

Design, Synthesis, and Biological Evaluation of Cosalane, a Novel Anti-HIV Agent Which Inhibits Multiple Features of Virus Reproduction

Mark Cushman,^{*,†} W. Marek Golebiewski,[†] James B. McMahon,[‡] Robert W. Buckheit, Jr.,[§] David J. Clanton,^{||} Owen Weislow,[⊥] Rudiger D. Haugwitz,[#] John P. Bader,[#] Lisa Graham,[∇] and William G. Rice^{*,∇}

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, Laboratory of Drug Discovery Research and Development, Division of Cancer Treatment, National Cancer Institute, Frederick, Maryland 21701-1201, Virology Research Group, Southern Research Institute-Frederick Research Center, Frederick, Maryland 21701, Anti-AIDS Virus Drug Screening Laboratory, Program Resources, Inc./DynCorp., National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201, SRA Technologies, Inc., 13 Taft Court, Rockville, Maryland 20850, Drug Synthesis and Chemistry Branch and Antiviral Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Executive Plaza North, Suite 837, 6130 Executive Boulevard, Rockville, Maryland 20852, and Laboratory of Antiviral Drug Mechanisms, Program Resources Inc./DynCorp., National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201.

Received April 25, 1994[®]

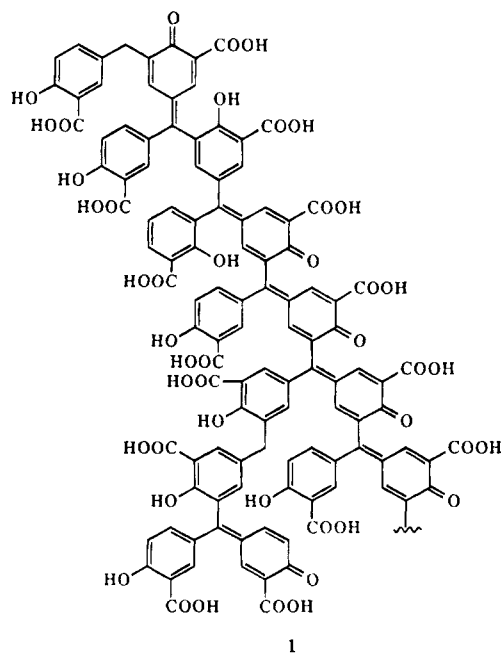
Cosalane (**3**), a novel anti-HIV agent having a disalicylmethane unit linked to C-3 of cholestane by a three-carbon linker, was synthesized from commercially available starting materials by a convergent route. Cosalane proved to be a potent inhibitor of HIV with a broad range of activity against a variety of laboratory, drug-resistant, and clinical HIV-1 isolates, HIV-2, and Rauscher murine leukemia virus. The cytotoxicity of cosalane is relatively low as reflected by an *in vitro* therapeutic index of >100. Although cosalane inhibits HIV-1 reverse transcriptase and protease, time of addition experiments indicate that it prevents the cytopathic effect of HIV by acting earlier than reverse transcription in the viral replication cycle. The available evidence indicates that the primary mechanism of action of cosalane involves inhibition of gp120–CD4 binding as well as inhibition of a postattachment event prior to reverse transcription.

Introduction

The design, synthesis, and evaluation of new potential therapeutic agents for the treatment of acquired immune deficiency syndrome (AIDS) is a significant challenge now facing the medical scientific community. The few drugs which inhibit HIV and are approved for the treatment of AIDS, zidovudine (AZT),^{1–3} dideoxycytidine (ddC),^{4,5} and dideoxyinosine (ddI),⁶ have been clinically useful only in delaying the progression of AIDS. Moreover, these nucleoside analogs have been limited in their clinical efficacy by their toxicities as well as by the emergence of drug-resistant viruses in treated patients.^{4,7–9} Similarly, the excitement generated by the discovery of a diverse class of potent non-nucleoside reverse transcriptase inhibitors^{10–16} was diminished when it was found that resistant virus isolates rapidly arose in treated patients.¹⁷ Although a number of other compounds or therapies are being evaluated clinically in AIDS patients, there is a clear need for continued identification and development of new agents with diverse antiviral mechanisms of action against HIV for use alone or in combination with other active agents.

Recent interest in aurintricarboxylic acid (ATA), a heterogeneous mixture of polymers that forms when salicylic acid is treated with formaldehyde, sulfuric acid, and sodium nitrite, has been stimulated by its ability to inhibit the cytopathic effect of HIV-1 and HIV-2 in a

variety of lymphocyte cell cultures.^{18,19} The anti-HIV potency of ATA polymer fractions was found to correlate with their average molecular weights,^{20,21} and a detailed structural analysis of polymeric ATA, as well as low molecular weight oligomers present in ATA, led to a schematic representation of the polymer as depicted in structure **1**.^{22,23} Although the higher molecular weight



ATA fractions displayed potent anti-HIV activity due to inhibition of gp120–CD4 binding,²⁰ interest in the potential development of ATA fractions for potential

[†] Purdue University.

[‡] Drug Discovery Research and Development.

[§] Virology Research Group.

^{||} Anti-AIDS Virus Drug Screening Laboratory.

[⊥] SRA Technologies, Inc.

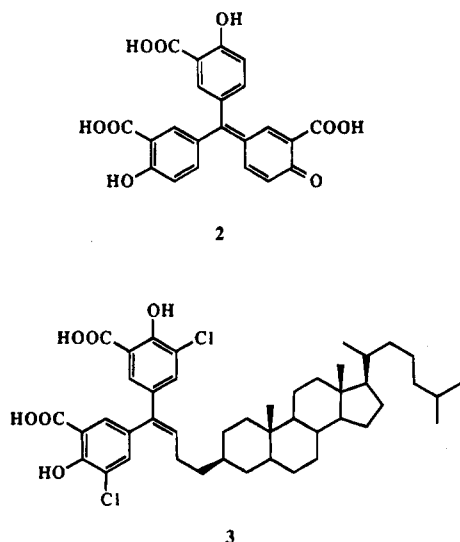
[#] Developmental Therapeutics Program.

[∇] Antiviral Drug Mechanisms.

[®] Abstract published in *Advance ACS Abstracts*, August 1, 1994.

therapeutic use was compromised significantly by the fact that ATA fractions are a heterogeneous mixture of polymers which are difficult to characterize. Additionally, the polymerization process yielding ATA is very sensitive to the exact reaction conditions, so that different polymer mixtures can result from the same procedure even under carefully controlled polymerization conditions.²⁰ As a result of these considerations, low molecular weight ATA components were obtained by fractionation and synthesis in an attempt to obtain structurally defined materials that would hopefully retain anti-HIV activity.^{22,24} These substances, including the ATA monomer **2**, were either inactive or had very low anti-HIV activity.^{22,24} An effort was therefore undertaken to synthesize ATA monomer analogs with increased potencies.

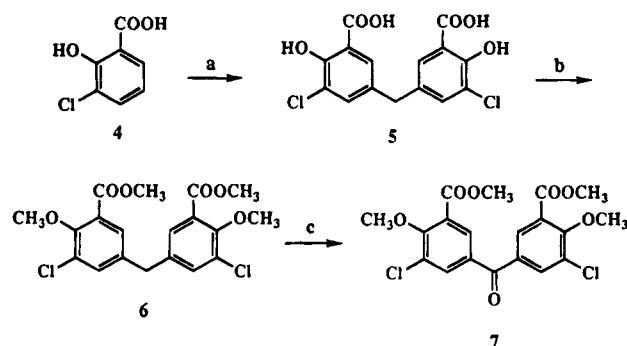
Cosalane (**3**), the subject of the present communication, was obtained conceptually by replacing the quinone methide moiety of the ATA monomer **2** with an alkene chain linked to cholestane and attaching two chlorine atoms ortho to the phenolic hydroxyl groups of the two salicylic acid units. It is one of the most potent of the more than 70 ATA monomer analogs synthesized to date. The design rationale was that the steroid might serve to direct the compound to the cell membrane and viral envelope, thus possibly enhancing the ability of this ATA monomer analog to prevent the binding of the virus to the cell membrane. The two chlorines would also be expected to enhance lipophilicity. As detailed below, cosalane (**3**) proved to be about as potent as the most active ATA polymer fractions. However, its mechanism of action appears to involve inhibition of postbinding fusion events as well as prevention of virus binding to the cell membrane. The synthesis of cosalane, as well as the evaluation of its range and mechanism of biological activity, was examined and is reported here.



Results

Synthesis of Cosalane. In order to maximize overall yield, a strategy utilizing a convergent route for cosalane synthesis was devised.²⁵ This involved the construction of a precursor to the disalicylmethane portion of cosalane as outlined in Scheme 1. The bis-(chlorosalicyl)methane intermediate **5** was prepared by a slight modification of a published procedure²⁴ in which 3-chlorosalicylic acid (**4**)²⁶ was treated with formalde-

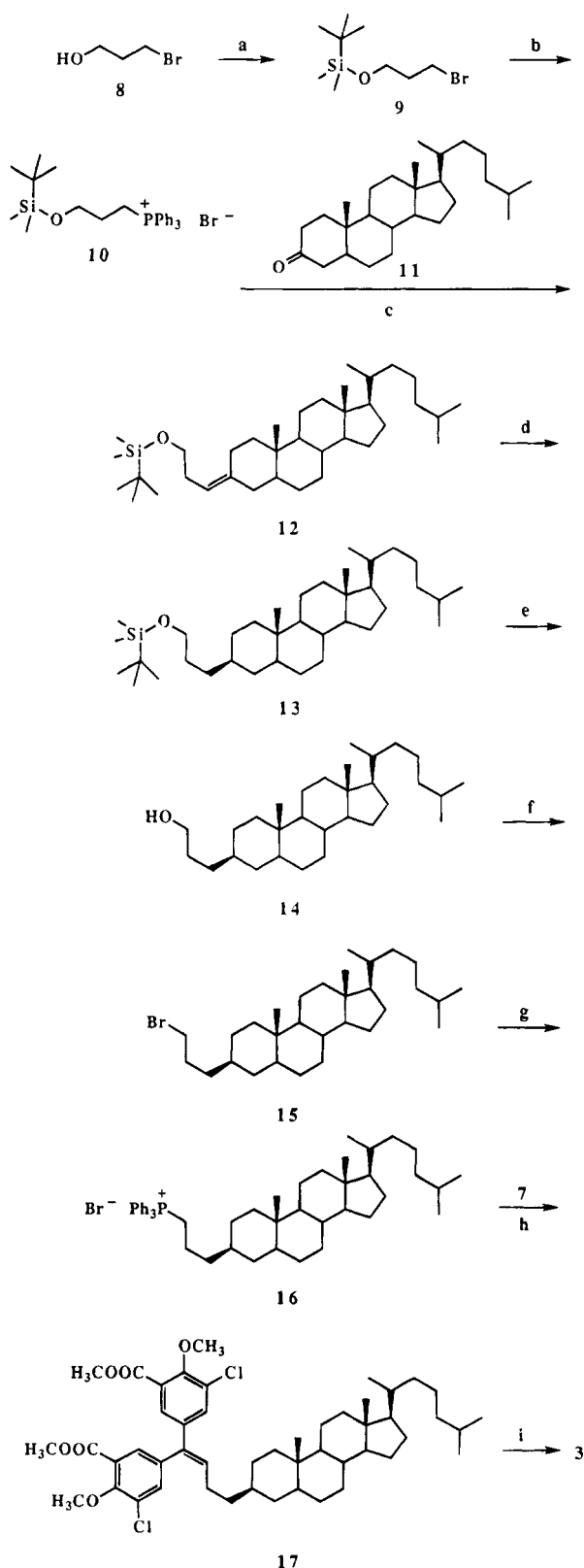
Scheme 1^a



^a (a) H_2CO , CH_3OH , H_2SO_4 , -78 – 23 °C (16 h); (b) K_2CO_3 , $(\text{CH}_3)_2\text{CO}$, $(\text{CH}_3)_2\text{SO}_4$, reflux (20 h); (c) CrO_3 , $(\text{CH}_3\text{CO})_2\text{O}$, 0 – 23 °C (14 h).

hyde under acidic conditions in methanol. Reaction of the two carboxylic acid and two phenolic groups of **5** with dimethyl sulfate using potassium carbonate as the base afforded the diester **6**. The methylene unit of **6** was oxidized with chromic anhydride in acetic anhydride to yield the substituted benzophenone **7**.

