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Synthesis and Anti-HIV Activity of Cosalane Analogues Incorporating Two Dichlorodisalicylmethane Pharmacophore Fragments

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Abstract—A new series of cosalane analogues incorporating two fragments of the dichlorodisalicylmethane pharmacophore has been synthesized. In order to identify the position for the attachment of the pharmacophore fragments to the steroid ring that results in the most potent analogues, two types of compounds were designed. In the first type, the two pharmacophore fragments were attached at C-3 and C-17 of the steroid ring by using appropriate linker units. In the second type, both pharmacophore groups were connected to C-3 of the steroid through an alkenyl chain containing an amide moiety. All of the new compounds displayed antiviral activity versus HIV-1_{RF}, HIV-1_{IIIB}, and HIV-2_{ROD} in cell culture. The relative potencies of the compounds resulting from the two attachment strategies were found to depend on the viral strain as well as the cell type. Overall, the attachment of the second pharmacophore did not result in either a large gain or a large loss in anti-HIV activity, and the results are therefore consistent with the hypothesis that the two pharmacophores act independently, and one at a time, with positively charged amino acid side chains present on the surface of gp120 and CD4.

The incorporation of additional aromatic rings containing carboxylic acid groups is one of the most recent strategies investigated for increasing anti-HIV potency in the cosalane series.[1–3] This new strategy is based in part on the results obtained during our work with aurintricarboxylic acid (ATA)[4–8] and the development of cosalane (**1**).[9,10] ATA is a mixture of polyanionic polymers that is obtained when salicylic acid is treated with formaldehyde in the presence of sulfuric acid and sodium nitrite. The anti-HIV activities of both cosalane and ATA have been linked to their ability to inhibit the binding of gp120 to CD4, and both ATA and cosalane bind to both gp120 and CD4.[8,10–12] During the isolation, structure elucidation, and anti-HIV evaluation of low-molecular-weight ATA components, a direct correlation between charge number and antiviral potency was observed.[5,7,8] Similar observations have been reported for other polyanionic classes of anti-HIV compounds.[13] This suggested that the antiviral potency of cosalane might be augmented by incorporating additional aromatic rings possessing carboxylate groups. Application of this strategy has already provided interesting results. Particularly, extension of the dichlorodisalicylmethane cosalane pharmacophore with additional benzoic acid rings afforded more potent agents.[1,2] The anti-HIV activity of compounds possessing two pharmacophore groups has been studied briefly. Only the tetraphenylethylene derivative **2** has been reported.[14] Compound **2** displayed an EC_{50} of 68 μM against HIV-1_{IIIB} in CEM cells. When comparing the anti-HIV potency of this compound with that of the cosalane pharmacophore, the dichlorinated disalicylmethane **3** (EC_{50} = 117 μM against HIV-1_{IIIB} in CEM cells),[8] a modest but noticeable increase in potency is observed for **2**.

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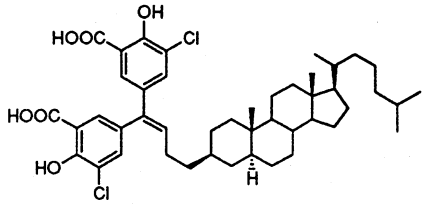

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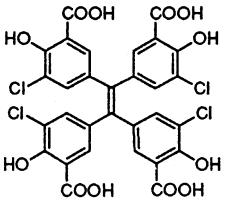

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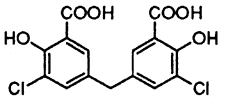

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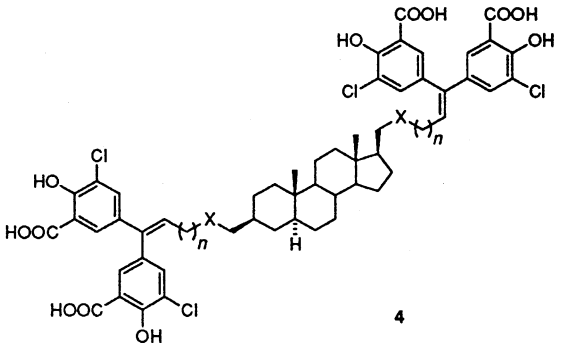
In this study, we considered the design of new analogues that incorporate additional carboxylic acid groups through attachment of two dichlorodisalicylmethane pharmacophore groups to the steroid ring. In this way, two different sets of congeners were planned. In the first group, represented by the general structure **4**, the pharmacophore groups would be attached at C-3 and C-17 of the steroid ring through appropriate linker chains. The length and composition of the linker unit could be modified so as to monitor the effects on the antiviral activity. In the second type of congener, represented by compound **5**, the pharmacophore groups would be joined by an alkenyl chain containing an amide group. The amide nitrogen atom serves to attach this fragment at C-3 of cholestane. In conjunction with our earlier studies, these two sets of compounds would provide valuable information regarding the most favorable constellation (spatial arrangement) of carboxylate groups for anti-HIV activity.



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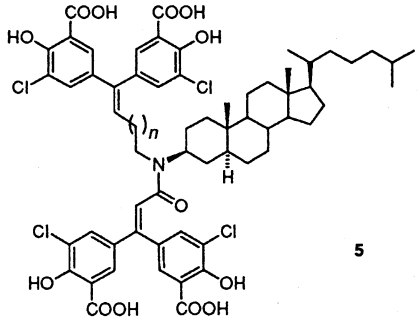

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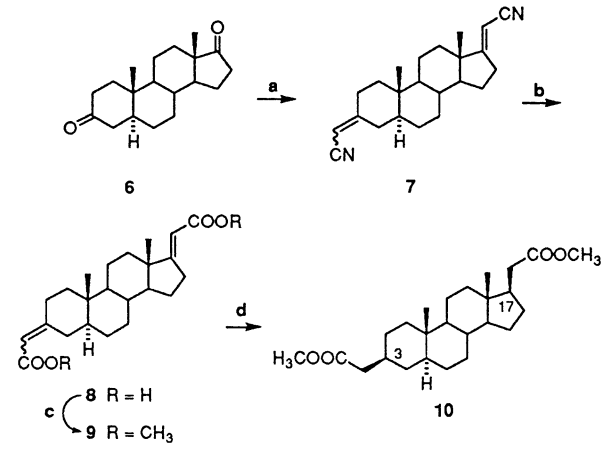
Chemistry

The synthesis of cosalane analogues of general structure **4** required the preparation of a steroid derivative properly functionalized at C-3 and C-17. The synthesis of the key intermediate **10** is displayed in Scheme 1. The α,β-unsaturated nitrile **7** was obtained as a mixture of E and Z double bond isomers from ketone **6** in excellent yield following a literature procedure.[15] Hydrolysis of nitrile **7** required drastic conditions that consisted of refluxing a mixture of **7** and 3 M NaOH in ethylene glycol for 72 h.[16] Acid **8** obtained from this reaction was then reacted with dimethylsulfate and potassium carbonate in refluxing acetone to afford the α,β-unsaturated methyl ester **9**. Both compounds **8** and **9** were also obtained as mixtures of alkene isomers. The reduction of ester **9** to **10** had to be carefully studied, as mixtures of β and α isomers at C-3 were initially obtained. Magnesium in methanol has been reported as a convenient method for the reduction of α,β-unsaturated esters,[17,18] and in fact this method has been applied to the reduction of an exocyclic double bond at C-17 of an α,β-unsaturated steroidal ester.[19] Disappointingly, application of these conditions led only to mixures of isomers at C-3. The results obtained with catalytic hydrogenation under atmospheric pressure were found to be solvent- and catalyst-dependent. Fortunately, reduction of **9** could be successfully carried out at atmospheric pressure in the presence of 10% Pd/C, using only ethyl acetate as solvent for 48 h, to give **10** as a single diastereomer with the desired 3β,17β stereochemistry. In order to be absolutely



Scheme 1. Reagents and conditions: (a) $(EtO)_2POCH_2CN$, $NaNH_2$, THF, 25 °C, 24 h, 96%; (b) NaOH, $HOCH_2CH_2OH$, reflux, 72 h, 66%; (c) $(MeO)_2SO_2$, K_2CO_3, CH_3COCH_3, reflux, 24 h, 65%; (d) H_2, 10% PD/C, EtOAc, 48 h, 98%.

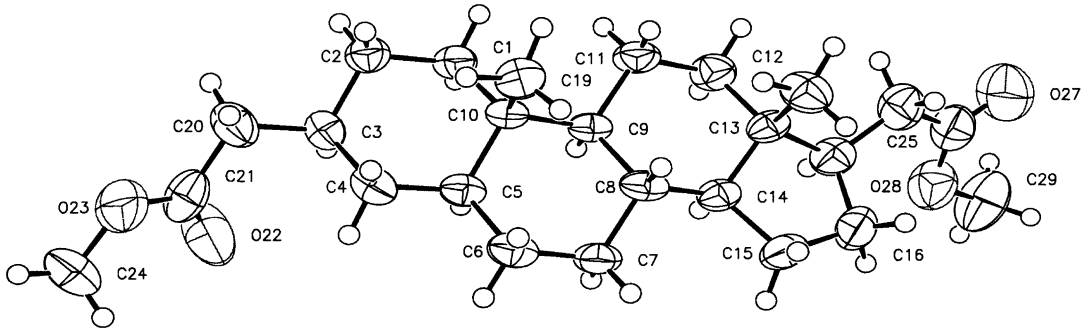


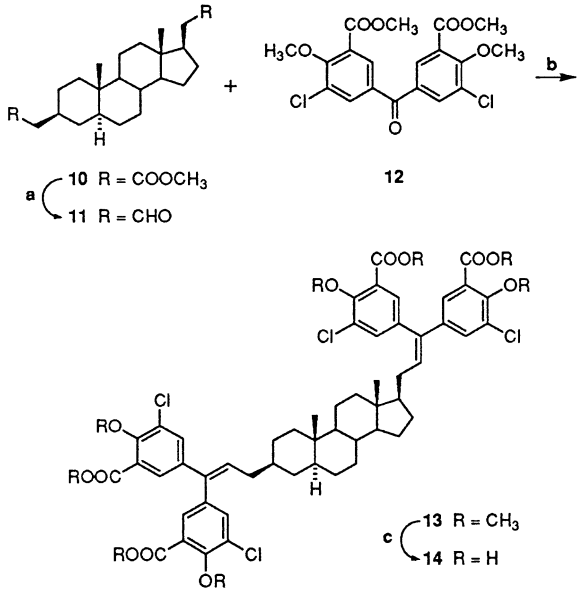
Figure 1. ORTEP diagram resulting from X-ray crystallography of **10**.

sure of the stereochemical assignment in the key intermediate **10**, the structure was determined by X-ray crystallography (Fig. 1).

