^{1}H NMR data are in accord with those previously reported.



5 **6**

4,7-Bis-(4-methoxy-benzyloxy)-2,2-dimethyl-hexahydro-benzo[1,3]dioxole-5,6-diol (6)

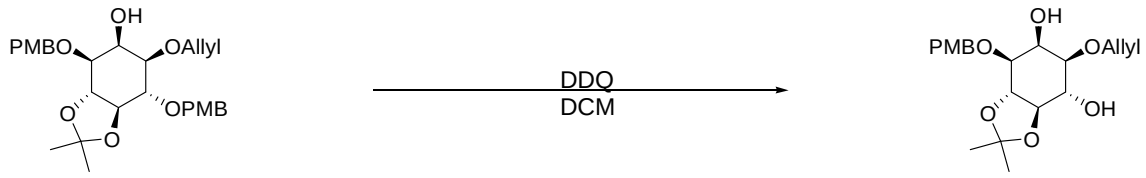
A solution of potassium ferrous cyanide (540 mg, 1.64 mmol), potassium carbonate (227 mg, 16.4mmol), methanesulfonamide (4.5 mg, 0.47 mmol), and quinuclidine (5.2 mg, 0.047 mmol) in water (2 mL) was stirred until it became homogeneous. Compound **5** (100 mg, 0.23 mmol) in *tert*-buthanol (3 mL) was added and followed by potassium osmate dehydrate (4.3 mg, 0.012 mmol). The mixture was stirred for 24 h at room temperature. The reaction mixture was quenched with saturated aqueous Na_2SO_3 (10 mL) and extracted with EtOAC (3 X 10 mL), washed with saturated aqueous NaCl, filtered, dried and concentrated. Flash chromatography (EtOAC : n-hexane = 1 : 1) furnished compound **6** as a white solid (79 mg, 74.6 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.29 (d, *J* = 8.6 Hz, 4 H), 6.86 (d, *J* = 8.6 Hz, 4 H), 4.85 (d, A of AB, J_{AB} = 11.5 Hz, 1H), 4.77 (d, B of AB, J_{AB} = 11.5 Hz, 1H), 4.62 (d, A of AB, J_{AB} = 2.6 Hz, 1H), 4.59 (d, B of AB, J_{AB} = 2.6 Hz, 1H), 4.16 (t, *J* = 2.6 Hz, 1H), 4.00 (t, *J* = 9.7 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.77 (t, *J* = 9.5 Hz, 1H), 3.56 (dd, *J* = 10.1, 3.1 Hz, 1H), 3.50 (dd, *J* =12.1, 4.8 Hz, 1H), 3.35 (t, *J* = 9.6 Hz, 1H), 2.55-2.53 (m, 2H), 1.47 (s, 3H), 1.45 (s, 3H). The ^{1}H NMR data are in accord with those previously reported.



6 **7**

6-Allyloxy-4,7-bis-(4-methoxy-benzyloxy)-2,2-dimethyl-hexahydro-benzo[1,3]dioxol-5-ol (7)

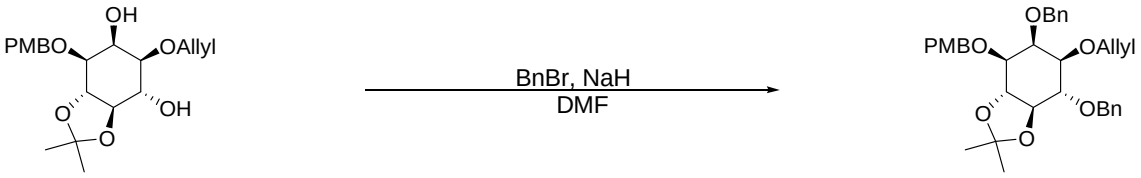
A solution of diol **6** (100 mg, 0.22 mmol) and dibutyltin oxide (54.1 mg, 0.22 mmol) in toluene (10 mL) was heated at reflux for 3 hours with azeotropic removal of water under argon. The solution was then cooled to 60 °C, and *tert*-butyl ammonium iodide (80.2 mg, 0.22 mmol) and allyl bromide (0.19 mL, 2.2 mmol) were added. The solution was stirred for 24 h at 60 °C under argon and concentrated under reduced pressure. The crude product was purified with flash chromatography (EtOAC : n-hexane = 1 : 2) to give compound **7** as a pale red oil (97 mg, 89.3 %) ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28 (d, J = 8.6 Hz, 4 H), 6.85 (d, J = 8.6 Hz, 4 H), 5.93-5.85 (m, 1H), 5.24 (d, J = 17.4 Hz, 1H), 5.15 (d, J = 10.0 Hz, 1H), 4.79 (d, A of AB, J_{AB} = 8.0 Hz, 1H), 4.76 (d, B of AB, J_{AB} = 8.0 Hz, 1H), 4.66 (d, A of AB, J_{AB} = 11.8 Hz, 1H), 4.63 (d, B of AB, J_{AB} = 11.8 Hz, 1H), 4.21-4.11 (m, 3H), 4.04 (t, J = 9.6 Hz, 1H), 3.89 (t, J = 8.4 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.52 (dd, J = 10.4, 3.2 Hz, 1H), 3.36-3.26 (m, 2H), 2.58 (s, 1H), 1.46 (s, 3H), 1.44 (s, 3H). The ^{1}H NMR data are in accord with those previously reported.



