Aromatic-Linked Polyamine Macrocyclic Compounds with Anti-HIV Activity

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Aromatic-linked polyamine macrocyclic compounds which show high selective activity against HIV. These compounds are macrocycles linked through methylene groups to an aromatic moiety and their complexes with bivalent metal ions exhibit potent and selective inhibition of human immunodeficiency virus (HIV)-1 and HIV-2 replication by a class of bicyclics interacting with a viral uncoating event.


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ABSTRACT

Polyamine macrocyclic compounds, e.g. of 10 to 15 ring members and 3 to 6 ring amine nitrogens, linked through methylene groups to an aromatic moiety, show high selective activity against HIV.

43 Claims, No Drawings
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AROMATIC-LINKED POLYAMINE MACROCYCLIC COMPOUNDS WITH ANTI-HIV ACTIVITY

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.


This invention concerns improvements in chemical compounds, more especially it concerns compounds and pharmaceutical compositions. In particular it concerns compositions and compounds having activity in vitro tests on Human Immunodeficiency Virus-infected cells.

The disease known as Acquired Immune Deficiency Syndrome (AIDS) caused by infection by HIV has attracted immense research effort because of the effects of the disease on infected individuals and the dangers of the disease spreading to a wider section of the population. In general, although various chemo-therapeutic treatments have been advocated, and some compounds have emerged as a potential basis for treatment, there is still a need for alternatives. In particular, most treatments such as the compound known as AZT have a high toxicity to cells, and it would be desirable to find compounds which are less toxic. In man, the development of resistance to AZT has been identified as an additional clinical problem.

We have found a group of compounds which show protective properties in vitro screens of cells challenged with HIV-1 and/or HIV-2, and are therefore useful for the treatment of AIDS and AIDS Related Complex and other viral and especially retroviral infections. Accordingly, the present invention provides the use of compounds defined below, in pharmaceutical compositions for treating HIV-infected patients. The invention further provides pharmaceutical compositions comprising a said compound in combination or association with a pharmaceutically acceptable diluent or excipient, for the treatment of HIV-infected patients. The invention may also be defined as the use of a said compound for the manufacture of a medicament for the treatment of HIV-infected patients. The invention further provides a process for the production of a pharmaceutical composition for the treatment of a HIV-infected patient, comprising the combination of a compound as defined below with a pharmaceutically acceptable diluent or excipient, and formulating said composition into a form suitable for administration to said patient. The invention also provides a method of treatment of an HIV-infected patient, comprising administering to said patient an effective dose of a said compound. It is to be understood that treatment includes prophylactic treatment of patients at risk, in view of the protective properties observed.

The use of the compounds may also be stated as a method of treating HIV-infected or HIV-challenged human cells to prevent or modulate the multiplication of the HIV, comprising administering to said cells an effective dose of a said compound. Whilst this description is especially directed to combating HIV, this invention includes other aspects in which other diseases may be treated, including for example microbial infections.

A 2,2’-dimer of cyclam has been reported as being isolated as a 2% by-product in the synthesis of cyclam (1,4,8,11-tetraaza-cyclooctadecane) (Burefeldt et al., J C S Chem Con (1981), 302). This compound was stated to be insoluble in water. We believe that the insoluble 2,2’-cyclam is a mixture of the 2R,2'R and 2S,2'S enantiomers; we have characterised a soluble dimer which we believe to be the meso, so 2R,2'S isomer. The 6,6'-cyclam isomer has been reported by Fabbrizzi et al., Inorg Chem 25, 2671 (1986). Certain N,N'-linked bicyclic compounds have been reported by Camplin et al., Inorg Chem 26, 3527 (1987). No biological activity has been suggested for such compounds.

U.S. Pat. No. 4,156,683 discloses monocyclic and bicyclic macrocyclic compounds, which [oxo] are said to have biological activity in regulating sodium, potassium and calcium levels in mammals. Additionally, a specific group of N-alkylated monocyclic compounds are said to possess activity against A3 influenza viruses in a modified Hermann test on chick fibroblast tissue. It is also said that the preferred compounds, which form complexes of greater stability, are those having three bridging chains between bridgehead nitrogen atoms, that is fused bicyclic compounds.

EP-A-0026522 discloses certain functionally modified cyclic polyamino ethers, including that known as "cyclam", which complexes with rhodium and may be bound to an antibody or antibody fragment. The aromatic-linked cyclic polyamino ethers which form the subject of the present invention are not disclosed, nor is any anti-vital activity.

EP-A-0035320 also discloses several modified cyclic polyamino ethers, but does not disclose identical cyclic polyamino ethers linked together.

WO-A-9105762 discloses polyamino acids useful for their multi-point chelating activity, but does not disclose linked cyclic polyamino acids.

WO-A-9216894 is in the same name as the present applicants, and discloses long-chain polyamino ethers, optionally linked to a cyclic polyamine, as agents active against HIV. No molecules having two cyclic polyamino ethers, linked through an aromatic linker are disclosed in this prior art.

Our U.S. Pat. No. 5,021,409 (equivalent to EP-A-0043585) describes linked cyclic compounds as being active against HIV-1 and HIV-2 in in vitro tests. We have now discovered that certain of the linked cyclic compounds exhibit surprisingly improved activity against HIV. Thus, the present invention concerns a selected group of the compounds taught in said USP, having activity of at least an order of magnitude greater than the compounds tested in said USP.

The present invention provides as active compounds linked cyclic polyamino ethers of the general formula

\[ \text{Z-R-A-R'-Y} \]  

in which Z and Y are identical cyclic polyamino moieties having from 9 to 20 ring members and from 3 to 6 amine nitrogens in the ring spaced by 2 or more carbon atoms from each other.

A is an aromatic or heteroaromatic moiety other than quinoline, and

R and R' are each methylene linked to an amine nitrogen in Z and in Y, the amine nitrogens being otherwise unsubstituted. The invention also encompasses acid addition salts and metal complexes of the compounds of formula 1.

In the above formula, the cyclic polyamino moieties may be substituted or unsubstituted, and suitable substituents are alkyl and/or aryl groups, eg of up to 10 carbon atoms, and any other atoms or groups which do not substantially adversely affect the activity or toxicity of the compounds. Preferred moieties are those of 10 to 15 ring members, and there are preferably 3 or 4 amine nitrogen atoms.
The aromatic or heteroaromatic moiety A tethers Y and Z through the linking groups R and R'. Moiety A may be phenyl or fused aromatic such as naphthyl, heterocyclic such as pyridyl or thiophenyl, fused heterocyclic or joined aromatic and/or joined heteroaromatic, for example biphenyl or bipyrindyl respectively. The moieties A may also be substituted at single or multiple non-linking positions with electron-donating groups, e.g. alkyl, thio, thioalkyl, hydroxyl, alkoxy, amino and derivatives thereof, or electron-withdrawing groups or atoms, e.g. nitro, halogen, carboxy, carboxamido, sulfonic acid and derivatives thereof.

The invention also includes what may be termed “prodrugs”, that is protected forms of the linked cyclic compounds, which release the compound after administration to a patient. For example, the compound may carry a protective group which is split off by hydrolysis in body fluids, e.g. in the bloodstream, thus releasing active compound. A discussion of pro-drugs may be found in “Smith and Williams’ Introduction to the Principles of Drug Design”, H. J. Smith, Wright, 2nd Edition, London 1988.