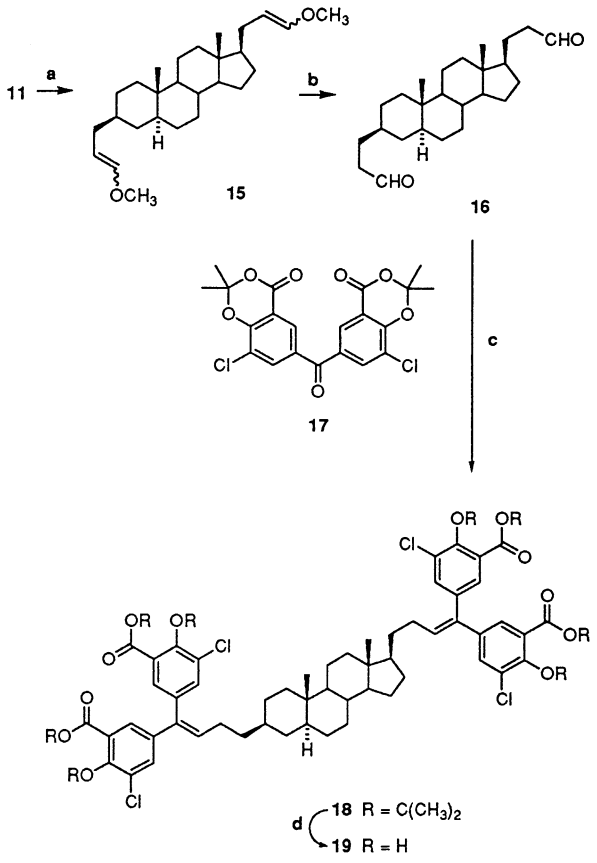
Ester **10** was then used for the preparation of analogue **14**, as shown in Scheme 2. Reduction of **10** with DIBAL-H in toluene-dichloromethane gave aldehyde **11**. The titanium-mediated coupling[20–22] of this aldehyde with benzophenone **12**[10] provided the desired cross-coupled product **13**. Removal of the methyl ester and methyl ether groups from **13** was achieved with boron tribromide–dimethyl sulfide complex to give **14**.

The synthesis of congener **19** is shown in Scheme 3. A one-carbon homologation of both aldehyde groups in **11** was accomplished in two steps. Wittig reaction of **11** with methoxymethyltriphenylphosphonium chloride,[23,24] using NaHMDS as the base, provided bis(enol ether) **15** as a mixture of double bond isomers. Reacting this intermediate with dilute acid provided the one-carbon homologuated dialdehyde **16**. This compound was then coupled to benzophenone **17**[25] using the McMurry reaction. The mild conditions required for removal of the protecting groups from **17** made the use of this compound attractive over benzophenone **12** during the coupling step.[25] In this way, the cross-coupled product **18** was obtained, which after deprotection with K_2CO_3 in refluxing dioxane, afforded the desired target compound **19**.

The preparation of amine **22**, used for the synthesis of cosalane congener **25**, was achieved in two steps from benzophenone **12**, as described in Scheme 4. McMurry coupling of **12** with commercially available Boc-protected



Scheme 2. Reagents and condiitons: (a) DIBAL-H (3.2 equiv), $PhCH_3$–CH_2Cl_2, −78 °C, 3 h, 54%; (b) $TiCl_4$–THF (1:2), Zn (0), THF reflux, 2 h, then **11** and **12**, reflux, 2.5 h, 56%; (c) BBr_3–$S(CH_3)_2$, $ClCH_2CH_2Cl$, reflux, 8 h, 59%.



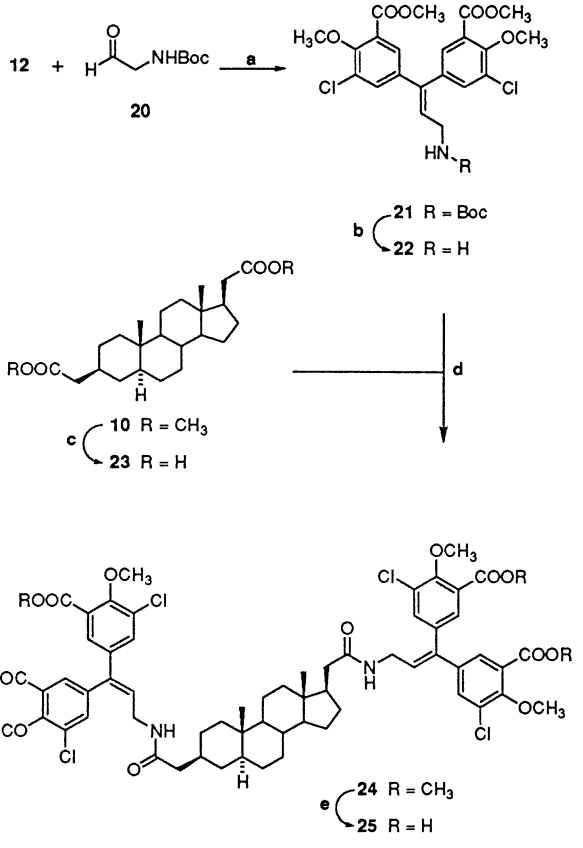
Scheme 3. Reagents and conditions: (a) $ClPh_3PCH_2OCH_3$, NaN$(SiMe_3)_2$, THF, 0 to 25 °C, then **11**, 25 °C, 3.5 h, 85%; (b) 0.5 N HCl, THF, 25 °C, 3 days, 78%; (c) $TiCl_4$–THF (1:2), Zn (0), THF, reflux, 2 h, then **16** and **17**, THF, reflux, 2.5 h, 43%; (d) 0.7 M K_2CO_3, 1,4-dioxane, reflux, 3 h, 96%.

amino aldehyde **20** afforded the cross-coupled product Boc-amine **21**. Removal of the nitrogen protecting group under standard conditions afforded amine **22**. The acid-sensitivity of the Boc group toward the McMurry conditions and the observed instability of amine **22** are probably the causes for the poor overall yield (38%) of the transformation from **12** to **22**. Saponification of ester **10** provided carboxylic acid **23**. EDCI-mediated coupling of amine **22** with acid **23** gave amide **24**. Sodium hydroxide hydrolysis of **24** in tetrahydrofuran afforded the desired amide **25**.

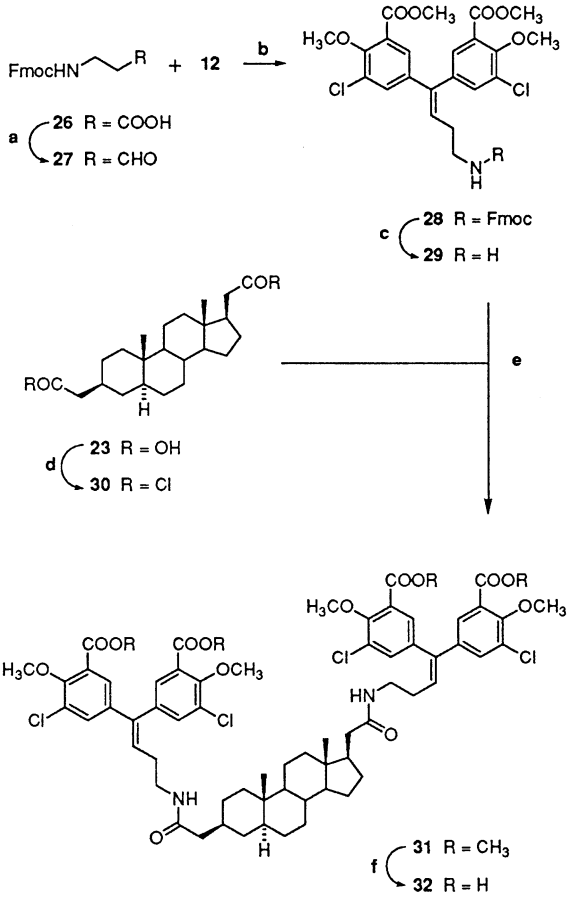
Amine **29** was needed for the preparation of analogue **32**. We previously reported a five-step synthesis of this compound starting from benzophenone **12**, which provided **29** with a 31% overall yield.[26] Now, we present a more efficient synthesis of amine **29** that departs from commercially available *N*-Fmoc-β-alanine (**26**) and is described in Scheme 5. Applying the methodology reported by Four and Guibe,[27] acid **26** was converted to the corresponding acid chloride using standard conditions. Reaction of this intermediate with $(Ph_3P)_4Pd$ and tributyltin hydride in THF provided aldehyde **27**. Cross-coupling of **27** with benzophenone **12** under the McMurry conditions gave Fmoc-amine **28.** Removal of the nitrogen protecting group was accomplished with piperidine in THF to afford amine **29**. Using this new synthetic pathway, the preparation of amine **29** was achieved in only two steps from **12** with a 65% overall yield.

Coupling of carboxylic acid **23** with amine **29** was carried out by first converting **23** to acid chloride **30** (Scheme 5). This intermediate was then reacted with amine **29** (2.2 equiv) and Et_3N in dichloromethane to afford bisamide **31**. Ester hydrolysis of **31** with 0.5 NaOH in THF gave amide **32**.

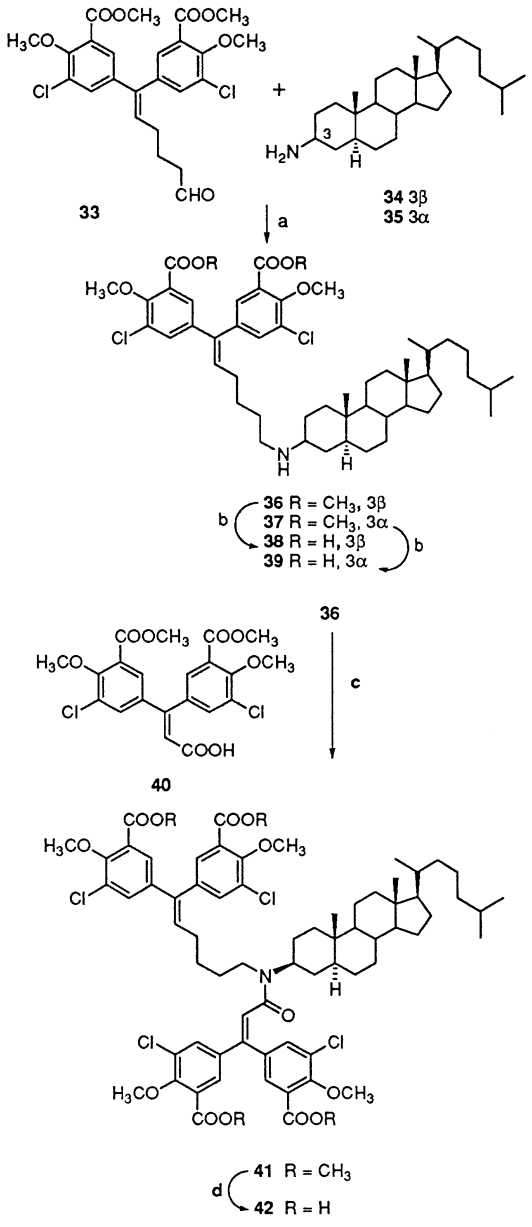
The preparation of analogue **42** begins with the reductive alkylation of the known aldehyde **33**[28] with 3β-aminocholestane (**34**)[29] as shown graphically in Scheme 6. This transformation was achieved using sodium triacetoxyborohydride in 1,2-dichlorethane to provide amine **36**.[30] EDCI-mediated coupling of amine **36** with carboxylic acid **40** gave amide **41**, which after cleavage of the methyl esters with 0.5 N NaOH in THF provided analogue **42**. In order to evaluate the anti-HIV activity of the amino component of analogue **42**, the ester groups from its precursor **36** were cleaved to provide amine **38**. The 3α-amine **39** was also prepared using 3α-aminocholestane (**35**) in the reductive alkylation step.



