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(71) Applicant *(for all designated States except US):* SYNPHAR LABORATORIES, INC. [CA/CA]; #24, Taiho Alberta Center, 4290-91A Street, Edmonton, Alberta T6E 5VE (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* LOWN, J., William [CA/CA]; 4704-117A Street, Edmonton, Alberta T6H 35L (CA). MICETICH, Ronald, George [CA/CA]; 12 Braeside Terrace, Sherwood Park, Alberta T6E 5V2 (CA).

(74) Agent: RICHES, McKENZIE & HERBERT; Suite 2900, 2 Bloor Street East, Toronto, Ontario M4W 3J5 (CA).

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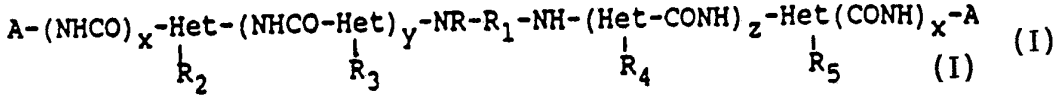
(54) Title: OLIGOPEPTIDE ANTIRETROVIRAL AGENTS



(57) Abstract

Oligopeptide antiretroviral agents are represented by formula (I), wherein A is a moiety bearing a positive charge and of a size which avoids steric inhibition of binding of said compound to nucleic acid sequences associated with the cellular activity of retroviruses; R_1 is a moiety derived from a dicarboxylic acid; Het is a five-membered heterocyclic moiety; y and z are independently 0, 1, 2 or 3; and x is 0 or 1. These compounds exhibit antiretroviral activity, especially against Human Immunodeficiency Virus (HIV).

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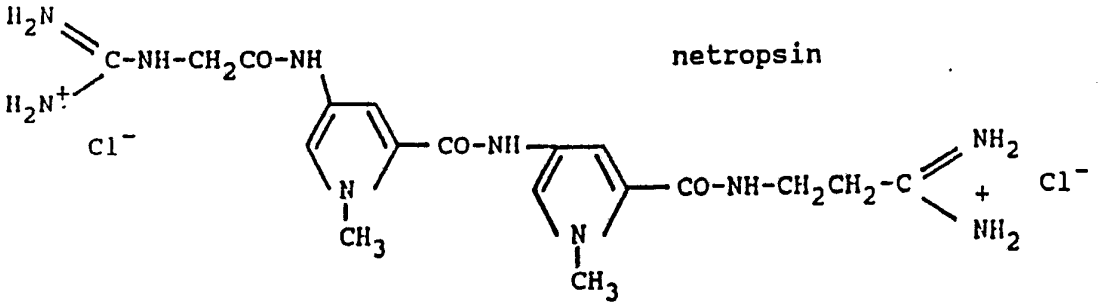
OLIGOPEPTIDE ANTIRETROVIRAL AGENTS

FIELD OF THE INVENTION

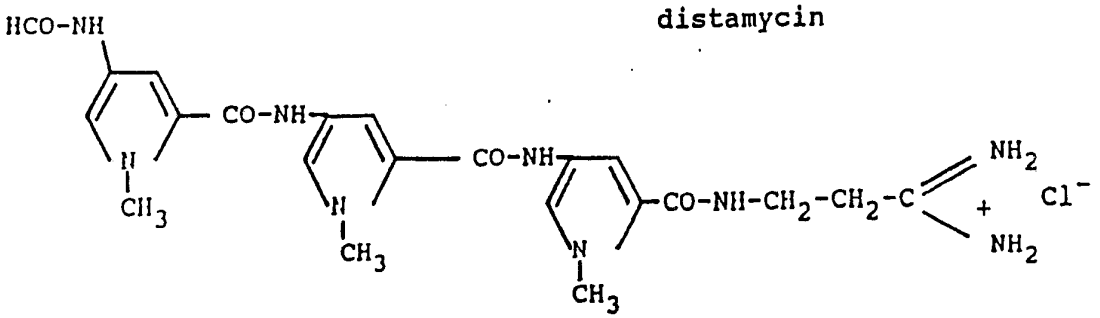
This invention relates to oligopeptides which are particularly useful as antiretroviral agents.

BACKGROUND OF THE INVENTION

Various oligopeptide derivatives have demonstrated various medicinal uses, such as enzyme inhibitors as disclosed in United States Patent 4,483,850. It is also known that various oligopeptides have anti-tumor activity as disclosed in United States Patents 4,216,208 and 4,314,999. Antibiotic activity of oligopeptides is disclosed in United States Patent 4,454,065. Naturally occurring oligopeptides, netropsin and distamycin, have been discovered as having antiviral and anti-tumor activity. The chemical formulas for netropsin and distamycin are as follows:



Compound 1



Compound 2

These oligopeptides are disclosed in Julia, M., Préau-Joseph, N., C.R. Hebd-Seances, Acad. Sci. 1963, 257. 1115 and Arcamone, F.; Orezzi, P.G.; Barbier, W.; Nicolella, V.; Penco,S.; Gazz. Chim. Ital., 1967, 97, 1097.

Netropsin and distamycin contain pyrrole moieties connected by peptide bonds and with side chains, at least one of which is positively charged; i.e., an amidine group, or a group of the guanidyl type.

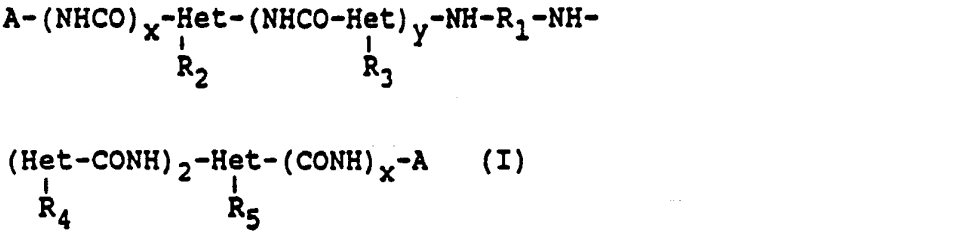
Only distamycin has been used as a therapeutic agent as commercialized and sold under the trade mark STALLIMYCIN HYDROCHLORIDE in the form of a 1% cream, ointment or paste. This composition has been used in the treatments of infections produced by herpes simplex, herpes zoster and vaccinia viruses. Topical application of distamycin has been limited due to its high cytotoxicity and a low therapeutic index which in the instance of treating the herpes virus is about 3.

U.S. Patent No. 4,912,199 discloses oligopeptides containing pyrrole moieties which demonstrated significantly enhanced antiviral and anticancer activities as compared to the oligopeptides of the prior art.

According to this invention oligopeptides have been developed which have significantly enhanced antiretroviral activity compared to prior types of oligopeptides.

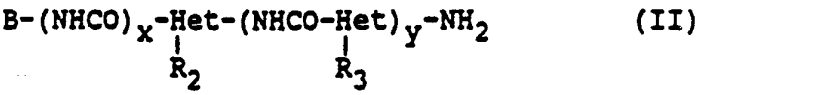
SUMMARY OF THE INVENTION

According to an aspect of the invention, a compound represented by the formula I:



wherein A is a moiety bearing a positive charge and of a size which does not inhibit binding of said compound to nucleic acid sequences associated with the cellular action of retroviruses; R_1 is a moiety derived from a dicarboxylic acid or a residue of carbonic acid; Het is a five-membered heterocyclic moiety; y and z are independently 0, 1, 2 or 3, x is 0 or 1, and pharmaceutically acceptable salts thereof, exhibit antiretroviral activity, especially against Human Immunodeficiency Virus and Hepititus B Virus.

A process for preparing such compounds comprises reacting a compound of the formula (II):



wherein x and y are as defined above; and B is the same as A or is a group with a nitrile, halogen or sulfide substituent; with a dicarboxylic acid of the formula (III):

wherein R_1 is as defined above and X is halogen, imidazolide or other reactive moiety and converting B to A to form said moiety bearing a positive charge.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing a correlation between DNA binding constants of linked oligopeptides (K_a,-) and observed inhibitory properties expressed in reciprocal ID_{50} values against Moloney Leukemia Virus reverse transcriptase (MlV-RT).

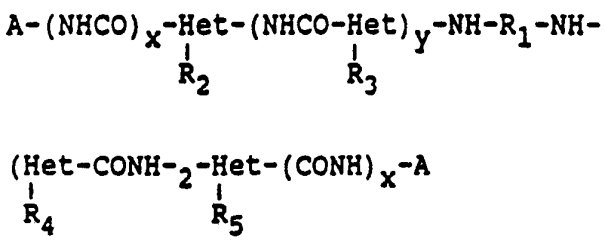
Figures 2-6 are graphs showing anti-HIV activity of several compounds of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Compounds according to this invention demonstrate significant antiretroviral activity. Although the actual biological mechanism of these compounds which cause antiretroviral activity is not fully understood, it is thought that the activity may be due to the compounds of this invention binding with nucleic acid sequence(s) associated with the cellular action of retroviruses to inactivate such nucleic acids which code for the retroviral activity. It has also been observed that the linked oligopeptides of the present invention are potent inhibitors of Moloney Leukemia Virus (MIV) reverse transcriptase, a potential indicator of anti-HIV activity. See Figure 1.

The compounds of this invention have heterocyclic moieties, which may be the same or different, linked by a dicarboxylic acid derivative. Such linked heterocyclic moieties of this invention have significant unexpected activity compared to unlinked pyrrole moieties such as the naturally occurring netropsin and distamycin.

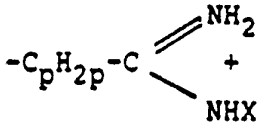
The compounds according to this invention are represented by the following formula:



wherein A is a moiety bearing a positive charge and of a size which does not inhibit binding of said compounds to deoxyribonucleic acid sequences associated with the cellular action of retroviruses; R_1 is a moiety derived from a dicarboxylic acid; Het is a five-membered heterocyclic moiety; R_2, R_3, R_4 and R_5 may be attached to a ring carbon atom or hetero ring atom and are independently selected from C_1-C_6 alkyl and CH_2-O-R_6, where R_6 is a C_1-C_6 alkyl; y and z are independently 0, 1, 2 or 3; x is 0 or 1; and pharmaceutically acceptable salts thereof.

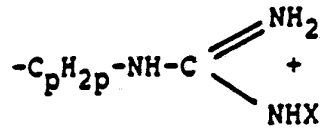
The positively charged moiety at each extremity of the compound and identified as group A is preferably selected from the group of derivatives consisting of an amidine, a guanidine, secondary ammonium salts, sulfonium salts and phosphonium salts.

The selected amidine may have one or both nitrogen atoms of the amidine as a member of a five-membered cyclic structure. More particularly, the amidine derivative is represented by the formula:



where p equals 0 to 5 and X is -H, -OH, $-NH_2$, $-CH_3$, $-C_2H_5$, $-C_3H_7$.

The selected guanidine for substituent A may be represented by the formula:



where p equals 0 to 5 and X equals -H, -OH, $-NH_2$, $-CH_3$, $-C_2H_5$, $-C_3H_7$.

When A is selected to be a quaternary, tertiary or secondary ammonium salt, it may be represented by the formula:

$$-C_pH_{2p}-{}^{+}NH_qX_{(3-q)}$$

where p equals 1 to 5 and q equals 0 to 3 and X is an alkyl or alkenyl group of 1 to 3 carbon atoms.

When A is selected as a sulfonium salt, it may be represented by the formula:

$$-C_pH_{2p}-SXY$$

where p equals 0 to 5 and X and Y are alkyl or alkenyl groups of 1 to 3 carbon atoms.

In the heterocyclic moieties, Het may be the same in each moiety or may be different. Preferably, the Het group is selected from the group consisting of a pyrrole, an imidazole, a triazole, a pyrazole, a thiazole, a thiophene, a furan, an oxazole and derivatives thereof.

Preferred ring carbon atom substituents are alkyl groups, and especially methyl groups, on the Het moiety, especially on thiazole rings.

Preferred Het substituents are N-alkyl pyrrole having 1 to 6 carbon atoms in the alkyl group; N-alkyl imidazole having 1 to 6 carbon atoms in the alkyl group; alkyl pyrazole having 1 to 6 carbon atoms in the alkyl group; and alkyl triazole having 1 to 6 carbon atoms in the alkyl group. Preferably the N-alkyl pyrrole has 1 to 4 carbon atoms in the alkyl group, and especially in N-methyl pyrrole. Also preferred Het substituents are N-linked alkoxymethyl groups. The choice of Het substituents will depend on their cellular uptake ability.

R_2, R_3, R_4 and R_5 are linked to the N or C atom of the Het moiety and are independently C_1-C_6 alkyl or $-CH_2-O-R_6$ where R_6 is C_1-C_6 alkyl. It has been found that the longer the alkyl group in either structure is, the better the cellular uptake of the compound. The choice of substituent will depend on solubility properties; solubility in pharmacologically acceptable solvents, such as water or DMSO, has been found to be higher with the methoxy substituents.

The linking group R_1 is a derivative from carboxylic acid. R_1 is represented generally by the formula:

$$-CO-C_pH_{2p}-CO-$$

where p equals any number from 1 to 22. Alternatively, R_1 may be a residue of carbonic acid, namely $-\overset{O}{\overset{\|}{C}}-$; or R_1 may be a residue of an aromatic dicarboxylic acid. The -CO- groups of the aromatic dicarboxylic acid residues may be in the ortho, meta or para positions on the ring. The aromatic residues may be 5 to 6 C membered rings. The aromatic dicarboxylic acid may also be a six membered heterocylic ring containing a nitrogen atom.

Other alternative structures for the linking group may be a residue of an unsaturated aliphatic dicarboxylic acid of the formula:

$$-CO-C_qH_{(2q-2)}-CO-$$

where q equals any number from 2 to 22.

R_1 may also be a residue of cycloalkane dicarboxylic acids of the formula:

$$-CO-C_rH_{(2r-2)}-CO-$$

where r equals any number from 3 to 7 and optionally may be fused to one or more three to seven C membered rings, preferably fused to one or two three to seven C membered rings.

R_1 may also be a residue of cycloalkane dicarboxylic acids of the formula:

$$-CO-C_sH_{(2s-4)}-CO-$$

where s equals any number from 3 to 7.

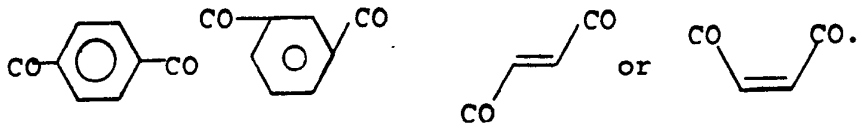
In a preferred compound of the present invention, A is a moiety selected from the group consisting of an amidine, a guanidine, secondary ammonium salts, tertiary ammonium salts, quaternary ammonium salts, sulfonium salts and phosphonium salts.

In another preferred compound of the present invention, R_2, R_3, R_4 and R_5 are each a C_1-C_6 alkyl or R_2, R_3, R_4 and R_5 are the same and are a C_1-C_6 alkyl group or R_2, R_3, R_4 and R_5 are each a methoxymethyl.

In another preferred compound of the present invention, R_1 is

or R_1 is a residue of a dicarboxylic acid of the formula $-CO-C_pH_{2p}-CO-$ where p equals 1 to 22. R_1 may also be preferably a residue of a dicarboxylic acid selected from the group consisting of: a residue of an unsaturated aliphatic dicarboxylic acid of the formula $-CO-C_q-H_{2q-2}-CO-$ where q equals 2; a residue of an aromatic dicarboxylic acid; and a residue of a cycloalkane dicarboxylic acid of the formula $-CO-C_r-H_{2r-2}-CO-$ where r equals 3 to 6.

In yet another preferred compound, R_1 is



Preferably, R_1 is a dicarboxylic acid residue of cyclopropane, a dicarboxylic acid residue of cyclopentane, or a dicarboxylic acid residue of cyclohexane.

The following are representative examples of the preferred compounds of the present invention.

N, N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] terephthalamide dihydrochloride.

N, N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] isophthalamide dihydrochloride.

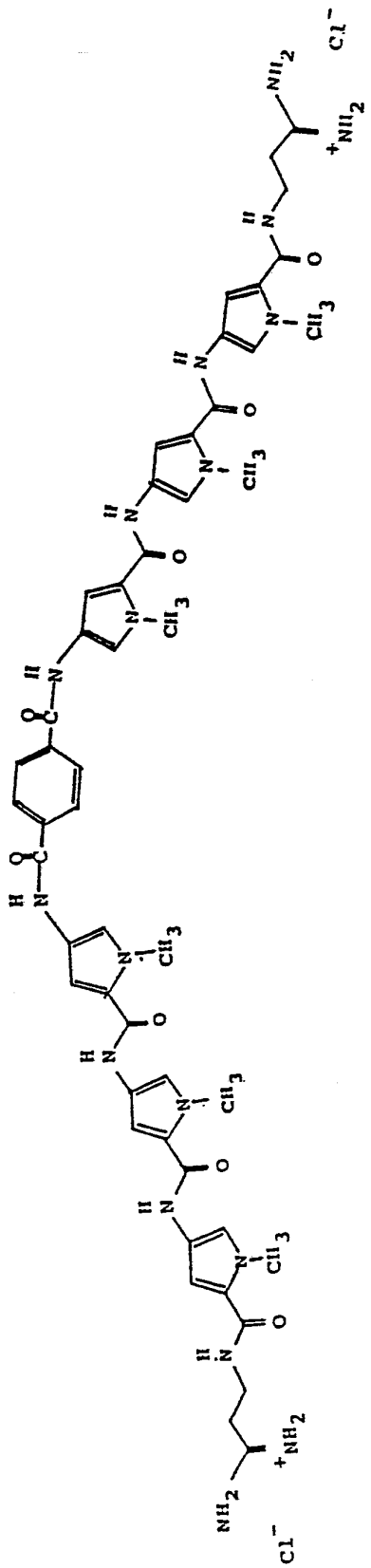
N, N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] fumaramide dihydrochloride.

N, N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] maleamide dihydrochloride.

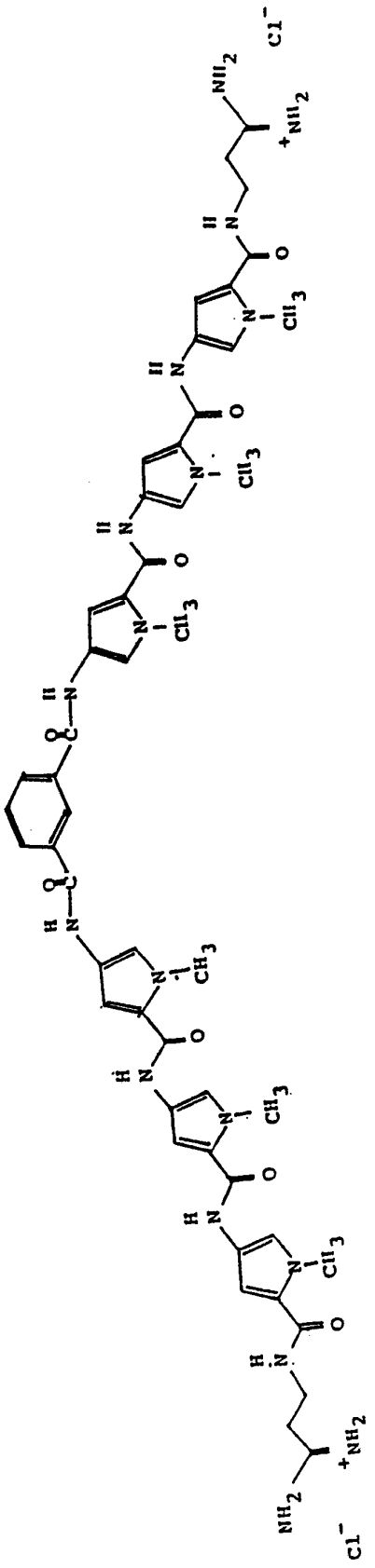
N, N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] trans 1,2-cyclobutanamide dihydrochloride.

N, N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] trans 1,2-cyclobutanamide dihydrochloride.

