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# 8-QUINOLYL NUCLEOSIDE 5'-PHOSPHATES AS A USEFUL INTERMEDIATE FOR THE SYNTHESIS OF NUCLEOSIDE 5'-DI- AND 5'-TRI-PHOSPHATES

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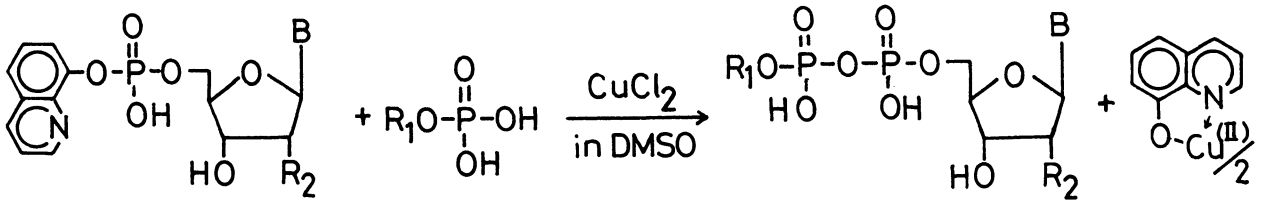
Nucleoside 5'-di- and 5'-tri-phosphates were obtained in high yields by the reaction of 8-quinolyl nucleoside 5'-phosphates with phosphoric or pyrophosphoric acids in the presence of cupric chloride.

Recently, it has been studied that 8-quinolyl group can be effectively used for protection of the phosphate of nucleotides in oligonucleotide synthesis.[1] This group was smoothly and selectively removed by a simple treatment with cupric chloride in a mixture of dimethyl sulfoxide (DMSO) and water. The reaction seems to proceed through a metaphosphate intermediate. Therefore, we examined the synthesis of unsymmetrical pyrophosphates[2] in the presence of phosphates as nucleophile.

In this communication, we wish to report the synthesis of nucleoside 5'-di- and 5'-triphosphates by the reaction of 8-quinolyl nucleoside 5'-phosphates with phosphoric acid or pyrophosphoric acid in the presence of cupric chloride as an activating reagent of 8-quinolyl group.

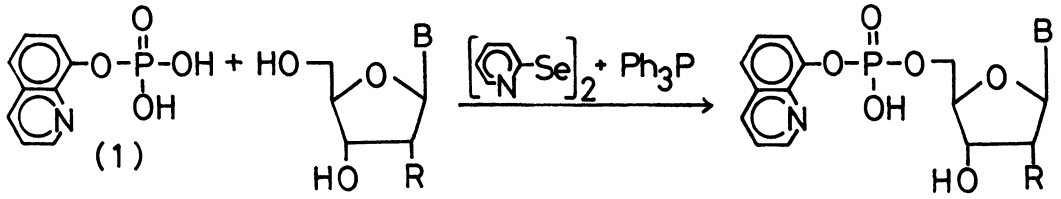



$R_1$ = H, or $(HO)_2\overset{O}{\overset{\|}{P}}$

$R_2$ = OH; B = uracil, cytosine, adenine, guanine

$R_2$ = H; B = thymine

First, we describe a selective phosphorylation of nucleosides[3] by means of 8-quinolyl phosphate (1) in the presence of triphenylphosphine ($Ph_3P$) and 2,2'-dipyridyl diselenide [$(PySe)_2$].[4]



(1)

B = nucleoside base; R = H or OH

To a mixture of thymidine (0.2 mmol), 1 (0.3 mmol), and $(PySe)_2$ (0.5 mmol) in dry pyridine $Ph_3P$ (0.5 mmol) was added at once at room temperature. It was stirred continuously for 6 hr. The reaction was quenched by addition of water (1 ml) and further stirred at room temperature for 2 hr. The solution was concentrated under reduced pressure and the residue was dissolved in water (10 ml). It was washed with three portions of chloroform (3 x 10 ml). The aqueous layer was concentrated and the residue was dissolved in small volume of water. It was chromatographed on DEAE cellulose column (20 x 2.5 cm) using a linear gradient with 0-0.2 M of triethylammonium bicarbonate (TEAB) solution [water (1 l) + 0.2 M TEAB (1 l)]. 8-Quinolyl thymidine 5'-phosphate was eluted and obtained in 87% yield estimated by spectrophotometrically.

Similarly, 8-quinolyl esters of common nucleoside 5'-phosphates were obtained as shown in Table 1.

The structures of the reported compounds were confirmed by uv spectra and periodate oxidation after removal of 8-quinolyl group by use of cupric chloride in a mixture of DMSO and water (5 : 1 v/v).

