Synthesis and Anti-HIV Activity of Cosalane Analogues with Substituted Benzoic Acid Rings Attached to the Pharmacophore through Methylene and Amide Linkers

Jeffrey A. Ruell,[†] Erik De Clercq,[‡] Christophe Pannecouque,[‡] Myriam Witvrouw,[‡] Tracy L. Stup,[§] Jim A. Turpin,[§] Robert W. Buckheit, Jr.,[§] and Mark Cushman*,[†]

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium, and Virology Research Group, Serquest, 431 Aviation Way, Frederick, Maryland 21701

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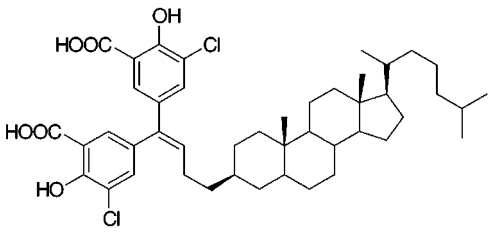
The cosalane pharmacophore has been extended by the attachment of two additional substituted benzoic acid rings through amide and methylene linkers. The resulting compounds display significant antiviral activity when tested in vitro for inhibition of the cytopathic effects of HIV-1_{RF} in CEM-SS cells and HIV-1_{IIIB} in MT-4 cells. The compound containing the methylene linker also shows moderate activity versus HIV-2_{ROD} in MT-4 cells. Because cosalane and related compounds containing extended pharmacophores inhibit the binding of gp120 to CD4, the presently described new compounds are assumed to act by a similar mechanism. A hypothetical model is proposed for the binding of the methylene-linked compound to CD4.

Introduction

Since the design and synthesis of cosalane (**1**),[1,2] a number of studies have been reported by us and others involving the development of new HIV inhibitors based on the cosalane framework.[3−8] These communications have provided information about the biological effects of (1) changing the length of the alkenyl linker chain between the cholestane and the disalicylmethane "pharmacophore" of the molecule, (2) altering the site of attachment of the linker chain to the steroid, (3) varying the chemical nature of the linker, and (4) alkylating the phenolic hydroxyl groups to prepare new inhibitors containing additional benzoic acid rings.



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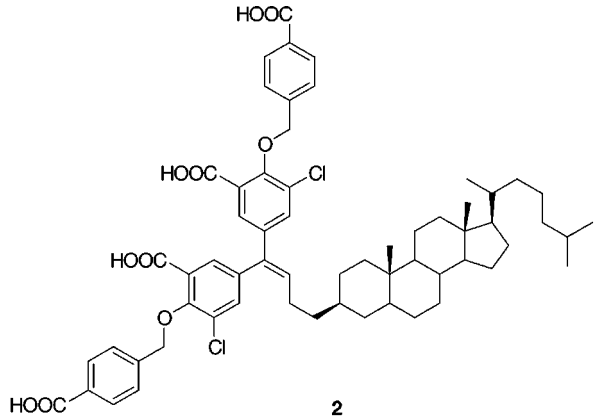

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From the latter study, it was demonstrated that extending the length of the cosalane (**1**) pharmacophore by functionalization with additional benzoic acid rings provided new compounds with enhanced anti-HIV potency, which could possibly be attributed to stronger cosalane/CD4 interactions. For example, it was found that the cosalane analogue **2** was more potent against HIV-1_{RF} in CEM-SS cells and HIV-1_{IIIB} in MT-4 cells than

[†] Purdue University.
[‡] Katholieke Universiteit Leuven.
[§] Serquest.

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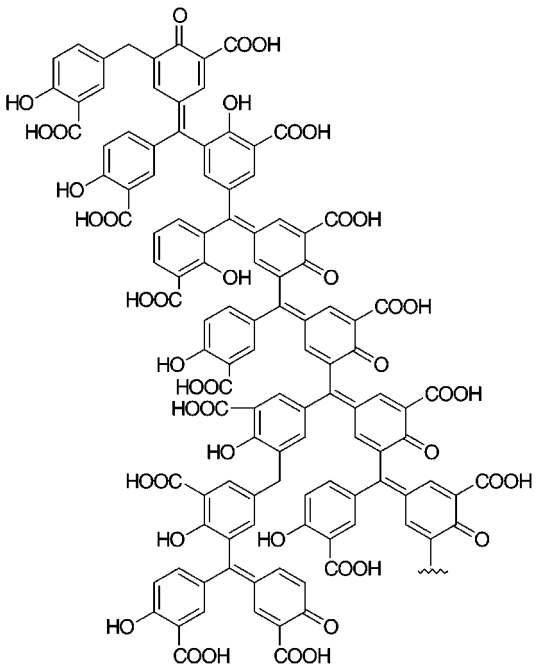
cosalane itself, and a hypothetical model was proposed for the binding of **2** to CD4.[4] This observation naturally led to a consideration of additional types of connections that could be made between the two terminal aromatic rings during the extension of the pharmacophore. An alteration of the chemical nature or length of these connections, as well as their sites of attachment to the central two aromatic rings, would be expected to have significant effects on important biological properties such as metabolic stability, pharmacokinetics, and receptor binding. The present report details the synthesis and anti-HIV activities of novel cosalane extended-pharmacophore analogues having amide and methylene linkers to the terminal substituted benzoic acid rings instead of the benzylic ether linkages reported previously for **2** and related compounds.

Rationale and Design

The disalicylmethane portion of cosalane is believed to be the pharmacophore of this compound.[7] This is supported by the observation that the dichlorodisalicylmethane fragment of cosalane has low but reproducible activity against HIV-1 in CEM cells.[9] In addition, low molecular weight components of the mixture of polyanionic polymers known as aurintricarboxylic acid (ATA, schematic representation shown in structure **3**) that are



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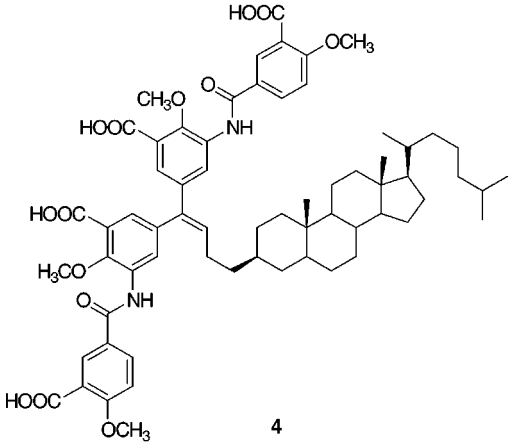
salicylic acid oligomers and are related in structure to the proposed cosalane pharmacophore have anti-HIV activity.[10] Also consistent with the proposal that the disalicylmethane portion of cosalane is the pharmacophore is the fact that the steroid moiety of cosalane can be replaced by normal alkyl chains and anti-HIV activity is retained, although potency is compromised.[5]

Cosalane binds to the HIV surface protein gp120 and its lymphocyte receptor CD4.[11] This finding suggests that cosalane may interact with one or both of these target molecules through anionic interactions of the carboxylates of the ligand with amino components of the protein. Because the D1D2 domain of CD4, the portion required for HIV-1 binding, has been crystallized and the structure is available for examination, a hypothetical model of cosalane interacting with this protein has been proposed.[4] The crux of this model is that the two carboxyl groups of cosalane could easily span the distance between the surface-exposed, adjacent arginines (Arg58 and Arg59) of CD4. These residues are believed to be important in HIV-1 binding to CD4.[12] Upon examination of the amino acids near this site, it was determined that other amino side chains are in close enough proximity to be reachable by additional anionic groups that could be appended to cosalane's pharmacophore. One such target amino acid would be Lys72. As revealed by the construction of models of the proposed compounds **4** and **5** bound to the CD4 molecule, both **4** and **5** appear to be capable of interacting with all three of these basic residues on the protein.



