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Synthesis of Some N^1-Methyl-2-substituted Inosines and Their 5′-Phosphates

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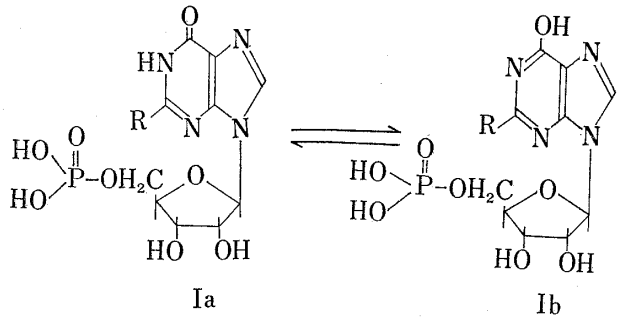
Some isopropylidene derivatives of N^1-methyl-2-substituted inosines were synthesized from 2′,3′-O-isopropylidene-N^1-methyl-2-methylthioinosine (VII). Phosphorylation of the isopropylidene derivatives (VIII and X) of N^1-methylinosine and N^1-methylguanosine followed by acidic treatment afforded N^1-methylinosine 5′-phosphate (XVIII) and N^1-methylguanosine 5′-phosphate (XIX), respectively, the flavoring activities of which were qualitatively investigated.

In the previous paper,[2] we have reported on the synthesis of some 2-substituted inosine 5′-phosphates in order to investigate the effect of a substituent in position 2 on flavoring activity, and proved that their flavoring strengths in synergistic effect with monosodium L-glutamate (MSG) varied significantly with the kind of 2-substituent. Kuninaka[3] reported that a structure essential to flavoring activity was purine 5′-mononucleotide with a hydroxy group in position 6. Since the 6-hydroxy group is capable of existing in lactam-lactim tautomers (Ia and Ib), it appears of interest to examine the flavoring activity of 5′-nucleotide in which 6-position is fixed to keto form, such as N^1-methylinosine 5′-phosphate (XVIII) and N^1-methylguanosine 5′-phosphate (XIX).



Ia Ib

It is also worthy of note that N^1-methyl purine nucleotides are occurring in transfer ribonucleic acid (RNA).

The present paper deals with the synthesis of N^1-methyl-2-substituted inosines and their 5′-phosphates, and presents further information on the flavoring activity of 5′-purine nucleotide.

There are two possible methods for the synthesis of N^1-methylpurine derivative. They involve (1) ring closure of the pyrimidine of imidazole derivative, and (2) direct methylation of purine derivative. As a preliminary experiment, we have attempted to prepare N^1-methylhypoxanthine (V) from 5-amino-4-imidazolecarboxamide (AICA) by the first method. AICA was refluxed in ethyl orthoformate to give 5-ethoxymethyleneamino-4-imidazolecarboxamide (II), in 64% yield, whose structure was confirmed by spectral properties and elemental analysis. This compound was fairy unstable to acid but could be readily converted to hypoxanthine (III) on treatment with acetic anhydride. Reaction of II with methylamine gave 5-methylaminomethyleneamino-4-imidazolecarboxamide (IV), which was submitted to ring closure in the presence of sodium ethoxide. However, contrary to our expectation that V would be obtained with the removal of ammonia, the only product isolated was III (see Chart 1).

1) Location: *Suzuki-cho, Kawasaki.*
2) A. Yamazaki, I. Kumashiro, and T. Takenishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 338 (1968).
3) A. Kuninaka, *Bull. Agr. Chem. Soc. Japan*, **34**, 489 (1960).

