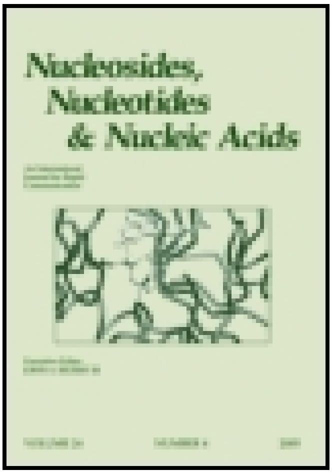

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Synthesis and Anti-HIV Evaluation of New 5-Substituted-2′,3′-Dideoxy-3′-thiauridine Nucleosides

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Synthesis and anti-HIV evaluation of new 5-substituted-2',3'-dideoxy-3'-thiauridine nucleosides

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Abstract: On the basis of molecular modeling calculations using GenMol software, new 5-substituted-2',3'-dideoxy-3'-thiauridine were designed as possible anti-HIV reverse transcriptase inhibitors. The synthesis of the key intermediate 5-carboxy-2',3'-dideoxy-3'-thiauridine was achieved through the condensation of the fully silylated 5-carboxyuracil on 2-benzoyl methyl-5-acetoxy-1,3-oxathiolane using trimethylsilyl triflate (TMSOTf). This latter compound was condensed with 2-(N-*tert*-Butoxycarbonyl)-1-aminoethane in the presence of N,N-diisopropylethylamine (DIEA). The subsequent carboxamide deprotection led to the final compounds. These new analogues were evaluated for their anti-HIV-1 activities on infected MT_4 cells but no significant protection was observed. Electronic and structural parameters considered in this model were not sufficient to predict any active anti-HIV molecular structures.

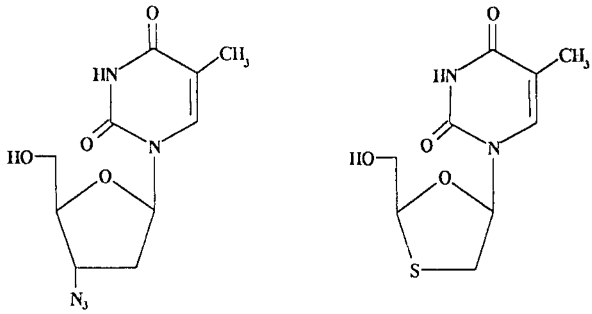
INTRODUCTION

Antiretroviral agents developed today have been primarily the inhibitors of HIV-reverse transcriptase. The HIV RT has been one of the most intensively studied viral targets for the development of anti-HIV drugs. 2',3'-dideoxy analogues can be metabolized to form potent RT. Chain terminator (1) have been identified to elicit potent antiretroviral activity.

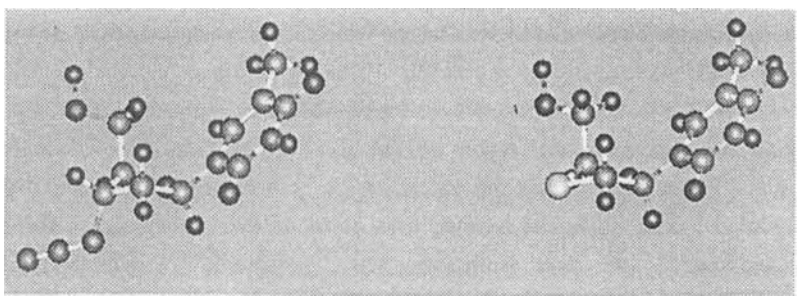
Dideoxynucleosides are of special interest since "simple" chemical modification of their sugar moiety can convert a normal substrate into a potent inhibitor for HIV replication. Analysis of the molecular structure of the most active anti-HIV nucleosides [AZT (Retrovir), ddI (Videx), ddC (Zalcitabine), d4T (Stavudine) and 3TC (Lamivudine)] reveals that for a given ribose ring only a few specific nucleic bases led to active anti viral drugs. Having actively participated to the discovery of 2',3'-dideoxy-3'-thiacytidine (3TC) (1,2,3) and related drugs (4,5,6,7), we have investigated the possibilities to use the promising heterocycle 1,3-oxathiolane as a ribose ring mimic coupled with modified uracil or thymine nucleic base. Molecular modeling calculations using GenMol software (5) were undertaken in order to evaluate the energetical differences between the lowest energy conformer of 2',3'-dideoxy-3'-azidothymidine (AZT) and its corresponding 2',3'-dideoxy-3'-thiathymidine (ddTT). From these calculations, we synthesized new 5-substituted 2',3'-dideoxy-3'-thiauridine, and proceed to their antiviral evaluation.

