Registry No.—**1**, 23462-75-1; **2**, 43152-89-2; **3**, 19090-03-0; **4**, 43152-90-5; **5**, 5519-50-6; **6**, 43152-92-7; **7**, 43152-93-8; **8**, 43152-94-9; **9**, 35890-61-0; **10**, 43152-95-0; **11**, 43152-96-1; **12**, 43152-97-2; **13**, 43152-98-3; **14**, 43152-99-4; **15**, 43153-00-0; **16**, 43153-01-1; 2-methyltetrahydropyran, 10141-72-7; 3-hydroxytetrahydropyran, 19752-84-2; 1,2-dimethylpiperidine, 671-36-3; 2-methylpiperidine, 109-05-7; formaldehyde, 50-00-0; 1-ethyl-2-methylpiperidine, 766-52-9; 3-hydroxypiperidine, 6859-99-0.

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Mechanism of Electron Impact Induced Elimination of Methylenimine from Dimethylamino Heteroaromatic Compounds[1]

D. L. von Minden, J. G. Liehr, M. H. Wilson, and James A. McCloskey*

Institute for Lipid Research and Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77025

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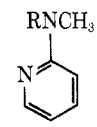
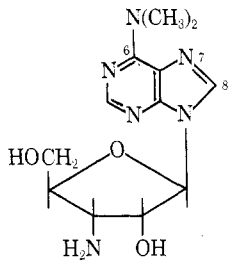
The characteristic expulsion of methylenimine from dimethylamino α-substituted N heterocycles was examined using models in which one methyl group is deuterium labeled, *e.g.*, 2-(*N*-methyl-*N*-methyl-d_3-amino)pyridine. Equal amounts of CD_2NH and CH_2ND are expelled, rather than CD_2ND and CH_2NH as expected from previously proposed mechanisms. A general mechanism is proposed in which the reaction is initiated by abstraction of methyl hydrogen by a charge-localized ring nitrogen, followed by skeletal rearrangement. Evidence from isomeric deazaadenosine derivatives suggests that N-7 rather than N-1 is the primary reactive site in N^6,N^6-dimethyladenine and related nucleosides. Complex hydrogen interchange reactions also occur during expulsion of methylenimine from analogous monomethyl-substituted heterocycles, but by a different reaction mechanism.

The expulsion of methylenimine is a common process observed in the mass spectra of many heteroaromatic compounds which bear methyl- or dimethylamino substituents,[2-15] *e.g.*, the simplest model **1**.[16,17] This structurally diagnostic reaction finds important use in the structural characterization of methylated purine bases or nucleosides, which often contain methyl- or dimethylamino groups. In the case of dimethylamino derivatives the mechanism of this reaction has been the subject of several investigations.[2-4,16,17] Eggers and coworkers postulated[2]



1a, R = H
b, R = CH_3

that expulsion of the elements of CH_3N from the base moiety of the puromycin nucleoside (**2**) occurs from the imidazole ring rather than the more obvious site at N^6.



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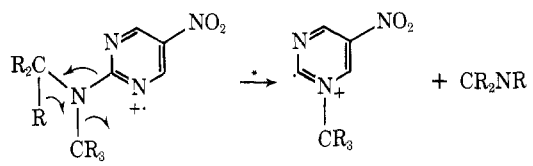
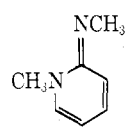
However, that mechanism was disproved by deuterium labeling at C-8.[7] The most reasonable proposal was that of Rahamim, *et al.*, who studied 2-dimethylamino-5-nitropyrimidine (**3a**) and 6-dimethylaminopurine and envisioned methyl migration to an endocyclic nitrogen (**3a** → ion a).[4]



3a, R = H
b, R = D

a

Their mechanism was supported by deuterium labeling (compound **3b**) and the observation that the reaction does not occur with *N,N*-dimethylaniline, thereby indicating participation of the adjacent ring nitrogen. In later studies, Neuner-Jehle[16] and Whittle[17] confirmed that the reaction is general for N-heteroaromatic molecules which possess the above structural requirements, and loss of the exocyclic nitrogen was confirmed by ^{15}N labeling.[17] They concurred with the earlier mechanism but added that it might be stepwise rather than concerted, based on the similarity of mass spectra of **1b** and **4**, the latter being a presumed intermediate in the general reaction shown above (**3a** → **a**).



