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A Convenient Synthesis of Nucleotides

The synthesis of nucleotides by the phosphorylation of properly protected nucleosides is a general route which has been widely applied during the recent years. In this respect, numerous phosphorylating agents have been investigated and reported in the literature. However, attempts to synthesize nucleotides by direct condensations of phosphorylated sugars with derivatives of pyrimidine or purine bases are very few[1,2] and have not been extensively explored.

In this communication, we wish to report a convenient synthetic procedure for natural and unnatural nucleotides by modifying the previously reported method[3a~d] for the synthesis of the pyrimidine and purine nucleosides starting from trimethylsilyl derivatives of the bases and acylhalogeno sugars.

When trimethylsilyl derivatives of various pyrimidines and purines were allowed to react at 90~100° with phosphorylated ribosyl or glucosyl bromide, then the protecting

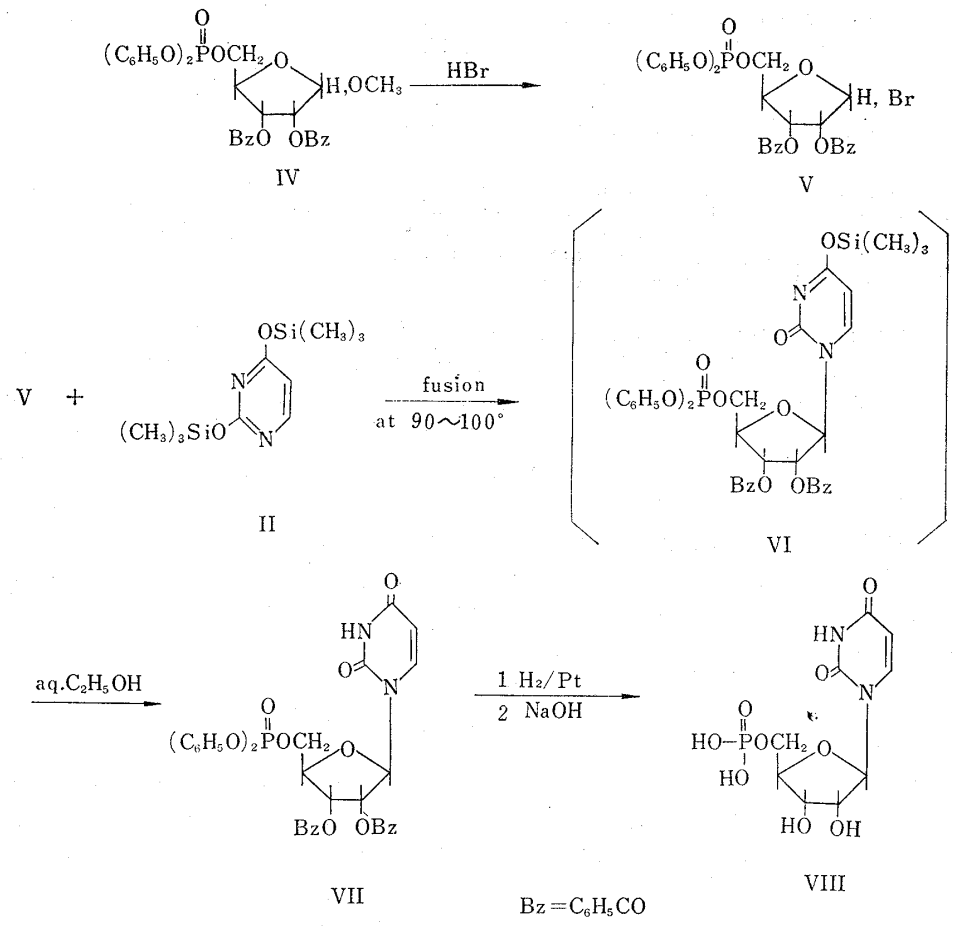

Chart 1.

1) T. Ukita, H. Hayatsu : J. Am. Chem. Soc., 84, 1879 (1962).
2) M. Matsui, A. Nobuhara : Agr. Biol. Chem., 27, 650 (1963).
3) *a*) T. Nishimura, B. Shimizu, I. Iwai : This Bulletin, 11, 1470 (1963); *b*) T. Nishimura, I. Iwai : *Ibid.*, 12, 352, 357 (1964); *c*) T. Nishimura, B. Shimizu : Agr. Biol. Chem., 28, 224 (1964); *d*) T. Nishimura, B. Shimizu, I. Iwai : This Bulletin, 12, 1471 (1964).

groups were removed, the corresponding nucleotides were produced in good yields. As a typical example of this reaction, uridine 5′-phosphate was prepared as follows.

Methyl 5-diphenylphosphoryl-D-ribofuranoside,[4] prepared by the phosphorylation of methyl D-ribofuranoside with diphenyl phosphorochloridate, was treated with benzoyl chloride to give methyl 5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranoside (IV). IV was converted into the corresponding 1-bromo derivative (V)[1] with hydrogen bromide in acetic acid by the usual manner. This bromide was coupled with bis (trimethylsilyl)uracil (II)[3a] by heating at 90~100° for 30 min. to give 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)-4-O-trimethylsilyluracil (VI), which was treated with aq. ethanol to afford 1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl) uracil (VII) in 10% yield (based on reacted uracil). The reaction was followed by the thin-layer chromatography.

