Correlation of Anti-HIV Activity with Anion Spacing in a Series of Cosalane Analogues with Extended Polycarboxylate Pharmacophores

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Cosalane and its synthetic derivatives inhibit the binding of gp120 to CD4 as well as the fusion of the viral envelope with the cell membrane. The binding of the cosalanes to CD4 is proposed to involve ionic interactions of the negatively charged carboxylates of the ligands with positively charged arginine and lysine amino acid side chains of the protein. To investigate the effect of anion spacing on anti-HIV activity in the cosalane system, a series of cosalane tetracarboxylates was synthesized in which the two proximal and two distal carboxylates are separated by 6−12 atoms. Maximum activity was observed when the proximal and distal carboxylates are separated by 8 atoms. In a series of cosalane amino acid derivatives containing glutamic acid, glycine, aspartic acid, β-alanine, leucine, and phenylalanine residues, maximum activity was displayed by the di(glutamic acid) analogue. A hypothetical model has been devised for the binding of the cosalane di(glutamic acid) conjugate to CD4. In general, the compounds in this series are more potent against HIV-1$_{RF}$ in CEM-SS cells than they are vs HIV-1$_{IIIB}$ in MT-4 cells, and they are least potent vs HIV-2$_{ROD}$ in MT-4 cells.

Introduction

The currently available chemotherapeutic agents for the treatment of AIDS include six nucleoside reverse transcriptase inhibitors (AZT, ddI, ddC, 3TC, d4T, and ABC), three nonnucleoside reverse transcriptase inhibitors (nevirapine, delavirdine, and efavirenz), and five protease inhibitors (saquinavir, ritonavir, indinavir, nelfinavir, and amprenavir). The recent trend toward early and aggressive intervention with combination chemotherapy has resulted in a significant decrease in the rate of disease progression in AIDS patients.[1−4] However, major problems remain in the chemotherapy of AIDS, including viral resistance and drug toxicity. Therefore, interest remains in the development of new anti-HIV agents that target additional steps in the replication of the virus. Low-molecular-weight CD4 ligands that inhibit the binding of the viral surface glycoprotein (gp120) to its cellular receptor (CD4) might circumvent the usual problems with the emergence of resistant viral strains because CD4 is not expected to mutate.

Cosalane (**1**), which was derived conceptually by attaching a membrane-interactive steroid to a dichlorinated disalicylmethane fragment of aurintricarboxylic acid (ATA, schematic representation shown in structure **2**), binds to both gp120 and CD4.[5,6] It inhibits both the attachment of gp120 to CD4 as well as an undefined postattachment event prior to reverse transcription.[7] Attempts to improve the potency of cosalane (EC$_{50}$: 5.1

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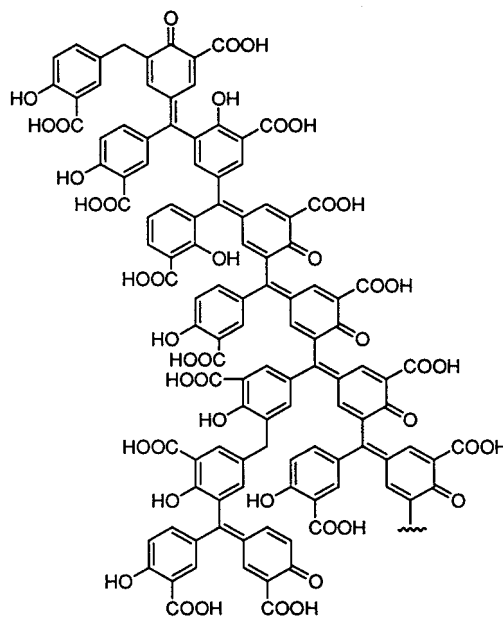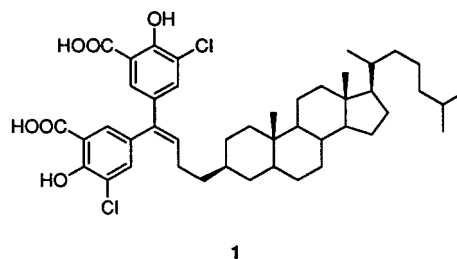
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μM vs HIV-1$_{RF}$ in CEM-SS cells) have resulted in several series of cosalane analogues, including those derived from attachment of the disalicylmethane pharmacophore to various regions of the steroid[8] and from modification of the linker chain.[9-11]

The available evidence indicates that the disalicylmethane moiety of cosalane (**1**) is the "pharmacophore" and that the steroid simply functions as a lipophilic accessory appendage. A hypothetical model for the binding of the cosalane "pharmacophore" to CD4 has been proposed.[12] According to this model, the two carboxylate groups of the disalicylmethane moiety of cosalane bind electrostatically to the Arg58 and Arg59 side chains of the protein. Inspection of the structure of the surrounding region[13,14] reveals several additional basic functional groups (e.g. the side chains of Lys46 and Lys72) that would be positively charged at physiological pH and could possibly bind to additional anionic groups that could be attached to the cosalane (**1**) "pharmacophore". An additional line of reasoning in support of the hypothesis that the incorporation of additional anionic groups would increase potency comes from examination of the biological activities of low-molecular-weight ATA components[15] and pharmacophore analogues.[16] Specifically, the activities of **3** and
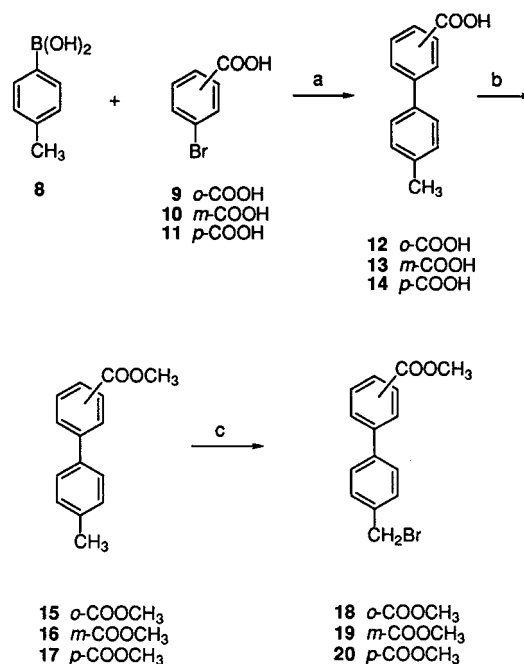
3 **4** **5** **6** **7**
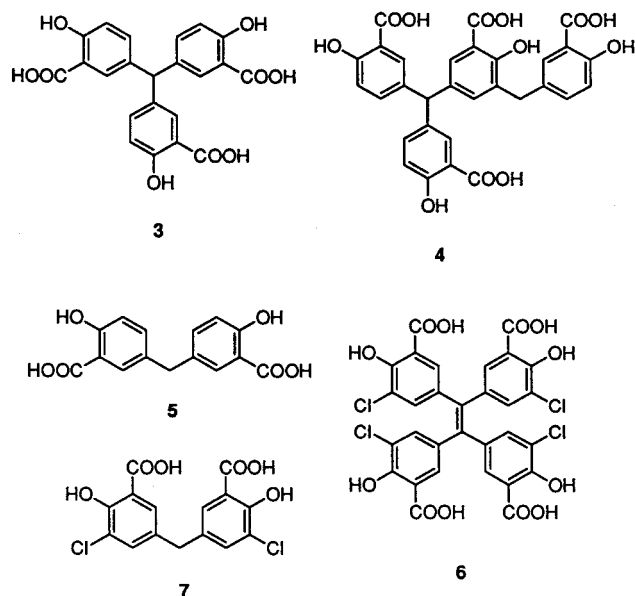
4 are higher than that of **5**, and **6** is more potent than **7**. Recent studies in selected systems have documented several examples in which moderate increases in anti-HIV potency resulted from the attachment of additional substituted benzoic acid rings onto the cosalane "pharmacophore".[17,18] The purpose of the present study was to define exactly how the constellation (spatial arrangement) and the constitution of additional anionic groups attached to the cosalane pharmacophore would affect the anti-HIV activity.

Chemistry

Several substituted biphenyls carrying a benzyl bromide alkylating group in one ring and a methyl ester group in the other were synthesized for attachment to the phenolic hydroxyl groups of cosalane (**1**) (Scheme 1). The tolyl-substituted benzoic acids **12-14** were
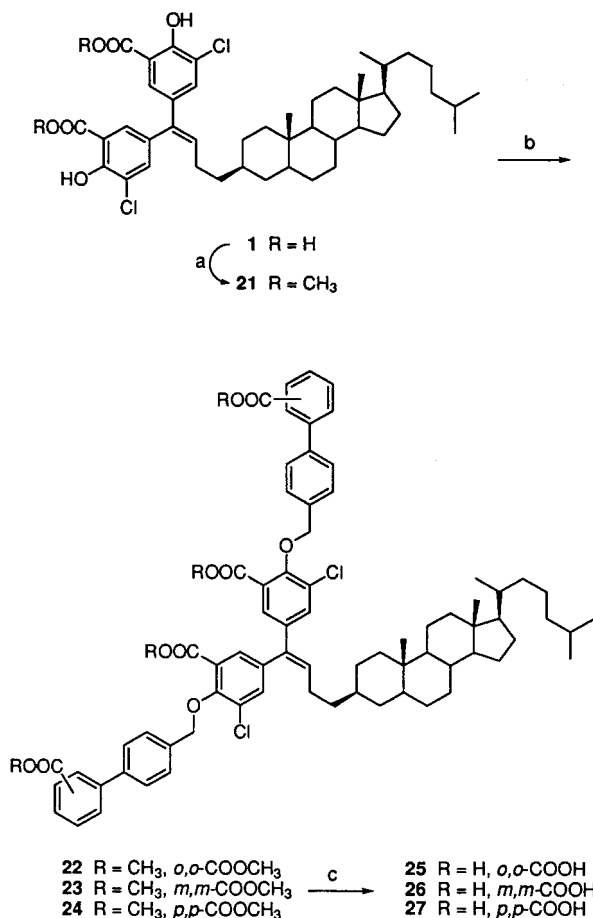
Scheme 1[a]

8

9 *o*-COOH
10 *m*-COOH
11 *p*-COOH

12 *o*-COOH
13 *m*-COOH
14 *p*-COOH

15 *o*-COOCH$_3$
16 *m*-COOCH$_3$
17 *p*-COOCH$_3$

18 *o*-COOCH$_3$
19 *m*-COOCH$_3$
20 *p*-COOCH$_3$

[a] Reagents and conditions: (a) PdCl$_2$, aq NaOH; (b) TMSCHN$_2$ in hexane, benzene and methanol; (c) NBS, (BzO)$_2$, CCl$_4$, heat (for **18**) or heat and irradiation (for **19** and **20**).

prepared by coupling 4-methylbenzeneboronic acid (**8**) with the appropriate *o*-, *m*-, and *p*-bromobenzoic acids **9-11** in the presence of palladium chloride and aqueous sodium hydroxide. The resulting carboxylic acids **12-14** were converted to the corresponding methyl esters **15-17** with (trimethylsilyl)diazomethane.[19] Free radical bromination of **15-17** in the presence of NBS and benzoyl peroxide afforded the desired benzyl bromides **18-20**. The free radical bromination of **16** and **17** to yield **19** and **20** was carried out by irradiation with a 200-W tungsten lamp, but the conversion of **15** to **18** was accomplished without irradiation.

As shown in Scheme 2, the two carboxylic acid groups of cosalane (**1**) were selectively methylated with (trimethylsilyl)diazomethane[19,20] to afford the diester **21**. Treatment of diphenol **21** with potassium carbonate in DMF afforded the diphenoxide dianions, which were alkylated with the benzyl bromides **18-20** to afford **22-24**. For conversion of the methyl esters of **23** and **24** to the acids **26** and **27**, the hydrolyses were performed using potassium carbonate in the presence potassium cyanide in hot aqueous ethanol. However, in the case of **22**, this method gave unsatisfactory yields, so its hydrolysis to afford **25** was performed with sodium hydroxide in a refluxing mixture of methanol and ethanol (5:1).

