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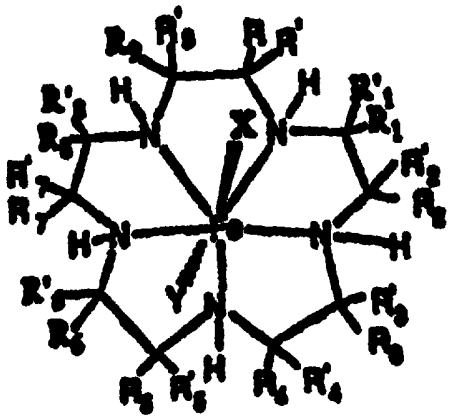
(57) Abstract

Pharmaceutical compositions of low molecular weight mimics of superoxide dismutase (SOD) represented by formula (I), wherein R, R', R_1, R'_1, R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8, R'_8, R_9, and R'_9, and X, Y, Z and n are as defined herein, useful as therapeutic agents for inflammatory disease states and disorders, ischemic/reperfusion injury, stroke, atherosclerosis, inflammatory bowel disease and all other conditions of oxidant-induced tissue damage or injury.



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IRON COMPLEXES OF NITROGEN-CONTAINING MACROCYCLIC LIGANDS EFFECTIVE AS CATALYSTS FOR DISMUTATING SUPEROXIDE

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of pending application Serial No. 08/397,469, filed March 1, 1995, which is a continuation of pending application Serial No. 08/231,599, filed April 22, 1994.

BACKGROUND OF THE INVENTION

The present invention relates to compounds effective as catalysts for dismutating superoxide and, more particularly, relates to iron(II) or iron(III) complexes of nitrogen-containing fifteen-membered macrocyclic ligands which catalytically dismutate superoxide. Application Serial No. 08/397,469 is hereby incorporated by reference herein in its entirety.

The enzyme superoxide dismutase catalyzes the conversion of superoxide into oxygen and hydrogen peroxide according to equation (1) (hereinafter referred to as dismutation). Reactive oxygen metabolites derived from superoxide are postulated to contribute to the tissue pathology in a number of

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H+ \rightarrow O_2 + H_2O_2 \qquad (1)$$

inflammatory diseases and disorders, such as reperfusion injury to the ischemic myocardium, inflammatory bowel disease, rheumatoid arthritis, osteoarthritis, atherosclerosis, hypertension, metastasis, psoriasis, organ transplant rejections, radiation-induced injury, asthma, influenza, stroke, burns and trauma. See, for example, Bulkley, G.B., Reactive oxygen metabolites and reperfusion injury: aberrant triggering of

reticuloendothelial function, *The Lancet*, Vol. 344, pp. 934-36, October 1, 1994; Grisham, M.B., Oxidants and free radicals in inflammatory bowel disease, *The Lancet*, Vol. 344, pp. 859-861, September 24, 1994; Cross, C.E. et al., Reactive oxygen species and the lung, *The Lancet*, Vol. 344, pp. 930-33, October 1, 1994; Jenner, P., Oxidative damage in neurodegenerative disease, *The Lancet*, Vol. 344, pp. 796-798, September 17, 1994; Cerutti, P.A., Oxy-radicals and cancer, *The Lancet*, Vol. 344, pp. 862-863, September 24, 1994 Simic, M. G., et al, Oxygen Radicals in Biology and Medicine, Basic Life Sciences, Vol. 49, Plenum Press, New York and London, 1988; Weiss J. Cell. Biochem., 1991 Suppl. 15C, 216 Abstract C110 (1991); Petkau, A., Cancer Treat. Rev. 13, 17 (1986); McCord, J. Free Radicals Biol. Med., 2, 307 (1986); and Bannister, J.V. et al, Crit. Rev. Biochem., 22, 111 (1987). The above-identified references from *The Lancet* teach the nexus between free radicals derived from superoxide and a variety of diseases. In particular, the Bulkley and Grisham references specifically teach that there is a nexus between the dismutation of superoxide and the final disease treatment.

It is also known that superoxide is involved in the breakdown of endothelium-derived vascular relaxing factor (EDRF), which has been identified as nitric oxide (NO), and that EDRF is protected from breakdown by superoxide dismutase. This suggests a central role for activated oxygen species derived from superoxide in the pathogenesis of vasospasm, thrombosis and atherosclerosis. See, for example, Gryglewski, R.J. et al., "Superoxide Anion is Involved in the Breakdown of Endothelium-derived Vascular Relaxing Factor", *Nature*, Vol. 320, pp. 454-56 (1986) and Palmer, R.M.J. et al., "Nitric Oxide Release Accounts for the Biological Activity of Endothelium Derived Relaxing Factor",

Nature, Vol. 327, pp. 523-26 (1987).

Clinical trials and animal studies with natural, recombinant and modified superoxide dismutase enzymes have been completed or are ongoing to demonstrate the therapeutic efficacy of reducing superoxide levels in the disease states noted above. However, numerous problems have arisen with the use of the enzymes as potential therapeutic agents, including lack of oral activity, short half-lives *in vivo*, immunogenicity with nonhuman derived enzymes, and poor tissue distribution.

The iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands that are low molecular weight mimics of superoxide dismutase (SOD) are useful as therapeutic agents and avoid many of the problems associated with SOD enzymes.

SUMMARY OF THE INVENTION

It is an object of the invention to provide iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands that are low molecular weight mimics of superoxide dismutase (SOD) which are useful as therapeutic agents for inflammatory disease states or disorders which are medicated, at least in part, by superoxide. It is a further object of the invention to provide iron complexes of nitrogen-containing fifteen-membered macro-cyclic ligands which are useful as magnetic resonance imaging (MRI) contrast agents. It is yet a further object of the invention to provide iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands that have unexpectedly improved stability compared to corresponding manganese complexes.

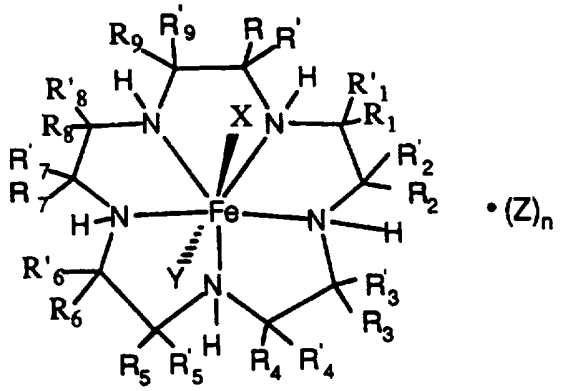
According to the invention, pharmaceutical compositions in unit dosage form useful for dismutating superoxide are provided comprising (a) a therapeutically or prophylactically effective amount of an iron complex

of the invention and (b) a nontoxic, pharmaceutically acceptable carrier, adjuvant or vehicle.

Further according to the invention, a method of preventing or treating a disease or disorder which is medicated, at least in part, by superoxide is provided comprising administering to a subject in need of such prevention or treatment, a therapeutically or prophylactically effective amount of an iron complex of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands which catalyze the conversion of superoxide into oxygen and hydrogen peroxide. These complexes are represented by the formula:



wherein R, R′, R_1, R'_1, R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8, R'_8, R_9, and R'_9 independently are selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cycloalkylcycloalkyl, cycloalkenylalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkenyl, heterocyclic,

aryl and aralkyl radicals and radicals attached to the α-carbon of α-amino acids; or R_1 or R'_1 and R_2 or R'_2, R_3 or R'_3 and R_4 or R'_4, R_5 or R'_5 and R_6 or R'_6, R_7 or R'_7 and R_8 or R'_8, and R_9 or R'_9 and R or R′ together with the carbon atoms to which they are attached independently form a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms; or R or R′ and R_1 or R'_1, R_2 or R'_2 and R_3 or R'_3, R_4 or R'_4 and R_5 or R'_5, R_6 or R'_6 and R_7 or R'_7, and R_8 or R'_8 and R_9 or R'_9 together with the carbon atoms to which they are attached independently form a nitrogen containing heterocycle having 2 to 20 carbon atoms provided that when the nitrogen containing heterocycle is an aromatic heterocycle which does not contain a hydrogen attached to the nitrogen, the hydrogen attached to the nitrogen as shown in the above formula, which nitrogen is also in the macrocyclic ligand or complex, and the R groups attached to the same carbon atoms of the macrocycle are absent.

X, Y and Z represent suitable ligands or charge-neutralizing anions which are derived from any monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof (for example benzoic acid or benzoate anion, phenol or phenoxide anion, alcohol or alkoxide anion). X, Y and Z are independently selected from the group consisting of halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxo, hydroperoxo, alkylperoxo, arylperoxo, ammonia, alkylamino, arylamino, heterocycloalkyl amino, heterocycloaryl amino, amine oxides, hydrazine, alkyl hydrazine, aryl hydrazine, nitric oxide, cyanide, cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl nitrile, aryl nitrile, alkyl isonitrile, aryl isonitrile, nitrate, nitrite, azido, alkyl sulfonic acid, aryl sulfonic acid, alkyl sulfoxide, aryl

sulfoxide, alkyl aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid, aryl thiol thiocarboxylic acid, alkyl carboxylic acid (such as acetic acid, trifluoroacetic acid, oxalic acid), aryl carboxylic acid (such as benzoic acid, phthalic acid), urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea,alkyl aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite, hydrosulfite, alkyl phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide, alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, alkyl phosphinic acid, aryl phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid, phosphate, thiophosphate, phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate, alkyl thiocarbamate aryl thiocarbamate, alkyl aryl thiocarbamate, alkyl dithiocarbamate, aryl dithiocarbamate, alkyl aryl dithiocarbamate, bicarbonate, carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite, tetrahalomanganate, tetrafluoroborate, hexafluorophosphate, hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate, and anions of ion exchange resins, or systems where one or more of X,Y and Z are independently attached to one or more of the "R" groups, wherein n is an integer from 0 or 1. The preferred ligands from which X, Y and Z are selected include halide, organic

acid, nitrate and bicarbonate anions.

As utilized herein, the term "alkyl", alone or in combination, means a straight-chain or branched-chain alkyl radical containing from 1 to about 22 carbon atoms, preferably from about 1 to about 18 carbon atoms, and most preferably from about 1 to about 12 carbon atoms. Examples of such radicals include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl, hexyl, octyl, nonyl, decyl, dodecyl, tetradecyl, hexadecyl, octadecyl and eicosyl. The term "alkenyl", alone or in combination, means an alkyl radical having one or more double bonds. Examples of such alkenyl radicals include, but are not limited to, ethenyl, propenyl, 1-butenyl, cis-2-butenyl, trans-2-butenyl, iso-butylenyl, cis-2-pentenyl, trans-2-pentenyl, 3-methyl-1-butenyl, 2,3-dimethyl-2-butenyl, 1-pentenyl, 1-hexenyl, 1-octenyl, decenyl, dodecenyl, tetradecenyl, hexadecenyl, cis- and trans- 9-octadecenyl, 1,3-pentadienyl, 2,4-pentadienyl, 2,3-pentadienyl, 1,3-hexadienyl, 2,4-hexadienyl, 5,8,11,14-eicosatetraenyl, and 9,12,15-octadecatrienyl. The term "alkynyl", alone or in combination, means an alkyl radical having one or more triple bonds. Examples of such alkynyl groups include, but are not limited to, ethynyl, propynyl (propargyl), 1-butynyl, 1-octynyl, 9-octadecynyl, 1,3-pentadiynyl, 2,4-pentadiynyl, 1,3-hexadiynyl, and 2,4-hexadiynyl. The term "cycloalkyl", alone or in combination means a cycloalkyl radical containing from 3 to about 10, preferably from 3 to about 8, and most preferably from 3 to about 6, carbon atoms. Examples of such cycloalkyl radicals include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and perhydronaphthyl. The term "cycloalkylalkyl" means an alkyl radical as defined

above which is substituted by a cycloalkyl radical as defined above. Examples of cycloalkylalkyl radicals include, but are not limited to, cyclohexylmethyl, cyclopentylmethyl, (4-isopropylcyclohexyl)methyl, (4-t-butyl-cyclohexyl)methyl, 3-cyclohexylpropyl, 2-cyclo-hexylmethylpentyl, 3-cyclopentylmethylhexyl, 1-(4-neopentylcyclohexyl)methylhexyl, and 1-(4-isopropylcyclohexyl)methylheptyl. The term "cycloalkylcycloalkyl" means a cycloalkyl radical as defined above which is substituted by another cycloalkyl radical as defined above. Examples of cycloalkylcycloalkyl radicals include, but are not limited to, cyclohexylcyclopentyl and cyclohexylcyclohexyl. The term "cycloalkenyl", alone or in combination, means a cycloalkyl radical having one or more double bonds. Examples of cycloalkenyl radicals include, but are not limited to, cyclopentenyl, cyclohexenyl, cyclooctenyl, cyclopentadienyl, cyclohexadienyl and cyclooctadienyl. The term "cycloalkenylalkyl" means an alkyl radical as defined above which is substituted by a cycloalkenyl radical as defined above. Examples of cycloalkenylalkyl radicals include, but are not limited to, 2-cyclohexen-1-ylmethyl, 1-cyclopenten-1-ylmethyl, 2-(1-cyclohexen-1-yl)ethyl, 3-(1-cyclopenten-1-yl)propyl, 1-(1-cyclohexen-1-ylmethyl)pentyl, 1-(1-cyclopenten-1-yl)hexyl, 6-(1-cyclohexen-1-yl)hexyl, 1-(1-cyclopenten-1-yl)nonyl and 1-(1-cyclohexen-1-yl)nonyl. The terms "alkylcycloalkyl" and "alkenylcycloalkyl" mean a cycloalkyl radical as defined above which is substituted by an alkyl or alkenyl radical as defined above. Examples of alkylcycloalkyl and alkenylcycloalkyl radicals include, but are not limited to, 2-ethylcyclobutyl, 1-methylcyclopentyl, 1-hexylcyclopentyl, 1-methylcyclohexyl, 1-(9-octadecenyl)cyclopentyl and 1-(9-

octadecenyl)cyclohexyl. The terms "alkylcycloalkenyl" and "alkenylcycloalkenyl" means a cycloalkenyl radical as defined above which is substituted by an alkyl or alkenyl radical as defined above. Examples of alkylcycloalkenyl and alkenylcycloalkenyl radicals include, but are not limited to, 1-methyl-2-cyclopentyl, 1-hexyl-2-cyclopentenyl, 1-ethyl-2-cyclohexenyl, 1-butyl-2-cyclohexenyl, 1-(9-octadecenyl)-2-cyclohexenyl and 1-(2-pentenyl)-2-cyclohexenyl. The term "aryl", alone or in combination, means a phenyl or naphthyl radical which optionally carries one or more substituents selected from alkyl, cycloalkyl, cycloalkenyl, phenyl, naphthyl, heterocycle, alkoxyaryl, alkaryl, alkoxy, halogen, hydroxy, amine, cyano, nitro, alkylthio, phenoxy, ether, trifluoromethyl and the like, such as phenyl, p-tolyl, 4-methoxyphenyl, 4-(tert-butoxy)phenyl, 4-fluorophenyl, 4-chlorophenyl, 4-hydroxyphenyl, 1-naphthyl, 2-naphthyl, and the like. The term "aralkyl", alone or in combination, means an alkyl or cycloalkyl radical as defined above in which one hydrogen atom is replaced by an aryl radical as defined above, such as benzyl, 2-phenylethyl, and the like. The term "heterocyclic" means ring structures containing at least one other kind of atom, in addition to carbon, in the ring. The most common of the other kinds of atoms include nitrogen, oxygen and sulfur. Examples of heterocyclics include, but are not limited to, pyrrolidinyl, piperidyl, imidazolidinyl, tetrahydrofuryl, tetrahydrothienyl, furyl, thienyl, pyridyl, quinolyl, isoquinolyl, pyridazinyl, pyrazinyl, indolyl, imidazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, benzoxadiazolyl, benzothiadiazolyl, triazolyl and tetrazolyl groups. The term "saturated, partially saturated or unsaturated cyclic" means fused ring structures in which 2 carbons of the ring are also part of the fifteen-membered macrocyclic ligand. The ring

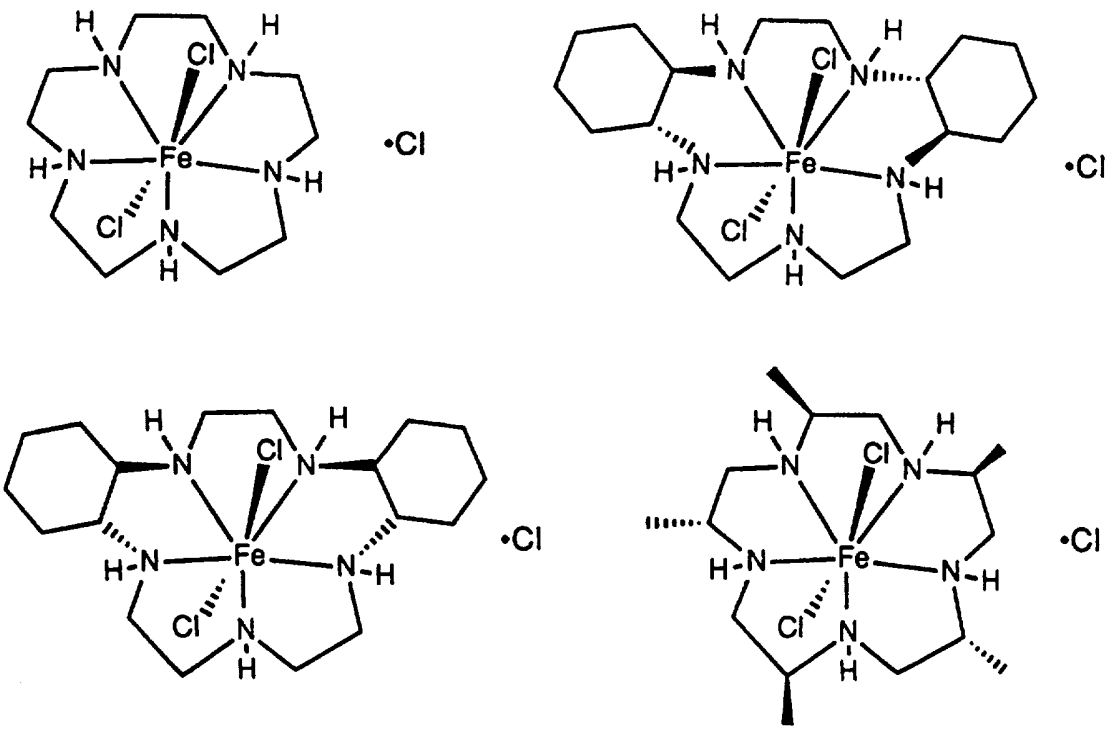
structure can contain 3 to 20 carbon atoms, preferably 5 to 8 carbon atoms, and can also contain one or more other kinds of atoms in addition to carbon. The most common of the other kinds of atoms include nitrogen, oxygen and sulfur. The ring structure can also contain more than one ring. The term "nitrogen containing heterocycle" means ring structures in which 2 carbons and a nitrogen of the ring are also part of the fifteen-membered macrocyclic ligand. The ring structure can contain 2 to 20, preferable 4 to 10 carbon atoms, can be partially or fully unsaturated or saturated and can also contain nitrogen, oxygen and/or sulfur in the portion of the ring which is not also part of the fifteen-membered macrocyclic ligand. The term "organic acid anion" refers to carboxylic acid anions having from about 1 to about 18 carbon atoms. The term "halide" means chloride or bromide.

