CHEMISTRY LETTERS, pp. 1283-1286, 1979. Published by the Chemical Society of Japan

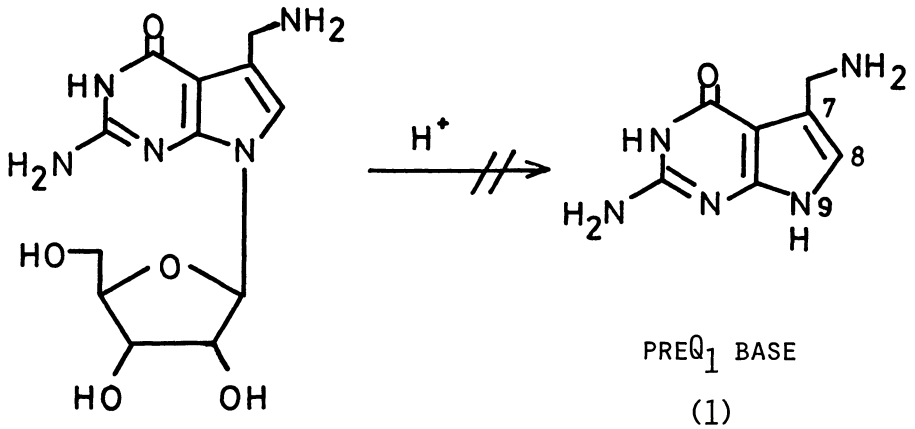
SYNTHESIS OF 7-AMINOMETHYL-7-DEAZAGUANINE, ONE OF THE NUCLEOSIDE Q (QUEUOSINE) PRECURSORS FOR THE POST-TRANSCRIPTIONAL MODIFICATION OF tRNA

Tadaaki OHGI, Tadao KONDO, and Toshio GOTO*

Department of Agricultural Chemistry, Nagoya University, Chikusa, Nagoya 464

7-Aminomethyl-7-deazaguanine, which is one of the precursors of nucleoside Q (queuosine) biosynthesis, was synthesized from 2-methylthio-6-methoxy-7-methyl-7-deazapurine in 13 steps.

Nishimura et al.[1,2] have found that modified nucleoside Q (queuosine), which is present in the first position of anticodon of $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asp}$, and $tRNA^{Asn}$,[3,4] is biosynthesized by post-transcriptional modification of the tRNA's; originally located guanine in the first position is replaced in the presence of a specific tRNA transglycosylase with precursors, one of which is assumed to be 7-aminomethyl-7-deazaguanine ($preQ_1$ base, 1) since $preQ_1$ nucleoside has been isolated from E. coli tRNA and its structure was determined to be 7-aminomethyl-7-deazaguanosine.[5] The $preQ_1$ base (1) could not be prepared by hydrolysis of the $preQ_1$ nucleoside since the glycosylic linkage of the deazaguanosine type nucleosides strongly resists towards acid hydrolysis. We carried out, therefore, a total synthesis of the $preQ_1$ base (1) to confirm the structure of this pre-



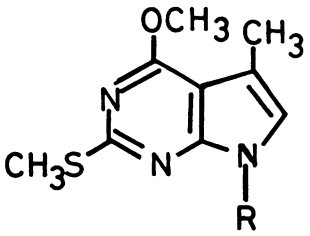
$PREQ_1$ NUCLEOSIDE

$PREQ_1$ BASE

(1)

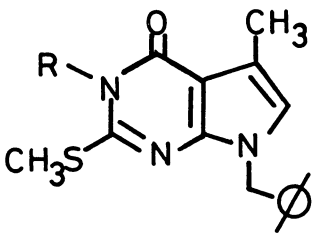
cursor and to use it for the post-transcriptional modification of tRNA.[2]

