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Studies on the Chemistry of Pyrimidine Derivatives with Dimethyldioxirane: Synthesis, Cytotoxic Effect and Antiviral Activity of New 5,6-Oxiranyl-5,6-dihydro and 5-Hydroxy-5,6-dihydro-6-substituted Uracil Derivatives and Pyrimidine Nucleosides.

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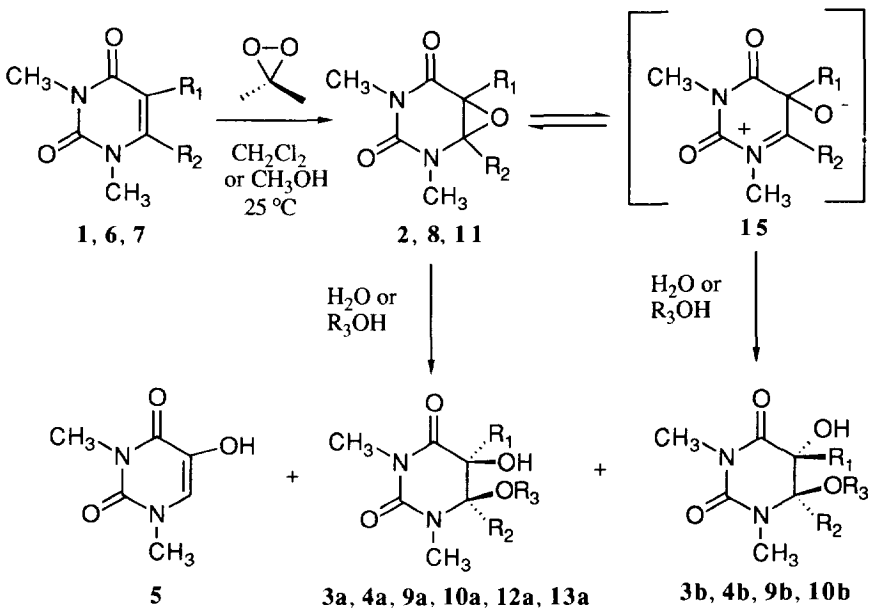
Abstract: The oxidation of uracil derivatives and pyrimidine nucleosides performed in CH_2Cl_2 with dimethyldioxirane afforded new 5,6-oxiranyl-5,6-dihydro and cis-/trans-5,6-dihydroxy-5,6-dihydro-derivatives. When the oxidations were performed in the presence of methanol as nucleophile cis- and trans-5-hydroxy-6-methoxy-5,6-dihydro derivatives were obtained in acceptable yields. Cis- and trans-1,3-dimethyl-5-hydroxy-6-alkylamino-5,6-dihydro uracils were obtained by nucleophilic ring opening of the 1,3-dimethyl-5,6-oxiranyl-5,6-dihydro uracil in the purified form. Interestingly some of the new title products revealed low cytotoxicity and selective antiviral activity against DNA and RNA Viruses. In particular, compound **17b** shows a strong and selective inhibition of the Sendai virus with lower effect on Herpes Simplex-1 virus. Compound **17b** is also able to slightly inhibit HIV-1 virus at high concentrations, but in this case a cytotoxic effect was observed.

Rational application of the selective chemical modifications of nucleic acids requires detailed knowledge of the mechanisms and of the reaction products with the monomeric units. Because of possible biological implications, the oxidative modifications of the nucleic acids and their components has been extensively studied.[1] It was noted[2] that in most cases the primary products of the oxidation are not well known because of the complexity of product mixture and the tendency of initial products to undergo further reactions.

As part of a project aimed to obtain selective modifications of nucleic acids, we have recently reported preliminary studies on the oxidation of pyrimidine[3] and purine derivatives[4] by dimethyldioxirane,[5] a powerful and selective oxidant which performs under strictly neutral conditions. Herein we describe extensively these data reporting the stereochemistry of the oxidation of pyrimidine derivatives in the presence of nucleophiles together with the interesting cytotoxic effect and antiviral activity of some of the new products obtained.

The oxidation of 1,3-dimethyl uracil **1** with a freshly prepared solution of dimethyldioxirane[6] performed in CH_2Cl_2 at 25°C afforded a mixture of three easily chromatographically separable products, 1,3-dimethyl-5,6-oxiranyl-5,6-dihydro uracil **2**, cis-1,3-dimethyl-5,6-dihydroxy-5,6-dihydro uracil **3a** and trans-1,3-dimethyl-5,6-dihydroxy-5,6-dihydro uracil **3b** (Scheme 1, Table 1, Entry 1). The isolation of the epoxide **2** is very interesting because Wang[7] showed that pyrimidine epoxides, never isolated before our recent synthesis,[3] may be formed as initial photooxidation products of nucleic acid components. The nucleophilic attack of pyrimidine epoxides offers an alternative mechanism for the formation of protein-nucleic acid cross-linkings probably involved carcinogenesis and mutagenesis. The formation of diols **3a-b** may result from the nucleophilic ring opening of epoxide **2** performed by the water present in the dioxirane solution. In fact, when the reaction was performed in presence of Na_2SO_4 as drying agent the yield of epoxide **2** increased (Table 1, Note b).

Scheme 1



1, 2: R_1=R_2= H. **3a-b**: R_1=R_2=R_3=H. **4a-b**: R_1=R_2= H, R_3= CH_3. **6, 8**: R_1= CH_3, R_2= H. **9a-b**: R_1= CH_3, R_2=R_3=H. **10a-b** : R_1=R_3= CH_3, R_2= H. **7, 11**: R_1= H, R_2= CH_3. **12a**: R_1=R_3=H, R_2=CH_3. **13a**: R_1= H, R_2=R_3= CH_3.

If the oxidative nucleophilic ring opening of epoxide **2** to afford 5-hydroxy-6-substituded-5,6-dihydro uracil derivatives was a general reaction, it might be a mild and efficient synthetic alternative to the known methods: in order to evaluate this point we studied the oxidation in the presence of alcohols and amines as nucleophiles. The oxidation of compound **1** in CH_3OH at 25°C yielded cis- and trans-1,3-dimethyl-5-hydroxy-6-methoxy-5,6-dihydro uracil derivatives **4a-b** as main products (Scheme 1, Table 1, Entry 2), and 1,3-dimethylisobarbituric acid **5** as by product (Table 1, Note c). Gentle heating of the cis-isomer **4a** in CH_2Cl_2 in the presence of a catalytic amount of pyridine afforded compound **5** in quantitative yield (Scheme 2), in accord

with the results reported by Duculomb[2a] on the dehydration of cis-5,6-dihydroxy-5,6-dihydro pyrimidines derivatives obtained by gamma irradiation of uridine in aqueous solution. The oxidation of **1** performed in CH_2Cl_2 at 25°C in the presence of CH_3NH_2 (2 mmol, 2.0 N solution in methyl alcohol) as nucleophile gave a complex mixture of reaction products; among them diols **3a-b**, and 6-methoxy derivatives **4a-b** were recovered in low yields. Probably, in this case the oxidation of the amine[8] proceded faster than that of the 5,6-double bond. These results are in contrast with the high selectivity that we have recently observed[9] in the oxidation of thionucleosides with dioxirane in the presence of alcohols and amines as nucleophiles.