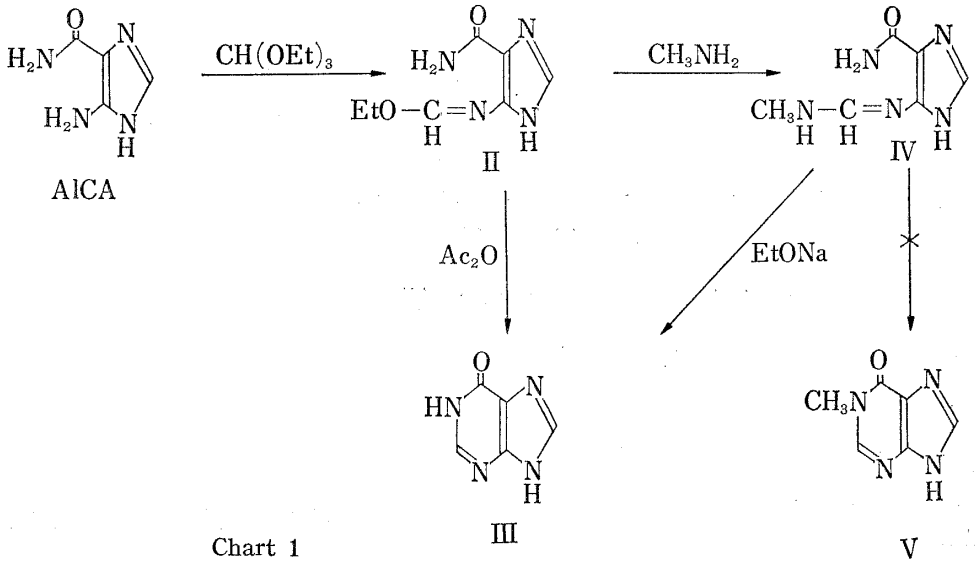


Chart 1

Although the conversion of AICA to V was found to be difficult, a successful preparation of N^1–methylpurine derivative was achieved by the second method. Previous workers[4,5] reported that the methylthio group of 2–methylthiohypoxanthine was resistant to the substitution with ammonia. Whereas the preparation of N^1–methylguanine from N^1–methyl–2–methylthiohypoxanthine was accomplished.[6] This suggests that the methylthio group adjacent to N^1–methyl group is more reactive, and as the most useful intermediate for the preparation of N^1–methyl–2–substituted inosine derivatives, 2′,3′–O–isopropylidene–N^1–methyl–2–methylthioinosine (VII) was chosen. This compound was synthesized by methylation of 2′,3′–O–isopropylidene–2–methylthioinosine (VI)[7] with methyl *p*–toluenesulfonate according to the procedure of Robins, *et al.*[9] The yield of VII was about 56% and its structure was confirmed by the nuclear magnetic resonance (NMR) spectrum and by desulfurization with Raney nickel to produce, after acidic treatment, N^1–methylinosine (XIV).[9]

Desulfurization of VII with Raney nickel yielded 2′,3′–O–isopropylidene–N^1–methylinosine (VIII), and, on treatment with 0.25 N sodium hydroxide, VII was converted to 2′,3′–O–isopropylidene–N^1–methylxanthosine (IX). Compound VII was allowed to react with ammonia in a sealed tube at 180° for 2 hr to give 2′,3′–O–isopropylidene–N^1–methylguanosine (X), in 49% yield. Compound X was hydrolyzed in acidic solution to N^1–methylguanosine (XVI).[10] Similar treatment of VII with methylamine afforded 2′,3′–O–isopropylidene–$N,^1N^2$–dimethylguanosine (XI) in 66% yield. The structure was supported by the NMR spectrum (in pyridine) which exhibited a singlet at 3.57 ppm due to the N^1–methyl group and a doublet at 3.02 ppm due to the $NHCH_3$ group splitted by the NH proton. Attempts to convert VII to 2′,3′–O–isopropylidene–N^1,N^2,N^2–trimethylguanosine (XII) with dimethylamine were unsuccessful under various conditions. This is probably due to the steric hindrance of N^1–methyl group, as in the case of reaction of 2–methylsulfinyladenine N^1–oxide with morpholine.[11]

4) G.B. Elion, W.H. Lange, and G.H. Hitchings, *J. Am. Chem. Soc.*, **78**, 217 (1956).
5) M. Ikehara, A. Yamazaki, and T. Fujieda, *Chem. Pharm. Bull.* (Tokyo), **10**, 1075 (1962).
6) "The Chemistry and Biology of Purines," CIBA Foundation Symposium, Churchill, London, 1957, p. 43.
7) This compound can be readily obtained by reaction[8] of 5–amino–1–(2′,3′–O–isopropylidene–β–D–ribofuranosyl)–4–imidazolecarboxamide with sodium methylxanthate followed by methylation of the resulting 2′,3′–O–isopropylidene–2–mercaptoinosine.
8) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **32**, 3032 (1967).
9) J.W. Jones and R.K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).
10) A.D. Broom, L.B. Townsend, J.W. Jones, and R.K. Robins, *Biochemistry*, **3**, 492 (1964).
11) R.M. Gresswell, and G.B. Brown, *J. Org. Chem.*, **28**, 2560 (1963).

