New Bicyclam−AZT Conjugates: Design, Synthesis, Anti-HIV Evaluation, and Their Interaction with CXCR-4 Coreceptor

Jean Dessolin,[†,‡] Pascale Galea,[‡] Patrick Vlieghe,[†,‡] Jean-Claude Chermann,[‡] and Jean-Louis Kraus*[,†,‡]

Laboratoire de Chimie Biomoléculaire, Faculté des Sciences de Luminy, case 901, Université de la Méditerranée, 163 avenue de Luminy, 13288 Marseille Cedex 9, France, and INSERM U322 "Rétrovirus et Maladies Associées", Campus Universitaire de Luminy, BP33, 13273 Marseille Cedex 9, France

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We report the synthesis of mono- and bis-tetraazamacrocycle−AZT conjugates. All new compounds were screened for their ability to inhibit HIV-1 replication in MT4 cell line and were compared to AZT alone. It appears that N-protected covalent prodrugs are equipotent to AZT as inhibitor of HIV replication, while N-deprotected analogues exhibit both higher activity and selectivity against HIV-infected cells. The most active antiviral compounds **27**, **28**, **34**, and **35** were then tested for their binding capability to CXCR-4 receptor. N-Boc analogues **27** and **34** were only weakly effective; in contrast, N-deprotected conjugates **28** and **35** were antagonists to 12G5 mAb binding until 0.05 and 5 μg/mL, respectively. The stability of compound **28** in human plasma was evaluated, and half-life was found to be approximately 8 h in the described conditions. All these results seem to demonstrate the confidence of our prodrug approach, with analogue **28** emerging as the best candidate as lead compound in HIV-1 polytherapy perspective.

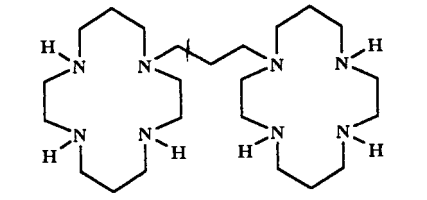
Introduction

The search for an effective chemotherapeutic treatment against human immunodeficiency virus (HIV) infection has led to the development of agents that target specific and critical events in the HIV replicative cycle. The most extensively studied of these agents are the 2′,3′-dideoxynucleoside analogues which terminate DNA synthesis during the reverse transcription reaction, the non-nucleoside reverse transcriptase (RT) inhibitors which interact at a specific site on HIV-1 RT, and the inhibitors of HIV protease, an essential proteolytic enzyme required for the assembly of fully infectious viral particles.[1] More recently, it has been pointed out that virus-induced cell fusion was a major hallmark of HIV infection both in vivo and in vitro; it represents a major mechanism of virus-induced cell killing.[2] These observations have led to the discovery of a novel class of highly potent antiviral compounds such as bis-tetraazamacrocycles[3−8] (**1**, **2**) or some tetrakis(ω-amino alkyl) tetraazamacrocycles[9] (**3**) (Figure 1).

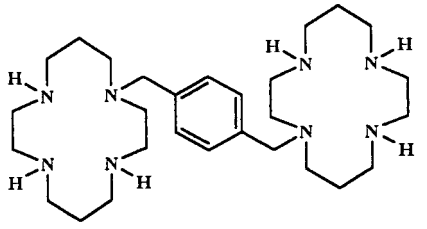
First, it was suggested that bicyclams such as JM 2763 (**1**) or JM 3100 (**2**) targeted the virus uncoating associated process.[10] Uncoating has to be preceded by the removal of the viral envelope and followed by the fusion process and then decapsidation (removal of capsid proteins). In recent papers,[11−13] it has been shown that bicyclams **1** and **2** were the first low molecular weight agents which interfered with the cellular coreceptor for T-cell tropic viruses, hence preventing cell infection.

At first, our interests were to explore the feasibility of increasing the delivery of various anti-HIV dideoxynucleosides to HIV-infected cells. For this purpose, we developed synthetic schemes in order to synthesize new cyclam− or bicyclam−nucleoside conjugates. Several observations supported the importance of the synthesis of these new covalent bicyclam−nucleoside conjugates:

(i) We have found that prodrugs of anti-RT nucleoside (2′,3′-dideoxy-3′-thiacytidine) bearing various polyaminated sidearms at the 5′-*O* or 4-*N* positions led to potent enhancement of the observed anti-HIV activity.[14] As it has been reported that nucleosides such as AZT cross



JM 2763 **1**



JM 3100 or AMD 3100 or SID 791 **2**

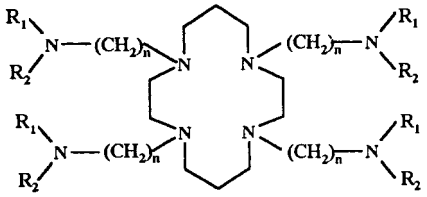


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Figure 1. Structures of bicyclam JM 2763 **1**, JM 3100 **2**, and tetrakis(ω-amino alkyl) tetraazamacrocycle **3** analogues.

* To whom correspondence should be addressed. E-mail: kraus@luminy.univ-mrs.fr. Tel: (33) 491 82 91 41. Fax: (33) 491 82 92 50.
† Université de la Méditerranée.
‡ INSERM U322.

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the cell membrane by non-facilitated diffusion and that its uptake was insensitive to inhibitors of nucleoside transport,[15,16] we have suggested that these new polyaminated prodrugs could interact with early events of the retrovirus replicative cycle.

(ii) Using a three-dimensional method of analysis,[3] it has been shown that when bicyclam **1** (JM 2763) was assayed against HIV-1 (IIIB) in combination with AZT, the compounds appeared to act in additive fashion at the concentrations used. This result was of interest since physical combination (mixture) of two or three drugs can act in several ways: additive combinations, which are distinct from synergistic combinations (e.g., TIBO plus AZT),[3] or from antagonistic combinations (e.g., AZT plus Ribavirin).[3] This study showed that AZT and bicyclam were not antagonistic drugs.

(iii) Covalent conjugate combination of two bicyclam-nucleoside drugs would differ subtly from physical combination of a mixture of two drugs in that the conjugate, transported initially as such into the cells, could present a specific antiviral activity profile. Indeed, an enzymic labile conjugate upon hydrolysis could gradually release the anti-HIV parent nucleoside to make its own antiviral combination superimposed on that of the bicyclam.

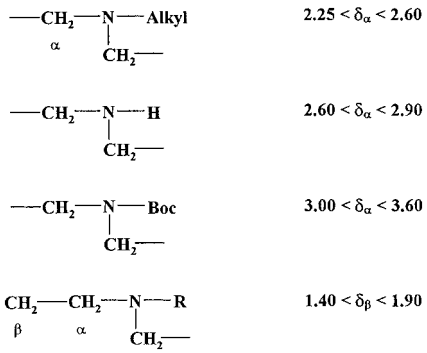
(iv) Several publications have shown the importance of chemokine receptors for HIV entry into target cells[12] and, in particular, the role of CXCR-4 receptor for the specific binding of T-tropic HIV strains.[17] In parallel, bicyclams were reported to interfere in HIV fusion events by targeting CXCR-4 receptors.[11]

Indeed, it was of great interest to study whether the new bicyclam-AZT conjugates could selectively target the CXCR-4 receptor and inhibit HIV infection of cell.

This paper describes the synthesis of cyclam− and bicyclam−AZT conjugates and their in vitro anti-HIV activities correlated to some of their biophysical properties and to their selective antagonization of the chemokine receptor CXCR-4.

Chemistry

All series of compounds required the synthesis of common intermediates as illustrated in Scheme 1. Tri-Boc compound (synthon **4**) and a mixture of di-Boc compounds were obtained in the same step, starting from the commercially available 1,4,8,11-tetraazacyclotetradecane (cyclam), following previously reported procedures.[18,19] Indeed, *N*-Boc protection of cyclam, being more suitable, was preferred to N-tosyl protection used in the preparation of bicyclam.[20] It should be emphasized that if the separation of the tri-Boc compound **4** was easily performed from the mixture of di-Boc compounds, the isolation and purification of N_1,N_{11}-di-Boc- and N_1,N_8-di-Boc-protected tetraazamacrocycle, in contrast, was difficult. This was particularly due to the polarity of the substrates. To avoid this time-consuming purification step, the crude mixture of di-Boc compounds was directly used in the next step consisting of an alkylation by ω-bromo ester in refluxing acetonitrile in the presence of potassium carbonate (Scheme 1). The resulting compounds were then easily purified. Compound **5** (monoalkylated N_4,N_8-di-Boc) was isolated in 65% yield, while the monoalkylated N_4,N_{11}-di-Boc **6** was obtained in 15% yield and the dialkylated products were isolated in small quantities (**7**: 7% yield; **8**: 3% yield). These four functionalized compounds were clearly identified by NMR ^{1}H COSY experiments. Depending on their relative position from the nitrogen atoms (α or β) and the substituents of these nitrogen atoms, the methylene groups of the azamacrocycles appear at different chemical shifts:



