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Synthesis of Anomeric Pyrimidine Nucleoside 5′-Phosphates of L-Ribose, α-D-Cytidine 5′-Phosphate and 3-β-L-Ribofuranosyladenine 5′-Phosphate

In the course of an investigation of the synthesis of unnatural nucleosides and their 5′-phosphates,[1,2] it became desirable to synthesize anomeric pyrimidine nucleoside 5′-phosphates of L-ribose for examination of their biological activities.

The present report deals with the synthesis of new L-α- and L-β-nucleoside 5′-phosphates as a continuation of our study on the synthesis of nucleotides.

Table I. Physical Properties of Anomeric Nucleoside 5′-Phosphates of L-Ribose and D-ribose

Configuration	α		β	
Optical isomer	D	L	D	L
Compound				
5′-Uridylic acid (5′-UMP)				
$[\alpha]_D$[a]	− 25.0	+ 26.9	− 12.9	+ 13.0
C. S.[b] ($J_{1',2'}$)	−148(4.0)	−148(4.0)	−135(4.5)	−138(5)
Rf[c]	0.24	0.24	0.24	0.24
Cotton effect (O.R.D.)	⊖	⊕	⊕	⊖
UV λ_{max} (ε) pH 1		263 (9600)		262.5(9350)
pH 7		263.5(9700)		262.5(9350)
pH 13		262.0(7300)		261.5(7100)
5′-Cytidylic acid (5′-CMP)				
$[\alpha]_D$[a]	− 86.8	+ 87.4	+ 6.9	− 6.8
C. S.[b] ($J_{1',2'}$)	−148(4.0)	−148(4.0)	−137(4.0)	−137(4.0)
Rf[c]	0.205	0.205	0.205	0.205
Cotton effect (O.R.D.)	⊖	⊕	⊕	⊖
UV λ_{max} (ε) pH 1	280 (14000)	280.5(14000)	279.0(13300)	279.0(13300)
pH 7	271.5(9500)	271.5(10000)	271.0(9100)	271.0(9350)
pH 13	271.5(9500)	271.5(9900)	271.0(9100)	271.5(9300)
Ribofuranosylthymine 5′-phosphate (5′-rTMP)				
$[\alpha]_D$[a]	− 41.0	+ 43.2	− 12.3(HCl)	+ 13.0
C. S.[b] ($J_{1',2'}$)	−146(4.5)	−147(4.5)	−136(5.0)	−137(5.0)
Rf[c]	0.31	0.31	0.31	0.31
Cotton effect (O.R.D.)	⊖	⊕	⊕	⊖
UV λ_{max} (ε) pH 1		269.0(10300)		267.5(9650)
pH 7		269.0(10200)		267.5(9550)
pH 13		268.0(7500)		267.0(7100)
3-isoAdenylic acid (3-isoAMP)				
$[\alpha]_D$[a]			− 6.6	+ 7.0
C. S.[b] ($J_{1',2'}$)			−250(1.0)	−249(1.0)
Rf[c]			0.20	0.20
Cotton effect (O.R.D.)			—	—
UV λ_{max} (ε) pH 1			275.0(16600)	275.0(18200)
pH 7			274.0(11800)	275.5(13800)
pH 13			275.0(11900)	275.5(13700)

a) Li salt (H_2O).
b) Chemical shift of C-1′ proton from dioxane as an internal standard in c.p.s.
c) Solvent system: iso-BuOH-H_2O-AcOH=5:3:2 (using Toyo Roshi No. 50 ascending at 20°).
d) Chemical shift of C-1′ proton from aceton in c.p.s.

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1) B. Shimizu, M. Asai, M. Miyaki, H. Okazaki: This Bulletin, **13**, 616 (1965). *Idem*: *Ibid.*, **15**, 1863 (1967).
2) T. Nishimura, B. Shimizu, M. Futai: Biochim. Biophys. Acta, **129**, 654 (1966).

