Cyclization of 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA-riboside): A Review

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The synthesis of various purine nucleosides by cyclization of AICA-riboside(5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide) is described. A variety of cyclization reactions provide new synthetic routes to inosine and guanosine. In this review, emphasis will be placed on the synthesis of the latter.

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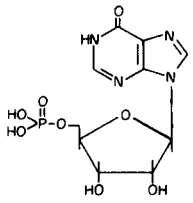
Introduction.

Inosinic acid was first reported by Liebig (1), who in 1847 isolated it from beef extracts but did not characterize it structurally. More than sixty years later, Kodama (2) stated that the histidine salt of inosinic acid was the principal flavor component of dried bonito stock. Structural studies, especially by Levene, *et al.* (3), demonstrated that muscle inosinic acid was inosine-5′-phosphate (**1**) which might be derived from adenosine-5′-triphosphate, and the result led to the complete elucidation of structure of inosinic acid derived from beef or bonito.



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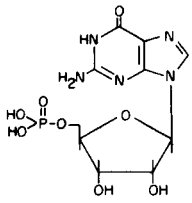
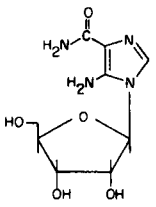


2

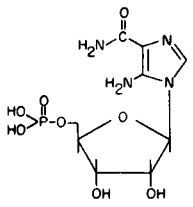
The first keystone to the relationship between chemical structures and flavor was the report by Kuninaka (4), who investigated systematically some naturally occurring nucleotides and discovered that a structural component essential to the flavoring activity was a purine-5′-mononucleotide with a 6-hydroxyl group capable of existing in lactim-lactam tautomers, such as **1** and guanosine-5′-phosphate (**2**). It was also shown (4) that **1** had a significant synergistic effect with monosodium glutamate (MSG) and that the flavor of **2** was about three times more intense than that of **1**. Based on these findings, both the nucleotides have been introduced into the food industry as flavoring agents. We have been interested in the development of a simple method for the preparation of inosine (**5**) and guanosine (**6**), which are the immediate precursors of **1** and **2**, respectively.

Fermentation has often given useful products which were not easily obtained by synthetic methods. Of special interest is 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA-riboside) (**3**) (5-9) which is a constituent of AICA-ribotide (**4**), a biosynthetic precursor of purine nucleotides. Recently, in our laboratories, **3** has become readily available from the culture broth of a mutant of *Bacillus subtilis* (10) and has served as the starting material for the present investigation.



(AICA-riboside)

3



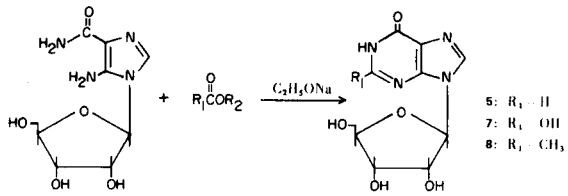
(AICA-ribotide)

4

Synthesis of Inosine.

The only example of the synthesis of a purine nucleoside from **3** is inosine (**5**) (11-13), which is obtained by formylation followed by cyclization. However, this method is of little preparative value. We first investigated the reaction of **3** with a carboxylic acid ester (14). Ethyl

Scheme 1

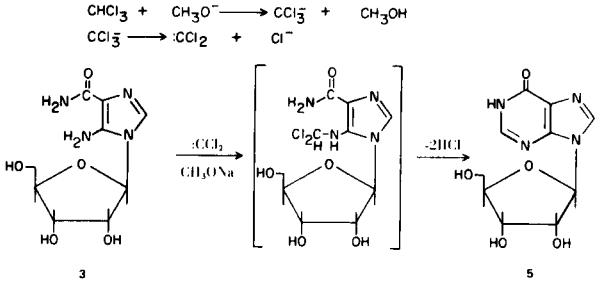


3

formate effects ring closure when it is heated with **3** in the presence of sodium ethoxide, giving **5** in 85% yield. This reaction is generally applicable; xanthosine (**7**) and 2-methylinosine (**8**) can be prepared in good yields using diethyl carbonate (15) and ethyl acetate (15), respectively. The formation of 2-substituted inosines appears to proceed *via* the N^5-acyl derivatives.

