Cosalane Analogues with Enhanced Potencies as Inhibitors of HIV-1 Protease and Integrase

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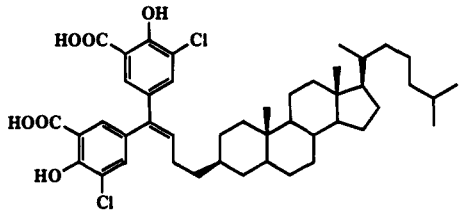
Several new analogues of the novel anti-HIV agent cosalane have been synthesized and evaluated as inhibitors of HIV-1 integrase and protease, HIV-1 replication, HIV-1 and HIV-2 cytopathicity, HIV-1- and HIV-2-mediated syncytium formation, and cytopathicity of a variety of human pathogenic viruses. The congeners displayed enhanced potencies relative to cosalane itself as inhibitors of HIV-1 integrase and protease. The two most potent analogues against HIV-1 integrase displayed IC_{50} values of 2.2 μM, while the three most potent compounds against HIV-1 protease had IC_{50} values in the 0.35–0.39 μM range. In addition to its activity against HIV-1 and HIV-2 cytopathicity, cosalane inhibited the cytopathic effects of herpes simplex virus-1, herpes simplex virus-2, and human cytomegalovirus at concentrations that were well below the cytotoxic concentrations. Potentially useful antiviral activities were also revealed for some of the new cosalane congeners against influenza virus, Junin virus, and Tacaribe virus.

Introduction

In the search for agents that will effectively combat HIV-1 infection, it is imperative that we consider the design of compounds that will act on multiple stages of the viral replication cycle because such an approach may prevent the rapid generation of drug-resistant mutant viral strains. Toward this end, we recently reported the design and synthesis of the noval anti-HIV agent cosalane (**1**).[1,2] Time course studies indicated quite clearly that the mechanism of anti-HIV activity of cosalane (**1**) was mainly due to interference with the gp120-CD4 binding interaction, as well as a postattachment event prior to reverse transcription.[2] However, experiments in cell-free systems demonstrated the ability of cosalane to also inhibit the enzymatic activities of HIV-1 reverse transcriptase and protease.[2]



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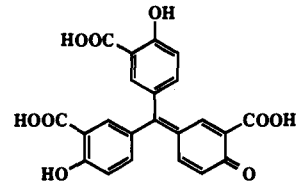
Recent interest in nonpeptide-based HIV-1 protease inhibitors has increased dramatically, as these molecules might display favorable bioavailability and metabolic stability properties relative to the peptide-based inhibitors.[3–9] Hence, a program was initiated to synthesize and screen analogues of cosalane as inhibitors of HIV-1 protease in an effort to identify nonpeptide enzyme inhibitors with enhanced potencies relative to cosalane (**1**). Likewise, prior studies have demonstrated the ability of aurintricarboxylic acid (ATA) and the ATA monomer **2**, the conceptual parent molecule of cosalane, to inhibit the *in vitro* enzymatic activity of the HIV-1 integration protein (integrase, IN).[10] The IN protein is required for insertion of the infectious double-stranded proviral DNA copy of the viral RNA genome into the host cellular DNA,[11] and this protein represents a prime target for antiviral therapy. In view of these findings, a decision was made to also evaluate cosalane and the synthetic analogues as inhibitors of the HIV-1 integration protein.



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As reported in the present communication, cosalane (**1**) and several structurally-related compounds in fact displayed significant inhibitory activity against HIV-1 protease and HIV-1 IN. The cosalane congeners selected for presentation were chosen from a large number of analogues because they proved to have enhanced potencies as inhibitors of HIV-1 IN and/or protease relative to cosalane (**1**) itself and because they exhibited inhibitory activity against several pathogenic viruses other than HIV.

Results

Chemistry. Cosalog-1 (**6**) was designed as a cosalane analogue lacking the linker chain between the disalicylmethane unit and the steroid. In compound **6**,

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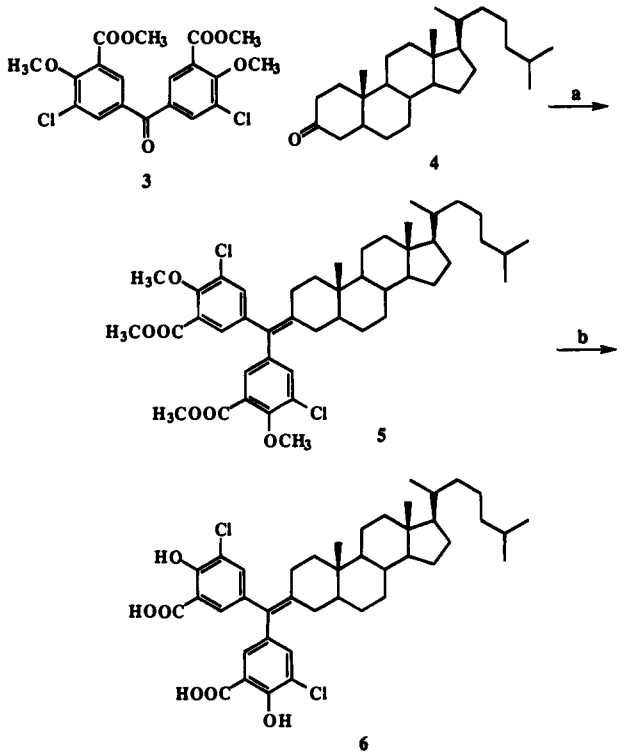
Scheme 1[a]



[a] (a) $TiCl_3$, ZnCu couple, DME, reflux (5.5 h); (b) $BBr_3 \cdot Me_2S$, $ClCH_2CH_2Cl$, 80 °C (3 h).

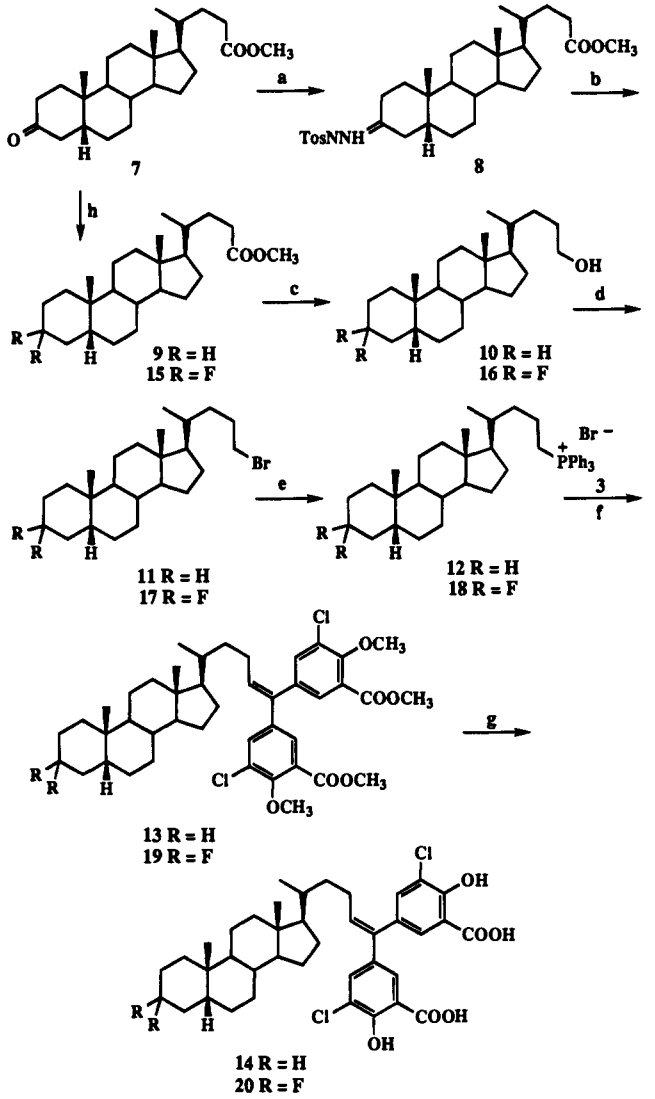
the diphenylmethane moiety is attached directly to the 3-position of the cholestane system by an alkene linkage. The synthesis of **6** was achieved in two steps from readily available starting materials as indicated in Scheme 1. McMurry coupling[12] of 3-cholestanone (**4**) with the substituted benzophenone **3** afforded intermediate **5**, which was demethylated with boron tribromide–dimethyl sulfide complex to yield cosalog-1 (**6**).

