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Synthesis of the "Minor Nucleotide" N^6-(γ,γ-Dimethylallyl)adenosine 5′-Phosphate and Relative Rates of Rearrangement of 1- to N^6-Dimethylallyl Compounds for Base, Nucleoside, and Nucleotide*

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ABSTRACT: 6-(γ,γ-Dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-phosphate (**6**) has been synthesized by three different routes. The most convenient method involved the alkylation of the monosodium salt of adenosine monophosphate (AMP) with γ,γ-dimethylallyl bromide to give the 1-substituted intermediate, which was then rearranged by heating in aqueous solution maintained at pH 7.5. A study of the relative rates of 1→N^6 rearrangement in concentrated ammonium hydroxide at 60° showed their order to be: 1-(γ,γ-dimethylallyl)adenosine → 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine (2-iPA) (**1**) and 1-(γ,γ-dimethylallyl)adenosine 5′-β-cyanoethyl phosphate (**8**) → 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-phosphate (2-iPA-5′-phosphate) (**6**) faster than 1-(γ,γ-dimethylallyl)adenine (**10**) → 6-(γ,γ-dimethylallylamino)purine (**11**) under these conditions. Under conditions that matched those which had been employed in the isolation of 2-iPA from serine transfer ribonucleic acid (tRNA), 1-(γ,γ-dimethylallyl)adenosine underwent partial rearrangement. The surviving 1-substituted derivative, however, would have been detectable at the mononucleotide stage if it had been present in the native tRNA. It has therefore been concluded that there is no evidence for the presence of appreciable 1-(γ,γ-dimethylallyl)-adenosine in serine tRNA as a source of 2-iPA by rearrangement.

6-(γ,γ-Dimethylallylamino)-9-β-D-ribofuranosylpurine (**1**) (also N^6-(γ,γ-dimethylallyl)adenosine, N^6-(Δ^2-isopentenyl)adenosine, or 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine)[1,2] has assumed importance because of its isolation and identification as a component of tRNA from yeast and calf liver (Hall *et al.*, 1966; Robins *et al.*, 1967; Skoog *et al.*, 1966) and its location adjacent to the anticodon se-

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[1] The shorter name, N^6-(γ,γ-dimethylallyl)adenosine, is used more frequently in the text since it points up the relation of this compound to adenosine.

[2] The abbreviation iPA or IPA has been used recently for **1**. In the past, however, there has been general acceptance of the "γ,γ-dimethylallyl" name for the internal double-bond moiety and reservation of the "isopentenyl" name for the terminal double-bond moiety (Bloch, 1965; Lynen *et al.*, 1958; Bloch *et al.*, 1959; Yuan and Bloch, 1959; Agranoff *et al.*, 1959; Rilling and Bloch, 1959; Cornforth, 1959; Johnson and Bell, 1960; Archer *et al.*, 1961). Accordingly, differentiation between the two isomeric side chains, especially in abbreviation, would be desirable for the case of their attachment to the N^6 of adenine. Dr. Waldo E. Cohn has suggested the use of 2-iPA (rather than iPA or γγA) for **1** and 3-iPA for the isomeric 6-(3-methyl-3-butenylamino)-9-β-D-ribofuranosylpurine (or N^6-(Δ^3-isopentenyl)adenosine), which has been obtained by synthesis in this laboratory by S. Hecht. AMP, adenosine 5′-phosphate; DMF, dimethylformamide; PDE, phosphodiesterase; PME, phosphomonoesterase; DCC, *N,N*-dicyclohexylcarbodiimide.

quence in serine tRNAs I and II from brewer's yeast (Zachau *et al.*, 1966a,b; Biemann *et al.*, 1966; Feldmann *et al.*, 1966) and in tyrosine tRNA from yeast (Madison *et al.*, 1967; Madison and Kung, 1967). Moreover, this riboside is a highly active cytokinin (Leonard *et al.*, 1966; Robins *et al.*, 1967; Skoog *et al.*, 1967). In the tobacco bioassay, it is somewhat less active than the base, 6-(γ,γ-dimethylallylamino)purine (**11**) (Cavé, 1962; Leonard and Fujii, 1964; Cavé *et al.*, 1962; Leonard, 1965; Rogozinska *et al.*, 1964; Beauchesne and Goutarel, 1963; Leonard *et al.*, 1966; Klämbt *et al.*, 1966; Helgeson and Leonard, 1966). We now wish to report the synthesis of the corresponding ribotide, 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-phosphate (also N^6-(γ,γ-dimethylallyl)-adenosine 5′-phosphate, or N^6-(Δ^2-isopentenyl)adenosine 5′-phosphate, or 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine 5′-phosphate) (**6**), as a requisite step for the preparation of other phosphate derivatives and oligonucleotides, as in the case of related N^6-methyl-substituted compounds (Griffin *et al.*, 1964; Griffin and Reese, 1963).

