Polyanion Inhibitors of HIV and Other Viruses. 7. Polyanionic Compounds and Polyzwitterionic Compounds Derived from Cyclodextrins as Inhibitors of HIV Transmission

Alain Leydet,[†] Céline Moullet,[†] Jean Pierre Roque,*[,†] Myriam Witvrouw,[‡] Christophe Pannecouque,[‡] Graciela Andrei,[‡] Robert Snoeck,[‡] Johan Neyts,[‡] Dominique Schols,[‡] and Erik De Clercq[‡]

Laboratoire de Chimie Organique Physique, Université Montpellier II, Place E. Bataillon, 34095 Montpellier Cedex 5, France, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received October 1, 1997

New polyanionic compounds were obtained from radical addition of thiomalic acid and mercaptopropionic acid onto perallylated cyclodextrins (CDs) under UV irradiation with a catalytic amount of α,α′-azobis(isobutyronitrile). All these polyanions, bearing 18−48 carboxylate groups, inhibited human immunodeficiency virus type 1 (HIV-1) strain III_B replication in MT-4 cells at a 50% inhibitory concentration (IC_{50}) of 0.1−2.9 μM, while not being toxic to the host cells at concentrations up to 62 μM. These compounds were also active against a clinical HIV-1 isolate (HE) at ≥4-fold higher concentrations. Only some compounds showed activity against the two HIV-2 strains (ROD and EHO) but at higher concentrations than those required to inhibit HIV-1 (III_B and HE) replication. In addition, these compounds were not active against the M-tropic HIV-1 strain BaL but were active against simian immunodeficiency virus [SIV (MAC_{251})]. These compounds were also inhibitory to the replication of human cytomegalovirus at an IC_{50} of 1−10 μM, but not herpes simplex virus (type 1 and type 2) or other (picorna-, toga-, reo-, orthomyxo-, paramyxo-, bunya-, rhabdo-, and poxvirus) viruses. Radical addition on perallylated CDs of a protected cysteine gave polyzwitterionic compounds. None of these last compounds proved inhibitory to the replication of HIV-1, HIV-2, or any of the other viruses tested.

Introduction

Several polyanionic compounds are markedly inhibitory to the replication of the human immunodeficiency virus (HIV).[1] They do so by blocking the interaction between the viral envelope glycoprotein gp120 and the host cell CD4 receptor, the first step of the replication process.[2] Such polyanionic inhibitors may be useful in the prophylaxis of AIDS by preventing virus transmission.[3]

However, most of the polyanions described so far as HIV inhibitors are polymers such as sulfated polysaccharides (dextran sulfate,[4] heparin,[5] lentinan sulfate,[6] etc.), aurintricarboxylic acid,[7] poly(vinyl alcohol) sulfate, and related compounds,[8] as well as polymerized surfactants.[9] Anti-HIV activity has also been reported for partially O-sulfated cyclodextrins.[10]

In the course of our studies on antiviral polyanions, we have recently shown that polyanions with a well-defined structure, based on a disaccharide core, are active against HIV provided that the number of anionic groups per molecule is high enough: antiviral activity was systematically observed when this number was equal to 16.[11] In such a case, their 50% inhibitory concentration (IC_{50}) for HIV-1 was in the range of 0.4−1.85 μM, while they were not toxic to the host cell (MT-4 or CEM-4) at concentration up to 50 μM or even higher. However when the molecules consisted of only 10 or 8 anionic groups, no activity was observed.

These results prompted us to examine if a higher inhibitory effect could result from an increase of the number of anionic groups per molecule. Therefore, we applied the synthetic route used for disaccharides to cyclodextrins (CDs) to access polyanions of well-defined structure, bearing from 18 to 48 carboxylic acid groups.

Synthesis

The syntheses consisted of perallylation of the α-, β-, and γ-cyclodextrins, followed by the radical addition of the mercapto acid as illustrated in Scheme 1. Using a suitably protected cysteine as the thiol derivative, we also prepared analogous zwitterionic compounds.

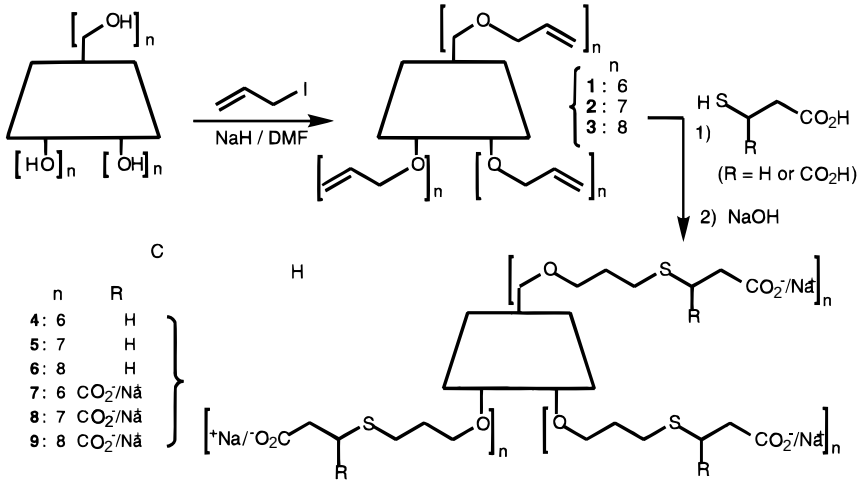
O-Perallylated Cyclodextrins. O-Perallylated CDs have not been described in the literature. The perallylation procedure involved allyl iodide as reagent in the presence of sodium hydride[12] in dimethylformamide under sonication at 0 °C. The use of allyl iodide instead of bromide led to better results. Nonoptimized yields ranged from 20% to 32%. The structures of the O-perallylated CDs **1**−**3** were proved by NMR spectroscopy as well as FAB mass spectrometry. In the latter case the $[M + Na]^+$ and $[M + H]^+$ ions were observed.

Polyanionic Derivatives. The O-perallylated CDs were allowed to react with the 3-mercaptopropanoic acid in THF, the radical addition being initiated with α,α′-azobis(isobutyronitrile) (AIBN) in catalytic amounts, under UV irradiation[13,14] (λ = 254 nm). The obtained polycarboxylic acids were transformed into their sodium salt by treatment with sodium hydroxide. The sodium polycarboxylates **4**−**6** possessed 18, 21, and 24 anionic groups, respectively.

