Potential Anti-AIDS Naphthalenesulfonic Acid Derivatives. Synthesis and Inhibition of HIV-1 Induced Cytopathogenesis and HIV-1 and HIV-2 Reverse Transcriptase Activities†

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Several naphthalenedi- and trisulfonic acids have been synthesized and evaluated for inhibitory potential against cytopathogenesis and purified recombinant human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) reverse transcriptase (RT). The most potent derivative that emerged from the anti-RT study was a small molecule **6** (MW = 840), a dipalmitoylated derivative of 2,7-naphthalenedisulfonic acid. Analog **6** demonstrated 50% inhibitory concentration (IC_{50}) values of 2.42 and 0.86 μM for HIV-1 and HIV-2 RT, respectively. The second most active compound was also a derivative of the same naphthalenedisulfonic acid but contained only one palmitoyl moiety. This compound **9** displayed IC_{50} values of 4.8 and 3.7 μM for HIV-1 and HIV-2 RT, respectively. Both analogs **6** and **9** are active at noncytotoxic doses, exhibit slightly higher potencies for the RT of HIV-2 over HIV-1, and demonstrate activities superior to the hexasulfonic acid derivative suramin (IC_{50} values of 9.4 and 15.5 μM for HIV-1 and HIV-2 RT, respectively). In the cytopathogenesis assay, the most active compound is a bis naphthalenedisulfonic acid derivative **17**, containing a flexible octamethylene spacer and exhibiting an in vitro therapeutic index of 29.7. Most striking, however, is the influence of the palmitoyl functionality in the naphthalenedisulfonic acid series to confer activity against both HIV-1 and HIV-2 RT.

The human immunodeficiency virus (HIV) reverse transcriptase (RT) is a viral encoded enzyme that is vital for viral replication. The RT from the AIDS virus type 1 (HIV-1) is a heterodimer consisting of two chains of 66 and 51 kD,[1] whereas the RT from the AIDS virus type 2(HIV-2) has two comparable chains of 68 and 55 kD.[2] Both RTs share a high degree of homology in their amino acid sequences, and possess RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, and ribonuclease H (RNase H) activities. However, they demonstrate differences in their K_m values for the DNA polymerizing activity, the RNase activity and other properties.[3] The crystal[4] and solution[5] secondary structures of the RNase H domain of HIV-1 have been elucidated, and considerable progress has been realized in obtaining the crystal structure of the intact protein.[6,7]

In spite of the lack of complete conformational data on both RTs, a myriad of HIV RT inhibitors are available. These include a wide array of nucleoside and non-nucleoside compounds, antisense oligonucleotides, and both small and large anionic molecules.[8] The most well-known and intensively studied HIV RT inhibitors are the nucleoside derivatives.[9] Three representatives of this group, 3′-azido-2′,3′-dideoxythymidine (AZT), 2′,3′-dideoxyinosine (DDI) and 2′,3′-dideoxycytidine (DDC) are the only federally licensed antivirals for the large-scale treat-

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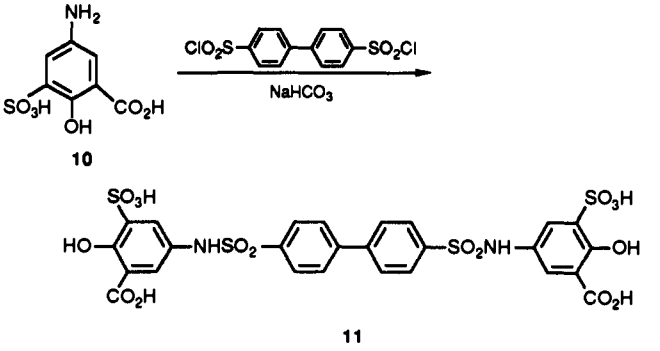
ment of AIDS and AIDS related complex (ARC). However, the demonstrated clinical toxicities of these agents and other members of this class, the established emergence of resistance to these agents,[10] and their inability to cure AIDS have propelled the search for different classes of RT inhibitors. These investigations have led to the discovery of many compounds, of both natural and synthetic origin, having diverse chemical structures and exhibiting highly potent and selective activity against RT. Unique amongst these are the agents belonging to the TIBO,[11] HEPT,[12] pyridinone,[13] Nevirapine,[14] and BHAP[15] classes. These derivatives are active against HIV-1 replication, interact with HIV-1 RT, but are inactive in the assays that measure inhibition of HIV-2 RT activity. It has been determined that many of these compounds bind to a site on HIV-1 RT that is distinct from the binding site of the anti-HIV-1 nucleosides.[16,17] In addition, it is remarkable that some of these chemically diverse agents bind to the same site on HIV-1 RT.[13] However, in vitro studies have revealed HIV-1 resistance to members of the pyridinone, TIBO, and Nevirapine classes of compounds.[18] We have evaluated a diverse group of natural products as inhibitors of HIV-1[19] and HIV-2[20] RT. As a result, certain structural features required for inhibition have been defined. Recently, the natural product psychotrine and its methyl ether have also demonstrated selective inhibition of HIV-1 RT with significantly less activity against other bacterial or mammalian DNA and RNA polymerases

Scheme I



and no effect on in vitro protein synthesis.[21] Interestingly, these alkaloids are approximately 4-fold more active with HIV-2 RT as compared with HIV-1 RT.[20]

In our continuing efforts to search for novel HIV inhibitors, we have evaluated a wide spectrum of synthetic naphthalenesulfonic acid derivatives for inhibitory activity against the purified recombinant RTs of both HIV-1 and HIV-2. The sulfonic acid class of derivatives were chosen for evaluation due to previously reported anti-HIV-1 RT activity of certain analogs of this family of compounds[22,23] and the known nonmutagenicity[24] of many parent naphthalenesulfonic acid molecules. In this report we describe the anti-RT activity of the most active compounds from a collection of over 40 small molecule sulfonic acid derivatives.

