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Synthesis and Conformational Analysis of Pyrimidine Nucleoside Analogues with a Rigid Sugar Moiety

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Abstract—In order to obtain rigidity within the sugar moiety of nucleosides, some bicyclic pyrimidine nucleoside analogues were synthesized starting from cyclopentanone. The C-3' is fused to C-4' via a propylene linker in order to obtain a [3.3.0]-bicyclic ring system wherein the sugar moiety should be restricted to mainly the C-1'-exo conformation.

Introduction

Over the last decade a large number of modified nucleosides have been synthesized and shown to inhibit the replication of human immunodeficiency virus (HIV).[1] The majority of these compounds are metabolized, *in vivo*, to their corresponding 5'-triphosphates and are, as such, inhibitors of reverse transcriptase and/or chain terminators of viral DNA synthesis.

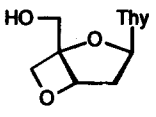
Owing to the complexity of nucleoside analogue metabolism, structure–activity relationships have, more or less, been limited to empirical generalizations.[2] Analysis of the solid state conformations of nucleoside analogues have led to the suggestion that C-3'-exo conformation is predictive for anti-HIV activity.[2,3] Using computational chemistry it has however been difficult to predict anti-HIV activity based on the sugar conformation in nucleoside analogues. Recently it has been postulated that 4'-substituted nucleoside analogues having anti-HIV activity have a strong preference for a C-4'-exo conformation.[4]

Bicyclic nucleoside analogues like the fused oxetane derivative of thymidine[5] (**1**) and the methylene derivative (**2**) have been synthesized and shown to inhibit HIV replication.[6,7] Both of these substances have in common that the sugar ring conformation is somewhat more flattened than in non-bicyclic nucleoside analogues.[8] It has been suggested that the sugar ring of nucleosides must be able to obtain a less puckered conformation in order to be active.[8]

For the purpose of obtaining further information regarding the correlation between sugar ring conformation and anti-viral activity, the cyclopentane fused pyrimidines **3** and **4** have been synthesized.

Result and Discussion

2-Propenylcyclopentanone[9] (**5**) was reacted with isopropoxydimethylsilylmethylmagnesium chloride[10] in tetrahydrofuran at 0°C followed by treatment with potassium fluoride, potassium hydrogen carbonate and hydrogen peroxide in tetrahydrofuran–methanol to give racemic 1-(hydroxymethyl)-2-(2-propenyl)-cyclopentanol (**6**) as a *cis/trans* mixture (4:1) in 48% yield. Introducing the hydroxymethyl group using [(methoxymethoxy)methyl]tributylstannane[11] in tetrahydrofuran at –78 °C gave racemic 1-[(methoxymethoxy)methyl]-2-(2-propenyl)-cyclopentanol in 60 % yield [(5:1) *cis/trans* ratio]. Although the yields were improved using this reagent, the limitations of having a predefined protective group of the stannane reagent made the Grignard reagent more flexible. Protection of the primary hydroxy group in **6** using benzoyl chloride in pyridine at 0°C and removal of the *trans*-isomer by silica gel chromatography gave the monobenzoylated compound **7** in 73% yield. *cis*-Hydroxylation of the olefinic bond in **7** using a catalytic amount of osmium tetroxide and *N*-methylmorpholine-*N*-oxide as oxidant[12] gave the corresponding diol which was cleaved using sodium periodate in aqueous tetrahydrofuran to produce an unstable furanose. Treatment of this furanose with methanol containing hydrochloric acid (2.5%, w/w) readily produced the methyl furanoside **8** as an enantiomeric mixture in 72% yield from **7**.