The first route to **6** was *via* the alkylation of adenosine (**2**) (Scheme I) with γ,γ-dimethylallyl bromide in dimethylformamide followed by rearrangement of the 1-γ,γ-dimethylallyl intermediate **3** to 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine (2-iPA) (**1**) (Leonard *et al.*, 1966, and references therein; Robins *et al.*, 1967). It was possible to interrupt the sequence and to convert the intermediate 1-(γ,γ-dimethylallyl)-adenosine hydrobromide (**3**) (Leonard, 1965) to the sulfate **3a**, which was characterized by analysis and ultraviolet absorption spectra (Leonard and Deyrup, 1962; Leonard *et al.*, 1965). After protection of the hydroxyl groups in **1** by the formation of the *O*-isopropylidene derivative, 6-(γ,γ-dimethylallylamino)-9-β-(2′,3′-*O*-isopropylidene-D-ribofuranosyl)purine (**4**), phosphorylation was carried out by the method of Tener (1961) through 6-(γ,γ-dimethylallylamino)-9-β-(2′,3′-*O*-isopropylidene-D-ribofuranosyl)purine 5′-β-cyanoethyl-phosphate (**5**) (not isolated) with deblocking to give the desired **6**.

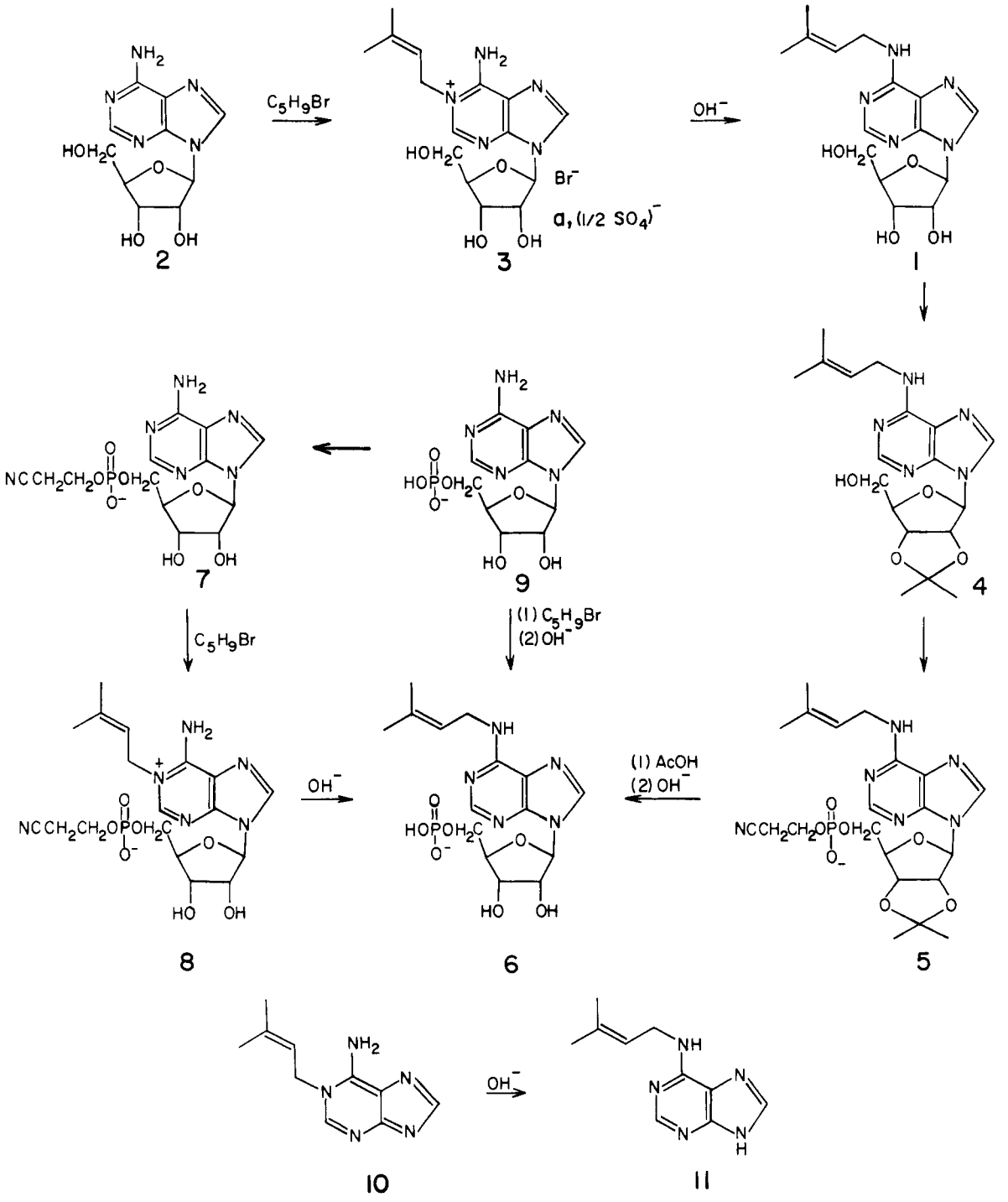
Another route to **6** started with the condensation of adenosine 5′-phosphate (AMP) (**9**) with 3-hydroxypropionitrile in the presence of dicyclohexylcarbodiimide to give adenosine 5′-β-cyanoethyl phosphate (**7**). With the phosphate group thus protected, alkylation with γ,γ-dimethylallyl bromide yielded 1-(γ,γ-dimethylallyl)adenosine 5′-β-cyanoethyl phosphate (**8**), which was purified through column chromatography using anion-exchange resin and was shown by its properties to be in the form of an internal salt. The phosphate diester was rearranged completely under the conditions employed for the conversion **2** → **3** → **1** (Leonard *et al.*, 1966), with partial cleavage of the β-cyanoethyl group. Concentrated ammonium hydroxide at 60° (see below) produced both rearrangement and cleavage with the formation of **6**.

