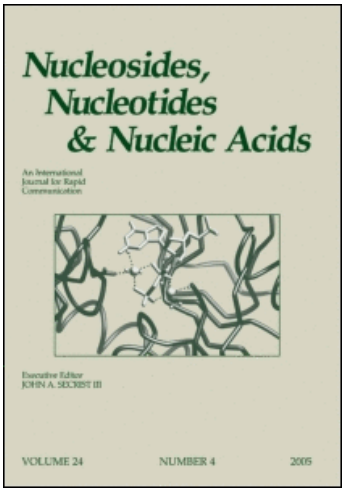

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PEPTIDE-NUCLEOSIDE CONJUGATES: SYNTHESIS AND ANTI-HIV ACTIVITIES

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Abstract: New peptide-nucleosides which consist of an anti-RT nucleoside and anti-protease peptide moieties, were designed in order to investigate their anti-HIV-1 properties. The synthesis of these new analogues was achieved using BOP coupling reagent which avoided the protection and deprotection steps of the 5'-OH group of the nucleoside part. These new compounds tested on MT_4 infected cells show no increase in anti-HIV activities compared to that of the component pieces. Interestingly we found that the 5'-(*tert*-butyldiphenyl)silyl analogues show anti-HIV activities equivalent to that of the 5'-free OH corresponding compounds. This result suggests a possible interaction of these compounds at a non-substrate binding site of the reverse transcriptase.

Many nucleoside and peptide derivatives are recognized as potent and selective inhibitors of the replication of Human Immunodeficiency Virus (HIV). In order to improve selectivity, efficiency and to overcome problems of resistance as well as toxicity [1,2] associated to the use of nucleoside or peptide agents, drug combination therapy [3] appears to be the most successful approach. Indeed, combinations of antiviral drugs having different viral targets or mechanism of action could potentially be additive, synergistic or antagonistic.[4,5] Combination treatment is generally accepted as having the potential to increase the activity of these two drugs at low concentrations. Such treatments have been suggested to decrease the development of resistance.[6] Various combinations have been tested such as combination of nucleoside and antiprotease peptides.[4,7,8] In numerous cases, synergistic effects have been reported.

Concentrations of various nucleosides with anti-protease inhibitor resulted in reduction of concentrations between 2 and 3 fold compared to those needed when inhibitors are used alone.[9] These features prompted us to synthesize and evaluate new peptide-nucleoside conjugates with the general structure shown in fig 1. These models could eventually inhibit both the HIV reverse transcriptase and the HIV-protease.

Since ddC (2',3'-dideoxycytidine, Zalcitabine) is one of the few nucleoside drugs clinically used, this was therefore selected for building the model shown in fig 1. The peptide moiety includes in its structure various residues which mimic the transitional state during HIV-protease hydrolysis.[10,11] The anti-HIV protease peptide (SR 141060A) was provided by SANOFI-RECHERCHES, Montpellier, FRANCE. It was found to be a potent *in vitro* inhibitor of HIV-protease, and was also active against HIV-replication in cell cultures. In this model the peptide moiety could be directly linked to the nucleosidic drug or through a spacer which could modify both entropy and membrane permeation properties of the resulting molecules.

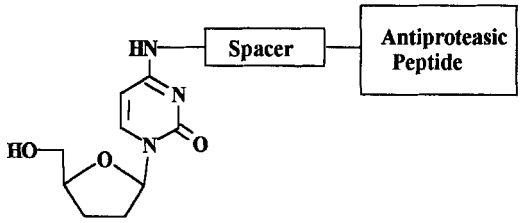
As long as the peptide-nucleoside is not quickly hydrolyzed extracellularly, both peptide and nucleoside parts of the molecule should target and be internalized within the same cell. In this hypothesis and if the required inhibition concentrations are reached, both HIV-RT and HIV-protease, belonging to the same cell could be inhibited. In this case, one can expect that the anti-HIV activity of this new model could be different from that of the simplest combination of the corresponding nucleoside and peptide drugs. Depending on the nature of the chemical bond between the peptide and the nucleoside, extra or intracellular hydrolysis could release the constituting anti-HIV moieties. In this view the model could act as a prodrug.[12,13] Improvements of the antiviral properties of these new molecules could be expected, mainly at the HIV resistance level [1,14] but also transport, delivery and bioavailability might be enhanced depending on the lipophilic character of these models.[15] We reported in this paper the synthesis and the antiretroviral properties of these new peptide-nucleosides: inhibition of HIV-1 replication in MT_4 cells.