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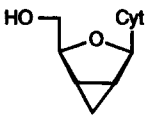

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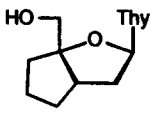

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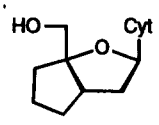

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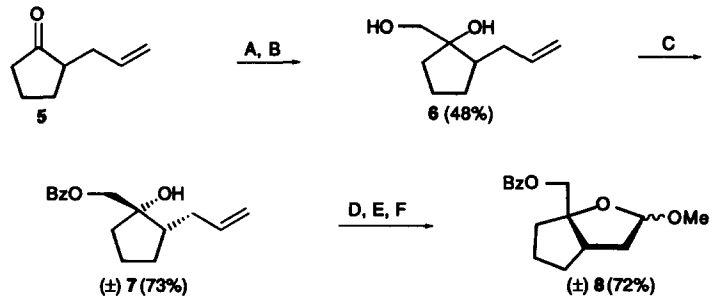
Glycosylation of **8** with bis(trimethylsilyl)thymine[13] gave (±)1-benzoyloxymethyl-3-α/β-(thymine-1-yl)-*cis*-2-oxabicyclo-[3.3.0]-octane (**9**) in 93% yield with an α/β ratio of 1:2.8. Glycosylation of **8** with bis(trimethylsilyl)uracil[13] gave (±)1-benzoyloxymethyl-3-α/β-(uracil-1-yl)-*cis*-2-oxabicyclo-[3.3.0]-octane (**10**) in 96% yield and an α/β ratio of 1:1.2.

Separation of the anomers by silica gel column chromatography were for both mixtures unsuccessful and as a consequence, separation was accomplished using chemical differentiation. Deprotection of the 5'-hydroxyl group by sodium methoxide in methanol gave compounds **11** and **12** in 98 and 92% yield, respectively. Converting the primary hydroxyl group to the corresponding tosylate by using *p*-toluenesulphonyl chloride in pyridine followed by refluxing in acetonitrile with added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave the 2,5'-anhydro compounds **13** and **14** in 52 and 43% yield, respectively, based on **11** and **12**. The 2,5'-anhydro compounds could easily be isolated by silica gel column chromatography. Compounds **13** and **14** were hydrolysed in 2M aqueous sodium hydroxide in dioxane to give (±)1-hydroxymethyl-3-β-(thymine-1-yl)-*cis*-2-oxabicyclo-[3.3.0]-octane (**15**) in 98% yield and (±)1-hydroxymethyl-3-β-(uracil-1-yl)-*cis*-2-oxabicyclo-[3.3.0]octane (**16**) in 78% yield. (±)1-Hydroxymethyl-3-β-(cytidine-1-yl)-*cis*-2-oxa-bicyclo[3.3.0]-octane (**17**) was prepared in 61% yield, by reacting the benzoylated **16** with triazole, phosphorus oxychloride and triethyl amine followed by methanolic ammonia at 40°C (Scheme 2).[14]

The determinations of the α/β ratios for **9** and **10** were done by ^{1}H-NMR and based on that the 5'-protons of the β-anomer showed a distinct splitting as the 5'-protons of the α-anomer appeared in a shape close to a singlet. With the exceptions of signals corresponding to the individual aglycones, the ^{1}H NMR spectra of compounds **15–17** were nearly identical.

