Robust Synthesis and Crystal-Structure Analysis of 7-Cyano-7-deazaguanine ($preQ_0$ Base) and 7-(Aminomethyl)-7-deazaguanine ($preQ_1$ Base)

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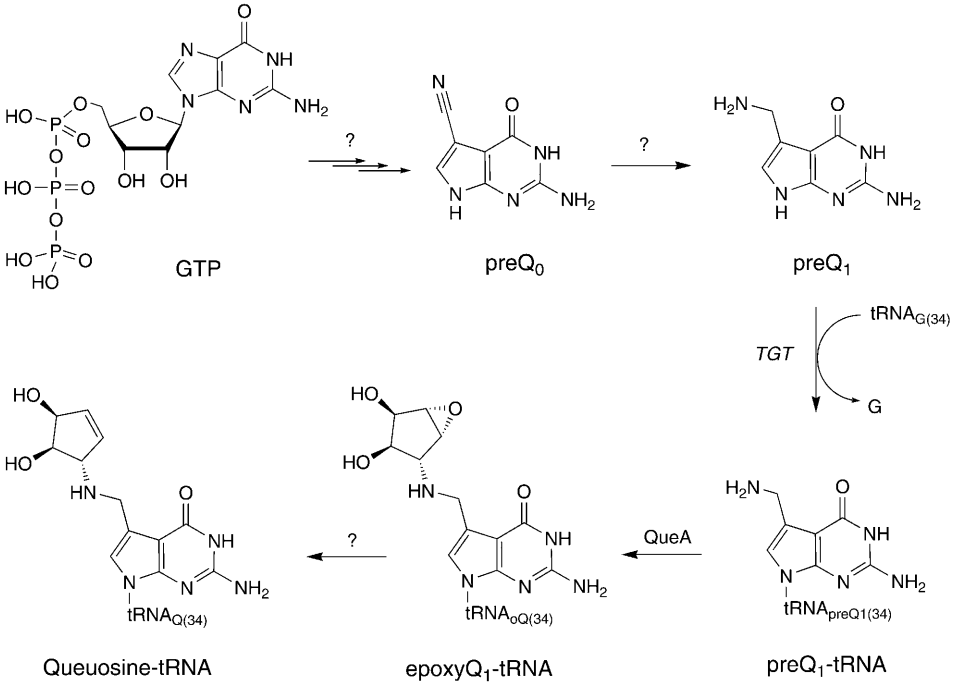
We describe robust and efficient synthetic methods for the synthesis of the $preQ_0$ and $preQ_1$ bases, which are the biosynthetic precursors of the hypermodified RNA nucleoside queuosine. The X-ray crystal-structure analysis of $preQ_1$ is also described.

Introduction. – 10–25% of all nucleotides present in tRNA are modified [1]. Today, more than 80 different nucleotide modifications are known [2]. The nature of the observed nucleotide modifications varies strongly. Frequently, methylations of the nucleobase or of the sugar moieties are found. Sometimes, however, also strongly altered nucleotide structures, so called 'hypermodifications' of the canonical bases, are observed. The biological impact of the modified bases in tRNA is unknown but it is generally assumed that the modifications are needed for the fine tuning of the translational process at the ribosome [3]. The biosynthesis of the hypermodified bases is also only rudimentarily understood. To gain insight into the biosynthesis and the function of the modified bases in tRNA, the chemical synthesis of the modified bases *via* robust, efficient, and high-yielding methods is required.