Chemistry

5-Amino-2-hydroxy-3-sulfobenzoic acid (**10**) when treated 4,4'-biphenyldisulfonyl chloride afforded the bis derivative **11** (Scheme I). Several substrates reacted with starting material **12** in pyridine to generate many target compounds. Reaction with benzoyl chloride yielded **14**, with glutaryl dichloride produced **15**, and with acetic anhydride at room temperature afforded the diacetylated derivative **13**. If the temperature of the acetic anhydride reaction was raised to 120–130 °C, the monoacetylated derivative **8**[23] was obtained. Similarly, treatment of **12** with sebacoyl dichloride at ambient temperature gave compound **16**, but at high temperature yielded the bis analog **17** (Scheme II). As previously documented, exclusive N-acylation is observed.[25] Unlike for the bis derivatives, the NMR of structres **15** and **16** did not show the characteristic downfield resonances for the amide, carboxylic, and phenol functionalities.[25] Negative ion fast atom bombardment mass spectrometry indicated the loss of carbon dioxide and confirmed the presence of the carboxylic group. The position of acylation was established

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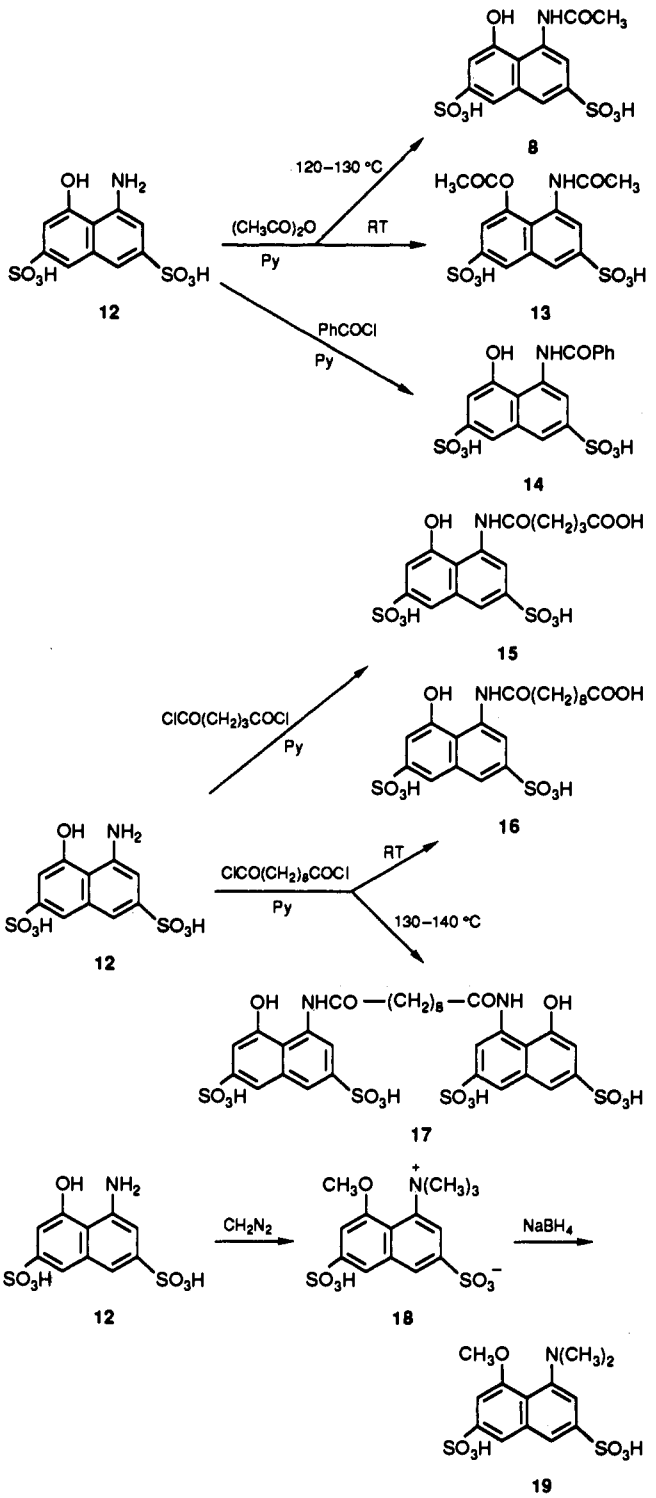
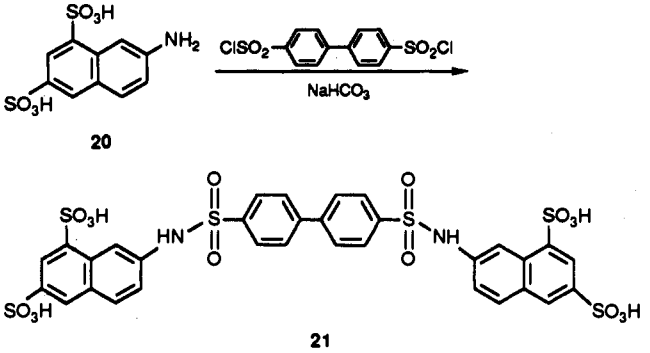
Scheme II

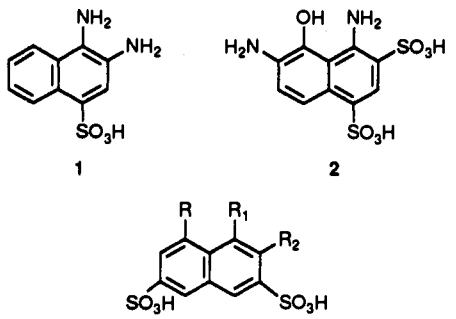


by individually treating compounds **15** and **16** with diazomethane. The appearance of an aromatic methoxy singlet and a downfield aromatic amide peak in the NMR spectrum of the products established the acylation position to be at the amino moiety.

In a separate reaction scheme, starting material **12** reacted with diazomethane to generate the quaternary amine ether derivative **18**, which when subjected to sodium borohydride[26] yielded the tertiary amine analog **19** (Scheme II). 7-Amino-1,3-naphthalenedisulfonic acid (**20**) on reaction with 4,4′-biphenyldisulfonyl chloride and sodium bicarbonate produced derivative **21** (Scheme III). The starting material for the trisulfonic acid series was 8-amino-1,3,6-naphthalenetrisulfonic acid (**22**) which in the presence of pyridine produced **23** with acetic anhydride and **24** with palmitoyl chloride. Reaction of **22** with 4,4′-biphenyldisulfonyl chloride and sodium bicarbonate produced the 1:2 condensation product, the bis derivative **26**. In this reaction we were also able to detect and isolate the 1:1 condensation product **25**. Treatment of **22** with dodecanedioyl dichloride and pyridine produced the bis compound **27** (Scheme IV). The syntheses of derivatives **1, 2, 4–9** have been previously reported.[23,25] Analogous to our previous work, most derivatives were isolated as sodium salts with varying amounts of water of crystallization.[23,25] A major hurdle in the synthetic arm of this investigation has been the isolation of the target compounds. Very often, the target compounds were isolated as highly hygroscopic products that were semisolid and presented difficulties at the stages of workup, separation, purification, and crystallization.

