(1 H, m, H-5'b), 2.56 (3 H, s, Me), 2.41 (2 H, t, ≡CCH_2(CH$_2$)$_2$CH$_3$), 1.60–1.35 (4 H, m, ≡CCH$_2$(CH_2)$_2$CH$_3$), 0.91 (3 H, t, ≡CCH$_2$-(CH$_2$)$_2$CH_3). Anal. ($C_{18}H_{25}N_7O_4 \cdot H_2O$) C, H, N.

5'-Deoxy-5'-(3-methylthioureido)-2-(1-hexyn-1-yl)-adenosine (31). Treatment of **26** (200 mg, 0.44 mmol) with TFA (10 mL) for 6 h at −20 °C gave **31** (96 mg, 53%, crystallized from EtOH): mp 131–135 °C; MS m/z 419 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.39 (1 H, s, H-8), 7.61 (1 H, br s, NH), 7.41 (3 H, br s, NH, 6-NH$_2$), 5.83 (1 H, d, H-1', $J_{1',2'}$ = 6.6 Hz), 5.47 (1 H, br s, 2'-OH), 5.28 (1 H, br s, 3'-OH), 4.59 (1 H, br s, H-2'), 4.15–4.00 (2 H, m, H-3', 4'), 3.90–3.60 (2 H, m, H-5'a,b), 2.81 (3 H, s, Me), 2.41 (2 H, t, ≡CCH_2(CH$_2$)$_2$CH$_3$), 1.60–1.36 (4 H, m, ≡CCH$_2$(CH_2)$_2$CH$_3$), 0.91 (3 h, t, ≡CCH$_2$(CH$_2$)$_2$CH_3). Anal. ($C_{18}H_{25}N_7O_3S \cdot H_2O$) C, H, N.

A_1 and A_2 Binding Assay. The A_1 and A_2 receptor binding affinities for the test compounds were measured in the presence of adenosine deaminase (type VI, Sigma) in triplicate by previously described methods.[8] A_1 receptor binding was measured in adenosine deaminase (ADA) pretreated rat brain (without cerebellum and brain stem) homogenates with 2.5 nM [^{3}H]CHA as a radioligand, and binding to the A2 receptor was measured in ADA-pretreated rat striatal membranes using 5 nM [^{3}H]NECA in the presence of 50 nM CPA. IC_{50} values were calculated by a computerized nonlinear, least-squares analysis program (SP-1.2.3, programmed by Dr. Ono, University of Tokyo, Tokyo, Japan).

Cardiovascular Effects. Effects of the compound on BP and HR after iv administration were measured in anesthetized male SHR as described previously.[8] Briefly, each test compound (0.03–100 μg/mL) was administered iv at 5-min intervals in a cumulative manner. The relative potency to decrease BP was estimated on the basis of the ED_{30} value, the mean dose that produced a 30% decrease in BP of SHR. Similarly, the relative potency to decrease HR was estimated on the basis of the ED_{10} value, the mean dose that produced a 10% decrease in HR of SHR.

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Registry No. 2, 35920-39-9; **10**, 35109-88-7; **11**, 141018-25-9; **12**, 141018-26-0; **13**, 142102-84-9; **14**, 142102-85-0; **15a**, 142103-01-3; **15b**, 142103-02-4; **15c**, 142103-03-5; **15d**, 142103-04-6; **15e**, 142103-05-7; **15f**, 142103-06-8; **16a**, 142103-07-9; **16b**, 142103-08-0; **16c**, 141018-30-6; **16d**, 142103-09-1; **16e**, 142103-10-4; **16f**, 142103-11-5; **17**, 142102-86-1; **18**, 142102-87-2; **19**, 142102-88-3; **20**, 142102-89-4; **21**, 142102-90-7; **22**, 142102-91-8; **23**, 142102-92-9; **24**, 142102-93-0; **25**, 142102-94-1; **26**, 142102-95-2; **27**, 142102-96-3; **28**, 142102-97-4; **29**, 142102-98-5; **30**, 142102-99-6; **31**, 142103-00-2; 1-hexyne, 693-02-7; methylamine, 74-89-5; ethylamine, 75-04-7; cyclopropylamine, 765-30-0; *n*-propylamine, 107-10-8; *n*-butylamine, 109-73-9; acetic formic anhydride, 2258-42-6; methyl isocyanate, 624-83-9; methyl isothiocyanate, 556-61-6.

Anti-HIV-I Activity of Linked Lexitropsins

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Five groups of lexitropsin oligopeptides have been synthesized that are structurally related to the natural antiviral agents netropsin and distamycin and bearing two such moieties joined by flexible or rigid linkers. Inhibitory activity of these types of agents against murine leukemia retrovirus (MuLV) led to an evaluation of their inhibition of HIV-I in cell culture. The antiretroviral activity of the five different classes of lexitropsins is discussed in terms of their structural differences.

Introduction

World Health Organization (WHO) data suggest that some 1.2 million cases of AIDS have occurred worldwide in the past 10 years and that 10 million people could be infected.[1] This last figure may rise to 30–40 million by the end of the century.[1] Although great strides have been made in understanding the molecular biology of the generally accepted causative agent, the human immunodeficiency viruses, HIV,[2,3] and some palliative agents are available clinically,[4,5] AIDS is still an incurable disease.

Wellcome's Zidovudine (AZT)[6] is the principal agent being used clinically for treating HIV infection,[4,5,7] although related nucleoside structures are anticipated to obtain U.S. Food and Drug Administration (FDA) approval soon. Experience with AZT has led to improved protocols such as lower doses—from daily doses of 1–2 g per day in early 1990, to about 0.5 g per day at the present time.[5] There has also been a trend to the earlier use of AZT to treat AIDS and ARC (AIDS related complex), although this may allow a reservoir of virus to build up in the population.[4,5,7] The challenge, fueled by the increasing resistance of HIV to AZT, is to develop alternative chemotherapeutic agents.

The majority of drugs being developed are structurally related to AZT, i.e. nucleoside derivatives that are reverse transcriptase (RT) inhibitors. Such agents, including dideoxyinosine (ddI), dideoxycytidine (ddC), and 3'-azidodideoxyuridine (AzdU) are likely to exhibit similar toxicity (pancreatitis and nerve damage) and resistance problems as AZT.[5] Therefore there is an urgent demand not only for alternative structures effective against HIV but also for agents that operate by different mechanisms. Tetrahydroimidazobenzodiazepinone (TIBO) is one such agent that has been uncovered by systematic screening procedures in Belgium.[5]

We describe the design and synthesis of certain extended oligopeptide lexitropsins that, based on their inhibition of

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0022-2623/92/1835-2890$03.00/0

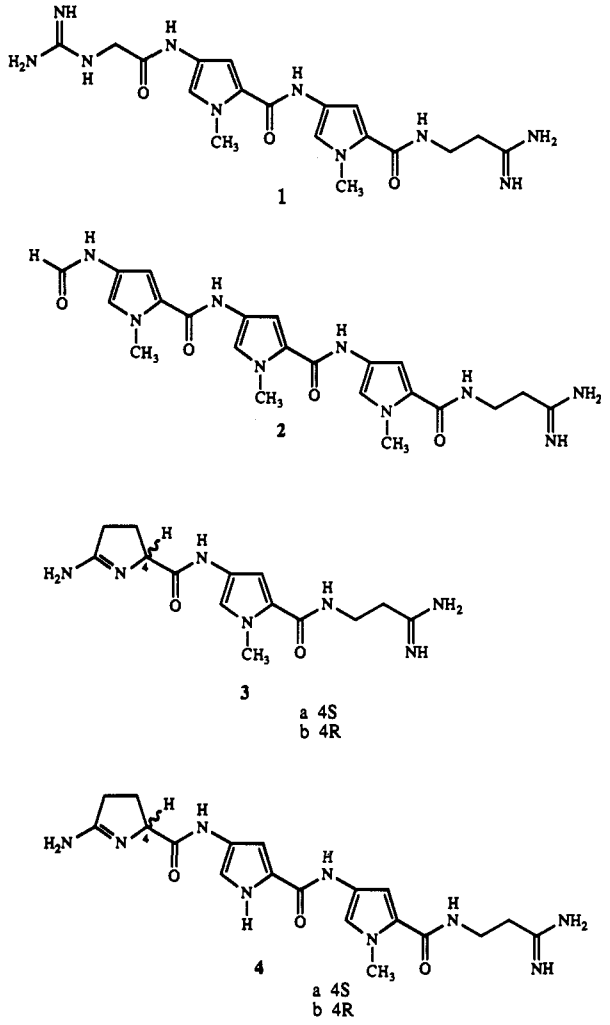
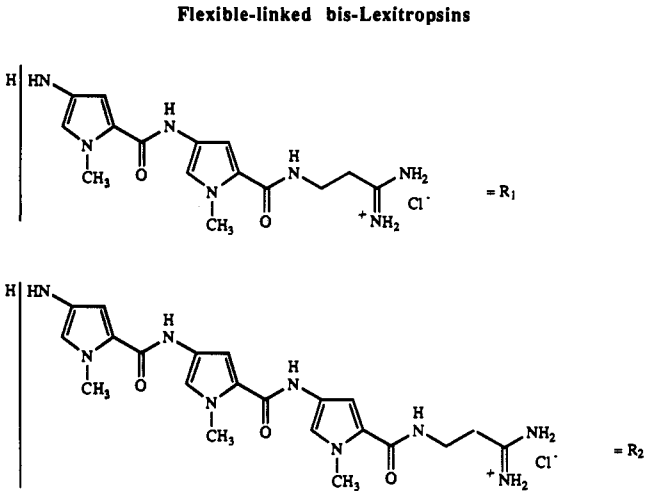


