Synthesis and Structure−Activity Relationships of Phenylenebis(methylene)-Linked Bis-tetraazamacrocycles That Inhibit Human Immunodeficiency Virus Replication. 2. Effect of Heteroaromatic Linkers on the Activity of Bicyclams

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A series of bicyclam analogs connected through a heteroaromatic linker have been synthesized and evaluated for their inhibitory effects on HIV-1 (III_B) and HIV-2 (ROD) replication in MT-4 cells. The activity of pyridine- and pyrazine-linked bicyclams was found to be highly dependent upon the substitution of the heteroaromatic linker connecting the cyclam rings. For example, 2,6- and 3,5-pyridine-linked bicyclams were potent inhibitors of HIV-1 and HIV-2 replication, whereas the 2,5- and 2,4-substituted pyridine-linked compounds exhibited substantially reduced activity and, in addition, were found to be highly toxic to MT-4 cells. We have subsequently discovered that these effects are not unique; amino-substituted linkers also have the potential to deactivate phenylenebis(methylene)-linked bicyclams. A model is proposed to explain the deactivating effects of the pyridine group in certain substitution patterns based on the ability of the pyridine nitrogen to participate in pendant conformations (complexation) with the adjacent azamacrocyclic ring, which may involve hydrogen bonding or coordination to a transition metal. The introduction of a sterically hindering group such as phenyl at the 6-position of the 2,4-substituted pyridine-linked bicyclam appears to prevent pendant conformations, providing an analog with comparable anti-HIV-1 and anti-HIV-2 activities to the parent *m*-phenylenebis(methylene)-linked bicyclam. The results of this study have been used to develop a quantitative structure−activity relationship model with improved predictive capability in order to aid the design of antiviral bis-azamacrocyclic analogs.

Introduction

Bicyclams are a novel class of antiviral agents that exhibit potent inhibitory effects on HIV-1 and HIV-2 replication with high selectivity.[1,2] From previous structure−activity relationship studies of aliphatic linked bicyclams such as JM2763 (**1**)[1] (Figure 1) and aromatic linked bis-tetraazamacrocycles that vary with ring size,[3] it was discovered that dimers of the 1,4,8,11-tetraazacyclotetradecane (cyclam) ring connected via a 1,4-phenylenebis(methylene) linker provided optimum anti-HIV-1 and anti-HIV-2 activity *in vitro*. Thus, JM3100 (**10d**, isolated as the octahydrochloride salt, Figure 1) was identified as a lead candidate for further structure−activity elaboration.[2,3]

The anti-HIV activity exhibited by bicyclams is particularly intriguing due to the possibility that binding to the molecular target at the HIV-inhibitory step is transition metal-mediated. For example, in coincidental but unrelated recent publications, Arnold and co-workers[4] have proposed that bis-transition metal complexes of **10d** can provide complementary coordinating sites for the chelating amino acid residues on a protein surface. This type of mechanism is not implausable in our system since **10d** will almost assuredly complex free transition metals *in vitro*, and a variety of preformed transition metal complexes of **10d** and several other

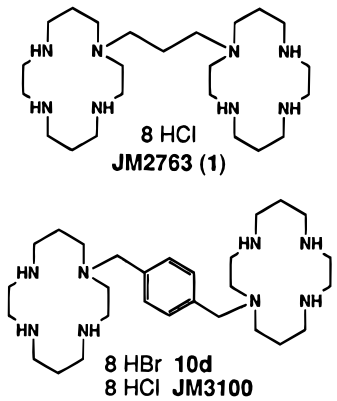

Figure 1. Structures of the bicyclam analogs JM2763 and JM3100.

bicyclams exhibit antiviral activity.[3,5] In this manner, **10d** could be viewed as a prodrug for a transition metal complex in a similar manner to the antitumor agent bleomycin.[6] However, definitively proving the involvement (or lack of involvement) of transition metal ions in the anti-HIV activity of bicyclams is complicated by the fact that upon protonation (which occurs at physiological pH) azamacrocycles can also complex anions.[7,8]

During the course of our ongoing investigations to determine the structural features required for potent anti-HIV activity in this class of compounds, we prepared a series of compounds in which the cyclam rings are connected by a heteroaromatic linker. Depending upon the substitution pattern of pyridine and pyrazine (and aniline) linkers, these analogs exhibited a striking

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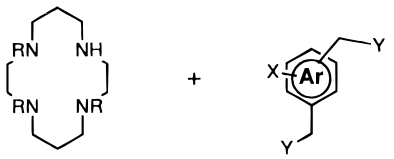
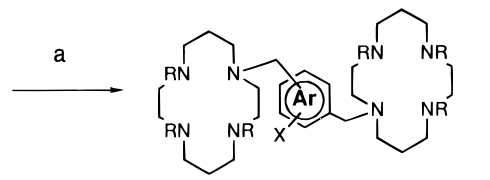
Scheme 1[a]



2a, R=Ts
2b, R=Dep,

3a, Ar=2,6-pyridyl, X=H, Y=Br
3b, Ar=3,5-pyridyl, X=H, Y=Br
3c, Ar=2,4-pyridyl, X=H, Y=Br
3d, Ar=2,5-pyridyl, X=H, Y=Br
3e, Ar=2,6-pyrazyl, X=H, Y=Cl
3f, Ar=2,5-pyrazyl, X=H, Y=Br
3g, Ar=1,4-xylyl, X=2-NO_2, Y=OMs
3h, Ar=1,4-xylyl, X=2-CO_2Me, Y=OMs
3i, Ar=1,3-xylyl, X=5-NO_2, Y=OMs
3j, Ar=1,3-xylyl, X=5-CO_2Me, Y=OMs



R =H

b,c

4a, Ar=2,6-pyridyl, X=H, R=Ts → **8a**
4b, Ar=3,5-pyridyl, X=H, R=Ts → **8b**
4c, Ar=2,4-pyridyl, X=H, R=Ts → **8c**
4d, Ar=2,5-pyridyl, X=H, R=Ts → **8d**

5a, Ar=2,6-pyrazyl, X=H, R=Ts → **9a**
5b, Ar=2,5-pyrazyl, X=H, R=Dep → **9b**

6a, Ar=1,4-xylyl, X=2-NO_2, R=Ts → **10a**, X=NH_2
6b, Ar=1,4-xylyl, X=2-CO_2Me, R=Ts → **10b**, X=CO_2H
6c, Ar=1,4-xylyl, X=2-CO_2Me, R=Dep → **10c**, X=CO_2Me

7a, Ar=1,3-xylyl, X=5-NO_2, R=Ts → **11a**, X=NH_2
7b, Ar=1,3-xylyl, X=5-CO_2Me, R=Ts → **11b**, X=CO_2H

[a] Reagents: (a) K_2CO_3, CH_3CN, reflux; (b) when X = NO_2, Fe, concentrated aqueous HCl, EtOH; (c) when R = Ts, 48% aqueous HBr, HOAc, reflux, when R = Dep, HBr/HOAc, room temperature.

anti-HIV structure−activity relationship, consistent with a model in which the heteroatom of the linker can assume a "pendant-coordinating"[9] conformation with the adjacent cyclam ring that is unfavorable with respect to anti-HIV activity. This intramolecular interaction may involve hydrogen bonding of the linker heteroatom with the azamacrocyclic ring, competition for the azamacrocyclic binding site, or, indeed, coordination to a transition metal complexed within the azamacrocyclic cavity.

In the present work, we propose a conformational model to explain the deleterious effects of certain heteroaromatic linkers on the anti-HIV activity of bicyclam analogs that is clearly a necessary consideration for further structural design.

Chemistry

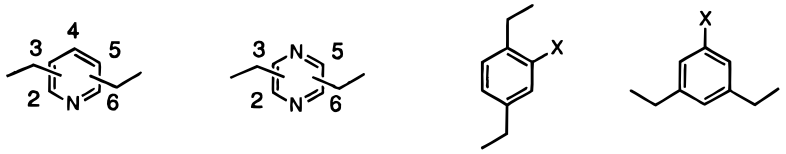
Bicyclam analogs (**8**−**11**; Table 1) were prepared by the reaction of the tris-N-protected cyclam derivatives with the appropriate aromatic or heteroaromatic bis-electrophiles as previously described (Scheme 1).[3] The bis(bromomethyl)pyridine electrophiles were obtained from commercially available pyridine diacids by BH_3·THF reduction to the corresponding diols followed by treatment with 48% aqueous hydrobromic acid/acetic anhydride according to literature procedures.[10] 2,6-Bis-(chloromethyl)pyrazine and 2,5-bis(bromomethyl)pyrazine were prepared by halogenation of dimethylpyrazine isomers with NCS or NBS, respectively. The amino analogs **10a** and **11a** were obtained from the nitro derivatives **6a** and **7a** by reduction with Fe/concentrated aqueous HCl[11] followed by deprotection.

