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Cyclic PNA hexamer-based compound: modelling, synthesis and inhibition of the HIV-1 RNA dimerization process

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Abstract—A cyclic molecule constituted by (i) a hexameric PNA moiety complementary to six among the nine residues of the dimerization initiation site loop of HIV-1 and (ii) a spacer tethering the N- to the C-extremities of the PNA, has been elaborated to inhibit the dimerization process of HIV-1 genome. This compound has been synthesized following a liquid-phase procedure (fully protected backbone approach). Preliminary agarose gel electrophoresis analyses have shown that the cyclic PNA conjugate is able to inhibit the HIV-1 dimerization.

1. Introduction

RNA hairpins are involved in site-specific recognition processes with both proteins and nucleic acids and play key roles in biological functions. Such single stranded stem-loop RNA structures can represent attractive targets for drug design. In the case of the HIV-1 genome, a duplex, involving two identical and auto-complementary stem-loops, is formed by their noncovalent association.[1] This HIV RNA dimerization process is mediated at the dimerization initiation site (DIS), a highly conserved sequence, located near the 5′ end of the RNA.[1,2] Such particular stem-loops structures, containing self-complementary sequences, are called palindromes. Palindrome 5′-GCGCGC-3′ (LAI strain) represents the major isoform found in natural isolates of HIV-1 (Fig. 1).[3]

This dimerization is an essential process in HIV-1 replication as it is thought to modulate several steps in the viral life cycle,[4] such as retrotranscription, packaging[5] as well as encapsidation.[6] Therefore, inhibition of RNA dimerization represents a promising alternative for efficient anti-HIV therapy.

The HIV RNA dimerization is a two step process, which is first initiated by base pairing between the palindromic sequence of each monomer. This transient 'kissing-loop' dimer then changes to a mature and linear duplex (Fig. 2).[7] It has been shown that small perturbations of the loop structure of DIS are detrimental for dimerization.[8]

Therefore, one can hypothesize that the inhibition of the kissing-loop complex formation would lead to a dramatic decrease of viral replication. Some natural or unnatural antisense oligonucleotides (9 and 16-mers) directed against

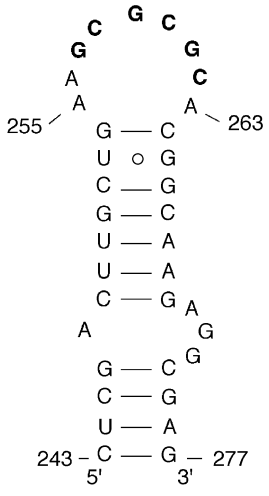

Figure 1. The dimerization initiation site (DIS) of HIV-1 LAI strain. The autocomplementary sequence is in bold. Watson–Crick base pairs are represented by dashes and the non canonical pairing is represented by open circle.

Keywords: PNA hexamer; HIV-1 dimerization process; synthesis.
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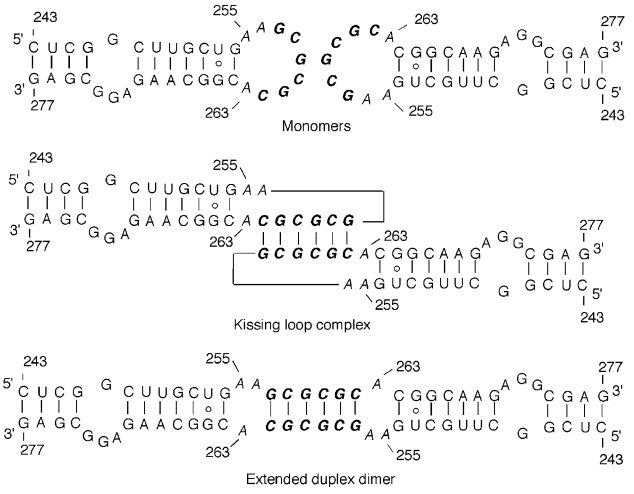


Figure 2. Proposed mechanism of LAI HIV-1 RNA dimerization.[12]

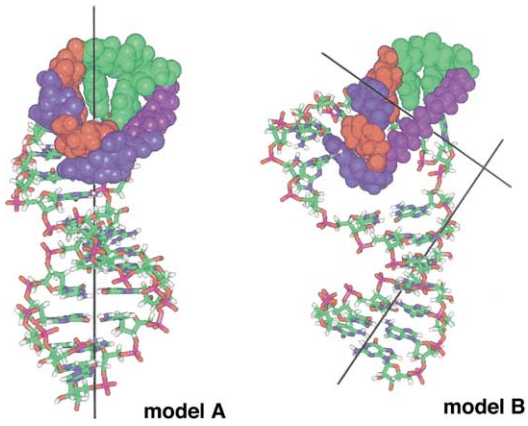


Figure 4. Molecular models of the interaction between compound **1** and DIS RNA of HIV-1. A: colinear model; B: perpendicular model. The DIS RNA ribbon is represented as sticks, compound **1** as balls. Guanine residues are in blue, cytosine in red, uracil in green and the linker is in purple.

the stem-loop sequence of DIS were found, in vitro, to be efficient inhibitors of RNA dimerization.[9] However, although promising, the use of such antisense agents in chemotherapy is limited, because of their poor cellular uptake and/or in vivo stability.

Kissing-loop interactions (involving two loops) have been reported to be more stable than complexes formed between a loop and a linear RNA.[10] Moreover, several studies have shown that kissing-loop interactions involving only six nucleotides per loop can be specific and selective.[10,11] Therefore, a strong interaction could result from a targeted hairpin loop of six residues and its complementary hexameric antisense which would be constrained (synthetically) as a loop to mimic a kissing-loop complex. This 'loop like' structure should afford several advantages over a linear analog such as: greater selectivity, better cellular uptake and enhanced enzymatic resistance. Thus, cyclic molecules composed of a sequence of six nucleotides (or analogs) complementary of six residues of the DIS loop appear to be promising candidates for inhibiting the initiation of the HIV RNA dimerization.

In this context and on the basis of molecular modelling studies, we designed the cyclic compound **1** constituted by an antisense polyamide nucleic acid (PNA) hexamer and a linker tethering the N- to the C-terminal extremities of the polymer backbone through amide bonds (Fig. 3). A PNA was chosen among the oligonucleotide analogs, because it is an achiral non-ionic mimic of oligonucleotides which recognizes its complementary sequence with a remarkable

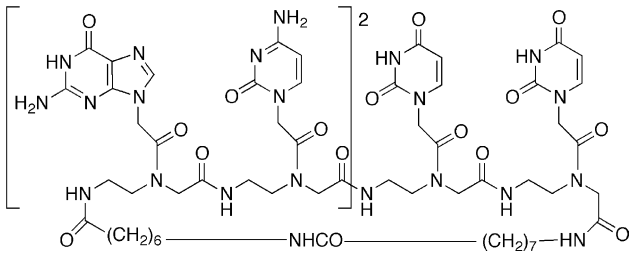


Figure 3. Structure of the target cyclic compound **1**.

high affinity and specificity. Moreover, the chemical stability, the enzymatic resistance to nucleases of PNAs make them more attractive than oligonucleotides for therapeutic use as gene targeting agents.[12]

The hexa-PNA moiety of **1**, i.e. H_2N-GCGCUU-CO_2H is antisense complementary of the $5'$-$_{255}$AAGCGC$_{260}$-$3'$ sequence of DIS (LAI strain). This last sequence was targeted rather than the $_{256}$AGCGCG$_{261}$, $_{257}$GCGCGC$_{262}$ or $_{258}$CGCGCA$_{263}$ ones because only four residues among six are self-complementary (versus five or six in the other cases), limiting thus, the risk of self-aggregation. The length of the linker (17 atoms) was optimized by molecular modelling to allow the 'kissing' interaction to occur between the synthetic cyclic loop **1** and the viral DIS loop.

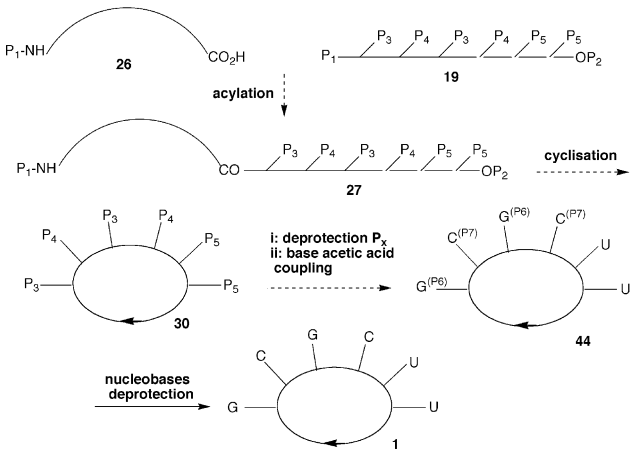
In this paper, we report on the molecular modelling of the cyclic PNA hexamer [CGCGUU]c **1** and its liquid phase synthesis following the FPB approach.[13,14] The ability of compound **1** to interfere with the dimerization process of HIV-1 (LAI) is also reported.

2. Dynamic molecular modelling studies

At the beginning of the study, no experimental models of a potential 'DIS RNA/inhibitor' complex were available. Thus, based on structural data of the DIS RNA Mal source[15] and on a resolved system (with a similar approach) involving a kissing loop interaction between an other RNA (TAR HIV RNA) with its complementary sequence,[11e,16] two models of the compound **1**/DIS RNA complex were built (Fig. 4).

In these models, axes formed by the stem and the DIS loop palindromic sequence are colinear (model A) or approximately perpendicular (model B).

In a first step, a complex resulting from the interaction of the linear PNA hexamer with the DIS RNA loop was designed for the two models of RNA. Docking assays were carried out, constraining some parameters, to allow correct base pairing between the DIS RNA loop and the linear PNA. In

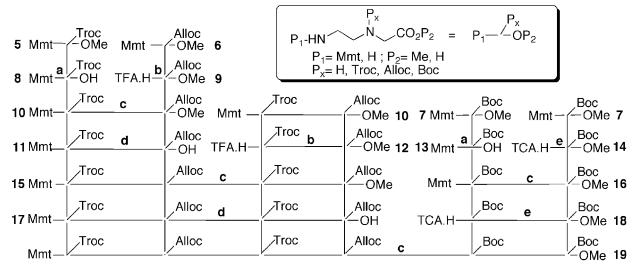


P_n	protecting group	cleavage
P_1	Mmt	5% TCA/CH_2Cl_2
P_2	Me	OH^-
P_3	Troc	Cd/AcOH
P_4	Alloc	$Pd[PPhe_3]_4$
P_5	Boc	50% TFA/CH_2Cl_2
P_6	Bn	50% TFA/CH_2Cl_2
P_7	Z	HBr/AcOH

Scheme 1. Synthesis of cyclic PNA hexamer **1**.

a second step, the linker was added to close the PNA and geometric optimization was applied to both models. The optimal length was found to be the same in the two cases (17 atoms). An amide function was introduced in view of allowing an additional hydrogen bond to be formed with the RNA. The potential energy of the system was minimized.