The overall charge-type of the complex can be varied from negative to positive by carbon substitution of the appropriate charged groups on the macrocyclic framework. By considering the dispositive nature of the iron metal center, the overall charge on the complex can be adjusted as needed to enhance desired pharmaceutical properties such as osmolality, tissue distribution and non-target clearance. For example, if the complex carries only charge neutral functionality, such as C-alkyl substitution, then the overall charge on the complex will be determined by the iron center and will be positive. Multi-positive complexes are available via the incorporation of pendant cations such as protonated aminoalkyl groups. These types of complexes can bind to endogenous anions, anionic proteins, cell membranes, and the like. If pendant anionic groups are attached, such as carboxylates, phenolate, phosphonates, sulfonates and the like, the overall charge on the complex can be envisioned as zero or positive, i.e. an anionic complex

will result. The pendant groups may be designed to axially chelate and formally displace the axial anions or they may be designed specifically to not chelate but retain a charge type.

Currently, preferred compounds are those wherein at least one, preferably at least two, of the "R" groups represent alkyl, or alkyl substituted with $-OR_{10}$ or $-NR_{10}R_{11}$ wherein R_{10} and R_{11} are independently hydrogen or alkyl, and the remaining R groups represent hydrogen, a saturated, partially saturated or unsaturated cyclic, or a nitrogen containing heterocycle, more preferably hydrogen or a saturated, partially saturated or unsaturated cyclic; those wherein at least one, preferably at least two, of R_1 or R'_1 and R_2 or R'_2, R_3 or R'_3 and R_4 or R'_4, R_5 or R'_5 and R_6 or R'_6, R_7 or R'_7 and R_8 or R'_8, and R_9 or R'_9 and R or R' together with the carbon atoms to which they are attached represent a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms and all the remaining "R" groups are hydrogen, nitrogen containing heterocycle, alkyl or alkyl substituted with $-OR_{10}$ or $-NR_{10}R_{11}$ groups, more preferably hydrogen, alkyl or alkyl substituted with $-OR_{10}$ or $-NR_{10}R_{11}$ groups; and those wherein at least one, preferably at least two, of R or R' and R_1 or R'_1, R_2 or R'_2 and R_3 or R'_3, R_4 or R'_4 and R_5 or R'_5, R_6 or R'_6 and R_7 or R'_7, and R_8 or R'_8 and R_9 or R'_9 together with the carbon atoms to which they are attached are bound to form a nitrogen containing heterocycle having 2 to 20 carbon atoms and all the remaining "R" groups are independently selected from hydrogen, saturated, partially saturated or unsaturated cyclic, alkyl or alkyl substituted with $-OR_{10}$ or $-NR_{10}R_{11}$ groups. As used herein, "R" groups means all of the R groups attached to the carbon atoms of the macrocycle, i.e., R, R', R_1, R'_1, R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6,

R'_6, R_7, R'_7, R_8, R'_8, R_9 and R'_9. Examples of complexes of the invention include, but are not limited to, compounds having the formulas:



The macrocyclic ligand wherein all R's are H can be prepared according to the general synthetic scheme A set forth below utilizing methods known in the art for preparation of certain intermediates and certain ligands. See, for example, Richman et al., *J. Am. Chem. Soc.*, 96, 2268 (1974); Atkins et al. *Org. Synth.*, 58, 86 (1978); and EP 287 465. Thus a triazaalkane is tosylated in a suitable solvent system to produce the corresponding tris(N-tosyl) derivative. Such derivative is then treated with a suitable base to produce the corresponding disulfonamide anion. The disulfonamide anion is then reacted with a di-O-tosylated di-N-tosylated diazaalkane diol to produce the corresponding pentatosylpentaazacycloalkane. The tosyl groups are then removed and the resulting compound is reacted with

an iron compound under essentially anhydrous and anaerobic conditions to form the corresponding iron pentaazacycloalkane complex.

The macrocyclic ligands useful in the complexes of the present invention, wherein R_1, R'_1, R_3, R'_3, R_5, R'_5, R_7, R'_7, R_9 and R'_9 can be H or any functionality as previously described, can be prepared according to the general peptide method shown in Scheme B set forth below. The procedure for preparing the cyclic peptide precursors from the corresponding linear peptides are the same or significant modifications of methods known in the art. See, for example, Veber, D.F. et al., J. Org. Chem., 44, 3101 (1979). The general method outlined in Scheme B below is an example utilizing the sequential solution-phase preparation of the functionalized linear pentapeptide from N-terminus to C-terminus. Alternatively, the reaction sequence to prepare the linear pentapeptide can be carried out by solid-phase preparation utilizing methods known in the art. The reaction sequence could be conducted from C-terminus to N-terminus and by convergent approaches such as the coupling of di- and tri-peptides as needed. Thus a Boc-protected amino acid is coupled with an amino acid ester using standard peptide coupling reagents. The new Boc-dipeptide ester is then saponified to the free acid which is coupled again to another amino acid ester. The resulting Boc-tri-peptide ester is again saponified and this method is continued until the Boc-protected pentapeptide free acid has been prepared. The Boc protecting group is removed under standard conditions and the resulting pentapeptide or salt thereof is converted to the cyclic pentapeptide. The cyclic pentapeptide is then reduced to the pentaazacyclopentadecane with lithium aluminum hydride or borane. The final ligand is then reacted with an iron compound under essentially anaerobic conditions to

form the corresponding iron pentaazacyclopentadecane complex.

The R groups in the macrocycles produced by the cyclic peptide route, i.e., R_1, R'_1, R_3, R'_3, R_5, R'_5, R_7, R'_7, R_9 and R'_9, could be derived from the D or L forms of the amino acids Alanine, Aspartic acid, Arginine, Asparagine, Cysteine, Glycine, Glutamic acid, Glutamine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Phenylalanine, Serine, Tryptophan, Threonine, Tyrosine, Valine and/or the R groups of unnatural α-amino acids such as alkyl, ethyl, butyl, tert-butyl, cycloalkyl, phenyl, alkenyl, allyl, alkynyl, aryl, heteroaryl, polycycloalkyl, polycycloaryl, polycycloheteroaryl, imines, aminoalkyl, hydroxyalkyl, hydroxyl, phenol, amine oxides, thioalkyl, carboalkoxyalkyl, carboxylic acids and their derivatives, keto, ether, aldehyde, amine, nitrile, halo, thiol, sulfoxide, sulfone, sulfonic acid, sulfide, disulfide, phosphonic acid, phosphinic acid, phosphine oxides, sulfonamides, amides, amino acids, peptides, proteins, carbohydrates, nucleic acids, fatty acids, lipids, nitro, hydroxylamines, hydroxamic acids, thiocarbonyls, borates, boranes, boraza, silyl, siloxy, silaza, and combinations thereof.

The macrocyclic ligands useful in the complexes of the present invention can also be prepared by the diacid dichloride route shown in Scheme C set forth below. Thus, a triazaalkane is tosylated in a suitable solvent system to produce the corresponding tris(N-tosyl) derivative. Such a derivative is treated with a suitable base to produce the corresponding disulfonamide anion. The disulfonamide anion is dialkylated with a suitable electrophile to produce a derivative of a dicarboxylic acid. This derivative of a dicarboxylic acid is treated to produce the dicarboxylic acid, which is then treated with a suitable reagent to form the

diacid dichloride. The desired vicinal diamine is obtained in any of several ways. One way which is useful is the preparation from an aldehyde by reaction with cyanide in the presence of ammonium chloride followed by treatment with acid to produce the alpha ammonium nitrile. The latter compound is reduced in the presence of acid and then treated with a suitable base to produce the vicinal diamine. Condensation of the diacid dichloride with the vicinal diamine in the presence of a suitable base forms the tris(tosyl)diamide macrocycle. The tosyl groups are removed and the amides are reduced and the resulting compound is reacted with an iron compound under essentially anhydrous and anaerobic conditions to form the corresponding substituted pentaazacycloalkane iron complex.

The vicinal diamines have been prepared by the route shown (known as the Strecker synthesis) and vicinal diamines were purchased when commercially available. Any method of vicinal diamine preparation could be used.

The macrocyclic ligands useful in the complexes of the present invention can also be prepared by the bis(haloacetamide) route shown in Scheme D set forth below. Thus a triazaalkane is tosylated in a suitable solvent system to produce the corresponding tris(N-tosyl) derivative. Such a derivative is treated with a suitable base to produce the corresponding disulfonamide anion. A bis(haloacetamide), e.g., a bis(chloroacetamide), of a vicinal diamine is prepared by reaction of the diamine with an excess of haloacetyl halide, e.g., chloroacetyl chloride, in the presence of a base. The disulfonamide anion of the tris(N-tosyl) triazaalkane is then reacted with the bis(chloroacetamide) of the diamine to produce the substituted tris(N-tosyl)diamide macrocycle. The tosyl groups are removed and the amides are reduced and the

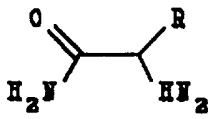
resulting compound is reacted with an iron compound under essentially anhydrous and anaerobic conditions to form the corresponding substituted pentaazacycloalkane iron complex.

The macrocyclic ligands useful in the complexes of the present invention, wherein R_1, R'_1, R_2, R'_2 are part of a *cis*- or *trans*- cycloalkyl ring system and R_5, R'_5, R_7, R'_7 and R_9, R'_9 can be H or any functionality previously described, can be prepared according to the pseudo-peptide method shown in Scheme E set forth below. A *cis*-1,2-Diaminocycloalkane or a *trans*-(R,R)-1,2-diaminocycloalkane or *trans*-(S,S)-1,2-diaminocycloalkane can be used in this method in combination with any amino acids. This allows the relative stereochemistry of the cycloalkane fused ring and substituent, R_5, R'_5, R_7, R'_7, R_9, R'_9, functionality and stereochemistry to be defined in any manner. As an example *trans*-(R,R)-1,2-diaminocyclhexane was monotosylated and reacted with Boc anhydride to afford the differentiated N-Boc, N-tosyl derivative. The sulfonamide was alkylated with methyl bromoacetate using sodium hydride as the base and saponified to the free acid. The cyclohexanediamine containing N-tosylglycine serves as a dipeptide surrogate in standard solution-phase peptide synthesis. Thus, coupling with a functionalized amino acid ester affords the corresponding pseudo-tripeptide. Two sequential TFA cleavage-couplings affords the pseudo-pentapeptide which can be N- and C-terminus deprotected in one step using HCl/AcOH. DPPA mediated cyclization followed by $LiAlH_4$ or Borane reduction affords the corresponding macrocylic ligand. This ligand system is reacted with an iron compound, such as iron (III) chloride under essentially anaerobic conditions to form the corresponding functionalized iron (III) pentaazacycloalkane complex.

The macrocyclic ligands useful in the complexes of the present invention, wherein R_1, R'_1, R_2, R'_2 and R_5, R'_5, R_6, R'_6, are part of a *cis*- or *trans*- cycloalkyl ring system and R_9, R'_9 can be H or any functionality previously described, can be prepared according to the iterative pseudo-peptide method shown in Scheme F set forth below. A *cis*-1,2-Diaminocycloalkane or a *trans*-(R,R)-1,2-diaminocycloalkane or *trans*-(S,S)-1,2-diaminocycloalkane can be used in any combination with each other using this method and in combination with any amino acids. This allows the relative stereochemistry of both cycloalkane fused rings and substituent, R_9, R'_9, functionality and stereochemistry to be defined in any manner. Thus, the (S,S)-1,2-diaminocyclohexyl-N-tosylglycine dipeptide surrogate, prepared from (S,S)-1,2-diaminocyclohexane exactly as in Scheme E in the case of (R,R)-1,2-diaminocyclohexane, can be coupled with a functionalized amino acid ester to afford the corresponding pseudo-tripeptide. TFA cleavage affords the pseudo-tripeptide TFA salt which is coupled with (R,R)-diaminocyclohexyl-N-tosylglycine. Saponification and TFA cleavage affords the bis-cyclohexano containing pseudo-pentapeptide. DPPA mediated cyclization followed by $LiAlH_4$ or Borane reduction affords the corresponding bis-cyclohexano-fused macrocylic ligand. This ligand system is reacted with an iron compound, such as iron (III) chloride under essentially anaerobic conditions to form the corresponding functionalized iron (III) pentaazacycloalkane complex.

The macrocyclic ligands useful in the complexes of the present invention can also be prepared according to the general procedure shown in Scheme G set forth below. Thus, an amino acid amide, which is the corresponding amide derivative of a naturally or non-naturally occurring α-amino acid, is reduced to form the corresponding substituted ethylenediamine. Such amino

acid amide can be the amide derivative of any one of many well known amino acids. Preferred amino acid amides are those represented by the formula:



wherein R is as previously defined. Most preferred are those wherein R represents hydrogen, alkyl, cycloalkylalkyl, and aralkyl radicals. The diamine is then tosylated to produce the di-N-tosyl derivative which is reacted with a di-O-tosylated tris-N-tosylated triazaalkane diol to produce the corresponding substituted N-pentatosylpentaazacycloalkane. The tosyl groups are then removed and the resulting compound is reacted with an iron compound under essentially anhydrous and anaerobic conditions to form the corresponding substituted iron pentaazacycloalkane complex.

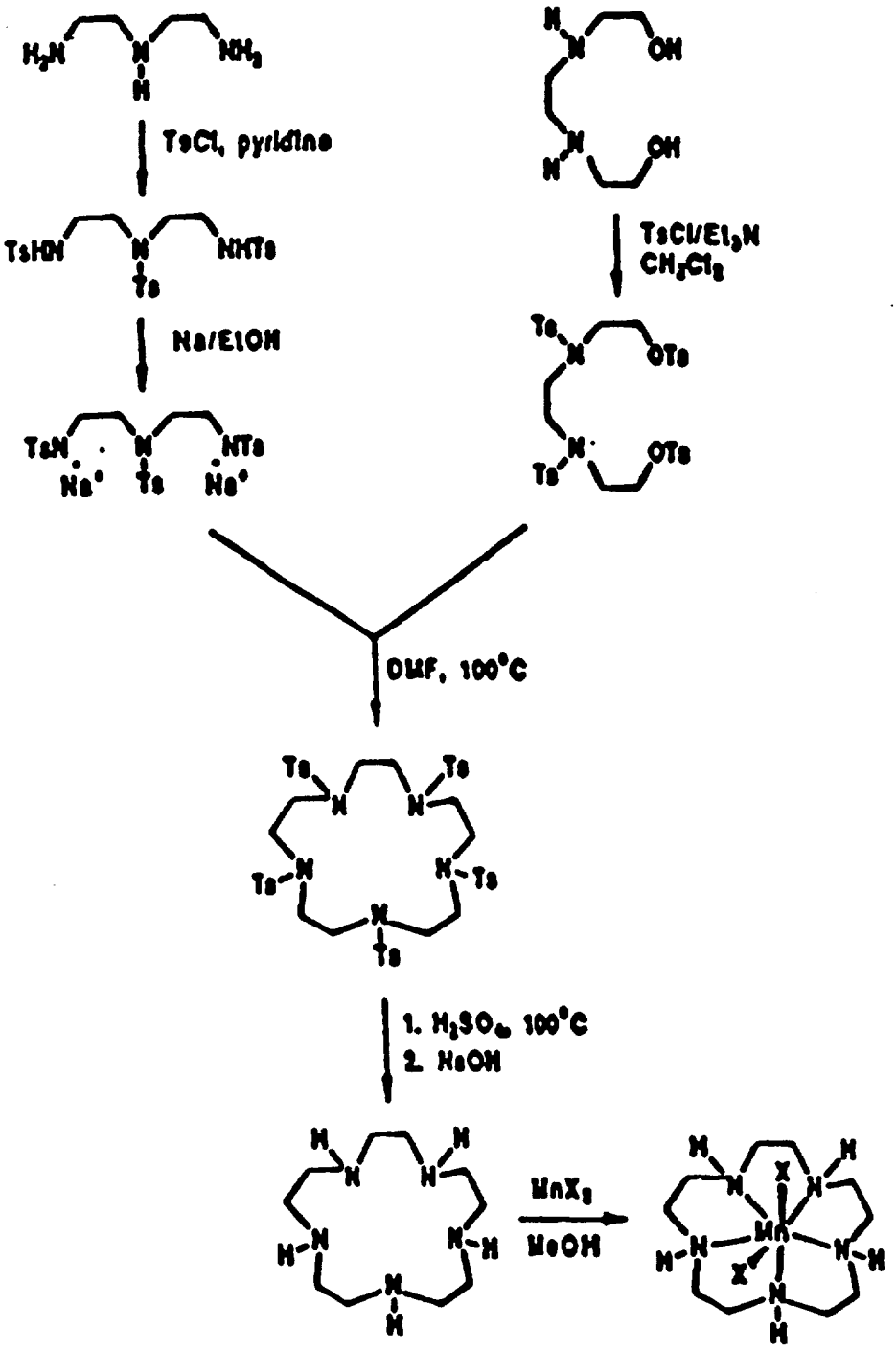
The complexes of the present invention, wherein R_9, and R_2 are alkyl, and R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8 and R'_8 can be alkyl, arylalkyl or cycloalkylalkyl and R or R′ and R_1 or R'_1 together with the carbon atoms they are attached to are bound to form a nitrogen containing heterocycle, can also be prepared according to the general procedure shown in Scheme H set forth below utilizing methods known in the art for preparing the iron pentaazabicyclo[12.3.1]octadecapentaene complex precursor. See, for example, Alexander et al., Inorg.

Nucl. Chem. Lett., 6, 445 (1970). Thus a 2,6-diketopyridine is condensed with triethylene tetraamine in the presence of an iron compound to produce the iron pentaazabicyclo[12.3.1]octadecapentaene complex. The iron pentaazabicyclo[12.3.1]octadecapentaene complex is hydrogenated with 5% rhodium on carbon at a pressure of 1000 psi to give the corresponding iron pentaazabicyclo[12.3.1]octadecatriene complex.

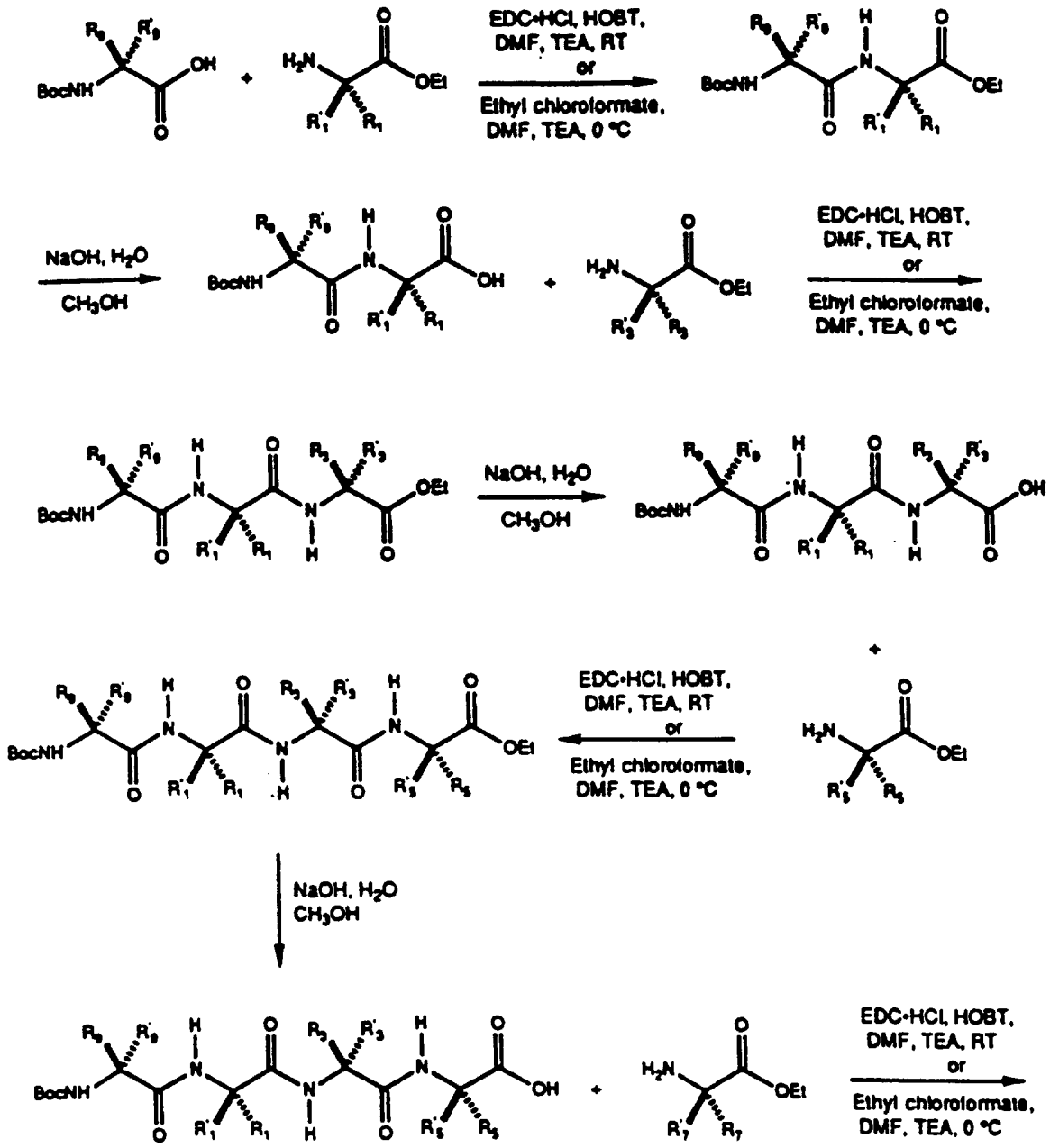
The macrocyclic ligands useful in the complexes of the present invention can also be prepared by the pyridine diamide route shown in Scheme I as set forth below. Thus, a polyamine, such as a tetraaza compound, containing two primary amines is condensed with dimethyl 2,6-pyridine dicarboxylate by heating in an appropriate solvent, e.g., methanol, to produce a macrocycle incorporating the pyridine ring as the 2,6-dicarboxamide. The pyridine ring in the macrocycle is reduced to the corresponding piperidine ring in the macrocycle, and then the diamides are reduced and the resulting compound is reacted with an compound under essentially anhydrous and anaerobic conditions to form the corresponding substituted pentaazacycloalkane iron complex.

