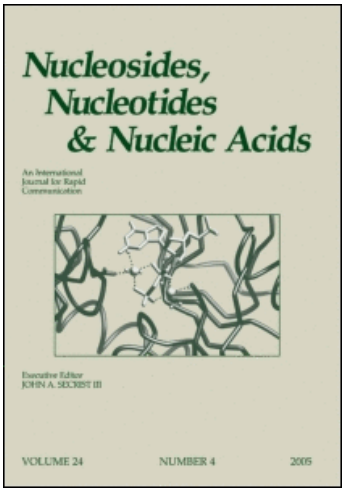

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of a New Class of HIV-1 Inhibitors

Audrey Farese-Di Giorgio[a]; Sandrine Pairot[a]; Nadia Patino[a]; Roger Condom[a]; Christophe Di Giorgio[a]; André Aumelas[b]; Anne-Marie Aubertin[c]; Roger Guedj[a]
[a] Laboratoire de Chimie Bio-Organique, CNRS ESA 6001 Université de Nice Sophia-Antipolis, Nice Cedex 2, France [b] Centre de Biochimie Structurale, CNRS UMR C9955, Faculté de Pharmacie, Montpellier Cedex, France [c] INSERM U74, Institut de Virologie, Strasbourg, France

To cite this Article Giorgio, Audrey Farese-Di , Pairot, Sandrine , Patino, Nadia , Condom, Roger , Giorgio, Christophe Di , Aumelas, André , Aubertin, Anne-Marie and Guedj, Roger(1999) 'Synthesis of a New Class of HIV-1 Inhibitors', Nucleosides, Nucleotides and Nucleic Acids, 18: 2, 263 — 275
To link to this Article: DOI: 10.1080/15257779908043073
URL: http://dx.doi.org/10.1080/15257779908043073

NUCLEOSIDES & NUCLEOTIDES, 18(2), 263-275 (1999)

SYNTHESIS OF A NEW CLASS OF HIV-1 INHIBITORS

Audrey Farese-Di Giorgio[+], Sandrine Pairot[+], Nadia Patino[+], Roger Condom[+]*, Christophe Di Giorgio[+], André Aumelas[$], Anne-Marie Aubertin[⊕], Roger Guedj[+]

[+] Laboratoire de Chimie Bio-Organique, CNRS ESA 6001
Université de Nice Sophia-Antipolis, F-06108 Nice Cedex 2, France
[$] Centre de Biochimie Structurale, CNRS UMR C9955, Faculté de Pharmacie,
15 av. Ch. Flahaut, F-34060, Montpellier Cedex, France
[⊕] INSERM U74, Institut de Virologie, 3 rue Koeberlé
67000 Strasbourg, France

ABSTRACT: A new family of molecules potentially inhibitors of the HIV-1 Tat-TAR complex was prepared. These compounds are constituted by dinucleotide analogs (PNA dimer) bound, through a linker, to an arginine residue. In this series, several molecules inhibit viral development in cell culture with a micromolar IC50 and without cellular toxicity until 200μM concentration.

INTRODUCTION

Transcription activation of HIV-1 gene expression by the Tat protein involves complex formation with an RNA target sequence termed TAR (Trans-Activation-Responsive-Element) that is located downstream of the transcription start site in the viral Long-Terminal-Repeat (LTR). TAR element is a nascent RNA transcript that has a stable stem loop structure formed by base-pair interactions between nucleotides +1 to +59. It contains a six-nucleotide loop (residues 30-35) and a three-nucleotide bulge (residues 23-25) that are both necessary for Tat function[(1)]. In the absence of Tat, only short RNA's ranging in size from 60 to 80 nucleotides are produced. The 86-amino acid Tat protein is produced from a doubly spliced mRNA formed by joining an exon preceding the *env* gene with a second exon within *env*. Mutational analyses suggest the presence of several functional domains within the Tat protein[(2)]. An acidic group of amino acids, present at

* Corresponding author.

the extreme amino terminus, consists of two glutamic acid residues, one aspartic acid and three sequences Pro-X-X-X-Pro. Amino acids 22 to 32 correspond to the highly flexible cysteine-rich region. The sixteen amino acid sequence of the core region (residues 32 to 47) is strictly conserved among the known Tat proteins. The basic domain (amino acids 49-57), a short region having six arginine and two lysine within nine residues, is required for both nuclear and nucleolar localization and for the ability to interact with RNA. The glutamine-rich region (residues 60-72) corresponds to a rigid substructure that is probably involved in TAR binding. The amino acids encoded by the second exon of Tat (residues 72 to 86) are dispensable for *in vitro* binding and *in vivo* transactivation. Tat is able to bind to TAR RNA to form a one to one complex. The basic region is directly involved in RNA binding (through, among others, an arginine residue), and Tat protein thus belongs to the family of arginine-rich motif (ARM) RNA binding proteins[2].

Several multidimensional heteronuclear NMR studies have been carried out to determine the structures of free TAR RNA and of Tat-TAR complex of HIV-1[3,4]. The first NMR study of TAR RNA has been reported by Puglisi et al.[3] who analysed a complex formed between the amino acid derivative argininamide and the apical portion of TAR RNA. Recently, Aboul-Ela et al.[4a,b] have studied a more realistic model because they characterized the interaction between TAR RNA and ADP-1, a Tat-derived peptide that binds TAR RNA with a high level of specificity and that includes both the basic and core regions of Tat. Concerning the structure of free TAR RNA, these two studies have shown that the three bulge residues (U23C24U25) are stacked. This bulge induces a bend in the RNA helix that distorts the local structure and widens the major groove of TAR RNA to expose hydrogen-bonding contacts that are important for binding of Tat. In the absence of Tat, U23 is stacked on A22. Upon complexation with argininamide[3] or with ADP-1[4], only the region around the bulge appears to be involved; there is an important requirement for uridine at the 5' most position of the bulge (+23). The stacked arrangement of the three bulge nucleotides (U23C24U25) is disrupted. The identity of several base-pairs surrounding the bulge is also important; in particular the two base-pairs immediately above the bulge G26-C39 and A27-U38 are essential. U23 moves in direct proximity of A27 and G26. The fixation site of Tat is found to be spatially close both to the bulge and to the six-nucleotide loop region.

We describe herein the preparation and the biological evaluation of molecules that could inhibit the formation of the Tat-TAR-cellular proteins complex. We targeted a TAR RNA in which the bulge sequence is constituted by the residues U23-C24-U25 and the

loop sequence (+30 to +35) by the residues C-U-G-G-G-A. This bulge sequence is present in most HIV-1 isolates (ie isolate IIIB) but the sequences U23-U24-U25 (ie isolate LAI) and U23-C24-A25 can also be seen[5]. In the other hand, five out of the six loop residues (+30 to +34) are highly conserved in most isolates of HIV-1 and HIV-2.

The concepts of the inhibitors are based on three elements :

- Tat protein interacts with TAR through, among others, an arginine residue.
- A free amino acid arginine binds specifically to the same site in TAR as does the Tat protein with a $K_i = 4.10^{-3}M$[6].
- This specific contact distorts the RNA structure; the two bulge residues C24 and U25 as well as the two loop nucleotides C30 and U31 are unstacked, they are free from interactions and thus they are accessible.

Compounds **4** and **5** described herein, are constituted by dinucleotide analogs (PNA dimer[7]) complementary to the residues C and U. These dimers are bound through linkers, to an arginine which should «drive» the whole molecule towards the Tat fixation site. According to the length of the linker, the dimer moiety should interact with the C and U residues either of the bulge or of the loop. In this context, **4** and **5** should have an affinity and a good specificity for TAR. Thus, such molecules could inhibit the formation of the Tat-TAR-cellular proteins complex, by binding to TAR at the fixation site of Tat and by inducing a steric hinderance.