To validate the design, molecular dynamic simulations in aqueous phase were performed. The global charge was neutralized with Mg^{2+} counter ions. All bond distances involving a hydrogen atom were constrained. Temperature was increased from 100 to 300 K during the first 80 ps of the simulation and then temperature and pressure were kept constant for 600 ps. At the same time, base pairs constraints were decreased. The detailed analysis of the trajectories for the two systems (A and B) showed a good recovering of all interactions. In the two cases, unpaired bases moved during the approach of system equilibrium to adopt and maintain a stable conformation. The three unpaired bases of the loop (A_{255}, A_{256} and A_{263}) were interiorized, minimizing thus the



Scheme 2. Synthesis of fully protected backbone **19**. *Reagents and conditions*: (a) 1 M NaOH, dioxane; (b) 50% TFA/CH_2Cl_2; (c) $ClCO_2iBu$, NMM, CH_2Cl_2; (d) 1 M LiOH, 0.8 M $CaCl_2$, *i*PrOH/H_2O 7/3; (e) 5% TCA/CH_2Cl_2.

interaction with the solvent, and were implied in π stacking interaction with Watson–Crick paired bases.

Based on energetic considerations, it can be concluded that the colinear model exhibits a better interaction mode, despite a solvent access surface minimized.

3. Chemistry

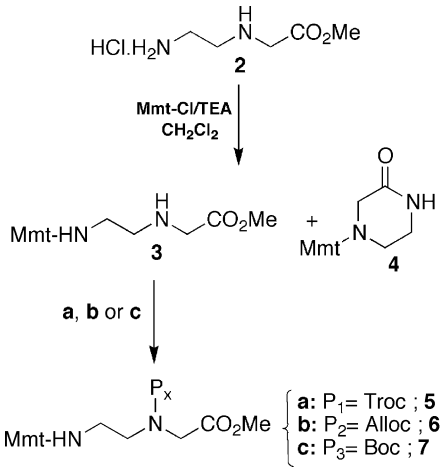
The FPB approach has been developed in our laboratory[13] to bring an alternative solution to the well establish solid-phase supported PNA syntheses. This strategy is more suitable in the case of smaller oligomers, less than decamers (higher overall yield, larger choices of solvents and reagents, better solubility of the different species). It consists of preparing a cyclic polyprotected backbone precursor, containing as many different and orthogonal protecting groups as there are different types of nucleic bases in the targeted PNA. A series of selective deprotection/coupling steps allows then the introduction of the required nucleobases on the cyclic framework.

The synthetic pathway of the target compound **1** is illustrated in Scheme 1. It consists first in condensing a suitable polyprotected hexameric backbone **19** with a linear amino acid spacer **26**. This condensation step is then followed by a cyclization reaction producing the synthetic loop **30**. The differently protected (guanine and cytosine) or unprotected (uracile) nucleobase acetic acid units are then introduced onto the cyclic backbone after sequential deprotection of the secondary amines. The last stage of the synthesis of **1** consists of nucleobases deprotection on **44**. As the target PNA **1** is constituted by three different nucleobases (cytidine, guanine and uracile), three orthogonal protecting groups (e.g. P_3, P_4 and P_5) have to be used for the protection of the secondary amines of the cyclic backbone. Moreover, they must be orthogonal with the protecting groups P_1 and P_2 of the amino and carboxy extremities of the linear precursors **19** and **27**. Furthermore, the guanine and cytidine acetic acid units have to be used in a protected form (e.g. P_6, P_7) in order to prevent side reactions and to improve solubility. This implies that the order of cleavage of P_3, P_4 and/or P_5 will also depend upon the nature of P_6 and/or P_7.

These requirements led us to select the acid-sensitive 4-methoxytrityl (Mmt) group as P_1, the base-labile methyl ester group as P_2 and P_3, P_4 and P_5 as respectively, the acid-stable trichloroethoxycarbonyl (Troc) and allyloxycarbonyl (Alloc), and the less acid-sensitive *tert*-butyloxycarbonyl Boc groups. The guanine and cytidine acetic acid units were protected by the benzyl (P_6: Bn) and benzyloxycarbonyl (P_7: Z) groups, respectively (Scheme 1). As the Bn and Boc groups are not orthogonal, this implies that the Boc protecting group should be removed prior to the *O*-benzyl-protected guanine unit introduction.

3.1. Synthesis of the linear polyprotected hexamer 19

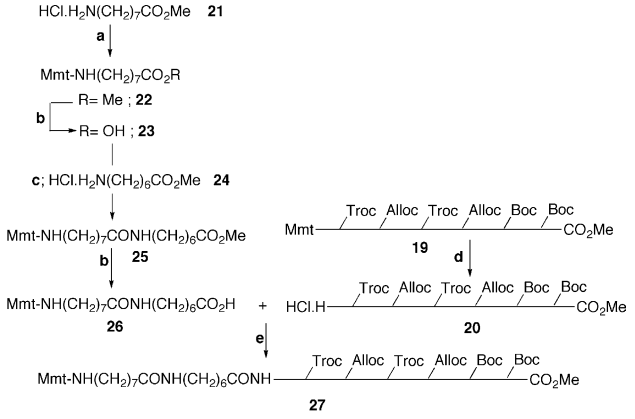
As shown in Scheme 2, a convergent process was applied to generate the key hexameric framework **19**. It required the synthesis of three monomers Mmt-[Troc]-OMe **5**, Mmt-[Alloc]-OMe **6** and Mmt-[Boc]-OMe **7** in order to



Scheme 3. Synthesis of protected monomers **5**, **6** and **7**. *Reagents and conditions*: (a) Troc-Cl or (b) Alloc-Cl or (c) $(Boc)_2O$, TEA, CH_2Cl_2.

prepare a set of three dimers, e.g. Mmt-[Troc-Alloc]-OH **11**, H-[Troc-Alloc]-OMe **12**, and H-[Boc-Boc]-OMe **18**. Condensation of the two preformed dimers **11** and **12** produced tetramer Mmt-[Troc-Alloc-Troc-Alloc]-OMe **15** which, after *C*-deprotection, was then coupled to the third dimer **18** to yield the target hexamer Mmt-[Troc-Alloc-Troc-Alloc-Boc-Boc]-OMe **19**.

The monomers **5**–**7** were synthesized from the Mmt-protected ester backbone **3** and from the respective reagents Troc-Cl, Alloc-Cl and $(Boc)_2O$ (97, 90, 95% yield, respectively) (Scheme 3). Particular precautions had to be taken when introducing the Mmt protection into backbone **2** with Mmt-Cl and triethylamine (TEA). Best results (90% yield of **3**) were obtained with dichloromethane as solvent and by adding TEA prior to Mmt-Cl to a cooled ($<-25°C$) solution of **2** (temperature was kept under $-25°C$ until completion). Without these precautions, by-product **4** was formed presumably by preferential monomethoxytritylation (after Mmt-Cl addition) of the secondary amine and subsequent intramolecular cyclization when TEA was added, or by intramolecular cyclization followed by Mmt-protection



Scheme 4. Synthesis of *N*-acylated fully protected hexamer **27**. *Reagents and conditions*: (a) Mmt-Cl, CH_2Cl_2, TEA; (b) 1 M LiOH, dioxane; (c) Bop, DIEA, CH_2Cl_2; (d) 0.115 M AcCl/MeOH; (e) HATU, HOAt, DIEA, CH_2Cl_2.

when the reaction was performed at a temperature above $-25°C$.[17]

The synthesis of dimers **10** and **16** required the acid-deprotected monomers **8** and **13** as well as the amine-deprotected **9** and **14**, respectively (Scheme 2). The acids **8** and **13** (82 and 90%, respectively) were obtained from their corresponding esters **5** and **7**, by saponification with 1 M LiOH in dioxane. Concerning the amine-deprotected derivatives **9** and **14**, they were prepared by cleavage of the Mmt group on the Alloc- and Boc-protected compounds **6** and **7**, respectively. Mmt-deprotection of **6** was performed almost quantitatively by means of a 50% TFA/dichloromethane solution. In the case of the acid-sensitive *N*-Boc derivative **7**, Mmt-deprotection was achieved by means of a weakly acidic 5% trichloroacetic acid (TCA)/dichloromethane solution. The corresponding TFA **9** and TCA **14** salts were obtained in 89 and 77% yields, respectively, after purification by silica gel chromatography. Finally, the condensation steps between **8** and **9**, and between **13** and **14** leading to dimers **10** and **16**, respectively, were performed with isobutylchloroformate in good yields (89 and 86%).

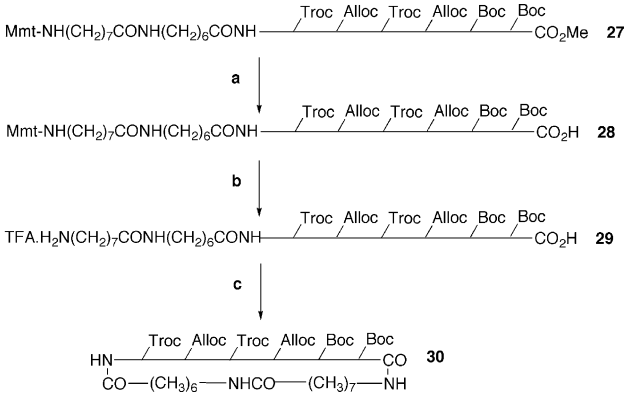
The synthesis of tetramer **15** was achieved in 81% yield by coupling the free acid and amine dimers **11** and **12** via the isobutylchloroformate method. These two compounds were obtained from **10** after methyl ester hydrolysis and Mmt-deprotection, respectively. Mmt-deprotection was achieved in 77% yield by submitting **10** to a 1/1 TFA/dichloromethane solution. Concerning the acid derivative **11**, its preparation could be significantly improved (91%, step d) when hydrolysis of **10** with 1 M LiOH was performed in a 0.8 M $CaCl_2$ solution of isopropanol/water. In the absence of $CaCl_2$, degradation was observed by HPLC monitoring. Such a 'protective' effect of $CaCl_2$ was also reported for the alkaline hydrolysis of Fmoc-protected peptide esters, $CaCl_2$ being efficient in preventing Fmoc cleavage.[18]

The synthesis of the targeted polyprotected hexamer **19** was achieved in 93% yield by isobutylchloroformate condensation of the tetrameric acid moiety **17** with the free amine dimer **18**. These two compounds were obtained by $CaCl_2$-catalyzed saponification of tetramer **15** (97%) and TCA-mediated Mmt-deprotection of dimer **16** (95% after silica gel chromatography), respectively.

