The high resolution mass spectrum of the tris-TMS derivative showed the parent ion at 598.3935 (calcd for $C_{21}H_{62}O_5Si_3$: 598.3905) with other ions at 583, 567, 527, 508, 493, and 477.

Acknowledgment. The authors gratefully acknowledge Mr. P. A. Meulman and Mr. R. J. Wnuk for the ir and mass spectra. We especially acknowledge Dr. H. A. Karnes and associates for the preparation of generous supplies of intermediates.

Intramolecular Mechanism of the Allylic Rearrangement from O^6 to C-8 in the Guanine Series. Double Labeling Experiments

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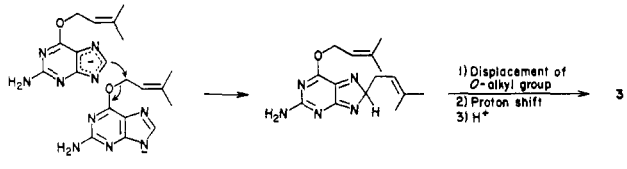
Abstract: The displacement reaction of 2-amino-6-chloropurine with the sodium salts of allylic alcohols proceeds through an intermediate O^6 ether to yield an 8-substituted guanine. The O^6 to C-8 rearrangement occurs with overall allylic retention, is partially controlled by the degree of methyl substitution on the allylic group and by the temperature, and proceeds with greatest facility through anionic species. In examining the general mechanism for the rearrangement of O^6-allylic guanines to 8-allylic guanines, several inter- and intramolecular pathways were considered. All were eliminated except for a double [3,3] sigmatropic shift *via* C-5. The intramolecular nature of the rearrangement was established by mass spectrometric analysis of the 8-substituted guanines formed from the reactions of 2-amino-6-chloropurine with the sodium salts of 3-methyl-2-buten-1-ol-^{18}O, 3-methyl-d_3-2-buten-1-ol-4,4,4-d_3, and mixtures of these having known ^{18}O and ^2H composition. Other intramolecular routes could be eliminated on the basis of the stability of postulated intermediates, *e.g.*, 7- or 9-substituted guanines, under the reaction conditions.

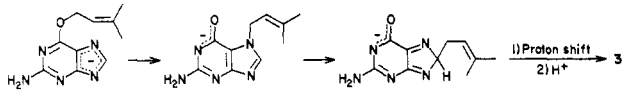
In an attempt to synthesize O^6-(3-methyl-2-butenyl)-guanine (**1**) by a substitution reaction of sodium 3-methyl-2-butenoxide upon 2-amino-6-chloropurine (**2**) in dioxane (101°), we obtained as the sole product 8-(3-methyl-2-butenyl)guanine (**3**).[1] The uniqueness of this $O^6 \rightarrow 8$ ring arrangement stimulated our curiosity concerning its mechanism. Complications are apparent both in proposing possible mechanisms and in showing which mechanisms could not be operative. The large number of heteroatoms forces the consideration of many possible pathways, and the rearrangement by our method occurs in heterogeneous phase. To keep the mechanistic considerations within reasonable bounds, two experimental facts should be noted. First, an overall retention of allylic structure of the isopentenyl side chain results from the rearrangement. Second, the initial displacement step places the oxygen of the alkoxide at the 6 position of the purine ring, and allylic C–O bond cleavage and rearrangement of the anion result in an 8-substituted guanine as the sole product. By contrast, the neutral O^6-allylic guanine requires higher temperatures for rearrangement and gives other products in addition to the 8-substituted guanine.[1]

One possible *intermolecular* rearrangement route can be visualized as involving a nucleophilic displacement upon the ether bond of first-formed **1** by the C-8 of another molecule of **1** as the anion, as illustrated in Scheme I. Two other possible intermolecular mechanisms involve alkylation at the N-7 or N-9 position of one purine by **1** with either retention or inversion of the allylic system, followed by movement of the isopentenyl group to C-8 while the C-8 hydrogen moves to neighboring nitrogen, in a manner similar to the final stages of the intramolecular pathways diagrammed in Schemes II and III.

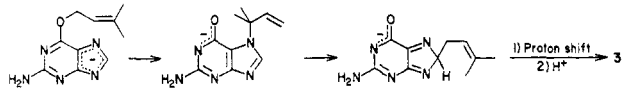
SCHEME I



SCHEME II



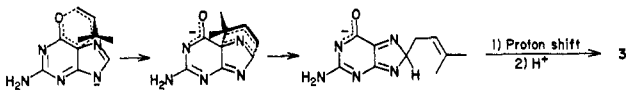
SCHEME III



Among the reasonable *intramolecular* rearrangement routes that can be visualized, the allylic side chain of **1** may be involved in an intramolecular alkylation of the nearby N-7 with either retention or inversion of the allylic group, followed by a transfer of the allylic group to C-8, as illustrated in Schemes II and III. A distinctly different mechanism starting with **1** would involve two consecutive [3,3] sigmatropic shifts *via* C-5, as illustrated in Scheme IV. The overall result resembes a para-Claisen rearrangement and can be viewed as a combined Claisen–Cope rearrangement. Local-

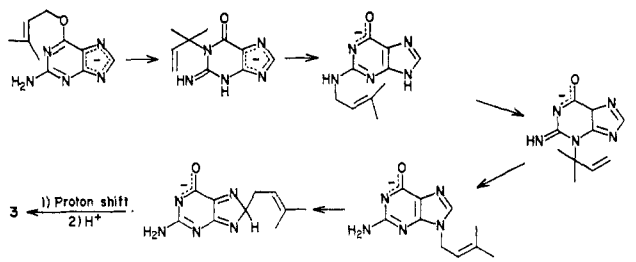
(1) C. R. Frihart and N. J. Leonard, *J. Amer. Chem. Soc.*, **95**, 7174 (1973); see especially ref 7; *cf.* the statement by B. S. Thyagarajan, *Advan. Heterocycl. Chem.*, **8**, 143 (1967): "Rearrangements of allyl ethers in the purines have been reported as early as 1935. However, there is little scope for unusual migrations in this ring system owing to the nonavailability of positions other than ring nitrogens for the attachment of the allyl moiety."

SCHEME IV



ization of the negative charge toward the 1,6,O^6-amide moiety would favor the first stage in the sequence. An extended series of [3,3] sigmatropic shifts might allow the lengthy pathway illustrated in Scheme V.

SCHEME V



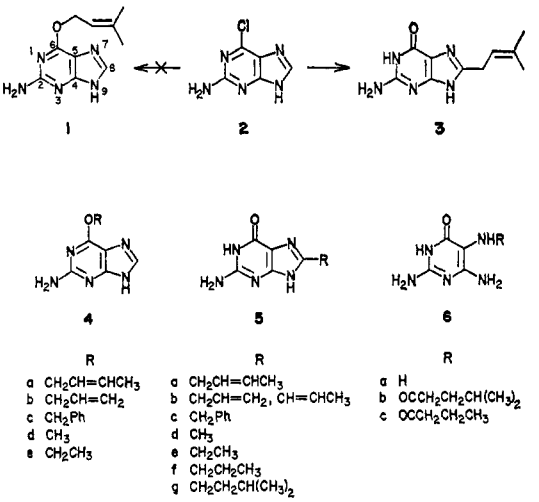
Results and Discussion

In order to be certain of the structure assignment as 8-(3-methyl-2-butenyl)guanine (**3**) of the product of the reaction of 2-amino-6-chloropurine (**2**) with 3-methyl-2-butenoxide,[1] we provided unambiguous proof by synthesis. Hydrogenation of **3** using palladium on charcoal yielded 8-(3-methylbutyl)guanine (**5g**). The same compound was obtained by unequivocal synthesis[2] involving acylation of 6-hydroxy-2,4,5-triaminopyrimidine (**6a**) with 4-methylvaleryl chloride to give 2,6-diamino-4-hydroxy-5-(4-methylvalerylamino)pyrimidine (**6b**), followed by basic cyclization of **6b** at 220–230° to **5g**.

Since none of the side-chain isomer, 8-(2-methyl-3-buten-2-yl)guanine, could be detected in the nmr spectrum of the rearrangement product **3** in crude form as initially isolated, the rearrangement must take place with overall allylic retention. The retention of the allylic structure during the rearrangement was confirmed in the reaction of sodium 2-butenoxide with **2** to yield 8-(2-butenyl)guanine (**5a**) as one of the products (see below), and no isomeric 8-(3-buten-2-yl)guanine could be detected in the nmr spectrum of the crude rearrangement product.

Since in the reaction of the sodium salt of isopentenyl alcohol with **2** only the rearranged product could be detected, the displacement reaction was carried out with the sodium salts of other allylic alcohols to obtain information as to intermediacy and scope. Under equivalent conditions, the reaction of sodium 2-propenoxide with **2** produced only O^6-(2-propenyl)guanine (**4b**). Compound **4b** was stable in refluxing dimethylformamide (153°) for 24 hr but was 50% rearranged in triglyme at 170° after 4 hr. The rearrangement mixture, which was not readily separable, showed six spots in tlc on a silica gel plate. After catalytic reduction of the mixture, followed by silica gel chromatography, a fraction was isolated that contained 8-propylguanine (**5f**). An authentic sample of **5f** was synthesized by condensation of butyryl chloride with 6-hydroxy-2,4,5-triaminopyrimidine (**6a**) to yield 5-butyrylamino-2,6-diamino-4-hydroxypyrimidine (**6c**), followed by ring closure of the sodium salt of the amide intermediate.

