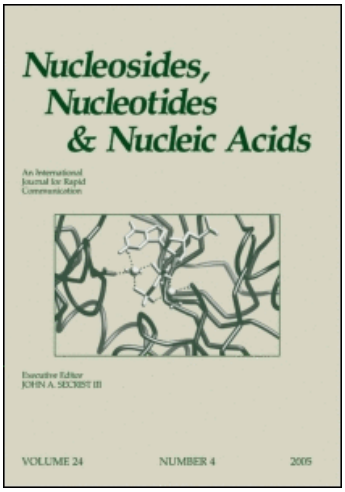

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SYNTHESIS OF SIX-MEMBERED NUCLEOSIDE ANALOGS. PART 1: PYRIMIDINE NUCLEOSIDES BASED ON THE 1,3-DIOXANE RING SYSTEM

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ABSTRACT: The synthesis of pyrimidine nucleosides, *cis-N*-1-[(2-hydroxymethyl)-1,3-dioxan-5-yl]uracil (**4**) *cis-N*-1-[(2-hydroxymethyl)-1,3-dioxan-5-yl]thymine (**5**) and *cis-N*-1-[(2-hydroxymethyl)-1,3-dioxan-5-yl]cytosine (**6**) and their corresponding *trans* isomers is described. Compound **4** showed modest, selective activity against human immunodeficiency virus in acutely infected primary human lymphocytes.

The success of antiviral drugs such as 3'-azido-3'-deoxythymidine (AZT)[1] and 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir)[2] has stimulated enormous interest in the field of nucleoside chemistry.[3] The last decade has seen the synthesis of a plethora of both sugar and aglycone modified nucleosides resulting in the discovery of several clinically useful compounds with activity against human immunodeficiency virus (HIV) and hepatitis B virus (HBV). Some of the most promising new candidates are encompassed within a group of nucleoside derivatives in which the 2-deoxyribose sugar is replaced by an oxathiolane or a dioxolane ring, e.g., (-)-β–(2*R*,5*S*)-1-[2-(hydroxymethyl)oxathiolan-5-yl]cytosine (3TC, **1**)[4], (-)-β–(2*R*,5*S*)-1-[2-(hydroxymethyl)oxathiolan-5-yl]-5-fluorocytosine [(-)-FTC, **2**][5] and (+)-β–(2*R*,5*R*)-9-[2-(hydroxymethyl)dioxolan-5-yl]guanine (DXG, **3**).[6] These and other related 2',3'-dideoxynucleosides possess activity against HIV and HBV *in vitro* and *in vivo*. Their triphosphates are potent and selective inhibitors

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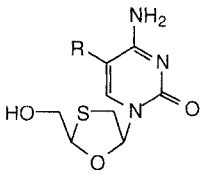
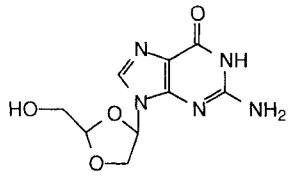
of the respective viral polymerases and act as viral DNA chain terminators.



1; R = H
2; R = F



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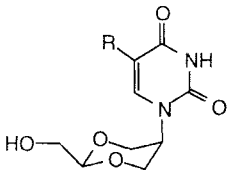
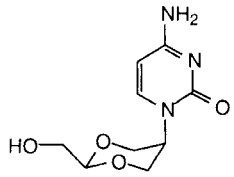


