Polyanion Inhibitors of Human Immunodeficiency Virus and Other Viruses. Part 2. Polymerized Anionic Surfactants Derived from Amino Acids and Dipeptides†

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A series of new polyanions was synthesized *via* γ-polymerization, in aqueous micellar solution, of ω-unsaturated anionic surfactants whose polar head was derived from amino acids or dipeptides. The obtained polyanions were evaluated for their activity against human immunodeficiency virus (HIV-1, HIV-2) and various other RNA and DNA viruses. All the test compounds proved active against HIV-1 and HIV-2, their 50% inhibitory concentration (IC_{50}) being in the range of 0.04−7.5 μg/mL, while they were not toxic to the host cells (CEM-4 or MT-4) at concentrations up to 100 μg/mL or higher. The HIV-inhibitory effect increased with the hydrophilic character of the amino acid moiety. The compounds were found to interact with both the viral envelope glycoprotein gp120 and the cellular CD4 receptor, thus blocking virus−cell binding and virus-induced syncytium formation. These polyanions also proved active against human cytomegalovirus at about the same IC_{50} as for HIV. In addition, they were also active, albeit at somewhat higher IC_{50} values (0.8−20 μg/mL), against other enveloped viruses such as respiratory syncytial virus and arenaviruses (Junin and Tacaribe). At yet higher IC_{50} values ($\geq$20 μg/mL), some of the compounds showed activity against influenza A virus. No activity was observed with any of the compounds against vesicular stomatitis virus, Sindbis virus, Semliki forest virus, influenza B, parainfluenza type 3, and the nonenveloped viruses Coxsackie type B4, polio type 1, and reovirus type 1.

Introduction

Some polyanions have an inhibitory effect against human immunodeficiency virus (HIV) and other viruses. Anti-HIV activity has been observed with sulfated polysaccharides such as heparin,[1] dextran sulfate,[2] pentosan polysulfate,[3] and lentinan sulfate.[4] Synthetic polyanions, such as sulfated poly(vinylic alcohol) or copolyanions from sulfated vinylic alcohol and acrylic acid[5] as well as polymeric aurintricarboxylic acid,[6] have also been reported to inhibit HIV replication.

Several polyanionic substances different from dextran sulfate are now being considered or pursued for clinical use. (i) PAVAS, a copolymer of acrylic acid with vinyl alcohol sulfate, has topical use as a vaginal microbicide against both HSV and HIV infection.[7] (ii) Some poly(oxometalate)s have a potent activity (superior to that of ribavirin) against respiratory syncitial virus (RSV) in cotton rats[8] and are now being further pursued for their potential against RSV, human cytomegalovirus (HCMV), and influenza virus infections. (iii) PRO 2000, a naphthalene sulfonate condensate,[9] has been shown to block the binding of HIV-1 gp120 to CD4 and has entered single-dose, as well as multiple-dose, clinical trials.[10] Recently,[11] we have shown that polyanions obtained by polymerization of unsaturated anionic surfactants in micellar solutions, according to Scheme 1, markedly inhibit the replication of HIV-1 and HIV-2 in cell culture.

We present here the synthesis, following the same scheme, of polyanions, in which the anionic groups (Y^-) were derived from an amino acid or dipeptide. Indeed, in our previous work, the polyanion obtained from β-alanine as anionic group appeared to be the most active. Copolyanions have also been prepared from equimolar mixtures of various pairs of monomers. The structures of the three types of polyanions thus obtained are shown in Chart 1. Antiviral activities of these compound have been determined.

Syntheses

Preparation of *N*-Undec-10′-enoyl-L-amino Acids. *N*-Undec-10′-enoyl-L-amino acids were obtained according to the Schotten−Baumann procedure[12,13] in two differents conditions. In method A, the coupling reaction was realized under Nagasawa's conditions[14] by direct reaction at 0 °C of undec-10-enoyl chloride (**1**)[15] on the amino acid dissolved in 4 N aqueous sodium hydroxide. The reaction started easily, but quickly a dense emulsion was formed, slowing down the reaction rate and leading to poor yields. By this method, the monomers **2m**, **3m**, and **4m**, derived from L-alanine, L-proline, and L-threonine, respectively, were prepared (Scheme 2). When method A was applied to L-threonine, side reactions occurred, especially O-acylation of the lateral chain of the amino acid.

Due to poor yields and difficulties encountered by this method, we improved method B in homogeneous medium, based on the *in situ* temporary protection of the carboxylic function by a trimethylsilyl ester group.[16] This method permitted the condensation of the undec-10-enoyl chloride in organic medium, thus avoiding the

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Scheme 1

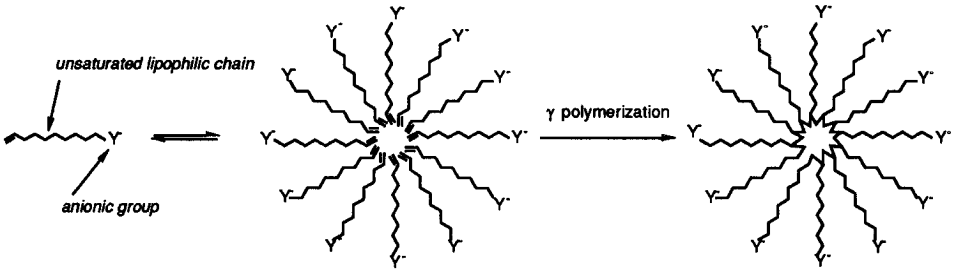
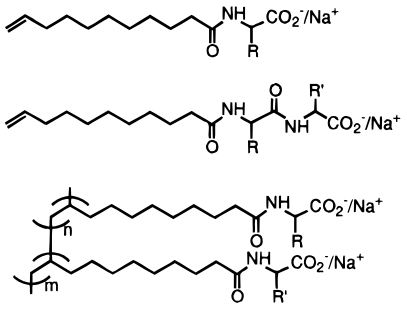
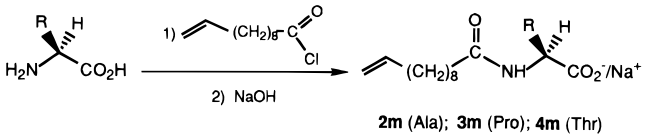


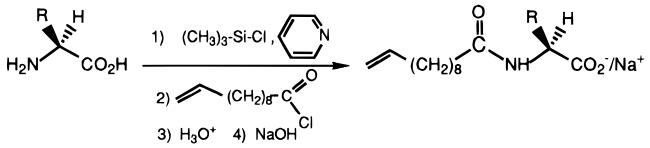
Chart 1



Scheme 2



Scheme 3



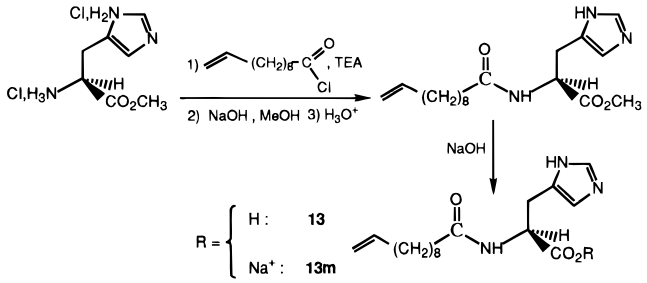
R = -CH_2-Ph	**5m**	= -CH_2-Ph-OH	**9m**
= -$CH(CH_3)_2$	**6m**	= -CH_2OH	**10m**
= -CH_2-$CH(CH_3)_2$	**7m**	= -CH_2-CH_2-S-CH_3	**11m**
= -$CH(CH_3)$-CH_2-CH_3	**8m**		

emulsion formation. Under these conditions, good yields were obtained (Scheme 3). The protecting step was achieved as soon as the dissolution of the amino acid in methylene chloride was completed; the reaction of the acid chloride **1**, followed by TLC, was then very fast (<15 min).

Whatever method used, N-acylated amino acids were purified as their acid form by either recrystallization or chromatography on silica gel. Neutralization of the acid forms, under nitrogen atmosphere, by an equimolar amount of sodium hydroxide gave the corresponding sodium salts. The latter were recovered by lyophilization. It is worthwhile to note that N-acylated L-proline (**3m**) and L-methionine (**11m**) sodium salts are poorly water soluble, the others being very soluble.

***N*-Undec-10′-enoyl-L-histidine.** The previous conditions applied to L-histidine led to a competitive acylation reaction of the imidazole ring. To decrease this side reaction, acid chloride **1** was reacted with L-histidine methyl ester dihydrochloride[17,18] in anhydrous chloroform in the presence of triethylamine. The acyl chloride and the triethylamine were added at 0 °C

Scheme 4



alternatively and successively by small fractions to induce a selective deprotonation of the primary amine,[19] thus favoring the formation of the monoacylated derivative (Scheme 4).

The monoacylated ester **12** was isolated as white crystals after chromatography on silica gel; then it was saponified by 1 N methanolic sodium hydroxide under sonication at room temperature. Finally, the corresponding acid **13** was transformed into its sodium salt **13m**, as previously mentioned.

