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Synthesis of 1′-aza-*C*-nucleosides from (3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol

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Abstract—Pyrimidine 1′-aza-*C*-nucleosides are synthesised by the fusion of 5-bromouracil, 5-bromocytosine and 5-bromoisocytosine with (3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol in 40–41% yield. A homologue of 1′-aza-Ψ-uridine is obtained in a Mannich reaction in 65% yield by treatment of the azasugar, paraformaldehyde and uracil. N-Alkylation of (3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol with 6-chloromethyluracil gives the 6-regioisomeric homologue. (3*R*,4*R*)-4-(Hydroxymethyl)pyrrolidin-3-ol is synthesised in 25% overall yield from diacetone-D-glucose via 3-*C*-(azidomethyl)-3-deoxy-D-allose which is subjected to an intramolecular reductive amino alkylation reaction to give (3*R*,4*S*)-4-[(1*S*,2*R*)-1,2,3-trihydroxypropyl]pyrrolidin-3-ol followed by Fmoc protection, oxidative cleavage of the triol group with further reduction of the obtained aldehyde and subsequent deprotection of the nitrogen atom.

1. Introduction

Since the discovery of pseudouridine (Ψ-uridine) **1** (Fig. 1) in nature in 1957,[1] a considerable number of *C*-nucleosides have been synthesised and found application as antibiotics and potential anticancer and/or antiviral agents.[2] Due to their structural relationship *C*-nucleosides can be incorporated into DNA/RNA instead of the natural occurring nucleosides. On the other hand the modification of the glycons is one of the main directions in the synthesis of nucleoside analogues. Among the possibilities are nucleosides with replacement of the natural glycons with polyhydroxylated cyclic amines known as azasugars or iminosugars. These sugars are also of interest as inhibitors of various glycosidases.[3] 3′-Aza-3′-deoxythymidine carboanalogue[4] and the pyrrolidinyl analogues of 2′,3′-dideoxycytidine[5] (**2**) were among the first representatives in this area. Lee and co-workers[6] have published the synthesis of 1′-aza carbacyclic thymidine analogues **3** from the racemic 1-benzyl-4-(hydroxymethyl)pyrrolidin-3-ol.[7]

Recently our group has presented a new class of nucleosides, called aza-*C*-nucleosides, in which a carbon in the heterocyclic base is linked to the nitrogen of the secondary cyclic amine.[8] For developing the synthesis of aza-*C*-nucleosides, the challenge is to synthesize pure enantiomeric azasugars. These can then be condensed with 5-bromopyrimidines to produce aza-*C*-nucleosides according to the procedure of Phillips for condensation of amines with 5-bromouracil.[9] According to this procedure the racemic 5-[3-hydroxy-4-(hydroxymethyl)pyrrolidin-1-yl]-uracil was prepared as a 1′-aza analogue of Ψ-uridine.[8] Racemic *trans*-3-hydroxy-4-hydroxymethyl-*N*-(6-uracilylmethyl)pyrrolidine, that is considered an analogue of inosine because it mimics the pyrimidine ring, could likewise be obtained by condensation with 6-(halogenomethyl)uracil.[10]

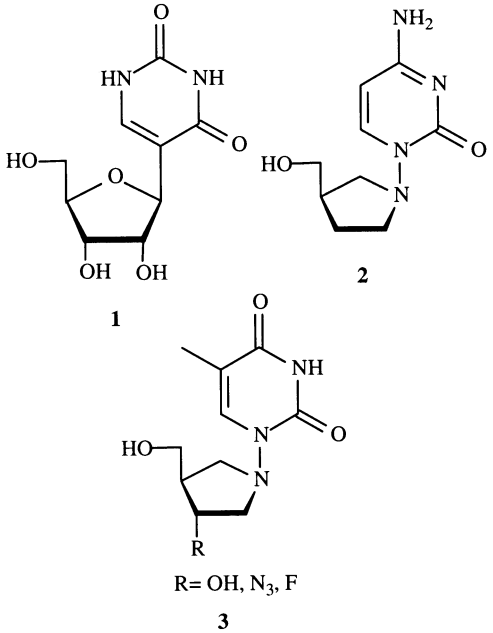

Figure 1.

Keywords: azasugar; iminosugar; reductive amination; (3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol; pseudonucleoside; aza-*C*-nucleoside.

