Polyanion Inhibitors of Human Immunodeficiency Virus and Other Viruses. 6. Micelle-like Anti-HIV Polyanionic Compounds Based on a Carbohydrate Core

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A new class of polyanionic compounds, inhibitors of human immunodeficiency virus, was obtained from radical addition of mercapto acid or mercapto ester on a perallylated carbohydrate under UV irradiation with a catalytic amount of AIBN. Unlike the polyanions that we have previously prepared by polymerization reactions, the compounds are structurally well defined. Polyanions bearing 16 carboxylate groups showed a 50% inhibitory concentration (IC_{50}) of 0.1−4.1 μg/mL against HIV-1 in MT-4 cells while not being toxic to the host cells at concentrations up to 125 μg/mL. The most potent polyanions also proved active against human cytomegalovirus at concentrations of 1−14 μg/mL. No activity was observed against any of the other viruses tested (i.e., herpes simplex virus, vesicular stomatitis virus, Sindbis, Semliki forest, parainfluenza-3, Junin, Tacaribe, Coxsackie B4, polio-1, reo-1, or vaccinia virus).

Introduction

Recently,[1] we have shown that polyanions obtained by γ-polymerization of aqueous micellar solutions of surfactants derived from undec-10-enoic acid (Scheme 1) markedly inhibit the replication of human immunodeficiency virus (HIV-1 and HIV-2) in cell culture. Their IC_{50} values were in the range of 0.1−3.6 μg/mL, while they were not toxic to the host cells (CEM-4 or MT-4) at concentrations up to 100 μg/mL or even higher.

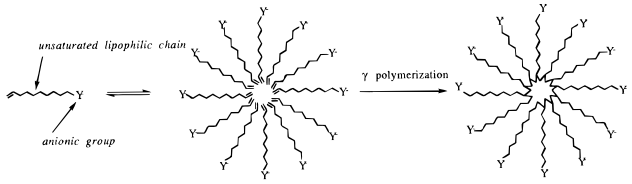
We have also shown[2,3] that the γ-polymerization of aqueous micellar solutions of ω-insaturated ionic surfactants led to polymers of relatively low molecular weight, the degree of polymerization being close to the aggregation number of the starting micelle. Their molecular weights ranged from 6000 to 10 000 Da according to the structure of the involved monomer.

Although these values are rather low for polymers, they are still too high to consider the *in vivo* use of these polyanions. Indeed, with such molecular weights these compounds could have a tendency to accumulate in tissues or organs.[4] Moreover, 5000 Da is considered as a limit for a drug to be able to pass through the various biological barriers.[5] In order to avoid undesirable effects and to improve the bioavailability of polyanionic compounds, we explored two different approaches. The first one consisted of the synthesis, through telomerization, of oligomers possessing a structure similar to that of the previous polyanions but with a molecular weight lower than 5000 Da. Such telomeric polyanions have proven to be effective against HIV.[6] The other approach is presented here.

Synthesis

This synthetic route consisted in mimicking the globular structure of polymerized anionic surfactants (Scheme 1) by functionalizing each hydroxyl group of a sugar, used as a polyol, with a hydrocarbon chain bearing an anionic group. As illustrated in the case of the methyl α-D-glucopyranoside (Scheme 2), it permitted the access to polyanions of well-defined structure, their anionic group number being strictly related to the number of hydroxyl groups in the starting carbohydrate. We also applied this strategy to D-glucose and the disaccharides cellobiose, maltose, lactose, and sucrose. To mimic the hydrophobic chains of the polymerized anionic micelles, we prepared the corresponding *O*-perallylic ethers. Then on each allyl function we condensed either a thioglycolate, a 3-mercaptopropanoate, a 2-mercaptosuccinate, or a 2-mercaptoethanesulfonate anionic group, by a radical mechanism.

Scheme 1



The *O*-perallylation reactions of sucrose[7] and cellobiose were performed with allyl bromide in the presence of potassium hydroxide in DMSO at room temperature. The yields of the allylation step ranged from 50% to 88%. The *O*-perallylations of the other carbohydrates were performed with allyl bromide in the presence of sodium hydride[8] in DMF under sonication at 0 °C.

Methyl 3-mercaptopropanoate, methyl thioglycolate, or diethyl 2-mercaptosuccinate were allowed to react with 1-*O*-methyl-2,3,4,6-tetra-*O*-allyl-α-D-glucopyranoside (**1**) in refluxing THF (Scheme 2), the radical addition being initiated with AIBN under UV irradiation[9] (λ = 254 nm). The use of AIBN alone led to poor results. On the contrary, the simultaneous use of AIBN and UV[7d] improved the yield of the coupling reactions (65−89%; see Table 1). The obtained esters **7**−**9** were purified by chromatography on silica gel (CH_2Cl_2−AcOEt). They were then saponified to their corresponding sodium salts **10**−**12**. This step gave good yields (73% and 72%, respectively) for compounds **10** and **11** but led to a poor result for compound **12** which bears eight ester groups (29% yield).

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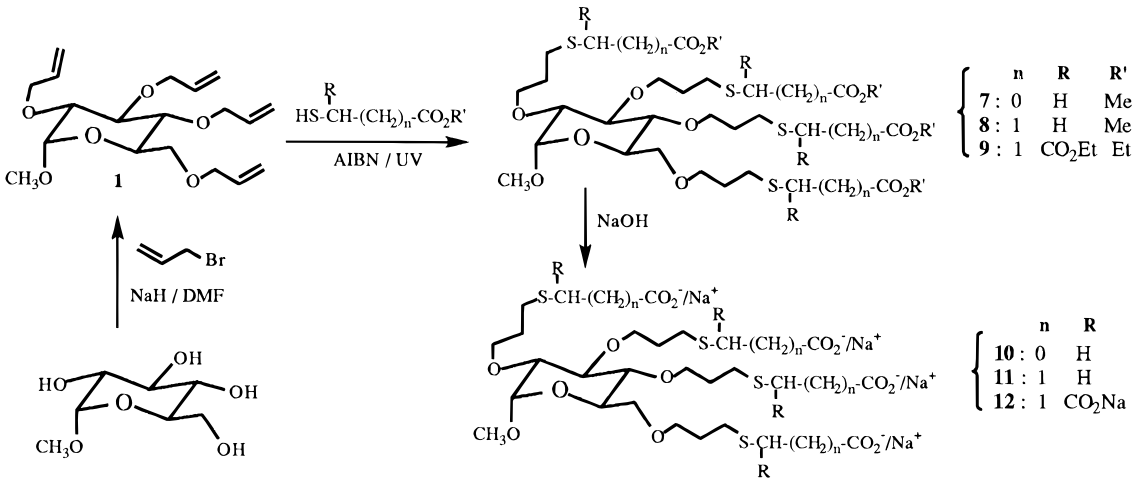
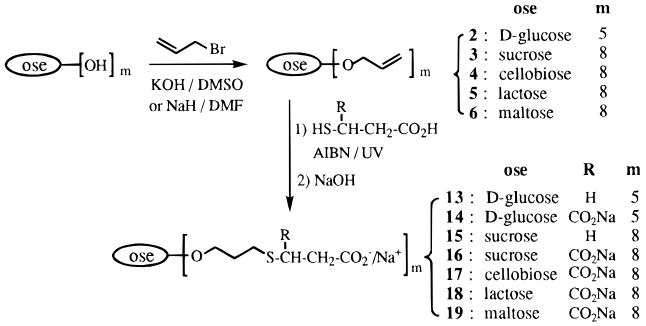
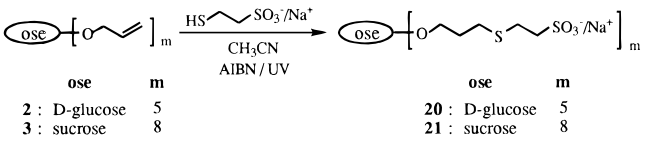
Scheme 2



Scheme 3



Scheme 4



In the same manner, the saponification reactions [10] of similar polyesters derived from D-glucose or sucrose and possessing a high number of ester functions were incomplete, and under forced conditions, destruction of the compounds occurred. We think that as soon as a sufficient number of ester groups has been saponified, the presence of the corresponding negative charges on the molecule strongly inhibits the saponification, by the hydroxide ion, of the remaining ester groups.

