50.3, 47.0, 38.0, 36.8, 36.6, 35.0, 34.8, 31.3, 31.1, 30.3, 27.6, 21.3, 20.6, 19.8, 19.1, 16.1; mass spectrum (70 eV), m/e 366 (9.20 parent – CH_3CO_2H, base), 351 (2.89), 253 (2.78). Anal. Calcd for $C_{27}H_{38}O_4$: C, 76.02; H, 8.98. Found: C, 75.86; H, 8.95.

Methyl (20*S*)-3β-Acetoxy-5α-cholanoate (3). A solution containing 30 mg of **2** and 5 mg of 5% PtO_2 in 12 mL of ethyl acetate was stirred under an atmosphere of hydrogen at ambient pressure for 12 h. The reaction mixture was filtered, the solvent was removed under reduced pressure, and the solid was recrystallized twice from methanol to produce white crystals, mp 134–135 °C [lit.[11] mp 136.0–137.5 °C]. The ^1H NMR spectrum showed the C-21 absorption at δ 0.83 (J = 6 Hz) [lit.[11] C-21, δ 0.83 (J = 6 Hz)].[12] The mass spectrum was identical with the literature values.[11]

Acknowledgment. This work was supported by Grant No. AM 00709, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. We are greatly indebted to Dr. Jaan Pesti for his assistance.

Registry No. 1, 29842-93-1; 2, 82660-25-1; 3, 1178-02-5; methyl propiolate, 922-67-8.

(12) The value for C-21 in the 20*R* series is δ 0.92 (J = 6 Hz).

Mass Spectrometry of Nucleic Acid Constituents. Trimethylsilyl Derivatives of Nucleosides[1,2]

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Trimethylsilylation of nucleosides provides derivatives which are thermally volatile and whose electron-ionization mass spectra are useful for structural characterization and for determination of chemically or biologically incorporated stable isotopes. The major reaction pathways and mechanisms of fragmentation of silylated nucleosides have been studied, on the basis of the mass spectra of a structural variety of nucleoside analogues and of uridine selectively labeled with deuterium (C-2′, C-3′, C-5′, $Si(CD_3)_3$) and oxygen-18 (O^2, O^4, O-2′, O-3′, O-5′). Formation of most of the major base-containing ions, which are even-electron species, involving rearrangement of hydrogen from the sugar skeleton to the ionized base. The site selectivity of some of the rearrangement processes indicates that base–H-2′ interactions are relatively important and that in those cases significant opening of the ribose ring does not occur prior to hydrogen abstraction by the base. The relative abundance of sugar H ions resulting from transfer of H-2′ to the base was found to be greater in derivatives of β-ribofuranosides compared with that for the corresponding α anomers, reflecting differences in steric accessibility of H-2′ to the base and providing a means of distinguishing α and β anomers. The determination of the site and extent of isotopic substitution in the sugar is better measured from fragment ions which contain the base plus portions of the sugar than from sugar ions which do not contain the base. This is a consequence of the multiple pathways of formation of most sugar-derived ions.

The structure elucidation of new nucleosides isolated from natural sources is often confounded by the combination of structural complexity and severe limitations in sample quantity. These problems are particularly prevalent in the case of transfer RNA, where modified nucleosides often occur at a rate of one residue per tRNA molecule, and the isolation of more than a few micrograms from grams or more of starting material is a demanding task.[3] Mass spectrometry has thus evolved as an important technique for the characterization of nucleosides,[4] largely as a consequence of high sensitivity compared with other methods.

Direct vaporization techniques[5–13] such as field desorption[14] or fast atom bombardment[15,16] can be useful for nucleosides that cannot be volatilized thermally. However, the lower abundances of information-bearing fragment ions produced make these methods generally less desirable as a single approach compared with microscale derivatization and electron ionization. Of the various derivatization procedures which have been used for mass spectrometry of nucleosides,[4,17] the most widely used has been trimethylsilylation,[18] which has been employed with partic-

(1) Taken in part from the Ph.D. thesis of H.P., University of Utah, 1982.

(2) A preliminary account of a portion of this work has been presented: Hattox, S. E.; Schram, K. H.; McCloskey, J. A. 23rd Annual Conference on Mass Spectrometry and Allied Topics, Houston May 1975, pp 435–436.

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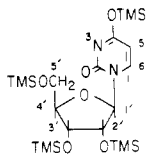
0022-3263/82/1947-3923$01.25/0

ular success for the characterization of naturally occurring nucleosides.[19] Structure assignments for the principal ions from trimethylsilylated nucleosides were established at an early date,[18] but subsequent reports have been largely descriptive in nature[4,20] and little effort has been devoted to mechanistic details. Knowledge of nucleoside fragmentation processes is an important aspect of providing a firm foundation for structural applications, for establishing its utility for location of substituents and isotopic labels[21] in the nucleoside skeleton, and in understanding the gaseous ion chemistry of polyfunctional molecules. Toward these goals, we have examined the fragmentation processes of a number of nucleoside Me_3Si derivatives,[22] with particular attention to uridine-$(Me_3Si)_4$ (1) as a



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general nucleoside model, selectively labeled with ^{18}O and deuterium.[23] Decomposition pathways have been measured by metastable ion techniques, and plausible ion structures have been deduced from isotopic labeling data and mechanistic considerations. Of particular interest have been those ions which may be useful for the structural characterization of nucleosides and in applications involving measurement of chemically or biologically incorporated stable isotopes.

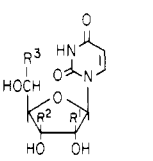
Results and Discussion

The electron ionization of nucleosides generally produces ion series which may conveniently be considered in three categories: those resulting from simple losses from the molecular ion, ions containing the intact heterocyclic base plus portions of the sugar, and the sugar moiety and its fragmentation products. A general rationale was earlier proposed in which many of the dissociation processes of nucleosides are initiated by abstraction of hydrogen from the sugar to the charge-localized base.[24] In the case of free nucleosides these hydrogens are transferred from sugar hydroxyls,[25] while in blocked derivatives they are rearranged almost exclusively from the ribose skeleton.[20,26] The selectivity of these reactions has been examined as a means of understanding the mechanistic origins of ions in nucleoside mass spectra and to establish their utility for location of stable isotopes in the base or sugar. For this purpose reliance has been placed on isotopically labeled uridine as a representative model, with the assumption that results can be qualitatively extended to nucleosides in general. The present study is concerned with the basic fragmentation processes of nucleosides, without regard to the additional influence of side-chain substitution. The presence of complex, heteroatom-containing side chains, as, for example, in the hypermodified nucleosides from tRNA,[3] can lead to lowered abundances of the basic series of nucleoside ions. In such cases, identification of the latter ions can often be made from their experimentally measured exact mass interrelationships.

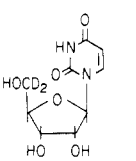
Table I. Molecular Ion Series from Trimethylsilylated Nucleosides

ion	composition	mass
M − 15	$M - CH_3$	M − 15.0235
M − 90	$M - C_3H_{10}OSi$	M − 90.0501
M − 103	$M - C_4H_{11}OSi$	M − 103.0579
M − 105	$M - C_4H_{13}OSi$	M − 105.0736
M − 118	$M - C_4H_{10}O_2Si$	M − 118.0450
M − 131	$M - C_5H_{11}O_2Si$	M − 131.0528
M − 180	$M - C_6H_{20}O_2Si_2$	M − 180.1002
M − 195	$M - C_7H_{23}O_2Si_2$	M − 195.1237

The supplementary material contains full mass spectra of 23 of the nucleoside derivatives examined in the present study and selected ion-abundance data of silylated derivatives of uridine (2) and 3–11, corrected for presence of natural heavy isotopes.



2, $R^1 = R^2 = R^3 = H$
3, $R^1 = D; R^2 = R^3 = H$
4, $R^2 = D; R^1 = R^3 = H$
5, $R^3 = D; R^1 = R^2 = H$



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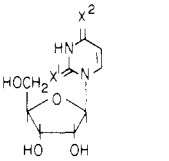
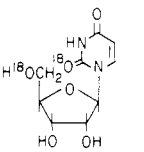


7, $X^1 = {}^{18}O; X^2 = O$
8, $X^1 = O; X^2 = {}^{18}O$



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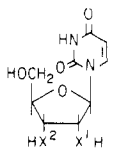

10, $X^1 = {}^{18}O; X^2 = O$
11, $X^1 = O; X^2 = {}^{18}O$

Ions Closely Related to the Molecular Ion. Mass spectra of trimethylsilylated nucleosides exhibit up to eight peaks representing simple losses from the molecular ion (Table I), of which the M − 15 and M − 90 ions are the most abundant and thus useful for identification of the molecular ion, M. Their origins as established from metastable ion measurements are shown in Scheme I. Molecular ion abundances were found to vary widely and are characteristically high for nucleosides of guanine and 1-methylpurines.[22]

The ubiquitous loss of a methyl radical occurs from both the base and sugar, as determined from the partially silyl-labeled derivative 12. The observed abundance ratio of $(M - CH_3)/(M - CD_3) = 2$ is similar to that of cyti-

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(22) See supplementary material.