In cosalog-2 (**14**), the disalicylmethane moiety was attached through a linker chain to the D ring of the steroid instead of the A ring as in cosalane (**1**). The synthesis of **14** proceeded from methyl 3-ketocholanate (**7**)[13] as outlined in Scheme 2. The starting ketone **7** was deoxygenated by reduction of the tosylhydrazone **8** with sodium cyanoborohydride in THF–sulfolane to afford the reduced product **9**.[14] Reduction of the methyl ester **9** with lithium aluminum hydride gave the alcohol **10**, which was converted to the primary bromide **11** with triphenylphosphine and carbon tetrabromide.[15] Conversion of the bromide **11** to the triphenylphosphonium salt **12** followed by reaction of the corresponding Wittig reagent with the substituted benzophenone **3** yielded intermediate **13**, which was then demethylated with boron tribromide–dimethyl sulfide complex to afford the product **14**.[16]

Cosalog-3 (**20**) is the 3,3-difluoro analogue of cosalog-2 (**14**). The two fluorines were introduced into the steroid framework by treatment of methyl 3-ketocholanate (**7**)[13] with (diethylamino)sulfur trifluoride (DAST) in benzene at room temperature to afford **15**. The subsequent conversion of intermediate **15** to cosalog-3 (**20**) was essentially identical to the previously-described conversion of **9** to **14** (Scheme 2).

In cosalog-4 (**25**), the steroid portion of cosalane (**1**) is replaced by an adamantyl substituent. The synthesis of cosalog-4 (**25**), which is depicted in Scheme 3, started from 1-adamantaneethanol (**21**), which was converted

Scheme 2[a]

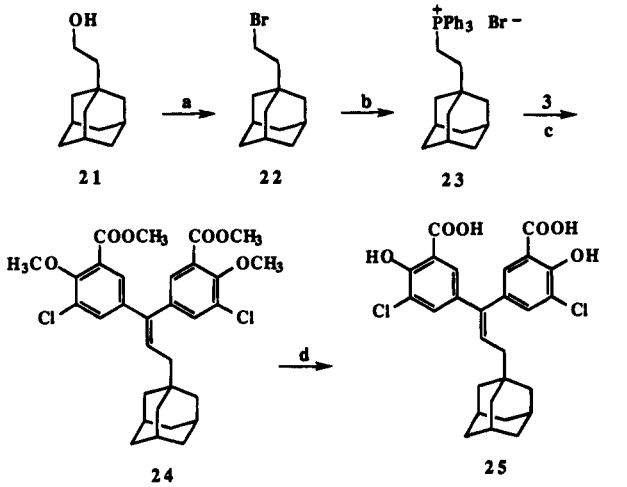


[a] (a) $TsNHNH_2$, AcOH, 23 °C (20 h); (b) $NaCNBH_3$, TsOH, DMF–sulfolane, 110 °C (3 h); (c) $LiAlH_4$, THF, 0 °C (1 h); (d) Ph_3P, CBr_4, CH_2Cl_2, 0 °C (10 min); (e) Ph_3P, PhCl, 140 °C (48 h); (f) (1) $NaN[Si(CH_3)_3]_2$, THF, 0 °C (30 min), (2) compound **3**, THF, 23 °C (48 h); (g) $BBr_3 \cdot (CH_3)_2S$, $ClCH_2CH_2Cl$, 70 °C (24 or 28 h); (h) DAST, benzene, 23 °C (48 h).

to the bromide **22** with triphenylphosphine and carbon tetrabromide.[15] Intermediate **22** was then converted to the corresponding triphenylphosphonium bromide salt **23**. Deprotonation of **23** and reaction of the resulting ylide with the substituted benzophenone **3** afforded the Wittig product **24**, which was demethylated with boron tribromide–dimethyl sulfide complex to yield the desired compound **25**.

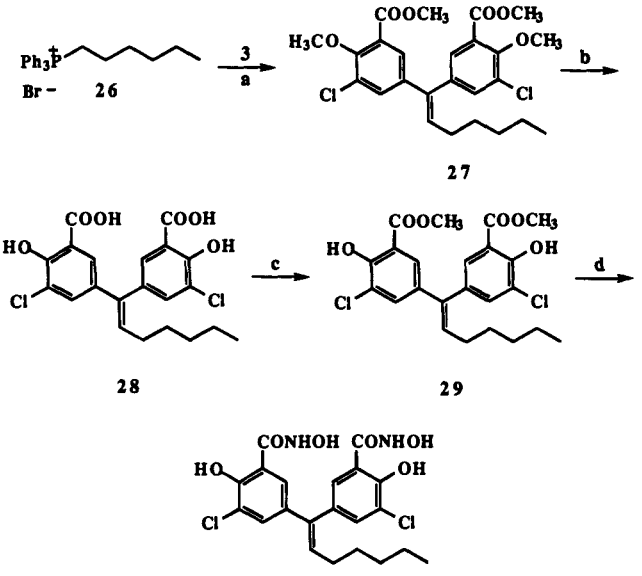
Cosalog-5 (**30**) is a hydroxamic acid-containing compound with a 6-carbon side chain appended to the disalicylmethane moiety. As outlined in Scheme 4, reaction of the Wittig reagent derived from hexyltriphenylphosphonium bromide (**26**) with intermediate **3** provided the expected alkene **27**, which was demethylated with boron tribromide in methylene chloride to afford compound **28**. Although the diacid **28** proved to be recalcitrant to Fischer esterification, the diester **29** could be obtained on prolonged heating in methanol under acidic conditions. The hydroxamic acid was then prepared by treatment of the diester **29** with hydroxylamine in aqueous dioxane.

Scheme 3[a]



[a] (a) PPh_3, CBr_4, CH_3CN, reflux (2.5 h); (b) PPh_3, C_6H_5Cl, reflux (4 days); (c) (1) $NaN[Si(CH_3)_2]_2$, THF, 15 min, (2) intermediate **3**, 70 °C (9 h); (d) $BBr_3 \cdot S(CH_3)_2$, $ClCH_2CH_2Cl$, 90 °C (5 h), then 23 °C (14 h).

Scheme 4[a]



[a] (a) (1) NaH, DMSO, 23 °C (15 min), (2) compound **3**, DMSO, 55 °C (27 h); (b) BBr_3, CH_2Cl_2, 23 °C (2 days); (c) CH_3OH, H_2SO_4, 90 °C (5 days); (d) H_2NOH, aq dioxane, 23 °C (20 h).

Inhibition of HIV-1 Enzyme Functions. Aurintricarboxylic acid (ATA), the conceptual precursor molecule for cosalane (**1**), was previously found to inhibit the enzymatic activity of the HIV-1 integration protein.[10] Retroviral integration proteins carry out a selective endonucleolytic cleavage of retroviral DNA's on the 3′ side of an A residue of a conserved CA dinucleotide, which is positioned, in general, two bases from the 3′ end of the minus strand of U3 and the plus strand of U5.[17,18] This 3′-processing reaction, in which two nucleotides are removed from the end of the strand, is required for insertion of the viral DNA into the host cellular DNA and can be modeled *in vitro* with synthetic double-stranded oligonucleotide mimetics of the viral DNA.[19-21] Utilizing this *in vitro* model, we compared the inhibitory activity of cosalane (**1**) to that of unfractionated, polymeric ATA against both the HIV-1 IN-mediated 3′-processing reaction and the strand transfer reaction. As displayed in Figures 1 and 2, cosalane

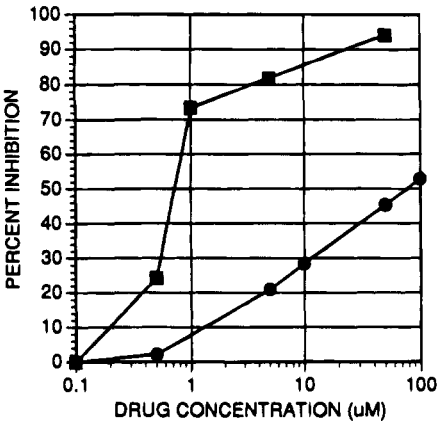


Figure 1. Inhibition of HIV-1 IN-catalyzed 3′ processing by cosalane (●) and unfractionated aurintricarboxylic acid (■). The concentration of ATA is based on the molecular weight of the monomer **2**.

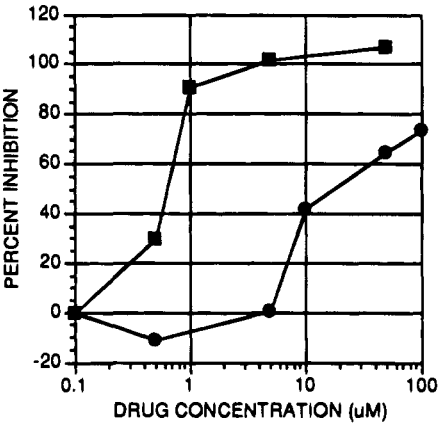


Figure 2. Inhibition of HIV-1 IN-catalyzed strand transfer by cosalane (●) and unfractionated aurintricarboxylic acid (■).

