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Synthetic Approaches To Heavily Lipidated Phosphoglyceroinositides

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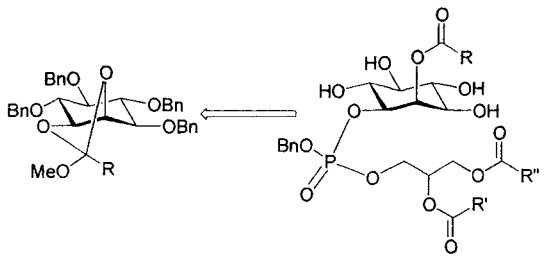
ABSTRACT



Naturally occurring phosphoinositide glycoconjugates are equipped with varied acyl residues that are important for their biological activity and biosynthesis. This paper reports that acylation at O2 of the *myo*-inositol moiety can be achieved by stereocontrolled ortho ester rearrangement. Coupling to homo- or heterodiacylated glycerols was achieved via phosphoramidite methods, and exhaustive debenzylation by transfer hydrogenation afforded the deprotected phosphoglyceroinositides. The latter can be kept in chloroform solution at room temperature for over two months without migration of the inositol acyl group.

Lipidated phosphatidylinositolglycerols are ubiquitous bioregulators that are associated not only with several dreaded diseases[1−3] but also with vital life processes, including calcium mobilization,[4] signal transduction,[5] and insulin stimulation.[6] Glycosylphosphatidylinositols (GPIs), summarized as **1a** and **b**, are a relatively new[7] class of glycoprotein/phosphoinositides that are associated with parasitic diseases, including African Sleeping Sickness[1] and malaria.[3] Landmark studies by Ferguson and co-workers resulted in the first structure elucidation of a GPI in 1988.[8] The chemoenzymatic methodology used was adopted so quickly that within five years, over 200 GPI-anchored

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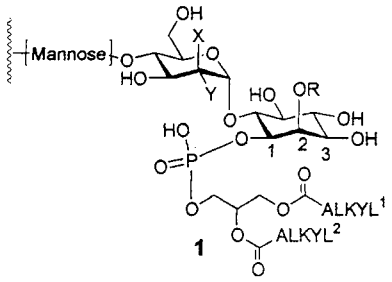
proteins had been identified[9] across the evolutionary array of eukaryotes[10] and, recently, a plant[11] and a virus.[12]

Lipoarabinomannans (LAMs), **1c**, associated with tuberculosis and leprosy,[13] carry mannose residues at inositols O2 and O6, the latter being part of an extended mannan that is eventually linked to an arabinan.[13]



(a) X = H; Y = NH_2; R = H (GPIs)
(b) X = H, Y = NH_2; R = COALYKL[3] (GPIs)
(c) X = OH; Y = H; R = Mannose α-1 (LAMs)

A fascinating feature of the phosphoinositide moiety of several parasite and mammalian GPIs[9] is an acyl group at O2 of the inositol moiety, e.g., **1b**, the presence of which can be deduced by the failure to produce a 1,2-cyclic phosphate upon treatment with phospholipase C (PLC).[14] Inositol O2 acylation gains further significance because it is essential for the addition of the mannose unit (Man-1) of **1a/b** in mammals,[15,16] but the process is optional in parasites, thereby providing a window for specific intervention in one or another pathway.[17] Furthermore, the acyl group may be cleaved and/or reinstalled at various stages of biosynthesis so that the final GPI may or may not be acylated.[9]

The glycerolipid moiety of **1** presents its own share of complexities. Biosynthetically in **1a** or **1b**, the unit begins with two long chain acyl groups; however, elaborate "fatty acid remodeling" processes subsequently take place, leading to hetero- or homodiacylation.[15,16] The sn2 position is of particular interest because this may eventually carry a free-OH or a saturated[18] or unsaturated[19] acyl group. Some of these events are presumed to enable the growing GPI to traverse the membrane of the endoplasmic reticulum under the agency of a flippase enzyme,[20] thus far unidentified.

