Regiospecific Synthesis of 2,6-Di-*O*-(α-D-mannopyranosyl)phosphatidyl-D-*myo*-inositol

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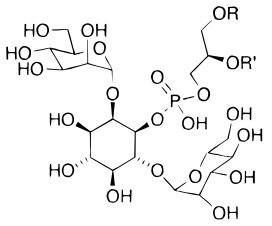
Glycosyl phosphatidylinositols (GPIs) in biological membranes have recently been shown to play a role in both the anchoring of cell-surface proteins to membranes and in signal transduction involving insulin action.[1] The chemical structures of mannosylated inositol phospholipids of *Mycobacterium* were elucidated 30 years ago, although their biological functions are still unclear.[2] Synthesis of 2-*O*-(mannopyranosyl)-[3] and 2,6-di-*O*-(mannopyranosyl)-1-phosphatidylinositol (**1**) (PIM_2)[4] was reported previously.



1, R, R'=fatty acid residue

Our investigation into synthetic methodologies based on phosphite chemistry has afforded new phosphorylation[5] and glycosylation[6] methods. Phosphite−phosphonium salt phosphorylation methodology,[5] which involves the reaction of a phosphite with an alcohol in the presence of pyridinium bromide perbromide and a *tertiary* amine, permits the regioselective introduction of a phosphoryl function to a 1,2-diol derivative of *myo*-inositol. No other phosphorylation methods,[7] except for that reported by Meek et al.,[8] have shown such regioselectivity. The new phosphorylation procedure[5] is quite useful for expeditious construction of inositol phospholipids. Hitherto, the introduction of phosphoryl functions at C-1 has been accomplished by sequential procedures involving temporary protection of OH-1 in a 3,4,5,6-protected inositol derivative, protection of OH-2, regeneration of the hydroxyl group at C-1, and then phosphorylation of the hydroxyl.[9] Glycosylation using a glycosyl phosphite as the glycosyl donor has been demonstrated to be useful for the synthesis of glycosyl inositols and sialides.[6,10]

Disiloxanyl derivatives such as **2** have been shown to be useful synthetic intermediates in the preparation of *myo*-inositol phosphates[11] and phosphoinositides.[12] Compound **2** was readily derived from *myo*-inositol by two sequential regioselective cyclohexylidenation and silylation reactions. The usefulness of the disiloxanyl group as the protecting group for inositol is partly based on *space-through* protection of the 5-hydroxyl group by the adjacent bulky silyl substituent. Thus, regioselective acylation of **2** could be accomplished at the 6 position.[11,12] In the present paper, we describe the synthesis of **1** using a combination of the two synthetic methods described above using synthetic intermediate **2**.

Glycosylation of racemic **2** using tetra-*O*-benzyl-D-mannopyranosyl phosphite **3a** in the presence of *N*-iodosuccinimide and trifluoromethanesulfonic acid in dichloromethane afforded a 1:1 diastereomeric mixture of 6-*O*-α-mannosylinositols **4a** [$\delta_{H(anomeric)}$ 5.40 (d, $J = 1.46$ Hz) and 5.50 (d, $J = 1.46$ Hz)][13] in 45% yield (Scheme 1). The reaction also yielded diastereomeric *β*-mannopyranosides that were isolated in 15% yield each [$\delta_{H(anomeric)}$ 5.75 (s) and 4.75 (s)] by flash chromatography. Using a combination of $ZnCl_2$ (0.2 equiv based on **3a**) and $AgClO_4$ (0.4 equiv) as the promoter in ethyl ether gave comparable results (α: 42% yield; *β*: 23% yield). When 2-*O*-acetylmannosyl phosphite **3b** replaced **3a** as the glycosyl donor in the presence of 0.2 equiv of $ZnCl_2$ and 0.4 equiv of $AgClO_4$, stereo- and regioselective reaction occurred, resulting in the formation of only the corresponding α-glycoside **4b** in 70% yield (inseparable mixture of two diastereomers in a 1:1 ratio).[13a] In both cases, 5-*O*-glycosylated products were not observed. The glycosylation site in **4a** was confirmed by analysis of ^1H NMR spectra that showed two broad triplets of diastereomeric H_5 protons as a result of coupling with the hydroxyl proton. Furthermore, experiments derivatizing **4b** and **10** to their corresponding triacetates by removal of the TIPDS group followed by acetylation elucidated the structures of the glycosides **4**. Although glycosidation of the acetylmannosyl phosphite **3b** produced a higher yield of **4b**, the inherent instability of the acetyl group under basic conditions resulted in a serious problem during a later stage. Therefore, **4a** was employed during the present synthesis. Treatment of **4a** with trifluoroacetic acid and methanol gave diastereomeric triols **5** which were then separated by flash chromatography to afford pure diastereomers in 44 and 45% yield, respectively.