(23) Me_3Si is used as an abbreviation for trimethylsilyl. For convenience, names such as uridine-$(Me_3Si)_4$ are used instead of $O^4,2',3',5'$-*O*-tetrakis(trimethylsilyl)uridine. As a consequence of the reaction conditions usually employed,[18,20] Me_3Si groups replace active hydrogens in the base and sugar, with the exception of exocyclic amino groups in the base, which bear only one Me_3Si function.

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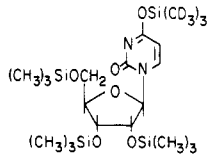

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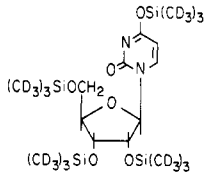
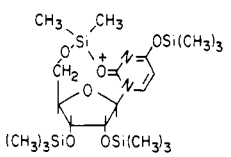


13

dine-$(Me_3Si)_4$ (2.6) and adenosine-$(Me_3Si)_4$ (3), showing loss from the base to be statistically favored or equal to that from the sugar. Although results from isotopic labeling (later sections) suggest that opening of the ribose ring does not occur in a significant population of the molecular ions, the even-electron M − 15 ion derived from the sugar silyl groups has several attractive possibilities for siliconium ion stabilization (e.g., **14** and **15**), a concept earlier discussed by Westmore.[27,28]



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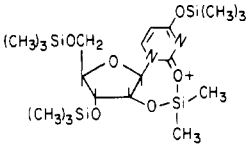
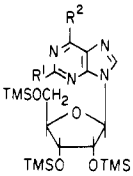


15

The ion abundance ratio M/(M − 15) < 1, which is widely observed for Me_3Si derivatives in general, also holds for most nucleosides, with the useful exception of guanosine[18] and its analogues. The effect is seen in selenoguanosine derivatives[29] and in members of the Q nucleoside family (7-deazaguanosines) in which mass spectrometry has played an important role in structural characterization.[30] Of 16 guanosine analogues examined (names given separately[22]), only 2′-deoxy-2′-aminoguanosine exhibited a M/(M − 15) ratio <1. A comparison of models which details these effects is listed in Table II. The first column represents N^6-substituted purine nucleosides, the second column shows 2-substituted adenosines and column three indicates the opposite substitution pattern for each entry in column two. These data show that the effect is associated with the presence of an amino function at C-2, while S, O, or halogen have relatively little influence. Similar correlations involving amino substitution at C-2 do not generally hold for pyrimidine nucleosides. Although the M, M − 15 abundance correlation is empirically useful in suggesting the presence of a guanosine analogue, it cannot be rigorously applied. For example, (M − 15) < M is observed in some adenosine analogues, particularly those substituted at N^6.

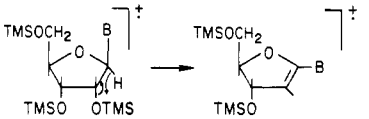
The elimination of trimethylsilanol to form ion M − 90 is common to all trimethylsilyl nucleoside derivatives such as uridine (Figure 1). The reaction is unusually slow, as indicated by the broad and intense metastable peak associated with the transition M → M − 90 and by its prominence at low ionizing energies, where increased molecular ion lifetimes pertain.[31] Below an 11-eV nominal ionizing energy the relative abundance of M − 90 from

Table II. Effect of Substituents at C-2 and C-6 of Purine Ribonucleosides on M/(M − 15) Abundance Ratios



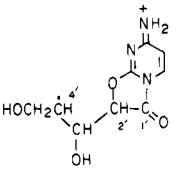
R′ = H		R² = NHTMS		R¹ = NHTMS	
R²	M/(M − 15) ratio	R¹	M/(M − 15) ratio	R²	M/(M − 15) ratio
H	0.04	H	0.15	H	1.5
$OSiMe_3$	0.12	$OSiMe_3$	0.29	$OSiMe_3$	1.8
Cl	0.24	Cl	0.17	Cl	1.8
SH	0.3	SH	0.15	SH	1.6
NHTMS	0.15	NHTMS	1.8	NHTMS	1.8

uridine rises sharply, having a relative intensity of 88% and a Σ of 10% at 10 eV. The trimethylsilyloxy group lost is derived exclusively from O-2′ (shown by **10**) while deuterium labeling (derivatives of **3–6** and **13**) show the leaving hydrogen to be from either C-1′ or C-4′. A simple 1,2-elimination (below) is favored on steric grounds (eq 1)



M (B = base) M − 90 (1)

although two factors favor the additional operation of a second and more complex mechanism involving 1′,2′-elimination. First, the further decomposition of M − 90 to ion B + 41 (discussed in a later section) by loss of C-3′, C-4′, and C-5′, supported by spectra of all labeled compounds, has direct analogy in the formation of ion B + 41 in the spectra of free cytidine following loss of H_2O.[32] That mechanism involves opening of the ribose ring in order to form the B + 41 ion (see later section). Second, further decomposition by loss of CO from M − 90 primarily involves oxygen from O-4′ (70%; the remainder from O-3′) as shown by ^{18}O-labeling patterns, producing ion M − 118. The intermediate structure for M − H_2O in free pyrimidines nucleosides[32] (**16**) is better suited to accommodate this loss than the closed-ring structure shown for M − 90 in eq 1.



16[32]

The selectivity of O-2′ elimination is reflected in the mass spectra of 2′-*O*-methyluridine-$(Me_3Si)_3$ and 2′-*O*-methyladenosine-$(Me_3Si)_3$[20] which show exclusive loss of CH_3OH and no trimethylsilanol. However, in derivatives of 3′-*O*-methyladenosine and N^6,N^6,3′-*O*-trimethyladenosine both are lost, so that the process is ambiguous with respect to establishment of the site of sugar O-methylation.

(27) Quilliam, M. A.; Ogilvie, K. K.; Sadana, K. L.; Westmore, J. B. *Org. Mass Spectrom.* **1980**, *15*, 207–219.
(28) Quilliam, M. A.; Ogilvie, K. K.; Westmore, J. B. *Org. Mass Spectrom.* **1981**, *16*, 129–138.
(29) Liehr, J. G.; Weise, C. L.; Crain, P. F.; Milne, G. H.; Wise, D. S.; Townsend, L. B.; McCloskey, J. A. *J. Am. Chem. Soc.* **1979**, *16*, 1263–1272.
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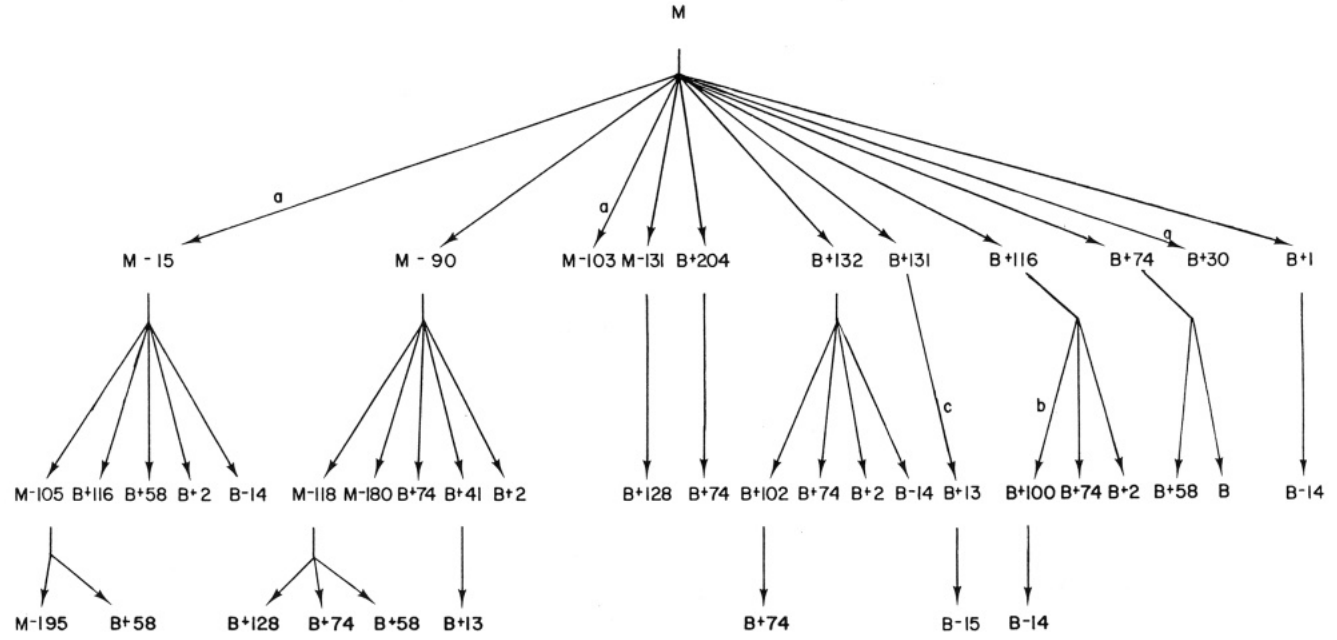
(32) Liehr, J. G.; von Minden, D. L.; Hattox, S. E.; McCloskey, J. A. *Biomed. Mass Spectrom.* **1974**, *1*, 281–285.