Another approach involves the reaction of **3** with a carbene (17). When **3** is treated with chloroform in the presence of sodium methoxide, **5** is formed in low yield (15%). This ring closure can be reasonably explained as follows.

Scheme 2



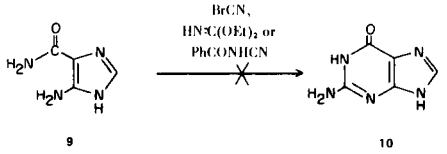
3 **5**

The use of carbon tetrachloride and hexachloroethane as carbene sources was also effective and gave a better yield of **5** (50-60%).

Synthesis (18) of Guanosine *via* Guanidino Derivative.

Several methods for the synthesis of **6** involving the condensation of the purine base and sugar have been reported by Davoll and coworkers (19-21). However, little is known about the synthesis of **6** from **3**. As a preliminary experiment, we investigated the direct preparation of guanine (**10**) from 5-aminoimidazole-4-carboxamide (**9**) (22-26). Failure has been recorded in some attempts (8) to prepare **10** from **9** by means of ring closing reagents such as cyanamide, guanidine, and *S*-methylisothiourea. Further attempts using reagents such as cyanogen bromide, ethyl imidocarbonate, and benzoyl cyanamide were unsuccessful. The unexpected difficulties encountered in

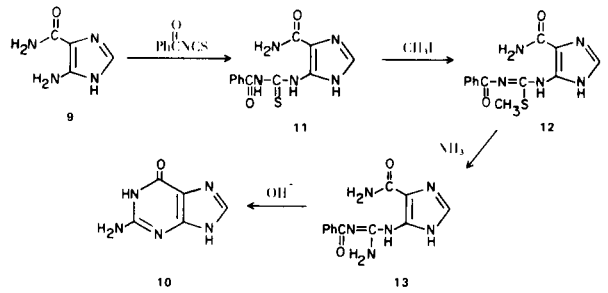
Scheme 3



9 10

the cyclization were probably due to the low basicity of the 5-amino group. Since the p*K*a value (27) of the amino group is 4.00, it seemed that it would be extremely difficult to convert **9** into **10** directly. Then an approach using benzoyl isothiocyanate was developed. This involved the sequence shown below, in which **9** reacted with the reagent in water to afford 5-(*N'*-benzoylthiocarbamoyl)-aminoimidazole-4-carboxamide (**11**). Treatment of the

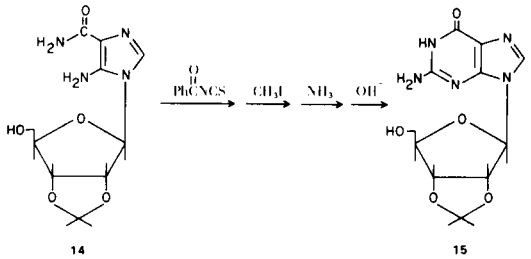
Scheme 4



9 11 12 13 10

latter with methyl iodide gives the methylthio derivative (**12**), from which the corresponding benzoylguanidino

Scheme 5



14 15

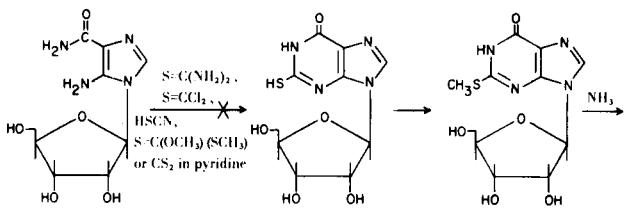
derivative (**13**) was easily prepared. As expected, **13** was cyclized to **10** upon treatment with alkali, with the elimination of benzoic acid and ammonia. The overall yield of **10** based on **9** is about 40%.

This method was extended successfully to the synthesis of 2′,3′-*O*-isopropylideneguanosine (**15**) from 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**14**) (28-30).

Synthesis (31) of Guanosine *via* 2-Mercaptoinosine.

