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DESIGN AND SYNTHESIS OF COSALANE, A NOVEL ANTI-HIV AGENT

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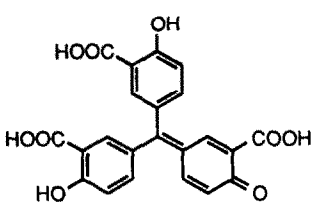
Abstract: Cosalane, a novel anti-HIV agent having a dichlorodisalicylmethane unit linked to C-3 of cholestane by a three-carbon linker, was synthesized by a convergent route.

The clinical efficacy of the drugs presently available for the treatment of AIDS, including AZT, ddC, and ddI, is limited by their toxicity as well as by the emergence of resistant viral strains.[1-9] The design and synthesis of new anti-HIV agents having novel mechanisms of action is therefore an important objective of current interest. Aurintricarboxylic acid (ATA), a heterogeneous mixture of polymers that forms when salicylic acid is treated with formaldehyde, sulfuric acid, and sodium nitrite, inhibits the cytopathic effect of HIV-1 and HIV-2 in a variety of lymphocyte cell cultures by blocking the attachment of the viral envelope to the cell membrane.[10-13] However, interest in the development of ATA as an anti-AIDS agent has been compromised significantly by the fact that it is a complex mixture which is difficult to characterize, and also by the fact that the polymerization process yielding ATA is very sensitive to reaction conditions, making it difficult to control the composition of the product. Low molecular weight ATA oligomers were therefore obtained by fractionation and synthesis in an attempt to obtain structurally defined materials that would retain anti-HIV activity.[12,14] These substances, including the ATA monomer **1**, had very low antiviral activity.[12,14] The design and synthesis of ATA monomer analogs was therefore undertaken in an attempt to obtain compounds with increased anti-HIV potencies.

The present communication details the synthesis of cosalane (**2**), which was obtained conceptually by replacing the quinone methide unit of the ATA monomer (**1**) with an alkene chain linked to C-3 of cholestane, and adding a chlorine atom to each of the two salicylic acid rings. The strategy was that the steroid might target the compound to the viral envelope and the cell membrane, where ATA polymers act, thus enhancing the ability of this monomer analog to prevent the initial binding of the virion to the cell membrane. As detailed below, cosalane (**2**) proved to be about as potent as the most active ATA fractions in inhibiting the cytopathic effects of HIV-1 and HIV-2 in lymphocyte cell cultures.



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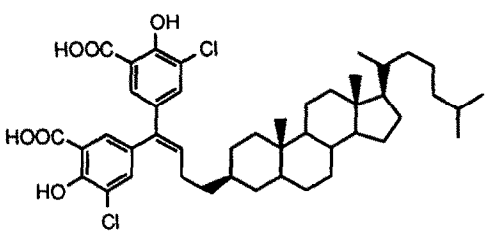

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Cosalane was synthesized by a convergent route as detailed in Schemes I and II. 3-Chlorosalicylic acid (**3**)[15] was treated with formaldehyde to afford the dichlorodisalicylmethane intermediate **4**.[14] Treatment of **4** with dimethylsulfate using potassium carbonate as the base gave the methylated compound **5**. The methylene group of **5** was oxidized with chromic anhydride, yielding the substituted benzophenone **6**. Deprotonation of intermediate **7** with *n*-butyllithium in 1,2-dimethoxyethane gave the corresponding ylide, which reacted with cholestanone (**8**) to afford the alkene **9** as a 1:1 mixture of (E)- and (Z)-isomers. Catalytic hydrogenation of the mixture **9** using platinum oxide as the catalyst in ethyl acetate at 50 °C provided intermediate **10** as one major isomer which crystallized from the crude hydrogenation product without contamination from the diastereomer having the opposite configuration at C-3. The *tert*-butyldimethylsilyl protecting group was removed from **10** with fluoride anion to afford the corresponding alcohol **11**[16], which was transformed into the corresponding bromide **12** with carbon tetrabromide and triphenylphosphine in either acetonitrile or methylene chloride. Reaction of the bromide **12** with triphenylphosphine in chlorobenzene at reflux gave the triphenylphosphonium bromide salt **13**, which on deprotonation with sodium bis(trimethylsilyl)amide in THF and Wittig reaction of the resulting ylide with the substituted benzophenone **6** afforded compound **14**. Final removal of the two ester and two ether methyl groups from **14** with boron tribromide-dimethylsulfide complex in methylene chloride yielded cosalane (**2**).[17]