Results and Discussion

Bicyclam analogs containing a pyridine linker (**8a**−**d**; Table 1) were tested for their inhibitory effects upon HIV-1 (III_B) and HIV-2 (ROD) replication in MT-4 cells according to known procedures.[3] Their anti-HIV activity was found to be highly dependent upon the substitution pattern of the pyridyl moiety connecting the cyclam rings. For example, both the 2,6- (**8a**) and 3,5- (**8b**) substituted pyridine analogs exhibited comparable anti-HIV-1 and anti-HIV-2 activity to the simple *m*-phenylenebis(methylene)-linked analog **11c** [50% effective concentrations (EC_{50}s) against HIV-1 for **8a**,**b** and **11c** were found to be 0.0245, 0.0316, and 0.0337 μM, respectively], whereas the 2,4- (**8c**) and 2,5- (**8d**) substituted pyridyl-linked analogs exhibited substantially reduced activity against HIV-1 and HIV-2. Both the 2,4-substituted (**8c**) and 2,5-substituted (**8d**) pyridine analogs were found to be 2 orders of magnitude less active against HIV-1 and HIV-2 replication than the parent phenylenebis(methylene) analogs **11c** and **10d**, respectively. Furthermore, **8c**,**d** also displayed a significantly increased cytotoxicity to MT-4 cells. We subsequently discovered that these effects were not unique to pyridine-linked bicyclams. Replacing the pyridine linker with the weaker base pyrazine (**9a**,**b**) (pK_a of pyridine is 5.25 and pK_{a_1} of pyrazine is 0.65[12]) or a nonheterocyclic base of comparable pK_a to pyridine such as aniline (pK_a of 4.65) (to give compounds **10a** and **11a**) gave similar results: The 2,6-pyrazine analog **9a** exhibited comparable anti-HIV-1 and anti-HIV-2 activity to **11c**, whereas the 2,5-analog **9b** was 3 orders of magnitude less active against HIV-1 and HIV-2 replication than **11c**. Although the effect of the aniline linkers was less pronounced, **10a** proved to be 44- and 15-fold less effective in inhibiting HIV-1 and HIV-2 replication than **11a**,**c**.

A model to explain the deactivating effects of heteroaromatic linkers in certain substitution patterns was obtained from reviewing the coordination chemistry literature. Azamacrocycles such as cyclam display a rich coordination chemistry, forming complexes with a variety of transition metals with high thermodynamic stability.[13] However, from a chelation standpoint, cyclam suffers from the disadvantage that it is unable to fill all of the vacant coordination sites on the majority of transition metals. One solution to this problem developed by several groups is the attachment of a single pendant-coordinating substituent such as an amine,[14,15] phenol,[16,17] pyridine,[18−20] imidazole,[21] or carboxylate[22] group to the periphery of the cyclam ring, which increases the ligating ability of the macrocycle by filling a remaining coordination site, thereby "wrapping up" the transition metal.[9] Furthermore, previous studies also indicate that the pendant-coordinating arm can assist in the kinetics of azamacrocyclic metal complex formation by rapid capture of the metal ion by the pendant arm preceeding the relatively slow step of transfer to the azamacrocyclic cavity, which is hindered, in part, by conformational changes in the ligand neces-

Table 1. Anti-HIV Activity of Heteroaromatic Linked Bis-Cyclams



I II III IV

compd	structure	subst	formula[a]	EC_{50} (μM)[b] HIV-1 (III_B)	EC_{50} (μM)[b] HIV-2 (ROD)	CC_{50}[c] (μM)
8a	I	2,6-	$C_{27}H_{53}N_9 \cdot 8HBr \cdot 3.5H_2O$	0.0245[d]	0.0654	>409
8b	I	3,5-	$C_{27}H_{53}N_9 \cdot 9HBr \cdot 2.5H_2O$	0.0316[d]	0.0710	>395
8c	I	2,4-	$C_{27}H_{53}N_9 \cdot 8HBr \cdot 4H_2O$	16.367	16.367	17
8d	I	2,5-	$C_{27}H_{53}N_9 \cdot 9HBr \cdot 5H_2O$	0.9081	2.1947	18
9a	II	2,6-	$C_{26}H_{52}N_{10} \cdot 8HBr \cdot H_2O \cdot 0.5HOAc$	0.0261	0.0174	>203
9b	II	2,5-	$C_{26}H_{52}N_{10} \cdot 8HCl \cdot 3.4H_2O \cdot HOAc$	138.53	105.88	>203
10a	III	X = NH_2	$C_{28}H_{55}N_9 \cdot 9HBr \cdot H_2O \cdot 1.5HOAc$	0.1869	0.0934	>179
10b	III	X = CO_2H	$C_{29}H_{54}N_8O_2 \cdot 8HBr \cdot HOAc$	200.30	200.30	>200
10c	III	X = CO_2Me	$C_{30}H_{56}N_8O_2 \cdot 8HBr \cdot 3H_2O$	0.6825	0.5904	>198
10d	III	X = H		0.0042	0.0059	>421[e]
11a	IV	X = NH_2	$C_{28}H_{55}N_9 \cdot 9HBr \cdot 2.2H_2O$	0.0468	0.0390	>194
11b	IV	X = CO_2H	$C_{29}H_{54}N_8O_2 \cdot 8HBr \cdot 2H_2O$	203.00	203.00	>200
11c	IV	X = H		0.0337	0.0422	>421[e]

[a] Microanalyses are within ±0.4 of theoretical values. All compounds tested as their hydrobromide salts unless otherwise indicated. [b] 50% antiviral effective concentration. [c] 50% cytotoxic concentration. The > symbol is used to indicate the highest concentrations at which the compounds were tested and still found to be noncytotoxic. [d] Anti-HIV-1 (III_B) data from ref 2. [e] Data from ref 3.

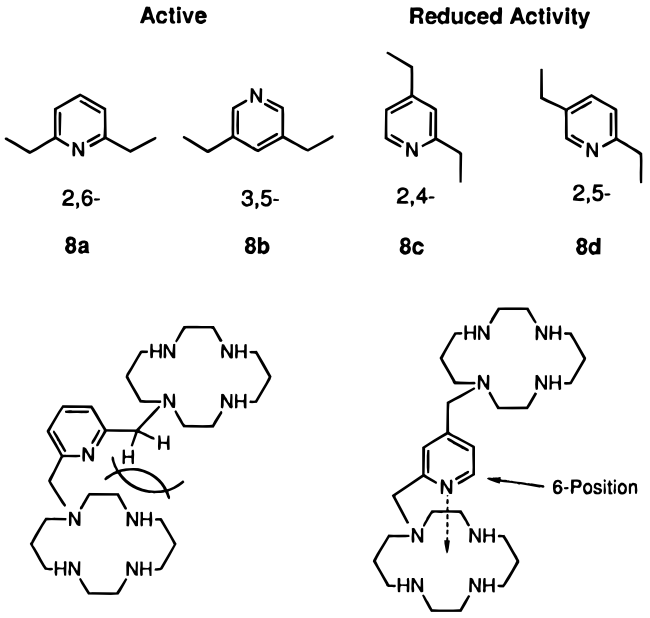


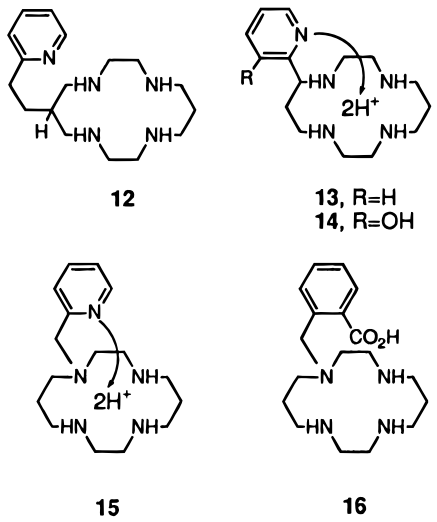
Figure 2. Proposed model of pyridine-linked bicyclams in which the 2,4- and 2,5-isomers can adopt pendant conformations.

sary for complexation.[20,23] Though the role of transition complexation at the molecular target for bicyclam activity at the HIV-inhibitory step has not been established, we found that these observations explained the reduced activity of particular bicyclam analogs by assuming that the heteroatom of the linker can participate in pseudopendant conformations (complexation) with the azamacrocycle ring dictated by the substitution pattern. Presumably, in cases where pendant conformations can occur, this results in the "wrong" molecular shape for binding with the target for **11c** and **10d**. Application of this hypothesis to the pyridyl-linked bicyclams is depicted in Figure 2.