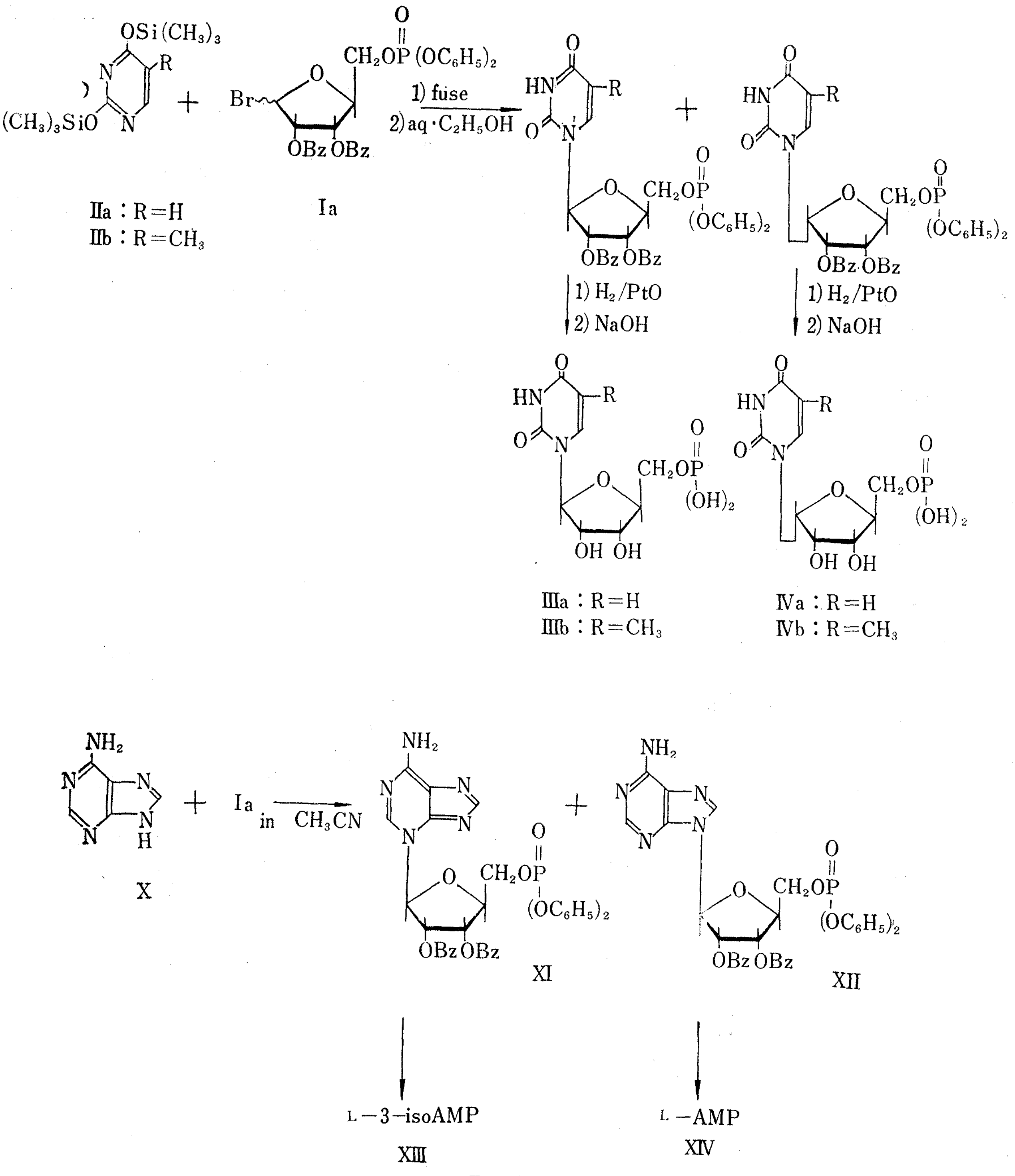

Fig. 1.

1-α-L-Ribofuranosyluracil 5′-monophosphate (α-L-UMP) and its β-anomer (β-L-UMP) were prepared by the "silyl method" starting from L-ribose[3] according to the procedure for α-D-UMP and its β-anomer.[2]

When the trimethylsilyluracil (IIa)[2] was allowed to react at 100～110° with 2.3-di-O-benzoyl-5-diphenylphosphoryl-L-ribofuranosyl bromide (Ia),[*1] then the protecting groups

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[*1] This bromide (Ia) was obtained from L-ribose[3] according to the procedure for the corresponding D-series (Ib).[4]

3) F. L. Humoller: Methods in Carbohydrate Chemistry, **I**, 83 (1962).

removed, α-L-UMP (Ⅳa) and β-L-UMP (Ⅲb) were produced in 34.5 and 18.0% yields based on the bromide, respectively (see Fig. 1).

Methods for the synthesis of L-α-ribofuranosylthymine 5′-phosphate (α-L-5′-rTMP) and its β-anomer (β-L-5′-rTMP) were virtually the same as those of the foregoing experiment. Starting from trimethylsilylthymine (Ⅱb)[2] and the phosphorylated sugar bromide (Ia), α-L-rTMP (Ⅳb) and β-L-rTMP (Ⅲb) were obtained in 34.3 and 17.0% yields based on the bromide, respectively (see Fig. 1).