Scheme III





3: R = R$_1$ = OH; R$_2$ = H
4: R = R$_1$ = OH; R$_2$ = NH$_2$
5: R = R$_1$ = OCOCH$_3$; R$_2$ = H
6: R = R$_1$ = OCO(CH$_2$)$_{14}$CH$_3$; R$_2$ = H
7: R = R$_1$ = OCOCH$_3$; R$_2$ = N$_2$Ph
8: R = OH; R$_1$ = NHCOCH$_3$; R$_2$ = H
9: R = OH; R$_1$ = NHCO(CH$_2$)$_{14}$CH$_3$; R$_2$ = H

Results

Inhibition of HIV-1 and HIV-2 RT by treatment with the test compounds synthesized in this study is summarized in Table I. The most active anti-RT inhibitor that emerged from this study was a diplamitoyl disulfonic acid derivative **6**, which demonstrated 50% inhibitory concentration (IC_{50}) values of 2.4 and 0.86 μM for HIV-1 and HIV-2, respectively. This activity was superior to that of suramin (IC_{50} values of 9 and 15 μM for HIV-1 and HIV-2 RT, respectively). However, the parent molecule **3** of derivative **6** showed corresponding IC_{50} values of 125 and

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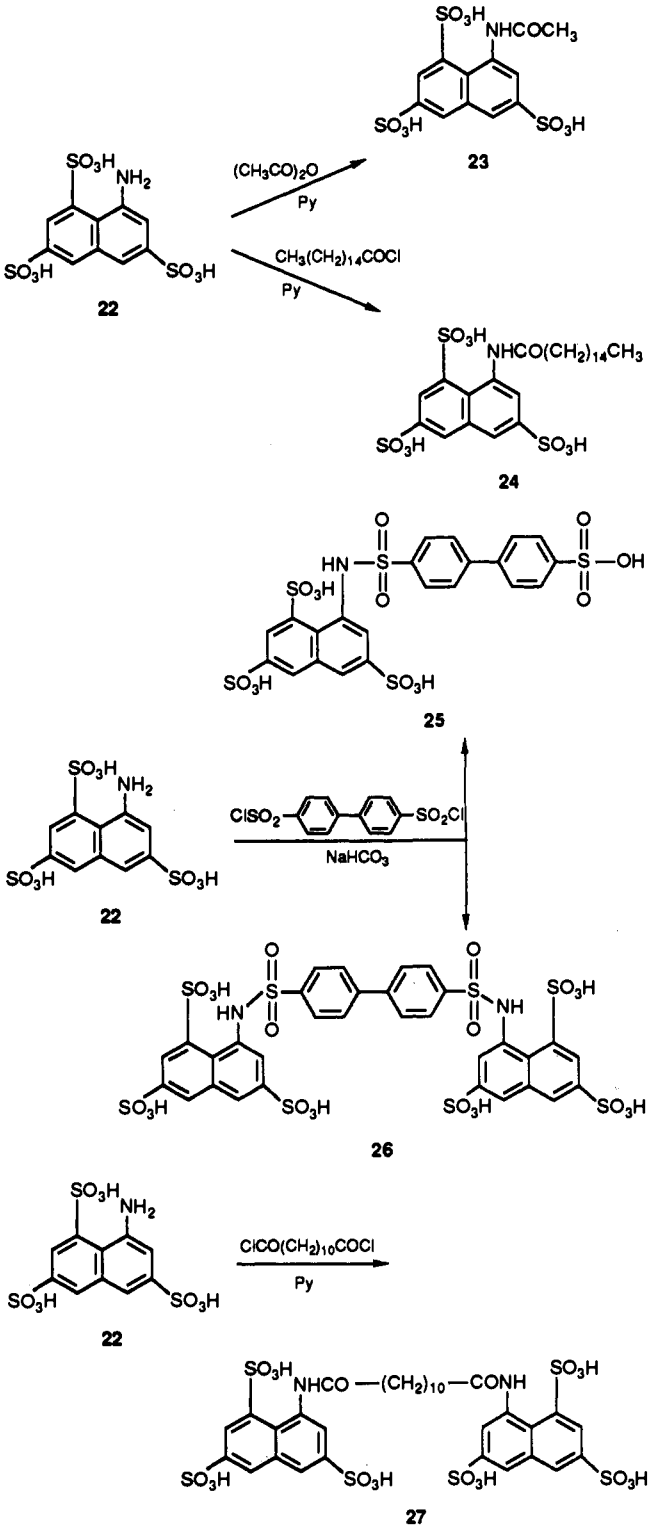
Scheme IV



201 μM for HIV-1 and HIV-2 RT, respectively, suggesting that the two free phenolic functionalities were not required to mediate an inhibitory response. To determine if esterification of the phenolic groups alone was sufficient to potentiate activity in the dihydroxy derivative **3**, we prepared the diacetyl derivative **5**. Evaluation of **5** revealed inactivity against the RT's of HIV-1 (15.7% inhibition at 1116 μM) and HIV-2 (0–10 % inhibition at 1116 μM), indicating a potentiating role played by the long-chain palmitoyl groups in derivative **6**.

The second most active analog was the *N*-palmitoyl derivative **9** (IC_{50} values of 4.8 and 3.7 μM for HIV-1 and HIV-2, respectively). The parent compound of **9**, the 4-amino-5-hydroxy derivative **12**, was inactive in both RT assays (inhibition values at 1392 μM: 24.7 and 0–10 % for HIV-1 and HIV-2, respectively), while the *N*-acetyl analog **8** (IC_{50} values of 243 and 584 μM for HIV-1 and HIV-2, respectively) and the *N*-benzoyl derivative **14** (IC_{50} values of 464 and 721 μM for HIV-1 and HIV-2, respectively) demonstrated low activity. The diacetyl analog **13** was inactive (inhibition values at 1116 μM: 0–10 and 0% for HIV-1 and HIV-2, respectively). In the naphthalenetrisulfonic acid class, the most potent analog was a simple N-palmitoylated derivative **24** which exhibited the same potencies for HIV-1 (IC_{50} = 55 μM) and HIV-2 (IC_{50} = 55 μM) RT.

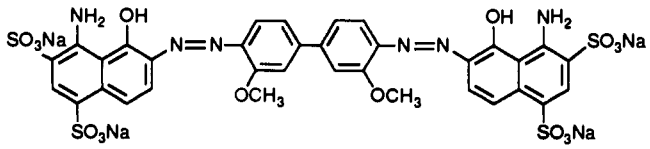
The amide carboxyl derivatives **15** and **16** showed a different activity profile. The trimethylene derivative **15** was inactive (inhibition values at 1984 μM: 26.8 and 17.5% for HIV-1 and HIV-2, respectively), while the octamethylene derivative **16** showed better activity against HIV-1 (IC_{50} = 135 μM) than HIV-2 (IC_{50} = 170 μM). Between derivatives **15** and **16**, it seems that a longer spacer between the amide and carboxyl moiety is favored for higher potency. Since all the active compounds in the 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid (**12**) series had both the phenolic and amide hydrogens free, we were eager to prepare simple derivatives that had substituents in place of these hydrogen atoms. Evaluation of derivatives **18** (0–10 % inhibition for HIV-1 RT at 2518 μM and 0–10 % inhibition for HIV-2 RT at 1259 μM) and **19** (inhibition values at 1275.5 μM: 0–10 and 0% for HIV-1 and HIV-2, respectively) revealed no activity.