RESULTS AND DISCUSSION

CHEMISTRY

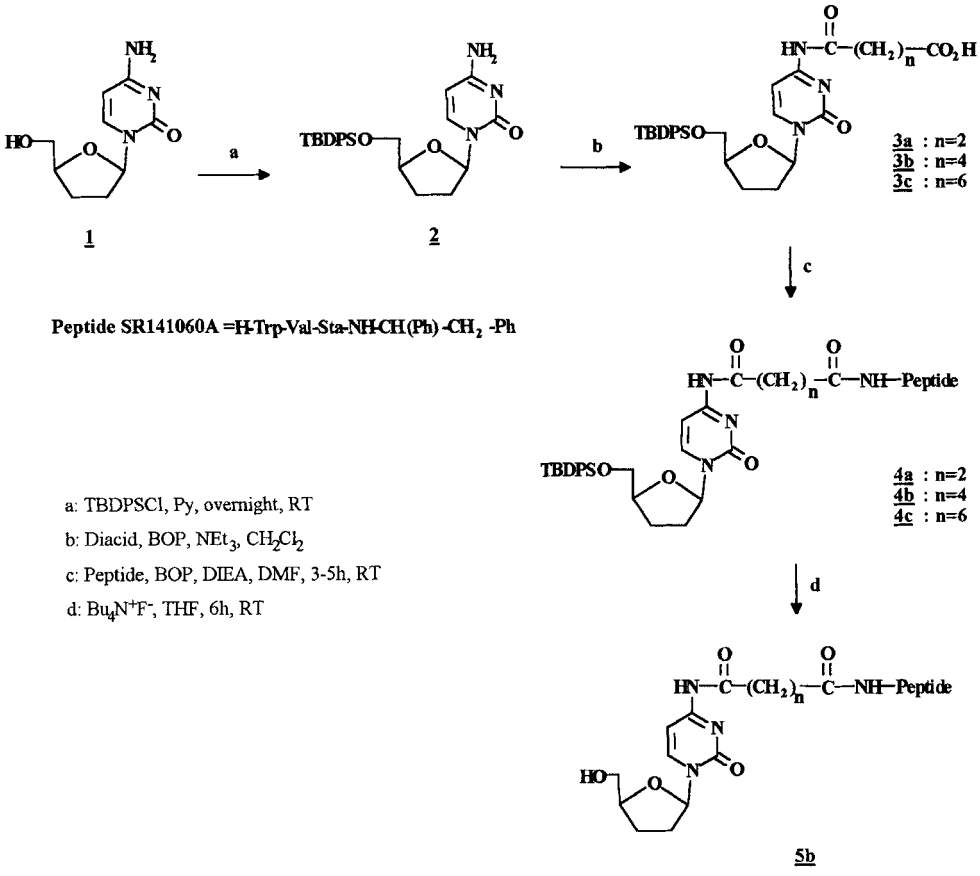
Starting from 2',3'-dideoxycytidine (1), two different synthetic routes were explored. Depending on the length of the linker between the nucleoside and the peptide, the linker should be first coupled to the protected nucleoside derivative. The resulting adduct is then coupled to the N-terminal of the peptide (step c, scheme 1). In the case of a C_6 carbon chain linker, the coupling occurs to the N-terminal of the peptide and the nucleoside moiety is then condensed to the resulting adduct 8 (step b, scheme 2).

