Extension of the Polyanionic Cosalane Pharmacophore as a Strategy for Increasing Anti-HIV Potency

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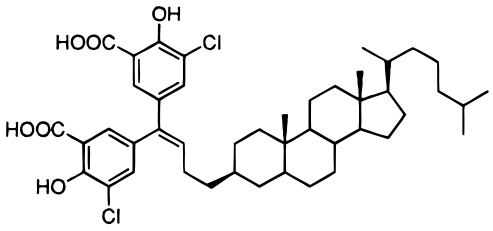
Received December 23, 1998

The anti-HIV agent cosalane inhibits both the binding of gp120 to CD4 as well as an undefined postattachment event prior to reverse transcription. Several cosalane analogues having an extended polyanionic "pharmacophore" were designed based on a hypothetical model of the binding of cosalane to CD4. The analogues were synthesized, and a number of them displayed anti-HIV activity. One of the new analogues was found to possess enhanced potency as an anti-HIV agent relative to cosalane itself. Although the new analogues inhibited both HIV-1 and HIV-2, they were more potent as inhibitors of HIV-1 than HIV-2. Mechanism of action studies indicated that the most potent of the new analogues inhibited fusion of the viral envelope with the cell membrane at lower concentrations than it inhibited attachment, suggesting inhibition of fusion as the primary mechanism of action.

Introduction

The binding of gp120 to CD4, as well as the subsequent postbinding fusion events, offers potentially useful targets for the development of new anti-HIV agents. A number of years ago, we reported the design and synthesis of cosalane **(1)**, a novel anti-HIV agent which inhibits both the initial attachment of the virus to the cell membrane as well as a postattachment event occurring prior to reverse transcription.[1,2] Although cosalane **(1)** has a number of positive features including synergy with AZT and activity against a wide spectrum of laboratory, drug-resistant, and clinical HIV-1 and HIV-2 isolates, its potency is moderate, ranging from an EC_{50} of 3.2 μM vs the IIIB strain in CEM-SS cells to 80.2 μM vs the VIHU (NSI) isolate in CEM-SS cells. A number of cosalane analogues have been synthesized, but none of them offer significantly enhanced potencies relative to cosalane **(1)** itself.[3−7] The present study was undertaken in order to design and synthesize more potent cosalane analogues.



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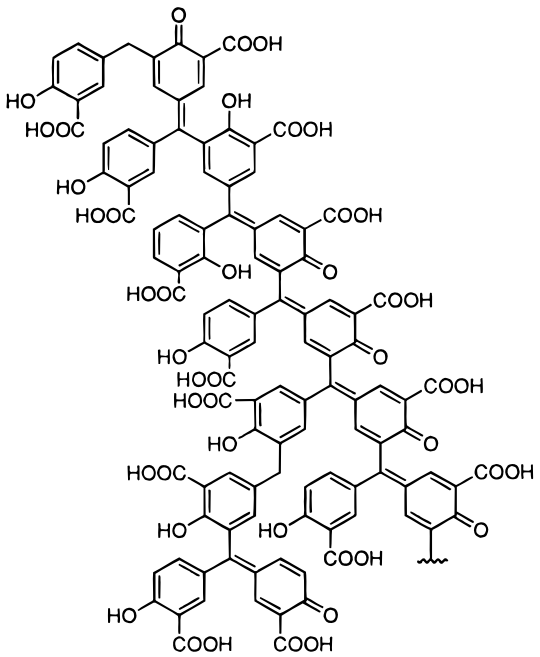

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Design

Prior studies have shown that the inhibition of gp120 to CD4 binding by cosalane (**1**) results from its ability to bind to both gp120 and CD4.[8] Since the structure of CD4 has been determined by X-ray crystallography,[9] it is possible to construct hypothetical models of the binding of cosalane (**1**) to CD4 and to use these models for designing more effective cosalane analogues.[10] Since the D1D2 region of CD4 contains residues that have been implicated in binding to gp120, it is logical to look

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10.1021/jm980727m CCC: $18.00
Published on Web 04/28/1999

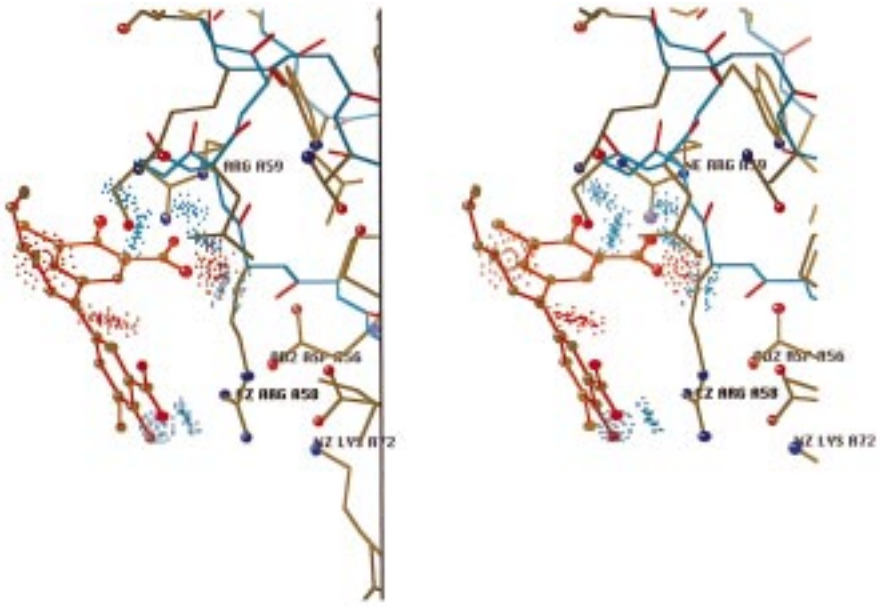


Figure 1. Hypothetical model of the binding of the cosalane "pharmacophore" to CD4 (programmed for walleyed viewing): blue dots, attraction; red dots, repulsion.

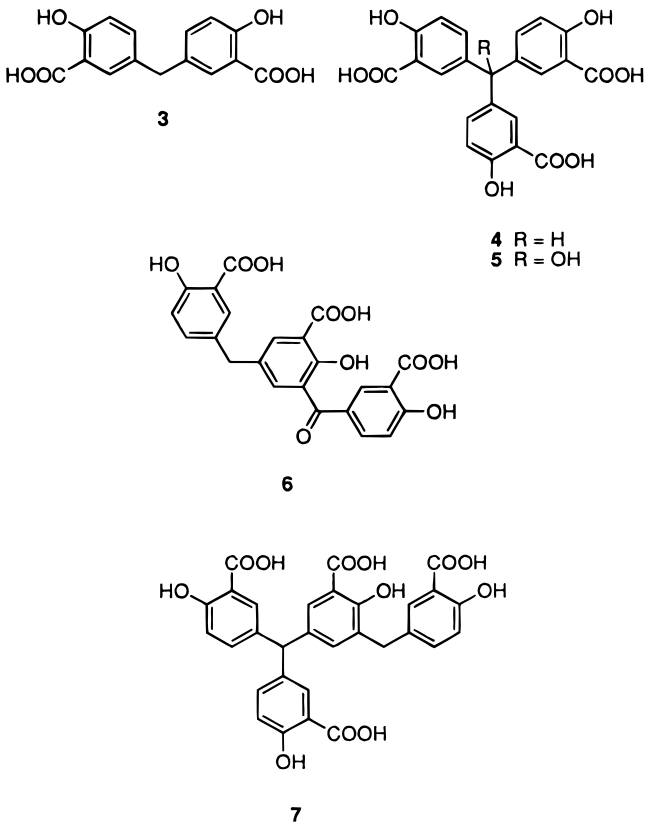
there for potential cosalane binding sites.[9,11−13] Residues Lys29, Lys35, Phe43, Leu44, Lys46, Gly47, and Arg59 of CD4 have been implicated as being directly involved in binding to gp120. In one study, it was reported that the alteration of Arg59 "dramatically disrupted the ability of CD4 to bind to gp120".[12] Prior studies have indicated that the disalicylmethane moiety of cosalane is the "pharmacophore", which makes it reasonable to look for possible modes of binding of this fragment to substructures within the D1D2 region of CD4. These considerations led to the conclusion that the two negatively charged carboxylate residues of cosalane may be binding to the two adjacent, positively charged Arg58 and Arg59 residues of CD4. A hypothetical model based on this idea is shown in Figure 1. The model was constructed by placing the two carboxylates of cosalane next to the guanidinium residues of Arg58 and Arg59 and "freezing" the structure of the protein while allowing the ligand to move (Sculpt software, Interactive Simulations, Inc.).

Although the model portrayed in Figure 1 is speculative, it provides a possible starting point for the design of more potent cosalane analogues. There is another basic residue, Lys72, in close proximity to the proposed binding site, and this could be targeted through the incorporation of additional anionic groups on aromatic rings that could be added to the structure of cosalane. Accordingly, in the present investigation, benzyl groups were attached to the two phenolic oxygens of cosalane through ether linkages, and carboxylic acid and nitro groups were attached in the ortho, meta, and para positions. The resulting compounds were tested for inhibition of the cytopathic effect of HIV-1_{RF} in CEM cell cultures and the activities compared with that of cosalane itself. This would allow an examination of the effect of the location of the new carboxyl groups and nitro groups on anti-HIV activity.

The idea to extend the cosalane pharmacophore to include additional aromatic rings with carboxyl groups is also supported by a second line of reasoning. Cosalane was originally designed on the basis of our work with aurintricarboxylic acid (ATA), a heterogeneous mixture of polymers that forms when salicylic acid is treated with formaldehyde in the presence of sulfuric acid and sodium nitrite.[14−18] The anti-HIV activity of ATA has been well-documented and results from its ability to inhibit the attachment of gp120 to CD4.[16,19,20] On the basis of the isolation and structure elucidation of low-molecular-weight ATA oligomers, a schematic representation (**2**) of the "structure" of ATA was proposed. This schematic representation does not depict the structure of ATA "literally", since ATA is a very complex heterogeneous mixture of polymers, and it is therefore impossible to know its "structure". Nevertheless, during the process of investigating the structures and anti-HIV activities of low-molecular-weight ATA components, several compounds were isolated which retained some of the anti-HIV activity of ATA, although their potencies were lower than that of ATA.[17] Specifically, some of the compounds isolated and their anti-HIV-1_{IIIB} activities in CEM cells were **3** (EC_{50} >350 μM), **4** (EC_{50} 77 μM), **5** (EC_{50} 110 μM), **6** (EC_{50} 380 μM), and **7** (EC_{50} 92 μM). Although these EC_{50} values are significantly higher than the corresponding value for cosalane (EC_{50} 3.2 μM), the comparison of the activities of **3** with **4**−**7** suggests that the potency of cosalane might be increased through the incorporation of additional aromatic rings having carboxylic acid groups. This idea, in conjunction with the molecular modeling results discussed above, provided a strong case for extension of the cosalane polyanionic pharmacophore as a possible method for increasing potency.

