A short and efficient synthesis of the tRNA nucleosides $PreQ_0$ and archaeosine

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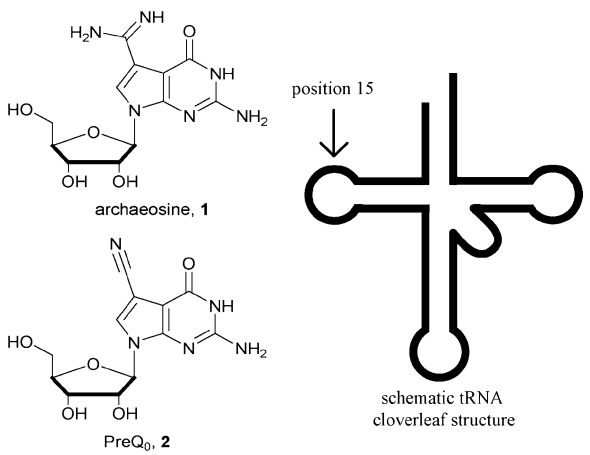
Modified nucleosides in tRNAs play an important role in the translational process. They fine tune the codon–anticodon interactions and they influence the folding and stabilisation of the tRNA structure. Herein, we present a novel synthetic route to the highly modified nucleosides $PreQ_0$ and archaeosine. The synthesis involves coupling of a protected 7-cyano-7-deazaguanosine nucleobase with a TBDMS and isopropylidene protected chloro-ribose unit yielding the $PreQ_0$ nucleoside after deprotection. This $PreQ_0$ nucleoside is then used as the starting material for the synthesis of archaeosine providing the first total synthetic access to this hypermodified RNA nucleoside.

Introduction

Transfer ribonucleic acids (tRNAs) contain more than one hundred modified nucleosides, which complement the four major RNA nucleosides A, C, G and U.[1] These modified nucleosides seem to be involved in the fine tuning of codon–anticodon interaction at the ribosome and also influence folding and stability of tRNAs.[2–10] However, in most cases the exact role of the modified RNA nucleosides is not known. We have recently started a synthetic program aimed at the preparation of the hypermodified tRNA nucleosides queuosine[11] and archaeosine in order to investigate in detail their biosyntheses and their function in the translational process. Herein, we report an efficient synthesis of the tRNA nucleosides archaeosine **1**, a hypermodified RNA nucleoside so far found only in archae bacteria,[12] and $PreQ_0$ **2**, which is a biosynthetic precursor for archaeosine.[5,7,10] Archaeosine occupies position 15 in the majority of archaeal tRNAs (Scheme 1).[7] It is consequently a quite abundant hypermodification, which seems to stabilize the 3-dimensional fold of these tRNAs. After being discovered in 1982[13] the structure of the nucleoside remained elusive until 1993, when Gregson *et al.* used mass spectrometric methods to elucidate the molecular structure.[12] These authors compared the mass spectrometric data of the natural product with data from a small synthetic sample of archaeosine (7 μg) obtained through transformation of 1 mg of the natural product toyocamycin into archaeosine.



Scheme 1

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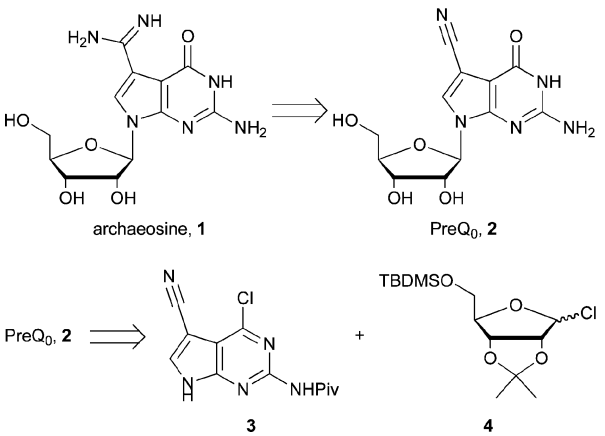
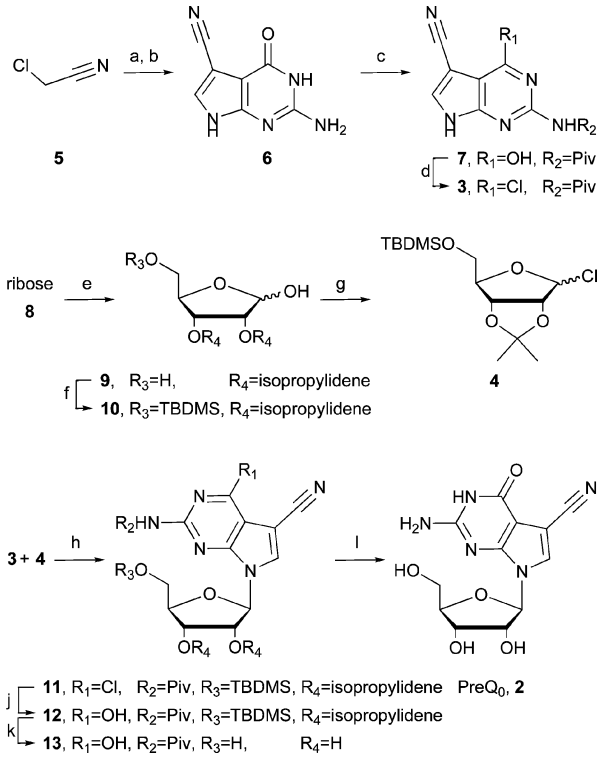
Results and discussion

