Synthesis and Structure–Activity Relationships of Phenylenebis(methylene)-Linked Bis-Tetraazamacrocycles That Inhibit HIV Replication. Effects of Macrocyclic Ring Size and Substituents on the Aromatic Linker

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Received August 30, 1994®

We have previously described the potent and selective inhibition of several strains of human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) by JM2763, an *n*-propyl-linked dimer of the 1,4,8,11-tetraazamacrocyclic (cyclam) ring system. Upon further investigation, we have also found that incorporating an aromatic rather than aliphatic linker leads to analogs with higher antiviral potency. The prototype, JM3100 (**19a**, isolated as the octahydrochloride salt), which contains a *p*-phenylenebis(methylene) moiety linking the cyclam rings, inhibited the replication of HIV-1 (III_B) and HIV-2 (ROD) at EC_{50}'s of 4.2 and 5.9 nM, respectively, while remaining nontoxic to MT-4 cells at concentrations exceeding 421 μM. In order to identify the structural features of bis-tetraazamacrocycles required for potent activity, we have prepared a novel series of phenylenebis(methylene)-linked analogs, in which the macrocyclic ring size was varied from 12 to 16 ring members. Depending upon the substitution of the phenylenebis-(methylene) linker (*para* or *meta*), sub-micromolar anti-HIV activity was exhibited by analogs bearing macrocycles of 12–14 ring members but with varying cytotoxicity to MT-4 cells. Furthermore, while we found that identical macrocyclic rings are not required for activity, substituting an acyclic polyamine equivalent for one of the cyclam rings in **19a** resulted in a substantial reduction in anti-HIV potency, clearly establishing the importance of the constrained macrocyclic structure. A short series of transition metal complexes of **19a** were also prepared and evaluated. Complexes of low kinetic stability such as the bis-zinc complex retained activity comparable to that of the parent compound. Finally, the activity of bicyclam analogs appears to be insensitive to the electron-withdrawing or -donating properties of substituents introduced onto the linker, but sterically hindering groups such as phenyl markedly reduced activity. As a result, several analogs with anti-HIV potency comparable to that of **19a** have been identified.

Introduction

The search for effective chemotherapeutic treatments for human immunodeficiency virus (HIV) infections has led to the development of agents that target specific and critical events in the HIV replicative cycle. The most extensively studied of these agents are the 2′,3′-dideoxynucleoside analogs AZT, DDC, and DDI, which terminate DNA synthesis during the reverse transcription (RT) reaction;[1,2] the non-nucleoside RT inhibitors,[3–5] which interact at a specific site on HIV-1 RT, designated the TIBO site;[6] and inhibitors of HIV protease, an essential proteolytic enzyme required for the assembly of fully infectious viral particles.[7–12] Mechanistic studies have revealed that the prototype bis-cyclams JM2763 (**1**) and JM3100 (**19a**, isolated as the octahydrochloride salt) (Figure 1) interact at an early stage in the HIV replicative cycle, tentatively assigned as a virus-associated uncoating process.[13–15] This process has previously been suggested as a target for anti-HIV agents, since inhibiting the release of viral RNA from the capsid proteins into the cells should disrupt the replicative cycle before reverse transcription can occur.[16]

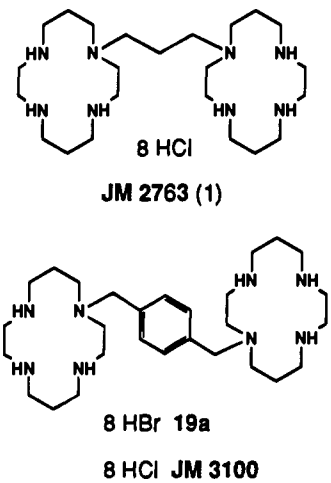

Figure 1. Structures of the bicyclam analogs JM2763 and JM3100.

In the present work, we report the synthesis and anti-HIV activity of a series of novel phenylenebis(methylene)-linked bis-tetraazamacrocyclic analogs. Systematic variations in the size of the azamacrocyclic ring and

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® Abstract published in *Advance ACS Abstracts*, January 1, 1995.

0022-2623/95/1838-0366$09.00/0

substituents on the linker have led to the discovery of a number of bis-tetraazamacrocycles with high potency and selectivity against HIV.

Chemistry

The phenylenebis(methylene)-linked bis-tetraazamacrocycles having 12–16 ring members (**16–21a–c**, Table 1) were prepared by reaction of the tris-N-protected tetraazamacrocycles (**2a**, **3**, **8a–d**, Scheme 1) with the appropriate aromatic bis-electrophiles (Scheme 2) according to literature procedures.[17,18] Two approaches were used to prepare the series of tris-N protected tetraazamacrocycles: (a) reaction of commercially available [14]aneN$_4$[19] (cyclam) and [12]aneN$_4$ tetraazamacrocycles with p-toluenesulfonyl chloride to give **2a** and **3**, respectively, using known methods[17,20] or (b) macrocyclization of the bis-sulfonates **5a–d** with the toluenesulfonamides **6a** and **6b** followed by a selective deprotection as summarized in Scheme 1. Using the strategy reported by Kaden,[21] the protected [*iso*-14]aneN$_4$ (isocyclam) macrocycle **8a** was prepared from the versatile bis-toluenesulfonamide precursor **6a** which contained a benzyl group targeted for selective deprotection. The requisite tris-*p*-toluenesulfonate portion (**5a**) was obtained by tosylation of diethanolamine (**4a**) in CH_2Cl_2 in the presence of Et_3N. A modified Richman–Atkins[22] cyclization of **5a** with the disodium salt of **6a** (prepared *in situ*, by reaction of **6a** with NaH in DMF) gave the benzyl compound **7a**, which was subjected to hydrogenolysis with $Pd(OH)_2$ in refluxing formic acid giving **8a**. In a similar manner, substituting dipropanolamine trimethanesulfonate (**5b**)[23] for **5a** in the cyclization reaction and subsequent hydrogenolysis of **7b** afforded the desired [16]aneN$_4$ macrocycle **8b**. Alternatively, synthesis of the appropriately protected [13]aneN$_4$ (**8c**) and [15]aneN$_4$ (**8d**) macrocycles was accomplished via the bis-methanesulfonates **5c** and **5d** in which a diethoxyphosphoryl (Dep) group[24] is targeted for the selective deprotection reaction. A two-step derivatization of the amino alcohols **4a** and **4b** with 1.0 equiv of diethyl phosphorochloridate (to give the phosphoramidate diols **4c** and **4d**), followed by 2.0 equiv of methanesulfonyl chloride, under standard conditions afforded **5c** and **5d** in a straightforward manner. Macrocyclization with tris(*p*-tolylsulfonyl)-*N*-(2-aminoethyl)-1,3-propanediamine (**6b**) in the presence of excess Cs_2CO_3[25] (or K_2CO_3[26]) gave **7c** and **7d**, respectively, in reasonable yields (50–55%) following purification by column chromatography on silica gel. Finally, selective removal of the phosphoryl group with 30% HBr/acetic acid at room temperature gave **8c** and **8d**.

With the series of tris-N protected tetraazamacrocycles in hand, we proceeded to the preparation of the *meta* and *para* phenylenebis(methylene)-linked dimers as illustrated in Scheme 2. Exclusive mono-N-alkylation of the available secondary amines with the corresponding dibromoxylene (0.5 equiv) gave the dimers **10–15a,b**. The *ortho* dimer of **2a** was prepared by bisacylation with phthaloyl dichloride to give the diamide **22**, which was reduced with BH_3·THF, affording **13c**. In the majority of cases, deprotection of the sulfonamido groups was accomplished by hydrolysis with concentrated sulfuric acid at 110 °C for 2–3 h followed by isolation of the free base and subsequent conversion to the octahydrobromide salt or by treatment with refluxing 48% aqueous HBr/acetic acid, which conveniently precipitates the octahydrobromide salt of the desired products in reasonable yields. However, repeated attempts at deprotection of **10a,b** ([12]aneN$_4$) and **11a,b** ([13]aneN$_4$) under these conditions resulted in significant cleavage of the single tetraazamacrocyclic ring from the dimer at the benzylic position. This problem was avoided by reductive removal of the tosyl groups with Na/Hg amalgam, affording **16a,b** and **17a,b**.

The preparation of compounds **28a,b**, **29**, **30**, and **32**, which contain nonidentical ring systems, is summarized in Scheme 3. In a typical synthesis, dropwise addition of **8a** into a large excess of **9a** avoided formation of the dimer **12a** in favor of the key bromo intermediate **24**. Subsequent alkylation of a second appropriately protected ring system such as **2a** afforded **25**. Deprotection with 48% aqueous HBr/acetic acid gave **28**. In order to prepare **35**, the 6,6′-carbon-linked dimer of the [14]aneN$_4$ (cyclam) ring system, we relied upon the previously reported strategy of malonate condensation with linear tetraamines, as shown in Scheme 4.[27–29] Thus, reaction of **9a** with 2 equiv of the sodium salt of diethyl malonate gave the tetraester **33**. Condensation with 1,4,8,11-tetraazaundecane in EtOH afforded the tetraamide **34**, which precipitated from the reaction mixture after 20 days at reflux. Reduction of **34** with BH_3·THF followed by aqueous HBr/acetic acid hydrolysis of the intermediate borane complex afforded **35**. Compounds **37–40** were prepared by derivatization/reaction of the free base of **19a** as indicated.

The bis-electrophiles required for the synthesis of the bicyclam analogs **46a–h** and **47a–f** (Table 2) were obtained via two general routes from commercially available aromatic derivatives as illustrated in Scheme 5 for the *para* linked compounds: (a) NBS bromination of a dimethyl aromatic derivative[30] or (b) BH_3·THF reduction of an aromatic diacid/diester to the diols **43a–h** followed by conversion to the corresponding dibromoxylenes using 48% aqueous HBr/acetic anhydride[31] or conversion to the bis-methanesulfonates using standard procedures. By analogy, 1,4-phenylenediacetic acid and 1,4-phenylenedipropionic acid were used as starting materials for the preparation of **48a,b**. The biphenyl intermediates, such as **44d**, were prepared from the appropriately substituted bromo aromatic derivative by palladium-catalyzed cross-coupling with phenylboronic acid according to known procedures.[32] Both dimerization of **2a** and detosylation were performed as previously described with the exception of **46d**, **46f**, and **47b**. These compounds proved extremely sensitive to the vigorous deprotection conditions (due to cleavage of the cyclam ring from the dimer, see above), and rather than switch to the Na/Hg amalgam procedure on this occasion, we completed their synthesis via the more readily deprotected tris-phosphoryl precursor **2b**.