Entry	Substrate	Product (s)	R_1	R_2	R_3	Ratio cis/trans[a]	Yield(%)
1	1	2	H	H	-		10 (50)[b]
		3a	H	H	H	3a/3b= 2/1	53
		3b	H	H	H		25
2	1[c]	4a	H	H	CH_3	4a/4b= 1/1	38
		4b	H	H	CH_3		35
3	6	8	CH_3	H	-		30
		9a	CH_3	H	H	9a/9b= 1/7	8
		9b	CH_3	H	H		57
4	6	10a	CH_3	H	CH_3	10a/10b= 1/5	45
		10b	CH_3	H	CH_3		40
5	7	11	H	CH_3	-		37
		12a	H	CH_3	H	only cis	53
6	7	13a	H	CH_3	CH_3	only cis	82

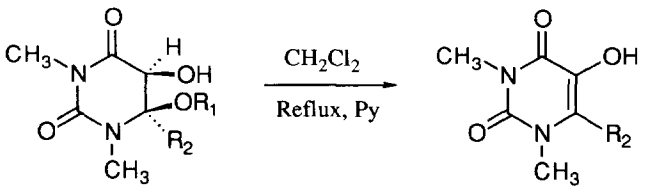
Table 1: Reaction of pyrimidine bases with dimethyldioxirane. All oxidations were performed with a freshy prepared solution of dioxirane (0.07 N acetone solution).[a] The ratio cis/trans was calculated after purification of the reaction mixture by flash-chromatography.[b] Yield of the 5,6-epoxide **2** obtained in the presence of Na_2SO_4 as drying agent in the reaction mixture.[c] 1,3-dimethyl isobarbituric acid **5** was obtained as by-product in 11% yield.

Attention was next turned to study the oxidation of 1,3,5-trimethyl uracil (1,3-dimethyl thymine) **6** and 1,3,6-trimethyl uracil **7**. The oxidation of **6** in CH_2Cl_2 at 25 °C afforded the 5,6-epoxide **8** and the trans-diol **9b** as main products, and the cis-diol **9a** as by-product (Scheme 1, Table 1, Entry 3). Moreover, the oxidation of **6** in CH_3OH at 25 °C gave a mixture of cis- and trans-1,3,5-trimethyl-5-hydroxy-6-methoxy-5,6-dihydro uracils **10a-b** (Scheme 1, Table 1, Entry 4). The oxidation of **7** performed in CH_2Cl_2 at 25°C yielded the 5,6-epoxide **11** and the cis-diol **12a** as only recovered products (Scheme 1, Table 1, Entry 5); while the oxidation of **7** performed in CH_3OH at 25 °C afforded cis-1,3,6-trimethyl-5-hydroxy-6-methoxy-5,6-dihydro uracil **13a** (Scheme 1, Table 1, Entry 6). Gentle heating of **13a** in CH_2Cl_2 in the presence of a catalytic amount of pyridine afforded 1,3,6-trimethyl-isobarbituric acid **14** in good yield (Scheme 2). Cis-diols **3a**, **9a**, and **12a** were found positive to metaperiodate/benzidine analysis,[2a] and their cis-stereochemistry was further confirmed by comparison with authentic samples prepared from **1**, **6**, and **7** by reaction with $KMnO_4$ or OsO_4 according to the methods reported in literature.[10]

It is noteworthy that the formation of the cis-products is inconsistent with the SN2 ring opening mechanism which should yield only trans-products. In accord with the previously reported hypothesis[11] it is

resonable to suggest that the reaction proceeds, in part, *via* an α-stabilized nitrogen cationic intermediate **15** (Scheme 1), in which a positive charge is localized on the C(6) and N(1) atoms of the uracil ring. Bond formation of C-6 with nucleophiles[12] would now be expected to proceed with energetically favorable cisoid[13] (gauche) stereochemistry to yield the cis-products.[14]

Scheme 2

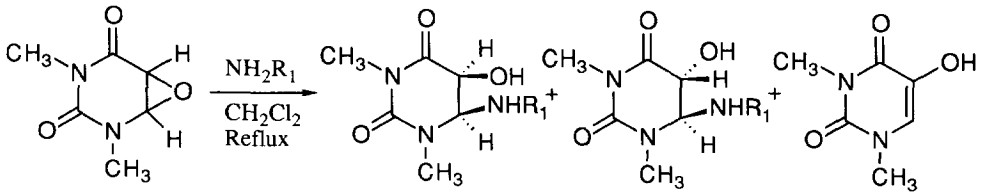


4a, 13a **5, 14**

4a: R_1=R_2= H. **5**: R_2= H. **13a**: R_1=R_2= CH_3. **14**: R_2= CH_3.

Moreover, some considerations about the effect of the methyl substituent on the 5,6-double bond on the reaction products might be done. The presence of a methyl group increases the stability of the 5,6-epoxides **8** and **11**, which were isolated in appreciable amounts even in absence of drying agents (Table 1), and further determines the stereochemistry of the oxiranyl ring opening. In fact, in the case of 5,6-epoxide **11** the nucleophilic attack on C-6 *via* SN2 mechanism is prevented by steric hindrance of the methyl group. Thus, the epoxide is stable enough to give cis-products as only isolated ring opening products. On the other hand, the 5,6-epoxide **8**, which lacks of C-6 substituent, is easily opened to give the trans-products, providing a better method than other previously reported procedures.[15]

Scheme 3



2 **16a** **16b, 17b** **5**

16a, 16b: R_1= CH_3. **17b**: R_1=CH_2CH_3.

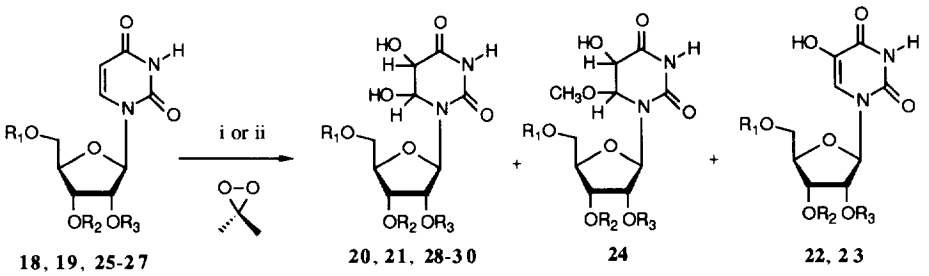
Finally, cis- and trans-1,3-dimethyl-5-hydroxy-6-alkylamino-5,6-dihydro uracils **16a-b** were obtained by nucleophilic ring opening of the epoxide **2** used in the purified form (Scheme 3). Treatment of epoxide **2** with methylamine and ethylamine in CH_2Cl_2 at reflux gave in the first case trans-1,3-dimethyl-5-hydroxy-6-

methylamino-5,6-dihydro uracil **16b** (42%) as main product, and cis-1,3-dimethyl-5-hydroxy-6-methylamino-5,6-dihydro uracil **16a** (28%) and **5** (23%) as by products; while only trans-1,3-dimethyl-5-hydroxy-6-ethylamino uracil **17b** (53%) and **5** (25%) were obtained in the second case. These data are in accord with the results reported by Yoneda[10] on the reaction of prepared *in situ* 1,3-dimethyl thymine epoxide with amines.

All new products obtained were tested as antiviral agents.[16] In particular, product **17b** shows an interesting broad antiviral activity against Sendai Virus (SV), Herpes Simplex-1 virus (HSV-1), and Human Immuno deficiency-1 Virus (HIV-1) [vide infra].

As a further approach to the study of the behaviour of dimethyldioxirane with more complex nucleic acid components we studied the oxidation of pyrimidine nucleosides **18** and **19** in order to enhance the synthetic utility of the procedure as well as to clarify the influence of the sugar moiety on the stereochemistry of the reaction. The oxidation of 2',3',5'-tri-O-acetyl uridine[17] **18** and 2',3',5'-tri-O-benzoyl uridine **19** performed in CH_2Cl_2 at 25 °C afforded cis-diols **20** and **21** (cis-diols were positive to metaperiodate/benzidine analysis and were recovered as chromatographically inseparable mixture of cis-(+) and cis-(-) isomers in the ratio cis-(+)/cis-(-)=1/1 determined by ^{1}H-NMR at 200 MHz) as main products and 2',3',5'-tri-O-acetyl- and 2',3',5'-tri-O-benzoyl- 5-hydroxy uridine derivatives **22** and **23** as by products (Scheme 4, Table 2, Entries 1 and 2).

