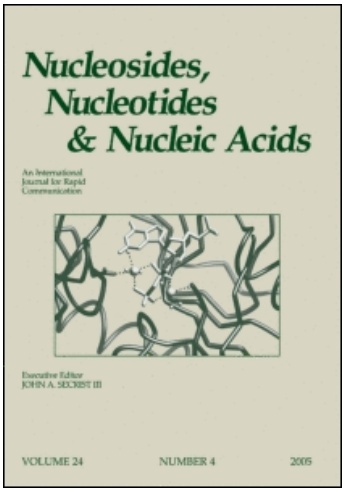

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Synthesis and anti-HIV evaluation of new 2',3'- dideoxy-3'-thiacytidine prodrugs

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Synthesis and anti-HIV evaluation of new 2',3'- dideoxy-3'-thiacytidine prodrugs

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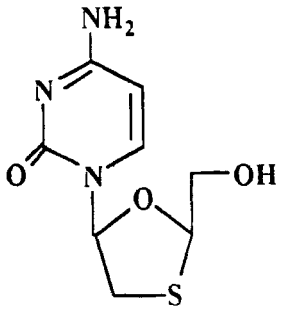
Abstract: A series of anti-HIV prodrugs possessing various polyaminated side arms have been developed. The incorporation of a N-Boc protected monoamine or diamine side arm into the backbone of the 2',3'-dideoxy-3'-thiacytidine **1** (BCH-189) provided an increase in antiviral potency, which could be several orders magnitude greater than the parent drug (**1**) depending on the cell culture systems used (MT-4 or MDMs). Twenty six 2',3'-dideoxy-3'-thiacytidine prodrugs which differ from each other by the length, the nature of the 5'-O function and the 5'-O or /and N-4 position on the

nucleoside moiety were synthesized. Among this new series of prodrugs, several congeners (**12c** and **12a**) were found to inhibit HIV-1 replication in cell culture with 50% effective concentrations EC_{50} of 10 and 50 nM respectively,in MT-4 cells. Compound **12c** was found more active on infected MDMs cells with 50% effective concentration of 0.01 nM. The synthesis and the antiviral properties of these compounds are discussed.

INTRODUCTION

The search for effective chemotherapeutic treatments against Human Immunodeficiency Virus (HIV) infections has led to the development of agents that target specific and critical events in the HIV replicative cycle. The most extensively studied of these agents are the 2',3'-dideoxynucleoside analogues : AZT (Retrovir®), ddC (Zalcitabine®), d4T (Zerit®), ddI (Videx®), 3TC (Epivir®), which terminate DNA synthesis during the reverse transcription (RT) reaction.[1,2] Several prodrugs of these FDA approved nucleosides based on different concepts have been reported. inducing the Bis (SATE) phosphotriester of AZT which bypasses the first activating phosphorylation step and produced promising results [3]. Other AZT prodrugs bearing various 5' O substitutents to enhance the uptake of the prodrugs by HIV-infected cells demonstrated a higher therapeutic index than AZT dihydropyridine carrier system. This carrier system has been used for sustained delivery of 2',3'-dideoxynucleosides to the brain and has been intensively studied [4,5] As a part of our efforts to design prodrugs of the 2',3'-dideoxy-3'-thiacytidine anti-HIV drug 1 (Figure 1), we have previously described some potent inhibitors of the cytopathicity of HIV-1 in MT-4 cells [6-11]. In this report, we described the synthesis and the anti-HIV activity of 2',3'-dideoxy-3'-thiacytidine derivatives obtained by substituting 5'-O and N-4 positions by various polyamines (Figure 2).

The initial idea was to design polyamine analogues of 2',3'-dideoxy-3'-thiacytidine, capable of using the polyamine transport apparatus for incorporation (Figure 2) [12-14]



1

Figure 1. Compound 1, 2',3'-dideoxy-3'-thiacytidine.

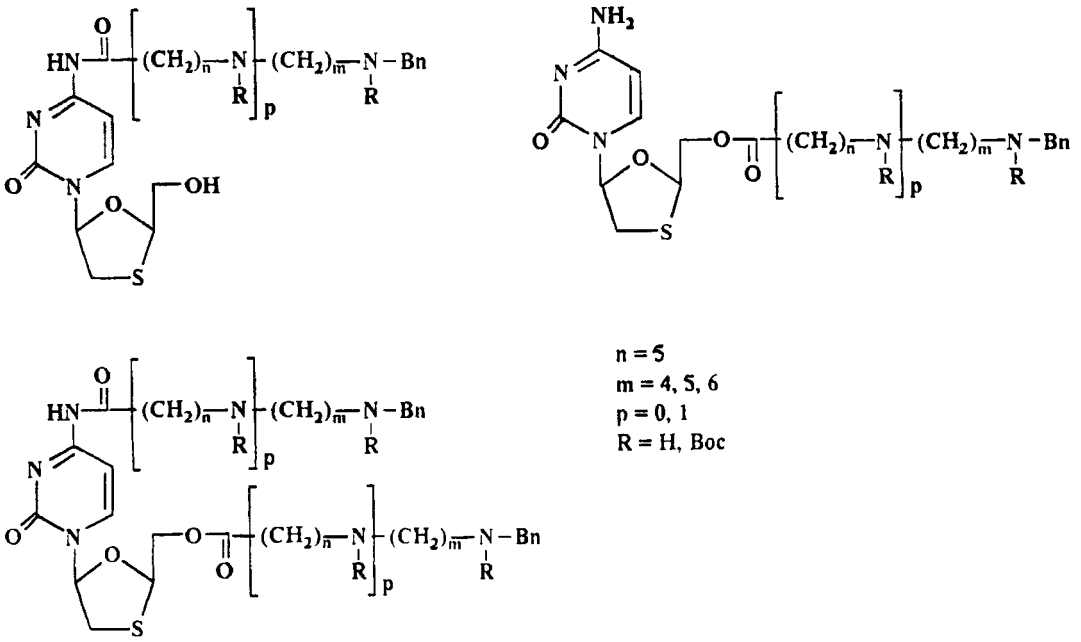


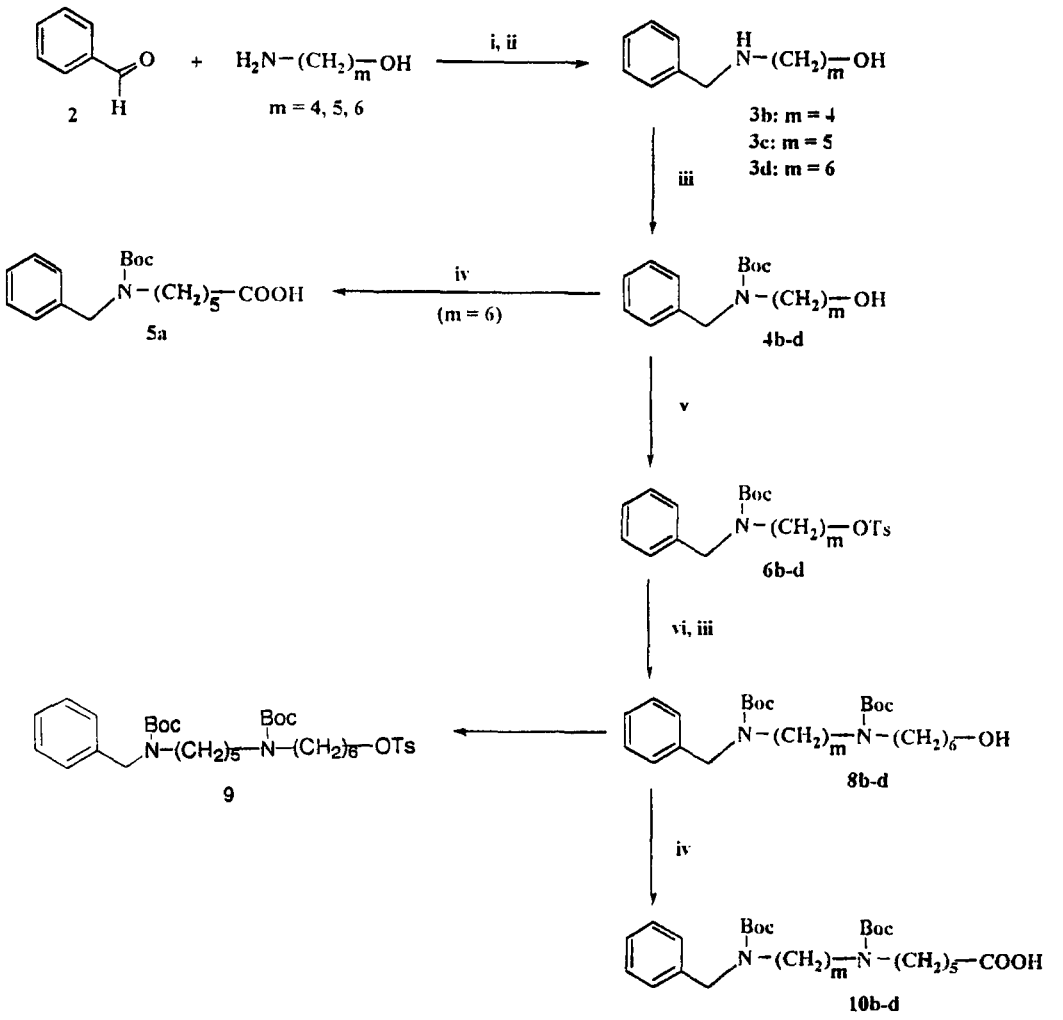
Figure 2. General structures of 2',3'-dideoxy-3'-thiacytidine polyamine analogues.

