Synthesis of Carrier Free [^{32}P] 5-Chloro-8-Quinolyl Phosphate as a Phosphorylating Agent

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The element phosphorus, a component of low molecular intermediate and nucleic acid, plays an important role in field of biology, genetics, chemistry and medical science. ^{32}P and ^{33}P, pure β emitters, have extensively been used in studies of biological mechanism and therapeutic agents. However, there has been much less attention for ^{32}P and ^{33}P labeled compounds and their application. It is because not much data are available in chemical reactions of phosphoric acid and its derivatives.

^{32}P can be produced by ^{32}S(n, P)^{32}P nucleic reaction and its final product is a form of H_3PO_4.[1] Its labeled compounds usually have a form of phosphoric ester into hydroxy group contained molecules, such as sugar and nucleoside. ^{32}P phosphorylation is divided into two kinds of methods; enzymatic and chemical methods. Enzymatic methods are usually performed with [^{32}P] ATP and various enzymes, but these methods restrict to organic chemist owing to difficulty to select and handle various enzymes. Chemical phosphorylation[2] with urea or trichloroacetonitrile is conventionally used for introducing ^{32}P monophosphate, but there are some drawbacks because higher temperature is required for urea phosphorylation besides of the fact that trichloroacetonitrile is highly reactive and sensitive with water. Another method is to use [^{32}P] $POCl_3$ prepared by ^{32}P exchange reaction,[3] which is accomplished by reacting [^{32}P] H_3PO_4 and $POCl_3$. This agent is of good value to [^{32}P] phosphorylation but its specific activity is very low for containing carriers, such as ^{31}P. 8-Quinolyl phosphate,[4] which selectively reacts with primary alcohols to form phosphoric esters, has been utilized in the synthesis of nucleotides (Fig. 1) and its derivative,[5] O-

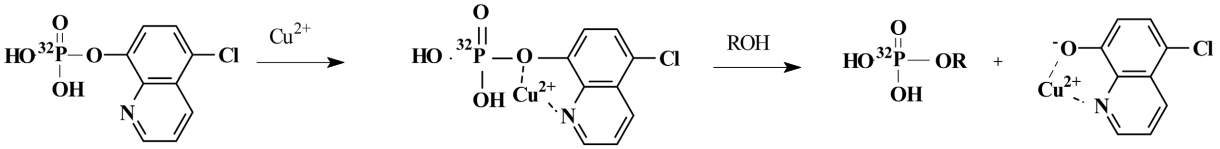
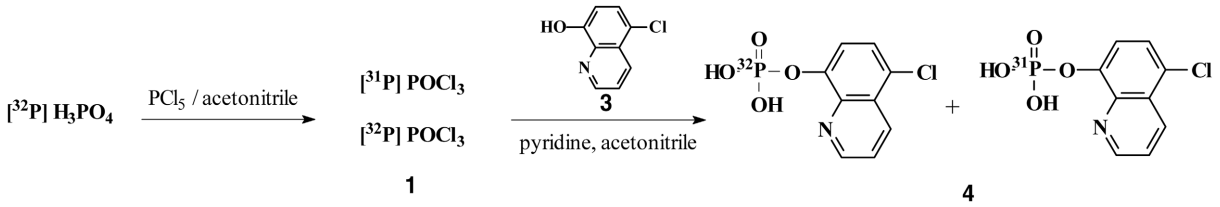
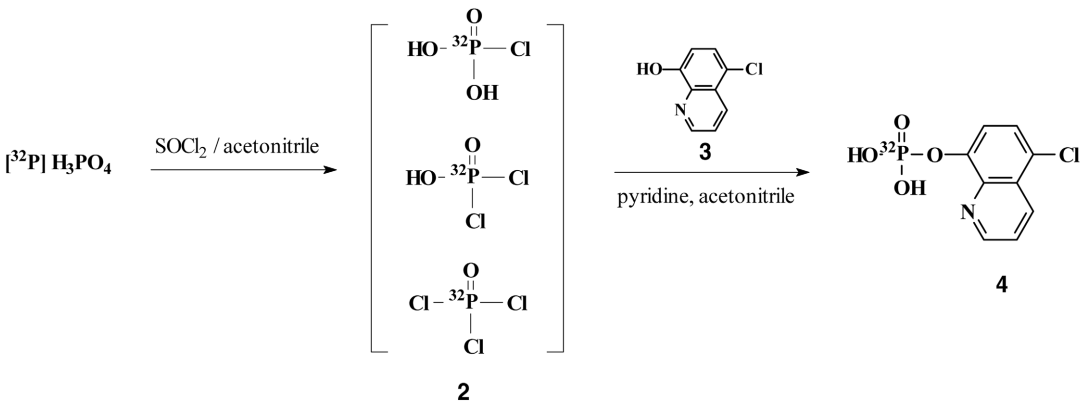


Figure 1. Phosphorylation using 8-Quinolyl Phosphate.





Scheme 1. Preparation scheme for 5-chloro-8-quinolyl phosphate.

8-(5-chloroquinolyl) S-phenyl phosphorothiate, is used as bifunctional coupling agent. Michael Taktakishvili and Vasu Nair developed a more reactive agent, 2-O-(4,4'-dimethoxytrityl) ethylsulfonylethan-2'-yl-phosphate.[6] Nevertheless, it has been restricted to prepare their labeled agents from [^{32}P] H_3PO_4 because these compounds are synthesized from $POCl_3$.