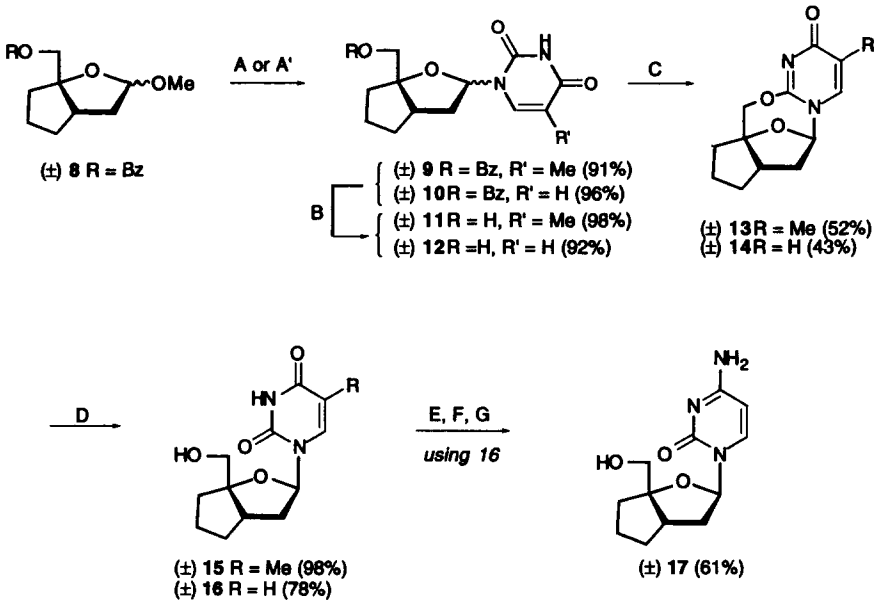
Conformational Analysis

For the purpose of detecting a possible equilibrium between different conformational states, proton NMR



A: Isopropoxydimethylsilylmethylmagnesium chloride, THF **B:** MeOH, THF, KF, $KHCO_3$, H_2O_2 **C:** BzCl, pyridine **D:**OsO_4, *N*-methylmorpholine-*N*-oxide **E:** $NaIO_4$ **F:** HCl/MeOH

Scheme 1.



A: Bismone(trimethylsilyl)thymine, CH_3CN/CH_2Cl_2, TBDMSOTf **A':** Bis(trimethylsilyl)uracil, CH_3CN/CH_2Cl_2, TMSOTf **B:** NaOMe **C:** a) TsCl, pyr. b) DBU, CH_3CN, Δ **D:** NaOH, dioxan **E:** BzCl, pyridine **F:** Triazole, $POCl_3$, Et_3N, CH_3CN **G:** $MeOH/NH_3$

Scheme 2.

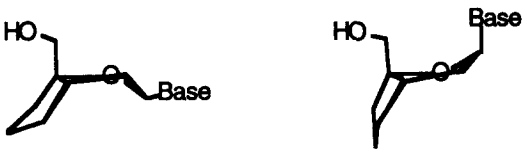
spectra (JEOL 400 MHz) of the cytidine derivative **17** were recorded in D_2O at various temperatures between 20 and 80°C. The protons in the furanose moiety are strongly coupled and the spectra were unsuitable for first-order analysis. The precise values for vicinal coupling constants ($^3J_{HH}$) and chemical shifts (δ) were obtained through simulation and iteration procedures by the geNMR program package.[15] The results are presented in Table 1. We could not find any relevant changes in coupling constants or chemical shift between the low and the high temperature spectra, indicating that no change in population between different conformers occurred under these conditions.

In order to confirm the assumed rigidity, the NMR results were also analysed using the program PSEUROT[16,17] by assuming an equilibrium mixture of two furanose conformations in order to find a best fit for the experimentally measured proton couplings. PSEUROT calculates the phase angles (*P*), puckering amplitudes (ψ) and mole fractions of the two conformers.

For the purpose of obtaining relevant starting conformations, the bicyclic cytidine derivative was analysed using the SYBYL graphics program (version 5.4; Tripos Associates, St Louis, MO) without taking electrostatic terms into account. In the first minimization series, the C-4'–C-5' bond was placed in γ (+) domain and the base was rotated in 30° steps around the glycosidic bond, from χ = −180° to χ = 180°. The 3" carbon was placed in a *exo* position. The second series differed only with respect to the C-4'–C-5' conformation, which was then placed in γ (*trans*). The same set of minimizations were repeated with the exception that the starting conformation of the 3" carbon was *endo*.

The global energy minimum obtained from SYBYL (*P*=130° and ψ=38°) were chosen as a starting point for the PSEUROT analysis, carried out assuming that only a single conformer is present, that being the conformer obtained from the minimizations. However, under these constraints, a solution having a good fit between the observed and the calculated coupling constants could not be found. As a consequence, the analysis were performed assuming a two-state equilibrium in which alternately the furanose puckering amplitudes or the phase angles of the two conformers were constrained. The minor conformer chosen was the conformer corresponding to the local minimum obtained in the minimizations (*P*=300° and ψ=42°). After several iteration cycles, the overall rms dropped to 0.06 Hz without using any constraints.

