Synthesis of Nucleoside Di- and Tri-phosphates *via* Nucleoside 5′-Phosphoric Di-n-butylphosphinothioic Anhydride Intermediates

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Summary Reaction of di-n-butylphosphinothioyl bromide with nucleotides afforded almost quantitatively the corresponding nucleoside phosphoric di-n-butylphosphinothioic anhydrides, which are quite stable even in the presence of water; in the presence of AgOAc they react readily with inorganic phosphate or pyrophosphate to give the corresponding nucleoside di- or tri-phosphates respectively.

METHODS of phosphorylation for the synthesis of nucleotide derivatives such as nucleotide coenzymes and oligonucleotides have been studied extensively. Mixed anhydrides consisting of phosphoric acid and inorganic oxyacids such as phosphoric sulphonic anhydrides[1] or unsymmetrical pyrophosphates[2] are known to be useful intermediates. Although the mixed anhydrides are formed rapidly, they are not isolated during the reactions because of their instability. Consequently, knowledge[5] about the mixed anhydrides is limited,[3] especially those containing nucleotide residue. We have found that a new type of mixed anhydride between a nucleotide and di-n-butylphosphinothioic acid can be obtained in high yield and isolated as a stable material.

We now report the synthesis and the reactions of nucleoside 5′-phosphoric di-n-butylphosphinothioic anhydrides.

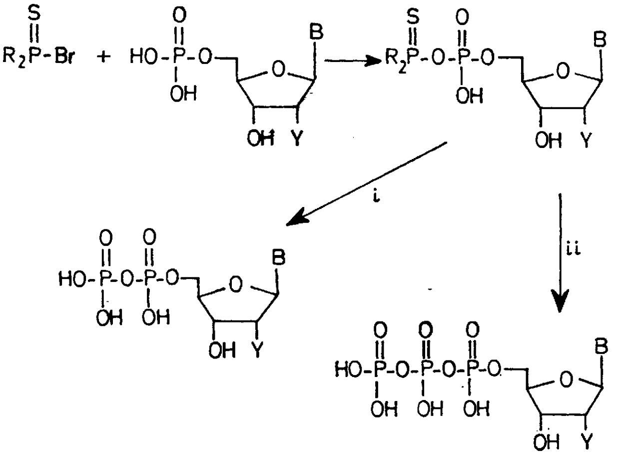
The reaction of thymidine 5′-phosphate (0·05 mmol)

Table. Preparation of nucleoside 5′-phosphoric di-n-butylphosphinothioic anhydrides and synthesis of nucleoside 5′-polyphosphates

Nucleoside 5′-phosphate	Yield (%)	Solvent	P.c.[a] A	P.c.[a] B	P.e.[b] C	P.e.[b] D	Spectral data[c] λ_{max}/nm (10^{-3} ϵ)	Spectral data[c] λ_{min}/nm	Yield of polyphosphate Triphosphate (%)	Yield of polyphosphate Diphosphate (%)
Thymidine	100	Pyridine	0·79	0·84	0·44	0·69	267(9·6)	237	88	86
Deoxyguanosine	95	Bu^tOH[d]	0·71	0·83	0·43	0·66	252(13·7)	224	—	—
Uridine	100	Pyridine	0·71	0·80	0·44	0·65	262(10·0)	233	75	77
Cytidine	100	α-Picoline	0·74	0·76	0·40	0·64	271(9·1)	250	88	86
Adenosine	100	Pyridine	0·77	0·83	0·42	0·70	259(15·4)	230	84	82
Guanosine	97	Bu^tOH[e]	0·70	0·82	0·42	0·69	252(13·7)	226	87	76

[a] P.c. = Paper chromatography, by the descending technique using Toyo Roshi No. 51 paper. Solvent systems: (A), Pr^iOH–NH_4OH–H_2O (7 : 1 : 2 v/v); (B), EtOH–1M NH_4OAc (7 : 3 v/v). [b] P.e. = Paper electrophoretic mobility relative to the corresponding nueleotide. Buffers: (C), phosphate (0·2 M, pH 8); (D), phosphate (0·2 M, pH 6). [c] In H_2O, pH 7. [d] Reaction carried out in the presence of Bu^n_3N (4 mol. equiv. relative to nucleotide).

with di-n-butylphosphinothioyl bromide[4] (0·1 mmol) in dry pyridine (0·5 ml) at room temperature for 3 h gave an almost quantitative yield of the corresponding thymidine 5′-phosphoric di-n-butylphosphinothioic anhydride.