(2) J. H. Lister, "Fused Pyrimidines," Part II, D. J. Brown, Ed., Wiley-Interscience, New York, N. Y., 1971, Chapter II.

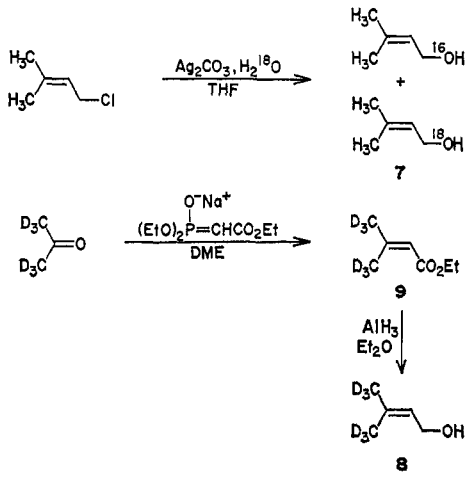


It was later observed that the anion of **4b** rearranged in diglyme at 150° during 5 hr to give a product which gave only one spot on silica gel tlc. This product was shown by nmr spectroscopy to consist of a mixture of α- and β-enes of 8-propenylguanine (**5b**) and could be reduced catalytically to 8-propylguanine (**5f**). These results indicate that the $O^6 \rightarrow 8$ rearrangement is facilitated by generation of the purine anion.

A crucial question is whether the rearrangement is allylic in nature, as required in Schemes III, IV and V, or not, as exhibited in Schemes I and II. Since some alkyl ethers are fairly good alkylating agents, we tested the stability of O^6-methylguanine (**4d**)[3] and O^6-ethylguanine (**4e**).[3] Since the sodium salts of these two compounds were completely stable in diglyme at 162° for 24 hr, it could be concluded that factors other than just the weakness of the ether linkage contributed to the rearrangement. Another factor that must be considered is whether the ability of the side chain to help stabilize a charge and therefore to increase the ease of dissociation of the ether bond plays a role in the rearrangement. Since a benzyl group cannot readily undergo an allylic rearrangement but can help stabilize charge, we tested the stability of O^6-benzylguanine (**4c**). The anion of this compound proved to be stable in diglyme at 162° for 24 hr; thus, on the basis of these selected experiments the rearrangement has been shown to be allylic in nature and Schemes I and II appear to be unlikely mechanisms for the rearrangement.

Although an intermolecular route seemed to be less likely mechanistically, we carried out a double labeling experiment to eliminate it conclusively. The experiment was based on the fact that in an intermolecular rearrangement the oxygen and the allylic side chain originate from separate antecedents while in an intramolecular rearrangement the oxygen and the allylic side chain must have a common antecedent. Accordingly, it was a matter of employing ^{18}O for ^{16}O in the original alcohol and labeling the side chain in the most convenient way with deuterium. Reaction of 2-amino-6-chloropurine (**2**) with a mixture of the sodium salt of 3-methyl-2-buten-1-ol-^{18}O (**7**) and the sodium salt of 3-methyl-d_3-2-buten-1-ol-4,4,4-d_3 (**8**), followed by examination of the rearranged 8-substituted guanine by mass spectrometry, would indicate whether the mechanism were mainly inter- or intra-

(3) R. W. Balsiger and J. A. Montgomery, *J. Org. Chem.*, 25, 1573 (1960).

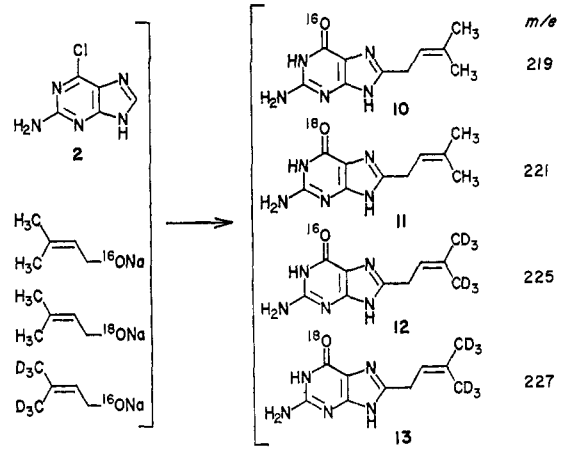


molecular, on the safe assumption that displacements and rearrangements based upon **7** and **8** would proceed at nearly equal rates. If the reaction proceeds by an intermolecular route the product peak at *m/e* 227 (which contains both ^{18}O and d_6 labels) would increase above the natural abundance[4] for either ^{18}O- or d_6-labeled product separately.

The d_6-labeled alcohol was readily prepared by the condensation of acetone-d_6 with the Wittig reagent generated from triethyl phosphonoacetate[5] in dimethoxyethane to yield the ester **9**, followed by its reduction with aluminum hydride[6] to yield hexadeuterated compound **8**. Determination of the most convenient synthesis of the ^{18}O-labeled alcohol **7** was more involved. Methods of synthesis of alcohols with high incorporation of ^{18}O label have not been very thoroughly investigated.[7] Moreover, the alcohol and its potential precursor acetate, as well as the commonly used reaction solvents dimethylformamide and diglyme, have similar boiling points. Attempts at displacement of the chloride of 1-chloro-3-methyl-2-butene using sodium hydroxide in diethyl ether, dimethyl sulfoxide, 1-methyl-2-pyrrolidone, and water or sodium acetate in dimethyl sulfoxide and 1-methyl-2-pyrrolidone failed to give respectable yields of the isopentenyl alcohol. However, by the use of a silver carbonate induced substitution of water on 1-chloro-3-methyl-2-butene,[8] we were able to obtain a relatively good yield of 3-methyl-2-buten-1-ol-^{18}O, although the ^{18}O content of the water was diluted, apparently by the unlabeled oxygens of the carbonate, and the alcohol contained an impurity which caused it to decompose faster than the pure alcohol. This route provided ^{18}O incorporation (15%) for our purpose, and the reaction with silver carbonate could be performed conveniently. Direct distillation through a short-path column provided a distillate that contained about 95% of the desired alcohol, as determined by glpc.

(4) N. C. Yang, W. Eisenhardt, and J. Libman, *J. Amer. Chem. Soc.*, **94**, 4030 (1972).
(5) J. Wolinsky and K. L. Erickson, *J. Org. Chem.*, **30**, 2208 (1965).
(6) The amount of saturated alcohol produced in the reduction can be cut from 10% to practically 0% by substituting aluminum hydride for the lithium aluminum hydride used by R. H. Hall and M. H. Fleysher in "Synthetic Procedures in Nucleic Acid Chemistry," W. W. Zorbach and R. S. Tipson, Ed., Wiley-Interscience, New York, N. Y., 1968, p 517.
(7) S. Oae and S. Tamagaki, "The Chemistry of the Hydroxyl Group," Part 2, S. Patai, Ed., Interscience, New York, N. Y., 1971, Chapter 15.
(8) C. M. McCloskey and G. H. Coleman, "Organic Syntheses," Collect. Vol. III, Wiley, New York, N. Y., 1955, p 434.

Control reactions were run in which each of the labeled alcohols **8** and **7** (both ^{16}O and ^{18}O alcohol) was caused to react separately with compound **2** to produce, respectively, **12** and a mixture of **10** and **11**. The



isotopic distribution in the product was compared with that of the starting alcohols as determined by the mass spectra, after subtracting the contribution to all the peaks from unlabeled (natural abundance) compounds. The results of the control reactions are given in columns 4, 5, 8, and 9 of Table IA. A mixture of the ^{18}O (some ^{16}O-) and d_6-labeled alcohols was made, and its isotopic distribution was determined by mass spectrometry (Table IA, column 6). Two aliquots of this mixture were separately treated, first with sodium hydride in dioxane and then with purine **2** to yield the labeled 8-substituted purine with the isotopic distribution given in columns 10 and 11 of Table IA. If the reaction were completely intramolecular, the labeled 8-substituted guanines produced in the reaction would be only **10**, **11**, and **12**, with no **13**, since there should be no interchange between the two labeled molecules. If, on the other hand, the rearrangement were intermolecular, some of the isopentenyl-d_6 side chain would become attached to a guanine with an ^{18}O label; thus, compound **13** would be present along with **10**, **11**, and **12**. By theoretical variation in the pathway from 100 to 0% intramolecular, the abundance of the peaks at *m/e* 219, 221, 225, and 227 can be calculated for these different possibilities (Table IB). From the inherent experimental errors in the abundance figures, our ability to detect an intermolecular pathway sets a minimum at about 10% for this route. To reinforce our observations and improve this limiting figure, we performed the entire procedure again using alcohol **7** that had a higher percentage incorporation of ^{18}O (25%). The observed and calculated isotope distribution figures are given in Table II and were derived in the same way as those in Table I (footnotes to Table I apply to both Tables). By the use of the higher ^{18}O incorporation, the limit for detection of an intermolecular rearrangement was lowered to 5%. Since a comparison of the data in Tables IB and IIB indicates that no intermolecular rearrangement was observed within our limits of detectability, we may safely state that the rearrangement must proceed at least 95% by an intramolecular route and we may further assume that the reaction is probably completely intramolecular.

