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LIPOPHILIC BIS-ARYLSULFONATES AS INHIBITORS OF THE CD4-GP120 INTERACTION

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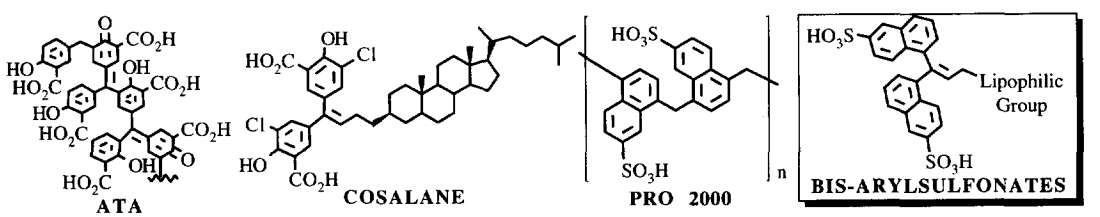
Abstract: A series of lipophilic bis-arylsulfonates was synthesized and evaluated as potential inhibitors of the CD4-gp120 interaction. Structure-activity studies suggested a direct relationship between the nature and extent of lipophilicity and inhibitory potency. Copyright © 1996 Elsevier Science Ltd

CD4 is a surface glycoprotein present on a subset of T lymphocytes that normally functions in concert with the T-cell receptor (TCR) to interact with antigen presented by class II MHC proteins.[1,2] Through this interaction, intracellular signaling events are initiated that result in a T-cell proliferation response. Human CD4 has also been shown to be the principal target for the human immunodeficiency virus (HIV).[3,4] The initial step in viral infection involves binding of the HIV surface glycoprotein, gp120 to CD4. Subsequent interaction of the third hypervariable (V3) domain of gp120 with additional cell surface components initiates the fusion of viral and T-cell membranes that ultimately leads to infection.[5,6]

Considerable effort has been expended in studying the CD4-gp120 interaction in order to identify agents that would interfere with this binding event. Such agents would be promising candidates for AIDS therapy, acting to prevent HIV infection. To date, several reports have indicated polyanionic compounds as a general class of antiviral agents acting at least in part, by interfering at the viral adsorption stage of infection. Thus, various sulfated polysaccharides such as dextran sulfate[7] and curdlan sulfate,[8] sulfonated polymers such as polyvinylsulfonate and polystyrenesulfonate,[9] polysulfonated azo dyes such as Evans Blue,[10] Direct Blue 1,[11] Direct Red 79[11] and quinobene[12] and the polysalicylate, aurintricarboxylic acid (ATA)[10] have all been reported to interfere either with CD4-gp120 binding and/or subsequent fusion events. Cushman has found that the lipophilic ATA analog, Cosalane, exhibits antiviral activity and appears to inhibit gp120-CD4 binding as well as post-binding HIV fusion steps and viral replication processes.[13,14]

We have recently reported on the anti-HIV properties of PRO 2000, a naphthalenesulfonate-formaldehyde condensate, that was identified as a highly potent inhibitor of the CD4-gp120 interaction *in vitro*.[15]

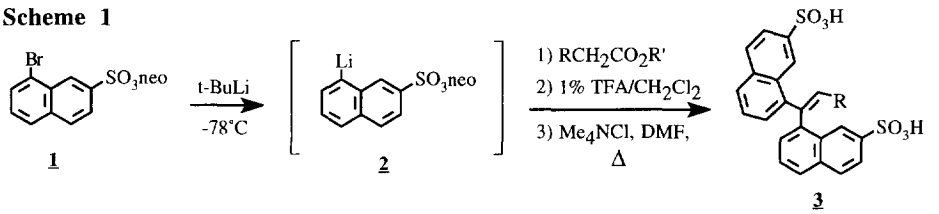


Our experience with arylsulfonates including PRO 2000, combined with Cushman's report of CD4-gp120 inhibition by Cosalane, prompted us to synthesize a series of bis-arylsulfonates that would be useful in investigating lipophilic and charge properties as they relate to this activity.

CHEMISTRY

In order to synthesize geminal di-(sulfonatoaryl) olefinic molecules of this type, we employed neopentyl ester protection for the sulfonate moiety (SO_3neo).[16] The protected arylsulfonate was then amenable to organometallic exchange chemistry as outlined in Scheme 1.[17] The intermediate aryl lithium **2** underwent double addition reactions with aliphatic esters to afford trisubstituted olefin **3**.