Synthesis

The syntheses of the three desired cosalane benzyl ethers **11a**−**c** containing ortho, meta, and para carboxylic acid groups, as well as the three nitro compounds **11d**−**f**, are outlined in Scheme 1. Bromination of methyl 2-methylbenzoate with *N*-bromosuccinimide and dibenzoyl peroxide in carbon tetrachloride afforded methyl 2-(bromomethyl)benzoate **8a**.[21,22] The remaining benzyl bromides **8b**−**f** were available commercially.



Reaction of cosalane **(1)** with the benzyl bromides **8a**−**f** in DMF in the presence of potassium carbonate afforded the corresponding products **9a**−**f** in which the two phenolic hydroxyl groups as well as the two carboxyl groups had been alkylated. The saponifications of the ester groups of **9a**−**f** were carried out in aqueous ethanol in the presence of potassium carbonate to afford **10a**−**f**. In the cases of **9a**−**c**, the hydrolysis reactions were catalyzed by potassium cyanide. The four acid groups present in **10a**−**c** and the two acid groups present in **10d**−**f** were converted to the corresponding sodium salts **11a**−**f** using sodium carbonate in methanol or ethanol.

Biological Results and Discussion

All of the new cosalane analogues were tested for their ability to inhibit the cytopathic effect of HIV-1_{RF} in CEM-SS cells, of HIV-1_{IIIB} in MT-4 cells, and of HIV-2-ROD in MT-4 cells, and the resulting EC_{50} values are listed in Table 1. Intermediates **9a**−**f** were all inactive as anti-HIV agents, and they are not listed in Table 1. The two most potent analogues proved to be compound **10c**, having two benzyl ether groups bearing para carboxylic acid groups, and the corresponding tetrasodium salt **11c**. These two compounds were more potent than cosalane itself against both HIV-1_{RF} in CEM-SS cells and HIV-1_{IIIB} in MT-4 cells. Compounds **10c** and **11c** were also active versus HIV-2-ROD in MT-4 cells, but they were less potent than they were versus the two HIV-1 strains tested. The meta acid **10b** and its salt **11b** were only slightly less active versus HIV-1_{IIIB} than the para acid and salt **10c** and **11c**, respectively, but they were approximately 10-fold less active versus HIV-1_{RF}. These meta isomers were intermediate in activity versus the para and ortho isomers when tested versus both HIV-1_{RF} and HIV-1_{IIIB}. On the other hand, the corresponding ortho acid and salt **10a** and **11a** were significantly less active than cosalane in all of the anti-HIV assays, and they were the least active of the regioisomers.

Table 1. Anti-HIV Activities of Cosalane Analogues

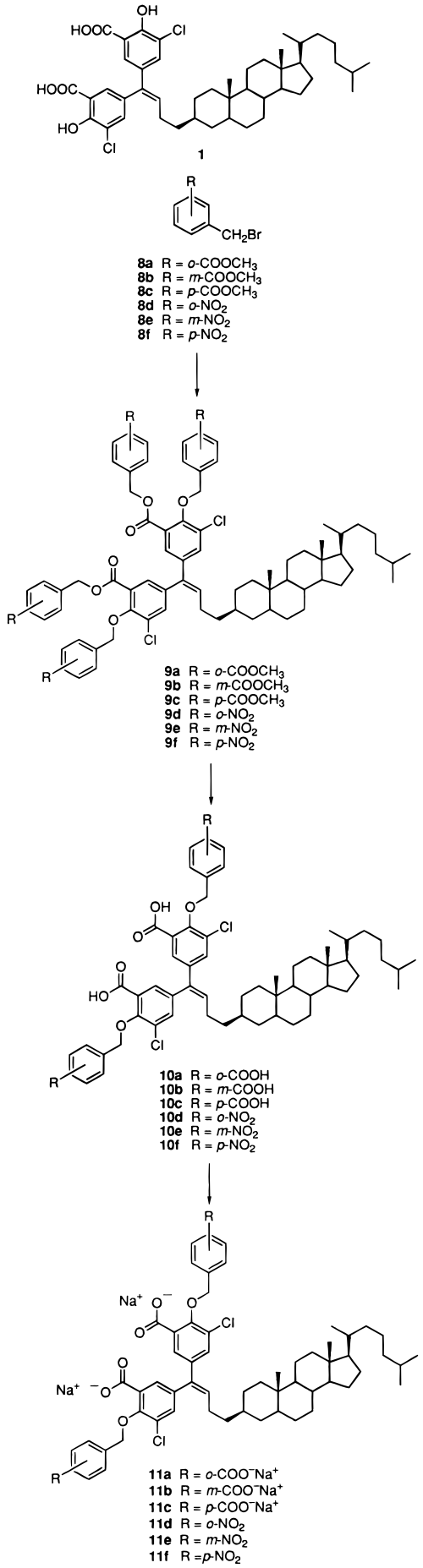
	EC_{50}[a] (μM)			cytotoxicity (μM)	
compd	HIV-1_{RF}[c]	HIV-1_{IIIB}[d]	HIV-2_{ROD}[d]	CEM-SS cells[b]	MT-4 cells[b]
1	5.1	3.0	4.0	>200	>125
10a	39.8	>37	>37	>316	37
10b	5.7	2.2	>29	>316	29
10c	0.5	1.7	22	72	88
10d	NA[e]	>125	>125	16	108
10e	NA[e]	>125	>125	66	97
10f	NA[e]	>83	>83	>316	83
11a	17.9	22.1	>52	>316	52
11b	7.6	2.4	>35	>316	35
11c	0.8	1.4	55	76	95
11d	12.2	>125	>125	>316	99
11e	21.5	48	29	>316	>125
11f	78.2	80	33	>316	>125

[a] The concentration required to reduce the cytopathic effect of the virus by 50%. [b] The concentration required for a 50% reduction in cellular viability in uninfected cells. [c] Determined in CEM-SS cells. [d] Determined in MT-4 cells. [e] No activity was observed up to a concentration of 125 μM.

With regard to the nitro acids **10d**−**f** and nitro salts **11d**−**f**, one might expect on the basis of the results with the acids **10a**−**c** and **11a**−**c** that the ortho nitro isomers **10d** and **11d** would be less active as anti-HIV agents than the para nitro isomers **10f** and **11f**. This expectation was borne out for the HIV-1_{IIIB} strain in MT-4 and for HIV-2_{ROD} in MT-4 cells, but not for HIV-1_{RF} in CEM-SS cells. In addition, the effect on potency resulting from replacement of the two carboxylic acid moieties on the benzyl groups with nitro groups is not consistent. For example, in the case of the para carboxylate **11c** versus HIV-1_{RF} in CEM-SS cells, the EC_{50} increases substantially from 0.8 to 78.2 μM in **11f** when the carboxylate group is replaced by a nitro group. On the other hand, replacement of the carboxylate group in **11a** with a nitro group resulted in a small decrease in the EC_{50} value from 17.9 μM in **11a** to 12.2 μM in **11d**.

To determine if the two most active compounds (**10c** and **11c**) could possibly interact with all three (Arg58, Arg59, and Lys72) residues of CD4, the structure of the "pharmacophore" of **10c** was docked on the surface of the protein. The structure of the protein was "frozen" and the energy of the bound complex was minimized using a procedure similar to that used to derive the model in Figure 1. This resulted in the structure shown in Figure 2. The positively charged guanidinium ions on the side chains of Arg58 and Arg59 interact by ionic bonding with two of the negatively charged carboxylates of the ligand. In addition, the oxygen atom of a third carboxylate residue is capable of ionic bonding to the positively charged amino group of Lys72. This model supports the possibility that three of the four carboxylates of the ligand could bind to the protein as depicted in Figure 2. In contrast, when the least potent of the three tetracarboxylate analogues (**10a**) was docked in a similar fashion as **10c**, it was calculated to be capable of binding ionically to Arg58 and Lys72, but the carboxylate on the "upper" ring in Figure 3 was clearly not positioned to bind to Arg59 (Figure 3). On the other

Scheme 1



hand, an alternative model can be constructed for the binding of **10a** to CD4 in which the four carboxylates are positioned to have possible attractive interactions with the side chains of Lys72, Arg58, and Arg59 and the backbone carbonyl of Arg59 (Figure 4). Therefore, it cannot be argued that the hypothetical models have any meaningful quantitative predictive value for in vitro antiviral potency, since the most potent of the tetracarboxylates **(10c)** as well as the least potent **(10a)** can both be docked favorably in the proposed binding site of CD4.

Although cosalane inhibits HIV-1 reverse transcriptase, integrase, and protease in cell-free systems, the available evidence indicates that the primary mechanism of the anti-HIV action of cosalane involves inhibition of gp120−CD4 binding as well as inhibition of a postattachment event prior to reverse transcription.[2] To gain some insight into the mechanism of action of the most potent analogue **10c** and its salt **11c**, both compounds were tested as inhibitors of HIV-1 attachment, fusion, reverse transcriptase, integrase, protease, and infection. The results of these mechanistic studies are presented in Figures 5 and 6 for analogues **10c** and **11c**, respectively. It is unlikely that inhibition of reverse transcriptase, integrase, and protease by these compounds would be responsible for their antiviral action, since **10c** and **11c** are inactive as inhibitors of protease and integrase and very weak inhibitors of reverse transcriptase with the concentration required for 50% inhibition approaching 100 μM. Both compounds are more potent inhibitors of attachment and fusion, but they are clearly more potent as inhibitors of a fusion event than attachment. The relative potencies of the compounds as inhibitors of attachment and fusion suggest that the inhibition of infection is due to inhibition of fusion rather than attachment. If fusion, rather than attachment, is the primary target for the cosalane analogues **10c** and **11c**, then they should be modeled on the coreceptors (CXCR4 or CCR5) and/or gp41/gp120.