(**1**) inhibited both the 3′-processing and the strand transfer functions of IN, although less effectively than the unfractionated ATA.

Analysis of the actions of the cosalane analogues (ammonium salt forms) on the 3′-processing reaction catalyzed by HIV-1 IN revealed marked differences in inhibitory activities as compared to cosalane (Table 1). The most potent of the cosalane analogues proved to be cosalog-2 (**14**) and cosalog-3 (**20**), which both displayed IC_{50} values of 2.2 μM. These analogues were approximately 1 order of magnitude more potent than cosalane itself (IC_{50} = 25 μM). Cosalog-5 (**30**), IC_{50} = 4 μM, and cosalog-4 (**25**), IC_{50} = 7 μM, were less potent than the most potent analogues, **14** and **20**, although both were more potent than cosalane itself. The results indicate that the HIV-1 IN inhibitory activity of cosalane (**1**) is not associated with a high degree of structural specificity within the side chain moiety, provided the disalicylic acid core structure was maintained.

Because cosalane, a nonpeptide molecule, was found to inhibit HIV-1 protease activity, we wanted to evaluate the cosalane analogues for their antiprotease activity. The results of the HIV-1 protease inhibition studies by the cosalane analogues are also listed in Table 1. The most potent inhibitors were cosalog-1 (**6**), IC_{50} = 0.35 μM, cosalog-4 (**25**), IC_{50} = 0.37 μM, and cosalog-3 (**20**), IC_{50} = 0.39 μM. All three of these compounds were approximately 4 times as potent as cosalane (**1**) itself, which displayed an IC_{50} of 1.5 μM. Cosalog-2 (**14**) was equipotent with cosalane (**1**), while cosalog-5 (**30**), IC_{50}

Table 1. Inhibition of HIV-I Integration Protein (IN), Protease, and p24 Production in PBL's by Cosalane and Cosalane Analogues[a]

	IC_{50} (μM)								CC_{50} (μM)		
				viral cytopathicity			giant cell assay		cytotoxicity[i]		
compd	IN[b]	pro-tease[c]	p24[d]	HIV-1[e]	HIV-2[f]	HIV-1[g]	HIV-1	HIV-2	PBL	MT-4	CEM
cosalane (1)	25	1.5	33	1.8	4.0	3.0	25	25	67	>125	>200
cosalog-1 (**6**)	>100	0.35	59.3	2.5	6.0	83.3	24	24	78	36.7	>200
cosalog-2 (**14**)	2.2	1.5	39	27.2	>31.3	28.0	24	40	135	31.4	69.5
cosalog-3 (**20**)	2.2	0.39	24	27.7	3.5	24.0	8	24	69.5	34.0	38.5
cosalog-4 (**25**)	7	0.37	148	>40	–	>110	>40	>40	185	>40	105
cosalog-5 (**30**)	4	58	112	21.9	>40	>200	8	40	148	>40	36.2

[a] All of the compounds were tested as their ammonium salts. [b] Inhibition of strand transfer. [c] Inhibition of HIV-1 protease. [d] Production determined in PBL's. [e] HTLV-III_B strain determined in MT-4 cells. [f] LAV-2_{ROD} strain determined in MT-4 cells. [g] RF strain determined in CEM cells. [h] Giant cell formation between persistently HIV-1 (HTLV-III_B) or HIV-2 (LAV-2_{ROD})-infected HUT-78 cells and uninfected MOLT-4 (clone 8) cells. [i] The values reported are based on reduction of cellular viability. Data represent mean values of at least two separate experiments.

Table 2. Antiviral Minimum Inhibitory Concentrations (IC_{50}, μg/mL) and Cytotoxicities (MCC, μg/mL) of Cosalane (**1**) and Cosalane Analogues[a]

	compound (IC_{50} or MCC)					
viruses and cells	1	**6**	**14**	**20**	**25**	**30**
herpes simplex virus-1 (KOS)[b]	10	>20	>20	20	>5	5
herpes simplex virus-2 (G)[g]	7.4	12	>20	>20	>20	>5
herpes simplex virus-1 TK^- (B2006)[b]	>20	>20	>20	>20	>5	5
herpes simplex virus-1 TK^-/TK^+ (VMW 1837)[b]	20	>20	>20	>20	>5	>5
vaccinia virus[b]	>20	>20	13	20	>5	5
vesicular stomatitis virus[b]	10	>20	>20	>20	>5	>20
human cytomegalovirus (strain Davis)[b]	2.7	2.35	>20	11	>11	>5
HEL cytotoxicity	>100	>50	50	50	50	20
influenza virus A (strain Ishikawa)[c]	–	14	>12	>12	>4	>4
influenza virus B (strain Singapore)[c]	–	>200	>20	>12	>4	>4
MDCK cytoxicity	–	>200	20	12	4	4
respiratory syncytial virus (strain Long)[d]	–	20	4	4	4	>4
HeLa cytotoxicity	–	100	100	20	20	4
Junin virus[e]	–	16	>5	>5	>20	>2
Tacaribe virus[e]	–	20	>5	>5	>20	>2
Vero cellular cytotoxicity	–	200	20	20	50	5

[a] The IC_{50} values reported are in concentrations (μg/mL) required to reduce virus-induced cytopathogenicity by 50%. Cytotoxicities are the minimum cytotoxic concentration (MCC, μg/mL) that causes a microscopically detectable alteration of normal cell morphology after 2 days of treatment. Compounds were added to the cells immediately after virus infection and remained thereafter. Virus cytopathogenicity was recorded at day 5 pi. [b] Determined in HEL cells. [c] Determined in MDCK cells. [d] Determined in HeLa cells. [e] Determined in Vero cells.

= 58 μM, was decidedly less potent than cosalane as an HIV-1 protease inhibitor. Thus, it appears that cosalane can be utilized as a template for development of more potent nonpeptide-based HIV-1 protease inhibitors.

Anti-retroviral Properties of Cosalane and the Cosalogs. Cosalane and the analogues were evaluated for their antiviral properties against HIV-1 and HIV-2 in a variety of systems. First, the compounds were screened for their ability to inhibit the cytopathic effect of HIV-1 and HIV-2 in cell cultures using the XTT cytoprotection assay. The compounds were tested against HIV-1 in both MT-4 and CEM cells, while the prevention of HIV-2 cytopathicity tests were determined exclusively in MT-4 cells (Table 2). Unfortunately, none of these cosalogs were as potent as cosalane (**1**) itself as an inhibitor of HIV-1 and HIV-2 cytopathicity, and the analogues tended to be more cytotoxic than cosalane in the MT-4 and CEM-SS cells.

The analogues were evaluated as inhibitors of syncytium formation between persistently HIV-1- or HIV-2-infected HUT-78 cells and uninfected MOLT-4 cells. Cosalog-3 (**20**), IC_{50} = 8 μM, and cosalog-5 (**30**), IC_{50} = 8 μM, were about 3 times more potent than cosalane (**1**), IC_{50} = 25 μM, as inhibitors of HIV-1-induced syncytium formation. In the other syncytial assays of the cosalane analogues, potencies equal to or less than that of cosalane were observed.

Finally, the compounds were also tested for inhibition of virion-associated p24 production in PBL cells. As shown in Table 1, none of the analogues were clearly superior to cosalane (**1**), IC_{50} = 33 μM, although cosalog-3 (**20**), IC_{50} = 24 μM, was slightly more potent than cosalane. Together, the biological data indicate that cosalane generally exerted superior antiviral properties over those of the cosalane analogues (Table 2), even though individual cosalogs might be better inhibitors of one or more of the features of HIV-1 replication (Table 1).

Besides testing against HIV-1 and HIV-2, all of the new compounds were screened against a variety of additional pathogenic viruses, and the results are presented in Table 2. Several of the compounds displayed interesting antiviral activities at concentrations that were significantly below their cytotoxic concentrations. Particularly notable were the minimum inhibitory concentrations (MIC's) observed for cosalog-2 (**14**) against respiratory syncytial virus (MIC = 4 μM) as well as those for cosalane (**1**) and cosalog-1 (**6**) against human cytomegalovirus (MIC's = 2.7 and 2.35 μM, respectively).