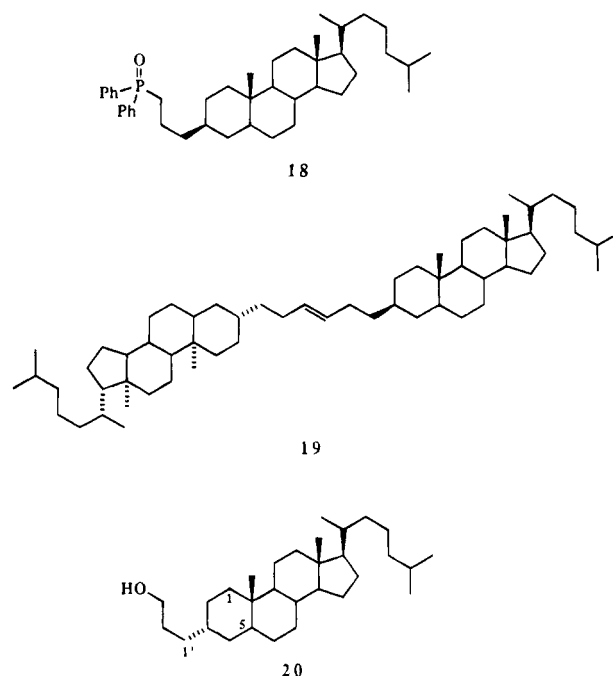
The attachment of the linker chain to the cholestane moiety and the subsequent attachment of the disalicylmethane unit are depicted in Scheme 2. Treatment of the *tert*-butyldimethylsilyl derivative **9** of 3-bromopropanol (**8**) with triphenylphosphine in refluxing acetonitrile yielded the phosphonium bromide **10**. Deprotonation of intermediate **10** with *n*-butyllithium in 1,2-dimethoxyethane gave the corresponding ylide, which underwent Wittig reaction with 3-cholestanone (**11**) to afford the alkene **12** as a mixture of *E*- and *Z*-isomers in *ca.* 1:1 ratio as evidenced by NMR analysis. This result is consistent with olefinations reported for 3-cholestanone and unstabilized ylides. For example, in the reaction of 3-cholestanone with ethyltriphenylphosphorane, using sodium hydride–DMSO or potassium *tert*-butoxide as the base, a mixture of *E*- and *Z*-olefins in a 3:2 ratio was obtained.²⁷ No attempts were made in the present case to improve the stereospecificity of this reaction because catalytic hydrogenation of the mixture of alkenes **12** using platinum oxide as the catalyst in ethyl acetate at 50 °C provided intermediate **13** as one major isomer. The hydrogen would be expected to add to the less sterically hindered side of the alkenes **12**, which is the side opposite to the two angular methyl groups. Pure **13** crystallized from the crude hydrogenation product without contamination from the diastereomer having the opposite configuration at C-3. The yield of **13** was somewhat erratic and seemed to depend on the activity of the catalyst, as variable amounts of a hydrogenolysis product, 3β -propylcholestanol, and the C-3 α -epimer of **13** were detected in the reaction mixture. Removal of the *tert*-butyldimethylsilyl protecting group from **13** with fluoride anion resulted in the formation of the corresponding alcohol **14**. The alcohol **14** was transformed into the corresponding bromide **15** with carbon tetrabromide and triphenylphosphine in either acetonitrile or methylene chloride. Reaction of the bromide **15** with triphenylphosphine in refluxing chlorobenzene gave the triphenylphosphonium bromide salt **16**, which on deprotonation with sodium bis(trimethylsilyl)amide in THF and Wittig reaction of the resulting ylide with the substituted benzophenone **7** afforded compound **17**. However, when

Scheme 2^a

^a (a) TBDMSCl, DIEA, DMAP, CH₂Cl₂, 23 °C (24 h); (b) Ph₃P, CH₃CN, reflux (30 h); (c) (1) *n*-BuLi, DME, 0 °C (80 min), (2) compound **10**, DME, 60 °C (22 h); (d) H₂, PtO₂, EtOAc, 50 °C (4 h); (e) (*n*-Bu)₄N⁺F⁻, THF, 23 °C (1 h); (f) CBr₄, Ph₃P, CH₂Cl₂, 0 °C (10 min); (g) Ph₃P, C₆H₅Cl, reflux (48 h); (h) (1) NaN[Si(CH₃)₃], THF, 23 °C (80 min), (2) compound **6**, 23 °C (20 h); (i) BBr₃·S(CH₃)₂, ClCH₂CH₂Cl, 90 °C (8 h).

the ylide was generated in a DMSO–DME mixture with sodium hydride, the reaction yielded a diphenylphosphine oxide, **18**, as the major product. Similar products

have been reported in reactions of phosphonium salts with sodium hydroxide.^{28,29} This suggests that decomposition of the phosphonium salt **16**, or the corresponding ylide, may be caused by moisture present in the solvent. Deprotonation of the phosphonium salt **16** with *n*-butyllithium in DME and reaction with **7** afforded, on the other hand, the alkene **17** in low yield and a dimeric olefin, **19**, in 11% yield as a result of ylide oxidation.³⁰ Final removal of the two ester and two ether methyl groups from **17** with boron tribromide–dimethyl sulfide complex in 1,2-dichloroethane yielded cosalane (**3**).



With regard to the stereochemical assignment of the C-3 side chain in intermediate **13** and the subsequent compounds in the synthesis, it was assumed that the major diastereomer resulting from catalytic hydrogenation of the double bond of **12** would have a β -configuration as shown in structure **13**, which would result from the delivery of hydrogen to the less hindered α -side of the steroid, opposite the two angular methyl groups. However, in order to gain additional evidence in support of this stereochemical assignment, a careful chromatographic separation of small amounts of diastereomer **20** from **14** after deprotection of crude **13** was performed. In agreement with the stereochemical assignment, C-1 and C-5 of **20** appear upfield at δ 33.25 and 40.34 in its ¹³C NMR spectrum relative to the corresponding carbons of **14**, which occur at δ 38.64 and 46.65, respectively. Carbons C-1 and C-5 are expected to be upfield by about 5 ppm in the diastereomer having the axial side chain at C-3 relative to the diastereomer with the equatorial side chain at C-3 due to γ -gauche interactions of C-1 and C-5 with C-1' of the axial side chain.^{31,32} The axial side chain also causes upfield absorption of the C-3 carbon atom of **20** at δ 32.96 compared to the C-3 resonance of the equatorial epimer **14** at δ 37.89.³³

Anti-HIV Activity of Cosalane. The microtiter XTT assay was used to determine the effect of cosalane (**3**) on the ability of HIV-1 to initiate a productive, cytopathic infection in CEM-SS cells. Cosalane reproducibly protected these cells from HIV-1-induced cyto-

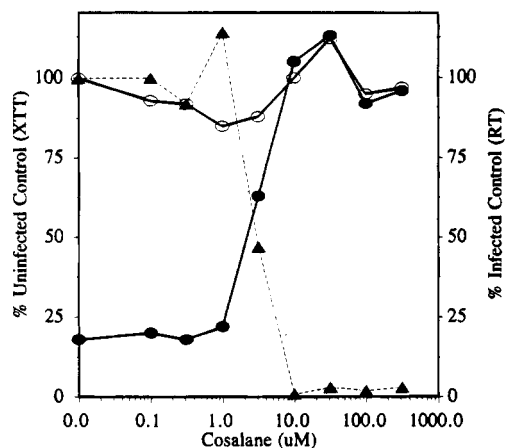


Figure 1. Effect of cosalane on HIV-induced cytopathicity. Effects of cosalane on growth and metabolic activity (XTT) of uninfected (○) and HIV-1-infected (●) CEM-SS cells assessed after 6 days in culture. Aliquots of culture supernatants were assayed for virion-associated RT (▲) as a measure of viral replication. Values represent the means of at least quadruplicate samples (SEM ≤ 10%).

Table 1. Range of Activity of Cosalane in Different Human Cell Lines

cells	phenotype ^a	EC ₅₀ (μM) ^b	
		cosalane	AZT
CEM-SS	T	5.1	0.095
MT-4	T (HTLV-1+)	20.0	0.085
174×CEM	(B×T)	6.1	0.055
AA5	B (EBV+)	6.6	0.001
U937	M	7.4	0.030
PBL	lymphocyte	44.8	0.029
macrophage	M	19.7	0.015

^a T = T-cell line; B = B-cell line; M = macrophage; HTLV-1 = human T-cell leukemia virus type 1; EBV = Epstein Barr virus.

^b Drug-induced inhibition of virus replication was quantitated by XTT assay in cytopathically infected cells and by RT activity assay or p24 ELISA in noncytopathically infected cells. HIV-1_{RF} was utilized in all antiviral testing involving cultured cell lines; fresh PBL and macrophage cells were infected with the clinical HIV-1 isolates WEJO and Ba-L, respectively.

pathicity, achieving 50% cellular protection (EC₅₀) with approximately 5 μM. This inhibitory concentration of cosalane was consistent with the inhibition of virus reproduction, as measured by the production of virion-associated RT from the cells (Figure 1). Microscopic examination of the cells in wells protected by effective doses of the compound confirmed the complete absence of giant-cell formation and other HIV-1-induced cytopathicity. Direct cytotoxic effects of cosalane on the CEM-SS cells occurred only at substantially higher concentrations (200–300 μM).