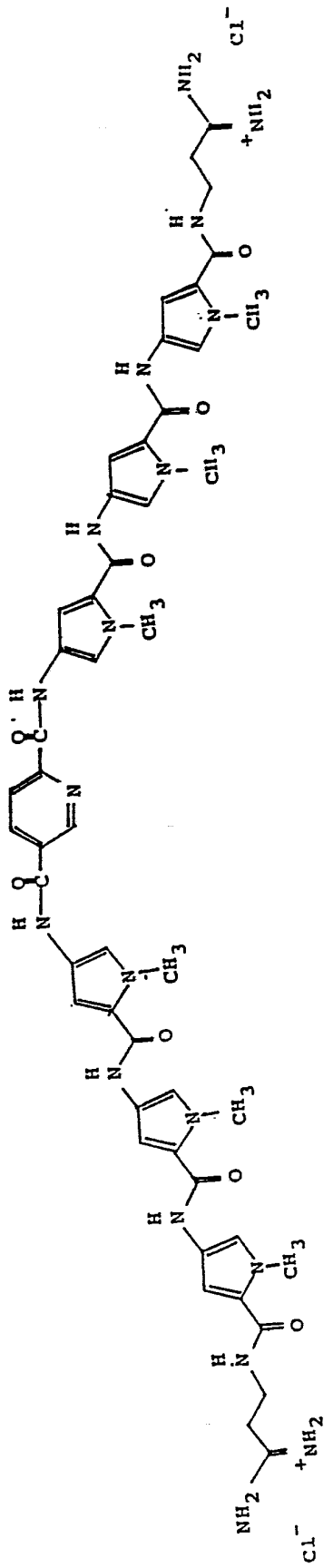
The compound:



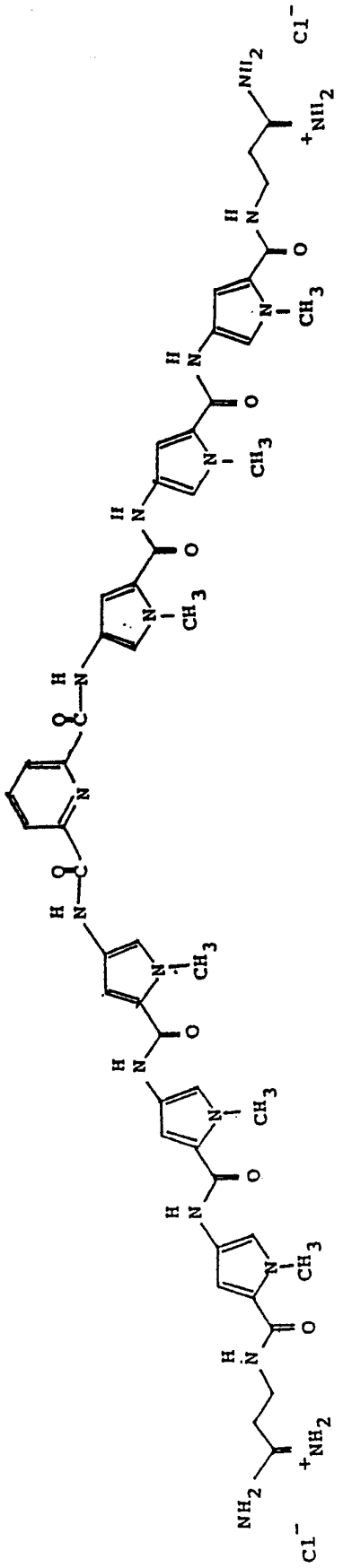
The compound:



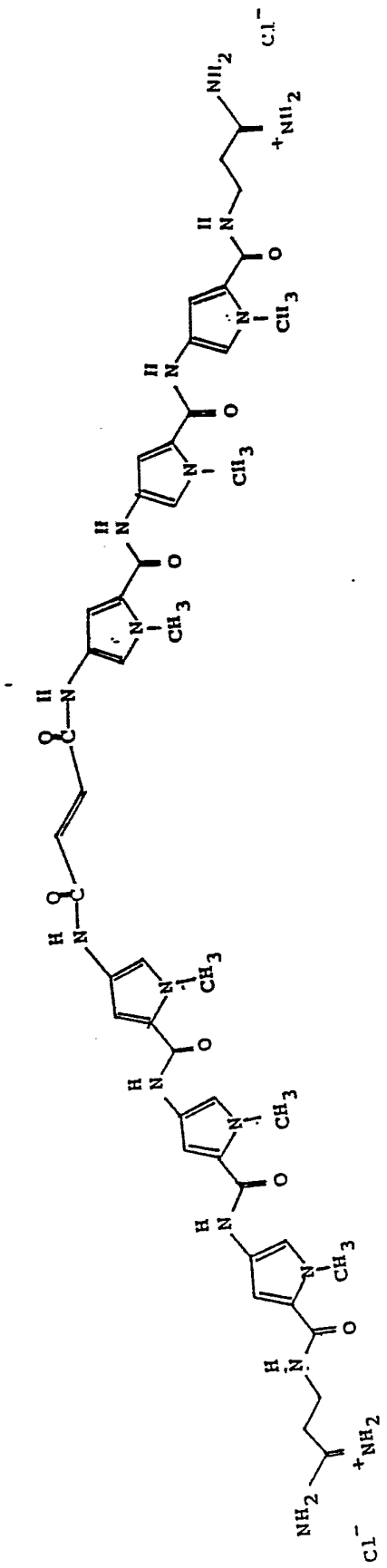
The compound:



The compound:

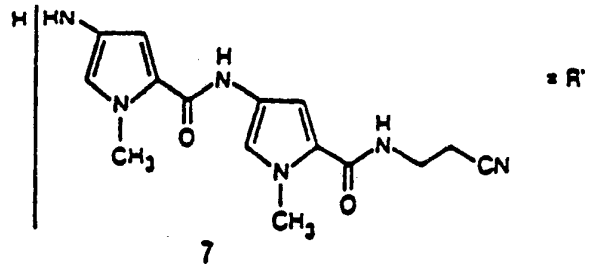


The compound:



In cases where R_1 is a dicarboxylic acid derivative of an aliphatic hydrocarbon, the linker is referred to as flexible. Rigid linkers refer to cases in which R_1 is carbonic acid or residues of aromatic, unsaturated aliphatic, cycloalkane and cycloalkene dicarboxylic acids. Most preferred are those compounds in which R_1 is a rigid linker. Examples of the flexible linked and rigid linked oligopeptides are set forth below.

FLEXIBLE LINKED OLIGOPEPTIDES



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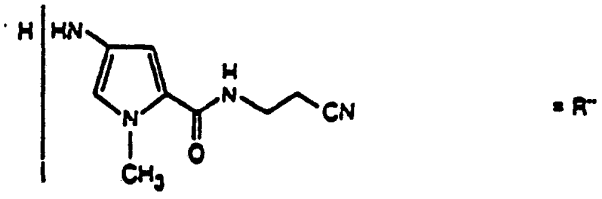

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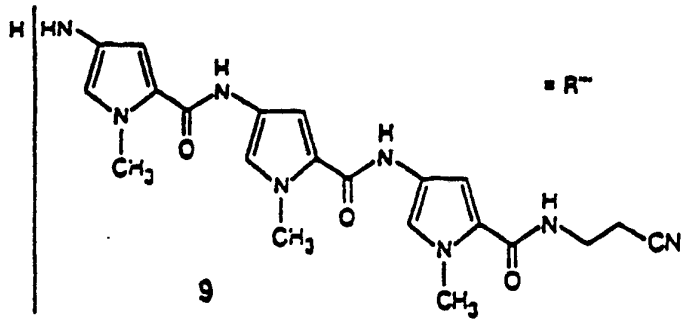

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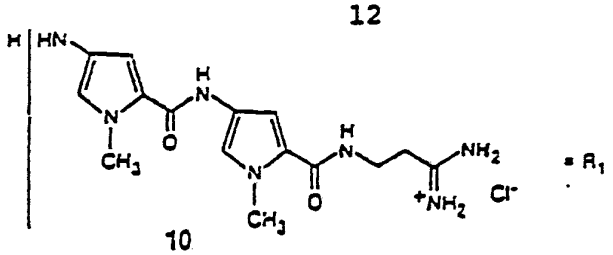
$R'—CO—(CH_2)_n—COR'$

$R''—COCH_2CH_2COR''$

$R'''—COCH_2CH_2COR'''$

12



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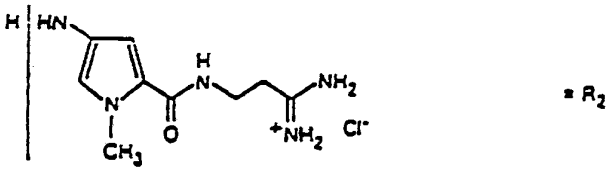

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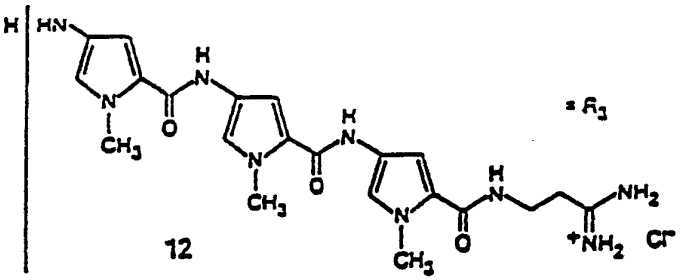


12

$R_1—CO—(CH_2)_n—COR_1$

$R_2—CO—CH_2CH_2—CO—R_2$

$R_3—CO—CH_2CH_2—CO—R_3$

3 $R_1—CO—R_1$

4 $R_1—COCH_2CO—R_1$

5 $R_1—CO(CH_2)_2CO—R_1$

6 $R_1—CO(CH_2)_3CO—R_1$

7 $R_1—CO(CH_2)_4CO—R_1$

8 $R_1—CO(CH_2)_5CO—R_1$

9 $R_1—CO(CH_2)_6CO—R_1$

10 $R_1—CO(CH_2)_7CO—R_1$

11 $R_1—CO(CH_2)_8CO—R_1$

12 $R_1—CO(CH_2)_9CO—R_1$

13 $R_1—CO(CH_2)_{10}CO—R_1$

14 $R_2—CO(CH_2)_2CO—R_2$

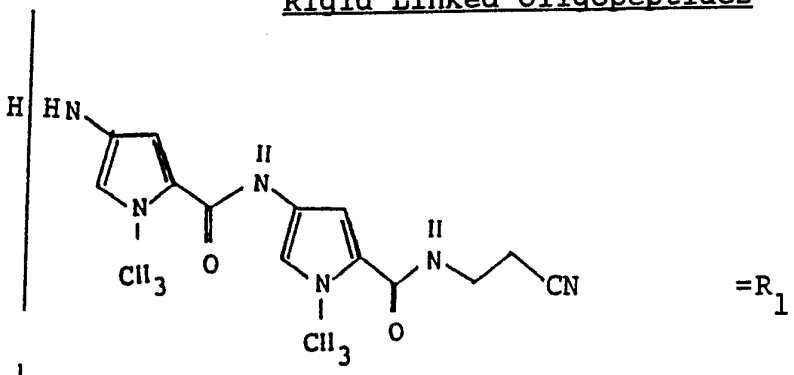
15 $R_3—CO(CH_2)_2CO—R_3$

16 $R_3—CO(CH_2)_6CO—R_3$

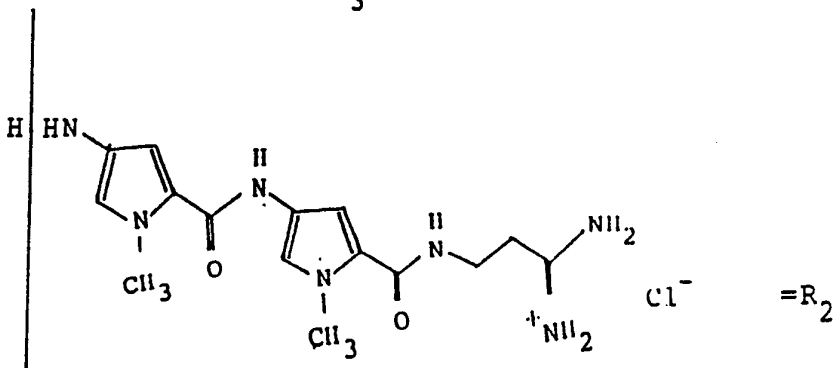
17 $R_3—CO(CH_2)_8CO—R_3$

18 $R_3—CO(CH_2)_{22}CO—R_3$

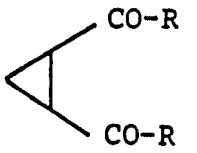
Rigid Linked Oligopeptides



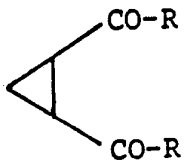
=R_1



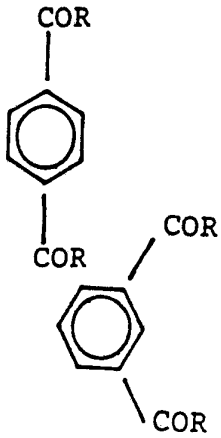
Cl^- =R_2

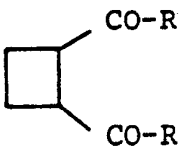


trans 23aR=R_1 23bR=R_2

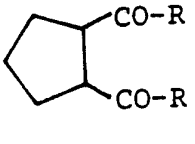


cis 24aR=R_1 24bR=R_2

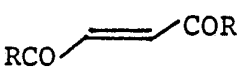


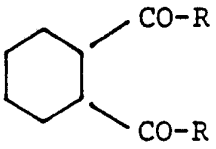


19aR=R_1 19bR=R_2 trans 25aR=R_1 25bR=R_2

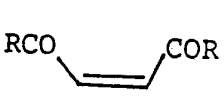


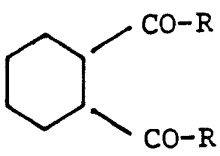
20aR=R_1 20bR=R_2 trans 26aR=R_1 26bR=R_2



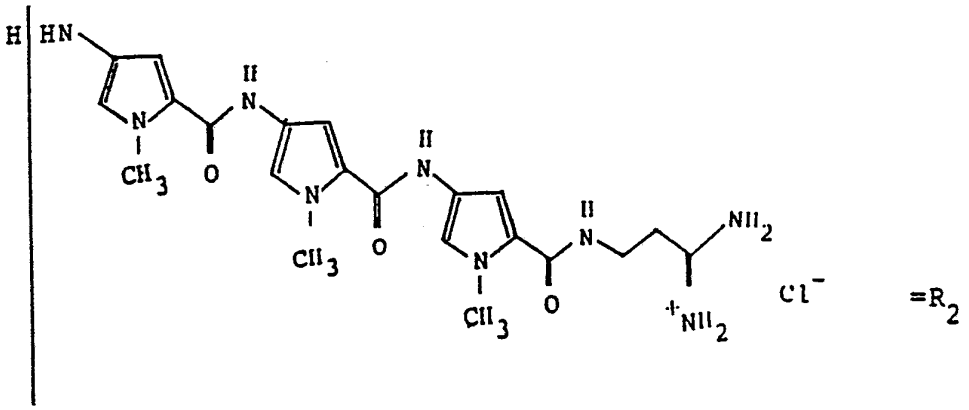


21aR=R_1 21bR=R_2 trans 27aR=R_1 27bR=R_2

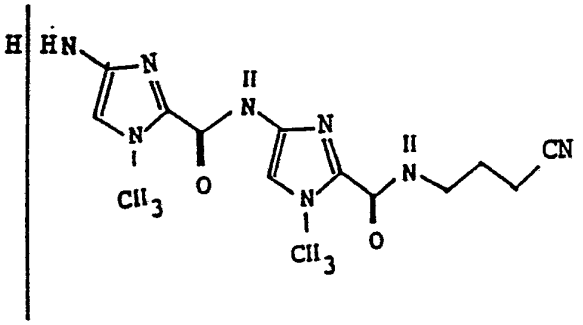




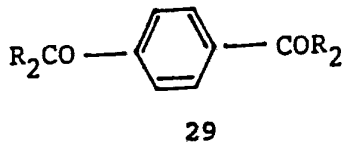
22aR=R_1 22bR=R_2 cis 28aR=R_1 28bR=R_2



=R_2



=R_3



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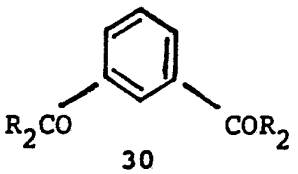

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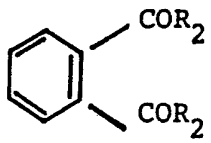

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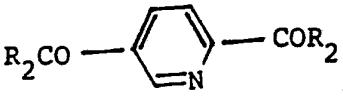

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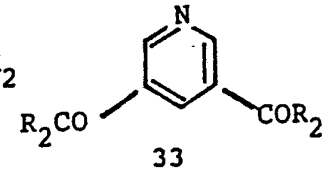

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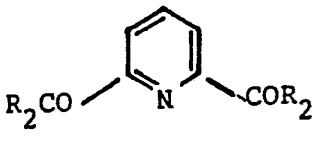

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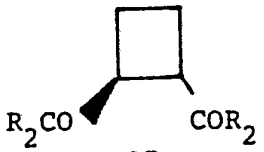

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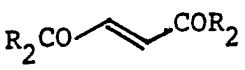

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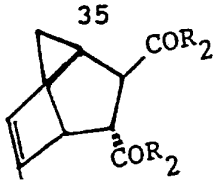

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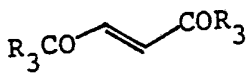

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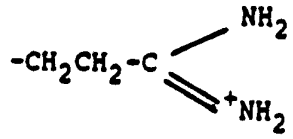
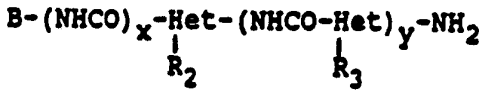
Other preferred compounds include compounds of formula I wherein Het is pyrrole and x is 1; A is:

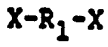


and R_1 is a rigid linker (as defined above).

The heterocyclic moiety of the compounds of this invention may be linked in accordance with various processes by use of the dicarboxylic acid derivatives. In accordance with one aspect of this invention, the process for providing such linkage comprises reacting a compound of the formula:

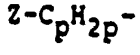


wherein x and y are as defined above; and B is the same as A or is a group with a nitrile, halogen or sulfide substituent; with a dicarboxylic acid of the formula:



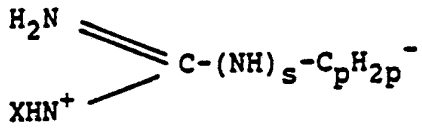
wherein R_1 is as defined above and X is halogen, imidazolide or other reactive moiety and converting B to A to form said moiety bearing a positive charge.

In the reactants, B may be generally represented by the formula:



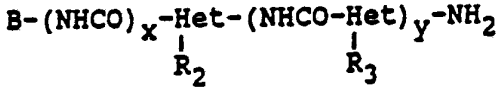
where Z is CN-, hal or XS; hal is a halogen ion, X is an alkyl or alkenyl group having 1 to 3 carbon atoms, and p equals 0 to 5.

It is to be appreciated that B may also be identical to A in providing a charge group, for example, a guanidinium end group. In that instance, B has the general formula:

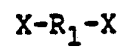


wherein X is an alkyl having 1 to 3 carbon atoms or alkenyl group having 2 or 3 carbon atoms and p equals 0 to 5 and s equals 0 or 1.

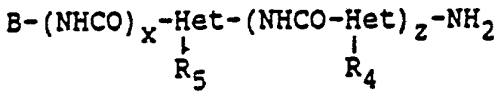
Compounds of the present invention which are asymmetrical around the linking group (i.e., wherein y and z are different in number) can be prepared by a two-step process, wherein the first step involves coupling a compound of the formula:



wherein B, x and z are as defined above, with a dicarboxylic acid of the formula:



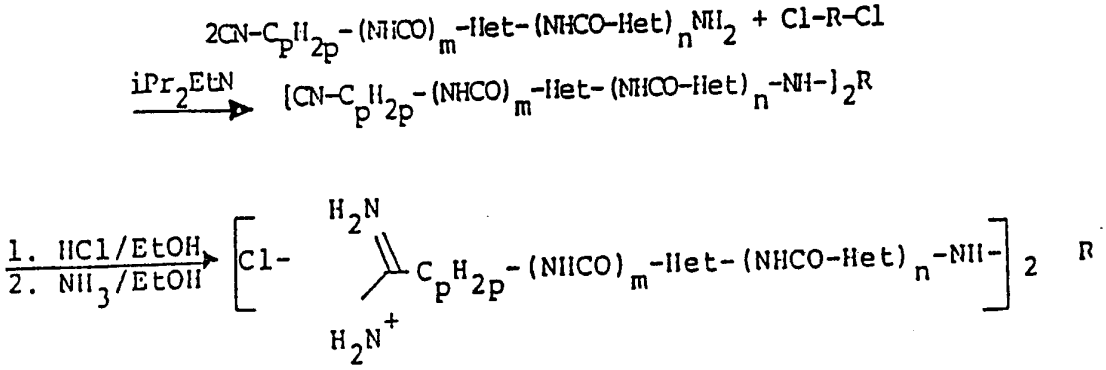
wherein R_1 and X are as defined above (this coupling is generally with the use of equimolar amounts of the reactants). This is followed by coupling of a compound of the formula:



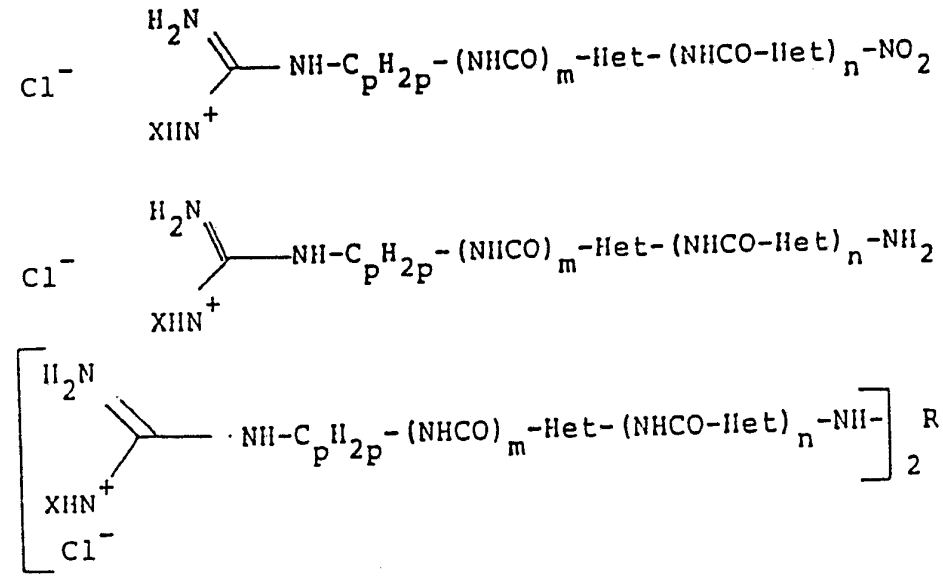
wherein B, x and z are as defined above, with the provisions that z is different than y.