To avoid this difficulty, the free mercaptoalcanoic acids were allowed to react, in the same conditions (AIBN/UV), with the *O*-perallylated sugars **2**−**6** (40−73% yield). The obtained polycarboxylic acids were transformed into their sodium salt by neutralization with 1 equiv of sodium hydroxide (Scheme 3). The sodium polycarboxylates **13**−**19** were purified by gel filtration chromatography (Sephadex G-10 or G-25, ultrapure water being used as eluent).

Polysulfonates derived from D-glucose or sucrose were prepared by radical addition of sodium 2-mercaptoethanesulfonate on the corresponding *O*-perallylated sugars with a 53−64% yield (Scheme 4). The sodium polysulfonates **20** and **21** were purified by gel filtration chromatography as indicated above.

Biological Results

The polyanions were evaluated for their inhibitory effects on human immunodeficiency virus, either HIV-1 (III_B) in CEM-4 cells (Table 1) or HIV-1 (III_B) and HIV-2 (ROD) in MT-4 cells (Table 2).

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

polyanion	charge number	CC_{50}[a] (μM)	IC_{50}[b] (μM)
10	4	>370	>370
11	4	>346	>346
12	8	>265	69.0
13	5	>98.0	>98.0
14	10	>74.0	>74.0
15	8	>59.3	>59.3
16	16	>45.1	3.02
20	5	>83.3	>83.3
21	8	>50.6	>50.6

[a] 50% cytotoxic concentration, or compound concentration required to reduce the viability of uninfected CEM-4 cells by 50%. [b] 50% inhibitory concentration, or compound concentration required to inhibit HIV-induced cytopathicity by 50%.

None of them proved to be cytotoxic, their CC_{50} being invariably higher than 50 μM. Only those compounds bearing 16 carboxylate groups inhibited HIV replication at low concentrations (IC_{50} = 0.04−1.85 μM). As we have previously reported,[6] a minimum charge number on the polyanion is required to obtain inhibition of HIV cytopathicity. As noted for other polyanions,[11] test compounds were 10−100-fold more inhibitory to HIV-induced cytopathicity in MT-4 cells than to syncytium formation between HIV-infected HUT-78 cells and uninfected MOLT-4 cells (Table 2). A similar correlation was found between the charge number on the polyanions and their inhibitory effect on HIV-induced giant cell formation as mentioned above for their inhibitory effect on HIV-induced cytopathicity. Only those compounds bearing 16 carboxylate groups were found to inhibit HIV-induced syncytium formation. All compounds tested proved to be at least 10 times more active against HIV-1-induced cytopathicity than HIV-2-induced cytopathicity.

Marked activity was noted with some of the compounds (**17**α+β and **17**β) against human cytomegalovirus (HCMV) (IC_{50} = 0.4−6.3 μM). No appreciable activity was noted with any of the compounds against various other viruses such as herpes simplex virus (HSV-1, HSV-2), vaccinia, vesicular stomatitis virus, parainfluenza-3, reovirus type 1, Sindbis, Semliki forest, Junin, Tacaribe, Coxsackie B4 and polio-1 (Table 3). The compounds (listed in Table 3) were not toxic (as assessed by either microscopically detectable alteration of normal cell morphology or inhibition of cell growth) to E_6SM,

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

polyanion	charge number	CC_{50}[a] (μM)	IC_{50}[b] (μM) cytopathicity HIV-1	IC_{50}[b] (μM) cytopathicity HIV-2	IC_{50}[c] (μM) syncytium formation HIV-1	IC_{50}[c] (μM) syncytium formation HIV-2
14	10	>185	85.9	>185	>185	>185
15	8	>148	29	>148	>148	>148
16	16	>113	0.18	2.89	22.6	22.6
17(α+β)	16	>56.4	0.04	4.17	1.80	54.2
17β[d]	16	>56.4	0.37	4.47	3.61	54.2
18	16	>56.4	1.85	>56.4	>113	>113
19	16	>56.4	0.67	>56.4	>113	>113
20	5	>208	99.1	>208	>208	>208
21	8	>127	>127	>127	>127	>127

[a] 50% cytotoxic concentration, or compound concentration required to reduce the viability of uninfected 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] 50% inhibitory concentration, or compound concentration required to inhibit by 50% HIV-induced syncytium formation upon coculturing persistently HIV-infected HUT-78 cells with MOLT-4 (clone 8) cells. [d] In the case of the cellobiose derivative, it has been possible to isolate a pure fraction of the β-anomer at the stage of the perallylated precursor (see experimental section).

HEL, HeLa, and Vero cells at concentrations up to 180 μM (Table 4).

Given the relative low number of compounds found to be active against HIV, a detailed structure−activity relationship (SAR) could not be made. It is obvious, however, that a minimum number of anionic charges seems to be required for anti-HIV activity. This corresponds to approximately seven negative charges per molecular mass fraction of 1000. From the compounds studied here, **17**α+β and **17**α emerged as the most active against HIV-1. These compounds were also active against HIV-1-induced syncytium formation, which points to their potential usefulness against SI (syncytium-inducing) variants of HIV-1. Moreover, **17**α+β and **17**α were also found to be active against CMV, a virus that often occurs in conjunction with HIV-1.

Experimental Protocols

Unless noted otherwise, all starting materials were obtained from commercial suppliers and used without further purification. *N*,*N*-Dimethylformamide was distilled under reduce pressure from calcium oxide and stored over 4 Å molecular sieves under nitrogen. Tetrahydrofuran (THF) was distilled over lithium aluminum hydride under nitrogen immediately prior to use.

Merck silica gel 60 F_{254} (0.25 mm) plates were employed for analytical TLC. Compounds were revealed by UV (254 nm), iodine, 20% aqueous sulfuric acid, or 10% methanolic ninhydrin sprayings.

Merck silica gel 60 H was used for silica gel column chromatography. Melting points were determined using a Bucchi 530 apparatus. Reactions under ultrasound were performed on a 72434 Bioblock Scientific Vibra cell apparatus. Infrared spectra were obtained on a IR-FT Bonem MB-100 spectrometer. ^{1}H and ^{13}C NMR were recorded on Bruker AC200 and WP200SY spectrometers, respectively. For ^{1}H and ^{13}C NMR we have used the numbering system presented in Chart 1.

Chemical shifts are expressed in ppm (δ) and coupling constants in hertz. Mass spectra were recorded on a Jeol DX 100 spectrometer. Used matrix was *m*-nitrobenzylic alcohol (NOBA) or thioglycerol (GT). Microanalyses were performed in the analytical department of the CNRS (ENSCM-Montpellier). C, H, and S elemental analysis was done for most of the compounds; the observed deviations to the indicated formula were always less than 0.4%.

Allylation: Method A. Sodium hydride (0.1 mol, 1.5 equiv per OH function) (60% in mineral oil) was washed under nitrogen with 2 × 10 mL of dry pentane. The hydride was suspended in 75 mL of dimethylformamide, and the carbohydrate was added in small portions. The reaction mixture under an inert atmosphere was put in an ultrasonic cleaning bath for 1 h; 0.1 mol of allyl bromide (1.5 equiv per OH function) in solution with 8 mL of DMF was added slowly at 0−5 °C. After 2 h of stirring at room temperature, the excess of sodium hydride was destroyed with methanol. The excess of allyl bromide and DMF was eliminated under reduced pressure. The residue was diluted in 150 mL of dichloromethane, and the mixture was washed twice with water. The organic layer was dried with sodium sulfate and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel with dichloromethane−ethyl acetate as eluent.