Table I. Inhibitory Effects of Naphthalenesulfonic Acid Derivatives on Purified Recombinant HIV-1 and HIV-2 Reverse Transcriptase

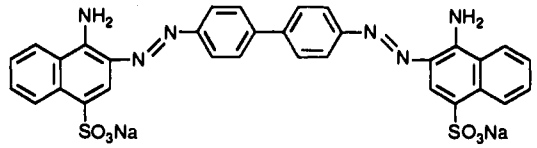
compd	IC_{50} (μM)[g] HIV-1	HIV-2
1	50	28
2	45	48
3[b]	125	201
4[a]	162	452
5[b]	>1000	>1000
6[b]	2.4	0.86
7[a]	>1000	>1000
8[a]	243	584
9[a]	4.8	3.7
10	nd[f]	nd
11[d]	427	478
12[a]	>1000	>1000
13[b]	>1000	>1000
14[b]	464	721
15[b]	>1000	>1000
16[c]	135	170
17[d]	52	122
18[a]	>1000	>1000
19[a]	>1000	>1000
20[b]	>1000	>1000
21[d]	125	374
22[c]	>1000	>1000
23[c]	>1000	>1000
24[c]	55	55
25[d]	>1000	>1000
26[e]	>849	>849
27[e]	136	214
suramin	9.4	15

[a] Monosodium salt. [b] Disodium salt. [c] Trisodium salt. [d] Tetrasodium salt. [e] Hexasodium salt. [f] nd = not determined. [g] Previous studies have demonstrated different IC_{50} values for **8**, **9**, and suramin.[19,20] This may be explained on the basis of the different assay conditions that were used in the respective studies. IC_{50} = 50% inhibitory antiviral concentration.

Amongst the monosulfonic acid derivatives, analog 1, the potential in vivo reduction product of the anti-HIV-1 dye Congo Red,[27] showed higher activity in the HIV-2 assay (IC_{50} = 28 μM) as compared to the HIV-1 assay (IC_{50} = 50 μM). A bis spacer derivative 11 containing sulfonic, carboxylic, and phenolic functionalities on the terminal phenyl groups was much less active (IC_{50} values of 427 and 478 μM for HIV-1 and HIV-2, respectively). A potential in vivo reduction product 2 of another anti-HIV-1 dye, Chicago Sky Blue 6B (or Evans Blue),[27] showed similar potencies in the HIV-1 (IC_{50} = 45 μM) and HIV-2 (IC_{50} = 48 μM) assays. The rationale to delete the spacers present in the anti-HIV-1 diazo dyes[27] has been previously addressed.[25] The diacetylated derivative 7 (IC_{50} values of >1 mM for both RTs) of the monoazo dye Chromotrope 2R was inactive. However, the reduction product 4 of the same dye demonstrated better inhibition properites against the RT of HIV-1 (IC_{50} = 162 μM) than HIV-2 (IC_{50} = 452 μM).



Chicago Sky Blue 6B

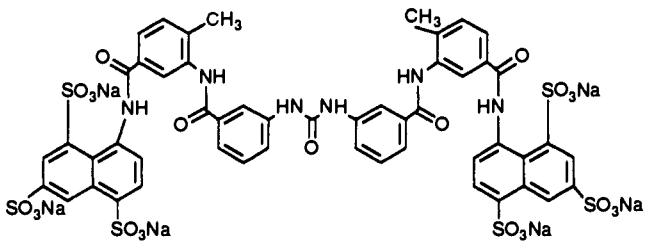


Congo Red

Since earlier studies had exhibited high anti-RT potencies in bis naphthalenedisulfonic acid derivatives,[22] we considered the synthesis of additional analogs in this series. The flexible octamethylene spacer derivative 17 (IC_{50} values of 52 and 122 μM for HIV-1 and HIV-2, respectively) was over 2-fold more potent as the biphenyl spacer derivative 21 (IC_{50} values of 125 and 374 μM for HIV-1 and HIV-2, respectively). The naphthalenedisulfonic acid unit 20 (11 and 13.6% inhibition at 1369 μM for HIV-1 and HIV-2, respectively), a potential in vivo hydrolysis product of the bis derivative 21, was inactive.

The parent trisulfonic acid compound 22 (0–10% inhibition at 1103.8 μM for both HIV-1 and HIV-2) or its acetyl derivative 23 (0–10% inhibition at 1018 μM for both HIV-1 and HIV-2) are both inactive. Derivative 25 demonstrated slight or no activity (44.3 and 0–10% inhibition at 622.2 μM for both HIV-1 and HIV-2 RT). Amongst the bis naphthalenetrisulfonic acid derivatives, the flexible decamethylene spacer derivative 27 (IC_{50} values of 136 and 214 μM for HIV-1 and HIV-2 RT, respectively) was more active than the biphenyl spacer derivative 26 (IC_{50} > 849 μM for both RTs).

Amongst the other active analogs, the most active compound in the cytopathogenesis assay is the bis naphthalenedisulfonic acid derivative 17 containing a flexible octamethylene spacer. The activity of compound 17 (EC_{50} = 9.7 μM) is similar to that of suramin (EC_{50} = 8.2 μM)



suramin

and exhibits an in vitro therapeutic index of 29.7 (ratio of CC_{50} to EC_{50}, Table II). The bis naphthalenedisulfonic acid derivative 21 (EC_{50} = 29.5 μM) was the next most active compound in this assay. The other naphthalenedisulfonic acid derivative that demonstrates activity at nontoxic concentrations is derivative 16 (EC_{50} = 134.5 μM), which contains the octamethylene spacer but is not a bis compound. Comparison of the activity of analogs 17 (EC_{50} = 9.7 μM) and 16 (EC_{50} = 134.5 μM) indicates the need for a bis naphthalenedisulfonic acid arrangement for higher potency. It should be noted that if the octamethylene chain in 16 is replaced with a trimethylene chain to produce analog 15, activity is observed only at toxic doses (Table II). The activity of 8 and 9 has been previously discussed.[23]

Discussion

The fact that the bis naphthalenetrisulfonic acid derivative suramin is a well-known potent inhibitor[28] of HIV-1 RT provided the impetus to investigate the anti-RT potential in other derivatives of this class. In general, it may be assumed that the negatively charged sulfonic acid moieties interact with positively charged sites on the enzyme. The high potencies of the palmitoyl derivatives 6 and 9 clearly indicate a governing role for the palmitoyl functionality. One explanation for the potency of these active derivatives may be their potential to chelate with metal ions. For example, it is known that HIV-1 RT has a Mg^{2+} preference for optimum activity.[29] The association of naphthalenesulfonic acid compounds containing chelating functionalities and their link with the ability to inhibit RT has been previously addressed by us.[22,25,30] However, the influential role of the lipophilic palmitoyl functionality remains unexplained at the present time.