According to preferred embodiments of the invention, the following reaction schemes demonstrate preferred chemical pathways to the compounds of this invention having the various desired end groups:

A - Preparation of Amidinium End Group



B - Preparation of Guanidinium End Groups



C - Preparation of Ammonium Salt in End Group

$$2\ Cl\text{-}C_pH_{2p}\text{-}(NHCO)_m\text{-}Het\text{-}(NHCO\text{-}Het)_n\text{-}NH_2 + Cl\text{-}R\text{-}Cl$$

$$\xrightarrow{iPr_2EtN} [Cl\text{-}C_pH_{2p}\text{-}(NHCO)_m\text{-}Het\text{-}(NHCO\text{-}Het)_n\text{-}NH\text{-}]_2R$$

$$\xrightarrow{HNH_qX_{(3-q)}} [X_{(3-q)}{}^{+}NH_q\text{-}C_pH_{2p}\text{-}(NHCO)_m\text{-}Het\text{-}(NHCO\text{-}Het)_n\text{-}NH\text{-}]_2R$$

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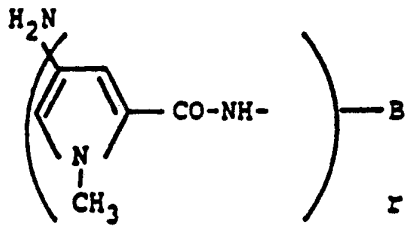
D - Preparation of Sulfonium Salts

$$2XS\text{-}C_pH_{2p}\text{-}(NHCO)_m\text{-}Het\text{-}(NHCO\text{-}Het)_n\text{-}NH_2 + Cl\text{-}R\text{-}Cl$$

$$\xrightarrow{iPr_2EtN} [XS\text{-}C_pH_{2p}\text{-}(NHCO)_m\text{-}Het\text{-}(NH\text{-}CO\text{-}Het)_n\text{-}NH\text{-}]_2R$$

$$\xrightarrow{YCl\ (or\ Br)} [XYS^{+}\text{-}C_pH_{2p}\text{-}(NHCO)_m\text{-}Het\text{-}(NHCO\text{-}Het)_n\text{-}NH\text{-}]_2R$$

Reference may be made to J.W. Lown and K. Krowicki, J. Org. Chem. 1985, 50, 3774 regarding the synthesis of related types of pyrrole moieties such as the synthesis of distamycin. The general synthesis of the compounds according to this invention are based on the total synthesis of distamycin. Dipyrrole or tripyrrole peptides bearing an amino group and a side-chain containing a group (B) which is the nitrile, ammonium or sulfide as represented by the following formula:



r = 2,3.

are allowed to react at a temperature of -35 to +10°C, preferably about -20°C, with a dicarboxylic acid dichloride in the presence of a base or with a diimidazolide of a dicarboxylic acid to give a bis-amide of the dicarboxylic acid. The resulting compound in the case of nitrile is allowed to react at a temperature of 0 to +35°C, preferable +15° to +25°C, more preferably about +20°C, with ethanol in the presence of hydrochloric acid and then at a temperature of 0 to +35°C, preferably +15 to +25°C, more preferably about +20°C, with ammonia (Pinner reaction) to generate an amidinium moiety in the final product, as exemplified by the above reaction scheme A. As with reaction scheme D, the sulfide is methylated at a temperature of 0 to +35°C, preferable +15 to +25°C, more preferably about +20°C, to produce the corresponding sulfonium salt.

The compounds of formula I, are useful as antiretroviral agents, especially against the Human Immunodeficiency Virus (HIV). Human patients suffering from diseases caused by, for example, HIV, can be treated by administering to the patient a pharmaceutically

effective amount of one or more of the present compounds optionally, but preferably in the presence of a pharmaceutically acceptable carrier or diluent. There may be also included pharmaceutically compatible binding agents and/or adjuvant materials. The active materials can also be mixed with other active materials which do not impair the desired action and/or supplement the desired action. The active materials according to the present invention can be administered by any route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, rectally or topically, in a liquid or solid form. For injection purposes, the medium used may be a sterile liquid. As an injection medium, it is preferred to use water which contains the stabilizing agents, solubilizing agents and/or buffers conventional in the case of injection solutions. Desirable additives include, for example, tartrate and borate buffers, ethanol, dimethylsulfoxide, complex forming agents (for example, ethylenediamine tetracetic acid), high molecular weight polymers (for example, liquid polyethylene oxide) for viscosity regulation or polyethylene derivatives of sorbitan anhydrides. Solid carrier materials include, for example, starch, lactose, mannitol, methylcellulose, talc, highly dispersed silicic acid, high molecular weight fatty acids (such as stearic acid), gelatin, agar, calcium phosphate, magnesium sterate, animal and vegetable fats or solid high molecular weight polymers (such as polyethylene glycol). Compositions suitable for oral administration can, if desired, contain flavoring and/or sweetening agents.

A preferred mode of administration of the compounds of this invention is oral. Accordingly, the compounds may be formulated into capsule form or tablet form.

The active materials according to the present invention can be employed in dosages and amounts which are conventional in the art. Thus, the materials can be used at a dosage range in humans of from about 1 to 200 mg/kg total body weight/day. A more preferred range lies between 1-30 mg/kg total body weight/day. The dosages may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

The in vitro anti-HIV screening test results, performed at the United States National Cancer Institute, have shown that 23 of the present compounds are active. Of the fifteen, ten are considered "active", and thirteen are determined "moderately active". Certain of the compounds screened for anti-AIDS activity at the NCI were determined to be "inactive". These compounds were ones wherein the R_1 is $-CO-(CH_2)_6-CO-$ or $-CO-(CH_2)_8-CO-$, A is amidine, x is 1, Het is methylpyrrole, and y and z are 1, as well as compounds 9, 11, 15, 16, 18 and 37.

The therapeutic index of a compound is determined by dividing the inhibitory or lethal concentration for 50% of the population (IC_{50}) by the effective concentration for 50% of the population (EC_{50}). The therapeutic indexes for the particularly active compounds of the present invention range from 1.46 to 161.

As used in this invention, antiretroviral activity refers to the ability of a compound to inhibit the growth of a retrovirus. The retrovirus of primary importance with respect to the present invention is HIV. However, the present compounds may also exhibit antiretroviral activity towards other retroviruses as would be apparent by the suspected mechanism of action and other viruses which replicate or exhibit reverse transcription.

The compounds of the present invention should also be therapeutically effective in the treatment of hepatitis B viral infection in mammals, especially humans. Similar to retroviruses (including HIV-1), the hepatitus B virus replicates by reverse transcription. In addition, hepatitus B virus putative viral polymerase share amino acid homology with reverse transcriptase of retroviruses and a comparison of the thirteen (13) hepadnavirus isolates determined that other conserved areas showing homolgy to corresponding regions of Type C retro virus. Miller et al., Proc.Natl.Acad.Sci. USA, Vol 83:2531-2535 (1986).

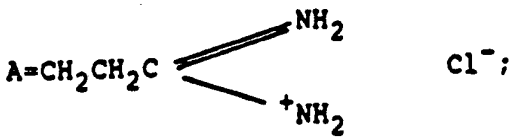
Since it is theorized that the activity of the compounds of the present invention may be due to the compounds binding with nucleic acid sequence(s) associated with the cellular action of retroviruses to inactivate such nucleic acids which code for the retroviral activity, the compounds are likely to inhibit binding with nucleic acid sequence(s) of the hepatitus B virus associated with the cellular action of reverse transcription to inactivate such nucleic acids which code for the retroviral-like activity. Therapeutically effective anti-hepatitus B dosages would be the same as anti-HIV-1 dosage levels as well as would the routes of administration.

The ability of a compound to inhibit HIV may be measured by various experimental techniques. One such technique, currently employed by the United States National Cancer Institute to screen potential anti-HIV compounds, involves the inhibition of the killing of HIV-infected T_4 lymphocytes. Compounds of the present invention have been tested for anti-HIV-1 activity in the NCI protocol; however, one skilled in the art would appreciate that the compounds should exhibit activity against HIV-2 as well.

Preferred embodiments of the invention are exemplified in the following Examples which are in no way to be construed as limiting the scope of the appended claims.

EXAMPLE 1

Compound of the formula I, where x=1, y and z each are 1;



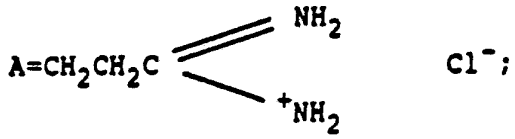
Cl^-;

R_1 equals $-COCH_2CH_2CO-$, was prepared. 1-Methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)-pyrrole-2-carboxamidopropionitrile (105 mg, 0.33 mmole) and 1-Pr_2EtN (diisopropylethylamine) (65µl, 0.16 mmole) in anhydrous THF (1 ml) was added and the mixture was allowed to reach room temperature. The solvents were evaporated to dryness and water was added. The resulting solid was collected and washed with hot MeOH to give 90 mg (77% yield) of the product m.p. 297°C. The latter was suspended in anhydrous EtOH and saturated with HCl while cooling. After 1.5 hours at room temperature, the solvent was removed in vacuo and the residue was washed with dry ether then ethanol was added followed by some ammonia condensed into the solution. After 1 hour at room temperature, the solvent was removed and the residue was washed with MeOH, EtOH and hexane to afford 80 mg of a solid. Recrystallization from a small volume of water gave a jelly-like precipitate which was washed with EtOH, hexane and dried to give 35 mg (35% yield) of pure product m.p. 283-285°C dec. ^{1}H-NMR (DSMO-d_6): δ 2.60 (m, 4H), 3.60 (m, 2H), 3.83 (s, 6H), 6.92 (d, 2H), 7.18 (d, 2H), 8.25 (t,

1H), 8.70 (bs, 2H), 9.02 (bs, 2H), 9.93 and 9.97 (2s, 2H), MS-FAB (m/z):745 $(M-Cl-HCl)^+$: Anal. Calcd. for $C_{34}H_{46}Cl_2N_{14}O_6$: C, 49.9, H. 5.7, N, 24.0, Cl, 8.7, Found: C, 50.3, H, 6.05, N, 22.9, Cl, 8.7.

EXAMPLE 2

Compound of the formula I, where x equals 1; y and z are each equal to 1;



Cl^-;

R_1 equals -CO- was prepared. 1-Methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)-pyrrole-2-carboxamidopropionitrile (315 mg, 1 mmole) and 81 mg of 1,1'-carbonyldiimidazole were dissolved in 10 ml of anhydrous CH_3CN and refluxed under argon for 5 minutes. A solid forms which was collected to give 302 mg (88.6% yield) of the pure product was treated with HCl in EtOH and then NH_3 (as in Example 1). After the reaction was completed, the mixture was decanted from an insoluble residue. The solvent was removed in vacuo and the residue was dissolved in 4 ml of MeOH and an excess of CH_3CN was added to precipitate the product which was collected and washed with 1 ml of cold water whereupon it became jelly-like. The product was redissolved in MeOH and reprecipitated with CH_3CN to give 216 mg (57% overall yield) of the pure compound m.p. 211-215°C; ^1H-NMR (DMS)-d_6): δ 2.64 (t, 2H), 3.52 (q, 2H), 3.84 (s, 6H), 6.82, 6.94, 7.03, 7.20 (4d, 4H), 8.25 (t, 1H), 8.73 (2s, 3H), 9.05 (s, 2H), 9.88 (s, 1H), MS-FAB: 690 $(M-Cl-HCl)^+$. Anal. Calcd. for $C_{31}H_{42}Cl_2N_{14}O_5$: C, 48.9, H, 5.6, Cl, 9.3, N, 25.7; Found C, 48.5, H, 5.7, Cl, 9.7, N, 25.3.

EXAMPLES 3(A) AND (B)

The following Examples illustrate the effect of altering the steric size of the terminal group (in these cases trialkylammonium) on the basic Het block of the general formula on the nucleic acid binding and antiviral efficacy. The effects were demonstrated on deoxyribonucleic acid to show that steric hindrance in the terminal group in DNA binding generally reduces antiviral activity of the compounds.

(A) 1-Methyl-4-(1-methyl-4-trimethylammonium-acetamidopyrrole-2-carboxamido)pyrrole-2-carboxyamidopriopionamidine chloride hydrochloride

A solution of the precursor 1-methyl-4-(1-methyl-4-trimethylammonium-acetamido-pyrrole-2-carboxamido)pyrrole-2-carboxyamidopriopionitrile chloride (347 mg, 0.07 mmoles) in 5 ml of absolute ethanol was treated with dry hydrogen chloride with cooling. After 2 hours, the solvent was removed in vacuo, 5 ml of absolute ethanol was added and dry NH_3 gas passed into the solution. The solid dissolved during 2 hours at room temperature, then the solution was evaporated to dryness and extracted with hot isopropyl alcohol (100 ml). The extract was concentrated to ca. 10 ml, acetone added and the resulting precipitate collected, washed with acetone, and dried to vacuo to give the product, 300 mg (85% yield) as an amorphous hygroscopic solid, no definite m.p.; ^{1}H-NMR (DMSO-d_6): δ 2.67 (t, 2H), 3.31 (s, 9H), 3.52 (q, 2H), 3.82 and 3.87 (2s, 6H), 4.44 (s, 2H), 6.97 (d, 1H), 7.02 (d, 1H), 7.24 (d, 1H), 7.29 (d, 1H), 8.31 (t, 1H), 8.82 (bs, 2H), 9.72 (bs, 2H), 10.06 (s, 1H), 11.23 (s, 1H), IR (Nujol) ν_{max}: 1260, 1377, 1405, 1453, 1531, 1582, 1643, 1685, 3247 cm^{-1}; MS-FAB (m/z) 430 $(M-HCl-Cl)^+$.

Sulfate. The sulfate corresponding to the product was prepared in order to obtain an analytically pure sample by precipitation from a methanolic solution of the above compound by means of a large excess of tetraethylammonium sulfate, m.p. 295°C: IR (Nujol) ν_{max}: 1255, 1377, 1405, 1462, 1525, 1560, 1580, 1640, 1670, 3280 cm^{-1}; MS-FAB (m/z) 431 $(M-HSO_4)^+$, 529 MH^+; Anal. Calcd. for $C_{20}H_{32}N_8O_7S$ (528.59), C, 45.4, H, 6.1, N, 21.1, S, 6.1. Found: C, 45.0, H, 6.0, N, 20.7, S, 5.8.

(B) 1-Methyl-4-(1-methyl-4-trimethylammonium-acetamidopyrrole-2-carboxamido)pyrrole-2-carboxyamidopriopionamidine chloride hydrochloride

A solution of the precursor analogous to that of Example 3(A) (173 mg, 035 mmoles) in 10 ml of absolute ethanol was treated with dry hydrogen chloride with cooling. After 2 hours, the solvent was removed in vacuo and the residue dissolved in 10 ml of absolute ethanol and treated with an excess of dry ammonia. After 2 hours at room temperature, the solvent was removed in vacuo and the residue dissolved in 5 ml of isopropyl alcohol; then the product was precipitated with ether. The solid was collected, washed with ether and dried at 100° in vacuo to afford the product 103 mg (59% yield) m.p. 180° (dec); ^1H-NMR (DMSO-d_6): δ 1.32 (t, 9H), 2.67 (t, 2H), 3.54 (m, 8H), 3.83 and 3.88 (2s, 6H), 4.32 (s, 2H), 6.96 (d, 1H), 7.01 (d, 1H), 7.21 (d, 1H), 7.30 (d, 1H), 8.28 (t, 1H) 8.80 and 9.10 (bs, 4H), 10.03 (s, 1H), 11.47 (s, 1H), IR (Nujol): 1376, 1404, 1462, 1531, 1581, 1646, 1684, 3250 cm^{-1}; MS-FAB (m/z): 981 $(2M-HCl-Cl)^+$, 473 $(M-HCl-Cl)^+$.

The activities of Examples 3(A) and 3(B) expressed as minimum inhibitory concentration (μg/ml) against vaccinia virus were 20 and 300 respectively illustrating the effects of steric hindrance in DNA binding on reducing agent activity. The larger the terminal group, as

demonstrated by compound 3(B), the lesser the activity; hence the terminal group is of a selected size which will maintain nucleic acid sequence bonding desired antiretroviral activity.

The compound numbers referred to in the following examples correspond to the numbered structures in the "Detailed Description of the Invention" section.

EXAMPLE 4

(A) 1-Methyl-4-[1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidopriopionitrile (Intermediate Compound)

1-Methyl-4-[1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamidopriopionitrile (Lown, W.J. and Krowicki, K., J. Org. Chem. Vol. 50, p. 3774 (1985) and Krowicki, K. and Lown, W.J., J. Org. Chem., Vol. 52, p. 3493 (1987) (420 mg, 0.9 mmol) was reduced over 5% palladium on charcoal (260 mg) in a mixture of DMF (15 ml) and methanol (5 ml) at 45°. After the reduction the solvents were evaporated under reduced pressure. The residue was dissolved in a small amount of acetonitrile (2 ml) and an excess of ethyl acetate (20-30 ml) as added to precipitate some impurities. The filtrate was treated with an excess of hexane to precipitate a white pure product 9 (250 mg, 63.5% yield), m.p. 155-160°. ^{1}H-NMR (DMSO-d_6): δ 2.74 (t, 2H), 3.42 (Q, 2H), 3.76 (s, 3H), 3.85 and 3.87 overlapped with a bs (3s, 8H), 6.27 (d, 1H), 6.40 (d, 1H), 6.95 (d, 1H), 7.04 (d, 1H), 7.24 (2d, 2H), 8.37 (t, 1H) 9.66 (s, 1H), 9.96 (s, 1H); IR (nujol): 1260, 1377, 1403, 1464, 1529, 1582, 1646, 2245, 3120, 3310 cm^{-1}; MS m/z 436.1981 (calcd. 436.1983). Analysis Calcd. for $C_{19}H_{26}ClN_6O_3$: C 52.3, H 6.0, Cl 8.1, N 22.5. Found: C 52.3, H 6.0, Cl 7.9, N 22.0.

EXAMPLE 5

(A) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamido (3-proprionitrile)-4-pyrrole]-4-pyrrolyl)succinamide

The intermediate compound (105 mg, 0.33 mmol) and i-Pr_2EtN (65 µL, 0.37 mmol) were dissolved in anhydrous acetonitrile (5 ml) and cooled to -20°C. Succinyl chloride (18 µL, 0.16 mmol) in anhydrous THF (1 ml) was added. The mixture was allowed to reach ambient temperature. The solvents were evaporated to dryness, water was added, and the resulting solid was collected and washed with hot MeOH. The product was dissolved in DMF and when placed on a TLC plate (SiO_2) with $CHCl_3$ + 15% MeOH system it gave one spot. For analytical purposes, the product was purified by dissolution in a small amount of DMF and precipitation with a large amount of EtOH to give 90 mg (77%) of 15 m.p. 292°. ^{1}H-NMR (DMSO-d_6): δ 2.58 (s, 4H) 2.74 (t, 4H), 3.42 (q, 4H), 3.83 (2s, 12H), 6.86, 6.93, 7.17 and 7.22 (4d, 2H each), 8.35 (t, 2H), 9.89 (s, 4H); IR (nujol): 1376, 1401, 1447, 1465, 1511, 1535, 1585, 1645, 2245, 3120, 3304 cm^{-1}; MS (m.z. rel. int.): 396.1543 (9.98) for $C_{19}H_{20}N_6O_4$ which is $(O{=}C{=}CH{-}M_{1/2})^+$. Analysis Calcd. for $C_{34}H_{38}N_{12}O_6$: C 57.5, H 5.4, N 23.6. Found: C 57.8, N 5.4, N 23.3.

(B) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamido (3-proprionamidine)-4-pyrrole]-4-pyrrolyl)succinamide dihydrochloride (Compound 5)

A suspension of the previous product (130 mg, 0.18 mmol) in 15 ml anhydrous EtOH was saturated with HCl with cooling. After 1.5 hr. at r.t., the solvent was evaporated under reduced pressure. The residue was washed with dry ether, then ethanol was added followed by some NH_3 condensed into the vessel. After 1 hr at r.t. the solvents were removed and the residue was washed with MeOH, EtOH and hexane to give 116 mg of a solid. The

latter was examined by TLC (SiO_2) with MeOH and a drop of formic acid and indicated formation of the product (Rf = 0.3) containing some more polar impurity. Recrystallization from a small amount of water gave a gel-like precipitate which was washed with EtOH and hexane and dried give to 50 mg (34% of pure 5a, m.p. 283-5° dec. ^{1}H-NMR (DMSO-d_6): δ 2.60 (m, 8H) 3.50 (m, 4H), 3.83 (s, 12H), 6.92 (d, 4H), 7.18 (d, 4H), 8.25 (t, 2H), 8.70 (bs, 4H), 9.02 (bs, 4H) 9.93 and 9.97 (2s, 4H); IR (nujol): 1352, 1377, 1464, 1521, 1576, 1638, 1700, 3260 cm^{-1}; MS-FAB (m/z): 745 $(M-Cl-HCl)^+$. Analysis Calcd. for $C_{34}H_{46}Cl_2N_{14}O_6$: C 49.94, H 5.67, N 23.98, Cl 8.67. Found: C 50.3, H 6.05, N 22.90, Cl 8.75.