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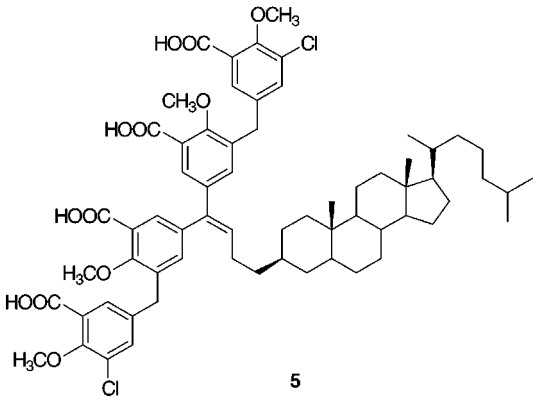

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A hypothetical model of the binding interaction is detailed in Figure 1. This model was created using the

(9) Cushman, M.; Kanamathareddy, S.; De Clercq, E.; Schols, D.; Goldman, M.; Bowen, J. A. Synthesis and Anti-HIV Activities of Low Molecular Weight Aurintricarboxylic Acid Fragments and Related Compounds. *J. Med. Chem.* **1991**, *34*, 337−342.

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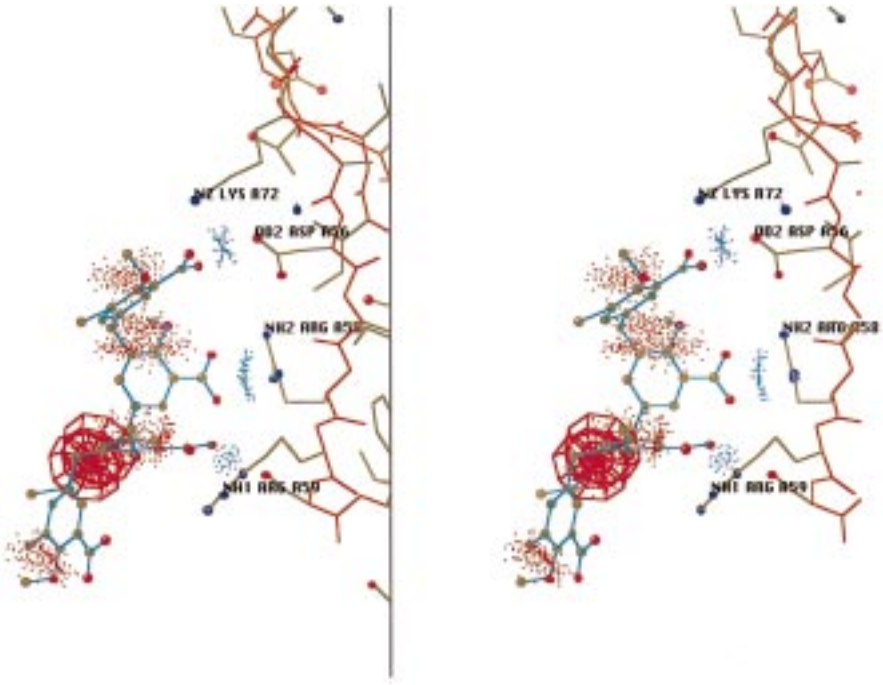

Figure 1. Hypothetical model of compound **5** binding to a portion of the CD4 protein (programmed for walleyed viewing). Blue dots: attraction; red dots: repulsion.

Sculpt molecular modeling program (Interactive Simulations, Inc.) by docking the cosalane analogue **5** onto the CD4 surface. Because free rotation exists about the methylene groups connecting the aromatic rings, it was possible to position this compound so that the carboxylates of the ligand contacted Arg58, Arg59, Lys72, and Asp56 of the protein by simple bond rotation. The protein structure was then "frozen", and the energy of the ligand was minimized.

Our decision to construct molecules **4** and **5** was influenced by three factors. First, it had been demonstrated that increasing the molecular weight of the polyanionic anti-HIV polymer aurintricarboxylic acid **(3)** by increasing the number of salicylic acid units resulted in increased anti-HIV potency in vitro.[10] Because cosalane incorporates the disalicylmethane structural unit of ATA as a part of its pharmacophore, addition of salicylic acid rings to the cosalane pharmacophore might be expected to increase antiviral potency. Second, molecules **4** and **5** were originally thought to be easily accessible from cosalane by dehalogenation followed by electrophilic substitution methods. Unfortunately, this did not prove to be the case, and other synthetic methods eventually had to be pursued. Third, the antiviral potency of low molecular weight ATA (**3**) oligomers increases as the number of salicylic acid rings increases.[13]

Chemistry

The desired analogues **4** and **5** were both expected to be synthesized by convergent routes. This would require the preparation of the aromatic pharmacophore region, as well as a functionalized cholestanyl component containing a precursor of the linker chain, and their eventual union late in the synthesis. The decision to execute the convergent step by McMurry olefination, which tolerates a wide range of functional groups, led us to prepare the pharmacophore regions in the form of substituted benzophenones.[14]

Several problems had to be overcome to achieve the synthesis of the required benzophenone derivative **14** (Scheme 2). The diamine derived from reduction of the two nitro groups of **12**[15] did not undergo acylation with acid chlorides or anhydrides but instead formed resins and intractable mixtures. Additionally, it was not possible to oxidize diphenylmethane **10**, accessible via standard chemistry outlined in Scheme 1, to **14** because of the insolubility of **10**. However, the tetraacid **11** derived from **10** proved to be valuable itself as a biological probe because it afforded a substance with which to determine the efficacy of a small, amide-containing compound as an inhibitor of HIV-1 infection. Our previous reports with small polyanionic molecules have not included amide derivatives.[10,16]

The benzophenone **14** was finally prepared according to Scheme 2. The diamino benzhydrol compound **13** was synthesized in one step by simultaneous reduction of the keto and nitro functionalities in **12.**[15] This route overcomes the inhibitory effect that the ketone functionality apparently imposes on acylating the *meta* amino groups of the compound resulting when only the two nitro groups of **12** are reduced to amino groups. Alcohol **13** proved to be very unstable and was normally acylated as soon as

(13) 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*, 329−336.

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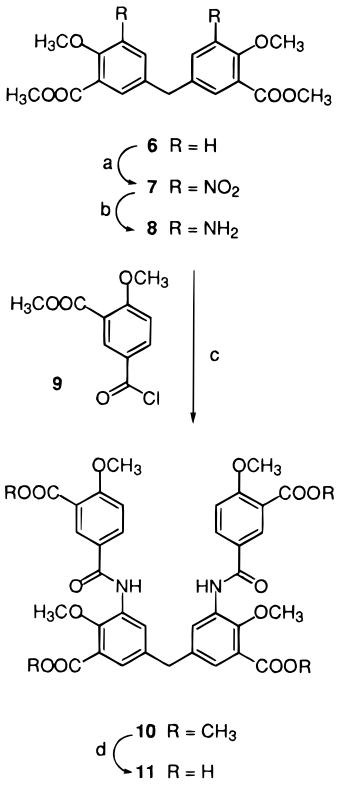
Scheme 1[a]

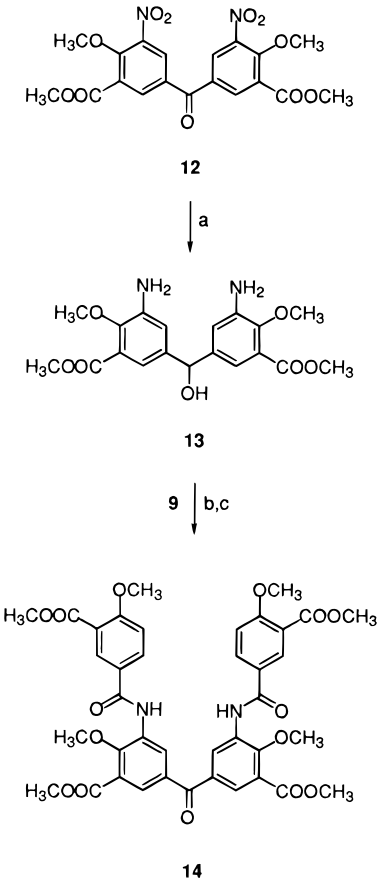


[a]Reagents and conditions: (a) 90% HNO_3, Ac_2O, 0 to 23 °C, 12 h. (b) H_2, PtO_2, EtOAc, 60 °C, 4 h. (c) Pyridine, 60 °C, 12 h. (d) K_2CO_3, aq EtOH, 95 °C, 12 h.

it was prepared. Thus, acylation with 4-methoxy-3-methoxycarbonylbenzoyl chloride **(9)**[17] followed by in situ Collins oxidation of the benzylic alcohol gave ketone **14** in 85% isolated yield.