The pyridyl-linked bicyclam analogs **8c**,**d**, exhibiting reduced anti-HIV activity, possess 2,4- and 2,5-substitution patterns connecting the tetraazamacrocyclic rings, which allow the pyridine group to participate in unfavorable (with respect to anti-HIV activity) pendant conformations without steric interference from the second ring (or the methylene group; see Figure 2). Conversely, the pyridyl-linked analog **8b** has a 3,5-substitution pattern which itself prevents pendant conformations. To complete the model, the 2,6-substitution pattern of **8a** must also prevent the pyridine nitrogen from approaching the macrocyclic ring as if it were the axial ligand of an octahedral metal complex. This is not unreasonable since, in fact, 2,6-disubstituted pyridines are poor ligands[24] and 2,6-lutidine is used as a hindered base for synthetic organic transformations. Similar arguments can be applied to the pyrazyl (**9a**,**b**) and aniline (**10a** and **11a**) analogs: Both the 2,6-pyrazyl (**9a**) and 3,5-disubstituted aniline (**11a**) analogs possess substitution patterns which would prevent pendant conformations (and therefore exhibit comparable activity to **11c**), whereas the corresponding 2,5-pyrazyl (**9b**) and 2,5-aniline (**10a**) analogs in which pendant conformations are allowed exhibit reduced anti-HIV activity. Moreover, the reduction in anti-HIV-1 and anti-HIV-2 potency of the 2,5-pyrazyl-linked analog **9b** compared to the 2,5-pyridine-linked analog **8d** and 1,4-phenylene-linked analog **10d** can be explained by assuming that both nitrogens of the pyrazine group of **9b** can participate in intramolecular pendant complexation with their respective, adjacent macrocyclic rings[25] (EC_{50}s against HIV-1 for **9b**, **8d**, and **10d** were 138.53, 0.9081, and 0.0042 μM, respectively). But what is the nature of the pendant interaction?

If the binding of bicyclams to the molecular target is not transition metal-mediated, then the pendant heteroatom of the linker may hydrogen bond to the protons shared between the nitrogen groups of the macrocyclic ring thereby inducing an unfavorable conformation with respect to anti-HIV activity and possibly competing for the azamacrocyclic binding site. Cyclam has four pK_as measured at 11.5, 10.3, 1.6, and 0.9,[13,26] which provide an overall charge on the macrocyclic ring under physi-

Scheme 2



ological conditions of +2/ring. The third and fourth protonations of cyclam become increasingly difficult (as indicated by their extremely low pK_a values) due to the mutually repulsive effects of the two positive charges already confined in a relatively constrained macrocyclic framework.[13] Kimura and co-workers have demonstrated that when a pyridine group is attached to the periphery of the cyclam ring in a pendant fashion, the pK_a of the pyridine nitrogen is dramatically reduced when appended in proximity to the macrocyclic ring. For example, the measured pK_{a_3} of the pyridine nitrogen in the 6-ethylpyridine cyclam derivative **12**[18] (Scheme 2) is 5.32 (which compares with pyridine, pK_a = 5.25), whereas the 5-pyridylcyclam derivatives **13**[19] and **14**[20] exhibit pK_{a_3}s for the pyridine nitrogen of <3 and 3.6, respectively. This observation (for **14**) has been interpreted as evidence for an intramolecular hydrogen bond between the pyridine N and a shared proton complexed within the azamacrocyclic cavity in aqueous solution.[20] On the basis of the assumption that the reduced pK_a value of the pyridine groups in **13** and **14** reflects the presence of an intramolecular hydrogen bond, we attempted to measure the pK_a of the pyridine moiety in **15** (Scheme 2), which represents the pendant-coordinating portion of the pyridyl analogs **8c**,**d**. Titration of an aqueous solution of **15** (0.1 mM, 50 mL) and 4 equiv of $HClO_4$ at 25 °C and I = 0.1 M ($NaClO_4$) with 0.1 M NaOH gave the titration curve shown in Figure 3A. From this curve, the pK_a of the pyridine group could only be estimated to be <3, a value indistinguishable from the pK_{a_3} of the cyclam ring. That protonation of the pyridine group only occurs at a pH of <3 was more accurately confirmed by monitoring the UV absorption spectrum of the pyridine moiety in **15** [0.17 mM at I = 0.1 M ($NaClO_4$)] at varying pHs (Figure 3B). The characteristic hyperchromic shift of the pyridine chromophore which occurs upon protonation begins to appear at pH 3 and increases with decreasing pH to a maximum at pH 1. Thus, the protonation constant of the pyridine moiety in **15** is entirely consistent with the hydrogen-bonding proposal of Kimura.[20]

Also of interest were the studies performed by Kaden et al.[22] on the chelating properties of the *o*-toluic acid cyclam derivative **16** (Scheme 2). In this case, the pK_a of the pendant carboxylic acid was found to be 3.85, which would provide a negatively charged carboxylate

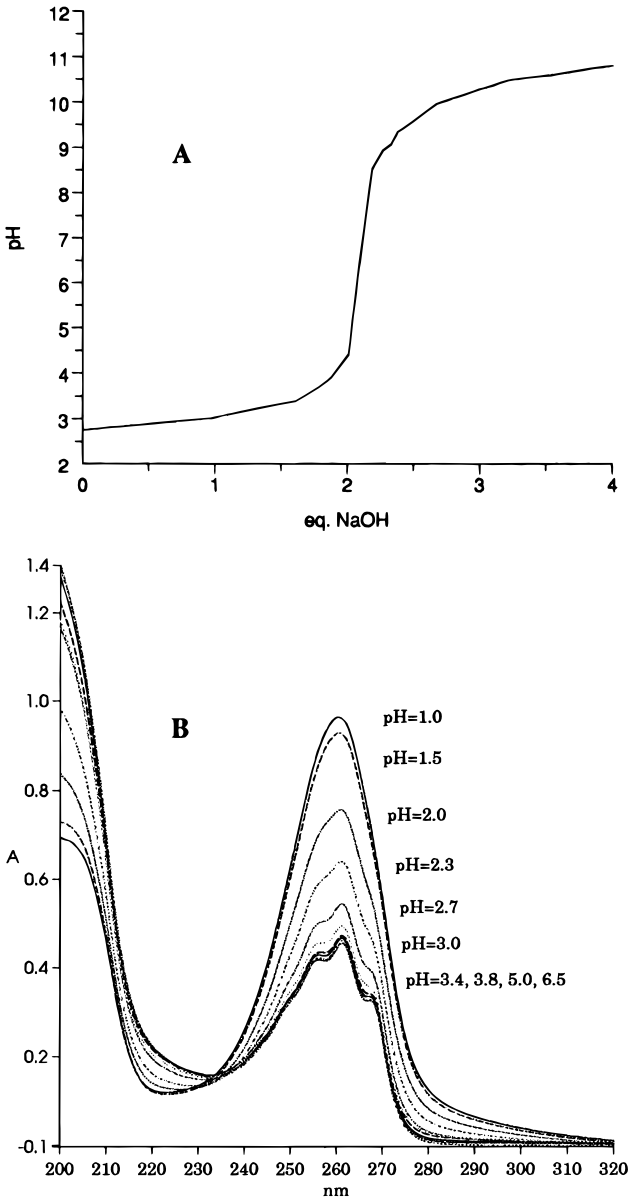
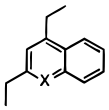
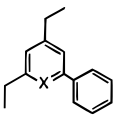


Figure 3. (A) pH titration curve of **15** (0.1 mM, 50 mL) and 4.0 equiv of $HClO_4$ at 25 °C and I = 0.1 M ($NaClO_4$) vs equiv of NaOH. (B) UV absorption spectrum of **15** (0.17 mM) at 25 °C and I = 0.1 M ($NaClO_4$) at varying pHs.

anion at physiological pH. Under basic conditions (pH 10−11), this derivative formed pendant-coordinated complexes with Cu(II) and a weak interaction of the carboxylate anion with the Ni(II) complex. The corresponding *p*-toluic acid derivative was, as expected, unable to form a complex in which the carboxylate is coordinated to Cu(II) or Ni(II). Furthermore, an X-ray crystal structure of a complex between four molecules of 4-*tert*-butylbenzoic acid and the cyclam ring has recently been published.[8] The detailed structure revealed that each of only two molecules of 4-*tert*-butylbenzoic acid interacts directly with the cyclam ring on opposing faces of the macrocycle through a network of hydrogen bonds (following a two-proton transfer to the macrocyclic ring) spanning diagonal amine groups. These combined results suggest that the carboxylate group has the propensity to pendant coordinate to a transition metal complex of the tetraazamacrocycle or simply form a direct complex with the protonated ring. By analogy, therefore, one would expect that bicyclam

Table 2. Anti-HIV Activity of Substituted Pyridine-Linked Bis-Cyclams[a]

compd	subst	structure	formula	EC_{50} (μM) HIV-1 (III_B)	EC_{50} (μM) HIV-2 (ROD)	CC_{50} (μM)
17a	X = N		$C_{31}H_{55}N_9$·9HBr·5.5H_2O	3.7716	4.2744	>419
17b	X = CH			0.1572	0.0786	207[b]
25a	X = N		$C_{33}H_{57}N_9$·9HBr	0.0398	0.0200	>203
25b	X = CH			0.2060	0.0246	>198[b]

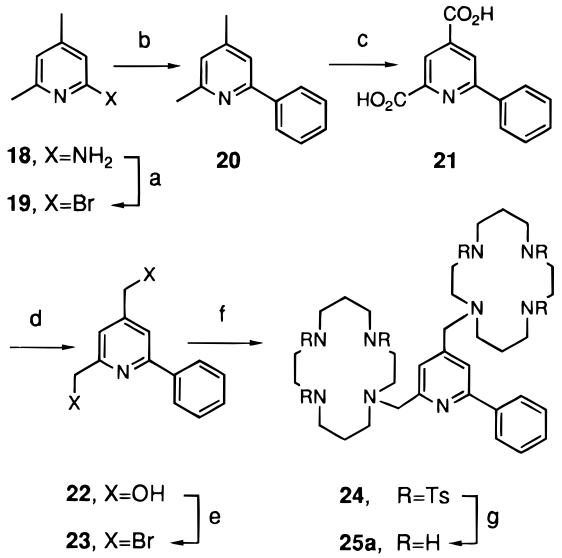
[a] See footnotes to Table 1. [b] Data from ref 3.

analogs containing a strategically placed carboxyl group on the aromatic linker would provide an identical anti-HIV structure−activity relationship to the pyridine-linked systems described above. Analogs **10b** and **11b** were prepared and tested for their inhibitory effects on HIV-1 and HIV-2 replication as shown in Table 1. To our surprise, neither **10b** (pendant conformation allowed) nor **11b** (pendant conformation disallowed) inhibited HIV replication in MT-4 cells at concentrations exceeding 200 μM. One possible explanation that could account for these results is that the negative charge of the carboxylate on the linker of **10b** or **11b** has a net repulsive interaction with the target for bicyclam activity. This was confirmed by testing the methyl ester derivative **10c** (Table 1) which inhibited HIV-1 and HIV-2 replication at 50% effective concentrations of 0.6825 and 0.5904 μM, respectively.

