Structure-Activity Relationship Studies with Symmetric Naphthalenesulfonic Acid Derivatives. Synthesis and Influence of Spacer and Naphthalenesulfonic Acid Moiety on Anti-HIV-1 Activity

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Symmetric bis(naphthalenesulfonic acid) derivatives containing a variety of spacers have been synthesized and evaluated for anti-HIV-1 activity in four assay systems. In the assay that measured inhibition of HIV-1-induced cytopathogenicity using a laboratory strain (HTLV-III_B), a hexamethylene and octamethylene spacer derivative of 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid emerged as the most potent derivatives. The hexamethylene spacer analog exhibited an in vitro therapeutic index that was >120. Selected derivatives were tested in the giant cell formation assay. In this assay, the most potent derivative was, again, the hexamethylene compound. Evaluation of selected derivatives against a clinical isolate of HIV-1 (HE strain) revealed that the hexamethylene derivative was the most potent compound. In the assay that measured the inhibition of HIV-1-induced cytopathogenesis in human peripheral blood lymphocytes, the hexamethylene compound emerged as the most active derivative, demonstrating a 50% inhibitory concentration of 1.3 μM. These studies clearly demonstrate that certain naphthalenesulfonic acid moieties when coupled to specific spacers were synergistic in producing anti-HIV-1 activity at nontoxic concentrations. In the 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid series, shortening of the spacer length, preferably with a flexible polymethylene chain, was highly beneficial for increasing anti-HIV-1 potency.

A series of reports from these laboratories has documented the anti-HIV potential in a variety of sulfonic acid derivatives.[1-4] In continuing endeavors to further delineate the structural parameters in this class of compounds, we have designed, synthesized, and evaluated several bis(naphthalenesulfonic acid) derivatives for their anti-HIV-1 activity. These symmetric derivatives containing different spacers were investigated for their ability to inhibit HIV-1-induced cytopathogenesis using a laboratory strain in both MT-4 cells and peripheral blood lymphocytes. We have also investigated the ability of these agents to suppress giant cell (syncytia) formation and replication of a clinical isolate of HIV-1 (HE strain).

The spacers for the bis derivatives were selected on the basis of our previous studies which documented the utility of biphenyl and the decamethylene spacer in the design of these agents.[1-3] These previous observations provided the impetus to design new analogs to answer two important questions. First, the role of different aromatic spacers with different sulfonic acid carrying moieties in influencing anti-HIV-1 activity and, second, to ascertain the need, if any, of a shorter polymethylene chain and its governing role in the anti-HIV-1 potential of these derivatives.

Chemistry

Representative reactions to obtain the target compounds are described as follows. A key substrate, 5-Amino-2-hydroxy-3-sulfobenzoic acid (1) was reacted under aqueous bicarbonate conditions with a variety of sulfonyl chlorides. Compound 1 was chosen as a sulfonic acid carrying moiety since a previously reported reaction with 4,4'-biphenyldisulfonyl chloride produced analog 2, which demonstrated anti-HIV-1 activity at nontoxic concentrations.[4] Reaction of 1 with 1,5-naphthalenedisulfonyl chloride yielded the bis sulfonamide derivative 3 (Scheme I). 7-Amino-1,3-naphthalenedisulfonic acid (20) and 1,5-naphthalenedisulfonyl chloride yielded analog 22 (Scheme II).

Reaction of the naphthol disulfonic acid 25 with 4,4'-biphenyldisulfonyl chloride in aqueous sodium bicarbonate media yielded the symmetrical derivative 26 (Scheme III). Since our earlier studies have documented the intriguing and beneficial role of the parent 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid (29) moiety which is present in the active symmetrical derivatives 30-32,[1,2,4] we have pondered over the outcome of further reducing the spacer distance between the naphthalene units. Reaction of 29 with suberoyl chloride in pyridine afforded the hexamethylene spacer compound 33 (Scheme IV). In accordance with our earlier work, sulfonylation was exclusively observed on the amino functionality and is substantiated by observing characteristic downfield ^1H resonances for both the phenolic and sulfonamide hydrogens.[1,3,4] In agreement with these previous observations, the target compounds were isolated as sodium salts containing varying amounts of water of crystallization.[1,3,4] It should be noted that these apparently straightforward synthetic procedures were often confronted with difficulties in their preparation. Due to the unusual physical and chemical properties of the sulfonic acids, the reaction products separate out as semisolid and gummy mixtures which offered considerable difficulties at the workup and purification stages. These problems have been circumvented by methodologies that are described in the Experimental Section.

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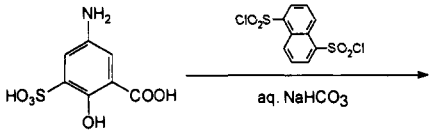
Scheme I



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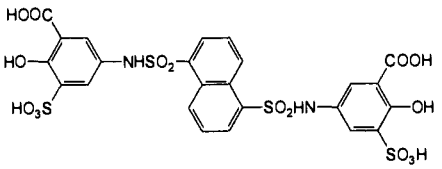

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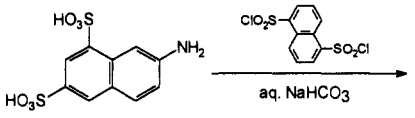
Scheme II



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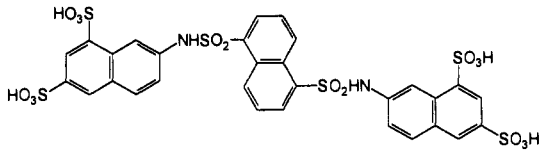

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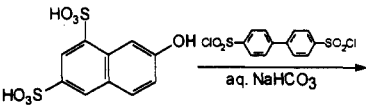
Scheme III



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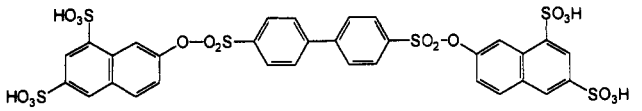

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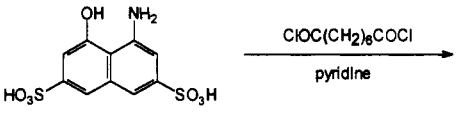
Scheme IV



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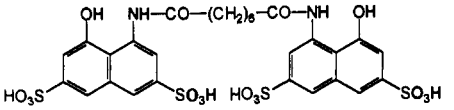

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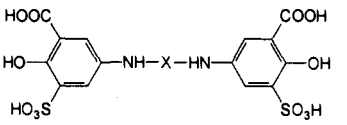
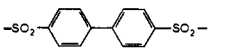
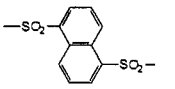
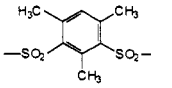
Results and Discussion