Finally, the most convenient method for preparing 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-phosphate (**6**) was derived from a study of the direct alkylation of unprotected adenosine 5′-phosphate (**9**), when it was found that the yield could be increased through the use of the sodium salt of AMP. The 1-substituted intermediate formed by the alkylation of **9** as the monosodium salt with γ,γ-dimethylallyl bromide in DMF was purified on a DEAE-cellulose column using a salt gradient of lithium chloride and was then maintained in heated aqueous solution at pH 7.5 by periodic addition of 0.1 N sodium hydroxide. The N^6-(γ,γ-dimethylallyl)adenosine 5′-phosphate (**6**) was isolated as the lithium salt.

It was of interest to compare the relative rates of 1→N^6 rearrangement for the base **10**, the nucleoside **3**, and the nucleotide from **8**. The reaction of each with concentrated ammonium hydroxide at 60° was followed during 3.5 hr by quantitative paper chromatography, with the results shown in Figure 1. After 3 hr the base had rearranged to the extent of 27%, the nucleoside to 100%, and the nucleotide to 92%. The faster rate of rearrangement observed for **3** and **8** over **10** is consistent with other observations on 1,X-disubstituted adenines (Taylor and Loeffler, 1960; Carraway, 1966) *vs.* 1-monosubstituted adenines and probably reflects the fact that the disubstituted derivatives in moderately to strongly basic solution will be in the 6-imino form, and thus more susceptible to attack and rearrangement to a more stable aromatic system in an equilibration process.

