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Synthetic Studies of Potential Antimetabolites. XII.[1] Synthesis of 4-Substituted 1-(β-D-Ribofuranosyl)-1H-imidazo[4,5-*c*]pyridines

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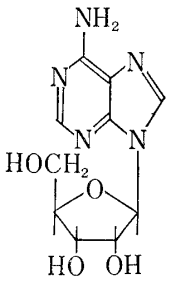
4-Hydroxy-1-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (3-deazainosine, XIV) was prepared by use of the corresponding 4-chloro derivative (IX) as a key intermediate. It was found that the reaction of IX with hydrazine afforded either 1-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (XIII) by de-chlorination or 4-hydrazino-1-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (X) depending upon conditions employed.

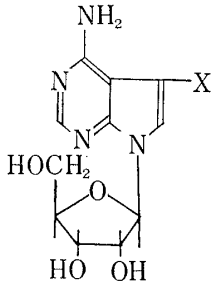
Improved synthesis of IX was achieved by an adaptation of Yamaoka, Matsuda and Aso's method. In addition to X and XIV, a number of other 4-substituted ribonucleosides and nucleotides having imidazo[4,5-*c*]pyridine (3-deazapurine) ring system, such as XII, XV, XVI and XVII were prepared.

A number of antibiotics of deaza-purine series have been isolated from various sources in recent years. Among them, viomycin,[3] tubercidin (II),[4] toyocamycin (III),[5] and formycin (IV),[6] possessing ring system isomeric or isosteric with purine are of special interest because of their structural uniqueness and their biological properties.[6,7]

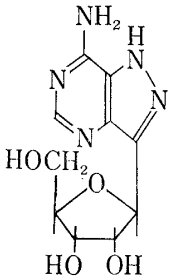
As a logical extention of our synthetic work on ribosyl derivatives of 3-deaza-purine,[8] the synthesis of 4-amino-1-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (V, 3-deaza-adenosine) and 4-hydroxy-derivative (XIV, 3-deazainosine) was undertaken by use of the corresponding 4-chloro-derivative (IX)[8] as a key intermediate. Among them, synthesis