The absolute configuration of **5** was confirmed by its transformation to fully deprotected mannosylinositol and

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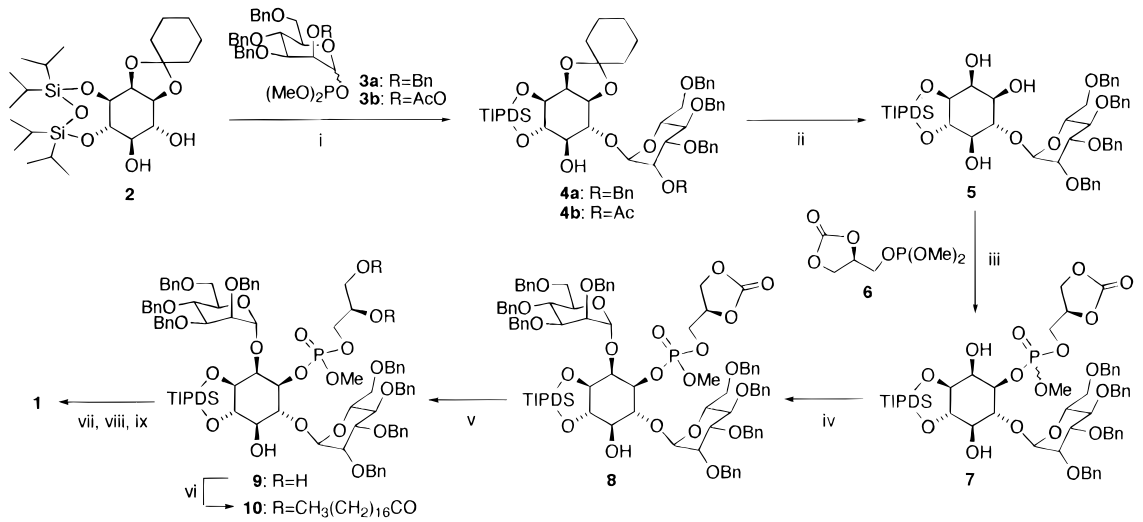
Scheme 1[a]



[a] Key: (i) NIS, TfOH, CH_2Cl_2; or $ZnCl_2$, $AgCl_4$, Et_2O; (ii) CF_3CO_2H, MeOH; (iii) $PyHBr_3$, Et_3N, CH_2Cl_2; (iv) **3a**, TMSOTf; (v) EtMgCl; (vi) *n*-$C_{17}H_{35}COCl$, Py; (vii) *n*-$Bu_4NF{\cdot}3H_2O$, $PhCO_2H$; (viii) PhSH, Et_3N; (ix) H_2, Pd−C. Abbreviations: Bn = benzyl; TIPDS = 1,1,3,3-tetraisopropyl-1,3-disiloxanyl; NIS = *N*-iodosuccinimide. Note: Although the inositol moieties in **2** and **4** are racemic, one isomer of each is illustrated.

comparison of its ^{13}C-NMR data with previously reported data.[13a] The stereochemistry of **5** was also supported by the empirical rule concerning the relationship between chemical shifts of inositol ^{13}C-3 and ^{13}C-5 and the absolute configuration of 4-α-glycosyl-*myo*-inositol.[14]