Cosalane Exhibits a Broad Range of Antiviral Activity. As shown in Table 1, cosalane was active in several human cell lines of different phenotype, including T-cells, B-cells, and macrophages. Cosalane was also found to be active against clinical strains of HIV-1 in fresh human peripheral blood lymphocytes (PBLs) and macrophages. In a comparative study with AZT, cosalane was tested against a wide variety of both laboratory and clinical isolates (Table 2). Cosalane was active against all of the HIV-1 isolates tested, including strains resistant to AZT and the pyridinone class of non-nucleoside RT inhibitors, as well as HIV-2. Cosalane was also active against a diverse panel of clinical isolates. Although the EC₅₀ values suggest that cos-

Table 2. Range of Activity of Cosalane against HIV-1 Isolates in Cell Culture-Based Assays

HIV strain	EC ₅₀ (μM) ^a	
	cosalane	AZT
Laboratory Strains		
III _B ^b	6.8	0.004
III _B ^c	3.4	0.003
214	7.1	0.009
205	6.7	0.009
MCK	2.1	0.002
PM16	6.6	0.011
TP1	6.5	0.006
G	2.2	0.002
SKI	20.6	0.013
RF	1.7	0.004
LAV	14.4	0.008
MN	8.8	0.004
Drug-Resistant Strains		
A17 (pyridinone-resistant)	4.3	0.002
G9106 (AZT-resistant)	6.6	>0.1
Clinical Isolates^d		
WEJO (SI) ^e	44.8	0.018
BAKI (SI)	48.1	0.010
WOME (SI)	69.9	0.006
VIHU (NSI)	80.2	0.020
Other		
MS (HIV-2)	22.5	0.002

^a EC₅₀ values determined by XTT-based cytopathicity assay.

^b Determined in CEM-SS cells unless otherwise noted. ^c Determined in MT-2 cells. ^d Determined in fresh human PBLs. ^e SI: syncytia inducing. NSI: nonsyncytia inducing.

alane is somewhat less effective in PBLs with clinical isolates, the effectiveness of the compound was dependent on the input titer of the virus, such that usage of lower virus titers yielded lower EC₅₀ values in the range of 5–25 μM. Furthermore, cosalane was fully active against HIV-1 when tested in the presence of human or dog plasma. Cosalane, like AZT, failed to inhibit HIV-1 production in chronically infected H9 cells, as assessed by the appearance of viral p24 antigen and virion-associated RT activity in culture fluids of treated cells (data not shown). Cosalane was also active against SIV (data not shown).

Mechanism of Action Studies. Preliminary studies indicated that treatment of HIV-1 virions with cosalane, using an infectivity titration assay previously described,³⁴ did not reduce viral infectivity if the cosalane was removed. Similarly, treatment of cells with cosalane failed to protect cells from HIV-1-induced killing if the compound was removed before infection (data not shown).

A series of time course experiments was then performed to determine at which stage(s) of the HIV-1 reproductive cycle cosalane acted. As shown in Figure 2, cosalane prevented HIV-1 DNA synthesis when included during the preincubation of cells with virus (pre condition), when preincubated and then added back at time zero following removal of unbound virus (pre/*t*₀ condition), or when added only at *t*₀ after virus attachment had already occurred (*t*₀ condition). No inhibition of HIV-1 DNA synthesis occurred when cosalane was added more than 30 min after virus was bound to the cells. In control experiments performed in parallel, ddC (a nucleoside inhibitor of RT) prevented HIV-1 DNA synthesis when added as late as 2 h after virus binding, unless the drug was removed from the culture. Dextran sulfate prevented HIV-1 DNA synthesis if present during the virus attachment phase (pre/*t*₀ and pre condi-

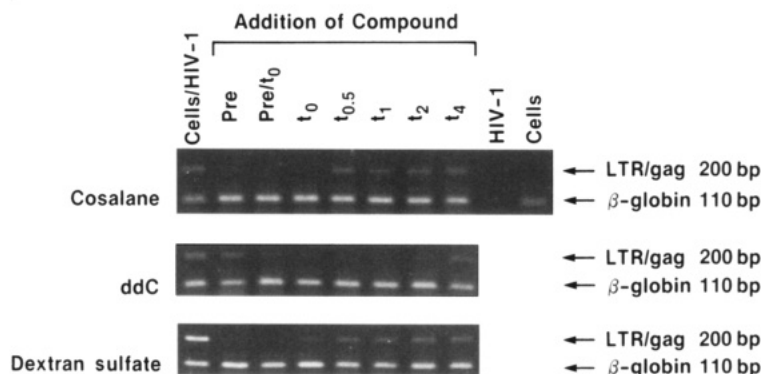


Figure 2. Effect of time of addition on cosalane activity. CEM-SS cells were preincubated with HIV-1_{IIIB} for 1 h at 0–4 °C, washed to remove residual virus, and warmed to 37 °C. Experimental drugs (cosalane at 50 μ M, ddC at 10 μ M, dextran sulfate at 100 μ g/mL) were included during the preincubation step only (pre), included during the preincubation and then added back after shifting to 37 °C (pre/ t_0), or added only at indicated times after shifting to 37 °C ($t = 0, 0.5, 1, 2$, or 4 h). Control lanes contain HIV-1 alone (no cells) or cells alone (no HIV-1) as controls. The band derived from the HIV-1 LTR/gag region corresponds to 200 bp, and the band derived from the cellular β -globin gene corresponds to 110 bp.

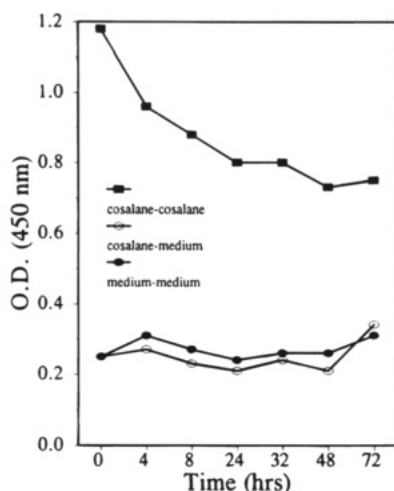


Figure 3. Reversibility of cosalane. CEM-SS cells were infected with HIV-1 in the presence or absence of cosalane. At various time intervals, cells were washed and reincubated in medium with or without cosalane. After a total of 6 days, cell viability was assessed by the XTT assay. Legend: cosalane–cosalane (■), cosalane–medium (○), medium–medium (●). Points represent the mean of quadruplicate samples (SEM $\leq 15\%$).

tions) but not when the drug was added following virus binding. Data from the time of addition study indicate that cosalane blocks the initial attachment of virus to cells as well as a postattachment event prior to reverse transcription. Assessment of cytoprotection by the XTT assay in delayed-addition experiments confirmed the PCR results (data not shown).

The requirement for the continuous presence of cosalane for antiviral activity was examined in a separate time course study in which HIV-infected cells were exposed to cosalane for various intervals and the progression of infection was monitored by the XTT assay. As shown in Figure 3, removal of cosalane at any time resulted in failure to protect the cells from subsequent cytopathicity, although readdition of cosalane continued to protect the cells. The action of cosalane, therefore, is reversible, and the continued presence of cosalane is required for protection. It can be noted that even a brief relief from cosalane during the experimental rinsing period was sufficient to allow some viral cytopathicity to occur in cosalane-treated controls.

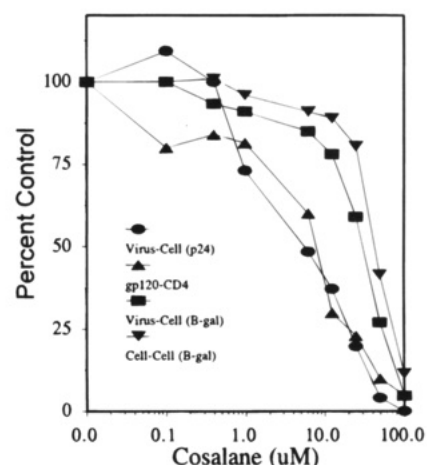


Figure 4. Effect of cosalane on binding interactions: attachment of HIV-1_{RF} to human PBLs, as quantitated by the association of virion p24 with the cells (●), binding of HIV-1_{IIIB} to HeLa CD4⁺/β-gal cells (■), binding of isolated HIV-1 gp120 to CD4 (▲), fusion of HeLa CD4/β-gal cells with HL 2/3 cells expressing *env* and *tat* proteins (▼). Values represent the results of two independent experiments.

Finally, the time course experiments indicated that cosalane exerted no action during reverse transcription or late stage viral protein processing, even though cosalane effectively inhibited the enzymatic activities of purified HIV-1 reverse transcriptase ($ID_{50} = 0.36 \mu$ M, mean of three separate experiments, standard error of the mean $\leq 15\%$) and HIV-1 protease ($ID_{50} = 0.55 \mu$ M, mean of three separate experiments, standard error of the mean $\leq 15\%$).

Effects of Cosalane on Virus Binding and Cell-to-Cell Fusion. The possible interference of cosalane with the binding of HIV to target cells was examined using two independent methods. Using the p24 ELISA to quantitate cell-associated virions, cosalane was observed to inhibit the binding of HIV-1_{RF} to freshly isolated PBLs in a concentration-dependent manner (Figure 4; $ID_{50} = 5.3 \mu$ M). The assay for β -galactosidase was used to determine the effect of cosalane on HIV-1_{IIIB} binding to HeLa-CD4-LTR-β-gal cells. The cosalane treatment effectively prevented the binding of virus and the induced expression of β -gal with an $ID_{50} = 21 \mu$ M (Figure 4). To further examine the specificity of the inhibition of the interactions between viral envelope components and target cells, a gp120–CD4

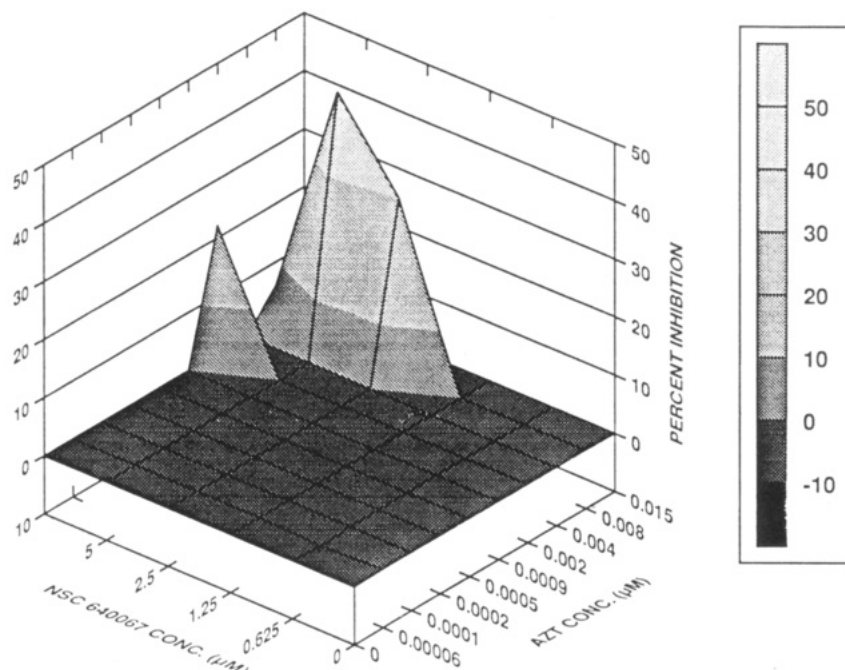


Figure 5. Combination antiviral activity of cosalane with AZT in CEM-SS cells infected with HIV-1 (IIIb): the three-dimensional synergy plot demonstrating the synergistic activity of the two compounds.

binding assay was performed. Cosalane inhibited the binding of soluble gp120 to CD4 (Figure 4; $ID_{50} = 7.8 \mu M$).