***N*-Undec-10′-enoyl Dipeptides.** Also we prepared monomer surfactants with a dipeptide as a polar group. The coupling of a second amino acid was expected either to induce a possible synergic effect between both amino acid residues or to improve the solubility of the monomers **3m** and **11m** by linking the hydrophilic L-glutamic acid. The condensation of the L-glutamic acid dimethyl ester[20] on monomers **3m** and **11m** was promoted by the dicyclohexylcarbodiimide/hydroxybenzotriazole couple[21] in methylene chloride (Scheme 5).

After saponification and acidification, we isolated the N-acylated dipeptides **14m** and **15m** under their acid form by chromatography on silica gel. Addition of two equimolar amounts of sodium hydroxide converted these two acids into their corresponding sodium salts, both of them being very water soluble. The monomer **16m** was obtained by the same method from *N*-acyl-L-phenylalanine (**5m**) and the *β*-alanine methyl ester (Scheme 5).

The structure of all monomers, as acid forms, was determined by IR, NMR (^{1}H), and mass (FAB^+) spectroscopy. Sodium salt structures were confirmed by IR, NMR (^{13}C), and mass (FAB^-) spectroscopy.

Polymerization Reaction

As we have previously shown,[11,22−24] the polymerization of aqueous micellar solution of *ω*-unsaturated surfactants leads to relatively monodipersed and low molecular weight polymers (MW being in the range of 6000−10 000, depending upon the structure of the monomer). At a 0.1 M aqueous solution, anionic monomers were polymerized by *γ*-irradiation (10 Mrad), the yield being generally quantitative.[11] Also some pairs

Scheme 5

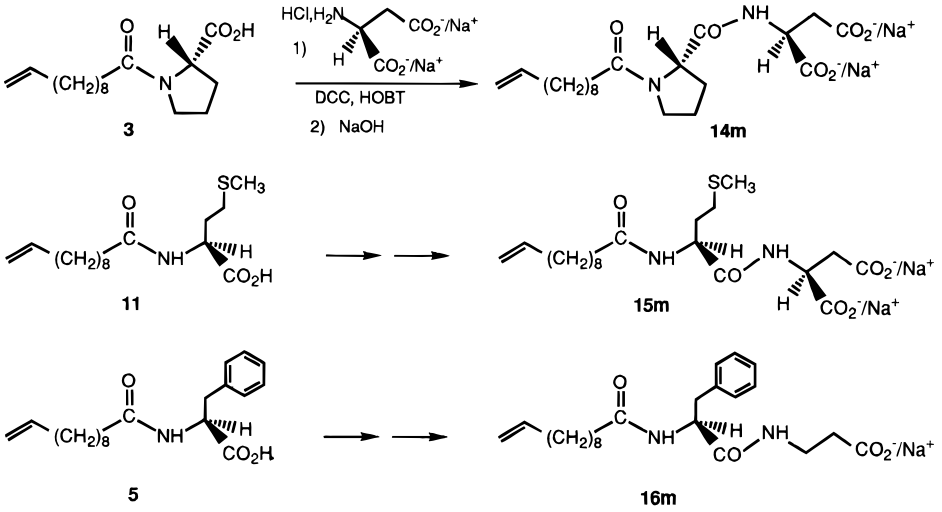


Table 1. Anti-HIV-1 Activity in CEM-4 Cells

compd	CC_{50}[a] (μg/mL)	IC_{50}[b] (μg/mL) MTT	IC_{50}[b] (μg/mL) RT	SI[c] MTT	SI[c] RT
		Y^- = Amino Acid			
2p (Ala)	>100	<0.07	0.2	>2500	>500
6p (Val)	>150	0.1	0.4	>1500	>375
8p (Ile)	>150	0.1	0.5	>1500	>300
7p (Leu)	>150	0.4	1	>375	>150
5p (Phe)	>150	0.6	3	>250	>50
10p (Ser)	>150	<0.07	0.1	>2150	>1500
4p (Thr)	>100	<0.07	0.1	>2500	>1500
13p (His)	>150	0.1	0.4	>1500	>375
9p (Tyr)	>150	0.2	1	>750	>150
		Y^- = Dipeptide			
14p (Pro-Glu)	>150	0.43	1	>350	>150
15p (Met-Glu)	>150	0.19	0.4	>800	>375
16p (Phe-βAla)	>150	0.1	0.4	>1500	>375
		Copolyanions			
17p (Ala/Phe)	>100	<0.04	0.8	>2500	>125
18p (Ala/βAla)	>100	<0.04	0.1	>2500	>1000
19p (βAla/Glu)	>100	<0.04	0.2	>2500	>500
20p (βAla/Phe)	>100	0.08	0.4	>1250	>250
21p (Phe/Glu)	>100	0.10	0.8	>1000	>125

[a] 50% cytotoxic concentration, or compound concentration required to reduce the viability of uninfected cells by 50% at 5 days of incubation in the presence of the compound. [b] 50% inhibitory concentration, or compound concentration required to inhibit by 50% HIV-1-induced cytopathicity (based on the MTT assay or RT activity). [c] 50% selectivity index, or ratio of CC_{50} to IC_{50}. All data represent the average values for at least two separate experiments.

of *N*-undec-10′-enoyl-L-amino acid sodium salts were copolymerized by γ-irradiation of an equimolar (0.5 M) mixture of both salts (Table 1).

Polyanions and copolyanions thus prepared were purified by gel permeation chromatography (Sephadex-G50) to remove trace of monomer. ^{1}H NMR spectroscopy in D_2O enabled us to verify the disappearance of vinylic protons and the absence of degradation during the γ-irradiation. For the copolymers, it also permitted to control that the two amino acid moieties were present in the 1:1 ratio.

Biological Activity

First of all it should be noted that no activity was observed with any of the tested monomers. The polyanions were evaluated against HIV in the T4-lymphocyte cell lines CEM-4 (Table 1) and MT-4 (Table 2) and also against a wide variety of DNA and RNA viruses in different cell lines (E_6SM, HEL, HeLa, MDCK, and Vero) (Table 4). Dextran sulfate was evaluated in parallel for comparison. In addition, cytotoxicity of the different compounds was determined for all the cell lines used (Table 5).

As shown in Table 1, all the polyanions derived from amino acids proved markedly inhibitory to HIV-1 in CEM-4 cells. The 50% inhibitory concentration (IC_{50}), based on either the MTT assay or reverse transcriptase (RT) production, fell in the range of 0.04−0.43 μg/mL (MTT) and 0.1−3 μg/mL (RT), respectively. For compounds derived from amino acids with an alkyl or aryl lateral chain, the anti-HIV-1 activity decreased with increasing the hydrophobicity of the lateral chain. The hydrophobicity, according to Delhaise, Whuilmart, and

Table 2. Anti-HIV Activity of Polyanions in MT-4 Cells

compd	CC_{50}[a] (μg/mL)	HIV-1 IC_{50}[b] (μg/mL)	HIV-1 SD[c]	HIV-1 SI[d]	HIV-2 IC_{50}[b] (μg/mL)	HIV-2 SD[c]	HIV-2 SI[d]
dextran sulfate	>250	0.1		>2500	0.08		>3125
2p	>250	0.37 (0.42−0.32)	0.07	>675	4.11 (2.27−5.94)	2.6	>60
6p	>250	0.70 (0.80−0.60)	0.14	>360	2.00 (1.64−2.36)	0.51	>125
8p	>250	0.83 (0.91−0.74)	0.12	>300	2.43 (1.86−3.00)	0.81	>100
7p	>250	2.20 (1.80−2.60)	0.57	>110	3.86 (3.04−4.68)	1.16	>65
5p	>250	2.41 (2.00−2.81)	0.57	>100	7.52 (12.4−2.66)	6.87	>35
10p	>250	0.40 (0.36−0.44)	0.06	>625	1.31 (1.78−0.83)	0.67	>190
4p	>250	0.10 (0.095−0.10)	0.00	>2500	3.41 (2.78−4.03)	0.88	>75
14p	>250	0.19 (0.25−0.13)	0.08	>1315	0.51 (0.32−0.70)	0.27	>490
16p	>250	0.67 (0.91−0.42)	0.35	>370	1.40 (0.74−2.05)	0.93	>180

[a] 50% cytotoxic concentration, or compound concentration required to reduced viability of mock-infected MT-4 cells by 50%. [b] 50% inhibitory concentration, or compound concentration required to inhibit HIV-induced cytopathicity in MT-4 cells by 50%. [c] Standard deviation; all data represent mean values for two separate experiments (the individual values are listed in parentheses). [d] Selective index: ratio of CC_{50} to IC_{50}.