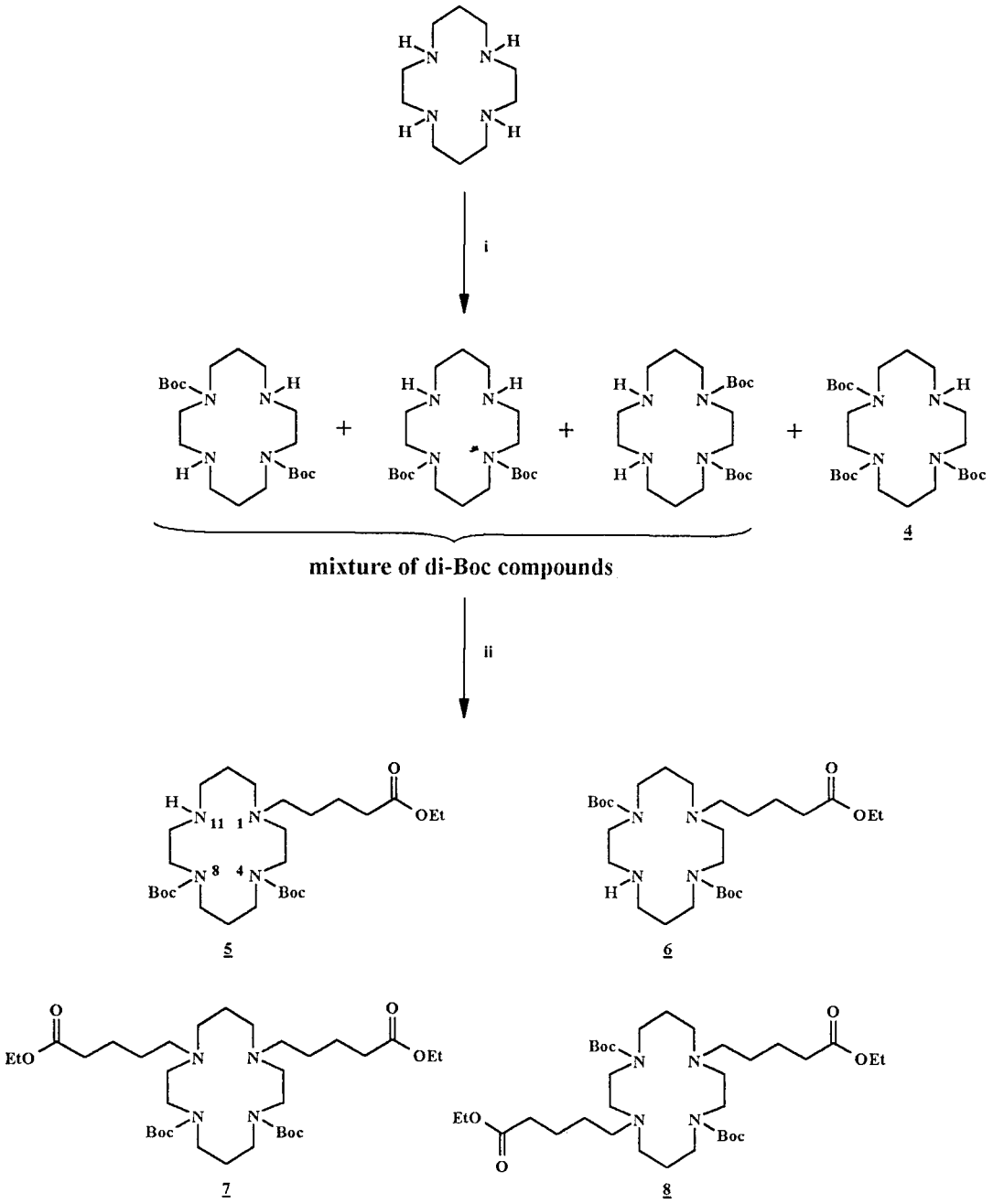
From COSY experiments, it can be observed that the protons of the methylene group in position 6 (β from nitrogen 4 and 8, see Scheme 1) is only coupled with the methylene 5 and 7 which are in α position from N_4 and N_8 Boc-substituted nitrogen atoms in compound **5**, while in compound **6** the corresponding protons in position 6 are conjugated with the methylene 5 and 7 which are, respectively, in α position from N_4 Boc and N_8 free nitrogen atoms. The same NMR argument was put forward to elucidate the structure of dialkyl compounds **7** and **8**.

Tetraazamacrocycle Conjugates. Starting from crucial synthon **4**, two series of mono-tetraazamacrocycle−nucleoside conjugates were prepared, and their synthesis are summarized in Scheme 2.

First Series: *N*-Alkyl Tetraazamacrocycle−Dideoxynucleoside Conjugates. Overnight condensation of the intermediate **4** with ethyl 5-bromo valerate afforded the corresponding alkylated polyazamacrocycle. Hydrolysis of the obtained ester was achieved in quantitative yield under heterogeneous basic conditions in tetrahydrofuran, which gave an overall yield of 28% for compound **9**. Condensations of the cyclam carboxylic acid derivative **9** with AZT were performed following different methods. Several assays with DCC/HOBt (*N*,*N*-dicyclohexyl carbodiimide/1-hydroxybenzotriazole) coupling reagent proved to be carboxylic acid analogue **9** consuming because of the concomitant formation of N-acyl urea derivatives as byproducts.[21,22] The use of PyBOP (benzotriazol-1-yloxy-tris(pyrrolidino) phosphonium hexafluorophosphate) as coupling reagent allowed the obtention of desired products in good yields. Final deprotection of the N-Boc-protected tetraazamacrocycle−nucleoside conjugate was possible under various experimental conditions. However, the obtention of solid chlorhydrate **11** without hydrolysis of the ester link favored the use of gaseous HCl in anhydrous diethyl ether.

Second Series: *N*-Acyl Tetraazamacrocycle−Dideoxynucleoside Conjugates. Acylation of compound **4** using glutaryl dichloride (Scheme 2) in Schotten−Baumann conditions afforded the expected acyl chloride **12**, along with the bis-polyazamacrocycle **15** resulting from the dimerization of compound **4** as a

Scheme 1

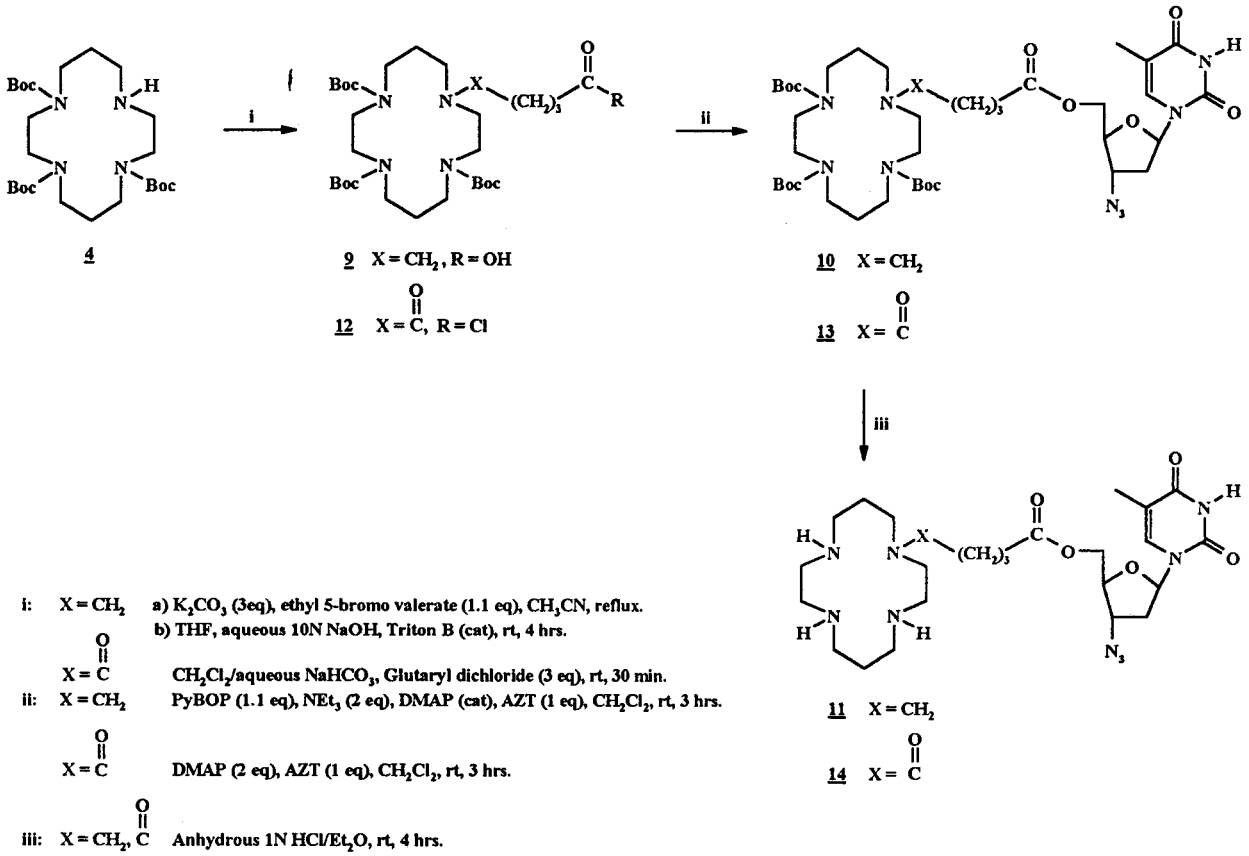


i: Boc_2O, CH_2Cl_2 ii: K_2CO_3 (3eq), ethyl 5-bromo valerate (0.5 eq), CH_3CN, reflux.

minor product (15% yield). The condensation of AZT with compound **12** using 4-DMAP (4-(dimethylamino)-pyridine) in dichloromethane afforded the desired ester in low yields (30%). Note that syntheses of compounds **4**, **9**, **10**, **12**, and **13** were already described elsewhere.[22]

Bis-tetraazamacrocycle Conjugates. The preparation of the different series of bis-tetraazamacrocycle−nucleoside conjugates was achieved using the following general procedure (Scheme 3): compounds **4** and **5** were condensed with an appropriate bis-electrophile (glutaryl dichloride for the first series, α,α′-dibromo-*p*-xylene and terephthaloyl dichloride for the second and the third series, respectively). The use of two different mono-polyazamacrocycles in condensation reaction with a bis-functionalized compound implied the possible formation of three different bis-polyazamacrocycles. We observed, for each of the three series, the concomitant formation of three different products detected by TLC. Due to the close R_f values, the purification of the obtained mixture appeared quite difficult. This problem was solved by isolation of the mixture of the three bis-tetraazamacrocycles (confirmed by NMR) from the crude reaction, followed by their saponification in tetrahydrofuran. The resulting compounds, possessing at this stage sufficiently different R_f values, allowed convenient and rapid purification of the desired unsymmetrical acids (**18**, **25**, **32**), symmetrical diacids (**19**, **26**, **33**), and the dimers (**15**, **22**, **29**). By analogy with the synthesis of the conjugate **10**, PyBOP coupling reagent in the presence of triethylamine in dichloromethane was used to prepare the bis-macrocycle−AZT conjugates (**20**, **27**, and **34**). These compounds proved to be as resistant

Scheme 2



versus *tert*-butyl carbamate deprotection as the corresponding monocyclic conjugates. Thus compounds **21**, **28**, and **35** were obtained from the respective N-Boc-protected esters **20**, **27**, and **34**. It should be noted that release of dideoxynucleoside did not occur in the described conditions.

