Highly Potent Synthetic Polyamides, Bisdistamycins, and Lexitropsins as Inhibitors of Human Immunodeficiency Virus Type 1 Integrase

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ABSTRACT

Alignment of the available human immunodeficiency virus type 1 (HIV-1) viral DNA termini [U5 and U3 long terminal repeats (LTRs)] shows a high degree of conservation and the presence of a stretch of five or six consecutive adenine and thymine (AT) sequences –10 nucleotides away from each LTR end. A series of AT-selective minor-groove binders, including distamycin and bisdistamycins, bisnetropsins, novel lexitropsins, and the classic monomeric DNA binders Hoechst 33258, 4'-diamino-2-phenylindole, pentamidine, berenil, spermine, and spermidine, were tested for their inhibitory activities against HIV-1 integrase (IN). Although netropsin, distamycin, and all other monomeric DNA binders showed weak activities in the range of 50–200 μM, some of the polyamides, bisdistamycins, and lexitropsins were remarkably active at nanomolar concentrations. Bisdistamycins were 200 times less potent when the conserved AAAAT stretch present in the US LTR was replaced with GGGGG, consistent with the preferred binding of these drugs to AT sequences. DNase I footprinting of the US LTR further demonstrated the selectivity of these bisdistamycins for the conserved AT sequence. The tested compounds were more potent in Mg\(^{2+}\) than in Mn\(^{2+}\) and inhibited IN\(^{50–212}\) deletion mutant in disintegration assays and the formation of IN/DNA complexes. The lexitropsins also were active against HIV-2 IN. Some of the synthetic polyamides exhibited significant antiviral activity. Taken together, these data suggest that selective targeting of the U5 and U3 ends of the HIV-1 LTRs can inhibit IN function. Polyamides might represent new leads for the development of antiviral agents against acquired immune deficiency syndrome.

The rapid emergence of HIV strains resistant to available drugs (Arts and Wainberg, 1996; De Clercq, 1996; Erickson and Burt, 1996) implies that effective treatment modalities will require the use of a combination of drugs targeting different sites of the HIV life cycle (Schinazi, 1991; Johnson, 1994; De Clercq, 1995; Larder et al., 1995). As part of a program to develop novel antiviral agents, we sought to determine the role of specific DNA binding agents as possible inhibitors of HIV-1 IN. IN is an important target for intervention by chemotherapeutics, and to date several inhibitors of this enzyme have been reported (for recent reviews, see Neamati et al., 1997c; Pommier et al., 1997). IN is responsible for the insertion of the viral DNA into a host chromosome. This process is essential for effective viral replication and can be reproduced in vitro using recombinant IN and short oligonucleotides (Katz and Skalka, 1994; Rice et al., 1996).

Integration takes place in two consecutive steps. Initially, IN processes the linear viral DNA by removing two nucleotides from each 3’-end, leaving the recessed 3’-OH termini. This reaction is followed by transesterification of phosphodiester bonds in which each processed viral 3’ terminus becomes linked to a 5’-phosphate of host DNA strand. These two steps, known as 3’-processing and 3’-end joining (strand transfer), can be easily measured in an in vitro assay using purified recombinant HIV-1 IN and an oligonucleotide corresponding to the U5 region of HIV LTR sequence.

IN binds to the viral DNA sequences located at both extremities of the LTRs. Because these sequences are highly conserved in all HIV genomes (Fig. 1), they could provide potential targets for the selective inhibition of integration. In addition, both the U5 and U3 LTRs contain a conserved AT-rich sequence ~10 base pairs from the viral ends (Fig. 1). Netropsin and distamycin (Fig. 2) bind tightly to AT sequences of B-DNA with little affinity for single-stranded nucleic acids, double-stranded RNA, or DNA/RNA hybrids.

