CHEMISTRY LETTERS, pp. 951-954, 1983.

INTRODUCTION OF "SOLUBILIZING GROUP" TO NUCLEOTIDE SYNTHESIS: STANNYL ESTER OF NUCLEOTIDES AS USEFUL INTERMEDIATE IN UNSYMMETRICAL ALKYL NUCLEOSIDE DIPHOSPHATE SYNTHESIS

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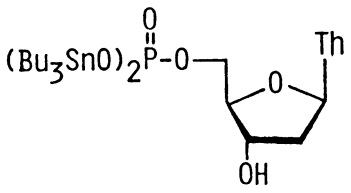
Tributylstannyl ester of 5'-guanylic acid and related compounds enhanced their solubility in pyridine. The stannyl phosphorothioate (5) was employed as synthetic intermediate for alkyl nucleoside diphosphate bond formation.

During the past ten years, several protecting groups of phosphate have been proposed and applied to synthesis of oligonucleotides through the phosphotriester approach.[1] In this field the protecting groups are of great importance since they not only play an original role of masking but also act as a solubilizing function. In our previous studies, the chemical synthesis of the 5'-terminus of a m-RNA from cytoplasmic polyhedrosis virus ($m^7G^{5'}$pppAmpGpU...) has been achieved by using a new type of diphosphorylating agent,[2] PhSppm^{7}G, which can be activated with iodine or silver salts.[3] However, there still remains an essential problem: solubilization of guanylic acid derivatives in aprotic solvents. The phosphotriester approach can not be applied to the synthesis of nucleoside polyphosphates and related compounds because the fully esterified polyphosphates are so reactive that they can not be isolated usually.[4]

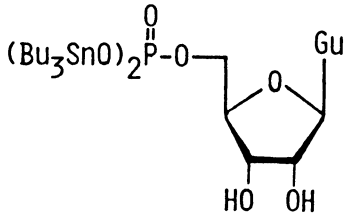
Recently, we have reported[5] the synthesis of acyl phosphates *via* stannyl phosphate intermediates that were found to be more soluble in organic solvents than the corresponding trialkylammonium salts. For example, the pyridinium or other ammonium salts of guanylic acid are quite insoluble or form gel in dry pyridine whereas the bis(tributylstannyl) ester of 5'-guanylic acid is easily dissolved.

In this paper, we wish to describe the utility of stannyl phosphate in the synthesis of unsymmetrical alkyl nucleoside diphosphates under neutral conditions starting from the stannylated species of isobutyl S-phenyl phosphorothioate and nucleoside 5'-phosphates.

Bis(tributylstannyl) esters of thymidine and guanosine 5'-phosphates (1a-b)[6] were prepared quantitatively according to a modification of the procedure of Moffatt[7] by employing bis(tributyltin) oxide in methanol and pyridine—water, respectively.

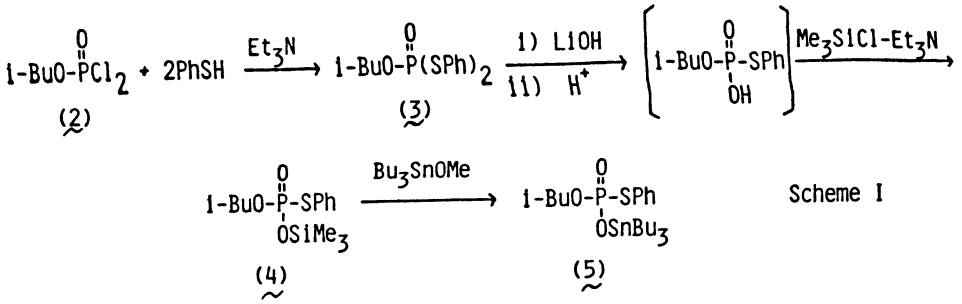


(1g)



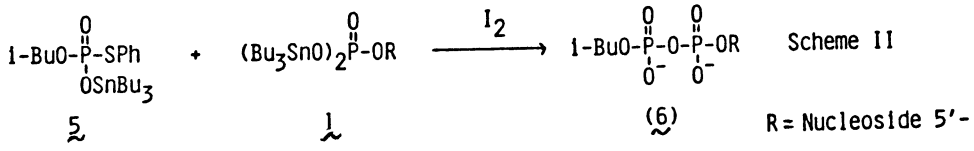
(1b)

