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RNA-Binding and Viral Reverse Transcriptase Inhibitory Activity of a Novel Cationic Diamino Acid-Based Peptide

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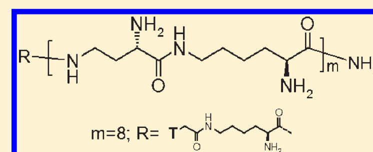
Supporting Information

ABSTRACT: A novel cationic peptide based on L-lysine and L-diaminobutyric acid was prepared for the first time by solid phase synthesis. After HPLC purification and ESI MS characterization, we studied by CD and IR spectroscopy the structural features of the novel basic peptide, which is able to form a β-turn-like structure. Furthermore, its interaction with DNA and RNA was investigated by CD and UV spectroscopy, which revealed a preferential RNA-binding ability of the sequential peptide, whereas its inhibitory activity toward HIV and Moloney murine leukemia virus (MMLV) reverse transcriptase action was evaluated by semiquantitative PCR. The cationic sequential peptide was able to inhibit the reverse transcriptase activity in both cases, even if our PCR data suggested a major activity in the case of HIV-RT, probably due to the stronger cationic peptide−protein interaction evidenced by UV spectroscopy.



INTRODUCTION

The binding of peptides to nucleic acids has been extensively studied leading to the identification of various types of interactions that might be responsible for the recognition of nucleic acid structures by proteins.[1,2] A key role in such recognition is played by electrostatic interactions between basic amino acids and the negatively charged phosphates of the nucleic acid. In particular, among the naturally occurring basic polyamides that are able to bind nucleic acids, it is worth mentioning ε-poly-L-lysine, a nonribosomally synthesized polyamino acid characterized by peptide bonds involving the carboxyl and the ε-amino groups of L-lysine residues. ε-Poly-L-lysine, which is produced by *Streptomyces albulus* with a molecular weight ranging from 2 to 4 kDa, is stable at high temperatures and under alkaline or acidic conditions and shows significant antimicrobial activity.[3,4] Owing to this feature, ε-poly-L-lysine is industrially produced as a food additive by a fermentation process using bacteria of the genus *Streptomyces*.[5,6] Other natural nonribosomally synthesized polyamino acids have been described, including poly(γ-glutamic acid), cyanophycin, a polymer containing L-arginine and L-aspartic acid residues,[7−9] poly(arginyl-histidine),[10,11] and γ-poly-L-diaminobutyric acid (Dab). Among these, γ-poly-L-Dab, which is coproduced with ε-poly-L-lysine by *Streptomyces* bacteria, as recently reported by Takehara and co-workers,[4] shows strong inhibitory activities against various types of yeast. Interestingly, no copolymers composed of L-Dab and L-lysine were found in the producer strains, probably because the two amino acids are polymerized by different enzymes.[4] Recently, we reported on an oligonucleotide analogue, having a γ,ε-nucleopeptide structure, which was assembled by nucleobase-modified L-lysine and L-diaminobutyric acid monomers. This oligonucleotide analogue was designed in order to have the same average distance among the nucleobase-bearing atoms as in natural nucleic acids and showed interesting DNA- and RNA-binding properties.[12] The use of cationic polyamino acids, such as L-lysine-based homopolymers, has important applications in both biotechnology and medicine owing to several features of these molecules, such as the ability of poly-ε-L-lysine to compact nucleic acids,[3,13,14] as well as the ability of L-lysine-based polycationic systems to deliver oligonucleotides.[15] Therefore, we planned to synthesize a novel sequential γ,ε-peptide, neither isolated nor synthesized previously, which corresponds to the backbone of the above-mentioned nucleopeptide. Here, we report preliminary studies on the structural characteristics of this novel sequential γ,ε-peptide, along with data on its nucleic acid-binding ability and other biological properties.

The replication of retroviruses relies on an important family of enzymes called reverse transcriptases (RTs), which are involved in the conversion of genomic viral RNA into proviral DNA. RTs are multifunctional enzymes presenting three activities including a DNA- and an RNA-dependent DNA polymerase activity and an RNase H activity that causes the cleavage of RNA in RNA−DNA hybrids.[16] An important goal for the treatment of diseases caused by retroviruses, such as human immunodeficiency virus type 1 (HIV-1) infection, is the inhibition of RT polymerase activity. RT inhibitors can be classified in two classes: nucleoside reverse transcriptase inhibitors (NRTIs), which are modified nucleotides or nucleosides, and non-nucleoside reverse transcriptase inhibitors (NNRTIs). The RT inhibitory activity of NRTIs is due to the fact that they are incorporated into DNA, following their conversion in vivo into triphosphate, and thereby block viral