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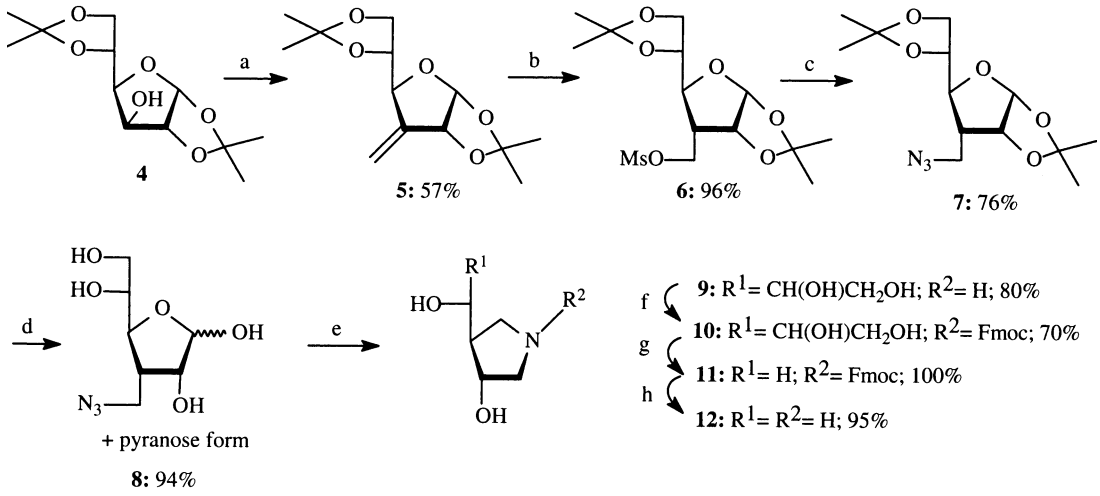
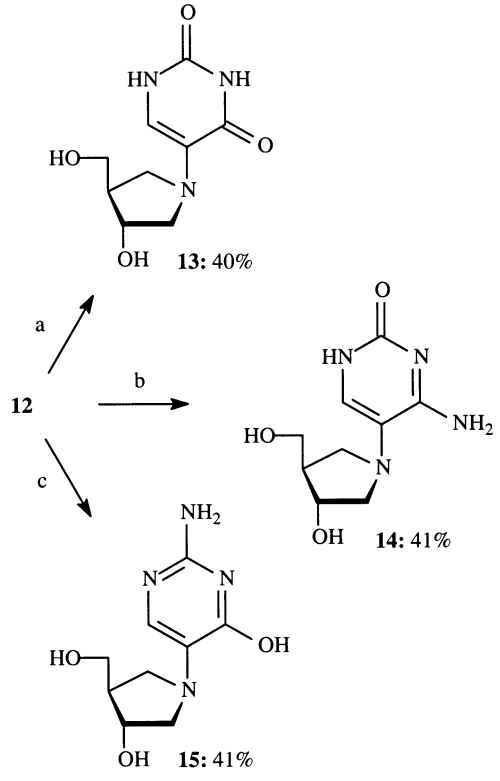


Scheme 1. *Reagents and conditions*: (a) (1) CrO_3/Py/Ac_2O/CH_2Cl_2, (2) $CH_3P(C_6H_5)_3Br$/*n*-BuLi/THF; (b) (1) BH_3/THF, (2) NaOH/H_2O_2, (3) MsCl/Py; (c) $(CH_3)_2NH\cdots HN_3$/DMF; (d) Amberlite IR-120(H^+); (e) H_2/200 psi/Pd–C/H_2O; (f) FmocCl/dioxane/10% aq. $NaHCO_3$; (g) (1) $NaIO_4$, (2) $NaBH_4$; (h) NEt_3/acetonitrile.

This paper describes the synthesis of several enantiomerically pure aza-*C*-nucleosides utilising a synthetic route for the required azasugar which is more easy and straightforward than the ones previously published.[11] We have synthesized azasugar analogues of 2-deoxy-D-hexofuranose and 2-deoxy-D-heptofuranose in a one-pot reaction by a catalytic reduction reaction of amino group precursors in aldosugars (3-*C*-cyano-3-deoxy-D-ribo-pentofuranose, 3-*C*-azidomethyl-3-deoxy-D-ribo-pentofuranose and 3-deoxy-3-*C*-nitromethyl-D-allose) followed by an in situ intramolecular reductive alkylation reactions.[11] Multistep reactions including multiple chromatographic purifications were needed in order to obtain the amino precursor sugars and in the end low overall yields (maximum 11%) were obtained of the azasugars.