Starting deazapurine 2[6] was N-benzylated[7] with benzyl bromide in the presence of sodium hydride in DMF to give 3 (80%), mp 95°C [λ_{max}^{MeOH} nm (ε) 288 (10,000), 247 (19,600)]. A solution of 3 in a mixture of 0.5 N HCl and dioxane (1:2) containing a trace of 4,4'-thiobis-(6-t-butyl-3-methylphenol)[9] was refluxed to afford 4 as a crystalline solid (84%), mp 236°C[8] [λ_{max}^{MeOH} 295 (9,580), 275sh (8,840), 227 (20,300); $\lambda_{max}^{MeOH-KOH}$ 283 (10,200), 227 (20,300)]. Isopropoxymethylation of 4 was carried out with sodium hydride and isopropoxymethyl chloride.[10] Silica gel preparative tlc of the reaction mixture gave 5 (83%), mp 114°C[8] [λ_{max}^{MeOH} 303 (10,900), 280sh (8,800), 227 (22,500)]. Displacement of the methylthio group with acetylamino group was done according to our previous papers[9] using sodium acetamide prepared <u>in situ</u>; the product 6 was obtained as needles (85%), mp 155°C[8] [λ_{max}^{MeOH} 302 (8,900), 270sh (6,100), 225 (19,600)]. The deazaguanine 6 was heated with acetic anhydride and pyridine (1:2) at 60°C and the reaction mixture was dried up completely to give the diacetamide 7[11] [PMR ($CDCl_3$) ppm 2.33 (6H, s, Ac_2N), 6.56 (1H, q, J=1 Hz)], which was treated with 1.2 equiv. of N-bromosuccinimide (NBS) and a trace of benzoyl peroxide in benzene at room temp. to afford the monobromide 8 as a syrup (91% after purification by prep. SiO_2 tlc)[11] [PMR ($CDCl_3$) 2.42 (3H, s, $CH_3C=C$)]. A suspension of 8, NBS (1.2 equiv.), K_2CO_3, and a trace of benzoyl peroxide in CCl_4 was refluxed under vigorous stirring for 3.5 h. After filtration and evaporation of the solvent, the residual syrup 9 [PMR ($CDCl_3$) 4.78 (2H, s)] was treated with sodium azide in anhydrous DMF at room temp. for 15 min with shaking. Addition of water and extraction with CH_2Cl_2 gave a product which was acetylated with acetic anhydride and pyridine (1:2). Purification by prep. tlc gave the azide 10 (76%) [PMR ($CDCl_3$) 4.60 (2H, s, CH_2-N_3)]. Hydrolysis of 10 with conc. ammonia-methanol (1:2) at room temp. gave 11 as crystals (89%), mp 158°C,[8] which was hydrogenated in methanol and benzene in the presence of 10% Pd-C giving 12[11] as its hydrobromide[12] in almost quantitative yield [λ_{max}^{MeOH} 285 (7,600), 265 (11,000)]. To a solution of 12 hydrobromide in liquid ammonia was added sodium under vigorous stirring at -78°C. After addition of ammonium chloride the mixture was evaporated and the residue was separated by prep. tlc using ammonia-saturated methanol and CH_2Cl_2 (3:17) to give 13 as white powder[11] (70%) [λ_{max}^{MeOH} 290sh, 260, 217; PMR (CD_3OD) 4.10 (2H, br.s, $C\underline{H}_2$-NH_2), 5.53 (2H, s, OCH_2N), 6.73 (1H, br.s, H-8)]. The protecting group of 13 was