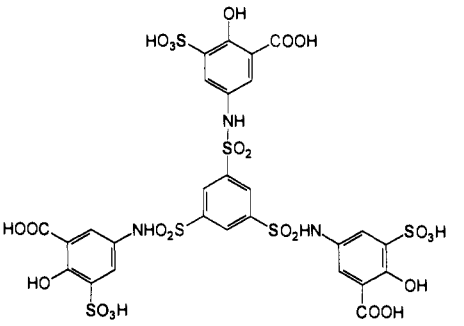
In the assay that measures inhibition of cytopathogenesis in MT-4 cells using a laboratory strain (HTLV-III$_B$) and considering the 5-amino-2-hydroxy-3-sulfobenzoic acid (1) series, replacing the biphenyl spacer (as in 2) with the shorter naphthalene spacer (as in 3, EC_{50} = 52.5 μM)



2 X = 

3 X = 

4 X = 



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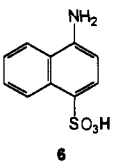
decreases toxicity (CC_{50} > 500). A further lessening of the spacer distance by utilizing a phenyl spacer (as in 4) slightly decreases the potency (Table I). The above derivatives fall into the class of monosulfonic acid derivatives and each of the phenyl units also possesses both a phenolic and a carboxylic moiety and therefore contains three anionic functionalities per phenyl unit. This substitution pattern in a bis derivative is not sufficient to further increase potency; however, if an additional sulfobenzoic acid unit is attached to a similar phenyl spacer to produce a novel tris derivative 5 (EC_{50} = 11 μM), potency is increased without a further increase in toxicity (CC_{50} > 500) (Table I).

In the naphthalenemonosulfonic acid derivatives not containing additional anionic functionalities, as exemplified by the 4-aminonaphthalene-1-sulfonic acid (6) series,



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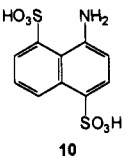

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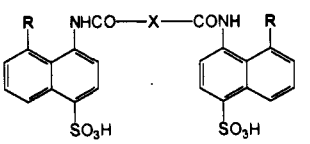


7 R = H, X = $(CH_2)_2$
8 R = H, X = $(CH_2)_6$
9 R = H, X = $(CH_2)_8$
11 R = SO_3H, X = $(CH_2)_5$
12 R = SO_3H, X = $(CH_2)_6$
13 R = SO_3H, X = $(CH_2)_8$
14 R = SO_3H, X = $(CH_2)_{10}$
15 R = SO_3H, X = 

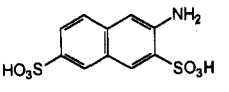
Table I. Inhibition of Sulfonic Acid Derivatives on the Replication of a Laboratory Strain of HIV-1 (HTLV-III_B) in MT-4 Cells[e]

compd	EC_{50}[f] (μM)	CC_{50}[g] (μM)	compd	EC_{50}[f] (μM)	CC_{50}[g] (μM)
2[c]	52.5	247	19[b]	43	>500
3[c]	52.5	>500	20[b]	>500	>500
4[c]	57	>500	21[c]	29.5	>500
5[d]	11	>500	22[c]	14	>500
6	>500	>500	24[b]	>100	100
7[b]	>500	>500	25	>500	>500
8[b]	>500	>500	26[c]	40	223.5
9[b]	>500	>500	27[b]	>55	144
10[a]	>500	>500	28[c]	>168	>500
11[c]	143	>500	29[a]	>529	>529
12[b]	242	>500	30[c]	8.3	320
13[b]	66.5	>500	31[b]	44	430
14[c]	46	>500	32[c]	3.9	>100
15[b]	>197	>500	33[c]	2.9	359.5
18[c]	41	>500	suramin[d]	8.2	250

[a] Monosodium salt. [b] Disodium salt. [c] Tetrasodium salt. [d] Hexasodium salt. [e] All data are the mean result of at least two separate experiments. Data for analogs 2, 21, and 32 were obtained from ref 4, and data for analogus 30 and 31 were obtained from ref 2. [f] 50% effective antiviral concentration. [g] 50% cytotoxic concentration.

its derivatives 7–9 containing spacers of two, six, and eight methylene units, produce activity at toxic doses. However, introduction of an additional sulfonic acid moiety to the parent compound 6 to produce the 8-amino-1,5-naphthalenedisulfonic acid (10) series demonstrates an overall beneficial effect in the preparation of analogs. Inspite of the fact that the parent compound 10 demonstrates activity at toxic concentrations, preparation of symmetrical bis compounds using polymethylene chains does modify activity. As the spacer length is increased from five (analog 11, EC_{50} = 143 μM) to six (12, EC_{50} = 242 μM) methylene units a change in activity is observed. Further increasing the spacer length to eight (13, EC_{50} = 66.5 μM) and to 10 methylene units (analog 14, EC_{50} = 46 μM) seems to increase potency in this series. A shorter spacer distance enforced with a phenyl ring, as is demonstrated in the activity of compound 15 (EC_{50} > 197 μM), produces less activity (Table I). The inconsistent gradation in activity that is observed suggests that additional analogs need to be evaluated to establish a definitive structure–activity relationship in the 8-amino-1,5-naphthalenedisulfonic acid (10) series.

In the 2,7-naphthalenesulfonic acid series, starting materials containing an amino (compound 16) and phenolic (compound 17) functionality were investigated to produce analogs 18 (EC_{50} = 41 μM) and 19 (EC_{50} = 43 μM), respectively, both having similar potency. However, using 7-amino-1,3-naphthalenesulfonic acid (20, EC_{50} > 500 μM) and decreasing the spacer length from a biphenyl analog 21 (EC_{50} = 29.5 μM) to a naphthalene analog 22 (EC_{50} = 14 μM) increased the potency 2-fold (Table I). Keeping the substitution pattern intact but substituting a phenolic group for the amino group and varying the sulfonic acid in the 3-position required the starting materials 23 and 25. Compound 23 was used to generate analogs 24 (EC_{50} > 100 μM) and 27 (EC_{50} > 55 μM). Starting material 25 possessing an additional 3-sulfonic acid substitution produced derivative 26 (EC_{50} = 40 μM) and the less active compound 28 (EC_{50} > 168 μM), demonstrating that, in this series also, a disulfonic acid naphthalene moiety was preferred over a monosulfonic acid pattern and the sulfonic acid carrying moiety should be coupled with a biphenyl spacer.



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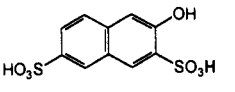

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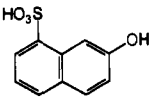

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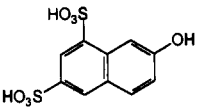

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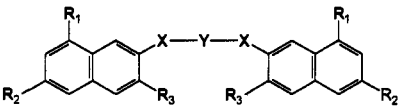


18 R_1 = H, R_2 = SO_3H, R_3 = SO_3H, X = NH, Y = $-O_2S-C_6H_4-C_6H_4-SO_2-$

19 R_1 = H, R_2 = SO_3H, R_3 = SO_3H, X = O, Y = $-O_2S-C_6H_4-C_6H_4-SO_2-$

21 R_1 = SO_3H, R_2 = SO_3H, R_3 = H, X = NH, Y = $-O_2S-C_6H_4-C_6H_4-SO_2-$

22 R_1 = SO_3H, R_2 = SO_3H, R_3 = H, X = NH, Y = $-O_2S-C_6H_4-SO_2-$

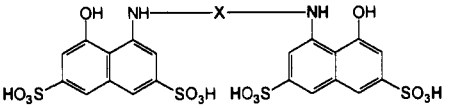
24 R_1 = SO_3H, R_2 = H, R_3 = H, X = O, Y = $-O_2S-C_6H_4-C_6H_4-SO_2-$

26 R_1 = SO_3H, R_2 = SO_3H, R_3 = H, X = O, Y = $-O_2S-C_6H_4-C_6H_4-SO_2-$

27 R_1 = SO_3H, R_2 = H, R_3 = H, X = O, Y = $-O_2S-C_{10}H_6-SO_2-$

28 R_1 = SO_3H, R_2 = SO_3H, R_3 = H, X = O, Y = $-O_2S-C_{10}H_6-SO_2-$

The 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid substitution pattern has previously produced several active derivatives like 30 (EC_{50} = 8.3 μM) and 31 (EC_{50} = 44 μM).[1-4] It is intriguing that, in this series, shortening the spacer length has the most beneficial effect on potency.