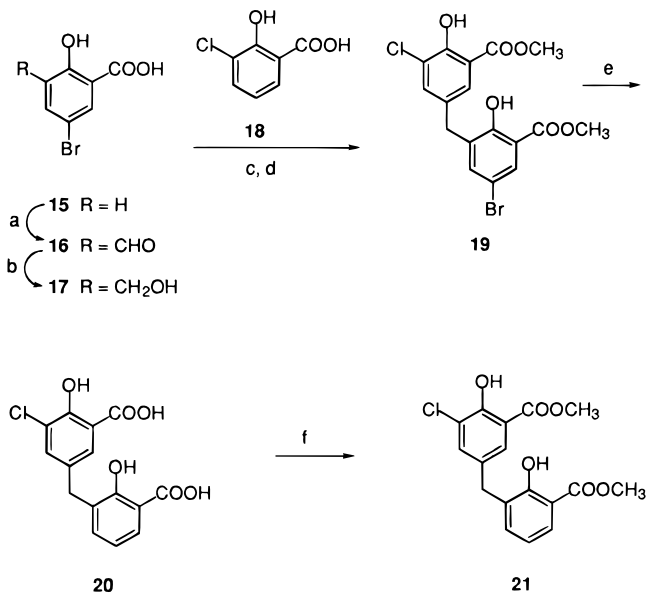
Benzophenone **24** was prepared by the methods outlined in Schemes 3 and 4. This synthesis makes use of chlorine as a protecting group to control the regiochemistry of aromatic substitution.[18] Thus, 5-bromosalicylic acid (**15**) was formylated to give aldehyde **16**. This formylation proved to be temperature-dependent. Under the conditions shown (90 °C, TFA, hexamethylenetetramine), the bond to the bromine atom was stable. At higher temperatures (~120 °C), the bromine was removed and a second formylation occurred. By comparison, the corresponding aromatic iodide was very unstable, and iodine sublimed up the condenser during the formylation reaction. Reduction of aldehyde **16** gave the benzyl alcohol **17**, which was used to alkylate 3-chlorosalicylic acid (**18**) to form a diacid that was esterified to yield **19**. The yield of the alkylation product was dependent on several variables. Chief among these was the solvent; alkylation proceeded in methanol/sulfuric acid, but only benzylic *O*-acetylation occurs in acetic acid/sulfuric acid. Additionally, a slight excess of the benzyl alcohol was needed. Finally, the concentrations of the reagents in the solvent were found to be very important. To obtain an analytically pure sample, the easiest method of purification was found to be Fisher esterification and isolation of the diester **19**. Reductive removal of the bromide with

Scheme 2[a]



[a]Reagents and conditions: (a) Zn, MeOH, AcOH, 45 °C, 1 h. (b) Pyridine, 23 °C, 12 h. (c) CrO_3, pyridine, 0 °C, 3 h.

Scheme 3[a]



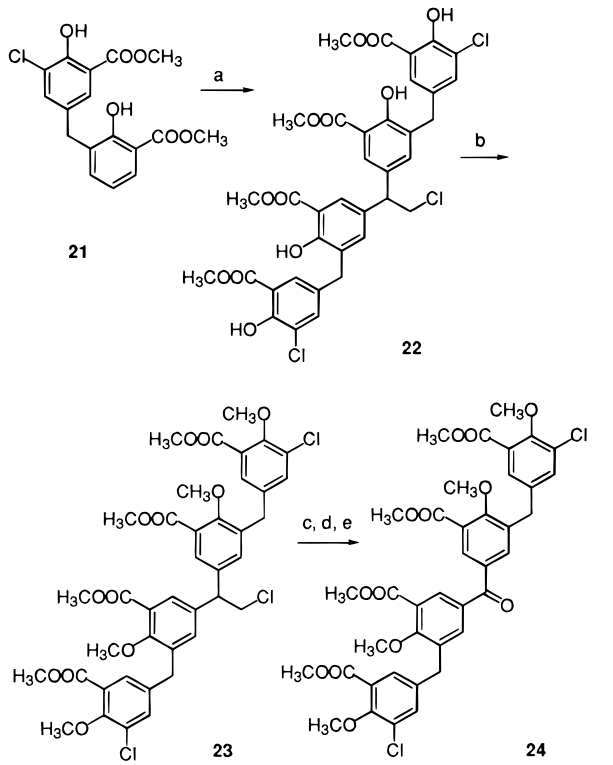
[a]Reagents and conditions: (a) Hexamethylenetetramine, TFA, 90 °C, 16 h. (b) $NaBH_4$, aq EtOH, NaOH, 0 °C, 4 h. (c) H_2SO_4, MeOH, 0-23 °C, 12 h. (d) MeOH, H_2SO_4, 78 °C, 12 h. (e) Zn, NaOH, H_2O, 23 °C, 12 h. (f) MeOH, H_2SO_4, 80 °C, 36 h.

zinc dust under basic conditions gave **20**, which could be used in ensuing reactions but was normally re-esterified to afford **21**. This was done so that **21** could be monitored by TLC analysis in the ensuing reactions. The selective

(17) Ninomiya, I.; Tada, Y.; Miyata, O.; Naito, T. A. A Formal Total Synthesis of the Alkaloid Yohimbine. *Heterocycles* **1980**, *5*, 631−633.

(18) Tashior, M. Selective Synthesis of Aromatic Compounds Using Positional Protective Groups. *Synthesis* **1979**, 921−936.

Scheme 4[a]



[a]Reagents and conditions: (a) $ClCH_2CH(OMe)_2$, H_2SO_4, AcOH, 0-23 °C, 12 h. (b) Me_2SO_4, K_2CO_3, DMF, 23 °C, 12 h. (c) KOH, aq MeOH, 78 °C, 12 h. (d) O_3, MeOH, -78 °C, 1 h. (e) Me_2SO_4, K_2CO_3, Me_2CO, 23 °C, 3 h.

removal of bromine from **19** left the chlorine atom to function as a protecting group and control the regiochemistry during the subsequent Friedel−Crafts reactions.