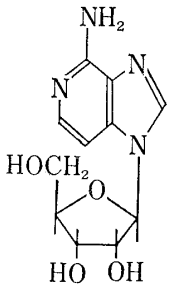
I



II : X=H
III : X=CN



IV



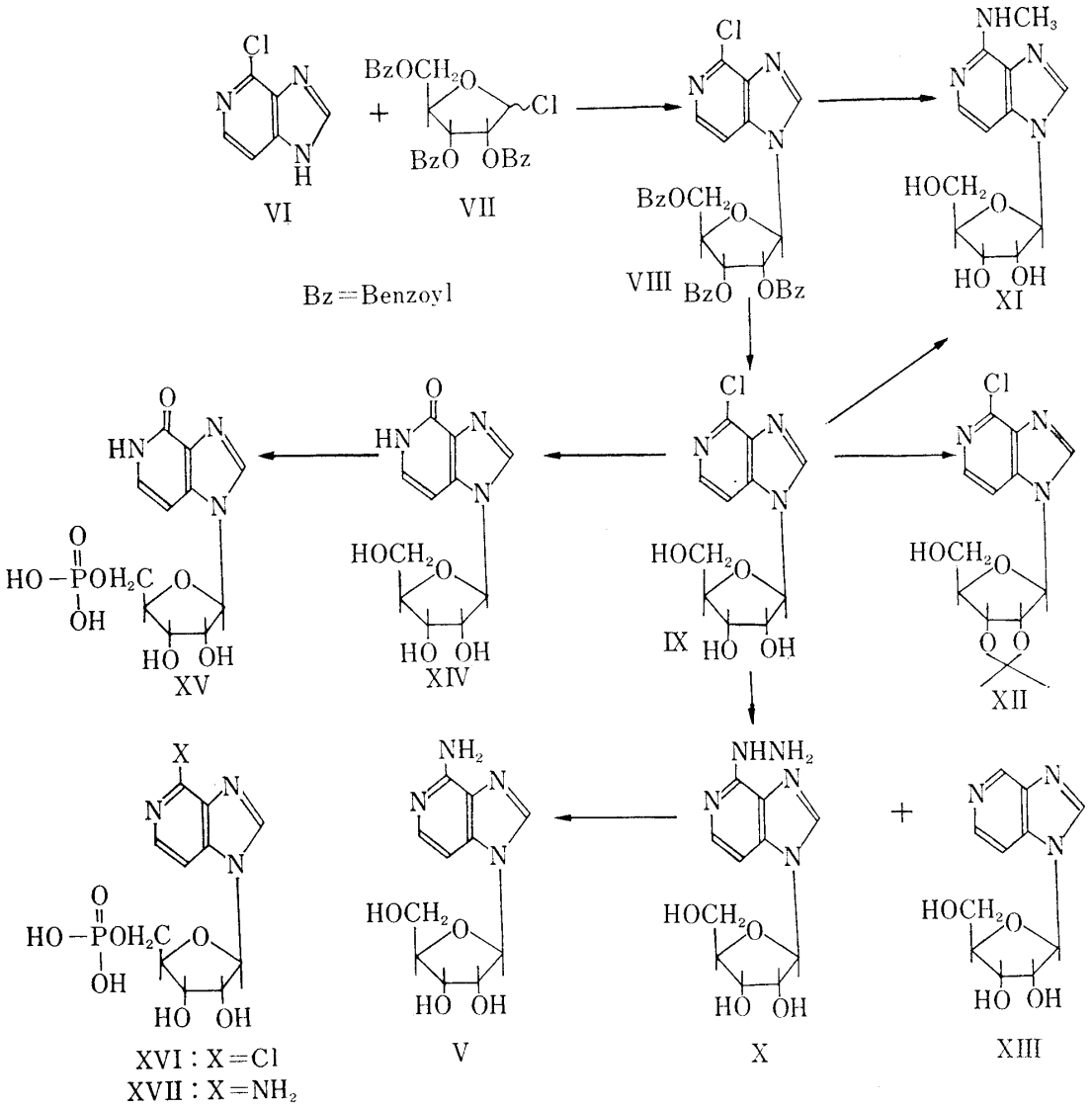
V

1) Paper XI: Y. Mizuno, T. Ueda, K. Ikeda, and K. Miura, *Chem. Pharm. Bull.* (Tokyo), **16**, 132 (1968).
2) Location: *Sapporo, Hokkaido.*
3) J.H. Bowie, A.W. Johnson, and G. Thomas, *Tetrahedron Letters*, **1964**, 863.
4) *a*) Anzai, G. Nakamura, and S. Suzuki, *J. Antibiotics* (Tokyo), **A10**, 201 (1957); *b*) Y. Mizuno, M. Ikehara, K.A. Watanabe, S. Suzaki, and T. Itoh, *J. Org. Chem.*, **28**, 3329 (1963).
5) *a*) N.H. Nishimura, K. Katagiri, K. Sato, M. Mayama, and N. Shimaoka, *J. Antibiotics* (Tokyo), **A9**, 343 (1961); *b*) K. Ohkuma, *ibid.*, **A14**, 343 (1961); *c*) E.C. Taylor and R.W. Henkes, *J. Am. Chem. Soc.*, **87**, 1995 (1965).
6) *a*) G. Koyama, K. Maeda, H. Umezawa, and Y. Iitaka, *Tetrahedron Letters*, **1966**, 597; *b*) R.K. Robins, L.B. Townsend, F. Cassidy, J.F. Garster, A.F. Lewis, and R.L. Miller, *J. Heterocyclic Chem.*, **3**, 110 (1966).
7) The biological activities of tubercidin (II) and toyocamycin (III) are well documented.[5c]
8) *a*) Y. Mizuno, T. Itoh, and K. Saito, *J. Org. Chem.*, **29**, 2611 (1964); *b*) Y. Mizuno, N. Ikekawa, T. Itoh, and K. Saito, *ibid.*, **30**, 4066 (1965).

of V has been reported by Rousseau, Townsend, and Robins during the course of this work.[9]

In this paper we report a synthesis of the 3-deazainosine (XIV) and related ribonucleosides along with a synthesis of the corresponding 5'-nucleotides.

Pertinent to the synthesis of the key intermediate (IX) is our work[8b] on gas chromatographic examination of the product distribution of the ribosylation of 4-chloro-1H-imidazo [4,5-*c*]pyridine (VI) which demonstrated that the ribosylation of VI showed a high preference for 1-isomer (VIII, 84%), rather than 3-isomer (16%). This result suggested that the reported yield of VIII (see Flow Sheet I) should be much improved provided the loss on isolation



Flow Sheet I

were reduced. This has been found the case. Thus, the condensation of VI with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride (VII) was carried out in the presence of mercuri cyanide, nitromethane serving as solvent.[10] VIII was isolated by crystallization from ethyl alcohol in satisfactory yield (47%), [10b] without the need for alumina column chromatographic separa-

9) R.J. Rousseau, L.B. Townsend, and R.K. Robins, *Biochemistry*, **5**, 756 (1966).