The importance of various structural parameters of the polyamine side chains could then be assessed. Analogues with different between the two Nα-Nβ nitrogen atoms, or between Nα-CO (distance between the Nα and the carbonyl function) were syntesized. Since the extracellular pH cell (pH = 7.4) differs from the intracellular pH (pH = 6.9),the penetration of various analogues as a function of their protonation state at various pH was studied (Figure 2). Indeed, the two secondary amino groups in these aliphatic side chains are sufficiently basic to be highly protonated at physiological pH, and this occurs at physiological pH predominately in the dicationic state [15,16].

CHEMISTRY

Compounds with mono and diaminated side arms **5a** and **10b-d** were prepared from benzaldehyde (**2**) and the appropriated commercially available aminoalcohol by the procedure summarized in Scheme 1. The oxidation of alcohol derivatives **4d** and **8b-d** afforded the best yields by using 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical (TEMPO) in a sodium hypochlorite solution [17-21]. Condensation with 6-amino-1-hexanol on tosylated derivatives **6b-d** was performed in basic conditions. Mono and diaminated acid side arms **5a-** and **10b-d** were coupled to 2',3'-dideoxy-3'-thiacytidine (**1**) by using the standard coupling reagent BOP (Benzotriazol-1-yloxyl-*tris*-(dimethylamino)-phosphonium hexafluorophosphate) in DMF at room temperature with Et_3N as summarized in Scheme 2.[22-24] Three series of condensation products were isolated and characterized : i. the 5'-O, N-4 disubstituted analogues **11a-d** ii. the 5'-O substituted analogues **12a-d** iii. the N-4 substituted analogues **13a-d**. It should be underlined that a large excess of triethylamine favoured the formation of the 5'-O substituted analogues versus the corresponding N-4 substituted derivatives. Removal of

Scheme 1

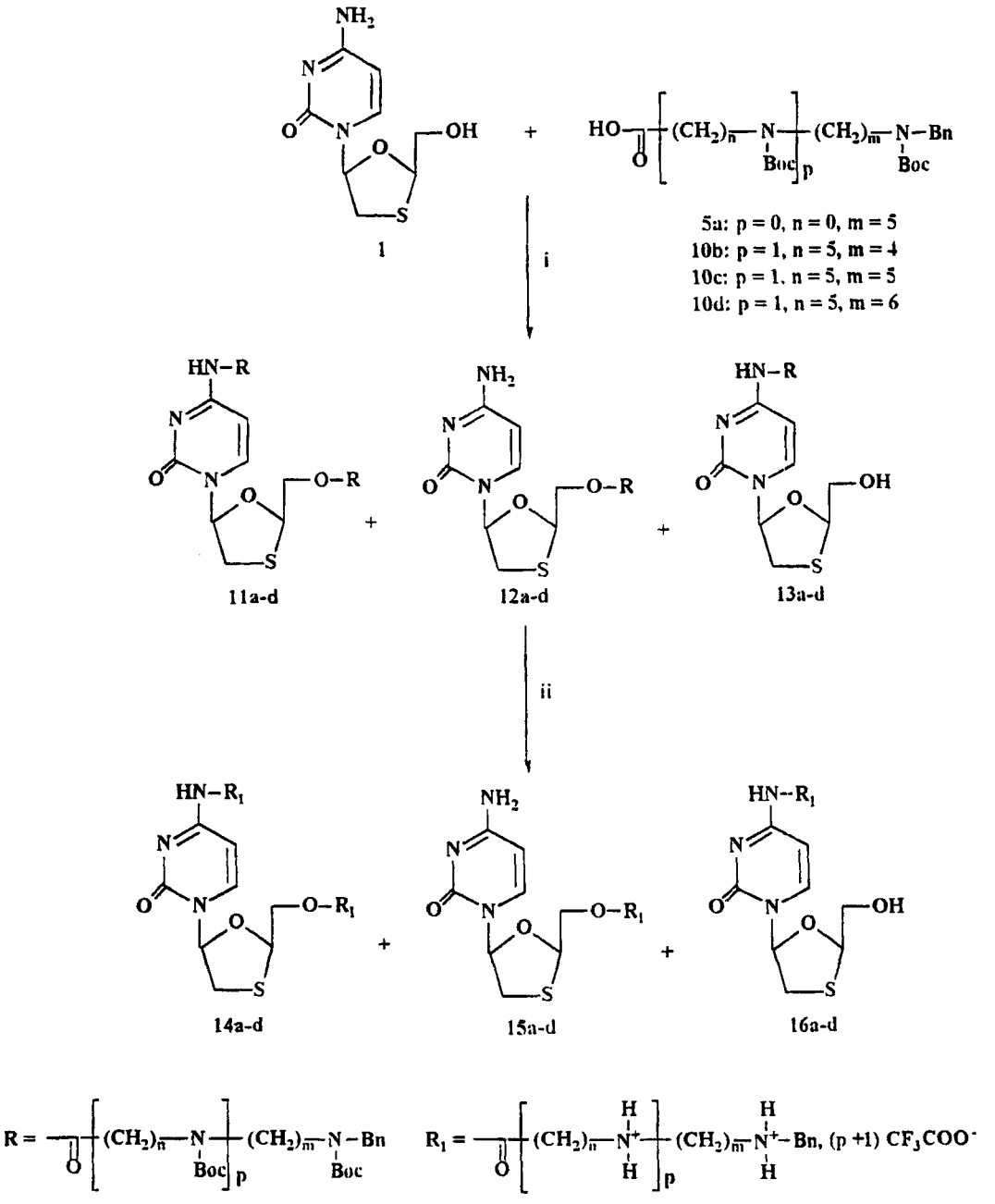


Reagents and conditions: i. H_2O/Et_2O 2:1; ii. $NaBH_4$, EtOH; iii. Boc_2O, CH_2Cl_2; iv. TEMPO, NaOCl, H_2O, CH_2Cl_2; v. TsCl, Et_2O, Et_3N; vi. HO-$(CH_2)_6$-NH_2, K_2CO_3, DMF.

Boc protecting group with trifluoroacetic acid (TFA) provided the free amine analogues **14a-d**; **15a-d** and **16a-d**.

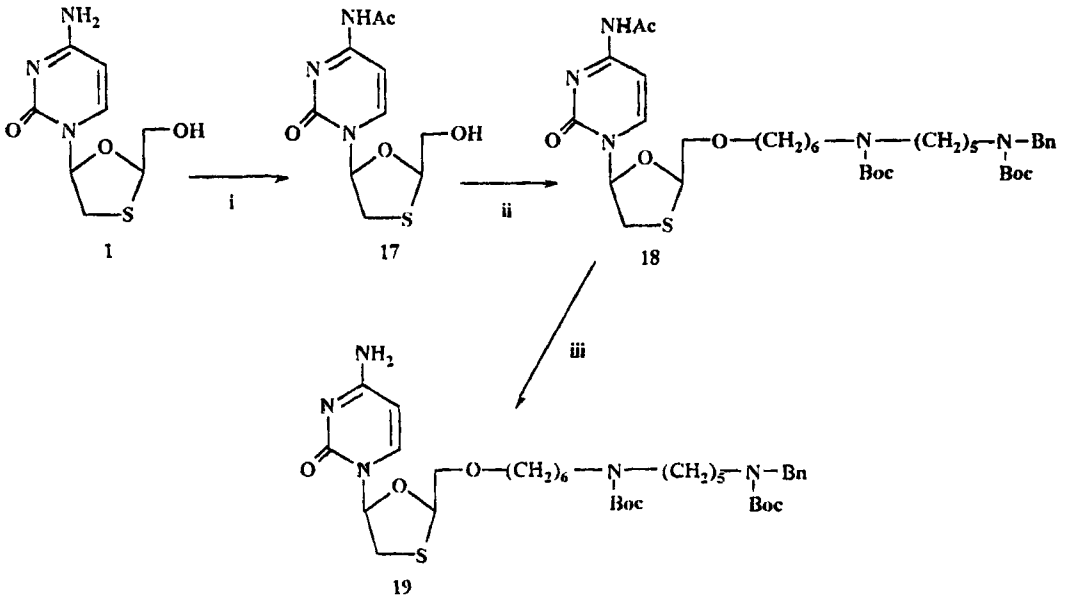
Preliminary anti-HIV results prompted us to synthesize a new series of compounds derived from the lead **12c** (m = n = 5; p =1, Scheme 2). Modifications concerning the

Scheme 2



Reagents and conditions: i. BOP/Et_3N/DMF; ii. TFA/CH_2Cl_2.

