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SYNTHESIS OF URIDINE OLIGONUCLEOTIDE BY THE REACTION OF UNPROTECTED URIDINE WITH TRI-(IMIDAZOLYL-(1))PHOSPHINE

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Summary : Uridine oligonucleotide was synthesized by the reaction of unprotected uridine with tri-(imidazolyl-(1))phosphine and the successive oxidation of the resulting phosphite with iodine and water.

Various applications of phosphorus compounds in nucleotide syntheses have been reported[1]. Recently, oligonucleotide synthesis *via* phosphite intermediate has been successfully achieved[2] and adapted to polymer supported oligonucleotide synthesis[3]. However tri-(imidazolyl-(1))phosphine has not been used for the phosphorylation of nucleoside because of the lack of its stability[4].

This communication describes the result on adapting tri-(imidazolyl-(1))-phosphine to uridine oligonucleotide synthesis. The synthetic procedure involves the following two step process; (1) the reaction of unprotected uridine with tri-(imidazolyl-(1))phosphine(within 60 min), (2) the *in situ* oxidation of the resulting phosphite with iodine and water(10 min). This approach is based on the observation that phosphorus compounds easily react with *cis*-vicinal diol, the resulting cyclic phosphite having P-X(X;Cl or amine) group can react rapidly with hydroxyl groups[5], and the phosphite triester can be oxidized by iodine and water quantitatively to yield the corresponding phosphate[2].

Tri-(imidazolyl-(1))phosphine was prepared in the following manner. The reaction of phosphorus trichloride(100 µl, 1.15 mmol) with imidazole(470 mg, 6.9 mmol) was carried out in 8 ml of THF at 0°C under nitrogen for 20 min. The supernatant separated from imidazolium hydrochloride was directly used as a phosphorylating reagent. As the supernatant did not contain chloride ion after treatment with water, it shows that phosphorus trichloride completely converted to the desired material. Tri-(imidazolyl-(1))phosphine decomposed in THF at 0°C within 1 day.

The reaction of uridine with tri-(imidazolyl-(1))phosphine was carried out in pyridine-THF at -78°C : The THF solution(1.2 ml) of tri-(imidazolyl-(1))-phosphine(approximately equimolar amount to uridine[6]) prepared immediatly before use was added to a 5:3 pyridine-THF solution(0.5 ml) of uridine(35.1 mg, 0.14

mmol), and then the mixture was stirred at -78°C. After the appropriate time interval, a 2:1 THF-water solution(1.2 ml) of iodine(equimolar amount to the phosphine) was added to the rection mixture and the resulting solution was stirred at 0°C for 10 min.

The analytical results of the reaction products by means of high pressure liquid chromatography, paper chromatography, and paper electrophoresis showed that the amount of oligonucleotide material formed did not significantly increase after 60 min. It is concluded that the polymerization reaction was almost completed within 60 min under the present condition. The products were separated on a DEAE-cellolose column(bicarbonate form). The elution pattern is shown in Figure 1 and the distribution of the nucleotide material is described in Table 1.

The polymerization products were classified into two series. One series consists of uridine oligonucleotides with a free 5'-OH end, $(Up)_n$, and the other consists of uridine oligonucleotides with free 5'-OH and 3'-OH ends, $(Up)_nU$.

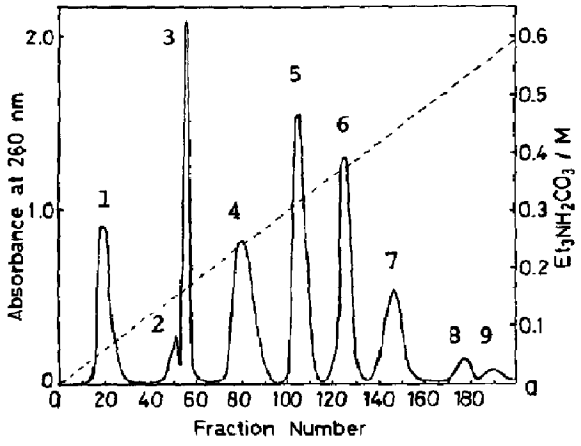


Figure 1. DEAE-cellulose column(28.0 cm x 3.4 cm) chromatography of the polymerization products by the reaction of uridine with tri-(imidazolyl-(1))phosphine. A linear gradient of triethylammonium bicarbonate, pH 7.5, from 0.001 M to 0.6 M was used for elution and 15 ml fractions per 15 min were collected.