Scheme 4. Reagents and conditions: (a) $TiCl_4$–THF (1:2), Zn (0), THF, reflux, 2 h, then **12** and **20**, THF, reflux, 2.5 h, 23%; (b) TFA, CH_2Cl_2, 38%; (c) NaOH, THF, reflux, 24 h, 86%; (d) EDCI–HCl, HOBt, Et_3N, DMF, 48 h, 22%; (e) 0.5 N NaOH, THF, reflux, 48%.



Scheme 5. Reagents and conditions: (a) (i) $(COCl)_2$, DMF (catalytic amount), THF; (ii) $(Ph_3P)_4Pd$, Bu_3SnH, THF, 89%; (b) $TiCl_4$–THF (1:2), Zn (0), THF, reflux, 2 h, then **12** and **27**m THF, reflux, 2.5 h, 72%; (c) piperidine, THF, 25 °C, 3 h, 91%; (d) $(COCl)_2$, DMF catalytic amount), THF; (e) **29** (2.2 equiv), Et_3N, 0→25 °C, 48 h, 53%; (f) N NaOH, THF, 60 °C, 24 h, 88%.



Scheme 6. Reagents and conditions: (a) **34** or **35**, Et_3N, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt, 8–24 h, **36**: 92%; **37**, 89%; (b) K_2CO_3, KCN, THF–MeOH–H_2O, 75 °C, 5 h, **38**: 72%, **39**: 98%; (c) EDCl–HCl, HOBt, Et_3N, DMF, 48 h, 84%; (d) NaOH, THF, 65 °C, 12 h, 89%.

Biological Results and Discussion

The incorporation of additional aromatic rings containing carboxylic acid groups has proven to be a moderately successful strategy for increasing the anti-HIV potency in the cosalane series.[1–3] The negatively charged carboxylates are thought to bind to multiple, positively charged, basic residues on the surface of CD4, including Arg58, Arg59, and Lys72.[2] In this study, cosalane analogues were prepared that incorporate two dichlorodisalicylmethane moieties. The new analogues were tested for inhibition of the cytopathic effect of HIV-1_{RF} in CEM-SS cells, of HIV-1_{IIIB} in MT-4 cells, and of HIV-2_{ROD} in MT-4 cells, and the resulting EC_{50} values are presented in Table 1. The new compounds were also evaluated for cytotoxicity in uninfected CEM-SS and MT-4 cells, and the CC_{50} values obtained from this screening are also displayed in Table 1. Except for amines **38** and **39**, all of the new analogues demonstrated anti-HIV activity against the replication of HIV-1 and HIV-2. The antiviral potency is dependent on the virus type and strain. The most potent analogue against HIV-1_{RF} was **19** ($EC_{50}=1.3$ μM), which showed an approximately 4-fold increase in potency over cosalane (**1**) ($EC_{50}=5.1$ μM). Amide **42** ($EC_{50}=6.9$ μM) was almost equipotent with cosalane against the replication of HIV-1_{RF}, while the other three new congeners **14** ($EC_{50}=12$ μM), and amides **25** ($EC_{50}=35$ μM) and **32** ($EC_{50}=38$ μM) were less potent than cosalane (**1**) against HIV-1_{RF}. In the case of HIV-1_{IIIB}, the most potent analogue was amide **42** ($EC_{50}=9.9$ μM), which turned out to be approximately 3-fold less potent than cosalane ($EC_{50}=3.0$ μM). Compound **14** ($EC_{50}=14$ μM) was the second most potent analogue against HIV-1_{IIIB}, followed by **19** ($EC_{50}=20$ μM) and the bisamides **25** ($EC_{50}=48$ μM) and **32** ($EC_{50}=47$ μM). Amide **42** ($EC_{50}=8.2$ μM) was also the most potent compound against HIV-2_{ROD}, but it was 2-fold less potent than cosalane ($EC_{50}=4.0$ μM). The other analogues were significantly less potent than cosalane (**1**) against HIV-2_{ROD}. Amide **42** was also the most cytotoxic analogue of this series in both cell lines.

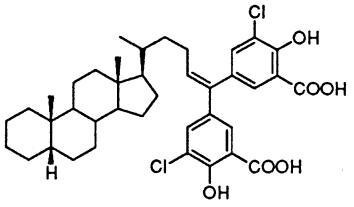
The incorporation of two pharmacophore groups attached at C-3 and C-17 of the steroid ring by means of an alkenyl chain provided two new compounds, **14** and **19**. Although there are differences in stereochemistry at C-5, the antiviral potency of these two analogues can be compared with that of the previously reported congener **43**, in which the pharmacophore group is appended at C-17 of the steroid ring by a four-carbon alkenyl chain.[31] The EC_{50} values of **43** against both strains of HIV-1 (RF and IIIB) and also against HIV-2_{ROD} are included in Table 1. Analogues **14** and **19** were more potent than **43** against both strains of HIV-1. Congener **14** can also be compared with analogue **44**, which was reported during our studies dealing with the exploration of the effects of linker chain modifications in the cosalane series.[32] Compound **44** has an EC_{50} of 25 μM against the replication of HIV-1_{RF} in CEM-SS cells. Both compounds contain a pharmacophore unit attached at C-3 of the steroid by a two-carbon alkenyl chain, and therefore they mainly differ in the second pharmacophore moiety attached at C-17. Although modest, there is a difference in anti-HIV-1 potency between these two analogues in favor of **14**, which contains four carboxylic acid groups. As mentioned previously, analogue **19** was approximately 4-fold more potent than cosalane against HIV-1_{RF}. Cosalane and **19** contain a pharmacophore group attached at C-3 by a three-carbon alkenyl chain, and, again, they mainly differ in the second pharmacophore unit at C-17. Taken together, the results observed with **14** and **19** indicate that there is a modest increase in potency against HIV-1_{RF} when a second dichlorodisalicylmethane unit is appended at C-17. This is not the situation for the antiviral activity against the replication of HIV-1_{IIIB} and

Table 1. Anti-HIV activities of cosalane (**1**) and analogues[a]

Compound	EC_{50} (μM)[b]			CC_{50} (μM)[c]	
	HIV-1_{RF}[d]	HIV-1_{IIIB}[e]	HIV-2_{ROD}[e]	CEM-SS cells	MT-4 cells
1	5.1±2.1	3.0±0.18	4.0±2.1	>200	>125
14	12±2.4	14±0.69	60±2.7	55±14	115
19	1.3±0.2	20±3.0	74±19	130±19.5	>125
25	35±3.5	48±12	70±40	133±20	>125
32	38±5.7	47±10	52±4.0	113±20	>125
38	>316	>150	>150	>316	>150
39	>200	>150	>150	>200	>150
42	6.9±1.7	9.9±0.90	8.2±1.8	58±12	33±12
43[f]	28±14	27±3.0	>31	70±23	31
44[g]	25±12	ND[h]	ND	>320	ND
47[i]	5.2±2.0	15±1.4	9.8±1.0	107±50	52±8.5

[a]Compounds were tested as their ammonium salts.
[b]Concentation required to reduce the cytophathic effect of the virus by 50%.
[c]Concentration required for a 50% reduction in cellular viability of uninfected cells.
[d]Determined in CEM-SS.
[e]Determined in MT-4 cells.
[f]Taken from ref 31.
[g]Taken from ref 32.
[h]ND, not determined.
[i]Taken from ref 29.

HIV-2_{ROD}, where the incorporation of a second pharmacophore group led to a decrease in antiviral potency. Several factors can be taken into account to explain these observations. It is important to consider the possible mode of action of the new analogues. It has been reported before that the mechanism of action of cosalane and analogues possessing an extended polyanionic pharmacophore involves interaction with both gp120 and CD4.[2,33] It might be expected that the new analogues would exert their antiviral action by interaction with these molecules as well. Therefore, the variations in antiviral potency observed in this study can partially be ascribed to differences of the gp120–CD4 interaction that are related to virus type and strain. This is consistent with the report that the affinity of the interaction of gp120 with CD4 is not the same among different strains of HIV-1. Particularly, it has been reported that gp120 of HIV-2_{ROD} shows at least a 15-fold lower affinity for CD4 than the corresponding glycoprotein derived from HIV-1_{IIIB}.[34] Strain-determined differences in the activity against HIV replication have been reported earlier for other classes of polyanionic compounds.[35]



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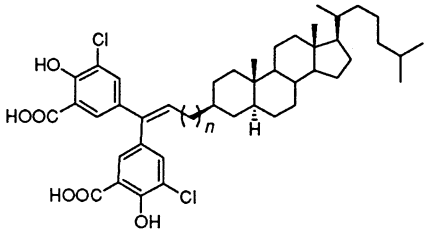
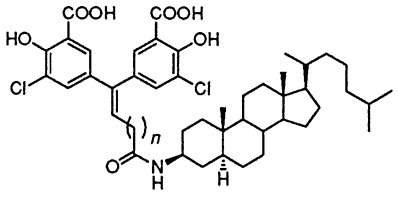


44 *n* = 1
45 *n* = 3



46 *n* = 1
47 *n* = 0

Amides **25** and **32** incorporate three modifications when compared with cosalane: (1) a second pharmacophore group was attached at C-17 of the steroid; (2) an amide group was inserted into the alkenyl chain; and (3) the length of the chain was increased by either two or three atoms. These changes led to a noticeable decrease in anti-HIV potency. The effects of incorporating a second pharmacophore group at C-17 have been already discussed. Therefore, it is important to consider the impact on the biological activity of incorporating an amide group into the alkenyl chain and the variations in the length of the chain. There has been limited work regarding the extension of the alkenyl chain of cosalane. Only compounds **45** and **46**, containing a one-atom extended linker chain when compared to that of cosalane (**1**), have been prepared and tested for inhibition of HIV cytopathicity.[29,32] Considering the results obtained with these analogues, it was difficult to predict the effects on the antiviral potency of lengthening the alkenyl chain in amides **25** and **32**. While **45** (EC_{50} = 3.4 μM) was equipotent with cosalane against HIV-1_{RF},[32] amide **46** (EC_{50} = 23 μM) was over 4-fold less potent than cosalane (**1**).[29] This difference in anti-HIV potency was attributed to alterations in conformation and rigidity introduced by the incorporation of the amide moiety,

resulting from the well-known restricted rotation around the amide bond. The results obtained with bisamides **25** and **32** indicate that lengthening the alkenyl chain does not have an effect on the antiviral potency, as both compounds displayed similar EC_{50} values against HIV-1 and HIV-2. Based on these considerations, it is proposed that the diminished antiviral potency of amides **25** and **32** relative to **14** and **19** is derived, at least partially, from the insertion of the amide groups into the linker chains. In addition, the length of the alkenyl chain required for optimal potency might have been surpassed.

Amide **42** is one of the most potent analogues in this series. It displayed similar potency as cosalane against both strains of HIV-1 (RF and IIIB) and against HIV-2_{ROD}. It is interesting to compare **42** with the previously reported amide **47**.[29] These two analogues only differ in the second pharmacophore group attached to the amide nitrogen. To facilitate the analysis, the EC_{50} values for amide **47** have been included in Table 1. Comparison of these results show that both compounds exhibit very similar EC_{50} values against HIV-1_{RF}, HIV-1_{IIIB}, and HIV-2_{ROD}. These observations tend to indicate that in this particular case, the incorporation of the second pharmacophore group does not provide a significant effect on anti-HIV potency. However, the length of the alkenyl chain utilized to attach the second pharmacophore group to the amide nitrogen could also be partially responsible for not having a noticeable increase in the antiviral potency. In addition, the amide group might not be the most appropriate group to link the pharmacophore groups. A reduction in conformation and rigidity has already been noted when an amide moiety is introduced in the linker unit. Also, the cytotoxicity tends to increase when this functionality is present in the molecule.