Scheme 4



18, **20**, **22**, **24**: R_1=R_2=R_3= Ac. **19**, **21**, **23**: R_1=R_2=R_3= Bz. **25**, **28**: R_1=R_3= Tr, R_2=H. **26**, **29**: R_1=R_2= Tr, R_3=H. **27**, **30**: R_1= Tr, R_2=R_3=H.

i: Dimethyldioxirane (0.07 N acetone solution), CH_2Cl_2, 25°C. ii: Dimethyldioxirane (0.07 N acetone solution), CH_3OH, 25°C.

No trans-diols or 5,6-epoxides were recovered even in the presence of drying agents. On the other hand, the oxidation of **18** performed in CH_3OH at 25 °C afforded cis-2',3',5'-tri-O-acetyl-5,6-dihydro-5-hydroxy-6-methoxy uridine **24** (cis-(+) and cis-(-) mixture) as main product and **22** as by-product (Scheme 4, Table 2, Entry 3). By these results it is possible to suggest that a fully protected sugar moiety does not influence the formation of the two possible epoxides on the Re and Si faces of the 5,6-double bond (cis-(+) and cis-(-) isomers were recovered in the ratio cis-(+)/cis-(-)=1/1) but strongly affects the epoxide opening mechanism since no trans-diols were recovered.

The presence of only cis-diols in the oxidation of acetylated uridine derivatives, that is in contrast with the results previously reported for the oxidation of 1,3-dimethyl uracil **1**, might be tentatively interpreted

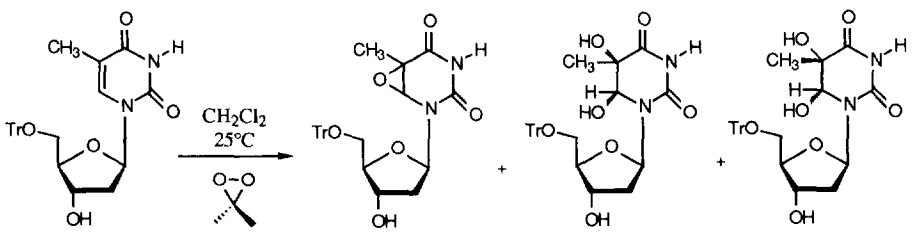
assuming that the sugar moiety might aid the oxiranyl ring opening. The effect of the C(5')-OH goup protected as methoxymethyl ether derivative (MOM) on the reactivity of pyrimidine nucleosides has been studied by Tanaka[18] who showed that this group is close enough to the C-6 position of the uracil ring to enhance its reactivity under lithiation conditions. It is resonable to suggest that a similar behaviour could be present also in the stabilization of the previously discussed α-nitrogen cationic intermediate to afford selectively cis-diols.

Entry	Substrate	Product (s)	R_1	R_2	R_3	Yield(%)
1	18	20	Ac	Ac	Ac	70
		22	Ac	Ac	Ac	18
2	19	21	Bz	Bz	Bz	65
		23	Bz	Bz	Bz	27
3	18	24	Ac	Ac	Ac	75
		22	Ac	Ac	Ac	11
4	25	28	Tr	H	Tr	63
5	26	29	Tr	Tr	H	69
6	27	30	Tr	H	H	57

Table 2: Reaction of pyrimidine nucleosides with dimethyldioxirane. All oxidations were performed with a freshy prepared solution of dioxirane (0.07 N acetone solution).

Furthermore, with the purpose to evaluate a possible influence of free C(2')-OH and/or C(3')-OH groups on the reaction pathway we studied the oxidation of partially protected 2',5'-O-ditrityl uridine **25**, 3',5'-di-O-trityl uridine **26**, and 5'-O-trityl uridine **27** easily prepared as described by Moffat.[19] The oxidation of **25**, **26** and **27** performed in CH_2Cl_2 at 25 °C gave cis-diols **28**, **29** and **30** (as mixture of cis-(+) and cis-(-) isomers in the ratio cis-(+)/cis-(-)=1/1) as only recovered products (Scheme 4, Table 2, Entries 4, 5, and 6). On the other hand, the oxidation of 5'-O-trityl thymidine[20] **31** performed in CH_2Cl_2 at 25 °C afforded the 5,6-epoxide **32** (10%), cis-diols **33** (43%), and trans-diols **34** (12%) [Scheme 5].[21]

Scheme 5



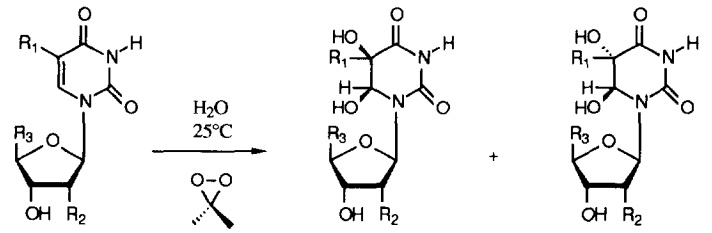
31 **32** (10%) **33** (43%) **34** (12%)

This result confirms that the presence of a methyl group as substituent on the C-5 position of the uracil ring stabilizes the epoxide enough to permit its isolation and the formation of the trans-diol, in accord with the results obtained for the oxidation of 1,3-dimethyl thymine **6**.

It is noteworthy that the reported oxidation of the 5,6-double bond was very selective. In fact, no C(3')-OH and/or C(2')-OH oxidation and the C(1')-H oxygen insertion on the ribosyl or 2'-deoxy ribosyl moieties were observed in spite of reported reactivity of alcohols[3] and acetal derivatives[22] with dioxirane.

Finally, with the purpose to investigate the influence of a free C(5')-OH on the stereochemistry of the oxidation, we started to study the reaction of unprotected uridine **35**, thymidine **36** and 5'-carboxy thymidine[23] **37** with dimethyldioxirane in water . The oxidation of uridine **35** by dioxirane in H_2O at 25 °C afforded the cis-diol **38** (Scheme 6, Table 3, Entry 1) as only recovered product as a chromatographically inseparable mixture of cis-(+) and cis-(-) isomers. However an unexpected diastereoselectivity was obtained in this case because the ratio between the cis-(+) and cis-(-) isomers, evaluated by ^{1}H-NMR (200 MHz) on the basis of the spectroscopic attribution reported by Duculomb,[2a] was cis-(+)/cis-(-)= 5/1.

Scheme 6



35, **38**: R_1=H, R_2= OH, R_3= CH_2OH. **36**, **39a-b**: R_1= CH_3, R_2= H, R_3= CH_2OH. **37**, **40a-b**: R_1= CH_3, R_2= H, R_3= COOH.

Probably, the presence of a free C(5')-OH on the ribosyl moiety is an important factor in the control of the trajiectory of the electrophilic approach of dioxirane[24, 25] to the 5,6-double bond of the uracil ring to give preferentially one of the two possible epoxides (not isolated in our case). The oxidation of thymidine **36** under similar reaction conditions gave the cis- and trans-diols **39a-b** (Table 3, Entry 2) in the ratio cis-/trans-=3/1. Moreover, the oxidation of the 5'-carboxy thymidine **37** afforded the cis- and trans-diols **40a-b** in the ratio cis-/trans- =1/1 (Table 3, Entry 3).[21]

Entry	Substrate	Product (s)	R_1	R_2	R_3	Ratio cis/trans[a]	Yield(%)
1	35	38	H	OH	CH_2OH	only cis	85
2	36	39a	CH_3	H	CH_2OH	39a/39b= 3/1	68
		39b	CH_3	H	CH_2OH		23
3	37	40a	CH_3	H	COOH	40a/40b= 1/1	37
		40b	CH_3	H	COOH		39

Table 3: Reaction of pyrimidine nucleosides with dimethyldioxirane in water. All oxidations were performed with a freshy prepared solution of dioxirane (0.07 N acetone solution).[a] The ratio cis/trans was calculated after purification of the reaction mixture by flash-chromatography.