With the rearrangement established as intramolecular, consideration may be limited to the pathways depicted

Table I. Isotopic Distribution Data

A. Experimental Data

Label present		Labeled alcohol[a]				Labeled purine[b]				
^{18}O	d_6	m/e	^{18}O, %	d_6, %	Mixture, %	m/e	^{18}O, %	d_6, %	Mixed reaction 1, %	Mixed reaction 2, %
No	No	86	84.8		48.7	219	86.0		47.5	48.6
Yes	No	88	15.2		7.9	221	14.0		7.7	7.5
No	Yes	92		100	43.4	225		100	44.8	43.8
Yes	Yes	94		0	0	227		0	0	0.1

B. Calculated for Product[c]

m/e	100% intra, %	95%, %	90%, %	85%, %	80%, %	0% intra, %
219	48.7	48.9	49.1	49.3	49.5	52.7
221	7.9	7.7	7.5	7.3	7.1	4.0
225	43.4	43.2	43.0	42.8	42.6	39.4
227	0	0.2	0.4	0.6	0.8	4.0

[a] The values given are the percentages for the isotopic composition for each sample after subtracting the natural abundance of the isotopes. The column headed by "Mixture" is a mixture of the ^{18}O- and d_6-labeled alcohols used in the double-labeling reaction. [b] The columns headed by "^{18}O" and "d_6" are the single-labeled reactions used as controls. The columns headed by "Mixed reaction 1" and "Mixed reaction 2" are the results of the double-labeling reactions using a mixture of the labeled alcohols given in column 6. [c] The label distribution for the rearranged purine is calculated from the isotopic distribution of the mixture of the labeled alcohols, which is given in part A, column 6. The 100% intra (for intramolecular) has the same distribution as the alcohol, and the 0% intra is a random distribution of the ^{18}O between molecules with d_6 and with no d_6.

Table II. Isotopic Distribution Data[a]

A. Experimental Data

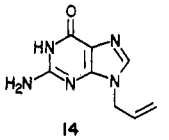
Label present		Labeled alcohol				Labeled purine				
^{18}O	d_6	m/e	^{18}O, %	d_6, %	Mixture, %	m/e	^{18}O, %	d_6, %	Mixed reaction 1, %	Mixed reaction 2, %
No	No	86	75.0		43.3	219	75.6		43.1	43.9
Yes	No	88	25.0		14.1	221	24.4		14.0	13.9
No	Yes	92		100	42.5	225		100	42.6	42.3
Yes	Yes	94		0	0	227		0	0.2	0

B. Calculated for Product

m/e	100% intra, %	97%, %	95%, %	90%, %	85%, %	80%, %	0% intra, %
219	43.3	43.5	43.7	44.0	44.4	44.7	50.3
221	14.1	13.9	13.7	13.4	13.0	12.7	7.0
225	42.5	42.3	42.1	41.8	41.4	41.1	35.5
227	0	0.2	0.4	0.7	1.1	1.4	7.0

[a] See footnotes for Table I.

in Schemes II–V. Of these, Scheme II has already been ruled unlikely since compounds **4c–4e** did not rearrange under the conditions employed. It is evident that for Schemes II, III, or V to be operative, the intermediate 7- or 9-allylic guanine would have to be capable of rearrangement to the 8 position under the same conditions that the O^6-allylic group rearranges to C-8. For studying such potential rearrangements, we selected the allyl group rather than crotyl or dimethylallyl to avoid the problem of allylic inversion. The sodium salt of 9-(2-propenyl)guanine (**14**) was unchanged in

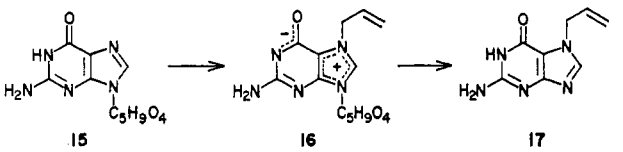


14

diglyme at 162° during 24 hr, except for isomerization of the double bond to give a mixture of the α- and β-enes.[9] Thus the 9-substituted guanine **14** is stable under conditions more strenuous than those required to rearrange **4b** completely into **5b**.

(9) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **30**, 3235 (1965).

For the synthesis of the 7-substituted compound, guanosine (**15**) was alkylated with allyl bromide in *N*,*N*-dimethylacetamide, followed by treatment of the salt with aqueous ammonium hydroxide and acetone to yield the zwitterionic 7-(2-propenyl)guanosine (**16**). Heating compound **16** in water caused the loss of the ribosyl group, yielding 7-(2-propenyl)guanine (**17**). When the sodium salt of **17** was heated in diglyme at



15 **16** **17**

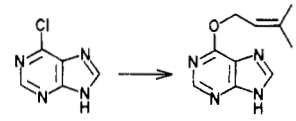
162° for 24 hr, no 8-allylguanine was produced. Thus, it is apparent that neither the 7- nor the 9-allylguanine, as the anion, is an intermediate in the rearrangement, and the sequences shown in Schemes II, III and V cannot account for the rearrangement.

We have thus eliminated all the pathways outlined except that given in Scheme IV. Whether this mechanism is compatible or not with the collected data on the rearrangement must be examined. Since the mechanism in Scheme IV resembles that of the para-Claisen

rearrangement, it was of interest to compare our results with those found with the aromatic-Claisen rearrangement, which has been extensively studied and reviewed.[10,11] We recognize that caution should be exercised in such a comparison of rearrangements occurring in heterocyclic and benzenoid rings having very different π systems, especially with the purine ring made up of a π-electron deficient pyrimidine portion and a π-electron rich imidazole portion.[12] Nevertheless, there are further similarities between our heterogeneous guanine ether rearrangement and the benzenoid-Claisen rearrangement which will become apparent.

The difference in the ease of rearrangement between the allyl and dimethylallyl case led us to examine whether the behavior of the crotyl compound lies between these two extremes. The reaction of 2-amino-6-chloropurine (**2**) with sodium 2-buten-1-oxide (2 equiv) at 100° in either 2-buten-1-ol or dioxane yielded a mixture of O^6-(2-butenyl)guanine (**4a**) and 8-(2-butenyl)-guanine (**5a**). The ratio of products was sensitive to the time of heating: **4a**–**5a** = 80:20 (74% yield) in 5 hr, 60:40 (70% yield) in 24 hr, and 10:90 (59% yield) in 48 hr. It was also sensitive to the temperature: **4a**–**5a** = 0:100 (70% yield) at 150° in diglyme in 5 hr. The sodium salt of **4a** was converted at 150° to **5a** (with its α-ene isomer) in 73% yield during 5 hr as the only isolable product. The rearrangement is thus facilitated by methyl groups at the γ position according to the comparison of the ease of rearrangement of the allyl, crotyl, and dimethylallyl compounds. No overall allylic inversion products, *i.e.*, 8-α-methylallylic products, were formed in the rearrangement of the crotyl and dimethylallyl compounds. The facilitation of the rearrangement by the γ-methyl groups has a parallel in the ortho-Claisen rearrangement. At least four examples have been reported in which an *O*-crotyl compound rearranges more readily than its O-allyl homolog.[13,14] The effect appears to be inductive rather than steric. In similar cases the effect of an α-methyl group is greater than that of a γ-methyl group, and this can be explained on steric grounds.[11] Contrary to earlier reports,[11c] γ,γ-dimethylallyl phenyl ether rearranges to give mainly the *p*-(γ,γ-dimethylallyl)phenol,[15] but the rate of rearrangement was not studied in comparison to the allyl and crotyl ethers so that the benzenoid case cannot be compared directly with the guanine case.

The 2-amino group in the initial purine **2** has an effect on the ease of rearrangement. This is apparent from the fact that the reaction of 6-chloropurine (**18**) with sodium 3-methyl-2-buten-1-oxide in dioxane at 100° yields O^6-(3-methyl-2-butenyl)hypoxanthine (**19**)



18 **19**

and not an 8-substituted hypoxanthine. Attempts to rearrange the sodium salt of **19** at 130 and 150° in diglyme yielded hypoxanthine as the only isolable purine (65% yield). It has been previously reported that electron donating groups such as amino in a benzenoid position para to the allylic ether accelerate the aromatic rearrangement.[14b,16] We plan to study the effects of 2- and 8-substituent groups on the 6-chloropurine nucleus, especially groups that might permit the trapping of the migrating allylic moiety at the C-5 bridgehead position.

The effects of the 2-amino group and the γ-methyl groups are both explicable in terms of electron donation to the electrocyclic transition state. In the same direction, conversion to the anion has a strong accelerating effect. The anion of the O^6-substituted guanines rearranges much more readily and cleanly than the neutral molecules, as determined in the case of **4b**. Early work by Claisen indicated that sodium carbonate facilitates the ortho rearrangement, but details are lacking.[11c] It has been reported that the anions of some allylic esters rearrange at much lower temperature than the neutral species,[17] and, more recently, that the lithium enolates of allyl esters undergo efficient rearrangement at −78° in tetrahydrofuran.[18] In contrast to the small amount of information on base catalysis of the Claisen rearrangement, acid catalysis has been thoroughly investigated.[19] The guanine ether rearrangement does not appear to be acid catalyzed, at least not for **4a** and **4b** with trifluoroacetic acid at 50° for 2 hr.