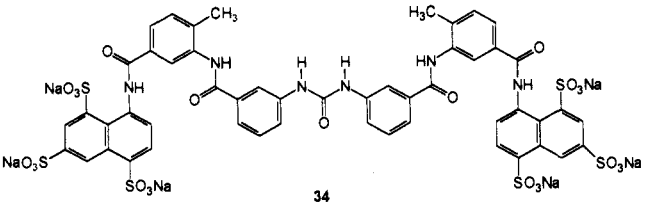


30 X = $-O_2S-C_6H_4-C_6H_4-SO_2-$

31 X = $-CO(CH_2)_{10}CO-$

32 X = $-CO(CH_2)_8CO-$

33 X = $-CO(CH_2)_6CO-$



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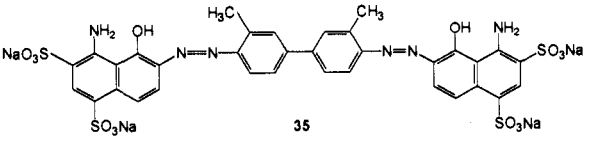

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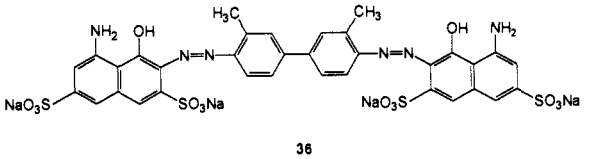

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Table II. Inhibition of HIV-1-Induced Giant Cell Formation by Sulfonic Acid Derivatives

	percent inhibition[c]				
compd	2[b]	21[b]	31[a]	32[b]	33[b]
500	100	100	100	100	100
100	94	100	100	100	100
20	22	34	40	35	96
4	0	21	11	30	31
0.8	3	6	0	0	6
IC_{50}[d] (μM)	37	29	26	29	6.4

[a] Disodium salt. [b] Tetrasodium salt. [c] All data is the mean result of at least two separate experiments. [d] 50% inhibitory concentration.

Table III. Inhibition of Sulfonic Acid Derivatives on the Replication of a Clinical Isolate of HIV-1 (HE strain) in MT-4 Cells[c]

compd	EC_{50}[e] (μM)	CC_{50}[f] (μM)	compd	EC_{50}[e] (μM)	CC_{50}[f] (μM)
2[b]	7.5	>100	32[a]	3.9	>100
21[b]	8.1	>100	33[a]	2.2	>100
31[a]	12.5	>100	suramin	8	47

[a] Disodium salt. [b] Tetrasodium salt. [c] All data is the mean result of at least two separate experiments. [e] 50% Effective antiviral concentration. [f] 50% Cytotoxic.

Using an octamethylene and hexamethylene spacer produces activity in compounds **32** (EC_{50} = 3.9 μM)[4] and **33** (EC_{50} = 2.9 μM) at concentrations far below their toxic concentrations. The most potent compound, **33**, demonstrates an in vitro therapeutic index that is >120 (Table I).

In the assay measuring the inhibition of HIV-1-induced syncytia formation, again, the most active derivative that emerged from this study was the hexamethylene compound **33** (IC_{50} = 6.4 μM, Table II). The decamethylene analog **31** (IC_{50} = 26 μM) was the next most active compound followed by analogs **21** (IC_{50} = 29 μM) and **32** (IC_{50} = 29 μM), which demonstrated equal potencies (Table II). Therefore, these results indicate that reducing the distance between the naphthalenesulfonic acid moieties is also beneficial for potentiating the inhibition of syncytia formation.

In evaluating derivatives **2**, **21**, and **31–33** in the assay that measured the inhibition of cytopathogenesis using a clinical isolate of HIV-1 (HE strain), once again the bis(naphthalenedisulfonic acid) derivative **33**, containing the hexamethylene spacer, emerged as the most potent derivative, demonstrating an EC_{50} value of 2.2 μM and a CC_{50} value of >100 μM (Table III). In this assay, it is interesting that extending the polymethylene chain by two carbon atoms to produce the octamethylene derivative **32** resulted in a slight lowering of potency (EC_{50} = 3.9 μM) (Table III). A further lengthening of the chain by incorporating two methylene groups to produce the decamethylene analog **31** resulted in still lower activity (EC_{50} = 12.5 μM, Table III). In the same assay, the naphthalenedisulfonic acid derivative **21** (EC_{50} = 8.1 μM) was almost equipotent to derivative **2** (EC_{50} = 7.5 μM) containing the same spacer with the sulfosalicylic acid moiety. However, both **21** and **2** were slightly more active than analog **31** (Table III).

The most active aromatic spacer derivative **30** and the most active polymethylene spacer analog **33** were evaluated for their inhibitory effects on the replication of HIV-1 in peripheral blood lymphocytes (PBL). In this assay, **33** (EC_{50} = 1.3 μM, CC_{50} = 20 μM) was more potent, but also more toxic, than **30** (EC_{50} = 9.2 μM, CC_{50} = 38 μM), as exemplified in Table IV. The increased toxicity of these derivatives in the PBL assay (Table IV) as compared to the cytopathogenesis assay (Table I) may be explained by considering the time of exposure of the cells to the target compounds. The PBL assay has a longer assay run time than the cytopathogenesis assay.

Table IV. Inhibition of Derivatives **30** and **33** on the Replication of HIV-1 ($HTLV\text{-}III_B$) in Peripheral Blood Lymphocytes[b]

compd	EC_{50}[c] (μM)	CC_{50}[d] (μM)
30[a]	9.2	38
33[a]	1.3	20

[a] Tetrasodium salt. [b] All data is the mean result of at least two separate experiments. [c] 50% Effective antiviral concentration. [d] 50% Cytotoxic concentration.