10) *a*) This is a procedure which has been developed recently by N. Yamaoka, K. Aso, and K. Matsuda, *J. Org. Chem.*, **30**, 149 (1965); *b*) J.A. Montgomery and K. Hewson, *J. Med. Chem.*, **9**, 105 (1965) have quite recently reported that fusion of VI with 1,2,3,5-tetra-O-acetyl-D-ribose gave a 40% yield of VIII.

tion. The direct amination of nucleoside (IX) to V was unsuccessful. Treatment of IX with aqueous ammonia in the presence of cupric sulfate[11] under conditions (160—180°, 6 hr) used for the conversion of VI to its corresponding 4-amino derivative (3-deaza-adenine) gave an intractable resinous mixture. The behavior of the 4-chloro atom as a poor leaving group in heteroaromatic nucleophilic displacement was not unexpected by analogy with the inertness of the 4-chloro atom in the pyrrolo[2,3-*d*]pyrimidine ring system.[12] This lack of reactivity of 4-chloro atom of IX suggested the use of the azide or hydrazino function as a method of introducing an amino group. The use of the hydrazino group for this purpose has been reported in purine[13] as well as the pyrimidine series.[14] However, IX failed to react with methanolic sodium azide at 120° (in a sealed tube). Treatment of IX with aqueous hydrazine gave a good yield of a de-chlorinated nucleosides, 1-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (XIII),[8,16] rather than V or X.[15] It was found that treatment of IX with anhydrous ethanolic hydrazine at 120° in a sealed tube gave a 68% yield of crystalline X. Compound X was subjected to hydrogenation over Raney nickel to afford "3-deaza-adenosine" (V) which was purified by silica gel chromatography, yield being 25% based on IX. Physical properties of our sample (V) were identical with reported properties.[9]

Reaction of VIII or IX with methylamine at 120° afforded a fair yield of XI. These result shows that, as one would expect, methylamine is better nucleophile than ammonia in the heteroaromatic nucleophilic substitution. For the preparation of 4-hydroxy-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (XIV 3-deazainosine), IX was treated with refluxing aqueous acetic acid. Under these conditions satisfactory results could not be obtained because of considerable breakdown of N-glycosidic bond. When IX was treated with acetic acid in the presence of sodium acetate, expected conversion of IX to XIV was observed. However XIV was contaminated with by-products, especially with partially acetylated XIV. Finally it was found that satisfactory results could be obtained when 4-chloro-1- (2,3,5-tri-O-acetyl-β-D-ribofuranosyl) -1H-imidazo [4,5-*c*] pyridine was treated with acetic acid in the presence of sodium acetate. De-blockng with methanolic ammonia followed by purification by the use of cellulose powder chromatography afforded a pure sample of XIV in 65% yield.

As part of our general program involving the synthesis of "deazapurine" nucleotides of potential biological interest 5'-phosphates of V, IX, and XIV were prepared: Firstly V, IX, and XIV were acetonated to the corresponding 2',3'-O-isopropylidene derivatives which in turn were phosphorylated by a Tener's general method.[17] Deblocking of the resulting phosphorylated 2',3'-O-isopropylidene derivatives was effected by treatment of formic acid or during purification by ion exchange chromatography. The 5'-phosphates obtained were completely hydrolyzed by crude snake venom 5'-nucleotidase to the corresponding nucleosides and inorganic phosphate.

11) *a*) F. Koegle, G.M. van der Want, and C.A. Salemink, *Rec. Trav. Chim.*, **68**, 1013 (1949).
12) J. Davoll, *J. Chem. Soc.*, **1960**, 131.
13) *a*) R.E. Holmes and R.K. Robins, *J. Am. Chem. Soc.*, **87**, 1773 (1965); *b*) T. Naito, K. Ueno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **12**, 951 (1964).
14) *a*) A. Albert and G.B. Barlin, *J. Chem. Soc.*, **1963**, 5156; *b*) J.F., McOmie and A.B. Turner, *ibid.*, **1963**, 5590.
15) Treatment of 2-amino-6-methylthio-9-ribofuranoside with anhydrous hydrazine gave rise to the corresponding 6-hydrazino derivative,[12b] whereas treatment of 8-bromoguanosine with aqueous hydrazine gave a good yield of 8-aminoguanosine rather than guanosine or its 8-hydrazinoderivatives.
16) A. Albert and G. Catterall, *J. Chem, Soc.*, **1967**, 1533.
17) G.M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).