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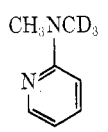
We have examined this interesting reaction in greater detail and find conclusive evidence that the earlier proposed mechanisms are incorrect. Several models were examined which contain a deuterium label in only one of the two methyl groups, *e.g.*, **5**, whose mass spectrum in Figure



5

1 can be compared with that of the unlabeled analog **1b** shown in Figure 2. The earlier proposed mechanisms[4,16,17] require that **5** expel approximately equal amounts of CH_3N (29 mass units) and CD_3N (32 mass units). However, as shown in Figure 1, neutral species of 30 (CH_2ND) and 31 (CD_2NH) mass units are lost instead. Similar results in which approximately equal amounts of CH_2ND and CD_2NH are lost were observed in the mass spectrum of N^6,*O*-3′,5′-tri(methyl-d_3)-N^6-methyl-2′-deoxyadenosine. These data indicate that the mechanism of methylenimine elimination proceeds by a more complex route involving discrete interchange of a single hydrogen between groups or atoms, as opposed to a randomization process

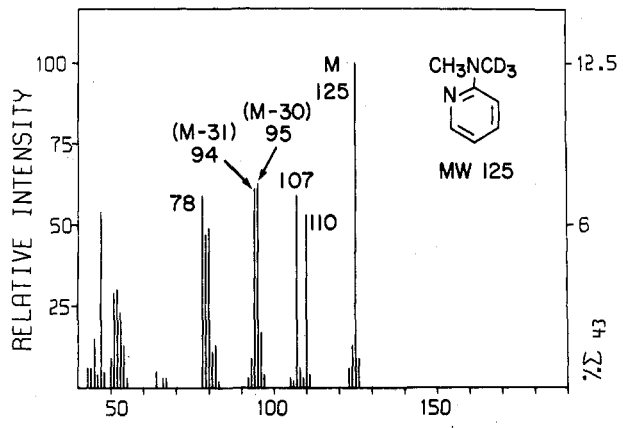
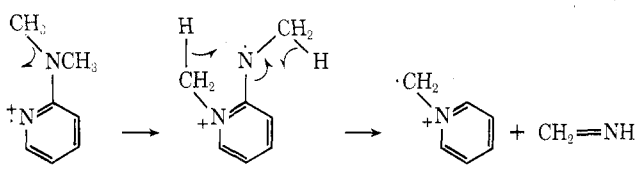


Figure 1. Mass spectrum of 2-(*N*-methyl-*N*-methyl-d_3-amino)pyridine.

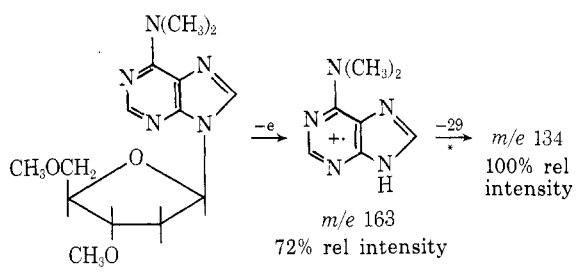
which would produce a more diffuse labeling pattern in the product ions.

If methyl migration is postulated as an initial step (**1b** → b), the remainder of the reaction could be rationalized in terms of subsequent hydrogen transfers, b → c. The va-



1b, *m/e* 122 b c, *m/e* 93

lidity of an initial methyl migration step has been rationally supported by the observation that the spectrum of **4**, which is formally equivalent to ion b, is similar to that of **1a**.[17] This similarity may be fortuitous, however, since other "intermediates" which we have examined do not show the required similarity, and in fact do not exhibit loss of methylenimine. For example, N^6,N^6-dimethyl-2′-deoxyadenosine and its *O*-3′,5′-dimethyl derivative (**6**)



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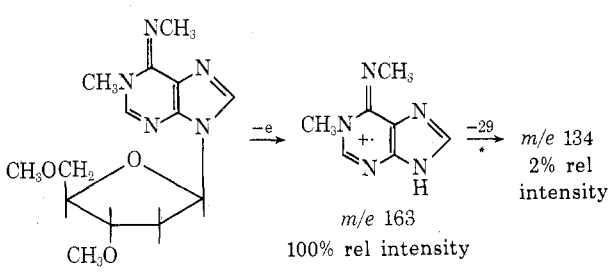
yield the usual abundant base + H ion species[18] which further undergoes facile loss of methylenimine. However, the potential intermediate **7** fails to undergo characteristic elimination of a 29 mass unit neutral from the base + H



7

ion. Likewise, the permethyl derivative of 3-methylcytosine (mol wt 125), which contains a similar methylimine moiety, shows essentially no loss (<2% rel intensity) of