ABBREVIATIONS: HIV-1, human immunodeficiency virus type-1; IN, integrase; LTR, long terminal repeat; ES, electrospray ionization; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TLC, thin layer chromatography; DMF, dimethylformamide; MES, 3-(N-morpholino)propanesulfonic acid.
These molecules have an intrinsic twist that favors insertion into the minor groove of B-DNA (Zimmer et al., 1977, 1983; Kopka et al., 1985; Kopka and Larsen, 1992). In a search for selective DNA binders with modified properties, novel lexitropsins were designed and synthesized (Lown, 1988, 1994; Lown et al., 1989). Recent studies demonstrated that polyamides can be synthesized to achieve highly selective recognition of the four Watson-Crick base pairs in the DNA minor groove (Helene, 1998; White et al., 1998). In addition, polymethylene-linked lexitropsins were shown to exhibit antiviral activity (Lown et al., 1989; Wang and Lown, 1992). However, their antiviral mechanism is not well understood.

The current report stems from our continuing efforts to identify novel IN inhibitors from compounds that exhibit antiviral activity in cellular assays. Herein, we show that synthetic polyamides that interact selectively with the conserved AT stretch present in the HIV LTRs can inhibit integrations at submicromolar concentrations.

**Materials and Methods**

**Chemistry.** All chemicals used were of reagent grade. The reactions were carried out in anhydrous tetrahydrofuran that was dried over sodium/benzophenone and distilled fresh at the time of reaction. Dimethylformamide and triethylamine were distilled and stored over molecular sieves (4 Å). The progress of the reaction was monitored by analytical TLC using silica gel (60F-254 mesh; Merck Research Labs, West Point, PA)-coated aluminum-backed plates. Preparative separations were performed by column chromatography on flash silica gel (70–230 mesh; Merck). Melting points were deter-

![Fig. 1](http://hiv-web.lanl.gov/).
mained on an electrothermal melting point apparatus (Fisher-John) and are uncorrected. $^1$H NMR spectra were recorded on a Bruker AM-300 spectrometer, the samples were prepared in dimethylsulfoxide-d6 unless otherwise specified, and the chemical shifts were reported in δ ppm with respect to tetramethylsilane as an internal standard. New products were characterized by elemental analysis, mass spectroscopic analysis, or both using ES on a Micromass Zab-Spec Hybrid Sector TOF.

**Representative synthesis:** $N,N'$-di-{1-methyl-2-[1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrrole]-carboxamido-4-pyrrrole} [carboxamido-4-pyrryl]-phenyl-1,4-dicarboxamide (26). Nitrodistamycin 7a (0.3 g, 0.6 mmol) was added to it followed by 0.5 ml of triethylamine. The reaction (61 mg, 0.3 mmol), predissolved in anhydrous tetrahydrofuran (4 ml), the solution cooled down to 0°, and 1,4-phenyl diacid dichloride under high vacuum to remove the traces of the solvent. The aminomethanol. The filtrate was evaporated, and the contents were dried for 1 hr at 22°. The catalyst was removed by filtration and washed with methanol. The filtrate was evaporated, and the contents were stirred for 2 hr at 22°, at which time the TLC showed complete consumption of 7b. The solvent was removed in vacuo, and the crude product was purified on a silica gel column using $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (6:4, v/v) as eluent to collect pure 26 (160 mg, 50%); melting point $>300°$ [1H NMR δ 1.60 [p, $J = 7.0$ Hz, 4 H, 2 $\text{CH}_2\text{CH}_2\text{CH}_2\text{NCH}_3\text{CH}_3\text{J}$], 2.46 [s, 12 H, 2 $\text{CH}_2\text{NCH}_3\text{J}$], 2.28 [t, $J = 7.0$ Hz, 4 H, 2 $\text{CH}_2\text{CH}_2\text{CH}_2\text{NCH}_3\text{CH}_3\text{J}$], 3.18 [dt, $J = 6.0$ Hz, $7.0$ Hz, 2 $\text{CH}_2\text{CH}_2\text{CH}_2\text{NCH}_3\text{CH}_3\text{J}$], 3.85, and 3.90 (3 s, 18 H, each for 6 protons of pyrrolyl N-$\text{CH}_2$), 6.84, 7.04, 7.14, 7.20, 7.25, and 7.38 (6 d, $J = 2.0$ Hz, 2 H, each for 2H of pyrrole ring), 8.08 (s, 4 H, phenyl protons), 8.09 (t, $J = 6.0$ Hz, two $\text{CONHCH}_2$ merged with phenylic protons), 9.9, 10.02, and 10.50 (3 s, 6 H, each s for 2 pyrrolyl NHCO); ES$^+$ calc. for $\text{C}_{31}\text{H}_{36}\text{N}_8\text{O}_6$, 587.57; found 587.60 ($M^+$, 100%).