Accordingly, certain of the compounds of formula I are novel. The invention accordingly provides novel linked cyclic polynamine compounds of general formula Ia,

\[ Z \cdot R \cdot A' \cdot R' \cdot Y \]  

in which Z, Y, R and R' are as defined above, with R and R' linked to nitrogen atoms in Z and Y, and

A' is an aromatic or heteroaromatic moiety which is unsubstituted or substituted, other than quinoline, provided that

A' is not unsubstituted phenylene when Z and Y are 14-membered tetraaza rings,

and their acid addition salts and metal complexes.

The invention further provides a method for the production of the compounds of formula Ia, which method comprises nuclophilic attack by cyclic polynamines Z' and Y' each having a single unprotected ring amine nitrogen, all other ring amine nitrogens being protected, on a compound of formula II

\[ X \cdot R \cdot A' \cdot R' \cdot X \]  

wherein R, R' and A' are as defined above, and
each X is an active substituent which can be displaced by the unprotected amine nitrogens of polynamines Z' and Y' and is preferably selected from Br, Cl, I, methanesulfonyl, 4-tolylsulfonate and trifluoromethane sulfonate, and subsequently deprotecting the ring amine nitrogens.

It is well within the capabilities and knowledge of the skilled synthetic chemist to protect the amine nitrogens of cyclic polynamines, and it is preferred to use substitution by methanesulfonil and/or 4-tolylsulfonil and/or diethylphosphoryl. The compounds of formula II are known.

The reaction is preferably carried out by reacting two equivalents of the protected polynamine with the compound of formula II in a solvent, such as acetonitrile or dimethylformamide, tetrahydrofuran or dioxane and in the presence of a base, for example sodium carbonate or potassium carbonate. The reaction generally takes place readily at room temperature to elevated temperature, to give a linked molecule having protected amine nitrogen atoms. In general, a mixture of products will be obtained, and we have found that chromatography on silica gel is a particularly convenient method of separation.

The deprotection step is suitably carried out by refluxing the protected molecule in a mixture of aqueous HBr or acetic acid or in the case of diethyl phosphoronyl in the presence of hydrogen chloride (gas) in THF or dioxane.

The compounds are indicated for the treatment of viral infections, especially retrovirus infections and particularly HIV infections, and the compounds of formula Ia are to be considered as active compounds for the pharmaceutical compositions, processes for making the same and methods of treatment mentioned above. In these aspects of the invention, it is to be understood that ase sets, enantiomers and resolved optically active forms of the compounds of formula Ia are included. Also, it is to be considered within the invention, compounds of formula Ia diluted with non-toxic or other active substances.

Acid addition salts, for example hydrochlorides, and non-toxic labile metal complexes of the compounds of formula Ia are also active compounds according to the present invention. Non-toxic in the present context has to be considered with reference to the prognosis for the infected patient without treatment. Copper and zinc complexes are preferred although other metals such as nickel may be considered, whereas less labile metal atoms such as cobalt and rhodium are less preferred because of likely lower selectivity.

The present invention will now be illustrated by the following preparative examples.

**Example 1**

a) 2,3,5,6-Tetrafluoro-p-xylene-\( \cdot X \cdot x'-\)diol

To a stirred solution of perfluoroethylphthalic acid (1.0 g, 4.2 mmol) in anhydrous THF (10 ml) under an atmosphere of dry argon was added Borane. THF solution (1.0M solution in THF, 10 equivalents, 42 ml) be relaxed, and the mixture stirred at room temperature overnight. The solution was evaporated under reduced pressure to give a colourless liquid and the excess Borane destroyed by addition of anhydrous methanol (40 ml) and evaporate (repeated three times). The residue was treated with 5% aqueous hydrochloric acid then the pH of the mixture was adjusted to pH9 with IN aqueous sodium hydroxide solution and extracted with dichloromethane (3×50 ml). The combined organic extracts were dried (MgSO4) and evaporated to give 2,3,5,6-tetrafluoro-p-xylene-\( \cdot X \cdot x'-\)diol (0.75 g, 86%) as a white solid. This was used without further purification.

b) 2,3,5,6-Tetrafluoro-p-xylene-\( \cdot X \cdot x'-\)dimeysylate

To a stirred solution of 2,3,5,6-tetrafluoro-p-xylene-\( \cdot X \cdot x'-\)diol (0.72 g, 3.4 mmol) in dichloromethane (40 ml) containing triethylamine (1.2 ml, 2.5 equivalents) was added methanesulfonyl chloride (0.58 ml, 2.2 equivalents) dropwise at 0°C, and the mixture was allowed to warm to room temperature overnight. The solution was washed with saturated aqueous sodium bicarbonate solution (2×20 ml) and brine (2×20 ml) then dried (MgSO4) and evaporated under reduced pressure. The residue was suspended in ether and filtered giving 2,3,5,6-tetrafluoro-p-xylene-\( \cdot X \cdot x'-\)dimeysylate (0.9 g, 72%) as a white solid.

c) 1,1'-(2,3,5,6-Tetrafluoro-1,4-phenylenebis-(methylene))-bis-tris(p-toluene sulfonfonyl)-1,4,8,11-tetraazacyclotetradecane

2,3,5,6-Tetrafluoro-p-xylene-\( \cdot X \cdot x'-\)dimeysylate (150 mg, 0.4 mmol), tris(p-toluene sulfonfonyl)-1,4,8,11-tetraazacyclotetradecane monohydrate 826 mg, 1.2 mmol, 3.0 equivalents) and potassium carbonate (252 mg, 3.0 equivalents) in anhydrous acetonitrile (20 ml) were heated to reflux with stirring under argon for 48 hours until all the
dimesylate starting material had been consumed; confirmed by TLC (silica gel, 2% methanol in dichloromethane as eluent). The mixture was evaporated under reduced pressure and the residue was dissolved in ethyl acetate (40 ml) and washed with saturated aqueous sodium bicarbonate solution (2×20 ml) and brine (2×20 ml) then dried (MgSO₄) and evaporated. The residue was purified by column chromatography on silica gel during with 2% methanol in dichloromethane giving a white foam identified by 'H NMR and FAB-MS as 1,1'-[2,3,5,6-tetrafluoro-1,4-phenylene-bis-(methylene)]-bis-tris-(p-toluenesulfonyl)-1,4,8,11-tetraazaacyclotetradecane (200 mg, 0.13 mmol) was dissolved in a mixture of acetic acid and hydrobromic acid (48%) in a ratio of approximately 3:2 by volume (10 ml) and heated to 100° C. for 24 hours during which time a white solid precipitated. The mixture was allowed to cool and the solid was filtered off and washed with acetic acid and ether and dried in vacuo giving a white solid identified by 'H NMR, FAB-MS and elemental analysis as 1,1'-[2,3,5,6-tetrafluoro-1,4-phenylene-bis-(methylene)]-bis-tris-(p-toluenesulfonyl)-1,4,8,11-tetraazaacyclotetradecane octahy-dro-bromide dihydrate (65 mg, 40%).

C₂₅H₂₄N₈O₈Br₂F₄ requires C, 26.73; H, 4.96; N, 8.90; found C, 26.84; H, 5.05; N, 8.21.