These results clearly suggest that certain naphthalenesulfonic acid moieties are more suitable than others for generating active compounds. The naphthalenedisulfonic acid compounds are the most promising for producing anti-HIV-1 activity. We have previously demonstrated that analogs prepared from a naphthalenetrisulfonic acid moiety are inactive as potential anti-HIV-1 agents.[4] However, in order to harness this activity in the naphthalenedisulfonic acids, the synthesis of a symmetrical bis derivative possessing an optimum distance between the naphthalenesulfonic acid moieties is preferred. For certain naphthalene moieties a shorter spacer is preferred while, for the others, a longer spacer is required.[5] The activity of these derivatives is not due to their potential hydrolysis products. Screening of the parent sulfonic acid moieties present in these derivatives demonstrated activities only at toxic doses. The spacer units showed little or no activity at nontoxic doses (data not shown), indicating that the activity in these compounds is due to the intact molecule.

Mechanism of Action

The detailed mechanism of action of these derivatives remains to be elucidated. However, due to the polar nature of these agents, they are unlikely to enter cells, and their activity may be attributed to the inhibition of viral binding as exhibited for several sulfonic acid dyes[6] and other analogs related to these compounds. The naphthalenesulfonic acid compounds have been shown to inhibit the binding of anti-gp120 monoclonal antibody to the envelope glycoprotein gp120.[7]

In this context, the V3 region in gp120 (amino acids 303–338), although variable, maintains a high degree of positive charge among many HIV strains.[8] Therefore, it could be reasoned that the anionic sulfonic acid groups interact with closely positioned cationic sites on gp120 and inhibit the viral binding and/or fusion event. Our studies reveal that among the mono-, di-, and trisulfonic acid naphthalene compounds, the naphthalenedisulfonic acid derivatives are the most active.[9] Molecular modeling studies conducted by us demonstrate that the decamethylene spacer analog **31**, containing a naphthalenedisulfonic acid moiety, can, like suramin, mimic the helical turn of B-DNA.[10] An added virtue of anionic sulfonate compounds is that, unlike the sulfate moiety which is prone to desulfation,[11] the sulfonic acid functionality undergoes little or no in vivo desulfonation.[12]

An apparent deterrent to the development of small sulfonic acid molecules has been their comparison to suramin (**34**), a drug that did not demonstrate any clinical

efficacy.[13] However, we[1-4] and others[14] have shown that it is possible to develop compounds that are more potent and less toxic than suramin. Further, suramin is distinctly different from our compounds since it is a hexasulfonic acid derivative possessing a large spacer and is a molecule that is endowed with unique properties. For example, it has a half-life of 44–54 days, one of the longest known for a drug, a fact that has been attributed to its protein binding properties.[15] Undoubtedly, this must be a major contributing factor to the in vivo toxicity of suramin. Nevertheless, this problem can be surmounted by the evaluation of analogs, since it is known that sulfonic acid containing fragments of suramin are easily cleared from the blood.[16] As another example, the symmetrical naphthalenedisulfonic acid dye Evans Blue (35) is also known to bind to proteins whereas its positional isomer, Trypan Blue (36), exhibits little or no binding and is rapidly excreted from the blood.[17]

In conclusion, these studies substantiate the anti-HIV-1 potential of the symmetric naphthalenedisulfonic acid class of compounds. Analog 33, a derivative of 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid possessing a hexamethylene spacer, is the most active compound in a variety of assays. This compound will be considered as a new lead for future studies. Further investigations to optimize activity and elucidate the detailed mechanism of action of these compounds are in progress and will be detailed in future reports.

Experimental Section

Synthetic Procedures. Melting points were determined on a Mel-Temp II apparatus and are uncorrected. NMR spectra were recorded on a Varian XL-300 (300 MHz) instrument in DMSO-d_6. Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard. The IR spectra were recorded on a MIDAC FT IR system, using KBr pellets, in the Department of Medicinal Chemistry and Pharmacognosy. Analytical thin-layer chromatography was performed with Baker-flex silica gel IB2-F sheets. Elemental analyses were carried out at Midwest Microlab (Indianapolis, IN). Pyridine was distilled from potassium hydroxide and stored over fresh potassium hydroxide. Gel permeation chromatography was performed using Spectra/Gel 05 (Fisher; Itasca, IL; filtration range 300–2500 MW) and using water under N_2 pressure as eluent.

5,5'-[1,5-Naphthalenediylbis(sulfonylamino)]bis(2-hydroxy-3-sulfobenzoic acid) (3). 5-Amino-2-hydroxy-3-sulfobenzoic acid (0.466 g, 2 mmol), 1,5-naphthalenedisulfonyl chloride (0.455 g, 1.4 mmol), and $NaHCO_3$ (0.336 g, 4 mmol) were stirred together in deionized water (25 mL) at 40–45 °C for 18 h, after which time the reaction mixture was filtered and concentrated to 10 mL. The concentrated filterate was loaded onto a gel permeation column in two portions and was eluted using deionized water. Appropriate fractions were pooled and evaporated to yield a pale beige powder isolated as the tetrasodium salt (0.159 g, 10%). An analytically pure sample was prepared by twice precipitating the product from its concentrated methanolic solution by ether: mp 283–284 °C dec; IR 3455, 3114, 1639, 1585, 1454, 1201, 1033, 809 cm^{-1}; NMR δ 17.90 (s, 2H, exchangeable with D_2O), 9.83 (br s, 2H, exchangeable with D_2O), 8.99 (d, 2H, J = 8.7 Hz), 8.13 (d, 2H, J = 7.3 Hz), 7.75 (t, 2H, J = 8.1 Hz), 7.38 (d, 2H, J = 2.9 Hz), 7.28 (d, 2H, J = 3.0 Hz). Anal. ($C_{24}H_{14}N_2O_{16}S_4Na_4$) C, H, N.

5,5'-[2,4-Mesitylenediylbis(sulfonylamino)]bis(2-hydroxy-3-sulfobenzoic acid) (4). 5-Amino-2-hydroxy-3-sulfobenzoic acid (0.466 g, 2 mmol), 2,4-Mesitylenedisulfonyl chloride (0.444 g, 1.4 mmol), and $NaHCO_3$ (0.336 g, 4 mmol) were stirred in deionized water (20 mL) for 15 h at 45–50 °C. The reaction mixture was filtered and concentrated to 10 mL. The concentrated filterate was loaded onto a gel permeation column in two portions and was eluted using deionized water. Appropriate fractions were pooled and evaporated to yield a pale brown solid as the tetrasodium salt (0.243 g, 15%). It was subjected to another purification step using a short gel permeation column yielding a pale brown solid (0.138 g). An analytically pure sample was prepared by precipitating the product twice from its methanolic solution with ether: mp 265–267 °C dec; IR 3463, 3286, 1647, 1591, 1460, 1190, 1041 cm^{-1}; NMR δ 17.65 (br s, 2H, exchangeable with D_2O), 9.76 (s, 2H, exchangeable with D_2O), 7.43 (d, 2H, J = 2.94 Hz), 7.41 (d, 2H, J = 2.91 Hz), 7.11 (s, 1H), 2.89 (s, 3H), 2.41 (s, 6H). Anal. ($C_{23}H_{18}O_{16}S_4N_2Na_4 \cdot 1.5H_2O$) C, H, N.