The range of antiviral activity of cosalane was tested against a number of HIV-1 strains in CEM-SS, MT-2, and MT-4 cells, as well as against HIV-2 in CEM-SS cells and the Rauscher murine leukemia virus (R-MuLV) in SC-1 cells (Table I).[18] The ability of cosalane (**2**) to prevent cytopathicity of all of the HIV strains was monitored, and the effect of cosalane (**2**) against R-MuLV was determined by measuring the inhibition of production of viral antigen (p30). Cosalane was active against all strains of HIV-1 tested, including varieties resistant to AZT (A012) and non-nucleoside reverse transcriptase inhibitors (A17). HIV-2 and the Rauscher murine leukemia virus were also inhibited by cosalane (**2**). The decrease in HIV-1 virus induced cytopathicity resulting from cosalane treatment was reflected by a coincident decrease in HIV-1 virus replication as monitored by decreased production of p24 antigen and virion-associated reverse transcriptase. With regard to the mechanism of action of cosalane, it may be pointed out that our previous studies on ATA demonstrated quite clearly that it inhibits syncytium formation between HIV-1 and HIV-2 infected HUT-78 cells and uninfected MOLT-4 cells, and prevents the binding of HIV-1 virions to MT-4 cells by binding to both gp120 and CD4.[11] Studies are presently being conducted to determine whether or not cosalane has a similar mechanism of action.

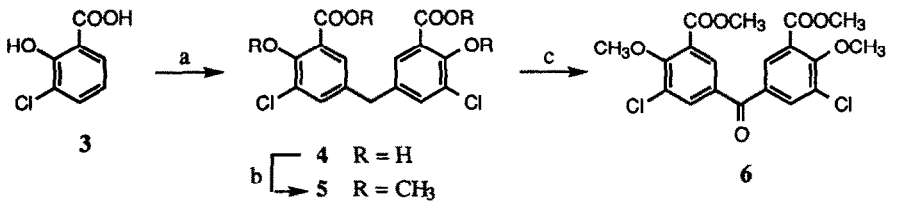
Table I. Range of Antiviral Activity of Cosalane (**3**)

Virus	Cells	EC_{50} (μM)	IC_{50} (μM)	TI_{50}
HIV-1 (RF)	CEM-SS	2-15	>300	>20
(IIIb)	CEM-SS	3	>300	>100
HIV-1 (A012)				
AZT-sens[a]	MT-2	8	200	25
AZT-res[b]	MT-2	10	200	20
HIV-1 (IIIb)	MT-4	20	>300	>15
(A17, NNRTIR)[c]	MT-4	20	>300	>15
HIV-2 (NIH-D2)	CEM-SS	5	>300	>60
R-MuLV[d]	SC-1 (mouse)[e]	4	>32	>8
	SC-1[f]	7	>32	>4

[a]An AZT resistant strain of HIV-1 (A012).[19] [b]An AZT sensitive strain of HIV-1 (A012).[19] [c]An HIV (IIIb) strain resistant to non-nucleoside reverse transcriptase inhibitors.[20] [d]Rauscher murine leukemia virus (R-MuLV). [e]Plaque assay. [f]p30 Production.