In order to test our hypothesis that the proximal pyridine nitrogen was responsible for the antiviral inactivation of the pyridine-linked bicyclams **8c**,**d**, we reasoned that incorporation of a sterically demanding group at the 6-position of the 2,4-pyridine-linked bicyclam **8c** should prevent this analog from adopting pendant conformations (giving a 2,6-disubstituted pyridine linker such as the 2,6-pyridyl analog **8a**; see Figure 2) thereby restoring anti-HIV activity comparable to that of the *m*-phenylenebis(methylene) analog **11c**. To this end, two analogs were synthesized, the 2,4-quinoline-linked analog in which the *peri*-hydrogen to the quinoline nitrogen has moderate blocking ability (**17a**; Table 2) and the phenyl analog (**25a**; Table 2) which, by examination of molecular models, should provide a total restriction on pendant conformations. The synthesis of **25a** was accomplished in seven steps from the commercially available 2-amino-4,6-dimethylpyridine precursor **18** as illustrated in Scheme 3. Diazotization[27] of **18** in the presence of bromine gave the bromide **19** which was subjected to palladium-catalyzed cross-coupling[28] with phenylboronic acid in order to introduce the phenyl substituent giving **20**. Conversion of **20** to the corresponding bis-electrophile **23** proved straightforward, and this reagent was used to prepare the bicyclam **25a** using previously established methodology (see also Scheme 1).

Upon anti-HIV-1 and anti-HIV-2 evaluation in MT-4 cells (Table 2), we found that activity modestly improved by incorporation of the 2,4-quinoline linker (**17a**) compared to the 2,4-pyridine-linked bicyclam analog **8c**. Compound **17a** was ca. 4-fold more effective at inhibiting HIV-1 and HIV-2 replication than **8c** but was still significantly less active than the nonheteroaromatic naphthyl analog **17b** (EC_{50}s for **17a**,**b** against HIV-1

Scheme 3[a]



[a] Reagents: (a) $NaNO_2$, Br_2, 48% aqueous HBr; (b) 1.1 equiv of $PhB(OH)_2$, 2.0 equiv of Na_2CO_3, toluene, EtOH, H_2O, 5 mol % $Pd(PPh_3)_4$, reflux; (c) 10.0 equiv of $KMnO_4$, *t*-BuOH, H_2O, reflux; (d) BH_3·THF; (e) 48% aqueous HBr, Ac_2O, reflux; (f) 2.0 equiv of **2a**, K_2CO_3, CH_3CN, reflux; (g) 48% aqueous HBr, HOAc, reflux.

were 3.7716 and 0.1572 μM, respectively). However, the 6-phenylpyridine analog **25a** proved equally potent at inhibition of HIV replication as **11c**, exhibiting EC_{50}s against HIV-1 and HIV-2 of 0.0398 and 0.0200 μM, respectively, which are around 400-fold lower than the concentration of **8c** required to inhibit viral replication by 50%. In addition, **25a** was also 11-fold less cytotoxic to the host cells than **8c**. The inactivity of the pyridine-linked bicyclams can therefore be directly assigned to the proximal pendant pyridine moiety.

In support of an alternate, transition metal-mediated anti-HIV inactivation mechanism for the proposed model, it was also necessary to demonstrate that formation of a pendant-coordinated transition metal complex is achievable for the pyridine analogs **8c**,**d**. Interestingly, of the pyridylcyclam literature examples shown in Scheme 2, only **13** and **14** formed pendant-coordinated complexes with Ni(II), consistent with several other examples of 14-[29] and 16-membered[30] tetraazamacrocyclic ligands featuring pendant amino or pyridine groups. The existence of a 5-coordinate, low-spin, d^7 Ni(III) complex of **12** in which the pyridine group was axially coordinated was only observed in the ESR spectrum when the **12**:Ni(II) complex was chemically oxidized.[18] Clearly, the conformational requirements for formation of a pendant transition metal complex are

rigid. Confirmation of the pendant-coordinating capabilities of the pyridyl analogs **8c**,**d** was accomplished by preparation of the Ni(II) complex of **15**. Addition of 1 equiv of Ni(II) perchlorate in *n*-butanol, which had been previously distilled to one-half volume to remove water, to an ethanol solution of **15** gave a bright purple precipitate which analyzed to the formula **15**:$Ni(ClO_4)_2$ (**26**). In full agreement with the literature,[14,29,30] the UV absorption spectrum of **26** (9.0 mM) in aqueous 1 M $NaClO_4$ (pH 6.9) gave three bands at 327 (sh), 531 (ϵ = 10) and 934 (ϵ = 9.1) nm, consistent with a 5-coordinate, high-spin Ni(II) complex in which the pyridine moiety is pendant-coordinated. Dissolving the solid **26** (9.0 mM) in 5 M aqueous perchloric acid gave an immediate color change from purple (**26**) to orange (**27**) (this concentration of $HClO_4$ was required for complete conversion of **26** to **27**), which was found to be reversible upon alternate additions of sodium hydroxide and perchloric acid. The UV absorption spectrum of the orange solution of **27** gave a new band at 462 (ϵ = 40) nm which is typical of low-spin Ni(II) tetraamine complexes,[14] while the high-spin chromophore was absent. This equilibrium represents a competition between the nickel cation and the proton for the pyridine nitrogen as illustrated in Figure 4 and establishes that formation of a pendant-coordinated transition metal complex can occur.

Finally, the anti-HIV virucidal properties of the pyridine (**8a**−**d**), pyrazine (**9a**,**b**), aniline (**10a** and **11a**), and phenyl (**10d**) analogs from Table 1 were tested. Rice and co-workers[31] have recently demonstrated that treating HIV-1 virions with a series of 3-nitrosobenzamide derivatives causes a loss of zinc ions and viral inactivation. Although the mechanism of this inactivation is not through metal chelation but oxidative damage of the cysteine thiolates of the NCp7 nucleocapsid protein, the ultimate result of treating free virus with chelating agents would be identical, that is, loss of metal ions and viral inactivation. Since, from a metal chelation standpoint, the pendant group can assist in the kinetics of metal complex formation and subsequently form complexes of higher kinetic stability then the parent unsubstituted cyclam (as described above), then it would be feasible that the observed anti-HIV EC_{50}s of the heteroaromatic linked bicyclam analogs of reduced activity reflected a virucidal effect caused by the extrusion of metal ions from free virus rather than a structure−activity relationship related to the molecular target for **10d** which has been previously shown not to exhibit a virucidal effect.[2] However, in all cases tested, preincubation of the bicyclam analogs with HIV-1 (III_B) at concentrations up to 50 μM failed to reduce the infectivity of the virus in MT-4 cells.[32] We conclude therefore that the observed structure−activity relationship of heteroaromatic linked bicyclams appears to be directly related to the target for the anti-HIV activity of **10d** (or JM3100; Figure 1) at the HIV-inhibitory step.

