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Anti-HIV Activity of a Series of Cosalane Amino Acid Conjugates

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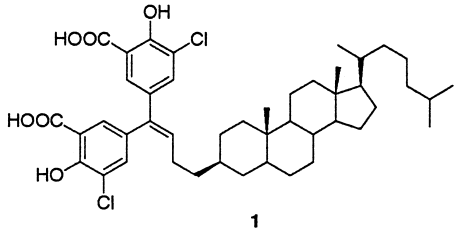
Abstract—The binding of the anti-HIV agent cosalane to CD4 is thought to involve ionic interactions of negatively charged carboxylates of the ligand with positively charged residues on the surface of the protein. The purpose of the present study was to examine the hypothesis that the two carboxyl groups of cosalane could be sacrificed through conjugation to amino acids, and the anti-HIV activity still be retained, provided that at least two new carboxyl groups are contributed by the amino acid residues.

The rate of disease progression in AIDS has been minimized through early and aggressive intervention with combination chemotherapy, but resistance and toxicity still remain as major problems during treatment with anti-HIV drugs.[1] New anti-AIDS agents are therefore needed that could possibly interact with viral proteins that are not targeted by the currently available therapies.

The anti-HIV agent cosalane (**1**) was designed by attaching a dichlorinated disalicylmethane fragment of aurintricarboxylic acid to C-3 of cholestane through an alkenyl linker chain.[2,3] The anti-HIV activity of cosalane (**1**) results from inhibition of the binding of gp120 to CD4, as well as from the inhibition of an unidentified post-attachment event prior to reverse transcription.[4] A hypothetical model (Fig. 1) has been proposed for the binding of the cosalane disalicylmethane 'pharmacophore' to CD4.[5] The model involves the binding of the two cosalane carboxylates to the positively charged Arg58 and Arg59 residues. Inspection of the surrounding region of the protein reveals several other positively charged residues that could be targeted by additional carboxylates of appropriately designed cosalane analogues, provided they are strategically placed.[5,6] The purpose of the present investigation was to examine the hypothesis that the two carboxyl groups of cosalane could be sacrificed through conjugation to amino acids, and the antiviral activity still retained, provided new carboxyl groups were contributed by the amino acid residues. If successful, this could lead to a strategy for modulating the spatial arrangement of carboxyl groups in cosalane analogues, since the structural diversity of currently available amino acids could be exploited. In addition, the number of carboxyl groups could be easily manipulated through incorporation of multiple amino acid residues in peptides containing acidic side chains.



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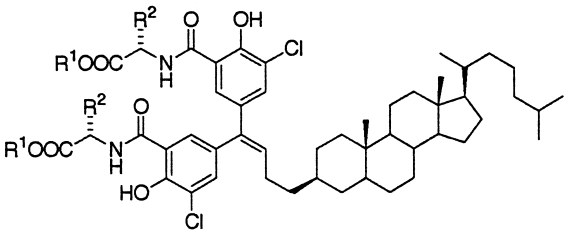
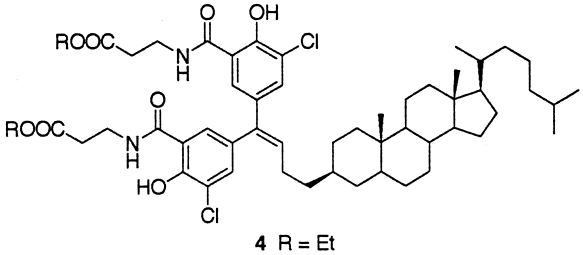
Attempts were therefore initiated to link several common amino acids to the two carboxyl groups of cosalane through amide bond formation. This strategy resulted in a novel series of amino acid conjugates of cosalane, including compounds **3** (derived from glycine), **5** (derived from β-alanine), **7** (derived from leucine), **9** (derived from phenylalanine), **11** (derived from aspartic acid), and **13** (derived from glutamic acid). A further variation in the structure of the glycine derivative **3** and the glutamic acid derivative **13** involved the corresponding dihydro compounds **15** and **17**, in which the alkene in the linker chain connecting the 'pharmacophore' to the steroid nucleus of **3** and **13** had been hydrogenated.