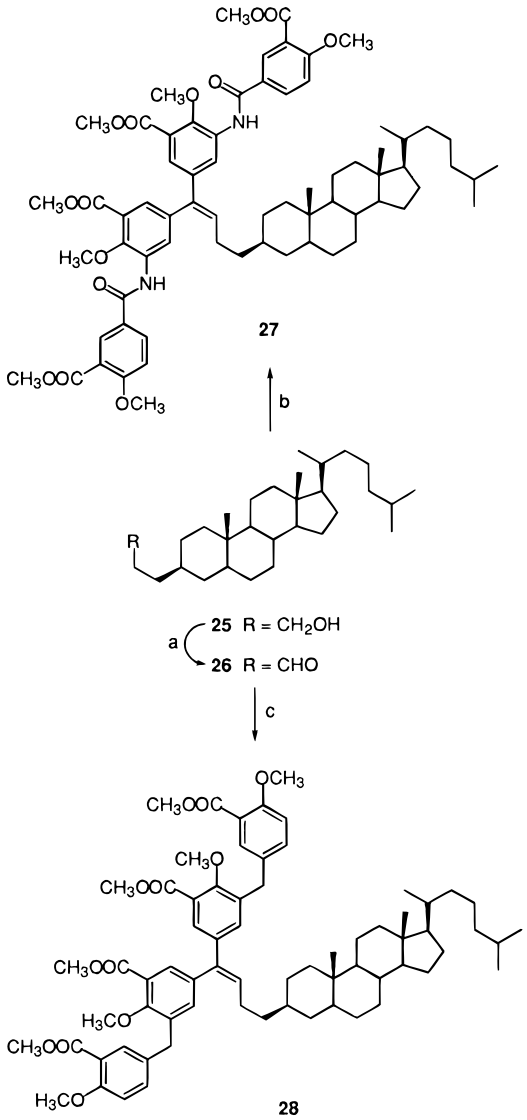
The electron-rich nature of **20** and **21** suggested that palladium carbonylation methods might prove difficult, so **24** was prepared by Friedel−Crafts alkylation followed by degradation chemistry. Treatment of **21** with 2 equiv of chloroacetaldehyde dimethyl acetal gave the diphenylethane **22** in fair yield.[19] Unlike the literature precedent,[19] **22** was without doubt undergoing some ester hydrolysis on workup. Attempts to find conditions that circumvented aqueous workup were not successful. Re-esterification of the crude material with diazomethane or by Fisher esterification led to a greater isolated yield. Regardless, there was also some loss of material by base-catalyzed reactions on the silica gel used to separate the compounds.

Etherification of **22** was then accomplished with dimethyl sulfate in DMF solution containing potassium carbonate as the base. It was necessary not to methylate until after **22** was isolated in pure form because it was not otherwise possible to separate the desired ether product **23** from unreacted starting material. Chloride **23** was obtained as an oil that was heat-sensitive and eliminated HCl slowly upon standing.

Intermediate **23** was converted to **24** through three reactions which were performed in series because the intermediates were difficult to isolate or unstable. The transformation of **23** to **24** was done as quickly as possible

(19) Xie, H.; Lee, D. A.; Wallace, D. M.; Senge, M. O. Syntheses and Some Chemistry of 1,2- and 1,1-Bis(2-pyrrolyl)ethenes. *J. Org. Chem.* **1996**, *61*, 8508−8517.

Scheme 5[a]



[a]Reagents and conditions: (a) PCC, CH_2Cl_2, Al_2O_3, 23 °C, 6 h. [b]Ketone **14**, THF, $TiCl_4$, Zn, 67 °C, 1 h. [c]Ketone **24**, THF, $TiCl_4$, Zn, 67 °C, 1 h.

to minimize rearrangement of the intermediary 1,1-disubstituted olefin. Thus, **23** underwent base-catalyzed elimination of HCl and ester hydrolysis using KOH in methanol to afford the corresponding 1,1-disubstituted olefin along with a small amount of the 1,2-rearrangement product (detected by ^1H NMR). Subsequent ozonolysis and re-esterification of the crude mixture provided **24**, which was purified free of aldehydes (obtained from the 1,2-rearranged product) by column chromatography.

Ketones **14** and **24** were appended to aldehyde **26** (Scheme 5). Esters **27** and **28** were readily hydrolyzed to give the free acids **4** and **5**. These compound were converted to their more water-soluble tetrasodium salts for biological evaluation.

Biological Results and Discussion

We have demonstrated that extended cosalane-type compounds are accessible by routes that do not utilize cosalane itself as an intermediate or a starting material. Despite the length of these syntheses, two different

Table 1. Anti-HIV Activities of Cosalane Analog

	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
2[e]	0.8	1.4	55	76	95
4[e]	28.6	8.4	>66	46.8	79.4
5[e]	2.9	12.5	19.9	48.9	41.0
10	NA[f]			>316	
11	NA[f]	NA[f]	NA[f]	>316	>125
27	NA[f]	NA[f]	NA[f]	84.0	78.2

[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] Tested as the tetrasodium salt. [f] No activity observed.

routes have been employed to prepare two classes of these compounds. Both **4** and **5** were found to inhibit the cytopathic effect of HIV-1 in vitro (Table 1). However, the amide compound **4** was not as potent as cosalane and had only moderate activity against HIV-1_{RF} in CEM-SS cells (EC_{50}: 28.6 μM) and HIV-1_{IIIB} in MT-4 cells (EC_{50}: 8.4 μM). It was inactive as an inhibitor of the cytopathic effect of HIV-2_{ROD} in MT-4 cells. Compound **11**, which is the pharmacophore portion of **4**, proved to be inactive. In general, all of the amide compounds had greater cytotoxicity than is normally seen with similar compounds.[10] As with cosalane,[2] the carboxylic acids appear to play a critical role in the antiviral activity, as the tetraester **27** was inactive. The methylene compound **5** appeared to be more potent than **4** against HIV-1_{RF} in CEM-SS cells (EC_{50}: 2.9 μM) and HIV-2_{ROD} in MT-4 cells (EC_{50}: 19.9 μM), but it was slightly less potent than **4** against HIV-1_{IIIB} in MT-4 cells (EC_{50}: 12.5 μM). Compound **5** was slightly more potent than **1** when tested against HIV-1_{RF} in CEM-SS cells, but it was less potent as an inhibitor of HIV-1_{IIIB} in MT-4 cells. In addition, compound **5** was less potent against both strains of HIV-1 than the compound **2** having a benzylic ether linkage.

As shown in the hypothetical model of the binding of **5** to CD4 displayed in Figure 1, the positively charged guanidinium ions of the side chains of Arg58 and Arg59 may interact with two of the carboxylates of the ligand **5** by ionic binding. The oxygen of a third carboxylate residue of the ligand is also capable of ionic bonding to the positively charged ammonium ion of Lys72, and it may also interact with the carboxylic acid residue of Asp56. A similar model has been proposed for the binding of the cosalane analogue **2** to CD4.[20] Thus, although the model proposed in Figure 1 is speculative, it has led to the design of several cosalane analogues that display significant anti-HIV activity in in vitro systems. However, the model has limited predictive value as a result of several factors. It is known that cosalane and structurally related compounds bind to gp120 and CD4, and the gp120 binding is also thought to contribute to the inhibition of the interaction of gp120 with CD4.[2,11,20] A more complete understanding of the mechanisms of action and potencies of the present compounds may therefore require a model for their binding to gp120 in addition to the CD4 binding model. It might be possible to propose such a model for gp120 binding in the future as a result of the recent publication of the X-ray crystal structure of gp120 in complex with the CD4 receptor and a neutralizing antibody.[21] In addition, recent mechanism of action studies on compound **2** have indicated that it inhibits both attachment of HIV-1_{RF} to CEM-SS cells and the subsequent fusion of the viral envelope with the cell membrane, but it is a more potent inhibitor of fusion than attachment.[20] It is likely that the present compounds **4** and **5** are acting by similar mechanisms, which would further complicate an interpretation of the observed inhibition of virus replication based on a model of CD4 binding. Thus, the CD4/cosalane binding model has provided information for the initial design of more effective cosalane analogues but requires further refinement to provide a more direct correlation of in vitro antiviral results to the interaction of cosalane with the complex CD4/gp120/fusion coreceptor target suggested by mechanistic studies for this class of compounds.

