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Synthesis and Anti-HIV Activity of Cosalane Analogues Incorporating Nitrogen in the Linker Chain

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Abstract—Introduction of an amido group or an amino moiety into the alkenyl linker chain of cosalane (**1**) provided a new series of analogues **3**–**8**. The new compounds were evaluated as inhibitors of the cytopathic effect of HIV-1 and HIV-2 in cell culture. The replacement of the 1′ and 2′ carbons in the linker chain of **1** by an amido group was generally tolerated. The length of the linker chain and the stereochemistry of the substituent at C-3 of the steroidal ring had significant effects on the antiviral activity and potency. Incorporation of an amino moiety into the linker completely abolished the anti-HIV activity. There are several steps in the HIV replication cycle that have been proposed as targets for the development of therapeutic agents (De Clercq, E. *J. Med. Chem.* **1995**, *38*, 2491; De Clercq, E. *Pure Appl. Chem.* **1998**, *70*, 567). However, currently approved anti-HIV drugs are only directed against the viral enzymes reverse transcriptase or protease (Carpenter, C. C. J.; Fischl, M. A.; Hammer, S. M.; Hirsch, M. S.; Jacobsen, D. M.; Katzenstein, D. A.; Montaner, J. S. G.; Richman, D. D.; Saag, M. S.; Schooley, R. T.; Thompson, M. A.; Vella, S.; Yeni, P. G.; Volberding, P. A. *JAMA* **1998**, *280*, 78). Drugs capable of interfering with other steps of the virus life cycle will be highly valuable in the antiretroviral therapy of AIDS, as they will have different patterns of resistance mutations than the drugs currently used clinically. In addition, their utilization in combination with other therapeutic agents could provide more potent drug 'cocktails' capable of completely suppressing virus replication. Consequently, there is an urgent need for the discovery of clinically useful anti-HIV agents possessing novel mechanisms of action.

Introduction

Cosalane (**1**) is a unique anti-HIV agent that acts primarily by inhibition of virus fusion and virus attachment.[1,2] Cosalane prevents the cytopathic effect of HIV-1(III_B) in CEM-SS cells with an EC_{50} of 3.4 μM, and it has been demonstrated to be active against a wide variety of HIV laboratory and clinical isolates.[2] A hypothetical model of the interaction of the disalicylmethane cosalane 'pharmacophore' with CD4 has been recently proposed.[3] In this model, the binding of **1** to CD4 was proposed to take place by two electrostatic interactions between the positively charged Arg-58 and Arg-59 residues of CD4 with the two negatively charged carboxylate groups of cosalane. There are additional residues near the proposed binding site of **1** to CD4, including Asp-56 and Lys-72, that could be targeted to generate analogues having higher affinity for this receptor. This strategy has been shown to be successful in the generation of analogues with improved potency over cosalane itself.[3] Besides alterations in the disalicylmethane 'pharmacophore',[3,4] the effects of changing the point of attachment of the linker chain to the steroid nucleus and the replacement of the steroid with other lipophilic appendages have been investigated.[5–7] Aside from the documented detrimental effect of phosphate incorporation into the linker chain connecting the steroid to the disalicylmethane 'pharmacophore',[7] there is little information available regarding either the biological effects of alterations in composition of the linker chain or the stereochemistry of its attachment at C-3 of the steroid. We have therefore decided to prepare a new series of cosalane analogues **3**–**8** in which an

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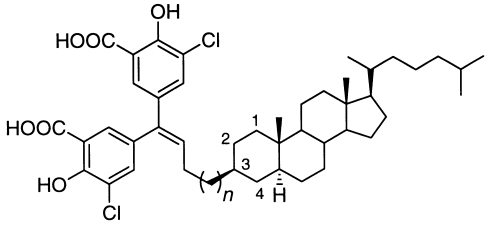
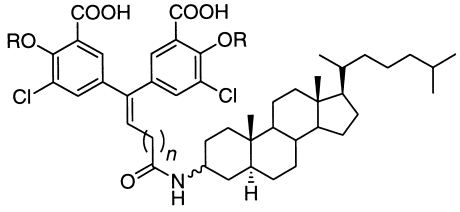
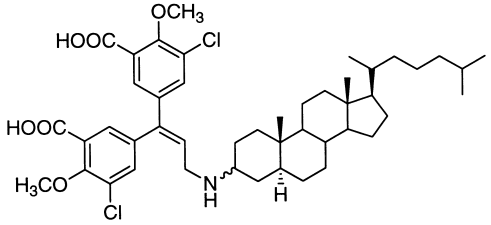
amido group or an amino moiety is incorporated into the alkenyl linker chain. The cosalane molecule can be viewed as a disalicylmethane 'pharmacophore' attached to a membrane-interactive steroid that may serve to anchor the molecule in a membrane, with the 'pharmacophore' protruding outward in an obstructive mode that might inhibit the fusion of the viral envelope with the cell membrane. According to this model, the incorporation of a positively-charged (at physiological pH) amino group might facilitate the anchoring of the molecule in the membrane through electrostatic interactions with the negatively-charged phosphates present there on the surface. Both amido and amino groups also are capable of acting as hydrogen bond donors or acceptors, and this might provide additional sites for interaction with residues on CD4. The preparation and evaluation of these new analogues as inhibitors of the cytopathic effect of HIV-1 and HIV-2 will be presented in this report.



1, $n = 1$
2, $n = 2$



3, $n = 0$, R = H, 3β
4, $n = 0$, R = H, 3α
5, $n = 1$, R = H, 3β
6, $n = 1$, R = CH_3, 3α

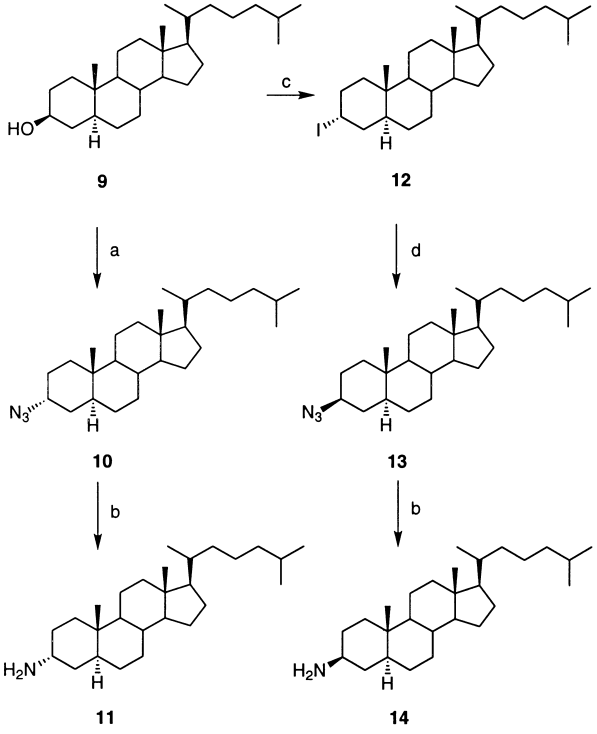


7, 3β
8, 3α

Chemistry

During the design of the new analogues, we decided to prepare compounds **3**, **4**, **7** and **8** having the same alkenyl chain length as that of cosalane. Compounds **5** and **6** have an extended alkenyl chain length, and were prepared based on the consideration that cosalane analogue **2** showed very similar anti-HIV activity as that of the parent compound.[7] In addition, we were interested in investigating the effect of the stereochemistry at C-3 of the steroidal ring on the anti-HIV activity. Compounds **3**, **5** and **7** have the C-3 substituent with an equatorial, β-stereochemistry, while in analogues **4**, **6** and **8** the substituent has an axial, α-stereochemistry. For the synthesis of analogues incorporating an amido group, we planned to couple amines having defined stereochemistry at C-3 of the steroidal ring with appropriate carboxylic acids. Reductive amination of these amines with a suitable aldehyde would provide analogues with amino functionality in the linker chain.