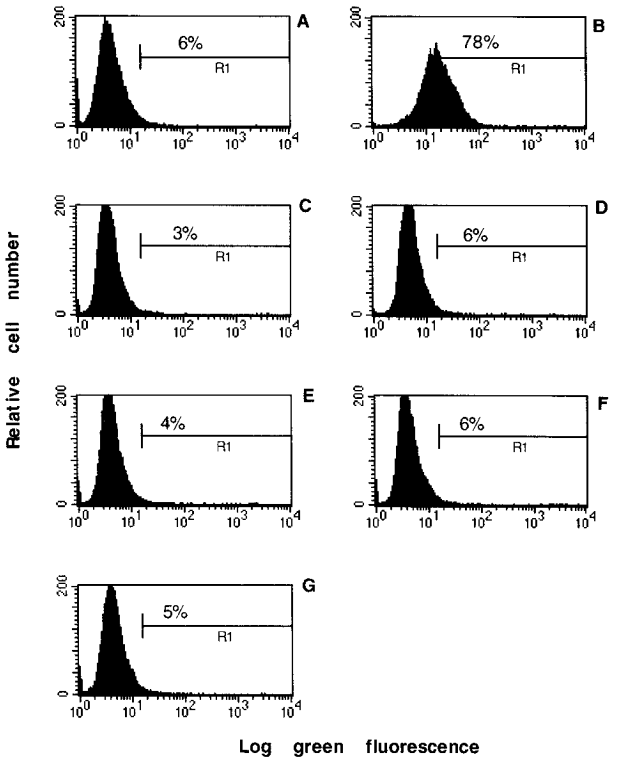


Figure 1. HIV-1-infected HUT-78 cells incubated with RaM-IgG(Fab′)$_2$-FITC only (A), anti-gp120 mAb and RaM-IgG-(Fab′)$_2$-FITC (B), or anti-gp120 mAb and RaM-IgG(Fab′)$_2$-FITC with compound **2p** (C), **4p** (D), **8p** (E), **14p** (F), and dextran sulfate (G) at 25 μg/mL. The method has been described in detail elsewhere.[27] The values in each histogram represent the percentage of fluorescent positive cells.

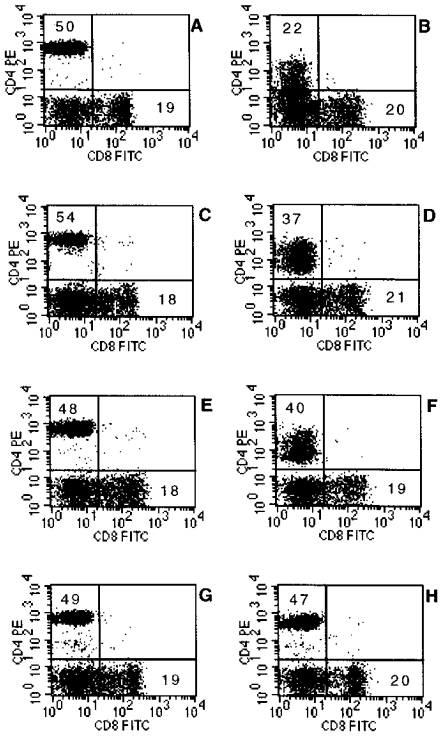


Figure 2. Peripheral blood lymphocytes incubated in PBS alone (A), with compounds **2p**, **4p**, **8p**, and **14p** (at 25 μg/mL) (B, D, F, H), or with **2p**, **4p**, and **8p**, (at 5 μg/mL) (C, E, G) at 4 °C, stained with anti-Leu3a mAb PE-labeled (CD4-PE) and anti-Leu2a mAb FITC-labeled (CD8-FITC) for 20 min at 4 °C, washed, fixed in 1% paraformaldehyde, and examined cytofluorometrically.[28] The values in each dot plot represent the percentage of CD4$^+$T cells (upper left) and CD8$^+$T cells (lower right).

Urbain,[25] increased in the order: alanine (0.5) < valine (1.5) < leucine (1.8) = isoleucine (1.8) < phenylalanine (2.5).

To evaluate the influence of the introduction of an alcohol function in the lateral chain on the antiviral activity, polyanions **10p**, **4p**, and **9p** were tested. These compounds, derived from serine, threonine, and tyrosine, respectively, showed a slightly higher activity against HIV-1 as compared to the corresponding polyanions with nonhydroxylated lateral chains (compounds **2p**, **6p**, and **5p**). The polyanions **14p**−**16p**, which were derived from dipeptides, also proved significantly active against HIV-1. The activity ($IC_{50(RT)}$ = 0.4 μg/mL) of compound **16p**, with β-alanine as the terminal amino acid, was identical with that of the polyanion derived from β-alanine alone. This means that the hydrophobic phenylalanine residue does not lower the antiviral activity of compound **16p** as it does with the compound **5p**; the terminal amino acid seems to play the predominant role.

The anti-HIV-1 activity of the copolyanions **17p**−**21p** was similar to those observed with the corresponding homopolyanions. No increase in activity was detected. For this reason, these compounds were not investigated further. As can be seen from Table 1, the cytotoxicity of the different compounds was rather low, so that the selectivity index exceeded 2500 in some cases, as based on the MTT assay.

Most of the homopolyanions were also evaluated against HIV-1 and HIV-2 in MT-4 cells, based on the MTT assay (Table 2). All proved inhibitory to HIV-1 and HIV-2. The IC_{50} values observed in MT-4 cells were slightly higher than those observed in CEM-4 cells, but the antiviral activity uniformly decreased with increasing the hydrophobic character of the amino acid. Compound **4p** showed the highest antiviral activity (IC_{50} = 0.1 μg/mL) and the highest selectivity index (>2500) for HIV-1 among all the polyanions tested. Compound **14p**, derived from the Pro-Glu dipeptide, was the most active against HIV-2 (IC_{50} = 0.5 μg/mL; SI > 500). As a rule, HIV-1 proved more sensitive to the inhibitory effects of the different polyanions than HIV-2.

Using a flow cytometric method,[26] it was found that compounds **2p**, **4p**, **8p**, and **14p** achieved an equally strong inhibition of HIV-1 binding to MT-4 cells as did dextran sulfate. Persistenly HIV-1 infected HUT-78 cells were found to express the viral glycoprotein gp120 as demonstrated with anti-gp120 mAb and flow cytometric analysis.[27] As shown in Figure 1, the compounds **2p**, **4p**, **8p**, and **14p** (25 μg/mL) had a marked inhibitory effect on the binding of the anti-gp120 mAb. Dextran sulfate was evaluated under the same conditions and gave similar results.

From the compounds **2p**, **4p**, **8p**, and **14p** tested, compounds **2p**, **4p**, and, to a lesser extent, **8p** selectively prevented the binding of anti-Leu3a mAb to the CD4 receptor on MT-4 cells (data not shown) and peripheral blood lymphocytes (Figure 2). Compound **2p** had a profound effect on CD4 expression at 25 μg/mL (Fig. 2B) but no effect at 5 μg/mL (Figure 2C). Also compounds **4p** and **8p** at 25 μg/mL were effective (Figure 2D,F), although less so than compound **2p**. At 5 μg/mL, compounds **4p** and **8p** were without effect (Figure 2E,G). Compound **14p** at 25 μg/mL had no effect on CD4 expression (Figure 2H). The percentage of CD8$^+$ T cells remained the same under all conditions. The

Table 3. Inhibitory Effect of Polyanions on HIV-Induced Giant Cell Formation

compd	EC_{50}[a] (μg/mL) HIV-1	HIV-2
2p	0.6	66
4p	0.03	26
6p	0.6	88
8p	2.7	66
10p	1	67
14p	1	75
dextran sulfate	14.8	0.04

[a] 50% effective concentration, based on the inhibition of giant cell formation upon coculturing persistently HIV-infected HUT-78 cells with MOLT-4 cells. All data represent mean values of two separate experiments.

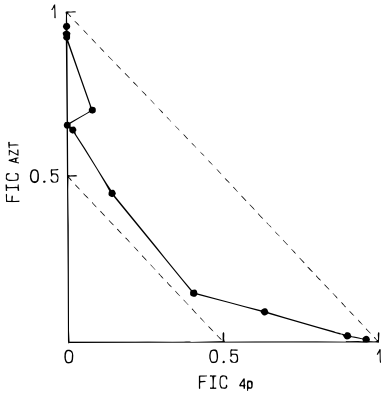


Figure 3. Isobologram representation of the inhibitory effects of compound **4p** in combination with AZT on HIV-1-induced cytopathicity in MT-4 cells. Broken lines represent the unity lines for FIC = 1 and 0.5, respectively. All results were confirmed in at least two independent experiments.

effect of these compounds on the CD4 receptor expression, however, did not appear to be correlated with their anti-HIV activity.

The polyanions proved capable of blocking giant cell formation and preventing destruction of the uninfected $CD4^+$ target cells by the HIV-infected HUT-78 cells (Table 3). The polyanions tested (**2p**, **4p**, **6p**, **8p**, **10p**, and **14p**) were 5−500-fold more active than dextran sulfate in inhibiting HIV-1-induced giant cell formation, while dextran sulfate proved to be more active against HIV-2-induced syncytium formation. These results correlated with the inhibitory effects on HIV-induced cytopathicity in MT-4 cells.