The lack of antiviral activity of amines **38** and **39** is not surprising and these results are in agreement with our previous work with the amine linker chains. We proposed earlier that the absence of anti-HIV activity in analogues containing an amino group in the alkenyl chain could be due to a compromise of the lipophilicity of the linker chain brought about by the positively-charged ammonium ion, which could disrupt the membrane interactive nature of the molecule.[29] It is noteworthy that by converting the nitrogen atom from an amine in **38** to an amide in **42**, the anti-HIV activity is fully recovered.

The new analogues **14**, **19**, **25** and **32** are not likely to interact with the viral envelope and cell membrane in the same way as proposed for cosalane. It has been postulated that the highly lipophilic steroid portion of cosalane might insert into the viral and cell membranes, while the pharmacophore group will remain protruding outward (Fig. 2).[2,32] With the analogues mentioned above, the incorporation of the additional pharmacophore group at C-17 of the steroid should eliminate, or at least alter, this mode of interaction of the molecule with the membrane. This is not the situation for amide **42**, which can still interact with the viral and cell membranes and in this case, not only one, but two dichlorodisalicylmethane pharmacophore groups will be pointing outward. This way of presenting the pharmacophore groups seems to be preferred for generating more potent compounds. However, there are other combinations of positions that have not been examined and could provide interesting results.

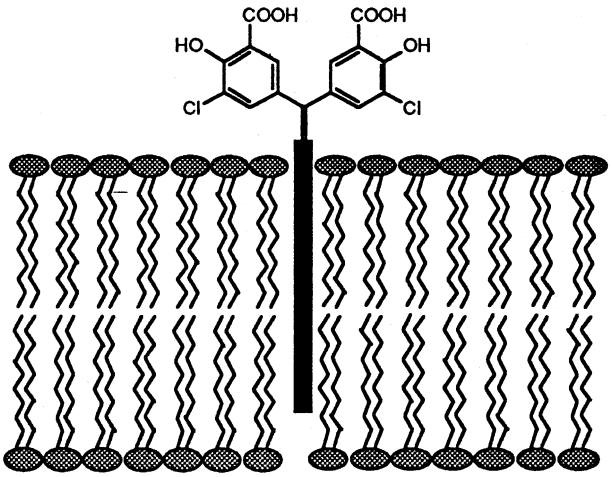


Figure 2. Schematic representation of a hypothetical model for the anchoring of the disalicylmethane moiety of cosalane and cosalane analogues to the cell membrane and viral envelope by the steroid fragment.

In summary, we have considered the appendage of an additional disalicylmethane moiety as a strategy to increase the anti-HIV potency of cosalane. This was implemented by attaching two pharmacophore groups through appropriate linker units to C-3 and C-17 or only C-3 of the steroid ring. At the outset, four possibilities existed for the effect of incorporating an additional pharmacophore: synergy, additivity, little of no effect, or cancellation of activity. While the results obtained from this study indicate that there is no significant gain in potency when a second pharmacophore unit is appended in the cosalane series, it is important to note that this strategy did not lead to inactive compounds. The results are consistent with the hypothesis that the two dichlorodisalicylmethane pharmacophore groups act independently, and that they interact one at a time with CD4 and gp120.

Experimental

General

Melting points were determined in capillary tubes on a Mel-Temp apparatus (Alrich Chemical Company, WI, USA) and are uncorrected. Spectra were obtained as follows: CI mass spectra on a Finnigan 4000 spectrometer (Thermo Finnigan, CA, USA); FAB mass spectra and EI mass spectra on a Kratos MS50 spectrometer (Kratos Analytical Inc., NY, USA); ^{1}H NMR spectra on Varian VXR-500S (Varian Inc., CA, USA) and Bruker ARX-300 (Bruker Analytik GmbH, Bremen,

Germany) spectrometers; and IR spectra on a Beckman IR-33 spectrometer (Beckman Coulter Inc.) or on a Perkin–Elmer 1600 series FTIR (Norwalk, CT, USA). 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.

(*E*)- and (*Z*)-5α-3,17-(Dicyanomethylene)androstane (7). This mixture of double bond isomers was obtained in excellent yield (96%) following the procedure reported by Bose et al.[15] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.06 (t, J=2 Hz) and 4.94 (q, J=2.5 Hz, total 1H), 5.00 and 4.98 (m, total 1H), 2.85–1.0 (m, 25H), 0.92 and 0.80 (s, total 3H), 0.89 (s, 3H).

(*E*)- and (*Z*)-5α-3,17-(Dicarboxymethylene)androstane (8). A solution of dinitrile **7** (1.00 g, 2.99 mmol) was suspended in ethylene glycol (100 mL) and 3 M aqueous sodium hydroxide (2.20 mL, 60.00 mmol) was added. The suspension, which turned into a colorless solution after heating, was stirred under reflux for 72 h (the reaction was monitored by placing a wet pH paper on top of the condenser to observe the evolution of ammonia). At this time, the reaction mixture was allowed to cool at room temperature and then water (300 mL) was added. The aqueous solution was acidified with 1 M HCl (100 mL) and the product extracted with chloroform (4×75 mL). The combined organic extracts were washed with brine (1×100 mL), dried over magnesium sulfate, filtered, and the solvent evaporated to give a purple solid. Recrystallization from methanol provided compound **8** as a white solid (0.72 g, 65%): mp 255–260 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.51 (s, 1H), 5.48 and 5.47 (s, total 1H), 2.95 (bs, 2H), 2.85–2.72 (m, 2H), 2.15–0.85 (m, 20H), 0.80 (s, 3H), 0.73 (s, 3H); IR (film) 3600–2700, 2912, 1681, 1415, 1217, 908 cm^{-1}; CIMS m/z 373 (MH^+) and 355 (MH^+-H_2O). Anal. calcd for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 73.87; H, 8.93.

(*E*)- and (*Z*)-5α-3,17-(Dimethoxycarbonylmethylene)androstane (9). Diacid **8** (0.8 g, 2.15 mmol) was dissolved in acetone (60 mL), followed by the addition of potassium carbonate (1.78 g, 12.9 mmol). Then, dimethyl sulfate (1.0 mL, 10.75 mmol) was added and the mixture was heated under reflux for 24 h. The mixture was allowed to cool at room temperature and the insoluble materials were separated by filtration, washing the filtrate with dichloromethane. The solvents were evaporated and then water (40 mL) was added to the residue. The product was extracted with dichloromethane (3×30 mL). The combined organic extracts were washed with brine (1×50 mL), dried over magnesium sulfate, filtered, and the solvent evaporated to give a yellowish oil. Purification by silica gel flash chromatography (50 g; column: 4×8 cm), using hexane-ethyl acetate (3:1) as eluant, afforded **9** as a yellowish solid (0.565 g, 65.6%). The analytical sample was recrystallized from ethyl acetate–methanol: mp 112–113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.52 (bs, 1H), 5.46 and 5.44 (s, total 1H), 3.67 (s, 3H), 3.66 (s, 3H), 3.00 (bs, 1H), 2.94 (bs, 2H), 2.83 and 2.80 (s, total 1H), 2.2–1.85 (m, 18H), 0.81 (s, 3H), 0.75 (s, 3H); IR (film) 2911, 2850, 1741, 1650, 1434, 1344, 1257, 1165, 1013, 865 cm^{-1}; CIMS m/z 401 (MH^+). Anal. calcd for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 75.02; H, 9.28.

5α-3β,17β-(Dimethoxycarbonylmethyl)androstane (10). Ester **9** (1.035 g, 2.584 mmol) was dissolved in ethyl acetate (85 mL) and 10% Pd/C (0.103 g) was added. The mixture was hydrogenated at atmospheric pressure and room temperature for 48 h. The catalyst was filtered off, and the solvent removed to provide compound **10** (1.04 g, 98%) as a white solid. The analytical sample was prepared by recrystallization from methanol: mp 89–90 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (s, 3H), 3.61 (s, 3H), 2.32 (dd, J=14.7 Hz and 5.1 Hz, 1H), 2.16 (d, J=6.6 Hz, 2H), 2.07 (dd, J=14.0 Hz and 9.6 Hz, 1H), 1.94–0.78 (m, 24H), 0.72 (s, 3H), 0.54 (s, 3H); IR (film) 2921, 2847, 1740, 1436, 1261, 1238, 1149 cm^{-1}; CI-MS m/z 405 (MH^+). Anal. calcd for $C_{25}H_{40}O_4$: C, 74.22; H, 9.97. Found: C, 74.29; H, 10.01.

5α-3β,17β-Di(formylmethyl)androstane (11). A solution of ester **10** (0.300 g, 0.742 mmol) in dichloromethane (10 mL) was cooled to −78 °C and put under Ar. A 1.0 M solution of DIBAL-H in toluene (1.6 mL, 1.6 mmol) was added and the mixture stirred for 2 h. More 1.0 M solution of DIBAL-H (0.8 mL, 0.8 mmol) was added and the mixture stirred for 1 h. At this time, a saturated solution of Rochelle salt (potassium sodium tartrate) (15 mL) was added and the mixture allowed to warm to rt. The mixture was diluted with dichloromethane (20 mL) and the phases were separated. The aqueous phase was extracted with dichloromethane (3×20 mL). The combined organic extracts were washed with brine (1×30 mL), dried over $MgSO_4$, filtered, and the solvent removed. Purification was achieved by flash chromatography on silica gel (40 g; column: 2×27.5 cm), eluting with hexanes–ethyl acetate (6:1), followed by hexanes–ethyl acetate (3:1). This purification afforded aldehyde **11** in moderate yield (0.137 g, 53.7%) as a white solid: mp 122–125 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.73 (s, 2H), 2.45 (ddd, J=2.3, 4.4, and 15.5 Hz, 1H), 2.27 (dd, J=2.3 and 6.9 Hz, 2H), 2.20 (ddd, J=2.4, 9.5, and 15.9 Hz, 1H), 2.0–0.8 (m, 24H), 0.74 (s, 3H), 0.56 (s, 3H); IR (film) 2914, 2846, 1704, 1445, 1284, 938 cm^{-1}; CI-MS m/z 345 (MH^+) and 327 (MH^+-H_2O). Anal. calcd for $C_{23}H_{36}O_2$: C, 80.18; H, 10.53. Found: C, 80.01; H, 10.46.