Finally, the intramolecular mechanism that we have proposed in Scheme IV, classified theoretically as involving two consecutive [3,3] sigmatropic shifts,[20] is consistent with all of the data collected thus far. Although there may be some concern about the ability of an allyl group to become attached to the C-5, the steric hindrance of the methyl groups in the isopentenyl case should be small since the side chain would be located above the planar hetero rings. Compounds of related bridgehead allylic or benzylic structure have been isolated from a reaction involving the alkylation of 5-acetyl-5,10-dihydro-1,3,7,8-tetramethylalloxazine.[21] The existence of such bridgehead-substituted compounds in the alloxazine series offers hope that C-5 intermediates may be isolable in the guanine series.

(10) (a) H.-J. Hansen and H. Schmid, *Chimia*, **24**, 89 (1970); (b) H.-J. Hansen and H. Schmid, *Chem. Brit.*, **5**, 108 (1969); (c) A. Jefferson and F. Scheinmann, *Quart. Rev., Chem. Soc.*, **22**, 391 (1968).

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Experimental Section

All melting points are uncorrected. The nmr spectra were recorded on Varian Associates A-60 and HA-100 spectrometers using tetramethylsilane (TMS) as an internal standard and $(CD_3)_2SO$ as the solvent unless otherwise stated. The ultraviolet spectra were obtained on a Cary Model 15 spectrophotometer. Microanalyses were performed by Mr. Josef Nemeth and associates who also weighed samples for the quantitative electronic absorption spectra. Low resolution mass spectra were obtained on a Varian-MAT CH-5 spectrometer and high resolution on a Varian-MAT 731 spectrometer, both coupled with a 620i computer and STATOS recorder.

The dioxane and diglyme used in the reactions described below were distilled from lithium aluminum hydride *in vacuo* just prior to use.

Thermal Stability of O^6-Methylguanine (4d) and O^6-Ethylguanine (4e). Sodium hydride (48 mg of a 51% oil dispersion, 1 mmol) and O^6-methylguanine[3] (165 mg, 1 mmol) were stirred in dry diglyme (10 ml). The solution was refluxed for 24 hr under nitrogen. After removing the diglyme *in vacuo*, the residue was dissolved in water, washed with ether, and acidified to pH 6 with 20% aqueous acetic acid. After cooling, the solid was removed by filtration and washed with water. Recrystallization from ethanol–water after using charcoal treatment yielded 132 mg (80%) of O^6-methylguanine. The silica gel tlc of the crude product and the purified product showed only one spot which had an R_f identical with that of the starting material, and the nmr spectra of the product and the starting material were identical. The same result was obtained with O^6-ethylguanine (**4e**).

3-Methyl-2-buten-1-ol. (a) The alcohol was made from 3-methyl-2-buten-1-oic acid using lithium aluminum hydride by the method of Hall and Fleysher,[6] except that 0.33 equiv of aluminum chloride was added to the lithium aluminum hydride suspension, to give 37.8 g (73%) of clear liquid, bp 84–85° (80 mm) (lit.[6] bp 84–86° (80 mm)); nmr (CCl_4) δ 1.63 (d, 3, J = 1 Hz, CH_3C), 1.68 (d, 3, J = 1 Hz, CH_3C), 4.06 (t, 2, J = 7 Hz, CCH_2O), 4.17 (s, 1, COH), 5.25 (m, 1, C=CHC). Glpc showed no trace of the saturated alcohol to be present using this AlH_3 reduction.

Anal. Calcd for $C_5H_{10}O$: C, 69.72; H, 11.70. Found: C, 69.38; H, 11.73.

(b) To a solution of 1-chloro-3-methyl-2-butene (5.2 g, 50 mmol) and water (0.9 g, 50 mmol) in dry tetrahydrofuran (50 ml) cooled to −5°, freshly prepared and dried silver carbonate (7.1 g, 26 mmol) was added over a 2-hr period. After stirring an additional hr, the mixture was allowed to come to room temperature under nitrogen. The mixture was filtered and the solid was washed with dry THF (100 ml). The solution was fractionally distilled to yield 2.05 g (45%) of the alcohol which had the same boiling point and nmr spectrum as the compound prepared in part a and was analytically pure.

8-(3-Methyl-2-butenyl)guanine (3). To a suspension of sodium hydride (0.50 g of a 51% oil dispersion; 11.7 mmol) in dioxane (20 ml), 3-methyl-2-butenol (1.00 g, 11.7 mmol) was added slowly. After the evolution of hydrogen gas had ceased, 2-amino-6-chloropurine (**2**) (1.00 g, 5.8 mmol) was added and the solution was refluxed for 24 hr. The dioxane was removed *in vacuo* and the residue was dissolved in water. After washing with ether, the aqueous solution was acidified to pH 6 with 20% aqueous acetic acid. After cooling, the solid was filtered, washed with water, and recrystallized from ethanol–water to give 0.92 g (74%) of white crystals, mp >360°; λ_{max} (H_2O) 246 nm (ϵ 9900), 278 (7400), (H_2O, 0.1 *N* HCl) 248 (12,400), 276 (sh, 8,200), (H_2O, 0.1 *N* NaOH) 276 (9500); nmr δ 1.67 (s, 6, $(CH_3)_2C$), 3.26 (d, 2, J = 7 Hz, $PuCH_2C$), 5.32 (t, 1, J = 7 Hz, C=CHC), 6.41–6.60 (s, 2, $PuNH_2$, exchanged by D_2O); mass spectrum at 10 eV showed peaks at m/e 219 (M^+), 204 ($M^+ - CH_3$), 178 ($M^+ - C_3H_7$), 165 ($M^+ - C_4H_6$), and 140 ($M^+ - C_6H_7$).

Anal. Calcd for $C_{10}H_{13}N_5O$: C, 54.78; H, 5.98; N, 31.95. Found: C, 54.70; H, 6.07; N, 32.25.

2,6-Diamino-4-hydroxy-5-(4-methylvalerylamino)pyrimidine (6b). After stirring a mixture of 6-hydroxy-2,4,5-triaminopyrimidine sulfate (2.4 g, 10 mmol) and sodium hydroxide (0.80 g, 20 mmol) in 30 ml of water (through which nitrogen had been bubbled for 18 hr) for 0.5 hr, 4-methylvaleryl chloride (1.3 g, 10 mmol) was added. The mixture was stirred for 4 hr and then neutralized with 1 *N* sodium hydroxide. The solid was removed by filtration and washed with water to yield 2.1 g (89%) of crude amide. Recrystallization from water yielded 1.9 g (81%) of white solid, mp 180° dec: nmr (TFA) δ 0.97 (d, 6, J = 5.5 Hz, $(CH_3)_2C$), 1.63 (m, 3, $CCH(C)_2$, CCH_2C), 2.52 (t, 2, J = 6.5 Hz, CCH_2CON), 7.2–7.7 (br, 2, NH's).

Anal. Calcd for $C_{10}H_{17}N_5O_2 \cdot H_2O$: C, 46.73; H, 7.44; N, 27.21. Found: C, 46.76; H, 7.21; N, 27.28.

8-(3-Methylbutyl)guanine (5g). (a) After dissolving 2,6-diamino-4-hydroxy-5-(4-methylvalerylamino)pyrimidine (**6b**) (0.5 g, 2 mmol) in aqueous NaOH (20 ml of 0.1 *N*, 2 mmol), the water was removed *in vacuo*. The solid was heated to 220–230° for 4 hr under nitrogen. After cooling, the residue was dissolved in water (20 ml) and then acidified to pH 6 using 20% aqueous acetic acid. The solid was removed by filtration and washed with water. The compound was purified on a silica gel column using $CHCl_3$–MeOH (1:1, v/v) and then recrystallized from the same solvent to yield 0.275 g (62%) of white crystals, mp >300°: λ_{max} (H_2O) 248 nm (ϵ 10,800), 277 (8400), (H_2O, 0.1 *N* HCl) 249 (12,700), 277 (8500), (H_2O, 0.1 *N* NaOH) 275 (9900); nmr (TFA) δ 1.09 (m, 6, $(CH_3)_2C$), 1.68 (m, 3, $CCH(C)_2$, CCH_2C), 3.06 (t, 2, J = 5 Hz, $PuCH_2C$); mass spectrum at 10 eV contained major peaks at m/e 221 (M^+), 206 ($M^+ - CH_3$), 192 ($M^+ - C_2H_5$), 178 ($M^+ - C_3H_9$), and 165 ($M^+ - C_4H_8$).

Anal. Calcd for $C_{10}H_{15}N_5O$: C, 54.28; H, 6.83; N, 31.65. Found: C, 54.58; H, 6.63; N, 31.74.

(b) After dissolving 8-(3-methyl-2-butenyl)guanine (44 mg, 2 mmol) as obtained from the rearrangement in absolute ethanol (300 ml), 5% Pd/C (100 mg) was added. The mixture was shaken for 18 hr under 3 atm of hydrogen. The mixture was filtered through Celite and thee thanol was removed. The residue was recrystallized from ethanol–water (4:1, v/v) to yield 31 mg (71%) of a white solid, mp >300°, which had the same uv, nmr, and mass spectra and R_f values on silica gel and cellulose tlc as the compound obtained by unequivocal synthesis.