We decided to synthesize the target compound archaeosine **1** from the nucleoside $PreQ_0$ **2** by conversion of the nitrile to an amidine. The synthesis of **2** was thought to be possible *via* a glycosylation of the heterocyclic precursor **3** with the protected sugar **4** as shown in Scheme 2.



Scheme 2

Synthesis of the heterocyclic building block **3** started from chloroacetonitrile **5**. Reaction of **5** with formic acid methylester and 2,6-diaminopyrimidine-4-one in a one pot procedure as established by Migawa *et al.* gave nitrile nucleobase **6** (Scheme 3).[14] Pivaloyl protection of **6** yielded compound **7**.[15] Subsequently, the heterocycle **7** was activated for the subsequent glycosylation reaction by replacement of the hydroxyl group at the aromatic system for chlorine.[16–19] By that route **3** was obtained in 21% overall yield in only four steps.

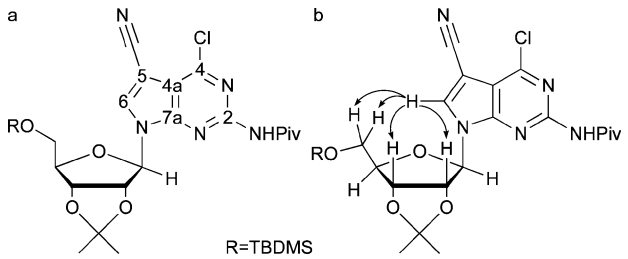


Scheme 3 Synthesis of $PreQ_0$ **2**: (a) HCOOMe, NaOMe, THF; (b) NaOAc, 2,6-diaminopyrimidine-4-one, water, THF, 38%; (c) trimethylacetyl chloride, pyridine, 75%; (d) $POCl_3$, TEBACl, dimethylaniline, acetonitrile, 75%; (e) acetone, conc. HCl, 87%; (f) imidazole, TBDMSCl, DMF, 83%; (g) tetrachloromethane, HMPA; (h) NaH, acetonitrile, 20%; (j) Et_3N, DABCO, NaOAc, DMF, 81%; (k) trifluoroacetic acid, water, 77%; (l) 28% ammonia in water, 99%.

The second building block **4** was synthesized starting from ribose **8**. Protection of the 2′,3′-OH groups with acetone gave compound **9**.[20,21] Treatment of the crude product with TBDMSCl provided the 5′-*O*-protected sugar **10** in 72% yield. Conversion of **10** into the glycosyl donor **4** was achieved with HMPA and CCl_4.[22] Due to the lability of compound **4**, it was used for the glycosylation reaction without further purification.

For the crucial step of the synthesis, the glycosylation, initially 1-acetoxyribose-2,3,5-tribenzoate as glycosyl donor was reacted with compound **6** under Lewis acid catalysis.[23] This route, however, turned out to give an inseparable mixture of two products. NMR measurements indicated formation of a nucleoside with the sugar attached to the exocyclic amino group. Additionally, when **3** was reacted under these conditions no conversion was observed. Ramasamy *et al.*[24] proposed a different glycosylation route using **4** and a twofold excess of NaH and 4-chloro-2-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine. Deprotonation of two equivalents of **3** with NaH and subsequent addition of **4** to the reaction mixture provided the nucleoside **11** in 16% yield. In our hands, the best yield (20%) was obtained when a 1.4 fold excess of NaH and of the glycosyl donor were used (Scheme 3). Slow addition of **4** at 0 °C and rigorously dried solvents and reagents are very important for the success of the reaction.

The configuration of the obtained product **11** was verified by HMBC and NOESY spectroscopy. Clear interactions could be observed between C(1′)H and C6, C5 and C7a in the HMBC spectrum. Furthermore, interactions between C(6)H and C1′ (Scheme 4a) were clearly detected. This unequivocally determines the constitution of the nucleoside **11**. The sugar and the nucleobase moiety are connected *via* a bond between C1′ and N7.