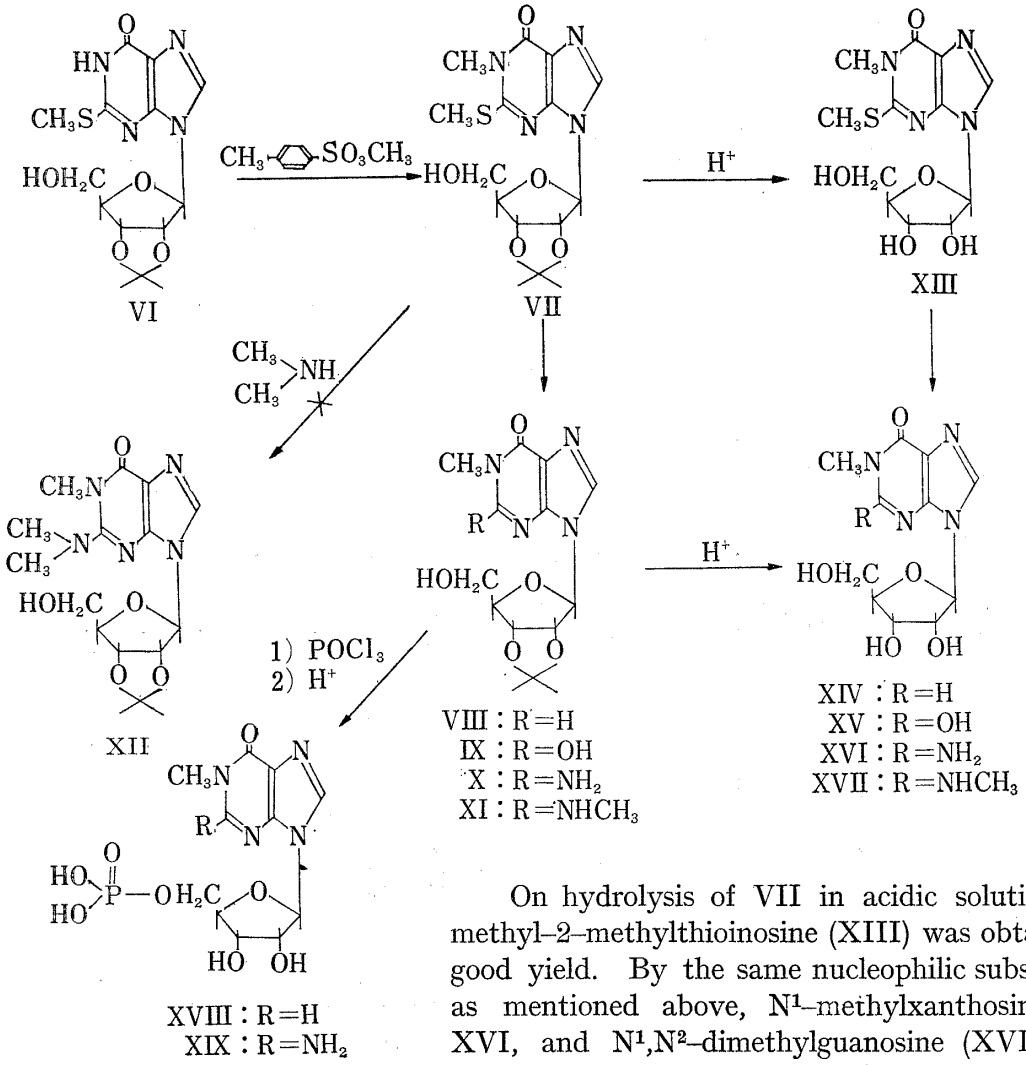
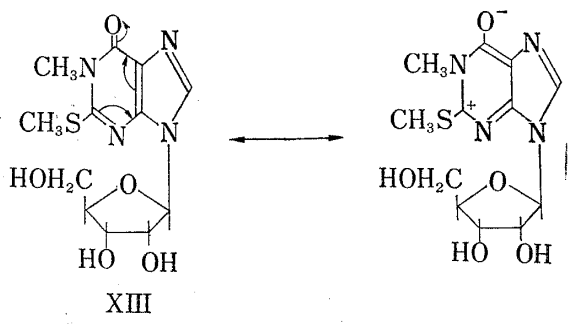