3.2. Synthesis of compound 27: introduction of the spacer

The next stage of the synthesis of **1** consisted in the preparation of the fully protected hexamer–spacer conjugate **27** (Scheme 4).

This required first Mmt-deprotection of **19** to produce **20** which was subsequently condensed with spacer **26** by means of the HATU/HOAt reagent to give **27** (76%). The Mmt-deprotection of **19** was performed selectively and smoothly (94% yield) using a 0.115 M methanolic solution of HCl, generated in situ by addition of acetyl chloride to methanol.[19] When a 5% TCA/dichloromethane solution was used, as described previously for the Mmt-deprotection of the Boc-containing compounds **7** and **16**, the corresponding



Scheme 5. Synthesis of fully protected cyclic backbone **30**. *Reagents and conditions*: (a) 1 M LiOH, 0.8 M $CaCl_2$, *i*PrOH/H_2O 7/3; (b) 0.1/10 TFA/CH_2Cl_2; (c) HATU, HOAt, DIEA, DMF.

trichloroacetate salt of **20** was obtained with moderate yields (40%). The spacer **26** was prepared starting from commercially available amino alkyl esters **21** and **24**. Mmt protection of **21** followed by saponification of the resulting ester **22** with 1 M LiOH, provided **23** in 80% overall yield. Coupling of **23** with **24** by means of the Bop activation procedure afforded the protected spacer **25**, which after saponification with 1 M LiOH, led to compound **26** (85% for the two steps).

3.3. Synthesis of the protected loop 30: cyclization of the spacer–hexamer conjugate 27

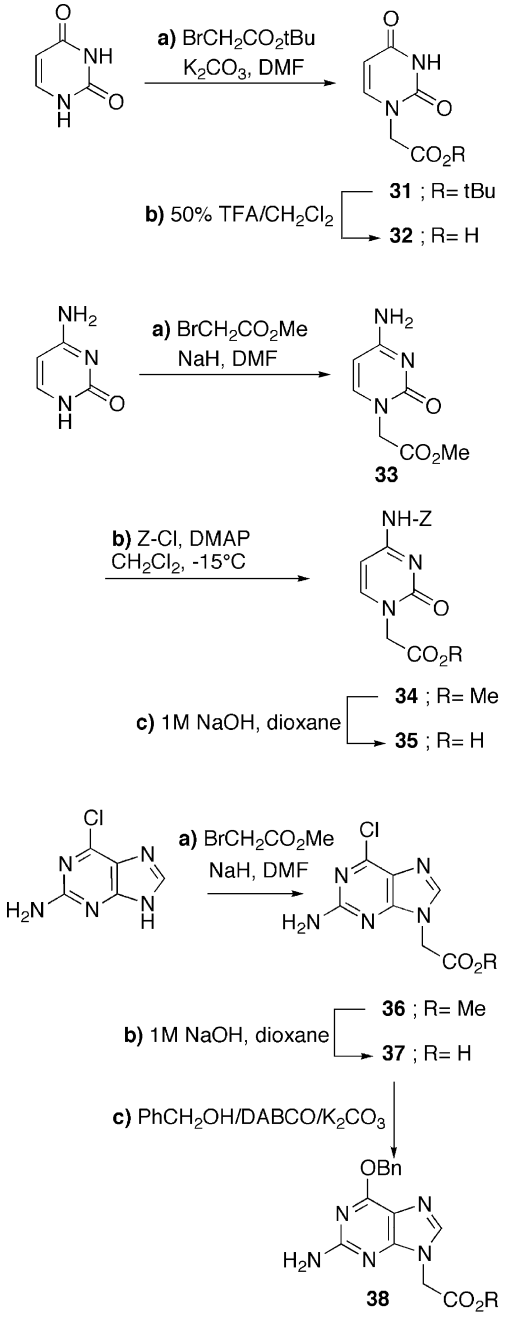
As shown in Scheme 5, the protected loop **30** was obtained by deprotection of the C- and N-terminal extremities of its linear precursor **27**, followed by head-to-tail cyclization of the resulting compound **29**.

$CaCl_2$-catalyzed saponification of **27** afforded the free carboxylic compound **28** in almost quantitative yield. The removal of the Mmt group on **28** was best performed with a 1% TFA/CH_2Cl_2 solution. TFA salt of **29** could be isolated with 88% yield after 6 h of reaction. The use of a dichloromethane solution of 0.115 M HCl, generated from stoichiometric amounts of acetyl chloride and methanol, gave also the chlorhydrate salt of **29** in good yield (82%) but the reaction progressed slower (35 h). The head-to-tail cyclization of **29** was accomplished in good yield (66%) using HATU/HOAt activation and semi-high dilution conditions ($\approx 10^{-2}$ M) in order to limit oligomerization. The key macrocycle **30** was isolated after a silica gel column chromatography, and its structure and purity were clearly attested by NMR, mass spectrometry and HPLC analyses.

3.4. Synthesis of the hexa-PNA loop 1

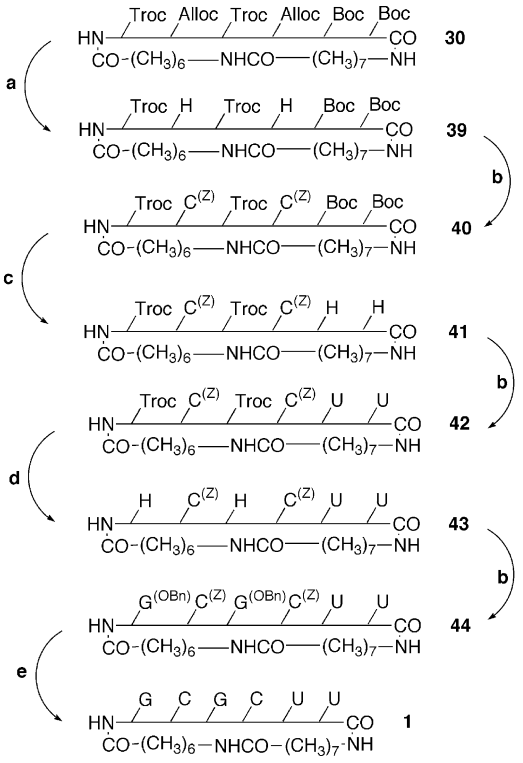
Conversion of the orthogonally hexa-protected cyclic precursor **30** into the target PNA loop **1** required the preparation of the three nucleobase acetic acid moieties **32**, **35** and **38** (Scheme 6).

The synthesis of the uracil compound **32** was carried out in 55% overall yield after (i) alkylation of uracil with *tert*-butyl bromoacetate and (ii) subsequent hydrolysis of the corresponding ester **31** with TFA. The Z-cytosine derivative **35**



Scheme 6. Synthesis of protected base acetic acid units.

was prepared in 43% overall yield from cytosine after (i) alkylation with methyl bromoacetate, (ii) Z-protection of the exocyclic amine of **33** using Z-Cl/DMAP reagents and (iii) saponification of the corresponding methyl ester **34**. The guanine derivative **38** was prepared in three steps starting from 2-amino-6-chloropurine in 48% overall yield. These steps consisted into (i) alkylation with methyl bromoacetate, (ii) saponification, (iii) replacement of the 6-chloro atom by the benzyloxy (OBn) group. This substitution, which is most commonly achieved using sodium benzyloxide,[20] could be improved when performed via a quaternary DABCO-purine salt,[21] which underwent facile displacement in refluxing benzylic alcohol, in presence of K_2CO_3. Indeed, compound **38** was isolated in 81% yield



Scheme 7. Synthesis of compound **1** from **30**. *Reagents and conditions*: (a) $Pd[PPhe_3]_4$, DEA, $CHCl_3$; (b) **35** or **32** or **38**, HATU, HOAt, DIEA, DMF; (c) 1/1 TFA/$CHCl_3$; (d) Cd/AcOH, DMF; (e) HBr/AcOH, DMF.

instead of 65% following the standard described protocol (i.e. BnONa).

Concerning the introduction of these three nucleobase acetic acid units onto the cyclic backbone **30** (Scheme 7), the unique requirement was that the Boc-protecting groups should be removed prior to the *O*-benzylated guanine units introduction, as the Bn protecting group would be cleaved in the conditions of Boc-deprotection (1/1 TFA/CH_2Cl_2). Taking this into account, we chose to replace first the two Alloc groups by two N-Z cytosine units, then the Boc groups by two uracile units, to finally introduce the *O*-Bn guanine units in the last stage after Troc-deprotection.

As shown in Scheme 7, the two Alloc protecting groups were cleanly removed from **30** with $Pd[PPh_3]_4$ as allyl acceptor and DEA, giving **39** in 84% yield after a silica gel column chromatography. The N-Z cytosine acetic acid units **35** were then condensed onto **39** by means of HATU/HOAt activation and **40** was isolated in 75% yield after purification by silica gel chromatography. The subsequent trifluoroacetic acid deprotection of the two Boc groups allowed to obtain the corresponding TFA salt of **41** in almost quantitative yield after precipitation with diethyl ether. Simultaneous attachment of two uracile acetic acid units **32** onto **41** via the HATU/HOAt activation afforded **42** (70%) after a LH20 Sephadex purification. The next step was the cleavage of the two Troc groups by means of cadmium in acetic acid. Although the conversion of **42** into **43** seemed to occur cleanly, as shown by HPLC monitoring, a tedious purification by LH20 and XAD-2 column chromatographies was necessary to remove the cadmium salts and to isolate the acetate salt of **43** in 40% yield. The HATU/HOAt-mediated condensation of two benzyl-protected guanine acetic acid units **38** onto **43** afforded the protected hexa-PNA loop **44** in 55% yield after purification (LH20 Sephadex chromatography).

The synthesis of the target compound **1** was achieved by submitting compound **44** to HBr/acetic acid which induced the simultaneous deprotection of both the cytosine and guanine nucleobases. The water-soluble compound **1** was isolated after purification by semi-preparative HPLC. Its purity was demonstrated by HPLC analyses and its structure confirmed by MALDI-TOF and HR-MS experiments.

