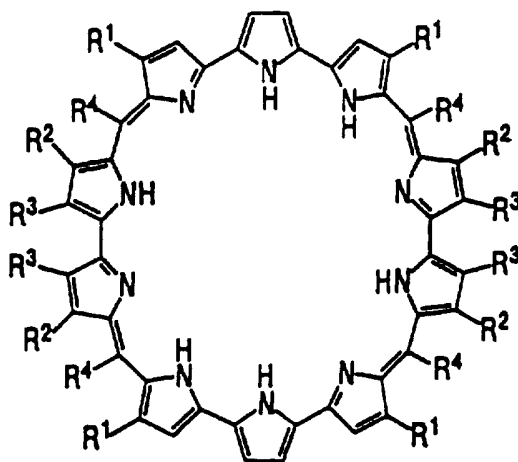




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(54) Title: TURCASARINS, NOVEL EXPANDED PORPHYRINS, AND USES THEREOF



(I)

(57) Abstract

Compounds of Formula (I), wherein R¹, R², R³ may be alkyl or a variety of functional groups and their use as radiation sensitizers.

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DESCRIPTION**TURCASARINS, NOVEL EXPANDED PORPHYRINS,
AND USES THEREOF**

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FIELD OF THE INVENTION

10 The present invention relates generally to the field of expanded porphyrins. More particularly, it concerns a decapyrrolic expanded porphyrin, named turcasarin, its characterization and its use as an anion chelating agent and as a photodynamic agent.

15

BACKGROUND OF THE INVENTION

 In recent years, the chemistry of large pyrrole-
20 containing macrocycles has been receiving increasing attention (References 1-8). Much of this interest reflects the fact this class of compounds, known generically as "expanded porphyrins", bears important analogies to the well-studied porphyrins, yet display
25 chemical properties such as anion binding, that have no parallel in the chemistry of porphyrins or other naturally occurring tetrapyrrolic macrocycles. Further interest in expanded porphyrins is derived from the study of extended π -conjugation pathways. Indeed, much
30 attention has been devoted to making ever larger π -conjugated systems that display far-red visible light absorption. For instance, the octavinyllogous porphyrin 1 prepared by Franck and coworkers (FIG. 2) has 34
 π -electrons in a conjugated path (Knübel and Franck,
35 1988). Also in the all-pyrrole category, expanded porphyrins with as many as six pyrrole subunits have been prepared (Sessler, J.L. et al., 1991 and Gossauer, A.,

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1983). One such example is rubyrin 2 which has 26 π -electrons in its conjugation pathway (Sessler, J.L. et al., 1991). Other even larger expanded porphyrins which rely on additional non-pyrrole subunits to define the macrocycle core have also been prepared. For instance, the eight-nitrogen torrand 3 (Bell, T.W. et al.) and the Schiff base derived macrocycle 4 (Sessler, J.L., et al.) each have extended π -conjugation pathways with visible absorptions considerably red shifted relative to the porphyrins.

To date, there remains relatively little documented information concerning the chemistry of the above-mentioned expanded porphyrin systems. Indeed, at present, structural information is available only for derivatives of sapphyrin (e.g., structure 5), rubyrin (e.g., structure 2) and pentaphyrin (e.g., structure 6) in the all-pyrrole series. Therefore, numerous fundamental questions concerning these molecules still remain to be answered, such as those pertaining to ring size, aromaticity, and effective macrocycle stability. The synthesis and structural characterization of larger macrocycles would be a particular advance in this area, allowing the answers to such inter-related questions to be elucidated.

It has long been appreciated that a considerable number of ionic (e.g. phosphorylated) nucleotide analogues exhibit antiviral activity in cell-free extracts, yet are inactive *in vivo* due to their inability to cross lipophilic cell membranes. For example, the anti-herpetic agent, acyclovir (FIG. 7A, structure a; 9-[(2-hydroxyethoxy)methyl]-9H-guanine), is typical in that it is able to enter the cell only in its uncharged nucleoside-like form. Upon gaining entry to the cytoplasm it is phosphorylated, first by a viral-encoded enzyme, thymidine kinase (FIG. 7A, structure b), and then

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by relatively nonspecific cellular enzymes to produce the active, ionic triphosphate nucleotide-like species (FIG. 7A, structure c). There, it functions both as an inhibitor of the viral DNA polymerase and as a chain terminator for newly synthesized herpes simplex DNA.

Many other potential antiviral agents, including, for instance, the anti-HIV agent, Xylo-G (FIG. 7B, structure d; 9-(β -D-xylofuranosyl)guanine), on the other hand, are not phosphorylated by a viral enzyme and are, therefore, largely or completely inactive. If, however, the active monophosphorylated forms of these putative drugs (such as in FIG. 7B, structure e) could be transported into cells, it would be possible to fight viral infections with a large battery of otherwise inactive materials. If such specific into-cell transport were to be achieved, it would therefore greatly augment the treatment of such debilitating diseases as, for example, AIDS, herpes, hepatitis and measles. Given the fact that AIDS is currently a major national health problem of frightening proportions, and that something so nominally benign as measles still claims over 100,000 lives per year world-wide, treatment of these diseases would be particularly timely and worthwhile.

There continues to exist a need for nucleotide transport agents. In early work, Tabushi was able to effect adenosine nucleotide transport using a lipophilic, diazabicyclooctane-derived, quaternary amine system. However, this same system failed to mediate the transport of guanosine 5'-monophosphate (GMP) or other guanosine-derived nucleotides. Since then, considerable effort has been devoted to the generalized problem of nucleic acid base ("nucleobase") recognition, and various binding systems have been reported.

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Currently known nucleotide binding systems include acyclic, macrocyclic, and macrobicyclic polyaza systems; nucleotide-binding bis-intercalands; guanidinium-based receptors; and various rationally designed H-bonding
5 receptors. These latter H-bonding receptors have been shown to be effective for the chelation of neutral nucleobase and/or nucleoside derived substrates but, without exception, have also all proved unsatisfactory for the important task of charged nucleotide recognition.
10 Large macrocyclic compounds, particularly macrocyclic compounds larger than sapphyrins, which could be relatively easily protonated could prove to be useful in anion binding and transport.

15 Despite intensive efforts in this field, there continues to exist a need for new synthetic systems capable of effecting the recognition and through-membrane transport of phosphate-bearing species such as anti-viral compounds. Furthermore, there continues to exist a need
20 for rationally designed receptors which are "tunable" for the selective complexation of a given nucleobase-derived system.

There is clearly, therefore, a need for novel drug
25 delivery systems to be developed. Compounds which would allow negatively-charged (anionic) structures, particularly specifically-recognized nucleotides, to be transported across naturally lipophilic cellular membranes would represent an important scientific and
30 medical advance. The development of such anionic carriers may also prove to be important in the clinical treatment of cystic fibrosis, in that such compounds would likely facilitate the out-of-cell diffusion of chloride anions. There also exists a continuing need for
35 new chelating molecules and, particularly, chelating molecules that bind metals for use in various procedures such as photodynamic therapy.

