Concise and Efficient Syntheses of preQ₁ base, Q base, and (ent)-Q Base

Supporting Information

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Concise schematic overview (Schemes S1 and S2) and brief discussion of previously reported procedures for the synthesis of preQ₁ and Q base

NMR-Spectra

¹H-NMR (DMSO-d₆) 1
¹H-NMR (CD₃OD) 1
¹³C-NMR (DMSO-d₆) 1
¹³C-NMR (D₂O/CD₃OD) 1
¹H-NMR (CD₃OD) 2
¹H-NMR (D₂O) 2
¹³C-NMR (CD₃OD) 2
¹H-NMR (CD₃OD) 3
¹H-NMR (D₂O) 3
¹³C-NMR (CD₃OD) 3
¹³C-NMR (D₂O/CD₃OD) 3
¹H-NMR (DMSO-d₆) 6
¹³C-NMR (DMSO-d₆) 6
¹H-NMR (DMSO-d₆) 7
¹³C-NMR (DMSO-d₆) 7
¹H-NMR (DMSO-d₆) 8
¹³C-NMR (DMSO-d₆) 8
¹H-NMR (DMSO-d₆) 9
¹³C-NMR (DMSO-d₆) 9
¹H-NMR (CDCl₃) 10b
\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) 11a
\textsuperscript{13}C-NMR (CDCl\textsubscript{3}) 11a
\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) 11b
\textsuperscript{13}C-NMR (CDCl\textsubscript{3}) 11b
\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) 12a
\textsuperscript{13}C-NMR (CDCl\textsubscript{3}) 12a
\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) 12b
\textsuperscript{13}C-NMR (CDCl\textsubscript{3}) 12b
\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) 13a
\textsuperscript{13}C-NMR (CDCl\textsubscript{3}) 13a
\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) 13b
\textsuperscript{13}C-NMR (CDCl\textsubscript{3}) 13b
\textsuperscript{1}H-NMR (CD\textsubscript{3}OD) 14a (HCl)
\textsuperscript{13}C-NMR (CD\textsubscript{3}OD) 14a (HCl)
\textsuperscript{1}H-NMR (CD\textsubscript{3}OD) 14a (base)
\textsuperscript{1}H-NMR (DMSO-d\textsubscript{6}) 14b (HCl)
\textsuperscript{1}H-NMR (CD\textsubscript{3}OD) 14b (HCl)
\textsuperscript{13}C-NMR (CD\textsubscript{3}OD) 14b (HCl)
\textsuperscript{1}H-NMR (CD\textsubscript{3}OD) 14b (base)
\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) 15
\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) 16
\textsuperscript{1}H-NMR (CD\textsubscript{3}OD) 17
\textsuperscript{1}H-NMR (CD\textsubscript{3}OD) 17
Overview and brief discussion of the most recently published procedures for preQ₁ by Carell et al.¹

The two most recent procedures suggested to access the modified guanine analogue preQ₁ base were reported by Carell et al. in 2005, both starting from pyrimidine 4 (Scheme S1).¹ The first one (Route A), being a short and seemingly straightforward five-step synthesis, involves intermediate formation of preQ₀ base from pyrimidine 4 and 2-chloro-3-oxopropanenitrile, followed by a subsequent hydrogenation step to yield preQ₁ base. However, this approach only yields 6.6 % of the desired nucleobase. The second route represents a higher-yielding, yet synthetically slightly more elaborate six-step sequence rendering preQ₁ base in 25 % overall yield. After an intermediate phthalimide deprotection step with hydrazine, the resulting product mixture is reprotected by treatment with (Boc)₂O in DMF to enable separation of the product from the simultaneously formed side-products (mainly Phthalhydrazide) by flash chromatography yielding 31 % of Boc-protected preQ₁ base. Final deprotection gives rise to the desired nucleobase 1.

![Scheme S1: Retrosynthetic approaches of most recent preQ₁ syntheses by Carell et al.¹](image)

Overview and brief discussion of all hitherto published synthetic approaches for Q base:

The first synthesis was reported by Goto et al.² in 1983. This rather lengthy and time-consuming route finally rendered Q base in 19 steps applying a reductive amination with cyclopentenylamin 19 as key step. In 1988, Akimoto et al.³ published a shorter, apparently more straightforward synthetic approach. The crucial step of their strategy is based on a regioselective Mannich reaction at the pyrrolo moiety of the previously prepared octanoyl-protected heterocyclic core 20 to introduce a dibenzylated aminomethyl side chain at position 5 of the heterocycle. The resulting intermediate 21 finally allows implementation of the required side chain moiety of queuine by an amine exchange reaction utilizing excessive amounts of the above-mentioned amine 19. Although the regioselectivity of the Mannich reaction (ratio of 5- versus 6-position substitution = 14.2 : 1) is quite high, the obtained product still might contain low amounts of the unwanted 6-substituted isomer. In addition, the synthesis of the required substrate 19 for the following amine exchange is rather challenging and, moreover, has to be applied in large excess (five-fold) in order to achieve a reasonable yield. In 2000, Grubb et al.⁴ described an alternative route utilizing a different disconnection approach. Herein, the key reaction is the ring closure via a cyclic condensation to install the pyrrolopyrimidine core of the nucleobase.
employing 2,6-diaminopyrimidine-4-one (4) and α-bromoaldehyde intermediate 22, prior to this derived via cyclopentenol 10 starting from D-(-)-ribose, and the fully protected 3-aminopropanol precursor 23.