When compound **4p** was combined with the reverse transcriptase inhibitor AZT and the inhibitory effect on HIV-1-induced CPE was evaluated by the isobologram method, all $FIC_{\mathbf{4p}} + FIC_{AZT}$ values fell between 0.5 and 1.0 (Figure 3), which means that the combination was additive to subsynergistic. The combination of **2p**, **8p**, and **14p** plus AZT was also examined for inhibitory effects on HIV-1-induced CPE (data not shown). In all cases, the minimum FIC indexes achieved were between 0.5 and 1.0, indicating an additive to subsynergistic effect for these combinations.

If addition of the compound **2p**, **4p**, **6p**, or **8p** to the HIV-1-infected MT-4 cells was deleted for 1 h, the inhibitory action of the compounds was reduced in a similar fashion as for dextran sulfate. For AZT the inhibitory effect was reduced only if the addition of the compound was deleted until 4−7 h postinfection (Figure 4). These "time of addition" studies thus indicate that the polyanions tested may have a mechanism of action similar to the known inhibitors of virus adsorption, such

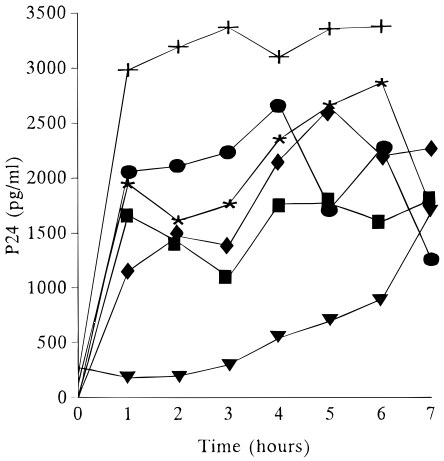


Figure 4. "Time of addition" experiment in MT-4 cells infected with $HIV\text{-}1_{IIIB}$ [MOI (multiplicity of infection) = 1]. The compounds were added at different times after infection as indicated by the differents symbols, and viral p24 antigen production was measured at 29 h postinfection: (+) dextran sulfate (50 μg/mL), (*) **2p** (40 μg/mL), (■) **4p** (10 μg/mL), (●) **6p** (80 μg/mL), (♦) **8p** (70 μg/mL), and (▼) AZT (0.5 μg/mL).

as dextran sulfate. As shown in Table 2, none of the polyanions derived from α-amino acids or dipeptides were toxic for MT-4 cells up to a concentration of 250 μg/mL.

In contrast to dextran sulfate, when evaluated for their inhibitory effects on the replication of herpes viruses, none of the polyanions tested proved inhibitory to herpes simplex virus type 1 (HSV-1) and thymidine kinase-deficient (TK^-) HSV-1 or HSV-2. However, all the polyanions tested were found to inhibit the replication of HCMV, their IC_{50}s being in the range of 0.1−3.3 μg/mL (Table 4) at nontoxic concentrations (Table 5). No activity was noted with any of the polyanions against vaccinia virus.

When evaluated for their inhibitory effects on the replication of RNA viruses other than HIV, some of the polyanions proved active against influenza A virus (**2p**, **8p**, **5p**, and **4p**) at a rather high IC_{50} (20−40 μg/mL). However, all compounds showed activity against RSV and the arenaviruses Junin and Tacaribe, within the IC_{50} range of 0.8−20 μg/mL. The polyanions **14p**, **10p**, and **4p** were the most active ones against RSV, while the polyanions **2p**, **8p**, **10p**, **4p**, and **14p** emerged as the most active ones against Junin and Tacaribe viruses (Table 4). Although vesicular stomatitis virus (VSV) and Sindbis virus were sensitive to the inhibitory effect of dextran sulfate, the replication of these viruses was not affected by any of the polyanions tested. No activity was noted with any of the compounds against Semliki forest virus, influenza B virus, parainfluenza type 3, and the nonenveloped viruses Coxsackie type B4, polio type 1, and reovirus type 1.

Although the antiviral activity spectrum of the test compounds could at first glance be considered as similar to that of dextran sulfate, closer inspection reveals that their activity spectrum is more restricted in that no activity was noted with any of the test compounds against HSV and VSV and only with some of them against influenza A virus. This differential activity spectrum points to a distinct molecular mode of action of the polymerized anionic surfactants and dextran sulfate. The precise nature of these molecular interactions is the subject of further study.

Table 4. Inhibitory Effect of Several Polyanions on the Replication of DNA and RNA Viruses

		IC_{50}[a] (μg/mL)									
virus	cell line	dextran sulfate	**2p**	**6p**	**8p**	**7p**	**5p**	**10p**	**4p**	**14p**	**16p**
HSV-1 (KOS)	E_6SM	2	>20	>40	>50	>200	>40	>40	>20	>50	>50
HSV-2 (G)	E_6SM	2	30	>40	40	70	70	70	50	15	30
HSV-1 TK^- (B2006)	E_6SM	2	>20	>40	>50	>200	>40	>40	>20	>50	>50
HCMV (AD169)	HEL	0.6	1.4	1.0	3.3	1	1	0.4	0.4	0.2	2.25
HCMV (Davis)	HEL	0.4	0.3	0.4	0.7	0.4	0.4	0.1	0.15	0.05	0.2
vaccinia	E_6SM	100	>50	>40	>50	>200	>40	>40	>50	>50	>50
vesicular stomatitis	E_6SM	2	>50	>40	>50	>200	>40	>40	>50	>50	>50
influenza A	MDCK	8	20	≥70	20	>100	40	≥100	30	>200	≥60
influenza B	MDCK	>200	>100	>100	>100	>100	≥100	>100	>100	>200	>100
respiratory syncytial	HeLa	4	14	20	20	20	20	4	6	1.6	20
parainfluenza 3	Vero	>400	>100	>40	>100	>100	>40	>200	>40	>400	>400
reovirus-1	Vero	>400	>100	>40	>100	>100	>40	>200	>40	>400	>400
Sindbis	Vero	40	>100	>40	>100	>100	>40	>200	>40	>400	>400
Semliki forest	Vero	>400	>100	>40	>100	>100	>40	>200	>40	>400	>400
Junin	Vero	8	2	42	3.2	16	7	2.5	0.9	4.3	7
Tacaribe	Vero	7	3.2	30	2.9	12	8	2.0	0.8	3.8	8
coxsackie B4	HeLa	>400	>400	>400	>200	>400	>200	>400	>100	>400	>400
polio-1	HeLa	>400	>400	>400	>200	>400	>200	>400	>100	>400	>400

[a] 50% inhibitory concentration, or compound concentration required to reduce virus-induced cytopathicity by 50%. Virus was added in the presence of the compounds, and the cells were further incubated until the cytopathogenic effect (CPE) was scored.

Table 5. Cytotoxicity of Polyanions for Different Cell Lines

cell	MCC[a] (μg/mL)									
culture	dextran sulfate	**2p**	**6p**	**8p**	**7p**	**5p**	**10p**	**4p**	**14p**	**16p**
E_6SM	>400	>200	≥100	>200	400	≥100	≥100	>200	>200	>200
HEL	>200	>200	60	>200	90	>200	70	>200	>200	>200
HeLa	>400	20	100	100	100	400	>400	100	>200	100
MDCK	>200	100	100	100	100	100	≥200	100	>200	100
Vero	>200	50	200	200	200	200	200	200	200	50

[a] Minimum cytotoxic concentration, or compound concentration required to cause a microscopically detectable alteration of normal cell morphology. For HEL cells, the MCC corresponds to the 50% inhibitory concentration required to inhibit cell growth by 50%.

Conclusion

The main fact emerging from this study was the marked activity of all polyanions tested against both HIV-1 and HIV-2 as well as HCMV, RSV, and arenaviruses. Moreover, a structure−activity relationship emerged, the less hydrophobic the amino acid moiety, the higher their virus-inhibitory effect. For this reason, synthesis and antiviral evaluation of polyanions with double-charged or multicharged anionic groups are underway.

Experimental Section

Synthesis. Melting points were determined using a Buchi 530 apparatus; they are uncorrected. C, H, and N elemental analyses have been realized for most of the monomers; the observed deviations to the indicated formula are always <0.4%. Merck silica gel 60 F_{254} (0.25 mm) plates were employed for analytical TLC. ^1H and ^{13}C NMR spectra used Bruker AC 250 and WP 200 SY spectrometers, respectively. For ^1H and ^{13}C NMR, we have used numbers 1′−11′ for the undecenoyl hydrocarbon chain and numbers 1, 2, 3... for the amino acid or peptide part of the molecule. Mass spectra used a JEOL DX 100 (FAB^+) instrument and *m*-nitrobenzylic alcohol as matrix for the acids and thioglycerol as matrix for the sodium salts. IR spectra used an IR-FT BONEM MB-100 instrument.

Undec-10-enoyl Chloride (1). To undec-10-enoic acid (62.6 g, 0.34 M) was dropwise added thionyl chloride (25.6 mL, 0.36 M) over a period of 45 min, under stirring. The mixture was refluxed for 2 h. The chloride **1** was distillated under reduced pressure (102 °C/2 mmHg): yield 88%; lit.[11] value 127.5−128 °C/13 mmHg.