Results and Discussion

In order to identify the key structural features of phenylenebis(methylene)-linked bis-tetraazamacrocycles that impart potent anti-HIV-1 and HIV-2 activity, a series of compounds were prepared in which the macrocyclic ring size was systematically varied between 12 and 16 members (**16–21a–c**, Table 1). Although these compounds broadly inhibited HIV-1 and HIV-2 replication (albeit at EC_{50}'s that vary over 4 orders of

Table 1. Anti-HIV Activity of Bis-Macrocycles with Varying Ring Size

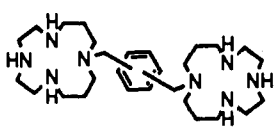
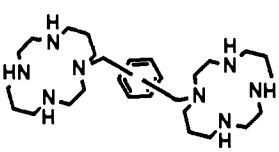
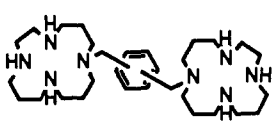
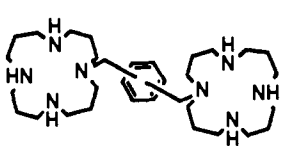
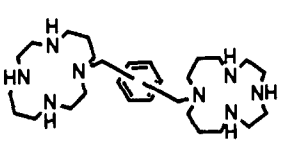
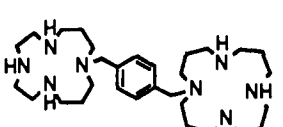
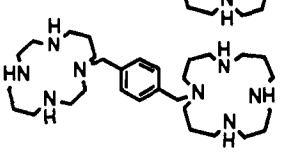
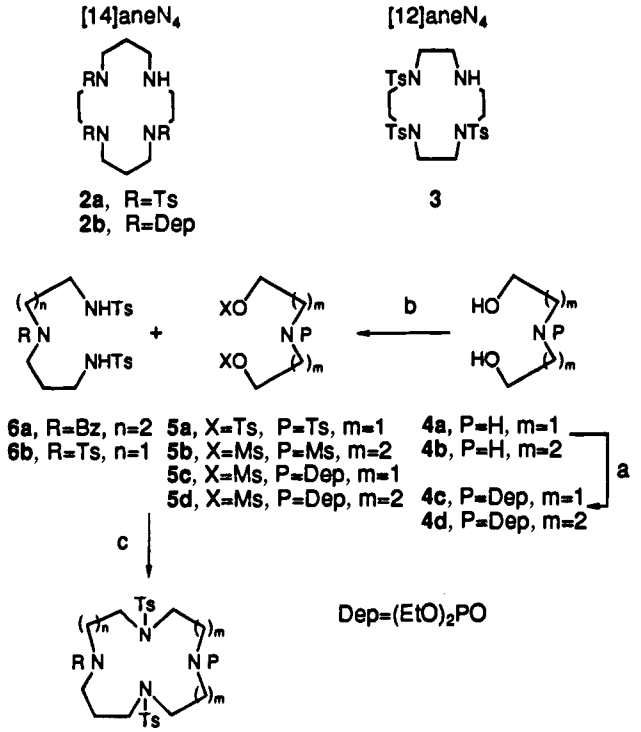
Compd.	Structure	Phenyl Subst.	Formula[a]	EC_{50} (μM)[b] HIV-1 (III_B)	EC_{50} (μM)[b] HIV-2 (ROD)	CC_{50}[c] (μM)
16a		para	$C_{24}H_{46}N_8.6HBr$	0.3218	2.3600	55
16b		meta	$C_{24}H_{46}N_8.6HBr$	0.0751	0.5364	20
17a		para	$C_{26}H_{50}N_8.8HBr.H_2O.HOAc$	0.1668	0.2341	> 208
17b		meta	$C_{26}H_{50}N_8.8HBr.HOAc$	0.0408	0.0618	> 184
18a		para	$C_{28}H_{54}N_8.8HBr.2H_2O$	0.0253	0.0590	> 421
18b		meta	$C_{32}H_{54}N_8.8HBr.2H_2O$	0.3226	0.6451	> 403
19a		para	$C_{28}H_{54}N_8.8HBr.2H_2O$	0.0042	0.0059	> 421
19b		meta	$C_{28}H_{54}N_8.8HBr.2H_2O$	0.0337	0.0422	> 421
19c		ortho	$C_{28}H_{54}N_8.8HBr.H_2O$	1.3574	3.1279	> 168
20a		para	$C_{30}H_{58}N_8.8HBr.4H_2O.HOAc$	1.6714	2.0072	171
20b		meta	$C_{30}H_{58}N_8.8HBr.4H_2O.HOAc$	2.7247	11.715	> 190
21a		para	$C_{32}H_{62}N_8.8HBr.3H_2O$	9.1301	13.695	48
21b		meta	$C_{32}H_{62}N_8.8HBr.2H_2O$	16.739	71.519	193
28a		para	$C_{28}H_{54}N_8.8HBr.2H_2O$	0.0079	0.0556	> 397
28b		meta	$C_{28}H_{54}N_8.8HBr$	0.0843	0.7588	> 421
29			$C_{30}H_{58}N_8.8HBr.H_2O$	0.3177	3.1767	101
30			$C_{30}H_{58}N_8.8HBr.H_2O$	2.4336	11.5535	> 209
32			$C_{28}H_{52}N_8.8HBr.H_2O.HOAc$	0.3730	0.7110	> 444
35			$C_{28}H_{54}N_8.8HBr.3.3H_2O.HOAc$	0.5059	0.6745	406
37			$C_{40}H_{78}N_8.8HBr.8H_2O$	7.7947	14.564	> 341
38			$Zn_2Cl_4C_{28}H_{54}N_8.H_2O$	0.0033	0.0024	> 251

Table 1 (Continued)

Compd.	Structure	Phenyl Subst.	Formula[a]	EC_{50} (μM)[b] HIV-1 (III_B)	HIV-2 (ROD)	CC_{50}[c] (μM)
39			$Cu_2(OAc)_4C_{28}H_{54}N_8.7H_2O$	0.0181	0.0272	> 201
40			$Pd_2(ClO_4)_4C_{28}H_{54}N_8.4H_2O$	31.548	59.299	> 210
41	N-(4-methyl)benzylcyclam		$C_{18}H_{32}N_4.4HBr.H_2O$	1.4169	1.1462	> 324
42	Cyclam[d]			399	150	> 1248

[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 greater than symbol (>) is used to indicate the highest concentrations at which the compounds were tested and still found to be noncytotoxic. [d] Available from Aldrich, tested as the free base.

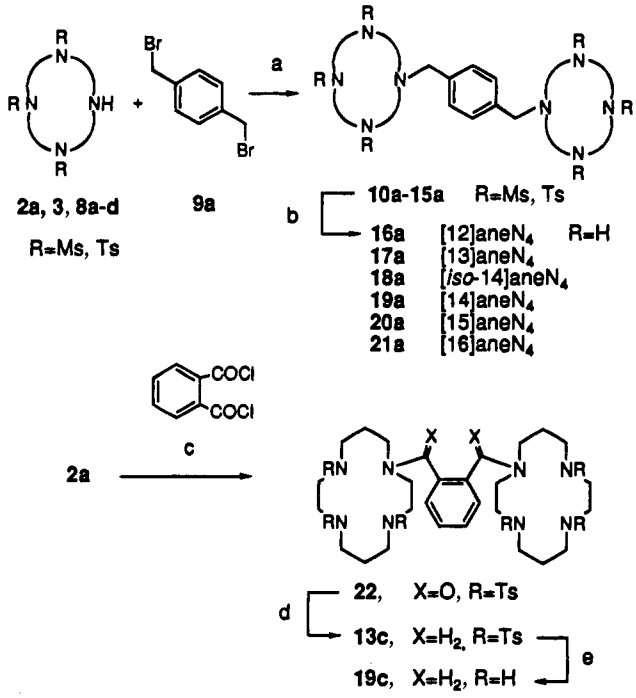
Scheme 1[a]



[a] Reagents: (a) $(EtO)_2POCl$, Et_3N, CH_2Cl_2; (b) Ts-Cl or Ms-Cl, Et_3N, CH_2Cl_2; (c) R = Bz: NaH, DMF, 100 °C; R = Dep: Cs_2CO_3, DMF, 55–60 °C; (d) $Pd(OH)_2$, HCO_2H, reflux; (e) HBr/HOAc, room temperature, 3h.

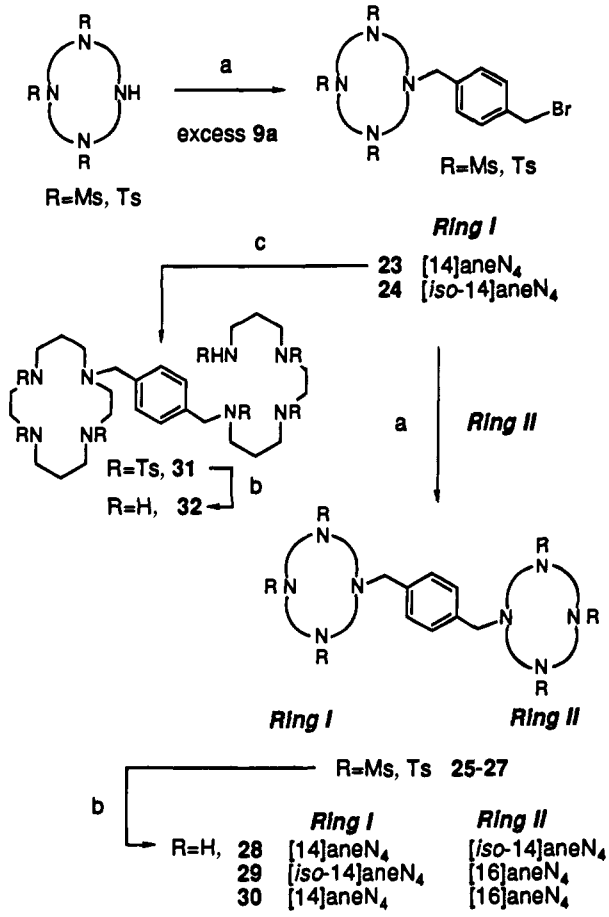
magnitude), potent activity was found to be specific for the size of the tetraazamacrocyclic rings and the substitution of the phenylenebis(methylene) linker which connects them. A comparison of the effects of macrocyclic ring size on anti-HIV potency was made for compounds in which the substitution of the phenylenebis(methylene) linker is identical. In general, increasing the size of the macrocyclic ring from 12 to 14 ring members resulted in increases in both the anti-HIV-1 and HIV-2 activity for the *para* series (**16**–**19a**) and the *meta* series (**16**–**19b**) while cytotoxicity decreased. A notable exception is the anti-HIV-1 and HIV-2 activity of compound **18b** ([*iso*-14]aneN4, isocyclam) among the series of *meta* analogs. In this case, compounds **16b** ([12]aneN4) and **17b** ([13]aneN4) proved more potent than **18b**, whereas the alternative 14-membered ring isomer **19b** ([14]aneN4, cyclam) exhibited the highest activity of the *meta* series. However, once the size of the macrocyclic ring exceeded 14 ring members, a substantial reduction in anti-HIV potency was observed. Using activity against HIV-1 as a representative example, the *para* [15]aneN4 dimer **20a** was approximately 400 times less potent than **19a** while the *meta* analog **20b** was 80 times less potent than **19b** and the 16-membered dimers (**21a**,**b**) exhibited EC_{50} values that were less than 12-fold lower than their CC_{50}'s in MT-4 cells.

Scheme 2[a]



[a] Reagents: (a) 0.5 equiv of α,α′-dibromo-*p*-xylene, K_2CO_3, CH_3CN, reflux; (b) deprotection: 48% aqueous HBr, HOAc, reflux or concentrated H_2SO_4, 110 °C or Na(Hg) THF/MeOH, Na_2HPO_4, reflux; (c) 0.5 equiv of phthaloyl dichloride, Et_3N, CH_2Cl_2; (d) BH_3·THF, reflux; (e) 48% aqueous HBr, HOAc, reflux.

Scheme 3[a]

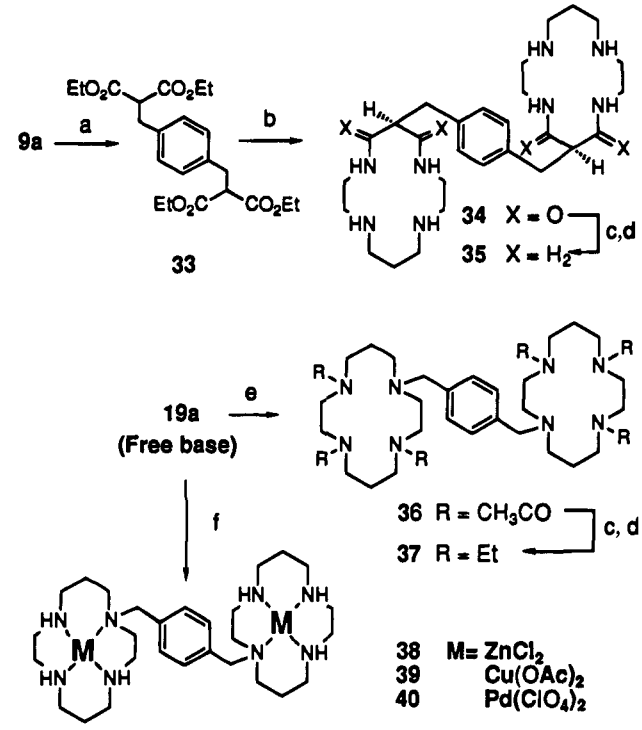


[a] Reagents: (a) K_2CO_3, CH_3CN, reflux; (b) 48% aqueous HBr, HOAc, reflux; (c) tetrakis-(*p*-tolylsulfonyl)-*N*,*N*'-bis(3-aminopropyl)ethylenediamine, K_2CO_3, CH_3CN, reflux.