The results obtained from PSEUROT reveals a major conformer having a phase angle of 112° and a puckering amplitude of 39°; and a minor conformer having a phase angle of 286° and a puckering amplitude of 44°. The mole fraction of the major conformer was found to be 0.79 regardless of the temperature. Thus, the best description of the solution conformation of the bicyclo cytidine derivative is a mixture of two conformers consisting in one C-1-*exo* and one C-1-*endo* conformation.



The sketch above shows the two conformers as obtained from the conformational analysis. The 3" carbon is depicted as proposed by molecular modelling.

Owing to the fact that the cyclopentane ring conformation is not taken into account in this analysis and that PSEUROT is designed to assume a two-state equilibrium between two furanose conformations, it is not excluded that the bicyclic cytidine derivative exists as a more complex conformational mixture, although a reasonable correspondence to the observed hydrogen coupling constants were found for the two-state model.

Biological Results

Compounds **17–19** were tested in an *in vitro* assay[18] for HIV-1 RT inhibition and in a XTT assay[19] for anti HIV-1 and cytopathic effects. All compounds were found to be inactive in the assays.

Experimental

General methods. All solvents were destilled prior to use. Thin layer chromatography was performed using silica gel 60 F-254 (Merck) plates with detection by UV and/or charring with 8% sulphuric acid. Column chromatography was performed on silica gel (Matrix Silica Si 60A, 35–70 μ, Amicon). Organic phases were dried over anhydrous magnesium sulphate. Concentrations were performed under reduced pressure.

Table 1. Vicinal coupling constants and chemical shifts

T (°C)	δ 1'	δ 2'a	δ 2'b	δ 3'	*J* 1'2'a	*J* 1'2'b	*J* 2'a3'	*J* 2'b3'
20	6.225	2.192	2.237	2.685	8.3	5.9	8.6	2.6
35	6.216	2.181	2.240	2.677	8.3	5.9	8.6	2.5
50	6.209	2.185	2.242	2.674	8.4	6.0	8.7	2.6
65	6.202	2.182	2.245	2.671	8.4	6.0	8.6	2.7
80	6.195	2.179	2.247	2.667	8.2	6.0	8.5	2.8

NMR spectra were recorded on a JEOL GSX-270, if not otherwise stated, shifts are given in ppm downfield from tetramethylsilane in $CDCl_3$ and CD_3OD.

1-(Hydroxymethyl)-2-(2-propenyl)-cyclopentanol (6). To a stirred and cooled (0°C) solution of isopropoxydimethylsilylmagnesium chloride (16.67 g, 100 mmol) in THF (120 mL) under an atmosphere of argon was added 2-propenylcyclopentanon (9.31 g, 75 mmol) over a period of 30 min. The reaction mixture was stirred for 30 min and a cooled solution of saturated aqueous NH_4Cl (100 mL) was added dropwise. The organic layer was separated and the aqueous layer extracted with Et_2O (4 × 25 mL). The combined organic layers were washed with brine, dried and concentrated below room temperature to give 1-[(isopropoxydimethylsilyl)metyl]-2-(2-propenyl)-cyclopentanol as a clear oil. The crude oil was dissolved in a round-bottomed flask containing THF (75 mL), MeOH (75 mL), $KHCO_3$ (7.5 g, 75 mmol) and KF (8.7 g, 105 mmol). To the stirred mixture was added 30% H_2O_2 (28 mL, 248 mmol) in one portion at room temperature. The reaction mixture was stirred for 2 h. Diethylether was added and the precipitate was filtered off and washed with Et_2O (3 × 20 mL). The combined filtrate and washes were concentrated until most of the water was removed. The remaining oil was diluted with Et_2O and washed with brine. The organic layer was separated, dried and concentrated. Purification by column chromatography (toluene:ethyl acetate, 1:1) gave **5** as a clear oil (5.62 g, 36 mmol, 48%). ^{13}C NMR ($CDCl_3$, 25°C): δ 21.5 (C-4), 30.7, 34.1, 37.6, 45.5 (C-4, C-2, C-1' and C-3), 69.1 (CH_2OH), 82.2 (C-1), 115.4 (C-3'), 138.3 (C-2').