5α-3β,17β-Di[3′,3′-(3″,3‴-dicarbomethoxy-5″,5‴-dichloro-4″,4‴-dimethoxydiphenyl)-2′-propenyl]androstane (13). A slurry of $TiCl_4$-THF (1:2) complex (0.687 g, 2.058 mmol) and Zn dust (0.27 g, 4.116 mmol) in THF (25 mL) was stirred and heated under reflux for 2 h. A solution of aldehyde **11** (0.118 g, 0.343 mmol) and benzophenone **12**[10] (0.32 g, 0.755 mmol) in THF (15 mL) was added. The black mixture was heated under reflux for 2.5 h. It was then cooled to room temperature and 1.0 N HCl (30 mL) was added. The mixture was stirred at room temperature overnight. The layers were separated and the aqueous one was extracted with EtOAc (3×20 mL). The combined organic extracts were washed with brine (1×20 mL), dried over $MgSO_4$, filtered, and the solvent removed. The residue was flash chromatographed on silica gel (70–75 g; column: 3×26 cm)

eluting with hexanes–ethyl acetate (3:1) to provide **13** as a glassy solid in moderate yield (0.216 g, 55.6%): mp 97–100 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, J = 2.4 Hz, 2H), 7.47 (d, J = 2.1 Hz, 1H), 7.46 (d, J = 2.1 Hz, 1H), 7.32 (d, J = 2.1 Hz, 1H), 7.30 (d, J = 2.1 Hz, 1H), 7.29 (d, J = 2.7 Hz, 1H), 7.28 (d, J = 2.4 Hz, 1H), 6.09 (t, J = 7.2 Hz, 1H), 6.07 (t, J = 6.9 Hz, 1H), 3.99 (s, 3H), 3.99 (s, 3H), 3.92 (s, 6H), 3.91 (s, 3H), 3.91 (s, 6H), 3.90 (s, 3H), 2.24–2.1 (m, 1H), 1.98 (t, J = 7.2 Hz, 2H), 1.94–1.78 (m, 1H), 1.76–0.8 (m, 24H), 0.72 (s, 3H), 0.46 (s, 3H); IR (film) 2933, 1737, 1476, 1435, 1264, 1206, 1000 cm^{-1}; PD-MS m/z 1135 (MH^+) and 1103 ($MH^+ - CH_3OH$). Anal. calcd for $C_{61}H_{68}Cl_4O_{12}$: C, 64.55; H, 6.04. Found: C, 64.80; H, 5.88.

5α-3β,17β-Di[3′,3′-(3″,3‴-dicarboxy-5″,5‴-dichloro-4″,4‴-dihydroxydiphenyl)-2′-propenyl]androstane (14). Boron tribromide–dimethyl sulfide complex (0.99 g, 3.108 mmol) was dissolved in 1,2-dichloroethane (4 mL) and put under Ar. A solution of compound **13** (0.176 g, 0.155 mmol) in 1,2-dichloroethane (15 mL) was added and the mixture was heated under reflux for 8 h. The mixture was cooled and water (25 mL) was added and the mixture stirred overnight. Ethyl acetate (30 mL) was added and the phases were separated. The aqueous one was extracted with chloroform (3×25 mL). The combined organic extracts were washed with brine (1×30 mL), dried over Na_2SO_4, filtered and the solvent removed. The residue was flash chromatographed on silica gel (65 g; column: 3×24.5 cm), eluting with $CHCl_3$–THF–formic acid (300:15:1), then (300:60:1), followed by $CHCl_3$–MeOH–formic acid (85:15:1). In this manner, compound **14** was obtained in moderate yield (94 mg, 59%) as an off-white solid: mp 190–194 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.71 (d, J = 2.2 Hz, 2H), 7.68 (d, J = 2.2 Hz, 2H), 7.56 (d, J = 2.2 Hz, 1H), 7.53 (d, J = 2.2 Hz, 1H), 7.47 (d, J = 2.2 Hz, 1H), 7.46 (d, J = 2.2 Hz, 1H), 6.21 (t, J = 7.6 Hz, 1H), 6.17 (t, J = 7.4 Hz, 1H), 1.8–0.8 (m, 24H), 0.72 (s, 3H), 0.48 (s, 3H); IR (film) 3500–2500, 2917, 1678, 1599, 1461, 1233, 1183, 1046 cm^{-1}; PD-MS m/z 1023 (MH^+). Anal. calcd for $C_{53}H_{52}Cl_4O_{12}$: C, 62.24; H, 5.12. Found: C, 62.44; H, 5.46.

5α-3β,17β-Di(*E*,*Z*-3′-methoxy-2-*n*-propenyl)androstane (15). Methoxymethyltriphenylphosphonium chloride (1.47 g, 4.288 mmol) was placed under Ar and dry THF (35 mL) was added. The suspension was cooled in an ice bath and a 1.0 M solution of $NaN(SiMe_3)_2$ in THF (4.3 mL, 4.3 mmol) was added. The red mixture was stirred at 0 °C for 20 min and then at 25 °C for 25 min. A solution of aldehyde **11** (0.369 g, 1.072 mmol) in THF (20 mL) was added. The mixture was stirred at room temperature for 3.5 h. It was quenched with satd NH_4Cl (35 mL). The phases were separated and the aqueous layer was extracted with EtOAc (3×30 mL). The combined organic layers were washed with brine (1×35 mL), dried over $MgSO_4$, filtered, and the solvent removed. Purification was achieved by flash chromatography on silica gel (75 g; column: 3×26.5 cm), eluting with hexanes–benzene (4:1 and then 2:1). This purification afforded a mixture of Z/E olefins **15** in 2:1 ratio as a clear oil in good yield (0.366 g, 85.3%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.24 (d, J = 12.6 Hz, 1H, major isomer), 6.22 (d, J = 12.5 Hz, 1H, major isomer), 5.85 (d, J = 17.2 Hz, 1H, minor isomer), 5.83 (d, J = 17.2 Hz, 1H, minor isomer), 4.68 (m, 1H, major isomer), 4.29 (m, 1H, minor isomer), 3.55 and 3.47 (s, total 3H), 3.48 (s, 3H), 2.2–0.8 (m, 28H), 0.72 (s, 3H), 0.57 and 0.55 (s, total 3H); IR (film) 2919, 2847, 1654, 1446, 1209, 1109, 932 cm^{-1}; FAB-MS m/z 401 (MH^+). Anal. calcd for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 81.27; H, 11.16.

5α-3β,17β-Di(formylethyl)androstane (16). Compound **15** (0.068 g, 0.170 mmol) was dissolved in THF (3 mL), 0.5 N HCl (1 mL) was added, and the mixture was stirred at room temperature for 3 days. The mixture was partitioned between EtOAc (8 mL) and 1.0 M $NaHCO_3$ (8 mL). The organic phase was separated and the aqueous layer was extracted with EtOAc (3×8 mL). The combined organic extracts were washed with brine (1×10 mL), dried over $MgSO_4$, filtered, and the solvent removed. Purification was achieved by flash chromatography on silica gel (35 g; column: 2×26.5 cm), eluting with hexanes-ethyl acetate (10:1). This purification afforded aldehyde **16** in moderate yield (49 mg, 78%) as a white solid: mp 70–74 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (s, 2H), 2.5–2.25 (m, 4H), 1.9–0.75 (m, 28H), 0.73 (s, 3H), 0.56 (s, 3H); IR (film) 2918, 2849, 1724, 1446, 1382 cm^{-1}. Anal. calcd for $C_{25}H_{40}O_2$: C, 80.59; H, 10.82. Found: C, 80.28; H, 10.64.

5α-3β,17β-Di[4′,4′-bis[(8″,8‴-dichloro-2″,2″,2‴,2‴-tetramethyl-4″,4‴-dioxo-6″,6‴-(1,3-benzodioxyl)]-3′-butenyl]-androstane (18). A slurry of $TiCl_4$–THF (1:2) complex (0.539 g, 1.614 mmol) and Zn dust (0.211 g, 3.228 mmol) in THF (20 mL) was stirred and heated under reflux for 2 h. A solution of aldehyde **16** (0.100 g, 0.269 mmol) and benzophenone **17**[25] (0.266 g, 0.591 mmol) in THF (15 mL) was added. The black mixture was heated under reflux for 2.5 h. It was then cooled to room temperature and 0.1 N HCl (25 mL) was added. The mixture was stirred for 15 min and the layers were separated. The aqueous phase was extracted with EtOAc (3×20 mL). The combined organic extracts were washed with brine (1×25 mL), dried over $MgSO_4$, filtered, and the solvent removed. The residue was flash chromatographed on silica gel (35 g; column: 2×27.5 cm), eluting with hexanes–ethyl acetate (4:1 to 3:1), to provide **18** as an off-white solid in moderate yield (0.14 g, 43%): mp 162–165 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, J = 1.1 Hz, 4H), 7.39–7.37 (m, 4H), 6.07 (t, J = 7.4 Hz, 1H), 6.06 (t, J = 7.4 Hz, 1H), 2.02 (m, 4H), 1.81 (s, 12H), 1.77 (s, 12H), 1.7–0.8 (m, 28H), 0.70 (s, 3H), 0.52 (s, 3H); IR (film) 2921, 2851, 1747, 1607, 1483, 1281, 1199, 1062 cm^{-1}; PD-MS m/z 1214 (MH^+) and 1155 ($MH^+ - CH_3COCH_3$). Anal. calcd for $C_{67}H_{72}Cl_4O_{12}$: C, 66.45; H, 5.99. Found: C, 66.20; H, 6.12.

5α-3β,17β-Di[4′,4′-(3″,3‴-dicarboxy-5″,5‴-dichloro-4″,4‴-dihydroxydiphenyl)-3′-butenyl]androstane (19). Acetonide **18** (0.133 g, 0.109 mmol) was dissolved in 1,4-dioxane (5.5 mL). A 0.7 M solution of K_2CO_3 (1.9 mL, 1.308 mmol) was added. The mixture was heated under reflux for 3 h. The mixture was allowed to cool, diluted with water (15 mL), and acidified with 2 N HCl. The

mixture was extracted with ethyl acetate (5×15 mL). The combined organic extracts were washed with brine (1×25 mL), dried over Na_2SO_4, filtered and the solvent removed. The residue was flash chromatographed on silica gel (35 g; column: 2×26.5 cm), eluting with $CHCl_3$–THF–formic acid (300:60:1), followed by $CHCl_3$–MeOH–formic acid (90:10:1 to 65:35:1). In this manner, compound **19** was obtained in excellent yield (0.11 g, 96%) as a pale brown solid. The analytical sample was obtained by precipitation from acetone–dichloromethane–hexanes: mp >200 °C (decomposition); ^{1}H NMR (300 MHz, acetone-d_6) δ 7.91 (d, J=2.1 Hz, 1H), 7.90 (d, J=2.0 Hz, 1H), 7.89 (d, J=1.9 Hz, 1H), 7.87 (d, J=2.6 Hz, 1H), 7.77 (d, J=2.8 Hz, 1H), 7.76 (d, J=2.6 Hz, 1H), 7.68 (d, J=1.3 Hz, 2H), 6.37 (t, J=7.5 Hz, 2H), 2.38 (m, 4H), 2.0–1.0 (m, 28H), 0.93 (s, 3H), 0.76 (s, 3H); IR (film) 3500–2500, 2920, 2850, 1681, 1600, 1455, 1232, 1182, 800 cm^{-1}; PD-MS m/z 1051 (MH^+) and 1074 (M + Na). Anal. calcd for $C_{55}H_{56}Cl_4O_{12}$: C, 62.86; H, 5.37. Found: C, 62.66; H, 5.44.