4. Inhibition of HIV-1dimerization

Compound **1** was evaluated for its ability to interfere in vitro with the dimerization process of the HIV-1 genome. A non competitive test was performed by incubating denatured 1–311 HIV-1 sequence with denatured compound **1** at different ratios, followed by agarose gel electrophoresis analyses (Fig. 5). An inhibition effect was observed for the complex RNA/PNA **1** at the ratio 1/50. This high needed excess could be attributed to a rapid PNA **1** self-aggregation (due to its palendromic nature) in the test being used. Further studies concerning the mechanism of action of compound **1** are currently in progress and the results will be disclosed in due course.

5. Conclusion

A loop containing a hexa-PNA sequence complementary of six of the nine residues of the RNA DIS loop of HIV-1, has been successfully synthesized following a liquid-phase FPB procedure, via a cyclic protected hexameric framework.

Preliminary biological results have revealed the ability of the cyclic PNA loop **1** to interfere with the dimerization process of the HIV-1 genome. This encouraging result indicates that this new kind of cyclic PNA conjugates of low molecular weight can be considered as good candidates in an 'antisense-like' approach for targeting RNA loops involved in crucial steps of different virus development. The original synthetic FPB strategy is currently exploited in our laboratory for the syntheses of cyclic PNA polymers targeting other essential non palindromic loops of the

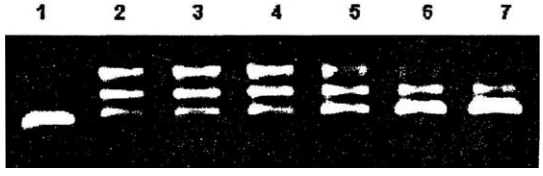


Figure 5. Agarose gel electrophoresis analyses of the inhibition of the 1–311 HIV-1 LAI dimerization by PNA **1**. Lane 1: incubation of 1–311 LAI RNA under low salt-no magnesium conditions (monomer reference). Lane 2: incubation of 1–311 LAI RNA under high salt-high magnesium conditions (dimer reference: the intermediate spot may represent the kissing loop complex). Lane 3–7: incubation of 1–311 LAI RNA in presence of 1, 10, 50, 100 and 134 equiv. of PNA **1** under dimerization conditions.

genomic HIV RNA as well as other viral RNA such as HCV.

6. Experimental

Unless otherwise noted, all commercial materials were used without further purification. ^{1}H and ^{13}C NMR spectra were obtained at 200 MHz in $CDCl_3$, CD_3OD or DMSO d_6 solutions. The NMR spectra of monomers displayed a doubling of signals caused by the presence of an equilibrium mixture of the *E* and *Z* isomers generated by the substituted amide bond. The minor form was indicated in italic. In the case of polymers '*N*-mers', the putative 2^N isomers could not be differentiated, the corresponding broad signals were then designated by the shift displacements of the beginning and the end of the signal. Thin layer chromatographies (TLC) were run on precoated silica gel plates (Merck, SDS 60F 254, 0.2 mm). Column chromatographies were carried out on silica gel (Merck, SDS 60A, 70–200 μm), on Sephadex (Sigma, LH20, 25–100 μm) or on Amberlite (Aldrich, XAD-2, 20–60 mesh). HPLC analyses were performed with a 996 photodiode array detector (195–290 nm), using a RP-18 (5 μm) Lichrospher 100 (250×4 mm) column as support. HPLC purifications were performed using a RP-18 (5 μm) Lichrospher (250×10 mm) column at 205 and 254 nm. Elution solvents A: H_2O (0.1% TFA), B: CH_3CN (0.1% TFA) and C: H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 60:40 were used with a flow of 1 mL/min for analyses or 2 mL/min for purifications.

The syntheses of backbone **2**, monomers Mmt-[Troc]-OMe **5**, Mmt-[Alloc]-OMe **6**, and TFA·H_2N-[Alloc]-OMe **9** have been previously described.[13,17]

Abbreviations used are as follow: AcOH, acetic acid; Alloc-Cl, allylchloroformate; $(Boc)_2O$, di-tert-butyl dicarbonate; Bop, benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium-hexafluorophosphate; DEA, diethylamine; DIPEA, diisopropylethylamine; Fmoc, fluoren-9-ylmethoxycarbonyl; HATU, *O*-(7-aza-1-benzotriazolyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexa-fluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; Mmt-Cl, 4-methoxytrityl chloride; NMM, *N*-methyl morpholine; TEA, triethylamine; Troc-Cl, trichloroethylchloroformate. Z-Cl, benzylchloroformate.

6.1. Dynamic molecular modelling studies

The two models A and B were built with Insight II molecular modelling package. Simulations were carried out with the AMBER Software. The AMBER 96 force field was chosen, the aqueous phase was elaborated from TIP3P water molecules.

6.2. Chemistry

6.2.1. Methyl *N*-[2-(monomethoxytrityl)aminoethyl] glycinate (3). Compound **2** (5 g, 24.4 mmol) was suspended in CH_2Cl_2 (300 mL) and the mixture was cooled to −40°C. The suspension was treated with TEA (10.2 mL, 73.2 mmol) with vigorous stirring then MmtCl (7.53 g, 24.4 mmol) was added portionwise so that the temperature was kept under −25°C. The mixture was stirred for 1 h at low temperature then allowed to warm to room temperature. MeOH (60 mL) was added and the solution was concentrated in vacuo. The residue was treated with EtOAc. The organic solution was washed with water then evaporated under reduced pressure. The residue was then chromatographed on silica gel (from EtOAc/hexane 1:1 to EtOAc) affording 9.0 g of **3** (91%). TLC (EtOAc/Hexane 1:1) R_f=0.26. ^{1}H NMR ($CDCl_3$) δ 7.60–6.80 (14H, m), 3.77 (3H, s), 3.71 (3H, s), 3.35 (2H, s), 2.73 (2H, t), 2.26 (2H, t), 1.86 (1H, bs). ^{13}C NMR ($CDCl_3$) δ 173.2, 158.0, 146.6, 138.5, 128.7, 127,9, 126.3, 112.3, 70.3, 55.4, 51.9, 50.7, 49.9, 43.2. MS (ESI+) *m/z* 427.2 $(M+Na)^+$.

6.2.2. Characterization of compound 4. TLC (EtOAc) R_f=0.43. ^{1}H NMR ($CDCl_3$) δ 7.60–6.80 (14H, m), 3.85 (3H, s), 3.50 (2H, m), 3.12 (2H, s), 2.50 (2H, m). ^{13}C NMR ($CDCl_3$) δ 171.0, 158.0, 148.0, 142.0, 130.6, 129.1, 128.0, 126.6, 113.3, 76.1, 55.3, 52.5, 45.0, 41.8. MS (ESI+) *m/z* 699.4 $(M+Na)^+$.

6.2.3. Methyl *N*-[2-(*N*-monomethoxytrityl)aminoethyl]-*N*-Boc glycinate (Mmt-[Boc]-OMe) (7). Compound **3** (2.20 g, 5.44 mmol) and TEA (0.210 mL, 1.48 mmol) were placed in CH_2Cl_2 (80 mL) at 0°C. $(Boc)_2O$ (1.18 g, 5.44 mmol) was then added and the mixture was stirred for 5 h at 0°C. The solvent was then evaporated in vacuo. The residue was taken up in EtOAc and the organic layer was washed with water and brine and finally dried over Na_2SO_4. After solvent evaporation, the residue was chromatographed on silica gel (EtOAc/hexane 4:6) to afford **7** as a yellow resin (2.60 g, 95%). TLC (EtOAc/hexane 6:4) R_f=0.54. ^{1}H NMR ($CDCl_3$) δ 7.40–6.70 (14H, m), 3.88, 3.81 (2H, m), 3.69 (3H, s), 3.60 (3H, s), 3.40–3.25 (2H, m), 2.25–2.15 (2H, m), 1.80 (1H, bs), 1.36, 1.33 (9H, s). ^{13}C NMR ($CDCl_3$) δ 170.8, 158.0, 156.0, 146.5, 138.4, 129.9, 128.7, 128.0, 126.4, 113.3, 80.4, 70.5, 55.3, 52.1, 49.6, 49.5, 49.1, 28.4. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=18.83 min, λ_{max}=210.9 nm.

6.3. Standard protocol for the synthesis of Mmt-[Troc]-OH (8) and Mmt-[Boc]-OH (13)

An aqueous 1 M LiOH solution (2 equiv.) was added to a stirred solution of **5** or **7** (1 equiv.) in dioxane (2 mL/mmol) at room temperature (rt). The reaction was stirred for 3 h then the solution was concentrated in vacuo. After dilution with water (10 mL/mmol), the pH was adjusted to 5 with an aqueous 5% citric acid solution. The aqueous solution was then extracted with EtOAc. The organic layer was washed with water and with brine, dried over Na_2SO_4 then evaporated in vacuo.

6.3.1. Mmt-[Troc]-OH (8). The above procedure was followed starting from **5** (3.85 g, 6.65 mmol). Purification on silica gel column gave **8** as a colorless resin (3.40 g, 90%). TLC (EtOAc/MeOH 8:2) R_f=0.48. ^{1}H NMR ($CDCl_3$) δ 7.65–6.75 (14H, m), 4.75, 4.62 (2H, s), 3.90–3.78 (2H, m), 3.72 (3H, s), 3.65–3.50 (2H, m), 3.05–2.75 (2H, m). ^{13}C NMR ($CDCl_3$) δ 174.8, 159.2, 159.1, 154.7, 141.6, 130.7, 128.8, 128.6, 127.8, 113.9, 95.4, 95.0, 75.7, 75.4, 74.0, 73.6, 55.4, 53.1, 52.8, 48.2, 47.6, 44.5, 43.4.

6.3.2. Mmt-[Boc]-OH (13). The above procedure was followed starting from **7** (2.16 g, 4.30 mmol). Purification on silica gel column gave **13** as a colorless resin (1.71 g, 82%). TLC (EtOAc/MeOH 8:2) R_f=0.41. ^{1}H NMR ($CDCl_3$) δ 7.55–6.65 (14H, m), 3.65 (5H, m), 3.49 (2H, m), 2.90–2.50 (2H, m), 1.38 (10H, m). ^{13}C NMR ($CDCl_3$) δ 175.7, 159.2, 156.0, 141.3, 132.7, 130.8, 128.8, 128.6, 127.9, 113.9, 80.8, 73.6, 55.4, 53.1, 47.3, 44.0, 29.8.