Several coupling reagents have been tested in order to optimize the overall yield of the synthesis. The use of BOP reagent ((benzotriazol-1-yloxy)tris-(dimethylamino)-phosphonium hexafluoro phosphonate) [16] appears to be the most suitable for this type of synthesis,[17] since the protection and the deprotection of the 5'-OH group of ddC are not required in that case. It could be also underlined that the coupling reagent used in peptide chemistry, DCC/HOBT,[18] also gave the desired compounds in lower yield. It should also be mentioned that isolation of the final derivatives with short linkers (n = 0,1,2) failed. During the final deprotection step of the silylated protecting group using standard conditions (tetrabutylammonium fluoride ($Bu_4N^+F^-$) in THF,[19] or triethylamine-trihydrofluoride solution [20]) the cleavage of the formed amide bond was observed for compound 4a leading to the starting nucleoside and peptide. In contrast longer linkers (n = 4,6) allowed the isolation and purification of the desired compounds. These observations were confirmed through NMR and MS (FAB^+) studies.

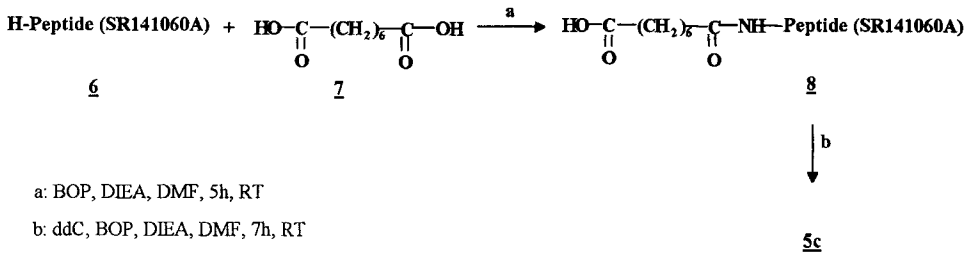


General structure of new 2',3'-dideoxycytidine-peptide

Fig.1



Scheme 1



Scheme 2

BIOLOGICAL RESULTS AND DISCUSSION

The potency of the synthetic peptide-nucleosides as inhibitors of HIV-1 replication was evaluated and the results are presented in table 1. The inhibition of HIV-replication is measured [21,22] by the formation of syncitia in HIV-1 infected MT_4 cells.[23] We observed for all the tested compounds listed in table 1 a dose-dependent relationship of this inhibition. Their IC_{50} values (concentration required to produce 50% inhibition of syncitia formation) are reported. The first observation which can be inferred from these results is that most of the antiviral activity of these peptide-nucleoside conjugates is lower than the component pieces nucleoside ddC (1) and peptide SR141060A (6). Secondly it can also be observed that the antiviral activity of the 5'-silylated compounds 4b and 4c is very similar to the corresponding 5'-deprotected compounds 5b and 5c. These results are surprising since previous studies,[12,13] have shown that the presence of various 5'-protected groups (ester or silylated groups) on anti-RT nucleosides are detrimental for anti-HIV potency. Indeed, reverse transcriptase inhibition requires enzymatic phosphorylation by cellular kinase [24,25,26] at the 5'-position, and it is obvious that free 5'-OH position analogues should elicit greater RT inhibitory potency. In contrast, 5'-protected compounds are more lipophilic than the 5'-OH corresponding analogues, and they should therefore penetrate the cellular membrane more easily. Some 5'-protected nucleosides have shown interesting in vitro anti-HIV activity.[27]

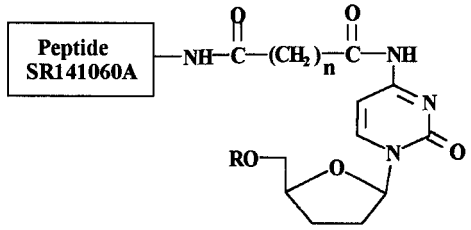
For instance, compound TSAO ([2',5'-bis-O-(tert-butyldimethylsilyl)-3"-spiro-5"-(4"-amino-1",2"-oxathiol-2",2"-dioxide)]-β-D-pentafuranosyl) is a nucleoside derivative of the HIV-1 inhibitor replication. It includes two silylated protecting groups in position 5' and 2' in order to target the nonsubstrate binding site of HIV-1 reverse transcriptase.[28,29,30] Current experiments should show if the silylated peptide-nucleosides (4b and 4c) could interact with a site of HIV-1 reverse trancriptase that is clearly distinct from the substrate binding site.