When the ligands or charge-neutralizing anions, i.e. X, Y and Z, are anions or ligands that cannot be introduced directly from the iron compound, the complex with those anions or ligands can be formed by conducting an exchange reaction with a complex that has been prepared by reacting the macrocycle with an iron compound.

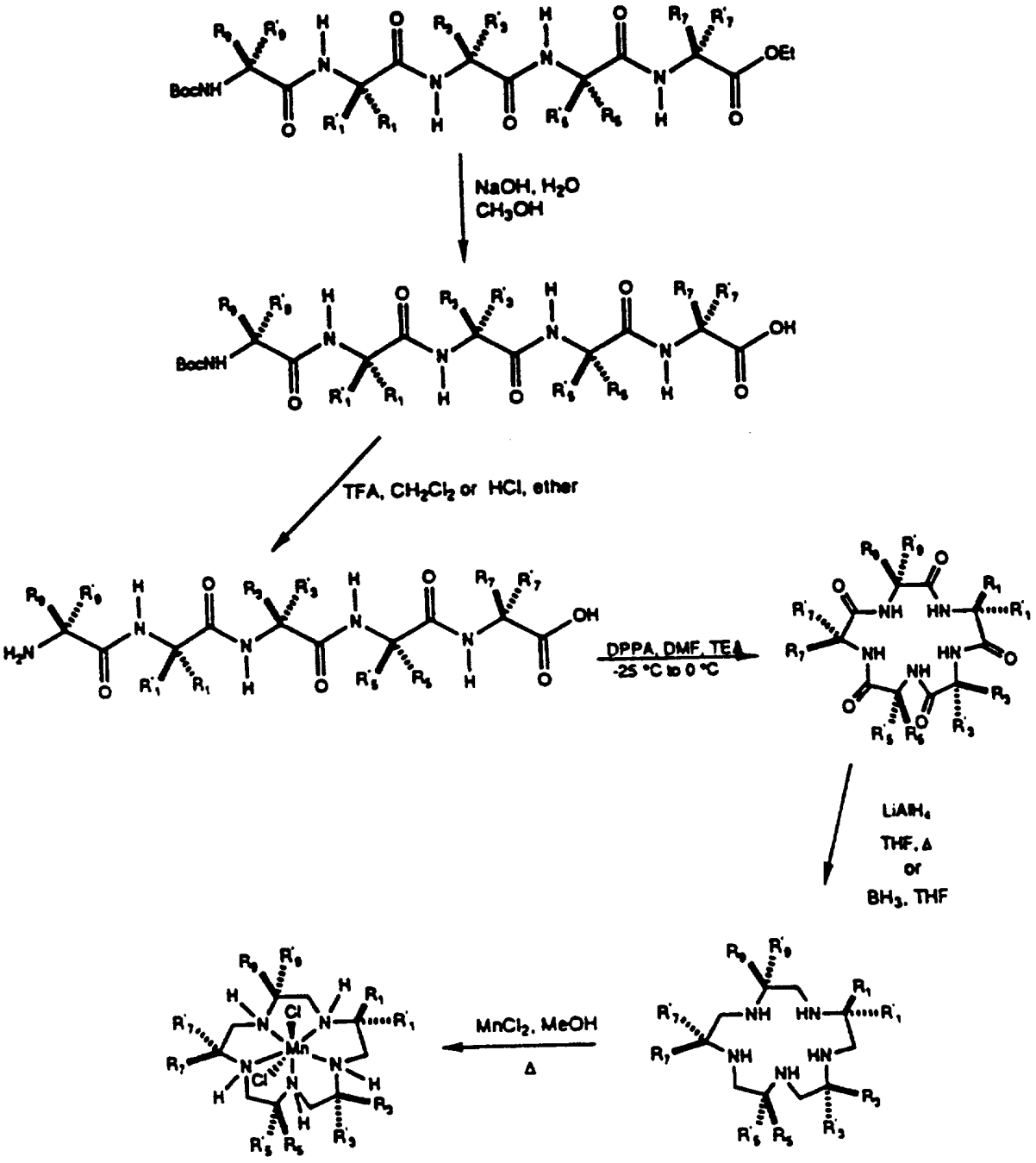
SCHEME A


TsCl, pyridine
Na/EtOH
TsCl/Et3N
CH2Cl2
DMF, 100°C
1. H2SO4, 100°C
2. NaOH
MnX2
MeOH

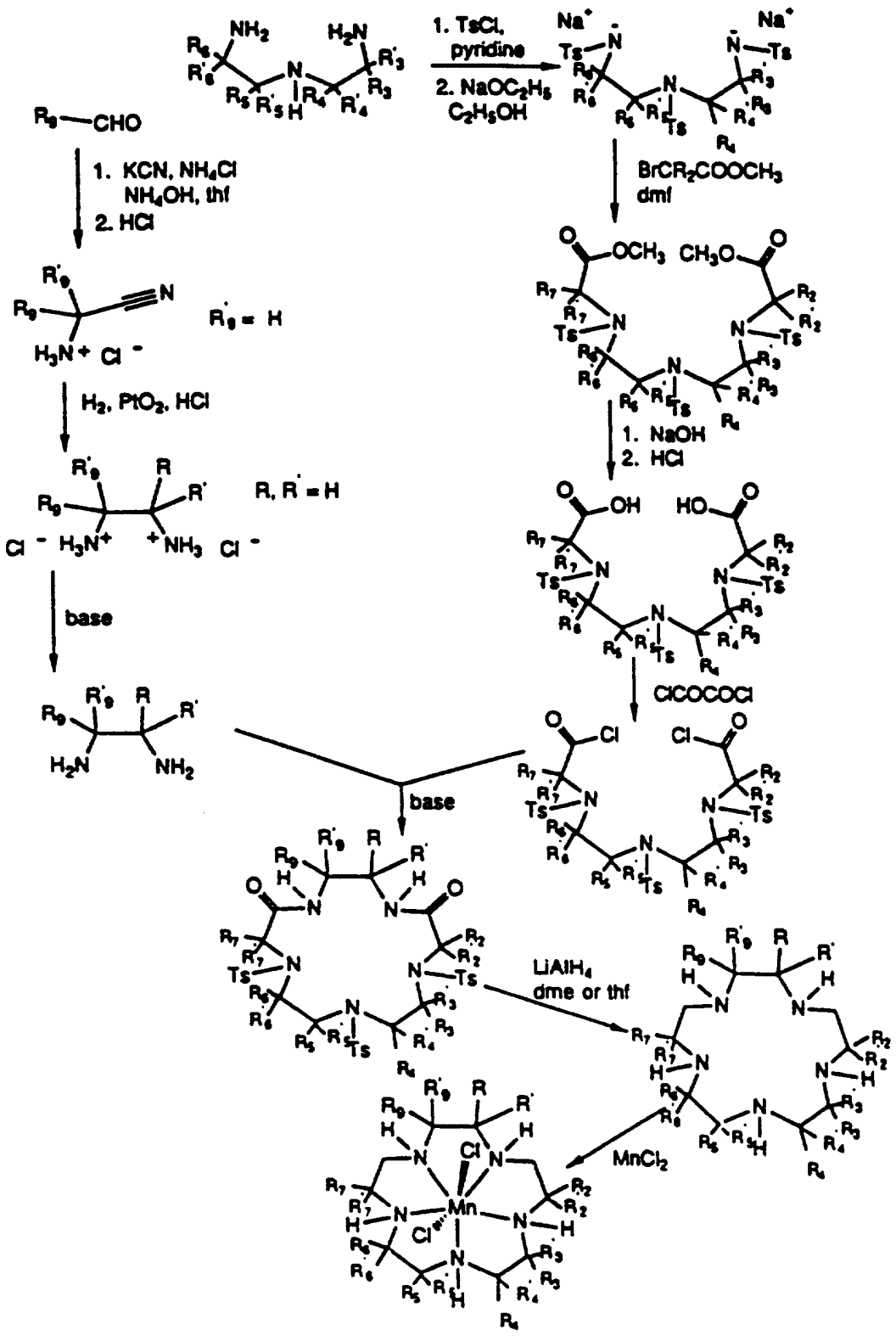
SCHEME B


EDC•HCl, HOBT, DMF, TEA, RT or Ethyl chloroformate, DMF, TEA, 0 °C
NaOH, H2O CH3OH
EDC•HCl, HOBT, DMF, TEA, RT or Ethyl chloroformate, DMF, TEA, 0 °C
NaOH, H2O CH3OH
EDC•HCl, HOBT, DMF, TEA, RT or Ethyl chloroformate, DMF, TEA, 0 °C
NaOH, H2O CH3OH
EDC•HCl, HOBT, DMF, TEA, RT or Ethyl chloroformate, DMF, TEA, 0 °C

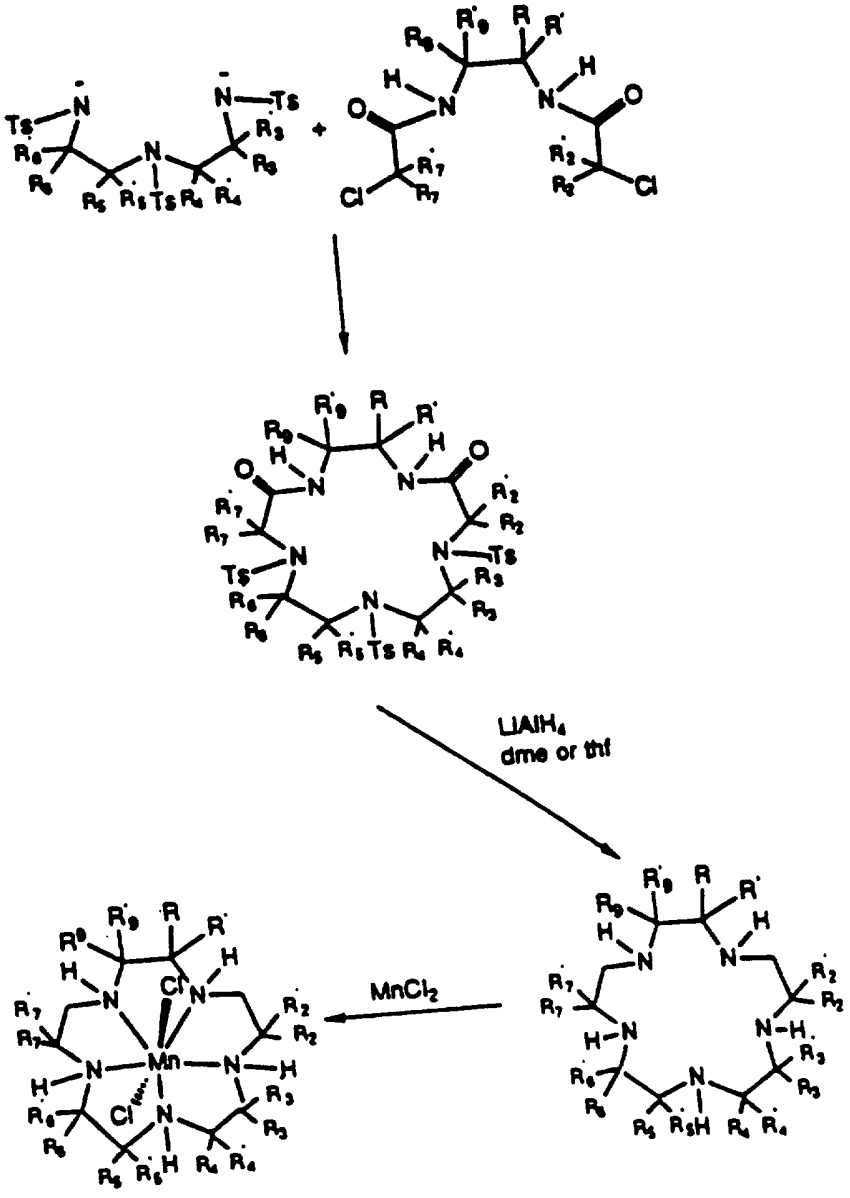
SCHEME B (Cont'd)



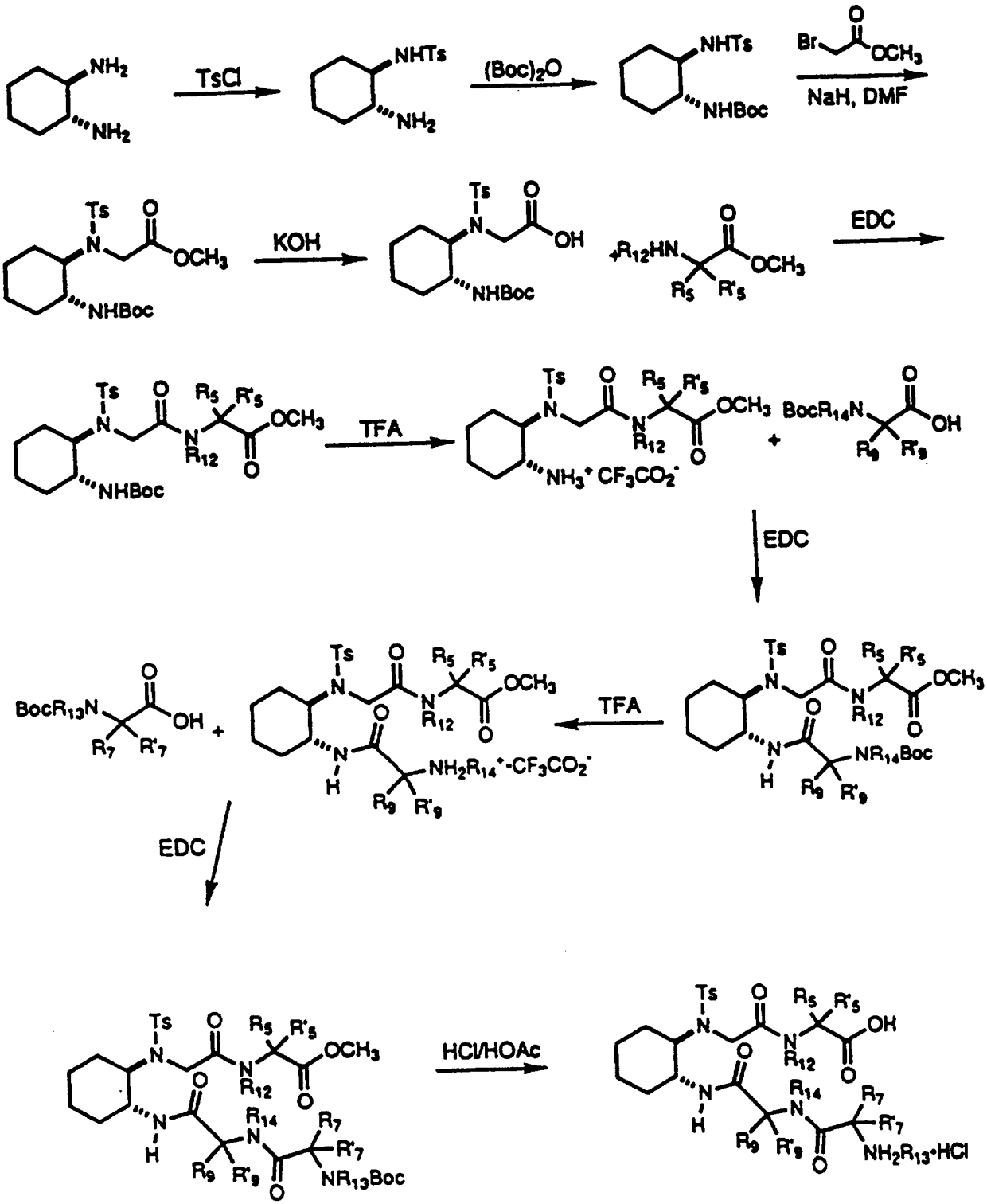
SCHEME C


1. TsCl, pyridine
2. NaOC2H5 C2H5OH
1. KCN, NH4Cl NH4OH, thf
2. HCl
R'9 = H
H2, PtO2, HCl
R, R' = H
base
BrCR2COOCH3 dmf
1. NaOH
2. HCl
ClCOCOCl
base
LiAlH4 dme or thf
MnCl2