6.4. Standard protocol for TCA-mediated Mmt deprotection. Synthesis of TCA salts 14 and 18

Compounds **7** or **16** (1 equiv.) was dissolved in a solution of CH_2Cl_2 (7 mL/mmol) containing TCA (4 equiv.). The solution was stirred for 4.5 h then MeOH (10 mL/mmol) was added. The solvent was removed under reduced pressure and the crude residue was purified on silica gel column chromatography.

6.4.1. TCA·H_2N-[Boc]-OMe (14). Following the above procedure and starting from **7** (1.50 g, 2.97 mmol) afforded **14** (900 mg, 77%) as an orange colored resin, after a chromatography purification (from EtOAc to MeOH). TLC (EtOAc/MeOH/AcOH 8:2:0.1) R_f=0.42. ^{1}H NMR ($CDCl_3$) δ 8.33 (3H, bs), 4.07, 4.01 (2H, s), 3.77 (3H, m), 3.66 (2H, m), 3.31 (2H, m), 1.47, 1.40 (9H, s). ^{13}C NMR ($CDCl_3$) δ 172.8, 165.9, 156.0, 96.2, 82.2, 51.3, 50.8, 47.8, 39.4, 28.3. MS (ESI+) *m/z* 233.0 $(M+H)^+$. *m/z* 255 $(M+Na)^+$. HPLC (A/B 60:40 to 0:100 over 30 min) R_t= 4.35 min, λ_{max}=202.7 nm.

6.4.2. TCA·H_2N-[Boc-Boc]-OMe (18). Following the above procedure and starting from **16** (1.35 g, 1.91 mmol) afforded **18** (1.07 g, 95%) as an colorless resin, after a chromatography purification (from EtOAc to MeOH 100%). TLC (EtOAc/MeOH/AcOH 8:2:0.1) R_f=0.41. ^{1}H NMR ($CDCl_3$) δ 7.60 (1H, m), 4.10–3.80 (4H, m), 3.75 (3H, s), 3.64 (2H, m), 3.55–3.20 (6H, m), 1.43, 1.40 (18H, s). ^{13}C NMR ($CDCl_3$) δ 172.2, 165.9, 156.0, 155.8, 96.3, 81.5, 81.4, 52.5, 52.0–39.0, 28.4. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=4.35 min, λ_{max}=202.7 nm.

6.5. Standard protocol for coupling with isobutyl chloroformate

Synthesis of Mmt-[Troc-Alloc]-OMe **(10)**, Mmt-[Boc-Boc]-OMe **(16)**, Mmt-[Troc-Alloc-Troc-Alloc]-OMe **(15)** and Mmt-[Troc-Alloc-Troc-Alloc-Boc-Boc]-OMe **(19)**. To a cooled solution (−15°C) of **8** or **13** or **11** or **17** (1 equiv.) and NMM (2 equiv.) in CH_2Cl_2 (10 mL/mmol) was added dropwise a solution of isobutyl chloroformate (1 equiv.) in CH_2Cl_2 (1 mL/mmol). After 15 min of stirring, **9** or **14** or **12** or **18** (1.2 equiv.) and NMM (1.5 equiv.) were added at −15°C. The mixture was stirred for 1 h at low temperature then the solvent was evaporated under reduced pressure. The residue was taken up in EtOAc and washed with water. The solution was dried over Na_2SO_4. Solvent evaporation afforded a crude product, which was subsequently purified using a silica gel chromatography.

6.5.1. Mmt-[Troc-Alloc]-OMe (10). The above procedure was followed starting from **8** (3.07 g, 5.43 mmol) and from **9** (2.15 g, 6.51 mmol). Purification by silica gel chromatography (EtOAc/hexane 1:1) afforded **10** (3.81 g, 89%) as an orange colored resin. TLC (EtOAc/hexane 7:3) R_f=0.43. ^{1}H NMR ($CDCl_3$) δ 7.40–6.65 (14H, m), 5.95–5.65 (1H, m), 5.30–5.05 (2H, m), 4.71, 4.61 (2H, m), 4.60–4.45 (2H, m), 4.00–3.85 (4H, m), 3.68 (3H, s), 3.65 (3H, s), 3.50–3.22 (6H, m), 2.30 (2H, m), 1.86 (1H, bs). ^{13}C NMR ($CDCl_3$) δ 174.0, 168.8, 158.1, 155.2, 155.0, 146.1, 138.0, 132.6, 129.8, 128.6, 128.0, 126.5, 118.0, 117.6, 113.4, 95.6, 95.3, 75.6, 75.3, 70.5, 66.8, 66.6, 55.3, 52.5, 51.4, 50.2, 49.6, 48.8, 42.3, 38.3. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=20.43 min, λ_{max}=206.2 and 232.0 nm.

6.5.2. Mmt-[Boc-Boc]-OMe (16). The above procedure was followed starting from **13** (1.10 g, 2.24 mmol) and from **14** (976 mg, 2.46 mmol). Purification by silica gel chromatography (EtOAc/hexane 6:4) afforded **16** (1.35 g, 86%) as a colorless resin. TLC (EtOAc) R_f=0.69. ^{1}H NMR ($CDCl_3$) δ 7.40–6.65 (14H, m), 3.90–3.75 (4H, m), 3.68 (3H, s), 3.64 (3H, s), 3.45–3.15 (6H, m), 2.40–2.15 (2H, m), 1.32 (18H, s). ^{13}C NMR ($CDCl_3$) δ 171.2, 170.0, 158.1, 155.9, 155.6, 146.3, 138.1, 129.9, 128.6, 128.0, 126.4, 113.3, 80.8, 80.6, 70.5, 55.3, 52.3, 53.6, 50.6, 49.3, 48.5, 42.4, 38.4, 28.5. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=22.15 min, λ_{max}=203.9 and 232.0 nm.

6.5.3. Mmt-[Troc-Alloc-Troc-Alloc]-OMe (15). The above procedure was followed starting from **11** (1.08 g, 1.44 mmol) and **12** (1.04 g, 1.73 mmol). Purification by silica gel chromatography (EtOAc/MeOH 8:2) afforded **15** (1.43 g, 81%) as a colorless resin. TLC (EtOAc/MeOH 8:2) R_f=0.52. ^{1}H NMR ($CDCl_3$) δ 7.60–6.75 (17H, m), 6.00–5.80 (2H, m), 5.40–5.10 (4H, m), 4.80–4.48 (8H, m), 4.10–3.30 (30H, m). ^{13}C NMR ($CDCl_3$) δ 175.0–166.0, 158.0, 156.1, 154.8, 145.9, 145.5, 132.4, 128.5, 128.0, 126.4, 113.3, 95.0, 73.3, 71.0, 65.9, 55.2, 52.5, 53.0–35.0. MS (ESI+) *m/z* 1223.8 $(M+H)^+$. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=22.64 min, λ_{max}=210.9 and 230.9 nm.

6.5.4. Mmt-[Troc-Alloc-Troc-Alloc-Boc-Boc]-OMe (19). The above procedure was followed starting from **17** (1.11 g, 0.92 mmol) and **18** (601 mg, 1.00 mmol). Purification by silica gel chromatography (EtOAc/MeOH 8:2) afforded **19** (1.38 g, 93%) as a colorless resin. TLC (EtOAc/MeOH 8:2) R_f=0.38. ^{1}H NMR ($CDCl_3$) δ 7.50–6.75 (19H, m), 6.05–5.75 (2H, m), 5.42–5.05 (4H, m), 4.05–3.25 (42H, m), 1.40 (18H, s). ^{13}C NMR ($CDCl_3$) δ 173.3–168.2, 157.9, 157.0, 156.3, 155.5, 154.5, 146.1, 145.9, 137.8, 132.7, 129.6, 128.4, 127.9, 126.4, 117.2, 113.2, 94.1, 80.7, 75.3, 70.4, 66.4, 65.9, 55.2, 52.3, 51.0–38.7, 28.3. MS (ESI+) *m/z* 1647.9 $(M+Na)^+$. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=24.15 min, λ_{max}=205.1 and 229.7 nm.

6.6. Standard protocol for $CaCl_2$-catalyzed saponification. Synthesis of compounds 11 and 17

Compound **10** or **15** (1 equiv.) was dissolved in a 0.8 M $CaCl_2$ solution of isopropanol/water 7:3 (15 mL/mmol). An aqueous solution of 1 M LiOH (2 equiv.) was then added at rt. After 6 h of stirring, the mixture was diluted with ice-cold water and then cautiously acidified until pH 5 using an aqueous solution of 5% citric acid. The aqueous layer was extracted with EtOAc and the organic solution

was washed with water then dried over Na_2SO_4. The solvent was evaporated in vacuo.

6.6.1. Mmt-[Troc-Alloc]-OH (11). The above procedure was followed starting from **10** (500 mg, 0.65 mmol). Silica gel chromatography purification (gradient from EtOAc to EtOAc/MeOH 1:1) afforded **11** (447 mg, 91%) as a yellow colored resin. TLC (EtOAc/MeOH/AcOH 8:2:0.1) R_f=0.48. ^{1}H NMR ($CDCl_3$) δ 7.55–6.86 (15H, m), 6.05–5.75 (1H, m), 5.42–5.05 (2H, m), 4.90–4.55 (4H, m), 4.35–4.05 (4H, m), 4.05–3.50 (13H, m), 2.82 (2H, m). ^{13}C NMR ($CDCl_3$) δ 174.8, 168.8, 158.3, 155.1, 145.0, 136.5, 132.8, 130.1, 128.7, 128.2, 126.9, 117.9, 117.5, 113.5, 95.4, 95.2, 71.0, 66.7, 66.5, 54.0–42.0. MS (ESI−) *m/z* 747.3 $(M-H)^-$. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=17.39 min, λ_{max}=205.1 and 232.0 nm.

6.6.2. Mmt-[Troc-Alloc-Troc-Alloc]-OH (17). The above procedure was followed starting from **15** (1.04 g, 0.85 mmol). **17** (980 mg, 95%) was obtained as a colorless resin. TLC (EtOAc/MeOH 8:2) R_f=0.34. ^{1}H NMR ($CDCl_3$) δ 7.45–6.75 (17H, m), 6.05–5.75 (2H, m), 5.45–5.05 (4H, m), 4.70–4.15 (8H, m), 4.05–3.25 (27H, m). ^{13}C NMR ($CDCl_3$) δ 171.1, 170.0–168.0, 158.0, 157.2, 156.4, 145.8, 139.2, 132.6, 129.2, 128.4, 127.9, 127.1, 117.8, 117.3, 113.2, 97.5, 76.2, 70.5, 65.9, 55.2, 52.3–37.4. MS (ESI−) *m/z* 1208.0 $(M-H)^-$. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=20.03 min, λ_{max}=208.6 and 230.9 nm.