***N*-Undec-10′-enoyl-L-amino Acids: Method A (Schotten−Baumann acylation).** To a solution of an L-amino acid (50 mM) in 20 mL of aqueous 4 N NaOH were alternately added at 0 °C three fractions of undec-10-enoyl chloride **1** (total 12.8 mL, 60 mM) and three fractions of aqueous 4 N NaOH (total 15 mL). The mixture was stirred for 30 min at 0 °C and then extracted with 75 mL of Et_2O. The aqueous phase was acidified at room temperature to pH = 2 with aqueous 5 N HCl and then extracted three times with 100 mL of $CHCl_3$. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure (θ < 40 °C).

Method B. To a suspension of an L-amino acid (10 mM) in 60 mL of dry CH_2Cl_2 were dropwise added trimethylsilyl chloride (30 mM) and pyridine (10 mM); the mixture was stirred and protected against moisture. When the amino acid was passed into solution, undec-10-enoyl chloride (**1**) (10 mM) was added. The reaction was followed by TLC. When the reaction was accomplished, the mixture was diluted with aqueous 1 N HCl and then saturated aqueous NaCl. The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure (θ < 40 °C). The crude product was purified by either recrystallization or chromatography on silica gel.

N-Undec-10′-enoyl-L-amino acids obtained by either method were transformed to their sodium salt by adding an equimolar amount of aqueous 0.5 N NaOH, at 0 °C and under nitrogen. The sodium salts were obtained after lyophilization as hygroscopic white powders.

***N*-Undec-10′-enoyl-L-alanine (2):** method A; yield 24%; mp 59−60 °C; R_f 0.8 (90/10 $CHCl_3$/MeOH); $[\alpha]^{25}_D = -20.8°$ (MeOH, c = 0.1 M); IR (KBr, ν, cm^{-1}) 3324 (N-H), 3070 (=C-H), 2919−2848 (C-H, CH_2-as and -s), 1727 (C=O, acid), 1651 (C=O, amide); ^1H NMR (90 MHz, $CDCl_3$) δ 1.4 (m, 12H, CH_2-3′−8′), 1.5 (d, 3H, CH_3-3, J = 8 Hz), 2.2 (m, 4H, CH_2-2′-9′), 4.7 (m, 1H, CH-2), 5.2 (m, 2H, CH_2=-11′), 6.0 (m, 1H, CH=-10′), 6.7 (d, 1H, NH, J = 8 Hz), 10.8 (s, 1H, CO_2H, exch); MS (FAB^+, NBA) *m/z* 256 $[M + H]^+$, 278 $[M + Na]^+$, 511 $[2M + H]^+$, 238 $[M + H - 18]^+$, 210 $[M + H - 46]^+$. Anal. ($C_{11}H_{24}NO_2$) C, H, N.

Sodium Salt 2m: IR (KBr, ν, cm^{-1}) 3340 (N-H), 3075 (=C-H), 2926−2858 (C-H, CH_2-as and -s), 1635 (C=O, amide), 1596 (CO_2^-); ^{13}C NMR (50.32, D_2O) δ 18.70 (C-3), 29.4−29.9 (C-4′−C-8′), 34.30 (C-9′), 36.76 (C-2′), 51.44 (C-2), 114.9 (C-11′), 139.9 (C-10′), 176.1 (C-1′), 180.8 (C-1); MS (FAB^-, TG) *m/z* 254 $[A^-]$, 276 $[A - H + Na]^-$, 531 $[2A + Na]^-$.

***N*-Undec-10′-enoyl-L-proline (3):** method A; yield 41%; oil; R_f 0.38 (80/20 $CHCl_3$/MeOH); $[\alpha]^{25}_D = -23.6°$ (MeOH, c = 0.1

M); IR (CCl_4, ν, cm^{-1}) 3076 (=C-H), 2928−2855 (C-H, CH_2-as and -s), 1747−1720 (C=O, acid) 1622 (C=O, amide); ^1H NMR (250 MHz, DMSO-d_6) δ 1.3 (m, 12H, CH_2-3′−8′), 2.1 (m, 4H, CH_2-2′ and -9′), 3.4 (m, 6H, CH_2-2−4), 4.2 (m, 1H, CH-1), 5.0 (m, 2H, CH_2=-11′), 5.8 (m, 1H, CH=−10′); MS (FAB$^+$, TG) *m/z* 282 [M + H]$^+$, 116 [M + H − 166], 70 (iminium ion). Anal. ($C_{16}H_{27}NO_3$) C, H, N.

***N*-Undec-10′-enoyl-L-threonine (4):** method A; yield 49%; mp 165−166 °C; R_f 0.66 (80/20 $CHCl_3$/MeOH); $[\alpha]^{25}_D = -14.4°$ (MeOH, c = 0.1 M); IR (KBr, ν, cm^{-1}) 3405 (O-H), 3318 (N-H), 3090 (=C-H), 1711 (C=O, acid), 1628 (C=O, amide); ^1H NMR (250 MHz, $CDCl_3$) δ 1.3 (m, 12H, CH_2-3′−8′), 1.7 (t, 3H, CH_3-4), 2.1 (m, 2H, CH_2-9′), 2.3 (t, 2H, CH_2-2′, J = 8 Hz), 4.4 (d, 1H, CH-2, J = 8.4 Hz), 4.5 (m, 1H, CH-3), 5.0 (m, 2H, CH_2=-11′), 5.8 (m, 1H, CH=-10′), 6.8 (d, 1H, NH, J = 8 Hz); MS (FAB$^+$, NBA) *m/z* 286 [M + H]$^+$, 308 [M + Na]$^+$, 571 [2M + H]$^+$, 268 [M + H − 18]$^+$, 120 [M + H − 166(ketene)]$^+$, 74 (iminium ion). Anal. ($C_{15}H_{27}NO_4$) C, H, N.

Sodium Salt 4m: IR (KBr, ν, cm^{-1}) 3410 (O-H), 3070 (=C-H), 2932−2856 (C-H, CH_2-as and -s), 1640 (C=O, amide), 1596 (CO_2^-); ^{13}C NMR (50.32 MHz, D_2O) δ 20.1 (C-4), 29.46−29.97 (C-4′−C-8′), 34.32 (C-9′), 36.88 (C-2′), 60.33 (C-2), 68.50 (C-3), 114.9 (C-11′), 139.8 (C-10′), 176.8 (C-1), 177.4 (C-1′); MS (FAB$^-$, TG) *m/z* 284 [A]$^-$, 306 [A + Na-H]$^-$, 591 [2A + Na]$^-$.

***N*-Undec-10′-enoyl-L-phenylalanine (5):** method B; yield 83%; mp 72−73 °C; R_f 0.46 (90/10 $CHCl_3$/MeOH); $[\alpha]^{25}_D$ = +13.8° (MeOH, c = 0.1 M); IR (KBr, ν, cm^{-1}) 3300 (N-H), 3072 (Ar-H), 3028 (=C-H), 2923−2851 (C-H, CH_2-as and -s), 1721 (C=O, acid), 1644 (C=O, amide); ^1H NMR (250 MHz, DMSO-d_6) δ 1.2 (m, 12H, CH_2-3′−8′), 2.0 (m, 4H, CH_2-2′ and -9′), 2.9 (m, 2H, CH_2-3), 4.4 (m, 1H, CH-2), 5.0 (m, 2H, CH_2=-10′), 5.8 (m, 1H, CH=-11′), 7.3 (s, 5H, Ar), 8.1 (d, 1H, NH, J = 8 Hz), 10.5 (s, 1H, CO_2H, exch); MS (FAB$^+$, NBA) *m/z* 332 [M + H]$^+$, 354 [M + Na]$^+$, 166 [M + H − 166(ketene)]$^+$, 120 (iminium ion). Anal. ($C_{20}H_{29}NO_3$) C, H, N.

Sodium Salt 5m: IR (KBr, ν, cm^{-1}) 3325 (N-H), 3072 (Ar-H), 3030 (=C-H), 2927−2854 (C-H, CH_2-as and -s), 1632 (C=O, amide), 1598 (CO_2^-); ^{13}C NMR (50.32 MHz, D_2O) δ 26.57 (C-3′), 29.3−30.2 (C-4′−C-8′), 34.55 (C-9′), 36.88 (C-2′), 38.51 (C-3), 56.59 (C-2), 115.0 (C-11′), 126.9 (C-7 Ar), 128.9 (C-6-8 Ar), 129.9 (C-5-9 Ar), 138.9 (C-4 Ar), 139.5 (C-10′), 175.8 (C-1), 179.2 (C-1′); MS (FAB$^-$, TG) *m/z* 330 [A]$^-$, 352 [A − H + Na]$^-$, 683 [2A + Na]$^-$.