5,5',5''-[1,3,5-Benzenetriyltris(sulfonylamino)]tris(2-hydroxy-3-sulfobenzoic acid) (5). 5-Amino-2-hydroxy-3-sulfobenzoic acid (0.932 g, 4 mmol), 1,3,5-benzenetrisulfonyl chloride (0.374 g, 1 mmol), and $NaHCO_3$ (0.672 g, 8 mmol) were stirred in deionized water (20 mL) at 45–50 °C for 16 h. The reaction was stopped and filtered. The filtrate was concentrated to *ca.* 15 mL and was loaded onto a gel permeation column in two portions. Elution with deionized water and evaporation of appropriate fractions afforded a green powder isolated as the hexasodium salt (0.332 g, 8%). Further purification was done by using a short gel permeation column in two batches. The solid obtained after evaporation of the appropriate fractions was dissolved in minimum volume of deionized water and precipitated by adding excess acetone (0.098 g). An analytically pure sample was prepared by twice reprecipitating the product from its concentrated methanolic solution with ether: mp 250–252 °C dec; IR 3421, 3408, 1655, 1587, 1458, 1198, 1043 cm^{-1}; NMR δ 17.96 (s, 3H, exchangeable with D_2O), 9.15 (br s, 3H, exchangeable with D_2O), 7.62 (s, 3H), 7.49 (d, 3H, J = 2.73 Hz), 7.43 (d, 3H, J = 2.64 Hz). Anal. ($C_{27}H_{15}O_{24}N_3S_6Na_6$) C, H, N.

4,4'-[1,2-Ethanediylbis(carbonylamino)]bis(1-naphthalenesulfonic acid) (7). 4-Amino-1-naphthalenesulfonic acid (0.300 g, 1.345 mmol) was heated to 100 °C for 15 min, succinyl chloride (0.12 g, 0.774 mmol) was added, and the mixture was stirred for another 15 min. Dry pyridine (3 mL) was added to this mixture and stirred for 5 h. Methanol (*ca.* 50 mL) was added into the mixture and filtered. The residue (*ca.* 0.200 g) was divided into three parts and loaded onto a short gel permeation column. Evaporation of pure fractions yielded a light brown powder (0.060 g, 16.9%): mp 306–307 °C dec; IR 3443, 3283, 1655, 1527, 1506, 1383, 1331, 1190, 1051, 761, 544 cm^{-1}; NMR δ 10.05 (s, 2H, exchangeable with D_2O), 8.87 (d, J = 8.6 Hz, 2H), 8.12 (d, J = 8.0 Hz, 2H), 7.92 (d, J = 7.7 Hz, 2H), 7.63 (d, J = 7.8 Hz, 2H), 7.52 (m, 4H), 2.89 (s, 4H). Anal. ($C_{24}H_{16}N_2O_8S_2Na_2 \cdot 2H_2O$) C, H, N.

4,4'-[1,6-Hexanediylbis(carbonylamino)]bis(1-naphthalenesulfonic acid) (8). 4-Amino-1-naphthalenesulfonic acid (6, 0.5 g, 2.2 mmol) and suberoyl chloride (0.3 g, 1.4 mmol) were heated to 120–125 °C. Dry pyridine (5 mL) was added and the mixture was refluxed for 4 h. Pyridine was evaporated, the solid was dissolved in methanol, and the methanolic solution was bubbled with ammonia gas for 10 s and evaporated to dryness. A further 30 mL of methanol was added to the solid and the mixture was stirred and filtered. The residue was dissolved in hot water (2 mL) and triturated with acetonitrile (50 mL) to yield the product (0.3 g, 21%). An analytical sample was prepared by recrystallizing the product one time from water: mp 347–348 °C dec; IR 3460, 3244, 3057, 2930, 2857, 1653, 1537, 1383, 1202, 1061, 853, 760, 692 cm^{-1}; NMR δ 9.90 (s, 2H, exchangeable with D_2O), 8.88 (m, 2H), 8.06 (m, 2H), 7.91 (d, 2H, J = 7.8 Hz), 7.60 (d, 2H, J = 7.7 Hz), 7.53 (m, 4H), 2.50 (m, 4H), 1.70 (m, 4H), 1.45 (br s, 4H). Anal. ($C_{28}H_{26}N_2O_8S_2Na_2 \cdot H_2O$) C, H, N.

4,4'-[1,8-Octanediylbis(carbonylamino)]bis(1-naphthalenesulfonic acid) (9). 4-Amino-1-naphthalenesulfonic acid (6, 0.5 g, 2.2 mmol) and octanedioyl dichloride (0.36 g, 1.5 mmol) were heated to a temperature of 120–125 °C. Dry pyridine (5 mL) was added and the mixture was refluxed for 3.5 h. The pyridine was evaporated and dissolved in methanol (30 mL). The methanolic solution was bubbled with ammonia gas for 10 s and evaporated to dryness. Another quantity of methanol (40 mL) was added to the solid and the mixture was filtered. The residue was dissolved in hot water (3 mL) and triturated with acetonitrile (50 mL) to yield the product (0.4 g, 27%). An analytical sample was prepared by recrystallizing the product one time from water: mp 359–360 °C dec; IR 3459, 3271, 2928, 2853, 1659, 1532, 1190, 1053, 760, 691 cm^{-1}; NMR δ 9.89 (s, 2H, exchangeable with D_2O), 8.87 (m, 2H), 8.04 (m, 2H), 7.9 (d, 2H,

J = 7.8 Hz), 7.59 (d, 2H, J = 7.6 Hz), 7.52 (m, 4H), 2.48 (m, 4H), 1.68 (m, 4H), 1.39 (m, 8H). Anal. ($C_{30}H_{30}O_6N_2S_2Na_2 \cdot 1.5H_2O$) C, H, N.

8,8'-[1,5-Pentanediylbis(carbonylamino)]bis(1,5-naphthalenedisulfonic acid) Tetrasodium Salt (11). 8-Amino-1,5-naphthalenedisulfonic acid monosodium salt (10, 0.70 g, 2.2 mmol) and pentanedioyl dichloride (0.30 g, 1.5 mmol) were heated to 120–125 °C. Dry pyridine (6 mL) was added and the mixture was refluxed for 20 h. The pyridine was evaporated and the solid mass was washed with chloroform (25 mL) and dissolved in methanol. The methanolic solution was bubbled with ammonia gas for 10 s and evaporated to dryness. The solid was washed with heptane (25 mL) and chloroform (30 mL). The residue was then dissolved in methanol (40 mL) and evaporated to dryness to yield a brown solid (0.8 g, 42%). Part of the solid (0.30 g) was dissolved in methanol (3 mL) and triturated with acetonitrile (75 mL). The separated solid was filtered and purified by gel permeation chromatography to yield a brown powder: mp 288–290 °C dec; IR 3454, 1945, 1649, 1535, 1404, 1319, 1192, 1040, 770 cm^{-1}; NMR δ 12.14 (s, 2H, exchangeable with D_2O), 9.05 (d, 2H, J = 8.5 Hz), 8.31 (d, 2H, J =7.3 Hz), 8.03 (d, 2H, J = 7.9), 7.97 (d, 2H, J = 7.8 Hz), 7.45 (t, 2H, J = 7.85), 2.44 (t, 4H, J = 7.0), 1.68 (m, 4H), 1.22 (m, 2H). Anal. ($C_{27}H_{22}N_2O_{14}S_4Na_4 \cdot 3H_2O$) C, H, N.