The action of cosalane on cell-cell fusion was investigated by coculturing HL 2/3 cells (env^+) with HeLa CD4 $^+$ /β-gal cells. Cosalane efficiently inhibited syncytium formation and activation of β-galactosidase (Figure 4; $ID_{50} = 33 \mu M$). In addition, cosalane inhibited the syncytia which formed when chronically infected CEM-SS cells were cocultured with uninfected cells (data not shown).

Combination Antiviral Activity with AZT. Cosalane was tested for anti-HIV activity in combination with AZT using the *in vitro* XTT anti-HIV assay. Five concentrations of cosalane were tested in all combinations with eight concentrations of AZT. Effects of the drug combination were calculated on the basis of the activity of the two compounds when tested alone. The results of these assays demonstrated that the combined antiviral activity was greater than that predicted for additivity (Figure 5). The peak above the plane represented a maximal antiviral activity (protection from HIV-induced killing) from a combination of cosalane and AZT which was nearly 50% greater than would have been expected if the antiviral effects were merely additive. No antagonism was detected at any concentration tested.

Discussion

Cosalane, a unique inhibitor of HIV reproduction and cytopathicity, was synthesized as part of a lead development effort after recognizing the anti-HIV activity of aurintricarboxylic acid, a heterogeneous mixture of polymers formed when salicylic acid is treated with formaldehyde, sulfuric acid, and sodium nitrite.^{18,20,22-24} The anti-HIV activities of ATA fractions were found to correlate with their average molecular weights in the range up to MW approximately 2500.^{20,22-24} Further increase in molecular weight beyond this point did not result in a further increase in potency.²³ Although

monomeric ATA had little activity against HIV, the hydrate of this molecule exhibited sufficient antiviral activity to encourage an effort to increase the activity of the monomer.^{22,24} Cosalane was designed conceptually by replacing the quinone methide moiety of the ATA monomer with an alkene chain linked to a cholestane unit and attaching two chlorine atoms ortho to the phenolic hydroxyl groups of the two salicylic acid rings. The resulting molecule, cosalane, proved to be a potent inhibitor of HIV with a broad range of activity against a variety of laboratory, drug-resistant, and clinical HIV-1 isolates, HIV-2, and Rauscher murine leukemia virus.²⁵

Cosalane failed to inactivate HIV when the drug was incubated with virus alone and when exogenous cosalane was removed from the culture medium, implying that the compound neither accumulated in nor bound to cells in concentrations sufficient to inhibit HIV. Time course experiments demonstrated that cosalane acted at an early stage of HIV reproduction. The simultaneous addition of cosalane and HIV-1 prevented attachment of virus to human PBLs, CD4-expressing HeLa cells, and CEM-SS cells; interference with soluble gp120 binding to isolated CD4 protein by cosalane was also demonstrated. At least a portion of the inhibitory action of cosalane, therefore, can be attributed to obstruction of virion gp120 interaction with the cellular CD4 receptor. Nonetheless, cosalane was shown to be effective even after attachment of virus, so its action is not limited to interference with attachment. Fusion of the viral envelope with the cell membrane may involve additional interactions of viral and cellular components after initial attachment, which are inhibited by cosalane. This line of reasoning is consistent with the finding that cosalane inhibited the reproduction of a murine leukemia virus that does not depend on gp120-CD4 binding. It is possible in this case that the steroid moiety of cosalane imbeds perpendicularly into the membrane, positioning the disalicylic acid moiety externally in an obstructive mode.

The inhibitory activities of cosalane against the HIV enzymes, reverse transcriptase and protease, further complicate an understanding of the anti-HIV mechanism of action of cosalane. The requirement for cosalane early in the reproductive cycle, and its failure to affect virus production from chronically infected cells, argues against the anti-protease action of cosalane in the biological system; several known protease inhibitors have been shown in our laboratory (unpublished observations) and others to prevent the production of infectious virus from chronically infected cells. The inability of cosalane to act like a viral protease inhibitor in infected cells suggests that cosalane fails to permeate the cell surface membrane and enter cells, which renders unlikely the possibility that cosalane acts on reverse transcriptase in its inhibition of HIV replication. The localization of cosalane in exposed cells is under examination in order to ascertain its intracellular antiviral potential.

Previous clinical trials with surface active agents, such as dextran sulfate and pentosan sulfate, resulted in disappointing outcomes, and this precedent has led to hesitance in further trials of such compounds. These polyanionic mixtures typically failed due to their poor bioavailability and pharmacokinetics and their adverse anticoagulant properties.³⁵⁻³⁹ However, cosalane was found to inhibit infection by HIV-1 clinical isolates in PBLs and macrophages. Likewise, cosalane has a monomeric structure and theoretically should not present adverse hematological properties of the polymeric anions.

The clinical potential of cosalane is encouraged by several other features shown by our results: (a) the effective concentrations of cosalane in a variety of cell types are low enough to suggest that these concentrations could be readily achieved in man, (b) the *in vitro* therapeutic index, *i.e.*, the antiviral efficacy compared with toxicity, is sufficiently high to suppose that *in vivo* toxicity may not be problematical, (c) the stability of cosalane in plasma suggests favorable pharmacologic properties, and (d) the synergistic activity of cosalane when used in combination with AZT suggests that the addition of cosalane could diminish the viral load in patients already undergoing treatment with AZT. Hence, preclinical development of cosalane, including definitive formulation, pharmacokinetic and toxicology studies, and continued congener synthesis, is underway. It is hoped that cosalane will represent a new class of compounds that can be utilized clinically against HIV, either alone or in combination with other approved agents.

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; ¹H 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 were within $\pm 0.4\%$ of the calculated compositions.

3,3'-Dicarboxy-5,5'-dichloro-4,4'-dihydroxydiphenylmethane (5). This compound was prepared by a slight modification of the published procedure.²⁴ 3-Chlorosalicylic acid²⁶ (32.4 g, 0.187 mmol) was placed in a 1 L three-necked, round-bottomed flask equipped with a mechanical stirrer, a

500 mL pressure equalizing dropping funnel, and a thermometer. The solid was dissolved in methanol (140 mL), water (25 mL) was added, and the mixture was vigorously stirred at dry ice-acetone bath temperature while concentrated sulfuric acid (325 mL) was added at such a rate to keep the temperature below 0 °C. The reaction mixture was stirred on an ice bath for 1 h and then cooled in a dry ice-acetone bath again. An aqueous solution of 37% formaldehyde (75 mL) was added at such a rate to keep the temperature below 0 °C. The mixture was stirred at 0 °C for 4 h and left overnight at room temperature. It was poured on crushed ice (1.5 kg), and the precipitate was filtered and dried, first at room temperature overnight and then in a vacuum desiccator, to afford a solid (34.5 g). The product was recrystallized from chloroform-methanol (2:1): mp 296 °C.

3,3'-Dicarbomethoxy-5,5'-dichloro-4,4'-dimethoxydiphenylmethane (6). 3,3'-Dicarboxy-5,5'-dichloro-4,4'-dihydroxydiphenylmethane (**5**; 8.55 g, 23.95 mmol) was placed in a 500 mL three-necked, round-bottomed flask equipped with a mechanical stirrer, a 50 mL pressure equalizing dropping funnel, and a reflux condenser connected to a drying tube. The acid was dissolved in acetone (240 mL; Mallinckrodt, AR), and ground anhydrous potassium carbonate (26.42 g, 191.16 mmol) was added followed by dimethyl sulfate (16.306 g, 12.23 mL, 129 mmol). The reaction mixture was vigorously stirred under reflux for 20 h. The mixture was filtered and inorganic salts were rinsed with methylene chloride (4 \times 15 mL). The solvents were removed *in vacuo*, water (50 mL) was added, and the mixture was extracted with methylene chloride (3 \times 30 mL). The combined organic extracts were washed with water, dried (sodium sulfate), and evaporated *in vacuo* to yield the product as a colorless solid, 9.15 g. The crude product was dissolved in boiling methylene chloride, hexane was added until the solution became turbid, and crystallization came to completion in the refrigerator, yielding pure product (8.22 g, 83%): mp 131–132 °C; IR (KBr) 2975, 2925, 1730, 1600, 1560, 1480, 1435, 1320, 1280, 1250, 1205, 1095, 1000, 925, 835, 790, 725 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.50 (d, *J* = 2.3 Hz, 2 H), 7.32 (d, *J* = 2.3 Hz, 2 H), 3.92 (s, 12 H), 3.87 (s, 2 H); CIMS *m/e* (relative intensity) 413 (MH⁺, 100), 381 (71). Anal. (C₁₉H₁₈Cl₂O₆) C, H.

3,3'-Dicarbomethoxy-5,5'-dichloro-4,4'-dimethoxybenzophenone (7). 3,3'-Dicarbomethoxy-5,5'-dichloro-4,4'-dimethoxydiphenylmethane (**6**; 24.99 g, 60.47 mmol) was placed in a 1000 mL one-necked, round-bottomed flask equipped with a Teflon-coated magnetic stirring bar and a reflux condenser connected to a drying tube. Compound **6** was partially dissolved in acetic anhydride (500 mL; Baker, AR). The mixture was cooled in an ice bath, and chromic anhydride (24.26 g, 242.6 mmol) was added in small portions over 0.5 h. The bath was removed, and the mixture was stirred at room temperature for 14 h. The chromium salts were filtered off and washed with methylene chloride (5 \times 20 mL). The solvent was removed *in vacuo*, and the solidified residue was flash chromatographed on silica gel (300 g). Elution with methylene chloride (2.5 L) afforded the benzophenone derivative **7** (18.78 g, 72.8%): mp 118–119 °C; IR (KBr) 1744, 1662, 1476, 1269, 988 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 8.07 (d, *J* = 2.3 Hz, 2 H), 7.97 (d, *J* = 2.3 Hz, 2 H), 4.03 (s, 6 H), 3.94 (s, 6 H); CIMS *m/e* (relative intensity) 427 (M⁺, 100), 381 (71). Anal. (C₁₉H₁₆Cl₂O₆) C, H.