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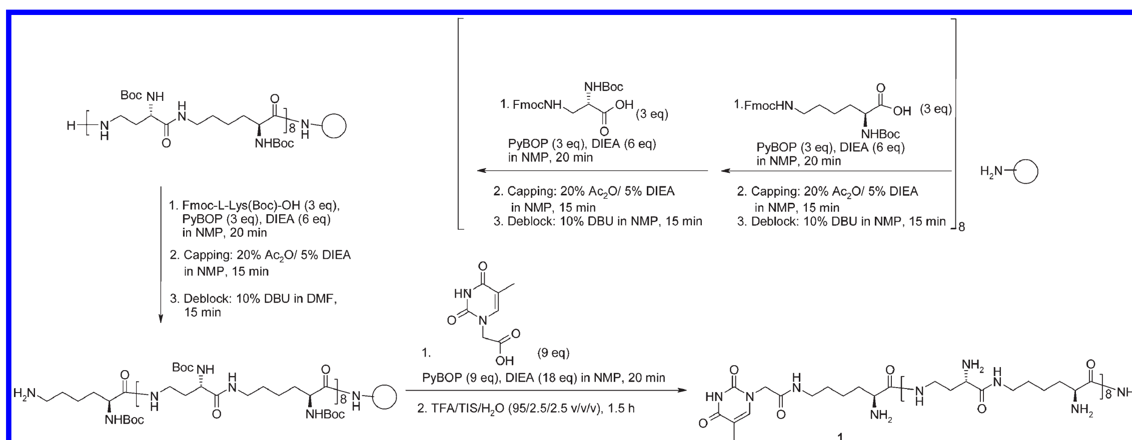
Scheme 1. Synthesis of Oligomer 1



DNA elongation. The family of NNRTIs comprises both synthetic and natural compounds, which, as reported in the literature, bind to a hydrophobic pocket about 10 Å away from the polymerase site that constitutes an allosteric site of the RT.[17−19] NNRTIs function as noncompetitive inhibitors of RT activity by preventing its binding to the DNA template−primer duplex.[20−22] Previously, we reported on an uncharged diamino acid-based nucleopeptide, unable to interact with natural nucleic acids, which inhibited MMLV reverse transcriptase activity in a dose-dependent manner, probably because of its direct interaction with the viral RT allosteric site.[23] In this study, we investigated the effect of the cationic diamino acid-based peptide on the viral reverse transcriptase activity of HIV and MMLV-RTs by semiquantitative PCR and UV spectroscopy.

RESULTS AND DISCUSSION

Solid Phase Synthesis of the L-Dab/L-Lysine-Based Heptadecapeptide. The synthesis of peptide **1** was performed in solid phase by using as starting materials (i) the commercial Boc-L-Lys(Fmoc)-OH and Boc-L-Dab(Fmoc)-OH amino acids for the realization of the γ,ε-peptide backbone and (ii) thymine-1-yl acetic acid, also commercially available, for the nucleobase derivatization of the oligomer. Indeed, this thymine residue was introduced at the N-terminus of the peptide as a chromophore in order to facilitate both quantitation and purification of the novel basic peptide. More in detail, we first synthesized an γ,ε-heptadecapeptide backbone by alternately coupling, on a solid support (Rink Amide MBHA), the commercial Boc-L-Lys(Fmoc)-OH and Boc-L-Dab(Fmoc)-OH derivatives, following an Fmoc peptide strategy (Scheme 1). Subsequently, the free N-terminal amino group was reacted with the commercial thymine-1-yl acetic acid under the same amidation conditions used for the peptide assembly, involving PyBOP as the activator agent and DIEA as the base.

After cleavage and Boc deprotection from the solid support, achieved under acid treatment (TFA/TIS/H_2O = 95/2.5/2.5 v/v/v), the product was precipitated from cold diethyl ether and purified by RP-HPLC. The thymine-labeled peptide **1**, easily identified by its UV spectrum and detection at 260 nm, was obtained in 1.4% yield. In the solid phase synthesis of peptide **1**, we found that the yields dramatically decreased after the tenth coupling, probably as a consequence of the high aggregation of the peptide chain. We tried different coupling conditions without significant improvements. Characterization of **1** was performed by LC-ESI MS, which confirmed the identity of the product (Figure S1, Supporting Information). The novel γ,ε-peptide did not present any tendency to self-aggregate and showed a good water solubility as expected owing to its positively charged nature at physiological pH. The simple route to the thymine-labeled peptide herein reported can be extended to other classes of chromophores and also to the other three DNA bases. This strategy allows one to obtain several nucleobase-labeled γ, ε-peptides by using commercial starting materials such as