Discussion

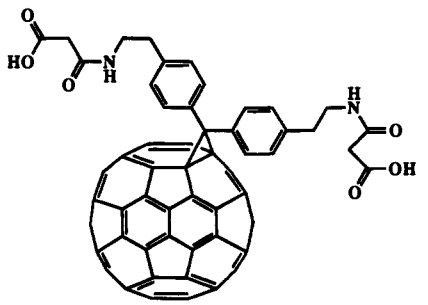
The integration of a double-stranded DNA copy of retroviral RNA into the host genome is an obligatory step in the retroviral replication cycle. The fact that

mutations in the region of the HIV-1 *pol* gene coding for IN result in noninfectious viral particles establishes IN as a legitimate target for anti-HIV drug development.[22] In spite of this fact, very little work has been reported on IN inhibitors, which reflects the limited availability of purified IN necessary for inhibitor identification and development. Recently, the expression of IN in bacterial[20] and insect[23] cells has allowed the development of effective assays necessary for evaluation of potential IN inhibitors.[24]

In 1992, we reported the polymeric mixture of substances known as aurintricarboxylic acid, as well as synthetic ATA monomers and structural analogues, as inhibitors of IN.[10] The related polyanions dextran sulfate and suramin have also been reported to inhibit IN.[25,26] A number of topoisomerase inhibitors, including actinomycin D, mithramycin A, doxorubicin, mitoxantrone, ellipticines, and quercetin, as well as the intercalator chloroquine and the bifunctional intercalator ditercalinium, are also active.[25,27] Various other compounds, including primaquine, 5,8-dihydroxy-1,4-naphthoquinone, caffeic acid phenethyl ester, AZT mono-, di-, and triphosphates, and several flavones, also display IN inhibitory activity.[27-29]

In the present investigation, several cosalane analogues were synthesized and found to be effective inhibitors of IN. These new compounds, of which cosalog-2 (**14**) and cosalog-3 (**20**) proved to be the most potent as IN inhibitors ($IC_{50} = 2.2\ \mu M$), are structurally related to ATA and the ATA monomer (**2**). ATA inhibits a variety of enzymes that process nucleic acids, including several RNA polymerases,[30,31] reverse transcriptases,[32-34] DNA polymerases,[32,35-38] and nucleases.[39-47] The fact that ATA inhibits these protein–nucleic acid interactions by binding to the protein, not the nucleic acid,[35,40] would lead one to suspect that the present cosalane analogues act by binding to IN as opposed to the DNA substrate. At physiological pH, the carboxylate anions present in ATA, cosalane (**1**), and the cosalane analogues may bind electrostatically to the protonated basic amino acid residues present in the C-terminal, DNA-binding domain of IN (residues 211–284).[48] This proposal is similar to the mechanism of action advanced recently for the polyanionic IN inhibitor suramin.[26] The noteworthy inactivity of cosalog-1 (**6**), in which the disalicylmethane moiety is attached directly to the steroid, indicates that the length of the linkage between these two structural moieties is of critical importance. If they are too close, the activity is abolished. The activity of cosalog-2 (**14**), cosalog-3 (**20**), and cosalog-4 (**25**) demonstrates that the exact nature of the hydrophobic moiety attached to the disalicylmethane fragment is not important. In addition, the activities of cosalog-2 (**14**) and cosalog-3 (**20**), when considered along with that of cosalane (**1**), show that the orientation of the steroid moiety relative to the disalicylmethane fragment is also of little importance in relation to IN inhibitory activity.

As detailed in Table 1, several of the new cosalane analogues were also effective HIV-1 protease inhibitors with IC_{50} values in the submicromolar range. Nonpeptide HIV protease inhibitors are of interest as a potential strategy to circumvent the limitations of peptide inhibitors, including hydrolysis by peptidases and poor oral bioavailability. The known HIV protease inhibitors which do not have apparent origins in peptide chemistry are increasing in number and now include several fullerene derivatives,[3,4] haloperidol derivatives,[5,49] cerulenin analogues,[50] boronated porphyrins,[51] benzophenones,[52] dimeric penicillin derivatives,[53] pyran-2-ones and pyran-4-ones,[54,55] diacids,[56-59] and a few natural products including certain tetronic acid homologues,[7] cerulenin,[9] cytochalasin L-696,474,[60] and didemnaketals A and B.[8] Of these, cosalane (**1**) and the active cosalane analogues listed in Table 1 most closely resemble the fullerene and benzophenone derivatives.[3,4] The fullerene derivative **31**, which inhibits HIV-1 protease with a K_i of 5.3 μM, has a diphenylmethylene fragment attached to a hydrophobic Bucky Ball moiety designed to fit the large cylindrical, hydrophobic surface at the active site of the protease.[3,4] The active cosalane analogues described here contain hydrophobic adamantyl or steroid moieties as well as a diphenylmethylene fragment which may be functioning similarly to the corresponding structural components of **31**. The structural diversity of the active cosalane analogues would be consistent with the hydrophobic steroid or adamantyl moieties occupying the cylinder lined by hydrophobic amino acid residues present at the active site of the enzyme. On the other hand, the disalicylmethane moiety of cosalane (**1**) and the active cosalane analogues also resembles the benzophenone derivatives including **32**, which also inhibit HIV-1 protease and were identified by computer substructure searching based on X-ray crystal structures of several HIV-1 protease–inhibitor complexes.[52] The cosalane analogues **6**, **20**, and **25**, as well as the boronated porphyrins[51] and certain 4-hydroxypyran-2-ones,[58,59] are among the most potent of the nonpeptide HIV protease inhibitors discussed here, with activities in the submicromolar range.



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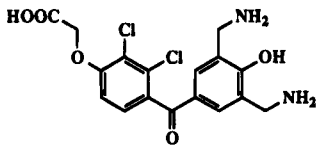

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Although cosalane and the cosalane analogues reported here have significant inhibitory activity against HIV-1 protease and integrase, the inhibition of these enzymes is most likely not the primary mechanism responsible for their prevention of HIV cytopathicity. Time of addition experiments with cosalane, as well as other available evidence, have demonstrated quite clearly that the primary mechanism of action of cosalane is inhibition of gp120-CD4 binding in addition to inhibition of a postattachment event prior to reverse transcription, and there is no compelling reason to suspect that the new cosalane analogues act differently.[2] This

view is consistent with the fact that even though some of the new cosalane analogues were more potent as inhibitors of IN and protease than cosalane itself, they were not as potent as cosalane in preventing HIV-1 and HIV-2 cytopathicity (Table 1). In addition, cosalog-4 **(25)**, which was active as an inhibitor of HIV-1 IN and protease but was inactive in the syncytial assay at the concentrations tested, was also inactive as an inhibitor HIV-1 cytopathicity. As a working hypothesis, it has been considered that cosalane **(1)** and the active cosalane congeners **6**, **14**, and **20** may act to prevent viral cytopathicity primarily through insertion of their steroid moieties into the cell and/or outer viral membranes with the disalicylmethane moieties protruding outward in an obstructive mode.[2] Our challenge will now be to direct the best analogues into the intracellular milieu to allow for inhibition of the multiple targets of the HIV-1 replication cycle.

In addition to being tested against HIV-1 and HIV-2, cosalane **(1)** and the new analogues were tested as inhibitors of the cytopathic effects of a variety of pathogenic viruses, and the results are listed in Table 2. Several of the compounds in this series inhibited the cytopathicity of a number of viruses at concentrations well below the cytotoxic concentrations in the cells in which the antiviral testing was performed. Particularly noteworthy are the IC_{50} values of cosalane **(1)** against herpes simplex virus-1 (IC_{50} = 10 μg/mL), herpes simplex virus-2 (IC_{50} = 7.4 μg/mL), and human cytomegalovirus (IC_{50} = 2.7 μg/mL) in human embryonic lung cells, which are well below cytotoxic concentrations of cosalane **(1)** in HEL cells (>100 μg/mL). Cosalog-1 **(6)** displayed similar activities against herpes simplex virus-1 and human cytomegalovirus, although it was less active than cosalane **(1)** against herpes simplex virus-1. Also noteworthy were the antiviral activities of cosalog-1 **(6)** against influenza virus (IC_{50} = 14 μg/mL) in MDCK cells (cytotoxicity > 200 μg/mL) and also against Junin virus (IC_{50} = 16 μg/mL) and Tacaribe virus (IC_{50} = 20 μg/mL) in Vero cells (cytotoxicity = 200 μg/mL). The IC_{50} value of 4 μg/mL of cosalog-2 **(14)** against respiratory syncytial virus in HeLa cells was also significantly below its cytotoxic concentration (100 μg/mL) in those cells.

In conclusion, certain novel cosalane analogues have displayed higher potencies than cosalane itself against HIV-1 integrase and protease. This may provide some insight of value in the future design of useful inhibitors of these important enzymes in the HIV replication cycle.

Experimental Section

Melting points were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. Spectra were obtained as follows: CI mass spectra on a Finnegan 4000 spectrometer; FAB mass spectra and EI mass spectra on a Kratos MS50 spectrometer; ^{1}H NMR spectra on Varian VXR-500S and XL-200A spectrometers; IR spectra on a Beckman IR-33 spectrometer or a Perkin Elmer 1600 series FTIR. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values are within ±0.4% of the calculated compositions.