The preparation of the required amines **11** and **14** was achieved from 3-cholestanol (**9**) in two, and three steps, respectively, using the Mitsunobu reaction to establish the stereochemistry at C-3 of the steroid (Scheme 1). In this way, azide **10**, prepared as previously described,[8] was reduced with LAH in refluxing ether to afford the amine **11**.[9,10] No C-3 epimer was detected in the ^{1}H NMR spectrum of this compound. Cholestane derivative **12** was prepared as described by Loibner and Zbiral.[11] Displacement of iodide with sodium azide in DMSO at



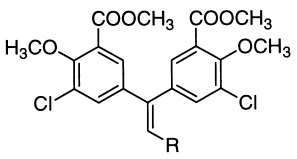
Scheme 1. Reagents and conditions: (a) PPh_3, DEAD, $(PhO)_2PON_3$, THF, 25 °C, 24 h, 51%; (b) $LiAlH_4$, Et_2O, reflux, **11**: 65%; **14**: 69.7%; (c) PPh_3, DEAD, CH_3I, THF, 25 °C, 76%; (d) NaN_3, DMSO, 90 °C, 5 h, 81%.

95 °C cleanly provided azide **13** as the sole isomer.[12] Reduction of the azido group as indicated above provided amine **14**.[13]

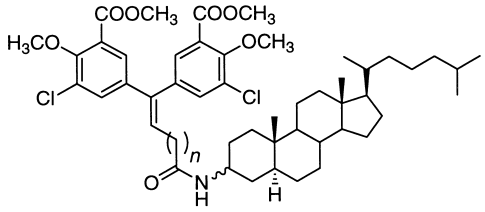
For the preparation of the new analogues possessing an amido group, carboxylic acids **15** and **17** were required. Compound **15** was prepared as described previously during our work on the synthesis of non-nucleoside reverse transcriptase inhibitors.[14] Compound **17** was obtained by an inverse-addition Jones oxidation of the known alcohol **16**,[14,15] which involved the slow addition of a solution of **16** in acetone to chromium trioxide-aqueous sulfuric acid. Having both intermediates on hand, the coupling of carboxylic acid **15** with amine **14** was performed with EDCI-HCl, HOBt, and Et_3N in DMF. In this way, compound **18** possessing the 3β-stereochemistry was obtained. A similar protocol, but starting with amine **11**, afforded compound **19**. Removal of the methyl esters and methyl ethers from **18** and **19** was initially attempted using boron tribromide-dimethyl sulfide complex in refluxing 1,2-dichloroethane, conditions that have been widely used in the synthesis of other cosalane analogues.[1,2,5,7] Unfortunately, these conditions provided only complex mixtures of products. Boron tribromide in dichloromethane is a milder agent for this type of transformation.[16] Using this reagent, deprotection of **18** was started at −78 °C, and continued at room temperature for 2 days, to afford amide **3**. Applying these conditions to **19** provided the expected amide **4**.

Similar chemistry was used for the preparation of **5** and **6**. Coupling the carboxylic acid **17** with amines **11** or **14** provided the desired amides **20** and **21**. Removal of the methyl ethers and methyl esters was attempted using the conditions mentioned above. This method proved only partially successful for the preparation of **5**, which was obtained in low yield. Our initial plan was to remove all methyl groups from **21** to obtain the fully deprotected analogue (**6**, R = H), but all our attempts to obtain this compound failed. Therefore, it was decided to cleave only the methyl esters. This transformation was achieved using potassium carbonate in ethanol-water to afford **6**. We were not particularly concerned about leaving the methyl ethers intact, as analogue **22** showed similar anti-HIV activity as that of cosalane (unpublished results).

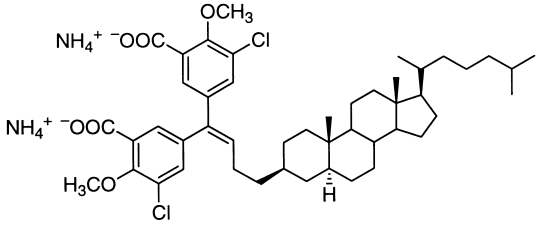
We planned to prepare analogues **7** and **8** by reductive amination of **11** and **14** with an appropriate aldehyde, which was obtained using Peterson olefination.[17] The anion derived from silyl imine **23** and LDA[18] was reacted with the known benzophenone **24**[2] at −78 °C to give an intermediate imine, which was hydrolyzed with aqueous citric acid to afford aldehyde **25**. The reductive amination reaction between **25** and amines **11** or **14** was first attempted in methanol–THF, using molecular sieves as the water scavenger, and sodium cyanoborohydride to reduce the intermediate imine.[19] However, formation of the imine was not observed under these conditions. Magnesium sulfate has been used to promote the formation of the imine between cinnamaldehyde and cyclohexylamine in benzene.[20] These conditions were



15, R = COOH
16, R = CH_2CH_2OH
17, R = CH_2COOH



18, *n* = 0, 3β
19, *n* = 0, 3α
20, *n* = 1, 3β
21, *n* = 1, 3α



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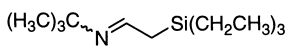
applied to our system, and reacting a mixture of amine **14**, aldehyde **25** and magnesium sulfate in dry benzene for 48 h provided the corresponding imine, which was reduced with methanolic sodium cyanoborohydride to afford **26**. Starting with amine **11**, a similar procedure afforded amine **27**. To finish the synthesis of the target compounds, cyanide-catalyzed ester hydrolysis with potassium carbonate in THF–MeOH–water afforded compounds **7** and **8**.[21] We also attempted to remove the methyl ethers from **7** and **8** using boron tribromide, but only complex mixtures of products were obtained.