Other bisdistamycins were synthesized in a similar way, and the related data are described below.

$N,N'$-Di-{1-methyl-2-[1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrrole]-carboxamido-4-pyrryl}-carboxamido-4-pyrryl]-phenyl-1,3-dicarboxamide (27). This product was isolated in same way as 26. Yield 225 mg (70%); melting point 295° $^1$H NMR δ 1.64 [p, $J = 7.0$ Hz, 4 H, two

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**Representative synthesis:** $N,N'$-di-{1-methyl-2-[1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrrole]-carboxamido-4-pyrryl]-phenyl-1,3-dicarboxamide (27). This product was isolated in same way as 26. Yield 225 mg (70%); melting point 295° $^1$H NMR δ 1.64 [p, $J = 7.0$ Hz, 4 H, two
using CH₂Cl₂/MeOH/NH₄OH (7:3:0.05) as eluent to give pure 29. Yield 80 mg (44%); melting point 220°C [1H NMR δ 1.60 (p, J = 7.0 Hz, 2 H, CH₂CH₂CH₂CH₂N(CH₃)₂), 2.20 (s, 6 H, N(CH₃)₂), 2.09 (t, J = 7.0 Hz, 2 H, CH₂CH₂CH₂CH₂N(CH₃)₂), 3.20 (dt, J = 6.0 Hz, J = 7.0 Hz, 2 H, CH₂CH₂CH₂CH₂N(CH₃)₂), 3.80, 3.88, and 3.98 (3 s, 9 H, each for 3 pyrrolid NCH₂), 6.82, 7.08, 7.20, 7.30, and 7.34, and 7.38 (6 d, J = 2.0 Hz, 6 H, each for one pyrrolid CH₂), 7.88-7.95 (m, 4 H, aromatic), 8.10 (t, j = 6.0 Hz, CONH-CH₃), 9.90 and 10.10 (2 s, 2 H, each for 2 pyrrolid NHCO); ES⁺ calc. for C₁₇H₂₇N₅O₆ 598.63, found 599.10 (% M⁺ 100%).

N,N'-Di[1-methyl-2-(1-methyl-2-carboxamidino(3-dimethylaminopropyl)-4-pyrrolyl)-carboxamido-4-pyrrolyl]-pyridine-2,5-dicarbamido (30). reaction of 2.5-pyridine dicarboxylic dihydrochloride (61 mg, 0.33 mmol) with amino diastemycin 7b (285 mg, 0.66 mmol) in the presence of triethylamine (0.5 ml), using anhydrous DMF as a solvent, gave the crude product. This material was purified the same way as described for 26. Yield 270 mg (85%); melting point >300°C [1H NMR δ 1.60 (p, J = 7.0 Hz, 4 H, 2 CH₂CH₂CH₂CH₂N(CH₃)₂), 2.14 [s, 12 H, 2 N(CH₃)₂], 2.25 [t, J = 7.0 Hz, 4 H, 2 CH₂CH₂CH₂CH₂N(CH₃)₂], 3.20 [dt, J = 6.0 Hz, J = 7.0 Hz, 7 H, 2 CH₂CH₂CH₂CH₂N(CH₃)₂], 3.78 and 3.85 (2 s, 12 H, each for 6 protons of pyrrolid N-CH₂), 3.90 and 3.92 (2 s, 6 H, each for 3 protons of N-CH₂), 6.84, 7.05, 7.12, 7.19, 7.26, 7.90, and 7.95 (7 d, J = 2.0 Hz, total 10 H of pyrrole ring) 8.10 (t, J = 6.0 Hz, 2 H, for 2 CONH-CH₂), 8.25 (d, J = 7.0 Hz, 1 H, pyridyl H-3), 8.50 (dd, J = 7.0 Hz, J = 2.0 Hz, 1 H, pyridyl H-4), 9.20 (d, J = 2.0 Hz, 1 H, pyridyl H-5), 9.70 and 9.100 (2 s, 4 H, each for 2 CONHCO), 10.75 and 10.90 (2 s, 2 H, each for 1 H of NHCO); ES⁺ calced. for C₃₀H₃₅N₉O₆ 618.64, found 618.64 (M⁺ 100%).

