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Regioselective phosphorylation of *myo*-inositol with BINOL-derived phosphoramidites and its application for protozoan lysophosphatidylinositol†

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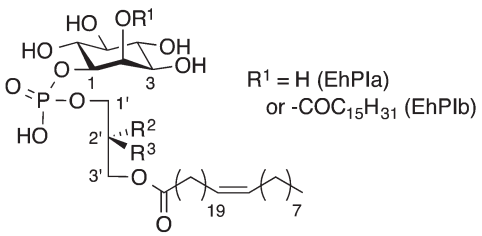
A regioselective phosphorylation method for *myo*-inositol was developed by utilizing readily preparable BINOL-derived phosphoramidites. The method also facilitated the complete separation of the diastereomeric products by simple chromatography. Based on this phosphorylation and Ni-catalyzed alkyl–alkyl cross-coupling reaction for long fatty acids, we achieved the first synthesis of a lysophosphatidylinositol, EhPIa having long fatty acid C30:1, as a partial structure of glycosylphosphatidylinositol (GPI) anchor from the cell membrane of a protozoa, *Entamoeba histolytica*.

Entamoeba histolytica is a protozoan parasite that causes the disease amebiasis and amebic liver abscess (ALA) in humans. Its cell surface contains a complex glycoconjugate, lipopeptidophosphoglycan (EhLPPG), which is composed of a glycosylphosphatidylinositol (GPI) anchor and the inositol phospholipid moieties, EhPIa and EhPIb (Fig. 1). The inositol phospholipids have been shown to stimulate natural killer T (NKT) cells and to induce selective production of IFN-γ but not IL-4 in a CD1d-restricted manner in murine systems in the case of EhPIb.[1] It has also been shown that EhLPPG has interesting biological activities, which induces characteristic selective IFN-γ production influenced by the host's testosterone.[2] We are particularly interested in the structure of its inositol phospholipid moieties, which have a characteristic lysophosphatidylinositol structure acylated by a long-chain fatty acid, although the 2′ position configuration of the glycerol moiety was not clearly determined by spectroscopic data and was inferred based on the previously reported configurations of similar types of protozoan glycerol moiety, as the sn-1 position of the glycerol is connected to a long fatty acid chain, as observed in **1a**.[1] We therefore synthesized both EhPIa stereoisomers to elucidate the further detailed biological activities, including the structure–activity relationships at the configuration of the glycerol moiety. Although numerous syntheses of phosphatidylinositol and phosphoinositides have been reported,[3] only a few examples of lysophosphatidylinositol synthesis have been reported.[4,5] The characteristic lysophosphatidylinositol structure of EhPIa has characteristic long fatty acids, such as C28:0 and C30:1, and it is also necessary to introduce a monophosphate to a certain position of the inositol moiety. Controlling selective phosphorylation is one of the fundamental issues in biomolecules, including carbohydrates, peptides/proteins and lipids, because of the importance of the site-specific phosphorylation for many physiological events.[6] There have thus been some investigations reported of the various roles of phosphates in natural[7–9] and designed molecules.[10,11]



1a (2′*R*) R^1 = H, R^2 = OH, R^3 = H: **EhPIa-C30:1**
1b (2′*S*) R^1 = H, R^2 = H, R^3 = OH: **2′-*epi*-EhPIa-C30:1**

Fig. 1 Inositol phospholipids, EhPIa and EhPIb, from *Entamoeba histolytica*.