6.6.3. TFA·H_2N-[Troc-Alloc]-OMe (12). Compound **10** (1.98 g, 2.59 mmol) was dissolved in a 1:1 TFA/CH_2Cl_2 solution (26 mL). The mixture was stirred for 2 h at rt then the solvent was evaporated under reduced pressure. The crude residue was purified using a silica gel column chromatography (from EtOAc to MeOH 100%) giving **10** as an orange colored resin (1.20 g, 77%). TLC (EtOAc/MeOH/AcOH 8:2:0.1) $R_{f=}$0.44. ^{1}H NMR ($CDCl_3$) δ 8.85–8.67 (3H, bs), 7.74–7.55 (1H, m), 5.94–5.79 (1H, m), 5.31–5.16 (2H, m), 7.72, 4.69 (2H, m), 4.61–4.54 (2H, m), 4.05–3.95 (4H, m), 3.76–3.72 (5H, m), 3.50–3.37 (4H, m), 3.30–3.20 (2H, m). ^{13}C NMR ($CDCl_3$) δ 171.8, 171.4, 154.4, 132.4, 117.7, 95.1, 75.6, 66.8, 52.6, 48.0–51.0, 36.8–31.7. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=4.52 min, λ_{max}=202.7 nm.

6.6.4. HCl·H_2N-[Troc-Alloc-Troc-Alloc-Boc-Boc]-OMe (20). Compound **19** (711 mg, 0.438 mmol) was dissolved at 0°C in a methanolic 0.115 M acetyl chloride solution (4.53 mL), prepared by adding acetyl chloride (100 μL, 1.4 mmol) to an ice cold solution of MeOH (12 mL). The mixture was stirred for 21 h at rt then ether (150 mL) was added. The resulting white precipitate was filtered off affording the salt **20** (573 mg, 94%). TLC (EtOAc/MeOH/AcOH 8:2:0.1) R_f=0.44. ^{1}H NMR ($CDCl_3$) δ 8.24 (3H, bs), 6.05–5.75 (2H, m), 5.40–5.10 (4H, m), 4.85–4.65 (4H, m), 4.65–4.45 (4H, m), 4.30–3.10 (39H, m), 1.37 (18H, m). ^{13}C NMR ($CDCl_3$) δ 170.9, 170.2, 169.8, 156.3, 155.6, 15.6, 154.3, 132.8, 117.8, 117.4, 95.4, 95.1, 81.0, 80.8, 75.5, 66.5, 52.3, 52.0–38.0, 28.4. MS (ESI+) *m/z* 1351.2 $(M+H)^+$. HPLC (A/B 60:40 to 0:100 over 30 min) R_t= 12.97 min, λ_{max}=202.7 nm.

6.6.5. Mmt-$(CH_2)_7$-COOMe (22). To a ice cold solution of **21** (500 mg, 2.38 mmol) in CH_2Cl_2 (7 mL) were added TEA (830 μL, 5.95 mmol) and Mmt-Cl (740 mg, 2.38 mmol) portionwise. The mixture was stirred for 1 h at 0°C then the solvent was evaporated in vacuo. The residue was taken up in EtOAc and the organic layer was washed with water then dried over Na_2SO_4. Evaporation of the solvent gave **22** (1.03 g, 97%) as a yellow colored resin. TLC (EtOAc/hexane 7:3) R_f=0.60. ^{1}H NMR ($CDCl_3$) δ 7.55–6.75 (14H, m), 3.80 (3H, s), 3.67 (3H, s), 2.30 (2H, m), 2.13 (2H, m), 1.85–1.20 (10H, m). ^{13}C NMR ($CDCl_3$) δ 174.3, 157.9, 146.7, 138.6, 129.9, 128.7, 127.8, 126.2, 113.2, 70.5, 55.2, 51.5, 43.7, 34.2, 30.9, 29.3, 29.2, 27.3, 25.0. HPLC (A/B 60:40 to 0:100 over 30 min) R_t= 15.64 min, λ_{max}=212.2 nm.

6.6.6. Mmt-$(CH_2)_7$-COOH (23). Compound **23** was prepared following the same procedure as for **8**, starting from **22** (2.04 g, 4.58 mmol). Purification on silica gel column chromatography (EtOAc/hexane: from 2:8 to 1:1) afforded **23** (1.58 g, 80%) as a yellow colored resin. TLC (EtOAc/hexane 1:1) R_f=0.76. ^{1}H NMR ($CDCl_3$) δ 7.55–6.75 (14H, m), 5.06 (1H, bs), 3.75 (3H, s), 2.31 (2H, m), 2.14 (2H, m), 1.70–1.15 (10H, m). ^{13}C NMR ($CDCl_3$) δ 179.9, 158.1, 146.3, 138.2, 130.0, 128.8, 127.9, 126.4, 113.3, 70.9, 55.3, 43.9, 34.5, 30.6, 29.3, 29.2, 27.3, 24.9. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=11.88 min, λ_{max}=210.9 and 232.0 nm.

6.6.7. Mmt-$(CH_2)_7$-CONH-$(CH_2)_6$-COOMe (25). To a solution of **23** (1.08 g, 2.5 mmol) and **24** (490.0 mg, 2.5 mmol) in CH_2Cl_2 (4 mL) were added DIEA (1.31 mL, 7.5 mmol) and Bop (1,10 g, 2.5 mmol). The mixture was stirred for 2 h at rt then the solvent was removed under reduced pressure. The crude residue was solubilized in EtOAc, washed with water, dried over Na_2SO_4 then evaporated in vacuo. The crude residue was chromatographed on silica gel (from EtOAc/hexane 6:4 to EtOAc) to furnish **25** (1.31 g, 92%) as an yellow colored resin. TLC (EtOAc/hexane 1:1) R_f=0.30. ^{1}H NMR ($CDCl_3$) δ 7.50–6.70 (14H, m), 5.52 (1H, m), 3.78 (3H, s), 3.65 (3H, s), 3.19 (2H, m), 2.29 (2H, m), 2.08 (4H, m), 1.94 (1H, bs), 1.70–1.10 (18H, m). ^{13}C NMR ($CDCl_3$) δ 174.2, 173.1, 157.9, 146.7, 138.6, 129.9, 128.7, 127.8, 126.1, 113.1, 70.4, 55.2, 51.5, 43.6, 39.4, 36.9, 34.0, 30.9, 29.5, 29.4, 29.3, 28.8, 27.3, 26.6, 25.8, 24.8. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=16.22 min, λ_{max}=206.2 nm.

6.6.8. Mmt-$(CH_2)_7$-CONH-$(CH_2)_6$-COOH (26). Compound **26** was prepared following the same procedure as for **23**, starting from **25** (430 mg, 0.75 mmol). Purification on silica gel column chromatography (from EtOAc/hexane 7:3 to EtOAc) afforded **26** (391 mg, 94%) as a colorless resin. TLC (EtOAc/hexane 7:3) R_f=0.54. ^{1}H NMR ($CDCl_3$) δ 7.55–6.80 (14H, m), 5.81 (1H, m), 5.65 (1H, m), 3.78 (3H, s), 3.21 (2H, m), 2.35–2.00 (6H, m), 1.25–1.10 (18H, m). ^{13}C NMR ($CDCl_3$) δ 176.4, 173.4, 157.8, 145.9, 137.8, 129.8, 128.5, 127.7, 126.2, 113.0, 70.7, 55.1, 43.7, 39.3, 36.7, 30.3, 29.3, 29.2, 29.1, 28.5, 27.1, 26.4, 25.7, 24.7. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=12.33 min, λ_{max}=205.1 and 230.9 nm.

6.6.9. Mmt-$(CH_2)_7$-CONH-$(CH_2)_6$-CONH-[Troc-Alloc-Troc-Alloc-Boc-Boc]-OMe (27). To an ice cold solution

of **26** (131 mg, 0.236 mmol) and **20** (328 mg, 0.236 mmol) in CH_2Cl_2 (5 mL) were added DIEA (246 μL, 1.41 mmol) HOAt (64.2 mg, 0.472 mmol) and HATU (108 mg, 0.283 mmol). The mixture was stirred for 2 h at rt then the solvent was removed under reduced pressure. The residue was solubilized in EtOAc, washed with water and with aqueous 5% $NaHCO_3$, dried over Na_2SO_4 then evaporated in vacuo. The crude residue was chromatographed on silica gel (from EtOAc to EtOAc/MeOH 1:1) to furnish **27** (340 mg, 76%) as a colorless resin. TLC (EtOAc/MeOH 8:2) R_f=0.62. ^{1}H NMR ($CDCl_3$) δ 7.55–6.70 (14H, m), 6.00–5.70 (2H, m), 5.35–5.10 (4H, m), 4.80–4.65 (4H, m), 4.65–4.45 (4H, m), 4.10–3.65 (18H, m), 3.65–3.25 (24H, m), 3.16 (2H, m), 2.20–2.00 (6H, m), 1.70–1.20 (36H, m). ^{13}C NMR ($CDCl_3$) δ 173.2–169.6, 157.8, 156.3, 155.5, 154.7, 146.7, 138.6, 132.7, 129.8, 128.6, 127.7, 126.1, 118.0, 117.3, 113.1, 95.4, 80.8, 75.4, 70.4, 66.4, 55.2, 52.3, 43.6, 39.4, 36.8, 36.4, 54.5–49.3, 40.0–37.3, 30.9, 29.4, 29.0, 28.8, 27.3, 26.5, 25.8, 25.5, 25.0, 28.3, 28.2. MS (ESI+) *m/z* 1893.1 $(M+H)^+$ and 1915.4 $(M+Na)^+$. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=20.29 min, λ_{max}=207.4 and 230.9 nm.

6.6.10. Mmt-$(CH_2)_7$-CONH-$(CH_2)_6$-CONH-[Troc-Alloc-Troc-Alloc-Boc-Boc]-OH (28). Compound **28** (388 mg, 90%) was obtained as a colorless resin, following the same protocol as for **11** and starting from **27** (435 mg, 0.230 mmol). TLC (EtOAc/MeOH 6:4) R_f=0.32. MS (ESI+) *m/z* 1879.65 $(M+H)^+$ and 1902.5 $(M+Na)^+$. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=18.18 min, λ_{max}=202.7 and 228.5 nm.

