Communications to the Editor

[Chem. Pharm. Bull. 35(10)4372—4374(1987)]

SYNTHESIS OF 7-METHYL-3-β-D-RIBOFURANOSYLWYE, THE MOST PROBABLE STRUCTURE FOR THE FLUORESCENT NUCLEOSIDE ISOLATED FROM ARCHAEBACTERIAL TRANSFER RIBONUCLEIC ACIDS

Taisuke Itaya
Faculty of Pharmaceutical Sciences, Kanazawa University,
Takara-machi 13-1, Kanazawa 920, Japan

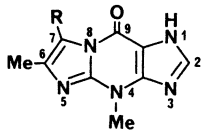
3-β-D-Ribofuranosylwye (**2a**) was converted into 7-methyl-3-β-D-ribofuranosylwye (**2b**) through the 2',3',5'-tri-*O*-acetate by formylation with $POCl_3$ and $HCONMe_2$ followed by catalytic hydrogenolysis.

KEYWORDS —— fluorescent nucleoside; tricyclic nucleoside; archaebacterium tRNA; Vilsmeier-Haack formylation; catalytic hydrogenolysis; glycosidic bond cleavage; acidic hydrolysis

Since the finding[1] of the fluorescent component at the position adjacent to the 3'-end of the anticodon of yeast phenylalanine transfer ribonucleic acid ($tRNA^{Phe}$), five tricyclic bases have been obtained from eukaryotic $tRNAs^{Phe}$ and their structures have been elucidated as **1a**,[2] **1c**,[3] **1d**,[4] **1e**,[5] and **1f**.[6,7] The parent nucleosides of **1a**[8] and **1c**[1,9] have been isolated from yeast $tRNAs^{Phe}$ and 3-β-D-ribofuranosylwye (**2a**), the most probable structure for the former, has been synthesized.[10] Recently, McCloskey and coworkers[11] found a new fluorescent component in unfractionated tRNAs of three extremely thermophilic archaebacteria and assigned 7-methylwye (**1b**) to the structure of the base by direct comparison with an authentic sample,[3c] which had been synthesized by us. Quite recently, Chattopadhyaya et al. reported the synthesis of 7-methyl-3-β-D-ribofuranosylwye (**2b**),[10e] the proposed structure[11] for the new fluorescent nucleoside. This paper presents an alternative synthesis of **2b**.

Probably, the most straightforward synthesis of **2b** is cyclocondensation of 3-methylguanosine[10a,b,c,12] with AcCHBrMe. This method, however, did not work effectively. We have reported the synthesis of **1b** by the Vilsmeier-Haack reaction of 1-benzylwye followed by reduction with $NaBH_4$ and hydrogenolysis over Pd–C.[3c] This procedure was now applied successfully to the nucleoside level as follows.

Acetylation of **2a** with Ac_2O and pyridine followed by treatment with a mixture of $POCl_3$ and $HCONMe_2$ at −25°C for 4 h gave the aldehyde **3** as a glass, ^{1}H-NMR (CDCl_3) δ: 10.83 (s, CHO), in 92% yield. Although reduction of **3** with $NaBH_4$ in tetra-



