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Michael T. Migawa [a b], Jack M. Hinkley [a b], Geoffrey C. Hoops [a b] & Leroy B. Townsend [a b]

[a] Department of Chemistry, College of Literature, Science, and Arts, Ann Arbor, Michigan, 48109-1065

[b] Department of Medicinal Chemistry, College of Pharmacy; The University of Michigan, Ann Arbor, Michigan, 48109-1065

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A TWO STEP SYNTHESIS OF THE NUCLEOSIDE Q PRECURSOR 2-AMINO-5-CYANOPYRROLO[2,3-*d*]PYRIMIDIN-4-ONE ($PreQ_0$)

Michael T. Migawa, Jack M. Hinkley, Geoffrey C. Hoops and Leroy B. Townsend*

Department of Chemistry, College of Literature, Science, and Arts; Department of Medicinal Chemistry, College of Pharmacy; The University of Michigan, Ann Arbor, Michigan 48109-1065

$PreQ_0$ (**1**) is prepared in a 70 % yield by the condensation of chloro(formyl)acetonitrile (**2**) with 2,6-diaminopyrimidin-4-one (**3**), and subsequently converted to its amide derivative **5**. Isolation of the intermediate **4** in the condensation reaction was also observed giving further insight into the proposed mechanism.

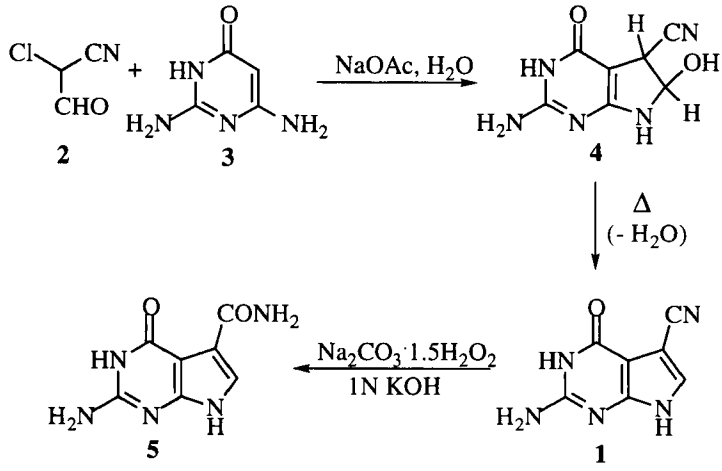
The isolation of nucleoside Q from *Escherichia coli* $tRNA^{Tyr}$, and the structural assignment of this nucleoside as 2-amino-5-(4,5-*cis*-dihydroxy-1-cyclopenten-3-yl-*trans*-aminomethyl)-7-(β-D-ribofuranosyl)pyrrolo[2,3-d] pyrimidine-4-one, prompted the synthesis of structurally related 7-deazaguanosines as potential precursors of nucleoside Q.[1,2] However, it was subsequently found that tRNA-Guanine transglycosylase (TGT) catalyses the exchange of guanine located in the first position of those anticodons possessing a GUN anticodon ($tRNA^{Asp}$, $tRNA^{Asn}$, $tRNA^{Tyr}$, and $tRNA^{His}$) for either 2-amino-5-(4,5-*cis*-dihydroxy-1-cyclopenten-3-yl-*trans*-aminomethyl)pyrrolo[2,3-d] pyrimidine-4-one (queuine) or the biological precursor, 2-amino-5-aminomethylpyrrolo[2,3-*d*]pyrimidin-4-one ($PreQ_1$).[3] This established that a

* To whom correspondence should be addressed

heterocycle rather than a nucleoside is the precursor for the hypermodified nucleoside Q. This finding has prompted a significant increase in the activity associated with the synthesis of 7-deazaguanine analogs. Subsequently, it has been found that 2-amino-5-cyanopyrrolo[2,3-*d*]pyrimidin-4-one (**1**, $PreQ_0$) is a possible biological precursor to queuine, $PreQ_1$, and some structurally related analogs.[3] Furthermore, $PreQ_0$ has been shown to act as a substrate for TGT from *Escherichia coli*..[4]

In addition to its biological importance, $PreQ_0$ could serve as a suitable synthetic precursor for nucleoside Q[5] and other natural products such as; cadeguomycin[6], kanagawamycin[7], archaeosine[8], archaeosine base[9], and echiguanine A and B[10]. Therefore, interest in $PreQ_0$ for its biological activity and its potential use as a synthetic precursor makes a facile route to $PreQ_0$ suitable for large scale synthesis very desirable. Although syntheses of $PreQ_0$ have been previously reported, the routes are either lengthy, involving at least 12 steps from a preformed pyrrolo[2,3-*d*]pyrimidine ring system[11], or involve low yields and the need to separate regioisomers[9]. Herein, we report a short and efficient synthesis of $PreQ_0$ from readily available and inexpensive starting material.