It was also of interest to deal with the problem posed by Hall *et al.* (1966) as to whether in a tRNA molecule containing a γ,γ-dimethylallyladenine moiety this moiety could exist as the 1 isomer and undergo rearrangement under the conditions of enzymic hydrolysis of tRNA and to approach indirectly the problem of the biogenetic timing of the alkylation of adenine with the γ,γ-dimethylallyl side chain (*cf.* Madison *et al.*, 1967; Dunn, 1961). It can be seen that complete rearrangement of any 1-(γ,γ-dimethylallyl)adenylate would occur under the vigorous conditions described above. However, the serine tRNA degradation conditions used by Zachau *et al.* (1966a,b) were milder and involved treatment with pancreatic RNase, T_1-RNase, and venom phosphodiesterase. Ion-exchange chromatography was carried out at 4° with NH_4CO_3 buffer as eluent. In order to determine the fate of 1-(γ,γ-dimethylallyl)adenosine (which tends to rearrange even faster than the adenylate (Figure 1)) when subjected to the degradation and isolation procedure as from tRNA, we treated it under the same conditions which has been employed in the isolation of 2-iPA from serine tRNA (Zachau *et al.*, 1966a,b; Melchers *et al.*, 1965; Feldmann *et al.*, 1966). The maximum percentages of 1→N^6 rearrangement occurring at each stage were as follows: column chromatography with ammonium carbonate (pH 8.9) at 4° for 2 days, <10%; conditions of T_1-RNase action, Tris buffer (pH 7.5) at 32° for 45 min, *ca.* 36%; conditions of venom phosphodiesterase (PDE) action, Tris buffer (pH 8.9) at 37° for 6 hr, *ca.* 46%. In all cases the extent of rearrangement was determined spectrophotometrically following paper chromatography in 1-

SCHEME I



butanol–water–acetic acid (15:4:1), and extraction with pH 7 buffer. In serine tRNAs I and II the N^6-(γ,γ-dimethylallyl)adenosine phosphate is attached to adenosine monophosphate on both sides. This requires that the diester bonds be split in the enzymic degradation in the last step, the treatment with venom phosphodiesterase. Thus, if any of the 1-substituted compound had been present in the native tRNA, about 50% of it would have survived this stage and would therefore have been detectable. We have been informed (H. Feldmann, D. Dütting, and H. G. Zachau, 1967, personal communication) that, although in the decanucleotide peak from T_1-RNase digestion no differences would have been detectable if both 2-iPA and 1-(γ,γ-dimethylallyl)adenosine were present, the tetranucleotide, Ap2-iPApApψ, always yielded one peak,

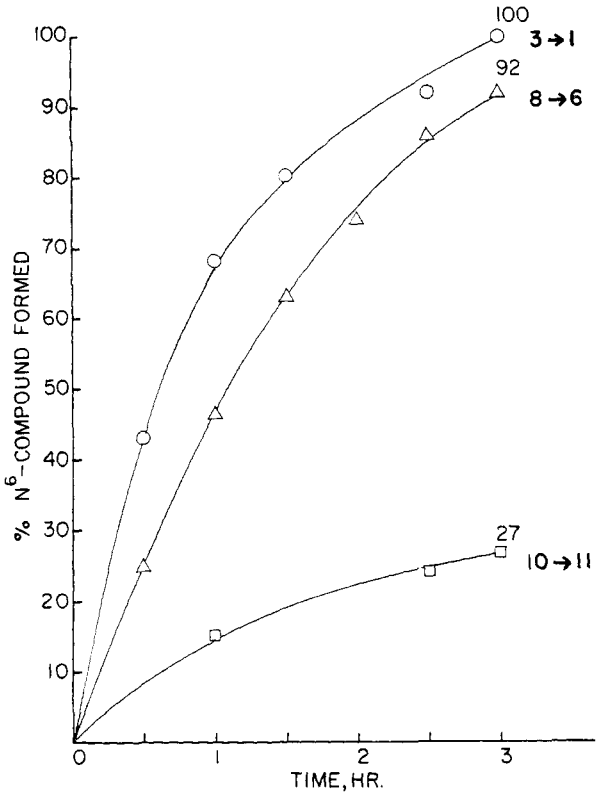


FIGURE 1: Relative rates of rearrangement, in concentrated NH_4OH at 60°, for 1-(γ,γ-dimethylallyl)adenosine (as **3a**), 1-(γ,γ-dimethylallyl)adenosine 5′-phosphate (as **8**), and 1-(γ,γ-dimethylallyl)adenine (**10**) to the corresponding N^6 derivatives.

whether determined by DEAE-cellulose chromatography or by paper electrophoresis. However, the single peak does not necessarily exclude heterogeneity at this level. Further degradation of this tetranucleotide with PDE or PDE–PME yielded only one compound, corresponding to *p*-2-iPA or 2-iPA, *viz.* We have been informed further that no contaminant showing the spectral characteristics of 1-(γ,γ-dimethylallyl)-adenosine was observed during paper electrophoresis, where 10–20% should have been detectable. In summary, we conclude that there is no evidence for the presence of appreciable 1-(γ,γ-dimethylallyl)-adenosine in serine tRNA as a source of 2-iPA by rearrangement. Robins *et al.* (1967) have come to the same conclusion on the basis of experiments employing mild acid hydrolysis of yeast tRNA and product analysis by ion-exchange chromatography.

