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(20) Compound **7a**: ^1H ($CDCl_3$) 1.63 and 1.70 (2 s, 9 H, allylic Me), 1.88 (m, 3 H, Me at C-3), 2.07 (br m, 8 H, allylic CH_2), 2.15 (s, 3 H, MeCO), 5.17 (m, 2 H, vinyl H), 5.60 ppm (t, J = 10 Hz, H, C-2 proton); ^{19}F NMR ($CFCl_3$ reference) 63.3 and 63.7 ppm (2 d, J = 10 Hz each, 2*E* and 2*Z* isomers); CIMS *m/e* 241 (MH^+ − HOAc). Anal. Calcd for $C_{17}H_{26}F_2O_2$: C, 67.97; H, 8.73. Found: C, 68.36; H, 8.75.
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(22) Compound **8a** (*E*): ^1H NMR ($CDCl_3$) 1.38 (d of t, J = 1, 7 Hz, 6 H, ethoxy Me), 1.63 and 1.70 (2 s, 9 H, allylic Me), 1.92 (*m*, 3 H, Me at C-3), 2.02 (m, 6 H, allylic CH_2), 2.13 (m, 2 H, C-4 protons), 4.25 (q, J = 7 Hz, 4 H, OCH_2), 5.15 (m, 2 H, vinyl), 5.58 ppm (t, J = 10 Hz, H, C-2 proton); ^{19}F NMR ($CFCl_3$ reference) 55.06 ppm (br m); CIMS *m/e* 395 (MH^+), 375 (MH^+ − HF). Anal. Calcd for $C_{19}H_{33}F_2PO_4$: C, 57.85; H, 8.43; P, 7.85. Found: C, 58.11; H, 8.28; P, 7.76.

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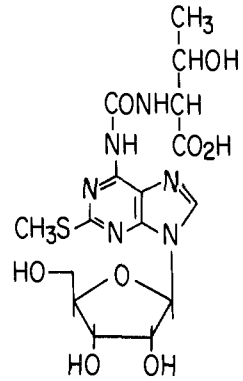
Structure Elucidation by High Resolution Mass Spectrometry of a Highly Modified Nucleoside from Mammalian Transfer RNA. *N*-[(9-β-D-Ribofuranosyl-2-methylthiopurin-6-yl)carbamoyl]threonine

Sir:

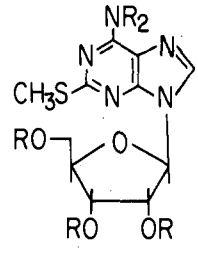
The chemical and conformational properties of modified nucleosides in tRNA, particularly those in the anticodon region, are thought to play a role in the biological functions of transfer RNA.[1,2] As interest in structure and function of eukaryotic tRNA grows, the structure elucidation of new nucleosides becomes more difficult owing to the complexity of structures encountered[2] and limitations in sample quantity. We report here the structure of the title compound as **1**,[3] based on mass spectrometry carried out on 35 μg of material.



1, 458.1203 (−1.7)

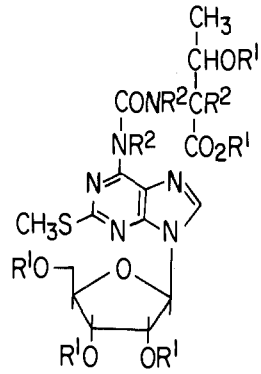
Unfractionated rabbit liver tRNA (5 g) was incubated with nuclease P_1 (pH 5.0, 37 °C, 2 h) and the resulting hydrolysate fractionated by DEAE-cellulose (DE-52) with a linear NaCl gradient (0–0.2 M, pH 7.5) in the presence of 7 M urea. Fractions containing the unknown nucleoside N as pNpA were converted into mononucleotides (snake venom phosphodiesterase, pH 7.5, 37 °C, 18 h) which were separated by DEAE Sephadex A-25 (pH 7.8, 0.1–0.7 M, triethylammonium bicarbonate gradient). Nucleotide pN (6 A_{260} units) was purified by two-dimensional paper electrophoresis and chromatography (first run, 30 V/cm for 5 h with 5% acetic acid (adjusted to pH 3.5 by pyridine); second run, isobutyric acid–0.5 M NH_3 (5:3 v/v)) and then dephosphorylated by alkaline phosphomonoesterase.[4]

Permethylation of the unknown nucleoside using methylsulfinyl carbanion with CD_3I or CH_3I[5] each yielded two



2, R = CD_3, 398.2558 (0.1)
3, R = CH_3, 383.1611 (−1.6)
8, R = H, 313.0831 (−1.4)

products which were completely fractionated by vaporization from the mass spectrometer probe at 100 (**2, 3**) and 150 °C (**4, 5**), while trimethylsilylation[6] produced a single derivative ($Si(CH_3)_3$, **6**; $Si(CD_3)_3$, **7**).