Figure 1. Structures of oligopeptide antibiotics netropsin (1), distamycin (2), dihydrokikumycins 3, and anthelvencins 4.

Moloney leukemic virus in cell culture, were tested and found to inhibit HIV-I in cell culture. Structure–activity relations are discussed on five subgroups of this general class of oligopeptidic agents.

Design and Synthesis of Linked Bis-*N*-methylpyrrole Dipeptides

Netropsin (1)[8] and distamycin (2)[9] (Figure 1) have served as prototype DNA sequence selective minor groove binding agents. Netropsin, having two *N*-methylpyrrole carboxamide repeating units and three amide groups (n = 3), binds four base pairs of DNA and distamycin (n = 4) binds five base pairs. The empirical "n + 1" rule[10] cannot be extended indefinitely in an attempt to bind longer sequences of DNA. This is because of the phenomenon of phasing or lack of dimensional correspondence between the oligopeptide repeating units and the oligonucleotide base spacing in the receptor.[11] As the number of repeating units increases, the mismatching becomes more severe and this is manifested by a trend of weaker binding with higher homologs.[12] One solution to this phasing problem is to



5	R_1—CO—R_1
5a	R_1—$COCH_2CO$—R_1
6	R_1—$CO(CH_2)_2CO$—R_1
6a	R_1—$CO(CH_2)_3CO$—R
6b	R_1—$CO(CH_2)_4CO$—R
7	R_1—$CO(CH_2)_5CO$—R_1
8	R_1—$CO(CH_2)_6CO$—R_1
9	R_1—$CO(CH_2)_7CO$—R_1
10	R_1—$CO(CH_2)_8CO$—R_1
11	R_1—$CO(CH_2)_9CO$—R_1
12	R_1—$CO(CH_2)_{10}CO$—R_1
13	R_2—$CO(CH_2)_2CO$—R_2
14	R_2—$CO(CH_2)_6CO$—R_2
15	R_2—$CO(CH_2)_8CO$—R_2
16	R_2—$CO(CH_2)_{22}CO$—R_2

Figure 2. Structures of the flexible polymethylene-linked lexitropsins.

employ a series of extended bis-oligopeptides bearing an appropriate linker[11] (Figures 2, 3). In this approach the size and binding properties of the extended molecules could, in principle, be controlled by different linkers with design features such as length, geometry, stereochemistry, and flexibility.[11,13,14] Bis-netropsins linked by various flexible and rigid linkers have been prepared and described previously.[11,14] Footprinting evidence on flexible linked bis-netropsins demonstrated that, depending on the length of the linkers, either mono- or bidentate binding occurs.[11,13] Antiviral and anticancer cytostatic experiments demonstrated that succinic, suberic, and sebacic linkers afforded the most potent bis-netropsins.[15]

Bis-netropsins bearing rigid linkers like fumaryl, cyclopropanedicarbonyl, and *p*-phenylenedicarbonyl exhibited the highest biological activities.[14] In cases where cis and trans isomers were compared, the trans isomers which confer bidentate binding were consistently more potent than the cis isomers in their antiviral activities, and for this reason, effective DNA binding was deemed to be a desirable design feature for these series of compounds.[14,15] Lipophilicity increased the potency of the dimeric compounds.[15]

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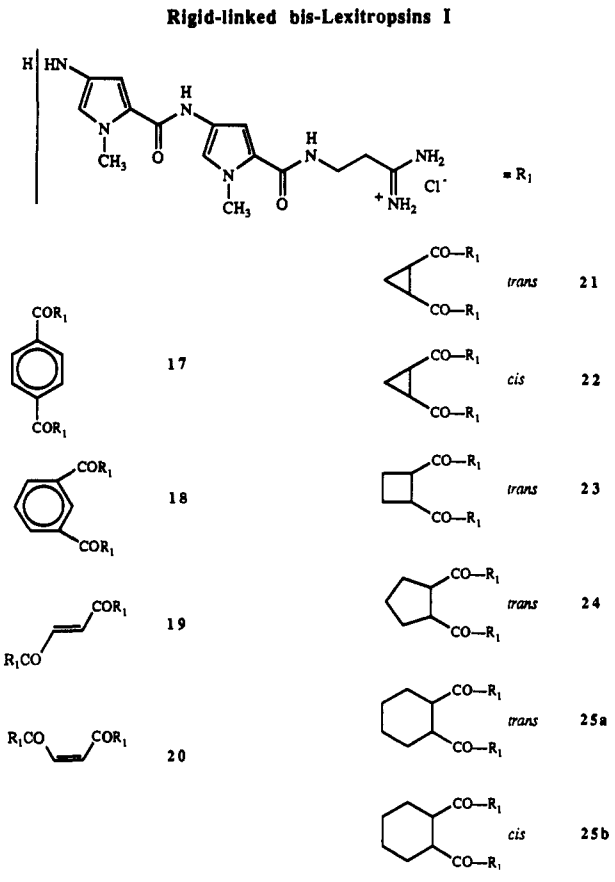

Figure 3. Structures of the rigid linked lexitropsins.

Table I. Analytical and Physical Data on New Linked Netropsins

compd	yield (%)	mp[a] (°C)	formula[b]	analysis
13	85	210	$C_{46}H_{58}H_{18}O_8Cl_2$	C, H, N, Cl
14	76	210	$C_{50}H_{60}N_{18}O_8Cl_2$	C, H, N, Cl
15	84	198–202	$C_{52}H_{64}N_{18}O_8Cl_2$	C, H, N, Cl
16	69	215	$C_{66}H_{92}N_{18}O_8Cl_2$	C, H, N, Cl
26	77	>300	$C_{50}H_{58}N_{18}O_8Cl_2$	C, H, N, Cl
27	68	240	$C_{50}H_{58}N_{18}O_8Cl_2$	C, H, N, Cl
28	83	245	$C_{50}H_{58}N_{18}O_8Cl_2$	C, H, N, Cl
29	88	250	$C_{49}H_{57}N_{19}O_8Cl_2$	C, H, N, Cl
30	74	260	$C_{49}H_{57}N_{19}O_8Cl_2$	C, H, N, Cl
31	54	260	$C_{49}H_{57}H_{19}O_8C_2$	C, H, N, Cl
32	78	230	$C_{46}H_{60}N_{18}O_8Cl_2$	C, H, N, Cl
33	33	255	$C_{46}H_{58}N_{18}O_8Cl_2$	C, H, N, Cl
34	67	280	$C_{46}H_{56}N_{18}O_8Cl_2$	C, H, N, Cl
35	53	260	$C_{51}H_{62}N_{18}O_8Cl_2$	C, H, N, Cl
36	58	260	$C_{72}H_{84}N_{27}O_{12}Cl_3$	C, H, N, Cl
37	73	250	$C_{34}H_{50}N_{16}O_8Cl_2$	C, H, N, Cl

[a] Uncorrected. [b] All compounds gave satisfactory elemental analyses within 0.4% of the calculated values and exhibited ^1H NMR, IR, and MS data consistent with the structure.