The large amount of cis-diols observed in the oxidation of compounds **36** and **37**, that is not in accord with the data obtained for 1,3-dimethyl thymine **6** and 5'-O-trytil thymidine **31**, togheter with the observed variation in the ratio between cis- and trans-diols depending on the oxidation state of the C-5' atom, suggest that the nature of this group is really an important factor in the control of the stereochemistry of the reaction even if other possible factors, such as the polarity of the solvent can play, in this case, some role. Further work in this area is in progress in our laboratories.

Biological part

Products **2**, **3a-b**, **4a-b**, **9a-b**, **10a-b**, **13a**, **14**, **16a-b** and **17b** did not express any cytotoxic activity on all cell lines and lymphocytes analysed. Among the different products tested, compound **17b** was noted to be strong inhibitor of Sendai virus (SV) production measured by decreased haemagglutinin titre (HAU). Sendai virus is an enveloped RNA virus with six major structural proteins among wich haemagglutinin-neuroaminidase (HN). HN glycoprotein is a multifunctional molecule involved in several aspects of infection including attachment of the virus to receptors on host cells, cell fusion and agglutination of erythrocytes (haemagglutinating activity). Then, **17b**-induced reduction of haemagglutinating activity in the supernatants of infected cells indicates a decreased release of mature viral particicles. The dose-response effect of **17b** is shown in Fig.1(panel A). **17b** concentrations lower than 1 mg/ml had not effect on haemagglutinin production. At higher concentrations, the inhibitions were dose-dependent and reached maximum values (87.5% inhibition) at a concentration of 100 mg/ml. The maximal antiviral dose was not toxic for the cell, as confirmed by microscopic examination of the monolayers and by vital dye exclusion.

Compounds	HIV-1	O. D.[a]	Activity(%)[b]
None	NO	0.524	n.a.
None	Yes	0.116	n.a.
2 (400 μg/ml)	Yes	0.048	0
3b (400 μg/ml)	Yes	0.057	0
9b (400 μg/ml)	Yes	0.062	0
10b (400 μg/ml)	Yes	0.050	0
13a (100 μg/ml)	Yes	0.053	0
14 (400 μg/ml)	Yes	0.046	0
17b (100 μg/ml)	Yes	0.148	6.1

Table 4: Antiviral activity against HIV-1 virus. C8166 cells were exposed to TCID50 of the HIV-1 preparation in the presence or absence of fixed concentrations of different compounds and HIV mediated cytopathic effect was assessed spectrophotometrically.[a] O. D.=optical density. [b] The percent activity was calculated as: number of live cells in compound treated sample/ number of live cells in the control (HIV infected).

The inhibitory activity of **17b** against Herpes Simplex-1 virus (HSV-1) is reported in Fig. 2 (panel A). At concentration of 100 mg/ml **17b** was able to inhibit HSV-1 replication by 98%, without any toxic effect on uninfected cells. Differently from Sendai virus (in which a dose of 10 mg/ml was able to give a 50% inhibition

of viral replication) HSV-1 seems to be quite more resistant. However, in both of the cases the comparison of the growth kinetics of viruses in control-infected and **17b**-treated cultures proved that the inhibition was stable as long as 72 hours post infection and it was not just a delay in virus burst (Fig. 1 and 2, panels B). The anti-HIV activity of some of the product tested is reported in Tab.4. Only product **17b** at the highest concentration is able to slightly inhibit HIV-1 replication in C8166 cells although at that concentration a cytotoxic effect was observed.

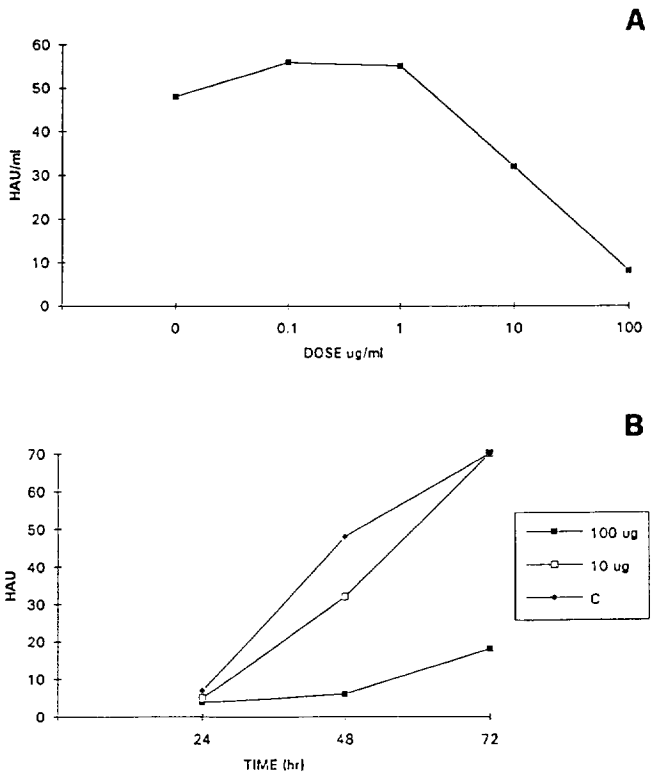


Fig. 1: Effect of compound **17b** on Sendai virus (SV) replication. Panel A: Dose-response curve at 48 hrs after virus challenge. Panel B: Time dependent activity at concentration of 10 μg/ml and 100 μg/ml. C= Control.

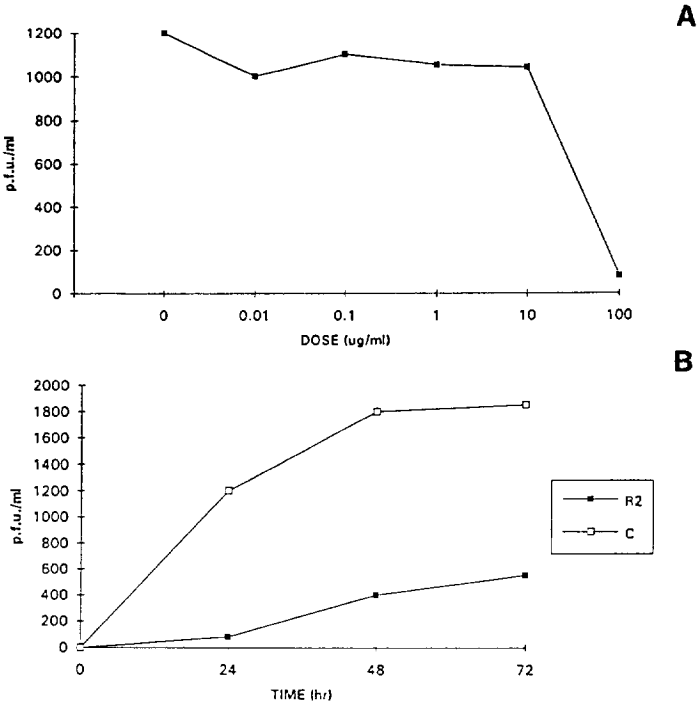


Fig. 2: Effect of compound **17b** on HSV-1 replication. Panel A: Dose-response at 24 hrs after virus challenge. Panel B: Time dependent activity. R2= concentration of 100 μg/ml. C= Control.