Scheme I



[a] No metastable ion observed. [b] B + 115 cannot be distinguished from B + 116 as the precursor of B + 100. [c] B + 132 cannot be excluded as a precursor of B + 13.

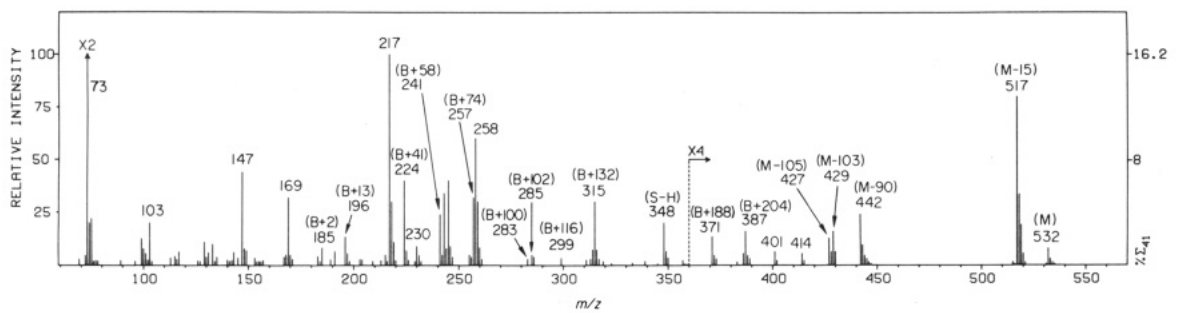
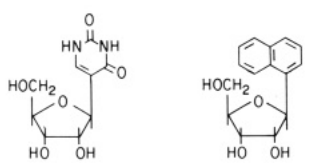


Figure 1. Mass spectrum of uridine–$(Me_3Si)_4$.

Expulsion of a second molecule of trimethylsilanol is normally a minor process (see Figure 1), except in the case of nucleosides which possess a C–C rather than C–N glycosidic bond.[20] A number of such nucleosides occur naturally,[33,34] the best studied of which is pseudouridine (**17**).



17 **18**

The prominence of M – 180, a useful test for recognition of the *C*-nucleoside linkage in new nucleosides,[19h] may be due in part to absence of numerous competing pathways associated with breakage of the C–C glycosidic bond, which is considerably stronger than the C–N form.[35,36] In the spectra of ten *C*-nucleosides examined, the M – 180 (or in some instances M – 179) ions are typically of 2–10% relative intensity,[22] but reach 30% in the case of 1-(β-D-ribofuranosyl)naphthalene-$(Me_3Si)_3$ (**18**).

The loss of trimethylsilanol was also found to be a very minor but common process from numerous ions in the molecular ion and base series. Brief comments on those ions and on the minor M series ions M – 131, M – 105 (M – 15 – 90), and M – 103 (M – 5′-CH_2OSiMe_3) are given in

Table III. Base-Ion Series from Trimethylsilylated Nucleosides

ion	composition	mass
B + 204	B + $C_8H_{20}O_2Si_2$	B + 204.1002
B + 188	B + $C_8H_{20}OSi_2$	B + 188.1053
B + 132[a]	B + $C_5H_{12}O_2Si$	B + 132.0607
B + 128	B + $C_6H_{12}OSi$	B + 128.0657
B + 116	B + $C_5H_{12}OSi$	B + 116.0658
B + 102	B + $C_4H_{10}OSi$	B + 102.0501
B + 100	B + C_4H_8OSi	B + 100.0344
B + 74	B + $C_3H_{10}Si$	B + 74.0552
B + 58	B + C_2H_6Si	B + 58.0239
B + 41	B + C_2HO	B + 41.0027
B + 30	B + CH_2O	B + 30.0106
B + 13	B + CH	B + 13.0078
B + 2	B + H_2	B + 2.0157
B + 1	B + H	B + 1.0078
B	B	B
B – 14	B – CH_2	B – 14.0157

[a] B + 131 in some mass spectra.

(33) Suhadolnik, R. J. "Nucleoside Antibiotics"; Wiley-Interscience: New York, 1970.
(34) Hall, R. H. "The Modified Nucleosides in Nucleic Acids"; Columbia University Press: New York, 1971.
(35) Townsend, L. B.; Robins, R. K. *J. Heterocycl. Chem.* **1969**, *6*, 459–464.
(36) Rice, J. M.; Dudek, G. O. *Biochem. Biophys. Res. Commun.* **1969**, *35*, 383–388.

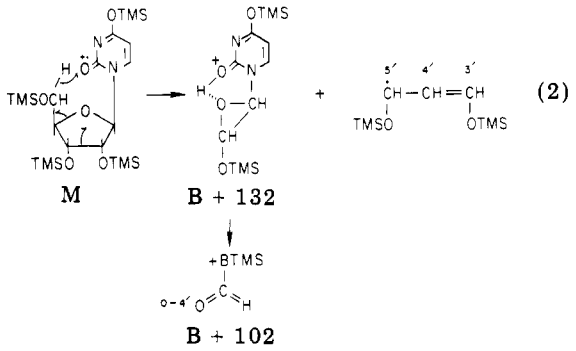
the supplementary material.

Ions of the Base Series. Ions containing the base plus portions of the sugar constitute the base series (Table III), which are useful in establishing the exact mass and elemental composition of the base[37] and the location of substituents in the sugar.[18] Fifteen such ions have been identified in the spectra of uridine (Figure 1), although six to ten are typically observed in the spectra of other simple nucleosides that do not provide competing pathways from side-chain fragmentation. Most ions listed in Table III require hydrogen rearrangement for formation. In some cases an analogous ion is also formed by transfer of trimethylsilyl, a common process,[38] and appears 72 mass units higher than the H-transfer species (B + 204, B + 132; B + 188, B + 116; B + 102, B + 30; B + 74, B + 2). Some ions in the base series are of low abundance but are readily recognized through their exact mass interrelationships and permit determination of an accurate exact mass value for the base moiety.[37]

Ions of the base series are derived in large part from the molecular ion rather than the even-electron M – CH_3 ion (Scheme I). This is in contrast to the behavior of *tert*-butyldimethylsilyl (and related) derivatives of nucleosides, studied in detail by Westmore and his colleagues,[27,28] in which almost all ions are formed from the analogous M – C_4H_9 species.

In accord with earlier reasoning,[24] the low ionization potential of the base relative to ribose oxygens suggests that transfer of H or Me_3Si to the charge-localized base is a major fragmentation-initiating step, although the present data do not exclude rearrangement of hydrogen within the sugar. This view is supported by multiple pathways of formation of B + 74 (base + H + Me_3Si) and B + 2 ions (Scheme I) in which the base bears hydrogen transferred from the sugar.

The B + 132 ion, which contains C-1′, C-2′, O-2′, O-4′, and H rearranged from the remainder of the sugar, is of major importance in structural characterization for recognition of methylation sites in the sugar.[19e] From the derivative of **6** it was established that the source of rearranged H is 82% from C-5′ and (by difference) 18% from C-4′. The latter source requires opening of the ribose ring, but in the case of C-5′, space-filling CPK models[39] show that direct transfer from C-5′ to the base can easily occur, without ring opening (eq 2). The concomitant loss of

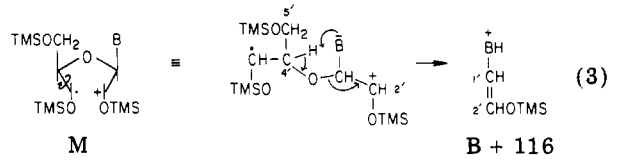


(2)

neutral C-3′, C-4′, C-5′ (217 mass units) has analogy in formation of the m/z 217 ion, discussed in the supplementary material.[22] In some instances (e.g., derivatives of 2-mercaptoadenosine, isocytidine) ribose cleavage occurs without hydrogen transfer to produce an ion of mass B + 131. No clear structural trend (purine vs. pyrimidine, aromaticity of the base, etc.) is evident which would allow prediction of which form will predominate. Daughter ions of B + 132 include B + 102, by elimination of formaldehyde from O-2′, and B + 74. The structurally related ion B + 204 contains the same skeletal atoms as B + 132 but differs by further decomposition to the minor species m/z 131 (1′-CHO – 2′-$CHOSiMe_3$)$_3$, and provides a minor contribution to m/z 103 (CH_2OSiMe_3; see Figure 1).