The general synthetic strategy for obtaining the linked bis-netropsins bearing either flexible or rigid linkers has been reported previously.[11,14,16] The new linked bis-distamycins described herein were synthesized from deformylated distamycin prepared from distamycin by a reported procedure.[17] It was found that diacid dichlorides give the most satisfactory results in coupling with deformyldistamycin. Compounds of satisfactory purity were obtained by recrystallization and bis-distamycins **26–34** (Figure 3) were obtained in this way. Bis-lexitropsin **29** was prepared similarly except that water was used as a cosolvent to dissolve the starting amine prior to the condensation process. In those cases where diacids were used for coupling mediated by carbodiimides, mixtures of products were usually obtained. Chromatography (methanol, chloroform, acetic acid, and water as eluent on silica gel) was required for purification of the final products in this procedure.

Representative procedures are given in the Experimental Section for the deformylation of distamycin and its coupling with various acid chlorides, to afford bis-distamycins **26–34** (Table I).

Results

Binding of Linked Bis-Lexitropsins to Duplex DNA. The naturally occurring oligopeptides **1**, **2**, **3a**, and **4a** and the unnatural enantiomeric forms of **3b** and **4b** together with both the flexible-linked and rigid-linked bis-lexitropsins bind to double helical DNA but not to single stranded DNA.[15] Relative binding constants of the ligands to calf thymus DNA or poly(dA-dT) were estimated and compared by measuring the decrease in fluorescence of an ethidium–DNA complex as a result of competitive displacement. The drug concentration that produced 50% suppression of fluorescence was taken to be inversely proportional to the binding constant.[18] None of the drugs tested interfered with the fluorescence measurements at the levels employed and all displaced ethidium from DNA with relative binding constants ranging

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Table II. MuLV (Retrovirus) Testing on Linked Bis-Distamycins

compd	toxicity, TD_{50}[a] ($\mu g\ mL^{-1}$)	activity, MIC_{50}[b] ($\mu g\ mL^{-1}$)	TI, TD_{50}/MIC_{50}
26	>100.00	3.98	>25.13
27	>100.00	>50.0	2.0
28	>100.00	79.63	>1.26
29	>100.00	15.93	>6.28
30	>100.00	>100.0	
31	>100.00	22.74	>4.40
32	83.50	>50.0	1.7
33	100.00	0.16	625.00
34	84.29	11.21	7.52
35	>100.00	22.04	>4.54
36	>100.00	62.79	>1.60
37	>100.00	>100.00	
AZT[c]	>100.00	0.0014	$>7.14 \times 10^5$
DDC[d]	>100.00	0.74	>135.14

[a] On day 5 the plates were examined for cell viability versus controls as a measure of drug toxicity. [b] MIC_{50} values were calculated using the formula: (% inhibition greater than 50% − 50%)/(% inhibition greater than 50% − inhibition less than 50%) to give the interpolative values between two dilutions. [c] Zidovudine. [d] Dideoxycytidine.

from 9.4×10^7 to $1.24 \times 10^6\ M^{-1}$. Effective binding of this class of drugs to the DNA template is implicated in their antiviral activity.[14,15] In addition a correlation exists between the extent of inhibition of Moloney leukemic virus (MuLV) in cell culture and DNA binding ability.[15]

A number of structural factors in the lexitropsins favor DNA binding including (i) increased length of ligand **4** vs **3**, (ii) isohelicity with right-handed DNA corresponding to appropriate absolute configuration of ligand, **3a** vs **3b**, **4a** vs **4b**, (iii) appropriate phasing in the flexible linked lexitropsins permitting effective bidentate binding **8** vs **7** or **9**, (iv) appropriate geometry in the case of rigid linked lexitropsins to permit effective bidentate binding of both portions of the ligand to the template **21** vs **22** and **19** vs **20**.[13,19]

Inhibition of Moloney Murine Leukemia (MuLV) Retrovirus by Linked Lexitropsins. The inhibition of Moloney murine leukemia virus (MuLV) activity by the flexible linked bis-lexitropsins was examined.[15] Inhibition was observed for the homologous series (n = 1–10) giving values for ID_{50} in the range 9.1–72.5 $\mu g/mL$.[15] The greatest extent of inhibition was observed in the following cases (order of decreasing activity): **12** > **11** > **10** > **8** > **6** > **9** > **5a** > **6a** > **6b** > **7**. A correlation is observed between the dependence of the DNA binding capacity as a function of the length of the linker and the inhibitory activity.[15]

A group of the rigid linked bis-lexitropsins was also tested for their inhibitory action on murine leukemia retrovirus. IC_{50} activity values ranged from 0.16 to >100 $\mu g/mL$ (Table II). The compounds showing the highest activities were [ligand (IC_{50})] **33** (0.16) > **26** (3.98) > **34** (11.21) > and **29** (15.93), and by determining their toxicities, the respective therapeutic indices (in parentheses) were **33** (625), **26** (>25), **34** (7.5), and **29** (>6). In these cases greater potency is associated with molecular geometry that permits more effective binding to the template,[13] e.g. **33** vs **34**, **27** vs **28**, and **31** vs **30**.

In the case of the rigid linked bis-lexitropsins examined, greater inhibition is seen with trans-linked structures (which permit bidentate binding[13]) than for the corresponding cis isomer: **21** vs **22** and **25a** vs **25b**.[14] In the case of the rigid 1,2-cycloalkane-linked structures, the inhibitory properties increase as the size of the ring decreases, i.e. **21** > **23** > **24** > **25a**. This result is consistent with force field calculations that predict more effective binding as the ring size of the linker decreases.[13]

Inhibition of HIV-I Virus in Cell Culture. The agents submitted to the NCI for anti HIV-I screening[20] fall into three main classes: (i) the small naturally occurring oligopeptide antibiotics dihydrokikumycin (**3a**) and anthelvencin (**4a**) together with their unnatural enantiomeric forms **3b** and **4b**, respectively (Table III, group A); (ii) flexible polymethylene-linked bis-netropsins **5–12** (Table III, group B) and bis-distamycins **13–16** (Table III, group C); (iii) rigid linked bis-netropsins **17–25** (Table III, group D) and bis-distamycins **26–34** (Table III, group E).

The inhibitory activities of the lexitropsins in HIV-I infected T4 lymphocytes are presented in Table III in numerical form of IC_{50} (μM), and EC_{50} (μM) values and the therapeutic indices are given as TI_{50} (IC/EC) values. The qualitative designations of activity from the NCI,[20] i.e. "active", "moderately active", and "inactive", are also included for the purposes of discussion. The designation "active" implies a compound that warrants further investigation. Since in the development of potential therapeutic agents the significant parameters is the activity of the drug against HIV-I relative to its toxicity against the host cell, relative ranking of the classes of agents is discussed in terms of the therapeutic indices TI_{50}.

In category A, the longer anthelvencin (**4**) shows moderate activity in contrast to the inactive and shorter kikumycins **3**. The two enantiomeric forms of **4** are closely comparable in activity, consistent with their closely similar DNA binding constants.[21]

In the second group of compounds (ii) the first subgroup B consisting of linked bis-netropsins **5–12** show higher activity (six moderate, two inactive) than the second subgroup C of linked bis-distamycins **13–16** (one moderate, three inactive). This suggests that increasing length of the ligand, per se, does not confer increased antiviral potency and that a more important drug design feature is effective template binding. In group (ii) the relative ranking of activities in terms of their therapeutic indices is **9** > **7** > **12** > **5** > **6**.