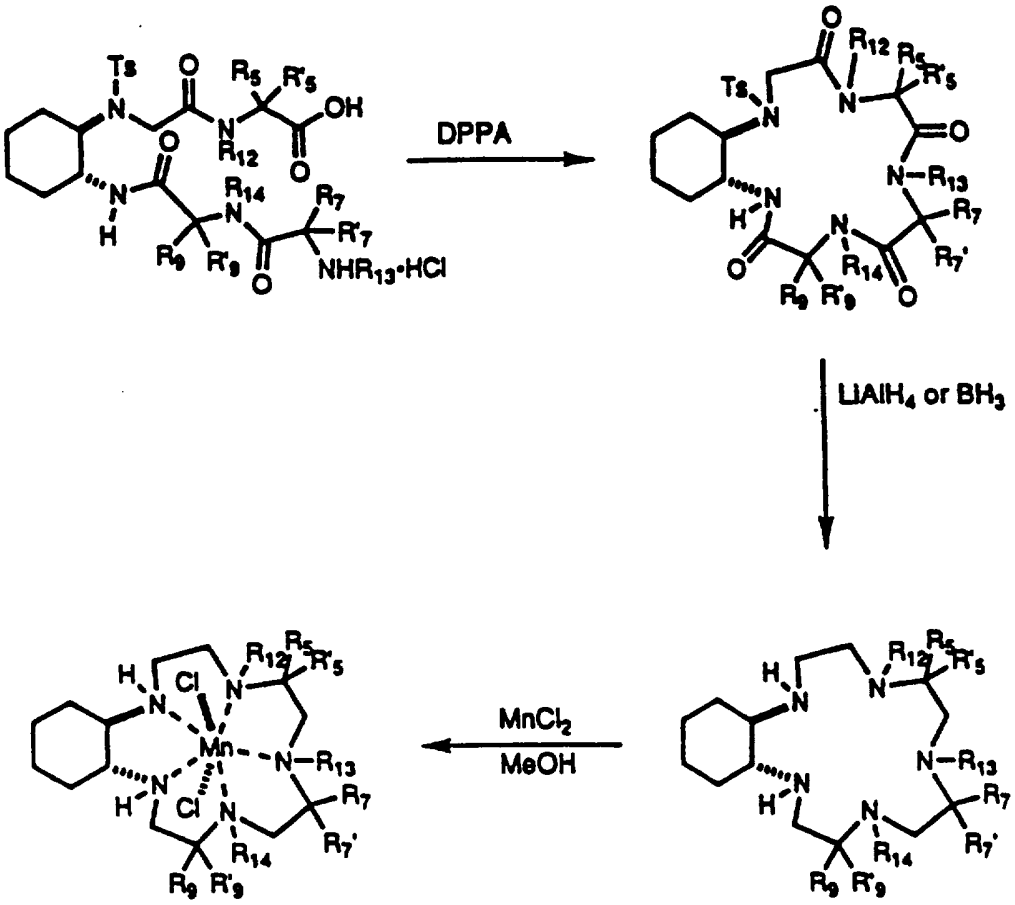
SCHEME D



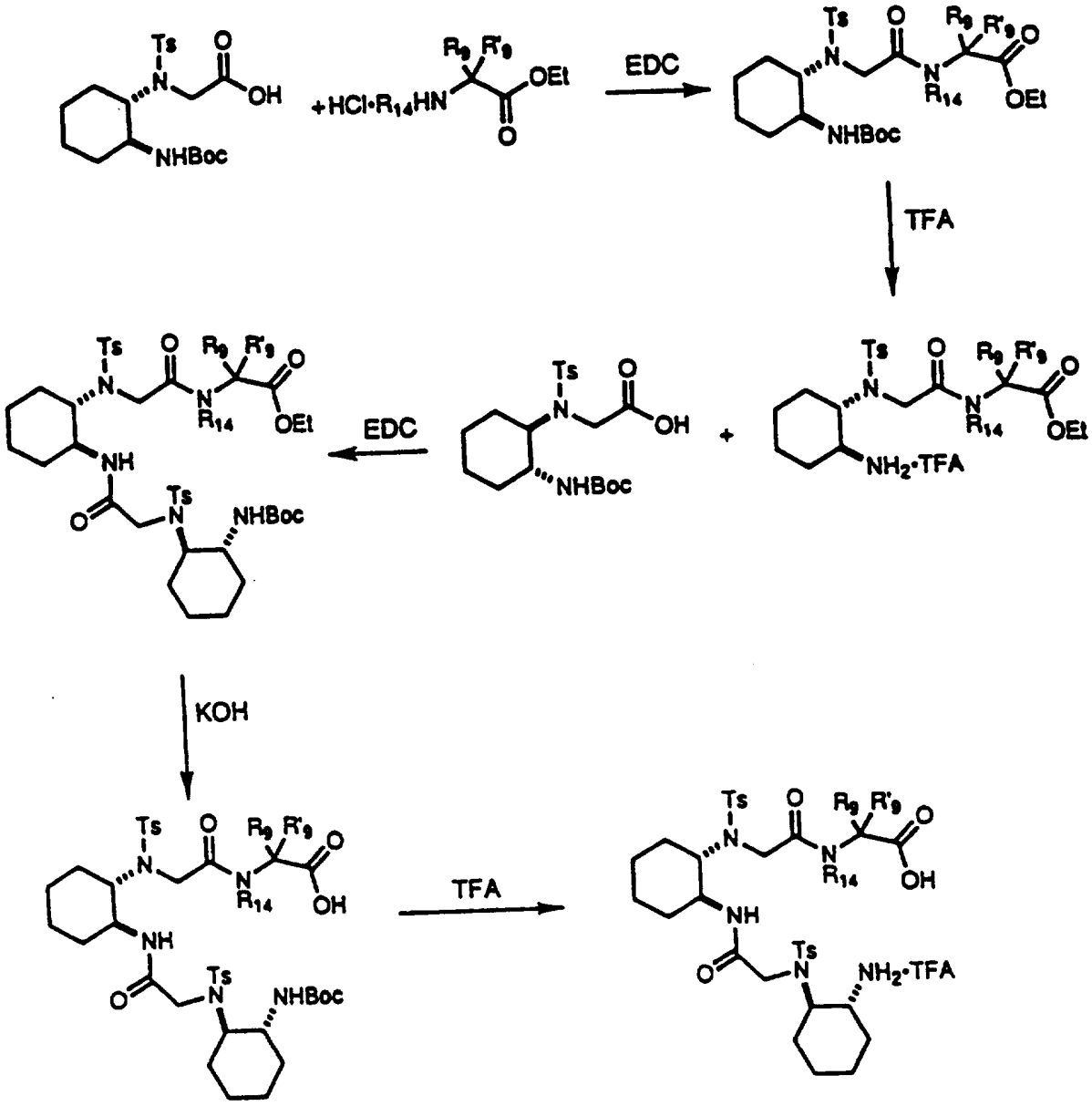
SCHEME E



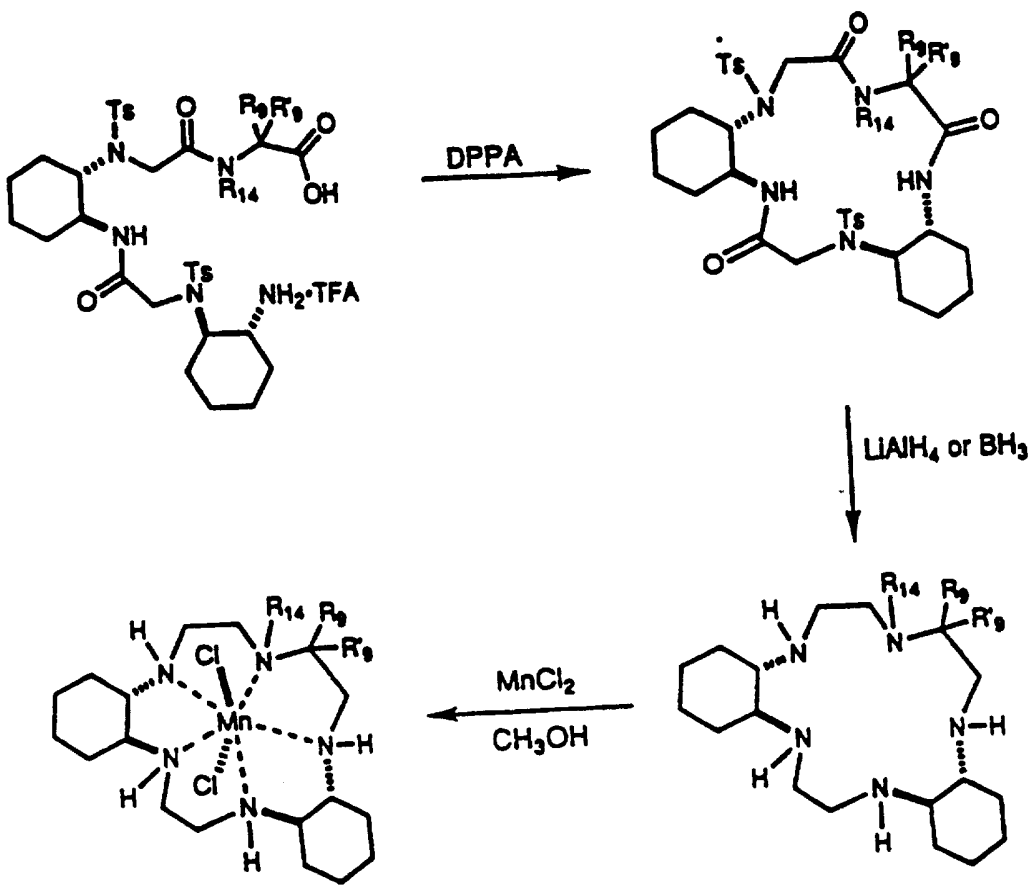
SCHEME E (Con't.)



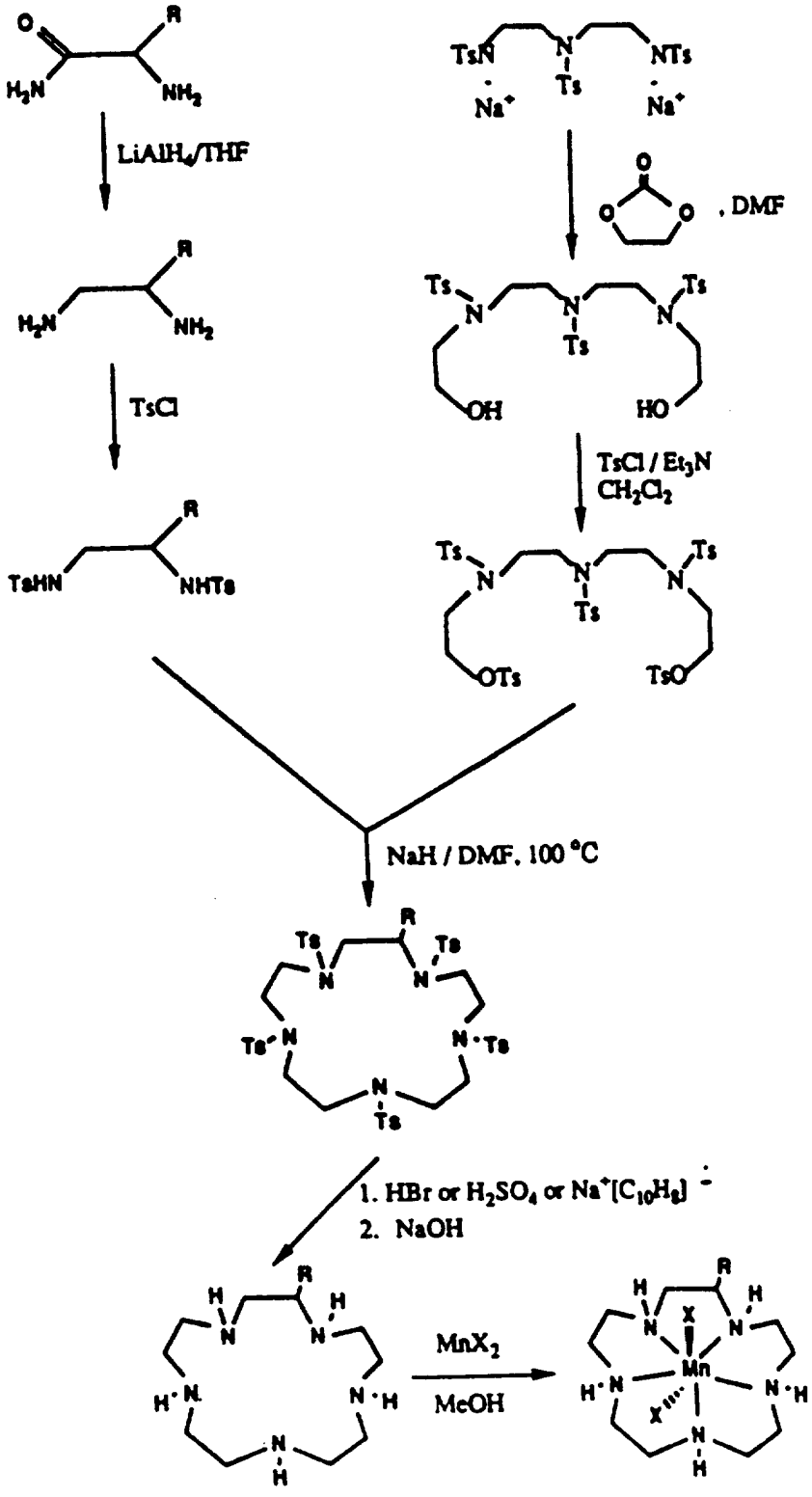
SCHEME F



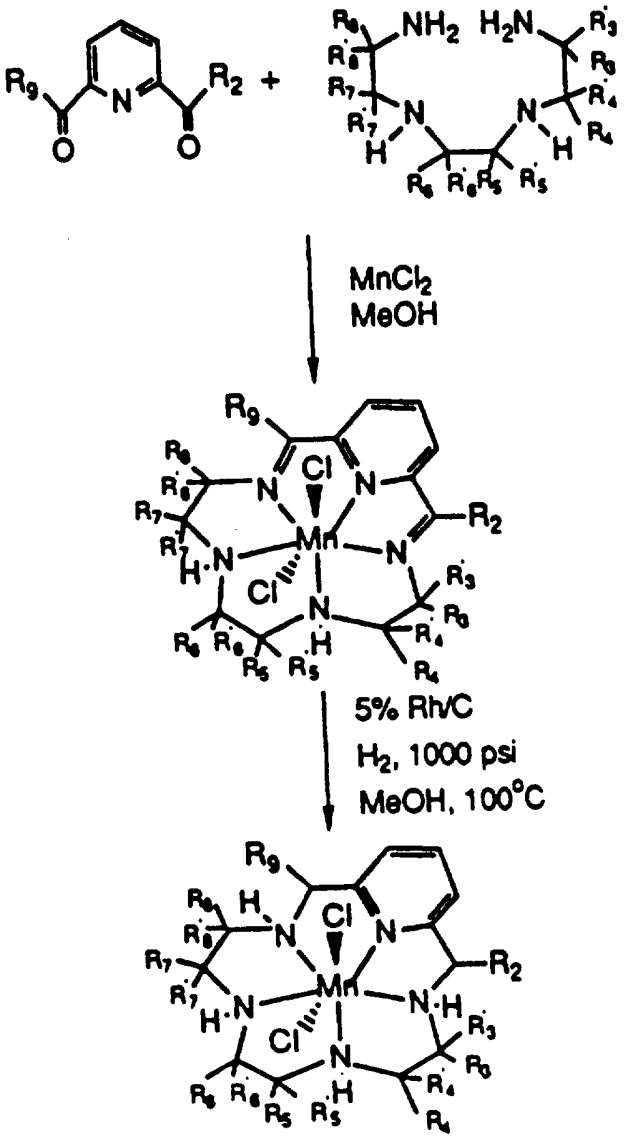
SCHEME F (Con't.)



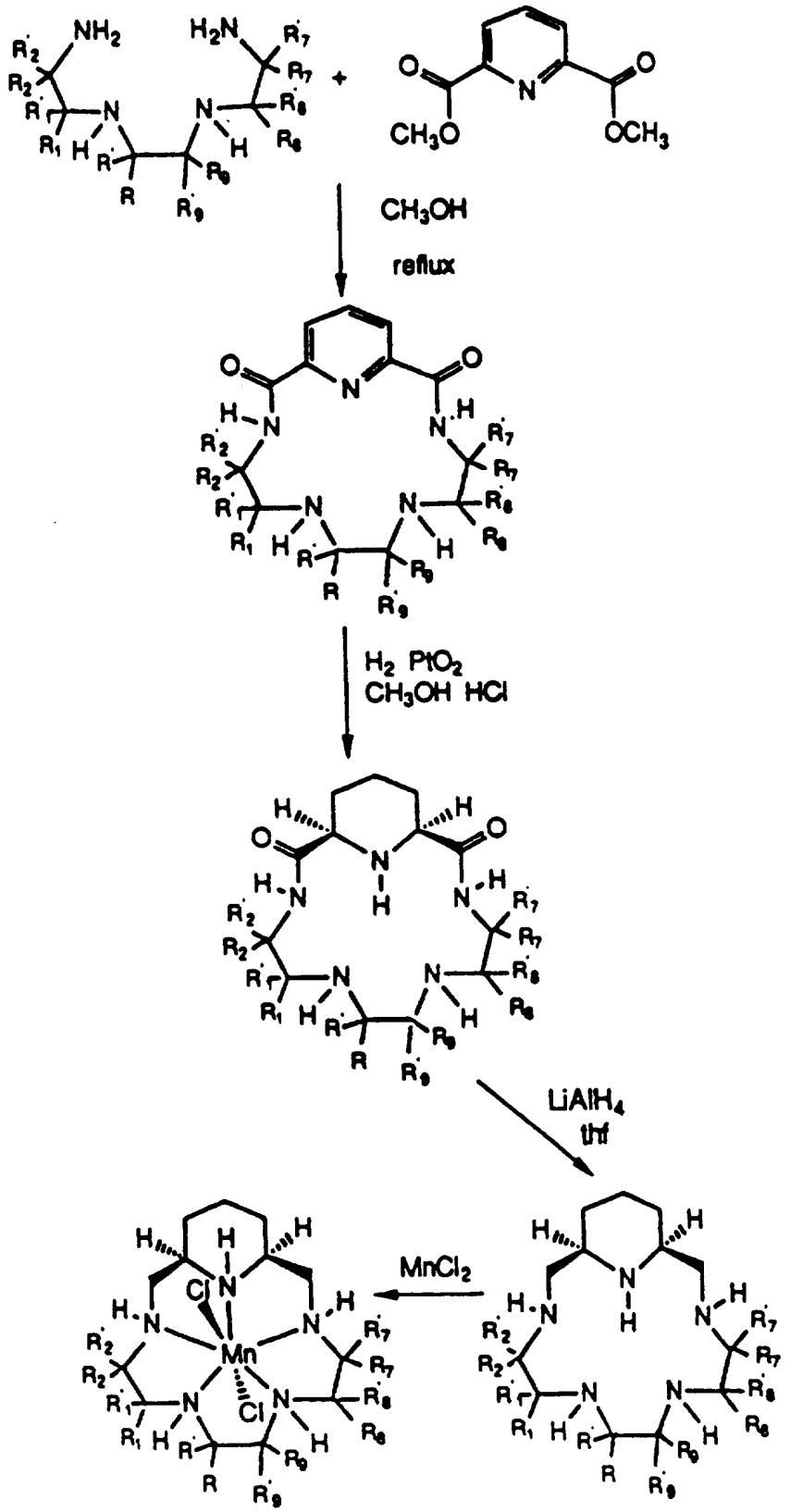
SCHEME G


LiAlH4/THF
TsCl
TsCl / Et3N
CH2Cl2
, DMF
NaH / DMF, 100 °C
1. HBr or H2SO4 or Na+[C10H8]-
2. NaOH
MnX2
MeOH

SCHEME II



SCHEME I



The pentaazamacrocycles of the present invention can possess one or more asymmetric carbon atoms and are thus capable of existing in the form of optical isomers as well as in the form of racemic or nonracemic mixtures thereof. The optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes, for example by formation of diastereoisomeric salts by treatment with an optically active acid. Examples of appropriate acids are tartaric, diacetyltartaric, dibenzoyltartaric, ditoluoyltartaric and camphorsulfonic acid and then separation of the mixture of diastereoisomers by crystallization followed by liberation of the optically active bases from these salts. A different process for separation of optical isomers involves the use of a chiral chromatography column optimally chosen to maximize the separation of the enantiomers. Still another available method involves synthesis of covalent diastereoisomeric molecules by reacting one or more secondary amine group(s) of the compounds of the invention with an optically pure acid in an activated form or an optically pure isocyanate. The synthesized diastereoisomers can be separated by conventional means such as chromatography, distillation, crystallization or sublimation, and then hydrolyzed to deliver the enantiomerically pure ligand. The optically active compounds of the invention can likewise be obtained by utilizing optically active starting materials, such as natural amino acids.

The compounds or complexes of the present invention can be utilized to treat numerous inflammatory disease states and disorders that are mediated, at least in part, by superoxide. For example, reperfusion injury to an ischemic organ, e.g., reperfusion injury to the ischemic myocardium, surgically-induced ischemia, inflammatory bowel disease, rheumatoid arthritis,

osteoarthritis, psoriasis, organ transplant rejections, radiation-induced injury, oxidant-induced tissue injuries and damage, atherosclerosis, thrombosis, platelet aggregation, metastasis, stroke, acute pancreatitis, insulin-dependent diabetes mellitus, disseminated intravascular coagulation, fatty embolism, adult and infantile respiratory distress, and carcinogenesis.