(cis)-1-(Benzoyloxymethyl)-2-(2-propenyl)-cyclopentanol (7). To an ice-cold mixture of compound **6** (9.70 g, 62.1 mmol) in pyridine (25 mL) and CH_2Cl_2 (100 mL) was added dropwise benzoyl chloride (9.13 g, 65 mmol). The reaction mixture was stirred for 45 min. Saturated $NaHCO_3$ (125 mL) was added and the organic layer was separated, concentrated, and residual volatiles were co-evaporated with added toluene. The residue was subjected to column chromatography (petroleum ether: EtOAc, 6:1) to give **7** as a clear oil (11.77 g, 45.2 mmol, 73%). ^1H NMR ($CDCl_3$, 25°C): δ 2.56 (*m*, 2H, H-4), 2.82 (*m*, 5H, H-2, H-3 and H-5), 2.38 (*m*, 2H, H-1'), 4.30 (*dd*, 2H, CH_2OBz), 5.0 (*m*, 2H, H-3'a and H-3'b), 5.83 (*m*, 1H, H-2'), 7.14–8.2 (*m*, 5H, Ar.). ^{13}C NMR ($CDCl_3$, 25°C): δ 21.5 (C-4), 30.6, 33.9, 38.8, 45.8 (C-4, C-2, C-1' and C-3), 70.8 (CH_2OBz), 80.9 (C-1), 115.6 (C-3'), 137.9 (C-2'), 166.7 (COPh).

(±)1-Benzoyloxymethyl-3-α/β-methoxy-cis-2-oxabicyclo-[3.3.0]octane (8). To an ice-cold mixture of compound **7** (11.77g, 45.2 mmol) and *N*-methylmorpholine *N*-oxide (10.6 g, 90.4 mmol) in tetrahydrofuran:water (3:1, 200 mL) was added osmium tetroxide (11.3 mL, 0.9 mmol, 2.5 wt% solution in *t*-butyl alcohol, stabilized with 1% *t*-butyl hydroperoxide), and after a few minutes, the ice-bath was removed, and the reaction mixture was stirred overnight at room temperature under argon atmosphere. Sodium hydrogen sulphite (4.4 g) was added, and the mixture was stirred for 30 min. The mixture was concentrated, and the residue was partitioned between EtOAc and 1 M HCl. The organic layer was washed with saturated aqueous $NaHCO_3$, dried and concentrated. The crude compound was dissolved in tetrahydrofuran:H_2O (3:1, 200 mL) and treated with sodium periodate (11.5 g, 54 mmol) at room temperature for 30 min. The mixture was concentrated, and the aqueous residue partitioned between saturated aqueous NaCl and EtOAc. The organic phase was dried, concentrated, and residual volatiles were co-evaporated with added toluene. The residue was treated with methanolic HCl (2.5%, w/w, 75 mL) for 15 min, neutralized using Amberlite resin IR-45 (OH), filtered and concentrated. The residue was purified by flash column chromatography (toluene) to give compound **8** as an anomeric mixture (8.9 g, 32.2 mmol, 72%). ^{13}C NMR ($CDCl_3$, 25°C): δ 23.7, 25.0, 33.3, 33.8, 36.7, 37.1, 40.3, 40.7, 43.6, 43.9, 54.4, 54.8 (C-2, C-3, C-1', C-2', C-3'), 54.4, 54.8 (OCH_3), 69.2, 70.1 (CH_2OBz), 94.3, 95.3 (C-4), 106.6, 107.3 (C-1), 128–133 (Arom.). Anal. calcd for $C_{16}H_{20}O_4$: C, 69.55; H, 7.30; Found: C, 69.40; H, 7.40.