EXAMPLE 6

(A) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamido (3-proprionitrile)-4-pyrrole]-4-pyrrolyl)malonamide

The intermediate compound (315 mg, 1 mmol), malonic acid (52 mg, 0.5 mmol) and DCC 206 mg, 1 mmol) were stirred in acetonitrile (6 ml) for 2 hr at room temperature and finally the mixture was heated briefly to boiling to complete the reaction. A solid which contained dicyclohexylurea was collected and the filtrate was extracted with DMF. The DMF solution was treated with water and the solid formed was recrystallized from a mixture of acetonitrile (2 ml) and methanol (2 ml) to give pure compound (140 mg, 40% yield), m.p. ~225-30°. ^{1}H-NMR (DMSO-d_6): δ 2.73 (t, 2x2H), ~2.40 (q+s overlapped, 2x2H+2H), 3.83 and 3.86 (2s, 2x6H), 6.91 (2d, 2x2H), 7.18 and 7.22 (2d, 2x2H), 8.35 (t, 2x1H), 9.91 (s, 2x1H), 10.09 (s, 2x1H); IR (nujol): 1200, 1264, 1290, 1376, 1401, 1464, 1511, 1532, 1585, 1638, 1662, 2250, 3120, 3305 cm^{-1}; MS-FAB (m/z): 697 (MH^+). Analysis Calcd. for $c_{33}H_{36}N_{12}O_6$: C 56.9, H 5.2, N 24.1. Found: C 56.6, H 5.4, N 23.9.

(B) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamido (3-proprionamide)-4-pyrrole]-4-pyrrolyl)malonamide dihydrochloride (Compound 4)

The compound of the previous synthesis (160 mg, 0.23 mmol) was suspended in dry ethanol and the mixture was saturated with dry halogen chloride. After 1.5 hr at room temperature, the solvent was removed under reduced pressure. The residue was treated with dry ethanol and dry ammonia. After 1 hr the solution was decanted from undissolved material and evaporated to dryness. The residue was dissolved in 2 ml of boiling water and an excess of acetonitrile was added to the hot solution. The precipitate was collected and washed with a small amount of water. The operation was repeated and pure compound 4 was collected, 100 mg (59% yield), m.p. 218-224°. The compound, if crystallized from water, precipitates in the form of a jelly. ^{1}H-NMR (DMSO-d_6): δ 2.63 (t, 2x2H), 3.35 (s overlapped with the peak of water), 3.50 (q, 2x2H), 3.80 and 3.83 (2s, 2x6H), 6.93 (s, 2x2H), 7.20 (s, 2x2H), 8.26 (t, 2x1H), 8.90 (bs, 2x4H), 9.96 (s, 2x1H), 10.28 (s, 2x1H). D_2O exchange experiment showed the presence of malonyl protons at δ3.30. IR (nujol): 1260, 1377, 1405, 1463, 1535, 1580, 1645, 3100, 3270 cm^{-1}; MS-FAB (m/z) 731 $(M-Cl-HCl)^+$. Analysis Calcd. for $C_{33}H_{44}N_{14}O_6Cl_2$: C 49.3, H 5.5, N 24.4, Cl 8.8. Found: C 49.0, H 5.7, N 27.0, Cl 9.0.

EXAMPLE 7

(A) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamido (3-proprionitrile)-4-pyrrole]-4-pyrrolyl)urea

The intermediate compound (365 mg, 1.16 mmol) and 1,1'carbonyldiimidazole (94 mg, 0.58 mmol) were allowed to react in boiling acetonitrile (3 ml). A solid which formed was collected, washed with acetonitrile to give 350 mg (88.6% yield) of pure product, m.p. 296-7°. ^{1}H-NMR

3.88 (s, 6H), 6.80 (d, 2H), 6.92 (d, 2H), 7.02 (d, 2H), 7.21 (d, 2H), 8.12 (s, 2H), 8.25 (t, 2H), 9.81 (s, 2H); IR (nujol): 1199, 1217, 1252, 1378, 1409, 1436, 1465, 1504, 1544, 1589, 1621, 1653, 1672, 2240, 3270, 3424 cm^{-1}; MS-FAB (m/z): 655 (MH^+). Analysis Calcd.: C 56.9, H 5.2, N 25.7. Found: C 56.6, H 5.4, N 25.5.

(B) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamido (3-propionamidine)-4-pyrrole]-4-pyrrolyl)urea dihydrochloride (Compound 3)

The compound synthesized in the previous step (116 mg, 0.25 mmol) was suspended in dry ethanol and the solution saturated with HCl. After 2 hr the solvent was evaporated in vacuo and the residue treated with dry ammonia in ethanol for 1 hour. The mixture was decanted from an insoluble residue and the solution evaporated to dryness. The residue was dissolved in 2 ml of methanol and an excess of acetonitrile was added to precipitate the product. The latter was collected and washed with 1 ml of water when it became jelly-like. It was redissolved in methanol and precipitated with acetonitrile to give the compound (3) (117 mg, 61.6% yield), m.p. 211-215°. ^{1}H-NMR (DMSO-d_6): δ 2.64 (t, 4H), 3.52 (q, 4H), 3.84 (2s, 12H), 6.82 (d, 2H), 6.94 (d, 2H), 7.03 (d, 2H), 7.20 (d, 2H), 8.73 (2s overlapped, 6H), 9.05 (s, 4H), 9.88 (s, 2H); IR (Nujol): 1264, 1377, 1402, 1439, 1489, 1531, 1583, 1640, 1689, 3088, 3279 cm^{-1}; MS-FAB (m/z): 690 $(M-Cl-HCl)^+$. Analysis Calcd. for $C_{31}H_{42}Cl_2N_{14}O_5$: C 48.9. H 5.6, Cl 9.3, N25.7. Found: C 48.5, H 5.7, Cl 9.7, N 25.3.

EXAMPLE 8

(A) N,N'-Di(1-methyl-2-carboxamido(3-proprionitrile)-4-pyrrole]-4-pyrrolyl}apidamide

Adipic acid (29.2 mg, 0.2 mmol) in acetonitrile (0.5 ml) was treated with pivaloyl chloride (50 µL, 0.4 mmol) and Hunig's base (160 µL, 0.9 mmol) and then compound 7 (126 mg, 0.42 mmol) in DMF (0.5 ml) was added. After a half hour at room temperature the mixture was evaporated to dryness under reduced pressure. The residue was washed with water and hot acetonitrile. The solid was dissolved in hot DMF and precipitated with an excess of acetonitrile to give the compound (95 mg, 61% yield), m.p. 244-46° dec. ^{1}H-NMR (DMSO-d_6): δ 1.60 (s, 4H), 2.27 (s, 4H), 2.74 (t, 4H), 3.40 (q, 4H), 3.83 (2s, 12H), 6.86 (s, 2H), 6.93 (s, 2H), 7.17 (s, 2H), 7.22 (s, 2H), 8.38 (t, 2H), 9.82 (s, 2H), 9.91 (s, 2H: IR (Nugol): 1376, 1400, 1464, 1513, 1533, 1585, 1641, 2258, 3294 cm^{-1}; MS-FAB (m/z): 738 (M^+), 739 (MH^+); Analysis Calcd. C 58.5, H 5.7, N 22.7. Found: C 58.9, H 5.9, N 22.5.

(B) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamido(3-proprionamidine)-4-pyrrole]-4-pyrrolyl}adipamide dihydrochloride (Compound 7)

The compound synthesized in the previous step (320 mg, 0.43 mmol) was treated under Pinner reaction conditions as in Example 3 above. After evaporation of solvents, water (3.5 ml) was added and a crystalline substance was collected to give (7) (215 mg, 58.7% yield), m.p. 195-6°. ^{1}H-NMR (DMSO-d_6): δ 1.60 (s, 4H), 2,27 (s, 4H), 2.62 (t, 4H), 3.52 (q, 4H), 3.80 (2s, 12H), 6.88 (d, 2H), 6.95 (d, 2H), 7.18 and 7.20 (2d, 4H), 8.25 (t, 2H) 8.70 (s, 4H), 9.00 (s, 4H), 9.00 (s, 4H), 9.92 (s, 2H); IR

(Nujol): 1208, 1261, 1377, 1404, 1463, 1531, 1579, 1641, 1691, 3256 cm^{-1}; MS-FAB m/z 773 $(M-HCl-Cl)^+$; Analysis Calcd.: C 51.1, H 6.0, N 23.2, Cl 8.4. Found: C 50.9, H 6.2, N 23.6, Cl 8.8.

EXAMPLE 9

(A) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamido(3-propionitrile)-4-pyrrole]-4-pyrrolyl)malemide

The intermediate compound (158 mg, 0.5 mmol) and maleic anhydride (49 mg, 0.5 mmol) were heated in acetonitrile (5 ml) at 50° for 3 minutes. Another portion of the intermediate compound (158 mg) was added and the solution was evaporated to dryness. The residual solid was dissolved in DMF (2 ml) and DCC (103 mg, 0.5 mmol) was added, and the mixture was set aside overnight at room temperature. Two drops of water were added and the solution was filtered. Then an excess of water precipitated the crude product. The product was collected and chromatographed on silica gel with chloroform and 15% of methanol providing yellow fractions. These were combined and evaporated, and the residue recrystallized from acetone to give the product (100 mg, 56.5% yield), m.p. 250-2°. Analytical data for this and related compounds is given in Table I.

(B) N,N'-Di(1-methyl-2-carboxamido(3-proprionamidine)-4-pyrrole]-4-pyrrolyl)maleamide dihydrochloride (Compound 14)

The product obtained in the previous step (170 mg, 0.24 mm) was treated under Pinner reaction conditions as in Example 3. The completed reaction mixture was evaporated to dryness and the residue dissolved in ethanol. Controlled addition of isopropanol provided selective precipitation of impurities. The mother liquor was evaporated and the residue was dissolved in methanol

and precipitation with acetonitrile gave pure compound (14) (166 mg, 85% yield), m.p. 217°.

EXAMPLE 10

(A) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamido(3-proprionitrile)-4-pyrrole]-4-pyrrolyl)trans-cyclopropyldicarboxamide (Compound 8a)

The synthesis and characterization of compounds 3, 4 and 5 have been reported (Krowicki, K. et al, J. Med. Chem., Vol. 31, p. 341 (1988)). Trans-cyclopropyldicarboxylic acid (59 mg, 0.45 mmole) and 1,1'-carbonyldiimidazole (146 mg, 0.7 mmole) in acetonitrile (2.5 ml) were heated under reflux until the evolution of carbon dioxide ceased. To the cooled solution the appropriate amine (284 mg, 0.9 mmole) and 0.8 ml of DMF were added and the mixture was stirred for 2 hr at room temperature (the product partially precipitated) and was evaporated to dryness under reduced pressure. The residue was washed with acetonitrile, aqueous K_2CO_3 then water to give 8a, 289 mg (88.6% yield) m.p. 312° dec.

(B) N,N'-Di(1-methyl-2-[1-methyl-2-carboxamide(3-proprionamidine)-4-pyrrole]-4-pyrrolyl)trans-cylcopropyldicarboxamide dihydrochloride (Compound 8b)

Compound 8a (216 mg, 0.3 mmole) was treated under Pinner reaction conditions as described previously. The final reaction mixture was evaporated to dryness and the residue was extracted with hot propanol (150 ml). The extract was evaporated to dryness and the residue dissolved in methanol 1 ml, and an excess of acetonitrile was added to precipitate the product 8b, 170 mg (68.5% yield) m.p. 210° (softens).

EXAMPLE 11

Commercially available acid chlorides for the linker groups were used directly without further purification. Otherwise, the appropriate acid chlorides were prepared from the acids according to the following procedure: An acid and a drop of dimethylformamide was heated in thionyl chloride (5 to 10 mole in excess) to 55-65°C for 30 to 45 min until a homogeneous liquid was obtained. The excess of the chlorinating agent was removed by evaporation. A small amount of methylene chlcride was added to the crude acid chloride then evaporated. The diacid dichloride was then dissolved in methylene chloride or THF and aliquots were taken and used for coupling reactions.

EXAMPLE 12

Distamycin A (50 mg, 0.09 mmol) was dissolved in 4 mL of methanol. To this yellow solution was added 100 µL of concentrated hydrochloric acid. The solution was stirred for 6-8 h and the reaction progress was followed by TLC (methanol:acetic acid, 100:5). The solvent was evaporated and the crude product was redissolved in methanol and precipitated with ether. The product was recrystallized in this way twice more. The supernatant was decanted and the residual solid was dried in vacuo. The final product was obtained as an off-white solid 50 mg (89% yield).

EXAMPLE 13

Bis-distamycin (Compound 15)

A solution of succinyl dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether.

The final product was obtained as a light yellow solid in 68% yield. m.p. 210°C; ^{1}H-NMR, 2.48 ($COCH_2CH_2CO$, 4H, s), 2.56 [2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 6 Hz], 3.50 (2x$CONHCH_2$, 4H, q, J = 6 Hz), 3.80 (2xNCH_3, 6H, S), 3.82 (2xNCH_3, 6H, s), 3.83 (2xNCH_3, 6H, s), 6.90 (2xpy-CH, 2H, d, J = 2 Hz), 6.94 (2xpy-CH, 2H, d, J = 2 Hz), 7.04 (2xpy-CH, 2H, d, J = 2 Hz), 7.14 (2xpy-CH, 2H, D, J = 2 Hz), 7.18 (2xpy-CH, 2H, d, J = 2 Hz), 7.22 (2xpy-CH, 2H, d, J = 2 Hz), 8.24 (2x$CONHCH_2$, 2H, tr, J = 6 Hz), 8.74 [2x$C(NH_2)_2Cl$, 4H, s], 9.04 [2x$C(NH_2)_2Cl$, 4H, S], 9.93 (5xpy-NHCO, 5H, s), 9.96 (py-NHCO, 1H, s); MS (FAB), 989 (M-2xCl-H, 0.34).

EXAMPLE 14

Bis-distamycin (Compound 16)

A solution of hexan-1,6-dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 78% yield. m.p., 210°C; ^{1}H-NMR, 1.28 (4,5-suber-CH_2, 4H, m), 1.57 (3,6-suber-CH_2, 4H, m), 2.23 (2m7-suber-CH_2, 4H, tr, J = 7 Hz), 2.63 (2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 6 Hz], 3.49 (2x$CONHCH_2$, 4H, m), 3.80 (2xNCH_3, 6H, s), 3.81 (2xNCH_3, 6H, s), 3.83 (2xNCH_3, 6H, s), 6.88 (2xpy-CH, 2H, d, J = 2Hz), 6.94 (2xpy-CH, 2H, d, J = 2Hz), 7.05 (2xpy-cH, 2H, d, J = 2 Hz), 7.15 (2xpy-CH, 2H, d, J = 2 Hz), 7.18 (2xpy-CH, 2H, d, J = 2 Hz), 7.23 (2xpy-CH, 2H, d, J = 2 Hz), 8.25 (2x$CONHCH_2$, 2H, m), 8.72 [2x$C(NH_2)_2Cl$, 4H, s], 9.03 [2x$C(NH_2)_2Cl$, 4H, s], 9.86 (2xpy-NHCO, 2H, s), 9.92 (4xpy-NHCO, 4H, s); MS (FAB), 1045 (M-2xCl-H, 0.38).

EXAMPLE 15

Bis-distamycin (Compound 17)

A solution of octan-1,8-dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 μL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min. a solution of Hunig's base (16 μL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 65% yield. m.p., 198-202°C; ^{1}H-NMR, 1.26 [(4,5,6,7-seba-CH_2, 8H, m), 4H, tr, J = 6 Hz], 1.55 [(3,8-seba-CH_2), 4H, m], 2.22 (2,9-seba-CH_2), 4H, tr, J = 8 Hz], 2.61 [2x$CH_2C(NH_2)_2Cl$, tr, J = 6 Hz], 3.48 (2x$CONHCH_2$, 4H, m), 3.80 (2xNCH_3, 6H, s), 3.81 (2xNCH_3, 6H, s), 3.83 (2xNCH_3, 6H, s), 6.89 (2xpy-CH, 2H, d, J = 2Hz), 6.95 (2xph-CH, 2H, d, J = 2Hz), 7.05 (2xpy-CH, 2H, d, J = 2 Hz), 7.15 (2xpy-CH, 2H, d, J = 2 Hz), 7.18 (2xpy-CH, 2H, d, J = 2 Hz), 7.22 (2xpy-CH, 2H, d, J = 2 Hz), 8.23 (2xCONHpy-2H, m), 8.65 [2x$C(NH_2)_2Cl$, 4H, s], 8.99 [2x$C(NH_2)_2Cl$, 4H, s], 9.82 (2xpy-NHCO, 2H, s), 9.91 (4xpy-NHCO, 4H, s); MS (FAB), 1074 (m-2xCl-H, 0.08).

EXAMPLE 16

Bis-distamycin (Compound 18)

A solution of docosane-1,22-dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 μL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 μL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a

light yellow solid in 73% yield. m.p., 215°C; ^{1}H-NMR, 1.23 (4,5,...20,21-tetraco-CH_2, 36H, s), 1.55 (3,22-tetraco-CH_2, 4H, m), 2.21 (2,23-tetraco-CH_2, 4H, tr, J = 7 Hz), 2.62 [2xCH_2C$(NH_2)_2$Cl, 4H, tr, J = 6 Hz], 3.50 (2xCONHCH_2, 4H, tr, J = 6 Hz), 3.80 (2xNCH_3, 6H, s), 3.82 (2xNCH_3, 6H, s), 3.84 (2xNCH_3, 6H, s), 6.89 (2xpy-CH, 2H, d, J = 2Hz), 6.94 (2xpy-CH, 2H, d, J = 2 Hz), 7.05 (2xpy-CH, 2H, d, J = 2 Hz), 7.15 (2xpy-CH, 2H, d, J = 2 Hz), 7.19 (2xpy-CH, 2H, d, J = 2 Hz), 7.23 (2xpy-CH, 2H, d, J = 2 Hz), 8.25 (2xCONHCH$_2$, 4H, tr, J = 6 Hz), 8.72 [2xC$(NH_2)_2$Cl, 4H, s], 9.02 [2xC$(NH_2)_2$Cl, 4H, s], 9.83 (2xpy-NHCO, 2H, s), 9.92 (4xpy-NHCO, 4H, s); MS (FAB), 1270 (M-2xCl-H, 0.10).

EXAMPLE 17

Bis-distamycin (Compound 29)

A solution of benzene-1,4-dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 77% yield. m.p., >300°C; ^{1}H-NMR, 2.63 [2xCH_2C$(NH_2)_2$Cl, 4H, tr, J = 6 Hz], 3.50 (2xCONHCH_2, 4H, tr, J = 6 Hz), 3.82 (2xNCH_3, 6H, s), 3.86 (2xNCH_3, 6H, s), 3.90 (2xNCH_3, 6H, s), 6.97 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.09 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.15 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.20 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.26 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.38 (2xpy-CH, 2H, d, J = 1.6 Hz), 8.10 (aromatic-CH, 4H, s), 8.25 (2xCONHCH$_2$, 2H, tr, J = 6 Hz), 8.65 [2xC$(NH_2)_2$Cl, 4H, s], 9.01 [2xC$(NH_2)_2$Cl, 4H, s], 9.95 (2xpy-NHCO, 2H, s), 10.03 (2xpy-NHCO, 2H, s), 10.57 (2xpy-NHCO, 2H, s); (CD_3OD), 2.71 [2xCH_2C$(NH_2)_2$Cl, 4H, tr, J =

7 Hz], 3.65 (2xCONHC$\underline{H}_2$, 4H, tr, J = 7 Hz), 3.87 (2xNCH_3, 6H, s), 3.91 (2xNCH_3, 6H, s), 3.95 (2xNCH_3, 6H, s), 6.90 (2xph-CH, 2H, d, J = 1.8 Hz), 6.98 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.07 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.16 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.20 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.34 (2xpy-CH, 2H, d, J = 1.8 Hz), 8.04 (aromatic-CH, 4H, s); MS (FAB), 1037 (M-2xCl-H, 0.05).

EXAMPLE 18

Bis-distamycin (Compound 30)

A solution of benzene-1,3-dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 68% yield. m.p., 240°C; ^{1}H-NMR, 2.61 [2xC$\underline{H}_2$C$(NH_2)_2$Cl, 4H, tr, J = 6 Hz], 3.48 (2xCONHC$\underline{H}_2$, 4H, tr, J = 6 Hz), 3.80 (2xNCH_3, 6H, s), 3.86 (2xNCH_3, 6H, s), 3.91 (2xNCH_3, 6H, s), 6.97 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.09 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.16 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.20 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.25 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.38 (2xpy-CH, 2H, d, J = 1.6 Hz), 7.66 (5-aromatic-CH, 1H, tr, J = 7.5 Hz), 8.10 (4,6-aromatic-CH, 2H, d, J^1 = 8 Hz); 8.21 (2-aromatic-CH, 1H, br, s); 8.21 (2xCON$\underline{H}$CH_2, 2H, br, s), 8.58 [2xC$\underline{H}_2$C$(NH_2)_2$Cl, 4H, tr, J = 7 Hz], 3.64 (2xCONHC$\underline{H}_2$, 4H, tr, J = 7 Hz), 3.88 (2xNCH_3, 6H, s), 3.90 (2xNCH_3, 6H, s), 3.94 (2xNCH_3, 6H, s), 6.89 (2xpy-CH, 2H, d, J = 1.8 Hz), 6.97 (2xph-CH, 2H, d, J = 1.8 Hz), 7.07 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.20 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.33 (2xpy-CH, 2H, d, J = 1.8 Hz),

7.65 (5-aromatic-CH, 1H, tr, J = 7.5 Hz), 8.08 (4,6-aromatic-CH, 2H, d,d, J_1 = 7.5 Hz, J_2 = 2 Hz), 8.47 (2-aromatic-CH, 1H, br, tr, J = 2 Hz); MS (FAB), 1037 (-2xCl-H, 0.43).