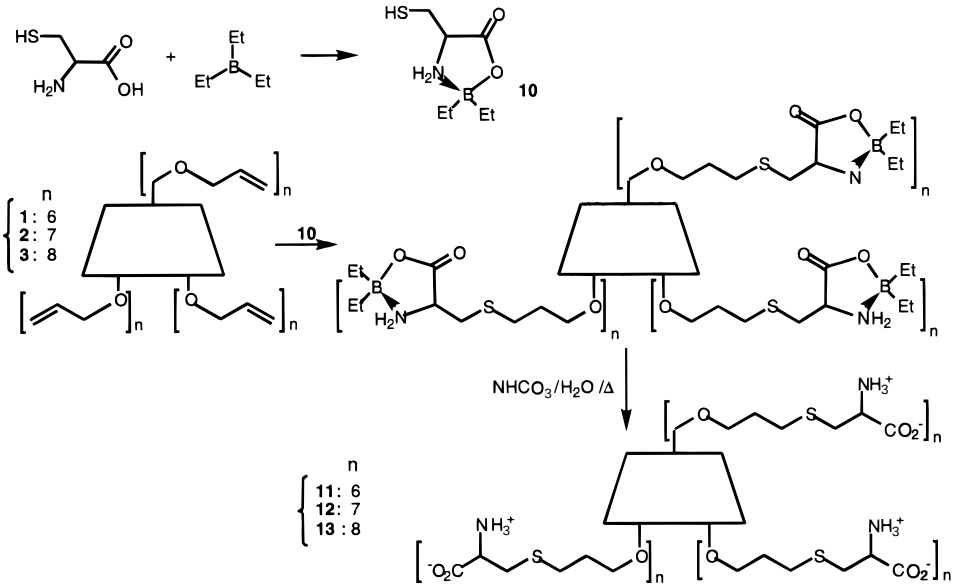
[†] Université Montpellier II.
[‡] Katholieke Universiteit Leuven.

10.1021/jm970661f CCC: $15.00
Published on Web 11/13/1998

Scheme 1



Scheme 2



To evaluate the influence on the anti-HIV activity of the local charge density or charge distribution, we pursued in the same way the addition of 2-mercaptosuccinic acid onto O-perallylated CDs. The corresponding polyanionic compounds thus obtained, **7**−**9**, bear 36, 42, and 48 carboxylate groups, respectively.

All sodium salts **4**−**9** were purified by gel permeation chromatography (Sephadex G-25, ultrapure water being used as eluent). Their structures were proved by ^{1}H and ^{13}C NMR spectroscopy. Characterization of polycarboxylates by ^{1}H and ^{13}C NMR depended on the disappearance of allyl protons and sp^2 carbon atoms of the allyl groups.

Polyzwitterionic Derivatives. Triethylborane was reacted with a suspension of cysteine in THF[15] to give the boron complex boroxazolidone **10** (Scheme 2). Thus both the amine function and the carboxylic function of the amino acid were protected. The zwitterionic compounds were obtained by the photochemical addition of the above protected cysteine on each of the perallylated CDs. Then the deprotection of the cysteine groups was run under very mild conditions, by refluxing in a methanolic solution of sodium hydrogenocarbonate,[16] leading to the polyzwitterions **11**−**13**.

Biological Results

The polyanionic and polyzwitterionic compounds were evaluated for their inhibitory effects on the replication of two different strains of HIV-1 (III_B and HE), two different strains of HIV-2 (ROD and EHO), and SIV (MAC_{251}) in MT-4 cells (Table 1).

A marked difference was observed between the two types of charged compounds. All polyanions **4**−**9** were active against different lymphocyte-tropic HIV-1 strains. They inhibited HIV-1(III_B) at low concentrations (IC_{50}: 0.1−2.9 μM), while their 50% cytotoxic concentration (CC_{50}) was rather high (>36 μM) in MT-4 cells. Detailed examination of the results revealed that compounds **7**−**9**, with anionic group numbers (36−48) twice that of compounds **4**−**6** (18−24), were at least 2-fold more inhibitory to HIV-1(III_B): IC_{50} ranging from 0.1 to 0.3 μM for compounds **7**−**9** and ranging from 0.7 to 1.4 μM for compounds **4**−**6** in MT-4 cells. The magnitude of the inhibitory effects of compounds **4**−**6** was similar to that of analogous polyanions derived from disaccharides and bearing 16 carboxylate groups.[11] Moreover, the anti-HIV-1 activity of polycarboxylates **7**−**9**, bearing 36−48 anionic groups, was comparable to

Table 1. Anti-HIV Activity of Polyanionic and Polyzwitterionic Compounds in Cell Culture

		IC_{50}[b] (μM)									
		HIV-1					HIV-2		SIV		
		III_B			HE	BaL	ROD	EHO	MAC_{251}	CC_{50}[c] (μM)	
compound	charge number[a]	MT-4	C8166	PBMC	MT-4	PBMC	MT-4	MT-4	MT-4	MT-4	PBMC
4	18	0.7	0.2	19.7	>31	>50	>31	>31	4.6	>62	50
5	21	2.9	0.3	6.1	10.4	>40	21.1	>27	2.9	>54	40
6	24	1.4	0.2	4.8	5.6	>37	12.4	19	2.6	>47	37
7	36	0.3	0.04	2.4	15.6	>5	14.8	>24	2.4	>48	5
8	42	0.3	0.01	2.1	6.8	>7	>21	>21	2.1	>41	7
9	48	0.1	0.01	1.3	5.0	>4	>18	>18	1.1	>36	4
11	36	>17					>17			17	
12	42	>15					>15			15	
13	48	>22					>22			22	
α-cyclodextrin dodecasulfate[d]	12	1.3					0.2			>1100	
β-cyclodextrin tetradecasulfate[d]	14	0.3					0.4			>1000	
γ-cyclodextrin hexadecasulfate[d]	16	0.1					0.1			>800	

[a] Total charge number including positive and negative charges in the case of zwitterionic compounds **11**−**13**. [b] 50% Inhibitory concentration, or compound concentration required to inhibit HIV-induced cytopathicity by 50%. [c] 50% Cytotoxic concentration, or compound concentration required to reduce the viability of uninfected MT-4 cells by 50%. [d] See Schols et al.[10b]

the anti-HIV-1 activity of β- and γ-cyclodextrin sulfates which bear only 14 and 16 anionic groups, respectively.

When we examined the inhibitory effect of these compounds (**4**−**9**) on the replication of HIV-1(III_B) in different cells lines, we noted a 4−30-fold higher activity when using C8166 cells, whereas HIV-1(III_B) in PBMCs was 2−30-fold less sensitive to the anti-HIV activity of the polyanions tested (**4**−**9**). Comparable activity was seen against the replication of a clinical isolate (HE) in MT-4 cells. The polyanions tested (**4**−**9**) did not show any inhibitory effect on the replication of a macrophage-tropic strain (BaL) in PBMCs. This means that the compounds may be inactive against HIV strains, using the CCR5 coreceptor to enter cells. When compounds are not active against macrophage-tropic strains, they might not be able to block transmission of HIV.