***N*-Undec-10′-enoyl-L-valine (6):** method B; yield 84%; mp 92−93 °C; R_f 0.7 (90/10 $CHCl_3$/MeOH); $[\alpha]^{25}_D = -42.4°$ (MeOH, c = 0.1 M); IR (KBr, ν, cm^{-1}) 3321 (N-H), 3070 (=C-H), 2921−2850 (C-H, CH_2-as and -s), 1725 (C=O, acid) 1648 (C=O, amide); ^1H NMR (250 MHz, $CDCl_3$) δ 1.0 (d, 6H, $2CH_3$-4), 1.3 (m, 10H, CH_2-4′−8′), 1.6 (m, 2H, CH_2-3′), 2.0 (m, 2H, CH_2-9′), 2.2 (m, 1H, CH-3), 2.3 (t, 2H, CH_2-2′), 4.6 (q, 1H, CH-2), 5.0 (m, 2H, CH_2=-11′), 5.8 (m, 1H, CH=-10′), 6.1 (d, NH, J = 8 Hz), 10.7 (s, 1H, CO_2H, exch); MS (FAB$^+$, NBA) *m/z* 284 [M + H]$^+$, 266 [M + H − H_2O]$^+$, 238 [266 − CO]$^+$, 72 (iminium ion). Anal. ($C_{16}H_{28}NO_3$) C, H, N.

Sodium Salt 6m: IR (KBr, ν, cm^{-1}) 3350 (N-H), 3070 (=C-H), 1650 (C=O, amide), 1597 (CO_2^-); ^{13}C NMR (50.32 MHz, D_2O) δ 17.7 and 18.98 ($2CH_3$-4), 25.77 (C-3′), 28.9−29.3 (C-4′−8′), 31.08 (C-3), 33.79 (C-9′), 57.07 (C-2), 114.2 (C-11′), 139.1 (C-10′), 174.3 (C-1), 175.3 (C-1′); MS (FAB$^-$, TG) *m/z* 282 [A]$^-$, 304 [A + Na − H]$^-$, 587 [2A + Na]$^-$.

***N*-Undec-10′-enoyl-L-leucine (7):** method B; yield 64%; mp 99−101 °C; R_f 0.4 (90/10 $CHCl_3$/MeOH); $[\alpha]^{25}_D$ = +14.8° (MeOH, c = 0.1 M); IR (KBr, ν, cm^{-1}) 3320 (N-H), 3070 (=C-H), 2919−2848 (C-H, CH_2-as and -s), 1724 (C=O, acid), 1648 (C=O, amide); ^1H NMR (250 MHz, DMSO-d_6) δ 0.9 (q, 6H, $2CH_3$-5), 1.4 (m, 12H, CH_2-3′−8′), 1.5 (m, 2H, CH_2-3), 1.6 (m, 1H, CH-4), 2.0 (m, 2H, CH_2-9′), 2.1 (t, 2H, CH_2-2′), 4.2 (m, 1H, CH-2), 5.0 (m, 2H, CH_2=-11′), 5.8 (m, 1H, CH=-10′), 8.0 (d, NH, J = 7 Hz); MS (FAB$^+$, NBA) *m/z* 298 [M + H]$^+$, 320 [M + Na]$^+$, 595 [2M + H]$^+$, 252 [M + H − 46]$^+$, 96 (iminium ion). Anal. ($C_{17}H_{30}NO_3$) C, H, N.

Sodium Salt 7m: IR (KBr, ν, cm^{-1}) 3330 (N-H), 3050 (=C-H), 2932−2852 (C-H, CH_2-as and -s), 1619 (C=O, amide), 1596 (CO_2^-); ^{13}C NMR (50.32 MHz, D_2O) δ 21.67 and 23.86 ($2CH_3$-5), 25.65 (C-3′), 26.71 (C-3), 29.6−30.16 (C-4′−8′), 34.38 (C-9′), 36.93 (C-2′), 41.62 (C-4), 54.17 (C-2), 114.9 (C-11′), 139.5 (C-10′), 176.2 (C-1′), 180.9 (C-1); MS (FAB$^-$, TG) *m/z* 296 [A]$^-$, 318 [A + Na − H]$^-$, 615 [2A + Na]$^-$.

***N*-Undec-10′-enoyl-L-isoleucine (8):** method B; yield 77%; mp 100−101 °C; R_f 0.40 (90/10 $CHCl_3$/MeOH); $[\alpha]^{25}_D$ = +3.37° (MeOH, c = 0.1 M); IR (KBr, ν, cm^{-1}) 3320 (N-H), 3070 (=C-H), 2920−2849 (C-H, CH_2-as and -s), 1720 (C=O, acid), 1648 (C=O, amide); ^1H NMR (250 MHz, DMSO-d_6) δ 0.9 (q, 6H, $2CH_3$-5), 1.4 (m, 12H, CH_2-3′−8′), 1.5 (m, 2H, CH_2-3), 1.6 (m, 1H, CH-4), 2.0 (m, 2H, CH_2-9′), 2.1 (t, 2H, CH_2-2′), 4.2 (m, 1H, CH-2), 5.0 (m, 2H, CH_2=-11′), 5.8 (m, 1H, CH=-10′), 8.0 (d, 1H, NH, J = 7 Hz); MS (FAB$^+$, NBA) *m/z* 298 [M + H]$^+$, 132 [M + H − 166(ketene)], 86 (iminium ion). Anal. ($C_{17}H_{30}NO_3$) C, H, N.

Sodium Salt 8m: IR (KBr, ν, cm^{-1}) 3340 (N-H), 3060 (=C-H), 2922−2852 (C-H, CH_2-as and -s), 1640 (C=O, amide); ^{13}C NMR (50.32 MHz, D_2O) δ 11.9 (CH_3-5), 16.5 (CH_3-6), 25.3 (C-4), 26.8 (C-3′), 29.6−30 (C-4′−8′), 34.38 (C-9′), 36.9 (C-2′), 37.9 (C-3), 60.3 (C-2), 114.8 (C-11′), 139.4 (C-10′), 176.1 (C-1′), 179.3 (C-1); MS (FAB$^-$, TG) *m/z* 296 [A]$^-$, 318 [A + Na − H]$^-$, 615 [2A + Na]$^-$.

***N*-Undec-10′-enoyl-L-tyrosine (9):** method B; yield 46%; mp 95−97 °C; R_f 0.27 (90/10 $CHCl_3$/MeOH); $[\alpha]^{25}_D$ = +20.75° (MeOH, c = 0.1 M); IR (KBr, ν, cm^{-1}) 3420 (O-H), 3321 (N-H), 3080 (=C-H), 2927−2852 (C-H, CH_2-as and -s), 1715 (C=O, acid), 1642 (C=O, amide); ^1H NMR (250 MHz, CD_3OD) δ 1.4 (m, 12H, CH_2-3′−8′), 2.1 (m, 2H, CH_2-9′), 2.2 (t, 2H, CH_2-2′, J = 7.3 Hz), 3.0 (2q, 2H, CH_2-3, AMX), 4.6 (m, 1H, CH-2), 5.0 (m, 2H, CH_2=-11′), 5.1 (s, 1H, OH), 5.9 (m, 1H, CH=-10′), 6.7 (d, 2H, Ar-ortho), 7.1 (d, 2H, Ar-meta); MS (FAB$^+$, GT) *m/z* 348 [M + H]$^+$, 182 [M + H − 166(ketene)]$^+$, 136 (iminium ion); 93 (phenonium ion). Anal. ($C_{20}H_{29}NO_4$) C, H, N.

Sodium Salt 9m: IR (KBr, ν, cm^{-1}) 3408 (O-H), 3351 (N-H), 3080 (Ar-H), 3032 (=C-H), 2924−2853 (C-H, CH_2-as and -s), 1590 (CO_2^-); ^{13}C NMR (50.32 MHz, D_2O) δ 26.35 (C-3′), 29.48−29.94 (C-4′−8′), 34.35 (C-9′) 36.87 (C-2′), 38.21 (C-3), 56.91 (C-2), 114.9 (C-11′), 116.3 (C-1″ Ar), 129.3 (C-2″ and -6″ Ar), 131.1 (C-3″ and -5″ Ar), 139.8 (C-10′), 156.1 (C-4″ Ar), 176.0 (C-1′), 179.1 (C-1); MS (FAB$^-$, TG) *m/z* 346 [A]$^-$, 368 [A + Na − H]$^-$, 715 [2A + Na]$^-$.

***N*-Undec-10′-enoyl-L-serine (10):** method B; yield 87%; mp 68−69 °C; R_f 0.30 (90/10 $CHCl_3$/MeOH); $[\alpha]^{25}_D$ = +9.96° (MeOH, c = 0.1 M); IR (KBr, ν, cm^{-1}) 3417 (O-H), 3315 (N-H), 3070 (=C-H), 2927−2854 (C-H, CH_2-as and -s), 1714 (C=O, acid), 1630 (C=O, amide); ^1H NMR (90 MHz, CD_3OD) δ 1.3 (m, 12H, CH_2-3′−8′), 2.1 (m, 2H, CH_2-9′), 2.3 (t, 2H, CH_2-2′, J = 8.4 Hz), 3.9 (m, 2H, CH_2-3), 4.6 (t, 1H, CH-2), 4.9 (s, 1H, OH), 5.1 (m, 2H, CH_2=-11′), 6.0 (m, 1H, CH=-10′); MS (FAB$^+$, NBA) *m/z* 272 [M + H]$^+$, 294 [M + Na]$^+$ 543 [2M + H]$^+$, 254 [M + H − H_2O], 226 (254 − CO), 106 [M + H − 166(ketene)]$^+$. Anal. ($C_{14}H_{24}NO_4$) C, H, N.