Additional studies were undertaken in order to pinpoint the mechanism of action of **10c** and **11c** in more detail. These involved investigation of the effects of the compounds on the binding of 12G5 antibody to the second extracellular loop of CXCR4, inhibition of binding of the Leu3a monoclonal antibody to the CD4 receptor, inhibition of the binding of recombinant gp120 to MT-4 cells, and inhibition of the binding of monoclonal antibody to the V3 loop of gp120 expressed in HIV-1-infected cells. The results of these investigations are detailed in Table 2. The results indicate that the compounds bind to CD4 and consequently inhibit the binding of gp120 to the cell. They also interact with the V3 loop of gp120, which is important for fusion, but they do not interact with the second extracellular loop of CXCR4. As the CD4 receptor is in close proximity with a chemokine receptor, an interaction with a chemokine receptor cannot be excluded when a compound interacts with CD4. However, a direct interaction with the second extracellular loop of CXCR4, as measured by the binding of a mAb (12G5) against CXCR4, was not observed. It is possible that **10c** and **11c** could bind to a domain other than the second extracellular loop of CXCR4, which would then prevent the proper association of the gp120−CD4 complex with the CXCR4 receptor. In any

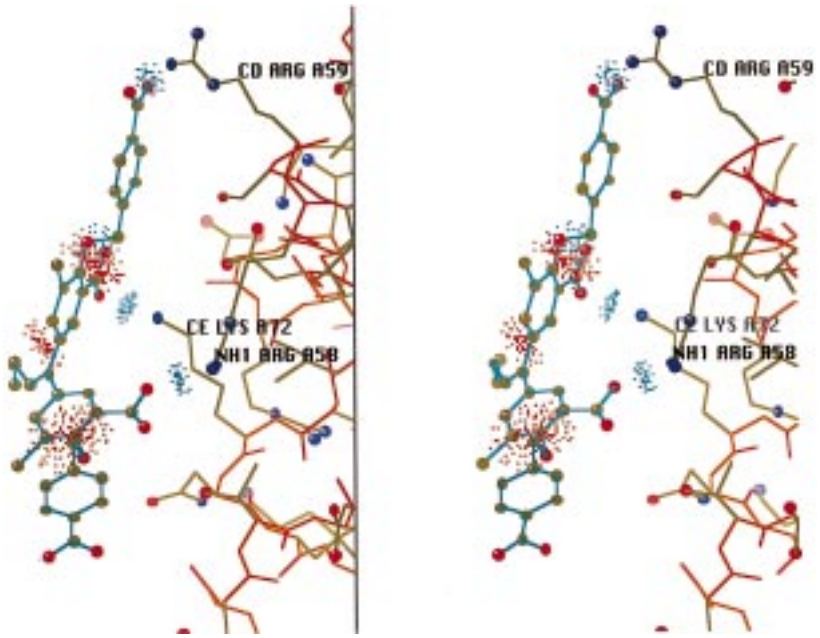


Figure 2. Hypothetical model of the binding of the **10c** "pharmacophore" to CD4 (programmed for walleyed viewing): blue dots, attraction; red dots, repulsion.

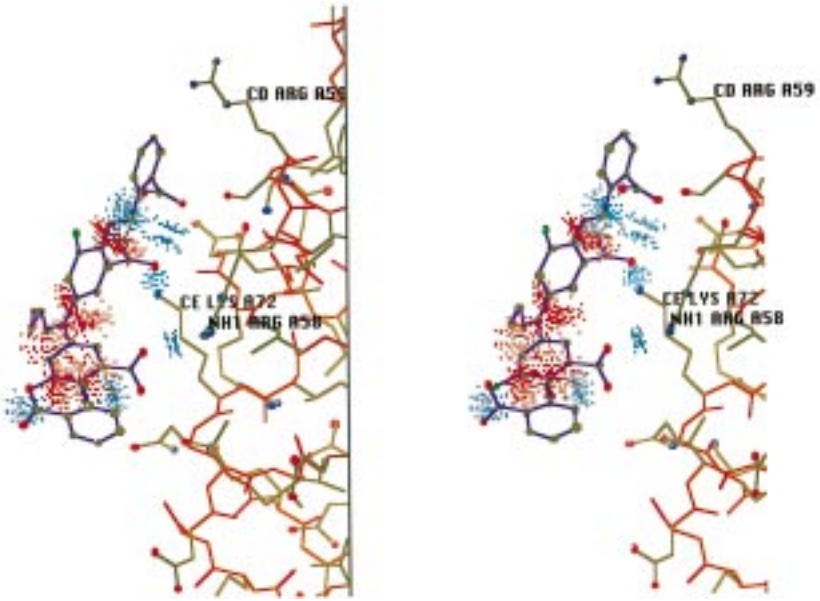


Figure 3. Hypothetical model of the binding of the **10a** "pharmacophore" to CD4 (programmed for walleyed viewing): blue dots, attraction; red dots, repulsion.

case, it appears likely that multiple mechanisms are operating. One mechanism may be a direct interference of the gp120 interaction with CD4, and the other mechanism may prevent the proper orientation of the gp120−CD4 complex with CXCR4 that is required for fusion.

In conclusion, the cosalane analogues **10c** and **11c** are the most potent analogues of cosalane synthesized to date. In contrast to cosalane itself, both compounds are either very weak or inactive as inhibitors of reverse transcriptase, integrase, and protease in cell-free systems, but they resemble cosalane in their ability to inhibit attachment and fusion. Studies are in progress on the mechanism of fusion inhibition observed with these compounds.

Experimental Section

Melting points are uncorrected. Nuclear magnetic resonance spectra for proton (^{1}H NMR) were recorded on a 300-MHz spectrometer. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as internal standard. Elemental analyses were performed by the Purdue Microanalytical Laboratory.

Methyl 2-(Bromomethyl)benzoate (8a). Methyl 2-methylbenzoate (1.50 g, 10 mmol) was dissolved in carbon tetrachloride (85 mL), and *N*-bromosuccinimide (1.81 g, 10 mmol) and dibenzoyl peroxide (52 mg) were added. The resulting mixture was heated at reflux for 12 h and stirred overnight. The precipitated succinimide was filtered off, and the filtrate was concentrated and flash chromatographed (SiO_2, 50 g; hexanes/ether, 9:1) to yield a colorless liquid (1.84 g, 80%) which turned yellow upon exposure to light: IR (neat) 3067, 3026, 2998, 2951, 2840, 1767, 1720, 1600, 1578, 1491, 1434, 1289, 1268, 1225, 1202, 1191, 1150, 1115, 1077, 1046, 965, 873, 840, 798, 761, 739, 708, 664, 610, 572 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, J = 7.6 Hz, 1 H), 7.53−7.44 (m, 2 H), 7.38 (m, 1 H), 4.96 (s, 2 H), 3.95 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 139.2, 132.5, 131.6, 131.2, 128.5, 52.2, 31.5. Anal. ($C_9H_9O_2Br$) C, H.

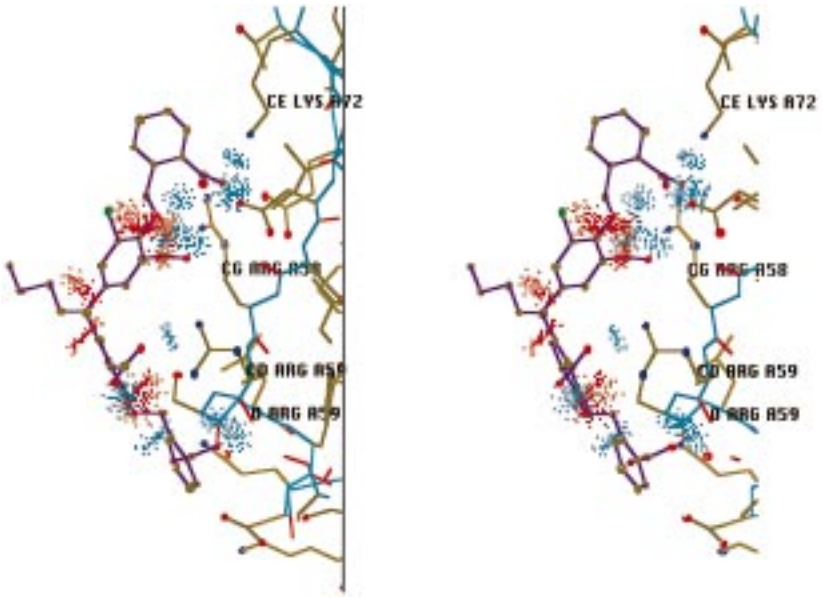


Figure 4. Hypothetical model of the binding of the **10a** "pharmacophore" to CD4 (programmed for walleyed viewing): blue dots, attraction; red dots, repulsion.

Table 2. Effects of Compounds **10c** and **11c** on the Binding of the mAb (12G5) to the CXCR4 Receptor, Binding of the mAb (Leu3a) to the CD4 Receptor, Binding of Recombinant gp120 (strain IIIB) to CD4+ T-Cell Lines, and Binding of the mAb (NEA9305) to the V3 Loop of gp120 Expressed on HIV-1-Infected MT-4 Cells

		CXCR4[a]		CD4[b]		gp120[b]		V3 loop interaction[b]	
compd	concn (μM)	% positive cells	% inhibn	% positive cells	% inhibn	% positive cells	% inhibn	% positive cells	% inhibn
10c	92	99.8	0	1.4	100	6.8	99	23.8	76
	18	99.9	0	1.2	100	8.3	98	34.4	58
	3.7	99.9	0	7.8	94	25.3	79	58.4	16
	0.73	99.4	0	99.1	4	88.2	9	65.9	3
	0.15	99.3	0	99.9	0				
11c	81	99.8	0	1.2	100	7.8	98	26.9	69
	16	99.9	0	2.1	100	6.9	99	33.6	58
	3.2	99.8	0	3.2	99	19.9	82	60.9	15
	0.65	99.2	0	99.9	0	76.2	22	66.6	5
	0.13	99.1	0	99.8	0				
controls	0	99.1	0	99.9	0	95.6	0	70.3	0
	isotype mAb	2.1		1.5		6.2		8.4	

[a] The assay was performed as previously described.[29] [b] The assay was performed as previously described.[16]

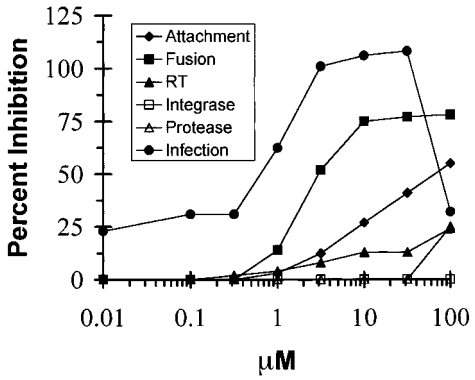


Figure 5. Mechanism of action studies of **10c**.