RESULTS AND DISCUSSION

We have synthesized eight molecules Z-diPNA-linker-ArgOR (**4** and **5**; FIG 2) that were biologically evaluated. Four of these molecules are methyl esters **4** (R=Me) whereas the four others are carboxylic acids **5** (R=H). Two diPNA sequences A-G and G-A were incorporated to be complementary with the C and U residues. A benzyloxycarbonyl group (Z) has been introduced at the terminal amine function of the diPNA both to further the formation of π-interactions with the nucleic bases of TAR RNA and to increase the lipophilicity of the molecules in order to facilitate their cellular penetration. Two linkers (n=2, 5) were used to allow the diPNA to interact with the U and C residues either of the bulge or of the loop.

Molecules **4** were synthesized by first condensing diPNA **1** with arginine derivative **2** to afford **3** (FIG 2), then by deprotecting **3**, on the nucleic bases of the diPNA and on the guanidinium moiety of arginine. Alkaline hydrolysis of **4** led to compounds **5.**

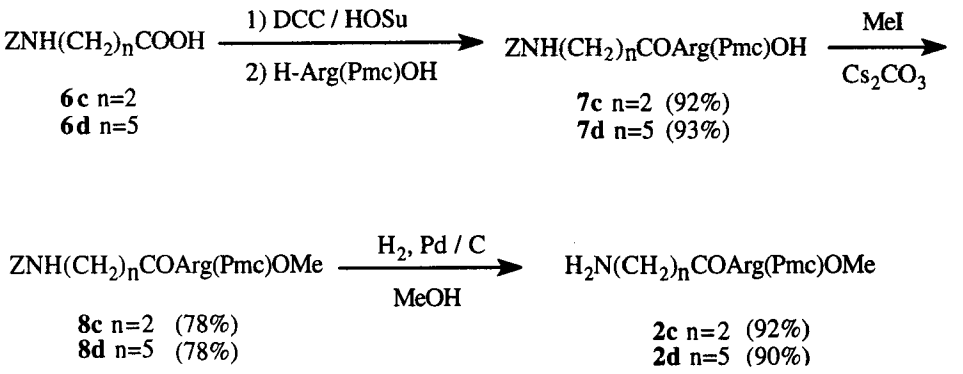

FIG. 1 : Synthesis of H-linker-Arg(Pmc)OMe compounds

The anti-HIV-1 and anti-HIV-2 activities of compounds **4** and **5** have been evaluated (TABLE 1). Several compounds appear to be active and non toxic, but some assays are in progress to confirm the target.

SYNTHESIS

The synthesis of compounds **1a** and **1b** (Z-diPNA-OH) was previously described[8]. The synthesis of compounds **2** (H-linker-Arg(Pmc)OMe; FIG 1) was carried out in three steps from commercially available N-benzyloxycarbonyl amino acids **6**. Condensation of **6** with N^G-Pmc-arginine was performed by first generating the activated ester of **6** by means of DCC/HOSu, then by adding H-Arg(Pmc)OH *in situ* to yield **7** (90%). Esterification of **7** with methyl iodide (MeI) and cesium carbonate (Cs_2CO_3) led to methyl ester **8** in approximately 80% yield. At last, the protecting benzyloxycarbonyl group was removed by hydrogenolysis in methanol with catalytic amounts of Pd/C (10%) (90% yield).

The synthesis of molecules **4** and **5** (Z-diPNA-linker-ArgOR) was carried out in two and three steps (FIG 2) from synthons **1** and **2**. Coupling of **1** with **2** via a DCC / HOSu activation led to polyprotected compound **3** in approximately 60% yield. Treatment of **3** with trifluoroacetic acid (TFA) in dichloromethane caused the cleavage of the protecting groups both of the nucleobases (Bn: benzyloxy; diBoc: bis-tertiobutyloxycarbonyl) and of arginine (Pmc: 2,2,5,7,8-penta-methylchroman-6-sulfonyl). Methyl ester **4** was then obtained in quasi-quantitative yield. **4** was saponified

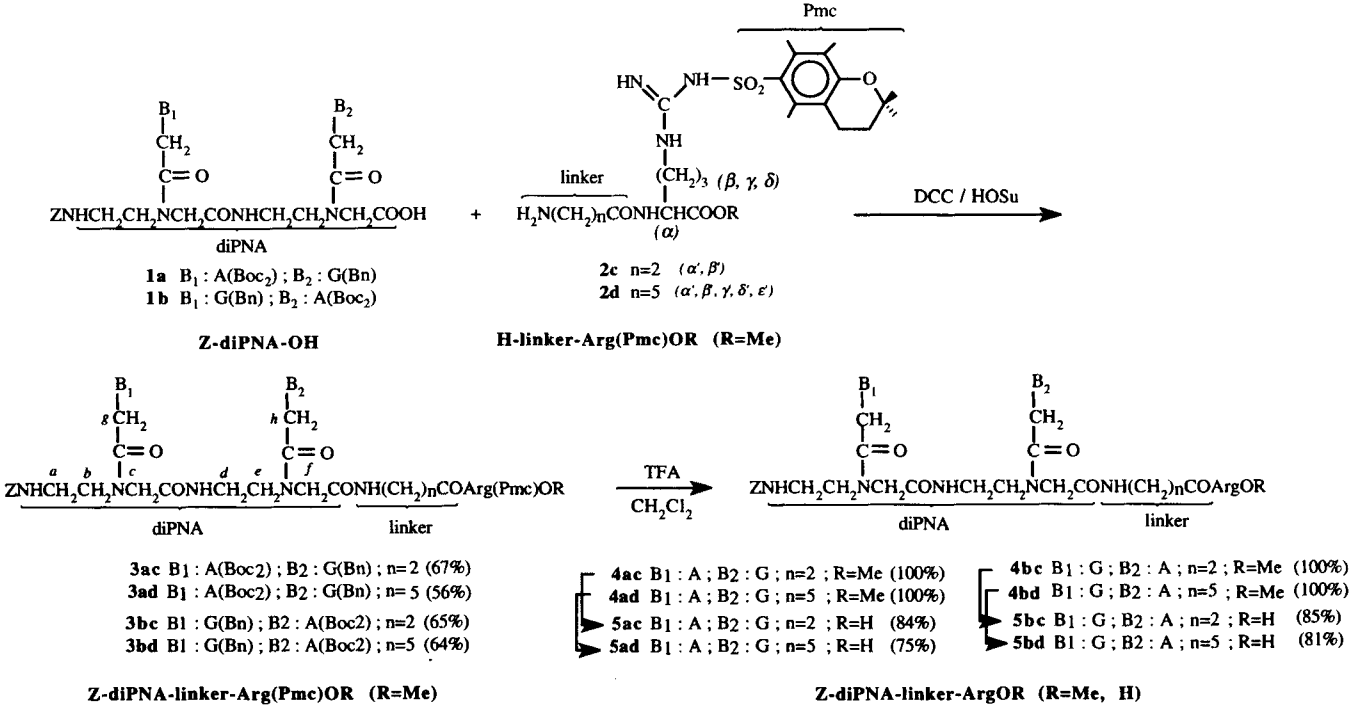

FIG. 2 : Synthesis of Z-diPNA-linker-ArgOR compounds (*NMR assigments are in italic*)

TABLE 1 : Anti-HIV-1 and 2 activities (IC_{50}) and cellular toxicities (CC_{50}) of compounds studied