EXAMPLE 19

Bis-distamycin (Compound 31)

A solution of benzene-1,2-dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 83% yield. m.p., 245°C; ^{1}H-NMR (CD_3OD), 2.71 [2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 6 Hz], 3.63 (2x$CONHCH_2$, 4H, tr, J = 6 Hz), 3.87 (2xNCH_3, 6H, s), 3.88 (2xNCH_3, 6H, s), 3.90 (2xNCH_3, 6H, s), 6.89 (2xpy-CH, 2H, d, J = 2 Hz), 6.91 (2xpy-CH, 2H, d, J = 2 Hz), 6.97 (2xpy-CH, 2H, d, J = 2 Hz), 7.15 (2xpy-CH, 2H, d, J = 2 Hz), 7.18 (2xpy-CH, 2H, d, J = 2 Hz), 7.24 (2xpy-CH, 2H, d, J = 2 Hz), 7.60 (2xm-aromatic-CH, 2H, q, J = 3 Hz), 7.68 (2xo-aromatic-CH, 2H, q, J = 3 Hz); MS (FAB), 1037 (m-2xCl-H, 0.65).

EXAMPLE 20

Bis-distamycin (Compound 32)

A solution of 3,5-pyridine dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was

evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid m.p. 250°C in 88% yield. m.p., 250°C; ^{1}H-NMR, 2.52 [2xCH_2C$(NH_2)_2$Cl, 4H, m], 3.48 (2xCONHCH_2, 4H, m), 3.81 (2xNCH_3, 6H, s), 3.85 (2xNCH_3, 6H, s), 3.88 (2xNCH_3, 6H, s), 3.90 (2xNCH_3, 6H, s), 6.96 (2xpy-CH, 2H, m), 7.09 (2xpy-CH, 2H, d, J = 2 Hz), 7.17 (py-CH, 1H, d, J = 2 Hz), 7.19 (2xpy-CH, 2H, d, J = 2 Hz), 7.25 (2xpy-CH, 2H, d, J = 2 Hz), 7.29 (py-CH, 1H, m), 7.40 (py-CH, 1H, m), 7.42 (py-CH, 1H, m), 8.23 (2xCONHCH$_2$, 2H, m), 8.25 (3-py-CH, 1H, d, J = 8 Hz), 8, 54 (4-py-CH, 1H, m), 8.64 [2xC$(NH_2)_2$Cl, 4H, s], 8.99 [2xC$(NH_2)_2$Cl, 4H, s], 9.20 (96-py-CH, 1H, m), 9.95 (2xpy-NHCO, 2H, s), 10.04 (2xpy-NHCO, 2H, s), 10.94 (py-NHCO, 1H, s), 11.00 (py-NHCO, 1H, s); (CD_3OD), 2.72 [2xCH_2C$(NH_2)_2$Cl, 4H, tr, J = 6 Hz], 3.65 (2xCONHCH$_2$, 4H, tr, J = 6 Hz), 3.87 (2xNCH_3, 6H, s), 3.91 (2xNCH_3, 6H, s), 3.94 (NCH_3, 3H, s), 3.954 (NCH_3, 3H, s), 6.90 (2xpy-CH, 2H, d, J = 2 Hz), 6.98 (2xpy-CH, 2H, d, J = 2 Hz), 7.07 (py-CH, 1H, d, J = 2 Hz), 7.10 (py-CH, 1H, d, J = 2 Hz), 7.15 (2xpy-CH, 2H, d, J = 2 Hz), 7.20 (2xpy-CH, 2H, d, J = 2 Hz), 7.34 (py-CH, 1H, d, J = 2 Hz), 7.41 (py-CH, 1H, d, J = 2 Hz), 8.27 (3-py-CH, 1H, d, J = 8 Hz), 8.44 (4-py-CH, 1H, m), 9.17 (6-py-CH, 1H, m); MS (FAB), 1038 (M-2xCl-H, 0.03).

EXAMPLE 21

Bis-distamycin (Compound 33)

A solution of pyridine-3,6-dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a

light yellow solid in 74% yield. m.p., 260°C; ^{1}H-NMR, 2.62 [2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 6Hz], 3.50 (2x$CONHCH_2$, 4H, q, J = 6Hz), 3.81 (2xNCH_3, 6H, s), 3.85 (2xNCH_3, 6H, s), 3.90 (2xNCH_3, 6H, s), 6.96 (2xpy-CH, 2H, d, J = 2 Hz), 7.08 (2xpy-CH 2H, d, J = 2 Hz, 7.16 (2xpy-CH, 2H, d, J = 2 Hz), 7.18 (2xpy-CH, 2H, d, J = 2 Hz), 7.26 (2xpy-CH, 2H, d, J = 2 Hz), 7.39 (2xpy-CH, 2H, d, J = 2 Hz), 8.23 (2x$CONHCH_2$, 2H, tr, J = 6 Hz), 8.59 [2x$C(NH_2)_2Cl$, 4H, s], 8.87 (4-py-CH, 1H, br,s), 8.98 [2x$C(NH_2)_2Cl$, 4H, s], 9.24 (2,6-py-CH, 2H, d, J = 2 Hz), 9.94 (2xpy-NHCO, 2H, s), 10.05 (2xpy-NHCO, 2H, s), 10.83 (2xpy-NHCO, 2H, s); (CD_3OD), 2.71 [2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 6 Hz], 3.64 (2x$CONHCH_2$, 4H, tr, J = 6 Hz), 3.87 (2xNCH_3, 6H, s), 3.99 (2xNCH_3, 6H, s), 4.02 (2xNCH_3, 6H, s), 6.88 (2xpy-CH, 2H, s), 6.96 (2xpy-CH, s), 7.07 (2xpy-CH, 2H, s), 7.15 (2xpy-CH, 2H, s), 7.19 (2xpy-CH, 2H, s), 7.35 (2xpy-CH, 2H, s), 8.82 (4-py-CH, 1H, s), 9.17 (2,5-py-CH, 2H, s); MS (FAB), (M-2xCL-H, 0.15).

EXAMPLE 22

Bis-distamycin (Compound 34)

A solution of pyridine-2,6-dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 54% yield. m.p., >260°C; ^{1}H-NMR, 2.62 [2x$CH_2(NH_2)_2Cl$, 4H, tr, J = 6 Hz], 3.50 (2x$CONHCH_2$, 4H, m), 3.82 (2xNCH_3, 6H, s), 3.86 (2xNCH_3, 6H, s), 3.90 (2xNCH_3, 6H, s), 6.97 (2xpy-CH, 2H, d, J = 2 Hz), 7.08 (2xpy-CH, 2H, d, J = 2 Hz), 7.15 (2xpy-CH, 2H, d, J = 2 Hz), 7.18 (2xpy-CH, 2H, d, J = 2 Hz), 7.25 (2xpy-CH, 2H,

d, J = 2 Hz), 7.39 (2xpy-CH, 2H, d, J = 2 Hz), 8.23 (2x$CONHCH_2$, 2H, tr, J = 6 Hz, 8.56 [2x$C(NH_2)_2Cl$, 4H, s], 8.85 (4-py-CH, 1H, tr, J = 2 Hz), 8.96 [2x$C(NH_2)_2Cl$, 4H, s], 9.24 (3,5-py-CH, 2H, d, J = 2 Hz), 9.94 (2xpy-NHCO, 2H, s), 10.04 (2xpy-NHCO, 2H, s), 10.81 (2xpy-NHCO, 2H, s); MS (FAB), 1038 (M-2xCl-H, 0.25).

EXAMPLE 23

Bis-distamycin (Compound 35)

A solution of trans-1,2-cyclobutane-dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 78% yield. m.p., >230°C; ^1H-NMR, 2.05 (3,4-cyclobutane-CH_2, 4H, m), 2.60 (2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 6 Hz], 3.38 (1,2-cyclobutane-CH, 2H, m), 3.49 (2x$CONHCH_2$, 4H, tr, J = 6 Hz), 3.79 (2xNCH_3, 6H, s), 3.84 (2xNCH_3, 6H, s), 3.85 (2xNCH_3, 6H, s), 6.88 (2xpy-CH, 2H, d, J = 1.8 Hz), 6.97 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.05 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.17 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.21 (2xpy-CH, 2H, d, J = 1.8 Hz), 7.23 (2xpy-CH, 2H, d, J = 1.8 Hz), 8.22 (2x$CONHCH_2$, 2H, tr, J = 6 Hz), 8.55 [2x$C(NH_2)_2Cl$, 4H, s], 8.96 [2x$C(NH_2)_2Cl$, 4H, s], 9.88 (2xpy-NHCO, 2H, s), 9.94 (4xpy-NHCO, 2H, s); (CD_3OD), 2.20 (3,4-cyclobutane-CH_2, 4H, m), 2.71 (2xCH_2, 4H, tr, J = 7 Hz), 3.49 (1,2-cyclobutane-CH, 2H, m), 3.64 [2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 7 Hz), 3.87 (2xNCH_3, 6H, s), 3.89 (2xNCH_3, 6H, s), 3.90 (2xNCH_3, 6H, s), 6.84 (2xpy-CH, 2H, d, J = 2 Hz), 6.89

(2xpy-CH, 2H, d, J = 2 Hz), 6.95 (2xpy-CH, 2H, d, J = 2 Hz), 7.15 (2xpy-CH, 2H, d, J = 2 Hz), 7.18 (2xpy-CH, 2H, d, J = 2 Hz), 7.20 (2xpy-CH, 2H, d, J = 2 Hz); MS (FAB), 1015 (M-2xCl-H, 1.06).

EXAMPLE 24

Bis-distamycin (Compound 36)

A solution of maleic-dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 μL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 μL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 33% yield. m.p., >255°C; ^{1}H-NMR, 2.61 [2x$CH_2C(NH_2)_2Cl$, 4H, tr, J =6 Hz], 3.50 (2x$CONHCH_2$, 4H, q, J = 6 Hz), 3.82 (2xNCH_3, 6H, s), 3.85 (2xNCH_3 6H, s), 3.87 (2xNCH_3 6H, s),6.97 (2xpy-CH, 2H, tr, J = 2 Hz), 7.07 (2xpy-CH, 2H, d, J = 2 Hz), 7.10 (-CH=CH-, 2H, s), 7.18 (2xpy-CH, 2H, s), 7.24 (2xpy-CH, 2H, d, J = 2 Hz), 7.35 (2xpy-CH, 2H, d, J = 2 Hz), 8.23 (2x$CONHCH_2$, 2H, tr, J = 6 Hz), 8.66 [2x$C(NH_2)_2Cl$, 4H, s], 8.94 [2x$C(NH_2)_2Cl$, 4H, s], 9.93 (2xpyNHCO, 2H, s), 9.99 (2xpy-NHCO, 2H, s), 10.54 (2xpy-NHCO, 2H, s),, (CD_3OD), 2.72 [2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 6 Hz], 3.65 (2x$CONHCH_2$, 4H, tr, J = 6 Hz), 3.88 (2xNCH_3, 6H, s), 3.90 (2xNCH_3, 6H, s), 3.92 (2xNCH_3, 6H, s), 6.91 (2xpy-CH, 2H, tr, J = 2 Hz), 6.98 (2xpy-CH, 2H, d, J = 2 Hz), 7.09 (-CH=CH-, 2H, s), 7.16 (2xpy-CH, 2H, d, J = 2 Hz), 7.19 (2xpy-CH, 2H, d, J = 2 Hz), 7.33 (2xpy-CH, 2H, d, J = 2 Hz); MS (FAB), 987 (M-2xCl-H, 0.27).

EXAMPLE 25

Bis-distamycin (Compound 37)

A solution of fumaroyl-dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent evaporated and the crude product was obtained as a light yellow solid in 67% yield. m.p., >280°C; ^{1}H-NMR, 2.61 [2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 6 Hz], 3.48 (2x$CONHCH_2$, 4H, tr, J = 6 Hz); 3.80 (2xNCH_3, 6H, s), 3.84 (2xNCH_3, 6H, s), 3.86 (2xNCH_3, 6H, s), 6.35 (-CH=CH-, 2H, s), 6.84-7.84 (12xpy-CH, 12H, m), 8.24 (2x$CONHCH_2$, 2H, tr, J = 6 Hz), 8.58-9.50 [2x$C(NH_2)_2Cl$, 8H, br, s], 9.93 (2xpy-NHCO, 2H, s), 9.97 (2xpy-NHCO, 2H, s), 9.98 (2xpy-NHCO, 2H, s); (CD_3OD), 2.66 [2x$CH_2C(NH_2)_2Cl$, 4H, tr, J = 6 Hz], 3.58 (2x$CONHCH_2$, 4H, tr, J = 6 Hz), 3.79 (2xNCH_3, 6H, s), 3.82 (2xNCH_3, 6H, s), 3.84 (2xNCH_3, 6H, s), 6.26 (-CH=CH-, 2H, s), 6.83 (2xpy-CH, 2H, d, J = 2 Hz), 6.87 (2xpy-CH, 2H, d, J = 2 Hz), 6.91 (2xpy-CH, d, J = 2 Hz), 7.13 (2xpy-CH, 2H, d, J = 2 Hz), 7.17 (2xpy-CH, 2H, d, J = 2 Hz), 7.27 (2xpy-CH, 2H, d, J = 2 Hz); MS (FAB), no M+1 peak.

EXAMPLE 26

Bis-distamycin 35 (Compound 38)

A solution of trans-5,6-bicyclo[2,2,1]-hept-2-ene dicarbonyl dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of deformyl distamycin (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 µL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 µL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the

crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 53% yield. m.p., 260°C; ^{1}H-NMR, 1.31 (7-bicyclohept, 1H, s), 1.86 (7-bicyclohept, 1H, d, J = 7 Hz), 2.76 (5-endo-bicyclohept, 1H, d, J = 8 Hz), 2.93 (4-bicyclohept, 1H, s), 3.35 (1-bicyclohept, 1H, s), 3.50 (6-exo-cyclohept, 1H, s), 3.50 [2x$CH_2C(NH_2)_2Cl$, 4H, m], 3.81 (3xNCH_3, 9H, s), 3.85 (3xNCH_3, 9H, s), 5.98 (3-bicyclohept, 1H, d,d, J = 2.5 Hz), 6.30 (2-bicyclohept, 1H, d,d, J = 2.5 Hz), 6.86 (py-CH, 1H, d, J = 2 Hz), 6.91 (py-CH, 1H, d, J = 2 Hz), 6.97 (2xpy-CH, 2H, d, J = 2 Hz), 7.06 (2xpy-CH, 2H, d, J = 2 Hz), 7.13 (py-CH, 1H, d, J = 2 Hz), 7.18 (2xpy-CH, 2H, d, J = 2 Hz), 7.19 (py-CH, 1H, d, J = 2 Hz), 7.23 (2xpy-CH, 2H, tr, J = 2 Hz), 8.24 (2x$CONHCH_2$, 2H, m), 8.57 [2x$C(NH_2)_2Cl$, 4H, m] 8.97 [23x$C(NH_2)_2Cl$, 4H, m], 9.88 (py-NHCO, 1H, m), 9.92 (4xpy-CH, 4H, m), 10.11 (Ipy-NHCO, 1H, m); (CD_3OD), 1.47 (7-bicyclohept, 1H, d, J = 8 Hz), 1.94 (7-bicyclohept, 1H, d, J - 8 Hz), 2.71 [2x$CH_2C(NH_2)_2Cl$, 4H, d, J = 6 Hz], 2.77 (5-endo-bicyclohept, 1H, d, J = 4 Hz), 3.04 (4-bicyclohept, 1H, s), 3.47 (6-exo-bicyclohept, 1H,), 3.64 (2x$CONHCH_2$, 4H, tr, J = 6 Hz), 3.87 (2xNCH_3, 9H, s), 3.89 (NCH_3, 3H, s), 3.90 (2xNCH_3, 6H, s), 6.08 (3-bicyclohept, 1H, d, J = 2.5 Hz), 6.37 (2-bicyclohept, 1H, d, J = 2.5 Hz), 6.82 (py-CH, 1H, d, J = 2 Hz), 6.83 (py-CH, 1H, d, J = 2 Hz), 6.89 (2xpy-CH, 2H, d, J = 2 Hz), 6.95 (2xpy-CH, 2H, d, J = 2 Hz), 7.11 (py-CH, 1H, d, J = 2 Hz), 7.14 (2xpy-CH, 2H, d, J = 2 Hz), 7.17 (3xpy-CH, 3H, tr, J = 2 Hz); MS (FAB), 1053 (M-2xCl-H, 0.21).

EXAMPLE 27

Bis-Lexitropsin (Compound 39)

A solution of maleic-dichloride (9.28 mg, 0.046 mmol) in 5 mL of tetrahydrofuran was added to a solution of 3-[1-methyl-4-(4-amino-1-methylimidazole-2-carboxamido)imidazole-2-carboxamido]propionamidine hydrochloride (48 mg, 0.09 mmol) and dissiopropylethylamine (Hunig's base, 16 μL, 0.09 mmol) in 3 mL of dimethylformamide cooled to 0°C. After 10 min, a solution of Hunig's base (16 μL, 0.09 mmol) in 3 mL of THF was added to the reaction solution. The resulting mixture was stirred overnight. The solvent was evaporated and the crude product was recrystallized from methanol and ether. The final product was obtained as a light yellow solid in 73% yield. m.p., >250°C; ^{1}H-NMR, 1.86 (2x$CH_2CH_2CH_2$, 4H, q, J = 8 Hz), 3.00 [2x$CH_2N(CH_3)_2$, 4H, tr, J = 8 Hz], 3.30 (2xCONHCH_2, 4H, m), 3.96 (2xNCH_3, 6H, s), 4.02 (2xNCH_3, 6H, s), 7.28 (-CH=CH-, 2H, s), 7.54 (2xim-CH, 2H, s), 7.67 (2xim-CH, 2H, s), 8.52 (2xCONHCH_2, 2H, tr, J = 6 Hz), 9.43 (2xpy-NHCO, 2H, s), 11.01 (2xpy-NHCO, 2H, s); MS (FAB), 777 (M-2xCl-H, 3.11).

Other compounds shown in Table I were similarily prepared and their analytical and physical data are summarized therein.

EXAMPLE 28

Drug-DNA binding constants of the compounds of the present invention were estimated. To 2 mL of Tris-EDTA buffer, pH 8, containing 1.3 μM ethidium bromide, calf thymus DNA was added to give a final concentration of 1.35 μM. The fluorescence was measured after equilibration for a few minutes, using a Turner model 430 spectrofluorometer (Turner Amsco Instruments, Carpinteria, CA) equipped with a 150 W xenon lamp, at an excitation wavelength of 525 nm and an emission wavelength of 600 nm. Aliquots of concentrated drug solutions were added and the

fluorescence measured. Controls were performed to show that the drugs themselves did not interfere with the fluorescence measurements at the levels employed. From a plot of the decreased fluorescence of the ethidium-DNA complex with increase dose of drug, the concentration of drug needed to reduce the fluorescence by 50% was determined and used to calculate a relative binding constant for the drug, given the binding constant of ethidium to be 10^7 M^{-1} under similar conditions.

The results of binding tests are shown in Table II and in Table III.

EXAMPLE 29

Compounds of the present invention were tested for anti-Moloney murine leukemia virus (MLV). The method utilized was adapted from Rowe et al (1970) and Lin et al (1987).

The following materials were utilized in the method:

- Retroviruses; rauscher - ATCC 998
  moloney LT(V) - ATCC 190
  Leukosis-sarcoma complex - ATCC 245

- cells; SC-1 - ATCC CRL 1404
  XC - ATCC CCL 165

- minimum essential medium (eagle) with Hanks Bss, supplemented with 10% fetal bovine serum, 100 1U ml^{-1} penicillin G, 100 $ugml^{-1}$ streptomycin, 2.5 $ugml^{-1}$ amphotericin B and non-essential amino acids (Sigma M2025).

- Dulbecco's modified eagles medium, supplemented with 10% fetal bovine with 5% fetal bovine serum, 100 $10ml^{-1}$ penicillin G, 100 $ugml^{-1}$ streptomycin and 2.5 $ugml^{-1}$ amphotericin B.

- minimum essential medium (eagle) with earles salt supplemented with 5% fetal bovine serum, 100 $10ml^{-1}$ penicillin G, 100 $ugml^{-1}$ streptomycin and non-essential aminoacids (Sigma M2025).

- phosphate buffered saline.

- crystal violet dye.

- 24 well plates.

- compounds dissolved in DMSO (or water) to 2-20 $ugml^{-1}$ then further diluted in 5% FBS-MEM.

Stock cell cultures were prepared in the 10% FBS-Dulbecco. To prepare 24 well plates for experiments, 0.8ml of 3.5×10^4 SC^1 cells ml^{-1} were added to each well one day in advance. This was using the 5% FBS-MEM. 0.1 ml of each compound dilution, in triplicate, was added to a well in the plate. 0.1 ml of 20-40 p.f.u. of moloney virus was added to each well of the plate. Those plates were shaken on a mechanical shaker at 0, 30 and 60 minutes. They were incubated for 5 days at 37°C in a 5% Co_2 incubator. The medium was removed and plates were subjected to ultraviolet light (175 W cm^2 at surface) for three minutes.