Predictive Model Based on a QSAR Study of Bistetraazamacrocycles. In an earlier study, a quantitative structure−activity relationship (QSAR-1) of bistetraazamacrocyclic compounds, including the potent anti-HIV-1 and anti-HIV-2 inhibitor **10d**, was described.[33] In this QSAR study, a correlation between several structural features of these compounds with anti-HIV activity was observed, resulting in a model

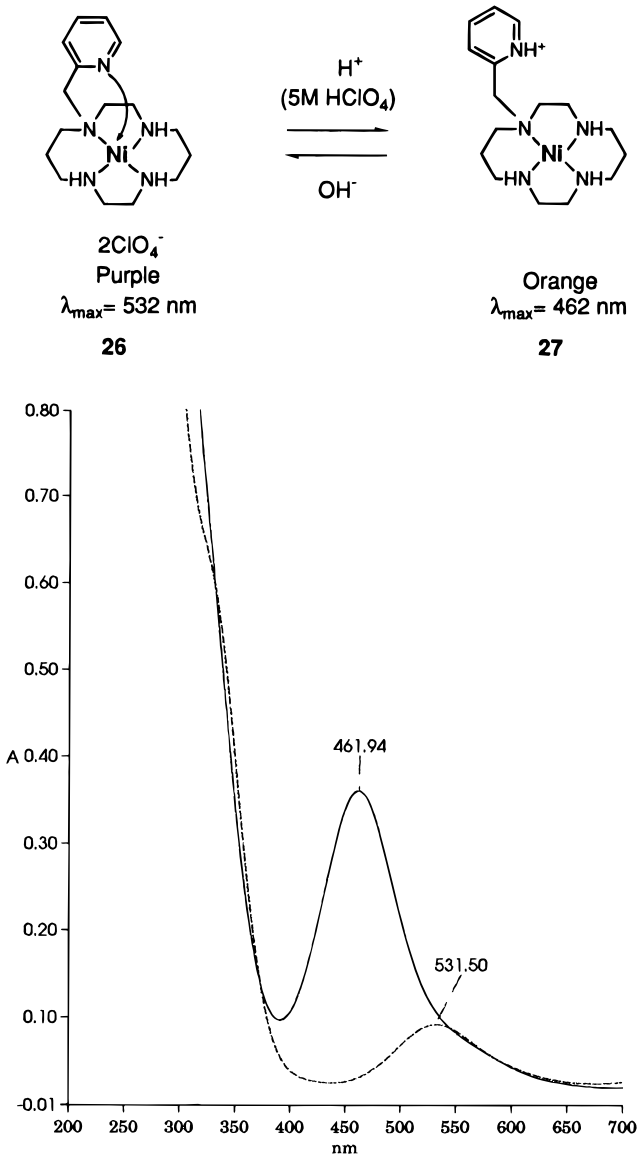


Figure 4. Reversible protonation equilibrium of **26**: (- - -) **26** (9.0 mM) in 1 M $NaClO_4$ (pH 6.9) and (—) **26** (9.0 mM) dissolved in 5 M $HClO_4$ (giving **27**).

with a high predictive capacity (as evident from the predictive r^2 value of 0.79). Partial least-squares (PLS) analysis of the descriptors used in this model led to the identification of a number of necessary features required for this class of compounds to display potent antiviral activity. For each analog included in the model, all sterically allowed conformations were included in the analysis, thus taking the flexibility of the bicyclams into account. The main descriptors used in the model are as follows. For each analog, a dummy atom was defined at the center of each macrocyclic ring to represent the position of a metal ion (or proton) complexed within the azamacrocyclic cavity. Planes were defined to represent the face of each macrocyclic ring (such that the four nitrogen atoms in each ring lay approximately in the plane), with the angles and torsions between these two planes subsequently being measured for every conformation generated in the conformational search (see Figure 5). These two descriptors are referred to as the "plane angle" and "plane torsion", respectively. In addition, the distance between the two dummy atoms (located at the metal coordination center of each macrocyclic ring) was measured for all conformations gener-

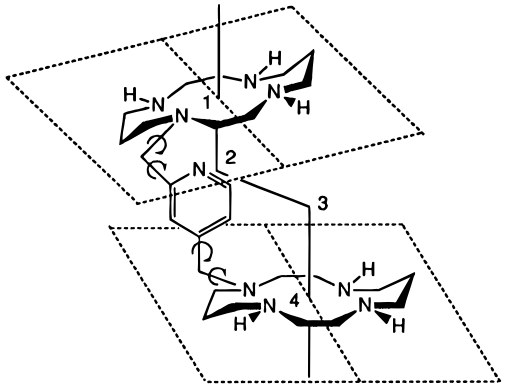


Figure 5. Schematic representation of compound **8c** showing the structural descriptors used for the QSAR analysis. (a) Plane angles—Planes were defined in which the four nitrogen atoms of an azamacrocyclic ring lie in the plane. The plane angle is the smallest angle formed upon intersection of the two planes. (b) Plane torsion—Normals to each plane were also defined, passing through a dummy atom placed at the center of each ring, having a length of 2 Å above and below the plane. This allowed measurement of the plane torsion (or twist) between the azamacrocyclic rings, illustrated by the connectivity 1-2-3-4 (using the shortest distance between the end points of the two normals (or points 2 and 3) to describe the torsion). (c) Metal−metal distance—Defined by the distance between the dummy atoms, points 1 and 4. Additional descriptors are defined in ref 33. The bonds in the linker region which were allowed to rotate during the conformational search are indicated by the curved arrows.

ated (referred to as the "metal−metal distance"). The binding affinity of each ring in the bis-azamacrocycles was included as further important descriptors (where zinc was chosen as the representative metal).

In the QSAR-1 model for antiviral activity (against HIV-1 and HIV-2), no provisions were made for analogs which might be able to adopt "pendant" conformations. It is clear, however, that if analogs capable of forming pendant complexes are included, a correction of the QSAR-1 model is required in order to retain its predictive capacity, taking into account both the metal affinity parameter and the reduction in conformational freedom. This was achieved by extention of the QSAR-1 model to include an additional indicator variable, indicating whether or not the analog in question can take up a "pendant" conformation. "Pendant" was defined as a conformation in which an additional complex between a donor atom (typically nitrogen) in the linker and the dummy atom in the center of the adjacent macrocyclic ring is possible. A value equal to the number of additional donor atoms was assigned to the pendant indicator variable for that analog giving a refined model, QSAR-2.

Analysis of the descriptors after a conformational search of compound **8c** using QSAR-1 reveals that this analog adopts conformations in which the metal−metal distance range is 9−10.4 Å, the plane angles are in the range 40−160° (with ca. 70% of all conformations having a plane angle in the range 80−110°), and the plane torsions are in the range −150−150°. For comparison, we repeated the conformational search of the *m*-phenylenebis(methylene)-linked analog **11c** using QSAR-1. Not surprisingly, **11c** exhibits similar conformational descriptors to **8c**: Metal−metal distances are in the range 8.8−10.4 Å, with the distribution maximum (including ~85% of all conformations) lying between 9.5 and 10.2 Å. Plane angles are in the range 15−175°, with a maximum in the distribution (ca. 40% of all conformations) being in the range 80−110°, and plane tortion angles are in the range −180−180°. However, since the N atom in the aromatic linker region of **8c** is capable of pendant complexation with the adjacent cyclam ring, the conformational search for sterically allowed conformations was repeated using the refined QSAR-2 model. A starting pendant conformation was chosen in which the distance between the dummy atom and the N atom in the linker was fixed at 2.11 Å (an average value for the N−Ni distance of the pendant group in several published crystal structures of pendant-coordinated complexes[15,19,20,29c] but which also approximates the length of a hydrogen bond), and only the two rotatable bonds of the linker to the cyclam ring not involved in pendant complexation were allowed to rotate at 10° increments (see compound **8c** in Figure 2). In striking contrast to the search results for **8c** using QSAR-1, the search results using QSAR-2 show that **8c** prefers conformations in which the metal−metal distance is 9.1−9.3 Å, the plane angles are in the range 70−120°, and the plane torsions are in the range 30−84°. The pendant conformation with the lowest energy is observed to have a metal−metal distance of 9.3 Å, a plane angle of 70°, and a plane torsion of 84°. Comparing these structural features with those of **11c** and the general descriptor requirements found to be necessary for potent antiviral activity in our earlier study with QSAR-1,[33] namely, (a) distance between metal-binding centers must be 9.5−11.5 Å, (b) plane torsions of −60−30° and 120−140° are allowed, and (c) plane angles of 40−70° and 110−140° are allowed, shows that none of these three structural features can be satisfied in the case of a pendant-complexed conformer of **8c**, and it would therefore be expected to have poor antiviral activity. Using the QSAR-2 model in which the pendant coordination indicator variable is given a value of 1, the EC_{50} for inhibition of HIV-1 by bicyclam **8c** was predicted to be 1 μM, while the experimentally observed activity is 16.4 μM (Table 3). A series of compounds from Table 1 were selected, and their predicted anti-HIV-1 EC_{50}s were calculated using the refined QSAR-2 model. The results show a strong correlation of the predicted versus the experimentally observed values (Table 3).

Table 3. Experimental vs Predicted EC_{50}s against HIV-1 Using the Refined QSAR-2 Model for Anti-HIV Bis-azamacrocycles

	EC_{50} (μM)	
compd	predicted	experimental
8a	0.06	0.02
8b	0.10	0.03
8c	1.00	16.4
8d	2.50	0.91
9a	0.04	0.03
9b	88.0	139
11c	0.10	0.03

The results for all bicyclam analogs using the refined QSAR-2 model which includes compounds able to take up pendant conformations resulted in an overall improved predictive model as reflected in an improvement in the cross-validated (predictive) r^2 value from 0.79 (QSAR-1) to 0.84 (QSAR-2). It is also evident that for the bicyclam analogs a quantitative relationship exists between the pendant indicator variable parameter and

antiviral activity and that all pendant conformations should be avoided if high antiviral activity is to be achieved.