N°	HIV-1								HIV-2
	PBMC/IIIB		CEM-SS/LAI		MT4/IIIB		MT4/LAI		PBMC/D194
	IC_{50}(μM)	CC_{50}(μM)	IC_{50}(μM)	CC_{50}(μM)	IC_{50}(μM)	CC_{50}(μM)	IC_{50}(μM)	CC_{50}(μM)	IC_{50}(μM)
4ac : Z-AG-(2)-ArgOMe	140	>200	>200	>200	>200	>200	-	-	>200
5ac : Z-AG-(2)-ArgOH	11	>200	16	>200	>200	>200	>200	>200	>200
4ad : Z-AG-(5)-ArgOMe	1	>200	2.5	>200	5.6	>200	6.5	>200	11
5ad : Z-AG-(5)-ArgOH	>200	>200	>200	>200	54	>200	>200	>200	>200
4bc : Z-GA-(2)-ArgOMe	>200	>200	>200	>200	>200	>200	>200	>200	>200
5bc : Z-GA-(2)-ArgOH	3.5	>200	10	>200	100	>200	>200	>200	>200
4bd : Z-GA-(5)-ArgOMe	20	45	20	90	>200	108	>200	>200	>200
5bd : Z-GA-(5)-ArgOH	>200	>200	>200	>200	>200	>200	-	-	>200

with 1N LiOH to lead to corresponding acid **5** (80% yield). The eight final compounds **4** and **5** were purified by semi-preparative HPLC to be tested.

BIOLOGICAL EVALUATION

The anti-HIV-1 activity of water soluble compounds **4** and **5** was biologically evaluated on PBMC/IIIB[9], CEM-SS/LAI[9], MT4/IIIB[10, 11], and MT4/LAI[10, 11] infected cells (TABLE 1). The anti-HIV-2 activity was also determined on PBMC/D194[9] infected cells (TABLE 1) as HIV-2 TAR RNA is similar to HIV-1 one.

TABLE 1 shows that only one compound (**4bd**) induces a cellular toxicity at the tested concentrations. Two molecules (**5ac** and **5bc**) are active against HIV-1 at micromolar concentration and one (**4ad**) is active both against HIV-1 and HIV-2, at the same concentration. The acid compounds containing a AG or GA dimer and a linker n=2 (**5ac** and **5bc**) show an anti-HIV-1 activity on PBMC/IIIB and CEM-SS/LAI infected cells but they are not active on MT4 cells. The corresponding methyl esters (**4ac** and **4bc**) do not display any activity whatever the cells. The methyl ester compound with a AG diPNA sequence and a linker n=5 (**4ad**) is the most active drug as it displays an activity in all cases. Contrary to the previous case, the corresponding carboxylic acid (**5ad**) does not produced any inhibitory effect, whereas the GA homolog (**4bd**) is cytotoxic.

These preliminary results seem to indicate the presence of two classes of compounds that have two different modes of action. Indeed, the molecules with n=2 (**5ac** and **5bc**) do not display any activity on MT4 HIV-1 infected cells while compound **4ad** with n=5 is active both on MT4 HIV-1 infected cells and on HIV-2 infected cells.

Structurally, this approach leads to new molecules that show an anti-HIV activity. The length of the linker as well as the nature of the diPNA seem to play an important role for the activity.

Some more experiments are in progress to establish a structure/activity relationship and to confirm that TAR RNA is the target.

EXPERIMENTAL SECTION

Unless otherwise stated, all reagents were obtained from commercial suppliers and were used without further purification. All solvents were freshly distilled. The following abbreviations are employed : 2,2,5,7,8-penta-methylchroman-6-sulfonyl (Pmc), N,N'-dicyclohexylcarbodiimide (DCC), N,N'-dicyclohexylurea (DCU), N-hydroxy succinimide (HOSu), trifluoroacetic acid (TFA), benzyloxy (Bn), bis-tertiobutyloxycarbonyl (diBoc). Melting points were determined using an electrothermal digital melting point apparatus IA900 Series and were uncorrected. Proton nuclear magnetic resonance spectra were recorded on a Brucker WP200 (200MHz) Fourier Transform spectrometer. TLC were performed on 0.25-

mm-thick silica gel plates (Merck, silica gel 60 F254). Merck grade 60 silica gel, 230-400 mesh was used for column chromatography. Mass measurements were carried out on a TSQ 7000 FINNIGAN MAT (ESI/MS) instrument. HPLC chromatograms were obtained using a HP1100 with a column (250×4mm) packed with Lichrospher 100 RP-18 (5µm), an UV detector set at 254nm (flow: 1ml/min) and using a gradient with water (0.1% TFA) as solvent A and acetonitrile (0.1% TFA) as solvent B. The elemental analyses of carbon, hydrogen and nitrogen were done at Vernaison (France) by CNRS.

Compounds **1a** and **1b** were synthesized as previously described[8].

ZNH(CH_2)$_2$COArg(Pmc)OH (7c)

In 5ml of DMF, at 0°C, 460mg (2.06mmol) of **6c** and 356mg (3.09mmol) of HOSu were placed. Then, 510mg (2.47mmol) of DCC were added. The mixture was stirred overnight at room temperature, then cooled at 0°C. After 15min, 1.0g (2.27mmol) of H-Arg(Pmc)OH, solubilized in 5ml of DMF, was added. The solution was stirred at room temperature overnight. DCU was filtered off at 0°C and DMF was evaporated under reduced pressure. The residue was taken up in a saturated aqueous solution of $NaHCO_3$ and washed with EtOAc. Then, the basic layer was acidified at 0°C until pH=3 with an aqueous solution of $KHSO_4$ (1M). The aqueous solution was extracted with EtOAc. The organic layers were washed with water, dried on $MgSO_4$ and the solvent was evaporated to dryness. **7c** (1.25g, 94%) was obtained as an amorphous compound. TLC (EtOAc/MeOH 7:3, v:v): Rf=0.38; ^{1}H RMN ($CDCl_3$) δ (ppm): 7.55 (1H, NH (arginine), bs), 7.20 (5H, 5CH (Z), s), 7.00-6.05 (3H, 3NH (guanidinium), m), 5.90 (1H, NH (Z), bs), 4.95 (2H, CH_2 (Z), s), 4.35 (1H, CH (α), m), 3.35 (2H, CH_2 (β'), m), 3.10 (2H, CH_2 (δ), m), 2.70-2.20 (12H, 2CH_3o (Pmc), 2CH_2 (Pmc), CH_2 (α'), m), 2.00 (3H, CH_3m (Pmc), s), 1.90-1.40 (4H, 2CH_2 (β, γ), m), 1.20 (6H, 2CH_3 gem (Pmc), s); ^{13}C RMN ($CDCl_3$) δ (ppm): 172.9, 171.2, 157.1, 156.6, 153.6, 136.7, 135.7, 134.9, 133.3, 128.6-128.0 (5C), 124.2, 118.2, 73.8, 67.1, 52.6, 40.8, 37.7, 36.1, 33.0, 29.3, 27.1, 26.9, 25.5, 21.4, 18.6, 17.6, 12.2; MS (ESI+) m/z 646.2 $(M+H)^+$, m/z 668.2 $(M+Na)^+$, m/z 684.2 $(M+K)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=16.0min; Anal. calcd for $C_{31}H_{43}N_5O_8S$ (645.77): C 57.66, H 6.71, N 10.84. Found: C 57.41, H 6.83, N 10.68.

