Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2149–2152

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Identification of Optimal Anion Spacing for Anti-HIV Activity in a Series of Cosalane Tetracarboxylates

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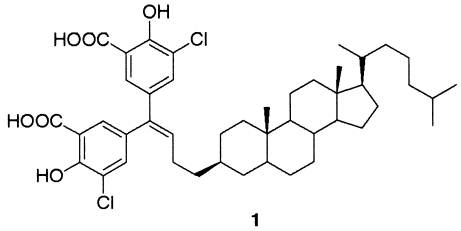
Received 5 June 2000; accepted 21 July 2000

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. An investigation of the optimal anion distances for anti-HIV activity in a series of cosalane tetracarboxylate analogues has been completed, and maximal activity results when the two proximal and the two distal carboxylates are separated by eight atoms.

Although early and aggressive intervention with combination chemotherapy has significantly slowed the rate of disease progression in AIDS, major problems still exist in AIDS treatment, including resistance and toxicity.[1–4] Interest therefore remains in the development of new anti-AIDS agents that would interact with viral proteins that are not targeted by the currently available anti-AIDS agents.

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.[5,6] 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 postattachment event prior to reverse transcription.[7] A hypothetical model (Fig. 1) has been proposed for the binding of the cosalane disalicylmethane 'pharmacophore' to CD4.[8] 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 additional positively charged residues that could be targeted by additional carboxylates of appropriately designed cosalane analogues, provided they are strategically spaced.[8,9] Accordingly, we designed a series of cosalane analogues of **2**–**4** in which the two distal carboxyl groups were placed *ortho*, *meta*, and *para* relative to the linkage to the cosalane skeleton. Maximal activity was found in compound **4**, in which the two carboxyl groups were *para*, followed by **3** and **2**. In order to precisely define how far the two distal carboxyl groups could be placed from the two proximal carboxyl groups and still increase activity, additional compounds in the series have now been synthesized in which the distance is further extended.



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We replaced each of the two distal carboxylic acid groups of **4** with phenyl substituents in order to provide two platforms for the attachment of carboxylic acid groups farther away from the two proximal carboxylic acid groups. The synthesis of the desired compounds was accomplished by the alkylation of the two phenoxide anions derived from cosalane (**1**) di(methyl ester) with the benzyl bromides **15**, **16**, and **17**. As shown in Scheme 1, coupling 4-methylbenzeneboronic acid (**5**) with the *o*-, *m*-, and *p*-bromobenzoic acids **6**, **7**, and **8** in the presence of palladium hydroxide and aqueous sodium hydroxide afforded the *o*-, *m*-, and *p*-tolylbenzoic

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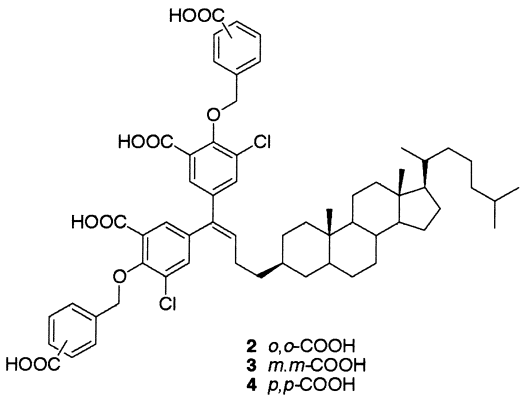


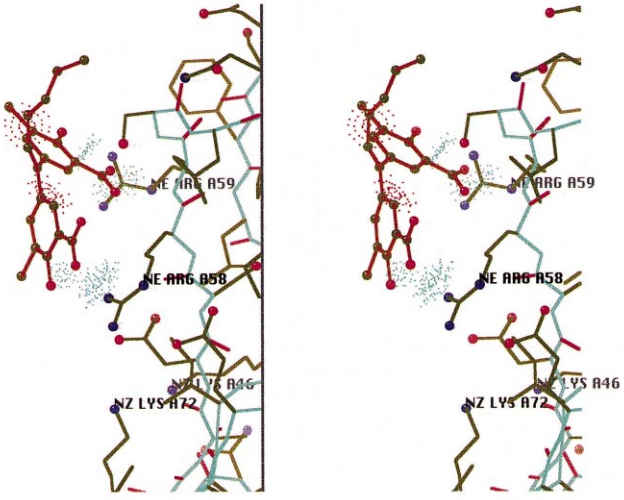
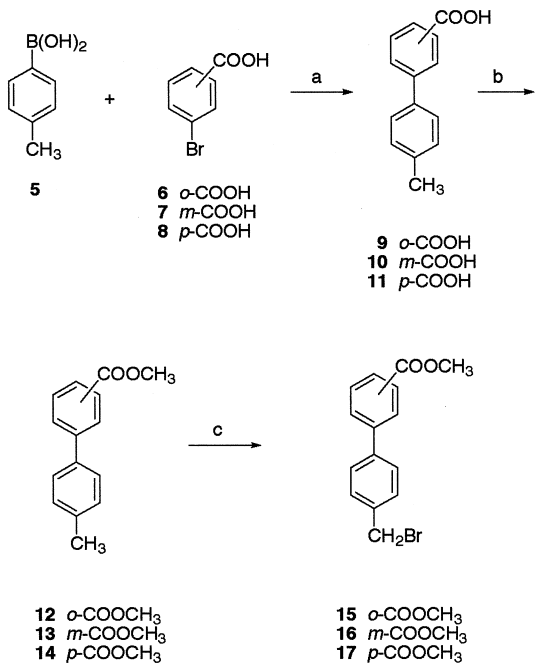