Experimental

NMR spectra were recorded on a Bruker (200 MHz) spectrometer and are reported in δ values. Infrared spectra were recorded on a Perkin Elmer 298 spectrophotometer using NaCl plates. Microanalyses were performed by C. Erba 1106 analyzer. Mass spectra were recorded on a VG 70/250S spectrometer with an electron beam of 70 eV. Melting points were obtained on a Reichert Kofler apparatus and are uncorrected. All solvents were ACS reagent grade and were redistilled and dried according to standard procedures. Chromatographic purifications were performed on columns packed with Merck silica gel 60, 230-400 mesh for flash technique. Thin-layer chromatography was carried out using Merck platten Kieselgel 60 F254.

Starting Compounds

Commercially available uridine **35** and thymidine **36** (Aldrich, Co.) were used without further purification. 1, 3-Dimethyl uracil **1**, 1,3,5-trimethyl uracil **6** and 1,3,6-trimethyl uracil **7** were synthesized according to the procedure reported by Allen;[26] 2',3',5'-tri-O-acetyl uridine **18** and 2',3',5'-tri-O-benzoyl uridine **19** were synthesized according to the procedure reported by Fox;[17] 2',5'-di-O-trityl uridine **25**, 3',5'-di-O-trityl uridine

26, and 5'-O-trityl uridine **27** were synthesized according to the procedure reported by Moffat;[19] 5'-carboxy thymidine **37** was synthesized according to the procedure reported by Zemlikca.[23]

Oxidation of compounds **1**, **6**, **7**, **18**, **19**, **25-27**, **31**, and **35-37** with dimethyldioxirane. General procedure. The dimethyldioxirane solution was prepared using the procedure reported by Adam[6] and the dioxirane content (Ca. 0.07N) was assayed with methyl-phenyl-sulfide yielding the corresponding sulfoxide; the latter being determined by ^{1}H-NMR. The reactions were carried out by adding freshly prepared solution of the dioxirane to solutions of the required substrate (2 mmol) in the appropriate solvent (5 ml) at 25°C, until the substrate disappeared (TLC chloroform:methanol=9.0:1.0), and the resulting solution was concentrated in vacuo. Cis-diols were positive to metaperiodate/benzidine analysis (0.1% sodium metaperiodate water solution, 70% benzidine water/ethanol solution).[2a, 27] The residue was purified by flash-chromatography using chloroform:methanol=9.0:1.0 as eluant.

1,3-Dimethyl-5,6-oxyranil-5,6-dihydro uracil **2**- (156 mg, 50%), oil. I.R. ($CHCl_3$) ν_{max}: 1650 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 3.12 (3H, s, CH_3), 3.16 (3H, s, CH_3), 4.30 (1H, d, J 3.0 Hz, H-5), 4.92 (1H, d, J 3.0 Hz, H-6). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ_C ppm: 171.1 (C), 153.2 (C), 80.2 (CH), 68.7 (CH), 34.6 (CH_3), 27.5 (CH_3); m/z 156 (M^+, 19%).

Cis-1,3-Dimethyl-5,6-dihydroxy-5,6-dihydro uracil **3a**- (184 mg, 53%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1680 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 3.05 (3H, s, CH_3), 3.20 (3H, s, CH_3), 4.40 (1H, d, J 6.0 Hz, H-5), 5.25 (1H, d, J 6.0 Hz, H-6). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ_C ppm: 170.1 (C), 151.4 (C), 80.3 (CH), 69.0 (CH), 34.9 (CH_3), 29.2 (CH_3); m/z 174 (M^+, 23%).

Trans-1,3-Dimethyl-5,6-dihydroxy-5,6-dihydro uracil **3b**- (87 mg, 25%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1680 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 3.05 (3H, s, CH_3), 3.15 (3H, s, CH_3), 4.12 (1H, d, J 4.0 Hz, H-5), 4.75 (1H, d, J 4.0 Hz, H-6). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ_C ppm: 170.1 (C), 152.4 (C), 81.5 (CH), 70.1 (CH), 32.9 (CH_3), 27.8 (CH_3); m/z 174 (M^+, 23%).

Cis-1,3-dimethyl-5-hydroxy-6-methoxy-5,6-dihydro uracil **4a**- (143 mg, 38%), oil, molecular distillation 129 °C/1 mm (bath temperature) [lit.;[7b] molecular distillation of the mixture 3a/3b 130 °C (bath temperature)]. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1650 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 3.16 (6H, s, CH_3), 3.48 (3H, s, OCH_3), 4.35 (1H, d, J 5.0 Hz, H-5), 4.56 (1H, d, J 5.0 Hz, H-6). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ_C ppm: 170.2 (C), 152.4 (C), 89.6 (CH), 68.1 (CH), 56.6 (OCH_3) 35.5 (CH_3), 27.6 (CH_3); m/z 188 (M^+, 16%).

Trans-1,3-dimethyl-5-hydroxy-6-methoxy-5,6-dihydro uracil **4b**- (132 mg, 35%), oil, molecular distillation 129 °C/1 mm (bath temperature) [lit.;[7b] molecular distillation of the mixture 3a/3b 130 °C (bath temperature)]. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1650 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 3.12 (3H, s, CH_3), 3.13 (3H, s, CH_3), 3.41 (3H, s, OCH_3), 4.18 (1H, d, J 3.5 Hz, H-5), 4.41 (1H, d, J 3.5 Hz, H-6). ^{13}C-NMR ($CDCl_3$, 200 MHz) δ_C ppm: 170.8 (C), 152.7 (C), 88.7 (CH), 69.1 (CH), 58.3 (OCH_3) 36.7

(CH_3), 27.8 (CH_3); m/z 188 (M^+, 22%).

1,3-Dimethyl isobarbituric acid **5**- (34 mg, 11%), m. p. 197-199 °C [lit.;[28] m.p. 198-199 °C]. Anal. Calcd. for $C_6H_8N_2O_3$: C, 46.15; H, 5.16; N, 17.94. Found: C, 46.19; H, 5.15; N, 17.77. I.R. ($CHCl_3$) ν_{max}: 3550 (OH), 1700 (CO) and 1640 (α,β-unsaturated ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 3.36 (3H, s, CH_3), 3.38 (3H, s, CH_3), 6.85 (1H, s, H-6); m/z 156 (M^+, 32%).

1,3,5-Trimethyl-5,6-oxyranil-5,6-dihydro uracil **8**- (102 mg, 30%), oil. I.R. ($CHCl_3$) ν_{max}: 1650 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.37 (3H, s, CH_3), 3.0 (3H, s, CH_3), 3.08 (3H, s, CH_3), 4.44 (1H, s, H-6); m/z 170 (M^+, 19%).

Cis-1,3,5-trimethyl-5,6-dihydroxy-5,6-dihydro uracil **9a**- (30 mg, 8%), m.p. 94-96 °C [lit.;[29] m.p. 95-96 °C]. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1680 (CO) cm^{-1}. Anal. Calcd. for $C_7H_{12}N_2O_4$: C, 44.67; H, 6.43; N, 14.89. Found: C, 44.73; H, 6.53; N, 14.93. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.39 (3H, s, CH_3), 3.14 (3H, s, CH_3), 3.17 (3H, s, CH_3), 4.65 (1H, s, H-6); m/z 188 (M^+, 32%).

Trans-1,3,5-trimethyl-5,6-dihydroxy-5,6-dihydro uracil **9b**- (214 mg, 57%), m.p. 94-96 °C [lit.;[29] m.p. 95-96 °C]. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1670 (CO) cm^{-1}. Anal. Calcd. for $C_7H_{12}N_2O_4$: C, 44.67; H, 6.43; N, 14.89. Found: C, 44.55; H, 6.40; N, 14.72. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.35 (3H, s, CH_3), 3.05 (3H, s, CH_3), 3.10 (3H, s, CH_3), 4.51 (1H, s, H-6); m/z 188 (M^+, 37%).