Chart 2

On hydrolysis of VII in acidic solution, N^1–methyl–2–methylthioinosine (XIII) was obtained in good yield. By the same nucleophilic substitution as mentioned above, N^1–methylxanthosine (XV), XVI, and N^1,N^2–dimethylguanosine (XVII) were prepared from XIII. Previously, the methylation of guanosine with dimethyl sulfate at pH 6—9 was reported by Bredereck, *et al.*[12] to yield XVII. The direct methylation was repeated, but no desired product was found in the reaction mixture. Under these conditions, the methylation would take place in position 7.[9]

In view of the above results, the methylthio group of XIII seems to be more susceptible to nucleophilic substitution than that of 2–methylthioinosine.[8] The cause of this enhancement in susceptibility may be explained by the deficiency of electron density in position 2, due to the electron–attracting property of the fixed keto group at position 6, as shown below.



By a method developed in our laboratories,[13] the compounds VIII and X were readily phosphorylated with phosphoryl chloride in trimethyl phosphate to afford, after removal of the isopropylidene group, XVIII and XIX, respectively. These com-

12) H. Bredereck, H. Haas, and A. Martini, *Ber.*, **81**, 307 (1948).
13) M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Letters*, **1967**, 5065.

Table I. Physical Properties and Analytical Data of Synthesized Compounds

Compd. No.	mp (°C)	$[\alpha]_D^{25}$	UV λ_{max}: mμ (ε) pH 1	pH 6	pH 13	Formula	Analysis (%) Calcd. C	H	N	P	Found C	H	N	P
I	220 (decomp.)			275(11700) (EtOH)		$C_7H_{10}O_2N_4$	46.15	5.50	30.77		46.25	5.81	31.36	
IV	195 (decomp.)			220(13400) 290(16700) (EtOH)		$C_6H_9ON_5$	43.11	5.43	41.90		43.30	5.36	42.01	
VII	119—122	−10.9° (c=1, 0.1 N HCl)	257(13800)	264(12000) 284(9800)	264(11900) 284(9700)	$C_{15}H_{20}O_5N_4S$	48.90	5.47	15.21		49.09	5.68	14.65	
VIII	173—174	−54.0° (c=1, 0.1 N NaOH)	252(10100)	252(9800)	252(9700)	$C_{14}H_{18}O_5N_4$	52.17	5.63	17.38		51.82	5.11	17.51	
IX	222	−62.0° (c=1, 0.1 N NaOH)	237(7600) 265(9400)	264(9500)	253(10900) 280(9700)	$C_{14}H_{18}O_6N_4$	49.70	5.36	16.56		49.33	5.80	16.56	
X	162—164	−25.0° (c=1, 0.1 N HCl)	260(11500) 286(7300)	257(13800) 274(10200)	258(12900) 276(8900)	$C_{14}H_{19}O_5N_5$	49.84	5.68	20.76		49.43	5.72	20.50	
XI	213—214	−8.9° (c=1, EtOH)	263(12700) 290(7200)	258(14500) 285(8200)	258(13700) 285(7400)	$C_{15}H_{21}O_5N_5$	51.27	6.02	19.93		51.30	5.82	20.16	
XIII	174—176		276(14800)	264(13100) 284(10500)	264(12600) 284(10900)	$C_{12}H_{16}O_5N_4S$	43.90	4.91	17.07		44.18	5.20	16.79	
XV	225—230 (decomp.)	−66.0° (c=1, 0.1 N NaOH)	239(7200) 264(9900)	243(8500) 265(9800)	253(10100) 280(9300)	$C_{11}H_{14}O_6N_4$	44.30	4.78	18.79		44.56	4.62	18.79	
XVII	198		263(14600) 290(8400)	257(11400) 283(9600)	258(14000) 285(7900)	$C_{12}H_{17}O_5N_5$	46.30	5.50	22.56		46.50	5.50	22.26	
XVIII		−29.8° (c=1.1, H_2O)	252(9300)	252(10000)	252(8700)	$C_{11}H_{15}O_8N_4P\cdot 2H_2O$	33.17	4.81	14.07	7.78	33.02	4.89	14.28	7.38
XIX	168—170 (decomp.)	−28.0° (c=1, 0.1 N NaOH)	260(12000) 286(7500)	257(13400) 274(10000)	258(14000) 276(9700)	$C_{11}H_{16}O_8N_5P\cdot H_2O$	33.42	4.59	17.72	7.84	33.47	4.60	17.78	7.86

