Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 10, pp 1637–1648, 1996

PII: S0968-0896(96)00159-9
0968-0896/96 $15.00+0.00

Exploration of the Effects of Linker Chain Modifications on Anti-HIV Activities in a Series of Cosalane Analogues

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Abstract—The effects of linker chain modifications were investigated in a series of cosalane analogues. The modifications investigated included: (1) shortening the three-carbon linker chain between the dichlorodisalicylmethane and the cholestane moiety by one carbon atom; (2) lengthening the linker chain by one carbon; (3) hydrogenation of the double bond in the linker chain; (4) changing the point of attachment of the linker chain from C-3 to C-6; (5) insertion of a phosphate between the steroid and the linker chain. With the exception of the phosphate modification, which abolished anti-HIV activity and increased cytotoxicity, the linker chain modifications produced relatively minor changes in anti-HIV potency. The steroid and attached linker chain of cosalane therefore appear only to provide a general lipophilic appendage for the dichlorodisalicylmethane pharmacophore.

Introduction

The anti-HIV agent cosalane (**1**) was designed conceptually by the attachment of a dichlorodisalicylmethane unit to a cholestane moiety by a three-carbon linker chain.[1] Mechanism of action studies have shown that cosalane (**1**) acts primarily by inhibition of the binding of gp120 to CD4, as well as by inhibition of a postattachment event prior to reverse transcription.[1] The exact postattachment event that is inhibited is not well defined, but the data are consistent with it being the fusion of the viral envelope with the cell membrane.[1] As a working hypothesis, the dichlorodisalicylmethane unit of cosalane may be considered the 'pharmacophore', while the cholestane unit acts as a lipophilic accessory appendage that aids in escorting the molecule to the lipid environment of the cell membrane and viral envelope. In agreement with this interpretation, the cholestane fragment of cosalane (**1**) has been replaced by straight-chain hydrocarbons of varying length, and the anti-HIV potencies of the resulting analogues have been found to correlate with chain length.[2] In addition, the dichlorodisalicylmethane fragment of cosalane has anti-HIV activity of its own, although its potency (EC_{50} 329 μM against HIV-1_{IIIB} in CEM cells) is low relative to that of cosalane (**1**) itself (EC_{50} 6.8 μM against HIV-1_{IIIB} in CEM cells).[1,3] The representation depicted in Figure 1 has been adopted as a hypothetical model for the interaction of cosalane (**1**) and related compounds with biological membranes.[2,4] According to this depiction, the cholestane moiety of cosalane (**1**) imbeds in the lipid bilayer, with the more hydrophilic disalicylmethane fragment protruding outward in an obstructive mode.

The present investigation was initiated in order to determine the effects of linker chain modifications on the anti-HIV activity of cosalane analogues. The chain

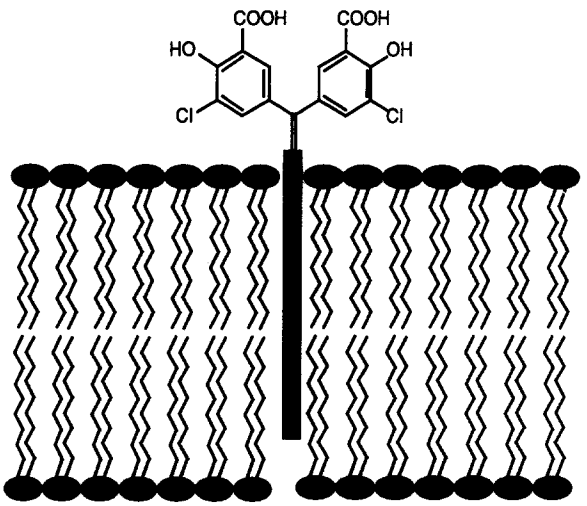
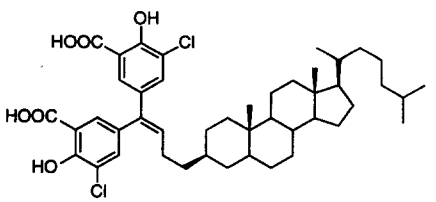


Figure 1. Schematic representation of the anchoring of the disalicylmethane moiety of cosalane and cosalane analogues to the cell membrane and viral envelope by the steroid fragment.



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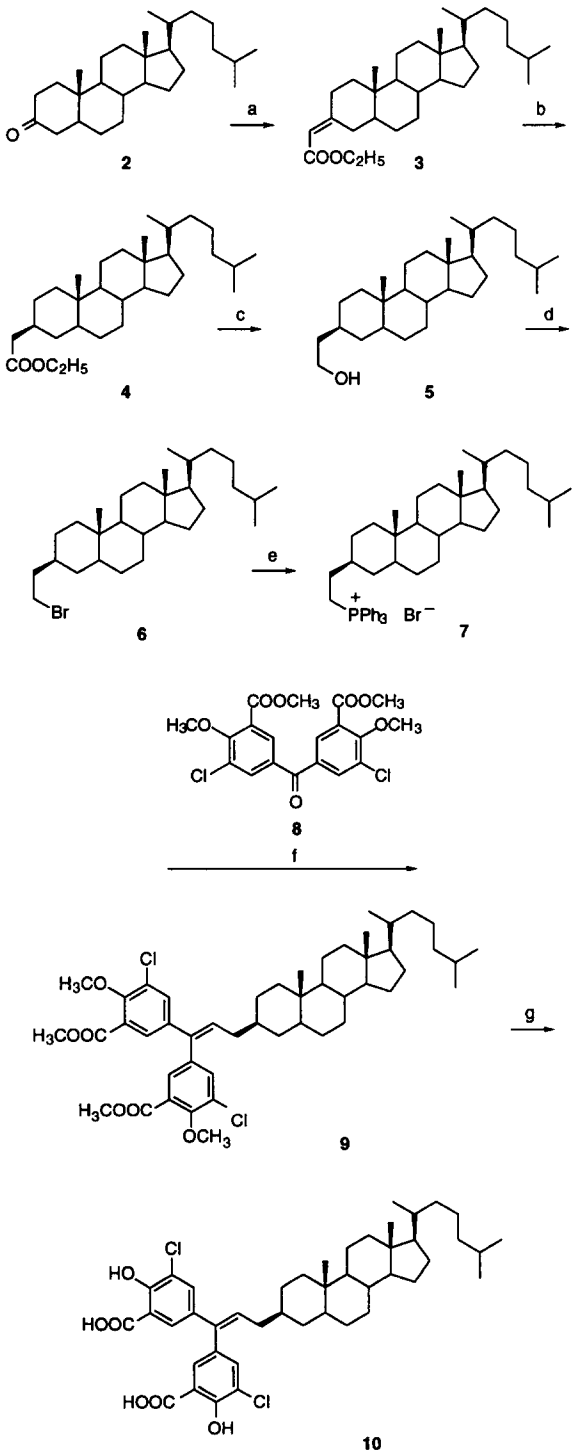
length may effect the depth of cholestane imbedding in the lipid bilayer, as well as the distance that the dichlorodisalicylmethane pharmacophore protrudes from the membrane. Cosalane analogues were therefore prepared having longer and shorter linker chains. The optimal geometry of the imbedded cholestane moiety of cosalane (**1**) relative to the lipid bilayer is also unknown, and the effect of the point of attachment of the linker chain to the steroid on the anti-HIV activity was probed by moving it from the 3-position to the 6-position. Another linker chain modification that was investigated involved the saturation of the double bond attached to the central carbon of the dichlorodisalicylmethane fragment of cosalane. Finally, the effect of the incorporation of a phosphate group into the linker chain on anti-HIV activity was investigated. A number of phospholipids have shown anti-HIV activity which is thought to be due to their insertion into cellular and viral membranes.[5-9] In addition, certain membrane-interactive phospholipids have been found recently to inhibit membrane fusion by an unidentified mechanism.[10] The incorporation of a phosphate group into the linker chain of cosalane (**1**) would be expected to make the resulting phosphodiester resemble more closely the structure of a phospholipid.