### Scheme S2: Retrosynthetic approach and key steps of previously reported syntheses of Q base

In total, this sequence requires 14 steps in a convergent approach applying several protection strategies to finally yield Q base (2) in an overall yield of 1.6 % from D-(-)-ribose. In 2010, Showalter et al.\textsuperscript{5} reported a significantly shorter strategy using the biochemical preQ\textsubscript{1} precursor preQ\textsubscript{0} base (24), available in two preceding steps from heterocycle 4,\textsuperscript{6} as starting material. Reductive amination of the trityl-protected formyl deazaguanine derivative 25, obtained from preQ\textsubscript{0} base 24, with cyclopentenylamine 19, furnishes, after a
final deprotection step, queuine (2) as monohydrochloride in 36 % over four steps from 24. Cyclopentenylamine 19 is accessible via the corresponding cyclopentenylazide 26 by a modified four-step protocol of Carell et al.\(^7\) in 14.5 % overall yield. However, a necessary requirement of this synthesis is the implementation of an intermediate silylation step with 24 thus gaining sufficient solubility of the heterocyclic intermediate for the successive nitrile reduction with DIBAL-H to form the trityl-protected derivative 25 at lower temperatures.

2-Amino-5-(aminomethyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (1, DMSO-d₆)
2-Amino-5-(aminomethyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (1, CD$_3$OD)
2-Amino-5-(aminomethyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (1, DMSO-d$_6$)
2-Amino-5-(aminomethyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (1, D₂O/CD₃OD)

![Chemical Structure](image)

**Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry**

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2-Amino-5([[1S,4S,5R]-4,5-dihydroxycyclopent-2-en-1-yl]amino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (2, CD₃OD)
2-Amino-5((((1S,4S,5R)-4,5-dihydroxycyclopent-2-en-1-yl)amino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (2, D₂O)
2-Amino-5(\([1S,4S,5R]-4,5\text{-dihydroxycyclopent-2-en-1-yl}amino\)methyl)-3,7-dihydro-4\text{H}-pyrrolo[2,3-\text{d}]pyrimidin-4-one dihydrochloride hydrate (2, CD\text{\textsubscript{3}OD})
2-Amino-5((((1R,4R,5S)-4,5-dihydroxycyclopent-2-en-1-yl)amino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (3, CD$_3$OD)
2-Amino-5(((1R,4R,5S)-4,5-dihydroxycyclopent-2-en-1-ylamino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (3, D$_2$O)
2-Amino-5((((1R,4R,5S)-4,5-dihydroxycyclopent-2-en-1-yl)amino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (3, CD3OD)
2-Amino-5(\{(1R,4R,5S)\}-4,5-dihydroxycyclopent-2-en-1-ylamino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one dihydrochloride hydrate (3, D2O/CD3OD)

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(R,S)-2-(2-Hydroxy-3-nitropropyl)-1H-isoindole-1,3(2H)-dione (6, DMSO-d$_6$)
(R,S)-2-(2-Hydroxy-3-nitropropyl)-1H-isooindole-1,3(2H)-dione (6, DMSO-d$_6$)
2-[(2E)-3-nitroprop-2-en-1-yl]-1H-isindole-1,3(2H)-dione (7, DMSO-d$_6$)
2-[(2E)-3-nitroprop-2-en-1-yl]-1H-isouindole-1,3(2H)-dione (7, DMSO-d$_6$)
(R,S)-2-[2-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-3-nitropropyl]-1H-isooindole-1,3(2H)-dione hydrate (8, DMSO-d₆)
(R,S)-2-[2-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-3-nitropropyl]-1H-isindole-1,3(2H)-dione hydrate (8, DMSO-d₆)
2-(((2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-5-yl)methyl)amino)carbonyl)benzoic acid dihydrate (9, DMSO-d$_6$)
2-([(2-Amino-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-5-yl)methyl]amino)carbonyl)benzoic acid dihydrate (9, DMSO-d$_6$)
(3′aR,4′R,6′aS)-4′,6′a-dihydro-3′aH-spiro[cyclohexane-1,2′-cyclopenta[d][1,3]dioxole]-4′-ol (10b, CDCl₃)
(3aS,4S,6aS)-2,2-dimethyl-2H,3aH,4H,6aH-cyclopenta[d][1,3]dioxol-4-yl 4-nitrobenzoate (11a, CDCl₃)
(3aS,4S,6aS)-2,2-dimethyl-2H,3aH,4H,6aH-cyclopenta[d][1,3]dioxol-4-yl 4-nitrobenzoate (11a, CDCl₃)
(3a'S,4'S,6a'S)-4',6a'-dihydro-3a'H-spiro[cyclohexane-1,2'-cyclopenta[d][1,3]dioxol]-4'-yl 4-nitro-benzoate (11b, CDCl₃)