5α-3-[Bis[5′-chloro-4′-methoxy-3′-(methoxycarbonyl)-phenyl]methylene]cholestane (5). Titanium trichloride–DME complex (1.141 g, 3.93 mmol) and Zn/Cu couple (1.075 g) were placed in a dry flask purged with argon followed by dry DME (20 mL). The mixture was stirred at reflux for 1 h 50 min and cooled to 40 °C, and a solution of cholestan-3-one (**4**) (190.6 mg, 0.493 mmol) and bis[5′-chloro-4′-methoxy-3′-(methoxycarbonyl)phenyl] ketone (**3**; 210.6 mg, 0.493 mmol) in dry DME (4 mL) was added dropwise. The black mixture was stirred at reflux for 5.5 h and cooled to room temperature, and hexane (5 mL) was added. The reaction mixture was filtered through a pad of Florisil followed by filtration through a pad of Celite. The solvent was evaporated *in vacuo*, and the residue was flash chromatographed on silica gel (20 g) using hexane–ethyl acetate (6:1) as the eluant. The first fractions yielded the cholestane dimer resulting from McMurry reaction of two molecules of the starting material **4**, followed by the cross-coupled alkene **5** (116 mg, 30%). The analytical sample was recrystallized from hexane–EtOH: mp 59–60 °C; IR (KBr) 2940, 2865, 1735, 1473, 1434, 1265, 1202, 1001 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38 (d, J = 2.5 Hz, 1 H), 7.38 (d, J = 2 Hz, 1 H), 7.22 (d, J = 2.5 Hz, 1 H), 7.21 (d, J = 2 Hz, 1 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 3.89 (s, 3 H), 3.89 (s, 3 H), 2.32 (d, J = 14.5 Hz, 1 H), 0.88 (s, 3 H), 0.87 (d, 3 H), 0.84 (dd, J = 6.8, 2.3 Hz, 6 H), 0.63 (s, 3 H); FABMS m/z (rel intensity) 837 (MH^+, 39), 805 (100). Anal. ($C_{50}H_{70}Cl_2O_6$) C, H.

5α-3-[Bis(3′-carboxy-5′-chloro-4′-hydroxyphenyl)methylene]cholestane (6). Boron tribromide–dimethyl sulfide complex (1 M solution in methylene chloride, 1.7 mL) was added dropwise to a stirred solution of diester **5** (0.170 g, 0.217 mmol) in dichloroethane (12 mL), and the mixture was stirred at 80 °C for 3 h. The reaction mixture was cooled in ice, the reaction quenched with water (5 mL), and the mixture stirred for 1 h. Ethyl acetate was added to dissolve a precipitate, and the organic layer was separated. The aqueous layer was extracted with chloroform (1 × 3 mL). The combined organic extracts were washed with water, dried (sodium sulfate), and evaporated to dryness to yield the product (0.159 g). The analytical sample was recrystallized from methylene chloride: mp 269 °C; IR (KBr) 3500–2600, 2931, 2852, 1673, 1455, 1232, 1178 cm^{-1}; ^{1}H NMR (acetone-d_6, 200 MHz) δ 7.66 (bs, 2 H), 7.43 (d, J = 2 Hz, 1 H), 7.42 (d, J = 2.1 Hz, 1 H), 0.96 (s, 3 H), 0.92 (d, J = 6.4 Hz, 3 H), 0.86 (d, J = 6.4 Hz, 6 H), 0.69 (s, 3 H); EIMS m/z (rel intensity) 728 (M^+ + 4, 20), 727 (M^+ + 3, 28), 726 (M^+ + 2, 64), 725 (M^+ + 1, 47), 724 (M^+, 100). Anal. ($C_{46}H_{62}Cl_2O_6 \cdot 1.5H_2O$) C, H. The ammonium salt had mp 248 °C.

Methyl 3-Ketocholanate Tosylhydrazone (8). p-Toluenesulfonohydrazide (3.05 g, 16.38 mmol) was added to a stirred solution of the oxosteroid **7** (3.18 g, 8.18 mmol) in acetic acid (50 mL). The mixture was stirred at room temperature for 20 h and then diluted with water (100 mL) with ice cooling. The precipitated white product (3.84 g, 84.3%) was filtered off, washed with water, and crystallized from methanol–methylene chloride: mp 184–185 °C; IR (KBr) 3442, 3218, 2938, 2866, 1715, 1446, 1380, 1166 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.84 (d, J = 8.2 Hz, 2 H), 7.31 (d, J = 8.2 Hz, 2 H), 7.07 (s, 1 H), 3.66 (s, 3 H), 2.43 (s, 3 H), 0.93 (s, 3 H), 0.895 (d, J = 6.2 Hz, 3 H), 0.64 (s, 3 H); FABMS m/z (rel intensity) 557 (MH^+, 100), 402 (68). Anal. ($C_{32}H_{48}N_2O_4 \cdot H_2O$) C, H, N.

Methyl Cholanate (9). A solution of the tosylhydrazone **8** (1.023 g, 1.837 mmol), sodium cyanoborohydride (577 mg, 9.185 mmol), and p-toluenesulfonic acid (100 mg) in DMF–sulfolane (10 mL, 1:1) was heated with stirring for 3 h at 110 °C. The reaction mixture was cooled in an ice bath, the reaction quenched with brine (10 mL), and the mixture extracted with ether (3 × 10 mL) and back-washed with brine (4 × 20 mL). The organic extract was dried (sodium sulfate) and evaporated to dryness, and the residue was flash chromatographed on silica gel (80 g). Elution with hexane–ethyl acetate (9:1) afforded the product, 0.484 g (70.4%), as a crystallizing oil. An analytical sample was prepared by crystallization from hexane–ethyl acetate (6:1): mp 87 °C; IR (KBr) 2928, 2858, 1736, 1444, 1377, 1209, 1172 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.64 (s, 3 H), 2.27 (m, 2 H), 0.895 (d, J = 6.4 Hz, 3 H), 0.89 (s, 3 H), 0.62 (s, 3 H); CIMS m/z (rel intensity) 375 (MH^+, 100).

24-Hydroxycholane (10). A solution of ester **9** (1.649 g, 4.402 mmol) in dry THF (12 mL) was added dropwise to a 1 M solution of LAH in ether (5.8 mL), and the mixture was stirred at 0 °C for 1 h. The reaction was quenched with water (0.22 mL), 15% sodium hydroxide (0.22 mL), and water (0.66 mL), the mixture was diluted with methylene chloride (2 mL) and filtered, and the precipitate on the filter was washed with methylene chloride. The filtrate was evaporated to dryness to afford alcohol **10** as a white solid (1.259 g, 82.5%): mp 130

°C; IR (KBr) 3354, 2929, 2860, 1446, 1377, 1052, cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.61 (m, 2 H), 0.92 (d, J = 5.5 Hz, 3 H), 0.91 (s, 3 H), 0.64 (s, 3 H); CIMS m/z (rel intensity) 346 (M^+, 61), 329 ($MH^+ - H_2O$, 100). Anal. ($C_{24}H_{42}O$) C, H.

24-Bromocholane (11). A solution of triphenylphosphine (1.427 g, 5.44 mmol) in dry methylene chloride (7 mL) was added dropwise with stirring to the solution of alcohol **10** (1.257 g, 3.63 mmol) and carbon tetrabromide (1.503 g, 4.538 mmol) in dry methylene chloride (12 mL) at 0 °C. The mixture was stirred in an ice bath for 10 min, the solvent was removed *in vacuo*, and the residue was extracted by stirring with ether (5 × 6 mL). The combined organic extracts were filtered, evaporated *in vacuo*, and flash chromatographed on silica gel (45 g), eluting with hexane–ethyl acetate (6:1), to afford the product (1.257 g, 85%). The analytical sample was obtained by crystallization from acetone: mp 85 °C; IR (KBr) 2928, 2858, 1447, 1377, 1007, 802, 690, 626, 557, 488 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.38 (m, 2 H), 0.91 (bs, 6 H), 0.64 (s, 3 H); CIMS m/z (rel intensity) 410 (M^+ + 2, 65), 409 (MH^+, 100), 408 (M^+, 69), 407 (69), 329 (M^+ – Br, 67). Anal. ($C_{24}H_{41}Br$) C, H.

24-(Triphenylphosphoniumyl)cholane Bromide (12). A solution of triphenylphosphine (855 mg, 3.26 mmol) and bromide **11** (1.335 g, 3.26 mmol) in chlorobenzene (3 mL) was heated at 140 °C for 48 h. The solvent was removed *in vacuo*, and the residue was triturated with hexane. The phosphonium salt was filtered, washed with hexane, and dried in a vacuum desiccator: mp 173–175 °C; FABMS m/z (rel intensity) 592 (MH^+, 74), 591 (M^+, 84), 262 (100). Anal. ($C_{42}H_{56}BrP$) C, H.