In the present study, we developed two key reactions for the synthesis of these inositol phospholipids; Ni-catalyzed alkyl–alkyl cross-coupling reaction and regioselective phosphorylation of *myo*-inositol using binaphthyl phosphoryl/phosphoramidite reagents. In many phosphatidylinositol syntheses, including the abovementioned lysophosphatidylinositol synthesis, one of main methods for introducing a phosphate group is that *myo*-inositol was first desymmetrized through

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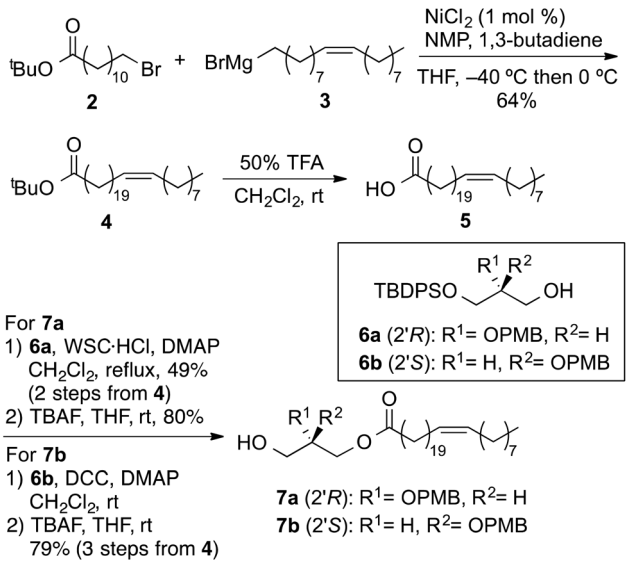
formation of diastereomeric derivatives.[12–14] A phosphoryl group was subsequently transferred to the liberated hydroxy group.[15] Miller and co-workers reported the regioselective phosphorylation using chiral peptide catalysis.[16,17] Utilizing molecular catalysts designed for those particular inositol moieties, the reaction displayed high selectivity. It has also been reported that chiral inositol derivative syntheses were enabled with using a commercially available chiral inositol building block, 2,3:5,6-bis-*O*-(1-methylethylidene)-D-*myo*-inositol 1,4-dibutanoate, as the starting material,[18,19] although the compound is quite costly and the protecting groups are not always convenient for the subsequent synthesis. Jessen and co-workers reported examples of desymmetrization of *meso*-inositol derivatives using a chiral phosphoramidite reagent containing mandelic acid derivative as the chiral auxiliary. However, no selectivity was obtained in the earlier reports[20,21] and then higher selectivity (1.0 : 2.5, in 66% yield) was reported in their latest research using pentafluorophenol (PFP)–phosphite trimester for higher reactivity.[22]

In the present study, we used BINOL as the chiral auxiliary for the phosphoramidite reagent for desymmetrization of *meso*-inositol. The reagent was readily prepared with the usual phosphoramidite synthesis and can be selectively cleaved with using simple cleavage conditions for the aryl phosphate linkage, while maintaining the alkyl phosphate linkage. It was also advantageous that BINOL recycling was relatively easy, which would be important in the case of a larger synthesis scales. In this study, concerning EhPIa synthesis, highly enantiomerically pure compounds are necessary for the biological studies. We thus developed the method for the regioselective phosphorylation using the chiral phosphorylation reagents with BINOL as the auxiliary, which facilitated the selective phosphorylation and also the complete separation of the diastereomeric products by simple chromatography.

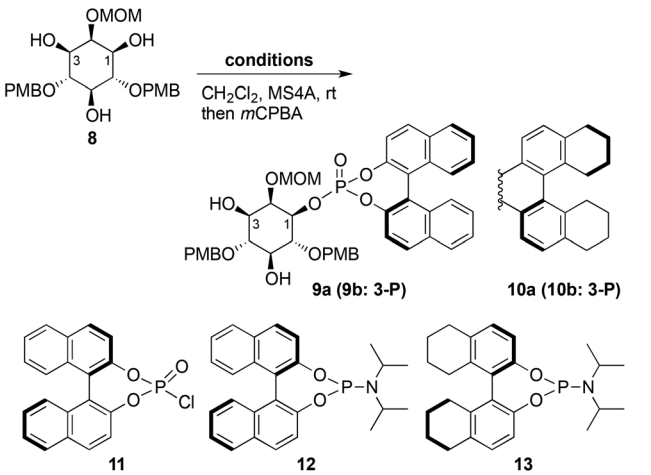
To prepare the characteristic long fatty acids, a Ni-catalyzed alkyl–alkyl cross-coupling reaction was also developed,[23] and applied to the total synthesis of EhPIa. To prepare the acylglycerol **7a** and **7b** (Scheme 1), the long fatty acid derivative **4** was synthesized with a Ni-catalyzed alkyl–alkyl cross-coupling reaction using bromide **2** and Grignard reagent **3** to obtain **4** with a 64% yield. Due to the very low solubility of the Grignard reagent **3** at lower temperature, the coupling reaction was performed through a reverse-addition procedure described in the previous report.[23] After cleaving the *t*Bu ester, condensation with the alcohol **6a** and **6b** was carried out. The TBDPS group was subsequently cleaved with TBAF to generate the acyl glycerols **7a** and **7b**.