Activity of the compounds or complexes of the present invention for catalyzing the dismutation of superoxide can be demonstrated using the stopped-flow kinetic analysis technique as described in Riley, D.P., Rivers, W.J. and Weiss, R.H., "Stopped-Flow Kinetic Analysis for Monitoring Superoxide Decay in Aqueous Systems," Anal. Biochem., 196, 344-349 (1991), which is incorporated by reference herein. Stopped-flow kinetic analysis is an accurate and direct method for quantitatively monitoring the decay rates of superoxide in water. The stopped-flow kinetic analysis is suitable for screening compounds for SOD activity and activity of the compounds or complexes of the present invention, as shown by stopped-flow analysis, correlate to treating the above disease states and disorders.

Total daily dose administered to a host in single or divided doses may be in amounts, for example, from about 1 to about 100 mg/kg body weight daily and more usually about 3 to 30 mg/kg. Dosage unit compositions may contain such amounts of submultiples thereof to make up the daily dose.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

The dosage regimen for treating a disease condition with the compounds and/or compositions of this invention is selected in accordance with a variety of

factors, including the type, age, weight, sex, diet and medical condition of the patient, the severity of the disease, the route of administration, pharmacological considerations such as the activity, efficacy, pharmacokinetic and toxicology profiles of the particular compound employed, whether a drug delivery system is utilized and whether the compound is administered as part of a drug combination. Thus, the dosage regimen actually employed may vary widely and therefore may deviate from the preferred dosage regimen set forth above.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administration may also involve the use of transdermal administration such as transdermal patches or iontophoresis devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In

addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols which are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, granules and gels. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

While the compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more compounds which are known to be effective against the specific disease state that one is targeting for treatment.

Contemplated equivalents of the general formulas set forth above for the compounds and derivatives as well as the intermediates are compounds otherwise corresponding thereto and having the same general

properties such as tautomers of the compounds and such as wherein one or more of the various R groups are simple variations of the substituents as defined therein, e.g., wherein R is a higher alkyl group than that indicated, or where the tosyl groups are other nitrogen or oxygen protecting groups or wherein the O-tosyl is a halide. Anions having a charge other than 1, e.g., carbonate, phosphate, and hydrogen phosphate, can be used instead of anions having a charge of 1, so long as they do not adversely affect the overall activity of the complex. However, using anions having a charge other than 1 will result in a slight modification of the general formula for the complex set forth above. In addition, where a substituent is designated as, or can be, a hydrogen, the exact chemical nature of a substituent which is other than hydrogen at that position, e.g., a hydrocarbyl radical or a halogen, hydroxy, amino and the like functional group, is not critical so long as it does not adversely affect the overall activity and/or synthesis procedure. Further, it is contemplated that iron (III) complexes will be equivalent to the subject iron (III) complexes.

The chemical reactions described above are generally disclosed in terms of their broadest application to the preparation of the compounds of this invention. Occasionally, the reactions may not be applicable as described to each compound included within the disclosed scope. The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art, e.g., by appropriate protection of interfering groups, by changing to alternative conventional reagents, by routine modification of reaction conditions, and the like, or other reactions disclosed herein or otherwise

conventional, will be applicable to the preparation of the corresponding compounds of this invention. In all preparative methods, all starting materials are known or readily preparable from known starting materials.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

EXAMPLES

All reagents were used as received without purification unless otherwise indicated. All NMR spectra were obtained on a Varian VXR-300 or VXR-400 nuclear magnetic resonance spectrometer. Qualitative and quantitative mass spectroscopy was run on a Finnigan MAT90, a Finnigan 4500 and a VG40-250T using m-nitrobenzyl alcohol(NBA) or m-nitrobenzyl alcohol/LiCl (NBA+Li). Melting points (mp) are uncorrected.

The following abbreviations relating to amino acids and their protective groups are in accordance with the recommendation by IUPAC-IUB Commission on Biochemical Nomenclature (Biochemistry, 11, 1726 (1972)) and common usage.

Ala	L-Alanine
DAla	D-Alanine
Gly	Glycine
Ppg	Propargylglycine
Tyr	L-Tyrosine
Bzl	Benzyl
Boc	*tert*-Butoxycarbonyl
Et	Ethyl
TFA	Trifluoroacetate
DMF	Dimethylformamide
HOBT•H_2O	1-Hydroxy-(1*H*)-benzotriazole monohydrate
EDC•HCl	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
TEA	Triethylamine
DMSO	Dimethylsulfoxide
THF	Tetrahydrofuran
DPPA	Diphenylphosphoryl azide
DMPU	Dimethylpropyleneurea
c	concentration, g/cc
DME	1,2-Dimethoxyethane

The abbreviation Cyc represents 1,2-cyclohexanediamine (stereochemistry, i.e. R_1R or S_1S, is indicated as such). This allows three letter code peptide nomenclature to be used in pseudopeptides containing the 1,2-cyclohexanediamine "residue".

Example 1

A. Synthesis of 1,4,7-Tris(p-toluenesulfonyl)-1,4,7-triazaheptane

This compound was synthesized following the procedure of Atkins, T. J.; Richman, J.E.; and Oettle, W.F.; *Org. Synth.*, 58, 86-98 (1978). To a

stirred solution of p-toluenesulfonyl chloride (618 g, 3.24 mole) in pyridine (1500 ml) at 0°C was added a solution of 1,4,7-triazaheptane (95.5 g, 0.926 mole) in pyridine (150 ml) under a dry argon atmosphere, maintaining the temperature ≤ 50°C. The addition required 30 minutes. After the mixture was allowed to cool to room temperature slowly while stirring for 3 h, H_2O(2 l) was slowly added to the cooled (ice bath) mixture. The heavy white precipitate which formed was filtered and washed thoroughly with H_2O. The pale yellow solid was dissolved in DMF (3 l) and 0.1 N HCl (4 l) was slowly added at 5°C. The slurry was filtered and the pale yellow solid was washed thoroughly with H_2O and dried *in vacuo* to give 486 g (93% yield) of the product: mp 180-1°C; ^{1}H NMR(DMSO-d_6) δ 2.39 (s,3 H), 2.40 (s, 6 H), 2.84 (m, 4 H), 3.04 (t, J=6.9 Hz, 4 H) 7.40 (d, J=8.1 Hz, 4 H), 7.59 (d, J=8.3 Hz, 2 H), 7.67 (m, 6 H).

B. Synthesis of 1,4,7-Tris(p-toluenesulfonyl)-1,4,7-triazaheptane-1,7-disodium Salt

This compound was synthesized following the procedure of Atkins, T.J.; Richman, J.E., and Oettle, W.F.; *Org. Synth.*, 58 86-98 (1978). To a mechanically stirred slurry of 1,4,7-tris(p-toluenesulfonyl)-1,4,7-triazaheptane prepared as in Example 1A (486 g, 0.859 mole) in ethanol (1150 ml) heated to reflux under a dry argon atmosphere was added a solution of sodium ethoxide (prepared by dissolving sodium metal (39.5 g, 1.72 mole) in absolute ethanol (1.0 l)) as rapidly as possible. The clear brown solution which formed rapidly was allowed to cool to room temperature and ethyl ether (1.0 l) was added. The crystals were filtered under a dry argon blanket, washed with 3:1 ethanol:ethyl ether and ethyl ether. The crystals were then dried *in vacuo* to give 509 g (97% yield) of the product as a white powder: ^{1}H NMR (DMSO-d_6) δ 2.30 (s 6 H), 2.36 (s, 3 H), 2.63 (t,

J=8.7 Hz, 4 H), 2.89 (t, J=7.2 Hz, 4 H) 7.11 (d, J=8.1 Hz, 4 H), 7.28 (d, J=8.0 Hz, 2 H), 7.46 (m, 6 H).

C. Synthesis of 3,6-Bis(p-toluenesulfonyl)-3,6-diazaoctane-1,8-di-p-toluenesulfonate

To a stirred solution of p-toluenesulfonyl chloride (566 g, 2.97 mole) and triethylamine (300 g, 2.97 mole) in CH_2Cl_2 (2.0 l) at 0°C under a dry argon atmosphere was added 3,6-diazaoctane-1,8-diol (100 g, 0.675 mole) in portions, maintaining the temperature <10°C. The addition required 30 minutes. The mixture was allowed to warm to room temperature while stirring an additional 18 h and was then poured onto ice (1000 g). The CH_2Cl_2 layer was separated, washed with 10% HCl, H_2O and saturated NaCl solution, and dried ($MgSO_4$). The solution was concentrated *in vacuo* to a volume of 1.5 l. Crystallization by the addition of hexane (4 l) gave 477 g (92% yield) of the product as colorless needles: mp 151-3°C; ^1H NMR ($CDCl_3$) δ 2.43 (s, 12 H), 3.29 (s, 4 H), 3.36 (t, J=5.2 Hz, 4 H) 4.14 (t, J=5.2 Hz, 4 H), 7.33 (d, J=7.8 Hz, 8 H), 7.71 (d, J=8.2 Hz, 4 H), 7.79 (d, J=8.3 Hz, 4 H).

D. Synthesis of 1,4,7,10,13-Penta(p-toluenesulfonyl)-1,4,7,10,13-pentaazacyclopentadecane

This compound was synthesized following the procedure of Richman, J.E., and Atkins, T.J., *J. Am. Chem. Soc.*, 96, 2268-70 (1974). To a stirred solution of 1,4,7-tris(p-toluenesulfonyl)-1,4,7-triazaheptane-1,7-disodium salt prepared as in Example 1B (146 g, 0.240 mole) in anhydrous DMF (2250 ml) was added dropwise over 3 h to a solution of 3,6-bis(p-toluenesulfonyl)-3,6-diazaoctane-1,8-di-p-toluenesulfonate prepared as in Example 1C (184 g, 0.240 mole) in anhydrous DMF (1020 ml) under a dry argon atmosphere, maintaining the temperature at 100°C. After stirring an

additional 1 h at 100°C, the solution was concentrated *in vacuo* to a volume of 1.5 l. H_2O (500 ml) was slowly added at 80°C to crystallize the product. The resulting slurry was slowly cooled to 0°C and additional H_2O (1250 ml) added. The solid was filtered, washed thoroughly with H_2O and then 90% ethanol and dried *in vacuo*. The off-white solid was dissolved in CH_2Cl_2, insoluble impurities were removed by filtration and the filtrate was washed with H_2O and then dried ($MgSO_4$). The solvent was removed *in vacuo* to give a yellow solid which was purified by recrystallization from CH_2Cl_2-hexane to give 164 g (69% yield) of the product as a white crystalline solid: mp 290-3°C; ^1H NMR ($CDCl_3$) δ 2.44 (s, 15 H) 3.27 (s, 20 H), 7.32 (d, J=8.3 Hz, 10 H), 7.66 (d, J=8.3 Hz, 10 H).

E. Synthesis of 1,4,7,10,13-Pentaazacyclopentadecane

A mixture of 1,4,7,10,13-penta(p-toluenesulfonyl)-1,4,7,10,13-pentaazacyclopentadecane prepared as in Example 1D (168 g, 0.170 mole) and concentrated H_2SO_4 (500 ml) was heated at 100°C with stirring under a dry argon atmosphere for 70 h. To the resulting dark brown solution ethanol (500 ml) was added dropwise with stirring at 0°C followed by ethyl ether (3 l). The white solid was filtered and washed with ethyl ether. The solid was then dissolved in H_2O (500 ml) and the resulting solution washed with ethyl ether. Upon reducing the volume of the solution *in vacuo* to 200 ml, the pH was adjusted to 10-11 with 10 N NaOH and the solvent was removed *in vacuo*. Ethanol (500 ml) was then added and removed *in vacuo* to dryness. The resulting tan oily solid was extracted with hot THF (2x500 ml) and filtered at room temperature. The filtrates were combined and the solvent removed *in vacuo* to give the crude product as a yellow crystalline solid which was

then redissolved in CH_3CN and filtered to remove insoluble impurities. Recrystallization from cold (-20°C) CH_3CN gave 11.3 g (31% yield) of the product as colorless needles: mp 108-9°C; ^1H NMR ($CDCl_3$) δ 1.74 (br s, 5 H), 2.73 (s, 20 H); Exact mass $(M+Li)^+$: calcd, 222.2270; Found, 222.2269 ($C_{10}H_{25}N_5Li$).

F. Synthesis of [Iron(III)dichloro(1,4,7,10,13-Pentaazacyclopentadecane)]hexafluorophosphate

Upon an inert atmosphere in a drybox, 108 mg (0.50 mmol) of the ligand, 1,4,7,10,13-tetraazacyclopentadecane, was dissolved in 15 ml of anhydrous methanol. To this solution was added with vigorous stirring 2 ml of a pyridine solution containing 0.50 mmol (80 mg) of anhydrous $FeCl_3$. The resultant dark solution was heated to reflux for two hours with stirring and then allowed to cool to room temperature and then filtered. To the filtrate was added 20 ml of a clear methanolic solution of NH_4PF_6 (163) mg). A yellow precipitate formed instantly and was collected by filtration, washed with diethyl ether and dried *in vacuo* overnight. The yield after drying was 170 mg (0.338 mmol) corresponding to a 68% theoretical yield. Anal. Calc. for $C_{10}H_{20}N_5Cl_2FeF_6P \cdot CH_3OH$: C, 25.07; H, 5.41: N, 13.92. Found: C, 25.18; H, 5.60; N, 13.89. Mass spectrum (FAB, NBA matrix): m/z 306 ($[Fe(L)Cl+e]^+$ and m/z 341 ($[Fe(L)Cl_2]^+$ were observed.

Example 2

A. Synthesis of N-(p-toluenesulfonyl)-(R,R)-1,2-diaminocyclohexane

To a stirred solution of (R,R)-1,2-diaminocyclohexane (300 g, 2.63 mole) in CH_2Cl_2 (5.00 l) at -10°C was added a solution of p-toluenesulfonylchloride (209 g, 1.10 mole) in CH_2Cl_2 (5.00 l) dropwise over a 7 h period, maintaining the

temp at -5 to -10 °C. The mixture was allowed to warm to room temp while stirring overnight. The mixture was concentrated *in vacuo* to a volume of 3 l and the white solid was removed by filtration. The solution was then washed with H_2O (10 x 1 l) and was dried over $MgSO_4$. Removal of the solvent *in vacuo* gave 286 g (97.5% yield) of the product as a yellow crystalline solid: ^1H NMR ($CDCl_3$) δ 0.98 - 1.27 (m, 4 H), 1.54 - 1.66 (m, 2 H), 1.81 - 1.93 (m, 2 H), 2.34 (dt, J = 4.0, 10.7 Hz, 1 H), 2.42 (s, 3 H), 2.62 (dt, J = 4.2, 9.9 Hz, 1 H), 7.29 (d, J = 8.1 Hz, 2 H), 7.77 (d, J = 8.3 Hz, 2 H); MS (LRFAB - DTT - DTE) m/z 269 $[M + H]^+$.

B. Synthesis of N-(p-toluenesulfonyl)-N'-(Boc)-(R,R)-1,2-diaminocyclohexane

To a stirred solution of N-(p-toluenesulfonyl)-(R,R)-1,2-diaminocyclohexane prepared as in Example 2A (256 g, 0.955 mole) in THF (1.15 l) was added a 1 N solution of aqueous NaOH (1.15 l, 1.15 mole). Di-t-butyldicarbonate (229 g, 1.05 mole) was then added and the resulting mixture was stirred overnight. The layers were separated and the aqueous layer was adjusted to pH 2 with 1 N HCl and saturated with NaCl. The aqueous solution was then extracted with CH_2Cl_2 (2 x 500 ml) and the extracts and THF layer were combined and dried over $MgSO_4$. The solvent was removed *in vacuo* to give a yellow solid. The crude product was purified by crystallization from a THF-ether-hexanes mixture to give 310 g (88.1% yield) of the product as a white crystalline solid: mp: 137 - 139 °C; ^1H NMR ($CDCl_3$) δ 1.04 - 1.28 (m, 4 H), 1.44 (s, 9 H), 1.61 - 1.69 (m, 2 H), 1.94 - 2.01 (m, 2 H), 2.43 (s, 3 H), 2.86 (brs, 1 H), 3.30 (br d, J = 9.6 Hz, 1 H), 4.37 (br d, J = 6.7 Hz, 1 H), 5.48 (br d, J = 4.6 Hz, 1 H), 7.27 (d, J = 9.7 Hz, 2 H), 7.73 (d, J = 8.1

Hz, 2 H); MS (LRFAB, NBA - Li) m/z 375 $[M + Li]^+$.

C. Synthesis of Boc-(R,R)-Cyc(Ts)-gly-OMe

To a stirred solution of N-(p-toluenesulfonyl)-N'-(Boc)-(R,R)-1,2-diaminocyclohexane prepared as in Example 2B (310 g, 0.841 mole) in anhydrous DMF (3.11 l) at 0 °C was added NaH (37.4 g - 60 % in oil, 0.934 mole) in portions and the resulting mixture was stirred for 30 min. Methyl bromoacetate (142 g, 0.925 mole) was then added dropwise over 45 min and the mixture was allowed to warm to room temp while stirring overnight. After stirring for 17 h, the solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate(3 l) and H_2O (1 l). The ethyl acetate solution was washed with saturated $NaHCO_3$ (1 l), saturated NaCl (500 ml) and was dried over $MgSO_4$. The solvent was removed *in vacuo* and the resulting oil was dissolved in ether. Crystallization by the addition of hexanes gave 364 g (98 % yield) of the product (TLC (98:2 $CHCl_3$-MeOH/silica gel/UV detn) showed that the product contained about 5% starting material) as colorless needles: mp of pure sample 151 - 2 °C ; ^1H NMR ($CDCl_3$) δ 1.11 - 1.22 (m, 4 H), 1.45 (s, 9 H), 1.64 - 1.70 (m, 3 H), 2.16 - 2.19 (m, 1 H), 2.43 (s, 3 H), 3.34 - 3.40 (m, 2 H), 3.68 (s, 3 H), 4.06 (ABq, J = 18.5 Hz, $\Delta\upsilon$ = 155 Hz, 2H), 4.77 (br s 1 H), 7.30 (d, J = 8.3 Hz, 2 H), 7.82 (d, J = 8.3 Hz, 2 H); MS (LRFAB, DTT - DTE) m/z 441 $[M + H]^+$.

D. Synthesis of Boc-(R,R)-Cyc(Ts)-Gly-OH

To a stirred solution of impure Boc-(R,R)-Cyc(Ts)-Gly-OMe prepared as in Example 2C (217 g, 0.492 mole) in MeOH (1.05 l) was slowly added a 2.5N solution of aqueous NaOH (295 ml, 0.737 mole) and the resulting solution was stirred for 2 h. The solvent was removed *in vacuo* and the residue was dissolved in H_2O (1.5 l).

The solution was filtered to remove a small amount of solid and was washed with ether (7 x 1 l) to remove the impurity (compound 1B) which upon drying of the combined washes over $MgSO_4$ and removal of the solvent *in vacuo* resulted in recovery of 8.37 g. The pH of the aqueous solution was then adjusted to 2 with 1 N HCl and the product was extracted with ethyl acetate (3 x 1 l). The extracts were combined, washed with saturated NaCl (500 ml) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residual ethyl acetate removed by coevaporation with ether (500 ml) and then CH_2Cl_2 (500 ml) to give 205 g (97.6% yield) of the product as a white foam: ^1H NMR ($CDCl_3$) δ 1.15 - 1.22 (m, 4 H), 1.48 (s, 9 H), 1.55 - 1.68 (m, 3 H), 2.12 - 2.15 (m, 1 H), 2.43 (s, 3 H), 3.41 - 3.49 (m, 2 H), 3.97 (ABq, J = 17.9 Hz, $\Delta\upsilon$ = 69.6 Hz, 2 H), 4.79 (br s, 1 H), 7.31 (d, J = 8.1 Hz, 2 H), 7.77 (d, J = 8.3 Hz, 2 H), 8.81 (br s, 1 H); MS (LRFAB, NBA - Li) m/z 433 $[M + Li]^+$.

