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Synthesis and Evaluation of Oligo-1,3-thiazolecarboxamide Derivatives as HIV-1 Reverse Transcriptase Inhibitors

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Abstract—A set of oligo-1,3-thiazolecarboxamide derivatives able to interact with the minor groove of nucleic acids was synthesized. These oligopeptides contained different numbers of thiazole units presenting dimethylaminopropyl or EDTA moieties on the C-terminus, and aminohexanoyl or EDTA moieties on the N-terminus. The inhibition of such compounds on HIV-1 reverse transcriptase activity was evaluated using different model template–primer duplexes: DNA·DNA, RNA·DNA, DNA·RNA and RNA·RNA. The biological properties of the thiazolecarboxamide derivatives were compared to those of distamycin, another minor groove binder which contains three pyrrole rings. Similar to distamycin, the thiazole containing oligopeptides were good inhibitors of the reverse transcription reaction in the presence of DNA·DNA. But in contrast to distamycin, the oligothiazolide derivatives were able to inhibit reverse transcription in the presence of RNA·DNA or DNA·RNA template–primers. Both distamycin and oligothiazolecarboxamides had low affinity for RNA·RNA duplexes. The inhibition obtained with the newly synthesized thiazolecarboxamides showed that these compounds were more powerful and versatile inhibitors of the RT-dependent polymerization than the natural minor groove binder distamycin.

Introduction

Most of the therapeutic agents used against HIV-1 induce the emergence of resistance strains.[1,2] That is why one of the most important lines in the pharmacology concerning HIV-1 is focused on the development of new, or on the modification of already existing drugs. Among the new and promising antivirals tools are compounds which interact reversibly with nucleic acids.[3] Minor groove binding molecules are capable of binding to the minor groove of a double-stranded DNA. Such molecules contain several small aromatic rings such as pyrrole, furan or benzene connected by bonds with torsional freedom. The DNA minor groove may "fit" aromatic molecules better at A·T-rich relative to G·C-rich regions.

The prototype minor groove binders are netropsin (**1**) and distamycin (**2**) (Fig. 1). Distamycin was first isolated from *Streptomyces distallicus*[4] and later synthesized.[5–7] It inhibits both Gram-positive and Gram-negative bacteria and replication of viral DNA[8] (vaccinia, herpes simplex virus). The synthesis of certain analogues of distamycin, containing either additional pyrrole units or derivatives in which the pyrrole rings were replaced by benzene, pyridine, thiophene, thiazole, imidazole, pyrazole, or triazole has been reported.[8–18] These compounds have been used as antitumor or antiviral drugs[8] or as tools to study the modulation of enzymes.[19–23] Several distamycin analogues possessing antitumor activity are undergoing clinical trials.[24,25]

The development of methods to synthesize minor groove molecules capable of binding with virtually any DNA sequence increased the interest for such compounds.[23,26] For example, lexitropsins have been designed to recognize A·T and also G·C base-pairs. Sulfonated and phosphonated distamycin derivatives

Abbreviations: MGB, minor groove binders; HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase.

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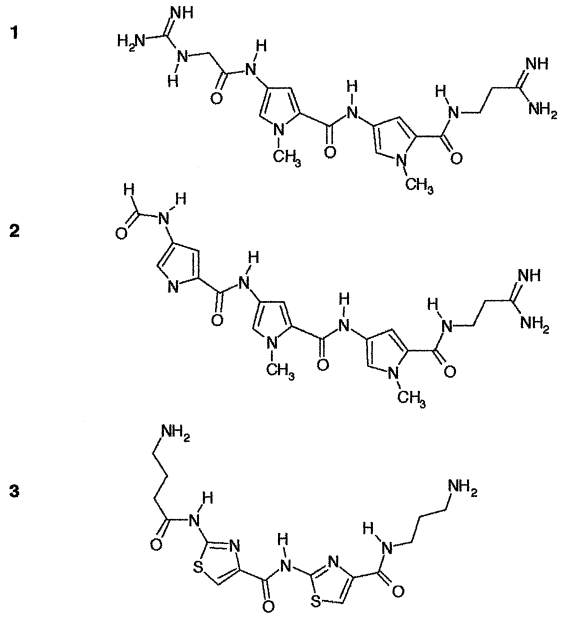


Figure 1. Structural formulas of netropsin (**1**), distamycin (**2**) and thiazolecarboxamides (**3**).

capable of suppressing HIV-1 replication are of potential therapeutic importance.[27] Of obvious interest are the analogues of distamycin and netropsin in which the *N*-methylpyrrole fragment is substituted with a thiazole cycle, since these compounds are likely to possess different site-specificity compared to oligopyrrolecarboxamides. Due to the low reactivity of the key oligomer 2-amino-4-carbonic acid-1,3-thiazole, studies concerning the minor groove binders which contain the thiazole fragment in their structure are not numerous.[28–31]

Heteroanalogues of distamycin and netropsin in which one of the methylpyrrole units is substituted by thiazole have been synthesized. Studies related to the site-specificity of a thiazole-containing oligopeptide analogue of netropsin (compound **3** in Fig. 1) demonstrated that this compound bound to 4–5 base pairs of the minor groove.[13,14] Compound **3** has higher binding affinity for sites with high content of G·C pairs;[13] in addition, it prefers an alternate pyrimidine sequence instead of a homopolymeric sequence. There are also reports showing the influence of the methyl substituents of a thiazole-containing lexitropsin on the mode of binding to DNA.[29]

HIV-1 reverse transcriptase (RT) is an enzyme playing a crucial role in the replication of the retrovirus.[32] RT catalyzes three reactions, all essential, to transform the single-stranded retroviral RNA genome into a double-stranded proviral DNA. The enzyme is multifunctional and exhibits three enzymatic activities: RNA-dependent DNA polymerase, DNA-dependent DNA polymerase and ribonuclease H (RNase H). HIV-1 RT starts minus-strand DNA synthesis from $tRNA^{Lys3}$ as primer and the plus-strand RNA genome as template. After digestion of the RNA template from the duplex RNA·DNA, the synthesis of the plus-strand DNA proceeds using the minus-strand DNA as template. Thus, during replication, the enzyme is first complexed with an RNA·RNA template–primer; then, with an RNA·DNA, and finally, at the second step of replication, with a DNA·DNA duplex.