Experimental Section

Melting points are corrected.

Paper Chromatography. The following R_F values were observed in solvent system A (ethanol–1 M NH_4OAc, 5:2) and B (1-butanol–water–acetic acid, 15:4:1), respectively; 1-(γ,γ-dimethylallyl)adenine (**10**), none and 0.65; 6-(γ,γ-dimethylallylamino)purine (**11**), none and 0.90; 1-(γ,γ-dimethylallyl)adenosine (**3**), 0.77 and 0.46; 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine (**1**), 0.86 and 0.83; adenosine 5′-phosphate (**9**), 0.12 and none; adenosine 5′-β-cyanoethyl phosphate (**7**), 0.27 and none; 1-(γ,γ-dimethylallyl)adenosine 5′-β-cyanoethyl phosphate (**8**), 0.68 and none; 1-(γ,γ-dimethylallyl)adenosine 5′-phosphate (not isolated, 0.34 and none; 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-β-cyanoethyl phosphate (not isolated), 0.79 and none; and 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-phosphate (**6**), 0.47 and none.

Electrophoresis. The following mobilities relative to AMP as 1.00 were observed at 250 v using 0.05 M NH_4OAc at pH 6.8: 1-(γ,γ-dimethylallyl)adenosine (**3**), −0.23 (positively charged); 1-(γ,γ-dimethylallyl)-adenosine 5′-β-cyanoethyl phosphate (**8**), 0.3; 1-(γ,γ-dimethylallyl)adenosine 5′-phosphate (not isolated), 0.48; 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosyl-purine 5′-β-cyanoethyl phosphate (not isolated), 0.67; 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-phosphate (**6**), 0.88; and adenosine 5′-β-cyanoethyl phosphate (**7**), 0.68.

6-(γ,γ-Dimethylallylamino)-9-β-D-ribofuranosylpurine (**1**). To a solution of 5.34 g (20 mmoles) of adenosine (**2**) in 175 ml of anhydrous dimethylformamide was added 4.32 g (29 mmoles) of freshly distilled γ,γ-dimethylallyl bromide. The reaction mixture was maintained at room temperature in the dark for 24 hr and was then evaporated to dryness under reduced pressure.

If the material was not solid at this stage it was advantageous to treat it with dry acetone and partially evaporate again (Grimm *et al.*, 1967). The acetone was decanted and the solid (**2** and **3**) was dissolved in 100 ml of water. The aqueous solution was adjusted to pH 7.5 and was heated on a steam bath for 1.5 hr while adding 0.1 N aqueous sodium hydroxide periodically to maintain the pH at 7.5 (Leonard *et al.*, 1966). The solution was then extracted four times with 100-ml portions of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to the initiation of crystallization. Recrystallization from ethanol–acetonitrile (1:1) gave colorless prisms of N^6-(γ,γ-dimethylallyl)adenosine (**1**): over-all yield 2.53 g, (38%); mp 146–147°; $[\alpha]_D^{28}$ −103° (*c* 0.14, ethanol). Melting points previously reported were 145–147 (Hall *et al.*, 1966; Robins *et al.*, 1967), 148 (Robins *et al.*, 1967), and 142–143° (Leonard *et al.*, 1966; Skoog *et al.*, 1967).

1-(γ,γ-Dimethylallyl)-9-β-D-ribofuranosyladenine as the Sulfate (**3a**). At the stage in the procedure described above using the same quantities, when the initial reaction mixture was evaporated to dryness, the mixture of adenosine (**2**) and 1-(γ,γ-dimethylallyl)-adenosine hydrobromide (**3**), which is hygroscopic, was dissolved in water and treated with Dowex 1-X8 (HCO_3^-) anion-exchange resin to remove most of the

