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Synthesis of 2-Methoxyinosine and Its 5′-Phosphate

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2,6-Dichloro-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (I), when treated with 0.5N barium hydroxide, afforded 2-chloroinosine (II), which was also obtained by reaction of I with 2 moles of methanolic sodium hydroxide followed by alkaline hydrolysis *via* 6-methoxy derivative (IV). Compound II was converted with sodium methoxide to 2-methoxyinosine (V). Alternatively, V was prepared by treating of I with the excess of methanolic sodium hydroxide. Compound V was allowed to react with acetone in the presence of 2,2-dimethoxypropane and then phosphorylated with phosphoryl chloride to give, after deacetonation, 2-methoxyinosine 5′-phosphate (VIII). The synergistic flavoring strength of VIII with monosodium L-glutamate (MSG) was evaluated as an analog of 2-methylthioinosine 5′-phosphate or xanthosine 5′-phosphate. Similarly, 2-chloroinosine 5′-phosphate (IX) was synthesized from II and its flavoring strength was evaluated.

Our interest in the relationship of structural features of purine nucleotides to flavoring activity has led us to synthesize some purine 5′-nucleotides.[2] Among them, 2-methylthioinosine 5′-phosphate[2a] was shown to have an extremely strong flavoring activity in synergistic effect with monosodium L-glutamate (MSG). In view of the facts that the methylthio group at 2-position enhances a flavoring strength and that xanthosine 5′-phosphate (5′-XMP) capable of keto–enol tautomeric forms at 2-position has a very weak flavoring activity (Table I), it became desirable to prepare 2-methoxyinosine 5′-phosphate (VIII), that is, 2-methoxyinosine 5′-phosphate, as an analog of 2-methylthioinosine 5′-phosphate or 5′-XMP.

The present work describes the synthesis of 2-methoxyinosine (V) and its 5′-phosphate VIII. To this end, the most attractive pathway seemed to be through 2-chloroinosine (II). This compound has been prepared by three methods, such as by deamination of 2-chloroadenosine derivative with nitrous acid,[3] by diazotization of 2-amino-9-β-D-ribofuranosyl-6-purinethione in the presence of concentrated hydrochloric acid followed by treatment with alkaline hydrogen peroxide,[4] and by the alkaline hydrolysis of 2,6-dichloro-9-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)purine.[5] In these cases, however, considerable difficulty seems to have been encountered in isolating II as pure crystals.

Ishido, *et al.*[6] have previously synthesized 2,6-dichloro-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine (I) by the fusion method. On repeating this reaction, I identical with an authentic sample[4,6] was obtained in 81% yield. In order to convert I to II, alkaline conditions were examined which were sufficiently mild so as not to prevent hydrolysis at 2-position of I. When I was treated with 0.5N barium hydroxide at room temperature for 24 hr, II accompanied by some by-products was formed and isolated by column chromatographies on

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[1] Location: *Suzuki-cho, Kawasaki.*

[2] *a*) A. Yamazaki, I. Kumashiro, and T. Takenishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 338 (1968); *b*) *Idem, ibid.*, **16**, 1561 (1968); *c*) *Idem, ibid.*, in press; *d*) S. Suzaki, A. Yamazaki, A. Kamimura, K. Mitsugi, and I. Kumashiro, *ibid.*, in preparation.

[3] J. Davoll, B. Lythgoe, and A.R. Todd, *J. Chem. Soc.*, **111**, 1685 (1949).

[4] J.F. Gerster and R.K. Robins, *J. Org. Chem.*, **31**, 3258 (1966).

[5] K. Imai, A. Nohara, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **12**, 1377 (1966).