The following compounds were prepared using analogous methods to those described above in steps a)-d):

5-Nitro-m-xylene, α,β-diol gave 1,1'-[5-Nitro-1,3-phenylene-bis-(methylene)]-bis-tris-(p-toluenesulfonyl)-1,4,8,11-tetraazaacyclotetradecane octahydrobromide dihydrate.

C₂₅H₂₄N₈O₈Br₂F₄ requires C, 27.31; H, 5.53; N, 10.24; found C, 27.49; H, 5.26; N, 9.75.

2,4,5,6-Tetrachloro-m-xylene, α,β-diol gave 1,1'-[2,4,5,6-tetrachloro-1,3-phenylene-bis-(methylene)]-bis-tris-(p-toluenesulfonyl)-1,4,8,11-tetraazaacyclotetradecane octahydrobromide dihydrate.

C₂₅H₂₄N₈O₈Cl₂Br₂ requires C, 25.40; H, 4.71; N, 8.46; found C, 25.72; H, 4.76; N, 8.05.

EXAMPEL 2

a) α,β-Dibromo-1,4-dimethylnaphthalene

To a solution of 1,4-dimethylnaphthalene (0.5 g, 3.2 mmol) and benzoyl peroxide (0.08 equivalents, 62 mg) in carbon tetrachloride (20 ml) was added N-bromosuccinimide (1.14 g, 2.0 equivalents) and the mixture was heated to reflux for 24 hours during which time a white solid precipitated. The mixture was filtered hot (to remove the succinimide by-product) and then allowed to cool over several hours during which time a white crystalline solid precipitated. The solid was filtered off and dried giving 1,4-Dimethylnaphthalene-α,β-dibromide (473 mg, 50%).

The following compounds were prepared using methods analogous to steps c) and d) of Example 1:

1,4-Dimethylnaphthalene-4,4'-dibromide gave 1,1'-[1,4-naphthylene-bis-(methylene)]-bis-tris-(p-toluenesulfonyl)-1,4,8,11-tetraazaacyclotetradecane octahydrobromide tetrahydrate.

C₂₃H₂₂N₈O₈Br₄ requires C, 30.20; H, 5.69; N, 8.81; found C, 30.28; H, 5.52; N, 8.66.

EXAMPEL 3

a) 1-Benzyl-5,13-di-(p-toluenesulfonyl)-9-methanesulfonyl-1,5,9,13-tetraazaacyclodecane

To a solution of N,N-bis-[3-(p-toluenesulfonylamidopropyl)-benzylamine hydrochloride (25 g) (NL Patent 6603655) in dry DMF (800 ml) under argon was added sodium hydride (10 equivalents) in small portions over 3 hours. When the addition was complete the solution was heated at 60° C. for 1 hour then allowed to cool and the excess sodium hydride was removed by filtration under argon. The filtrate was transferred to another dry flask and the solution was heated to 100°-110° C. for a further 16 hours, allowed to cool then the mixture was poured into iced water (1500 ml) and the resulting off-white precipitate that formed was collected by filtration. The solid was dissolved in dichloromethane (250 ml) and the solution was washed with water (5×50 ml), then dried (MgSO₄) and evaporated under reduced pressure to give a yellow oil. Trituration with ethanol (200 ml) gave a white crystalline solid which was filtered off, washed with a small volume of ethanol, then ether and dried in vacuo to give 1-benzyl-5,13-di-(p-toluenesulfonyl)-9-methanesulfonyl-1,5,9,13-tetraazaacyclodecane (45%), identified by 'H NMR and FAB-MS.

b) 1,9-Di-(p-toluenesulfonyl)-5-methanesulfonyl-1,5,9,13-tetraazaacyclodecane

To a solution of 1-benzyl-5,13-di-(p-toluenesulfonyl)-9-methanesulfonyl-1,5,9,13-tetraazaacyclodecane 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 hours with stirring. The mixture was allowed to cool, then filtered through celite and the filtrate was evaporated under reduced pressure. The colourless oil which remained was dissolved in dichloromethane (50 ml) and washed with 10% aqueous sodium hydroxide solution (2×20 ml) and water (2×20 ml) then dried (MgSO₄) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with 3% methanol in dichloromethane giving a white solid identified by 'H NMR and FAB-MS as 1,9-di-(p-toluenesulfonyl)-5-methanesulfonyl-1,5,9,13-tetraazaacyclodecane.

The mono-deprotected tetraazaacyclodecane macrocycle described in step b) was used as described in Example 1 steps c) and d) to prepare tetraazaacyclodecane dimers.

The following compounds were prepared in this manner.

4,4'-Dibromo-m-xylene gave 1,1'-[1,3-phenylene-bis-(methylene)]-bis-tris-(p-toluenesulfonyl)-1,4,8,11-tetraazaacyclodecane octahydrobromide hexahydrate.

C₂₃H₂₂N₈O₈Br₄ requires C, 29.2; H, 6.15; N, 8.54; found C, 29.37; H, 5.50; N, 8.07.

4,4'-Dibromo-m-xylene gave 1,1'-[1,4-phenylene-bis-(methylene)]-bis-tris-(p-toluenesulfonyl)-1,4,8,11-tetraazaacyclodecane octahydrobromide hexahydrate.

C₂₃H₂₂N₈O₈Br₄ requires C, 29.29; H, 6.15; N, 8.54; found C, 28.96; H, 5.47; N, 7.96.

Other compounds which may be made according to the invention are:

1,1'-[1,3-phenylenebis(methylene)]-bis-1,5,9,13-tetraazaacyclodecane

1,1'-[1,3-phenylenebis(methylene)]-bis-1,5,9,13-triazacyclodecane

1,1'-[1,4-phenylenebis(methylene)]-bis-1,5,9,13-triazacyclodecane

EXAMPEL 4

Synthesis of Compound F

1,1'-[1,4-Phenylenbis(methylene)]-bis-1,4,8,11-tetraazaacyclotetradecane zine dichloride monohydrate

To a stirred solution of 1,1'-[1,4-phenylene-bis-(methylene)]-bis-1,4,8,11-tetraazaacyclotetradecane (1 g) in
methanol (25 ml) was added zinc(II) chloride (0.54 g, 2.0 eq) in methanol (5 ml). Towards the end of the addition a white precipitate formed. Sufficient methanol and water were added to give a homogenous solution and the mixture was then evaporated in vacuo. The solid residue was suspended in a mixture of methanol/ether and filtered giving 1,1’-[1,4-phenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclododecane zinc dichloride monohydrate (1.45 g, 94%) as a white powder. 

$$C_{45}H_{70}Cl_2O_2Zn$$ requires: C, 42.38; H, 7.71; N, 14.12; Cl, 17.88; found: C, 42.64; H, 7.14; N, 14.18; Cl, 17.89.

**EXAMPLE 5**

Synthesis of Compound G

1,1’-[1,4-phenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclododecane copper diacetate hexahydrate

To a stirred solution of 1,1’-[1,4-phenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclododecane (100 mg) was added copper(II) acetate (72 mg, 2.0 eq) in one portion. The solution became dark blue/purple in colour almost immediately. The mixture was stirred for 1 hour then triturated with ether to give a blue precipitate. The blue solid was filtered off and dried giving 1,1’-[1,4-phenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclododecane copper diacetate hexahydrate (80 mg, 46%).

$$C_{45}H_{70}N_4O_8Cu_2$$ requires: C, 45.38; H, 8.13; N, 11.29; found: C, 45.24; H, 7.88; N, 11.13.