4,4'-[1,6-Hexanediylbis(carbonylamino)]bis(1,5-naphthalenedisulfonic acid) (12). 4-Amino-1,5-naphthalenedisulfonic acid monosodium salt (0.300 g, 0.93 mmol) was heated to 120 °C for 15 min, suberoyl chloride (0.15 mL, 0.83 mmol) was added, and the mixture was stirred for another 15 min. Dry pyridine (3 mL) was added to this mixture and the resulting mixture was stirred for 3 h and evaporated to dryness. The solid was dissolved in methanol (20 mL) and filtered, to yield a bright yellow solid which was passed through a short gel permeation column. Evaporation of the pure fractions yielded a pale yellow powder (0.097 g, 12.5%): mp 290 °C dec; IR 3462, 3287, 2940, 2859, 1647, 1535, 1404, 1318, 1190, 1042, 770, 612 cm^{-1}; NMR δ 12.14 (s, 2H, exchangeable with D_2O), 9.04 (d, J = 8.5 Hz, 2H), 8.30 (d, J = 6.5 Hz, 2H), 8.03 (d, J = 8.2 Hz, 2H), 7.96 (d, J = 8.55 Hz, 2H), 7.44 (t, J = 7.9 Hz, 2H), 2.42 (t, J = 6.9 Hz, 4H), 1.65 (s, 4H), 1.38 (s, 4H). Anal. ($C_{28}H_{26}N_2O_{14}S_4Na_2 \cdot 2.5H_2O$) C, H, N.

4,4'-[1,8-Octanediylbis(carbonylamino)]bis(1,5-naphthalenedisulfonic acid) (13). 4-Amino-1,5-naphthalenedisulfonic acid monosodium salt (0.26 g, 0.80 mmol) was heated to 120 °C for 15 min, sebacoyl chloride (0.1 mL, 0.51 mmol) was added, and the mixture was stirred for another 15 min. Dry pyridine (2 mL) was added and the reaction mixture was stirred further for 2 h. The reaction mixture was evaporated to dryness and the resulting solid was dissolved in methanol and filtered. The obtained yellow solid (0.16 g) was divided into three portions and loaded onto a short gel permeation column. Evaporation of pure fractions yielded the product (110 mg, 33.6%): mp 310–311 °C dec; IR 3460, 3302, 2922, 2853, 1647, 1528, 1468, 1404, 1319, 1194, 1043, 607 cm^{-1}; NMR δ 12.14 (s, 2H, exchangeable with D_2O), 9.04 (d, J = 8.6 Hz, 2H), 8.29 (d, J = 7.8 Hz, 2H), 8.02 (d, J = 8.3 Hz, 2H), 7.95 (d, J = 8.2 Hz, 2H), 7.43 (t, J = 8.3 Hz, 2H), 2.42 (t, J = 7.0 Hz, 4H), 1.66 (s, 4H), 1.36 (s, 8H). Anal. ($C_{30}H_{30}N_2O_{14}S_4Na_2 \cdot 2H_2O$) C, H, N.

8,8'-[1,10-Decanediylbis(carbonylamino)]bis(8-amino-1,5-naphthalenedisulfonic acid) Tetrasodium Salt (14). 8-Amino-1,5-naphthalenedisulfonic acid (10, 0.50 g, 1.5 mmol) and dodecanoyl chloride (0.27 g, 1.0 mmol) were heated to a temperature of 120–125 °C. Dry pyridine (5 mL) was added and refluxed for 3.75 h. The pyridine was evaporated and the solid mass was washed with chloroform (25 mL). The solid was then dissolved in methanol (30 mL) and bubbled with ammonia gas for 10 s. The solution was evaporated to dryness and the solid was washed with heptane (25 mL) and chloroform (30 mL). The residue was then dissolved with methanol (40 mL) and evaporated to dryness to yield a yellow solid as the desired product (0.6 g, 43%). An analytical sample was prepared by loading a portion of the product (0.050 g) onto a short gel permeation column to yield the product: mp 325–326 °C dec; IR 3459, 2926, 2853, 1653, 1535, 1319, 1190, 1042, 770, 611 cm^{-1}; NMR δ 12.13 (s, 2H, exchangeable with D_2O), 9.04 (d, 2H, J = 8.7 Hz), 8.30 (d, 2H, J = 6.7 Hz), 8.03 (d, 2H, J = 8.1 Hz), 7.97 (d, 2H, J = 8.1 Hz), 7.44 (t, 2H, J = 7.9 Hz), 2.39 (t, 4H, J = 7.3 Hz), 1.61 (m, 4H), 1.27 (m, 12H). Anal. ($C_{32}H_{32}N_2O_{14}S_4Na_4 \cdot 2H_2O$) C, H, N.

4,4'-[1,4-Benzenediylbis(carbonylamino)]bis(1,5-naphthalenedisulfonic acid) (15). 4-Amino-1,5-naphthalenedisulfonic acid monosodium salt (0.20 g, 0.62 mmol) was heated at 100–110 °C for 30 min. After this time, terephthaloyl chloride (0.06 g, 0.30 mmol) was added and stirring was continued for 15 min. Dry pyridine (3 mL) was added and the reaction mixture was further stirred for 24 h. The mixture was evaporated under vacuum to remove pyridine, washed with heptane (3 × 10 mL), dissolved in methanol (20 mL), and filtered. The filtrate was bubbled with ammonia gas and then evaporated to dryness to afford a yellow solid (0.150 g). The solid was dissolved in water (3 mL) and introduced onto a gel permeation column. Evaporation of pure fractions yielded a solid which was further purified by passing twice through a short gel permeation column. Evaporation of pure eluate yielded a pale yellow powder (0.030 g, 13%): mp 312–313 °C; IR 3462, 1655, 1535, 1400, 1339, 1190, 1039, 725, 605 cm^{-1}; NMR δ 12.75 (s, 2H, exchangeable with D_2O), 9.13 (d, J = 8.4 Hz, 2H), 8.33 (d, J = 7.5 Hz, 2H), 8.26 (s, 4H), 8.06 (m, 4H), 7.49 (t, J = 7.3 Hz, 2H), 2.39 (t, 4H, J = 7.3 Hz), 1.61 (m, 4H), 1.27 (m, 12H). Anal. ($C_{28}H_{16}N_2O_{14}S_4Na_2 \cdot 3H_2O$) C, H, N.