ZNH(CH_2)$_5$COArg(Pmc)OH (7d)

548mg (2.06mmol) of **6d**, 356mg (3.09mmol) of HOSu and 510mg (2.47mmol) of DCC were dissolved in 5ml of DMF at 0°C. Compound **7d** was obtained following the above procedure as an amorphous compound (1.29g, 91%). TLC (EtOAc+εAcOH): Rf=0.15; ^{1}H RMN ($CDCl_3$) δ (ppm): 7.40-7.15 (6H, NH (arginine), 5CH (Z), m), 6.45-5.85 (4H, NH (Z), 3NH (guanidinium), m), 5.00 (2H, CH_2 (Z), s), 4.40 (1H, CH (α), m), 3.25-2.95 (4H, 2CH_2 (ε', δ), m), 2.65-2.35 (8H, CH_2 (α'), 2CH_3o (Pmc), m), 2.30-2.00 (7H, 2CH_2 (Pmc), CH_3m (Pmc), m), 1.90-1.45 (10H, 5CH_2 (β, γ, β', γ', δ'), m), 1.20 (6H, 2CH_3 gem (Pmc), s); ^{13}C RMN ($CDCl_3$) δ (ppm): 175.1, 174.9, 156.9, 156.5, 153.9, 136.7, 135.6, 135.5, 133.0, 128.8-128.0 (5C), 124.3, 118.2, 73.9, 66.7, 52.6, 41.0, 40.8, 35.9, 32.9, 29.7, 26.9, 26.2, 25.3, 24.5, 21.3, 18.6, 17.6, 12.3; MS (ESI+) m/z 688.3 $(M+H)^+$, m/z 710.3 $(M+Na)^+$, m/z 726.3 $(M+K)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=16.4min; Anal. calcd for $C_{34}H_{49}N_5O_8S$ (687.85): C 59.37, H 7.18, N 10.18. Found: C 59.19, H 7.21, N 10.29.

ZNH(CH_2)$_2$COArg(Pmc)OMe (8c)

7c (1.10g, 1.70mmol) and Cs_2CO_3 (556mg, 1.70mmol) were placed in 5ml de DMF. After 10min stirring, MeI (120µl, 1.87mmol) was added and the solution was stirred overnight at room temperature. The solvent was then evaporated *in vacuo* and the residue was taken up in an aqueous solution of Na_2CO_3 (10%). The product was extracted with EtOAc, the organic layers were washed with brine, dried on $MgSO_4$ and finally the solvent was evaporated under reduced pressure to dryness. The crude product was purified on a column chromatography to isolate **8c** (880mg, 78%) as an amorphous compound. TLC (EtOAc 100%): Rf=0.33; ^{1}H RMN ($CDCl_3$) δ (ppm): 7.25 (5H, 5CH (Z), m), 6.80 (1H, NH (arginine), d), 6.20-6.00 (3H, 3NH (guanidinium), bs), 5.55 (1H, NH (Z), bs), 5.00 (2H, CH_2 (Z), s), 4.45 (1H, CH (α), m), 3.65 (3H, CH_3 (ester), s), 3.35 (2H, CH_2 (β') m), 3.10 (2H, CH_2 (δ), m), 2.65-2.30 (12H, 2CH_3o (Pmc), 2CH_2 (Pmc), CH_2 (α'), m), 2.05 (3H, CH_3m (Pmc), s), 1.85-1.60 (4H, 2CH_2 (β, γ), m), 1.20 (6H,

$2CH_3$ gem (Pmc), s); ^{13}C RMN ($CDCl_3$) δ (ppm): 172.7, 171.8, 156.8, 156.4, 153.8, 136.7, 135.6, 134.9, 133.4, 128.6-128.0 (5C), 124.3, 118.2, 73.8, 66.8, 52.6, 52.1, 40.7, 37.5, 36.6, 33.0, 29.4, 26.9, 25.5, 21.5, 18.6, 17.6, 12.2; MS (ESI+) m/z 660.3 $(M+H)^+$, m/z 682.3 $(M+Na)^+$, m/z 698.1 $(M+K)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=18.0min; Anal. calcd for $C_{32}H_{45}N_5O_8S$ (659.80): C 58.25, H 6.87, N 10.61. Found: C 58.47, H 6.82, N 10.49.

ZNH$(CH_2)_5$COArg(Pmc)OMe (8d)

7d (1.18mg, 1.72mmol), Cs_2CO_3 (560mg, 1.72mmol) and 118µl (1.89mmol) of MeI were dissolved in 5ml of DMF. Following the above procedure, **8d** was obtained as an amorphous compound (843mg, 70%). TLC (EtOAc 100%): Rf=0.45; ($CDCl_3$) ^1H RMN ($CDCl_3$) δ (ppm): 7.25 (5H, 5CH (Z), m), 6.70 (1H, NH (arginine), bd), 6.30-6.05 (3H, 3NH (guanidinium), bs), 5.15 (1H, NH (Z), s), 5.00 (2H, CH_2 (Z), s), 4.45 (1H, CH (α), m), 3.60 (3H, CH_3 (ester), s), 3.20-3.00 (4H, $2CH_2$ (ε', δ), m), 2.65-2.45 (8H, CH_2 (α'), 2CH3o (Pmc), m), 2.20-1.95 (7H, $2CH_2$ (Pmc), CH3m (Pmc), m), 1.85-1.35 (10H, $5CH_2$ (β, δ, β', γ', δ'), m), 1.25 (6H, $2CH_3$ gem (Pmc), s); ^{13}C RMN ($CDCl_3$) δ (ppm): 173.7, 172.9, 156.8, 156.4, 153.8, 136.8, 135.6, 134.9, 133.5, 128.6-128.0 (5C), 124.2, 118.1, 73.8, 66.7, 52.6, 51.9, 41.0, 40.7, 36.0, 33.0, 29.7, 26.9, 26.2, 25.9, 25.5, 25.2, 21.6, 18.7, 17.6, 12.3; MS (ESI+) m/z 702.3 $(M+H)^+$, m/z 724.3 $(M+Na)^+$, m/z 740.4 $(M+K)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=18.8min; Anal. calcd for $C_{35}H_{51}N_5O_8S$ (701.88): C 59.89, H 7.32, N 9.98. Found: C 60.04, H 7.40, N 9.76.

$NH_2(CH_2)_2$COArg(Pmc)OMe (2c)

A solution of **8c** (500mg, 0.75mmol) in methanol (30ml) and catalytic amount of 10% Pd/C (50mg) was hydrogenated at room temperature for 2 hours. Pd/C (10%) was then filtered off on celite and methanol was evaporated to dryness. After trituration in Et_2O, **2c** (384mg, 96%) was obtained as white crystals. TLC (MeOH/1-PrOH/H_2O/NH_4OH 250:6:3:1, v:v:v:v): Rf=0.36; mp 150-152°C; ^1H RMN (CD_3OD) δ (ppm): 4.35 (1H, CH (α), m), 3.65 (3H, CH_3 (ester), s), 3.15 (2H, CH_2 (δ), m), 2.80-2.45 (12H, $2CH_2$ (Pmc), CH_2 (α'), 2CH3o (Pmc), m), 2.10 (3H, CH3m (Pmc), s), 1.90-1.50 (6H, $3CH_2$ (β, γ, β'), m), 1.25 (6H, $2CH_3$ gem (Pmc), s); ^{13}C RMN (CD_3OD) δ (ppm): 173.9, 172.4, 157.9, 154.5, 136.3, 135.9, 134.6, 124.8, 119.2, 74.7, 53.6, 52.7, 45.5, 41.2, 36.9, 33.7, 32.8, 29.5, 26.8 (2C), 22.2, 18.8, 17.8, 12.2; MS (ESI+) m/z 526.1 $(M+H)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=13.3min; Anal. calcd for $C_{24}H_{39}N_5O_6S$ (525.66): C 54.84, H 7.48, N 13.32. Found: C 55.00, H 7.53, N 13.21.