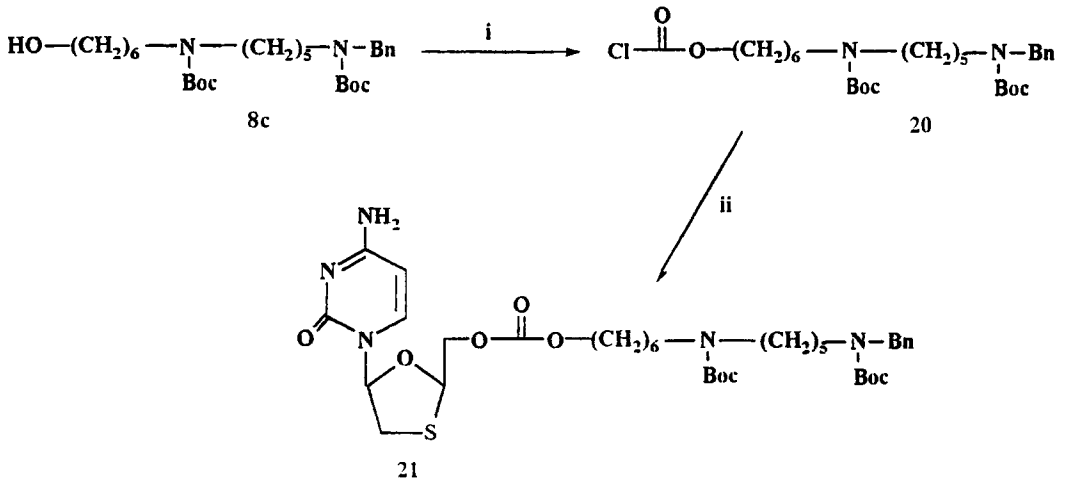
Scheme 3



Reagents and conditions: i. Ac_2O/DMF; ii. Compound 9/NaH/DMF; iii. NH_3/MeOH.

replacement of the chemical function between the 5'-O position of the nucleoside and the polyamine side chain were undertaken. Therefore, the ester was replaced by an ether or a carbonate function. The ether derivative **19** was synthesized according to a procedure related by Doshi et al. (Scheme 3). [25] 2',3'-dideoxy-3'-thiacytidine **1** was acetylated with acetic acid anhydride. The resulting derivative **17** was treated with sodium hydride at 0°C, heated to 50°C and condensed to the tosylated derivative **9** obtained from **8c** to afford after purification compound **18** (Scheme 3). This later was then treated with saturated methanolic ammonia solution to give the final ether derivative **19**. The synthesis of carbonate **21** is summarized in Scheme 4. The alcohol **8a** was treated with a solution of phosgene in toluene and the resulting chloroformate

Scheme 4



Reagents and conditions: i. Phosgene/toluene/N,N-Dimethylaniline; ii. 2',3'-dideoxy-3'-thiacytidine.

intermediate directly added to a solution of 2',3'-dideoxy-3'-thiacytidine (**1**) in DMF. After purification, the final compound **21** was obtained.

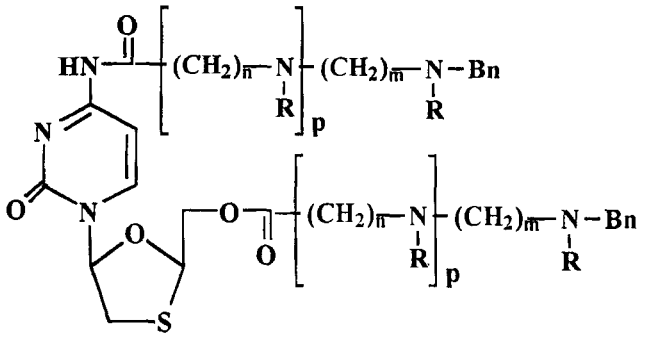
ANTIVIRAL EVALUATIONS AND DISCUSSION

The primary objective of this study was to design and synthesize prodrugs of 2',3'-dideoxy-3'-thiacytidine with enhanced transport facility across cell membrane independently of the nucleoside transport system. This was realized bylinking mono or di-amine side chains located at the 5'-O and/or N-4 positions of the nucleoside **1** to evaluate the anti-HIV activity of the obtained prodrugs. Like AZT prodrugs, in the absence of an active nucleoside transport system, the lipophilic character of the side chains at the 5'-O position should influence their ability to cross the cell membrane by a diffusion.[4,26] The compounds described in this article were evaluated as inhibitors of HIV-1 replication by the use of an assay measuring the formation of *syncytia* in HIV-1

infected MT-4 cells. [27-29] A dose-dependent relationship of the inhibition was found for the tested compounds. The EC_{50} values (concentration required to produce 50% inhibition of *syncytia* formation) and CC_{50} values (concentration required to produce 50% death of uninfected MT-4 cells) were determined (Tables 1-5). A large number of the tested compounds broadly inhibited HIV-1 replication although the EC_{50} values varied over 3 orders of magnitude. Anti HIV potency was affected by the position, length and terminal chemical functionality connecting the polyamine side chain to the parent nucleoside **1**. Compounds in which the polyamine side chain is linked to the 5'-O nucleosidic position through an ester function were the most potent derivatives (Table 2). 5'-O, N-4-disubstituted or N-4 substituted analogues showed a substantial reduction in anti-HIV potency (Table 1 and 3). Two different side chains characterized by one or two amine functions were inserted on to the 5'-O position on the nucleoside **1** (Table 2). In both cases, monoamino compounds **12a** and diamino compounds **12b** and **12c** exhibited sub-micromolar EC_{50} values 10 to 100 fold lower than the EC_{50} found for the parent nucleoside 2',3'-dideoxy-3'-thiacytidine (**1**). Furthermore, Boc protection of the amine functions led to more active compounds.

Compound **12c** is about five hundred times more active than the corresponding N-4 free amine analogue **15c**. Since the distance between the nitrogen charged centers of the side arms may be critical for the recognition of the polyamine analogues by the transport apparatus, several compounds incorporating various side chains were synthetized. Distances (d_1) between Nα-Nβ nitrogen atoms, along with Nα-CO (distance d_2 between the Nα nitrogen atom and the carbonyl function) were also varied. Compounds having one or two nitrogens Nα, Nβ separated by a distance d_1 represented by 4 or 5 methylene groups and/or distances d_2 (Nα-CO) constituted by 5 methylene

Table 1. Effective and cytotoxicity concentrations (EC_{50} and CC_{50} μM) against HIV of 5'-O-and N-4 nucleoside derivatives.



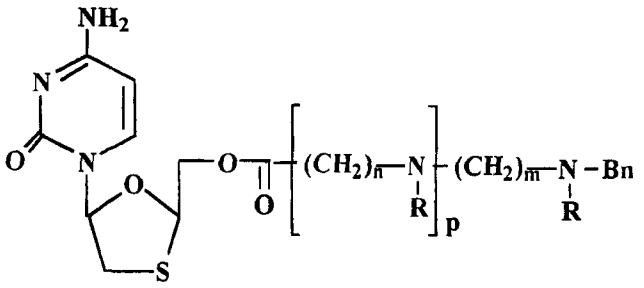
Compound	n	m	p	R	Log P [a]	EC_{50} (μM)[b]	CC_{50} (μM)[c]
11a	-	5	0	Boc	5.85 ± 0.96	20.0 ± 10	100.0
11b	5	4	1	Boc	8.89 ± 1.0	inactive	10.0
11c	5	5	1	Boc	9.35 ± 1.09	1.0 ± 0.5	10.0
11d	5	6	1	Boc	10.41 ± 1.09	inactive	10.0
14a	-	5	0	H	N/A	10.0	>50.0
14b	5	4	1	H	N/A	10.0	>50.0
14c	5	5	1	H	N/A	10.0	>50.0
14d	5	6	1	H	N/A	inactive	10.0
1	-	-	-	-	- 1.02 ± 0.60	1.0 ± 0.5	>50.0

[a] Log P determinations were performed using ACD software (Advanced Chemistry Development, Inc.) log P 1.0 base calculations.

[b] Concentration required to inhibit *syncitia* formation by 50% on MT-4 cells.

[c] Concentration required to produce 50% death of uninfected MT-4 cells.

Table 2. Effective and cytotoxicity concentrations (EC_{50} and CC_{50} μM) against HIV of 5'-O-nucleoside derivatives.



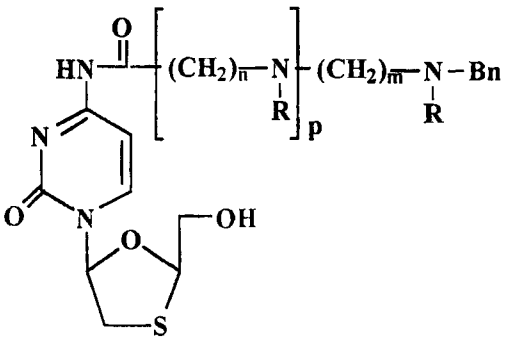
Compound	n	m	p	R	Log P [a]	EC_{50} (μM)[b]	CC_{50} (μM)[c]
12a	-	5	0	Boc	2.79 ± 0.70	0.05 ± 0.01	100.0
12b	5	4	1	Boc	4.31 ± 0.79	0.1 ± 0.05	10.0
12c	5	5	1	Boc	4.54 ± 0.78	0.01 ± 0.01	50.0
12d	5	6	1	Boc	5.07 ± 0.78	inactive	10.0
15a	-	5	0	H	2.15 ± 0.62	10.0 ± 5	>50.0
15b	5	4	1	H	1.80 ± 0.63	10.0 ± 5	>50.0
15c	5	5	1	H	2.03 ± 0.63	5.0 ± 1.0	>10.0
15d	5	6	1	H	2.56 ± 0.63	inactive	50.0
1	-	-	-	-	- 1.02 ± 0.6	1.0 ± 0.5	>100

[a] Log P determinations were performed using ACD software (Advanced Chemistry Development, Inc.) log P 1.0 base calculations.