(±) 1-Benzoyloxymethyl-3-α/β-(thymine-1-yl)-cis-2-oxabicyclo[3.3.0]-octane (9). To a cooled (0°C) solution of silylated thymine[13] (2.63 g, 9.75 mmol) in CH_2Cl_2 and acetonitrile (30 mL, 4:1), under an atmosphere of argon, was added a solution of **8** (1.8 g, 6.5 mmol) in the same solvent as above (10 mL). *t*-Butyldimethylsilyl triflate (2.1 mL, 9.1 mmol) was added and the reaction mixture was stirred for 30 min at 0°C and then at room temperature overnight. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (125 mL) and the solution was extracted with two 100 mL portions of CH_2Cl_2, dried and concentrated. The residue was subjected to column chromatography ($CHCl_3$–MeOH, gradient 1–5% MeOH) to give **9** as an anomeric mixture, α/β ratio 1:1 (2.23 g, 6.0 mmol, 93%). ^1H NMR (selected signals)($CDCl_3$, 25°C): δ 4.37 (*m*, 2H, H-5'α), 4.39 (*d*, $J_{5'-5'}$=11.8 Hz, 1H, H-5'β), 4.63 (*d*, $J_{5'-5'}$=11.8 Hz, 1H, H-5'β). Anal. calcd for $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.95; H, 6.09; N, 7.45.

(±)1-Hydroxymethyl-3-α/β-(thymine-1-yl)-cis-2-oxabicyclo[3.3.0]octane (11). Compound **9** (2.22g, 6.0 mmol) was dissolved in 50 mL MeOH and NaOMe (5 mmol, 1 M solution) was added. The reaction mixture was stirred for 20 h at room temperature when TLC indicated completed reaction. The reaction mixture was neutralized by the addition of Dowex H^+. The solution was filtered and concentrated to give a white foam which was purified by column chromatography ($CHCl_3$: MeOH, gradient 0–4% MeOH) to give **11** (1.59 g, 5.89 mmol, 98%) as a white solid. ^1H NMR (selected signals) ($CDCl_3$, 25°C): δ 3.59 (*d*, $J_{5'-5'}$=11.5 Hz, 1H, H-5'β), 3.62 (*m*, 2H, H-5'α), 3.88 (*d*, $J_{5'-5'}$=11.5 Hz, 1H, H-5'β).

β-Bicyclo-2,5'-anhydro-thymidine (13). To a solution of **11** (425 mg, 1.6 mmol) in pyridine (10 mL) was added

p-toluenesulphonyl chloride (365 mg, 1.9 mmol) and the mixture was stirred overnight at room temperature. Saturated $NaHCO_3$ (40 mL) was added and the mixture was extracted with two 25 mL portions of CH_2Cl_2, dried, concentrated and filtered through a pad of silica gel ($CHCl_3$:MeOH, 19:1). The crude product was dissolved in dry acetonitrile and DBU (135 μL, 0.96 mmol) was added. The reaction mixture was refluxed for 24 h, concentrated and purified by column chromatography ($CHCl_3$:MeOH, gradient 1–5% MeOH) to give **13** (207 mg, 0.83 mmol, 52%) (71% calculated on β-anomer). ^1H NMR ($CDCl_3$, 25°C): δ 1.41–2.13 (*m*, 9H, Thy-CH_3, H-1", H-2"and H-3"), 2.43 (*m*, 2H, H-2'), 2.85 (*m*, 1H, H-3'), 4.25 (*s*, 2H, H-5'), 5.60 (*dd*, 1H, H-1'), 7.12 (*s*, 1H, H-6). Anal. calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.89; H, 6.54; N, 11.13.