Conformational studies

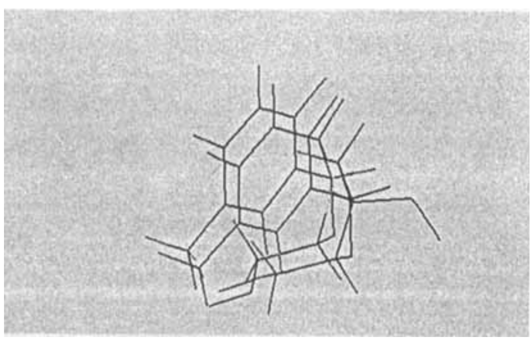
All the molecules with modified or unmodified ribose are considered in the C3' exo conformation as deduced form X-Ray analysis by Van Roey and al. (2). According to these authors, C3' exo conformation was the one active on the kinase and RT receptors. In order to have a chance to fit into the kinase or RT active site, the nucleoside drug must have an envelope (related to conformation) in terms of both geometry and electrostatic potential equivalent to that of the natural substrate. This is required to be complementary to the active site envelope (3). Therefore necessary energy to put the new 5-substituted-2',3'-dideoxythiauridine into cytidine-like geometry was calculated. The difference between strain energy of an analogue in the cytidine-like geometry and the minimum strain energy of this analogue in C3' conformation, corresponds to the increase of the transition state energy with cytidine. Its effect on the kinetic constant of the fit was calculated. These calculations based on Force Field and MEP (Molecular Electrostatic Potential) (4), were performed with GenMol software (5). GenMol is a 3.10^5 instructions software, with an original Force Field able to treat atomic and molecular systems containing up to 10^5 atoms of 96 different types. The lowest energy conformers of AZT, 2',3'-dideoxy-3'-thiathymidine (ddTT) and 5-substituted-2',3'-dideoxythiauridine derivatives were examined and characterized. As shown on Fig.1, the lowest energy conformation of both AZT and ddTT was found to be very similar. Force Field and Molecular Electrostatic Potential (MEP) calculations indicated in the case of AZT that a 3'-electron with drawing substituent N3 induced a slightly positive MEP. ddTT with a sulfur atom in 3' position displayed also a weak negative MEP in the same region. In contrast MEP effect disappeared in the case of the corresponding 3'-deoxyribose thymidine. Since both AZT and Thymidine fits the reverse transcriptase active site, this result could indicate that the active site has a weak MEP selectivity. *In vitro* anti-HIV experiments on infected MT_4 cells showed that ddTT was inactive (8), while AZT was one of the most active anti-HIV drug. This



AZT ddTT

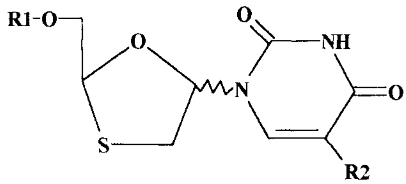


1a Stereoviews of AZT (left) and ddTT (2',3'-dideoxy-3'-thiathymidine, right) in their lowest conformation energy.



1b Overlay of AZT and ddTT as found in the surimposed structure determinations in their HIV-RT bound conformations.

Fig. 1- Lowest energy conformation of AZT and its 3'-oxathiolane derivative.



	R1	R2
2α, 2β :	PhCO	I
3α, 3β :	PhCO	CH_3
4α, 4β :	H	I
5α, 5β :	H	CH_3
6α, 6β :	PhCO	COOH
7α, 7β :	PhCO	CONH-$(CH_2)_2$-NH-Boc
8α, 8β :	H	CONH-$(CH_2)_2$-NH-Boc
9α, 9β :	H	CONH-$(CH_2)_2$-NH_2