The present results in both the RT and cytopathogenesis assays indicate that the naphthalenedisulfonic acid series should be considered for further structural modification in terms of future development. Due to the unlikely entry of these agents into cells, the activity of derivative 17, the most potent analog in the cytopathogenesis assay, is probably due to inhibition of virus adsorption as established for various anionic derivatives.[8] Indeed, we have confirmed this mode of action for other analogs of 17 which have shown inhibition of viral adsorption by specifically

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Table II. Inhibitory Effects of Naphthalenesulfonic Acid Derivatives on HIV-1 Induced Cytopathogenesis in MT-4 Cells

compd	EC_{50}[g,h] (μM)	CC_{50}[g,i] (μM)
1	>189	189
2	>7.7	7.7
3[b]	>1175	1175
4[a]	>12	12
5[b]	>461	461
6[b]	>15	15
7[a]	>500	>500
8[a]	53	402
9[a]	37	250
10	nd[f]	nd
11[d]	52.5	247
12[a]	>529	529
13[b]	>500	>500
14[b]	357.5	>500
15[b]	>500	>500
16[c]	134.5	>500
17[d]	9.7	288.5
18[a]	>500	>500
19[a]	>500	>500
20[b]	>500	>500
21[d]	29.5	>500
22[c]	>100	>100
231[c]	>100	>100
24[c]	>42	42
25[d]	>100	>100
26[e]	36	>100
27[e]	>51	>100
suramin	8.2	250

[a] Monosodium salt. [b] Disodium salt. [c] Trisodium salt. [d] Tetrasodium salt. [e] Hexasodium salt. [f] nd = not determined. [g] Data represent mean values for at least two separate experiments. Data for compounds 1–9 and 12 are taken from refs 22 and 23. [h] 50% antiviral effective concentration based on inhibition of HIV-1 (HTLV-III_B) induced cytopathogenicity. [i] 50% cytotoxic concentration based on reduction of viability of mock-infected cells.

interacting with the viral envelope glycoprotein, gp 120.[31] The in vitro evaluation of the RT inhibitory potential of polar derivatives is justified because if activity is ascertained at nontoxic concentrations, as in this study, and RT is the desired target for development, prodrug modifications may be initiated to enhance cellular entry. Overall, we have discovered several naphthalenesulfonic acid derivatives which can be considered as novel leads for the development of putative anti-AIDS agents.

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 tetramethyl silane as internal standard. IR spectra were recorded on an IBM FT IR-32 system 9000, using KBr pellets, in the Department of Chemistry. The IR spectra for compounds 11 and 21 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. Amberlite IRA-94 weakly basic anion exchange resin and Amberlite IR-122 (H^+) strongly acidic cation exchange resin (Sigma, St. Louis, MO) were used for ion exchange chromatography. 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.

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5,5′-[4,4′-Biphenyldiylbis(sulfonylamino)]bis(2-hydroxy-3-sulfobenzoic acid) (11). 5-Amino-2-hydroxy-3-sulfobenzoic acid (10) (tech., 0.433 g, 1.86 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 50 °C for 18 h. The reaction mixture was filtered and concentrated to 10 mL. The concentrated filtrate was divided into two equal portions. One portion was loaded on Spectra/Gel filteration column. Elution with water and evaporation of pure fractions gave a light brown powder isolated as a tetrasodium salt (0.153 g, 10%). An analytical sample was prepared by precipitating the product from its methanolic solution by adding ether: mp 248–250 °C dec; IR 3482, 3098, 1638, 1591, 1454, 1325, 1199, 1041, 719, 627 cm^{-1}; NMR δ 18.03 (s, 2 H, exchangeable with D_2O), 9.70 (br s, 2 H, exchangeable with D_2O), 7.93 (d, 4 H, J = 7.8 Hz), 7.79 (d, 4 H, J = 7.8 Hz), 7.51 (s, 2 H), 7.50 (s, 2 H). Anal. ($C_{26}H_{18}N_2O_{16}S_4Na_4 \cdot 3H_2O$) C, H, N.

4-Amino-5-hydroxy-2,7-naphthalenedsulfonic Acid (12). A commerical sample of 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid (12), monosodium salt (Aldrich), after one recrystallization from methanol–water (5:1), was dissolved in water and introduced on to a column of weakly basic anion-exchange resin. The compound was eluted with 1 N NaOH. The solution was neutralized with 1 N HCl and was passed through a column of strongly acidic cation-exchange resin. After evaporation of water, the residue was recrystallized from methanol–water (5:1) to give white crystals of 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid (12) disodium salt, which was used as starting material without further purification.

4-(Acetylamino)-5-acetoxy-2,7-naphthalenedisulfonic Acid (13). 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid (12) disodium salt (0.363 g, 1 mmol) in dry pyridine (2 mL) was stirred with acetic anhydride (0.5 mL, 5 mmol) at room temperature for 1 h. The reaction mixture was stirred with heptane (20 mL) and filtered. The residue was washed several times with heptane, dissolved in methanol (10 mL), and filtered, and the filtrate was triturated with ether (50 mL). The separated solid was filtered to yield the product as the disodium salt, a white powder (0.256 g, 57%): mp 310–312 °C dec; IR 3457, 1757, 1651, 1543, 1377, 1210, 1046, 666, 619 cm^{-1}; NMR δ 9.90 (s, 1 H, exchangeable with D_2O), 8.03 (s, 2 H), 7.85 (s, 1 H), 7.37 (s, 1 H), 2.34 (s, 3 H), 2.11 (s, 3 H). Anal. ($C_{14}H_{11}NO_9S_2Na_2 \cdot 1.5H_2O$) C, H, N.

4-(Benzoylamino)-5-hydroxy-2,7-naphthalenedisulfonic Acid (14). 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid (12) disodium salt (0.182 g, 0.5 mmol) in dry pyridine (0.5 mL) was stirred for 15 min at 70–80 °C. Benzoyl chloride (0.07 mL, 0.6 mmol) was added and the mixture was stirred further for 8 h. The resulting mixture was stirred with heptane (20 mL) and filtered. The residue was washed several times with $CHCl_3$, dissolved in methanol (10 mL), and filtered, and the filtrate was triturated with ether (50 mL). The separated solid was filtered to yield the product as the disodium salt, a pale yellow powder (0.145 g, 62%): mp 318–320 °C dec; IR 3455, 1630, 1563, 1393, 1289, 1186, 1063, 1044, 671, 583 cm^{-1}; NMR δ 12.04 (s, 1 H, exchangeable with D_2O), 11.74 (s, 1 H, exchangeable with D_2O), 8.99 (s, 1 H), 7.98 (d, J = 7.6 Hz, 2 H), 7.78 (s, 1 H), 7.62 (m, 4 H), 7.24 (s, 1 H). Anal. ($C_{17}H_{11}NO_8S_2Na_2 \cdot H_2O$) C, H, N.