Scheme 4 (a) Nucleoside **11** highlighting the systematic numbering of the nucleobase and of C(1′)H; (b) nucleoside **11** displaying the sugar and the nucleobase protons together with observed NOESY interactions.

The corresponding NOESY spectrum proved the *β*-configuration of the obtained nucleoside **11**. A strong interaction between C(6)H and C(2′)H was observed. Additional interactions (C(6)H–C(3′)H and C(6)H–C(5′)H) usually observed for *β*-configured nucleosides and the absence of typical interactions (C(6)H–C(4′)H) in *α*-nucleosides further supported the stereochemical assignment (Scheme 4b). Another indication for the *β*-configuration of **11** is the small C(1′)H–C(2′)H coupling constant (1 Hz), which is in the typical range for *β*-configured nucleosides. *α*-Configured nucleosides usually show larger coupling constants. These spectroscopic data, in our eyes, prove the formation of the glycosidic bond between C1′ and N7 and the *β*-configuration of the obtained nucleoside **11**.

Conversion of the chloro to the needed oxo substituent in position 4 of the nucleoside **11** was achieved with DABCO, Et_3N and NaOAc yielding **12** (Scheme 3).[25] The protecting groups at the sugar moiety of the nucleoside **12** were removed using a water–trifluoroacetic acid mixture to give **13**. The best method for the deprotection of the amine in position 2 of the nucleoside **13** proved to be 28% ammonia in water. By this method the conversion of **13** to $PreQ_0$ **2** was quantitative. The transformation of **11** to $PreQ_0$ **2** was therefore possible with 62% yield in only three steps. The analytical data obtained from compound **2** compared very well with those published by Kondo *et al.*[26] and Cheng *et al.*[27,28]

The final transformation of $PreQ_0$ to archaeosine turned out to be the second critical step. A synthesis of the archaeosine base only was reported by Hashizume and McCloskey.[29] They converted the nitrile nucleobase **6** into the corresponding amidine with trimethylaluminium and ammonium chloride. This method, however, failed in our hands with compound **2** as the starting material. Also, the conversion of the nitrile to the amidine with hydroxylamine followed by reduction with palladium on charcoal or Raney nickel[24,30,31] gave no conversion to compound **1**. We finally used the classical Pinner reaction[32–36] for the desired transformation. Treatment of a solution of **2** in methanol with HCl (g) for 3 h, followed by the removal of the solvent and stirring of the resulting white solid in 7 N ammonia in methanol indeed gave the title compound archaeosine **1** as a white solid (Scheme 5). **1** was purified by reversed phase HPLC with a 0.1 M triethylammonium acetate buffer. The resulting white solid turned out to be archaeosine obtained as its acetate salt plus a small amount of additional

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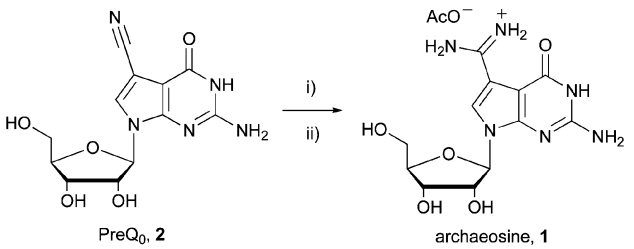

Scheme 5 Synthesis of archaeosine **1**: (i) HCl (g), methanol (ii) 7 N ammonia in methanol, 30%.

triethylammonium acetate. Attempts to completely remove the excess triethylammonium acetate under high vacuum caused degradation of archaeosine **1** partly back to $PreQ_0$ **2**. However, the analytical data obtained for the product were in excellent agreement with those presented by Gregson *et al.*[12]

In summary, we were able to establish a short and efficient synthesis of $PreQ_0$ (2% overall yield, 11 steps) and archaeosine (0.6% overall yield, 12 steps).

Together with our recently presented work on the synthesis of queuosine,[11] these syntheses allow preparation of most of the deazaguanosine modified tRNA nucleosides in reasonable quantities. Our future aim is to incorporate these deazaguanosine derived nucleosides into RNA strands *via* RNA solid phase synthesis in order to unravel their functions in tRNA.