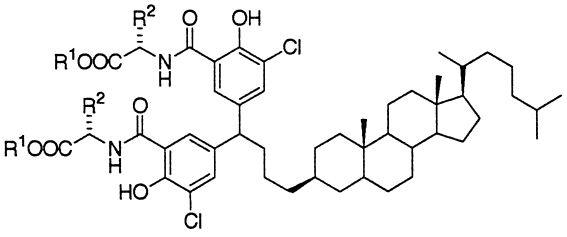
In general, amide bond formation during the coupling of cosalane to the amino acid derivatives was performed by reacting the hydrochloride salts of the amino acid esters with cosalane or dihydrocosalane in the presence of BOP and triethylamine in THF. The dicarboxylic acids **7** and **9** were synthesized by coupling cosalane with the methyl esters of leucine and phenylalanine to afford the protected intermediates **6** and **8**, respectively. Hydrolysis of the methyl esters of **6** and **8** yielded the desired analogues **7** and **9**. The aspartic acid derivative **11** and the glutamic acid derivative **13** were synthesized by reaction of cosalane with the corresponding di-*tert*-butyl esters of the amino acids, followed by saponification of the four *tert*-butyl ester groups. The dicarboxylic acid **3**, having two glycine residues, was prepared similarly by coupling cosalane to the *tert*-butyl ester of glycine to afford **2**, followed by hydrolysis. The β-alanine derivative **5** was prepared by coupling cosalane to β-alanine ethyl ester, resulting in intermediate **4**, followed by hydrolysis of the ester groups. The dihydro analogues **15** and **17** were prepared by coupling dihydrocosalane[7]



2 R^1 = *t*-Bu, R^2 = H
3 R^1 = H, R^2 = H
6 R^1 = CH_3, R^2 = $CH_2CH(CH_3)_2$
7 R^1 = H, R^2 = $CH_2CH(CH_3)_2$
8 R^1 = CH_3, R^2 = CH_2Ph
9 R^1 = H, R^2 = CH_2Ph
10 R^1 = *t*-Bu, R^2 = CH_2COOt-Bu
11 R^1 = H, R^2 = CH_2COOH
12 R^1 = *t*-Bu, R^2 = CH_2CH_2COOt-Bu
13 R^1 = H, R^2 = CH_2CH_2COOH



4 R = Et
5 R = H



14 R^1 = *t*-Bu, R^2 = H
15 R^1 = H, R^2 = H
16 R^1 = *t*-Bu, R^2 = CH_2CH_2COOt-Bu
17 R^1 = H, R^2 = CH_2CH_2COOH

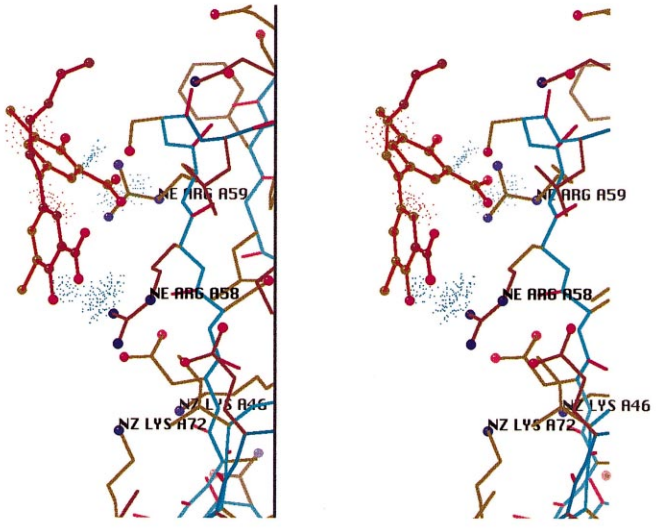


Figure 1. Hypothetical model of the binding of the cosalane 'pharmacore' to CD4 (programmed for walleyed viewing). Blue dots: attraction; red dots: repulsion.

with the corresponding di-*tert*-butyl esters of glycine and glutamic acid to afford **14** and **16**, followed by hydrolysis.

The new cosalane and dihydrocosalane amino acid conjugates were examined for antiviral activity against two strains of HIV-1 (IIIB and RF) and one strain of HIV-2 (ROD). Specifically, they were tested for inhibition of the cytopathic effect of HIV-1_{RF} in CEM-SS cells, HIV-1_{IIIB} in MT-4 cells, and HIV-2_{ROD} in MT-4 cells. The cytotoxicities of the compounds in uninfected CEM-SS cells and in MT-4 cells were also determined. The results of the antiviral and cytotoxicity testing are displayed in Table 1.

The first hypothesis to be tested was that the two carboxyl groups of cosalane could be sacrificed through

amide bond formation, and anti-HIV activity still maintained, provided two new carboxyl groups were added with the amino acid residues. This hypothesis was first examined through the synthesis of the glycine derivative **3**, which proved to be more active than cosalane itself when examined versus HIV-1_{RF} in CEM-SS cells (EC_{50}: 5.1 μM for **1** versus 3.4 μM for **3**). However, the glycine derivative **3** was significantly less active than **1** when tested versus HIV-1_{IIIB} in MT-4 cells (EC_{50} values of 3.0 μM for **1** versus 50.8 μM for **3**). Evidently, the qualitative terms, antiviral activity can be maintained through amino acid conjugation, but potency is effected differently, depending on the strain of virus tested and the cell line used. The requirement for two free carboxylic acid groups for anti-HIV activity is apparent from the inactivity displayed by the di-*tert*-butyl ester **2** when evaluated versus HIV-1_{IIIB} in MT-4 cells, and it is also in agreement with results seen with other cosalane diesters. The inactivity seen with the diester **2** is also consistent with the importance of the proposed ionic bonding of cosalane derivatives to positively charged residues of CD4 (Fig. 1). Like many of the other cosalane amino acid derivatives in the present series, the glycine conjugate **3** was inactive when tested versus HIV-2_{ROD} in MT-4 cells.