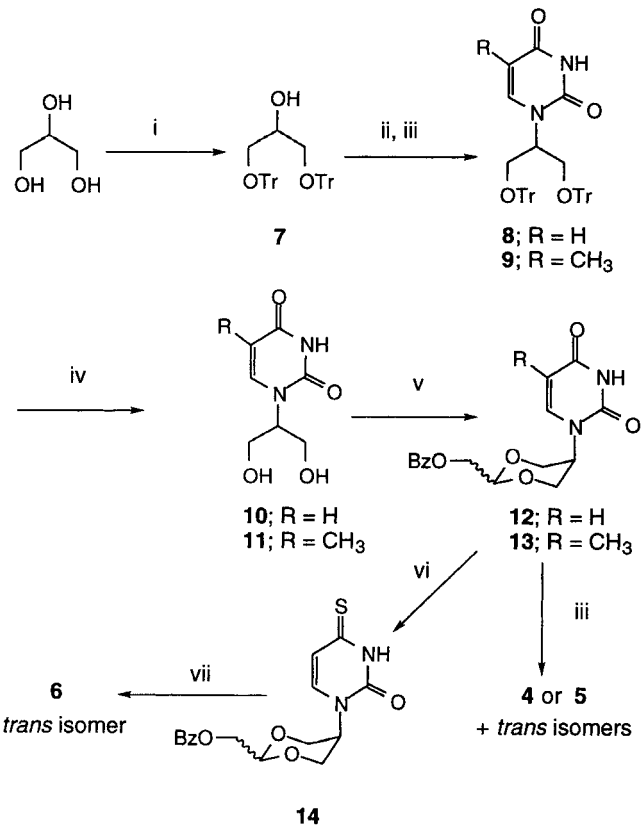
4; R = H
5; R = CH_3



6

As part of our ongoing efforts in the field of antiviral nucleoside chemistry the synthesis of a new class of pyrimidine nucleoside derivatives based on the 1,3-dioxane ring system (compounds **4-6**) is described. These compounds can be thought of as 6-membered analogs[8] of the above mentioned oxathiolane and dioxolane nucleosides without the potential issue of stereoisomerism. Although the vast majority of active nucleoside analogs have a *cis* relationship between the 5'-hydroxy (or equivalent) functionality and the base, a method of synthesis resulting in a mixture of *cis* and *trans* 2,5-substituted 1,3-dioxanes was developed since it should permit a fuller evaluation of any structure-activity relationship present in this new class of nucleosides.

We envisaged that the dioxane nucleosides **4-6**, could be accessed expediently from the bis(hydroxymethyl)methylthymine and uracil derivatives. There have been several recent reports that alkylation of heterocyclic bases (specifically nucleoside aglycones) can be achieved under Mitsunobu conditions.[9] Coupling of a protected uracil or thymine moiety with a suitably protected glycerol would lead directly to the desired intermediates. To this end, condensation of easily obtained 1,3-di-*O*-tritylglycerol[10] (**7**) with either *N*-3-benzoyluracil or *N*-3-benzoylthymine[11] in the presence of DEAD and triphenylphosphine (TPP) followed by removal of the protecting groups gave the desired *N*-1-uracil or *N*-1-thymine alkylated derivatives **10** and **11** in high overall yield (scheme 1). No products resulting from *O*-2 alkylation of the pyrimidine bases were observed.



Scheme 1: *Reagents and Conditions*: i, trityl chloride, pyridine, cat. DMAP, CH_2Cl_2, rt, 24 h; ii, 3-*N*-benzoyluracil or 3-*N*-benzoylthymine, DEAD, TPP, dioxane, rt, 20 h; iii, 8 M ethanolic methylamine, rt, 3 h; iv, AcOH-H_2O (4:1 v/v), reflux, 40 min; v. benzoyloxyacetaldehyde, TFA, CH_2Cl_2, rt, 24 h; vi, Lawesson's reagent, toluene, reflux, 90 min; vii, sat. NH_3-MeOH, 100 °C, 16 h.

Assembly of the dioxane ring system was accomplished by acid-mediated condensation of **10** or **11** with benzoyloxyacetaldehyde[12] to give compounds **12** or **13**, respectively, as the expected mixtures of their *cis* and *trans* isomers. In both cases the reaction proceeded stereoselectively giving the *cis* and *trans* isomers in a 2:1 ratio. Assignment of configuration was made on the basis of an X-ray structure (see below). Whether this selectivity results from a thermodynamic equilibration process which prevails under the acidic conditions of the reaction or from kinetic control of product formation has not been determined. Why the major isomer should have the *cis* geometry, when the

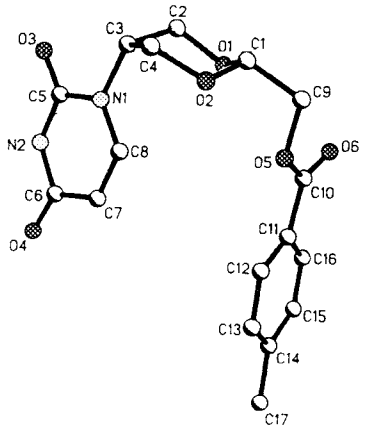


FIG 1: Single-crystal X-ray structure of *cis-N*-1-{[2-(4-methylbenzoyl)oxymethyl]-1,3-dioxan-5-yl}uracil (**15**). See experimental for details.

transition state leading to the *trans* product could benefit by having both substituents equatorial, is unclear. This phenomenon is not without precedent; a similar outcome was noted by Schreiber and Wang, although the origins of the selectivity observed in our case would appear to differ from those previously postulated.[13] Finally, treatment with ethanolic methylamine gave the desired fully deprotected compounds **4** and **5**, which could be separated into their respective *cis* and *trans* isomers by preparative tlc. Alternatively treatment of **12** with Lawesson's reagent[14] followed by ammonolysis of the formed 4-thio derivative **14** with methanolic ammonia at 100 °C[15] gave the *cis* and *trans* cytosine derivatives **6** which were again separated by preparative tlc.

Assignment of the *cis* configuration to *cis-N*-1-[(2-hydroxymethyl)-1,3-dioxan-5-yl]uracil (**4**) was made on the basis of a single-crystal X-ray structure of its 4-methoxybenzoyl derivative **15** (Figure 1).