Experimental[18]

Improved Synthesis of 4-Chloro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (VIII)——To an azeotropically dried suspension of 4-chloro-1H-imidazo[4,5-*c*]pyridine[11,19] (VI, 2.20 g, 14.0 mmoles) in 100 ml of dry nitromethane was added successively 3.6 g of $Hg(CN)_2$, 5.0 g of anhydrous $CaSO_4$, and an azeotropically-dried nitromethane solution (50 ml) of 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride (VII),[20] prepared from 6.84 g (13.5 mmoles) of dried 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribose. The suspension was refluxed for 4 hr with stirring and with exclusion of moisture. The reaction mixture was filtered while hot. The filter cake was extracted with two 10 ml portions of hot nitromethane. The combined filtrate and washings were concentrated to dryness. The residue (18.6 g) was dissolved in 50 ml of $CHCl_3$. The $CHCl_3$ solution was washed with five 30 ml portions of 30% KI solution, then with water, and finally dried over $MgSO_4$. The salt was filtered off and washed with $CHCl_3$. The combined filtrate and washings were again concentrated to dryness. Crystallization of the residue (8.0 g) from EtOH afforded a pure sample of VIII; Yield 4.66 g (47%), mp 110—111° (reported mp 110—102°[8]). UV λ_{max}^{EtOH} mμ: 273.5. *Rf* in solvent A: 0.83. Combustion values and spectral properties were similar to those reported for VIII. *Anal.* Calcd. for $C_{32}H_{24}O_7N_3Cl$: C, 64.27; H, 4.05; N, 7.03. Found: C, 64.25; H, 4.12; N, 7.22.

4-Chloro-1-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (IX)——To a dry methanolic solution (100 ml) of 2.45 g (3.5 moles) of VIII was added 12.3 g (124 mmoles) of cyclohexylamine. The resulting solution was kept with exclusion of moisture at room temperature for two days. The solution was then refluxed for 30 min. The solvent was removed to afford a residue. The residue was freed of residual cyclohexylamine by co-distillation with four 10 ml portions of MeOH to afford 2.35 g of gummy substrate. Crystallization from EtOH gave white needles, mp 200—201° (reported mp[7] 189—190°), $[\alpha]_D^{20°}$ −41.7 (c=1.25 in MeOH). *Rf* in solvent A: 0.30. *Anal.* Calcd. for $C_{11}H_{12}O_4N_3Cl$: C, 46.22; H, 4.22; N, 14.71. Found: C, 46.32; H, 4.23; N, 15.05.

4-Hydroxy-1-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (XIV)——Nucleoside (IX, 2.13 g, 8 mM) was treated with 80 ml of acetic anhydride in the presence of 6.56 g of NaOAc at the refluxing temperature for 30 min . After making sure that IX was almost completely converted into its corresponding 2′,3′,5′-tri-O-acetyl derivatives (2′,3′,5′-tri-O-acetylated XIV) on thin-layer chromatogram (silica gel: EtOH—$CHCl_3$ 2:1), 80 ml of glacial acetic acid was added to the resulting mixture. The solution was refluxed. The process was followed by thin-layer chromatography (silica gel: EtOH—$CHCl_3$ 2:1). Before the 2′,3′,5′-tri-O-acetylated XIV completely disappeared, 4-hydroxyimidazo[4,5-*c*]pyridine (3-deazahypoxanthine) began to appear on the chromatogram. The reaction was stopped at this stage, (it required 4.5 hr). The solution was concentrated to dryness. The residue was extracted with four 50 ml portions of EtOAc. After removal of the solvent the residue was dissolved in dry MeOH saturated with ammonia. The solution was kept at room temperature for two days. After evaporation of solvent crude product was purified by cellulose powder chromatography (350 g of cellulose: 4×76 cm): The column was washed with C_3H_7OH—H_2O (4:1) and 10 ml of eluate was collected as one fraction. Fractions ranging from 82 to 115 contained 3-deazainosine (*Rf* 0.30 in C_3H_7OH—H_2O) and were pooled. Removal of the solvent, followed by crystallization from water afforded 1.31 g (65%). mp 218—219°, $[\alpha]_D^{25°}$ −39.2 (c=0.92 in H_2O). UV $\lambda_{max}^{H_2O}$ mμ: 259; $\lambda_{min}^{H_2O}$ mμ: 227.5. pK_a, determind spectrally: 1.5. *Anal.* Calcd. for $C_{11}H_{11}O_5N_3$: C, 49.44; H, 4.87; N, 15.73. Found: C, 49.47; H, 4.81; N, 15.86.