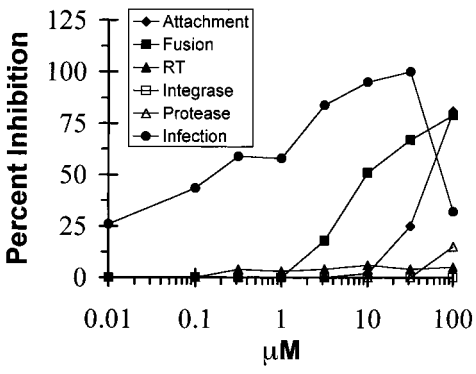


Figure 6. Mechanism of action studies of **11c**.

3″,3‴-Dichloro-4″,4‴-bis(*o*-carboxybenzyloxy)-5″,5‴-bis(*o*-carboxybenzyloxycarbonyl)-4′,4′-diphenyl-3β-[1-(3′-butenyl)]cholestane Tetramethyl Ester (9a). A mixture of cosalane (**1**) (308 mg, 0.4 mmol), potassium carbonate (280 mg, 2.03 mmol), and methyl 2-(bromomethyl)benzoate **(8a)** (404 mg, 1.76 mmol) in DMF (4 mL) was stirred at ambient temperature for 16 h. The solution was poured into iced water (14 mL) and extracted with EtOAc (4 × 10 mL). The organic layers were pooled, dried with Na_2SO_4, and evaporated, and the residue was purified by flash chromatography on silica gel (100 g), eluting with hexanes−ethyl acetate (5:2), to give a white foamy solid (466 mg, 86%): mp (softens at 43 °C) 92−94 °C; IR ($CHCl_3$) 3022, 2924, 2850, 1766, 1727, 1602, 1580, 1490, 1467, 1437, 1405, 1371, 1267, 1216, 1168, 1138, 1085, 1052, 997, 895, 767, 668 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07−7.92 (m, 4 H), 7.89 (dd, J = 7.4, 1.6 Hz, 2 H), 7.69 (d, J = 2.4 Hz, 1 H), 7.65 (d, J = 2.1 Hz, 1 H), 7.54 (dd AB pattern, J = 7.6, 6.6 Hz, 2 H), 7.47−7.22 (m, 8 H), 7.40 (d, J = 2.3 Hz, 1 H), 7.36 (d, J = 2.3 Hz, 1 H), 6.13 (t, J = 7.5 Hz, 1 H), 5.67 (s, 2 H), 5.64 (s, 2 H), 5.56 (s, 2 H), 5.48 (s, 2 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 2.16 (q, J = 7.3 Hz, 2

H), 1.94 (d, J = 12.0 Hz, 1 H), 1.88−1.71 (m, 1 H), 1.71−0.95 (m, 32 H), 0.89 (d, J = 6.6 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.72 (s, 3 H), 0.63 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 165.2, 164.8, 153.9, 153.6, 140.0, 139.8, 139.0, 137.3, 136.8, 135.9, 135.3, 134.1, 132.6, 132.3, 131.4, 130.8, 130.4, 130.1, 129.8, 128.4, 128.3, 127.7, 127.2, 127.1, 126.8, 73.9, 73.8, 65.6, 56.6, 56.3, 54.7, 52.1, 51.9, 46.6, 42.6, 40.2, 39.5, 38.6, 37.6, 37.3, 36.2, 36.1, 35.8, 35.6, 32.1, 29.0, 28.9, 28.3, 28.0, 27.4, 24.2, 23.9, 22.8, 22.6, 21.0, 18.7, 12.3, 12.1. Anal. ($C_{81}H_{92}Cl_2O_{14}$) C, H.

3″,3‴-Dichloro-4″,4‴-bis(*m*-carboxybenzyloxy)-5″,5‴-bis(*m*-carboxybenzyloxycarbonyl)-4′,4′-diphenyl-3β-[1-(3′-butenyl)]cholestane Tetramethyl Ester (9b). Cosalane (**1**) (308 mg, 0.4 mmol), potassium carbonate (280 mg), and methyl 3-(bromomethyl)benzoate (**8b**) (403 mg, 1.76 mmol) in DMF (4 mL) were stirred at room temperature for 16 h. The solution was poured into iced water (14 mL) and extracted with EtOAc (4 × 10 mL). The organic layers were pooled, dried with Na_2SO_4, and evaporated, and the residue was purified by flash chromatography on silica gel (40 g), eluting with hexanes−ethyl acetate (9:4), to give a white foamy solid (456 mg, 84%): mp (softens at 43 °C) 92−94 °C; IR ($CHCl_3$) 3020, 2951, 2926, 2850, 1722, 1593, 1468, 1450, 1437, 1372, 1289, 1212, 1167, 1109, 1088, 973, 752, 668 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J = 13.4 Hz, 2 H), 8.04−7.91 (m, 6 H), 7.68−7.57 (m, 4 H), 7.57−7.47 (m, 2 H), 7.47−7.30 (m, 6 H), 6.12 (t, J = 7.5 Hz, 1 H), 5.35 (s, 2 H), 5.33 (s, 2 H), 5.14 (s, 2 H), 5.06 (s, 2 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 3.88 (s, 6 H), 2.11 (q, J = 7.3 Hz, 2 H), 1.94 (d, J = 12.0 Hz, 1 H), 1.87−1.70 (m, 1 H), 1.70−0.94 (m, 32 H), 0.89 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 6 H), 0.71 (s, 3 H), 0.63 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 166.4, 165.1, 164.8, 153.4, 153.0, 138.9, 137.0, 136.5, 136.0, 135.7, 135.3, 134.2, 132.6, 132.2, 131.3, 130.8, 130.5, 130.2, 130.0, 129.7, 129.5, 129.3, 129.2, 128.7, 128.6, 128.4, 128.3, 127.1, 126.9, 75.1, 66.7, 56.5, 56.3, 54.6, 52.0, 46.5, 42.5, 40.0, 39.5, 38.5, 37.5, 37.1, 36.1, 36.0, 35.7, 35.5, 32.1, 28.9, 28.8, 28.2, 27.9, 27.3, 24.1, 23.8, 22.7, 22.5, 21.0, 18.6, 12.2, 12.0. Anal. ($C_{81}H_{92}Cl_2O_{14}$) C, H.

3″,3‴-Dichloro-4″,4‴-bis(*p*-carboxybenzyloxy)-5″,5‴-bis(*p*-carboxybenzyloxycarbonyl)-4′,4′-diphenyl-3β-[1-(3′-butenyl)]cholestane Tetramethyl Ester (9c). Cosalane (**1**) (616 mg, 0.8 mmol), potassium carbonate (560 mg), and *p*-carboxymethylbenzyl bromide (**8c**) (806 mg, 3.52 mmol) in DMF (8 mL) were stirred at room temperature for 24 h. The solution was poured into iced water (30 mL) and extracted with EtOAc (4 × 20 mL). The organic layers were pooled, dried with Na_2SO_4, and evaporated, and the residue was purified by flash chromatography on silica gel (120 g), eluting with hexanes−ethyl acetate (2:1), to give a white foamy solid (1.05 g, 96%): mp (starts softening at 59 °C) 132 °C; IR ($CHCl_3$) 3027, 2953, 2929, 2850, 1722, 1616, 1470, 1437, 1417, 1374, 1283, 1194, 1179, 1168, 1111, 1021, 970 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, J = 6.5 Hz, 2 H), 7.99 (d, J = 6.5 Hz, 2 H), 7.94 (d, J = 7.8 Hz, 4 H), 7.61−7.56 (m, 2 H), 7.52−7.42 (m, 4 H), 7.42−7.33 (m, 6 H), 6.13 (t, J = 7.6 Hz, 1 H), 5.34 (s, 2 H), 5.32 (s, 2 H), 5.15 (s, 2 H), 5.07 (s, 2 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.91 (s, 3 H), 3.89 (s, 3 H), 2.09 (q, J = 7.6 Hz, 2 H), 1.95 (d, J = 12.0 Hz, 1 H), 1.88−1.70 (m, 1 H), 1.70−0.92 (m, 32 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 6 H), 0.71 (s, 3 H), 0.64 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 166.4, 165.0, 164.7, 153.4, 153.1, 141.6, 140.2, 139.0, 136.5, 136.0, 135.4, 134.3, 132.6, 131.3, 130.1, 129.8, 129.7, 129.6, 128.3, 127.8, 127.4, 127.0, 126.8, 75.1, 66.6, 56.6, 56.3, 54.6, 52.1, 52.0, 46.5, 42.6, 40.1, 39.5, 38.5, 37.5, 37.2, 36.2, 36.0, 35.8, 35.5, 32.1, 29.0, 28.8, 28.2, 28.0, 27.3, 24.2, 23.8, 22.7, 22.5, 21.0, 18.6, 12.3, 12.0. Anal. ($C_{81}H_{92}Cl_2O_{14}$) C, H.

5α-3β-[4,4-(3′,3″-Bis(carbo-*o*-nitrobenzyloxy)-5′,5″-dichloro-4′,4″-bis(*o*-nitrobenzyloxy)diphenyl)buten-3-yl]cholestane (9d). Cosalane (**1**) (308 mg, 0.4 mmol), potassium carbonate (280 mg), and *o*-nitrobenzyl bromide (**8d**) (380 mg, 1.76 mmol) in DMF (3 mL) were stirred at room temperature for 16 h. The solution was poured into iced water (20 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were pooled, dried with Na_2SO_4, and evaporated, and the residue was purified by flash chromatography on silica gel (100 g), eluent hexane/ethyl acetate, 2:1, to give a pale-yellow foamy solid (491 mg, 94%): mp (starts softening at 65 °C) 102−103 °C; IR ($CHCl_3$) 3021, 2926, 2851, 1732, 1614, 1579, 1529, 1467, 1373, 1344, 1278, 1247, 1215, 1166, 1094, 1050, 1002, 896, 860, 754, 669 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20−8.05 (m, 4 H), 8.02−7.94 (m, 2 H), 7.68 (tdd, J = 7.7, 4.5, 1.0 Hz, 2 H), 7.65 (d, J = 2.2 Hz, 1 H), 7.63 (d, J = 2.4 Hz, 1 H), 7.55−7.36 (m, 10 H), 6.16 (t, J = 7.6 Hz, 1 H), 5.63 (s, 2 H), 5.60 (s, 2 H), 5.53 (s, 2 H), 5.45 (s, 2 H), 2.17 (q, J = 7.4 Hz, 2 H), 1.92 (d, J = 12.1 Hz, 1 H), 1.83−1.70 (m, 1 H), 1.70−0.91 (m, 32 H), 0.87 (d, J = 6.5 Hz, 3 H), 0.84 (d, J = 6.6 Hz, 6 H), 0.70 (s, 3 H), 0.61 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.4, 164.2, 153.2, 146.3, 139.4, 136.4, 135.8, 134.8, 134.0, 133.7, 133.6, 133.0, 131.6, 131.4, 131.2, 130.2, 130.0, 129.4, 129.3, 129.1, 129.0, 128.7, 128.5, 128.0, 126.6, 126.3, 125.0, 124.7, 72.2, 64.1, 56.6, 56.3, 54.7, 46.6, 42.6, 40.1, 39.5, 38.6, 37.6, 37.2, 36.2, 36.1, 35.8, 35.6, 32.2, 29.1, 28.9, 28.3, 28.0, 27.4, 24.2, 23.9, 22.8, 22.6, 21.0, 18.7, 12.3, 12.1. Anal. ($C_{73}H_{80}Cl_2N_4O_{14}$) C, H, N.