$NH_2(CH_2)_5$COArg(Pmc)OMe (2d)

Following the above procedure, **2b** was obtained from **8d** (708mg, 1.0mmol) as white crystals (555mg, 97%). TLC (MeOH/1-PrOH/H_2O/NH_4OH 250:6:3:1, v:v:v:v): Rf=0.21; mp 135-137°C; ^1H RMN ($CDCl_3$) δ (ppm): 7.25 (1H, NH (arginine), bs), 6.80 (2H, NH_2, m), 6.60-6.25 (3H, 3NH (guanidinium), m), 4.40 (1H, CH (α), m), 3.60 (3H, CH_3 (ester), s), 3.25-3.00 (4H, $2CH_2$ (ε', δ), m), 2.70-2.40 (10H, $2CH_2$ (Pmc), 2CH3o (Pmc), m), 2.15 (2H, CH_2 (α'), m), 2.00 (3H, CH3m (Pmc), s), 1.80-1.10 (16H, $5CH_2$ (β', γ', δ', β, γ), $2CH_3$ gem (Pmc), m); ^{13}C RMN ($CDCl_3$) δ (ppm): 174.1, 173.1, 156.5, 153.7, 135.5, 134.9, 133.5, 124.1, 118.1, 73.8, 52.5, 52.1, 41.3, 40.7, 35.8, 32.9, 31.5, 29.3, 26.9 (2C), 26.1, 25.7, 25.3, 21.5, 18.6, 17.6, 12.3; MS (ESI+) m/z 568.2 $(M+H)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=11.1min; Anal. calcd for $C_{27}H_{45}N_5O_6S$ (567.74): C 57.12, H 7.99, N 12.34. Found: C 56.96, H 8.07, N 12.04.

Z-PNA(A-diBoc)-PNA(G-Bn)-NH$(CH_2)_2$COArg(Pmc)OMe (3ac)

1a (234mg, 0.232mmol), **2c** (135mg, 0.255mmol) and HOSu (41mg, 0.348mmol) were placed in 10ml of DMF. Then, DCC (58mg, 0.278mmol) was added at 0°C. The mixture was stirred at room temperature for 12 hours, then DCU was filtered at 0°C. DMF was evaporated under reduced pressure and the residue was taken up in EtOAc. The organic layer was washed by an aqueous solution of citric acid (10%), then by a saturated aqueous solution of $NaHCO_3$ and finally by water. After drying on $MgSO_4$, the solvent was evaporated to dryness. The crude residue was purified on a column chromatography and **3ac** was obtained (67% yield) as a white powder. TLC (EtOAc/MeOH 6:4, v:v): Rf=0.42; mp 205-207°C (dec); ^1H RMN

(CD_3OD) δ (ppm): 8.45-8.05 (3H, 3CH (adenine, guanine), m), 7.45-7.00 (10H, 10CH (Z, OBn), m), 5.50-4.70 (8H, $4CH_2$ (Z, OBn, g, h), m), 4.35-3.80 (5H, $2CH_2$ (c, f), CH (α), m), 3.70-3.05 (15H, $6CH_2$ (a, b, d, e, δ, β'), CH_3 (ester), m), 2.60-2.20 (12H, $3CH_2$ (α', Pmc), $2CH_3o$ (Pmc), m), 2.00 (3H, CH_3m (Pmc), s), 1.75-1.15 (28H, $2CH_2$ (β, γ), $8CH_3$ (Boc, Pmc) m); ^{13}C RMN (CD_3COCD_3) δ (ppm): 171.3, 171.0, 170.0, 169.4, 168.5, 168.1, 161.6, 160.8, 157.8, 156.9, 155.7, 154.6, 154.2, 152.3, 151.2, 150.4, 148.1, 141.7, 138.2, 137.9, 136.1, 135.7, 134.1, 129.0-128.0 (10C), 127.6, 124.9, 118.8, 115.1, 84.0, 74.5, 68.0, 67.0, 53.2, 52.7, 51.1, 50.1-47.4 (3C), 45.6, 44.0, 41.5, 39.5, 38.5, 38.0, 36.8, 33.9, 30.1, 27.4 (2C), 27.0 (6C), 26.3, 22.3, 19.4, 18.4, 13.1; MS (ESI+) m/z 1517.1 $(M+H)^+$, m/z 1539.0 $(M+Na)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=21.5min; Anal. calcd for $C_{71}H_{93}N_{19}O_{17}S$ (1516.70): C 56.23, H 6.18, N 17.55. Found: C 56.44, H 6.04, N 17.69.

Z-PNA(A-diBoc)-PNA(G-Bn)-NH(CH_2)$_5$COArg(Pmc)OMe (3ad)

3ad was obtained as a white powder (56% yield) from **1a** (250mg, 0.248mmol) and **2d** (155mg, 0.273mmol) following the above procedure. TLC (EtOAc/MeOH 6:4, v:v): Rf=0.30; mp 193-195°C (dec); ^1H RMN (CD_3OD) δ (ppm): 8.60 (1H, CH (adenine), s), 8.05 (1H, CH (adenine), s), 7.60 (1H, CH (guanine), s), 7.45-7.05 (10H, 10CH (Z, OBn), m), 5.50-4.70 (8H, $4CH_2$ (OBn, Z, g, h), m), 4.50-3.65 (8H, $2CH_2$ (c, f) CH (α), CH_3 (ester), m), 3.60-3.05 (12H, $6CH_2$ (a, b, d, e, ε', δ), m), 2.60-2.25 (10H, $2CH_2$ (Pmc) $2CH_3o$ (Pmc), m), 2.15 (2H, CH_2 (α'), m), 2.05 (3H, CH_3m (Pmc), s), 1.85-1.20 (34H, $5CH_2$ (β', γ', δ', β, γ), $8CH_3$ (Boc, Pmc), m); ^{13}C RMN (CD_3COCD_3) δ (ppm): 171.3, 171.0, 170.0, 169.4, 168.5, 168.1, 161.6, 160.8, 157.8, 156.9, 155.7, 154.6, 154.2, 152.3, 151.2, 150.4, 148.1, 141.7, 138.2, 137.9, 136.1, 135.7, 134.1, 129.0-128.0 (10C), 127.6, 124.9, 118.8, 115.1, 84.0, 74.5, 68.0, 67.0, 53.2, 52.7, 51.1, 50.1-47.4 (3C), 45.6, 44.0, 41.5, 40.7, 39.5, 38.0, 36.9, 33.9, 30.1, 27.4 (2C), 27.0 (6C), 27.0-26.1 (4C), 22.3, 19.4, 18.4, 13.1; MS (ESI+) m/z 1559.1 $(M+H)^+$, m/z 1581.0 $(M+Na)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=22.1min; Anal. calcd for $C_{74}H_{99}N_{19}O_{17}S$ (1558.78): C 57.02, H 6.40, N 17.07. Found: C 57.29, H 6.29, N 16.89.