hydrogen bromide. The solution was evaporated to about 50 ml and was then added to the top of a 2.5 × 60 cm column of Dowex 50W-X8 (Na^+) which was eluted with 1 N sodium sulfate. Initially the uncharged adenosine was eluted, followed by the desired product (Grimm *et al.*, 1967). The fractions containing **3a** were combined and evaporated to about 60 ml. Ethanol was added to cause the precipitation of sodium sulfate, which was removed by filtration. The mother liquor was evaporated and treated with ethanol as before. The ethanolic filtrate was evaporated and the product was taken up in water and lyophylized. This material was dissolved in boiling methanol, and the solution was allowed to stand overnight. The precipitate was collected by filtration, dissolved in water, and again lyophilized. These steps, which were employed to remove sodium sulfate and then water, produced a colorless product (**3a**), which was pulverized under exclusion of moisture in a drybox and dried at 65° overnight *in vacuo* over phosphorus pentoxide: yield 3.92 g (51%); mp 150° dec; λ_{max}^{EtOH} 259 mμ (ε 13,250) and sh 265 mμ; λ_{min}^{EtOH} 236.5 mμ (ε 5200), $\epsilon_{290}/\epsilon_{260}$ 0.219; $\lambda_{max}^{H_2O}$ (pH 7) 258 mμ (ε 13,600), λ_{min} 235 mμ (ε 5900); $\lambda_{max}^{H_2O}$ (0.1 N NaOH) 259 mμ (ε 14,250) and sh 265 mμ, λ_{min} 236.5 mμ (ε 5450), $\epsilon_{290}/\epsilon_{260}$ 0.308; $\lambda_{max}^{H_2O}$ (0.1 N HCl) 257.5 mμ (ε 13,900), λ_{min} 235 mμ (ε 6100). *Anal.* Calcd for $C_{15}H_{22}N_5O_4 \cdot 0.5SO_4$: C, 46.87; H, 5.73; N, 18.22. Found: C, 46.60; H, 5.88; N, 17.89.

6-(γ,γ-Dimethylallylamino)-9-β-(2′,3′-O-isopropylidene-D-ribofuranosyl)purine (**4**). The general method of Hampton (1961) was used for the conversion of **1** to the 2′,3′-*O*-isopropylidene derivative. To a solution of 1.44 g (4.3 mmoles) of carefully dried 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine in 35 ml of anhydrous acetone was added 3.2 g (31 mmoles) of 2,2-dimethoxypropane and then 1.7 g (5.0 mmoles) of di-*p*-nitrophenyl hydrogen phosphate. When the solution had been allowed to stand 5 hr in the dark at room temperature the presence of only a trace of starting material was indicated by thin layer chromatography. Anion-exchange resin (HCO_3^-) was added to exchange the phosphate, and after 1 hr the resin was filtered and the solvent was evaporated. Crystallization of N^6-(γ,γ-dimethylallyl)-2′,3′-*O*-isopropylideneadenosine (**4**) was induced from a chloroform–cyclohexane solution: mp 93°; yield 1.24 g (77%). *Anal.* Calcd for $C_{18}H_{25}N_5O_4$: C, 57.58; H, 6.71; N, 18.66. Found: C, 57.77; H, 6.95; N, 18.19.

Phosphorylation of 6-(γ,γ-Dimethylallylamino)-9-β-(2′,3′-O-isopropylidene-D-ribofuranosyl)purine (**4**) *to Obtain 6-(γ,γ-Dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-Phosphate* (**6**). All of the reagents were carefully dried to effect the phosphorylation (Tener, 1961; Schaller *et al.*, 1963). To a solution of pyridine containing excess (4.2 mmoles) β-cyanoethyl phosphate as the pyridinium salt was added 0.946 g (2.5 mmoles) of **4** in 10 ml of pyridine, followed by 2.58 g (12.5 mmoles) of dicyclohexylcarbodiimide. The solution was allowed to stand at room temperature for 3 days in the dark with exclusion of moisture. Then 5 ml of water was added with cooling. After 2 hr the mixture was evaporated to dryness, the residue was taken up in 30 ml of water, and the dicyclohexylurea was collected by filtration. The isopropylidene protecting group was removed by treatment with 70% acetic acid for 1 hr on the steam bath. The β-cyanoethyl group was removed by treatment with 0.3 N sodium hydroxide for 10 min at room temperature. Dowex 50 WX-8 (pyridinium form) was added to neutralize the base. Further purification could be effected as outlined under the alkylation and rearrangement procedure (**7** → **8** → **6**) (see below). The identity of the 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-phosphate (**6**) obtained from **4** with that obtained by the other route was checked by thin layer chromatography.