The synthesis of the tetraacid **30**, in which the two terminal acids are closer to the cosalane nucleus than they are in **25-27**, is shown in Scheme 3. The required benzyl bromide **28** was made by methylation of commercially available 4-(bromomethyl)phenylacetic acid with (trimethylsilyl)diazomethane.[19] Deprotonation of the phenolic hydroxyl groups of **21** with potassium carbonate in DMF afforded the dianion, which was alkylated with **28** to yield intermediate **29**. The four ester groups of **29** were then hydrolyzed with potassium carbonate in the presence of potassium cyanide in aqueous ethanol.

Scheme 2[a]

| 22 R = CH₃, *o,o*-COOCH₃		25 R = H, *o,o*-COOH
23 R = CH₃, *m,m*-COOCH₃	c	26 R = H, *m,m*-COOH
24 R = CH₃, *p,p*-COOCH₃	→	27 R = H, *p,p*-COOH

[a] Reagents and conditions: (a) TMSCHN₂ in hexane, benzene and methanol, 25 °C (25 min); (b) **18**, **19** or **20**, K₂CO₃, DMF; (c) for **25**: aq NaOH, MeOH, EtOH, reflux (14 h); for **26**: KCN, K₂CO₃, aq EtOH, 95 °C (20 h) followed by H₂O, 80 °C (2 h); for **27**: KCN, K₂CO₃, aq EtOH, 90 °C (20 h) followed by H₂O, 80 °C (1 h).

To explore the importance of the geometrical constraint afforded by the alkene double bond in the linker chain connecting the steroid to the "pharmacophore", the hydrogenated product **34** was also synthesized (Scheme 4). Dihydrocosalane[9] was converted to the diester **32** with (trimethylsilyl)diazomethane.[19] Alkylation of the dianion derived from **32** with methyl *p*-bromomethylbenzoate afforded the dialkylated product **33**. Hydrolysis of the four ester groups yielded the tetraacid **34**.

A new series of cosalane derivatives was also made in which the two carboxyl groups of cosalane were linked to amino acid residues through amide bond formation. We felt that the successful implementation of this strategy would allow access to a wide variety of polyanionic compounds which could be used as a tool to observe the effects of anion spacing, as well as the effects of the nearby amino acid side chains, on biological activity. This in fact resulted in a novel array of dicarboxylic acids and tetracarboxylic acids including compounds **36** (derived from glycine), **38** (derived from β-alanine), **40** (derived from leucine), **42** (derived from phenylalanine), **44** (derived from aspartic acid), and **46** (derived from glutamic acid). A further variation in the structure of the glycine derivative **36** and the glutamic acid derivative **46** involved the corresponding dihydro

Scheme 3[a]
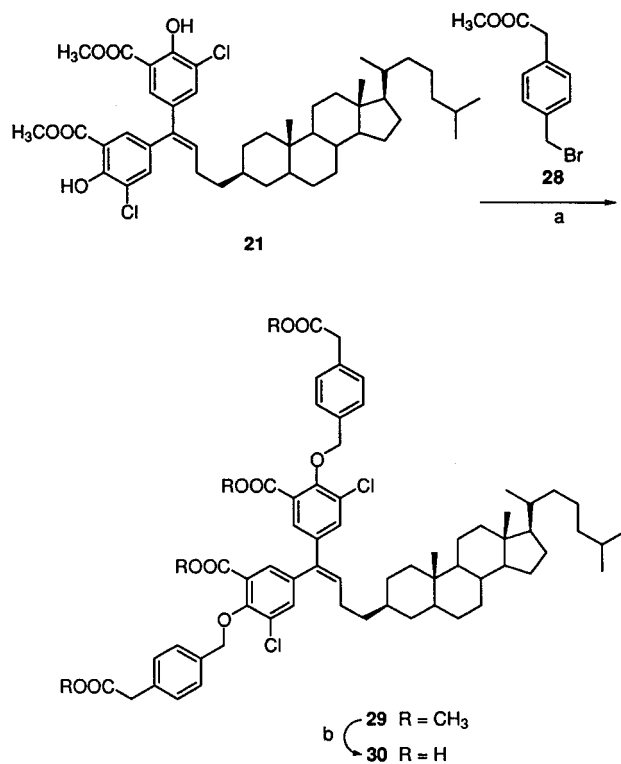
[a] Reagents and conditions: (a) K₂CO₂, DMF, 23 °C (22 h); (b) K₂CO₃, KCN, aq EtOH, 80 °C (14 h), then H₂O, 80 °C (2 h).

Scheme 4[a]
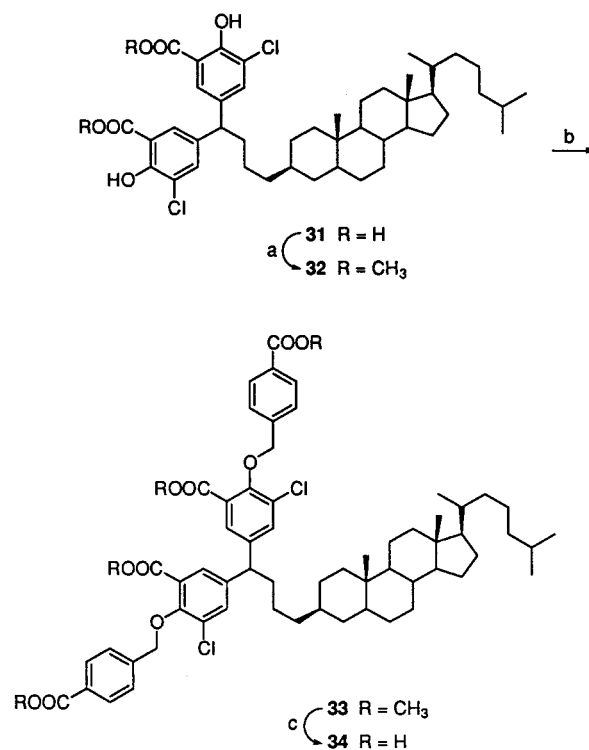


[a] Reagents and conditions: (a) TMSCHN₂ in hexane, benzene and methanol; (b) K₂CO₃, methyl 4-(bromomethyl)phenylacetate, DMF, 23 °C (20 h); (c) K₂CO₃, KCN, aq EtOH, 80 °C (12 h), then H₂O, 80 °C (2 h).
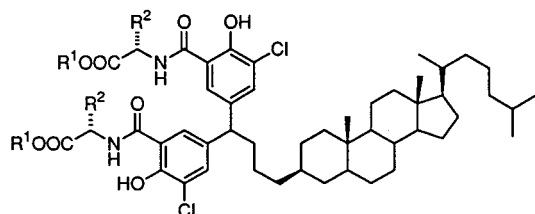
compounds **48** and **50**, in which the alkene in the linker chain connecting the "pharmacophore" to the steroid nucleus of **44** and **46** had been hydrogenated.
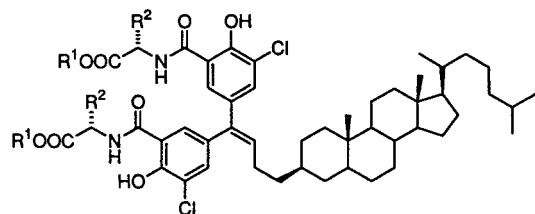
35 R^1 = t-Bu, R^2 = H
36 R^1 = H, R^2 = H
39 R^1 = CH$_3$, R^2 = CH$_2$CH(CH$_3$)$_2$
40 R^1 = H, R^2 = CH$_2$CH(CH$_3$)$_2$
41 R^1 = CH$_3$, R^2 = CH$_2$Ph
42 R^1 = H, R^2 = CH$_2$Ph
43 R^1 = t-Bu, R^2 = CH$_2$COOt-Bu
44 R^1 = H, R^2 = CH$_2$COOH
45 R^1 = t-Bu, R^2 = CH$_2$CH$_2$COOt-Bu
46 R^1 = H, R^2 = CH$_2$CH$_2$COOH
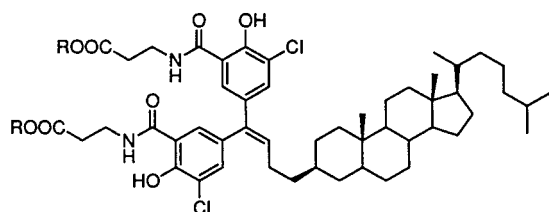
37 R = Et
38 R = H

47 R^1 = t-Bu, R^2 = H
48 R^1 = H, R^2 = H
49 R^1 = t-Bu, R^2 = CH$_2$CH$_2$COOt-Bu
50 R^1 = H, R^2 = CH$_2$CH$_2$COOH

In general, amide bond formation during the coupling of cosalane to the amino acid derivatives was performed by reacting the hydrochloride salts of the amino acids with cosalane or dihydrocosalane in the presence of BOP and triethylamine in THF. The dicarboxylic acids **40** and **42** were synthesized by coupling cosalane with the methyl esters of leucine and phenylalanine to afford the protected intermediates **39** and **41**, respectively. Hydrolysis of the methyl esters of **39** and **41** yielded the desired analogues **40** and **42**. The aspartic acid derivative **44** and the glutamic acid derivative **46** were synthesized by reaction of cosalane with the corresponding di-*tert*-butyl esters of the amino acids, followed by hydrolysis of the four *tert*-butyl ester groups. The dicarboxylic acid **36**, having two glycine residues, was prepared similarly by coupling cosalane to the *tert*-butyl ester of glycine to afford **35**, followed by hydrolysis. The β-alanine derivative **38** was prepared by coupling cosalane to β-alanine ethyl ester, resulting in intermediate **37**, followed by hydrolysis. The dihydro analogues **48** and **50** were prepared by coupling dihydrocosalane (**31**)[9] with the corresponding di-*tert*-butyl esters of glycine and glutamic acid to afford **47** and **49**, followed by deprotection.

Biological Results and Discussion

The new cosalane derivatives were evaluated for inhibition of the cytopathic effect of HIV-1$_{RF}$ in CEM-SS cells and HIV-1$_{IIIB}$ and HIV-2$_{ROD}$ in MT-4 cells. Cytotoxicities in uninfected CEM-SS cells and MT-4 cells were also determined. The results are listed in Table 1.

In general, the new cosalane polycarboxylates synthesized in the present study were more potent against HIV-1$_{RF}$ in CEM-SS cells than they were vs HIV-1$_{IIIB}$ in MT-4 cells. This difference in activity was more pronounced with some of the compounds in the amino acid series (e.g. **36** and **46**) than in the biphenyl series (**25** and **26**) or in the benzyl compound **30**. All new cosalane derivatives were also either inactive or displayed very low antiviral activity against HIV-2$_{ROD}$ in

Table 1. Anti-HIV Activities of Cosalane Analogues

compd	EC$_{50}$^{a} (μM)			CC$_{50}$^{b} (μM)	
	HIV-1$_{RF}$	HIV-1$_{IIIB}$	HIV-2$_{ROD}$	CEM-SS	MT-4
1	5.1	3.0	4.0	>200	>125
25	5.2 ± 0.3	10.1 ± 1.6	>125	>200	>125
26	6.1 ± 0.9	12.0 ± 3.0	>95	>200	79 ± 15
27	>100	>100	>100	>100	100
30	1.8 ± 0.1	4.1 ± 2.6	83.8 ± 30.4	133 ± 21	>125
34^c	4.8 ± 0.6	5.1 ± 2.1	70.4 ± 25.6	>200	>125
35	NT^d	>125	>125	>125	>125
36	3.4 ± 0.4	57.6 ± 10.7	>125	>200	>125
38	34.7 ± 0.3	73.8 ± 3.1	120	>200	>125
40	40.0 ± 2.0	63.4 ± 13.1	>125	>200	>125
42	>200	>62	>62.0	>200	62
44	10.7 ± 1.2	16.6 ± 7.2	122	90 ± 13	>125
46	1.1 ± 0.1	8.9 ± 2.9	69.6 ± 32.0	82 ± 11	>125
48	4.5 ± 1.0	39.4 ± 34	>125	>200	>125
50	11.6 ± 1.0	10.3 ± 3.9	73.0 ± 25.0	34 ± 4	>125
51	39.8 ± 3.2	>37	>37.0	>316	37
52	5.7 ± 0.7	2.2 ± 0.1	>29.0	>316	76 ± 33
53	0.5 ± 0.1	1.7 ± 0.3	22.0	72 ± 14	89 ± 22
54^c	28.6 ± 4.3	8.4	>66.0	47 ± 6	81 ± 17
55^c	2.9 ± 0.1	12.5 ± 6.3	19.9 ± 3.8	50 ± 4	41 ± 3

^{a} Concentration required to reduce the cytopathic effect of the virus by 50%. The HIV-1$_{RF}$ assays were performed in CEM-SS cells, while the HIV-1$_{IIIB}$ and HIV-2$_{ROD}$ assays were carried out in MT-4 cells. ^{b} Concentration required for a 50% reduction in cellular viability of uninfected cells. ^{c} Tested as the tetrasodium salt. ^{d} Not tested.