(±)1-Hydroxymethyl-3-β-(thymine-1-yl)-cis-*2-oxabicyclo-[3.3.0]octane (15).* To a solution of dioxane (5 mL) and aqueous NaOH (5 mL, 1 M) was added **13** (157 mg, 0.63 mmol) and the reaction mixture was stirred for 30 min at room temperature. After neutralisation with Dowex H^+ the mixture was filtrated and the Dowex washed twice with MeOH. Concentration of the filtrate, lyophylisation and column chromatography ($CHCl_3$: MeOH, gradient 1–4% MeOH) gave **15** as a white foam (165 mg, 0.62 mmol, 98%). ^1H NMR (CD_3OD, 25°C): δ 1.52–2.04 (*m*, 9H, Thy-CH_3, H-1", H-2" and H-3"), 2.21 (*m*, 2H, H-2'), 2.72 (*m*, 1H, H-3'), 3.59 (*d*, $J_{5'-5'}$=11.4 Hz, 1H, H-5'), 3.89 (*d*, $J_{5'-5''}$=11.5 Hz, 1H, H-5"), 6.14 (*dd*, 1H, H-1'), 7.57 (s, 1H, H-6). Anal. calcd for $C_{13}H_{18}N_2O_4$: C, 58.63; H, 6.81; N, 10.52. Found: C, 58.42; H, 6.81; N, 10.34.

(±)1-Benzoyloxymethyl-3-α/β-uracil-1-yl-cis-*2-oxabicyclo[3.3.0]octane (10).* To a cooled (0°C) solution of silylated uracil (2.56 g, 10 mmol) in CH_2Cl_2 and acetonitrile (30 mL, 4:1) under an atmosphere of argon, was added a solution of **7** (1.38 g, 5 mmol) in the same solvent as above (5 mL). Trimethylsilyl triflate (1.16 mL, 6 mmol) was added and the reaction mixture was stirred for 30 min at 0°C and then at room temperature overnight. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (100 mL) and the solution extracted with two 100 mL portions of CH_2Cl_2, dried and concentrated. The residue was subjected to column chromatography (CH_2Cl_2:MeOH, gradient 1–5% MeOH) to give **10** as an anomeric mixture, α/β ratio 1:1.2 (1.71 g, 4.8 mmol, 96%). ^1H NMR (selected signals) ($CDCl_3$, 25°C): δ 4.39 (s, 2H, H-5'-α), 4.46 (*d*, $J_{5'-5''}$=11.6 Hz, 1H, H-5'β), 4.57 (*d*, $J_{5''-5}$=11.6 Hz, 1H, H-5'β), 5.54 (*dd*, 1H, H-5), 5.80 (*dd*, 1H, H-5). Anal. calcd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.66; N, 7.86. Found: C, 63.88; H, 5.72; N, 7.71.

β-Bicyclo-2,5'-anhydro-uridine (14). Compound **10** (1.50 g, 4.2 mmol) was dissolved in 9 mL MeOH and NaOMe (1 mmol, 1 M solution) was added. The reaction mixture was stirred for 20 h at room temperature when TLC indicated uncompleted reaction and another portion of NaOMe was added (1 mmol, 1 M solution). After another 20 h, the reaction mixture was neutralised by the addition of Dowex H^+. The solution was filtered and concentrated to give a white foam which was purified by column chromatography ($CHCl_3$:MeOH, gradient 1–5% MeOH) to give **12** (0.98 g, 3.9 mmol, 92%) as a white solid.

To a solution of **12** (0.98 g, 3.9 mmol) in pyridine (10 mL) was added *p*-toluenesulphonyl chloride (0.89 g, 4.7 mmol) and the mixture was stirred overnight at room temperature. Saturated $NaHCO_3$ (100 mL) was added and the mixture was extracted with two 50 mL portions of CH_2Cl_2, dried and concentrated. The crude product was dissolved in dry acetonitrile and DBU (307 μL, 1.98 mmol) was added. The reaction mixture was refluxed for 24 h when TLC indicated approximately 50% conversion corresponding to the anomeric ratio of the β-anomer. The reaction mixture was concentrated and purified by column chromatography to give **14** (0.40 g, 1.69 mmol, 43%) (79% calculated on β-anomer). ^1H NMR ($CDCl_3$, 25°C): δ 1.42–2.45 (*m*, 8H, H-2', H-1", H-2" and H-3"), 2.86 (*m*, 1H, H-3'), 4.28 (*s*, 2H, H-5'), 5.63 (*dd*, 1H, H-5), 6.06 (*d*, 1H, H-1'), 7.28 (*d*, 1H, H-6). Anal. calcd for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.02; N, 11.96. Found: C, 62.22; H, 6.18; N, 11.56.