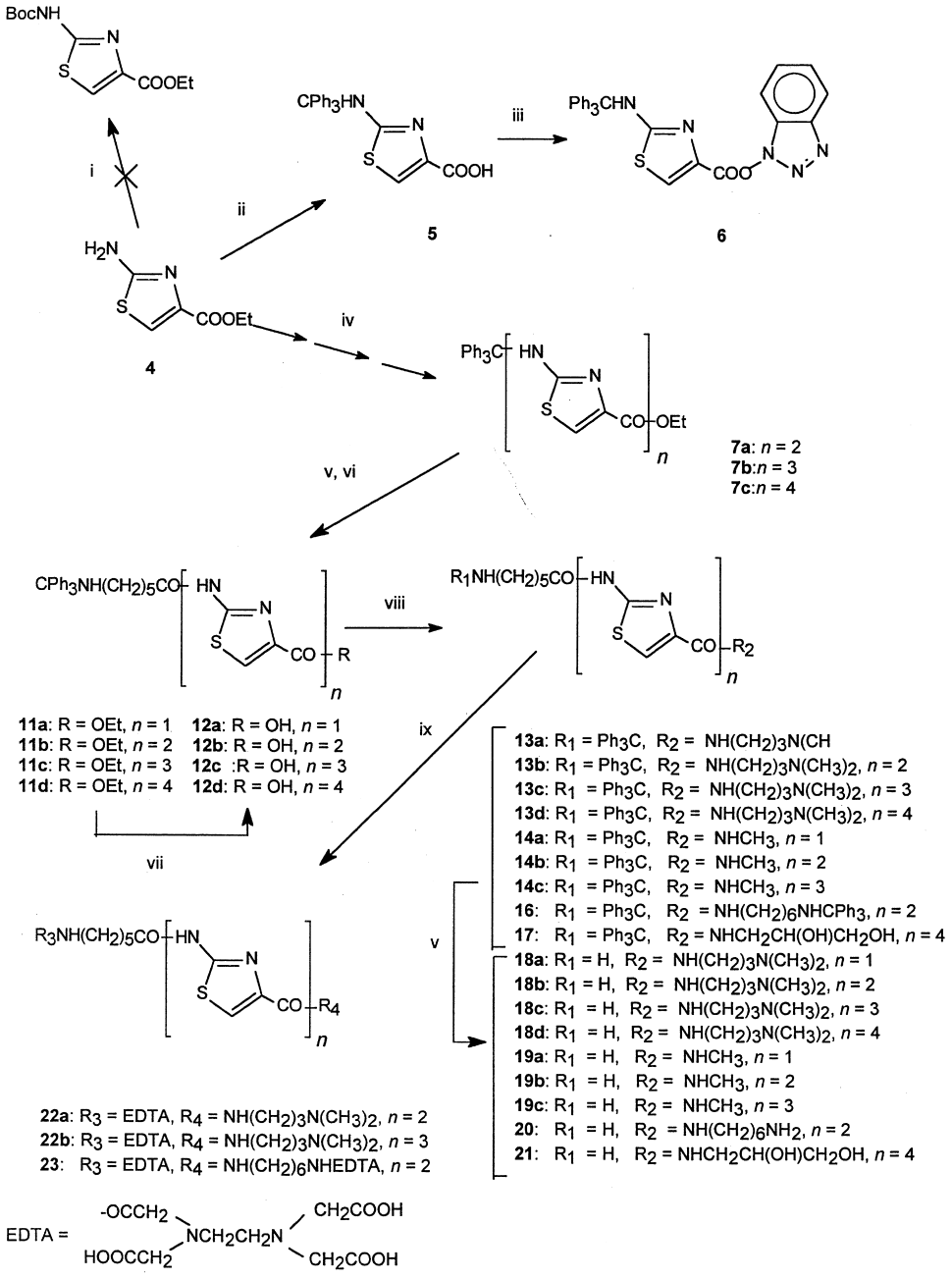
Here we describe the synthesis of a new type of minor groove binder. They are thiazole-containing oligopeptides of various lengths (up to 4 thiazole rings), containing different groups on their C- and N-termini. The effect of these oligocarboxamides on the activity of HIV-1 reverse transcriptase was determined.

Results and Discussion

Synthesis of oligo-1,3-thiazolecarboxamide

The basic scheme of the synthesis of oligo-1,3-thiazolecarboxamide derivatives is shown in Figure 2. The ethyl-2-amino-1,3-thiazole-4-carboxylate (**4**) was selected as the starting material to obtain the target products. The amino group-protected 1-hydroxy-1,2,3-benzotriazole ester of 2-amino-1,3-thiazole-4-carboxylate was used as a synthon. Attempts to obtain the (*tert*-butyloxy)-carbonyl (Boc)-derivative were unsuccessful, possibly due to the extremely low nucleophilicity of the heterocyclic amino group. Hence, we used trityl group for the protection of the amino group. The 1-hydroxy-1,2,3-benzotriazole ester **6** was readily synthesized from equimolar amounts of acid **5** and 1-hydroxy-1,2,3-benzotriazole by employing dicyclohexylcarbodiimide (DCC) as a coupling reagent. Compound **6** is sufficiently stable and can be stored for several months at 0–4 °C without any alteration.

The cycle of oligocarboxamide chain lengthening comprised detritylation under the effect of trifluoroacetic acid (TFA) in chloroform with consequent combination of the amine formed hydroxy-1,2,3-benzotriazole ester **6** in CH_2Cl_2–DMF, since the amine solubility decreased with the growth of the chain. The compounds synthesized were purified by chromatography on silica gel. First, the residue of *N*-tritylaminohexanoic acid was attached to the N-termini of the obtained di-, tri-, and tetrathiazolecarboxamide derivatives **12** followed by condensation of the C-terminal carboxyl group with *N*,*N*-dimethyl-2-aminopropane, methylamine, or *N*-triphenylmethyl-1,6-diaminohexane. At the last stage, detritylation of the synthesized compounds by TFA was carried out. EDTA-derivatives of di- and trithiazolecarboxamides were obtained. As a result, compounds with different numbers of thiazolecarboxamide units in the peptide and the substitutes in the side chain were obtained. The number of thiazole units in the synthesized oligopeptides was estimated by NMR spectroscopy by comparing the integrals of proton signals of thiazole and those of the additional groups attached to the N- or C-terminus of the compounds. The elution time of the compounds on reverse-phase chromatography gradually increased with the lengthening of the oligopeptides. The structures of the thiazolecarboxamide analogues are given in Figure 2. We did not try to prepare longer oligocarboxamides (penta- and hexathiazolecarboxamides) because of their low solubility in



[a] (i) Boc_2O/i-PrOH/K_2CO_3/DMAP; (ii) Ph_3CCl/Py; (iii) Hobt/DCC/CH_2Cl_2/4 h; (iv) **6**/DMF/NEt_3/50°C/17 h; (v) TFA/$CHCl_3$/1 h; (vi) **10**/NEt_3/DMF/60°C/10 h; (vii) 2M NaOH/70% EtOH/50°C/1 h; (viii) $NH_2(CH_2)N(CH_3)_2$ or $NH_2CH_2CH(OH)CH_2OH$ or NH_2CH_3 or **15** /Hobt/DCC/DMF/60°C /3-10 h; (ix) bisanhydrid EDTA/DMF/Py

Figure 2. Basic scheme of the synthesis of oligo-1,3-thiazolecarboxamide derivatives.

organic solvents and water. According to preliminary DNA footprinting data, oligothiazolecarboxamides interacted with DNA sites containing both A·T and G·C pairs (to be published elsewhere).