Figure 1. Hypothetical model of the binding of the cosalane 'pharmacophore' to CD4 (programmed for walleyed viewing). Blue dots:

acids **9**, **10**, and **11**. These three carboxylic acids were converted to their corresponding methyl esters **12**, **13**, and **14** with trimethylsilyldiazomethane.[10] Free radical bromination of **12**, **13**, and **14** in the presence of *N*-bromosuccinimide and dibenzoyl peroxide yielded the desired benzyl bromides **15** (90% yield from **9**), **16** (90% yield from **10**), and **17** (84% yield from **11**). The free radical bromination of **13** and **14** to yield **16** and **17** was carried out during irradiation with a 200-W tungsten lamp. Conversion of **12** to **15** was accomplished without irradiation.

Selective methylation of the two carboxylic acid groups of cosalane (**1**) with trimethylsilyldiazomethane afforded the di(methyl ester) **18** (100% yield, Scheme 2).[10] The two phenolic hydroxyl groups of **18** were deprotonated using potassium carbonate as the base in DMF, and both of the resulting phenoxide anions were alkylated with the benzyl bromides **15**, **16**, or **17** to afford the corresponding cosalane derivatives **19** (60% yield), **20** (60% yield), or **21** (46% yield). Hydrolysis of all four esters present in these compounds yielded the corresponding tetracarboxylic acids **22** (60% yield), **23** (38% yield), or **24** (42% yield).

The synthesis of the prior series of compounds **2**–**4** provided compounds in which the proximal and distal carboxyl groups were spaced by 6–8 atoms, and the



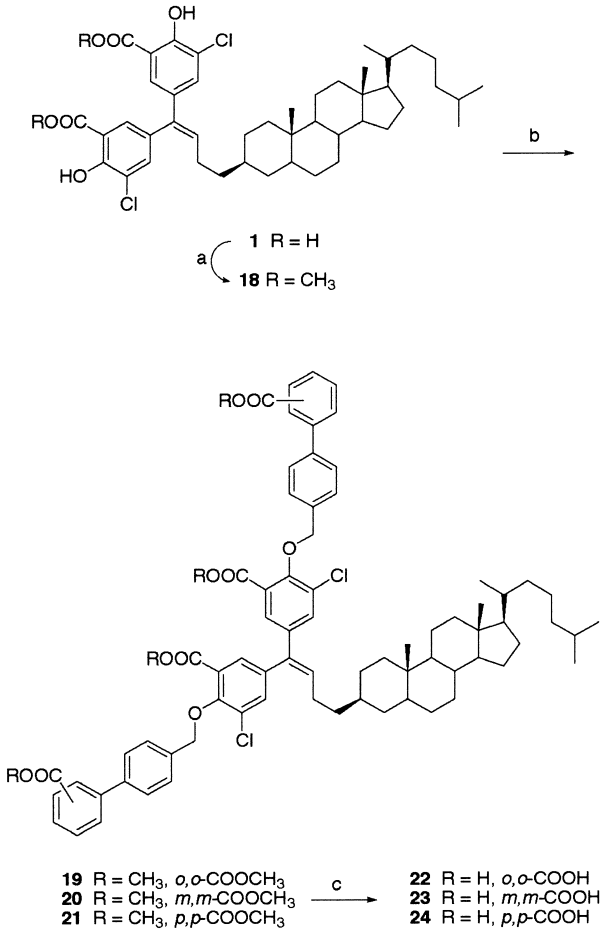
Scheme 1. Reagents and conditions: (a) $PdCl_2$, aq NaOH; (b) $TMSCHN_2$ in hexane, C_6H_6 and MeOH; (c) NBS, $(BzO)_2$, CCl_4.

present series **22**–**24** contributes three additional analogues in which the spacing is 10–12 atoms. In order to complete this series, we were therefore interested in having an analogue in which the proximal and distal carboxyl groups were spaced by nine atoms. The synthesis of the desired compound **27** is outlined in Scheme 3. The starting material **25** was made by methylation of commercially available 4-(bromomethyl)phenylacetic acid with trimethylsilyldiazomethane.[10] Deprotonation of the two phenolic hydroxyl groups of cosalane di(methyl ester) (**18**) with potassium carbonate, and alkylation of the resulting anions with the benzyl bromide **25**, yielded intermediate **26** (44% yield). Hydrolysis of the four methyl esters with potassium carbonate and a catalytic amount of potassium cyanide afforded the target compound **27** (42% yield).