[b] Concentration required to inhibit *syncitia* formation by 50% on MT-4 cells.

[c] Concentration required to produce 50% death of uninfected MT-4 cells.

Table 3. Effective and cytotoxicity concentrations (EC_{50} and CC_{50} μM) against HIV of N-4- nucleoside derivatives.



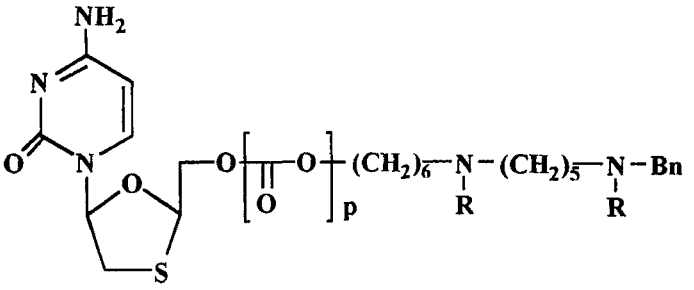
Compound	n	m	p	R	Log P [a]	EC_{50} (μM)[b]	CC_{50} (μM)[c]
13a	-	5	0	Boc	1.75 ± 0.89	20.0 ± 10	100.0
13b	5	4	1	Boc	3.27 ± 0.96	inactive	10.0
13c	5	5	1	Boc	3.50 ± 0.96	inactive	10.0
13d	5	6	1	Boc	4.03 ± 0.96	inactive	10.0
16a	-	5	0	H	0.58 ± 0.83	10.0 ± 5	>50.0
16b	5	4	1	H	0.76 ± 0.84	20.0 ± 10	>50.0
16c	5	5	1	H	0.99 ± 0.83	10.0 ± 5	>50.0
16d	5	6	1	H	1.52 ± 0.83	inactive	10.0
1	-	-	-	-	- 1.02 ± 0.60	1.0 ± 0.5	>50.0

[a] Log P determinations were performed using ACD software (Advanced Chemistry Development, Inc.) log P 1.0 base calculations.

[b] Concentration required to inhibit *syncitia* formation by 50% on MT-4 cells.

[c] Concentration required to produce 50% death of uninfected MT-4 cells.

Table 4. Effective and cytotoxicity concentrations (EC_{50} and CC_{50} μM) against HIV of 5'-O- ether and carbonate nucleoside derivatives **19** and **21**.



Compound	p	Log P [a]	EC_{50} (μM)[b]	CC_{50} (μM)[c]
19	0	4.81 ± 0.78	Inactive	5
21	1	5.04 ± 0.82	1.0 ± 0.5	10
1	-	- 1.02 ± 0.60	1 ± 0.5	>50

[a] Log P determinations were performed using ACD software (Advanced Chemistry Development, Inc.) log P 1.0 base calculations.

[b] Concentration required to inhibit *syncitia* formation by 50% on MT-4 cells.

[c] Concentration required to produce 50% death of uninfected MT-4 cells.

groups (**12a-c**) appeared to be the most active compounds. This indicated that the lengths for d_1, d_2 did not constitute a very specific requirement to gain anti-HIV activity. These new analogues showed similar cytotoxicities compared to the parent drug **1**.

Given the role and function of macrophages in HIV infection, it is crucial to be able to inhibit HIV multiplication in these cells. In contrast to proliferating MT-4 cells, macrophages, which are non-dividing cells and demonstrate limited metabolism. The

Table 5. Effects of selected 2',3'-dideoxy -3'-thiacytidine prodrugs (**12c**, **13c**, **15c**, **16c**) on HIV-1 infected human monocyte-derived macrophages (MDMs).

Compound	EC_{50} (nM)[a]	CC_{50} (nM)[b]
12c	0.01 ± 0.05	>5000
15c	1 ± 0.50	>1000
13c	50 ± 10	1000
16c	100 ± 50	>5000
1	10 ± 5	>50000

[a] Concentration required to inhibit *syncitia* formation by 50% on MDMs cells.
[b] Concentration required to produce 50% death of uninfected MDMs cells.

relatively low enzyme activity implicated in DNA synthesis, usually correlate with low levels of endogenous nucleotides. Therefore, the anti-HIV activity of the studied 2',3'-dideoxy-3'-prodrugs could differ from those determined in MT4-cell. [30] Prodrugs **12c**, **13c**, **15c**, **16c**, and the parent drug **1** were tested (Table 5) for their ability to inhibit viral replication in monocyte-derived macrophages (MDMs). The antiviral activity of the prodrug **12c** was three orders magnitude higher than that of the parent drug **1**. The IC_{50} values for **12c** and **1** were 0.01 nM and 10 nM respectively. This observation is in agreement with the results of Perno et *al.* in the case of AZT prodrugs.[30] The current study clearly shows that 5'-O-polyaminated 2',3'-didoxy3'-thiacytidine prodrugs were more efficient than the parent drug **1**. Compound **12c** was 2 and 3 orders of magnitude more active than the parent drug 1 in MT4 and MDMs cell cultures, infected with HIV-1.

The usefulness of the prodrugs of 2',3'-dideoxy-3'-thiacytidine should depend not only on the stability of the prodrug for its transport across the cell membrane but also upon its reversion to the parent compound intracellularly, especially in the virally infected cells. Therefore, to test the validity of our prodrug hypothesis, the corresponding ether and carbonate prodrugs **19** and **21** were synthesized and evaluated. From the preliminary anti-HIV tests, the ether derivative **19** was inactive while the carbonate **21** was as potent as the parent drug **1** but less active than the lead compound **12c** (Table 4). Ether prodrugs of the nucleoside AZT have already proved their efficiency as anti-HIV drugs. [25, 31] The observed abolition of anti-HIV activity related to the ether derivative **19** compared to the ester analogue **12c** could be correlated to either a higher chemical stability under the test conditions or with the release of inactive species from degradation. These observations would not be valid for the carbonate derivative **21** which can be easily hydrolyzed. However the ester derivatives proved to be the most potent candidates.

The biological results do not support the primary hypothesis concerning the use of polyamine transport apparatus for the incorporation of anti-RT nucleoside. Boc-protected mono or diamino compounds were found to be the most active derivatives. MonoBoc or diBoc compounds like **12a** and **12c** are at least 20 fold more active than the parent drug 2',3'-dideoxy-3'-thiacytidine **1** in infected MT-4 cell cultures and 1000 more active in infected MDMs for the derivative **12c**. The partition coefficient of 2'3'-dideoxy-3'-thiacytidine prodrugs may have a significant effect on cellular transport. Partition coefficients (log P) were determined for all prodrugs and log P values ranged from 10.41 to –1.02 (Table 1-4). As expected, these values were greater than the value of the parent drug **1** (Log P= -1.02). The observed increase of anti-HIV activity of these 2',3'-dideoxy-3'-thiacytidine prodrugs compared to that of the parent drug may be

related to their improved permeability through the cell membrane , allowing higher concentrations, of the prodrug accumulated in HIV target cells, T-lymphocytes and macrophages. Lipophilicity is probably not the only factor that influences the antiviral activity of these enzyme-labile prodrugs. For example compounds **12a** and **15d** which have similar Log P values (2.79 and 2.56) are active and inactive respectively with the respect to inhibition of HIV replication.

The high anti-HIV activities of prodrugs bearing polyamine moiety at the sugar 5'-O function compared to the corresponding N-4 derivatives may be explained by the preferential cleavage of the ester bond under mild slightly alkaline hydrolysis at 37 °C (pH = 7.4). The amide bond cleavage required more basic conditions.[32] Furthermore, since sensitivity to enzymatic hydrolysis of the prodrugs are likely to be crucial, stability studies were performed on two representative prodrugs compounds **12a** and **15d.** The half-life ($t_{1/2}$) of hydrolysis of these two prodrugs determined in human plasma by HPLC were very similar, respectively 120 min and 112 min. These results suggest that these two prodrugs have very closed sensibilities to plasma esterases.Hydrolysis may be independent of the polarity of the side arm. Abolition of anti-HIV activity of 5'-O ether **19** compared to the ester or carbonate derivatives seems to validate the prodrug behaviour of the new synthesized analogues. The ester function could afford the best results in terms of transport facility and regeneration of the parent drug **1** inside the infected cells.

In conclusion, compounds **12a** and **12c** are clearly more potent than the parent drug **1** in inhibiting viral replication may have a promising candidates for clinical use. Further studies are in progress in order to explain the mechanism of action of these prodrugs and to generalize the polyamine approach to other anti-HIV drugs such as AZT, d4T, ddC or ddI.

EXPERIMENTAL SECTION

Mass spectra were recorded on a Finnigan SSQ 70 spectrometer by Zambon Group. Nuclear magnetic resonance spectra were recorded on a Bruker WH-250 (250 MHz) spectrometer. The chemical shift values are expressed in ppm (parts per million) relative to tetramethysilane as internal standard; s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Elemental analysis was performed by the Service Central d' Analyse du CNRS (Vernaison, France) and were within 0.4% of the theoretical values. Thin layer chromatography (TLC) was performed on POLYGRAM Sil G/UV_{254} silica gel plates with fluorescent indicator, and the spots were visualized under 254 and 366 nm illumination. Proportions of solvents used for TLC are by volume. All solvents and chemicals were purchased from Aldrich Chemical Co. and were used as received. Partition coefficients (log P) were determined for all prodrugs using ACD/logP software from ChemCAD.