Biological Results and Discussion

The new cosalane analogues **3**–**8** were evaluated for inhibition of the cytopathic effect of HIV-1 and HIV-2 in cell culture, as well as for cytotoxicity in uninfected cells. The results are listed in Table 1. The compounds were tested against HIV-1 in both CEM-SS and MT-4 cells, while for prevention of the cytophatic effect of HIV-2 only MT-4 cells were used. In general, the amides **3**–**5** were found to possess anti-HIV-1 or anti-HIV-2 activity, while compounds having an amino group (**7** and **8**), as well as the amide **6**, were completely inactive



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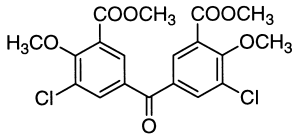

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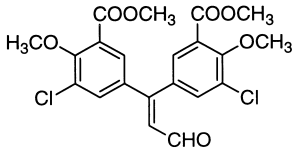

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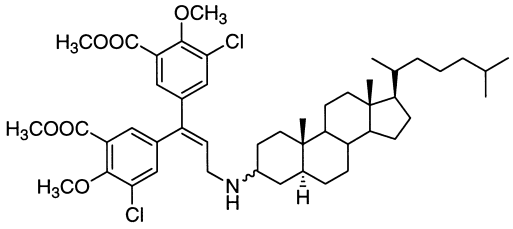
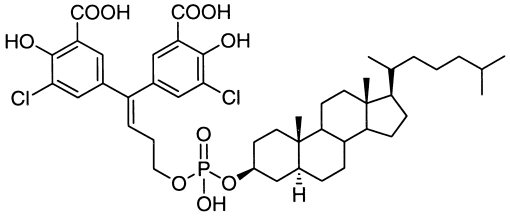


26, 3β
27, 3α



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in both systems. The anti-HIV activities of the new compounds were dependent on the virus strain. The 3-β amides **3** ($EC_{50}=5.2\,\mu M$) and **5** ($EC_{50}=22.6\,\mu M$) were the only active compounds against HIV-1_{RF}, with compound **3** being equipotent with cosalane ($EC_{50}=5.1\,\mu M$). For the III_B strain in MT-4 cells, the 3-β amides **3** ($EC_{50}=15.1\,\mu M$) and **5** ($EC_{50}=35.8\,\mu M$) were again the only compounds that displayed activity. However, both of them were less potent than cosalane ($EC_{50}=1.8\,\mu M$). The 3-α amide **4** ($EC_{50}=5.1\,\mu M$) was equipotent to cosalane ($EC_{50}=5.1\,\mu M$) against HIV-2 (ROD) in MT-4 cells, with the 3-β amides **3** ($EC_{50}=9.8\,\mu M$) and **5** ($EC_{50}=14.2\,\mu M$) being slightly less potent. Compounds **6**, **7** and **8**, the last two possessing an amino group in the alkenyl linker chain, did not inhibit the cytopathic effect of HIV-1 and HIV-2 at concentrations lower than the cytotoxic concentrations. All of the new compounds were more cytotoxic than cosalane ($CC_{50}=>200\,\mu M$) for CEM-SS cells with CC_{50} values ranging from 13.5 μM (analogue **6**) to 161 μM (analogue **5**). The new analogues were also more cytotoxic than **1** ($CC_{50}=>125\,\mu M$) against MT-4 cells, with **6** ($CC_{50}=6.0\,\mu M$) being the most cytotoxic compound.

In comparing the active analogues against HIV-1_{RF} and HIV-1_{IIIB}, it seems that there is a decrease in potency relative to the length of the linker chain. Analogue **3** possesses a three atom linker, which is identical in length to that of cosalane, while **5** has a four atom chain. Data from our previous work indicated that analogue **2** ($EC_{50}=3.4\,\mu M$), possessing a four atom linker similar to that present in **5**, was equipotent with **1** versus HIV-1_{RF}.[5] Therefore, it would be expected that increasing the length of the linker by one atom would not produce a significant effect on the antiviral potency. The results observed with these analogues are probably due to changes in conformation and rigidity introduced by incorporation of the amido group.

The stereochemistry of the substituent at C-3 of the steroidal ring was important for activity against HIV-1, but not HIV-2. Both of the active amides **3** and **5** have the alkenyl linker chain with a C-3 β-stereochemistry

Table 1. Anti-HIV activities of cosalane analogues

Compound	EC_{50}[a] (μM)			Cytotoxicity (μM)	
	HIV-1_{RF}[c]	HIV-1_{IIIB}[d]	HIV-2_{ROD}[d]	CES-SS cells[b]	MT-4 cells[b]
1	5.1 ± 1.6	1.84 ± 0.07	5.1 ± 2.8	> 200	> 125
3	5.2	15.1 ± 2.08	9.8 ± 1.0	107 ± 50.2	52.2 ± 9.4
4	NA[e]	NA[e]	5.1 ± 0.1	26.6 ± 0.7	24.4 ± 5.9
5	22.6 ± 5.8	35.8 ± 5.1	14.2 ± 10.5	160.7 ± 39.2	> 60
6	NA[e]	NA[e]	NA[e]	13.5 ± 4.5	6.0 ± 3.2
7	NA[e]	NA[e]	NA[e]	> 50	> 150
8	NA[e]	NA1e[c]	NA[e]	61.7 ± 2.2	87 ± 27.2
22	13.9 ± 6.33	—	—	> 300	—

[a]Concentration required to reduce the cytopathic effect of the virus by 50%.
[b]Concentration required for 50% reduction in cellular viability of uninfected cells.
[c]Determined in CEM-SS cells.
[d]Determined in MT-4 cells.
[e]No activity was observed up to a concentration of 125 μM.

(equatorial), while the alkenyl linker chain is axial in **4**. It could be argued that in the corresponding C-3 α-epimers **4** and **6** the steroidal moiety is imposing steric hindrance to the carboxylate groups to interact with the positively charged residues on CD4. However, this interpretation is not in agreement with the antiviral activity of **4** against HIV-2, because the interaction with CD4 would also be involved in that case as well. Prior studies demonstrated that cosalane binds to both gp120 and CD4,[22] and it can be assumed that the new analogues would interact with both molecules as well. A more complete understanding of the mechanisms of action and potencies of the present compounds may therefore require a model for their interaction with gp120 in addition to the CD4 binding model. Distinct modes of interaction of analogues **3**, **4** and **5** with gp120 could explain the differences observed in the anti-HIV-1 versus anti-HIV-2 activity. In this regard, it is interesting that changing the configuration of the linker chain from equatorial in **3** to axial in **4** abolishes antiviral activity against both strains of HIV-1, but it actually increases activity against HIV-2_{ROD} (Table 1). This intriguing result is not easily explained by any simple molecular model. The relative configuration of the side chain also has an effect on cytotoxicity in uninfected cells, with the axial configuration **4** being more cytotoxic than the equatorial configuration **3** in both CEM-SS cells and MT-4 cell. The lack of antiviral activity in analogues **7** and **8** incorporating an amino group provides new insights into the functionality tolerated in this part of the molecule. Previously, we reported a series of cosalane analogues, including **28**, that incorporated a phosphate group into the linker chain.[7] This modification completely abolished the anti-HIV activity. Under the assay conditions, the amino groups of compounds **7** and **8** are protonated. The positively charged amino functionality and the phosphate moiety are both polar groups that reduce the lipophilicity of the alkenyl linker chain. This factor has been related before to the antiviral potency.[6] The presence of a polar group next to the steroid is probably obstructing the imbedding of this moiety into the membrane. Although the model that we proposed for the interaction of compounds **7** and **8** with a membrane is definitively not correct, this general model should still be valid for other analogues, provided that the lipophilicity of the linker chain is not disrupted.