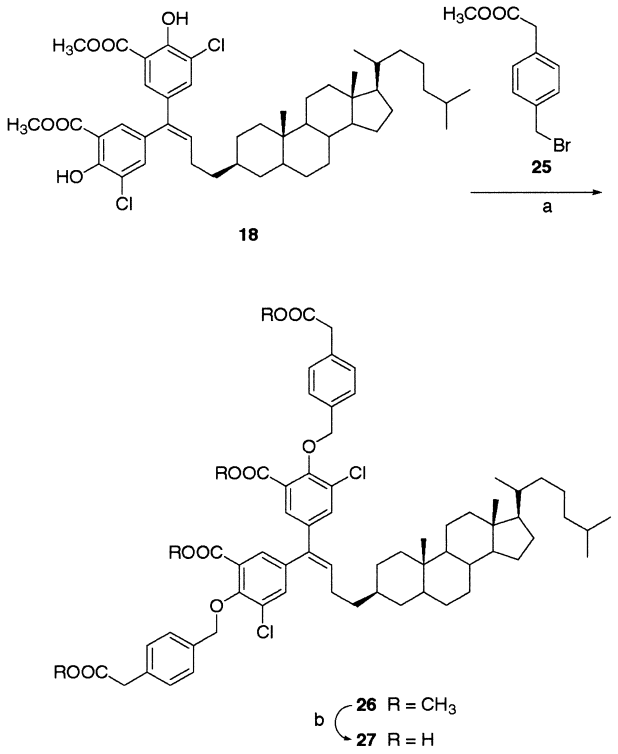
The new cosalane derivatives were evaluated for inhibition of the cytopathic effect of HIV-1_{RF} in CEM-SS cells, HIV-1_{IIIB} in MT-4 cells, and HIV-2_{ROD} in MT-4 cells. Cytotoxicities in uninfected CEM-SS cells and MT-4 cells were also determined. The results are listed in Table 1.

The new cosalane polycarboxylates **22**, **23**, **24**, and **27** synthesized in the present study were generally more potent against HIV-1_{RF} in CEM-SS cells than they were versus HIV-1_{IIIB} in MT-4 cells. They were also either inactive or displayed very low antiviral activity against HIV-2_{ROD} in MT-4 cells. The low activity of the compounds in the present series versus HIV-2_{ROD} is not totally unexpected, because the previously reported polyanions **3** and **4**, also having extended polyanionic pharmacophores, were less active versus HIV-2_{ROD} than they were versus HIV-1_{RF} or HIV-1_{IIIB}.[9]

Movement of the two distal carboxyl groups farther away from the two proximal carboxyl groups in the



Scheme 2. Reagents and conditions: (a) $TMSCHN_2$ in hexane, benzene and methanol, 25 °C (25 min); (b) **15**, **16**, or **17**, K_2CO_3, DMF; (c) for **22**: aq NaOH, MeOH and EtOH, reflux (14 h); for **23**: KCN, K_2CO_3, aq EtOH, 95 °C (20 h) followed by H_2O, 80 °C (2 h); for **24**: KCN, K_2CO_3, aq EtOH, 90 °C (20 h) followed by H_2O, 80 °C (1 h).



Scheme 3. Reagents and conditions: (a) K_2CO_3, DMF, 23 °C (22 h). (b) K_2CO_3, KCN, aq EtOH, 80 °C (14 h), and then H_2O, 80 °C (2 h).

Table 1. Anti-HIV activity of cosalane analogues[a]

Compound	EC_{50} (μM)			CC_{50} (μM)	
	HIV-1_{RF}	HIV-1_{IIIB}	HIV-2_{ROD}	CEM-SS cells	MT-4 cells
1	5.1	3.0	4.0	>200	>200
2	39.8	>37	>37	>316	37
3	5.7	2.2	>29	>316	29
4	0.5	1.7	22	72	88
22	5.17	12.1	>125	>200	>125
23	6.11	14.5	>95	>200	95
24	>100	>80	>80	>100	80
27	1.78	4.40	124.1	133	>125

[a]The antiviral EC_{50} values are the concentrations required to reduce the cytopathic effect of the virus by 50%, while the 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.[11,12] The values are the averages of at least two determinations.

biphenyl series **22**, **23**, and **24** resulted in a progressive decrease in antiviral activity against both HIV-1 strains. This effect is the reverse of what has been seen in the benzyl series **2**, **3**, and **4**. The most active of the new compounds was **27**, which was slightly more active than **22**, but less active than **4**. A plot of the antiviral activity versus HIV-1_{RF} as a function of the number of atoms separating the proximal and distal carboxyl groups is shown in Figure 2.

