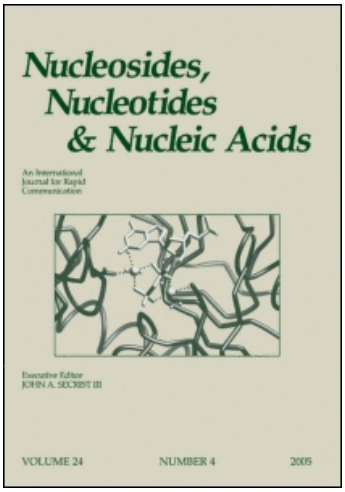

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Tri-N-Boc-Tetraazamacrocycle-Nucleoside Conjugates : Synthesis and anti-HIV activities

J. Dessolin,[1,2] P. Vlieghe,[1,2] M. Bouygues,[1,2] M. Medou,[1,2] G. Quéléver,[1,2] M. Camplo,[1,2] J.C. Chermann[2] and J.L. Kraus*[1,2]

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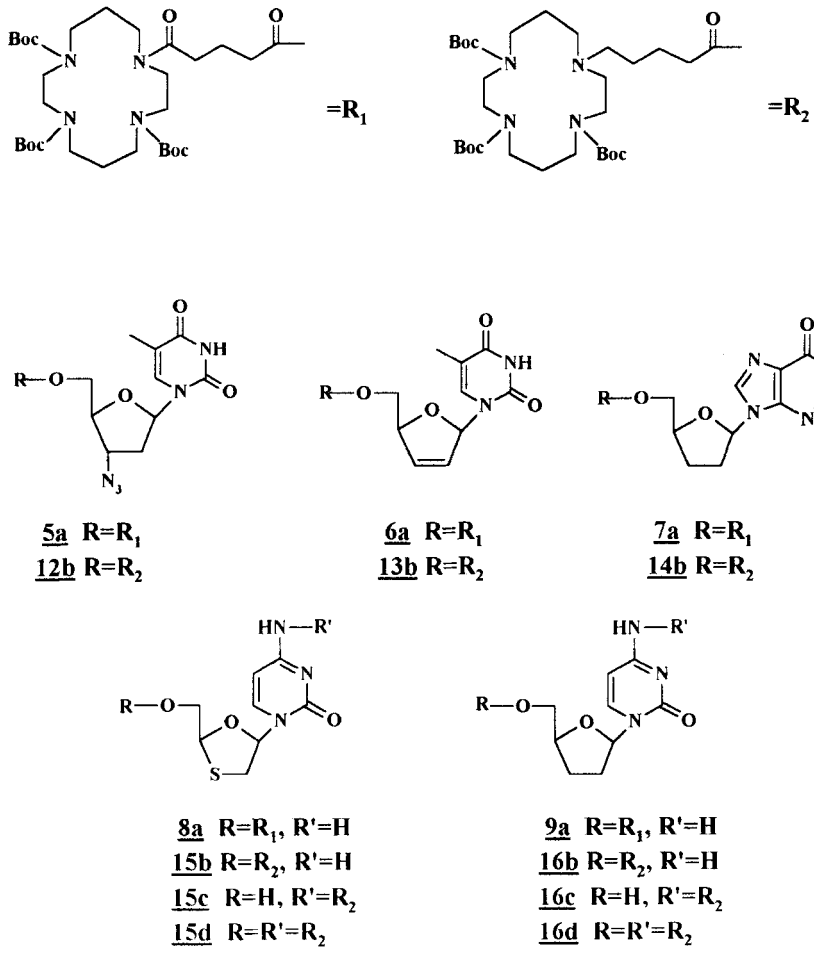
Abstract.

As far as linear N-Boc-polyamines conjugates elicited remarkable anti-HIV activity, the synthesis and anti-HIV properties of cyclic N-Boc-polyamines conjugates such as tetraazamacrocycle-nucleoside were studied. These new conjugates include an ester linkage between the two moieties. They were synthesized using Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate coupling reagent, in the case of N-alkyl polyazamacrocycle derivatives, or through direct condensation of the acyl chloride derivative with nucleoside in the case of N-acyl polyazamacrocycle compounds. None of the new conjugates presented anti-HIV activity greater than that of the corresponding parent nucleosides.

Introduction.

The search for effective chemotherapeutic treatments for 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 reverse transcriptase (RT) inhibitors 2',3'-dideoxynucleoside analogues (ddN's) such as AZT (Retrovir®), ddC (Zalcitabine®), d4T (Zerit®), ddI (Videx®), 3TC (Epivir®).[1,2] As a part of our efforts to design prodrugs of ddN's, we found that linear N-Boc-protected polyamine-3TC conjugates allowed an increased anti-HIV activity compared to that of the parent nucleoside, while the corresponding deprotected analogues were found less active.[3] Following these encouraging results, we decided to replace the linear N-Boc-protected polyamines by N-Boc cyclic ones. Several reasons support the design of bipartate prodrug : i- as long as the tri-N-Boc-tetraazamacrocycle-nucleoside conjugate is not hydrolysed extracellularly, both structural moieties could be targeted and internalized within the same cell. ii- transport, delivery and bioavailability might also be enhanced depending on the lipophilic character of the new model. We described in this report the synthesis and the anti-HIV activities of new ddN's prodrugs bearing N-protected polyazamacrocycles at various positions of the anti-RT nucleosides (Figure 1). The modifications described in this paper were focused on the introduction of N-



