Polyanion Inhibitors of Human Immunodeficiency Virus and Other Viruses. 1. Polymerized Anionic Surfactants†

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A series of polyanionic compounds was synthesized and evaluated for their activity against human immunodeficiency virus (HIV-1, HIV-2) and various other RNA and DNA viruses. Several compounds, i.e., **2p, 3p, 8p, 13p, 14p, 15p, 17p, 18p**, and **19p**, proved active against HIV-1 within the concentration range of 0.1–3 μg/mL while not being toxic to the host cells (CEM, MT-4) at concentrations up to 100 μg/mL or higher. As a rule, these polyanionic compounds proved also active, albeit at somewhat higher concentrations than those required for HIV-1 inhibition, against a number of other enveloped viruses, including HIV-2, human cytomegalovirus, influenza A virus, respiratory syncytial virus, and arenaviruses (Junin and Tacaribe). Among the most potent HIV-1 inhibitors ranked compounds **18p** and **19p**, the sodium salts of *N*-methylamides obtained by polymerization of monomers prepared starting from 10-undecenoyl chloride and ω-aminoalkanoic acids.

Introduction

The AIDS (acquired immune deficiency syndrome) epidemic has resulted in continuous and intensive efforts to find effective chemotherapeutic agents against the human immunodeficiency virus (HIV), the causative agent of the disease. Of the various steps in the replicative cycle of HIV that could be considered as adequate targets for chemotherapeutic intervention, most of the work performed has been directed toward reverse transcriptase and protease.[1-3] The inhibitory effects of polyanionic molecules on the replication of herpes simplex virus (HSV) and other viruses were reported 30 years ago, but this observation did not give rise to further development until the suggestion by De Clercq in 1986 that polyanions can act as potent anti HIV-1 agents.[4,6] It is now established that sulfated polysaccharides such as heparin,[7,8] dextran sulfate,[7-10] pentosan polysulfate,[11] or lentinan sulfate[12] are potent in vitro inhibitors of HIV replication.

In 1990, Baba et al. showed that sulfated polyvinyl alcohol and sulfated copolymers of acrylic acid with vinyl alcohol display potent anti-HIV activity in cell culture.[13] More recently, the same team reported the anti-HIV activity of various salts of aurintricarboxylic acid (ATA). Interestingly, analogues of ATA polymers in which the carboxylic acid moiety is replaced by a sulfonic acid or phosphonic acid group were also found to prevent the cytopathic effect of HIV-1 and HIV-2 in MT-4 cells and HIV-1 in CEM cells.[14]

Although the results of the first clinical trials with dextran sulfate or pentosan polysulfate were disappointing,[15] polyanions may still prove clinically useful, for instance, if combined with other inhibitors of HIV replication[16,17] or if applied topically so as to facilitate their inhibitory effect on virus–cell binding. It should also be recognized that various polyanionic substances have been found to inhibit enveloped viruses other than HIV, such as herpes simplex virus (HSV), human cytomegalovirus (HCMV), vesicular stomatitis virus (VSV), influenza A virus, and respiratory syncytial virus (RSV).

In the course of work dealing with the improvement of the physicochemical properties of micelles,[18-20] we have synthesized a new class of polyanions via γ-ray-induced polymerization of unsaturated micelle-forming surfactants according to the general Scheme 1.

The anti-HIV activity detected in the case of such polyanions bearing a CO_2^- or a OSO_3^- group[21] prompted us to explore in more detail this new class of polyanionic compounds varying in particular the nature of the anionic group. We wish to disclose here the chemical synthesis of a set of 15 polyanions as well as the antiviral effects associated with these compounds.

Chemistry

According to the nature and the structure of the polar part, three classes of polyanionic compounds in this study can be distinguished. The first one originates from monomers of a C_{11} linear chain bearing at one extremity a carbon–carbon double bond and at the other end a monofunctional simple polar head (**1m**–**4m**).

The monomer **1m** was obtained by neutralization of the commercially available 11-undecenoic acid. As shown in Scheme 2, the reaction of undec-10-en-1-ol (**5m**) with sulfur trioxide pyridine complex, according

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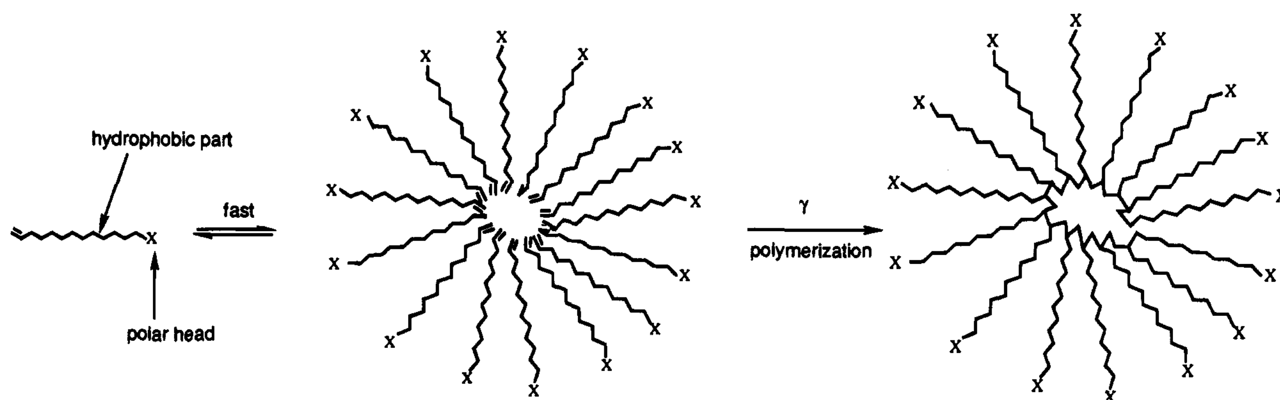
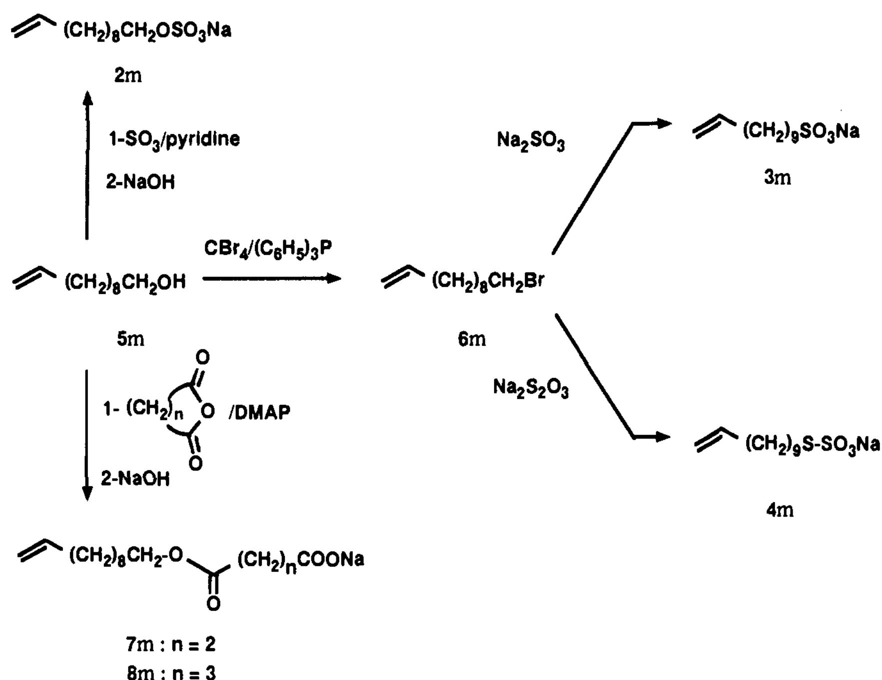
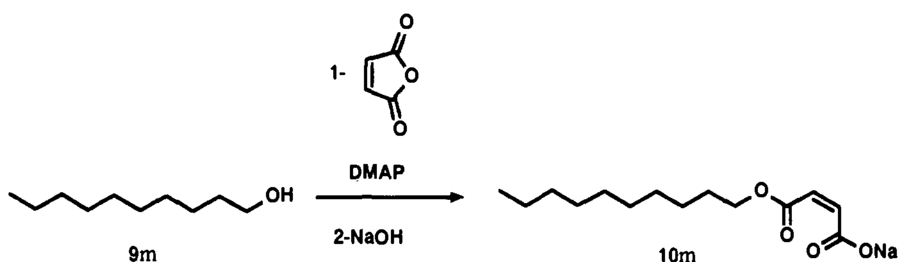
Scheme 1