It was reported (21) that the amination of 2-methylmercaptoinosine (**17**), prepared by the mercury salt method, gave rise to **6**. If 2-mercaptoinosine (**16**) could be obtained from **3**, a new route for the synthesis of **6** would be available by methylation of **16** followed by

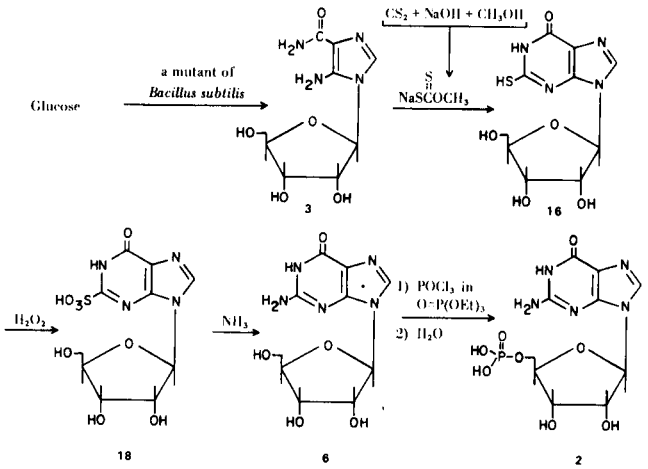
Scheme 6



3 16 17

amination. All attempts to cyclize **3** with thiourea, thiophosgen, thiocyanic acid, *O*,*S*-dimethyl xanthate, or carbon disulfide in pyridine failed. Although there was difficulty in finding a suitable cyclizing reagent, sodium methylxanthate was eventually found to be satisfactory. Compound **3** was treated with sodium methylxanthate prepared *in situ* to give **16**. Compound **3** is almost insoluble in methanol but was found to dissolve in the presence of alkali. Thus, **3** was dissolved in methanolic sodium hydroxide, carbon disulfide was added, and the mixture was heated in an autoclave, affording **16** (32) in almost quantitative yield. Compound **16** was then methylated with methyl iodide to give **17** which, on treatment with *N*-chlorosuccinimide (34), was converted to 2-methylsulfonyl-

Scheme 7



3 16 18 6 2

inosine in order to facilitate nucleophilic displacement on the methylthio group. Without isolation, the methylsulfonyl derivative was aminated with ammonia, giving **6** in good yield.

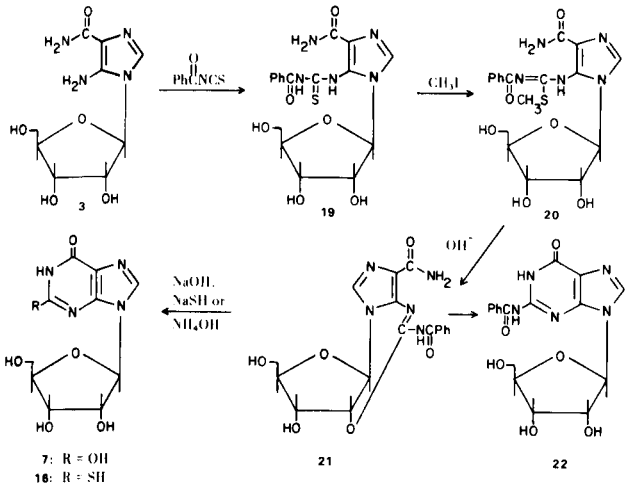
The successful preparation of **6** offered a new route for the production (35-37) of **2** from glucose by a combination of fermentation and chemical synthesis. Without isolating the intermediates, ring closure, oxidation, and amination reactions were carried out successively. Hydrogen peroxide was employed in the oxidation of **16** instead of *N*-chlorosuccinimide, giving inosine-2-sulfonic acid (**18**). By the selective phosphorylation method (38) developed in our laboratories, **6** was treated with phosphoryl chloride in triethyl phosphate to afford **2**.

Synthesis (39) of Guanosine *via* Cycloimidazole Nucleoside.

As further extention of the benzoyl isothiocyanate method, we have reinvestigated the reaction between **3** and the reagent, and developed an additional synthesis of **6** *via* a new type of cycloimidazole nucleoside. The reported examples (40-42) of cycloimidazole are limited to some N^3,5′-cyclonucleosides, which are of little practical use as synthetic intermediates.