It can be seen that compound **15** exists in the chair conformation with the 2-(4-methoxybenzoyl)oxymethyl group residing in an equatorial position. This is in agreement with previous work on 2,5-substituted dioxanes[16] in which it was shown that the equatorial conformation at position 2 is highly favored even if this results in a larger substituent at C-5 assuming an axial orientation. This preference is thought to arise from the relatively short C-O bond distance which would bring an axial group at C-2 close to the

syn-axial hydrogens at C-4 and C-6. It should also be mentioned that an axial group at C-5 in a dioxane ring has a much lower conformational energy than the same group in a cyclohexane ring due to the absence of any axial hydrogens at C-1 and C-3. In the dioxane ring system this interaction is replaced by the less destabilizing *syn*-axial relationship between the C-5 substituent and the axial lone pair of the O-1 and O-3 atoms. As the corresponding cytosine derivative **6** was synthesised from the isomeric mixture of protected uracil derivatives **12**, assignment of the *cis* configuration to the major isomer obtained from ammonolysis of **14** is appropriate. Stereochemical assignment of the thymine derivative **5** was made by comparison of ^{1}H NMR spectra. A portion of the ^{1}H NMR spectra of **4** and its minor *trans* isomer (spectra A and B respectively) next to spectra of compound **5** and its minor isomer (spectra C and D respectively) is shown in Figure 2.

Inspection of the resonance at δ 4.3, corresponding to the dioxane C-5 proton in *cis* **4** (proton assignments were made unambiguously using COSY NMR spectroscopy), indicates two small coupling constants between H-5 and the H-4 (and H-6) protons; this pattern is mirrored in the spectrum of the major isomer of compound **5**. Analogously, both minor isomers (spectra B and D) have a large and a small coupling constant between H-5 (δ 4.5) and the H-4 (and H-6) protons. Therefore, it appears consonant to assign the *cis* configuration to the major isomer of compound **5**.

The ^{1}H NMR spectra of the *cis* isomers of compounds **4** and **5** are consistent with the configuration shown in Figure 1. The small coupling constants observed between H-5 and both the axial and equatorial H-4 (and H-6) protons indicate that the former is equatorial leaving the base moiety axial and the hydroxymethyl group at C-2 equatorial. Conversely, in the case of the *trans* isomers the presence of a large and a small coupling constant indicates that H-5 must be axial and the base and the hydroxymethyl group equatorial. These results are again in good accord with other NMR studies on the conformation of 2,5-disubstituted dioxanes.[16]

Compounds were evaluated in primary human peripheral blood mononuclear (PBM) cells acutely infected with HIV-1_{LAI}. Virus yield was quantitated by measuring the levels of HIV-1 reverse transcriptase (RT) activity in virions obtained from the supernatant from cells exposed to the compounds.[17] Compound **4** had an EC_{50} of 18.8 μM against HIV-1_{LAI} in acutely infected human peripheral blood mononuclear cells. Other compounds, *trans* **4**, *cis* and *trans* **5** and **6**, were essentially inactive when tested against HIV-1. All the compounds demonstrated no toxicity in human PBM, CEM and Vero cells up to 100 μM. It should be noted that none of the compounds synthesized had greater potency than 3'-

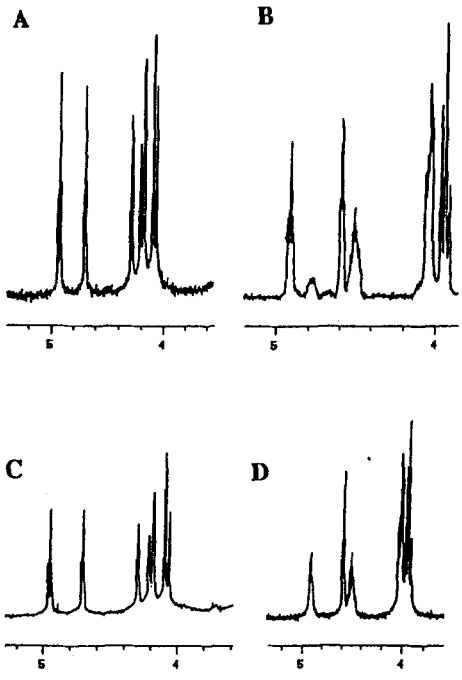


Figure 2: 400 MHz ^{1}H NMR spectra of A, *cis* **4**; B, *trans* **4**; C, *cis* **5**; D, *trans* **5**. In spectra A and C the dioxane H-5 is the narrow resonance at *ca.* δ 4.3, in spectra B and D it is the wider resonance at *ca* δ 4.5.

azido-3'-deoxythymidine (AZT) against HIV-1 in human PBM cells (EC_{50} = 0.004 μM). Similarly, all the compounds were inactive against herpes simplex virus type 1 (strain F) when tested up to 100 μM in a plaque reduction assay in Vero cells.[18] Finally, evaluation of compound **6**, the 6-membered analog of 3TC, in HBV transfected HepG2 cells demonstrated no activity at 10 μM.[19] In uninfected HepG2 cells it had no cytotoxicity with an IC_{50} of 1,953 μM. The general lack of antiviral activity and cytotoxicity of these analogs in various systems suggests that the presence of 6-membered ring may prevent the molecule from being activated by cellular or viral kinases.