Sodium Salt 10m: IR (KBr, ν, cm^{-1}) 3415 (O-H), 3348 (N-H), 3072 (=C-H), 2919−2849 (C-H, CH_2-as and -s), 1637 (C=O, amide), 1598 (CO_2^-); ^{13}C NMR (50.32 MHz, D_2O) δ 26.28 (C-3′), 29.45−29.93 (C-4′−8′), 34.32 (C-9′), 36.83 (C-2′), 57.69 (C-2), 63.23 (C-3), 114.9 (C-11′), 140.0 (C-10′), 176.7 (C-1′), 177.3 (C-1); MS (FAB$^-$, TG) *m/z* 270 [A]$^-$, 292 [A + Na − H]$^-$, 563 [2A + Na]$^-$.

***N*-Undec-10′-enoyl-L-methionine (11):** method B; yield 90%; mp 65−66 °C; R_f 0.24 (80/20 $CHCl_3$/MeOH); $[\alpha]^{25}_D$ = −7.62° (MeOH, c = 0.1 M); IR (KBr, ν, cm^{-1}) 3310 (N-H), 3073 (=C-H), 2924−2850 (C-H, CH_2-as and -s), 1721 (C=O, acid), 1648 (C=O, amide); ^1H NMR (90 MHz, $CDCl_3$) δ 1.4 (m, 12H, CH_2-3′−8′), 1.6 (m, 2H, CH_2-3), 2.1 (m, 2H, CH_2-9′), 2.2 (s, 3H, CH_3-S), 2.3 (t, 2H, CH_2-2′, J = 8 Hz), 2.6 (t, 2H, CH_2-4), 4.8 (m, 1H, CH-2), 5.1 (m, 2H, CH_2=-11′), 6.0 (m, 1H, CH=-10′), 6.5 (d, 1H, NH, J = 8 Hz); MS (FAB$^+$, NBA) *m/z* 316 [M + H]$^+$, 338 [M + Na]$^+$, 631 [2M + H]$^+$, 268 [M + H − $HSCH_3$]$^+$, 150 [M + H − 166(ketene)]$^+$, 104 (iminium ion). Anal. ($C_{16}H_{29}NO_2S$) C, H, N.

***N*-Undec-10′-enoyl-L-histidine (13).** To a suspension of L-histidine methyl ester dihydrochloride (5 g, 20.7 mM) in 50 mL of dry $CHCl_3$ were dropwise added at 0 °C TEA (5.8 mL) and then undec-10-enoyl chloride (**1**) (2.2 mL, 10.3 mM). After 20 min under stirring at 0 °C to the mixture in the same conditions were added new fractions of TEA (2.9 mL) and chloride **1** (2.2 mL). Stirring was maintained for 30 min at 0 °C and then for 2 h at room temperature. The mixture was

diluted with 150 mL of $CHCl_3$ and washed three times with water. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure ($\theta < 40$ °C). The residue was purified by chromatography on silica gel (95/5 $CHCl_3$/MeOH); 4.3 g of a white powder was obtained (yield 62%).

Ester **12** (12.5 mM), previously prepared, was dissolved in 25 mL of CH_3OH; 27.5 mL of aqueous 1 N NaOH was added at room temperature. Stirring was maintained for 6 h. CH_3OH was evaporated under reduced pressure, and the aqueous solution was acidified with 15 mL of aqueous 1 N HCl. After 12 h at 5 °C, the precipitate was filtered, washed with water, and then dried over P_2O_5; 3.14 g of the acid **13** was obtained as a white powder: yield 78%; mp 71−72 °C; R_f 0.20 (70/30 $CHCl_3$/MeOH); $[\alpha]^{25}_D = -1.86°$ (MeOH, $c = 0.1$ M); IR (KBr, ν, cm^{-1}) 3330 (N-H), 3072 (=C-H), 2923−2850 (C-H, CH_2-as and -s), 1724 (C=O, acid), 1625 (C=O, amide), 1560 (C=N, imidazole); ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.3 (m, 12H, CH_2-3′−8′), 2.1 (m, 4H, CH_2-2′ and -9′), 3.0 (m, 2H, CH_2-3), 4.5 (m, 1H, CH-2), 5.0 (m, 2H, CH_2=-11′), 5.8 (m, 1H, CH=-10′), 7.2 (s, 1H, CH-4 Im), 8.1 (s, 1H, CH-5 Im), 8.2 (d, 1H, NH, $J = 8$ Hz); MS (FAB$^+$, NBA) m/z 322 $[M + H]^+$, 344 $[M + Na]^+$, 156 [M + H − 166(ketene)], 110 (iminium ion). Anal. ($C_{17}H_{26}N_3O_3$) C, H, N.

Sodium Salt 13m: IR (KBr, ν, cm^{-1}) 3341 (N-H), 3077 (=C-H), 2924−2852 (C-H), 1639 (C=O, amide), 1580 (CO_2^-), 1563 (C=N, Im); ^{13}C NMR (50.32 MHz, D_2O) δ 26.27 (C-3′), 29.39−29.83 (C-4′−8′) 30.24 (C-3), 34.26 (C-9′), 36.77 (C-2′), 55.68 (C-2), 114.9 (C-11′), 118.2 (C-Im), 134.0−136.1 (2C-Im), 139.9 (C-10′), 176.2 (C-1′), 178.8 (C-1); MS (FAB$^-$, TG) m/z 320 $[A]^-$, 342 $[A + Na - H]^-$, 663 $[2A + Na]^-$.

***N*-Undec-10′-enoyl Dipeptides.** To a suspension of 15 mM L-glutamic acid dimethyl ester (or β-alanine methyl ester) in 120 mL of dry CH_2Cl_2 was first dropwise added diisopropylamine (1.1 equiv) to fix the pH value between 8 and 9. Hydroxybenzotriazole (15 mM) and *N*-undec-10′-enoyl-L-amino acid were added under stirring at 0 °C to the reaction mixture followed by the addition of dicyclohexylcarbodiimide (15 mM). Stirring was maintained for 1 h at 0 °C and then for 24 h at room temperature. After filtration to remove dicyclohexylurea, the filtrate was concentrated under reduced pressure. The obtained residue was dissolved in 200 mL of AcOEt, and the solution washed successively by aqueous 1 M HCl (50 mL × 3), H_2O (50 mL × 3), saturated aqueous $NaHCO_3$ (20 mL × 3), and H_2O (50 mL × 3). The organic phase was dried ($MgSO_4$), filtered, and concentrated. The crude obtained oil was purified by column silica gel chromatography (AcOEt).

Saponification: To a solution of *N*-acyl dipeptide methyl ester (10 mM) in 30 mL of CH_3OH was added aqueous 1 N NaOH (20 mL). Methanol was evaporated and the aqueous phase acidified at 0−5 °C by aqueous 5 N HCl (12 mL). The precipitate was filtered and purified by chromatography on silica gel (AcOEt). The *N*-acyl dipeptides were transformed to their corresponding sodium salts by addition under nitrogen at 0−5 °C of aqueous 0.5 N NaOH (1 equiv). The sodium salts were obtained after lyophilization as hygroscopic white powders.

***N*-Undec-10′-enoyl-L-prolyl-L-glutamic acid (14):** oil; R_f 0.18 (85/15 $CHCl_3$/MeOH); yield 60%; IR (CCl_4, ν, cm^{-1}) 3322 (N-H), 3072 (=C-H), 2929−2856 (C-H, CH_2-as and -s), 1758−1717 (C=O, acid), 1624 (C=O, amide); ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.3 (m, 12H, CH_2-3′−8′), 2.0 (m, 8H, CH_2-9′, Pro, Glu), 2.2 (m, 4H, CH_2-2′,Glu), 3.4 (m, 2H, CH_2-N, Pro), 4.2 (m, 2H, CH*-Pro, Glu), 5.0 (m, 2H, CH_2=-11′), 5.8 (m, 1H, CH=-10′), 8.2 (m, 1H, NH-Glu); MS (FAB$^+$, NBA) m/z 411 $[M + H]^+$, 433 $[M + Na]^+$, 455 $[M + 2Na - H]^+$, 236 $[M + H - 175]^+$, 70 (proline iminium ion). Anal. ($C_{21}H_{34}N_2O_6$) C, H, N.