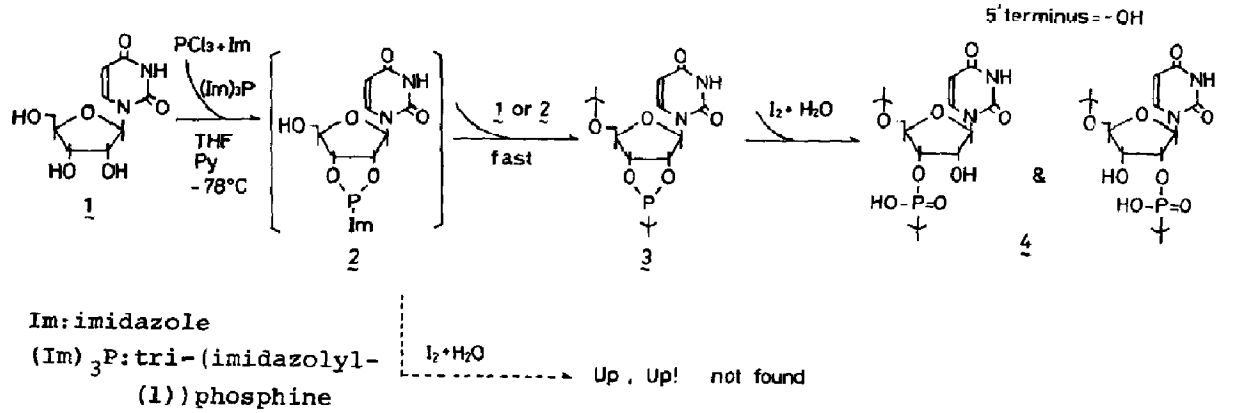
Table 1. Distribution of Nucleotide Material in the Peaks of Figure 1.

Peak	Fractions pooled	% of total nucleotide material	composition of peaks
1	17- 27	11.3	uridine
2	47- 54	3.2	unidentified
3	55- 58	16.1	UpU
4	74- 93	20.0	$(Up)_2U$
5	100-114	22.8	$(Up)_3U$ [9.2 %], $(Up)_2$ [13.6 %]
6	119-136	16.1	$(Up)_4U$ [7.6 %], $(Up)_3$ [8.5 %]
7	137-156	8.2	$(Up)_5U$ [5.5 %], $(Up)_4$ [2.7 %]
8	173-180	1.5	$(Up)_5$
9	185-193	0.8	$(Up)_6$

In the products, however, uridine monophosphate and uridine 2',3'-cyclic phosphate were not detected.

The phosphodiester linkage in formed UpU and UpUpU was investigated by snake venom phosphodiesterase, spleen phosphodiesterase, and alkaline(KOH) degradations[7]. UpU and UpUpU were completely hydrolyzed by both snake venom phosphodiesterase and KOH. It is noteworthy that the phosphodiester linkage in UpU and UpUpU is composed of both 3'-5'linkage and 2'-3'linkage but not 5'-5' linkage and 3'-3'linkage. In addition, as the higher oligonucleotides were completely degraded by KOH, a possibility of the formation of 5'-5'phosphodiester linkage is denied. That a 32 % of UpU was hydrolyzed by spleen phosphodiesterase shows the ratio of the 3'-5'linkage to the 2'-5'linkage in UpU to be 1:2(mol/mol). Consequently, it is considered that tri-(imidazolyl-(1))phosphine does not attack to 5'-OH of unprotected uridine in the first phosphorylation step and the polymerization reaction proceeds *via* uridine 2',3'-cyclic phosphorimidazole 2 which is formed by the selective attack of tri-(imidazolyl-(1))phosphine to 2'-OH and 3'-OH of uridine. Generally, the reaction of phosphorus compound with *cis*-vicinal diol gives only the cyclic phosphite ester[5]. This observation supports the existence of the intermediate having cyclic phosphite bond, 2. Shimilar consideration of a nucleoside 2',3'-cyclic phosphite ester intermediate has been given in the reaction of ribonucleoside with triethyphosphine[1-e]. However, in the products there were no uridine 2'(3')-monophosphate and uridine 2',3'-cyclic phosphate resulted from an oxidation of uridine 2',3'-cyclic phosphorimidazole 2. This fact indicates that uridine 2',3'-cyclic phosphorimidazole 2, the intermediate of this reaction, is highly reactive and it reacts rapidly with 1 or 2 at 5'-OH soon after its formation.

Uridine oligonucleotide 4 were easily obtained by the *in situ* oxidation of the oligomer 3 with iodine and water. This process involves the oxidation of cyclic phosphite triester and the ring-opening reaction of the resulting cyclic phosphate. The type of the phosphodiester linkage is resulted from the cleavage



of either 3'-O-P or 2'-O-P in the ring-openig step. No cleavage of 5'-O-P occurred, because uridine monophosphate was not found in the products. The results of enzymatic degradations show that the produced uridine oligonucleotide contain both 3'-5'linkage and 2'-5'linkage.

Owing to the simplicity of this method, after appropriate improvements of reaction conditions, this process may have some merits to be used as a convenier procedure for the synthesis of short-chain homooligoribonucleotides starting frc unprotected ribonucleoside.

Further research to evaluate the potential of this synthetic method for the preparation of homooligoribonucleotides containing all kinds of nucleotides is in progress.

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