**EXAMPLE 6**

1,1’-[3’-Biphenylene-bis-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclododecane

A mixture of 3,3’-bis-(bromomethyl)-1,1’-biphenyl [W. Wenner, J. Org. Chem. (1952), 17, 525–528], (200 mg, 0.59 mmol), anhydrous potassium carbonate (325 mg, 2.35 mmol, 4 eq) and tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclododecane (801 mg, 1.18 mmol, 2 eq) in anhydrous acetonitrile (15 ml) was stirred at 50°C under argon. After 6 hours the reaction mixture was allowed to cool; dichloromethane (75 ml) was added and the resulting solution filtered. The filtrate was evaporated in vacuo to yield a glassy white solid. Chromatography of the crude product on a column of silica gel (2.5 cm x 20 cm), eluting with methanol/dichloromethane 1:160 v/v gave a white solid, identified by 1H NMR as 1,1’-[3,3’-biphenylene-bis-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclododecane (655 mg, 76%).

Synthesis of Compound J

1,1’-[3,3’-Biphenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclododecane octahydrobromide tetrahydrate

The per-tosylate derivative from above (450 mg, 0.30 mmol) was dissolved in glacial acetic acid (9 ml). Hydrobromic acid (~48% w/w, Aldrich, 3.5 ml) was added and the resulting mixture heated to reflux. After 24 hours the dark brown solution was cooled in an ice bath over 2 hours during which time an off-white precipitate formed. The precipitate was collected by centrifugation and washed with glacial acetic acid (3x10 ml) followed by diethyl ether (4x10 ml) then dried overnight in vacuo to give a white powder, identified by 1H NMR and elemental analysis as 1,1’-[3,3’-biphenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclododecane octahydrobromide tetrahydrate (194 mg, 50%).

$$C_{45}H_{70}N_4Br_2O_2$$ requires: C, 31.46; H, 5.70; N, 8.63; found: C, 31.30; H, 5.68; N, 8.60.

**EXAMPLE 7**

1,1’-[4,4’-(2,2’-Bipyridine)-bis-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclododecane

A mixture of 4,4’-bis-(bromomethyl)-2,2’-bipyridine [T.J. Meyer, Inorg. Chim. Acta. (1991), 30, 2942–2949], (200 mg, 0.57 mmol), anhydrous potassium carbonate (314 mg, 2.27 mmol, 4 eq) and tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclododecane (774 mg, 1.14 mmol, 2 eq) in anhydrous acetonitrile (20 ml) was stirred at 50°C under argon for 2 hours. The mixture was allowed to cool and dichloromethane (100 ml) was added and the resulting solution filtered through celite. The filtrate was evaporated in vacuo to give a yellow glassy solid which was purified by column chromatography on silica gel (3x20 cm column) using triethylamine/methanol/dichloromethane 1:100 v/v as eluent. A glassy white solid was obtained, identified by 1H NMR as 1,1’-[4,4’-(2,2’-bipyridine)-bis-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclododecane (600 mg, 70%).

Synthesis of Compound K

1,1’-[4,4’-(2,2’-Bipyridine)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclododecane decahydrobromide pentahydrate

The per-tosylate derivative from above (570 mg, 0.38 mmol) was dissolved in glacial acetic acid (6.5 ml). Hydrobromic acid (~48% w/w, Aldrich, 3.0 mmol) was added and the mixture heated to reflux for 24 hours. The resulting dark brown solution was cooled in an ice bath over 2 hours) during which time an off-white precipitate formed. The precipitate was collected by centrifugation and washed with glacial acetic acid (3x10 ml) followed by diethyl ether (5x10 ml) and dried overnight in vacuo to give a white powder identified by 1H NMR and elemental analysis as 1,1’-[4,4’-(2,2’-bipyridine)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclododecane decahydrobromide pentahydrate (450 mg, 81%).

$$C_{45}H_{70}N_4Br_2O_2$$ requires: C, 25.97; H, 5.17; N, 9.46; found: C, 26.07; H, 4.57; N, 9.47.

**EXAMPLE 8**

1,1’-[2,9-(1,10-Phenanthroline)-bis-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclododecane

A mixture of 2,9-(bromomethyl)-1,10-phenanthroline [C.J. Chandler, J. Heterocycl. Chem. (1981), 18, 599–601], (300 mg, 0.54 mmol), anhydrous potassium carbonate (300 mg, 2.17 mmol, 4 eq) and axis-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclododecane (740 mg, 1.09 mmol, 2 eq) in anhydrous acetonitrile (20 ml) were stirred at 50°C under argon for 3 hours. The mixture was allowed to cool and dichloromethane (100 ml) was added and the resulting solution filtered through celite. The filtrate was evaporated in vacuo to give a yellow glassy solid which was purified by column chromatography on silica gel (3x20 cm column) using triethylamine/methanol/dichloromethane 1:3:100 v/v eluent. A pale yellow solid was obtained, identified by 1H NMR as 1,1’-[2,9-(1,10-phenanthroline)-bis-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclododecane (575 mg, 69%).
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Synthesis of Compound L

11-[[2.9-(1,10-Phenanthroline)-bis-(methylene)-bis-1,4,8,11-tetraazacyclotetradecane decahydrobromide trihydrate

The per-tosylated derivative from above (400 mg, 0.26 mmol) was dissolved in glacial acetic acid (8 mL). Hydrobromic acid (48% w/v, Aldrich, 3.5 mL) was added and the mixture was heated to reflux for 16 hours. The resulting dark brown solution was cooled in an ice bath over 2 hours during which time an off-white precipitate formed. The precipitate was collected by centrifugation, then purified by reprecipitation from a mixture of hydrobromic acid (48% w/v, 2 mL) and water (3 mL) with glacial acetic acid (5 mL). The white solid was again collected by centrifugation, washed with glacial acetic acid (3×10 mL) and diethyl ether (4×10 mL) and finally dried overnight in vacuo to give a white powder, identified by 'H NMR and elemental analysis as 11.11'-[2.9-(1,10-Phenanthroline)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane decahydrobromide trihydrate (80 mg, 21%).

C32H26N6O9Br2 requires: C, 28.35; H, 5.61; N, 9.45; found: C, 27.82; H, 4.94; N, 9.54; N, 9.17.

EXAMPLE 9


11.11'-[1,4-Phenylene-bis-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,7,11-tetraazacyclotetradecane

A mixture of α,α'-dibromo-p-xylene (249 mg, 0.94 mmol), anhydrous potassium carbonate (652 mg, 4.71 mmol, 5 eq) and tris(p-toluenesulphonyl)-1,4,7,11-tetraazacyclotetradecane [T. A. Kaden, Heur. Chim. Acta., (1983), 66, 861–870) (1.25 g, 1.89 mmol, 2 eq) in anhydrous acetonitrile (15 mL) was heated at 50°C with stirring under argon for 18 hours. The reaction mixture was allowed to cool and dichloromethane (50 mL) was added and the resulting solution filtered through celite. The filtrate was evaporated in vacuo to give a white foam which was purified by column chromatography on silica gel using methanol/dichloromethane (1:40 v/v) as eluent. A white solid was obtained, identified by 'H NMR as 11.11'-[1,4-phenylene-bis-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,7,11-tetraazacyclotetradecane (1.0 g, 74%).