One of the most significant hypermodifications is the nucleoside queuosine (Q; (=7-{[(3,4-*trans*-4,5-*cis*-4,5-dihydroxycyclopent-1-en-3-yl)amino]methyl}-7-deazaguanosine), which is generally found in the anticodon loop of the tRNAs encoding for the amino acids aspartate, asparagine, histidine, and tyrosine [4]. The nucleoside Q was initially discovered by *Harada* and *Nishimura* in 1972 [5]. The proposed biosynthesis of this unusual nucleoside *via* its biosynthetic precursors $preQ_0$ and $preQ_1$ is depicted in *Scheme 1* [6][7]. Today, it is clear that bacteria produce Q-nucleobase-modified tRNA directly by using the unmodified precursor tRNA as the substrate. The enzyme tRNA-guanine transglycosylase (TGT) removes in a first step a specific guanine from the tRNA by cleaving the glycosidic bond between the ribose and the nucleobase [8]. The abasic site, which is thus created, reacts in a second step with the $preQ_1$ nucleobase, which is biosynthesized by a recently discovered enzyme QueF from $preQ_0$ (=7-cyano-7-deazaguanine) to generate a $preQ_1$ (=7-(aminomethyl)-7-deazaguanine)-containing tRNA [9]. The tRNA containing $preQ_1$ base is subsequently further modified to give first epoxyqueuosine-containing tRNA by the action of the *S*-adenosylmethionine-dependant enzyme QueA. Final conversion of epoxyqueuosine to queuosine is carried out by an unknown enzyme in a possibly vitamin B_{12}-dependant reaction [10].

Scheme 1. *Proposed Biosynthesis of Queuosine-Containing tRNA*

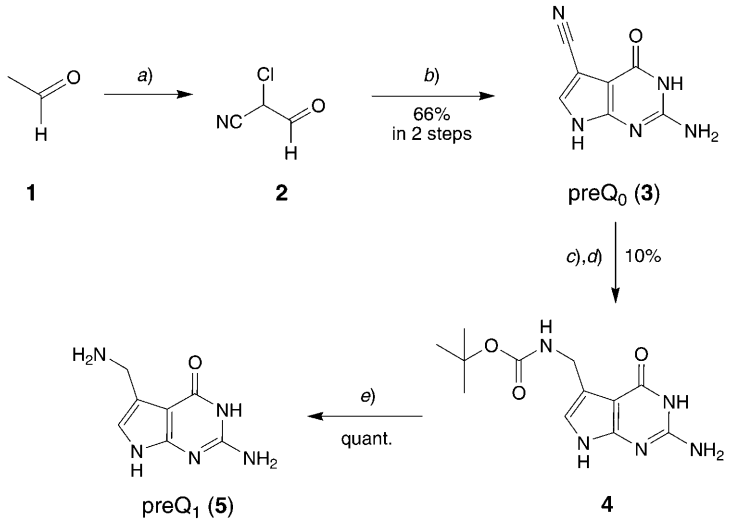


One of the most interesting steps in the biosynthesis is clearly the replacement of a specific guanine base by the $preQ_1$ base on the level of the whole tRNA. To study this process in more detail, it is essential to have an efficient synthetic route to the $preQ_1$ base. Herein, we describe two convenient synthetic strategies together with the crystal structure of $preQ_1$ [11].

Results and Discussion. – The preparation of the $preQ_1$ base *via* a route providing also access to the $preQ_0$ base is possible by the short five-step synthesis depicted in *Scheme 2*. Starting material is methyl format (**1**), which was converted after enolization and reaction with $ClCH_2CN$ into the 2-chloro-3-oxopropanenitrile (**2**) (*Scheme 2*).

Condensation of the nitrile **2** with 2,6-diaminopyrimidin-4-one furnished 7-cyano-7-deazaguanine (**3**, $preQ_0$) in an excellent yield [12]. For the synthesis of $preQ_1$, we wanted to directly hydrogenate (H_2, Pd/C) the CN group to give the NH_2CH_2 group of $preQ_1$ [13]. The reaction afforded $preQ_1$, however, only as a crude reaction product, from which the isolation of $preQ_1$ (**5**) turned out to be very difficult. To accomplish the tedious purification, we reacted the crude reaction mixture with $(Boc)_2O$. The *N*-Boc-protected $preQ_1$ **4** was then purified by flash chromatography. Final deprotection with TFA afforded $preQ_1$ (**5**) in high purity. The reaction yield, however, was with only 10% rather low. Attempts to improve the final yield of compound **5**, *e.g.*, by performing the critical hydrogenation at elevated pressure, failed.