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One of the main motivations, then, justifying the preparation of new metal-chelating molecules is to obtain macrocycles designed such that metal coordination may be facilitated through rational structural design. Such molecules may be used to form strong, non-labile complexes of many of the metals or semi-metals on the Periodic Table, including large metal ions, such as lanthanides and actinides. Such a goal derives in part from the general lack of understanding that continues to exist for the metal coordination of the lanthanides and actinides. Also, molecules of sufficiently large size and/or suitable shape may act as bimetallic or even oligometallic receptors.

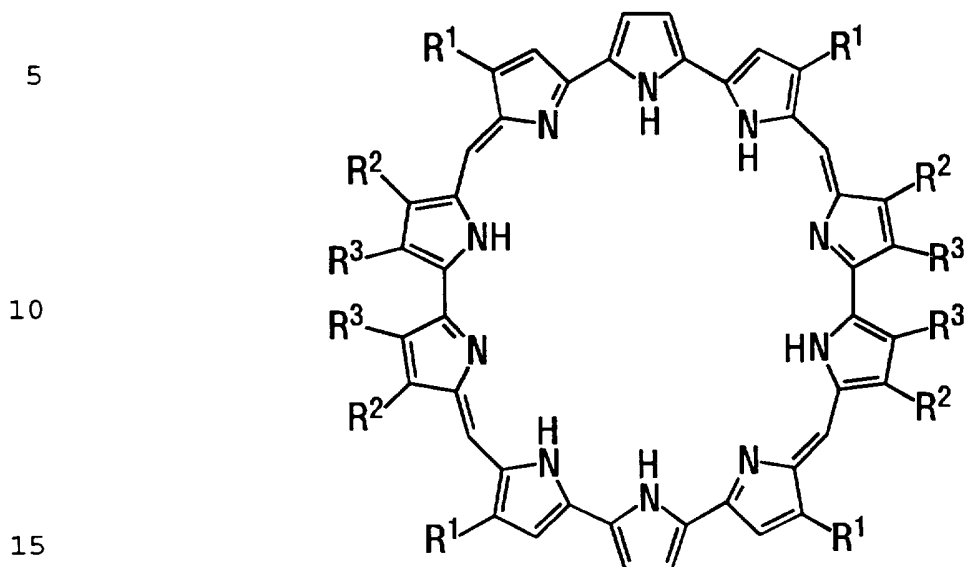
Such metal coordinating molecules may also find use in applications involving physiological (i.e., aqueous) environments. One such application is as a photosensitizer in the photodynamic therapy (PDT) of tumors. For example, by virtue of tumor localizing capability, singlet oxygen production, and long wavelength (i.e., 700-800 nanometer) absorption of visible light (where bodily tissues are readily transparent), metal complexes of these novel molecules would likely be well suited for such an application.

SUMMARY OF THE INVENTION

The present invention provides a new class of expanded porphyrins containing 10 pyrrole units. The present invention also provides uses of and a method of manufacture for this new class of macrocycles.

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In a first respect, the present invention is a compound of formula I:



wherein, individually in each occurrence, each of R^1 , R^2 or R^3 is a monoradical moiety of hydrogen, alkyl, alkyl halide, alkene, alkyne, aryl, amino, hydroxyl, alkoxy, carboxy, carboxamide, ester, amide, sulfonate, hydroxy substituted alkyl, glycol, polyglycol, alkoxy substituted alkyl, carboxy substituted alkyl, amino substituted alkyl, sulfonate substituted alkyl, ester substituted alkyl, amide substituted alkyl, substituted aryl, substituted alkyl, substituted ester, substituted ether, substituted amide, thiol, alkyl thiol, alkoxy carbonyl, aryloxy carbonyl, aldehyde, ketone, carboxylic acid, phosphate, phosphate substituted alkyl, phosphonate, phosphonate substituted alkyl, sulfate, sulfate substituted alkyl, or is of the formula $(CH_2)_m-A-(CH_2)_n-B$ wherein A is CH_2 , O, S, NH or NR^4 or wherein A is a diradical moiety selected from any of the R^{1-3} groups oxy, sulfide, carbonyl, alkene, alkyne, alkyl halide, hydroxyalkyl, glycol, polyglycol, alkyl thiol, substituted alkyl, phosphate, phosphonate, sulfate, phosphate substituted alkyl, phosphonate substituted alkyl, sulfate substituted alkyl, carboxy, carboxamide,

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thiol-substituted carboxamide, derivatized carboxamide of formula CONR^4 where R^4 may be alkyl, alkyl halide, hydroxyalkyl, glycol, polyglycol, or alkyl thiol; wherein B independently in each occurrence is any of the R^{1-3} groups, nucleobase, saccharide, nucleotide, an expanded porphyrin, steroid, amino acid, peptide or polypeptide, turcasarin or polymeric or solid support matrix, wherein n is 0-10, wherein m is 0-10, and wherein the total number of carbon atoms in each of R^{1-4} is less than or equal to 20, or salt thereof.

The present invention provides a new class of expanded porphyrins exemplified by the 10-pyrrole containing macrocycle 11 (FIG. 1) ([40]decaphyrin-[1.0.1.0.0.1.0.1.0.0]). This compound 11 is prepared by the acid catalyzed condensation of 4,4'-diethyl-5,5'-diformyl-3,3'-dimethyl-2,2'-bipyrrole 10 (Sessler, et al., 1990) with 2,5-bis(4-propyl-2-pyrrolyl)pyrrole 9. Compound 11, depending on its state of protonation, displays an intense purple to blue to green color in organic solution. Thus, in view of its color, compound 11 is termed "turcasarin" (from Middle English turcas) and, as used herein, this class of compounds is referred to as "turcasarins." Thus, for instance, compound 11, having a systematic name of 15,20,37,42-tetraethyl-16,19,38,41-tetramethyl-2,11,24,33-tetrapropyl-45,46,47,48,49,50,51,52,53,54-decaazaundecacyclo-[40.2.1.1^{5,8}.1^{9,12}.1^{14,17}.1^{18,21}.1^{23,26}.1^{27,30}.1^{31,34}.1^{36,39}.1^{40,43}]-tetrapentaconta-1,3,5,7,9(47),10,12,14,16,18(49),19,21,23,25,27,29,31(52),32,34,36,38,40(54),41,43-tetracosane, can be renamed as 15,20,37,42-tetraethyl-16,19,38,41-tetramethyl-2,11,24,33-tetrapropylturcasarin.

Turcasarin 11 itself is a macrocycle which can be generally characterized by the presence of ten pyrrolic subunits contained within a 40 π -electron macrocyclic

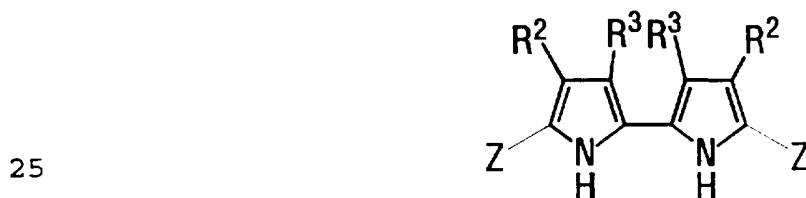
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framework and by UV/VIS absorption bands that are considerably red-shifted as compared to that of other porphyrins.