In summary, a route for the synthesis of the novel 1,3-dioxane based nucleosides **4-6** from easily accessible starting materials was developed. None of the compounds described was shown to possess any significant antiviral activity in the assays so far performed. However, it is often the case that the true potential of a modification to the sugar portion of a nucleoside is unrealized until all four base analogs have been evaluated,

the salutary case of the oxathiolane nucleosides, where only the cytosine analogs have any real potency and selectivity,[4,5] being a case in point. With this in mind, work is in progress toward the synthesis of the corresponding purine derivatives of compounds **4-6**.

Experimental: Melting points (mp) were measured on an Electrothermal melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Plus 400 spectrometer at 400 MHz and 100.6 MHz, respectively. Tetramethylsilane was used as an internal standard and *J*-values are given in Hz. HRMS (FAB+) was performed on a JEOL MS-SX102/SX102A/E mass spectrometer by the Emory University Mass Spectrometry Center, Atlanta, GA. Microanalyses were performed by Atlantic Microlab, Norcross, GA. Whatman silica gel HP-KF glass-backed silica gel plates were developed in solvent systems A [$CHCl_3$-MeOH (19:1 v/v)], B [$CHCl_3$-MeOH (9:1 v/v)], C [$CHCl_3$-MeOH (4:1 v/v)] and D [hexane-ethyl acetate (7:3 v/v)]. Preparative tlc was performed on Whatman PK-5F glass-backed tlc plates. Merck silica gel (grade 10184) was used for flash column chromatography. Anhydrous solvents were purchased from the Aldrich Chemical Company, Milwaukee, WI, and used without further purification or drying. Trifluoroacetic acid was distilled at atmospheric pressure prior to use. X-ray crystallographic data on compound **15** was collected at the Department of Chemistry, Emory University.

1,3-Di-*O*-tritylglycerol (7).[10] Triphenylmethyl chloride (41.4 g, 150 mmol) was added to an emulsion of glycerol (6.90 g, 75 mmol) in anhydrous CH_2Cl_2 (300 ml) containing pyridine (40 ml). DMAP (0.88 g, 7.2 mmol) was added and the mixture allowed to stir at rt for 20 h. The products were washed with water (250 ml), 1 M HCl (250 ml) and saturated aqueous $NaHCO_3$ (250 ml), the organic phase dried (Na_2SO_4) then evaporated under reduced pressure. The residue was suspended in MeOH (100 ml) and the *title compound*, a colorless solid (34.7 g, 60 mmol, 80%), collected by filtration.
R_f 0.63 (system D); ^1H-NMR [$(CD_3)_2SO$] δ 3.02 (m, 2H), 3.14-3.18 (m, 2H), 3.82 (m, 1H), 5.01 (d, *J* 5.8, ex., 1H), 7.20-7.35 (m, 30H); ^{13}C-NMR [$(CD_3)_2SO$] 64.82, 68.90, 85.97, 127.21, 128.06, 128.53, 144.08.

***N*-1-(Bistrityloxymethyl)methyluracil (8).** DEAD (5.7 ml, 36 mmol) was added, at 0 °C in the dark, to a stirred suspension of **7** (10.4 g, 18 mmol), 3-*N*-benzoyluracil[11] (7.35 g, 36 mmol) and triphenylphosphine (9.44 g, 36 mmol) in anhydrous 1,4-dioxane (250 ml). After 10 min the formed solution was allowed to warm to rt and stirring continued for a further 20 h. Water (10 ml) was added and the solvent removed *in vacuo*. A solution of the residue in $CHCl_3$ (300 ml) was washed with saturated aqueous $NaHCO_3$ (200 ml), dried (Na_2SO_4) and evaporated under reduced pressure. The residue was

purified by column chromatography on silica gel, combination and evaporation *in vacuo* of the fractions eluted with hexane-ethyl acetate (1:1 v/v) gave *N*-1-(bistrityloxymethyl)-methyl-*N*-3-benzoyluracil which contained traces of a lower R_f impurity. The above material was suspended in 8 M ethanolic methylamine (30 ml, 240 mmol) and the mixture stirred at rt for 2 h. The products were concentrated and the residue resuspended in MeOH (50 ml). The *title compound*, a colorless solid, (10.4 g, 15.8 mmol, 88%) was collected by filtration. R_f 0.72 (system B); ^{1}H-NMR [$(CD_3)_2SO$] δ 3.24-3.36 (m, 4H), 4.83 (m, 1H), 5.52 (d, *J* 7.7, 1H), 7.21-7.32 (m, 30H), 7.41 (d, *J* 7.7, 1H), 11.32 (s, ex, 1H): ^{13}C-NMR [$(CD_3)_2SO$] δ 61.09, 86.18, 100.72, 127.16, 127.94, 128.04, 143.19, 143.34, 151.11, 163.09.