Experimental

General procedures

All reactions were performed with dry solvents in dried Schlenk flasks fitted with a septum under a positive pressure of nitrogen, unless water was used as a solvent. Air- and moisture-sensitive liquids and solutions were transferred *via* syringes. Organic solutions were concentrated by rotary evaporation below 40 °C under appropriately reduced pressure. Thin layer chromatography (TLC) was carried out with precoated Merck F254 silica gel plates. Flash chromatography (FC) was carried out with Merck Silica gel 60 (0.040–0.063 mm). Reagents, dry solvents and starting materials were obtained from commercial suppliers, and used without further purification unless otherwise indicated. IR spectra were recorded on a Bruker IFS 25 and a Bruker IFS 88 spectrometer. IR data are reported as wave numbers with w (weak), s (strong) and vs (very strong) indicating the intensity of the absorption bands. ^{1}H NMR and ^{13}C NMR spectra were recorded with Varian Mercury 200 (200 MHz and 75 MHz), Bruker AMX 400 (400 MHz and 100 MHz) and Bruker AMX 600 (600 MHz and 150 MHz) spectrometers. Chemical shifts for ^{1}H NMR are reported in ppm relative to Me_4Si as internal standard. Data for ^{1}H NMR are reported as follows: chemical shift (δ, ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz) and assignment. Data for ^{13}C NMR are reported in terms of chemical shifts. Mass spectrometry was performed with Finnigan MAT 95Q, Finnigan MAT 90 and Thermo Finnigan LTQ-FT mass spectrometers.

2-Amino-5-cyano-3,4-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-one 6

6 was synthesised according to literature.[14] ν_{max}/cm^{-1} 3362 s (NH), 3105 s (NH), 2229 s (conj. CN), 1627 vs (NH_2), 1592 vs and 1260 s; δ_H (600 MHz, [D$_6$]DMSO) 6.40 (2 H, s, NH_2), 7.63 (1 H, s, C(6)H), 10.72 (1 H, s, N(7)H) and 12.00 (1H, s, N(3)H); δ_C (150 MHz, [D$_6$]DMSO) 90.7 (C5), 105.0 (C4a), 121.7 (CN), 133.7 (C6), 157.7 (C7a), 159.7 (C2) and 163.8 (C4); *m/z* (ESI−) 174.0426 (M^-, $C_7H_4N_5O^-$ requires 174.0421).

5-Cyano-3,4-dihydro-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-one 7

6 (1.5 g, 8.6 mmol) was dissolved in pyridine (10 cm^3) and slowly treated with trimethylacetyl chloride (3.1 g, 26 mmol). The resulting suspension was stirred for 2 h at 85 °C. After cooling to room temperature a white solid precipitated, which was removed by filtration. The resulting solution was neutralised with 7 N ammonia in methanol and left to stand at 4 °C for 16 h. The resulting precipitate was collected and washed with ethanol (20 cm^3) and diethyl ether (10 cm^3) to give the pivaloyl protected nucleobase **7** (1.7 g, 75%). R_f = 0.2 (DCM–MeOH, 20 : 1); ν_{max}/cm^{-1} 3125 vs (NH), 2228 s (CN), 1684 s (CO), 1646 vs (CC), 1608 vs, 1579 vs (NH), 1409 vs (CH_3), 1239 s (CN), 1176 s (CN), 938 w (CH) and 782 s (CH); δ_H (600 MHz, [D$_6$]DMSO) 1.25 (9 H, s, CH_3), 7.93 (1 H, s, C(6)H), 11.00 (1 H s, N(7)H), 12.11 (1 H, s, N(3)H) and 12.65 (1 H, s, NHPiv); δ_C (150 MHz, [D$_6$]DMSO) 27.0 (C(CH_3)$_3$), 40.7 (C(CH_3)$_3$), 86.9 (C5), 103.7 (C4a), 115.9 (CN), 130.9 (C6), 149.1 and 149.1 (C2 and C7a), 156.4 (C4) and 181.7 (COC(CH_3)$_3$); *m/z* (ESI+) 259.1088 (M^+, $C_{12}H_{13}N_5O_2^+$ requires 259.1069).

4-Chloro-5-cyano-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine 3

7 (200 mg, 0.77 mmol) was suspended in acetonitrile (1.2 cm^3) and dimethylaniline (0.41 cm^3, 3.28 mmol), triethylbenzylammonium chloride (TEBACl) (89 mg, 0.39 mmol) and phosphoryl chloride (0.72 cm^3, 7.70 mmol) were added. The suspension was heated to 90 °C for 1 h. The cooled down suspension was concentrated *in vacuo* and the resulting oil was cautiously treated with ice. The acidic mixture was set to pH = 4 with conc. ammonia in methanol. The precipitate was collected by filtration and washed with water. Purification by column chromatography (isohexane–ethyl acetate 3 : 2) gave nucleobase **3** (160 mg, 75%) as a white solid. R_f = 0.65 ($CHCl_3$–MeOH, 9 : 1); ν_{max}/cm^{-1} 3448 w (NH), 3160 vs (NH), 2231 s (CN), 1708 s (CO), 1563 vs (NH), 1521 s (NH), 1445 vs (CH_3), 1419 vs (CH_3), 1271 s (CN), 1164 s (CN) and 785 s; δ_H (400 MHz, [D$_6$]DMSO) 1.22 (9 H, s, C(CH_3)$_3$), 8.50 (1 H, s, C(6)H), 10.27 (1 H, s, N(7)H) and 13.35 (1 H, s, NHPiv); δ_C (100 MHz, [D$_6$]DMSO) 27.4 (C(CH_3)$_3$), 40.3 (C(CH_3)$_3$), 84.1 (C5), 111.8 and 115.1 (C4a and CN), 138.2 (C6), 151.4 (C4), 153.5 (C7a), 153.8 (C2) and 176.5 (COC(CH_3)$_3$); *m/z* (ESI+) 278.0796 (M^+, $C_{12}H_{13}ClN_5O^+$ requires 278.0806).