In group (iii) the linkers are rigid and this group consists of the subgroup of linked bis-netropsins **17–25** (Table III, group D) and that of the linked bis-distamycins **26–34** (Table III, group E). The data in Table III reveal that incorporation of rigid linkers (which confers strong DNA binding properties) also generally enhance HIV-I inhibitory properties. Table III shows in the first subgroup **17–25** (five active and two moderate) and in the second subgroup (five active, four moderate, and only one inactive). Not only do rigid linkers confer increased likelihood of HIV-I inhibitory activity within a given group of agents but the potency and therapeutic indices are generally enhanced. For example, within the subgroup D **17–25**, the therapeutic index reaches 566 in the case of **20**, and on the basis of TI values, the relative activities are **20** > **23** > **25** > **18** > **19** > **17** > **22**. In the subgroup E of linked bis-distamycins **26–34** the potency is generally increased relative to the flexible linked counterparts **13–16**, which is interpreted in terms of the rigid linker promoting more effective DNA binding. A carbon–carbon double bond linker again leads to good activity (and therapeutic index) as in the case of the first subgroup, although the reason for the reversal of

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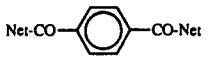
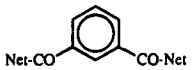
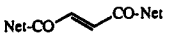
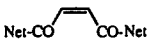
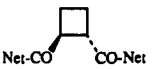
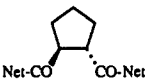
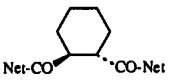
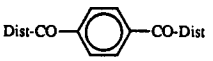
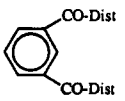
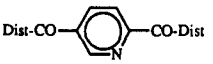
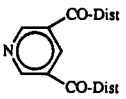
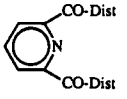
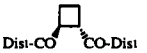
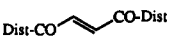
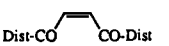
Table III. Anti-HIV-I Data on Linked Lexitropsins

group	structure	no.	NSC	IC_{50} (μM)	EC_{50} (μM)	TI_{50} (IC/EC)	activity
A	(4*S*)-dihydrokikumycin	**3a**	624526	>127			inactive
	(4*R*)-dihydrokikumycin	**3b**	624527	>127			inactive
	(4*S*)-anthelvencin	**4a**	624528	750	88	8.5	moderate
	(4*R*)-anthelvencin	**4b**	624529	750	91	8.2	moderate
B	Net-CO-Net[a]	**5**	632329	83.5	11.9	7.01	moderate
	Net-CO$(CH_2)_2$CO-Net	**6**	637124	75.3	12	6.3	moderate
	Net-CO$(CH_2)_5$CO-Net	**7**	633769	64.8	5.3	12.1	moderate
	Net-CO$(CH_2)_6$CO-Net	**8**	633963				inactive
	Net-CO$(CH_2)_7$CO-Net	**9**	633964	51.1	2.1	24.1	moderate
	Net-CO$(CH_2)_8$CO-Net	**10**	635076				inactive
	Net-CO$(CH_2)_9$CO-Net	**11**	634698	57	3.9	14.6	moderate
	Net-CO$(CH_2)_{10}$CO-Net	**12**	634699	78	6.6	11.7	moderate
C	Dist-CO$(CH_2)_2$CO-Dist[b]	**13**	646025	41	41	1.0	inactive
	Dist-CO$(CH_2)_6$CO-Dist	**14**	646026	>100			inactive
	Dist-CO$(CH_2)_8$CO-Dist	**15**	646027	29	14	2	moderate
	Dist-CO$(CH_2)_{22}$CO-Dist	**16**	646028	>120			inactive
D		**17**	634554	17.9	1.21	14.8	active
		**18**	634555	284	3.55	80	active
		**19**	634556	33	1.37	24.1	active
		**20**	634557	199	0.35	566	active
		**21**	634558	9.3	3.44	2.7	moderate
		**22**	634559	257	42.5	6.1	moderate
		**23**	634560	68.2	0.42	161	active
		**24**	634561	168	46.3	3.6	moderate
		**25**	634562	181	5.6	32.4	moderate
E		**26**	643399	4.7	0.39	12	active
		**27**	643401	140	21	6.6	active
		**29**	643404	69	1.6	43	active
		**30**	643403	69	9.8	7.0	moderate
		**31**	643402	140	13	11	active
		**32**	643400	71	16	4.5	moderate
		**33**	646086	207	10.4	19.8	active
		**34**	646087	35			inactive

[a] Net stands for the netropsin moiety (see Figures 1 and 2). [b] Dist stands for the distamycin moiety (see Figures 1 and 2).

preference for cis and trans linkers is not apparent. In terms of the TI_{50} values the relative ranking of the drugs in group E is **29 > 33 > 26 > 31 > 30 > 27 > 32**.

Discussion

The results indicate that certain minor groove DNA binding agents that exhibit inhibitory activity against MuLV murine retrovirus in cell culture also exhibit inhibition of HIV-I in cell culture. The results obtained on five separate classes of lexitropsins permit identification of some of the structural parameters that contribute to enhanced activity. First, effective binding to DNA in these series of linked bis-lexitropsins, which is comparable ($\sim 10^7$ M^{-1}) both within the series and to that of the parent natural products netropsin (**1**), distamycin (**2**), kikumycin (**3**), and anthelvencin (**4**), is a necessary but not sufficient

condition to permit inhibition of HIV-I. Second, differences in sequence selectivity are unlikely to be a dominant factor given the AT binding preference of all the *N*-methylpyrrole-based lexitropsins.[19,22] The strong AT sequence preference was also found to be an important determinant of antiviral and anticancer activity in other classes of lexitropsin agents.[19,22] This is especially evident in the case of lexitropsins that exhibit potent inhibition of topoisomerase II in intact nuclei and whole cells and which show a strict sequence homology with matrix associated region (MAR) AT-rich conserved sequence elements.[23]

A more significant pharmacological factor is likely to be the increased lipophilicity promoting cellular uptake as a result of introducing hydrophobic linkers.[15] Increased length of the ligand beyond the minimum length represented by kikumycin, per se, does not correlate with enhanced anti-HIV-I activity. However the effect of the nature of the linker is pronounced with rigid linkers conferring a higher incidence of active anti HIV-I agents in groups D and E compared with B and C.

Recognizing the fact that relatively few examples are available, the activity data presented in Table II on the inhibition of murine leukemia retrovirus correlate well with the anti HIV-I data in Table III in that five out of six agents that show significant activity against MuLV correspond to the NCI designation of "active" against HIV-I. In addition the ID_{50} values against MuLV for **21**, **22**, **23**, **24**, and **25** correspond in their relative ranking of activity to **21** > **23** > **25** > **22** > **24**, whereas the corresponding ranking of ID_{50} values against HIV-I is **21** > **23** > **24** > **25** > **22**.[14] This suggests that activity against MuLV in cell culture could be a useful predictor of anti-HIV-I activity in these types of DNA minor groove binding agents. This suggests a possible similarity in the mechanisms of inhibitory action of the rigid linked lexitropsins against MuLV and HIV-I retroviruses. However definite inferences in this regard are clearly premature and further investigation of the mechanism of action of these agents is warranted. Such studies involving purified HIV-I reverse transcriptase are in progress and will be reported in due course. As we have seen, effective DNA binding generally leads to more potent antiviral activity. However a reviewer has pointed out that strong DNA binding might also lead to toxicities to rapidly proliferating cells in vivo. For example the bis-lexitropsins are effective inhibitors of topoisomerase II[23] which may lead to myelosuppression.

In summary this first examination of certain linked lexitropsins has identified agents that show significant anti-HIV-I activity and therefore may represent a new class of agents that function by different cellular mechanisms than existing nucleoside-based drugs.

Experimental Section

Melting points were measured on an Electrothermal melting point apparatus and are uncorrected. Samples for NMR measurements were prepared in DMSO-d_6 unless otherwise specified. The NMR spectra were recorded on a Bruker AM-300 instrument in units of ppm against an internal reference. Mass spectra (FAB, high and low reolutions) were determined on Associated Electrical Industries MS-9, AEI/Kratos MS-50 and AEI MS-12 and recorded as relative abundance. Accurate molecular weight measurements were performed on a Microgram Balance (E. Mettler and distributed by Fisher Scientific Co.). Reagent grade solvents and other chemicals, such as distamycin A (Sigma) and various diacid dichlorides, were used as received.

Synthesis. The synthesis and characterization of the enantiomeric forms of dihydrokikumycin (3) and of anthelvencin (4) have been reported.[21,24] Similarly the synthesis, characterization, and properties of the flexible linked bis-netropsins **5**–**12**[15] and of the first group of rigid linked bis-netropsins **17**–**25**[14] have been reported.

Preparation of Acid Chlorides. Commercially available acid chlorides were used directly without further purification. Otherwise, the acid chlorides were prepared from the acids according to the following procedure: An acid and a drop of dimethylformamide was heated in thionyl chloride (5–10 mol in excess) to 55–65 °C for 30–45 min until a homogeneous liquid was obtained. The excess of the chlorinating agent was removed by evaporation. A small amount of methylene chloride was added to the crude acid chloride and then evaporated. The diacid dichloride was then dissolved in methylene chloride or THF, and aliquots were taken and used for coupling reactions.