Following the strategy of Noell and Robins[12], we observed that the condensation of chloro(formyl)acetonitrile (**2**) with 2,6-diaminopyrimidin-4-one (**3**) would lead directly to $PreQ_0$ (Scheme). Modifying a known procedure[13] for the formylation of chloroacetonitrile to accommodate a large scale synthesis, we prepared and then immediately condensed **2** with **3**. The resultant precipitate was collected by filtration and converted to its potassium salt with 6N KOH. Reprecipitation of the salt with concentrated HCl furnished a 70% yield of $PreQ_0$. It was of considerable interest that when the reagents were added at 0 °C, and then stirred at room temperature for 12 hours, the intermediate **4** was obtained along with $PreQ_0$ in a ratio of 6:1, respectively, as determined by ^1H nmr (some starting material was also present). Although the diastereomeric configuration of **4** could not be determined by ^1H nmr, only one diastereomer was observed. The isolation of this intermediate allows us to speculate that the loss of water is the last step in the general reaction mechanism. However, it is not yet known whether the carbonyl carbon of **2** becomes bonded to the 6-NH_2 of **3** before or after the formation of the carbon-carbon bond between **2** and **3**.[14] Furthermore, subsequent heating of the intermediate in water at reflux for one hour, or treatment with an acid catalyst, afforded a complete conversion of this



Scheme

intermediate into $PreQ_0$. $PreQ_0$ was converted to the amide **5** using sodium percarbonate[15] in 1N KOH after attempts to prepare **5** using H_2O_2 in NH_4OH[16] failed. Both the amide derivative (**5**) and $PreQ_0$ were found to be identical (^1H nmr, ir and tlc comparisons) to that reported in the literature.[6, 11, 13]

EXPERIMENTAL

Melting points were determined on an Electrothermal IA9100 capillary melting point apparatus and are uncorrected. The ^1H-nmr spectra were recorded on a Bruker 300 or 360 MHz instrument, and ^{13}C-nmr spectra were recorded on a Bruker 90 MHz instrument. Chemical shifts (δ) are in ppm relative to tetramethylsilane. Infrared spectra (ir) were recorded on a Perkin-Elmer spectrometer. Ultraviolet spectra (uv) were recorded in a quartz cell on a Kontron 860 spectrophotometer. High resolution mass spectra (hrms) was performed by the University of Michigan.

Chloro(formyl)acetonitrile (2). While maintaining a temperature of 0 °C, methyl formate (438 g, 7.7 moles) was added to a stirred mixture of sodium

methoxide (357 g, 6.6 moles) in 6 L of THF. This was followed by the dropwise addition of chloroacetonitrile (500 g, 6.6 moles) over a 1 hour period. The mixture was allowed to stir for an additional 3 hours, and then 607 mL of 12M HCl was added dropwise, while maintaining the temperature below 10 °C, to give **2**. This resultant solution was reduced *in vacuo* at 40 °C to 1.5 L and then placed in an addition funnel to be used in the synthesis of **1**.

2-Amino-5-cyanopyrrolo[2,3-d]pyrimidin-4-one (1, PreQ$_0$). $NaOAc{\cdot}3H_2O$ (1.4 kg, 11moles) was dissolved in 11 L of distilled H_2O. 2,6-Diaminopyrimidin-4-one (**3**, 666 g, 5.28 moles) was added and the mixture was heated to 50 °C, at which time the 1.5 L of **2** (prepared above) was added over a 1 hour period. The solution was allowed to stir for 12 hours, and then heated to first remove the THF, and then heated at reflux for 1 hour. The mixture was allowed to cool to room temperature and then filtered. The solid was washed with copious amounts of water and acetone to yield **1**. Compound **1** was purified by converting it to its potassium salt with 5 L of 6N KOH, treating the resultant solution with charcoal, filtering, and then bringing the pH to 6 with 30 % HCl. The resultant solid was collected by filtration and dried in a vacuum oven at 50 °C for 24 hours to yield 647 g (70 %) of PreQ$_0$. A ^{1}H nmr spectrum of the crude compound showed **1** as the only product. An analytical sample was prepared by a Soxlet extraction with methanol containing charcoal; subsequent removal of the charcoal by filtration, evaporation of the solvent, and drying as before yielded a pure white solid. mp >350 °C, Lit[11] > 360 °C; ^{1}H nmr (DMSO-d$_6$): δ 11.97 (bs, 1H, 3-H), 10.70 (bs, 1H, 7-H), 7.61 (s, 1H, 6-H), 6.38 (s, 2H, NH_2); ^{13}C nmr (DMSO-d$_6$): δ 157.6 (C-4), 153.8 (C-2), 151.7 (C-7a), 127.8 (C-6), 115.9 (CN), 98.8 (C-4a) and 85.5 (C-5); ir (potassium bromide): ν 2228 cm^{-1} (CN), Lit.[11] 2240 cm^{-1} (CN); uv (e): (pH1) 222 nm (50093), 257 (26448); (pH7) 229 (13252), 262 (104531), 287 (8722); (pH11) 226 (32437), 271 (Shoulder), 291 (11560); Lit.[11] (0.1N NaOH) 225 (16900), 245 (10100), 292 (7200).