5a R=R1
12b R=R2

6a R=R1
13b R=R2

7a R=R1
14b R=R2

8a R=R1, R'=H
15b R=R2, R'=H
15c R=H, R'=R2
15d R=R'=R2

9a R=R1, R'=H
16b R=R2, R'=H
16c R=H, R'=R2
16d R=R'=R2

Figure 1 : General structure of new N-Boc protected tetraazamacrocycle-nucleoside conjugates

Boc protected polyazamacrocycle moieties at the 5'-O or/and 4N positions of the ddN's. Such modifications could be of interest, since these N-protected polyazamacrocycle moieties could also contribute to inhibit HIV-induced membrane fusion. Indeed, anti-HIV betulinic acid derivatives bearing at the C_{17} carboxylic position various linear diamido side chains[4] were reported as agents inhibiting HIV induced membrane fusion as well as RT inhibitors.

Chemistry

Two series of nucleoside-polyazamacrocycle conjugates have been synthesized. N-acyl modified polyazamacrocycles are included in the A Series (compounds **5a** - **9a**), while the B Series include the N-alkyl modified polyazamacrocycles (compounds **12b** - **16d**).
Both series of compounds required the synthesis of the N,N',N''-tri-Boc protected 1,4,8,11-tetraazacyclotetradecane **2** intermediate. This was achieved starting from the commercially

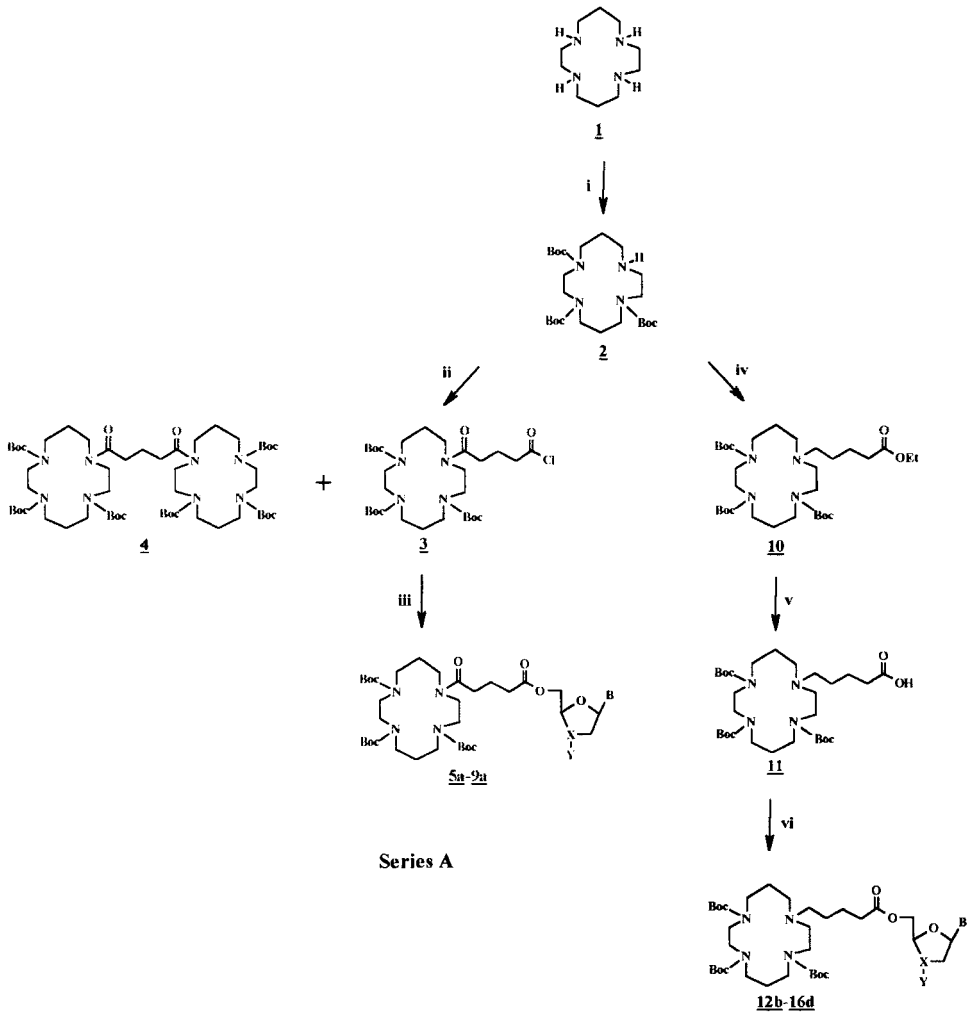
available 1,4,8,11-tetraazacyclotetradecane **1** according to a procedure already reported.[5] The tri-Boc tetraazamacrocycle intermediate **2** was monoacylated through a Schötten-Baumann like reaction by addition of an excess of glutaryl dichloride in a biphasic dichloromethane/$NaHCO_3$ solution. The mono N-acylated acid chloride **3** was isolated in 68% yield, while the expected di-N-acylated bispolyazamacrocycle side product was formed in low yield. The coupling of nucleosides (AZT, d4T, ddI) on the N-Boc protected polyazamacrocycle acid chloride **3** was achieved using dimethylaminopyridine in dichloromethane, affording the corresponding products in low yields. When the same reaction was attempted on cytosinyl nuleosides (3TC, ddC), three products could be formed corresponding to 5'-O or 4N monosubstituted compounds and 5'-O, 4N disubstituted analogues. In fact, only the 5'-O substituted analogues **8a** and **9a** were isolated. The corresponding 4N substituted expected analogues were not detected in the above coupling conditions.