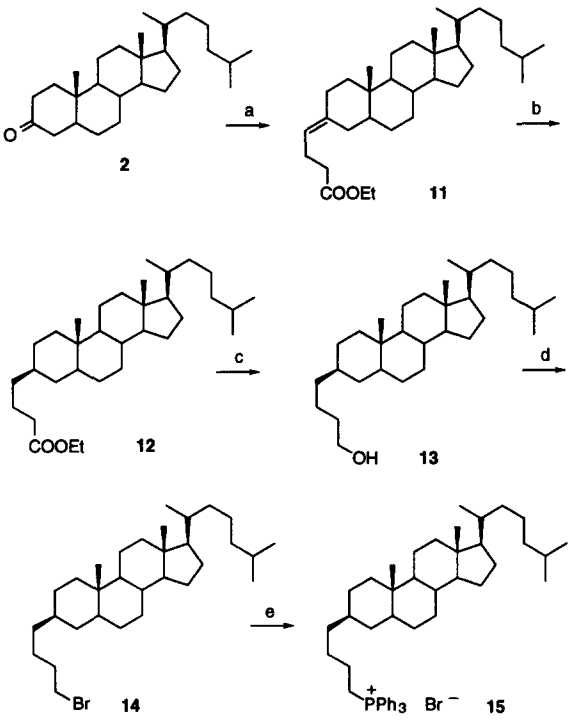
Chemistry

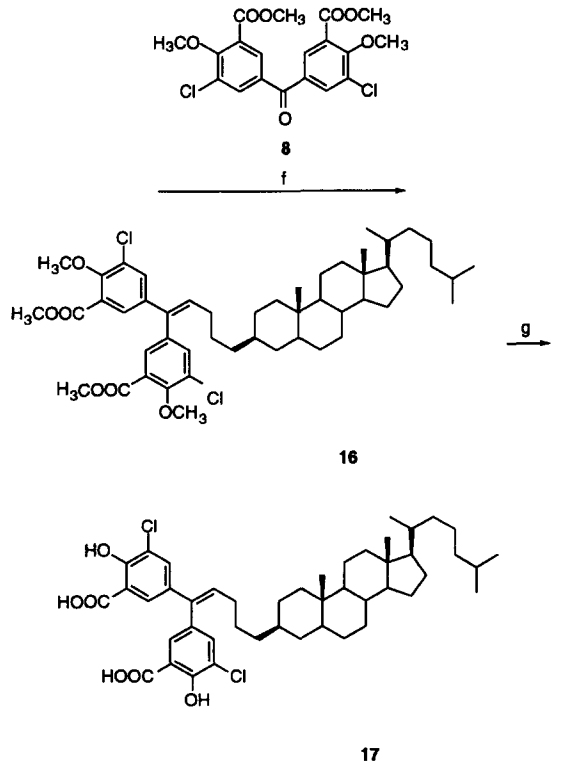
The synthesis of the cosalane analogue **10** having a linker chain shortened by one carbon relative to cosalane (**1**) is outlined in Scheme 1. Treatment of 3-cholestanone (**2**) with the Horner–Emmons reagent derived from deprotonation of diethyl ethoxycarbonylmethylphosphonate afforded 5α-3-ethoxycarbonylmethylenecholestane (**3**).[11] Hydrogenation of **3** using platinum oxide as the catalyst gave 5α-3β-ethoxycarbonylmethylcholestane (**4**), derived from delivery of hydrogen to the sterically less hindered α-side of the steroid. The primary alcohol **5** was obtained by lithium aluminum hydride reduction of the ester **4**. The conversion of the alcohol **5** to the corresponding bromide **6** was carried out with triphenylphosphine and carbon tetrabromide in methylene chloride.[12,13] Reaction of the bromide **6** with triphenylphosphine in refluxing chlorobenzene yielded the phosphonium bromide **7**. Deprotonation of **7** with sodium bis(trimethylsilyl)amide in tetrahydrofuran gave the corresponding ylide, which was reacted with the substituted benzophenone derivative **8**[1] to provide the Wittig reaction product **9**. The four *O*-methyl groups were removed from **9** with boron tribromide–dimethyl sulfide complex on heating in 1,2-dichloroethane to afford the desired final product **10**.

The synthesis of the cosalane congener **17**, having a linker chain that is lengthened by one carbon atom relative to cosalane itself, is detailed in Scheme 2. Reaction of 3-cholestanone (**2**) with the Wittig reagent formed by deprotonation of triphenyl-3-ethoxycarbonylpropylphosphonium bromide resulted in the expected intermediate **11**. Hydrogenation of **11** at room temperature over platinum oxide catalyst afforded a mixture of **12** and its C-3 epimer in a 4:1 ratio, respectively, which was reduced with lithium aluminum hydride. The resulting diastereomeric mixture of alcohols was separated by recrystallization from ethanol to give the pure 3-β epimer **13**. The remainder

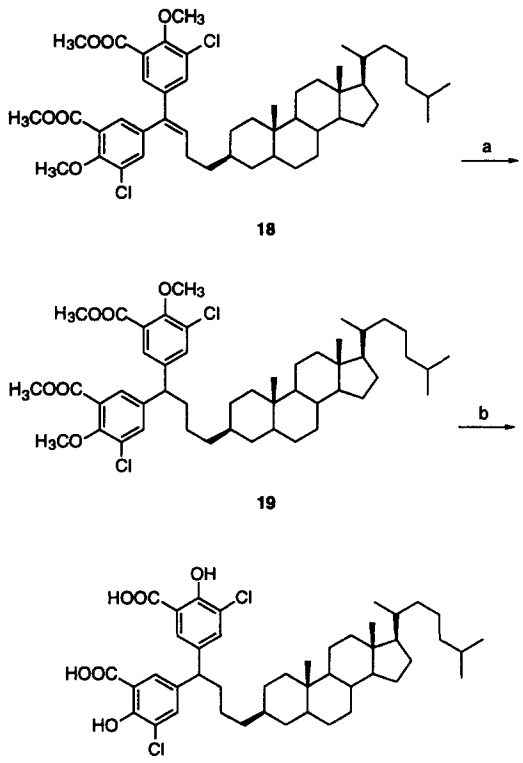


Scheme 1. Reagents and conditions: (a) (1) $(EtO)_2POCH_2COOEt$, NaOEt, DMF, THF, 23 °C (1 h); (2) cholestanone (**1**), 23 °C (3 days); (b) H_2, PtO_2, EtOAc, 23 °C (1.5 h), 50 °C (2 h); (c) $LiAlH_4$, THF, Et_2O, 0 °C (1 h); (d) Ph_3P, CBr_4, CH_2Cl_2, 0 °C (10 min); (e) Ph_3P, PhCl, 140 °C (24 h); (f) (1) Compound **7**, $NaN(SiMe_3)_2$, THF, 0 °C (30 min); (2) compound **8**, 23 °C (2 days); (g) $BBr_3 \cdot Me_2S$, $Cl(CH_2)_2Cl$, 60 °C (24 h).





Scheme 2. Reagents and conditions: (a) (1) $Ph_3P^+(CH_2)_3COOEt\ Br^-$, NaH, DMF, 23 °C (1 h); (2) cholestanone (**1**), 70 °C (4 h); (b) H_2, PtO_2, EtOAc, 23 °C (4 h); (c) $LiAlH_4$, THF, Et_2O, 0 °C (1 h); (d) Ph_3P, CBr_4, CH_2Cl_2, 0 °C (35 min); (e) Ph_3P, PhCl, 140 °C (24 h); (f) (1) Compound **15**, $NaN(SiMe_3)_2$, THF, 0 °C (30 min); (2) compound **8**, 23 °C (2 days); (g) $BBr_3 \cdot Me_2S$, $Cl(CH_2)_2Cl$, 70 °C (16 h).



Scheme 3. Reagents and conditions: (a) H_2, PtO_2, EtOAc, 23 °C (45 min); (b) $BBr_3 \cdot Me_2S$, $Cl(CH_2)_2Cl$, 70 °C (8 h), 23 °C (12 h).

of the synthesis to afford **17** closely paralleled the conversion of **3** to **10**, which has already been outlined.

The preparation of the cosalane analogue **20**, in which the double bond of cosalane is hydrogenated, is depicted in Scheme 3. Compound **18**,[1] obtained as an intermediate in the synthesis of cosalane (**1**), was subjected to catalytic hydrogenation over platinum oxide to yield the reduction product **19**. The ester and ether groups were demethylated with boron tribromide–dimethyl sulfide complex to yield the desired product **20**.

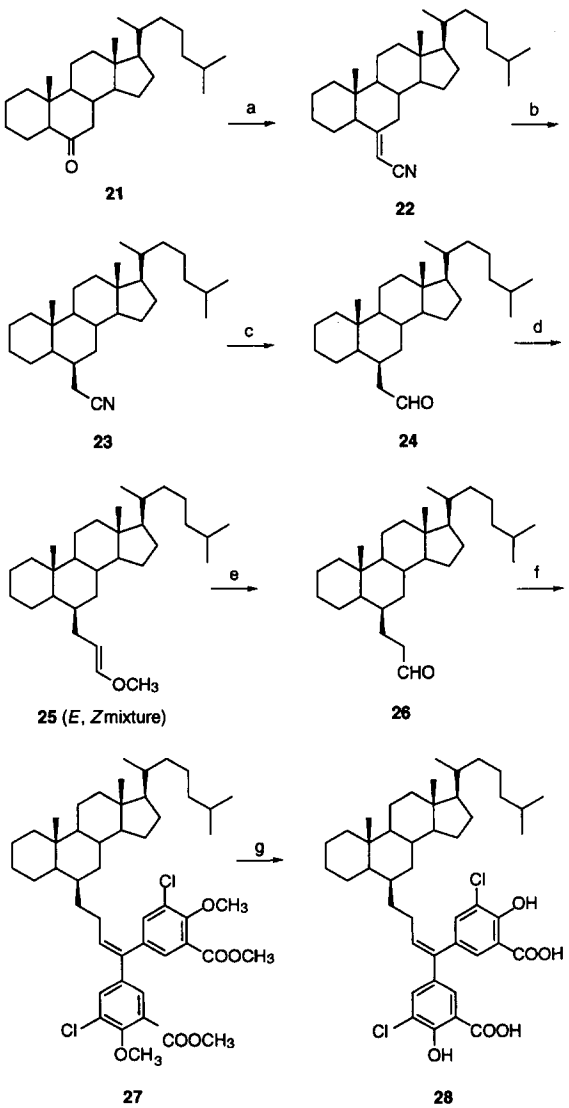
The synthesis of the cosalane analogue **28**, in which the linker chain is attached to C-6 of the steroid, is described in Scheme 4. The synthetic approach outlined in Scheme 4 is fundamentally different from the preparation of **10** and **17** shown in Schemes 1 and 2. It was designed with the idea that the availability of a series of homologous aldehydes (e.g., **24** and **26**), obtained by a number of identical iterative aldehyde homologation reactions, would in principle allow the preparation of a series of analogues in which the linker chain is 'grown' one carbon at a time, provided the McMurry reaction of the aldehydes with **8** could be used effectively to introduce the dichlorodisalicylmethane fragment. We decided to test this strategy by first synthesizing the cosalane analogue **28**, having a linker chain of the same length as that of cosalane (**1**) itself. Reaction of the ylide derived by deprotonation of diethyl cyanomethylphosphonate with 6-cholestanone (**21**) afforded 6-cyanomethylenecholestane (**22**). The *E*