(±)1-Hydroxymethyl-3-β-(uracil-1-yl)-cis-*2-oxabicyclo-[3.3.0]octane (16).* To a solution of dioxane (5 mL) and aqueous NaOH (5 mL, 1 M) was added **12** (0.40 g, 1.69 mmol) and the reaction mixture was stirred for 30 min at room temperature. After neutralisation with Dowex H^+ the mixture was filtrated and the Dowex washed twice with methanol. Concentration of the filtrate, lyophylization and column chromatography ($CHCl_3$: MeOH, gradient 1–4% MeOH) gave **16** as a white solid (332 mg, 1.32 mmol, 78%). ^1H NMR (CD_3OD, 25°C): δ 1.54–2.18 (*m*, 8H, H-2', H-1", H-2" and H-3"), 2.69 (*m*, 1H, H-3'), 3.55 (*d*, $J_{5'-5'}$=11.7 Hz, 1H, H-5'), 3.76 (*d*, $J_{5'-5}$=11.7 Hz, 1H, H-5'), 5.67 (*dd*, 1H, H-5), 6.17 (*t*, 1H, H-1'), 8.08 (*d*, 1H, H-6). Anal. calcd for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.13; H, 6.42; N, 10.90.

(±)1-Hydroxymethyl-3-β-(cytidine-1-yl)-cis-*2-oxa-bicyclo[3.3.0]octane (17).* To a solution of **16** (421 mg, 1.67 mmol) in pyridine (10 mL) was added benzoyl chloride (230 μL, 2.0 mmol) and the reaction mixture was stirred for 2 h at room temperature. The mixture was partitioned between $CHCl_2$ and saturated aqueous $NaHCO_3$. The organic layer was separated and concentrated. Toluene (50 mL) was added and the solution was dried, filtered and concentrated. The remaining pyridine was removed by co-evaporation with added toluene to give (±) 1-hydroxymethyl-3-β-(cytidine-1-yl)-*cis*-2-oxabicyclo[3.3.0]octane (600 mg, 99%) after purification by column chromatography ($CHCl_3$:MeOH, gradient 0–2% MeOH).

This material (400 mg, 1.12 mmol) was dissolved in triethylamine (1.56 mL, 11.2 mmol) and acetonitrile (10 mL) and added to a stirred and cooled (0°C) mixture of triazole (814 mg, 11.8 mmol) and phosphorous oxychloride (379 mg, 2.47 mmol) in acetonitrile (8

mL). The reaction mixture was allowed to rise to room temperature and then stirred for 2 h. Triethylamine (1.01 mL) and H_2O (510 μL) were added and the reaction mixture was concentrated. The residue was portioned between CH_2Cl_2 and H_2O and the organic layer separated, dried and concentrated. The residue was dissolved in MeOH saturated with NH_3 and stirred at 40°C for 48 h to give **17** (173 mg, 0.69 mmol, 61%) after column chromatography ($CHCl_3$:MeOH, 5:1). ^1H NMR ($CDCl_3$, 25°C): δ 1.49–2.23 (*m*, 8H, H-2', H-1", H-2", H-3"), 2.64 (*m*, 1H, H-3'), 3.52 (*d*, $J_{5'-5''}$=11.7 Hz, 1H, H-5'), 3.72 (*d*, $J_{5'-5''}$=11.7 Hz, 1H, H-5'), 5.88 (*d*, 1H, H-5), 6.17 (*dd*, 1H, H-1'), 8.08 (*d*, 1H, H-6). Anal. calcd for $C_{12}H_{17}N_3O_3$: C, 57.36; H, 6.82; N, 16.72. Found: C, 57.24; H, 6.79; N, 16.68.

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