Eur J Med Chem (1995) 30, 789–800

Synthesis and anti-human immunodeficiency virus type 1 activities of new peptido-nucleoside analogues

M Camplo[1,2], V Niddam[1,2], P Barthélémy[1,2], P Faury[1,2], N Mourier[1,2], V Simon[1,2], AS Charvet[1,2], C Trabaud[1,2], JC Graciet[1,2], JC Chermann[2], JL Kraus[1,2]*

[1]*Laboratoire de chimie biomoléculaire, Faculté des sciences de Luminy, Case 901, 13288 Marseille Cedex 9;*
[2]*INSERM Unité U322, Unité des rétrovirus et maladies associées,*
Campus universitaire de Luminy, BP 33, 13273 Marseille Cedex 9, France

(Received 20 February 1995; accepted 14 June 1995)

Summary — In order to investigate whether antiproteasic peptides coupled to anti-reverse transcriptase nucleosides can act as inhibitors at the different stages of the HIV life cycle, various peptido-nucleosides were synthesized using methodologies involving (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as a coupling reagent between the N^4-cytosinyl moiety and the peptide carboxy terminus. The anti-HIV-1 activity in MT_4 cells of this new class of compounds and their anti-HIV protease activities were determined. Fourteen peptido-nucleosides have been synthesized and six act against both the HIV-protease and viral replication *in vitro*. Although the activity of the most potent compounds against HIV was found to be one order of magnitude lower than that of the parent nucleoside drug 2',3'-dideoxy-3'-thiacytidine, this new class of compound could be of biological interest. Indeed, since the *in vitro* half-lives ($t_{1/2}$) of the hydrolysis of the most potent compounds in human plasma were found to be longer than 2.5 h, these analogues could reach the infected cells in their structural integrity. This observation does not exclude that these compounds may exert their antiviral effects as combined prodrugs through extracellular or intracellular hydrolysis.

peptido-nucleoside / HIV / 2',3'-dideoxythiacytidine / HIV-antiprotease inhibitor / reverse transcriptase inhibitor

Introduction

In the treatment of acquired immunodeficiency syndrome (AIDS) [1], the targeting of the viral enzymes necessary for viral replication represents one of the most successful rational approaches of modern antiviral chemotherapy. Thus, many nucleoside and peptide derivatives have been recognized as potent and selective inhibitors of the replication of the human immunodeficiency virus (HIV). Only four drugs have been approved by the Food and Drugs Administration (FDA) for the treatment of AIDS patients: 3'-azido-3'-deoxythymidine (AZT, Zidovudine, Retrovir®) [2]; 2',3'-dideoxyinosine (ddI, Videx®) [3]; 2',3'-dideoxycytidine (ddC, Zalcitabine®) [4]; and recently 2',3'-didehydro-3'-deoxythymidine (d4T, Stavudine®) [5, 6]. These drugs inhibit viral-encoded reverse transcriptase (RT). One of the other most studied fields of research in HIV/AIDS chemotherapy involves the peptide-involving inhibitors of the viral encoded protease of HIV [7, 8]. Indeed, HIV protease, a member of the aspartyl protease family, plays a key role in processing the HIV polyproteins gag and gag-pol [7] which are produced directly after translation. The result of these steps is the production of viral particles which are morphologically immature and noninfectious [9]. Although AZT and nucleoside combinations [10] slow down the progress of the AIDS disease, serious side effects, particularly bone marrow suppression, are severe and often require cessation of the treatment [11, 12]. As regards anti-HIV protease, numerous compounds appear very promising, such as Ro 318959 (Roche) [13], A-77003 (Abbott) [14], Sc-52151 (Monsanto-Searle) [15] and L-735,524 (Merck) [16], but the duration of the clinical trials is not yet long enough to provide a suitable conclusion on their possible use in HIV chemotherapy. Therefore, it is imperative to search for new anti-HIV drugs with improved selectivity and efficiency, and which could overcome problems of resistance as well as toxicity [17, 18].

Towards this goal, one of the most successful approaches is combination drug therapy [10]. Indeed combinations of antiviral drugs with different viral targets or mechanisms of action could potentially be additive, synergistic or antagonistic [19, 20]. Com-

*Correspondence and reprints

bined treatment is generally accepted as having the potential to increase the activity of the two drugs at low concentrations. Such treatments have been suggested to decrease the development of resistance [21]. Various combinations have been tested, *eg*, the combination of nucleosides and anti-protease peptides, such as AZT and SK&F-108922 (Smith-Kline Beecham) [19], and between nucleosides, such as AZT, ddI or ddC [22, 23]. In numerous cases, synergistic effects have been reported. Combinations of various nucleosides (AZT, ddC) with anti-protease inhibitors like Ro 31-8959 results in the reduction of concentrations between two- and threefold compared with those needed when inhibitors are used alone [20]. Moreover, Busso *et al* [24] and Schinazi *et al* [25] showed that several nucleotide dimers were potentially useful as antiretroviral agents and inhibited HIV-1-induced cytopathic effects. These features prompted us to synthesize and evaluate new peptido-nucleosidic analogs with the general structure shown in figure 1. These models could inhibit both the HIV reverse transcriptase (RT) and the HIV protease. (±) 3TC [BCH-189] (2',3'-dideoxy-3'-thiacytidine) **1** was first synthesized by Belleau *et al* [26–28] and has since proved to be very promising [29]. It was therefore selected for the new model. Indeed (±) 3TC showed potent anti-HIV activity (EC_{50} = 0.73 μM) in MT_4 cells. It was ten times less toxic than AZT in the same cell system and also exhibited potent anti-HIV activity (EC_{50} = 0.02–0.06 μM) in human peripheral mononuclear blood (PMB) cells [30, 31]. The peptidic moiety includes various residues in its structure which mimic the transitional state during HIV-protease hydrolysis [8, 14]. Most of the anti-HIV protease peptides were provided by Sanofi Recherches, Montpellier, France. The syntheses of some of them have previously been published [32, 33]. They were all found to be potent *in vitro* inhibitors of the HIV-protease, and some of them were also active against HIV-replication in cell culture.

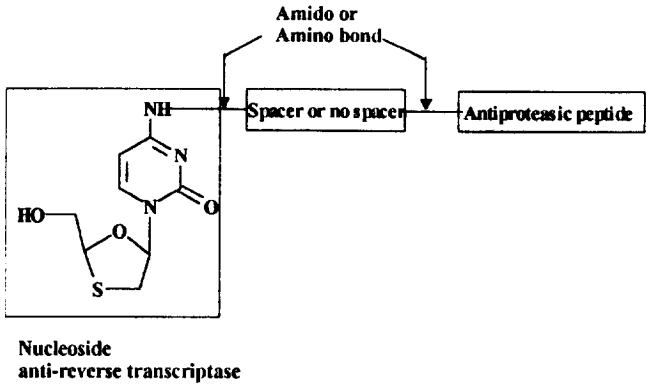


Fig 1. General structure of the new peptidic-nucleoside model.

Several reasons support the design of these peptido-nucleosidic compounds. (a) As long as the peptido-nucleoside is not hydrolyzed extracellularly, both structural moieties should be targeted and internalized within the same cell. In this hypothesis, HIV-RT and HIV-protease belonging to the same cell could be inhibited. In this case, the activity of this new model on virus replication cycle could be different from that of the simplest combination of the nucleoside and peptide moieties. (b) From the literature reports mentioned above, some synergistic effects on the inhibition of HIV replication could be expected. (c) Depending on the nature of the chemical bond between the peptide and the nucleoside, we can expect some extra- or intracellular hydrolysis which could release the constituting moieties. In this view, the model will act as a prodrug [34, 35]. (d) Since RT inhibition requires enzymatic phosphorylation by cellular kinase [36–38] at the 5'-position, the coupling between the peptide and the nucleoside occurred at the N_4-nitrogen atom of cytosinyl group. (e) Most nucleosidic drugs develop HIV resistance, whereas the peptido-nucleosidic compounds could represent an improvement on this [17, 39]. (f) Transport, delivery and bioavailability might also be enhanced, depending on the lipophilic character of these new models [40].

In this paper, we describe the synthesis and the antiretroviral properties of new peptido-nucleosides, which incorporate dual antiprotease and anti-reverse transcriptase inhibitory activities within the same molecule.

Chemistry

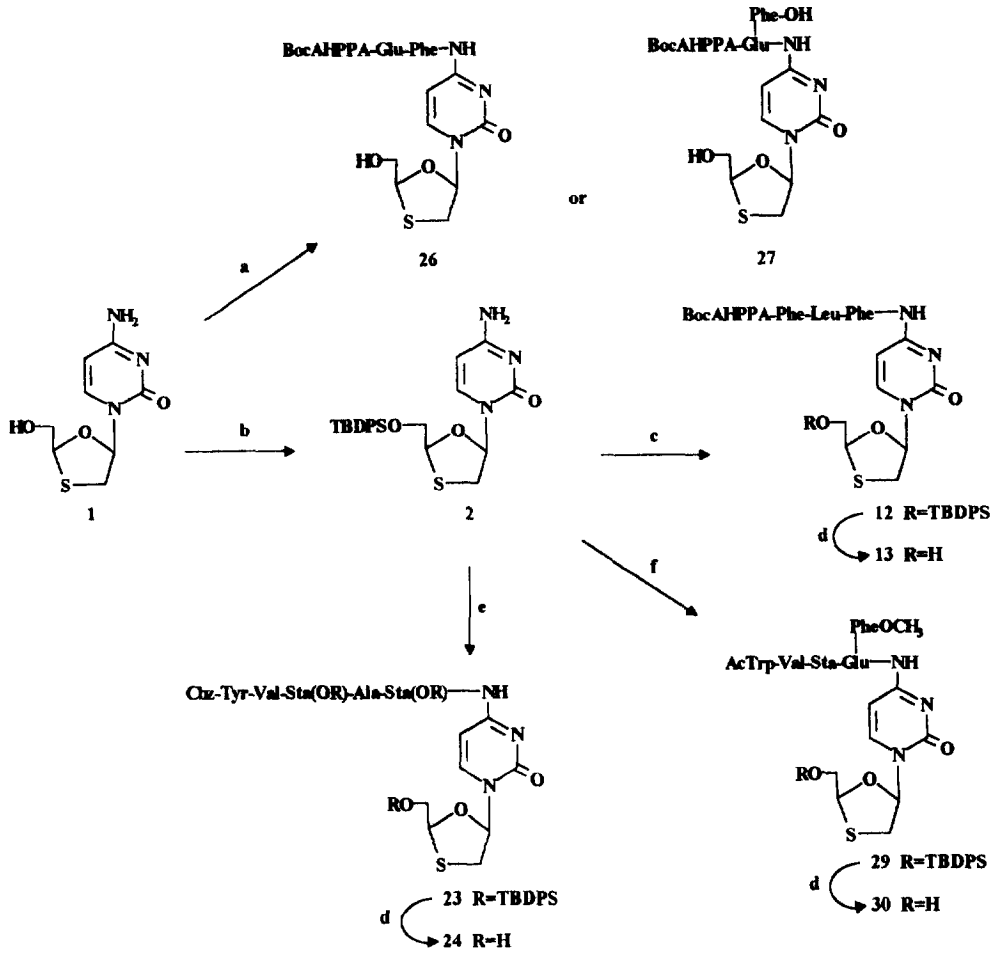
The HIV-protease peptide inhibitors used in this work are listed in table I. For those provided by Sanofi the syntheses have already been reported [32, 33]. The synthesis of the others is reported here. The synthesis of **1** was achieved according to a previously published procedure [26, 27, 31, 41].