5α-3β-[4,4-(3′,3″-Bis(carbo-*m*-nitrobenzyloxy)-5′,5″-dichloro-4′,4″-bis(*m*-nitrobenzyloxy)diphenyl)buten-3-yl]-cholestane (9e). Cosalane **(1)** (308 mg, 0.4 mmol), potassium carbonate (280 mg, 2.03 mmol), and *m*-nitrobenzyl bromide (**8e**) (380 mg, 1.76 mmol) in DMF (3 mL) were stirred at room temperature for 16 h. The solution was poured into iced water (14 mL) and extracted with EtOAc (4 × 10 mL). The organic layers were pooled, dried with Na_2SO_4, and evaporated, and the residue was purified by flash chromatography on silica gel (80 g), eluent hexane/ethyl acetate, 2:1, to give a white foamy solid (507 mg, 97%): mp (starts softening at 53 °C) 128 °C; IR ($CHCl_3$) 3028, 2927, 2851, 1731, 1532, 1470, 1352, 1247, 1195, 1166, 1094, 1013, 980, 896, 806, 760, 753, 731, 691, 668 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (s, 1 H), 8.28 (s, 1 H), 8.22−8.15 (m, 4 H), 8.15 (s, 1 H), 8.12 (s, 1 H), 7.79 (t, J = 8.2 Hz, 2 H), 7.68 (t, J = 7.8 Hz, 2 H), 7.62 (t apparent, J = 2.5 Hz, 2 H), 7.59−7.44 (m, 4 H), 7.44 (d, J = 2.2 Hz, 1 H), 7.42 (d, J = 2.4 Hz, 1 H), 6.16 (t, J = 7.6 Hz, 1 H), 5.41 (s, 2 H), 5.40 (s, 2 H), 5.22 (s, 2 H), 5.14 (s, 2 H), 2.13 (q, J = 7.5 Hz, 2 H), 1.95 (d, J = 12.1 Hz, 1 H), 1.87−1.70 (m, 1 H), 1.70−0.92 (m, 32 H), 0.89 (d, J = 6.5 Hz, 3 H), 0.86 (d, J = 6.7 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.71 (s, 3 H), 0.63 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.7, 164.4, 153.3, 153.0, 148.4, 139.3, 138.7, 137.4, 136.4, 136.3, 135.8, 134.9, 134.0, 133.5, 133.4, 133.0, 131.4, 130.2, 129.9, 129.7, 129.4, 128.5, 126.7, 126.5, 123.4, 123.1, 122.9, 122.5, 74.2, 65.9, 56.6, 56.4, 54.7, 46.6, 42.6, 40.1, 39.5, 38.6, 37.6, 37.2, 36.2, 36.1, 35.8, 35.6, 32.2, 29.1, 28.9, 28.2, 28.0, 27.4, 24.2, 23.9, 22.8, 22.6, 21.0, 18.7, 12.3, 12.1. Anal. ($C_{73}H_{80}Cl_2N_4O_{14}$) C, H, N.

5α-3β-[4,4-(3′,3″-Bis(carbo-*p*-nitrobenzyloxy)-5′,5″-dichloro-4′,4″-bis(*p*-nitrobenzyloxy)diphenyl)buten-3-yl]cholestane (9f). Cosalane **(1)** (230 mg, 0.3 mmol), potassium carbonate (210 mg), and *p*-nitrobenzyl bromide **(8f)** (285 mg, 1.32 mmol) in DMF (2 mL) were stirred at room temperature for 16 h. The solution was poured into iced water (10 mL) and extracted with EtOAc (3 × 10 mL). The organic layers were pooled, dried with Na_2SO_4, and evaporated, and the residue was purified by flash chromatography on silica gel (60 g), eluent hexane/ethyl acetate, 2:1, to give a white foamy solid (389 mg, 99%): mp (starts softening at 71 °C) 106−109 °C; IR ($CHCl_3$) 3113, 3082, 3031, 2926, 2852, 1732, 1608, 1523, 1496, 1470, 1404, 1348, 1281, 1248, 1195, 1166, 1110, 1092, 1014, 857 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (d, J = 7.8 Hz, 2 H), 8.20 (d, J = 7.8 Hz, 2 H), 8.13 (d, J = 8.4 Hz, 4 H), 7.69−7.40 (m, 12 H), 6.18 (t, J = 7.6 Hz, 1 H), 5.40 (s, 2 H), 5.37 (s, 2 H), 5.24 (s, 2 H), 5.15 (s, 2 H), 2.14 (q, J = 7.2 Hz, 2 H), 1.94 (d, J = 12.1 Hz, 1 H), 1.87−1.70 (m, 1 H), 1.70−0.92 (m, 32 H), 0.88 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 6 H), 0.71 (s, 3 H), 0.63 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.3, 164.1, 153.3, 153.0, 147.7, 147.6, 143.7, 142.4, 142.3, 139.2, 136.3, 136.1, 135.8, 134.7, 132.8, 131.3, 130.2, 129.9, 128.4, 128.3, 128.0, 126.5, 126.2, 123.8, 123.6, 123.5, 74.3, 65.8, 65.7, 56.5, 56.2, 54.6, 46.5, 42.5, 40.0, 39.4, 38.5, 37.5, 37.2, 36.1, 36.0,

35.7, 35.5, 35.4, 32.1, 29.0, 28.8, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.0, 18.6, 12.2, 12.0. Anal. ($C_{73}H_{80}Cl_2N_4O_{14}$) C, H, N.

3″,3‴-Dichloro-4″,4‴-bis(*o*-carboxybenzyloxy)-5″,5‴-dicarboxy-4′,4′-diphenyl-3*β*-[1-(3′-butenyl)]cholestane (10a). Hexaester **9a** (402 mg, 0.3 mmol), K_2CO_3 (1.55 g), and KCN (12 mg) were suspended in ethanol (10 mL) and water (2 mL). The mixture was heated on an oil bath (80 °C) for 8 h. Ethanol was distilled off, water (10 mL) was added, and the mixture was stirred at 80 °C for 2 h. After cooling to room temperature, the aqueous solution was washed with ethyl acetate (2 × 10 mL), acidified with 1 N HCl, and extracted with ethyl acetate (3 × 15 mL). The organic layers were pooled, dried over Na_2SO_4, concentrated by evaporation, and purified by precipitation from acetone to afford a white solid (98 mg, 32%): mp 217−220 °C; IR ($CHCl_3$) 3021, 2917, 2840, 2657, 2549, 1683, 1598, 1580, 1468, 1444, 1424, 1369, 1268, 1216, 1151, 1106, 1004, 928, 906, 854, 771, 670 cm^{-1}; ^1H NMR (300 MHz, C_5D_5N) δ 8.54 (apparent t, J = 6.5 Hz, 2 H), 8.41 (dd, J = 6.6, 5.4 Hz, 2 H), 8.26 (d, J = 2.2 Hz, 1 H), 8.24 (d, J = 2.1 Hz, 1 H), 7.82 (d, J = 2.2 Hz, 1 H), 7.73 (d, J = 2.3 Hz, 1 H), 7.68−7.60 (m, 2 H), 7.40 (t apparent, J = 7.6 Hz, 2 H), 6.39 (s, 2 H), 6.36 (s, 2 H), 6.34 (t, J = 7.2 Hz, 1 H), 2.29 (q, J = 7.2 Hz, 2 H), 1.91 (d, J = 12.1 Hz, 1 H), 1.86−1.69 (m, 1 H), 1.69−0.98 (m, 32 H), 0.94 (d, J = 6.4 Hz, 3 H), 0.88 (d, J = 6.6 Hz, 6 H), 0.71 (s, 3 H), 0.62 (s, 3 H); ^{13}C NMR (75 MHz, C_5D_5N) δ 169.8, 169.7, 168.0, 154.5, 154.2, 149.2, 142.3, 140.9, 139.4, 138.5, 137.9, 136.5, 136.2, 134.8, 134.2, 133.4, 132.4, 132.1, 131.9, 131.2, 130.4, 130.3, 130.2, 130.0, 129.7, 128.9, 127.8, 127.2, 124.2, 122.8, 75.1, 75.0, 56.7, 56.6, 54.8, 46.7, 42.8, 40.4, 39.8, 38.8, 37.6, 36.5, 36.3, 36.1, 35.9, 35.7, 32.4, 29.3, 28.5, 28.3, 27.7, 24.4, 24.2, 23.0, 22.7, 21.3, 18.9, 12.5, 12.3. Anal. ($C_{61}H_{72}Cl_2O_{10}$·$4H_2O$) C, H.