Analogues belonging to the B series were synthesized according to the following synthetic pathway. Condensation of 5-bromopentanoic ethyl ester on the tri-Boc protected tetraazamacrocycle **2** in acetonitrile, in the presence of potassium carbonate, led to the corresponding N-pentanoic ethyl ester tetraazamacrocycle **10** in 25% yield. After saponification, the resulting corresponding acid **11** was condensed on the nucleosides (AZT, d4T, ddI, 3TC, ddC) using BOP [Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate] reagent in the presence of a mixture of NEt_3/DMAP in dichloromethane. Under these coupling conditions, in the case of the cytosinyl nucleosides (3TC, ddC), the three expected 5'-O or 4N monosubstituted and 5'-O, 4N disubstituted analogues were isolated and characterized. It should be underlined that the use of dicyclohexylcarbodiimide (DCC) in the presence of 1-hydroxybenzotriazole (HOBt) and triethylamine did not improved the yield of coupling products, since the simultaneous formation of N-acyl urea derivatives, as byproducts, was observed[6]. The synthesis of both series of compounds A and B are summarized on Scheme 1.

Results and discussion.

The primary objective of this study was to design and synthesize anti-RT prodrugs with improved permeability properties. The N-protected tetraazamacrocycle moiety linked to the nucleosides contributes on the one hand to increase the lipophilicity of the resulting prodrug and on the other hand to inhibit HIV-induced membrane fusion.

The antiviral activity of the compounds against HIV-1 was determined *in vitro* in MT4 lymphocytes. Inhibition of HIV replication was measured[7,8] through the formation of *syncitia* in MT4 infected cells.[9] We observed for all the tested compounds a dose-dependant relationship of this inhibition. Their EC_{50} values (concentration required to produce 50% inhibition of *syncitia* formation) and their CC_{50} values (concentration required to produce 50% death of uninfected MT4 cells) were determined. The obtained results are presented on Table 1. From these results, it can be observed that the antiviral activity of the new nucleoside-tetraazamacrocycle conjugates is nearly equal or lower to their corresponding parent drug. The differences in anti-HIV potencies depend on the type of nucleoside linked to the tetraazamacrocycle. AZT (**5a**), ddC (**16b**, **16c**, **16d**) and 3TC (**15d**) derivatives elicited an anti-HIV activity equipotent to that of the corresponding parent drug; in contrast, ddI (**7a**, **14b**) and d4T (**6a**, **13b**) derivatives were less active than the parent drug. The different ddC-tetraazamacrocycle conjugates belonging to the B series elicited significant anti-HIV activity whatever the position of the tetraazamacrocycle substituent on the nucleoside moiety. These results are of interest since it has been reported that 4N acylation of the cytidine base in ddC[10] or 3TC[11] derivatives reduced significantly anti-HIV activity. With the exception of compound **5a**, in the reported tested conditions the selectivity indexes for the other synthesized conjugate



i : Boc_2O (1.8eq), CH_2Cl_2. ii : Glutaryl dichloride (3eq), CH_2Cl_2/$NaHCO_3$aq. iii : Nucleoside (1eq), DMAP (2eq), CH_2Cl_2. iv : Ethyl-5-Bromovalerate (1.1eq), K_2CO_3 (3eq), CH_3CN, 85°C. v : NaOH 10N, THF. vi : Nucleoside (1eq), BOP (1.2eq), NEt_3 (3eq), DMAP(0.25eq), CH_2Cl_2.

Scheme 1

Table 1 : Anti-HIV activities of various N-Boc protected tetraazamacrocycle-nucleoside conjugates

N°	Series[a]	Anti-RT nucleoside	EC_{50}[b] (μM)	CC_{50}[c] (μM)	SI[d]
5a	A	AZT	0.1	50	500
6a	A	d4T	10	50	5
7a	A	ddI	inactive	50	-
8a	A	3TC	5	100	50
9a	A	ddC	5	50	10
12b	B	AZT	1	50	50
13b	B	d4T	10	50	5
14b	B	ddI	10	50	5
15b	B	3TC	10	50	5
15c	B	3TC	10	10	1
15d	B	3TC	1	50	50
16b	B	ddC	0.5	10	50
16c	B	ddC	0.05	1	20
16d	B	ddC	1	10	10
AZT	-	-	0.05	>100	>2000
d4T	-	-	1	>100	>100
ddI	-	-	5	>100	>20
3TC	-	-	0.5	>100	>500
ddC	-	-	0.5	>100	>200

a - Series A compounds correspond to derivatives in which the tetraazamacrocycles are linked to the anti-RT nucleoside spacer through an amide bond. Series B compounds correspond to derivatives in which the tetraazamacrocycles are linked to the anti-RT nucleoside spacer through an amine bond.