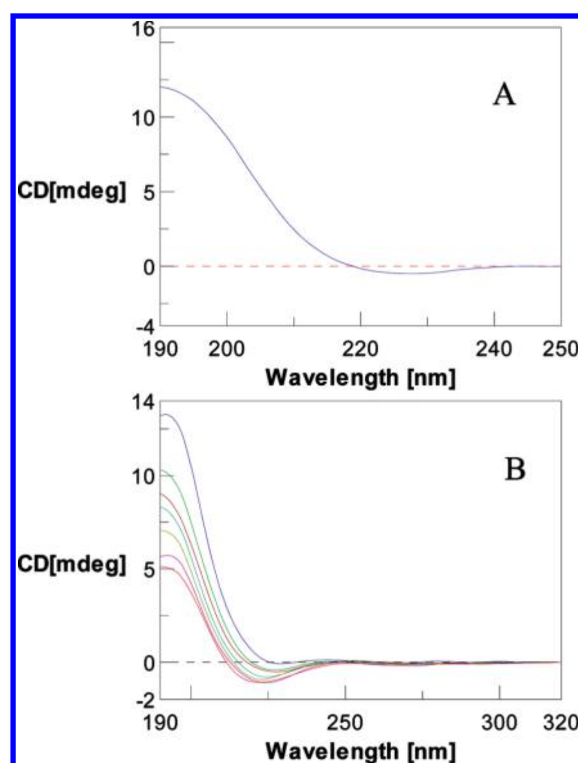


Figure 1. CD spectra relative to an 82 μM solution of peptide **1** in 10 mM phosphate buffer, pH 7.5, recorded at 10 °C (A) and at different temperatures (B): 5 (blue), 20 (green), 35 (brown), 50 (azure), 65 (kaki), 80 (violet), and 95 °C (red).