The compounds (**4**−**9**) did not inhibit the binding of a specific mAb to CXCR4, the main coreceptor for T-tropic viruses, not even at a concentration up to 100 μM (data not shown). This suggests that the compounds do not interfere with the coreceptor as such but probably target other membrane-mediated binding/fusion events.

The polycarboxylates derived from cyclodextrins differ markedly from the cyclodextrin sulfates in their inhibitory effect on HIV-2 replication. While the polycarboxylates inhibited HIV-1 replication, they did not inhibit or only very weakly inhibited the cytopathic effect of two different strains of HIV-2 (ROD and EHO) in MT-4 cells. In contrast, the sulfated cyclodextrins inhibited HIV-1(III_B) and HIV-2(ROD) at comparable concentrations. It thus appears that the structural requirements for inhibition of HIV-2-induced cytopathicity are more stringent than those for inhibition of HIV-1-induced cytopathicity. In contrast with the polyanionic compounds (**4**−**9**), the polyzwitterionic compounds (**11**−**13**) did not inhibit HIV-1 (or HIV-2) replication, at subtoxic concentrations in MT-4 cells (Table 1). The polyanionic compounds (**4**−**9**) were also active against the replication of SIV(MAC_{251}) at concentrations varying from 1.1 to 4.6 μM.

The compounds were also evaluated for their activity against a number of viruses other than HIV, such as parainfluenza-3, reovirus type 1, Sindbis, Coxsackie B4, Punta Toro, vesicular stomatitis virus, respiratory syncytial virus, herpes simplex virus (HSV-1, HSV-2), vaccinia, human cytomegalovirus (CMV), and influenza (Table 2). No appreciable activity was noted with any of the compounds against any of the viruses except for the polyanion series **4**−**9** and in particular polyanions **5** and **6** that showed activity against CMV in the 1−10 μM concentration range. None of the compounds tested were cytotoxic at concentrations below 10 μM (Table 3), except for compound **7** that was toxic to HeLa and E6SM cells at a CC_{50} of about 1 and 0.2 μM, respectively.

Experimental Section

All chemicals were purchased from Aldrich and used as received. Cyclodextrins were dried before use over P_4O_{10} at 110 °C under vacuum.

N,*N*-Dimethylformamide was distilled under reduced 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, and 20% aqueous sulfuric acid spraying. Merck silica gel 60H was used for silica gel column chromatography. Melting points were determined using a BUCHI 530 apparatus. Reactions under ultrasound were performed on a Bransonic 220 ultrasonic cleaning bath. Infrared spectra were obtained on a IR-FT BONEM MB-100 spectrometer. ^1H and ^{13}C NMR were recorded on Bruker AC200 and WP200SY spectrometers, respectively. For ^1H and ^{13}C NMR we used a numbering system as presented in Chart 1. Chemical shifts are expressed in ppm (δ). Mass spectra were recorded on a JEOL DX 100 spectrometer. The matrix used was *m*-nitrobenzylic alcohol (NOBA). Microanalyses were performed in the analytical department of the CNRS (Vernaison, Rhône, France). C, H, and S elemental analyses were done for most of the compounds.

Allylation. Sodium hydride (0.1 mol, 3 equiv per OH function) (60% in mineral oil) was washed under nitrogen with 2 × 10 mL of dry pentane. The hydride was suspended in 40 mL of dimethylformamide, and the cyclodextrin was added in a small portion. The reaction flask was put in an ultrasonic cleaning bath during 1 h under inert atmosphere; then 0.17 mol of allyl iodide (5 equiv per OH function) in solution with 20 mL of DMF was added slowly at 0−5 °C. After 2 h of stirring at room temperature, the excess of sodium hydride was annihilated with methanol. The excess of allyl iodide and DMF was eliminated under reduced pressure. The residue was diluted in 150 mL of ethyl acetate, and the mixture was washed twice with water. The organic layer was dried with

Table 2. Activity of Polyanionic and Polyzwitterionic Compounds against Several DNA and RNA Viruses, Other than HIV

		IC_{50} (μM)[a]									
virus	cell line	dextran sulfate	**4**	**5**	**6**	**7**	**8**	**9**	**11**	**12**	**13**
parainfluenza-3	Vero	>20	>50	>43	>37	>4	>33	>29	>123	>44	>39
reovirus-1	Vero	>20	>50	>43	>37	>4	>33	>29	>123	>44	>39
Sindbis	Vero	>20	>50	>43	>37	>4	>33	>29	>123	>44	>39
Coxsackie B4	Vero	>20	>50	>43	>37	>4	>33	>29	>123	>44	>39
	HeLa	>20	>25	>21	>37	>1	>16	>29	>31	>11	>10
Punta Toro	Vero	10	>50	>43	>37	>4	>33	>29	>123	>44	>39
vesicular stomatitis	HeLa	>20	>25	>21	>37	>1	>16	>29	>31	>11	>10
	E6SM	0.2	>25	>21	>19	>0.1	>16	>14	>61	>22	>39
respiratory syncytial	HeLa	4.0	>25	>21	>37	>0.1	>16	>29	>31	>11	>10
HSV-1 (KOS)	E6SM	0.2	>25	>21	>19	>0.1	>16	>14	>61	>22	>39
HSV-1 (TK-B2006)	E6SM		>25	>21	>19	>0.1	>16	>14	>61	>22	>39
HSV-1 (VMW1837)	E6SM		>25	>21	>19	>0.1	>16	>14	>61	>22	>39
HSV-2 (G)	E6SM	0.2	>25	>21	>19	>0.1	>16	14	>61	>22	>39
vaccinia	E6SM	10	>25	>21	>19	>0.5	>16	>14	>61	>22	>39
HCMV (AD-169)	HEL	0.06	7	2	0.7	8	>8	6	>13	>11	>10
HCMV (Davis)	HEL	0.08	12	5	2	10	>8	>7	>13	>11	>10
influenza (H_2N_2)	MDCK	8	>62	>11	>9	>10	>41	>36	>65	>55	>48
influenza (B)	MDCK	>200	>62	>11	>9	>10	>41	>36	>65	>55	>48
influenza (H_3N_2)	MDCK	8	>62	>11	>9	>10	>41	>36	>65	>55	>48

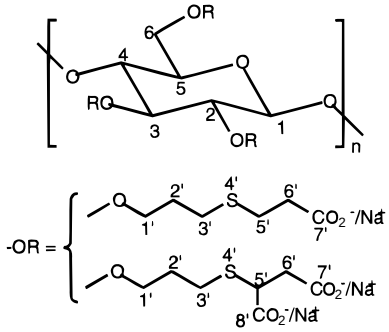
[a] 50% Inhibitory concentration, or compound concentration required to reduce virus-induced cytopathicity by 50%. Virus was added in the presence of the compounds, and the cells were further incubated until the cytopathic effect (CPE) was scored.