MT-4 cells. The low activity of the compounds in the present series vs HIV-2$_{ROD}$ is not totally unexpected, because the previously reported polyanions **51**−**55**, also having extended polyanionic pharmacophores, were less active vs HIV-2$_{ROD}$ than they were vs HIV-1$_{RF}$ or HIV-1$_{IIIB}$.[17,18] However, the generally greater potency in the presently reported cosalane derivatives against HIV-1$_{RF}$ in comparison to HIV-1$_{IIIB}$ was not expected, either on the basis of the activity of cosalane (**1**) or in the **51**−**55** series.

51 R = *o*-COOH
52 R = *m*-COOH
53 R = *p*-COOH

54

55
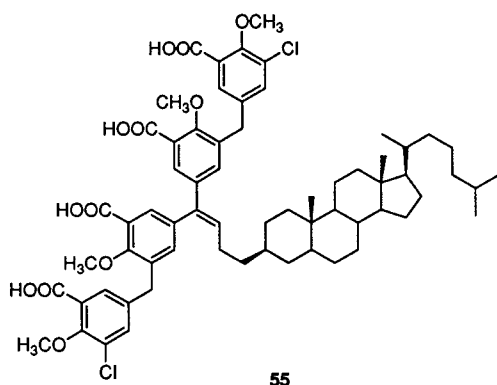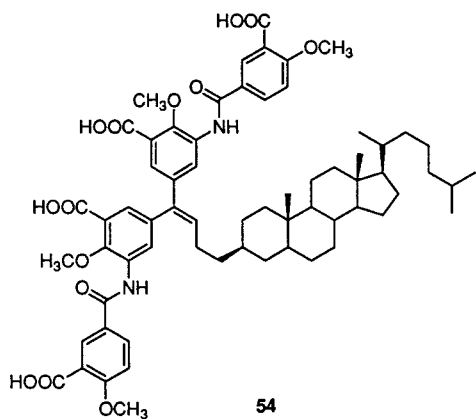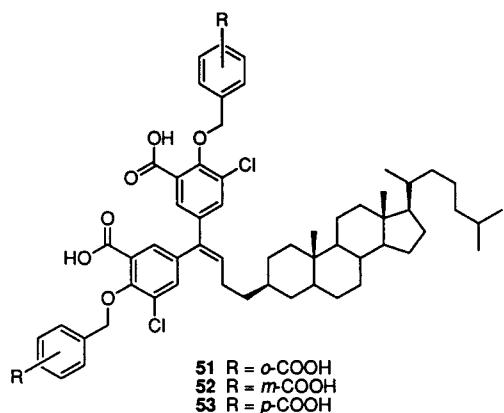
Turning to the biphenyl series of compounds **25**−**27**, the activities of **25** and **26** are very close, but in general, a decline in activity vs both HIV-1 strains is observed as the two terminal carboxyl groups are moved farther away from the internal carboxyl groups, eventually resulting in the inactive compound **27**. This effect is in contrast to the relative activities seen in the benzyl series containing **51**−**53**, in which the activity increased
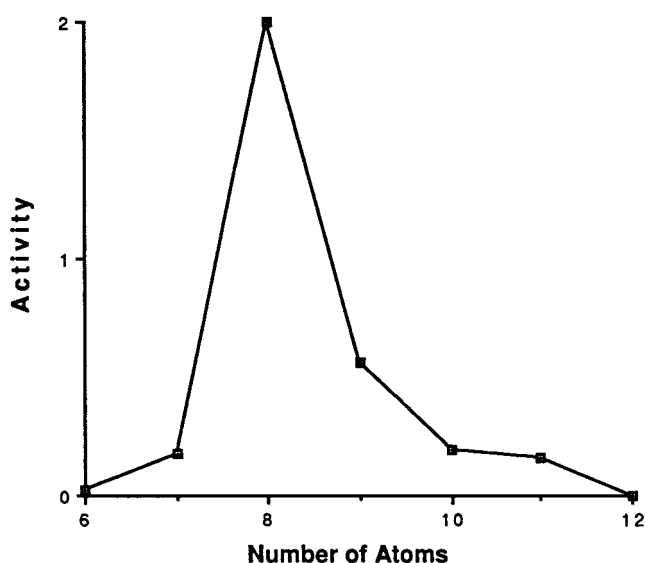

Figure 1. Relationship between anti-HIV activity (1/EC$_{50}$, μM, vs HIV-1$_{RF}$ in CEM-SS cells) and the number of atoms separating the internal and external carboxylates in the series of compounds **51** (6 atoms), **52** (7 atoms), **53** (8 atoms), **30** (9 atoms), **25** (10 atoms), **26** (11 atoms), and **27** (12 atoms).

as the two terminal carboxyl groups are moved farther away from the two internal carboxyl groups. Evidently, there is an optimal distance between the internal and external carboxyl groups that is approximated by the distances in the analogue having structure **53** (Figure 1). Consistent with this hypothesis, the most active of the new compounds presently being reported is **30**, which is slightly less active than **53** but slightly more active than **25**.

The correlation of the distance separating the internal and external carboxyl groups and antiviral activity is apparent for both HIV-1$_{RF}$ in CEM-SS cells (Figure 1) as well as HIV-1$_{IIIB}$ in MT-4 cells, although it is less apparent vs HIV-2$_{ROD}$ in MT-4 cells because of the low activities observed vs HIV-2$_{ROD}$. The optimal spacing of the carboxyl groups may reflect a requirement for interaction with basic amino acid side chains on the surface of the CD4 molecule. A hypothetical model for the binding of **53** to CD4, involving ionic bonding of three of the carboxylates of the ligand with the Arg58, Arg59, and Lys72 side chains of the protein, has been proposed.[17]

Considering the amino acid derivatives **36**, **38**, **40**, **42**, **44**, and **46**, the first hypothesis to be examined was that the two carboxyl groups of cosalane could be sacrificed and activity still maintained as long as two new carboxyl groups were generated in the process. This was first tested by synthesizing the glycine derivative **36**, which proved to be slightly more active than cosalane (**1**) itself when evaluated vs HIV-1$_{RF}$ in CEM-SS cells (EC$_{50}$: 5.1 μM for **1** vs 3.4 μM for **36**). However, the glycine derivative **36** was significantly less active than cosalane (**1**) when tested vs HIV-1$_{IIIB}$ in MT-4 cells (EC$_{50}$: 3.0 μM for **1** vs 57.6 μM for **36**). The requirement for two free carboxyl groups of **36** for activity was apparent by the inactivity of the di-*tert*-butyl ester **35** when evaluated vs HIV-1$_{IIIB}$. The inactivity of the diester **35** is consistent with the importance of the proposed hypothetical ionic binding of the cosalane derivitives to the protein and is in agreement with the general observa-
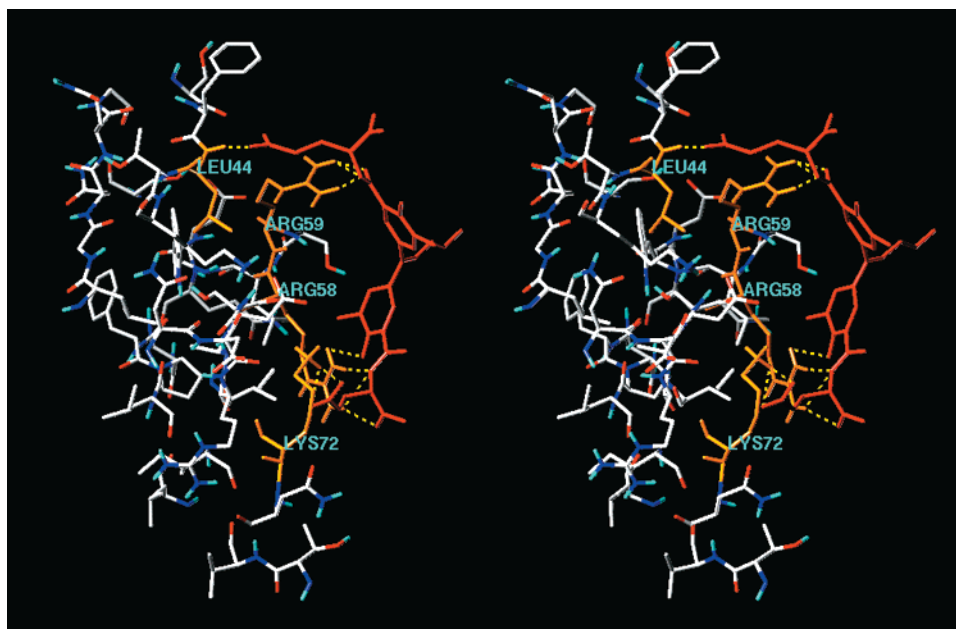
Figure 2. Hypothetical model of the binding of the **46** "pharmacophore" to CD4 (programmed for walleyed viewing): red, **46** "pharmacophore"; orange, CD4 protein residues involved in hydrogen bonding; yellow, hydrogen bonds.

tion that the ester precursors of the carboxylic acids of cosalane derivatives are inactive as antiviral agents. Like many of the polyanions in the present series, **36** was inactive when tested against HIV-2$_{ROD}$ in MT-4 cell culture (EC$_{50}$: >125 μM), even though cosalane (**1**) was active vs HIV-2$_{ROD}$ (EC$_{50}$: 4.0 μM).

The effect of extending the distance between the amide nitrogens of **36** and the two carboxyl groups was determined by synthesizing the β-alanine derivative **38**. Compound **38** was less active than **36** vs both HIV-1 strains. However, **38** did display low activity against HIV-2$_{ROD}$, in contrast to **36**, which was inactive.