***N-tert*-Butoxycarbonyl-3′,3″-dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-3,3-diphenyl-2-propenylamine (21).** A slurry of $TiCl_4$–THF 1:2 complex (0.47 g, 1.407 mmol) and Zn dust (0.184 g, 2.814 mmol) in THF (15 mL) was heated under reflux for 2 h. A solution of benzophenone **12** (0.200 g, 0.469 mmol) and aldehyde **20** (0.112, 0.703 mmol) in THF (10 mL) was then added. The black mixture was heated at reflux for 3 h. It was cooled and 10% aq K_2CO_3 (30 mL) was added, and the mixture stirred overnight at rt. The mixture was filtered through a Celite pad and washed with ethyl acetate (2×20 mL). The layers were separated and the aqueous one was extracted with ethyl acetate (2×15 mL). The combined organic extracts were washed with brine (1×20 mL), dried over $MgSO_4$, filtered, and the solvent removed. Purification by flash chromatography on silica gel (30 g; column: 2×24 cm), eluting with hexanes–ethyl acetate (3:1), afforded **21** (61 mg, 23%) as a thick oil in low yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J=2.1 Hz, 1H), 7.44 (d, J=1.7 Hz, 1H), 7.30 (d, J=2.1 Hz, 1H), 7.29 (d, J=2.5 Hz, 1H), 6.04 (t, J=6.5 Hz, 1H), 4.61 (bs, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.77 (t, J=6.7 Hz, 2H), 1.42 (s, 9H); IR (film) 3386, 2951, 1734, 1715, 1477, 1250, 1167, 998 cm^{-1}; PD-MS m/z 554 (MH^+) and 498 ($MH^+ - C_4H_8$)$^+$. Anal. calcd for $C_{26}H_{29}Cl_2NO_8$: C, 56.33; H, 5.27; N, 2.53. Found: C, 56.49; H, 5.49; N, 2.86.

3′,3″-Dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-3,3-diphenyl-2-propenylamine (22). The crude product from the McMurry coupling obtained as described above was stirred with TFA–CH_2Cl_2 (1:2 mL) at room temperature for 1 h. Solvent and excess TFA were evaporated off. The residue was purified by flash chromatography on silica gel (30 g; column: 2×30 cm), eluting with 100% CH_2Cl_2, and then CH_2Cl_2–MeOH–NH_4OH (200:10:0.1 to 200:20:0.1). Amine **22** (82 mg, 38%) was obtained as an orange thick oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J=2.2 Hz, 1H), 7.44 (d, J=2.2 Hz, 1H), 7.30 (d, J=1.5 Hz, 1H), 7.28 (d, J=2.2 Hz, 1H), 6.13 (t, J=6.9 Hz, 1H), 4.40 (bs, 2H, exchangeable with D_2O), 3.96 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.46 (d, J=6.6 Hz, 2H); IR (film) 2952, 1732, 1477, 1436, 1266, 1204, 997 cm^{-1}.

5α-3β,17β-(Dicarboxymethyl)androstane (23). Ester **10** (0.152 g, 0.376 mmol) was dissolved in THF (10 mL), 8 N NaOH (1.0 mL) was added, and the mixture was heated under reflux for 24 h. The solvent was evaporated and the residue was diluted with water (15 mL), washed with ethyl acetate (2×10 mL), and acidified with concd HCl. The mixture was refrigerated overnight. The precipitated solid was separated by filtration and washed with water. The product was dried under high vacuum to afford **23** (0.122 g, 86.2%) as a white solid. The analytical sample was prepared by recrystallization from methanol: mp 234–237 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 2.32 (dd, J=14.5 and 4.8 Hz, 1H), 2.16 (d, J=6.8 Hz, 2H), 2.07 (dd, J=14.4 and 10.0 Hz, 1H), 2.0–0.83 (m, 24H), 0.78 (s, 3H), 0.60 (s, 3H); IR (film) 3200–2500, 2914, 2846, 1723, 1702, 1409, 1286, 1242, 900 cm^{-1}; PD-MS m/z 376 (MH^+). Anal. calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64. Found: C, 73.06; H, 9.41.

5α-3β,17β-Di[*N*-3′,3′-(3″,3‴-dicarbomethoxy-5″,5‴-dichloro-4″,4‴-dimethoxydiphenyl)-2′-propenyl-acetamido]-androstane (24). A solution of acid **23** (0.061 g, 0.162 mmol) and Et_3N (0.27 mL, 1.944 mmol) in dry DMF (5 mL) was stirred under Ar. HOBt (0.087 g, 0.648 mmol) was added, followed by amine–HCl **22** (0.166 g, 0.341 mmol). EDCI-HCl (0.124 g, 0.648 mmol) was then incorporated. The reaction mixture was stirred at room temperature for 24 h. The mixture was partitioned between water (60 mL) and Et_2O–EtOAc (30:15 mL). The aqueous layer was extracted with a 2:1 mixture of Et_2O–EtOAc (4×30 mL). The combined organic extracts were washed with brine (1×30 mL), dried over $MgSO_4$, filtered, and the solvent removed. Purification by flash chromatography on silica gel (30 g; column: 2×25.5 cm), eluting with ethyl acetate–hexanes (1:1, followed by 2:1) afforded 24 as a yellowish solid in low yield (45 mg, 22%): mp 172–175 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J=2.2 Hz, 2H), 7.45 (d, J=2.1 Hz, 2H), 7.32 (d, J=2.1 Hz, 2H), 7.28 (d, J=2.3 Hz, 2H), 6.02 (t, J=6.8 Hz, 2H), 5.59 (t, J=5.3 Hz, 2H), 3.96 (s, 6H), 3.90 (s, 6H), 3.89 (s, 6H), 3.88 (s, 6H), 2.35–0.80 (m, 24H), 2.24 (dd, J=13.7 and 4.5 Hz, 1H), 2.14 (d, J=2.1 Hz, 2H), 2.03 (dd, J=7 and 2.3 Hz, 4H), 1.89 (dd, J=14.4 and 10.0 Hz, 1H), 0.72 (s, 3H), 0.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 172.4, 165.6, 165.4, 155.5, 155.2, 139.3, 137.4, 134.8, 134.2, 132.6, 130.8, 130.0, 129.6, 128.3, 126.9, 126.7, 62.1, 62.0, 55.3, 54.6, 52.6, 47.4, 46.3, 44.6, 42.2, 38.7, 38.2, 37.5, 37.4, 35.8, 35.7, 35.5, 35.3, 31.9, 28.7, 28.2, 24.5, 20.7, 12.7, 12.3; IR (film) 3287, 2932, 1735, 1643, 1537, 1477, 1435, 1264, 1207, 998, 738 cm^{-1}; PD-MS m/z 1249 (MH^+). Anal. calcd for $C_{65}H_{74}Cl_4N_2O_{14}$: C, 62.50; H, 5.97; N, 2.24. Found: C, 62.42; H, 5.98; N, 2.27.

5α-3β,17β-Di[*N*-3′,3′-(3″,3‴-dicarboxy-5″,5‴-dichloro-4″,4‴-dimethoxydiphenyl)-2′-propenyl-acetamido]androstane (25). Ester **24** (0.073 g, 0.058 mmol) was dissolved in THF (8 mL). A 0.5 N NaOH solution (2 mL) was added. The reaction mixture was stirred at 65 °C overnight. The solvent was removed, the residue diluted with

water (10 mL), and the mixture extracted with EtOAc (1×10 mL). The aqueous phase was acidified with concd HCl and extracted with EtOAc (4×10 mL). The organic extracts were washed with brine (1×10 mL), dried over Na_2SO_4, filtered and the solvent removed. Purification was achieved by flash chromatography on silica gel (30 g; column: 2×25.5 cm). Elution started with $CHCl_3$, followed by $CHCl_3$–MeOH (95:5), and then $CHCl_3$–MeOH–formic acid (95:5:0.3 to 85:15:0.3). This purification afforded pure **25** as a yellowish solid in moderate yield (33 mg, 48%): mp 220–221 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.61 (d, J=1.8 Hz, 4H), 7.58 (d, J=2.1 Hz, 2H), 7.47 (d, J=2.1 Hz, 2H), 6.22 (t, J=6.8 Hz, 2H), 3.95 (s, 6H), 3.90 (s, 6H), 2.22 (dd, J=13.7 and 4.5 Hz, 1H), 1.9–0.80 (m, 27H), 0.74 (s, 3H), 0.57 (s, 3H); IR (film) 3500–2500, 2928, 1713, 1477, 1435, 1248, 998 cm^{-1}. Anal. calcd for $C_{61}H_{66}Cl_4N_2O_{14}\cdot 1H_2O$: C, 60.50; H, 5.66; N, 2.31. Found: C, 60.66; H, 5.78; N, 2.25.

_N_-Fluorenylmethyloxycarbonyl-3-aminopropanal (27). _N_-Fmoc-β-alanine (**26**) (1.5 g, 4.8 mmol) was dissolved in dry THF (10 mL). Oxalyl chloride (0.6 mL, 6.745 mmol) was added, followed by DMF (1 drop). The mixture was stirred for 2 h at rt. The solvent and excess oxalyl chloride were removed. The residue was dissolved in dry THF (10 mL) and $(Ph_3P)_4Pd$ (0.056 g, 0.048 mmol) was added. Tributyltin hydride (1.55 mL, 5.782 mmol) was added dropwise over 5 min. The mixture was stirred at room temperature for 1 h. The solvent was removed and the residue was washed with hexanes. The solids were separated by filtration, dissolved in dichloromethane, adsorbed on silica gel (15 g) and applied to a silica gel column (100 g; column: 4×16 cm). Elution with hexanes–ethyl acetate (1:1) afforded **27** in excellent yield (1.27 g, 89%). The analytical sample was obtained by recrystallization from ethyl acetate–hexanes: mp 110.5–111.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.80 (s, 1H), 7.76 (d, J=7.4 Hz, 2H), 7.57 (d, J=7.3 Hz, 2H), 7.38 (t, J=7.3 Hz, 2H), 7.30 (t, J=7.3 Hz, 2H), 5.15 (s, 1H), 4.38 (d, J=6.7 Hz, 2H), 4.18 (t, J=6.6 Hz, 1H), 3.47 (dd, J=11.1 and 5.4 Hz, 2H), 2.73 (t, J=5.4 Hz, 2H); IR (film) 3324, 2944, 1692, 1537, 1265, 737 cm^{-1}; PD-MS m/z 295 (MH^+). Anal. calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 72.93; H, 5.72; N, 4.67.

4-(_N_-Fluorenylmethyloxycarbonyl)amino-3′,3″-dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-butene (28). A slurry of $TiCl_4$–THF (1:2) complex (0.705 g, 2.112 mmol) and Zn dust (0.28 g, 4.224 mmol) in THF (25 mL) was stirred and heated under reflux for 2 h. A solution of benzophenone **12** (0.300 g, 0.704 mmol) and aldehyde **27** (0.312 g, 1.056 mmol) in THF (15 mL) was added. The black mixture was heated under reflux for 2.5 h. It was then cooled to room temperature and water (30 mL) was added. The mixture was stirred at room temperature overnight. It was filtered through a pad of Celite and washed with EtOAc (3×20 mL). The layers were separated and the aqueous one was extracted with EtOAc (2×20 mL). The combined organic extracts were washed with brine (1×30 mL), dried over $MgSO_4$, filtered, and the solvent removed. The residue was flash chromatographed on silica gel (70 g; column: 3×27 cm) eluting with hexanes–ethyl acetate (2:1) to provide **28** as a white foam in good yield (0.35 g, 72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J=7.5 Hz, 2H), 7.66 (d, J=7.5 Hz, 2H), 7.50 (d, J=1.9 Hz, 3H), 7.45 (d, J=2.0 Hz, 1H), 7.37 (t, J=7.4 Hz, 2H), 7.25 (t, J=7.4 Hz, 2H), 6.63 (t, J=5.6 Hz, 1H), 6.27 (t, J=7.4 Hz, 1H), 4.34 (d, J=7.0 Hz, 2H), 4.19 (t, J=6.9 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.31 (q, J=6.3 Hz, 2H), 2.36 (q, J=6.7 Hz, 2H); IR (film) 3388, 2949, 1731, 1477, 1435, 1252, 1208, 998 cm^{-1}; PD-MS m/z 691 (MH^+). Anal. calcd for $C_{37}H_{33}Cl_2NO_8$: C, 64.35; H, 4.82; N, 2.03. Found: C, 64.22; H, 4.85 ; N, 2.02.