***N*-1-(Bishydroxymethyl)methyluracil (10).** Compound **8** (10.4 g, 15.8 mmol) was suspended in acetic acid-H_2O (4:1 v/v, 100 ml) and the products heated under reflux for 30 min. The formed solution was allowed to cool and then concentrated under reduced pressure. The residue was co-evaporated with benzene (3 x 20 ml) then triturated with CH_2Cl_2 to remove tritanol. The residue was crystallized from MeOH-$CHCl_3$ to give the *title compound* (1.95 g, 10.5 mmol, 67%) as colorless crystals. R_f 0.25 (system C); mp 147-148 °C; Found: C, 45.09; H, 5.35; N, 14.99. $C_7H_{10}N_2O_4$ requires C, 45.16; H, 5.41; N, 15.05. ^{1}H-NMR [$(CD_3)_2SO$] δ 3.56-3.65 (m, 4H), 4.42 (m, 1H), 4.93 (t, *J* 5.6, 2H), 5.52 (d, *J* 7.6, 1H), 7.58 (d, *J* 7.6, 1H), 11.16 (s, ex. 1H); ^{13}C-NMR [$(CD_3)_2SO$] δ 58.97, 59.03, 100.23, 143.70, 151.55, 163.32.

***N*-1-(Bistrityloxymethyl)methylthymine (9).** DEAD (9.6 ml, 60.8 mmol) was added, at 0 °C in the dark, to a stirred suspension of **7** (17.5 g, 30.4 mmol), 3-*N*-benzoylthymine[11] (13.3 g, 60.8 mmol) and triphenylphosphine (15.95 g, 60.8 mmol) in anhydrous dioxane (400 ml). After 10 min, the formed solution was allowed to warm to rt and stirring continued for a further 20 h. Water (10 ml) was added and the solvent removed *in vacuo*. A solution of the residue in $CHCl_3$ (400 ml) was washed with saturated aqueous $NaHCO_3$ (200 ml), dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel, combination and evaporation *in vacuo* of the fractions eluted with hexane-ethyl acetate (1:1 v/v) gave *N*-1-(bistrityloxymethyl)methyl-*N*-3-benzoylthymine which contained traces of a lower R_f impurity. The above material was suspended in 8 M ethanolic methylamine (40 ml, 320 mmol) and the mixture stirred at rt for 2 h. The products were concentrated and the residue resuspended in MeOH (75 ml). The *title compound*, a colorless solid, (17.5 g, 26 mmol, 85%) was collected by filtration. R_f 0.75 (system B) ^{1}H-NMR [$(CD_3)_2SO$] δ 1.65 (s, 3H), 3.26-3.37 (m, 4H), 4.87 (m, 1H), 7.16 (s, 1H) , 7.21-7.32 (m, 30H), 10.82 (s, ex,

***N*-1-(Bishydroxymethyl)methylthymine (11).** Compound **9** (4.0 g, 6.05 mmol) was suspended in acetic acid-H_2O (4:1 v/v, 100 ml) and the products heated under reflux for 30 min. The formed solution was allowed to cool and then concentrated under reduced pressure. The residue was co-evaporated with benzene (3 x 20 ml), then suspended in CH_2Cl_2 (100 ml) and the *title compound*, a colorless solid (1.0 g, 4.99 mmol, 82%), collected by filtration. To obtain an analytical sample, a portion of the product was crystallized from MeOH-$CHCl_3$. R_f 0.33 (system C); mp 229-230 °C; Found: C, 48.08; H, 5.99; N, 13.90. $C_8H_{12}N_2O_4$ requires C, 48.00; H, 6.04; N, 14.00. ^{1}H-NMR [$(CD_3)_2SO$] δ 1.77 (s, 3H), 3.60-3.66 (m, 4H), 4.42 (m, 1H), 4.89 (t, *J* 5.1, ex, 2H), 7.46 (s, 1H), 11.15 (s, ex, 1H); ^{13}C-NMR [$(CD_3)_2SO$] δ 12.10, 58.75, 59.09, 107.68, 139.21, 151.52, 163.87.

Benzoyloxyacetaldehyde. The *title compound* was prepared according to the method of Hashiguchi *et al.*[12] and isolated in similar yield. bp 74-75 °C/1 mm Hg, lit.[12] 85-88 °C/2 mm Hg; ^{1}H-NMR ($CDCl_3$) δ 4.8 (s, 2H), 7.45 (m, 2H), 7.58 (m, 1H), 8.08 (m, 2H), 9.70 (s, 1H).