Scheme 2



Scheme 3



to the already published procedure,[18] led, after sodium hydroxide neutralization, to the monomer **2m**. The smooth reaction of **5m** with tetrabromomethane in the presence of triphenylphosphine[22] led, in quantitative yield, to the bromo derivative **6m**.[23] The latter is the key intermediate in the synthesis of both the sulfonate **3m**[24] and the Bunte salt **4m**.[25,26]

Representatives of the second group are polyanions derived from monomers **7m**, **8m**, and **10m**. They all have in common an ester function within the chain together with a terminal carboxylate moiety.

Compounds **7m** and **8m** were prepared in quantitative yield by reacting undec-10-en-1-ol (**5m**) in the presence of DMAP with succinic or glutaric anhydride, respectively[27,28] (Scheme 2). In order to locate the polymerizable double bond in the vicinity of the polar head, compound **10m** was synthesized by reacting the alcohol **9m** with maleic anhydride in the presence of DMAP. Careful sodium hydroxide neutralization of the intermediate acid led to the corresponding sodium salt **10m**.

The third type of polyanions studied are the amide derivatives obtained from monomers **12m**–**19m** (Scheme 4). Amides **12m** and **13m** were obtained by reacting under Schotten–Baumann conditions[29,30] undec-10-enoyl chloride (**11**) respectively with glycine and *N*-methylglycine. The reaction of acid chloride **11** with β-alanine, the sodium salt of 2-aminomethansulfonic

Scheme 4

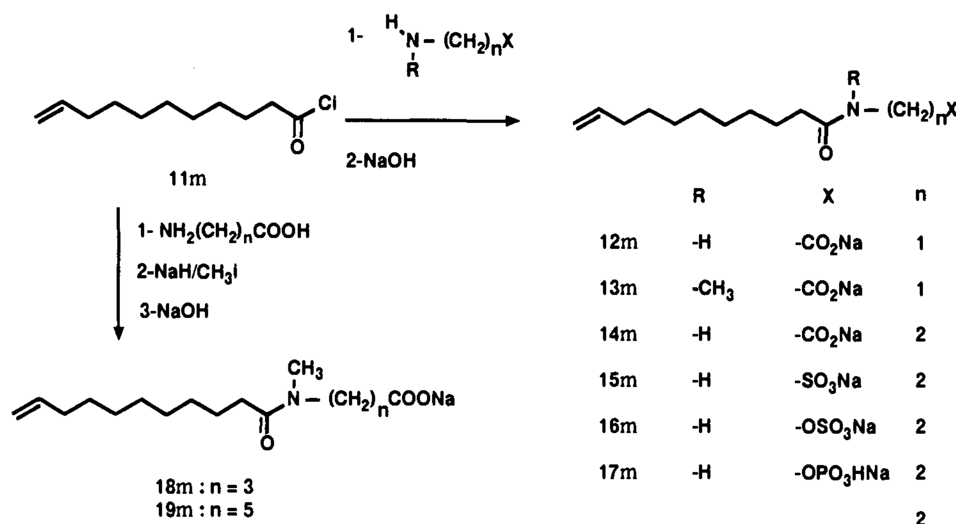


Table 1. Activities of the Polymers against HIV-1 in CEM-4 Cells[a]

X⁻ / Na⁺ → X⁻ / Na⁺

monomers (m) polymers (p)

compd	X⁻	CC_{50}[b] (μg/mL)	IC_{50} (μg/mL)[c] MTT	RT	SI[d] MTT	RT
1p	$-CO_2^-$	100	ND[e]	3.5	ND	30
2p	$-CH_2OSO_3^-$	50	ND	0.5	ND	100
3p	$-CH_2SO_3^-$	>100	ND	0.3	ND	>333
4p	$-CH_2S\text{-}SO_3^-$	>100	ND	2.0	ND	>50
7p	$-OCO(CH_2)_2CO_2^-$	>100	ND	0.75	ND	>133
8p	$-OCO(CH_2)_3CO_2^-$	>100	ND	0.6	ND	>150
10p[f]	$-OCOCH{=}CHCO_2^-$	>100	ND	50	ND	>2
12p	$-CONHCH_2CO_2^-$	>100	ND	0.5	ND	>200
13p	$-CON(CH_3)CH_2CO_2^-$	>100	0.8	0.8	>125	>125
14p	$-CONH(CH_2)_2CO_2^-$	770	0.1	0.4	7700	1925
15p	$-CONH(CH_2)_2SO_3^-$	>100	0.8	2	>125	>50
16p	$-CONH(CH_2)_2OSO_3^-$	>100	11	8	>9	>12
17p	$-CONH(CH_2)_2OPO_3H^-$	>100	ND	>100	ND	ND
18p	$-CON(CH_3)(CH_2)_3CO_2^-$	>50	0.37	0.1	>135	>500
19p	$-CON(CH_3)(CH_2)_5CO_2^-$	>50	0.15	0.1	>333	>500

[a] All data represent the average value of at least two separate experiments. [b] 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. [c] 50% inhibitory concentration or compound concentration required to inhibit by 50% HIV-induced cytopathicity [based on the MTT assay or reverse transcriptase (RT) activity]. [d] SI: selectivity index or ratio of CC_{50} to IC_{50}. [e] ND: not determined. [f] This compound does not correspond to the general structure. The hydrocarbon chain is saturated, and the polymerization occurs near the anionic group.

acid (taurine), the sodium salt of 2-aminoethane sulfate, or 2-aminoethanephosphoric acid (*O*-phosphocolamine) led to molecules **14m–17m** with nonoptimized yields ranging from 41% to 82%. As the sodium salt of 4-aminobutanoic and 6-aminohexanoic acid derivatives were found to be poorly soluble in water, we converted them into the more soluble tertiary amides **18m** and **19m** by action of sodium hydride and methyl iodide.