4-Methylamino-1-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (XI)–Method A——To a solution of IX (300 mg, 1.05 mmoles) in 20 ml of absolute MeOH was introduced dry methylamine gas until 9.14 g of the amine was absorbed. The reaction mixture was heated in a sealed tube at 120° for 4 hr. The cooled solution was concentrated to dryness. The residue was dissolved in 50 ml of MeOH and the solvent removed. The process was repeated until the residue was free of methylamine. The residue (361 mg) was crystallized

18) Melting points are corrected. Ultraviolet absorption spectra were run with a Hitachi recording spectrophotometer. Paper chromatography was performed by use of the ascending technique. Solvent system employed were (a), N-butylalcohol–H_2O (86:14); (b) H_2O, adjusted to pH 10 with NH_4OH, (c) iso-C_3H_7OH—NH_4OH—H_2O (7:1:2). pK_a values were determined spectrally essentially according to D. Shugar and J.J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).

19) Y. Mizuno, T. Itoh, and K. Saito, *Chem. Pharm. Bull.* (Tokyo), **12**, 866 (1964).

20) VII was prepared essentially according to H.M. Kissmann, C.P. Pidacks, and B.R. Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955) with slight modifications: to a suspension of 6.84 g of 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribose in 250 ml of dry ether was added 5 ml of freshly distilled acetyl chloride. Through the suspension was introduced dry hydrogen chloride gas, until the ether solution was saturated with hydrogen chloride gas. The vessel was tightly stoppered, stored in ice-box (0—2°) for two weeks. Ether was removed with exclusion of moisture at room temperature. The residue was freed of a trace of hydrogen chloride by repeated co-distillation with four 20 ml portions of dry benzene. The residue was dissolved in *ca.* 50 ml of dry nitromethane and used for the subsequent step.

with charcoaling from EtOH. Yield 54 mg (18.4%), mp 153—154°. UV λ_{max}^{MeOH} mμ: 274; $\lambda_{max}^{pH\ 1}$ mμ; 268; $\lambda_{max}^{pH\ 10}$ mμ: 274. *Rf* in A: 0.60.

Method B——A solution of 5.02 (7.1 mmoles) of VIII in 20.5 g of liquid methylamine was heated in a sealed tube at 120° for 5 hr. The methylamine was removed with co-distillation with EtOH as described in Method A. The residue was dissolved in 20 ml of H_2O. The aqueous solution was concentrated to half its volume to afford crystals. Recrystallization from H_2O afforded pure sample, mp 153—154°. *Rf* in A: 0.60. $[\alpha]_D^{20}$ −60.0 (*c*=1.0 in pyridine). Yield, 1.6 g (36%). *Anal.* Calcd. for $C_{12}H_{15}O_4N_4$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.74; H, 5.74; N, 19.71.

Formation of XIII by Reaction of IX with Aqueous Hydrazine: To a solution of 57 mg of IX in 4 ml of 80% hydrazine was added three drops of acetic acid. The solution was heated in a sealed tube for 2 hr at 120°. The excess hydrazine was removed *in vacuo* and a trace of residual hydrazine was removed by repeated co-distillation with MeOH. The residue was crystallized from 98% EtOH. Yield 35 mg (76%), mp and mixed mp with an authentic sample of XIII was 198—199°. *Anal.* Calcd. for $C_{11}H_{13}O_4N_3$: C, 52.58; H, 5.22; N, 16.73. Found: C, 52.67; H, 5.40; N, 16.28.

A similar treatment of 114 mg of 4-chloro-3-(β-D-ribofuranosyl)-3H-imidazo[4,5-*c*]pyridine[8] with 5 ml of hydrazine hydrate in the presence of three drops of acetic acid afforded 71 mg of de-chlorinated nucleoside, mp and mixed mp with authentic sample of XIII was 198—200.° UV $\lambda_{max}^{pHI.3}$ mμ: 252 (5000) and 283 (7200); $\lambda_{min}^{pH1.3}$ mμ (ε): 261 (2800); $\lambda_{min}^{pH12.0}$ mμ (ε): 242 (5600) and 275 (5500); $\lambda_{min}^{pH12.6}$ mμ (ε): 257 (3100). *Rf* in B: 0.70. These spectral and chromatographic properties were identical with those of an authentic sample of XIII.[7]