The activatable compound, isobutyl tributylstannyl S-phenyl phosphorothioate (5), was prepared as follows: Isobutyl phosphorodichloridate (2) (13.0 g, 68.2 mmol) and benzenethiol (15.0 g, 137 mmol) were mixed in the presence of triethylamine (13.8 g, 136 mmol) in dry benzene (200 ml) and the mixture was stirred at room temperature for 2 h. The usual workup followed by column chromatography gave isobutyl S,S-diphenyl phosphorodithioate (3)[7] as an oily material in 93% yield. Compound 3 (30.7 g, 91 mmol) was dissolved in pyridine (100 ml) and treated with 0.5 M LiOH (365 ml) at room temperature for 10 min. After removal of the solvent under reduced pressure, the residue was acidified with 0.1 M HCl (400 ml) and extracted three times with CH_2Cl_2. The extracts were combined and dried (Na_2SO_4). The solvent was removed in vacuo. The residual oil was silylated with 2 equiv. each of trimethylsilyl chloride and triethylamine in dry benzene at room temperature for 1 h to afford isobutyl trimethylsilyl S-phenyl phosphorothioate (4)[9] in 69% yield (bp 124-126 °C/0.06 mmHg, ^{31}P NMR[10] δ-10.7). Compound 4 was further treated with 1 equiv. of tributyltin methoxide in CCl_4 at room temperature for 2 h to give 5 quantitatively (^{31}P NMR δ-6.9).[10] A change in ^{31}P NMR chemical shifts (δ-10.7→δ-6.9) obviously indicates transesterification.



Scheme I

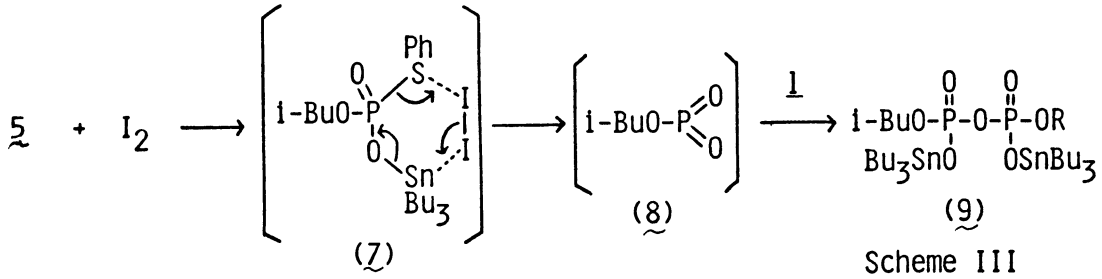
The reaction of 1a-b with 5 was performed by activation of the PhS group with iodine as shown in Scheme II. When 1 equiv. of bis(tributylstannyl) ester of



Scheme II

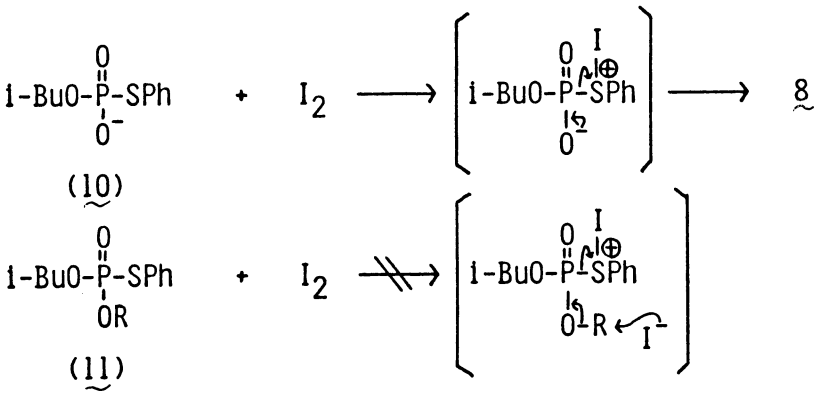
thymidine 5'-phosphate 1a was allowed to react with 5 in the presence of 1 equiv. of iodine in dry pyridine, the corresponding P^1-isobutyl P^2-thymidine 5'-diphosphate (6a)[11] was obtained in 53% yield [% conversion (time): 26% (5 min); 29% (15 min); 33% (60 min); 53% (20 h)]. However, use of 5 equiv. of iodine

increased the yield of 6a up to 70% [58% (5 min); 62% (15 min); 70% (30 min)]. Addition of more excess of iodine did not affect the yield of 6. In a similar manner, P^1-isobutyl P^2-guanosine 5'-diphosphate (6b)[11] was obtained in 42% yield. The reaction seems to proceed as shown in Scheme III.