Scheme 2



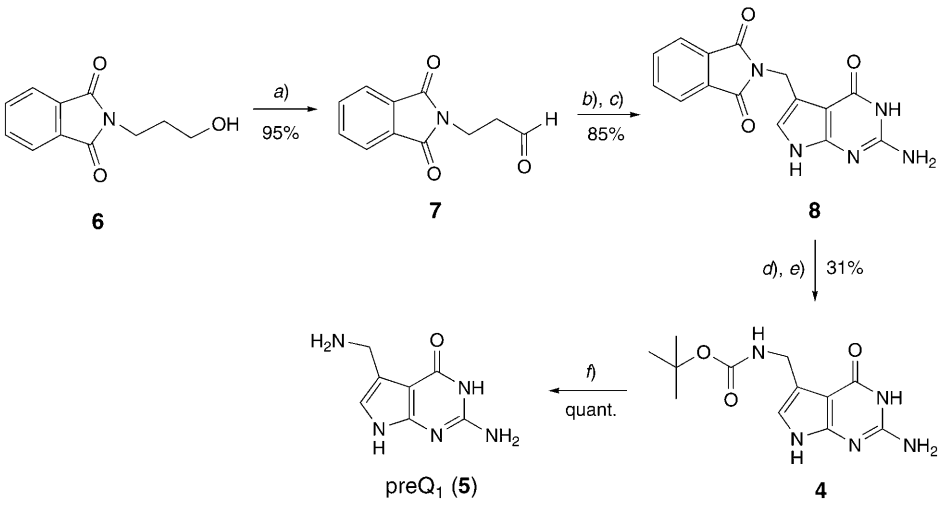
a) MeONa, $ClCH_2CN$, THF, 4 h, 0° – r.t. *b*) AcONa·3 H_2O, 2,6-diaminopyrimidin-4-one, H_2O/MeCN 1:1), 12 h; 66% (two steps). *c*) H_2 (50 psi), Pd/C, DMF, 20 h. *d*) $(Boc)_2O$, DMF, r.t., 16 h; 10%. *e*) CF_3COOH (TFA), r.t., 2 h; quant.

To achieve the synthesis of $preQ_1$ (**5**) in larger quantities as required, *e.g.*, for crystallographic studies, we developed the alternative synthetic pathway depicted in *Scheme 3*. Starting compound was *N*-(3-hydroxypropyl)phthalimide, which was oxidized with *Dess–Martin* periodinane to give the aldehyde **7** [14]. α-Bromination with Me_3SiBr, and subsequent reaction of the product without purification, with 2,6-diaminopyrimidin-4-one furnished directly the phthalimido-protected $preQ_1$ base **8** with a yield of 85% [15]. Deprotection was finally performed with NH_2NH_2 [16]. The purification of $preQ_1$ was again performed, after reaction of the reaction mixture with $(Boc)_2$ O, followed by flash chromatographic purification of **4**. Cleavage of the Boc protecting group to give **5** was performed as described above. This synthetic route is slightly longer, allows, however, the synthesis of preQ (**5**) base in an overall yield of 25%.

Single-Crystal X-Ray Diffraction Analysis of preQ1. The synthesis depicted in *Scheme 3* afforded $preQ_1$ (**5**) in amounts sufficient to perform crystallographic investigations. A yellow crystal of **5** suitable for X-ray diffraction analysis was grown by heating ($T=40°$) a saturated aqueous solution of **5**. Analysis of the crystal by X-ray diffraction revealed the surprising fact that the crystal cell obtained has twice the volume than expected. Closer inspection of the crystal structure revealed that the monocation and the dication of the $preQ_1$ salt co-existed in the crystal arrangement. In the crystal, both compounds are surrounded by three trifluoroacetate molecules (*Fig. 1*). The measurement clearly shows the protonation of the monocation at the amino group N(10). The dicationic species is protonated at N(2) and N(5) (Numbering according to *Fig. 1*).

An intramolecular H-bond between the H-atom of the protonated amino function and the C=C function is observed in both bases. The length of the H-bonds between

Scheme 3



a) *Dess–Martin* periodinane, CH_2Cl_2, r.t., 2 h; 95%. *b*) Me_3SiBr (TMSBr), DMSO, MeCN, 0° – r.t., 4 h. *c*) 2,6-Diaminopyrimidin-4-one, H_2O, 12 h; 85% (two steps). *d*) NH_2NH_2, MeOH, reflux – r.t., 10 h. *e*) $(Boc)_2O$, DMF, r.t., 16 h; 31% (two steps). *f*) TFA, r.t., 2 h; quant.