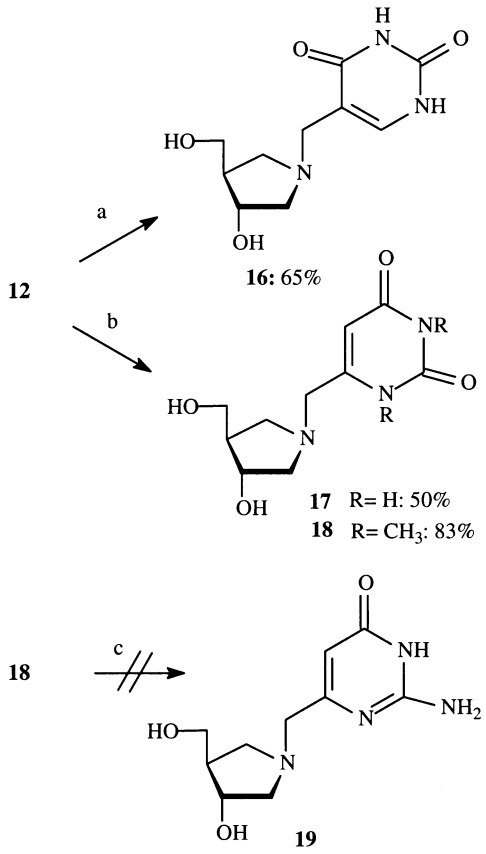


Scheme 2. *Reagents and conditions*: (a) 5-bromouracil, fusion, 145–150°C, 10 min; (b) 5-bromocytosin, fusion, 145–150°C, 10 min; (c) 5-bromoisocytosin, fusion, 145–150°C, 10 min.

2. Results and discussion

We found it attractive to synthesise (3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol (**12**) through 3-*C*-azidomethyl-3-deoxy-D-allose (**8**) starting from diacetone-D-glucose (**4**) (Scheme 1).

In order to avoid contamination with sulfur compounds which later on could poison the catalyst during the reductive amination,[11] the methylene derivative **5** was obtained from diacetone-D-glucose (**4**) by oxidation with a complex of CrO_3/acetic anhydride/pyridine[12] followed by treatment with the Wittig reagent $MePPh_3Br$/*n*-BuLi in dry THF.[13] Scale up of the Wittig reaction to 30 g led to a decreased yield (30%). The best yield (57%) was obtained from 20 g of 1,2:5,6-di-*O*-isopropylidene-α-D-ribo-hexofuranos-3-ulose. Compound **6** was prepared in three steps from **5**[13,14] and was then converted to compound **7** by treatment with 1.5 equiv. of dimethylammonium azide[11,15] which in this investigation was prepared in situ. Deprotection of compound **7** under acidic conditions afforded the azido sugar **8** in an overall yield of 39% from **4**. The required azasugar **9** was obtained as a brown oil in 80% yield by a reductive amination reaction using hydrogen over 10% Pd/C in water in an autoclave for 16 h. Fmoc protection of the primary amine followed by oxidative cleavage of the triol group in the corresponding Fmoc-azasugar **10** gave **11**. Then, reduction with sodium borohydride and deprotection with NEt_3 in acetonitrile[11] afforded (3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol (**12**) in 67% yield from **9**. In this synthetic route the purification of products using silica gel column chromatography is necessary only in three steps (oxidation by CrO_3, the Wittig reaction, synthesis of azide) and this makes this scheme more practically useful than the ones previously published.



Scheme 3. *Reagents and conditions*: (a) uracil, $(CH_2O)_n$, EtOH, reflux, 24 h; (b) 6-chloromethyluracil (for **17**), 6-chloromethyl-1,3-dimethyluracil (for **18**), $(i\text{-Pr})_2$EtN, MeOH, rt, three days; (c) guanidine, fusion, 100°C, 10 h.