E. Synthesis of N-(p-toluenesulfonyl)-(S,S)-1,2-diaminocyclohexane

To a stirred solution of (S,S)-1,2-diaminocyclohexane (300 g, 2.63 mole) in CH_2Cl_2 (5.00 l) at -10 °C was added a solution of p-toluenesulfonylchloride (209 g, 1.10 mole) in CH_2Cl_2 (5.00 l) dropwise over a 8 h period, maintaining the temp at -5 to -10 °C. The mixture was allowed to warm to RT while stirring overnight. The mixture was concentrated *in vacuo* to a volume of 3 l and the white solid was removed by filtration. The solution was then washed with H_2O (10 x 1 l) and was dried over $MgSO_4$. Removal of the solvent *in vacuo* gave 289 g (98.3% yield) of the product as a yellow crystalline solid: ^1H NMR ($CDCl_3$) δ 0.98 - 1.27 (m, 4 H), 1.55 - 1.66 (m, 2 H), 1.81 - 1.94 (m, 2 H), 2.32 (dt, J = 4.0, 10.9 Hz, 1 H),

2.42 (s, 3 H), 2.61 (dt, J = 4.0, 9.9 Hz, 1 H), 7.30 (d, J = 7.9 Hz, 2 H), 7.77 (d, J = 8.3 Hz, 2 H); MS (LRFAB,GT - HCl) m/z 269 $[M + H]^+$.

F. Synthesis of N-(p-toluenesulfonyl)-N'-(Boc)-(S,S)-1,2-diaminocyclohexane

To a stirred solution of N-(p-toluenesulfonyl)-(S,S)-1,2-diaminocyclohexane prepared as in Example 2E (289 g, 1.08 mole) in THF (1.29 l) was added a 1 N solution of aqueous NaOH (1.29 l, 1.29 mole). Di-t-butyldicarbonate (258 g, 1.18 mole) was then added and the resulting mixture was stirred overnight. The solid was removed by filtration and washed with THF. The THF layer was separated and the aqueous layer was adjusted to pH 2 with 1 N HCl and saturated with NaCl. The aqueous solution was then extracted with CH_2Cl_2 (2 x 500 ml) and the extracts and THF layer were combined, washed with saturated NaCl (500 ml) and dried over $MgSO_4$. The solvent was removed *in vacuo* to give a yellow slurry. Crystallization with the addition of ether gave 364 g (91.9% yield) of the product as colorless needles: mp 137 - 139 °C; ^1H NMR ($CDCl_3$) δ 1.06 - 1.31 (m, 4 H), 1.44 (s, 9 H), 1.60 - 1.69 (m, 2 H), 1.95 - 1.99 (m, 2 H), 2.42 (s, 3 H), 2.86 (br s, 1 H), 3.30 (br d, J = 2.6 Hz, 1 H), 4.41 (br d, J = 7.3 Hz, 1 H), 5.54 (br d, J = 5.4 Hz, 1 H), 7.28 (d, J = 8.1 Hz, 2 H), 7.73 (d, J = 8.3 Hz, 2 H); MS (LRFAB, NBA - HCl) m/z 369 $[M + H]^+$.

G. Synthesis of Boc-(S,S)-Cyc(Ts)-gly-OMe

To a stirred solution of N-(p-toluenesulfonyl)-N'-(Boc)-(S,S)-1,2-diaminocyclohexane prepared as in Example 2F (364 g, 0.989 mole) in anhydrous DMF (3.66 l) at 0 °C was added NaH (47.4 g - 60% in oil, 1.19 mole) in portions and the resulting mixture was stirred for 1.5 h. The mixture was warmed to room temp and stirred an additional 30 min and then cooled back to 0 °C. Methyl

bromoacetate (189 g, 1.24 mole) was added dropwise over 30 min and the mixture was allowed to warm to RT while stirring overnight. After stirring for 17 h, the solvent was removed *in vacuo* and the residue was dissolved in a mixture of ethyl acetate(3 l) and H_2O (1 l). The layers were separated and the ethyl acetate solution was washed with saturated $NaHCO_3$ (1 l), H_2O (1 l), saturated NaCl (2 x 500 ml) and was dried over $MgSO_4$. The solvent was removed *in vacuo* and the resulting oil was dissolved in ether. Crystallization by the addition of hexanes gave 290 g of the crude product as yellow needles. Another 180 g was recovered from the filtrate as an oil. TLC (98:2 $CHCl_3$-MeOH/silica gel/UV detn) showed that both the solid and the oil contained starting material. ^1H NMR ($CDCl_3$) δ 1.06 - 1.29 (m, 4 H), 1.44 (s, 9 H), 1.58 - 1.66 (m, 3 H), 2.17 - 2.19 (m, 1 H), 2.43 (s, 3 H), 3.28 - 3.43 (m, 2 H), 3.68 (s, 3 H), 4.25 (ABq, J = 18.5 Hz, $\Delta \upsilon$ = 115 Hz, 2H), 4.76 (br s 1 H), 7.31 (d, J = 8.3 Hz, 2 H), 7.83 (d, J = 8.3 Hz, 2 H); MS (LRFAB, NBA - Li) m/z 447 $[M + H]^+$.

H. Synthesis of Boc-(S,S)-Cyc(Ts)-Gly-OH

To a stirred solution of impure Boc-(S,S)-Cyc(Ts)-Gly-OMe prepared as in Example 2G (197 g, 0.447 mole) in MeOH (925 ml) was slowly added a 2.5N solution of aqueous NaOH (268 ml, 0.670 mole) and the resulting solution was stirred for 2 h. The solvent was removed *in vacuo* and the residue was dissolved in H_2O (1 l). The solution was washed with ether (4 x 1 l) to remove the impurity (compound 2F) which upon drying of the combined washes over $MgSO_4$ and removal of the solvent *in vacuo* resulted in recovery of 14.8 g. The pH of the aqueous solution was then adjusted to 2 with 1 N HCl and the product was extracted with ethyl acetate (3 x 1 l). The extracts were combined, washed with saturated NaCl

and dried over $MgSO_4$. The solvent was removed *in vacuo* to give 171 g (89.7% yield) of the product as an oil which crystallized on standing: ^1H NMR ($CDCl_3$) δ 1.10 - 1.22 (m, 4 H), 1.45 (s, 9 H), 1.55 - 1.68 (m, 3 H), 2.13 - 2.16 (m, 1 H), 2.43 (s, 3 H), 3.39 - 3.41 (m, 2 H), 4.00 (ABq, J = 18.1 Hz, $\Delta\upsilon$ = 80.4 Hz, 2 H), 4.82 (br s, 1 H), 7.31 (d, J = 8.3 Hz, 2 H), 7.75 (d, J = 8.3 Hz, 2 H), 9.28 (br s, 1 H); MS (LRFAB, NBA - Li) m/z 433 $[M + Li]^+$.

I. Synthesis of Boc-(S,S)-Cyc(Ts)-Gly-Gly-OEt

To a stirred solution of Boc-(S,S)-Cyc(Ts)-Gly-OH prepared as in Example 2H (26.7 g, 62.5 mmole) in degassed anhydrous DMF (690 ml) was added HOBT (10.1 g, 75.0 mmole) and EDC-HCl (14.4 g, 75.0 mmole). After the resulting solution was stirred for 30 min, glycine ethyl ester hydrochloride (9.60 g, 68.8 mmole) was added and the pH adjusted to 8 with TEA. After stirring for 2.75 days the solvent was removed *in vacuo*. The residue was dissolved in a mixture of ethyl acetate (1 l) and H_2O (1 l) and the layers were separated. The aqueous layer was extracted with ethyl acetate (1 l) and the extracts were combine. The ethyl acetate solution was washed with 0.1 N HCl (1 l), saturated $NaHCO_3$ (1 l), saturated NaCl (500 ml) and was dried over $MgSO_4$. The solvent was removed *in vacuo* to give 30.2 g (94.4% yield) of the product as a white foam: ^1H NMR ($CDCl_3$) δ 1.19 - 1.23 (m, 3 H), 1.28 (t, J =7.05 Hz, 3 H), 1.42 (s, 11 H), 1.63 - 1.71 (m, 2 H), 2.16 - 2.18 (m, 1 H), 2.43 (s, 3 H), 3.50 - 3.57 (m, 2 H), 3.83 (ABq, J = 17.7 Hz, delta v = 35.7 Hz, 2 H), 4.01 (dABq, J = 6.05, 17.92 Hz, $\Delta\upsilon$ = 28.9 Hz, 2 H), 4.20 (q, J = 7.3 Hz, 2 H), 4.88 (br s, 1 H), 7.31 (d, J = 8.3 Hz, 2 H), 7.36 (br s, 1 H), 7.73 (d, J = 8.3 Hz, 2 H); MS (LRFAB, NBA - HCl) m/z 512 $[M + H]^+$.

J. Synthesis of (S,S)-Cyc(Ts)-Gly-Gly-OEt TFA salt

To a stirred solution of Boc-(S,S)-Cyc(Ts)-Gly-Gly-OEt prepared as in Example 2I (30.1 g, 58.8 mmole) in CH_2Cl_2 (265 ml) was added TFA (63 ml) and the resulting solution was stirred for 30 minutes. The solvent was removed *in vacuo* and residual TFA was coevaporated with CH_2Cl_2 (2 x 1 l) and ether (1 l). The oil was then triturated with ether (2 x 1 l) and the ether decanted. The resulting foam was dried *in vacuo* to give 33.7 g (assumed quantitative yield) of the product as a tan powder: ^1H NMR ($CDCl_3$) δ 0.96 - 1.23 (m, 4 H), 1.25 (t, J = 7.3 Hz, 3 H), 1.51 - 1.66 (m, 3 H), 2.12 - 2.26 (m, 1 H), 2.41 (s, 3 H), 2.98 - 3.10 (brs, 1 H), 3.67 - 3.71 (m, 1 H), 4.04 (ABq, J = 17.7 Hz, $\Delta\upsilon$ = 154 Hz, 2 H), 4.04 (d, J = 4.4 HZ, 2 H), 4.17 (q, J = 7.3 Hz, 2 H), 7.29 (d, J = 8.3 Hz, 2 H), 7.70 (d, J = 8.3 Hz, 2 H), 8.04 (br s, 1 H), 8.14 (br s, 3 H) MS (LRFAB, NBA - HCl) m/z 412 $[M + H]^+$.

K. Synthesis of Boc-(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OEt

To a stirred solution of Boc-(R,R)-Cyc(Ts)-Gly-OH prepared as in Example 2D (25.1 g, 58.8 mmole) in degassed anhydrous DMF (650 ml) was added HOBT (9.54 g, 70.6 mmole) and EDC-HCl (13.5 g, 70.6 mmole). After the resulting solution was stirred for 30 min (S,S)-Cyc(Ts)-Gly-Gly-OEt TFA salt prepared as in Example 1J (33.6 g, 58.8 mmole) was added and the pH was adjusted to 8 with TEA. After stirring for 2.75 days, the solvent was removed *in vacuo*. The residue was dissolved in a mixture of ethyl acetate (1 l) and H_2O (1 l) and the layers were separated. The ethyl acetate solution was washed with 0.1 N HCl (2 x 1 l), saturated $NaHCO_3$ (2 x 1 l), saturated NaCl (500 ml) and was dried over $MgSO_4$. The solvent was removed *in vacuo* to give 47.5 g (98.4%

yield) of the product as a tan foam: ^{1}H NMR ($CDCl_3$) δ 1.12 - 1.83 (m, 26 H), 2.21 - 2.24 (m, 2 H), 2.42 (s, 3 H), 2.43 (s, 3 H), 3.36 - 3.51 (br s, 2 H), 3.68 -3.96 (m, 6 H), 4.00 (d, J = 5.4 Hz, 2 H), 4.19 (q, J = 7.1 Hz, 2 H), 4.72 (br s, 1 H), 6.78 (br s, 1 H), 7.31 (d, J = 8.1 Hz, 4 H), 7.46 (br s, 1 H), 7.79 (m, 4 H); MS (LRFAB, NBA - HCl) m/z 820 $[M + H]^+$.

L. Synthesis of Boc-(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OH

To a stirred solution of Boc-(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OEt prepared as in Example 2K (47.4 g, 57.8 mmole) in MeOH (240 ml) was added a 2.5 N solution of aqueous NaOH (34.7 ml, 86.7 mmole) and the resulting solution was stirred for 2 h. The solvent was removed *in vacuo* and the residue was dissolved in H_2O (1 l). The aqueous solution was washed with ether (2 x 1 l) and the pH was adjusted to 2 with 1 N HCl. The solution was then saturated with NaCl and extracted with ethyl acetate (3.x 1 l). The combined extracts were dried over $MgSO_4$ and the solvent was removed *in vacuo*. The residual ethyl acetate was removed by coevaporation with CH_2Cl_2 and the resulting foam was dried *in vacuo* to give 45.7 g (99.7% yield) of the product as a tan powder: ^{1}H NMR ($CDCl_3$) δ 1.16 - 1.75 (m, 23 H), 2.13 - 2.17 (m, 2 H), 2.41 (s, 3 H), 2.42 (s, 3 H), 3.49 - 4.16 (m, 10 H), 4.53 (br s, 1 H), 7.01 (br s, 1 H), 7.30 (d, J = 8.1 Hz, 4 H), 7.40 (br s, 1 H), 7.79 (d, J = 8.1 Hz, 2 H), 7.86 (d, J = 7.7 Hz, 2 H), 10.40 (br s, 1 H); MS (LRFAB, NBA - HCl) m/z 792 $[M + H]^+$.

M. Synthesis of (R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OH TFA salt

To a stirred solution of Boc-(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OH prepared as in Example 2L (45.5

g, 57.5 mmole) in CH_2Cl_2 (260 ml) was added TFA (60 ml). The resulting solution was stirred for 30 min and the solvent was removed *in vacuo*. Residual TFA was removed by coevaporation with CH_2Cl_2 (3 x 1 l) and trituration of the resulting foam with ether (1 l, 2 x 750 ml), decanting the ether each time. After desiccation *in vacuo*, 47.4 g (100% yield) of the product was obtained as an off white powder: ^1H NMR ($CDCl_3$) δ 1.05 - 1.31 (m, 9 H), 1.48 - 1.63 (m, 5H), 2.11 - 2.21 (m, 2 H), 2.40 (s, 3 H), 2.42 (s, 3 H), 3.25 (br s, 1 H), 3.60 - 3.80 (m, 3 H), 3.83 - 4.19 (m, 6 H), 6.94 (br s, 1 H), 7.31 (m, 4 H), 7.69 (m, 4 H), 7.83 (br s, 3 H), 13.17 (br s, 2 H); MS (LRFAB, DTT - DTE) m/z 692 $[M + H]^+$.

N. Synthesis of Cyclo-[(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-]

To a stirred solution of (R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly-OH TFA salt prepared as in Example 2M (32.2 g, 40.0 mmole) in degassed anhydrous DMF (10.0 l) at -78 °C was added DPPA (13 4 g, 48.8 mmole). The pH of the solution was then adjusted to 8 with TEA and the solution was allowed to stand for 6 h at -78 °C. The pH was readjusted to 8 with TEA and the solution was warmed to -45 °C for 24 h. After readjusting the pH as before, the solution was allowed to warm to -40 °C for 24 h. The pH was adjusted as before and the solution was allowed to stand at -20 °C for 24 h. The pH was readjusted as before and the solution was allowed to warm to 2 °C over 24 h. The pH had dropped only slightly. The pH was readjusted as before and the solution was allowed to stand at 2 °C for another 24 h after which time the pH had not changed. The solution was divided equally among 6 - 4 l beakers and H_2O (1.1 l) was added to each. Then added a total of 5.00 kg mixed-bed ion exchange resin to the solution (divided equally among the 6 beakers) and stirred the mixtures for 6 h. The resin was then

filtered and washed with DMF. The solvent was then removed *in vacuo* and the solid residue was dissolved in MeOH (100 ml) and filtered to remove finely divided solids. The solution was then concentrated *in vacuo* to a volume of 25 ml and ether was added periodically as the crystallization proceeded to give 22.2 g (82.5 % yield) of the product as colorless needles; mp 190 - 200 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 - 2.13 (m, 16 H), 2.41 (s, 3 H), 2.45 (s, 3 H), 3.56 - 3.97 (m, 10 H), 6.66 (br s, 1 H), 7.18 (br s, 1 H), 7.34 (d, J = 8.1 Hz, 4 H), 7.65 (br s, 1 H), 7.71 (d, J = 7.3 Hz, 2 H), 7.89 (d, J = 7.3 Hz, 2 H); MS (LRFAB, NBA - Li) m/z 680 $[M + Li]^+$.

O. Synthesis of 2,3-(R,R)-8,9-(S,S)-Bis-cyclohexano-1,4,7,10,13-pentaazacyclopentadecane

To a stirred solution of Cyclo-[(R,R)-Cyc(Ts)-Gly-(S,S)-Cyc(Ts)-Gly-Gly] prepared as in Example 2N (19.4 g, 28.8 mmole) in anhydrous THF (475 ml) was added a solution of 1.0 M $LiAlH_4$ in THF (345 ml, 345 mmole) dropwise over 30 min. The yellow homogeneous solution was refluxed for 20 h (by which time it had become heterogeneous) and was then cooled to 0 °C. The mixture was then quenched by the dropwise addition of a 10% $NaSO_4$ solution (50 ml) while cooling in an ice bath. The solids were removed by filtration under an Ar blanket and the THF was removed *in vacuo* to give an oil which rapidly crystallized. The solids were then refluxed with anhydrous THF (1 l) for 1 h and the mixture was filtered and the solvent removed *in vacuo* as before. The solids were then refluxed with a mixture of THF (1 l) and MeOH (500 ml) for 1 h and worked up as before. The residues from the extractions were then dissolved in anhydrous THF, combined and solids were removed by filtration. The solvent was removed *in vacuo* and the yellow foam dried by azeotroping H_2O with toluene

(1.75 l) in vacuo at 90°C. Then refluxed the solids with hexanes (1 l) for 30 min and transferred the hot solution to a tared flask and removed the solvent *in vacuo* to give 6.1 g of an oil which crystallized on standing. The remaining solids were refluxed with hexanes as before and obtained 1.4 g of an oil which crystallized on standing. The solids were then dissolved in MeOH and toluene (1 l) was added. The solvent was removed *in vacuo* and any remaining H_2O was removed by azeotroping with toluene (1 l) and then hexanes (3 x 1 l). The resulting fine powder was refluxed with hexanes (1 l) for 2 h under argon and filtered into a tared flask. The solvent was removed *in vacuo* to give 1.7 g oil which crystallized on standing. The crystalline residues from the 3 extracts were dissolved in hexanes and combined. A small amount of haziness was removed by filtration and the solution was concentrated to give 5.3 g (57% yield) of product as a pale yellow crystalline solid. Recrystallization from acetonitrile gave 4.47 g (48.0% yield) of a colorless crystalline solid: mp 107 - 8°C; ^1H NMR ($CDCl_3$) δ 0.95 - 1.01 (m, 4 H), 1.19 - 1.24 (m, 4 H), 1.70 - 1.73 (m, 4 H), 1.97 (br s, 5 H), 2.08 - 2.14 (m, 8 H), 2.49 - 2.68 (m, 6 H), 2.74 - 2.80 (m, 2 H), 2.85 - 2.90 (m, 2 H), 2.94 - 2.99 (m, 2 H); MS (LRFAB, NBA) m/z 324 $[M + H]^+$; Anal. calcd. for $C_{18}H_{37}N_5$: C, 66.83; H, 11.53; N, 21.65. Found: C, 66.80; H, 11.44; N, 21.71.