4-Amino-1-(β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (3-Deaza-adenosine (V))——The followinf0 procedure was essentially that of Robins and coworkers.[9] To an ethanolic solution (100 ml) of 1.15 g (4g mmoles) of IX was added 3 ml of anhydrous hydrazine. The solution was heated in a sealed tube at 12 or 40 hr. The solution was cooled and concentrated to dryness.[21] The residue was dissolved in 20 ml of° 50% EtOH. To the solution was added 5 ml of Raney nickel, wetted with EtOH. The suspe nsion wa refluxed for 1.5 hr. Since paper chromatography of the reaction mixture at this stage still showed the presence of unreacted X,[21] a further amount of Raney nickel (3 ml) was added to the suspension and heating was continued for another 1.5 hr. Paper chromatography of the reaction mixture in solvent C showed the presence of a main spot, *Rf* 0.44, in addition to a faint spot (*Rf* 0.69) (XIII).[22] The catalyst was filtered off, and washed with a small amount of H_2O. The combined filtrate and washings were concentrated to dryness. The residue was purified by silica gel chromatography (weight of silical gel: 6 g; column 24×1.3 in diam.), solvent C serving as a developing solvent. Fifteen ml of eluate were collected as one fraction. Fractions 3—8 contained only V (on the basis of paper chromatographic examination). The combined fractions were concentrated to dryness after Norit treatment. The residue was crystallized from 95% iso-C_3H_7OH. Yield 266 mg (25%). mp 225—226°. $[\alpha]_D^{26}$ −38.6° (*c*=0.99 in H_2O). Paper chromatography: R_{Ad} in B 0.87; R_{Ad} in C 0.88; R_{Ad} in A 0.57.[23] UV $\lambda_{max}^{pH1.0}$ mμ (ε): 262 (10000) and λ_{max}^{pH7} mμ (ε): 262 (8730); $\lambda_{min}^{pH11.7}$ mμ (ε): 265 (10600); $\lambda_{min}^{pH11.7}$ mμ (ε): 235 (4150). *Anal.* Calcd. for $C_{11}H_{14}O_4N_4$: C, 49.62; H, 5.26; N, 21.05. Found: C, 49.62; H, 5.31; N, 21.12.

4-Chloro-1-(2,3-O-isopropylidene-β-D-ribofuranosyl)-1H-imidazo[4,5-*c*]pyridine (XII)——Compound (XII) was prepared by a standard procedure,[24] yield 48%. *Rf* in thin layer chromatography on silica gel using $CHCl_3$—EtOH (35:5) as eluent 0.40 (single spot), mp 173—174° (after recrystallization from EtOH). *Anal.* Calcd. for $C_{14}H_{16}O_4N_3Cl$: C, 51.61; H, 4.91; N, 12.90. Found: C, 51.53; H, 4.99; N, 12.83.

Preparation of XVI by Phosphorylation of XII——To a pyridine solution (10 ml) of XII (325.5 mg, 1 mmole) was added 4 ml of pyridine solution of 2-cyanoethyl phosphate[17] (4 mmoles). The solvent was removed *in vacuo*. The residue was dissolved in 10 ml of dry pyridine and the solvent removed. The process was repeated three times. The residue was again dissolved in 10 ml of pyridine. To the solution was added 2 g of N,N-dicyclohexylcarbodiimide (DCC). The solution was kept stirring magnetically at room temperature for 2 hr H_2O (1 ml) was added with stirring. Stirring was continued for one hr. The solution was then concentrated to dryness. To the residue was added 20 ml of H_2O and the solution was filtered. Filter-cake was throughly washed with a small amount of H_2O. The combined filtrate and washing were concentrated to dryness *in vacuo*. The residue was dissolved in 40 ml of 0.4 N LiOH solution. The solution was stirred magnetically for one hr, filtered. The filtrate was applied on the top of a column of an ion exchange column (Dowex 50H+; column: 9.5×3 cm). The column was washed with 1 liter of H_2O until the effluent was neutral. The combined eluates were adjusted to pH 6 with 0.3 Ba $(OH)_2$ solution. The solution was concentrated

21) Paper chromatography in solvent B at this stage showed the presence of a spot *Rf* 0.58 (main) and a faint spot *Rf* 0.66 (*Rf* of XIII 0.63). The main spot was cut out and extracted with H_2O. UV of the extract: $\lambda_{max}^{H_2O}$ mμ: 267.5; $\lambda_{min}^{H_2O}$ mμ: 236; λ_{max}^{pH2} mμ: 267; λ_{min}^{pH2} mμ: 236. Assuming an ε_{max} of 10000 for the product (X), the yield as estimated spectrohotometrically was 68%.

22) *Rf* values in C of IX, X, and XIII were 0.77, 0.33, and 0.69, respectively.

23) R_{Ad} is referred as to relative *Rf* value of a substance with respect of *Rf* value of adenosine (I).