When **3** was reacted with benzoyl isothiocyanate, 5-(*N*′-benzoylthiocarbamoyl)aminoimidazole-4-carboxamide derivative (**19**) was obtained and then methylated with methyl iodide, affording the methylthio derivative (**20**). When treated with alkali, **20** gave cycloimidazole nucleoside (**21**) having an *O*-anhydro linkage. Support for the

Scheme 8

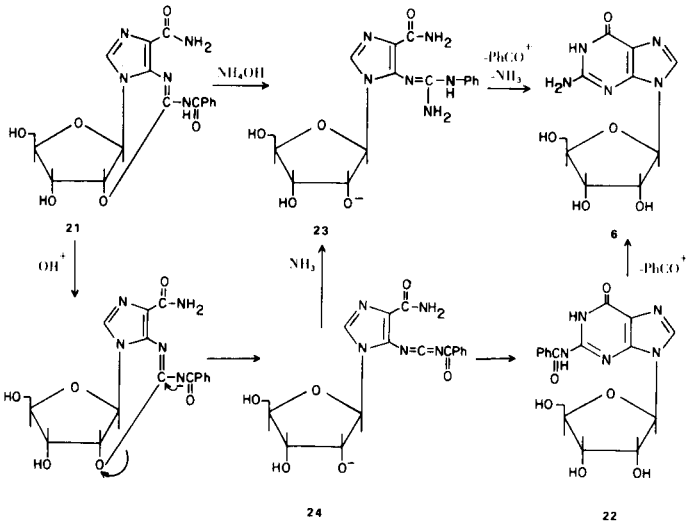


structure was provided by elemental analysis, periodide test (negative), and acetylation to give the 3′,5′-diacetyl derivative. The most compelling evidence for **21** was given by the nmr data. Of considerable interest are the mechanisms of formation and ring-opening of **21**. At *p*H values above 12, the 2′-hydroxyl group of **20** dissociates and cyclization could proceed by nucleophilic attack of the 2′-hydroxyl anion on the carbon atom attached to the methylthio group.

Most interesting is the ring-opening reaction of **21** by a nucleophile. Heating of **21** in solutions of sodium hydroxide and sodium hydrogen sulfide gave **7** and **16**, respectively. It should be noted that **6** could be synthesized in 72% yield by heating **21** with aqueous ammonia. Without isolation of **19**, **20**, and **21**, compound **6** was obtained in 68% yield based on **3**. On brief treatment of **21** with ethanolic sodium ethoxide, N^2-benzoylguanosine (**22**) was formed, and, as expected, could be hydrolyzed with alkali to **6**.

The formation of **6** can be satisfactorily explained if one considers the alkaline susceptibility of **21**, which could be converted to the intermediate benzoylguanidino (**23**) or possibly carbodiimido derivative (**24**) by the indicated anhydro bond cleavage.

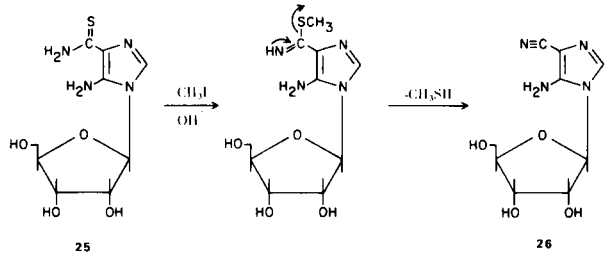
Scheme 9



Synthesis (43) of Guanosine and Isoguanosine *via* a Cyanamide Derivative.

It has been reported by Desai and co-workers (44) that treatment of *O*-hydroxyphenylthiocarbamide with mercuric oxide gave 2-aminobenzoxazole. Presumably, *O*-hydroxyphenylcyanamide was formed initially as an intermediate in the cyclization. Of additional interest is the fact (45) that the methylation of 5-amino-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (**25**) with methyl iodide in an alkaline solution resulted in the liberation of