2′,3′-*O*-Isopropylidene-α/β-D-ribofuranose 9[20,21]

D-Ribose **8** (25.0 g, 150 mmol) was suspended in acetone. Conc. HCl (0.025 cm^3) was added at 0 °C and the mixture was stirred for 2 h. The resulting suspension was filtered through Celite and activated charcoal. Concentration of the resulting solution gave the isopropylidene protected sugar **9** (33.0 g, 87%). R_f = 0.29 (DCM–MeOH, 20 : 1); δ_H (200 MHz, [D$_6$]DMSO) 1.31 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 3.72 (2 H, m, C(5′)H_2), 4.40 (1 H, t, *J* 2, C(4′)H), 4.57 (1 H, d, *J* 6, C(3′)H), 4.83 (1 H, d, *J* 6, C(2′)H) and 5.41 (1 H, s, C(1′)H).

5′-*O*-[(1,1-Dimethylethyl)dimethylsilyl]-2′,3′-*O*-isopropylidene-α/β-D-ribofuranose 10

9 (33.0 g, 174 mmol) and imidazole (29.0 g, 422 mmol) were dissolved in DMF (60 cm^3). *tert*-Butyldimethylsilyl chloride (TBDMSCl) (27.4 g, 182 mmol) was added and the solution was stirred at room temperature for 16 h. The reaction mixture was treated with water (100 cm^3) and extracted with ethyl acetate (3 × 250 cm^3). The combined organic phases were washed with water (2 × 120 cm^3) and dried with $MgSO_4$. Removal of the solvent *in vacuo* yielded the protected sugar **10** (44 g, 83%) as a white solid, which was used without purification in the next step. An analytically pure sample was obtained by column chromatography (isohexane–ethyl acetate, 7 : 1). $R_f = 0.6$ ($CHCl_3$–MeOH 10 : 1); mp 49.8 °C; ν_{max}/cm^{-1} 3345 s (OH), 2939 s (CH), 2861 s (CH), 1382 w (CH_3), 1368 w (CH_3), 1258 s (COC), 1084 vs (COC) and 1062 vs (COC); δ_H (600 MHz, [D_6]DMSO) 0.06 (6 H, s, $SiC(CH_3)_3(CH_3)_2$), 0.88 (9 H, s, $SiC(CH_3)_3(CH_3)_2$), 1.25 (3 H, s, CH_3), 1.37 (3 H, s, CH_3), 3.59 (2 H, d, *J* 7, C(5′)H_2), 3.95 (1 H, t, *J* 7, C(4′)H), 4.46 (1 H, d, *J* 6, C(2′)H), 4.63 (1 H, d, *J* 6, C(3′)H), 5.19 (1 H, d, *J* 5, C(1′)H) and 6.43 (1 H, d, *J* 5, OH); δ_C (150 MHz, [D_6]DMSO) −5.5 and −0.54 ($SiC(CH_3)_3(CH_3)_2$), 17.9 ($SiC(CH_3)_3(CH_3)_2$), 24.7 ($C(CH_3)_2$), 25.8 ($SiC(CH_3)_3(CH_3)_2$), 26.3 ($C(CH_3)_2$), 64.2 (C5′), 81.7 (C3′), 85.5 (C2′), 85.8 (C4′), 101.7 (C1′) and 111.1 ($C(CH_3)_2$); *m/z* (ESI−) 303.1626 (M^-, $C_{14}H_{27}O_5Si^-$ requires 303.1633).

1′-Chloro-5′-*O*-[(1,1-dimethylethyl)dimethylsilyl]-2′,3′-*O*-isopropylidene-α/β-D-ribofuranose 4

10 (1.12 g, 3.7 mmol) and CCl_4 (0.5 cm^3, 5.92 mmol) were dissolved in THF (10 cm^3). Hexamethylphosphoramide (HMPA) (0.8 cm^3, 4.44 mmol) was added dropwise at −78 °C over a period of 5 min. After stirring at −78 °C for 2 h, the cooling was removed upon which a white solid precipitated. The obtained suspension containing **4** was used in the subsequent reaction *in situ*.