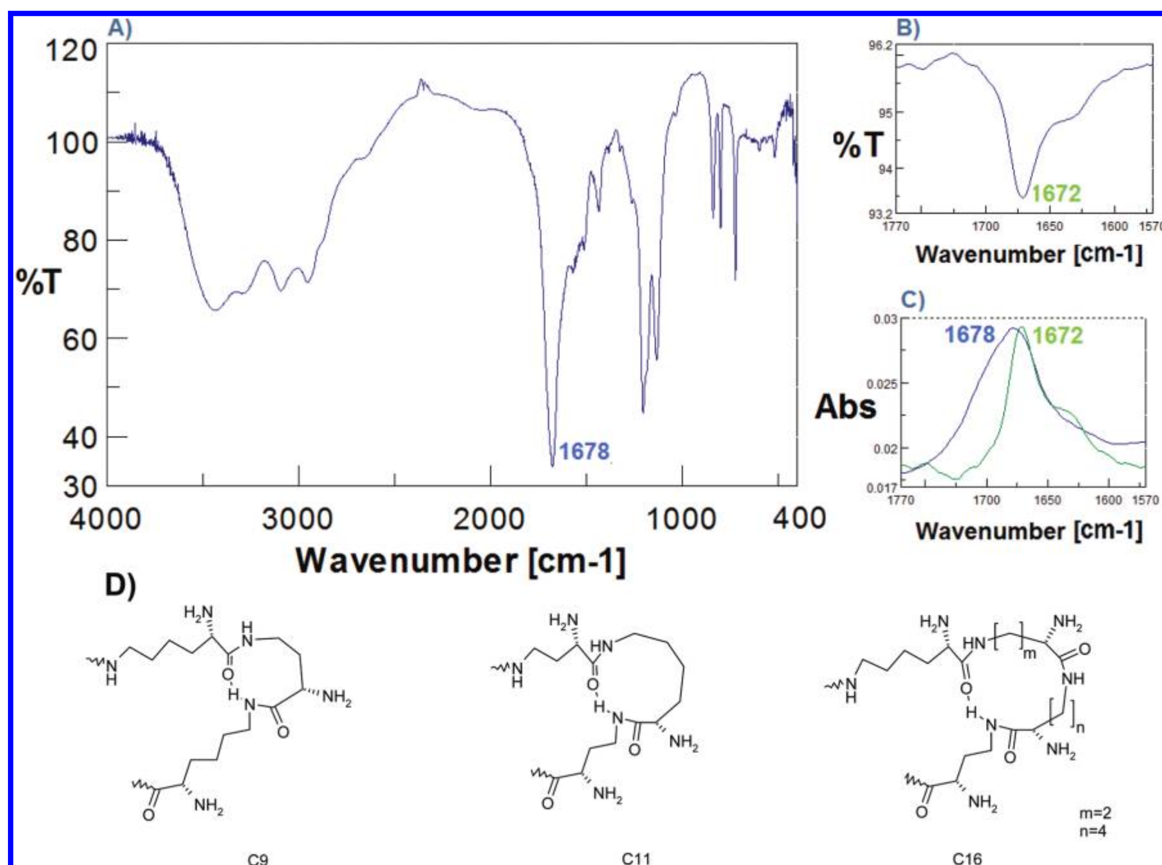


Figure 2. IR spectra relative to the cationic peptide in (A) the KBr pellet and (B) the 0.1 mM D_2O solution. (C) Comparison of the amide I/I′ IR absorbance bands obtained in solid state (blue line) and solution (green line). (D) Schematic representation of three possible γ,ε-peptide turns (B).

Boc-L-Lys(Fmoc)-OH, Boc-L-Dab(Fmoc)-OH, and nucleobase-1-yl acetic acids.

CD and IR Conformational Studies. The structural characteristics of the L-lysine/L-Dab-based peptide were investigated by CD and UV spectroscopy. First, the CD profile of the monomeric peptide in 10 mM phosphate buffer, pH 7.5, was analyzed in order to evaluate eventual preorganization of the heptadecapeptide. By examining the CD behavior of the peptide in the 190−250 nm range at different temperatures (5, 10, 20, 35, 50, 65, 80, and 95 °C), we observed a strong positive band at 192 nm and a small negative band at about 225 nm. These spectra are formally similar to those indicative of β-turns in α-peptides with a small amount of disordered comformations,[24,25] the contribution of which increased at higher temperatures, causing a decrease in the intensity of the positive band. Thus, the analysis of the CD spectral features of our γ,ε-peptide allows one to deduce the presence of a β-turn-like secondary structure that is relatively stable over the temperature (Figure 1A and B).

As already reported in the literature, also IR spectroscopy can be used for the conformational study of peptides different from α-peptides. For example, by assigning the IR bands of ε-peptides in comparison with α-polyamino acids, it was possible to conclude that poly-ε-lysines adopt a β-sheet conformation.[26]

IR spectral data on the γ,ε-peptide support the presence of a β-turn-like secondary structure. Indeed, the spectra, recorded in KBr pellet (Figure 2A) and in D_2O solution (Figure 2B), exhibited an amide I/I′ band located in the region 1690−1660 cm^{-1} which is assigned to β-turns.[27] Furthermore, in addition to the amide A band at around 3400 cm^{-1} expected for the free NH group, peptide **1** exhibited also a band at around 3300 cm^{-1}, which is assigned to intramolecularly hydrogen-bonded conformations.[28]

CD and UV Nucleic Acid-Binding Studies. CD and UV experiments on the heptadecapeptide in the presence of DNA and RNA were then performed in order to evaluate its potential as a cationic compacting agent. In particular, direct mixing of dT_{12} DNA and **1**, at 5 °C in 10 mM phosphate buffer (pH 7.5), led to a slight reduction of the magnitude of the CD spectrum (Figure 3A).

Since the novel peptide **1** did not show any significant absorption in the 248−300 nm wavelength range, this effect can be ascribed only to a little conformational change of DNA. Moreover, a small hypochromism in the UV absorption band of DNA was observed in the presence of the novel peptide in the same experimental conditions, confirming this hypothesis. However, as evidenced by the subsequent CD and UV experiments (Figure 4A and B), direct mixing of peptide **1** with poly(U) RNA at 5 °C in the presence of 10 mM phosphate buffer (pH 7.5), led to a strong interaction which could be associated with the conversion of the polynucleotide to its ordered form in analogy to previous reports.[1] This transition was reflected in both CD and UV absorption spectra which showed a strong increase in the positive CD band of RNA and a significant UV hypochromic effect (Figure 4A and B). The spectral changes induced by peptide **1** were analogous to those induced by polylysine, Mg^{2+}, and Cs^{+}.[29]

Biological Properties. The biological activity of the sequential peptide was determined by analyzing the effect of peptide **1** in a MMLV or HIV reverse-transcription reaction using total RNA