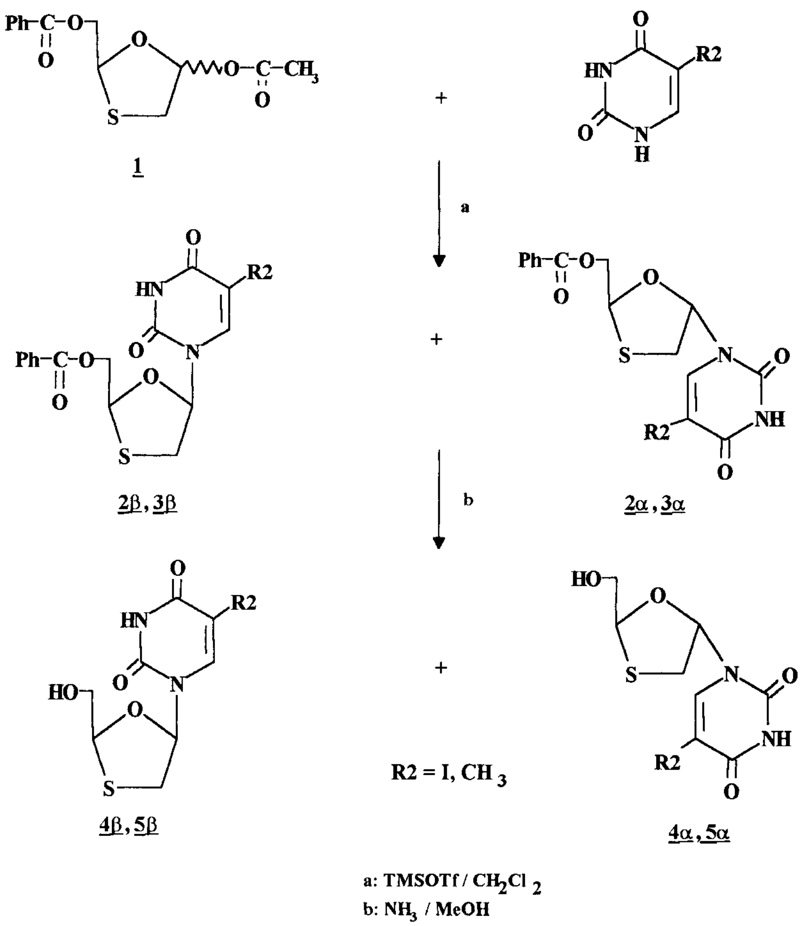
Fig 2

biological observation indicates that parameters others than conformation energy, or geometrical effects are involved in the mechanism which confer an antiretroviral activity. Anti-HIV activity of dideoxynucleosides is critically dependent of triphosphorylation mediated by cellular kinase (9,10,11), but also depends on the bioavailability of the drug inside the infected cells. Taking into account results report by Van Roey (2) that the C3' exo conformation deduced froml X-Ray analysis was the one active on both kinase and RT active site, then biodelivery of the drug inside the infected cells could be the limiting factor for anti-HIV activity. Consequently, we have synthesized new 5-substituted-2',3'-dideoxy-3'-thiauridine which were submitted to anti-HIV evaluation. The 5-substituents were selected for their large difference in polarity (COOH, CONH-$(CH_2)_2$-NH-Boc, CONH-$(CH_2)_2$-NH_2, CH_3, I) which can confer to the resulting molecules differents lipophilic characters.

Chemistry

The following new 2',3'-dideoxy-3'-thia C-5 substituted uracylnucleosides were synthesised (Fig.2).

The synthesis of these new oxathiolane nucleosides is illustrated on scheme 1 and scheme 2. The key intermediate 1,3-oxathiolane (1) was synthesised according to standard procedures already reported (12,13,14). In an attempt to find the optimal conditions for the condensation of 2-benzoyloxymethyl-5-acetoxy-1,3-oxathiolane (1) with the silylated 5-carboxy uracil base, various Lewis acid (TMSOTf, $SnCl_4$ and $TiCl_4$), solvents (CH_3CN, CH_2Cl_2, $ClCH_2CH_2Cl$) and reaction thermodynamic conditions were investigated. We were finally able to synthesize the corresponding α and β forms of 5-substituted uracil derivatives (6α and 6β) using TMSOTf in dichloromethane at room temperature for 8h. The 1:1 mixture of α and β isomers was separated using column chromatography (eluent: CH_2Cl_2/ CH_3OH 9:1) with an overall yield of 90%. The next step involved the coupling between a mono-protected ω-diamine and the 5-carboxylic function. As a preliminary assay, aminoethylcarboxamide prepared according to a described procedure (15) was coupled to 7α and 7β. Several coupling