When applying the modified Phillips's procedure[8] to obtain enantiomerically pure 1′-aza-Ψ-uridine from 5-bromouracil and azasugar **9** under reflux in pyridine for 24 h, neither products of coupling nor starting azasugar were observed in the black reaction mixture. The presence of the vicinal hydroxyl groups makes the structure **9** too sensitive for heating. Full solubility of **9** is possible only in highly polar solvents (H_2O, DMF), but as earlier observed,[8] DMF is undesirable as solvent because of side reactions with dimethylamine. The sugar **12** dissolves completely in pyridine and in alcohols in contrast to the partly soluble compound **9**. However, our attempts to obtain the aza-*C*-nucleoside **13** from **12** under the same conditions also failed. The problem could be solved by decreasing the reaction time and increasing the polarity of the solvent. Therefore, returned to the original Phillips's method, i.e. fusion of a large excess of an amine with a bromoheterocycle.[9] 5-[(3*R*,4*R*)-3-Hydroxy-4-(hydroxymethyl)pyrrolidin-1-yl]-uracil (**13**) was isolated in 40% yield after fusion of 3 equiv. of the azasugar **12** and 5-bromouracil followed by a silica gel column chromatography and subsequent recrystallization (Scheme 2). Excess of pyrrolidine **12** was also isolated from the residue and this is important because **12** is not easily available.

To obtain 1′-aza-*C*-cytosine analogues we used the same methodology. The reaction of 5-bromoisocytosine with primary and secondary amines have been examined by Phillips[16] in 1953 and less reactivity of 5-bromoisocytosine was found when compared with 5-bromouracil. According to the literature, 5-bromocytidine could be converted to the corresponding 5-aminocytidine in liquid ammonia.[17] The fusion of the azasugar **12** with 5-bromocytosine and 5-bromoisocytosine at 145–150°C for 10 min led to the 1′-aza-Ψ-isocytosine **14** and 1′-aza-Ψ-cytosine **15**, respectively, both in 41% yield.

The NMR spectra were confirmed by comparison with calculated spectra using ACD/HNMR predictor and ACD/CNMR predictor.[18] A characteristic feature of the cytosine derivative is the lower field signals of C-5 (128.2) and C-6 (150.8) in **15** compare to the signals of C-5 (120.1, 117.6) and C-6 (125.2, 131.4) in **13** and **14**, respectively. This is explained by a shift in the tautomeric form of compound **15** to the OH-form and this group appears in the ^{1}H NMR spectrum at 6.95 ppm compare to the NH signal at 10.3 ppm in compound **14**. Spectra for all the possible tautomers of the compounds **13**–**15** were calculated and the tautomers in Scheme 2 are those giving the best fit with the calculated ones.

An interesting property of the nucleosides **13**–**15** is their potential ability of existing both as α and β-anomers due to flipping of the ring nitrogen. This was confirmed by NOE spectra. Thus the irradiation of β and α protons at H-2′ and H-5′ gave both 3–4% NOE in H-6 of **13** and 2% NOE in H-6 of **14**. On irradiation of H-6 3–4% NOE was observed in H-2′ and H-5′ for both β and α hydrogens in compound **13**.

Uracil was reacted according to the procedure of Motawia et al.[19] with paraformaldehyde and the secondary amine **12** to give the Mannich base **16** in 65% yield. This compound is considered a homologue of 1′-aza-Ψ-uridine (Scheme 3).

(3*R*,4*R*)-3-Hydroxy-4-hydroxymethyl-*N*-(6-uracilylmethyl)-pyrrolidine (**17**) was prepared in 50% yield by N-alkylation of azasugar **12** with 6-chloromethyluracil and Hünig's base in MeOH.

We attempted the synthesis of the guanosine analogue **19** by nucleophilic displacement of the N_1–C_2–N_3 fragment in **18** by a 1,3-ambident nucleophile using the procedure Hirota et al.[20] They found that refluxing of 1,3,6-trimethyluracil with guanidine for 6 h led to the 6-methylisocytosine in 45% yield. However, treatment of (3*R*,4*R*)-3-hydroxy-4-hydroxymethyl-*N*-(1,3,6-trimethyluracil-6-yl)pyrrolidine (**18**) and guanidine under reflux at 100°C for 10 h did not result in any conversion of **18**.