O^6-(2-Propenyl)guanine (4b). To a suspension of sodium hydride (0.51 g of a 51% oil dispersion, 12 mmol) in dry dioxane (40 ml), allyl alcohol (0.70 g, 12 mmol) was added under a nitrogen atmosphere. After the evolution of hydrogen gas had ceased, 2-amino-6-chloropurine (1.0 g, 5.9 mmol) was added. The mixture was refluxed for 18 hr. After cooling to room temperature, the solvent was removed *in vacuo*. The residue was dissolved in water (20 ml) and extracted twice with ether (40 ml). The water layer was then acidified to pH 6 with 20% aqueous acetic acid. After cooling, the solid was removed by filtration and recrystallized once from ethanol–water (1:1, v/v) and then from dry tetrahydrofuran–*tert*-butyl alcohol (9:1, v/v) to yield 0.78 g (70%), mp 205° dec: λ_{max} (H_2O) 239 nm (ϵ 7900), 281 (8400), (H_2O, 0.1 *N* HCl) 230 (sh, 6000), 285 (11,000), (H_2O, 0.1 *N* NaOH) 245 (sh, 4900), 283 (8800); nmr, δ 5.02 (d, J = 5 Hz, of t, J = 1.5 Hz, 2, CCH_2O), 5.18–5.60 (m, 2, CH_2=C), 5.82–6.45 (m, 1, C=CHC), 6.25–6.35 (br s, 2, $PuNH_2$, exchanged by D_2O), 7.95 (s, 1, PuC_8H).

Anal. Calcd for $C_8H_9N_5O$: C, 50.25; H, 4.74; N, 36.63. Found: C, 50.21; H, 4.89; N, 36.52.

8-(1-Propenyl)- and (2-Propenyl)guanine (5b). To a suspension of sodium hydride (0.58 g of a 51% oil dispersion, 12 mmol) in dry diglyme (40 ml), allyl alcohol (0.70 g, 12 mmol) was added slowly. After the evolution of hydrogen gas ceased, 2-amino-6-chloropurine (1.0 g, 6 mmol) was added and the mixture was heated at 150° for 5 hr under nitrogen. After removing the solvent *in vacuo*, the residue was dissolved in water, washed with ether, and acidified to pH 6 with 20% aqueous acetic acid. The solid was filtered and recrystallized twice from ethanol–water to yield 0.75 g (67%) of a mixture of 8-(1-propenyl)guanine and 8-(2-propenyl)guanine, mp >300°: the nmr spectrum was complex due to overlapping peaks of the isomers; the mass spectrum had major peaks at 10 eV at m/e 191 (M^+), 165 ($M^+ - C_2H_2$), and 151 ($M^+ - C_3H_5$). The structure proof is given below.

Anionic Rearrangement of O^6-(2-Propenyl)guanine. A suspension of O^6-(2-propenyl)guanine (**4b**) (191 mg, 1 mmol) and sodium hydride (48 mg of a 51% oil dispersion, 1 mmol) in dry diglyme (10 ml) was stirred for 2 hr at room temperature and then heated at 150° for 5 hr under nitrogen. After removal of the solvent *in vacuo*, the residue was dissolved in water, washed with ether, and then acidified to pH 6 using 20% aqueous acetic acid. The solid was removed by filtration and recrystallized twice from ethanol–water to yield 124 mg (64%) of a mixture of 8-(1-propenyl)guanine and 8-(2-propenyl)guanine, mp >300°; the nmr spectrum was complex due to overlapping peaks of the isomers.

Anal. Calcd for $C_8H_9N_5O$: C, 50.25; H, 4.74; N, 36.63. Found: C, 50.62; H, 5.00; N, 36.33.

This mixture was dissolved in 300 ml of 95% ethanol, and 10% Pd/C (100 mg) was added. The mixture was hydrogenated at 1 atm for 8 hr and then filtered through Celite. After removal of the solvent, the residue was recrystallized twice from aqueous ethanol to yield 93 mg (75%) of white solid, mp >300°, with the same uv,

Table III. Averaged Mass Spectral Relative Intensity Readings

Unlabeled compd			
Alcohol		8-Substituted guanine	
m/e	Rel I	*m/e*	Rel I
84	0.5 ± 0.1	217	1.1 ± 0.1
85	10.1 ± 0.6	218	18.2 ± 0.8
86	100	219	100
87	8.2 ± 0.45	220	16.1 ± 0.9
88	1.1 ± 0.16	221	2.1 ± 0.11
		222	0.1 ± 0.04

Alcohol (^{18}O)		8-Substituted guanine	
m/e	Rel I	*m/e*	Rel I
85	24.7 − 10.1 = 14.6	217	1.3 − 1.1 = 0.2
86	100 = 100.	218	26.4 − 18.2 = 8.2
87	9.6 − 8.2 = 1.4	219	100 = 100.
88	19.0 − 1.1 = 17.9	220	18.8 − 16.1 = 2.7
89	1.0 − 0 = 1.0	221	18.3 − 2.1 = 16.2
		222	2.6 − 0.1 = 2.5
		223	0.4 − 0 = 0.4

$$^{16}O = \frac{100}{100 + 18} = 84.8\%$$

$$^{18}O = \frac{17.9}{100 + 18} = 15.2\%$$

$$^{16}O = \frac{100}{100 + 16} = 86.0\%$$

$$^{18}O = \frac{16.2}{100 + 16} = 14.0\%$$

Double-Label Reaction

Alcohol (^{18}O and d_6)	
m/e	Rel I
83	1.4
84	3.3 − 0.5 = 2.8
85	9.5 − 10.1 = (−)0.5
86	100 = 100
87	8.0 − 8.2 = (−)0.2
88	17.3 − 1.1 = 16.2
89	3.6 − (0.886 × 7.4) = (−)2.9
90	4.4 − (0.886 × 5.1) = (−)0.1
91	9.8 − (0.886 × 12.8) = (−)1.2
92	88.6 = 88.6
93	12.2 − (0.886 × 15.0) = (−)1.0
94	0.8 − (0.886 × 0.9) = 0.0 = 0.0

$$^{16}O = \frac{100}{100 + 16 + 89 + 0} = 48.7\%$$

$$^{18}O = \frac{16.2}{100 + 16 + 89 + 0} = 7.9\%$$

$$d_6,^{16}O = \frac{98.6}{100 + 16 + 89 + 0} = 43.4\%$$

$$d_6,^{18}O = \frac{0.0}{100 + 16 + 89 + 0} = 0.0\%$$

Alcohol (^{18}O)		8-Substituted guanine	
m/e	Rel I	*m/e*	Rel I
84	1.8	217	2.0 − 1.1 = 0.9
85	2.5 − 10.1 = (−)7.6	218	1.4 − 18.2 = (−)16.8
86	100 = 100	219	100 = 100
87	14.7 − 8.2 = 6.5	220	16.6 − 16.1 = 0.5
88	34.3 − 1.1 = 33.2	221	34.3 − 2.1 = 32.2
89	4.8 − 0 = 4.8	222	5.2 − 0.1 = 5.1
90	0.1	223	0.7

$$^{16}O = \frac{100}{100 + 33} = 75.0\%$$

$$^{18}O = \frac{33.2}{100 + 33} = 25.0\%$$

$$^{16}O = \frac{100}{100 + 32} = 75.6\%$$

$$^{18}O = \frac{32.2}{100 + 32} = 24.4\%$$

Double-Label Reaction

Alcohol (^{18}O and d_6)	
m/e	Rel I
84	1.3 − 0.5 = 0.8
85	2.4 − 10.1 = (−)7.7
86	100 = 100
87	13.0 − 8.2 = 4.8
88	33.6 − 1.1 = 34.8
89	4.4 − (0.980 × 7.4) = (−)2.9
90	1.2 − (0.980 × 5.1) = (−)3.8
91	8.4 − (0.980 × 12.8) = (−)4.1
92	98.0 = 98.0
93	14.7 − (0.980 × 15.0) = (−)0.1
94	1.0 − (0.980 × 0.9) = 0.1 = 0.1

$$^{16}O = \frac{100}{100 + 33 + 98} = 43.3\%$$

$$^{18}O = \frac{32.5}{100 + 33 + 98} = 14.1\%$$

$$d_6,^{16}O = \frac{98.0}{100 + 33 + 98} = 42.5\%$$

nmr, and mass spectra and R_f values on silica gel tlc in three solvent systems as the compound synthesized from hydroxytriaminopyrimidine (see below).