Interaction of derivatives with HIV-1 RT

While distamycin and its analogues are known to interact predominantly with the minor groove of the B-form DNA·DNA duplexes,[7,26,31,33] there are no reports concerning the interaction of such compounds with RNA·RNA or RNA·DNA duplexes. As HIV-1 RT interacts with various template–primers, such as RNA·RNA, RNA·DNA and DNA·DNA during viral replication, it seemed interesting to study the interaction of the thiazolecarboxamides with all these nucleic acids. This was done by determining the efficiency of the interaction between the thiazolecarboxamides and HIV-1

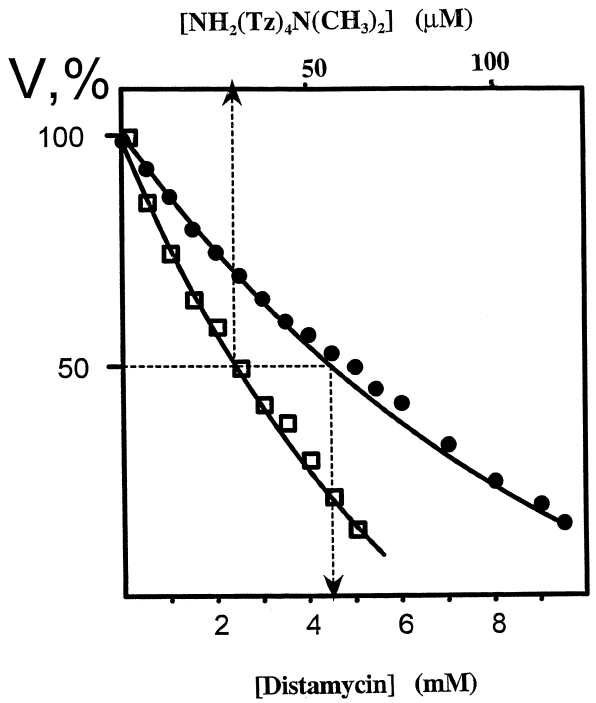


Figure 3. Reverse transcription in the presence of thiazolecarboxamides or distamycin. Relative rates of RT-catalyzed polymerization (**V**) versus concentration of distamycin (●) or tetrathiazole carboxamide $NH_2(Tz)_4N(CH_3)_2$ (compound **18d**) (□), in the presence of poly(A)-d(pT)$_{16}$ template–primer complex. Arrows indicate the IC_{50} values.

RT in the presence of different duplexes used as model of nucleic acids: poly(A)·oligo(U), poly(A)·oligo(dT), poly(dA)·oligo(U), poly(dA)·oligo(dT) and activated DNA.

The efficiency of the interaction was determined by estimating the concentration of the thiazolecarboxamide giving 50% inhibition (IC_{50}) of the RT-dependent polymerization. Experiments were done in parallel in the presence of distamycin used as reference of a small groove binding molecule. A typical experiment on the determination of the IC_{50} value for the thiazole-containing oligopeptides is shown in Figure 3. The IC_{50} values for all the compounds analyzed are given in Table 1. The thiazole-containing oligopeptides were better inhibitors of reverse transcription than distamycin in the presence of a template–primer DNA·DNA, as shown by the results with activated DNA or poly-(dA)·d(pT)$_{16}$.

In the presence of RNA·DNA [poly(A)·d(pT)$_{16}$] or DNA·RNA [poly(dA)·(pU)$_{16}$] template–primers, the inhibition of reverse transcription was less important than in the case of DNA·DNA, although compounds **18c** and **18d** showed good inhibitory properties (Table 1). The affinity of the thiazole-containing compounds for nucleic acids depended essentially on the structure of the additional groups at the N- and C-termini of oligocarboxamides, and on the number of thiazole cycles. For tri- and tetrameric oligomers (compounds **18c** and **18d**), the inhibition was enhanced by one order of magnitude.

In the case of a template–primer RNA·RNA such as poly(A)·(pU)$_{16}$, the inhibitory effect of the thiazole-containing derivatives in the RT-catalyzed reaction was extremely low (IC_{50} = 1.5–7.7 mM). Apparently, this inhibition was independent of either the number of amino acid units in the oligopeptide or the structure of the additional groups within these compounds.

Our results showed that the effects of the thiazole derivatives were similar to those of distamycin only in the case of DNA·DNA and RNA·RNA template–primer duplexes. A different behavior was found, however, between the thiazole derivatives and distamycin in the case of RNA·DNA or DNA·RNA: some thiazole derivatives inhibited the polymerization reaction on poly-(dA)·d(pT)$_{16}$ and poly(dA)·(pU)$_{16}$ at concentrations that were 1–2 orders of magnitude lower than that of distamycin.

We compared logically our values to results described for a compound of the same nature. Anyway, the IC_{50} values for thiazole compounds are higher than what is found for a standard inhibitor of the HIV-1 RT like AZTTP.

The features governing the interaction of DNA with different compounds in solution cannot always be extrapolated to the interaction of DNA with an enzyme, as the latter causes substantial structural changes in the nucleic acid regions situated within the protein globule (for a review see ref 34). For example oligonucleotides containing the phenazinium group at the 5′-end

Table 1. Effect of thiazolecarboxamide derivatives on HIV-1 RT[a]

Compound	N° thiazole units	IC_{50} (μM)				
		Activated DNA	Poly(dA)·d(pT)$_{16}$	Poly(A)·d(pT)$_{16}$	Poly(dA)·(pU)$_{16}$	Poly(A)·(pU)$_{16}$
18b	2	10	970	250	460	7700
18c	3	10	700	40	20	5500
18d	4	10	10	20	40	6800
19b	2	50	20	350	500	6000
19c	3	10	10	180	870	2700
20	2	50	350	250	360	2800
21	4	50	650	9000	120	5000
Distamycin	—	50	860	5000	7500	4800

[a]Reverse transcription was performed as described in the Experimental using different template–primers as indicated. IC_{50} corresponds to the concentration of thiazolcarboxamide derivative inhibiting 50% of the HIV-1 RT activity. IC_{50} values for the distamycin are indicated.

demonstrated a similar affinity for complementary DNA and RNA in solution, while the affinity of the ethidium derivative of $d(pT)_8$ was about 2 orders of magnitude higher for poly(A) than that for poly(dA) templates in their complexes with HIV RT.[35] This seems to result as a consequence of a change in the structure of the nucleic acid after binding to the enzyme.

The formation of a complex between ds DNA and enzymes involved in replication substantially diminishes intrastrand complementary interactions, apparently as a result of an increase of the distance between the complementary strands and alteration of their structural characteristics as compared with those in solution. Such a change of the complex is promoted by binding of the template and primer on the enzyme through effective interactions with the protein. It is interesting to recall that there is a correlation between the interaction of distamycin with ds DNA in solution, and in the complex with HIV-1 RT.

Why can thiazole-containing oligopeptides interact better with RNA·DNA complexes than distamycin? And why do all compounds analyzed have a low affinity for the RNA·RNA duplex?

Different reports have focused on the significance of HIV-1 RT primer–template interactions. It is known that DNA·DNA and DNA·RNA duplexes in solution can be either in B or in A form, whereas A-form (11 residues per turn) is characteristic of RNA·RNA.[36,37] Based on kinetic and thermodynamic data it can be assumed that a duplex containing a template in A-like form and a primer in B-like form is optimal for RT or DNA polymerases such as mammalian DNA polymerase β.[38,39] X-ray analysis data of HIV-1 RT and polymerase β complexes with nucleic acids strongly support this idea.[40–42] A template–primer in complex with HIV-1 RT binds to the enzyme in a catalytically relevant fashion. The template–primer conforms more closely in structure to a classical A-form DNA near the polymerase active site and to B-form DNA near the RNase H active site. RT must be able to induce a significant bend in the DNA·DNA duplex such that the helical axes of the A-form and B-form portions make an angle of about 40–45°. For DNA polymerase β a similar situation was found: the DNA duplex presented an A-form in the vicinity of the active site, while double stranded DNA outside the active site presented a B-form. Thus, the adaptation of DNA or RNA to the optimal conformation for the enzyme may be a very fine process.