b - EC_{50} : concentration required to inhibit *syncitia* formation by 50% on MT4 cells. Data are means of four replicate samples.

c - CC_{50} : concentration required to cause 50% death of uninfected MT4 cells.

d - SI : selective index = CC_{50}/ EC_{50}

analogues are 10 to 100-fold lower than those of the parent drugs. These results indicate that there is no synergistic effect between a possible inhibitory activity against HIV-induced membrane fusion and an anti-RT activity. Indeed, an effective prodrug must achieve a balance between two opposing tendencies : it must have sufficient stability versus extracellular esterases, and must be sensitive to those one in the infected cells.[12] It can be deduced from the above results that ddC-tetraazamacrocycle conjugates (**16b**, **16c**, **16d**), and to lesser extend AZT-tetraazamacrocycle conjugate (**5a**), are more sensitive to cellular hydrolases than the other ddI, d4T, or 3TC-tetraazamacrocycle conjugates. Details to precise the mechanism of the bipartate prodrugs **5a**, **16b**, **16c** and **16d** are in progress and will be published in a next paper.

Experimental section.

Chemistry

Nuclear magnetic resonance spectra were recorded with a Bruker AC-250 (^{1}H NMR). Chemical shift values were expressed in δ values (part per million) relative to TMS as internal standard. FAB$^+$ mass spectra were obtained on a Jeol DX-100 mass spectrometer (Laboratoire de Mesures Physiques-RMN, Dr Astier, USTL, Montpellier, France) using a caesium ion source. Preparative flash column chromatography was performed using Merck G60 230-240 mesh silica gel. Analytical thin-layer chromatography was performed on $60F_{254}$ silica gel aluminium plates of 0.2mm thickness (Merck, Darmstadt). All reagents were of commercial quality (Aldrich Company) from freshly opened containers.

1,4,8-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradecane **2**
To a solution of 1.00 g of cyclam **1** (5.00 mmol, 1 eq) in 125 mL of dichloromethane were added 2.00 g of di-*tert*-butyl dicarbonate (9.00 mmol, 1.8 eq). The solution was stirred 4 hrs at room temperature. After solvent evaporation, the crude yellow oil was purified by flash column chromatography ($MeOH/CH_2Cl_2$ 5:95), to give the desired product as a white-yellow foam (24% yield, 0.60 g). R_f = 0.47 ($MeOH/CH_2Cl_2$ 1:9)
^{1}H NMR ($CDCl_3$) δ : 1.31 (s, 27H, t-Bu), 1.64 (m, 2H, H-13), 1.86 (m, 2H, H-6), 2.54 (t, J = 5.30 Hz, 2H, H-12), 2.71 (t, J = 5.20 Hz, 2H, H-10), 3.10-3.40 (m, 12H, CH_2-NBoc).
MS (FAB$^+$) : 501 $(M+H)^+$

5-[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-oxo-1-pentanoyl chloride **3**
Compound **2** (0.22 g, 0.44 mmol, 1 eq) was dissolved in a biphasic mixture of dichloromethane and saturated aqueous sodium carbonate (respectively 100 mL:50 mL). Glutaryl dichloride (0.22 g, 1.32 mmol, 3 eq) was added and the reaction mixture was stirred 30 min, until disappearance of the starting material. Aqueous layer was separated and extracted 3 times with dichloromethane. The combined organic extracts were dried over Na_2SO_4, and concentrated. The crude white foam was flash chromatographied ($MeOH/CH_2Cl_2$ 5:95) to give the desired product **3** as a yellow-white foam (68% yield, 0.19 g), plus compound **4** resulted from dimerisation. R_f = 0.38 ($MeOH/CH_2Cl_2$ 1:9)
^{1}H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.69 (m, 4H, H-6 & H-13), 1.90 (m, 2H, CO-CH_2-CH_2-CH_2-CO), 2.35 (m, 4H, CO-CH_2-CH_2-CH_2-CO), 3.15-3.49 (m, 16H, CH_2-N-CO).
MS (FAB$^+$) : 633 $(M+H)^+$

1,1'-(1,5-dioxo-pentane)-bis[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradecane] **4**

Compound **4** was obtained as a white foam (14% yield, 0.07 g). R_f = 0.53 ($MeOH/CH_2Cl_2$ 1:9)
^1H NMR ($CDCl_3$) δ : 1.39 (s, 54H, t-Bu), 1.69 (m, 8H, H-6 & H-13 & H-6' & H-13'), 1.89 (m, 2H, CO-CH_2-CH_2-CH_2-CO), 2.34 (m, 4H, CO-CH_2-CH_2-CH_2-CO), 3.12-3.48 (m, 32H, CH_2-N-CO).
MS (FAB^+) : 1101 $(M+H)^+$

A. General procedure for the coupling of compound **3** with nucleosides
Compound **3** (0.17 g, 0.26 mmol, 1 eq) was dissolved in dichloromethane (10 mL), to give a greenish solution. DMAP (0.06 g, 0.52 mmol, 2 eq) and 1 eq of nucleoside were then added. The reaction mixture was stirred for 8 hrs. The resulting solution was successively washed with 5% aqueous citric acid and water, dried over Na_2SO_4 and concentrated. Purification was performed by flash column chromatography using $MeOH/CH_2Cl_2$ 5:95 as eluent.