Table 3. Cytotoxicity of the Polyanionic and Polyzwitterionic Compounds for Different Cell Lines

cell line	MCC (μM)[a]								
	4	**5**	**6**	**7**	**8**	**9**	**11**	**12**	**13**
E6SM	50	>21	>19	0.2	33	29	>52	44	>39
HeLa	>25	43	>37	1	>16	>29	>13	22	19
Vero	>50	>43	>37	>4	>33	>29	>52	>44	>39
HEL	>12	>11	>9	>10	>8	>7	31	29	24

[a] Minimum cytotoxic concentration, or compound concentration 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%. For MT-4 cells, the cytotoxicity data are presenred in Table 1.

Chart 1



sodium sulfate and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel with cyclohexanes−ethyl acetate as eluent.

Per-*O*-allyl-α-cyclodextrin, 1: yield 24%; R_f 0.42 (cyclohexane/AcOEt, 75/25 v/v); IR (cyclohexane, ν, cm^{-1}) 3075 (Csp^2-H), 2931−2856 (Csp^3-H), 1649 (C═C); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.2−4.6 (m, 72H, $6H_2$, $6H_3$, $6H_4$, $6H_5$, $12H_6$, $36H_{1'}$), 4.9−5.3 (m, 42H, $6H_1$, $36H_{3'}$), 5.6−6.2 (m, 18H, $18H_{2'}$); ^{13}C NMR (50.32 MHz, CD_3CN) δ 37.80 (C_6), 71.03 (C_5), 69.27, 71.68, 74.12 ($C_{1'}$), 79.50, 79.63, 79.73 (C_2, C_3, C_4), 98.49 (C_1), 114.97, 115.69, 115.99 ($C_{3'}$), 135.36, 135.71, 136.83 ($C_{2'}$) MS (FAB$^+$, NBA) *m*/*z* 1715 (M + Na)$^+$, 1693 (M + H)$^+$, 1652 (M + H − CH_3−CH═CH_2)$^+$, 1635 (M + H − $HOCH_2$−CH═CH_2)$^+$.

Per-*O*-allyl-β-cyclodextrin, 2: yield 32%; R_f 0.38 (cyclohexane/AcOEt, 75/25 v/v); IR (cyclohexane, ν, cm^{-1}) 3083 (Csp^2-H), 2929−2856 (Csp^3-H), 1645 (C═C); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.3−4.6 (m, 84H, $7H_2$, $7H_3$, $7H_4$, $7H_5$, $14H_6$, $42H_{1'}$), 4.9−5.3 (m, 49H, $7H_1$, $42H_{3'}$), 5.7−6.1 (m, 21H, $21H_{2'}$); ^{13}C NMR (50.32 MHz, CD_3CN) δ 54.28 (C_6), 71.04 (C_5), 69.15, 71.65, 74.07 ($C_{1'}$), 78.31, 79.44, 79.95 (C_2, C_3, C_4), 97.87 (C_1), 115.09, 115.77, 115.86 ($C_{3'}$), 135.27, 135.62, 136.59 ($C_{2'}$); MS (FAB$^+$, NBA) *m*/*z* 1998 (M + Na)$^+$, 1976 (M + H)$^+$, 1948 (M + H − CH_2═CH_2)$^+$, 1934 (M + H −CH_3−CH═CH_2)$^+$, 1918 (M + H − $HOCH_2$−CH═CH_2)$^+$.

Per-*O*-allyl-γ-cyclodextrin, 3: yield 20%; R_f 0.40 (cyclohexane/AcOEt, 75/25 v/v); IR ($CHCl_3$, ν, cm^{-1}) 3078 (Csp^2-H), 2928−2862 (Csp^3-H), 1648 (C═C); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.1−4.7 (m, 96H, $8H_2$, $8H_3$, $8H_4$, $8H_5$, $16H_6$, $48H_{1'}$), 4.7−5.3 (m, 56H, $8H_1$, $48H_{3'}$), 5.5−6.1 (m, 24H, $24H_{2'}$); ^{13}C NMR (50.32 MHz, CD_3CN) δ 37.82 (C_6), 70.99 (C_5), 69.05, 71.61, 74.00 ($C_{1'}$), 77.56, 79.62, 79.98 (C_2, C_3, C_4), 97.54 (C_1), 115.17, 115.88, 116.46 ($C_{3'}$), 134.98, 135.23, 135.60 ($C_{2'}$); MS (FAB$^+$, NBA) *m*/*z* 2280 (M + Na)$^+$, 2257 (M + H)$^+$, 2230 (M + H − CH_2═CH_2)$^+$, 2198 (M + H − $HOCH_2$−CH═CH_2)$^+$, 2174 (M + H − 2CH_2═CH_2)$^+$.

Radical Addition of Mercapto Acid on the Perallylated Cyclodextrins. General Method: In a quartz reactor was placed 1.0 mM perallylated cyclodextrin dissolved in 40 mL of peroxide-free THF. The solution was degassed with nitrogen during 15 min, and 10 equiv per allyl function of mercaptopropionic acid or mercaptosuccinic acid was added with 50 mg of AIBN as radical initiator. The mixture was irradiated during 6 h with UV light (λ = 254 nm; Rayonet apparatus) under nitrogen atmosphere.

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

Per-*O*-(3-((sodium oxycarbonyl)ethylthio)propyl)-α-cyclodextrin, 4: yield 16%; mp > 250 °C dec; R_f 0.85 (2/2/0.5/1.5 water/AcOH/acetone/AcOEt, v/v); $[\alpha]^{22}_D$ = +33.6° (*c* = 1.4, H_2O); IR (KBr, ν, cm^{-1}) 2920−2869 (Csp^3-H), 1599 (CO_2-); ^{1}H NMR (400 MHz, D_2O) δ 1.7−1.9 (m, 36H, $36H_{6'}$), 2.3−2.7 (m, 108H, $36H_{5'}$, $36H_{3'}$, $36H_{2'}$), 3.4−3,9 (m, 72H, $6H_2$, $6H_3$, $6H_4$, $6H_5$, $12H_6$, $36H_{7'}$), 4.9−5.1 (m, 6H, $6H_1$); ^{13}C NMR (50.32 MHz, D_2O) δ 28.69 ($C_{5'}$), 29.53 ($C_{3'}$), 30.21 ($C_{2'}$), 38.44 ($C_{6'}$), 70.71 ($C_{1'}$), 73.69 (C_6), 81.52 (C_2, C_3, C_4, C_5), 98.25 (C_1), 179.60−179.77 (CO_2−). Anal. ($C_{144}H_{222}O_{66}S_{18}Na_{18}$·2$H_2O$) C, H, S.