1

a: R = H wye

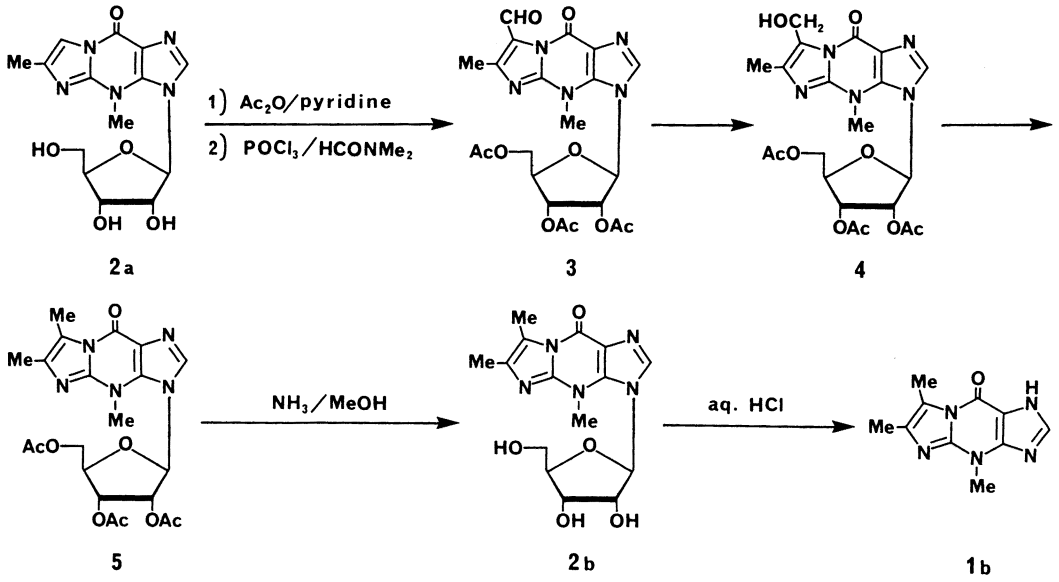
b: R = Me

c: R = CH_2CH_2–C(CO$_2$Me)(H)(NHCO$_2$Me)

d: R = CH_2CH(OH)CHCO$_2$Me (NHCO$_2$Me)

e: R = CH_2CH(O$_2$H)CHCO$_2$Me (NHCO$_2$Me)

f: R = CH_2CH(OH)CHCO$_2$H (NH_2)



hydrofuran gave the alcohol **4**, the yield was poor, probably owing to partial migration of an acetyl group to the oxygen of the 7-hydroxymethyl group of **4**. Compound **3** was then directly submitted to catalytic reduction over Pd–C in EtOH to give **5** as a glass, ^{1}H-NMR ($CDCl_3$) δ: 2.11 (s, Ac), 2.15 (s, Ac), 2.18 [s and q, Ac and C(6)Me], 2.63 [q, J = 0.7 Hz, C(7)Me], 4.10 (s, NMe), 4.31 [d, J = 3 Hz, C(5')H_2], 4.49 [m, J = 3 and 4 Hz, C(4')H], 5.48 [d-d, J = 4 and 5 Hz, C(3')H], 5.85 [d-d, J = 5 and 6 Hz, C(2')H], 6.21 [d, J = 6 Hz, C(1')H], 7.66 [s, C(2)H]; CD (H_2O) $[\theta]^{20}_{232}$ +6700, in 32% yield. Deprotection of **5** was performed by treating with saturated methanolic NH_3 at 0°C for 5 h to afford **2b** as colorless needles (from H_2O), mp ca. 190°C (dec.); UV $\lambda^{95\%\ EtOH}_{max}$ 239 nm (ε 28800), 298 (5700); $\lambda^{H_2O}_{max}$ (pH 2) 233 (31300), 279 (10000); $\lambda^{H_2O}_{max}$ (pH 7) 241 (29800), 302 (5300); $\lambda^{H_2O}_{max}$ (pH 13) 241 (30800), 302 (5500); ^{1}H-NMR [$(CD_3)_2SO$] δ: 2.11 [q, J = 0.7 Hz, C(6)Me], 2.56 [q, J = 0.7 Hz, C(7)Me], 3.63 [m, C(5')H_2], 3.98 [m, C(4')H], 4.01 (s, NMe), 4.12 [m, C(3')H], 4.45 [m, C(2')H], 5.11 (br, 5'-OH), 5.30 (d, J = 5 Hz, 3'-OH), 5.69 (d, J = 6 Hz, 2'-OH), 6.08 [d, J = 5 Hz, C(1')H], 8.17 [s, C(2)H]; CD (H_2O) $[\theta]^{20}_{244}$ −7300, in 72% yield. Compound **2b** underwent hydrolysis of the glycosidic bond at the rate (rate constant 4.7×10^{-1} min^{-1}, half-life 88 s) comparable to that (4.4×10^{-1} min^{-1})[10b,c] of **2a** in 0.1 N aq. HCl at 25°C to give **1b**[3c] in 86% yield, confirming the correctness of the structure **2b**.

Although the ultimate identification of the nucleoside from natural sources[11] was difficult owing to the extremely minute amounts available, it was identical with the synthetic one according to fast atom bombardment mass spectrometry and high performance liquid chromatography.[13,14] These results further support the proposal that the structure of the fluorescent nucleoside from the archaebacterial tRNAs is **2b**.[11]

It appears that analogues of **2b** oxidized at the 7-methyl group to a variable extent may be components of tRNAs of some species. The present synthesis has an advantage over the reported procedure[10e] in that it comprises a potential for synthesizing these nucleosides.

