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Formation of Interribonucleoside Phosphate Bond by the Use of Reagent Formed by the Reaction of 2-Chlorophenyl Phosphorodichloridate with 5-Nitrobenzotriazole and Preparation of Anticodon Triplet of Yeast $tRNA^{Lys}$

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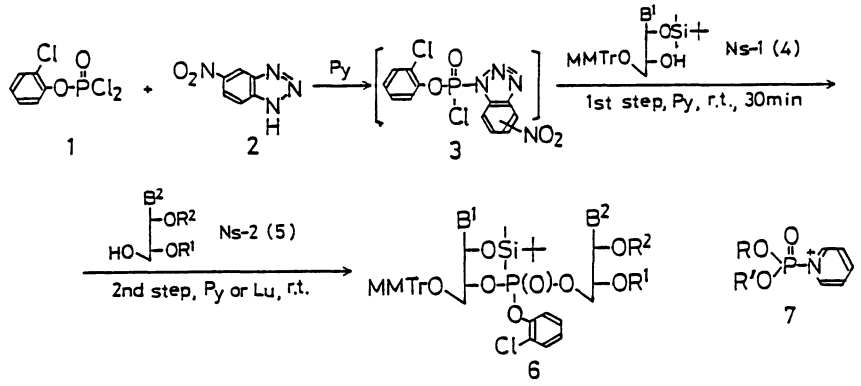
The reagent formed by the reaction of 2-chlorophenyl phosphorodichloridate with an equimolar amount of 5-nitrobenzotriazole was treated with protected ribonucleosides having free 3'-OH, followed by protected ribonucleosides having free 5'-OH in pyridine for 8 h (the second step), the corresponding dinucleoside phosphates being obtained in 55-84% yields. When the second step was carried out in the presence of 2,6-lutidine, the yields were increased to 80-90% within 2 h. By this procedure, the anticodon triplet of yeast $tRNA^{Lys}$ was prepared in 71% yield.

The development of simple procedures for the synthesis of oligoribonucleotides having defined sequence is of great importance in the investigation of RNA. Of a variety of phosphorylating systems reported so far, those utilizing bifunctional phosphorylating reagents are attractive because (3'-5')-internucleotide phosphate linkage can be formed by one-pot procedure.[1,2] However, only a few have sufficient reactivity and selectivity to be applied to the synthesis of oligoncleotides.

In this paper, we wish to report the preparation of diribonucleoside phosphates by the use of a bifunctional phosphorylating reagent formed by the reaction of 2-chlorophenyl phosphorodichloridate (1) with one equivalent of 5-nitrobenzotriazole (2),[3] and preparation of the anticodon triplet of yeast $tRNA^{Lys}$.

When 1 (0.6 mmol) was allowed to react with 2 (0.6 mmol) in pyridine at room temperature for 15 min, followed by treatment with 2'-O-t-butyldimethylsilyl-5'-O-monomethoxytritylribonucleoside (Ns-1; 4a-4e, 0.5 mmol)[4] at room temperature (the first step), the Ns-1 was completely converted into products with zero mobility within 30 min as indicated by thin layer chromatography. The resulting mixture reacted with protected ribonucleosides having a free 5'-hydroxy group (Ns-2; 5a and 5b, 0.5 mmol) for 8 h (the second step) to afford the corresponding protected dinucleoside phosphates (6a-6h) in 55-84% yields with varied amounts of recovered Ns-2 (Scheme 1; Table 1, entries 1-8).

Dedicated to Professor Teruaki Mukaiyama on the occasion of his 60th birthday.



Scheme 1. (For B^1, B^2, R^1, R^2, Py, and Lu, see Table 1).

Table 1. Preparation of Dinucleoside Phosphates (Scheme 1)

Entry	1st Step[a] Ns-1 No.	B^1	2nd Step[a] Ns-2 No.	B^2	R^1	R^2	Solvent, time / h	Product No.	Yield / %	Recovery Ns-2 / %
1	4a	Ura	5a	Ura	H	TBDMS	Py, 8	6a	73	18
2	4b	bzCyt						6b	78	14
3	4c	bzAde						6c	61	24
4	4d	ibGua						6d	55	45
5	4a	Ura	5b	bzAde	H	TBDMS	Py, 8	6e	70	19
6	4b	bzCyt						6f	84	15
7	4c	bzAde						6g	72	22
8	4e	ibGua						6h	61	32
9	4a	Ura	5c	Ura	Bz	Bz	Py, 16	6i	70	29
10	4a	Ura	5c	Ura	Bz	Bz	Lu-Py, 2	6i	80	8
11	4a	Ura	5d	bzUra	Bz	Bz	Lu-Py, 2	6j	91[b]	-
12	4c	bzAde	5e	bzAde	TBDMS	TBDMS	Lu-Py, 2	6k	90	-

a) The first step was carried out in pyridine except for entry 12 where 2,6-lutidine was used. Ura = uracil-1-yl, bzUra = N^3-benzoyluracil-1-yl, bzCyt = N^4-benzoylcytosin-1-yl, bzAde = N^6-benzoyladenin-9-yl, ibGua = N^2-isobutyrylguanin-9-yl, Py = pyridine, Lu = 2,6-lutidine, TBDMS = t-butyldimethylsilyl.