Per-*O*-(3-((sodium oxycarbonyl)ethylthio)propyl)-β-cyclodextrin, 5: yield 69%; mp 290 °C dec; R_f 0.75 (2/2/0.5/1.5 water/AcOH/acetone/AcOEt, v/v); $[\alpha]^{22}_D$ = +44° (*c* = 1.5,

H_2O); IR (KBr, ν, cm^{-1}) 2923−2872 (Csp^3-H), 1565 (CO_2-); ^1H NMR (400 MHz, D_2O) δ 1.7−1.9 (m, 42H, 42$H_{6'}$), 2.3−2.7 (m, 126H, 42$H_{5'}$, 42$H_{3'}$, 42$H_{2'}$), 3.2−4.1 (m, 84H, 7H_2, 7H_3, 7H_4, 7H_5, 14H_6, 42$H_{7'}$), 4.95−5.2 (m, 7H, 7H_1); ^{13}C NMR (50.32 MHz, D_2O) δ 28.76 ($C_{5'}$), 29.73 ($C_{3'}$), 30.37 ($C_{2'}$), 38.32 ($C_{6'}$), 70.69 ($C_{1'}$), 73.79 (C_6), 71.94−81.31 (C_2, C_3, C_4, C_5), 98.15 (C_1), 181.28−181.58 (CO_2−). Anal. ($C_{168}H_{259}O_{77}S_{21}Na_{21}\cdot 3H_2O$) C, H, S.

Per-*O*-(3-((sodium oxycarbonyl)ethylthio)propyl)-γ-cyclodextrin, 6: yield 36%; mp > 265 °C dec; R_f 0.78 (2/2/0.5/1.5 water/AcOH/acetone/AcOEt, v/v); $[\alpha]^{22}_D = +43.5°$ (c = 1.5, H_2O); IR (KBr, ν, cm^{-1}) 2927−2872 (Csp^3-H), 1582 (CO_2−); ^1H NMR (400 MHz, D_2O) δ 1.7−1.9 (m, 48H, 48$H_{6'}$), 2.3−2.65 (m, 144H, 48$H_{5'}$, 48$H_{3'}$, 48$H_{2'}$), 3.4−4.0 (m, 96H, 8H_2, 8H_3, 8H_4, 8H_5, 16H_6, 48$H_{7'}$), 5.0−5.2 (m, 8H, 8H_1); ^{13}C NMR (50.32 MHz, D_2O) δ 28.53 ($C_{5'}$), 29.42 ($C_{3'}$), 30.33 ($C_{2'}$), 38.05 ($C_{6'}$), 70.65 ($C_{1'}$), 73.79 (C_6), 71.98−81.36 (C_2, C_3, C_4, C_5), 98.11 (C_1), 180.53−181.31 (CO_2−). Anal. ($C_{192}H_{296}O_{88}S_{24}Na_{24}\cdot 2H_2O$) C, H, S.

Per-*O*-(3-(1′,2′-bis(sodium oxycarbonyl)ethylthio)-propyl)-α-cyclodextrin, 7: yield 17%; mp > 250 °C dec; R_f 0.81 (2/2/0.5/1.5 water/AcOH/acetone/AcOEt, v/v); IR (KBr, cm^{-1}) 2927−2865 (Csp^3-H), 1589 (CO_2−); ^1H NMR (400 MHz, D_2O) δ 1.7−2.1 (m, 36H, 36$H_{6'}$), 2.3−2.8 (m, 72H, 36$H_{2'}$, 36$H_{5'}$), 3.15−4.2 (m, 90H, 6H_2, 6H_3, 6H_4, 6H_5, 12H_6, 36$H_{7'}$, 18$H_{3'}$), 4.9−5.1 (m, 6H, 6H_1); ^{13}C NMR (50.32 MHz, D_2O) δ 28.52 ($C_{3'}$), 29.99 ($C_{2'}$), 47.91 ($C_{5'}$), 41.27 ($C_{6'}$), 71.13 ($C_{1'}$), 73.91 (C_6), 71.33−81.43 (C_2, C_3, C_4, C_5), 98.90 (C_1), 179.34−180.61 (CO_2−). Anal. ($C_{162}H_{204}O_{102}S_{18}Na_{36}\cdot 2H_2O$) C, H, S.

Per-*O*-(3-(1′,2′-bis(sodium oxycarbonyl)ethylthio)-propyl)-β-cyclodextrin, 8: yield 47%; mp 255 °C dec; R_f 0.73 (2/2/0.5/1.5 water/AcOH/acetone/AcOEt, v/v); $[\alpha]^{22}_D = +32.6°$ (c = 1.5, H_2O); IR (KBr, ν, cm^{-1}) 2920−2870 (Csp^3-H), 1588 (CO_2−); ^1H NMR (400 MHz, D_2O) δ 1.7−2.0 (m, 42H, 42$H_{6'}$), 2.3−2.75 (m, 84H, 42$H_{2'}$, 42$H_{5'}$), 3.35−4.2 (m, 105H, 7H_2, 7H_3, 7H_4, 7H_5, 14H_6, 42$H_{7'}$, 21$H_{3'}$), 4.9−5.1 (m, 7H, 7H_1); ^{13}C NMR (50.32 MHz, D_2O) δ 28.58 ($C_{3'}$), 29.86−30.54 ($C_{2'}$), 48.10 ($C_{5'}$), 41.43 ($C_{6'}$), 70.39−70.93 ($C_{1'}$), 73.69 (C_6), 70.93−81.03 (C_2, C_3, C_4, C_5), 98.50 (C_1), 178.92−180.0 (CO_2−). Anal. ($C_{189}H_{238}O_{119}S_{21}Na_{42}\cdot 2H_2O$) C, H, S.

Per-*O*-(3-(1′,2′-bis(sodium oxycarbonyl)ethylthio)-propyl)-γ-cyclodextrin, 9: yield 29%; mp > 265 °C dec; R_f 0.77 (2/2/0.5/1.5 water/AcOH/acetone/AcOEt, v/v); $[\alpha]^{22}_D = +34.6°$ (c = 1.5, H_2O); IR (KBr, cm^{-1}) 2927−2872 (Csp^3-H), 1582 (C═O); ^1H NMR (400 MHz, D_2O) δ 1.7−2.1 (m, 48H, 48$H_{6'}$), 2.3−2.9 (m, 96H, 48$H_{2'}$, 48$H_{5'}$), 3.2−4.1 (m, 120H, 8H_2, 8H_3, 8H_4, 8H_5, 16H_6, 48$H_{7'}$, 24$H_{3'}$), 4.85−5.2 (m, 8H, 8H_1); ^{13}C NMR (50.32 MHz, D_2O) δ 29.74 ($C_{3'}$), 30.56 ($C_{2'}$), 41.34 ($C_{6'}$), 47.48 ($C_{5'}$), 70.48−70.81 ($C_{1'}$), 73.50 (C_6), 72.50−82.00 (C_2, C_3, C_4, C_5), 94.43 (C_1), 179.93−179.98 (CO_2−). Anal. ($C_{216}H_{272}O_{136}S_{24}Na_{48}\cdot 2H_2O$) C, H, S.