Assuming that thiazole oligopeptides have an increased affinity for the minor groove of the RNA·DNA duplexes when complexed to RT, the low affinity of these compounds for RNA·RNA can be explained by the inability of the latter to effectively adapt to the RT-dependent specific conformation. Thus, it seems possible to use the thiazole-containing oligopeptides for detection of specific nucleic acid conformation (A- and B-forms) after binding with enzymes.

Conclusion

We synthesized different oligothiazolecarboxamide derivatives containing up to four thiazole cycles. These compounds were inhibitors of HIV-1 RT when assayed in the presence of different template–primers: DNA·DNA, DNA·RNA and RNA·DNA. Under the same conditions, distamycin inhibited the enzyme only with DNA·DNA duplexes. The results presented here implicate that interactions of HIV-1 RT with the secondary structures of the primer–template may play a significant role in its inhibition by thiazole derivatives.

Experimental

Chemistry

TLC analysis was carried out on precoated plates of silica gel 60F254 (Merck). Purification was perfomed on silica gel (40–60 μm). ^{1}H NMR spectra were recorded with a Bruker AM-200 apparatus. Reverse phase HPLC was performed with Altex dual-pump system and 2.5×25 cm with Diasorb 130 C16T (BioChemMack). All the chemicals used were obtained from Fluka. EDTA-bisanhydride was prepared according to ref 43.

Ethyl 2-amino-1,3-thiazole-4-carboxylate (4). This was prepared as described in ref 44. After recrystallization from EtOH the yield of pure compound was 62%. ^{1}H NMR ($CDCl_3$) δ 7.42 (s, 1H), 7.02 (b s, 2H), 4.26 (q, 2H, J = 7.0 Hz), 1.29 (t, 3H, J = 7.0 Hz).

2-Triphenylmethylamino-4-carboxy-1,3-thiazol (5). A mixture of compound **4** (7.4 g, 43 mmol) and triphenylmethyl chloride (12.0 g, 43 mmol) was dissolved in 60 mL of dry pyridine and stirred for 16 h at room temperature. The precipitate was filtered off, washed with pyridine (2×10 mL), and the filtrate was evaporated under reduced pressure up to 40–60 mL. Ten grams of NaOH in 100 mL of 60% EtOH was added to the residue. The mixture was stirred until precipitate formation and left at room temperature for 20 h. The mixture was cautiously acidified with acetic acid to pH 5–6. The precipitate was collected by filtration, washed with water (10×50 mL) and acetone (2×25 mL). The yield of product **5** was 12.6 g (72%).^{1}H NMR ($CDCl_3$) δ 9.46 (bs, 1H), 7.27–7.40 (m, 15H), 7.27 (s, 1H).

1,2,3-Benzotriazol-1-yl-2-triphenylmethylamino-1,3-thiazol-4-carboxylate (6). Compound **5** (7.20 g, 18.6 mmol) was rapidly stirred with 1-hydroxy-1,2,3-benzotriazole (2.52 g, 18.6 mmol) in dry dichloromethane (200 mL), and DCC (3.90 g, 19.0 mmol) was added to the mixture in small portions at room temperature. After stirring the mixture for 4 h, the precipitated urea was filtered off and solution was poured into hexane (200 mL). The precipitate was collected and dried in vacuo over SiO_2. The resulting crude product **6** (9.0 g, 96%) was sufficiently pure and could be used directly for synthesis. ^{1}H NMR ($CDCl_3$) δ 7.65 (s, 1H), 7.28–7.65 (m, 4H), 7.32 (s, 15H), 7.08 (s, 1H).

Ethyl-2-[2-(triphenylmethylamino)-1,3-thiazol-4-carboxamido]-1,3-thiazole-4-carboxylate (7a). A mixture of compound **4** (1.83 g, 10.6 mmol), compound **6** (5.35 g, 10.6 mmol) and NEt_3 (2.6 mL, 18 mmol) in dry DMF (25 mL) was stirred for 1 h at 50 °C. The greenish mixture was allowed to react at 50 °C without stirring for 16 h. The mixture was evaporated under reduced pressure. The residue was purified by column chromatography; the resulting product **7a** was eluted by chloroform: hexane (1:1) (5.0 g, 88%). ^{1}H NMR ($CDCl_3$), δ 10.40 (bs, 2H), 7.81 (s, 1H), 7.31 (s, 15H), 7.26 (s, 1H), 6.60 (s, 1H), 4.38 (q, 2H, J = 7.0 Hz), 1.37 (t, 3H, J = 7.0 Hz).

Ethyl-2-[2-[2-(triphenylmethylamino)-1,3-thiazol-4-carboxamido]-1,3-thiazol-4-carboxamido]-1,3-thiazole-4-carboxylate (7b). Compound **7a** (2.6 g, 4.8 mmol) dissolved in $CHCl_3$ (30 mL) was treated with TFA (15 mL) for 1 h at room temperature. The mixture was evaporated under reduced pressure. Twenty milliliters of water were added to the residue. The precipitate was filtered, washed with 5% $NaHCO_3$ (20 mL), rinsed with water (4×20 mL), dried on a filter, washed with ether and dried in vacuo over SiO_2. The resulting amine (1.03 g), compound **6** (1.82 g, 3.5 mmol) and NEt_3 (0.7 mL, 5.0 mmol) in dry DMF (5 mL) were stirred for 1 h at 50 °C. The greenish mixture was allowed to react at 50 °C without stirring for 16 h. The mixture was evaporated under reduced pressure. The residue was purified by column chromatography using chloroform as eluent giving the product **7b** (1.76 g, 55%). ^{1}H NMR ($(CD_3)_2CO$) δ 10.90 (bs, 1H), 9.80 (bs, 1H), 8.14 (s, 1H), 8.21 (s, 1H), 8.04 (s, 1H), 7.25–7.56 (m, 16H), 4.32 (q, 2H, J = 7.0 Hz), 1.34 (t, 3H, J = 7.0 Hz).

Ethyl-2-[2-[2-[2-(triphenylmethylamino)-1,3-thiazol-4-carboxamido]-1,3-thiazol-4-carboxamido]-1,3-thiazol-4-carboxamido]-1,3-thiazole-4-carboxylate (7c). Compound **7b** (0.88 g, 1.3 mmol) in $CHCl_3$ (15 mL) was treated with TFA (7 mL) for 1 h at room temperature. The mixture was evaporated under reduced pressure. Ten milliliters of water were added to the residue. The precipitate was filtered, washed with 5% $NaHCO_3$ (5 mL), rinsed with plenty of water, dried on a filter, washed with ether (4×5 mL) and dried in vacuo over SiO_2. The resulting amine (0.51 g), compound **6** (1.55 g, 3.1 mmol) and NEt_3 (0.6 mL, 4.2 mmol) in dry DMF (6 mL) were stirred for 1 h at 50 °C. The greenish mixture was allowed to react without stirring at 50 °C for 16 h. The mixture was evaporated under reduced pressure. The residue was purified by column chromatography using chloroform:DMF (9:1) as eluent giving the product **7c** (0.49 g, 48%). ^{1}H NMR ($CDCl_3$:$(CD_3)_2SO$ = 1:1) δ 7.84 (s, 1H), 7.81 (s, 1H), 7.68 (s, 1H), 7.62 (s, 1H), 7.05–7.25 (m, 16H), 4.17 (q, 2H, J = 7.0 Hz)), 1.20 (t, 3H, J = 7.0 Hz).