3,3'-[4,4'-Biphenyldiylbis(sulfonylamino)]bis(2,7-naphthalenedisulfonic acid) (18). 3-Amino-2,7-naphthalenedisulfonic acid monosodium salt (0.759 g, 2 mmol), 4,4'-biphenyldisulfonyl chloride (0.490 g, 1.4 mmol), and $NaHCO_3$ (0.336 g, 4 mmol) were stirred in deionized water (20 mL) at 40–45 °C for 10 h. Since thin-layer chromatography of the mixture showed the presence of parent sulfonic acid, additional disulfonyl chloride (0.070 g, 0.2 mmol) was added and stirring was continued. After 24 h, disulfonyl chloride (0.140 g, 0.4 mmol) was again added and the reaction was further stirred for 2 days. The reaction mixture was filtered and concentrated to *ca.* 10 mL and was loaded onto a gel permeation column in two portions. Elution with deionized water and evaporation of pure fractions afforded a pale brown powder isolated as a tetrasodium salt (0.087 g, 5%). An analytically pure sample was prepared by precipitating the product twice from its concentrated methanolic solution was ether: mp 317–319 °C dec; IR 3480, 1637, 1443, 1371, 1199, 1041, 862 cm^{-1}; NMR δ 10.53 (br s, 2H, exchangeable with D_2O), 8.15 (d, 2H, J = 5.94 Hz), 8.03 (s, 2H), 7.99 (d, 4H, J = 9.87 Hz), 7.72 (m, 10H). Anal. ($C_{32}H_{20}O_{16}N_2S_8Na_4 \cdot 2H_2O$) C, H, N.

2,2'-[4,4'-Biphenyldiylbis(sulfonyloxy)]bis(3,6-naphthalenedisulfonic acid) (19). 2-Hydroxy-3,6-naphthalenedisulfonic acid (0.608 g, 2 mmol), 4,4'-biphenyldisulfonyl chloride (0.492 g, 1.4 mmol), and $NaHCO_3$ (0.336 g, 4 mmol) were stirred in deionized water (25 mL) at 45–50 °C for 18 h. Since thin-layer chromatography of the reaction mixture showed the presence of parent sulfonic acid, an additional quantity of 4,4'-biphenyldisulfonyl chloride (0.105 g, 0.3 mmol) was added to the reaction mixture and it was stirred. After stirring for another 72 h, more 4,4'-biphenyldisulfonyl chloride (0.105 g, 0.3 mmol) and $NaHCO_3$ (0.168 g, 2 mmol) were added, and the reaction mixture was stirred for another 48 h, after which time the reaction mixture was filtered and concentrated. The concentrated aqueous solution was divided into two portions and was loaded onto a long gel permeation column. Elution by deionized water and evaporation of appropriate fractions afforded a thin aqueous film which was triturated with acetone (20 mL) to yield a pale yellow solid. The solid was further evaporated to dryness with acetone (5 × 5 mL). The resulting product was precipitated twice by adding excess ether to the concentrated methanolic solution to afford a pale yellow powder isolated as the disodium salt (0.128 g, 7%). Further purification was performed by using a short gel permeation column and ether precipitation of the concentrated methanolic solution (81 mg): mp 310–312 °C dec; IR 3466, 1593, 1445, 1372, 1103, 1038, 864 cm^{-1}; NMR δ 8.35 (d, 6H, J = 8.28 Hz), 8.14 (s, 2H), 8.03 (d, 4H, J = 8.55 Hz), 7.99 (s, 2H), 7.96 (d, 2H, J = 8.73 Hz), 7.77 (d, 2H, J = 9.30 Hz). Anal. ($C_{32}H_{20}O_{18}S_6Na_2 \cdot 4H_2O$) C, H.

7,7'-[1,5-Naphthalenediylbis(sulfonylamino)]bis(1,3-naphthalenedisulfonic acid) (22). 7-Amino-1,3-naphthalenedisulfonic acid monopotassium salt (tech., 0.683 g, 2 mmol), 1,5-naphthalenedisulfonyl chloride (0.455 g, 1.4 mmol), and $NaHCO_3$ (0.336 g, 4 mmol) were stirred in deionized water (20 mL) at

40–45 °C for 9 h. Since thin-layer chromatography showed the presence of parent sulfonic acid, additional disulfonyl chloride (0.098 g, 0.3 mmol) and $NaHCO_3$ (0.084 g, 1 mmol) were added and the reaction was stirred for another 30 h. The reaction mixture was filtered and concentrated to *ca.* 10 mL and was loaded onto a gel permeation column in two portions. Elution with deionized water and evaporation of appropriate fractions yielded a beige powder isolated as the tetrasodium salt (0.289 g, 15%). An analytically pure sample was prepared by twice precipitating the product from the concentrated methanolic solution with ether: mp 292–294 °C dec; IR 3472, 3108, 1628, 1510, 1336, 1201, 1054, 1026, 663 cm^{-1}; NMR δ 10.87 (br s, 2H, exchangable with D_2O), 9.06 (d, 2H, J = 8.6 Hz), 8.61 (s, 2H), 8.50 (d, 2H, J = 7.3 Hz), 8.17 (s, 2H), 7.90 (s, 2H), 7.77 (t, 2H, J = 8.1 Hz), 7.68 (d, 2H, J = 8.9 Hz), 7.08 (d, 2H, J = 8.8 Hz). Anal. ($C_{30}H_{18}O_{16}N_2S_6Na_4 \cdot 5H_2O$) C, H, N.

2,2′-[4,4′-Biphenyldiylbis(sulfonyloxy)]bis(8-naphthalenesulfonic acid) (24). A mixture of 2-hydroxy-8-naphthalenesulfonic acid monosodium salt (0.800 g, 3.33 mmol), 4,4′-biphenyldisulfonyl chloride (0.300 g, 0.85 mmol), and $NaHCO_3$ (0.800 g, 9.52 mmol) in water (20 mL) was stirred at 45–50 °C for 48 h. A further quantity of 4,4′-biphenyldisulfonyl chloride (0.300 g, 0.85 mmol) was added during the course of the reaction time. The resulting white solid that formed in the orange reaction mixture was filtered and dissolved in hot methanol (20 mL). The methanolic solution was evaporated to dryness and the obtained white solid was recrystallized two times by adding the solid to hot water (7 mL) followed by dropwise addition of ethanol up to the cloud point. The resulting white powder (0.224 g, 17.9%) was isolated as the disodium salt: mp 268–269 °C dec; IR 3458, 3096, 1626, 1595, 1506, 1441, 1365, 1184, 1095, 1049, 989, 914, 837, 729, 658, 619 cm^{-1}; NMR δ 8.67 (d, J = 2.3 Hz, 2H), 8.05 (s, 8H), 7.96 (m, 6H), 7.50 (t, J = 7.7 Hz, 2H), 7.16 (d, J = 9.0 Hz, 2H). Anal. ($C_{32}H_{20}O_{12}S_4Na_2 \cdot 1.5H_2O$) C, H.

2,2′-[4,4′-Biphenyldiylbis(sulfonyloxy)]bis(6,8-naphthalenedisulfonic acid) (26). A mixture of 2-hydroxy-6,8-naphthalenedisulfonic acid (0.500 g, 1.64 mmol), 4,4′-biphenyldisulfonyl chloride (0.300 g, 0.85 mmol), and $NaHCO_3$ (0.800 g, 9.52 mmol) in water (20 mL) was stirred at 45–50 °C for 48 h. A further quantity of 4,4′-biphenyldisulfonyl chloride (0.100 g, 0.28 mmol) was added during the course of the reaction time. The resulting mixture was filtered and the filtrate was evaporated to dryness to yield a brown residue (1.85 g). A portion of this residue (0.300 g) was loaded onto a gel permeation column. Evaporation of pure fractions yielded the product which was further purified by passing througyh a short gel permeation column two times to afford a pale brown powder as the tetrasodium salt (0.120 g, 16.5%): mp 305–306 °C; IR 3464, 3134, 1624, 1500, 1365, 1194, 1136, 1041, 928, 845, 656, 615 cm^{-1}; NMR δ 8.65 (s, 2H), 8.27 (s, 2H), 8.13 (s, 2H), 8.05 (br s, 8H), 8.01 (m, 2H), 7.74 (s, 2H), 7.16 (d, J = 8.7 Hz, 2H). Anal. ($C_{32}H_{18}O_{18}S_6Na_4 \cdot 3H_2O$) C, H.