24) A. Hampton and D.I. Magrath, *J. Am. Chem. Soc.*, **79**, 3250 (1957).

to 50 ml. The solution was adjusted to pH 7.5.[25] The barium phosphate precipitated was removed by centrifugation. The salt was washed with 200 ml of H_2O. The upper layer on centrifugation and washings were concentrated to 50 ml. The solution was filtered. To the filtrate was added with stirring 100 ml of EtOH. Nucleoside–5′ phosphate (XVI) which precipitated was collected by centrifugation, washed with two 5 ml portions of EtOH, twice with 5 ml of acetone and finally with 10 ml of dry ether to afford a white powder (265 mg). The barium salt of XVI was treated with 5 ml of Dowex 50 resin (H^+ form) and the resin was filtered off. The filtrate was adjusted to pH 7.8 with 1N NaOH. The solution was treated with 1% NH_4OH in aqueous EtOH (EtOH—H_2O 1:1) and was concentrated to 2 ml. To the concentrated solution was added acetone until no more precipitate was observed. The precipitates were collected by centrifugation, washed successively with EtOH, then with acetone, and finally with ehter, (18 mg). Paper chromatography in solvent A: *Rf* 0.10; *Rf* in C 0.24.[26] Paper electrophoresis in NH_4HCO_3 buffer (pH 7.5) showed that the nucleotide migrated 10.8 cm when 5′–AMP showed 10.5 cm $\varepsilon(P)_{260}=6970$.[27] UV $\lambda_{max}^{H_2O}$ $m\mu$ (ε): 256 (7000), 266 (shoulder), and 274 (shoulder).

3-Deaza-adenosine-5′phosphate——To a suspension of 3–deaza–adenosine (V, 799 mg, 3mM) in 85 ml of acetone was added 5.67 g of *p*–toluenesulfonic acid and 0.312 g (3 mM) of 2,2–dimethoxypropane. The mixture was stirred at room temperature. After 30 min, voluminous precipitate was formed. Stirring was continued for 15 hr. The reaction mixture was poured into a solution of 20 g of $NaHCO_3$ in ice–water (500 ml). After making sure that the solution was alkaline the solution was concentrated to dryness. The residue was extracted with five 120 ml portions of refluxing $CHCl_3$. Removal of the solvent, followed by crystallization from EtOH gave 280 mg (32%) of 2′,3′–O–isopropylidene derivative. TLC (silica gel, EtOH—$CHCl_3$ 2:1) 0.44. *Rf* value of 3–deaza–adenosine in the same conditions 0.23. To a solution of 274 mg (0.896 mM) of 2′,3′–O–isopropylidene–3–deaza–adenosine in 10 ml of dry pyridine was added 3.6 ml of pyridine solution of 2–cyanoethyl phosphate (whose concentration was 1 mM in 1 ml of pyridine). Pyridine was removed *in vacuo*. The residue was dissolved in 10 ml of pyridine and the solution was concentrated to dryness *in vacuo*. The process was repeated three times. The residue was again dissolved in 20 ml of dry pyridine. DCC (2.5 g) was added with stirring to the solution. The solution was kept at room temperature for 24 hr and then 0.5 g of DCC was again added. The mixture was kept for further 45 hr H_2O (2 ml) was added to the reaction mixture. After one hr of stirring, the precipitate was removed and the filtrate was concentrated to dryness. The residue was dissolved in 20 ml of H_2O. Residual dicyclohexylurea was extracted with three 20 ml portions of ether. Aqueous layer was filtered. The solution was rendered alkaline with 2N NH_4OH and refluxed for 2 hr. After cooling the solution was concentrated to dryness. The residue was dissolved in 10 ml of 98% formic acid. The solution was kept at room temperature for 3 hr. On the basis of *Rf* values and periodate oxidation, it was found that deaceonation had took place by the above procedure. The solution was concentrated to dryness until the residue was freed of formic acid. The final residue was dissolved in 10 ml of H_2O. The solution was adjusted to PH 8. At the pH total optical density of the solution was 8180. The solution was applied to column of Dowex 1 (1×8) chloride form, 200—400 mesh, 1.8×23 cm). The column was washed with a linear gradient of 1 liter of H_2O and 1 liter of 0.5 M LiCl. Each 15 ml of eluate was collected as one fraction. Fractions ranging from 75 to 84 were pooled (TOD: 4950, 56%). The combined fractions were concentrated to a volume of 10 ml. The solution was filtered. EtOH (150 ml) was added to the filtrate. The precipitate formed was collected by centrifugation. The precipitate was successively washed with EtOH, acetone and ether. 130 mg of white powder were obtained (41%). *Rf* (iso-C_3H_7OH–NH_3–H_2O 7:1:2) 0.12. Paper electrophoresis (0.05 M triethylammonium bicarbonate buffer, 700v, 2.5 mA, 1.5 hr) 11.3 R_{AMP} 0.97. UV was quite the same as those of 3–deaza–adenosine. ε (P)=9850. The sample was free of inorganic phosphate.