6.6.11. TFA·H_2N-$(CH_2)_7$-CONH-$(CH_2)_6$-CONH-[Troc-Alloc-Troc-Alloc-Boc-Boc]-OH (29). Compound **28** (168 mg, 0.089 mmol) was treated with a 0.1:10 TFA/CH_2Cl_2 solution (4.10 mL) for 6 h. Adding ether gave an insoluble resin which was first isolated then dissolved in MeOH. After evaporation in vacuo, compound **29** (134 mg, 88%) was obtained as a colorless resin. ^{1}H NMR (CD_3OD) δ 6.15–5.80 (2H, m), 5.40–5.05 (4H, m), 4.85–4.65 (4H, m), 4.65–4.45 (4H, m), 4.12–3.75 (12H, m), 3.60–3.22 (24H, m), 3.13 (2H, m), 2.90 (2H, m), 2.15 (4H, m), 1.80–1.20 (36H, m). ^{13}C NMR (CD_3OD) δ 176.3, 176.1, 172.4, 172.0, 171.4, 172.5, 171.8, 158.0, 157.4, 157.3, 156.2, 156.1, 134.2, 134.1, 118.3, 117.8, 96.8, 81.9, 76.6, 67.8, 67.5, 40.8, 40.3, 37.2, 37.0, 30.3, 30.0, 29.9, 29.8, 28.6, 28.5, 27.7, 27.0, 26.9, 53.0–51.5, 39.8–38.2, 28.7. MS (ESI+) *m/z* 1628.04 $(M+H)^+$ and 1606.03 $(M+Na)^+$. HPLC (A/B 60:40 to 0:100 over 30 min) R_t=9.98 min, λ_{max}=201.5 nm.

6.6.12. Troc-Alloc-Troc-Alloc-Boc-Boc]c (30). To a solution of **29** (133 mg, 0.077 mmol) in DMF (7.5 mL) were added DIEA (81 μL, 0.460 mmol), HOAt (21 mg, 0.154 mmol) and HATU (44 mg, 0.115 mmol), and the mixture was stirred for 15 h at rt and in the dark. The reaction mixture was concentrated under vacuum, solubilized in $CHCl_3$, and washed with aqueous 10% $NaHCO_3$, water and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to furnish a resin, which was chromatographed on silica gel (EtOAc/MeOH from 8:2 to 7:3). Compound **30** was obtained (75 mg, 66%) as a colorless resin. ^{1}H NMR ($CDCl_3$) δ 6.75 (1H, m), 6.12 (1H, bs), 6.00–5.75 (2H, m), 5.40–5.10 (4H, m), 4.80–4.63 (4H, m), 4.60–4.45 (4H, m), 4.12–3.65 (12H, m), 3.65–3.28 (24H, m), 3.16 (4H, m), 2.09 (4H, m), 1.65–1.05 (36H, m). ^{13}C NMR ($CDCl_3$) δ 172.0–169.0, 156.4, 155.6, 154.7, 132.7, 118.0, 117.5, 95.4, 80.7, 75.4, 66.4, 39.6, 39.3, 36.5, 36.4, 54.0–48.5, 40.2–37.5, 28.4, 29.8, 29.7, 29.0, 28.7, 26.5, 25.4. MS (ESI+) *m/z* 1609.4 $(M+Na)^+$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=19.51 min, λ_{max}=205.1 nm.

6.7. Synthesis and characterization of nucleobase acetic acids 32, 35 and 38

6.7.1. *tert*-Butyl uracil-1-yl acetate (31). To a suspension of uracil (2.0 g, 17.84 mmol) and K_2CO_3 (2.48 g, 17.84 mmol) in DMF (30 mL) was added *tert*-butyl bromoacetate (2.8 mL, 17.84 mmol) at 0°C. The mixture was stirred for 5 h at rt then filtered off and evaporated in vacuo. The solid residue was treated with water (100 mL) and the aqueous phase was extracted with EtOAc. The combined extracts were washed with water, dried over Na_2SO_4 then evaporated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane 2:8), affording the *N*-alkylated uracil intermediate **31** in 57% yield as a white amorphous powder (2.32 g). TLC (EtOAc) R_f=0.50. ^{1}H NMR ($CDCl_3$) δ 9.75 (1H, bs), 7.10 (1H, d), 5.75 (1H, d), 4.35 (2H, s); 1.45 (9H, s). ^{13}C NMR ($CDCl_3$) δ 166.6, 163.9, 151.0, 144.7, 102.6, 83.7, 49.5, 28.1. MS (ESI+) *m/z* 227.3 $(M+H)^+$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=8.68 min, λ_{max}=210.9 and 261.5 nm.

6.7.2. Uracil-1-yl acetic acid (32). Compound **31** (2.32 g, 10.6 mmol) was dissolved in a 1:1 TFA/CH_2Cl_2 solution (30 mL) and the mixture was stirred at rt for 3 h. The solvent was then evaporated under reduced pressure and the crude residue was triturated with EtOAc. The amorphous solid was filtered off to give 1.65 g (94%) of compound **32**. TLC (EtOAc/MeOH 1:1) R_f=0.52. ^{1}H NMR (d_6 DMSO) δ 11.35 (1H, bs), 7.60 (1H, d), 5.60 (1H, d), 4.40 (2H, s); ^{13}C NMR (d_6 DMSO) δ 169.1, 163.1, 151.6, 145.6, 100.4, 48.2. MS (ESI−) *m/z* 169.0 $(M-H)^-$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=2.1 min, λ_{max}=207.4 and 260.3 nm.

6.7.3. Methyl cytosin-1-yl acetate (33). To a suspension of cytosine (5.0 g, 45.0 mmol) in DMF (100 mL) was added NaH (1.08 g, 45.0 mmol) under N_2 at 0°C. The mixture was stirred for 2 h at rt then methyl bromoacetate (4.3 mL, 45.0 mmol) was added. The mixture was stirred for 48 h at rt. The solvent was then evaporated in vacuo. The crude residue was triturated with water (100 mL) and the precipitate was filtered off. Recrystallization from MeOH/H_2O gave 5.27 g (64%) of **33** as white crystals (mp: 225–227°C). TLC (EtOAc/MeOH 1:1) R_f=0.55. ^{1}H NMR (d_6 DMSO) δ 8.40 (1H, bs), 7.75 (1H, bs), 7.90 (1H, d), 6.00 (1H, d), 4.60 (2H, s), 3.70 (3H, s). ^{13}C NMR (d_6 DMSO) δ 168.5, 164.5, 154.0, 147.0, 93.5, 52.0, 49.5. MS (ESI+) *m/z* 184.2 $(M+H)^+$; HPLC (A/B 100:0 to 0:100 over 40 min) R_t=10.42 min, λ_{max}=217.9 and 273.3 nm.

6.7.4. Methyl (N^4-benzyloxycarbonyl) cytosin-1-yl acetate (34). Cbz-Cl (3.12 mL, 21.8 mmol) and DMAP (2.67 g, 21.8 mmol) were dissolved at −15°C in CH_2Cl_2 (20 mL). The mixture was stirred for 15 min then **33**

(2.0 g, 10.9 mmol) was gradually added. After stirring for 15 min at −15°C then 5 h at rt, the mixture was evaporated in vacuo and the crude residue was taken up in $CHCl_3$. The organic layer was washed with 1 M HCl, with water then dried over Na_2SO_4 and lastly evaporated under reduced pressure. Trituration of the crude residue in ether gave a white precipitate which was then filtered off, affording 2.65 g (77%) of **34** as an amorphous powder. TLC (EtOAc/MeOH 1:1) R_f=0.79. ^{1}H NMR (d_6 DMSO) δ 8.10 (1H, d), 7.40 (5H, m), 7.00 (1H, d), 5.20 (2H, s), 4.70 (2H, s), 3.70 (3H, s). ^{13}C NMR (d_6 DMSO) δ 168.0, 163.0, 154.5, 152.7, 149.9, 135.5, 128.0–127.5, 93.8, 66.1, 51.8, 50.1. MS (ESI+) *m/z* 318.3 $(M+H)^+$; HPLC (A/B 100:0 to 0:100 over 40 min) R_t=13.8 min, λ_{max}=236.7 and 291.1 nm.

6.7.5. (N^4-Benzyloxycarbonyl) cytosin-1-yl acetic acid (35). 34 (2.0 g, 6.3 mmol) was dissolved in dioxane (50 mL) and aqueous 1 M NaOH (8.82 mL) was added. The mixture was stirred 5 h at rt and then concentrated under reduced pressure. The residue was taken up in aqueous 1 M $KHSO_4$. The resultant white precipitate was isolated by filtration, washed with water then dried in a vacuum desiccator affording 1.66 g of **35** (87%). TLC (EtOAc/MeOH 1:1) R_f=0.10. ^{1}H NMR (d_6 DMSO) δ 8.10 (1H, d), 7,40 (5H, m), 7.00 (1H, d), 5.20 (2H, s), 4.60 (2H, s). ^{13}C NMR (d_6 DMSO) δ 169.0, 163.0, 154.7, 152.8, 150.0, 135.6, 128.1–127.6, 93.7, 66.2, 50.2. MS (ESI−) *m/z* 302.1 $(M-H)^-$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=11.62 min, λ_{max}=217.9 and 239.1 nm.

6.7.6. Methyl (2-amino-6-chloropurin-9-yl) acetate (36). To an ice cold solution of 2-amino-6-chloro purine (2.0 g, 11.8 mmol) in anhydrous DMF (35 mL) was added NaH (284.2 mg, 11.8 mmol). After 20 min, methyl bromoacetate (1.15 mL, 12 mmol) was added. The mixture was stirred 24 h at rt under nitrogen then the solvent was evaporated in vacuo. The crude residue was triturated with water and the resulting white precipitate was filtered off, washed with water and dried in a vacuum desiccator, affording 2.27 g of **36** (83%). TLC (EtOAc) R_f=0.41. ^{1}H NMR (d_6 DMSO) δ 8.20 (1H, s), 7.11 (2H, s), 5.10 (2H, s), 3.79 (3H, s). ^{13}C NMR (d_6 DMSO) δ 167.8, 159.6, 153.9, 149.1, 143.1, 122.5, 52.2, 43.5. MS (EI) *m/z* 241.0 (M, $^{35}Cl)^+$, *m/z* 243.0 (M, $^{37}Cl)^+$. HPLC (A/B 90:10 to 770:30 over 40 min) R_t=9.76 min, λ_{max}=221.0 and 245 nm.