Z-PNA(G-Bn)-PNA(A-diBoc)-NH(CH_2)$_2$COArg(Pmc)OMe (3bc)

3bc was obtained as a white powder (65% yield) from **1b** (200mg, 0.20mmol) and **2c** (115.5mg, 0.22mmol) following the above procedure. TLC (EtOAc/MeOH 8:2, v:v): Rf=0.30; mp 207-209°C (dec); ^1H RMN (CD_3OD) δ (ppm): 8.60-8.00 (3H, 3CH (adenine, guanine), m), 7.50-7.00 (10H, 10CH (Z, OBn), m), 5.45-4.70 (8H, $4CH_2$ (Z, OBn, g, h), m), 4.35-3.80 (5H, $2CH_2$ (c, f), CH (α), m), 3.70-3.00 (15H, $6CH_2$ (a, b, d, e, β', δ), CH_3 (ester) m), 2.60-2.20 (12H, $3CH_2$ (α', Pmc), $2CH_3o$ (Pmc), m), 2.05 (3H, CH_3m (Pmc), s), 1.75-1.20 (28H, $2CH_2$ (β, γ), $8CH_3$ (Boc, Pmc), m); ^{13}C RMN (CD_3COCD_3) δ (ppm): 171.5, 170.9, 170.0, 169.3, 168.5, 168.0, 161.5, 160.9, 157.9, 156.7, 155.7, 154.6, 154.3, 152.3, 151.3, 150.4, 148.2, 141.7, 138.1, 137.9, 136.1, 135.6, 134.1, 129.0-128.0 (10C), 127.5, 124.9, 118.7, 115.1, 84.1, 74.5, 68.1, 67.0, 53.2, 52.8, 51.2, 50.1-47.4 (3C), 45.5, 44.0, 41.5, 39.4, 38.5, 38.0, 36.7, 33.9, 30.2, 27.4 (2C), 27.0 (6C), 26.4, 22.3, 19.4, 18.4, 13.1; MS (ESI+) m/z 1517.0 $(M+H)^+$, m/z 1538.9 $(M+Na)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=21.6min; Anal. calcd for $C_{71}H_{93}N_{19}O_{17}S$ (1516.70): C 56.23, H 6.18, N 17.55. Found: C 56.37, H 6.09, N 17.41.

Z-PNA(G-Bn)-PNA(A-diBoc)-NH(CH_2)$_5$COArg(Pmc)OMe (3bd)

3bd was obtained as a white powder (64% yield) from **1b** (190mg, 0.188mmol) and **2d** (117mg, 0.207mmol) following the above procedure. TLC (EtOAc/MeOH 1:1, v:v): Rf=0.24; mp 195-197°C (dec); ^1H RMN (CD_3OD) δ (ppm): 8.65 (1H, CH (adenine), s), 8.00 (1H, CH(adenine), s), 7.60 (1H, CH (guanine), s), 7.45-6.90 (10H, 10CH (Z, OBn), m), 5.55-4.70 (8H, $4CH_2$ (Z, OBn, g, h), m), 4.40-3.70 (8H, $2CH_2$ (c, f), CH (α), CH_3 (ester), m), 3.60-3.00 (12H, $6CH_2$ (a, b, d, e, ε', δ), m), 2.60-2.20 (10H, $2CH_2$ (Pmc) $2CH_3o$ (Pmc), m), 2.15 (2H, CH_2 (α'), m), 2.10 (3H, CH_3m (Pmc), s), 1.80-1.25 (34H, $5CH_2$ (β', γ', δ', β, γ), $8CH_3$ (Boc, Pmc), m); ^{13}C RMN (CD_3COCD_3) δ (ppm): 171.3, 171.1, 169.9, 169.3, 168.5, 168.2, 161.7, 160.8, 157.7, 156.8, 155.7, 154.6, 154.3, 152.3, 151.1, 150.3, 148.1, 141.7, 138.1, 137.9, 136.2, 135.7, 134.2, 129.0-128.0 (10C), 127.6, 124.8, 118.8, 115.2, 84.0, 74.5, 68.0, 67.1, 53.2, 52.7, 51.1, 50.1-47.4 (3C), 45.5, 44.0, 41.5, 40.6, 39.5, 38.1, 36.9, 33.9, 30.2, 27.4 (2C), 27.0 (6C), 27.0-26.1 (4C), 22.2, 19.4, 18.4, 13.2; MS (ESI+) m/z 1559.0 $(M+H)^+$, m/z 1580.9 $(M+Na)^+$; HPLC (A/B 80:20 to 0:100 over 30min): Rt=22.2min; Anal. calcd for $C_{74}H_{99}N_{19}O_{17}S$ (1558.78): C 57.02, H 6.40, N 17.07. Found: C 57.31, H 6.29, N 16.97.

Z-PNA(A)-PNA(G)-NH(CH_2)$_2$COArgOMe (4ac)

3ac (220mg, 0.145mmol) was dissolved in 3ml of a solution of CH_2Cl_2 / TFA (1:1, v:v) and the mixture was stirred at room temperature for 6 hours. The solvent was evaporated under reduced pressure and the residue was taken up in water. The aqueous layer was washed with EtOAc and water was evaporated to dryness. **4ac** was obtained (139mg, 100%) as a beige powder. mp 218-220°C (dec); ^{1}H RMN (DMSO-d^6) δ (ppm): 8.60 (1H, NH (guanidinium), bs), 8.40-8.05 (4H, 3CH (adenine, guanine), NH (arginine), m), 8.00-7.70 (2H, 2NH (guanidinium, β'), m), 7.45-7.00 (7H, 5CH (Z), 2NH (amide, (Z)), m), 6.50 (2H, NH_2 (guanine), bs), 5.50-4.80 (6H, 3CH_2 (Z, g, h), m), 4.30-3.90 (5H, 2CH_2 (c, f), CH (α), m), 3.65-3.20 (15H, 6CH_2 (a, b, d, e, δ, β'), CH_3 (ester), m), 2.35 (2H, CH_2 (α'), m), 1.75-1.15 (4H, 2CH_2 (β, γ), m); ^{13}C RMN (DMSO-d^6) δ (ppm): 170.5, 170.2, 169.3, 168.6, 168.3, 167.4, 165.8, 160.8, 157.0, 155.0, 152.6, 150.5, 149.7, 148.8, 147.3, 141.0, 137.2, 128.0-127.1 (5C), 126.9, 114.8, 66.3, 52.5, 52.0, 50.4, 49.4-46.8 (3C), 45.0, 43.3, 40.8, 39.0, 37.6, 37.0, 36.0, 29.4, 25.6; MS (ESI+) m/2z 480.8; HPLC (A/B 90:10 to 70:30 over 40min): Rt=19.6min; Anal. calcd for $C_{40}H_{53}N_{19}O_{10}$.2CF_3COOH (1188.03): C 44.48, H 4.67, N 22.40. Found: C 44.61, H 4.72, N 22.29.

Z-PNA(A)-PNA(G)-NH(CH_2)$_5$COArgOMe (4ad)

4ad was prepared from **3ad** (216mg, 0.138mmol) following the procedure described above. **4ad** was obtained as a beige powder (100% yield). mp 160-162°C (dec); ^{1}H RMN (DMSO-d^6) δ (ppm): 10.90 (1H, NH (guanine), bs), 8.60 (1H, NH (guanidinium), bs), 8.40-7.80 (6H, 3CH adenine, guanine), 3NH (guanidinium, arginine, ε'), m), 7.40-7.10 (7H, 5CH (Z), 2NH (amide, Z), m), 6.50 (2H, NH_2 (guanine), bs), 5.40-4.70 (6H, 3CH_2 (Z, g, h), m), 4.30-3.70 (5H, 2CH_2 (c, f), CH (α), m), 3.65 (3H, CH_3 (ester), s), 3.60-3.05 (12H, 6CH_2 (a, b, d, e, δ, ε'), m), 2.15 (2H, CH_2 (α'), m), 1.85-1.60 (4H, 2CH_2 (β, γ), m), 1.50-1.20 (6H, 3CH_2 (β', γ', δ'), m); ^{13}C RMN (DMSO-d^6) δ (ppm): 170.4, 170.2, 169.4, 168.5, 168.3, 167.4, 165.7, 160.8, 157.1, 155.0, 152.6, 150.6, 149.7, 148.7, 147.3, 141.0, 137.3, 128.0-127.1 (5C), 126.8, 114.8, 66.3, 52.6, 52.0, 50.3, 49.4-46.8 (3C), 45.0, 43.2, 40.9, 40.0, 39.0, 37.1, 36.1, 29.5, 26.2-25.4 (4C); MS (ESI+) m/2z 502.0; HPLC (A/B 90:10 to 70:30 over 40min): Rt= 26.3min; Anal. calcd for $C_{43}H_{59}N_{19}O_{10}$.2CF_3COOH (1230.11): C 45.89, H 5.00, N 21.63. Found: C 46.01, H 5.13, N 21.77.