4, $R^1 = R^2 = CD_3$
5, $R^1 = R^2 = CH_3$, 570.2448 (−2.4)
6, R^1 = Me_3Si (TMS), R^2 = H, 818.3184 (−1.2)

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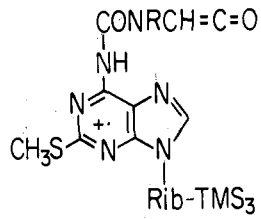
Complete high resolution mass spectra were photographically recorded.[7] Exact molecular masses of permethyl[5] and trimethylsilyl[8] derivatives were established by calculating mean values derived from experimentally measured molecular ion and base series of ions,[9] in which the unknown parameter (the exact mass of the base) is associated in each measurement with known mass differences (e.g., M − CH_3, base + H). Determination of the number of blocking groups introduced by comparison of molecular weights (**2** vs. **3**, **6** vs. **7**) then permitted calculation of the masses of **1** and its degradation product **8**, as shown.

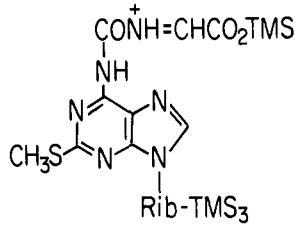
The structure elucidation of nucleoside N followed the characterization of **8** based on the premise that the identity of **8** would permit selection of the basic skeleton from the four major bases. The exact mass of **2** results in 11 computer generated candidates for composition, which can be narrowed by application of a set of restrictions which are generally applicable to nucleosides: (1) total number of rings and double bonds between 4 and 12, (2) oxygen ≥4, (3) nitrogen ≥2, (4) the nitrogen rule. As a result, no candidates emerge which contain only C, H, D, N, and O, while inclusion of S results in a single possibility for **2**: $C_{16}H_{10}D_{15}N_5O_4S$. The mass spectrum of **2** shows an unmodified sugar (*m/e* 149, 183),[5] an ion of *m/e* 120 ($C_6H_4D_5O_2$) generally characteristic of adenosine derivatives,[5] and loss of CD_2ND from the base + H species, thus revealing **8** to have a free exocyclic amino group.[5,10] These data, plus a detailed analysis of the high resolution mass spectra of **2** and **3**, lead to structure **8**. An isomer bearing a methylenethiol function is excluded by the extent of methylation (5 instead of 6); substitution of C-8 is excluded by incorporation of one deuterium (D_2O, 100 °C for 1 h, then cold H_2O).

The mass difference between **1** and **8** (146.0467) permits three compositions within the limits $C_{\leq 10}H_{\leq 20}N_{\leq 5}O_{\leq 6}S_{\leq 1}$ (±0.004 amu): $C_6H_4N_5$, $C_3H_8N_5S$, and $C_5H_8NO_4$. The N_5



R = H, 612.2020 (−1.7)
R = Me_3Si, 684.2415 (−1.8)

candidates are rejected as structurally implausible. The third corresponds to threonine plus CO, leading to structure **1**. Support for the N^6-carbamoylthreonine structure is gained from the high resolution spectra of **6** and **7**, which includes fragment ions of *m/e* 612, 684, and 701,[11] which have analogy in the mass spectra of *N*-[(9-β-D-ribofuranosylpurin-6-yl)-carbamoyl]threonine (t^6A)[12] and its N^6-methyladenosine analogue (mt^6A).[13]



701.2462 (0.2)

The present finding is the first case in which the methylthio group has been found in mammalian tRNA,[14,15] while t^6A and mt^6A occur in both prokaryotic and eukaryotic sources.[16] The new nucleoside (**1**, ms^2t^6A) has recently been located adjacent to the 3′ position of the anticodon in $tRNA_3^{Lys}$ from rabbit liver.[17] Its presence thus satisfies the empirical rule that tRNAs which contain t^6A or its derivatives recognize messenger RNA codons which begin with A.[1,2]

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Silyl Ketone Chemistry. A New Regiospecific Route to Silyl Enol Ethers

Sir:

The reversible rearrangement of silyl groups from carbon to oxygen in α-silyl alkoxides (**1** ⇌ **2**)[1] provides an unusual route to potentially useful carbanions. We are investigating several synthetic applications of this rearrangement and report here the development of a new regiospecific silyl enol ether

1 2

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