Adenosine 5′-β-Cyanoethyl Phosphate (**7**). Adenosine 5′-phosphate (**9**) (8 g, 22 mmoles) was lyophilized and partly dissolved in 250 ml of dry pyridine, then 60 ml of 3-hydroxypropionitrile was added and finally 25 g (116 mmoles) of DCC, which was warmed to 40° and added as a melt. The reaction mixture became clear after 5 hr and turned yellowish after 1 day. After 3 days, 10 ml of water was added with cooling. The dicyclohexylurea was removed by filtration, but additional urea precipitated when more water was added. The combined precipitates were treated with 10% pyridine solution and again filtered. The filtrate was evaporated to about 150 ml and extracted three times with 50 ml of cyclohexane. The aqueous solution was evaporated as far as possible without destruction of the diester and was then applied to a silica gel column. Ethyl acetate was used for elution of the 3-hydroxypropionitrile, and methanol was used for the diester. The solvent was evaporated, and ethanol was added to precipitate the diester. Filtration and further precipitation provided additional material, and the total was dried *in vacuo* over P_2O_5: yield 7.2 g (82%) of adenosine 5′-β-cyanoethyl phosphate (**7**), uniform by thin layer chromatography, paper chromatography (R_F 0.27 in ethanol–1 M NH_4OAc, 5:2), and electrophoresis (mobility relative to AMP (**1**) = 0.68). Compound **7** was alkylated directly with γ,γ-dimethylallyl bromide.

1-(γ,γ-Dimethylallyl)adenosine 5′-β-Cyanoethyl Phosphate (**8**). A solution of 5.56 g (14 mmoles) of adenosine 5′-β-cyanoethyl phosphate and 3.1 g (21 mmoles) of γ,γ-dimethylallyl bromide in 200 ml of dimethylformamide was maintained at room temperature for 12 hr, 1.49 g (10 mmoles) additional of the bromide was added, and after 36 hr the DMF and unreacted bromide were evaporated and the residue was taken up in 50 ml of water. The 290 mμ:260 mμ absorbance ratio of 0.12 indicated about 36% yield of 1-alkylated product. Dowex resin (1-X8 SO_4^{2-} form, 100–200 mesh) was washed thoroughly with water, methanol, and water. A 60 × 2.5 cm column of the anion-exchange resin was washed with water (2 l.). The product was added to the column, followed by water (100 ml) and 0.1 M Na_2SO_4 solution. Fractions (25 ml) were collected at the rate of 25 ml/10 min. Fractions 4–15 contained pure product and fractions 16–28 contained a mixture of alkylated and unalkylated diester. Fraction 15 was

lyophilized, extracted with methanol, evaporated, dissolved in water, and relyophilized for the preparation of an analytical sample. The 1-(γ,γ-dimethylallyl)-adenosine 5′-β-cyanoethyl phosphate, which was very hygroscopic, was of use mainly as an intermediate. The total yield was 32%; R_F 0.68 (ethanol–1 M NH_4OAc, 5:2), mobility 0.3 relative to AMP in electrophoresis; $\lambda_{max}^{H_2O}$ (pH 7 buffer) 258 mμ (ϵ 13,700), λ_{min} 235 mμ (ϵ 5700); $\lambda_{max}^{H_2O}$ (0.1 N NaOH) 260 mμ (ϵ 14,300) and sh 265 mμ, λ_{min} 236 mμ (ϵ 5650), $\epsilon_{290}/\epsilon_{260}$ 0.312; $\lambda_{max}^{H_2O}$ (0.1 N HCl) 257.5 mμ (ϵ 13,900) and 234 mμ (ϵ 5600). *Anal.* Calcd for $C_{18}H_{25}N_6O_7P$: C, 46.15; H, 5.38; N, 17.95. Found: C, 45.56; H, 5.34; N, 18.00.

Rearrangement of 1-(γ,γ-Dimethylallyl)adenosine 5′-β-Cyanoethyl Phosphate (**8**). Under the rearrangement conditions previously applied for the conversion of 1-(γ,γ-dimethylallyl)adenine (**10**) to 6-(γ,γ-dimethylallylamino)purine (**11**) (Leonard *et al.*, 1966), the phosphate diester **8** could be rearranged completely but the β-cyanoethyl group was partially split off. Accordingly, the rearrangement to 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-phosphate (**6**) was carried out in concentrated ammonium hydroxide at 60° (see below), whereby both rearrangement and protective group cleavage could be effected. For preparative purposes, however, a simpler method of obtaining **6** was by alkylation of the sodium salt of AMP, described in the following section.