To enable the selective monophosphorylation of *myo*-inositol with three non-protected hydroxy groups of compound **8**,[24] BINOL-containing chiral phosphorylation reagents were designed. Phosphorylation using (*R*)-BINOL-phosphoryl chloride (**11**)[25] with several *myo*-inositol derivatives under various reaction conditions obtained unsatisfactory results; *e.g.*, the conditions shown in Table 1 (entry 1) obtained no selectivity. We then used the phosphoramidite reagent with (*R*)-BINOL (**12**)[26] using tetrazole as the additive (entry 2) to obtain **9a** in



Scheme 1 Synthesis of acyl glycerol **7a** and **7b** *via* Ni catalyzed alkyl–alkyl cross-coupling reaction.

Table 1 Phosphorylation of *myo*-inositol with liberated 1,3,5-hydroxy groups using BINOL-containing phosphorylation reagents



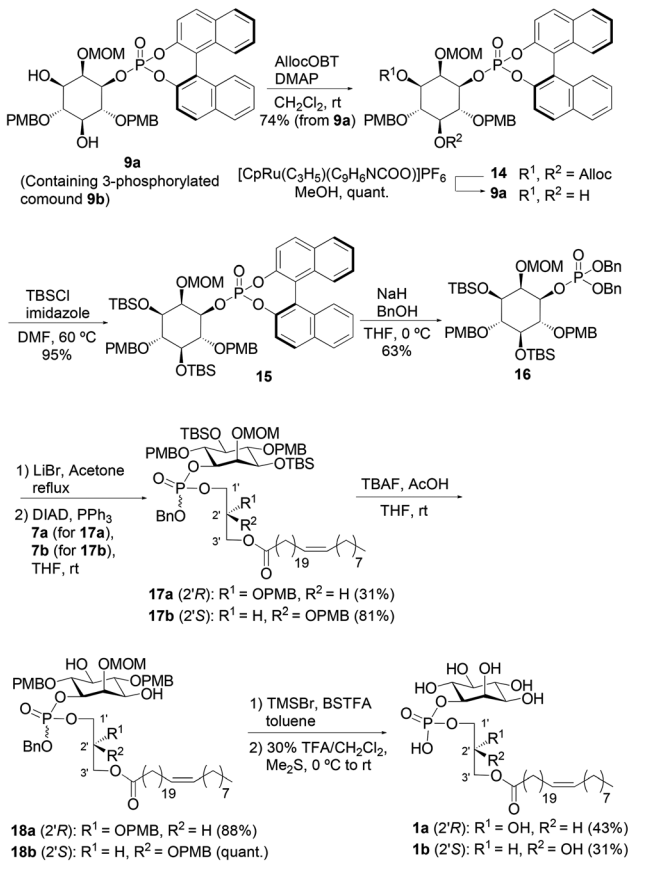
Entry	Reagent (eq.)	Time	Product, yield, dr(1-P : 3-P)[a]
1	**11** (5.0), Et_3N (2.0), DMAP (0.5)	1 d	**9a**, 40%, dr (50 : 50)
2	**12** (5.0), tetrazole (5.0)	1 d	**9a**, 74%, dr (79 : 21)[b]
3	**12** (3.0), HOBt (6.0)	12 h	**9a**, 79%, dr (67 : 33)
4	**12** (3.0), CF_3-HOBt (6.0)	1 h	**9a**, 53%, dr (75 : 25)
5	**13** (5.0), tetrazole (5.0)	2.5 h	**10a**, 49%,[c] dr (90 : 10)

[a] Determined by HPLC analysis. [b] Determined by NMR analysis. [c] Contains *ca.* 50% of diphosphate.