Removal of phenyl groups by catalytic hydrogenation and subsequent deacylation, converted VII into 1-β-D-ribofuranosyluracil 5′-phosphate (UMP) (VIII) in 50% yield based on VII.

Furthermore, trimethylsilyl derivatives of purines[3a] were similarly reacted with 5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl bromide by the same procedure described above. The aqueous alkaline treatment of the condensation products gave 9-(5-phenylphosphoryl-D-ribofuranosyl)purines which liberated the remaining phenyl group by incubation with phosphodiesterase*1 obtained from *trimeresurus flavoviridis*

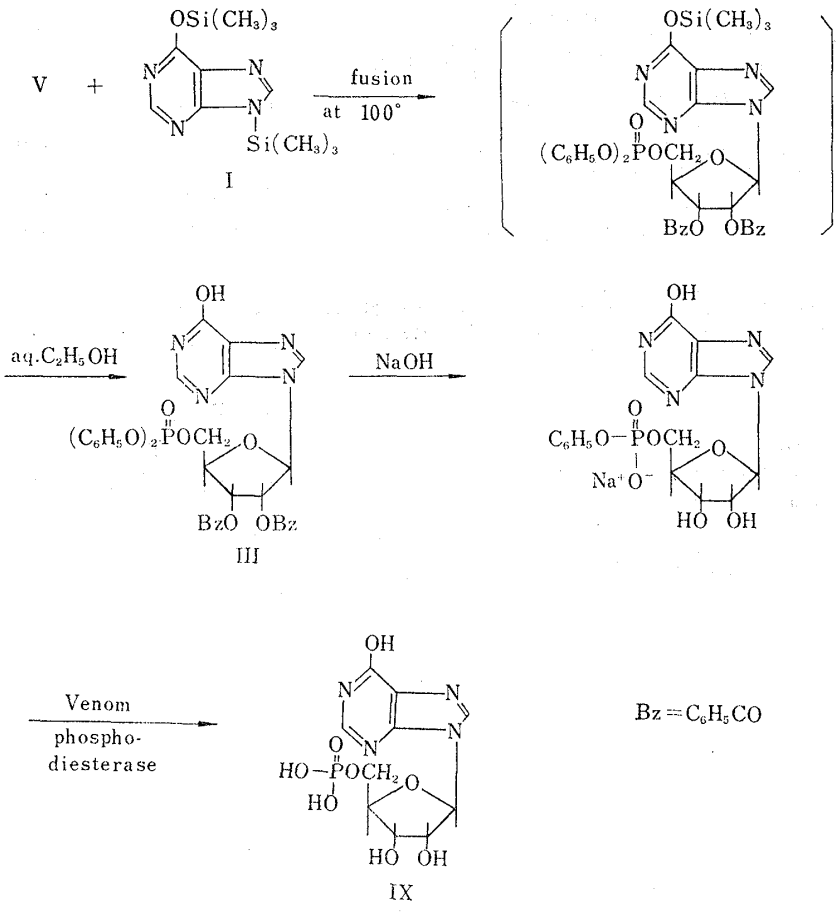

Chart 2.

*1 The enzyme preparation was obtained through courtesy of Dr. H. Ikezawa of the National Institute of Health, Japan.

4) G. M. Tenner, H. G. Khorana : J. Am. Chem. Soc., 80, 1999 (1958).

(HALLOWELL) to yield the corresponding ribofuranosylpurine 5′-phosphates in good yields.

The structural confirmation of thus obtained nucleotides was achieved by chromatographic and spectral analyses, as well as comparison with the authentic samples prepared by the known methods.

The scope of reactions was investigated by applying several RNA and DNA bases, with phosphorylribosyl bromide and phosphorylglucosyl bromide. The results of such investigations are shown in Table I.

TABLE I. Physical Properties and Yields of the Synthetic Nucleotides and their Intermediate Compounds

	m.p.[a] (°C)	$[\alpha]_D$	Yield[b] (%)
1-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)thymine	144	−51.3	48.1
Barium 1-β-D-ribofuranosylthymine 5′-phosphate*2	—	−12.25	33.5
1-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl) uracil	amorph.	−51.0	10.0
Barium 1-β-D-ribofuranosyluracil 5′-phosphate	—	−15	50
1-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)-4-ethoxy-2(1*H*)-pyrimidinone[c]	147～148	−83.8	35.0
6-Benzamido-9-(5-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)purine	amorph.	—	37.6
Barium 9-β-D-ribofuranosyladenine 5′-phosphate	188	−45.5	47.3
9-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)hypoxanthine	amorph.	−65.7	59.2
Barium 9-β-D-ribofuranosylhypoxanthine 5′-phosphate	—	−18.5	36.0
7-(5-Diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl)theophylline	154～156	−30.2	73.3
Barium 7-β-D-ribofuranosyltheophylline 5′-phosphate	—	+20.0	34.2
1-[6-Bis(*p*-nitrophenyl)phosphoryl-2,3,4-tri-O-acetylglucopyranosyl]uracil	amorph.	—	18.5
Barium 1-β-D-glucopyranosyluracil 6′-phosphate	—	− 2.5	27.0

a) Melting points are uncorrected.
b) Yields of the intermediates based on the reacted silyl compounds.
c) UMP was produced as a minor component in 2.7% yield.

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