The effect of attaching various amino acid side chains to **36** was examined next. The analogue having a leucine side chain (**40**) was less active against both HIV-1$_{RF}$ (EC$_{50}$: 40.0 μM vs 3.4 μM) and HIV-1$_{IIIB}$ (EC$_{50}$: 63.4 μM vs 57.6 μM) than **36**. Incorporation of the phenylalanine side chain (**42**) led to an inactive compound. The compound having the aspartic acid side chain (**44**) was more active than either the leucine or phenylalanine derivatives. Compound **44** proved to be less active than the glycine derivative **36** against HIV-1$_{RF}$ (EC$_{50}$: 10.7 μM vs 3.4 μM), but it was more active than **36** vs HIV-1$_{IIIB}$ (EC$_{50}$: 16.6 μM vs 57.6 μM). In addition, **44** had low activity against HIV-2$_{ROD}$ (EC$_{50}$: 122 μM), in contrast to the inactive glycine derivative **36**. Finally, the glutamic acid derivative **46** was more active than the glycine derivative **36** in all three systems tested (EC$_{50}$: 1.1 μM vs 3.4 μM against in HIV-1$_{RF}$, 8.9 μM vs 57.6 μM against HIV-1$_{IIIB}$, and 69.6 μM vs >125 μM against HIV-2$_{ROD}$). This indicates that when spaced properly, the addition of two carboxyl groups to the two already present in the glycine derivative **36** does offer an increase in anti-HIV activity. In summary, the rank order of the di(amino acid) derivatives vs HIV-1$_{RF}$ in CEM-SS cells was glutamic acid (EC$_{50}$: 1.1 μM), glycine (EC$_{50}$: 3.4 μM), aspartic acid (EC$_{50}$: 10.7 μM), β-alanine (EC$_{50}$: 34.7 μM), leucine (EC$_{50}$: 40.0 μM), and phenylalanine (EC$_{50}$: >200 μM). The rank order of these derivatives vs HIV-1$_{IIIB}$ in MT-4 cells was basically the

same, except the aspartic acid derivative (EC$_{50}$: 16.6 μM) was more potent than the glycine derivative (EC$_{50}$: 57.6 μM) and the leucine derivative (EC$_{50}$: 63.4 μM) was slightly more potent than the β-alanine derivative (EC$_{50}$: 73.8 μM).

A computer graphics molecular modeling study was performed using Sybyl software (Tripos, Inc.) in order to investigate how the most active conjugate **46** might interact with CD4. Gasteiger–Hückel charges were calculated for cosalane, and the charges from the Kollman united-atom force field were loaded for the enzyme portion of the complex. The "pharmacophore" of the di(glutamic acid) conjugate **46** was then docked on the surface of CD4[13] in the site previously proposed for the binding of cosalane.[17] During the docking procedure, the protein was "frozen" and cosalane was allowed to move. Hydrogen bonds between the ligand and the protein were then displayed, with the maximum acceptable distance between the donor and acceptor atoms for a hydrogen bond specified at 2.8 Å and the minimum angle at 120°. The results are pictured in Figure 2. According to the hypothetical model, the "upper" carboxyl group at the end of the chain of the cosalane analogue forms a hydrogen bond with the amide NH of Leu44, while the amide moiety of the "upper chain" of the ligand bonds through both C=O and NH groups to the guanidine moiety of Arg59. The "lower" carboxyl group at the end of the chain of the cosalane analogue bonds to the amino group of Lys72 of CD4. The Arg58 guanidine group of CD4 is bonded to the proximal carboxyl group of the "lower" chain of the ligand, as well as to the amide NH and the phenolic hydroxyl group of cosalane. The hypothetical model shown in Figure 2 makes it plausible that **46** could bind in the site proposed for cosalane, with both of the glutamic acid moieties of the cosalane analogue participating in hydrogen bonding to the protein.

To investigate the effect of increased conformational flexibility of the linker chain attached to the two substituted benzene rings in the cosalane analogues, the

double bond in the linker chain was reduced. The three cases studied were the glycine derivatives **36** vs **48**, the glutamic acid derivatives **46** vs **50**, and the benzyl derivatives **53** vs **34**. When investigated in the HIV-1$_{IIIB}$ and HIV-2$_{ROD}$ systems, the activity decreased in two cases when the double bond was reduced (**46** vs **50** and **53** vs **34**), but it increased in one case (**36** vs **48**). In the HIV-1$_{RF}$ system, the activity consistently decreased in all three cases when the double bond was reduced. The general decrease in antiviral activity accompanying the reduction of the double bond in the linker chain is consistent with the previously reported activity of cosalane (**1**) vs HIV-1$_{RF}$ in CEM-SS cells (EC$_{50}$: 5.1 μM) in comparison with the activity of dihydrocosalane (**31**) (EC$_{50}$: 20 μM) in the same system.[9] Overall, the data show that the conformational restriction provided by the double bond does contribute to the antiviral activity.

Mechanism of action studies have been performed on both cosalane (**1**) as well as the most potent of the tetraanions, compound **53**.[7,17] These investigations have established that both inhibition of binding of gp120 to CD4 and inhibition of an unidentified postbinding fusion event play a role in the inhibition of the cytopathic effect of the virus by cosalane and related compounds. In the case of **53**, inhibition of fusion occurs at a lower concentration than inhibition of attachment.[17] More detailed studies have shown that **53** binds to both CD4 as well as gp120, but a direct interaction with CXCR4 was not observed.[17] It is possible that **53** prevents the proper orientation of the gp120−CD4 complex with CXCR4 that is required for binding. We assume that the additional polyanions in the present series have a similar mechanism of action, involving inhibition of both viral attachment and fusion.

It is clear from the data presented in Table 1 that there are differences in the potencies of the cosalane analogues when they are examined vs HIV-1$_{RF}$ in CEM-SS cells compared with HIV-1$_{IIIB}$ in MT4 cells. In general, the compounds appear to be less potent in the HIV-1$_{IIIB}$ system than they are in the HIV-1$_{RF}$ system, although in four cases (**1**, **50**, **52**, and **54**), the compounds were more active vs HIV-1$_{IIIB}$. These differences in potency may be ascribed to both differences in the cell types as well as differences between the viral strains. Whereas the MT-4 cells are co-infected with HTLV-1, CEM-SS cells are not. This can affect potency, since in the MT-4 cells, there is a second source of antiviral targets in addition to HIV-1. Since the cosalanes are both attachment and fusion inhibitors, their potencies may also be affected by differences in the end sequences between HIV-1$_{RF}$ and HIV-1$_{IIIB}$. The main point to notice from the data presented in Table 1 is that some of the compounds (e.g. **1**, **30**, **34**, **46**, **52**, and **53**) are active against both HIV-1 strains, while others (e.g. **36**, **48**, and **54**) are more selective and are therefore more questionable as anti-HIV agents.

In the cosalane series we have created low to submicromolar inhibitors of HIV-1 replication (e.g. **53**). Since the current paradigm for antiviral therapy is the use of antiviral agents in combination, one of the current goals for the development of new antivirals is to obtain new compounds with novel mechanisms of action that complement the existing reverse transcriptase and protease

inhibitors. This can be seen, for example, in the proposed use and initial success of the pentafuside (T-20), a 36-amino acid fragment of gp41 that inhibits membrane fusion.[21−23] The cosalanes may offer some of the same advantages resulting from an alternative antiviral target. Although the cosalanes are not as potent as anti-HIV agents that inhibit reverse transcriptase or protease, their antiviral potencies are consistent with current inhibitors of HIV-cell fusion.

The potential therapeutic use of cosalane is compromised by its poor oral bioavailability, which may be ascribed to poor enterohepatic transport and sequestration in liver parenchymal cells.[24] However, many drugs that are short peptides, are peptide-derived, or are structurally related to peptides are able to utilize the peptide transport system in the gastrointestinal tract for absorption.[25] The cosalane amino acid conjugates synthesized here therefore also offer the prospect of enhanced oral bioavailability through utilization of peptide transporters.

Experimental Section

General. 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; liquid SIMS mass spectra and EI mass spectra on a MAT 95 XL spectrometer; ^{1}H NMR spectra on Varian VXR-500S and Bruker ARX-300 spectrometers; IR spectra on a Perkin-Elmer 1600 series FTIR. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of the calculated compositions. Silica gel used for column chromatography was 230−400 mesh.

Methyl 4′-(Bromomethyl)biphenyl-2-carboxylate (18). A solution of 2-(*p*-tolyl)benzoic acid (**12**)[26,27] (250 mg, 1.18 mmol) in benzene (8 mL) and methanol (4 mL) was esterified with excess (trimethylsilyl)diazomethane[19] (2.0 M solution in hexane) to afford the corresponding methyl ester **15**:[28,29] ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.79 (bd, J = 7.6 Hz, 1 H), 7.51 (dt, J = 1.6 and 7.6 Hz, 1 H), 7.38 (t, J = 7.5 Hz, 2 H), 7.21 (bs, 4 H), 3.66 (s, 3 H), 2.39 (s, 3 H). A solution of ester **15** (301 mg, 1.33 mmol), *N*-bromosuccinimide (260 mg, 1.46 mmol) and benzoyl peroxide (20 mg, 0.083 mmol) in CCl$_4$ (5 mL) was heated under reflux for 3 h. After cooling, the mixture was filtered. The filtrate was concentrated under vacuum to obtain a liquid residue that was used for the next step without further purification. From proton NMR the conversion to bromide **18**[28] was estimated to be greater than 90%: ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.82 (d, J = 8.2 Hz, 1 H), 7.58 (d, J = 8.2 Hz, 1 H), 7.50−7.26 (m, 6 H), 4.54 (s, 2 H), 3.64 (s, 3 H).

Methyl 4′-(Bromomethyl)biphenyl-3-carboxylate (19). A solution of 3-(*p*-tolyl)benzoic acid (**13**)[30] (250 mg, 1.18 mmol) in benzene (8 mL) and methanol (4 mL) was esterified with excess (trimethylsilyl)diazomethane[19] (2.0 M solution in hexane) to afford the corresponding methyl ester **16**:[29,31,32] ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.26 (t, J = 1.6 Hz, 1 H), 8.00 (td, J = 1.5 and 7.6 Hz, 1 H), 7.77 (td, J = 1.6 and 7.6 Hz, 1 H), 7.52 (d, J = 7.5 Hz, 1 H), 7.50 (AB quartet, J = 7.0 Hz, 2 H), 7.26 (d, J = 7.5 Hz, 2 H), 3.94 (s, 3 H), 2.40 (s, 3 H). A solution of ester **16** (301 mg, 1.33 mmol), *N*-bromosuccinimide (260 mg, 1.46 mmol) and benzoyl peroxide (20 mg, 0.083) in CCl$_4$ (5 mL) was irradiated with a 200-W tungsten lamp while being heated under reflux for 3 h. After cooling, the mixture was filtered. The filtrate was concentrated under vacuum to obtain a liquid residue that was used for the next step without further purification. From proton NMR, the conversion to bromide **19**[31] was estimated to be greater than 90%: ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.26 (t, J = 1.2 Hz, 1 H), 8.04 (td, J = 1.1 and 8.2 Hz, 1 H), 7.78 (td, J = 1.1 and 8.2 Hz, 1 H), 7.60 (d, J = 8.2 Hz, 2 H), 7.54−7.46 (m, 3 H), 4.54 (s, 2 H), 3.94 (s, 3 H).

Methyl 4′-(Bromomethyl)biphenyl-4-carboxylate (20). A solution of 4-(*p*-tolyl)benzoic acid[30,33,34] (**14**) (250 mg, 1.18

mmol) in benzene (8 mL) and methanol (4 mL) was esterified with excess (trimethylsilyl)diazomethane[19] (2.0 M solution in hexane) to afford the corresponding methyl ester **17**:[29,35] ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.09 (d, J = 8.4 Hz, 2 H), 7.64 (d, J = 8.4 Hz, 2 H), 7.52 (d, J = 8.1 Hz, 2 H), 7.27 (d, J = 8.1 Hz, 2 H), 3.93 (s, 3 H), 2.40 (s, 3 H). A solution of ester **17** (301 mg, 1.33 mmol), *N*-bromosuccinimide (260 mg, 1.46 mmol) and benzoyl peroxide (20 mg) in CCl$_4$ (5 mL) was irradiated with a 200-W tungsten lamp while being heated under reflux for 3 h. After cooling, the mixture was filtered. The filtrate was evaporated off to leave a solid residue that was purified by crystallization from hexane to give white crystals of **20** (0.34 g, 84%): mp 113–114 °C; IR (CHCl$_3$) 3011, 2952, 2842, 1732, 1614, 1435, 1341, 1260, 1229, 1203 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.11 (d, J = 8.2 Hz, 2 H), 7.64 (d, J = 8.2 Hz, 2 H), 7.59 (d, J = 8.2 Hz, 2 H), 7.48 (d, J = 8.2 Hz, 2 H), 4.54 (s, 2 H), 3.93 (s, 3 H); HRMS for C$_{15}$H$_{13}$BrO$_2$ calcd 305.0177, found 305.0166.