Method B. Disaccharide (10 mmol) was suspended under an inert atmosphere in 40 mL of dimethyl sulfoxide with vigorous stirring at 15 °C; 13.9 mL of allyl bromide (160 mmol, 2 equiv per OH group) and 9.0 g of potassium hydroxide were added slowly. The reaction mixture was allowed to stir for

Table 3. Activity (IC_{50}, μM)[a] of Polyanions against Several DNA and RNA Viruses, Other than HIV

virus	cell line	dextran sulfate	**14**	**15**	**16**	**17**α+β	**17**β	**18**	**19**	**20**	**21**
HSV-1TK$^-$ (B2006)	E_6SM		>296	>237	>181	45	68	>181	>181	>333	>203
VMW1837	E_6SM		>296	>237	>181	>181	>181	>181	>181	>333	>101
(KOS)	E_6SM	0.2	>296	>237	>181	>181	136	>181	>181	>333	>101
(F)	E_6SM		>296	>237	>181	ND[b]	ND	ND	ND	>333	>101
(McIntyre)	E_6SM		>296	>237	>181	ND	ND	ND	ND	>333	>101
HSV-2(G)	E_6SM	0.2	>296	>237	>181	45	136	>181	>181	>333	>101
(196)	E_6SM		>296	>237	>181	ND	ND	ND	ND	>333	>101
(Lyons)	E_6SM		>296	>119	>135	ND	ND	ND	ND	>333	>101
HCMV(AD-169)	HEL	0.06	>37	>30	>22	1.4	6.3	>22	>22	>42	>25
(Davis)	HEL	0.08	>37	>30	>22	0.4	3.6	>22	>22	>42	>25
vaccinia	E_6SM	10	>296	>237	>181	>181	>181	>181	>181	>333	>101
	E_6SM	0.2	>296	>237	>181	>181	>181	>181	>181	>83	>101
vesicular stomatitis	HeLa	>20	>148	>119	>90	>181	>181	>181	>181	>167	>51
parainfuenza-3	Vero	>20	>148	>59	>90	>181	>181	>181	>181	>167	>101
reovirus-1	Vero	>20	>148	>59	>90	>181	>181	>181	>181	>167	>101
Sindbis	Vero	>20	>148	>59	>90	>181	>181	>181	>181	>167	>101
Semliki forest	Vero	>20	>148	>59	>90	ND	ND	ND	ND	>167	>101
Junin	Vero	0.7	>37	>12	>22	>22	>22	>22	>22	>42	>25
Tacaribe	Vero	0.7	>37	>12	>22	>22	>22	>22	>22	>42	>25
Coxsackie B4	Vero	>20	>148	>59	>90	>181	>181	>181	>181	>167	>101
	HeLa	>20	>148	>119	>90	>181	>181	>181	>181	>167	>51
polio-1	HeLa	>20	>148	>119	>90	ND	ND	ND	ND	>167	>51

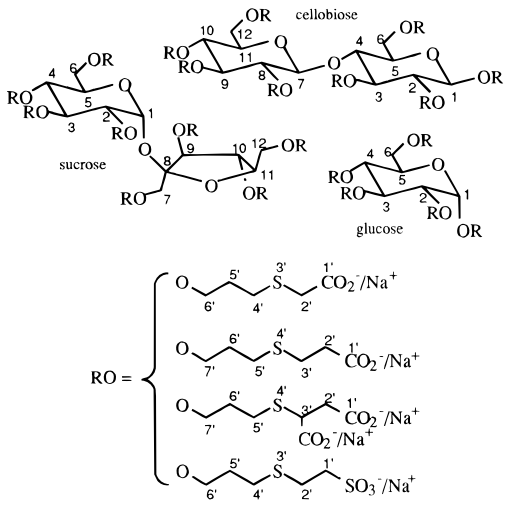
[a] 50% inhibitory concentration, or compound concentration required to reduce virus-induced cytopathicity by 50%. Virus was added in the presence of the compounds, and the cells were further incubated until the cytopathic effect (CPE) was scored. [b] ND, not determined.

Table 4. Cytotoxicity of the Polyanions for Different Cell Lines[a]

cell line	dextran sulfate	**14**	**15**	**16**	**17**α+β	**17**β	**18**	**19**	**20**	**21**
E6SM	>40	>296	>237	>181	>181	>181	>181	>181	>333	>203
HEL	>20	>37	>30	>22	>22	>22	>22	>22	>42	>25
HeLa	>20	>148	>119	>90	>181	>181	>181	>181	>167	>101
Vero	>20	>148	>119	>90	>181	>181	>181	>181	>167	>101

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

Chart 1



2.5 h at room temperature. The mixture was filtered using a Buchner funnel, and the filtrate was evaporated under reduced pressure to eliminate the excess of allyl bromide and solvent. The residue was dissolved in 200 mL of dichloromethane, and the mixture was washed twice with water. The organic layer was dried with sodium sulfate and evaporated under reduced pressure. The oily residue was purified on a silica gel column with hexane−ethyl acetate as eluent.

Methyl 2,3,4,6-tetra-*O*-allyl-α-D-glucopyranoside (1): method A; yield 88%; R_f 0.37 (CH_2Cl_2/AcOEt, 95/5, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.41 (m, 5H, $3H_8$, H_6, H_7), 3.67 (m, 4H, H_2, H_3, H_4, H_5), 4.18 (m, 8H, $8H_{1'}$), 4.77 (d, 1H, H_1, J_{1-2} = 3.51 Hz), 5.22 (m, 8H, $8H_{3'}$), 5.94 (m, 4H, $4H_{2'}$); MS (FAB$^+$, NBA) *m*/*z* 353 (M − H)$^+$, 323 (M + H − MeOH)$^+$, 265 (M + H − MeOH − allylOH)$^+$.

Penta-*O*-allyl-D-glucopyranose (2): method A; yield 73% (α/β: 7/3); α, R_f 0.68 (CH_2Cl_2/AcOEt, 95/5, v/v); β, R_f 0.70 (CH_2Cl_2/AcOEt, 95/5, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.24 (m, 4H, H_2, H_3, H_4, H_5), 3.53 (q, 1H, H_7, J_{5-7} = 3.1 Hz, J_{6-7} = 11 Hz), 3.63 (q, 1H, H_6, J_{5-6} < 1 Hz, J_{6-7} = 10.8 Hz), 4.09 (m, 11H, $10H_{1'}$, H_1), 5.17 (m, 10H, $10H_{3'}$), 5.8 (m, 5H, $5H_{2'}$); MS (FAB$^+$, NBA) *m*/*z* 379 (M − H)$^+$, 323 (M + H − allylOH)$^+$, 265 (M + H − 2allylOH)$^+$.

Octa-*O*-allylsucrose (3): method B; yield 48%; R_f 0.48 (CH_2Cl_2/AcOEt, 98/2, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.35 (m, 9H, 9 glycosidic H), 4.15 (m, 20H, 4 glycosidic H, $16H_{1'}$), 5.25 (m, 16H, $16H_{3'}$), 5.5 (d, 1H, H_1, J_{1-2} = 3.38 Hz), 5.95 (m, 8H, $8H_{2'}$); MS (FAB$^+$, NBA) *m*/*z* 661 (M − H)$^+$, 547 (M + H − 2allylOH)$^+$, 323 (M + H − $C_{18}H_{27}O_6$)$^+$.