The nucleotides thus obtained were identical with those of the corresponding D-series except that the specific rotations were opposite in sign having the same numerical values, (see Table I).

The synthesis of α-D-cytidine 5′-phosphate was not reported yet. An attempt was made successfully to prepare the nucleotide. Bistrimethylsilyl N-acetylcytosine (Ⅴ) was similarly treated with 5-O-diphenylphosphoryl-2,3-di-O-benzoyl-D-ribofuranosyl bromide (Ib)[4] by the same procedure described above, followed by treatment of the reaction mixture with aqueous ethanol to give protected anomeric nucleotides. Chromatography of the crude product mixture on a silicagel column afforded 1-(5-O-diphenylphosphoryl-2,3-di-O-benzoyl-α-D-ribofuranosyl)-N-acetylcytosine (Ⅶ) m.p., 202.5° and its β-anomer (Ⅵ), m.p., 176.5°, in 32.1 and 15.6% yield respectively (see Fig. 2). The aqueous alkaline (LiOH) treatment of the products (Ⅲc and Ⅳc) gave 1-(5-phenylphosphoryl-α-D-ribofuranosylcytosine and its β-anomer which liberated the remaining phenyl group by incubation with phosphodiesterase obtained from trimeresurus flavoviridis (Hallowell) to yield the corresponding α-D-5′-CMP (Ⅸ) and its β-anomer (β-D-5′-CMP) (Ⅷ) in good yield respectively. Characterization of the former (Ⅸ) as α-D-CMP(5′) was readily achieved as follows: The mobilities of the former (Ⅸ) on paper electrophoresis (acetate buffer, pH, 3.5) and thin-layer chromatography (DEAE-cellulose, 0.02M HCl) were almost identical to those of 5′-CMP (D). The ultraviolet absorption spectra curves for the product (Ⅸ) at various pH values were closely similar to those for the β-anomer but the absorption maximum exhibited a slight bathochromic shift (approx., 1 mμ) as compared with β-anomer. Furthermore, the product (Ⅸ) consumed 1 mole of metaperiodate. In NMR spectra, the signals due to the C-1′ proton of this product appeared at a lower magnetic field than that for the β-anomer[5,6] (see Table I). Recently, the usefulness of O.R.D. measurement was reported by Ulbricht[7] for the assignment of the configuration at the anomeric carbon; α-pyrimidine nucleotides show the negative Cotton effect and β-anomers the positive effect. (In the case of α-nucleotides prepared previously, almost same results were obtained with α-UMP and α-rTMP giving the negative Cotton curves. In the case of the product (α-D-CMP), it also shows the negative Cotton effect and β-anomer the positive effect.[*2])

The latter compound (Ⅷ) was identical with the authentic sample of natural 5′-cytidylic acid (5′-CMP) in its physical properties.

In the same manner α-L-5′-CMP and β-L-5′-CMP were obtained starting from L-ribose. The nucleotides thus obtained were indicated to be α-L- and β-L-compound in physical properties as shown in Table I.

In addition, 3-β-L-ribofuranosyladenine 5′-phosphate (XIII) (L-3-isoAMP) was prepared by coupling of the bromosugar phosphate (Ia) with adenine (X), according to the procedure for that of D-isomer.[8,9] When adenine (X) was allowed to react at 40° with the bromide

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[*2] Details of the ORD studies on the anomeric nucleotides will be published elsewhere.

4) T. Ukita, H. Hayatsu: J. Am. Chem. Soc., **84**, 1879 (1962).

5) T. Nishimura, B. Shimizu, I. Iwai: This Bulletin, **12**, 1471 (1964).

6) H.G. Gassen, H. Witzel: Biochim. Biophys. Acta, **95** 244 (1965).

7) T.L.V. Ulbricht, J.P. Jennings, P.M. Scopes, W. Klyne: Tetrahedron Letters, **1964**, 695.

8) M. Asai, M. Miyaki, B. Shimizu: Agr. Biol. Chem., **29**, 170 (1965); *Idem*: *Ibid.*, **31**, 319 (1967).

9) N.J. Leonard, R.A. Laursen: Biochemistry, **4**, 354 (1965).