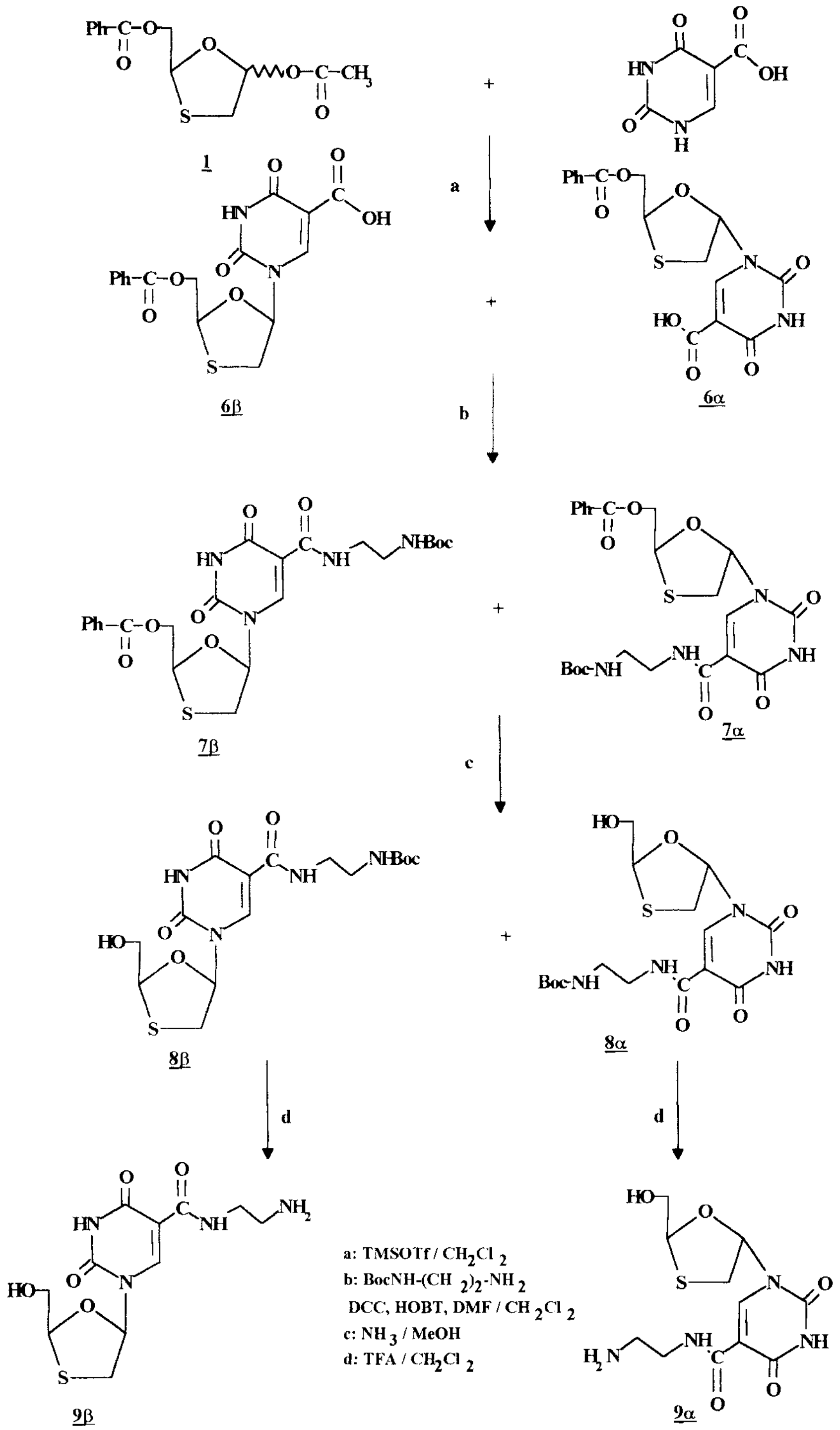


Scheme 1

conditions were investigated:

- BOP (benzotriazol-1-yloxy-tris (dimethylamino) phosphonium-hexafluoro-phosphate) in DMF (21,22,4) in the presence of TEA.
- DCC (4-dicyclohexylcarbodiimide), HOBT (hydroxybenzotriazole) in DMF, and DCC/HOBT in the presence of DIEA.

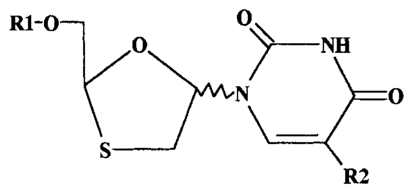
We found that the best yields (65%) were obtained using DCC/HOBT in CH_2Cl_2 with DIEA. Deprotection of compounds 7α and 7β using saturated methanolic ammonia solution led to the corresponding analogues 8α and 8β in a quantitative yield. In order to avoid oxathiolane ring opening, very mild acidic conditions (98% formic acid or trifluoroacetic acid) were used at room temperature to remove Boc aminoprotecting group (16,17). Final compounds 9α and 9β were obtained in 68% and 98% yield, respectively. The determination


1
6β
6α
7β
7α
8β
8α
9β
9α
a: TMSOTf / CH2Cl2
b: BocNH-(CH2)2-NH2
DCC, HOBT, DMF / CH2Cl2
c: NH3 / MeOH
d: TFA / CH2Cl2

Scheme 2

Table 1

Anti-HIV-1 activity of 5-substituted 2',3'-dideoxy-3'-thiacytidine.