Cosalane Dimethyl Ester (21). Cosalane (**1**) (384 mg, 0.5 mmol) was dissolved under nitrogen in a mixture of methanol (4.0 mL) and benzene (8.0 mL). (Trimethylsilyl)diazomethane[19] (2.0 M in hexane, 0.8 mL, 1.6 mmol) was added dropwise. After stirring for 25 min, the solvent was evaporated off to leave a white foamy solid of the desired diester in quantitative yield: mp (softens at 88 °C) 161–162 °C; IR (CHCl$_3$) 3160, 2926, 2852, 2362, 1682, 1606, 1465, 1443, 1324, 1286, 1243, 1198, 1175 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 11.31 (s, 1 H), 11.23 (s, 1 H), 7.47 (d, J = 2.1 Hz, 1 H), 7.44 (d, J = 2.3 Hz, 1 H), 7.33 (d, J = 2.2 Hz, 1 H), 7.27 (d, J = 2.1 Hz, 1 H), 5.90 (t, J = 7.6 Hz, 1 H), 3.89 (s, 3 H), 3.87 (s, 3 H), 1.99 (q, J = 7.4 Hz, 2 H), 1.88 (d, J = 12.2 Hz, 1 H), 1.82–1.65 (m, 1 H), 1.65–0.88 (m, 32 H), 0.82 (d, J = 6.6 Hz, 3 H), 0.79 (d, J = 6.6 Hz, 6 H), 0.65 (s, 3 H), 0.57 (s, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 170.2, 156.5, 137.0, 134.4, 134.1, 132.1, 130.7, 129.4, 126.7, 122.3, 122.2, 113.5, 113.2, 56.6, 56.3, 54.7, 52.8, 46.6, 42.6, 40.1, 39.5, 38.5, 37.5, 37.4, 36.2, 36.1, 35.8, 35.5, 32.1, 29.0, 28.9, 28.2, 28.0, 27.2, 24.2, 23.8, 22.8, 22.5, 21.0, 18.7, 12.3, 12.1. Anal. (C$_{47}$H$_{64}$Cl$_2$O$_6$) C, H.

3″,3‴-Dichloro-5″,5‴-di(methoxycarbonyl)-4″,4‴-di[*p*-(2-methoxycarbonylphenyl)benzyloxy]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (22). A mixture of cosalane dimethyl ester (**21**) (360 mg, 0.45 mmol), potassium carbonate (700 mg) and methyl 4′-(bromomethyl)biphenyl-2-carboxylate (**18**; 368 mg, 1.2 mmol) in DMF (7 mL) was stirred at 80 °C for 24 h. The solution was cooled, poured into ice–water (15 mL) and extracted with EtOAc (4 × 20 mL). The organic layers were combined, dried over Na$_2$SO$_4$, evaporated and the residue purified by flash chromatography on silica gel (80 g), eluting with hexanes–ethyl acetate (5:1), to give a white foamy solid (0.336 g, 60%): mp 70–72 °C; IR (CHCl$_3$) 3027, 2925, 2849, 1725, 1608, 1463, 1278, 1106 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.82 (bd, J = 7.7 Hz, 2 H), 7.64–7.50 (m, 8 H), 7.45–7.32 (m, 10 H), 6.10 (t, J = 7.6 Hz, 1 H), 5.20 (s, 2 H), 5.12 (s, 2 H), 3.87 (s, 3 H), 3.86 (s, 3 H), 3.67 (s, 3 H), 3.66 (s, 3 H), 2.11 (q, J = 7.6 Hz, 2 H), 1.95 (bd, J = 12.0 Hz, 1 H), 0.88 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 6.6 Hz, 6 H), 0.72 (s, 3 H), 0.63 (s, 3 H). Anal. (C$_{77}$H$_{88}$Cl$_2$O$_{10}$) C, H.

3″,3‴-Dichloro-5″,5‴-di(methoxycarbonyl)-4″,4‴-di[*p*-(3-methoxycarbonylphenyl)benzyloxy]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (23). A mixture of cosalane dimethyl ester (**21**) (360 mg, 0.45 mmol), potassium carbonate (700 mg) and methyl 4′-(bromomethyl)biphenyl-3-carboxylate (**19**; 368 mg, 1.2 mmol) in DMF (7 mL) was stirred at 80 °C for 24 h. The solution was cooled, poured into ice–water (15 mL) and extracted with EtOAc (4 × 20 mL). The organic layers were combined, dried over Na$_2$SO$_4$, evaporated and the residue purified by flash chromatography on silica gel (80 g), eluting with hexanes–ethyl acetate (5:1), to give a white foamy solid (0.336 g, 60%): mp 83–85 °C; IR (CHCl$_3$) 3027, 2925, 2849, 1725, 1608, 1463, 1278, 1106 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.32–8.28 (m, 2 H), 8.02 (dd, J = 1.4 and 7.7 Hz, 2 H), 7.82–7.77 (m, 2 H), 7.70–7.61 (m, 8 H), 7.55–7.49 (m, 4 H), 7.37 (dd, J = 2.3 and 8.5 Hz, 2 H), 6.11 (t, J = 7.6 Hz, 1 H), 5.22 (s, 2 H), 5.13 (s, 2 H), 3.95 (s, 3 H), 3.95 (s, 3 H), 3.87 (s, 3 H),

3.86 (s, 3 H), 2.11 (q, J = 7.6 Hz, 2 H), 1.95 (bd, J = 12.0 Hz, 1 H), 0.88 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 6.6 Hz, 3 H), 0.84 (d, J = 6.5 Hz, 3 H), 0.71 (s, 3 H), 0.62 (s, 3 H). Anal. (C$_{77}$H$_{88}$Cl$_2$O$_{10}$) C, H.

3″,3‴-Dichloro-5″,5‴-di(methoxycarbonyl)-4″,4‴-di[*p*-(4-methoxycarbonylphenyl)benzyloxy]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (24). A mixture of cosalane dimethyl ester (**21**) (239 mg, 0.30 mmol), potassium carbonate (200 mg) and methyl 4′-(bromomethyl)biphenyl-4-carboxylate (**20**; 215 mg, 0.70 mmol) in DMF (5 mL) was stirred at room temperature for 36 h. The solution was poured into ice–water (10 mL) and extracted with EtOAc (4 × 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, evaporated and the residue purified by flash chromatography on silica gel (45 g), eluting with hexanes–ethyl acetate (5:1), to give a white foamy solid (0.173 g, 46%): mp 89–91 °C; IR (CHCl$_3$) 3027, 2925, 2849, 1725, 1608, 1463, 1278, 1106 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.11 (d, J = 8.3 Hz, 2 H), 8.10 (d, J = 8.3 Hz, 2 H), 7.71–7.65 (m, 12 H), 7.55 (d, J = 2.3 Hz, 1 H), 7.52 (d, J = 2.1 Hz, 1 H), 7.38 (d, J = 2.1 Hz, 1 H), 7.36 (d, J = 2.3 Hz, 1 H), 6.10 (t, J = 7.6 Hz, 1 H), 5.22 (s, 2 H), 5.13 (s, 2 H), 3.94 (s, 3 H), 3.94 (s, 3 H), 3.87 (s, 3 H), 3.86 (s, 3 H), 2.08 (q, J = 7.6 Hz, 2 H), 1.95 (bd, J = 12.0 Hz, 1 H), 0.88 (d, J = 6.5 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 3 H), 0.71 (s, 3 H), 0.62 (s, 3 H). Anal. (C$_{77}$H$_{88}$Cl$_2$O$_{10}$·H$_2$O) C, H.

3″,3‴-Dichloro-5″,5‴-dicarboxy-4″,4‴-di[*p*-(2-carboxyphenyl)benzyloxy]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (25). Tetraester **22** (210 mg, 0.16 mmol) was suspended in a mixture (5:1) of methanol–ethanol (5 mL) and aqueous NaOH (5 M, 2 mL) was added. The mixture was heated at reflux for 14 h to obtain a clear solution. The solvent was evaporated and water (10 mL) was added. The solution was extracted with ethyl acetate (2 × 6 mL). The aqueous layer was acidified with dilute HCl. The precipitate was collected by filtration, washed with water and dried under vacuum to afford the acid **25** (120 mg, 60%): mp 152–154 °C; IR (CHCl$_3$) 2925, 2849, 1697, 1469, 1245 cm^{-1}; ^{1}H NMR (300 MHz, CD$_3$-SOCD$_3$) δ 7.74 (d, J = 8.0 Hz, 2 H), 7.62–7.50 (m, 8 H), 7.50–7.32 (m 10 H), 6.27 (t, J = 7.6 Hz, 1 H), 5.12 (s, 2 H), 5.04 (s, 2 H), 0.86 (d, J = 7.0 Hz, 3 H), 0.82 (d, J = 7.5 Hz, 6 H), 0.67 (s, 3 H), 0.59 (s, 3 H). Anal. (C$_{73}$H$_{80}$Cl$_2$O$_{10}$·H$_2$O) C, H.

3″,3‴-Dichloro-5″,5‴-dicarboxy-4″,4‴-di[*p*-(3-carboxyphenyl)benzyloxy]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (26). Tetraester **23** (230 mg, 0.18 mmol), KCN (7.0 mg) and K$_2$CO$_3$ (400 mg) were suspended in ethanol (7 mL) and water (1.5 mL). The mixture was heated on an oil bath (95 °C) for 20 h. Ethanol was evaporated off and water (10 mL) was added. The aqueous solution was stirred at 80 °C for 2 h. After cooling to room temperature, the solution was extracted with ethyl acetate (2 × 12 mL). The aqueous layer was acidified with concentrated HCl and diluted with water. The precipitate was collected by filtration and dried under vacuum to yield the acid (82 mg, 38%): mp (softens at 182 °C) 196–198 °C; IR (CHCl$_3$) 3400, 2923, 2854, 1696, 1606, 1453, 1250 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.33 (td, J = 1.5 and 5.3 Hz, 2 H), 8.03 (dd, J = 1.5 and 7.5 Hz, 2 H), 7.95 (bd, J = 7.9 Hz, 2 H), 7.78–7.59 (m, 12 H), 7.57 (d, J = 2.5 Hz, 2 H), 6.34 (t, J = 7.6 Hz, 1 H), 5.28 (s, 2 H), 5.20 (s, 2 H), 0.91 (d, J = 7.0 Hz, 3 H), 0.85 (d, J = 7.5 Hz, 6 H), 0.75 (s, 3 H), 0.66 (s, 3 H). Anal. (C$_{73}$H$_{80}$Cl$_2$O$_{10}$·3H$_2$O) C, H.

3″,3‴-Dichloro-5″,5‴-dicarboxy-4″,4‴-di[*p*-(4-carboxyphenyl)benzyloxy]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (27). Tetraester **24** (200 mg, 0.16 mmol), KCN (5.4 mg) and K$_2$CO$_3$ (300 mg) were suspended in ethanol (6 mL) and water (1.5 mL). The mixture was heated on an oil bath (90 °C) for 20 h. Ethanol was distilled off and water (10 mL) was added. The aqueous solution was stirred at 80 °C for 1 h. After cooling to room temperature, the solution was extracted with ethyl acetate (3 × 10 mL). The aqueous layer was acidified with concentrated HCl and diluted with water. The precipitate was collected by filtration and dried under vacuum to afford an almost pure acid (100 mg). It was further purified by crystallization from ethyl acetate–hexane mixture to obtain the pure sample (70 mg, 42%): mp 210–212 °C; IR (CHCl$_3$)

3390, 3039, 2923, 2852, 1690, 1606, 1454, 1277 cm^{-1}; ^{1}H NMR (300 MHz, CD$_3$COCD$_3$) δ 8.14 (d, J = 2.5 Hz, 2 H), 8.12 (d, J = 2.5 Hz, 2 H), 7.88–7.66 (m, 14 H), 7.58 (d, J = 2.5 Hz, 2 H), 6.35 (t, J = 7.6 Hz, 1 H), 5.27 (s, 2 H), 5.20 (s, 2 H), 0.91 (d, J = 7.0 Hz, 3 H), 0.85 (d, J = 7.5 Hz, 6 H), 0.75 (s, 3 H), 0.66 (s, 3 H). Anal. (C$_{73}$H$_{80}$Cl$_2$O$_{10}$·1.5H$_2$O) C, H.