ACKNOWLEDGMENT The author is grateful to Professors J. A. McCloskey and T. Hashizume, University of Utah, for mass spectral and chromatographic comparison of **2b** with the natural nucleoside.

REFERENCES AND NOTES

1) U. L. RajBhandary, R. D. Faulkner, and A. Stuart, J. Biol. Chem., 243, 575 (1968).
2) a) H. Kasai, M. Goto, S. Takemura, T. Goto, and S. Matsuura, Tetrahedron Lett., 1971, 2725; b) H. Kasai, M. Goto, K. Ikeda, M. Zama, Y. Mizuno, S. Takemura, S. Matsuura, T. Sugimoto, and T. Goto, Biochemistry, 15, 898 (1976).
3) a) K. Nakanishi, N. Furutachi, M. Funamizu, D. Grunberger, and I. B. Weinstein, J. Am. Chem. Soc., 92, 7617 (1970); b) R. Thiebe, H. G. Zachau, L. Baczynskyj, K. Biemann, and J. Sonnenbichler, Biochim. Biophys. Acta, 240, 163 (1971); c) T. Itaya and A. Mizutani, Tetrahedron Lett., 26, 347 (1985).
4) H. Kasai, Z. Yamaizumi, Y. Kuchino, and S. Nishimura, Nucleic Acids Res., 6, 993 (1979).
5) a) K. Nakanishi, S. Blobstein, M. Funamizu, N. Furutachi, G. Van Lear, D. Grunberger, K. W. Lanks, and I. B. Weinstein, Nature, New Biol., 234, 107 (1971); b) S. H. Blobstein, D. Grunberger, I. B. Weinstein, and K. Nakanishi, Biochemistry, 12, 188 (1973); c) D. Yoshikami and E. B. Keller, ibid., 10, 2969 (1971); d) A. M. Feinberg, K. Nakanishi, J. Barciszewski, A. J. Rafalski, H. Augustyniak, and M. Wiewiórowski, J. Am. Chem. Soc., 96, 7797 (1974); e) A. Mochizuki, Y. Omata, and Y. Miyazawa, Bull. Chem. Soc. Jpn., 53, 813 (1980); f) M. Barciszewska, M. Kaminek, J. Barciszewski, and M. Wiewiórowski, Plant Sci. Lett., 20, 387 (1981).
6) Y. Kuchino, H. Kasai, Z. Yamaizumi, S. Nishimura, and E. Borek, Biochim. Biophys. Acta, 565, 215 (1979).
7) For a recent review, see T. Itaya, Yuki Gosei Kagaku Kyokai Shi, 45, 431 (1987).
8) S. Takemura, H. Kasai, and M. Goto, J. Biochem., 75, 1169 (1974).
9) a) R. Thiebe and H. G. Zachau, Eur. J. Biochem., 5, 546 (1968); b) S. H. Blobstein, R. Gebert, D. Grunberger, K. Nakanishi, and I. B. Weinstein, Arch. Biochem. Biophys., 167, 668 (1975).
10) a) S. Nakatsuka, T. Ohgi, and T. Goto, Tetrahedron Lett., 1978, 2579; b) T. Itaya, T. Watanabe, and H. Matsumoto, J. Chem. Soc., Chem. Commun., 1980, 1158; c) T. Itaya, H. Matsumoto, T. Watanabe, and T. Harada, Chem. Pharm. Bull., 33, 2339 (1985); d) B. Golankiewicz and W. Folkman, Nucleic Acids Res., 11, 5243 (1983); e) H. Bazin, X-X. Zhou, C. Glemarec, and J. Chattopadhyaya, Tetrahedron Lett., 28, 3275 (1987).
11) J. A. McCloskey, P. F. Crain, C. G. Edmonds, R. Gupta, T. Hashizume, D. W. Phillipson, and K. O. Stetter, Nucleic Acids Res., 15, 683 (1987).
12) T. Itaya and K. Ogawa, Tetrahedron Lett., 1978, 2907.
13) The comparison was done by J. A. McCloskey's group at the University of Utah.
14) Liquid chromatographic analysis was carried out according to reported procedure: M. Buck, M. Connick, and B. N. Ames, Anal. Biochem., 129, 1 (1983).

(Received September 3, 1987