3-Bromo-1-[(*tert*-butyldimethylsilyl)oxy]propane (9). Diisopropylethylamine (25.488 g, 34.35 mL, 0.197 mol) was added dropwise to a solution of 3-bromopropanol (25 g, 0.18 mol), *tert*-butyldimethylsilyl chloride (29.81 g, 0.197 mol), and DMAP (0.880 g, 7.2 mmol) in methylene chloride (250 mL) and placed in a dry 500 mL three-necked, round-bottomed flask kept at ice bath temperature and equipped with a Teflon-coated magnetic stirring bar, a 50 mL pressure equalizing dropping funnel, a stopper, and a reflux condenser connected to an argon flow line. The reaction mixture was stirred at room temperature for 24 h and washed successively with water (2 \times 150 mL), dilute ammonium chloride solution (1 \times 100 mL), and brine (2 \times 150 mL). The organic solution was dried (sodium sulfate), the solvent was removed *in vacuo*, and the residual oil was distilled, yield 37.5 g (81%): bp 62–64 °C (2

mmHg); ^1H NMR (CDCl_3 , 200 MHz) δ 3.71 (t, J = 6.1 Hz, 2 H), 3.46 (t, J = 7.0 Hz, 2 H), 2.00 (m, 2 H), 0.87 (s, 9 H), 0.06 (s, 6 H); CIMS m/e 253.

Triphenyl[3-[(*tert*-butyldimethylsilyl)oxy]propyl]-phosphonium Bromide (10). 3-Bromo-1-[(*tert*-butyldimethylsilyl)oxy]propane (**9**; 2.7 g, 10.75 mmol) and triphenylphosphine (3.8 g, 10.75 mmol) were placed in a 25 mL two-necked, round-bottomed flask equipped with a Teflon-coated magnetic stirring bar, a rubber septum, and a reflux condenser connected to an argon flow line. Acetonitrile (3 mL) was added, and the solution was heated under reflux for 30 h. The reaction mixture was cooled to room temperature, the solvent removed *in vacuo*, and the oily residue triturated with hexane until crystallization came to completion. The white salt (4.995 g, 93%) was filtered off on the next day, washed with hexane (3×5 mL), and dried in a vacuum desiccator: mp 137–139 °C; ^1H NMR ($\text{DMSO}-d_6$, 200 MHz) δ 7.9–7.8 (m, 15 H), 3.68 (t, J = 5.9 Hz, 2 H), 3.53 (m, 2 H), 1.67 (m, 2 H), 0.84 (s, 9 H), 0.02 (s, 6 H); IR (KBr) 2931, 2858, 1587, 1438, 1254, 1110, 838, 746, 692 cm^{-1} ; EIMS m/e 435 ($\text{M}^+ - \text{Br}$).

5 α -3-[3'-[(*tert*-Butyldimethylsilyl)oxy]propylidene]-cholestane (12). Phosphonium salt **10** (3.896 g, 7.56 mmol) was placed in a dry 250 mL three-necked, round-bottomed flask equipped with a Teflon-coated magnetic stirring bar, a 50 mL pressure equalizing dropping funnel capped with a rubber septum, a reflux condenser connected to an argon flow line, and a rubber septum. The apparatus was flushed with argon, and an argon atmosphere was maintained throughout the reaction. Dry DME (40 mL) was added, the suspension was stirred for 5 min and cooled in an ice bath, and *n*-butyllithium (2.5 M in hexanes, 3.06 mL; Aldrich) was added dropwise with vigorous stirring. The red reaction mixture was stirred for 80 min, and a solution of freshly dried 3-cholestanone (**11**) (2.47 g, 6.3 mmol) in DME (40 mL) was added dropwise. The reaction mixture was heated at 60 °C for 22 h. The reaction mixture was cooled to room temperature and the reaction quenched with a solution of ammonium chloride (0.8 g) in water (10 mL). The organic layer was separated and the aqueous layer extracted once with ether (1×10 mL). The combined organic extracts were washed with brine (2×50 mL) and dried (sodium sulfate), and the solvent was removed *in vacuo* to afford a semisolid (6 g) which was triturated with hexane (20 mL). The mixture was stirred for 5 min and filtered, and the procedure was repeated three times. An oil obtained after evaporation of the solvent was flash chromatographed on silica gel (80 g). Elution with hexane–ethyl acetate (6:1) yielded the starting ketone (58–332 mg) and olefin **12** (3.46–2.785 g, 99–80.3%): mp 60–61 °C (benzene–ethanol); IR (neat) 2926, 2853, 1668, 1470, 1445, 1383, 1363, 1254, 1100, 835, 775 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 4.99 (t, J = 6.9 Hz, 1 H), 3.53 (m, 2 H), 0.872 (s, 9 H), 0.87 (d, J = 6.0 Hz, 3 H), 0.84 (dd, J = 7.0, 2.5 Hz, 6 H), 0.83 (s, 3 H), 0.63 (s, 3 H), 0.03 (s, 6 H); CIMS m/e (relative intensity) 543 (MH^+ , 10), 485 ($\text{MH}^+ - t\text{-Bu}$, 29), 411 ($\text{MH}^+ - \text{TBDMSOH}$, 73). Anal. ($\text{C}_{36}\text{H}_{66}\text{OSi}$) C, H.

5 α -3 β -[3'-[(*tert*-Butyldimethylsilyl)oxy]propyl]-cholestane (13). The Wittig olefin 3-[3-[(*tert*-butyldimethylsilyl)oxy]propylidene]cholestane (**12**; 2.596 g, 4.78 mmol) was dissolved in ethyl acetate (25 mL) and hydrogenated over platinum oxide (0.486 g; Aldrich) at 50 °C, atmospheric pressure, for 4 h. The catalyst was filtered off on a Buchner funnel and washed with ethyl acetate (3×3 mL). The solvent was removed *in vacuo* and the colorless product crystallized. The yield of pure **13** was approximately 85% and seemed to vary with the activity of the catalyst. The analytical sample was prepared by recrystallization from an ethanol–benzene (4:1) mixture: mp 38–39 °C; IR (neat) 2927, 2854, 1468, 1383, 1254, 1102, 836, 776 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 3.56 (t, J = 6.3 Hz, 2 H), 0.875 (d, J = 6.8 Hz), 0.87 (s, 9 H), 0.84 (dd, J = 6.5, 2.0 Hz), 0.72 (s), 0.62 (s, 3 H), 0.02 (s, 6 H); ^{13}C NMR (CDCl_3 , 126 MHz) δ 63.77, 56.62, 56.31, 54.70, 46.69, 42.63, 40.16, 39.55, 38.69, 37.95, 36.22, 36.15, 35.86, 35.83, 35.60, 33.50, 32.25, 30.37, 29.12, 29.02, 28.31, 28.06, 26.05, 24.26, 23.89, 22.87, 22.62, 21.08, 18.73, 18.45, 12.40, 12.14, –5.15; CIMS m/e (relative intensity) 545 (MH^+ , 51), 487 (11), 413 (34). Anal. ($\text{C}_{36}\text{H}_{68}\text{OSi}$) C, H.

5 α -3 β -(3'-Hydroxypropyl)cholestane (14). The silyl ether **13** (2.232 g, 4.095 mmol) was dissolved in THF (18 mL), and a 1 M solution of tetrabutylammonium fluoride in THF (8.7 mL) was added. The solution was stirred at ambient temperature for 1 h. THF was removed *in vacuo*, brine (25 mL) was added, and the mixture was extracted with a 1:1 mixture of hexane and benzene (3×15 mL). The combined extracts were washed with brine (5×20 mL) and dried (sodium sulfate), and the product obtained after evaporation of the solvent was crystallized from absolute ethanol to afford the solid (1.56 g, 88%): mp 130 °C; IR (KBr) 3302, 2931, 2850, 1466, 1381, 1051 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 3.60 (t, J = 6.6 Hz, 2 H), 1.93 (dt, J = 12, 3.3 Hz, 2 H), 0.87 (d, J = 6.9 Hz, 3 H), 0.835 (dd, J = 6.7, 2.5 Hz, 6 H), 0.716 (s, 3 H), 0.617 (s, 3 H); ^{13}C NMR (CDCl_3 , 126 MHz) δ 63.46, 56.61, 56.30, 54.68, 46.64, 42.62, 40.14, 39.54, 38.64, 37.89, 36.21, 36.13, 35.84, 35.75, 35.58, 33.37, 32.21, 30.25, 29.10, 28.97, 28.30, 28.05, 24.25, 23.89, 22.87, 22.61, 21.07, 18.72, 12.38, 12.13; EIMS m/e (relative intensity) 430 (M^+ , 4), 415 (6), 275 (45), 207 (30). Anal. ($\text{C}_{30}\text{H}_{54}\text{O}$) C, H.

5 α -3 β -(3'-Bromopropyl)cholestane (15). Alcohol **14** (1.402 g, 3.255 mmol) and carbon tetrabromide (1.349 g, 4.069 mmol) were placed in a dry 50 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. Methylene chloride (20 mL, dried over phosphorus pentoxide) was added, the solution was cooled in an ice bath, and a solution of triphenylphosphine (1.195 g, 4.557 mmol) in methylene chloride (8 mL) was added dropwise over 1 min. The yellow solution was stirred at 0 °C for 10 min, and the solvent was removed *in vacuo*. The reaction mixture was triturated and stirred with hexane for 10 min (4×20 mL). The extracts were filtered and evaporated *in vacuo*. The solid residue was flash chromatographed on silica gel (90 g). Elution with hexane–ethyl acetate (39:1) afforded the light sensitive product as a crystallizing oil (1.69–1.52 g, 100–90%). Crystallization from acetone afforded the analytical sample of bromide: mp 85–86 °C; IR (KBr) 2931, 2850, 1464, 1381, 1237 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 3.37 (t, J = 6.9 Hz, 2 H), 0.875 (d, J = 6.8 Hz, 3 H), 0.84 (dd, J = 6.8, 2.5 Hz, 6 H), 0.72 (s, 3 H), 0.62 (s, 3 H); ^{13}C NMR (CDCl_3 , 126 MHz) δ 56.59, 56.29, 54.65, 46.60, 42.62, 40.12, 39.54, 38.57, 37.46, 36.20, 36.11, 35.93, 35.84, 35.64, 35.57, 34.49, 34.19, 30.47, 29.06, 28.89, 28.30, 28.05, 24.25, 23.88, 22.87, 22.87, 22.61, 21.06, 18.72, 12.38, 12.13; CIMS m/e (relative intensity) 493 (MH^+ , 100), 413 (91). Anal. ($\text{C}_{30}\text{H}_{53}\text{Br}$) C, H.