N°	R1	R2	Stereochemistry	IC_{50}*µM
4α	H	I	α	>100
4β	H	I	β	>100
5α	H	CH_3	α	>200
5β	H	CH_3	β	>200
7α	PhCOO	$CONH(CH_2)_2NHBoc$	α	>200
7β	PhCOO	$CONH(CH_2)_2NHBoc$	β	>200
9α	H	$CONH(CH_2)_2NH_2$	α	>200
9β	H	$CONH(CH_2)_2NH_2$	β	>200
AZT	-	-	β	0.05
±3TC	H	H	β	0.1

* IC_{50} : concentration required to produce 50% inhibition of syncitia formation on MT_4 cells.

of the anomeric configuration of compounds 9α and 9β was confirmed by NMR NOESY experiments. Structural assigments of the compounds described in the experimental part were based on elemental analysis, ^{1}H, ^{13}C NMR and mass spectra data. ^{13}C NMR signal assigments were confirmed by DEPT-135 experiments.

Anti-HIV activity and discussion

The potency of the new synthesised 2',3'-dideoxy-5-substituted-3'-thiauridine as inhibitors of HIV-replication was evaluated. The anti-HIV-1 activity was determined through the formation of syncitia (18,19) in infected MT_4 cells (20). IC_{50} values (concentration of drugs required to produce 50% inhibition of the syncitia formation) are reported in Table 1. The first observation which can be inferred from these results is that none of the new derivatives in their α or β forms elicited anti-HIV activity. Moreover, as already reported (8), ddTT (5β) which represents the corresponding analogue of (±) 3TC is denied of anti-HIV activity. The observed anti-HIV inactivity of these 5-substituted 2',3'-dideoxy-3'-thiauridine derivatives suggest the following comments. Substitution at the C5 position of the uracil base by chemical groups having different lipophilic characters did not confer to the resulting molecule any anti-HIV activity. Therefore, the lowest energy conformation C3' exo, selected by the modeling molecular calculations to be the one active on both kinase and RT enzyme active sites is not suitable in the case of new 5-substituted 2',3'-dideoxy-3'-thiauridine. This result seems to indicate a greater sensitivity of the thymine kinases to the thymidine structure modifications.

In conclusion, the synthesis of new 5-substituted-2',3'-dideoxy-3'-thiauridine derivatives has been achieved. These compounds were designed in order to elicit anti-HIV properties. The introduction of heteroatoms like sulfur in the backbone sugar ring could lead to active anti-HIV nucleosides, (3TC). At the opposite the replacement of cytosine of 2',3'-dideoxy-3'-thiacytidine by different uracils substituted at C-5 by groups having different lipophilic characters seems to abolish the anti-HIV properties.

EXPERIMENTAL SECTION

Proton magnetic spectra were recorded on a Bruker AMX 200 spectrometer. Chemical shifts were reported as values in parts per million. Coupling constants were expressed in hertz (Hz). Elemental microanalysis were determined by Service Central d'Analyse CNRS Vernaison-Lyon France and gave combustion values for C, H, N within 0.4% of theoritical values. Analytical thin layer chromatographies (TLC) were carried out on aluminium precoated TLC plates with silica gel Kieselgel 60 F_{254nm} 0.2 mm thickness (Merck and Co, Darmstadt). Column flash chromatographies were performed with Merck silica gel (230-400 mesh). Preparative layer chromatographies were carried out on silica gel 60 F_{254nm} precoated PLC plates (20 x 20 cm layer thickness 1 or 2 mm). Melting points were determined with a MEL-TEMP capillary apparatus. FAB+ mass spectra were recorded on a JEOL DX-100 mass spectrometer at the Laboratoire de Mesures Physiques-RMN, USTL, Montpellier, France.

Chemistry

2',3'-dideoxy-3'-thiathymidine α and β (5α and 5β) forms have been synthesized according to a procedure described in reference 13.

2',3'-dideoxy-5'-benzoyloxymethyl-5-iodo-3'-thiauridine 2α and 2β :

A solution of silylated 5-iodouracil (1eq, 1.7 g, 3.6 mmol) in dry dichloromethane (10 mL) was added to a solution of compound 1 (1eq, 1.0 g, 3.6 mmol) in dry dichloromethane (10 mL).