5 Generally, the turcasarins of the present invention may be prepared by acid catalyzed condensation of a 5,5'-diformyl-bipyrrole with a terpyrrole. A generalized scheme of this condensation is shown in FIG. 3 wherein a substituted or unsubstituted 5,5'-diformyl-bipyrrole **12** and a substituted or unsubstituted terpyrrole **13** are
 10 condensed in an acid catalyzed reaction to prepare a turcasarin **14** corresponding to a compound of formula I. The condensation is typically run in an organic solvent such as an alcohol solution.

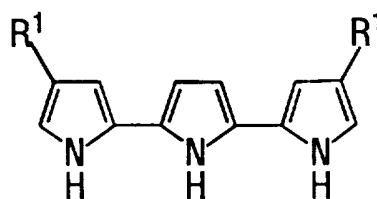
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Thus, in a second respect, the present invention is a process for the production of turcasarins, comprising condensing a bipyrrole and a terpyrrole in the presence of an acid under conditions such that a turcasarin is
 20 formed. In this aspect of the invention, the bipyrrole may be of formula II:



wherein R^{2-3} are as defined above; and wherein Z is H or -CHO, and the terpyrrole can be of formula III:

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wherein R^1 is as defined above.

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The present invention provides a solution to the needs described above by providing a novel composition for use in specific anion binding and transport.

5 Turcasarin and turcasarin analogues are able to bind negatively charged substances, anions, at near-neutral pH, and would be expected to have the ability to transport anionic compounds across cell membranes. The turcasarin macrocyclic compounds of this invention are particularly contemplated for use in transporting
10 antiviral nucleotide analogues into cells, and in facilitating chloride anion exit from cells. They may thus find use in the treatment of a variety of viral diseases and also cystic fibrosis.

15 It is contemplated that the compounds of this invention have a wide variety of uses. In particular, the turcasarins may be useful as a chelating agent and as a photodynamic agent. For example, the turcasarins are useful as chelating agents of metal cations such as
20 uranium oxide or zinc cations. In Example 4, the use of turcasarin 11 to form a uranyl complex is demonstrated. Advantageously, the turcasarin is capable of binding two uranyl cations. This provides another advantage of this invention since the chelating capacity is double that of
25 most molecules. Thus, the turcasarins may be used to treat solutions contaminated with uranium cations to thereby remove the uranium cations from solution. Additionally, uranium is diamagnetic and is itself useful in photodynamic therapy; therefore, one skilled in this
30 art would expect that the turcasarin/uranyl complex would be useful in photodynamic therapy also. In addition, both the red-shifted wavelength of the lower energy absorption and the high extinction coefficient for turcasarin 11 indicates that turcasarin is a likely
35 candidate for use as a photodynamic agent and, accordingly, the turcasarins may have utility as photodynamic agents per se. In addition, the macrocycles

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may also be useful as carriers for the through-membrane transport of nucleotide monophosphates such as guanosine-5' monophosphate.

5 Turcasarin and analogues thereof may be further characterized by the ability to undergo facile protonation at the pyrrolic nitrogens and, once protonated, by an ability to form complexes with anions. It will also be understood that any of the above
10 turcasarin macrocycles may be either singly, doubly, triply or four-fold protonated. An advantageous functional characteristic of turcasarin and analogues thereof is the ability to bind anions at near-neutral pH and possibly to transport anions across lipophilic
15 structures such as biological membranes. Preferred compounds of the present invention therefore have the capacity to bind anions and yet the ability to retain overall supramolecular charge neutrality. In particular, the fact that turcasarins are larger than sapphyrins, the
20 only other class of compounds known to display such behavior, makes them considerably easier to protonate and thus much more effective in the recognition and transport of anions than this other class of molecules. This then represents a significant advance embodied in the present
25 invention.

A range of compounds with a wide variety of substituents in the R^{1-3} groups fall within the scope of the present invention. In one embodiment of this
30 invention, at least one of R^1 , R^2 or R^3 is other than alkyl of 1 to 3 carbons. The precursor molecules may be derivatized or the turcasarin macrocycle may be modified after condensation to yield turcasarins having a variety of substituents. For example, when an R^{1-3} group is an
35 ester, the ester may be converted through well known synthetic procedures to produce a carboxylic acid which itself may be converted to a cyano group, an ether,

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another ester by transesterification, an aldehyde, an alcohol, a halide or an amide which itself can be converted to an amine. The α -positions of the starting bipyrrroles may be carboxyl-, ester-, formyl-, and/or unsubstituted. Likewise, the alpha-position on the terpyrroles may be carboxyl-, ester-, formyl-, and/or unsubstituted.

It is contemplated that one of skill in the art will be able to prepare various turcasarin conjugates, including those listed above, without undue experimentation given the readily-available starting materials and in light of the synthetic methodology disclosed in the present application.

In certain embodiments, turcasarin compounds are contemplated that contain the turcasarin macrocycle core for phosphate binding and also nucleobase "appendages" for specific nucleic acid recognition. These are referred to as turcasarin-nucleobase conjugates, which term is intended to include any conjugate formed by the covalent conjugation of any turcasarin macrocycle to any nucleic acid base ("nucleobase"). The synthesis of such conjugates is described in Example 8 and specific turcasarin-nucleobase conjugates are exemplified in structures 51, 55, 59 and 63 of Schemes F through I.

The term "nucleobase", as used herein, refers generally to any moiety that includes within its structure a purine or pyrimidine, a nucleic acid, nucleoside, nucleotide, or any derivative of any of these, such as a "protected" nucleobase. Thus, the term nucleobase includes adenine, cytosine, guanine, thymidine, uridine, inosine, and the like; bases, nucleotides or nucleosides, as well as any base, nucleotide or nucleoside derivative based upon these or related structures.

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Nucleotides can be readily coupled onto a turcasarin through manipulation of amide, ether and thioether linkages. For example, a turcasarin of formula I wherein R^1 , R^2 and/or R^3 contains a functional group of formula -
5 Y-CO-CH₂Br, wherein Y is NH or O, may be reacted with an hydroxyl group of an oligonucleotide in the presence of a Lewis acid such as FeBr₃ to form an ether linkage between the turcasarin linker and the oligonucleotide. Alternatively, oligonucleotide analogues containing one
10 or more thiophosphate or thiol groups are selectively alkylated at the sulfur atom with an alkyl halide derivative of the turcasarin complex.

Oligonucleotides are used to bind selectively
15 compounds which include the complementary nucleotide or oligo- or poly- nucleotides containing substantially complementary sequences. As used herein, a substantially complementary sequence is one in which the nucleotides generally base pair with the complementary nucleotide and
20 in which there are very few base pair mismatches. The oligonucleotide may be large enough to bind probably at least 9 nucleotides of complementary nucleic acid.

A particular example of a useful nucleobase are the
25 so-called antimetabolites that are based upon purine or pyrimidine structure. These structures typically exert their biological activity as antimetabolites through competing for enzyme sites and, thereby, inhibiting vital metabolic pathways. However, in the context of the
30 present invention, the term "antimetabolite nucleobase" is used quite broadly to refer to any purine or pyrimidine-based molecule that will effect an anticellular, antiviral, antitumor, antiproliferative or antienzymatic effect, regardless of the underlying
35 mechanism. Exemplary structures are shown in Table 1, and include the antimetabolites FU, AraC, AZT, ddI, xylo-GMP, Ara-AMP, PFA and LOMDP.