In conclusion, the present study reveals that the incorporation of an amido group into the linker chain of cosalane provides analogues exhibiting anti-HIV-1 and anti-HIV-2 activity. The range of activity and the potency of these compounds is related to the length of the linker chain, as well as to the stereochemistry of the substituent at C-3 of the steroidal ring. Introduction of an amino group into the linker chain completely abolishes the antiviral activity. We are currently contemplating the preparation of new analogues in which the nitrogen atom of the amido functionality in the present series could be used to attach a second unit of the cosalane "pharmacophore" through an appropriate linker. Results of these studies will be presented shortly.

Experimental

General

Melting points (mp) were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. Spectra were obtained as follows: CI mass spectra on a Finnegan 4000 spectrometer; FAB mass spectra and EI mass spectra on a Kratos MS50 spectrometer; ^{1}H NMR spectra on Varian VXR-500S and Bruker 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, and all values were within ±0.4% of the calculated compositions. Silica gel used for column chromatography was 230–400 mesh.

5α,3α-Azidocholestane (10). This compound was prepared in 51% yield following a literature procedure:[8] mp 54–56 °C (lit.[8] mp 62.5–63 °C); IR (film) 2950, 2866, 2100, 1653, 1465, 1381 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.88 (t, J = 2.5 Hz, 1H), 0.90 (d, J = 6.5 Hz, 3H), 0.86 (dd, J = 7.5 Hz and J = 1.1 Hz, 6H), 0.78 (s, 3H), 0.64 (s, 3H); CIMS m/z (relative intensity) 413 (M^+, 24), 387 ($MH_2^+ - N_2$, 100).

3α-Amino-5α-cholestane (11). This compound was prepared from 5α,3α-azido cholestane (**10**) in 65% yield following the method described by Bose et al.:[9] mp 100–101 °C (lit.[9] mp 87–88 °C; lit.[10] mp 104.5–105.5 °C); IR (film) 3450, 3350, 2933, 2866, 2850, 1560, 1467, 1381, 844, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.16 (b.s., 1H), 1.63 (m, 2H, D_2O exchange), 0.87 (d, J = 6.5 Hz, 3H), 0.83 (dd, J = 6.6 Hz and J = 1.3 Hz, 6H), 0.75 (s, 3H), 0.62 (s, 3H); CIMS m/z (relative intensity) 388 (MH^+, 100), 390 (21), 386 (17).

3α-Iodo-5α-cholestane (12). This compound was obtained in 76% yield as a colorless solid following the method described by Loibner and Zbiral:[11] mp 104.5–106 °C (lit.[11] mp 109–112 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.95 (s, 1H), 0.90 (d, J = 6.5 Hz, 3H), 0.86 (dd, J = 6.6 Hz and J = 1.2 Hz, 6H), 0.79 (s, 3H), 0.65 (s, 3H); CIMS m/z (relative intensity) 497 (MH^+, 8), 371 (M^+ – HI, 100).

5α,3β-Azidocholestane (13). A solution of compound **12** (1.0 g, 2.00 mmol) and sodium azide (1.3 g, 20 mmol) in dry DMSO (50 mL) was stirred at 90 °C for 5 h. The reaction mixture was cooled at room temperature and water (50 mL) was added. The product was extracted with ethyl ether (4×30 mL). The combined organic extracts were washed with brine (1×100 mL), dried over sodium sulfate, filtered and the solvent evaporated to give a yellowish solid (0.82 g, 99%). The product was recrystallized from ether/methanol to afford **13** (0.67 g, 81%) as a white crystalline solid: mp 61–62.5 °C (lit.[12] mp 65–66 °C); IR (film) 2932, 2866, 2090, 1653, 1540, 1507, 1465, 1381, 1252 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.23 (tt, J = 11.7 Hz and J = 4.6 Hz, 1H), 0.87 (d, J = 6.5 Hz, 3H), 0.84 (dd, J = 6.6 Hz and J = 1.2 Hz, 6H), 0.77 (s, 3H), 0.62 (s, 3H); CIMS (relative intensity) m/z 414 (MH^+, 60), 388 (100).

3β-Amino-5α-cholestane (14). A solution of 3β-azide **13** (0.45 g, 1.09 mmol) in dry ether (20 mL) was placed under argon. A 1.0 M solution of $LiAlH_4$ in ether (4.4 mL, 4.4 mmol) was added dropwise. A reflux condenser was adapted and the reaction mixture was refluxed for 3 h. After cooling the reaction at room temperature, wet ether (10 mL) was slowly added followed by water (20 mL), which was added very slowly. The layers were separated and the ethereal phase was washed with brine (1×20 mL), dried over sodium sulfate and filtered. To the dry solution, a 1.0 M solution of HCl in ether (2 mL) was added. The precipitate was separated by vacuum filtration, washed with dry ether and dried. The dry salt was suspended in 10% aqueous KOH and extracted with ether. After evaporation of the solvent, free amine **14** (0.29 g, 69.7%) was obtained as a white solid: mp 104–106 °C (lit.[13] mp 118 °C); IR (film) 3400, 3277, 2933, 2848, 1540, 1466, 1382 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.61 (tt, J = 10.9 Hz and J = 4.3 Hz, 1H), 1.38 (m, 2H, D_2O exchange), 0.86 (d, J = 6.5 Hz, 3H), 0.83 (dd, J = 6.6 Hz and J = 1.2 Hz, 6H), 0.75 (s, 3H), 0.61 (s, 3H); CIMS m/z (relative intensity) 388 (MH^+, 100), 386 (26).