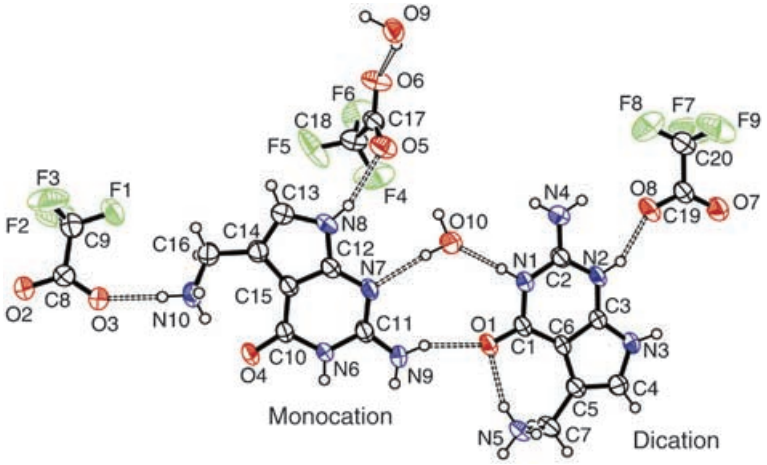


Fig. 1. *X-Ray structure of preQ$_1$·trifluoroacetate salt in the crystal, showing the co-existence of the monocation and the dication.* Ellipsoids represent 30% probability levels.

N(10) and O(4) is 1.944 Å, and between O(1) and N(5) is 1.994 Å. Two direct interactions can be found between the cationic bases. A direct H-bonding exists between N(9) and O(1) with a length of 2.086 Å, and a H_2O molecule mediated H-bond connects the two heterocycles between N(1) and N(7). The H_2O molecule forms H-bonds from O(10) to N(7) with a length of 2.017 Å and to N(1) with 1.887 Å. The second H_2O molecule shows a H-bond from O(9) to O(6) of a trifluoroacetate anion with a length of 1.868 Å.

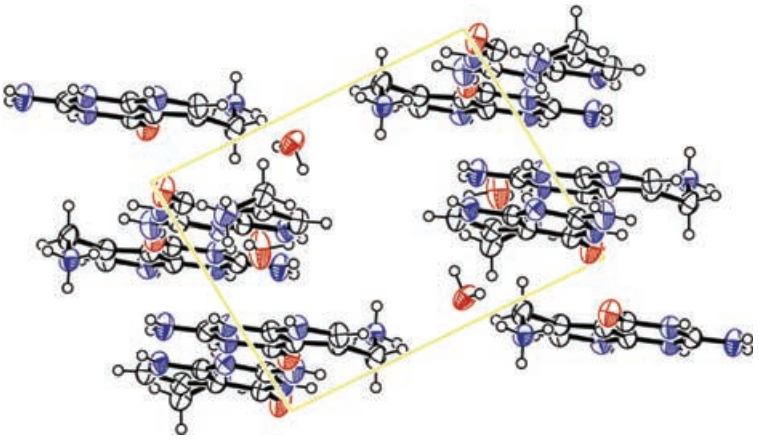


Fig. 2. *Packing of the preQ$_1$ cations* (projection normal (001)) *in the crystal*. Ellipsoids represent 30% probability levels.

In *Fig. 2*, the crystal packing of the mono- and the dication is shown. Two bases exist in a dimer-like form and appear in a planar stacked orientation. The zigzag chain of the monocation N(3)–C(3)–N(2)–C(2)–N(4) lies above the zigzag chain of the dication C(13)–N(8)–C(12)–N(7)–C(11) in such a way that alternatively a N-atom lies over a C-atom and *vice versa*, indicating π-π interactions. The distances between these atoms vary from 3.269 to 3.356 Å. The trifluoroacetate anions are bridged to the monocation and the dication, thus forming a lattice with a calculated density of 1.625 g cm^{-3} for preQ$_1$ (**5**), which is a fairly high value.