Dimeric compounds such as **15**, **22**, and **29** were Boc-deprotected to give the symmetric bis-polyazamacrocycles **36**, **37**, and **38**, respectively. These latter were used as anti-HIV references since dimer **37** was found to be an anti-HIV compound.[4]

Results

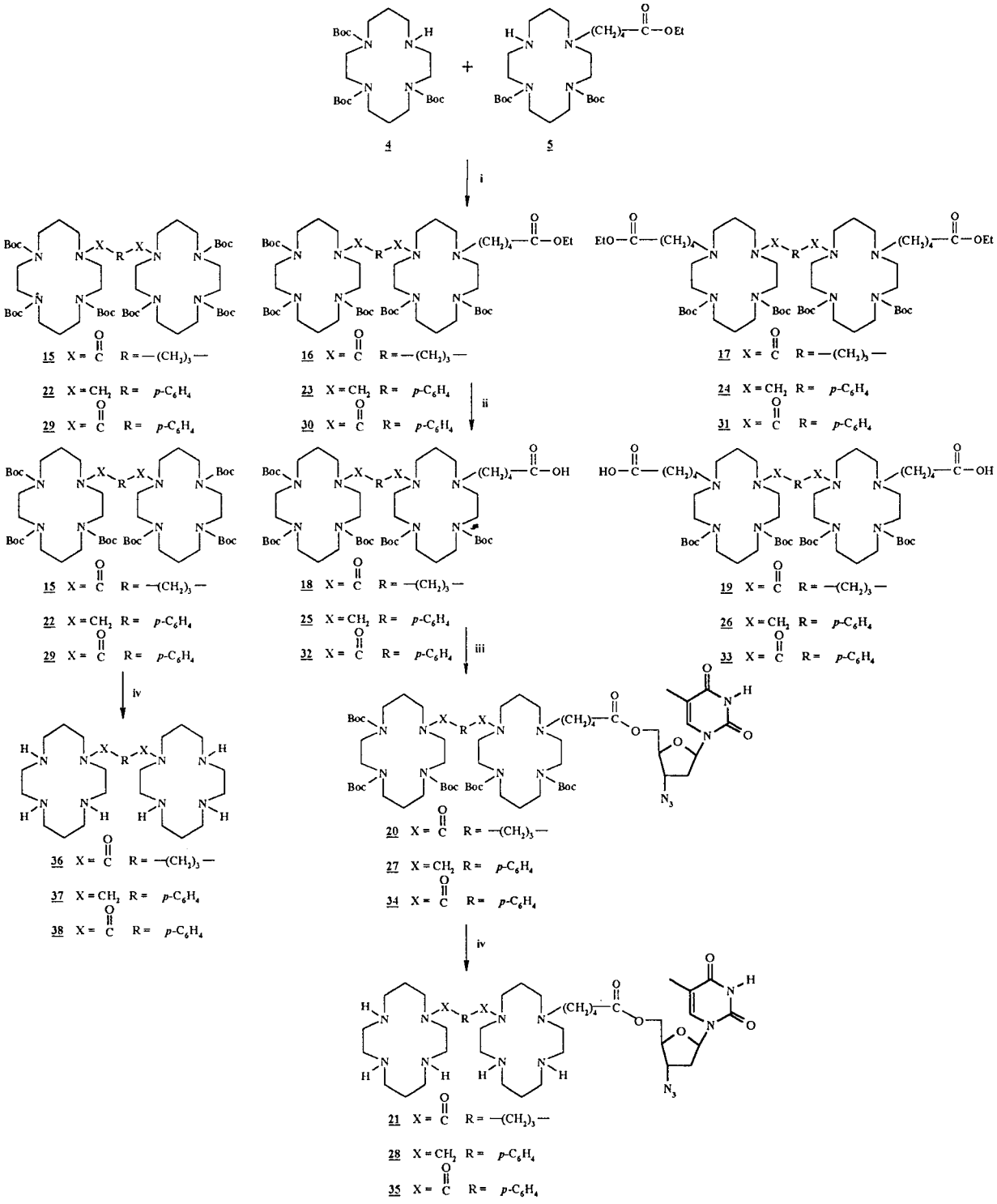
The first objective of this project was to design and synthesize covalently linked bicyclam−AZT conjugates with improved anti-HIV activities. The design of these new conjugates was based on the following finding: bicyclam moiety are known to interact specifically with the CXCR-4 coreceptor[12,23] used by T-tropic viruses to infect target cells and then they could help to achieve higher AZT concentration in infected cells. In this perspective, the effects of the new bicyclam−AZT conjugates on HIV cytopathicity, their interaction with CXCR-4 chemokine receptor, and the correlation of their antiviral activities with lipophilicity and biological stability properties were studied.

Antiviral Activity Measurements. AZT, cyclam/bicyclam intermediates, and cyclam− or bicyclam−AZT conjugates were evaluated for their inhibitory effects on HIV replication in MT4 cell culture (Table 1). Under assay conditions, all the tested cyclam− or bicyclam−AZT prodrugs elicited anti-HIV activity with EC_{50} values ranging from 0.005 to 0.1 μM. In the same testing conditions, AZT alone inhibited *syncytia* formation with $EC_{50} = 0.05$ μM.

Monocyclam−AZT conjugates were found to be slightly less active than the corresponding bicyclam−AZT analogues. Analogues **27** and **28** appeared to be 1 order of magnitude more active than the parent drug AZT, while compounds **34** and **35** were equipotent to AZT. The lower EC_{50} values for **27** and **28** compared to that of the parent molecule AZT could be tentatively attributed to an increase in cellular uptake followed by intracellular release of AZT. The prodrugs appeared to be lipophilic enough to cross the cellular membrane by simple diffusion as demonstrated by the antiviral activities.

Binding Capability of the New Drugs to the CXCR-4 Receptor and Correlation with Their Biophysical Properties. Compounds **27**, **28**, **34**, **35**, and reference compound **2** were studied for their ability to bind to the CXCR-4 receptor as already reported.[24] SDF-1α was also included in the study as the natural biological ligand of the CXCR-4 receptor. By using cytometry of flux, drugs were tested for their power to inhibit the fixation of 12G5 mAb, which specifically recognizes cellular CXCR-4 receptor. As shown in Figure 2, JM 3100 (**2**) and N-deprotected analogues **28** and **35** prevented 12G5 mAb fixation to CXCR-4 receptor as attested by the extinction of the specific fluorescent signal. Fixation of control mAb B1G6 was not affected by drug treatment of cells, indicating a specific action of compounds **28**, **35**, and JM 3100 on CXCR-4 molecules. Compounds **2** and **28** were the most efficient compounds to compete with 12G5 mAb fixation, as they decreased mAb fixation from 81% and 69%, respectively,

Scheme 3



i: X = C=O R = $(CH_2)_3$ Glutaryl dichloride (1 eq), CH_2Cl_2 / $NaHCO_3$, rt, 30 min.

X = CH_2 R = p-C_6H_4 K_2CO_3 (3 eq), Dibromo-*p*-xylene (1 eq), CH_3CN, reflux, 8 hrs.

X = C=O R = p-C_6H_4 Terephtaloyl dichloride (1 eq), CH_2Cl_2 / $NaHCO_3$, rt, 30 min.

ii: THF, aqueous 10N NaOH, Triton B (cat), rt, 4 hrs.

iii: PyBOP (1.1 eq), NEt_3 (3 eq), DMAP (cat), AZT (1 eq), CH_2Cl_2, rt, 3 hrs.

iv: Anhydrous 1N HCl / Et_2O, rt, 4 hrs.

at a concentration of 5 μg/mL; the inhibitory effect was dose-dependent: at 0.05 μg/mL the inhibitory effect was maintained at 82% for JM 3100 versus 47% for compound **28**. Compound **35**, whose structure includes a phthaloyl linker between the two cyclam rings, was also efficient in competing for the binding to the CXCR-4 receptor but at higher concentrations, as 52% of inhibition was observed at 25 μg/mL versus 26% at 5 μg/mL.

Table 1. Antiviral Evaluation of Cyclam/Bicyclam−AZT Conjugates

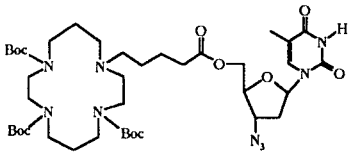
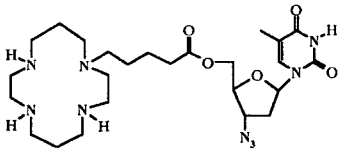
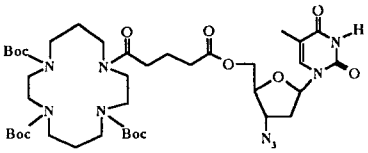
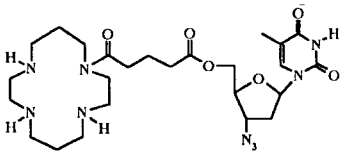
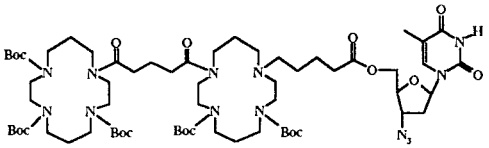
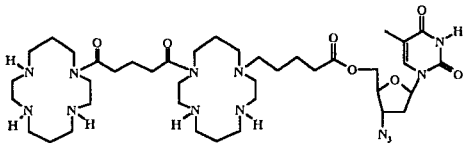
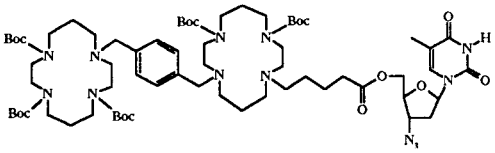
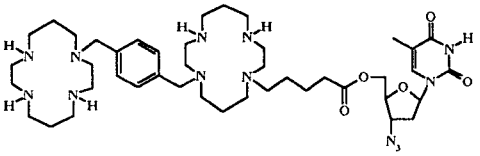
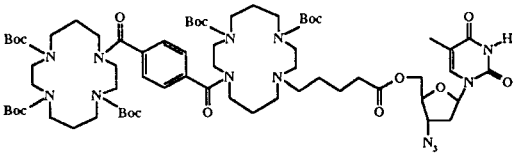
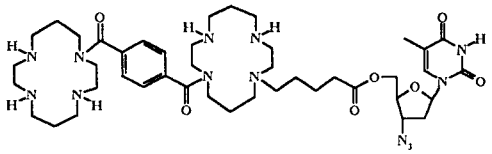
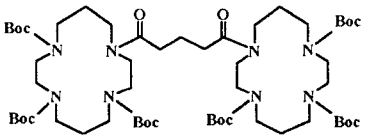
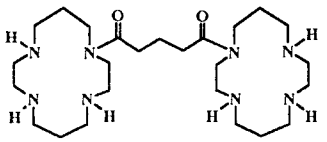
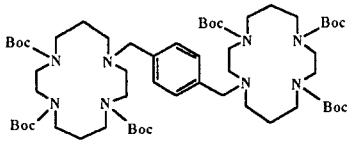
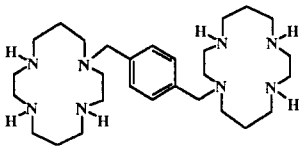
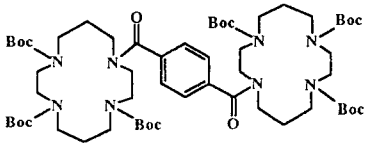
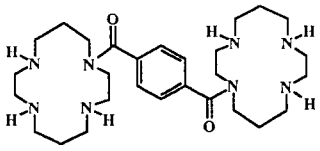
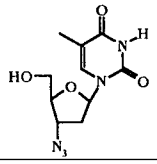
Compound	Structure	EC_{50}[a]	CC_{50}[b]	SI[c]	Log P[d]
10		0.1	30	300	2.34
11		0.01	30	3000	-0.21
13		0.05	50	1000	2.68
14		0.01	100	10000	0.14
20		0.05	5	100	5.85
21		0.05	75	1500	1.81
27		0.005	5	1000	5.70
28		0.005	75	15000	1.65
34		0.03	0.5	17	2.69
35		0.01	75	7500	-1.35
15		> 50	10	-	6.14

Table 1 (Continued)

Compound	Structure	EC_{50}[a]	CC_{50}[b]	SI[c]	Log P[d]
36		10	50	5	1.05
22		0.5	5	10	6.47
37		10	50	5	0.89
29		1	10	10	3.46
38		50	100	2	-2.11
AZT		0.05-0.01	50	> 1000	-0.88

[a] EC_{50}: concentration in μM required to inhibit *syncytia* formation by 50% on MT4 cells. [b] CC_{50}: concentration in μM required to cause 50% death of uninfected MT4 cells. [c] SI: selective index = CC_{50}/EC_{50}. [d] log *P* determinations were performed using ACD (Advanced Chemistry Development, Inc.)/log *P* 1.0 base calculations.