Conversion of AMP (**9**) *to 6-(γ,γ-Dimethylallylamino)-9-β-D-ribofuranosylpurine 5′-Phosphate* (**6**) *by Alkylation and Rearrangement.* Adenosine 5′-phosphate (9.46 g, 27.3 mmoles) was transformed into the monosodium salt, which was lyophilized and suspended in 600 ml of anhydrous dimethylformamide, and 8 g of γ,γ-dimethylallyl bromide was added. The suspension was stirred at 25° for 15 hr, at which time some material remained undissolved. Accordingly, 300 ml of DMF and 1 g of γ,γ-dimethylallyl bromide (60 mmoles in all) were added and stirring was continued. After 32 hr the ratio of ultraviolet absorbance at 290 mμ:260 mμ was 0.097, equivalent to 34% of alkylated AMP (290:260 = 0.31 = 100%). The DMF and excess γ,γ-dimethylallyl bromide were removed *in vacuo*, and the residual yellow oil was dissolved in water and applied to a DEAE-cellulose column. The column was eluted with 4 l. of salt gradient of LiCl (0–0.1 M LiCl), and fractions of 25 ml were collected. Fractions 33–36 were combined, representing a yield (by ultraviolet analysis) of 7.6 mmoles (28%) of 1-(γ,γ-dimethylallyl)adenosine 5′-phosphate, as the salt. Fractions 37–47 contained further alkylated AMP in addition to starting material. Paper chromatography indicated three spots (ethanol–1 M NH_4OAc, 5:2): R_F 0.12, AMP (5%); R_F 0.34, 1-γ,γ-dimethylallyl-AMP (92%); R_F 0.69, not determined (3%). The 1-γ,γ-dimethylallyl-AMP was not purified further but was rearranged to the N^6 compound. The pH of the aqueous solution was adjusted to about 7.5, and the solution was heated on the steam bath for 2.5 hr while 0.1 N NaOH was added from time to time to maintain the pH at 7.5. The yield of 6-(γ,γ-dimethylallylamino)-9-β-D-ribofuranosyl purine 5′-phosphate was 6.4 mmoles. The brownish solution was treated with a small amount of charcoal at room temperature for about 10 min, then the solution was filtered. The final purification was done on a cellulose column by elution with isopropyl alcohol–ammonium hydroxide–water (7:1:2). Combined fractions yielded 5.1 mmoles (19% over-all) of N^6-(γ,γ-dimethylallyl)adenosine 5′-phosphate, pure by chromatography using two solvent systems (R_F 0.47 in ethanol–1 M NH_4OAc, 5:2, R_F 0.44 in isopropyl alcohol–ammonium hydroxide–water (7:1:2)) and by electrophoresis (relative mobility to AMP = 0.88). For analysis it was transformed into the dilithium salt, lyophilized, treated with absolute methanol, and relyophilized. *Anal.* Calcd for $C_{15}H_{20}Li_2N_5O_7P \cdot H_2O$: C, 40.46; H, 4.98; N, 15.73. Found: C, 40.46; H, 5.01; N, 15.51. Comparative pH values indicated this was the dilithium salt, and comparative permanganate tests (positive) with **1** indicated that the hydrate was part of the crystal lattice and not an integral part of the side chain. The ultraviolet spectra were as follows: $\lambda_{max}^{H_2O}$ (pH 7 buffer) 267 mμ (ϵ 19,200), λ_{min} 293 mμ (ϵ 3300); $\lambda_{max}^{H_2O}$ (0.1 N NaOH) 268 mμ (ϵ 19,000), λ_{min} 232 mμ (ϵ 3300); $\lambda_{max}^{H_2O}$ (0.1 N HCl) 264 mμ (ϵ 20,900) and 232.5 mμ (ϵ 5150).

Relative Rates of 1→N^6 Rearrangement. A weighed quantity, near 20 mg, of 1-(γ,γ-dimethylallyl)adenine (**10**), 1-(γ,γ-dimethylallyl)adenosine (as salt **3a**), and 1-(γ,γ-dimethylallyl)adenosine 5′-β-cyanoethyl phosphate (**8**) was dissolved separately in 5 ml of concentrated ammonium hydroxide and heated for 3 hr at 60°. At 30-min intervals 15-μl samples were removed and subjected to paper chromatography. The spots were cut out as equal sections of paper and eluted with 3 ml of ethanol or 3 ml of pH 7 buffer. A reference spot was used as a blank. The absorbance values were converted to ϵ_{max} values with the results shown in Figure 1. The curves were checked by quantitative thin layer chromatography.

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