3–Deaza–inosine–5′ Phosphate: To a suspension of XIV (710 mg, 2.66 mM) in 40 ml of acetone was added 10 ml of 2,2–dimethoxypropane and then 4.58 g of *p*–toluenesulfonic acid. The suspension was stirred for 2 days at room temperature. On the TLC (EtOH—$CHCl_3$ 35:1, silica gel) some of the starting material remained unreacted. Acetone (10 ml) and 1.0 g of *p*–toluenesulfonic acid was added. The solution was kept for another 2 days. The reaction mixture was poured into an aqueous solution of 20 g of $NaHCO_3$. After making sure that the mixture was slightly basic, the solution was concentrated to dryness. The residue

25) The solution at this stage contained a product *Rf* in solvent C 0.23. The spot *Rf* 0.23 on the paper chromatogram could be detected under the ultraviolet lamp and by the Hanes and Isherwood reagent for phosphorous (C.S. Hanes and F.A. Isherwood, *Nature*, **164**, 1107 (1953)) as well as by the metaperiodate reagent.

26) *Rf* value in solvent A and C of adenosine–5′ phosphate (5′–AMP) were 0.30 and 0.11, whereas *Rf* values in the same solvents of IX were 0.64 and 0.61, respectively.

27) a) $\varepsilon(P)_{260}$ A/C.D where ε, A, and are molar extinction coefficient, optical density, and internal cell length in centimeters; C is measured in gramatoms of phosphorous per liter; b) also see G.H. Beaven, E.R. Holiday, and E.A. Johnson, "Nuclic Acids," E. Chargaff and J.N. Davidson, Ed., Academic Press, Inc., New York 1955, pp. 495—517; c) Since molar extinction coefficient of IX was *ca.* 7000, this value showed that our sample was quite pure.

was codistilled with benzene. The residue was refluxed with the 50 ml portions of $CHCl_3$. The solvent was removed *in vacuo* to give a gummy substance: *Rf* (silica gel EtOH—$CHCl_3$ 35:10) 0.72 (single spot). The 2′,3′-O-isopropylidene derivative was dissolved in dry pyridine. To a pyridine solution was added 8 mM of 2-cyanoethyl phosphate (pyridinium salt). The residue was dissolved in 20 ml of pyridine. To the solution was added 4 g of DCC. The solution was stirred magnetically for 4 days. H_2O was added. The dicyclohexylurea precipitated was filtered off. The filtrate was concentrated to dryness. The residue was dissolved in 20 ml of H_2O. The aqueous solution was extracted with 20 ml portions of ether. The aqueous layer was concentrated to dryness. The residue was dissolved in 10 ml of 98% formic acid, and then formic acid was removed *in vacuo*. The residue was treated with 9 M NH_4OH for 2 hr at the refluxing temperature. The solution was concentrated to dryness. Paper electrophoresis of the residue (NH_4OAc buffer, pH 7.1, 700 volt, 5.5 mA, 2 hr) 9.4 cm R_{AMP} 1.2 with some tailing. TOD of the solution at pH 7.1 (259 mμ) 7660. The residue was dissolved in 1 ml of H_2O and was applied on the top of Dowex 1 column (1×8, 200—400 mesh, Cl-form) (1.6×29 cm). The column was washed with H_2O, then a linear gradient (500 ml of H_2O and 500 ml of 0.5 LiCl), then finally avlinear gradient of 0.5M LiCl and 500 ml of 1M LiCl. 10 ml of eluate was collected as one fraction. The fraction eluted by a linear gradient (500 ml of H_2O and 0.5 M LiCl) was pooled, and concentrated to 30 ml. To the resulting solution was added 60 ml of EtOH. The solution was kept at ice-box and the precipitate formed was collected by centrifugation, and washed with EtOH (two times), acetone (two times) and finally ether (two times). White powder (67mg) was obtained (Yield 5%). Paper electrophoresis (triethylammonium bicarbonate buffer pH 7.1, 700 volt, 2.5 mA, 1.5 hr) R_{AMP} 0.97. ε (P) 10000 at 259 mμ (at pH 7). The sample was free of inorganic phosphate.

Test of the Nucleoside 5′-Phosphates (XV, XVI, and XVII) as substrate for Venom 5′-nucleotidase.[28] The test conditions were quite similar to those reported before.[28]

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28) Y. Mizuno, M. Ikehara, T. Ueda, A. Nomura, E. Ohtsuka, F. Ishikawa, and Y. Kanai, *Chem. Pharm. Bull.* (Tokyo), **9**, 338 (1961).