***cis/trans*-*N*-1-[(2-Benzoyloxymethyl)-1,3-dioxan-5-yl]uracil (12).** Trifluoroacetic acid (8.1 ml, 106 mmol) was added to a suspension of **10** (2.44 g, 13.2 mmol) and benzoyloxyacetaldehyde (10.8 g, 66 mmol) in anhydrous CH_2Cl_2 (45 ml). The formed yellow solution was stirred at rt for 24 h then poured into ice-cold saturated aqueous $NaHCO_3$ (300 ml). The products were extracted with $CHCl_3$ (2 x 100 ml) and the organic layer dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel, combination and evaporation *in vacuo* of the fractions eluted with $CHCl_3$-MeOH (19:1 v/v) gave the *title compound* (3.58 g, 10.8 mmol, 82%) as an amorphous solid. R_f 0.5 (system B); ^{1}H-NMR ($CDCl_3$) δ 4.26 (m, 4H), 4.42 (m, 0.7H), 4.43-4.52 (m, 2H), (m, 0.3H), 4.97 (m, 0.3H), 5.07 (m, 0.7H), 5.61 (d, *J* 8.8, 0.7H), 5.75 (d, *J* 8.8, 0.3H), 7.19 (d, J 8.8, 0.3H), 7.43-7.49 (m, 2H), 7.57 (m, 1H), 8.06 (m, 2H), 8.30 (d, *J* 8.8, 0.7H), 9.52 (s, ex, 1H).

***cis* and *trans*-*N*-1-[(2-Hydroxymethyl)-1,3-dioxan-5-yl]uracil (4).**
Compound **12** (0.50 g, 1.51 mmol) was dissolved in 8 M ethanolic methylamine (10 ml, 80 mmol) and the mixture stirred at rt for 16 h. The products were concentrated *in vacuo* and the residue purified by column chromatography on silica gel. Combination and concentration under reduced pressure of the fractions eluted with $CHCl_3$-MeOH (9:1 v/v) gave the *title compound* (0.22 g, 0.98 mmol, 65%) as a colorless solid. A portion of the product (0.10 g, 0.49 mmol) was further purified by preparative tlc, development in

system B gave samples of the *cis* (0.055 g) and the *trans* isomer (0.028 g). Crystallization from MeOH-$CHCl_3$ gave samples for microanalysis. *Cis* isomer: R_f 0.26 (system B); mp 200-201 °C; Found: C, 47.47; H, 5.32; N, 12.34. $C_9H_{12}N_2O_5$ requires C, 47.37; H, 5.30; N, 12.27. ^{1}H-NMR [$(CD_3)_2SO$] δ 3.40 (m, 2H), 4.10 (m, 2H), 4.19 (m, 2H), 4.29 (m, 1H), 4.70 (m, 1H), 4.94, (t, *J* 6.0, ex, 1H), 5.60 (d, *J* 7.6, 1H), 8.17 (d, *J* 7.6, 1H), 11.33 (s, ex, 1H); ^{13}C-NMR [$(CD_3)_2SO$] δ 47.57, 62.68, 67.86, 100.74, 101.27, 143.67, 151.08, 163.25. *Trans* isomer: R_f 0.16 (system B); mp 258 °C (dec); Found: C, 47.10; H, 5.33; N, 12.01. $C_9H_{12}N_2O_5$ requires C, 47.37; H, 5.30; N, 12.27. ^{1}H-NMR [$(CD_3)_2SO$] δ 3.39 (m, 2H), 3.95 (m, 2H), 4.05 (m, 2H), 4.50 (m, 1H), 4.60 (m, 1H), 4.91, (t, J 6.8, ex, 1H), 5.56 (d, *J* 7.6, 1H), 7.71 (d, J 7.6, 1H), 11.36 (s, ex, 1H); ^{13}C-NMR[$(CD_3)_2SO$] δ 47.97, 62.52, 66.73, 101.18, 101.84, 143.10, 151.17, 163.46.

***cis/trans-N*-1-[(2-Benzoyloxymethyl)-1,3-dioxan-5-yl]thymine (13).**

Trifluoroacetic acid (3.1 ml, 40 mmol) was added to a suspension of **11** (1.0 g, 5.0 mmol) and benzoyloxyacetaldehyde (4.10 g, 25 mmol) in anhydrous CH_2Cl_2 (30 ml). The formed yellow solution was stirred at rt for 24 h then poured into ice-cold saturated aqueous $NaHCO_3$ (300 ml). The products were extracted with $CHCl_3$ (2 x 100 ml) and the organic layer dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel, combination and evaporation *in vacuo* of the fractions eluted with $CHCl_3$-MeOH (19:1 v/v) gave the *title compound* (1.55 g, 4.47 mmol, 90%) as a colorless solid. R_f 0.55 and 0.58 (system B); ^{1}H-NMR ($CDCl_3$) δ 1.84 (s, 1.7H), 1.92 (s, 1.3H), 4.05-4.65 (m, 7H), 4.97 (m, 0.4H), 5.06 (m, 0.6H), 6.98 (s, 0.6H), 7.38-7.61 (m, 3.4H) 8.07 (m, 2H), 9.18 (s, ex, 0.6H), 9.21 (s, ex, 0.4H).