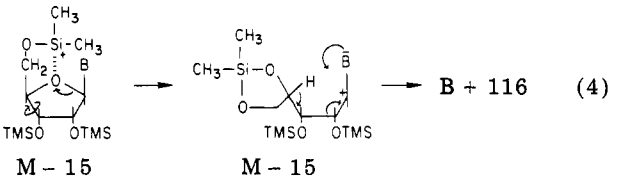
Similar to the structurally related ion B + 30,[18,25] the B + 102 ion is characteristically abundant in the spectra of C-nucleosides as shown in Table IV. By contrast, its contribution is minor from normal nucleosides such as uridine (Figure 1).

Sites of sugar modifications can also be established from the B + 116 ion, which formally differs from B + 132 by absence of O-4′.[18] The spectra derived from selectively deuterated derivatives **4–6** and **13** reveal no mass shifts[22] due to transferred deuterium, showing the site of abstraction to be C-4′. Two precursors were identified by their metastable ions: M and M – 15. In both cases, steric accessibility of H-4′ to the base requires opening of the sugar ring. The molecular-ion-derived product can be readily accounted for by eq 3, supported by the mass



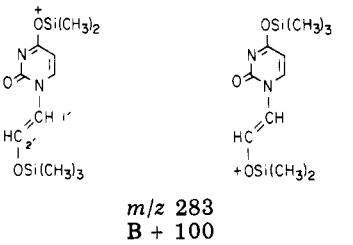
(3)

spectra of all labeled uridines examined. Formation of B + 116 from the even-electron M – 15 ion requires initial loss of CH_3 specifically from O-3′ or O-5′, for which cyclic stabilization analogous to that proposed by Westmore et al.[27,28] (eq 4) provides an attractive intermediate. The



(4)

rearranged hydrogen from C-4′ is lost with a silyl methyl group as CH_4 in a subsequent step, producing ion B + 100. From **12** it was determined that the CH_3 moiety involved is approximately equally derived from the base and O-2′ silyl groups:



m/z 283
B + 100

The compositionally related (H vs. Me_3Si) ion B + 188 was observed in 83% of the mass spectra examined and is likewise a useful indicator of sugar substitution. Unlike the apparently simple analogy between ions B + 132 and B + 204, ion B + 188 differs both structurally and mechanistically from B + 116. Its major precursor in uridine is the minor ion M – 131 (M – [4′-O-1′-CH-2′-CHO-$SiMe_3$]);[22] a metastable peak of low intensity showed its production from M – 15, apparently the major pathway in other (nontrimethylsilyl) alkylsilyl nucleosides.[27] Thirty

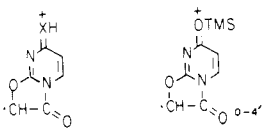
(37) Crain, P. F.; Yamamoto, H.; McCloskey, J. A.; Yamaizumi, Z.; Nishimura, S.; Limburg, K.; Raba, M.; Gross, H. J. *Adv. Mass Spectrom.* **1980**, *8*, 1135–1141.
(38) For leading references see: Draffan, G. H.; Stillwell, R. N.; McCloskey, J. A. *Org. Mass Spectrom.* **1968**, *1*, 669–685.
(39) Koltun, W. L. *Biopolymers* **1965**, *3*, 665–679.

Table IV. Abundance of B + 30 and B + 102 Ions from Trimethylsilylated C-Nucleosides

parent compd	B + 30 m/z	B + 30 rel intens/ % Σ_{70}	B + 102 m/z	B + 102 rel intens/ % Σ_{70}
5-(β-D-ribofuranosyl)uracil-$(Me_3Si)_5$ (pseudouridine-$(Me_3Si)_5$)	285	0.9/0.2	357	21/4.8
5-(α-D-ribofuranosyl)uracil-$(Me_3Si)_5$	285	0.8/0.2	357	21/4.8
4-(β-D-ribofuranosyl)pyrazole-3-carboxamide-$(Me_3Si)_5$	284	1.8/0.6	356	1.9/0.6
4-(β-D-ribofuranosyl)pyrazole-3-carboxylic acid methyl ester-$(Me_3Si)_4$	227	4.5/1.2	299	6.2/1.7
5-(β-D-ribofuranosyl)isocytidine-$(Me_3Si)_5$	284	2.1/0.5	356	18/4.2
5-(α-D-ribofuranosyl)isocytidine-$(Me_3Si)_5$	284	3.4/0.9	356	14/3.7
8-(β-D-ribofuranosyl)adenine-$(Me_3Si)_5$	308	28/7.0	380	1.8/0.4
2-(β-D-ribofuranosyl)adenine-$(Me_3Si)_5$	308	66/12	380	4.9/0.7
4-hydroxy-3(5)-(β-D-ribofuranosyl)pyrazole-5(3)-carboxamide-$(Me_3Si)_6$ (pyrazomycin-$(Me_3Si)_6$)	372	23/3.2	444	3.5/0.5
1-(β-D-ribofuranosyl)naphthalene-$(Me_3Si)_3$	157	1.0/0.2	229	16/3.3

percent of the B + 188 population was found to contain O-5′ (from **9**), with H-2′ (from **3**) but without hydrogen from C-5′ (**5**, **6**). These data demonstrate the occurrence of trimethylsilyloxy migration from C-5′, thus placing a limitation on the use of the B + 188 ion for location or measurement of ^{18}O.

An ion of mass equal to B + 41 is characteristically abundant in the mass spectra of cytidine and many of its derivatives;[32,40] in cytidine-$(Me_3Si)_4$ its relative intensity is 90%. Its presence in the mass spectra of other pyrimidines such as uridine-$(Me_3Si)_4$ (Figure 1) is reduced, previously interpreted[32] as being due to reduced influence of the heteroatom at C-4 (O vs. N) in initiating the reaction and stabilizing the final product **19**. In the present study,

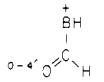


19, X = NH, O **20**, *m/z* 224

B + 41

metastable ion evidence shows a pathway of formation (M → M − 90 → B + 41) analogous to that of free cytidine (M → M − 18 → B + 41), while isotopic labeling (**3**) indicates retention of H-2′. The mechanism of formation is therefore directly analogous to that in free nucleosides,[32] leading to **20**.

The B + 30 ion includes C-1′ and O-4′ from the sugar.[18]



B + 30

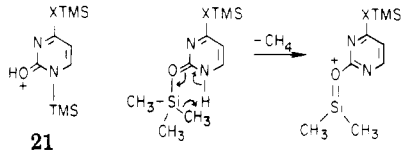
Its abundance, which is often high in purine nucleosides, makes it useful for identification of members of the base series. Probable multiple modes of formation are reflected in the complex labeling pattern for the rearranged hydrogen in the case of uridine: C-3′, 20% (from **4**), trimethylsilyl 20% from **13**, none from C-2′ (from **3**) or C-5′ (from **6**), and 60% (by difference) from C-4′. It is structurally, though perhaps not mechanistically, related to ion B + 102 (eq 2). The absence of an observed metastable ion species (Scheme I) suggests its formation to be unusually rapid in uridine, in spite of the multiple paths of formation shown by deuterium labeling.

Cleavage of the C–N glycosidic bond is often accompanied by transfer of H or Me_3Si in various combinations to yield ions B + H, B + 2H and B + H + Me_3Si.[18] The latter even-electron ion (B + 74) is most prevalent in the spectra

Table V. Abundance of B + H Ion from Trimethylsilylated Adenosine Derivatives

compd	B + 1 abundance rel intens	B + 1 abundance % Σ_{70}
23	2.8	0.42
24	7.5	0.92
25	25	5.4
26	40	14

of purine nucleosides that do not bear side chains which offer competing sites for fragmentation. The rearranged hydrogen in B + 74 from uridine is from several sources (55% C-5′, 23% C-2′, 22% C-1′ + C-4′ by difference) in keeping with its multiple paths of formation. The rearranged H and the Me_3Si group are assumed to reside at N-1 and O^2 (**21** or **22**), on the basis of their spatial proximity to the base prior to transfer. Further expulsion of CH_4 (ion B + 58) occurs specifically from the rearranged silyl group methyl and hydrogen, as determined from **12** and the analogous cytidine derivative. These data further support either structure **21** or **22**, which are well suited



21 **22** *m/z* 241

B + 74 B + 58

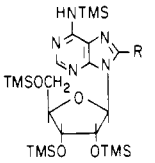
X = O, NH

sterically for a loss of CH_4 not involving the silyloxy group at C-4. Some other precursors of B + 58 (Scheme I) are even-electron ions in which a silyl methyl has already been lost (M − 15, M − 105, and M − CH_3 − 2 Me_3SiOH), implying a different mechanism involving electrophilic attack of the sugar siliconium ion on the base.

