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

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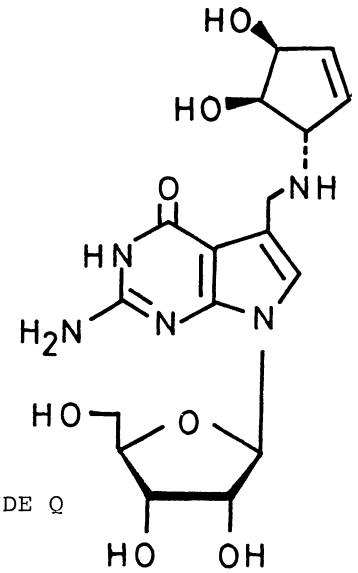
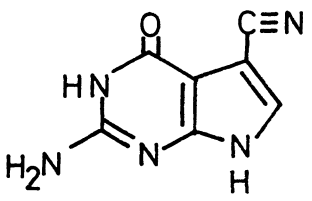
7-Cyano-7-deazaguanine, which is one of the precursors of nucleoside Q (queuosine) biosynthesis, was synthesized from 2-methylthio-6-methoxy-7-methyl-7-deazapurine; methoxymethyl protecting group at 9 position could be removed by transformation to acetoxymethyl group followed by hydrolysis with aq. ammonia.

It was found[1] that hypermodified nucleoside Q (queuosine), which is located at the first position of the anticodon of E.coli $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asp}$, and $tRNA^{Asn}$, is biosynthesized in the presence of a transglycosylase by exchange guanine in these tRNA's with a precursor, which is then modified to Q. One of the precursors, $preQ_1$ base, was assumed to be 7-aminomethyl-7-deazaguanine and confirmed by a total synthesis.[2,3] Another precursor, $preQ_0$ base, was presumed



NUCLEOSIDE Q

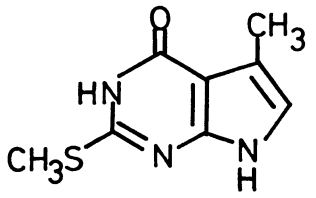


PRE Q_0 BASE (1)

by Okada et al.[4] to be 7-cyano-7-deazaguanine since $preQ_0$ nucleoside was isolated from tRNA of an E. coli mutant and its structure was determined to be 7-cyano-7-deazaguanosine.[5] This paper deals with a total synthesis of 7-cyano-7-deazaguanine (1), which is to be used for the post-transcriptional modification of tRNA.

Our synthetic strategy of the $preQ_0$ base is basically similar to the synthesis of the $preQ_1$ base already reported,[3] but the most elaborated point is the development of a new method of removing the protecting group.[10] The glycosidic linkage of 7-deazapurine nucleosides strongly resists acid hydrolysis and, therefore, deazapurine bases cannot be obtained from the corresponding nucleosides. When methoxymethyl group was used for protection on the pyrrole nitrogen in the starting deazapurine, it could not be removed by acid treatments. Protection of the nitrogen with benzyl group was very useful in the synthesis of $preQ_1$ base,[3] but in the case of $preQ_0$ base, removal of benzyl group with sodium in liq. ammonia was accompanied by hydrogenolysis of the nitrile group.[6] We found that the methoxymethyl group could be converted with acetic anhydride in the presence of acid to acetoxymethyl group, which is easily removable with aq. ammonia. By applying this method the total synthesis of the $preQ_0$ base could be accomplished.

5-Methyl-2-methylthiopyrrolo[2,3-d]pyrimidin-4-one (2)[7] was methoxymethylated with sodium hydride and chloromethyl methyl ether in dimethoxyethane at room temp. to give the di(methoxymethyl) derivative 3 (68%), mp 116°C,[8] which was then heated in acetamide containing excess sodium acetamide to 120°C for 1 h. The solvent was removed in vacuo and the residue was acidified with acetic acid to afford the deazaguanine 4 (75%), mp 140.5°C.[8] A mixture of 4, acetic anhydride, and pyridine was heated at 80°C and then dried up completely to afford the diacetamide 5 [nmr[9] 2.38 (6H, s)]. A solution of 5 and N-bromosuccinimide (NBS)(1.08 eq.) in CCl_4 containing a trace of benzoyl peroxide was refluxed for 30 min to give the monobromide 6 (74%), mp 147-149°C (dec).[8] A suspension of 6, NBS (1.78 eq.), and anhydrous K_2CO_3 in CCl_4 containing a trace of benzoyl peroxide was refluxed for 4 h under occasional monitoring by tlc. Filtration and evaporation of the mixture in vacuo below 50°C gave the dibromide 7 [nmr[9] 4.76 (2H, s, $BrCH_2$-)], which was dissolved in water-dioxane(1:10) and treated with silver carbonate at room temp. for 48 h. The mixture was filtered, the filtrate dried up, and the residue was dissolved in a mixture of water and dioxane(1:3) containing triethylamine.