6.7.7. (2-Amino-6-chloropurin-9-yl) acetic acid (37). Compound **36** (1.0 g, 4.13 mmol) was dissolved in dioxane (20 mL) and aqueous 1 M LiOH (12.5 mL) was added. After stirring for 4 h at rt, the mixture was neutralized by the addition of aqueous 1 M HCl. The solution was concentrated under reduced pressure then was poured into an aqueous solution of 10% $NaHCO_3$. After extraction with EtOAc, the aqueous layer was acidified to pH=2 by addition of aqueous 1 M HCl. The resulting white precipitate was filtered off, washed with water, then dried in a vacuum desiccator giving 672 mg (71%) of compound **37**. TLC (EtOAc/MeOH 6:4) R_f=0.30. ^{1}H NMR (d_6 DMSO) δ 8.10 (1H, s), 6.99 (2H, bs), 4.88 (2H, s). ^{13}C NMR (d_6 DMSO) δ 168.6, 159.5, 153.9, 149.1, 143.2, 122.6, 43.7. MS (EI) *m/z* 227.0 (M, $^{35}Cl)^+$, *m/z* 229.0 (M, $^{37}Cl)^+$. HPLC (A/B 90:10 to 70:30 over 40 min) R_t=5.32 min, λ_{max}=221.0 and 245.0 nm.

6.7.8. (2-Amino-6-(benzyloxy)purin-9-yl) acetic acid (38). To a mixture of **37** (1.14 g, 3.34 mmol) in benzyl alcohol (10 mL) was added K_2CO_3 (696 mg, 5.01 mmol) and DABCO (75.1 mg, 0.67 mmol). The mixture was heated at 85°C for 18 h. Subsequently, water (50 mL) was added to the reaction. The resulting aqueous solution was washed with EtOAc and the pH adjusted to 3 with an aqueous 1 M solution of $KHSO_4$, at 0°C. The resulting aqueous solution was extracted with EtOAc, and the combined organic extracts were washed with brine then dried over Na_2SO_4. The solvent was evaporated in vacuo and the crude residue was triturated with ether. The white precipitate was filtered off to give 810 mg of **38** (81%). TLC (EtOAc/MeOH 1:1) R_f=0.37. ^{1}H NMR (d_6 DMSO) δ 7.90 (1H, s), 7.60–7.40 (5H, m), 6.60 (2H, bs), 5.60 (2H, s), 4.85 (2H, s). ^{13}C NMR (d_6 DMSO) δ 169.3, 159.7, 159.3, 154.4, 140.4, 128.0, 127.7, 112.9, 66.5, 44.3. MS (ESI−) *m/z* 298.0 $(M-H)^-$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=9.98 min, λ_{max}=216.8 and 243.8 and 287.5 nm.

6.8. Deprotection-couplings steps: synthesis of compounds 39–44, 1

6.8.1. [Troc-H-Troc-H-Boc-Boc]c (39). To an ice cold solution of **30** (90 mg, 0.057 mmol) and DEA (176 μL, 1.7 mmol) in $CHCl_3$ was added $Pd[P(Phe)_3]_4$ (6.5 mg, 0.0057 mmol). The mixture was stirred for 30 min at rt then the solvent was removed under reduced pressure. The crude residue was chromatographed on silica gel column (from EtOAc to MeOH/H_2O 9:1) to afford **39** (67 mg, 84%) as a colorless resin. TLC (EtOAc/MeOH/NH_4OH 8:2:1) R_f=0.16. ^{1}H NMR ($CDCl_3$) δ 4.75–4.60 (4H, m), 4.10–3.70 (8H, m), 3.70–3.30 (16H, m), 3.30–3.05 (8H, m), 2.72 (4H, m), 2.54 (4H, m), 2.12 (4H, m), 1.70–1.25 (36H, m). MS (ESI+) *m/z* 1441.4 $(M+Na)^+$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=15.99 min, λ_{max}=202.7 and 230.9 nm.

6.8.2. [Troc-$C^{(Z)}$-Troc-$C^{(Z)}$-Boc-Boc]c (40). To an ice cold solution of **39** (67.0 mg, 0.047 mmol) and **35** (37.2 mg, 0.122 mmol) in DMF (1.5 mL) were added DIPEA (83 μL, 0.47 mmol), HOAt (19.2 mg, 0.141 mmol) and HATU (50.0 mg, 0.132 mmol). The mixture was stirred for 2 h at rt then the solvent was removed under reduced pressure. The residue was solubilized in $CHCl_3$, washed with water and with aqueous 10% $NaHCO_3$, dried over Na_2SO_4 then evaporated in vacuo. Sephadex LH-20 purification using MeOH as eluant furnished **40** (70 mg, 75%) as a colorless resin. TLC (EtOAc/MeOH/NH_4OH 7:3:1) R_f=0.17. MS (ESI+) *m/z* 2012.3 $(M+Na)^+$, (ESI−) *m/z* 1987.2 $(M-H)^-$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=21.23 min, λ_{max}=205.1 and 239.1 nm.

6.8.3. [Troc-$C^{(Z)}$-Troc-$C^{(Z)}$-H-H]c·2TFA (41). To a solution of **40** (86.7 mg, 0.0435 mmol) in $CHCl_3$ was added TFA (0.5 mL, 6.49 mmol) at rt. After 1 h stirring, evaporation under vacuum gave a crude residue which was triturated with ether. The resulting white solid was filtered off, affording **41** (86 mg) in almost quantitative yield. MS (ESI+) *m/z* 1789.1 $(M+H)^+$ and *m/z* 1811.0 $(M+Na)^+$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=18.04 min, λ_{max}=202.7 and 239.1 nm.

6.8.4. [Troc-C$^{(Z)}$-Troc-C$^{(Z)}$-U-U]c (42). This compound was prepared following the same protocol as for **40**, starting from **41** (87.0 mg, 0.0438 mmol) and **32** (19.4 mg, 0.114 mmol). Sephadex LH-20 purification using MeOH as eluant furnished **42** (63 mg, 70%) as a colorless resin. TLC (EtOAc/MeOH/NH_4OH 5:5:1) R_f=0.24. MS (ESI+) *m/z* 2118.0 $(M+Na)^+$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=16.75 min, λ_{max}=201.5 and 242.6 nm.

6.8.5. [H-C$^{(Z)}$-H-C$^{(Z)}$-U-U]c·2AcOH (43). Cadmium (723 mg, 6.43 mmol) and AcOH (741 μL, 12.88 mmol) were added to a solution of **42** (135 mg, 0.0644 mmol) in DMF (500 μL). The reaction mixture was stirred for 13 h at rt then the remaining cadmium was filtered off and washed with a minimum amount of DMF. Ether was subsequently added at 0°C to the filtrate, causing precipitation of the product as a resin. Isolation of this crude residue followed by purification using a XAD-2 Amberlite (from H_2O to MeOH) furnished **43** (48 mg, 40%) as a pale yellow resin. MS (ESI+) *m/z* 1766.6 $(M+Na)^+$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=13.05 min, λ_{max}=205.1 and 242.6 nm.

6.8.6. [G$^{(OBn)}$-C$^{(Z)}$-G$^{(OBn)}$-C$^{(Z)}$-U-U]c (44). This compound was prepared following the same protocol as for **40**, starting from **43** (31.0 mg, 0.017 mmol) and **38** (12.4 mg, 0.041 mmol). Sephadex LH-20 purification using MeOH as eluant furnished **44** (21 mg, 55%) as a colorless resin. MS (ESI+) *m/z* 2329.33 $(M+Na)^+$. HPLC (A/B 80:20 to 0:100 over 30 min) R_t=16.05 min, λ_{max}=208.6 nm; (A/B 97:3 to 40:60 over 40 min) R_t=28.78 min, λ_{max}=205.1 and 243.8 nm; (A/C 95:5 to 0:100 over 30 min) R_t= 21.93 min, λ_{max}=213.3 and 274.5 nm.

6.8.7. [GCGCUU]c (1). Compound **44** (18 mg, 7.8 μmol) was dissolved in DMF (0.3 mL) and glacial HBr/AcOH (400 μL) was added. The mixture was stirred for 2 h at rt and the solvent was evaporated under reduced pressure.

6.9. Purification of compound 1 by semi-preparative HPLC and purity assessment

The crude product was purified by HPLC, using a column RP-18 (5 μm) Lichrospher (250×10 mm). A linear gradient of B in A (A/B 97:3 to 40:60 over 60 min) and a flow rate of 2 mL/min were used for elution. The absorbency was detected both at 205 and 254 nm. The mixture was first concentrated in vacuo, then the remaining solvent was removed by lyophilization. Compound **1** (3 mg, 17% from the protected PNA **44**) was obtained as a colorless resin. The purity of the purified material was assessed by analytical reverse phase HPLC. A single peak at 19.31 min was recorded for the system A/B 97:3 to 40:60 over 40 min (λ_{max}=206.2 and 265.0 nm) or at 15.91 min for the system A/C 95:5 to 0:100 over 30 min (λ_{max}=217.9 and 256.8 nm). MS (ESI+) *m/z* 1859.0 $(M+H)^+$. MALDI-TOF MS calculated average mass for $C_{77}H_{104}O_{22}N_{34}$: 1857.85; found: positive mode: *m/z* 1858.87 $(M+H)^+$, negative mode: *m/z* 1856,16 $(M-H)^-$. HR-MS found 1902.78476 $(M+2Na)^+$, calculated monoisotopic mass for $C_{77}H_{104}O_{22}N_{34}Na_2$: 1902.78601, Δ=0.63 ppm.

6.10. Inhibition of the dimerization

In a standard experiment, 0.42 μg of 1–311 RNA dissolved in 7 μL of water was heated for 2 min at 90°C and chilled for 2 min on ice. 2 μL of buffer A (for monomer reference) or 2 μL of buffer B (for dimer reference) or 2 μL of buffer B+1 μL of denatured PNA **1** at different ratios (134.5, 100, 50, 10 or 1 equiv. of PNA) were added, and the samples were incubated at 37°C for 20 min. The samples were cooled in ice, mixed with 2 μL of bromophenol blue and loaded on a 1.2% agarose gel. Electrophoreses were run at 4°C at 8 V/cm.

Buffer A was constituted by 50 mmol of 1 M sodium cacodylate (3.75 mL), 40 mmol of 3 M KCl (1 mL) and 10.25 mL of water. Buffer B was constituted by 50 mmol of 1 M sodium cacodylate (3.75 mL), 300 mM of 3 M KCl (7.5 mL), 5 mM of 1 M $MgCl_2$ (3.75 μM) and 3.75 mL of water. 1.2% Agarose gel was prepared as follow: 2.5 mL of Tris–borate TB25X, 10 μL of 1 M $MgCl_2$, 97.5 mL of water, 1.2 g of agarose gel and 5 μL of ethidium bromide.

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