0.8 ml of 2×10^5 XC cells ml^{-1} were added to each well using the 10% FBS-Hanks mem. The plates were incubated at 37°C, 5% CO_2 for 4 days, but the medium was replaced after 2 days. The medium was removed, the wells were washed with pbs and 0.25 ml of 0.05% crystal violet was added to each well for 2 hours. The plates were washed, dried and the plaques counted.

MIC_{50} values were calculated using the formula -

$$\frac{\%\text{ inhibition greater than } 50\% - 50\%}{\%\text{ inhibition greater than } 50\% - \%\text{ inhibition less than } 50\%}$$

to give the interpolative values between two dilutions.

The results of the test are shown in Tables IV and V and demonstrate comparative anti-MLV activity between compounds of the present invention and AZT and DDC.

EXAMPLE 30

Compounds of the present invention were tested for anti-HIV activity by the National Cancer Institute (NIH, Bethesda). The procedure used by the National Cancer Institute is described in Weislow, O.W. et al, J. Natl. Cancer Inst., Vol. 81, pages 577-586 (1989). NCI uses this procedure to test for agents active against Human Immunodeficiency Virus (HIV) and is designed to detect agents acting at any stage of the virus reproductive cycle. The assay basically involves the killing of T4 lymphocytes by HIV. Small amounts of HIV are added to cells, and a complete cycle of virus reproduction is necessary to obtain the required cell killing. Agents that interact with virions, cells, or virus gene-products to interfere with viral activities will protect cells from cytolysis. The system is automated in several features to accommodate large numbers of candidate agents and is generally designed to detect anti-HIV activity. However, compounds that degenerate or are rapidly metabolized in the culture conditions may not show activity in this screen. All tests are compared with at least one positive (e.g., AZT-treated) control done at the same time under identical conditions. The procedure is set forth below:

1. Candidate agent is dissolved in dimethyl sulfoxide (unless otherwise instructed) then diluted 1:100 in cell culture medium before preparing serial half-$\log_{10}$ dilutions. T4 lymphocytes (CEM cell line) are added and after a brief interval HIV-1 is added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls.

2. Cultures are incubated at 37° in a 5% carbon dioxide atmosphere for 6 days.

3. The tetrazolium salt, XTT, is added to all wells, and cultures are incubated to allow formzan color development by viable cells.

4. Individual wells are analyzed spectrophotometrically to quantitate formazan production, and in addition are viewed microscopically for detection of viable cells and confirmation of protective activity.

5. Drug-treated virus-infected cells are compared with drug-tested noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells, etc.) on the same plate.

6. Data are reviewed in comparison with other tests done at the same time and a determination about activity is made.

The test results for five of the active compounds are set forth in the Figures 2-6 and the corresponding Tables VI-X below and test results of the compounds of the present invention are compilated in Table XI.

Table I. Analytical and physical data on linked netropsins and their precursors

Comp.	Yield(5)	m.p.[a]	Formula	Analysis
15	85	210°	$C_{46}H_{58}H_{18}O_8Cl_2$	C,H,N,Cl
16	76	210°	$C_{50}H_{60}N_{18}O_8Cl_2$	C,H,N,Cl
17	84	198-202	$C_{52}H_{64}N_{18}O_8Cl_2$	C,H,N,CL
18	69	215	$C_{66}H_{92}N_{18}O_8Cl_2$	C,H,N,CL
19a	99	305-6°	$C_{38}H_{38}N_{12}O_6$	C,H,N
19b	64	262-8°	$C_{38}H_{46}N_{14}O_6Cl_2$	C,H,N,Cl
20a	95	278-82°	$C_{38}H_{38}N_{12}O_6$	C,H,N,
20b	78	248-50°	$C_{38}H_{46}N_{14}O_6Cl_2$	C,H,N,Cl
21a	84.7	289-90°	$C_{34}H_{36}N_{12}O_6$	C,H,N
21b	58	295°	$C_{34}H_{44}N_{14}O_6Cl_2$	C,H,N,Cl
22a	56.5	250-2°	$C_{34}H_{36}N_{12}O_6$	C,H,N,
22b	85	217°	$C_{34}H_{44}N_{14}O_6Cl_2$	C,H,N,Cl
23a	88.6	312° (dec)	$C_{35}H_{38}N_{12}O_6$	C,H,N
23b	68.5	210° (softens)	$C_{35}H_{46}N_{14}O_6Cl_2$	C,H,N,Cl
24a	59	175°	$C_{35}H_{38}N_{12}O_6$	C,H,N
24b	70.6	204° (softens)	$C_{35}H_{46}N_{14}O_6Cl_2$	C,H,N,Cl
25a	69	172° (softens)	$C_{36}H_{40}N_{12}O_6$	C,H,N,
25b	77	238° (softens)	$C_{36}H_{48}N_{14}O_6CL_2$	C,H,N,Cl
26a	70	165-8°	$C_{37}H_{42}N_{12}O_6$	C,H,N,
26b	46	231°	$C_{37}H_{50}N_{14}O_6Cl_2$	C,H,N,Cl
27a	82.6	189°	$C_{38}H_{44}N_{12}O_6$	C,H,N
27b	61	201° (softens)	$C_{38}H_{52}N_{14}O_6Cl_2$	C,H,N,Cl
28a	54	175°	$C_{38}H_{44}N_{12}O_6$	C,H,N
28b	23	198°	$C_{38}H_{52}N_{14}O_6Cl_2$	C,H,N,Cl
29	77	>300	$C_{50}H_{58}N_{18}O_8Cl_2$	C,H,N,CL
30	68	240	$C_{50}H_{58}N_{18}O_8Cl_2$	C,H,N,CL
31	83	245	$C_{50}H_{58}N_{18}O_8Cl_2$	C,H,N,CL
32	88	250	$C_{49}H_{57}N_{19}O_8Cl_2$	C,H,N,CL
33	74	260	$C_{49}H_{57}N_{19}O_8Cl_2$	C,H,N,CL
34	54	260	$C_{49}H_{57}N_{19}O_8Cl_2$	C,H,N,CL
35	78	230	$C_{48}H_{60}N_{18}O_8Cl_2$	C,H,N,CL
36	33	255	$C_{46}H_{56}N_{18}O_8Cl_2$	C,H,N,CL
37	67	280	$C_{46}H_{56}N_{18}O_8Cl_2$	C,H,N,CL
38	53	260	$C_{51}H_{62}N_{18}O_8Cl_3$	C,H,N,CL
39	73	250	$C_{34}H_{50}N_{16}O_6Cl_2$	C,H,N,CL

a. Uncorrected.

b. All compounds gave satisfactory elemental analyses within 0.4% of the calculated values and exhibited ^{1}H-NMR, IR and MS data consistent with the structures.

TABLE II. Relative binding constants for natural and linked oligopeptides $R_1CO(CH_2)_nCO-R_1$ to calf thymus DNA determined by ethidium displacement assay.[a]

Compound	n[b]	DNA Binding Constant (M^{-1})
1	--	1.9×10^7
2	--	0.8×10^7
3	0	5.6×10^7
4	1	3.6×10^7
5	2	7.2×10^7
8	5	1.2×10^7
9	6	2.5×10^7
10	7	0.9×10^7
11	8	1.7×10^7
12	9	1.9×10^7
13	10	2.2×10^7

[a]Based on a binding constant of ethidium of 10^7 M^{-1} under similar conditions of temperature, pH and ionic strength. Binding constant values represent the average of repeat measurements.

[b]Number of CH_2 units in the linker in R_1-$CO(CH_2)_nCO-R_1$.

TABLE III. Relative binding constants for cis and trans bis-netropsins to poly(dA-dT) determined by the ethidium displacement assay.[a]

Compound	K_{app} (M^{-1})
1	9.4×10^7
2	6.3×10^7
19b	4.3×10^7
20b	4.9×10^7
21b	4.9×10^7
22b	3.8×10^7
23b	5.3×10^7
24b	4.4×10^7
25b	5.6×10^7
26b	3.1×10^7
27b	4.0×10^7

[a]Based on a binding constant of ethidium of 9.5×10^6 M^{-1} under similar conditions of temperature, pH and ionic strength. Binding constants represent the average of repeat measurements.

TABLE IV.

Compound	Toxicity, TD_{50} (ug mL^{-1})	Activity, MIC50(ug mL^{-1})	T.I., TD_{50}/ MIC_{50}
29	>100.00	3.98	>25.13
30	>100.00	>50.0	2.0
31	>100.00	79.63	>1.26
32	>100.00	15.93	>6.28
33	>100.00	>100.00	--
34	>100.00	22.74	>4.40
35	83.50	>50.0	1.7
36	100.00	0.16	625.00
37	84.29	11.21	7.52
38	>100.00	22.04	>4.54
39	>100.00	>100.00	--
AZT	>100.00	0.0014	$>7.14 \times 10^5$
DDC	>100.00	0.74	>135.14

TABLE V. Inhibition of Moloney murine leukemia (MLV) associated reverse transciptase activity by linked.

Compound	n[a]	ID_{50}[b] (ug/mL) (average±SD)
4	1	39.0 ± 13.9
4	2	25.2 ± 11.4
8	5	72.5 ± 7.69
9	6	21.3 ± 6.1
10	7	34.2 ± 0.9
11	8	20.3 ± 9.2
12	9	10.3 ± 7.5
13	10	9.1 ± 6.7
23b	--	7.0 ± 3.6
24b	--	30.4 ± 19.3
25b	--	21.8 ± 9.2
26b	--	45.9 ± 11.3
27b	--	29.1 ± 6.0
28b	--	63.8 ± 41.0
Aurintricarboxylic acid		1.42 ± 0.26

[a]Number of CH_2 groups in linker in R_1-$CO(CH_2)_nCO$-R_1.

[b]50% inhibitory dose, measured after 120 min incubation of the reaction mixtures. [MLV: lot 804-845-8A; (^{3}H-methyl)dTTP at 10 μC; (specific activity: 30 Curies/mmol) per 250 μL of reaction mixture.)

TABLE VI

Results of the compound N,N'-di[1-methyl-2-[1-methyl-2-carboxamido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] terephthalamide dihydrochloride.

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	% of Control	% of Control
IC50 (Molar)	$>1.79 \times 10^{-5}$	5.68×10^{-9}	39.64	88.62
EC50 (Molar)	2.08×10^{-6}	1.79×10^{-8}	33.00	92.56
TI50 (IC/EC)	$>8.59 \times 10^{0}$	5.68×10^{-8}	25.70	94.69
		1.79×10^{-7}	29.97	92.72
		5.67×10^{-7}	28.86	88.21
		1.79×10^{-6}	51.73	136.50
		5.66×10^{-6}	134.54	164.46
		1.79×10^{-5}	167.25	192.17

TABLE VII

Results of the compound N,N'-di[1-methyl-2-[1-methyl-2-carboxamido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] isophthalamide dihydrochloride.

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	% of Control	% of Control
IC50 (Molar)	2.84×10^{-4}	4.28×10^{-7}	48.47	116.38
EC50 (Molar)	3.55×10^{-6}	1.35×10^{-6}	29.25	131.95
TI50 (IC/EC)	$8.00 \times 10^{+1}$	4.27×10^{-6}	64.76	123.64
		1.35×10^{-5}	117.45	117.76
		4.26×10^{-5}	120.68	123.27
		1.34×10^{-4}	63.57	142.80
		4.26×10^{-4}	0.02	-0.29
		1.34×10^{-3}	4.26	10.39

TABLE VIII

Results of the compound N,N'-di[1-methyl-2-[1-methyl-2-carboxamido(3-propionamidine)-4-pyrrole]-4-4pyrrolyl] fumaride dihydrochloride.

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	% of Control	% of Control
IC50 (Molar)	$>3.30 \times 10^{-5}$	1.05×10^{-8}	18.00	83.05
EC50 (Molar)	1.67×10^{-6}	3.32×10^{-8}	22.96	87.56
TI50 (IC/EC)	$>1.97 \times 10^{+1}$	1.05×10^{-7}	26.15	90.26
		3.31×10^{-7}	28.00	88.21
		1.04×10^{-6}	38.90	83.87
		3.31×10^{-6}	96.78	151.83
		1.04×10^{-5}	150.24	154.79
		3.30×10^{-5}	132.20	138.63

TABLE IX

Results of the compound N,N'-di[1-methyl-2-[1-methyl-2-carboxamido(3-propionamidine)-4-pyrrole]-4-pyrrolyl]maleamide dihydrochloride.

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	% of Control	% of Control
IC50 (Molar)	1.63×10^{-4}	3.04×10^{-7}	28.75	100.16
EC50 (Molar)	4.04×10^{-6}	9.61×10^{-7}	32.14	100.02
TI50 (IC/EC)	$4.04 \times 10^{+1}$	3.03×10^{-6}	44.03	103.19
		9.59×10^{-6}	115.19	112.20
		3.03×10^{-5}	114.32	112.05
		9.58×10^{-5}	84.02	94.18
		3.02×10^{-4}	-0.29	-0.86
		9.57×10^{-4}	-0.14	5.40

TABLE X

Results of the compound N,N'-di[1-methyl-2-[1-methyl-2-carboxamido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] trans 1,2-cyclobutaneamide dihydrochloride.

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	% of Control	% of Control
IC50 (Molar)	1.67×10^{-4}	2.94×10^{-7}	42.92	90.95
EC50 (Molar)	1.39×10^{-6}	9.29×10^{-7}	59.07	102.41
TI50 (IC/EC)	$1.20 \times 10^{+2}$	2.93×10^{-6}	77.00	116.36
		9.28×10^{-6}	80.26	145.18
		2.93×10^{-5}	110.05	174.62
		9.27×10^{-5}	90.52	101.95
		2.92×10^{-4}	0.04	0.54
		9.25×10^{-4}	9.72	8.29

TABLE XI

The following Table XI shows the results of anti-HIV-1 data on the oligopeptides of the present invention and their anti-HIV-1 activity is designated as inactive, moderate or active. Compounds 19b, 20b, 21b, 25b, 29, 30, 32, 34 and 36 are designated as active.

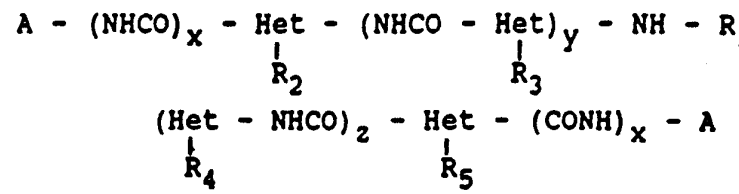
TABLE XI Anti-HIV-1 Activity

Compound	IC_{50} (μM)	EC_{50}	TI_{50}	Activity*
3	83.5	11.9	7.01	Moderate
5	75.3	12	6.3	Moderate
8	64.8	5.3	12.1	Moderate
9	--	--	--	Inactive
10	51.1	2.1	24.1	Moderate
11	--	--	--	Inactive
12	57	3.9	1.46	Moderate
13	78	6.6	11.7	Moderate
15	41	41	1.0	Inactive
16	>100	--	--	Inactive
17	29	14	2	Moderate
18	>120	--	--	Inactive
19b	17.9	1.21	14.8	Active
20b	284	3.55	80	Active
21b	33	1.37	24.1	Active
22b	199	0.35	566	Active
23b	9.3	3.44	2.7	Moderate
24b	257	42.5	6.1	Moderate
25b	68.2	0.42	161	Active
26b	168	46.3	3.6	Moderate
27b	181	5.6	32.4	Moderate
29	4.7	0.39	12	Active
30	140	21	6.6	Active
32	69	1.6	43	Active
33	69	9.8	7.0	Moderate
34	140	13	11	Active
35	71	16	4.5	Moderate
36	207	10.4	19.8	Active
37	35	--	--	Inactive

* National Cancer Institute Designation

IN THE CLAIMS:

1. A method for treating a patient infected with a retrovirus, comprising administering to the patient an antiretroviral effective amount of a compound of the formula:



wherein:

A is a moiety bearing a positive charge and of a size which does not inhibit binding of said compound to nucleic acid sequences associated with the cellular action of retroviruses;

R_1 is a moiety derived from a residue of carbonic acid or a residue of a dicarboxylic acid selected from the group consisting of:

(i) a residue of a dicarboxylic acid of the formula $-CO-C_pH_{2p}-CO$ where p equals 1 to 22;

(ii) a residue of an unsaturated aliphatic dicarboxylic acid of the formula $-CO-C_qH_{2q-2}-CO-$ where q equals 2 to 22;

(iii) a residue of an aromatic dicarboxylic acid;

(iv) a residue of a cycloalkane dicarboxylic acid of the formula $-CO-C_rH_{2r-2}-CO-$ where r equals 3 to 7, optionally fused to one or more three to seven membered C rings; and

(v) a residue of a cycloalkene dicarboxylic acid of the formula $-CO-C_sH_{2s-4}-CO$ where s equals 3 to 7;

Het is a five membered heterocyclic moiety selected from the group consisting of a pyrrole, an imidazole, a triazole, a pyrazole, a thiazole, a thiophene, a furan and an oxazole;

x is 0 or 1;

y is 0, 1, 2 or 3;

z is 0, 1, 2 or 3;

R_2, R_3, R_4 and R_5 are attached to a ring atom other than carbon and are independently selected from the group consisting of C_1-C_6 alkyl and $-CH_2-O-R_6$, where R_6 is a C_1-C_6 alkyl;

and salts thereof.

2. The method of claim 1, wherein A is a moiety selected from the group consisting of an amidine, a guanidine, secondary ammonium salts, tertiary ammonium salts, quaternary ammonium salts, sulfonium salts and phosphonium salts.

3. The method of claim 1, wherein R_2, R_3, R_4 and R_5 are each a C_1-C_6 alkyl.

4. The method of claim 1, wherein R_2, R_3, R_4 and R_5 are the same and are a C_1-C_6 alkyl group.

5. The method of claim 1, wherein R_2, R_3, R_4 and R_5 are each a methoxymethyl.

6. The method of claim 1, wherein R_1 is

7. The method of claim 1, wherein R_1 is a residue of a dicarboxylic acid of the formula $-CO-C_pH_{2p}-CO-$ where p equals 1 to 22.

8. The method of claim 1, wherein R_1 is a residue of a dicarboxylic acid selected from the group consisting of: a residue of an unsaturated aliphatic dicarboxylic acid of the formula $-CO-C_q-H_{2q-2}-CO-$ where q equals 2; a residue of an aromatic dicarboxylic acid; and a residue of a cycloalkane dicarboxylic acid of the formula $-CO-C_r-H_{2r-2}-CO-$ where r equals 3 to 6.

9. The method of claim 1, wherein the compound is N,N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] terephthalamide dihydrochloride.

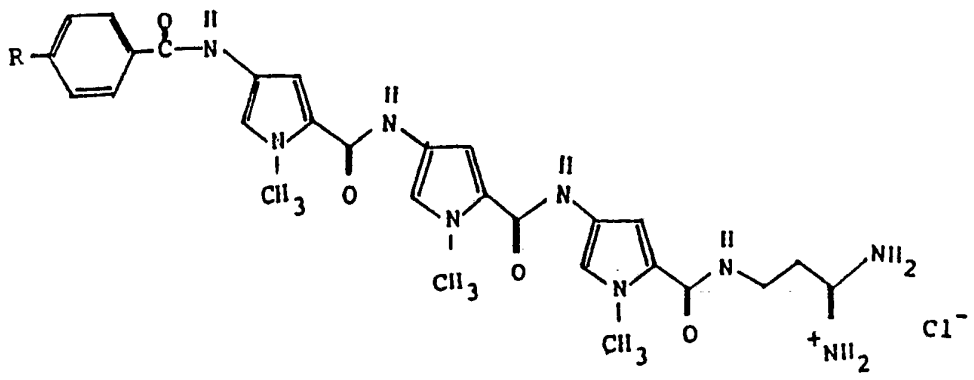
10. The method of claim 1, wherein the compound is N,N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] isophthalamide dihydrochloride.

11. The method of claim 1, wherein the compound is N,N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] fumaramide dihydrochloride.

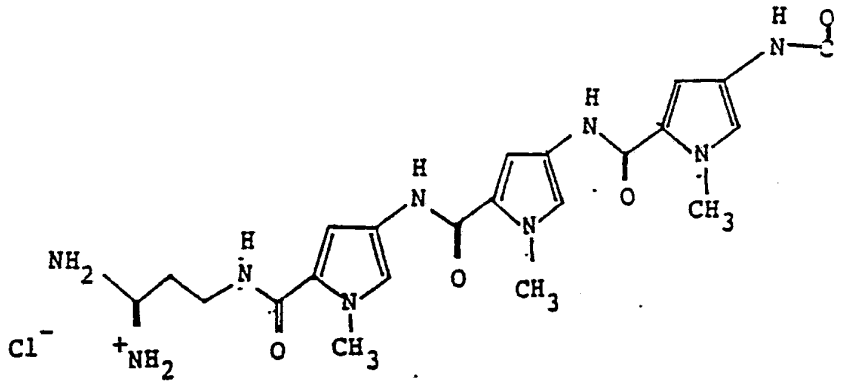
12. The method of claim 1, wherein the compound is N,N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] maleamide dihydrochloride.

13. The method of claim 1, wherein the compound is N,N'-di[1-methyl-2-[1-methyl-2-carboximido(3-propionamidine)-4-pyrrole]-4-pyrrolyl] trans 1,2-cyclobutanamide dihydrochloride.

