PERKIN 1

Synthesis and anti-HIV-1 activity of novel bicyclic nucleoside analogues restricted to an *S*-type conformation

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(1*S*,3*R*,4*S*)-3-Hydroxymethyl-1-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane **9** and the corresponding cytosine derivative **10**, nucleoside analogues with a novel bicyclic nucleoside structure **3**, were synthesized in a few steps from the known 1-(3′-deoxy-β-D-psicofuranosyl)uracil **4**. NOE experiments verified the bicyclic nucleosides to be restricted to the expected *S*-type furanose conformation while the nucleobase is in an *anti*-conformation. Both nucleosides proved to be devoid of anti-HIV activity in MT-4 cells, which further supports the hypothesis that conformational flexibility of the furanose ring in a nucleoside analogue is necessary to obtain both intracellular 5′-triphosphorylation and inhibition of HIV-1 reverse transcriptase.

Introduction

The synthesis and evaluation of novel nucleoside analogues, which, as their triphosphates, can act as inhibitors of HIV 1 reverse transcriptase (HIV-1 RT), continue to be of immense importance in AIDS therapy.[1–3] 3′-Azido-3′-deoxythymidine (AZT) was the first nucleoside analogue to show effective inhibition of HIV-1 RT[4] and, together with another five nucleoside analogues, AZT is at present approved by the United States Food and Drug Administration (FDA) for use in the treatment of AIDS.[5,6] To interact successfully with HIV-1 RT, the nucleoside analogue has to be triphosphorylated in the 5′-position, which also appears to be the rate-limiting intracellular step since different known HIV-1 RT inhibitors showed a similar inhibitory effect when triphosphorylated *in vitro*.[7] When triphosphorylated, the nucleoside analogue acts either as a competitive inhibitor (*i.e.*, prevents chain incorporation of natural substrates) or as a substrate, thus causing viral DNA chain termination since no 3′-hydroxy group is present for chain elongation.[8]

The conformation of the furanose ring as described by the pseudorotation cycle[9] seems to be of major importance for anti-HIV activity. It has been proposed that the preference of AZT for an extreme *S*-type ($_3E$) conformation is responsible for both its efficient triphosphorylation and potent anti-HIV activity,[10,11] while later NMR studies of AZT and thymidine triphosphates when actually bound to HIV-1 RT revealed both nucleosides to adopt an *N*-type ($_4E$) conformation.[12] Marquez and co-workers have recently reported the synthesis of two conformationally restricted carbocyclic nucleoside analogues **1** and **2** (Fig. 1) locked into an *N*-type ($_2E$) and an *S*-type ($_3E$) conformation, respectively, and demonstrated that neither of them showed anti-HIV activity. However, when converted *in vitro* to their 5′-triphosphates, the triphosphate of the *N*-type analogue **1** showed an inhibitory effect similar to that of AZT 5′-triphosphate. These results were generalized to the hypothesis that an *S*-type conformation is necessary for triphosphorylation while an *N*-type conformation is required for successful interaction with HIV-1 RT.[13]

However, the potential activity of a nucleoside analogue restricted to an *S*-type conformation can still not be completely refuted, since conformational analysis of **2** by X-ray and NMR studies proved the nucleobase to exist in an unusual *syn*-conformation[13] which might be unfavourable for both intracellular triphosphorylation and/or HIV-1 RT inhibition. We therefore decided to further investigate the above-mentioned hypothesis proposed by Marquez *et al.* by synthesis of bicyclic pyrimidine furanosides of the type **3** (Fig. 1) expected to be restricted entirely to an *S*-type conformation by the additional 3′,1′-oxymethylene bridge. An additional desirable property of **3** is its presumed stability towards hydrolysis of the C–N glycosylic bond, since formation of a planar oxonium ion is unfavourable at the bridgehead in such a small bicyclic system.

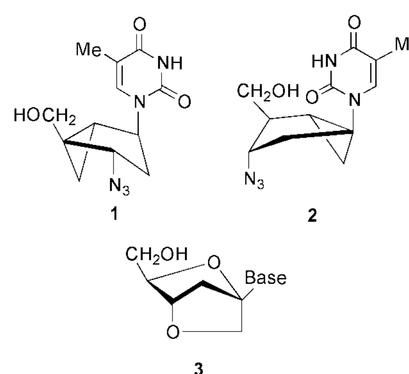

Fig. 1 Structures of conformationally restricted bicyclic nucleosides. **1** is restricted to an *N*-type whilst **2** and **3** are restricted to an *S*-type conformation.