24-[Bis[5′-chloro-4′-methoxy-3′-(methoxycarbonyl)phenyl]methylene]cholane (13). A solution of the phosphonium salt **12** (1.567 g, 2.33 mmol) in dry THF (30 mL) was treated with sodium bis(trimethylsilyl)amide (1 M solution, 2.33 mL) for 30 min at 0 °C. A solution of the ketone **3** (0.996 g, 2.33 mmol) in dry THF (5 mL) was added dropwise, and the mixture was stirred at room temperature for 48 h. The reaction was quenched with a solution of ammonium chloride (300 mg) in water (10 mL). The organic layer was separated, and the aqueous layer was extracted with ether (1 × 5 mL). The combined organic extracts were washed with brine (2 × 20 mL) and dried (sodium sulfate), and the solvent was evaporated *in vacuo* to afford 2.5 g of a semisolid, which was flash chromatographed on silica gel (120 g). Elution with hexane–ethyl acetate (6:1) yielded alkene **13** as a glass (1.370 g, 75.6%), which failed to crystallize: IR (KBr) 2932, 2860, 1736, 1597, 1477, 1438, 1264, 1207, 1094, 1001 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.495 (dd, J = 2.4, 0.7 Hz, 1 H), 7.48 (dd, J = 2.2, 1.4 Hz, 1 H), 7.33 (dd, J = 2.2, 0.7 Hz, 1 H), 7.27 (dd, J = 2.3, 0.8 Hz, 1 H), 6.07 (t, J = 7.7 Hz, 1 H), 4.00 (s, 3 H), 3.93 (s, 3 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 0.91 (s, 3 H), 0.84 (d, J = 6.5 Hz, 3 H), 0.625 (s, 3 H); FABMS m/z (rel intensity) 739 (MH^+, 19), 707 (MH^+ – MeOH, 52). Anal. ($C_{43}H_{56}Cl_2O_6$) C, H.

24-[Bis(3′-carboxy-5′-chloro-4′-hydroxyphenyl)methylene]cholane (14). Boron tribromide–dimethyl sulfide complex (1 M solution in methylene chloride, 13.56 mL) was added dropwise to a stirred solution of diester **13** (1.318 g, 1.694 mmol) in dichloroethane (25 mL), and the mixture was stirred at 70 °C for 24 h and at room temperature overnight. It was cooled in ice, the reaction quenched with water (10 mL), and the mixture stirred for 1 h. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with water, dried (sodium sulfate), and evaporated to dryness. The solid residue (1.4 g) was flash chromatographed on silica gel (52 g). Elution with chloroform–THF–97% formic acid (150:30:0.5) yielded pure diacid **14** (1.066 g, 87.5%). The analytical sample was prepared by crystallization from a methylene chloride–acetone mixture: mp 262–263 °C; IR (KBr) 3500–2600, 2930, 2861, 1668, 1607, 1446, 1231, 1177, 711 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) δ 7.70 (d, J = 2.1 Hz, 1 H), 7.675 (d, J = 2.3 Hz, 1 H), 7.56 (d, J = 2.3 Hz, 1 H), 7.49 (d, J = 2.1 Hz, 1 H), 6.18 (t, J = 7.8 Hz, 1 H), 0.915 (s, 3 H), 0.855 (d, J = 6.6 Hz, 3 H), 0.65 (s, 3 H); FABMS m/e (rel intensity) 687 (MH^+ + 4, 0.6), 686 (MH^+ + 3, 1.1), 685 (MH^+ + 2, 2.4), 684 (MH^+ + 1, 2.8), 683 (MH^+, 4.4), 136 (100); High-resolution FABMS calcd for $C_{39}H_{49}Cl_2O_6$ m/z 683.2906, found 683.2895.

Methyl 3,3-Difluorocholanate (15). DAST (2.7 mL, 20.43 mmol) was added dropwise to a stirred solution of methyl 3-ketolithocholate (**7**; 4.33 g, 11.14 mmol) in dry benzene (35 mL) at room temperature under an argon atmosphere, and the solution was stirred for 48 h. It was cooled in an ice bath and the reaction quenched with ice followed by addition of 0.63 M sodium bicarbonate (10 mL). The benzene layer was separated, and the aqueous layer was extracted with benzene (1 × 10 mL). The combined organic extracts were dried (sodium sulfate) and evaporated to dryness. The solid residue was flash chromatographed on silica gel (200 g). Elution with hexane–ethyl acetate (6:1) yielded the difluoro derivative **15** (4.46 g (97.5%). The analytical sample was prepared by crystallization from hexane–ethyl acetate (6:1): mp 111–112 °C; IR (KBr) 2936, 2887, 2867, 1740, 1453, 1373, 1216, 1178, 1108, 1079, 1025, 955 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.67 (s, 3 H), 0.97 (s, 3 H), 0.91 (d, J = 6.2 Hz, 3 H), 0.65 (s, 3 H); CIMS m/z (rel intensity) 411 (MH^+, 100), 391 (MH^+ – HF, 25), 371 ($MH^+ - 2 \times HF$). Anal. ($C_{25}H_{40}F_2O_2$) C, H.

3,3-Difluoro-24-hydroxycholane (16). A solution of ester **15** (189 mg, 0.419 mmol) in dry THF (1 mL) was added dropwise to a 1 M solution of LAH in ether (0.55 mL), and the mixture was stirred at 0 °C for 1 h. The reaction was quenched with water (1 drop), 15% sodium hydroxide (1 drop), and water (3 drops), the mixture was diluted with methylene chloride (2 mL) and filtered, and the precipitate on the filter was washed with methylene chloride. The filtrate was evaporated to dryness, and the oil obtained was flash chromatographed on silica gel (10 g). Elution with hexane–ethyl acetate (3:1) afforded alcohol **16** as a crystallizing oil, 152 mg (86.3%): mp 100 °C; IR (KBr) 3377, 2936, 2867, 1451, 1370, 1275, 1181, 1056, 1026, 955 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.62 (t, J = 5.9 Hz, 2 H), 0.97 (s, 3 H), 0.925 (d, J = 6.4 Hz, 3 H), 0.66 (s, 3 H); CIMS m/z (rel intensity) 382 (M^+, 26), 365 (46), 363 (48), 343 (100). Anal. ($C_{24}H_{40}F_2O$) C, H.

3,3-Difluoro-24-bromocholane (17). A solution of triphenylphosphine (573 mg, 2.185 mmol) in dry methylene chloride (2 mL) was added dropwise with stirring to the solution of alcohol **16** (557 mg, 1.456 mmol) and carbon tetrabromide (603.6 mg, 1.82 mmol) in dry methylene chloride (10 mL) at 0 °C. The mixture was stirred in an ice bath for 10 min, the solvent was removed *in vacuo*, and the residue was extracted with ether (4 × 5 mL). The combined organic extracts were filtered, evaporated *in vacuo*, and flash chromatographed on silica gel (50 g), eluting with hexane–ethyl acetate (6:1), to yield the product as a colorless, crystallizing oil (570 mg, 87.7%). The analytical sample was recrystallized from acetone: mp 90 °C; IR (KBr) 2933, 2873, 1445, 1369, 1261, 1102, 956 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.39 (m, 2 H), 0.97 (s, 3 H), 0.925 (d, J = 6.5 Hz, 3 H), 0.66 (s, 3 H); CIMS m/z (rel intensity) 446 (M^+ + 2, 52), 445 (M^+ + 1, 28), 444 (M^+, 52), 425 (M^+ – F, 100), 407 (28), 405 (M^+ – F – HF, 24), 365 (M^+ – Br, 34).

3,3-Difluoro-24-(triphenylphosphoniumyl)cholane Bromide (18). A solution of triphenylphosphine (288.7 mg, 1.101 mmol) and bromide **17** (491.5 mg, 1.101 mmol) in chlorobenzene (1.5 mL) was heated at 140 °C for 48 h. The solvent was removed *in vacuo*, and the residue was triturated with hexane. The phosphonium salt (697 mg, 89%) was filtered, washed with hexane, and dried *in vacuo*: mp 135–137 °C; FABMS m/z (rel intensity) 627 (M^+, 92), 607 (M^+ – HF, 55), 136 (100).