As a conclusion, we have achieved the synthesis of new peptide-nucleosidic compounds, which include an anti-HIV protease moiety coupled to the well-known nucleoside analogue ddC. These new compounds elicit no improvement of the anti-HIV activity in syncitia formation in comparison with the specific anti-HIV activity of the component pieces. In contrast, we found that the 5'-O-silylated peptide-nucleosidic analogues present an anti-HIV activity in syncitia formation very similar to the corresponding 5'-OH free analogues. More detailed virology and biochemical studies are necessary to understand the mode of action of these compounds.

EXPERIMENTAL SECTION

Proton magnetic spectra were recorded on a Bruker AMX 200 spectrometer. Chemical shifts are reported as values in parts per million downfield from internal tetramethylsilane. Coupling constants are expressed in Hertz (Hz). Elemental microanalysis were determined by Service

Table 1: Anti HIV-1 activities of various peptido-nucleoside compounds



Peptide SR141060A= H-Trp-Val-Sta-NH-CH(Ph)-CH_2-Ph

Compound	n	R	IC_{50} μM Syncitia Formation [a]	TI [b]=ID_{50}/IC_{50}
4a	2	TBDPS	10 ±5	_
4b	4	TBDPS	10 ± 5	10
4c	6	TBDPS	0.5 ± 0.3	100
5b	4	H	10 ±5	10
5c	6	H	1	100
1	ddC		0.1 ±0.01	_
6	Peptide SR141060A		5	_

a- IC_{50}: Concentration required to produce 50% inhibition of syncitia formation on MT_4 cells.
b-TI: Therapeutic Index. ID_{50}: Concentration required to cause 50% death of uninfected MT_4 cells.

Central d'Analyse CNRS Vernaison-Lyon France, and gave combustion values for C, H, N within 0.4% of theoritical values. Analytical thin layer chromatographies (TLC) were carried out on aluminium based sheets precoated with Kieselgel 60 F_{254nm} 0.2 mm thickness (Merck Co, Darmstadt). Column flash chromatographies were performed with Merck silica gel (230-400 mesh). Preparative layer chromatographies were carried out on silicagel 60 F_{254nm} precoated PLC plates (20 x 20 cm layer thickness 1 or 2 mm). Mass spectra were recorded on a NERMAG 10-10C mass spectrometer (SANOFI-Recherches, Montpellier, FRANCE) using a glycerol / HCl matrix.

5'-[(*tert*-Butyldiphenyl)silyl]-2',3'-dideoxycytidine (2).
ddC (1 eq, 0.50 g, 2.37 mmol) was partially dissolved under nitrogen atmosphere, in anhydrous pyridine (6 mL). Following the addition of *tert*-butylchlorodiphenylsilane (2.1 eq, 5.12 mmol, 1.60 mL), the mixture became clear and was stirred overnight. The solvent was removed under reduced pressure, then the residue hydrolyzed (20 mL) and extracted with EtOAc (3x5 mL). Organic phases were dried over Na_2SO_4, filtered, and the solvent evaporated in order to give a white solid which was recrystallized in CH_2Cl_2. Yield (72%). TLC (EtOAc/MeOH 9:1) Rf =0.31. ^1H NMR ($CDCl_3$) δ : 1.06 (s, 9H, tBu), 1.75-2.35 (m, 4H, 2H-2' and 2H-3'), 3.65-3.70 (m,1H, Ha-5' or Hb-5'), 3.95-4.10 (m, 2H, Ha-5' or Hb-5' and H-4'), 5.60 (d, J =7.4 Hz, 1H, H-5), 6.05 (dd, 1H, H-1'), 7.30-7.40 (m, 6H, ArH), 7.60-7.70 (m, 4H, ArH), 7.90 (d, J =7.4 Hz, 1H, H-6). Anal. ($C_{25}H_{31}N_3O_3Si$) C, H, N.