R = Bu^n; Y = H, OH; B = nucleoside base. Reagents: i, $H_2PO_4^-$ + AgOAc; ii, $H_2P_2O_4^{2-}$ + AgOAc.

Other nucleoside 5′-phosphoric di-n-butylphosphinothioic anhydrides were obtained similarly (Table).† The structures were confirmed by u.v. spectroscopy, periodic oxidation, and elemental analysis. These anhydrides are quite stable even in the presence of water, but in the presence of silver acetate, they react readily with nucleophiles such as inorganic phosphate and inorganic pyrophosphate.[5]‡

For example, adenosine 5′-triphosphate (ATP) was obtained when adenosine 5′-phosphoric di-n-butylphosphinothioic anhydride (0·1 mmol) was treated with bis(tri-n-butylammonium) pyrophosphate (0·5 mmol) in the presence of silver acetate (0·4 mmol) in dry pyridine at room temperature for 2 h. The ATP was isolated by passing H_2S through the mixture to precipitate Ag^+, filtering off Ag_2S, concentration under reduced pressure and chromatography on DEAE cellulose (30 × 1·7 cm column), with linear gradient elution (0—0·2M) with Et_3NHHCO_3 solution. After elution of adenosine 5′-diphosphate (ADP), ATP was obtained in 84% yield, and was homogeneous on paper chromatography.

Uridine 5′-triphosphate (UTP), guanosine 5′-triphosphate (GTP), cytidine 5′-triphosphate (CTP), and thymidine 5′-triphosphate (dTTP) were obtained similarly (Table).

In the above experiments, when the mono(tri-n-butylammonium) phosphate salt (0·5 mmol) was used in place of pyrophosphate, the corresponding nucleoside diphosphates such as ADP, uridine 5′-diphosphate (UDP), guanosine 5′-diphosphate (GDP), cytidine 5′-diphosphate (CDP), and thymidine 5′-diphosphate (dTDP) were obtained (Table). The structures of the nucleoside di- and tri-phosphates were confirmed by comparison by paper electrophoresis, paper chromatography, and u.v. spectroscopy with commercial samples. We thank Professor Teruaki Mukaiyama for encouragement and discussions, and Miss Setsuko Mori for the elemental analyses.

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† The mixed anhydrides were isolated by washing with Et_2O and then extracted with a mixture of Bu^nOH and CH_2Cl_2.

‡ For the synthesis of nucleoside polyphosphates, the mixed anhydrides were used without isolation after removal of excess of di-*n*-butylphosphinothioyl bromide by washing with ether.

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[2] A. M. Michelson, *J. Chem. Soc.*, 1959, 1371, 3655; F. Cramer and R. Wittmann, *Angew. Chem.*, 1960, **72**, 628.
[3] F. Cramer and R. Wittmann, *Chem. Ber.*, 1961, **94**, 322, 328.
[4] Di-n-butylphosphinothioyl bromide was prepared in 87% yield, b.p. 143—144 °C at 6 mmHg, by a modification of the procedure of H. J. Harwood and K. A. Pollart, *J. Org. Chem.*, 1963, **28**, 3430.
[5] The synthesis of unsymmetrical pyrophosphates containing nucleoside residues has been studied extensively. Nucleoside phosphoromorpholidates are the most widely used reagent for the synthesis of unsymmetrical pyrophosphates: J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, 1961, **83**, 663; A. Adams and J. G. Moffatt, *ibid.*, 1966, **88**, 838; J. G. Moffatt, *Canad. J. Chem.*, 1964, **42**, 599; J. G. Moffatt, in 'Methods in Enzymology,' eds. L. Grossman and K. Moldave, Academic Press, New York, 1967, vol. 12 (Part A), pp. 182.