Cis-1,3,5-trimethyl-5-hydroxy-6-methoxy-5,6-dihydro uracil **10a**- (166 mg, 45%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1680 (C0) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.44 (3H, s, CH_3), 3.13 (3H, s, CH_3), 3.17 (3H, s, CH_3), 3.43 (3H, s, OCH_3), 4.22 (1H, s, H-6); m/z 202 (M^+, 24%).
Trans-1,3,5-trimethyl-5-hydroxy-6-methoxy-5,6-dihydro uracil **10b**- (147 mg, 40%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1680 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.41 (3H, s, CH_3), 3.20 (6H, s, CH_3), 3.52 (3H, s, OCH_3), 4.26 (1H, s, H-6); m/z 202 (M^+, 31%).

1,3,6-Trimethyl-5,6-oxyranil-5,6-dihydro uracil **11**- (126 mg, 37%), oil. I.R. ($CHCl_3$) ν_{max}: 1650 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 2.20 (3H, s, CH_3), 3.30 (3H, s, CH_3), 3.36 (3H, s, CH_3), 5.59 (1H, s, H-5); m/z 170 (M^+, 39%).

Cis-1,3,6-trimethyl-5,6-dihydroxy-5,6-dihydro uracil **12a**- (199 mg, 53%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1675 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.63 (3H, s, CH_3), 3.08 (3H, s, CH_3), 3.20 (3H, s, CH_3), 4.07 (1H, s, H-5); m/z 188 (M^+, 32%).

Cis-1,3,6-trimethyl-5-hydroxy-6-methoxy-5,6-dihydro uracil **13a**- (302 mg, 82%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1675 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.64 (3H, s, CH_3), 3.11 (3H, s, CH_3), 3.17 (3H, s, CH_3), 3.18 (3H, s, OCH_3), 4.10 (1H, s, H-5); m/z 202 (M^+, 15%).

Cis-2',3',5'-tri-O-acetyl-5,6-dihydroxy-5,6-dihydro uridine **20**- (566 mg, 70%), oil; m/z 404 (M^+, 43%).

Cis-diol **20** was obtained as chromatographically inseparable mixture of cis-(+) and cis-(-) in the ratio cis-(+)/cis-(-)=1/1 calculated by ^{1}H-NMR. ^{1}H-NMR of one isomer of **20**: (CDCl3, 200 MHz) δ_H ppm: 2.0 (9H, s, CH3), 4.11-4.18 (3H, m, H-4',5',5"), 4.29 (1H, d, J 3.6 Hz, H-6), 5.16 (1H, d, J 3.6 Hz, H-5), 5.18-5.24 (1H, m, H-3'), 5.30-5.40 (1H, m, H-2'), 5.84 (1H, d, J 5.1 Hz, H-1'). ^{1}H-NMR of the other isomer of **20**: (CDCl3, 200 MHz) δ_H ppm: 2.0 (9H, s, CH3), 4.11-4.18 (3H, m, H-4',5',5"), 4.24 (1H, d, J 3.7 Hz, H-6), 5.11 (1H, d, J 3.7 Hz, H-5), 5.18-5.24 (1H, m, H-3'), 5.40-5.90 (1H, m, H-2'), 5.78 (1H, d, J 5.5 Hz, H-1').

Cis-2',3',5'-tri-O-benzoyl-5,6-dihydroxy-5,6-dihydro uridine **21**- (705 mg, 65%), oil; m/z 558 (M^+, 27%). Cis-diol **21** was obtained as chromatographically inseparable mixture of cis-(+) and cis-(-) in the ratio cis-(+)/cis-(-)=1/1 calculated by ^{1}H-NMR. ^{1}H-NMR of one isomer of **21**: (CDCl3, 200 MHz) δ_H ppm: 4.36 (1H,d, J 4.0 Hz, H-6), 4.58-4.74 (3H, m, H-4',5',5"), 5.42 (1H, d, J 3.6 Hz, H-5), 5.82 (1H, t, J= 4,8 Hz, H-3'), 5.83-5.88 (1H, m, H-2'), 6;27 (1H, d, J 2.4 Hz, H-1'), 7.20-8.00 (15H, m, Ph). ^{1}H-NMR of the other isomer of **21**: (CDCl3, 200 MHz) δ_H ppm: 4.44 (1H, d, H-5), 4.58-4.74 (3H, m, H-4',5',5"), 5.36 (1H, d, J 3.5 Hz, H-5), 5.79 (1H, t, J 5.1 Hz, H-3'), 5.83-5.88 (1H, m, H-2'), 6.24 (1H, d, J 2.2 Hz, H-1'), 7.20-8.00 (15H, m, Ph).

2',3',5'-tri-O-acetyl-5-hydroxy uridine **22** and 2',3',5'-tri-O-benzoyl-5-hydroxy uridine **23**- Products 22 and 23 were characterized as 5-hydroxy uridine after deacylation with ammonia (2N methanol solution) in CH2Cl2 at 25 °C. m.p. 238-240 °C [lit.;[30] m.p. 238-240 °C]. Anal. Calcd. for $C_9H_{12}N_2O_7$: C, 41.54; H, 4.65; N, 10.76. Found: C, 41.60; H, 4.65; N, 10.80 ; m/z 260 (M^+, 13%).

Cis-2',3',5'-tri-O-acetyl-5,6-dihydro-5-hydroxy-6-methoxy uridine **24**- (627 mg, 75%), oil; m/z 418 (M^+, 31%). Product **24** was obtained as chromatographically inseparable mixture of cis-(+) and cis-(-) in the ratio cis-(+)/cis-(-)=1/1 calculated by ^{1}H-NMR. ^{1}H-NMR of one isomer of **24**: (CDCl3, 200 MHz) δ_H ppm: 2.10 (9H, s, CH3), 3.40 (3H, s, CH3), 4.18-4.30 (3H, m, H-4',5',5"), 4.40 (1H, d, J 4.0 Hz, H-5), 5.18-5.30 (3H, m, H-6, 3', 2'), 5.80 (1H, d, J 4.0 Hz, H-1'). ^{1}H-NMR of the other isomer of **24**: (CDCl3, 200 MHz) δ_H ppm: 2.10 (9H, s, CH3), 3.50 (3H, s, CH3), 4.18-4.30 (3H, m, H-4',5',5"), 4.43 (1H, d, J 4.0 Hz, H-5), 5.18-5.30 (3H, m, H-6, 3', 2'), 5.65 (1H, d, J 4.0 Hz, H-1').

Cis-2',5'-di-O-trityl-5,6-dihydroxy-5,6-dihydro uridine **28**- (960 mg, 63%), oil; m/z 762 (M^+, 74%). Cis-diol **28** was obtained as chromatographically inseparable mixture of cis-(+) and cis-(-) in the ratio cis-(+)/cis-(-)=1/1 calculated by ^{1}H-NMR. ^{1}H-NMR of one isomer of **28**: (CDCl3, 200 MHz) δ_H ppm: 2.93-3.10 (3H, m, H-4', 5', 5"), 3.67-3.70 (1H, m, H-3'), 4.0 (1H, d, J 5.0 Hz, H-5), 4.44-4.52 (1H, m, H-2'), 5.16 (1H, d, J 5.0 Hz, H-6), 6.56 (1H, d, J 7.3 Hz, H-1'), 7.15-7.51 (30H, m, Ph). ^{1}H-NMR of the other isomer of **28**: (CDCl3, 200 MHz) δ_H ppm: 2.93-3.10 (3H, m, H-4', 5', 5"), 3.67-3.70 (1H, m, H-3'), 4.0 (1H, d, J 5.0 Hz, H-5), 4.44-4.52 (1H, m, H-2'), 5.10 (1H, d, J 5.0 Hz, H-6), 6.60 (1H, d, J 7.3 Hz, H-1'), 7.15-7.51 (30H, m, Ph).