4-[[(3-Carboxypropyl)carbonyl]amino]-5-hydroxy-2,7-naphthalenedisulfonic Acid (15). 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid (12) disodium salt (0.363 g, 1 mmol) in dry pyridine (2 mL) was stirred for 15 min at 70–80 °C. Glutaryl dichloride (0.08 mL, 0.6 mmol) was added and the mixture was stirred further for 4 h. The reaction mixture was stirred with heptane (20 mL) and filtered. The residue was washed several times with $CHCl_3$, dissolved in methanol (10 mL), and filtered. The filtrate was triturated with $CHCl_3$ (50 mL) and the separated solid was filtered. This solid was dissolved in water (5 mL) and was introduced on to a Spectra/Gel filtration column. The column was eluted with water and aqueous fractions containing the product were concentrated in vaccuo and triturated with acetone. The separated solid was filtered to give the product as the disodium salt, a beige powder (0.251 g, 53%): mp 314–316 °C dec; IR 3451, 1628, 1563, 1395, 1194, 1061, 1044, 679 cm^{-1}; NMR δ 8.68 (s, 1 H), 7.61 (s, 1 H), 7.35 (s, 1 H), 7.03 (s, 1 H), 2.43 (t, J = 7.3 Hz, 2 H), 2.29 (t, J = 7.3 Hz, 2 H), 1.89 (m, 2 H); (–)-

FABMS *m/e* (relative intensity) 454 ([M − Na]⁻, 59), 432 ([M − 2Na + H]⁻, 100), 410. Anal. ($C_{15}H_{13}NO_{10}S_2Na_2 \cdot 1.5H_2O$) C, H, N.

4-[[(8-Carboxyoctyl)carbonyl]amino]-5-hydroxy-2,7-naphthalenedisulfonic Acid (16). 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid (12) disodium salt (0.341 g, 1 mmol) in dry pyridine (1 mL) was stirred for 15 min at room temperature. Sebacyl chloride (0.13 mL, 0.6 mmol) was added and the mixture was stirred further for 8 h. The reaction mixture was stirred with heptane (20 mL) and filtered. The residue was washed several times with heptane, dissolved in methanol (10 mL), and filtered, and the filtrate was triturated with ether (50 mL). The separated solid was filtered, dissolved in water (5 mL), and introduced on to a Spectra/Gel filtration column. The column was eluted with water, and the aqueous fractions containing the product were concentrated in vaccuo and triturated with acetone. The separated solid was filtered to yield the product as the disodium salt, a light brown powder (0.235 g, 45%): mp 284–285 °C dec; IR 3467, 2928, 1659, 1628, 1555, 1393, 1196, 1059, 1044, 679 cm⁻¹; NMR δ 8.69 (s, 1 H), 7.59 (s, 1 H), 7.30 (s, 1 H), 7.01 (s, 1 H), 2.30 (t, *J* = 7.3 Hz, 2 H), 2.19 (t, *J* = 7.0 Hz, 2 H), 1.68 (m, 2 H), 1.55 (m, 2 H), 1.31 (br s, 8 H); (+)-HRFABMS *m/e* 548.0622, calcd for $[C_{20}H_{23}NO_{10}S_2Na_2 + H]^+$ 548.0637.

4,4'-(1,10-Sebacyldiamino)bis(5-hydroxy-2,7-naphthalenedisulfonic acid) (17). 4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid (12) disodium salt (0.341 g, 1 mmol) in dry pyridine (1 mL) was heated to a temperature of 130–140 °C, after which time sebacyl chloride (0.13 mL, 0.6 mmol) was added and the mixture was 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, dissolved in water (5 mL), and introduced on to a Spectra/Gel column. The column was eluted with water, and the aqueous fractions containing product were concentrated in vaccuo and triturated with acetone. The separated solid was filtered to yield the product as the tetrasodium salt, a light brown powder (0.322 g, 38%): mp 330–332 °C dec; IR 3453, 2932, 1655, 1626, 1561, 1393, 1190, 1059, 1044, 681 cm⁻¹; NMR δ 11.41 (s, 2 H, exchangeable with D_2O), 11.08 (s, 2 H, exchangeable with D_2O), 8.75 (s, 2 H), 7.69 (s, 2 H), 7.52 (s, 2 H), 7.18 (s, 2 H), 2.38 (t, *J* = 7.2 Hz, 4 H), 1.67 (m, 4 H), 1.36 (br s, 8 H). Anal. ($C_{30}H_{30}N_2O_{16}S_4Na_4 \cdot 2H_2O$) C, H, N.

4-(*N,N,N*-Trimethylammonio)-5-methoxy-2,7-naphthalenedisulfonic Acid (18). A solution of 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid (12) disodium salt (0.726 g, 2 mmol) in methanol (10 mL) was added to an ethereal solution containing ca. 3 g (71 mmol) of CH_2N_2, and the resulting mixture was stirred at room temperature for 24 h. Excess CH_2N_2 was destroyed with AcOH and the mixture was evaporated to yield a white residue which was recrystallized from methanol–water (10:1) as white crystals (0.594 g, 75%): mp 354–356 °C dec; IR 3449, 1358, 1267, 1244, 1198, 1094, 1055, 1046, 673 cm⁻¹; NMR δ 8.30 (s, 1 H), 8.25 (s, 1 H), 7.94 (s, 1 H), 7.48 (s, 1 H), 4.10 (s, 3 H), 3.89 (s, 9 H). Anal. ($C_{14}H_{18}NS_2O_7Na$) C, H, N.

4-(*N,N*-Dimethylamino)-5-methoxy-2,7-naphthalenedisulfonic Acid (19). A solution of 4-(*N,N,N*-trimethylammonio)-5-methoxy-2,7-naphthalenedisulfonic acid (12) monosodium salt (0.397 g, 1 mmol) in dry DMSO (1 mL) was heated to 100 °C, $NaBH_4$ (0.151 g, 4 mmol) was added, and the mixture was stirred for 6 h. The cooled reaction mixture was washed several times with CH_2Cl_2, dissolved in methanol (10 mL), and filtered. The filtrate on trituration with acetone (50 mL) gave a white precipitate which was recrystallized from glacial acetic acid–methanol (5:1) to give the product as the monosodium salt (0.267 g, 70%): mp 350–352 °C dec; IR 3505, 1364, 1269, 1204, 1100, 1045, 675, 646 cm⁻¹; NMR δ 8.26 (s, 1 H), 8.19 (s, 1 H), 7.90 (s, 1 H), 7.38 (s, 1 H), 4.11 (s, 3 H), 3.41 (s, 6 H). Anal. ($C_{13}H_{14}NO_7S_2Na \cdot 0.5H_2O$) C, H, N.