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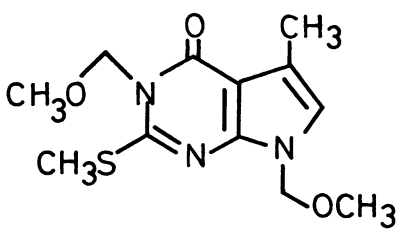

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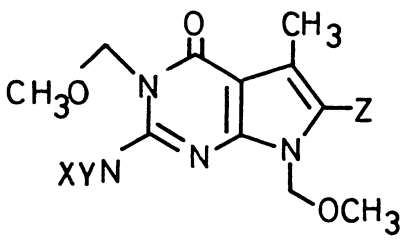
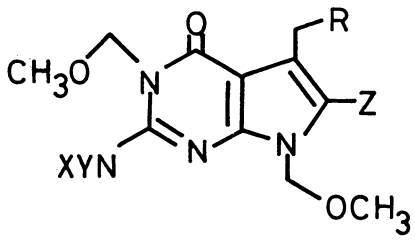


4 X=Ac, Y=Z=H

5 X=Y=Ac, Z=H

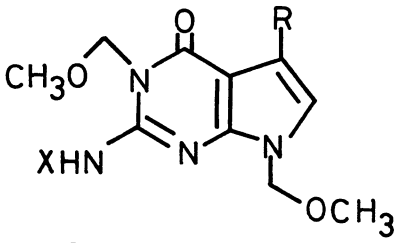
6 X=Y=Ac, Z=Br



7 X=Y=Ac, R=Z=Br

8 R=OH, X=Ac, Y=H, Z=Br

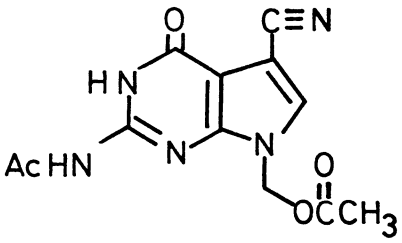
9 R=OH, X=Ac, Y=Z=H



10 R=CHO, X=Ac

11 R=-CH=NOH, X=Ac

12 R=-CN, X=H



13

After allowing to stand at room temp. for 36 h, the solution was worked up as usual to give the 7-hydroxymethyl-8-bromodeazaguanine 8 (84%), mp 137°C[8] [nmr[9] 4.68 (2H, br.s, $-CH_2OH$)]. Removal of the bromine atom of 8 was carried out by hydrogenolysis in aq. methanol containing potassium acetate in the presence of 10% palladium on charcoal under H_2 atmosphere to afford the 7-hydroxymethyl-7-deazaguanine 9 (94%), mp 124°C[8] [nmr[9] 4.72 (2H, br.s, $-CH_2OH$), 6.81 (1H, s, arom.)]. A solution of 9 in dichloromethane containing pyridine was treated with chromic anhydride-pyridine complex at 40°C for 24 h to give the aldehyde 10 (71%), mp 167-168°C[8] [nmr[9] 7.65 (1H, s, arom.), 10.37 (1H, s, -CHO)]. Oxime of 10 was prepared in ethanol with free hydroxylamine made from hydroxylamine hydrochloride and excess silver carbonate. The solution, when dried up, gave the oxime 11 [nmr[9] ($CDCl_3$-CD_3OD, 1:1) 8.05 (1H, -CH=NOH)], which was treated with acetic anhydride and pyridine (3:7) at 55°C for 12 h. Evaporation of the

reagents followed by deacetylation with conc. ammonia in methanol gave the nitrile 12 (58%), mp 191°C[8] [ir ($CHCl_3$) 2230 cm^{-1}; nmr (CD_3OD) 3.31, 3.39 (each 3H, s, CH_3O), 4.85, 5.49 (each 2H, s, $-OCH_2N$), 7.60 (1H, s, arom.); uv(MeOH)nm (ε) 300 (sh, 8200), 290 (9690), 270 (12,600)]. Deprotection of 12 to $preQ_0$ base (1) was carried out by heating in a mixture of acetic anhydride and trifluoroacetic acid (1:1) at 60°C for 2 h to form the acetoxymethyl derivative 13 [nmr ($CDCl_3$-CD_3OD) 2.06 (3H, s), 2.23 (3H, s), 5.98 (2H, s), 7.62 (1H, s)], which was hydrolyzed by treatment with a mixture of conc. ammonia and dioxan (1:1) at 60°C for 2 h. Purification of the product 1 by paper chromatography using n-BuOH:H_2O: conc. ammonia (86:14:5) yielded crystals of 1 (62% from 12), mp above 360°C[8] [exact mass 175.0487 (calc. 175.0494); nmr (DMSO-d_6) 7.52 (1H,s,arom.); λmax (0.1N NaOH) nm (ε) 225 (16900), 245 (10100), 292 (7200); νmax (KBr) 2240 cm^{-1}.

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4. N. Okada, S. Noguchi, H. Kasai, N. Shindo-Okada, T. Ohgi, T. Goto, and S. Nishimura, J. Biol. Chem., 254, 3067 (1979).
5. S. Noguchi, Z. Yamaizumi, T. Ohgi, T. Goto, Y. Nishimura, Y. Hirota, and S. Nishimura, Nucleic Acids Res., 5, 4215 (1978).
6. Cf. T. Ohgi, T. Kondo, and T. Goto, Nucleic Acids Res., Spec. Publ. No. 5, s285 (1978). $preQ_0$ base (1) could also be obtained by this route, but the overall yield was extremely low since oxidation of the unprotected 1-isopropoxymethyl-7-hydroxymethyl-7-deazaguanine with active MnO_2 proceeded poorly.
7. T. Kondo, T. Ohgi, and T. Goto, Agric. Biol. Chem., 41, 1501 (1977).
8. Satisfactory elemental analysis and pmr, uv, and mass spectra were obtained.
9. Nmr were measured in $CDCl_3$ unless otherwise stated, and expressed in ppm from internal tetramethylsilane.
10. S. Nakatsuka, H. Miyazaki, and T. Goto, unpublished results.

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