24-[Bis[5′-chloro-4′-methoxy-3′-(methoxycarbonyl)phenyl]methylene]-3,3-difluorocholane (19). A solution of the phosphonium salt **18** (581 mg, 0.820 mmol) in dry THF (12 mL) was treated with sodium bis(trimethylsilyl)amide (1 M solution, 0.82 mL) for 30 min at 0 °C. A solution of the ketone **3** (350 mg, 0.82 mmol) in dry THF (5 mL) was added dropwise, and the mixture was stirred at room temperature for 48 h. Usual workup afforded an oil (0.87 g) which was flash chromatographed on silica gel (45 g). Elution with hexane–ethyl acetate (6:1) yielded the alkene **19** as a glass (472 mg, 74%) which failed to crystallize: IR (KBr) 2938, 2868, 1735, 1477, 1438, 1367, 1265, 1208, 1095, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.47 (d, J = 2.3 Hz, 1 H), 7.46 (d, J = 2.1 Hz, 1 H), 7.305 (d, J = 2.1 Hz, 1 H), 7.27 (d, J = 2.3 Hz, 1 H),

6.04 (t, J = 7.7 Hz, 1 H), 3.98 (s, 3 H), 3.91 (s, 3 H), 3.89 (s, 6 H), 0.94 (s, 3 H), 0.815 (d, J = 6.3 Hz, 3 H), 0.61 (s, 3 H); FABMS m/z (rel intensity) 779 (MH^+ + 4, 0.9), 778 (MH^+ + 3, 1.8), 777 (MH^+ + 2, 4.4) 776 (MH^+ + 1, 6.1), 775 (MH^+, 11.6), 725 (M^+ − HF, 19.3), 136 (100). Anal. ($C_{43}H_{54}Cl_2F_2$) C, H.

24-[Bis(3′-carboxy-5′-chloro-4′-hydroxyphenyl)methylene]-3,3-difluorocholane (20). Boron tribromide−dimethyl sulfide complex (1 M solution in methylene chloride, 4.3 mL) was added dropwise to a stirred solution of diester **19** (410 mg, 0.529 mmol) in dichloroethane (8 mL), and the mixture was stirred at 70 °C for 28 h and at room temperature overnight. It was cooled in ice, the reaction quenched with water (10 mL), and the mixture stirred for 1 h and then extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were washed with water, dried (sodium sulfate), and evaporated to dryness. The solid residue was flash chromatographed on silica gel (30 g). Elution with chloroform−THF−formic acid (97%) (150:30:0.5) yielded pure diacid **20** (230 mg, 61%). The analytical sample was prepared by crystallization from a methylene chloride−acetone mixture: mp 180−182 °C; IR (KBr) 3400−2600, 2933, 2866, 1669, 1607, 1444, 1232, 1178 cm^{-1}; ^1H NMR (acetone-d_6, 200 MHz) δ 7.725 (d, J = 1.9 Hz, 1 H), 7.70 (d, J = 2.2 Hz, 1 H), 7.57 (d, J = 2.2 Hz, 1 H), 7.505 (d, J = 1.9 Hz, 1 H), 6.20 (t, J = 7.6 Hz, 1 H), 0.99 (m, 3 H), 0.875 (d, J = 6.3 Hz, 3 H), 0.69 (s, 3 H); FABMS m/z (rel intensity) 760 ($MH^+ + H_2O$ + Na, 4), 154 (100). The product was converted to the dihydrate of its diammonium salt for elemental analysis. Anal. ($C_{39}H_{56}Cl_2F_2N_2O_6$) C, H, N.

1-(2-Bromoethyl)adamantane (22). A solution of 1-adamantaneethanol (**21**; 1.915 g, 10.62 mmol) and carbon tetrabromide (7.04 mg, 21.24 mmol) in dry acetonitrile (170 mL) was heated under reflux with stirring, and a solution of triphenylphosphine (8.356 g, 31.86 mmol) in dry acetonitrile (150 mL) was added dropwise over 5 min. The mixture was heated under reflux for 2.5 h and cooled, the solvent was removed *in vacuo*, and the residue was extracted with benzene (3 × 10 mL). The combined extracts were filtered, and the solvent was removed *in vacuo*. The residue was flash chromatographed on silica gel (250 g; hexane−ethyl acetate, 6:1) to afford the bromide **22** (2.15 g, 83%). The analytical sample was recrystallized from acetone: mp 68 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.41 (m, 2 H), 1.96 (bs, 2 H), 1.67 (m, 9 H), 1.51 (bs, 6 H); CIMS m/z (rel intensity) 243 [$(M - H)^+$ + 2, 14], 241 [$(M - H)^+$, 13], 163 (MH^+ − HBr, 30), 135 (MH^+ − HBr − C_2H_4, 100). Anal. ($C_{12}H_{19}Br$) C, H.

1-[2-(Triphenylphosphoniumyl)ethyl]adamantane Bromide (23). Bromide **22** (1.703 g, 7.003 mmol) and triphenylphosphine (1.838 g, 7.003 mmol) were dissolved in chlorobenzene (9 mL), and the solution was heated at reflux for 4 days under argon with stirring. The solvent was removed *in vacuo*. The residue was triturated with hexane, filtered, and washed with hexane to afford a solid (2.12 g, 60%): mp 265 °C; ^1H NMR (CD_3OD) δ 7.89 (m, 15 H), 3.35 (m, 2 H), 1.98 (bs, 3 H), 1.71 (m, 5 H), 1.55 (m, 6 H), 1.38 (m, 3 H); FABMS m/z (rel intensity) 425 (M^+, 100). Anal. ($C_{30}H_{34}BrP$).

1-[3,3-Bis[5′-chloro-4′-methoxy-3′-(methoxycarbonyl)-phenyl]-2-propen-1-yl]adamantane (24). 1-[2-(Triphenylphosphoniumyl)ethyl]adamantane bromide (**23**) (451 mg, 0.892 mmol) was suspended in dry THF (12 mL), the mixture was cooled in an ice bath, and sodium bis(trimethylsilyl)amide (1 M solution in THF, 0.9 mL) was added dropwise. The mixture was stirred for 15 min, and a solution of bis[5-chloro-4-methoxy-3-(methoxycarbonyl)phenyl] ketone (**3**) (0.479 g, 1.12 mmol) in THF (7 mL) was added dropwise. The ice bath was removed, and the reaction mixture was stirred for 9 h at 70 °C. The reaction was quenched with ammonium chloride solution, the THF phase was separated, and the aqueous phase was extracted with ether. The combined organic extracts were dried (sodium sulfate) and evaporated to dryness. Flash chromatography of the residue on silica gel (80 g; hexane−ethyl acetate, 4:1) yielded solid product (0.324 g, 70.4%): mp 123 °C (ethanol); IR (KBr) 2903, 2845, 1728, 1478, 1436, 1362, 1284, 1207, 1003, 745 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.47 (d, J = 2.5 Hz, 1 H), 7.46 (d, J = 2.5 Hz, 1 H), 7.315 (d, J = 2.5 Hz, 1 H), 7.305 (d, J = 2.5 Hz, 1 H), 6.20 (t, J = 7.7 Hz, 1 H), 4.01 (s, 3 H), 3.93 (s, 9 H), 1.96 (bs, 2 H), 1.86 (d, J = 7.7 Hz, 2 H), 1.67 (m, 6 H), 1.50 (m, 6 H); FABMS m/z (rel intensity) 576 (M^+ + 4, 16), 575 (M^+ + 3, 27), 574 (M^+ + 2, 46), 573 (M^+ + 1, 44), 572 (M^+, 58), 541 (MH^+ − MeOH, 60), 135 (100). Anal. ($C_{31}H_{34}Cl_2O_6$).

1-[3,3-Bis(3′-carboxy-5′-chloro-4′-hydroxyphenyl)-2-propen-1-yl]adamantane (25). A solution of boron tribromide−dimethyl sulfide complex (1 M, 3 mL) was placed in a dry 25 mL two-necked, round-bottomed flask equipped with a Teflon-coated magnetic stirring bar, a reflux condenser connected to an argon flow line, and a rubber septum. A solution of ester **24** (214 mg, 0.373 mmol) in 1,2-dichloroethane (12 mL) was added dropwise. The mixture was stirred at 90 °C (oil bath) for 5 h and at room temperature for 14 h. Water (5 mL) was added with ice bath cooling, and the mixture was stirred at room temperature for 1 h. Ethyl acetate was added to dissolve the product, the organic phase was separated, and the aqueous phase was extracted with chloroform (5 mL). The combined organic extracts were washed with brine and dried (sodium sulfate). The crude product obtained after evaporation of the solvent *in vacuo* was crystallized from an acetone−chloroform mixture to afford a solid (176 mg, 86%): mp 287−288 °C; IR (KBr) 3500−2500, 2902, 2846, 1666, 1446, 1232, 1183, 898, 802, 711 cm^{-1}; ^1H NMR (acetone-d_6, 200 MHz) δ 7.71 (d, J = 2 Hz, 1 H), 7.67 (d, J = 2.2 Hz, 1 H), 7.61 (d, J = 2.2 Hz, 1 H), 7.47 (d, J = 2 Hz, 1 H), 6.31 (t, J = 8.8 Hz, 1 H), 1.95 (m, 6 H), 1.63 (m, 11 H); FABMS (negative ion mode) m/z (rel intensity) 519 [$(M - H^+)$ + 4, 15], 518 [$(M - H^+)$ + 3, 22], 517 [$(M - H^+)$ + 2, 73], 516 [$(M - H^+)$ + 1, 37], 515 [$(M - H^+)$, 100]; high-resolution FABMS (positive ion mode) calcd for $C_{27}H_{26}Cl_2O_6$ 516.1106, found 516.1106. The ammonium salt was prepared by dissolving the diacid in ammonia (1 mL) and evaporation of the solution *in vacuo*: mp 198 °C.

