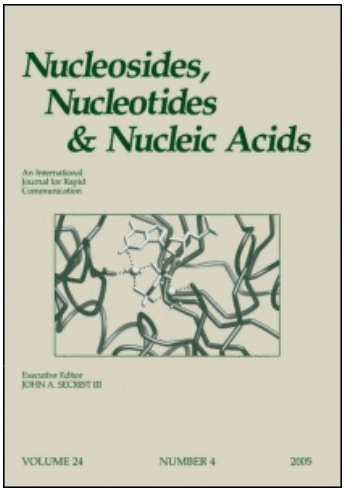



**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# Synthesis of the Selenium Analog of the Cytokinin 6-(3-Methyl-2-Butenylamino)-2-Methylthio-9-(β-D-Ribofuranosyl)Purine

Dean S. Wise Jr.[a]; Leroy B. Townsend[a]
[a] Department of Medicinal Chemistry College of Pharmacy and Department of Chemistry, The University of Michigan, Ann Arbor, MI

**To cite this Article** Wise Jr., Dean S. and Townsend, Leroy B.(1986) 'Synthesis of the Selenium Analog of the Cytokinin 6-(3-Methyl-2-Butenylamino)-2-Methylthio-9-(β-D-Ribofuranosyl)Purine', Nucleosides, Nucleotides and Nucleic Acids, 5: 5, 511 — 516
**To link to this Article: DOI:** 10.1080/07328318608068693
**URL:** http://dx.doi.org/10.1080/07328318608068693

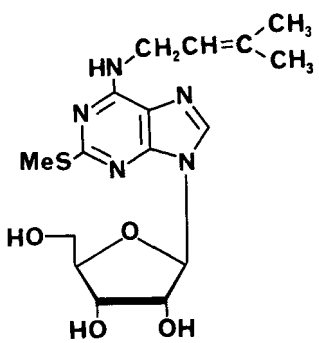
NUCLEOSIDES & NUCLEOTIDES, 5(5), 511-516 (1986)

# SYNTHESIS OF THE SELENIUM ANALOG OF THE CYTOKININ 6-(3-METHYL-2-BUTENYLAMINO)-2-METHYLTHIO-9-(β-D-RIBOFURANOSYL)PURINE

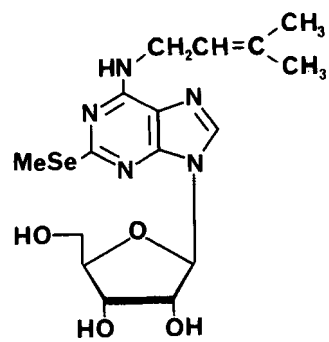
Dean S. Wise, Jr. and Leroy B. Townsend*
Department of Medicinal Chemistry
College of Pharmacy
and Department of Chemistry
The University of Michigan
Ann Arbor, MI 48109-1065



6-(3-Methyl-2-butenylamino)-2-methylthio-9-(β-D-ribofuranosyl)purine (1) is a naturally occurring nucleoside with potent cytokinin activity. It has been isolated and identified in the t-RNA of E. coli,[1,2] the t-RNA from wheat germ[3,4] and from Staphylococcus epidermidis.[5] In addition, compound 1 has been found in t-RNA species corresponding to each of six amino acids whose codons start with uridine, i.e., t-RNA$^{Cys}$,



1



2

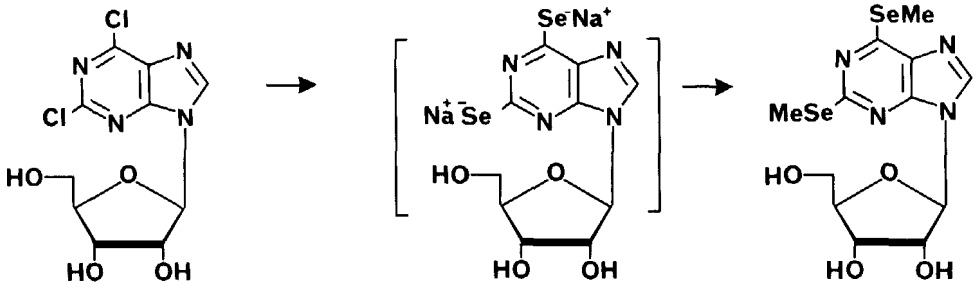
t-RNA$^{Leu}$, t-RNA$^{Phe}$, t-RNA$^{Ser}$, t-RNA$^{Trp}$ and t-RNA$^{Tyr}$.[6] However, it is not present in all sub-species which respond to these codons.[6] It



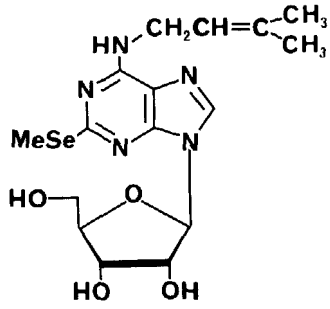


0732-8311/86/0505-0511$3.50/0

has been determined to be positioned next to the 3'-end of the anti-codon in E. coli t-RNA$^{Tyr}$.[7,8] Incorporation of selenium into t-RNA molecules has been observed in several bacterial species.[9-11] Recently, a selenium containing t-RNA was isolated from cultured mouse leukemia cells which had been grown in medium supplemented with $H_2^{75}SeO_3$ [12]. Although this nucleoside was not characterized, it was thought that it was most likely a 6-threonine derivative of 2-methylseleno-9-(β-D-ribofuranosyl)purine. Due to the above finding, one might expect that a selenium substituted congener of 1 may yet be found




3 4 5




2

in nature. This prompted us to prepare 6-(3-methyl-2-butenylamino)-2-methylseleno-9-(β-D-ribofuranosyl)purine (2).