Results and discussion

Starting from the known 1-(3′-deoxy-β-D-psicofuranosyl)-uracil **4**[14,15] (Scheme 1), selective protection of the 6′-hydroxy group was a significant obstacle in the synthesis. Even though 4′,6′-*O*-1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) protection of **4** has been reported in 53% yield,[15] later authors were only able to reproduce this reaction in 24–26% yield.[16] Alternatively, 4,4′-dimethoxytrityl (DMT) protection of the thymine analogue of **4** was reported to occur selectively at the 6′-position in 56% yield,[17] in an approach which also left the secondary hydroxy group at C-4′ unprotected. Triol **4** showed only a moderate selectivity when treated with various amounts of DMTCl in a pyridine–dichloromethane (DCM) mixture at

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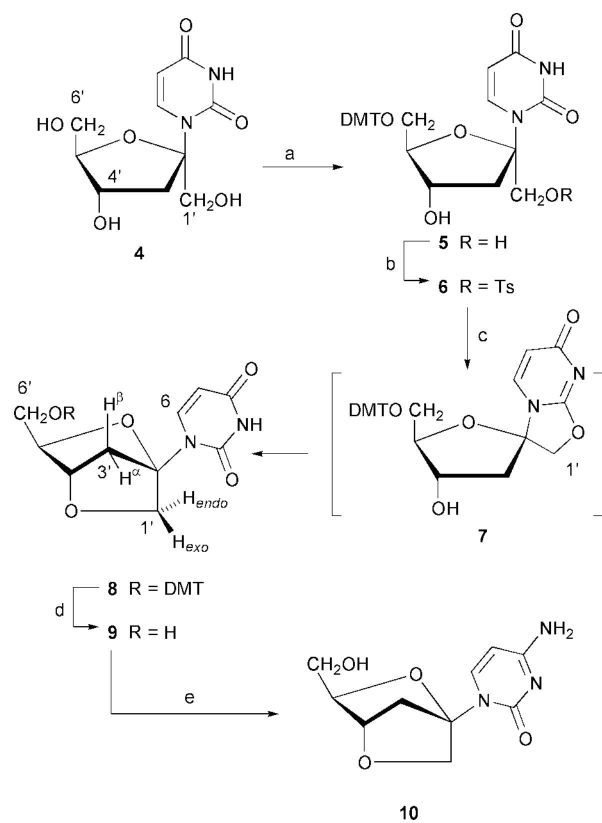
0 °C, and the highest yield obtained of the desired 6′-*O*-DMT-protected isomer **5** was 37%, using 1.1 mole equivalent of DMTCl. Surprisingly, when treating **4** with more than two mole equivalents of DMTCl, even the secondary hydroxy group reacted to afford the tris-DMT-protected isomer in considerable yields. This result was the more surprising since the subsequent selective 1′-tosylation of **5** using 3 mole equivalents of TsCl in a pyridine–dichloromethane mixture at room temperature furnished the monotosyl derivative **6** in 77% yield. Cyclization to the bicyclic structure **8** was accomplished in 93% yield by treatment with excess of sodium hydride in anhydrous DMF for 3 days. The reaction was shown to proceed *via* the 2,1′-anhydronucleoside **7**, since the latter was the main product, isolable in 41% yield, after reaction overnight.[18] The structure of **7** was supported by NMR data; in particular, the signals for C-2 and C-4 (δ_C 173.1 and 160.4) were similar in chemical shift to the values we observed (δ 170.6 and 159.1) for 2,3′-anhydro-1-[1,4,6-tri-*O*-benzoyl-β-D-fructofuranosyl]uracil,[14] an intermediate in the synthesis of **4**, but more different from the values typically observed for normal uracil nucleosides ($\delta \approx 165$ and 151). Additionally, the signals for the diastereotopic protons at C-1′ resonated at δ 4.74 and 5.20, deshielded relative to the equivalent signals (δ 4.15 and 4.20) in the spectrum of **8**. Removal of the DMT protecting group from **8** proceeded in 91% yield using 80% AcOH to give the final uracil analogue **9** as the first nucleoside of the bicyclic structure **3**. Derivatization to afford the cytosine analogue **10** *via* a 4-*O*-*p*-nitrophenyl intermediate was accomplished by a one-pot method reported by Reese and co-workers.[19] Thus, nucleoside **9** was successively treated with trimethylsilyl chloride, triflic anhydride and *p*-nitrophenol in a mixture of *N*-methylpyrrolidine and acetonitrile, followed by conc. ammonia in 1,4-dioxane at 50 °C, to give **10** in 80% yield (Scheme 1).