74% yield with appropriate selectivity (1-P : 3-P = 79 : 21) with (*R*)-BINOL. When the reaction was continued for more than one day, the 1,3-bisphosphorylated compound quantity was gradually increased, which is shown in entry 2.

To increase the reactivity, the additive was changed to HOBt.[27] The reaction was faster than with tetrazole, but the selectivity was somewhat decreased (1-P : 3-P = 67 : 33) (entry 3). HOBt-CF_3 was used to further investigate the additive. HOBt-CF_3 enhanced the reactivity but the yield was low (53%) due to the additional phosphorylation (entry 4). In the case of the phosphoramidite reagent having (*R*)-H_8-BINOL (**13**) and using tetrazole as the additive (entry 5), the reactivity and selectivity was higher (1-P : 3-P = 90 : 10) than the (*R*)-BINOL (**12**) (entry 2), but additional phosphorylation also proceeded and the diphosphates were difficult to separate. Therefore, the condition of entry 2 was used for the following EhPIa total synthesis. The configuration of **9a** was determined by cleavage of protecting groups obtaining the enantiomeric inositol-1-phosphate for which the optical rotation is known[28] (see ESI†).

The total EhPIa synthesis is shown in Scheme 2. First, two hydroxyl groups of phosphorylated inositol **9** (**9a** : **9b** = 79 : 21) were protected with allyloxycarbonyl (alloc) groups for easier separation using SiO_2 middle-pressure column chromatography. The protecting group was subsequently cleaved with Ru complex[29] to obtain compound **9a** as the single isomer. After protecting the hydroxyl groups with TBS, BINOL was exchanged with the benzyl group to obtain **16** with 63% yield. The cleaved BINOL could be easily recycled. One of the benzyl groups of benzyl phosphate was cleaved with LiBr in acetone.[30] Acyl glycerol (**7a** or **7b**) was then introduced using the Mitsunobu reaction[31] to obtain compound **17a** and **17b** with 31% and 81% yields (for the two steps), respectively. The TBS groups were cleaved with TBAF to obtain **18a** in 88% yield and **18b** quantitatively. Final deprotection of all protecting groups with TMSBr and BSTFA and then 30% TFA in CH_2Cl_2 led to successful production of the desired inositol phospholipid **1a** and 2′-epimer **1b** (43% for **1a** and 31% for **1b**).



Scheme 2 Synthetic scheme for the inositol phospholipid, EhPIa. Compound **9** was a mixture of 1- and 3-phosphates in the ratio of 79 : 21, and the diastereomer was removed while purifying compound **14**.

In conclusion, we developed a regioselective phosphorylation method for *myo*-inositol by utilizing readily preparable BINOL-derived phosphoramidites, which led to higher selectivity for a desymmetrization method obtained to date. The obtained diastereomeric mixture was separable by standard chromatography and the chiral auxiliary, BINOL, could be easily recycled. We also developed the Ni-catalyzed alkyl–alkyl cross-coupling reaction for preparing long-chain fatty acids. Based on these phosphorylation and alkyl–alkyl cross-coupling reactions, we achieved the first synthesis of immunomodulatory lysophosphatidylinositols, EhPIa and its epimer, as the partial structure of the glycosylphosphatidylinositol (GPI) anchor from the cell membrane of a protozoa, *Entamoeba histolytica*. The synthetic methods would be also applicable for various types of inositol phospholipids.

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