Biological Activity

First of all, it is noteworthy that none of the tested monomers exhibit an antiviral activity (data not shown), while most of the polymers are active. As shown in Table 1, the polyanions were nontoxic for CEM-4 cells at concentrations up to 50–100 μg/mL or higher. Compound **14p** was even nontoxic at a concentration up to 770 μg/mL.

The simplest anionic compounds (**1p–4p**) displayed various patterns of antiviral activity against HIV-1 in CEM-4 cells. The highest activities were observed with a sulfate or sulfonate group terminating the lateral chain (**2p**, **3p**). The presence of a terminal thiosulfate or carboxylate moiety (**1p**, **4p**) led to a 10-fold loss in anti-HIV activity in CEM cells. The introduction of a glutaric or succinic acid moiety at the end of the chain (**7p**, **8p**) restored the anti-HIV-1 activity to the level observed with **2p** and **3p**. As demonstrated by **12p** and **14p**, the ester function as present in compounds **7p** and **8p** can be replaced by a secondary amide group without significant change of anti-HIV-1 activity.

To evaluate the influence of the nature of the terminal anionic group on the anti-HIV-1 activity, polymers **15p–17p** were tested. Compared to molecule **14p**, the presence of a sulfonate (**15p**) or sulfate (**16p**) group diminished anti-HIV-1 activity. Furthermore, a complete loss of activity was observed when the carboxylic acid function is replaced by a monohydrogenophosphate group (**17p**).

Whereas the sarcosine derivative **13p** was equipotent compared to the glycine derivative **12p**, increasing the

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

compd	IC_{50}[a] (μg/mL)		CC_{50}[b] (μg/mL)	SI[c]	
	HIV-1	HIV-2		HIV-1	HIV-2
1p	3.6	13.4	130	36	10
2p	0.9	2.4	180	200	75
3p	2.6	11.1	>250	>96	>23
4p	ND[d]	ND	ND	ND	ND
7p	ND	ND	ND	ND	ND
8p	1.3	4.6	>250	>192	>54
10p	ND	ND	ND	ND	ND
12p	ND	ND	ND	ND	ND
13p	2.8	12.8	150	54	12
14p	0.2	7.2	180	900	25
15p	0.2	6.9	>250	>1250	>36
16p	90	214	>250	>3	>1.2
17p	2.7	19.2	>250	>93	>13
18p	0.6	4.2	>250	>417	>60
19p	0.8	4.5	100	125	22
dextran sulfate	0.1	0.08	>250	>2500	>3125

[a] 50% inhibitory concentration or compound concentration required to inhibit HIV-induced cytopathicity in MT-4 cells by 50%. [b] 50% cytotoxic concentration or compound concentration required to reduce the viability of mock-infected MT-4 cells by 50%. [c] Selectivity index: ratio of CC_{50} to IC_{50}. [d] Not determined. All data represent mean values of two separate experiments.

distance between the internal amide and the terminal carboxylic acid function by two or four methylene groups led to a significant increase of the antiviral activity leading for both compounds **18p** and **19p** to an IC_{50} value of 0.1 μg/mL. Although this structural modification led to a slight increase of the cytotoxicity, these polymers achieved a selectivity index greater than 500.

For compounds **13p**–**18p**, the IC_{50} values based on MTT assays were similar to those based on RT measurements, which points to a close correlation between their potency to inhibit the cytopathicity of the virus and to inhibit viral production.

The compounds were also evaluated against a wide variety of DNA and RNA viruses in five different cell lines (E_6SM, HEL, HeLa, MDCK, and Vero) and against HIV in the T4-lymphocytic cell line MT-4 (Tables 2 and 3). Dextran sulfate was evaluated in parallel for comparison. In addition, cytotoxicity of the different compounds was determined for all the cell lines used (Table 4).

The simplest anionic derivative tested (**1p**, **2p**, **3p**) proved markedly inhibitory to HIV-1 and HIV-2: their 50% inhibitory concentration (IC_{50}) fell within the range of 0.9–3.6 and 2.4–13.4 μg/mL for HIV-1 and HIV-2, respectively (Table 2). Compound **2p**, containing a sulfate group in the lateral chain, was the most active among these molecules. However, its IC_{50} value was 9- and 30-fold higher than that of dextran sulfate for HIV-1 and HIV-2, respectively.

The anti-HIV activity of compound **7p**, containing a terminal succinic acid moiety in the chain, was comparable to that of compounds **1p**, **2p**, and **3p**. The anti-HIV activity was maintained if the ester function present in compound **7p** was replaced by a secondary amide group (compound **14p**).

As for CEM-4 cells, a significant loss of activity was observed when the carboxylate moiety (**14p**) was replaced by a sulfate group (**16p**), which contrasts to what was observed with compound **2p** relative to **3p**. The presence of a carboxylate (**14p**) or sulfonate group (**15p**) resulted in an equally optimal activity against HIV, while the presence of a terminal phosphate group (**17p**) weakened the antiviral activity.

N-Methylation of the amide moiety, as in **13p**, **18p**, and **19p**, was compatible with anti-HIV activity; extension of the side chain by two (**18p**) or four (**19p**) methylene groups led to a slight increase in anti-HIV activity, as compared to the sarcosine derivative **13p**. These structural modifications only slightly altered [increased (**19p**) or decreased (**18p**)] the cytotoxicity for MT-4 cells.

Using a flow cytometric method,[31] it was found that compounds **18p** and **19p** achieved an equally strong inhibition of HIV-1 binding to MT-4 cells as did dextran sulfate (data not shown). Also, when the compounds were examined for their interaction with the binding of monoclonal antibody to gp120 to persistently HIV-infected cells, expressing the viral gp120 that is involved in virus–cell binding,[32] they exerted a comparable inhibitory effect on the binding of the anti-gp120 mAb (data not shown).