The hydroxyl function of compound **1** was first protected with *tert*-butyldiphenylsilylchloride (TBDPSCl) leading to the corresponding protected nucleoside **2** (scheme 1). The *N*-protected peptide was then condensed to **2**, in the presence of (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphonate (BOP) [42, 43] or dicyclohexylcarbodiimide and 1-hydroxybenzotriazol (DDC/HOBT) [44] in DMF/TEA. The adduct obtained was then desilylated to produce the final peptido-nucleoside compound. The suitable AHPPA (4-amino-3-hydroxy-5-phenylpentanoic acid) analogs **7** and **8** were prepared in a concise manner as shown in scheme 2. L-Phenylalanine methyl ester hydrochloride

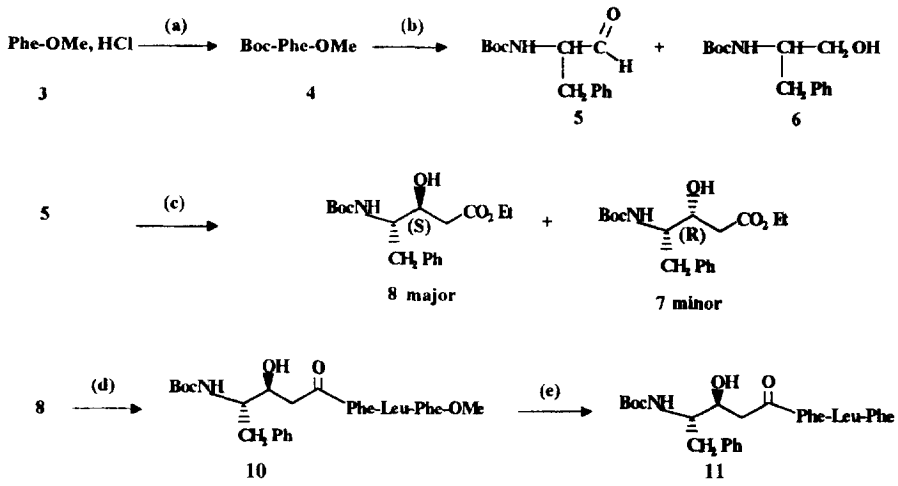
Table I. Anti HIV-1 activity of various peptides.

Compound	*Peptide*	*IC_{50}[a] (μM) aspartyl protease*	*IC_{50}[b] (μM) syncitia formation*
10	BocAHPPA-Phe-Leu-Phe-OCH_3	Inactive	Inactive
19	CbzTyr-Val-Sta-Ala-Sta-OH	0.06	Inactive
25	BocAHPPA-Glu-Phe-OH	0.2	Inactive
28	AcTrp-Val-Sta-Glu-Phe-OCH_3	0.01	Inactive
31	Trp-Val-Sta-NH-CH(Ph)-CH_2Ph	0.02	5
35	Trp-Val-Sta-Glu(OChex)-Phe-OCH_3	0.05	Inactive

[a]IC_{50} = concentration required to inhibit 50% of the enzymatic activity. [b]Concentration under which there is syncitia apparition.



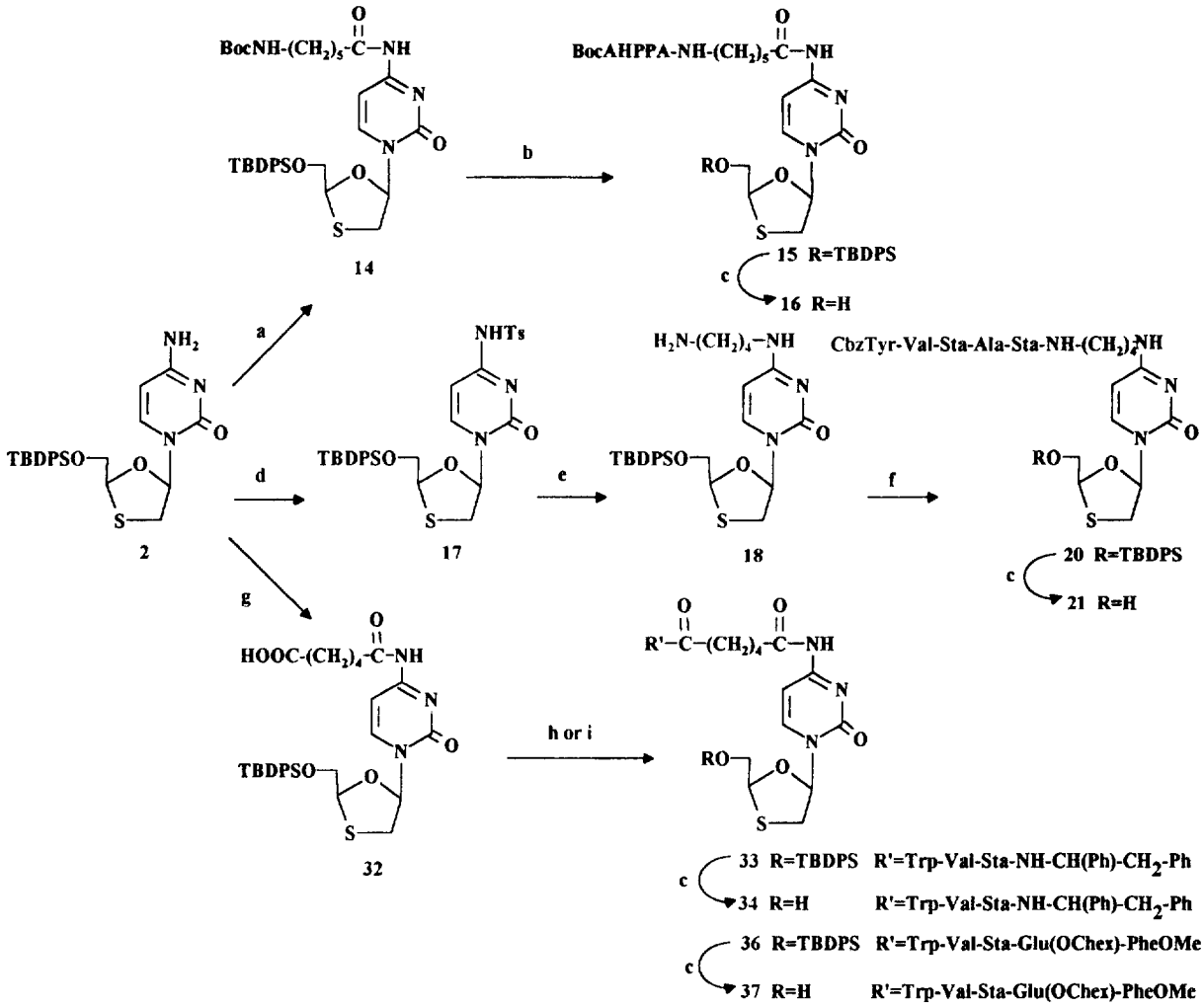
Scheme 1. Reagents: (a) BocAHPPA-Glu-PheOH **25**, DDC, HOBT, CH_2Cl_2; (b) TBDPSCl, pyridine; (c) BocAHPPA-Phe-Leu-PheOH **11**, BOP, DMF, TEA; (d) TBAF/THF; (e) CbzTyr-Val-Sta(OTBDMS)-Ala-Sta(OTBDMS) **22**, BOP, TEA, CH_2Cl_2; (f) AcTrp-Val-Sta-Glu-PheOCH_3 **28**, DDC, HOBT, CH_2Cl_2, DMF/TEA.



Scheme 2. Reagents: (a) $(Boc)_2O/CH_2Cl_2$, TEA; (b) DIBAL/CH_2Cl_2 –70°C; (c) Zn/$BrCH_2CO_2Et$/THF reflux; (d) NaOH/THF, Phe-Leu-Phe-OMe **9**, BOP, TEA/DMF; (e) NaOH/THF.

(**3**) was *N*-protected with di-*tert*-butyldicarbonate [45] to give **4** which was then reduced with diisobutylaluminium hydride (DIBAL) into *N*-(*tert*-butyloxycarbonyl)-L-phenylalaninal **5** and a small amount of *N*-(*tert*-butyloxycarbonyl)-L-phenylalaninol **6**. A Reformatsky reaction between Boc-phenylalaninal **5** and ethylbromoacetate in the presence of activated Zinc under refluxing THF gave the major enantiomeric adduct **8**. Thaisrivongs *et al* [46] have reported that under these reaction conditions, the yield was 87% and the formation of adduct **8** was favored over adduct **7** both kinetically and thermodynamically. This feature was important since it has been shown that the *S* configuration was the preferred stereochemistry of the C_3 carbon of **8** for an optimal inhibition of the aspartyl protease [47]. After separation of compounds **7** and **8** by flash chromatography, compound **8** was saponified with NaOH in THF and the resulting adduct was immediately coupled with the peptide Phe-Leu-Phe-OMe **9**. This reaction was performed using BOP as a coupling reagent to produce compound **10**. Compound **10** was saponified with NaOH/THF to give **11**, whereas **1** was protected in the 5' position with 2 equiv of TBDPSCl in pyridine to give **2** (scheme 1). The latter was then coupled with peptide **11** to produce peptido-nucleoside **12**. To our knowledge, this is the first time that the BOP reagent has been used for coupling between a peptide and a nucleoside. It was usually successfully applied in terms of yields ease of use and specificities. This was not the case with DCC, DCC/HOBT, EDCI (1-ethyl-3-[3-(dimethylaminopropylcarbodiimide)]) or oxalyl chloride for similar reactions [48, 49]. Further desilylation of **12** with tetra-*N*-butylammonium fluoride (TBAF) in THF allowed the formation of **13**.