Conformational analysis of the nucleoside analogues **8**–**10** by NOE spectroscopy supported the assumption of a rigid bicyclic structure in which the furanose ring was locked in an *S*-type conformation. NOE difference spectroscopy experiments on **8** revealed one of the hydrogen atoms at C-3′ to be close to 6-H (an NOE of 3% after irradiation of the 3′-H) and to one of the hydrogen atoms at C-6′ (NOEs of 2% for both irradiations). NOESY spectra of **9** and **10** also confirmed the proximity of 3′β-H and the protons at C-6′, whilst 3′α-H interacted with 1′-*exo*-H. HMBC (Heteronuclear Multiple-Bond Connectivity) spectra of **9** and **10** showing two- and three-bond H–C correlations were another verification of the expected cyclization since the pairs of atoms 1′-H and C-4′, and 4′-H and C-1′, appeared in each case to be maximally three bonds apart. The absence of any coupling constant between 4′-H and 5′-H in compounds **8**–**10** also indicated the conformations of the furanose rings to be exclusively *S*-type, even though caution must be taken since this description does not allow for deviations from the *N*/*S* two-state model.[20]

In contrast to the *syn* conformation of the carbocyclic nucleoside **2** (Fig. 1), the nucleobases of **9** and **10** were shown to adopt *anti* conformations, since significant NOE interactions were observed in each case between 3′β-H and 6-H. Structural deviations between **3** and naturally occurring nucleosides should thus be minimized. Both the uracil and the cytosine nucleoside analogues, **9** and **10** respectively, were tested for antiviral activity against HIV-1 in MT-4 cells (refer to Experimental section for further details) but the results showed both compounds to be completely devoid of anti-HIV activity. This result further supports the hypothesis of Marquez and co-workers that a flexible furanose conformation is required to obtain both efficient triphosphorylation (*S*-type) and inhibition of HIV-1 Reverse Transcriptase (*N*-type).[13]



Scheme 1 *Reagents and conditions* (*and yields*): (a) DMTCl (1.1 equiv.), pyridine, DCM (37%); (b) TsCl, pyridine, DCM (77%); (c) NaH, DMF, 17 h (41% **7**) or 3 days (93% **8**); (d) 80% AcOH (91%); (e) (i) TMSCl, *N*-methylpyrrolidine, acetonitrile; (ii) Tf_2O, 0 °C; (iii) *p*-nitrophenol; (iv) NH_3, 1,4-dioxane, 50 °C (80%).

Conclusions

In summary, a successful synthesis of the novel conformationally restricted bicyclic nucleosides **9** and **10** has been accomplished. Both were shown by NOE experiments to be confined to the expected *S*-type furanose conformation while the base adopted an *anti* conformation as also seen for naturally occurring nucleosides. Their complete lack of anti-HIV activity provides further support to the theory proposed by Marquez *et al.*[13] about conformational flexibility of the furanose ring being essential to obtain both efficient triphosphorylation and HIV-1 RT inhibition.

Experimental

All reagents were obtained from commercial suppliers and were used without further purification. Light petroleum of distillation range 40–60 °C was used and the silica gel (0.035–0.070 mm) used for column chromatography was purchased from Fischer Chemicals. After column chromatography, fractions containing product were pooled, evaporated under reduced pressure, and the residue was maintained overnight under high vacuum to give the product. NMR spectra were recorded on Bruker WH 400 or 200 SY spectrometers. ^1H Spectra were obtained at 400 MHz and ^{13}C spectra at 100 MHz, with assignments based on 2D experiments, unless otherwise stated. Coupling constants (*J*) are measured in Hz. Assignments are in accordance with standard carbohydrate/nucleoside nomenclature (*i.e.* the furanose skeleton numbered 1′ to 6′) even though the systematic compound names are given according to IUPAC nomenclature. Fast-atom bombardment and electrospray mass spectra (FAB-MS and ES-MS, respectively) were recorded in positive-ion mode.