2,2′-[1,5-Naphthalenediylbis(sulfonyloxy)]bis(8-naphthalenesulfonic acid) (27). A mixture of 2-naphthanol-8-sulfonic acid monosodium salt (0.60 g, 2.50 mmol), 1,5-naphthalenedisulfonyl chloride (0.20 g, 0.62 mmol), and $NaHCO_3$ (0.60 g, 7.14 mmol) in water (20 ml) was stirred at 45–50 °C for 72 h. A further quantity of 1,5-naphthalenedisulfonyl chloride (0.20 g, 0.62 mmol) was added during the course of the reaction time. The resulting solid that formed in the pale brown reaction mixture was filtered, dissolved in a hot methanol–water mixture (8:2), and evaporated to dryness. The obtained white solid was recrystallized two times from methanol–water (8:2) to yield the product as a white powder (190 mg, 20.9%): mp >355 °C; IR 3439, 3069, 2363, 1626, 1504, 1443, 1364, 1204, 1053, 989, 920, 845, 696, 623, 582, 528 cm^{-1}; NMR δ 9.15 (d, J = 8.6 Hz, 2H), 8.69 (d, J = 2.4 Hz, 2H), 8.38 (d, J = 7.5 Hz, 2H), 8.04 (t, J = 8.1 Hz, 2H), 7.97 (d, J = 7.3 Hz, 2H), 7.87 (d, J = 9.0 Hz, 4H), 7.48 (t, J = 7.6 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H). Anal. ($C_{30}H_{18}O_{12}S_4Na_2 \cdot H_2O$) C, H.

2,2′-[1,5-Naphthalenediylbis(sulfonyloxy)]bis(6,8-naphthalenedisulfonic acid) (28). A mixture of 2-hydroxynaphthalene-6,8-disulfonic acid (0.500 g, 1.64 mmol), 1,5-naphthalenedisulfonyl chloride (0.200 g, 0.62 mmol), and $NaHCO_3$ (0.600 g, 7.14 mmol) in water (20 mL) was stirred at 45–50 °C for 48 h. A further quantity of 1,5-naphthalenedisulfonyl chloride (0.200 g, 0.62 mmol) was added during the course of the reaction time. The resulting mixture was filtered and the filtrate evaporated to dryness. Hot methanol (50 mL) was added to the residue and the solution filtered. The filtrate was evaporated to dryness to yield the crude product (1.60 g). A portion of the product (0.250 g) was passed through a long gel permeation column. Pure fractions were collected, evaporated to dryness, and further purified by passing twice through a short gel permeation column. Evaporation of the pure fractions yielded a pale brown product as the tetrasodium salt (0.150 g, 19.2%): mp 310–311 °C dec; IR 3453, 3107, 1626, 1502, 1371, 1204, 1134, 1041, 928, 849, 796, 658 cm^{-1}; NMR δ 9.16 (d, J = 8.8 Hz, 2H), 8.68 (d, J = 2.2 Hz, 2H), 8.41 (d, J = 7.7 Hz, 2H), 8.26 (d, J = 1.6 Hz, 2H), 8.10 (s, 2H), 8.05 (t, J = 8.2 Hz, 2H), 7.97 (d, J = 9.2 Hz, 2H), 6.96 (d, J = 9.0 Hz, 2H). Anal. ($C_{30}H_{16}O_{18}S_6Na_4 \cdot H_2O$) C, H.

4,4′-[1,6-Hexanediylbis(carbonylamino)]bis(5-hydroxy-2,7-naphthalenedisulfonic acid) (33). 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid disodium salt (0.341 g, 1 mmol) in dry pyridine (1 mL) was heated to a temperature of 130–140 °C, after which time suberoyl chloride (0.11 mL, 0.6 mmol) was added to the reaction mixture and heated under reflux for 2 h. The resulting mixture was stirred with heptane (20 mL) and filtered. The residue was dissolved in methanol (10 mL) and filtered and the filtrate was triturated with ether (50 mL). The separated solid was filtered and dissolved in deionized water (5 mL) and was introduced onto a gel permeation column. The column was eluted with water, and appropriate fractions were concentrated. This concentrated aqueous solution was triturated with acetone, which after filtration afforded a light brown powder (0.342 g, 42%) as the tetrasodium salt: mp 315–317 °C dec; IR 3458, 2932, 1657, 1626, 1554, 1390, 1188 cm^{-1}; NMR δ 8.73 (s, 2H), 7.67 (s, 2H), 7.48 (s, 2H), 7.18 (s, 2H), 2.38 (t, J = 7.2 Hz, 4H), 1.69 (m, 4H), 1.41 (br s, 4H). Anal. ($C_{28}H_{24}N_2O_{16}S_4Na_4 \cdot 3H_2O$) C, H, N.

Antiviral Assay Procedures. Cytopathogenesis Assay. Activity of the compounds against the replication of HIV-1 was determined on the basis of the inhibition of virus-induced cytopathogenicity in MT-4 cells, as previously described.[16] Briefly, MT-4 cells were infected with either $HTLV\text{-}III_B$ (a laboratory strain of HIV-1) or $HIV\text{-}1_{HE}$ (a clinical isolate of HIV-1[19]) at a multiplicity of infection of 0.02 and incubated in the presence of various concentrations of test compounds. After a 4-day incubation, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) method, as previously described.[18]

Peripheral Blood Lymphocyte Assay. Phytohemagglutinin-stimulated peripheral blood lymphocytes (PBL) (1×10^5 cells per mL) were infected with HIV-1 (HE strain) at a multiplicity of infection of 0.2. After virus adsorption for 2 h, the cells were extensively washed to remove unabsorbed virus particles and cultured in the presence of varying concentration of the test compounds. On day 4 after virus infection, the cells were subcultured at a ratio of 1:5 with fresh culture medium containing appropriate concentrations of the compounds. The amount of HIV-1 p24 antigen in the culture supernatant was determined on day 8 using a sandwich ELISA kit (Abbott). Cytotoxicity of the compounds was evaluated in parallel with their anti-HIV-1 activity. It was based on the viability of mock-infected cells as determined by the MTT method.

Giant Cell Formation Assay. The giant cell formation assay was carried out according to an established protocol.[20] MOLT-4 cells (5×10^4/well) were cultured with an equal number of MOLT-4/$HTLV\text{-}III_B$ cells in a microtiter tray containing various concentrations of the test compounds. After a 24 h cocultivation period, the cell clusters were broken by gentle pipetting, and the number of giant cells was determined microscopically.

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