5 α -3 β -[3'-(Triphenylphosphonio)propyl]cholestane Bromide (16). Bromide **15** (5.663 g, 11.47 mmol) and triphenylphosphine (3.009 g, 11.47 mmol) were dissolved in chlorobenzene (16 mL), and the solution was heated at reflux for 2 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 (7.97 g, 92%). The analytical sample was prepared by crystallization from acetone: mp 274 °C; IR (KBr) 3055, 2927, 2865, 1438, 1190, 1118, 751, 722, 696 cm^{-1} ; ^1H NMR (CD_3OD , 200 MHz) δ 7.9–7.7 (m, 15 H), 3.35 (m, 2 H), 0.90 (d, J = 8.5 Hz, 3 H), 0.886 (d, J = 6.3 Hz, 6 H), 0.74 (s, 3 H), 0.66 (s, 3 H); PDMS m/e (relative intensity) 676 (MH^+ , 100). Anal. ($\text{C}_{48}\text{H}_{88}\text{BrP}$) C, H.

5 α -3 β -[4',4'-(3',3''-Dicarbomethoxy-5'',5''-dichloro-4'',4'''-dimethoxydiphenyl)-3'-butenyl]cholestane (17). The phosphonium salt **16** (5.82 g, 7.70 mmol) was placed in a dry 250 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. The apparatus was flushed with argon, and the argon atmosphere was maintained throughout the reaction. THF (90 mL, freshly distilled from sodium–benzophenone) was added via the septum, the suspension of the phosphonium salt was cooled in an ice bath, and a 1 M solution of sodium bis(trimethylsilyl)amide in THF (7.7 mL) was added dropwise. The ice bath was removed and the mixture stirred at room temperature until a homogeneous solution was obtained (*ca.* 80 min). The orange solution was cooled again in the ice bath, and a solution of the ketone **7** (3.585 g, 8.47 mmol) in THF (25 mL) was added dropwise via the septum/syringe. The bath was removed, and the reaction

mixture was stirred at room temperature for 20 h. The reaction was quenched with a solution of ammonium chloride (1 g) in water (20 mL). The organic layer was separated and the aqueous phase once extracted with ether (1 × 10 mL). The combined organic extracts were dried (sodium sulfate), and the solvent was removed *in vacuo* to yield a yellowish solid (9.5 g) which was flash chromatographed on silica gel (300 g). Elution with a hexane-ethyl acetate mixture (6:1) afforded an alkene dimer (95 mg), 1,1-di(3 β -cholestanyl)-3-hexene (**19**), and Wittig product **17** (5.53 g, 87.2%). The dimer **19** was crystallized from an acetone-methylene chloride mixture: mp 140 °C; IR (KBr) 2924, 2850, 1638, 1464, 1380 cm⁻¹; ¹H NMR (CDCl₃, 200 MHz) δ 5.28 (t, J = 4.5 Hz, 1 H), 1.97 (m, 2 H), 0.88 (d, J = 7.4 Hz, 3 H), 0.84 (d, J = 6.8 Hz, 6 H), 0.72 (s, 3 H), 0.63 (s, 3 H); PDMS 825 (MH⁺). Anal. (C₆₀H₁₀₄) C, H. The olefin **17** was crystallized from methyl ethyl ketone-ethanol: mp 152–153 °C; IR (KBr) 2930, 2852, 1735, 1656, 1474, 1438, 1254, 1210, 1092, 1001, 745 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 7.49 (d, J = 2.3 Hz, 1 H), 7.475 (d, J = 2.1 Hz, 1 H), 7.32 (d, J = 2.1 Hz, 1 H), 7.295 (d, J = 2.3 Hz, 1 H), 6.06 (t, J = 7.6 Hz, 1 H), 4.00 (s, 3 H), 3.93 (s, 3 H), 3.92 (s, 3 H), 3.915 (s, 3 H), 2.045 (m, 2 H), 1.95 (dm, 1 H), 0.89 (d, J = 7.0, 3 H), 0.86 (dd, J = 7.0, 2.2 Hz, 6 H), 0.72 (s, 3 H), 0.64 (s, 3 H); FABMS m/e (relative intensity) 823 (MH⁺, 5). Anal. (C₄₉H₆₈Cl₂O₆) C, H.

5 α -3 β -[4',4'-(3'',3''-Dicarboxy-5'',5''-dichloro-4'',4''-dihydroxydiphenyl)-3'-butenyl]cholestane (3). A solution of boron tribromide-dimethyl sulfide complex (1 M in CH₂Cl₂, 3.2 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. Dry 1,2-dichloroethane (12 mL) was added followed by a solution of ester **17** (311 mg, 0.377 mmol) in 1,2-dichloroethane (4 mL). The mixture was stirred at 90 °C (oil bath) for 8 h and at room temperature for 30 min. Water (10 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 once extracted with chloroform (5 mL), and the combined extracts were dried (sodium sulfate). The crude product (290 mg) obtained after evaporation of the solvent *in vacuo* was crystallized from an acetone-chloroform mixture to afford the first crop (135 mg). The mother liquors were concentrated, and the residue (150 mg) was flash chromatographed on silica gel (15 g). Elution with chloroform-THF-97% formic acid (300:15:1) afforded another 126 mg of pure diacid (overall yield 90%): mp 265–267 °C; IR (KBr) 3500–2500, 2925, 2852, 1664, 1606, 1444, 1378, 1231, 1179, 899, 798 cm⁻¹; ¹H NMR (acetone-*d*₆, 500 MHz) δ 7.70 (d, J = 2.5 Hz, 1 H), 7.68 (d, J = 2.5 Hz, 1 H), 7.58 (d, J = 2.5 Hz, 1 H), 7.50 (d, J = 2.5 Hz, 1 H), 6.18 (t, J = 7.5 Hz, 1 H), 2.17 (m, 2 H), 0.92 (d, J = 6.5 Hz, 3 H), 0.86 (dd, J = 6.5, 2.0 Hz, 6 H), 0.74 (s, 3 H), 0.66 (s, 3 H); ¹³C NMR (acetone-*d*₆, 126 MHz) δ 172.18, 157.78, 157.61, 137.93, 137.70, 134.90, 134.82, 132.68, 131.47, 130.99, 128.13, 122.47, 122.39, 114.62, 114.29, 57.43, 57.15, 55.55, 47.35, 43.35, 40.97, 40.24, 39.39, 38.12, 37.91, 36.95, 36.81, 36.62, 36.39, 36.29, 32.89, 30.10, 29.75, 28.95, 28.70, 27.81, 24.87, 24.54, 23.10, 22.85, 21.75, 19.12, 12.70, 12.47; FABMS m/e (relative intensity) 767 (MH⁺, 10), 749 (13). Anal. (C₄₅H₆₀Cl₂O₆) C, H. The ammonium salt was prepared by dissolving the diacid in ammonia (1 mL) and evaporation of the solution *in vacuo*.

5 α -3 α -(3'-Hydroxypropyl)cholestane (20). Crude **13** (9.900 g, 18.17 mmol) was dissolved in THF (80 mL), and a 1 M solution of tetrabutylammonium fluoride in THF (38.5 mL) was added. The solution was stirred at ambient temperature for 1 h. THF was removed *in vacuo*, brine (100 mL) was added, and the mixture was extracted with a benzene-hexane 1/1 mixture (3 × 50 mL). The combined extracts were washed with brine (5 × 100 mL) and dried (sodium sulfate), and the product obtained after evaporation of the solvent was dissolved in a small amount of absolute ethanol and slowly crystallized at room temperature. The crystallization was completed in the refrigerator to afford the pure **14** (4.48 g). The mother liquors were concentrated, and the obtained oil (4.10 g) was flash chromatographed on silica gel (400 g) eluting with a

hexane-ethyl acetate (6:1) mixture to afford **20** (0.841 g) and more of 3 β -alcohol **14** (1.543 g): mp 114 °C (ethanol); ¹H NMR (CDCl₃, 500 MHz) δ 3.62 (t, J = 6.8 Hz, 2 H), 1.93 (dt, J = 12, 3.3 Hz, 2 H), 0.87 (d, J = 6.9 Hz, 3 H), 0.835 (dd, J = 6.7, 2.5 Hz, 6 H), 0.76 (s, 3 H), 0.615 (s, 3 H); ¹³H NMR (CDCl₃, 126 MHz) δ 63.43, 56.65, 56.28, 54.74, 42.62, 40.33, 40.12, 39.54, 36.47, 36.20, 35.84, 35.55, 33.25, 32.96, 32.95, 32.17, 31.45, 29.05, 28.29, 28.05, 28.02, 25.55, 24.22, 23.89, 22.87, 22.61, 20.82, 18.71, 12.13, 11.89; CIMS m/e (relative intensity) 430 (M⁺, 55), 413 (MH⁺ - H₂O, 100). Anal. (C₃₀H₅₄Cl₂O₆) C, H.

Cell Lines and Viruses. The CEM-SS lymphocyte cell line was obtained from Peter Nara.⁴⁰ H9 cells chronically infected with HIV-1_{IIIB} were obtained from Makoto Matsukura. MT-4 and MT-2 cells were obtained from the NIAID AIDS Research and Reference Reagent Program, a contribution of Douglas Richman. All cells were maintained in RPMI 1640 medium without phenol red and supplemented with 5% fetal bovine serum, 2 mM L-glutamine, and 50 μ g of gentamycin/mL (complete medium).

The HIV-1 isolates included the common laboratory HIV-1 strains (RF, III_B, LAV, MN, and others), as well as a panel of HIV-1 clinical isolates obtained from patients at The University of Alabama at Birmingham Children's Hospital. The biological and biochemical properties of these isolates have been described previously.^{41,42} The HIV-2 isolate MS⁴³ and the AZT-resistant HIV-1 isolate G910-6⁴⁴ were obtained from the NIAID AIDS Research and Reference Program, and the pyridone-resistant HIV-1 A17 isolate⁴⁵ was obtained from Emilio Emimi at Merck Sharpe and Dohme Laboratories.

The initial anti-HIV screening was performed as previously described.⁴⁶ This microtiter assay quantitates drug-induced protection from the killing of CD4(+) lymphoid cells by HIV-1_{RF}. Briefly, cosalane or 3'-azido-3'-deoxythymidine (AZT, NSC 602670), used as a reference compound, was serially diluted in complete medium and added to 96-well test plates. Exponentially growing target cells were pelleted, suspended in complete medium, and added at 5000 cells/well. Frozen virus stock solutions were thawed immediately before use, suspended in complete medium to yield the desired multiplicity of infection (MOI = 0.01), and added to the microtiter wells. Test plates were incubated at 37 °C in 5% CO₂ for 6 days. On day 6, aliquots of cell-free supernatant were removed from each well and analyzed for reverse transcriptase (RT) activity, p24 antigen, and/or infectious virions. Cellular growth or viability was estimated with the remaining contents of each well by the XTT assay.⁴⁷ Effective antiviral concentrations (EC₅₀) and cellular growth inhibitory concentrations (IC₅₀) were calculated.

Chronically infected cell lines (H9-HIV-1_{IIIB}) were obtained from the outgrowth of HIV-infected, virus-producing cells following acute infection of H9 cells. These cells were cultured in the presence of serial, one-log dilutions of cosalane. Cell-free supernatant samples were collected daily and analyzed for virus content by RT assay, p24 ELISA, and CEM-SS infectivity assays as described above.