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It is contemplated that turcasarin-nucleobase conjugates will have a wide variety of applications, including their use as carriers for the delivery of antiviral drugs to a particular body or even subcellular locale. In the case of antimetabolite nucleobases, it is known that many nucleobase antimetabolites can not be readily employed in therapy due to the fact that their charged nature inhibits their uptake by target cells, or otherwise inhibits or suppresses their unencumbered movement across biological membranes. Typically, this shortcoming is due to the presence of charged structures such as phosphates, phosphonates, sulfates or sulfonates on the nucleobase that, due to their charged nature, prevents or inhibits their crossing of a biological membrane. It is proposed that the turcasarins of the present invention may be employed as transport agents for carrying such nucleobases across membranes, (whether the nucleobase is directly conjugated to the macrocycle or simply complexed with it). This point is elaborated in further detail in Sessler et al. (1992), (reference 8m) incorporated herein by reference.

Generally speaking, in the context of turcasarin-nucleobase constructs designed for drug delivery, it will usually be the case that one will employ only one or two nucleobase-containing substituents for each turcasarin macrocycle. Turcasarin derivatives with a single nucleobase are termed "ditopic receptors", whereas those with two nucleobases are termed "tritopic receptors". However, the invention is not limited to compounds containing one or two nucleobase units, indeed, mono- or di-substituted turcasarin-nucleobase conjugates may have any number of nucleobases or nucleobase oligomers or polymers attached. The ultimate number of such residues that are attached will, of course, depend upon the application. One may employ a turcasarin derivative with 10 or so bases attached to bind and transport

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complementary oligo- or poly-nucleotides. Of course, there is no limitation to the particular position(s) within the turcasarin macrocycle to which the nucleobase(s) may be attached to construct a conjugate.

5

Further examples of turcasarin derivatives or conjugates encompassed by the present invention are turcasarin saccharide derivatives, wherein the macrocycle is appended to a saccharide-based unit, such as a sugar, sugar derivative or polysaccharide. The synthesis of turcasarin-saccharide compounds is described in Example IX and specific turcasarin saccharide conjugates are represented by structures 66, 68, 76 and 84 in reaction schemes J, K, M and O, respectively. A non-exhaustive, exemplary list of sugars which may be conjugated to turcasarin in this manner is set forth in Table 2. Of course, any sugar or modified sugar may be employed including sugars having additional phosphate, methyl or amino groups, and the like. Moreover, the use of both D- and L-forms, as well as the α and β forms, are also contemplated. The use of sugars such as glucose, galactose, galactosamine, glucosamine and mannose is particularly contemplated. Turcasarin-saccharide conjugates are envisioned to be of use where one would like to control, or otherwise modify, the water solubility of the resultant turcasarin-based compound, for example, in exploiting its novel properties in connection with human or animal applications.

30 In still further embodiments, the invention concerns compositions which are composed of a turcasarin derivative in accordance with any one of the embodiments discussed above complexed to a second substance, wherein the second substance includes within its structure a negatively charged moiety. More particularly, the second substance will include a negatively charged component such as a chloride, phosphate, phosphonate, sulfate, or

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sulfonate moiety, of which, turcasarin-chloride ion complexes are a particular example.

5 In other preferred embodiments, the second substance will include a purine or pyrimidine, or an analog of either, within its structure. As mentioned above, these nucleobase structures include, for example, adenine, cytosine, guanine, thymidine, uridine and inosine; various antimetabolic or antienzymatic nucleobase
10 compounds; and also oligo or polynucleotides such as DNA or RNA. Antimetabolic and antienzymatic compounds include those with antitumor, anticellular, antiproliferative and antiviral activity, examples of which are represented in Table 1.

15

In further related embodiments, the invention concerns a method for forming a complex between a turcasarin macrocycle and a negatively charged second substance, or selected agent. The method involves
20 preparing a turcasarin or turcasarin analogue or conjugate, such as any one of the turcasarin derivatives as described above, and contacting this turcasarin or turcasarin derivative with a negatively charged substance or selected agent under conditions effective to allow the
25 formation of a complex between the turcasarin macrocycle and the negatively charged substance.

This method is appropriate for binding, or
30 complexing, a range of negatively charged substances or selected agents, such as for example, chloride ions and other halides, pseudohalides such as azide or cyanide anions, and anionic clusters such as ferricyanide. The complexing of phosphate-containing compounds, including, simple alkyl or aryl phosphates, nucleotides, oligo- and
35 polynucleotides, such as DNA, RNA and anti-sense constructs, and nucleotide analogues is particularly contemplated. Even more preferable, is the complexing of

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antiviral compounds such as phosphonate derivatives and simple species such as the pyrophosphate derivatives PFA and COMDP; the antiviral agents of FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E and FIG. 7F and Table 1, and particularly, acyclovir monophosphate, Xylo-GMP, and Ara-AMP. Here, as throughout this invention, a key embodiment is the high basicity of the turcasarin, which allows this class of compounds and its derivatives to act as vastly improved anion chelators or carriers in comparison to expanded porphyrins such as sapphyrins or any other extant system.

Still further methodological embodiments concern methods for the cellular transport of a given substance, generally a negatively charged substance. This may be employed as a means of, for example, successfully introducing a negatively charged substance into a cell, or alternatively, as a means of facilitating the removal of a negatively charged substance from a cell. To achieve this, one would prepare a turcasarin macrocycle in accordance with the present invention, contact the macrocycle with the negatively charged substance under conditions effective to allow complex formation, and then simply contact the cell, either *in vitro* or *in vivo*, with the macrocycle-bound substance.

Any one of a variety of negatively charged substances may be delivered to a cell, including a cell within an organism, in this manner. The delivery of oligonucleotides, including anti-sense constructs, and nucleotide analogues, such as antiviral compounds, is particularly envisioned. One example concerns the introduction of a turcasarin-complex composition which includes an antiviral antimetabolic or antienzymatic compound into a cell suspected of being a virally infected target cell. Another example is the introduction of an antitumor antimetabolic or

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antienzymatic compound into a cell suspected of being a tumor or proliferating cell.

Of course, it is contemplated that such target cells
5 may be located within an animal or human patient, in
which case an effective amount of the complex, in
pharmacologically acceptable form, would be administered
to the patient. Generally speaking, it is contemplated
that useful pharmaceutical compositions of the present
10 invention will include the selected turcasarin derivative
in a convenient amount that is diluted in a
pharmacologically or physiologically acceptable buffer,
such as, for example, phosphate buffered saline. The
route of administration and ultimate amount of material
15 that is administered to the patient or animal under such
circumstances will depend upon the intended application
and will be apparent to those of skill in the art.

The capacity of turcasarin and analogues thereof to
20 effect specific into-cell transport of anti-viral
compounds is contemplated to be of use against a wide
variety of debilitating diseases such as AIDS, herpes,
hepatitis and measles. As a mediator of DNA import,
turcasarins may conceivably be employed in the treatment
25 of any disease in which the delivery of an
oligonucleotide or DNA fragment would be advantageous,
such as in supplying a functioning gene, or in inhibiting
an aberrant gene, for example, by employing an antisense
DNA construct. As discussed above, the larger size, high
30 basicity, and relative ease with which turcasarins may be
protonated, renders them particularly effective molecules
for use in anion transport.