Synthesis of Compound N

11.11'-[1,4-Phenylene-bis-(methylene)]-bis-1,4,7,11-tetraazacyclotetradecane octahydrobromide hexahydrate

The per-tosylated derivative from above (115 mg, 0.08 mmol) was dissolved in glacial acetic acid (3 mL). Hydrobromic acid (48% w/v, Aldrich, 1.5 mL) was added and the mixture was heated to reflux for 48 hours. The resulting dark brown solution was cooled in an ice bath and a white precipitate formed. The solid was collected by centrifugation and washed with glacial acetic acid (2×10 mL) followed by diethyl ether (4×10 mL) and dried overnight in vacuo to give a white powder, identified by 'H NMR and elemental analysis as 11.11'-[1,4-phenylene-bis-(methylene)]-bis-1,4,7,11-tetraazacyclotetradecane octahydrobromide hexahydrate (71 mg, 75%).

C32H26N6O9Br2 requires: C, 28.35; H, 5.61; N, 9.45; found: C, 28.34; H, 5.42; N, 9.02.

EXAMPLE 10

11.11'-[1,4-Phenylene-bis-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,7,11-tetraazacyclotetradecane

A mixture of α,α'-dibromo-p-xylene (3.98 g, 15.1 mmol, 10 eq) and anhydrous potassium carbonate (417 mg, 3.02 mmol, 2 eq) in anhydrous acetonitrile (20 mL) was heated to 50°C. With rapid stirring a solution of tris(p-toluenesulphonyl)-1,4,7,11-tetraazacyclotetradecane (1.0 g, 1.51 mmol) in anhydrous acetonitrile (20 mL) was added dropwise over 4 hours. After a further 1 hour the reaction mixture was allowed to cool and the solvent evaporated in vacuo. The residue was purified by column chromatography on silica gel (5×20 cm), eluting with a gradient of dichloromethane to methanol/dichloromethane 1:20 v/v over 2 liters total elution volume. The resulting colourless glass was dried in hexane (150 mL) and the mixture was heated to reflux then allowed to cool to room temperature. The precipitate which formed was filtered, washed with hexane (3×10 mL) followed by diethyl ether (20 mL) and dried overnight in vacuo to give the title compound as a white powder (710 mg, 53%).

11.11'-[1,4-Phenylene-bis-(methylene)]-tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclotetradecane

A mixture of 11-[(1-methylene-4-bromomethylene)-phenylene]-tris-(p-toluenesulphonyl)-1,4,7,11-tetraazacyclotetradecane (350 mg, 0.41 mmol), anhydrous potassium carbonate (230 mg, 1.66 mmol, 4 eq) and tris(p-toluenesulphonyl)-1,4,8,11-tetraazacyclotetradecane (422 mg, 0.62 mmol, 1.5 eq) in anhydrous acetonitrile (20 mL) were heated with stirring at 50°C under argon for 7 hours. The reaction mixture was allowed to cool and the solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel (2.5×25 cm column) using methanol/dichloromethane (1:90 v/v) as eluent, followed by preparative thin layer chromatography on silica gel (eluent methanol/dichloromethane 1:40 v/v, 20 mg/plate) to give a colourless glass, identified by 'H NMR as the title compound (130 mg, 30%).

Synthesis of Compound N

11.11'-[1,4-Phenylene-bis-(methylene)]-1,4,8,11-tetraazacyclotetradecane octahydrobromide hexahydrate

EXAMPLE 11

11.11'-[2,6-Pyridinebisc-(methylene)]-bis-tris-(p-toluenesulphonyl)-1,4,8,11-tetraazacyclotetradecane

A stirred solution of 2,6-bis(bromomethyl)pyridine hydrobromide [M. E. Haeg, B. J. Whitlock and H. W. Whit-
lock Jr, J. Ant. Chem. Soc., (1989), 111, 692], (131 mg, 0.378 mmol), tris-(p-toluene-sulphonyl)-1,4,8,11-tetraazacyclotetradecane (500 mg, 0.755 mmol) and potassium carbonate (400 mg, 2.88 mmol) in anhydrous acetonitrile (15 ml) was heated at 80° C. for 22 hours under an atmosphere of argon. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. The residue was purified by column chromatography on silica gel using 3% methanol in dichloromethane as eluent thus affording a pale white solid which was identified by H NMR, FAB-MS and elemental analysis as 1,1'-[2,6-pyridinebis-(methylene)]-bis-tris(p-toluene-sulphonyl)-1,4,8,11-tetraazacyclotetradecane (500 mg, 93%).

Mass spectrum (FAB); m/e (relative intensity); 1428 (M+1, 100), 1272 (35)

Synthesis of Compound O

1,1'-[2,6-Pyridinebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane octahydrorhodidine tetrahydrate

To a stirred solution of 1,1'-[2,6-pyridinebis-(methylene)]-bis-tris(p-toluene-sulphonyl)-1,4,8,11-tetraazacyclotetradecane (500 mg, 0.355 mmol) in acetic acid (16 ml) was added 48% hydrobromic acid (12 ml) and the solution heated to 110° C. for 48 hours during which time a white solid precipitated. The reaction mixture was allowed to cool to room temperature and the solid was filtered off, washed with acetic acid followed by ether and dried in vacuo thus affording a white solid which was identified by H NMR, 13C NMR, FAB-MS and elemental analysis as 1,1'-[2,6-pyridinebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane octahydrorhodidine tetrahydrate (230 mg, 65%).

C22H22N2O2Br requires; C, 56.70; H, 5.64; N, 10.31; Br, 25.29; found C, 56.11; H, 5.31; N, 10.08; Br, 25.99. Mass spectrum (FAB); m/e (relative intensity); 586 (M+HBr), 584 (M+HBr), 504 (M+1, 100), 201 (60).

EXAMPLE 12

1,1'-[3,5-Pyridinebis-(methylene)]-bis-tris(p-toluene-sulphonyl)-1,4,8,11-tetraazacyclotetradecane

A stirred solution of 3.5-bis(bromomethyl)pyridine hydrobromide (M. Momenteau, J. Mis piller, B. Lacko and J. M. Hoste, J. Chem. Soc. Perkin Trans. 1, (1985), 61) (151 mg, 0.37 mmol), tris-(p-toluene-sulphonyl)-1,4,8,11-tetraazacyclotetradecane (500 mg, 0.755 mmol) and potassium carbonate (400 mg, 2.88 mmol) in anhydrous dimethylformamide (15 ml) were heated at 70° C. for 21 hours under an atmosphere of argon. The reaction mixture was allowed to cool to room temperature and concentrated in vacuo. The residue was purified by column chromatography on silica gel using 2% methanol in dichloromethane as eluent thus affording a white foamy solid which was identified by H NMR and FAB-MS as 1,1'-[3,5-pyridinebis-(methylene)]-bis-tris(p-toluene-sulphonyl)-1,4,8,11-tetraazacyclotetradecane (320 mg, 78%).

Mass spectrum (FAB); m/e (relative intensity); 1428 (M+1, 100), 1272 (45).