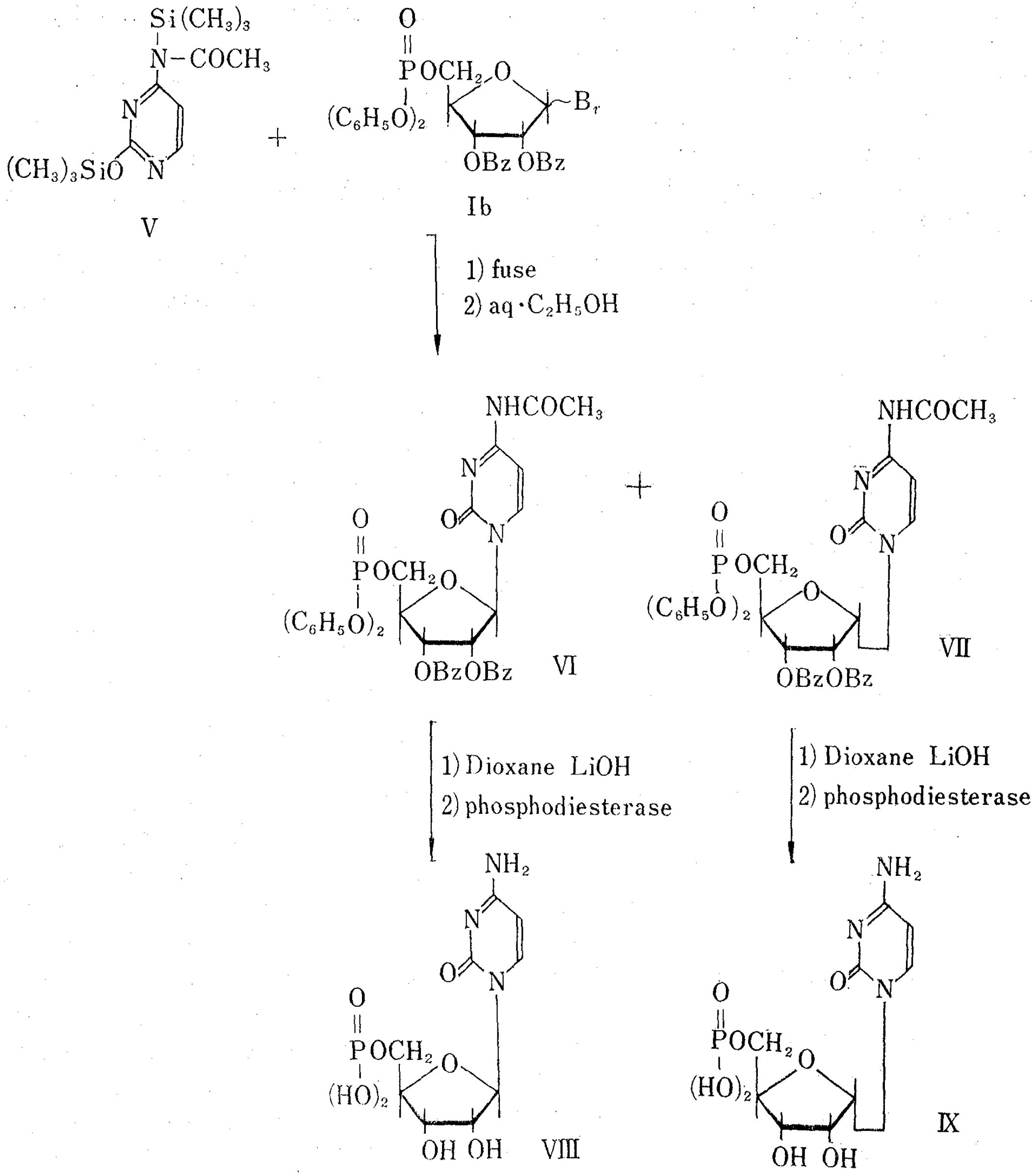

Fig. 2.

(Ia) in acetonitrile (see Fig. 1), then the protecting groups removed, by usual method, 3β-L-ribofuranosyladenine 5′-phosphate (XII) and L-5′-adenylic acid (XIV) were obtained in 14.8% and 8.8% yield based on the bromide (Ia), respectively. As shown in Table I, the former (XIII) was indicated to be 3-β-L-AMP in physical properties.

The results thus obtained are listed in Table I. Studies on biochemical activities of these nucleotides are in progress in the laboratories.

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Central Research Laboratories Sankyo Co., Ltd.
2-58, 1-chome, Hiromachi,
Shinagawa-ku, Tokyo

Bunji Shimizu (清水文治)
Akio Saito (斎藤彰夫)
Takuzo Nishimura (西村卓三)
Michiko Miyaki (宮木美知子)

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