5'-[(*tert*-Butyldiphenyl)silyl]-N^4-succinyl-2',3'-dideoxycytidine (3a).
To the silylated nucleoside 2 (1 eq, 0.10 g, 0.22 mmol) in dry DMF (5 mL) was added under nitrogen atmosphere succinic anhydride (1.5 eq, 0.37 g, 0.33 mmol). The solution was stirred at room temperature for 48 hours and the reaction mixture concentrated under vaccum. The residue dissolved in EtOAc (15 mL), washed with water (3x5 mL), and dried over Na_2SO_4 gave after flash column chromatography using EtOAc/MeOH (9:1) as eluent the compound 3a. Yield (73%). TLC (EtOAc/MeOH 3:1) Rf =0.20. ^1H NMR ($CDCl_3$) δ : 1.10 (s, 9H, tBu), 1.80-2.50 (m, 4H, 2H-2' and 2H-3'), 2.65 (m, 4H, -CH_2-CH_2-), 3.70-3.80 (m, 1H, Ha-5' or Hb-5'), 4.10-4.30 (m, 2H, Ha-5' or Hb-5' and H-4'), 5.50 (d, J =7.4 Hz, 1H, H-5), 6.10 (dd, 1H, H-1'), 7.40-7.80 (m, 10H, ArH), 8.10 (d, J =7.4 Hz, 1H, H-6). Anal. ($C_{29}H_{35}N_3O_6Si$) C, H, N.

5'-[(*tert*-Butyldiphenyl)silyl]-N^4-adipyl-2',3'-dideoxycytidine (3b).
The title compound was prepared in 34% yield (0.21 g) from 2 (1 eq, 0.5 g, 1.1 mmol) dissolved in anhydrous CH_2Cl_2 under nitrogen atmosphere, followed by the addition of BOP (1.1 eq, 0.49 g, 1.1 mmol), triethylamine (4.4 eq, 0.65 mL, 4.4 mmol) and adipic acid (1.1 eq, 0.16 g, 1.1 mmol). The reaction mixture was stirred at room temperature overnight. After evaporation, an aqueous solution of 5% citric acid (20 mL) and EtOAc (3x5 mL) were added. The isolated organic layers were washed with water (3x10 mL), dried over Na_2SO_4, filtered and finally evaporated to give after purification by flash-chromatography with CH_2Cl_2/MeOH (9:1) as eluent, the compound 3b. TLC (CH_2Cl_2/MeOH 9:1) Rf =0.20. ^1H NMR ($CDCl_3$) δ : 1.30 (s, 9H, tBu), 1.70 (m, 4H, -CH_2-CH_2-), 2.15 (m, 4H, 2[CH_2-CO]), 2.20-2.60 (m, 4H, 2H-2' and 2H-3'), 3.65-3.70 (m, 1H, Ha-5' or Hb-5'), 3.95-4.10 (m, 2H, Ha-5' or Hb-5' and H-4'), 5.50 (d, J =7.4 Hz, 1H, H-5), 6.05 (dd, 1H, H-1'), 7.30-7.55 (m, 6H, ArH), 7.60-7.80 (m, 4H, ArH), 8.15 (d, J =7.4 Hz, 1H, H-6). Anal. ($C_{31}H_{39}N_3O_6Si$) C, H, N.

5'-[(*tert*-Butyldiphenyl)silyl]-N^4-suberyl-2',3'-dideoxycytidine (3c).
To the silylated nucleoside 2 (1 eq, 0.20 g, 0.44 mmol) in dry CH_2Cl_2 was added under nitrogen atmosphere a solution of suberic acid (1.1 eq, 0.08 g, 0.49 mmol) and BOP (1.1 eq, 0.22 g, 0.49 mmol) in dry CH_2Cl_2 with triethylamine (4 eq, 0.17 mmol, 0.25 mL). The reaction mixture was stirred at room temperature for 48 hours and concentrated under vaccum. The