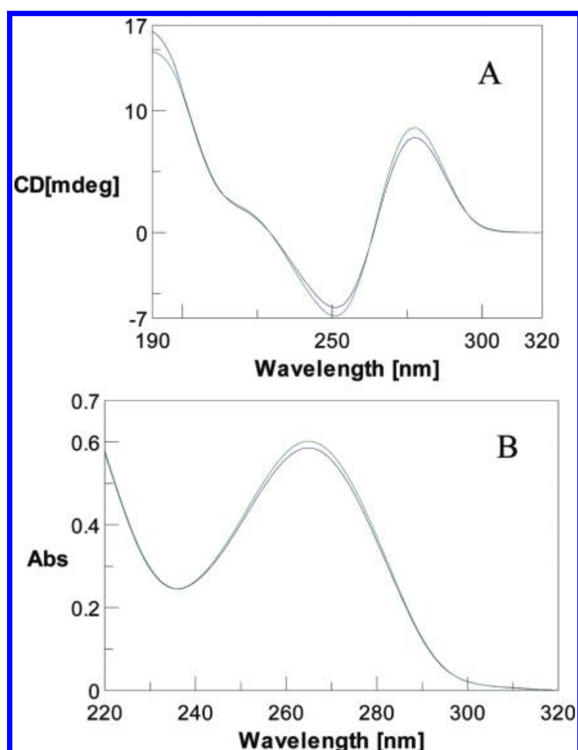


Figure 3. "Sum" (green line) and "mix" (blue line) CD (A) and UV (B) spectra, recorded at 5 °C by a Tandem cell, relative to dT_{12} (60 nmol in T) and peptide **1** (120 nmol in $-NH_3^+$) dissolved each in 1 mL of 10 mM phosphate buffer at pH 7.5. The final volume of solution was 2 mL.

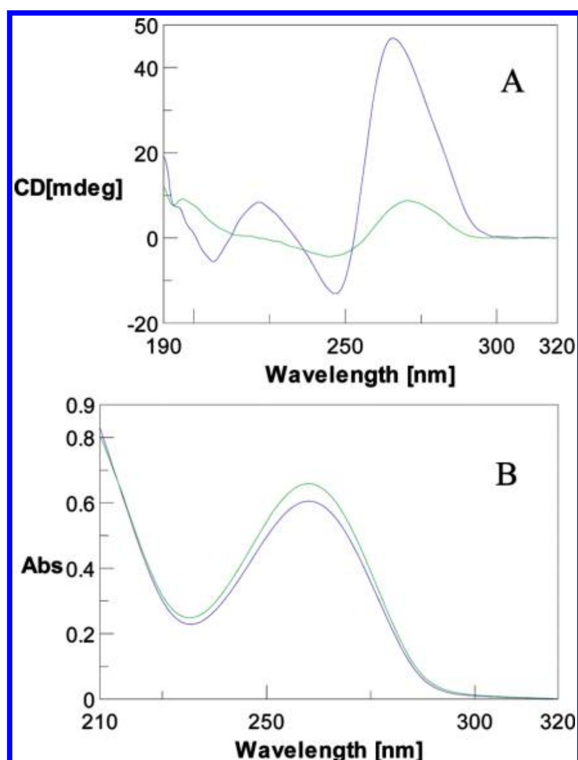


Figure 4. "Sum" (green line) and "mix" (blue line) CD (A) and UV (B) spectra, recorded at 5 °C by a Tandem cell, relative to poly(U) (60 nmol in U) and peptide **1** (120 nmol in $-NH_3^+$) dissolved each in 1 mL of 10 mM phosphate buffer at pH 7.5. The final volume of the solution was 2 mL.

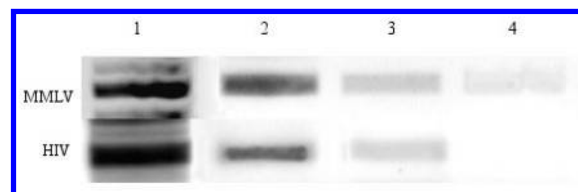


Figure 5. Effect of peptide **1** addition on semiquantitative RT-PCR reaction. After reverse transcription, performed with MMLV or HIV retrotranscriptase, cDNA sequences were PCR amplified using primers specific for GAPDH in the presence of 0.5 or 1 μg (lane 3 and 4, respectively) of peptide **1**. Molecular markers are in lane 1.