[6] Y. Ishido, A. Hosono, S. Isome, A. Maruyama, and T. Sato, *Bull. Chem. Soc. Japan*, **37**, 1389 (1964).

decolorizing resin and ion exchange vresin. Despite such relatively mild conditions, the yield of II was only 32%. In an attempt to avoid side reactions, it seemed possible that, by changing the more susceptible 6-chlorine atom to alkoxy group followed by hydrolyzing in alkaline solution, better yield of II might be obtained. The reaction of I with 2 moles of sodium hydroxide in refluxing aqueous methanol gave 6-methoxy-2-chloro-9-β-D-ribofuranosylpurine (IV), which in turn was hydrolyzed with 1N sodium hydroxide to yield II in good yiled. When less than 2 moles of sodium hydroxide was employed, the substitution at 6-position was incomplete. On the other hand, an increase in the molar ratio of sodium hydroxide markedly decreased the yield of II: in this case, V and/or 2,6-dimethoxy-9-β-D-ribofuranosylpurine were detected in the reaction mixture. It is noteworthy that using 2 moles of sodium hydroxide was optimum for effecting the replacement of 6-chlorine atom by methoxy group. The compound II was isolated in 54% yield based on I. Interestingly, the same conditions were employed between I and ethanolic sodium hydroxide to give not 6-ethoxy derivative but II.

Attention in our laboratories has been focused on the synthesis[7] of guanosine (III) which is the key substance for preparing guanosine 5′-phosphate (5′-GMP). 2-Mercaptoinosine[7b] previously reported was found to be an excellent intermediate for producing III. Compound II was also of interest to us since it would be a versatile intermediate to III or 2-substituted inosines. The compound was then aminated with ammonia in a sealed tube at 150° for 3 hr, giving III in quantitative yield. When the reaction of I with methanolic sodium hydroxide followed by amination was carried out without isolating the intermediates, there was obtained III in an overall yield of 47%.

Treatment of II with sodium methoxide provided the crystalline V,[4] mp 180—181°, in 66% yield. This was also prepared by reaction of I with methanolic sodium hydroxide in a sealed tube at 100° for 4 hr. The compounds, II and V, were treated with acetone in the presence of 2,2-dimethoxypropane and dry hydrochloric acid[8] to yield 2′,3′-O-isopropylidene-2-chloroinosine (VI) and 2′,3′-O-isopropylidene-2-methoxyinosine (VII), in 61 and 58% yields, respectively. These compounds were phosphorylated[9] with phosphoryl chloride in trimethyl phosphate to afford, after deacetonation, 2-chloroinosine 5′-phosphate (IX) and VIII, respectively, which were isolated as pure crystals.

Table I shows the values of the synergistic flavoring strengths of some 5′-nucleotides with MSG and the values are given by a comparison with the flavoring activity of inosine 5′-phosphate (5′-IMP). Both VIII and IX were found to have flavoring activities and, in synergistic

Table I. Synergistic Flavoring Strengths of 5′-Nucleotides with MSG

Nucleotide	Relative strength
5′-IMP·2Na·7.5H_2O	1.0
5′-GMP·2Na·7H_2O	2.3[10]
5′-XMP·2Na·2.8H_2O	0.6[11]
2-CH_3S-5′-IMP·2Na·6H_2O	8.0[12]
VIII·2Na·H_2O	4.2[13]
IX·Na·NH_3·$\frac{3}{2}H_2O$	3.1[13]

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[7] *a*) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **32**, 1825 (1967); *b*) *Idem, ibid.*, **32**, 3032 (1967); *c*) I. Kumashiro, A. Yamazaki, T. Meguro, T. Takenishi, and T. Tsunoda, *Biotechnol. Bioeng.*, **10**, 303 (1968).

[8] Y. Tsuchiya, T. Takenishi, T. Kato, I. Kumashiro, and T. Mori, Japan. Patent 12345 (1964).

[9] M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Letters*, **1967**, 5065.

[10] M. Ohara, T. Ninomiya, S. Ikeda, S. Yamaguchi, and T. Yoshikawa, *J. Agr. Chem. Soc. Japan*, **40**, 169 (1966).

[11] Private communication by Miss S. Yamaguchi.