Scheme 1

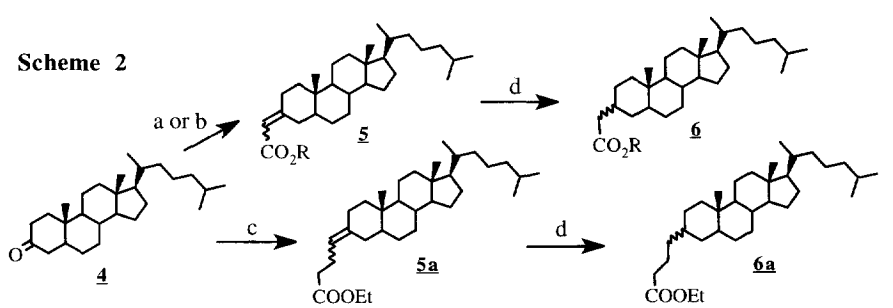


1 **2** **3**

With this general strategy validated for benzene as well as naphthalene systems, we embarked on our series development initially targeting the direct naphthalenesulfonate hybrid analog of PRO 2000 and cosalane as a means of establishing bioactivity for the series prototype. Also investigated in this study were limited structure-activity relationships with respect to the nature of the aromatic group, its substitution pattern, the length of the chain linking the arylsulfonates to the cholestane ring systems and the cholestane system itself.

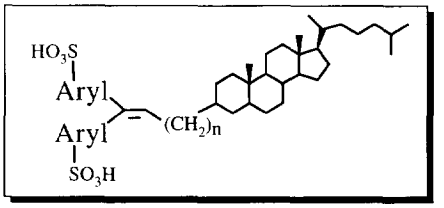
Cholestane esters were prepared according to Scheme 2 following Wittig or modified Wittig methodology. Thus, reaction of 5α-cholestan-3-one (**4**) with either methyl triphenylphosphoranylideneacetate (contrary to the report of Bose and Dahill[17]) or triethylphosphonoacetate provides unsaturated ester **5** as an enriched geometric isomer mixture (E/Z ~ 5:1) in very good yield. Similarly, Wittig reaction of **4** with ethyl 4-triphenylphosphonium butyrate affords olefinic ester **5a**. Hydrogenation of **5** and **5a** over platinum oxide provides saturated esters **6** and **6a**, respectively, as epimeric mixtures at C_3. (In the case of **6**, proton NMR analysis indicated the epimeric ratio of 3β:3α to also be ~ 5:1 as determined from the methyl ester signals.[18]) At this stage of our study, we did not attempt to separate isomers or optimize the reduction in terms of diastereomeric selectivity; thus, biological analyses were carried out on enriched epimeric mixtures.

Scheme 2



(a) $Ph_3P{=}CHCO_2Me$, PhCl, 140 °C, 48 h; 96% (b) $Et_2O_3PCH_2CO_2Et$, NaH, THF; 87% (c) $Ph_3P(CH_2)_3CO_2Et$, $KN(TMS)_2$, PhCl, 140 °C; 64% (d) H_2, PtO_2, 50psig, 3 h; 99%.

Table 1. Inhibition of CD4-gp120 binding; effects of aryl group and linker



Compound	Aryl Group	SO3H Position	n	ELISA IC50 (uM)	Cellular IC50 (uM)
1357	Phenyl	4 -	0	10	39 (56T)†
1399	Phenyl	4 -	2	13	47 (135T)†
1489	Phenyl	3 -	0	62	Flat D. R.‡
1400	1-Naphthyl	6 -	0	16	6
1401	1-Naphthyl	6 -	2	9	7
1402	1-Naphthyl	7 -	0	10	25 (125T)†
1488	1-Naphthyl	7 -	2	10	7 (120T)†
1751*	1-Naphthyl	6 -	2	18	9
PRO 2000±	1,4-Naphthyl	6 -		0.4±	2±
Cosalane	5-(3-Chloro)-salicyl		2	18	5

* Reduced olefin; saturated analog of 1401
† T indicates cytotoxic dose (CD_{50})
‡ Flat dose response
± PRO 2000 5K; activities are ug/mL

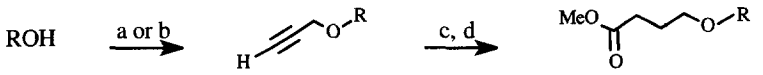
The abilities of our target compounds[19] to inhibit the CD4-gp120 interaction was evaluated in ELISA[20] and cellular[21] formats as presented in Table 1.[22] In general, the activities of these analogs were much less than observed with PRO 2000, but were similar to that of cosalane. With the exception of the 3-sulfonatophenyl analog, **1489**, all compounds were essentially equipotent as determined in the ELISA assay. In the cellular assay, some distinctions could be made amongst the series members: (1) the phenyl analogs and the 7-naphthyl analogs showed cytotoxic effects at higher doses; (2) the linker chain length did not play a critical role for activity; and (3) reduction of the olefinic bond did not affect activity. Given these results, we next chose to examine the lipophilic requirements for bioactivity by preparing a series wherein the aryl group and chain linker remained constant (6-sulfonato-1-naphthyl; n = 0) and the cholestane ring system was modified or replaced with other lipophilic groups.

