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A Convenient Method for the Synthesis of Nucleoside Phosphorothioates

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Recently, interest in polymers of nucleoside phosphorothioates was caused by strong induction effect of interferon[1] production. Eckstein[2] first prepared thymidine- and uridine-phosphorothioates by way of thiophosphorylation using tri-imidazoyl-1-phosphinesulfide and reported the enzymatic activities of dinucleoside phosphorothioates. Murray and Atkinson[3] synthesized adenosine 5′-phosphorothioate and tested the activity for some enzymes as a nucleotide analog.

In the present experiment, a general synthetic procedure for the preparation of nucleoside 5′-phosphorothioates starting from nucleoside and thiophosphoryl chloride, which is a readily available and inexpensive starting material, was investigated. For example, one equivalent of 2′,3′-*O*-isopropylidene uridine was allowed to react with two equivalent of thiophosphoryl chloride in dry pyridine at 0°C for 30 min. After hydrolysis of the resulting mixture under cooling, 2′,3′-*O*-isopropylidene uridine 5′-phosphorothioate was obtained in 85% yield. The structure was confirmed by elemental analysis, paper electrophoresis and UV spectrum.

In a similar manner, various nucleoside 5′-phosphorothioates were prepared in high yields as shown in Table 1.

It was found that phosphorothioate group was detected sensitively on paper chromatogram as a

1) S. Baron and B. Levy, "Annual Review of Microbiology," Vol. 20, Annual Reviews, Inc., Palo Alto, California, U.S.A. (1966), p. 291.

2) F. Eckstein, *J. Amer. Chem. Soc.*, **88**, 4292 (1966); *Tetrahedron Lett.*, **1967**, 1157, 3495.

3) A. W. Murray and M. R. Atkinson, *Biochemistry*, **7**, 4023 (1968).

Table 1. Preparation of protected nucleoside 5′-phosphorothioates

Nucleoside		Product	
	(g)	Yield (%)	R_f value*
2,′3′-*O*-isopropylidene adenosine	1.54	81	0.23
2′,3,-*O*-isopropylidene guanosine	1.64	89	0.10
2′,3′-*O*-isopropylidene inosine	1.54	85	0.11
2′,3′-*O*-isopropylidene uridine	1.42	85	0.22
2′,3′-di-*O*-benzoyl uridine	2.26	73**	0.10***
3′-*O*-acetyl thymidine	1.42	83	0.18***

* Paper chromatography was carried out by descending technique using Toyo Roshi No. 51 paper. Solvent system used was: isopropyl alcohol-concentrated ammonium hydroxide-water (7 : 1 : 2 *v/v*).
** The reaction time was 1 hr.
*** The R_f value shows that after removal of protecting group.

brownish spot by spraying 10% aqueous copper(II) sulfate solution. It was further detected by Isherwood Reagent.[4]

In the above reactions, it was found that the yields of nucleoside 5′-phosphorothioates considerably decreased when just one equivalent of thiophosphoryl chloride was used, or when the reaction was carried out without ice-cooling.

In conclusion, it is noted that this procedure has two advantageous points over the previous method, namely, (1) satisfactory yields are obtained with respect to various nucleosides, (2) products can be handled by conventional organic technique on a relatively large scale without using column chromatography as described in the experimental part.

Experimental

Materials. 2′,3′-*O*-Isopropylidene adenosine,[5] 2′,3′-*O*-isopropylidene inosine,[6] 2′,3′-*O*-isopropylidene guanosine,[7] 2′,3′-*O*-isopropylidene uridine,[8] 2′,3′-di-*O*-benzoyl uridine[9] and 3′-*O*-acetyl thymidine[10] were prepared by literature procedures.

Preparation of Protected Nucleoside 5′-Phosphorothioate. *(A general procedure).* To a solution of freshly distilled thiophosphoryl chloride (1 m*l*, 10 mmol) in 25 m*l* of dry pyridine was added, portionwise, a protected nucleoside (5 mmol) (see Table 1) with stirring at 0°C. The stirring was continued for 30 min under cooling. Then, the reaction mixture was poured with stirring into 50 m*l* of ice water. After the addition of 5 m*l* of triethylamine (*ca.* 92 mmol), the resulting aqueous solution was concentrated under reduced pressure at a temperature below 30°C until white crystals appeared. The crystals, triethylammonium chloride, was filtered off by using Toyo Roshi No. 131 paper (for fine precipitates) and the filtrate was concentrated to dryness. The residual oil was still contaminated by triethylammonium chloride and then it was diluted with dry pyridine. The precipitate was filtered off by the No. 131 paper, and the filtrate was concentrated to dryness. The corresponding nucleoside phosphorothioate was isolated as triethylammonium salt by means of either method A or method B. Uridine- and thymidine-phosphorothioates were obtained according to method A and the other nucleoside phosphorothioates, such as adenosine, guanosine and inosine derivatives were isolated by method B.

Method A. The syrup was dissolved in 100 m*l* of dry chloroform and it was allowed to stand overnight in a refrigerator. Triethylammonium salt of inorganic thiophosphate was successively precipitated. After removal of the salt by centrifuge, (if the resulting supernant was a slightly cloudy solution, it should be again filtered by the No. 131 paper) the chloroform was removed by evaporation. Mono-triethylammonium salt of 2′,3′-*O*-isopropylidene uridine 5′-phosphorothioate (2.05 g, 4.26 mmol) was obtained in 85% yield (based on 2′,3′-*O*-isopropylidene uridine) as white powder.

Found: C, 44.43; H, 5.80; N, 8.48; S, 6.98%. Calcd for $C_{18}H_{32}N_3O_8PS$: C, 44.87; H, 6.65; N, 8.72; S, 6.65%. Ratio uridine : phosphorus, Found: 1.00 : 0.93. Required: 1 : 1.

Method B. The syrup was dissolved in 100 m*l* of isopropyl alcohol. A white precipitate soon appeared in the solution. The precipitate was collected by filtration. Mono-triethylammonium salt of 2′,3′-*O*-isopropylidene guanosine phosphorothioate (2.31 g, 4.44 mmol) was obtained in 89% yield (based on 2′,3′-*O*-isopropylidene guanosine) as white powder. Repeated reprecipitations by means of isopropyl alcohol and a small amount of methanol afforded white powder for analytical sample.

Found: C, 41.90; H, 5.90; N, 16.29; S, 6.25%. Calcd for $C_{19}H_{33}N_6O_7PS$: C, 43.85; H, 6.35; N, 16.15; S, 6.14%. Ratio guanosine : phosphorus, Found: 1.00 : 0.99. Required: 1 : 1.

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4) C. A. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).
5) E. C. Taylor and W. A. Ehrhart, *J. Amer. Chem. Soc.*, **82**, 3138 (1960).
6) N. Nagasawa, I. Kumashiro and T. Takenishi, *J. Org. Chem.*, **31**, 2685 (1966).
7) A. Hampton, *J. Amer. Chem. Soc.*, **83**, 3640 (1961).
8) N. C. Yung and J. J. Fox, *ibid.*, **83**, 3060 (1961).
9) R. Lohrmann and H. G. Khorana, *ibid.*, **86**, 4188 (1964).
10) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, **1953**, 954.