Conclusions. – We described efficient methods for the preparation of the preQ$_0$ (**3**) and preQ$_1$ (**5**), which are the biosynthetic precursors of the hypermodified base queuosine. The crystal structure of preQ$_1$ revealed rather high basicity of the NH_2CH_2 group and of the N-atom N(3), giving rise to a structure in which the mono- and dications coexist.

The synthetic access to both compounds now allows a deeper crystallographic investigation of how guanine is replaced by preQ$_1$ (**5**) in the enzymatic process catalyzed by the TGT enzyme.

Experimental Part

General. Solvents were dried by standard procedures, and reactions requiring anh. conditions were run under N_2. TLC: precoated *Merck F254* silica-gel plates. Flash chromatography (FC): *Merck* silica gel *60* (0.040–0.063 mm). ^{1}H-NMR Spectra: *Varian Mercury 200* spectrometer at 200 MHz, and a *Bruker AMX 600* spectrometer at 600 MHz; chemical shifts in ppm relative to Me_4Si as internal standard. ^{13}C-NMR Spectra: *Bruker AMX 600* spectrometer at 150 MHz. MS: *Finnigan MAT 95Q* and *Finnigan MAT 90* apparatus.

*2-Amino-4-oxopyrrolo[2,3-*d*]pyrimidin-5-carbonitrile (=7-Cyano-7-deazaguanine, preQ$_0$*; **3**). MeONa (3.57 g, 66 mmol) was suspended in THF (abs., 60 ml). At 0°, HCOOMe (4.38 g, 77 mmol) was added dropwise over 1 h. After stirring for 15 min, $ClCH_2CN$ (5 g, 66 mmol) was added dropwise over 1 h. The soln. was stirred for 2 h at 0°, and for 2 h at r.t. Conc. aq. HCl (6 ml) was added dropwise to the soln., so that the temp. of the mixture did not exceed 10°. After filtration, the soln. was concentrated to 15 ml and used directly in the following reaction.

AcONa·3 H_2O (14 g, 110 mmol) and 2,6-diaminopyrimidin-4-one (6.66 g, 5.28 mmol) were solved in H_2O (110 ml), and the soln. was heated to 50°. With a syringe pump, 2-chloro-2-oxopropanenitrile was added dropwise over 1 h. The suspension was stirred overnight. THF was subsequently evaporated *in vacuo*, and the suspension was heated for 1 h to reflux. The suspension was filtered, and the residual material was washed with acetone (3×50 ml) and H_2O (3×50 ml). The bright yellow solid was dissolved in KOH soln. (6N, 50 ml). After the addition of activated charcoal, the suspension was filtered, and the filtrate was brought to pH 6 with aq. HCl (30%). The precipitate was filtered and dried 24 h under vacuum at 50° to give **3** (7 g, 66 %). White powder. M.p. >250°. IR (KBr): 3437*m*, 2229*s*, 1685*s*, 1510*m*, 1162*w*, 883*w*, 610*w*. ^{1}H-NMR (600 MHz, (D_6)DMSO): 6.40 (*s*, NH_2); 7.63 (*s*, CH); 10.72 (*s*, NH); 12.00 (*s*, NH). ^{13}C-NMR (150 MHz, (D_6)DMSO): 78.0; 84.4; 97.6; 114.8; 126.6; 150.5; 152.7; 156.4. HR-EI-MS (pos.): 175.0479 ($[M+H]^+$, $C_7H_5N_5O^+$; calc. 175.0494).