The relative abundances of ions B, B + 1, and B + 2 vary widely but tend to fall in the relative intensity range of 0–15% for pyrimidine and 0–100% for purine nucleosides, reflecting greater accommodation of the charge in the purine nucleus. As shown in Table V, a strong correlation in purines is observed between the ion abundance and the electronegativity of substituents at C-8. Stabilization through electron-donating ability of substituents at C-8 (**23–26**) results in an increase in the B + 1 relative abundance with a decrease in substituent electronegativity.

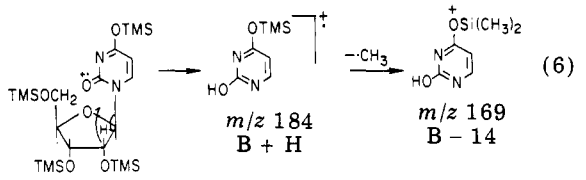
In the mass spectrum of uridine-$(Me_3Si)_4$ the B + 1 ion is too low in abundance for an accurate determination of the source of rearranged hydrogen. However, some insight into its origin can be gained from its principal daughter ion base + H − CH_3 (B − 14), even though it is also produced by other routes (Scheme I). C-2′ is a major source of hydrogen in B − 14, so that direct transfer of H to O^2

(40) Sochacki, M.; Sochacka, E., Malkiewicz, A. *Biomed. Mass Spectrom.* **1980**, *7*, 257–258.



23, R = Br
24, R = H
25, R = OH (oxo)
26, R = $NHCH_3$

through a six-membered transition state analogous to the McLafferty rearrangement is a likely contributor to the formation of B + H (eq 6). Abstraction of H from the



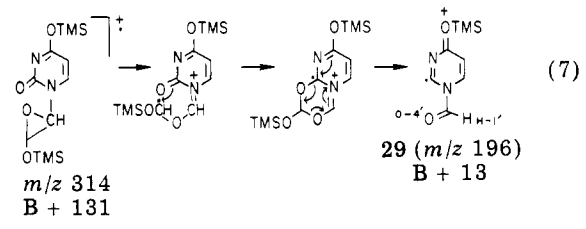
(6)

M

secondary 2′-carbon is shown by CPK models to be sterically feasible and further[26,27] confirms the importance of base–2′ interactions in the mass spectra of nucleosides.

The deuterium labeling pattern of the two hydrogens transferred in the B + 2 ion from uridine was found to be largely nonselective (28% C-2′, 42% C-3′, and 48% C-5′ of 200% total), reflecting its multiple paths of formation.

The B + 13 ion, equivalent in composition to base + CH (m/z 196.0668, $C_8H_{12}N_2O_2Si$), was observed in none of the purine spectra but in approximately half of the pyrimidines, including uridine-$(Me_3Si)_4$ (Figure 1), cytidine-$(Me_3Si)_4$ (16% relative intensity), 5-methylcytidine-$(Me_3Si)_4$ (6.7%), 5-azacytidine-$(Me_3Si)_4$ (9%), 5-methyluridine-$(Me_3Si)_4$ (**27**, 2.4%), 6-methyluridine-$(Me_3Si)_4$ (**28**, 5%), and 2-thiouridine-$(Me_3Si)_4$ (4%). The isotopic (compounds **7–11** and [2-^{14}C]uridine) and substituent (**27**, **28**) labeling patterns unexpectedly revealed the absence of O^2 of the uracil ring, as well as O-2′ and O-3′, but showed the presence of C-2, C-5 and C-6. Approximately 86% of H from C-1′ and 14% from C-2′ is retained but none from C-3′, C-5′, or the sugar Me_3Si methyl groups. Metastable ion evidence shows B + 13 to be derived in part from ions B + 131 and B + 41. These data are consistent with a mechanism (eq 7) involving stabilization by the lone pair



(7)

on the C-4 heteroatom, thus accounting for retention of H-1′ and the increased abundance of B + 13 in derivatives of cytidine compared with uridine.[41] A second pathway from ion B + 41 (**20**) requires expulsion of CO (C-2′ + O^2) with retention of H-2′. Ion B + 13 is potentially useful for selective measurement of ^{18}O in the uracil nucleus, but caution must be exercised in its use for establishing the position of thiation in an unknown pyrimidine nucleoside. The ion was observed in the spectrum of 4-thiouridine-$(Me_3Si)_4$, while a different mechanism operates in the case of 2-thiouridine-$(Me_3Si)_4$ and 2-thiocytidine-$(Me_3Si)_4$, as

Table VI. Sugar Ion Series from Trimethylsilylated Nucleosides

ion	mass, m/z pentose	mass, m/z 2′-O-methylpentose
S	349.1687	291.1448
S – H	348.1609	290.1370
S – CH_3OH		259.1186
S – Me_3SiOH	259.1186	
S – H – Me_3SiOH	258.1108	200.0869
S – H – CH_2OSiMe_3	245.1030	187.0791
S – CH_4 – Me_3SiOH	243.0873	185.0634
$C_4H_4O_2(Me_3Si)_2$	230.1159	230.1159
$C_5H_7O_2Me_3Si$		172.0920
$C_3H_3O_2(Me_3Si)_2$	217.1081	217.1081
$C_4H_6O_2Me_3Si$		159.0842
S – 2 Me_3SiOH	169.0685	
CH_2OSiMe_3	103.0580	103.0580

evidenced by its appearance at mass values (m/z 212, 211) consistent with inclusion of the intact base, with sulfur at C-2.

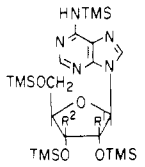
Ions of the Sugar Series. Only three sugars are so far known in all nucleic acids: ribose, 2′-*O*-methylribose, and 2′-deoxyribose, although a great structural variety of modified sugars are found in nucleosides from other natural sources.[33,42] From the standpoint of characterization of RNA-derived nucleosides, identification of the sugar moiety can thus be made from the sugar (S) or S – H fragment ions, corroborated by the difference in mass between the base and molecular ion mass values. The S series of ions, listed in Table VI, were found in the present study to generally arise by multiple pathways which produce isomeric ion mixtures, as determined by complex isotopic labeling patterns. As a result, the present report is limited mainly to the S – H ion, which is of low abundance but of considerable importance for structural applications and for understanding the decomposition processes of nucleosides in general. Unlike most other ions of the sugar series, the S – H ion from labeled uridines gives a relatively clear and consistent picture regarding its mechanistic origin and structure–abundance correlations in the mass spectra of other silylated nucleosides. A summary of isotopic labeling patterns and brief comments on some other members of the sugar series are given in the supplementary material.

Ion S – H is formed directly from the molecular and M – 15 ions, and the mass spectrum of the trimethylsilyl derivative of **3** shows the hydrogen lost exclusively from C-2′. Hydrogen H-2′ is therefore transferred directly to the base which is lost as a neutral species. A similar conclusion was reached in the case of permethylated nucleosides, on the basis of circumstantial evidence without the benefit of deuterium labeling.[26] Space-filling models show that H-2′ and the base readily come within bonding distance during rotation of the glycosidic bond. The selectivity of transfer from C-2′ as opposed to other secondary ribose carbons therefore suggests that significant ring-opening in the sugar does not precede hydrogen rearrangement. Base–H-2′ interactions are also shown by the greatly diminished abundance of S – H when H-2′ is replaced by methyl (**30**) compared with the 3′-methyl isomer **31** (Table VII), in part reflecting the decreased likelihood of methyl rearrangement compared with hydrogen.

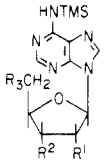
The relative abundances of S – H in the spectra of other nucleosides further suggest a role in S – H formation

(41) Several rational structures can be visualized for ion B + 13; **29** is speculative and is written for convenience.

(42) Suhadolnik, R. J. "Nucleosides as Biological Probes"; Wiley-Interscience: New York, 1979.