[12] S. Yamaguchi, T. Yoshikawa, S. Ikeda, and T. Ninomiya, *Agr. Biol. Chem.*, **32**, 797 (1968).

[13] S. Yamaguchi, T. Yoshikawa, S. Ikeda, and T. Ninomiya, *Agr. Biol. Chem.*, in press.

effect, showed higher activities than that of 5′-GMP. The replacement of 2-hydroxy group by methoxy gorup in 2-methylthioinosine 5′-phosphate caused a considerable decrease of the activity. However, VIII exhibited a far higher activity than that of 5′-XMP. The high activity of VIII, as compared with 5′-XMP, is somewhat surprizing. Accordingly, it seems that there is a differenece between the two compounds in the configuration of the nucleotide molecules. From the fact that all the synthesized 2-substituted inosine 5′-phosphates have flavoring activities more or less, it might be concluded that the 2-position of 5′-purine nucleotide is not a functionally important part for the site of a taste receptor.

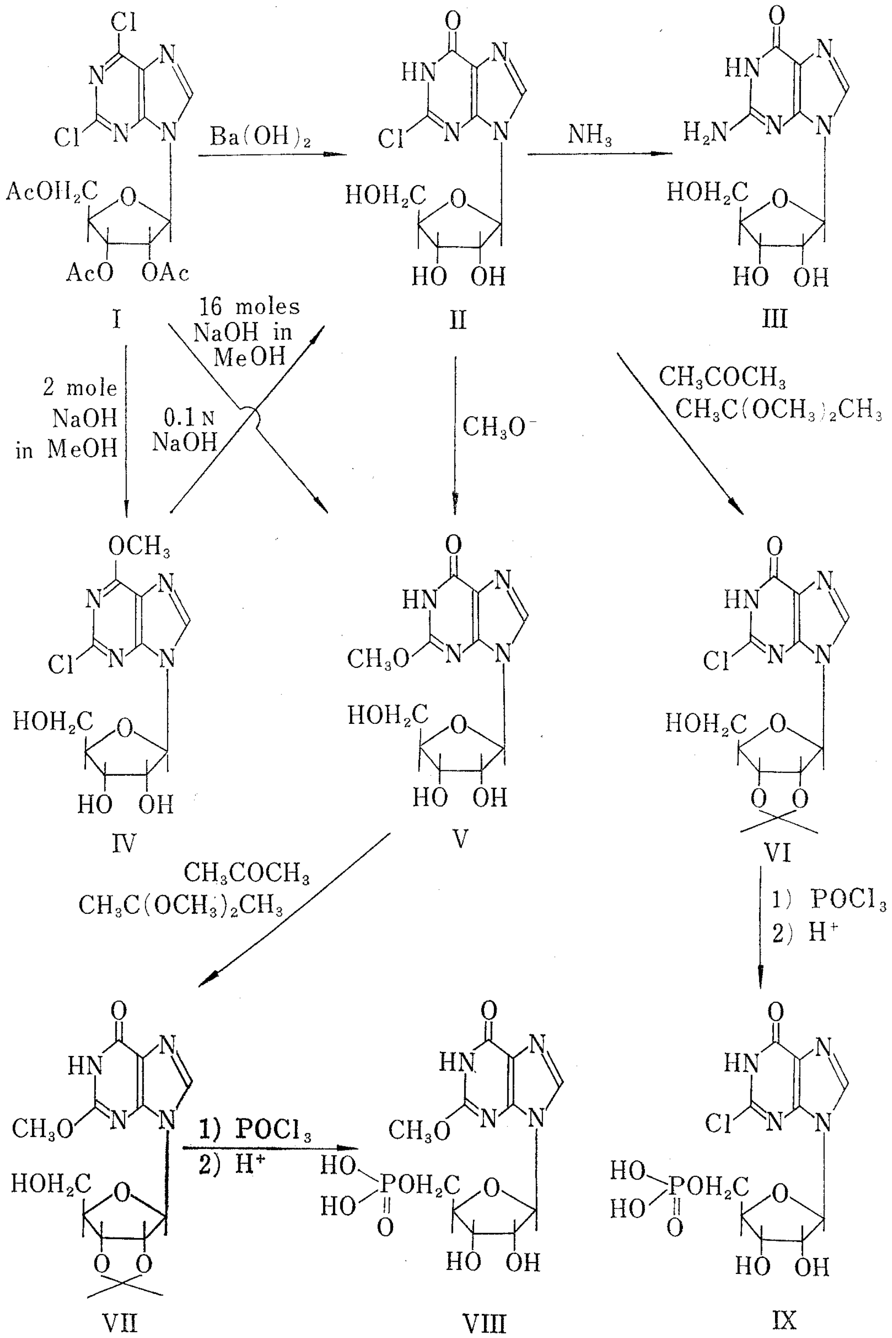

Chart 1

Experimental[14]

2,6-Dichloro-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine (I)——A mixture of 2,6-dichloropurine[15] (5.76 g), 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose[16] (10 g), and anhydrous zinc chloride (0.2 g) was heated at the temperature of from 130 to 135° for 20 min under aspirator vacuum. The reaction mixture was cooled to room temperature, dissolved in 120 ml of absolute methanol, and triturated. The crystalline solid that precipitated was collected and recrystallized from methanol affording 11 g (81%) of I. The melting point and specific rotation were identical with those reported;[4] mp 158—159°; $[\alpha]_D^{25}$ −3.0° (c=1, chloroform).

2-Chloroinosine (II)——A) Fifty grams of I was added to a solution of barium hydroxide (141 g) in water (894 ml) and the mixture was stirred at room temperature for 50 hr. After the solution was adjusted to pH 2 with sulfuric acid, the resulting precipitate, barium sulfate, was removed by filtration. The filtrate was passed through a column of about 1 liter of decolorizing