geometry of the double bond in **22** was established by X-ray crystallography. Selective reduction of the olefin unit of the α,β-unsaturated nitrile moiety present in **22** was accomplished using a sodium hydridocuprate complex, formed by reaction of sodium bis(2-methoxyethoxy)aluminum hydride with cuprous bromide in the presence of 2-butanol, as a proton donor, in tetrahydrofuran.[14,15] The crude product consisted of a mixture of 6β and 6α isomers in a 15:1 ratio, reflecting stereoselective hydride delivery to the side of the steroid opposite to the C-10 angular methyl group. Crystallization of the mixture of diastereomers from a mixture of methanol and ether afforded the pure 6β epimer **23**. Reduction of the nitrile **23** to the aldehyde **24** was accomplished with diisobutylaluminum hydride in toluene.[16] The one-carbon homologation of the aldehyde **24** was initiated by the Wittig reaction with methoxymethylene–triphenylphosphorane to yield a 1:1 mixture of *E*- and *Z*-enol ethers (**25**).[17] Hydrolysis of the mixture of enol ethers **25** with ethereal perchloric acid gave the desired intermediate aldehyde **26**.[18] A McMurry reaction of the aldehyde **26** with the substituted benzophenone **8** in the presence of titanium trichloride–DME complex and zinc–copper couple afforded the cross-coupled compound **27**.[19] Demethylation of **27** by heating it with boron tribromide–dimethylsulfide complex in 1,2-dichloroethane afforded the target compound **28**.

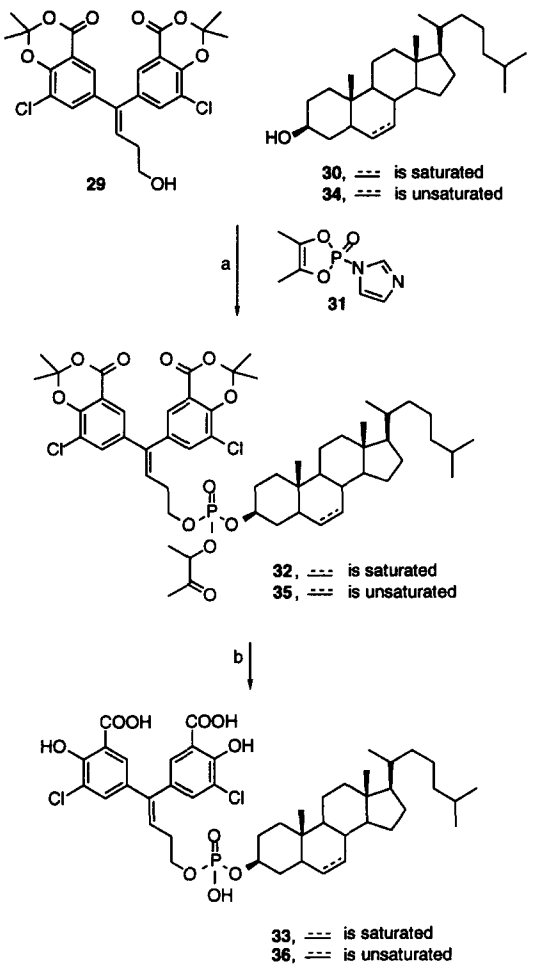
The synthesis of the cosalane analogue **33** having a phosphodiester linkage inserted into the linker chain is outlined in Scheme 5. This pathway relies on the use of the acetoin enediol cyclophosphoimidazole reagent **31**, which is ideally suited for the preparation of unsymmetrical phosphodiesters.[20] Sequential treatment of **31** with the primary alcohol **29**,[2] followed by 3β-cholestanol (**30**), afforded the phosphotriester **32**. The 3-keto-2-butyl group, as well as the two acetonide protecting groups, were then removed from **32** on heating with potassium carbonate in refluxing dioxane. An identical synthesis starting from cholesterol (**34**) yielded the related cosalane congener **36** (Scheme 5).

Biological Results and Discussion

Since a number of phospholipids have displayed anti-HIV activity,[5-9] a phosphodiester moiety was inserted into the linker chain of cosalane (**1**) in order to convert it to the phospholipid analogue **33**. The anti-HIV activities of phospholipids are thought to be due to their incorporation into biological membranes, and it was reasoned that the cholestane and attached phosphate of **33** might imbed in the viral envelope and cell membrane with the dichlorodisalicylmethane fragment protruding outward as depicted in Figure 1. Also, the inhibition of postbinding fusion may be involved in the mechanism of action of cosalane, and a number of membrane-interactive phospholipids have been found to inhibit membrane fusion.[10] For these reasons, it was hoped that the cosalane-phospholipid analogue **33** would have enhanced antiviral activity relative to cosalane itself. However, this was decidedly not the case, as it was found that the insertion of a phosphodiester group into the linker chain of cosalane resulting in **33** completely abolished the anti-HIV activity (Table 1). In addition, besides failing to protect the cells against the cytopathic effect of HIV, the phosphodiester **33** proved to be a cytotoxic agent in CEM cell culture (IC_{50} 40 μM), in contrast to cosalane (**1**), which is not cytotoxic even at a concentration of 300 μM. Identical results were obtained with the analogue **36** having a cholesterol moiety instead of a cholestane unit. The results with **33** and **36** are similar to those encountered previously with the phosphodiesters **37–41**, which were also cytotoxic and were either inactive as anti-HIV agents or were less potent than their counterparts lacking the phosphodiester insertion.[2] In summary, the phosphodiester modification leads to a loss of anti-HIV activity and an increase in



Scheme 4. Reagents and conditions: (a) (1) $(EtO)_2POCH_2CN$, $NaNH_2$, THF, 23 °C (3 h); (2) ketone **21**, THF, 23 °C (20 h); (b) CuBr, THF, $Na(CH_3OCH_2CH_2O)_2AlH_2$, $PhCH_3$, 2-BuOH, 0 °C (2 h), 23 °C (5 h); (c) DIBAL, $PhCH_3$, −78 °C (1.5 h); (d) (1) $Ph_3P^+CH_2OCH_3$ Cl^-, $NaN(SiMe_3)_2$, THF, CH_2Cl_2, 0 °C (25 min), 23 °C (30 min); (2) **24**, THF, 0 °C (2.5 h); (e) $HClO_4$, Et_2O, 23 °C (1.5 h); (f) (1) $TiCl_3$–DME, Zn–Cu, DME, reflux (90 min); (2) **26**, **8**, DME, reflux (1 h); (g) BBr_3–SMe_2, $ClCH_2CH_2Cl$, 80 °C (3 h).



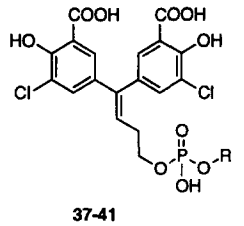
Scheme 5. Reagents and conditions: (a) $CHCl_3$, −10 °C (15 min), 23 °C (2.5 h); (b) (1) aq. K_2CO_3, 1,4-dioxane, reflux (3 h); (2) 3 N HCl, 23 °C (1.5 h).

cytotoxicity, and one may wonder whether these phosphates are simply acting as surfactants. The phosphodiester moiety might also restrict the sliding motion of the linker chain in the membrane (Fig. 1), thus making it more difficult to achieve optimal positioning of the compound in the membrane for biological activity.

Table 1. Anti-HIV activities of cosalane analogues

Compd	EC_{50}[a] (μM)	IC_{50}[b] (μM)
1	3.0	>300
10	25	>320
17	3.4	>200
20	20	>310
28	19	>100
33	NA[c]	40
36	NA[c]	39

[a]The EC_{50} is the 50% inhibitory concentration for cytopathicity of HIV-1_{RF} in CEM-SS cells. The values are the averages of at least two determinations. All compounds were tested as their ammonium salts.
[b]The IC_{50} is the 50% cytotoxic concentration for mock-infected CEM-SS cells. The values are the averages of at least two determinations. All compounds were tested as their ammonium salts.
[c]No antiviral activity was observed at the IC_{50} concentration.

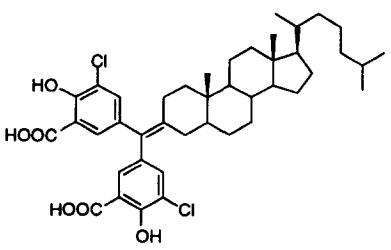


37-41

37 R = $(CH_2)_{13}CH_3$
38 R = $(CH_2)_{15}CH_3$
39 R = $(CH_2)_{17}CH_3$
40 R = *cis,cis*-$(CH_2)_6CH{=}CH(CH_2)_3CH{=}CH(CH_2)_3CH_3$
41 R = *cis*-$(CH_2)_8CH{=}CH(CH_2)_7CH_3$

The effect of linker chain length was also probed by examining the anti-HIV activities of analogue **10**, having a linker chain shortened by one carbon relative to cosalane (**1**), and congener **17**, in which the linker chain is one carbon atom longer than that of cosalane (**1**). The anti-HIV potency of **17** (EC_{50} 3.4 μM), having the longer linker chain, was essentially identical with that of cosalane (**1**) itself (EC_{50} 3.0 μM) against HIV-1_{RF} in CEM-SS cell culture. The analogue **10** (EC_{50} 25 μM) with the shorter linker chain was less potent than cosalane by approximately one order of magnitude. The elimination of the linker chain altogether results in compound **42**, which has previously been synthesized and found to have an EC_{50} value of 83.3 μM for the inhibition of HIV-1_{RF} cytopathicity in CEM-SS cells.[4] Taken together, the results seem to indicate a reduction in activity when the linker chain is shortened or eliminated, and no change in activity accompanying chain lengthening by one carbon atom.