Neutral Rearrangement of O^6-(2-Propenyl)guanine. A solution of O^6-(2-propenyl)guanine (60 mg, 0.3 mmol) in triglyme (distilled from calcium hydride and then from sodium) (300 ml to give 10^{-3} *M* solution) was heated at 170° for 4 hr under nitrogen. After cooling, ether (500 ml) was added to the solution and it was extracted with aqueous NaOH (three times with 10 ml of 1 *N*). The aqueous solution was extracted twice with 100 ml of ether and then acidified to pH 6 with 20% HOAc. The solid was composed of ~50% 8-allylguanine, ~20% guanine, and ~30% of four other compounds as determined by silica gel tlc. Since the 8-allylguanine could not be separated from the other products, the entire sample was hydrogenated. The solid was dissolved in ethanol (300 ml) and 10% Pd/C (100 mg) was added. The mixture was hydrogenated at 1 atm for 8 hr and then filtered through Celite. After removing the solvent, the 8-propylguanine was purified on a silica gel column followed by recrystallization from methanol–water (1:1, v/v) to yield 20 mg (31%) of a white solid, mp >300°, with the same uv,

d_6-Labeled compd: Alcohol m/e	Rel I	8-Substituted guanine m/e	Rel I
89	7.4 ± 0.1	220	1.6 ± 0.2
90	5.1 ± 0.1	221	3.2 ± 0.6
91	12.8 ± 0.4	222	2.4 ± 0.4
92	100	223	9.8 ± 0.4
93	15.0 ± 0.2	224	10.3 ± 0.5
94	0.94 ± 0.04	225	100
95	0.73 ± 0.06	226	24.8 ± 0.8
		227	3.1 ± 0.05

I (See Table I)

Mixed reaction 1 m/e	Rel I	Mixed reaction 2 m/e	Rel I
217	1.2 − 1.1 = 0.1	217	0.9 − 1.1 = (−)0.2
218	22.0 − 18.2 = 3.8	218	13.8 − 18.2 = (−)4.4
219	100 = 100	219	100 = 100
220	23.4 − (0.942 × 1.6) = 21.9 − 16.1 = 5.8	220	25.8 − (0.907 × 1.6) = 24.4 − 16.1 = 8.3
221	21.3 − (0.942 × 3.2) = 18.2 − 2.1 = 16.2	221	20.4 − (0.907 × 3.2) = 17.5 − 2.1 = 15.4
222	4.9 − (0.942 × 2.4) = 2.6 − 0.1 = 2.5	222	5.8 − (0.907 × 2.4) = 3.6 − 0.1 = 3.5
223	9.9 − (0.942 × 9.8) = 0.7	223	9.4 − (0.907 × 9.8) = 0.5
224	19.6 − (0.942 × 10.3) = 9.8	224	16.8 − (0.907 × 10.3) = 7.4
225	94.2 = 94.2 = 94.2	225	90.7 = 90.7 = 90.7
226	21.7 − (0.942 × 24.8) = (−)1.6	226	24.6 − (0.907 × 24.8) = 2.0
227	2.7 − (0.942 × 3.1) = (−)0.2 = (−)0.2	227	3.4 − (0.907 × 3.1) = 0.3 = 0.3

$$^{16}O = \frac{100}{100 + 16 + 94 + 0} = 47.5\%$$

$$^{18}O = \frac{16.2}{100 + 16 + 94 + 0} = 7.7\%$$

$$d_6,^{16}O = \frac{94.2}{100 + 16 + 94 + 0} = 44.8\%$$

$$d_6,^{18}O = \frac{0}{100 + 16 + 94 + 0} = 0\%$$

$$^{16}O = \frac{100}{100 + 15 + 91 + 0.3} = 48.6\%$$

$$^{18}O = \frac{15.4}{100 + 15 + 91 + 0.3} = 7.5\%$$

$$d_6,^{16}O = \frac{90.7}{100 + 15 + 91 + 0.3} = 43.8\%$$

$$d_6,^{18}O = \frac{0.3}{100 + 15 + 91 + 0.3} = 0.1\%$$

II (See Table II)

Mixed reaction 1 m/e	Rel I	Mixed reaction 2 m/e	Rel I
217	1.0 − 1.1 = (−)0.1	217	1.6 − 1.1 = 0.5
218	1.8 − 18.2 = (−)16.4	218	1.9 − 18.2 = (−)16.3
219	100 = 100	219	100 = 100
220	30.3 − (0.987 × 1.6) = 28.7 − 16.1 = 12.6	220	23.0 − (0.966 × 1.6) = 21.5 − 16.1 = 5.4
221	37.8 − (0.987 × 3.2) = 34.6 − 2.1 = 32.5	221	37.0 − (0.966 × 3.2) = 33.9 − 2.1 = 31.8
222	4.7 − (0.987 × 2.9) = 2.3 − 0.1 = 2.2	222	2.6 − (0.966 × 2.4) = 0.3 − 0.1 = 0.2
223	11.5 − (0.987 × 9.8) = 1.7	223	8.1 − (0.966 × 9.8) = (−)1.4
224	12.4 − (0.987 × 10.3) = 2.1	224	8.1 − (0.966 × 10.3) = (−)1.9
225	98.7 = 98.7 = 98.7	225	96.6 = 96.6
226	29.4 − (0.987 × 24.8) = 4.9	226	23.0 − (0.966 × 2.48) = (−)0.7
227	3.6 − (0.987 × 3.1) = 0.5 = 0.5	227	2.8 − (0.966 × 3.1) = (−)0.2

$$^{16}O = \frac{100}{100 + 32 + 99 + 0.5} = 43.1\%$$

$$^{18}O = \frac{32.5}{100 + 32 + 99 + 0.5} = 14.0\%$$

$$d_6,^{16}O = \frac{98.7}{100 + 32 + 99 + 0.5} = 42.6\%$$

$$d_6,^{18}O = \frac{0.5}{100 + 32 + 99 + 0.5} = 0.2\%$$

$$^{16}O = \frac{100}{100 + 32 + 96 + 0} = 43.9\%$$

$$^{18}O = \frac{31.8}{100 + 32 + 96 + 0} = 13.9\%$$

$$d_6,^{16}O = \frac{96.6}{100 + 32 + 96 + 0} = 42.3\%$$

$$d_6,^{18}O = \frac{0}{100 + 32 + 96 + 0} = 0\%$$

nmr, and mass spectra and R_f values on silica gel and cellulose tlc as the compound synthesized from hydroxytriaminopyrimidine (see below).

5-Butyrylamino-2,6-diamino-4-hydroxypyrimidine (6c). After stirring a mixture of 6-hydroxy-2,4,5-triaminopyrimidine sulfate (2.4 g, 10 mmol) and sodium hydroxide (0.80 g, 20 mmol) in 30 ml of water (through which nitrogen had been bubbled for 18 hr) for 30 min, butyryl chloride (1.06 g, 10 mmol) was added. The mixture was stirred for 4 hr and then neutralized with 1 *N* sodium hydroxide. The solid was removed by filtration and washed with water (40 ml) to yield 2.0 g (95%) of crude amide. Recrystallization from water yielded 1.8 g (86%) of white solid, mp 185° dec: nmr (TFA) δ 1.10 (t, 3, J = 6 Hz, CH_3C), 1.88 (m, 2, CCH_2C) 2.64 (t, 2, J = 7 Hz, CCH_2CON), 7.9 (br, 2, NH's).

Anal. Calcd for $C_8H_{13}N_5O_2 \cdot H_2O$: C, 41.91; H, 6.59; N, 30.55. Found: C, 42.05; H, 6.65; N, 30.24.

8-Propylguanine (5f). After dissolving 5-butyrylamino-2,6-diamino-4-hydroxypyrimidine (0.42 g, 2 mmol) in aqueous sodium hydroxide (20 ml of 0.1 *N*, 2 mmol), the water was removed *in vacuo* and the solid was heated at 220–230° for 4 hr under nitrogen.

After cooling, the residue was dissolved in water (20 ml) and then acidified to pH 6 using 20% aqueous acetic acid. The solid was removed by filtration, washed with water, and placed on a 20-g silica gel column. The product was eluted with chloroform–methanol (1:1, v/v) and then recrystallized from methanol–water (1:1, v/v) to yield 0.25 g (66%) of white solid, mp >300°; λ_{max} (H_2O) 248 (ε 10,800), 278 (8400), (H_2O, 0.1 *N* HCl) 249 (13,200), 276 (8300), (H_2O, 0.1 *N* NaOH) 276 nm (9000); nmr (TFA) δ 1.17 (t, 3, *J* = 6.5 Hz, CH_3C), 2.04 (m, 2, CCH_2C), 3.22 (t, 2, *J* = 7 Hz, $PuCH_2C$); the mass spectrum at 10 eV showed major peaks at *m/e* 193 (M^+), 178 ($M^+ - CH_3$), and 165 ($M^+ - C_2H_4$).

Anal. Calcd for $C_8H_{11}N_5O$: C, 49.73; H, 5.74; N, 36.25. Found: C, 49.67; H, 5.89; N, 36.02.