Boroxazolidone Cysteine Complex 10. Finely ground cysteine (10 mM) was suspended in freshly distilled THF (10 mL), then a 1 M solution of triethylborane (12 mmol) in THF (12 mL) was added, and the mixture was stirred until the cysteine dissolved (about 30 min). THF was eliminated under reduced pressure. The obtained oil was treated with cyclohexane; the product was collected by filtration and washed with cyclohexane: yield 93%; mp 90 °C; R_f 0.65 (AcOEt); $[\alpha]^{22}_D$ −4.8° (c = 1, DMF); IR (KBr, ν, cm^{-1}) 3230, 3180, 3030 (N−H), 1700 (C═O); ^1H NMR (250.13 MHz, $CDCl_3$) δ 0.30−0.50 (sext, 4H, 2 CH_2), 0.70−0.90 (m, 6H, 2 CH_3), 1.40−1.55 (q, 1H, SH), 2.75−2.90 (m, 1H, CH_{2a}), 3.3−3.5 (dt, 1H, CH_{2b}), 4.05−4.20 (m, 1H, CH), 4.60−4.80 (m, 1H, NH_2), 5.60−5.80 (m, 1H, NH_2); ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 11.26−11.40 ($CH_{2\ ethyl}$), 14.84−15.06 (CH_3), 28.21 (CH_2), 58.34 (C*H), 176.29 (C═O); MS (FAB$^+$, NBA) m/z 190 (M + H)$^+$. Anal. ($C_7H_{16}BNO_2S$).

Radical Addition of the Boroxazolidone on the Perallylated Cyclodextrins. In a quartz reactor was placed the perallylated cyclodextrin (0.5 mmol) dissolved in 40 mL of peroxide-free THF. The solution was degassed with nitrogen during 15 min, and 2 equiv per allyl function of the boroxazolidone was added with 50 mg of AIBN as radical initiator. The mixture was irradiated during 8 h with UV light (λ = 254 nm; Rayonet apparatus) under nitrogen atmosphere. The solvent was evaporated under reduced pressure, and the residue was directly deprotected, without any further treatment and characterization. The product was dissolved in 10 mL of a 50/50 mixture of saturated aqueous $NaHCO_3$/methanol and refluxed for about 2.5 h. The crude product was poured onto crushed ice and neutralized with 2 N aqueous HCl. Then, 50 mL ethanol was added. The precipitate formed was washed with THF and EtOH, then filtered, and dried under reduced pressure.

Per-*O*-(3-(2-amino-2-carboxyethyl)thiopropyl)-α-cyclodextrin, 11: yield 35%; mp 200 °C dec; $[\alpha]^{22}_D = -13.5°$ (c = 1.4, H_2O); IR (KBr, ν, cm^{-1}) 2920, 3033 (N−H), 1599 (C═O); ^1H NMR (400 MHz, D_2O) δ 1.70−1.85 (m, 36H, 36$H_{2'}$), 2.45−2.60 (m, 36H, 36$H_{3'}$), 2.80−3.10 (m, 36H, 36$H_{5'}$), 3.15−3.30 (m, 18H, 18$H_{6'}$), 3.40−3.80 (m, 72H, 36$H_{\alpha-CD}$, 36$H_{1'}$); ^{13}C NMR (50.32 MHz, D_2O) δ 28.88 ($C_{3'}$), 33.50 ($C_{2'}$), 38.77 ($C_{5'}$), 54.06−54.33 ($C_{6'}$), 70.00 ($C_{1'}$), 71.53 (C_6), 82.77−83.82 (4C, C_2, C_3, C_4, C_5), 94.54 (C_1), 173.67 (COO^-). Anal. ($C_{144}H_{240}N_{18}O_{66}S_{18}\cdot 4H_2O$) C, H, S.

Per-*O*-(3-(2-amino-2-carboxyethyl)thiopropyl)-β-cyclodextrin, 12: yield 32%; mp 200 °C dec; $[\alpha]^{22}_D = -41.1°$ (c = 1.4, H_2O); IR (KBr, ν, cm^{-1}) 2920, 3037 (N−H), 1618 (C═O); ^1H NMR (400 MHz, D_2O) δ 1.80−2.00 (m, 42H, 42$H_{2'}$), 2.50−2.80 (m, 42H, 42$H_{3'}$), 2.80−3.20 (m, 42H, 42$H_{5'}$), 3.20−3.40 (m, 21H, 21$H_{6'}$), 3.45−3.90 (m, 84H, 42$H_{\beta-CD}$, 42$H_{1'}$); ^{13}C NMR (50.32 MHz, D_2O) δ 28.91−30.00 ($C_{3'}$), 33.49 ($C_{2'}$), 39.93 ($C_{5'}$), 54.45−54.99 ($C_{6'}$), 70.33 (2C, C_6, $C_{1'}$), 80.96 (4C, C_2, C_3, C_4, C_5), 94.58 (C_1), 172.93−173.42 (COO^-). Anal. ($C_{168}H_{280}N_{21}O_{77}S_{21}\cdot 3H_2O$) C, H, S.

Per-*O*-(3-(2-amino-2-carboxyethyl)thiopropyl)-γ-cyclodextrin, 13: yield 49%; mp 200 °C dec; (c = 1.4, H_2O); ^1H NMR (400 MHz, D_2O) δ 1.75−1.90 (m, 48H, 48$H_{2'}$), 2.50−2.65 (m, 48H, 48$H_{3'}$), 2.90−3.10 (m, 48H, 48$H_{5'}$), 3,15−3.30 (m, 24H, 24$H_{6'}$), 3.40−3.90 (m, 96H, 48$H_{\gamma-CD}$, 48$H_{1'}$); ^{13}C NMR (50.32 MHz, D_2O) δ 28.23 ($C_{3'}$), 36.33 ($C_{2'}$), 39.66 ($C_{5'}$), 56.72 ($C_{6'}$), 67.32 ($C_{1'}$), 70.28 (C_6), 84.28 (4C, C_2, C_3, C_4, C_5), 94.52 (C_1), 162.70 (COO^-). Anal. ($C_{192}H_{320}N_{24}O_{88}S_{24}\cdot 5H_2O$) C, H, S.