Additionally, certain turcasarins with advantageous
35 chloride ion transporting properties may be employed as
synthetic carriers capable of facilitating out-of-cell
diffusion of chloride anions, and are therefore

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contemplated for use as therapeutic agents for the treatment of cystic fibrosis.

5 **BRIEF DESCRIPTION OF THE DRAWINGS**

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention
10 may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1 shows a synthetic scheme for the synthesis of
15 turcasarin 11.

FIG. 2 shows structures of expanded porphyrins described in the Background of the Invention.

20 FIG. 3 shows a generalized synthetic scheme for the synthesis of turcasarin macrocycles of the present invention.

FIG. 4 shows a schematic representation of the
25 interconversion between the two limiting enantiomeric conformations of turcasarin 11. These conformations may be considered as left- or right-handed twists along the long axis of a presumed open, pseudo-circular intermediate.

30
FIG. 5 shows a two-dimensional H,H-correlated spectrum (H,H COSY) of the alkyl region of turcasarin 11 that allowed definitive peak assignments to be made. Paired sets of crosspeaks indicate near C_2 symmetry in the
35 molecule and provide evidence for diastereotopic protons in the propyl side chains.

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FIG. 6 shows the top and side views of the tetraprotonated form of turcasarin 11 ($(C_{68}H_{74}N_{10})^{4+}(Cl^-)_4$) with partial labelling showing the conformation of the macrocycle and the binding of the Cl^- ions. At the point of "ribbon crossing" the interplane separation is 3.268 Å. Further important distances (Å) and angles (°) are: N1-H1a ... Cl1, N ... Cl 3.133(11), H ... Cl 2.442(11), N-H ... Cl 133.9(10); N40-H40a ... Cl1, N ... Cl 3.054(10), H ... Cl 2.178(10), N-H ... Cl 164.3(11); N45-H45a ... Cl1, N ... Cl 3.194(10), H ... Cl 2.370(10), N-H ... Cl 152.4(11); N8-H8a ... Cl2, N ... Cl 3.190(10), H ... Cl 2.353(10), N-H ... Cl 154.8(11); N51-H51a ... Cl2, N ... Cl 3.116(11), H ... Cl 2.334(11), N-H ... Cl 145.2(11); N24-H24a ... Cl3, N ... Cl 3.133(11), H ... Cl 2.349(11), N-H ... Cl 145.5(11); N35-H35a ... Cl3, N ... Cl 3.161(11), H ... Cl 2.280(11), N-H ... Cl 166.0(11); N13-H13a ... Cl4, N ... Cl 3.069(10), H ... Cl 2.183(10), N-H ... Cl 167.8(11); N18-H18a ... Cl4, N ... Cl 3.194(11), H ... Cl 2.375(11), N-H ... Cl 151.4(11); N29-H29a ... Cl4, N ... Cl 3.132(10), H ... Cl 2.459(10), N-H ... Cl 131.9(10). Because the hydrogen atoms are at idealized positions, the resulting estimated standard deviations for quantities involving them are underestimated. Thermal ellipsoids were scaled to the 30% probability level. For clarity, only N-H hydrogen atoms are shown.

FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E and 7F show the structures for potential antiviral agents a through i.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The turcasarins of the present invention are of formula I shown hereinabove. The structure has

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substituent groups R^{1-3} , each of R^{1-3} being defined as above.

Representative examples of alkanes used as such
5 monoradicals include methane, ethane, straight-chain,
branched or cyclic isomers of propane, butane, pentane,
hexane, heptane, octane, nonane and decane, with methane,
ethane and propane being preferred. Representative
examples of alkenes useful as such monoradicals or
10 diradicals include ethene, straight-chain, branched or
cyclic isomers of propene, butene, pentene, hexene,
heptene, octene, nonene and decene, with ethene and
propene being preferred. Representative examples of
alkynes useful as such monoradicals or diradicals include
15 ethyne, straight-chain, branched or cyclic isomers of
propyne, butyne, pentyne, hexyne, heptyne, octyne, nonyne
and decyne, with ethyne and propyne being preferred.
Among the halides, chlorine and bromine are preferred in
the practice of this invention. Representative examples
20 of alkyl halides used in this invention include halides
of methane, ethane, propane, butane, pentane, hexane,
heptane, octane, nonane and decane, with halides,
preferably chlorides or bromides, of methane, ethane and
propane being preferred. Representative examples of
25 hydroxyalkyls include alcohols of methane, ethane,
straight-chain, branched or cyclic isomers of propane,
butane, pentane, hexane, heptane, octane, nonane and
decane, with alcohols of methane, ethane or propane being
preferred. Representative examples of useful glycols of
30 this invention include diols of ethane, straight-chain,
branched or cyclic isomers propane, butane, pentane,
hexane, heptane, octane, nonane and decane, with diols of
ethane or propane being preferred. Representative
examples of polyglycols include polyethylene glycol,
35 polypropylene glycol and polybutylene glycol as well as
polyalkylene glycols containing combinations of ethylene,
propylene and butylene. Representative examples of

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alkyl thiols include thiols of ethane, thiols of straight-chain, branched or cyclic isomers of propane, butane, pentane, hexane, heptane, octane, nonane and decane, with thiols of ethane (ethanethiol, C_2H_5SH) or propane (propanethiol, C_3H_7SH) being preferred.

Representative examples of substituted alkyls include alkyls substituted by two or more functional groups described herein. Representative examples of phosphates include phosphate or polyphosphate groups.

Representative examples of phosphate substituted alkyls include alkyls as described above substituted by one or more phosphate or polyphosphate groups. Representative examples of phosphonate substituted alkyls include alkyls as described above substituted by one or more phosphonate groups. Sulfate substituted alkyls include alkyls as described above substituted by one or more sulfate groups, a representative example of which is diethyl sulfate ($(C_2H_5)_2SO_4$). Representative examples of carboxy groups include carboxylic acids of the alkyls described above as well as aryl carboxylic acids such as benzoic acid. Representative examples of carboxyamides include primary carboxyamides ($CONH_2$), secondary ($CONHR^4$) and tertiary ($CONR^4R^4$) R^4 carboxyamides where R^4 is a functional group as described herein. Representative examples of useful amines include a primary, secondary or tertiary amine of an alkyl as described hereinabove.

Representative examples of useful oligonucleotides include nucleotides, oligonucleotides and polynucleotides composed of adenine, cytosine, guanine, thymine or uracil bases. It is understood that the term nucleotide as used herein refers to both naturally-occurring and synthetic nucleotides, poly- and oligonucleotides and to analogs and derivatives thereof such as methylphosphonates, phosphotriesters, phosphorothioates and phosphoramidates.