3″,3‴-Dichloro-4″,4‴-bis(*m*-carboxybenzyloxy)-5″,5‴-dicarboxy-4′,4′-diphenyl-3*β*-[1-(3′-butenyl)]cholestane (10b). Hexaester **9b** (276 mg, 0.203 mmol), KCN (7.9 mg), and K_2CO_3 (1.06 g) were suspended in ethanol (7 mL) and water (1.4 mL). The mixture was heated on an oil bath at 80 °C for 8 h. Ethanol was distilled off, water (7 mL) was added, and the mixture was stirred at 80 °C for 2 h. After cooling to room temperature, the aqueous solution was washed with ethyl acetate (2 × 10 mL), acidified with 1 N HCl, and extracted with ethyl acetate (3 × 30 mL). The organic layers were pooled, dried over Na_2SO_4, concentrated by evaporation, and purified by precipitation from acetone to afford a white solid (125 mg, 60%): mp (softens at 201−203 °C) 217−219 °C; IR ($CHCl_3$) 2923, 2849, 2643, 1693, 1594, 1552, 1466, 1418, 1380, 1283, 1238, 1216, 1170, 1104, 980, 934, 912, 816, 753 cm^{-1}; ^1H NMR (300 MHz, C_5D_5N) δ 8.95−8.88 (m, 2 H), 8.40 (d, J = 7.0 Hz, 2 H), 8.26 (d, J = 2.1 Hz, 1 H), 8.25 (d, J = 2.0 Hz, 1 H), 8.02 (t apparent, J = 6.9 Hz, 2 H), 7.82 (d, J = 2.0 Hz, 1 H), 7.79 (d, J = 2.3 Hz, 1 H), 7.52−7.43 (m, 2 H), 6.35 (t, J = 7.5 Hz, 1 H), 5.52 (s, 2 H), 5.49 (s, 2 H), 2.31 (q, J = 7.4 Hz, 2 H), 1.93 (d, J = 12.0 Hz, 1 H), 1.88−1.69 (m, 1 H), 1.69−0.98 (m, 32 H), 0.95 (d, J = 6.3 Hz, 3 H), 0.88 (d, J = 6.6 Hz, 6 H), 0.73 (s, 3 H), 0.63 (s, 3 H); ^{13}C NMR (75 MHz, C_5D_5N) δ 169.0, 168.3, 168.0, 139.6, 138.4, 137.8, 136.7, 134.9, 134.3, 132.9, 132.7, 132.2, 132.0, 130.3, 130.2, 129.9, 129.1, 128.8, 122.8, 75.9, 75.8, 75.6, 56.7, 56.5, 54.8, 46.6, 42.8, 40.3, 39.7, 38.8, 37.7, 37.5, 36.5, 36.3, 36.1, 35.8, 35.7, 32.4, 29.2, 28.5, 28.3, 27.7, 24.4, 24.2, 22.9, 22.7, 21.3, 18.9, 12.5, 12.3. Anal. ($C_{61}H_{72}Cl_2O_{10}$·$2H_2O$) C, H.

3″,3‴-Dichloro-4″,4‴-bis(*p*-carboxybenzyloxy)-5″,5‴-dicarboxy-4′,4′-diphenyl-3*β*-[1-(3′-butenyl)]cholestane (10c). Hexaester **9c** (1.05 g, 0.77 mmol), KCN (30 mg), and K_2CO_3 (4.02 g) were suspended in ethanol (28 mL) and water (6 mL). The mixture was heated on an oil bath (80 °C) for 8 h. Ethanol was distilled off, and water (20 mL) was added. The aqueous solution was stirred at 80 °C for 2 h. After cooling to room temperature and washing with ethyl acetate (2 × 15 mL), the mixture was acidified with 1 N HCl and extracted with ethyl acetate (3 × 15 mL). The organic layers were pooled, dried over Na_2SO_4, concentrated by evaporation, and purified by precipitation from acetone to afford a white solid (762 mg, 96%): mp 223−225 °C; IR ($CHCl_3$) 3373, 3019, 2918, 2849, 2660, 2556, 1694, 1615, 1597, 1580, 1556, 1514, 1471, 1422, 1381, 1314, 1288, 1250, 1214, 1179, 1127, 1103, 1045, 973, 959, 779, 746, 669 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 8.13−8.02 (m, 4 H), 7.75−7.62 (m, 6 H), 7.59−7.54 (m, 2 H), 6.35 (t, J = 7.6 Hz, 1 H), 5.29 (s, 2 H), 5.22 (s, 2 H), 2.22 (q, J = 7.2 Hz, 2 H), 1.98 (d, J = 13.7 Hz, 1 H), 1.89−1.74 (m, 1 H), 1.74−0.96 (m, 32 H), 0.93 (d, J = 8.3 Hz, 3 H), 0.87 (d, J = 6.7 Hz, 6 H), 0.76 (s, 3 H), 0.68 (s, 3 H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 167.5, 166.3, 154.2, 153.8, 143.1, 139.9, 137.8, 137.1, 135.8, 134.8, 132.7, 132.4, 131.2, 130.5, 130.2, 129.3, 129.0, 128.8, 75.9, 62.4, 57.5, 57.2, 55.6, 47.4, 43.4, 41.0, 40.2, 39.4, 38.2, 37.7, 37.0, 36.8, 36.6, 36.4, 36.3, 32.9, 28.9, 28.6, 27.9, 24.8, 24.5, 23.0, 22.8, 21.7, 19.1, 12.7, 12.4. Anal. ($C_{61}H_{72}Cl_2O_{10}$·$3H_2O$) C, H.

5α-3*β*-[4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-bis(*o*-nitrobenzyloxy)diphenyl)buten-3-yl]cholestane (10d). Diester **9d** (392 mg, 0.3 mmol) and K_2CO_3 (720 mg, 5.22 mmol) were suspended in ethanol (6 mL) and water (1.2 mL). The mixture was heated on an oil bath (105−110 °C) for 8 h. The reaction mixture was concentrated to remove ethanol. KOH (500 mg) was added, and the alkaline solution was washed with CH_2Cl_2 (5 mL) and acidified with concentrated HCl. The acidified suspension was extracted with ethyl acetate (3 × 20 mL). The organic layer was separated, dried over Na_2SO_4, and chromatographed (SiO_2, 80 g; hexanes:ethyl acetate:CH_2Cl_2, 1:1:1 containing 1% acetic acid) to afford a pale-yellow solid (228 mg, 73%): mp (starts softening at 109 °C) 128−136 °C; IR (KBr) 3071, 2924, 2851, 2656, 1701, 1611, 1596, 1570, 1528, 1466, 1448, 1376, 1341, 1275, 1239, 1167, 1102, 1049, 1000, 896, 859, 789, 727, 679 cm^{-1}; ^1H NMR (300 MHz, C_6D_6) δ 8.10−7.95 (m, 2 H), 7.90 (d, J = 1.8 Hz, 1 H), 7.84 (dd, J = 4.2, 2.2 Hz, 1 H), 7.75 (td, J = 9.3, 4.2 Hz, 2 H), 7.49 (d, J = 2.1 Hz, 1 H), 7.43 (dd, J = 3.3, 2.0 Hz, 1 H), 7.11 (t, J = 8.4 Hz, 2 H), 6.79 (q apparent, J = 7.4 Hz, 2 H), 5.96 (q apparent, J = 7.3 Hz, 1 H), 5.62 (s, 2 H), 5.59 (s, 2 H), 2.15−1.96 (m, 2 H), 1.94−1.78 (m, 1 H), 1.78−1.65 (m, 2 H), 1.65−0.83 (m, 31 H), 1.02 (d, J = 6.2 Hz, 3 H), 0.93 (d, J = 6.6 Hz, 6 H), 0.79 (s, 3 H), 0.71 (s, 3 H); ^{13}C NMR (75 MHz, C_6D_6) δ 169.5, 169.4, 154.7, 154.3, 147.0, 139.3, 136.8, 136.6, 136.5, 134.8, 133.8, 133.5, 132.7, 130.7, 130.5, 129.7, 129.1, 129.0, 126.2, 125.9, 124.7, 72.6, 56.9, 56.8, 55.0, 46.8, 43.0, 40.5, 39.9, 38.9, 37.8, 37.5, 36.7, 36.4, 36.3, 36.0, 35.9, 32.5, 30.2, 29.4, 28.7, 28.4, 27.6, 24.6, 24.4, 23.0 22.8, 21.5, 19.0, 12.6, 12.4. Anal. ($C_{59}H_{70}Cl_2N_2O_{10}$) C, H, N.

5α-3*β*-[4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-bis(*m*-nitrobenzyloxy)diphenyl)buten-3-yl]cholestane (10e). Diester **9e** (392 mg, 0.3 mmol) and K_2CO_3 (720 mg, 5.2 mmol) were suspended in ethanol (6 mL) and water (1.2 mL). The mixture was heated on an oil bath (105−110 °C) for 8 h. KOH (450 mg) was added, and the alkaline solution was washed with CH_2Cl_2 (15 mL) and acidified with concentrated HCl (3 mL). The acidified suspension was extracted with ethyl acetate (2 × 40 mL). The organic layer was separated, dried over Na_2SO_4, and chromatographed (SiO_2, 150 g; hexanes:ethyl acetate:CH_2Cl_2, 1:1:1 containing 1% acetic acid) to afford a white foamy solid (280 mg, 90%): mp (starts softening at 102 °C) 126−128 °C; IR ($CHCl_3$) 3076, 3022, 2926, 2851, 2644, 2565, 1698, 1621, 1595, 1556, 1531, 1470, 1444, 1380, 1352, 1248, 1221, 1098, 980, 930, 898, 859, 807, 775, 732, 690, 669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, J = 8.1 Hz, 2 H), 8.19 (d, J = 8.0 Hz, 2 H), 7.86 (dd, J = 7.67, 4.2 Hz, 2 H), 7.72 (d, J = 2.1 Hz, 1 H), 7.69 (d, J = 2.4 Hz, 1 H), 7.55 (td, J = 8.0, 2.8 Hz, 2 H), 7.51−7.44 (m, 2 H), 6.18 (t, J = 7.6 Hz, 1 H), 5.29 (s, 2 H), 5.21 (s, 2 H), 2.14 (q, J = 7.2 Hz, 2 H), 1.92 (d, J = 11.9 Hz, 1 H), 1.85−1.68 (m, 1 H), 1.68−0.91 (m, 32 H), 0.88 (d overlapped, 3 H), 0.86 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 3 H), 0.71 (s, 3 H), 0.62 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3, 168.2, 153.7, 153.4, 148.6, 139.6, 138.4, 138.3, 136.7, 136.6, 136.4, 135.3, 134.3, 134.2, 133.8, 132.5, 130.2, 129.9, 129.7, 129.5, 125.9, 125.5, 123.5, 123.4, 123.3, 74.9, 56.8, 56.6, 54.9, 46.8, 42.8, 40.3, 39.7, 38.7, 37.7, 37.3, 36.4, 36.3, 36.0, 35.8, 32.3, 29.2, 29.1, 28.4, 28.2, 27.5, 24.4, 24.1, 22.9, 22.7, 21.2, 18.8, 12.5, 12.2. Anal. ($C_{59}H_{70}Cl_2N_2O_{10}$) C, H, N.