Methyl 4-(Bromomethyl)phenylacetate (28). 4-(Bromomethyl)phenylacetic acid (0.50 g, 2.19 mmol) was dissolved in a mixture of methanol (4 mL) and benzene (6 mL). A hexane solution of (trimethylsilyl)diazomethane[19] (2.0 M, 1.5 mL) was added dropwise to the acid solution until the solution turned light yellow. After 20 min of stirring, glacial acetic acid was added in small portions until the yellow color disappeared. The colorless solution was concentrated by evaporation and dried in vacuo to yield **28**[36] as a colorless oily liquid (0.51 g): IR (CHCl$_3$) 3011, 2952, 2842, 1732, 1614, 1435, 1341, 1260, 1229, 1203, 1069, 1036, 1014 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.35 (d, J = 8.2 Hz, 2 H), 7.25 (d, J = 8.2 Hz, 2 H), 4.47 (s, 2 H), 3.68 (s, 3 H), 3.61 (s, 2 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 171.6, 136.6, 134.2, 129.7, 129.2, 52.0, 40.8, 33.1. Anal. (C$_{10}$H$_{11}$BrO$_2$) C, H.

3″,3‴-Dichloro-5″,5‴-di(methoxycarbonyl)-4″,4‴-di[*p*-[(methoxycarbonyl)methyl]benzyloxy]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (29). Cosalane dimethyl ester (**21**) (180 mg, 0.23 mmol), potassium carbonate (140 mg) and methyl 4-(bromomethyl)phenylacetate (**28**) (190 mg, 0.78 mmol) in DMF (5 mL) were stirred at room temperature for 22 h. The solution was poured into ice–water (8 mL) and extracted with EtOAc (4 × 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, evaporated and the residue purified by flash chromatography on silica gel (70 g), eluting with hexanes–ethyl acetate (4:1), to give a white foamy solid (0.112 g, 44%): mp 56–58 °C; IR (CHCl$_3$) 3027, 2953, 2926, 2850, 1736, 1465, 1251, 1161, 1021, 975 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.58–7.46 (m, 6 H), 7.40–7.30 (m, 6 H), 7.42–7.33 (m, 6 H), 6.09 (t, J = 7.6 Hz, 1 H), 5.13 (s, 2 H), 5.05 (s, 2 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 3.69 (s, 3 H), 3.68 (s, 3 H), 3.65 (s, 2 H), 3.64 (s, 2 H), 2.08 (q, J = 7.6 Hz, 2 H), 1.95 (bd, J = 12.0 Hz, 1 H), 0.88 (d, J = 6.5 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 3 H), 0.71 (s, 3 H), 0.63 (s, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 171.9, 165.8, 165.5, 153.5, 153.1, 138.9, 136.8, 135.8, 135.6, 135.2, 134.0, 132.4, 131.2, 130.0, 129.8, 129.4, 128.7, 128.8, 128.1, 127.3, 127.2, 77.2, 75.7, 56.6, 56.3, 54.6, 52.5, 52.0, 46.5, 42.6, 41.0, 40.0, 39.5, 38.5, 37.4, 37.2, 36.1, 36.0, 35.8, 35.5, 32.1, 29.0, 28.8, 28.2, 28.0, 27.2, 24.2, 23.8, 22.8, 22.5, 18.6, 12.3, 12.0. Anal. (C$_{67}$H$_{84}$Cl$_2$O$_{10}$) C, H.

3″,3‴-Dichloro-5″,5‴-dicarboxy-4″,4‴-di[*p*-(carboxymethyl)benzyloxy]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (30). Tetraester **29** (175 mg, 0.156 mmol), KCN (4.2 mg) and K$_2$CO$_3$ (240 mg) were suspended in ethanol (5 mL) and water (1.2 mL). The mixture was heated on an oil bath (80 °C) for 14 h. Ethanol was distilled off and water (8 mL) was added. The aqueous solution was stirred at 80 °C for 2 h. After cooling to room temperature, the solution was washed with ethyl acetate (2 × 5 mL). The aqueous layer was acidified with 1 N HCl and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated by evaporation to afford a white solid (120 mg). It was further purified by crystallization from ethyl acetate–hexane mixture to give pure **30** (70 mg, 42%): mp 142–144 °C; IR (CHCl$_3$) 3390, 3039, 2923, 2854, 1580, 1380 cm^{-1}; ^{1}H NMR (500 MHz, CD$_3$COCD$_3$) δ 7.68 (d, J = 2.5 Hz, 1 H), 7.67 (d, J = 2.5 Hz, 1 H), 7.60–7.53 (m, 6 H), 7.40–7.35 (m, 4 H), 6.35 (t, J = 7.6 Hz, 1 H), 5.20 (s, 2 H), 5.13 (s, 2 H), 3.68 (s, 2 H), 3.67 (s, 2 H), 0.93 (d, J = 7.0 Hz, 3 H), 0.88 (d, J = 7.5 Hz, 3 H), 0.87 (d, J = 6.5 Hz, 3 H), 0.77 (s, 3 H), 0.68 (s, 3 H). Anal. (C$_{63}$H$_{76}$Cl$_2$O$_{10}$·H$_2$O) C, H.

Dihydrocosalane Dimethyl Ester (32). Dihydrocosalane (**31**) (200 mg, 0.26 mmol) was dissolved under nitrogen atmosphere in a mixture of methanol (4.0 mL) and benzene (8.0 mL). (Trimethylsilyl)diazomethane[19] (Aldrich, 2.0 M in hexane) (0.4 mL, ~0.8 mmol) was added dropwise to the above solution. The reaction was instantaneous and a slight excess

of the solution was added (a light yellow color should persist). After 20–30 min of stirring, the solvent was evaporated off to leave a white residue that was purified by flash chromatography on silica gel (40 g silica, 10:1 hexanes–ethyl acetate) to obtain a white foamy solid (145 mg, 70% overall yield): mp 144–146 °C; IR (CHCl$_3$) 3160, 2930, 2852, 1681, 1611, 1462, 1443, 1382, 1335, 1283, 1249, 1198, 1176 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 11.14 (s, 2 H), 7.50 (d, J = 2.1 Hz, 2 H), 7.29 (d, J = 2.2 Hz, 2 H), 3.90 (s, 6 H), 3.66 (t, J = 7.6 Hz, 1 H), 1.90–1.78 (m, 3 H), 1.78–1.62 (m, 1 H), 1.62–0.86 (m, 34 H), 0.82 (d, J = 6.6 Hz, 3 H), 0.79 (d, J = 6.5 Hz, 6 H), 0.65 (s, 3 H), 0.57 (s, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 170.1, 155.9, 135.7, 135.0, 127.0, 122.4, 113.4, 56.6, 56.3, 54.7, 52.7, 48.9, 46.6, 42.6, 40.1, 39.5, 38.6, 37.7, 37.1, 36.2, 36.1, 35.8, 35.7, 35.5, 35.4, 32.1, 31.6, 29.7, 29.0, 28.2, 28.0, 24.9, 24.2, 23.8, 22.8, 22.5, 21.0, 18.6, 12.3, 12.1. Anal. (C$_{47}$H$_{66}$Cl$_2$O$_6$) C, H.

3″,3‴-Dichloro-5″,5‴-di(methoxycarbonyl)-4″,4‴-di[*p*-(methoxycarbonyl)benzyloxy]-4′,4′-diphenyl-3β-(1′-butyl)cholestane (33). Dimethyl dihydrocosalane (**32**) (181 mg, 0.23 mmol), potassium carbonate (138 mg, 1 mmol) and methyl *p*-bromomethylbenzoate (114 mg, 0.50 mmol) in DMF (4 mL) were stirred at room temperature for 20 h. The solution was poured into iced water (10 mL) and extracted with EtOAc (4 × 15 mL). The organic layers were combined, dried over Na$_2$SO$_4$, evaporated and the residue purified by flash chromatography on silica gel (75 g), eluting with hexanes–ethyl acetate (7:1 to 9:4), to give a white foamy solid (0.194 g, 78%): mp 70–72 °C; IR (CHCl$_3$) 3026, 2953, 2928, 1724, 1455, 1277, 1194, 1105 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.06 (d, J = 8.2 Hz, 4 H), 7.58 (d, J = 8.2 Hz, 4 H), 7.56 (d, J = 2.2 Hz, 2 H), 7.40 (d, J = 2.2 Hz, 2 H), 5.12 (s, 4 H), 3.92 (s, 6 H), 3.83 (s, 6 H), 3.90 [t, (burried under singlets), 1 H], 0.88 (d, J = 6.6 Hz, 3 H), 0.85 (d, J = 6.6 Hz, 3 H), 0.84 (d, J = 6.6 Hz, 3 H), 0.71 (s, 3 H), 0.62 (s, 3 H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 168.8, 165.6, 152.6, 141.9, 141.1, 133.2, 130.0, 129.7, 129.1, 127.6, 127.1, 75.1, 56.5, 56.2, 54.6, 52.5, 52.1, 49.5, 46.5, 42.5, 40.0, 39.4, 38.5, 37.6, 37.0, 36.1, 36.0, 35.7, 35.6, 35.5, 35.3, 32.1, 29.0, 28.9, 28.2, 27.9, 24.9, 24.1, 23.8, 22.8, 22.5, 20.9, 18.6, 12.3, 12.0. Anal. (C$_{65}$H$_{82}$Cl$_2$O$_{10}$) C, H.

3″,3‴-Dichloro-5″,5‴-dicarboxy-4″,4‴-di(*p*-carboxybenzyloxy)-4′,4′-diphenyl-3β-(1′-butyl)cholestane (34). Tetraester **33** (0.227 g, 0.207 mmol), KCN (5.4 mg) and K$_2$CO$_3$ (0.23 g) were suspended in ethanol (7 mL) and water (1.5 mL). The mixture was heated on an oil bath (80 °C) for 12 h. Ethanol was distilled off and water (8 mL) was added. The aqueous solution was stirred at 80 °C for 2 h. After cooling to room temperature, the solution was washed with ethyl acetate (2 × 6 mL). The aqueous layer was acidified with concentrated HCl and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried over Na$_2$SO$_4$, and concentrated by evaporation to obtain the desired acid **34** (150 mg, 70%) as a white solid: mp 146–148 °C; IR (CHCl$_3$) 3373, 3019, 2924, 1685, 1615, 1471, 1420, 1293 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.06 (d, J = 8.2 Hz, 4 H), 7.84 (d, J = 2.2 Hz, 2 H), 7.77 (d, J = 2.2 Hz, 2 H), 7.68 (d, J = 8.2 Hz, 4 H), 5.19 (s, 4 H), 4.20 (t, J = 7.71 Hz, 1 H), 2.15 (q, J = 7.2 Hz, 2 H), 0.91 (d, J = 6.4 Hz, 3 H), 0.85 (d, J = 6.7 Hz, 6 H), 0.75 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.1, 167.4, 166.4, 153.3, 143.1, 142.9, 133.9, 132.7, 130.9, 130.4, 130.3, 128.7, 75.8, 60.5, 57.4, 57.1, 55.5, 50.0, 47.4, 43.3, 40.9, 40.2, 39.4, 38.4, 37.7, 36.9, 36.8, 36.6, 36.4, 36.3, 35.7, 32.9, 25.6, 24.8, 24.5, 23.0, 22.8, 21.7, 20.8, 19.0, 14.4, 12.6, 12.4; MS (electrospray, negative ion mode) m/z 1035 (M − H, 100), 991 (10), 947 (8), 901 (45), 765 (10). Anal. (C$_{61}$H$_{74}$Cl$_2$O$_{10}$) C, H.