Cis-3',5'-di-O-trityl-5,6-dihydroxy-5,6-dihydro uridine **29**- (1.05 g, 69%), oil; m/z 762 (M^+, 59%). Cis-diol **27** was obtained as chromatographically inseparable mixture of cis-(+) and cis-(-) in the ratio cis-(+)/cis-(-)=1/1

calculated by ^{1}H-NMR. ^{1}H-NMR of one isomer of **29**: ($CDCl_3$, 200 MHz) δ_H ppm: 2.50-3.17 (3H, m, H-4', 5', 5"), 3.90-4.10 (2H, m, H-2', 3'), 4.29 (1H, d, J 3.6 Hz, H-5), 5.18 (1H, d, J 3,6 Hz, H-6), 5.93 (1H, m, H-1'), 7.0-7.60 (30H, m, Ph). ^{1}H-NMR of the other isomer of **29**: ($CDCl_3$, 200 MHz) δ_H ppm: 2.50-3.17 (3H, m, H-4', 5', 5"), 3.90-4.10 (2H, m, H-2', 3'), 4.21 (1H, d, J 3.7 Hz, H-5), 5.29 (1H, d, J 3.7 Hz, H-6), 5.82 (1H, d, J 5.12 Hz, H-1'), 7.0-7.60 (30H, m, Ph).

Cis-5'-O-trityl-5,6-dihydroxy-5,6-dihydro uridine **30**- (593 mg, 57%), oil; m/z 520 (M^+, 59%). Cis-diol **30** was obtained as chromatographically inseparable mixture of cis-(+) and cis-(-) in the ratio cis-(+)/cis-(-)=1/1 calculated by ^{1}H-NMR. ^{1}H-NMR of one isomer of **30**: ($CDCl_3$, 200 MHz) δ_H ppm: 3.19-3.33 (3H, m, H-4', 5', 5"), 3.90-4.25 (3H, m, H-2', 3', 5), 5.23 (1H, d, J 4.0 Hz, H-6), 5.73 (1H, d, J 4.0 Hz, H-1'), 7.19-7.48 (15H, m, Ph). ^{1}H-NMR of the other isomer of **30**: ($CDCl_3$, 200 MHz) δ_H ppm: 3.19-3.33 (3H, m, H-4', 5', 5"), 3.90-4.25 (3H, m, H-2', 3', 5), 5.60 (1H, d, J 3.6 Hz, H-6), 5.77 (1H, d, J 4.0 Hz, H-1'), 7.19-7.48 (15H, m, Ph).

5'-O-Trityl-5,6-oxiranyl-5,6-dihydro thymidine **32**- (94.8 mg, 10%), oil. I.R. ($CHCl_3$) ν_{max}: 1720 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.40 (3H, s, CH_3), 2.20 (2H, m, H-2', 2"), 3.28-3.35 (2H, m, H-5', 5"), 3.75 (1H, m, H-4'), 4.30 (1H, m, H-3'), 6.05 (2H, m, H-1', 6), 7.10-7.50 (15H, m, Ph). m/z 500 (M^+, 5%).

Cis-5'-O-trityl-5,6-dihydroxy-5,6-dihydro thymidine **33**- (414 mg, 43%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1720 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.43 (3H, s, CH_3), 2.01 (2H, m, H-2', 2"), 3.28-3.35 (2H, m, H-5', 5"), 3.75 (1H, m, H-4'), 4.33 (1H, m, H-3'), 6.15 (2H, m, H-1', 6), 7.10-7.50 (15H, m, Ph). m/z 518 (M^+, 77%).
Trans-5'-O-trityl-5,6-dihydroxy-5,6-dihydro thymidine **34**- (116 mg, 12%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 1720 (CO) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.43 (3H, s, CH_3), 2.15 (2H, m, H-2', 2"), 3.28-3.35 (2H, m, H-5', 5"), 3.75 (1H, m, H-4'), 4.30 (1H, m, H-3'), 6.05 (2H, m, H-1', 6), 7.23-7.42 (15H, m, Ph). m/z 518 (M^+, 23%).

Cis-5,6-dihydroxy-5,6-dihydro uridine **38**- (473 mg, 85%), oil; m/z 278 (M^+, 11%). Cis-diol **38** was obtained as chromatographically inseparable mixture of cis-(+) and cis-(-) in the ratio cis-(+)/cis-(-)=5/1 calculated by ^{1}H-NMR on the basis of data reported by Ducolomb.[2a] ^{1}H-NMR of cis-(+) isomer of **38**: (D_2O, 200 MHz) δ_H ppm: 3.70-4.68 (2H, m, H-5',5"), 4.07 (1H, m, H-4'), 4.19 (1H, m, H-3'), 4.30 (1H, m, H-2'), 4.68 (1H, d, J 3.5 Hz, H-5), 5.39 (1H, d, J 3.5 Hz, H-6), 5.74 (1H, d, J 5.78 Hz, H-1') . ^{1}H-NMR of the cis-(-) isomer of **38**: (D_2O, 200 MHz) δ_H ppm: 3.73-4.60 (2H, m, H-5',5"), 4.01 (1H, m, H-4'), 4.18 (1H, m, H-3'), 4.39 (1H, m, H-2'), 4.60 (1H, d, J 3.5 Hz, H-5), 5.36 (1H, d, J 3.5 Hz, H-6), 5.72 (1H, d, J 5.78 Hz, H-1') .

Cis-5,6-dihydroxy-5,6-dihydro thymidine **39a**- Product **39a** was active to metaperiodate/benzidine[2a] analysis and was identical to an authentic sample prepared by oxidation of thymidine with $KMnO_4$;[10] (375 mg, 68%), m. p. 190-192 °C (dec.) [lit.;[31, 16] m.p. 191-193 °C (dec.)]. Anal. Calcd. for $C_{10}H_{16}N_2O_7$: C, 40.92; H, 6.10; N, 10.60. Found: C, 41.05; H, 6.18; N, 10.47. ^{1}H-NMR (CD_3OD, 200 MHz) δ_H ppm: 1.45 (3H, s, CH_3),

2.10-2.50 (2H, m, H-2', 2"), 3.60-3.90 (3H, m, H-4', 5', 5"), 4.30 (1H, m, H-3'), 4.90 (1H, s, H-6), 6.05-6.40 (1H, dd, J 6.0 Hz and J 8.0 Hz, H-1'). m/z 276 (M^+, 13%).

Trans-5,6-dihydroxy-5,6-dihydro thymidine **39b**- Product **39b** was not active to metaperiodate/benzidine[2a] analysis; (127 mg, 23%), m. p. 198-200 °C (dec.) [lit.;[31, 16] m.p. 196-200 °C (dec.), 195-197 °C].Anal. Calcd. for $C_{10}H_{16}N_2O_7$: C, 40.92; H, 6.10; N, 10.60. Found: C, 41.11; H, 6.15; N, 10.72. ^{1}H-NMR (CD_3OD, 200 MHz) δ_H ppm: 1.33 (3H, s, CH_3), 2.10-2.50 (2H, m, H-2', 2"), 3.60-3.90 (3H, m, H-4', 5', 5"), 4.30 (1H, m, H-3'), 5.0 (1H, s, H-6), 6.10-6.40 (1H, dd, J 6.87 Hz and J 6.9 Hz, H-1'). m/z 276 (M^+, 24%).