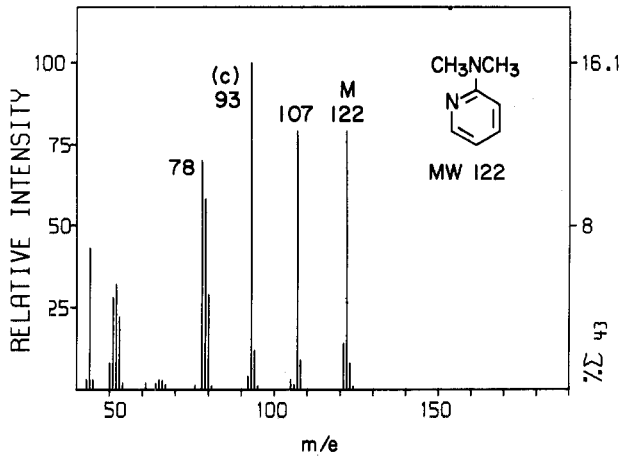
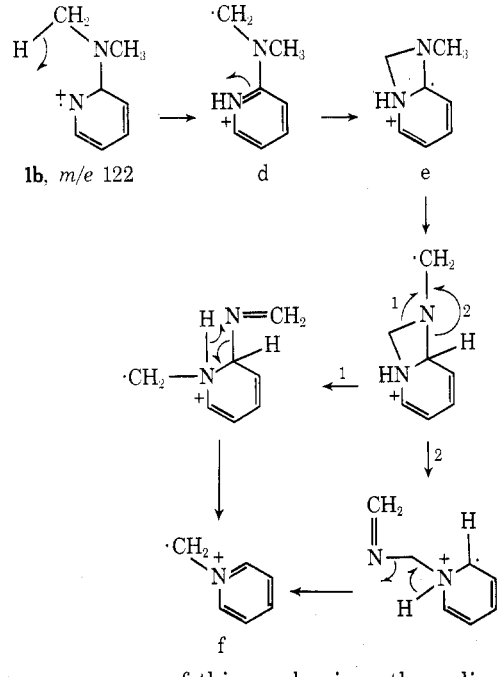


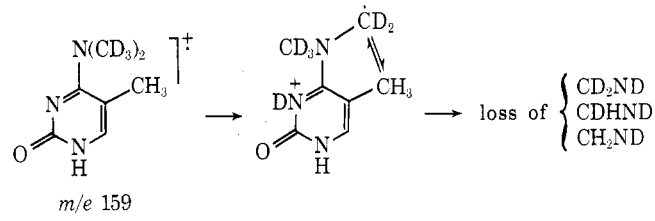
Figure 2. Mass spectrum of 2-dimethylaminopyridine.

methylenimine. Although comparisons of the behavior of molecular ions with isomeric fragment ions from more complex molecular ions may seem logical, such evidence must still be regarded as circumstantial, since the energy contents of isomeric ions from different sources can in principle differ.

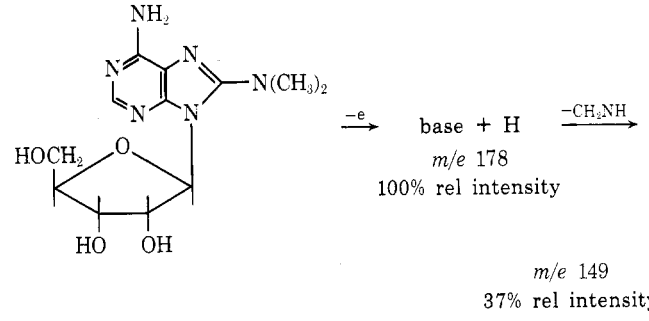
We propose a general mechanism in which the decomposition sequence is initiated by transfer of methyl hydrogen to the charge-localized ring nitrogen (**1b** → d). Reactions of this type between side-chain hydrogens and ring heteroatoms have been previously reported.[19] In addition, reviews of the literature show that hydrogen rearrangement[20] is in general a far more common process than methyl migration.[21] By way of the cyclic intermediate e and its reopening by either route 1 or 2, the final highly stabilized species f can be formed.