In contrast, N-Boc-protected analogue **27** was totally ineffective whereas compound **34** weakly inhibited 12G5 mAb binding (30% of inhibition at 25 μg/mL).

These results suggest that selective competition for the binding to the CXCR-4 receptor requires a prototype molecule presenting required structural parameters: (i) the number of free nitrogen atoms in the bicyclam moiety should be maximum, and (ii) the preferred linker between the two bicyclams should be a xylyl or alkyl group, the phthaloyl linker being less effective. The presence of only one sidearm bearing the AZT moiety slightly decreased the binding affinity to the CXCR-4 receptor (compound **28** versus reference compound **2**).

Lipophilicity, Stability, and Decomposition Studies. Previous studies have reported that AZT crossed the cell membrane by nonfacilitated diffusion and that its uptake was insensitive to inhibitors of nucleoside transport.[16] This indicates that the partition coefficient of AZT analogues might have a significant role in their diffusion. In this perspective, partition coefficients (log *P*) were determined for all conjugates using ACD/LogP software from ChemCAD.

From the results shown in Table 1, log *P* values for Boc-protected derivatives ranged from 2.68 to 2.34 in the cyclam series and from 5.85 to 2.69 for conjugates belonging to the bicyclam series. As expected, these values were greater than the those obtained for the corresponding deprotected cyclam or bicyclam conjugates. For example, in the case of AZT-conjugate congeners, log *P* values ranged from 1.81 to −1.35, while the log *P* value for AZT itself was −0.88. It has been reported[21] that prodrugs of AZT in which the 5′-hydroxyl group was esterified with various terminal carboxylic ligands had anti-HIV activity in peripheral blood lymphocytes, greater than that of AZT alone. Most of these AZT prodrugs that had higher partition coefficients than that of AZT diffused into the cells at a higher level than AZT itself, suggesting an important influence of partition coefficients on the diffusion of AZT analogues into the cells. In the case of cyclam/bicyclam−AZT prodrugs, conjugates having greater log *P* values (**13**, **14**, **10**, **11**, **34**, **27**, **28**, **20**, **21**) as well as those having lower log *P* values (**35**) than the one of AZT elicited higher or at least equal anti-HIV activity compared to that of AZT alone. Therefore, from the results presented in Table 1, lipophilicity is probably not the only factor that influences the antiviral activity of these enzyme-labile prodrugs. Starting from the hypothesis that enzymatic

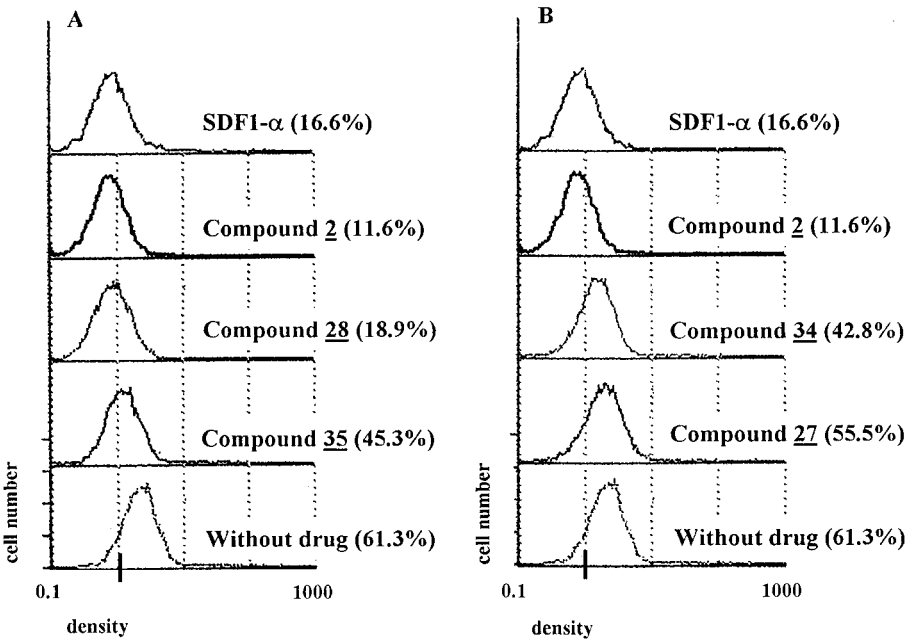


Figure 2. Effect of N-deprotected and N-Boc-protected drugs on CXCR-4 expression. After a 20 min incubation of drugs with MT4 cells, cells were incubated with anti-CXCR-4 mAb and then washed and counterstained with PE-GAM as described in Material and Methods. The histograms show the surface expression of CXCR-4 receptor on MT4 cells incubated (A) with 5 μg/mL of N-deprotected compounds **28** and **35** in comparison with control compound **2** and natural ligand SDF1-α used at 5 μg/mL, and (B) with 25 μg/mL of Boc-protected compounds **27** and **34** in comparison with control compound **2** and natural ligand SDF1-α used at 5 μg/mL. The vertical line indicates the cutoff at which fluorescence was less than 1% of cells stained with a negative control mAb. In parentheses are expressed the percentages of positive cells. Data from one representative experiment out of three are shown. Figure 2 was treated with Adobe Photoshop software in order to increase the contrast.

hydrolysis sensibilities and stabilities of the prodrugs are likely to be crucial, we have performed stability and decomposition studies.

The usefulness of cyclam/bicyclam−AZT prodrugs depends not only on the stability of the prodrug for its transport across cell membrane but also upon its intracellular reversion to the parent compound, especially in HIV-infected cells.[21] The half-life ($t_{1/2}$) of hydrolysis of two selected AZT conjugates, **14** and **28** was determined in human plasma, as well as under acidic and basic aqueous conditions. Prodrugs **14** and **28** were found chemically stable in basic or acidic aqueous media (pH 8.3 and 2.2, respectively) up to 24 h at 37 °C. In contrast, in human serum, the stability determined by HPLC showed that the half-life values of compounds **14** and **28** were, respectively, 7 and 8 h. These results suggest that cyclam/bicyclam−AZT prodrugs are only slightly sensitive to plasma esterases. Therefore, these prodrugs may have increased plasma $t_{1/2}$ under in vivo conditions.

Discussion

The new synthesized cyclam/bicyclam−AZT prodrugs differ noticeably from known 5′-*O*-AZT prodrugs in several ways: no apparent correlation appears between lipophilicity and antiviral activity, the highly lipophilic N-Boc-protected analogues (**13**, **10**, **34**, **27**, **20**) and the corresponding hydrophilic analogues (**14**, **11**, **35**, **28**, **21**) eliciting similar antiviral activities. This result suggests that passive diffusion of the prodrugs into the cells is not the rate-limiting step. Since esters have been shown to undergo extensive first-pass metabolism,[12] the stability of the new prodrugs of AZT was studied. The relative good enzymatic stability found for the tested prodrugs, in comparison with other AZT prodrugs, suggests that their intracellular hydrolysis under in vitro conditions could limit release of AZT inside infected cells. In this case, one can expect a decrease in anti-HIV activity of the AZT prodrugs in comparison to AZT itself. In order to explain the increased anti-HIV activity of bicyclam−AZT conjugates **27** and **28** which blocked *syncytium* formation at concentrations 2- to 10-fold lower than the corresponding concentration required when AZT is used alone, several facts can be highlighted:

(i) It has been reported that bicyclams are inhibitory to *syncytium* formation at concentrations that were about 10- to 100-fold higher than their EC_{50} in viral replication assays.[4] Thus, the bicyclam moiety could partake weakly in the inhibition of *syncytium* formation in addition to that of AZT.

(ii) It has been found that linear polyamine covalently coupled to nucleoside anti-reverse transcriptase drugs potentialized *syncytium* formation inhibition, specifically in the case of macrophage-tropic strains of HIV.[14] Thus, the bicyclam moiety could enhance the transport and/or the internalization of AZT in target cells.

(iii) The bicyclam moiety of compound **28** interacts with the CXCR-4 receptor which mediates entry of T-tropic HIV strains, while the released AZT (intra- or extracellulary) interacts with the enzyme reverse transcriptase as chain terminator. At concentrations that are effective in inhibiting viral cytopathicity, bicyclam has been reported to fail to inhibit the formation of giant cells in a direct *syncytium* formation assay.[11] In these hypotheses, only the inhibition of *syncytium* formation caused by AZT (inhibition of replication) could be observed, while the possible effect of the bicyclam moiety on the inhibition of virus infection[3] would not. This

means that conjugate **28** behaves as a prodrug of AZT, delivering the nucleoside to infected cells at higher concentration.

Other antiviral activity assays, such as viral antigen expression have to be performed in order to evaluate the EC_{50} for inhibiting viral cytopathicity caused by the bicyclam moiety.

Moreover, the cytotoxicity of AZT and the prodrugs, determined against MT4 cells by the Trypan blue dye exclusion method for cell viability, revealed that N-protected prodrugs were more cytotoxic than AZT, suggesting that this increase in toxicity may be due to the partial release of the terminal carboxylic N-Boc cyclam or bicyclam moiety.