Furthermore, within each group of compounds containing identical ring systems, a variation in the substitution of the phenylenebis(methylene) linker necessary for high potency was discovered. For example, the *meta*-linked analog of the [12]aneN$_4$ bis-macrocycle **16b** and the [13]aneN$_4$ bis-macrocycle **17b** were more active against HIV-1 and HIV-2 replication than the corresponding *para* analogs **16a** and **17a**. In striking contrast, the substitution of the phenylenebis(methylene) linker required for high potency in the series of [*iso*-14]aneN$_4$ analogs (**18a**,**b**) and [14]aneN$_4$ analogs (**19a**–**c**) [and throughout the 15- and 16-membered bis-macrocycles (**20a**,**b** and **21a**,**b**)] was clearly reversed. Compounds **18a** and **19a** were 8–13-fold more potent against HIV-1 replication than the corresponding *meta*-linked analogs **18b** and **19b**. Although the majority of compounds containing macrocycles of 12–14 ring members inhibit HIV replication at sub-micromolar concentrations, the optimum selectivity was achieved with **19a**, which inhibited the replication of HIV-1 and HIV-2 with EC_{50} values of 0.0042 and 0.0059 μM, respectively, but remained nontoxic to MT-4 cells at a CC_{50} exceeding 421 μM. Compound **19a** was therefore selected as our lead for further structure–activity elaboration.

To assess the symmetry requirements for activity of **19a**, a series of compounds that feature nonidentical ring systems (**28a**,**b**, **29**, **30**, Table 1) was also evaluated. Compounds **28a**,**b** differ from **19a**,**b** in that one of the [14]aneN$_4$ ring systems has been replaced by the [*iso*-14]aneN$_4$ macrocycle. Consistent with the activity of

Scheme 4[a]



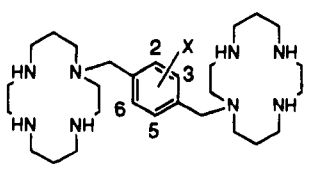
[a] Reagents: (a) diethyl malonate, NaH, DMF; (b) *N*,*N*'-bis(2-aminoethyl)-1,3-propanediamine, EtOH, reflux; (c) BH_3·THF, reflux; (d) 48% aqueous HBr, HOAc, reflux; (e) Ac_2O, 55 °C, 18 h; (f) transition metal salt, MeOH, H_2O.

compounds in which the ring systems are identical, **28a** was 2-fold less active against HIV-1 replication and 9-fold less active against HIV-2 replication than **19a** while the substitution of the phenylenebis(methylene) linker required for highest activity remained *para*. By analogy, somewhat predictable reductions in activity were observed for compounds **29** and **30** which contain the [16]aneN$_4$ macrocycle coupled to a 14-membered ring system. This demonstrates that identical tetraazamacrocycles are not a requirement for activity.

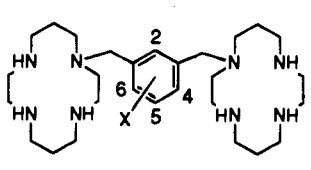
In view of these results, we additionally prepared the pharmacophore of **19a** in which the second [14]aneN$_4$ (cyclam) ring was replaced by the acyclic polyamine equivalent, giving **32** (structure shown in Scheme 3). Compound **32** proved 89-fold less effective against HIV-1 replication than **19a**, which suggests that the more rigid array of nitrogen donors/acceptors is necessary for potent activity. Furthermore, both the 6,6'-carbon-linked analog **35** (possessing all secondary amines) and the hexa-*N*-ethyl analog **37** (all tertiary amines) were 2–3 orders of magnitude less active than **19a**. In the absence of a characterized binding site for bis-tetraazamacrocycles, we are unable to provide a satisfactory explanation for the importance of intraring nitrogen–nitrogen distance, donor–acceptor requirements, or linker substitution upon activity.

Since the metal complexation properties of tetraazamacrocycles are well-established, a short series of compounds **38**–**40** were prepared to explore the activity of divalent transition metal complexes of **19a**. Activity appeared to inversely correlate with the stability of the metal complex: the kinetically labile bis-zinc complex (**38**) exhibited a similar EC_{50} value against HIV-1 replication compared to the free ligand **19a** but was 2-fold more potent against HIV-2. The bis-copper

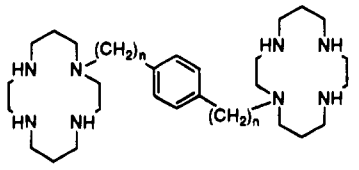
Table 2. Anti-HIV Activity of Bicyclam Analogs[a]



I



II

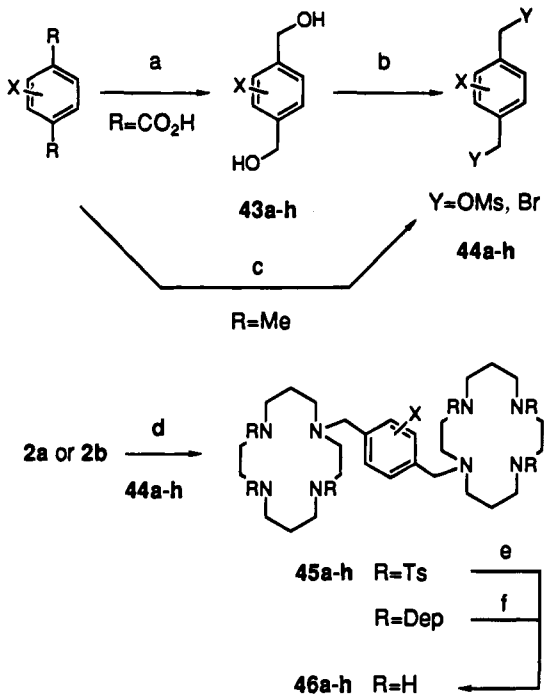


III

compd	structure	X	formula	EC_{50} (μM) HIV-1 (IIIB)	EC_{50} (μM) HIV-2 (ROD)	CC_{50} (μM)
46a	I	2,5-dimethyl	$C_{30}H_{58}N_8 \cdot 8HBr \cdot H_2O$	0.0064	0.0011	>208
46b	I	2,5-dichloro	$C_{28}H_{52}Cl_2N_8 \cdot 8HBr \cdot 1/2HOAc$	0.0107	0.0025	58
46c	I	2-bromo	$C_{28}H_{53}BrN_8 \cdot 8HBr$	0.0061	0.0035	>203
46d	I	2-phenyl	$C_{34}H_{58}N_8 \cdot 8HBr \cdot 2H_2O$	0.1062	0.0800	>198
46e	I	2-nitro	$C_{28}H_{53}N_9O_2 \cdot 8HBr \cdot 2H_2O$	0.0650	0.0731	>203
46f	I	2,5-dimethoxy	$C_{30}H_{58}N_8O_2 \cdot 8HBr$	0.0058	0.0066	>206
46g	I	2,3,5,6-tetrafluoro	$C_{28}H_{50}F_4N_8 \cdot 8HBr \cdot 4.5H_2O$	0.0079	0.0079	47
46h	I	1,4-naphthyl	$C_{32}H_{56}N_8 \cdot 8HBr \cdot 4H_2O$	0.0550	0.0393	55
47a	II	1,3-naphthyl	$C_{32}H_{56}N_8 \cdot 8HBr \cdot 4.5H_2O \cdot HOAc$	0.1572	0.0786	207
47b	II	5-phenyl	$C_{34}H_{58}N_8 \cdot 8HBr \cdot 2H_2O$	0.2060	0.0246	>198
47c	II	2-bromo	$C_{28}H_{53}BrN_8 \cdot 8HBr \cdot 4H_2O$	0.1383	0.2459	>144
47d	II	5-bromo	$C_{28}H_{53}BrN_8 \cdot 8HBr \cdot 5H_2O$	0.0845	0.0538	>192
47e	II	5-nitro	$C_{28}H_{53}N_9O_2 \cdot 8HBr \cdot 2.75H_2O$	0.0406	0.0569	44
47f	II	2,4,5,6-tetrachloro	$C_{28}H_{50}Cl_4N_8 \cdot 8HBr \cdot 1/2HOAc$	0.5287	1.9638	9
47g	II	2-fluoro	$C_{28}H_{53}FN_8 \cdot 8HBr \cdot 4H_2O$	0.0347	0.0734	>201
48a	III ($n = 2$)		$C_{30}H_{58}N_8 \cdot 8HBr \cdot HOAc$	14.852	69.713	>201
48b	III ($n = 3$)		$C_{32}H_{62}N_8 \cdot 8HBr \cdot 2H_2O$	0.4025	14.489	283

[a] See footnotes to Table 1.

Scheme 5[a]



[a] Reagents: (a) BH_3·THF; (b) Ms-Cl, Et_3N, CH_2Cl_2 or 48% aqueous HBr, Ac_2O; (c) NBS, BzO_3H, CCl_4, reflux; (d) K_2CO_3, CH_3CN, reflux; (e) 48% aqueous HBr, HOAc, reflux; (f) HBr/HOAc, room temperature, 3 h.

complex (**39**) proved 4−5-fold less active against HIV-1 and HIV-2 replication than **19a**, and the inert bis-palladium complex (**40**) was inactive. A more extensive study of the anti-HIV properties of a variety of cyclam derivatives and their metal complexes has recently been reported by Kimura et al.[33] Alternatively, one can envisage a mechanism of action of **19a** involving chelation to an endogenous metal complex. For example, Rice and co-workers have reported the inhibition of HIV-1 infectivity by a series of aromatic C-nitroso compounds which eject zinc from isolated HIV-1 nucleocapsid zinc fingers and intact HIV-1 virions.[34] This particular mechanism appears unlikely for bis-tetraazamacrocycles for two reasons: JM2763 (**1**) and JM3100 (**19a**, isolated as the octahydrochloride salt) have been previously shown not to directly inactivate the virus[13,14] and, on a molar basis, cyclam compounds (**42** and **41**) are equally capable of metal ion extrusion but are relatively inactive. At present, it is unclear what role, if any, metal chelation plays in the anti-HIV activity of bis-tetraazamacrocycles. Finally, it is worth noting that *N*-(4-methylbenzyl)cyclam (**41**) proved more potent against HIV-1 replication than cyclam (**42**). On the basis of the assumption that **41** inhibits HIV replication at the identical mechanistic stage as **19a**, these results suggest that the phenyl ring is involved in the binding of **19a** to its target, rather than simply providing the appropriate intramolecular distance between the tetraazamacrocycles.