The mixture treated with trimethylsilyl triflate became clear after stirring for 3h, under nitrogen atmosphere at room temperature. It was then evaporated under reduced pressure. An aqueous solution (5%) $NaHCO_3$ was added and the product was extracted with dichloromethane. The organic phases were washed with H_2O and dried over Na_2SO_4 to give after solvent evaporation a white solid (1.514 g). Yield (91%). TLC (CH_2Cl_2/MeOH 95:5) Rf = 0.40.

2α isomer: ^1H NMR ($CDCl_3$) δ: 3.13-3.30 (m, 1H, H-2'a or H-2'b); 3.54-3.71 (m, 1H, H-2'a or H-2'b); 4.41-4.60 (m, 1H, H-5'a or H-5'b); 4.70-4.77 (m, 1H, H-5'a or H-5'b); 5.52 (t, 1H, J= 3.85 Hz, H-4'); 6.34 (t, 1H, J= 5.10 Hz, H-1'); 7.47-8.15 (m, 6H, H arom.); 9.29 (s br, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 37.9 (C-2'); 66.7 (C-5); 69.4 (C-5'); 84.8 (C-4'); 88.5 (C-1'); 129.0-134.0 (C arom.); 144.4 (C-6); 150.1 (C-4); 160.3 (COPh); 166.5 (C-2).

2β isomer: ^1H NMR ($CDCl_3$) δ: 3.13-3.30 (m, 1H, H-2'a or H-2'b); 3.54-3.71 (m, 1H, H-2'a or H-2'b); 4.41-4.60 (m, 1H, H-5'a or H-5'b); 4.70-4.77 (m, 1H, H-5'a or H-5'b); 5.90 (pseudo d, 1H, H-4'); 6.55 (dd, 1H, H-1'); 7.47-8.15 (m, 6H, H arom. and H-6); 9.29 (s br, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 37.9 (C-2'); 64.9 (C-5); 68.7 (C-5'); 83.6 (C-4'); 86.9 (C-1'); 129.0-134.0 (C arom.); 144.3 (C-6); 150.1 (C-4); 160.1 (COPh); 166.4 (C-2). Anal. ($C_{15}H_{13}N_2O_5SI$) C, H, N. MS (FAB+) m/z 461 MH^+.

2',3'-dideoxy-5-iodo-3'-thiauridine 4α and 4β :

Compounds 2α and 2β (0.70 g) were dissolved in a solution of NH_3/MeOH (12 mL) and stirred overnight at room temperature. After solvent evaporation, the two isomers were separated over silica gel by flash column chromatography using CH_2Cl_2/MeOH (95:5) as eluent. It gave 0.405 g of compounds 4α and 4β as white solid. Yield (75%). TLC (CH_2Cl_2/MeOH 95:5) Rf = 0.21. mp = 193-195°c for 4α and mp = 169-170°c for 4β.

4α isomer: ^1H NMR (CD_3COCD_3) δ: 3.33-3.44 (m, 2H, 2H-2'); 3.51-3.73 (m, 2H, 2H-5'); 4.62 (t, 1H, J= 5.66 Hz, OH); 5.33 (t, 1H, J= 3.54 Hz, H-4'); 6.27 (dd, 1H, H-1'); 8.06 (s, 1H, H-6); 10.43 (s br, 1H, NH).

4β isomer: ^1H NMR (CD_3COCD_3) δ: 3.31-3.53 (m, 2H, 2H-2'); 3.57-3.62 (m, 2H, 2H-5'); 4.28 (t, 1H, J= 6.22 Hz, OH); 5.71 (t, 1H, J= 4.81 Hz, H-4'); 6.46 (dd, 1H, H-1'); 8.63 (s, 1H, H-6); 10.41 (s br, 1H, NH). Anal. ($C_8H_9N_2O_4SI$) C, H, N. MS (FAB+) m/z 357 MH^+.

2',3'-dideoxy-5'-benzoyloxymethyl-3'-thiauridine- 5-carboxylic acid 6α and 6β:

A suspension of 5-carboxyuracil (1.3 eq, 0.5 g, 3.2 mmol) and trimethylsilyl chloride (13eq, 2.7 mL, 32.0 mmol) containing a catalytic amount of $(NH_4)_2SO_4$ in 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 10 mL) was refluxed under nitrogen until a clear solution was obtained (3h). The solution was allowed to cool to room temperature and the HMDS removed under reduced pressure. The resulting solid was resuspended in anhydrous 1,2 dichloromethane (5 ml) and 1 (1.0 eq, 0.7 g, 2.5 mmol) was added. This suspension was treated with trimethylsilyl triflate (0.5 mL). The reaction mixture which became gradually a clear solution was allowed to stir for 8h. It was then evaporated under reduced pressure. An aqueous solution (5%) $NaHCO_3$ was added and the product extracted with ethyl acetate. The organic phases were washed with H_2O and dried over Na_2SO_4 to give after evaporation 0.83 g of white solid which was recrystallized in CH_2Cl_2. Yield (90%). TLC (CH_2Cl_2/MeOH 9:1) Rf = 0.15.