As evidenced by the anti-HIV activity of **20** (EC_{50} 20 μM), the saturation of the double bond of cosalane resulted in a small decrease in potency. A loss of activity of comparable magnitude was also seen when the linker chain was moved from C-3 in cosalane (**1**) to C-6 in **28** (EC_{50} 19 μM). This observation, in conjunction with the previously reported anti-HIV activities of compounds **43** (IC_{50} 28 μM) and **44** (IC_{50} 24 μM), indicates that the orientation of the steroid moiety relative to the dichlorodisalicylmethane pharmacophore and the configuration of the A/B ring fusion are not of critical importance for the anti-HIV activity.[4] Indeed, replacement of the steroid and linker chain of cosalane (**1**) with a C-30 straight-chain hydrocarbon results in the active anti-HIV agent **45** (IC_{50} 13 μM).[2]



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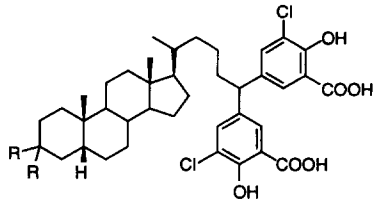
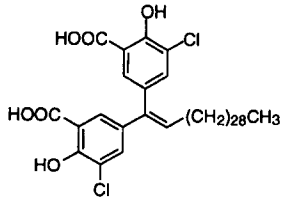


43 R = H
44 R = F



45

The function of the steroid moiety of cosalane, therefore, appears to be only to provide a lipophilic appendage for the dichlorodisalicylmethane pharmacophore that increases its potency dramatically. Prior work has demonstrated that the potency of cosalane congeners related to **45**, with straight-chain lipophilic groups, correlates with the length and therefore with the lipophilicity of the hydrocarbon chain.

Experimental

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; FABMS and EIMS on a Kratos MS50 spectrometer; ^{1}H NMR spectra on Varian VXR-500S, XL-200A, and Brüker ARX-300 spectrometers; IR spectra on a Beckman IR-33 spectrometer or on a Perkin–Elmer 1600 series FTIR. Microanalyses were performed at the Purdue Microanalysis Laboratory.

5α-3-Ethoxycarbonylmethylenecholestane (3). This compound was prepared in 94% yield according to a published procedure,[11] except THF was used as a solvent for **1** instead of DMF: mp 73–74 °C; lit.[11] mp 83–85 °C; IR (KBr): 2929, 2858, 1649, 1462, 1382, 1219, 1147, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.58 (m, 1 H), 4.13 (q, J=7 Hz, 2 H), 1.27 (t, J=7.0 Hz, 3 H), 0.904 (s, 3 H), 0.895 (d, J=6.5 Hz, 3 H); 0.862 (d, J=6.5 Hz, 3 H), 0.858 (d, J=6.5 Hz, 3 H), 0.654 (s, 3 H), 0.56 (s, 3 H); CIMS *m/z* (relative intensity): 456 (MH^+, 88).

5α-3β-Ethoxycarbonylmethylcholestane (4). The olefin **3** (4.22 g, 9.24 mmol) was hydrogenated over PtO_2 (0.8 g) in ethyl acetate (30 mL) at room temperature for 1.5 h and at 50 °C for 2 h. The catalyst was filtered off, washed with ethyl acetate, and the filtrate was evaporated in vacuo to yield an oil (4.109 g, 97%). The analytical sample was prepared by crystallization from a mixture of methanol and ethyl acetate: mp 81–83 °C; IR (KBr): 2930, 2853, 1731, 1465, 1379, 1299, 1175, 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.10 (q, J=7.0 Hz, 2 H), 1.23 (t, J=7.0 Hz, 3 H), 0.87 (d, J=6.5 Hz, 3 H), 0.842 (d, J=6.5 Hz, 3 H), 0.836 (d, J=6.5 Hz, 3 H), 0.76 (s, 3 H), 0.62 (s, 3 H); CIMS *m/z* (relative intensity): 459 (MH^+, 100). Anal. calcd for $C_{31}H_{54}O_2$: C, 81.16; H, 11.86. Found: C, 81.10; H, 12.05%.

5α-3β-(2′-Hydroxyethyl)cholestane (5). A solution of ester **4** (4.324 g, 9.422 mmol) in dry THF (30 mL) was added dropwise to a 1 M solution of LAH in ether (12.4 mL, 12.4 mmol) and the mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched with water (0.48 mL), 15% sodium hydroxide (0.48 mL), and water (1.43 mL), diluted with methylene chloride (10 mL), filtered, and the precipitate on the filter was washed with methylene chloride (3×5 mL). The filtrate was evaporated to dryness to afford alcohol **5** as a white solid (3.594 g, 91%). The analytical sample was recrystallized from ethanol: mp 94 °C; IR (KBr): 3332, 2926, 2853, 1462, 1378, 1299, 1062 cm^{-1}; ^{1}H NMR of the major isomer ($CDCl_3$, 200 MHz) δ: 3.66 (t, J=6.7 Hz, 2 H), 0.89 (d, J=6.7 Hz, 6 H), 0.86 (d, J=6.7 Hz, 3 H), 0.78 (s, 3 H), 0.64 (s, 3 H); CIMS *m/z* (relative intensity): 371 ($MH^+ - H_2O - H_2C{=}CH_2$, 8), 262 (100). Anal. calcd for $C_{29}H_{52}O$: C, 83.58; H, 12.58. Found: C, 83.26; H, 12.72.

5α-3β-(2′-Bromoethyl)cholestane (6). A solution of triphenylphosphine (3.370 g, 12.85 mmol) in dry methylene chloride (16 mL) was added dropwise with stirring to the solution of alcohol **5** (3.594 g, 8.624 mmol) and carbon tetrabromide (3.575 g, 10.78 mmol) in dry methylene chloride (12 mL) at 0 °C. The mixture was stirred in an ice bath for 10 min, the solvent was removed in vacuo, and the residue was stirred with ether (5×6 mL). The combined organic extracts were filtered, evaporated in vacuo, and flash chromatographed on silica gel (250 g), eluting with hexane:ethyl acetate (19:1) to afford the product **6** (3.85 g, 93%). An analytical sample was obtained by crystallization from acetone: mp 75–76 °C; IR (KBr): 2930, 2856, 1461, 1377, 1260 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.39 (t, J=6.7 Hz, 2 H), 0.88 (d, J=7 Hz, 3 H), 0.892 (d, J=6.5 Hz, 3 H), 0.888 (d, J=6.5 Hz, 3 H), 0.76 (s, 3 H), 0.616 (s, 3 H); CIMS *m/z* (relative intensity): 479 (MH^+, 81), 399 ($MH^+ - HBr$). Anal. calcd for $C_{29}H_{51}Br$: C, 72.62; H, 10.72. Found: C, 72.81; H, 11.10%.

(5α)-3β-(2′-Triphenylphosphoniumethyl)cholestane bromide (7). A solution of triphenylphosphine (1.908 g, 7.276 mmol) and bromide **6** (3.490 g, 7.276 mmol) in chlorobenzene (7 mL) was heated at 140 °C (oil bath temperature) for 24 h. The solvent was removed in vacuo and the residue was triturated with hexane. The phosphonium salt **7** (4.249 g, 79%) was filtered, washed

with hexane, and dried in a vacuum desiccator: mp 150 °C. IR (KBr): 2928, 2861, 1626, 1439, 1378, 1190, 744, 692 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.9–7.8 (m, 15 H), 3.75 (m, 2 H), 0.86 (d, $J=5.3$ Hz, 3 H), 0.83 (d, $J=6.3$ Hz, 6 H); 0.69 (s, 3 H), 0.585 (s, 3 H); FABMS *m/z* (relative intensity): 661 (M^+, 100).

5α-3β-[3′,3′-(3″,3‴-Dicarbomethoxy-5″,5‴-dichloro-4″,4‴-dimethoxydiphenyl)-2′-propenyl]cholestane (9). A solution of the phosphonium salt **7** (0.378 g, 0.51 mmol) in dry THF (9 mL) was treated with sodium bis(trimethylsilyl)amide (1 M solution, 0.51 mL) for 30 min at 0 °C. A solution of the ketone **8** (0.218 g, 0.51 mmol) in dry THF (5 mL) was added dropwise and the mixture was stirred at room temperature for 2 days. The reaction mixture was quenched with a solution of ammonium chloride (60 mg) in water (3 mL). The organic layer was separated and the aqueous layer extracted with ether (5 mL). The combined organic extracts were washed with brine (2 × 5 mL), dried (sodium sulfate) and the solvent was evaporated in vacuo to afford a semisolid (0.58 g) which was flash chromatographed on silica gel (28 g). Elution with hexane:ethyl acetate (6:1) yielded the alkene **9** as a glass (0.24 g, 64%), which was crystallized twice from a mixture of hexane, ethyl acetate, and ethanol: mp 96–98 °C; IR (KBr): 2932, 2862, 1735, 1475, 1438, 1261, 1206, 1001 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.47 (m, 1 H), 7.45 (m, 1 H), 7.30 (m, 1 H), 7.27 (m, 1 H), 6.07 (t, $J=7.6$ Hz, 1 H), 3.99 (s, 3 H), 3.91 (s, 6 H), 3.913 (s, 3 H), 3.906 (s, 3 H), 0.86 (d, $J=6.5$ Hz, 3 H), 0.845 (d, $J=6.5$ Hz, 3 H), 0.840 (d, $J=6.5$ Hz, 3 H), 0.72 (s, 3 H), 0.59 (s, 3 H); FABMS *m/z* (relative intensity): 809 (MH^+, 19), 777 ($MH^+ - CH_3OH$, 100). Anal. calcd for $C_{48}H_{66}Cl_2O_6$: C, 71.18; H, 8.21. Found: C, 71.19; H, 8.25%.