The triol **5** with the desired absolute configuration was then subjected to phosphorylation with glyceryl dimethyl phosphite **6**, derived from *sn*-glycerol 1,2-carbonate[15] and dimethyl *N*,*N*-diisopropylphosphoramidite, in the presence of pyridinium bromide perbromide and triethylamine. This resulted in the formation of 1-*O*-phosphate **7** in 83% yield in a completely regioselective manner, without affecting the two other hydroxyl groups at C-2 and C-5. Two epimers of the product **7** due to the asymmetric phosphorus atom could be separated by silica gel chromatography and characterized to be regiochemically homogeneous on the basis of their TLC and NMR data. However, their phosphorylation sites were not clearly determined at this stage because the H_2 resonance signal overlapped with other signals. Fortunately, this problem was solved using the NMR (^{1}H and ^{31}P) analysis on each diastereomeric dimannoside **8** obtained by a subsequent procedure (*vide infra*). Thus, glycosylation of diastereomeric **7** with mannosyl phosphite **3a** in the presence of TMSOTf (0.1 equiv) occurred at C-2 to afford stereo- and regioselectively α-glycoside **8** in 74% yield. The product could then be separated into two diastereomers due to the asymmetric phosphorus atom at C-1 by flash chromatography [R_f 0.39 and 0.30 (AcOEt/*n*-C_6H_{14}, 5:6)], and each diastereomerically pure compound was used in the following procedures. The anomeric protons of these isomers had the following chemical shifts and coupling constants: 5.08 (d, J = 1.4 Hz) and 5.30 (d, J = 1.4 Hz) ppm for the faster running product and 5.19 (d, J = 1.96 Hz) and 5.35 (d, J = 1.95 Hz) ppm for the slower, indicating α-configuration.[13] The appearance of dt for H_5 (t in the presence of D_2O), t for H_2, and dt for H_1 protons in the inositol ring of the each diastereomer supported the structure of **8**. In general, the axial hydroxyl group at C-2 in the *myo*-inositol ring is much less reactive than other equatorial ones. However, in the case of **7** bearing the 1,3-disiloxanyl group, glycosylation occurred exclusively at C-2 without affecting OH-5. In relation to this phenomena, attempts to acetylate the 5-hydroxyl group in **4b** failed.

Deprotection of the glyceryl moiety in **8** was performed with a large excess of ethylmagnesium chloride without migration or decomposition of the phosphate function to afford **9** in 81% yield. We previously reported that deprotection of carboxylic esters using a Grignard reagent did not cause migration of neighboring phosphate and silyl groups.[16] The structure of **9** was supported by its chemical transformation with phosgene in pyridine into the corresponding cyclic carbonate **8**. The reaction of **9** with stearoyl chloride in the presence of DMAP afforded **10** in 73% yield, keeping OH-5 free.

During the final stage, deprotection of **10** was accomplished by removal of the disiloxanyl group with tetrabutylammonium fluoride and benzoic acid, deprotection of the phosphate function with benzenethiol and triethylamine, and then hydrogenolysis of the benzyl ethers. In order to confirm the position of the hydroxyl groups that have no substituents, the triol, which was derived by the removal of the siloxanyl group from one diastereomer **10**, was acetylated to produce 3,4,5-tri-*O*-acetate.[17] This indicated the precise introduction of the desired substituents to the inositol ring. The structure of the final product was characterized by NMR (^{1}H, ^{13}C, and ^{31}P) and FAB-MS [negative, *m*/*z* 1189.7 $(M - 1)^+$] data.

Finally, it should be noted that three necessary substituents at C-1, -2, and -6 were introduced in a completely regioselective manner using only two protecting groups, cyclohexylidene and disiloxanyl, for the protection of the inositol moiety.

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Supporting Information Available: Characterization data and procedures (7 pages).

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