P. Synthesis of [Iron (III) dichloro (2,3-(R,R)-8,9-(S,S)-Bis-cyclohexano-1,4,7,10,13-pentaazacyclopentadecane] chloride

Under an inert atmosphere in a drybox, 199 mg (0.615 mmol) of the ligand, 2,3-(R,R)-8,9-(S,S)-bis-cyclohexano-1,4,7,10,13-tetraazacyclopentadecane, was dissolved in 10 ml of an anhydrous methanol solution containing 0.615 mmol (100 mg) of anhydrous $FeCl_3$. The

resultant dark yellow-orange solution was heated to reflux for one-half hour with stirring and then allowed to cool to room temperature and then filtered. The filtrate was reduced to dryness and redissolved in 25 cc of hot abs. Ethanol and then filtered through Celite®. The ethanol solution was reduced to ~ 10 ml volume. To this warm ethanol solution was added diethyl ether to the cloud point. The solution was allowed to sit undisturbed for 16 hours upon which a yellow microcrystalline precipitate had formed. The yellow solid was isolated by filtration, washed with diethyl ether, and dried *in vacuo* overnight. The yield after drying was 235 mg (0.486 mmol) corresponding to a 79% theoretical yield. Anal. Calc. for $C_{10}H_{20}N_5FeCl_3 \cdot CH_3CH_2OH$: C, 45.25; H, 8.16: N, 13.19; Cl, 20.03. Found: C, 44.97; H, 8.07; N, 13.01; Cl, 19.88. Mass spectrum (FAB, NBA matrix): m/z 449 ($[Fe(L)Cl+e]^+$ and m/z 431 ($[Fe(L)Cl_2]^+$ were observed.

A. Synthesis of N,N'-Bis(chloroacetyl)1R,2R-diaminocyclohexane

1R, 2R-(-)-Diaminocyclohexane (6.98 g, 61.13 mmol) was dissolved in 75 ml of alcohol free $CHCl_3$ in a 4 neck 2000 ml round bottom flask along with 37 ml H_2O under argon. Two Normag dropping funnels were connected to the reaction flask, and charged separately with, chloroacetyl chloride (15 ml, 188.3 mmol) in alcohol free $CHCl_3$ (88 ml), and K_2CO_3 (24.1 g, 174.4 mmol) in 918 ml H_2O. An internal thermometer was inserted into the reaction flask. After cooling the two phase mixture in the reaction flask to 0 °C in an ice bath, the additions from the dropping funnels were started in such a way as to keep the proportion of each solution added approximately equal over a 1 h 20 min period. During the addition, an ice salt bath was used to moderate the

temperature, keeping it between 3 and -3 °C. A shell of ice formed on the inside of the reaction flask which didn't seem to impede the stirring. The reaction flask was removed from the ice bath at the end of the addition and was stirred for 2 h 20 min. The lower chloroform layer appeared to have a considerable quantity of a light solid in it at ice bath temperature, but it dissolved as the reaction warmed. The reaction mixture was placed in a separatory funnel, some additional chloroform added, and the layers were separated. The aqueous layer was extracted with another portion of $CHCl_3$, and the combined chloroform layers were washed with water, then saturated NaCl, dried (Na_2SO_4) and stripped down to a brownish white solid. This solid was stirred overnight with about 450 ml of ether, then filtered, much of the color staying in the ether, giving a beige solid, 13.68 g, 51.60 mmol, 84.4% yield. ^1H NMR ($CDCl_3$, 400 MHz) d 1.34 (m, 4H), 1.80 (m, 2H), 2.08 (m, 2H), 3.74 (m, 2H), 3.99 (ABq, J = 15.1 Hz, dn = 8.2 Hz, 4H), 7.26 (br s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) d 24.59, 32.07, 42.45, 53.94, 166.65; MS (FAB, NBA-LiCl matrix): *m/z* (relative intensity) 273 (100) $[M+ Li]^+$, 275 (71) $[M+ Li]^+$.

B. Synthesis of N-Tosylglycyl-1R,2R-diaminocyclohexane

1R, 2R-Diaminocyclohexane (10.0 g, 87.57 mmol) was dissolved in dry DMF (150 ml) under argon and cooled to -10°C. Separately, N-tosylglycine (10.04 g, 43.62 mmol), 1-hydroxybenzotriazole (6.75 g, 44.08 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (8.45 g, 44.05 mmol) were dissolved in dry DMF (150 ml), and cooled to -10°C under argon. The latter solution was added to the diaminocyclohexane solution at -10°C via cannula. After 2 hours at this temperature, water (8 ml) was added and the reaction was allowed to warm to 0°C over one hour, then to room

temperature over the next half hour. The solvent was removed on the rotary evaporator under reduced pressure. The residue was heated to 40 to 42°C with water (150 ml) added in small portions with stirring. After 25 minutes this solution was filtered. The white precipitate was largely the bis adduct (5.55 g). Exactly 68 ml of the filtrate was worked up by repeated extraction with dichloromethane (9 x 50 ml). The combined organic phase was dried (sodium sulfate), filtered and the solvent was removed. The resulting white solid which contained some residual DMF was redissolved in dichloromethane (30 ml) and added dropwise to a stirred solution of 9: 1 ether: hexane (250 - 300 ml) giving an immediate precipitate which was stirred overnight and then filtered. This procedure was repeated, stirring for three hours instead of overnight. After drying the white product on the vacuum line, 2.36 g, 7.25 mmol were obtained, equivalent to a 36.7% yield for the entire reaction. ^{1}H NMR ($CDCl_3$, 400 MHz) d 1.10 - 1.34 (m, 4H), 1.70 (d, J = 9.7 Hz, 2H), 1.81 - 1.97 (2 m, 2H), 2.41 (s, 3H), 2.51 (td, J = 10.2, 3.8 Hz, 1H), 3.53 (m + ABq, J = 16.9 Hz, dn = 51.6 Hz, 3H), 3.69 (br s, 3H), 6.84 (d, J = 9.1 Hz, 1H), 7.30 (d, J = 8.3 Hz, 2H), 7.73 (d, J = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) d 21.48, 24.87, 24.97, 32.08, 35.16, 46.09, 54.85, 55.78, 127.15, 129.85, 136.02, 143.84, 168.69; MS (GTHCl): *m/z* 326 (100) $[M+ H]^+$.

C. Synthesis of 2R,3R,8R,9R-Bis(cyclohexano)-13-p-toluenesulfonyl-1,4,7,10,13-pentaazacyclopentadecan-6,11,15-trione

N-p-Toluenesulfonylglycyl-1R,2R-diaminocyclohexane (1.11 g, 3.42 mmol) and N, N'-bis(chloroacetyl)-1R,2R-diaminocyclohexane (0.913 g, 3.42 mmol) were combined in a one liter flask and dry N,N-dimethylacetamide (650 ml) was added. The flask was inerted. After 10 minutes, the sodium hydride was added

directly to the homogeneous mixture. The reaction flask was placed in a 70°C oil bath. After the internal temperature reached 45-50°C, gas evolution became constant. The oil bath temperature was stabilized at about 65°C with some excursions from about 60 to 75°C. Overnight, the reaction mixture became homogeneous. After heating for 17 hours the reaction flask was removed from the bath and allowed to cool. The solvent was removed under reduced pressure, and the yellowish oil was placed on the vacuum line. The residue was treated with dichloromethane (300 ml) and washed with water (40 ml) and twice with saturated sodium chloride (40 ml each). After combining, the aqueous layers were backwashed with dichloromethane (100 ml). The combined organic layers were dried over sodium sulfate, filtered, and stripped down to a viscous yellow oil which was placed on the vacuum line, 2.14 g. This residue was chromatographed using 0.5% NH_4OH/ 9% CH_3OH/ 90.5% CH_2Cl_2. On tlc on silica using the same system, R_f = 0.25. Fractions containing the correct spot were combined and evaporated down to a slightly off white solid, 0.89 g, 1.71 mmol, 50.1% yield. ^1H NMR ($CDCl_3$, 300 MHz) d 0.92 - 2.1 (several m, 15H), 2.27 (m, 1H), 2.41 (s, 3H), 3.10 (ABq, J = 16 Hz, dn = 34.2 Hz, 2H), 3.39 (m, 1H), 3.58 (m, 3H), 3.83 (m, 1H), 4.08 (d, J = 17.6 Hz, 1H), 4.39 (d, J = 17.4 Hz, 1H), 7.30 (m, 3H), 7.44 (d, J = 5.9 Hz, 1H), 7.76 (d, J = 7.8 Hz, 2H), 8.05 (d, J = 8.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) d 21.39, 24.20, 24.69, 24.87 (double intensity), 31.49, 31.54, 31.58, 32.43, 47.01, 52.19, 52.25, 52.49, 52.97, 55.63, 58.36, 127.65, 129.67, 135.28, 143.97, 167.52, 170.04, 172.84; MS (FAB, NBA-LiCl matrix): *m/z* (relative intensity) 526 (100) $[M+ Li]^+$, 370 (29) $[M+ Li -Ts]^+$.

D. Synthesis of 2R,3R,8R,9R-Bis(cyclohexano)-1,4,7,10,13-pentaazacyclopentadecane

2R,3R,8R,9R-Bis(cyclohexano)-13-p-toluenesulfonyl-1,4,7,10,13-pentaazacyclopentadecan-6,11,15-trione (4.072 g, 7.84 mmol) was placed in a 1 liter flask under an argon atmosphere, and dry 1,2-dimethoxyethane (dme, 220 ml) was added. The powder fused, and did not appreciably dissolve. It was partially broken up with a spatula, and stirred in a cold water bath while lithium aluminum hydride (0.5 M in dme, 140 ml, 70 mmol) was added in portions over a 10 minute period. Initially, the solution became cloudy, and undissolved chunks of compound were present. After about 70 ml had been added, the solution was fairly homogeneous, with only a few undissolved pieces remaining, which appeared to dissolve with gas evolution. Heating was started after a few minutes, and the solution rapidly became heterogeneous and yellow. The reaction mixture was refluxed overnight. Reflux was ended after 16.5 hours. The reaction mixture was cooled in a cold water bath, then in a -18°C bath. Water (2.2 ml) was added *cautiously* in small quantities over a 5 to 10 minute period, followed more rapidly by 15% NaOH (2.2 ml), then by water (6.6 ml). Stirring was continued for 2 hours in the ice bath. Tetrahydrofuran (thf, 210 ml) was added and stirring was continued for about an hour. The thick white suspension was allowed to settle, and was filtered with a filter transfer device (#1 Whatman paper). The filtrate was stripped. The white residue was stirred with thf (150 ml) and filtered onto the stripped first filtrate. The solvent was removed under reduced pressure, and the residue was placed on the vacuum line. The resulting yellow-white solid was extracted with hot dry hexane (initially 70 ml, 65°C; then an additional 15 ml) and filtered through a filter transfer device (#50 Whatman paper), and the

solvent was removed under reduced pressure. This crude product, weight about 1.5 g, was dissolved in hot (>70°C) dry acetonitrile (about 60 ml), filtered (filter transfer device, #50 Whatman paper), concentrated by more than half, reheated to dissolve all of the white solid, then allowed to cool slowly to room temperature. White crystals were obtained, 0.923 g, 2.85 mmol, 36.4% yield. ^{1}H NMR (C_6D_6, 300 MHz) d 0.75 - 1.21 (several m, 8H), 1.23 - 2.19 (several m, 17H), 2.36 - 2.61 (several H, 6H), 2.61 - 2.73 (m, 2H), 2.74 - 2.85 (m, 2H), 2.90 (d, J = 7.5 Hz, 2H); ^{13}C NMR (C_6D_6, 75 MHz) d 25.48, 25.56, 32.41, 32.48, 46.50, 47.82, 49.56, 61.86, 62.88; Anal. calcd. for $C_{18}H_{37}N_5$: C, 66.83; H, 11.54; N, 21.65. Found: C, 66.66; H, 11.46; N, 21.78.

Example 3

E. Synthesis of [Iron(III)dichloro(2,3-(R,R)-8,9-(R,R)-bis-cyclohexano-1,4,7,10,13-pentaazacyclopentadecane)] hexafluorophosphate

Upon an inert atmosphere in a drybox, 97 mg (0.30 mmol) of the ligand, 2R, 3R, 8R, 9R-Bis(cyclohexano)-1,4,7,10,13-tetraazacyclopentadecane, was dissolved in 15 ml of anhydrous methanol. To this solution was added with vigorous stirring 2 ml of a pyridine solution containing 0.30 mmol (48 mg) of anhydrous $FeCl_3$. The resultant dark brown solution was heated to reflux for three hours with stirring and then allowed to cool to room temperature and then filtered. To the filtrate was added 20 ml of a clear methanolic solution of NH_4PF_6 (120) mg). This solution was evaporated to dryness and 2 ml of anhydrous acetonitrile was added to the resultant solid. This mixture was stirred vigorously for two hours and then filtered and the resultant yellow filtrate was evaporated to dryness. The resultant yellow solid was dissolved in hot ethanol and filtered.

The solution was evaporated to dryness and the resultant yellow solid collected by filtration from a diethyl ether wash. The yellow precipitate was dried *in vacuo* overnight. The yield after drying was 75 mg corresponding to a 42% theoretical yield. Anal. Calc. for $C_{18}H_{37}N_5Cl_2F_6FeP$: C, 36.35; H, 6.28: N, 11.78. Found: C, 36.37; H, 6.34; N, 11.58.

Example 4

A. Synthesis of Boc-DAla-Ala-OEt

To a solution of Boc-DAla (25.0 g, 132.1 mmol) in DMF (1450 ml) was added HOBT•H_2O (19.8 g, 129.3 mmol) and EDC•HCl (28.0 g, 146.3 mmol) and the resulting solution was allowed to stir at RT for 30 min. To this solution was added Alanine ethyl ester hydrochloride (20.3 g, 132.1 mmol) and TEA (20.4 ml, 146.3 mmol) and the reaction was allowed to stir for 3 days (for convenience). The DMF was evaporated and the residue was partitioned between water (500 ml) and ethyl acetate (500 ml). The ethyl acetate solution was washed with 1N $NaHSO_4$ (250 ml), water (250 ml), saturated $NaHCO_3$ (250 ml), brine (250 ml) and dried over Na_2SO_4. Filtration and concentration afforded 31.7 g (83% yield) of the desired dipeptide as a white foam: ^1H NMR (DMSO-d_6) δ 1.14 (d, J = 7.4 Hz, 3 H), 1.16 (t, J = 7.4 Hz, 3 H), 1.24 (d, J = 7.0 Hz, 3 H), 1.36 (s, 9 H), 3.96 - 4.09 (m, 3 H), 4.17 - 4.22 (apparent quintet, J = 7.4 Hz, 1 H), 6.77 (d, J = 7.7 Hz, 1 H), 8.09 (d, J = 7.0 Hz, 1 H); MS (LRCI, CH_4) m/z (relative intensity) = 317 (5) [M + C_2H_5]$^+$, 289 (60) [M + H]$^+$.

B. Synthesis of Boc-Ala-Ala-OH

To a suspension of the dipeptide (15.0 g, 93.6 mmol) in THF (192 ml) was added 0.5 N NaOH solution (192

ml). To the resulting solution was added di-t-butyldicarbonate (26.6 g, 121.7 mmol) at once. The pH of the reaction was maintained at ~10 for 5 h and the mixture was then allowed to stir overnight. The pH of the reaction was again adjusted to ~10 and the solution was extracted with ethyl acetate (2 x 100 ml). The pH of the aqueous layer was adjusted to ~3.5 with aqueous potassium bisulfate and this mixture was extracted with ethyl acetate (3 x 100 ml). The combined extracts were dried ($MgSO_4$), filtered and concentrated to afford 20.7 g (85% yield) of the desired product as a white powder: ^1H NMR (DMSO-d_6) δ 1.16 (d, J = 6.8 Hz, 3 H), 1.28 (d, J = 7.3 Hz, 3 H), 1.38 (s, 9 H), 3.95 - 4.09 (m, 1 H), 4.20 (quintet, J = 7.3 Hz, 1 H), 6.87 (d, J = 8.0 Hz, 1 H), 8.00 (d, 7.3 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 267.1557 [M + Li]+; 267.1532 calcd for $C_{11}H_{20}N_2O_5Li$.

C. Synthesis of DAla-Ala-OEt•TFA

The protected dipeptide (31.4 g, 109 mmol) was dissolved in methylene chloride (200 ml) and TFA (66 ml) was added. The resulting solution was allowed to stir for 30 min at RT and concentrated. The residue was coevaporated with methylene chloride (2 x 200 ml), dissolved in ether and oiled out with the addition of excess hexanes. The solvents were decanted and the residue was pumped at high vacuum for 12 h to afford 39.6 g (100% yield, contains residual TFA) of the desired TFA salt as an orange oil: ^1H NMR (DMSO-d_6) δ 1.16 (t, J = 7.0 Hz, 3 H), 1.28 (d, J = 7.0 Hz, 3 H), 1.34 (d, J = 7.0 Hz, 3 H), 3.86 (bs, 1H), 4.07 (q, J = 7.0 Hz, 2 H), 4.26 (quintet, J = 7.0 Hz, 1 H), 8.21 (bs, 3 H), 8.86 (d, J = 7.4 Hz, 1 H); MS (LRCI, CH_4) m/z (relative intensity) 217 (5) $[M + C_2H_5]^+$, 189 (40) $[M+H]^+$.

D. Synthesis of Boc-Ala-Ala-DAla-Ala-OEt

To a solution of Boc-Ala-Ala-OH (20.1 g, 77.2 mmol) in DMF (850 ml) was added HOBT•H_2O (13.1 g, 85.4 mmol) and EDC•HCl (16.4 g, 85.4 mmol). To this solution was added DAla-Ala-OEt•TFA (23.3 g, 77.2 mmol) followed by TEA (11.9 ml, 85.4 mmol) and the resulting mixture was stirred for 12 h thereafter. The DMF was evaporated and the residue was dissolved in ethyl acetate (300 ml) and washed with 1 N potassium bisulfate (150 ml), water (150 ml), saturated sodium bicarbonate (150 ml) and brine (150 ml). The ethyl acetate layer was dried ($MgSO_4$), filtered and concentrated to half volume and crystallization was allowed to proceed. Isolation by filtration afforded 20.5 g (62% yield) of the desired tetrapeptide as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.13 (d, J = 7.0 Hz, 3 H), 1.17 (two coincidental d, J = 7.0 Hz, 6 H), 1.25 (d, J = 7.4 Hz, 3 H), 3.91 - 4.30 (m, 6 H), 6.87 (d , 7.0 Hz, 1 H), 7.92 (d, J = 6.3 Hz, 1 H), 8.07 (d, J = 7.3 Hz, 1 H), 8.09 (d, J = 6.6 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 437.2600 $[M + Li]^+$; 437.2588 calcd for $C_{19}H_{34}N_4O_7Li$.

E. Synthesis of Boc-Ala-Ala-DAla-Ala-OH

A solution of Boc-Ala-Ala-DAla-Ala-OEt (10.9 g, 25.3 mmol) in methanol (100 ml) was treated with 2.5 M sodium hydroxide (20.0 ml, 50.0 mmol) and the resulting solution was allowed to stir for 2 h at RT. At this time the pH of the solution was lowered to ~3 with the addition of aqueous potassium bisulfate and the resulting mixture was extracted with ethyl acetate (3 x 100 ml). The combined extracts were dried ($MgSO_4$), filtered and concentrated to afford 6.8 g (67% yield of the desired acid as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.17 (d, J = 7.2 Hz, 3 H), 1.20 (two coincidental d, J = 7.1 Hz, 6 H), 1.28 (d, J = 1.3 Hz, 3 H), 1.38 (s, 9 H),

3.90 - 4.00 (m, 1 H), 4.17 - 4.30 (m, 3 H), 6.93 (d, J = 6.7 Hz, 1 H), 7.96 (d, J = 6.7 Hz, 1 H), 8.04 (d, J = 7.4 Hz, 1 H), 8.07 (d, J = 7.8 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 409.2331 $[M + Li]^+$; 409.2353 calcd for $C_{17}H_{30}N_4O_7Li$.