***O*⁶- and 8-(2-Butenyl)guanine (4a and 5a, Respectively).** To a suspension of sodium hydride (0.58 g of a 51% oil dispersion, 12 mmol) in dry dioxane (40 ml), crotyl alcohol (0.87 g, 12 mmol) was added slowly. After the evolution of hydrogen had ceased, 2-amino-6-chloropurine (1.0 g, 6 mmol) was added and the mixture was refluxed for 24 hr under nitrogen. After removing the solvent *in vacuo*, the residue was dissolved in water, washed with ether, and acidified to pH 6 with 20% aqueous acetic acid. The solid was filtered and applied to a 50-g silica gel column. The fraction eluted with chloroform–methanol (9:1, v/v) contained *O*⁶-(2-butenyl)guanine (**4a**). Recrystallization from tetrahydrofuran–*tert*-butyl alcohol (9:1, v/v) yielded 0.51 g (43%) of a white solid, mp above 210° dec: λ_{max} (H_2O) 240 (ε 7400), 280 (8100); (H_2O, 0.1 *N* HCl) 232 (sh, 5500), 286 (11,500), (H_2O, 0.1 *N* NaOH) 245 (sh, 4300), 283 nm (7900); nmr δ 1.71 (d, 3, *J* = 6 Hz, CH_3C), 4.90 (m, 2, CCH_2O), 5.83 (m, 2, C=CHC), 6.15 (br s, 2, $PuNH_2$, exchanged by D_2O), 7.86 (s, 1, PuC_8H).

Anal. Calcd for $C_9H_{11}N_5O$: C, 52.67; H, 5.40; N, 34.13. Found: C, 52.37; H, 5.34; N, 34.02.

The fraction from the silica gel column eluted with chloroform–methanol (1:1, v/v) contained 8-(2-butenyl)guanine (**5a**). Recrystallization from ethanol–water (1:1, v/v) yielded 0.34 g (28%) of a yellowish solid, mp >300°: λ_{max} (H_2O) 248 (ε 10,400), 276 (7700), (H_2O, 0.1 *N* HCl) 249 (12,900), 278 (8400), (H_2O, 0.1 *N* NaOH) 276 nm (9000); nmr, δ 1.69 (m, 3, CH_3C), 3.31–3.49 (m, 2, $PuCH_2C$), 5.51–5.72 (m, 2, C=CHC), 6.08–6.29 (br s, 2, $PuNH_2$, exchanged by D_2O).

Anal. Calcd for $C_9H_{11}N_5O$: C, 52.67; H, 5.40; N, 34.13. Found: C, 52.50; H, 5.39; N, 34.27.

8-(1-Butenyl)- and -(2-Butenyl)guanine. (a) To a suspension of sodium hydride (0.58 g of a 51% oil dispersion, 12 mmol) in dry diglyme (40 ml), crotyl alcohol (0.87 g, 12 mmol) was added slowly. After the evolution of hydrogen ceased, 2-amino-6-chloropurine (1.0 g, 6 mmol) was added and the mixture was heated at 150° for 5 hr under nitrogen. After removal of the solvent *in vacuo*, the residue was dissolved in water, washed with ether, and acidified to pH 6 with 20% aqueous acetic acid. The solid was filtered and recrystallized twice from ethanol–water to yield 0.843 g (70%) of a mixture of 8-(1-butenyl)guanine and 8-(2-butenyl)guanine, mp >300°; the nmr spectrum was very complex due to overlapping peaks of the isomers.

(b) A suspension of *O*⁶-(2-butenyl)guanine (205 mg, 1 mmol) and sodium hydride (48 mg of a 51% oil dispersion, 1 mmol) in dry diglyme (10 ml) was stirred for 2 hr and then heated at 150° for 5 hr under nitrogen. After removal of the solvent *in vacuo*, the residue was dissolved in water, washed with ether, and acidified to pH 6 using 20% acetic acid. The solid was filtered and recrystallized twice from ethanol–water to yield 0.150 g (73%) of a mixture of 8-(1-butenyl)guanine and 8-(2-butenyl)guanine, mp >300°; the nmr spectrum was complex due to overlapping peaks of the isomers.

Anal. Calcd for $C_9H_{11}N_5O$: C, 52.67; H, 5.40; N, 34.13. Found: C, 52.50; H, 5.39; N, 34.27.

Thermal Stability of *O*⁶-Benzylguanine (4c). Sodium hydride (48 mg of a 51% oil dispersion, 1 mmol) and *O*⁶-benzylguanine[22] (241 mg, 1 mmol) were stirred in dry diglyme (10 ml). The solution was heated at reflux for 24 hr under nitrogen. After removal of the diglyme *in vacuo*, the residue was dissolved in water, washed with ether, and acidified to pH 6 using 20% aqueous acetic acid. After cooling, the solid was removed by filtration and washed with water. Recrystallization from ethanol–water yielded 198 mg (82%) of *O*⁶-benzylguanine, mp 200–201°. The silica gel tlc of the crude product and the purified product showed only one spot which had an R_f identical with that of the starting material, and the nmr spectra of the product and the starting material were identical.

***O*⁶-(3-Methyl-2-butenyl)hypoxanthine or 6-(3-Methyl-2-butenyloxy)purine (19).** To a suspension of sodium hydride (0.63 g of a 51% oil dispersion, 13 mmol) in dry dioxane (40 ml), 3-methyl-2-buten-1-ol (1.11 g, 13 mmol) was added slowly. After the evolution of hydrogen ceased, 6-chloropurine (1.0 g, 6.5 mmol) was added and the solution was refluxed for 24 hr. After removal of the dioxane *in vacuo*, the residue was dissolved in water, washed with ether, and acidified to pH 6 using 20% aqueous acetic acid. After cooling, the solid was filtered and recrystallized from ethanol–water to give 0.95 g (72%) of white solid, mp 210° dec: λ_{max} (H_2O) 249 (ε 11,300), (H_2O, 0.1 *N* HCl) 247 (11,600), (H_2O, 0.1 *N* NaOH) 262 nm (12,400); nmr δ 1.81 (s, 6, $(CH_3)_2$), 5.09 (d, 2, *J* = 7 Hz, CCH_2O), 5.45–5.60 (m, 1, CCHC), 8.38 and 8.54 (2s, 2, PuC_2H and PuC_8H), 13.0–13.8 (very br, 1, PuN_9H, exchanged by D_2O). The sodium salt yielded only hypoxanthine when heated at 130 or 150° in diglyme.

Anal. Calcd for $C_{10}H_{12}N_4O$: C, 58.81; H, 5.92; N, 27.43. Found: C, 58.84; H, 5.82; N, 27.20.

9-(2-Propenyl)guanine (14). To a suspension of guanine (4.53 g, 30 mmol) in dry *N*,*N*-dimethylformamide (500 ml), sodium hydride (1.32 g, 30 mmol) was added. After stirring for 3 hr, allyl chloride (2.30 g, 30 mmol) in dry *N*,*N*-dimethylformamide was added over a 2-hr period. After stirring for 18 hr, the suspension was cooled to −20° and then filtered to yield 4.55 g of white solid containing 9-(2-propenyl)guanine and guanine. Purification on a silica gel column using ethyl acetate–ethanol (1:1, v/v) to elute the product yielded 2.57 g (45%) of white crystals, mp >300°: λ_{max} (H_2O) 253 (ε 13,200), 270 (sh, 10,000), (H_2O, 0.1 *N* HCl) 254 (13,100), 276 (9800); (H_2O, 0.1 *N* NaOH) 256 (10,700), 268 nm (11,500); nmr δ 4.64 (d, *J* = 5 Hz, of t, *J* = 1.5 Hz, 2, $PuCH_2C$), 4.80–5.35 (m, 2, $C=CH_2$), 5.87–6.39 (m, 1, C=CHC), 6.55 (s, 2, $PuNH_2$, exchanged by D_2O), 7.80 (s, 1, PuC_8H).

Anal. Calcd for $C_8H_9N_5O$: C, 50.25; H, 4.74; N, 36.63. Found: C, 50.11; H, 4.71; N, 36.36.

Thermal Stability of 9-(2-Propenyl)guanine. Sodium hydride (48 mg of a 51% oil dispersion, 1 mmol) and 9-(2-propenyl)guanine (191 mg, 1 mmol) were stirred in dry diglyme (10 ml). The solution was refluxed for 24 hr under nitrogen. After removal of the diglyme *in vacuo*, the residue was dissolved in water, washed with ether, and acidified to pH 6 using 20% aqueous acetic acid. The solid was removed by filtration and recrystallized from water to yield 172 mg (90%) of a white solid, mp 300°. The silica gel tlc of the crude and purified product showed only one spot which had an R_f the same as the starting material and no detectable spot (<5%) which had the same R_f as 8-propenylguanine. The nmr spectrum of the product showed it to be a mixture of **14** with 9-(1-propenyl)guanine.

7-(2-Propenyl)guanosine 16. Guanosine (**15**) (11.2 g, 40 mmol) and 3-bromopropene (12.0 g, 100 mmol) in *N*,*N*-dimethylacetamide (100 ml) were heated at 50° for 30 hr under nitrogen. After adding Celite (4 g), the mixture was filtered. Ethanol (300 ml) and then petroleum ether (1200 ml of bp 65–110°) were added to the filtrate. After decanting the supernatant from the oil, acetone (500 ml) was added. After decanting the acetone from the very gummy solid, it was dissolved in water cooled to 5°. After adjusting the pH to 9.5 with concentrated ammonium hydroxide, acetone (250 ml) was immediately added and the pH readjusted to pH 8. Filtration yielded 7.5 g (58%) of white solid, mp 110° dec; λ_{max} (H_2O) 275 (pH >3) 256, 275 (sh), (pH >10) 265 nm; nmr δ 9.40 (s, 1, PuC_8H). This is the same procedure by which 7-methylguanosine was made.[23] The product was used directly in the following reaction.