Synthesis of Compound P

1,1'-[3,5-Pyridinebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane nonahydrorhodidine dihydrate

To a stirred solution of 1,1'-[3,5-pyridinebis-(methylene)]-bis-tris(p-toluene-sulphonyl)-1,4,8,11-tetraazacyclotetradecane nonahydrorhodidine dihydrate (20 ml, 95%) was bubbled HBr gas for 15 minutes resulting in an immediate white precipitate. The white solid was filtered off, washed with ethanol and ether and immediately dried in vacuo for 48 hours thus affording a white solid which was identified by 1H NMR, 13C NMR, FAB-MS and elemental analysis as 1,1'-[3,5-pyridinebis-(methylene)]-bis-1,4,8,11-tetraazacyclododecan nonahydrorhodidine dihydrate (130 mg, 63%).
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C_{6}H_{4}N_{2}Br_{2} requires C, 30.92; H, 5.62; N, 12.02; Br, 51.43; found C, 31.06; H, 5.80; N, 11.90; Br, 51.17. Mass spectrum (FAB); m/e (relative intensity); 529 (M+HBr), 53, 527 (M+HBr), 55, 447 (M+1, 100), 277 (40), 185 (35).

EXAMPLE 14

1,1′-[1,4-Phenylenbis-(methylene)]-bis-tris-(p-toluene sulphonyl)-1,4,7,10-tetraazacyclododecane

A stirred solution of α,α’-dibromo-p-xylene (99 mg, 0.374 mmol), tris-(p-toluene sulphonyl)-1,4,7,10-tetraazacyclododecane (475 mg, 0.748 mmol) and potassium carbonate (320 mg, 2.24 mmol) in anhydrous acetonitrile (15 ml) was heated at reflux for 1.4 hours under an atmosphere of argon. The resulting cloudy white solution was allowed to cool to room temperature and the solids collected by filtration and washed with acetonitrile. The solid residue was dissolved in a mixture of dichloromethane (120 ml) and water (15 ml). The organic phase was separated and washed with water (15 ml), dried (MgSO_{4}) and concentrated under reduced pressure. The residue was dried in vacuo thus affording a white solid which was identified by 'H NMR as 1,1′-[1,4-phenylenebis-(methylene)]-bis-tris-(p-toluene sulphonyl)-1,4,7,10-tetraazacyclododecane (360 mg, 70%).

Mass spectrum (FAB); m/e (relative intensity); 1371 (IV1+1, 12), 1217 (8).

Synthesis of Compound R

1,1′-[1,4-Phenylenbis-(methylene)]-bis-1,4,7,10-tetraazacyclododecane hexahydrate bromide

To a stirred solution of 1,1′-[1,4-phenylenebis-(methylene)]-bis-tris-(p-toluene sulphonyl)-1,4,7,10-tetraazacyclododecane (360 mg, 0.262 mmol) in anhydrous methanol/dimethyl sulfoxide (1:5, 18 ml) was added 3% sodium amalgam (23 g) and dibasic sodium phosphate (400 mg). The reaction mixture was vigorously stirred under argon at 100°C for 4 hours then allowed to cool to room temperature and the supernatant solution was separated from the solids by decantation and concentrated in vacuo. Chloroform (50 ml) and water (5 ml) were added to the residue and the aqueous phase was extracted with chloroform (3×50 ml). Concentration of the combined organic fractions afforded quantitatively a foamy white solid which was identified by 'H NMR as 1,1′-[1,4-phenylenebis-(methylene)]-bis-1,4,7,10-tetraazacyclododecane.

Into a stirred solution of 1,1′-[1,4-phenylenebis-(methylene)]-bis-1,4,7,10-tetraazacyclododecane in ethanol (15 ml, 95%) was bubbled HBr gas for 15 minutes resulting in an immediate white precipitate. The white solid was filtered off, washed with ethanol and ether and immediately dried in vacuo for 48 hours thus affording a white solid which was identified by 'H NMR, 13C NMR, FAB-MS and elemental analysis as 1,1′-[1,4-phenylenebis-(methylene)]-bis-1,4,7,10-tetraazacyclododecane hexahydrate bromide (115 mg, 44%).

C_{18}H_{16}Br_{2}N_{2} requires C, 30.92; H, 5.62; N, 12.02; Br, 51.43, found C, 30.90; H, 5.83; N, 11.83; Br, 51.19. Mass spectrum (FAB); m/e (relative intensity); 529 (M+HBr), 53, 527 (M+HBr), 55, 447 (M+1, 18), 237 (40), 185 (35).

EXAMPLE 15

1,1′-[2,5-Thiophenebis-(methylene)]-bis-tris-(p-toluene sulphonyl)-1,4,8,11-tetraazacyclotetradecane

To a solution of tris-p-toluene sulphonyl-1,4,8,11-tetraazacyclotetradecane monohydrate (1.0 g, 1.5 mmol) and potassium carbonate (300 mg, 2.2 mmol) in acetonitrile (20 ml) was added 2,5-dichloromethyl thiophene [J. M. Griffing, L. F. Salisbury, J. Am. Chem. Soc., (1948), 70, 3416-3419], (157 mg, 0.76 mmol) and the mixture was heated to reflux overnight with rapid stirring. The mixture was allowed to cool and the solid was filtered off. The filtrate was evaporated in vacuo and the residue partitioned between methyl chloride (50 ml) and water (25 ml). The organic layer was separated, dried (Na_{2}SO_{4}) and evaporated in vacuo to give the crude product as a light brown solid. Column chromatography [silica gel; methylene chloride/methanol (40:1)] was used to isolate a white solid identified by 1H NMR and FAB-MS as 1,1′-[2,5-thiophenebis-(methylene)]-bis-tris-(p-toluene sulphonyl)-1,4,8,11-tetraazacyclotetradecane (315 mg, 29%).

Mass spectrum (FAB); m/e (relative intensity); 1434 (M+1, 49), 1277 (31), 772 (100), 616 (30), 508 (24).

Synthesis of Compound T

1,1′-[2,5-Thiophenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane octahydrobromide

To a solution of 1,1′-[2,5-thiophenebis-(methylene)]-bis-tris-(p-toluene sulphonyl)-1,4,8,11-tetraazacyclotetradecane (177 mg, 0.12 mmol) in acetic acid (6 ml) was added hydrobromic acid (Aldrich 48% aqueous, 4 ml) and the mixture was heated to reflux with stirring for 16 hours during which time a light brown solid precipitated from a dark brown solution. On cooling, a further portion of acetic acid was added (10 ml) and the solids were filtered off, washed with acetic acid (10 ml) and ether (20 ml) and dried in vacuo giving a white solid identified by 1H NMR and FAB-MS as 1,1′-[2,5-thiophenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane octahydrobromide (82 mg, 97%).

Mass spectrum (FAB); m/e (relative intensity); 591 (M+HBr), 589 (M+HBr) 26, 589 (M+HBr), 569 (M+1, 22) 311 (23), 201 (71), 185 (100).

The compounds of the invention were tested in a screen by the MTT method (1 Vixol Methods 120:309-321 [1988], MT-4 cells (2.5×10^4/well) were challenged with HIV-1 (HTLV-IIIB) or HIV-2 (LAB-2 ROD) at a concentration of 100 CCID_{50} and incubated in the presence of various concentrations of the test compounds, which were added immediately after challenge with the virus. After 5 days culture at 37°C in a CO_{2} incubator, the number of viable cells was assessed by the MTT (tetrazolium) method. Anti-viral activity and cytoxicity of the compounds are expressed in the table below as IC_{50} (µg/ml) and CC_{50} (µg/ml), respectively. The potential therapeutic usefulness was assessed by calculating a Selectivity Index (SI) corresponding to the ratio of CC_{50} to IC_{50}. A control test was performed using the known anti-HIV treatment AZT.