Biological Methods. 1. Viruses. The virus strains used were as follows:[17] herpes simplex virus type 1 (HSV-1 strain KOS) and type 2 (HSV-2 strain G), thymidine kinase-deficient (TK^-) HSV-1 (B2006), human cytomegalovirus (CMV) [strain Davis (ATCC VR-807) and strain AD-169 (ATCC VR-538)], vesicular stomatitis virus (VSV), Coxsackie B4, parainfluenza virus type 3 (ATCC VR-93), reovirus type 1 (ATCC VR-230), Sindbis virus, Punta Toro virus (ATCC VR-559), influenza A virus [(H_3N_2) X_{31} strain, H_2N_2 (A_2/Japan/305/57)], influenza B virus (strain Hong Kong/5/72), respiratory syncytial virus (RSV strain Long), human immunodeficiency virus [HIV-1 (III_B, HE, BaL) and HIV-2 (ROD, EHO)], and simian immunodeficiency virus [SIV (MAC_{251})].

2. Antiviral Assays. In all the assays the compounds were added to the cell cultures together with the virus inoculum. For all viruses (except for CMV, influenza virus, RSV, and HIV), confluent cultures of human embryonic skin muscle (E_6-SM), HeLa, or Vero cells in microtiter trays were inoculated with virus at 100 times the $CCID_{50}$ (50% cell culture infective dose) per well. Virus-induced cytopathicity was recorded at 1−2 days postinfection (pi) for VSV, at 2 days for Coxsackie virus, at 2−3 days for HSV-1, HSV-2, TK^- HSV-1, and Sindbis virus, and at 5 days for reovirus and Punta Toro virus. For the anti-CMV assays, human embryonic lung (HEL) fibroblasts in microtiter trays were infected with 100 PFU (plaque-forming units) of CMV per well in the presence of compounds. Virus-induced cytopathicity was recorded at 7 days pi. Anti-influenza virus activity was assessed in Madin-Darby canine kidney (MDCK) cells, and anti-RSV activity was assessed in HeLa cells, both following infection with 20 $CCID_{50}$ of virus. Evaluation of the anti-HIV activity of the compounds in MT-4, C8166, and PBMC was assessed as described previously.[17c−f]

3. Cytotoxicity Measurements. Measurements were based on either microscopically detectable alteration of normal

cell morphology (E_6SM, Vero, HeLa, MDCK) or inhibition of cell growth (HEL) or reduction of cell viability (MT-4 and PBMC).

Acknowledgment. We are grateful to Wacker Chimie S.A. for the generous gift of cyclodextrin samples. These investigations were supported in part by the Biomedical Research Programme of the European Commission and by grants from the Belgian National Fonds voor Wetenschappelijk Onderzoek and the Belgian Geconcerteerde Onderzoeksacties. We thank Anita Camps, Sandra Claes, Frieda Demeyer, Kristien Erven, Erik Fonteyn, Cindy Heens, and Anita Van Lierde for excellent technical assistance.

References

(1) (a) Baba, M.; Nakajima, M.; Schols, D.; Pauwels, R.; Balzarini, J.; De Clercq, E. Pentosanpolysulfate, a Sulfated Oligosaccharide, is a Potent and Selective Anti-HIV agent in vitro. *Antiviral Res.* **1988**, *9*, 335−343. (b) Haseltine, W. B. Replication and Pathogenesis of AIDS Virus. *J. Acquir. Immune Defic. Syndr.* **1988**, *1*, 217−240. (c) De Clercq, E. Chemotherapeutic Approaches to the Treatment of the Acquired Immune Deficiency Syndrome (AIDS). *J. Med. Chem.* **1986**, *29*, 1561−1569.

(2) (a) Baba, M.; Pauwels, R.; Balzarini, J.; Desmyter, J.; De Clercq, E. Mechanism of Inhibitory Effect of Dextran Sulphate and Heparin on Replication of Human Immunodeficiency Virus in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6132−6136. (b) Mitsuya, H.; Looney, D. J.; Kuno, S.; Ueno, R.; Wong-Staal, F.; Broder, S. Dextran Sulphate Suppression of Virus in the HIV Family: Inhibition of Virion Binding to CD4+ cells. *Science* **1988**, *240*, 646−649.

(3) Neyts, J.; De Clercq, E. Effect of polyanionic compounds on intracutaneous and intravaginal herpes virus infection in mice: impact on the search for vaginal microbicides with anti-HIV activity. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **1995**, *10*, 8−12.

(4) Ueno, R.; Kuno, S. Dextran Sulfate, a Potent Anti-HIV Agent in vitro having Synergism with Zidovudine. *Lancet* **1987**, *1*, 1379.

(5) (a) Ito, M.; Baba, M.; Pauwels, R.; De Clercq, E.; Shigeta, S. Inhibitory Effect of Dextran Sulfate and Heparin on the Replication of Human Immunodeficiency Virus (HIV) in vitro. *Antiviral Res.* **1987**, *7*, 361−367. (b) Cushman, M.; Wang, P.; Chang, S. H.; Wild, C.; De Clercq, E.; Schols, D.; Goldman, M. E.; Bowen, J. A. Preparation and Anti-HIV Activities of Aurintri-carboxylic Acid Fractions and Analogues: Direct Correlation of Antiviral Potency with Molecular Weight. *J. Med. Chem.* **1991**, *34*, 329−337.

(6) Yoshida, O.; Nakashima, H.; Toshida, T.; Kanedo, Y.; Matsuzaki, K.; Uryu, T.; Yamamoto, N. Sulfatation of the Immunomodulating Polysaccharide Lentinan: a Novel Strategy for Antivirals to Human Immunodeficiency Virus (HIV). *Biochem. Pharmacol.* **1988**, *37*, 2887−2889.

(7) (a) Balzarini, J.; Mitsuya, H.; De Clercq, E.; Broder, S. Aurintrocarboxylic Acid and Evans Blue Represent Two Different Classes of Anionic Compounds wich Selectively Inhibit the Cytopathogenicity of Human T-cell Lymphotropic Virus Type III/ Lymphadenopathy-associated Virus. *Biochem. Biophys. Res. Commun.* **1986**, *136*, 64−71. (b) Cushman, M.; Wang, P.; Chang, S. H.; Wild, C.; De Clercq, E.; Schols, D.; Goldman, M. E.; Bowen, J. A. Preparation and Anti-HIV Activities of Aurintricarboxylic Acid Fractions and Analogues: Direct Correlation of Antiviral Potency with Molecular Weight. *J. Med. Chem.* **1991**, *34*, 329−337.

(8) Baba, M.; Schols, D.; De Clercq, E.; Pauwels, R.; Nagy, M.; Györgyi-Edelényi, J.; Löw, M.; Görög, S. Novel Sulfated Polymers as Highly Potent and Selective Inhibitors of Human Immunodeficiency Virus Replication and Giant Cell Formation. *Antimicrob. Agents Chemother.* **1990**, *34*, 134−138.