N,N'-Di[1-methyl-2-(1-methyl-2-carboxamido(3-dimethylaminopropyl)-4-pyrrolyl)-carboxamido-4-pyrrolyl]-pyridine-2,4,6-dicarbamido (31). This product was obtained following the synthetic procedure as described for 26. The chromatographic purification, using CH₂Cl₂/MeOH/NH₄OH (7:3:0.05, v/v/v) as eluent afforded 240 mg (75%) of pure 31 as yellow solid; melting point 200°C [1H NMR δ 1.60 (p, J = 7.0 Hz, 4 H, 2 CH₂CH₂CH₂CH₂N(CH₃)₂), 2.18 [s, 12 H, 2 N(CH₃)₂], 2.26 [t, J = 7.0 Hz, 4 H, 2 CH₂CH₂CH₂CH₂N(CH₃)₂], 3.20 [dt, J = 6.0 Hz, J = 7.0 Hz, 7 H, 2 CH₂CH₂CH₂CH₂N(CH₃)₂], 3.80, 3.86, 3.89, and 3.91 (4 s, 24 H, each for 6 protons of pyrrolid N-CH₂), 6.84, 7.06, 7.16, 7.18, 7.26, 7.42, and 7.44 (7 d, J = 2.0 Hz, total 12 H of 6 pyrrole rings) 8.10 (m, 3 H, 2 H, for 2 CONH-CH₂, and 1 H of pyridyl H-5), 8.62 (d, J = 1.5 Hz, 1 H, pyridyl H-3), 8.90 (d, J = 5.5 Hz, 2 H, pyridyl H-6), 9.90 and 10.02, 10.88, and 10.91 (4 s, 4 H, 1 H for each NHCO); ES⁺ calced. for C₃₀H₃₅N₉O₆ 618.64, found 618.64 (M⁺ 100%).

Preparation of radiolabeled DNA substrates, IN proteins and assays, electrophoresis and quantification, and anti-HIV assays in cultured cell lines. These methods were performed essentially as described previously (Neamati et al., 1997b). The Mg²⁺-based assays were carried in the presence of 5% polyethylene glycol as described previously (Engelman and Craigie, 1995). The anti-HIV drug testing were performed at National Cancer Institute essentially as described by Weislow et al. (1989).

Schiff base formation and chemical trapping. IN was incubated with an oligonucleotide-containing an abasic site (see Fig. 7A) in reaction buffer as described above (Mazumder et al., 1996a) for 2 min at room temperature. A freshly prepared solution of borohydride (0.1 M final concentration) was added, and reaction was continued for an additional 5 min. An equal volume (16 μl) of 2× SDS-polyacrylamide gel electrophoresis buffer (100 mEq Tris, pH 6.8, 4% 2-mercaptoethanol, 4% SDS, 0.2% bromphenol blue, 20% glycerol) was added to each reaction, and the reaction was heated at 95°C for 3 min before loading a 20-μl aliquot on a 12% SDS-polyacrylamide gel. The gel was run at 120 V for 1.5 hr, dried, and exposed in a Phosphormager cassette. For inhibition of DNA binding experiments, IN (200 nm) was preincubated with the inhibitor (at the indicated concentration) for 30 min at 30°C before the subsequent addition of the radiolabelled viral DNA substrate (20 nM) and borohydride. Gels were analyzed using a Molecular Dynamics PhosphorImager (Sunnyvale, CA).