In Table 1 below, the compounds screened were:

AZT: known anti-HIV compound
A: 1,1′-[1,3-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane
B: 1,1′-[1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane
C: 1,1′-[5-nitro-1,3-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane
D: 1,1′-[2,3,5,6-tetrafluoro-1,3-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane
E: 1,1′-[4-naphthylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane
F-V: See preceding preparative Examples.
W: 1,1′-[2,5-dimethyl-1,4-phenylenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane
It can readily be seen that the compounds according to the invention are highly active against HIV-1 and -2, with low toxicity, in the in vitro tests used.

The compound B, which is the most preferred compound of the invention, was further tested for antiviral effects on different laboratory strains of HIV-1 in MT-4 cells, using the MIT assay. Compound B was found to have an IC50 in the range of 2–5 ng/ml against IIIB, RF, HE and NDK strains, showing that its high activity is remarkably strain-independent.

T4-lymphocytes and monocytes are targets for HIV-1 infection in vivo. The following test method showed that compound B inhibits virus replication also in primary T4 cells and primary monocytes in culture.

Primary T4 lymphocytes were purified from human spleens obtained from healthy donors by using a commercial kit ("Lympho-Kwik") which combines reaction of cells with specific monoclonal antibodies and density gradient centrifugation to separate the cells. Preparations obtained by this procedure contained 60–80% CD4 positive cells as analysed by FACS. Cells were stimulated with 2 μg/ml PHA for 24 hours. Then they were spun down and infected with HIV-1, strain IIIB, by suspending the cells 10-fold concentrated in virus solution. Adsorption was allowed for 2 hours at 37°C. The inoculum was removed by centrifugation and the cells were re-suspended at their original concentration in fresh culture medium containing II-2 (40 IE/ml). Test compound was added after stimulation and virus adsorption. Every 3 to 4 days post infection half of the supernatant of the infected cultures was removed and replaced by fresh medium containing the test compound at the particular concentration. The concentration of viral p24 antigen was determined in the supernatant by means of a commercial ELISA kit (Coulter) and served as a parameter for virus production. Compound B does not interfere with the p24 Elisa test (highest concentration tested: 100 μg/ml).

Mononuclear cells were isolated from healthy, HIV-negative donors using Ficoll density separation. Cells (4x10^6/ml) were incubated for 5 days in 48 well plates (Costar) in monocyte medium consisting of RPMI1640, supplemented with 20% FCS and 10% human serum. On day 5 non-adherent cells were washed out four times with warm PBS containing 2% human serum. Preparations obtained by the procedure were >95% positive for non-specific esterase (Sigma) and cell viability (as determined by trypan blue exclusion) was always >95%.

The monocytes were seeded in 10% FBS, and were used for the infection of these monocyte preparations (Pemo et al., Exp. Med., 169, 933, 1989).

Adherent monocytes were exposed to 50 μg/well of a 1:30 dilution of HIV-1, Bal, for 30 minutes subsequently, monocyte medium was added to 1 ml well. Adsorption was allowed for 24 hours at 37°C. Then the wells were washed twice in order to remove excess virus and were cultivated in the presence of different drug concentrations. Thus, test compounds were added after adsorption. Every 3 to 4 days post infection the supernatant of the infected cultures was removed and replaced by fresh medium containing the test compound at the particular concentration. The concentration of viral p24 antigen was determined as described above.

IC50 and IC90 values were calculated by comparing the p24 antigen concentrations in supernatant of treated, infected cells and untreated, infected cells at days 11 and 14 post infection.

Table 2 shows that Compound B is a potent inhibitor of HIV-1 replication in both primary cell types, with IC90 values of 1–2 ng/ml. At the highest concentration tested, 100 ng/ml, no cytotoxicity was observed.

Using the same methods, it was also shown that Compound B was a strong inhibitor of viral replication in primary T4 cells infected with low-passage primary clinical isolates of HIV-1 from three different geographical locations (K51, Zaire, D370, California, and K62, Germany).

The low cytotoxicity of Compound B was also shown by incubation of exponentially growing cells with Compound B or with AZT and determining cell numbers 2, 3 and 4 days after seeding. Compound B did not inhibit growth of MT4, MOLT4, HUT178, Jurkat cells (all T cell lines) nor the growth of the monocytic U937 cell line at concentrations below 300 μg/ml. With the exception of the HUT178 cells, AZT was in all cases more cytotoxic than Compound B with TC50 values (μg/ml) of 23, 37, 184 and 5 for MT4, MOLT4, Jurkat and U937 respectively.

In contrast to HIV-protease inhibitors, the compounds of the invention do not block virus production from chronically infected cells, indicating that the antiviral target is in the early part of the infection process, before or at, integration of the provirus. To pinpoint the stage at which the com-
pounds interact with the HIV replicative cycle, a time-of-addition experiment was carried out on MT4 cells infected with HIV-1 strain IIIb at high virus multiplicity to ensure that the virus replicative steps would be synchronised in the whole cell populations. Test compounds were added 1, 2, 3, 18, 22, 23, 24 hours after infection, and viral p24 antigen production determined 29 hours after infection. Depending on the stage at which compounds interact and the need for intracellular metabolism, addition of the compound could be delayed for a number of hours without loss of activity. Dextran sulphate, which acts at the virus adsorption step, must be added together with the virus (n=0) to be active. For AZT, which, following its intracellular phosphorylation, acts at the reverse transcriptase step, addition to the cells could be delayed until 4 hours (n=4) after infection. For the TIBO derivative (R82913), which does not need intracellular transformation before it can interact with reverse transcriptase the addition could be delayed by another 2 hours (n=6). The protease inhibitor Ro31-8959 which interacts with a late event in the virus cycle (assembly of mature virus) was still effective if added as late as 12 hours after infection (n=12). From the time-of-addition experiment appeared that for Compound B, n=1 or 2, so that the compound must interact with a process following virus adsorption but preceding reverse transcription, for example, virus-cell fusion and/or uncoating.

To obtain further evidence for the inhibitory effect of Compound B on HIV uncoating (or fusion), experiments were designed whereby the viral RNA harvested from cells that had just been infected was monitored for its sensitivity to degradation by RNase. It was reasoned that if uncoating (fusion) was hampered, the viral capsid (or envelope) proteins would remain associated with the viral RNA genome and thus the RNA should be protected against RNase attack. When MT4 cells were exposed to radiolabelled viral particles at a very high multiple of infection and then treated with different concentrations of Compound B, viral RNA harvested from the cells 4 hours after infection showed resistance to degradation by RNase. Vital RNA harvested from HIV-infected cells treated with other anti-HIV agents (ie AZT, DDI, R82913, or Ro31-8959) did not show this increased resistance to degradation by RNase.

In addition, Compound B was also found to inhibit fusion, which is the mechanism by which viruses enter cells and by which virus-encoding material is transmitted from cell to cell. Syncytium formation between chronically infected cells and uninfected cells reflects the gp120/41 mediated fusion process of viral entry. The syncytium inhibition assay (Baba et al, J AIDS 3 493, 1990) using HIV-1 IIIb infected HUT78 cells with MOLT4 cells indicates that Compound B is at least as potent as dextran sulphate in inhibition of fusion. The concentrations required (approximately 1 μg/ml) are considerably higher than the antiviral IC_{50} values, but are well below cytotoxicity levels.