Summary

The anti-HIV activity of a series of heteroaromatic linked bicyclam analogs has been evaluated. Depending upon the substitution pattern of the linker connecting the tetraazamacrocyclic rings, we observed a striking anti-HIV structure−activity relationship, which is consistent with a model in which the heteroatom of the linker can participate in pendant conformations (complexation) with the adjacent azamacrocyclic ring. We suggest that this interaction provides the wrong molecular shape for binding to the target for bicyclam activity at the HIV-inhibitory step. We have provided preliminary evidence that the intramolecular interaction responsible for the inactivation may be due to the involvement of transition metal chelation or hydrogen bonding of the linker heteroatom with the shared protons of the azamacrocyclic ring at physiological pH. In either event, this is clearly an important molecular design consideration. Further work is in progress to resolve the apparent mechanistic paradox.

Experimental Section

General experimental procedures for the synthesis of bicyclams have been previously published.[3]

General Procedure A. 1,1′-[2,6-Pyridylbis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Trihydrate (8a). To a solution of 1,1′-[2,6-pyridylbis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (**4a**) (500 mg, 0.35 mmol) in acetic acid (16 mL) was added 48% aqueous HBr (Aldrich; 12 mL), and the mixture was heated to 110 °C with stirring for 48 h during which time a white crystalline solid precipitated from a dark brown solution. Upon cooling, the solid was collected by filtration, washed with acetic acid and then ether, and dried *in vacuo* to give **8a** (280 mg, 65%) as a white solid: mp 229−231 °C dec; ^{1}H NMR (D_2O) δ 1.89 (m, 4H), 2.01 (m, 4H), 2.69 (m, 4H), 2.90 (m, 4H), 3.04−3.45 (m, 24H), 3.98 (s, 4H), 7.68 (d, 2H, J = 8 Hz), 8.19 (t, 1H, J = 8 Hz); ^{13}C NMR (D_2O) δ 19.35, 19.78, 37.77, 37.95, 39.07, 41.47, 41.70, 41.89, 46.17, 49.41, 58.23, 126.35, 143.22, 151.02; FAB MS *m/z* (rel intensity) 586 (MH + H^{81}Br, 47), 584 (MH + H^{79}Br, 50), 504 (M + H, 100), 306 (19), 201 (60). Anal. ($C_{27}H_{53}N_9$·8HBr·3.5H_2O) C, H, N, Br.

1,1′-[3,5-Pyridylbis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Nonahydrobromide Dihydrate (8b). Using general procedure A, 1,1′-[3,5-pyridylbis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (**4b**) (350 mg, 0.245 mmol) gave **8b** (150 mg, 53%) as a white solid: mp 252−254 °C dec; ^{1}H NMR (D_2O) δ 1.92 (m, 4H), 2.04 (m, 4H), 2.57 (m, 4H), 2.74 (m, 4H), 3.07−3.46 (m, 24H), 3.92 (s, 4H), 8.58 (s, 1H), 8.69 (s, 2H); ^{13}C NMR (D_2O) δ 20.11, 22.09, 38.89, 39.45, 42.26, 42.56, 42.73, 43.36, 47.42, 49.75, 54.38, 136.80, 141.92, 149.55; FAB MS *m/z* (rel intensity) 586 (MH + H^{81}Br, 40), 584 (MH + H^{79}Br, 41), 504 (M + H, 60), 305 (20), 201 (100). Anal. ($C_{27}H_{53}N_9$·9HBr·2.5H_2O) C, H, N, Br.

1,1′-[2,4-Pyridylbis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Tetrahydrate (8c). Using general procedure A, 1,1′-[2,4-pyridylbis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (**4c**) (270 mg, 0.189 mmol) gave **8c** (120 mg, 52%) as a white solid: mp 220−222 °C dec; ^{1}H NMR (D_2O) δ 1.79−1.96 (m, 4H), 2.03−2.21 (m, 4H), 2.39 (m, 4H), 2.78−2.95 (m, 4H), 3.04−3.56 (m, 24H), 3.93 (s, 2H), 4.02 (s, 2H), 7.86 (d, 1H, J = 8 Hz), 7.92 (s, 1H), 8.59 (d, 1H, J = 8 Hz); ^{13}C NMR (D_2O) δ 19.88, 20.01, 22.18, 22.38, 38.74 (2C), 38.79, 38.99, 39.19, 42.06, 42.13, 42.42, 42.55, 42.72, 42.95, 43.28, 47.73, 47.95, 50.43, 50.89, 56.15, 57.41, 127.14, 128.29, 142.20, 152.66, 158.89; FAB MS *m/z* (rel intensity) 586 (MH + H^{81}Br, 39), 584 (MH + H^{79}Br, 40), 504 (M + H, 76), 305 (40), 201 (100). Anal. ($C_{27}H_{53}N_9$·8HBr·4H_2O) C, H, N, Br.

1,1′-[2,5-Pyridylbis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Nonahydrobromide Pentahydrate (8d). Using general procedure A, 1,1′-[2,5-pyridylbis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (**4d**) (350 mg, 0.245 mmol) gave **8d** (180 mg, 56%) as a white solid: mp 241−243 °C dec; ^{1}H NMR (D_2O) δ 1.89 (m, 4H), 2.10 (m, 4H), 2.61−2.72 (m, 4H), 2.82−2.97 (m, 4H), 3.12−3.58 (m, 24H), 3.96 (s, 2H), 4.02 (s, 2H), 7.87 (d, 1H, J = 9 Hz), 8.39 (d, 1H, J = 9 Hz), 8.67 (s, 1H); ^{13}C NMR (D_2O) δ 19.09, 19.89, 20.91, 20.97, 38.29, 38.71 (3C), 39.02, 40.67, 41.81, 42.07, 42.26, 42.42, 42.46, 43.13, 46.59, 47.81, 49.24, 50.81, 54.72, 56.28, 128.03, 132.87, 144.10, 148.17, 153.14; FAB MS *m/z* (rel intensity) 586 (MH + H^{81}Br, 25), 584 (MH + H^{79}Br, 27), 504 (M + H, 68), 306 (37), 201 (100). Anal. ($C_{27}H_{53}N_9$·9HBr·5H_2O) C, H, N, Br.

1,1′-[2,6-Pyrazylbis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Monohydrate (9a). Using general procedure A, 1,1′-[2,6-pyrazylbis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (**5a**) (500 mg, 0.35 mmol) gave **9a** (95 mg, 23%) as a white solid: mp 214−217 °C dec; ^{1}H NMR (D_2O) δ 1.83 (m, 4H), 1.91 (m, 4H), 2.63 (m, 4H), 2.71 (m, 4H), 2.82−3.32 (m, 24H), 3.82 (s, 4H), 8.42 (s, 2H); ^{13}C NMR (D_2O) δ 20.25, 20.92, 38.71, 39.65, 41.13, 42.56, 42.78 (2C), 47.27, 49.86, 55.84, 144.27, 150.35; FAB MS *m/z* (rel intensity) 587 (MH + H^{81}Br, 60), 585 (MH + H^{79}Br, 66), 505 (M + H, 100). Anal. ($C_{26}H_{52}N_{10}$·8HBr·H_2O·0.5HOAc) C, H, N, Br.

1,1′-[2,5-Pyrazylbis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrochloride Trihydrate (9b). To a stirred solution of 1,1′-[2,5-pyrazylbis(methylene)]bis[4,8,11-tris(diethoxyphosphoryl)-1,4,8,11-tetraazacyclotetradecane] (**5b**) (240 mg, 0.181 mmol) in THF (10 mL) was bubbled HCl_g, and the pale yellow solution was stirred at room temperature for 20 h, during which time a solid precipitated. The reaction mixture was concentrated to dryness, and the residue was partitioned between H_2O (10 mL) and CH_2Cl_2 (10 mL). The aqueous phase was then separated and treated with charcoal (100 mg) with heating to 80 °C for 30 min. The hot solution was filtered through Celite, and the filtrate was concentrated to ca. 1 mL, after which acetic acid was added resulting in the immediate formation of a white precipitate. The solid was collected by filtration to give **9b** (80 mg, 50%): ^{1}H NMR (D_2O) δ 1.78−2.10 (m, 8H), 2.71 (m, 8H), 2.89−3.37 (m, 22H), 3.42 (s, 2H), 3.81 (s, 4H), 8.58 (s, 2H); ^{13}C NMR (D_2O) δ 19.13, 21.71, 38.30, 40.37, 41.97, 42.43, 43.82, 44.55, 49.41, 51.89, 55.38, 145.04, 151.18; FAB MS *m/z* (rel intensity) 542 (MH + H^{37}Cl, 58), 506 (M + H, 44), 306 (32), 201 (100), 93 (100). Anal. ($C_{26}H_{52}N_{10}$·8HCl·3.4H_2O·HOAc) C, H, N, Cl.