Octa-*O*-allyl-β-D-cellobiose (4): method B; yield 38% (α + β); β anomer isolated by silica gel column chromatography (cyclohexane/AcOEt, 85/15−75/25, v/v); R_f 0.54 (cyclohexane/AcOEt, 75/25, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3−3.4 (m, 7 glycosidic H), 3.5−3.75 (m, 5 glycosidic H), 3.85−4.4 (m, 17H, $16H_{1'}$, $1H_1$), 4.9−5.2 (m, 16H, $16H_{3'}$), 5.25 (d, 1H, $1H_7$), 5.7−6.0 (m, 8H, $8H_{2'}$); ^{13}C NMR (25.178 MHz, CD_3CN) δ 67.75−73.55 (C_6, C_{12}, $8C_{1'}$), 74.45−81.86 (C_2, C_8, C_3, C_5, C_{11}), 101.08 (C_1), 102.05 (C_7), 114.9−117.12 ($8C_{3'}$), 134.26−136.14 ($8C_{2'}$); MS (FAB$^+$, NBA) *m*/*z* 663 [M + H]$^+$, 685 [M + H]$^+$, 685 [M + Na]$^+$, 701 [M + Na]$^+$, 323 [M + H − $C_{18}H_{27}O_6$]$^+$, 605 [M + H − allylOH]$^+$, 547 [M + H − 2allylOH]$^+$, 489 [M + H − 3allylOH]$^+$.

Octa-*O*-allyl-D-lactose (5): method A; yield 48%; R_f 0.59 (cyclohexane/AcOEt, 75/25, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.1−3.8 (12 glycosidic H), 3.85−4.5 (17H, $16H_{1'}$, $1H_1$), 5−5.4 (17H, $16H_{3'}$, $1H_7$), 5.75−6.15 (8H, $8H_{2'}$); MS (FAB$^+$, NBA) *m*/*z* 685 [M + Na]$^+$, 605 [M + H − allylOH]$^+$, 547 [M + H − 2allylOH]$^+$, 489 [M + H − 3allylOH]$^+$, 323 [M + H − $C_{18}H_{27}O_6$]$^+$.

Octa-*O*-allyl-D-maltose (6): method A; yield 64% (α + β); R_f 0.59 (cyclohexane/AcOEt, 75/25, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.2−3.7 (m, 12 glycosidic H), 3.9−4.5 (m, 16H, $16H_{1'}$), 4.85 (d, 1H), 5−5.3 (m, 16H, $16H_{3'}$), 5.6 (dd, 1H), 5.75−6.0 (m, 8H, $8H_{2'}$); ^{13}C NMR (25.178 MHz, CD_3CN) δ 67.86−68.82 (C_6, C_{12}), 72−73 ($8C_{1'}$), 95.9 (C_1), 102.08 (C_7), 115.19−116.15 ($8C_{3'}$), 134.3−135.9 ($8C_{2'}$); MS (FAB$^+$, NBA) *m*/*z* 663 [M + H]$^+$, 685 [M + Na]$^+$, 605 [M + H − allylOH]$^+$, 547 [M + H − 2allylOH]$^+$, 489 [M + H − 3allylOH]$^+$, 323 [M + H−$C_{18}H_{27}O_6$]$^+$.

Radical Addition of Mercapto Esters on the Methyl 2,3,4,6-Tetra-*O*-allyl-α-D-glucopyranoside. General Method. In a quartz reactor was disposed 1.0 mmol of tetraallyl derivative **1** dissolved in 40 mL of THF, peroxide free. The solution was degassed with nitrogen for 15 min; 6.0 mmol of mercapto ester was added (1.5 equiv per allyl function) with 50 mg of AIBN as radical initiator. The mixture was irradiated for 3 h with UV light (λ = 254 nm; Rayonet apparatus) under an inert atmosphere. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography of silica gel eluted with a mixture of dichloromethane and ethyl acetate.

Methyl 2,3,4,6-tetra-*O*-(3-(((methoxycarbonyl)methyl)-thio)propyl)-α-D-glucopyranoside (7): obtained by the addition of 0.6 mL of methyl thioglycolate (6.8 mmol) on 0.4 g (1.1 mmol) of **1** under UV irradiation for 2 h; yield 89%; R_f 0.32 (CH_2Cl_2/AcOEt, 90/10, v/v); ^{1}H NMR (250 MHz, CDCl33) δ 1.83 (m, 8H, $8H_{5'}$), 2.65 (t, 8H, $8H_{4'}$, $J_{4'-5'}$ = 6.4 Hz), 3.16 (s, 8H, $8H_{2'}$), 3.18 (m, 1H, H_5), 3.31 (s, 3H, $3H_8$), 3.67 (s, 12H, $4COOCH_3$), 3.76−3.4 (m, 15H, H_2, H_3, H_4, H_6, H_7, $8H_{6'}$), 4.71 (d, 1H, H_1, J_{1-2} = 3.46 Hz); ^{13}C NMR (25.178 MHz, CD_3CN) δ 28.84−29.9 ($4C_{5'}$, $4C_{4'}$), 32.99−33.0 ($4C_{2'}$), 51.81 ($4OCH_3$), 54.32 (C_8), 71.27, 70.77, 69.39, 69.25, 68.63 ($4C_{6'}$, C_6), 70.89, 77.8, 80.42, 81.53 (C_2, C_3, C_4, C_5), 97.5 (C_1), 170.9 ($C_{1'}$); MS (FAB$^+$, NBA) *m*/*z* 779 [M + H]$^+$, 747 [M + H − CH_3OH]$^+$, 641 [M + H − CH_3OH − $HSCH_2CO_2CH_3$]$^+$, 615 [M + H − $HO(CH_2)_3$-$SCH_2CO_2CH_3$]$^+$, 583 [M + H − CH_3OH − $HO(CH_2)_3SCH_2CO_2$-CH_3]$^+$.

Methyl 2,3,4,6-tetra-*O*-(3-(((methoxycarbonyl)ethyl)-thio)propyl)-α-D-glucopyranoside (8): obtained by the addition of 0.68 mL of methyl 3-mercaptopropanoate (6.2 mmol) on 0.4 g (1.1 mmol) of **1** under UV irradiation for 2 h; yield 75%; R_f 0.41 (CH_2Cl_2/AcOEt, 80/20, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.82 (m, 8H, $8H_{6'}$), 2.54 (m, 16H, $8H_{3'}$, $8H_{5'}$), 2.71 (t, 8H, $8H_{2'}$, $J_{2'-3'}$ = 7.3 Hz), 3.2 (m, 1H, H_5), 3.31 (s, 3H, $3H_8$), 3.63 (s, 12H, $4COOCH_3$), 3.78−3.42 (m, 15H, H_2, H_3, H_4, H_6, H_7, $8H_{7'}$), 4.71 (d, 1H, H_1, J_{1-2} = 3.37 Hz); ^{13}C NMR (25.178 MHz, CD_3CN) δ 26.5 ($4C_{3'}$), 28.04−30.43 ($4C_{5'}$, $4C_{6'}$), 34.36 ($4C_{2'}$), 51.15 ($4COOCH_3$), 54.29 (C_8), 71.39, 70.84, 69.37, 69.28, 68.7 ($4C_{7'}$, C_6), 70.12, 77.83, 80.45, 81.52 (C_2, C_3, C_4, C_5), 97.5 (C_1), 172.2 ($C_{1'}$); MS (FAB$^+$, NBA) *m*/*z* 833 [M − H]$^+$, 747 [M + H − $CH_3CH_2CO_2CH_3$]$^+$, 659 [M + H − $2CH_3CH_2CO_2CH_3$]$^+$, 625 [M + H − CH_3OH − $HO(CH_2)_3S(CH_2)_2$ − CO_2CH_3]$^+$, 447 [M + H − CH_3OH − $2HO(CH_2)_3S(CH_2)_2CO_2CH_3$]$^+$.