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-4,4-diphenyl-3-butenoic acid (17). A solution of CrO_3 (1.073 g, 10,73 mmol) and 0.75 N sulfuric acid (15.5 mL, 23.2 mmol) was stirred in an ice bath. A solution of alcohol **16**[14] (0.837 g, 1.79 mmol) in acetone (80 mL) was added over 6 h, while maintaining the temperature between 0 and 10 °C. After the addition was complete, the reaction mixture was stirred at room temperature for 6 h. Then, the solvents were removed and the residue diluted with water (100 mL). The aqueous mixture was extracted with ethyl ether (4×50 mL). The combined organic fractions were washed with water (1×50 mL), brine (1×50 mL), dried over magnesium sulfate and filtered. The solvent was removed and the residue was purified by flash chromatography on silica gel (105 g, column: 4 cm×7.5 inch), eluting with hexanes/ethyl acetate (3/1 to 1/2). Compound **17** was obtained as a light-yellow solid (0.365 g, 42.3%). The analytical sample was recrystallized from acetone/hexanes: mp 138–140 °C; IR (film) 3500–2500, 2952, 1732, 1477, 1258, 1210, 996, 744 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.49 (d, J = 2.3 Hz, 1H), 7.46 (d, J = 2.2 Hz, 1H), 7.32 (d, J = 2.2 Hz, 1H), 7.31 (d, J = 2.5 Hz, 1H), 6.20 (t, J = 7.4 Hz, 1H), 3.95 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.14 (d, J = 7.4 Hz, 2H); FABMS m/z (relative intensity): 483 (MH^+, 10) and 451 (MH^+ – CH_3OH, 15). Anal. calcd for $C_{22}H_{20}Cl_2O_8$: C: 54.67, H: 4.17. Found: C: 54.45, H: 4.06.

***N*-(5α,3β-Cholestanyl)-3,3-(3′,3″-dicarbomethoxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-2-propenamide (18).** A solution of the acid **15**[14] (0.121 g, 0.259 mmol), triethylamine (0.22 mL, 1.6 mmol) in dry DMF (12 mL) was stirred at room temperature. HOBt (0.070 g, 0.52 mmol) was then added, followed 5 min later by 3β-aminocholestane (**14**) (0.150 g, 0.388 mmol). A solution of EDCI·HCl (0.099 g, 0.52 mmol) in dry DMF (5 mL) was incorporated. Dry dichloromethane (3 mL) was added to facilitate dissolution of amine. The reaction mixture was stirred at room temperature for 48 h. Water (135 mL) was added and the mixture stirred for 5 min. The product was then extracted with dichloromethane (5×35 mL). The combined organic extracts were washed with brine (1×50 mL), dried over magnesium sulfate, filtered, and the solvent removed. Purification was achieved by flash chromatography on silica gel (~50 g; column: 3 cm×9.5 inch), eluting with hexanes/ethyl acetate 2/1, to yield pure **18** as a light-tan solid (0.165 g, 76.3%): mp 137–139 °C; IR (film) 3290, 2932, 2866, 1737, 1636, 1476, 1260, 1207, 999 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J = 2.4 Hz, 1H), 7.53 (d, J = 2.2 Hz, 1H), 7.43 (d, J = 2.2 Hz, 1H), 7.33 (d, J = 2.3 Hz, 1H), 6.26 (s, 1H), 5.07 (d, J = 8.4 Hz, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.67 (bm, 1H), 0.67 (s, 3H), 0.60 (s, 3H); FABMS m/z 838 (MH^+) and 451 [MH^+ – 387(R-NH)]. Anal. calcd for $C_{48}H_{65}Cl_2NO_7$: C, 68.72; H, 7.81; N, 1.67. Found: C, 68.55; H, 7.63; N, 1.43.

***N*-(5α,3α-Cholestanyl)-3,3-(3′3″-dicarbomethoxy-5′,5″-dichloro-4′4″-dimethoxydiphenyl)-2-propenamide (19).** A solution of the acid **15**[14] (0.203 g, 0.434 mmol) and triethylamine (0.36 mL, 2.6 mmol) in dry DMF (20 mL) was stirred at room temperature. HOBt (0.117 g, 0.868 mmol) was then added, followed 5 min later by 3α-aminocholestane (**11**) (0.252 g, 0.651 mmol). A solution of EDCI·HCl (0.166 g, 0.868 mmol) in dry DMF (10 mL) was incorporated. The reaction mixture was stirred at room temperature for 3 h. Dry dichloromethane (5 mL) was added to facilitate the dissolution of the amine. The resulting cloudy solution was stirred at room temperature for 48 h. Water (100 mL) was added and the mixture stirred for 5 min. The product was then extracted with ethyl ether (4×45 mL). The combined organic extracts were washed with brine (1×40 mL), dried over magnesium sulfate, filtered, and the solvent removed. Purification was achieved by flash chromatography on silica gel (37 g; column: 3 cm×7 inch), eluting with hexanes/ethyl acetate (2/1), to yield pure **19** as a white solid (0.324 g, 89.1%): mp 86–88 °C; IR (film) 3316, 2934, 1734, 1646, 1474, 1436, 1263, 1205, 996 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 2.4 Hz, 1H), 7.56 (d, J = 2.3 Hz, 1H), 7.47 (d, J = 2.2 Hz, 1H), 7.33 (d, J = 2.4 Hz, 1H), 6.34 (s, 1H), 5.56 (d, J = 8.1 Hz, 1H), 4.06 (m, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 0.69 (s, 3H), 0.60 (s, 3H); FABMS m/z: 838 (MH^+). Anal. calcd for $C_{48}H_{65}Cl_2NO_7$: C, 68.72; H, 7.81; N, 1.67. Found: C, 68.51; H, 7.85; N, 2.01.

***N*-(5α,3β-Cholestanyl)-3,3-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dihydroxydiphenyl)-2-propenamide (3).** A 1.0 M solution of BBr_3 in dichloromethane (0.36 mL, 0.36 mmol) was stirred under argon in a dry ice–acetone bath. Dry dichloromethane (4 mL) was added. A solution of amide **18** (0.050 g, 0.059 mmol) in dry dichloromethane (4.5 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h. The dry ice-acetone bath was removed, allowing the temperature to reach room temperature, and the mixture was stirred at this temperature for 2 days. Additional 1.0 M solution of BBr_3 (0.2 mL) was added on the second day. Water (10 mL) was

added, followed by 10% NaOH (20 mL), and the mixture was stirred overnight. The basic solution was washed with Et_2O (2×20 mL), followed by acidification with conc HCl. The product was then extracted with Et_2O (3×25 mL) and the organic extracts were washed with brine (1×30 mL), dried over magnesium sulfate and filtered. The solvent was removed in vacuo to give a pale brown residue. Purification was achieved by column chromatography on silica gel (10 g, column: 1.2 cm ×8.5 inch), eluting with $CHCl_3$:MeOH:formic acid (95:5:0.5), to afford **3** as a pale-tan solid (32.2 mg, 69%): mp 100–102.5 °C; IR (film) 3500–2500, 2928, 1667, 1463, 1233, 1191, 978 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 7.58 (d, J = 2.4 Hz, 1H), 7.56 (d, J = 2.3 Hz, 1H), 7.47 (d, J = 2.2 Hz, 1H), 7.33 (d, J = 2.4 Hz, 1H), 6.34 (s, 1H), 5.56 (d, J = 8.1 Hz, 1H), 4.06 (m, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 0.69 (s, 3H), 0.60 (s, 3H); FABMS m/z 782 (MH^+). Anal. calcd for $C_{44}H_{57}Cl_2NO_7 \cdot 0.7\ H_2O$: C, 66.44; H, 7.40; N, 1.76. Found: C, 66.11; H, 7.30; N, 1.73.