5'-[5-[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-oxo-1-pentanoyl]- 3'-azido-3'-dideoxythymidine **5a**
The coupling of 0.07 g of AZT (0.26 mmol) with compound **3** afforded the title product (0.07 g, 30% yield) following general procedure **A**. R_f = 0.45 ($MeOH/CH_2Cl_2$ 1:9)
^1H NMR ($CDCl_3$) δ : 1.39 (s, 27H, t-Bu), 1.69 (m, 4H, H-13 & H-6), 1.86 (s, 3H, CH_3Thy), 1.92 (m, 2H, CO-CH_2-CH_2-CH_2-CO), 2.25-2.50 (m, 6H, CO-CH_2-CH_2-CH_2-CO & H-2'), 3.15-3.50 (m, 16H, CH_2-N), 3.58 (m, 1H, H-4'), 3.99 (m, 1H, H-3'), 4.26 (m, 2H, H-5'), 6.00 (t, J = 6.50 Hz, 1H, H-1'), 7.15 (d, J = 1.10 Hz, 1H, H-6Thy), 8.42 (brs, 1H, NH).
MS (FAB^+) : 864 $(M+H)^+$

5'-[5-[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-oxo-1-pentanoyl]-2',3'-didehydro-3'-deoxythymidine **6a**
Compound **3** was reacted with 0.06 g of d4T (0.26 mmol) according to general procedure **A**. The title compound was obtained in a 28% yield (0.06 g) after purification. R_f = 0.47 ($MeOH/CH_2Cl_2$ 1:9)
^1H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.68 (m, 4H, H-13 & H-6), 1.84 (s, 3H, CH_3Thy), 1.90 (m, 2H, CO-CH_2-CH_2-CH_2-CO), 2.25-2.45 (m, 4H, CO-CH_2-CH_2-CH_2-CO), 3.15-3.50 (m, 16H, CH_2-N), 4.10-4.42 (m, 2H, H-5'), 4.97 (brs, 1H, H-4'), 5.83 (m, 1H, H-2'), 6.22 (m, 1H, H-3'), 6.95 (brd, J = 1.85 Hz, 1H, H-1'), 7.14 (d, J = 1.10 Hz, 1H, H-6Thy), 8.83 (brs, 1H, NH).
MS (FAB^+): 821 $(M+H)^+$

5'-[5-[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-oxo-1-pentanoyl]-2',3'-dideoxyinosine **7a**
ddI (0.07 g, 0.26 mmol) and compound **3** were condensed according to general procedure **A**. After purification, the desired compound was obtained in 46% yield (0.08 g). R_f = 0.42 ($MeOH/CH_2Cl_2$ 1:9)
^1H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.69 (m, 4H, H-13 & H-6), 1.88 (m, 2H, CO-CH_2-CH_2-CH_2-CO), 2.10 (m, 2H, H-2'), 2.25-2.40 (m, 4H, CO-CH_2-CH_2-CH_2-CO), 2.50 (m, 2H, H-3'), 3.15-3.50 (m, 16H, CH_2-N), 4.10-4.40 (m, 3H, H-4' & H-5'), 6.21 (t, J = 6.45 Hz, 1H, H-1'), 8.01 (s, 1H, H-2Ino), 8.14 (s, 1H H-8Ino), 12.8 (brs, 1H, NH).
MS (FAB^+) : 833 $(M+H)^+$

5'-[5-[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-oxo-1-pentanoyl]-2',3'-dideoxy-3'-thiacytidine **8a**
Condensation of compound **3** with 3TC (0.06 g) afforded 0.06 g of the title compound in 24% yield. R_f = 0.38 ($MeOH/CH_2Cl_2$ 1:9)

^{1}H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.69 (m, 4H, H-6 & H-13), 1.92 (m, 2H, CO-CH_2-C$\underline{H}_2$-CH_2-CO), 2.20-2.50 (m, 4H, CO-C$\underline{H}_2$-CH_2-C$\underline{H}_2$-CO), 3.30-3.50 (m, 18H, CH_2-N & H-2'), 4.35-4.55 (m, 2H, H-5'), 5.28 (t, J = 3.20 Hz, 1H, H-4'), 5.82 (d, J = 7.50 Hz, 1H, H-5Cyt), 6.28 (t, J = 6.50 Hz, 1H, H-1'), 7.73 (d, J = 7.40 Hz, 1H, H-6Cyt).
MS (FAB$^+$) : 826 $(M+H^+)$

5'-[5-[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-oxo-1-pentanoyl]-2',3'-dideoxycytidine **9a**
Compound **3** and 0.07 g of ddC (0.26 mmol) were condensed, giving 0.03 g (yield 10%) of the title compound. R_f = 0.40 ($MeOH/CH_2Cl_2$ 1:9)
^{1}H NMR ($CDCl_3$) δ : 1.39 (s, 27H, t-Bu), 1.69 (m, 4H, H-6 & H-13), 1.90 (m, 2H, CO-CH_2-C$\underline{H}_2$-CH_2-CO), 2.10-2.30 (m, 4H, H-2' & H-3'), 2.30-2.60 (m, 4H, CO-C$\underline{H}_2$-CH_2-C$\underline{H}_2$-CO), 3.20-3.60 (m, 16H, CH_2-N), 4.15-4.30 (m, 3H, H-4' & H-5'), 5.85 (d, J = 7.30 Hz, 1H, H-5Cyt), 6.06 (t, J = 6.40 Hz, 1H, H-1'), 7.80 (d, J = 7.25 Hz, 1H, H-6Cyt).
MS (FAB$^+$) : 808 $(M+H)^+$