Table 1. Preparation of 8-Quinolyl Nucleoside 5'-Phosphates

| Nucleoside | Yield (%) | Rf value* | Spectral Data (at pH 7) $\lambda_{max}^{H_2O}$ ($\varepsilon \times 10^{-3}$) | $\lambda_{min}^{H_2O}$ (nm) |
|---|---|---|---|---|
| uridine | 78 | 0.55 | 262(10.0), 234 | 246 |
| cytidine | 70 | 0.47 | 271 (9.1), 232 | 250 |
| adenosine | 81 | 0.59 | 259(15.4), 233 | 245 |
| guanosine | 76 | 0.48 | 252(13.7), 233 | 245 |
| thymidine | 87 | 0.60 | 267 (9.6), 233 | 243 |

*Solvent system used was: Isopropyl alcohol-concentrated ammonia-water (7 : 1 : 2 v/v).

Next, the reaction of 8-quinolyl nucleoside 5'-phosphates prepared in the above experiments with phosphoric acid in the presence of cupric chloride was tried.

For example, guanosine 5'-diphosphate (GDP) was prepared as follows: Mono(4-morpholino-N,N'-dicyclohexylformamidinium) salt of 8-quinolyl guanosine 5'-phosphate (0.1 mmol) was treated with mono(tri-n-butylammonium) phosphate (0.4 mmol) in the presence of cupric chloride (0.4 mmol) in dry DMSO (4 ml) at room temperature for 6 hr. The solvent was coevaporated by addition of water and the oily residue was suspended in water. 8-Hydroxyquinoline-copper complex precipitated. After removal of the precipitate by filtration, the filtrate was treated with Dowex 50W-X2 (pyridinium form). The resin was washed with water and the filtrate was concentrated to small volume. It was chromatographed on DEAE cellulose column (40 x 1.5 cm) using a linear gradient with 0-0.3 M of TEAB solution [water (2 l) + 0.3 M TEAB (2 l)].[5] GDP was separated from phosphoric acid[6] and obtained in 88% yield. It was homogeneous on paper chromatography.

In a similar manner, uridine 5'-diphosphate (UDP), adenosine 5'-diphosphate (ADP), cytidine 5'-diphosphate (CDP), and thymidine 5'-diphosphate (TDP) were obtained in 72%, 81%, 76%, and 75% yields, respectively.

Further, nucleoside 5'-triphosphates were obtained similarly from the reactions of 8-quinolyl nucleoside 5'-phosphates (0.2 mmol) and bis(tri-n-butylammonium) pyrophosphate (0.8 mmol) in the presence of cupric chloride (0.8 mmol) in dry DMSO (2 ml).

By this method, adenosine 5'-triphosphate (ATP), guanosine 5'-triphosphate (GTP), cytidine 5'-triphosphate (CTP), uridine 5'-triphosphate (UTP), and thymidine 5'-triphosphate (TTP) were obtained in 77%, 76%, 80%, 73%, and 83% yields, respectively.

Table 2. Chromatographical Data of Nucleoside Di- and Tri-phosphates

| | Rf values | | |
|---|---|---|---|
| | Solvent A | Solvent B | Solvent C |
| AMP | 0.08 | 0.16 | —— |
| ADP | 0.03 | 0.10 | —— |
| ATP | 0.02 | 0.05 | —— |
| GMP | 0.03 | 0.10 | 0.23 |
| GDP | —— | 0.03 | 0.07 |
| GTP | —— | —— | 0.03 |
| CMP | 0.06 | 0.17 | —— |
| CDP | 0.03 | 0.09 | —— |
| CTP | 0.02 | 0.04 | —— |
| UMP | 0.07 | 0.22 | —— |
| UDP | 0.04 | 0.12 | —— |
| UTP | 0.04 | 0.08 | —— |
| TMP | 0.15 | 0.36 | —— |
| TDP | 0.09 | 0.23 | —— |
| TTP | 0.06 | 0.14 | —— |

Solvent systems:

Solvent A = isopropyl alcohol-concentrated ammonia-water(7:1:2 v/v);
Solvent B = ethanol-1 M ammonium acetate (5:2 v/v, pH 7.5);
Solvent C = ethanol-0.05 M ammonium acetate (5:2 v/v, pH 3.8).

The structures of the nucleoside di- and tri-phosphates were characterized by Rf values on paper chromatography, mobility on paper electrophoresis, and uv spectra after elution of the spots from the chromatograms. Chromatographical data of nucleoside di- and tri-phosphates are given in Table 2.

In conclusion, it is noteworthy that nucleoside di- and tri-phosphates were prepared in high yields by two steps without the use of the protecting groups on hydroxyl groups of sugar moiety and amino groups of nucleoside base during all of the synthetic procedures and the method is invaluable since these nucleoside polyphosphates can be prepared simply from the unprotected nucleosides.

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6) In order to separate completely GDP from $H_3PO_4$, the eluate was concentrated and rechromatographed.

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