In order to functionalize the protected nucleoside **2**, we used different strategies which led to nucleosides possessing different spacers on their N^4-cytosine functions. One of these nucleosides **14** (scheme 3) was produced by coupling the 6-*N*-(*tert*-butoxycarbonyl)-hexanoic acid **4** with the 5'-*O*-silylated 2',3'-dideoxy-3'-thiacytidine **2** using the BOP reagent. The Boc group of **14** was then removed with trifluoroacetic acid (TFA) and the resulting adduct coupled with Boc-AHPPA **8** using the BOP reagent again to give **15**. Compound **15** was then desilylated with TBAF in THF to give **16** (scheme 3). Another useful intermediate was the compound **18** (scheme 3) on which a spacer had been introduced through an *N*-alkylation. Several attempts had to be investigated to attain this goal. Previous articles have described several methods of the synthesis of N^4-alkylated cytosines [50–52]. However, most of these methods used thymine or uracil derivatives as starting materials. The first attempt was direct N^4-alkylation using halogenated derivatives by analogy with N^4-methyl cytosine compounds [53]. These reactions failed so we used another method which consisted of using N^4-tosyl cytosine derivatives of **2** first. Indeed, Markiewicz *et al* [54] have shown that substituting the N^4-amino cytosine function of some nucleosides by methanesulfonic acid or *p*-toluenesulfonic acid could lead to compound like **18** (after alkylation by primary or secondary amines). This successful approach was therefore employed. Compound **2** was first tosylated with *p*-toluenesulfonyl chloride in pyridine at 60°C to



Scheme 3. Reagents: (a) $BocNH(CH_2)_5COOH$ **4**, BOP, TEA, CH_2Cl_2; (b) TFA/CH_2Cl_2 then BocAHPPA, BOP, TEA, CH_2Cl_2; (c) TBAF/THF; (d) TsCl/pyridine, 60°C; (e) $H_2N(CH_2)_4NH_2$, lutidine, 90°C; (f) CbzTyr-Val-Sta-Ala-StaOH **19**, BOP, TEA, DMF; (g) adipic acid, BOP, TEA, CH_2Cl_2; (h) Trp-Val-Sta-NH-CH(Ph)-CH_2-Ph **31**, BOP, TEA, CH_2Cl_2; (i) Trp-Val-Sta-Glu(OChex)-$PheOCH_3$ **35**, BOP, TEA, DMF.

give after purification **17**. 1,4-Diaminobutane in lutidine was then added at 90°C to give the N^4-alkylated nucleoside **18**. The latter was coupled with the peptide Cbz-Tyr-Val-Sta-Ala-Sta-OH **19**, using BOP reagent to produce **20**, which gave **21** after desilylation with TBAF in THF (scheme 3).

For the synthesis of compound **24** (scheme 1), the hydroxyl functions of the statine moiety of peptide **19** (CbzTyr-Val-Sta-Ala-Sta-OH) were silylated before the coupling step. Although this protection procedure is not always required we observed many improvements in terms of yield and ease of use following such silylations. Indeed, as has been mentioned in the literature [55, 56], this precaution allowed us to remove the reactivity of the hydroxyl functions and increase the hydrophobic properties of the peptide **19**. The latter was protected with an excess of *tert*-butyldimethylsilyl chloride in DMF/imidazole. Since the silylation also occurred at the carboxy-terminus group of **19**, the resulting silylated product was immediately allowed to stir with methanol in order to deprotect this function to give **22** [55]. Compound **22** was coupled with the nucleoside **2** to give **23** (scheme 1). Deprotection of **23** with TBAF in THF produced the final compound **24**.

The peptide **25** was coupled with **1** (scheme 1). Using BOP reagent in the coupling reaction in this case failed, whereas DCC/HOBT was employed in a

successful manner. From these coupling experiments, several products were theoretically possible. Products resulting from a 5'-esterification products were rejected on the basis of a structural analysis and bibliographic search [49]. However, we were not able to establish the correct structure of the compounds formed and structures **26** and **27** were both possible (scheme 1).

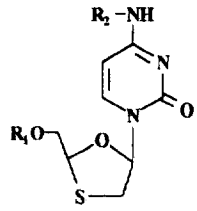
Since the use of BOP was unsuccessful, peptide **28** was coupled to silylated nucleoside **2** using DCC/HOBT as coupling reagent to produce **29**, which was deprotected to give **30** (scheme 1).

The synthesis of **33** (scheme 3) was performed by coupling adipic acid to the 5'-silylated nucleoside **2** and then by coupling the resulting intermediate **32** with the peptide **31**. Both steps required the use of the BOP reagent in the presence of triethylamine in methylene chloride. Deprotection of compound **33** using TBAF in THF, produced the peptido-nucleoside **34**. In the same manner, compound **37** (scheme 3) was obtained by coupling the peptide **35** with the modified nucleoside **32** leading to the intermediate **36** which after deprotection led to compound **37**.

Biological results

The potency of the synthetic peptido-nucleosidic analogs as inhibitors of both HIV-1 protease and viral replication was evaluated and the results are presented in table II. Table I shows the inhibition of HIV-1 protease and viral replication by the peptidic moieties alone. The IC_{50} values were determined for the inhibition of the HIV protease [32]. The inhibition of HIV-1 replication is measured [57, 58] by the formation of syncitia in HIV-1-infected MT_4 cells [59]. For all the tested compounds listed in table I we observed a dose-dependent relationship of this inhibition. Their IC_{50}

Table II. Anti-HIV-1 and anti-aspartyl protease activities of various peptido-nucleosidic compounds.



Compound	R_1	R_2	IC_{50}[a,b] *(μM) aspartyl protease*	IC_{50}[c] *(μM) syncitia formation*	*TI*[d] *(ID_{50}/IC_{50}[c])*
12	TBDPS	BocAHPPA-Phe-Leu-Phe-	30	Inactive	–
13	H	BocAHPPA-Phe-Leu-Phe-	30	1	500
16	H	BocAHPPA-NH$(CH_2)_5$CO-	–	100	–
20	TBDPS	CbzTyr-Val-Sta-Ala-Sta-NH$(CH_2)_4$-	1	Inactive	–
21	H	CbzTyr-Val-Sta-Ala-Sta-NH$(CH_2)_4$-	0.6	Inactive	–
23	TBDPS	CbzTyr-Val-Sta(OTBDMS)-Ala-Sta(OTBDMS)-	10	Inactive	–
24	H	CbzTyr-Val-Sta-Ala-Sta-	0.2	50	10
26 or 27	H	BocAHPPA-Glu-Phe- or BocAHPPA-Glu-Phe	10	100	10
29	TBDPS	AcTrp-Val-Sta-Glu-PheOCH_3	0.06	Inactive	–
30	H	AcTrp-Val-Sta-Glu-PheOCH_3	0.02	10	50
33	TBDPS	-CO$(CH_2)_4$COTrp-Val-Sta-NHCH(Ph)CH_2Ph	0.45	–	–
34	H	-CO$(CH_2)_4$COTrp-Val-Sta-NHCH(Ph)CH_2Ph	0.4	10	500
36	TBDPS	-CO$(CH_2)_4$COTrp-Val-Sta-Glu(OChex)-PheOCH_3	2	Inactive	50
37	H	-CO$(CH_2)_4$COTrp-Val-Sta-Glu(OChex)-PheOCH_3	0.01	100	–

[a]The assay was performed according to the published procedure listed under references [31, 63]. [b]Concentration needed to inhibit at 50%, cleavage of standard peptide; see reference [64] for comparison to standards. [c]IC_{50} = concentration required to inhibit syncitia formation by 50% on MT_4 cells. [d]TI = therapeutic index. ID_{50} = concentration required to cause 50% death of uninfected MT_4 cells. The enzyme and antiviral activities of several structurally unrelated HIV protease inhibitors were compared using HIV protease and HIV-1 cell culture assays described in this paper; ED_{50}/IC_{50} ratios ranged from 4.7 to 21.7 for Ro 31-8959 (Roche) (IC_{50} = 0.85 (< 1.4) nM; ED_{50} = 4 (2) nM) [13], A-77003 (Abbott) (IC_{50} = 2.3 (< 1) nM; ED_{50} = 50 (200) nM) [14], Sc-52151 (Monsanto-Searle) (IC_{50} = 4.0 (6.0) nM; ED_{50} = 20 (21) nM) [15], and L-735,524 (Merck) (IC_{50} = 1.1 (0.4) nM; ED_{50} = 10 (50) nM)) [16]. The first set of values quoted are from Barrish *et al* [8], while the values in parentheses were reported separately by other authors.

values (concentration required to produce 50% inhibition of syncitia formation) are reported in table I. The results of the experiments presented in table II showed that six of the 14 peptido-nucleoside analogs (**13**, **24**, **26** and **27**, **30**, **34** and **37**) inhibit both HIV-1 aspartyl protease and viral replication. Their chemical structures have the following characteristics: (i) the hydroxyl function in 5' position is free; and (ii) the peptidic and nucleosidic moieties are linked by the intermediate amide bond which involves the N^4-amino group.

Compounds **20**, **29** and **36** are HIV protease active but inactive with respect to viral replication. These compounds present a 5'-OH function, which is protected by *tert*-butyldiphenylsilyl ester.

The biological tests of compound **34** result in the following remarks. Peptide **31** is active on HIV protease and in viral replication, and so we could expect compound **34** to have a higher potency due to a possible synergistic effect of the two activities. In the tested *in vitro* experimental conditions, we did not observe such an effect. Compounds **20** and **21** can be considered to result from amide bond reduction of the N^4-cytosine atom. These compounds are inactive as HIV replication inhibitors. The efficiency of **20** and **21** for inhibiting the HIV protease is lower than that of the parent compound **19**.

Compounds **16**, **33**, **34** and **37** present a diamide spacer on the amino N^4-cytosine function and it is difficult to determine the effect of this spacer on the antiviral activity. For example, compounds **36** and **37** maintain their antiprotease activities relatively close to that of the single peptide **35**. The half-lives ($t_{1/2}$) of the hydrolysis of the most potent compounds **13** and **34** were determined in human plasma and were found to be 6 and 2.5 h, respectively. (The $t_{1/2}$ values of hydrolysis of compounds **13** and **34** were determined according to a previously published procedure [34].) These results suggest that these compounds may have increased plasma $t_{1/2}$ under *in vivo* conditions. Indeed, active antiviral compounds with *in vivo* $t_{1/2}$ higher than 30 min are considered as potential candidates [60].