7-(2-Propenyl)guanine (17). A solution of 7-(2-propenyl)guanosine (1.6 g, 5 mmol) in water (100 ml) was heated on a steam bath for 1 hr. After cooling, the brown solid was removed by filtration and recrystallized from water using charcoal to yield 0.76 g (80%), mp >300°; λ_{max} (H_2O) 247 (ε 6200), 284 (7900), (H_2O, 0.1 *N* HCl) 250 (10,900), 270 (sh, 7400), (H_2O, 0.1 *N* NaOH) 240 (sh, 7000), 279 nm (7700); nmr (TFA) δ 5.20 (d, 2, *J* = 5.5 Hz, $PuCH_2C$), 5.38–6.45 (m, 3, $CH_2=C$, C=CHC), 8.75 (s, 1, PuC_8H).

Anal. Calcd for $C_8H_9N_5O$: C, 50.25; H, 4.74; N, 36.63. Found: C, 50.21; H, 4.75; N, 36.40.

Thermal Stability of 7-(2-Propenyl)guanine. Sodium hydride (48 mg of a 51% oil dispersion, 1 mmol) and 7-(2-propenyl)guanine (191 mg, 1 mmol) were stirred in dry diglyme (10 ml). The solution was refluxed for 24 hr under nitrogen. After removing the diglyme *in vacuo*, the residue was dissolved in water, washed with

(22) W. A. Bowles, F. H. Schneider, L. R. Lewis, and R. K. Robins, *J. Med. Chem.*, **6**, 471 (1963); see ref 19 in ref 1.

(23) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, **85**, 193 (1963).

ether, and acidified to pH 6 using 20% aqueous acetic acid. The solid was removed by filtration and recrystallized from water to yield 43 mg (90%) of a white solid, mp >300°. The silica gel tlc of the crude and purified products showed only one spot which had an R_f the same as the starting material and no detectable spot (<5%) which had an R_f the same as 8-propenylguanine. The nmr spectrum of the product was the same as that of the starting material.

Ethyl 3-Methyl-d_3-2-butenoate-4,4,4-d_3 (9). Sodium hydride (7.2 g of a 51% oil dispersion, 0.17 mol) was suspended in dimethoxyethane (DME) (150 ml distilled from $LiAlH_4$). While cooling in an ice bath, triethylphosphonoacetate[5] (40.4 g, 0.18 mol) was slowly added. After stirring for an additional hr, acetone-d_6 (11.5 g of 99.5% minimum isotopic purity, 0.18 mol) in DME (20 ml) was slowly added over a 2-hr period. The mixture was allowed to come to room temperature and then heated at 50° for 15 min. After cooling, water (20 ml) and ether (100 ml) were added and the layers were separated. The aqueous layer was washed twice with ether (100 ml) and the combined ethereal fractions were washed with water (100 ml). After drying over Na_2SO_4, the ethereal solution was fractionally distilled to yield 19.3 g (80%) of the ester, bp 75–76° (70 nm); nmr δ ($CDCl_3$) 1.26 (t, 3, CH_3C), 4.15 (q, 2, CCH_2O), 5.68 (s, 1, C=CHC).

3-Methyl-d_3-2-buten-1-ol-4,4,4-d_3 (8). To a suspension of lithium aluminum hydride (5.7 g, 150 mmol) in ether (200 ml), aluminum chloride (6.6 g, 50 mmol) was added. After stirring for 0.5 hr, ethyl 3-methyl-d_3-2-butenoate-4,4,4-d_3 (26.8 g, 200 mmol) in ether (100 ml) was added over a 4-hr period and stirring was continued for 2 hr more. After the addition of water to destroy excess hydride, 10% aqueous sulfuric acid was added until the precipitate dissolved. The layers were separated and the aqueous layer was extracted with ether. The combined ethereal layers were washed with water and dried over sodium sulfate. Fractional distillation yielded 10.8 g (58%) of the alcohol, bp 82–85° (70 nm): nmr δ ($CDCl_3$) 3.39 (s, 1, COH), 4.08 (d, 2, J = 7 Hz, CCH_2O), 5.39 (t, 1, C=CHC).

3-Methyl-2-buten-1-ol-^{18}O (7). To a solution of redistilled 1-chloro-3-methyl-2-butene (5.2 g, 50 mmol) and water-^{18}O (0.95 g of 50% ^{18}O, 50 mmol) in tetrahydrofuran (50 ml distilled from lithium aluminum hydride) cooled to −5° under nitrogen, fresh silver carbonate (7.1 g, 26 mmol) was added over a 2-hr period. After stirring an additional hour, the mixture was allowed to come to room temperature. The mixture was filtered through Cacelite pad and the solid was washed with dry tetrahydrofuran (100 ml). The solution was dried with calcium hydride, filtered under nitrogen, and fractionally distilled to yield 2.0 g (43%) of **7**, bp 85–86° (80 mm). From the mass spectrum it was determined that the alcohol contained 84.8% ^{16}O and 15.2% ^{18}O.

This reaction was repeated using 0.95 g of water containing 98.5% ^{18}O to yield after distillation 2.1 g (45%) of the alcohol, bp 84–86° (80 mm). From the mass spectrum the isotopic distribution was calculated to be 75.0% ^{16}O and 25.0% ^{18}O.

A reasonable (~50%) yield of 3-methyl-2-buten-1-ol-^{18}O could be isolated from the silver carbonate reaction when 1 equiv of labeled water was used. In attempts to increase the incorporation of ^{18}O into the alcohol by using 2 equiv of labeled water, the amount of alcohol that could be isolated was drastically reduced (<25% yield).

8-(3-Methyl-2-butenyl)guanine-^{18}O or d_6 Labeled. To a suspension of sodium hydride (52 mg of a 51% oil dispersion, 1.2 mmol) in dry dioxane (5 ml), 3-methyl-2-buten-1-ol containing an ^{18}O label (**7**) or a d_6 label (**8**) or a mixture of the two (110 mg, 1.2 mmol) was added; After 2 hr, 2-amino-6-chloropurine (**2**) (100 mg, 0.6 mmol) was added and the mixture was refluxed for 24 hr. After removing the diglyme *in vacuo*, the residue was dissolved in water, quickly washed with ether, and acidified to pH 6 using 20% aqueous acetic acid. After filtering, the solid was washed with water and recrystallized from ethanol to yield 90 mg (67%) of white solid, mp >300°. The ratio of isotopically labeled compounds making up the 8-substituted guanine product was determined by the mass spectrum.

Table IV. Thin-Layer Chromatographic Data

Compd	R_f[a,c]	R_f[b,c]
2-Amino-6-chloropurine (**2**)	0.25	0.48
O^6-Methylguanine (**4d**)	0.32	0.53
O^6-Ethylguanine (**4e**)	0.34	0.58
O^6-(2-Propenyl)guanine (**4b**)	0.36	0.60
O^6-(2-Butenyl)guanine (**4a**)	0.38	0.65
O^6-Benzylguanine (**4c**)	0.54	0.66
Guanine (**5**, R = H)	0.03	0.09
8-Methylguanine (**5d**)	0.04	0.14
8-Propylguanine (**5f**)	0.08	0.30
8-(2-Butenyl)guanine (**5a**)	0.11	0.33
8-(3-Methyl-2-butenyl)guanine (**3**)	0.11	0.35
8-(3-Methylbutyl)guanine (**5g**)	0.12	0.36
N^2-Benzylguanine[1]	0.14	0.37
7-Allylguanine (**17**)	0.13	0.42
9-Allylguanine (**14**)	0.14	0.44

[a] R_f values are for silica gel plates using chloroform–ethanol (9:1, v/v) as the solvent. [b] R_f values are for silica gel plates using chloroform–methanol (8:2, v/v) as the solvent. [c] Numerical values are difficult to reproduce accurately in several cases and should be considered to be only indicators of relative mobilities.

Determination of Isotopic Distribution. Since guanines are excellent bases, they pick up free protons very well upon electron bombardment. This protonation causes the peak one mass unit higher than M to be larger than would be expected from the calculated natural abundance for M + 1. Since for the same reason the peak at M + 2 is also larger than would be expected, the enrichment in ^{18}O cannot be calculated merely by subtracting the expected natural abundance contribution to this peak. Another way to calculate the enrichment in ^{18}O is to subtract the portions of the peaks that are derivative of the unlabeled compound. As shown in Table III, the contributions to all the peaks from the unlabeled guanine were subtracted from the observed peaks for the ^{18}O-labeled guanine. The value of the observed peak was calculated from three runs and the average deviation was 10%. For internal comparison purposes the ^{18}O and ^{16}O isotopic distribution calculated by the formula ^{16}O or $^{18}O/(^{16}O + {}^{18}O)$ served as a check. All of the calculations were done in a similar manner. For the double-labeled experiment, the background spectra for both the unlabeled compound and the d_6 labeled were subtracted from the observed spectrum for the double-labeled compound.

Thin-layer chromatography on silica gel provided the best general method for analyzing the products of rearrangement and for following the reactions. The R_f values are given in Table IV. Since, in general, the guanine derivatives decompose on heating and 8-substituted guanines melt >300°, melting points do not offer meaningful criteria of purity in this series.

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