1-[3′-Deoxy-6′-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]-uracil 5

A solution of triol **4** (839 mg, 3.25 mmol) in anhydrous pyridine (50 ml) was concentrated to *ca.* half volume under reduced

pressure, anhydrous DCM (30 ml) was added and the mixture was cooled to 0 °C. 4,4′-Dimethoxytrityl chloride (DMTCl) (1.215 g, 3.58 mmol) was added under an atmosphere of nitrogen and, after 4 h at 0 °C, the mixture was allowed slowly to warm to room temperature. After 21 h, MeOH (4 ml) and DCM (100 ml) were added and the mixture was washed successively with saturated aq. $NaHCO_3$ (40 ml) and water (40 ml). The combined organic phase was evaporated under reduced pressure, coevaporated with anhydrous acetonitrile (2 × 30 ml) and the residue was subjected to silica gel column chromatography (8.8 × 5.5 cm) using a gradient of pyridine–MeOH–DCM (0.5 : 0.5–4 : 99–95.5 v/v/v) as eluent to give dimethoxytrityl derivative **5** (673 mg, 37%) as a yellowish foam, R_f 0.45 (MeOH–DCM 1 : 9), after coevaporation with acetonitrile (6 × 15 ml) and DCM (2 × 15 ml); ES-MS *m/z* 561 $[M + H]^+$, 583 $[M + Na]^+$; FAB-MS *m/z* 560 $[M]^+$; δ_H ($CDCl_3$) 9.87 (1H, br s, NH), 7.86 (1H, d, *J* 8.2, 6-H), 7.32–7.15 (9H, m, DMT), 6.79 (4H, dd, *J* 1.3, 8.8, DMT), 5.47 (1H, d, *J* 8.2, 5-H), 4.45 (1H, m, 5′-H), 4.35 (2H, br s, OH), 4.22 (1H, br s, 4′-H), 4.06 (1H, d, *J* 11.2, 1′-H^a), 3.76 (6H, s, OMe), 3.58 (1H, d, *J* 11.2, 1′-H^b), 3.15 (1H, dd, *J* 4.2, 10.6, 6′-H^a), 3.05 (1H, dd, *J* 5.6, 10.4, 6′-H^b), 2.82 (1H, d, *J* 15.2, 3′-H^a), 2.71 (1H, dd, *J* 5.6, 15.2, 3′-H^b); δ_C ($CDCl_3$) 163.89 (C-4), 158.47 (DMT), 150.38 (C-2), 144.10 (DMT), 140.94 (C-6), 135.13, 135.11, 129.78, 129.75, 127.78, 127.70, 126.91, 113.08 (DMT), 100.98 (C-5), 100.10 (C-2′), 89.40 (C-5′), 86.74 (Ar_2PhC), 72.93 (C-4′), 64.98 (C-1′), 63.25 (C-6′), 55.11 (OMe), 44.08 (C-3′).

1-[3′-Deoxy-6′-*O*-(4,4′-dimethoxytrityl)-1′-*O*-(*p*-tolylsulfonyl)-β-D-psicofuranosyl]uracil 6

The diol **5** (443 mg, 0.790 mmol) was dissolved in anhydrous pyridine (15 ml) and the solution was concentrated to *ca.* half volume under reduced pressure. Anhydrous DCM (25 ml) was added and the mixture was cooled to 0 °C. Toluene-*p*-sulfonyl chloride (447 mg, 2.34 mmol) was added and the mixture was stirred at room temperature under an atmosphere of nitrogen for 2 days. EtOAc (100 ml) was added and the mixture was washed successively with saturated aq. $NaHCO_3$ (25 ml) and water (2 × 25 ml). The organic phase was evaporated to dryness under reduced pressure, coevaporated with acetonitrile (2 × 40 ml) and the residue was subjected to silica gel column chromatography (12.5 × 3.3 cm) using a gradient of pyridine–MeOH–DCM (0.5 : 1–2 : 98.5–97.5 v/v/v) as eluent to give the tosyl ester **6** (432 mg, 76.5%) as a yellowish foam, R_f (EtOAc–DCM, 3 : 1) 0.50, after coevaporation with acetonitrile (5 × 15 ml) and DCM (2 × 15 ml); ES-MS *m/z* 737 $[M + Na]^+$; FAB-MS *m/z* 714 $[M]^+$; δ_H ($CDCl_3$) 8.78 (1H, d, *J* 1.9, NH), 7.73 (1H, d, *J* 8.3, 6-H), 7.70 (2H, d, *J* 8.6, Ts), 7.30–7.17 (11H, m, DMT, Ts), 6.79 (4H, d, *J* 9.0, DMT), 5.48 (1H, dd, *J* 2.3, 8.3, 5-H), 4.55 (1H, d, *J* 10.7, 1′-H^a), 4.43 (1H, d, *J* 10.7, 1′-H^b), 4.30 (1H, br s, 4′-H), 4.28 (1H, m, 5′-H), 3.76 (6H, s, OMe), 3.21 (1H, dd, *J* 4.1, 10.7, 6′-H^a), 3.15 (1H, dd, *J* 4.4, 10.7, 6′-H^b), 2.74 (1H, dd, *J* 5.8, 15.3, 3′-H^a), 2.58 (1H, dd, *J* 1.8, 15.1, 3′-H^b), 2.41 (3H, s, Ts), 2.32 (1H, d, *J* 3.4, OH); δ_C ($CDCl_3$) 163.41 (C-4), 158.67 (DMT), 149.77 (C-2), 145.35 (Ts), 144.19 (DMT), 141.29 (C-6), 135.20, 135.14 (DMT), 132.51 (Ts), 129.91, 129.14, 127.94, 127.85, 127.78, 127.09 (DMT, Ts), 113.2 (DMT), 100.86 (C-5), 96.96 (C-2′), 88.67 (C-5′), 86.87 (Ar_2PhC), 72.54 (C-4′), 70.70 (C-1′), 63.01 (C-6′), 55.25 (OMe), 44.56 (C-3′), 21.66 (Ts).