5-[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoic ethyl ester **10**
To 0.48 g of compound **2** (0.96 mmol) dissolved in 15 mL of dry acetonitrile were added 0.38 g of potassium carbonate (2.71 mmol, 3 eq) and 170 µL of ethyl-5-bromovalerate ester (1.05 mmol, 1.1 eq). The reaction mixture was refluxed 8 hrs under nitrogen atmosphere, and then allowed to cool to room temperature. After filtration, the solvent was removed and the oily residue dissolved in ethyl acetate. After washing with 5% aqueous citric acid, then with water, the organic layer was dried over Na_2SO_4 and concentrated. Flash column chromatography ($MeOH/ CH_2Cl_2$ 4:96) afforded 0.19 g of a mixture of two products and 0.35 g of compound **2**. The mixture of products was purified by flash column chromatography ($MeOH/ CH_2Cl_2$ 2:98) to give the desired compound (0.15 g, 25% yield) and the residual valerate ester.
R_f = 0.71 ($MeOH/CH_2Cl_2$ 1:9)
^{1}H NMR ($CDCl_3$) δ : 1.02 (t, J = 7.20 Hz, 3H, O-CH_2-C$\underline{H}_3$), 1.22 (s, 27H, t-Bu), 1.25-1.51 (m, 6H, N-CH_2-C$\underline{H}_2$-C$\underline{H}_2$-CH_2-CO & H-13), 1.63 (m, 2H, H-6), 2.06 (t, J = 7.30 Hz, 2H, N-CH_2-CH_2-CH_2-C$\underline{H}_2$-CO), 2.14 (m, 4H, H-14 & N-C$\underline{H}_2$-CH_2-CH_2-CH_2-CO), 2.32 (m, 2H, H-2), 2.97 (m, 2H, H-3), 3.02-3.20 (m, 10H, H-5 & H-7 & H-9 & H-10 & H-12), 3.89 (q, J = 7.20 Hz, 2H, O-C$\underline{H}_2$-CH_3).
MS (FAB$^+$) : 629 $(M+H)^+$

5-[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoic acid **11**
Compound **10** (0.43 g) was dissolved in 10 mL of tetrahydrofuran, 10 mL of aqueous 10N sodium hydroxide were added with a few drops of triton B. The biphasic resulting mixture was stirred at room temperature during 4 hrs. The separated organic layer was washed with 5% aqueous citric acid until pH>7. The aqueous phase was thoroughthly neutralized with HCl, then extracted 3 times with dichloromethane. Collected organic phases were dried over Na_2SO_4 and concentrated to give 0.40 g of the title compound as a white solid in quantitative yield. R_f = 0.28 ($MeOH/CH_2Cl_2$ 1:9)
^{1}H NMR ($CDCl_3$) δ : 1.40 (s, 27H, t-Bu), 1.58 (m, 4H, N-CH_2-C$\underline{H}_2$-CH_2-CH_2-CO & H-13), 1.77 (m, 4H, N-CH_2-CH_2-C$\underline{H}_2$-CH_2-CO & H-6), 2.36 (t, J = 7.30 Hz, 2H, N-CH_2-CH_2-CH_2-C$\underline{H}_2$-CO), 2.75 (m, 4H, H-14 & N-CH_2-CH_2-CH_2-C$\underline{H}_2$-CO), 2.92 (m, 2H, H-2), 3.15-3.50 (m, 12H, CH_2-NBoc), 9.80 (brs, 1H, OH).
MS (FAB$^+$) : 601 $(M+H)^+$

B. General procedure for the coupling of compound **11** with nucleosides

To a solution of 0.20 g of compound **11** (0.33 mmol, 1 eq) in 8 mL of dichloromethane was added a catalytic amount of DMAP (0.01 g, 0.25 eq), a large excess of NEt_3 (230 µL, 5 eq), 1 eq of nucleoside dissolved in 2 mL of dimethylformamide and 0.17 g of BOP reagent (0.40 mmol, 1.2 eq) was added. The reaction mixture was allowed to stir 8 hrs at room temperature under nitrogen atmosphere. The solvent was then removed and the residue was dissolved in EtOAc. The organic layer was successively washed with 5% aqueous citric acid, with water, and then dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave a crude product which was then purified by flash column chromatography using $MeOH/CH_2Cl_2$ 5:95 as eluent.

5'-[[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoyl]-3'-azido-3'-deoxythymidine **12b**

AZT (0.09 g) was reacted with compound **11** according to general procedure **B**, to afford the title compound as a white solid (0.08 g, 28% yield). R_f = 0.47 ($MeOH/CH_2Cl_2$ 1:9)

^1H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.45-1.70 (m, 6H, H-13 & N-CH_2-$C\underline{H}_2$-$C\underline{H}_2$-CH_2-CO), 1.78 (m, 2H, H-6), 1.90 (s, 3H, CH_3Thy), 2.20-2.50 (m, 8H, N-$C\underline{H}_2$-CH_2-CH_2-$C\underline{H}_2$-CO & H-2' & H-12), 2.52 (m, 2H, H-10), 3.08-3.40 (m, 12H, CH_2-NBoc), 4.00 (m, 1H, H-4'), 4.20 (m, 1H, H-3'), 4.28 (m, 2H, H-5'), 6.06 (t, J = 6.50 Hz, 1H, H-1'), 7.17 (d, J = 1.10 Hz, 1H, H-6Thy), 9.58 (brs, 1H, NH).