pounds, which were of current interest as the minor constituents[14,15] of transfer RNA, have been found to show flavoring activities and to exhibit less flavoring strengths in synergistic effect with MSG than their original nucleotides (5′-inosinic and 5′-guanylic acids), respectively. The details of their flavoring strengths will be reported later. The physical properties and the analytical data of the newly synthesized compounds are summarized in Table I.

In biological assay, the compounds XIII, XIV, and XV did not exhibit antitumor activity.

Experimental[16]

Paper Chromatography——All chromatographies were carried out on Toyo No. 51 filter paper by the ascending method. Solvent systems: A, *n*-butyl alcohol–acetic acid–water, 4:1:1 (v/v); B, *n*-propyl alcohol–ammonia (28%)–water, 20:12:3 (v/v); C, isopropyl alcohol–sat. ammonium sulfate–water, 2:79:19 (v/v).

5-Ethoxymethyleneamino-4-imidazolecarboxamide (II)——A solution of 5-amino-4-imidazolecarboxamide (AICA, 30 g)[17] in ethyl orthoformate (500 ml) was heated under reflux for 2 hr. In several minutes, the reaction mixture became celar and a precipitate began to form. The resulting precipitates were separated by filtration and recrystallized from EtOH containing a small amount of ethyl *ortho*-formate to give 28 g (64%) of pure product. When this compound was heated in 1N HCl, paper chromatography of the solution showed complete hydrolysis to AICA. The NMR spectrum in DMSO-d_6 revealed the presence of the ethoxy group.

5-Methylaminomethyleneamino-4-imidazolecarboxamide (IV)——Three grams of II was dissolved in 70 ml of hot EtOH, and the solution was saturated with methylamine at room temperature. The mixture was then allowed to stand at that temperature for 1 hr. Concentration of the solution gave crude product, which was recrystallized from EtOH to afford 1.84 g (64%) of colorless crystals.

Hypoxanthine (III)——a) A solution of II in acetic anhydride was heated at 80° for 2 hr with stirring. EtOH was added and the solvent was removed *in vacuo* to leave a residue, which was crystallized from water to give hypoxanthine (III).

b) To a solution of EtONa (prepared from 1 g of Na and 25 ml of EtOH) was added 1 g of II. The mixture was then refluxed for 2 hr. After 50 ml of H_2O was added, the resulting clear solution was neutralized by adding Amberlite IR-120 (H^+ form) portionwise. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to give crude crystals, which were recrystallized from H_2O to afford 0.28 g of III. This compound was confirmed to be identical with an authentic sample by comparison of their ultraviolet and infrared absorption spectra.