4-Chloro-5-cyano-7-[2′,3′-*O*-isopropylidene-5′-*O*-[(1,1-dimethylethyl)dimethylsilyl]-β-D-ribofuranosyl]-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine 11

Acetonitrile (100 cm^3) was dried over molecular sieves for 1 h before addition of **3** (1.10 g, 3.97 mmol) and NaH (95%, 220 mg, 5.56 mmol). The solution was stirred at room temperature for 1 h. **4** (1.80 g, 5.56 mmol) was added dropwise over a period of 20 min at 0 °C. The resulting solution was stirred at room temperature for 16 h. After removal of the solvent *in vacuo* the product was treated with water (50 cm^3) and ethyl acetate (50 cm^3). The layers were separated and the water layer was extracted with ethyl acetate (2 × 100 cm^3). The combined organic layers were washed with brine (200 cm^3) and dried with $MgSO_4$. The crude mixture was purified by column chromatography (isohexane–ethyl acetate 7 : 1) to yield nucleoside **11** (441 mg, 20%). An analytically pure sample was obtained *via* HPLC (gradient; eluent A: isohexane, eluent B: ethyl acetate, gradient: 100% A, 0% B → 30% A, 70% B in 45 min, retention time = 24.9 min, Nucleodur 100–5). $R_f = 0.85$ (isohexane–ethyl acetate, 1 : 1); δ_H (400 MHz, [D_6]DMSO) −0.16 (3 H, s, $SiC(CH_3)_3(CH_3)_2$), −0.13 (3 H, s, $SiC(CH_3)_3(CH_3)_2$), 0.73 (9 H, s, $SiC(CH_3)_3(CH_3)_2$), 1.22 (9 H, s, $COC(CH_3)_3$), 1.32 (3 H, s, *exo*-$C(CH_3)_2$), 1.51 (3 H, s, *endo*-$C(CH_3)_2$), 3.64 (1 H, dd, *J* 11 and 5, C(5′)H_a), 3.67 (1 H, dd, *J* 11 and 6, C(5′)H_b), 4.12 (1 H, ddd, *J* 6, 5 and 4, C(4′)H), 5.21 (1 H, dd, *J* 6 and 4, C(3′)H), 5.45 (1 H, dd, *J* 6 and 1, C(2′)H), 6.25 (1 H, d, *J* 1, C(1′)H), 8.64 (1 H, s, C(6)H) and 10.51 (1 H, s, NHPiv); δ_C(100 MHz, [D_6]DMSO) −4.9 ($SiC(CH_3)_3(CH_3)_2$), −4.8 ($SiC(CH_3)_3(CH_3)_2$), 18.7 ($SiC(CH_3)_3(CH_3)_2$), 25.9 (*exo*-$C(CH_3)_2$), 26.4 ($SiC(CH_3)_3(CH_3)_2$), 27.3 ($COC(CH_3)_3$), 27.6 (*endo*-$C(CH_3)_2$), 40.6 ($COC(CH_3)_3$), 64.1 (C5′), 81.3 (C3′), 84.4 (C2′), 84.9(C5), 88.7 (C4′), 91.3 (C1′), 113.6 ($C(CH_3)_2$), 112.2 and 114.3 (C4a and CN), 138.9 (C6), 151.7 (C7a), 151.9 and 153.8 (C2 and C4) and 176.6 ($COC(CH_3)_3$); *m/z* (ESI+) 564.2368 (M^+, $C_{26}H_{39}ClN_5O_5Si^+$ requires 564.2404).

5-Cyano-3,4-dihydro-7-[2′,3′-*O*-isopropylidene-5′-*O*-[(1,1-dimethylethyl)dimethylsilyl]-β-D-ribofuranosyl]-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-one 12