Ethyl ester-6-aminohexanoic acid hydrochloride (8). Thionyl chloride was added dropwise to the solution of 6-aminohexanoic acid (13.1 g, 100 mmol) in dry ethanol (120 mL) at temperature 0–5 °C. The mixture was stirred for 1 h at room temperature, filtered off and the filtrate was evaporated. The crude product was recrystallized from EtOH. The yield of pure compound **8** was 18.6 g (95%). ^{1}H NMR ($(CD_3)_2CO$) δ 7.05–7.43 (m, 15H), 4.02 (q, 2H, J = 7.0 Hz), 2.19 (t, 2H, J = 7.0 Hz), 2.08 (t, 2H, J = 7.0 Hz), 1.28–1.60 (m, 6H), 1.20 (t, 3H, J = 7.0 Hz).

6-Triphenylmethylaminohexanoic acid (9). Triphenylmethyl chloride (26.8 g, 97 mmol) was added to a solution of compound (**8**) (18.5 g, 95 mmol) and NEt_3 (30 mL) in dry CH_2Cl_2 at 0 °C. The mixture was stirred for 3 h at room temperature. The precipitate was filtered off, the filtrate was washed with water (2×50 mL) and then evaporated. The residue was dissolved in ethanol (150 mL), and a mixture of NaOH (27 g) and H_2O (30 mL) was added dropwise at room temperature. The mixture was stirred for 4 h at room temperature, evaporated to 100 mL, water (200 mL) was added to the residue and the mixture was cautiously acidified with acetic acid to pH 5–6. The product was extracted with $CHCl_3$ (2×100 mL), the organic layer was washed with water (3×200 mL) and dried over Na_2SO_4. After evaporation, practically pure **9** was obtained (24.8 g, 65%). ^{1}H NMR ($(CD_3)_2CO$) δ 7.12–7.52 (m, 15H), 2.26 (t, 2H, J = 7.0 Hz), 2.10 (t, 2H, J = 7.0 Hz), 1.28–1.67 (m, 6H).

1,2,3-Benzotriazol-1-yl-6 triphenylmethylaminohexylcarboxylate (10). DCC (3.70 g, 17.9 mmol) in small portions was added to compound **9** (6.36 g, 17.0 mmol) and 1-hydroxy-1,2,3-benzotriazole (2.42 g, 17.9 mmol) in dry dichloromethane (100 mL) at room temperature. After the mixture was stirred for 3 h, the precipitated urea was filtered off. Filtrate was evaporated and dried in vacuo over SiO_2. The resulting crude product **10** (8.27 g, 99%) was sufficiently pure and could be used directly for synthesis. ^{1}H NMR ($(CD_3)_2CO$) δ 7.12–7.91 (m, 19H), 2.25 (t, 2H, J = 7.0 Hz), 2.16 (t, 2H, J = 7.0 Hz), 1.28–1.67 (m, 6H).

6-Triphenylmethylaminohexylcarboxy derivatives (11a–d). Compound **7a–c** (1.0 mmol) was dissolved in the mixture of $CHCl_3$ (15 mL) and TFA (7 mL). After 1 h at room temperature the mixture was evaporated under reduced pressure, and water (10 mL) was added to the residue. The precipitate was filtered, washed with 5% $NaHCO_3$ (5 mL), rinsed with plenty of water, dried on filter, washed with ether (4×10 mL) and dried in vacuo over SiO_2. The resulting amine or 1.0 mmol compound **4**, compound **10** (1.02 g, 2.1 mmol) and NEt_3 (0.3 mL) in dry DMF (6 mL) were stirred for 10 h at 60 °C. The brownish mixture was evaporated under reduced pressure. The residue was purified by column chromatography using chloroform as eluent. The yield of pure compounds **11a**, **11b**, **11c**, **11d** was 85, 76, 58 and 63%, respectively.

^{1}H NMR of compound **11a** ($(CD_3)_2CO$) δ 11.07 (bs, 1H), 8.02 (s, 1H), 7.1–7.5 (m, 15H), 4.30 (q, 2H, J = 7.0 Hz), 2.91 (bs, 2H), 2.57 (t, 2H, J = 7.0 Hz), 1.46–1.80 (m, 6H), 1.32 (t, 3H, J = 7.0 Hz).

^{1}H NMR of compound **11b** ($(CD_3)_2CO$) δ 11.29 (bs, 1H), 10.77 (bs, 1H), 8.09 (s, 1H), 8.01 (s, 1H), 7.1–7.5 (m, 16H), 4.29 (q, 2H, J = 7.0 Hz), 2.93 (bs, 2H), 2.58 (t, 2H, J = 7.0 Hz), 1.49–1.79 (m, 6H), 1.33 (t, 3H, J = 7.0 Hz).

^{1}H NMR of compound **11c** ($(CD_3)_2CO$) δ 11.44 (bs, 1H), 10.85 (bs, 2H), 8.19 (s, 1H), 8.10 (s, 1H), 8.03 (s, 1H), 7.1–7.5 (m, 16H), 4.30 (q, 2H, $J=7.0$ Hz), 2.90 (bs, 2H), 2.60 (t, 2H, $J=7.0$ Hz), 1.30–1.84 (m, 6H), 1.33 (t, 3H, $J=7.0$ Hz).

^{1}H NMR of compound **11d** (DMF-d_7) δ 11.30 (bs, 1H), 10.60 (bs, 1H), 8.44 (s, 1H), 8.39 (s, 1H), 8.30 (s, 1H), 8.20 (s, 1H), 7.1–7.5 (m, 16H),4.35 (q, 2H, $J=7.0$ Hz), 2.90 (bs, 2H), 2.60 (t, 2H, $J=7.0$ Hz), 1.20–1.90 (m, 6H), 1.36 (t, 3H, $J=7.0$ Hz)

Preparation of acids 12a–d. 4 M NaOH (10 mL) was added to the solution of ethyl ester (**11a–d**) (3 mmol) in ethanol (20 mL). The mixture was stirred for 12 h at 20 °C, diluted with water (30 mL) and cautiously acidified with HCl to pH 5–6. The precipitated acid was collected, washed with water (6×10 mL) and dried in vacuo over SiO_2. The resulting acids (90–95%) were sufficiently pure and could be used directly for synthesis.