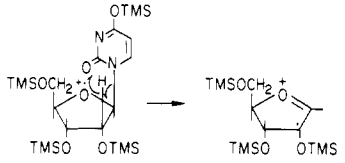
30, $R^1 = CH_3$; $R^2 = H$
31, $R^1 = H$; $R^2 = CH_3$



33, $R^1 = H$; $R^2 = R^3 = OSiMe_3$
34, $R^1 = R^3 = OSiMe_3$; $R^2 = H$
35, $R^1 = R^2 = OSiMe_3$; $R^3 = H$

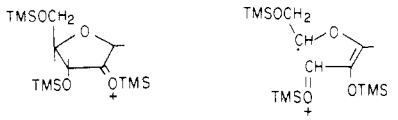
played by O-2′: (1) S – H is generally more abundant from ribonucleosides than 2′-deoxyribonucleosides (e.g., **1** vs. **32** and **24** vs. **33**; see Table VII). (2) The abundance of S – H is significantly lower in 2′-deoxyadenosine (**33**) compared with that in the other deoxy isomers **34** and **35**, showing the influence of O-2′ in stabilization of the resulting odd-electron S – H ion.

The foregoing data are consistent with the mechanism shown in eq 8 in which a sugar-localized charge plays a role



M m/z 348 S – H (8)

in S – H formation. A similar mechanism can be rationalized by utilizing an initial charge on O-2′. It is noted that loss of H-2′ results in stabilization through charge delocalization on O-2′ (**36**) or O-3′ (**37**), in addition to O-4′ (eq

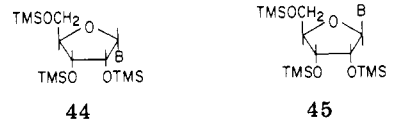


36 **37**

m/z 348

8). The generally greater abundance of S – H and of sugar ions in the spectra of pyrimidine nucleoside derivatives compared with purines is viewed as being due to a relatively greater degree of charge localization in the sugar than the base. Initiation of H-2′ transfer by the charged base (eq 6) as opposed to the charged sugar (eq 8) is considered likely to result in retention of the charge in the base moiety, i.e., the B + H ion.

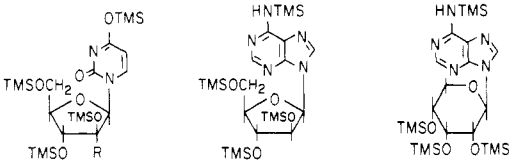
The mechanism shown in eq 8 implies that the abundance of S – H ions will be decreased in nucleosides having restricted steric access of H-2′ to the base. This correlation is observed in the spectra of α,β-anomeric pairs shown in Table VII (e.g., **1** vs. **38**, **39** vs. **40**, **41** vs. **42**, and **32** vs. **43**). In all cases, the abundance of S – H is lower in the α anomer (**44**) than the β anomer (**45**). If both anomers are



44 **45**

available for examination, this suggests that S – H ion abundance may be useful in assignment of anomeric configuration, which is often a problem encountered in nucleoside synthesis involving fusion reactions.[43]

The facility of transfer of H-2′ to the base (eq 8) is also influenced by the steric orientation of the hydroxyl (silyl ether) group at C-2′. The effect is shown by the arabinose derivatives **46** and **47** relative to the ribose analogues **1** and



46, R = H
48, R = D

47 **49**

24 (Table VII). Interestingly, D is lost in the case of **48** to form m/z 348. CPK models show that H-2′ in **46** has steric access to the base without opening of the ribose ring, by rotation of the glycosidic bond, albeit less than when H-2′ is "up" in **1**.

Also noteworthy from the sugar series is m/z 103 ($Me_3SiO^+{=}CH_2$), which occurs extensively in the mass spectra of silylated carbohydrates,[44,45] and nucleotides,[46] and was earlier assigned to the C-5′ silyloxy group.[18] Our experience has shown that this ion, when formed in abundance, is a good indicator of the presence of a 5′-silyloxy group. However, deuterium labeling at C-5′ (**6**) reveals that only 80% is derived from C-5′ of uridine, the remaining being from O-2′ and O-3′ with rearrangement of hydrogen. The appearance of m/z 103 in low abundance in the spectra of the 5′-deoxynucleoside **35** (2.2% relative intensity), 9-(β-D-ribopyranosyl)adenine (**49**, 7.3% relative intensity), and the Me_3Si derivative of adenosine 5′-monophosphate (2.2% relative intensity)[46] therefore dictates caution in its use regarding structural assignments involving C-5′.

Experimental Section

Materials. *N,O*-Bis([$^2H_9$]trimethylsilyl)acetamide and [$^2H_9$]trimethylchlorosilane was purchased from Merck Isotopes, St. Louis, MO. Other reagents for silylation were from Pierce Chemical Co., Rockford, IL.

Sodium borodeuteride (99 atom %) was purchased from Stohler Isotope Chemicals, Waltham, MA. $H_2{}^{18}O$ (98 atom %) was from Norsk-Hydro Sales, New York, NY.

Thin-layer chromatography was performed by using silica gel GF plates (250 μm), and preparative thin-layer chromatography utilized silica gel GF plates (2000 μm, 20 × 20 cm) obtained from Analtech, Inc., Newark, DE.

Other reagents and solvents were purchased from commercial sources and were used without further purification.

Mass Spectrometry. Mass spectra were acquired by using an LKB 9000S gas chromatograph–mass spectrometer: 70-eV ionizing energy; ion source and carrier gas separator temperatures 250–270 °C; sample introduction by gas chromatograph [uridine-$(Me_3Si)_4$ and most other samples], 3-ft 1% OV-17 column. Structures of all ions shown are supported by measurements of exact mass made by peak matching or magnetic scanning at R ≃ 15000 by using a Varian MAT 731 instrument. A Varian MAT 112S mass spectrometer was used to obtain the mass-analyzed ion kinetic energy spectra in which the electric sector voltage was scanned at a fixed magnetic field and accelerating voltage. Metastable ion measurements made by scanning the accelerating voltage with a fixed magnetic field and electric sector voltage were obtained by using a Varian MAT 731 spectrometer. For data acquired with the Varian instrument, samples were introduced by direct probe: ion source temperatures 220 °C; 70-eV ionizing energy.

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Table VII. Abundance of S – H Ion from Selected Trimethylsilylated Nucleosides

parent compd	m/z of S – H	S – H abundance	
		rel intens	% Σ_{70}
2′-*C*-methyladenosine-$(Me_3Si)_4$ (**30**)	362	0.6	0.06
3′-*C*-methyladenosine-$(Me_3Si)_4$ (**31**)	362	10	2.1
uridine-$(Me_3Si)_4$ (**1**)	348	10	1.6
2′-deoxyuridine-$(Me_3Si)_3$ (**32**)	260	<0.1	<0.1
adenosine-$(Me_3Si)_4$ (**24**)	348	9.5	1.0
2′-deoxyadenosine-$(Me_3Si)_3$ (**33**)	260	1.0	0.18
3′-deoxyadenosine-$(Me_3Si)_3$ (**34**)	260	15	3.1
5′-deoxyadenosine-$(Me_3Si)_3$ (**35**)	260	33	6.7
1-(α-D-ribofuranosyl)uracil-$(Me_3Si)_4$ (**38**)	348	1.0	0.24
1-(α-D-ribofuranosyl)cytosine-$(Me_3Si)_4$ (**39**)	348	1.2	0.29
cytidine-$(Me_3Si)_4$ (**40**)	348	20	2.3
9-(β-D-xylofuranosyl)adenine-$(Me_3Si)_4$ (**41**)	348	6.0	1.2
9-(α-D-xylofuranosyl)adenine-$(Me_3Si)_4$ (**42**)	348	1.0	0.19
2′-deoxy-9-(α-D-ribofuranosyl)adenine-$(Me_3Si)_3$ (**43**)	260	<0.1	<0.1
1-(β-D-arabinofuranosyl)uracil-$(Me_3Si)_4$ (**46**)	348	2.0	0.4
9-(β-D-arabinofuranosyl)adenine-$(Me_3Si)_4$ (**47**)	348	<0.1	<0.1

Preparation of Trimethylsilyl Derivatives. Dried nucleoside (50–100 μg) was heated with *N,O*-bis(trimethylsilyl)-trifluoroacetamide, or *N,O*-bis(trimethylsilyl)acetamide, trimethylchlorosilane, and pyridine (100:1:10) for 1 h at 100 °C.