The results indicate an optimal spacing of the proximal and distal carboxyl groups that is approximated by the distances in the cosalane analogue **4**. This may reflect a requirement for optimal interaction of the anionic polycarboxylates with cationic amino acid side chains present on the surface of CD4. A hypothetical model of the binding of **4** with CD4, involving ionic bonding interactions of three of the carboxylates with the side chains of the Arg58, Arg59, and Lys72 residues is displayed in Figure 3.[9]

Mechanism of action studies on both cosalane (**1**) and the most active of the tetracarboxylates **4** have revealed that both of these antiviral agents bind to gp120 as well as CD4. Both compounds also inhibit the binding of gp120 to CD4, as well as a postbinding event prior to reverse transcription.[7,9] A detailed analysis of **4** indicated that inhibition of fusion occurs at lower concentrations than inhibition of attachment.[9] Like cosalane (**1**), the cosalane analogue **4** binds to both gp120 as well as CD4, and a direct interaction with the HIV co-receptor CXCR4 was not observed. It is possible that the interaction of these compounds with gp120 and CD4 inhibits the attainment of the proper orientation of the complex that is required for binding to CXCR4. We assume that the new compounds reported in the present study are

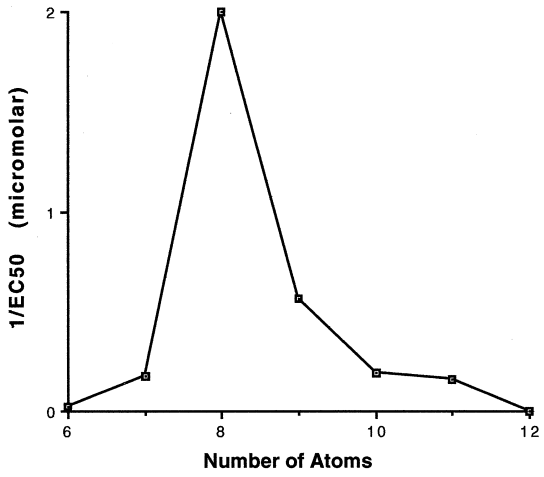


Figure 2. The relationship between anti-HIV activity (vs HIV-1_{RF} in CEM-SS cells) and the number of atoms separating the proximal and distal carboxylates in the series of compounds **2** (6 atoms), **3** (7 atoms), **4** (8 atoms), **27** (9 atoms), **22** (10 atoms), **23** (11 atoms), and **24** (12 atoms).

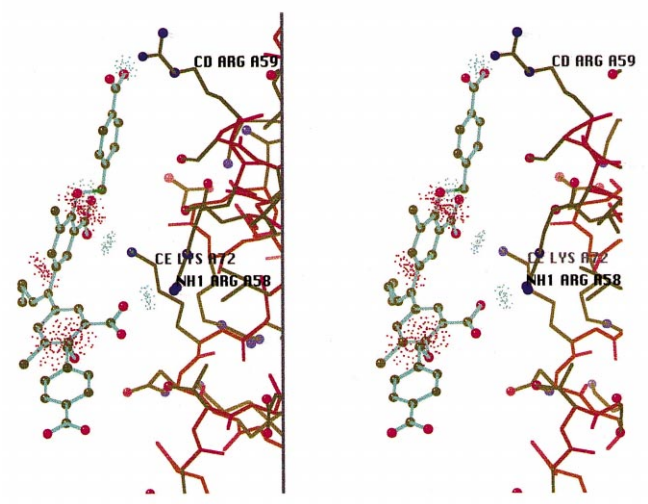


Figure 3. Hypothetical model of the binding of the **4** 'pharmacophore' to CD4 (programmed for walleyed viewing). Blue dots: attraction, red dots: repulsion.

acting similarly to cosalane (**1**) and its more potent analogue **4**.

Acknowledgements

This research was made possible by NIH grant 36624, and by grants from the Fonds voor Wetenschappelijk Onderzoek (FWO) Vlaanderen, the Belgian Geconcerteerde Onderzoekacties and the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (FGWO). We would like to thank Kristien Erven for excellent technical assistance.

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