5α-3β-[3′,3′-(3″,3‴-Dicarboxy-5″,5‴-dichloro-4″,4‴-dihydroxydiphenyl)-2′-propenyl]cholestane (10). Boron tribromide–dimethyl sulfide complex (1 M solution in methylene chloride, 3.4 mL) was added dropwise to a stirred solution of diester **9** (0.177 g, 0.219 mmol) in dichloroethane (12 mL) and the mixture was stirred at 60 °C for 24 h. The reaction mixture was cooled on an ice bath, quenched with water (5 mL), and stirred for 1 h. Ethyl acetate was added to dissolve the precipitate and the organic layer was separated. The aqueous layer was extracted with chloroform (1 × 5 mL). The combined organic extracts were washed with water, dried (sodium sulfate), and evaporated to dryness. The solid residue was flash chromatographed on silica gel (15 g). Elution with a mixture of chloroform, THF, and 97% formic acid (150:30:0.5) yielded the product **10** (97 mg, 60%). The analytical sample was prepared by crystallization from a mixture of methylene chloride and acetone: mp 278 °C. IR (KBr): 3500–2600, 2927, 2856, 1668, 1607, 1446, 1232, 1181, 714 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz): δ 7.71 (d, $J=2.0$ Hz, 1 H), 7.68 (d, $J=2.0$ Hz, 1 H), 7.58 (d, $J=2.0$ Hz, 1 H), 7.47 (d, $J=2.0$ Hz, 1 H), 6.20 (t, $J=7.6$ Hz, 1 H), 0.92 (d, $J=6.5$ Hz, 3 H), 0.85 (dd, $J=6.5$ and 2.0 Hz, 6 H), 0.75 (s, 3 H), 0.67 (s, 3 H); FABMS *m/z* (relative intensity): 683 (MH^+, 20), 735 (MH^+-H2O, 51). Anal. calcd for $C_{44}H_{58}Cl_2O_6 \times 3/2H_2O$: C, 67.68; H, 7.87. Found: C, 67.46; H, 7.84%. The diammonium salt showed mp 156 °C.

Triphenyl-3-ethoxycarbonylpropylphosphonium bromide. A solution of triphenylphosphine (34.80 g, 32.68 mmol) and ethyl γ-bromobutyrate (25.88 g, 32.68 mmol) in acetonitrile (35 mL) was stirring at reflux for 40 h. The solvent was removed in vacuo and the residue was triturated with hexane. The phosphonium salt was filtered, washed with dry ether, and dried in a vacuum desiccator: mp 182 °C; IR (KBr): 3407, 3012, 2888, 1726, 1622, 1439, 1379, 1324, 1243, 1185, 1116, 750, 694 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz): δ 7.9–7.7 (m, 15 H), 4.05 (q, $J=7.1$ Hz, 2 H), 3.56 (m, 2 H), 1.75 (m, 2 H), 1.15 (t, $J=7.1$ Hz, 3 H); FABMS *m/z* (relative intensity): 377 (M^+, 100); Anal. calcd ($C_{24}H_{26}BrPO_2$) C, H.

(5α)-3-[3-Ethoxycarbonylpropylene]cholestane (11). A solution of triphenyl-3-ethoxycarbonylpropylphosphonium bromide (9.159 g, 20.03 mmol) in dry DMF (50 mL) was added to a suspension of sodium hydride (50%, 801 mg, 20.03 mmol) in dry DMF (15 mL) and the deoxygenated mixture was stirred at room temperature under an argon atmosphere for 1 h. A solution of cholestan-3-one (**2**; 3.867 g, 10 mmol) in dry THF (20 mL) was added dropwise and the mixture was stirred at 70 °C for 3.5 h. The reaction mixture was poured on an ice–water mixture (400 g) and extracted with ether (2 × 150 mL). The combined organic extracts were washed with 0.5 M HCl (1 × 100 mL) and water (4 × 200 mL) and were dried (sodium sulfate). The semisolid residue obtained after evaporation of the solvent was stirred with hexane (40 mL), and the precipitate was filtered off and washed with hexane (5 × 10 mL). The oil (5.26 g) obtained after evaporation of the solvent was purified by flash chromatography on silica gel (300 g). The column was washed with a 39:1 mixture of hexane and ethyl acetate, and the product (4.085 g) was eluted with a 6:1 mixture of hexane and ethyl acetate to afford an oil which solidified in the refrigerator. The analytical sample was prepared by crystallization from a mixture of ethyl acetate and methanol: mp 88–90 °C; IR (KBr): 2930, 2867, 1739, 1466, 1445, 1374, 1162, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 5.00 (m, 1 H), 4.10 (q, $J=7.2$ Hz, 2 H), 1.23 (t, $J=7.2$ Hz, 3 H), 0.872 (d, $J=6.5$ Hz, 3 H), 0.837 (d, $J=6.5$ Hz, 3 H), 0.333 (d, $J=6.5$ Hz, 3 H), 0.826 (s, 3 H), 0.624 (s, 3 H); FABMS *m/z* (relative intensity): 485 (MH^+, 100). Anal. calcd for $C_{33}H_{56}O_2$: C, 81.76; H, 11.64. Found: C, 81.43; H, 11.79%.

5α-3β-(3′-Ethoxycarbonylpropyl)cholestane (12) and 5α-3α-(ethoxycarbonylpropyl)cholestane (4:1 mixture). The olefin **11** (2.243 g, 6.36 mmol) was hydrogenated over PtO_2 (0.416 g) in ethyl acetate (16 mL) at room temperature for 4 h. The catalyst was filtered off, washed with ethyl acetate, and the filtrate was evaporated in vacuo to yield an oil (2.221 g) containing a mixture of 3β and 3α diastereoisomers in 4:1 ratio: IR

(neat): 2931, 2850, 1739, 1462, 1376, 1176, 1040 cm^{-1}; ^1H NMR of the major 3β isomer ($CDCl_3$, 500 MHz): δ 4.10 (q, $J=7.0$ Hz, 2 H), 2.24 (t, $J=7.3$ Hz, 2 H), 1.23 (t, $J=7.0$ Hz, 3 H), 0.87 (d, $J=6.5$ Hz, 3 H), 0.839 (d, $J=6.5$ Hz, 3 H), 0.834 (d, $J=6.5$ Hz, 3 H), 0.71 (s, 3 H), 0.62 (s, 3 H); CIMS *m/z* (relative intensity): 487 (MH^+, 100). Anal. calcd for $C_{33}H_{58}O_2$: C, 81.42; H, 12.01. Found: C, 81.30; H, 12.31%.

5α-3β-(4′-Hydroxybutyl)cholestane (13). A solution of the 4:1 diastereomeric mixture **12** and its 3α isomer (2.218 g, 4.556 mmol) in dry THF (15 mL) was added dropwise to a 1 M solution of LAH in ether (6 mL) and the mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched with water (0.25 mL), 15% sodium hydroxide (0.25 mL), and water (0.75 mL), diluted with methylene chloride (5 mL), filtered, and the precipitate on the filter was washed with methylene chloride (3 × 5 mL). The filtrate was evaporated to dryness to afford alcohol **13** and its 3α isomer as a white solid (1.922 g, 94.8%). The pure 3β isomer was obtained after recrystallization of the solid three times from ethanol: mp 100 °C; IR (KBr): 3347, 2926, 2856, 1459, 1378, 1056 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 3.62 (t, $J=6.5$ Hz, 2 H), 0.87 (d, $J=6.5$ Hz, 3 H), 0.839 (d, $J=6.5$ Hz, 3 H), 0.834 (d, $J=6.5$ Hz, 3 H), 0.72 (s, 3 H), 0.62 (s, 3 H); CIMS *m/z* (relative intensity): 444 (M^+, 53), 443 (100), 427 ($MH^+ - H_2O$, 46). Anal. calcd for $C_{31}H_{56}O$: C, 83.71; H, 12.69. Found: C, 83.51; H, 12.98%.