A variety of bicyclam analogs derived from compounds **19a,b** are detailed in Table 2. High activity appears to be independent of the electron-withdrawing or -donating properties of the substituents in the *p*-phenylenebis(methylene)-linked series since the dimethyl (**46a**), dichloro (**46b**), bromo (**46c**), dimethoxy (**46f**), and tetrafluoro (**46g**) analogs displayed comparable anti-HIV-1 and HIV-2 EC_{50} values to **19a**. However, analogs bearing multiple halogen substituents exhibited a markedly higher cytotoxicity to MT-4 cells. Both the dichloro (**46b**) and tetrafluoro (**46g**) analogs were approximately 4-fold more cytotoxic than either **46a** or **46f**. In contrast, activity is adversely effected by the incorporation of a sterically demanding substituent, which most likely restricts rotation of the cyclam ring around the benzylic position. This is exemplified by **46d**, a 2-phenyl-substituted analog which inhibits HIV-1 replication at an EC_{50} around 25-fold higher than that of **19a**, whereas, **47b**, a *meta*-linked analog containing a 5-phen-

yl substituent in a nonrestricting position exhibits an EC_{50} against HIV-1 which is only 6-fold higher than that of **19b**. Similarly, the 2-nitro *para*-linked analog (**46e**) was 15-fold less active against HIV-1 replication than **19a** whereas the 5-nitro *meta*-linked analog (**47e**) and **19b** were of equal activity. The activity of the *p*- and *m*-naphthyl compounds were both reduced, but **46h** was 4-fold more cytotoxic than **47a**. Among the series of *meta*-linked analogs, the introduction of a fluoro substituent at the 2-position (**47g**) did not affect activity, but the larger bromo substituent (**47c**) reduced the activity 4-fold against HIV-1 and 5-fold against HIV-2 compared to **19b**. In addition, several compounds, namely **46a,b**, and **47a,b**, displayed a 2–10-fold higher potency against HIV-2 replication than HIV-1. Finally, increasing the distance between the cyclam ring and the phenylene group of the linker by incorporating additional methylene groups markedly influenced antiviral potency. The phenylenebis(ethylene) analog (**48a**) was 3–4 orders of magnitude less antivirally effective than **19a** against HIV-1 and HIV-2 replication while the phenylenebis(propylene) analog (**48b**) was around 36-fold more potent than **48a** against HIV-1. However, compound **48b** displayed a uniquely high selectivity for HIV-1 over HIV-2: the EC_{50} value for **48b** against HIV-1 replication was also 36-fold lower than the EC_{50} value against HIV-2. It is clear that the optimum spacer between the cyclam ring and the phenylene moiety is a single methylene group.

Comparing the activity data for all compounds displayed in Tables 1 and 2, we found a close correlation between activity against HIV-1 and HIV-2 (Figure 2); the correlation coefficients were 0.918 and 0.844 for Tables 1 and 2, respectively. The weaker correlation coefficient for analogs in Table 2 can be explained in part by the unusual activity of **48b** described above. When the data for this analog is removed, the correlation coefficient for Table 2 increases to 0.890.

In summary, the following conclusions can be made regarding the structure–activity relationship of phenylenebis(methylene) linked bis-tetraazamacrocycles. Potent anti-HIV activity and low cytotoxicity to MT-4 cells is highly dependent upon the substitution of the linker connecting macrocycles having 12–14 ring members. For example, the substitution of the phenylenebis(methylene) linker required for high potency in dimers of 12- and 13-membered macrocycles was found to be *meta*, whereas a *para*-substituted linker was preferred for dimers of 14-membered macrocycles. Compounds featuring macrocycles of two distinct ring sizes remained active against HIV-1 and HIV-2 replication, indicating that identical macrocyclic rings are not a requirement for activity. However, the macrocyclic ring structure was found to be important for potent activity: replacing a single cyclam ring in **19a** with an acyclic polyamine equivalent gave an analog with markedly reduced activity. The 6,6′-carbon analog, bearing all secondary amines and the hexaethyl analog, featuring all tertiary amines were both significantly less active than **19a**. The importance of these structural requirements is still unclear.

Though the role of transition metal complexation in the activity of bis-tetraazamacrocycles is not established, a short series of transition metal complexes of

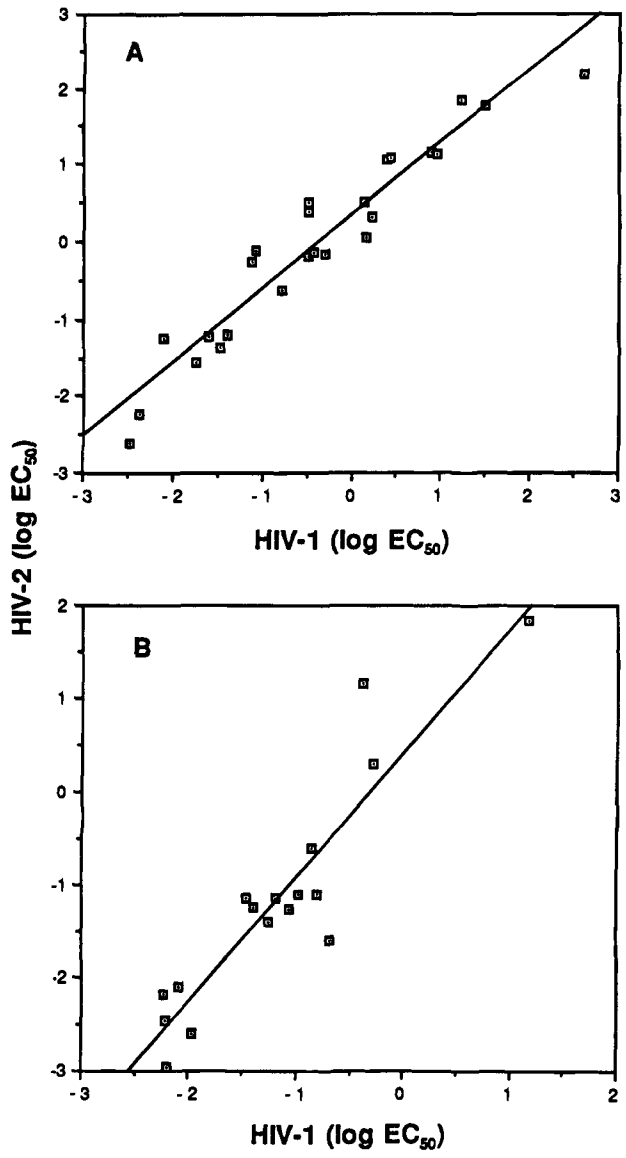


Figure 2. Correlation of anti-(HIV-1) and HIV-2 activity by linear regression for bis-tetraazamacrocyclic analogs: (A) data for compounds from Table 1 and (B) data for compounds from Table 2.

19a was prepared. Complexes of low kinetic stability remained potent inhibitors of HIV replication.

The introduction of electron-withdrawing or -donating substituents on the *p*-phenylenebis(methylene) linker of **19a** had little influence on the antiviral potency. Bulky groups such as phenyl reduced the activity. Consequently, we have been able to identify several analogs of **19a** with comparably high potency and selectivity against HIV.[35]

Experimental Section

General Methods. Linear triamines and tetramines were purchased from Aldrich and derivatized with *p*-toluenesulfonyl chloride according to literature procedures.[36] Melting points were determined with a Thomas-Hoover or Electrothermal melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-300 spectrometer operating at 300 and 75 MHz, respectively. Chemical shifts are expressed as δ units downfield from TMS (in $CDCl_3$) or TSP [3-(trimethylsilyl)propionic acid-d_4 sodium salt in D_2O]. Fast atom bombardment mass spectral analysis was carried out by M-Scan (West Chester, PA) on a VG Analytical ZAB 2-SE high-field spectrometer operating at V_{acc} = 8 kV using

a *m*-nitrobenzyl alcohol (MNBA) or glycerol/thioglycerol (1:1) matrix. Mass calibration was performed using cesium iodide. IR spectra were recorded on a Mattson FTIR 5000 spectrometer. Microanalyses for C, H, N, and halogen were carried out by Atlantic Microlabs (Norcross, GA) and were within ±0.4% of the theoretical values. The presence and approximate stoichiometry of acetic acid in a number of final products was confirmed by ^{1}H NMR.

Thin-layer chromatography (TLC) was carried out on silica gel plates (Merck 60 F_{254}). Column chromatography was performed on silica gel (Merck, 230–400 mesh). Analytical HPLC to determine final compound purity was carried out on a Waters 600E instrument using the following conditions: 4.6 × 250 mm PLRP-S column (100 Å, 5 μM available from Polymer Laboratories, Amherst, MA); mobile phases, A = 0.1% TFA in H_2O, B = 0.1% TFA in CH_3CN; gradient 10–40% B over 15 min; flow rate, 1 mL/min; UV detection at 230 nm.

Chemistry. *N*-(Diethoxyphosphoryl)diethanolamine (4c). To a solution of diethanolamine (**4a**) (5.0 g, 48 mmol) and Et_3N (8.0 mL) in CH_2Cl_2 (75 mL) was added dropwise with stirring under argon a solution of diethyl phosphorochloridate (8.2 g, 48 mmol) in CH_2Cl_2 (25 mL) over approximately 15 min, and the reaction mixture was then stirred at room temperature overnight. The solution was washed with brine (50 mL) and then dried (Na_2SO_4) and evaporated *in vacuo* to give the crude product as a viscous oil. The oil was dissolved in Et_2O (100 mL) and the white solid which precipitated was removed by filtration (Et_3N·HCl). The filtrate was evaporated *in vacuo*, giving **4c** (6.2 g, 54%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.27 (td, 6H, J = 7.2 Hz, $^4J_{P-H}$ = 0.6 Hz), 3.22 (dt, 4H, $^2J_{P-H}$ = 11.6 Hz, J = 5.1 Hz), 3.72 (t, 4H, J = 5.1 Hz), 4.08 (m, 4H).

***N*-(Diethoxyphosphoryl)-*O,O'*-bis(2-methylsulfonyl)diethanolamine (5c).** To a solution of **4c** (3.0 g, 12 mmol) and Et_3N (5.2 mL) in CH_2Cl_2 (50 mL), cooled to 0–5 °C, was added dropwise with stirring a solution of methanesulfonyl chloride (3.0 g, 26 mmol) in CH_2Cl_2 (25 mL), and the reaction mixture was then stirred at room temperature overnight. The solution was washed with saturated aqueous ammonium chloride (50 mL) and brine (50 mL) and then dried (Na_2SO_4) and evaporated *in vacuo* to give **5c** (4.0 g, 81%) as a light brown oil: ^{1}H NMR ($CDCl_3$) δ 1.34 (td, 6H, J = 7.2 Hz, $^4J_{P-H}$ = 0.9 Hz), 3.06 (s, 6H), 3.45 (dt, 4H, $^2J_{P-H}$ = 11.8 Hz, J = 5.6 Hz), 4.08 (qd, 4H, J = 7.2 Hz, $^3J_{P-H}$ = 2.9 Hz), 4.33 (t, 4H, J = 5.6 Hz).

***N*-(Diethoxyphosphoryl)-*O,O'*-bis(3-methylsulfonyl)dipropanolamine (5d).** Using identical procedures to those described for the preparation of **5c**, dipropanolamine[23] (**4b**) (12.9 g, 0.097 mol) gave **5d** (32.5 g, 79%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.31 (td, 6H, J = 7.2 Hz, $^4J_{P-H}$ = 0.7 Hz), 1.98 (quint, 4H, J = 6.1 Hz), 3.06 (s, 6H), 3.15 (m, 4H), 4.05 (m, 4H), 4.25 (t, 4H, J = 6.1 Hz).

1-Benzyl-5,13-bis(*p*-tolylsulfonyl)-9-(methylsulfonyl)-1,5,9,13-tetraazacyclohexadecane (7b). To a solution of 1,9-bis-(*p*-tolylsulfonyl)-5-benzyl-1,5,9-triazanonane hydrochloride[37] (**6a**) (25 g, 0.044 mol) in dry DMF (800 mL) under argon was added NaH (10.6 g, 0.44 mol, 10 equiv) in small portions over 3 h. When the addition was complete, the solution was heated at 60 °C for 1 h and then allowed to cool and the excess NaH was removed by filtration under argon. The filtrate was transfered to a second dry flask and the solution was then heated to 100–110 °C and bis(propanolamine) trimethanesulfonate[23] **5b** (1.0 equiv) in DMF (500 mL) was added dropwise over 8 h with rapid stirring. The temperature was maintained at 100–110 °C for a further 16 h, the mixture was allowed to cool and then poured into iced water (1500 mL), and the resulting off-white precipitate that formed was collected by filtration. The solid was dissolved in CH_2Cl_2 (250 mL) and the solution was washed with H_2O (5 × 50 mL) and then dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow oil. Trituration with EtOH (200 mL) gave a white crystalline solid which was collected by filtration, washed with a small volume of EtOH and then Et_2O, and dried *in vacuo* to give **7b** (14.0 g, 45%): ^{1}H NMR ($CDCl_3$) δ 1.70 (quint, 4H, J = 7.2 Hz), 2.00 (quint, 4H, J = 7.2 Hz) 2.42 (s, 6H), 2.48 (t, 4H, J = 7.0 Hz), 2.80 (s, 3H), 3.00 (t, 4H, J = 7.8 Hz), 3.08 (t, 4H, J = 7.0 Hz), 3.14 (t, 4H, J = 7.0 Hz), 3.50 (s, 3H), 7.18–7.35 (m, 9H), 7.60 (d, 4H, J = 7.8 Hz).