Methyl 2,3,4,6-tetra-*O*-(3-((1,2-bis(ethoxycarbonyl)eth-yl)thio)propyl)-α-D-glucopyranoside (9): obtained by the addition of 1.36 g of diethyl 2-mercaptosuccinate (6.6 mmol) on 0.33 g (0.92 mmol) of **1** under UV irradiation for 3 h; yield 55%; R_f 0.65 (CH_2Cl_2/AcOEt, 85/15, v/v); ^{1}H NMR (250 MHz,

$CDCl_3$) δ 1.2 (q, 24H, CH_3 ester), 1.81 (m, 8H, $8H_{6'}$), 2.6 (m, 12H, $4H_{2'}$, $8H_{5'}$), 2.91 (q, 4H, $4H_{2'}$, $J_{2'gem}$ = 16.9 Hz, $J_{2'-3'}$ = 10.1 Hz), 3.2 (m, 2H, H_6, H_7), 3.2 (s, 3H, $3H_8$), 3.63 (s, 8H, $4COOCH_2$), 3.44−3.72 (m, 16H, H_2, H_3, H_4, H_5, $4H_{3'}$, $8H_{6'}$), 4.71 (d, 1H, H_1, J_{1-2} = 3.36 Hz); ^{13}C NMR (25.178 MHz, CD_3CN) δ 13.5 ($8CH_3$ ester), 27.91, 28, 28.1 ($4C_{5'}$), 29.3−30.19 ($4C_{6'}$), 36.35 ($4C_{2'}$), 54.3 ($C_{8'}$), 60.6, 61.0 ($4COOCH_2CH_3$), 68.57−71.23 ($4C_{7'}$, C_6), 70.1, 77.78, 81.45, 81.5 (C_2−C_5), 97.5 (C_1), 170.5, 171.6 (COO-Et); MS (FAB$^+$, NBA) *m/z* 1177 [M − H]$^+$, 1147 [M + H − CH_3OH]$^+$, 1005 [M + H − ethyl succinate]$^+$, 883 [M + H − CH_3OH − $HO(CH_2)_3SCH(CO_2CH_2CH_3)CH_2CO_2CH_2CH_3$]$^+$.

Saponification of Esters 7−9. Polyester (0.30 mmol) was dissolved in a mixture of 4 mL of THF and 3 mL of a 1 M methanolic solution of sodium hydroxide (3 mmol). After 3 h of stirring at room temperature, the tetracarboxylate was filtered off. The precipitate was dissolved in a small amount of water and purified by column chromatography on a silanized gel column with distilled water as eluent. The fractions were collected and lyophilized.

Methyl 2,3,4,6-tetra-*O*-(3-(((sodium oxycarbonyl)methyl)thio)propyl)-α-D-glucopyranoside (10): yield 73%; mp 250 °C dec; R_f 0.52 (acetone/ethyl acetate/acetic acid/water, 38/60/1/1, v/v); ^{1}H NMR (250 MHz, D_2O) δ 1.74 (m, 8H, $H_{5'}$), 2.49 (t, 8H, $8H_{4'}$, $J_{4'-5'}$ = 7.1 Hz), 3.05 (s, 8H, $8H_{2'}$), 3.24 (s, 3H, $3H_8$), 3.33−3.75 (m, 14H, $8H_{6'}$, H_2−H_7), 4.78 (d, 1H, H_1, J_{1-2} = 3.37 Hz); MS (FAB$^-$, TG) *m/z* 787 [M − Na]$^-$, 674 (M − Na − $HSCH_2CO_2Na$]$^-$, 615 [M − Na − $HO(CH_2)_3SCH_2CO_2Na$]$^-$, 501 [M − Na − $HO(CH_2)_3SCH_2CO_2Na$ − $HSCH_2CO_2Na$]$^-$. Anal. ($C_{27}H_{42}O_{14}S_4Na_4$) C, H, S.

Methyl 2,3,4,6-tetra-*O*-(3-(((sodium oxycarbonyl)ethyl)thio)propyl)-α-D-glucopyranoside (11): yield 72%; mp 232 °C dec; R_f 0.4 (acetone/ethyl acetate/acetic acid/water, 38/60/1/1); ^{1}H NMR (250 MHz, D_2O) δ 1.86 (m, 8H, $H_{6'}$), 2.42 (t, 8H, $8H_{2'}$, $J_{2'-3'}$ = 7.2 Hz), 2.6 (m, 8H, $8H_{5'}$), 2.69 (t, 8H, $H_{3'}$), 3.28 (s, 3H, $3H_8$), 3.33−3.75 (m, 14H, $8H_{6'}$, H_2−H_7), 4.78 (d, 1H, H_1, J_{1-2} = 3.37 Hz); ^{13}C NMR (25.178 MHz, D_2O) δ 28.8−30.1 ($C_{3'}$, $C_{5'}$, $C_{6'}$), 38.6 ($C_{2'}$), 56.03 (C_8), 69.75, 70.7, 72.7, 73.1 ($4C_{7'}$, C_6), 70.4, 78.5, 80.42, 82.00 (C_2, C_3, C_4, C_5), 98.2 (C_1), 179.46 ($C_{1'}$); MS (FAB$^-$, TG) *m/z* 843 [M − Na]$^-$, 821 [M + H − 2Na]$^-$. Anal. ($C_{31}H_{50}O_{14}S_4Na_4$) C, H, S.

Methyl 2,3,4,6-tetra-*O*-(3-((1,2-bis(sodiumoxycarbonyl)ethyl)thio)propyl)-α-D-glucopyranoside (12): yield 29%; mp 250 °C dec; R_f 0.24 (acetone/ethyl acetate/acetic acid/water, 3/5/1/1, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.74 (m, 8H, $8H_{6'}$), 2.26 (m, 4H, $4H_{2'}$), 2.51 (m, 12H, $4H_{2'}$, $8H_{5'}$), 3.24 (s, 3H, $3H_8$), 3.44−3.72 (m, 18 H, H_2, H_3, H_4, H_5, H_6, H_7, $4H_{3'}$, $8H_{6'}$), 4.71 (d, 1H, H_1, J_{1-2} = 3.36 Hz); MS (FAB$^-$, TG) *m/z* 1107 [M − Na]$^-$, 1085 [M + H − 2Na]$^-$, 1063 [M + H − 3Na]$^-$, 923 [M + H − $2NaCO_2(CH_2)_2CO_2Na$]$^-$. Anal. ($C_{35}H_{46}O_{22}S_4Na_8$) C, H. S.

Radical Addition of Mercapto Acid on the Perallylated Carbohydrate. General Method. In a quartz reactor was disposed 1.0 mmol of perallylated derivative dissolved in 40 mL of THF, peroxide free, or a mixture of THF/water (1/1, v/v) in the case of the 2-mercaptosuccinic acid. The solution was degassed with nitrogen for 15 min, and 1.5−2.0 equiv per allyl function of mercapto acid was added with 50 mg of AIBN as radical initiator. The mixture was irradiated for 3 h with UV light (λ = 254 nm; Rayonet apparatus) under an inert atmosphere.

The solvent was evaporated under reduced pressure, and the residue was treated with 0.5 mol of aqueous sodium hydroxide (1.2 equiv per acid group). Vigorous stirring continued until residue dissolution. The pH of the solution was adjusted to 8.5−9.0 with acidic resin (Dowex 50W2), and then the mixture was lyophilized. The solid obtained was purified by exclusion column chromatography with Sephadex G25 eluted with ultrapure water.

Penta-*O*-(3-(((sodium oxycarbonyl)ethyl)thio)propyl)-β-D-glucopyranose (13): obtained by the addition of 0.46 mL of 3-mercaptopropanoic acid (5.28 mmol) on 0.26 g (0.68 mmol) of **2** under UV irradiation for 3.5 h; yield 63%; mp 261 °C dec; R_f 0.4 (acetone/ethyl acetate/acetic acid/water, 38/60/1/1, v/v); ^{1}H NMR (250 MHz, D_2O) δ 1.86 (m, 10H, $10H_{6'}$), 2.45 (t, 10H, $10H_{2'}$, $J_{2'-3'}$ = 7.14 Hz), 2.61 (m, 10H, $10H_{5'}$), 2.72 (t, 10H, $10H_{3'}$, $J_{2'-3'}$ = 7.15 Hz), 3.07 (m, 1H, H_5), 3.39 (m, 2H, H_6, H_7), 3.78 (m, 13H, H_2, H_3, H_4, $10H_{7'}$), 4.19 (d, 1H, H_1, J_{1-2} = 7.69 Hz); ^{13}C NMR (25.178 MHz, D_2O) δ 28.24−28.46 ($C_{3'}$), 29.29−30.2 ($C_{5'}$, $C_{6'}$), 37.7 ($C_{2'}$), 69.57, 69.85, 70.50, 72.53, 72.62, 73.18 ($5C_{7'}$, C_6), 74.39, 78.60, 82.23, 84.45 (C_2, C_3, C_4, C_5), 103.11 (C_1), 181 ($C_{1'}$); MS (FAB$^-$, TG) *m/z* 975 [M + H − 2Na]$^-$, 953 [M + 2H − 3Na]$^-$, 931 [M + 3H − 4Na]$^-$, 909 [M + 4H − 5Na]$^-$, 803 [M + H − 2Na − $HO(CH_2)_3S(CH_2)_2CO_2Na$]$^-$. Anal. ($C_{36}H_{57}O_{16}S_5Na_5$) C, H, S.