Footprinting experiments. DNase I footprinting was performed in buffer containing 50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM diethiothreitol, 10% glycerol (w/v), 7.5 mM MgCl₂, 0.1 mg/ml bovine serum albumin, 10 mM 2-mercaptoethanol, 25 mM MES, pH 7.2, and 10 mM oligonucleotide. Lexitropsins were added to the reaction mixture and incubated at room temperature for 5 min. Digestion was achieved by the addition of DNase I (3 units/ml) for 1 min. The reaction was quenched with EDTA and 2× SDS-polyacrylamide gel electrophoresis buffer (100 mM Tris, pH 6.8, 4% 2-mercaptoethanol).
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<th>Compound No.</th>
<th>Structure</th>
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<th>EC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>TI</th>
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<td>7.5 ± 2.5</td>
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<sup>a</sup> Second independent experiment.
<sup>b</sup> Numbers in parantheses refer to GC rich DNA duplex.
<sup>c</sup> NR, not reached.
TABLE 2
Inhibition of HIV-1 and HIV-2 IN catalytic activities and inhibition of HIV-1 replication in CEM cells by a series of novel lexitropsins

<table>
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<tr>
<th>Compound No.</th>
<th>Structure</th>
<th>HIV-1 IC$_{50}$ (µM)</th>
<th>HIV-2 IC$_{50}$ (µM)</th>
<th>Cellular anti-HIV-1 data</th>
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Values with the standard deviations are from three independent experiments; the others are from two independent experiments. NR, not reached due to cytotoxicity.
to ethanol, 4% SDS, 0.2% bromphenol blue, 20% glycerol), and an aliquot (5 μl) was electrophoresed on a denaturing 20% polyacrylamide gel as described above.

Results

Synthesis of Dimethylaminopropylamido Analogs of Distamycin (Novel Lexitropsins)

Preparation of aryl acid chlorides. Except for commercially available diacid chlorides, these linkers were prepared fresh, before their coupling with distamycin, from diacids. The acids were taken in thionyl chloride (5–10 mol in excess) with 2 or 3 drops of anhydrous dimethylformamide, and the mixture was heated at 65° for 2–3 hr. Excess of thionyl chloride was removed in vacuo, the contents were coevaporated with anhydrous methylene chloride, and the diacid chloride formed was used as such in its coupling with aminodistamycin.

3-{1-Methyl-4-[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}dimethylaminopropane (distamycin, 7a). Distamycin (7a) was synthesized according to a reported procedure (Nishiwaki et al., 1988). Distamycin (7a) was the key intermediate for the synthesis (Figs. 3 and 4) of new rigidly linked bislexitropsins (26–35).

Targeting HIV-1 LTR as an Alternative Strategy to Inhibit IN Function

The viral DNA sequences that are required and sufficient for recognition by IN are the distal ends of the LTRs. To determine how conserved are the HIV-1 LTRs among all the reported HIV-1 strains, we aligned the sequences from the Los Alamos HIV sequence Database (http://hiv-web.lanl.gov/). The termini of the HIV-1 LTRs seemed highly conserved among 43 available HIV-1 strains (Fig. 1). It is noticeable that a conserved AT stretch was identified; 10 bases away from both ends of the U3 and U5 LTRs. Thus, we envisaged that lexitropsins, or information-reading molecules, with a predictable base and sequence recognition capability could target these sites and block integration.

Inhibition of IN Catalytic Activities by a Series of Polyamides

Netropsin and distamycin analogs. Thirty-five polyamides were tested for anti HIV-1 IN activity (Tables 1 and 2) using a dual assay measuring both 3′-processing and 3′-end joining (strand transfer or integration) (Fig. 5). The bisnetropsin (1–6) and bisdistamycin (8–19) derivatives composed of two netropsin or distamycin molecules separated by aliphatic or aromatic linkers with different size and geometry were examined (Table 1). The result presented in Table 1 attests to the remarkable relationship between the structure of various derivatives and their IC50 values against IN. For example, the striking difference in potency shown by distamycin 7 compared with the potency of bisdistamycin derivatives suggests the importance of the dimeric structure. The linkers separating the two distamycin moieties contribute to potency, and the geometry of the linker seems critical for

Fig. 4. General synthetic scheme to linked bislexitropsins.

Fig. 3. Catalytic reduction of nitrolexitropsin 7a to aminolexitropsin reagent 7b.