3. Conclusion

In the present investigation we have devised a simple way of synthesising one of the enantiomers of a 1-aza analogue of 2-deoxy-D-ribofuranose from diacetone-D-glucose (**4**). 1′-Aza-*C*-nucleosides **13**–**15** were obtained by the fusion of the azasugar **12** and 5-bromopyrimidines in 40–41% yield. The homologues of 1′-aza-Ψ-uridine **16** and **17** were obtained from the azasugar **12** in the Mannich reaction

with uracil and by alkylation with 6-chloromethyluracil in 65 and 50% yield, respectively.

4. Experimental

4.1. General

NMR spectra were recorded on a Bruker AC-300 FT NMR spectrometer at 300 MHz for ^{1}H NMR and at 75.5 MHz for ^{13}C NMR. Internal standards used in ^{1}H NMR spectra were TMS (δ: 0.00) for $CDCl_3$, CD_3OD, Me_2SO-d_6; in ^{13}C NMR were $CDCl_3$ (δ: 77.0), CD_3OD (δ: 49.0), Me_2SO-d_6 (δ: 39.5). ^{1}H NMR steady-state NOE difference spectroscopy experiments were carried out on compounds **13** and **14** with a Bruker AC-250 spectrometer. Accurate ion mass determinations were performed using the 4.7 Tesla Ultima Fourier transform (FT) mass spectrometer (IonSpec, Irvine, CA). The $[M+H]^+$ and $[M+Na]^+$ ions were peakmatched using ions derived from the 2,5-dihydroxybenzoic acid matrix. Thin layer chromatography (TLC) analyses were carried out with use of TLC plates 60 F_{254} purchased from Merck and were visualized in an UV light (254 nm) and/or with a 5% solution of H_2SO_4 in methanol for sugar derivatives and/or with a ninhydrin spray reagent (0.3 g ninhydrin in 100 mL butan-1-ol and 3 mL HOAc) for azasugars and its derivatives. Microanalysis were performed by Atlantic Microlab, USA. The silica gel (0.063–0.200) used for column chromatography was purchased from Merck. All solvents were distilled before use. The reagents used were purchased from Aldrich, Sigma or Fluka.

4.1.1. 3-*C*-Azidomethyl-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (7). Compound **6**[14] (10.0 g, 28.4 mmol) and 1.5 equiv. of dimethylamine hydrochloride (3.5 g, 42.6 mmol) and NaN_3 (2.8 g, 42.6 mmol) in DMF (100 mL) were heated with stirring at 95°C for 3 h. After cooling insoluble NaCl was filtered off, and the mother liquor was evaporated to dryness in vacuo, co-evaporated with toluene (30 mL). The residue was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O (3×50 mL), dried over Na_2SO_4, and evaporated to give a syrup which was purified using silica gel column chromatography with cyclohexane/EtOAc (0⟶10% EtOAc) to afford compound **7** (6.5 g, 76%). The R_f and NMR data agreed with previous data.[14]

4.1.2. 3-*C*-Azidomethyl-3-deoxy-D-allofuranose (8). A solution of azide **7** (7.8 g, 26.0 mmol), freshly washed Amberlite IR-120(H^+) (25 g) in H_2O (100 mL) was heated at 60°C for 3.5 h. After allowing the mixture to cool, the resin was filtered off and the solvent was removed in vacuo. The residue was purified by chromatography on a silica gel column with 20% MeOH in CH_2Cl_2 to give **8** (4.0 g, 94%) as a colourless oil: ^{1}H NMR (Me_2SO-d_6) δ 2.43 (m, 1H, H-3), 3.40–3.56 (m, 4H, 4×OH), 3.86 (m, 2H, CH_2N_3), 4.41 (t, J=5.4 Hz, 1H, C*H*OH), 4.75 (d, J=4.7 Hz, 1H, H-4), 4.98 (m, 2H, CH_2OH), 5.21 (d, J=4.4 Hz, 1H, H-2), 6.22 (d, J=4.4 Hz, 1H, H-1); ^{13}C NMR (Me_2SO-d_6) δ 44.5 (C-3), 48.0 (CH_2N_3), 63.4 (CH_2OH), 75.0 (C-2), 75.4 (C-4), 79.6 (*C*HOH), 102.1 (C-1); FAB-MS: m/z 242 $[M+Na]^+$; Anal. Calcd for $C_7H_{13}N_3O_5{\cdot}0.75H_2O$ (232.7): C, 36.14; H, 6.28; N, 18.06. Found: C, 36.38; H, 6.45; N, 18.00.