5α-3β-[4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-bis(*p*-nitrobenzyloxy)diphenyl)buten-3-yl]cholestane (10f). Diester **9f** (130 mg, 0.1 mmol) and K_2CO_3 (240 mg, 1.74 mmol) were suspended in ethanol (2 mL) and water (0.4 mL). The mixture was heated on an oil bath (105−110 °C) for 6−8 h. The reaction mixture was concentrated to remove ethanol. KOH (150 mg) was added, and the alkaline solution was washed with CH_2Cl_2 (5 mL) and acidified with concentrated HCl (1 mL). The acidified suspension was extracted with ethyl acetate (2 × 10 mL). The organic layer was separated, dried over Na_2SO_4, and chromatographed (SiO_2, 15 g; hexanes:ethyl acetate:CH_2Cl_2, 1:1:1 containing 1% acetic acid) to afford a white foamy solid (80 mg, 77%): mp (starts softening at 110 °C) 179−182 °C; IR ($CHCl_3$) 3032, 2926, 2852, 1698, 1608, 1558, 1524, 1470, 1348, 1249, 1178, 1109, 1013, 856 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.26−8.17 (m, 4 H), 7.74−7.65 (m, 6 H), 7.48 (d, *J* = 2.3 Hz, 1 H), 7.47 (d, *J* = 2.5 Hz, 1 H), 6.18 (t, *J* = 7.6 Hz, 1 H), 5.29 (s, 2 H), 5.21 (s, 2 H), 2.14 (q, *J* = 7.2 Hz, 2 H), 1.93 (d, *J* = 12.0 Hz, 1 H), 1.85−1.70 (m, 1 H), 1.70−0.92 (m, 32 H), 0.87 (d, *J* = 6.5 Hz, 3 H), 0.85 (d, *J* = 6.5 Hz, 6 H), 0.72 (s, 3 H), 0.62 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 153.8, 153.4, 147.8, 143.5, 143.4, 139.4, 136.5, 136.4, 136.1, 135.1, 133.7, 132.2, 130.2, 129.9, 129.2, 128.5, 128.4, 125.6, 125.3, 123.7, 74.6, 56.6, 56.3, 54.7, 46.6, 42.6, 40.1, 39.5, 38.5, 37.5, 37.1, 36.2, 36.1, 35.8, 35.5, 32.1, 29.7, 29.0, 28.9, 28.2, 28.0, 27.4, 24.2, 23.9 22.8, 22.5, 21.0, 18.7, 12.3, 12.1. Anal. ($C_{59}H_{70}Cl_2N_2O_{10}$·$2H_2O$) C, H. N.

3″,3‴-Dichloro-4″,4‴-bis(*o*-carboxybenzyloxy)-5″,5‴-di-*o*-carboxy-4′,4′-diphenyl-3β-[1-(3′-butenyl)]cholestane Tetrasodium Salt (11a). Tetraacid **10a** (36 mg, 0.034 mmol) was dissolved in a mixture of ethanol (2 mL) and a 1.016 N solution of Na_2CO_3 (0.135 mL). The resulting solution was concentrated and dried in vacuo to yield a white solid (39 mg, 100%): mp (darkens above 200 °C) >300 °C; IR (KBr) 3418, 2924, 2845, 1570, 1462, 1381, 1281, 1264, 1241, 1212, 1097, 946, 888, 810, 744, 664 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.93−7.80 (m, 2 H), 7.63 (dd, *J* = 7.4, 6.6 Hz, 2 H), 7.42−7.16 (m, 6 H), 7.08 (d, *J* = 1.7 Hz, 1 H), 7.36 (d, *J* = 2.1 Hz, 1 H), 6.07 (t, *J* = 7.4 Hz, 1 H), 5.49 (s, 2 H), 5.43 (s, 2 H), 2.17 (q, *J* = 7.0 Hz, 2 H), 1.97 (d, *J* = 12.2 Hz, 1 H), 1.90−1.73 (m, 1 H), 1.73−0.94 (m, 32 H), 0.91 (d, *J* = 6.4 Hz, 3 H), 0.87 (d, *J* = 6.6 Hz, 6 H), 0.75 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 177.7, 177.0, 174.9, 174.7, 152.4, 152.2, 140.2, 140.0, 138.5, 138.2, 137.3, 137.1, 134.9, 133.0, 132.6, 132.2, 131.9, 131.7, 129.9, 129.8, 129.6, 129.4, 129.1, 129.0, 127.9, 126.9, 126.7, 75.8, 75.7, 58.0, 57.7, 56.2, 43.8, 41.5, 40.7, 39.9, 38.9, 38.6, 38.5, 37.4, 37.2, 37.1, 37.0, 36.8, 36.7, 33.4, 30.2, 30.0, 29.3, 29.1, 28.2, 25.2, 24.9, 23.2, 22.9, 22.1, 19.2, 12.8, 12.5. Anal. ($C_{61}H_{68}Cl_2Na_4O_{10}$·$5H_2O$) C, H.

3″,3‴-Dichloro-4″,4‴-bis(*m*-carboxybenzyloxy)-5″,5‴-dicarboxy-4′,4′-diphenyl-3β-[1-(3′-butenyl)]cholestane Tetrasodium Salt (11b). Tetraacid **10b** (48 mg, 0.046 mmol) was dissolved in a mixture of ethanol (2 mL) and a 1.016 N solution of Na_2CO_3 (0.18 mL). The resulting solution was concentrated and dried in vacuo to yield a white solid (56 mg, 98%): mp (darkens above 220 °C) >300 °C; IR ($CHCl_3$) 3415, 2925, 2855, 1719, 1600, 1570, 1459, 1450, 1404, 1377, 1286, 1232, 1212, 1121, 1103, 966, 934, 905, 881, 813, 767, 742, 674, 645 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.14 (s, 1 H), 8.10 (s, 1 H), 8.00−7.79 (m, 2 H), 7.70 (t, *J* = 7.5 Hz, 2 H), 7.44−7.18 (m, 3 H), 7.20 (d, *J* = 2.0 Hz, 1 H), 7.08 (d, *J* = 2.1 Hz, 1 H), 7.03 (d, *J* = 2.2 Hz, 1 H), 6.08 (t, *J* = 7.5 Hz, 1 H), 5.20 (s, 2 H), 5.13 (s, 2 H), 2.17 (q, *J* = 6.7 Hz, 2 H), 1.97 (d, *J* = 12.0 Hz, 1 H), 1.90−1.74 (m, 1 H), 1.74−0.97 (m, 32 H), 0.91 (d, *J* = 6.4 Hz, 3 H), 0.87 (d, *J* = 6.6 Hz, 6 H), 0.75 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.4, 175.0, 174.8, 174.7, 151.8, 151.6, 140.4, 139.9, 139.3, 138.7, 138.4, 138.3, 137.5, 133.2, 131.8, 131.6, 130.4, 130.3, 129.9, 129.7, 129.5, 129.4, 129.2, 129.0, 128.6, 126.5, 77.0, 58.0, 57.7, 56.2, 48.1, 43.8, 41.5, 40.7, 39.9, 38.9, 38.5, 37.4, 37.2, 37.1, 37.0, 36.8, 33.4, 30.2, 30.1, 29.3, 29.1, 28.2, 25.2, 25.0, 23.2, 22.9, 22.1, 19.2, 12.8, 12.5. Anal. ($C_{61}H_{68}Cl_2Na_4O_{10}$·$6H_2O$) C, N.

3″,3‴-Dichloro-4″,4‴-bis(*p*-carboxybenzyloxy)-5″,5‴-dicarboxy-4′,4′-diphenyl-3β-[1-(3′-butenyl)]cholestane Tetrasodium Salt (11c). Tetraacid **10c** (100 mg, 0.097 mmol) was dissolved in a mixture of methanol (2 mL) and a 1.016 N solution of Na_2CO_3 (0.38 mL). The resulting solution was concentrated and dried in vacuo to yield a white solid (112 mg, 93%): mp > 300 °C; IR ($CHCl_3$) 3390, 3020, 2924, 2849, 2400, 1600, 1564, 1556, 1463, 1455, 1384, 1284, 1214, 1102, 972, 957, 887, 844, 780, 741, 669 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.96 (d, *J* = 8.1 Hz, 2 H), 7.94 (d, *J* = 8.2 Hz, 2 H), 7.58 (d, *J* = 8.4 Hz, 2 H), 7.55 (d, *J* = 8.3 Hz, 2 H), 7.36 (d, *J* = 2.3 Hz, 1 H), 7.21 (d, *J* = 2.1 Hz, 1 H), 7.09 (d, *J* = 2.1 Hz, 1 H), 7.04 (d, *J* = 2.3 Hz, 1 H), 6.08 (t, *J* = 7.5 Hz, 1 H), 5.20 (s, 2 H), 5.13 (s, 2 H), 2.17 (dd, *J* = 7.4, 7.0 Hz, 2 H), 1.98 (d, *J* = 12.2 Hz, 1 H), 1.90−1.73 (m, 1 H), 1.73−0.94 (m, 32 H), 0.91 (d, *J* = 6.4 Hz, 3 H), 0.87 (d, *J* = 6.6 Hz, 6 H), 0.75 (s, 3 H), 0.67 (s, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.4, 175.0, 174.8, 151.8, 151.6, 140.9, 140.4, 139.9, 138.8, 138.6, 138.3, 137.4, 133.2, 131.8, 130.2, 129.7, 129.3, 129.1, 128.9, 128.6, 126.5, 76.6, 76.5, 58.0, 57.7, 56.2, 48.0, 43.8, 41.5, 40.7, 39.9, 38.8, 38.5, 37.4, 37.2, 37.1, 37.0, 36.8, 33.4, 30.2, 30.1, 29.3, 29.1, 28.2, 25.2, 25.0, 23.2, 22.9, 22.1, 19.2, 12.8, 12.5. Anal. ($C_{61}H_{68}Cl_2Na_4O_{10}$·$7H_2O$) C, H.