General procedure A (Scheme 1). Synthesis of the mono and diaminated acid side arms 5a and 10b-d.

Step 1 (Derivatives 3b-d) To 1.0 equiv of the appropriate aminoalcohol dissolved in water was added 1.1 eq of benzaldehyde (2)dissolved in diethyl ether (100ml). The mixture was stirred overnight at room temperature. After extraction with diethyl ether (2x50mL), the resulting organic phase was dried (Na_2SO_4) and evaporated to give oily residue. Then, to 1.0 equiv of the crude appropriate imine dissolved in EtOH was added 1 eq of $NaBH_4$ at 0°C. The mixture was stirred for 2h at 0°C and 2h at room temperature. After evaporation of EtOH the residue was washed with water and

extracted with EtOAc. The organic layer was dried (Na_2SO_4) and purified by flash column chromatography to give benzylaminoalcohol derivatives **3b-d**.

Step 2 (Derivatives 4b-d)

To 1.0 equiv of the appropriate benzylaminoalcohol **3b-d** dissolved in dichloromethane was added 1.1 equiv of di-*tert*-butyldicarbonate in CH_2Cl_2 at 0°C. The mixture was allowed to stir at room temperature for 3h. After solvent evaporation, the crude residues **4b-d** were directly used for the next step. These alcohols were etheir oxidized (step 5) etheir tosylated according to the following method (step 3).

Step 3 (derivatives 6b-d, 9)

To 1.0 equiv of **4b-d** and **8c** derivatives were added 1.1 equiv of tosylchloride dissolved in diethyl ether. A large excess of Et_3N (30.0 equiv) was added to this solution. The mixture reaction was stirred overnight at room temperature and 2h under reflux. After cooling solvent evaporation *in vacuo* gave a residue washed with H_2O (2x50mL) and extracted with CH_2Cl_2. (2x50mL). The combined organic layers were dried (Na_2SO_4), filtrated and evaporated to give the crude product which was purified by flash column chromatography (eluent CH_2Cl_2) to give compounds **6b-d** and **9**.

Step 4 (Derivatives 8b-d) To 1.0 equiv of derivatives **6b-d** dissolved in dry DMF was added 1.1 equiv of the 6-amino-1-hexanol and 5.0 equiv of K_2CO_3. The solution was heated at 100°C for 6h. After filtration, DMF was evaporated under *vacuo*. The residue was then washed with H_2O (2x50mL), extracted with EtOAc (3x30mL), dried over Na_2SO_4 and purified by flash column chromatography using ETOAc as eluent. The resulting compound was then protected with Boc_2O using the method described in step 3 to give derivatives **8b-d.**

Step 5 (Derivatives 5a, 10b-d) : At 0°C, a solution containing 1.0 equiv of derivatives **8b-d** and **4a**, a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO), a catalytic amount of aliquat tricaprylylmethylamonium chloride and KBr, one portion of saturated $NaHCO_3$ solution and 3 portions of CH_2Cl_2 was prepared. To this mixture was added a solution of sodium hypochlorite (pH=8) at 0°C. Addition of NaOCl was performed over a period of 5h until the starting material was consumed. Brine and CH_2Cl_2 were then added and the product extracted. After drying over Na_2SO_4, filtration and evaporation, the crude product was purified by flash column chromatography to give the acid derivatives **10b-d**. and **5a.**

N-benzyl-N-*tert*-butoxycarbonyl-6-aminohexanoic acid 5a : ^{1}H-NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.70-1.90 (m, 17H), 2.25-2.38 (m, 2H), 3.10-3.33 (m, 2H), 4.41 (s, 2H), 7.15-7.36 (m, 5H).

N-(N'-benzyl-N'-*tert*-butoxycarbonyl-4-aminobutyl)-N-*tert*-butoxycarbonyl-6-aminohexanoic acid 10b : ^{1}H-NMR ($CDCl_3$) δ 1.10-1.70 (m, 28H), 2.29-2.35 (m, 2H), 3.00-3.25 (m, 6H), 4.39 (s, 2H), 7.15-7.33 (m, 5H).

N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxycarbonyl-6-aminohexanoic acid 10c: ^{1}H-NMR ($CDCl_3$) δ 1.14-1.68 (m, 30H), 2.20 (bs, 2H), 3.05 (bs, 6H), 4.40 (s, 2H), 7.17-7.29 (m, 5H).

N-(N'-benzyl-N'-*tert*-butoxycarbonyl-6-aminohexyl)-N-*tert*-butoxycarbonyl-6-aminohexanoic acid 10d : ^{1}H-NMR ($CDCl_3$) δ 1.10-1.70 (m, 32H), 2.28-2.39 (m, 2H), 3.10-3.20 (m, 6H), 4.35 (s, 2H), 7.15-7.35 (m, 5H).

General procedure B: Synthesis of 2',3'-dideoxy-3'-thiacytidine analogues

Step 1 : (coupling to 1) A mixture of 1.0 equiv of acid derivatives **5a** or **10b-d**, 1.5

equiv of BOP reagent and 3.0 equiv of triethylamine were dissolved in dry DMF. Then was added. portionwise 1.0 equiv of 2',3'-dideoxy-3'-thiacytidine (1) The resulting mixture was stirred at room temperature for 3-5h. After evaporation to dryness, brine and EtOAc were added. The combined organic layers were dried over Na_2SO_4, filtered and evaporated. The residue was purified by flash column chromatography using EtOAc as eluent to give the derivatives in equal proportions. **11a-d, 12a-d** and **13a-d**

Step 2 : (Removal of the Boc protecting group).

This deprotection was performed according to standard procedure using an excess of TFA in CH_2Cl_2. After evaporation, the product was purified by flash column chromatography to give compounds **14a-d, 15a-d** and **16a-d**. The yields are ranging from 20 to 65%.

(±)-4-(N-benzyl-N-*tert*-butoxycarbonyl-6-aminohexyl)amino]-1-[2-(N-benzyl-N-*tert*-butoxycarbonyl-6-aminohexanoyloxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 11a : ^{1}H-NMR ($CDCl_3$) δ 1.16-1.68 (m, 30H), 2.18-2.38 (m, 4H), 2.88-3.12 (m, 5H), 3.49-3.58 (m, 1H), 4.34 (bs, 5H), 4.49-4.57 (m, 1H), 5.28 (pseudo q, J_1 = 3.1 Hz, J_2 = 4.8 Hz, 1H), 6.22 (pseudo q, J_1 = 3.0 Hz, J_2 = 5.1 Hz, 1H), 7.10-7.26 (m, 10H), 7.37 (d, J = 7.5 Hz, 1H), 8.03 (d, J = 7.5 Hz, 1H), 9.39 (bs, 1H). MS (EI) m/z 836 (M^+). Anal. calcd for $C_{44}H_{61}N_5O_9S$: C, 63.20, H, 7.37; N, 8.38. Found C, 63.31, H, 7.39 ; N, 8.40.

(±)-4-[[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-4-aminobutyl)-N-*tert*-butoxycarbonyl-6-amino-hexanoyl]amino]-1-[2-[N-(N'-benzyl-N'-*tert*-butoxycarboyl-4-aminobutyl)-N-*tert*-butoxycarbonyl-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 11b : ^{1}H-NMR ($CDCl_3$) δ 1.15-1.80 (m, 56H), 2.25-2.50 (m, 4H), 3.00-3.30 (m, 12H), 3.60-3.70 (m, 1H), 4.35-4.50 (bs, 5H), 4.60-4.70 (m, 1H), 5.35 (pseudo t, 1H), 6.30 (pseudo t, 1H), 7.15-7.40 (m, 10H), 7.50 (d, J = 7.5 Hz, 1H), 8.15

(d, J = 7.5 Hz, 1H), 10.25 (bs, 1H). MS (EI) m/z 1178 (M^+). Anal. calcd for $C_{62}H_{95}N_7O_{13}S$: C, 63.17, H, 8.14; N, 8.32. Found C, 63.31, H, 8.35 ; N, 8.34.

(±)-4-[[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxycarbonyl-6-amino-hexanoyl]amino]-1-[2-[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxy-carbonyl-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 11c : ^{1}H-NMR ($CDCl_3$) δ 1.09-1.77 (m, 60H), 2.40 (q, J_1 = 7.0 Hz, J_2 = 12.0 Hz, 4H), 2.99-3.24 (m, 12H), 3.58-3.67 (m, 1H), 4.40-4.46 (m, 5H), 4.58-4.67 (m, 1H), 5.38 (pseudo t, 1H), 6.31 (pseudo q, 1H), 7.23-7.36 (m, 10H), 7.43 (d, J = 7.5 Hz, 1H), 8.10 (d, J = 7.5 Hz, 1H). MS (EI) m/z 1206 (M^+). Anal. calcd for $C_{64}H_{99}N_7O_{13}S$: C, 63.69, H, 8.29; N, 8.13. Found C, 63.74, H, 8.34 ; N, 8.15.