4.1.3. (3*R*,4*S*)-4-[(1*S*,2*R*)-1,2,3-Trihydroxypropyl]pyrrolidin-3-ol (9). 3-*C*-Azidomethyl-3-deoxy-D-allose (**8**, 3.0 g, 13.3 mmol) was dissolved in 125 mL H_2O, and 10% Pd/C (2.1 g) was added. The solution was hydrogenated in an autoclave for 16 h at 200 psi. The solution was filtered through Celite® and washed thoroughly with H_2O and evaporated in vacuo giving the title compound **9** (1.9 g, 80%) as a brown oil. R_f and NMR data agreed with previous data.[11]

4.1.4. *N*-Fmoc-(3*R*,4*S*)-4-[(1*S*,2*R*)-1,2,3-trihydroxypropyl]pyrrolidin-3-ol (10). (3*R*,4*S*)-4-[(1*S*,2*R*)-1,2,3-Trihydroxypropyl]pyrrolidin-3-ol (**9**, 1.0 g, 5.9 mmol) was dissolved in a mixture of 10% aqueous $NaHCO_3$ (25 mL) in dioxane (25 mL). The mixture was cooled at 0–5°C and 9-fluorenylmethyl chloroformate (2.3 g, 8.9 mmol) was added. The resulting solution was stirred at rt for 3 h, treated with H_2O (50 mL) and extracted with EtOAc (3×75 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under diminished pressure to give an oil that was purified by silica gel column chromatography with CH_2Cl_2/MeOH (0⟶10% MeOH) to afford **10** (1.6 g, 70%) as an oil: R_f 0.37 (10% MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD) δ 2.42 (m, 1H, H-4), 3.20 (m, 1H, H-5), 3.35 (m, 1H, H-5), 3.50–3.80 (m, 6H, H-2, [C*H*(OH)]$_2$CH_2OH,), 4.23 (m, 1H, CH [Fmoc]), 4.40 (m, 3H, CH_2 [Fmoc], H-3), 4.85 (br.s, 4H, 4×OH), 7.25–7.83 (m, 8H, Fmoc); ^{13}C NMR (CD_3OD) δ 48.1, 48.9 (C-4), 49.2 (Fmoc), 49.6, 49.8 (C-5), 54.1, 54.3 (C-2), 64.7 (CH [Fmoc]), 68.4, 68.5, 70.8, 71.5, 72.4 ([CHOH]$_2$CH$_2$OH), 75.1 (C-3), 120.9, 126.1, 128.1, 128.8, 142.6, 145.3, 156.6 (Fmoc); FAB-MS: m/z 400 $(M+H)^+$.

4.1.5. *N*-Fmoc-(3*R*,4*R*)-4-(hydroxymethyl)pyrrolidin-3-ol (11). To a cooled solution of compound **10** (1.4 mg, 3.5 mmol) in EtOH (50 mL) a solution of $NaIO_4$ (1.6 g, 7.7 mmol) in H_2O (10 mL) was added under vigorous stirring. After 30 min $NaBH_4$ (139 mg, 3.85 mmol) was added. The reaction mixture was stirring for 30 min and the solution was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (4×40 mL). The combined organic layers were dried (Na_2SO_4), and evaporated under reduced pressure to give pure **11** (1.2 g, 100%). R_f and NMR data agreed with previous data.[11]

4.2. General procedure for the synthesis of compounds 13–15

The azasugar **12** and 5-bromopyrimidines were mixed in the molar ratio 3:1. The mixture was fused in a preheated oil bath at 145–150°C for 10 min. After cooling, the residue was purified on a silica gel column with CH_2Cl_2/MeOH (0⟶25% MeOH) to afford the aza-*C*-nucleosides **13–15**. The excess of starting azasugar **12** was eluted with MeOH/25% aq. NH_3 (0⟶25%).