In our initial attempts to prepare the target compound 2, 2,6-dichloro-9-(β-D-ribofuranosyl)purine[13] (3) was reacted with sodium hydrogen selenide, and after no starting material could be detected by TLC, the reaction was treated with Dowex-50($H^+$) resin in an attempt to isolate the 2,6-di-selenoxo intermediate 4. However, this product proved to be unstable in our hands. Therefore, we decided

against the isolation of this intermediate and instead elected to treat the crude sodium salt of 4 with methyl iodide. The precipitated crude 4 was collected, suspended in ethanol and then treated with enough 0.1 N sodium methylate to effect a clear solution. The subsequent reaction of crude 4 with methyl iodide furnished, after chromatography, a white solid which was characterized as 2,6-bis-methylseleno-9-(β-D-ribofuranosyl)purine (5). That alkylation had occurred at the 2- and 6-selenoxo groups rather than at a ring nitrogen was established by the chemical shift for the methyl protons in the $^1$H NMR spectrum and by the hypsochromic shift (from 363 nm for 4 to 317 nm for 5) observed in the UV spectrum.

Treatment of the bis-methylseleno-derivative 5 with 3-methyl-2-butenylamine hydrochloride in the presence of triethylamine afforded a smooth conversion of 5 to the desired cytokinin analog 6-(3-methyl-2-butenylamino)-2-methylseleno-9-(β-D-ribofuranosyl)purine (2) in 67% yield. The ultraviolet spectra of 2 displayed a nearly direct comparison with the parent methylthio derivative 1.[14] In the mass spectra of 2, the presence of the methylseleno function had little influence upon the basic fragmentation behavior reported for 1 other than an appropriate upward shift in mass ions which contain the adenine nucleus.[15]



## EXPERIMENTAL

Proton nuclear magnetic resonance ($^1$H NMR) spectra were obtained on a Varian EM-390 spectrophotometer with chemical shift values reported in δ units relative to an internal standard (tetramethylsilane). Ultraviolet absorption spectra (UV) were recorded on a Beckman Acta C-III spectrophotometer. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Analytical thin layer chromatography was performed on Analtech SilicAR GF plates. Compounds of interest were detected either by an ultraviolet lamp (254 nm) or treatment with sulfuric acid followed by charring. Open-bed column chromatography was carried out on SilicAr CC7 (Mallinckrodt) using gravity flow. Solvent proportions are given by volume. Evaporations were performed under reduced pressure (water aspirator) or in vacuo at 40° with a rotary evaporator. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

2,6-Bis-methylseleno-9-(β-D-ribofuranosyl)purine (5)

2,6-Dichloro-9-(β-D-ribofuranosyl)purine[3] (3, 3.4 g, 9.8 mmole) was dissolved in 250 mL of 95% ethanol. Nitrogen was bubbled through this solution for 0.5 hr prior to the addition of any reagents and then throughout the rest of the reaction sequence. To this solution was added 80 mL of abs. ethanol containing NaSeH (54.4 mmole) prepared from sodium metal (1.25 g, 54.4 mmole) and subsequent saturation of the sodium ethoxide solution with $H_2Se$. The orange reaction mixture was heated at reflux temperature for 4 hr, then cooled to room temperature and subsequently stored at 0° for 14 hr. The precipitate was collected by filtration (1.8 g), added to ethanol (50 mL), and then enough 1 N sodium methylate was added to effect a clear solution. Methyl iodide (2.82 g, 20 mmole), was added to this solution and the reaction mixture was stirred at room temperature for 10 hr during which time the solution became colorless. The reaction mixture was neutralized to pH 7 by the addition of $CO_2$ and then evaporated onto ≈ 3 g of SilicAR CC-7. This material was added to the top of a 2.5 x 20 cm SilicAR CC-7 dry column and eluted with a methanol:chloroform (1:9 v:v) mixture. The fractions containing the product, as determined by TLC in an identical solvent system, were pooled and evaporated in vacuo to afford 0.85 g (20%) of the bis-methylseleno-derivative 5, m.p. 104-106°; UV $\lambda_{max}$ nm, ($\varepsilon \times 10^3$); (MeOH) 235 (12.1), 277 (16.9), 316 (11.4); (pH 1) 279 (16.9), 317 (12.7); (pH 11), 235 (11.8), 267 (15.8), 317 (11.8). $^1$H NMR (DMSO-$d_6$) δ 8.68 (1 H, $C_8H$, s); 5.91 (1 H, $C_{1'}$-H, d, $J_{1',2'}$ = 8 Hz); 2.56, 2.51 (6 H, $CH_3Se$, s). Anal. Calcd. for $C_{12}H_{16}N_4O_4Se_2$: C, 32.89; H, 3.68; N, 12.78. Found: C, 33.04; H, 3.63; N, 12.72.