2′,3′-O-Isopropylidene-N^1-methyl-2-methylthioinosine (VII)——To a suspension of 2.35 g (17.0 mmoles) of anhydrous potassium carbonate in 30 ml of N,N-dimethylacetamide was added 5 g (14.1 mmoles) of 2′,3′-O-isopropylidene-2-methylthioinosine[8] at 80° with stirring. After 2.7 g (14.5 mmoles) of methyl *p*-toluenesulfonate was added, the mixture was heated at 100° for 2 hr. The insoluble material was removed and the filtrate was evaporated to dryness in reduced pressure. A small amount of H_2O was added and the solution was extracted with three 50 ml portions of $CHCl_3$. The combined extracts, dried with Na_2SO_4, were evaporated *in vacuo* to dryness. The resulting residue was crystallized from EtOH to give 2.9 g (55.8 %) of the product. NMR (in pyridine): singlet at 3.50 ppm (N^1–C$\underline{H}_3$), singlet at 2.55 ppm (S–C$\underline{H}_3$), and two singlets at 1.44 and 1.67 ppm ((C$\underline{H}_3$)$_2$C$\langle$).

2′,3′-O-Isopropylidene-N^1-methylinosine (VIII)——To a solution of VII (2 g) in 50% aqueous EtOH (50 ml) was added Raney nickel[18] (10 ml) with stirring. The solution was heated at 50° for 2 hr. The catalyst was filtered off, the filtrate evaporated to dryness, and the resulting residue crystallized from EtOH; yield 0.95 g (51.6%). NMR (in pyridine): singlet at 3.71 ppm (N^1–C$\underline{H}_3$).

2′,3′-O-Isopropylidene-N^1-methylxanthosine (IX)——Three grams of VII was refluxed in 100 ml of 0.1 N NaOH for 2 hr. After the solution was neutralized with Amberlite IR-120 (H^+ form), the resin was filtered off and the filtrate was evaporated to a small volume. The resulting precipitate was collected by filtration and recrystallized from H_2O to afford 1.3 g (47%) of the pure product. NMR (in DMSO-d_6): singlet at 3.21 ppm (N^1–C$\underline{H}_3$).

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14) R.H. Hall, *Biochem. Biophys. Res. Comm.*, **13**, 394 (1964).
15) M. Adler, B. Weissmann, and A.B. Gutman, *J. Biol. Chem.*, **230**, 717 (1958).
16) All melting points are uncorrected. Ultraviolet absorption spectra were taken with a Hitachi Type EPS-2 automatic recording spectrophotometer. The NMR spectra were measured with a Varian A-60 using tetramethylsilane as internal standard.
17) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **32**, 3258 (1967).
18) A. Domingcez, I.C. Lorez, and R. Franco, *J. Org. Chem.*, **26**, 1625 (1961).

2′,3′-O-Isopropylidene-N¹-methylguanosine (X)——Two grams of VII was added to 50 ml of EtOH saturated with NH_3 at O°, and the mixture was heated in an autoclave at 180° for 4—5 hr. The solution turned to dark brown but showed a single spot on a paper chromatogram. The reaction mixture was concentrated *in vacuo* and the resulting residue was dissolved in H_2O. Upon standing in a refrigerator overnight, dark brown crystals were obtained. These were recrystallized thrice from H_2O, giving 0.9 g (49%) of colorless crystals. NMR (in pyridine): singlet at 3.78 ppm (N^1–$C\underline{H}_3$).

2′,3′-O-Isopropylidene-N¹, N²-dimethylguanosine (XI)——One gram of VII was added to 15 ml of EtOH saturated with methylamine at 0° and the mixture was heated in an autoclave at 130—140° for 3 hr. The solvent was removed *in vacuo* to afford a crude product. Recrystallization from EtOH gave 0.63 g (66%) of pure sample.

N¹-Methyl-2-methylthioinosine (XIII)——Two grams of VII was dissolved in 100 ml of H_2O on warming. After being adjusted to pH 1.5, the solution was heated on a steam bath at 70° for 40 min, cooled, and neutralized with Amberlite IRA-410 (OH^- form). The resin was removed and the filtrate was concentrated to dryness. The residue was crystallized from EtOH; yield 1.2 g (67.4%).

N¹-Methylinosine (XIV)——This compound was obtained in a manner similar to that described for XIII. Physical properties were identical with those reported by Robins, *et al.*[9]

N¹-Methylxanthosine (XV)——One gram of XIII was treated in a manner similar to that described for IX to yield a crude product. An analytically pure sample was obtained by recrystallization from H_2O; yield 0.49 g (53.4%).