Deformylation of Distamycin A.[17] Distamycin A (50 mg, 0.09 mmol) was dissolved in 4 mL of methanol. To this yellow solution was added 100 μL of concentrated hydrochloric acid. The solution was stirred for 6–8 h and the reaction progress was followed by TLC (methanol/acetic acid, 100:5). The solvent was evaporated and the crude product was redissolved in methanol and precipitated with ether. The product was recrystallized in this way twice more. The supernatant was decanted and the residual solid was dried in vacuo. The final product was obtained as an off-white solid (50 mg, 89% yield).

Representative Synthesis. (3,5-Pyridinedicarbonyl)didistamycin (29). A solution of 3,5-pyridinedicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyldistamycin (48 mg, 0.09 mmol) and diisopropylethylamine (Hunig's base, 16 μL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0 °C. After 10 min, a solution of Hunig's base (16 μL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid, mp 250 °C, in 88% yield.

Bis-lexitropsin 33. The nitro-*N*-methylpyrrole derivative (100 mg, 0.241 mmol) in DMF and methanol (4 mL of each) was hydrogenated (Pd, 300 mg, 0.028 mmol) at 1 atm and room temperature for 6 h. The reaction mixture was filtered and the filtrate was evaporated to remove methanol. The amine residue was recrystallized from DMF and was collected as a white solid (70 mg, 73% yield). A solution of the amine (in 2 mL of DMF and 100 μL of water) was cooled to 0 °C and then treated with a solution of fumaroyl chloride (7.0 μL, 0.06 mmol) in 6 mL of THF. The resulting light brown solution was stirred for 15 h as it warmed to room temperature. The solvent was evaporated to give a crude product which was stirred with methanol. The insoluble portion was collected and the NMR showed it to be the desired product, mp 225 °C (15 mg, 33% yield). The portion soluble in methanol (30 mg) contained the desired product and an impurity in a 1:1 mole ratio.

Spectroscopic and Analytical Data on Linked Lexitropsins. Bis-distamycin 13: mp 210 °C; ^{1}H NMR δ 2.48 ($COCH_2CH_2CO$, 4 H, s), 2.56 [2 $CH_2C(NH_2)_2Cl$, 4 H, tr, J = 6 Hz], 3.50 (2 $CONHCH_2$, 4 H, q, J = 6 Hz), 3.80 (2 NCH_3, 6 H, s), 3.82 (2 NCH_3, 6 H, s), 3.83 (2 NCH_3, 6 H, s), 6.90 (2 py-CH, 2 H, d, J = 2 Hz), 6.94 (2 py-CH, 2 H, d, J = 2 Hz), 7.04 (2 py-CH, 2 H, d, J = 2 Hz), 7.14 (2 py-CH, 2 H, d, J = 2 Hz), 7.18 (2 py-CH, 2 H, d, J = 2 Hz), 7.22 (2 py-CH, 2 H, d, J = 2 Hz), 8.24 (2 $CONHCH_2$, 2 H, tr, J = 6 Hz), 8.74 [2 $C(NH_2)_2Cl$, 4 H, s], 9.04 [2 $C(NH_2)_2Cl$, 4 H, s], 9.93 (5 py-NHCO, 5 H, s), 9.96 (py-NHCO, 1 H, s); MS (FAB), 989 (M − 2Cl − H, 0.34).

Bis-distamycin 14: mp 210 °C; ^{1}H NMR δ 1.28 (4,5-suber-CH_2, 4 H, m), 1.57 (3,6-suber-CH_2, 4 H, m), 2.23 (2,7-suber-CH_2, 4 H, tr, J = 7 Hz), 2.63 (2 $CH_2C(NH_2)_2Cl$, 4 H, tr, J = 6 Hz), 3.49 (2 $CONHCH_2$, 4 H, m), 3.80 (2 NCH_3, 6 H, s), 3.81 (2 NCH_3, 6 H, s), 3.83 (2 NCH_3, 6 H, s), 6.88 (2 py-CH, 2 H, d, J = 2 Hz), 6.94 (2 py-CH, 2 H, d, J = 2 Hz), 7.05 (2 py-CH, 2 H, d, J = 2 Hz), 7.15 (2 py-CH, 2 H, d, J = 2 Hz), 7.18 (2 py-CH, 2 H, d, J

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(24) Lee, M.; Lown, J. W. *J. Org. Chem.* **1987**, *562*, 5717–5721.

= 2 Hz), 7.23 (2 py-CH, 2 H, d, J = 2 Hz), 8.25 (2 CONHCH$_2$, 2 H, m), 8.72 (2 C(NH$_2$)$_2$Cl, 4 H, s], 9.03 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.86 (2 py-NHCO, 2 H, s), 9.92 (4 py-NHCO, 4 H, s); MS (FAB) 1045 (M - 2Cl - H, 0.38).

Bis-distamycin 15: mp 198–202 °C; ^{1}H NMR δ 1.26 [(4,5,6,7-seba-CH$_2$, 8 H, m), 4 H, tr, J = 6 Hz], 1.55 [(3,8-seba-CH$_2$), 4 H, m], 2.22 [(2,9-seba-CH$_2$), 4 H, tr, J = 8 Hz], 2.61 [2 CH$_2$C(NH$_2$)$_2$Cl, tr, J = 6 Hz], 3.48 (2 CONHCH$_2$, 4 H, m), 3.80 (2 NCH$_3$, 6 H, s), 3.81 (2 NCH$_3$, 6 H, s), 3.83 (2 NCH$_3$, 6 H, s), 6.89 (2 py-CH, 2 H, d, J = 2 Hz), 6.95 (2 aromatic-CH, 2 H, d, J = 2 Hz), 7.05 (2 py-CH, 2 H, d, J = 2 Hz), 7.15 (2 py-CH, 2 H, d, J = 2 Hz), 7.18 (2 py-CH, 2 H, d, J = 2 Hz), 7.22 (2 py-CH, 2 H, d, J = 2 Hz), 8.23 (2 CONHpy, 2 H, m), 8.65 [2 C(NH$_2$)$_2$Cl, 4 H, s], 8.99 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.82 (2 py-NHCO, 2 H, s), 9.91 (4 py-NHCO, 4 H, s); MS (FAB) 1074 (M - 2Cl - H, 0.08).

Bis-distamycin 16: mp 215 °C; ^{1}H NMR δ 1.23 (4,5,...20,21-tetraco-CH$_2$, 36 H, s), 1.55 (3,22-tetraco-CH$_2$, 4 H, m), 2.21 (2,23-tetraco-CH$_2$, 4 H, tr, J = 7 Hz), 2.62 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.50 (2 CONHCH$_2$, 4 H, tr, J = 6 Hz), 3.80 (2 NCH$_3$, 6 H, s), 3.82 (2 NCH$_3$, 6 H, s), 3.84 (2 NCH$_3$, 6 H, s), 6.89 (2 py-CH, 2 H, d, J = 2 Hz), 6.94 (2 py-CH, 2 H, d, J = 2 Hz), 7.05 (2 py-CH, 2 H, d, J = 2 Hz), 7.15 (2 py-CH, 2 H, d, J = 2 Hz), 7.19 (2 py-CH, 2 H, d, J = 2 Hz), 7.23 (2 py-CH, 2 H, d, J = 2 Hz), 8.25 (2 CONHCH$_2$, 4 H, tr, J = 6 Hz), 8.72 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.02 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.83 (2 py-NHCO, 2 H, s), 9.92 (4 py-NHCO, 4 H, s); MS (FAB) 1270 (M - 2Cl - H, 0.10).

Bis-distamycin 26: mp >300 °C; ^{1}H NMR δ 2.63 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.50 (2 CONHCH$_2$, 4 H, tr, J = 6 Hz), 3.82 (2 NCH$_3$, 6 H, s), 3.86 (2 NCH$_3$, 6 H, s), 3.90 (2 NCH$_3$, 6 H, s), 6.97 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.09 (2 py-CH, 2 H, d, J = 16 Hz), 7.15 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.20 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.26 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.38 (2 py-CH, 2 H, d, J = 1.6 Hz), 8.10 (aromatic-CH, 4 H, s), 8.25 (2 CONHCH$_2$, 2 H, tr, J = 6 Hz), 8.65 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.01 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.95 (2 py-NHCO, 2 H, s), 10.03 (2 py-NHCO, 2 H, s), 10.57 (2 py-NHCO, 2 H, s), (CD$_3$OD) 2.71 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 7 Hz], 3.65 (2 CONHCH$_2$, 4 H, tr, J = 7 Hz), 3.87 (2 NCH$_3$, 6 H, s), 3.91 (2 NCH$_3$, 6 H, s), 3.95 (2 NCH$_3$, 6 H, s), 6.90 (2 py-CH, 2 H, d, J = 1.8 Hz), 6.98 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.07 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.16 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.20 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.34 (2 py-CH, 2 H, d, J = 1.8 Hz), 8.04 (aromatic-CH, 4 H, s); MS (FAB) 1037 (M - 2Cl - H, 0.05).