***N*-(5α,3α-Cholestanyl)-3,3-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dihydroxydiphenyl)-2-propenamide (4).** A 1.0 M solution of BBr_3 in dichloromethane (1.07 mL, 1.07 mmol) was stirred under argon in an dry ice-acetone bath. Additional dry dichloromethane (4 mL) was added. A solution of amide **19** (0.150 g, 0.179 mmol) in dry dichloromethane (8.0 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h. The dry ice-acetone bath was removed, allowing the temperature to reach room temperature, and the mixture was stirred at this temperature for 2 days. Additional 1.0 M solution of BBr_3 (0.6 mL, 0.6 mmol) was added on the second day. Water (10 mL) was added, followed by 10% KOH (30 mL), and the mixture was stirred for 48 h. The basic solution was washed with Et_2O (2×20 mL), followed by acidification with conc HCl. The product was then extracted with Et_2O (4×30 mL) and the organic extracts were washed with brine (1×30 mL), dried over magnesium sulfate and filtered. The solvent was removed in vacuo to give a brownish residue. Purification was achieved by column chromatography on silica gel (26 g, column: 2.0 cm×8.25 inch), eluting with 100% $CHCl_3$, followed by $CHCl_3$:MeOH:formic acid (95:5:0.5). The fractions containing pure product were pooled and the solvent removed. The residue was then triturated with dichloromethane. The solid obtained in this way was separated by vacuum filtration and washed with additional dichloromethane. Compound **4** was obtained as a white solid in moderate yield (93.7 mg, 67.4%): mp 206–211 °C; IR (film) 3500–2500, 2928, 1667, 1463, 1233, 1191, 978 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 7.80 (d, J = 2.2 Hz, 1H), 7.79 (d, J = 2.2 Hz, 1H), 7.58 (d, J = 2.0 Hz, 1H), 7.57 (d, J = 2.2 Hz, 1H), 7.56 (d, J = 7.3 Hz, 1H), 6.52 (s, 1H), 4.02 (m, 1H), 0.76 (s, 3H), 0.66 (s, 3H); FABMS m/z 782 $(MH)^+$. Anal. calcd for $C_{44}H_{57}Cl_2NO_7 \cdot 0.3\ H_2O$: C, 67.05; H, 7.37; N, 1.78. Found: C, 66.68; H, 7.42; N, 1.65.

***N*-(5α,3β-Cholestanyl)-4,4-(3′,3″-dicarbomethoxy-5′,5″-dichloro-4′4″-dimethoxydiphenyl)-3-butenamide (20).** A solution of the acid **17** (0.070 g, 0.14 mmol) and triethylamine (0.12 mL, 0.87 mmol) in dry DMF (8 mL) was stirred at room temperature under Ar. HOBt (0.039 g, 0.290 mmol) was then added, followed by aminocholestane **14** (0.084 g, 0.22 mmol). A solution of EDCI·HCl (0.056 g, 0.29 mmol) in dry DMF (4.0 mL) was incorporated. The reaction mixture was stirred at room temperature for 48 h. Water (70 mL) was added and the mixture stirred for 5 min. Ethyl ether was added (30 mL) and the layers were separated. The aqueous fraction was then extracted with ethyl ether (3×30 mL). The combined organic extracts were washed with brine (1×50 mL), dried over magnesium sulfate, filtered, and the solvent removed. Purification was achieved by flash chromatography on silica gel (30 g, column dimensions: 2 cm×8.25 inch), eluting with hexanes:ethyl acetate (3:1 to 2:1). Compound **20** was obtained as a light tan solid (92 mg, 75%): mp 87–95 °C; IR (film) 3302, 2933, 1736, 1636, 1476, 1257, 1209, 999, 737 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.51 (d, J = 2.1 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.34 (d, J = 2.2 Hz, 1H), 7.31 (d, J = 2.5 Hz, 1H), 6.29 (t, J = 7.5 Hz, 1H), 5.25 (d, J = 8.1 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.73 (m, 1H), 2.93 (d, J = 7.5 Hz, 2H), 0.75 (s, 3H), 0.61 (s, 3H); FABMS m/z (relative intensity): 852 (MH^+, 90). Anal. calcd for $C_{49}H_{67}Cl_2NO_7$: C: 69.00, H: 7.92, N: 1.64. Found: C: 69.23, H: 8.02, N: 1.65.

***N*-(5α,3α-Cholestanyl)-4,4-(3′,3″-dicarbomethoxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-3-butenamide (21).** A solution of the acid **17** (0.120 g, 0.249 mmol), triethylamine (0.21 mL, 1.5 mmol) in dry DMF (13 mL) was stirred at room temperature under Ar. HOBt (0.067 g, 0.5 mmol) was then added, followed by aminocholestane **11** (0.134 g, 0.346 mmol). A solution of EDCI·HCl (0.095 g, 0.5 mmol) in dry DMF (7.0 mL) was incorporated. The reaction mixture was stirred at room temperature for 2.5 h. Dry CH_2Cl_2 (5 mL) was added and the mixture stirred at room temperature for 38 h. Water (100 mL) was added and the mixture stirred for 5 min. Ethyl ether was added (35 mL) and the layers were separated. The aqueous fraction was then extracted with ethyl ether (3×35 mL). The combined organic extracts were washed with brine (1×60 mL), dried over magnesium sulfate, filtered, and the solvent removed. Purification was achieved by flash chromatography on silica gel (45 g, column dimensions: 2 cm×12.5 inch), eluting with hexanes:ethyl acetate (3:1 to 2:1). Compound **21** was obtained as a white solid (0.19 g, 89.6%): mp 167.5–169.5 °C; IR (film) 3330, 2932, 1737, 1477, 1259, 1208, 999, 742 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 2.3 Hz, 1H), 7.47 (d, J = 2.2 Hz, 1H), 7.36 (d, J = 2.1 Hz, 1H), 7.33 (d, J = 2.3 Hz, 1H), 6.33 (t, J = 7.4 Hz, 1H), 5.67 (d, J = 7.6 Hz, 1H), 4.11 (bs, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 3.00 (d, J = 7.4 Hz, 2H), 0.77 (s, 3H), 0.62 (s, 3H); FABMS m/z (relative intensity): 852 (MH^+, 40). Anal. calcd for $C_{49}H_{67}Cl_2NO_7$: C: 69.00, H: 7.92, N: 1.64. Found: C: 69.22, H: 7.89, N: 1.68.