Conclusion

This is the first time to our knowledge that the synthesis and antiviral properties of covalently bounded (AZT)−cyclam/bicyclam prodrug conjugates are reported. From the obtained results, the relative stability under acidic and basic conditions, as well as in serum, indicates that these prodrugs might enhance AZT bioavailability.

Among the cyclam− or bicyclam−AZT conjugate series, derivatives **27** and **28** emerged as the two most active antiviral compounds, but **28** had the highest selective index. Furthermore, compound **28** elicited the greatest binding affinity to the CXCR-4 coreceptor (with an extinction coefficient of 69% at 5 μg/mL) whereas compound **27** did not inhibit binding to CXCR-4. This indicates that although both compounds were effective in inhibiting viral replication, they did not use the same ways to enter into the cells. Hence, compound **28**, by targeting the CXCR-4 receptor at the cell surface, probably selectively drove AZT compounds into the cells, increasing its efficiency of action (less cytotoxic for the same EC_{50}).

The presented new bipharmacophore drugs could represent the first prototype drug associating in a single molecule an anti-reverse transcriptase inhibitor covalently linked to a fusion inhibitor. Antiviral activity of these new prototypes on AZT-resistant virus strains is under investigation. Improvement of the selective index of such a prototype could have clinical potential in AIDS combinotherapy.

Experimental Section

Nuclear magnetic resonance spectra were recorded with a Bruker AC-250 spectrometer (^{1}H NMR); chemical shifts are expressed as δ units (part per million) downfield from TMS. Fast atom bombardment mass spectral analysis were obtained by Dr. Astier (Laboratoire de Mesures Physiques-RMN, USTL, Montpellier, France) on a JEOL DX-100 using a cesium ion source and glycerol/thioglycerol (1:1) or *m*-nitrobenzyl alcohol (NOBA) as matrix. Mass calibration was performed using cesium iodide. Microanalyses were carried out by Service Central d'Analyses du CNRS (Venaison, France) and were within 0.4% of the theoretical values. Thin-layer chromatography (TLC) and preparative layer chromatography (PLC) were performed using silica gel plates 0.2, 1, or 2 mm thick ($60F_{254}$ Merck). Preparative flash column chromatography was carried out on silica gel (230−240 mesh, G60 Merck). Analytical HPLC was performed on a Waters 600E instrument with a M991 detector using the following conditions: 4.6 × 150 mm column (Waters Spherisorb S5 ODS2, 5 μM); mobile phases: A = 0.1% TFA in H_2O, B = 0.1% TFA in CH_3CN; flow rate 1.5 mL/min. All reagents were of commercial quality (Aldrich Company) from freshly opened containers. Preparation and structural data of compounds **4**, **9**, **10**, **12**, and **13** are available elsewhere.[22]

Cell Lines, Virus, and Cell Culture. The CEM cell line and the T-leukaemia virus type one (HTLV-I) CD4-positive T-cell line, MT4, were cultured in RPMI/10% FCS and refeeded twice a week.

The laboratory-adapted strain HIV^{LAV} clade B stock was prepared from the supernatant of the infected CEM cell line, and aliquots were kept frozen at −80 °C until use.[25]

Anti-HIV Activity Assay. Anti-HIV activity was monitored by the efficiency of drug compounds to inhibit *syncytia* formation after HIV infection of MT4 as already described.[26,27] Briefly, 3 × 10^5 MT4 cells were first preincubated with 100 μL of various concentrations of drug compounds dissolved in DMSO or in H_2O and then diluted in phosphate buffer saline solution for 1 h at 37 °C. Then 100 μL of an appropriate virus dilution was added to the mixture, and another 1 h incubation period at 37 °C was done. After three washes, cells were resuspended in culture medium in the presence or not of drug compounds. Cultures were then continued for 7 days at 37 °C under 5% CO_2 atmosphere and refeeded at day 3 postinfection with culture medium supplemented or not with drug compounds. Each culture well was done in duplicate. The appearance of *syncytia* was followed each day with an inverted optical microscope. Typically, the virus dilution used in this assay (multiplicity of infection of 0.1 TCID50/cell) allowed *syncytia* formation at day 5 postinfection. The inhibitory concentration of drug compounds was expressed as the concentration that caused 50% inhibition of *syncytia* formation (EC_{50}) without direct toxicity for the cells. The cytotoxic concentration (CC_{50}) of drug compounds was monitored on growth of noninfected cells by Trypan blue exclusion assay and corresponded to the concentration required to cause 50% of cell death. It should be emphasized that when compounds required the addition of DMSO to be solubilized in water (compounds **10**, **13**, **15**, **20**, **22**, **27**, **29**, **34**), the concentration in volume of DMSO used was in many cases inferior to 10% with respect to water (the final concentration of DMSO in MT4 cells incubation medium being less than 2 μM). As far as DMSO could affect the antiviral activity of the tested drugs,[28] antiviral assays in which solutions containing equal concentration of DMSO in water were performed and used as standard assays for each tested drugs. EC_{50} and CC_{50} reported values were then calculated from these standard assays. In any case, final DMSO concentrations (1/1000) are very far from the percentages[28] which induced an enhancement of in vitro HIV-1 infection of T-cells.

CXCR-4 Immunofluorescence Cell Staining. Monoclonal Antibodies (mAb) and SDF-1α Used. Anti-CXCR-4 mAb 12G5 was from R&D Systems (England). Positive control mAb B1G6 directed against the β2-microglobulin, irrelevant mAb (1G11), and secondary antibody (PE-conjugated goat anti mouse IgG) were purchased from Immunotech-Coultronics (France). SDF-1α, the natural ligand for CXCR-4, was purchased from R&D Systems (England).

Cell Staining. MT4 cells were preincubated with various concentrations of drug products (prepared as already stated for antiviral assays) or in medium alone for 20 min at room temperature. Then anti-CXCR-4, positive control B1G6, and irrelevant mAbs were added to the cells, and incubation was performed at 4 °C for 30 min. After 2 washes, cells were then incubated with PE-conjugated secondary antibody for another 30 min at 4 °C. After being washed, cells were fixed in 0.1 mL of 1% paraformaldehyde PBS solution. Cell surface molecule expression was then analyzed by flow cytometry (ELITE-Coultronics).

Results are expressed as percentage of positive cells for the studied molecules. Fixation of the drug compound on the CXCR-4 receptor was followed by the extinction of the specific fluorescence signal in comparison to CXCR-4 expression on nontreated cells. The specificity of drug compound action on CXCR-4 was evaluated in comparison to the constant B1G6 mAb fixation to cells in the presence or not of drugs.

Hydrolysis of the Compounds 14 and 28 in Acid Solution, in Basic Solution, and in Human Serum. A solution (0.5 mL) of one of the esters (**14**: 13 mg/mL; **28**: 18 mg/mL in H_2O) was added to 2 mL of normal human serum (NHS), or a saturated aqueous $NaHCO_3$ solution (pH 8.9), or a 5% aqueous citric acid solution (pH 2.2), and the mixture was incubated at 37 °C in a water bath. The samples (100 μL) were withdrawn and added immediately to ice-cold water (400 μL) during the first 8 h every 60 min and then every 12 h until 48 h. The resulting samples were centrifuged, and the supernatants were filtered through nylon filters (0.45 μm) and then analyzed by HPLC using the following method: 0% → 100% of solvent B in 15 min.

As previous experiments demonstrated 267 nm to correspond to the λ_{max} of each compound, this wavelength was used for the detection. The peak retention time for the three compounds was shown to be: 8.8 min (AZT), 9.6 min (**14**), and 10.4 min (**28**).

Area variation during the time of experiment allowed us to estimate $t_{1/2}$ for compounds **14** and **28** as being, respectively, 7 and 8 h in normal human serum.

Chemical Synthesis. 1,4,8-Tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecane 4. To a solution of 1.00 g of cyclam **1** (5.00 mmol, 1 equiv) in ice-cold CH_2Cl_2 (125 mL) was added 2.00 g of di-*tert*-butyl dicarbonate (9.00 mmol, 1.8 equiv). The solution was stirred for 4 h 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 ("tri-Boc") as a white−yellow foam (0.60 g, 24% yield) and a mixture of products (0.77 g) identified by NMR as "di-Boc" compounds (relative to the integration of the *t*-Bu signal).

5-[4,8-Bis(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecanyl] Pentanoic Ethyl Ester 5. To the previously obtained mixture of products (0.77 g, 1.93 mmol) and an excess of K_2CO_3 (0.80 g, 5.77 mmol, 3 equiv) in CH_3CN (40 mL) was added 0.5 equiv of ethyl 5-bromo valerate (0.20 g, 0.96 mmol). The reaction mixture was refluxed overnight while stirring and then allowed to cool, and the solid carbonate was removed by filtration. The solvent was evaporated, and the residue was partitioned between EtOAc and brine. The organic layer was separated, washed twice with brine, dried (Na_2SO_4), and concentrated to give a brown−yellow oil. Purification by flash column chromatography (MeOH/CH_2Cl_2 5:95) afforded compound **5** as a light yellow oil (0.66 g, 65% yield) and its isomer **6** as minor product. R_f = 0.41 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, J = 7.10 Hz, −O−CH_2−CH_3), 1.46 (brs, 20H, *t*-Bu and −CH_2−$(CH_2)_2$−CO_2Et), 1.49−1.77 (m, 4H, HN−CH_2−CH_2−CH_2−N and −CH_2−CH_2−CO_2Et), 1.85 (m, 2H, BocN−CH_2−CH_2−CH_2−NBoc), 2.29 (t, 2H, J = 7.35 Hz, −CH_2−CO_2Et), 2.39 (t, 2H, J = 7.25 Hz, N−CH_2−$(CH_2)_3$−CO_2Et), 2.44−2.60 (m, 4H, N−$(CH_2)_2$), 2.78 (t, 2H, J = 5.25 Hz, HN−CH_2−CH_2−CH_2−N), 2.85 (t, 2H, J = 5.10 Hz, HN−CH_2−CH_2−NBoc), 3.10−3.30 (m, 4H, −CH_2−NBoc), 3.34 (t, 2H, J = 8.00 Hz, BocN−CH_2−CH_2−CH_2−NBoc), 3.42 (t, 2H, J = 4.90 Hz, N−CH_2−CH_2−NBoc), 4.12 (q, 2H, J = 7.10 Hz, −O−CH_2−CH_3). MS (FAB$^+$): 529 $(M + H)^+$.