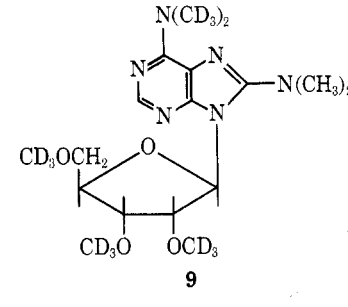
As a consequence of this mechanism, the radical site in intermediate ion d is potentially free to initiate reactions with other sterically accessible hydrogens. Recent data obtained from per(methyl-d_3) derivatives of 5-methylcytosine and related nucleosides give evidence for interactions of this type.[14] Hydrogen interchange between the N^4-alkyl radical and the methyl function at C-5 was found to result in extensive isotopic scrambling in the ion generated by loss of methylenimine. The occurrence of reactions of this type suggests that they may be useful in establishing the proximity of *C*-methyl and amino groups in the nucleic acid bases and their analogs.



Also, amino and alkylamino groups within the same molecule can be distinguished by the use of labeled reagents for derivatization prior to mass spectrometry. For example, the spectrum of 8-dimethylaminoadenosine (8) shows expulsion of CH_2NH from the base + H ion as a major process. Conversion to the *N,O*-per(methyl-d_3) de-



rivative[14] (**9**, Figure 3) results in the analogous ion (*m/e* 183) and an additional product resulting from loss of CD_2ND (*m/e* 180) from the newly formed di(methyl-d_3)amino group at position C-6. The product ion associated with the substituent at C-6 predominates, evidently owing to more favorable resonance stabilization afforded by the pyrimidine moiety in the intermediate and final species.



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Initiation of the expulsion reaction (*e.g.*, **1b** → d) requires the steric availability of nitrogen for hydrogen abstraction.[17] In derivatives of 6-dimethylaminopurine, both N-1 and N-7 are competitive acceptor sites, *via* five- and six-membered transition states, respectively. To gain some insight into the extent of participation of either nitrogen, the *N,O*-permethyl derivatives of 1-deazaadenosine[22] (**10**, Figure 4) and 7-deazaadenosine (**11**, Figure 5) were employed as models. Their mass spectra exhibit general fragment ions characteristic of this type of derivative;[14] the process of interest is the decomposition of *m/e* 162 (base + H) to *m/e* 133 by loss of CH_2NH. Using the ratio of reactants to products (*i.e.*, % Σ *m/e* 162:% Σ *m/e* 133) as a criterion, the 1-deaza analog **10** (ratio 0.29) shows a substantially greater tendency to undergo loss of methylenimine than the 7-deaza model **11** (ratio 2.1), and is similar to the analogous ratio derived from N,O-permethylated adenosine (0.45).[14] These results show that substantial inhibition of the reaction results from the absence of N-7. Although it might be argued that the observed differences are due simply to altered reaction rates

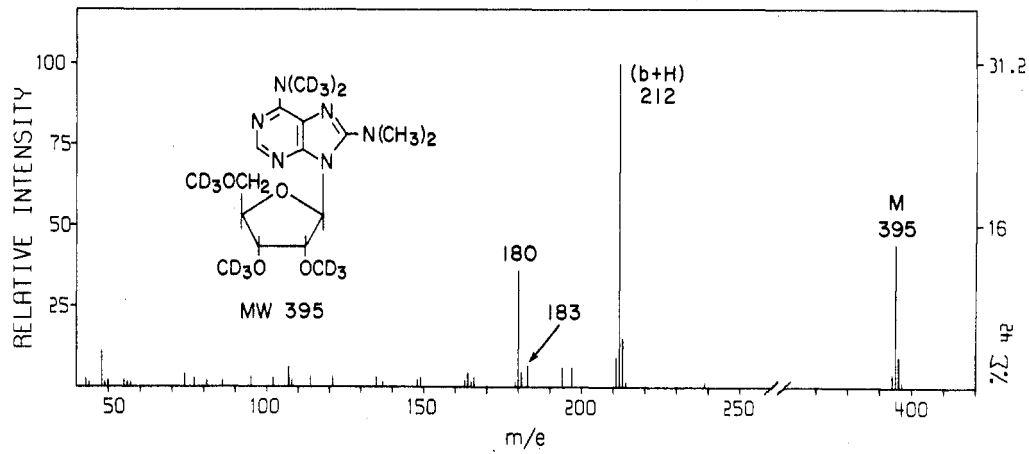


Figure 3. Mass spectrum of N^6,N^6,O-2′,3′,5′-penta(methyl-d_3)-8-dimethylaminoadenosine.