***N*,*N*-Dimethyl-1,3-propylendiamine derivatives (13a–d) and methylamine derivatives.** DCC (0.4 g, 2.0 mmol) in small portions was added to the mixture of acid **12a–c** (1.0 mmol) and 1-hydroxy-1,2,3-benzotriazole (0.3 g, 2.0 mmol) in dry DMF (3 mL) at room temperature. After stirring for 2 h, *N*,*N*-dimethyl-1,3-diaminopropane (0.2 mL) or methylamine hydrochloride (0.4 g, 6 mmol) and NEt_3 (5 mL) was added and reaction mixture was left for 12 h. The precipitate was filtered off, washed with CH_2Cl_2 (5 mL). Combined filtrates diluted with $CHCl_3$ (50 mL) were washed with 5% $NaHCO_3$ (2×15 mL), water (3×25 mL), dried over Na_2SO_4 and evaporated. The yield of products **13a**, **13b**, **13c**, **13d** and **14a**, **14b**, **14c** after column chromatography (0–20% gradient of EtOH in $CHCl_3$:NEt_3 (95:5) was 60, 49, 55, 35, 65, 52 and 40%, respectively.

^{1}H NMR of compound **13a** ($(CD_3)_2CO$) δ 8.10 (bs, 1H), 7.87 (s, 1H), 7.15–7.55 (m, 15H), 3.54 (bt, 2H), 2.64 (bs, 2H), 2.49 (bt, 2H), 2.17 (s, 6H), 1.10–2.10 (m, 10H).

^{1}H NMR of compound **13b** ($(CD_3)_2CO$) δ 8.17 (bs, 1H), 7.96 (s, 1H), 7.86 (s, 1H), 7.15–7.55 (m, 15H), 3.54 (bt, 2H), 2.64 (bs, 2H), 2.49 (bt, 2H), 2.17 (s, 6H), 1.10–2.10 (m, 10H).

^{1}H NMR of compound **13c** ($(CD_3)_2CO$) δ 8.00 (s, 1H), 7.90 (s, 1H), 7.85 (s, 1H), 7.15–7.55 (m, 15H), 3.58 (b.quin, 2H), 2.60 (bm, 2H), 2.33 (bm, 2H), 2.07 (s, 6H), 1.10–2.10 (m, 10H).

^{1}H NMR of compound **13d** (DMF-d_7) δ 8.40 (s, 1H), 8.31 (s, 1H), 8.28 (s, 1H), 8.10 (s, 1H), 7.1–7.5 (m, 15H), 3.83 (bs, 2H), 2.55 (bs, 2H), 2.30 (bs, 2H), 2.13 (s,6H), 1.1–2.0 (m, 10H).

^{1}H NMR of compound **14a** (CCl_4) δ 10.78 (bs, 1H), 7.82 (bs, 1H), 7.74 (s, 1H), 7.20–7.50 (m, 15H), 3.27 (s, 3H), 2.80 (bt, 2H), 2.35 (bt, 2H), 2.01 (bm, 2H), 1.77 (bm, 4H).

^{1}H NMR of compound **14b** ($(CD_3)_2CO$) δ 11.49 (bs, 1H), 10.62 (bs, 1H), 8.35 (bs, 1H), 7.78 (bs, 1H), 7.75 (s, 1H), 7.15–7.50 (m, 15H), 3.25 (s, 3H), 2.75 (t, 2H), 2.33 (t, 2H), 1.90–2.10 (m, 2H), 1.70–1.80 (m, 4H).

^{1}H NMR of compound **14c** (DMF-d_7) δ 8.38 (s, 1H), 8.03 (bs, 1H), 7.83 (s, 1H), 7.15–7.55 (m, 15H), 2.61 (t, 2H), 2.11 (s, 3H), 2.09 (t, 2H), 1.3–1.8 (m, 6H).

1-Triphenylmethyl-1,6-diaminohexane (15). Triphenylmethyl chloride (6.6 g, 24 mmol) in 50 mL dry CH_2Cl_2 was added to a solution of 1,6-diaminohexane (16.6 mg, 140 mmol) in dry CH_2Cl_2 (100 mL). The mixture was stirred for 4 h at 20 °C. Product was purified by column chromatography using chloroform:EtOH:NEt_3 (94:5:1) as eluent. The yield of compound **15** was 2.73 g (7.7 mmol, 32%).

^{1}H NMR ($(CD_3)_2CO$) δ 7.10–7.60 (m, 15H), 3.09 (t, 7.84, 2H, $J=7.2$ Hz), 2.10 (t, 7.84, 2H, $J=7.2$ Hz), 1.52 (bm, 4H), 1.32 (bm, 4H).

Preparation of compound 16. To the mixture of acid **12c** (145 mg, 0.23 mmol) and 1-hydroxy-1,2,3-benzotriazole (40 mg, 0.26 mmol) in dry CH_2Cl_2 (5 mL) was added DCC (54 g, 0.26 mmol) at room temperature. After stirring for 4 h, the amine **15** (93 mg, 0.26 mmol) and 70 μl NEt_3 were added and the reaction mixture was boiled for 4 h. The yield of pure compound **16** after column chromatography (0–5% gradient of ethanol in chloroform) was 187 mg (0.19 mmol, 84%).

^{1}H NMR ($(CD_3)_2CO$) δ 11.46 (bs, 1H), 10.50 (bs, 1H), 7.84 (s, 1H), 7.10–7.50 (m, 30H), 7.98 (s, 1H), 3.40 (q, 2H, $J=6.5$ Hz), 2.84 (t, 2H, $J=7.0$ Hz), 2.64 (t, 2H, $J=7.0$ Hz), 2.14 (t, 2H, $J=7.0$ Hz), 0.94–1.90 (m, 14H).

Preparation of compound 17. DCC (93 g, 0.45 mmol) was added to the mixture of acid **12d** (260 mg, 0.30 mmol) and 1-hydroxy-1,2,3-benzotriazole (69 mg, 0.45 mmol) in dry DMF (5 mL) at room temperature. After stirring for 4 h, $NH_2CH_2CH(OH)CH_2OH$ (120 mg, 1.30 mmol) was added and the reaction mixture was left for 4 h at 60°C. The yield of pure compound **17** after column chromatography (0–15% gradient of EtOH in $CHCl_3$:NEt_3 (95:5)) was 110 mg (0.12 mmol, 40%).

^{1}H NMR ($(CD_3)_2CO$) δ 11.48 (bs, 1H), 10.75 (bs, 2H), 10.30 (bs, 1H), 8.18 (s, 1H), 8.09 (bs, 1H), 8.02 (s, 1H), 8.01 (s, 1H), 7.82 (s, 1H), 7.10–7.50 (m, 15H), 4.51 (bs, 1H), 3.88 (bs, 1H), 3.60 (m, 4H), 2.60 (t, 2H, $J=7.0$ Hz), 2.15 (t, 2H, $J=7.0$ Hz), 1.70 (m, 4H), 1.45 (m, 2H).

Preparation of compounds 18–21. Compounds **13**, **14**, **16** or **17** (100 mg) were dissolved in the mixture of $CHCl_3$ (2 mL) and TFA (1 mL) and, after 1 h at room temperature, the mixture was evaporated under reduced pressure and washed with ether (4×5 mL), dissolved in 0.1% TFA and purified by HPLC (0–15% gradient CH_3CN, 0.1% TFA 50–80%). The yield of products **18a**, **18b**, **18c**, **18d**, **19a**, **19b**, **19c**, **20**, **21** after column chromatography (0–20% gradient of EtOH in $CHCl_3$: NEt_3 (95:5) was 80, 75, 71, 50, 72, 60, 55, 51 and 45%, respectively.