MS (FAB^+) : 850 $(M+H)^+$

5'-[[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoyl]-2',3'-didehydro-3'-deoxy thymidine **13b**

According to general procedure **B**, 0.09 g of d4T were coupled with compound **11**, to give after purification 0.07 g of the title compound as a white solid (27% yield). R_f = 0.49 ($MeOH/CH_2Cl_2$ 1:9)

^1H NMR ($CDCl_3$) δ : 1.41 (s, 27H, t-Bu), 1.45-1.88 (m, 8H, H-6 & H-13 & N-CH_2-$C\underline{H}_2$-$C\underline{H}_2$-CH_2-CO), 1.88 (s, 3H, CH_3Thy), 2.20-2.65 (m, 8H, N-$C\underline{H}_2$-CH_2-CH_2-$C\underline{H}_2$-CO & H-10 & H-12), 3.08-3.55 (m, 12H, CH_2-NBoc), 4.15-4.38 (m, 2H, H-5'), 5.00 (s, 1H, H-4'), 5.87 (d, J = 5.90 Hz, 1H, H-2'), 6.25 (d, J = 5.90 Hz, 1H, H-3'), 6.96 (brd, J = 1.80 Hz, 1H, H-1'), 7.16 (d, J = 1.10 Hz, 1H, H-6Thy), 8.85 (brs, 1H, NH).

MS (FAB^+) : 807 $(M+H)^+$

5'-[[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoyl]-2',3'-dideoxyinosine **14b**

ddI (0.08 g) was reacted with compound **11**, to give the title compound as a white solid (0.05 g, 18%). R_f = 0.45 ($MeOH/CH_2Cl_2$ 1:9)

^1H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.35-1.50 (m, 4H, N-CH_2-$C\underline{H}_2$-$C\underline{H}_2$-CH_2-CO), 1.59 (m, 2H, H-13), 1.79 (m, 2H, H-6), 1.90-2.20 (m, 2H, H-3'), 2.20-2.40 (m, 6H, N-$C\underline{H}_2$-CH_2-CH_2-$C\underline{H}_2$-CO & H-12), 2.40-2.60 (m, 4H, H-2' & H-10), 3.00-3.40 (m, 12H, CH_2-NBoc), 4.15-4.45 (m, 3H, H-4' & H-5'), 6.22 (t, J = 4.60 Hz, 1H, H-1'), 8.02 (s, 1H, H-2Ino), 8.09 (s, 1H H-8Ino), 12.50 (brs, 1H, NH).

MS (FAB^+) : 819 $(M+H)^+$

5'-[[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoyl]-2',3'-dideoxy-3'-thiacytidine **15b**

According to general procedure **B**, 0.08 g of 3TC were reacted with compound **11** to afford the title compound in 11% yield (0.03 g). R_f = 0.42 ($MeOH/CH_2Cl_2$ 1:9)

^1H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.40-1.70 (m, 6H, H-13 N-CH_2-$C\underline{H}_2$-$C\underline{H}_2$-CH_2-

CO), 1.79 (m, 2H, H-6), 2.19-2.45 (m, 6H, N-C<u>H</u>$_2$-CH$_2$-CH$_2$-C<u>H</u>$_2$-CO & H14), 2.45-2.67 (m, 2H, H-2), 3.00-3.42 (m, 12H, CH$_2$-NBoc), 3.45-3.60 (m, 2H, H-2'), 4.29-4.65 (m, 2H, H-5'), 5.28 (t, J = 3.30 Hz, 1H, H-4'), 5.77 (d, J = 7.40 Hz, 1H, H-5Cyt), 6.22 (t, J = 6.50 Hz, 1H, H-1'), 7.83 (d, J = 7.50 Hz, 1H, H-6Cyt).
MS (FAB$^+$) : 812 (M+H)$^+$

4N-[[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoyl]-2',3'-dideoxy-3'-thiacytidine **15c**:
After purification, compound **15c** was obtained as white solid in a 11% yield (0.03 g).
R_f = 0.45 (MeOH/CH_2Cl_2 1:9)
^{1}H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.42-1.70 (m, 6H, H-13 & N-CH$_2$-C<u>H</u>$_2$-C<u>H</u>$_2$-CH$_2$-CO), 1.78 (m, 2H, H-6), 2.21-2.55 (m, 8H, N-C<u>H</u>$_2$-CH$_2$-CH$_2$-C<u>H</u>$_2$-CO & H-14 & H-2), 3.08-3.39 (m, 12H, CH$_2$-NBoc), 3.50-3.62 (m, 2H, H-2'), 3.84-4.12 (m, 2H, H-5'), 5.29 (t, J = 3.00 Hz, 1H, H-4'), 6.28 (t, J = 6.45 Hz, 1H, H-1'), 7.35 (d, J = 7.50 Hz, 1H, H-5Cyt), 8.35 (d, J = 7.50 Hz, 1H, H-6Cyt), 8.82-8.97 (brs, 1H, NH).
MS (FAB$^+$) : 812 (M+H)$^+$