Discussion

Six of the 14 peptido-nucleosides synthesized act against both HIV protease and viral replication. Compound **34** is particularly interesting since it inhibits both HIV-1 protease (with an IC_{50} = 0.4 μM) and viral replication *in vitro* (IC_{50} = 10 μM). This suggests that this kind of compound could act separately on the two targeted enzymes, reverse transcriptase and the HIV protease, but not necessarily simultaneously. Because these compounds have relatively long half-lives in human plasma ($t_{1/2}$ more than 120 min), they could be considered as potential *in vivo* candidates [60] thus targeting the same infected cell.

This represents an advantage in comparison to mixtures made from both constituents, the peptide and the nucleoside. In contrast to studies reported on the potentiation of antiviral effects by a combination of HIV protease (SK&F 1089282 or Ro 631-8959) and RT inhibitors (AZT, ddI) [19, 20], our results show that no increase of these antiviral effect was observed. One explanation is that these conjugates may act as prodrugs, thereby releasing the active antiviral agent by hydrolysis. Our results also show that the reduction in activity of the peptido-nucleoside conjugates compared with their components is not in a fixed ratio. Therefore, it is possible that some of these peptido-nucleosides adducts themselves have antiviral activities. Indeed, if the observed anti-HIV activity in syncitia formation was only due to the release of **1** during the hydrolysis process, all the prodrug analogs should be active in the experimental conditions. Our present results and some previous observations [43, 61, 62] have clearly shown that some N^4-substituted 2',3'-dideoxy-3'-thiacytidine conjugates are not active. Importantly, on the basis of the *in vitro* studies presented here, it is likely that peptido-nucleoside analogs such **24**, **26** and **27**, which incorporate in their structure an anti-protease moiety that is not active alone in viral replication in cell cultures, are active in both biological assays. This observation could be of interest if the half-lives of these analogs are long enough to reach an infected cell in their structural integrity. In this case, both anti-protease and anti-RT moieties should targeted and internalized in the same cell. Consequently, each component could act at the different stages of the viral life cycle at which their antiviral activities function.

Our results confirm the fact that the 5'-OH function of the peptido-nucleosidic compound should be left free so that it can be phosphorylated by cellular kinase and therefore inhibit the viral replication [36–38]. Our results also showed that the bond between the peptide and the nucleoside should preferably be an amide. Indeed, the reduction of this function seems to abolish its potency on viral replication inhibition and decreases that of its inhibition on the HIV protease. This fact could be explained by the fact that compounds with an amide bond can be hydrolyzed intracellularly and therefore have an activity toward the hydrolyzed products.

In summary, we have developed the synthesis of new peptido-nucleosidic compounds. Some of them have properties that inhibit both HIV protease *in vitro* and the viral replication in culture cell and could therefore be of clinical importance. Obviously more detailed biochemical studies are necessary to understand the mode of action of these compounds. Toward

this goal, research on these models may become useful in the future.

Experimental protocols

Chemistry

Nuclear magnetic resonance (NMR) spectra were recorded with a Varian XL-200 (^{1}H-NMR, ^{13}C-NMR) spectrometer. Chemical shifts values were expressed in δ values (part per million) relative to tetramethylsilane as an internal standard for ^{1}H. Multiplicity is abbreviated as s (singlet), d (doublet), t (triplet), br (broad), or m (multiplet). Coupling constants are expressed in hertz (Hz). FAB$^+$ mass spectra were obtained on a NERMAG 10-10C mass spectrometer (Sanofi Recherches, Montpellier, France) using a glycerol/HCl matrix. Ultraviolet spectra were obtained using an UVIKON 930 (Kontron Instruments) spectrophotometer. Elemental microanalysis were determined by Service Central d'Analyse CNRS, Vernaison, Lyon, France, and gave combustion values for C, H, N within 0.4% of the theoretical values. Preparative flash column chromatography was performed using silica-gel Merck G60 230-240 mesh. Analytical thin-layer chromatography (TLC) was performed on silica-gel plates $60F_{254}$ aluminium (Merck, Darmstadt) of 0.2 mm thickness. Preparative layer chromatographies (PLC) were carried out on silica-gel $60F_{254}$ pre-coated PLC plates (20 x 20 cm layer, thickness 1 or 2 mm). Optical rotations were determined on a JASCO DIP-370 polarimeter. All solvents were used from sealed bottles purchased from the Aldrich Company. All amino acid used as starting materials were of the L configuration. *N,N'*-Dicyclohexylcarbodiimide (DCC) was recrystallized from ether.

The synthesis of the key intermediate **1** ((±) 3TC) was performed according to a procedure reported previously [26, 27, 31, 41].

General procedure A. Coupling reactions using (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent)

The peptide (1.0 equiv) was dissolved in CH_2Cl_2 or DMF (1–2 mL). (±) 3TC or silylated (±) 3TC was added, followed by a solution of BOP (1.5 equiv) and triethylamine (3 equiv). The reaction mixture was allowed to stir at room temperature for 3–5 h. After evaporation to dryness, brine and EtOAc were added. After extraction, the organic phase was dried over Na_2SO_4 and the peptido-nucleoside was purified by flash-chromatography.

General procedure B. Desilylation of protected peptido-nucleosidic compounds

A solution of protected peptido-nucleosides (1.0 equiv) and tetra-*n*-butylammonium fluoride (1 M in THF) (2 equiv) in THF (3–5 mL) was stirred at room temperature until TLC indicated the disappearance of starting material (0.5–2 h). The reaction mixture was concentrated under reduced pressure and the residue was purified by silica-gel flash column chromatography or PLC using the appropriate solvent.

cis-Isomer of 2-[(tert-butyldiphenyl)silyloxymethyl]-5-(cytosin-1'-yl)-1,3-oxathiolane 2

(±) 3TC **1** (100 mg, 0.43 mmol) was partially dissolved in 6 mL anhydrous pyridine under a nitrogen atmosphere. After the addition of *tert*-butylchlorodiphenylsilane (135 µL, 0.52 mmol), the mixture became clear and was stirred overnight. The solvent was removed under reduced pressure, the residue was hydrolyzed and extracted with EtOAc (3 x 5 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated to give a solid which was recrystallized in EtOAc to give a white solid (159 mg) in 80% yield. TLC (EtOAc/MeOH 2:1) R_f 0.62. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (s, 9H, *t*Bu), 3.2 (dd, J_{gem} = 12.4 Hz, J = 2.9 Hz, 1H, C_4H), 3.5 (dd, J_{gem} = 12.4 Hz, J' = 4.4 Hz, 1H, C_4H), 4.08 (dd, 2H, C_2CH_2O), 5.25 (t, J = 6.1 Hz, J' = 5.7 Hz, 1H, C_2H), 5.51 (d, J = 7.6 Hz, 1H, C_5H), 6.37 (dd, J = 5.4 Hz, J' = 2.9 Hz, 1H, C_5H), 7.44 (m, 5H, Ar*H*), 7.69 (m, 5H, Ar*H*), 8.04 (d, J = 7.6 Hz, 1H, C_6H).

N-(tert-Butoxycarbonyl)-L-phenylalaninemethylester 4

At 0°C, di-*tert*-butoxydicarbonate (7 mL, 30 mmol) was added dropwise under a nitrogen atmosphere to a solution of L-phenylalanine methylester hydrochloride **3** (5 g, 23 mmol) and triethylamine (5.5 mL, 56 mmol) in CH_2Cl_2 (120 mL). The resulting mixture was stirred at room temperature for 1 d. After solvent evaporation, EtOAc (150 ml) was added and the solution washed with 5% citric acid (100 mL), then with 5% $NaHCO_3$ (100 ml) and finally with H_2O (50 mL). The organic layer was dried over Na_2SO_4 and evaporated to give an oily residue (7.4 g, quantitative). TLC (EtOAc/hexane 3:1) R_f 0.61. ^{1}H-NMR ($CDCl_3$) δ: 1.4 (s, 9H, *t*Bu), 3.1 (m, 2H, $CH_2\beta$ Phe), 3.7 (s, 3H, OCH_3), 4.6 (q, 1H, $CH\alpha$ Phe), 5.0 (m, 1H, N*H*), 7.1–7.4 (m, 5H, Ph).

N-(tert-Butoxycarbonyl)-L-phenylalaninal 5

To a stirred solution of **4** (7 g, 25 mmol) in dry CH_2Cl_2 (140 mL) was added diisobutylaluminium hydride (1 M) solution in hexane at –78°C under a nitrogen atmosphere. After 6 min, the reaction was quenched with MeOH (8 mL) and Rochelle salt solution was added immediately. The mixture was allowed to warm to 25°C and CH_2Cl_2 (50 mL) was added. The organic layer was separated and combined with CH_2Cl_2 extracts of aqueous layer. The combined layers were dried over $MgSO_4$ and concentrated under reduced pressure to give the crude product **5** (6 g, 96%).

N-(tert-Butoxycarbonyl)-4(S)-amino-3(S)-hydroxy-5-phenyl-pentanoic acid ethyl ester 8

To a stirred suspension of activated zinc (1.7 g, 26 mmol) in THF (13 mL) under nitrogen in an oil bath at 75°C was added ethyl bromoacetate (83 µL, 0.75 mmol). After 1 min, a solution of **5** (2.6 g, 10.5 mmol) and ethyl bromoacetate (2.7 mL, 24 mmol) in THF (13 mL) was slowly added. After complete addition, the resulting mixture was allowed to reflux for an additional 45 min. The cooled reaction mixture was partitioned between CH_2Cl_2 (2 x 30 ml) and a 5% solution of $NaHCO_3$ (50 mL). The organic phase was dried over Na_2SO_4 and then concentrated. The residue was flash-chromatographed on silica gel to give **8** (291 mg, 8%). TLC (EtOAc/hexane 3:7) R_f 0.28. Mp = 85°C. $[\alpha]_D$ = –35.2 (1.0 M, CH_3OH at 25°C). ^{1}H-NMR ($CDCl_3$) δ: 1.2 (t, J = 7.1 Hz, 3H, OCH_3), 1.4 (s, 9H, *t*Bu), 2.2–2.6 (m, 2H, J_{gem} = 16.9 Hz, J = 2.7 Hz, J' = 10.1 Hz, $-CH_2COOEt$), 2.85 (d, J = 7.6 Hz, 2H, CH_2Ph), 3.7 (m, 1H, *CH*OH), 3.95 (m, 1H, *CH*NH), 4.1 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 4.9 (d, J = 9.5 Hz, 1H, N*H*), 7.2 (m, 5H, Ar*H*).