N¹-Methylguanosine (XVI)——This compound was obtained from XIII (1 g) by the same procedure as that described for X. Yield 0.6 g (65%). Physical properties were identical with those previously reported.[10]

N¹,N²-Dimethylguanosine (XVII)——One gram of XIII was treated with methylamine as described for the preparation of XI. A crude product was recrystallized from H_2O to give 0.6 g (63.4%) of a pure sample.

N¹-Methylinosine 5′-Phosphate (XVIII)——Phosphoryl chloride (4.26 ml, 46.5 mmoles) was mixed with 20 ml of trimethyl phosphate cooled at −10° in a three-necked flask equipped with a thermometer and a silica gel drying tube. To this solution was added VIII (5 g, 15.5 mmloes) with stirring, and the mixture was stirred at −5° for 3 hr. The reaction mixture was then poured into 750 ml of ice water to decompose unchanged phosphoryl chloride. After being adjusted to pH 1.5 with 2N NaOH, the solution was heated at 70° for 40 min with stirring to remove the isopropylidene group. Paper chromatography showed two spots which, on elution, corresponded to XVIII and XIV. A major spot was that of XVIII. The above solution was adjusted to pH 2 with 2 N NaOH and passed through a column (3 × 60 cm) of 180 ml. of decolorizing resin[19] to absorb XVIII. The column was washed with 2 liter of H_2O and XVIII was eluted with 0.5 N NH_4OH until eluate became free from ultraviolet absorbing material. An aliquot of the eluate exhibited a single spot on a paper chromatogram in solvent C. After the eluate was concentrated to dryness, the residue was dissolved in 100 ml of H_2O and passed through a column of 60 ml of Dowex 1–XI ($HCOO^-$ form). The column was washed with H_2O, the nucleotide eluted with 0.5 N HCOOH, and the eluate concentrated at 30—40° which a rotary evaporator. The resulting gummy product was triturated with EtOH containing a small amount of H_2O. A somewhat hygroscopic product was obtained. This was dried *in vacuo* over phosphorus pentoxide at 60° for 4 hr; yield 1.96 g (31.7%); the migrating distance in paper electrophoresis (10% acetic acid buffer, 800 V/cm, 2 hr): 9.4 cm; paper chromatography: *Rf* 0.04 (solvent A), 0.22 (solvent B) and 0.61 (solvent C); NMR (in deuterium oxide): singlet at 3.73 ppm (N^1–$C\underline{H}_3$);

N¹-Methylguanosine 5′-Phosphate (XIX)——To a solution of phosphoryl chloride (2.8 ml, 30.5 mmoles) in 13 ml of trimethyl phosphate being cooled at −10°, X (3.4 g, 10.1 mmoles) was added, and the mixture was stirred at −5° for 3 hr. The clear solution was poured into 500 ml of ice water, adjusted to pH 1.5 with 2 N NaOH, and heated at 70° for 40 min with stirring. After cooling, the solution was adjusted to pH 2 and applied to a column of the decolorizing resin (120 ml). The column was washed with 2 liter of H_2O and the nucleotide was eluted with 0.5 N NH_4OH. Paper chromatogram of this eluate showed a single spot in solvent C. After the eluate was concentrated to dryness, the residue (1.9 g) was dissolved in 50 ml of H_2O and passed through a column of Dowex 1–XI ($HCOO^-$ form, 40 ml). The column was washed with H_2O, the nucleotide was eluted with 0.5 N HCOOH, and the eluate was concentrated *in vacuo* at 30—40°. To this, 50% aqueous EtOH was added and the solution was concentrated. This procedure was repeated several times. The resulting crystals were collected and dried *in vacuo* at 70°; yield 1.4 g (36%); paper chromatography :*Rf* 0.01 (solvent A), 0.17 (solvent B), and 0.43 (solvent C); the migrating distance in paper electrophoresis (10% acetic acid buffer, 800 V/cm, 2 hr); 5.5 cm; NMR (1 N NaOD): singlet at 3.35 ppm (N^1–CH_3);

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19) This resin was prepared in our Laboratories by copolymerization of metaphenylenediamine, resorcin and formalin.