2,1′-Anhydro-1-[3′-deoxy-6′-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]uracil 7

A solution of sulfonate **6** (240 mg, 0.336 mmol) in anhydrous DMF (3 ml) was added to a suspension of NaH [60% (w/w); 45 mg, 1.13 mmol] in anhydrous DMF (5 ml) at 0 °C under a nitrogen atmosphere. After stirring of the mixture at room temperature for 19 h, water (25 ml) and saturated aq. $NaHCO_3$ (10 ml) were added and the mixture was extracted with EtOAc (40 + 3 × 20 ml). The combined organic phase was washed successively with saturated aq. $NaHCO_3$ (20 ml) and water (20 ml), evaporated to dryness under reduced pressure and coevaporated with anhydrous acetonitrile (2 × 20 ml). The residue was purified by silica gel column chromatography (9 × 3.3 cm) and elution with a gradient of pyridine–MeOH–DCM (0.5 : 2–6 : 97.5–93.5 v/v/v) to give anhydronucleoside **7** (74 mg, 41%) as a light brownish foam, R_f 0.29 (MeOH–DCM 7 : 93) after coevaporation with anhydrous acetonitrile (4 × 15 ml); ES-MS *m/z* 543 $[M + H]^+$, 565 $[M + Na]^+$, 606 $[M + CH_3CN + Na]^+$; FAB-MS *m/z* 543 $[M + H]^+$; δ_H ($CDCl_3$; 200 MHz) 7.40–7.20 (10H, m, DMT, 6-H), 6.85 (4H, d, DMT), 5.68 (1H, d, *J* 7.5, 5-H), 5.28 (1H, br s, 4′- or 5′-H), 5.20 (1H, d, *J* 10.5, 1′-H^a), 4.85 (1H, m, 5′- or 4′-H), 4.74 (1H, d, *J* 10.5, 1′-H^b), 4.30 (1H, br s, OH), 3.80 (6H, s, OMe), 3.45 (1H, dd, *J* 3.0, 10.5, 6′-H^a), 3.31 (1H, dd, *J* 2.5, 10.5, 6′-H^b), 2.79 (1H, dd, *J* 4.3, 14.0, 3′-H^a), 2.70 (1H, br d, *J* 14, 3′-H^b); δ_C [CD_3OD–$CDCl_3$ (1 : 1 v/v); 50 MHz] 173.09 (C-2), 160.37 (C-4), 158.51 (DMT), 143.78 (DMT), 134.96, 134.68, 134.38 (DMT, C-6), 130.00, 128.04, 127.82, 127.07, 113.07 (DMT), 109.40 (C-5), 98.21 (C-2′), 87.67 (C-5′), 87.00 (Ar_2PhC), 78.80 (C-1′), 71.68 (C-4′), 63.63 (C-6′), 55.02 (OMe), 43.01 (C-3′).