7-Amino-1,3-naphthalenedisulfonic Acid (20). A commercial sample of 7-amino-1,3-naphthalenedisulfonic acid monopotassium salt (tech., Aldrich) was dissolved in water, and the insoluble residue was filtered. The aqueous solution of the sulfonic acid was introduced on to a column of a weakly basic anion-exchange resin. The column was washed thoroughly with deionized water. The sulfonic acid was eluted by 1 N NaOH solution. The eluted solution was passed through a column of strongly acidic cation-exchange resin. After evaporation of water, the residue was dissolved in methanol and was precipitated by ether. The precipitated solid was recrystallized from ethanol–methanol (8:2) to give pale pink crystals of 7-amino-1,3-naphthalenedisulfonic acid as the disodium salt: mp >350 °C. Anal. ($C_{15}H_7NO_6S_2Na_2 \cdot H_2O$) C, H, N.

7,7-[4,4-Biphenyldiylbis(sulfonylamino)]bis(1,3-naphthalenedisulfonic acid) (21). A mixture of 7-amino-1,3-naphthalenedisulfonic acid monopotassium salt (tech., 0.341 g, 1 mmol), 4,4'-biphenyldisulfonyl chloride (0.245 g, 0.7 mmol), and $NaHCO_3$ (0.336 g, 4 mmol) in deionized water (20 mL) was stirred at 50–55 °C for 24 h. The reaction mixture showed the presence of unreacted naphthalenedisulfonic acid. More 4,4'-biphenyldisulfonyl chloride (0.035 g, 0.1 mmol) was added and the reaction mixture was again stirred at 50–55 °C for 24 h. The reaction was stopped and filtered. The filtrate was concentrated and was divided into two equal portions. One of the portion was loaded on to the Spectra/Gel filtration column. Elution with water and evaporation of appropriate fractions gave a pale pink powder isolated as the tetrasodium salt (0.136 g, 14%). An analytical sample was prepared by precipitating the product two times from its methanolic solution by adding ether: mp 332–334 °C dec; IR 3477, 3156, 1626, 1503, 1201, 1050, 956, 814 cm⁻¹; NMR δ 10.63 (s, 2 H, exchangeable with D_2O), 8.68 (s, 2 H), 8.22 (s, 2 H), 8.01 (s, 2 H), 7.98 (d, 4 H, *J* = 8.2 Hz), 7.83 (d, 2 H, *J* = 4.1 Hz), 7.80 (d, 2 H, *J* = 3.3 Hz), 7.32 (d, 4 H, *J* = 8.6 Hz). Anal. ($C_{32}H_{20}N_2O_{16}S_8Na_4 \cdot 4.5H_2O$) C, H, N.

8-Amino-1,3,6-naphthalenetrisulfonic Acid (22). A commercial sample of 8-amino-1,3,6-naphthalenetrisulfonic acid (Chem Service), after one recrystallization from methanol–water (10:1), was dissolved in water and introduced on to a column of weakly basic anion-exchange resin. The compound was eluted with 1 N NaOH. The solution was neutralized with 1 N HCl and was passed through a column of strongly acidic cation-exchange resin. After evaporation of water, the residue was recrystallized from methanol–water (10:1) to give white crystals of 8-amino-1,3,6-naphthalenetrisulfonic acid trisodium salt. Anal. ($C_{10}H_6NO_9S_3Na_3 \cdot H_2O$) C, H, N.

8-(Acetylamino)-1,3,6-naphthalenetrisulfonic Acid (23). 8-Amino-1,3,6-naphthalenetrisulfonic acid (22) trisodium salt (0.449 g, 1 mmol) in dry pyridine (2 mL) was stirred for 15 min at 70–80 °C. Acetic anhydride (0.5 mL, 5 mmol) was added and the mixture was stirred further for 8 h. The reaction mixture was stirred with heptane (20 mL) and filtered. The residue was washed several times with $CHCl_3$, dissolved in MeOH (10 mL), and filtered, and the filtrate was triturated with ether (50 mL). The separated solid was filtered and stirred with glacial acetic acid (10 mL) and filtered again. The residue was dissolved in MeOH (10 mL) and filtered, and the filtrate was triturated with ether (50 mL). The separated solid was filtered to yield the product as the trisodium salt, a white powder (0.301 g, 61%): mp 332–334 °C dec; IR 3499, 1673, 1563, 1391, 1294, 1215, 1148, 1055, 677, 629 cm⁻¹; NMR δ 12.05 (s, 1 H, exchangeable with D_2O), 8.55 (s, 1 H), 8.35 (s, 1 H), 8.06 (s, 1 H), 7.88 (s, 1 H), 2.09 (s, 3 H). Anal. ($C_{12}H_8NO_{10}S_3Na_3$) C, H, N.

8-(Palmitoylamino)-1,3,6-naphthalenetrisulfonic Acid (24). 8-Amino-1,3,6-naphthalenetrisulfonic acid (22) trisodium salt (0.449 g, 1 mmol) in dry pyridine (2 mL) was heated to 70–80 °C, and to this mixture was added palmitoyl chloride (0.8 mL, 2 mmol). The reaction mixture was stirred for 8 h followed by addition of heptane (20 mL) and filtered. The residue was dissolved in MeOH (10 mL) filtered and the filtrate was triturated with ether (50 mL). The separated solid was filtered to yield the product as the trisodium salt, a white powder (0.314 g, 46%): mp 311–312 °C dec; IR 3467, 2923, 2853, 1665, 1640, 1549, 1202, 1148, 1048, 677 cm⁻¹; NMR δ 12.03 (s, 1 H, exchangeable with D_2O), 8.55 (s, 1 H), 8.39 (s, 1 H), 8.06 (s, 1 H), 7.87 (s, 1 H), 2.38 (t, 2 H, *J* = 7.3 Hz), 1.65 (m, 2 H), 1.24 (br s, 24 H), 0.85 (t, 3 H, *J* = 6.7 Hz); (−)-HRFABMS *m/e* 664.1298, calcd for $[C_{28}H_{36}NO_{10}S_3Na_3 - Na]^-$ 664.1297.

8-[[(4'-Sulfobiphenyl-4-yl)sulfonyl]amino]-1,3,6-naphthalenetrisulfonic Acid (25) and 8,8'-[4,4'-Biphenyldiylbis(sulfonylamino)]bis(1,3,6-naphthalenetrisulfonic acid) (26). A mixture of 8-amino-1,3,6-naphthalenetrisulfonic acid (22) trisodium salt (0.449 g, 1 mmol), 4.4'-biphenyldisulfonyl chloride

(0.210 g, 0.6 mmol) and $NaHCO_3$ (0.336 g, 4 mmol) in water (20 mL) was stirred for 24 h at 40–45 °C. The reaction mixture was passed through the acidic cation-exchange resin. The solution was concentrated and half of it was introduced on to a Spectra/Gel filtration column. Elution of the column with water gave first **26** as the hexasodium salt (0.099 g, 34%) and then **25** as the tetrasodium salt (0.116 g, 30%).