These results strongly indicate that the compounds of the invention inhibit primarily the uncoating step and also to some extent the fusion step of the viral replicative cycle. This is a unique mode of action for anti-HIV agents, and the involvement of two distinct target steps makes it less likely that resistance to the drug will develop rapidly in treated patients.

Although no suitable animal models exist for the testing of in vivo efficacy of anti-HIV agents, testing of drug serum levels in the rabbit was carried out, and after se administration of 10 mg/kg of Compound B, samples of rabbit serum were taken. Measurement of anti-HIV activity in the serum showed levels of the drug exceeding the in vitro IC_{50} level by a factor of a hundred for at least 6 hours after administration. This indicates that the compound would have anti-HIV activity in humans or an animal susceptible to infection by HIV.

The compounds of Formula I are therefore useful for the treatment and/or prophylaxis of HIV infection, alone or in combination with other active agents. The appropriate dosage will, of course, vary depending upon, for example, the compound of Formula I employed, the host, the mode of administration and the nature and severity of the conditions being treated. However, in general, satisfactory results in humans are indicated to be obtained at daily dosages from about 0.01–20 mg/kg of body weight. An indicated daily dosage in humans is in the range from about 0.7 mg to about 1400 mg of a compound of Formula I conveniently administered for example in divided doses up to four times a day.

The compounds of Formula I may be administered by any conventional route, particularly enterally, preferably orally, eg in the form of tablets or capsules or in liquid form, eg as a syrup, or parenterally, eg in the form of solutions or suspensions for iv or sc administration.

Compound B is the preferred compound of Formula I. In view of its activity in the test methods as described above, it is indicated that Compound B may be administered to humans at daily dosages of from 1 to 200 mg, preferably 10 to 70 mg, by parenteral administration, eg by subcutaneous injection.

The compounds of Formula I may be administered in free base form or in pharmaceutically acceptable acid addition salt or metal complex form. Such salts and complexes may be prepared in conventional manner as described in the Examples, and exhibit the same order of activity as the free bases. Pharmaceutical compositions containing compounds of Formula I may be prepared in conventional manner. Unit dosage forms contain for example from about 0.5 mg to about 100 mg of a compound of Formula I in a single dosage form, or pharmaceutically acceptable addition salt form.

We claim:

1. A pharmaceutical composition active against HIV comprising as an active ingredient a linked cyclic compound of formula I,

   \[
   \text{Z-RA-RY} 
   \]

   in which Z and Y are identical cyclic polyamine moieties having from 10 to 15 ring members and from 3 to 9 amine nitrogens in the ring spaced by 2 or more carbon atoms from each other, said amine nitrogens being the only ring heteroatoms.

   A is an unsubstituted aromatic or heteroaromatic moiety other than quinoline,

   R and R' are each methylene linked to nitrogen atoms in Z and Y,

   the amine nitrogen atoms being otherwise unsubstituted, in admixture or association with a pharmaceutically acceptable diluent or carrier.

2. A composition according to claim 1, wherein in the compound of formula I, each moiety Z and Y has 14 ring members and 4 amine nitrogens in the ring.

3. A pharmaceutical composition according to claim 1, wherein the compound active against HIV comprising as an active ingredient \( [\text{I}] \), \( 1,1'-(1,3-\text{phenylene})\)-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

4. A pharmaceutical composition according to claim 1, wherein the compound active against HIV comprising as an active ingredient \( [\text{I}] \), \( 1,1'-(1,3-\text{phenylene})\)-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.
ingredient is 1,1’-[1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

5. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is a bis-zinc complex of 1,1’-[1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

6. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 1,1’-[1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

7. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 1,1’-[3,3’-biphenylene-bis-methylene]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

8. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 11,11’-[1,4-phenylenebis(methylene)]-bis-1,4,7,11-tetraazacyclotetradecane 11,11’-[1,4-phenylene-bis(methylene)]-bis-1,4,7,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

9. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 11,11’-[1,4-phenylene-bis(methylene)]-1,4,8,11-tetraazacyclotetradecane 1,4,7,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

10. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 11,11’-[2,6-pyridine-bis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

11. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 11,11’-[2,5-pyridine-bis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

12. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 11,11’-[2,5-thiophene-bis-(methylen)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

13. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 11,11’-[4,4’-(2,2’-bipyridine)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

14. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 11,11’-[2,9-(1,10-phenanthroline)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

15. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 11,11’-[1,3-phenylenebis(methylene)]-bis-1,4,7,10-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

16. A pharmaceutical composition according to claim 1, wherein the active against HIV comprising as an active ingredient is 11,11’-[1,4-phenylene-bis-(methylene)]-bis-1,4,7,10-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

17. The compound of claim 1, which is A pharmaceutical composition active against HIV comprising as an active ingredient 1,1’-[5-nitro-1,3-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

18. The compound of claim 1, which is 11,11’-[2,4,5,6-tetrachloro-1,3-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane A pharmaceutical composition active against HIV comprising as an active ingredient 11,11’-[2,4,5,6-tetrachloro-1,3-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

19. The compound of claim 1, which is A pharmaceutical composition active against HIV comprising as an active ingredient 1,1’-[2,3,5,6-tetrafluoro-1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

20. The compound of claim 1, which is A pharmaceutical composition active against HIV comprising as an active ingredient 11,11’-[1,4-naphtylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

21. The compound of claim 1, which is A pharmaceutical composition active against HIV comprising as an active ingredient 11,11’-[1,3-phenylenebis-(methylene)]-bis-1,5,9-triazacyclodecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

22. The compound of claim 1, which is 11,11’-[1,4-phenylene-bis-(methylene)]-1,5,9-triazacyclodecane A pharmaceutical composition active against HIV comprising as an active ingredient 11,11’-[1,4-phenylene-bis-(methylene)]-bis-1,3,9-triazacyclodecane in acid addition salt form in admixture or association with a pharmaceutically acceptable diluent or carrier.

23. The compound of claim 1, which is a bis-zinc complex of 11,11’-[1,4-phenylenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane.

24. The compound of claim 1, which is 11,11’-[3,3’-biphenylene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form.

25. The compound of claim 1, which is 11,11’-[2,6-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form.

26. The compound of claim 1, which is 11,11’-[3,5-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form.

27. The compound of claim 1, which is 11,11’-[2,5-thiophene-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form.

28. The compound of claim 1, which is 11,11’-[4,4’-(2,2’-bipyridine)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in acid addition salt form.
The compound of claim 1, which is 1,1'-[2,9-(1,10-phenanthroline)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,5-dimethyl-1,4-phenylenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,3,5-triiodo-1,4-phenylenebis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,9-(1,10-phenanthroline)-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,3,5,6-tetrachloro-1,3-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,3,5,6-tetrafluoro-1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,6-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[3,5-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,3,5,6-tetrafluoro-1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,6-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,3,5,6-tetrafluoro-1,4-phenylenebis(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[2,6-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[3,5-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.

A pharmaceutical composition active against HIV comprising as an active ingredient 1,1'-[3,5-pyridine-bis-(methylene)]-bis-1,4,8,11-tetraazacyclotetradecane in admixture or association with a pharmaceutically acceptable diluent or carrier.