Penta-*O*-(3-((1,2-bis(sodium oxycarbonyl)ethyl)thio)propyl)-β-D-glucopyranose (14): obtained by the addition of 0.98 g of 2-mercaptosuccinic acid (6.53 mmol) on 0.25 g (0.66 mmol) of **2** under UV irradiation for 5 h; yield 73%; mp 252 °C dec; R_f 0.26 (acetone/ethyl acetate/acetic acid/water, 3/5/1/1, v/v); ^{1}H NMR (250 MHz, D_2O) δ 1.75 (m, 10H, $10H_{6'}$), 2.32 (m, 5H, $5H_{2'}$), 2.56 (m, 15H, $5H_{2'}$, $10H_{5'}$), 2.94 (m, 1H, H_5), 3.17 (m, 2H, H_6, H_7), 3.78 (m, 18H, H_2, H_3, H_4, $5H_{3'}$, $10H_{7'}$), 4.26 (d, 1H, H_1, J_{1-2} = 7.8 Hz); ^{13}C NMR (25.178 MHz, D_2O) δ 28.33−30.26 ($C_{5'}$, $C_{6'}$), 40.6−40.69 ($C_{2'}$), 47.36 ($C_{3'}$), 69.76, 70.12, 70.80, 72.67, 73.23 ($5C_{7'}$, C_6), 74.40, 78.61, 82.23, 84.48 (C_2, C_3, C_4, C_5), 103.16 (C_1), 179.05, 179.53, 180.3 ($C_{1'}$, CO_2^-); MS (FAB$^-$, TG) *m/z* 1305 [M + H − 2Na]$^-$, 1283 [M + 2H − 3Na]$^-$, 1261 [M + 3H − 4Na]$^-$, 1239 [M + 4H − 5Na]$^-$, 1123 [M + H − 2Na − $NaCO_2CHCHCO_2Na$]$^-$, 1101 [M + 2H − 3Na − $NaCO_2CHCHCO_2Na$]$^-$. Anal. ($C_{41}H_{52}O_{26}S_5Na_{10}$) C, H, S.

Octa-*O*-(3-(((sodium oxycarbonyl)ethyl)thio)propyl)-D-sucrose (15): obtained by the addition of 0.525 mL of 3-mercaptopropanoic acid (6.0 mmol) on 0.25 g (0.38 mmol) of octa-*O*-allylsucrose (**3**) under UV irradiation for 4 h; yield 56%; mp 259 °C dec; R_f 0.65 (acetone/ethyl acetate/acetic acid/water, 6/2/1/1, v/v); ^{1}H NMR (250 MHz, D_2O) δ 1.75 (m, 10H, $10H_{6'}$), 2.45 (t, 10H, $10H_{2'}$, $J_{2'-3'}$ = 7.4 Hz), 2.51 (m, 10H, $10H_{5'}$), 2.62 (t, 10H, $10H_{3'}$, $J_{2'-3'}$ = 6.3 Hz), 3.2−4.05 (m, 29H, H_2−H_{12}, $10H_{7'}$), 5.39 (d, 1H, H_1, J_{1-2} = 3.27 Hz); ^{13}C NMR (25.178 MHz, D_2O) δ 28.43 ($C_{3'}$), 29.43−30.3 ($C_{5'}$, $C_{6'}$), 38.3 ($C_{2'}$), 69.49, 70.64, 70.72, 70.92, 71.07, 72.36, 72.62, 73.08 ($8C_{7'}$, C_6, C_7, C_{12}), 71.46, 78.23, 79.46, 80.28, 81.81, 82.63, 84.06 (C_2, C_3, C_4, C_5, C_9, C_{10}, C_{11}), 90.76 (C_1), 104.8 (C_8), 181.56 ($C_{1'}$); MS (FAB$^-$, TG) *m/z* 1663 [M − Na]$^-$, 1641 [M + H − 2Na]$^-$, 1619 [M + 2H − 3Na]$^-$, 1597 [M + 3H − 4Na]$^-$, 1575 [M + 4H − 5Na]$^-$, 1493 [M + H − 2Na − $H(CH_2)_3S(CH_2)_2CO_2H$]$^-$, 1471 [M + H − 2Na − $H(CH_2)_3S(CH_2)_2CO_2Na$]$^-$. Anal. ($C_{60}H_{94}O_{27}S_8Na_8$) C, H, S.

Octa-*O*-(3-((1,2-bis(sodium oxycarbonyl)ethyl)thio)propyl)-D-sucrose (16): obtained by the addition of 0.91 g of 2-mercaptosuccinic acid (6.0 mmol) on 0.26 g (0.39 mmol) of octa-*O*-allylsucrose (**3**) under UV irradiation for 7 h; yield 58%; mp 195 °C dec; R_f 0.26 (acetone/ethyl acetate/acetic acid/water, 3/5/1/1, v/v); ^{1}H NMR (250 MHz, D_2O) δ 1.9 (m, 16H, $16H_{6'}$), 2.5 (m, 8H, $8H_{2'}$), 2.65 (m, 24H, $8H_{2'}$, $16H_{5'}$), 3.25 (m, 4H, 2 glycosidic CH_2), 3.6 (m, 33H, 9 glycosidic H, $8H_3$, $16H_7$), 5.5 (m, 1H, H_1); ^{13}C NMR (25.178 MHz, D_2O) δ 28.50−30.6 ($C_{5'}$, $C_{6'}$), 41.84 ($C_{2'}$), 48.07 ($C_{3'}$), 69.31−73.17 ($5C_{7'}$, C_6, C_7, C_{11}), 74.58, 78.18−83.68 (C_2, C_3, C_4, C_5, C_9, C_{10}, C_{11}), 91.03 (C_1), 103.16 (C_8), 179.05, 179.53, 180.3 ($C_{1'}$, CO_2^-). MS (FAB$^-$, TG) *m/z* 1861 [M + 15H − 16Na]$^-$. Anal. ($C_{68}H_{86}O_{43}S_8Na_{16}$) C, H, S.

Octa-*O*-(3-((1,2-bis(sodium oxycarbonyl)ethyl)thio)propyl)-D-cellobiose (17): obtained by the addition of 0.70 g of 2-mercaptosuccinic acid (4.67 mmol) on 0.20 g (0.30 mmol) of octa-*O*-allylcellobiose (**4**) under UV irradiation for 8 h; yield 40%; mp 270 °C dec; R_f 0.4 (acetone/ethyl acetate/acetic acid/water, 1/1/1/1, v/v); IR (KBr, ν cm^{-1}) 1580 (C=O), 1415 (C−O); ^{1}H NMR (250 MHz, D_2O) δ 1.6−2.2 (m, 16H, $16H_{6'}$), 2.3−2.6 (m, 8H, $8H_{2'}$), 2.5−3.0 (m, 24H, $8H_{2'}$, $16H_{5'}$), 3.3−4 (m, 38H, $8H_{3'}$, $16H_{7'}$, 14 glycosidic H); ^{13}C NMR (25.178 MHz, D_2O) δ 28.17−34.9 ($C_{5'}$, $C_{6'}$), 41.3 ($C_{2'}$), 48.07 ($C_{3'}$), 68.4−72.4 (C_6, C_{12}, $8C_{7'}$), 73.36−82.80 (C_2, C_3, C_4, C_5, C_8, C_9, C_{10}, C_{11}), 96.5 (C_1), 103.2 (C_7), 180.25−180.8 ($C_{1'}$, CO_2^-); MS (FAB$^-$, TG) *m/z* 1861 [M + 15H − 16Na]$^-$. Anal. ($C_{68}H_{86}O_{43}S_8Na_{16}$) C, H, S.