3-Phthalimidopropanal (**7**). To a soln. of 3-phthalimidopropan-1-ol (0.5 g, 24 mmol) in CH_2Cl_2 (15 ml), *Dess–Martin* periodinane (1.5 g, 36 mmol) was added. After stirring, the soln. was diluted with CH_2Cl_2 (30 ml), extracted with sat. aq. NaS_2O_3 (20 ml), $NaHCO_3$ (20 ml), and brine (20 ml). The org. extract was evaporated and dried. After removal of the solvents, the resulting residue was purified by FC (hexane/AcOEt 1:1) to give **7** (0.51 g, 95%). White solid. M.p. 121–123°. TLC (hexane/EtOAc 1:2): R_f 0.45. IR (KBr): 3456*m*, 1768*s*, 1706*s*, 1441*s*, 1403*s*, 1369*s*, 1324*m*, 1031*s*, 721*s*, 532*m*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.81 (*t*, CH_2(2)); 3.98 (*t*, CH_2(3)); 7.63–7.66 (*m*, 3 arom. H); 7.75–7.79 (*m*, 2 arom. H); 9.75 (*s*, CH(1)). ^{13}C-NMR (150 MHz, $CDCl_3$): 31.8; 42.5; 123.5; 132.1; 134.1; 168.1; 199.5. EI-MS (pos.): 160 (100), 203 (8, M^+). HR-EI-MS (pos.): 203.0573 ($[M+Na]^+$, $C_{11}H_9NO_3^+$; calc. 203.0582).

*2-[(2-Amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)methyl]-1,3-dihydro-2*H*-isoindole-1,3-dione* (**8**). A soln. of **7** (5 g, 24.6 mmol) in MeCN (80 ml) was cooled to 0°. DMSO (1.84 ml, 25.9 mmol) was added dropwise, and Me_3SiBr (3.35 ml, 35.9 mmol) was added slowly. The yellow colored soln. was stirred for 4 h at r.t.. A suspension of 2,6-diaminopyrimidin-4-one (3.1 g, 24.6 mmol) and AcOH·3 H_2O (3.5 g, 25.1 mmol) in H_2O (80 ml) was added slowly. The mixture was stirred overnight. A yellow precipitate was formed. The bright yellow suspension was filtered and washed with acetone (3×80 ml) and H_2O (3×80 ml). The solid was dried to yield **8** (6.5 g, 85%). Bright yellow solid. M.p. >250°. IR (KBr): 3387*s* (br.), 2926*m*, 1770*m*, 1711*s*, 1627*s*, 1444*m*, 1361*s*, 1116*w*, 950*w*, 719*m*, 532*m*. ^{1}H-NMR (200 MHz, (D_6)DMSO): 4.82 (*s*, CH_2N); 6.06 (*s*, NH_2); 6.36 (*s*, CH); 7.75–7.90 (*m*, 4 arom. H); 10.21 (*s*, NH); 10.80 (*s*, NH). ^{13}C-NMR (150 MHz, (D_6)DMSO): 27.9; 28.3; 35.0; 37.9; 98.4; 114.0; 123.2; 123.4; 132.1; 134.6; 151.7; 152.8; 163.7; 168.2. HR-FAB-MS (pos.): 310.0943 ($[M+H]^+$, $C_{14}H_{14}N_4O_2^+$; calc. 310.0940).

tert-*Butyl [(2-Amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)methy]carbamate* (**4**). *Method A*. Compound **3** (100 mg, 0.57 mmol) was suspended in DMF (5 ml), Pd/C (20 mg) was added, and the mixture was stirred under H_2 atmosphere (50 psi) for 24 h. The mixture was filtered and washed with DMF, and the solvents were removed *in vacuo*. The red colored residue was dissolved in DMF (8 ml), and $(Boc)_2O$ (73 mg, 0.33 mmol) and Et_3N (46 µl, 0.33 mmol) were added. After stirring for 16 h, the soln. was washed with aq. $NaHCO_3$ soln. (10 ml), and the aq. phase was re-extracted with CH_2Cl_2 (4×10 ml). The combined org. fractions were washed with brine (10 ml) and dried ($MgSO_4$). The solvent was evaporated, and, after FC (MeOH/$CHCl_3$ 1:10), **4** (10 mg, 10%) was obtained. White powder.

Method B. Compound **8** (800 mg, 2.64 mmol) was suspended in EtOH (30 ml), and $NH_2NH_2·H_2O$ (1.3 ml, 26.4 mmol) was added. The suspension was heated at reflux for 2 h at 80° and stirred for another 8 h at r.t. (the reaction was monitored by TLC (BuOH/AcOH/H_2O 2:1:1): R_f (**4**) 0.4). The solvent was evaporated, and $preQ_1$ (crude) was obtained as a white solid, which was used without further purification.