1,1′-[2-Amino-1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Nonahydrobromide Monohydrate (10a). To a solution of 1,1′-[2-nitro-1,4-phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (**6a**) (900 mg, 0.61 mmol) in ethanol (75 mL) were added iron powder (900 mg) and concentrated HCl (1 mL), and the mixture was heated to reflux for 2.5 h with rapid stirring. The hot solution was made basic by the dropwise addition of aqueous NaOH solution (10 M). The solution was filtered hot and the solvent evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (75 mL), boiled with charcoal (50 mg), and filtered and the solvent removed under reduced pressure to give 1,1′-[2-amino-1,4-phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (680 mg, 77%) as a pale yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.55−1.75 (m, 4H), 1.85−2.05 (m, 4H), 2.25−2.45 (m, 22H), 2.55−2.75 (m, 4H), 2.85−3.35 (m, 24H), 3.47 (s, 2H), 3.57 (s, 2H), 6.55 (d, 1H, J = 7.5 Hz), 6.59 (s, 1H), 6.89 (d, 1H, J = 7.5 Hz), 7.25−7.45 (m, 12H), 7.50−7.80 (m, 12H); FAB MS *m/z* (rel intensity) 1443 (M + H, 27), 1287 (18), 708 (100), 663 (41), 624 (25). This was used without further purification.

The amino compound from above (500 mg, 0.35 mmol) was deprotected using general procedure A to give **10a** (250 mg, 58%) as a white solid: mp 255−260 °C dec; ^{1}H NMR (D_2O) δ

1.75−2.15 (m, 8H), 2.75−3.45 (m, 32H), 3.89 (s, 2H), 4.06 (s, 2H), 7.11−7.25 (m, 2H), 7.34 (d, 1H, J = 8.2 Hz); ^{13}C NMR (D_2O) δ 23.57, 23.67, 24.15, 25.35, 41.89, 42.08, 42.35 (3C), 43.67, 45.64, 45.76, 46.04 (2C), 46.22, 46.50, 49.04, 50.05, 52.38, 53.16, 60.47, 63.12, 128.62, 130.07, 132.15, 136.14, 138.06, 143.07; FAB MS m/z (rel intensity) 601 (MH + H^{81}Br, 91), 599 (MH + H^{79}Br, 91), 519 (M + H, 77), 473 (55). Anal. ($C_{28}H_{55}N_9$·9HBr·H_2O·1.5HOAc) C, H, N, Br.

1,1′-[2-Carboxy-1,4-phenylenebis(methylene)]bis-[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide (10b). Using general procedure A, 1,1′-[2-carbomethoxy-1,4-phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (**6b**) (280 mg, 0.189 mmol) gave **10b** (160 mg, 68%) as a white solid: mp 247−251 °C dec; IR (CsI) ν 1705, 1615, 1576, 1456, 1208, 1076 cm^{-1}; ^{1}H NMR (D_2O) δ 1.92−2.07 (m, 8H), 2.97 (m, 2H), 3.02−3.43 (m, 26H), 3.47 (s, 4H), 4.05 (s, 2H), 4.36 (s, 2H), 7.50 (m, 2H), 7.81 (s, 1H); ^{13}C NMR (D_2O) δ 19.01, 19.20, 19.50, 19.58, 37.82 (4C), 37.98, 38.28, 41.38, 41.70 (3C), 42.12, 42.27, 45.39, 45.61, 48.51, 48.65, 58.42, 59.01, 131.91, 133.17, 133.98, 134.73, 135.44, 135.97, 171.25; FAB MS m/z (rel intensity) 629 (MH + H^{81}Br, 5), 627 (MH + H^{79}Br, 5), 547 (M + H, 82), 349 (40), 201 (100). Anal. ($C_{29}H_{64}N_8O_2$·8HBr·HOAc) C, H, N, Br.

General Procedure B. 1,1′-[2-Carbomethoxy-1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Trihydrate (10c). To a stirred solution of 1,1′-[2-carbomethoxy-1,4-phenylenebis(methylene)]-bis[4,8,11-tris(diethoxyphosphoryl)-1,4,8,11-tetraazacyclotetradecane] (**6c**) (250 mg, 0.182 mmol) in acetic acid (2 mL) was added 30% HBr in acetic acid (Aldrich; 6 mL), and the solution was stirred at room temperature for 3 h. The resulting precipitate was collected by filtration, washed with acetic acid and ether, and dried *in vacuo* giving **10c** (130 mg, 57%) as a white powder: mp 212−214 °C dec; IR (CsI) ν 1715, 1615, 1575, 1476, 1215, 1078 cm^{-1}; ^{1}H NMR (D_2O) δ 1.90−1.99 (m, 8H), 2.83 (m, 2H), 2.93−3.64 (m, 30H), 3.87 (s, 3H), 3.97 (s, 2H), 4.54 (s, 2H), 7.59 (m, 2H), 8.02 (s, 1H); ^{13}C NMR (D_2O) δ 18.65, 19.24, 19.81, 20.07, 37.38, 37.55, 37.65, 38.65, 39.17 (2C), 41.13, 41.43 (2C), 41.92, 42.39, 42.83, 45.31, 46.15, 48.48, 48.92, 54.10, 57.95, 58.99, 131.63, 134.37, 134.66, 135.55, 136.42 (2C), 168.75; FAB MS m/z (rel intensity) 643 (M + H^{81}Br, 25), 641 (M + H^{79}Br, 24), 562 (M + H, 66), 363 (100). Anal. ($C_{30}H_{53}N_8O_2$·8HBr·$3H_2O$) C, H, N, Br.

1,1′-[5-Amino-1,3-phenylenebis(methylene)]bis-[1,4,8,11-tetraazacyclotetradecane] Nonahydrobromide Dihydrate (11a). In a similar manner to the preparation of **10a** described above, 1,1′-[5-nitro-1,3-phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (**7a**) (300 mg, 0.2 mmol) gave 1,1′-[5-amino-1,3-phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (240 mg, 83%) as a pale yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.65−1.85 (m, 4H), 1.90−2.05 (m, 4H), 2.25−2.55 (m, 22H), 2.65−2.75 (m, 4H), 2.95−3.35 (m, 24H), 3.42 (s, 4H), 6.49 (s, 1H), 6.55 (s, 2H), 7.15−7.45 (m, 12H), 7.55−7.75 (m, 12H); FAB MS m/z (rel intensity) 1442 (M, 100), 1286 (64), 1130 (13), 663 (36). This was used without further purification.

The 5-amino compound from above (120 mg, 0.08 mmol) was deprotected using general procedure A to give **11a** (80 mg, 77%) as a white solid: mp 235−237 °C dec; ^{1}H NMR (D_2O) δ 1.85−2.15 (m, 8H), 3.15−3.65 (m, 32H), 4.36 (s, 4H), 7.52 (s, 2H), 7.67 (s, 1H); ^{13}C NMR (D_2O) δ 18.99, 19.09, 37.44, 37.59, 37.71, 38.00, 41.28, 41.66, 44.89, 48.09, 57.78, 126.96, 132.91, 133.32, 133.69; FAB MS m/z (rel intensity) 600 (M + H^{81}Br, 19), 598 (M + H^{79}Br, 19), 519 (M + H, 24), 320 (18), 201 (100). Anal. ($C_{28}H_{55}N_9$·9HBr·$2.2H_2O$) C, H, N, Br.

1,1′-[5-Carboxy-1,3-phenylenebis(methylene)]bis-[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Dihydrate (11b). Using general procedure A, 1,1′-[5-carbomethoxy-1,3-phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (**7b**) (810 mg, 0.543 mmol) gave **11b** (350 mg, 52%) as a white solid: mp 234−237 °C dec; IR (CsI) ν 1715, 1609, 1576, 1454, 1202, 1065 cm^{-1}; ^{1}H NMR (D_2O) δ 2.03 (m, 8H), 3.04−3.66 (m, 32H), 4.35 (s, 4H), 7.81 (s, 1H), 8.07 (s, 2H); ^{13}C NMR (D_2O) δ 18.71, 19.06, 37.41, 37.61 (2C), 41.12 (2C), 41.65, 44.69, 48.00, 58.23, 131.43, 132.53, 133.78, 138.30, 168.84; FAB MS m/z (rel intensity) 629 (M + H^{81}Br, 14), 627 (M + H^{79}Br, 15), 548 (M + H, 58), 185 (45), 93 (100). Anal. ($C_{29}H_{54}N_8O_2$·8HBr·$2H_2O$) C, H, N, Br.

1,1′-[2,4-Quinolinylbis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Nonahydrobromide Pentahydrate (17a). Using general procedure B, 1,1′-[2,4-quinolinylbis-(methylene)]bis[4,8,11-tris(diethoxyphosphoryl)-1,4,8,11-tetraazacyclotetradecane] (170 mg, 0.13 mmol) gave **17a** (0.15 g, 94%) as a white solid: mp 222−224 °C dec; ^{1}H NMR (D_2O) δ 1.75−2.15 (m, 8H), 2.55−3.45 (m, 32H), 4.24 (s, 2H), 4.41 (s, 2H), 7.80 (t, 1H, J = 7.5 Hz), 7.96 (t, 1H, J = 7.8 Hz), 8.13 (s, 1H), 8.22 (d, 1H, J = 8.4 Hz), 8.31 (d, 1H, J = 8.4 Hz); FAB MS m/z (rel intensity) 636 (MH + H^{81}Br, 11), 634 (MH + H^{79}Br, 11), 554 (M + H, 13), 356 (14), 229 (37), 201 (100). Anal. ($C_{31}H_{55}N_9$·9HBr·$5.5H_2O$) C, H, N, Br.