For the series presented in Tables 2 and 3, ester precursors were either commercially available or easily prepared from the corresponding ketones according to the methods outlined in Scheme 2 for cholestan-3-one. For the preparation of γ-alkoxyesters, a propargylation and homologation sequence was used as outlined in Scheme 3.

With respect to steroidal analogs, stereochemistry at the 5-position did not seem to be an important factor for biological activity, as seen with cis-fused isomer **3434**. Removal of the C_8 tail from the D-ring also did not adversely affect activity (**1766**). However, when this deletion was accompanied by the incorporation of a polar

functionality such as keto (**3093**) or hydroxyl (**3003**), there was a significant loss of inhibitory activity as measured in the ELISA format; in the cellular context, the trend is much less pronounced. Etherification of the hydroxyl functionality with an allyl group restored much of the activity (**3002**). Incorporation of an ether functionality in the linker chain region was similarly tolerated, as demonstrated with analog **2850**.

Scheme 3



(a) aliphatic ROH: propargyl bromide/toluene, 50% NaOH, CH_2Cl_2, $Bu_4N^+HSO_4^-$, 4d, 30-35% (b) phenolic ROH: propargyl bromide/toluene, K_2CO_3, acetone, 16h, 65-70% (c) n-BuLi, THF, -78 °C; MeO_2CCl, RT, 6h, 60-65% (d) H_2, PtO_2, 60psig, 12h, 75-90%.

Table 2. Inhibition of CD4-gp120 binding; lipophilic analogs

Compound	ELISA*	Cellular*	Log P‡	Compound	ELISA	Cellular	Log P‡
1400	16	6	9.18	**2851**	25	5	10.62
3434	26	13	9.18	**3435**	41	23	7.25
1766	26	17	6.27	**1347**	192	NA#	7.42
3093	107	35	5.82	**1344**	56	NA#	6.90
3003	83	24	5.30	**1496**	>350	>170	4.49
3002	33	12 (100T)†	6.31	**1989**	130	52	6.46
2850	31	6	8.88	**1990**	>400	150	4.47
1765	292	49	5.60				

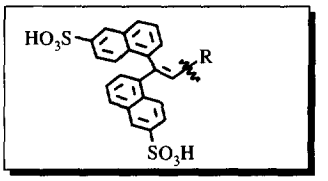
* IC_{50} (uM)
‡ Log P values were generated using the program Cerius^{2TM}, developed by BIOSYM/Molecular Simulations; ref. 23
# Not Available
† T indicates cytotoxic dose

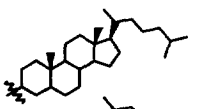
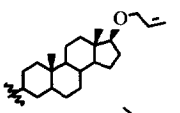
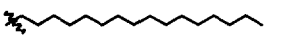
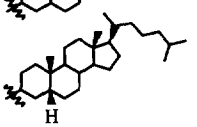
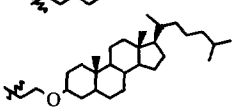
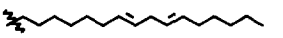
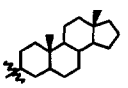
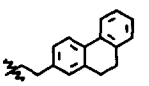
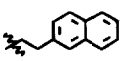
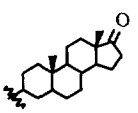
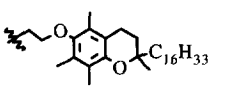
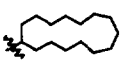
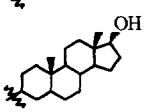
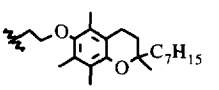
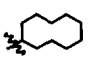
Departure from the steroid nucleus resulted in more dramatic effects upon bioactivity which suggested that a combination of lipophilicity and conformational rigidity are required for activity. When the steroid was replaced with a straight chain aliphatic group (C_{16}) as in **1347**, activity decreased more than 10-fold (ELISA). Introduction of limited conformational restriction as brought about by the incorporation of unsaturation in the chain restored a significant amount of activity (**1344**). Further conformational rigidity, as incorporated in the partially aromatic tricyclic dihydrophenanthryl group (**1765**; also C_{16}), did not provide the same increase in activity as measured in the ELISA format. Comparison of **1989** and **1990** provided further evidence of a correlation between lipophilicity and bioactivity. Introduction of a combination of aromatic and non-aromatic lipophilicity by way of vitamin E analog **2851** was well tolerated. Truncation of the tocopheryl lipid tail (**3435**) resulted in only a slight weakening of activity.