![NMR spectrum diagram]

X : parts per Million : 1H

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(3a'S,4'S,6a'S)-4',6a'-dihydro-3a'H-spiro[cyclohexane-1,2'-cyclopenta[d][1,3]dioxol]-4'-yl 4-nitro-benzoate (11b, CDCl₃)
(3aR,4S,6aS)-2,2-dimethyl-2H,3aH,4H,6aH-cyclopenta[d][1,3]dioxol-4-ol (12a, CDCl₃)
(3aR,4S,6aS)-2,2-dimethyl-2H,3aH,4H,6aH-cyclopenta[d][1,3]dioxol-4-ol (12a, CDCl₃)
(3'aR,4'S,6'aS)-4',6'a-dihydro-3'aH-spiro[cyclohexane-1,2'-cyclopenta[d][1,3]dioxole]-4'-ol (12b, CDCl₃)
(3′aR,4′S,6′aS)-4′,6′a-dihydro-3′aH-spirocyclohexane-1′,2′-cyclopenta[d][1,3]dioxole-4′-ol (12b, CDCl₃)
(3aS,4R,6aS)-4-bromo-2,2-dimethyl-2H,3aH,4H,6aH-cyclopenta[d][1,3]dioxole (13a, CDCl₃)
(3aS,4R,6aS)-4-bromo-2,2-dimethyl-2H,3aH,4H,6aH-cyclopenta[d][1,3]dioxole (13a, CDCl₃)
(3aS,4R,6aS)-4-bromo-4,6a-dihydro-3aH-spiro[cyclohexane-1,2-cyclopenta[d][1,3]dioxole (13b, CDCl₃)
(3aS,4R,6aS)-4-bromo-4,6a-dihydro-3aH-spiro[cyclohexane-1,2-cyclopenta[d][1,3]dioxole (13b, CDCl₃)
2-Amino-5(((3a'R,4'S,6a'S)-2,2-dimethyl-4,6a'-dihydro-3a'H-cyclopenta[d][1,3]dioxol-4-yl)amino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one hydrochloride (14a, CD$_3$OD)
2-Amino-5(((3a'R,4'S,6a'S)-2,2-dimethyl-4,6a’-dihydro-3a’H-cyclopenta[d][1,3]dioxol-4-ylamino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one hydrochloride (14a, CD$_3$OD)
2-Amino-5(((3a’R,4’S,6a’S)-2,2-dimethyl-4,6a’-dihydro-3a’H-cyclopenta[d][1,3]dioxol-4-ylamino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one (14a, CD$_3$OD)
5-((3'aR, 4'S,6'aS)-4',6'a-dihydro-3'aH-spiro[cyclohexane-1,2'-cyclopenta[d][1,3]dioxole]-4'-ylamino)methyl)-2-amino-3H,4H,7H-pyrrolo[2,3-d]pyrimidin-4-one hydrochloride (14b, CD$_3$OD)
5-(((3'R, 4'S,6'aS)-4',6'a-dihydro-3'aH-spiro[cyclohexane-1,2'-cyclopenta[d][1,3]dioxole-4'-yl]amino)methyl)-2-amino-3H,4H,7H-pyrrolo[2,3-d]pyrimidin-4-one hydrochloride (14b, DMSO-d₆)

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5-(((3'R, 4'S, 6'a'R)-4', 6'a-dihydro-3'aH-spiro[cyclohexane-1, 2'-cyclopenta[d][1, 3]dioxole-4'-yl]amino)methyl)-2-amino-3H, 4H, 7H-pyrrolo[2, 3-d]pyrimidin-4-one hydrochloride (14b, CD₃OD)
5-((((3'R, 4'S,6'aS)-4',6'a-dihydro-3'aH-spiro[cyclohexane-1,2'-'cyclopenta[d][1,3]dioxole]-4'-yl]amino)methyl)-2-amino-3H,4H,7H-pyrrolo[2,3-d]pyrimidin-4-one (14b, CD,OD)
(3aS,4R,6aR)-2,2-dimethyl-2H,3aH,4H,6aH-cyclopenta[d][1,3]dioxol-4-ol (15, CDCl₃)
(3aR,4S,6aR)-4-bromo-2,2-dimethyl-2H,3aH,4H,6aH-cyclopenta[d][1,3]dioxole (16, CDCl₃)
2-Amino-5(((3a’S,4’R,6a’R)-2,2-dimethyl-4,6a’-dihydro-3a’H-cyclopenta[d][1,3]dioxol-4-yl]amino)methyl)-3,7-dihydro-4H-pyrrolo[2,3-d]pyrimidin-4-one hydrochloride (17, CD$_3$OD)
2-Amino-5(\([(3a'S,4'R,6a'R\)-2,2-dimethyl-4,6a'-dihydro-3a'H-cyclopenta[d][1,3]dioxol-4-ylamino)methyl]-3,7-dihydro-4H-pyrrolo[2,3-\(d\)]pyrimidin-4-one hydrochloride (17, CD\(3\)OD)