(±)-4-[[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-6-aminohexyl)-N-*tert*-butoxycarbonyl-6-amino-hexanoyl]amino]-1-[2-[N-(N'-benzyl-N'-*tert*-butoxycarboyl-6-aminohexyl)-N-*tert*-butoxy-carbonyl-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 11d : ^{1}H-NMR ($CDCl_3$) δ 1.06-1.80 (m, 64H), 2.28-2.41 (m, 4H), 2.95-3.17 (m, 12H), 3.52-3.61 (m, 1H), 4.34-4.41 (m, 5H), 4.52-4.61 (m, 1H), 5.32 (pseudo d, 1H), 6.25 (pseudo q, 1H), 7.12-7.34 (m, 10H), 7.38 (d, J = 7.5 Hz, 1H), 8.04 (d, J = 7.5 Hz, 1H). MS (EI) m/z 1234 (M^+).Anal. calcd for $C_{66}H_{103}N_7OS$: C, 64.19, H, 8.42; N, 7.94. Found C, 64.35, H, 8.46 ; N, 7.92.

(±)-4-amino-1-[2-(N-benzyl-N-*tert*-butoxycarbonyl-6-aminohexanoyloxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 12a : ^{1}H-NMR ($CDCl_3$) δ 1.10-1.66 (m, 15H), 2.26 (m, J = 7.5 Hz, 2H), 2.96-3.07 (m, 3H), 3.42-3.51 (m, 1H), 4.26-4.32 (m, 3H), 4.43-4.52 (m, 1H), 5.25 (q, J_1 = 3.2 Hz, J_2 = 5.0 Hz, 1H), 5.72 (bs, 1H), 6.25 (pseudo t, 1H), 7.10-7.40 (m, 5H), 7.67 (d, J = 7.3 Hz, 1H). MS (EI) m/z 533 (M^+). Anal. Calcd for $C_{26}H_{36}N_4O_6S$: C, 58.62, H, 6.83, N, 10.52. Found C, 58.74; H, 6.86 ; N, 10.53.

(±)-4-amino-1-[2-[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxycarbonyl-5-aminopentanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 12b : ^{1}H-NMR ($CDCl_3$) δ 1.10-1.70 (m, 28H), 2.30-2.41 (m, 2H), 3.00-3.55 (m, 8H), 4.30-4.60 (m, 4H), 5.28-5.35 (m, 1H), 5.75-5.95 (bd, 1H), 6.29-6.33 (m, 1H), 7.12-7.35 (m, 5H), 7.60-7.82 (bs, 1H). MS (EI) m/z 704 (M^+). Anal. Calcd.for $C_{35}H_{53}N_5O_8S$: C, 59.71 ; H, 7.60 ; N, 9.95. Found: C, 59.58, H, 7.63 ; N 9.97.

(±)-4-amino-1-[2-[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxycarbonyl-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 12c : ^{1}H-NMR ($CDCl_3$) δ 1.72-1.55 (m, 32H), 2.35 (t, J = 7.3 Hz, 2H), 3.02-3.10 (m, 6H), 3.46-3.55 (m, 2H), 4.32-4.56 (m, 4H), 5.31 (pseudo q, 1H); 5.83 (bs, 1H), 6.33 (pseudo t, 1H), 7.17-7.34 (m, 5H), 7.67 (d, J = 5.8 Hz , 1H). MS (EI) m/z 718 (M^+). Anal. calcd.for $C_{36}H_{55}N_5O_8S$: C, 60.22; H, 7.74; N, 9.76. Found : C, 60.12; H , 7.76; N, 9.77.

(±)-4-amino-1-[2-[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-6-aminohexyl)-N-*tert*-butoxycarbonyl-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 12d : ^{1}H-NMR ($CDCl_3$) δ 1.18-1.88 (m, 28H), 2.26-2.43 (m, 4H), 2.84-2.97 (m, 12H), 3.16-3.20 (m, 1H), 3.51-3.60 (m, 1H), 4.09 (s, 4H), 4.30-4.37 (m, 1H), 4.52-4.61 (m, 1H), 5.38 (pseudo d, 1H), 6.21 (pseudo d, 1H), 7.32-7.40 (m, 10H), 8.20 (d, J = 7.5 Hz, 1H). MS (EI) m/z 732 (M^+). Anal. calcd. for $C_{37}H_{57}N_5O_8S$: C, 60.70 ; H, 7.86 ; N, 9.57. Found : C, 6.62 ; H, 7.84 ; N, 9.58.

(±)-4-[(N-benzyl-N-*tert*-butoxycarbonyl-6-aminohexyl)-amino]-1-[2-hydroxymethyl-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 13a : ^{1}H-NMR ($CDCl_3$) δ 1.12-1.67 (m, 15H), 2.34 (t, J = 7.5 Hz, 2H), 3.06-3.12 (m, 3H), 3.45-3.54 (m, 1H), 3.80-4.06 (m, 2H), 4.30 (bs, 2H), 5.22 (t, J = 3.0 Hz, 1H), 6.22 (pseudo d, 1H), 7.08-7.25 (m, 5H), 7.33 (d, J = 7.5 Hz, 1H), 8.33 (d, J = 7.5 Hz, 1H), 9.62 (bs, 1H). MS (EI)

m/z 533 (M^+). Anal. calcd for $C_{26}H_{36}N_4O_6S$: C, 58.62 ; H, 6.83 ; N, 10.52. Found C, 58.74 ; H, 6.82 ; N, 10.53.

(±)-4-[[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-4-aminobutyl)-N-*tert*-butoxycarbonyl-6-amino-hexanoyl]-amino]-1-[2-hydroxymethyl-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 13b : ^{1}H-NMR ($CDCl_3$) δ 1.15-1.80 (m, 56H), 2.25-2.50 (m, 4H), 3.00-3.30 (m, 12H), 3.60-3.70 (m, 1H), 4.35-4.50 (bs, 5H), 4.60-4.70 (m, 1H), 5.35 (pseudo t, 1H), 6.30 (pseudo t, 1H), 7.15-7.40 (m, 10H), 7.50 (d, J = 7.5 Hz, 1H), 8.15 (d, J = 7.5 Hz, 1H), 10.25 (bs, 1H). MS (EI) m/z 704 (M^+). Anal. calcd. for $C_{35}H_{53}N_5O_8S$: C, 59.71 ; H, 7.60 ; N, 9.95. Found : C, 59.66 ; H, 7.65 ; N, 9.98.

(±)-4-[[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxycarbonyl-6-amino-hexanoyl]-amino]-1-[2-hydroxymethyl-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 13c : ^{1}H-NMR ($CDCl_3$) δ 1.72-1.55 (m, 32H), 2.35 (t, J = 7.3 Hz, 2H), 3.02-3.10 (m, 6H), 3.46-3.55 (m, 2H), 4.32-4.56 (m, 4H), 5.31 (pseudo q, 1H), 5.83 (bs, 1H), 6.33 (pseudo t, 1H), 7.17-7.34 (m, 5H), 7.67 (d, J = 5.8 Hz, 1H). MS (EI) m/z 718 (M^+). Anal. calcd. for $C_{36}H_{55}N_5O_8S$: C, 60.22 ; H, 7.74 ; N, 9.76. found : C, 60.28, H, 7.76 ; N, 9.77.

(±)-4-[[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-6-aminohexyl)-N-*tert*-butoxycarbonyl-6-amino-hexanoyl]-amino]-1-[2-hydroxymethyl-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 13d : ^{1}H-NMR ($CDCl_3$) δ 1.05-1.86 (m, 32H), 2.10-2.41 (m, 4H), 2.87-3.16 (m, 7H), 3.53-3.62 (m, 1H), 4.34-4.41 (m, 3H), 4.52-4.61 (m, 1H), 5.32 (pseudo q, 1H), 6.25 (pseudo d, 1H), 7.12-7.29 (m, 5H), 7.40 (d, J = 7.5 Hz, 1H), 8.10 (d, J = 7.5 Hz, 1H). MS (EI) m/z 732 (M^+). Anal. calcd. for $C_{37}H_{57}N_5O_8S$: C, 60.70 ; H, 7.86 ; N, 9.57. Found : C, 60.75 ; H, 7.89 ;N, 9.59.

(±)-4-[N-(N-benzyl-6-aminohexyl)amino]-1-[2-(N-(N-benzyl-6aminohexanoyloxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 14a : ^{1}H-NMR ($CDCl_3$) δ 1.28-1.72 (m, 6H), 2.40-2.50 (t, J = 6.9 Hz, 2H), 2.91-3.28 (m, 3H), 3.53-3.69 (m, 1H), 4.19 (bs, 2H),

4.35-4.70 (m, 2H), 5.46-5.50 (m, 1H), 6.27 (pseudo q, 1H), 7.44 (bs, 10H). MS (EI) m/z 636 (M^+). Anal. calcd for $C_{38}H_{45}N_5O_9SF_6$: C, 52.95 ; H, 5.27, N, 8.13. Found : C, 52.85 ; H, 5.24 ; N, 8.16.