Polyanions are known to exert their anti-HIV activity by a shielding of gp120/V3 through an interaction of the negative charges of the polyanions, for example, the sulfate groups of dextran sulfate, with the positively charged amino acid residues in the V3 loop.[11] The early inhibitory effect of polyanionic compounds may be the result of a disruption of the ionic interactions between the charged regions of viral surface glycoproteins, including gp120, and the membrane phospholipids and/or the receptor molecules at the cell surface (i.e., CD4 and chemokine receptors including CXCR4 and CCR5). Enveloped viruses that bud from cells carry part of the cell membrane and therefore may interact more effectively with the negatively charged cell surface if the outer part of the envelope protein contains regions of high positive charge density. The V3 loop in HIV may provide this function, and the binding of polyanions to the loop not only neutralizes positive charges but may also add an additional negative potential, thereby electrostatically preventing the interaction between the virion and the cell. Prior studies have demonstrated that cosalane inhibits the binding of a specific anti-gp120 monoclonal antibody, directed to the V3 loop of HIV-1 gp120, to persistently HIV-1_{IIIB}-infected HUT-78 cells, thus establishing the possible involvement of the V3 loop of gp120 as a target in the anti-HIV activity of cosalane.[11]

The synthesis of **24** offers new methodology for the preparation of structurally defined aromatic oligomers related to ATA in a controlled fashion. Recent interest in the synthesis of functionalized diphenylmethane oligomers stems from their potential use in combinatorial chemistry strategies as platforms to support functional groups employed in the search for new pharmacophores.[22]

Experimental Section

General. 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

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as internal standard; s = singlet, d = doublet, m = multiplet, and bs = broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of the calculated compositions. Silica gel used for column chromatography was 230−400 mesh.

1,1-Bis(4-methoxy-3-methoxycarbonyl-5-nitrophenyl)-methane (7). A solution of 1,1-bis(4-methoxy-3-methoxycarbonylphenyl)methane **(6)**[15] (0.17 g, 0.5 mmol) in acetic anhydride (4 mL) was cooled to 0 °C while 90% HNO_3 (2 mL) was added. The mixture was stirred overnight while warming to room temperature. Ethanol (10 mL) was added, and the solvents were removed in vacuo. Methanol (20 mL) was added to the residue, and the solid was filtered off and air-dried. This gave 0.087 g, but more product (0.04 g) precipitated out of the methanol solution to give a total of 0.127 g (60%): mp 138−140° C; IR (neat) 1729, 1535 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, J = 2.3 Hz, 2 H), 7.69 (d, J = 2.3 Hz, 2 H), 4.03 (s, 2 H), 3.98 (s, 6 H), 3.94 (s, 6 H). Anal. Calcd for $C_{19}H_{18}N_2O_{10}$: C, 52.54; H, 4.18; N, 6.45. Found: C, 52.24; H, 4.04; N, 6.32.

1,1-Bis(3-amino-4-methoxy-5-methoxycarbonylphenyl)-methane (8). A solution of **7** (1.1 g, 2.5 mmol) in ethyl acetate (100 mL) was purged with nitrogen. Solid PtO_2 (0.15 g, 0.6 mmol) was added, and the solution was heated to 60 °C while stirring under a hydrogen atmosphere until TLC indicated complete consumption of the starting material. The solvents were removed at reduced pressure, and the residue was dissolved in EtOAc (10 mL) and chromatographed on silica gel (50.0 g, 230−400 mesh) eluting with EtOAc to give 0.73 g (78%) of the product as a thick oil: IR (neat) 3467, 3367, 2945, 1719, 1614 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.99 (d, J = 2.0 Hz, 2 H), 6.64 (d, J = 2.0 Hz, 2 H), 3.86 (s, 6 H), 3.78 (s, 6 H), 3.69 (s, 2 H). Anal. Calcd for $C_{19}H_{22}N_2O_6$: C, 60.95; H, 5.93; N, 7.48. Found: C, 60.67; H, 5.55; N, 7.25.

1,1-Bis[3-(4′-methoxy-3′-methoxycarbonylbenzamido)-4-methoxy-5-methoxycarbonylphenyl]methane (10). A solution of **8** (0.41 g, 1.0 mmol) in dry pyridine (4 mL) was added, via cannulation under nitrogen, to a nitrogen-purged flask containing a stirring bar and 4-methoxy-3-methoxycarbonylbenzoyl chloride **(9)**[17] (0.73 g, 3.2 mmol). The addition initially gave a homogeneous solution, but a solid soon separated out. More pyridine (4 mL) was added, and the mixture was stirred overnight at 60 °C. The addition of water (50 mL) gave a solid (0.71 g, 93%). Recrystallization of this material from acetone gave the bisamide as a white solid: mp 209−210 °C; IR (neat) 3419, 3010, 2897, 1724, 1673 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 2.1 Hz, 2 H), 8.31 (d, J = 2.4 Hz, 2 H), 8.03 (dd, J = 2.3, 8.7 Hz, 2 H), 7.42 (d, J = 2.0 Hz, 2 H), 7.08 (d, J = 8.9 Hz, 2 H), 4.00 (s, 2 H), 3.97 (s, 3 H), 3.91 (s, 6 H), 3.89 (s, 3 H). Anal. Calcd for $C_{39}H_{38}N_2O_{14}\cdot H_2O$: C, 60.31; H, 5.19; N, 3.61. Found: C, 60.64; H, 4.88; N, 3.49.

1,1-Bis[3-(3′-carboxy-4′-methoxybenzamido)-5-carboxy-4-methoxyphenyl]methane (11). A suspension of **10** (0.30 g, 0.4 mmol) in EtOH (8 mL) and water (2 mL) containing K_2CO_3 (1.0 g) was heated to 95 °C for 12 h. The solution was cooled to room temperature and poured into 1 N HCl (100 mL). Extraction with ethyl acetate (2 × 100 mL) and evaporation of the solvent gave the product (0.129 g, 45%): mp 226−229 °C (dec). IR (KBr) 3419, 2534 (b), 1717, 1680 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 9.78 (s, 2 H, N*H*), 8.27 (d, J = 2.1 Hz, 2 H), 8.12 (dd, J = 2.1, 8.8 Hz, 2 H), 7.75 (d, J = 2 Hz, 2 H), 7.43 (d, J = 1.8 Hz, 2 H), 7.24 (d, J = 9 Hz, 2 H), 3.96 (s, 2 H), 3.88 (s, 6 H), 3.70 (s, 6 H). The analytical sample was prepared as the tetrasodium salt, which was obtained as a glass that decomposed when heated above 100 °C. Anal. Calcd for $C_{35}H_{26}N_2O_{14}\cdot H_2O\cdot 2NaOH$: C, 45.64; H, 3.68; N, 3.04. Found: C, 45.96; H, 4.02; N, 2.91.

1,1′-Bis(3-amino-4-methoxy-5-methoxycarbonylphenyl)-methanol (13). A mixture of 4,4′-dimethoxy-3,3′-dimethoxycarbonyl-5,5′-dinitrobenzophenone **(12)**[15] (2.2 g, 4.9 mmol) was stirred in acetic acid (10 mL) and methanol (10 mL) and heated to 45 °C. Then, zinc dust (2.4 g, 37 mmol) was added, and the mixture was stirred at 45 °C until TLC (EtOAc, SiO_2) indicated that all of the starting material had been consumed (∼1 h). The zinc salts were filtered off, and the solution was concentrated to give an oil that was flash chromatographed on silica gel (50.0 g, 230−400 mesh), eluting with ethyl acetate/hexanes/triethylamine (100:50:1), to give the product as a foamy, low-melting solid (1.1 g, 58%): IR (neat) 3457, 3367, 1714 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, J = 1.9 Hz, 2 H), 6.93 (d, J = 1.8 Hz, 2 H), 5.54 (s, 1 H), 4.31 (bs, OH), 3.84 (s, 6 H), 3.76 (s, 6 H). Anal. Calcd for $C_{19}H_{22}N_2O_7\cdot 0.2CH_3CO_2H$: C, 57.91; H, 5.71; N, 6.96. Found: C, 57.90; H, 5.68, N, 6.60.