The β-alanine derivative **5** was synthesized in order to determine the effect of extending the distance between the amide nitrogens of **2** and the carboxyl groups. As seen from the results in Table 1, this structural change resulted in a decrease in activity versus both HIV-1 strains. However, in contrast to **3**, the β-alanine derivative **5** did display low activity versus HIV-2_{ROD}.

The biological effect of attaching a variety of amino acid side chains to the basic structure of the glycine derivative **3** was examined next. The leucine derivative **7** was less active against HIV-1_{RF} (EC_{50}: 40.0 μM) than the glycine analogue **3** (EC_{50}: 3.4 μM), and **7** was also less active versus HIV-1_{IIIB} (EC_{50}: 63.0 μM) than **3** (EC_{50} 50.8 μM). Like the glycine conjugate **3**, the leucine derivative **7** was inactive versus HIV-2_{ROD}.

The phenylalanine derivative **9** was inactive against both HIV-1 strains tested, as well as HIV-2. The inactivity of **9** may indicate a limit to the size of the side chain that can be tolerated for antiviral activity.

The aspartic acid analogue **11** was more active than either the phenylalanine derivative **9** or the leucine derivative **7**. Compound **11** proved to be less active than the glycine conjugate **3** when tested against HIV-1_{RF} (EC_{50}: 10.7 μM for **11** versus 3.4 μM for **3**), but it was more active than **3** versus HIV-1_{IIIB} (EC_{50}: 16.6 μM for **11** versus 50.8 μM for **3**). Also, the aspartic acid analogue **11** had low activity against HIV-2_{ROD}, in contrast to the inactive glycine conjugate **3**.

The glutamic acid congener **13** proved to be the most active new compound in the series. It was more active than the glycine derivative **3** in all three systems tested (EC_{50}: 1.1 μM versus 3.4 μM against HIV-1_{RF}, 9.2 versus 50.8 μM against HIV-1_{IIIB}, and 71.6 versus >125 μM against HIV-2_{ROD}). This indicates that the addition of two carboxyl groups to those already present in the glycine derivative **3** results in a general increase in antiviral potency, provided the spacing between the carboxyl groups is correct. In summary, the rank order of the di(amino acid) derivatives versus HIV-1_{RF} in CEM-SS cells was glutamic acid (EC_{50}: 1.13 μM), glycine (EC_{50}: 3.44 μM), aspartic acid (EC_{50}: 10.7 μM), β-alanine (EC_{50}: 34.7 μM), leucine (EC_{50}: 40.0 μM), and phenylalanine (EC_{50}: >200 μM). The rank order of these derivatives versus HIV-1_{IIIB} in MT-4 cells was basically the same, except that the aspartic acid derivative (EC_{50}: 16.6 μM) was more potent than the glycine derivative (EC_{50}: 50.8 μM), and the leucine derivative (EC_{50}: 63.0 μM) was slightly more potent than the β-alanine derivative (EC_{50}: 67.2 μM).

The effect of increasing the conformational flexibility of the linker chain connecting the steroid to the two aromatic rings was examined in two cases. The glycine derivative **3** and the glutamic acid derivative **13** were compared with their corresponding dihydro derivatives **15** and **17**. Except in the case of **3** versus **15** in HIV-1_{RF}, there was a general decrease in antiviral activity accompanying the reduction of the double bond, which is consistent with the previously reported activity of cosalane (**1**) versus HIV-1_{RF} in CEM-SS cells (EC_{50}: 5.1 μM) in comparison with the activity of dihydrocosalane (**21**) (EC_{50}: 20 μM) in the same system.[7] When considered as a whole, the data show that the conformational restriction provided by the double bond does contribute to the antiviral activity.