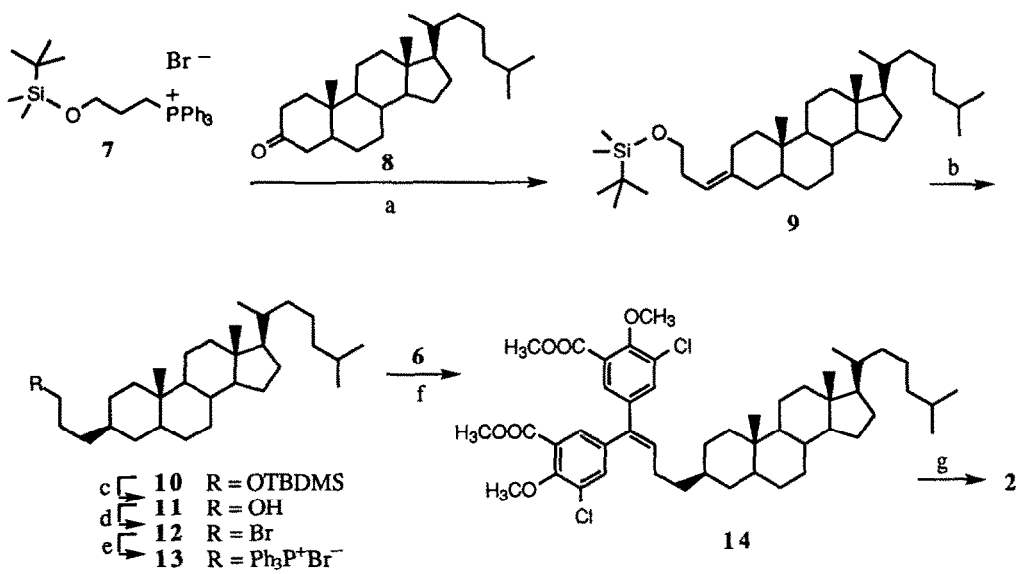
In summary, cosalane (**2**) is a unique anti-HIV agent. It was one of the most potent of the more than 70 ATA monomer analogs that have been prepared to date. Detailed bioavailability and toxicology studies in animal models are presently being carried out.

Scheme I



[a]H_2CO, CH_3OH, H_2SO_4, -78 to 23 °C (16 h). [b]K_2CO_3, $(CH_3O)_2CO$, reflux (20 h). [c]CrO_3, $(CH_3CO)_2O$, 23 °C (20 h).

Scheme II



[a](1)*n*-BuLi, DME. (2) Compound **8**. [b]H_2, PtO_2, EtOAc, 50 °C (4 h). [c](*n*-Bu)$_4N^+F^-$, THF 23 °C. [d]CBr_4, Ph_3P, CH_2Cl_2, 0 °C (10 min). [e]Ph_3P, C_6H_5Cl, reflux (72 h). [f](1) NaN[Si$(CH_3)_3$], THF. (2) Compound **6**. [g]BBr_3·S$(CH_3)_2$, $ClCH_2CH_2Cl$, 90 °C (8 h).

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(16) mp 130 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.60 (t, J = 6.6 Hz, 2 H), 1.93 (dt, J = 12;3.3 Hz, 2 H), 0.87 (d, J = 6.9 Hz, 3 H), 0.835 (dd, J = 6.7;2.5 Hz, 6 H), 0.716 (s, 3 H), 0.617 (s, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 63.46, 56.61, 56.30, 54.68, 46.64, 42.62, 40.14, 39.54, 38.64, 37.89, 36.21, 36.13, 35.84, 35.75, 35.58, 33.37, 32.21, 30.25, 29.10, 28.97, 28.30, 28.05, 24.25, 23.89, 22.87, 22.61, 21.07, 18.72, 12.38, 12.13.

(17) mp 265-267 °C ; ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.70 (d, J = 2.5 Hz, 1 H), 7.68 (d, J = 2.5 Hz, 1 H), 7.58 (d, J = 2.5 Hz, 1 H), 7.50 (d, J = 2.5 Hz, 1 H), 6.18 (t, J = 7.5 Hz, 1 H), 2.17 (m, 2 H), 0.92 (d, J = 6.5 Hz, 3 H), 0.86 (dd, J = 6.5; 2.0 Hz, 6 H), 0.74 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (acetone-d6, 126 MHz) δ 172.18, 157.78, 157.61, 137.93, 137.70, 134.90, 134.82, 132.68, 131.47, 130.99, 128.13, 122.47, 122.39, 114.62, 114.29, 57.43, 57.15, 55.55, 47.35, 43.35, 40.97, 40.24, 39.39, 38.12, 37.91, 36.95, 36.81, 36.62, 36.39, 36.29, 32.89, 30.10, 29.75, 28.95, 28.70, 27.81, 24.87, 24.54, 23.10, 22.85, 21.75, 19.12, 12.70, 12.47.

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