***N*-(5α,3β-Cholestanyl)-4,4-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dihydroxydiphenyl)-3-butenamide (5).** A 1.0 M solution of BBr_3 in dichloromethane (0.65 mL, 0.65 mmol) was stirred under argon. Dry dichloromethane (3 mL) was added, and the solution was cooled

at −78 °C. A solution of amide **20** (0.092 g, 0.11 mmol) in dry dichloromethane (6 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h. The dry ice–acetone bath was removed, allowing the temperature to reach 25 °C, and the mixture was stirred at this temperature for 2 days. Additional 1.0 M solution of BBr_3 was added (0.49 mL, 0.49 mmol) after 24 h. An ^1H NMR of the crude product run after 48 h indicated incomplete reaction. More 1.0 M BBr_3 was added (0.5 mL, 0.5 mmol) and the mixture was stirred at 25 °C for another 24 h. Water (10 mL) was added, followed by 10% KOH (10 mL), and the mixture stirred for 7 h. The basic solution was washed with Et_2O (2×15 mL), followed by acidification with conc HCl. The product was then extracted with Et_2O (4×25 mL) and the organic extracts were washed with brine (1×20 mL), dried over magnesium sulfate and filtered. The solvent was removed in vacuo to give a pale brown residue. Purification was achieved by double column chromatography on silica gel (40 g, column: 2 cm×12 inch), eluting with a gradient of $CHCl_3$:MeOH:formic acid (100:0:0 to 90:10:0.1), to afford **5** as a pale beige solid (0.020 g, 23.3%): mp 188–190 °C; IR (film) 3500–2500, 2929, 1677, 1610, 1461, 1235, 1183, 801 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 7.74 (d, J=1.6 Hz, 1H), 7.72 (d, J=2.1 Hz, 1H), 7.50 (d, J=1.7 Hz, 1H), 7.46 (d, J=2.0 Hz, 1H), 7.27 (d, J=7.9 Hz, 1H), 6.33 (t, J=7.5 Hz, 1H), 3.73 (m, 1H), 3.09 (d, J=7.5 Hz, 2H), 0.91 (d, J=6.5 Hz, 3H), 0.85 (d, J=6.5 Hz, 6H), 0.77 (s, 3H), 0.66 (s, 3H); FABMS m/z: 796 (MH^+). Anal. calcd for $C_{45}H_{59}Cl_2NO_7 \cdot H_2O$: C, 66.39; H, 7.56; N, 1.72. Found: C, 66.45; H, 7.69; N, 1.52.

***N*-(5α,3α-Cholestanyl)-4,4-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-3-butenamide (6).** A mixture of **21** (0.065 g, 0.076 mmol), K_2CO_3 (0.182 g, 1.32 mmol), EtOH (2 mL) and water (0.5 mL) was stirred and heated at 60 °C for 4 days. At the end of this time, the solvent was removed, and the residue was diluted with water (15 mL) and washed with ethyl acetate (10 mL). The aqueous phase was acidified with conc HCl and extracted with EtOAc (4×10 mL). The combined organic extracts were washed with brine (1×15 mL), dried over Na_2SO_4, filtered and the solvent removed. The crude product was recrystallized from acetone:acetonitrile to provide **6** as an off-white solid (0.052 g, 82.5%): mp 164–166 °C; IR (film) 3500–2500, 2931, 1697, 1477, 1254, 999 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J=2.2 Hz, 1H), 7.74 (d, J=2.0 Hz, 1H), 7.46 (d, J=2.0 Hz, 1H), 7.43 (d, J=2.1 Hz, 1H), 6.36 (t, J=7.3 Hz, 1H), 5.79 (d, J=7.5 Hz, 1H), 4.12 (m, 1H), 4.09 (s, 3H), 4.02 (s, 3H), 3.05 (d, J=7.3 Hz, 2H), 0.85 (s, 6H), 0.82 (s, 3H), 0.77 (s, 3H), 0.61 (s, 3H); FABMS m/z 824 (MH^+). Anal. calcd for $C_{47}H_{63}Cl_2NO_7$: C, 68.43; H, 7.70; N, 1.70. Found: C, 68.60; H, 7.93; N, 1.61.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-3,3-diphenyl-2-propenal (25). A solution of diisopropylamine (0.43 mL, 3.1 mmol) in dry THF (5 mL) was cooled to 0 °C and put under Ar. A 1.6 M solution of BuLi in hexanes (1.8 mL, 2.9 mmol) was added slowly. The mixture was stirred at 0 °C for 10 min. A solution of silyl imine **23**[18] (0.625 g, 2.93 mmol) in dry THF (2 mL) was added. The red mixture was stirred at 0 °C for 15 min. Then, it was cooled to −78 °C and a solution of ketone **24**[2] (1.00 g, 2.35 mmol) in THF (8 mL) was added. The reaction mixture was warmed to room temperature over 3 h. A 20% aq solution of citric acid was added to adjust the pH to 4. The mixture was then stirred at room temperature overnight. The mixture was poured into brine (100 mL) and the layers were separated. The aqueous fraction was extracted with ethyl ether (3×40 mL). The combined organic extracts were washed with sat $NaHCO_3$ (1×40 mL) and dried over $MgSO_4$. The solvent was removed and the residue was purified by flash chromatography on silica gel (∼120 g; column: 4×24 cm), eluting with hexanes:ethyl acetate 3:1. Compound **25** was obtained as a pale yellow solid in moderate yield (0.476 g, 44.9%): mp 101–103 °C; IR (film) 2953, 1732, 1668, 1477, 1282, 1209, 994, 745 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.50 (d, J=7.8 Hz, 1H), 7.65 (d, J=2.3 Hz, 1H), 7.61 (d, J=2.2 Hz, 1H), 7.45 (d, J=2.6 Hz, 1H), 7.44 (d, J=2.7 Hz, 1H), 6.52 (d, J=7.8 Hz, 1H), 4.01 (s, 3H), 3.96 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H); FABMS m/z 453 (MH^+) and 421 (MH^+-CH_3OH). Anal. calcd for $C_{21}H_{18}Cl_2O_7$: C, 55.65; H, 4.00. Found: C, 55.80; H, 4.08.

***N*-(5α,3β-Cholestanyl)-3,3-(3′,3″-dichloro-5′,5″-dimethoxy-4′,4″-dimethoxydiphenyl)-2-propenylamine (26).** A mixture of 3β-aminocholestane hydrochloride (**14**) (0.135 g, 0.320 mmol), aldehyde **25** (0.100 g, 0.221 mmol) and Et_3N (0.045 mL, 0.32 mmol) was partially dissolved in benzene:THF (3:3 mL). Anhydrous magnesium sulfate (0.053 g, 0.44 mmol) was added and the mixture was stirred at room temperature under Ar for 24 h. Additional magnesium sulfate (0.053 g, 0.44 mmol) was added at this time, and the mixture was stirred for 24 h at room temperature. A solution of $NaCNBH_3$ (0.042 g, 0.66 mmol) in MeOH (2 mL) was added. The mixture was stirred for 6 h at room temperature. More solid $NaCNBH_3$ (0.014 g) was added and the mixture was stirred overnight. The solvents were removed and the residue was partitioned between water (15 mL) and EtOAc (20 mL). The phases were separated and the aqueous phase was extracted with EtOAc (4×15 mL). The combined organic extracts were washed with brine (1×30 mL), dried over magnesium sulfate, filtered and the solvent removed. Purification was achieved by flash chromatography on silica gel (∼38 g; column: 2×26 cm), eluting with hexanes:ethyl acetate 2:1 containing 1% Et_3N, followed by hexanes:ethyl acetate 1:1 containing 1% Et_3N. Compound **26** was obtained as a white solid in good yield (0.149 g, 82%): mp 152–154 °C; IR (film) 2930, 2865, 1736, 1475, 1435, 1264, 1206, 999, 742 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J=1.8 Hz, 1H), 7.46 (d, J=1.7 Hz, 1H), 7.33 (d, J=2.0 Hz, 1H), 7.30 (d, J=1.8 Hz, 1H), 6.14 (t, J=6.7 Hz, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.28 (d, J=6.9 Hz, 2H), 2.40 (m, 1H), 0.74 (s, 3H), 0.61 (s, 3H); FABMS m/z 824 (MH^+). Anal. calcd for $C_{48}H_{67}Cl_2NO_6$: C, 69.88; H, 8.19; N, 1.70. Found: C, 70.04; H, 8.27; N, 1.76.