3,3′-Bis(4-methoxy-3-methoxycarbonylbenzamido)-4,4′-dimethoxy-5,5′-dimethoxycarbonylbenzophenone (14). Diamine **13** (0.388 g, 1 mmol) and 4-methoxy-3-methoxycarbonylbenzoyl chloride **(9)**[5] (0.258 g, 2.1 mmol) were stirred in dry pyridine (5 mL) and CH_2Cl_2 (5 mL) overnight under nitrogen. Solid CrO_3 (0.6 g, 6 mmol) was added, and the mixture was stirred at 0 °C for 3 h. The CH_2Cl_2 was removed in vacuo, and the solution was poured into water (100 mL). The solid produced was filtered off and air-dried to give crude material (1.2 g). Purification by silica gel column chromatography (20 g, 230−400 mesh), eluting with ethyl acetate/hexanes (2:1, v/v), gave the product as an amorphous glass (0.45 g, 85%): IR (neat) 3421, 3003, 3338, 1730, 1675 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.07 (d, J = 2.0 Hz, 2 H), 8.65 (s, 2 H, NH), 8.32 (d, J = 2.5 Hz, 2 H), 8.09 (d, J = 2.2 Hz, 2 H), 8.03 (dd, J = 8.8, 2.5, 2 H), 7.09 (d, J = 8.8 Hz, 2 H), 4.03 (s, 6 H), 3.97 (s, 6 H), 3.95(s, 6 H), 3.91(s, 6 H); FABMS m/z (relative intensity) 772 (MH^+, 16.3), 193 (100). Anal. Calcd for $C_{39}H_{36}N_2O_{15}\cdot 0.5H_2O$: C, 59.92; H, 4.77; N, 3.58. Found: C, 59.89; H, 4.65; N, 3.37.

3-Bromo-5-formyl-6-hydroxybenzoic acid (16). A mixture of hexamethylenetetramine (10.0 g, 71 mmol) and 5-bromosalicylic acid (3.25 g, 15 mmol) was heated in trifluoroacetic acid (30 mL) to 90 °C for 16 h. The homogeneous mixture was poured into 1 N HCl (100 mL) and stirred for 6 h. The precipitate was collected by filtration and recrystallized from ethanol/water to afford the product (2.7 g, 72%): mp 204−206 °C; IR (KBr) 1673 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 10.26 (s, 1 H), 8.11 (d, J = 2.7 Hz, 1 H), 7.96 (d, J = 2.7 Hz, 1 H). Anal. Calcd for $C_8H_5BrO_4\cdot H_2O$: C, 36.53; H, 2.68. Found: C, 36.93; H, 2.68.

3-Bromo-6-hydroxy-5-hydroxymethylbenzoic Acid (17). A solution of **16** (1.34 g, 5 mmol) in ethanol (50 mL), water (20 mL), and 1 N NaOH (20 mL) was cooled to 0 °C by means of stirring in an ice bath. Solid $NaBH_4$ (0.18 g, 5 mmol) was added, and the mixture was stirred at 0 °C for 4 h, after which the bright yellowish green color of the aldehyde had disappeared. Acetone (10 mL) was added, and the solvents were removed in vacuo. The solid paste was dissolved in water (10 mL) and poured into 1 N HCl (100 mL) to give the product (1.2 g, 90%) as a white solid: mp 178−180 °C; IR (KBr) 3418, 2569 (bs), 1688 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 7.75 (s, J = 2.2 Hz, 1 H), 7.66 (d, J = 2.4 Hz, 1 H), 4.50 (s, 2 H). Anal. Calcd for $C_8H_7BrO_4$: C, 38.89; H, 2.86. Found: C, 38.68; H, 2.78.

1,1-Bis[3-bromo-3-′chloro-4′,6-dihydroxy-5,5′-dimethoxycarbonyl diphenyl]methane (19). A mixture **17** (5.0 g, 20 mmol) and 3-chlorosalicylic acid **(18)** (2.69 g, 15 mmol) dissolved in MeOH (15 mL) was cooled to 0 °C. Concentrated sulfuric acid (60 mL) was added dropwise at 0 °C. The mixture was stirred while warming to ambient temperature for 12 h and then poured into ice water (200 mL). The solid was filtered off, washed with water, and air-dried overnight. The crude product was heated to reflux temperature in a mixture of methanol (70 mL) and H_2SO_4 (10 mL) overnight. A solid slowly precipitated out of the mixture. The cooled solution was filtered and washed with cold methanol (20 mL) to give the product as a white solid (2.6 g, 32%): mp 166−168 °C; IR (neat) 3090, 1677, 1608 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 11.20 (s, OH), 11.03 (s, OH), 7.85 (d, J = 2.6 Hz, 1 H), 7.63 (d, J = 2.1 Hz, 1 H), 7.42 (d, J = 2.1 Hz, 1 H), 7.32 (d, J = 2.4 Hz, 1 H), 3.94 (s, 3 H), 3.93 (s, 3 H), 3.85 (s, 2 H). Anal. Calcd for $C_{17}H_{14}BrClO_6$: C, 47.52; H, 3.28. Found: C, 47.82; H, 3.32.

1,1-Bis[3-chloro-3′,5-dicarboxy-2,4′-dihydroxydiphenyl]-methane (20). A solution of **19** (0.408 g, 1 mmol) dissolved in 5% NaOH (50 mL) was stirred overnight in the presence of

zinc dust (3.0 g). The zinc was removed by filtration through a pad of Celite, and acidification of the aqueous solution with concentrated HCl to pH 1 gave a white solid (0.256 g, 77%). The solid was air-dried to give the product: mp 263−265 °C; IR (KBr) 2936, 1660, 1613 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 11.27 (s, 1 H), 7.25 (dd, J = 1.5, 7.9 Hz, 1 H), 7.19 (d, J = 1.9 Hz, 1 H), 7.12 (d, J = 1.9 Hz, 1 H), 7.01 (dd, J = 1.1, 7.3 Hz, 1 H), 6.44 (t, J = 7.7 Hz, 1 H), 3.44 (s, 2 H). Anal. Calcd for $C_{15}H_{11}ClO_6 \cdot 0.7H_2O$: C: 53.73; H: 3.73. Found: C: 53.76. H: 4.00

1,1-Bis[3-chloro-4,6′-dihydroxy-5,5′-dimethoxycarbonyldiphenyl]methane (21). A mixture of **20** (2.62 g, 8.1 mmol) in methanol (200 mL) containing sulfuric acid (20 mL) was heated to 80 °C for 1.5 days. The solvents were concentrated to one-half the initial volume, and the solution was allowed to stand at room temperature overnight. The solid product (1.9 g, 65%) was collected by filtration: mp 108−109 °C; IR (neat) 3139, 2952, 1675 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.73 (dd, J = 1.5, 8.0 Hz, 1 H), 7.65 (d, J = 2.0, 1 H), 7.43 (d, J = 2.0 Hz, 1 H), 7.26 (m, 1 H), 6.81 (t, J = 7.7 Hz, 1 H), 3.93 (s, 3 H), 3.92 (s, 3 H). Anal. Calcd for $C_{17}H_{15}ClO_6$: C, 58.21; H, 4.31. Found: C, 58.26; H, 4.25.