(±)-4-[[N-(N'-benzyl-4-aminobutyl)-6-aminohexanoyl]amino]-1-[2-[N-(N'-benzyl-4-amino-butyl)-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 14b : ^{1}H-NMR ($CDCl_3$) δ 1.10-1.70 (m, 20H), 2.20-2.45 (m, 4H), 3.10-3.55 (m, 14H), 3.85-4.15 (m, 3H), 4.25-4.45 (m, 1H), 5.25-5.30 (m, 1H), 6.05-6.18 (m, 1H), 7.10 (d, J = 7.5 Hz, 1H), 7.20-7.45 (m, 10H), 8.05 (d, J = 7.5 Hz, 1H). MS (EI) m/z 778 (M^+). Anal. calcd for $C_{57}H_{63}N_7O_{13}SF_{12}$: C, 48.81 ; H,5.17., N, 7.97. Found : C, 48.78 ; H, 5.12 ; N, 7.94.

(±)-4-[[N-(N'-benzyl-5-aminopentyl)-6-aminohexanoyl]amino]-1-[2-[N-(N'-benzyl-5-amino-pentyl)-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 14c ^{1}H-NMR ($CDCl_3$) δ 1.15-1.55 (m, 24H), 2.06-2.27 (m, 4H), 2.63-2.80 (m, 12H), 3.00-3.10 (m, 1H), 3.30-3.50 (m, 1H), 3.91 (s, 4H), 4.12-4.38 (m, 2H), 5.20 (pseudo q, 1H), 6.02 (pseudo d, 1H), 7.12-7.23 (m, 10H), 8.09 (d, J = 7.5 Hz, 1H). MS (EI) m/z 806 (M^+). Anal. calcd for $C_{52}H_{67}N_{13}O_{13}SF_{12}$: C, 46.63 ; H, 5.38, N, 7.79. Found : C, 49.59; H, 5.42 ; N, 7.77.

(±)-4-[[N-(N'-benzyl-6-aminohexyl)-6-aminohexanoyl]amino]-1-[2-[N-(N'-benzyl-6-amino-hexyl)-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 14d ^{1}H-NMR ($CDCl_3$) δ 1.18-1.88 (m, 28H), 2.26-2.43 (m, 4H), 2.84-2.97 (m, 12H), 3.16-3.21 (m,1H), 3.51-3.60 (m, 1H), 4.09 (s, 4H), 4.30-4.37 (m, 1H), 4.52 (pseudo d, 1H), 5.38 (pseudo d, 1H), 6.21 (pseudo d, 1H), 7.32-7.40 (m, 10H), 8.20 (d, J = 7.5 Hz, 1H). MS (EI) m/z 834 (M^+). Anal. calcd for $C_{54}H_{71}N_7O_{13}SF_{12}$: C, 50.42 ; H, 5.57, N, 7.62. Found : C, 50.49 ; H, 5.60 ; N, 7.63.

(±)-4-amino-1-[2-(N-benzyl-6-aminohexanoyloxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 15a : ^{1}H-NMR ($CDCl_3$) δ 1.20-1.50 (m, 15H), 2.26 (m, J = 7.5 Hz, 2H), 2.96-3.07 (m, 3H), 3.42-3.51 (m, 1H), 4.26-4.32 (m, 3H),-4.43-4.52 (m, 1H), 5.25 (q, J_1 = 3.2 Hz, J_2 = 5.0 Hz, H), 5.72 (bs, 1H), 6.25 (pseudo t, 1H), 7.10-7.40 (m, 5H), 7.67 (d, J = 7.3 Hz, 1H). MS (EI) m/z 433 (M^+). Anal. calcd for $C_{23}H_{28}N_4O_6SF_3$: C, 50.63 ; H, 5.18, N, 10.27. Found : C, 50.74 ; H, 5.22 ; N,10.24.

(±)-4-amino-1-[2-[N-(N'-benzyl-5-aminopentyl)-5-aminopentanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 15b : ^{1}H-NMR ($CDCl_3$) δ 1.20-1.60 (m, 10H), 2.30-2.41 (m, 2H), 3.00-3.55 (m, 8H), 4.30-4.60 (m, 4H), 5.28-5.35 (m, 1H), 5.75-5.95 (bd, 1H), 6.29-6.33 (m, 1H), 7.12-7.35 (m, 5H), 7.60-7.82 (bs, 1H). MS (EI) m/z 504 (M^+). Anal. calcdfor $C_{29}H_{37}N_5O_8SF_6$: C, 47.73; H, 5.12, N, 9.60. Found : C, 47.86 ; H, 5.15 ; N, 9.62.

(±)-4-amino-1-[2-[N-(N'-benzyl-5-aminopentyl)-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 15c : ^{1}H-NMR ($CDCl_3$) δ 1.09-1.67 (m, 12 H), 2.09 (t, J = 7.1 Hz, 2H), 2.62-2.75 (m, 6H), 2.95-3.07 (m, 3H), 3.23-3.32 (m, 1H), 3.86 (bs, 2H), 4.02-4.10 (m, 1H), 4.25-4.34 (m, 1H), 5.12 (pseudo q, 1H), 5.81 (d, J = 7.9 Hz, 1H), 5.96 (pseudo q, 1H), 7.08-7.18 (m, 5H), 7.84 (d, J = 7.9 Hz, 1H). MS (EI) m/z 518 (M^+). Anal. calcd for $C_{30}H_{39}N_5O_8SF_6$: C, 48.44 ; H, 5.30, N, 9.42. Found : C, 48.48 ; H, 5.34 ; N, 9.44.

(±)-4-amino-1-[2-[N-(N'-benzyl-6-aminohexyl)-6-aminohexanoyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 15d : ^{1}H-NMR ($CDCl_3$) δ 1.21-1.89 (m, 14H), 2.31 (t, J = 6.9 Hz, 2H), 2.74-2.86 (m, 6H), 3.05-3.10 (m, 1H), 3.38-3.47 (m, 1H), 3.66-3.89 (bs, 2H), 3.97 (s, 2H), 5.12 (t, J = 3.2 Hz, 1H), 6.09 (pseudo d, 1H), 7.02-7.27 (m, 6H), 8.55 (d, J = 7.5 Hz, 1H). MS (EI) m/z 532 (M^+). Anal. calcd for $C_{31}H_{41}N_5O_8SF_6$: C, 49.13 ; H, 5.46, N, 9.24. Found : C, 49.26 ; H, 5.50 ; N, 9.21.

(±)-4-[(N-benzyl-6-aminohexyl)-amino]-1-[2-hydroxymethyl-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 16a : ^{1}H-NMR ($CDCl_3$) δ 1.12-1.50 (m, 6H), 2.34 (t, J = 7.5 Hz,

2H), 3.06-3.12 (m, 3H), 3.45-3.54 (m, 1H), 3.80-4.06 (m, 2H), 4.30 (bs, 2H), 5.22 (t, J = 3.0 Hz, 1H), 6.22 (pseudo d, 1H), 7.08-7.25 (m, 5H), 7.33 (d, J = 7.5 Hz, 1H), 8.33 (d, J = 7.5 Hz, 1H), 9.62 (bs, 1H). MS (EI) m/z 533 (M^+). Anal. calcd for $C_{23}H_{28}N_5O_8SF_3$: C, 50.63 ; H, 5.18, N, 10.27. Found : C, 50.70 ; H, 5.20 ; N, 10.29.

(±)-4-[[N-(N'-benzyl-4-aminobutyl)-6-aminohexanoyl]-amino]-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 16b : ^{1}H-NMR ($CDCl_3$) δ 1.25-1.85 (m, 10H), 2.40-2.60 (m, 2H), 2.80-3.10 (bs, 7H), 3.20-3.30 (m, 1H), 3.60-3.80 (m, 2H), 4.20 (bs, 2H), 5.30-5.40 (pseudo t, 1H), 6.20-6.35 (m, 1H), 7.25 (d, J = 7.5 Hz, 1H), 7.40-7.65 (m, 5H), 8.05 (d, J = 7.5 Hz, 1H). MS (EI) m/z 504 (M^+). Anal. calcd for $C_{29}H_{37}N_4O_6SF_6$: C, 47.73 ; H, 5.12, N, 9.60. Found : C, 47.78 ; H, 5.14 ; N, 9.62

(±)-4-[[N-(N'-benzyl-5-aminopentyl)-6-aminohexanoyl]-amino]-1-[2-hydroxymethyl-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 16c : ^{1}H-NMR ($CDCl_3$) δ 1.20-1.61 (m, 12H), 2.30 (t, J = 7.1 Hz, 2H), 2.73-2.86 (m, 6H), 3.03-3.10 (m, 3H), 3.37-3.46 (m, 1H), 3.66-3.86 (m, 2H), 3.97 (s, 2H), 5.12 (t, J = 3.2 Hz, 1H), 6.08 (pseud q, 1H), 7.00 (d, J = 3.2 Hz, 1H), 7.19-7.29 (m, 5H), 8.53 (d, J = 7.5 Hz, 1H). MS (EI) m/z 518 (M^+). Anal. calcd for $C_{30}H_{39}N_5O_8SF_6$: C, 48.44 ; H, 5.30, N, 9.42. Found : C, 48.57 ; H, 5.32 ; N, 9 .43.