***N*-(5α,3α-Cholestanyl)-3,3-(3′,3″-dichloro-5′,5″-dimethoxy-4′,4″-dimethoxydiphenyl)-2-propenylamine (27).** A mixture

of 3α-aminocholestane hydrochloride (**11**) (0.205 g, 0.484 mmol), aldehyde **25** (0.151 g, 0.334 mmol) and Et_3N (0.07 mL, 0.5 mmol) was partially dissolved in dry benzene (7 mL). Anhydrous magnesium sulfate (0.080 g, 0.67 mmol) was added and the mixture was stirred at room temperature under Ar for 24 h. Additional magnesium sulfate (0.040 g, 0.33 mmol) was added at this time, and the mixture was stirred for 24 h at room temperature. The solvent was partially removed, and a solution of $NaCNBH_3$ (0.063 g, 1.002 mmol) in MeOH (2 mL) was added. The cloudy mixture was stirred for 1 h at room temperature. The solvents were removed and the residue was partitioned between water (15 mL) and EtOAc (20 mL). The phases were separated and the aqueous phase was extracted with EtOAc (4×15 mL). The combined organic extracts were washed with brine (1×30 mL), dried over magnesium sulfate, filtered and the solvent removed. Purification was achieved by flash chromatography on silica gel (~38 g; column: 2×29 cm), eluting with hexanes:ethyl acetate 4:1 containing 1% Et_3N, followed by hexanes:ethyl acetate 3:1 containing 1% Et_3N. Compound **27** was obtained as an off-white solid in good yield (0.238 g, 86.5%): mp 76–77 °C; IR (film) 2930, 2867, 1737, 1476, 1434, 1264, 1206, 1000, 743 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 1.9 Hz, 1H), 7.45 (d, J = 1.8 Hz, 1H), 7.36 (d, J = 1.8 Hz, 1H), 7.31 (d, J = 1.5 Hz, 1H), 6.18 (m, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.21 (d, J = 4.8 Hz, 2H), 2.80 (m, 1H), 0.74 (s, 3H), 0.61 (s, 3H); FABMS m/z 824 (MH^+). Anal. calcd for $C_{48}H_{67}Cl_2NO_6 \cdot 0.3\ H_2O$: C, 69.43; H, 8.21; N, 1.69. Found: C, 69.08; H, 8.27; N, 1.57.

***N*-(5α,3β-Cholestanyl)-3,3-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-2-propenylamine (7).** Amine **26** (0.108 g, 0.131 mmol) was partially dissolved in THF:MeOH:water (4:3:1.5 mL). K_2CO_3 (0.326 g, 2.36 mmol) was added, followed by KCN (~2.0 mg, 0.026 mmol). The mixture was heated at 75 °C for 5 h. The solvents were removed and more water was added (10 mL). The aqueous solution was then heated at 75 °C for 1 h. It was allowed to cool and washed with EtOAc (2×15 mL). Then, it was acidified with conc HCl, and extracted with EtOAc (5×15 mL). The combined organic extracts were washed with brine (1×30 mL), dried over Na_2SO_4, filtered and the solvent removed. Compound **7** was obtained as an off-white solid in excellent yield (0.102 g, 97.9%): mp 205–210 °C; IR (film) 3500–2500, 2934, 1714, 1477, 1257, 1000 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 7.72 (s, 1H), 7.71 (s, 1H), 7.70 (s, 1H), 7.51 (s, 1H), 6.68 (t, J = 7.0 Hz, 1H), 3.95 (s, 3H), 3.88 (s, 3H), 3.84 (m, 2H), 3.16 (m, 1H), 0.75 (s, 3H), 0.65 (s, 3H); FABMS m/z 796 (MH^+). Anal. calcd for $C_{46}H_{63}Cl_2NO_6 \cdot 2.0\ H_2O$: C, 66.33; H, 8.11; N, 1.68. Found: C, 66.37; H, 7.84; N, 1.68.

***N*-(5α,3α-Cholestanyl)-3,3-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-2-propenylamine (8).** Amine **27** (0.184 g, 0.223 mmol) was dissolved in THF:MeOH: water (4.5:3.5:2.0 mL) and K_2CO_3 (0.556 g, 4.02 mmol) was added, followed by KCN (3.0 mg, 0.045 mmol). The mixture was heated at 70–75 °C for 4 h. Solvents were removed and more water (10 mL) was added. The aqueous solution was then heated at 75 °C for 1 h. It was allowed to cool and washed with EtOAc (2×15 mL). Then, it was acidified with conc HCl and extracted with EtOAc (6×15 mL). The combined organic extracts were washed with brine (1×30 mL), dried over Na_2SO_4, filtered and the solvent removed. Drying under high vaccum afforded **8** (0.174 g, 98.1%) in excellent yield. The analytical sample was obtained by recrystallization from THF–acetonitrile: mp 205–210 °C; IR (film) 3500–2500, 2934, 2867, 1704, 1477, 1255, 999 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6–$CDCl_3$) δ 7.58 (d, J = 2.2 Hz, 1H), 7.56 (d, J = 2.2 Hz, 1H), 7.47 (d, J = 2.2 Hz, 1H), 7.45 (d, J = 2.1 Hz, 1H), 6.64 (t, J = 7.1 Hz, 1H), 3.95 (s, 3H), 3.87 (s, 3H), 3.71 (m, 2H), 3.38 (bs, 1H), 0.73 (s, 3H), 0.57 (s, 3H); FABMS m/z 796 (MH^+). Anal. calcd for $C_{46}H_{63}Cl_2NO_6 \cdot 1.0\ H_2O$: C, 67.80; H, 8.04; N, 1.72. Found: C, 67.68; H, 8.31; N, 1.53.

In vitro anti-HIV assay. Evaluations of the antiviral activity of compounds against HIV-1_{RF}, HIV-1_{IIIB}, and HIV-2_{ROD} infection in CEM-SS and MT-4 cells were performed as previously described.[24,25]

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