11 (74.0 mg, 0.13 mmol) was dissolved in DMF (2 cm^3). Et_3N (39.7 mg, 0.05 cm^3, 0.39 mmol), NaOAc (32.0 mg, 0.39 mmol) and 1,4-diazabicyclo[2.2.2]octane (DABCO) (15.0 mg, 0.13 mmol) were added. The mixture was stirred at room temperature for 48 h. Afterwards, water (1 cm^3) was added and the solution was stirred for 1 h. The mixture was extracted with ethyl acetate (3 × 10 cm^3). The combined organic phases were washed with brine (20 cm^3) and dried with $MgSO_4$. Purification *via* column chromatography (isohexane–ethyl acetate 3 : 1) gave nucleoside **12** (58 mg, 81%). $R_f = 0.56$ (isohexane–ethyl acetate 1 : 1); mp 46.5 °C; ν_{max}/cm^{-1} 3163 s (NH), 2933 vs (CH), 2232 vs (CN), 1671 vs, 1606 vs, 1542 s (NH), 1250 s, 1077 vs, 833 vs and 780 vs; δ_H (400 MHz, [D_6]DMSO) 0.02 (6 H, s, $SiC(CH_3)_3(CH_3)_2$), 0.85 (9 H, s, $SiC(CH_3)_3(CH_3)_2$), 1.25 (9 H, s, $COC(CH_3)_3$), 1.32 (3 H, s, *exo*-$C(CH_3)_2$), 1.51 (3 H, s, *endo*-$C(CH_3)_2$), 3.69 (1 H, dd, *J* 11 and 5, C(5′)H_a), 3.73 (1 H, dd, *J* 11 and 4, C(5′)H_b), 4.14 (1 H, ddd, *J* 5, 4 and 4, C(4′)H), 4.96 (1 H, dd, *J* 6 and 4, C(3′)H), 5.13 (1 H, dd, *J* 6 and 2, C(2′)H), 6.25 (1 H, d, *J* 2, C(1′)H), 8.14 (1 H, s, C(6)H), 11.03 (1 H, s, N(3)H) and 12.24 (1 H, s, NHPiv); δ_C(100 MHz, [D_6]DMSO) −5.62 ($SiC(CH_3)_3(CH_3)_2$), −5.59 ($SiC(CH_3)_3(CH_3)_2$), 18.0 ($SiC(CH_3)_3(CH_3)_2$), 25.2 (*exo*-$C(CH_3)_2$), 25.7 ($SiC(CH_3)_3(CH_3)_2$), 26.2 ($COC(CH_3)_3$), 26.9 (*endo*-$C(CH_3)_2$), 40.6 ($COC(CH_3)_3$), 63.0 (C5′), 80.4 (C3′), 83.9 (C2′), 86.3 (C4′), 87.2 (C5), 88.2 (C1′), 103.0 (C4a), 113.3 (CN), 114.2 ($C(CH_3)_2$), 129.7 (C6), 147.8 (C2), 148.9 (C7a), 155.3 (C4) and 181.1 ($COC(CH_3)_3$); *m/z* (ESI+) 568.2572 ([M + Na]$^+$, $C_{26}H_{39}N_5NaO_6Si^+$ requires 568.2562).

5-Cyano-3,4-dihydro-2-pivaloylamino-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-one 13

12 (18.0 mg, 0.03 mmol) was dissolved in water (0.15 cm^3) and trifluoroacetic acid (0.35 cm^3) was added at 0 °C. The mixture was stirred at 0 °C for 4 h before removing the solvent *in vacuo* at 0 °C. The resulting solid was dissolved in water (2 cm^3) and stirred at room temperature over a period of 10 min. Concentration yielded a colourless oil, which was purified *via* column chromatography (DCM–MeOH 10 : 1). Nucleoside **13** (10 mg, 77%) was obtained as a white solid. $R_f = 0.38$ (DCM–MeOH 10 : 1); mp >210 °C (decomposition); ν_{max}/cm^{-1} 3482 s, 3417 vs, 3152 s (NH), 2966 s (CH), 2884 s (CH), 2233 s (CN), 1693 vs, 1659 vs, 1598 s (NH), 1557 s (NH), 1538 s (NH), 1411 s (CH), 1091 s (COC) and 775 s; δ_H (400 MHz, [D_6]DMSO) 1.2

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(9 H, s, $COC(CH_3)_3$), 3.5 (1 H, dd, *J* 12 and 4, $C(5')H_a$), 3.6 (1 H, dd, *J* 12 and 4, $C(5')H_b$), 3.9 (1 H, ddd, *J* 4, 4 and 3, C(4′)H), 4.1 (1 H, dd, *J* 5 and 3, C(3′)H), 4.3 (1 H, dd, *J* 6 and 5, C(2′)H), 6.1 (1 H, d, *J* 6, C(1′)H), 8.2 (1 H, s, C(6)H), 11.1 (1 H, s, N(3)H) and 12.2 (1 H, s, NHPiv); δ_C (100 MHz, [D_6]DMSO) 26.9 ($COC(CH_3)_3$), 40.7 ($COC(CH_3)_3$), 61.9 (C5′), 71.0 (C3′), 74.9 (C2′), 86.1 (C4′), 87.0 (C1′), 87.7 (C5), 103.6 (C4a), 115.2 (CN), 130.3 (C6), 149.3 (C7a), 149.7 (C2), 156.1 (C4) and 182.0 ($COC(CH_3)_3$); *m/z* (ESI+) 414.1363 ([M + Na]$^+$, $C_{17}H_{21}N_5NaO_6^+$ requires 414.1384).