5″,5‴-Di[(*tert*-butoxycarbonylmethyl)carbamoyl]-3″,3‴-dichloro-4″,4‴-dihydroxy-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (35). Cosalane (77 mg, 0.1 mmol), glycine *tert*-butyl ester hydrochloride (38 mg, 0.22 mmol) and BOP (90 mg, 0.2 mmol) were taken in a flask and purged with argon for 15 min. The mixture was dissolved in THF (4 mL). To this solution was added Et$_3$N (0.22 mL, 0.3 mmol) and the reaction mixture was stirred at ambient temperature for 16 h. The reaction mixture was diluted with ethyl acetate (25 mL) and was washed with 5% aqueous HCl (2 × 10 mL), water (2 × 10

mL) and brine (1 × 10 mL). The organic layer was dried (anhydrous MgSO$_4$). Concentration and rapid purification by flash chromatography using hexanes−ethyl acetate (4:1) as eluent to give the product **35** (65 mg, 65%): mp 124−132 °C dec; ^{1}H NMR (300 MHz, CDCl$_3$) δ 13.43 (s, 1 H), 13.34 (s, 1 H), 8.62 (bs, 2 H), 7.67 (d, $J = 2$ Hz, 1 H), 7.62 (d, $J = 2$ Hz, 1 H), 7.50 (d, $J = 2$ Hz, 1 H), 7.15 (d, $J = 2$ Hz, 1 H), 7,43 (d, $J = 2$ Hz, 1 H), 6.12 (t, $J = 7.5$ Hz, 1 H), 4.03−4.01 (dd, $J = 6$ Hz, 1.5 Hz, 2 H), 3.96 (dd, $J = 6$ Hz, 1.5 Hz, 2 H), 2.19−1.50 (m, 10 H), 1.43 (s, 9 H), 1.42 (s, 9 H), 1.39−0.8 (m, 35 H), 0.74 (s, 3 H), 0.66 (s, 3 H); IR (KBr) 3358, 2916, 1734, 1644, 1548 cm^{-1}; PDMS m/z 994 (MH$^+$). Anal. (C$_{57}$H$_{82}$Cl$_2$N$_2$O$_8$) C, H, N.

5′′,5′′′-Di[(carboxymethyl)carbamoyl]-3′′,3′′′-dichloro-4′′,4′′′-dihydroxy-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (36). Ester **35** (50 mg, 0.05 mmol) was dissolved in CF$_3$COOH (2 mL). The mixture was stirred at room temperature for 5−10 min. A white precipitate was formed. After the reaction was complete, the solvent was evaporated and the solid obtained was dissolved in ethyl acetate (20 mL). The organic layer was washed with water (2 × 20 mL) and brine (1 × 20 mL) and dried (anhydrous MgSO$_4$). Concentration under vacuum furnished the product (43 mg, 98%): mp 220−226 °C dec; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.42 (s, 1 H), 13.35 (s, 1 H), 8.72−8.66 (m, 2 H), 7.68 (d, $J = 2$ Hz, 1 H), 7.63 (d, $J = 2$ Hz, 1 H), 7.50 (d, $J = 2$ Hz, 1 H), 7.44 (d, $J = 2$ Hz, 1 H), 6.12 (t, $J = 7.5$ Hz, 1 H), 4.12 (d, $J = 6$ Hz, 2 H), 4.06 (d, $J = 6$ Hz, 2 H), 2.20−0.8 (m, 45 H), 0.74 (s, 3 H), 0.66 (s, 3 H); IR 3386, 2927, 2849, 1735, 1648, 1593. cm^{-1}; PDMS m/z 882 (MH$^+$). Anal. (C$_{49}$H$_{66}$O$_8$N$_2$Cl$_2$·0.7CF$_3$COOH) C, H, N.

5′′,5′′′-Di[[2-(ethoxycarbonyl)ethyl]carbamoyl]-3′′,3′′′-dichloro-4′′,4′′′-dihydroxy-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (37). Cosalane (77 mg, 0.1 mmol), β-alanine ethyl ester hydrochloride (35 mg, 0.22 mmol), BOP (90 mg, 0.2 mmol), and Et$_3$N (0.22 mL, 0.3 mmol) in a similar procedure as that described for **35** gave pure **37** (60 mg, 62%): mp 134−138 °C dec; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.88 (s, 1 H), 13.56 (s, 1 H), 8.47−8.41 (m, 2 H), 7.61 (d, $J = 2$ Hz, 1 H), 7.53 (d, $J = 2$ Hz, 1 H), 7.42 (d, $J = 2$ Hz, 1 H), 7.38 (d, $J = 2$ Hz, 1 H), 6.08 (t, $J = 7.5$ Hz, 1 H), 4.11−4.03 (q, $J = 6$ Hz, 4 H), 3.67−3.55 (m, 4 H), 2.65−2.56 (m, 4 H), 2.20−0.84 (m, 51 H), 0.73 (s, 3 H), 0.66 (s, 3 H); IR 3348, 2926, 1729, 1639, 1588 cm^{-1}; PDMS m/z 965 (MH$^+$).

5′′,5′′′-Di[(2-carboxyethyl)carbamoyl]-3′′,3′′′-dichloro-4′′,4′′′-dihydroxy-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (38). Ester **37** (48 mg, 0.05 mmol) was dissolved in ethanol (4 mL). Sodium hydroxide solution (1 N, 0.3 mL) was added. The reaction mixture was stirred at ambient temperature for 16−20 h. The solvent was removed under vacuum and HCl (1 N, 0.5 mL) was added. The precipitated product was dissolved in ethyl acetate (20 mL) and the organic solution was briefly washed with brine (1 × 10 mL) and dried (anhydrous MgSO$_4$). Concentration and recrystallization from the mixture of ethanol−ether−hexane (5:3:2) gave the product **38** (32 mg, 72%): mp 224−229 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.70 (s, 1 H), 13.62 (s, 1 H), 8.45−8.40 (m, 2 H), 7.65 (d, $J = 2$ Hz, 1 H), 7.55 (d, $J = 2$ Hz, 1 H), 7.40 (d, $J = 2$ Hz, 1 H), 7.38 (d, $J = 2$ Hz, 1 H), 6.07 (t, $J = 7.5$ Hz, 1 H), 3.66−3.55 (m, 4 H), 2.66−2.58 (m, 4 H), 2.14−0.77 (m, 45 H), 0.73 (s, 3 H), 0.66 (s, 3 H); IR (KBr) 3363, 2922, 1712, 1639, 1589 cm^{-1}; PDMS m/z 909 (M + H)$^+$. Anal. (C$_{51}$H$_{70}$O$_8$N$_2$Cl$_2$·0.5H$_2$O) C, H, N.

3′′,3′′′-Dichloro-4′′,4′′′-dihydroxy-5′′,5′′′-di[[1-(methoxycarbonyl)isopentyl]carbamoyl]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (39). Cosalane (77 mg, 0.1 mmol), L-leucine methyl ester hydrochloride (40 mg, 0.22 mmol), BOP (90 mg, 0.2 mmol), and Et$_3$N (0.22 mL, 0.3 mmol) in a similar procedure as that described for **35** gave pure **39** (72 mg, 70%): mp 112−118 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.37 (s, 1 H) 13.25 (1 H), 8.49 (m, 2 H), 7.80 (bs, 1 H), 7.69 (bs, 1 H), 7.43−7.41 (m, 2 H), 6.09 (t, $J = 7.5$ Hz, 1 H), 4.70 (m, 2 H), 2.01−0.8 (m, 63 H), 0.77 (s, 3 H), 0.68 (s, 3 H); IR (KBr) 3367, 2957, 1751, 1723, 1640, 1545 cm^{-1}; PDMS m/z 1022 (MH$^+$) .

3′′,3′′′-Dichloro-4′′,4′′′-dihydroxy-5′′,5′′′-di[[1-carboxyisopentyl]carbamoyl]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)-

cholestane (40). Ester **39** (51 mg, 0.05 mmol) and sodium hydroxide solution (1 N, 0.3 mL) in a similar procedure as described for **38** gave pure **40** (32 mg, 75%): mp 224−229 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.42 (s, 1 H), 13.32 (s, 1 H), 8.47−8.38 (m, 2 H), 7.79 (d, $J = 2$ Hz, 1 H), 7.68 (d, $J = 2$ Hz, 1 H), 7.39 (bs, 2 H), 6.04 (t, $J = 7.5$ Hz, 1 H), 4.76−4.69 (m, 4 H), 2.14−0.80 (m, 63 H), 0.75 (s, 3 H), 0.66 (s, 3 H); IR (KBr) 3328, 2936, 1714, 1639, 1588, cm^{-1}; PDMS m/z 994 (MH$^+$). Anal. (C$_{57}$H$_{82}$O$_8$N$_2$Cl$_2$·H$_2$O) C, H, N.

3′′,3′′′-Dichloro-4′′,4′′′-dihydroxy-5′′,5′′′-di[[(1-methoxycarbonyl-2-phenylethyl)carbamoyl]-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (41). Cosalane (77 mg, 0.1 mmol), L-phenylalanine methyl ester hydrochloride (54 mg, 0.22 mmol), BOP (90 mg, 0.2 mmol), and Et$_3$N (0.22 mL, 0.3 mmol) in a similar procedure as described for **35** gave pure **41** (66 mg, 62%): mp 110−118 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.15 (s, 1 H), 12.98 (s, 1 H), 8.50 (d, $J = 9$ Hz, 1 H) 8.42 (d, $J = 9$ Hz, 1 H), 7.60 (d, $J = 2$ Hz, 1 H), 7.56 (d, $J = 2$ Hz, 1 H), 7.41 (d, $J = 2$ Hz, 1 H), 7.38 (d, 1 H), 7.28−7.17 (m, 10 H), 6.03 (t, $J = 7.5$ Hz, 1 H), 4.98−4.84 (m, 2 H), 3.69 (s, 3 H), 3.66 (s, 3 H), 3.28−3.22 (m, 2 H), 3.10−2.97 (m, 2 H), 2.00−0.8 (m, 45 H), 0.75 (s, 3 H), 0.66 (s, 3 H); IR (film) 3385, 2932, 1750, 1723, 1644, 1591 cm^{-1}; PDMS m/z 1090 (MH$^+$).

5′′,5′′′-Di[(1-carboxy-2-phenylethyl)carbamoyl]-3′′,3′′′-dichloro-4′′,4′′′-dihydroxy-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (42). Ester **41** (55 mg, 0.05 mmol) and sodium hydroxide solution (1 N, 0.3 mL) in a similar procedure as that described for **38** gave pure **42** (63 mg, 60%): mp 240−246 °C dec; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.20 (s, 1 H), 12.95 (s, 1 H), 8.5−83 (m, 2 H) 7.60 (d, $J = 2$ Hz, 1 H), 7.64 (d, $J = 2$ Hz, 1 H), 7.48 (d, $J = 2$ Hz, 1 H), 7.40−7.22 (m, 12 H), 6.04 (t, $J = 7.5$ Hz, 1 H), 4.84−4.60 (m, 2 H), 3.44−2.80 (m, 4 H), 2.00−0.8 (m, 45 H), 0.76 (s, 3 H), 0.67 (s, 3 H); IR (KBr) 3367, 2924, 1719, 1643, 1589 cm^{-1}; PDMS m/z 1062 (MH$^+$). Anal. (C$_{63}$H$_{78}$O$_8$N$_2$Cl$_2$·H$_2$O) C, H, N.