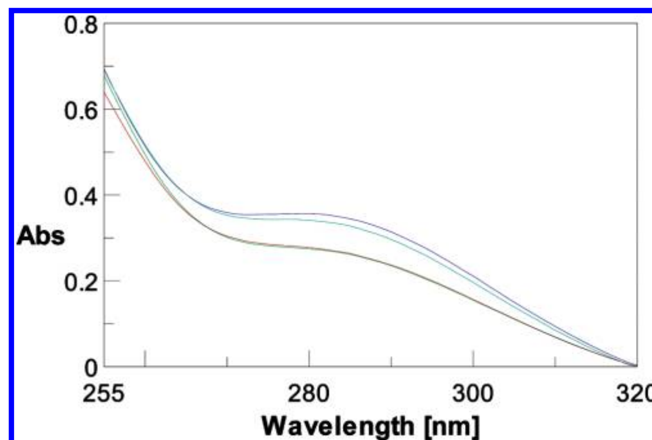


Figure 6. "Sum" (green line, HIV; red line, MMLV) and "mix" (purple line, HIV; teal line, MMLV) UV spectra, recorded at 42 °C by a tandem cell (Hellma), relative to HIV or MMLV-RT and oligomer **1** (in the same concentration of lane 4) dissolved each in 0.8 mL of reaction buffer (see Experimental Section). The final volume of the solution was 1.6 mL.

from HeLa cells. In detail, following the reverse transcription, which was performed using the dT_{12} primer and peptide **1**, a PCR reaction was carried out using specific primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The results showed a reduction in gene expression in the presence of a 1:5 or 1:10 base/base ratio of primer/peptide **1** (Figure 5). In particular, when MMLV-RT was used, a 30% and 18% inhibition was observed in the presence of 0.5 and 1 μg of the sequential peptide, respectively, calculated by comparing PCR amplification in the absence of the peptide (lanes 2, Figure 5). When the experiment was carried out with HIV-RT as retrotranscriptase, the results showed 60% inhibition or total gene suppression when we used 0.5 μg or 1 μg of peptide **1**, respectively.

By our previous CD and UV experiments, we concluded that the cationic peptide can interact strongly with RNA (but not with DNA) in a 1:2 phosphate/positive charge ratio. In the in vitro experiments, the 1:10 base/base ratio of primer/peptide, corresponding to a 1:5 phosphate/positive charge ratio of RNA/peptide (calculated on the basis of total RNA), was used in the case of the total inhibitory effect toward HIV-RT (lane 4). However, the only partial reverse transcriptase inhibition found with the 1:2.5 phosphate/positive charge ratio (1:5 base/base ratio of primer/peptide, lane 3), as well as the different response of HIV and MMLV reverse transcriptases, suggests a mechanism for the inhibition of the reverse transcriptases that is not exclusively dependent on RNA complexation. In other

words, a direct interaction with the enzyme could be involved in the inhibitory mechanism of the cationic peptide and it could be also responsible for the different behavior of the HIV and MMLV-RTs. In order to explain the major effect of the sequential peptide on the inhibition of HIV-RT, we performed UV studies on both enzymes and on their complexes with the cationic peptide.

In particular, the reverse transcriptase−peptide interaction was studied in the same conditions of concentration, buffer, and temperature employed in the semiquantitative PCR experiments using a tandem cell. These studies, as shown in Figure 6, revealed a clear interaction between the enzyme and the peptide, which was stronger with HIV-RT than MMLV-RT. Finally, it is interesting to point out that the reverse transcriptase-peptide binding causes an absorbance increase in the 255−320 nm wavelength range. This effect is associable with an increased exposure to the solvent of the aromatic amino acids present in the hydrophobic pocket, which constitutes the reverse transcriptase allosteric site, as a consequence of the binding of the inhibitor peptide to the enzyme.

CONCLUSIONS

To the best of our knowledge, this is the first report on the γ,ε-L-Dab,L-Lys sequential peptide, an artificial peptide which, owing to its similarity with ε-poly-L-lysine and γ-poly-L-diaminobutyric acid, can be expected to have biomedical applications similar to those of the naturally occurring polyamino acids.[14,30] In this preliminary work, a heptadecapeptide was prepared in solid phase by an efficient Fmoc strategy, purified by RP-HPLC and characterized by ESI-MS. Subsequently, we investigated the occurrence of secondary structures of the single-stranded peptide and its ability to form complexes with natural nucleic acids. In particular, the CD and IR spectral data suggested the presence of a β-turn-like preorganization of the sequential peptide in water solution, which was not substantially altered by temperature changes in the 5−95 °C range. It is also evident from the CD and UV results, that the γ,ε-L-Dab,L-Lys sequential peptide, which can interact weakly with dT_{12} DNA, induces a large conformational change of poly(U) RNA, probably from the single stranded random coil to an ordered form.[1] This novel cationic peptide could act as a nucleic acid compacting device potentially useful in oligonucleotide delivery strategies, for example, for the delivery of siRNAs or interferon inducer RNAs. Furthermore, our peptide could be of particular interest in RNA isolation from cellular extracts owing to its selective RNA versus DNA binding ability. Biological studies were also performed by using semiquantitative PCR in order to investigate the inhibitory ability of the peptide toward two retroviral RTs. A dose-dependent inhibition of the reverse transcriptase activities was detected in both cases. However, a major effect on HIV-RT was evidenced probably due to the stronger HIV-RT-peptide interaction, revealed by UV studies, as compared to the MMLV-RT-peptide recognition. In conclusion, the γ,ε-L-Dab,L-Lys sequential peptide can be expected to act as an oligoribonucleotide delivery agent in analogy to other basic peptides and, due to its in vitro reverse transcriptase inhibitory activity, can be regarded as a potential antiviral drug to be further investigated for innovative applications in medicine.