(1*S*,3*R*,4*S*)-3-(4,4′-Dimethoxytrityloxymethyl)-1-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane 8

To a solution of sulfonate **6** (277 mg, 0.388 mmol) in anhydrous DMF (10 ml) at 0 °C under a nitrogen atmosphere was added NaH [60% (w/w); 62 mg, 1.55 mmol] and the mixture was stirred at room temperature for 3 days. EtOAc (100 ml) was added, the mixture was washed with water (2 × 30 ml) and the organic phase was evaporated to dryness under reduced pressure and coevaporated successively with acetonitrile (50 ml) and *n*-hexane (2 × 50 ml). The residue was purified by silica gel column chromatography (11 × 3.3 cm) with a gradient of pyridine–MeOH–DCM (0.5 : 0.5–2 : 99–97.5 v/v/v) as eluent to give the bicyclonucleoside **8** (196 mg, 93.3%) as a light yellowish foam, R_f 0.49 (MeOH–DCM 7 : 93), after coevaporation with anhydrous acetonitrile (4 × 15 ml), *n*-hexane–DCM (1 : 4; 2 × 15 ml) and DCM (2 × 15 ml); *m/z* (FAB) 542 $[M]^+$, 543 $[M + H]^+$, 565 $[M + Na]^+$; Found: *m/z* (FAB) 542.20762 (M^+), 543.21302 (MH^+). Calc. for $C_{31}H_{30}N_2O_7$: *M*, 542.20530. Calc. for $C_{31}H_{31}N_2O_7$: *m/z*, 543.21313; δ_H ($CDCl_3$) 9.14 (1H, br s, NH), 7.55 (1H, d, *J* 8.2 , 6-H), 7.40–7.20 (9H, m, DMT), 6.84–6.81 (4H, m, DMT), 5.73 (1H, d, *J* 8.2, 5-H), 4.48 (1H, d, *J* 2.2, 4′-H), 4.35 (1H, dd, *J* 4.6, 6.0, 5′-H), 4.20 (1H, d, *J* 7.9, 1′-H^a), 4.15 (1H, d, *J* 7.7, 1′-H^b), 3.78 (6H, s, OMe), 3.21 (1H, dd, *J* 4.5, 10.4, 6′-H^a), 3.03 (1H, dd, *J* 6.4, 10.3, 6′-H^b), 2.57 (1H, dd, *J* 2.3, 10.0, 3′-H^a), 2.22 (1H, d, *J* 10.1, 3′-H^b); δ_C ($CDCl_3$) 162.85 (C-4), 158.45 (DMT), 149.38 (C-2), 144.23 (DMT), 141.46 (C-6), 135.41, 135.36, 129.84, 127.92, 127.75, 126.83, 113.05 (DMT), 102.49 (C-5), 95.76 (C-2′), 86.40 (Ar_2PhC), 83.92 (C-5′), 77.67 (C-4′), 72.97 (C-1′), 63.40 (C-6′), 55.10 (OMe), 37.19 (C-3′).

(1*S*,3*R*,4*S*)-3-Hydroxymethyl-1-(uracil-1-yl)-2,5-dioxabicyclo-[2.2.1]heptane 9

A solution of the dimethoxytrityl derivative **8** (101 mg, 0.186 mmol) in AcOH–water (4 : 1; 5 ml) was stirred for 40 min, after which the solution was evaporated to dryness under reduced pressure and the residue was coevaporated with anhydrous toluene (2 × 10 ml). The residue was purified by silica gel column chromatography (7.3 × 3.3 cm) with MeOH–DCM (1 : 19 to 1 : 9) as eluent to give the diol **9** (41 mg, 91%) as a white solid, R_f 0.32 (MeOH in DCM, 1 : 9); *m/z* (NH_3 CI) 241 $[M + H]^+$, 258 $[M + NH_4]^+$; *m/z* (EI, perfluorotributylamine reference) 240.074597 (M^+). Calc. for $C_{10}H_{12}N_2O_5$: *M*, 240.074622; δ_H (CD_3OD–$CDCl_3$ 1 : 1) 7.79 (1H, d, *J* 8.2, 6-H), 5.72 (1H, d, *J* 8.2, 5-H), 4.51 (1H, d, $J_{4',3'\alpha}$ 1.8, 4′-H), 4.33 (1H, d, *J* 7.9, 1′-*endo*-H), 4.18 (1H, dd, *J* 4.7, 6.1, 5′-H), 4.14 (1H, d, *J* 7.9, 1′-*exo*-H), 3.56 (1H, dd, *J* 4.7, 12.1, 6′-H^a), 3.49 (1H, dd, *J* 6.1,

12.1, 6′-H[b]), 2.47 (1H, d, J 10.0, 3′β-H), 2.43 (1H, dd, J 2.3, 10.0, 3′α-H); δ_C (CD_3OD–$CDCl_3$ 1:1) 165.63 (C-4), 151.39 (C-2), 143.91 (C-6), 102.92 (C-5), 97.19 (C-2′), 86.48 (C-5′), 78.35 (C-4′), 74.16 (C-1′), 62.66 (C-6′), 37.87 (C-3′).