As shown in Table 2, all the compounds exhibited a similar toxicity for MT-4 cells with CC_{50} values in the range of 100–250 μg/mL, except for **3p**, **8p**, **15p**, **16p**, **17p**, and **18p**, which showed no toxicity at the highest concentration tested (250 μg/mL). As a rule, HIV-1 proved more sensitive to the inhibitory effects of the different polyanions than HIV-2.

In contrast to dextran sulfate, when evaluated for their inhibitory effects on the replication of herpes viruses, none of the tested polyanions proved inhibitory to herpes simplex virus type 1 (HSV-1) or thymidine kinase-deficient (TK^-) HSV-1 or HSV-2. However, several polyanions (**1p**, **8p**, **13p**, **14p**, **15p**, **18p**, and **19p**) were found to inhibit the replication of human cytomegalovirus (HCMV) (Table 3) at concentrations that were not toxic to the host cells (Table 4). No activity was noted with any of the polyanions tested against vaccinia virus.

When evaluated for their inhibitory effects on the replication of RNA viruses other than HIV, some of the polyanions were found to be active against influenza A virus (**1p**, **8p**, **13p**, **17p**, and **19p**), respiratory syncytial virus (RSV) (**2p**, **3p**, **14p**, and **15p**), and the arenaviruses Junin and Tacaribe (**8p**, **13p**, **14p**, **15p**, and **18p**). 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 tested polyanions. No activity was noted with any of the compounds against Semliki forest virus, influenza B virus, parainfluenza virus type 3, and the nonenveloped viruses Coxsackie type B4, polio type 1, and reovirus type 1.

Conclusion

The series of polyanions described here is characterized by their potent antiviral activity against enveloped viruses such as HIV or CMV, based on inhibition of virus entry process into the target cells. This had led to the concept that these classes of anionic compounds could be used topically for the prevention of sexually transmitted viral diseases. Anionic derivatives in combination with other antivirals and spermicides could be applied onto the vaginal mucosa before sexual intercourse in order to prevent HIV transmission.

Table 3. Inhibitory Effect of Several Polyanions on the Replication of DNA and RNA Viruses

		IC_{50}[a] (μg/mL)											
virus	cell line	dextran sulfate	**1p**	**2p**	**3p**	**8p**	**13p**	**14p**	**15p**	**16p**	**17p**	**18p**	**19p**
HSV-1 (KOS)	E_6SM	2	>40	>10	>40	>10	>50	>40	70	>200	>10	>50	>50
HSV-2 (G)	E_6SM	2	>40	>10	>40	>10	>50	>40	70	>200	>10	>50	40
HSV-1 TK^- (B2006)	E_6SM	2	>40	>10	>40	>10	>50	>40	70	>200	>10	>50	>50
HCMV (AD169)	HEL	0.6	11	>1	>20	2.2	8.1	1.1	0.8	>20	>20	5.0	5.6
HCMV (Davis)	HEL	0.4	8	>1	>20	1.1	5.6	0.5	0.5	>20	>20	0.9	1.1
vaccinia	E_6SM	100	>40	>10	>40	>10	>50	>40	>200	>200	>10	>50	>50
vesicular stomatitis	E_6SM	2	>40	>10	>40	>10	>50	>40	300	>200	>10	>50	>50
influenza A	MDCK	8	20	>20	100	20	30	>100	>100	>200	4	60	20
influenza B	MDCK	>200	100	>20	100	100	>100	>100	>100	>200	>200	>100	>100
respiratory syncytial	HeLa	4	>20	8	20	20	>20	9	10	>200	150	>20	>20
parainfluenza-3	Vero	>400	>100	>40	>400	>40	>100	>40	>400	>400	>40	400	>400
reovirus-1	Vero	>400	>100	>40	>400	>40	>100	>40	>400	>400	>40	>400	>400
sindbis	Vero	40	>100	>40	>400	>40	>100	>40	>400	>400	>40	>400	>400
Semliki forest	Vero	>400	>100	>40	300	>40	>100	>40	>400	>400	>40	>400	>400
Junin	Vero	8	10	>4	>5	2.7	1.15	10	12	>20	>5	3.2	32
Tacaribe	Vero	7	>10	>4	>5	2.0	1.4	10	10	>20	>5	10	27
Coxsackie B4	HeLa	>400	150	>100	300	>100	200	300	>400	>400	>200	300	>200
polio-1	HeLa	>400	>200	>100	>400	>100	>200	>40	>400	>400	>200	200	>200

[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 4. Cytotoxicity of the Polyanions for Different Cell Lines

	MCC[a] (μg/mL)											
cell culture	dextran sulfate	**1p**	**2p**	**3p**	**8p**	**13p**	**14p**	**15p**	**16p**	**17p**	**18p**	**19p**
E_6SM	>400	100	40	100	40	>200	100	400	400	40	>200	200
HEL	>200	80	10	110	15	>200	25	20	>200	>200	>200	200
HeLa	>200	20	20	100	20	20	100	>200	>200	>200	20	20
MDCK	>200	200	20	100	100	100	100	100	100	>200	100	100
Vero	>200	40	10	20	50	50	40	50	50	20	200	200

[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%.

Experimental Protocols

Chemistry. Melting points were determined using a Buchi 530 apparatus and are uncorrected. Microanalysis were performed for most of the monomers. The observed deviations to the theoretical values were always less than 0.4%. Merck silica gel 60 F_{254} (0.25 mm) plates were employed for analytical TLC. ^1H and ^{13}C NMR were recorded on Bruker AC250 and WP200SY spectrometers, respectively. Mass spectra were recorded on a JEOL DX 100 spectrometer. Infrared spectra were obtained on a IR-FT BONEM MB-100 spectrometer. Undec-10-enoic acid and undec-10-enol were gratuitously provided by Atochem.

1-Bromoundec-10-ene (6m). To a suspension of undec-10-en-1-ol (10.1 mL, 50 mM), potassium carbonate (50.3 g, 5 equiv), and tetrabromomethane (33.2 g, 100 mM) at 0–5 °C in 200 mL of CH_2Cl_2 was added triphenylphosphine (26.3 g, 100 mM). The mixture was kept for 30 min at 0–5 °C and then filtered twice on a short silica gel column (hexane): yield 95%; bp 89–90 °C/2 mmHg (lit.[12] bp 117–8 °C/8 mmHg); R_f 0.57 (20/80 AcOEt/hexane, v/v); d_{15} 1.070; ^1H NMR (250 MHz, $CDCl_3$) δ 1.3 (m, 14H, H_2 to H_8), 1.7 (m, 2H, H_3), 2.0 (q, 2H, H_9, J = 7.5 Hz), 3.4 (t, 2H, H_1, J = 6.9 Hz), 4.9 (m, 2H, H_{11}, J_{trans} = 17 Hz, J_{cis} = 10 Hz), 5.7 (m, 1H, H_{10}, J_{trans} = 17 Hz, J_{cis} = 10 Hz, J = 7.5 Hz).