EXPERIMENTAL SECTION

Chemicals. Boc-L-Lys(Fmoc)-OH was purchased from Advanced Biotech (Italy), while Boc-L-Dab(Fmoc)-OH was from Iris Biotech Gmbh (Germany). Anhydroscan NMP was from LabScan (Poland). Solvents for HPLC chromatography, and acetic anhydride were from Reidel-de Haën (Germany). Diethyl ether was from Carlo Erba (Italy). DBU and TIS were purchased from Sigma Aldrich (U.S.). dT_{12} (DNA) was from Biomers, TFA, thymine-1-yl acetic acid and poly(U) (RNA) were from Fluka (Switzerland). Deuterium oxide was purchased from Armar Chemicals (Switzerland).

Apparatus. Centrifugations were performed on a Z 200 A Hermle centrifuge (Wehingen, Germany). The product was analyzed and characterized by LC-MS on an MSQ mass spectrometer (ThermoElectron, Milan, Italy) equipped with an ESI source operating at 3 kV needle voltage and 320 °C, and with a complete Surveyor HPLC system, comprising a MS pump, an autosampler, and a PDA detector, by using a Jupiter C18 300 Å (5 μm, 4.6 × 150 mm) column (Phenomenex, Torrance, CA, U.S.). Gradient elution was performed by using increasing amounts of acetonitrile (0.05% TFA, B) in water (0.05% TFA, A), with a linear gradient of 5% (for 5 min) to 40% B in A over 10 min (t_R = 11.7 min), monitoring at 260 nm, with a flow rate of 0.8 mL/min. Semipreparative purification was performed on a Hewlett-Packard 1100 series HPLC (Agilent Technologies, Santa Clara, CA, U.S.), equipped with a diode array detector, by using a Phenomenex Jupiter C18 300 Å (10 μm, 10 × 250 mm) column. Gradient elution was performed by building up a gradient starting with buffer A (0.1% TFA in water) and applying buffer B (0.1% TFA in acetonitrile) with a flow rate of 4 mL/min (monitoring at 260 nm). Samples were lyophilized in a FD4 Freeze-Dryer (Heto Lab Equipment, Birkerod, Denmark) for 16 h. UV and CD spectra were recorded on a UV−vis Jasco (Easton, MD, U.S.) model V-550 spectrophotometer equipped with a Peltier ETC-505T temperature controller and a CD Jasco model J-715 spectrophotometer equipped with a Peltier PTC-423/15.

Solid Phase Synthesis of T-ε-L-lys-(γ-L-Dab-ε-L-lys)$_8$-NH$_2$ (1, Scheme 1). Solid phase synthesis was carried out in a short PP column (4 mL) equipped with a PTFE filter, a stopcock, and a cap on a Rink amide resin using the peptide-like Fmoc chemistry. Oligomer **1** was assembled on Rink Amide MBHA resin (0.54 mmol/g, 54 mg, 29 μmol) using the synthetic strategy described in Scheme 1. In particular, a solution of Boc-L-Lys(Fmoc)-OH or Boc-L-Dab(Boc)-OH (87 μmol, 3 equiv), PyBOP (87 μmol, 3 equiv), and DIEA (30 μL, 174 μmol, 6 equiv) in 0.7 mL of NMP was added to the NH_2-resin, and the mixture was reacted for 20 min under stirring. Each condensation step was followed by capping performed with 20% Ac_2O/5% DIEA in NMP for 15 min, whereas Fmoc deprotection was achieved with 10% DBU in NMP (15 min). After the final Fmoc removal (UV Fmoc test: 8 μmol, 28% yield), the N-terminal free amino group was functionalized with thymine nucleobase by reaction with thymine-1-yl acetic acid (42 mg, 232 μmol, 8 equiv), PyBOP (120 mg, 232 μmol, 8 equiv), and DIEA (79 μL, 464 μmol, 16 equiv), in 0.7 mL of NMP at room temperature for 20 min. The peptide was then cleaved from the solid support by treatment with TFA/TIS/H_2O (95/2.5/2.5 v/v/v), over 1.5 h and recovered by precipitation with cold diethyl ether, centrifugation, and lyophilization. Oligomer **1** was purified by semipreparative HPLC using a linear gradient of 5% (for 5 min) to 40% B in A over 15 min: t_R = 13.2 min. The compound was ≥95% pure by HPLC analysis. UV quantification of the purified product gave 401 nmol of **1**; ESI-MS (S1) 1070.17 (found), 1069.87 (expected for $[C_{93}H_{181}N_{37}O_{20}+2H]^{2+}$); 714.31 (found), 713.58 (expected for $[C_{93}H_{181}N_{37}O_{20}+3H]^{3+}$).