Bis-distamycin 27: mp 240 °C; ^{1}H NMR δ 2.61 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.48 (2 CONHCH$_2$, 4 H, tr, J = 6 Hz), 3.80 (2 NCH$_3$, 6 H, s), 3.86 (2 NCH$_3$, 6 H, s), 3.91 (2 CH$_3$, 6 H, s), 6.97 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.09 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.16 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.20 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.25 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.38 (2 py-CH, 2 H, d, J = 1.6 Hz), 7.66 (5-aromatic-CH, 1 H, tr, J = 7.5 Hz), 8.10 (4,6-aromatic-CH, 2 H, d, J^1 = 8 Hz), 8.21 (2-aromatic-CH, 1 H, br s), 8.21 (2 CONHCH$_2$, 2 H, br s), 8.58 [2 CY$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 7 Hz], 3.64 (2 CONHCH$_2$, 4 H, tr, J = 7 Hz), 3.88 (2 NCH$_3$, 6 H, s), 3.90 (2 NCH$_3$, 6 H, s), 3.94 (2 NCH$_3$, 6 H, s), 6.89 (2 py-CH, 2 H, d, J = 1.8 Hz), 6.97 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.07 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.20 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.33 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.65 (5-aromatic-CH, 1 H, tr, J = 7.5 Hz), 8.08 (4,6-aromatic-CH, 2 H, dd, J_1 = 7.5 Hz, J_2 = 2 Hz), 8.47 (2-aromatic-CH, 1 H, br tr, J = 2 Hz); MS (FAB) 1037 (M - 2Cl - H, 0.43).

Bis-distamycin 28: mp 245 °C; ^{1}H NMR (CD$_3$OD) δ 2.71 [2 CH$_2$(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.63 (2 CONHCH$_2$, 4 H, tr, J = 6 Hz), 3.87 (2 NCH$_3$, 6 H, s), 3.88 (2 NCH$_3$, 6 H, s), 3.90 (2 NCH$_3$, 6 H, s), 6.89 (2 py-CH, 2 H, d, J = 2 Hz), 6.91 (2 py-CH, 2 H, d, J = 2 Hz), 6.97 (2 py-CH, 2 H, d, J = 2 Hz), 7.15 (2 py-CH, 2 H, d, J = 2 Hz), 7.18 (2 py-CH, 2 H, d, J = 2 Hz), 7.24 (2 py-CH, 2 H, d, J = 2 Hz), 7.60 (2 m-aromatic-CH, 2 H, q, J = 3 Hz), 7.68 (2 aromatic-CH, 2 H, q, J = 3 Hz); MS (FAB) 1037 (M - 2Cl - H, 0.65).

Bis-distamycin 29: mp 250 °C; ^{1}H NMR δ 2.52 [2 CH$_2$(NH$_2$)$_2$Cl, 4 H, m], 3.48 (2 CONHCH$_2$, 4 H, m), 3.81 (2 NCH$_3$, 6 H, s), 3.85 (2 NCH$_3$, 6 H, s), 3.88 (NCH$_3$, 3 H, s), 3.90 (NCH$_3$, 3 H, s), 6.96 (2 py-CH, 2 H, m), 7.09 (2 py-CH, 2 H, d, J = 2 Hz), 7.17 (py-CH, 1 H, d, J = 2 Hz), 7.19 (2 py-CH, 2 H, d, J = 2 Hz), 7.25 (2 py-CH, 2 H, d, J = 2 Hz), 7.29 (py-CH, 1 H, m), 7.40 (py-CH, 1 H, m), 7.42 (py-CH, 1 H, m), 8.23 (2 CONHCH$_2$, 2 H, m), 8.25 (3-py-CH, 1 H, d, J = 8 Hz), 8.54 (4-py-CH, 1 H, m), 8.64 [2 C(NH$_2$)$_2$Cl, 4 H, s], 8.99 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.20 96-py-CH, 1 H, m), 9.95 (2 py-NHCO, 2 H, s), 10.04 (2 py-NHCO, 2 H, s), 10.94 (py-NHCO, 1 H, s), 11.00 (py-NHCO, 1 H, s), (CD$_3$OD) 2.72 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.65 (2 CONHCH$_2$, 4 H, tr, J = 6 Hz), 3.87 (2 NCH$_3$, 6 H, s), 3.91 (2 NCH$_3$, 6 H, s), 3.94 (NCH$_3$, 3 H, s), 3.954 (NCH$_3$, 3 H, s), 6.90 (2 py-CH, 2 H, d, J = 2 Hz), 6.98 (2 py-CH, 2 H, d, J = 2 Hz), 7.07 (py-CH, 1 H, d, J = 2 Hz), 7.10 (py-CH, 1 H, d, J = 2 Hz), 7.15 (2 py-CH, 2 H, d, J = 2 Hz), 7.20 (2 py-CH, 2 H, d, J = 2 Hz), 7.34 (py-CH, 1 H, d, J = 2 Hz), 7.41 (py-CH, 1 H, d, J = 2 Hz), 8.27 (3-py-CH, 1 H, d, J = 8 Hz), 8.44 (4-py-CH, 1 H, m), 9.17 (6-py-CH, 1 H, m); MS (FAB) 1038 (M - 2Cl - H, 0.03).

Bis-distamycin 30: mp 260 °C; ^{1}H NMR δ 2.62 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.50 (2 CONHCH$_2$, 4 H, q, J = 6 Hz), 3.81 (2 NCH$_3$, 6 H, s), 3.85 (2 NCH$_3$, 6 H, s), 3.90 (2 NCH$_3$, 6 H, s), 6.96 (2 py-CH, 2 H, d, J = 2 Hz), 7.08 (2 py-CH, 2 H, d, J = 2 Hz), 7.16 (2 py-CH, 2 H, d, J = 2 Hz), 7.18 (2 py-CH, 2 H, d, J = 2 Hz), 7.26 (2 py-CH, 2 H, d, J = 2 Hz), 7.39 (2 py-CH, 2 H, d, J = 2 Hz), 8.23 (2 CONHCH$_2$, 2 H, tr, J = 6 Hz), 8.59 [2 C(NH$_2$)$_2$Cl, 4 H, s], 8.87 (4-py-CH, 1 H, br s), 8.98 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.24 (2,6-py-CH, 2 H, d, J = 2 Hz), 9.94 (2 py-NHCO, 2 H, s), 10.05 (2 py-NHCO, 2 H, s), 10.83 (2 py-NHCO, 2 H, s), (CD$_3$OD) 2.71 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.64 (2 CONHCH$_2$, 4 H, tr, J = 6 Hz), 3.87 (2 NCH$_3$, 6 H, s), 3.99 (2 NCH$_3$, 6 H, s), 4.02 (2 NCH$_3$, 6 H, s), 6.88 (2 py-CH, 2 H, s), 6.96 (2 py-CH, s), 7.07 (2 py-CH, 2 H, s), 7.15 (2 py-CH, 2 H, s), 7.19 (2 py-CH, 2 H, s), 7.35 (2 py-CH, 2 H, s), 8.82 (4-py-CH, 1 H, s), 9.17 (2,5-py-CH, 2 H, s); MS (FAB) (M - 2Cl - H, 0.15).