Representative examples of useful steroids include any of the steroid hormones of the following five categories: progestins (e.g. progesterone), glucocorticoids (e.g.,

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cortisol), mineralocorticoids (e.g., aldosterone), androgens (e.g., testosterone) and estrogens (e.g., estradiol). Representative examples of useful amino acids include amino acids with simple aliphatic side chains (e.g., glycine, alanine, valine, leucine, and isoleucine), amino acids with aromatic side chains (e.g., phenylalanine, tryptophan, tyrosine, and histidine), amino acids with oxygen and sulfur-containing side chains (e.g., serine, threonine, methionine, and cysteine), amino acids with side chains containing carboxylic acid or amide groups (e.g., aspartic acid, glutamic acid, asparagine, and glutamine), and amino acids with side chains containing strongly basic groups (e.g., lysine and arginine), and proline. Representative examples of useful peptides include any of both naturally occurring and synthetic di-, tri-, tetra-, and pentapeptides derived from any of the above described amino acids. Representative examples of useful polypeptides include both naturally occurring and synthetic polypeptides (e.g., insulin, ribonuclease, and endorphins) derived from the above described amino acids and peptides. Representative examples of polymeric or solid support matrixes include resin-type polystyrene derived solid support matrixes, aminopropyl-functionalized silica gel or natural polymers such as polysaccharides. Representative examples of nucleobases useful in this invention include those shown in Table 1 hereinbelow. Representative examples of sugars useful in the practice of this invention include those shown in Table 2 hereinbelow. Representative examples of useful chelates include EDTA, EGTA, DTPA, DOTA, ethylene diamine, bipyridine, 1,10-phenanthralene, crown ether, aza crown and catechols.

35 The bipyrrroles that are used in the practice of this invention to prepare turcasarins contain two formyl groups at the alpha positions. The R²⁻³ substituents may

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be introduced on the pyrrole ring before or after the condensation reaction to synthesize a turcasarin depending on the nature of the substituent. If the substituent impairs the formation of the turcasarin macrocycle, for example, due to steric hindrance, then the substituent may be incorporated after the condensation. The bipyrrroles are well known compounds which can be readily prepared by conventional techniques well known to those of skill in the art.

10

The terpyrroles used in the practice of this invention may be unsubstituted at the alpha positions. The terpyrroles used in this invention are well known materials which can be readily prepared by conventional techniques well known to those skill in the art. One method of terpyrrole preparation involves the synthesis as shown in Example 2. Other methods include those described by Chierici et al., Gaz. Chim. Ital., volume 86, pages 1278-1283 (1956).

15
20

In the condensation to form the turcasarins of this invention, the most preferred acid is hydrogen chloride. Suitable solvents for the condensation include ethanol or methanol with cosolvents such as chloroform or methylene chloride. The condensation is generally run at room temperature.

25

The turcasarin and turcasarin analogues of the present invention are characterized by the capacity to bind anions and yet retain overall supramolecular charge neutrality. A particular advantage to turcasarin molecules is their large size. This property renders turcasarins easier to protonate than other macrocycles and makes them considerably more effective at anion recognition and transport than any other classes of expanded porphyrins, including the sapphyrins. The increased basicity of turcasarin relative to sapphyrin is

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thus of considerable importance and represents a significant advance relative to the existing art.

5 Additionally, several other turcasarin analogues are contemplated by the present invention. Different combinations of bipyrrroles and terpyrrroles may be employed in the condensation step to yield a variety of different macrocyclic products. The generation of further turcasarin analogues is particularly described in 10 Example VII, and represented in reaction schemes A through E. A range of compounds with a wide variety of alkyl and/or aryl substituents in the *meso* and/or β -pyrrolic positions, as exemplified by structures 27, 35, 40 and 47, thus fall within the scope of the present 15 invention.

It is contemplated that turcasarins of the present invention will be of use as drug delivery agents. It is contemplated that they will find utility in mediating the 20 cross-membrane transport of negatively charged compounds or molecules, including halides, pseudohalides, such as azide or cyanide anions, or anionic clusters such as ferricyanide. The anion carrying properties of turcasarins make them ideal candidates for the 25 development of synthetic carriers capable of facilitating out-of-cell diffusion of chloride anions, and hence for use as therapeutic agents for the treatment of cystic fibrosis.

30 Importantly, turcasarin and analogues thereof are contemplated for transporting phosphate-containing compounds into cells. Phosphate-containing compounds which may be transported in this manner include, for example, simple alkyl or aryl phosphate, nucleotides such 35 as AMP or GMP, oligonucleotides and DNA or RNA, including anti-sense DNA or RNA constructs, and more particularly, antiviral compounds such as those depicted in FIG. 7A,

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FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E and FIG. 7F structures b,e,f,g,h,i, Table 1, and equivalents thereof.

5
TABLE 1
MODIFIED NUCLEOSIDE/NUCLEOTIDE
ANALOGUE ANTI-METABOLITES

AraC	Erythrohydroxynonyladenine
AraAMP	Floxuridine
10 Azaribine	Fluorouracil (5-FU)
Azathioprine	Idoxuridine
Azauridine	LOMPD
AZT	Mercaptopurine
Bromodeoxyuridine	PFA
15 Chlorodeoxyuridine	Thioguanine
Cytarabine	Trifluoromethylde-oxyuridine
Deoxyuridine	Xylo-GMP
DideoxyInosine DDI	

20 Any one of a variety of antiviral agents may be delivered to a cell using turcasarin or a turcasarin analogue in accordance herewith. These agents include, for example, the anti-HSV and anti-HIV agents acyclovir monophosphate, Xylo-GMP, Ara-AMP, and/or phosphonate
 25 derivatives that also have documented anti-HSV and anti-HIV activity *in vitro* (e.g., FIG. 7F and FIG. 7G), and simple species such as the pyrophosphate derivatives PFA (FIG. 7E) and COMDP (FIG. 7F) that have demonstrated anti-HIV reverse transcriptase activity in cell-free
 30 media.

As mentioned above, the large size, increased basicity and ease of protonation of turcasarins makes this class of macrocycles considerably more effective at
 35 the recognition and transport of anions than other classes of molecules. The capacity of turcasarin, and

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analogues thereof, to effect specific into-cell transport of anti-viral compounds is contemplated to be of advantageous use against a wide variety of debilitating diseases, including, for example, herpes, hepatitis, measles, and AIDS. Such diseases are of major medical and economic importance with AIDS being an international health problem and even measles claiming over 100,000 lives per year world-wide.

Furthermore, it is contemplated that the turcasarin compounds of the present invention may be rendered useful as nucleotide transporters if one or more nucleobase recognition units were to be "appended" directly onto the phosphate-chelating macrocyclic core. This would impart a further degree of nucleotide specificity to binding and transport reactions. Accordingly, turcasarin-nucleobase conjugates which have been derivatized by the addition of one or more nucleobase compounds form an important aspect of the present invention.

Turcasarin mononucleobase derivatives may include any of the naturally-occurring purine or pyrimidine nucleobases, namely, cytosine, guanine, thymidine, adenine, uridine or inosine. Equally, they may include modified versions of any of these, such as the heterocyclic components of those nucleoside/nucleotide analogues listed in Table 1. Also included within the invention are the turcasarin mononucleobase derivatives including chemically modified nucleobases such as "protected" bases. Protecting groups are used to protect reactive groups, such as amino and carboxyl groups, from inappropriate chemical reactions. Turcasarin-nucleobase conjugates with protected bases include, for example, conjugates wherein one or more base has a protecting group, such as 9-fluorenylmethylcarbonyl, benzyloxycarbonyl, 4-methoxyphenacyloxycarbonyl, t-butylloxycarbonyl, 1-adamantylloxycarbonyl, benzoyl, N-

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triphenylmethyl or N-di-(4-methoxyphenyl)phenylmethyl on the amino group of the nucleobase.