(1*S*,3*R*,4*S*)-1-(Cytosin-1-yl)-3-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane 10

The uracil derivative **9** (35 mg, 0.146 mmol) was dissolved in anhydrous acetonitrile (3 ml) containing *N*-methylpyrrolidine (0.5 ml, 4.79 mmol) under a nitrogen atmosphere and trimethylsilyl chloride (0.10 ml, 0.79 mmol) was added. After 1 h, the reaction mixture was cooled to 0 °C and triflic anhydride (0.10 ml, 0.59 mmol) was added. After 25 min, additional triflic anhydride (0.10 ml, 0.59 mmol) was added and, after a total of 70 min, *p*-nitrophenol (110 mg, 0.79 mmol) was added and the mixture was stirred at room temperature for 4.5 h. Saturated aq. $NaHCO_3$ (10 ml) was added and the mixture was extracted with DCM (3 × 10 ml). The combined organic phase was evaporated to dryness under reduced pressure and coevaporated with anhydrous acetonitrile (2 × 10 ml). The residue was dissolved in 1,4-dioxane (5 ml) containing conc. aq. ammonia (1.5 ml) and the solution was heated in a sealed flask at 50 °C for 24 h. The mixture was evaporated to dryness under reduced pressure, coevaporated with abs. EtOH (4 × 5 ml) and anhydrous acetonitrile (10 ml), and the residue was purified by silica gel column chromatography (11.5 × 2 cm) and elution with MeOH–DCM (1:9 to 1:4) to give the cytosine analogue **10** (28 mg, 80%) as a light brownish solid, R_f 0.25 (MeOH–DCM 1:4); *m*/*z* (FAB, NBA + PEG300 matrix) 240.0988 (MH^+). Calc. for $C_{10}H_{14}N_3O_4$: *m*/*z*, 240.0984; δ_H (CD_3OD) 7.76 (1H, d, J 7.6, 6-H), 5.84 (1H, d, J 7.6, 5-H), 4.40 (1H, d, $J_{4',3'\alpha}$ 2.1, 4′-H), 4.21 (1H, d, J 7.7, 1′-*endo*-H), 4.06 (1H, t, $J \approx 5.5$, 5′-H), 4.05 (1H, d, J 7.8, 1′-*exo*-H), 3.44 (1H, dd, J 4.9, 12.0, 6′-H[a]), 3.38 (1H, dd, J 6.2, 12.0, 6′-H[b]), 2.39 (1H, dd, J 2.4, 10.0, 3′α-H), 2.29 (1H, d, J 10.0, 3′β-H); δ_C (CD_3OD) 167.51 (C-4), 158.16 (C-2), 144.92 (C-6), 98.15 (C-2′), 96.52 (C-5), 86.85 (C-5′), 78.66 (C-4′), 74.44 (C-1′), 63.03 (C-6′), 38.04 (C-3′).

Virus and cells

The HIV-1 strain HTLV-IIIB[21] was propagated in H9 cells[22] at 37 °C in air containing 5% CO_2, using RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (growth medium). The culture supernatant was filtered (0.45 nm), aliquotted and stored at −80 °C until use.

Inhibition of HIV-1 replication

Compounds were examined for possible antiviral activity against HIV-1 using MT-4 cells as target cells. For screening studies, MT-4 cells were incubated with virus [0.005 multiplicity of infection (MOI)] for 2 h, washed and thereafter added in the ratio of 1:10 to uninfected cells, which had been pre-incubated in growth medium containing the test compound for 2 h. Cultures were maintained with the test compound for 6 days in parallel with virus-infected control cultures without compound added. Expression of HIV in the culture medium was quantitated by HIV-1 antigen detection ELISA.[23] Compounds mediating less than 30% reduction of antigen expression were considered without biological activity.

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