1,9-Bis(*p*-tolylsulfonyl)-5-(methylsulfonyl)-1,5,9,13-tetraazacyclohexadecane (8b). To a solution of **7b** (925 mg, 1.31 mmol) in formic acid (20 mL) was added palladium hydroxide on carbon (Pearlmans catalyst, 4.0 g), and the resulting suspension was heated to reflux for 72 h with stirring. The mixture was allowed to cool and then filtered through Celite, and the filtrate was evaporated under reduced pressure. The colorless oil which remained was dissolved in CH_2Cl_2 (50 mL) and washed with 10% aqueous NaOH solution (2 × 20 mL) and H_2O (2 × 20 mL) and then dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 97:3), giving **8b** (506 mg, 63%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.76 (quint, 4H, J = 6.5 Hz), 1.99 (quint, 4H, J = 7.1 Hz), 2.42 (s, 6H), 2.74 (t, 4H, J = 6.4 Hz), 2.81 (s, 3H), 3.00–3.19 (m, 8H), 3.20–3.33 (m, 4H), 7.30 (d, 4H, J = 8.2 Hz), 7.65 (d, 4H, J = 8.2 Hz); FAB MS *m/z* 615 (M + H, 100), 459 (17).

4-(Diethoxyphosphoryl)-1,7,10-tris(*p*-tolylsulfonyl)-1,4,7,10-tetraazacyclotridecane (7c). To a stirred solution of tris(*p*-tolylsulfonyl)-*N*-(2-aminoethyl)-1,3-propanediamine (**6b**) (5.7 g, 9.8 mmol) in DMF (250 mL) containing cesium carbonate (11.2 g, 34 mmol) maintained at 55 °C was added a solution of **5c** (3.9 g, 9.8 mmol) in DMF (100 mL) dropwise over a period of 16–18 h. The reaction mixture was stirred at 55 °C for a total of 30 h and then allowed to cool to room temperature and evaporated *in vacuo*. The brown residue was partitioned between CH_2Cl_2 (750 mL) and brine (500 mL) and the aqueous layer was separated and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were dried (Na_2SO_4) and evaporated *in vacuo* to give the crude product as a pale yellow solid. Purification by column chromatography on silica gel (CH_2Cl_2/MeOH, 97:3) gave **7c** (4.2 g, 55%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.35 (td, 6H, J = 7.2 Hz, $^4J_{P-H}$ = 0.7 Hz), 2.00 (quint, 2H, J = 6.2 Hz), 2.43 (s, 3H), 2.45 (s, 6H), 3.13 (t, 2H, J = 6.4 Hz), 3.15–3.38 (m, 14H), 4.05 (m, 4H), 7.31–7.36 (m, 6H), 7.63–7.67 (m, 6H).

8-(Diethoxyphosphoryl)-1,4,12-tris(*p*-tolylsulfonyl)-1,4,8,12-tetraazacyclopentadecane (7d). In a similar manner, macrocyclization of **6b** with **5d** gave **7d** (55%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 6H, J = 7.2 Hz), 1.70–1.86 (m, 4H), 1.93 (quint, 2H, J = 6.7 Hz), 2.43 (s, 3H), 2.44 (s, 3H), 2.45 (s, 3H), 3.05–3.35 (m, 16H), 3.99 (qd, 4H, J = 7.2 Hz, $^3J_{P-H}$ = 2.9 Hz), 7.27–7.36 (m, 6H), 7.64 (d, 2H, J = 8.2 Hz), 7.70 (d, 2H, J = 8.2 Hz), 7.75 (d, 2H, J = 8.2 Hz).

1,7,10-Tris(*p*-tolylsulfonyl)-1,4,7,10-tetraazacyclotridecane (8c). To a stirred solution of **7c** (1.5 g, 1.91 mmol) in glacial acetic acid (10 mL) was added 30% HBr/acetic acid (Aldrich, 5 mL) and the reaction mixture stirred at room temperature for 2.5 h. Ether (100 mL) was added to precipitate a white solid which was allowed to settle to the bottom of the flask and the supernatant solution was decanted off. The solid was then washed by decantation with Et_2O three times and the remaining traces of Et_2O removed by evaporation under reduced pressure. The solid was partitioned between NaOH solution (10 N, 10 mL) and CH_2Cl_2 (150 mL) and the organic layer was separated, dried (Na_2SO_4), and evaporated *in vacuo* to give **8c** (910 mg, 76%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.93 (m, 2H), 2.42 (s, 3H), 2.44 (s, 3H), 2.45 (s, 3H), 2.88 (m, 4H), 3.06 (m, 4H), 3.16–3.27 (br m, 4H), 3.39 (m, 2H), 3.54 (m, 2H), 7.27–7.36 (m, 6H), 7.61–7.66 (m, 4H), 7.74 (d, 2H, J = 8.2 Hz); FAB MS *m/z* 649 (M + H, 100), 495 (54), 337 (20), 239 (20).

1,4,12-Tris(*p*-tolylsulfonyl)-1,4,8,12-tetraazacyclopentadecane (8d). In a similar manner, **7d** (1.4 g, 1.72 mmol) gave **8d** (996 mg, 86%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.68 (quint, 2H, J = 6.7 Hz), 1.79–1.98 (m, 4H), 2.42 (s, 3H), 2.44 (s, 3H), 2.45 (s, 3H), 2.66 (m, 2H), 3.01–3.40 (m, 14H), 7.28–7.35 (br m, 6H), 7.64 (d, 2H, J = 8.2 Hz), 7.70–7.76 (m, 4H); FAB MS *m/z* 677 (M + H, 54), 523 (100), 367 (17), 155 (30).

General Procedure A: Dimerization. To a solution of the appropriately protected tetraazamacrocycles in dry CH_3CN (15–20 mL/mmol of macrocycle) were added the aromatic bis-electrophile (0.5 equiv) and potassium carbonate (3.0 equiv), and the mixture was heated to reflux for 18 h with

rapid stirring. The reaction mixture was allowed to cool to room temperature and then concentrated and the residue was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was separated and extracted with two further portions of CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and evaporated, and the residue was purified by column chromatography on silica gel using CH_2Cl_2/MeOH or ethyl acetate/hexanes as eluent giving the fully protected bis-tetraazamacrocycles.

1,1'-[1,4-Phenylenebis(methylene)]bis[4,7,10-tris(*p*-tolylsulfonyl)-1,4,7,10-tetraazacyclododecane] (10a). Using general procedure A, 1,4,7-tris(*p*-tolylsulfonyl)-1,4,7,10-tetraazacyclododecane[20] (**3**) (600 mg, 0.95 mmol), and α,α'-dibromo-*p*-xylene (**9a**) (125 mg, 0.47 mmol) gave **10a** (490 mg, 76%) as a white flaky solid: ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 12H), 2.45 (s, 6H), 2.73 (m, 8H), 3.14 (m, 8H), 3.37–3.51 (m, 16H), 3.63 (s, 4H), 7.10 (s, 4H), 7.26 (d, 8H, J = 8.2 Hz), 7.34 (d, 4H, J = 8.2 Hz), 7.59 (d, 8H, J = 8.2 Hz), 7.69 (d, 4H, J = 8.2 Hz); FAB MS *m/z* 1371 (M + H, 11), 789 (17), 635 (57), 481 (45), 157 (100).

1,1'-[1,3-Phenylenebis(methylene)]bis[4,7,10-tris(*p*-tolylsulfonyl)-1,4,7,10-tetraazacyclododecane] (10b). Using general procedure A, **3** (600 mg, 0.95 mmol), and α,α'-dibromo-*m*-xylene (**9b**) (125 mg, 0.47 mmol) gave **10b** (490 mg, 76%) as a white flaky solid: ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 12H), 2.45 (s, 6H), 2.73 (m, 8H), 3.15 (m, 8H), 3.39 (m, 16H), 3.63 (s, 4H), 7.04–7.09 (m, 3H), 7.25 (d overlapping s, 9H, J = 8.1 Hz), 7.32 (d, 4H, J = 8.1 Hz), 7.59 (d, 8H, J = 8.1 Hz), 7.67 (d, 4H, J = 8.1 Hz); FAB MS *m/z* 1371 (M + H, 100), 1215 (57).

4,4'-[1,4-Phenylenebis(methylene)]bis[1,7,10-tris(*p*-tolylsulfonyl)-1,4,7,10-tetraazacyclotridecane] (11a). Using general procedure A, **8c** (600 mg, 0.93 mmol), and **9a** (123 mg, 0.47 mmol) gave **11a** (420 mg, 65%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 2.04 (m, 4H), 2.40 (s, 6H), 2.42 (s, 6H), 2.45 (s, 6H), 2.66 (m, 8H), 3.12–3.31 (br m, 16H), 3.34 (m, 8H), 3.58 (s, 4H), 7.15 (s, 4H), 7.24–7.32 (m, 8H), 7.33 (d, 4H, J = 8.2 Hz), 7.59–7.62 (m, 8H), 7.69 (d, 4H, J = 8.2 Hz); FAB MS *m/z* 1400 (M + H, 100), 1245 (58), 1090 (21).

1,1'-[1,4-Phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (13a). Using general procedure A, 1,4,8-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane[17] (**2a**) (1.0 g, 1.46 mmol), and **9a** (193 mg, 0.73 mmol) gave **13a** (0.7 g, 67%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.76 (m, 4H), 1.95 (m, 4H), 2.38–2.50 (m, 22H), 2.70 (m, 4H), 2.98–3.24 (m, 24H), 3.55 (s, 4H), 7.16 (s, 4H), 7.22–7.37 (m, 12H), 7.58 (d, 4H, J = 8.3 Hz), 7.64 (d, 4H, J = 8.3 Hz), 7.70 (d, 4H, J = 8.3 Hz); FAB MS *m/z* 1428 (M + H, 85), 1274 (90), 1120 (47), 964 (17), 767 (100).

1,1'-[1,3-Phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (13b). Using general procedure A, **2a** (0.9 g, 1.32 mmol), and **9b** (174 mg, 0.66 mmol) gave **13b** (0.92 g, 98%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.73 (m, 4H), 1.94 (m, 4H), 2.38–2.46 (m, 22H), 2.78 (m, 4H), 2.99–3.24 (br m, 24H), 3.54 (s, 4H), 7.11–7.38 (br m, 16H), 7.55 (d, 4H, J = 8.3 Hz), 7.62 (d, 4H, J = 8.3 Hz), 7.69 (d, 4H, J = 8.2 Hz); FAB MS *m/z* 1427 (M, 100), 1272 (77), 1115 (14), 764 (11).

8,8'-[1,4-Phenylenebis(methylene)]bis[1,4,12-tris(*p*-tolylsulfonyl)-1,4,8,12-tetraazacyclopentadecane] (14a). Using general procedure A, **8d** (996 mg, 1.50 mmol), and **9a** (195 mg, 0.74 mmol) gave **14a** (850 mg, 79%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.58–1.77 (m, 8H), 1.93 (quint, 4H, J = 7.1 Hz), 2.41 (s, 6H), 2.43 (s, 6H), 2.45 (s, 6H), 2.41–2.45 (m, 4H), 2.51 (t, 4H, J = 7.8 Hz), 2.99–3.17 (m, 16H), 3.21 (m, 8H), 3.50 (s, 4H), 7.14 (s, 4H), 7.28 (d, 4H, J = 7.9 Hz), 7.30 (d, 4H, J = 7.9 Hz), 7.34 (d, 4H, J = 7.9 Hz), 7.62 (d, 4H, J = 7.9 Hz), 7.66 (d, 4H, J = 7.9 Hz), 7.71 (d, 4H, J = 7.9 Hz); FAB MS *m/z* 1455 (M + H, 100), 1299 (37), 1143 (10).