Sodium Salt 14m: $[\alpha]^{25}_D = -20.3°$ (H_2O, $c = 0.1$ M); IR (KBr, ν, cm^{-1}) 3350 (N-H), 3050 (=C-H), 2956−2858 (C-H, CH_2-as and -s), 1630 (C=O, amide), 1598 (CO_2^-); ^{13}C NMR (50.32 MHz, D_2O) δ 25.12−25.51 (C-3′, CH_2-Glu, Pro), 29.0−29.96 (C-4′−8′), 30.40 (CH_2-Glu, Pro), 33.96 (C-9′), 35.09 (C-2′), 56.06 (CH*-Glu), 61.05 (CH*-Pro), 114.9 (C-11′), 140.7 (C-10′), 174.5−176.1 (CO-NH), 179.3 (CO_2^--γ), 183.0 (CO_2^--α); MS (FAB$^-$, TG) m/z 411 $[A^{2-} + H]^-$, 432 $[A^{2-} + Na]^-$, 455 $[A^{2-} + 2Na - H]^-$.

***N*-Undec-10′-enoyl-L-methionyl-L-glutamic acid (15):** mp 100−101 °C; R_f 0.15 (80/20 $CHCl_3$/MeOH); yield 71%; IR (KBr, ν, cm^{-1}) 3332 (N-H), 3070 (=C-H), 2926−2851 (C-H, CH_2-as and -s), 1726 (C=O, acid), 1643 (C=O, amide); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.3 (m, 10H, CH_2-4′−8′), 1.6 (m, 2H, CH_2-3′), 2.0 (m, 4H, CH_2-CH*), 2.1 (s, 3H, CH_3-S), 2.2 (m, 2H, CH_2-9′), 2.4 (m, 2H, CH_2-2′), 2.6 (m, 2H, CH_2-γGlu), 3.5 (q, 2H, CH_2-S, $J = 14$ Hz), 4.6 (m, 2H, 2CH*, Met, Glu), 5.0 (m, 2H, CH_2=-11′), 5.8 (m, 1H, CH=-10′), 6.3 (d, 1H, NH, exch, $J = 7.8$ Hz), 7.0 (d, 1H, NH, exch, $J = 7.7$ Hz); MS (FAB$^+$, NBA) m/z 445 $[M + H]^+$, 467 $[M + Na]^+$, 397 $[M + H - HSCH_3]^+$, 298 $[M + H - Glu]^+$, 104 (iminium ion). Anal. ($C_{21}H_{36}N_2O_6S$) C, H, N.

Sodium Salt 15m: IR (KBr, ν, cm^{-1}) 3340 (N-H), 3050 (=C-H), 2952−2856 (C-H, CH_2-as and -s), 1628 (C=O, amide), 1599 (CO_2^-); ^{13}C NMR (50.32 MHz, D_2O) δ 15.13 (CH_3-S), 26.19 (C-3′), 29.12−29.49 (C-4′−8′), 30.40−31.30 (CH_2-Glu, Met), 34.05 (C-9′), 36.45 (C-2′), 53.27 (CH*-Glu), 56.05 (CH*-Met), 114.9 (C-11′), 140.8 (C-10′), 173.6−177.8 (CO-NH), 179.1 (CO_2^--γ), 182.8 (CO_2^--α); MS (FAB$^-$, TG) m/z 443 $[A^{2-} + H]^-$, 465 $[A + Na]^-$, 487 $[A + 2Na - H]^-$.

***N*-Undec-10′-enoyl-L-phenylalanyl-β-alanine (16):** mp 113−114 °C; R_f 0.12 (80/20 $CHCl_3$/MeOH); yield 72%; IR (KBr, ν, cm^{-1}) 3310 (N-H), 3073 (C=-H), 2923−2847 (C-H, CH_2-as and -s), 1715 (C=O, acid), 1637 (C=O, amide); ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.3 (m, 12H, CH_2-3′−8′), 1.9 (m, 2H, CH_2-9′), 2.0 (m, 4H, CH_2-2′, CH_2-CO_2H, β-Ala), 2.3 (t, 2H, CH_2-N, β-Ala), 2.9 (m, 2H, CH_2-Ar), 4.4 (m, 1H, CH*), 5.0 (m, 2H, CH_2=-11′), 5.8 (m, 1H, CH=-10′), 7.2 (m, 5H, Ar), 8.1 (m, 2H, 2NH); MS (FAB$^+$, NBA) m/z 403 $[M + H]^+$, 425 $[M + Na]^+$, 447 $[M + 2Na - H]^+$, 805 $[2M + H]^+$, 314 $[M + H - \beta\text{-Ala}]^+$, 120 (Phe iminium ion). Anal. ($C_{23}H_{34}N_2O_4$) C, H, N.

Sodium Salt 16m: IR (KBr, ν, cm^{-1}) 3330 (N-H), 3060 (=C-H), 2956−2858 (C-H, CH_2-as and -s), 1630 (C=O, amide), 1595 (CO_2^-); ^{13}C NMR (50.32 MHz, D_2O) δ 26.52 (C-3′), 29.41−30.25 (C-4′−8′), 34.53 (C-9′), 36.7 (C-2′), 37.53−38.33 (CH_2, β-Ala), 55.46 (CH*), 114.9 (C-11′), 127.2−138.0 (CH-Ar), 139.5 (C-10′), 173.6−176.2 (CO-N), 180.8 (CO_2^-); MS (FAB$^-$, TG) m/z 401 $[A]^-$, 423 $[A + Na - H]^-$, 445 $[A + 2Na - 2H]^-$.

Polymerization. After γ-irradiation of a 0.1 M aqueous solution of the monomer, the polyanion obtained was purified by gel permeation chromatography on a Sephadex G-50 column to remove any trace of monomer. Distilled water (Elgastat, UHQ PS apparatus) was used as eluent (flow, 0.25 mL/min; column, 25 mm diameter, 500 mm length; 500 mg of sample). The polyanions were recovered by lyophilization of the corresponding aqueous fraction. Except for a slight signal broadening and the disappearance of vinylic protons, the ^{1}H NMR spectra of the polymers were identical with those of the analogous monomers.

Biological Methods. The flow cytometric methods used to demonstrate inhibition of virus adsorption to the cells and interactions of monoclonal antibodies with the viral glycoprotein gp120 and cellular CD4 receptor have been described previously.[26−28]

The different compounds were evaluated for their antiviral activity according to well-established procedures.[11,29,30] The origin of the viruses [herpes simplex type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK$^-$) HSV-1 strain B2006, herpes simplex type 2 (HSV-2) strain G, human cytomegalovirus (HCMV) strains AD169 and Davis, human immunodeficiency virus type 1 (HIV-1) strain HTLV-III$_B$/LAI, human immunodeficiency virus type 2 (HIV-2) strain LAV-2$_{ROD}$, vaccinia virus (VV), vesicular stomatitis virus (VSV), influenza virus A strain Ishikawa, influenza virus B strain Singapore, respiratory syncytial virus (RSV) strain Long, parainfluenza virus type 3, reovirus type 1, Junin virus, Tacaribe virus, Sindbis virus, Semliki forest virus, Coxsackie virus type 4, and poliovirus type 1] has been described previously.[29,30]

Cytotoxicity measurements were based on either microscopical examination of detectable alteration, normal cell morphology, or inhibition of cell growth. MT-4, Vero, HeLa, MDCK (Madin Darby canine kidney), human embryonic lung (HEL) fibroblasts, and human embryonic skin-muscle (E_6SM) fibroblasts were used for both the antiviral activity and the cytotoxicity assays.

The combined inhibitory effects on HIV-1-induced cytopathic effect (CPE) in MT-4 cells were examined by checkerboard combination of various concentrations of the test compounds. When the combination consisted of two effective compounds,

the combined effect was analyzed by the isobologram method, as previously described.[31] In this analysis, the 50% effective concentration (EC_{50}), which is the concentration of compound required to protect 50% of MT-4 cells against HIV-1-induced CPE, was used for calculation of the fractional inhibitory concentration (FIC). When the minimum FIC index, which corresponds to the FIC of compounds combined (e.g., $FIC_x + FIC_y$), is equal to 1.0, the combination is additive; when it is between 1.0 and 0.5, the combination is subsynergistic; when it is <0.5, it is synergistic. On the other hand, when the minimum FIC index is between 1.0 and 2.0, the combination is subantagonistic, and when it is >2.0, the combination is antagonistic.[32] To assess the inhibitory effects of the compounds on giant cell formation, MOLT-4 (clone 8) cells (1.8×10^6 cells mL^{-1}) were cultured with persistently HIV-1- or HIV-2-infected HUT-78 cells (2×10^5 cells mL^{-1}) in microtiter tray wells containing various concentrations of the test compounds. After a 24 h cocultivation period, the number of giant cells (syncytia) was recorded microscopically, as described previously.[33]

In the time of addition experiments, compounds **2p**, **4p**, **6p**, **8p**, dextran sulfate (DS5000), or AZT was added to MT-4 cells infected with HIV-1_{IIIB} (MOI = 1) at various times postinfection. Test compounds were added at a concentration that was 100-fold higher than the IC_{50} for each drug in the standard assay using MT-4 cells and the IIIB strain of HIV-1 (Table 2). Viral antigen (p24) production was measured at 24 h postinfection.

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