2-Amino-5-cyano-3,4-dihydro-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-one ($PreQ_0$) 2

13 (18.0 mg, 0.046 mmol) was dissolved in 28% ammonia in water (4 cm^3). The solution was stirred at 60 °C for 17 h. Subsequent removal of the solvent *in vacuo* resulted in a colourless oil, which was purified *via* column chromatography (ethyl acetate–MeOH 7 : 1). $PreQ_0$ **2** (14 mg, 99%) was obtained as a white solid. R_f = 0.30 (ethyl acetate–MeOH 5 : 1); mp >260 °C (decomposition); ν_{max}/cm^{-1} 3363 vs, 3165 vs (NH), 2231 s (CN), 1651 vs, 1623 vs, 1576 s (NH), 1448 s (CH) and 1398 vs; δ_H (400 MHz, [D_6]DMSO) 3.53 (1 H, dd, *J* 12 and 4, $C(5')H_a$), 3.59 (1 H, dd, *J* 12 and 4, $C(5')H_b$), 3.85 (1 H, ddd, *J* 4, 4 and 4, C(4′)H), 4.06 (1 H, dd, *J* 5 and 4, C(3′)H), 4.25 (1 H, dd, *J* 6 and 5, C(2′)H), 5.86 (1 H, d, *J* 6, C(1′)H), 6.65 (2 H, s, NH_2), 7.92 (1 H, s, C(6)H) and 10.89 (1 H, s, N(3)H); δ_C (150 MHz, [D_6]DMSO) 61.9 (C5′), 70.9 (C3′), 74.8 (C2′), 85.8 (C4′), 87.0 and 87.2 (C1′ and C5), 99.3 (C4a), 116.0 (CN), 128.2 (C6), 152.1 (C7a), 154.7 (C2) and 157.9 (C4); *m/z* (ESI−) 306.0837 (M^-, $C_{12}H_{12}N_5O_5^-$ requires 306.0844).

2-Amino-3,4-dihydro-4-oxo-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboximidamide (archaeosine) 1

2 (10.0 mg, 0.03 mmol) was dissolved in methanol (2 cm^3) and treated with gaseous HCl for 3 h. Subsequently, nitrogen was bubbled through the solution to remove the solvent. The resulting white solid was stirred in 7 N ammonia in methanol (2 cm^3) for 16 h. Removal of the solvent *in vacuo* yielded a white solid, which was purified by HPLC (eluent A: 0.1 M triethylamine–AcOH in water, eluent B: 0.1 M triethylamine–AcOH in 20% water and 80% acetonitrile, gradient: 100% A, 0% B → 80% A, 20% B in 45 min, retention time = 22.2 min, Nucleosil 100–7 C18). The solvent was removed *in vacuo* to give a 2 : 1 mixture of nucleoside **1** and triethylammonium acetate (4 mg, 30%). R_f = 0.57 (isopropanol–water–AcOH 5 : 1 : 1); mp >215 °C (decomposition); λ_{max} (HPLC buffer)/nm 306; ν_{max}/cm^{-1} 3368 vs, 3176 vs (NH), 2931 s, 1660 vs, 1559 s (NH), 1518 s, 1411 s (CH), 1058 vs (CO) and 1023 vs; δ_H (600 MHz, [D_6]D_2O) 1.93 (3 H, s, H_3CCOO^-), 3.84 (1 H, dd, *J* 4 and 12, $C(5')H_a$), 3.90 (1 H, dd, *J* 3 and 12, $C(5')H_b$), 4.24 (1 H, m, C(4′)H), 4.38 (1 H, dd, *J* 4 and 5, C(3′)H), 4.61 (1 H, dd, *J* 5 and 6, C(2′)H), 6.06 (1 H, d, *J* 6, C(1′)H) and 8.03 (1 H, s, C(6)H) [lit.,[12] 3.6 (m, $C(5')H_2$), 4.0 (m, C(4′)H), 4.1 (dd, C(3′)H), 4.4 (dd, C(2′)H), 5.8 (d, C(1′)H) and 7.8 (s, C(6)H)]; δ_C (150 MHz, [D_6]D_2O) 23.4 (CH_3COO^-), 61.5 (C5′), 70.5 (C3′), 74.2 (C2′), 85.3 (C4′), 87.9 (C1′), 98.1 (C4a), 107.4 (C5), 125.3 (C6), 153.4, 154.7, 159.2 and 162.2 (C7a, $C_{amidine}$, C2, C4); *m/z* (ESI−) 323.1116 (M^-. $C_{12}H_{15}N_6O_5^-$ requires 323.1109).

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