1,1'-[1,4-Phenylenebis(methylene)]bis[5,13-bis(*p*-tolylsulfonyl)-9-methylsulfonyl]-1,5,9,13-tetraazacyclohexadecane (15a). Using general procedure A, **8b** (600 mg, 0.907 mmol), and **9a** (129 mg, 0.49 mmol) gave **15a** as a white solid (300 mg, 46%): ^{1}H NMR ($CDCl_3$) δ 1.73 (quint, 8H, J = 6.7 Hz), 1.98 (quint, 8H, J = 6.6 Hz), 2.40 (s, 12H), 2.48 (t, 8H, J = 6.7 Hz), 2.80 (s, 6H), 2.97–3.16 (m, 16H), 3.22 (t, 8H, J = 7.2 Hz), 3.50 (s, 4H), 7.16 (s, 4H), 7.28 (d, 8H, J = 8.1 Hz), 7.60 (d, 8H, J = 8.3 Hz); FAB MS *m/z* 1331 (M + H, 100), 1175 (33), 716 (38).

General Procedure B: Amalgam Deprotection. To a stirred solution of the fully protected bis-tetraazamacrocycle (0.1–1.0 mmol) in a mixture of anhydrous THF (or DMSO depending upon solubility, 15 mL) and anhydrous MeOH (3 mL) were added dibasic sodium phosphate (250 mg, 1.76 mmol) and freshly prepared 2% sodium amalgam (23 g). The reaction mixture was stirred at 100 °C under argon and checked periodically by ^{1}H NMR of an evaporated aliquot until the deprotection was complete. This usually requires a reaction time of 24–72 h. The reaction mixture was then allowed to cool to room temperature and the supernatant solution was decanted from the amalgam and evaporated to dryness. The residue upon evaporation was partitioned between $CHCl_3$ (20 mL) and brine (5 mL), the organic layer was separated and washed with additional brine (2x), and the combined organic extracts were dried (K_2CO_3) and concentrated to give the crude free base.

The crude solid is then dissolved in EtOH (10 mL) and a freshly prepared solution of saturated HBr in EtOH (5 mL) is added. A solid precipitates immediately upon addition and is collected by filtration, washed with EtOH and Et_2O, and dried *in vacuo*.

1,1'-[1,4-Phenylenebis(methylene)]bis[1,4,7,10-tetraazacyclododecane] Hexahydrobromide (16a). Using general procedure B, **10a** (360 mg, 0.26 mmol) gave **16a** (115 mg, 47%) as a white solid: mp 198–202 °C dec; ^{1}H NMR (D_2O) δ 2.78 (t, 8H, J = 5.1 Hz), 2.87 (br m, 8H), 3.02 (t, 8H, J = 5.1 Hz), 3.09 (br m, 8H), 3.75 (s, 4H), 7.26 (s, 4H); ^{13}C NMR (D_2O) δ 42.02, 42.16, 44.41, 47.79, 56.23, 130.72, 134.71; FAB MS *m/z* 529 (MH + H^{81}Br, 48), 527 (MH + H^{79}Br, 47), 447 (M + H, 55), 277 (52), 201 (55), 185 (100). Anal. ($C_{24}H_{52}N_8Br_6$) C, H, N, Br.

1,1'-[1,3-Phenylenebis(methylene)]bis[1,4,7,10-tetraazacyclododecane] Hexahydrobromide (16b). Using general procedure B, **10b** (330 mg, 0.24 mmol) gave **16b** (130 mg, 58%) as a white solid: mp 146–151 °C dec; ^{1}H NMR (D_2O) δ 2.79 (br m, 8H), 2.88 (br m, 8H), 3.03 (br m, 8H), 3.09 (br m, 8H), 3.79 (s, 4H), 7.19–7.30 (m, 3H), 7.30–7.41 (m, 1H); ^{13}C NMR (D_2O) δ 42.33, 42.48, 44.73, 48.22, 56.96, 130.06, 130.32, 132.58, 135.62; FAB MS *m/z* 529 (MH + H^{81}Br, 52), 527 (MH + H^{79}Br, 54), 447 (M + H, 100), 277 (39), 185 (36). Anal. ($C_{24}H_{52}N_8Br_6$) C, H, N, Br.

General Procedure C: Sulfuric Acid Deprotection. The fully protected bis-tetraazamacrocycle (0.1–1.0 mmol) was dissolved in concentrated H_2SO_4 (1.5–4.0 mL) and stirred rapidly at 100 °C for 2–3 h. The mixture was allowed to cool and carefully made basic with a solution of NaOH (10 N, 10 mL). The resulting aqueous solution was then extracted with CH_2Cl_2 (2 × 50 mL), and the combined organic extracts were dried (Na_2SO_4) and evaporated to give the crude free base. An alternative procedure for conversion to the hydrobromide salt follows: The solid was dissolved in acetic acid (5.0 mL), and HBr/acetic acid (30%, Aldrich, 0.5 mL) was added. Addition of Et_2O precipitated the product, which was allowed to settle to the bottom of the flask, and the supernatant solution was decanted off. The solid was washed by decantation with Et_2O three times, and the remaining traces of Et_2O were removed by evaporation under reduced pressure followed by drying *in vacuo* overnight.

4,4'-[1,4-Phenylenebis(methylene)]bis[1,4,7,10-tetraazacyclotridecane] Octahydrobromide Monohydrate (17a). Using general procedure C, **11a** (300 mg, 0.21 mmol) gave **17a** (130 mg, 54%) as a white solid: mp 220–225 °C dec; ^{1}H NMR (D_2O) δ 2.02 (m, 4H), 2.75–3.55 (m, 32H), 3.82 (s, 4H), 7.26 (s, 4H); ^{13}C NMR (D_2O) δ 21.03, 41.82, 42.23, 42.32, 42.55, 42.63, 44.21, 47.78, 48.53, 54.31, 129.92, 131.88; FAB MS *m/z* 557 (MH + H^{81}Br, 12), 555 (MH + H^{79}Br, 12), 475 (M + H, 20), 291 (100). Anal. ($C_{26}H_{58}N_8Br_8 \cdot H_2O \cdot HOAc$) C, H, N.

1,1'-[1,4-Phenylenebis(methylene)]bis[1,5,9,12-tetraazacyclopentadecane] Octahydrobromide Tetrahydrate (20a). Using general procedure C, **14a** (130 mg, 0.09 mmol) gave **20a** (57 mg, 49%) as a white solid: mp 280–285 °C dec; ^{1}H NMR (D_2O) δ 2.03 (m, 12H), 3.18–3.23 (m, 24H), 3.45 (s, 8H), 4.37 (s, 4H), 7.49 (s, 4H); FAB MS *m/z* 613 (MH + H^{81}Br,

26), 611 (MH + $H^{79}Br$, 26), 531 (M + H, 50), 413 (9), 319 (100), 215 (87). Anal. ($C_{30}H_{66}N_8Br_8 \cdot 4H_2O \cdot HOAc$) C, H, N, Br.

General Procedure D: HBr/Acetic Acid Deprotection. A rapidly stirred solution of the fully protected bis-tetraazamacrocycle (0.1–1.0 mmol) in acetic acid/HBr (Aldrich, 48% aqueous) (3:2, 5–15 mL) was heated at 100–110 °C for 18–48 h, during which time a crystalline solid precipitated from the dark brown solution. Upon cooling, the solid is collected by filtration and washed with acetic acid and then Et_2O and dried *in vacuo* overnight.

1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Dihydrate (19a). Using general procedure D, **13a** (0.25 g, 0.18 mmol) gave **19a** (173 mg, 86%) as a white solid: mp 239–241 °C dec; ^1H NMR (D_2O) δ 1.95 (br m, 8H), 2.96–3.40 (br m, 32 H), 4.08 (s, 4H), 7.37 (s, 4H); ^{13}C NMR (D_2O) δ 18.68, 19.34, 37.78 (3C), 41.47 (2C), 41.98, 44.86, 48.00, 58.55, 131.45, 132.16; FAB MS *m/z* 585 (MH + $H^{81}Br$, 41), 583 (MH + $H^{79}Br$, 44), 503 (M + H, 38), 385 (20), 305 (100). Anal. ($C_{28}H_{62}N_8Br_8 \cdot 2H_2O$) C, H, N, Br.

1,1′-[1,3-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Dihydrate (19b). Using general procedure D, **13b** (0.5 g, 0.35 mmol) gave **19b** (250 mg, 62%) as a white solid: mp 237–240 °C dec; ^1H NMR (D_2O) δ 1.97 (br s, 8H), 3.18–3.29 (m, 16H), 3.38–3.58 (m, 16H), 4.34 (s, 4H), 7.48 (m, 3H), 7.55 (s, 1H); ^{13}C NMR (D_2O) δ 18.49, 19.01, 37.31, 37.38, 37.56, 41.08, 41.23, 41.63, 44.42, 47.80, 58.86, 130.28, 131.04, 133.16, 133.78; FAB MS *m/z* 585 (MH + $H^{81}Br$, 82), 583 (MH + $H^{79}Br$, 86), 503 (M + H, 100), 385 (22), 305 (62). Anal. ($C_{28}H_{62}N_8Br_8 \cdot 2H_2O$) C, H, N, Br.

1,1′-[1,4-Phenylenebis(methylene)]bis[1,5,9,13-tetraazacyclohexadecane] Octahydrobromide Trihydrate (21a). Using general procedure D, **15a** (300 mg, 0.23 mmol) gave **21a** (160 mg, 74%) as a white solid: mp 271–274 °C dec; ^1H NMR (D_2O) δ 1.80–2.10 (m, 16H), 3.04–3.45 (m, 32H), 4.38 (s, 4H), 7.50 (s, 4H); ^{13}C NMR (D_2O) 18.10, 18.93, 40.29, 40.60, 47.48, 58.57, 131.08, 132.31; FAB MS *m/z* 641 (M + $H^{81}Br$, 29), 639 (M + $H^{79}Br$, 33), 560 (M + H, 47), 333 (45), 229 (100). Anal. ($C_{32}H_{70}N_8Br_8 \cdot 3H_2O$) C, H, N.

1,1′-[1,2-Phenylenebis(oxomethylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (22). Phthaloyl dichloride (63 μL, 0.44 mmol) was added to a stirred solution of **2a** (600 mg, 0.88 mmol) in Et_3N/CH_2Cl_2 (1:5, 12 mL) cooled to −10 °C under argon. The mixture was stirred at −10 °C for 1 h and then at room temperature for 12 h. The solvent was evaporated to dryness and the residue was purified by column chromatography on silica gel ($MeOH/CH_2Cl_2$, 1:160) to give a colorless oil. Trituration with Et_2O (50 mL) gave **22** as a white powder (454 mg, 70%): IR (IBr) 3452 (br), 2927 (s), 1640 (s), 1598 (s), 1494, 1456, 1424, 1342, 1159, 1121, 1091, 816, 721, 692 cm^{-1}.

1,1′-[1,2-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Monohydrate (19c). To a stirred solution of **22** (434 mg, 0.30 mmol) in anhydrous THF (10 mL) under argon was added BH_3·THF (Aldrich, 1.0 M in THF, 6.0 mL, 6.0 mmol) and the mixture was heated to reflux for 24 h. After cooling, the excess borane was destroyed by addition of MeOH (20 mL) and evaporation (repeated three times). The residue was dissolved in CH_2Cl_2 (50 mL) and the solution was washed with aqueous NaOH (10 N, 10 mL) followed by H_2O (2 × 10 mL) and then dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel ($MeOH/CH_2Cl_2$, 1:60) to give a colorless oil. Trituration with Et_2O (30 mL) gave **13c** as a white solid (142 mg, 33%).