4-Amino-3′,3″-dichloro-4′,4″-dimethoxy-5′,5″-bis(methoxycarbonyl)-1,1-diphenyl-1-butene (29).[26] Fmoc amine **28** (0.142 g, 0.206 mmol) was dissolved in dry THF (10 mL). Piperidine (2 mL) was added and the reaction mixture was stirred at room temperature for 3 h. The solvent and excess piperidine were removed and the residue was flash chromatographed on silica gel (40 g; column: 2×31 cm), eluting with $CHCl_3$ and then $CHCl_3$–MeOH–NH_4OH (200:10:0.2, and then 100:10:0.2 to 100:20:0.2). In this way, compound **29** was obtained as a yellowish oil in excellent yield (87 mg, 91%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (d, J=2.3 Hz, 1H), 7.47 (d, J=2.1 Hz, 1H), 7.33 (d, J=2.1 Hz, 1H), 7.30 (d, J=2.3 Hz, 1H), 6.07 (t, J=7.4 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 2.83 (bs, 2H), 2.26 (q, J=7.0 Hz, 2H), 2.10 (bs, 2H, D_2O exchange).

5α-3β,17β-Di[_N_-4′,4′-(3″,3‴-dicarbomethoxy-5″,5‴-dichloro-4″,4‴-dimethoxydiphenyl)-3′-butenyl-acetamido]-androstane (31). Acid **23** (0.062 g, 0.166 mmol) was dissolved in dry THF. Oxalyl chloride (0.04 mL, 0.415 mmol) was added, followed by DMF (1 drop). The mixture was stirred at room temperature for 5 h. The solvent and excess oxalyl chloride were removed. The residue was dissolved in dichloromethane (2.5 mL) and added dropwise to a solution of amine **29** (0.171 g, 0.366 mmol) and Et_3N (0.06 mL, 0.415 mmol) in dichloromethane (1.5 mL) cooled in an ice bath. After the addition was finished, the bath was removed and the mixture was stirred at room temperature for 48 h. At this time, the mixture was diluted with dichloromethane (40 mL), washed with 1.0 N HCl (1×20 mL) and brine (1×20 mL), dried over $MgSO_4$, filtered and the solvent removed. The residue was purified by flash chromatography on silica gel (35 g; column: 2×25.5 cm), eluting with hexanes–ethyl acetate (1:1), followed by hexanes–ethyl acetate (1:1 containing 5% MeOH). Compound **31** was obtained as a white foam in moderate yield (112 mg, 53%): mp 111–113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.43 (m, 4H), 7.28 (d, J=1.8 Hz, 4H), 6.03 (t, J=7.3 Hz, 1H), 6.01 (t, J=7.1 Hz, 1H), 5.46 (d, J=3.0 Hz, 2H), 3.97 (s, 6H), 3.90 (s, 6H), 3.88 (s, 6H), 3.87 (s, 6H), 3.36–3.32 (m, 4H), 2.28 (q, J=7.0 Hz, 4H), 2.1–0.75 (m, 28H), 0.69 (s, 3H), 0.52 (s, 3H); IR (film) 3291, 2933, 1732, 1644, 1537, 1477, 1435, 1256, 1208, 999 cm^{-1}. Anal. calcd for $C_{67}H_{78}Cl_4N_2O_{14}\cdot 2H_2O$: C, 61.28; H, 6.29; N, 2.13. Found: C, 61.39; H, 5.96; N, 2.24.

5α-3β,17β-Di[*N*-4′,4′-(3″,3‴-dicarboxy-5″,5‴-dichloro-4″,4‴-dimethoxydiphenyl)-3′-butenyl-acetamido]androstane (32). Bisamide **31** (0.070 g, 0.055 mmol) was dissolved in THF (5 mL). A 0.5 N NaOH solution (2 mL) was added. The reaction mixture was stirred at 55–60 °C for 24 h. The solvent was removed, the residue diluted with water (13 mL), and the mixture extracted with EtOAc (1×10 mL). The aqueous phase was acidified with concd HCl and extracted with EtOAc (4×10 mL). The organic extracts were washed with brine (1×10 mL), dried over Na_2SO_4, filtered and the solvent removed. Purification was achieved by flash chromatography on silica gel (35–40 g; column: 2×31 cm). Elution started with $CHCl_3$, followed by $CHCl_3$–MeOH (95:5), and then $CHCl_3$–MeOH–formic acid (98:2:0.3 to 90:10:0.3). In this manner, compound **32** was obtained as an off-white solid in good yield (50 mg, 88%): mp 180–185 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.58–7.47 (m, 8H), 6.28 (d, J=7.3 Hz, 1H), 6.27 (t, J=7.3 Hz, 1H), 3.95 (s, 6H), 3.89 (s, 6H), 3.40–3.28 (m, 4H), 2.34 (q, J=7.0 Hz, 4H), 2.24–0.8 (m, 28H), 0.71 (s, 3H), 0.54 (s, 3H); IR (film) 3500–2500, 3290, 2932, 1713, 1621, 1555, 1477, 1428, 1253, 999 cm^{-1}; PD-MS m/z 1222 (MH^+). Anal. calcd for $C_{63}H_{70}Cl_4N_2O_{14}\cdot 2H_2O$: C, 60.19; H, 5.93; N, 2.23. Found: C, 59.90; H, 5.53; N, 2.20.

***N*-(5α,3β-Cholestanyl)-6,6-(3′,3″-dicarbomethoxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-5-hexenylamine (36).** 3β-Aminocholestane hydrochloride (**34**)[29] (0.093 g, 0.220 mmol), aldehyde **33**[28] (0.075 g, 0.152 mmol) and Et_3N (0.03 mL, 0.220 mmol) were dissolved in 1,2-dichloroethane (3 mL) and put under Ar. Sodium triacetoxyborohydride (0.045 g, 0.213 mmol) was added and the reaction mixture stirred at room temperature for 24 h. A saturated solution of $NaHCO_3$ (15 mL) was added and the mixture stirred for 15 min. EtOAc (15 mL) was added and the phases were separated. The aqueous phase was extracted with EtOAc (4×15 mL). The combined organic extracts were washed with brine (1×25 mL), dried over magnesium sulfate, filtered and the solvent removed. Purification was achieved by flash chromatography on silica gel (32 g; column: 2 26 cm), eluting with ethyl acetate-hexanes (5:1 containing 1% Et_3N). Compound **36** was obtained as a thick oil in good yield (0.117 g, 89.3%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, J=2.3 Hz, 1H), 7.44 (d, J=2.2 Hz, 1H), 7.29 (d, J=2.2 Hz, 1H), 7.26 (d, J=2.3 Hz, 1H), 6.03 (t, J=7.5 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 2.56 (bt, 2H), 2.40 (m, 1H), 2.08 (bq, J=6.9 Hz, 2H), 2.00–1.88 (m, 1H), 1.88–0.90 (m, 34H), 0.87 (d, J=6.6 Hz, 3H), 0.83 (d, J=6.3 Hz, 6H), 0.74 (s, 3H), 0.61 (s, 3H); IR (film) 2928, 2853, 1736, 1475, 1259, 1207, 1001, 738 cm^{-1}; PD-MS m/z 866 (MH^+). Anal. calcd for $C_{51}H_{73}Cl_2NO_6$: C, 70.65; H, 8.49; N, 1.62. Found: C, 70.94; H, 8.43; N, 1.55.

***N*-(5α,3α-Cholestanyl)-6,6-(3′,3″-dicarbomethoxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-5-hexenylamine (37).** 3α-Aminocholestane hydrochloride (**35**)[29] (0.124 g, 0.293 mmol), aldehyde **33**[28] (0.100 g, 0.202 mmol) and Et_3N (0.041 mL, 0.293 mmol) were partially dissolved in 1,2-dichloroethane (4 mL) and the solution put under Ar. Sodium triacetoxyborohydride (0.060 g, 0.283 mmol) was added and the reaction mixture stirred at room temperature for 8 h. A saturated solution of $NaHCO_3$ (15 mL) was added and the mixture stirred for 15 min. EtOAc (15 mL) was added and the phases were separated. The aqueous phase was extracted with EtOAc (4×15 mL). The combined organic extracts were washed with brine (1×25 mL), dried over magnesium sulfate, filtered and the solvent removed. Purification was achieved by flash chromatography on silica gel (30 g; column: 2×24.5 cm), eluting with ethyl acetate–hexanes (2:1 containing 1% Et_3N). Compound **37** was obtained as a thick oil in very good yield (0.16 g, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, J=2.3 Hz, 1H), 7.45 (d, J=2.2 Hz, 1H), 7.30 (d, J=2.2 Hz, 1H), 7.27 (d, J=2.3 Hz, 1H), 6.05 (t, J=7.5 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.89 (s, 3H), 2.75 (bs, 1H), 2.48 (bt, 2H), 2.09 (bq, J=7.2 Hz, 2H), 2.00–1.88 (m, 1H), 1.88–1.68 (m, 1H), 1.68–0.93 (m, 33H), 0.88 (s, 3H), 0.84 (d, J=6.3 Hz, 6H), 0.75 (s, 3H), 0.61 (s, 3H); IR (film) 2930, 1737, 1477, 1263, 1207, 1001 cm^{-1}; PD-MS m/z 866 (MH^+). Anal. calcd for $C_{51}H_{73}Cl_2NO_6$: C, 70.65; H, 8.49; N, 1.62. Found: C, 70.73; H, 8.41; N, 1.59.

***N*-(5α,3β-Cholestanyl)-6,6-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-5-hexenylamine (38).** Amine **36** (0.109 g, 0.126 mmol) was dissolved in a mixture of THF (4 mL), MeOH (3 mL), and water (1.5 mL). K_2CO_3 (0.313 g, 2.268 mmol) was added, followed by KCN (2.0 mg, 0.025 mmol). The mixture was heated at 75 °C for 4 h. The solvents were removed and the residue was diluted with water (10 mL). The aqueous solution was then heated at 75 °C for 1 h. It was allowed to cool and then washed with EtOAc (2×15 mL). Then, it was acidified with concd HCl and extracted with EtOAc (5×15 mL). The combined organic extracts were washed with brine (1×25 mL), dried over Na_2SO_4, filtered and the solvent removed. Compound **38** was obtained as an off-white solid in moderate yield (76 mg, 72%). The analytical sample was obtained by recrystallization from THF–acetonitrile: mp 215–217 °C; ^{1}H NMR (300 MHz, THF-d_8) δ 7.58 (d, J=1.8 Hz, 1H), 7.57 (d, J=1.4 Hz, 1H), 7.46 (d, J=2.2 Hz, 1H), 7.44 (d, J=2.1 Hz, 1H), 6.21 (t, J=7.4 Hz, 1H), 3.95 (s, 3H), 3.88 (s, 3H), 3.07 (m, 1H), 2.96 (m, 2H), 2.18 (q, J=7.2 Hz, 2H), 2.00–0.97 (m, 35H), 0.92 (d, J=6.3 Hz, 3H), 0.86 (d, J=6.6 Hz, 6H), 0.85 (s, 3H), 0.67 (s, 3H); IR (film) 3500–2500, 2934, 2864, 1701, 1475, 1250, 998, 845 cm^{-1}; FAB-MS m/z 838 (MH^+). Anal. calcd for $C_{49}H_{69}Cl_2NO_6\cdot 2.0H_2O$: C, 67.26; H, 8.41; N, 1.60. Found: C, 67.32; H, 8.19; N, 1.58.