5α-3β-(4′-Bromobutyl)cholestane (14). A solution of triphenylphosphine (0.945 g, 3.601 mmol) in dry methylene chloride (5 mL) was added dropwise with stirring to the solution of alcohol **13** (1.144 g, 2.572 mmol) and carbon tetrabromide (1.066 g, 3.215 mmol) in dry methylene chloride (10 mL) at 0 °C. The mixture was stirred in an ice bath for 35 min, the solvent was removed in vacuo, and the residue was stirred with hexane (4 × 10 mL). The combined organic extracts were filtered, evaporated in vacuo and flash chromatographed on silica gel (60 g) to afford the product (1.24 g, 99%): mp 62 °C; IR (KBr): 2928, 2855, 1460, 1378, cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 3.38 (t, $J=7$ Hz, 2 H), 0.874 (d, $J=6.5$ Hz, 3 H), 0.840 (d, $J=6.5$ Hz, 3 H), 0.836 (d, $J=6.5$ Hz, 3 H), 0.72 (s, 3 H), 0.62 (s, 3 H); CIMS *m/z* (relative intensity): 507 (MH^+, 96), 427 (34). Anal. calcd for $C_{31}H_{55}Br$: C, 73.34; H, 10.92. Found: C, 73.57; H, 11.15.

5α-3β-(4′-Triphenylphosphoniumbutyl)cholestane bromide (15). A solution of triphenylphosphine (109 mg, 0.416 mmol) and bromide **14** (210.7 mg, 0.416 mmol) in chlorobenzene (1 mL) was heated at 140 °C for 24 h. The solvent was removed in vacuo and the residue was triturated with hexane. The phosphonium salt (165 mg, 51.4%) was filtered, washed with hexane, and dried in a vacuum desiccator: mp 135–140 °C; IR (KBr): 2931, 2861, 1636, 1439, 1112, 727, 691 cm^{-1}; ^1H NMR (CD_3OD, 200 MHz): δ 7.9–7.7 (m, 15 H), 0.91 (d, $J=6.6$ Hz, 3 H), 0.87 (d, $J=6.7$ Hz, 6 H); 0.75 (s, 3 H), 0.67 (s, 3 H); FABMS *m/z* (relative intensity): 689 (M^+, 31).

5α-3β-[5′,5′-(3″,3‴-Dicarbomethoxy-5″,5‴-dichloro-4″,4‴-dimethoxydiphenyl)-4′-pentenyl]cholestane (16). This intermediate was obtained using a procedure similar to that used for the preparation of **9**: mp 62 °C (hexane: ethanol); IR (KBr): 2933, 2862, 1734, 1474, 1260, 1207, 1004 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.463 (d, $J=2.4$ Hz, 1 H), 7.45 (d, $J=2.1$ Hz, 1 H), 7.30 (d, $J=2.1$ Hz, 1 H), 7.27 (d, $J=2.4$ Hz, 1 H), 6.04 (t, $J=7.0$ Hz, 1 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H), 0.87 (d, $J=6.5$ Hz, 3 H), 0.838 (d, $J=6.5$ Hz, 3 H), 0.834 (d, $J=6.5$ Hz, 3 H), 0.70 (s, 3 H), 0.61 (s, 3 H); FABMS *m/z* (relative intensity): 836 (M^+, 8), 805 ($MH^+ - CH_3OH$, 100). Anal. calcd for $C_{50}H_{70}Cl_2O_6$: C, 71.66; H, 8.42. Found: C, 71.52; H, 8.67%.

5α-3β-[5′,5′-(3″,3‴-Dicarboxy-5″,5‴-dichloro-4″,4‴-dihydroxydiphenyl)-2′-propenyl]cholestane (17). Boron tribromide–dimethyl sulfide complex (1 M solution in methylene chloride, 1.4 mL) was added dropwise to a stirred solution of diester **16** (0.138 g, 0.165 mmol) in dichloroethane (8 mL) and the mixture was stirred at 70 °C for 16 h. The reaction mixture was cooled in an ice bath, quenched with water (5 mL), and stirred for 1 h. Ethyl acetate was added to dissolve a precipitate and the organic layer was separated. The aqueous layer was extracted with chloroform (1 × 3 mL). The combined organic extracts were washed with water, dried (sodium sulfate), and evaporated to dryness. The solid residue was crystallized from methylene chloride: mp 257 °C; IR (KBr): 3500–2600, 2929, 2861, 1667, 1606, 1445, 1232, 1179, 713 cm^{-1}; ^1H NMR (acetone-d_6, 200 MHz): δ 7.70 (m, 2 H), 7.60 (d, $J=2.6$ Hz, 1 H), 7.51 (d, $J=2.4$ Hz, 1 H), 6.20 (t, $J=7.6$ Hz, 1 H), 0.92 (d, $J=6.5$ Hz, 3 H), 0.86 (d, $J=6.5$ Hz, 6 H), 0.75 (s, 3 H), 0.67 (s, 3 H); FABMS *m/z* (relative intensity): 780 (MH^+, 4). Anal. calcd for $C_{46}H_{62}Cl_2O_6 \times 5/2H_2O$: C, 66.81; H, 8.17. Found: C, 67.07; H, 8.01%. The diammonium salt showed mp 236 °C.

5α-3β-[4′,4′-(3″,3‴-Dicarbomethoxy-5″,5‴-dichloro-4″,4‴-dimethoxydiphenyl)butyl]cholestane (19). The Wittig olefin **18** (68 mg, 0.082 mmol) was hydrogenated over PtO_2 (18 mg) in ethyl acetate (8 mL) at room temperature for 45 min. The catalyst was filtered off, washed with ethyl acetate, and the filtrate was evaporated in vacuo to yield a solid (67 mg, 99%). An analytical sample was prepared by crystallization from a mixture of hexane and ethyl acetate: mp 113–114 °C; IR (KBr): 2931, 2858, 1733, 1473, 1259, 1198, 1001 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.45 (d, $J=2.0$ Hz, 2 H), 7.275 (d, $J=2$ Hz, 2 H), 3.86 (s, 6 H), 3.84 (s, 6 H), 3.75 (t, $J=7.6$ Hz, 1 H), 0.82 (d, $J=6.5$ Hz, 3 H), 0.79 (d, $J=6.5$ Hz, 6 H); 0.65 (s, 3 H), 0.56 (s, 3 H); FABMS *m/z* (relative intensity): 824 (M^+, 9), 136 (100). Anal. calcd for $C_{49}H_{70}Cl_2O_6$: C, 71.25; H, 8.54. Found: C, 71.48; H, 8.87%.

5α-3β-[4′,4′-(3″,3‴-Dicarboxy-5″,5‴-dichloro-4″,4‴-dihydroxydiphenyl)butyl]cholestane (20). Boron tribromide–dimethyl sulfide complex (1 M solution in methylene chloride, 1.54 mL) was added dropwise to a stirred solution of diester **19** (0.108 g, 0.131 mmol) in dichloroethane (9 mL) and the mixture was stirred at 70 °C for 8 h and at room temperature overnight. The reaction mixture was cooled on an ice bath, quenched with water (5 mL), and stirred for 1 h. Ethyl acetate was added to dissolve the precipitate and the organic layer was separated. The aqueous layer was extracted with chloroform (1 × 5 mL). The combined organic extracts were washed with water, dried (sodium sulfate), and evaporated to dryness. The solid residue (0.11 g) was purified by flash chromatography on silica gel (10 g). Elution with chloroform:THF:97% formic acid (150:30:0.5) yielded the pure diacid **20** (77 mg, 76%). The analytical sample was prepared by crystallization from methylene chloride: mp 252 °C; IR (KBr): 3500–2600, 2928, 2852, 1667, 1609, 1443, 1236, 1182, 712 cm^{-1}; ^1H NMR (acetone-d_6, 200 MHz): δ 7.84 (broad s, 2 H), 7.67 (broad s, 2 H), 4.05 (t, J = 8.0 Hz, 1 H), 0.915 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 6.5 Hz, 6 H), 0.74 (s, 3 H), 0.66 (s, 3 H); FABMS *m/z* (relative intensity): 791 ($MH^+ + Na$, 5), 768 (MH^+, 1.5), 752 ($MH^+ - H_2O$, 1.5), 136 (100). Anal. calcd for $C_{45}H_{62}Cl_2O_6$: C, 70.20; H, 8.12. Found: C, 70.49; H, 8.28%. The ammonium salt showed mp 242 °C.

5(α)-*E*-6-Cyanomethylenecholestane (22). Diethyl cyanomethylphosphonate (7.19 g, 6.57 mmol) was added to a solution of sodium amide (1.80 g, 46.1 mmol) in THF (40 mL). The mixture was stirred at room temperature for 3 h. A solution of 6-cholestanone (3.92 g, 10.14 mmol) in THF (150 mL) was added dropwise to the solution over a period of 30 min, and the mixture was stirred at room temperature for 20 h. The THF was evaporated, cold water (40 mL) was added, and the product was extracted with ether (3 × 30 mL). The ether was evaporated and the solid residue recrystallized from ether (10 mL) to afford the product **22** (3.60 g, 87%): mp 86–87 °C; IR (KBr): 2943, 2860, 2214, 1618, 1457, 1378 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 5.28 (s, 1 H), 2.88 (dd, J = 12.9; 4 Hz, 1 H), 1.975 (dt, J = 12.9; 6.9 Hz, 1 H), 0.88 (d, J = 6.5 Hz, 3 H); 0.844 (d, J = 6.5 Hz, 3 H), 0.840 (d, J = 6.5 Hz, 3 H), 0.626 (s, 6 H); CIMS *m/z* (relative intensity): 410 (MH^+, 100). Anal. calcd for $C_{29}H_{47}N$: C, 85.02; H, 11.56; N, 3.42. Found: C, 84.79; H, 11.85; N, 3.13%.