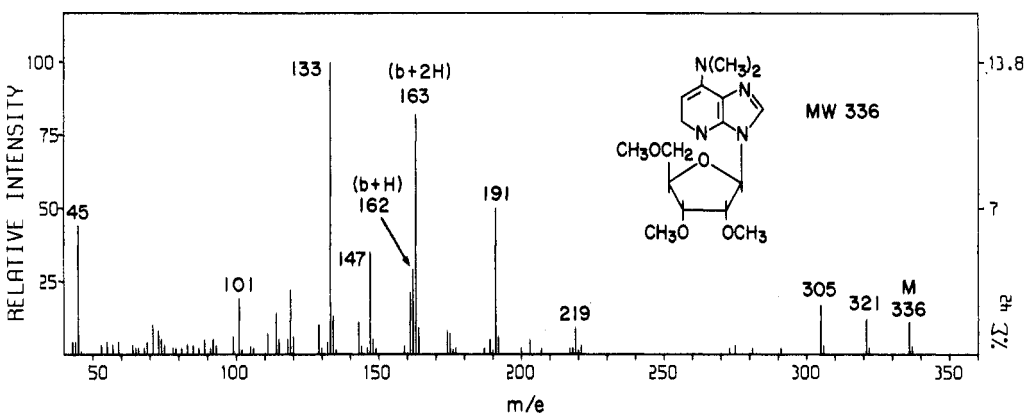


Figure 4. Mass spectrum of N^6,N^6,O-2′,3′,5′-pentamethyl-1-deazaadenosine.

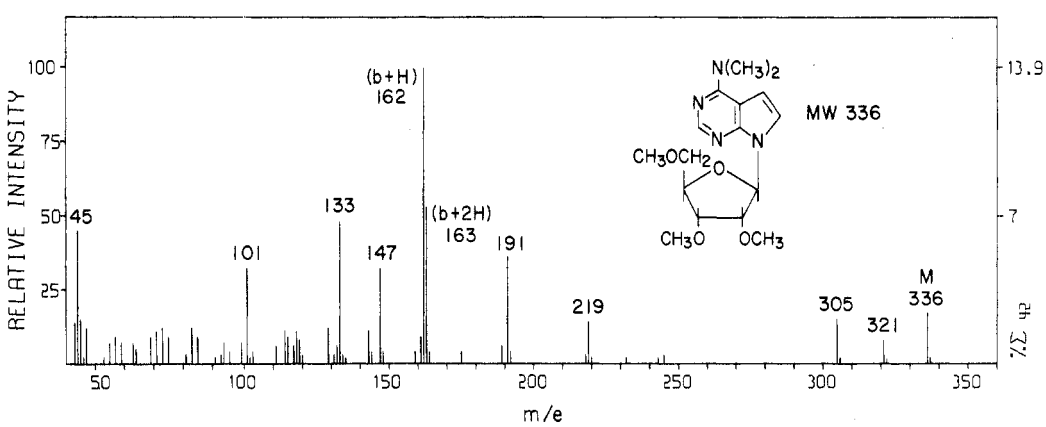
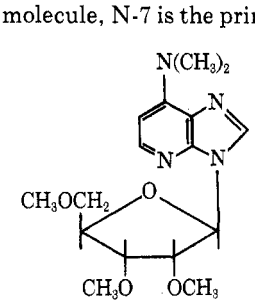


Figure 5. Mass spectrum of N^6,N^6,O-2′,3′,5′-pentamethyl-7-deazaadenosine.

of pathways associated with different structures (**10** *vs.* **11**), the otherwise great similarity of these spectra (Figures 4, 5) leads us to conclude that this comparison is valid and that, when both nitrogen atoms are present in the molecule, N-7 is the primary hydrogen-acceptor site.



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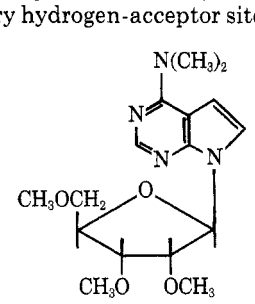
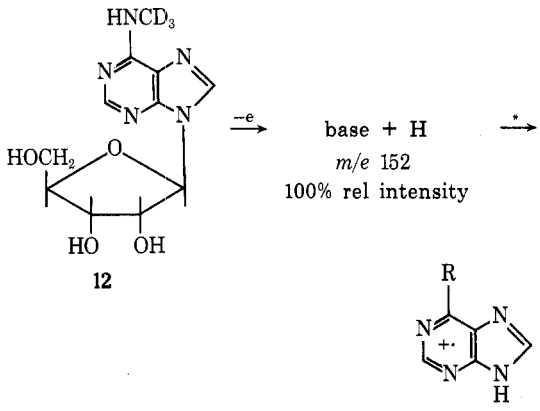