5-[4,11-Bis(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecanyl] Pentanoic Ethyl Ester 6. Compound **6** was obtained as a slightly yellow oil during alkylation of the mixture of di-Boc compounds (0.15 g, 15% yield). R_f = 0.51 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, J = 7.10 Hz, −O−CH_2−CH_3), 1.44 (brs, 20H, *t*-Bu and −CH_2−$(CH_2)_2$−CO_2Et), 1.48−1.70 (m, 4H, HN−CH_2−CH_2−CH_2−NBoc and −CH_2−CH_2−CO_2Et), 1.80 (qt, 2H, BocN−CH_2−CH_2−CH_2−N), 2.29 (t, 2H, J = 7.35 Hz, −CH_2−CO_2Et), 2.35−2.60 (m, 6H, −$(CH_2)_2$−N−CH_2−$(CH_2)_3$−CO_2Et), 2.64 (t, 2H, J = 5.65 Hz, HN−CH_2−CH_2−CH_2−NBoc), 2.79 (t, 2H, J = 5.05 Hz, HN−CH_2−CH_2−NBoc), 3.19−3.47 (m, 8H, −CH_2−NBoc), 4.12 (q, 2H, J = 7.10 Hz, −O−CH_2−CH_3). MS (FAB$^+$): 529 $(M + H)^+$.

5-[4,8-Bis(*tert*-butoxycarbonyl)-11-pentanoic Ethyl Ester-1,4,8,11-tetraazacyclotetradecanyl] Pentanoic Ethyl Ester 7. Compound **7** is a byproduct (di-alkylated compound) obtained during alkylation of the mixture of di-Boc compounds and appears as bright yellow oil (0.09 g, 7% yield). R_f = 0.62 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, J = 7.10 Hz, −O−CH_2−CH_3), 1.46 (brs, 22H, *t*-Bu and −CH_2−$(CH_2)_2$−CO_2Et), 1.53−1.74 (m, 6H, N−CH_2−CH_2−CH_2−N and −CH_2−CH_2−CO_2Et), 1.86 (qt, 2H, BocN−CH_2−CH_2−CH_2−NBoc), 2.30 (t, 2H, J = 7.20 Hz, −CH_2−CO_2Et), 2.33−2.61 (m, 8H, −CH_2−N−CH_2−$(CH_2)_3$−CO_2Et), 2.57 (m, 4H, N−CH_2−CH_2−NBoc), 3.14−3.35 (m, 8H, −CH_2−NBoc), 4.11 (q, 2H, J = 7.10 Hz, −O−CH_2−CH_3). MS (FAB$^+$): 657 $(M + H)^+$.

5-[4,11-Bis(*tert*-butoxycarbonyl)-8-pentanoic Ethyl Ester-1,4,8,11-tetraazacyclotetradecanyl] Pentanoic Ethyl Ester 8. Compound **8** is a byproduct (di-alkylated compound) obtained during alkylation of the mixture of di-Boc compounds and appears as bright yellow oil (0.04 g, 3% yield). R_f = 0.69 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, J = 7.10 Hz, −O−CH_2−CH_3), 1.43 (brs, 22H, *t*-Bu and −CH_2−$(CH_2)_2$−CO_2Et), 1.65 (m, 4H, −CH_2−CH_2−CO_2Et), 1.79 (m, 4H, BocN−CH_2−CH_2−CH_2−N), 2.32 (t, 4H, J = 7.30 Hz, −CH_2−CO_2Et), 2.35−2.63 (m, 8H, −CH_2−N−CH_2−$(CH_2)_3$−CO_2Et), 2.60 (m, 4H, N−CH_2−CH_2−NBoc), 3.11−3.33 (m, 8H, −CH_2−NBoc), 4.10 (q, 2H, J = 7.10 Hz, −O−CH_2−CH_3). MS (FAB$^+$): 657 $(M + H)^+$.

General Procedure A for the Coupling of Tetraazamacrocycle Acids with Nucleosides. To a solution of tetraazamacrocycle acid (1 equiv) in CH_2Cl_2 and a catalytic amount of DMAP, were added a large excess of NEt_3 (3 equiv), then 1 equiv of nucleoside dissolved in a mixture of DMF/CH_2Cl_2, and 1.2 equiv of PyBOP reagent. The reaction mixture was allowed to stir for 8 h 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 and water and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave a crude product which was then purified by flash column chromatography or PLC, using MeOH/CH_2Cl_2 as eluent.

General Procedure B for the Deprotection of N-Boc Conjugates. Anhydrous 1 N HCl in Et_2O was added (10 mL/mmol) to the mono-tetraazamacrocycle or bis-tetraazamacrocycle conjugate, and the resulting solution was stirred until complete disappearance of the starting conjugate. The solution was then concentrated to give the hydrochloride conjugate.

3′-Azido-3′-deoxy-5′-*O*-{5-[1,4,8,11-tetrahydrochloride-1,4,8,11-tetraazacyclotetradecanyl]pentanoyl}thymidine 11. According to the general carbamate deprotection procedure B, the reaction of compound **10** (0.05 g, 0.06 mmol) afforded the title compound **11** as a slightly yellow solid, in quantitative yield (0.03 g, 96% yield). R_f = 0.10 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR (D_2O): 1.64−2.03 (m, 7H, −CH_2−CH_2−CH_2−CO_2Nu and CH_3Thy), 2.18 (m, 4H, N−CH_2−CH_2−CH_2−N), 2.27−2.65 (m, 4H, −CH_2−CO_2Nu and H-2′), 3.10−3.88 (m, 18H, −CH_2−N), 3.95−4.07 (m, 1H, H-4′), 4.10−4.48 (m, 3H, H-3′ and H-5′), 6.17 (m, 1H, H-1′), 7.42 (brs, 1H, H-6Thy). MS (FAB$^+$): 550, $(M + H)^+$.

General Procedure C for the Coupling of Compound 12 with Nucleosides. Compound **12** (0.17 g, 0.26 mmol, 1 equiv) was dissolved in CH_2Cl_2 (10 mL) giving a greenish solution, and DMAP (0.06 g, 0.52 mmol, 2 equiv) and 1 equiv of nucleoside were then added. The reaction mixture was stirred for 8 h at room temperature. The resulting solution was successively washed with 5% aqueous citric acid and water and dried over Na_2SO_4 and concentrated. Purification was performed by flash column chromatography or PLC using MeOH/CH_2Cl_2 as eluent.

3′-Azido-3′-deoxy-5′-*O*-{5-oxo-5-[4,8,11-trihydrochloride-1,4,8,11-tetraazacyclotetradecanyl]pentanoyl}thymidine 14. According to the general carbamate deprotection procedure B, reaction of compound **13** (0.04 g, 0.05 mmol) afforded the title compound **14** as a yellow solid in quantitative yield (0.02 g, 94% yield). R_f = 0.10 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR (D_2O): 1.72−1.98 (m, 2H, CO−CH_2−CH_2−CH_2−CO_2Nu), 2.00−2.36 (m, 7H, N−CH_2−CH_2−CH_2−N and CH_3Thy), 2.39−2.57 (m, 6H CO−CH_2−CH_2−CH_2−CO_2Nu and H-2′), 3.08−3.96 (m, 16H, −CH_2−N), 4.14 (m, 1H, H-4′), 4.43−4.62 (m, 3H, H-3′

and H-5′), 6.21 (m, 1H, H-1′), 7.47 (brs, 1H, H-6Thy). MS (FAB^+): 564 $(M + H)^+$.

1,1′-(1,5-Dioxo-pentane)-bis[4,8,11-tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecane] 15. Compound **15** was obtained as a byproduct during the synthesis of ester **16** and was purified after the saponification of the whole mixture (0.08 g, 17% yield). This compound was also obtained during the synthesis of acyl chloride **12**. R_f = 0.53 (MeOH/CH_2Cl_2 1:9). ^1H NMR ($CDCl_3$): 1.39 (s, 54H, *t*-Bu), 1.69 (m, 8H, N−CH_2−CH_2−CH_2−N), 1.89 (m, 2H, CO−CH_2−CH_2−CH_2−CO), 2.34 (m, 4H, CO−CH_2−CH_2−CH_2−CO), 3.21−3.50 (m, 32H, −CH_2−N−CO). MS (FAB^+): 1097 $(M + H)^+$.

5-[4,8-Bis(*tert*-butoxycarbonyl)-11-{5-oxo-5-[4,8,11-tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecanyl]pentanoyl}-1,4,8,11-tetraazacyclotetradecanyl] Pentanoic Acid 18. In a mixture of compound **4** (0.22 g, 0.44 mmol, 1 equiv) and **5** (0.23 g, 0.44 mmol, 1 equiv) in the biphasic CH_2Cl_2/$NaHCO_3$ (30 mL: 15 mL), was added glutaryl dichloride (0.07 g, 0.44 mmol, 1 equiv). The reaction mixture was stirred for 30 min until disappearance of the starting tetraazamacrocycles. The aqueous layer was partitioned and extracted three times with CH_2Cl_2, and the combined organic phases were dried over Na_2SO_4 and concentrated. The NMR spectrum of the obtained crude white foam confirmed the existence of at least two different products. This mixture was submitted to an aqueous 5 N NaOH treatment in THF in the presence of Triton B (catalytic amount) during 4 h. This last reaction allowed us to separate the three different compounds by flash chromatography: compound **15** was eluted with MeOH/CH_2Cl_2 5:95, compound **18** was eluted with MeOH/CH_2Cl_2 10:90, and compound **19** was eluted with MeOH/CH_2Cl_2 20:80. Acid **18** was finally obtained as a white foam (0.29 g, 61% yield). R_f = 0.36 (MeOH/CH_2Cl_2 5:95). RMN ^1H ($CDCl_3$) δ: 1.44 (brs, 47H, *t*-Bu and −CH_2−$(CH_2)_2$−CO_2H), 1.58−1.75 (m, 6H, −CH_2−CH_2−CO_2H and CO−CH_2−CH_2−CH_2−CO and BocN−CH_2−CH_2−CH_2−N−$(CH_2)_4$−CO_2H), 1.82−2.00 (m, 6H, BocN−CH_2−CH_2−CH_2−NBoc), 2.19 (t, J = 7.30 Hz, 2H, −CH_2−CO_2H), 2.25−2.68 (m, 10H, −$(CH_2)_2$−N−CH_2−$(CH_2)_3$−CO_2H and N−CO−CH_2−), 3.00−3.40 (m, 28H, −CH_2−N−CO and −CH_2−NBoc), 10.25 (brs, 1H, −OH). MS (FAB^+): 1097 $(M + H)^+$. MS (FAB^-): 1095 $(M - H)^-$.