Z-PNA(G)-PNA(A)-NH(CH_2)$_2$COArgOMe (4bc)

4bc was prepared from **3bc** (196mg, 0.129mmol) following the procedure described above. **4bc** was obtained as a beige powder (100% yield). mp 216-218°C (dec); ^{1}H RMN (DMSO-d^6) δ (ppm): 8.45 (1H, NH (guanidinium), bs), 8.40-7.75 (6H, 3CH (adenine, guanine), 3NH (arginine, guanidinium, β'), m), 7.35-7.00 (7H, 5CH (Z), 2NH (Z, amide), m), 6.35 (2H, NH_2 (guanine), bs), 5.50-4.80 (6H, 3CH_2 (Z, g, h), m), 4.40-3.80 (5H, 2CH_2 (c, f), CH (α), m), 3.65-3.60 (5H, CH_2 (e), CH_3 (ester), m), 3.35-3.10 (10H, 5CH_2 (a, b, d, β', δ), m), 2.35 (2H, CH_2 (α'), m), 1.80-1.50 (4H, 2CH_2 (β, γ), m); ^{13}C RMN (DMSO-d^6) δ (ppm): 170.6, 170.3, 169.3, 168.5, 168.3, 167.4, 165.7, 160.8, 157.1, 155.0, 152.6, 150.4, 149.7, 148.7, 147.3, 141.1, 137.2, 128.0-127.1 (5C), 126.9, 114.7, 66.3, 52.4, 52.0, 50.4, 49.4-46.7 (3C), 45.4, 43.3, 40.8, 39.0, 37.7, 37.0, 36.1, 29.4, 25.6; MS (ESI+) (m/2z) 480.8; HPLC (A/B 90:10 to 70:30 over 40min): Rt=19.1min; Anal. calcd. for $C_{40}H_{53}N_{19}O_{10}$.2CF_3COOH (1188.03): C 44.48, H 4.67, N 22.40. Found: C 44.64, H 4.61, N 22.51.

Z-PNA(G)-PNA(A)-NH(CH_2)$_5$COArgOMe (4bd)

4bd was prepared from **3bd** (187mg, 0.120mmol) following the procedure described above. **4bd** was obtained as a beige powder (100% yield). mp 171-173°C (dec); ^{1}H RMN (DMSO-d^6) δ (ppm): 10.90 (1H, NH (guanine), bs), 8.60 (1H, NH (guanidinium), bs), 8.40-7.80 (6H, 3CH (adenine, guanine), 3NH (guanidinium, arginine, ε'), m), 7.40-7.10 (7H, 5CH (Z), 2NH (amide, Z), m), 6.50 (2H, NH_2 (guanine), bs), 5.40-4.70 (6H, 3CH_2 (Z, g, h), m), 4.40-3.70 (5H, 2CH_2 (c, f), CH (α), m), 3.65 (3H, CH_3 (ester), s), 3.60-3.10 (12H, 6CH_2 (a, b, d, e, ε', δ), m), 2.15 (2H, CH_2 (α'), m), 1.85-1.60 (4H, 2CH_2 (β, γ), m), 1.50-1.20 (6H, 3CH_2 (β', γ', δ'), m); ^{13}C RMN (DMSO-d^6) δ (ppm): 170.4, 170.3, 169.5, 168.5, 168.2, 167.4, 165.8, 160.8, 157.0, 155.1, 152.6, 150.5, 149.8, 148.7, 147.3, 141.1, 137.3, 128.1-127.1 (5C), 126.8, 114.9, 66.3, 52.5, 51.9, 50.3, 49.4-46.8 (3C), 45.0, 43.2, 41.0, 39.9, 39.0, 37.0, 36.1, 29.5, 26.3-25.4 (4C); MS (ESI+) m/2z 501.9; HPLC (A/B 90:10 to 70:30 over 40min): Rt=26.7min; Anal. calcd. for $C_{43}H_{59}N_{19}O_{10}$.2CF_3COOH (1230.11): C 45.89, H 5.00, N 21.63. Found: C 45.69, H 5.05, N 21.51.

Z-PNA(A)-PNA(G)-NH(CH_2)$_2$COArgOH (5ac)

In 3ml of THF containing 78mg (0.081mmol) of **4ac**, 0.8ml of an aqueous solution of LiOH (1M) was added. The mixture was stirred at room temperature for 4 hours and an aqueous solution of HCl (1M) was added until pH=3. THF was evaporated under reduced pressure to dryness and the residue was taken up in a minimum of water to be purified on a XAD-2 Amberlite (20-60 mesh). **5ac** was obtained (64mg, 84%) as an amorphous compound. ^{1}H RMN (DMSO-d^6) δ (ppm): 10.85 (1H, NH (guanine), bs), 8.55 (1H, NH (guanidinium), bs), 8.40-7.75 (6H, 3CH (adenine, guanine), 3NH (β', arginine, guanidinium), m), 7.40-7.00 (7H, 5CH (Z), 2NH (amide, Z), m), 6.55 (2H, NH_2 (guanine), bs), 5.50-4.80 (6H, 3CH_2 (Z, g, h), m), 4.40-3.80 (5H, 2CH_2 (c, f), CH (α), m), 3.60-3.20 (12H, 6CH_2 (a, b, d, e, β', δ), m), 2.35 (2H, CH_2 (α'), m), 1.75-1.15 (4H, 2CH_2 (β, γ), m); ^{13}C RMN (DMSO-d^6) δ (ppm): 170.6, 170.2, 169.8, 169.3, 168.5, 167.4, 165.9, 160.8, 157.1, 155.0, 152.6, 150.4, 149.7, 148.8, 147.3, 141.2, 137.2, 128.0-127.1 (5C), 126.7, 114.8, 66.4, 52.5, 50.4, 49.4-46.8 (3C), 45.0, 43.3, 40.7, 39.0, 37.6, 37.1, 36.0, 29.4, 25.5; MS (ESI+) m/2z 473.7; HPLC (A/B 90:10 to 70:30 over 40min): Rt=17.2min; Anal. calcd for $C_{39}H_{51}N_{19}O_{10}$.2HCl (1018.88): C 45.98, H 5.24, N 26.12. Found: C 46.19, H 5.30, N 26.31.