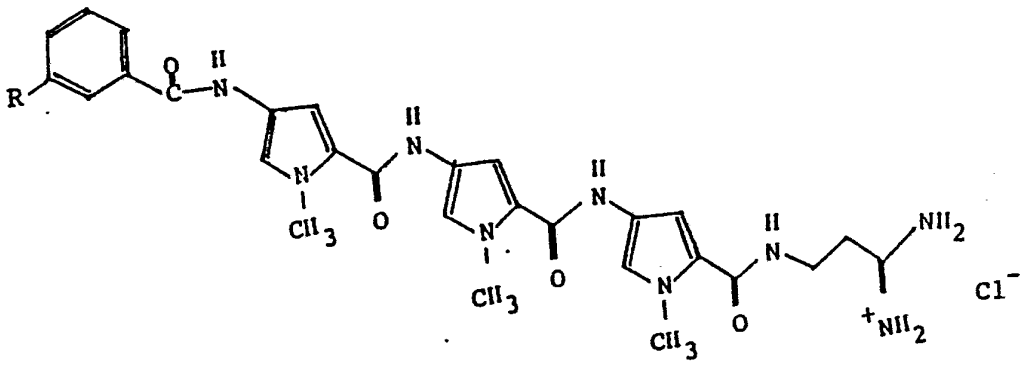
14. The method of claim 1, wherein the compound is:



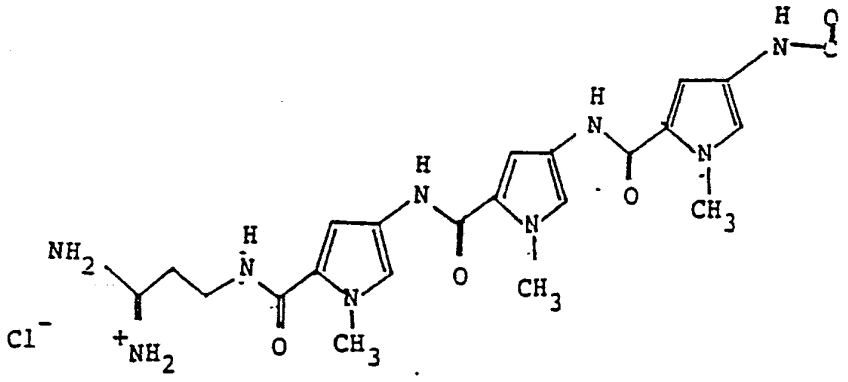
and R is



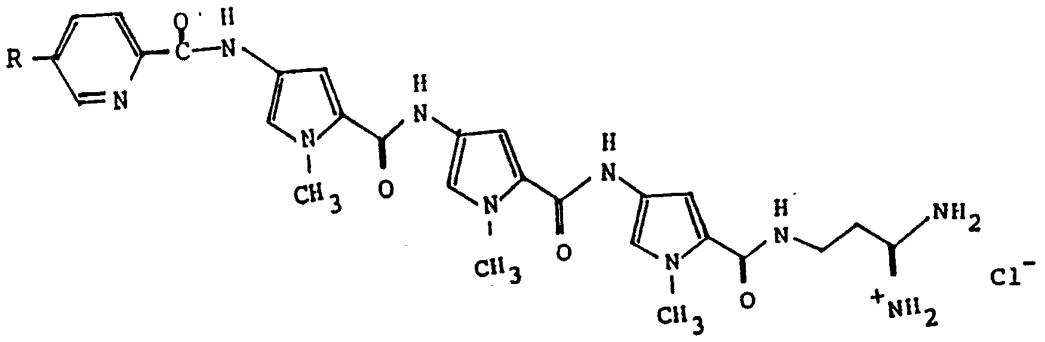
15. The method of claim 1, wherein the compound is:



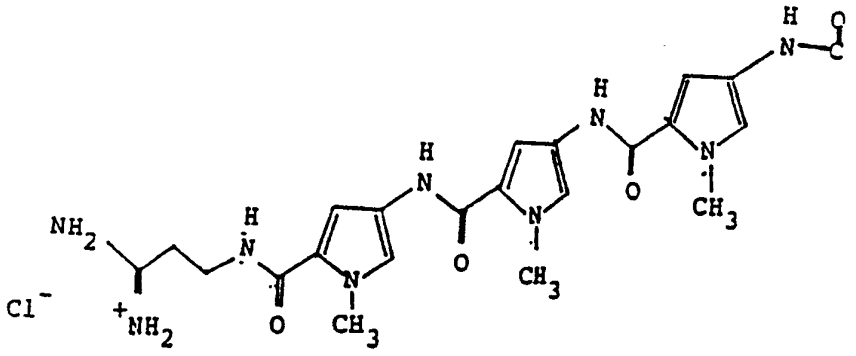
and R is



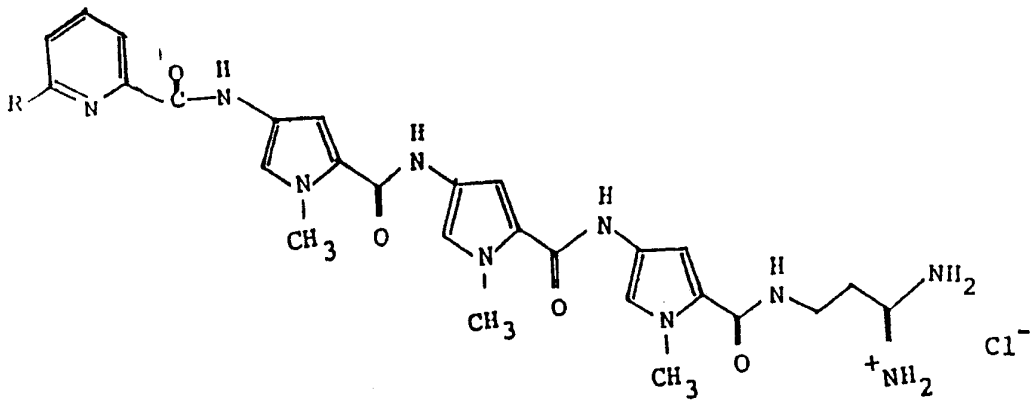
16. The method of claim 1, wherein the compound is:



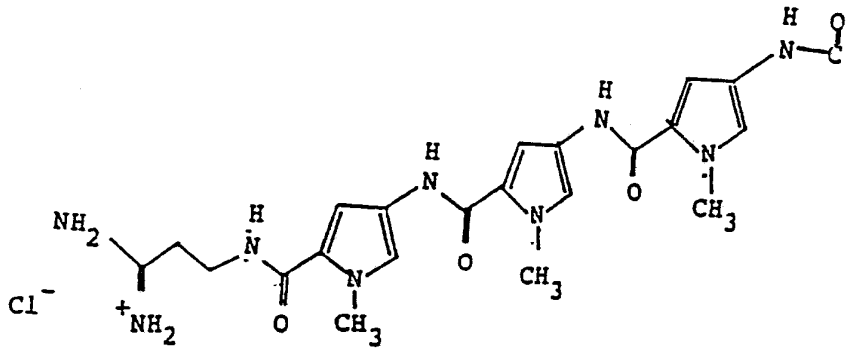
and R is



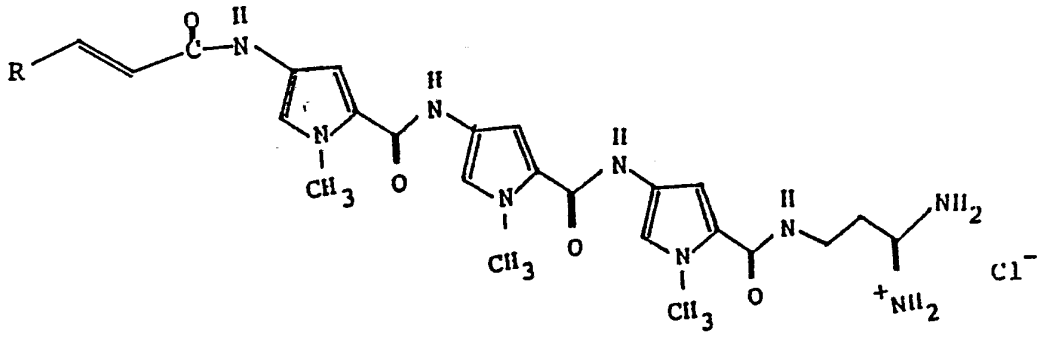
17. The method of claim 1, wherein the compound is:



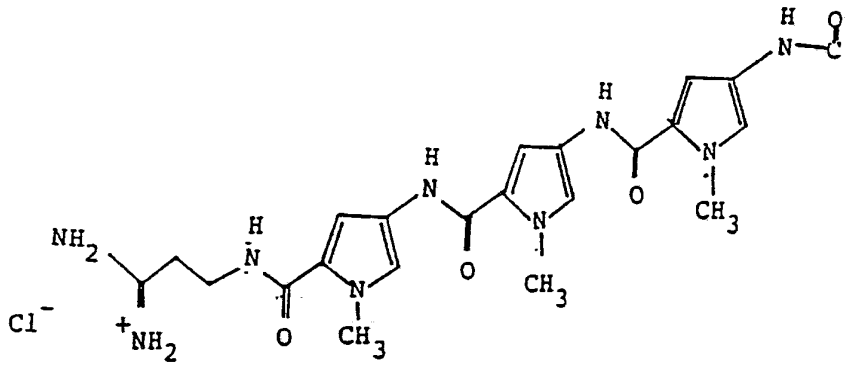
and R is



18. The method of claim 1, wherein the compound is:



and R is



19. The method of claim 1, wherein said retrovirus is Human Immunodeficiency Virus.

20. The method of claim 1, wherein the antiretroviral effective dose is in a range of 1 to 200 mg/kg body weight per day.

21. The method of claim 1, wherein the compound is administered intravenously or orally.

22. A compound exhibiting activity against retroviruses, represented by the formula:

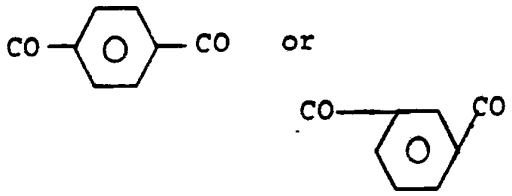
$$A - (NHCO)_x - \underset{R_2}{Het} - (NHCO - \underset{R_3}{Het})_y - NH - R_1 - NH -$$

$$(\underset{R_4}{Het} - NHCO)_x - \underset{R_5}{Het} - (CONH)_x - A$$

wherein R_1 is a moiety derived from a residue of a dicarboxylic acid selected from the group consisting of: a residue of a C_6 aromatic dicarboxylic acid; a residue of

an unsaturated aliphatic dicarboxylic acid of the formula $CO-C_q-H_{2q-2}-CO-$ where q equals 2; a residue of a cycloalkane dicarboxylic acid of the formula $CO-C_r-H_{2r-2}-CO$ where r equals 3 to 6 optionally fused to one or more three to seven C membered rings, and A, R_2, R_3, R_4, R_5 and x, y and z are as defined in claim 1; and salts thereof.

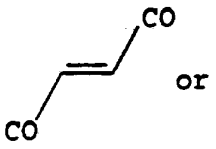
23. The compound of claim 22, wherein R_1 is



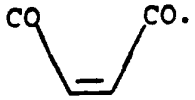
or

.

24. The compound of claim 22, wherein R_1 is



or



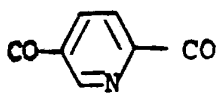
.

25. The compound of claim 22, wherein R_1 is a dicarboxylic acid residue of cyclopropane.

26. The compound of claim 22, wherein R_1 is a dicarboxylic acid residue of cyclopentane.

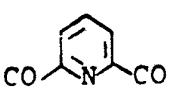
27. The compound of claim 22, wherein R_1 is a dicarboxylic acid residue of cyclohexane.

28. The compound of claim 22, wherein R_1 is



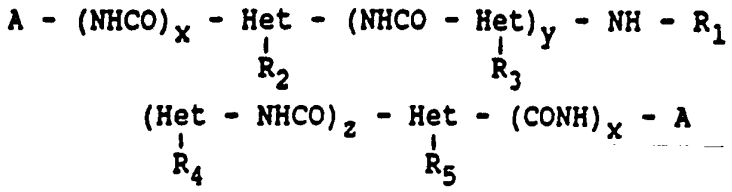
.

29. The compound of claim 22, wherein R_1 is



.

30. A pharmaceutical composition suitable for the treatment of retroviral infections, comprising a compound of the formula:



wherein:

A is a moiety bearing a positive charge and of a size which does not inhibit binding of said compound to nucleic acid sequences associated with the cellular action of retroviruses;

R_1 is a moiety derived from a residue of carbonic acid or a residue of a dicarboxylic acid selected from the group consisting of:

(i) a residue of a dicarboxylic acid of the formula $-CO-C_p-H_{2p}-CO$ where p equals 1 to 16;

(ii) a residue of an unsaturated aliphatic dicarboxylic acid of the formula $-CO-C_q-H_{2q-2}-CO-$ where q equals 2 to 16;

(iii) a residue of an aromatic dicarboxylic acid;

(iv) a residue of a cycloalkane dicarboxylic acid of the formula $-CO-C_r-H_{2r-2}-CO-$ where r equals 3 to 7 optionally fused to one or more three to six C membered rings; and

(v) a residue of a cycloalkene dicarboxylic acid of the formula $-CO-C_s-H_{2s-4}-CO$ where s equals 3 to 7;

Het is a five membered heterocyclic moiety selected from the group consisting of a pyrrole, an imidazole, a triazole, a pyrazole, a thiazole, a thiophene, a furan and an oxazole;

x is 0 or 1;

y is 0, 1, 2 or 3;

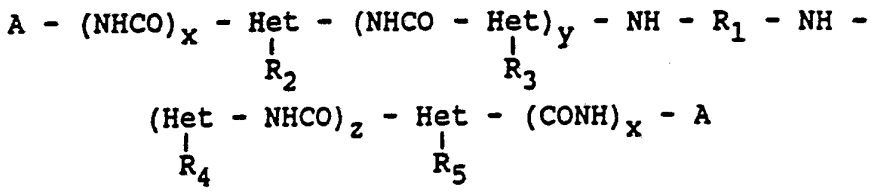
z is 0, 1, 2 or 3;

R_2, R_3, R_4 and R_5 are attached to a ring atom other than carbon and are independently selected from the group

consisting of C_1-C_6 alkyl and $-CH_2-O-R_6$ is a C_1-C_6 alkyl;

and salts thereof, in a pharmaceutically acceptable carrier.

31. A process for the preparation of a compound of the formula:



wherein:

A is a moiety bearing a positive charge and of a size which does not inhibit binding of said compound to nucleic acid sequences associated with the cellular action of retroviruses;

R_1 is a moiety derived from a residue of carbonic acid or a residue of a dicarboxylic acid selected from the group consisting of:

(i) a residue of a dicarboxylic acid of the formula $-CO-C_p-H_{2p}-CO$ where p equals 1 to 16;

(ii) a residue of an unsaturated aliphatic dicarboxylic acid of the formula $-CO-C_q-H_{2q-2}-CO-$ where q equals 2 to 16;

(iii) a residue of an aromatic dicarboxylic acid;

(iv) a residue of a cycloalkane dicarboxylic acid of the formula $-CO-C_r-H_{2r-2}-CO-$ where r equals 3 to 7 optionally fused to a three to seven C membered ring; and

(v) a residue of a cycloalkene dicarboxylic acid of the formula $-CO-C_s-H_{2s-4}-CO$ where s equals 3 to 7;

Het is a five membered heterocyclic moiety selected from the group consisting of a pyrrole, an imidazole, a triazole, a pyrazole, a thiazole, a thiophene, a furan and an oxazole;

x is 0 or 1;

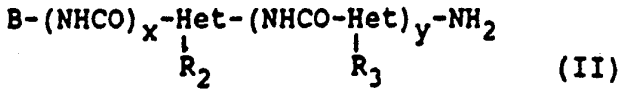
y is 0, 1, 2 or 3;

z is 0, 1, 2 or 3;

R_2, R_3, R_4 and R_5 are attached to a ring atom other than carbon and are independently selected from the group consisting of C_1-C_6 alkyl and $-CH_2-O-R_6$ is a C_1-C_6 alkyl;

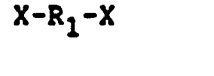
and salts thereof, comprising the steps of:

reacting a compound of the formula (II)



(II)

with a dicarboxylic acid of the formula (III)



(III)

and converting B to A to form said moiety bearing a positive charge,

wherein;

x, y and R_1 are as defined above;

B is the same as A or is a group with a nitrile, halogen or sulfide substituent; and

X is a halogen, imidazolide or other reactive moiety.

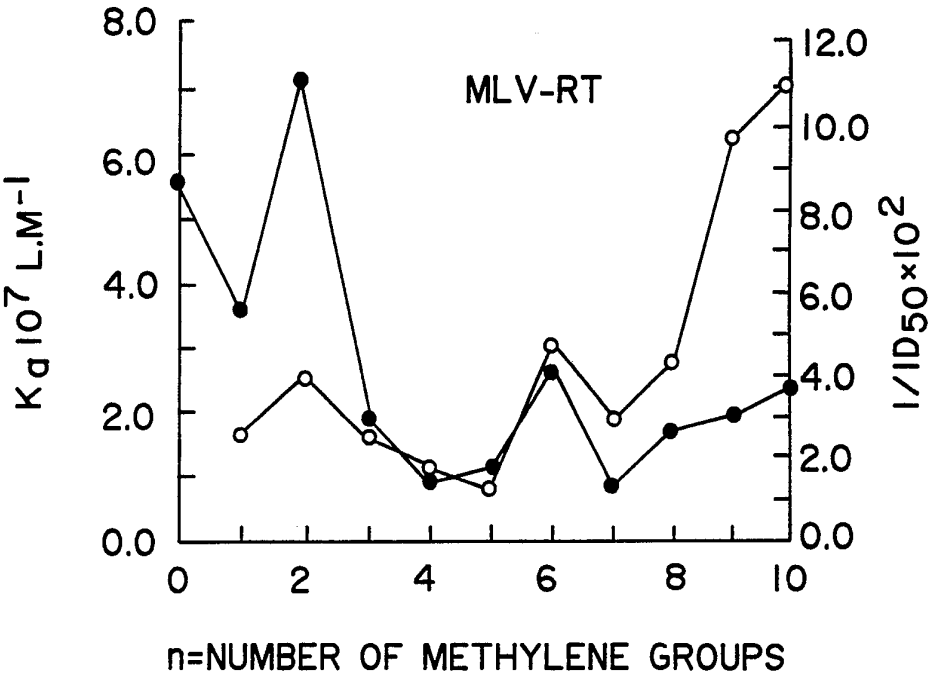

MLV-RT
Ka 10^7 L.M^-1
1/ID50x10^2
8.0
6.0
4.0
2.0
0.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
2
4
6
8
10
n=NUMBER OF METHYLENE GROUPS

Fig.1

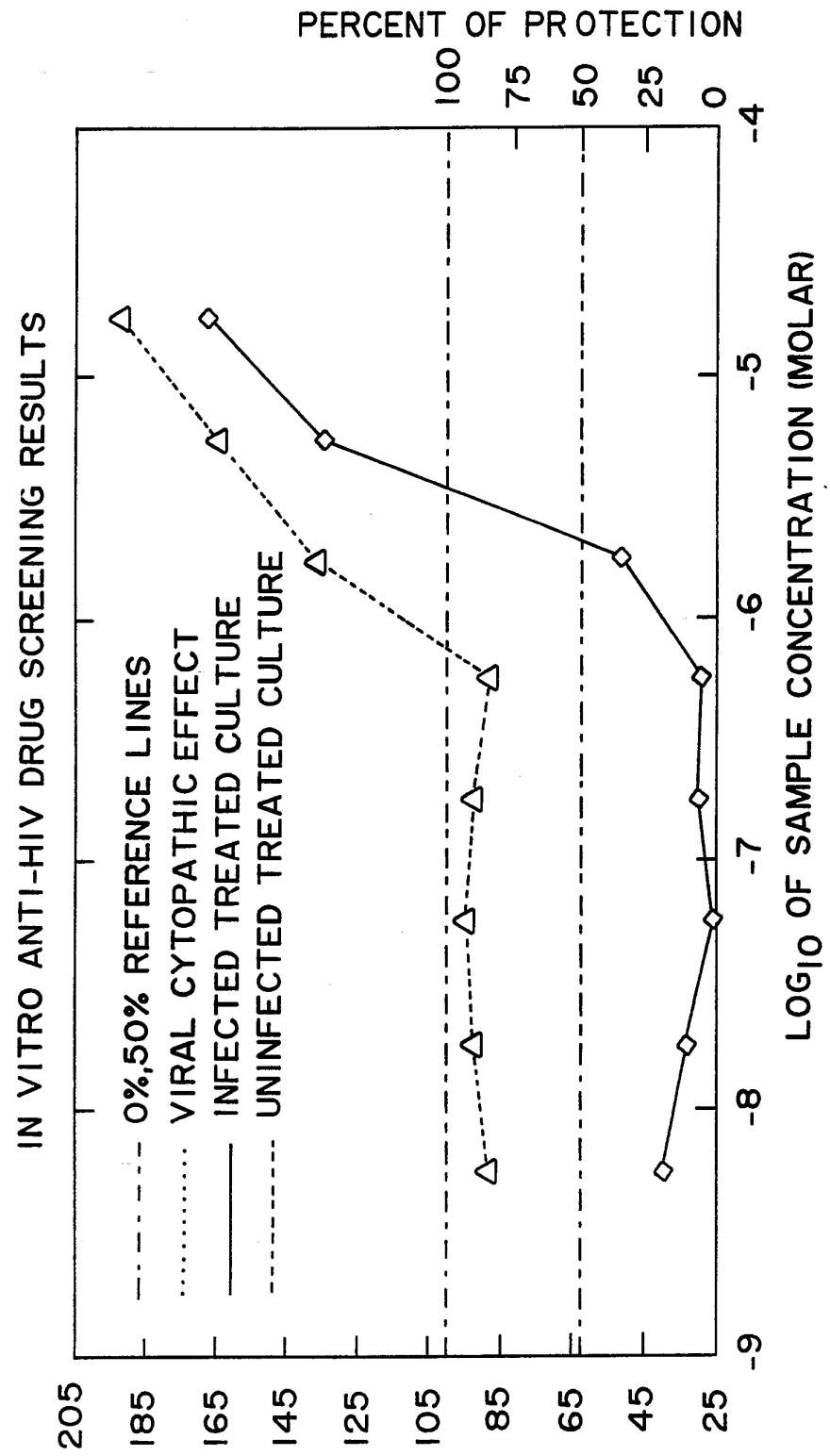

PERCENT OF PROTECTION
100
75
50
25
0
-4
-5
-6
-7
-8
-9
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
IN VITRO ANTI-HIV DRUG SCREENING RESULTS
0%,50% REFERENCE LINES
VIRAL CYTOPATHIC EFFECT
INFECTED TREATED CULTURE
UNINFECTED TREATED CULTURE
205
185
165
145
125
105
85
65
45
25
PERCENT OF UNINFECTED UNTREATED CONTROL CULTURE