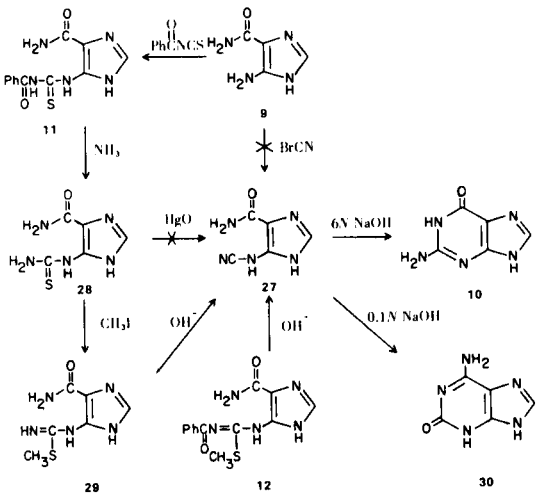
Scheme 10



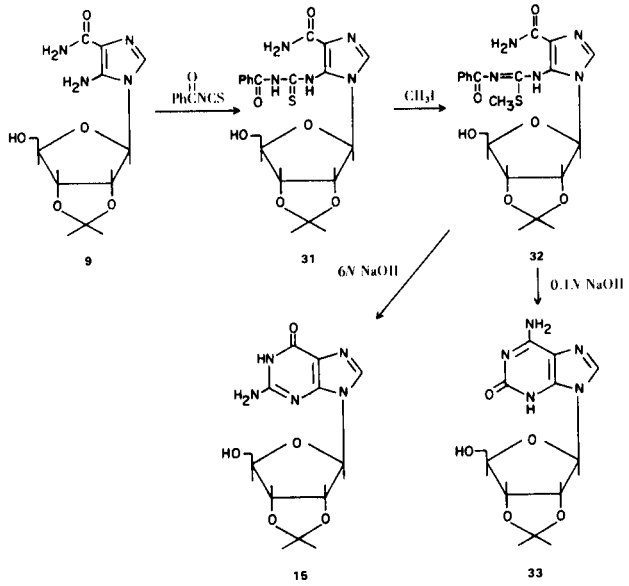
methyl mercaptan, giving 5-amino-4-cyanoimidazole riboside (**26**). On the basis of these facts, our initial efforts involved attempts to generate 4-cyanamidoimidazole-5-carboxamide (**27**), which could lead to **10**. Reaction of **9** with cyanogen bromide was attempted, but did not give **27**. Attempted preparation of **10** *via* **27** by treatment of the thiocarbamoyl derivative (**28**) (18) with mercuric oxide also failed. The preparation of **27** was eventually accomplished by heating the *S*-methylthiocarbamoyl derivative (**29**), obtained from **28**, in 0.1*N* sodium hydroxide for 2-3 minutes. A strong band at 2190 cm^{-1} in the infrared spectrum of **27** was assigned to a cyano group.

As expected, **27** was converted to **10** upon heating in 6*N* sodium hydroxide but to isoguanine (**30**) in dilute alkaline solution. Under the same conditions, **10** and **30** were also synthesized from **12** through the intermediate **27**.

Scheme 11



Scheme 12



The extention of the synthetic procedures for **10** and **30** to their nucleosides proved highly successful. The *S*-methylthio derivative (**32**), when heated in 6*N* sodium hydroxide, furnished **15** in 56% yield. Without isolating the intermediates (**31** and **32**), **9** was converted successfully to **15** in 50% yield. Compound **32** was reacted with 0.1*N* sodium hydroxide to give 2′,3′-*O*-isopropylideneisoguanosine (**33**).

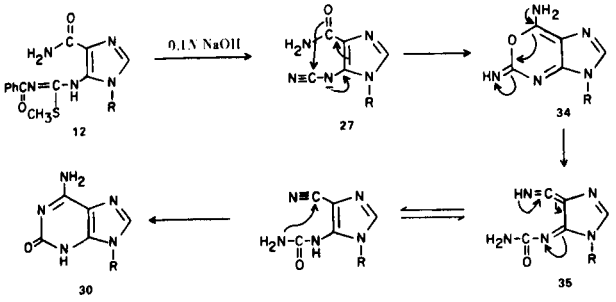
A suggested mechanism for the formation of **30** involves the initial cyclization by the carboxamide oxygen of **27** to give the intermediate oxazine derivative (**34**). Subsequent ring opening would provide the ureido derivative (**35**), which could be rationalized as giving rise to the ring-closed compound **30**. In a strongly alkaline solution, the base abstracted a proton from the carboxamide, and nucleophilic attack by carboxamide nitrogen would occur on the cyanamide carbon atom prior to the attack of carboxamide oxygen, giving **10**.