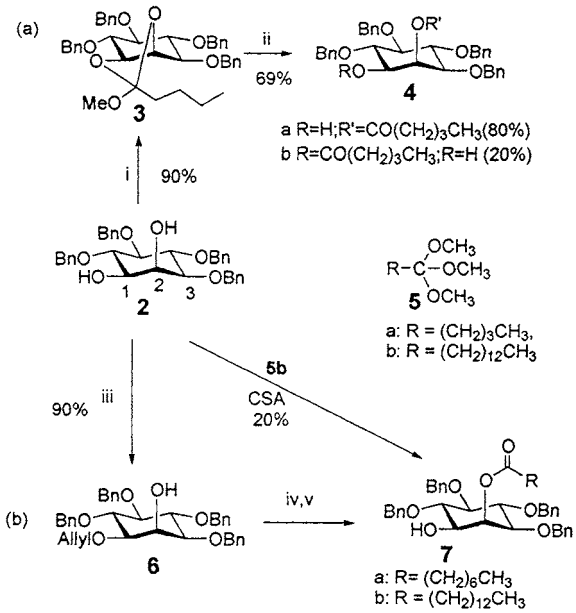
The above brief summary indicates that the lipid residues of substructure **1** individually and/or collectively play dominant roles in the biology of phosphoglycero inositides. Accordingly, we have developed synthetic approaches that allow each of the units to be modified as needed.

Inositol Moiety. 3,4,5,6-Tetra-*O*-benzyl inositol, **2**, which is readily available in racemic[21] or optically pure[22] form, was a convenient starting material. The seminal work of King and Allbutt on stereoelectronically controlled decomposition of cyclic ortho esters on a cyclohexano scaffold[23] provided a ready procedure for differentiating between the hydroxyls of **2** with simultaneous installation of the desired O2-acyl group. Commercially available trimethyl orthovalaerate **5a** afforded the cyclic counterpart **3** quantitatively. Rearrangement with camphorsulfonic acid then gave the desired axial O2-esters **4a**, but only in a modest 20−30% excess over the equatorial option **4b**.

We have recently reported on the efficiency of ytterbium(III) triflate for promoting the glycosidation reactions of *n*-pentenyl ortho esters.[24] This reagent was found to produce a higher ratio of **4a** to **4b** with improved yields, in much shorter reaction times.

Interestingly, long-chain alkyl trimethyl ortho esters such as the myristoyl derivative **5b**, which had to be prepared from the corresponding nitrile,[25] bypassed an isolable cyclic intermediate, leading directly to the stereoelectronic controlled axial product **7b**. Unfortunately, the overall yield was poor, and therefore we were forced to take the alternative,

Scheme 1[a]



[a] Conditions: (i) **5a**, CSA, CH_3CN, 18 h (90%); (ii) CH_2Cl_2, $Yb(OTf)_3$, 0 °C, 1 h, (69%), **4a**:**4b** =4:1; (iii) Bu_2SnO, toluene, then DMF, −10 °C allyl bromide, CsF, 18 h, (90%); (iv) pyridine, acyl chloride, (100%); (v) $PdCl_2$, NaOAc, H_2O (30−35%).

longer route shown in Scheme 1b involving tin-mediated alkylation[26] of the equatorial OH to give **6a** or **6b** followed