1,1-Bis[5′-chloro-4′-methoxy-3′-(methoxycarbonyl)-phenyl]-1-heptene (27). Sodium hydride (54 mg, 1.35 mol, 60% dispersion in mineral oil) was washed with n-hexane (3 × 5 mL). Dimethyl sulfoxide (2 mL) was introduced *via* a syringe, and the mixture was heated at 75 °C until the evolution of hydrogen ceased. The clear solution was cooled in an ice−water bath, and a solution of the n-hexyltriphenylphosphonium bromide (**26**; 576 mg, 1.35 mmol) in DMSO (3 mL) was added dropwise. The resulting solution was stirred at room temperature for 15 min. A solution of the ketone **3** (0.86 mmol, 369 mg) in warm DMSO (6 mL) was added dropwise, and the reaction mixture was heated at 55 °C for 27 h. It was cooled in an ice−water bath. A solution of ammonium chloride (143 mg) in water (5 mL) was added, and the reaction mixture was extracted with ethyl ether (5 × 5 mL). The organic extracts were washed twice with brine, dried over sodium sulfate, and evaporated *in vacuo*. Flash chromatography on silica gel (230−400 mesh), eluting with n-hexane−ethyl acetate (4:1), yielded starting ketone (39 mg) and heptene **27** (320 mg, 75%): mp 88 °C; IR (KBr) 3046, 1732, 1595, 1364, 1250, 1211, 1092, 998 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.49 (d, J = 2.4 Hz, 1 H), 7.48 (d, J = 2.2 Hz, 1 H), 7.32 (d, J = 2.2 Hz, 1 H), 7.30 (d, J = 2.4 Hz, 1 H), 6.07 (t, J = 7.6 Hz, 1 H), 4.00 (s, 3 H), 3.93 (s, 3 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 2.08 (m, 2 H), 1.44 (m, 2 H), 1.27 (m, 4 H), 0.88 (t, J = 6.9 Hz, 3 H); CIMS m/z (rel intensity) 499 (MH^+ + 4, 7), 197 (MH^+ + 2, 95), 495 (MH^+, 100). Anal. ($C_{25}H_{28}Cl_2O_6$).

1,1-Bis(3′-carboxy-5′-chloro-4′-hydroxyphenyl)-1-heptene (28). Boron tribromide (1 M solution in methylene chloride, 1.6 mL) was cooled in a dry ice−acetone bath, and a solution of methoxy ester **27** (148.6 mg, 0.3 mmol) in dry methylene chloride (2.5 mL) was added *via* septum in an argon atmosphere with magnetic stirring. The cooling bath was removed after 2 h and stirring continued at ambient temperature for 2 days. More BBr_3 (0.8 mL) was added on the second day. The reaction was quenched with water (2 mL), stirring continued for 30 min, and the product extracted with 20% KOH. The alkaline solution was acidified on cooling with concentrated HCl and the product extracted with ethyl acetate. The organic extracts were washed with brine, dried (sodium sulfate), and concentrated *in vacuo*. The product was crystallized from methylene chloride: mp 234−236 °C; IR (KBr) 3500−2500, 2926, 2856, 1670, 1600, 1443, 1232, 1179, 901, 799, 715 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.68 (m, 2 H), 7.50 (d, J = 1.6 Hz, 1 H), 7.41 (d, J = 1.6 Hz, 1 H), 6.13 (t, J

= 7.2 Hz, 1 H), 2.11 (m, 2 H), 1.48 (m, 2 H), 1.28 (m, 14 H), 0.85 (t, J = 6.3 Hz, 3 H); FABMS m/z (rel intensity) 442 (M^+ + 4, 0.4), 440 (M^+ + 2, 1.8), 438 (M^+, 2.3), 421 (0.6), 154 (100). The ammonium salt showed mp 150 °C. Anal. ($C_{21}H_{20}Cl_2O_6$·$^1/_2H_2O$).

1,1-Bis[5′-chloro-4′-hydroxy-3′-(methoxycarbonyl)-phenyl]-1-heptene (29). Concentrated sulfuric acid (5 drops) was added to a solution of phenol acid **28** (98 mg, 0.11 mmol) in dry methanol (4 mL), and the mixture was heated at 90 °C for 5 days. The reaction mixture was cooled in an ice bath, water was added, and the product was extracted with methylene chloride (3 × 3 mL). The combined extracts were washed once with 5% sodium bicarbonate solution and twice with brine and then dried (sodium sulfate). The solvent was removed *in vacuo*, and the residue was flash chromatographed on silica gel (4 g; hexane–ethyl acetate, 4:1) to yield the ester as a crystallizing oil: mp 118 °C (methanol–ether); IR (KBr) 3422, 3125, 2952, 2927, 2858, 1679, 1604, 1442, 1334, 1237, 1200, 1168, 797, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 11.37 (s, 1 H), 11.28 (s, 1 H), 7.53 (d, J = 2.1 Hz, 1 H), 7.50 (d, J = 2.2 Hz, 1 H), 7.38 (dd, J = 2.2, 0.3 Hz, 1 H), 7.33 (bd, J = 2.1 Hz, 1 H), 5.96 (t, J = 7.5 Hz, 1 H), 3.93 (s, 3 H), 3.92 (s, 3 H), 2.03 (m, 2 H), 1.43 (m, 2 H), 1.23 (m, 6 H), 0.85 (t, J = 5.1 Hz, 3 H); FABMS m/z (rel intensity) 470 (M^+ + 4, 2), 468 (M^+ + 2, 20), 467 (M^+ + 1, 10), 466 (M^+, 28), 465 (MH^+ − 2 H, 3), 433 (3), 281 (100). Anal. ($C_{23}H_{24}Cl_2O_6$).

1,1-Bis[5′-chloro-4′-hydroxy-3′-(*N*-hydroxycarbamoyl)-phenyl]-1-heptene (30). Sodium hydroxide (3 M, 3.07 mL) was added to a solution of hydroxylamine hydrochloride (275 mg, 3.95 mmol) in water (2.8 mL) followed by a solution of the dimethyl ester **29** (74 mg, 0.158 mmol) in dioxane (2 mL). The cloudy mixture was stirred at room temperature for 20 h, cooled in an ice bath, acidified with 3 M HCl (2 mL), and extracted with ethyl acetate. The combined extracts were washed twice with brine and dried (sodium sulfate). The solvent was removed *in vacuo*, and the residue crystallized on trituration with methylene chloride to afford a solid (67 mg, 90%): mp 137–138 °C; IR (KBr) 3340, 3160, 2925, 1648, 1470, 1108, 978 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 11.21 (bs, 2 H), 8.65 (bs, 2 H), 7.53 (d, J = 2.1 Hz, 1 H), 7.47 (d, J = 2.1 Hz, 1 H), 7.45 (d, J = 2.1 Hz, 1 H), 7.38 (d, J = 2.1 Hz, 1 H), 6.14 (t, J = 7.5 Hz, 1 H), 2.05 (m, 2 H), 1.49 (m, 2 H), 1.27 (m, 4 H), 0.84 (t, J = 6.8 Hz, 3 H); FABMS m/z (rel intensity) 473 (MH^+ + 4, 14), 472 (MH^+ + 3, 13), 471 (MH^+ + 2, 52), 470 (MH^+ + 1, 51), 469 (MH^+, 79), 436 (MH^+-NH_2OH, 100). Anal. ($C_{21}H_{22}N_2Cl_2O_6$).

Enzymatic Assays. The effects of compounds on the *in vitro* enzymatic activity of purified HIV-1 protease were quantitated by a reverse phase HPLC assay utilizing the Ala-Ser-Glu-Asn-Tyr-Pro-Ile-Val-Glu-amide substrate (Multiple Peptide Systems, San Diego, CA) as previously described.[61] The 3′ cleavage and integration activities of purified HIV-1 integrase were quantitated as previously described.[27]

Antiviral Assays. 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.[62] Antiviral assays with HIV-1_{WEJO} in phytohemagglutinin-stimulated PBL's were performed as previously described.[61] The methodology used to monitor antiviral activity in the remaining assay systems was also described previously.[63]

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