***cis* and *trans-N*-1-[(2-Hydroxymethyl)-1,3-dioxan-5-yl]thymine (5).**

Compound **13** (0.50 g, 1.44 mmol) was dissolved in 8 M ethanolic methylamine (10 ml, 80 mmol) and the mixture stirred at rt for 16 h. The products were concentrated *in vacuo* and the residue purified by column chromatography on silica gel. Combination and concentration under reduced pressure of the fractions eluted with $CHCl_3$-MeOH (9:1 v/v) gave the *title compound* (0.252 g, 1.04 mmol, 72%) as a colorless solid. A portion of the product (0.20 g) was further purified by preparative tlc, development in system B gave samples of the *cis* (0.102 g) and *trans* (0.054 g) isomers. Crystallization of the isomers from MeOH-$CHCl_3$ and wet isopropanol respectively, gave samples for microanalysis. *Cis* isomer: R_f 0.33 (system B); mp 232-233 °C; Found: C, 49.67; H, 5.82; N, 11.64. $C_{10}H_{14}N_2O_5$ requires C, 49.58; H, 5.83; N, 11.56. ^{1}H-NMR [$(CD_3)_2SO$] δ 1.78 (s, 3H), 3.43 (m, 2H), 4.08 (m, 2H), 4.20 (m, 2H), 4.30 (m, 1H), 4.71 (m, 1H), 4.96 (t, *J* 6.0, ex, 1H), 8.08 (s, 1H), 11.32 (s, ex, 1H); ^{13}C-NMR [$(CD_3)_2SO$] δ 12.47, 47.19,

62.65, 67.95, 101.1, 108.01, 139.54, 151.05, 163.78. *Trans* isomer: R_f 0.22 (system B); mp 210 °C; Found: C, 49.53; H, 5.82; N, 11.51. $C_{10}H_{14}N_2O_5$ requires C, 49.58; H, 5.83; N, 11.56. ^{1}H-NMR [$(CD_3)_2SO$] δ 1.77 (s, 3H), 3.40 (m, 2H), 3.87-4.06 (m, 4H), 4.52 (m, 1H), 4.62 (m, 1H), 4.92 (t, ex, *J* 6.0, 1H), 7.61 (s, 1H), 11.34 (s, ex, 1H); ^{13}C-NMR [$(CD_3)_2SO$] δ 12.03, 47.15, 62.44, 66.49, 101.01, 109.10, 138.06, 150.85, 163.53.

***cis/trans*-*N*-1-[(2-Benzoyloxymethyl)-1,3-dioxan-5-yl]-4-thiouracil (14).** Compound **12** (0.30 g, 0.9 mmol) was co-evaporated with anhydrous toluene (5 ml) then resuspended in toluene (10 ml) and Lawesson's reagent[14, 15] (0.22 g, 0.54 mmol) added. The mixture was heated under reflux for 90 min then allowed to cool and the solvent removed under reduced pressure. A solution of the residue in $CHCl_3$ (50 ml) was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, combination and evaporation under reduced pressure of the fractions eluted with $CHCl_3$-MeOH (98:2 v/v) gave the *title compound* (0.31 g, 0.9 mmol, 100%) as a yellow solid. R_f 0.67 (system B); ^{1}H-NMR [$(CD_3)_2SO$] δ 4.05-4.39 (m, 6.6H), 4.57 (m, 0.4H), 5.01 (m, 0.4H), 5.14 (m, 0.6H), 6.18 (d, *J* 7.6, 0.6H), 6.28 (d, *J* 7.6, 0.4H), 7.5-8.0 (m, 5.4H) 8.09 (d, *J* 7.6, 0.6H), 12.74 (s, ex, 0.6H), 12.76 (s, ex, 0.4H).