Sodium Undec-10-enethiosulfate (4m). To a solution of 1-bromoundec-10-ene (**6m**) (2.18 g, 9.4 mM) in 25 mL of 95% ethanol was added sodium thiosulfate (2.33 g, 9.4 mM) dissolved in a minimum of water. The mixture was refluxed for 2 h. The solvent was eliminated under reduced pressure, and the residue was recrystallized (ethanol:ether, 50:50); 1.036 g of white needles was isolated for **4m**: yield 38%; mp 103–105 °C; ^1H NMR (90 MHz, D_2O) δ 1.3 (m, 12H, H_3 to H_8), 1.6 (m, 2H, H_2), 2.0 (m, 2H, H_9, J_{9-10} = 6 Hz), 3.1 (t, 2H, H_1, J_{1-2} = 7 Hz), 4.9 (m, 1H, H_{11a}, J_{11a-10} = 10.5 Hz), 5.05 (m, 1H, H_{11b}, J_{10-11b} = 18 Hz), 5.8 (m, 1H, J_{10-11b} = 18 Hz, J_{10-11a} = 10.5 Hz, J = 6 Hz); MS (FAB^-, G) m/z 553 (2M − Na)$^-$, 265 (M − Na)$^-$. Anal. ($C_{11}H_{21}O_3S_2Na$) C, H.

Monomers Obtained from Cyclic Anhydrides. General Procedure. To a solution of the fatty alcohol (2 mM, 1 equiv) in 100 mL of dry dichloromethane were successively added the cyclic dicarboxylic anhydride (2.2 mM, 1.1 equiv), triethylamine (2.84 mL; 2 mM, 1 equiv), and 0.24 g of 4-*N,N*-(dimethylamino)pyridine (DMAP). The mixture, protected against moisture, was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, and the residual oil was dissolved in 200 mL of chloroform. This solution was washed three times with 60 mL of aqueous 1 N HCl and then four times with 50 mL of water. The organic phase was dried ($MgSO_4$), and chloroform was evaporated. The obtained oil was exactly neutralized under nitrogen at 5–10 °C by aqueous 1 N NaOH. A white powder was obtained after lyophilization.

Sodium 4-oxo-4-(undec-10′-enoxy)butanoate (7m): yield 60%; mp 105–107 °C, R_f 0.12 (acid) (40/60 AcOEt/hexane, v/v); IR (KBr, ν, cm^{-1}) 2924–2853 (C-H), 1730 (C=O, ester), 1590 (C=O, CO_2^-); ^1H NMR (90 MHz, D_2O) δ 1.4 (m, 12H, CH_2, $H_{3'}$ to $H_{8'}$), 1.7 (m, 2H, $H_{2'}$), 1.8 (m, 2H, CH_2, $H_{9'}$), 2.3 (m, 4H, CH_2, H_2, H_3), 4.2 (t, 2H, CH_2, $H_{1'}$, $J_{1'-2'}$ = 5.5 Hz), 5.2 (m, 2H, CH_2, $H_{11'}$), 6.0 (m, 1H, CH, $H_{10'}$); ^{13}C NMR (50.32 MHz, D_2O) δ 26.6–34.47 ($C_{2'}$ to $C_{9'}$, $C_{2'}$, C_3), 65.8 ($C_{1'}$), 114.8 ($C_{11'}$), 139.4 ($C_{10'}$), 176.1 (C_4), 180.7 (C_1); MS (FAB^-, G) m/z 269 (M)$^-$, 168 (M − 152)$^-$. Anal. ($C_{15}H_{25}O_4Na$) C, H.

Sodium 5-oxo-5-(undec-10′-enoxy)pentanoate (8m): yield 51%; mp 161–163 °C, R_f 0.25 (acid) (40/60 AcOEt/hexane, v/v); IR (KBr, ν, cm^{-1}) 2929–2854 (C-H), 1731 (C=O, ester), 1582 (C=O, CO_2^-); ^1H NMR (90 MHz, D_2O) δ 1.4 (m, 16H, CH_2, H_3, $H_{2'}$ to $H_{8'}$), 1.9 (m, 2H, CH_2, $H_{9'}$), 2.2 (m, 4H, CH_2, H_2, H_4), 4.2 (t, 2H, CH_2, $H_{1'}$, $J_{1'-2'}$ = 5.5 Hz), 5.2 (m, 2H, CH_2, $H_{11'}$), 5.9 (m, 1H, CH, $H_{10'}$); ^{13}C NMR (50.32 MHz, D_2O) δ 22.21–37.51 ($C_{3'}$ to $C_{9'}$, C_2, C_3, C_4), 65.68 ($C_{1'}$), 114.9 ($C_{11'}$), 139.4 ($C_{10'}$), 176.3 (C_5), 182.5 (C_1); MS (FAB^-, G) m/z 283 (M)$^-$, 131 (M − 152)$^-$. Anal. ($C_{16}H_{27}O_4Na$) C, H.

Sodium 4-oxo-4-(decyloxy)but-2-enoate (10m): yield 50%; mp 135–137 °C; R_f 0.75 (EtOH/aqueous 20% NH_3/H_2O,

100/16/12, v/v); IR (KBr, ν, cm^{-1}) 2923–2852 (C-H), 1710 (C=O, ester), 1570 (C=O, CO_2^-); ^1H NMR (90 MHz, D_2O) δ 0.8 (t, 3H, CH_3, $H_{10'}$, $J_{10'-9'}$ = 5 Hz), 1.2 (m, 16H, CH_2, $H_{2'}$ to $H_{8'}$), 4.0 (t, 2H, CH_2, $H_{1'}$, $J_{1'-2'}$ = 5.5 Hz), 5.7 (d, 1H, H_3, J_{3-2} = 9.9 Hz), 6.5 (d, 1H, CH, H_2, J_{3-2} = 9.9 Hz); MS (FAB⁻, G) m/z 255 (M)⁻, 211 (M − 44)⁻. Anal. ($C_{14}H_{23}O_4Na$) C, H.

Sodium (*N*-Undec-10′-enoylamino)alcanoates. General Procedure. The amino acid (35 mM, 1 equiv) was dissolved under nitrogen in a three-necked round-bottomed flask in 18 mL of aqueous 2 N NaOH. The solution was magnetically stirred for 15 min; then undec-10-enoyl chloride (**11m**) (9.03 mL, 42 mM, 1.2 equiv) was added dropwise simultaneously with 21 mL of aqueous 2 N NaOH. The mixture was magnetically stirred at room temperature for 30 min and then acidified to pH 1 by adding aqueous 1 N HCl and extracted three times with 100 mL of dichloromethane. The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The obtained white solid was washed with hexane, and then exactly neutralized under nitrogen at 5–10 °C by aqueous 1 N NaOH. The sodium salt was isolated as a white powder after lyophilization.