^{1}H NMR of compound **18a** ($(CD_3)_2SO$) δ 11.48 (bs, 1H), 7.84 (s, 1H), 3.56 (t, 2H, $J=7.0$ Hz), 2.64 (t, 2H, $J=7.0$ Hz), 2.53 (t, 2H, $J=7.0$ Hz), 2.20 (s, 6H), 2.10 (t, 2H, $J=7.0$ Hz), 1.40–1.90 (m, 8H).

^{1}H NMR of compound **18b** ($(CD_3)_2SO$) δ 12.30 (bs, 1H), 11.48 (bs, 1H), 7.98 (s, 1H), 7.84 (s, 1H), 3.54 (t, 2H, $J=7.0$ Hz), 2.65 (t, 2H, $J=7.0$ Hz), 2.53 (t, 2H, $J=7.0$ Hz), 2.17 (s, 6H), 2.11 (t, 2H, $J=7.0$ Hz), 1.40–1.90 (m, 8H).

^{1}H NMR of compound **18c** ($(CD_3)_2SO$) δ 12.40 (bs, 2H), 11.48 (bs, 1H), 7.99 (s, 1H), 7.98 (s, 1H), 7.84 (s, 1H), 3.55 (t, 2H, $J=7.0$ Hz), 2.65 (t, 2H, $J=7.0$ Hz), 2.52 (t, 2H, $J=7.0$ Hz), 2.18 (s, 6H), 2.10 (t, 2H, $J=7.0$ Hz), 1.40–1.90 (m, 8H).

^{1}H NMR of compound **18d** ($(CD_3)_2SO$) δ 12.40 (bs, 2H), 12.20 (bs, 1H), 11.48 (bs, 1H), 8.01 (s, 1H), 7.99 (s, 1H), 7.98 (s, 1H), 7.84 (s, 1H), 3.56 (t, 2H, $J=7.0$ Hz), 2.63 (t, 2H, $J=7.0$ Hz), 2.54 (t, 2H, $J=7.0$ Hz), 2.18 (s, 6H), 2.11 (t, 2H, $J=7.0$ Hz), 1.40–1.90 (m, 8H).

^{1}H NMR of compound **19a** (D_2O) δ 7.69 (s, 1H), 3.00 (t, 2H, $J=7.0$ Hz), 2.86 (s, 3H), 2.51 (t, 2H, $J=7.0$ Hz), 1.69 (bruin, 4H, $J=7.0$ Hz), 1.43 (bruin, 4H, $J=7.0$ Hz).

^{1}H NMR of compound **19b** ($(CD_3)_2SO$) δ 12.43 (bs, 1H), 11.95 (bs, 1H), 8.33 (s, 1H), 8.00 (bs, 1H), 7.85 (s, 1H), 4.30 (bs, 2H), 2.81 (s, 3H), 2.16 (t, 2H, $J=7.0$ Hz), 1.68 (m, 4H, $J=7.0$ Hz), 1.39 (m, 4H, $J=7.0$ Hz).

^{1}H NMR of compound **19c** ($(CD_3)_2SO$) δ 12.57 (bs, 1H), 12.43 (bs, 1H), 11.95 (bs, 1H), 8.34 (s, 1H), 8.33 (s, 1H), 8.00 (bs, 1H), 7.85 (s, 1H), 4.40 (bs, 2H), 2.79 (s, 3H), 2.12 (t, 2H, $J=7.0$ Hz), 1.61 (m, 4H, $J=7.0$ Hz), 1.35 (m, 4H, $J=7.0$ Hz).

^{1}H NMR of compound **20** ($(CD_3)_2SO$) δ 12.40 (bs, 1H), 12.00 (bs, 1H), 8.19 (s, 1H), 7.88 (s, 1H), 3.48 (t, 2H, $J=7.0$ Hz), 3.02 (t, 2H, $J=7.0$ Hz), 2.52 (t, 2H, $J=7.0$ Hz), 2.14 (t, 2H, $J=7.0$ Hz), 1.20–1.80 (m, 14H).

^{1}H NMR of compound **21** ($(CD_3)_2CO$:$D_2O=1:1$) δ 7.94 (s, 1H), 7.92 (s, 1H), 7.90 (s, 1H), 7.73 (s, 1H), 3.81 (bs, 1H), 3.51 (m, 4H), 2.94 (t, 2H, $J=7.0$ Hz), 2.38 (t, 2H, $J=7.0$ Hz), 1.63 (m, 4H) 1.37 (m, 2H).

Reverse transcriptase assay

Nucleotides, polynucleotides, salts and distamycin were obtained from Sigma. [^{3}H]dTTP was obtained from Radioisotop (Russia) or ICN Biomedicals (France). All oligodeoxyribonucleotides were synthesized as in ref 45 and were homogeneous according to reverse-phase and ion-exchange chromatography. Oligoribonucleotides were prepared as reported before.[46] Calf thymus DNA was hydrolyzed by DNase I to obtain activated DNA.[48] Electrophoretically homogeneous HIV-1 RT p66/p51 was prepared as previously described.[47]

RT assay was performed at 30 °C. The reaction mixture (50 μL) contained 50 mM Tris–HCl, pH 8.0, 80 mM KCl (in the case of DNA·DNA and DNA·RNA template–primer duplexes, KCl was omitted), 0.5 mM EDTA, 5 mM $Mg(OAc)_2$, 1 mM dithiothreitol, 40 μM [^{3}H]dTTP (1.50 μCi) or in case of activated DNA all four dNTPs: dATP, dCTP, dGTP and [^{3}H]dTTP. The polymerization reaction was initiated by addition of RT (2–5 nM). Samples were taken from the reaction mixture at 2–10 min intervals and further treatment of the reaction was carried out as described.[48]

Reaction mixtures contained each one of the template–primer duplexes in the following optimal concentrations[38] expressed as A_{260} units/mL: 0.5 poly(A)·$d(pT)_{16}$, 1.5 poly(A)·$(pU)_{16}$, 1.5 poly(dA)·$(pU)_{16}$, 2.0 poly(dA)·$d(pT)_{16}$ or 2.0 activated DNA. The ratio of the primer to template concentration (mononucleotide concentration) was 1:5 in all cases except for activated DNA.

Initial rates of the polymerization reaction were determined from the tangents of the data curves at zero time. The oligopeptide concentration inhibiting the polymerization rate to 50% (IC_{50}) was estimated from the dependencies of the initial rates. The statistical error was estimated using the data from 3–4 experiments.