4.2.1. 5-[(3*R*,4*R*)-3-Hydroxy-4-(hydroxymethyl)pyrrolidin-1-yl]uracil (13). Colorless powder, 40% yield, mp 236–239°C (ethanol/few drops of H_2O), R_f 0.11 (10% MeOH/CH_2Cl_2); ν_{max} (neat) 3417–2700 (br), 1745, 1665 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.06 (m, 1H, H-4′), 2.78 (dd, J=5.8, 9.6 Hz, 1H, H-5′ (β)), 2.88 (dd, J=4.2, 10.2 Hz, 1H, H-2′ (β)), 3.22 (m, 2H, CH_2OH), 3.31 (m, 1H, H-2′ (α)), 3.45 (m, 1H, H-5′ (α)), 3.92 (t, J=4.8 Hz,

1H, H-3′), 4.60 (t, J=5.0 Hz, 1H, CH_2OH), 4.88 (d, J=4.7 Hz, 1H, 3′-OH), 6.43 (d, J=2.6 Hz, 1H, H-6), 10.30 (s, 1H, NH), 10.95 (s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 49.0 (C-4′), 51.7 (C-5′), 57.8 (C-2′), 61.8 (CH_2OH), 71.1 (C-3′), 120.1 (C-5), 125.2 (C-6), 150.1 (C-2), 161.5 (C-4); HRMS: m/z $(M+Na)^+$ found 250.0793, $C_9H_{13}N_3O_4Na$ requires 250.0798.

4.2.2. 5-[(3*R*,4*R*)-3-Hydroxy-4-(hydroxymethyl)pyrrolidin-1-yl]cytosine (14). Light-brown crystals, 41% yield, mp 138°C (EtOH/5% CH_2Cl_2), R_f 0.10 (30% MeOH/CH_2Cl_2); ν_{max} (neat) 3352–2800, 1664 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.10 (m, 1H, H-4′), 2.58 (dd, J=5.4, 9.1 Hz, 1H, H-5′ (β)), 2.69 (dd, J=3.0, 9.3 Hz, 1H, H-2′ (β)), 2.96 (dd, J=5.6, 9.6 Hz, 1H, H-2′ (α)), 3.06 (t, J=8.3 Hz, 1H, H-5′ (α)), 3.40 (m, 2H, CH_2OH), 3.90 (t, J=2.3 Hz, 1H, H-3′), 4.60 (br.s, 1H, CH_2OH), 4.90 (br.s, 1H, OH), 6.60 (br.s, 2H, NH_2), 7.10 (s, 1H, H-6), 10.30 (br.s, 1H, NH); ^{13}C NMR (Me_2SO-d_6) δ 50.1 (C-4′), 53.7 (C-5′), 59.8 (C-2′), 62.0 (CH_2OH), 71.9 (C-3′), 117.6 (C-5), 131.4 (C-6), 155.8 (C-2), 164.2 (C-4); HRMS: m/z $(M+H)^+$ found 227.1121, $C_9H_{15}N_4O_3$ requires 227.1139.

4.2.3. 5-[(3*R*,4*R*)-3-Hydroxy-4-(hydroxymethyl)pyrrolidin-1-yl]isocytosine (15). Light-brown crystals, 41% yield, mp 182–183°C (MeOH), R_f 0.06 (20% MeOH/CH_2Cl_2); ν_{max} (neat) 3366–2800, 1647 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.06 (m, 1H, H-4′), 2.83 (dd, J=5.8, 9.5 Hz, 1H, H-5′ (β)), 2.92 (dd, J=4.1, 9.8 Hz, 1H, H-2′ (β)), 3.20–3.52 (m, 4H, H-2′, H-5′ (α), CH_2OH), 3.90 (m, 1H, H-3′), 4.80 (br.s, 2H, CH_2OH, 3′-OH), 6.20 (br.s, 2H, NH_2), 6.68 (s, 1H, H-6), 6.95 (s, 1H, OH); ^{13}C NMR (Me_2SO-d_6) δ 49.1 (C-4′), 51.6 (C-5′), 57.7 (C-2′), 62.0 (CH_2OH), 71.2 (C-3′), 128.2 (C-5), 150.8 (C-6), 156.0 (C-2), 156.9 (C-4); HRMS: m/z $(M+H)^+$ found 227.1139, $C_9H_{15}N_4O_3$ requires 227.1135.