$preQ_1$ (crude; 200 mg, 1.12 mmol) was suspended in DMF (8 ml), Et_3N (93 µl, 0.67 mmol) and $(Boc)_2O$ (146 mg, 0.67 mmol) were added. After stirring for 8 h at r.t., more $(Boc)_2O$ (146 mg, 0.67 mmol) and Et_3N (93 µl, 0.67 mmol) were added. After 16 h, the soln. was washed with $NaHCO_3$ (50 ml), and the aq. phase was re-extracted with CH_2Cl_2 (4×50 ml). The combined org. fractions were extracted with brine (40 ml) and dried ($MgSO_4$). The solvent was evaporated, and, after FC (MeOH/$CHCl_3$ 1:10), **4** (50 mg, 31%) was obtained. White powder. M.p. >250°. TLC ($CHCl_3$/MeOH 5:1): R_f 0.40. IR (KBr): 3429*s*, 2978*m*, 2933*m*, 1673*s*, 1592*s*, 1535*m*, 1249*w*, 1169*w*. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.33 (*s*, 3 Me); 4.13 (*s*, CH_2); 6.08 (*s*, NH_2); 6.46 (*s*, CH); 7.04 (*s*, NH); 10.33 (*s*, NH); 10.79 (*s*, NH). ^{13}C-NMR (150 MHz, (D_6)DMSO): 27.7; 37.0; 78.1; 98.9; 113.7; 113.9; 116.3; 151.9; 152.7; 155.7; 160.1. EI-MS (pos.): 178 (100), 279 (6, $[M+H]^+$). HR-EI-MS (pos.): 279.1359 ($[M+H]^+$, $C_{12}H_{17}N_5O_3^+$; calc. 279.1331).

7-(Aminomethyl)-7-deazaguanine (=preQ$_1$; **5**). Compound **4** (13 mg, 0.046 mmol) was dissolved at 0° in TFA (1 ml). The soln. was stirred for 2 h at r.t. MeOH (5 ml) was added dropwise at 0°, and the volatiles were evaporated *in vacuo*. The trifluoroacetate salt of **5** (19 mg, quant.) was obtained. Bright yellow powder.

M.p. 220° (dec.). TLC (BuOH/AcOH/H_2O 2 : 1 : 1): R_f 0.40. IR (KBr): 3402*m*, 1684*s*, 1204*s*, 802*w*, 724*m*. ^{1}H-NMR (200 MHz, CD_3OD): 4.15 (*s*, CH_2); 6.81 (*s*, CH). ^{13}C-NMR (150 MHz, CD_3OD): 38.2; 100.9; 112.6; 119.3; 154.7; 155.5; 163.5. EI-MS (pos.): 164 (100), 178 (20, M^+). HR-EI-MS (pos.): 179.0807 ($[M+H]^+$, $C_7H_9N_5O^+$; calc. 179.0807).

X-Ray Crystal Structure of preQ$_1$ (**5**). $C_{20}H_{25}F_9N_{20}O_{10}$, M_r 736.50, habit: yellow plate, crystal size (mm) = 0.13 × 0.43 × 0.47, triclinic, space group $P\bar{1}$ (no. 2), a = 8.5290(17), b = 10.870(2), c = 16.646(3) Å, α = 88.01(3), β = 78.82(3), γ = 84.02(3)°, V = 1505.6(5) Å^3, T = 295(2)K, Z = 2, λ(MoK_α) = 0.71073 Å, 5587 unique reflections (R_i = 0.0174) and 3635 observed reflections with $I > 2\sigma(I)$. The final R values are $R1$ = 0.0581 and $wR2$ ($F2$) = 0.1311 for the observed reflections with 529 parameters and 144 restraints. The CF_3 groups were split and refined with thermal and distance restraints. Programs used were SHELXS-90 and SHELXL-97 [17][18].

CCDC-265111 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/conts/retrieving.html.

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