BocAHPPA-Phe-Leu-Phe-OCH$_3$ 10

Compound **8** was first saponified according to the following procedure. NaOH (1 N) was added dropwise to a solution of **8** (110 mg, 0.3 mmol) in THF (5 mL) until pH 10. The mixture was stirred for 2 h and then evaporated under reduced pressure. H_2O (20 mL) was added and the solution acidified with 1 N HCl until pH 2–3. After extraction with EtOAc (3 x 20 mL) the organic layer was dried over Na_2SO_4 and

evaporated to give the saponified product (103 mg, 98%), which was directly used in following step. The compound (103 mg, 0.3 mmol), BOP (141 mg, 0.3 mmol) and NEt_3 (180 μL, 1.2 mmol) were added to a solution of Phe-Leu-Phe-OCH_3 **9** (140 mg, 0.3 mmol) in DMF (4 mL) and the reaction stirred for 1 d. H_2O (50 mL) was then added and the solution extracted with EtOAc (2 x 30 mL). The organic layer was dried over Na_2SO_4 and evaporated to give **10** (137 mg, 58%). TLC (EtOAc/MeOH 9:1) R_f 0.7. ^{1}H-NMR (DMSO-d_6) δ: 0.9 (dd, J = 6.3 Hz, 6H, 2CH_3 Leu), 1.3 (s, 9H, *t*Bu), 1.4–1.6 (m, 3H, $(CH_3)_2CHCH_2$ Leu), 2.2 (d, J = 6.4 Hz, 2H, CH_2CO), 3.3 (d, J = 4.8 Hz, 6H, 3CH_2Ph), 3.6 (s, 3H, OCH_3 ester), 3.65 (m, 1H, CHNH), 3.8 (m, 1H, CHOH), 4.3–4.6 (m, 3H, 3CHCONH), 6.6 (d, J = 9.0 Hz, 1H, N*H*), 7.2 (m, 15H, 3 Ar*H*), 8.0 (dd, J = 10.0 Hz, 2H, 2N*H*), 8.3 (d, J = 7.3 Hz, 1H, N*H*). MS: MH$^+$/FAB *m/z* 731.4. Anal $C_{40}H_{54}N_4O_8$ (C, H, N).

BocAHPPA-Phe-Leu-Phe ***11***
This compound results from the saponification of **10** according to the same procedure used with compound **8**.

cis-Isomer of 2-(tert-butyldiphenylsilyloxymethyl)-5-[N⁴-(Boc-AHPPA-Phe-Leu-Phe)cytosin-1'-yl]-1,3-oxathiolane ***12***
To a solution of **11** (110 mg, 0.15 mmol) in CH_2Cl_2 (10 mL) and DMF (1 mL) were successively added **2** (80 mg, 0.17 mmol), NEt_3 (42 μL, 0.3 mmol) and BOP (132 mg, 0.3 mmol). The mixture was stirred at room temperature for 3 h. Brine (10 mL) was then added and the solution extracted with EtOAc (2 x 15 mL). The organic layer was dried over Na_2SO_4 and evaporated to give a residue which was purified by flash chromatography to give **12** (67 mg, 38%). TLC (toluene/MeOH 4:1) R_f 0.5. ^{1}H-NMR (CD_3OD) δ: 0.8 (m, 6H, 2CH_3 Leu), 1.1 (s, 9H, *t*Bu), 1.3 (s, 9H, Boc), 1.5–1.7 (m, 3H, $(CH_3)_2CHCH_2$ Leu), 2.3–2.5 (q, J = 7.0 Hz, J_{gem} = 15.0 Hz, 2H, -COCH_2CHOH-), 3.0–3.4 (m, 6H, 3CH_2Ph), 3.5 (m, 2H, C_4H_2), 3.9 (m, 1H, CH_2CHOH), 4.0–4.2 (m, 2H, C_2CH_2O), 4.2–4.7 (m, 3H, CHNHCO), 5.25 (t, J = 5.6 Hz, 1H, C_2H), 5.9 (d, J = 7.5 Hz, 1H, C_5H), 6.3 (t, J = 5.5 Hz, 1H, C_5H), 7.2 (m, 15H, 3 Ph), 7.4–7.7 (m, 10H, 2 Ph), 8.15 (d, J = 7.5 Hz, 1H, C_6H). MS: MH$^+$/FAB *m/z* 1166.5.

cis-Isomer of 2-hydroxymethyl-5-[N⁴-(BocAHPPA-Phe-Leu-Phe)cytosin-1'-yl]-1,3-oxathiolane ***13***
The title compound was prepared in 94% yield (crude) by *General procedure B*. TLC (toluene/MeOH 4:1) R_f 0.13. ^{1}H-NMR (CD_3OD) δ: 0.8 (m, 6H, 2 CH_3 Leu), 1.3 (s, 9H, Boc), 1.5–1.7 (m, 3H, $(CH_3)_2CHCH_2$ Leu), 2.3–2.5 (q, J = 7.0 Hz, J_{gem} = 15.0 Hz, 2H, -COCH_2CHOH), 3.0–3.3 (m, 6H, 3CH_2Ph), 3.5 (m, 2H, C_4H_2), 3.9 (m, 1H, CH_2CHOH), 4.0–4.2 (m, 2H, C_2CH_2O), 4.2–4.7 (m, 3H, 3 CHNHCO), 5.2 (t, J = 5.5 Hz, 1H, C_2H), 5.9 (d, J = 7.4 Hz, 1H, C_5H), 6.3 (t, J = 5.5 Hz, 1H, C_5H), 7.2 (m, 15H, 3Ph), 8.1 (d, J = 7.4 Hz, 1H, C_6H). MS: MH$^+$ (FAB) *m/z* 928.5. Anal $C_{48}H_{63}N_7O_{10}S$ (C, H, N).

cis-Isomer of 2-(tert-butyldiphenylsilyloxymethyl)-5-[N⁴-(Boc-1-oxo-6-aminopentyl)cytosin-1'-yl]-1,3-oxathiolane ***14***
The title compound was prepared in a quantitative yield (156 mg) from **2** (108 mg, 0.23 mmol) and *N*-boc-6-amino-hexanoic acid (53 mg, 0.23 mmol) by *General procedure A*. The crude product was used without any purification in the following step.

cis-Isomer of 2-(tert-butyldiphenylsilyloxymethyl)-5-[N⁴-(Boc-AHPPA-1-oxo-6-aminopentyl)cytosin-1'-yl]-1,3-oxathiolane ***15***
Removal of the *tert*-butoxycarbonyl group of compound **14** was achieved according to the following procedure. To a solution of **14** (156 mg, 0.23 mmol) in CH_2Cl_2 (3 mL) was added trifluoroacetic acid (382 μL, 5 mmol). The resulting mixture was stirred at room temperature until TLC indicated the disappearance of starting material (2 h). After evaporation the crude compound was directly used in the following step *via General procedure B*. The title compound **15** was obtained in 55% yield (110 mg) from deprotected compound **14** (133 mg, 0.23 mmol) and saponified product **10** (71 mg, 0.23 mmol). TLC (CH_2Cl_2/MeOH 19:1) R_f 0.33. ^{1}H-NMR ($CDCl_3$) δ: 1.1 (s, 9H, *t*Bu), 1.3 (s, 9H, Boc), 1.6–2.4 (m, 10H, -$(CH_2)_4$CONH- and CHOHCH_2CONH), 2.9 (m, 2H, CONHCH_2), 3.15 (m, 2H, CH_2Ph), 3.5–3.8 (m, 2H, C_4H_2), 3.9 (m, 1H, CH_2CHOH), 4.1 (m, 2H, C_2CH_2O), 5.2 (br s, 1H, C_2H), 5.35 (d, J = 6.6 Hz, 1H, C_5H), 6.25 (br s, 1H, C_5H), 7.15 (m, 5H, Ph), 7.4–7.8 (m, 10H, 2 Ph), 8.35 (d, J = 6.6 Hz, 1H, C_6H).

cis-Isomer of 2-hydroxymethyl-5-[N⁴-(BocAHPPA-1-oxo-6-aminopentyl)cytosin-1'-yl]-1,3-oxathiolane ***16***
The title compound was prepared in 84% yield (31 mg) from **15** (50 mg, 0.06 mmol). TLC (EtOAc/MeOH 4:1) R_f 0.5. ^{1}H-NMR ($CDCl_3$) δ: 1.3 (s, 9H, Boc), 1.6–2.4 (m, 10H, $(CH_2)_4$CONH- and CHOHCH_2CONH), 2.9 (m, 2H, CONHCH_2), 3.2 (m, 2H, CH_2Ph), 3.6–3.8 (m, 2H, C_4H_2), 3.9–4.2 (m, 3H, CH_2CHOH and C_2CH_2O), 5.2–5.4 (m, 2H, C_2H and C_5H), 6.3 (br s, 1H, C_5H), 7.2 (m, 5H, Ph), 8.4 (d, J = 7.0 Hz, 1H, C_6H). MS: MH$^+$ (FAB) *m/z* 634. Anal $C_{30}H_{43}O_8N_5S$ (C, H, N).

cis-Isomer of 2-(tert-butyldiphenylsilyloxymethyl)-5-[N⁴-(tosyl)-cytosin-1'-yl]-1,3-oxathiolane ***17***
To a solution of **2** (56 mg, 0.12 mmol) at 60°C in pyridine (3 mL) was added under nitrogen *para*-toluenesulfonyl chloride (46 mg, 0.24 mmol). The mixture was stirred for 30 h. The solution was then evaporated, a 5% citric acid aqueous solution and EtOAc were added. The organic layer was washed with H_2O, dried over Na_2SO_4 and finally evaporated. The residue was separated by flash-chromatography to give **17** (48 mg, 64%). TLC (EtOAc/MeOH 3:1) R_f 0.8. ^{1}H-NMR ($CDCl_3$) δ: 1.1 (s, 9H, *t*Bu), 2.45 (s, 3H, CH_3 tosyl), 3.15–3.55 (m, 2H, C_4H_2), 3.9–4.1 (m, 2H, C_2CH_2O-), 5.25 (br s, 1H, C_2H), 6.3 (t, J = 5.6 Hz, 1H, C_5H), 7.3 (d, J = 7.5 Hz, 1H, C_5H), 7.4–7.7 (m, 14H, ArH), 8.05 (d, J = 7.5 Hz, 1H, C_6H).

