Comparison of Cyclic and Acyclic Phosphites by Selective Phosphorylation. Synthesis of Phosphatidylinositol 4-Phosphate

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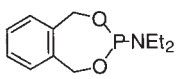
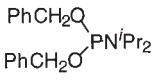
To compare the synthetic utility of cyclic xylylene *N*,*N*-diethylphosphoramidite and its acyclic analog, dibenzyl ester, both phosphites derived from them with dipalmitoylglycerol were subjected to the selective phosphorylation at the OH-3 position in 1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol in the presence of pyridinium tribromide, and the cyclic version showed to be much superior to the acyclic. The 3-*O*-phosphorylated product was conveniently converted into phosphatidylinositol 4-phosphate.

We reported xylylene *N*,*N*-diethylphosphoramidite (XEPA, **1a**) as a promising phosphitylating agent for the synthesis of phosphoric monoesters[1] and diesters.[2] The *N*,*N*-dimethyl[3] and *N*,*N*-diisopropyl[4] versions also appeared after our first report. These reagents have been used for phosphorylation of inositols[5] and sugars,[6] and for glycosylation.[7] Their acyclic analog, dibenzyl phosphoramidite[8] **1b** has been more widely utilized than the cyclic ones. However, preparation[9] and purification of XEPA are much easier than those of **1b**, and both reagents have an identical reactivity.[10] Furthermore, a cyclic phosphate derived from **1a** is particularly prone to crystallize. These advantages of XEPA prompted us to demonstrate its efficiency in terms of selective phosphorylation of diol **4** with the phosphite **3a** derived from **1a** using the phosphite-pyridinium tribromide method,[11] that has been well documented to give selectively 1-*O*-phosphates starting from 1,2-free inositol derivatives.[12] We describe here the comparison of the selective phosphorylation and transformation of the resultant 1-*O*-phosphate **5** to phosphatidylinositol 4-phosphate {PI(4)P}, which is known to be a metabolic precursor of biologically important phosphatidylinositol 4,5-bisphosphate.[13]



1a



1b

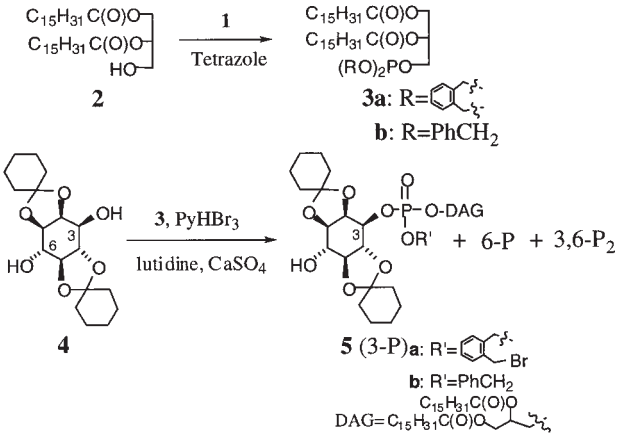
The attempts to selectively introduce a phosphate function at the OH-3 position in DL-1,2 : 4,5-di-*O*-cyclohexylidene-*myo*-inositol[14] were made using the reaction with DL-dipalmitoylglycerol phosphites **3** in the presence of pyridinium tribromide (PTB), 2,6-lutidine, and anhydrous calcium sulfate. Both phosphites were derived respectively by the reaction of **1a** and **1b** with racemic 1,2-di-*O*-palmitoylglycerol **2** in the presence of 1*H*-tetrazole. Cyclic phosphite **3a** gave a better yield of the desired 3-*O*-phosphorylated product **5a** with higher selectivity (Table 1). In contrast to the result, acyclic dibenzyl phosphite **3b** yielded fair amounts of 3,6-diphosphate, even when a limited amount of the phosphite was used.

The difference in selectivity of the phosphorylation between the cyclic and acyclic derivatives can not be explained in terms of the steric bulkiness of a protecting group, because the dibenzyl

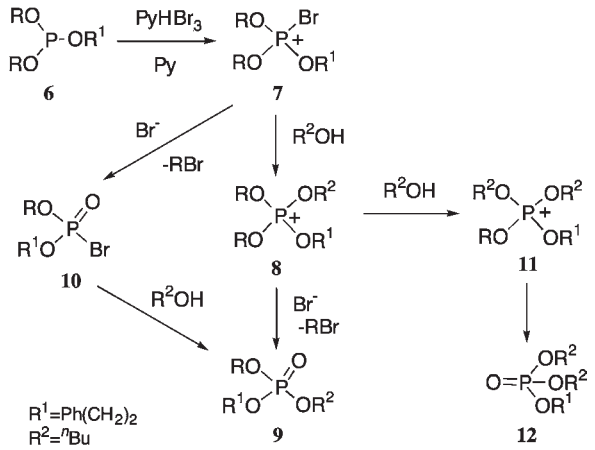
Table 1. Comparison of phosphorylation

1	2 (3),[a] equiv[b]	Reaction conditions	R'	Yield, % 3-P	6-P	3,6-P_2
a	1.4	-42 °C, 15 min then 0 °C, 1.5 h		84	6	-
b	1.4	-42 °C, 15 min then -18 °C, 1.5 h	$PhCH_2$-	43	-	17[c]
b	2.0	-42 °C, 15 min then -18 °C, 1.5 h	$PhCH_2$-	55	-	36

[a]Phosphites **3** were used without purification. Since **3** were prepared quantitatively using a slight excess of phosphoramidites, the equivalent of **2** is almost identical with that of **3**. [b]The molar equivalent is based on **4**. [c]The starting material **4** was recovered in 23%.