CD, UV, and IR Studies. Circular dichroism (CD) and ultraviolet (UV) spectra were recorded using a Hellma quartz cell with a light path of 1 cm and a Hellma Tandem quartz cell 2 × 0.4375 cm.[31,32] The quantitation of the heptadecapeptide was performed by UV absorbance

measurements on solutions of the purified oligomer dissolved in a known amount of Milli-Q water ($T = 85$ °C, absorbance value at $\lambda = 260$ nm). The molar extinction coefficient value used for the quantitation of the thymine-functionalized oligomer **1** was that corresponding to the thymine PNA monomer (8.6 mM^{-1}). IR spectra were recorded in KBr pellets and nujol mull solvent or in a 0.1 mM D_2O solution using a CaF_2 cell (Hellma, Jena, Germany) with a path length of 40 μm and a volume of 50 μL in the region 500−4000 cm^{-1} on a Jasco FT IR-430 spectrometer (Tokyo, Japan).

Cell Culture. HeLa cells (ATCC, U.S.) were grown in RPMI1640 medium supplemented with 10% fetal bovin serum (FBS), 1% glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, U.S.), at 37 °C and 5% CO_2.

RT-PCR. Total RNA was extracted from the cellular lysate by using Tri-reagentTM (Sigma Aldrich) according to the manufacturer's instructions. Reverse transcription was performed using 0.5 μg of total RNA, 200 U of MMLV (Finnzymes, Finland), or HIV Reverse Transcriptase RNase H (Ambion, U.S.), and 250 ng of dT_{12} primer (Biomers, Germany) in the presence of 1 or 10 μg of peptide **1**. A preincubation mixture containing RNA and **1** was performed for 10 min. Reaction temperature was set at 42 °C for 1 h. The reaction buffer was 50 mM Tris HCl at pH 8.3; 75 mM KCl; 3 mM $MgCl_2$; and 5 mM DTT. After reverse transcription, PCR assay of GAPDH transcripts was carried out using the following primers: forward primer, 5′-ATGGGGAAGGT-GAAGGTC-3′; reverse primer, 5′-GTCATGGATGACCTTGGC-3′ (purchased by Sigma-Genosys Ltd.). The PCR protocol was as follows: 5 min at 95 °C followed by 25 cycles of 1 min at 95 °C, 1 min at 58 °C, and 1 min at 72 °C. PCR products were then analyzed on 1% agarose gel in TAE buffer and visualized by ethidium bromide staining. Gel images were captured by a ChemiDocTM XRS and analyzed by Quantity-One software (Biorad, Hercules, CA, U.S.).

■ ASSOCIATED CONTENT

S Supporting Information. Figure S1 with the LC-ESI MS (positive ions) characterization of the cationic peptide. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

Ac_2O, acetic anhydride; Boc, tertbutoxycarbonyl; D_2O, deuterium oxide; Dab, L-2,4-diaminobutyric acid; DBU, 1,8-diazabicyclo-[5.4.0]undecene; DIEA, *N*,*N*-0.022w?>diisopropylethylamine; DTT, dithiothreitol; FBS, fetal bovine serum; Fmoc, 9-fluorenyl-methoxycarbonyl; MBHA, 4-methylbenzhydrylamine; NMP, 4-methylpyrrolidone; PyBOP, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; PTFE, polytetrafluoroethylene; RT, reverse transcriptase; TFA, trifluoroacetic acid; TIS, triisopropyl silane; TAE, Tris-acetate-EDTA

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