Phytohemagglutinin-stimulated human peripheral blood lymphocytes (PBLs) were prepared according to the method of Saag *et al.*¹⁷ Antiviral assays in PBLs were performed as described previously.⁴⁸ Culture supernatants were assayed for HIV RT and/or p24 by an antigen capture ELISA as described by the manufacturer (Coulter Immunology, Hialeah, FL). Fresh monocyte-macrophage cultures were prepared from blood of uninfected donors (American Red Cross, MD). The adherent cells were incubated for 3 days in complete medium and then washed twice with Hank's balanced salt solution. The cultures were treated with various concentrations of antiviral compound for 1 h prior to infection with the Ba-L strain of HIV-1 (MOI = 0.5). Following 7 days in culture at 37 °C in complete medium, the supernatant levels of p24 were determined.

Time Course Experiments. In order to identify the stage(s) of HIV infection affected by cosalane, the compound was evaluated in a high-MOI acute-phase time of addition assay. CEM-SS cells (10⁵) were preincubated with HIV_{IIIB} (MOI = 1.0) at 0–4 °C for 1 h to allow attachment of virus to cells but not fusion or reverse transcription. Samples were then washed

three times with ice cold media to remove unbound virus, after which the samples were rapidly warmed to 37 °C (at time zero, t_0), allowing the infectious cycle to proceed. Cosalane (50 μ M) was included during the preincubation step only (pre), included during the preincubation step and then added back at t_0 (pre/ t_0) following removal of residual virus, or added to samples only at t_0 or at various times after warming to 37 °C ($t = 0.5, 1, 2$, or 4 h postwarming). Dextran sulfate (100 μ g/mL, NSC 620255) and ddC (10 μ M, NSC 606170) served as controls for inhibitors of virus attachment and reverse transcriptase, respectively. After a 24 h incubation, the cells were collected by centrifugation, lysed in QuickLyse buffer (10 mM Tris, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 0.1 mg/mL gelatin, 0.45% Nonidet P-40, 0.45% Tween-20) containing 100 μ g/mL proteinase K, incubated at 56 °C for 2 h, and boiled for 20 min. Products of viral reverse transcription were amplified by PCR using LTR/gag primer pairs (M667/M661; Recombinant DNA Laboratory, PRI/DynCorp., NCI-FCRDC, Frederick, MD) and the cellular β -globin primer pairs.⁴⁹ Amplified products were analyzed by electrophoresis in 2% agarose gels and visualized by ethidium bromide staining. The specificity of products was verified by restriction enzyme cleavage and Southern blot hybridization.

To determine the reversibility of the antiviral action of cosalane, limited-treatment experiments were performed. CEM-SS cells were pelleted and infected in bulk with HIV-1_{RF} in complete media with or without cosalane (10 μ M). The infection proceeded for 45 min at 22 °C with constant agitation. After infection, cells were suspended in media with or without 10 μ M cosalane. After intervals of 0, 4, 8, 24, 48, or 72 h, aliquots of cells were removed, washed free of cosalane, and plated into individual wells of a 96-well microtiter plate containing media with or without 10 μ M cosalane. Plates were then incubated for 6 days from the time of initial infection, and cellular viability was assessed by the XTT assay.

Virus Attachment and Fusion Assays. Binding of HIV-1_{RF} to PBLs was measured by a p24-based assay.⁴⁸ Briefly, 5×10^5 PBLs were incubated with a concentrated stock of virus for 30 min at 37 °C, the unbound virus was washed away, and the cell-associated virus was solubilized in 1% Triton X-100, 1% BSA and analyzed by the p24 antigen capture assay as previously described.

HeLa CD4+ cells harboring a β -galactosidase gene attached to the HIV-1 promoter were used to quantitate the binding of infectious virus to cells, as well as to study HIV-specific membrane fusion interactions. For infectivity determinations, cells in microtiter wells were infected with HIV-1 (III_B) and incubated for 48 h. Successfully infected cells expressed *tat* protein, which activated the expression of β -galactosidase in this system. Foci of cells expressing β -galactosidase can be observed microscopically after staining with the reagent X-gal. HeLa cells (HL 2/3) expressing HIV-1 *env* protein on the cell surface, as well as *tat* protein in the cytoplasm, can fuse with the CD4+/ β -gal cells, activating β -galactosidase in the syncytium. Effects of cosalane on infectivity and fusion interactions were examined by exposing the appropriate cells to cosalane before mixing with virus or the HL 2/3 cells.

Binding and Enzymatic Assays. The binding of gp120 to CD4 was analyzed using an antigen capture ELISA (DuPont). All steps of the assay were carried out according to the manufacturer's protocols. The effects of drugs on the *in vitro* activity of purified RT was determined by measurement of incorporation of [³H]TTP onto the artificial poly(rA):oligo(dT) homopolymer primer/template. Samples (5 μ L) were blotted onto DE81 paper, washed with 5% dibasic sodium phosphate as previously described,¹⁶ and then quantitated on a Packard Matrix 9600 direct beat counter. 3'-Azido-3'-deoxythymidine-5'-triphosphate served as a positive control for inhibition of RT. HIV-1 protease activity was quantitated by a reversed 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.^{48,50} Binding and enzymatic assays were performed with three separate preparations of cosalane, and the concentration of drug providing 50% inhibition (ID₅₀) in each assay was calculated.

Combination Antiviral Analysis. Analysis of drug com-

bination assay was performed utilizing the XTT assay described above, with statistical evaluations performed according to the method of Prichard and Shipman.⁵¹ Combination antiviral XTT assays were performed with CEM-SS cells utilizing HIV-1 (III_B). The standard anti-HIV assay was altered for combination analysis by increasing the multiplicity of infection 3-fold, allowing greater statistical consistency in these assays.

Acknowledgment. We are grateful to Ming Bu and Catherine A. Schaeffer for their helpful discussions and their performance of various laboratory procedures and to Judy Duears for preparation of the manuscript and figures. This work was supported by the National Cancer Institute under contract N01-CO-74102 with PRI/DynCorp., under contract N01-CM-37818 to Southern Research Institute, and under contract N01-CM-17513 to Purdue Research Foundation. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

References

- (1) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Nisnoff Lehrman, S.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. 3'-Azido-3'-deoxythymidine (BW A509U): An antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096-7100.
- (2) Yarchoan, R.; Klecker, R. W.; Weinhold, K. J.; Markham, P. D.; Lyerly, H. K.; Durak, D. T.; Gelmann, E.; Nisnoff-Lehrman, S.; Blum, R. M.; Barry, D. W.; Shearer, G. M.; Fischl, M. A.; Myers, C. M.; Broder, S. Administration of 3'-Azido-3'-deoxythymidine, an Inhibitor of HTLV-III/LAV Replication, to Patients with AIDS or AIDS-related Complex. *Lancet* **1986**, 575-580.
- (3) Fischl, M. A.; Richman, D. D.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Schooley, R. T.; Jackson, G. G.; Durack, D. T.; King, D. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. *N. Engl. J. Med.* **1987**, *317*, 185-191.
- (4) Yarchoan, R.; Thomas, R. V.; Allain, J.-P.; McAtee, N.; Dubinsky, R.; Mitsuya, H.; Lawley, T. J.; Safai, B.; Myers, C. E.; Perno, C. F.; Klecker, R. W.; Wills, R. J.; Fischl, M. A.; McNeely, M. C.; Pluda, J. M.; Leuther, M.; Collins, J. M.; Broder, S. Phase I studies of 2',3'-dideoxycytidine in severe human immunodeficiency virus infection as a single agent and alternating with AZT. *Lancet* **1988**, *1*, 76-81.
- (5) Yarchoan, R.; Mitsuya, H.; Thomas, R. V.; Pluda, J. M.; Hartman, N. R.; Perno, C.-F.; Marczyk, K. S.; Allain, J.-P.; Johns, D. G.; Broder, S. *In vivo* activity against HIV and favorable toxicity profile of 2',3'-dideoxyinosine. *Science* **1989**, *245*, 412-415.
- (6) Ahluwalia, G.; Conney, D. A.; Mitsuya, H.; Fridland, A.; Flora, K. P.; Hao, Z.; Dalal, M.; Broder, S.; Johns, D. G. Initial Studies on the Cellular Pharmacology of 2,3-Dideoxyinosine, an Inhibitor of HIV Infectivity. *Biochem. Pharmacol.* **1987**, *36*, 3797-3800.
- (7) Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S.; Jackson, G. G.; Durack, D. T.; Nisnoff-Lehrman, S. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. *N. Engl. J. Med.* **1987**, *317*, 192-197.
- (8) Cooper, J. A.; Gould, K. L.; Cartwright, C. A.; Hunter, T. Tyr527 in regulatory domain of pp60^{src} is phosphorylated. *Science* **1986**, *231*, 1431-1434.
- (9) St. Clair, M. H.; Martin, J. L.; Tudor-Williams, G.; Bach, M. C.; Vavro, C. L.; King, D. M.; Kellam, P.; Kemp, S. D.; Larder, B. A. Resistance to ddI and Sensitivity to AZT Induced by a Mutation in HIV-1 Reverse Transcriptase. *Science* **1991**, *245*, 1557-1559.
- (10) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. Potent and Selective Inhibition of HIV-1 Replication *In vitro* by Novel Series of TIBO Derivatives. *Nature* **1990**, *343*, 470.