(9) (a) Leydet, A.; Barthélémy, P.; 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 Others Viruses. Part I.- Polymerized Anionic Surfactants. *J. Med. Chem.* **1995**, *38*, 2433. **(**b) Leydet, A.; El Hachemi, H.; Boyer, B.; Lamaty, G.; Roque, J. P.; Schols, D.; Snoeck, R.; Andrei, G.; Ikeda, S.; Neyts, J.; Reymen, D.; Este, J.; Witvrouw, M.; De Clercq, E. Polyanion inhibitors of human immunodeficiency virus and others viruses. Part II. Polymerized anionic surfactants derived from amino acids and dipeptides. *J. Med. Chem.* **1996**, *39*, 1626. (c) Leydet, A.; Jeantet-Seconds, C.; Barthélémy, P.; Boyer, B.; Roque, J. P. Polyanion inhibitors of human immunodeficiency virus. Part III.- Polymerized anionic surfactants derived from glucose. *Recl. Trav. Chim. Pays-Bas* **1996**, *115*, 421−426. *(*d) Leydet, A.; Barthélémy, P.; Boyer, B.; Lamaty, G.; Roque, J. P.; Witvrouw, M.; De Clercq, E. Polyanion 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.

(10) (a) Anand, R.; Nayyar, S.; Pitha, J.; Merril, C. R. Sulphated Sugar Alpha-Cyclodextrin Sulphate, a Uniquely Potent Anti-HIV Agent, also Exhibits Marked Synergism with AZT, and Lymphoproliferative Activity. *Antiviral Chem. Chemother.* **1990**, *1*, 41−46. (b) Schols, D.; De Clercq, E.; Witvrouw, M.; Nakashima, H.; Snoeck, R.; Pauwels, R.; Van Schepdael, A.; Claes, P. Sulphated Cyclodextrins Are Potent Anti-HIV Agents Acting Synergistically with 2′,3′-Dideoxynucleoside Analogues. *Antiviral Chem. Chemother.* **1991**, *2*, 45−53. (c) Moriya, T.; Kurita, H.; Matsumoto, K.; Otake, T.; Mori, H.; Morimoto, M.; Ueba, N.; Kunita, N. Potent Inhibitory Effect of a Series of Modified Cyclodextrin Sulfates (mCDS) on the Replication of HIV-1 *in vitro*. *J. Med. Chem.* **1991**, *34*, 2301−2304. (d) Moriya, T.; Saito, K.; Kurita, H.; Matsumoto, K.; Otake, T.; Mori, H.; Morimoto, M.; Ueba, N.; Kunita, N. A New Candidate for an Anti-HIV-1 Agent: Modified Cyclodextrin sulfate (mCDS71). *J. Med. Chem.* **1993**, *36*, 1674−1677. (e) Otake, T.; Schols, D.; Witvrouw, M.; Naesens, L.; Nakashima, H.; Moriya, T.; Kurita, H.; Matsumoto, K.; Ueba, N.; De Clercq, E. Modified Cyclodextrin Sulphates (mCDS11) Have Potent Inhibitory Activity against HIV and Oral Bioavailability. *Antiviral Chem. Chemother.* **1994**, *5*, 155−161.

(11) Leydet, A.; Jeantet-Segonds, C.; Bouchitté, C.; Moullet, C.; Boyer, B.; Roque, J. P.; Witvrouw, M.; Este, J.; Snoeck, R.; Andrei, G.; De Clercq, E. Polyanion Inhibitors of Human Immunodeficiency Virus and Others Viruses. Part VI − Micelle Like Anti-HIV Polyanionic Compounds Based on a Carbohydrate Core. *J. Med. Chem.* **1997**, *40*, 350−356.

(12) Brimacombes, J. S.; Jones, B. D.; Stacey, M.; Willard, J. Alkylation of Carbohydrates using Sodium Hydride. *J. Carbohydr. Res.* **1966**, *2*, 167−169.

(13) Pallaud, J. F.; Pallaud, R. On the Radical Addition of Thiols on Allylic Compounds with Diterbutylperoxide Initiator. *C. R. Acad. Sci. (Fr.)* **1970**, *270*, 2150−2153.

(14) 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.

(15) Nefkens, G. H. L.; Zwanenburg, B. Boroxazolidones as Simultaneous Protection of the Amino and Carboxyl Group in α-Amino Acids. *Tetrahedron* **1983**, *39*, 2995−2998.

(16) Yang, L.; Weber, A. E.; Greenlee, W. J.; Patchett, A. A. Macrocyclic Renin Inhibitors: Synthesis of a Subnanomolar, Orally Active Cysteine Derived Inhibitor. *Tetrahedron Lett.* **1993**, *34*, 44, 7035−7038.

(17) (a) Ikeda, S.; Neyts, J.; Verma, S.; Wickramasinghe, A.; Mohan, P.; De Clercq, E. In vitro and in vivo inhibition of ortho- and paramyxovirus infections by a new class of sulfonic acid polymers interacting with virus-cell binding and/or fusion. *Antimicrob. Agents Chemother.* **1994**, *38*, 256−259. (b) Schols, D.; De Clercq, E.; Balzarini, J.; Baba, M.; Witvrouw, M.; Hosoya, M.; Andrei, G.; Snoeck, R.; Neyts, J.; Pauwels, R.; Nagy, M.; Györgi-Edelényi, J.; Machovich, R.; Horwath, I.; Löw, M.; Görög, S. Sulphated polymers are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, respiratory syncytial virus, and toga-, and arena- and retroviruses. *Antiviral Chem. Chemother.* **1990**, *1*, 233−240. (c) Vandamme, A.-M.; Witvrouw, M.; Pannecouque, C.; Balzarini, J.; Schmit, J.-C.; Desmyter, J.; De Clercq, E. Evaluating clinical isolates for their phenotypic and genotypic resistance against anti-HIV drugs. In *Methods in Molecular Biology: Antiviral Testing*; Kinchington, D., Shinazi, R. F., Eds.; The Humana Press: Totowa, NJ; in press, 42p. (d) 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. (e) Witvrouw, M.; Balzarini, J.; Pannecouque, C.; Jhaumeer-Laulloo, S.; Esté, J. A.; Schols, D.; Cherepanov, P.; Schmit, J.-C.; Debyser, Z.; Vandamme, A.-M.; Desmyter, J.; Ramadas, S. R.; De Clercq, E. SRR-SB3, a disufide-containing macrolide that inhibits a late stage of the replicative cycle of human immunodeficiency virus. *Antimicrob. Agents Chemother.* **1997**, *41*, 262−268. (f) 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.

JM970661F