Fig.2

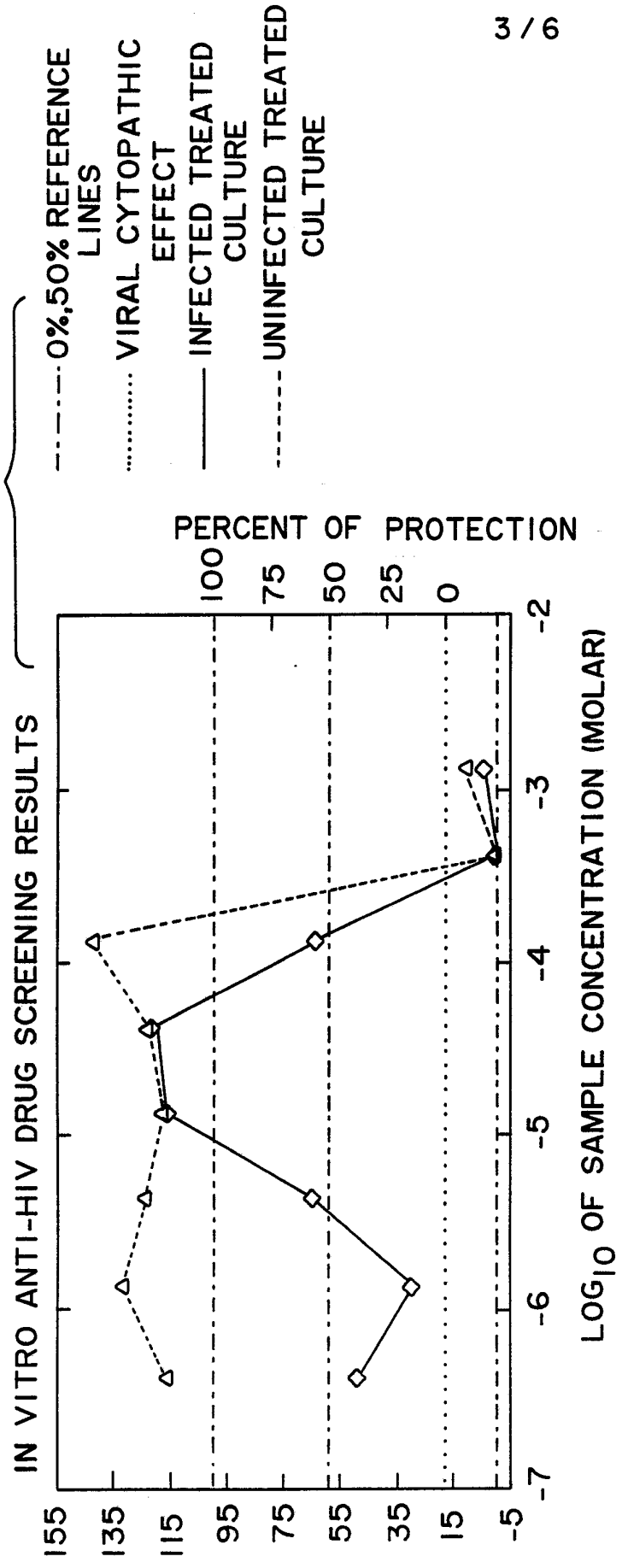

0%,50% REFERENCE LINES
VIRAL CYTOPATHIC EFFECT
INFECTED TREATED CULTURE
UNINFECTED TREATED CULTURE
PERCENT OF PROTECTION
100
75
50
25
0
IN VITRO ANTI-HIV DRUG SCREENING RESULTS
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
-2
-3
-4
-5
-6
-7
155
135
115
95
75
55
35
15
-5
PERCENT OF UNINFECTED UNTREATED CONTROL CULTURE

Fig.3

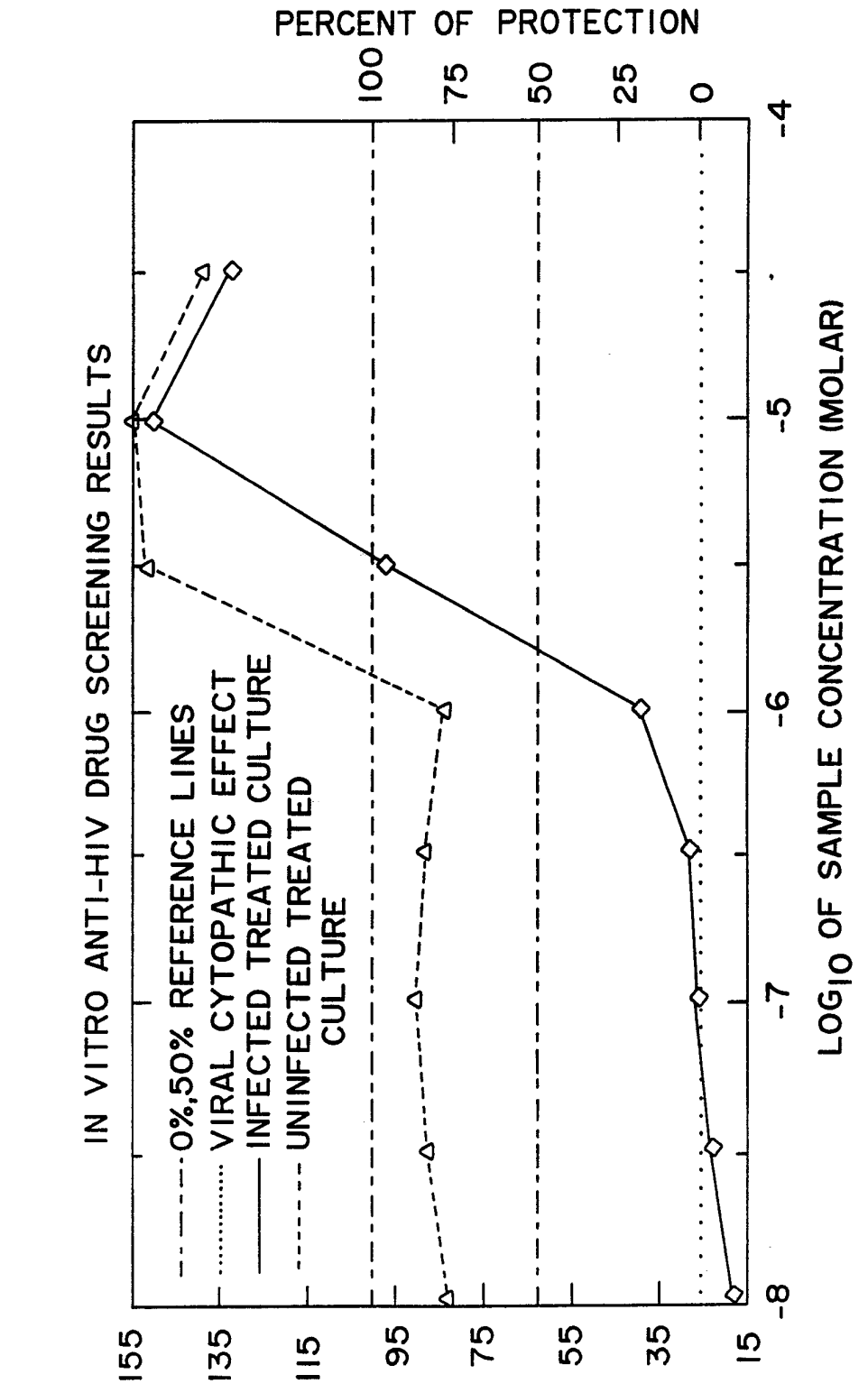

PERCENT OF PROTECTION
100
75
50
25
0
-4
-5
-6
-7
-8
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
IN VITRO ANTI-HIV DRUG SCREENING RESULTS
0%,50% REFERENCE LINES
VIRAL CYTOPATHIC EFFECT
INFECTED TREATED CULTURE
UNINFECTED TREATED CULTURE
155
135
115
95
75
55
35
15
PERCENT OF UNINFECTED UNTREATED CONTROL CULTURE

Fig.4

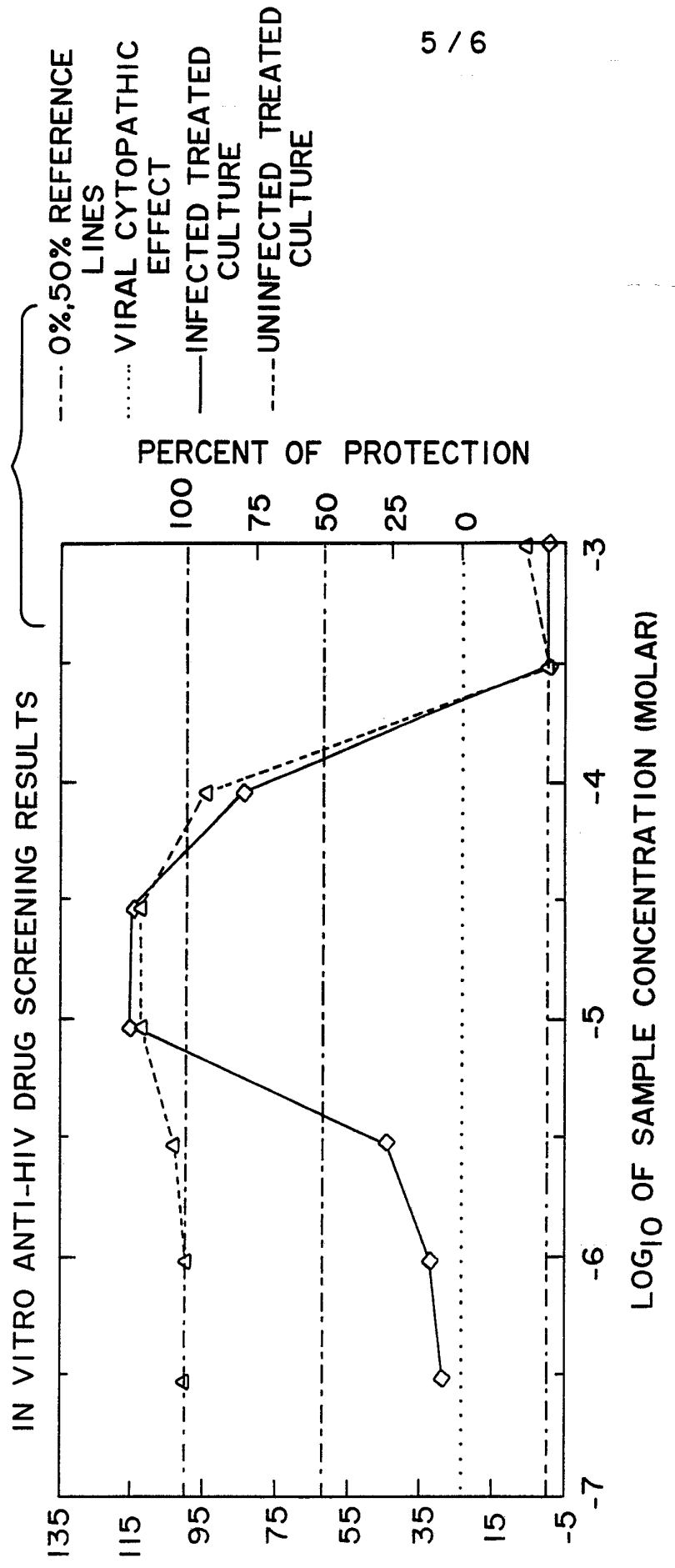

0%,50% REFERENCE LINES
VIRAL CYTOPATHIC EFFECT
INFECTED TREATED CULTURE
UNINFECTED TREATED CULTURE
PERCENT OF PROTECTION
100
75
50
25
0
IN VITRO ANTI-HIV DRUG SCREENING RESULTS
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
-3
-4
-5
-6
-7
135
115
95
75
55
35
15
-5
PERCENT OF UNINFECTED UNTREATED CONTROL CULTURE

Fig.5

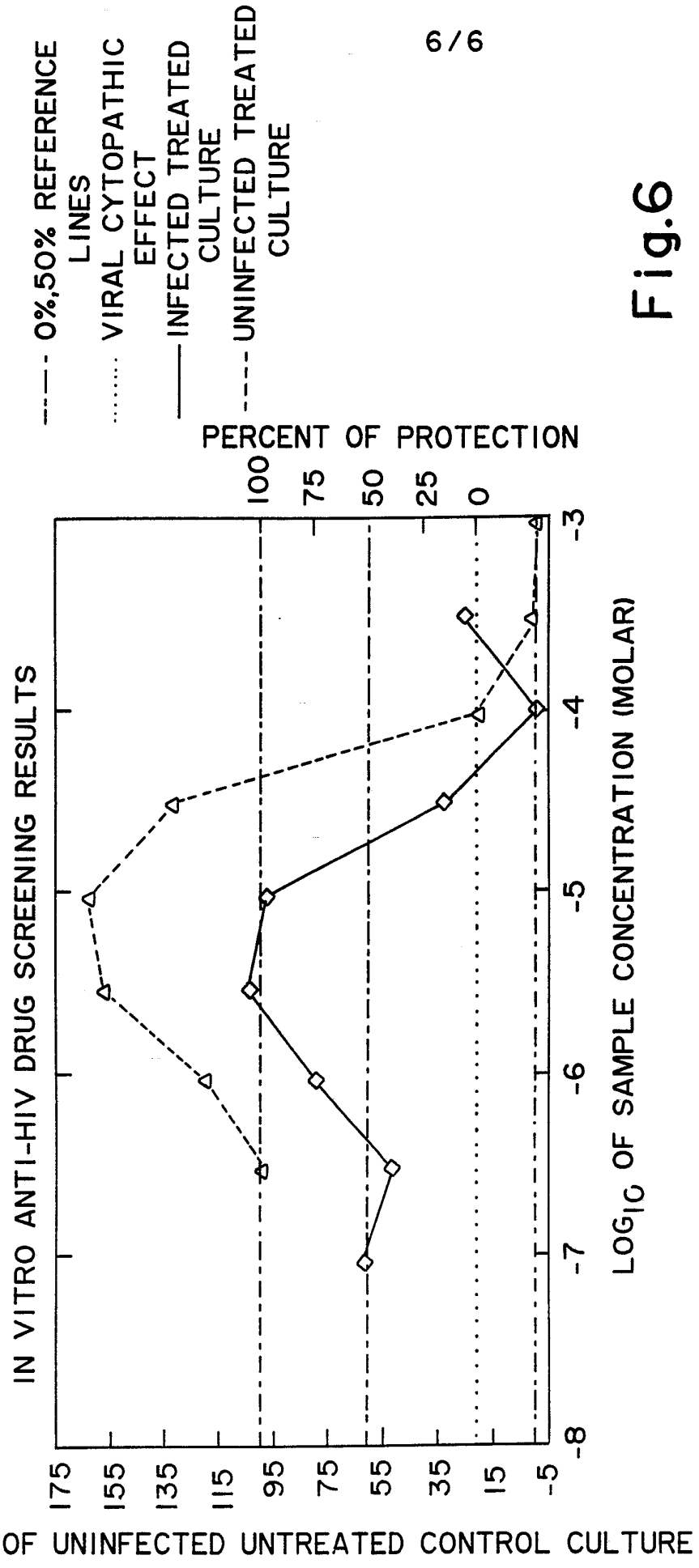

---- 0%,50% REFERENCE LINES
..... VIRAL CYTOPATHIC EFFECT
—— INFECTED TREATED CULTURE
---- UNINFECTED TREATED CULTURE
PERCENT OF PROTECTION
100
75
50
25
0
IN VITRO ANTI-HIV DRUG SCREENING RESULTS
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
-3
-4
-5
-6
-7
-8
175
155
135
115
95
75
55
35
15
-5
PERCENT OF UNINFECTED UNTREATED CONTROL CULTURE

Fig.6

INTERNATIONAL SEARCH REPORT

International Application No PCT/CA 92/00051

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 D 207/34 C 07 D 401/12 C 07 D 233/90
A 61 K 31/40 A 61 K 31/415

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 D 207/00 C 07 D 233/00 C 07 D 401/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	Chemical Abstracts, vol. 98, 1983, (Columbus, Ohio, US), A.A. KHORLIN et al.: "DNA base pair sequence specific ligands. VIII. Synthesis of AT-specific ligands built of two netropsin-like molecules linked symmetrically by residues of different aliphatic dicarboxylic acids", see page 686, abstract no. 72699f, & BIOORG. KHIM. 1982, 8(10), 1358-64, see entire abstract ---	1-31
A	Chemical Abstracts, vol. 93, 1980, (Columbus, Ohio, US), A.A. KHORLIN et al.: "A new type of AT-specific ligand constructed of two netropsin-like molecules", see page 224, abstract no. 199447z, & FEBS LETT. 1980, 118(2), 311-14, see entire abstract --- -/-	1-31

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27-03-1992	27 APR 1992
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: MISS I. TAZELAAR

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	US,A,4665184 (P.B. DERVAN et al.) 12 May 1987, see RN 116845-82-4: Heptanediamide, N,N'-bis[5-[[[5-[[[5-[[[3-(dimethylamino)propyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-	1-31
X	J. Med. Chem., vol. 32, 1989, American Chemical Society, J.L. LOWN et al.: "Novel linked antiviral and antitumor agents related to netropsin and distamycin: Synthesis and biological evaluation", pages 2368-2375, see entire publication	1-31
X	US,A,4912199 (LOWN et al.) 27 March 1990, see entire document (cited in the application)	1-31
A	WO,A,9110649 (CARLO ERBA) 25 July 1991, see entire document	1-31
A	J. Med. Chem., vol. 31, no. 2, February 1988, American Chemical Society, K. KROWICKI et al.: "Novel DNA groove binding alkylators: Design, synthesis, and biological evaluation", pages 341-345, see entire publication (cited in the application)	1-31
A	GB,A,2178037 (CARLO ERBA) 4 February 1987, see entire document	1-31
A	GB,A,2178036 (CARLO ERBA) 4 February 1987, see entire document	1-31
P,X	J. Org. Chem., vol. 56, no. 2, 1991, American Chemical Society, K.W. RAO et al.: "Sequence-selective DNA binding by linked Bis-N-methylpyrrole dipeptides: An analysis by MPE footprinting and force field calculations", pages 786-797, see entire publication	1-31
X	Chemical Abstracts, vol. 116, 1992, (Columbus, Ohio, US), K.E. RAO et al.: "Novel linked antiviral and antitumor agents related to netropsin - 2: synthesis and biological evaluation", see page 844, abstract no. 42027h, & ACTUAL. CHIM. THER. 1991, 18 (RENCONTRES INT. CHIM. THER., 26TH, 1990), 21-42, see entire abstract	1-31

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 14 is directed to a method of treatment of (diagnostic method practised on) the human /animal body the search has been carried out and based on the alleged effects of the compound/composition."

2. [X] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

see next page

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Lack of conciseness

The definition of the following substituent(s) is too general and/or encompasses too broad a range of totally different chemical groups, only partly supported by examples given in the descriptive part of the application:

A:"a moiety bearing a positive charge and of a size which does not inhibit binding of said compound to nucleic acid sequences associated with the cellular action of retroviruses".

x.y,z

Het: a five membered heterocyclic ring

The vast number of theoretically conceivable compounds resulting from the combination of all claimed sustituents of above list precludes a comprehensive search. Guided by the spirit of the application and the inventive concept as disclosed in the descriptive part of the present application the search has been limited to the following case(s):

A: amidines, guanidines, ammonium salts

x,y,z not equal 0

Het: pyrrole, imidazole

Obscurity:

Formula I is unclear: (CONH)x and attachment of amido/carboxamido groups to Het

Searched: 4-carboxamido-Het-2-carboxamides(4-CO-NH-Het-2-CO-NH-Het)

(Cf. Arts. 6, 15 and Rule 33 PCT, guidelines Exam. Part B, Chapt III, 3.6, 3.7)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. CA 9200051 SA 56216

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 21/04/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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