6-(3-Methyl-2-butenylamino)-2-methylseleno-9-(β-D-ribofuranosyl)-purine (2)

2,6-Bis-methylseleno-9-(β-D-ribofuranosyl)purine (5, 0.44 g, 1 mmole), 3-methyl-2-butenylammonium chloride (0.266 g, 2.2 mmole) and triethylamine (0.84 mL, 6 mmole) were added to a solution of 20 mL of methanol and 20 mL of water. The reaction was heated at reflux temperature for 48 hr. The reaction was cooled to room temperature, 3 g of SilicAR-CC7 was added and the solvent removed in vacuo. The resulting dried SilicAr, containing the absorbed product, was placed on the top of a SilicAr-CC7 dry column (2.5 x 30 cm) and eluted with methanol:chloroform (1:9, v:v). The fractions which contained product,

as determined by TLC, were combined and evaporated. The resulting residue was recrystallized from isopropyl alcohol to yield pure 2; 0.29 g (67%); m.p. 190-191°decomposed; UV $\lambda_{max}$ nm, ($\varepsilon$ x $10^3$); (MeOH) 249 (22.3), 286 (17.1); (pH 1) 240 (19.7), 290 infl (17.5); (pH 11), 243 (20.8 ), 286 (17.1). $^1$H NMR (DMSO-$d_6$) $\delta$ 8.18 (1 H, $C_8$ H, s); 7.9 (1 H, N-H, s); 5.77 (1 H, $C_{1'}$-H, d, $J_{1',2'}$ = 6 Hz); 2.43 ( 3 H, $CH_3$-Se, s); $\delta$ 1.73 (6 H, $CH_3$, s). Anal. Calcd. for $C_{16}H_{23}N_5O_4Se$: C, 44.86; H, 5.41; N, 16.35. Found: C, 44.67; H, 5.37; N, 16.48.

ACKNOWLEDGMENT

This work was supported by Research Grants CA-11147 and CA-12585, awarded by the National Cancer Institute, DHEW. We thank Ms Deanna VanSickle for her assistance in the preparation of this manuscript.



REFERENCES

1. W. J. Burrows, D. J. Armstrong, F. Skoog, S. M. Hecht, J. T. A. Boyle, N. J. Leonard and J. Occolowitz, Science, 161, 691 (1968).

2. W. J. Burrows, D. J. Armstrong, F. Skoog, S. M. Hecht, J. T. A. Boyle, N. J. Leonard and J. Occolowitz, Biochem., 8, 3071 (1969).

3. S. M. Hecht, N. J. Leonard, W. J. Burrows, D. J. Armstrong, F. Skoog, J. Occolowitz, Science, 166, 1271 (1969).

4. W. J. Burrows, D. J. Armstrong, M. Kaminek, F. Skoog, R. M. Bock, S. M. Hecht, L. G. Dammann, N. J. Leonard, and J. Occolowitz, Biochem., 9, 1867 (1970).

5. D. J. Armstrong, P. K. Evans, W. J. Burrows, F. Skoog, J.-F. Petit, J. L. Dahl, T. Steward, J. L. Strominger, N. J. Leonard, S. M. Hecht, and J. Occolowitz, J. Biol. Chem., 245, 2922 (1969).

6. J. Bartz, D. Soll, W. J. Burrows and F. Skoog, Proc. Natl. Acad. Sci., U.S.A, 67, 1448 (1970).

7. F. Harada, H. J. Gross, F. Kimura, S. H. Chang, S. Nishimura and U. L. Raj Bhandary, Biochem. Biophys. Res. Commun., 33, 294 (1968).

8. M. L. Gefter and R. L. Russell, J. Molec. Biol., 39, 145 (1969).

9. C.-S. Chen, T. C. Stadtman, Proc. Natl. Acad. Sci., U.S.A, 77, 1403 (1980).

10. W.-M. Ching, A. J. Wittwer, L. Tsai and T. C. Stadtman, Proc. Natl. Acad. Sci. U.S.A, 81, 57 (1984).

11. A. Wittwer, J. Biol. Chem., 258, 8637 (1983).

12. W.-M. Ching, Proc. Natl. Acad. Sci., U.S.A, 81, 3010 (1984).

13. T. Sato in "Synthetic Procedures in Nucleic Acid Chemistry," W. W. Zorbach and R. S. Tipson, eds, Wiley Interscience, NY, 1968, Vol. 1, p. 264.

14. R. Y. Schmitz, F. Skoog, S. M. Hecht and R. M. Bock, Phyto. Chem., 11, 1603 (1972).

15. J. G. Liehr, C. L. Weise, P. F. Crain, G. H. Milne, D. S. Wise, L. B. Townsend and J. A. McCloskey, J. Heterocyclic Chem., 16, 1263 (1979).

Received March 21, 1986.