5α-3β-[4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-bis(*o*-nitrobenzyloxy)diphenyl)buten-3-yl]cholestane Disodium Salt (11d). Diacid **5d** (44 mg, 0.042 mmol) was dissolved in a mixture of ethanol (2 mL) and a 1.016 N solution of Na_2CO_3 (0.083 mL). The resulting solution was concentrated and dried in vacuo to yield a pale-yellow foam (47 mg, 98%): mp (darkens above 196 °C) 241 °C with dec; IR (KBr) 3423, 2925, 2851, 1608, 1580, 1527, 1462, 1410, 1376, 1344, 1304, 1287, 1228, 1102, 1049, 1003, 886, 861, 816, 789 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.26 (d, *J* = 7.9 Hz, 1 H), 8.22 (d, *J* = 7.9 Hz, 1 H), 8.12 (dd, *J* = 8.1, 1.1 Hz, 1 H), 8.11 (dd, *J* = 8.1, 1.1 Hz, 1 H), 7.76 (ddd ABX pattern, J_{AB} = 7.6 Hz, J_{AB} = 5.2 Hz, J_{AX} = 1.1 Hz, 2 H), 7.53 (tdd, *J* = 7.8, 3.5, 1.2 Hz, 2 H), 7.42 (d, *J* = 2.2 Hz, 1 H), 7.25 (d, *J* = 2.1 Hz, 1 H), 7.11 (d, *J* = 2.1 Hz, 1 H), 7.04 (d, *J* = 2.3 Hz, 1 H), 6.12 (t, *J* = 7.5 Hz, 1 H), 5.58 (s, 2 H), 5.51 (s, 2 H), 2.18 (dd, *J* = 7.2, 6.7 Hz, 2 H), 1.97 (d, *J* = 12.1 Hz, 1 H), 1.88−1.72 (m, 1 H), 1.72−0.94 (m, 32 H), 0.91 (d, *J* = 6.4 Hz, 3 H), 0.87 (d, *J* = 6.5 Hz, 3 H), 0.87 (d, *J* = 6.6 Hz, 3 H), 0.75 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.7, 174.4, 151.6, 151.3, 148.3, 140.7, 139.7, 137.8, 135.5, 134.9, 133.5, 131.9, 130.5, 129.9, 129.5, 129.2, 128.7, 126.5, 125.4, 73.0, 58.0, 57.7, 56.2, 48.0, 43.8, 41.5, 40.7, 39.9, 38.8, 38.4, 37.4, 37.2, 37.1, 37.0, 36.8, 33.4, 30.2, 30.1, 29.3, 29.1, 28.2, 25.2, 25.0, 23.2, 22.9, 22.1, 19.2, 12.8, 12.5. Anal. ($C_{59}H_{68}Cl_2N_2Na_2O_{10}$·$3H_2O$) C, H, N.

5α-3β-[4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-bis(*m*-nitrobenzyloxy)diphenyl)buten-3-yl]cholestane Disodium Salt (11e). Diacid **10e** (10 mg, 0.0096 mmol) was dissolved in a mixture of ethanol (0.5 mL) and a 1.016 N solution of Na_2CO_3 (0.019 mL, 0.019 mmol). The resulting solution was concentrated and dried in vacuo to yield a white solid (10.6 mg, 98%): mp (starts softening at 184 °C) dec at 252 °C; IR (KBr) 3448, 2925, 2850, 1604, 1579, 1531, 1462, 1411, 1377, 1352, 1285, 1237, 1100, 1002, 894, 807, 734, 693, 672 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.47 (s, 1 H), 8.44 (s, 1 H), 8.23−8.13 (m, 2 H), 7.99 (d, *J* = 8.2 Hz, 1 H), 7.96 (d, *J* = 9.1 Hz, 1 H), 7.62 (t, *J* = 7.9 Hz, 1 H), 7.60 (t, *J* = 7.9 Hz, 1 H), 7.43 (d, *J* = 2.2 Hz, 1 H), 7.25 (d, *J* = 2.0 Hz, 1 H), 7.09 (d, *J* = 2.1 Hz, 1 H), 7.01 (d, *J* = 2.2 Hz, 1 H), 6.11 (t, *J* = 7.5 Hz, 1 H), 5.30 (s, 2 H), 5.22 (s, 2 H), 2.16 (dd, *J* = 7.5, 7.1 Hz, 2 H), 1.97 (d, *J* = 12.2 Hz, 1 H), 1.88−1.72 (m, 1 H), 1.72−0.97 (m, 32 H), 0.91 (d, *J* = 6.4 Hz, 3 H), 0.87 (d, *J* = 6.6 Hz, 6 H), 0.75 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.6, 174.4, 151.5, 151.3, 141.3, 140.6, 139.7, 138.4, 138.2, 137.7, 135.4, 135.3, 133.5, 131.9, 130.5, 130.0, 129.5, 129.0, 128.8, 126.6, 123.8, 123.7, 123.6, 75.3, 58.0, 57.7, 56.2, 48.0, 43.8, 41.5, 40.7, 39.9, 38.8, 38.4, 37.4, 37.2, 37.1, 37.0, 36.7, 33.4, 30.2, 30.0, 29.3, 29.1, 28.2, 25.2, 24.9, 23.1, 22.9, 22.1, 19.2, 12.8, 12.5. Anal. ($C_{59}H_{68}Cl_2N_2Na_2O_{10}$·$2H_2O$) C, H, N.

5α-3β-[4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-bis(*p*-nitrobenzyloxy)diphenyl)buten-3-yl]cholestane Disodium Salt (11f). Diacid (**10f**) (56 mg, 0.054 mmol) was dissolved in a mixture of ethanol (2 mL) and a 1.016 N solution of Na_2CO_3

(0.106 mL). The resulting solution was concentrated and dried in vacuo to yield a pale-yellow solid (60 mg, 97%): mp (starts softening at 224 °C) 230 °C with dec; IR ($CHCl_3$) 3419, 2926, 2854, 1606, 1581, 1525, 1463, 1408, 1375, 1348, 1284, 1236, 1105, 1010, 887, 854, 739 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 8.24 (d, J = 8.8 Hz, 2 H), 8.23 (d, J = 8.8 Hz, 2 H), 7.81 (t, apparent, J = 8.4 Hz, 4 H), 7.37 (s br, 1H), 7.23 (d, J = 2.1 Hz, 1 H), 7.08 (d, J = 2.0 Hz, 1 H), 7.02 (s br, 1 H), 6.10 (t, J = 7.5 Hz, 1 H), 5.30 (s, 2 H), 5.23 (s, 2 H), 2.16 (q, J = 7.2 Hz, 2 H), 1.97 (d, J 12.2 Hz, 1 H), 1.90−1.73 (m, 1 H), 1.73−0.94 (m, 32 H), 0.91 (d, J = 6.4 Hz, 3 H), 0.87 (d, J = 6.5 Hz, 3 H), 0.87 (d, J = 6.6 Hz, 3 H), 0.74 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.7, 174.4, 151.5, 151.3, 148.9, 146.6, 140.6, 139.7, 138.5, 138.3, 137.7, 133.4, 131.8, 129.8, 129.7, 129.4, 129.0, 128.7, 126.6, 124.3, 75.3, 58.0, 57.7, 56.2, 48.0, 43.8, 41.5, 40.7, 39.9, 38.8, 38.4, 37.4, 37.2, 37.1, 37.0, 36.8, 33.4, 30.2, 30.0, 29.3, 29.1, 28.2, 25.2, 25.0, 23.2, 22.9, 22.1, 19.2, 12.8, 12.5. Anal. ($C_{59}H_{68}Cl_2N_2Na_2O_{10}\cdot 4H_2O$) C, H, N.

In Vitro Anti-HIV Assay. Evaluation of the antiviral activity of compounds against HIV-1_{RF} infection in CEM-SS cells was performed using the XTT cytoprotection assay as previously described.[23] This cell-based microtiter assay quantitates the drug-induced protection from the cytopathic effect of HIV-1. Data are presented as the percent control of XTT values for the uninfected, drug-free control. EC_{50} values reflect the drug concentration that provides 50% protection from the cytopathic effect of HIV-1 in infected cultures, while the CC_{50} reflects the concentration of drug that causes 50% cell death in the uninfected cultures. XTT-based results were confirmed by measurement of cell-free supernatant reverse transcriptase and p24 levels. All XTT cytoprotection data were derived from triplicate tests on each plate, with two separate sister plates. Thus, the EC_{50} value from each plate represents the average of triplicates, and the two EC_{50} values from sister plates were averaged. The variation from the mean averaged less than 10%. The methodology used to monitor antiviral activity in the remaining assay systems was also described previously.[24] Evaluation of the antiviral activity of compounds against HIV-1_{IIIB} or HIV-2_{ROD} infection in MT-4 cells was performed using the MTT cytoprotection assay as previously described.[25]

Mechanism of Antiviral Action Studies. Binding of HIV-1_{RF} to CEM-SS cells was measured by a p24-based assay as previously based.[23] The ability of compounds to block fusion was measured using a surrogate fusion assay as previously described.[23] This assay employs the HeLa-CD4-LTR-β-gal (MAGI) cells and the HIV-1 Tat- and Env-expressing HL2/3 cells. The HL2/3 cells, which express HIV-1 Env protein on the cell surface and Tat protein in the cytoplasm, can fuse with the MAGI cells and activate the β-galactosidase expression in the syncytium. Cell monolayers were fixed for 5 min with 2% formaldehyde-2% glutaraldehyde, washed twice with cold phosphate-buffered saline, and then stained with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) substrates for 50 min at 37 °C. The blue-stained cells in each well were then counted as an indicator of the relative levels of fusion events. The effects of the compounds on the in vitro activity of purified HIV-1 p66/51 RT (a kind gift of S. Hughes, ABL Basic Research, NCI−FCRDC, Frederick, MD) were determined by measurement of incorporation of [^{32}P]TTP onto the poly(rA):oligo(dT) (rAdT) or [^{32}P]GTP onto the poly(rC):oligo(dG) homopolymer template systems.[26] HIV-1 protease activity was quantitated by a reversed-phase HPLC assay utilizing the Ala-Ser-Glu-Asn-Tyr-Pro-Ile-Val-Glu-amide artificial protease substrate as previously described.[27] The in vitro effects of compounds on HIV-1 integrase (a kind gift of S. Hughes, ABL Basic Research, NCI−FCRDC, Frederick, MD) activity were determined as previously described.[28]

Acknowledgment. This research was made possible by NIH Grant NO1-AI-36624 and by grants from the Fonds voor Wetenschappelijk Onderzoek (FWO) Vlaanderen, the Belgian Geconcerteerde Onderzoekacties, and the Belgiun Fonds voor Geneeskundig Wetenschappelijk Onderzoek (FGWO). We would also like to thank Sandra Claes, Kristien Erven, and Erik Fonteyn for excellent technical assistance.

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JM980727M