resin,[17] which was adjusted to pH 2 prior to adsorption. The column was washed with water until eluate became pH 4, and then the product was eluted with 5N ammonium hydroxide. Fractions containing ultraviolet absorbing material were collected and the combined eluate was evaporated under reduced pressure to *ca.* 200 ml. The solution was applied to a column of about 500 ml of Amberlite IR-120 (H^+ form). On eluting with water, the desired product II emerged in the fraction of pH 3.5 to 4. Concentration of the eluate afforded a crystalline solid, which was recrystallized from water with charcoal treatment to give 10 g (32%) of white crystals; mp 123°; *Rf*: 0.58 (solvent A); 0.38 (solvent B); UV $\lambda_{max}^{pH\,1}$ mμ (ε): 253.5 (10200); $\lambda_{max}^{pH\,13}$ mμ (ε): 258 (11400). *Anal.* Calcd. for $C_{10}H_{11}O_5N_4Cl$: C, 39.68; H, 3.66; N, 18.51. Found: C, 39.62; H, 4.06; N, 18.36.

In another run, compound II, adsorbed on a column of decolorizing resin as described above, was eluted with 5N ammonium hydroxide. The eluate was concentrated *in vacuo* to give the brown residue, which was crystallized from methanol. Four crystallizations furnished the ammonium salt of II, mp 188—188.5° (decomp.). This compound gave a brown precipitate with Nessler's reagent. *Anal.* Calcd. for $C_{10}H_{11}O_5N_4Cl \cdot NH_3$: C, 37.56; H, 4.41; N, 21.90; Cl, 11.09. Found: C, 38.05; H, 4.53; N, 21.51; Cl, 10.65.

B) Compound I (4.47 g) was treated with methanolic sodium hydroxide in a manner similar to that described for the preparation of IV. After reaction, the mixture was concentrated *in vacuo*, and to this, 80 ml of 1N sodium hydroxide was added. After being refluxed for 20 min, the solution was adjustded to pH 2 with concentrated hydrochloric acid and applied to columns of decolorizing resin and subsequent Amberlite IR-120 as described for A. Concentration of the eluate followed by crystallization gave 1.6 g (54%) of pure crystals. This was identical with that obtained by method A.