We describe herein a method for carrier free ^{32}P derivatives and a synthetic technique for carrier free ^{32}P phosphorylating agent, [^{32}P] 5-chloro-8-Quinolyl Phosphate (Scheme 1). Carrier free compounds, only containing ^{32}P not ^{31}P, showed a good quality and higher specific activity. All of standard compounds were prepared according to scheme 1 and comparative experiments were accomplished with [^{32}P] H_3PO_4.

3 reacted with $POCl_3$ in Pyridine / dioxan (1 : 1 v/v) at 0 to give standard **4** in quantitative yield. Standard **4** was recrystalized with Pyridine / Water and its yield was 63%.[7] Standard **4** was also obtained by treating **3** with $POCl_3$ resulting from the reaction of 99.99% H_3PO_4 and PCl_5. These were confirmed with TLC and HPLC. **4** was synthesized from [^{32}P] H_3PO_4 and PCl_5 and its radiochemical yield was 96%.[8] However, this experiment contains carrier and theoretical specific activity dropped to 25%.[3]

$$[^{32}P]\ H_3PO_4 + 3PCl_5 \rightarrow [^{32}P]\ POCl_3 + 3\ POCl_3 + 3\ HCl$$

Standard **2** was formed from the reaction of 1 eq H_3PO_4 and 2.5 eq $SOCl_2$. So far, its reaction pathways have not been well understood. **2** was proposed as an intermediate to exist in this reaction. Sticky liquid was identified when this reaction would be continued at least 48 hours. This may become polymer slowly. The mixture of standard **2** and **3** in pyridine / acetonitrile gave standard **4** in 90% yield. The reaction of **2** and bulky **3** selectively gave monosubstituted product **4**. **4** using [^{32}P] H_3PO_4 resulted in a radiochemical yield of 92% and radiochemical purity was quantitative after silica sep-pak filter.[9] (Fig. 2)

Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	-0.6	7.3	4.5	0.074	325.0	162.5	0.95	1.07
Rgn 2	8.1	12.5	9.9	0.165	332.0	166.0	0.97	1.09
Rgn 3	18.6	27.3	23.4	0.390	1497.0	748.5	4.38	4.92
Rgn 4	36.0	56.1	45.3	0.754	28254.0	14127.0	82.76	92.92
Bkg 1	59.6	75.3	64.8	1.081				
Bkg 2	75.3	86.6	80.0	1.334				
4 Peaks					30408.0	15204.0	89.07	100.00

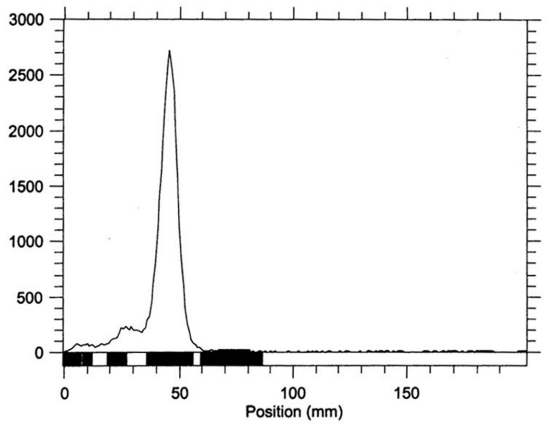


Figure 2. Radiochromatogram of [^{32}P] 5-chloro-8-quinolyl phosphate. Crude Cpds (**4**) : (Eluents (*n*-BuOH : H_2O : MeOH : NH_4OH = 1 : 1 : 1 : 1), Distance from solvent front : 60 mm, R_f = 0.75).

These reactions showed a wide range of solvent effects. To prepare **1** and **2**, a highly polar aprotic solvent, which can dissolve [^{32}P] H_3PO_4, should be selected. We have found that these reactions do not proceed in other aprotic solvents (*e.g.* DMSO, DMF, or HMPA), while using dried acetonitrile gives products **1** and **2**. It may be because the product has good solubility and stability in acetonitrile. In order to prepare **4**, pyridine was used as a solvent which can easily remove Cl ions.

In conclusion, The value of this result is that **4**, *i.e.* [^{32}P] 5-chloro-8-quinolyl phosphate can be easily used by both organic chemists as well as biochemists for carrier free ^{32}P phosphorylation.

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7. TLC (*n*-BuOH : MeOH : H_2O : NH_4OH = 1 : 1 : 1 : 1): R_f = 0.75. mp = 127. ^{1}H NMR (DMSO): δ = 9.02 (dd; 1H, Ar-H), 8.55 (dd; 1H, Ar-H), 7.73 (m; 3H, 3Ar-H), 5.32 (broad; 2H, 2 P-OH) MS (FAB, M^++1): $C_9H_7ClNO_4P$ cald. for 259.98 found 260.07.
8. 0.2 mCi of [^{32}P] H_3PO_4 was rendered anhydrous by 3 times of evaporation with acetonitrile at 15 °C and dissolved in dried acetonitrile. This solution was transferred to another reaction vial containing 20 mg of PCl_5. After 10 min, **3** was added. The reaction mixture was kept for 30 min and water added at −18 °C. **4** was obtained at radiochemical yield of 96%.
9. 20 μL of $SOCl_2$ was added into 0.2 mCi of dried [^{32}P] H_3PO_4 solution and kept to stir for 10 min. 30 mg of **3** in 0.3 mL of pyridine was added at −18 °C and stirred for 30 min. After add 100 μL of H_2O, this mixture was evaporated under reduced pressure at 20 °C and filtered with silica sep-pak.