The increase in antiviral activity seen with the tetracarboxylate **13** versus the dicarboxylate **3** is in agreement with some of the general observations previously made with cosalane derivatives. For example, the tetracarboxylate **18** has been found to be an order of magnitude more potent than cosalane (**1**) itself when tested versus HIV-1_{RF}, and a modest increase in potency was also observed with the tetracarboxylate **19** versus cosalane.[6,8]

Table 1. Anti-HIV and cytotoxic activities of cosalane analogues[a]

Compound	EC_{50} (μM)			CC_{50} (μM)	
	HIV-1_{RF}	HIV-1_{IIIB}	HIV-2_{ROD}	CEM-SS	MT-4
1	5.1	3.0	4.0	>200	>200
2	NT[b]	>125	>125	>125	>125
3	3.44±0.4	50.8±9.4	>125	>200	>125
5	34.7±0.3	75.3±2.8	120±5	>200	>125
7	40.0±2.0	63.0±13	>125	>200	>125
9	>200	>62	>62	>200	62±10
11	10.7±1.1	16.6±7.2	122	90±13	>125
13	1.1±0.1	9.2±3.0	71.6±33	82±11	>125
15	4.5±1.0	34.8±30	>125	>200	>125
17	11.6±1.0	10.6±4.0	75.2±26	34±4	>125

[a]The antiviral EC_{50} values are the concentrations required to reduce the cytopathic effect of the virus by 50%, while the cytotoxicity CC_{50} values are the concentrations required for 50% reduction in cellular viability in uninfected cells. Antiviral testing versus HIV-1_{RF} was performed in CEM-SS cells, while antiviral testing versus HIV-1_{IIIB} and HIV-2_{ROD} was carried out in MT-4 cells. The values are the averages of at least two determinations.
[b]Not tested.

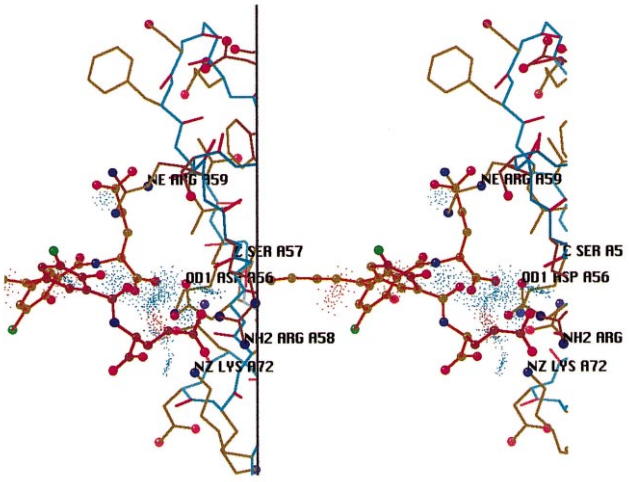
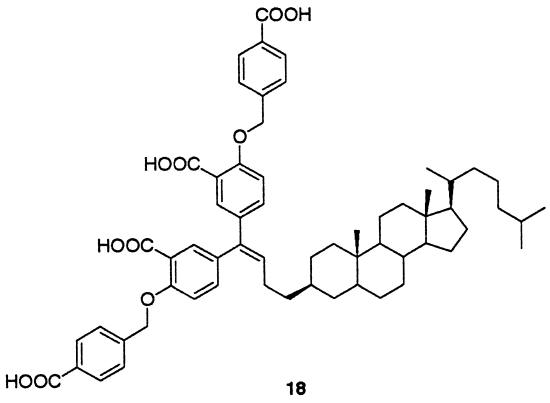


Figure 2. Hypothetical model of the binding of the 'pharmacophore' of the glutamic acid conjugate **13** to CD4 (programmed for walleyed viewing). The ligand is displayed in orange. Blue dots: attraction; red dots, repulsion.



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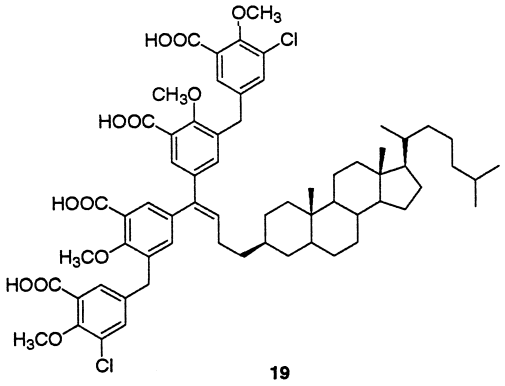

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In order to look for possible interactions between the glutamic acid conjugate **13** and CD4, the 'pharmacophore' of the conjugate was docked on the surface of the protein in the site previously proposed for cosalane.[6] The structure of the protein was 'frozen' and the energy of the complex minimized while the ligand was allowed to move, using Sculpt® software. As shown in the hypothetical model displayed in Figure 2, there are a number of attractive interactions possible between the ligand and the protein. In the model structure of the complex, one of the distal (primary) carboxylic acid groups of **13** binds electrostatically to the positively charged Arg59 residue, while the other distal (primary) carboxyl group binds to the backbone carbonyl of Ser57 and the side chain carboxyl of Asp56. Both of the proximal (secondary) carboxyl groups can form hydrogen bonds to the Asp56 residue, while one of them is also involved in bonding to Lys72. This model supports the idea that all four of the carboxylic acid groups of **13** could participate readily in binding to the protein.

Acknowledgements

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