6α isomer: ^{1}H NMR (DMSO d_6) δ : 3.30-3.60 (m, 2H, 2H-2'), 4.20-4.30 (m, 1H, Ha-5' or Hb-5'), 4.50-4.60 (m, 1H, Ha-5' or Hb-5'), 5.50 (t, 1H, J= 3.78 Hz, H-4'), 6.20 (pseudo t, 1H, H-1'), 7.40-7.90 (m, 5H, H arom.), 8.30 (s, 1H, H-6), 12.00 (br s, 1H, COOH)

6β isomer: ^{1}H NMR (DMSO d_6) δ : 3.30-3.60 (m, 2H, 2H-2'), 4.20-4.30 (m, 1H, Ha-5' or Hb-5'), 4.50-4.60 (m, 1H, Ha-5' or Hb-5'), 5.90 (pseudo t, 1H, H-4'), 6.40 (dd, 1H, H-1'), 7.40-7.90 (m, 5H, H arom.), 8.50 (s, 1H, H-6), 12.00 (br s, 1H, COOH). Anal. ($C_{16}H_{14}N_2O_7S$) C, H, N. MS(LS/MS)$^+$ m/z 379 MH$^+$.

5-(2-N-*tert*-Butoxycarbonyl)-ethylamido-2',3'-dideoxy-5'-benzoyloxymethyl-3'-thiauridine 7α and 7β:

The 6α and 6β isomers (1 eq, 0.20 g, 0.52 mmol) were dissolved in anhydrous CH_2Cl_2 (5 mL). DCC (1.1 eq, 0.12 g, 0.58 mmol) and HOBT (1.1 eq, 0.08 g, 0.58 mmol) were added. The mixture was allowed to stir for 4h at room temperature under nitrogen. When a precipitation occured, aminoethylcarboxamide (1.1eq, 0.10 mL, 0.58 mmol) and N,N'-diisopropylethylenediamine (4eq, 0.48 mL, 2.40 mmol) were added. The solution was stirred for 5h, concentrated under reduced pressure, it was washed with H_2O (25 mL) and extracted with ethyl acetate. The resulting organic phases were successively washed with a 5% solution of citric acid (50 mL), a 10% sodium chloride solution (50 mL), and then dry with Na_2SO_4. The solution was concentrated under reduced pressure. Compounds 7α and 7β were obtained in a ratio 1:1 after purification by flash chromatography over silica gel using CH_2Cl_2/MeOH (96:4) as eluent. It gave 0.18 g of a white solid. Yield (65%). TLC (CH_2Cl_2/MeOH 9:1) Rf = 0.50. mp = 103-105°c.

7α isomer: ^{1}H NMR ($CDCl_3$) δ : 1.25 (s, 9H, tBu), 3.20-3.70 (m, 6H, 2H-2' and CH_2-CH_2), 4.30-4.55 (m, 1H, Ha-5' or Hb-5'), 4.65-4.80 (m, 1H, Ha-5' or Hb-5'), 4.90 (br s, 1H, NHCO), 5.50 (t, 1H, J= 4.43 Hz, H-4'), 6.35 (t, 1H, J= 5.13 Hz, H-1'), 7.40-8.10 (m, 5H, H arom.), 8.55 (s, 1H, H-6), 9.00 (s, 1H, NH-3).

7β isomer: ^{1}H NMR ($CDCl_3$) δ : 1.25 (s, 9H, tBu), 3.20-3.70 (m, 6H, 2H-2' and CH_2CH_2), 4.30-4.55 (m, 1H, Ha-5' or Hb-5'), 4.65-4.80 (m, 1H, Ha-5' or Hb-5'), 4.90 (br s, 1H, NHCO), 5.95 (pseudo t, 1H, H-4'), 6.50 (dd, 1H, H-1'), 7.40-8.10 (m, 5H, H arom.), 8.80 (s, 1H, H-6), 9.05 (s, 1H, NH-3).