2-Methoxyinosine (V)——A) To a solution of 80 ml of 2N methanolic sodium hydroxide was added 4.47 g (10 mmoles) of I. The mixture was heated in a sealed tube at 100° for 4 hr, and then concentrated *in vacuo* to give a residue, which was dissolved in a small amount of water. The solution was adjusted to pH 2 and passed through a column of decolorizing resin (500 ml). The column was washed with water, the product was eluted with 5N ammonium hydroxide until effluent became free from ultraviolet absorbing material, and the effluent was concentrated to dryness *in vacuo*. After the residue was dissolved in a small amount of water, the solution was applied again to a column of 100 ml of Amberlite IR-120 (H^+ form). After washing, the product was eluted with 1N ammonium hydroxide. The fractions containing ultraviolet absorbing material were collected and evaporated to dryness *in vacuo*. The residue was crystallized from water, giving 745 mg (25%) of V. Additional recrystallization afforded an analytically pure crystal; mp 180—181°,[19] *Rf*: 0.53 (solvent A); 0.35 (solvent B); UV $\lambda_{max}^{pH\,1}$ mμ: 253; $\lambda_{max}^{pH\,13}$ mμ: 261. *Anal.* Calcd. for $C_{11}H_{14}O_6N_4$: C, 44.30; H, 4.73; N, 18.79. Found: C, 43.84; H, 4.66; N, 18.58.

B) Two grams of II was added to a solution of sodium methoxide prepared from 1.53 g of sodium in 80 ml of absolute methanol. The solution was refluxed for 1 hr, diluted with one volume of water, and neutralized by adding Amberlite IR-120 (H^+ form) portionwise with stirring. The resin was removed by filtration and the filtrate was concentrated to dryness. The residue was crystallized from water to give pure crystals, which were collected by filtration and dried, affording 1.3 g (66%) of V.

6-Methoxy-2-chloro-9-β-D-ribofuranosylpurine (IV)——To a solution of 40 ml of 0.5N methanolic sodium hydroxide and 16 ml of water, 4.47 g (10 mmoles) of I was added and the mixture was heated in a sealed tube at 100° for 3 hr. The solution was then evaporated *in vacuo* to give a solid, which was dissolved

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14) All melting points are uncorrected. Ultraviolet absorption spectra were taken with a Hitachi EPS-2 automatic recording spectrophotometer, infrared absorption spectra were measured with a Jasco Model IR-S spectrophotometer, nuclear magnetic resonance (NMR) spectar were measured with a Varian A-60 using tetramethylsilane as internal standard, and specific rotations were measured with a Jasco Model DIP-SL automatic polarimeter. All chromatographies were performed on Toyo No. 51 filter paper by the ascending technique. Solvent system: A, n-PrOH–NH_3 (28%)–H_2O (20:12:3, v/v); B, n-BuOH–AcOH–H_2O (4:1:1, v/v); C, iso-PrOH–sat. $(NH_4)_2SO_4$–H_2O (2:79:19, v/v).

15) H. Kawashima and I. Kumashiro, *Bull. Chem. Soc. Japan*, **40**, 639 (1967).

16) H. Zinner, *Ber.*, **83**, 153 (1950); *idem*, *ibid.*, **86**, 817 (1953).

17) This decolorizing resin was prepared in our laboratories by copolymerization of metaphenylenediamine, resorcin, and formalin. It is of interest to note that the decolorizing resin adsorbs not only nucleotides[18] but also limited kinds of nucleosides, *e.g.* II and V.

18) Y. Tsuchiya, I. Hayashi, T. Kato, M. Yoshikawa, T. Mori, and S. Miyasaka, Japan. Patent 12343 (1964).