Cyclization with Some Other Reagents (46).

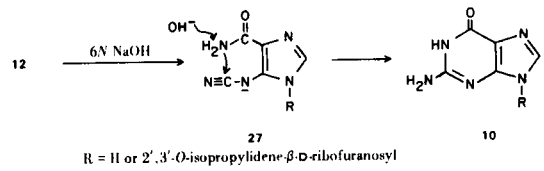
Trichloroethylene in the presence of sodium methoxide cyclizes **9** to 2′,3′-*O*-isopropylidene-2-methoxymethylinosine (**38**). This reaction presumably proceeds through

Scheme 13

Mechanism of Formation of Isoguanine and Its Riboside



Mechanism of Formation of Guanine and Its Riboside



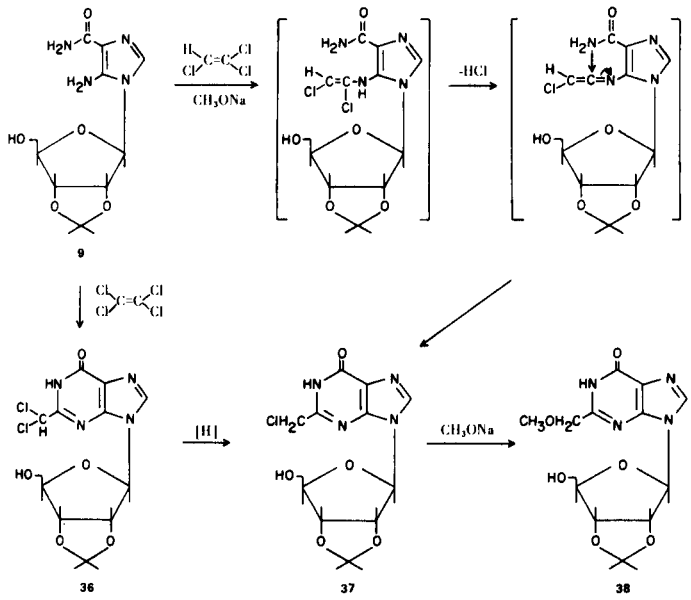
R = H or 2′,3′-*O*-isopropylidene-β-D-ribofuranosyl

the 2-chloromethyl derivative (**37**). Other potential ring closing reagents, such as ethyl chloroacetate, chloroacetamide, and tetrachloroethane, are also successful, and give the same compound. A similar reaction takes place with tetrachloroethylene. Compound **9**, on treatment with the reagent, cyclizes readily to the 2-dichloromethylinosine derivative (**36**), from which **37** can be prepared by catalytic hydrogenation. Compound **38** can also be obtained by reaction of **37** with sodium methoxide.

Biologically Active Nucleosides and Nucleotides.

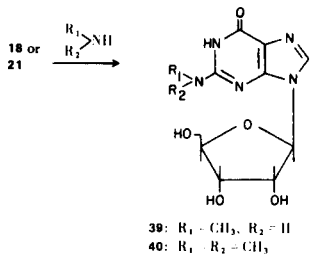
Compound **3** is not only of interest as a precursor for the preparation of **1** and **2**, but also as intermediate for

Scheme 14



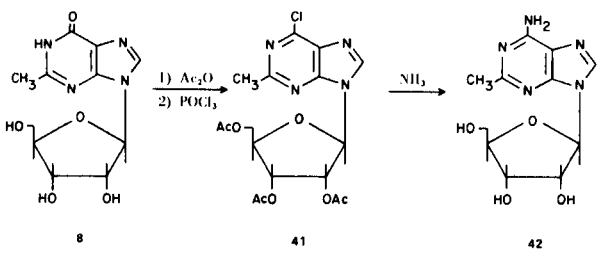
the preparation of various purine nucleosides. The above mentioned methods appear to provide the preferred routes to 2-substituted purine nucleosides, which have often been difficult to obtain. Substitution of methylamine and dimethylamine for ammonia in the guanosine synthesis *via* **18** easily afforded N^2-methylguanosine (**39**) (31) and N^2-dimethylguanosine (**40**) (31), respectively. Alternatively, both the compounds were prepared (39) from **21**.