cis-Isomer of 2-(tert-butyldiphenylsilyloxymethyl)-5-[N⁴-(4-aminobutyl)cytosin-1'-yl]-1,3-oxathiolane ***18***
To a solution of **17** (400 mg, 0.63 mmol) in lutidine (4 mL) at 90°C was added 1,4-diaminobutane (380 μL, 0.63 mmol) under nitrogen. The resulting mixture was stirred for 2 d and then evaporated to give after purification by flash chromatography **18** (338 mg, quantitative). TLC (EtOAc/pyridine/acetic acid/H_2O 8:4:1:2) R_f 0.32. ^{1}H-NMR (DMSO-d_6) δ: 1.0 (s, 9H, *t*Bu), 1.45 (br s, 4H, -$(CH_2)_2$- diaminobutane), 2.6 (br s, 2H, CH_2NH_2), 3.2 (br s, 2H, NHCH_2), 3.1 and 3.4 (m, J = 4.8 Hz, J' = 5.0 Hz, J_{gem} = 11.7 Hz, 2H, C_4H_2), 3.8 (br s, 2H, NH_2), 4.0 (m, 2H, C_2CH_2O), 5.3 (t, J = 4.0 Hz, 1H, C_2H), 5.65 (d, J = 7.5 Hz, 1H, C_5H), 6.2 (t, J = 4.8 Hz, J' = 5.0 Hz, 1H, C_5H), 7.45–7.65 (m, 10H, Ar*H*), 7.8 (d, J = 7.5 Hz, 1H, C_6H).

cis-Isomer of 2-(tert-butyldiphenylsilyloxymethyl)-5-[N⁴-(CbzTyr-Val-Sta-Ala-Sta-4-aminobutyl)cytosin-1'-yl]-1,3-oxathiolane ***20***
The title compound was prepared in 56% yield (64 mg) from **18** (47 mg, 0.09 mmol) and **19** (125 mg, 0.16 mmol) by *General procedure A*. TLC (EtOAc/pyridine/acetic acid/H_2O 12:4:1:2) R_f 0.5. ^{1}H-NMR (CD_3OD) δ: 0.9 (m, 12H, 2 [$(CH_3)_2$ Sta]), 1.1 (s, 9H, *t*Bu), 1.5 (m, 20H, $(CH_3)_2CH$ Val, CH_3 Ala, 2 [$(CH_3)_2CHCH_2$ Sta] and -$(CH_2)_2$-), 2.2–3.0 (m, 8H, 2 [COCH_2CHOH], CH_2NH_2 and NHCH_2), 3.4–4.7 (m, 10H,

C_4H_2, 2 [CH_2CHOH], C_2CH_2O and 5[CHNHCO]), 5.0 (s, 2H, OCH_2Ph), 5.3 (t, J = 5.5 Hz, 1H, C_2H), 5.65 (d, J = 7.6 Hz, 1H, C_5H), 6.3 (m, 1H, C_5H), 6.7 and 7.5 (m, 4H, ArH Tyr), 7.3 (m, 5H, ArH Cbz), 7.4–7.7 (m, 10H, ArH Ph), 7.85 (d, J = 7.6 Hz, 1H, C_6H). MS: MH$^+$/FAB m/z 1319.67.

cis-Isomer of 2-hydroxymethyl-5-[N⁴-(CbzTyr-Val-Sta-Ala-Sta-4-aminobutyl)cytosin-1'-yl]-1,3-oxathiolane ***21***

The title compound was prepared in 35% yield (18 mg) from **20** (50 mg, 0.05 mmol) by *General procedure B*. TLC (EtOAc/pyridine/acetic acid/H_2O 16:4:1:2) R_f 0.22. ^{1}H-NMR (CD_3OD) δ: 0.8–1.1 (m, 18H, $(CH_3)_2$ Val, 2 [$(CH_3)_2$ Sta)], 1.2–1.8 (m, 14H, $(CH_3)_2CH$ Val, CH_3 Ala, 2[$(CH_3)_2CHCH_2$ Sta], -$(CH_2)_2$-), 2.0–3.0 (m, 8H, 2 [$COCH_2CHOH$], CH_2NH_2, CH_2NH), 3.3–3.7 (m, 11H, C_4-H_2, 2[CH_2CHOH], C_2CH_2O- and 5[CHNHCO]), 5.0 (s, 2H, OCH_2Ph), 5.3 (t, J = 5.5 Hz, 1H, C_2H), 5.65 (d, J = 7.6 Hz, 1H, C_5H), 6.3 (m, 1H, C_5H), 6.7 and 7.5 (m, 4H, ArH Tyr), 7.3 (m, 5H, ArH Cbz), 7.85 (d, J = 7.6 Hz, 1H, C_6H). MS: MH$^+$/FAB m/z 1081.55. Anal $C_{53}H_{79}N_9O_{13}S$ (C, H, N).

CbzTyr-Val-Sta(OTBDMS)-Ala-Sta(OTBDMS) ***22***

To a solution of **19** (140 mg, 0.12 mmol) and imidazole (200 mg, 3 mmol) in DMF (5 mL) was added under nitrogen *tert*-butyldimethylsilyl chloride (450 mg, 3 mmol). The resulting mixture was stirred for 18 h at room temperature before adding imidazole (200 mg, 3 mmol) and *tert*-butyldimethylsilyl chloride (100 mg, 0.66 mmol). The reaction mixture was then allowed to stir 60 h. After evaporation MeOH (5 mL) was added. The resulting mixture was stirred during 1 h at room temperature and then washed with a 5% solution of citric acid, H_2O and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated to give **22** after purification by flash chromatography (52 mg, quantitative). TLC (EtOAc) R_f 0.47.

cis-Isomer of 2-hydroxymethyl-5-[N⁴-(CbzTyr-Val-Sta-Ala-Sta)-cytosin-1'-yl]-1,3-oxathiolane ***24***

To a solution of **22** (51 mg, 0.05 mmol) in CH_2Cl_2 under nitrogen was added **2** (19 mg, 0.04 mmol). The mixture was stirred at room temperature for 2 d and then evaporated and purified by flash chromatography to give **23** (45 mg, 61%) the *cis* isomer of 2-(*tert*-butyldiphenylsilyloxymethyl)-5-[N^4-(CbzTyr-Val-Sta(OTBDMS)-Ala-Sta(OTBDMS) cytosin-1'-yl]-1,3-oxathiolane. TLC (EtOAc) R_f 0.48. This compound was desilylated in 76% yield (23 mg) by *General procedure B* using 4 equiv of tetrabutylamoniumfluoride to give **24**. TLC (EtOAc/MeOH 9:1) R_f 0.46. ^{1}H-NMR (CD_3OD) δ: 0.95 (m, 12H, 2 [$(CH_3)_2$ Sta]), 1.3 and 1.7 (m, 16H, $(CH_3)_2CH$ Val, 2[$(CH_3)_2CHCH_2$ Sta] and CH_3Val), 2.2–2.8 (m, 4H, 2 [$COCH_2CHOH$]), 3.1–3.2 (m, 4H, CH_2 Tyr, C_4H_2), 3.6 (m, 2H, 2 [CHOH Sta]), 3.9–4.1 (m, 6H, C_2CH_2O, 4[CONHCH]) 4.4 (m, 1H, CH Tyr), 5.0 (s, 2H, CH_2Cbz), 5.35 (t, J = 3.4 Hz, 1H, C_2H), 6.3 (t, J = 3.5 Hz, 1H, C_5H), 6.7 and 7.1 (m, 4H, ArH Tyr), 7.3 (m, 5H, ArH Cbz), 7.45 (d, J = 7.5 Hz, 1H, C_5H), 8.6 (d, J = 7.5 Hz, 1H, C_6H). MS: MH$^+$ (FAB) m/z 1011. Anal $C_{49}H_{70}N_8O_{13}S$ (C, H, N).

cis-Isomer of 2-hydroxymethyl-5-[N⁴-(BocAHPPA-Glu-Phe)-cytosin-1'-yl]-1,3-oxathiolane ***26*** *or BocAHPPA-Glu-ω-N⁴-[cis-isomer of 2-hydroxymethyl-5-(cytosin-1'-yl)-1,3-oxathiolane]-Phe* ***27***

To a solution of **25** (51 mg, 0.09 mmol) in CH_2Cl_2 at 0°C was added under nitrogen, DCC (20 mg, 0.1 mmol) and HOBT (13 mg, 0.1 mmol). The mixture was stirred for 5 h at 0°C before adding (±) 3TC (20 mg, 0.09 mmol). The solution was allowed to warm to room temperature and stirred for 1 d. The mixture was then evaporated, H_2O was added and the solution diluted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated to give after purification by flash chromatography **26** or **27** (19 mg, 27%). TLC (EtOAc/MeOH 19:1) R_f 0.4. ^{1}H-NMR (DMSO-d_6) δ: 1.25 (s, 9H, Boc), 2.2 (m, 2H, -CHOHCH_2CO- (AHPPA)), 2.4–3.2 (m, 10H, CH_2 Phe, CH_2Ph (AHPPA), $(CH_2)_2$ Glu and C_4H_2), 3.6–4.0 (m, 4H, NHCHC-HOH (AHPPA), C_2CH_2O-), 4.65 (m, 2H, NH-CH Phe, NH-CH Glu), 5.25 (t, J = 3.5 Hz, 1H, C_2H), 6.25 (q, J = 3.5 Hz, 1H, C_5H), 6.6 (d, J = 7.3 Hz, 1H, C_5H), 7.2 (m, 10H, ArH), 8.4 (d, J = 7.3 Hz, 1H, C_6H). MS: MH$^+$ (FAB) m/z 797. Anal $C_{38}H_{48}N_6O_{11}S$ (C, H, N).