1,1-Bis[3,3-(3′-chloro-4′-hydroxy-5′-methoxycarbonylbenzyl)-4-hydroxy-5-methoxycarbonylphenyl)]-2-chloroethane (22). A mixture of **21** (2.34 g, 7.2 mmol), chloroacetaldehyde dimethyl acetal (1.14 mL, 10 mmol), and acetic acid (15 mL) was stirred at 0 °C while concentrated sulfuric acid (60 mL) was added dropwise. The mixture slowly became red. After stirring overnight while warming to room temperature, the brown mixture was poured into ice (200 g), and the grayish solid produced was filtered and air-dried. Flash chromatography on SiO_2 (200 g, 230−400 mesh) eluting with hexane/ethyl acetate (4:1, v/v) gave recovered **21** (0.31 g, 0.9 mmol) followed by **22** (0.82 g, 1.1 mmol, 30%) as a glass: IR (neat) 3140, 2958, 1677, 1610 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 11.15 (s, OH), 11.03 (s, OH), 7.60 (d, J = 1.98 Hz, 2 H), 7.54 (d, J = 2.1 Hz, 2 H), 7.33 (d, J = 1.9 Hz, 2 H), 7.03 (d, J = 1.8 Hz, 2 H), 4.19 (t, J = 7.43, 1 H), 3.93 (d, J = 7.5 Hz, 2 H), 3.90 (s, 12 H), 3.85 (s, 4 H). Anal. Calcd for $C_{36}H_{31}Cl_3O_{12}$: C, 56.75; H, 4.10. Found: C, 56.71; H, 3.99.

1,1-Bis[3,3-(3′-chloro-4′-methoxy-5′-methoxycarbonylbenzyl)-4-methoxy-5-methoxycarbonylphenyl]-2-chloroethane (23). A mixture of **22** (0.8 g, 1.1 mmol), K_2CO_3 (1.0 g), and dimethyl sulfate (2 mL, 48 mmol) was stirred in DMF (25 mL) overnight at room temperature. The solution was poured into water, extracted with ethyl acetate (2 × 100 mL), washed with brine, and dried over Na_2SO_4. The solvents were removed at reduced pressure, and the oil was flash chromatographed on SiO_2 (100 g, 230−400 mesh), eluting with hexanes/ethyl acetate (3:1, v/v), to give the product as an oil (0.51 g, 56.8%): IR (neat) 2950, 2828, 1729, 1600 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 2 H), 7.47 (s, 2 H), 7.26 (s, 2 H), 7.12 (s, 2 H), 4.25 (t, 1 H, J = 7.4 Hz), 3.88 (m, 20 H), 3.69 (s, 6 H). Anal. Calcd for $C_{40}H_{39}Cl_3O_{12} \cdot CHCl_3$: C, 52.53; H, 4.30. Found: C, 52.39; H, 4.27.

3,3′-Di(3′-chloro-4′-methoxy-5′-methoxycarbonyl-benzyl)-4,4′-dimethoxy-5,5′-dimethoxycarbonylbenzophenone (24). A solution of **23** (0.51 g, 0.62 mmol) in MeOH (50 mL) containing KOH (1.0 g) was heated to reflux temperature for 12 h. The solvents were removed at reduced pressure, and the solid material was triturated in 1 N HCl (100 mL), extracted with ethyl acetate (100 mL), and dried over $MgSO_4$. The solvents were evaporated to give the product as a glass (0.398 g, 90% recovery). This material was inspected by NMR, which indicated the presence of the desired compound **23** with the following shifts: ^1H NMR (300 MHz, acetone-d_6) δ 7.72 (d, J = 2.3 Hz, 2 H), 7.63 (d, J = 2.0 Hz, 2 H), 7.50 (d, J = 1.9 Hz, 2 H), 7.48 (d, J = 2.2 Hz, 2 H), 5.50 (s, 2 H), 4.07 (s, 4 H), 3.85 (s, 6 H), 3.83 (s, 6 H). The crude material was dissolved in MeOH (200 mL), and ozonized air was bubbled through the solution at −78 °C for 1 h. The reaction was quenched at −78 °C with the addition of 5% KI solution (2 mL). The solvents were removed in vacuo, and the residue was partitioned between ethyl acetate (100 mL) and 5% Na_2SO_3. The organic layer was dried over $MgSO_4$ and evaporated to give crude ketone. This material was immediately esterified by stirring a solution of the crude ketone in acetone (50 mL) containing dimethyl sulfate (2 mL) and K_2CO_3 for 3 h at room temperature. The solid mass was filtered off, and the solvents were evaporated at reduced pressure to give an oil. The oil was flash chromatographed on SiO_2 (25 g, 230−400 mesh), eluting with hexanes/ethyl acetate (1:1, v/v), to give **24** (0.12 g, 45%) as an oil: IR (neat) 2950, 2828, 1731, 1659 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J = 2.2 Hz, 2 H), 7.73 (d, J = 2.2 Hz, 2 H), 7.49 (d, J = 2.2 Hz, 2 H), 7.32 (d, J = 2.2 Hz, 2 H), 3.98 (s, 4 H), 3.87 (s, 6 H), 3.85 (s, 6 H), 3.84 (s, 6 H), 3.79 (s, 6 H). Anal. Calcd for $C_{39}H_{36}Cl_3O_{13} \cdot H_2O$: C, 58.43; H, 4.78. Found: C, 58.50; H, 4.53.

3β-(3-Oxopropanyl)-5a-cholestane (26). The alcohol **25**[2] (0.32 g, 0.75 mmol) was dissolved in CH_2Cl_2 (5 mL), and neutral alumina (0.5 g) was added. Solid pyridinium chlorochromate (0.22 g, 1 mmol) was then added, and the mixture was stirred at room temperature for 6 h. The residue produced was placed atop a column of silica gel (30.0 g, 230−400 mesh) and flash chromatographed using ethyl acetate/hexanes (4:1) to give the aldehyde as an oil that crystallized to afford a solid (0.22 g, 69%) upon cooling: mp 58−60 °C; IR (neat) 2717, 1723 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.76 (s, 1 H, CHO), 2.43 (td, J = 7.53, 1.7 Hz, 2 H), 1.34 (m, 6 H), 1.2 (m, 6 H), 0.7 (s, 3 H), 0.64 (s, 5 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.1, 56.6, 56.3, 54.6, 46.5, 41.6, 40.1, 39.5, 38.4, 37.6, 36.2, 39.5, 38.4, 37.6, 36.2, 35.8, 35.5, 35.4, 32.1, 29.3, 28.9, 28.7, 28.2, 28.0, 24.2, 23.8, 22.8, 22.5, 21.0, 18.7, 12.3, 12.1. Anal. Calcd for $C_{30}H_{52}O$: C, 84.04; H, 12.23. Found: C, 84.00; H, 12.49.