Compound **13c** (130 mg, 0.10 mmol) was deprotected using general procedure D, giving **19c** (40 mg, 38%) as a white powder: mp 233–235 °C dec; ^1H NMR (D_2O) δ 2.00–2.20 (m, 8H), 3.08–3.64 (m, 32H), 4.25 (s, 4H), 7.42–7.68 (m, 4H). FAB MS *m/z* 585 (MH + $H^{81}Br$, 14), 583 (MH + $H^{79}Br$, 15), 503 (M + H, 15), 201 (100). Anal. ($C_{28}H_{62}N_8Br_8 \cdot H_2O$) C, H, N.

11-[1-Methylene-4-(bromomethylene)phenylene-1,4,7-tris(*p*-tolylsulfonyl)-1,4,7,11-tetraazacyclotetradecane (24). To a stirred solution of **9a** (3.98 g, 15.1 mmol) and K_2CO_3 (417 mg, 3.02 mmol) in anhydrous CH_3CN (20 mL) maintained at 50 °C was added dropwise a solution of **8a** (1.0 g, 1.51 mmol) in anhydrous CH_3CN (20 mL). The reaction mixture was allowed to stir for a further 1 h at 50 °C and then cooled and the solvent evaporated *in vacuo*. The residue was purified by column chromatography on silica gel ($MeOH/CH_2Cl_2$, 1:20) to give a viscous oil which solidified upon trituration with hot hexane (150 mL). The solid was collected by filtration, washed with hexane (3 × 10 mL) followed by Et_2O (20 mL), and dried *in vacuo* to give **24** as a white powder (710 mg, 53%): ^1H NMR ($CDCl_3$) δ 1.55–1.70 (m, 4H), 2.35–2.50 (m, 13H), 3.00–3.10 (m, 4H), 3.15–3.25 (m, 4H), 3.30–3.40 (m, 4H), 3.46 (s, 2H), 4.47 (s, 2H), 7.15–7.38 (m, 8H), 7.56–7.78 (m, 8H).

11-[[4,8,11-Tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecanyl]-1,4-phenylenebis(methylene)]-1,4,7-tris(*p*-tolylsulfonyl)-1,4,7,11-tetraazacyclotetradecane (25). To a stirred solution of **24** (350 mg, 0.41 mmol) and anhydrous K_2CO_3 (130 mg, 1.66 mmol) in anhydrous CH_3CN (20 mL) was added **2a** (422 mg, 0.62 mmol) and the mixture was heated at 60 °C for 7 h. The solvent was then evaporated *in vacuo* and the residue was purified by column chromatography on silica gel ($MeOH/CH_2Cl_2$, 1:160) followed by preparative thin layer chromatography on silica gel ($MeOH/CH_2Cl_2$, 1:40) to give **25** (130 mg, 30%) as a white solid: ^1H NMR ($CDCl_3$) δ 1.56–1.75 (m, 6H), 1.86–2.00 (m, 2H), 2.36–2.40 (m, 24H), 2.98–3.28 (m, 20H), 3.30–3.40 (m, 4H), 3.50 (s, 2H), 3.52 (s, 2H), 7.15 (s, 4H), 7.22–7.37 (m, 12H), 7.55–7.75 (m, 12H).

11-[1,4,8,11-Tetraazacyclotetradecanyl-1,4-phenylenebis(methylene)]-1,4,7,11-tetraazacyclotetradecane Octahydrobromide Dihydrate (28a). Using general procedure D, **25** (115 mg, 0.08 mmol) gave **28a** (71 mg, 75%) as a white powder: mp 269–271 °C dec; ^1H NMR (D_2O) δ 2.05–2.38 (m, 8H), 3.10–3.65 (m, 32H), 4.30 (s, 2H), 4.50 (s, 2H), 7.55–7.72 (m, 4H); FAB MS *m/z* 585 (MH + $H^{81}Br$, 35), 583 (MH + $H^{79}Br$, 34), 503 (M + H, 40), 305 (30), 201 (100). Anal. ($C_{28}H_{62}N_8Br_8 \cdot 2H_2O$) C, H, N.

Tetraethyl [1,4-Phenylenebis(methylene)]bismalonate (33). A solution of diethyl malonate (5.0 g, 0.03 mol) in dry DMF (10 mL) was added dropwise with stirring to a suspension of NaH (95%, 0.95 g, 1.2 equiv) in dry DMF (10 mL) cooled to 0–5 °C under an atmosphere of dry argon. When the addition was complete, the solution was stirred at room temperature for 1 h. To this solution was added dropwise a solution of **9a** (4.12 g, 0.016 mol) in dry DMF (30 mL) and the mixture was heated at 55 °C for a further 2 h. The solvent was evaporated *in vacuo* and the residue was partitioned between ethyl acetate (100 mL) and aqueous HCl (0.1 N, 50 mL). The organic phase was separated, washed with saturated aqueous sodium bicarbonate solution (50 mL) and brine (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure to give the crude product as a pale yellow oil. Purification by column chromatography on silica gel (Et_2O/hexane, 1:3) gave **33** (2.6 g, 40%) as a white solid: ^1H NMR ($CDCl_3$) δ 1.20 (t, 12H, J = 7.1 Hz), 3.16 (d, 4H, J = 7.8 Hz), 3.65 (t, 2H, J = 7.8 Hz), 4.16 (q, 8H, J = 7.1 Hz), 7.11 (s, 4H).

6,6′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane-5,7-dione] (34). To a stirred solution of **33** (2.57 g, 6.10 mmol) in absolute EtOH (100 mL), under argon, was added dropwise, a solution of *N,N′*-bis(2-aminoethyl)-1,3-propanediamine (Aldrich, 1.95 g, 12.2 mmol) in absolute EtOH (50 mL). When the addition was complete, the solution was heated to reflux for 20 days, during which time a white solid precipitated. The mixture was allowed to cool and the solid was collected by filtration, washed with EtOH (10 mL), and dried *in vacuo*, giving **34** (138 mg, 4%) as a white solid: IR (KBr) ν 3293 (s), 2926, 2818, 1666, 1556, 1341, 1132, 739 cm^{-1}; ^1H NMR (TFA/D_2O, 1:1) δ 1.99 (quint, 4H, J = 5.7 Hz), 2.95 (d, 4H, J = 7.2 Hz), 3.05–3.29 (m, 24H), 3.50 (t, 2H, J = 7.2 Hz), 6.89 (s, 4H); FAB MS *m/z* 559 (M + H, 100). This was used without further purification.

6,6′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide (35). To a stirred solution of **34** (64 mg, 0.12 mmol) in anhydrous THF (10 mL) under an atmosphere of dry argon was added BH_3·THF (1.0 M solution in THF, 5 mL) dropwise, and the mixture was

heated to reflux overnight. The mixture was allowed to cool and the excess borane was destroyed by addition of anhydrous MeOH (50 mL) and evaporation (repeated three times). The white residue was dissolved in glacial acetic acid (2.0 mL), hydrobromic acid (Aldrich, 48% aqueous, 1.5 mL) was added, and the mixture was heated at 110 °C with stirring for 1 h, during which time a white amorphous solid precipitated. On cooling, a further portion of acetic acid (2 mL) was added, and the solids were collected by filtration, washed with acetic acid (2 mL) and then Et_2O (10 mL), and dried *in vacuo*, giving **35** (45 mg, 35%) as a white powder: ^1H NMR (D_2O) δ 1.95 (m, 4H), 2.25 (m, 2H), 2.65 (d, 4H), 2.75–3.15 (m, 32H), 7.15 (s, 4H); FAB MS *m/z* 585 (MH + $H^{81}Br$, 53), 583 (MH + $H^{79}Br$, 58), 504 (M + H, 100), 331 (20), 305 (40). Anal. ($C_{28}H_{62}N_8Br_8 \cdot 3.3H_2O \cdot HOAc$) C, H, N, Br.

1,1′-[1,4-Phenylenebis(methylene)]bis[4,8,11-triacetyl-1,4,8,11-tetraazacyclotetradecane] (36). 1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] (**19a**, free base) (200 mg, 0.39 mmol) was stirred in acetic anhydride (5.0 mL) at 55 °C for 18 h. After cooling, Et_2O (100 mL) was added, precipitating a pale yellow solid. The solid was collected by filtration, dissolved in CH_2Cl_2 (100 mL), and washed with H_2O (50 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated *in vacuo*, giving **36** (118 mg, 40%) as a white amorphous solid: ^1H NMR ($CDCl_3$) δ 1.85 (m, 8H), 2.11 (s, 18H), 2.45–2.75 (m, 8H), 3.42–3.54 (m, 28H), 7.21 (s, 4H). This was used without further purification.

1,1′-[1,4-Phenylenebis(methylene)]bis[4,8,11-triethyl-1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Octahydrate (37). Using the procedures described for the $BH_3 \cdot THF$ reduction of **35** and subsequent hydrolysis of the intermediate borane complex (**36**) (115 mg, 0.15 mmol) gave **37** (145 mg, 73%) as a white powder: mp 265–270 °C dec; ^1H NMR (D_2O) δ 0.91–1.01 (m, 18H), 1.81 (m, 8H), 2.93–3.42 (m, 44H), 4.02 (s, 4H), 7.28 (s, 4H). Anal. ($C_{40}H_{88}N_8Br_8 \cdot 8H_2O$) C, H, N, Br.

1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Zinc Dichloride Monohydrate (38). To a stirred solution of **19a** (free base) (1.0 g, 2.0 mmol)) in MeOH (25 mL) was added a solution of zinc(II) chloride (0.54 g, 4.00 mmol, 2.0 equiv) in MeOH (5 mL) during which time a white precipitate formed. Sufficient MeOH and H_2O were added to give a homogeneous solution, and the mixture was then evaporated *in vacuo*. The solid residue was suspended in a mixture of MeOH/Et_2O and filtered giving **38** (1.45 g, 94%) as a white powder: IR (KBr) ν 3472 (br), 3226 (s), 2927, 2869, 1620, 1463, 1428, 1100, 1065, 987 cm^{-1}. Anal. ($Zn_2Cl_4 \cdot C_{28}H_{54}N_8 \cdot H_2O$) C, H, N, Cl.

1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Copper Diacetate Heptahydrate (39). To a stirred solution of **19a** (free base) (100 mg, 0.20 mmol) was added copper(II) acetate (72 mg, 2.0 equiv) in one portion. The solution became dark blue almost immediately. The mixture was stirred for 1 h and then triturated with Et_2O to give a blue precipitate, which was collected by filtration and dried *in vacuo*, giving **39** (80 mg, 46%) as a blue powder: IR (KBr) ν 3410 (br), 3167, 2925, 2871, 1573, 1405, 1099, 1068, 1006, 648 cm^{-1}. Anal. ($Cu_2(OAc)_4 \cdot C_{28}H_{54}N_8 \cdot 7H_2O$) C, H, N.

1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Palladium Diperchlorate Tetrahydrate (40). To a refluxing solution of **19a** (free base) (114 mg, 0.22 mmol) in MeOH/H_2O (1:1, 20 mL) was added dropwise with stirring a solution of sodium tetrachloropalladate trihydrate (Aesar, 174 mg, 0.50 mmol, 2.2 equiv) in H_2O (20 mL), during which time a black precipitate formed. The mixture was heated to reflux for 1 h and then allowed to cool to room temperature and filtered through Celite, and the filtrate was evaporated. The residue upon evaporation was dissolved in a small volume of H_2O, filtered to remove insoluble solids and excess sodium perchlorate was added precipitating a pale yellow solid. The solid was collected by filtration, washed with H_2O and dried *in vacuo* to give **40** (90 mg, 33%) as a yellow powder. IR (KBr) ν 3443 (br), 3227 (s), 2885, 1471, 1103, 636 cm^{-1}. Anal. ($Pd_2(ClO_4)_4 \cdot C_{28}H_{54}N_8 \cdot 4H_2O$) C, H, N, Cl.