Fig. 5. HIV-1 IN catalytic assays. A, A 21-mer blunt-end oligonucleotide corresponding to the U5 end of the HIV-1 proviral DNA, 5′-end-labeled with 32P, is reacted with purified HIV-1 IN. The initial step involves nucleolytic cleavage of two bases from the 3′-end, resulting in a 19-mer oligonucleotide. The second step, 3′-end joining or strand transfer, involves joining of this recessed 3′-end to the 5′-end of an IN-induced break in another identical oligonucleotide, which serves as the target DNA. B, Concentration-dependent inhibition of HIV-1 IN by compounds 8, 11, 13, and 12. Lane 1, DNA alone. Lanes 2, 13, and 24, DNA plus IN. Lanes 3–23, DNA and IN in the presence of indicated concentrations (μM) of drugs.
effective inhibition. The 1,4 disubstituted para derivative 8 exhibited markedly higher potency than the 1,2-ortho and 1,3-disubstituted meta derivative 9 and 10, respectively. A similar observation was made when the pyridinyl derivative with para substitution (compound 11) was compared with the meta substituted derivative 12. In addition, the linear 1,2-trans substituted compound 13 was 30–50 times more potent than the more rigid cyclobutanyl and norbornyl derivatives 14 and 15, respectively. Compound 12 exhibited remarkable selectivity for the 3′-end joining (strand transfer) step (Fig. 5B). Moreover, the length of the linker also contributes to potency. The dimers with the short dimethylene linker (derivative 16) or a long aliphatic chain linker (derivative 19) exhibited a significantly reduced potency compared with compound 17 or 18 with hexamethylene or octamethylene linkers, respectively.

**Novel lexitropsins.** A series of novel polyamides also was examined (Table 2). As in the case of the bisdistamycins presented in Table 1, a common structural feature required for potency seems to be the para substitution. For example, the 1,4-disubstituted derivative 26 was markedly more potent than its corresponding 1,3-disubstituted analog, 27. In addition, the 1,4-disubstituted pyridinyl derivative 30 was more potent than its corresponding 1,3-disubstituted derivatives 31, 32, and 33. In accord with the results for the monosubstituted minor-groove binders presented in Table 1, the monosubstituted lexitropsins 28, 34, and 35 were practically inactive. We also found that the novel lexitropsins were active against HIV-2 IN (Table 2).

**Classic minor-groove binders.** Netropsin and distamycin (Fig. 2) are natural oligopeptide antibiotics with antitumor, antiviral, and antibacterial activities (Zimmer et al., 1977, 1983; Kopka et al., 1985; Kopka and Larsen, 1992). Both antibiotics are known to bind to AT-rich regions in the minor groove of B-DNA in nonintercalative fashion (Zimmer et al., 1977, 1983; Kopka et al., 1985; Kopka and Larsen, 1992). Netropsin, distamycin, Hoechst 33258, 4′-diamino-2-phenylindole, pentamidine, and berenil (Fig. 2), which possess a crescent shape, bind noncovalently in the DNA minor groove without insertion between the base pairs (Zimmer et al., 1977, 1983; Kopka et al., 1985; Kopka and Larsen, 1992). None of these monomeric groove binders (compounds 20-25) exhibited significant activity at 100 μM against IN (Table 1). Distamycin 7 was only weakly active with an IC_{50} value of ~50 μM. Thus, the ability of the polyamides examined to inhibit IN varied considerably. The difference in activity exceeded 2–3 orders of magnitude.

### Probing the Mechanism of IN Inhibition

#### Effect of the polyamides on the HIV-1 IN core region.

To examine the mechanism of inhibition of IN, we used an IN deletion mutant, IN^{30–212}, which lacks the amino-terminal zinc-binding region and the carboxy-terminal DNA-binding domain (Chow et al., 1992; Bushman et al., 1993). This mutant can catalyze an apparent reversal of the integration reaction known as disintegration (Chow et al., 1992) (Fig. 6A). In the disintegration assay, the lexitropin 26 was markedly more potent than lexitropin 34 (Fig. 6B). The bisdistamycins 16, 17, 18, and 19 exhibited IC_{50} values of 2.3, 0.009, 0.03, and 3.7 μM (data not shown). The activity of the novel lexitropsins 27–36 in the disintegration assay with IN^{30–212} are indicated in Table 2. These results demonstrate that polyamides can interfere with the activity of the IN core region and that their inhibitory activity does not require the presence of the zinc-binding and carboxy-terminal domains of IN.