residue was washed with 5% aqueous citric acid solution (20 mL), extracted with EtOAc (3x5 mL) and dried over Na_2SO_4 to give, after solvent evaporation, the compound 3c. This compound was purified on a silica gel column using EtOAc/MeOH (95:5) as eluent. Yield (44%). TLC (EtOAc/MeOH 9:1) Rf =0.46. ^1H NMR ($CDCl_3$) δ : 1.10 (s, 9H, tBu), 1.30 (m, 4H,-C$\underline{H}_2$-C$\underline{H}_2$-), 1.45 (m, 4H, 2[C$\underline{H}_2$-CH$_2$-CO]), 2.15 (m, 4H, 2[C$\underline{H}_2$-CO]), 2.20-2.60 (m, 4H, 2$\underline{H}$-2' and 2$\underline{H}$-3'), 3.70-3.80 (m, 1H, $\underline{Ha}$-5' or $\underline{Hb}$-5'), 4.10-4.30 (m, 2H, $\underline{H}$-4' and $\underline{Ha}$-5' or $\underline{Hb}$-5'), 5.50 (d, J =7.4 Hz, 1H, $\underline{H}$-5), 6.10 (dd, 1H, $\underline{H}$-1'), 7.40-7.80 (m, 10H, ArH), 8.10 (d, J =7.4 Hz, 1H, $\underline{H}$-6). Anal. ($C_{33}H_{39}N_3O_6Si$) C, H, N.

5'-[(*tert*-Butyldiphenyl)silyl]-N^4-(1,4-dioxobutyl-Trp-Val-Sta-CH(Ph)-CH$_2$Ph)-2',3'-dideoxycytidine (4a).

To a solution of 3a (1 eq, 0.09 g, 0.16 mmol) in dry DMF (1 mL) with BOP (1 eq, 0.08 g, 0.16 mmol) was added a mixture of peptide 6 (1 eq, 0.12 g, 0.16 mmol), N,N-diisopropylethylamine (2 eq, 0.1 mL, 0.32 mmol) and anhydrous DMF (1 mL). The reaction mixture was stirred for 5h at room temperature under nitrogen atmosphere. Evaporation of the solvent gave a residue which was washed with 5% aqueous citric acid solution (20 mL) and extracted with EtOAc (2x5 mL). The organic layers were dried over Na_2SO_4, concentrated under reduced pressure and the compound 4a was purified by flash column chromatography over silica gel using EtOAc/MeOH (95:5) as eluent. Yield (16%). TLC (EtOAc/MeOH 9:1) Rf =0.50. ^1H NMR ($CDCl_3$) δ : 0.90 (m, 12H, 2[C$\underline{H}_3$ γ Val] and 2[C$\underline{H}_3$ Sta]), 1.10 (s, 9H, tBu), 1.40-1.70 (m, 2H, C$\underline{H}_2$ Sta), 1.75-2.20 (m, 4H, 2$\underline{H}$-2' and 2$\underline{H}$-3'), 2.30-2.50 (m, 2H, C$\underline{H}_2$ α Sta), 2.65 (m, 4H, -C$\underline{H}_2$-C$\underline{H}_2$-), 2.90-3.15 (m, 4H, C$\underline{H}_2$-Ar and C$\underline{H}_2$ β Trp), 3.70-4.20 (m, 4H, C$\underline{H}$-OH, C$\underline{H}$ α Val and 2$\underline{H}$-5'), 5.25 (dd, 1H, $\underline{H}$-1'), 6.80-7.80 (m, 27H, ArH and $\underline{H}$-5), 8.40 (d, J =7.4 Hz, 1H, $\underline{H}$-6). Anal. ($C_{67}H_{82}N_8O_9Si$) C, H, N. MS(LS/MS)$^+$ m/z 1171.7 MH$^+$.

5'-[(*tert*-Butyldiphenyl)silyl]-N^4-(1,6-dioxohexyl-Trp-Val-Sta-CH(Ph)-CH$_2$Ph)-2',3'-dideoxycytidine (4b).