Octa-*O*-(3-((1,2-bis(sodium oxycarbonyl)ethyl)thio)propyl)-D-lactose (18): obtained by the addition of 0.78 g of 2-mercaptosuccinic acid (5.20 mmol) on 0.215 g (0.32 mmol) of octa-*O*-allyllactose (**5**) under UV irradiation for 5.5 h; yield 60%; mp >265 °C dec; R_f 0.55 (acetone/ethyl acetate/acetic acid/water, 1/3/1/1, v/v); IR (KBr, ν cm^{-1}) 1585 (C=O), 1410 (C−O); ^{1}H NMR (250 MHz, D_2O) δ 1.8−2.1 (m, 16H, $16H_{6'}$), 2.4−

2.6 (m, 8H, $8H_{2'}$), 2.6−2.85 (m, 24H, $8H_{2'}$, $16H_{5'}$), 3.4−4.1 (m, 37H, $8H_{3'}$, $16H_{7'}$, 13 glycosidic H, H_7); MS (FAB$^-$, TG) *m*/*z* 1861 [M + 15H − 16Na]$^-$. Anal. ($C_{68}H_{86}O_{43}S_8Na_{16}$) C, H, S.

Octa-*O*-(3-((1,2-bis(sodium oxycarbonyl)ethyl)thio)-propyl)-D-maltose (19): obtained by the addition of 0.78 g of 2-mercaptosuccinic acid (5.20 mmol) on 0.375 g (0.57 mmol) of octa-*O*-allylmaltose (**6**) under UV irradiation for 5 h; yield 60%; mp 250 °C dec; R_f 0.30 (acetone/ethyl acetate/acetic acid/water, 1/3/1/1, v/v); IR (KBr, ν cm^{-1}) 1576 (C═O), 1417 (C−O); ^{1}H NMR (250 MHz, D_2O) δ 1.7−2.1 (m, 16H, $16H_{6'}$), 2.4−2.55 (m, 8H, $8H_{2'}$), 2.6−2.9 (m, 24H, $8H_{2'}$, $16H_{5'}$), 3.4−4.1 (m, 37H, $8H_{3'}$, $16H_{7'}$, 13 glycosidic H, H_7); ^{13}C NMR (25.178 MHz, D_2O) δ 28.45−29.55 ($C_{5'}$, $C_{6'}$), 41.74 ($C_{2'}$), 48.05 ($8C_{3'}$), 69.2−73.73 (C_6, C_{12}, $C_{7'}$), 78.0−85.19 (C_2, C_3, C_4, C_5, C_8, C_9, C_{10}, C_{11}), 179.0−180.58 ($C_{1'}$, CO_2^-); MS (FAB$^-$, TG) *m*/*z* 1861 [M + 15H − 16Na]$^-$. Anal. ($C_{68}H_{86}O_{43}S_8Na_{16}$) C, H, S.

Radical Addition of Sodium 2-Mercaptoethanesulfonate on Perallylated Carbohydrate. General Method. The perallylated carbohydrate (0.50 mmol) was dissolved in 25 mL of acetonitrile in a quartz reactor; the sodium 2-mercaptoethanesulfonate (1.5 equiv per allyl function) was added in solution with 10 mL of water. The mixture was degassed for 15 min with nitrogen, and 50 mg of AIBN was added. The reaction mixture was irradiated for 4 h with UV light (λ = 254 nm). The end of the reaction was determined by TLC, the solvents were eliminated by evaporation under reduced pressure, a byproduct of AIBN was discarded by filtration, and the filtrate was lyophilized. The residue was purified by exclusion column chromatography with Sephadex gel G10 or G25 eluted with pure water.

Penta-*O*-(3-(((sodium oxysulfonyl)ethyl)thio)propyl)-*β*-D-Glucopyranose (20): yield 64%; mp >265 °C dec; R_f 0.31 (acetone/ethyl acetate/acetic acid/water, 5/3/2/2, v/v); ^{1}H NMR (250 MHz, D_2O) δ 1.89 (m, 10H, $H_{5'}$), 2.65 (t, 10H, $H_{4'}$), 2.87 and 3.1 (2t, 2 × 10H, $H_{1'}$, $H_{2'}$), 3.7 (m, 16H, H_2−H_7, $10H_{7'}$), 4.38 (d, 1H, H_1, J_{1-2} = 7.9 Hz); ^{13}C NMR (25.178 MHz, D_2O) δ 26.3 ($C_{2'}$), 28.7−30.1 ($C_{5'}$), 51.9 ($C_{1'}$), 69.76−73.05 (C_6, $5C_{6'}$), 74.52, 78.67, 82.29, 84.51 (C_2, C_3, C_4, C_5), 103.17 (C_1); MS (FAB$^-$, NOBA) *m*/*z* 1177 [M − Na]$^-$, 1155 [M + H − 2Na]$^-$, 1133 [M + 2H − 3Na]$^-$. Anal. ($C_{31}H_{57}O_{21}S_{10}Na_5$) C, H, S.

Octa-*O*-(3-(((sodium oxysulfonyl)ethyl)thio)propyl)sucrose (21): yield 53%; mp >265 °C dec; R_f 0.23 (acetone/ethyl acetate/acetic acid/water, 5/3/2/2, v/v); ^{1}H NMR (250 MHz, D_2O) δ 1.78 (m, 16H, $H_{5'}$), 2.57 (t, 16H, $H_{4'}$, $J_{4'-5'}$ = 3.0 Hz), 2.76, 3.0 (2q, 2 × 16H, $H_{1'}$, $H_{2'}$), 3.65 (m, 19H, H_2−H_7, H_{10}−H_{12}, $10H_{7'}$), 4.06 (d, 1H, H_9, J_{9-10} = 7.75 Hz), 5.42 (d, 1H, H_1, J_{1-2} = 3.37 Hz); ^{13}C NMR (25.178 MHz, D_2O) δ 26.37 ($C_{2'}$), 28.6−30.2 ($C_{5'}$), 51.9 ($C_{1'}$), 69.54−72.92 (C_6, C_7, C_{12}, $8C_{6'}$), 71.2, 78.2−84.11 (C_2, C_3, C_4, C_5, C_9, C_{10}, C_{11}), 90.76 (C_1), 104.8 (C_8); MS (FAB$^-$, NOBA) *m*/*z* 1951 [M − Na]$^-$, 1929 [M + H − 2Na]$^-$. Anal. ($C_{52}H_{94}O_{35}S_{16}Na_8$) C, H, S.