5α,3β-[4′,4′-(3″,3″-Bis[3‴-methoxy-4‴-methoxycarbonylbenz-amido]-4″,4″-dimethoxy-5″,5″-dimethoxycarbonyldiphenyl)-3′-butenyl]cholestane (27). A suspension of $TiCl_4 \cdot 2THF$ (0.5 g, 1.5 mmol) and zinc dust (0.1 g, 1.5 mmol) in dry THF (5 mL) was heated to reflux under Ar for 30 min. A solution of ketone **14** (0.231 g, 0.3 mmol) and aldehyde **26** (0.183 g, 0.4 mmol) dissolved in dry THF (5 mL) was added by cannulation. The mixture was heated for 1 h at reflux temperature and then cooled to room temperature. Ethyl acetate (50 mL) was added, and the suspension was filtered through silica gel (10.0 g, 230−400 mesh). The silica gel was washed with ethyl acetate (50 mL), and the filtrate was concentrated to an oil. The oil was chromatographed (30.0 g SiO_2, 230−400 mesh), eluting with benzene/THF (10:1), to give the product as a glassy solid (0.120 g, 25%): IR (neat) 3423, 3332, 1738, 1731, 1714, 1681 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.63 (s, 1 H, NH), 8.59 (d, J = 1.6 Hz, 1 H), 8.54 (s, 1 H, NH), 8.52 (d, J = 1.7 Hz, 1 H) 8.31 (d, J = 2.4 Hz, 2 H), 8.03 (s, 1 H), 8.01 (s, 1 H), 7.45 (d, J = 1.4 Hz, 1 H), 7.31 (d, J = 1.9 Hz, 1 H), 7.06 (d, J = 8.8 Hz, 2 H), 6.11 (t, J = 7.4 Hz, 1 H), 4.03 (s, 3 H), 3.97 (s, 3 H), 3.89 (s, 3 H), 3.83 (s, 3 H), 2.12 (q, J = 7.5 Hz, 2 H), 1.60−1.00 (m, 40 H), 0.86 (s, 3 H), 0.71 (s, 3 H), 0.54 (s, 3 H); FABMS m/z (relative intensity) 1169.25 (MH^+, 77.3), 1152.25 (50.4), 1151.25 (64.2), 945.25 (54.5), 757.0 (60.4), 565 (100). Anal. Calcd for $C_{69}H_{88}N_2O_{14} \cdot H_2O$: C, 69.79; H, 7.64; N, 2.36. Found: C, 69.91; H, 7.50; N, 2.22.

5α,3β-4′,4′-[3″,3″-Bis(3‴-carboxy-4‴-methoxybenzamido)-5″,5″-dicarboxy-4″,4″-dimethoxydiphenyl]-3′-butenyl]-cholestane (4). Alkene **27** (0.1 g, 0.08 mmol) was stirred in 0.5 N NaOH (2 mL, 1 mmol) and THF (2 mL) overnight in an argon atmosphere. The solution was acidified by the addition of TFA (1 mL), and the solvents were removed in vacuo. The residue was chromatographed on SiO_2 (20.0 g, 230−400 mesh), eluting with CH_2Cl_2/MeOH/TFA (100:10:1, v/v), to give the product (87 mg, 90%) as a glass. IR (KBr) 3287, 2554 (b), 1733, 1720, 1702, 1686, 1653 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 8.50 (m, J = 2.25, 2.25 Hz, 5 H), 8.21 (t, J = 8.3 Hz, 1 H), 8.20 (t, J = 8.6 Hz, 1 H), 7.50 (d, J = 2.1 Hz, 1 H), 7.48 (d, J = 2.2 Hz, 1 H), 7.34 (d, J = 8.8 Hz, 1 H), 7.32 (d, J = 8.8 Hz, 1 H), 6.22 (t, J = 7.6 Hz, 1 H), 4.05 (s, 3 H), 4.03 (s, 3 H), 4.01 (s, 3 H), 3.92 (s, 3 H) 2.2 (q, J = 7.1 Hz, 2 H), 1.60−1.00 (m, 40 H), 0.90 (s, 3 H), 0.69 (s, 3 H), 0.55 (s, 3 H). Anal. Calcd for $C_{65}H_{80}N_2O_{14} \cdot H_2O$: C, 69.01; H, 7.31; N, 2.48. Found: C, 69.04; H, 7.64; N, 2.11. The tetrasodium salt was obtained as a glass that decomposed slowly upon heating above 100 °C: FTIR (KBr) 3419, 2925, 1658, 1613, 1573 cm^{-1}. Anal. Calcd for

$C_{65}H_{76}N_2O_{14}Na_4 \cdot 8H_2O$: C, 58.03; H, 6.89; N, 2.08. Found: C, 57.70; H, 7.06; N, 1.72.

5α,3β-[4,4-(3′,3″-(3‴-Dichloro-4‴-dimethoxy-5‴-dimethoxycarbonylbenzyl)-4′,4″dimethoxy-5′,5″-dimethoxycarbonylphenyl)-buten-3-yl]cholestane (28). A suspension of $TiCl_4 \cdot 2THF$ (0.339 g, 1 mmol) and Zn (0.24 g, 4 mmol) was heated in THF (10 mL) under argon at reflux for 1 h. A solution of ketone **24** (0.25 g, 0.32 mmol) and aldehyde **26** (0.2 g, 0.5 mmol) in THF (20 mL) was added under argon, and the mixture was heated at reflux for 1 h. The reaction was stopped by pouring it into 1 N HCl (100 mL) and extracting with ethyl acetate (100 mL). The organic layer was dried over $MgSO_4$ and evaporated to afford an oil. The oil was flash chromatographed on silica gel (230−400 mesh, 20 g), eluting with hexanes/ethyl acetate (2:1, v/v), to give the product (0.13 g, 33%) as an oil: IR (neat) 2929, 2848, 1731, 1600 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, J = 1.9 Hz, 1 H), 7.50 (t, J = 2.4 Hz, 2 H), 7.47 (d, J = 2.1 Hz, 1 H), 7.44 (t, J = 2.3 Hz, 2 H), 7.33 (d, J = 2.2 Hz, 1 H), 7.31 (d, J = 2.2 Hz, 1 H), 6.12 (t, J = 7.5 Hz, 1 H), 4.08 (s, 2 H), 4.00 (s, 2 H), 3.84 (s, 18 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 3.75 (s, 3 H), 2.08 (q, J = 7.5 Hz, 2 H). Anal. Calcd for $C_{69}H_{88}Cl_2O_{12} \cdot C_6H_{14}$: C,71.12; H, 8.12. Found: C, 70.94; H, 8.34.

5α,3β-[4,4-(3′,3″-Dicarboxy-5′,5″-(3‴-dicarboxy-5‴-dichloro-4‴-dimethoxybenzyl)-4′,4″-dimethoxyphenyl)-buten-3-yl]cholestane (5). The tetraester (0.13 g, 0.11 mmol) was heated in THF (10 mL) containing 0.5 N NaOH (2 mL) at reflux temperature overnight. The solution was cooled to room temperature and poured into ethyl acetate (50 mL). Acidification with 1 N HCl (50 mL) and evaporation of the organic layer gave the product (0.12 g, 97%) as a glass: IR (KBr) 3236, 2658 (b), 1699, 1600; ^1H NMR (300 MHz, acetone-d_6) δ 7.66 (d, J = 1.8 Hz, 1 H), 7.61 (s, 2 H), 7.57 (d, J = 1.7 Hz, 1 H), 7.48 (d, J = 1.8 Hz, 1 H), 7.45 (d, J = 1.9 Hz, 1 H), 7.37 (d, J = 2.1 Hz, 1 H), 7.33 (d, J = 1.9 Hz, 1 H), 6.15 (t, J = 7.7 Hz, 1 H), 4.11 (s, 2 H), 4.03 (s, 2 H), 3.86 (s, 6 H), 3.84 (s, 3 H), 3.80 (s, 3 H), 2.13 (dd, J = 14.1, 7.3 Hz, 2 H). Anal. Calcd for $C_{69}H_{80}Cl_2O_{12} \cdot 0.75CH_3OH$: C, 68.77; H, 7.29. Found: C, 69.09; H, 7.66. The tetrasodium salt was obtained as a lyophilized powder: IR (KBr) 2926, 1604, 1579 cm^{-1}. Anal. Calcd for $C_{65}H_{76}Cl_2O_{12}Na_4 \cdot 2H_2O \cdot CH_3OH$: C, 62.18; H, 6.67. Found: C, 61.93; H, 6.95.

In Vitro Anti-HIV Assays. Evaluations of the antiviral activity of compounds against HIV-1_{RF}, HIV-1_{IIIB}, and HIV-2_{ROD} infection in CEM-SS and MT-4 cells were as previously described.[23,24]

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