19) No melting point is given in the literature.[4]

in water. The solution was neutralized with Amberlite IR-120 with stirring and concentrated under reduced pressure. The residue was crystallized from absolute methanol. Three times recrystallizations from the same solvent afforded an analytically pure sample; yield 885 mg (28%); mp 124—125° (decomp.); UV $\lambda_{\max}^{\mathrm{pH}\,1}$ mμ: 261.5; $\lambda_{\max}^{\mathrm{pH}\,13}$ mμ: 261.5. *Anal.* Calcd. for $C_{11}H_{13}O_5N_4Cl$: C, 41.71; H, 4.14; N, 17.69. Found: C, 41.20; H, 3.93; N, 17.68.

Guanosine (III)——Compound II (1 g) was added to 40 ml of methanol saturated with ammonia at 0° and the mixture was heated in a sealed tube at 150° for 3 hr. Paper chromatography showed a single spot of III. The spot was cut and eluted with 0.1N sodium hydroxide. The measurement of the absorbancy of the eluate showed the formation of III in almost quantitative yield. The above reaction mixture was concentrated to give a crystalline mass, which was recrystallized from water, providing 0.7 g (74%) of pure product. *Anal.* Calcd. for $C_{10}H_{13}O_5N_5 \cdot H_2O$: C, 39.89; H, 5.02; N, 23.25. Found: C, 40.21; H, 5.32; N, 23.65.

2′,3′-O-Isopropylidene-2-chloroinosine (VI)——Compound II (5 g, 16.5 mmoles) was added with stirring to a solution of acetone (44 ml), ethanol (20 ml), 2,2-dimethoxypropane (10 g), and 9.5N ethanolic hydrochloric acid (11.5 ml). The mixture was allowed to react for 1 hr, poured into 200 ml of 5N ammonium hydroxide, and concentrated *in vacuo* to *ca.* 30 ml, during which time crystallization occurred. The resulting crystals were collected and recrystallized from water to yield 3.3 g (58%) of white needles; mp 192—193° (decomp.); *Rf*: 0.79 (solvent A); 0.71 (solvent B); UV $\lambda_{\max}^{\mathrm{pH}\,1}$ mμ (ε): 253.5 (12200); $\lambda_{\max}^{\mathrm{pH}\,13}$ mμ (ε): 258 (13200). *Anal.* Calcd. for $C_{13}H_{15}O_5N_4Cl$: C, 45.55; H, 4.38; N, 16.35. Found: C, 45.61; H, 4.62; N, 16.14.

2′,3′-O-Isopropylidene-2-methoxyinosine (VII)——To a mixture of V (4.5 g, 15.2 mmoles), acetone (40 ml), ethanol (9.9 ml), and 2,2-dimethoxypropane (9 g), 21% (w/w%) of ethanolic hydrochloric acid (18.2 g, 102.3 mmoles) was added with stirring at room temperature. Within a few minutes, the mixture became clear. After 1 hr, the yellowish solution was poured into 200 ml of 5N ammonium hydroxide with stirring. Evaporation of the solvent *in vacuo* left a residue, 20 ml of water was added, and the product was extracted with three 50 ml portions of chloroform. The combined extracts, dried with sodium sulfate, were evaporated *in vacuo* to dryness. The residue was dissolved in a small amount of water and crystallized, giving 4.5 g (61%) of a white crystal; mp 89—90°, 183—184° (double melting points); *Rf*: 0.75 (solvent A); 0.69 (solvent B); $\lambda_{\max}^{\mathrm{pH}\,1}$ mμ (ε): 253.5 (10600); $\lambda_{\max}^{\mathrm{pH}\,13}$ mμ (ε): 261 (10300); NMR (in pyridine) ppm: 3.95 (3H, singlet, $OC\underline{H}_3$). *Anal.* Calcd. for $C_{14}H_{18}O_6N_4 \cdot 1/2H_2O$: C, 48.42; H, 5.48; N, 16.14. Found: C, 48.33; H, 5.59; N, 16.63.