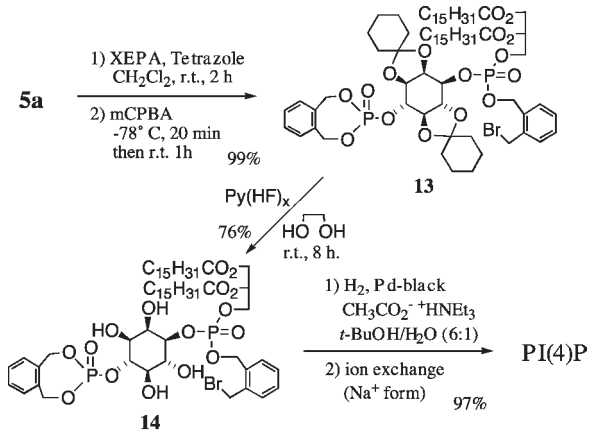
phosphoryl group seems to be sterically more hindered rather than the xylylene. A more plausible explanation for the selectivity may be mainly put forward by the consideration of the reactive intermediate.[15] The phosphorylation first forms the bromophosphonium salt **7**, that reacts with an alcohol and then decomposes to the final phosphorylation product **9** (Scheme 1). The other pathway involves the first decomposition of **7** into the phosphorobromidate **10** and then the phosphorylation of the alcohol. When both phosphites **3** were treated respectively with 3 molar equivalents of butyl alcohol (R^2OH in Scheme 1) in the presence of PTB and pyridine in CH_2Cl_2, **3a** gave a fair amount of the ester exchange product **12** (31%) accompanied with the normal product **9** (47%). Since **9** did not react with butyl alcohol under the identical conditions, the major pathway for the cyclic phosphite involves the reaction of the phosphonium salt **7** with the alcohol without decomposition to the phosphorobromidate, to form **8**, which, when further reacts with R^2OH, is converted to the undesired product[16] **12**. In the case of **3b**, the ester exchange reaction scarcely took place. Therefore, an acyclic phosphite such as dibenzyl ester would likely be transformed via the phosphorobromidate pathway. These results shows that the acyclic **7** is more



Scheme 1. Mechanistic pathways.

sensitive toward the bromide attack than the cyclic intermediate in accordance with the report.[17] These also suggest that, since the selectivity depends principally on the reactivity, a phosphorobromidate is more reactive than a phosphonium salt.

The XEPA-derived 3-phosphate **5a** thus obtained was used for the synthesis of PI(4)P. Phosphorylation of **5a** using XEPA gave **13** quantitatively. The deprotection procedure was first carried out by removal of the cyclohexylidene groups. To prevent the migration of the phosphate functions, we applied the method using pyridinium poly(hydrogen fluoride), which was recently demonstrated to efficiently deprotect acetals,[18] giving **14** in 76% yield. The structure of **14** was chemically confirmed by its transformation to the tetrakis(chloroacetyl) derivative and the NMR analysis. The final deprotection by catalytic hydrogenolysis on Pd black in the presence of triethylammonium acetate followed by the cation exchange to the Na^+ form gave a racemic and diastereomeric mixture of PI(4)P sodium salt[19] in 97%. The reaction in the absence of the acetate considerably conducted the decomposition of the diester moiety in the product, presumably because hydrogen bromide generated during the reaction caused hydrolysis of the function.



Scheme 2. Synthesis of phosphatidylinositol 4-phosphate.

In this paper, difference in the synthetic merit between seven-membered cyclic phosphite, XEPA and acyclic dibenzyl has been first shown.

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This paper is dedicated to Professor Teruaki Mukaiyama on the occasion of his 75th birthday.

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19 ^{1}H-NMR (400 MHz,$CDCl_3$) δ 0.79–0.95 (6H, brt, CH_3), 1.04–1.49 (48H, complex, CH_2), 1.51–1.68 (4H, m, β-CH_2), 2.22–2.48 (4H, m, α-CH_2), 3.34–3.78 (4H, complex, $InsH_3$, H_5, $GlyH_3$), 3.80–4.45 (6H, complex, $InsH_1$, H_2, H_4, H_6, $GlyH_1$), 5.04–5.12 (0.3H, m, $GlyH_2$), 5.20–5.30 (0.7H, m, $GlyH_2$); ^{31}P-NMR (162 MHz, $CDCl_3$) 3.73 (P_1), 6.36 (P_4); MS (FAB$^-$, triethanolamine, M: $C_{41}H_{78}O_{16}P_2Na_2$) m/z 911(M-Na), 889 (M-2Na + H).