[2′-^{2}H]Uridine (3) and 1-(β-D-[2-^{2}H]Arabinofuranosyl)-uracil (48). To a solution of 3′,5′-di-*O*-trityl-2′-oxouridine[47] (340 mg, 0.47 mM) in ethanol (10 mL) was added sodium borodeuteride ($NaBD_4$, 300 mg). After being stirred at room temperature for 1 h, the reaction mixture was partitioned between chloroform ($CHCl_3$) (25 mL) and water (25 mL). The $CHCl_3$ layer was removed and dried over solid sodium sulfate. The aqueous layer was extracted three additional times with $CHCl_3$ (10 mL), and the extracts were combined and dried over sodium sulfate. Removal of $CHCl_3$ under reduced pressure afforded 310 mg of an off-white foam. The foam was redissolved in $CHCl_3$ (2 mL) and applied, evenly distributed, to ten preparative thin-layer plates. After seven consecutive developments of the plates in carbon tetrachloride–acetone (5:1), two closely spaced bands were evident. Separation of the faster moving band and elution of the silica gel with acetone afforded 28 mg (0.038 mmol, 8% yield) of 3′,5′-di-*O*-trityl[2′-^{2}H]uridine identical with an authentic sample (unlabeled) by thin-layer chromatography (chloroform-ethyl acetate (1:1)).[47] Detritylation of the 3′,5′-di-*O*-trityl[2′-^{2}H]uridine was effected by treatment with 80% acetic acid (5 mL) at 100 °C for 4 h. The reaction mixture was extracted with $CHCl_3$ (2 × 5 mL), and the aqueous phase was applied to a preparative thin-layer plate. Following development of the plate in ethyl acetate–1-propanol–water (4:1:2; upper phase), the single band in the region with an R_f of ~0.4 was separated and the silica gel eluted with methanol until no UV-absorbing fractions were observed. Removal of the methanol under reduced pressure afforded approximately 5 mg (0.023 mmol, 60% yield) of **3** which was shown by gas chromatography[48] to be free of any of the arabino analogue **48**. Compound **48** was isolated in 40% yield (47 mg) from the starting ditrityl compound by isolation of the slower moving band from the preparative thin-layer plate (during the carbon tetrachloride–acetone separation step) by using the same procedure as used for **3**.

[3′-^{2}H]Uridine (4). Preparation of **4** followed exactly the same procedure as described above for **3** except that 2′,5′-di-*O*-trityl-3′-oxouridine (340 mg) was used for the starting material. The yield of **4** was 22 mg (20% from the ditrityl starting material). The purity of **4** was established by thin-layer chromatography at the 2′,5′-di-*O*-trityluridine and uridine stages and by gas chromatography.

[5′-^{2}H]Uridine (5). Preparation of **5** has been previously described.[49]

[5′,5′-$^2H_2$]Adenosine (50). Ethyl adenosine-5′-carboxylate (1.54 g) was suspended in absolute ethanol (30 mL), and the solution was cooled to 20 °C. Sodium borodeuteride (0.24 g) was then added in small portions. The solution was stirred at 20 °C for an additional 20 h and then evaporated to dryness in vacuo. The residue was dissolved in water (50 mL) and cooled to 0 °C, and the pH was carefully adjusted to 6.5 with dilute hydrochloric acid. The solid was collected by filtration, washed with a small amount (5 mL) of cold water and then recrystallized from water to yield 1.06 g of product, mp 234–236.

1,2,3,5-Tetra-*O*-acetyl-β-D-[5,5-$^2H_2$]ribofuranose (51). [5′,5′-$^2H_2$]Adenosine (**50**, 1.34 g) was heated in acetic anhydride (5 mL) and acetic acid (0.5 mL) at reflux temperature for 14 h. The solution was allowed to stand at 5 °C for 18 h, and the precipitated N^6-acetyladenine was then removed by filtration. The filtrate was evaporated in vacuo to afford a syrup. This syrup was poured onto ice and then allowed to stand at 0–5 °C for 12 h. The solid was collected by filtration and recrystallized twice from methanol to afford 1.158 g of the desired product, mp 80–82 °C.

[5′,5′-$^2H_2$]Uridine (6). A mixture of uracil (1.0 g) in 40 mL of hexamethyldisilizane containing a catalytic amount of ammonium sulfate (2 mg) was heated to reflux temperature. After 2 h a clear solution was effected, and heating was continued for an additional 2 h. The solvent was removed in vacuo, and the remaining residue was dissolved in 30 mL of 1,2-dichloroethane. 1,2,3,5-Tetra-*O*-acetyl-β-D-[5,5-$^2H_2$]ribofuranose (**51**, 3.0 g) was dissolved in 30 mL of 1,2-dichloroethane, and 2 mL of stannic chloride was then added. This mixture was stirred at room temperature for 30 min and then added to the above solution. This reaction mixture was then stirred for 18 h, at which time a single UV-absorbing compound was observed by TLC. Pyridine (2 mL) was added to the reaction mixture, the mixture was stirred for another 30 min, and the solution was then filtered through a Celite pad. The filtrate was washed with a saturated sodium bicarbonate solution (2 × 50 mL) and then water (2 × 50 mL). The solution was then evaporated to afford a syrup which was dissolved in 40 mL of methanolic ammonia (saturated solution). This solution was allowed to stand at 5 °C for 18 h and evaporated to dryness. The residue was dissolved in a small amount of methanol which was applied to the top of a silica gel column (50 g). The product was eluted with a mixture of methanol–chloroform (20:80 v/v) to yield 880 mg of **6**.

[*O*^{2}-^{18}O]Uridine (7) and [*O*^{2},5′-$^{18}O_2$]uridine (9) were synthesized by ring opening of *O*^{2},5′-anhydro-2′,3′-*O*-isopropylideneuridine as described elsewhere.[50]

[*O*^{4}-^{18}O]Uridine (8) was synthesized by exchange using $H_2{}^{18}O$ in 1 N HCl by a previously reported procedure.[51]

[2′-^{18}O]Uridine (10). A solution of 3′-5′-di-*O*-trityl-2′-oxouridine (300 mg, 0.41 mM) in methanol (3 mL) containing 0.01 N HCl–$H_2{}^{18}O$ (150 μL) was agitated at 45 °C for 18 h. Following removal of the solvents under reduced pressure, the foam was dissolved in ethanol (9 mL), sodium borohydride (265 mg) was

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added, and the reaction was allowed to proceed at room temperature for 1 h. At this stage, the isolation and deblocking procedure described above in the preparation of **3** was followed exactly to provide less than 1 mg of **10** (yield not determined). The product was shown to be free of arabino isomer by using thin-layer and gas chromatography and was used without further purification.

[3′-^{18}O]Uridine (11). A sample of 2′,5′-di-*O*-trityl-3′-oxouridine (300 mg) was treated as described above in the preparation of **10** to give approximately 2 mg (yield not determined) of **11** shown to be free of any xylo isomer by thin-layer and gas chromatography.

***O*^{4}-([^{2}H$_9$]Trimethylsilyl)-2′,3′,5′-tris-*O*-(trimethylsilyl)uridine (12).** A mixture of hexamethyldisilazane, trimethylchlorosilane, and pyridine (50 μL, 10:5:1) was added to **2** (50 μg), heated 1 h at 100 °C, and then dried in vacuo.[52] A mixture of *N*,*O*-bis([^{2}H$_9$]trimethylsilyl)acetamide, [^{2}H$_9$]trimethylchlorosilane, and pyridine (50 μL, 100:1:10) was added and the solution heated for 1.2 h at 100 °C.

***O*^{4},2′,3′,5′-*O*-Tetrakis([^{2}H$_9$]trimethylsilyl)uridine (13)** was prepared from **2** by using deuterated reagents. Resulting solutions of **12** and **13** were submitted directly to gas chromatography–mass spectrometry.

1-(β-D-Ribofuranosyl)naphthalene, from which the trimethylsilyl derivative **18** was derived, was prepared by the procedure of Ohrui and co-workers.[53]

The following were prepared earlier in the authors' laboratories: 3′-*O*-methyladenosine and 5′-deoxyadenosine;[24] 8-(methylamino)adenosine;[54] 9-(β-D-ribopyranosyl)adenine.[55] N^6,N^6,$O^{3'}$-Trimethyladenosine was previously obtained as a byproduct during preparation of related compounds.[24] All other nucleosides were obtained from commercial sources, with the exception of the following: 8-oxoadenosine, Dr. A. D. Broom, University of Utah; 9-(β-D-xylofuranosyl)adenine, 9-(β-D-arabinofuranosyl)-adenine, 8-bromoadenosine, and 6-methyluridine, Cancer Chemotherapy National Service Center of the National Institutes of Health; 2′-amino-2′-deoxyadenosine and 2-thioadenosine, Dr. M. Ikehara, Osaka University; 2-thiouridine, Dr. T. Hashizume, Tokyo University of Agriculture and Technology; 2′-*C*-methyladenosine and 3′-*C*-methyladenosine, Dr. F. W. Holly, Merck, Sharp, and Dohme; 2-(β-D-ribofuranosyl)adenine and 8-(β-D-ribofuranosyl)adenine, Dr. J. Igolen, Institut Pasteur, Paris; 4-hydroxy-3(5)-(β-D-ribofuranosyl)pyrazole-5(3)-carboxamide (pyrazomycin), Dr. K. Gerzon, Indiana University School of Medicine, Indianapolis; 9-(α-D-xylofuranosyl)adenine and 9-(α-D-2′-deoxyribofuranosyl)adenine, Dr. L. Goodman, University of Rhode Island; 4-(β-D-ribofuranosyl)pyrazole-3-carboxamide and 4-(β-D-ribofuranosyl)pyrazole-3-carboxylic acid methyl ester, 5-(α-D-ribofuranosyl)isocytidine and 5-(β-D-ribofuranosyl)isocytidine, Dr. J. J. Fox, Sloan Kettering Institute for Cancer Research.