It is contemplated that various chemical means may be used to covalently bond nucleobases to turcasarin macrocycles. Various spacers may be used for the binding, such as, for example, oligomethylene bridges with terminal amino, or hydroxy function, which allow formation of an amide or ester bond for the connection of the turcasarin and nucleobase units. This bridge may also be modified, for example, by the reduction of the amide bond to give the amine function. Specific examples of the synthesis of turcasarin-nucleobase conjugates are described in Example 7 and the resultant compounds are represented by structures 51, 55, 59 and 63 of reaction schemes F through I.

Turcasarin nucleobase conjugates would be useful as antiviral agents, capable of binding and solubilizing nucleotides and of effecting their selective through-membrane transport at or near physiologic pH. Turcasarin nucleobase conjugates with appended oligonucleotides are also contemplated by the present invention, and would be of use in binding and transporting oligo- or polynucleotides, including antisense constructs, into cells. As a mediator of DNA import, turcasarins may conceivably be employed in the treatment of any disease in which the delivery of an oligonucleotide or DNA fragment would be advantageous, such as in supplying a functioning gene, or in inhibiting an aberrant gene, for example, by employing an antisense DNA construct.

Another class of turcasarin derivatives or conjugates contemplated are the turcasarin saccharide derivatives which comprise a turcasarin macrocycle conjugated to a sugar, sugar derivative or polysaccharide. The synthesis of turcasarin-saccharide

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compounds, as represented by structures 66, 68, 76 and 84 in reaction schemes J, K, M and O, respectively, is described in Example IX. It will be understood that any one of a variety of individual sugar units, such as those set forth in Table 2, or polymers thereof, may be conjugated to turcasarin in accordance herewith. Table 2 is intended to include modified versions of the sugar units, such as sugars having additional phosphate, methyl or amino groups and the like, and also includes D- and L-isomers and α and β forms.

TABLE 2
EXAMPLES OF SUGARS AND SUGAR DERIVATIVES

15	Ribose	Fructose
	Arabinose	Sorbose
	Xylose	Tagatose
	Lyxose	Fucose
	Allose	
20	Altrose	Methylglucoside
	Glucose	Glucose 6-phosphate
	Mannose	
	Gulose	N-Acetylgalactosamine
	Idose	N-Acetylglucosamine
25	Galactose	Sialic Acid
	Talose	
	Ribulose	
	Xylulose	
30	Psicose	

In addition to the turcasarin analogues described above and the nucleobase and saccharide conjugates, it will be appreciated that a variety of other substituents, of desirable chemical function, may be appended to a functionalized turcasarin moiety to construct a

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turcasarin-based conjugate. It is contemplated that the synthesis of turcasarin conjugates includes, for example: metal chelator moieties such as EDTA, EGTA, 1,10-phenanthralene, DTPA, DOTA, crown ether, azacrown, catecholate and ethylene diamine; alkylating agents such as ethylene diamine, epoxide and bromoacetamide; steroids and steroid derivatives; amino acids, peptides and polypeptides; other turcasarins, turcasarin derivatives, polymeric turcasarin, or other macrocyclic compounds such as sapphyrins, texaphyrins or derivatives thereof; and polymeric matrices or solid supports such as polymers, glasses, agarose, polyacrylamide, controlled pore glass, silica gel, polystyrene and sepharose.

Even though the invention has been described with a certain degree of particularity, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art in light of the foregoing disclosure. For example, it is anticipated that the open beta positions on the residual terpyrrole on the turcasarin ring, once the ring is formed, can be functionalized using standard Friedal-Crafts alkylation or acylation, with the resulting compounds potentially being further manipulated to prepare functionalized turcasarins. Accordingly, it is intended that all such alternatives, modifications, and variations which fall within the spirit and the scope of the invention be embraced by the defined claims.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in

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light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1**SYNTHESIS OF TERPYRROLE TETRAESTER 8**

The synthesis of terpyrrole tetraester **8** (FIG. 1) was carried out as follows. Dipyrroylbutanedione **7** (23.54 g., 40 mmol), prepared according to the procedure of Johnson *et al.* (1992), ammonium acetate (vacuum dried at 50°C, 91.1g, 1 mol), acetic anhydride (32 mL, 0.33 mol) and glacial acetic acid (150 mL) were held at reflux under argon for 18 hours, poured into stirred ice water (400 mL), and stirred for 1 hour. The solid was collected by filtration, washed with water, and dissolved in CH₂Cl₂. The CH₂Cl₂ solution was washed with water, diluted with methanol, and concentrated on the rotary evaporator until the volume was approximately 50 mL. The flask contents were cooled in ice for 1 hour and the solid collected by filtration, washed (MeOH, Et₂O), and dried to give the pure product as light yellow needles or powder (17.85 g, 31.3 mmol, 78%).

Characterization Data for **8**:

Mp 150-152 °C. ¹H NMR (300 MHz, CDCl₃) δ = 0.99 (t, J_{HH} = 7.4 Hz, 6H, CH₂CH₂CH₃); 1.36 (t, J_{HH} = 7.1 Hz, 6H, OCH₂CH₃); 1.40 (t, J_{HH} = 7.1 Hz, 6H, OCH₂CH₃); 1.62 (sextet, J_{HH} = 7 Hz, 4H, CH₂CH₂CH₃); 3.06 (m, 4H, CH₂CH₂CH₃); 4.37 (q, J_{HH} = 7.1 Hz, 8H, OCH₂CH₃) 6.61 (d, J_{HH} = 2.5 Hz, 2H, pyrrole CH); 9.05 (bs, 2H, NH); 9.10 (bs, 1H, NH). ¹³C NMR (75.5 MHz, CDCl₃) δ = 14.2, 14.3, 14.4, 24.5, 28.0, 60.5, 60.5, 109.4, 111.9, 119.0, 124.3, 132.2, 135.7, 161.3, 166.0 MS (CHCl₃): 569 (100%), 523

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(83%), 477 (64%). HRMS: Calculated for $C_{30}H_{39}N_3O_8$: m/z = 569.2720. Found: 569.2720.

EXAMPLE 2

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SYNTHESIS OF TERPYRROLE 9

The synthesis of terpyrrole 9 (FIG. 1) was carried out as follows. Terpyrrole tetraester 8 (5.7 g, 10 mmol), NaOH (10 g, 400 mmol), and ethylene glycol (100 mL) were placed in a three-necked 250 mL round bottomed flask equipped with an argon inlet, thermometer, condenser, and stir bar, and brought to reflux under a gentle flow of argon through the flask. When the solution reached 180°C, the flask contents were heated for another 30 minutes and cooled. When the solution reached 100-120°C, the flask contents were diluted with water (70 mL, degassed by the freeze-pump-thaw method) and allowed to cool to room temperature. The flask contents were diluted to 400 mL with ice water. The solid so obtained was separated by filtration under an argon blanket (an aluminum foil cover and a pipette blower works fine), washed with water, and dried in vacuo to give the pure product as a tan to light green powder (2.62 g, 9.3 mmole, 93%).