Z-PNA(A)-PNA(G)-NH(CH_2)$_5$COArgOH (5ad)

4ad (50mg; 0.05mmol) was treated following the above procedure to yield **5ad** (75%) as a colorless resin. ^{1}H RMN (DMSO-d^6) δ (ppm): 10.90 (1H, NH (guanine), bs), 8.60 (1H, NH (guanidinium), bs), 8.40-7.80 (6H, 3CH (adenine, guanine), 3NH (ε', arginine, guanidinium), m), 7.40-7.10 (7H, 5CH (Z), 2NH (Z, amide), m), 6.50 (2H, NH_2(guanine) bs), 5.35-4.80 (6H, 3CH_2 (Z, g, h), m), 4.30-3.05 (17H, 8CH_2 (a, b, c, d, e, f, ε', δ), CH (α), m), 2.15 (2H, CH_2 (α'), m), 1.85-1.20 (10H, 5CH_2,(β', γ', δ', β, γ), m); ^{13}C RMN (DMSO-d^6) δ (ppm): 170.8, 170.4, 169.9, 169.4, 168.5, 167.3, 165.7, 160.7, 157.1, 155.1, 152.6, 150.6, 149.8, 148.7, 147.2, 141.0, 137.3, 128.0-127.0 (5C), 126.8, 114.8, 66.3, 52.5, 50.3, 49.5-46.8 (3C), 45.0, 43.3, 40.9, 40.0, 39.0, 37.2, 36.1, 29.5, 26.3-25.4 (4C); MS (ESI+) m/2z 494.9; HPLC (A/B 90:10 to 70:30 over 40min): Rt=23.3min; Anal. calcd. for $C_{42}H_{57}N_{19}O_{10}$.2HCl (1060.96): C 47.55, H 5.61, N 25.08. Found: C 47.79, H 5.66, N 25.21.

Z-PNA(G)-PNA(A)-NH(CH_2)$_2$COArgOH (5bc)

4bc (32mg; 0.033mmol) were treated following the above procedure to yield **5bc** (85%) as a colorless resin. ^{1}H RMN (DMSO-d^6) δ (ppm): 10.90 (1H, NH (guanine), bs), 8.45 (1H, NH (guanidinium), bs), 8.40-7.70 (6H, 3CH (adenine, guanine), 3NH (β', arginine, guanidinium), m), 7.35-7.00 (7H, 5CH (Z), 2NH (amide, Z), m), 6.50 (2H, NH_2 (guanine), bs), 5.50-4.80 (6H, 3CH_2 (Z, g, h), m), 4.40-3.80 (5H, 2CH_2 (c, f), CH (α), m), 3.60 (2H, CH_2 (e), m), 3.35-3.10 (10H, 5CH_2 (a, b, d, β', δ), m), 2.35 (2H, CH_2 (α'), m), 1.80-1.50 (4H, 2CH_2 (β, γ), m); ^{13}C RMN (DMSO-d^6) δ (ppm): 170.7, 170.3, 169.8, 169.3, 168.4, 167.4, 166.0, 160.8, 157.0, 155.0, 152.5, 150.4, 149.6, 148.8, 147.2, 141.2, 137.1, 128.0-127.1 (5C), 126.8, 114.6, 66.4, 52.5, 50.5, 49.4-46.8 (3C), 45.1, 43.3, 40.7, 39.1, 37.6, 37.1, 36.2, 29.4, 25.5; MS (ESI+) m/2z 473.9; HPLC (A/B 90:10 to 70:30 over 40min): Rt=16.2min; Anal. calcd for $C_{39}H_{51}N_{19}O_{10}$.2HCl (1018.88): C 45.98, H 5.24, N 26.12. Found: C 45.82, H 5.21, N 26.06.

Z-PNA(G)-PNA(A)-NH(CH_2)$_5$COArgOH (5bd)

4bd (67mg; 0.067mmol) were treated following the above procedure to yield **5bd** (81%) as a colorless resin. ^{1}H RMN (DMSO-d^6) δ (ppm): 10.90 (1H, NH (guanine), bs), 8.60 (1H, NH (guanidinium), bs), 8.40-7.80 (6H, 3CH (adenine, guanine), 3NH (ε', arginine, guanidinium), m), 7.40-7.10 (7H, 5CH (Z), 2NH (amide, Z), m), 6.50 (2H, NH_2 (guanine) bs), 5.35-4.80 (6H, 3CH_2 (Z, g, h), m), 4.30-3.10 (17H, 8CH_2 (a, b, c, d, e, f, ε', δ), CH (α), m), 2.15 (2H, CH_2 (α'), m), 1.85-1.50 (4H, 2CH_2 (β, γ), m), 1.60-1.20 (6H, 3CH_2 (β', γ', δ'), m); ^{13}C RMN (DMSO-d^6) δ (ppm): 170.8, 170.3, 169.8, 169.2, 168.5, 167.4, 165.7, 160.8, 157.1, 155.3, 152.6, 150.5, 149.8, 148.7, 147.2, 141.0, 137.3, 128.0-127.1 (5C), 126.8, 114.8, 66.5, 52.5, 50.3, 49.5-46.8 (3C), 45.2, 43.3, 40.9, 40.1, 39.0, 37.2, 36.1, 29.6, 26.3-25.4 (4C); MS (ESI+) m/2z 494.8; HPLC (A/B 90:10 to 70:30 over 40min): Rt=23.7min; Anal. calcd for $C_{42}H_{57}N_{19}O_{10}$.2HCl (1060.96): C 47.55, H 5.61, N 25.08. Found: C 47.40, H 5.70, N 25.26.

Acknowledgements : This work was supported by the Agence Nationale de Recherches sur le SIDA (ANRS) and by SIDACTION.

REFERENCES

1. Frankel, A. D. *Curr. Opin. Genet. Dev.* **1992**, 2, 293-298.
2. Bayer, P.; Kraft, M.; Ejchart, A.; Westendorp, M.; Frank, R.; Rösch, P. *J. Mol. Biol.* **1995**, 247, 529-535.
3. (a) Puglisi, J. D.; Tan, R.; Calnan, B. J.; Frankel, A. D.; Williamson, J. R. *Science.* **1992**, 257, 76-80. (b) Puglisi, J. D.; Chen, L.; Frankel, A. D.; Williamson, J. R. *Proc. Natl. Acad. Sci. USA 90.* **1993**, 3680-3684.
4. (a) Aboul-ela, F.; Karn, J.; Varani, G. *J. Mol. Biol.* **1995**, 253, 313-332. (b) Aboul-Ela, F.; Karn, J.; Varani, G. *Nucl. Acids Res.* **1996**, 24, 3974-3981.
5. Berkhout, B. *Nucl. Acids Res.* **1992**, 20, 27-31.
6. Tao, J.; Frankel, A. D. *Proc. Natl. Acad. Sci. USA.* **1992**, 89, 2723-2726.
7. We have chosen Polyamide Nucleic Acids (PNA) as structural nucleotide analogs because of their high affinity for DNA or RNA. They are more stable towards nucleases and more lipophilic than their natural homologs. For more informations see (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science.* **1991**, 254, 1497-1500. (b) Egholm, M.; Nielsen, P. E.; Buchardt, O.; Berg, R. H. *J. Am. Chem. Soc.* **1992**, 114, 9677-9678. (c) Nielsen, P. E.; Egholm, M.; Buchardt, O. *Bioconjugate Chem.*, **1994**, 5, 3-7.
8. (a) Farese, A.; Patino, N.; Condom, R.; Dalleu-Pairot, S.; Guedj, R. *Tetrahedron Lett.* **1996**, 37 (9), 1413-1416. (b) Farese, A.; Pairot, S.; Patino, N.; Ravily, V.; Condom, R.; Aumelas, A.; Guedj, R. *Nucl. & Nucl.* **1997**, 16, 1893-1906.
9. Moog, C.; Wick, A.; Le Ber, P.; Kirn, A.; Aubertin, A. M. *Antiviral Res.* **1994**, 24, 275-288.
10. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods.* **1988**, 20, 309-321.
11. Genu-Dellac, C.; Gosselin, G.; Puech, F.; Henry, J. C.; Aubertin, A. M.; Aubert, G.; Kirn, A.; Imbach, J. L. *Nucl. & Nucl.* **1991**, 10, 1345-1376.

received 9/4/98
accepted 11/23/98