1,1′-(1,5-Dioxo-pentane)-bis[4,8-bis(*tert*-butoxycarbonyl)-11-pentanoic Acid-1,4,8,11-tetraazacyclotetradecane] 19. Diester **19** was obtained as a byproduct during the synthesis of ester **16**. After saponification of the whole mixture, the diacid **19** was obtained as a yellow foam (0.10 g, 20% yield). R_f = 0.12 (MeOH/CH_2Cl_2 5:95). RMN ^1H ($CDCl_3$) δ: 1.41 (m, 40H, *t*-Bu and −CH_2−$(CH_2)_2$−CO_2H), 1.58−1.77 (m, 8H, −CH_2−CH_2−CO_2H and CO−N−CH_2−CH_2−CH_2−N), 1.79−2.05 (m, 6H, BocN−CH_2−CH_2−CH_2−NBoc and CO−CH_2−CH_2−CH_2−CO), 2.26 (m, 4H, −CH_2−CO_2H), 2.29−2.62 (m, 16H, N−CH_2− and CO−CH_2−CH_2−CH_2−CO), 3.07−3.60 (m, 24H, −CH_2−N−CO and −CH_2−NBoc). MS (FAB^+): 1097 $(M + H)^+$. MS (FAB^-): 1095 $(M - H)^-$.

3′-Azido-3′-deoxy-5′-*O*-{5-[4,8-bis(*tert*-butoxycarbonyl)-11-{5-oxo-5-[4,8,11-tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecanyl]pentanoyl}-1,4,8,11-tetraazacyclotetradecanyl]pentanoyl}thymidine 20. According to general procedure **A**, condensation of AZT (0.02 g, 0.08 mmol) with acid **18** (0.08 g, 0.08 mmol) afforded after purification the title product as a white foam (0.04 g, 42% yield). R_f = 0.46 (MeOH/CH_2Cl_2 1:9). ^1H NMR ($CDCl_3$): 1.40 (s, 27H, *t*-Bu and −CH_2−CH_2−CH_2−CO_2−Nu), 1.53−2.05 (m, 12H, N−CH_2−CH_2−CH_2−N and CO−CH_2−CH_2−CH_2−CO and −CH_2−CH_2−CO_2−Nu), 1.93 (d, J = 1.00 Hz, 3H, CH_3Thy), 2.30−2.57 (m, 14H, CO−CH_2−CH_2−CH_2−CO and −$(CH_2)_2$−N−CH_2−CH_2−CH_2−CH_2−CO_2−Nu and H-2′), 3.12−3.56 (m, 28H, −CH_2−N−CO and −CH_2−NBoc), 4.07 (m, 1H, H-4′), 4.19−4.44 (m, 3H, H-3′ and H-5′), 6.09 (t, J = 6.20 Hz, 1H, H-1′), 7.21 (d, J = 1.10 Hz, 1H, H-6Thy), 9.15 (brs, 1H, NH). MS (FAB^+): 1346 $(M + H)^+$.

3′-Azido-3′-deoxy-5′-*O*-(5-{1,4,8-trihydrochloride-11-[5-oxo-5-(4,8,11-trihydrochloride-1,4,8,11-tetraazacyclotetradecanyl)pentanoyl]-1,4,8,11-tetraazacyclotetradecanyl}-pentanoyl)thymidine 21. According to the general carbamate deprotection procedure B, reaction of compound **20** (0.04 g, 0.03 mmol) afforded the title compound **21** as a white solid in quantitative yield (0.03 g, 95% yield). R_f = 0.10 (MeOH/CH_2Cl_2 1:9). ^1H NMR (D_2O): 1.57−1.91 (m, 9H, −CH_2−CH_2−CH_2−CO_2Nu and CH_3Thy and CO−CH_2−CH_2−CH_2−CO), 1.91−2.23 (m, 8H, N−CH_2−CH_2−CH_2−N), 2.27−2.59 (m, 8H, CO−CH_2−CH_2−CH_2−CO and −CH_2−CO_2Nu and H-2′), 3.07−3.79 (m, 34H, −CH_2−N), 4.03 (m, 1H, H-4′), 4.21−4.36 (m, 3H, H-3′ and H-5′), 6.09 (m, 1H, H-1′), 7.37 (brs, 1H, H-6Thy). MS (FAB^+): 846 $(M + H)^+$.

1,1′-Xylyl-bis[4,8,11-tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecane] 22. Compound **22** was obtained as a byproduct during the synthesis of ester **23** and was purified after the saponification of the whole mixture to give a white solid (0.17 g, 20% yield). R_f = 0.84 (MeOH/CH_2Cl_2 1:9). ^1H NMR ($CDCl_3$): 1.46 (brs, 54H, *t*-Bu), 1.67 (m, 4H, BocN−CH_2−CH_2−CH_2−N−Ph), 1.91 (m, 4H, BocN−CH_2−CH_2−CH_2−NBoc), 2.37 (m, 4H, Ph−N−CH_2−CH_2−CH_2−NBoc), 2.61 (m, 4H, Ph−N−CH_2−CH_2−NBoc), 3.13−357 (m, 24H, BocN−CH_2−), 3.51 (brs, 4H, Ph−CH_2−), 7.17 (s, 4H, −Ph−). MS (FAB^+): 1103 $(M + H)^+$.

5-[4,8-Bis(*tert*-butoxycarbonyl)-11-(4-{[4,8,11-tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecanyl]-methyl}benzyl)-1,4,8,11-tetraazacyclotetradecanyl] Pentanoic Acid 25. In a mixture of compounds **4** (0.40 g, 0.80 mmol, 1 equiv) and **5** (0.43 g, 0.80 mmol, 1 equiv) in CH_3CN were added K_2CO_3 (0.33 g, 2.41 mmol, 3 equiv) and α,α′-dibromo-*p*-xylene (0.21 g, 0.80 mmol). The reaction mixture was refluxed for 8 h under a nitrogen atmosphere and then allowed to cool to room temperature; the solid carbonate was removed by filtration. The solvent was evaporated, and the oily residue was dissolved in EtOAc, then successively washed with aqueous 5% citric acid and with water, dried over Na_2SO_4, and concentrated. NMR spectrum of the obtained crude white foam confirmed the presence of at least two different products. Thus the mixture was submitted to an aqueous 5 N NaOH treatment in THF in the presence of Triton B (catalytic amount) during 4 h. This last reaction allowed us to separate the three different compounds by flash chromatography: compound **22** was eluted with MeOH/CH_2Cl_2 5:95, compound **25** was eluted with MeOH/CH_2Cl_2 10:90, and compound **26** was eluted with MeOH/CH_2Cl_2 20:80. Acid **25** was finally obtained as a white foam (0.50 g, 56% yield). R_f = 0.38 (CH_2Cl_2/MeOH 95:5). RMN ^1H ($CDCl_3$) δ: 1.43 (brs, 47H, *t*-Bu and −CH_2−$(CH_2)_2$−CO_2H), 1.51−1.74 (m, 6H, −CH_2−CH_2−CO_2H and Ph−N−CH_2−CH_2−CH_2−N), 1.76−1.96 (m, 4H, BocN−CH_2−CH_2−CH_2−NBoc), 2.25 (t, 2H, J = 7.25 Hz, −CH_2−CO_2H), 2.27−2.67 (m, 14H, −CH_2−N), 3.12−3.42 (m, 20H, −CH_2−NBoc), 3.48 (d, J = 8.30 Hz, 4H, −CH_2−Ph), 7.16 (m, 4H, −Ph−). MS (FAB^+): 1103 $(M + H)^+$. (FAB^-): 1101 $(M - H)^-$.

1,1′-Xylyl-bis[4,8-bis(*tert*-butoxycarbonyl)-11-pentanoic Acid-1,4,8,11-tetraazacyclotetradecane] 26. Diester **24** was obtained as a byproduct during the synthesis of ester **23**. After saponification of the whole mixture, diacid **26** was obtained as a yellow−white foam (0.13 g, 15% yield). R_f = 0.13 (MeOH/CH_2Cl_2 5:95). RMN ^1H ($CDCl_3$) δ: 1.39 (brs, 40H, *t*-Bu and −CH_2−$(CH_2)_2$−CO_2H), 1.43−1.73 (m, 8H, −CH_2−CH_2−CO_2H and Ph-N−CH_2−CH_2−CH_2−N), 1.74−2.13 (m, 4H, BocN−CH_2−CH_2−CH_2−NBoc), 2.23 (m, 8H, −CH_2−CO_2H), 2.26−2.53 (m, 12H, −CH_2−N), 3.08−3.39 (m, 16H, −CH_2−NBoc), 3.46 (brs, 4H, −CH_2−Ph), 7.14 (m, 4H, −Ph−). MS (FAB^+): 1103 $(M + H)^+$. MS (FAB^-): 1101 $(M - H)^-$.

3′-Azido-3′-deoxy-5′-*O*-{5-[4,8-bis(*tert*-butoxycarbonyl)-11-(4-{[4,8,11-tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecanyl]methyl}benzyl)-1,4,8,11-tetraazacyclotetradecanyl]pentanoyl}thymidine 27. According to general procedure A, condensation of AZT (0.04 g, 0.15 mmol) with acid **25** (0.17 g, 0.15 mmol) afforded, after purification, the title product as a white solid (0.07 g, 35% yield). R_f = 0.42 (MeOH/CH_2Cl_2 1:9). ^1H NMR ($CDCl_3$): 1.42 (s, 47H, *t*-Bu and −CH_2−CH_2−CH_2−CO_2Nu), 1.44−1.72 (m, 6H, −CH_2−CH_2−CO_2Nu and Ph−CH_2−N−CH_2−CH_2−CH_2−N), 1.74−2.02 (m,

7H, CH_3Thy and BocN−CH_2−CH_2−CH_2−NBoc), 2.26−2.70 (m, 10H, −(CH_2)$_2$−N−CH_2−CH_2−CH_2−CH_2−CO_2Nu and H-2′), 3.17−3.46 (m, 20H, −CH_2−NBoc), 3.49−3.70 (d, J = 7.60 Hz, 4H, −CH_2−Ph−CH_2−), 4.03 (m, 1H, H-4′), 4.15−4.34 (m, 3H, H-3′ and H-5′), 6.04 (t, J = 6.30 Hz, 1H, H-1′), 7.15 (m, 5H, −Ph− and H-6Thy), 8.90 (brs, 1H, NH). MS (FAB$^+$): 1352 (M + H)$^+$.