***N*-(5α,3α-Cholestanyl)-6,6-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-5-hexenylamine (39).** Amine **37** (0.155 g, 0.179 mmol) was dissolved in a mixture of THF (4 mL), MeOH (3.5 mL), and water (2 mL). K_2CO_3 (0.445, 3.222 mmol) was added, followed by KCN (2.0 mg, 0.036 mmol). The mixture was heated at 75 °C for 4 h. The solvents were removed and the residue was diluted with water (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 concd HCl and extracted with EtOAc (5×15 mL). The combined organic extracts were washed with brine (1×25 mL), dried over Na_2SO_4, filtered and the solvent removed. Compound **39** was obtained as an off-white solid in excellent yield (147 mg, 98%): mp 210–215 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J=2.2 Hz, 1H), 7.69 (d, J=2.0 Hz, 1H), 7.27 (d, J=1.9 Hz, 1H), 7.26 (d, J=1.7 Hz, 1H), 6.04 (t, J=7.4 Hz, 1H), 4.03 (s, 3H), 3.97 (s, 3H), 3.30 (bs, 1H), 2.91 (m, 2H), 2.2–1.0 (m, 35H), 2.10 (q, J=7.3 Hz, 2H), 0.95 (s, 3H), 0.83 (d, J=6.6 Hz, 6H), 0.74 (s, 3H), 0.58 (s, 3H); IR (film) 3500–2500, 2934, 2864, 1703, 1475, 1250, 998 cm^{-1}; PD-MS m/z 840 (MH^+). Anal. calcd $C_{49}H_{69}Cl_2NO_6{\cdot}2H_2O$: C, 67.26; H, 8.41; N, 1.60. Found: C, 67.35; H, 8.41; N, 1.49.

***N*-(5α,3β-Cholestanyl)-*N*-[6,6-(3′,3″-dicarbomethoxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-5-hexenyl]-3,3-(3′,3″-dicarbo-methoxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-2-propenamide (41).** A solution of acid **40**[26] (0.027 g, 0.058 mmol) and Et_3N (0.05 mL, 0.348 mmol) in dry DMF (4 mL) was stirred under Ar. HOBt (0.016 g, 0.116 mmol) was added, followed by amine 36 (0.050 g, 0.058 mmol). A solution of EDCI–HCl (0.022 g, 0.116 mmol) in dry DMF (1 mL) was then added. The reaction mixture was stirred at room temperature for 48 h. The mixture was diluted with Et_2O (25 mL) and water (50 mL). The phases were separated and the aqueous one was extracted with Et_2O (4×20 mL). The combined organic extracts were washed with brine (1×25 mL), dried over $MgSO_4$, filtered and the solvent removed. Purification was accomplished by flash chromatography on silica gel (30 g; column: 2×20 cm), eluting with hexanes–ethyl acetate (2:1). Compound **41** was obtained as an off-white foam in good yield (64 mg, 84%): mp 97–100 °C; ^{1}H NMR (300 MHz, $CDCl_3$) major rotamer δ 7.55 (d, J=2.3 Hz, 1H), 7.48 (d, J=2.3 Hz, 1H), 7.45 (m, 2H), 7.42 (d, J=2.3 Hz, 1H), 7.39 (d, J=2.3 Hz, 1H), 7.31 (d, J=2.2 Hz, 1H), 7.28 (d, J=2.3 Hz, 1H), 6.31 (s, 1H), 6.02 (t, J=7.2 Hz, 1H), 3.98 (s, 3H), 3.95 (s, 3H), 3.90 (s, 12H), 3.88 (s, 3H), 3.81 (s, 3H), 3.61–3.53 (m, 1H), 3.06–3.00 (m, 2H), 2.07 (q, J=7.0 Hz, 2H), 2.0–1.88 (m, 1H), 1.88–0.93 (m, 30H), 0.87 (s, 3H), 0.83 (dd, J=6.6 and 1.2 Hz, 6H), 0.70 (s, 3H), 0.61 (s, 3H); IR (film) 2935, 2866, 1736, 1626, 1476, 1435, 1261, 1207, 998 cm^{-1}; PD-MS m/z 1320 (MH^+). Anal. calcd for $C_{72}H_{89}Cl_4NO_{13}$: C, 65.60; H, 6.80; N, 1.06. Found: C, 65.45; H, 6.86; N, 1.06.

***N*-(5α,3β-Cholestanyl)-*N*-[6,6-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-5-hexenyl]-3,3-(3′,3″-dicarboxy-5′,5″-dichloro-4′,4″-dimethoxydiphenyl)-2-propenamide (42).** A solution of amide **41** (0.125 g, 0.095 mmol) in THF (10 mL) was stirred at room temperature and 0.5 N NaOH (3 mL) was added. The mixture was heated at 65 °C overnight. The mixture was cooled and the solvent removed. The residue was diluted with water (10 mL) and washed with EtOAc (2×15 mL). The aqueous solution was then acidified with concd HCl and extracted with EtOAc–THF (3:1) (3×20 mL). The combined organic extracts were washed with brine (1×25 mL), dried over Na_2SO_4, filtered and the solvent removed. Further purification was accomplished by flash chromatography on silica gel (30 g; column: 2×24 cm). Elution started with 100% CH_2Cl_2, followed by CH_2Cl_2–MeOH (95:5), and then CH_2Cl_2–MeOH–formic acid (95:5:0.3 to 85:15:0.3). In this way, compound **42** was obtained as a pale yellow solid in good yield (0.106 g, 88.6%): mp 165–170 °C; ^{1}H NMR (300 MHz, $CDCl_3$) major rotamer δ 7.81 (d, J=1.8 Hz, 1H), 7.76 (d, J=2.3 Hz, 1H), 7.69 (d, J=1.8 Hz, 1H), 7.67 (d, J=1.8 Hz, 1H), 7.38 (m, 2H), 7.31 (d, J=1.8 Hz, 1H), 7.29 (d, J=1.8 Hz, 1H), 6.45 (s, 1H), 6.06 (t, J=7.0 Hz, 1H), 4.04 (s, 3H), 3.99 (s, 6H), 3.95 (s, 3H), 3.77–3.60 (m, 1H), 3.30–3.00 (m, 2H), 2.09 (m, 2H), 2.00–0.9 (m, 31H), 0.90 (s, 3H), 0.82 (d, J=6 Hz, 6H), 0.72 (s, 3H), 0.59 (s, 3H); IR (film) 3500–2500, 2934, 1702, 1593, 1476, 1429, 1261, 998 cm^{-1}; PD-MS m/z 1261 (MH^+). Anal. calcd for $C_{68}H_{83}Cl_4NO_{14}{\cdot}1H_2O$: C, 63.80; H, 6.53; N, 1.09. Found: C, 63.80; H, 6.46; N, 1.09.

Anti-HIV assays

The HIV-inhibitory activity of compounds was evaluated as previously described for HIV-1_{Rf} in CEM-SS[36] cells and HIV-1_{IIIB} and HIV-2_{Rod} in MT-4 cells.[37] These are microtiter assays which quantitate the ability of a compound to inhibit HIV-1 or HIV-2-induced cell killing via syncytium formation. Antiviral and toxicity data are reported as the concentration of compound required to inhibit 50% virus-induced cell killing [50% effective concentration (EC_{50})] and the concentration of compound required to reduce cell viability by 50% (cytotoxicity). All data are derived from three tests.

For the assays involving HIV-1_{IIIB} and HIV-2_{ROD}, stock solutions (10×final concentration) of test compounds were added to 25 μL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat bottom 96-well plastic microtiter trays using a Biomek 2000 robot (Beckman Instruments, Fullerton, CA, USA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1_{IIIB}[38] or HIV-2_{ROD}[39] stock (50 μL) at 100–300 $CCID_{50}$ (cell culture infectious dose) or culture medium was added to either the infected or mock-infected well of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compounds on uninfected cells and to determine the concentration at which the test compounds were cytotoxic. Exponentially growing MT-4 cells[40] were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, using slight magnetic stirring, and 50 μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells were examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2.5diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically acive

cells to a blue formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiskan Ascent Reader, Labsystems, Helsinki, Finland), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of extract that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

X-ray crystallography of compound 10

Data collection. A colorless plate of compound **10** having approximate dimensions of $0.25\times0.22\times0.08$ mm was mounted on a glass fiber in a random orientation. Preliminary examination and data collection were performed with Mo K_α radiation ($\lambda = 0.71073$ Å) on a Nonius KappaCCD. Cell constants and an orientation matrix for data collection were obtained from least-squares refinement, using the setting angles of 5391 reflections in the range $4 < \theta < 20°$. The orthorhombic cell parameters and calculated volume are: a = 6.916 (0), b = 11.881 (1), c = 27.919 (2) Å, V = 2294.0 Å^3. For Z = 4 and F.W. = 404.60, the calculated density is 1.30 g/cm^3. The space group was determined by the program ABSEN.[41] From the systematic presences of h00 h = 2n, 0k0 k = 2n, 001 1 = 2n, and from least-squares refinement, the space group was determined to be $P2_12_12_1$ (# 19). The data were collected at a temperature of 296 ± 1 K. Data were collected to a maximum 2θ of 41.8°.

Data redection. A total of 5391 reflections were collected, of which 2348 were unique. Lorenz and polarization corrections were applied to the data. The linear absorption coefficient is 0.7/cm for Mo *K* radiation. No absorption correction was made. Intensities of equivalent reflections were averaged. The agreement factor for the averaging was 5.0% based on intensity.

Structure solution and refinement. The structure was solved by direct methods using SIR97.[42] The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were included in the refinement but restrained to ride on the atom to which they are bonded. The structure was refined in full-matrix least-squares where the function minimized was $\Sigma w(|Fo|^2-|Fc|^2)^2$ and the weight w is defined as $w = 1/[\sigma^2(Fo^2) + (0.0649P)^2 + 0.0266P]$ where $P = (Fo^2 + 2Fc^2)/3$. Scattering factors were taken from the 'International Tables for Crystallography'.[43] 2348 Reflections were used in the refinements. However, only reflections with $Fo^2 > 2\sigma(Fo^2)$ were used in calculating R. The final cycle of refinement included 265 variable parameters and converged (largest parameter shift was 0.02 times its esd) with unweighted and weighted agreement factors of: $R1 = \Sigma|Fo - Fc|/Fo = 0.060$, $R2 = SQRT(\Sigma w(Fo^2 - Fc^2)^2/\Sigma w(Fo^2)^2) = 0.130$. The standard deviation of an observation of unit weight was 1.06. The highest peak in the final difference Fourier had a height of 0.26 e/A^3. The minimum negative peak had a height of −0.13 e/A^3. The absolute structure was determined from a known fragment within the molecule. Refinement was performed on a Alpha Server 2100 using SHELX-97.[44] Crystallographic drawings were done using programs ORTEP[45] and PLUTON[46] and/or Xtal_GX.[47]

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