AcTrp-Val-Sta-Glu-ω-N⁴-[cis-isomers of 2-(tert-butyldiphenylsilyloxymethyl)-5-(cytosin-1'-yl)-1,3-oxathiolane]-Phe-OCH₃ ***29***

To a solution of **28** (100 mg, 0.13 mmol) in DMF/CH_2Cl_2 at 0°C were added under nitrogen. DCC (28 mg, 0.14 mmol) and HOBT (19 mg, 0.14 mmol). The mixture was stirred for 4 h at 0°C before to add **2** (58 mg, 0.13 mmol). The solution was allowed to warm to room temperature and stirred for 1 d. The mixture was then evaporated, a solution of 5% citric acid and EtOAc was added. The organic layer was washed with H_2O, dried over Na_2SO_4 and evaporated to give after purification by PLC **29** (30 mg, 19%). TLC (EtOAc/MeOH 9:1) R_f 0.4. ^{1}H-NMR ($CDCl_3$) δ: 0.9 (m, 12H, 2 CH_3 Val and 2 CH_3 Sta), 1.1 (s, 9H, *t*Bu), 1.15 and 1.7 (m, 8H, $(CH_2)_2$ Glu, $(CH_3)_2CHCH_2$ Sta and $(CH_3)_2CH$ Val), 2.0 (s, 3H, H acetyl), 2.2 (m, 2H, $COCH_2CHOH$), 2.8–3.6 (m, 6H, CH_2 Ph, CH_2 Trp and C_4H_2), 3.65 (s, 3H, OCH_3), 3.8–4.2 (m, 6H, NHCH Val, NHCH Glu and C_2CH_2O-), 4.7–4.8 (m, 2H, NHCH Phe and NHCH Trp), 5.3 (br s, 1H, C_2H), 6.25 (br s, 11H, C_5H), 7.0–7.4 (m, 21H, 3 ArH Ph and ArH Trp), 7.6 (d, J = 7.5 Hz, 1H, C_5H), 8.15 (m, J = 7.5 Hz, 1H, C_6H). MS: MH$^+$ (FAB) m/z 1242.

AcTrp-Val-Sta-Glu-ω-N⁴-[cis-isomers of 2-hydroxymethyl-5-(cytosin-1'-yl)-1,3-oxathiolane]-Phe-OCH₃ ***30***

The title compound was prepared in quantitative yield (20 mg) from **29** (25 mg, 0.02 mmol) by *General procedure B*. TLC (EtOAc/MeOH 9:1) R_f 0.25. ^{1}H-NMR ($CDCl_3$) δ: 0.9 (m, 12H, 2 CH_3 Val and 2 [CH_3 Sta]), 1.15 and 1.7 (m, 8H, $(CH_2)_2$ Glu, $(CH_3)_2CHCH_2$ Sta and $(CH_3)_2CH$ Val), 2.0 (s, 3H, CH_3 acetyl), 2.2 (m, 2H, $COCH_2CHOH$), 2.8–3.6 (m, 6H, CH_2 Ph, CH_2 Trp and C_4H_2), 3.65 (s, 3H, OCH_3), 3.8–4.2 (m, 6H, NHCHCHOH Sta, NHCH Val, NHCH Glu and C_2CH_2O-), 4.7–4.8 (m, 2H, NHCH Phe, NHCH Trp), 5.3 (br s, 1H, C_2H), 6.25 (br s, 1H, C_5H), 7.0–7.4 (m, 11H, ArH Phe and ArH Trp), 7.6 (d, J = 7.5 Hz, 1H, C_5H), 8.15 (m, J = 7.5 Hz, 1H, C_6H). MS: MH$^+$ (FAB) m/z 1004. Anal $C_{49}H_{65}N_9O_{12}S$ (C, H, N).

cis-Isomer of 2-(tert-butyldiphenylsilyloxymethyl)-5-[N⁴-(adipyl)-cytosin-1'-yl]-1,3-oxathiolane ***32***

The title compound was prepared in 30% yield (60 mg) from **2** (159 mg, 0.34 mmol) and adipic acid (50 mg, 0.34 mmol) by *General procedure A*. TLC (CH_2Cl_2/MeOH 9:1) R_f 0.4. ^{1}H-NMR (CD_3OD) δ: 0.95 (s, 9H, *t*Bu), 1.5 (m, 4H, -$(CH_2)_2$-), 2.15 and 2.35 (m, 4H, 2 [CH_2CO]), 3.15 and 3.5 (m, 2H, C_4H_2), 3.85 and 4.1 (m, 2H, C_2CH_2O), 5.2 (m, 1H, C_2H), 6.1 (br s, 1H, C_5H), 7.1–7.5 (m, 11H, ArH, C_5H), 8.35 (d, J = 7.6 Hz, 1H, C_6H).

cis-Isomer of 2-(tert-butyldiphenylsilyloxymethyl)-5-[N⁴-(1,6-dioxohexyl-Trp-Val-Sta-NH-CHPh-CH₂-Ph)cytosin-1'-yl]-1,3-oxathiolane ***33***

The title compound was prepared in 32% yield (13 mg) from **32** (35 mg, 0.025 mmol) and **31** (23 mg, 0.03 mmol) by

General procedure A. TLC (toluene/MeOH 9:1) R_f 0.22. ^{1}H-NMR ($CDCl_3$) δ: 0.9 (m, 12H, 2 [CH_3 γ Val] and 2 [CH_3 Sta]), 1.1 (s, 9H, *t*Bu), 1.7 (m, 6H, CH_2 Sta, -$(CH_2)_2$-), 2.35 (m, 4H, $(CH_2CO)_2$), 2.5 (m, 2H, CH_2α Sta), 3.0–3.25 (m, 6H, CH_2 Ar, CH_2β Trp and C_4H_2), 3.55–3.75 (m, 2H, CHOH, CHα Val), 3.8–4.2 (m, 2H, C_2CH_2O), 5.25 (t, *J* = 3.4 Hz, 1H, C_2H), 6.25 (q, 1H, C_5H), 7.0–7.7 (m, 27H, C_5H, Ar*H*), 8.25 (d, *J* = 7.5 Hz, 1H, C_6H). MS: MH$^+$ (FAB) *m/z* 1217.

cis-Isomer of 2-hydroxymethyl-5-[N^4-(1,6-dioxohexyl-Trp-Val-Sta-NH-CHPh-CH$_2$-Ph)cytosin-1'-yl]-1,3-oxathiolane **34**
The title compound was prepared in 50% yield (5 mg) from **33** (13 mg, 0.01 mmol) by *General procedure B*. TLC (CH_2Cl_2/MeOH 9:1) R_f 0.45. ^{1}H-NMR ($CDCl_3$) δ: 0.9 (m, 12H, 2 [CH_3γ Val] and 2 [CH_3 Sta]), 1.65 (m, 6H, CH_2 Sta, $(CH_2)_2$-), 2.3 (m, 4H, $(CH_2CO)_2$), 2.5 (m, 2H, CH_2α Sta), 3.1–3.3 (m, 6H, CH_2-Ar, CH_2β Trp, C_4H_2), 3.6–3.8 (m, 2H, CHOH and CHα Val), 3.8–4.2 (m, 2H, C_2CH_2O), 5.2 (t, *J* = 3.3 Hz, 1H, C_2H), 6.35 (m, 1H, C_5H), 7.0–7.4 (m, 17H, ArH, C_5H), 8.1 (d, *J* = 7.5 Hz, 1H, C_6H). MS: MH$^+$ (FAB) *m/z* 979. Anal $C_{52}H_{66}N_8O_9S$ (C, H, N).

cis-Isomer of 2-(tert-butyldiphenylsilyloxymethyl)-5-[N^4-(1,6-dioxohexyl-Trp-Val-Sta-Glu(OChex)-Phe-OCH$_3$)cytosin-1'-yl]-1,3-oxathiolane **36**
The title compound was prepared in 85% yield (72 mg) from **32** (36 mg, 0.34 mmol) and **26** (47 mg, 0.05 mmol) by *General procedure A*. TLC (CH_2Cl_2/MeOH 9:1) R_f 0.31. ^{1}H-NMR ($CDCl_3$) δ: 0.9 (m, 12H, 2 [CH_3γ Val] and 2 [CH_3 Sta]), 1.1 (s, 9H, *t*Bu), 1.3 (m, 11H, Chex), 1.65 (m, 8H, -$(CH_2)_2$-, CH_2βCH_2γ Glu), 2.3 and 2.5 (m, 4H, 2 [CH_2CO]), 3.0–3.3 (m, 4H, CH_2β Phe, C_4H_2 and CH_2β Trp), 3.7 (s, 3H, OCH_3), 3.8–4.25 (m, 5H, CHOH, CHαVal, C_2CH_2O and CHαPhe), 4.7 (m, 1H, CHα Leu), 4.8 (m, 1H, CHα Trp), 5.25 (br s, 1H, C_2H), 6.23 (m, 1H, C_5H), 7.2–7.4 (m, 26H, Ar*H* and N*H*CO), 8.3 (d, *J* = 7.5 Hz, 1H, C_6H). MS: MH$^+$ (FAB) *m/z* 1410.8.

cis-Isomer of 2-hydroxymethyl-5-[N^4-(1,6-dioxohexyl-Trp-Val-Sta-Glu(OChex)-Phe-OCH$_3$)cytosin-1'-yl]-1,3-oxathiolane **37**
The title compound was prepared in quantitative yield (29 mg) from **36** (35 mg, 0.025 mmol) by *General procedure B*. TLC (CH_2Cl_2/MeOH 19:1) R_f 0.1. ^{1}H-NMR ($CDCl_3$) δ: 0.9 (m, 12H, 2 [CH_3γVal] and 2 [CH_3Sta]), 1.3 (m, 11H, Chex), 1.65 (m, 8H, -$(CH_2)_2$-, -CH_2βCH_2γ Glu), 2.0 and 2.4 (m, 6H, CH_2α Sta, 2[CH_2CO]), 3.0–3.3 (m, 6H, CH_2βPhe, CH_2βTrp and C_4H_2), 3.6 (m, 1H, CH-OH), 3.7 (s, 3H, OCH_3), 4.0–4.1 (m, 4H, C_2CH_2O, CHα Val and CHα Phe), 4.7 (m, 1H, CHα Leu), 4.8 (m, 1H, CHα Trp), 5.35 (q, 1H, C_2H), 6.25 (q, 1H, C_5H), 7.0–7.4 (m, 15H, Ar*H*, C_5H and N*H*CO), 8.4 (m, 1H, C_6H). MS: MH$^+$ (FAB) *m/z* 1172.6. Anal $C_{59}H_{81}N_9O_{14}S$ (C, H, N).