Bis-distamycin 31: mp >260 °C; ^{1}H NMR δ 2.62 [2 CH$_2$(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.50 (2 CONHCH$_2$, 4 H, m), 3.82 (2 NCH$_3$, 6 H, s), 3.86 (2 NCH$_3$, 6 H, s), 3.90 (2 NCH$_3$, 6 H, s), 6.97 (2 py-CH, 2 H, d, J = 2 Hz), 7.08 (2 py-CH, 2 H, d, J = 2 Hz), 7.15 (2 py-CH, 2 H, d, J = 2 Hz), 7.18 (2 py-CH, 2 H, d, J = 2 Hz), 7.25 (2 py-CH, 2 H, d, J = 2 Hz), 7.39 (2 py-CH, 2 H, d, J = 2 Hz), 8.23 (2 CONHCH$_2$, 2 H, tr, J = 6 Hz), 8.56 [2 C(NH$_2$)$_2$Cl, 4 H, s], 8.85 (4-py-CH, 1 H, tr, J = 2 Hz), 8.96 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.24 (3,5-py-CH, 2 H, d, J = 2 Hz), 9.94 (2 py-NHCO, 2 H, s), 10.04 (2 py-NHCO, 2 H, s), 10.81 (2 py-NHCO, 2 H, s); MS (FAB) 1038 (M - 2Cl - H, 0.25).

Bis-distamycin 32: mp >230 °C; ^{1}H NMR δ 2.05 (3,4-cyclobutane-CH$_2$, 4 H, m), 2.60 (2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.38 (1,2-cyclobutane-CH, 2 H, m), 3.49 (2 CONHCH$_2$, 4 H, tr, J = 6 Hz), 3.79 (2 NCH$_3$, 6 H, s), 3.84 (2 NCH$_3$, 6 H, s), 3.85 (2 NCH$_3$, 6 H, s), 6.88 (2 py-CH, 2 H, d, J = 1.8 Hz), 6.97 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.05 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.17 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.21 (2 py-CH, 2 H, d, J = 1.8 Hz), 7.23 (2 py-CH, 2 H, d, J = 1.8 Hz), 8.22 (2 CONHCH$_2$, 2 H, tr, J = 6 Hz), 8.55 [2 C(NH$_2$)$_2$Cl, 4 H, s], 8.96 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.88 (2 py-NHCO, 2 H, s), 9.94 (4 py-NHCO, 2 H, s), (CD$_3$OD) 2.20 (3,4-cyclobutane-CH$_2$, 4 H, m), 2.71 (2 CH$_2$, 4 H, tr, J = 7 Hz), 3.49 (1,2-cyclobutane-CH, 2 H, m), 3.64 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 7 Hz), 3.87 (2 NCH$_3$, 6 H, s), 3.89 (2 NCH$_3$, 6 H, s), 3.90 (2 NCH$_3$, 6 H, s), 6.84 (2 py-CH, 2 H, d, J = 2 Hz), 6.89 (2 py-CH, 2 H, d, J = 2 Hz), 6.95 (2 py-CH, 2 H, d, J = 2 Hz), 7.15 (2 py-CH, 2 H, d, J = 2 Hz), 7.18 (2 py-CH, 2 H, d, J = 2 Hz), 7.20 (2 py-CH, 2 H, d, J = 2 Hz); MS (FAB) 1015 (M - 2Cl - H, 1.06).

Bis-distamycin 33: mp >255 °C; ^{1}H NMR δ 2.61 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.50 (2 CONHCH$_2$, 4 H, q, J = 6 Hz), 3.82 (2 NCH$_3$, 6 H, s), 3.85 (2 NCH$_3$, 6 H, s), 3.87 (2 NCH$_3$, 6 H, s), 6.97 (2 py-CH, 2 H, tr, J = 2 Hz), 7.07 (2 py-CH, 2 H, d, J = 2 Hz), 7.10 (-CH=CH-, 2 H, s), 7.18 (2 py-CH, 2 H, s), 7.24 (2 py-CH, 2 H, d, J = 2 Hz), 7.35 (2 py-CH, 2 H, d, J = 2 Hz), 8.23 (2 CONHCH$_2$, 2 H, tr, J = 6 Hz), 8.66 [2 C(NH$_2$)$_2$Cl, 4 H, s], 8.94 [2 C(NH$_2$)$_2$Cl, 4 H, s], 9.93 (2 pyNHCO, 2 H, s), 9.99 (2 py-NHCO, 2 H, s), 10.54 (2 py-NHCO, 2 H, s), (CD$_3$OD) 2.72 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.65 (2 CONHCH$_2$, 4 H, tr, J = 6 Hz), 3.88 (2 NCH$_3$, 6 H, s), 3.90 (2 NCH$_3$, 6 H, s), 3.92 (2 NCH$_3$, 6 H, s), 6.91 (2 py-CH, 2 H, tr, J = 2 Hz), 6.98 (2 py-CH, 2 H, d, J = 2 Hz), 7.09 (-CH=CH-, 2 H, s), 7.16 (2 py-CH, 2 H, d, J = 2 Hz), 7.19 (2 py-CH, 2 H, d, J = 2 Hz), 7.33 (2 py-CH, 2 H, d, J = 2 Hz); MS (FAB) 987 (M - 2Cl - H, 0.27).

Bis-distamycin 34: mp >280 °C: ^{1}H NMR δ 2.61 [2 CH$_2$C(NH$_2$)$_2$Cl, 4 H, tr, J = 6 Hz], 3.48 (2 CONHCH$_2$, 4 H, tr, J = 6

Hz), 3.80 (2 NCH_3, 6 H, s), 3.84 (2 NCH_3, 6 H, s), 3.86 (2 NCH_3, 6 H, s), 6.35 (–CH=CH–, 2 H, s), 6.84–7.84 (12 py-CH, 12 H, m), 8.24 (2 CON*H*CH_2, 2 H, tr, J = 6 Hz), 8.58–9.50 [2 $C(NH_2)_2Cl$, 8 H, br, s], 9.93 (2 py-NHCO, 2 H, s), 9.97 (2 py-NHCO, 2 H, s), 9.98 (2 py-NHCO, 2 H, s), (CD_3OD) 2.66 [2 $CH_2C(NH_2)_2Cl$, 4 H, tr, J = 6 Hz], 3.58 (2 CONHCH_2, 4 H, tr, J = 6 Hz), 3.79 (2 NCH_3, 6 H, s), 3.82 (2 NCH_3, 6 H, s), 3.84 (2 NCH_3, 6 H, s), 6.26 (–CH=CH–, 2 H, s), 6.83 (2 py-CH, 2 H, d, J = 2 Hz), 6.87 (2 py-CH, 2 H, d, J = 2 Hz), 6.91 (2 py-CH, d, J = 2 Hz), 7.13 (2 py-CH, 2 H, d, J = 2 Hz), 7.17 (2 py-CH, 2 H, d, J = 2 Hz), 7.27 (2 py-CH, 2 H, d, J = 2 Hz); MS (FAB) no M + 1 peak.

Bis-distamycin 35: mp 260 °C; ^{1}H NMR δ 1.31 (7-bicycloheptt, 1 H, s), 1.86 (7-bicycloheptt, 1 H, d, J = 7 Hz), 2.76 (5-endo-bicycloheptt, 1 H, d, J = 8 Hz), 2.93 (4-bicycloheptt, 1 H, s), 3.35 (1-bicycloheptt, 1 H, s), 3.50 (6-exo-cycloheptt, 1 H, s), 3.50 [2 $CH_2C(NH_2)_2Cl$, 4 H, m], 3.81 (3 NCH_3, 9 H, s), 3.85 (3 NCH_3, 9 H, s), 5.98 (3-bicycloheptt, 1 H, dd, J = 2.5 Hz), 6.30 (2-bicycloheptt, 1 H, dd, J = 2.5 Hz), 6.86 (py-CH, 1 H, d, J = 2 Hz), 6.91 (py-CH, 1 H, d, J = 2 Hz), 6.97 (2 py-CH, 2 H, d, J = 2 Hz), 7.06 (2 py-CH, 2 H, d, J = 2 Hz), 7.13 (py-CH, 1 H, d, J = 2 Hz), 7.18 (2 py-CH, 2 H, d, J = 2 Hz), 7.19 (py-CH, 1 H, d, J = 2 Hz), 7.23 (2 py-CH, 2 H, tr, J = 2 Hz), 8.24 (2 CON*H*CH_2, 2 H, m), 8.57 [2 $C(NH_2)_2Cl$, 4 H, m], 8.97 [23 $C(NH_2)_2Cl$, 4 H, m], 9.88 (py-NHCO, 1 H, m), 9.92 (4 py-CH, 4 H, m), 10.11 (Ipy-NHCO, 1 H, m), (CD_3OD) 1.47 (7-bicycloheptt, 1 H, d, J = 8 Hz), 1.94 (7-bicycloheptt, 1 H, d, J = 8 Hz), 2.71 [2 $CH_2C(NH_2)_2Cl$, 4 H, d, J = 6 Hz], 2.77 (5-endo-bicycloheptt, 1 H, d, J = 4 Hz), 3.04 (4-bicycloheptt, 1 H, s), 3.47 (6-exo-bicycloheptt, 1 H), 3.64 (2 CONHCH_2, 4 H, tr, J = 6 Hz), 3.87 (3 NCH_3, 9 H, s), 3.89 (NCH_3, 3 H, s), 3.90 (2 NCH_3, 6 H, s), 6.08 (3-bicycloheptt, 1 H, d, J = 2.5 Hz), 6.37 (2-bicycloheptt, 1 H, d, J = 2.5 Hz), 6.82 (py-CH, 1 H, d, J = 2 Hz), 6.83 (py-CH, 1 H, d, J = 2 Hz), 6.89 (2 py-CH, 2 H, d, J = 2 Hz), 6.95 (2 py-CH, 2 H, d, J = 2 Hz), 7.11 (py-CH, 1 H, d, J = 2 Hz), 7.14 (2 py-CH, 2 H, d, J = 2 Hz), 7.17 (3 py-CH, 3 H, tr, J = 2 Hz); MS (FAB) 1053 (M – 2Cl – H, 0.21).