5',4N-bis[[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoyl]-2',3'-dideoxy-3'-thiacytidine **15d**:
After purification, compound **15d** was obtained as white solid in a 5% yield (0.03 g).
R_f = 0.48 (MeOH/CH_2Cl_2 1:9)
^{1}H NMR ($CDCl_3$) δ : 1.44 (s, 54H, t-Bu), 1.55-1.97 (m, 16H, H-13 & H-6 & N-CH$_2$-C<u>H</u>$_2$-C<u>H</u>$_2$-CH$_2$-CO), 2.21-2.68 (m, 16H, N-C<u>H</u>$_2$-CH$_2$-CH$_2$-C<u>H</u>$_2$-CO & H-14 & H-2), 3.10-3.54 (m, 24H, CH$_2$-NBoc), 3.63 (m, 2H, H-2'), 4.39-4.70 (m, 2H, H-5'), 5.38 (t, J = 3.20 Hz, 1H, H-4'), 6.32 (t, J = 6.50 Hz, 1H, H-1'), 7.47 (d, J = 7.50 Hz, 1H, H-5Cyt), 8.10 (d, J = 7.50 Hz, 1H, H-6Cyt), 8.55-8.82 (brs, 1H, NH).
MS (FAB$^+$) : 1394 (M+H)$^+$

5'-[[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoyl]-2',3'-dideoxycytidine **16b**
ddC (0.04 g) was condensed with compound **11** following general procedure **B**, to give 0.01 g (yield 8%) of the title compound. R_f = 0.42 (MeOH/CH_2Cl_2 1:9)
^{1}H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.49-1.70 (m, 6H, H-13 & N-CH$_2$-C<u>H</u>$_2$-C<u>H</u>$_2$-CH$_2$-CO), 1.70-2.00 (m, 4H, H-6 & H-3'), 2.03-2.58 (m, 10H, N-C<u>H</u>$_2$-CH$_2$-CH$_2$-C<u>H</u>$_2$-CO & H-14 & H-2 & H-2'), 3.07-3.43 (m, 12H, CH$_2$-NBoc), 4.18-4.42 (m, 3H, H-4' & H-5'), 5.73 (d, J = 7.50 Hz, 1H, H-5Cyt), 5.99 (t, J = 6.45 Hz, 1H, H-1'), 6.00-6.28 (brs, 2H, NH$_2$Cyt), 7.80 (d, J = 7. 50 Hz, 1H, H-6Cyt).
MS (FAB$^+$) : 794 (M+H)$^+$

4N-[[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoyl]-2',3'-dideoxycytidine **16c**
After purification, compound **16c** was obtained as white solid in a 11% yield (0.01 g).
R_f = 0.46 (MeOH/CH_2Cl_2 1:9)
^{1}H NMR ($CDCl_3$) δ : 1.38 (s, 27H, t-Bu), 1.48-1.65 (m, 6H, H-13 & N-CH$_2$-C<u>H</u>$_2$-C<u>H</u>$_2$-CH$_2$-CO), 1.68-1.94 (m, 4H, H-6 & H-3'), 2.02-2.55 (m, 10H, N-C<u>H</u>$_2$-CH$_2$-CH$_2$-C<u>H</u>$_2$-CO & H-14 & H-2 & H-2'), 3.03-3.40 (m, 12H, CH$_2$-NBoc), 3.64-4.06 (m, 2H, H-5'), 4.19 (m, 1H, H-4'), 6.02 (t, J = 6.40 Hz, 1H, H-1'), 7.32 (d, J = 7.40 Hz, 1H, H-5Cyt), 8.42 (d, J = 7.30 Hz, 1H, H-6Cyt), 9.00 (brs, 1H, NH).
MS (FAB$^+$) : 794 (M+H)$^+$

5',4N-bis[[4,8,11-tris(*tert*-butyloxycarbonyl)-1,4,8,11-tetraazacyclotetradec-1-yl]-1-pentanoyl]-2',3'-dideoxycytidine **16d**

After purification, compound **16d** was obtained as white solid in a 8% yield (0.01 g).

R_f = 0.48 ($MeOH/CH_2Cl_2$ 1:9)

^1H NMR ($CDCl_3$) δ : 1.39 (s, 54H, t-Bu), 1.50-1.70 (m, 12H, H-13 & N-CH_2-CH_2-CH_2-CH_2-CO), 1.71-1.93 (m, 6H, H-6 & H-3'), 2.00-2.58 (m, 18H, N-CH_2-CH_2-CH_2-CH_2-CO & H-14 & H-2 & H-2'), 3.01-3.48 (m, 24H, CH_2-NBoc), 4.12-4.40 (m, 3H, H-4' & H-5'), 5.98 (t, J = 6.45 Hz, 1H, H-1'), 7.34 (d, J = 7.45 Hz, 1H, H-5Cyt), 8.04 (d, J = 7. 50 Hz, 1H, H-6Cyt), 8.76 (brs, 1H, NH).

MS (FAB^+) : 1376 $(M+H)^+$

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