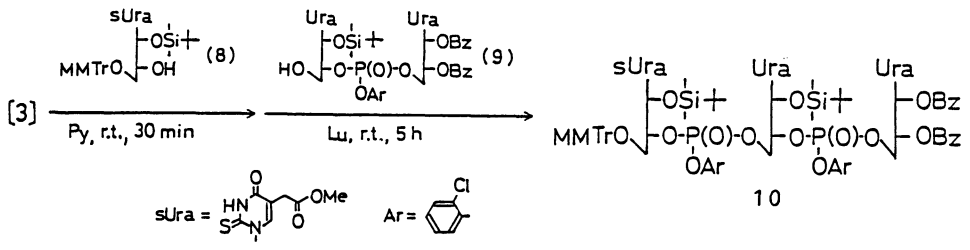
b) 4a and 5d were used in a molar ratio of 5:4.

As described above, the first step of the reaction proceeded smoothly with complete consumption of Ns-1, while the second step required a period of about 8 h with a considerable amount of recovered Ns-2. Although the marked difference in reactivity between the first and the second phosphorus-activating group bonds in the bifunctional phosphorylating reagent (3) is essential to avoid the undesirable formation of 3'-3'- and/or 5'-5'-phosphate bond, the activation of the second step after the completion of the first step is desirable from the synthetic point of view.

N-Phosphorylpyridinium salt (7) has been assumed as a reactive intermediate in phosphorylation reaction.[5] Although many phosphorylating systems reported so far have been carried out in pyridine, however, the addition of considerable amounts of azoles has been recommended to accelerate the internucleosidic phosphate bond formation. This fact suggests that 7 is not so reactive as to facilitate the formation of internucleosidic phosphate bond. It could therefore be assumed that a reactive phosphorylating species formed in the first step of the present reaction (Scheme 1) was partly converted into less reactive N-phosphorylpyridinium salt by the nucleophilic attack of pyridine.

In order to retard the formation of any N-phosphorylpyridinium salt, the reaction using 2,6-lutidine as a solvent was tried. The preparation of 3 (0.6 mmol) and subsequent reaction with 4a (0.5 mmol) was carried out in pyridine (0.8 ml). After 30 min at room temperature, 2',3'-di-O-benzoyluridine (5c; 0.5 mmol) in 2,6-lutidine (1.0 ml) was added. As expected, the second step was completed within 2 h giving uridilyluridine derivative (6i) in 80% yield (Table 1, entry 10; see also entry 9). Inspection of the crude reaction mixture by thin layer chromatography revealed the formation of a trace amount of more polar product which could not be identified. When 0.4 mmol of N^3,2',3'-O-tribenzoyluridine (5d) was used in the place of 5c in the above reaction, protected uridilyluridine derivative (6j) was obtained in 91% yield (Table 1, entry 11).[6] The reaction of 3 (0.68 mmol) with 0.57 mmol each of 4c and N^6-benzoyl-2',3'-di-O-t-butyldimethylsilyladenosine (5e) in 2,6-lutidine afforded the corresponding fully protected adenylyladenosine (6k) in 90% yield (Table 1, entry 12).

Based on the findings described above, the preparation of the anticodon triplet of yeast $tRNA^{Lys}$ was attempted.[7] Thus, 2'-O-t-butyldimethylsilyl-5'-O-monomethoxytrityl-5-methoxycarbonylmethyl-2-thiouridine[8] (8; 0.337 mmol) was allowed to react with 3 (0.404 mmol) in pyridine (0.8 ml) for 30 min, followed by the reaction with uridilyluridine derivative, 9,(0.337 mmol), prepared by demonomethoxytritylation of 6i (2% trifluoroacetic acid in CH_2Cl_2; at room temperature for 5 min), in 2,6-lutidine (1.3 ml) for 5 h at room temperature to give the expected fully protected triplet (10) in 71% isolated yield with 17% recovery of 9 (Scheme 2). The structure of 10 was confirmed by enzymatic degradation of the deprotected product [i) pyridine-2-aldoximate (room temperature, 2 h), ii) MeONa in MeOH (room temperature, 16 h), and then iii) 0.1 M HCl (pH 2, room temperature 6 h)].



Scheme 2.

The work described in this paper demonstrates the utility of the reagent (3) formed by the reaction of 1 with an equimolar amount of 2 as a bifunctional phosphorylating reagent for the formation of interribonucleoside phosphate bond by one-pot procedure. It was found that the use of 2,6-lutidine as a cosolvent facilitates the reaction.

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