(±)-4-[[N-(N'-benzyl-6-aminohexyl)-6-aminohexanoyl]-amino]-1-[2-hydroxymethyl-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 16d : ^{1}H-NMR ($CDCl_3$) δ 1.18-1.82 (m, 14H), 2.24-2.55 (m, 2H), 2.82-2.98 (m, 6H), 3.10-3.22 (m, 1H), 3.51-3.60 (m, 1H), 4.09 (s, 2H), 4.33-4.39 (m, 1H), 4.51-4.60 (m, 1H), 5.38 (pseudo d, 1H), 6.20 (pseudo d, 1H), 7.14-7.40 (m, 6H), 8.22 (d, J = 7.5 Hz, 1H). MS (EI) m/z 532 (M^+). Anal. calcd for $C_{31}H_{41}N_5O_8SF_6$: C, 49.13 ; H, 5.46, N, 9.24. Found : C, 49.26 ; H, 5.50 ; N, 9.26.

2-hydroxymethyl-5-(N4-acetylcytosin-1'-yl)-1,3-oxathiolane 17

Compound **1** (470mg, 2.0 mmol) was dissolved in DMF (8ml) and acetic acid anhydride

(212 μl, 2.25 mmol) was added. The solution was stirred under N_2 for 5h and then concentrated in vacuo. The residue was flash chromatographed on silica gel eluting with 2% MeOH in ethyl acetate to give **17** (400mg, 74%). ^{1}H-NMR ($CDCl_3$) δ 2.10 (s, 3H), 3.0-3.40 (2dd, 2H), 3.70-4.0 (2dd, 2H), 5.10 (t, 1H), 6.10 (m, 1H), 7.20 (d, 1H, J = 7.5 Hz), 8.30 (d, 1H, J = 7.5 Hz).

N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxycarbonyl-6-aminopentannoyl-1-toluene –4-sulfonic acid 9

To a solution of **8c** (150 mg, 1.5 mmol) in Et_2O (3ml) was added a solution of tosyl chloride (450 mg, 2.3 mmol) in Et_2O/NEt_3 (3/2 15 ml). The reaction mixture was heated at 60□C stirred for 5 h and 3 h at room temperature. The mixture was concentrated *in vacuo*. Ethyl acetate was then added and the organic phasis extracted successively with 5% citric acid, H_2O, 5% $NaHCO_3$ and finally H_2O. The organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using 15% EtOAc/Hexane to give **9** (624mg, 42%). ^{1}H-NMR ($CDCl_3$) δ 1.10-1.50 (m, 32H), 2.40 (2dd, 3H), 3.0-3.20 (bs, 6H), 3.90 (t, 3H, J = 6.4 Hz), 4.30 (s, 2H), 7.15 (m, 5H), 7.25 (m, 2H).

(±)-4-acetylamino-1-[2-[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxycarbonyl-6-aminohexanyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 18

To a solution of **17** (95 mg, 0.35 mmol) in dry THF at 0□C under N_2 was added sodium hydride (120 mg, 5.2 mmol). The reaction mixture was then allowed to stir at 60□C for 1h and **9** (252 mg, 0.39 mmol) was added. The mixture was stirred for 20h and THF was evaporated under *vacuo*. EtOAc was added and the organic phase extracted with H_2O. The organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel to give **18** (150 mg, 61%). ^{1}H-NMR ($CDCl_3$) δ 1.1-1.5 (m, 32H), 2.0 (s, 3H), 3.0-3.10 (m, 8H), 3.50 (m, 2H), 3.70 and 3.90 (m, 2H), 4.30 (bs, 2H), 5.30 (t, 1H, J = 3.4 Hz), 6.30 (m, 1H), 7.20 (m, 5H), 7.35 (d, 1H, J = 7.5 Hz), 8.45 (d, 1H, J = 7.5 Hz)

(±)-4-amino-1-[2-[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxycarbonyl-6-aminohexanyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 19

Compound **18** (36 mg, 0.05 mmol) was treated under N_2 with a saturated ammonia solution in methanol (2ml). The reaction mixture was stirred for 10h. The solution was then evaporated under *vacuo* and the residue purified by preparative layer chromatography with using 10% MeOH in EtOAc to give **19** (22mg, 65%). ^{1}H-NMR ($CDCl_3$) δ 1.1-1.6 (m, 32H), 3.0-3.1 (m, 8H), 3.50 (m, 2H), 3.6 0and 3.90 (m, 2H), 4.30 (bs, 2H), 5.20 (t, 1H, J = 3.15 Hz), 6.30 (m, 1H), 7.20 (m, 5H), 7.20 (m, 6H), 8.05 (br, 1H). MS (EI) m/z 704 (M^+). Anal. calcd for $C_{36}H_{57}N_5O_7S$: C, 61.41; H, 8.18, N, 9.95. Found : C, 61.45; H, 8.43 ; N, 9.97.

(±)-4-amino-1-[2-[N-(N'-benzyl-N'-*tert*-butoxycarbonyl-5-aminopentyl)-N-*tert*-butoxycarbonyl-6-aminohexanyloxycarbonyloxymethyl]-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone 21

N,N-Dimethylaniline was added to a stirred solution of **8g** (296 mg, 0.6 mmol) and phosgene (20% solution in toluene, 310μl, 0.6 mmol) in dry toluene (1ml) at 0□C. The mixture was stirred for 1h and evaporated to remove the unreacted phosgene. The resulting solution was added to a solution of **1** (128 mg, 0.6 mmol) in dry DMF (1ml). Triethylamine (84μl) was added and the resulting mixture stirred at room temperature for 4h. The solution was then evaporated *in vacuo* and flash chromatographed to give among the N-4 and di-substituted derivatives the desired compound **21** (20mg). ^{1}H-NMR ($CDCl_3$) δ 1.2-1.6 (m, 26H), 3.0-3.1 (bs, 12H), 3.50 (dd, 2H), 4.01 (t, 2H, J = 5Hz), 4.30 (bs, 2H), 4.40 (s, 2H), 5.30 (t, 1H, J = 2.5 Hz), 5.10 (bs, 1H), 6.40 (t, 1H, J = 5Hz), 7.0-7.30 (m, 5H), 7.70 (bs, 1H). MS (EI) m/z 748 (M^+). Anal. calcd for $C_{37}H_{39}N_5O_9S$: C, 59.40 ; H, 7.70, N, 9 ;36. Found : C, 59.49 ; H, 7.74 ; N, 9.38.

VIROLOGY

Cell lines, Monocyte-derived Macrophages, Virus and Cell Cultures. The T-leukaemia virus type one (HTLV-1) CD4-positive T-cell line, MT4, was cultured in RPMI supplemented with 10% fetal calf serum (FCS) and refeeded twice a week. Monocyte-derived macrophages (MDMs) were derived from peripheral blood mononuclear cells (PBMC) of healthy blood donors. Following ficoll separation, PBMC in Hepes- buffered RPMI supplemented with 10% FCS plus 5% human normal pool sera were allowed to adhere for 2h at 37°C. After removal of non-adherent cells by extensive washing, adherent cells were cultured in RPMI supplemented with 10% FCS plus 5% human normal pool sera and 50 U/ml of recombinant GM-CSF (Genzyme). In order to eliminate any residual non-adherent cells, washing was repeated at day 1 post-isolation. Two different strains of HIV were used in this study: the T cell laboratory-adapted HIV-LAV strains [33] and the macrophage-tropic HIV-PAR strain [34] Stocks of HIV-LAV and HIV-PAR were prepared in CEM cells and cord blood lymphocytes, respectively.

Anti-HIV Activity Assay.

MT4 Cell line. Anti-HIV activity was monitored by the efficiency of drug compounds to inhibit *syncytia* formation after HIV infection of MT4 as already described. [27-28] Briefly, 3 x 10^5 MT4 cells were first preincubated for 1 h at 37°C with 100 µL of various concentrations of drug compounds, first dissolved in DMSO or in H_2O and then diluted in phosphate buffer saline solution (PBS). Then, 100 µL of an appropriate virus dilution was added to the mixture and another 1h incubation period at 37°C was done. After three washes, cells were resuspended in culture medium in the presence or not of drug compounds. Cultures were then continued for 7 days at 37°C, 5% CO_2 and

refeeded at day 3 postinfection with culture medium supplemented or not with drug compounds . Each culture well was done in duplicate. The appearance of *syncytia* was followed each day with an inverted optical microscope. Typically, the virus dilution used in this assay (multiplicity of infection of 0.1 TCID50/cell) allowed *syncytia* formation at day 5 postinfection. The inhibitory concentration of drug compounds was expressed as the concentration that caused 50% inhibition of *syncytia* formation (EC_{50}) without direct toxicity for the cells.

MDMs antiviral assay. At day 4 post-isolation, MDMs were preincubated with 100 µL of various concentrations of drug compounds (first dissolved in DMSO or in water and then diluted in PBS). The day after, HIV-1 PAR (RT activity 7 x 10^4 cpm/mL) was added to cells for 2h at 37°C. Unbound particles were removed by washing and culture was continue in RPMI 10% FCS plus 5% human normal pooled sera supplemented with 50 U/ml of GM-CSF. Culture supernatant was tested every 3-4 days for viral production by standard reverse transcription assay [28]. The inhibitory concentration of drug compounds was expressed as the concentration that caused 50% inhibition of RT activity (EC_{50}) without direct toxicity for the cells.

Cytotoxicity Assay The cytotoxic concentration (CC_{50}) of drug compounds was monitored on growth of non-infected cells by Trypan blue exclusion assay and corresponded to the concentration required to caused 50% of cell death.

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