3′-Azido-3′-deoxy-5′-*O*-[5-(1,4,8,11-tetrahydrochloride-11-{4-[(1,4,8,11-tetrahydrochloride-1,4,8,11-tetraazacyclotetradecanyl)methyl]benzyl}-1,4,8,11-tetraazacyclotetradecanyl)pentanoyl]thymidine 28. According to the general carbamate deprotection procedure B, reaction of compound **27** (0.06 g, 0.04 mmol) afforded the title compound **28** as a white solid, in quantitative yield (0.04 g, 97% yield). R_f = 0.10 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR (D_2O): 1.59 (m, 2H, −CH_2−CH_2−CO_2Nu), 1.70 (m, 2H, −CH_2−CH_2−CH_2−CO_2Nu), 1.78 (brs, 1H, CH_3Thy), 2.11 (m, 8H, −N−CH_2−CH_2−CH_2−N−), 2.29−2.60 (m, 4H, −CH_2−CO_2Nu and H-2′), 3.08−3.97 (m, 34H, −CH_2−N), 4.08 (m, 1H, H-4′), 4.21−4.58 (m, 7H, −CH_2−Ph−CH_2− and H-3′ and H-5′), 6.08 (m, 1H, H-1′), 7.42 (brs, 1H, H-6Thy), 7.56 (brs, 4H, −Ph−). MS (FAB$^+$): 852 (M + H)$^+$.

1,1′-Terephthaloyl-bis[4,8,11-tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecane] 29. Compound **29** was obtained as a byproduct during the synthesis of ester **30** and was purified after the saponification of the whole mixture to give a brown−white solid (0.21 g, 27% yield). R_f = 0.56 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR ($CDCl_3$): 1.42 (brs, 54H, *t*-Bu), 1.74 (m, 8H, N−CH_2−CH_2−CH_2−N−), 3.08−3.75 (m, 32H, CO−N−CH_2−), 7.40 (brs, 4H, CO−Ph−CO). MS (FAB$^+$): 1131 (M + H)$^+$.

5-[4,8-Bis(*tert*-butoxycarbonyl)-11-(4-{[4,8,11-tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecanyl]-carbonyl}benzoyl)-1,4,8,11-tetraazacyclotetradecanyl] Pentanoic Acid 32. In a mixture of compound **4** (0.35 g, 0.70 mmol, 1 equiv) and **5** (0.37 g, 0.70 mmol, 1 equiv) in the biphasic CH_2Cl_2/$NaHCO_3$ was added terephthaloyl dichloride (0.14 g, 0.70 mmol, 1 equiv). The reaction mixture was stirred for 30 min until disappearance of the starting tetraazamacrocycles. The aqueous layer was partitioned and extracted three times with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and concentrated. NMR spectrum of the obtained crude white foam confirmed the presence of at least two different products. This mixture was submitted to an aqueous 5 N NaOH treatment in THF in the presence of Triton B (catalytic amount) during 4 h. This last reaction allowed to separate the three different compounds by flash chromatography: compound **29** was eluted with MeOH/CH_2Cl_2 5:95, compound **32** was eluted with MeOH/CH_2Cl_2 10:90, and compound **33** was eluted with MeOH/CH_2Cl_2 20:80. Acid **32** was finally obtained as a white foam (0.38 g, 48% yield). R_f = 0.29 (CH_2Cl_2/MeOH 95:5). RMN ^{1}H ($CDCl_3$) δ: 1.45 (brs, 47H, *t*-Bu and −CH_2−(CH_2)$_2$−CO_2H), 1.49−1.78 (m, 6H, −CH_2−CH_2−CO_2H and Ph−CO−N−CH_2−CH_2−CH_2−N), 1.79−2.05 (m, 4H, BocN−CH_2−CH_2−CH_2−NBoc), 2.23 (m, 4H, −CH_2−CO_2H and BocN−CH_2−CH_2−N−(CH_2)$_4$−CO_2H), 2.50 (m, 4H, −CH_2−N−CH_2−(CH_2)$_3$−CO_2H), 3.10−3.58 (m, 24H, −CH_2−NBoc and BocN−CH_2−CH_2−CH_2−N−CO−Ph), 3.57−3.83 (m, 4H, BocN−CH_2−CH_2−N−CO−Ph), 7.44 (m, 4H, CO−Ph−CO). MS (FAB$^+$): 1131 (M + H)$^+$. MS (FAB$^-$): 1129 (M − H)$^-$.

1,1′-Terephthaloyl-bis[4,8-bis(*tert*-butoxycarbonyl)-11-pentanoic acid-1,4,8,11-tetraazacyclotetradecane] 33. Diester **31** was obtained as a byproduct during the synthesis of ester **30**. After saponification of the whole mixture, diacid **33** was obtained as a yellow foam (0.13 g, 16% yield). R_f = 0.13 (MeOH/CH_2Cl_2 5:95). RMN ^{1}H ($CDCl_3$) δ: 1.41 (brs, 40H, *t*-Bu and −CH_2−(CH_2)$_2$−CO_2H), 1.43−1.75 (m, 8H, −CH_2−CH_2−CO_2H and Ph−CO−N−CH_2−CH_2−CH_2−N), 1.76−2.10 (m, 4H, BocN−CH_2−CH_2−CH_2−NBoc), 2.25 (m, 8H, −CH_2−CO_2H and N−CH_2−), 2.47 (m, 8H, N−CH_2−), 3.02−3.50 (m, 20H, −CH_2−NBoc and Ph−CO−N−CH_2−CH_2−NBoc), 3.53−3.75 (m, 4H, Ph−CO−N−CH_2−CH_2−CH_2−NBoc), 7.41 (m, 4H, CO−Ph−CO). MS (FAB$^+$): 1131 (M + H)$^+$. MS (FAB$^-$): 1129 (M − H)$^-$.

3′-Azido-3′-deoxy-5′-*O*-{5-[4,8-bis(*tert*-butoxycarbonyl)-11-(4-{[4,8,11-tris(*tert*-butoxycarbonyl)-1,4,8,11-tetraazacyclotetradecanyl]carbonyl}benzoyl)-1,4,8,11-tetraazacyclotetradecanyl]pentanoyl}thymidine 34. According to general procedure A, condensation of AZT (0.03 g, 0.11 mmol) with acid **33** (0.13 g, 0.11 mmol) afforded, after purification, the title product as a white foam (0.10 g, 66% yield). R_f = 0.44 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR ($CDCl_3$): 1.42 (s, 47H, *t*-Bu and −CH_2−CH_2−CH_2−CO_2Nu), 1.45−1.70 (m, 6H, −CH_2−CH_2−CO_2−Nu and Ph−CO−N−CH_2−CH_2−CH_2−N), 1.72−2.00 (m, 7H, CH_3Thy and BocN−CH_2−CH_2−CH_2−NBoc), 2.12−2.64 (m, 10H, −(CH_2)$_2$−N−CH_2−CH_2−CH_2−CH_2−CO_2Nu and H-2′), 3.03−3.50 (m, 24H, BocN−CH_2−CH_2−N−CO−Ph and −CH_2−NBoc), 3.52−3.70 (m, 4H, BocN−CH_2−CH_2−CH_2−N−CO−Ph), 3.99 (m, 1H, H-4′), 4.12−4.44 (m, 3H, H-3′ and H-5′), 6.01 (t, J = 6.20 Hz, 1H, H-1′), 7.39 (brs, 1H, H-6Thy), 7.39 (brs, 1H, CO−Ph−CO), 8.86 (brs, 1H, NH). MS (FAB$^+$): 1380 (M + H)$^+$.

3′-Azido-3′-deoxy-5′-*O*-[5-(1,4,8-trihydrochloride-11-{4-[(4,8,11-trihydrochloride-1,4,8,11-tetraazacyclotetradecanyl)carbonyl]benzoyl}-1,4,8,11-tetraazacyclotetradecanyl)pentanoyl]thymidine 35. According to the general carbamate deprotection procedure B, reaction of compound **34** (0.06 g, 0.05 mmol) afforded the title compound **35** as a white solid in quantitative yield (0.04 g, 97% yield). R_f = 0.08 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR (D_2O): 1.60 (m, 2H, −CH_2−CH_2−CO_2-Nu), 1.65−1.90 (m, 5H, CH_3Thy and −CH_2−CH_2−CH_2−CO_2Nu), 2.01 (m, 4H, N−CH_2−CH_2−CH_2−N), 2.15 (m, 4H, Ph−CO−N−CH_2−CH_2−CH_2−N), 2.36−2.68 (m, 4H, −CH_2−CO_2Nu and H-2′), 3.05−3.99 (m, 34H, −CH_2−N), 4.09 (m, 1H, H-4′), 4.24−4.44 (m, 3H, H-3′ and H-5′), 6.11 (m, 1H, H-1′), 7.42 (brs, 1H, H-6Thy), 7.54 (brs, 4H, CO−Ph−CO). MS (FAB$^+$): 880 (M + H)$^+$.

1,1′-(1,5-Dioxo-pentane)-bis[4,8,11-trihydrochloride-1,4,8,11-tetraazacyclotetradecane] 36. According to the general carbamate deprotection procedure B, reaction of compound **15** (0.06 g, 0.05 mmol) afforded the title compound **36** as a yellow solid in quantitative yield (0.02 g, 97% yield). R_f = 0.05 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR (D_2O): 1.82 (m, 2H, CO−CH_2−CH_2−CH_2−CO), 1.89−2.43 (m, 8H, N−CH_2−CH_2−CH_2−N), 2.53 (t, J = 7.20 Hz, 4H, CO−CH_2−CH_2−CH_2−CO), 3.26−4.05 (m, 32H, −CH_2−N). MS (FAB$^+$): 497 (M + H)$^+$.

1,1′-Xylyl-bis[4,8,11-trihydrochloride-1,4,8,11-tetraazacyclotetradecane] 37. According to the general carbamate deprotection procedure B, reaction of compound **22** (0.08 g, 0.07 mmol) afforded the title compound **37** as a white solid in quantitative yield (0.03 g, 95% yield). R_f = 0.05 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR (D_2O): 2.12 (brs, 8H, N−CH_2−CH_2−CH_2−N), 2.97−3.68 (m, 32H, N−CH_2−), 4.39 (s, 4H, Ph−CH_2−), 7.29 (s, 4H, −Ph−). MS (FAB$^+$): 503 (M + H)$^+$.

1,1′-Terephthaloyl-bis[4,8,11-trihydrochloride-1,4,8,11-tetraazacyclotetradecane] 38. According to the general carbamate deprotection procedure B, reaction of compound **29** (0.07 g, 0.06 mmol) afforded the title compound **38** as a slightly yellow solid in quantitative yield (0.03 g, 92% yield). R_f = 0.05 (MeOH/CH_2Cl_2 1:9). ^{1}H NMR ($CDCl_3$): 2.05 (m, 4H, N−CH_2−CH_2−CH_2−N), 2.19 (m, 4H, CO−N−CH_2−CH_2−CH_2−N−CO), 2.82−4.00 (m, 32H, N−CH_2−), 7.56 (brs, 4H, CO−Ph−CO). MS (FAB$^+$): 531 (M + H)$^+$.

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