**Global nucleophilic inhibition.** The 3′-processing reaction involves hydrolysis of a single phosphodiester bond 3′ of the conserved CA-3′ dinucleotide (Fig. 7A). However, in addition to this hydrolysis reaction, retroviral INs can use glycerol or the hydroxyl group of the viral DNA terminus as the nucleophile in the 3′-processing reaction, yielding a glycerol esterified to the 5′-phosphate, a circular dinucleotide or trinucleotide, respectively (Engelman et al., 1991; Vink et al., 1991; Mazumder et al., 1996b) (Fig. 7A). To examine the effect of synthetic polyamides on the choice of nucleophiles in the 3′-processing reaction, a substrate DNA labeled at the 3′-end with 32P-cordycepin was used (Mazumder et al., 1996b). Compounds 8, 11, 13, and 12 inhibited glycerolysis, hydrolysis, and circular nucleotide formation similarly (Fig. 7B). This result indicates that lexitropsins block indiscriminately all the IN nucleophilic reactions.

**Divalent ion effects.** Although in vitro assays are generally more efficient with Mn^{2+} as a cofactor, it has been proposed that the physiological cation is Mg^{2+}. We compared the extent of 3′-processing and strand transfer for two representative derivatives, 8 and 11, in the presence of Mg^{2+} and Mn^{2+}. Both compounds were more potent with Mg^{2+} than with Mn^{2+} (Fig. 8). This suggests that in contrast to polyhydroxylated aromatics (Fesen et al., 1994; Hazuda et al., 1997b; Neamati et al., 1997a), polyamides do not interact selectively with the divalent metal of the IN catalytic site.

**Inhibition of DNA IN binding by polyamides.** A recently described DNA IN cross-linking assay (Mazumder et al., 1994; Hazuda et al., 1997b; Neamati et al., 1997a), polyamides do not interact selectively with the divalent metal of the IN catalytic site.

**Fig. 6.** HIV-1 IN disintegration assay using the core truncated mutant IN^{30–212}. A, The substrate oligonucleotide mimics a strand transfer step product (i.e., a Y oligonucleotide containing a 15-mer oligonucleotide 5′-end labeled with ^32P). HIV-1 IN-mediated disintegration generates a 30-mer oligonucleotide. B, Concentration-dependent inhibition of HIV-1 IN-mediated disintegration by compounds 26 and 34.
al., 1996a) was used. Briefly, a 21-mer oligonucleotide in which uracil was substituted for adenine in the conserved CA dinucleotide on the distal end of the U5 LTR was treated with uracil DNA glycosylase to generate an abasic site (Fig. 9A, U). Subsequent incubation with IN and stabilization with sodium borohydride of the Schiff base formed between free aldehyde on the DNA backbone and the amino group of lysines in the IN produced a cross-linked product running as a 39-kDa band after electrophoresis (Fig. 9B, lane 2). Compound 26 effectively inhibited the formation of IN/DNA complexes, and the lexitropsin 27 was considerably less potent (Fig. 9C). These results are in accord with the inhibition of DNA integration by these compounds (Table 2) and suggest that lexitropsins inhibit IN activity by blocking IN binding to its DNA substrate.

**DNA sequence selectivity of polyamides.** Because minor-groove binders such as distamycin and netropsins preferentially bind to AT sequences, we mutated the U5 DNA substrate to replace the conserved AT sequence with GC (Fig. 10A). This replacement decreased the efficiency of 3’-processing by ~30%, whereas 3’-end joining became almost undetectable. The two most potent bisdistamycins, 17 and 18, were compared for their ability to inhibit 3’-processing using the two DNA substrates. For both polyamides, inhibitory activity was ~200-fold less with the GC-containing oligonucleotide than with the physiological 21-mer oligonucleotide containing the conserved AT stretch (Fig. 10B). These results are consistent with the importance of DNA binding for IN inhibition by polyamides.

**DNase I footprinting.** A DNase I footprinting experiment using a labeled 21-mer was performed to examine further the binding of the highly potent bisdistamycins 17 and 18 to the U5 LTR (Fig. 11). Protection of the AT-rich domain was observed with both compounds, and this protection tended to extend toward the U5 end at higher concentrations with 17. No such protection was observed using the GC oligonucleotide (see Fig. 10A and data not shown), which is consistent with the selective binding of lexitropsins to AT stretches.