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Registry No. 1, 51432-30-5; **12**, 82741-03-5; **13**, 53502-53-7; **23**, 60091-09-0; **25**, 82741-04-6; **26**, 82741-05-7; **27**, 53294-30-7; **28**, 82741-06-8; **30**, 82741-07-9; **31**, 82741-08-0; **32**, 51432-34-9; **33**, 76223-08-0; **34**, 82741-09-1; **35**, 82752-43-0; **38**, 53941-24-5; **39**, 82741-10-4; **40**, 51476-19-8; **41**, 82741-11-5; **42**, 82741-12-6; **43**, 82741-13-7; **46**, 53294-28-3; **47**, 82741-14-8; **48**, 82752-44-1; **49**, 82741-15-9; 3′,5′-di-*O*-trityl-2′-oxouridine, 16731-30-9; 3′,5′-di-*O*-trityl[2′-^{2}H]uridine, 82741-16-0; 2′,5′-di-*O*-trityl-3′-oxouridine, 16731-37-6; [5′,5′-^{2}H$_2$]adenosine, 82741-17-1; ethyl adenosine-5′-carboxylate, 35803-57-7; 1,2,3,5-tetra-*O*-acetyl-β-D-[5,5-^{2}H$_2$]ribofuranose, 82741-18-2; uracil, 66-22-8; 9-[2,3,5-tris(trimethylsilyl)-β-D-ribofuranosyl)-purine, 53294-27-2; 6-[(trimethylsilyl)oxy]-9-[2,3,5-tris(trimethylsilyl)-β-D-ribofuranosyl]purine, 53294-31-8; 6-chloro-9-[2,3,5-tris-(trimethylsilyl)-β-D-ribofuranosyl]purine, 60091-06-7; 6-mercapto-9-[2,3,5-(trimethylsilyl)-β-D-ribofuranosyl]purine, 82741-19-3; 2′,3′,5′,N^6-tetrakis(trimethylsilyl)adenosine, 53294-33-0; 2-[(trimethylsilyl)oxy]-2′,3′,5′,N^6-tetrakis(trimethylsilyl)adenosine, 82741-20-6; 2-chloro-2′,3′,5′,N^6-tetrakis(trimethylsilyl)adenosine, 82741-21-7; 2-mercapto-2′,3′,5′,N^6-tetrakis(trimethylsilyl)adenosine, 82752-45-2; 2-[(trimethylsilyl)amino]-2′,3′,5′,N^6-tetrakis(trimethylsilyl)-adenosine, 82741-22-8; 2-[(trimethylsilyl)amino]-9-[2′,3′,5′-tris(trimethylsilyl)-β-D-ribofuranosyl]purine, 82752-46-3; 2-[(trimethylsilyl)amino]-6-[(trimethylsilyl)oxy]-9-[2′,3′,5′-tris(trimethylsilyl)-β-D-ribofuranosyl]purine, 53294-38-5; 2-[(trimethylsilyl)amino]-6-chloro-9-[2′,3′,5′-tris(trimethylsilyl)-β-D-ribofuranosyl]purine, 82752-47-4; 6-mercapto-2-[(trimethylsilyl)amino]-9-[2′,3′,5′-tris(trimethylsilyl)-β-D-ribofuranosyl]purine, 82741-23-9; 5-(β-D-ribofuranosyl)uracil-$(Me_3Si)_5$, 82741-24-0; 5-(α-D-ribofuranosyl)uracil-$(Me_3Si)_5$, 53294-25-0; 4-(β-D-ribofuranosyl)pyrazole-3-carboxamide-$(Me_3Si)_5$, 82752-48-5; 4-(β-D-ribofuranosyl)pyrazole-3-carboxylic acid methyl ester-$(Me_3Si)_4$, 82741-25-1; 5-(β-D-ribofuranosyl)isocytidine-$(Me_3Si)_5$, 82741-26-2; 5-(α-D-ribofuranosyl)isocytidine-$(Me_3Si)_5$, 82741-27-3; 8-(β-D-ribofuranosyl)adenine-$(Me_3Si)_5$, 82752-49-6; 2-(β-D-ribofuranosyl)adenine-(Me_3Si), 82741-28-4; 4-hydroxy-3(5)-(β-D-ribofuranosyl)pyrazole-5(3)-carboxamide-$(Me_3Si)_6$, 72360-96-4; 5-(α-D-ribofuranosyl)-2-oxo-4-aminopyrimidine-$(Me_3Si)_5$, 82741-29-5; 5-(β-D-ribofuranosyl)-2-oxo-4-aminopyrimidine-$(Me_3Si)_5$, 82741-30-8; [2-hydroxy-1-(9-guanine)]ethyl 1,3-dihydroxy-2-propyl ether-$(Me_3Si)_5$, 82741-31-9; N^2,N^2,7-trimethyl-8-oxoguanosine-$(Me_3Si)_4$, 82752-50-9; 2′-*O*-methylguanosine-$(Me_3Si)_4$, 82741-32-0; 5′-deoxyguanosine-$(Me_3Si)_3$, 82741-33-1; 5′-deoxyguanosine-$(Me_3Si)_4$, 82741-34-2; 6-thioguanosine-$(Me_3Si)_4$, 82741-35-3; 7-methyl-8-oxoguanosine-$(Me_3Si)_5$, 82741-36-4; 7-(aminomethyl)-7-deazaguanosine-$(Me_3Si)_6$, 82741-37-5; 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine-$(Me_3Si)_8$, 82741-38-6; 7-cyano-7-deazaguanosine-$(Me_3Si)_5$, 82741-39-7; 8-aminoguanosine-$(Me_3Si)_6$, 82741-40-0; 8-bromoguanosine-$(Me_3Si)_5$, 82741-41-1; guanosine-$(Me_3Si)_5$, 53294-38-5; N^2-methylguanosine-$(Me_3Si)_4$, 53294-42-1; N^2,N^2-dimethylguanosine-$(Me_3Si)_5$, 53294-41-0; 1-methyladenosine-$(Me_3Si)_3$, 53294-40-9; 1-methylinosine-$(Me_3Si)_3$, 55556-96-2; N^6-furfuryladenosine-$(Me_3Si)_3$, 82741-42-2; 2′-*O*-methyluridine-$(Me_3Si)_3$, 53359-14-1; 3′-*O*-methyladenosine-$(Me_3Si)_3$, 82741-43-3; N^6,N^6,*O*-3′-trimethyladenosine-$(Me_3Si)_2$, 82741-44-4; 2-thiouridine-$(Me_3Si)_4$, 82741-45-5; 4-thiouridine-$(Me_3Si)_3$, 53294-34-1; 4-thiouridine-$(Me_3Si)_4$, 82752-51-0; α-pseudouridine-$(Me_3Si)_5$, 53294-26-1; 1-(α-D-ribofuranosyl)uracil-$(Me_3Si)_4$, 53941-24-5; 6-thioguanosine-$(Me_3Si)_5$, 82752-52-1; 7-methyl-8-oxoguanosine-$(Me_3Si)_6$, 82741-46-6.

Supplementary Material Available: Discussion of isotopic labeling patterns and structure assignments to ions from uridine-$(Me_3Si)_4$ not discussed in the primary text, mass-abundance tables for selected ions from the mass spectra of isotopically labeled uridine-$(Me_3Si)_4$, abundances of M − 90 and M − 180 ions from *C*-nucleosides; scheme of fragmentation pathways for ions of the sugar series, names of 16 guanosine analogues examined for M/(M − 15) ratio correlation, and complete mass spectra of 23 trimethylsilylated nucleosides from which data were reported (53 pages). Ordering information is given on any current masthead page.

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