5′′,5′′′-Di[(1,2-di-*tert*-butoxycarbonylethyl)carbamoyl]-3′′,3′′′-dichloro-4′′,4′′′-dihydroxy-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (43). Cosalane (77 mg, 0.1 mmol), L-aspartic acid di-*tert*-butyl ester hydrochloride (62 mg, 0.22 mmol), BOP (90 mg, 0.2 mmol), and Et$_3$N (0.22 mL, 0.3 mmol) in a similar procedure as described for **35** gave pure **43** (92 mg, 75%): mp 148−152 °C dec; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.3 (bs, 2 H), 8.45 (t, $J = 6$ Hz, 2 H), 7.64 (bs, 1 H), 7.58 (bs, 1 H), 7.41 (bs, 2 H), 6.07 (t, $J = 7.5$ Hz, 1 H), 4.89−4.78 (m, 2 H), 2.92−2.69 (m, 4 H), 2.14−0.80 (m, 81 H), 0.74 (s, 3 H), 0.66 (s, 3 H); IR (KBr) 3350, 2929, 2837, 1737, 1639, 1547 cm^{-1}; PDMS m/z 1223 (MH$^+$).

5′′,5′′′-Di[(1,2-dicarboxyethyl)carbamoyl]-3′′,3′′′-dichloro-4′′,4′′′-dihydroxy-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (44). Ester **43** (62 mg, 0.05 mmol) and CF$_3$COOH (2 mL) in a similar procedure as that described for **36** gave pure **44** (94 mg, 95%): mp 242−246 °C dec; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.27 (s, 1 H), 13.18 (s, 1 H), 8.64 (m, 2 H), 7.76 (bs, 1 H), 7.63 (bs, 1 H), 7.42 (d, $J = 2$ Hz, 1 H), 7.39 (d, $J = 2$ Hz, 1 H), 6.08 (t, $J = 7.5$ Hz, 1 H), 5.10−4.96 (m, 2 H), 3.07−2.83 (m, 4 H), 2.20−0.81 (m, 45 H), 0.74 (s, 3 H), 0.67 (s, 3 H); IR (KBr) 3350, 2929, 1716, 1644, 1542 cm^{-1}; PDMS m/z 998 (MH$^+$). Anal. (C$_{53}$H$_{70}$O$_{12}$N$_2$Cl$_2$·0.8CF$_3$COOH) C, H, N.

5′′,5′′′-Di[[1,2-di(*tert*-butoxycarbonyl)propyl]carbamoyl]-3′′,3′′′-dichloro-4′′,4′′′-dihydroxy-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (45). Cosalane (77 mg, 0.1 mmol), l-glutamic acid di-*tert*-butyl ester hydrochloride (66 mg, 0.22 mmol), BOP (90 mg, 0.2 mmol), and Et$_3$N (0.22 mL, 0.3 mmol) in a similar procedure as that described for **35** gave pure **45** (95 mg, 76%): mp 154−159 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 13.31 (bs, 2 H), 8.50 (d, $J = 9$ Hz) 8.45 (d, $J = 9$ Hz, 1 H), 7.83 (d, $J = 2$ Hz, 1 H), 7.67 (bs, 1 H), 7.40 (d, $J = 2$ Hz, 1 H), 7.36 (d, $J = 2$ Hz, 1 H) 6.10 (t, $J = 7.5$ Hz, 1 H), 4.60−4.52 (m, 2 H), 2.21−2.37 (m, 4 H), 2.2−0.84 (m, 85 H), 0.74 (s, 3 H), 0.66 (s, 3 H); IR (KBr) 3338, 2926, 1729, 1644, 1542 cm^{-1}; PDMS m/z 1248 (MH$^+$).

5′′,5′′′-Di[(1,2-di-*tert*-carboxypropyl)carbamoyl]-3′′,3′′′-dichloro-4′′,4′′′-dihydroxy-4′,4′-diphenyl-3β-(3′-buten-1′-yl)cholestane (46). Ester **45** (63 mg, 0.05 mmol) and CF$_3$-

COOH (2 mL) in a similar procedure as that described for **36** gave pure **46** (49 mg, 98%): mp 236−242 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.84 (d, $J = 2$ Hz, 1 H), 7.71 (bs, 1 H), 7.40 (bs, 2 H), 6.11 (t, $J = 7.5$ Hz, 1 H), 4.74−4.67 (m, 2 H), 2.60−0.84 (m, 49 H), 0.74 (s, 3 H), 0.66 (s, 3 H); IR (KBr) 3360, 2930, 2847, 1716, 1639, 1542 cm^{-1}; PDMS m/z 1026 (MH$^+$). Anal. (C$_{55}$H$_{74}$O$_{12}$N$_2$Cl$_2$·CF$_3$COOH) C, H, N.

5″,5‴-Di[(1,2-di-*tert*-butoxycarbonylethyl)carbamoyl]-3″,3‴-dichloro-4″,4‴-dihydroxy-4′,4′-diphenyl-3β-(1′-butyl)cholestane (47). Dihydrocosalane (78 mg, 0.1 mmol), glycine *tert*-butyl ester hydrochloride (34 mg, 0.22 mmol), BOP (90 mg, 0.2 mmol), and Et$_3$N (0.22 mL, 0.3 mmol) in a similar procedure as that described for **35** gave pure **47** (32 mg, 31%): ^{1}H NMR (500 MHz, acetone-d_6) δ 8.60 (bs, 2 H), 7.2 (bs, 2 H), 7.46 (bs, 2 H), 4.05−4.04 (m, 4 H), 3.62 (t, $J = 6$ Hz, 1 H), 2.20−0.93 (m, 54 H), 0.92 (d, $J = 4$ Hz, 3 H), 0.84 (d, $J = 4$ Hz, 6 H), 0.72 (s, 3 H), 0.62 (s, 3 H); IR (CHCl$_3$) 3369, 2923, 1736, 1648, 1591 cm^{-1}; PDMS m/z 995 (MH$^+$).

5″,5‴-Di[(1,2-dicarboxyethyl)carbamoyl]-3″,3‴-dichloro-4″,4‴-dihydroxy-4′,4′-diphenyl-3β-(1′-butyl)cholestane (48). Ester **47** (50 mg, 0.05 mmol) and CF$_3$COOH (2 mL) in a similar procedure as that described for **36** gave pure **48** (39 mg, 90%): ^{1}H NMR (300 MHz, acetone-d_6) δ 13.3 (s, 2 H), 8.68 (bs, 2 H), 7.8 (bs, 2 H), 7.52 (bs, 2 H), 4.20−4.22 (m, 4 H), 3.80 (t, $J = 6$ Hz, 1 H), 2.10−0.90 (m, 36 H), 0.88 (d, $J = 4$ Hz, 3 H), 0.84 (d, $J = 4$ Hz, 6 H), 0.72 (s, 3 H), 0.62 (s, 3 H); IR (CHCl$_3$) 3378, 2932, 1725, 1644, 1595 cm^{-1}; PDMS m/z 886 (MH$^+$). Anal. (C$_{55}$H$_{74}$O$_{12}$N$_2$Cl$_2$·1.3CF$_3$COOH) C, H, N.

5″,5‴-Di[[1,2-di(*tert*-butoxycarbonyl)propyl]carbamoyl]-3″,3‴-dichloro-4″,4‴-dihydroxy-4′,4′-diphenyl-3β-(1′-butyl)cholestane (49). Dihydrocosalane (78 mg, 0.1 mmol), L-glutamic acid di-*tert*-butyl ester hydrochloride (66 mg, 0.22 mmol), BOP (90 mg, 0.2 mmol), and Et$_3$N (0.22 mL, 0.3 mmol) in a similar procedure as described for **35** gave pure **49** (45 mg, 36%): ^{1}H NMR (300 MHz, acetone-d_6) 8.30 (bs, 2 H), 7.70 (d, $J = 2$ Hz, 2 H), 7.32 (d, $J = 2$ Hz, 2 H), 4.48−4.44 (m, 2 H), 3.81 (t, $J = 7.5$ Hz, 1 H), 2.24−2.22 (m, 4 H), 2.10−0.84 (m, 86 H), 0.76 (s, 3 H), 0.65 (s, 3 H); IR (film) 3369, 2923, 1736, 1648, 1591 cm^{-1}; PDMS m/z 1249 (MH$^+$).

5″,5‴-Di[(1,2-di-*tert*-carboxypropyl)carbamoyl]-3″,3‴-dichloro-4″,4‴-dihydroxy-4′,4′-diphenyl-3β-(1′-butyl)cholestane (50). Ester **49** (50 mg) and CF$_3$COOH (2 mL) in a similar procedure as that described for **36** gave pure **50** (34 mg, 88%): ^{1}H NMR (300 MHz, acetone-d_6) δ 13.00 (s, 2 H) 8.53 (bs, 2 H), 7.87 (bs, 1 H), 7.84 (bs, 1 H), 7.51 (d, $J = 1.5$ Hz, 1 H), 7.50 (bs, 1 H) 4.72−4.68 (m, 2 H), 3.89 (t, $J = 7.5$ Hz, 1 H), 2.60 (m, 4 H) 2.20−0.90 (m, 36 H), 0.92 (d, $J = 4$ Hz, 3 H), 0.85 (d, $J = 4$ Hz, 6 H), 0.74 (s, 3 H), 0.66 (s, 3 H); IR 3376, 2930, 1722, 1648, 1597 cm^{-1}; PDMS m/z 1029 (MH$^+$). Anal. (C$_{55}$H$_{76}$Cl$_2$N$_2$O$_{12}$·0.25CF$_3$COOH) C, H, N.

In Vitro Anti-HIV Assays. Evaluation of the antiviral activity of compounds against HIV-1$_{RF}$ infection in CEM-SS cells was performed using the MTS cytoprotection assay as previously described.[37] This cell-based microtiter assay quantitates the drug-induced protection from the cytopathic effect of HIV-1. Briefly, exponentially growing CEM-SS cells (obtained from the AIDS Research and Reference Reagent Repository, Bethesda, MD) were collected by centrifugation and resuspended in fresh tissue culture medium. A pretitered aliquot of HIV-1$_{RF}$ (AIDS Research and Reference Reagent Repository, Bethesda, MD), 5×10^3 cells and serially diluted compounds were placed into 0.2-cm round-bottomed microtiter plates (final volume 200 μL). Each plate contained cell control wells (cells only), virus control wells (cells plus virus), drug toxicity control wells (cells plus drug only), drug colorimetric control wells (drug only) as well as experimental wells (drug plus cells plus virus). Cultures were incubated for 6 days at 37 °C, 5% CO$_2$. At assay termination, the assay plates were stained with the soluble tetrazolium-based dye MTS (CellTiter Reagent, Promega) to determine HIV cytoprotection and quantify compound toxicity. 20 μL of MTS reagent was added per well and allowed to develop overnight at 37 °C. Prior to quantitation, the formazan product was mixed and the plate was read spectrophotometrically at 490 nm. Activity was

confirmed by both macroscopic and microscopic observation of the assay. Data are presented as the percent control of MTS values for the uninfected, drug-free control. EC$_{50}$ values reflect the drug concentration that provides 50% protection from the cytopathic effect of HIV-1 in infected cultures, while the CC$_{50}$ reflects the concentration of drug that causes 50% cell death in the uninfected cultures. All MTS cytoprotection data were derived from triplicate tests on each plate, with two separate sister plates. Thus, the EC$_{50}$ value from each plate represents the average of triplicates, and the two EC$_{50}$ values from sister plates were averaged.

Evaluation of the antiviral activity of the compounds against HIV-1$_{IIIB}$ and HIV-2$_{ROD}$ in MT-4 cells was performed using the MTT cytoprotection assay as previously described.[38] Stock solutions (10× final concentration) of test compounds were added in 25-μL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottom 96-well plastic microtiter trays using a Biomek 2000 robot (Beckman Instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1$_{IIIB}$[39] or HIV-2$_{ROD}$[40] stock (50 μL) at 100−300 CCID$_{50}$ (cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compounds on uninfected cells and to determine the concentration at which the test compounds were cytotoxic. Exponentially growing MT-4 cells[41] were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, using slight magnetic stirring, and 50-μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiskan Ascent Reader, Labsystems, Helsinki, Finland), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells. The 50% cytotoxic concentration (CC$_{50}$) was defined as the concentration of extract that reduced the absorbance (OD$_{540}$) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC$_{50}$).

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