5-(2-N-*tert*-Butoxycarbonyl)-ethylamido-2',3'-dideoxy-3'-thiauridine 8α and 8β:

A mixture of compounds 7α and 7β (0.083 g) was dissolved in a solution of NH_3/MeOH (5 mL) and stirred overnight at room temperature. After solvent evaporation the two isomers were separated over silica gel by flash column chromatography using CH_2Cl_2/MeOH (49:1) as eluent. It gave 0.060 g of colourless oil. Yield (87%).

<u>8α isomer</u>: TLC (CH_2Cl_2/MeOH 9:1) Rf = 0.39. ^{1}H NMR ($CDCl_3$) δ: 1.25 (s, 9H, tBu), 3.10-3.80 (m, 6H, 2H-2', 2H-5' and CH_2-CH_2), 5.20 (br s, 1H, NHCO), 5.50 (s, 1H, H-4'), 6.35 (pseudo t, 1H, H-1'), 8.55 (pseudo d, 1H, H-6), 9.00 (s, 1H, NH-3).
<u>8β isomer</u>: TLC (CH_2Cl_2/MeOH 9:1) Rf = 0.46. ^{1}H NMR ($CDCl_3$) δ: 1.25 (s, 9H, tBu), 3.20-3.50 (m, 6H, 2H-2' and -$(CH_2)_2$-), 3.80-4.20 (m, 2H, 2H-5'), 5.15 (s br, 1H, NHCO), 5.30 (pseudo t, 1H, H-4'), 6.30 (pseudo d, 1H, H-1'), 8.80 (s, 1H, H-6), 9.05 (s, 1H, NH-3). Anal. (C_{16} H_{24} N_4 O_7 S_1) C, H, N. MS(FAB+) m/z 417 MH^+.

5-(2-ethylamine)-amido-2',3'-dideoxy-3'-thiauridine <u>9α</u> and <u>9β</u>:
Compounds <u>8α</u> (0.018 g) and <u>8β</u> (0.020 g) were separately dissolved in CH_2Cl_2 (5 mL) with an excess of trifluoroacetic acid (2 mL). The solution was stirred for 1h under nitrogen until the starting material disappeared. It was then concentrated under reduced pressure to give a brown oil which was triturated with dichloromethane/hexane to give compounds <u>9α</u> (0.008 g), 68% and <u>9β</u> (0.015 g), 98%.
<u>9α isomer</u>: TLC (CH_2Cl_2/MeOH 9:4) Rf = 0.38. ^{1}H NMR (DMSO-d_6) δ: 2.85 (s br, 2H, NH_2), 3.15-3.60 (m, 8H, 2H-2', 2H-5' and CH_2-CH_2), 5.15 (br s, 1H, NHCO), 5.40 (t, 1H, J= 4.82 Hz, H-4'), 6.34 (dd, 1H, H-1'), 8.30 (s, 1H, H-6), 8.76 (t, 1H, J= 5.76 Hz, NH-3), 11.94 (s, 1H, OH).
<u>9β isomer</u>: TLC (CH_2Cl_2/MeOH 9:4) Rf = 0.50. ^{1}H NMR (DMSO-d_6) δ: 2.80 (s br, 2H, NH_2), 3.20-3.80 (m, 8H, 2H-2', 2H-5' and -$(CH_2)_2$-), 5.14 (t, 1H, J= 4.95 Hz, H-4'), 5.31 (br s, 1H, NHCO), 6.16 (pseudo d, 1H, H-1'), 8.58 (s, 1H, H-6), 8.75 (t, 1H, J= 5.85 Hz, NH-3), 11.92 (s, 1H, OH). Anal. ($C_{11}H_{16}N_4O_5S$) C, H, N. MS(FAB+) m/z 317 MH^+.

Virology

The representative compounds were tested *in vitro* for their abilities to inhibit HIV-1 infection in MT_4 cells culture. The fusogenic effect of HIV in the MT_4 cell line was determined as described by Rey *et al* (21,22). A total of 3×10^5 MT_4 cells were infected with 100 μl of diluted virus for 1 h at 37°C. After three washes, the infected cells were cultured in 24-well cell culture plates in the presence of the inhibitor. The appearance of syncitia was measured with an inverted optical microscope, 5 days after infection. The inhibitory concentration was expressed as the concentration of the tested compound which causes 50% inhibition of syncitia formation (IC_{50}) but was not toxic for the cells. For toxicity testing, three replication cultures of each uninfected MT_4 cells (2×10^5 cells) were incubated with various concentrations of 2',3'-dideoxy-3'-thiacytidine analogues. Cell viability was determined 6 days from drug addition by trypan blue exclusion.

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