Biological Methods. Antiviral Assays. Cytotoxicity measurements were based on inhibition of cell growth. The cell lines used for both the anti-HIV activity and cytotoxicity assays were CEM-4 and MT-4 cells. The different compounds were evaluated for their anti-HIV activity according to well-established procedures.[1,12,13] The origin of the viruses (human immunodeficiency virus type 1 (HIV-1) (strain HTLV-III$_B$/LAI), HIV-2 (strain LAV$_{ROD}$), herpes simplex virus type 1 (HSV-1) (strains KOS, F, and McIntyre), thymidine kinase-deficient (TK$^-$) HSV-1 (strain B2006), TK$^-$/TK$^+$ HSV-1 (strain VMW 1837), herpes simplex virus type 2 (HSV-2) (strains G, Lyons, and 196), human cytomegalovirus (HCMV) (strains AD169 and Davis), vaccinia virus, vesicular stomatitis virus, parainfluenza virus type 3, reovirus type 1, Junin virus, Tacaribe virus, Sindbis virus, Semliki forest virus, Coxsackie B4 virus, and poliovirus type 1) has been described previously.[12,13]

Syncytium Formation. MOLT-4 (clone 8) cells (1 × 10^6 cells/mL) were cultured with an equal number of HUT-78/HIV-1 or HUT-78/HIV-2 cells in microtiter tray wells containing various concentrations of the test compounds. After a 24 h cocultivation period, the number of giant cells was recorded microscopically, as described previously.[14]

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References

(1) (a) Leydet, A.; Barthelemy, Ph.; Boyer, B.; Lamaty, G.; Roque, J. P.; Bousseau, A.; Evers, M.; Henin, Y.; Snoeck, R.; Andrei, G.; Ikeda, S.; Reymen, D.; De Clercq, E. Polyanion Inhibitors of Human Immunodeficiency Virus and Other Viruses. Part I.− Polymerized Anionic Surfactants. *J. Med. Chem.* **1995**, *38*, 2433−2440. (b) Leydet, A.; El Hachemi, H.; Boyer, B.; Lamaty, G.; Roque, J. P.; Snoeck, R.; Andrei, G.; Ikeda, S.; Reymen, D.; Witvrouw, M.; De Clercq, E. Polyanion Inhibitors of Human Immunodeficiency Virus and Other Viruses. Part II − Polymerized Anionic Surfactants derived from Amino Acids and Dipeptides. *J. Med. Chem.* **1996**, *39*, 1626−1636. (c) Leydet, A.; Jeantet-Second, C.; Barthelemy, Ph.; Boyer, B.; Roque, J. P. Polyanion Inhibitors of Human Immunodeficiency Virus. Part III. − Polymerized Anionic Surfactants Derived from D-Glucose. *Recl. Trav. Chim. Pays-Bas* **1996**, *115*, 421−426. (d) Leydet, A.; Barthelemy, Ph.; Boyer, B.; Lamaty, G.; Roque, J. P.; Witvrouw, M.; De Clercq, E. Polyanions Inhibitors of Human Immunodeficiency Virus. Part IV. − Polymerized Anionic Surfactants: Influence of the density and distribution of Anionic Groups on the Antiviral Activity. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 397−402.

(2) (a) Boutevin, B.; Robin, J. J.; Boyer, B.; Roque, J. P.; Senhaji, O. Study of the telomerization reaction of an anionic surfactant monomer. *Macromol. Chem. Phys.* **1994**, *195*, 129−137. (b) Boutevin, B.; Robin, J. J.; Boyer, B.; Roque, J. P.; Senhaji, O. Study of the telomerization reaction of a cationic surfactant monomer. *Macromol. Chem. Phys.* **1994**, *195*, 2177−2185.

(3) (a) Boutevin, B.; Robin, J. J.; Boyer, B.; Roque, J. P.; Senhaji, O. Comparative study of polymerization reaction in solution of methacrylic surfactants. *Eur. Polym. J.* **1996**, *32*, 821−825. (b) Hebrant, M.; Toumi, L.; Tondre, C.; Roque, J. P.; Leydet, A.; Boyer, B. Kinetics of metal ion complex formation in micellar media. A comparison between normal and polymerized micelles. *Colloid Polym. Sci.* **1996**, *274*, 453−460.

(4) Kopecek, J.; Ulbrich, K. Biodegradation of biomedical polymers. *Prog. Polym. Sci.* **1983**, *9*, 1−58.

(5) Azori, M.; Szinai, I.; Veres, Z.; Pato, J.; Otvos, L., Jr.; Tudos, F. Polymeric prodrugs. 3. Synthesis, elimination, and whole-body distribution of carbon-14-labeled drug carrier. *Makromol. Chem.* **1986**, *187*, 297−302.

(6) Leydet, A.; Barragan, V.; Boyer, B.; Lamaty, G.; Montero, J. L.; Roque, J. P.; Witvrouw, M.; Este, J.; Snoeck, R.; Andrei, G.; De Clercq, E. Polyanion Inhibitors of Human Immunodeficiency Virus and Other Viruses. 5. Telomerized Anionic surfactants derived from amino acids. *J. Med. Chem.* **1997**, *40*, 342−349.

(7) (a) Nichols, P. L., Jr.; Yanovsky, E. Allyl Ethers of Carbohydrates. I. Preparation and Polymerization of Tetra-allyl-α-Methyl Glucoside. *J. Am. Chem. Soc.* **1944**, *66*, 1625−1627. (b) Gigg, J.; Gigg, R. The Allyl Ether as a Protecting Group in Carbohydrate Chemistry. *J. Chem. Soc. C* **1966**, 82−86. (c) Lakhimiri, R.; Lhoste, P.; Sinou, D. An improved Synthesis of Allyl Ethers of Carbohydrates. *Synth. Commun.* **1990**, *20*, 1551−1554. (d) Seto, C. T.; Mathias, J. P.; Whitesides, G. M. Molecular Self-Assembly through Hydrogen Bonding: Aggregation of five molecules to form a discrete Supramolecular Structure. *J. Am. Chem. Soc.* **1993**, *115*, 1321−1329.

(8) (a) Brimacombe, J. S.; Jones, B. D.; Stacey, M.; Willard, J. Alkylation of carbohydrates using sodium hydride. *J. Carbohydr. Res.* **1966**, *2*, 167−169. (b) Corey, E. J.; Suggs, J. W. Selective Cleavage of Allyl Ethers under mild Conditions by Transition metal Reagents. *J. Org. Chem.* **1973**, *38*, 3224.

(9) (a) Pallaud, J. F.; Pallaud, R. On the radical addition of thiols on allylic compounds with diterbutyl peroxide initiator. *C. R. Acad. Sci. (Fr).* **1970**, *270*, 2150−2153. (b) For a review on the subject, see: Griesbaum, K. Problems and Possibilities of the Free-Radical Addition of Thiols to Unsaturated Compounds. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 273−287.

(10) (a) Honma, K.; Nakazima, K.; Uematsu, T.; Hamada, A. A facile O-Glycosidation using Stannic Chloride. *Chem. Pharm. Bull.* **1976**, *24*, 394−399. (b) For a review on the subject, see: Salomon, C. J.; Mata, E. G.; Mascaretti, O. A. Recent Developments in Chemical Deprotection of Ester Functional Groups. *Tetrahedron* **1993**, *49*, 3691−3748.

(11) Witvrouw, M.; Desmyter, J.; De Clercq, E. Antiviral portrait series: 4. Polysulfates as inhibitors of HIV and other enveloped viruses. *Antiviral Chem. Chemother.* **1994**, *5*, 345−359.

(12) Witvrouw, M.; Schols, D.; Andrei, G.; Snoeck, R.; Ikeda, S.; Pauwels, R.; Van Schepdael, A.; Arnout, J.; Claes, P.; Desmyter, J.; De Clercq, E. New polyacetal polysulphate active against human immunodeficiency virus and other enveloped viruses. *Antiviral Chem. Chemother.* **1992**, *3*, 351−360.

(13) Yamamoto, N.; Schols, D.; De Clercq, E.; Debyser, Z.; Pauwels, R.; Balzarini, J.; Nakashima, H.; Baba, M.; Hosoya, M.; Snoeck, R.; Neyts, J.; Andrei, G.; Murrer, B.; Theobald, B.; Bossard, G.; Henson, G.; Abrams, M.; Picker, D. Mechanism of anti-human immunodeficiency virus action of polyoxometalates, a class of broad-spectrum antiviral agents. *Mol. Pharmacol.* **1992**, *42*, 1109−1117.

(14) Baba, M.; Schols, D.; Pauwels, R.; Nakashima, H.; De Clercq, E. Sulfated polysaccharides as potent inhibitors of HIV-induced syncytium formation: a new strategy toward AIDS chemotherapy. *J. Acquired Immune Defic. Syndr.* **1990**, *3*, 493−499.

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