(5α)-6β-Cyanomethylcholestane (23). Copper(I) bromide (2.10 g, 14.6 mmol) was placed in a three-necked flask purged with argon and equipped with a reflux condenser, a mechanical stirrer and a septum. Dry oxygen-free THF (20 mL) was added, the mixture was cooled in an ice-bath, and a solution of Red-Al [sodium bis(2-methoxyethoxy)aluminum hydride] in toluene (65%, 8.8 mL) was added dropwise. The dark solution was stirred 30 min and was cooled in a dry ice–acetone bath. Anhydrous 2-butanol (2.69 mL) was slowly added dropwise, followed by a solution of (5α)-6-cyanomethylenecholestane (**22**; 600 mg, 1.46 mmol) in THF (10 mL). After 2 h the cooling bath was removed and the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was cooled in a dry ice–acetone bath, and saturated ammonium chloride solution (6.8 mL) was added dropwise, followed by brine (20 mL). The bath was removed and the mixture was stirred for 10 min. The organic layer was decanted, ether (15 mL) was added, and the solid residue was stirred for 5 min. The procedure was repeated twice. The combined organic extracts were washed with brine (2 × 30 mL) and dried (magnesium sulfate). The solvents were removed in vacuo and the residue (0.645 g) was chromatographed on silica gel (25 g, hexane:ethyl acetate, 19:1) to yield the product as a yellowish solid (382 mg), which consisted of mixture of 6β/6α isomers in a 15:1 ratio. Crystallization from a methanol–ether mixture yielded the 6β epimer (320 mg, 53%): mp 103–104 °C; IR (KBr): 2930, 2860, 2244, 1445, 1381, 1261, 1095, 1024, 804 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 0.87 (d, J = 6.5 Hz, 3 H), 0.884 (d, J = 6.5 Hz, 3 H), 0.879 (d, J = 6.5 Hz, 3 H), 0.76 (s, 3 H), 0.64 (s, 3 H); CIMS *m/z* (relative intensity): 412 (MH^+, 60), 369 ($MH^+ - CH_3CN$, 100); HRCIMS: calcd for M^+: 411.3869; found: 411.3869. Anal. calcd for $C_{29}H_{49}N$: C, 84.60; H, 11.00; N, 3.40. Found: C, 84.58; H, 12.31; N, 3.39%.

(5α)-6β-Formylmethylcholestane (24). The nitrile **23** (411.6 mg, 1 mmol) was dissolved in toluene (12 mL, dried over the molecular sieves 4 Å) under argon, the solution was cooled in a dry ice–acetone bath, and DIBAL (1.5 mL, 1 M solution in toluene, 1.5 mmol) was added dropwise. The mixture was stirred for 1.5 h and ethyl acetate (1 mL) was added followed by a satd soln of ammonium chloride (5 mL). The cooling bath was removed and the reaction mixture was stirred for 15 min. HCl (0.5 M, 6 mL) was added and stirring was continued for 10 min. The organic phase was removed and the remaining white salts were dissolved in water (10 mL) and the solution was extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with brine (2 × 30 mL) and dried (magnesium sulfate). The solvents were removed in vacuo and the obtained oil (0.50 g) was chromatographed on silica gel (18 g, hexane:ethyl acetate, 19:1) to yield the product as a colorless oil, 387 mg (93.3%): IR (KBr): 2951, 2854, 2710, 1727, 1468, 1445, 1383, 1265, 1022, 739 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 9.63 (m, 1 H), 0.87 (d, J = 6.5 Hz, 3 H), 0.841 (d, J = 6.5 Hz, 3 H), 0.837 (d, J = 6.5 Hz, 3 H), 0.81 (s, 3 H), 0.64 (s, 3 H); CIMS *m/z* (relative intensity): 415 (MH^+, 100), 370 ($M^+ - CH_3CHO$, 96). Anal. for the 2,4-DNP derivative: calcd for $C_{35}H_{54}N_4O_4 \times 1/2H_2O$: C, 69.62; H, 9.18; N, 9.28. Found: C, 69.89; H, 9.25; N, 9.04%.

(5α)-6β-(*E*,*Z*-3′-Methoxy-2-*n*-propenyl)cholestane (25). Methoxymethyltriphenylphosphonium chloride (670 mg, 1.95 mmol) was placed in a dry 50 mL two-neck flask and the apparatus was purged with argon. Dry oxygen-free THF (17 mL) was added, the mixture was cooled in an ice-bath, and sodium bis(trimethylsilyl)amide (1 M solution in methylene chloride, 1.9 mL, 1.9

mmol) was added dropwise. The red solution was stirred at 0 °C for 25 min and 30 min at room temperature. The reaction mixture was cooled again in the ice-bath. A solution of the aldehyde **24** (387 mg, 0.933 mmol) in dry THF (14 mL) was added dropwise and the mixture was stirred for 2.5 h at 0 °C. The reaction was quenched with satd ammonium chloride soln (5 mL), the organic phase was separated, and the aqueous phase was diluted with water (10 mL) and was extracted with ethyl acetate (3 × 5 mL). The combined organic extracts were washed with brine (2 × 20 mL) and dried (magnesium sulfate). The solvents were removed in vacuo and the obtained semisolid (1.00 g) was chromatographed on silica gel (35 g, hexane:ethyl acetate, 39:1) to afford a colorless oil (0.51 g) containing a mixture of *Z/E* olefins **25** in 2:1 ratio: IR (neat): 3031, 2926, 1652, 1469, 1445, 1384, 1268, 1213, 1111, 932, 742 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): of the major isomer δ 5.86 (d, $J = 16.3$ Hz, 1 H), 4.23 (m, 1 H), 3.53 (s, 3 H), 0.88 (d, 3 H), 0.86 (s, 3 H), 0.83 (d, $J = 6.1$ Hz, 6 H), 0.65 (s, 3 H); CIMS *m/z* (relative intensity): 443 (MH^+, 89), 409 ($MH^+ - CH_3OH$, 59); 371 ($MH^+ - C_3H_5OMe$, 64).

(5α)-6β-(2′-Formylethyl)cholestane (26). Perchloric acid (35%, 0.4 mL) was added to a solution of the above enol mixture **25** (0.51 g, 0.12 mmol) in ether (6 mL). The solution was stirred at room temperature for 1.5 h. Brine (5 mL) was added and the mixture was neutralized with aq sodium bicarbonate. The organic phase was separated and the aqueoeus phase was extracted with ethyl ether (2 × 5 mL). The combined organic extracts were washed with brine (2 × 10 mL) and dried (magnesium sulfate). The solvent was removed in vacuo and the resulting oil (0.45 g) was chromatographed on silica gel (18 g, hexane:ethyl acetate, 19:1) to afford a colorless oil (0.365 g, 91% overall yield from **24**); IR (neat): 2927, 2856, 1728, 1468, 1444, 1383 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 9.73 (m, 1 H), 0.88 (d, $J = 6.5$ Hz, 3 H), 0.84 (dd, $J = 6.5$ Hz, 6 H), 0.83 (s, 3 H), 0.65 (s, 3 H); CIMS *m/z* (relative intensity): 429 (MH^+, 30), 369 ($MH^+ - C_2H_5CHO$, 59); 371 ($MH^+ - C_3H_5OMe$, 64). Anal. for the 2,4-DNP derivative: calcd for $C_{36}H_{56}N_4O_4 \times 1/2H_2O$: C, 69.98; H, 9.30; N, 9.07. Found: C, 70.29; H, 9.65; N, 8.82%.

(5α)-6β-[4′,4′-(3″,3‴-Dicarbomethoxy-5″,5‴-dichloro-4″,4‴-dimethoxydiphenyl)-3′-butenyl]cholestane (27). Titanium trichloride–DME complex (207 mg) was placed in a dry argon purged two-necked flask, followed by zinc–copper couple (195 mg).[19] Dry DME (6 mL) was added and the black mixture was stirred at reflux for 90 min. The bath was removed and after 10 min a solution of the aldehyde **26** (35.5 mg, 0.828 mmol) and ketone **8** (35.4 mg, 0.028 mmol) in DME (2 mL) was added dropwise. The mixture was stirred at reflux for 1 h and 0.5 h at room temperature. Hexane (20 mL) was added and the mixture was stirred for 20 min. The contents of the flask were filtered through a Florisil column (3 g, 1 cm wide) and the flask and the column were washed with dry ether (3 × 3 mL). The solvents were removed in vacuo and the residue (108 mg) was chromatographed on silica gel (6 g, hexane: ethyl acetate, 6:1 and 3:1). The first fraction afforded a symmetrical olefin (10 mg) formed from the aldehyde **26**: IR (neat): 3054, 2923, 2863, 1468, 1435, 1379, 1119, 1027, 743, 695 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 5.33 (m, 1 H), 0.87 (d, $J = 7.1$ Hz, 3 H), 0.83 (d, $J = 6.4$ Hz, 6 H), 0.81 (s, 3 H), 0.64 (s, 3 H); the next fractions gave the cross-coupled product **27** (39.5 mg, 57.8%) as an oil: IR (neat): 2928, 2856, 1738, 1477, 1436, 1264, 1207, 1002, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.47 (d, $J = 2.4$ Hz, 1 H), 7.44 (d, $J = 2.2$ Hz, 1 H), 7.30 (d, $J = 2.2$ Hz, 1 H), 7.27 (d, $J = 2.4$ Hz, 1 H), 6.05 (t, $J = 7.2$ Hz, 1 H), 3.97 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 6 H), 0.87 (d, $J = 6.3$ Hz, 3 H), 0.84 (d, $J = 6.6$ Hz, 6 H), 0.79 (s, 3 H), 0.63 (s, 3 H). The last fractions afforded a symmetrical dimer (13 mg) formed from the ketone **8**.