2-Methoxyinosine 5′-Phosphate (VIII)——phosphoryl chloride (3.65 ml, 39.2 mmoles) was added to 20 ml of trimethyl phosphate cooled at −10° in a three-necked flask equipped with a thermometer and a drying tube. To this, 5.2 g (15.7 mmoles) of VII was added portionwise and the mixture was stirred at −5° for 3 hr. Within about 30 min, it became clear. The solution was then poured into stirred ice water (800 ml) to decompose the unreacted phosphoryl chloride, adjusted to pH 1.5 with alkaline solution, and heated at 70° for 40 min to deacetonate. The solution was adjusted to pH 2 with alkaline solution and passed through a column (3 × 100 cm) of decolorizing resin. The column was washed with water and the product was eluted with 0.5N ammonium hydroxide. An aliquot of the eluate exhibited a single spot on a paper chromatogram in solvent C. Evaporation of the above eluate under reduced pressure gave a solid, which was dissolved in 20 ml of water. Addition of five volume of ethanol afforded the gummy ammonium salt of VIII, which, after filtration, was dissolved in 100 ml of water. The solution was adjusted to pH 8 with 2N sodium hydroxide and refluxed for 10 min. This procedure was repeated several times until the pH became 8. The solution was concentrated to *ca.* 20 ml and two volume of ethanol was added to give a crystalline product, which was recrystallized from aqueous ethanol. The product was dried and weighed 4.3 g (54.7%); mp 130—135° (decomp.); $[\alpha]_{\mathrm{D}}^{25}$ −27.5° (c=1, water); *Rf*: 0.01 (solvent A); 0.18 (solvent B); 0.46 (solvent C); UV $\lambda_{\max}^{\mathrm{pH}\,1}$ mμ (ε): 253—254 (9200); $\lambda_{\max}^{\mathrm{pH}\,6}$ mμ (ε): 248 (10000), 264 (inflection); $\lambda_{\max}^{\mathrm{pH}\,13}$ mμ (ε): 261 (11900). NMR (in deuterium oxide) ppm: 4.3 (3H, singlet, $OC\underline{H}_3$). *Anal.* Calcd. for $C_{11}H_{13}O_9N_4Na_2P \cdot H_2O$: C, 30.00; H, 3.41; N, 12.73; P, 7.05. Found: C, 30.33; H, 3.61; N, 12.56; P, 6.95.

2-Chloroinosine 5′-Phosphate (IX)——Compound VI (2.2 g) wastreated with phosphoryl chloride (1.8 ml) in trimethyl phosphate (10 ml), and after deacetonation, the reaction mixture was worked up in the same manner as that described for the preparation of VIII. The product, eluted with 0.5N ammonium hydroxide from a column of decolorizing resin, was dissolved in 50 ml of water. The solution was adjusted to pH 7.5 and heated to reflux for 10 min. This procedure was repeated and two volume of ethanol was added. On cooling, white crystals formed. Yield 0.95 g (32.9%); mp 164—167° (decomp.); $[\alpha]_{\mathrm{D}}^{25}$ −29.0° (c=1, water); *Rf*: 0.03 (solvent A); 0.02 (solvent B); 0.40 (solvent C); UV $\lambda_{\max}^{\mathrm{pH}\,1}$ mμ (ε): 253 (11500); $\lambda_{\max}^{\mathrm{pH}\,6}$ mμ (ε): 255 (11400); $\lambda_{\max}^{\mathrm{pH}\,13}$ mμ (ε): 258 (13400). *Anal.* Calcd. for $C_{10}H_{14}O_8N_5ClNaP \cdot 3/2H_2O$: C, 26.76; H, 3.79; N, 15.61; P, 6.91. Found: C, 26.46; H, 4.26; N, 15.64; P, 6.92.

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