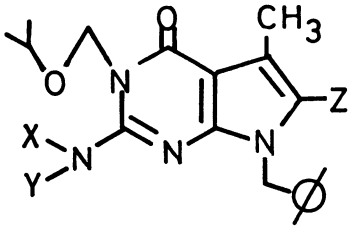
2: R = H

3: R = $CH_2Ø$



4: R = H

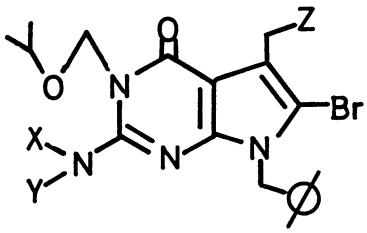
5: R = $CH_2OCH(CH_3)_2$



6: X = H, Y = Ac, Z = H

7: X = Y = Ac, Z = H

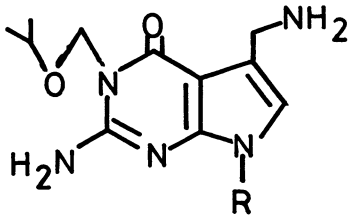
8: X = Y = Ac, Z = Br



9: X = Y = Ac, Z = Br

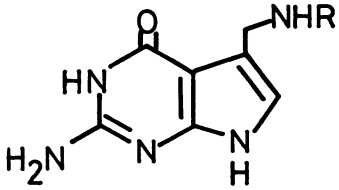
10: X = Y = Ac, Z = N_3

11: X = Y = H, Z = N_3



12: R = $CH_2Ø$

13: R = H



1: R = H

14: R = Ac

removed by heating with 2N HCl at 70°C for 5 h; $preQ_1$ base (1) was obtained by evaporation of the solvent as its hydrochloride (amorphous). Free $preQ_1$ base (1) was isolated as colorless solid (33% from 13) by neutralization with Amberlite IR-420 [Avicel tlc Rf 0.38 (BuOH:HOAc:H_2O, 4:2:1); field desorption mass spec. m/e 180 (M+1); PMR (D_2O, external TMS) 4.26 (2H, s), 6.97 (1H, br.s, H-8); λ_{max}^{MeOH} 285sh, 260, 217; $\lambda_{max}^{MeOH-HCl}$ 280, 260, 217]. $PreQ_1$ base (1) was further characterized as its monoacethyl derivative 14, which was prepared by acetylation of 1 [acetic anhydride and pyridine (1:2)] followed by treatment with ammonium hydroxide in methanol [exact mass m/e calcd. for $C_9H_{11}N_5O_2$: 221.0913; found: 221.0935; PMR ($CDCl_3$:CD_3OD, 1:1) 1.98 (3H, s, AcNH), 4.40 (2H, s, CH_2NHAc), 6.56 (1H, s, H-8); λ_{max}^{MeOH} 286sh, 260, 218; $\lambda_{max}^{MeOH-HCl}$ 262, 220; $\lambda_{max}^{MeOH-KOH}$ 262].

The experiment[2] using this synthetic $preQ_1$ base (1) has clearly shown that 1 is indeed a precursor for the post-transcriptional modification of the first position of the anticodon of undermodified $tRNA^{Tyr}$ and $tRNA^{Asn}$.[2]

Acknowledgements — We thank Dr. S. Nishimura, National Cancer Center Research Institute, Tokyo, for valuable advice. This work was partly supported by Grant-in-Aid for Special Project Research, The Ministry of Education, Science and Culture, and Grant from the Naito Foundation.

REFERENCES AND FOOTNOTES

1. Y. Kuchino, H. Kasai, K. Nihei, and S. Nishimura, Nucleic Acids Res., 3, 393 (1976); N. Okada, F. Harada, and S. Nishimura, Nucleic Acids Res., 3, 2593 (1976); N. Okada, T. Yasuda, and S. Nishimura, Nucleic Acids Res., 4, 4063 (1977).
2. N. Okada, S. Noguchi, H. Kasai, N. Shindo-Okada, T. Ohgi, T. Goto, and S. Nishimura, J. Biol.Chem., 254, 3067 (1979).
3. H. Kasai, Z. Ohashi, F. Harada, S. Nishimura, N. J. Oppenheimer, P. F. Crain, J. G. Liehr, D. L. von Minden, and J. A. McCloskey, Biochemisty, 14, 4198 (1975).
4. H. Kasai, Y. Kuchino, K. Nihei, and S. Nishimura, Nucleic Acids Res., 2, 1931 (1975).
5. N. Okada, S. Noguchi, S. Nishimura, T. Ohgi, T. Goto, P. F. Crain, and J. A. McCloskey, Nucleic Acids Res., 5, 2289 (1978).
6. T. Kondo, T. Ohgi, and T. Goto, Agric. Biol. Chem., 41, 1501 (1977).
7. For protection of N-9 position of 7-deazapurines, we have tried to use alkoxymethyl and acyl groups, but the former could not be removed even in strongly acidic conditions and the latter were too labile to acids and bases; cf. T. Ohgi, T. Kondo, and T. Goto, Nucleic Acids Res., Spec. Publ. No. 2, 83 (1976).
8. Satisfactory elemental analysis and PMR spectra were obtained.
9. T. Ohgi, T. Kondo, and T. Goto, Tetr. Letters, 4051 (1977); T. Ohgi, T. Kondo, and T. Goto, J. Am. Chem. Soc., 101, 3629 (1979).
10. The isopropoxymethyl protecting group could be removed in a condition milder than that used in the case of methoxymethyl group[6] affording product in a pure form.
11. Satisfactory exact mass values (within ± 3 millimass units) and PMR spectra were obtained.
12. The salt was formed with HBr that was produced by debromination of 11.

(Received August 16, 1979)