Scheme 15



2-Methyladenosine (**42**) (47) was obtainable *via* 2-methyl-6-chloropurine derivative (**41**) from **8**. The isopropylidene derivatives of **39**, **40**, and **42** were subjected to phosphorylation with phosphoryl chloride to furnish, after

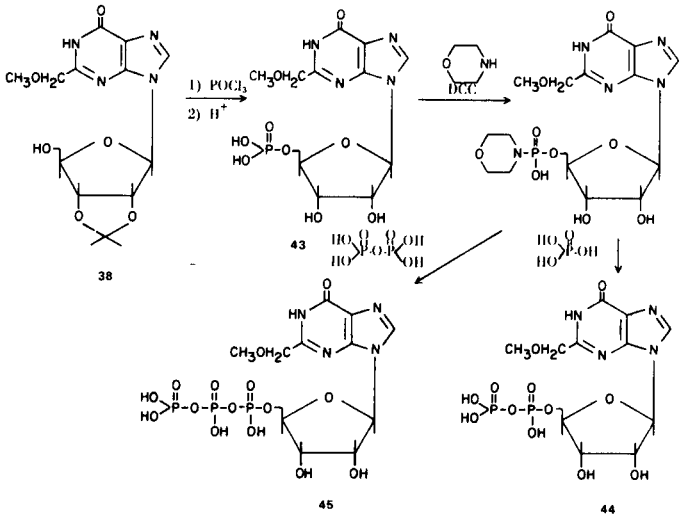
Scheme 16



acidic treatment, the corresponding 5′-mononucleotides. These nucleosides or nucleotides are well known as the minor components of various transfer RNA's: it is noteworthy that the facile preparation of such minor components could contribute to studies of the biological, chemical, and physical properties of transfer RNA.

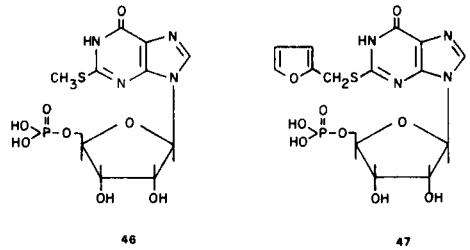
Compound **38** can be phosphorylated and then deacetonated to give 2-methoxymethylinosine-5′-phosphate (**43**), which may be further converted to the corresponding 5′-diphosphate (**44**) and 5′-triphosphate (**45**) by the method

Scheme 17



of Moffatt and Khorana (48). Interestingly enough, **44** was found to be a specific inhibitor (49) of Qβ-replicase at a low concentration.

The overall chemical structure essential for flavoring activity was elucidated by Kuninaka (4), but the influence of the 2-substituent was not studied in detail. Since compound **2** has stronger activity as a flavoring agent than **1** and the difference depends on the presence or the absence of amino group in the 2-position, it seemed that the introduction of a suitable 2-substituent might produce a compound having much stronger activity. Therefore, we prepared a number of 2-substituted purine nucleotides, among which 2-methylthioinosine-5′-phosphate (**46**) (50) was shown (51,52) to be about 8 times more active than **1**. Replacement of the methylthio group with a furfuryl-



thio group leads to a compound (**47**) having extremely strong activity, which has been reported (33) to be about 17 times as active as **1**. Studies on the physicochemical properties (53) of **46** and **47** have been reported in relation to their structure-activity relationships.

Conclusion.

We have focused our attention on the synthesis of **5** and **6** from **3**. A few of the methods of ring closure which we have developed should be applicable to the preparation of 2-[^{14}C]-labeled inosine and guanosine, whose phosphates are important in biochemical research. The methods developed for the synthesis of **6** should also have great potential for the preparation of analogs of **6** and **10**; for instance, 1-hydroxyguanine (54) was synthesized by the benzoyl isothiocyanate method. We conclude that **3** is a useful synthetic intermediate since its cyclization leads to new types of biologically active purine derivatives as well as naturally occurring purine nucleosides.

Acknowledgment.

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