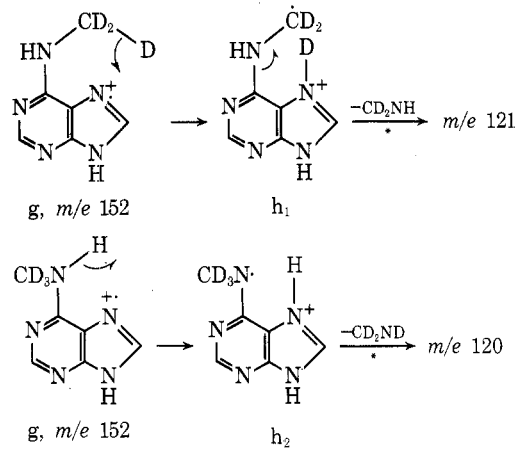
11

Expulsion of the elements of methylenimine also occurs from the corresponding monomethylamines, *e.g.*, N^6-methyladenosine[6,7] and related compounds.[3,7,9,10,12,13] Deuterium labeling in the methyl group (**12**) leads to isotopic interchange in the products *m/e* 120 and 121. These results[1,14] are in agreement with those of Grønneberg,[13] who reported the mass spectrum of 2-*N*-methylaminopyridine (**1a**) and its *N*-d_1 analog. The reaction involved is categorically different from that of the *N,N*-dimethyl analogs because only hydrogen (or deuterium) transfer, as opposed to skeletal rearrangement, occurs. However, the reaction may be initiated in the same manner, namely by hydrogen or deuterium migration from nitrogen or carbon to a charge-localized nitrogen (g → h), followed by loss of methylenimine (h → *m/e* 120 or 121) essentially as proposed by Grønneberg.[13] Loss of isotopic identity could



R = H *m/e* 120, 18% rel intensity
R = D *m/e* 121, 50% rel intensity

also reasonably occur by H–D interchange between N^6 and N-7 in ion h_1. Although g → h_2 might *a priori* be expected to be more favorable than g → h_1 (abstraction



from N *vs.* C), the lower abundance of *m/e* 120 compared with *m/e* 121 may reflect the added burden of CD_3N rearrangement to CD_2=ND in ion h_2 during formation of *m/e* 120.

Experimental Section

Mass spectra were recorded using an LKB 9000 instrument, with sample introduction through the gas chromatographic inlet system (6 in. or 3 ft 1% OV-1). Ion source and carrier gas separator temperatures were 250°; ionizing energy was 70 eV.

Materials. The following compounds were obtained from commercial sources as indicated: 2-dimethylaminopyridine (**1b**), Aldrich Chemical Co., Milwaukee, Wis.; 2-methylaminopyridine, RSA Corp., Ardsley, N. Y.; 3-methylcytosine, 2′-deoxyadenosine, and 1-methyl-2′-deoxyadenosine, Sigma Chemical Co., St. Louis, Mo.; methyl-d_3 iodide (99% D), Merck Sharp and Dohme of Canada, Montreal, Canada.

1-Deazaadenosine[22] was obtained from Dr. Y. Mizuno, Hokkaido University, Sapporo, Japan; 7-deazaadenosine (tubercidin) and 8-dimethylaminoadenosine were from Dr. L. B. Townsend, University of Utah, Salt Lake City, Utah.

N^6-(Methyl-d_3)adenosine (**12**) had been prepared in conjunction with a previous study by base-catalyzed rearrangement of 1-(methyl-d_3)adenosine.[23]

***N,O*-Permethyl Derivatives.** 2-(*N*-Methyl-*N*-methyl-d_3-amino)pyridine (**5**), N^6,N^6,O-3′,5′-tetramethyl-2′-deoxyadenosine (**6**), 1,N^6,O-3′,5′-tetramethyl-2′-deoxyadenosine (**7**), N^6,N^6,O,-2′,3′,5′-penta(methyl-d_3)-8-dimethylaminoadenosine (**9**) N^6,N^6,O-2′,3′,5′-pentamethyl-1-deazaadenosine (**10**), N^6,N^6,O,-2′,3′,5′-pentamethyl-7-deazaadenosine (**11**), and the di(methyl-d_3) derivative of 3-methylcytosine were prepared on a scale of 50–100 μg using methylsulfinyl carbanion and CH_3I or CD_3I as described previously.[14] Sites of methylation under these reaction conditions were established in an earlier study.[14,24] Purity and identity of the products were checked by gas chromatography–mass spectrometry.

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