4.2.4. (3*R*,4*R*)-3-Hydroxy-4-hydroxymethyl-*N*-(5-uracilylmethyl)pyrrolidine (16). The azasugar **12** (50 mg, 0.43 mmol), uracil (53 mg, 0.47 mmol) and paraformaldehyde (15.4 mg, 0.51 mmol) in 20 mL EtOH were refluxed for 24 h. Solvent was evaporated under diminished pressure. The residue was purified on a silica gel column with CH_2Cl_2/MeOH (10→50% MeOH) to afford compound **16** (67 mg, 65%) as an oil: R_f 0.63 (aq. 25% NH_3 /1,4-dioxane: 50/50); ^{1}H NMR (CD_3OD) δ 2.20 (m, 1H, H-4′), 2.38 (m, 1H, H-5′ (β)), 2.70 (m, 2H, H-2′), 2.95 (t, J=8.5 Hz, 1H, H-5′ (α)), 3.35 (s, 2H, 5-CH_2N), 3.60 (m, 2H, CH_2OH), 4.05 (m, 1H, H-3′), 4.95 (br.s, 4H, 2×NH, 2×OH), 7.44 (s, 1H, H-6); ^{13}C NMR (CD_3OD) δ 51.5 (C-4′), 51.4 (C-5′), 56.8 (C-2′), 62.6 (CH_2OH), 64.0 (5-CH_2N), 74.1 (C-3′), 110.5 (C-5), 142.7 (C-6), 153.7 (C-2), 166.7 (C-4); HRMS: m/z $(M+H)^+$ found 242.1123, $C_{10}H_{16}N_4O_3$ requires 242.1150.

4.2.5. (3*R*,4*R*)-3-Hydroxy-4-hydroxymethyl-*N*-(6-uracilylmethyl)pyrrolidine (17). The azasugar **12** (144 mg, 1.23 mmol) was dissolved in 1 mL (i-Pr)$_2$EtN and 2.5 mL MeOH and 6-uracilylmethylchloride (237 mg, 1.48 mmol) was added. The mixture was stirred at rt for three days. The solvent was removed in vacuo and the residue was purified using silica gel column chromatography with CH_2Cl_2/MeOH (0→10% MeOH) to afford compound **17** (140 mg, 50%) as an oil: R_f 0.35 (20% MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD) δ 2.20 (m, 2H, H-4′, H-5′ (β)), 2.64 (m, 2H, H-2′), 2.95 (t, J=8.0 Hz, 1H, H-5′ (α)), 3.38 (s, 2H, 6-CH_2N), 3.53 (m, 2H, CH_2OH), 4.10 (m, 1H, H-3′), 4.90 (br.s, 4H, 2×NH, 2×OH), 5.59 (s, 1H, H-5); ^{13}C NMR (CD_3OD) δ 51.4 (C-4′), 56.5 (C-5′), 56.8 (C-2′), 62.8 (CH_2OH), 63.9 (6-CH_2N), 74.3 (C-3′), 99.7 (C-5), 153.5 (C-6), 156.0 (C-2), 167.2 (C-4); HRMS: m/z $(M+H)^+$ found 242.1123, $C_{10}H_{16}N_4O_3$ requires 242.1135.

4.2.6. (3*R*,4*R*)-3-Hydroxy-4-hydroxymethyl-*N*-(1,3,6-trimethyluracil-6-yl)pyrrolidine (18). This compound was prepared by the same methodology as described for the synthesis of **17** using 1,3-dimethyluracil-6-ylmethyl chloride.[21] Yield 83%, R_f 0.41 (20% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 2.36 (m, 2H, H-4′, H-5′ (β)), 2.65 (dd, J=4.1, 9.7 Hz, 1H, H-2′ (β)), 2.92 (m, 2H, H-2′, H-5′ (α)), 3.34 (s, 3H, NCH_3), 3.41 (s, 2H, 6-CH_2N), 3.50 (s, 3H, NCH_3), 3.65 (m, 4H, CH_2OH, 2×OH), 4.24 (m, 1H, H-3′), 5.80 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$) δ 27.9, 31.2 (NCH_3), 50.1 (C-4′), 55.6 (C-5′), 56.9 (C-2′), 61.7 (CH_2OH), 63.8 (6-CH_2N), 73.7 (C-3′), 101.7 (C-5), 151.3 (C-6), 152.7 (C-2), 162.7 (C-4); HRMS: m/z $(M+H)^+$ found 270.1440, $C_{12}H_{20}N_3O_4$ requires 270.1448.

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