Bis-distamycin 36: mp >260 °C; ^{1}H NMR δ 2.63 [3 $CH_2C(NH_2)_2Cl$, 6 H, tr, J = 6 Hz], 3.50 (3 CONHCH_2, 6 H, q, J = 6 Hz), 3.81 (3 NCH_3, 9 H, s), 3.84 (3 NCH_3, 9 H, s), 3.89 (3 NCH_3, 9 H, s), 6.96 (3 py-CH, 3 H, s), 7.08 (3 py-CH, 3 H, s), 7.20 (3 py-CH, 3 H, s), 7.23 (3 py-CH, 3 H, s), 7.27 (3 py-CH, 3 H, s), 7.42 (3 py-CH, 3 H, s), 8.23 (3 CON*H*CH_2, 3 H, tr, J = 6 Hz), 8.70 [3 $C(NH_2)_2Cl$, 6 H, s], 8.76 (2,4,6-aromatic-CH, 3 H, s), 9.01 [3 $C(NH_2)_2Cl$, 6 H, s], 9.93 (3 py-NHCO, 3 H, s), 10.07 (3 py-NHCO, 3 H, s), 10.98 (3 py-NHCO, 3 H, s), (CD_3OD) 2.71 [3 $CH_2(NH_2)_2Cl$, 6 H, tr, J = 6 Hz], 3.64 (3 CONHCH_2, 6 H, m), 3.86 (3 NCH_3, 9 H, s), 3.90 (3 NCH_3, 9 H, s), 3.94 (3 NCH_3, 9 H, s), 6.89 (3 py-CH, 3 H, s), 6.96 (3 py-CH, 3 H, s), 7.08 (3 py-CH, 3 H, s), 7.14 (3 py-CH, 3 H, s), 7.18 (3 py-CH, 3 H, s), 7.37 (3 py-CH, 3 H, s), 8.62 (2,4,6-aromatic-CH, 3 H, s); MS (FAB) no M + 1 peak, 108 (M – 544, 0.05).

Bis-distamycin 37: mp >250 °C; ^{1}H NMR δ 1.86 (2 $CH_2CH_2CH_2$, 4 H, q, J = 8 Hz), 3.00 [2 $CH_2N(CH_3)_2$, 4 H, tr, J = 8 Hz], 3.30 (2 CONHCH_2, 4 H, m), 3.96 (2 NCH_3, 6 H, s), 4.02 (2 NCH_3, 6 H, s), 7.28 (–CH=CH–, 2 H, s), 7.54 (2 im-CH, 2 H, s), 7.67 (2 im-CH, 2 H, s), 8.52 (2 CON*H*CH_2, 2 H, tr, J = 6 Hz), 9.43 (2 py-NHCO, 2 H, s), 11.01 (2 py-NHCO, 2 H, s); MS (FAB) 777 (M – 2Cl – H, 3.11).

Anti-HIV Drug Testing System. The procedure used in the National Cancer Institute's test for agents active against human immunodeficiency virus (HIV) is designed to detect agents acting at any stage of the virus reproductive cycle. The assay involves the killing of T4 lymphocytes by HIV. Small amounts of HIV are added to cells, and a complete cycle of virus reproduction is necessary to obtain the required cell killing. Agents that interact with virions, cells, or virus gene-products to interfere with viral activities will protect cells from cytolysis. The system is automated in several features to accommodate large numbers of candidate agents and is generally designed to detect anti-HIV activity. However, compounds that degenerate or are rapidly metabolized in the culture conditions may not show activity in this screen. All tests are compared with at least one positive (e.g., AZT-treated) control done at the same time under identical conditions.

Procedure.[20] Candidate agent is dissolved in dimethyl sulfoxide (unless otherwise indicated) and then diluted 1:100 in cell culture medium before preparing serial half-log dilutions. T4 lymphocytes (CEM cell line) are added, and after a brief interval HIV-I is added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls. Cultures are incubated at 37 °C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt XTT is added to all wells, and cultures are incubated to allow formazan color development by viable cells. Individual wells are analyzed spectrophotometrically to quantify formazan production, and in addition are viewed microscopically for detection of viable cells and confirmation of protective activity. Drug-treated virus-infected cells are compared with drug-treated noninfected cells and with other appropriate controls (untreated infected and untreated uninfected cells, drug-containing wells without cells, etc.) on the same plate. Data are reviewed in comparison with other tests done at the same time, and a determination about activity is made.

Antiretroviral (MuLV) Plaque Reduction Assay. The method was adapted from Rowe et al. (1970)[25] and Lin et al. (1987).[26]

Materials. The following materials were used. retroviruses: rauscher-ATCC 998; moloney LT(V)-ATCC 190; Leukosis-sarcoma complex-ATCC 245.

Cells: SC-1-ATCC CRL 1404; XC-ATCC CLC 165.

Minimum essential medium (eagle) with Hanks'Bss, supplemented with 10% fetal bovine serum, 100 IU mL^{-1} penicillin G, 100 μg mL^{-1} streptomycin, 2.5 μg mL^{-1} amphotericin B, and nonessential amino acids (Sigma M2025).

Dulbecco's modified eagles medium, supplemented with 10% fetal bovine serum, 100 10 mL^{-1} penicillin G, 100 μg mL^{-1} streptomycin, and 2.5 μg mL^{-1} amphotericin B.

Minimum essential medium (Eagle) with Earles salt supplemented with 5% fetal bovine serum, 100 10 mL^{-1} penicillin G, 100 μg mL^{-1} streptomycin, and nonessential amino acids (Sigma M2025). Phosphate-buffered saline. Crystal violet dye. Twenty-four-well plates were employed.

Compounds were dissolved in DMSO (or water) to 2–20 μg mL^{-1} and then further diluted in 5% FBS-MEM.

Procedure. Stock cell cultures were prepared in the 10% FBS-Dulbecco. To prepare 24-well plates for experiments, 0.8 mL of 3.5×10^4 SC^1 cells mL^{-1} were added to each well 1 day in advance. This was using the 5% FBS-MEM. A 0.1-mL amount of each compound dilution, in triplicate, was added to a well in the plate. A 0.1-mL amount of 20–40 pfu of moloney virus was added to each well of the plate. Those plates were shaken on the mechanical shaker at 0, 30 and 60 minutes. They were incubated for 5 days at 37 °C in a 5% CO_2 incubator. At this stage the plates were examined for extent of cell viability versus controls as a measure of toxicity of the drugs. Then the medium was removed. The plates were subjected to ultraviolet light (175 W cm^2 at surface) for 3 min.

A 0.8-mL amount of 2×10^5 XC cells mL^{-1} was added to each well using the 10% FBS-Hanks MEM. The plates were incubated at 37 °C, 5% CO_2 for 4 days, but the medium was replaced after 2 days. The medium was removed, the wells were washed with pbs, and 0.25 mL of 0.5% crystal violet was added to each well for 2 h. The plates were washed and dried, and the plaques were counted.

MIC_{50} values were calculated using the formula

$$\frac{\%\text{ inhibition greater than } 50\% - 50\%}{\%\text{ inhibition greater than } 50\% - \text{inhibition less than } 50\%}$$

to give the interpolative values between two dilutions.

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