Cis-5'-carboxy-5,6-dihydroxy-5,6-dihydro thymidine **40a**- Product **40a** was active to metaperiodate/benzidine analysis;[2a, 27] (215 mg, 37%), m. p. 200-202 °C (dec.). Anal. Calcd. for $C_{10}H_{14}N_2O_8$: C, 38.85; H, 5.07; N, 10.07. Found: C, 38.73; H, 5.15; N, 9.96. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.32 (3H, s, CH_3), 2.20 (2H, m, H-2', 2"), 3.60-3.85 (1H, m, H-4'), 4.30 (1H, m, H-3'), 4.95 (1H, s, H-6), 6.20 (1H, t, J 7.7 Hz, H-1'). m/z 290 (M^+, 34%).

Trans-5'-carboxy-5,6-dihydroxy-5,6-dihydro thymidine **40b**- Product **40b** was not active to metaperiodate/benzidine analysis;[2a, 27] (226 mg, 39%), m. p. 198-200 °C (dec.). Anal. Calcd. for $C_{10}H_{14}N_2O_8$: C, 38.85; H, 5.07; N, 10.07. Found: C, 38.78; H, 5.20; N, 10.18. ^{1}H-NMR (CD_3OD, 200 MHz) δ_H ppm: 1.35 (3H, s, CH_3), 2.15 (2H, m, H-2', 2"), 3.36-3.39 (1H, m, H-4'), 4.33 (1H, m, H-3'), 4.80 (1H, s, H-6), 6.25 (1H, t, J 7.7 Hz, H-1'). m/z 290 (M^+, 21%).

Synthesis of 1,3-dimethyl isobarbituric acid **5** and 1,3,6-trimethyl isobarbituric acid **14**-General procedure. Cis-isomer **4a** and **13a** (1 mmol) in in CH_2Cl_2 (10 ml) was refluxed until complete disappearance of substrate (TLC eluent chloroform:methanol=9.0:1.0) and the residue was purified by flash-chromatography using chloroform:methanol=9.0:1.0 as eluant.

1,3,6-Trimethyl-isobarbituric acid **14**- (309 mg, 91%), oil. I.R. ($CHCl_3$) ν_{max}: 3550 (OH), 1700 (CO) and 1640 (α,β-unsaturated ketone) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 2.25 (3H, s, CH_3), 2.75 (3H, s, CH_3), 3.05 (3H, s, CH_3); m/z 170 (M^+, 42%).

Synthesis of cis- and trans-1,3-dimethyl-5-hydroxy-6-alkylamino-5,6-dihydro uracils **16a-b** and **17b**. General procedure. The amine (2.0 mmol) was added to a solution of 1,3-dimethyl-5,6-oxyranil-5,6-dihydrouracil **2** (156 mg, 1.0 mmol) in CH_2Cl_2 (10 ml) at reflux until complete disappearance of substrate (TLC eluent chloroform:methanol=9.0:1.0). The solution was evaporated to dryness. The residue was purified by flash-chromatography using chloroform:methanol=9.0:1.0 as eluant.

Cis-1,3-dimethyl-5-hydroxy-6-methylamino-5,6-dihydro uracil **16a**- (52 mg, 28%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 3300 (NH), 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 3.30 (3H, s, CH_3), 3.42 (3H, s, CH_3), 3.54 (1H, d, J 4.0 Hz, H-5), 3.75 (3H, s, CH_3), 4.60 (1H, d, J 4.0 Hz, H-6); m/z 187 (M^+, 37%).

Trans-1,3-dimethyl-5-hydroxy-6-methylamino-5,6-dihydro uracil **16b**- (147 mg, 42%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 3300 (NH), 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 3.30 (3H, s, CH_3), 3.38-3.45 (4H, m, CH_3 and H-5), 3.75 (3H, s, CH_3), 4.64 (1H, b.s., H-6); m/z 187 (M^+, 24%).

Trans-1,3-dimethyl-5-hydroxy-6-ethylamino-5,6-dihydro uracil **17b**- (106 mg, 53%), oil. I.R. ($CHCl_3$) ν_{max}: 3600 (OH), 3300 (NH), 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H ppm: 1.57 (3H, m, CH_3), 2.79 (2H, m, CH_2), 3.26 (3H, s, CH_3), 3.34 3.26 (3H, s, CH_3), 3.75 (1H, b.s., H-5), 4.71 (1H, b.s., H-6); m/z 201 (M^+, 12%).

Biological methods

Materials and methods

C8166 is a T4 cell line infected with human T lymphocytotropic virus type 1 (HTLV-I) particularly sensitive to the cytopathic effect of HIV-1, Jurkat is a human T cell line, HL60 is a human promyelocytic cell line, U937 is a human histiocytic lymphoma, Madin Darby (MDCK) is a canine kidney cell line. A lymphocytotropic strain of HIV-1 (HTLV strain IIIB, also called HIVIIIB) was used to infect C8166 cells. The strain is available through the AIDS Research and Reference Reagent Program (NIH, Bethesda, MD). Stock of Parainfluenza-1 (Sendai) virus were prepared by allantoic inoculation of 10-days-old embryonated eggs with 0.2 ml of a 1:1000 dilution of infected allantoic fluid, which was harvested after 72 hours at 37°C, clarified by centrifugation and stored at - 80°C. Virus HSV type 1 clinically isolatyte TV1 was grown and titered in MDCK cells.

Cells lines and lymphocyte preparation and activation

Cell lines in exponential phase were washed twice in RPMI 1640, resuspensed in RPMI 1640 with 50 units/ml, 50 μg/ml streptomycin and 2 mM L-glutamine (all reagents from Flow Laboratories, Milan, Italy), supplemented with 10% endoxin-free fetal calf serum (Hyclone Laboratories, Logan UT) at a concentration of 200,000 cell/ml and plated out in 96/well microtiter plates at a final concentration of 20,000 cells/well in presence of serial dilutions of different compounds in DMSO. Control samples with DMSO alone were also performed. Each point was performed in quadruplicate. One or two days later, 1 μCi/well tritiated thymidine (Amersham International plc, Amersham, U.K.) in 20 μl complete culture medium was added to each well. Eight hours later, cells were harvested by a semiautomated cell harvester. Radioactivity was measured as counts per minute (CPM) in a liquid scintillation counter (LKB Wallac, Turku, finland). Alternatively, some wells from all groups were daily harvested and living cells were counted in a haemocytometer. Each experiment was performed not less than three times with consistent results. Peripheral blood samples collected from healthy donors were separated from heparinized whole blood on a Ficoll-Hypaque gradient as described by Boyum.[32] Mononuclear cells collected from the interface were washed twice in RPMI 1640 containing 2% FCS and suspended in complete medium. Proliferation test in the presence of products were performed as described by Scatena.[33]

Virus infection and assay for Antiviral activity

For the assessment of drug anti HIV activity in C8166 cells, $2x10^5$ cells were seeded into 15-ml

polyethylene tubes (Falcon, Basel, Switzerland) in 1 ml of complete medium, in a humidified atmosphere enriched with 5% CO_2. The cells were then exposed to HIV (100 TCID50), in the absence or in the presence of the componds. One and half hour later the cells were washed twice in PBS to remove excess virus and cultured in a 96 well plate (Falcon, Basel, Switzerland), at a concentration of $5x10^4$ cells/well in the presence of the same concentrations of the compounds as before. The antiviral activity of the different compounds in C8166 cells was assessed by evaluation of the virus-induced cytopathic effect 4-5 days after infection, with a colorimetric method, the MTT assay. Details of this method are described by Pauwels.[34] The inhibitory activity against Sendai virus and Herpes Simplex-1 virus was performed as described by Garaci et al.[35]

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