- (11) Merluzzi, V. J.; Hargrave, K. D.; Labidia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih, C. K.; Eckner, K.; Hattox, S.; Adams, J.; Roshthal, A. S.; Faanes, R.; Eckner, R. J.; Koup, R. A.; Sullivan, J. L. Inhibition of HIV-1 Replication by a Non-nucleoside Reverse Transcriptase Inhibitor. *Science* **1990**, *250*, 1411–1413.
- (12) Goldman, M. E.; Nunberg, J. H.; O'Brien, J. A.; Quintero, J. C.; Schleif, W. A.; Freund, K. F.; Gaul, S. L.; Saari, W. S.; Wai, J. S.; Hoffman, J. M.; Anderson, P. S.; Hupe, D. J.; Emini, E. A.; Stern, A. M. Pyridone Derivatives: Specific Human Immunodeficiency Virus Type 1 Reverse Transcriptase Inhibitors with Antiviral Activity. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6863–6867.
- (13) Romero, D. L.; Busso, M.; Tan, C. K.; Reusser, F.; Palmer, J. R.; Poppe, S. M.; Aristoff, P. A.; Downey, K. M.; So, A. G.; Resnick, L.; Tarpley, W. G. Nonnucleoside Reverse Transcriptase Inhibitors that Potently and Specifically Block Human Immunodeficiency Virus Type 1 Replication. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8806–8810.
- (14) Klunder, J. M.; Hargrave, K. D.; West, M.; Cullen, E.; Pal, K.; Behnke, M. L.; Kapadia, S. R.; McNeil, D. W.; Wu, J. C.; Chow, G. C.; Adams, J. Novel Non-nucleoside Inhibitors of HIV-1 Reverse Transcriptase. 2. Tricyclic Pyridobenzoxazepinones and Dibenzoxazepinones. *J. Med. Chem.* **1992**, *35*, 1887–1897.
- (15) McMahon, J. B.; Gulakowski, R. J.; Weislow, O. S.; Schultz, R. J.; Narayanan, V. L.; Clanton, D. J.; Pedemonte, R.; Wassmundt, F. W.; Buckheit, R. W.; Decker, W. D.; White, E. L.; Bader, J. P.; Boyd, M. Diarylsulfones, a New Class of Nonnucleoside Antiviral Inhibitors of Human Immunodeficiency Virus Type 1 Reverse Transcriptase. *Antimicrob. Agents Chemother.* **1993**, *37*, 754–760.
- (16) Buckheit, R. W., Jr.; Hollingshead, M. G.; Germany-Decker, J.; White, E. L.; McMahon, J. B.; Allen, L. B.; Roxx, L. J.; Decker, W. D.; Westbrook, L.; Shannon, W. M.; Bader, J. P.; Boyd, M. R. Thiazolobenzimidazole: Biological and Biochemical Antiretroviral Activity of a New Nonnucleoside Reverse Transcriptase Inhibitor. *Antiviral Res.* **1993**, *21*, 247–265.
- (17) Saag, M. S.; Emini, E. A.; Laskin, O. L.; Douglas, J.; Lapidus, W. I.; Schleif, W. A.; Whitley, R. J.; Hildebrand, S.; Byrnes, V. W.; Kappas, J. C.; Anderson, K. W.; Massari, F. E.; Shaw, G. M. A Short-term Clinical Evaluation of 1-697,661, a Non-nucleoside Inhibitor of HIV-1 Reverse Transcriptase. *N. Engl. J. Med.* **1993**, *329*, 1065–1072.
- (18) Balzarini, J.; Mitsuya, H.; De Clercq, E.; Broder, S. Aurintricarboxylic Acid and Evans Blue Represent Two Different Classes of Anionic Compounds which Selectively Inhibit the Cytopathicity of Human T-Cell Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus. *Biochem. Biophys. Res. Commun.* **1986**, *136*, 64–71.
- (19) Baba, M.; Schols, D.; Pauwels, R.; Balzarini, J.; De Clercq, E. Fuchsin Acid Selectively Inhibits Human Immunodeficiency Virus (HIV) Replication *In Vitro* Biochem. Biophys. Res. Commun. **1988**, *155*, 1404–1411.
- (20) Cushman, M.; Wang, P.; Chang, S. H.; Wild, C.; De Clercq, E.; Schols, D.; Goldman, M. E.; Bowen, J. A. Preparation and Anti-HIV Activities of Aurintricarboxylic Acid Fractions and Analogues: Direct Correlation of Antiviral Potency with Molecular Weight. *J. Med. Chem.* **1991**, *34*, 329–337.
- (21) 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.
- (22) Wang, P.; Kozlowski, J.; Cushman, M. Isolation and Structure Elucidation of Low Molecular Weight Components of Aurintricarboxylic Acid (ATA). *J. Org. Chem.* **1992**, *57*, 3861–3866.
- (23) Cushman, M.; Wang, P.; Stowell, J. G.; Schols, D.; De Clercq, E. Structural Investigation and Anti-HIV Activities of High Molecular Weight ATA Polymers. *J. Org. Chem.* **1992**, *57*, 7241–7248.
- (24) Cushman, M.; Kanamathareddy, S. Synthesis of the Covalent Hydrate of the Incorrectly Assumed Structure of Aurintricarboxylic Acid (ATA). *Tetrahedron* **1990**, *46*, 1491–1498.
- (25) Golebiewski, M.; Bader, J. P.; Cushman, M. Design and Synthesis of Cosalane, a Novel Anti-HIV Agent. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1739–1742.
- (26) Goldberg, A. A. Hydrolysis of Substituted *o*-Chlorobenzoic Acids. The Mechanism of the Reaction between *o*-Halogenobenzoic Acids and Nucleophilic Reagents. *J. Chem. Soc.* **1952**, 4368–4373.
- (27) Kirk, D. N.; Mullins, R. J. Optical Rotatory Dispersion and Circular Dichroism. Part LXXX. Circular Dichroism of the Ethylidenecyclohexane System. *J. Chem. Soc., Perkin Trans. I* **1974**, 14–18.
- (28) Wittig, G.; Eggers, H.; Duffner, P. Benzocycloheptatriene, Benzocyclooctatriene und Benzocyclooctatetraene. *Justus Liebigs Ann. Chem.* **1958**, *619*, 10–27.
- (29) Grayson, J. L.; Warren, S. J. Acyl Anion Equivalents: Synthesis of Ketones and Enones from α -Phenylthioalkylphosphine Oxides. *J. Chem. Soc., Perkin Trans. I* **1977**, 2263–2272.
- (30) Bestmann, H. J.; Kratzer, O. Olefins and Ketones by Autooxidation of Alkenylphosphines. *Chem. Ber.* **1963**, *96*, 1899–1908.
- (31) Grant, D. M.; Cheney, B. V. Carbon-13 Magnetic Resonance. VII. Steric Perturbation of the Carbon-13 Chemical Shift. *J. Am. Chem. Soc.* **1967**, *89*, 5315–5318.
- (32) Dalling, D. K.; Grant, D. M. Carbon-13 Magnetic Resonance. IX. The Methylcyclohexanes. *J. Am. Chem. Soc.* **1967**, *89*, 6612–6622.
- (33) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*, 3rd ed.; Verlag Chemie: Weinheim, 1987; pp 316.
- (34) Rice, W. G.; Schaeffer, C. A.; Harten, B.; Villinger, F.; South, T. L.; Summers, M. F.; Henderson, L. E.; Bess, J. W., Jr.; Arthur, L. O.; McDougal, J. S.; Orloff, S. L.; Mendeleyev, J.; Kun, E. Inhibition of HIV-1 Infectivity by Zinc-ejecting Aromatic C-Nitroso Compounds. *Nature* **1993**, *361*, 473–475.
- (35) Abrams, D. I.; Kuno, S.; Wong, R.; Jeffords, K.; Nash, M.; Molaghan, J. B.; Gorter, R.; Ueno, R. Oral Dextran Sulfate (UA001) in the Treatment of the Acquired Immunodeficiency Syndrome (AIDS) and AIDS-related Complex. *Ann. Intern. Med.* **1989**, *110*, 183–188.
- (36) Lorentsen, K. J.; Hendrix, C. W.; Collins, J. M.; Kornhauser, D. M.; Petty, B. G.; Klecker, R. W.; Flexner, C.; Eckel, R. H.; Lietman, P. S. Dextran Sulfate is Poorly Absorbed After Oral Administration. *Ann. Intern. Med.* **1989**, *111*, 561–566.
- (37) Foster, B. C.; Gallicano, K. D.; Whitehouse, L. W.; McGilveray, I. J.; Khan, S. R. Dextran Sulfate Disposition in the Rat. *Biopharm. Drug Dispos.* **1990**, *1990*, 595–606.
- (38) Hartman, N. R.; Johns, D. G.; Mitsuya, H. Pharmacokinetic Analysis of Dextran Sulfate in Rats as Pertains to its Clinical Usefulness for Therapy of HIV Infection. *AIDS Res. Hum. Retroviruses* **1990**, *6*, 805–812.
- (39) Mathes, L. E.; Hayes, K. A.; Swenson, C. L.; Polas, P. J.; Weisbrode, S. E.; Kociba, G. J. Evaluation of Antiviral Activity and Toxicity of Dextran Sulfate in Feline Leukemia Virus-infected Cats. *Antimicrob. Agents Chemother.* **1992**, *35*, 2147–2150.
- (40) Nara, P. L.; Fischinger, P. J. Quantitative Infectivity Assay for HIV-1 and HIV-2. *Nature* **1988**, *332*, 468–470.
- (41) Cloyd, M. W.; Moore, B. E. Spectrum of Biological Properties of Human Immunodeficiency Virus (HIV-1) Isolates. *Virology* **1989**, *174*, 103–116.
- (42) Buckheit, R. W., Jr.; Swanstrom, R. I. HIV-Heterogeneity: Characterization of HIV-1 Isolates Displaying an Apparent Absence of Virion-associated Reverse Transcriptase Activity. *AIDS Res. Hum. Retroviruses* **1991**, *7*, 295–302.
- (43) Kanki, P.; Barin, F.; Essex, M. Proceedings of the Fourth International Conference of AIDS, Stockholm, Sweden; Abstract No. 1659.
- (44) Larder, B. A.; Darby, G.; Richman, D. D. HIV with Reduced Sensitivity to Zidovudine (AZT) Isolated During Prolonged Therapy. *Science* **1989**, *243*, 1731–1734.
- (45) Nunberg, J. H.; Schleif, W. A.; Boots, F. J.; O'Brien, J. A.; Quintero, S. C.; Hoffman, J. M.; Emini, B. A.; Goldman, M. E. Viral Resistance to Human Immunodeficiency Virus Type 1-Specific Pyridinone Reverse Transcriptase Inhibitors. *J. Virol.* **1991**, *65*, 4887–4892.
- (46) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. New Soluble-formazan Assay for HIV-1 Cytopathic Effects: Application to High-flux Screening of Synthetic and Natural Products for AIDS-antiviral Activity. *J. Natl. Cancer Inst.* **1989**, *81*, 577–586.
- (47) Gulakowski, R. J.; McMahon, J. B.; Staley, P. G.; Moran, R. A.; Boyd, M. R. A Semiautomated Multiparameter Approach for anti-HIV Drug Screening. *J. Virol. Methods* **1991**, *33*, 87–100.
- (48) Rice, W. G.; Schaeffer, C. A.; Graham, L.; Bu, M.; McDougal, J. S.; Orloff, S. L.; Villiger, F.; Young, M.; Oroszlan, S.; Fesen, M. R.; Pommier, Y.; Mendelev, J.; Kun, E. The Site of Action of 3-Nitrosobenzamide on the Infectivity Process of Human Immunodeficiency Virus in Human Lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9721–9724.
- (49) Zack, J. A.; Arrigo, S. J.; Weitsman, S. R.; Go, A. S.; Haislip, A.; Chen, I. S. Y. HIV-1 Entry into Quiescent Primary Lymphocyte: Molecular Analysis Reveals a Labile, Latent Viral Structure. *Cell* **1990**, *61*, 213–222.
- (50) Wondrack, E. M.; Louis, J. M.; Mora, P. T.; Oroszlan, S. Purification of HIV-1 Wild-type Protease and Characterization of Proteolytically Inactive HIV-1 Protease Mutants by Pepstatin A Affinity Chromatography. *FEBS Lett.* **1991**, *280*, 347–350.
- (51) Prichard, M.; Shipman, C. J. A Three-dimensional Model to Analyze Drug-drug Interactions. *Antiviral Res.* **1990**, *14*, 181–206.