(5α)-6β-[4′,4′-(3″,3‴-Dicarboxy-5″5‴-dichloro-4″,4‴-dihydroxydiphenyl)-3′-butenyl]cholestane (28). A solution of boron tribromide–dimethyl sulfide complex (1 M, 1.2 mL, 1.2 mmol) 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 (6 mL) was added, followed by a solution of dimethyl ester **27** (110 mg, 0.134 mmol) in 1,2-dichloroethane (2 mL). The mixture was stirred at 80 °C (oil bath) for 3 h. Brine (3 mL) was added with ice-bath cooling and the mixture was stirred at room temperature for 30 min. Ethyl acetate was added to dissolve the product, the organic phase was separated, and the aqueous phase was extracted with chloroform (3 mL). The combined organic extract was dried (sodium sulfate). The crude product (150 mg) obtained after evaporation of the solvent in vacuo was triturated with methylene chloride to afford the product **27** (44 mg, 43%): mp 192–194 °C; IR (KBr): 3500–2500, 2931, 2860, 1667, 1606, 1442, 1365, 1231, 1179 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.67 (d, $J = 2.1$ Hz, 1 H), 7.66 (d, $J = 2.3$ Hz, 1 H), 7.56 (d, $J = 2.3$ Hz, 1 H), 7.48 (d, $J = 2.1$ Hz, 1 H), 6.21 (t, $J = 7.6$ Hz, 1 H), 0.91 (d, $J = 6.4$ Hz, 3 H), 0.86 (d, $J = 5.8$ Hz, 6 H), 0.84 (s, 3 H), 0.66 (s, 3 H); FABMS *m/z* (relative intensity): 767 (MH^+, 5), 749 ($MH^+ - H_2O$, 7). Anal. calcd for $C_{45}H_{60}Cl_2O_6 \times H_2O$: C, 68.78; H, 7.95. Found: C, 68.89; H, 8.05%.

General procedure for the synthesis of phosphotriesters 32 and 35

Phosphorylating reagent **31** (0.146 g, 0.728 mmol) was dissolved in anhyd $CHCl_3$ (1.2 mL) and placed under an argon atmosphere. The solution was cooled to −10 °C in an ice–salt bath and then a solution of alcohol **29** (0.275 g, 0.560 mmol) dissolved in anhyd $CHCl_3$ (1 mL) was added dropwise over a 40–50 min period. Once the addition was complete, the reaction was stirred at −10 °C for 15 min and then at room temperature for 2.5 h. The reaction mixture was cooled back to −10 °C and a solution of **30** or **34** (0.560

mmol) dissolved in anhyd $CHCl_3$ (1.0 mL) was slowly added. The cooling bath was removed and stirring was continued for 24 h. Saturated NH_4Cl (5 mL) was then added. This solution was extracted with ethyl acetate (3 × 25 mL). The combined extracts were washed with distilled water (1 × 50 mL) followed by brine (1 × 50 mL). The extracts were dried (Na_2SO_4), filtered, and the solvent removed in vacuo. Purification by flash chromatography eluting with 50% EtOAc/50% hexanes gave pure **32** or **35**.

4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-dihydroxydiphenyl)-3-buten-1-yl 2-keto-1-methylpropyl 3-cholestanyl phosphate (32). Compound **32** was obtained as a mixture of diasteromers in 60% yield: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.69 (d, J = 2.0 Hz, 1 H), 7.67 (d, J = 2.2 Hz, 1 H), 7.50 (d, J = 2.3 Hz, 0.5 H), 7.49 (d, J = 2.2 Hz, 0.5 H), 7.45 (t, J = 1.9 Hz, 1 H), 6.15 (m, 1 H), 4.76 (m, 1 H), 4.15 (m, 3 H), 2.53 (m, 2 H), 2.25 (s, 3 H), 1.86 (s, 6 H), 1. 81 (s, 6H), 1.48 (m, 3 H), 0.90 (d, J = 6.6 Hz, 3 H) 0.87 (d, J = 6.6 Hz, 6 H), 0.81 (s, 3 H), 0.65 (s, 3 H). IR (neat): 2939, 1748, 1607, 1483, 1380, 1282, 1199, 1062, 1002, 876, 777, 730 cm^{-1}; FABMS *m/z* (relative intensity): 1035 (MNa^+, 75), 966 (100), 900 (85). Anal. calcd for $C_{55}H_{75}Cl_2PO_{11}$: C, 65.14; H, 7.45. Found C, 64.80; H, 7.17%.

4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-dihydroxydiphenyl)-3-buten-1-yl 2-keto-1-methylpropyl 3-(6,7-dehydrocholestanyl) phosphate (35). Compound **35** was obtained as a mixture of diastereomers in 67% yield: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.69 (d, J = 2.0 Hz, 1 H), 7.67 (d, J = 2.0 Hz, 1 H), 7.50 (d, J = 2.2 Hz, 0.5 H), 7.49 (d, J = 2.2 Hz, 0.5 H), 7.45 (t, J = 1.5 Hz, 1 H), 6.15 (m, 1 H), 5.23 (m, 1 H), 4.76 (m, 3 H), 2.52 (m, 2 H), 2.24 (d, J = 1.7 Hz, 3 H), 1.84 (s, 6 H), 1.80 (s 6 H), 1.46 (m, 3 H), 0.99 (s, 3 H), 0.91 (d, J = 6.4 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 0.67 (s, 3 H); IR (neat): 2943, 1749, 1608, 1483, 1381, 1282, 1199, 1062, 1002, 937, 876, 777 cm^{-1}; FABMS *m/z* (relative intensity): 1033 (MNa^+, 55), 985 (15), 617 (45), 665 (100), 607 (70). Anal. calcd for $C_{55}H_{73}Cl_2PO_{11}$: C, 65.27; H, 7.27. Found C, 64.90; H, 7.58%.

General procedure for the synthesis of phosphates 33 and 36

Phosphotriester **32** or **35** (0.293 mmol) was dissolved in 1,4-dioxane (4 mL). A 0.74 M solution of aq K_2CO_3 (0.242 g, 1.75 mmol) was added. The yellow solution heated at reflux for 3 h. Distilled water (10 mL) was added and the solution was acidified with 3 N HCl. The solution was extracted with ethyl acetate (5 × 25 mL). The combined extracts were washed with brine (1 × 50 mL). The extracts were dried ($MgSO_4$), filtered, and the solvent removed in vacuo. Purification by preparative TLC, eluting with *n*-BuOH:H_2O:HOAc (4:1:1) gave the salt of **33** or **36**. Distilled water (10 mL) was added, and the mixture was acidified with 3 N HCl and stirred at room temperature for 1.5 h. The solution was extracted with EtOAc (5 × 25 mL). The combined extracts were washed with brine (1 × 50 mL), dried ($MgSO_4$), and filtered. Concentration of the filtrate gave **33** or **36**.

4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-dihydroxydiphenyl)-3-buten-1-yl 3-cholestanyl phosphate (33). Yield 41%: ^{1}H NMR (CD_3OD, 300 MHz): δ 7.54 (t, J = 2.1 Hz, 2 H), 7.53 (d, J = 2.5 Hz, 1 H), 7.45 (d, J = 2.0 Hz, 1 H), 7.33 (d, J = 1.7 Hz, 1 H), 6.12 (t, J = 7.2 Hz, 1 H), 4.0 (m, 3 H), 2.37 (q, J = 6.6 Hz, 2 H), 0.83 (d, J = 6.4 Hz, 3 H), 0.80 (s, 3 H), 0.77 (s, 3 H), 0.66 (s, 3 H), 0.56 (s, 3 H). IR (KBr): 2944, 1684, 1602, 1461, 1363, 1232, 1182, 1021, 900, 801, 708 cm^{-1}. Anal. calcd for $C_{45}H_{61}Cl_2PO_{10} \times 1H_2O$: C, 61.29; H, 7.20. Found C, 61.14; H, 7.42%.

4,4-(3′,3″-Dicarboxy-5′,5″-dichloro-4′,4″-dihydroxydiphenyl)-3-buten-1-yl 3-(6,7-dehydrocholestanyl) phosphate (36). Yield 23%: ^{1}H NMR (CD_3OD, 300 MHz): δ 7.54 (t, J = 2.0 Hz, 2 H), 7.50 (d, J = 2.2 Hz, 1 H), 7.40 (d, J = 2.2 Hz, 1 H), 7.32 (d, J = 2.0 Hz, 1 H), 6.08 (t, J = 7.3 Hz, 3 H), 5.10 (d, J = 4.5 Hz, 1 H), 3.99 (q, J = 5.0 Hz, 2 H), 3.84 (m, 1 H), 2.37 (q, J = 6.7 Hz, 2 H), 0.84 (s, 3 H), 0.82 (s, 3 H), 0.77 (d, J = 6.4 Hz, 3 H). IR (KBr): 2949, 1686, 1599, 1460, 1375, 1233, 1182, 1026, 901, 801, 706 cm^{-1}. FABMS *m/z* (relative intensity): 883 ($MHNa^+$, 100), 759 (40), 669 (100). Anal. calcd for $C_{45}H_{59}Cl_2PO_{10} \times 1H_2O$: C, 61.43; H, 6.99. Found C, 61.87; H, 7.15%.

Acknowledgment

This research was made possible by NIH grant RO1-AI-36624 and contract NO1-CM-17513.

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(*Received in U.S.A.* 29 *April* 1996; *accepted* 17 *June* 1996)