***cis* and *trans*-*N*-1-[(2-Hydroxymethyl)-1,3-dioxan-5-yl]cytosine (6).** Compound **14** (0.303 g, 0.87 mmol) was dissolved in saturated methanolic ammonia (20 ml) and the solution heated at 100 °C for 16 h in a sealed bomb.[15] The mixture was allowed to cool and then concentrated under reduced pressure. The residue was purified by preparative tlc; development in system B gave the *cis* (0.10 g, 0.44 mmol) and *trans* (0.060 g, 0.26 mmol) isomers as colorless solids. Crystallization of the isomers from aqueous isopropanol gave samples for microanalysis. *Cis* isomer: R_f 0.50 (system C) ; mp 238 °C ; Found: C, 47.40; H, 5.74; N, 18.44. $C_9H_{13}N_3O_4$ requires C, 47.57; H, 5.77; N, 18.49. ^{1}H-NMR [$(CD_3)_2SO$] δ 3.40 (m, 2H), 4.03 (m, 2H), 4.18 (m, 2H), 4.31 (m, 1H), 4.69 (m, 1H), 4.90 (t *J* 6.0, ex, 1H), 5.70 (d, J 7.6, 1H), 7.10 (br, ex, 2H), 8.11 (d, *J* 7.6, 1H); ^{13}C-NMR [$(CD_3)_2SO$] δ 47.95, 62.73, 67.99, 79.15, 92.99, 144.06, 155.54, 165.48. *Trans* isomer: R_f 0.35 (system C) ; mp 239-241 °C (dec.); HRMS (FAB+) calc. for $C_9H_{13}N_3O_4Li$ 234.1066, found 234.1073; ^{1}H-NMR [$(CD_3)_2SO$] δ 3.37 (m, 2H), 3.90 (m, 2H), 4.00 (m, 2H), 4.57 (m, 2H), 4.91 (t, *J* 6.3, ex, 1H), 5.66 (d, *J* 7.5, 1H), 7.11 (br, ex, 2H), 7.61 (d, *J* 7.5, 1H); ^{13}C-NMR [$(CD_3)_2SO$] δ 48.53, 62.62, 67.36, 94.48, 101.25, 143.41, 156.02.

***cis*-*N*-1-{[2-(4-Methylbenzoyl)oxymethyl]-1,3-dioxan-5-yl}uracil (15).** Compound **4** (0.023 g 0.105 mmol) was co-evaporated with dry pyridine (2 ml) then redissolved in dry pyridine (3 ml) and 4-methylbenzoyl chloride (0.017 ml, 0.126 mmol) added. The mixture was stirred at rt for 16 h then water (0.2 ml) added. The solvent was removed under reduced pressure and a solution of the residue in $CHCl_3$ (25 ml) washed with saturated aqueous $NaHCO_3$ (20 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, combination and evaporation of the fractions eluted with $CHCl_3$-MeOH (95:5 v/v) gave the *title compound* (0.030 g, 0.087 mmol, 82%) as a colorless solid. Crystallization of the solid from $CHCl_3$-MeOH gave samples for microanalysis and X-ray crystallography. R_f 0.52 (system B) ; mp 219-220 °C; Found: C, 59.04; H, 5.28; N, 8.01. $C_{17}H_{18}N_2O_6$ requires C, 58.96; H, 5.24; N, 8.09. ^{1}H-NMR [$(CD_3)_2SO$] δ 2.40 (s, 3H), 4.15 (m, 2H), 4.28 (m, 2H), 4.34 (m, 3H), 5.10 (m, 1H), 5.45 (d, J 7.6, 1H), 7.37 (m, 2H), 7.86 (m, 2H), 8.21 (d, J 7.6, 1H), 11.32 (s, ex, 1H).

X-ray Analysis: A colorless needle of compound **15** (0.13 x 0.3 x 0.7 mm) was mounted on a glass fiber and centered on a Siemens P4 four circle diffractometer equipped with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). A total of 4976 reflections ($2\theta_{max} = 55°$) were collected using ω scan mode, of which 4279 were unique. The structure was solved by direct methods and refined by full-matrix-least-squared-on-F^2 techniques (4267 reflections with $F^2 > 2\sigma(Fo^2)$, using anisotropic temperature factors for all non-hydrogen atoms. All hydrogen atoms were placed at idealized positions and refined as fixed contributors. The R value was 0.0428 at final convergence. The atomic coordinates, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates and isotropic parameters and observed and calculated structure factors have been submitted to the Cambridge Crystallographic Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

Antiviral and Cytotoxicity Assays: Anti-HIV-1 activity of the compounds was determined in human PBM cells as described previously.[17] Stock solutions (10-40 mM) of the compounds were prepared in sterile DMSO and then diluted to the desired concentration. Other compounds such as AZT were dissolved in water. Cells were infected with the prototype HIV-1_{LAI} at a multiplicity of infection of 0.01. Virus obtained from the cell supernatant was quantitated on day 6 after infection by a reverse transcriptase assay using poly(rA)$_n$.oligo(dT)$_{12-18}$ as template-primer. The DMSO present in the diluted solution (< 0.1%) had no effect on the virus yield. The toxicity of the compounds was

assessed in human PBM, CEM, and Vero cells, as described previously.[17] Evaluation of the compounds against herpes simplex virus in Vero cells and hepatitis B virus in transfected HepG2 cells (also known as 2.2.15 cells) was performed as described previously.[18,19] The antiviral EC_{50} and cytotoxicity IC_{50} was obtained from the concentration-response curve using the median effective method described by Chou and Talalay.[20]

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