Biological methods

HIV-1 protease assay
An initial evaluation of these compounds as HIV protease inhibitors was carried out with recombinant HIV-1 aspartyl protease which was expressed in *Escherichia coli* at a high level and purified by HPLC [64]. The protease activity was measured by a scintillation proximity assay [HIV Proteinase [^{125}I]] SPA Enzyme Assay System (IMK 8939) developed by Amersham International PLC, Cardiff, UK. Ac-Tyr[^{125}I]-Arg-Ala-Arg-Val-Phe-Phe-Val-Arg-Ala-Lys-biotin-fluoromicrosphere was used as substrate. The reaction mixture was composed of 9.2 mM sodium acetate buffer pH 5.5, 18.5 mM sodium citrate buffer pH 5.5, 4.15 mM dithiothreitol, 0.92 mM EDTA, 7.4% (wt/vol) glycerol and 1.48 M urea. The test compounds were added in DMSO (7.4% final concentration). The reaction was started by addition of enzyme. After incubation at 30°C for 60 min, the reaction was stopped by adding 0.2 mL of stop reagent. The tubes were placed in scintillation vials and the fluorescence was measured in a scintillation counter. Fluorescence quenching of the tests compounds was evaluated in the absence of enzyme. This test was validated by comparison with a HPLC test described elsewhere [31].

In vitro inhibition of HIV-1 replication in MT$_4$ blood lymphocytes
Representative compounds were tested for their ability to inhibit HIV-1 infection in cell culture. The fusogenic effect of HIV in the MT_4 cell line [58] was determined as described by Rey *et al* [56, 57]. A total of 3 x 10^5 MT_4 cells were infected with 100 µL of diluted virus for 1 h at 37°C. After three washes, the infected cells were cultured in 24-well cell culture plates in the presence of the inhibitor. The appearance of syncitia was measured with an inverted optical microscope 5 d after infection. The inhibitory concentration was expressed as the concentration of the tested compound which causes 50% inhibition of syncitia formation (IC_{50}) but was not toxic for the cells.

For toxicity testing, three replication cultures of each uninfected MT_4 cells (2 x 10^5 cells) were incubated with various concentrations of 2',3'-dideoxy-3'-thiacytidine analogs. Cell viability was determined 6 d from drug addition by trypan blue exclusion.

Acknowledgments

We are very grateful to D Nisato, B Chomier and JA Ferentz from Sanofi Recherches for the gift of some anti-proteasic peptides. We are indebted to E Abdili for technical assistance in antiviral activity evaluation. We thank Dr Noailly (Faculté de pharmacie, Université Aix-Marseille II) for the determination of NMR data. This research was financially supported by Sanofi Recherches, by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Agence Nationale pour la Valorisation de la Recherche (ANVAR) and by the Conseil Régional de la Région Provence Alpes-Côte-d'Azur.

References

1 Barré-Sinoussi F, Chermann JC, Rey F *et al* (1983) *Science* 220, 868–871
2 Mitsuya H, Weinhold K, Furman P *et al* (1985) *Proc Natl Acad Sci USA* 82, 7096–7100
3 Mitsuya H, Broder S (1986) *Proc Natl Acad Sci USA* 83, 1911–1915
4 Yarchoan R, Thomas R, Allain JP *et al* (1988) *Lancet* 1, 76–81
5 Balzarini J, Kang G, Dalal M *et al* (1987) *Mol Pharmacol* 32, 162–167
6 Hamamoto Y, Nakashima H, Matsui T, Matsuda A, Ueda T, Yamamoto N (1987) *Antimicrob Agents Chemother* 31, 907–910
7 Norbeck DW, Kempf DJ (1991) *Ann Rep Med Chem* 26, 141–150
8 Barrish JC, Gordon E, Alam M *et al* (1994) *J Med Chem* 37, 1758–1768
9 Kohl WE, Emini EA, Schleif WA *et al* (1988) *Proc Natl Acad Sci USA* 85, 4686–4690
10 Husson RN, Mueller BU, Farley M *et al* (1994) *Pediatrics* 93, 316–322
11 Yarchoan R, Klecker RW, Weinhold KJ *et al* (1986) *Lancet* 1, 575–580
12 Klecker RW, Collins JM, Yarchoan R *et al* (1987) *Clin Pharmacol Ther* 41, 407–412
13 Roberts NA, Martin JA, Kinchington D *et al* (1990) *Science* 248, 358–361
14 Kempf DJ, Codacovi L, Wang XC *et al* (1993) *J Med Chem* 36, 320–330
15 Getman DP, DeCrecenzo GA, Heintz RM *et al* (1993) *J Med Chem* 36, 288–291

16 Vacca JP, Dorsey BD, Darke P, Emini E, Lin J, IXth Int Conf AIDS/IVth World Congress, Abst PO-B26-2023
17 St Clair M, Martin J, Tudor-Williams G *et al* (1991) *Science* 253, 1557–1559
18 Lopezgalindez C, Rojas JM, Najera B, Najera R, Richman D, Perocho M (1992) *AIDS Res Hum Retroviruses* 8, 865
19 Lambert DM, Bartus H, Fernandez A *et al* (1993) *Antiviral Res* 21, 327–342
20 Craig JC, Duncan L, Wittaker L, Roberts N (1993) *Antiviral Chem Chemother* 4, 161–166
21 Rideout DC, Chou TC (1991) *Synergism and Antagonism in Chemotherapy* (Chou TC, Rideout DC, eds), Academic Press, New York, USA, 3–60
22 Kageyama S, Weinstein JN, Shirasaka T *et al* (1992) *Antimicrob Agents Chemother* 36, 926–933
23 Johnson VA, Merill DP, Chou TC, Hirsch M (1992) *J Inf Dis* 166, 1143–1146
24 Busso M, Mian AM, Hahn EF, Resnick L (1988) *AIDS Res Hum Retroviruses* 4, 449–455
25 Schinazi RF, Sommadossi JR, Saalmann V *et al* (1990) *Antimicrob Agents Chemother* 34, 1061–1067
26 Belleau B, Dixit D, Nguyen-Ga N, Kraus JL (1989) Vth Int Conf on AIDS, Montreal, Canada, June 4-9, paper TCOI
27 Soudeyns H, Yao Q, Belleau B *et al* (1991) *Antimicrob Agents Chemother* 35, 1386–1390
28 Belleau B, Nguyen-Ba N, Kraus JL *et al* (1992) *Trends in Receptor Research* (Angeli P, Gulini U, Quaglia W, eds), Elsevier Science, New York, USA
29 Jeong L, Schinazi F, Beach JW *et al* (1993) *J Med Chem* 36, 181–195
30 Schinazi R, Chu C, Peck A *et al* (1992) *Antimicrob Agents Chemother* 36, 672–676
31 Coates JA, Cammack N, Jenkinson HJ *et al* (1992) *Antimicrob Agents Chemother* 36, 202–205
32 Fehrentz JA, Chomier B, Bignon E, Venaud S, Chermann JC, Nisato D (1992) *Biochem Biophys Res Commun* 188, 865–872
33 Venaud S, Yahi N, Fehrentz J *et al* (1992) *Res Virol* 143, 311–319
34 Aggarwal SK, Gogu SR, Rangan SR, Agrawal KC (1990) *J Med Chem* 33, 1505–1510
35 Charvet AS, Camplo M, Faury P *et al* (1994) *J Med Chem* 37, 2216–2223
36 St Clair M, Richards CA, Spector T *et al* (1987) *Antimicrob Agents Chemother* 31, 1972–1977
37 Furman P, Fyfe J, St Clair M *et al* (1986) *Proc Natl Acad Sci USA* 83, 8333–8337
38 Greenberg M, Allaudeen H, Hershfield M (1990) *Ann NY Acad Sci* 616, 517–518
39 Snoeck R, De Clercq E (1991) *La lettre de l'infectiologue* VI, 15, 507–510
40 Mirchandani H, Chien YW (1993) *Int J Pharm* 95, 1–21
41 Storer R, Clemens I, Lamont B, Noble S, Williamson C, Belleau B (1993) *Nucleosides Nucleotides* 12, 225–236
42 Castro B, Dormoy J, Dourtoglou B, Eving G, Selve C, Ziegler J (1976) *Synthesis* 751–752
43 Camplo M, Charvet AS, Faury P, Wondrak E, Chermann JC, Kraus JL (1993) *Med Chem Res* 3, 87–95
44 Mikolajczyk M, Kielbasinski P (1981) *Tetrahedron* 37, 233–284
45 Bodansky M (1984) *Principles of Peptide Chemistry*, Springer-Verlag, New York, USA, 90–102
46 Thaisrivongs S, Pals D, Kati W, Turner S, Thomasco L, Watt W (1986) *J Med Chem* 29, 2080–2087
47 Raju B, Deshpande MS (1991) *Biochem Biophys Res Commun* 180, 187–190
48 Hartgers W, De Boer R, Wanner M, Koomen G (1992) *Nucleosides Nucleotides* 11, 1325–1332
49 Balajthy Z, Aradi J, Kiss I, Elodi P (1992) *J Med Chem* 35, 3344–3349
50 Hayashi M, Yamauchi K, Kinoshita M (1980) *Bull Chem Soc Jpn* 53, 277–278
51 Ziff E, Fresco J (1968) *J Am Chem Soc* 90, 7338–7342
52 Krazewenski A, Delort A, Teoule R (1986) *Tetrahedron Lett* 27, 861–864
53 Ogilvie K, Kader H (1983) *Nucleosides Nucleotides* 2, 345–350
54 Markiewicz W, Kierzek R, Hernes B (1987) *Nucleosides Nucleotides* 6, 269–272
55 Vacca J, Guare J, Desolms S *et al* (1991) *J Med Chem* 34, 1225–1228
56 Desolms S, Giuliani E, Guare J *et al* (1991) *J Med Chem* 35, 2852–2857
57 Rey F, Barré-Sinoussi F, Schmidtmayerova H, Chermann JC (1987) *J Virol Methods* 16, 239–249
58 Rey F, Donker G, Hirsch I, Chermann JC (1991) *Virology* 181, 165–171
59 Harada S, Koyanagi Y, Yamamoto N (1985) *Science* 229, 563–566
60 Yarchoan R, Broder S (1987) *In: AIDS, Modern Concepts and Therapeutic Challenges* (Broder S, ed), Marcel Dekker Inc, New York, USA, 335–360
61 Camplo M, Faury P, Charvet AS, Lederer F, Chermann JC, Kraus JL (1993) *Nucleosides Nucleotides* 12, 631–641
62 Kraus JL (1993) *Nucleosides Nucleotides* 12, 157–162
63 Le Grice S, Mills J, Mous J (1988) *EMBO* 78, 2547–2553
64 Meek TD, Dayton BD, Metcalf BW *et al* (1989) *14th International Congress of Biochemistry, Prague, Czechoslovakia, July 10-15* (Kostka V, ed), Walter de Gruyter, Berlin, Germany, 73