2,4-Dimethyl-6-phenylpyridine (20). To a solution of 2-bromo-4,6-dimethylpyridine (**19**) (3.0 g, 16.1 mmol), phenylboric acid (2.16 g, 17.7 mmol, 1.1 equiv), and sodium carbonate (3.59 g, 33.9 mmol, 2.1 equiv) in toluene (200 mL), ethanol (50 mL), and water (50 mL) was added $Pd(PPh_3)_4$ (932 mg, 5 mol %), and the mixture was heated to reflux overnight with rapid stirring. Upon cooling, the reaction mixture was diluted with CH_2Cl_2, washed with a solution of saturated aqueous sodium bicarbonate, dried ($MgSO_4$), and evaporated to dryness. The residue was dissolved in boiling hexane, and the solid which formed was removed by filtration. The filtrate was allowed to cool, during which time a white solid precipitated (triphenylphosphine) which was also removed by filtration. This procedure was repeated until all of the triphenylphosphine had been removed giving **20** as a light yellow liquid upon evaporation (2.7 g, 90%): ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 2.58 (s, 3H), 7.33 (s, 1H), 7.37−7.47 (m, 3H), 7.95 (dd, 2H, J = 8.3, 1.6 Hz). This was used without further purification.

6-Phenyl-2,4-pyridinedicarboxylic Acid (21). To a solution of **20** (2.0 g, 10.9 mmol) in a mixture of H_2O (30 mL) and *t*-BuOH (60 mL) maintained at a temperature of 100 °C with stirring was added $KMnO_4$ (10.34 g, 6.0 equiv) in one portion. The reaction mixture was heated overnight, during which time a brown solid precipitated which was removed by hot filtration through Celite. The filtrate was evaporated to a small volume and then acidified to pH 4 with concentrated aqueous HCl which precipitated a white solid. The solid was collected by filtration and dried *in vacuo* to give **21** (0.95 g, 3.91 mmol, 36%): ^{1}H NMR (DMSO-d_6) δ 7.42−7.60 (m, 3H), 8.20 (dd, 2H, J = 7.9, 1.4 Hz), 8.32 (d, 1H, J = 1.2 Hz), 8.46 (d, 1H, J = 1.2 Hz).

6-Phenyl-2,4-pyridinedimethanol (22). To a solution of **21** (0.92 g, 3.79 mmol) in anhydrous THF (15 mL) with stirring was added BH_3·THF (Aldrich; 1.0 M solution in THF, 37.8 mL, 10.0 equiv), and the mixture was heated to 60 °C with stirring overnight. The mixture was evaporated to dryness and the residue dissolved in anhydrous methanol and evaporated once again (repeated three times). The residue was dissolved in 1 N HCl and then made basic with 10 N sodium hydroxide to pH 14, during which time a white solid precipitated. The aqueous solution was extracted with CH_2Cl_2 (3 × 50 mL) and then dried ($MgSO_4$) and evaporated to give **22** as a white solid (0.75 g, 3.49 mmol, 93%): ^{1}H NMR (DMSO-d_6) δ 4.60−4.62 (m, 4H), 5.45 (m, 2H), 7.38−7.48 (m, 4H), 7.70 (s, 1H), 8.05 (d, 2H, J = 7.8 Hz).

6-Phenyl-2,4-bis(bromomethyl)pyridine Hydrobromide (23). To a solution of the diol **22** (160 mg, 0.74 mmol) in acetic anhydride (6 mL) cooled to 0 °C was added hydrobromic acid (Aldrich; 48% aqueous solution, 5 mL) dropwise with stirring, and the mixture was then heated to reflux overnight. Upon cooling the solution was triturated with ether to give **23** as a white crystalline solid (150 mg, 0.36 mmol, 47%): ^{1}H NMR (DMSO-d_6) δ 4.74 (s, 2H), 4.77 (s, 2H), 7.40−7.56 (m, 3H), 7.59 (s, 1H), 7.96 (s, 1H), 8.10 (m, 2H).

1,1′-[6-Phenyl-2,4-pyridylbis(methylene)]bis[4,8,11-tris-(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (24). Dimerization of **2a** (444 mg, 0.65 mmol) with **23** (125 mg, 0.3 mmol) under standard conditions[3] and purification of the crude product by column chromatography on silica gel (CH_2Cl_2/MeOH/Et_3N, 98:2:1) gave **24** (420 mg, 0.28 mmol, 94%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.78 (m, 2H), 1.92 (m, 2H),

2.32−2.43 (multiple s, 18H), 2.50−2.80 (m, 8H), 3.11−3.23 (m, 24H), 3.66 (s, 2H), 3.81 (s, 2H), 7.16 (d, 2H, J = 7.8 Hz), 7.22−7.69 (m, 27H), 7.98 (m, 2H); FAB MS m/z (rel intensity) 1503 (M + H, 100), 1348 (18).

1,1′-[6-Phenyl-2,4-pyridylbis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Nonahydrobromide (25a). Deprotection of **24** (400 mg, 0.27 mmol) using general procedure A gave **25a** (55 mg, 16%) as a white solid: ^{1}H NMR (D_2O) δ 1.65 (m, 2H), 1.90 (m, 6H), 2.60 (m, 2H), 2.72 (m, 2H), 2.80 (m, 6H), 2.98 (m, 8H), 3.11−3.21 (m, 12H), 3.29 (m, 2H), 3.89 (m, 4H), 7.49 (m, 3H), 7.55 (s, 1H), 7.63−7.65 (m, 3H); FAB MS m/z (rel intensity) 662 (MH + H^{81}Br, 17), 660 (MH + H^{79}Br, 16), 580 (M + H, 57), 382 (38), 201 (100). Anal. ($C_{33}H_{57}N_9$·9HBr) C, H, N, Br.

1-(2-Pyridylmethylene)-1,4,8,11-tetraazacyclotetradecane Nickel Diperchlorate (26). To a stirred solution of 1-(2-pyridylmethylene)-1,4,8,11-tetraazacyclotetradecane (**15**) (236 mg, 0.81 mmol) in dry ethanol (5 mL) was added a solution of nickel(II) perchlorate (296 mg, 1.0 equiv) in *n*-butanol (which had been partially distilled to remove most of the hydrated water, leaving ca. 5 mL). During the addition a purple precipitate formed from the virtually colorless solution. The mixture was allowed to stir for 30 min, and the solid was then collected by filtration, washed with ethanol and then ether, and dried *in vacuo* giving **26** (380 mg, 86%) as a purple powder: IR (KBr) ν 3427, 3269, 3151, 2924, 2868, 1606, 1442, 1141, 1111, 1087, 763, 626, 426 cm^{-1}. Anal. ($C_{16}H_{29}N_5$-$NiCl_2O_8$) C, H, N, Cl.

Potentiometric Titrations. An aqueous (50 mL) solution of the compound (0.1 mM) and 4 equiv of $HClO_4$ at 25 °C and I = 0.1 M ($NaClO_4$) was titrated with 0.1 M NaOH according to literature procedures.[20] The pH values were read with a Corning 345 pH meter. The electrode was calibrated with pH 4.00 and 7.00 buffer solutions and checked with a theoretical titration curve of 0.4 mM $HClO_4$ and I = 0.1 M ($NaClO_4$) with 0.1 M NaOH solution. The perchloric acid was calibrated by titration against standard NaOH solution.

UV Absorption Spectra at Varying pHs. A stock solution of **15** (3.4 mM) in 0.1 M $NaClO_4$ was prepared. Aliquots of 100 μL of the stock solution were diluted with varying amounts of 0.4 M $HClO_4$ (5−500 μL), and the mixture was brought to an overall volume of 2 mL with 0.1 M $NaClO_4$. This gave pH values in the range 1−7 and kept the concentration of **15** at 0.17 mM. The UV absorption spectra of these solutions were recorded on a Perkin-Elmer Lambda 2 UV/vis double-beam spectrometer with a cell path length of 1 cm and a scan range of 400−200 nm.

Anti-HIV Activity Assays. The human immunodeficiency virus strains used were HIV-1 (III_B) and HIV-2 (ROD) whose origin has been described previously.[1] Anti-HIV activity and cytotoxicity measurements were carried out in parallel. They were based on the viability of MT-4 cells that had been infected with HIV and then exposed to various concentrations of the test compounds. After the MT-4 cells were allowed to proliferate for 5 days, the number of viable cells was quantified by a tetrazolium-based colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) procedure in 96-well microtrays.[34] In all of these assays, viral input (viral multiplicity of infection, MOI) was 0.01, or 100 times the 50% cell culture infective dose ($CCID_{50}$). The 50% antivirally effective concentration (EC_{50}) was defined as the compound concentration required to protect 50% of the virus-infected cells against viral cytopathicity. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration required to reduce the viability of mock-infected cells by 50%. The > symbol is used to indicate the highest concentration at which the compounds were tested and still found noncytotoxic. Average EC_{50} and CC_{50} values for several separate experiments are presented as defined above. As a rule, the individual values did not deviate by more than 2-fold up or down from the EC_{50} and CC_{50} values indicated in the tables.

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