To a solution of the peptide 6 (1 eq, 0.06 g, 0.08 mmol) in dry DMF (3 mL) was added 3b (1 eq, 0.05 g, 0.08 mmol) followed by a solution of BOP (1 eq, 0.04 g, 0.08 mmol) and N,N-diisopropylethylamine (0.1 mL). The reaction mixture was allowed to stir at room temperature, under nitrogen atmosphere for 3-5h. After evaporation, the residue was washed with a 5% citric acid aqueous solution (15 mL) and extracted with EtOAc (3x5 mL). The organic layers were dried over Na_2SO_4 and filtered to give after purification by flash chromatography, using EtOAc/MeOH (99:1) as eluent, the compound 4b. Yield (45%). TLC (EtOAc/MeOH 95:5) Rf =0.40. ^1H NMR ($CDCl_3$) δ : 0.90 (m, 12H, 2[C$\underline{H}_3$ γ Val] and 2[C$\underline{H}_3$ Sta]), 1.10 (s, 9H, tBu), 1.30-1.70 (m, 6H, C$\underline{H}_2$ Sta and -C$\underline{H}_2$-C$\underline{H}_2$-), 1.70-2.30 (m, 4H, 2$\underline{H}$-2' and 2$\underline{H}$-3'), 2.35 (m, 4H, 2[C$\underline{H}_2$-CO]), 2.50 (m, 2H, C$\underline{H}_2$ α Sta), 3.00-3.25 (m, 4H, C$\underline{H}_2$ Ar and C$\underline{H}_2$ β Trp), 3.55-3.75 (m, 2H, C$\underline{H}$-OH and C$\underline{H}$ α Val), 3.80-4.20 (m, 2H, 2$\underline{H}$-5'), 5.25 (dd, 1H, $\underline{H}$-4'), 6.25 (dd, 1H, $\underline{H}$-1'), 7.00-7.70 (m, 27H, ArH and $\underline{H}$-5), 8.25 (d, J =7.4 Hz, 1H, $\underline{H}$-6). Anal. ($C_{69}H_{86}N_8O_9Si$) C, H, N. MS(ESI$^+$) m/z 1200 MH$^+$.

5'-[(*tert*-Butyldiphenyl)silyl]-N^4-(1,8-dioxooctyl-Trp-Val-Sta-CH(Ph)-CH$_2$Ph)-2',3'-dideoxycytidine (4c).

A solution of 3c (1 eq, 0.12 g, 0.2 mmol) in dry DMF (1 mL) with BOP (1.1 eq, 0.11 mg, 0.22 mmol) was added the peptide 6 (1.1 eq, 0.12 g, 0.16 mmol), N,N-diisopropylethylamine (4 eq, 0.26 mL, 0.8 mmol) and anhydrous DMF (1 mL). The reaction mixture was stirred for 18h at room temperature under nitrogen atmosphere. Evaporation of the mixture gave a residue which was washed with a 5% aqueous citric acid solution (10 mL) and extracted with EtOAc (2x5 mL). The organic layers were dried over Na_2SO_4, concentrated under reduced pressure and the compound 4c purified by flash column chromatography over silica gel using

EtOAc/MeOH (98:2) as eluent. Yield (36%). TLC (EtOAc/MeOH 9:1) Rf =0.42. Anal. ($C_{71}H_{90}N_8O_9Si$) C, H, N. MS(LS/MS)$^+$ m/z 1227.7 MH^+.