Sodium 2-(*N*-undec-10′-enoylamino)ethanoate (12m): yield 41%, mp >200 °C; R_f 0.82 (EtOH/aqueous 20% NH_3/H_2O, 60/35/5, v.v); IR (KBr, ν, cm^{-1}) 3400 (N-H), 2926–2853 (C-H), 1570–1407 (C=O, CO_2^-); ^1H NMR (90 MHz, D_2O) δ 1.2 (m, 12H, CH_2, $H_{3'}$ to $H_{8'}$), 1.8 (m, 2H, $H_{9'}$), 2.2 (t, 2H, CH_2, $H_{2'}$, $J_{2'-3'}$ = 8 Hz), 3.7 (s, 2H, CH_2, H_2), 5.0 (m, 2H, CH_2, $H_{11'}$), 5.8 (m, 1H, CH, $H_{10'}$); MS (FAB⁻,G) m/z 503 (2M − 2H + Na)⁻, 354 (M − 2H + G + Na)⁻, 240 (M − H)⁻. Anal. ($C_{13}H_{22}NO_3Na$) C, H, N.

Sodium 2-(*N*-methyl-*N*-undec-10′-enoylamino)ethanoate (13m): yield 81%; mp 63–65 °C, R_f 0.6 (EtOH/aqueous 20% NH_3/H_2O, 100/16/12, v/v); IR (KBr, ν, cm^{-1}) 2927–2850 (C-H), 1630 (C=O, amide), 1560 (C=O, CO_2^-); ^1H NMR (250 MHz, D_2O) δ 1.2 (m, 10 H, $H_{4'}$ to $H_{8'}$), 1.6 (m, 2H, $H_{3'}$), 2.05 (q, 2H, $H_{9'}$, $J_{9'-10'}$ = 6.5 Hz), 2.2–2.5 (m, 2H, $H_{2'}$), 3.0 (2s, 3H, N-CH_3 syn and trans forms), 3.85 (d, 2H, H_2, syn and trans), 4.9 (m, 2H, $H_{11'}$, $J_{11b'-10'}$ = 18 Hz, $J_{11a'-10'}$ = 10.7 Hz), 5.8 (m, 1H, $H_{10'}$, $J_{10'-11a'}$ = 10.7 Hz, $J_{10'-11b'}$ = 18 Hz, $J_{10'-9'}$ = 6.5 Hz); ^{13}C NMR (50.32 MHz, D_2O) δ 25.8–37.5 ($C_{2'}$ to $C_{9'}$, N-CH_3), 52.7 and 55.1 (C_2, syn and trans), 114.9 ($C_{11'}$), 139.6 ($C_{10'}$), 176.3–177.1 ($C_{1'}$, C_1, syn and trans); MS (FAB⁻, G) m/z 531 (2M − 2H + Na)⁻, 254 (M − H)⁻. Anal. ($C_{14}H_{24}NO_3Na$) C, H, N.

Sodium 3-(*N*-undec-10′-enoylamino)propanoate (14m): yield 82%; mp 205–207 °C, R_f 0.46 (dioxane); IR (KBr, ν, cm^{-1}) 3400 (N-H), 2928–2854 (C-H), 1570–1407 (CO_2^-); ^1H NMR (250 MHz, D_2O) δ 1.1 (m, 12H, CH_2, $H_{3'}$ to $H_{8'}$), 1.9 (q, 2H, $H_{9'}$, $J_{9'-10'}$ = 6.8 Hz, $J_{9'-8'}$ = 6.8 Hz), 2.2 (t, 2H, $H_{2'}$, $J_{2'-3'}$ = 7.3 Hz), 2.2 (t, 2H, H_2, J_{3-2} = 7 Hz), 3.2 (2H, H_3, J_{3-2} = 7 Hz), 4.8 (m, 2H, $2H_{11'}$, $J_{11b'-10'}$ = 16.7 Hz, $J_{11a'-10'}$ = 10.4 Hz), 5.7 (m, 1H, $H_{10'}$, $J_{10'-11a'}$ = 10.4 Hz, $J_{10'-11b'}$ = 16.7 Hz, $J_{10'-9'}$ = 6.8 Hz); ^{13}C NMR (50.32 MHz, D_2O) δ 26.30 ($C_{3'}$), 29.31–29.72 ($C_{4'}$ to $C_{8'}$), 34.21 ($C_{9'}$), 36.78 ($C_{2'}$), 37.45 (C_2), 37.71 (C_3), 114.9 ($C_{11'}$), 140.3 ($C_{10'}$), 177.1 ($C_{1'}$), 180.9 (C_1); MS (FAB⁺, NBA) m/z 278 (M + Na)⁺, 256 (M − H)⁺, 185 (M − 71 + H)⁺. Anal. ($C_{14}H_{24}NO_3Na$) C, H, N.

Sodium 2-(*N*-undec-10′-enoylamino)ethanesulfonate (15m): yield 75%; mp 220–222 °C; R_f 0.56 (40/60 MeOH/$CHCl_3$, v/v); IR (KBr, ν, cm^{-1}) 3477–3414 (N-H), 2924–2852 (C-H), 1640 (C=O, amide), 1200 (S=O, sulfonate); ^1H NMR (90 MHz, D_2O) δ 1.2 (m, 12H, CH_2, $H_{3'}$ to $H_{8'}$), 1.9 (m, 2H, $H_{9'}$), 2.1 (m, 2H, $H_{2'}$), 2.9 (t, 2H, H_1, J_{1-2} = 6.7 Hz), 3.5 (t, 2H, H_2, J_{2-1} = 6.7 Hz), 4.9 (m, 2H, $H_{11'}$, $J_{11b'-10'}$ = 18 Hz, $J_{11a'-10'}$ = 10.5 Hz), 5.7 (m, 1H, $H_{10'}$, $J_{10'-11b'}$ = 18 Hz, $J_{10'-11a'}$ = 10.5 Hz); ^{13}C NMR (50.32 MHz, D_2O) δ 25.96 ($C_{3'}$), 28.99–29.34 ($C_{4'}$ to $C_{8'}$); 33.95 ($C_{9'}$), 35.67 (C_2), 36.51 ($C_{2'}$), 50.46 (C_1), 114.7 ($C_{11'}$), 140.5 ($C_{10'}$), 177.4 ($C_{1'}$); MS (FAB⁻, G) m/z 312 (M − H)⁻, 290 (M − Na)⁻, 603 (2M − Na)⁻. Anal. ($C_{13}H_{24}NO_4SNa$) C, H, N.