PreQ$_0$ Intermediate (4). The intermediate **4** was prepared on a 59 mmolar scale as above, except the addition of **2** was carried out at 0 °C and then stirred at room temperature before filtering. Attempts to purify this intermediate were unsucessful. ^{1}H nmr (DMSO-d$_6$): δ 9.96 (bs, 1H), 7.57 (s, 1H), 6.64 (d, J = 7.1 Hz, 1H), 6.54 (bs, 2H), 5.32 (t, J = 7.2 Hz, 1H), 4.22 (d, J = 7.3, 1H); ir

(potassium bromide): ν 2256 cm^{-1} (CN); hrms (60 ev w/ DCI probe) m/z: calcd. for $C_7H_7N_5O_2$: 193.0600. Found: 193.0604.

2-Aminopyrrolo[2,3-d]pyrimidin-4-one-5-carboxamide (**5**). Compound **1** (3.57 g, 20 mmoles) was dissolved in 200 mL of 1N KOH, and then sodium percarbonate (3.50 g, 22.5 mmoles) was added in one portion. After stirring for 6 hours at room temperature the reaction mixture was brought to pH=6 with 3N HCl. The resultant precipitate was collected by filtration and dried in a vacuum oven at 50 °C to yield 2.18 g (56 %) of **5** as a yellow powder. mp > 370 °C, Lit.[6] > 300 °C (dec.); ^{1}H nmr (DMSO-d_6): δ 11.55 (br s, 1H, 3-H), 10.85 (br s, 1H, 7-H), 9.55 and 6.95 (2s, 2H, $CONH_2$), 7.22 (s, 1H, 6-H), 6.40 (s, 2H, NH_2). Lit.[6] (DMSO-d_6): δ 11.60 (br s, 1H, 3-H), 10.83 (br s, 1H, 7-H), 9.55 and 7.03 (2s, 2H, $CONH_2$), 7.22 (s, 1H, 6-H), 6.34 (s, 2H, NH_2); ir (potassium bromide): ν 3459 cm^{-1}, 3353 and 3107 (NH, NH_2), 1631 and 1588 (C=O); Lit.[6]: 3300-3120 (NH, NH_2), 1620 and 1590 (C=O).

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REFERENCES

1. Townsend, L. B.; Tolman, R. L.; Robins, R. K.; Milne, G. H. *J. Heterocycl. Chem.*, **1976**, *13*, 1363.

2. Cheng, C. S.; Hinshaw, B. C.; Panzica, R. P.; Townsend, L. B. *J. Am. Chem. Soc.* **1976**, *98*, 7870.

3. Shindo-Okada, N.; Okada, N.; Ohgi, T.; Goto, T.; Nishimura, S. *Biochemistry*, **1980**, *19*, 395.

4. Okada, N.; Noguchi, S.; Kasi, H.; Shindo-Okada, N.; Ohgi, T.; Goto, T.; Nishimura, S. *J. Biol. Chem.*, **1979**, *8*, 3067.

5. Ohgi, T.; Kondo, T.; Goto, T. *Tetrahedron Lett.*, **1977**, *46*, 4051.

6. Ramasamy, K.; Joshi, R. V.; Robins, R. K.; Revankar, G. R. *J. Chem. Soc. Perkin Trans. I,* **1989**, 2375.
7. Naruto, S.; Uno, H.; Tanaka, A.; Kotani, H.; Takase, Y. *Heterocycles*, **1983**, *20*, 27.
8. Gregson, J. M.; Crain, P. F.; Edmonds, C. G.; Gupta, R.; Hashizume, T.; Phillipson, D. W.; and McCloskey, J. A. *J. Biol. Chem.,* **1993**, *268*, 10076.
9. Hashizume, T.; McCloskey, J. A. *Nucleic Acids Symposium Series No. 31*, **1994**, 137.
10. Shih, C.; Hu, Y. *Tetrahedron Lett.,* **1994**, *35*, 4677.
11. Kondo, T.; Nakatsuka, S.; Goto, T. *Chem. Lett.*, **1980**, 559.
12. Noell, C. W.; Robins, R. K. *J. Heterocycl. Chem.,* **1964**, *1*, 34.
13. Lozinskii, M. O.; Gershkovich, A. A. *Zh. Organ. Khim.*, **1974**, *10*, 1482.
14. Secrist III, J. A.; Liu, P. S. *J. Org. Chem.,* **1978**, *43*, 3937.
15. Kabalkla, G. W.; Deshpande, S, M.; Wadgankar, P. P; Chatla, N. *Synth. Commun.*, **1990**, *20*, 1445.
16. Swayze, E. S.; Shannon, W. M.; Buckheit, R. W.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *Nucleosides and Nucleotides,* **1992**, *11*, 1507.

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