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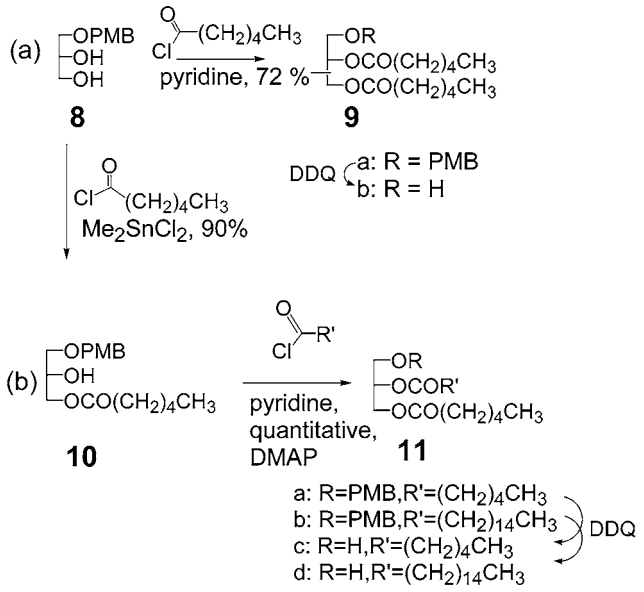
by acylation and deprotection to give **7**. While our work was underway, a similar strategy for the route in Scheme 1b was published by Xue and Guo.[27]

Glycerol Moiety. Commercially available (*R*)-(−)-2,2-dimethyl 1,3-dioxolane-4-methanol was *p*-methoxybenzylated and then deacetonated routinely to obtain diol **8**. Homodiacyl derivatives such as **9a** were obtained under standard conditions, and the deprotected modifications such as **9b** were prepared routinely.

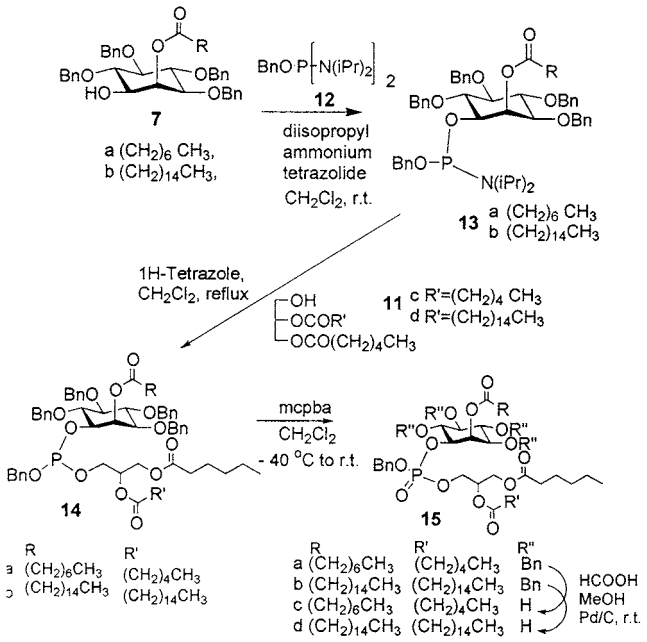
Heterodiacyl analogues such as **11** are of special interest in view of Gowda's findings concerning the dependence of bioactivity the sn2 acyl substituents.[19] Attempts at selective acylation of **8** at the sn1 position did not give acceptable yields of **10**, because copious diacylation products could not be suppressed. Standard stannylene methodology[26] was not successful; however, dimethyltin dichloride, recently introduced by Maki et al.,[28] solved the problem, affording the sn1 acylation product, **10**, in excellent yield. The second acylation to give **11a** and **11b** and then the deprotected analogues **11c** and **11d**, respectively, were accomplished without problems under standard conditions.

Scheme 2



Phosphoinositides. For converting phosphoramidite **12**[29] into phosphotriesters, e.g., **14**, it was better to connect the inositol first (giving **13**) followed by the glycerol, the preferred catalysts for the two stages being *N*,*N*-diisopropylammonium tetrazolide[30] and **1**-*H*-tetrazole, respectively. As shown in Scheme 3, these protocols afforded lipidated inositols **15a** and **15b**.

Scheme 3



Exhaustive debenzylation to obtain **15c** and **15d** was conveniently carried out by transfer hydrogenation using formic acid in methanol at room temperature. We note further that samples of **15c** and **15d** have not given any evidence for O2→O3 acyl migration upon standing in $CDCl_3$ solution for over two months.

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Supporting Information Available: Experimental details for all new compounds and ^{1}H NMR spectra for compounds **3**, **7,** and **15c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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