Sodium 2-(*N*-undec-10′-enoylamino)ethanesulfate (16m): yield 70%; mp 169–171 °C; R_f 0.6 (40/60 MeOH/$CHCl_3$, v/v); IR (KBr, ν, cm^{-1}) 2925–2854 (C-H), 1636 (C=O, amide) 1400 (S=O, sulfate); ^1H NMR (90 MHz, D_2O) δ 1.2 (m, 12H, $H_{3'}$ to $H_{8'}$), 1.9 (m, 2H, $H_{9'}$), 2.1 (m, 2H, $H_{2'}$), 3.4 (t, 2H, H_2, J_{2-1} = 5.2 Hz), 4.1 (t, 2H, H_1, J_{1-2} = 5.2 Hz), 5.0 (m, 2H, $H_{11'}$, $J_{11b'-10'}$ = 18 Hz, $J_{11a'-10'}$ = 9 Hz), 5.7 (m, 1H, $H_{10'}$, $J_{10'-11b'}$ = 18 Hz, $J_{10'-11a'}$ = 9 Hz); ^{13}C NMR (50.32 MHz, D_2O) δ 26.22 ($C_{3'}$) 29.05–29.38 ($C_{4'}$ to $C_{8'}$), 34.04 ($C_{9'}$), 36.66 ($C_{2'}$), 39.62 (C_2), 67.49 (C_1), 115.3 ($C_{11'}$), 140.9 ($C_{10'}$), 177.6 ($C_{1'}$); MS (FAB⁺, NBA) m/z 681 (2M − Na)⁺, 352 (M + Na)⁺, 330 (M + H)⁺, 308 (M − Na + 2H)⁺. Anal. ($C_{13}H_{24}NO_5SNa$) C, H, N.

[2-(*N*-Undec-10′-enoylamino)ethyl]phosphoric acid: yield 94%; mp 153–155 °C, R_f 0.36 (EtOH/aqueous 20% NH_3/H_2O, 100/16/12, v/v); IR (KBr, ν, cm^{-1}) 2923–2851 (C-H), 1636 (C=O, amide), 1076 (P-O-C); ^1H NMR (90 MHz, DMSO-d_6) δ 1.2 (m, 12H, $H_{3'}$ to $H_{8'}$), 2.0 (m, 4H, $H_{2'}$, $H_{9'}$), 3.2 (q, 2H, H_2, $J_{2\text{-NH}}$ = 6 Hz, J_{2-1} = 6 Hz), 3.8 (q, 2H, H_1, $J_{1\text{-P}}$ = 7.5 Hz, J_{1-2} = 6 Hz), 5.1 (m, 2H, $H_{11'}$), 5.8 (m, 1H, $H_{10'}$), 8.1 (t, 1H, N-H, $J_{2\text{-NH}}$ = 6 Hz), 9.9 (m, 2H, P(O-H)$_2$); ^{13}C NMR (50.32 MHz, DMSO-d_6) δ 25.12 ($C_{3'}$), 28.18–28.67 ($C_{4'}$ to $C_{8'}$), 33.1 ($C_{9'}$), 35.25 ($C_{2'}$), 39.1 (C_2), 63.1 (C_1), 114.5 ($C_{11'}$), 138.7 ($C_{10'}$), 172.2 ($C_{1'}$); MS (FAB⁻, NBA) m/z 613 (2M − H)⁻, 306 (M − H)⁻.

Sodium 2-(*N*-undec-10′-enoylamino)ethanephosphate (17m): mp 228–230 °C; ^1H NMR (250 MHz, D_2O) δ 1.24 (m, 12H, $H_{3'}$ to $H_{8'}$), 2.0 (q, 2H, $H_{9'}$, $J_{9'-8'}$ = 6.7 Hz, $J_{9'-10'}$ = 6.7 Hz), 2.2 (t, 2H, $H_{2'}$, $J_{2'-3'}$ = 7.5 Hz), 3.34 (t, 2H, H_2, J_{2-1} = 5.5 Hz), 3.79 (q, 2H, H_1, J_{2-1} = 5.5 Hz, $J_{1\text{-P}}$ = 6 Hz), 4.89 (m, 1H, $H_{11a'}$, $J_{11a'-10'}$ = 10.3 Hz, $J_{11a'-11b'}$ = 1.5 Hz), 4.96 (m, 1H, $H_{11b'}$, $J_{11b'-11a'}$ = 1.5 Hz, $J_{11b'-10'}$ = 17.1 Hz), 5.8 (m, 1H, $H_{10'}$, $J_{10'-9'}$ = 6,7 Hz, $J_{10'-11b'}$ = 17.1 Hz, $J_{10'-11a'}$ = 10.3 Hz). Anal. ($C_{13}H_{25}NO_5PNa$) C, H, N.

Sodium 4-(*N*-Methyl-*N*-undec-10′-enoylamino)butanoate (18m). This salt was obtained by N-methylation of the compound synthesized by condensation of the fatty acid chloride **11m** to the 4-aminopropanoic acid. To a suspension of sodium hydride (0.52 g, 13 mM) in 30 mL of dimethylformamide was added the amide (1.5 g, 5.2 mM) under nitrogen. After a 2 h sonication, methyl iodide (20.4 mM) was added dropwise at 0–5 °C. Sonication was maintained for 2 h; 10 mL of MeOH was added to destroy the hydride excess, and the solvent was removed under reduced pressure. The residue was dissolved in 100 mL of chloroform. The obtained solution was washed twice with 10 mL of water and then twice with 10 mL of aqueous 1 N HCl and finally three times with 10 mL of water. The elimination of chloroform under reduced pressure gave an oil which was purified by silica gel column chromatography ($CHCl_3$). The obtained oil was saponified by 4 mL of aqueous 4 N NaOH under sonication during 15 mn; 20 mL of water was added, and the pH value was adjusted at 8 with an acidic resin (Dowex 50W-2X). After filtration and lyophilization, 0.97 g of the salt **18m** (white powder) was isolated: yield 62%; mp 48–50 °C, R_f 0.35 (acid) (Et_2O); IR (KBr, ν, cm^{-1}) 3080 (=C-H), 2928–2855 (C-H), 1650 (C=O, amide), 1560 (C=O, CO_2^-); ^1H NMR (250 MHz, D_2O) δ 1.2 (m, 10H, CH_2, $H_{4'}$ to $H_{8'}$), 1.6 (m, 2H, $H_{3'}$), 1.85 (m, 2H, H_3), 2.1 (q, 2H, $H_{9'}$, J = 6.6 Hz), 2.2 (m, 2H, H_2), 2.4 (m, 2H, $H_{2'}$), 2.9–3.1 (2s, 3H, N-CH_3 syn and trans), 3.4 (m, 2H, H_4 syn and trans) 4.9 (m, 2H, $H_{11'}$, J_{trans} = 19 Hz, J_{cis} = 10.8 Hz), 5.9 (m, 1H, $H_{10'}$, $J_{10'-9'}$ = 6.6 Hz, J_{trans} = 19 Hz, J_{cis} = 10.8 Hz); ^{13}C NMR (62.8 MHz, D_2O) δ 24.4–26.4 (C_3, $C_{3'}$), 29.6–30.2 ($C_{4'}$ to $C_{8'}$), 33.5–34.2 (N-CH_3 syn and trans), 35.2–35.6 (C_2 syn and trans), 36.4 ($C_{2'}$), 48.6–49.8 (C_4 syn and trans), 114.8 ($C_{11'}$), 139.7 ($C_{10'}$), 183.0–182.3 ($C_{1'}$ cis and trans), 182.3 and 183 (C_1 cis and trans); MS (FAB⁻, G) m/z 282 (M)⁻. Anal. ($C_{16}H_{28}NO_3Na$) C, H, N.