N^4-(1,6-dioxohexyl-Trp-Val-Sta-CH(Ph)-CH_2Ph)-2',3'-dideoxycytidine (5b).
To a solution of protected peptide-nucleoside 4b (1 eq, 0.03 g, 0.025 mmol) in anhydrous THF (1 mL) was added a solution of tetra-N-butylammonium fluoride 1M in THF (9 eq, 0.21 mL,0.22 mmol). The mixture was stirred under nitrogen atmosphere for 6h, and then concentrated under reduced pressure. The residue was purified by PLC using EtOAc/MeOH (9:1) as eluent. Yield (68%). TLC (EtOAc/MeOH 3:1) Rf =0.45. ^1H NMR ($CDCl_3$) δ: 0.90 (m, 12H, 2[C$\underline{H}_3$ γ Val] and 2[C$\underline{H}_3$ Sta]), 1.30-1.70 (m, 6H, C$\underline{H}_2$ Sta and C$\underline{H}_2$-C$\underline{H}_2$-), 1.70-2.30 (m, 4H, 2$\underline{H}$-2' and 2$\underline{H}$-3'), 2.35 (m, 4H, 2[C$\underline{H}_2$-CO]), 2.50 (m, 2H, C$\underline{H}_2$ α Sta), 3.00-3.25 (m, 4H, C$\underline{H}_2$ Ar and C$\underline{H}_2$ β Trp), 3.55-3.75 (m, 2H, C$\underline{H}$-OH and C$\underline{H}$ α Val), 3.80-4.20 (m, 2H, 2$\underline{H}$-5'), 5.25 (dd, 1H, $\underline{H}$-4'), 6.25 (dd, 1H, $\underline{H}$-1'), 7.00-7.45 (m, 17H, ArH and $\underline{H}$-5), 8.25 (d, J =7.4 Hz, 1H, $\underline{H}$-6). Anal. ($C_{51}H_{64}N_8O_9$) C, H, N. MS(ESI$^+$) m/z 961 MH^+.

(Suberyl-Trp-Val-Sta-CH(Ph)-CH_2Ph) acid (8).
To a solution of suberic acid (1 eq, 0.02 g, 0.12 mmol) and BOP (1 eq, 0.06 g, 0.12 mmol) in dry DMF (2 mL) was added the peptide 6 (1 eq, 0.08 g, 0.12 mmol) and N,N-diisopropylethylamine (10 eq, 0.4 mL, 1.2 mmol). The resulting mixture was stirred for 6h under nitrogen atmosphere. When the starting material was consumed, the solution was concentrated under reduced pressure and the residue was washed with a 5% aqueous citric acid solution (20 mL) and extracted with EtOAc (20 mL). Organic layers were dried over Na_2SO_4, filtered and evaporated to give an oily residue which was chromatographed on a silica gel column using CH_2Cl_2/MeOH (9:1) as eluent. Yield (28%). TLC (CH_2Cl_2/MeOH 9:1). Rf =0.41. ^1H NMR ($CDCl_3$) δ: 0.90 (m, 12H, 2[C$\underline{H}_3$ γ Val] and 2[C$\underline{H}_3$ Sta]), 1.30-1.60 (m, 10H, C$\underline{H}_2$ Sta ;-C$\underline{H}_2$-C$\underline{H}_2$- and 2[C$\underline{H}_2$-CH$_2$-CO]), 2.00-2.30 (m, 6H, 2[C$\underline{H}_2$-CO] and C$\underline{H}_2$ α Sta), 3.30-3.60 (m, 4H, C$\underline{H}_2$ Ar and C$\underline{H}_2$ β Trp), 3.65-3.80 (m, 2H, C$\underline{H}$-OH and C$\underline{H}$ α Val), 6.90-7.40 (m, 16H, ArH). Anal. ($C_{46}H_{61}N_5O_7$) C, H, N.

N^4-(1,8-dioxooctyl-Trp-Val-Sta-CH(Ph)-CH_2Ph)-2',3'-dideoxycytidine (5c).
To a solution of the peptide-acid 8 (1 eq, 0.03 g, 0.03 mmol) in dry DMF (1 mL), BOP (1.1 eq, 0.02 g, 0.04 mmol) and N,N-diisopropylethylamine (4 eq, 0.04 mL, 0.07 mmol) in dry DMF (1 mL) with ddC (1.2 eq, 0.01 g, 0.04 mmol) were added. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 7h. After evaporation the residue was washed with a 5% $NaHCO_3$ aqueous solution (15 mL) and extracted with EtOAc (3x5 mL). The organic layers were dried over Na_2SO_4, and filtered to give the peptide-nucleoside after purification by flash-chromatography using EtOAc/MeOH (9:1) as eluent. TLC (EtOAc/MeOH 9:1). Rf =0.16. Yield (24%). Anal. ($C_{55}H_{72}N_8O_9$) C, H, N. MS(LS/MS)$^+$ m/z 989 MH^+.

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