**Discussion**

Earlier work showed that DNA intercalators can effectively inhibit HIV-1 integration (Carteau et al., 1993; Fesen et al., 1993; for review, see Neamati et al., 1997c). However, intercalators have limited sequence specificity because they primarily interact with two adjacent base pairs. Thus, they would be expected to bind to numerous DNA sites, to affect cellular metabolism relatively indiscriminately, and to be too toxic for anti-acquired immune deficiency syndrome therapy. In the current study, we examined the activity of synthetic polyamides that can recognize with high selectivity DNA sequences from the DNA minor groove and exhibit antiviral activity.

Selective minor-groove binding to the conserved AT sequences in the LTRs seems to be the primary mechanism of inhibition of IN by the polyamides studied. First, the polyamides inhibited the binding of IN to its DNA substrate in the Schiff base assay at concentrations that effectively inhib-
ited integration. Second, DNase I footprinting demonstrated the binding of polyamides to the U5 LTR in the conserved AT stretch. Third, changing the AT stretch into a GC stretch reduced the inhibitory activity of the bisdistamycins by 2 orders of magnitude.

The difference in anti-integration potency among the polyamides tested exceeded 2–3 orders of magnitude. This provided a basis for structure-activity relationships. Monomers were relatively inactive even at micromolar concentrations, thus suggesting a requirement of a dimeric form (bilextropsins) for potent anti-IN activity. Among the bisdistamycins and novel lexitropsins, isomers with para substituted aromatic linkers consistently exhibited higher potency than ortho or meta substituted derivatives (Tables 1 and 2), implying the importance of a linear versus a folded arrangement for activity. Actually, the bisdistamycins with rigid linkers that tended to fold the molecule into a hairpin configuration (compounds 9, 10, 14, and 15) were not more potent inhibitors than distamycin (compound 7). The greater activity of the linear bisdistamycins suggests that drug binding to an extended DNA segment is essential for inhibition of integration. Compounds with an aliphatic flexible linker composed of a hexamethylene or an octamethylene chain (compounds 17 and 18) also were remarkably active, implying the importance of the size of the aliphatic chain linker for optimum potency. Finally, the finding that lexitropsins with substitutions on the distamycin moiety (compare compounds in Tables 1 and 2) remained active inhibitors of IN suggests that further chemical modifications can be made to improve antiviral activity.

In contrast to other IN inhibitors, such as catechol-containing compounds (Hazuda et al., 1997a; Mazumder et al., 1997), the lexitropsins were more active in Mg$^{2+}$-based assays than in the Mn$^{2+}$-based assays commonly used in vitro. Thus, if Mg$^{2+}$ is more physiologically relevant than Mn$^{2+}$, it seems that polyamides are potential candidates to block integration in vivo.

Targeting of the conserved AT stretches of the LTRs (Bouzagiane et al., 1996) with lexitropsins (current study) represents a clear strategy for interfering with provirus integration and retrovirus replication. The remarkable conservation of the AT stretches in all the available HIV-1 LTRs (Fig. 1) suggests that synthetic polyamides tested exhibited significant antiviral activity (Table 1). Independent experiments demonstrated that such compounds inhibited reverse transcriptase only at high concentrations (150–200 μM for compounds 1, 2, 5, 6, 10, and 16) (Lown JW, unpublished observations). Such concentrations are much higher than those required to inhibit integration. Further studies are warranted to determine whether integration is a prime mechanism for the antiviral activity of synthetic polyamides and to investigate whether other sites of the HIV genome and other viral processes are targeted by lexitropsins.

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![Fig. 10. Importance of the conserved AT sequence for HIV-1 IN inhibition by bisdistamycins. A. The 21-mer blunt-end oligonucleotides where the AT stretch was replaced with GC (underlined). B. Concentration-dependent inhibition of HIV-1 IN by compounds 18 (■) and 17 (○) using native 21-mer oligonucleotide (○) or the GC-modified oligonucleotide (■).](image1)

![Fig. 11. DNase I footprinting of the 21-mer oligonucleotide corresponding to the U5 end of the HIV-1 proviral DNA, 5' end-labeled with $^{32}$P in the presence of indicated concentrations of lexitropsins 17 and 18.](image2)
References


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