In order to examine these effects more quantitatively, partition coefficients (P) were calculated for the lipophilic portions of these molecules.[23] With respect to the more constrained analogs (steroidal, aryl and tocopheryl-based), good activities are seen in compounds whose log P is 6 or greater; below this apparent threshold (**3093**, **3003**, **1765** and **1496**) activities weaken. That compounds whose log P values are greater than 6 require the additional element of rigidity for activity is also evident from the comparisons of virtually inactive **1347** (log P = 7.42) with **1344** (log P = 6.90) and **3435** (log P = 7.25). It would appear that within

this lipophilic range, rigidity would appear to be the more significant determinant for bioactivity. Clearly more examples would be needed to further substantiate this hypothesis or extend it to better define the rigidity requirements for this class of molecules.

Table 3. Lipophilic Analog Series



Compound	R Group	Compound	R Group	Compound	R Group
1400		3002		1347	
3434		2850		1344	
1766		1765		1496	
3093		2851		1989	
3003		3435		1990	

In summary, we have demonstrated CD4-gp120 inhibitory activity for a series of lipophilic bis-arylsulfonates. Activity, particularly in the context of cellular presentation of CD4, is somewhat dependent on the nature and point of substitution of the aryl group, with the 1-naphthyl-6-sulfonato substitution pattern being preferred. The nature of the lipophilic group, both in terms of the extent of lipophilicity and conformational rigidity, appears to be a more significant determinant for this activity.

Acknowledgement: We are grateful to Dr. Mark Cushman, who kindly provided us with reference samples of cosalane.

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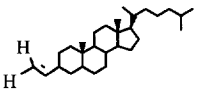
18. The assignment of β-configuration to the major isomer is made by analogy to Cushman's observations and analysis (ref. 11) of the structurally similar cosalane intermediate.
19. All final compounds were purified by preparative HPLC and characterized by NMR and MS (FAB^-).
20. For the ELISA assay, the HIV gp120/CD4 receptor EIA kit, available from DuPont-NEN, Boston, MA was used. In this format, gp120 binding to plated recombinant 4-domain CD4 is detected by an envelope-specific monoclonal antibody (NEA-9205) coupled to horseradish peroxidase. Data reported represent the mean of at least two assays, each run in duplicate. IC_{50} values from different experiments were within a factor of 2 or less.
21. Cellular assay: CEM cells (American Culture Collection, Rockville, MD; 3 x 10^6 cells per ml) were suspended in RPMI 1640 (Whittaker-Bioproducts, Walkersville, MD) with 10% fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS) plus sodium azide. 100 uL of the suspension were added to individual tubes. Generally, test compounds were dissolved in water to a concentration of 4 mg/mL. Various dilutions of the compounds (1:40, 1:100, 1:200, 1:400, 1:800) were added to the tubes which were then incubated for 2 h at 25 °C. After this time, gp120 (American Bio-Technologies, Inc., Cambridge, MA), diluted in RPMI 1640 buffer, was added to a final concentration of 10 nM and the resulting mixture was incubated overnight (~ 16 hrs.) at 37 °C. Cells were then washed thoroughly with phosphate-buffered saline containing 10% FBS and 0.1% sodium azide. Bound gp120 was detected using a monoclonal antibody specific for gp120 (DuPont-NEN, NEA-9284 or NEA-9205) which was incubated with the cells at a concentration of 1 ug/mL (100ul per tube) for 30 min. on ice. The cells were then washed thoroughly as before; bound NEA-9284 was detected by staining with goat anti-mouse immunoglobulin (Boehringer Mannheim Biochemicals, Indianapolis, IN) which was labeled with fluorescein (50 uL per tube) for 30 min. on ice. The washed cells were analyzed for fluorescence on a FACScan™ instrument (Becton Dickinson).
22. Since activities are determined indirectly by V3 loop antibody capture, compounds that bind to the V3 region with slow dissociation may not be distinguished from true CD4-gp120 inhibitors in these formats. Thus, for the more potent compounds, inhibition was confirmed independently by a C-terminal detecting antibody.
23. The naphthalenesulfonate systems were excluded from these calculations (replaced with hydrogen atoms) for simplification purposes. Thus, for example, the log P value calculated for the hydrophobic portion of compound 1400 is based on structure *i*:



i

Assuming that the naphthylenesulfonates do not interact with the linkers, contributions from these groups to the partition constant would be uniform throughout the series and would be expected to be additive.

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