Sodium 4-(*N*-methyl-*N*-undec-10′-enoylamino)hexanoate (19m): same procedure as for compound **18m**; yield 58%; mp 121–122 °C, R_f 0.4 (acid) (Et_2O); IR (KBr, ν, cm^{-1}), 2929–2856 (C-H), 1650 (C=O, amide), 1560 (C=O, CO_2^-); ^1H NMR (250 MHz, D_2O), δ 1.2 (m, 12H, CH_2), 1.5 (m, 6H, CH_2), 2.0 (q, 2H, $H_{9'}$, J = 6.5 Hz), 2.1 (t, 2H, H_2, J = 7.4 Hz), 2.3 (q, 2H, $H_{2'}$, J = 7.5 Hz), 2.8–2.9 (2s, 3H, N-CH_3 syn and trans), 3.2 (m, 2H, H_6 syn and trans), 4.8 (m, 2H, $H_{11'}$, J_{trans} = 19 Hz, J_{cis} = 10 Hz), 5.7 (m, 1H, $H_{10'}$, $J_{10'-9'}$ = 6.5 Hz, J_{trans} = 19 Hz, J_{cis} = 10 Hz); ^{13}C NMR (62.8 MHz, D_2O) δ 25.8–27.2 (C_3 to C_5, $C_{3'}$), 29.5–30.0 ($C_{4'}$ to $C_{8'}$), 33.4–34.0 (N-CH_3 syn and trans), 36.2–38.3 (C_2, $C_{2'}$), 36.3 ($C_{9'}$), 48.6–50.9 (C_6 syn and trans), 114.8 ($C_{11'}$), 139.4 ($C_{10'}$), 184.0–183.4 ($C_{1'}$ cis and trans), 182.4 and 184 (C_1 cis and trans); MS (FAB⁻, G) m/z 310 (M)⁻, 643 (2M⁻ + Na⁺)⁻. Anal. ($C_{18}H_{32}NO_3Na$) C, H, N.

Polymerization Reaction. As we have previously shown,[37–39] the polymerization of aqueous micellar solution of

ω-unsaturated surfactants leads to relatively monodispersed and low molecular weight polymers. It has been observed that their degree of polymerization is close to the aggregation number of the original micelles. Using ω-unsaturated anionic surfactants, we were thus allowed to prepare polyanions whose molecular weights were in the range of 6000–10 000 Da, 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 was generally quantitative. However, to remove any trace of monomer, the polyanions thus obtained were purified by gel permeation chromatography on a Sephadex G-50 column using distilled water as eluent (flow, 0.25 mL/min; column, 25 mm diameter, 500 mm length, and 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 to those of the analogous monomers.

Biological Methods. Anti-HIV-1 Assays (Table 1). The CEM-4 cell line, a subclone enriched in CD_4 receptors, was obtained from the CEM T-lymphoblastoid tumor cell line. CEM was originally isolated from a child with acute lymphoblastic leukemia. Cells were grown at 37 °C in a CO_2 incubator (5%) in RPMI 1640 medium, supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 IU/mL), glutamine (100 μg/mL), streptomycin (100 μg/mL), and polybrene (Sigma; 2 μg/mL). HIV-1 strain HTLV-$III_{B/LAI}$ stocks were obtained from filtered (0.45 μm pore size) supernatants of infected CEM-4 cells. The titer of the HIV-1 preparations was around 10^4 $CCID_{50}$ (50% cell culture infective doses/mL). The compounds were tested for their cytotoxicity and their ability to inhibit the cytopathic effect induced by HIV-1 replication. They were dissolved at 100 mg/mL in 100% dimethylformamide (DMF) and then diluted in phosphate-buffered saline (PBS) just before use. The maximum final concentration of DMF added to the cell cultures was 0.1% at the highest concentration of the test compound. At this concentration, DMF did not interfere with cell viability. Assays were carried out in 96-well microtiter plates. The HIV-1-induced cytopathic effect (CPE) was monitored by the MTT viability assay.[33] To follow HIV replication (see below), we measured reverse transcriptase activity in the culture supernatant. In the routine microplate test, 25 μL of a 10-fold diluted concentration of the compounds or 25 μL of a 10-fold diluted concentration of the reference compound AZT or PBS alone was distributed in each well. A CEM-4 cell suspension (125 μL of 1.10^5 cells/mL) was then added, and cultures were incubated for 1 h at 37 °C (5% CO_2). Cells were infected with 100 μL of virus suspension (100–200 $CCID_{50}$) and cultured for at least 5 days. Mock-infected cultures were carried out in parallel to determine the cytotoxicity of the compounds by the addition of 100 μL of supplemented RPMI only. After 5 days, 30 μL of the supernatant was removed for reverse transcriptase (RT) assay.

The determination of reverse transcriptase activity was determined in cell supernatants as a marker of HIV replication, and reverse transcriptase activity was measured by a poly(rA) RT [^{3}H]SPA (scintillation proximity assay) enzyme assay (NK9020 from Amersham, U.K.) and expressed in counts/min. Results are expressed according to the formula: % inhibition = cpm in treated infected cells (50 μL)/cpm in infected cells (50 μL). Concomitantly, 100 μL of cell suspension was transferred into another microplate and mixed with 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay)[34] for determination of the cell viability. The results are expressed according to the formula: % viable cells = OD of treated infected cells/OD of mock-infected cells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of compound that reduced the absorbance of the mock-infected control sample by 50%. The concentration giving 50% protection was defined as the 50% inhibitory concentration (IC_{50}). The selectivity index was defined as the ratio CC_{50}/IC_{50}. All assays were carried out in triplicate.

Antiviral Activity and Cytotoxicity Assays (Tables 2–4). The different compounds were evaluated for their antiviral activity according to well-established procedures.[35,36] 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.[35,36] 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.

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