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References

1. Larder, B. A.; Darby, G.; Richman, D. D. *Science* **1989**, *243*, 1731.
2. Larder, B. A.; Kemp, S. D. *Science* **1989**, *246*, 1155.
3. Bailly, C.; Chaires, J. B. *Bioconjug. Chem.* **1998**, *9*, 513.
4. Di Marco, A.; Gaetani, M.; Orezzi, P.; Arcamone, F. *Cancer Chemother. Rep.* **1962**, *18*, 15.
5. Julia, M.; Preau-Joseph, N. P. *Bull. Soc. Chim. Fr.* **1967**, *97*, 4348.
6. Penco, S.; Redaelli, S.; Arcamone, F. *Gazz. Chim. Ital.* **1967**, *97*, 1110.
7. Arcamone, F.; Penco, S.; Delle Monache, F. *Gazz. Chim. Ital.* **1969**, *99*, 620.
8. Lown, J. W. *Antiviral Res.* **1992**, *17*, 179.
9. Krowicki, K.; Lown, J. W. *J. Org. Chem.* **1987**, *52*, 3493.
10. Rao, K. E.; Dusgupta, D.; Sasisekharan, V. *Biochemistry* **1988**, *27*, 3018.
11. Rao, K. E.; Krowicki, K.; Burckhart, G.; Zimmer, C.; Lown, J. W. *Chem. Res. Toxicol.* **1991**, *4*, 241.
12. Ding, L.; Grehn, L.; De Clercq, E.; Andrei, G.; Snoeck, R.; Balzarini, J.; Fransson, B.; Ragnarsson, U. *Acta Chem. Scand.* **1994**, *48*, 498.
13. Plouvier, B.; Bailly, C.; Houssier, R.; Rao, K. E.; Lown, J. W.; Heinichart, J. P. *Nucleic Acids Res.* **1991**, *19*, 5821.
14. Plouvier, B.; Bailly, C.; Houssier, R.; Heinichart, J. P. *Heterocycles* **1991**, *32*, 693.

15. Wade, W. S.; Mrksich, M.; Dervan, P. B. *Biochemistry* **1993**, *32*, 11385.
16. Rao, K. E.; Sasisekharan, V. *Indian J. Chem.* **1990**, *29B*, 503.
17. Rao, K. E.; Sasisekharan, V. *Indian J. Chem.* **1990**, *29B*, 508.
18. Rao, K. E.; Bathini, Y. J. W. *J. Org. Chem.* **1990**, *55*, 728.
19. Mote, Jr, J.; Ghanouni, P.; Reines, D. A. *J. Mol. Biol.* **1994**, *236*, 725.
20. Filipowsky, M. E.; Kopka, M. L.; Brazilzison, M.; Lown, J. W.; Dickerson, R. E. *Biochemistry* **1996**, *35*, 15397.
21. Chen, A. Y.; Yu, C.; Gatto, B.; Liu, L. F. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8131.
22. Kittler, L.; Bell, A.; Baguley, B. C.; Lober, G. *Bioch. Mol. Biol. Int.* **1996**, *40*, 263.
23. Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Nature* **1996**, *382*, 559.
24. Volpe, D. A.; Du, D. L.; Zurlo, M. G.; Mongelli, N.; Murphy, M. J. *Invest. New Drugs* **1992**, *10*, 255.
25. Broggini, M.; Coley, H. M.; Mongelli, N.; Pesenti, E.; Wyatt, M. D.; Hartley, J. A.; Dincalci, M. *Nucleic Acids Res.* **1995**, *23*, 81.
26. Lown, J. W. *Drug Dev. Res.* **1995**, *34*, 145.
27. Clanton, D. J.; Buckheit, R. W.; Terpening, S. J.; Kiser, R.; Mongelli, N.; Borgia, A. L.; Schultz, R.; Narayanan, V.; Bader, J. P.; Rice, W. G. *Antiviral Res.* **1995**, *27*, 335.
28. Baraldi, P. G.; Beria, I.; Cacciari, B.; Capolongo, L.; Cozzi, P.; Mongelli, N.; Romagnoli, R.; Spalluto, G. *Bioorg. Med. Chem. Lett.* **1996**, *11*, 1247.
29. Plouvier, B.; Houssin, R.; Helbecque, N.; Colson, P.; Houssier, C.; Henichart, J. P.; Bailly, C. *Anti-Cancer Drug Design* **1995**, *10*, 155.
30. Plouvier, B.; Houssin, R.; Hecquet, B.; Colson, P.; Houssier, C.; Waring, M. J.; Henichart, J. P.; Bailly, C. *Bioconjug. Chem.* **1994**, *5*, 475.
31. Lown, J. W. *J. Mol. Recognit.* **1994**, *7*, 79.
32. *Retroviruses*; Coffin, J. M.; Hughes, S. H.; Varmus, H. E., Eds.; Cold Spring Harbor Laboratory Press, 1997.
33. Wyatt, M. D.; Garbiras, B. J.; Lee, M.; Forrow, S. M.; Hartley, J. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 801.
34. Nevinsky, G. A. *Mol. Biol.(Mosk.)* **1995**, *29*, 6.
35. Khodyreva, S. N.; Podust, V. N.; Sergeev, D. S.; Ivanova, E. M.; Frolova, E. I.; Koshkin, A. A.; Godovikova, T. S.; Zarytova, V. F.; Richetti, M.; Lavrik, O. I. *Mol. Biol. Rep.* **1993**, *18*, 43.
36. Reid, R. S.; Stuart, A. L.; Gupta, S. V.; Latimer, L. J.; Haug, B. L.; Lee, J. S. *Nucleic Acids Res.* **1987**, *15*, 4325.
37. Matthew, J. B.; Richards, F. M. *Biopolymers* **1984**, *23*, 2743.
38. Nevinsky, G. A.; Andreola, M-L.; Yamkovoy, V. I.; Levina, A. S.; Barr, P. J.; Tarrago-Litvak, L.; Litvak, S. *Eur. J. Biochem.* **1992**, *207*, 351.
39. Kolocheva, T. I.; Nevinsky, G. A. *Mol. Biol. (Mosk.)* **1993**, *27*, 1368.
40. Jacobo-Molina, A.; Ding, J.; Nanni, R.; Clark, A.; Lu, X.; Tantillo, C.; Williams, R.; Kamer, G.; Ferris, A.; Clark, P.; Hizi, A.; Hughes, S.; Arnold, E. *Proc. Natl. Acad. Sci. USA* **1993**, *49*, 6320.
41. Patel, P.; Jacobo-Molina, A.; Ding, J.; Tantillo, C.; Clark, A.; Raag, R.; Nanni, R.; Hughes, S.; Arnold, E. *Biochemistry* **1995**, *34*, 5351.
42. Pelletier, H.; Sawaya, M. R.; Kumar, A.; Wilson, S. H.; Kraut, J. *Science* **1994**, *264*, 1891.
43. Eckelman, W. C.; Karesh, S. M.; Reba, R. C. *J. Pharm. Sci.* **1975**, *64*, 704.
44. Sprague, J. M.; Lincoln, R. M.; Ziegler, C. *J. Am. Chem. Soc.* **1946**, *68*, 266.
45. Zarytova, V. P.; Ivanova, E. M.; Romanenko, V. P. *Bioorg. Chem. (Mosk.)* **1985**, *11*, 815.
46. Mudrakovskaya, A. V.; Yamkovoy, V. I. In *The Enzymes of Microorganisms and Biopolymer Degradation*; Debabov, V. G., Gordon, I. O., Eds.; NPO Medbioeconomics: Moscow, 1990; pp 199–206.
47. Sallafranque-Andreola, M. L.; Robert, D.; Barr, P. J.; Fournier, M.; Litvak, S.; Sarih-Cottin, L.; Tarrago-Litvak, L. *Eur. J. Biochem.* **1989**, *184*, 367.
48. Nevinsky, G. A.; Veniaminova, A. G.; Levina, A. S.; Podust, V. N.; Lavrik, O. I.; Holler, E. *Biochemistry* **1990**, *29*, 1200.