1,1′-[2,5-Dimethyl-1,4-phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (45a). Using general procedure A, **2a** (500 mg, 0.75 mmol), and 2,5-dimethyl-α,α′-dibromo-*p*-xylene (**44a**) (110 mg, 0.38 mmol) gave **45a** (530 mg, 97%) as a white solid: ^1H NMR ($CDCl_3$) δ 1.60–1.75 (m, 4H), 1.81–1.99 (m, 4H), 2.21 (s, 6H), 2.40 (s, 6H), 2.42 (s, 6H), 2.44 (s, 6H), 2.40–2.47 (m, 4H), 2.63–2.75 (m, 4H), 2.82–2.93 (m, 4H), 3.04 (m, 4H), 3.07–3.22 (br m, 16H), 3.51 (s, 4H), 6.99 (s, 2H), 7.23 (d, 4H, J = 8.2 Hz), 7.28 (d, 4H, J = 8.2 Hz), 7.33 (d, 4H, J = 8.2 Hz), 7.49 (d, 4H, J = 8.2 Hz), 7.65 (d, 4H, J = 8.2 Hz), 7.69 (d, 4H, J = 8.2 Hz); FAB MS *m/z* 1456 (M + H, 100), 1300 (20), 793 (18).

1,1′-[2,5-Dichloro-1,4-phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (45b). Using general procedure A, **2a** (500 mg, 0.75 mmol), and 2,5-dichloro-α,α′-dibromo-*p*-xylene (**44b**) (125 mg, 0.38 mmol) gave **45b** (555 mg, 98%) as a white solid: ^1H NMR ($CDCl_3$) δ 1.68–1.82 (m, 4H), 1.82–2.00 (m, 4H), 2.40 (s, 6H), 2.42 (s, 6H), 2.44 (s, 6H), 2.52 (m, 4H), 2.76 (m, 4H), 2.97–3.27 (br m, 24H), 3.63 (s, 4H), 7.19–7.41 (m, 14H), 7.59 (d, 4H, J = 8.2 Hz), 7.66 (d, 4H, J = 8.2 Hz), 7.69 (d, 4H, J = 8.2 Hz); FAB MS *m/z* 1497 (M + H, 100), 1341 (80), 663 (48), 507 (38).

1,1′-[2-Bromo-1,4-phenylenebis(methylene)]bis[4,8,11-tris(*p*-tolylsulfonyl)-1,4,8,11-tetraazacyclotetradecane] (45c). Using general procedure A, **2a** (500 mg, 0.75 mmol), and 2-bromo-α,α′-dibromo-*p*-xylene (**44c**) (130 mg, 0.38 mmol) gave **45c** (560 mg, 98%) as a white solid: ^1H NMR ($CDCl_3$) δ 1.72 (m, 4H), 1.83–2.01 (m, 4H), 2.40 (s, 6H), 2.42 (s, 6H), 2.44 (s, 6H), 2.42–2.57 (m, 4H), 2.65–2.80 (m, 4H), 2.99–3.28 (m, 24H), 3.53 (s, 2H), 3.63 (s, 2H), 7.18 (d, 1H, J = 7.7 Hz), 7.22–7.38 (m, 13H), 7.42 (s, 1H), 7.53–7.74 (m, 12H); FAB MS *m/z* 1507 ($M^{81}Br$ + H, 100), 1505 ($M^{79}Br$ + H, 95), 1351 (73), 1195 (16), 925 (24), 844 (30), 691 (46), 507 (31).

1,1′-[2,5-Dimethyl-1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Monohydrate (46a). Using general procedure D, **45a** (530 mg, 0.36 mmol) gave **46a** (230 mg, 54%) as a white solid: mp 235–240 °C dec; ^1H NMR (D_2O) δ 1.92–2.10 (m, 8H), 2.26 (s, 6H), 3.03–3.51 (m, 32H), 4.14 (s, 4H), 7.22 (s, 2H); ^{13}C NMR (D_2O) δ 18.48, 18.82, 18.98, 37.32 (3C), 40.94, 41.23, 41.64, 44.74, 48.11, 56.94, 129.90, 134.70, 137.44; FAB MS *m/z* 613 (M + $H^{81}Br$, 45), 611 (M + $H^{79}Br$, 47), 532 (M + H, 36), 333 (100), 201 (100). Anal. ($C_{30}H_{66}N_8Br_8 \cdot H_2O$) C, H, N, Br.

1,1′-[2,5-Dichloro-1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide (46b). Using general procedure D, **45b** (550 mg, 0.37 mmol) gave **46b** (130 mg, 29%) as a white solid: mp 258–263 °C dec; ^1H NMR (D_2O) δ 1.88–2.05 (m, 4H), 2.98 (br s, 4H), 3.03–3.50 (m, 32H), 4.07 (s, 4H), 7.61 (s, 2H); ^{13}C NMR (D_2O) δ 18.67, 19.00, 37.29, 37.77, 38.84, 41.01 (2C), 41.56, 45.63, 48.09, 54.26, 132.04, 132.83, 132.95; FAB MS *m/z* 653 (M + $H^{81}Br$, 96), 651 (M + $H^{79}Br$, 74), 571 (M + H, 70), 307 (44), 201 (100). Anal. ($C_{28}H_{60}N_8Cl_2Br_8 \cdot 1/2HOAc$) C, H, N, Br.

1,1′-[2-Bromo-1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide (46c). Using general procedure D, **45c** (560 mg, 0.37 mmol) gave **46c** (320 mg, 69%) as a white solid: mp 214–218 °C dec; ^1H NMR (D_2O) δ 2.01 (br m, 8H), 3.01–3.59 (m, 32H), 4.18 (s, 2H), 4.30 (s, 2H), 7.45 (d, 1H, J = 7.7 Hz), 7.55 (d, 1H, J = 7.7 Hz), 7.75 (s, 1H); ^{13}C NMR (D_2O) δ 18.75, 19.00, 19.11 (2C), 37.66 (6C), 41.29 (4C), 41.74 (2C), 44.80, 45.57, 48.06, 48.41, 57.91, 59.05, 126.77, 133.21, 131.74, 133.58, 134.37, 136.19; FAB MS *m/z* 665 ($M^{81}Br$ + $H^{81}Br$, 50), 663 ($M^{81}Br$ + $H^{79}Br$/$M^{79}Br$ + $H^{81}Br$, 100), 661 ($M^{79}Br$ + $H^{79}Br$, 50), 583 ($M^{81}Br$ + H, 66), 581 ($M^{79}Br$ + H, 65), 429 (60), 383 (66). Anal. ($C_{28}H_{61}N_8Br_9$) C, H, N, Br.

4,8,11-Tris(diethoxyphosphoryl)-1,4,8,11-tetraazacyclotetradecane (2b). To a stirred solution of 1,4,8,11-tetraazacyclotetradecane (5.0 g, 0.025 mol) and Et_3N (7.65 mL, 0.055 mol, 2.2 equiv) in $CHCl_3$ (300 mL) cooled to 0–5 °C under argon was added dropwise a solution of diethyl phosphorochloridate (7.57 mL, 0.052 mol, 2.1 equiv) in $CHCl_3$ (30 mL) over 1 h and the reaction mixture was then stirred overnight at room temperature. The solution was washed with saturated aqueous sodium bicarbonate solution and brine and then dried

($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 9:1), giving **2b** (3.7 g, 35%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.27–1.33 (m, 18H), 1.74 (m, 2H), 1.88 (m, 2H), 2.72 (m, 2H), 2.80 (m, 2H), 3.00–3.26 (m, 12H), 3.90–4.10 (m, 12H).

1,1'-[2-Phenyl-1,4-phenylenebis(methylene)]bis[4,8,11-tris(diethoxyphosphoryl)-1,4,8,11-tetraazacyclotetradecane] (45d). Using general procedure A, **2b** (358 mg, 0.59 mmol), and 2-phenyl-α,α'-dibromo-*p*-xylene (**44d**) (100 mg, 0.29 mmol) gave **45d** (160 mg, 39%) as a viscous oil: ^1H NMR ($CDCl_3$) δ 1.23–1.31 (m, 36H), 1.51–1.61 (m, 2H), 1.62–1.77 (m, 4H), 1.78–1.92 (m, 2H), 2.17 (m, 2H), 2.27–2.42 (m, 4H), 2.60 (m, 2H), 2.78–3.29 (m, 24H), 3.35 (s, 2H), 3.48 (s, 2H), 3.88–4.03 (m, 24H), 7.04 (s, 1H), 7.23 (d, 1H, J = 6.6 Hz), 7.25–7.36 (m, 6H); FAB MS *m/z* 1395 (M + H, 100), 786 (46), 607 (20), 180 (52).

1,1'-[2-Phenyl-1,4-phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide Dihydrate(46d). To a stirred solution of **45d** (160 mg, 0.12 mmol) in acetic acid (3 mL) was added 30% HBr in acetic acid (Aldrich, 5 mL) and the solution was stirred at room temperature for 14 h. The resulting precipitate was collected by filtration and washed with acetic acid and then Et_2O. The solid was then dissolved in H_2O (3 mL) and treated with charcoal (100 mg) and the mixture was heated to 80 °C for 30 min. The hot solution was filtered through Celite and the filtrate was concentrated to approximately 1 mL, after which acetic acid was added, resulting in the immediate formation of a white precipitate. The white solid was collected by filtration, giving **46d** (70 mg, 48%): mp 208–212 °C dec; ^1H NMR (D_2O) δ 1.61–1.73 (m, 2H), 1.84–2.19 (m, 6H), 2.96–3.63 (m, 32H), 4.38 (s, 4H), 7.36–7.52 (m, 6H), 7.55 (d, 1H, J = 7.8 Hz), 7.68 (d, 1H, J = 7.8 Hz); ^{13}C NMR (D_2O) δ 18.87, 19.13, 19.24, 19.45, 37.88 (2C), 38.06 (4C), 41.52 (2C), 41.79 (2C), 42.10 (2C), 45.20 (2C), 48.45 (2C), 55.52, 58.45, 129.01, 129.67, 129.78, 129.94, 130.89, 131.63, 132.95, 133.64, 138.99, 144.60; FAB MS *m/z* 661 (MH + $H^{81}Br$, 6), 659 (MH + $H^{79}Br$, 6), 579 (M + H, 12), 381 (94), 201 (73). Anal. ($C_{34}H_{66}N_8Br_8 \cdot 2H_2O$) C, H, N, Br.

1,1'-[2,5-Dimethoxy-1,4-phenylenebis(methylene)]bis-[1,4,8,11-tetraazacyclotetradecane] Octahydrobromide (46f). In a manner similar to that of **46d**, 1,1'-[2,5-dimethoxy-1,4-phenylenebis(methylene)]bis[4,8,11-tris(diethoxyphosphoryl)-1,4,8,11-tetraazacyclotetradecane] (**45f**) (350 mg, 0.25 mmol) gave **46f** (205 mg, 69%) as a white solid: mp 292–297 °C dec; ^1H NMR (D_2O) δ 1.92–2.18 (m, 8H), 3.19–3.62 (m, 32H), 3.78 (s, 6H), 4.33 (s, 4H), 7.13 (s, 2H); ^{13}C NMR (D_2O) δ 18.64, 19.32, 37.74 (2C), 38.01, 41.34, 41.64, 42.09, 45.12, 48.43, 54.95, 56.85, 116.45, 120.61, 152.48; FAB MS *m/z* 645 (M + $H^{81}Br$, 28), 643 (M + $H^{79}Br$, 29), 564 (M + H, 29), 365 (66), 201 (100). Anal. ($C_{30}H_{66}N_8O_2Br_8$) C, H, N, Br.

Anti-HIV Activity Assays. The human immunodeficiency virus strains used were HIV-1 (III_B) and HIV-2 (ROD) the origins of which have been described previously.[13] 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.[38] 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 greater than symbol (>) is used to indicate the highest concentrations at which the compounds were tested and still found to be 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.

Acknowledgment. The authors gratefully acknowledge the technical contributions of Forrest Gaul and Alice Seidel and the editorial assistance of Susan O'Hara (JM). Investigations at the Rega Institute were supported by the Biomedical Research Programme of the European Community, the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek, the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek, the Belgian Geconcerteerde Onderzoeksacties, and the Janssen Research Foundation. We thank Hilde Azijn, Barbara Van Remoortel, and Patrick Seldeslachts for excellent technical assistance. The constructive suggestion of activity correlation by a reviewer is acknowledged.

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JM9405699