7 **8**

5-Allyloxy-7-(4-methoxy-benzyloxy)-2,2-dimethyl-hexahydro-benzo[1,3]dioxole-4,6-diol (8)

A solution of compound **7** (80 mg, 0.16 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (43.5 mg, 0.19 mmol) in CH_2Cl_2 (30 mL) was stirred at 0 °C for 24 h under argon. The resulting solution was quenched with saturated aqueous $NaHCO_3$ (20 mL) and washed saturated aqueous NaCl (1 X 20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash chromatography (EtOAC : n-hexane = 1 : 1) afforded compound **8** as a white solid (10 mg, 16.4 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30 (d, *J* = 7.6 Hz, 2 H), 6.87 (d, *J* = 8.0 Hz, 2 H), 5.94-5.87 (m, 1H), 5.28 (d, *J* = 17.6 Hz, 1H), 5.21 (d, *J* = 10.0 Hz, 1H), 4.79 (d, A of AB, J_{AB} = 11.6 Hz, 1H), 4.63 (d, B of AB, J_{AB} = 11.6 Hz, 1H), 4.19 (dd, *J* = 16.8, 9.2 Hz, 2H), 4.09-4.03 (m, 3H), 3.79 (s, 3H), 3.56 (d, *J* = 9.6 Hz, 1H), 3.31 (t, *J* = 9.4 Hz, 1H), 3.18 (d, *J* = 8.4 Hz, 1H), 2.47 (s, 2H), 1.46 (s, 6H).



8 **9**

5-Allyloxy-4,6-bis-benzyloxy-7-(4-methoxy-benzyloxy)-2,2-dimethyl-hexahydro-benzo[1,3]dioxole (9)

To a solution of compound **8** (35 mg, 0.092 mmol) in DMF (10 mL) was added 60 % dispersion sodium hydride (4.42 mg, 0.18 mmol). After the solution was cooled to 0 °C, benzyl bromide (0.028 mL, 0.23 mmol) was added. The solution was stirred for 24 h at room temperature under argon. The solution was quenched with water and extracted with CH_2Cl_2 (3 X 10 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash

chromatography (EtOAC : n-hexane = 1 : 4) to give compound **9** as a brown oil (3.86 m g, 74.8 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36 (d, *J* = 6.4 Hz, 4 H), 7.30 (d, *J* = 6.8 Hz, 4 H), 7.23 (d, *J* = 8.4 Hz, 2 H), 6.84 (d, *J* = 8.8 Hz, 2 H), 5.89 -5.81 (m, 1H), 5.24 (d, *J* = 17.2 Hz, 1H), 5.12 (d, *J* = 10.8 Hz, 1H), 4.87-4.66 (m, 5H), 4.53 (d, *J* = 11.6 Hz, 1H), 4.12-3.95 (m, 5H), 3.77 (s, 3H), 3.50 (d, *J* = 10.0 Hz, 1H), 3.33 (t, *J* = 9.2 Hz, 1H), 3.26 (d, *J* = 8.8 Hz, 1H), 1.43 (s, 3H), 1.41 (s, 3H).

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