Scheme III

The diester type of phosphorothioate such as (10) is well known to be activated with iodine.[2,12] On the contrary, we have known that the triester (11) carrying an alkyl group (R) could not be activated under the same conditions.



It seems that, in the case of the stannylated triester 5, iodonium anion can push out the stannyl group at six-membered transition state (7) and the generated methaphosphate ester (8) reacts with distannyl ester 1 to afford the corresponding diphosphate (9). The driving force of this reaction is due to the preferable bond formation of Bu_3Sn-I. Moreover, since all of the phosphate esters were of the stannylated triester-type, the reactions could be kept homogeneous. The stannyl ester bonds were cleaved during paper chromatography with basic medium [i-ProOH—concd. ammonia—water (7:1:2, v/v/v)] and the products were isolated as the corresponding ammonium salts. In the above reaction the corresponding P^1,P^2-dithymidine 5'-diphosphate[12] was detected as a byproduct in 10% yield.

In conclusion, the stannylation of insoluble phosphate derivatives described here would provide a useful method for the alkyl nucleoside diphosphate synthesis because the reaction can be carried out homogeneously under neutral conditions. This methodology might be applied to the synthesis of nucleoside coenzymes or cap structures consisting of the di- or triphosphate bridge. Further study is now in progress.

We thank Mr. Y. Nakamura for ^{31}P NMR measurements.

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6) 1a: Anal. Found: C, 45.58; H, 7.98; N, 3.19%. Calcd for $C_{34}H_{68}N_2O_8PSn_2$: C, 45.31; H, 7.60; N, 3.11%. ^{31}P NMR (methanol): δ +3.10 (external standard: 85% H_3PO_4).
1b: Anal. Found: C, 43.15; H, 7.33; N, 7.30%. Calcd for $C_{34}H_{66}N_5O_8PSn_2$: C, 43.39; H, 7.07; N, 7.44%. ^{31}P NMR (pyridine): δ +6.15 (external standard: 85% H_3PO_4).

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8) ^1H NMR (CCl_4): δ 0.88 (d, 6H, J= 6 Hz, $-CH(CH_3)_2$), 1.90 (m, 1H, $-CH(CH_3)_2$), 3.87 (dd, 2H, J_{H-H}= 6 Hz, J_{P-H}= 8 Hz, P-O-CH_2-), 7.27(m, 10H, aromtic). Anal. Found: C, 56.74; H, 5.63; S, 19.13%. Calcd for $C_{16}H_{19}O_2S_2P$: C, 56.79; H, 5.66; S, 18.95%.

9) ^1H NMR ($CDCl_3$): δ 0.28 (s, 9H, $Si(CH_3)_3$), 0.97 (d, 6H, J= 7 Hz, $-CH(CH_3)_2$), 1.95 (m, 1H, $-CH(CH_3)_2$), 3.85 (m, 2H, P-O-CH_2-), 7.35 (m, 5H, aromatic). Anal. Found: C, 48.85; H, 7.20; S, 9.89%. Calcd for $C_{13}H_{23}O_3SPSi$: C, 49.03; H, 7.28; S, 10.07%.

10) The ^{31}P NMR spectrum was measured in C_6D_6 with an external standard of 85% H_3PO_4.

11) 6a: UV[a] λ_{max} 266 nm, λ_{min} 236 nm; Rf[b] 0.71; Rm[c] 1.61.
6b: UV[a] λ_{max} 252 nm, λ_{min} 226 nm; Rf[b] 0.30; Rm[c] 1.40.
a) UV spectra were measured in 0.05 M phosphate buffer at pH 7.0.
b) Paper chromatographic separation was carried out by using Whatman 3MM papers with i-PrOH--conc. ammonia--water (6:1:3, v/v/v).
c) Rm: Paper electrophoretic mobility relative to that of the corresponding nucleoside 5'-phosphate. 0.2 M Phosphate buffer adjusted to pH 6.0 was used.

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(Received March 17, 1983)