F. Synthesis of Boc-Ala-Ala-DAla-Ala-DAla-OBzl

To a solution of Boc-Ala-Ala-DAla-Ala-OH (6.5 g, 16.3 mmol) in DMF (180 ml) was added HOBT•H_2O (2.86 g, 18.7 mmol) and EDC•HCl (3.58 g, 18.7 mmol). The resulting solution was allowed to stir for 15 min at RT and treated with DAla-OBzl p-toluenesulfonate salt (6.57 g, 18.7 mmol) and TEA (2.6 ml, 18.7 mmol). This mixture was allowed to stir for 12 h thereafter. The DMF was evaporated and the residue was partitioned between ethyl acetate (300 ml) and water (300 ml). The ethyl acetate layer was washed with 1 N potassium bisulfate (150 ml), water (150 ml), saturated sodium bicarbonate (150 ml) and brine (150 ml). The ethyl acetate layer was then dried ($MgSO_4$), filtered and concentrated to afford 9.0 g (100% yield) of the desired compound as a white powder: ^1H NMR (DMSO-d_6) δ 1.17 (d, J = 7.3 Hz, 3 H), 1.21 (two coincidental d, J = 7.0 Hz, 6 H), 1.22 (d, J = 7.0 Hz, 3 H), 1.32 (d, J = 7.3 Hz, 3 H), 1.37 (s, 9 H), 3.90 - 4.09 (m, 1 H), 4.18 - 4.34 (m, 4 H), 5.13 (ABq, J = 12.7, $\Delta\nu$ = 10.5 Hz, 2 H), 6.94 (d, J = 7.3 Hz, 1 H), 7.30 - 7.41 (m, 5 H), 7.97 (d, J = 7.0 Hz, 1 H), 8.10 - 8.18 (m, 2 H), 8.25 (d, J = 6.9 Hz, 1 H); MS (HRFAB, NBA - Li) m/z = 570.3140 $[M + Li]^+$; 570.3115 calcd for $C_{27}H_{41}N_5O_8Li$.

G. Synthesis of Ala-Ala-DAla-Ala-DAla•HCl

Boc-Ala-Ala-DAla-Ala-DAla-OEt (10.4 g, 18.7 mmol) was dissolved in acetic acid (225 ml) and treated with concentrated hydrochloric acid (75 ml). The resulting

solution was allowed to stir at RT for 14 h thereafter. At this time the reaction was concentrated, coevaporated with water (50 ml) and azeotropically dried by toluene coevaportation (2 x 100 ml) to afford 7.8 g (96% yield) of the deprotected pentapeptide hydrochloride as a white powder: ^{1}H NMR (D_2O) δ 1.29 - 1.39 (m, 12H), 1.47 (d, J = 7.0 Hz, 3 H), 4.06 (q, J = 7.0 Hz, 1 H), 4.18 - 1.38 (m, 4 H); MS (LRFAB, NBA - HCl) 374 $[M + H]^+$.

H. Synthesis of *Cyclo*-(Ala-Ala-DAla-Ala-DAla-)

To a solution of Ala-Ala-DAla-Ala-DAla•HCl (7.8 g, 19.0 mmol) in DMF (2400 ml) at -40 °C was added DPPA (6.29 g, 22.8 mmol) and enough TEA to adjust the "pH" to ~8 (measured by spotting the reaction mixture on moistened hydrion paper). This solution was allowed to stand at -23 °C for 48 hours and at 8 °C for 48 hours. During this time the "pH" was again maintained at ~8 with the periodic addition of TEA. At the end of this period the reaction mixture was poured into water (2400 ml) and stirred with mixed-bed ion exchange resin (1200 g) for 6 h. The resin was removed by filtration and the filtrate was concentrated to a volume of ~ 100 ml. Ether (500 ml) was added and the precipitated white solid was isolated by filtration and washed with more ether (250 ml). The solid was then triturated by stirring with THF (100 ml) for 12 h (to remove traces of DMF), filtered and thoroughly dried to afford 3.15 g (47% yield) of the desired cyclic peptide as a fine white powder: ^{1}H NMR (DMSO-d_6) δ 1.08 - 1.25 (m, 12 H), 1.24 (d, J = 7.3 Hz, 3 H), 4.00 - 4.10 (m, 1 H), 4.26 - 4.30 (m, 2 H), 4.34 (q, J = 7.2 Hz, 1 H), 4.41 (q, J = 7.6 Hz, 1 H), 7.58 (d, J = 7.0 Hz, 1 H), 7.83 (d, J = 8.4 Hz, 1 H), 8.22 (d, J = 6.2 Hz, 1 H), 8.33 (d, J = 7.81, 1 H), 8.49 (d, J = 6.8 Hz, 1 H); MS (HRFAB, NBA - HCl) m/z 356.1989 $(M + H)^+$; 356.1934 calcd for $C_{15}H_{25}N_5O_5$ $(M + H)^+$.

I. Synthesis of (2S, 5R, 8S, 11R, 14S)-Pentamethyl-1,4,7,10,13-pentaazacyclopentadecane

To a stirred suspension of *cyclo*-(Ala-Ala-DAla-Ala-DAla-) (3.10 g, 8.70 mmol) in THF (70 ml) at RT was added lithium aluminum hydride (108 ml of a 1.0 M solution in THF, 108 mmol). The resulting mixture was stirred at RT for 2 h and heated to reflux for 16 h thereafter. The mixture was then cooled to ~-20 °C and quenched with the dropwise addition of saturated sodium sulfate (~30 ml). The resulting mixture was concentrated to a dry white powder and this powder was triturated with ether (2 x 150 ml). The combined triturates were concentrated and recrystallized form acetonitrile to afford 1.10 g (44 % yield) of the desired ligand as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.96 (d, J = 5.2 Hz, 3 H), 1.00 (two coincidental d, J = 5.0 Hz, 6 H), 1.02 (two coincidental d, J = 5.0 Hz, 6 H), 1.30 - 1.55 (bm, 2 H), 1.85 - 2.15 (bs, 3 H), 2.05 - 2.19 (m, 5 H), 2.42 - 3.00 (complex m, 12 H); MS (HRFAB, NBA - HCl) m/z = 286.3013 $(M + H)^+$; 286.2971 calcd for $C_{15}H_{36}N_5$.

J. Synthesis of [Iron (III)dichloro-(2S, 5R, 8S, 11R, 14S)-Pentamethyl-1,4,7,10,13-pentaazacyclopentadecane]hexafluorophosphate

This complex was prepared in a fashion entirely analogous to that described previously in Example 3. After recrystallization of the crude yellow solid from ethanol, yellow crystals were obtained in a 40% yield. Analysis calc. for $C_{15}H_{35}N_5Cl_2FeF_6P$: C, 32.37; H, 6.34: N, 12.59. Found: C, 32.44; H, 6.30; N, 12.40.

Example 5

Stopped-Flow Kinetic Analysis

Stopped-flow kinetic analysis has been utilized to determine whether a compound can catalyze the dismutation

of superoxide (Riley, D.P., Rivers, W.J. and Weiss, R.H., "Stopped-Flow Kinetic Analysis for Monitoring Superoxide Decay in Aqueous Systems," Anal. Biochem, 196, 344-349 [1991]). For the attainment of consistent and accurate measurements all reagents were biologically clean and metal-free. To achieve this, all buffers (Calbiochem) were biological grade, metal-free buffers and were handled with utensils which had been washed first with 0.1 N HCl, followed by purified water, followed by a rinse in a 10^4 M EDTA bath at pH 8, followed by a rinse with purified water and dried at 65°C for several hours. Dry DMSO solutions of potassium superoxide (Aldrich) were prepared under a dry, inert atmosphere of argon in a Vacuum Atmospheres dry glovebox using dried glassware. The DMSO solutions were prepared immediately before every stopped-flow experiment. A mortar and pestle were used to grind the yellow solid potassium superoxide (~100 mg). The powder was then ground with a few drops of DMSO and the slurry transferred to a flask containing an additional 25 ml of DMSO. The resultant slurry was stirred for 1/2 h and then filtered. This procedure gave reproducibly ~2 mM concentrations of superoxide in DMSO. These solutions were transferred to a glovebag under nitrogen in sealed vials prior to loading the syringe under nitrogen. It should be noted that the DMSO/superoxide solutions are extremely sensitive to water, heat, air, and extraneous metals. A fresh, pure solution has a very slight yellowish tint.

Water for buffer solutions was delivered from an in-house deionized water system to a Barnstead Nanopure Ultrapure Series 550 water system and then double distilled, first from alkaline potassium permanganate and then from a dilute EDTA solution. For example, a solution containing 1.0 g of potassium permanganate, 2 liters of water and additional sodium hydroxide necessary to bring the pH to 9.0 were added to a 2-liter flask

fitted with a solvent distillation head. This distillation will oxidize any trace of organic compounds in the water. The final distillation was carried out under nitrogen in a 2.5-liter flask containing 1500 ml of water from the first still and 1.0×10^{6} M EDTA. This step will remove remaining trace metals from the ultrapure water. To prevent EDTA mist from volatilizing over the reflux arm to the still head, the 40-cm vertical arm was packed with glass beads and wrapped with insulation. This system produces deoxygenated water that can be measured to have a conductivity of less than 2.0 nanomhos/cm^2.

The stopped-flow spectrometer system was designed and manufactured by Kinetic Instruments Inc. (Ann Arbor, MI) and was interfaced to a MAC IICX personal computer. The software for the stopped-flow analysis was provided by Kinetics Instrument Inc. and was written in QuickBasic with MacAdios drivers. Typical injector volumes (0.10 ml of buffer and 0.006 ml of DMSO) were calibrated so that a large excess of water over the DMSO solution were mixed together. The actual ratio was approximately 19/1 so that the initial concentration of superoxide in the aqueous solution was in the range 60-120 μM. Since the published extinction coefficient of superoxide in H_2O at 245 nm is ~2250 M^{-1} cm^{-1} (1), an initial absorbance value of approximately 0.3-0.5 would be expected for a 2-cm path length cell, and this was observed experimentally. Aqueous solutions to be mixed with the DMSO solution of superoxide were prepared using 80 mM concentrations of the Hepes buffer, pH 8.1 (free acid + Na form). One of the reservoir syringes was filled with 5 ml of the DMSO solution while the other was filled with 5 ml of the aqueous buffer solution. The entire injection block, mixer, and spectrometer cell were immersed in a thermostatted circulating water bath with a temperature of 21.0 ± 0.5°C.

Prior to initiating data collection for a superoxide decay, a baseline average was obtained by injecting several shots of the buffer and DMSO solutions into the mixing chamber. These shots were averaged and stored as the baseline. The first shots to be collected during a series of runs were with aqueous solutions that did not contain catalyst. This assures that each series of trials were free of contamination capable of generating first-order superoxide decay profiles. If the decays observed for several shots of the buffer solution were second-order, solutions of iron (III) complexes could be utilized. In general, the potential SOD catalyst was screened over a wide range of concentrations. Since the initial concentration of superoxide upon mixing the DMSO with the aqueous buffer was ~1.2 x 10^{-4} M, we wanted to use a iron (III) complex concentration that was at least 20 times less than the substrate superoxide. Consequently, we generally screened compounds for SOD activity using concentrations ranging from 5 x 10^{-7} to 8 x 10^{-6} M. Data acquired from the experiment was imported into a suitable math program (e.g., Cricket Graph) so that standard kinetic data analyses could be performed. The catalytic rate constant for dismutation of superoxide by the iron (III) complexes of Examples 1-4 were determined from the linear plot of observed rate constants (k_{obs}) versus the concentration of the iron (III) complexes. k_{obs} values were obtained from the liner plots of ln absorbance at 245 nm versus time for the dismutation of superoxide by the iron (III) complex. The k_{cat} ($M^{-1}sec^{-1}$) of the iron (III) complexes of Examples 1-4 are shown in Table I.

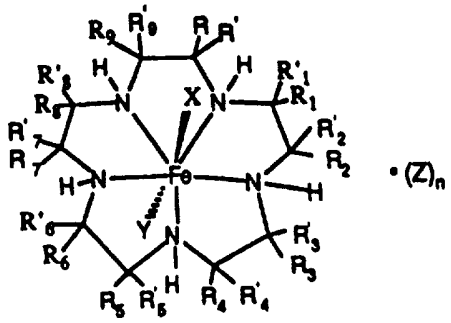
The iron (III) complexes of the nitrogen-containing macrocyclic ligands in Examples 1-4 are effective catalysts for the dismutation of superoxide, as can be seen from the k_{cat} data in Table I.

TABLE I

Compound Example No.	k_{cat} @ pH=7.6, 21°C (M^{-1} sec^{-1})
1	1.06×10^7
2	0.96×10^7
3	1.60×10^7
4	2.94×10^7

WHAT IS CLAIMED IS:

1. Pharmaceutical composition in unit dosage form useful for dismutating superoxide comprising (a) a therapeutically or prophylactically effective amount of a complex represented by the formula:



wherein R, R′, R_1, R'_1, R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8, R'_8, R_9, and R'_9 independently are selected from the group consisting of hydrogen and alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cycloalkylcycloalkyl, cycloalkenylalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkenyl, heterocyclic, aryl and aralkyl radicals and radicals attached to the α-carbon of α-amino acids; or R_1 or R_1' and R_2 or R'_2, R_3 or R'_3 and R_4 or R'_4, R_5 or R'_5 and R_6 or R'_6, R_7 or R'_7 and R_8 or R'_8 and R_9 or R'_9 and R or R′ together with the carbon atoms to which they are attached independently form a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms; or R or R′ and R_1 or R'_1, R_2 or R'_2 and R_3 or R'_3, R_4 or R'_4 and R_5 or R'_5, R_6 or R'_6 and R_7 or R'_7, and R_8 or R'_8 and R_9 or R'_9 together with the carbon atoms to which they are attached independently form a nitrogen containing heterocycle having 2 to 20 carbon atoms provided that when the nitrogen containing heterocycle is an aromatic heterocycle which does not contain a hydrogen

attached to the nitrogen, the hydrogen attached to the nitrogen in said formula, which nitrogen is also in the macrocycle and the R groups attached to the same carbon atoms of the macrocycle are absent;

wherein X, Y and Z are ligands independently selected from the group consisting of halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxo, hydroperoxo, alkylperoxo, arylperoxo, ammonia, alkylamino, arylamino, heterocycloalkyl amino, heterocycloaryl amino, amine oxides, hydrazine, alkyl hydrazine, aryl hydrazine, nitric oxide, cyanide, cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl nitrile, aryl nitrile, alkyl isonitrile, aryl isonitrile, nitrate, nitrite, azido, alkyl sulfonic acid, aryl sulfonic acid, alkyl sulfoxide, aryl sulfoxide, alkyl aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid, aryl thiol thiocarboxylic acid, alkyl carboxylic acid, aryl carboxylic acid, urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea,alkyl aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite, hydrosulfite, alkyl phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide, alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, alkyl phosphinic acid, aryl phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid, phosphate, thiophosphate, phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate, alkyl thiocarbamate, aryl thiocarbamate, alkylaryl thiocarbamate, alkyl dithiocarbamate, aryl dithiocarbamate, alkylaryl dithiocarbamate, bicarbonate,

carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite, tetrahalomanganate, tetrafluoroborate, hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate, and anions of ion exchange resins, or the corresponding anions thereof, or X, Y and Z are independently attached to one or more of the "R" groups and n is an integer from 0 to 1, and (b) a nontoxic, pharmaceutically acceptable carrier, adjuvant or vehicle.

2. Composition of Claim 1 wherein at least one of R, R', R_1, R'_1, R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8, R'_8, R_9 and R'_9 are alkyl or alkyl substituted with $-OR_{10}$ or $-NR_{10}R_{11}$ wherein R_{10} and R_{11} are independently hydrogen or alkyl; and the remaining "R" groups are hydrogen or form part of a saturated, partially saturated or unsaturated cyclic, or form part of a nitrogen containing heterocycle.

3. Composition of Claim 2 wherein at least two of R, R', R_1, R'_1, R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8, R'_8, R_9 and R'_9 are alkyl or alkyl substituted with $-OR_{10}$ or $-NR_{10}R_{11}$ groups and said remaining "R" groups are hydrogen.

4. Composition of Claim 1 wherein at least one of R_1 or R_1' and R_2 or R_2', R_3 or R_3' and R_4 or R_4', R_5 or R_5' and R_6 or R_6', R_7 or R_7' and R_8 or R_8', and R_9 or R_9' and R or R' together with the carbon atoms to which they are attached form a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms; and the remaining "R" groups are hydrogen, alkyl or alkyl substituted with $-OR_{10}$ or $-NR_{10}R_{11}$ groups or form part of a nitrogen containing heterocycle; wherein R_{10} and R_{11} are independently hydrogen or alkyl.

5. Composition of Claim 4 wherein said remaining "R" groups are hydrogen, alkyl or alkyl substituted with $-OR_{10}$ or $-NR_{10}R_{11}$ groups.

6. Composition of Claim 5 wherein at least one of R_1 or R_1' and R_2 or R_2', R_3 or R_3' and R_4 or R_4', R_5 or R_5' and R_6 or R_6', R_7 or R_7' and R_8 or R_8', and R_9 or R_9' and R or R' together with the carbon atoms to which they are attached is a cyclohexano group.

7. Composition of Claim 6 wherein at least two of R_1 or R_1' and R_2 or R_2', R_3 or R_3' and R_4 or R_4', R_5 or R_5' and R_6 or R_6', R_7 or R_7' and R_8 or R_8', and R_9 or R_9' and R or R' together with the carbon atoms to which they are attached are cyclohexano groups.

8. Composition of Claim 1 wherein at least one of R or R' and R_1 or R_1', R_2 or R_2' and R_3 or R3', R_4 or R_4' and R_5 or R_5', R_6 or R_6' and R_7 or R_7', and R_8 or R_8' and R_9 or R_9' together with the carbon atoms to which they are attached are bound to form a nitrogen containing heterocycle; and the remaining "R" groups are hydrogen, alkyl or alkyl substituted with $-OR_{10}$ or $-NR_{10}R_{11}$ groups or form part of a saturated, partially saturated or unsaturated cyclic; wherein R_{10} and R_{11} are independently hydrogen or alkyl.

9. Composition of Claim 1 wherein X,Y and Z are independently selected from the group consisting of halide, organic acid, nitrate and bicarbonate anions.

10. Method of preventing or treating a disease or disorder which is mediated, at least in part, by superoxide comprising administering to a subject in need of such prevention or treatment, a therapeutically or prophylactically effective amount of a complex of Claim 1.

11. Method of Claim 10 wherein said disease or disorder is selected from the group consisting of reperfusion injury to an ischemic organ, surgically-

induced ischemia, inflammatory bowel disease, rheumatoid arthritis, osteoarthritis, psoriasis, organ transplant rejections, radiation-induced injury, oxidant-induced tissue injuries and damage, atherosclerosis, thrombosis, platelet aggregation, metastasis, stroke, acute pancreatitis, insulin-dependent diabetes mellitus, disseminated intravascular coagulation, fatty embolism, adult and infantile respiratory distress and carcinogenesis.

12. Method of Claim 11 wherein said disease or disorder is selected from the group consisting of reperfusion injury to an ischemic organ, surgically-induced ischemia, stroke, atherosclerosis and inflammatory bowel disease.

INTERNATIONAL SEARCH REPORT

Inter ial Application No
PCT/US 97/03348

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/555 C07F15/02 C07D259/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 28968 A (MONSANTO CO ;SEARLE & CO (US)) 2 November 1995 see page 4, line 34 - page 8, line 10 see page 44, line 8 - page 46, line 15	1-7,9
A	EP 0 524 161 A (MONSANTO CO) 20 January 1993 see claims 1,8-10	1-12
P,X	WO 96 39396 A (MONSANTO CO ;NEUMANN WILLIAM L (US); RILEY DENNIS P (US); WEISS RA) 12 December 1996 see claims 18-21	1-12

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"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 when the document is taken alone

"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

2

Date of the actual completion of the international search	Date of mailing of the international search report
24 June 1997	11. 07. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Seufert, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Ir ational application No.
PCT/US 97/03348

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 10-12
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
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:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ 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 claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr al Application No
PCT/US 97/03348

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