8-[[(4'-Sulfobiphenyl-4-yl)sulfonyl]amino]-1,3,6-naphthalenetrisulfonic acid (25): mp 342–344 °C; IR 3468, 1642, 1196, 1130, 1049, 1001, 621, 596 cm^{-1}; NMR δ 12.94 (s, 1 H, exchangeable with D_2O), 8.51 (s, 1 H), 8.17 (s, 1 H), 8.05 (d, 2 H, J = 8.4 Hz), 7.99 (s, 1 H), 7.75 (s, 1 H), 7.65 (m, 6 H). Anal. ($C_{22}H_{17}NO_{16}S_5Na_4 \cdot 2.5H_2O$) C, H; N: calcd, 1.72; found, 2.33.

8,8'-[4,4'-Biphenyldiylbis(sulfonylamino)]bis(1,3,6-naphthalenetrisulfonic acid) (26): mp 318–320 °C dec; IR 3466, 1640, 1308, 1202, 1094, 1049, 677 cm^{-1}; NMR δ 12.94 (s, 2 H, exchangeable with D_2O), 8.50 (s, 2 H), 8.15 (s, 2 H), 8.00 (d, 4 H, J = 8.4 Hz), 7.98 (s, 2 H), 7.75 (s, 2 H), 7.70 (d, 4 H, J = 8.4 Hz). Anal. ($C_{32}H_{18}N_2O_{22}S_8Na_6 \cdot 0.5H_2O$) C, N; H: calcd 1.60; found, 2.05.

8,8'-[1,10-Decanediylbis(carbonylamino)]bis(1,3,6-naphthalenetrisulfonic acid) (27). 8-Amino-1,3,6-naphthalenetrisulfonic acid (**22**) trisodium salt (0.449 g, 1 mmol) in dry pyridine (2 mL) was stirred for 15 min at 70–80 °C. Dodecanedioyl dichloride (0.15 mL, 0.6 mmol) was added and the mixture was stirred further for 8 h. The resulting mixture was stirred with heptane (20 mL) and filtered. The residue was washed several times with $CHCl_3$, stirred with glacial acetic acid (10 mL), and filtered. The residue was dissolved in MeOH (10 mL) and filtered, and the filtrate was triturated with ether (50 mL). The separated solid was filtered to yield the product as its hexasodium salt, a pale yellow powder (0.298 g, 55%): mp 288–290 °C dec; IR 3457, 2928, 1659, 1547, 1393, 1197, 1146, 1048, 679, 621 cm^{-1}; NMR δ 12.02 (s, 2 H, exchangeable with D_2O), 8.57 (s, 2 H), 8.39 (s, 2 H), 8.08 (s, 2 H), 7.89 (s, 2 H), 2.38 (t, 4 H, J = 7.3 Hz), 1.62 (m, 4 H), 1.26 (br s, 12 H). Anal. ($C_{32}H_{30}N_2O_{20}S_6Na_6 \cdot 1.5H_2O$) C, H; N: calcd, 2.50; found, 3.08.

Antiviral Assay Procedures. Cytopathogenesis Assay. Activity of the compounds against the replication of HIV-1 was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells, as previously described.[28] Briefly, MT-4 cells were infected with $HTLV\text{-}III_B$ 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.[32] Suramin was obtained from Bayer AG (Wuppertal, Germany).

Reverse Transcriptase Assays. Preparation of HIV-1 (p66/p51) and HIV-2 (p68/p55) RTs. Dimeric HIV-1 and HIV-2 RT are recombinant enzymes synthesized in *Escherichia coli* expression systems using genetically engineered plasmids. HIV-1 RT was prepared by a modification of a previously described pocedure.[33] HIV-2 RT was purified by using a recently published protocol.[34]

HIV-1 and HIV-2 RT Assays. Identical conditions were utilized for the HIV RT assays in view of the conservation of biological properties in both enzymes.[3] The standard assay mixture was adapted from the optimal conditions developed for the detection of RT in AIDS associated virions[27] and contained the following: 50 mM Tris·HCl buffer (pH 8.0), 150 mM KCl, 5 mM $MgCl_2$, 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 5 mM dithiothreitol, 0.3 mM glutathione, 41 μM poly(rA) [ϵ_{260} (mM) = 7.8], 9.5 μM oligo$(dT)_{12-18}$ [ϵ_{265} (mM) = 5.6], 20 μM TTP, and 0.5 μCi of [*methyl*-^{3}H]TTP. Test substance was dissolved in 10 μL of DMSO or H_2O. Control experiments were performed with the solvent only. The reaction was initiated by the addition of 10 μL (0.08 μg) of HIV-1 or HIV-2 RT and the mixture (final volume, 100 μL) was incubated at 37 °C for 1 h. Reactions were terminated by the addition of 25 μL of 0.1 M EGTA followed by chilling in ice. Aliquots of each reaction mixture (100 μL) were then spotted onto circular 2.5 cm DE-81 (Whatman) filters and washed four times with 5% aqueous Na_2HPO_4 and twice with H_2O. Finally, the filters were dried and subjected to liquid scintillation counting. With these reaction conditions, the uninhibited rates of incorporation were 2.5 and 1.3 μmol of TMP/mg of protein per 10 min for HIV-1 and HIV-2 RTs, respectively.

An initial prescreen was conducted utilizing three concentrations of the test compounds (50, 200, and 400 μg/mL). Compounds demonstrating >50% inhibition at concentrations less than or equal to 400 μg/mL were then subjected to complete dose–response evaluations. The median inhibitory concentration (IC_{50}) was calculated from a linearly regressed dose–response plot of percent control activity versus concentration or log concentration of compound, utilizing at least five concentrations of each compound. Each data point represents the average of duplicate tests. Suramin was used as the positive-control substance in routine testing. This compound had an IC_{50} value of 22.1 μg/mL (15.5 μM) and 13.4 μg/mL (9.37 μM) in the HIV-1 and HIV-2 RT systems, respectively.

Polymerization Assay Conditions. Preliminary experiments were conducted to ensure that product incorporation was linear with respect to incubation time and that each set of assay conditions used represented subsaturation levels with respect to the template, substrate, and enzyme concentrations. The effect of inhibitors were evaluated at the same region on the saturation curves of HIV-1 and HIV-2 RT as an attempt to standardize the polymerization activity of each system and hence their susceptibility to inhibitors.

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