25

Characterization Data for 9:

M.P.: (sealed tube) 141-144 °C. 1H NMR (300 MHz, DMSO- d_6) δ = 0.91 (t, J_{HH} = 7.4 Hz, 6H, $CH_2CH_2CH_3$); 1.53 (sextet, J_{HH} = 7.4 Hz, 4H, $CH_2CH_2CH_3$); 2.35 (t, J_{HH} = 7.4 Hz, 4H, $CH_2CH_2CH_3$); 6.13 (m, 2H, $CHC(n-Pr)CHNH$); 6.44 (s, 2H, CH); 10.47 (s, 2H, NH); 10.56 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO- d_6) δ = 14.0, 23.9, 28.9, 103.1, 103.3, 114.1, 123.3, 125.5, 125.7. MS ($CHCl_3$): 281 (100%), 252 (25%), 238 (8%). HRMS: Calculated for $C_{18}H_{23}N_3$: m/z = 281.1892. Found: 281.1878.

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EXAMPLE 3
SYNTHESIS OF TURCASARIN 11

The synthesis of turcasarin 11 (FIG. 1) was carried
5 out as follows. Bipyrrrole 10 (72.5 mg, 2.67×10^{-4} mol)
was dissolved in 20 mL absolute ethanol with the aid of a
heat gun. CHCl_3 was added (500 mL), followed by
terpyrrole 9 (74.9 mg, 2.67×10^{-4} mol). HCl gas was
10 bubbled into the stirred reaction mixture for five
minutes. The reaction was then stirred under an argon
blanket for four hours. The CHCl_3 was removed using a
rotary evaporator. The ethanolic residue was treated
with concentrated aqueous NaHCO_3 until effervescence
15 ceased, and then extracted into chloroform in a
separatory funnel. The organic layer was separated off,
dried over Na_2SO_4 , and taken to dryness *in vacuo*. The
residue was taken up in a minimum amount of CH_2Cl_2 , and
 NH_3 gas bubbled into the solution for one minute. The
resulting solution was purified chromatographically on a
20 pre-basified 2 cm x 20 cm silica column (achieved by
bubbling NH_3 through a CH_2Cl_2 -silica slurry before
packing). The first major purple band was collected
using CH_2Cl_2 as the eluent placed in a separatory funnel,
and washed first with water (2 x 25 mL) and then 1N HCl
25 (3x 25 mL). The organic layer was then separated, dried
over Na_2SO_4 , and taken to dryness *in vacuo*.
Recrystallization from CH_2Cl_2 layered with hexanes gives
pure 11 as shiny green metallic crystals (62.9 mg, $5.34 \times$
 10^{-5} mol, 20%).

30

Characterization data for 11:

M.P.: dec. above 215 °C; ^1H NMR (500 MHz, CDCl_3): $d =$
0.99 (t, $J_{\text{HH}} = 7$ Hz, 6H, CH_2CH_3), 1.05 (t, $J_{\text{HH}} = 7$ Hz, 6H,
35 CH_2CH_3), 1.08 (t, $J_{\text{HH}} = 7$ Hz, 6H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.27 (t, $J_{\text{HH}} =$
7 Hz, 6H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.77 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.85 (s,
6H, CH_3), 1.89 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.06 (s, 6H, CH_3), 2.10

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(m, 2H, CH₂CH₂CH₃), 2.19 (m, 2H, CH₂CH₂CH₃), 2.25 (q, $J_{\text{HH}} = 7$ Hz, 4H, CH₂CH₃), 2.36 (m, 4H, CH₂CH₃), 2.64 (m, 2H, CH₂CH₂CH₃), 2.87 (m, 2H, CH₂CH₂CH₃), 2.89 (m, 2H, CH₂CH₂CH₃), 3.13 (m, 2H, CH₂CH₂CH₃), 6.73 (s, 2H, meso-H), 6.84 (s, 2H, meso-H), 7.00 (s, 2H, pyrrole CH), 7.65 (bs, 2H, pyrrole CH), 8.68 (s, 2H, pyrrole CH), 9.73 (bs, 2H, pyrrole CH), 14.32 (s, 2H, NH), 14.56 (s, 2H, NH), 14.75 (s, 2H, NH), 15.37 (s, 2H, NH), 15.74 (s, 2H, NH); ¹³C NMR (75.5 MHz, CDCl₃): $\delta = 11.4, 11.5, 14.1, 14.4, 14.9, 15.0, 18.0, 18.2, 23.0, 23.2, 28.2, 28.9, 119.1, 119.7, 121.4, 122.8, 123.7, 124.7, 125.1, 127.0, 128.6, 132.7, 133.0, 133.9, 136.4, 137.2, 137.6, 139.6, 141.6, 143.1, 147.8, 148.17, 149.0, 151.3$; UV/vis (CH₂Cl₂): λ_{max} [nm] (ϵ) = 369 (46 500), 399 (49 000), 445 (51 500), 642 (312 500); HRMS (FAB): Calculated for C₆₈H₇₉N₁₀: $m/z = 1035.6489$. Found: 1035.6505 ((M + 1)⁺ - 4 HCl); Elemental Analysis: Calculated for C₆₈H₈₂Cl₄N₁₀•H₂O: C 68.10, H 7.06, N 11.68, Cl 11.82. Found: C 68.55, H 7.08, N 11.55, Cl 11.79.

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Crystallographic summary for 11 (C₆₈H₇₄N₁₀)⁴⁺ (Cl⁻)₄ - 3CH₂Cl₂ - 3/2 (C₆H₁₄), Mr = 1557.29; crystallized as dark green blocks from methylene chloride layered with n-hexane in the triclinic, space group P $\bar{1}$ (no. 2), with $a = 14.221(6)$, $b = 15.374(3)$, $c = 20.531(5)$ Å, $\alpha = 100.03(2)$, $\beta = 101.71(3)$, $\gamma = 106.23(3)$ °, $V = 4091(2)$ Å³, $\rho_{\text{calc}} = 1.26$ g-cm⁻³ for $Z = 2$, $F(000) = 1642$, m (Mo Ka) = 3.888 cm⁻¹, $\lambda = 0.71073$ Å. FIG. 6 shows top and side views of turcasarin 11, the views derived from the crystallographic data. Data were collected on a Nicolet R3 diffractometer at -90°C, equipped with a Nicolet LT-2 low-temperature device, using the omega scan technique at 5 - 12°/min out to a 2θ limit of 45°. A total of 12144 reflections were collected, of which 10699 were unique. The R for averaging symmetry equivalent reflections was equal to 0.034. Data were corrected for L_p effects and decay but not for absorption. The structure was solved

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by the direct methods and refined by full-matrix least-squares using SHELXTL-Plus (Sheldrick, 1991). The hydrogen atoms were calculated in ideal positions, with Uiso set to $1.2 \times U_{eq}$ of the relevant atom. One molecule of n-hexane and one of methylene chloride are disordered. The tetrahydrochloric acid salt of 11, $(C_{68}H_{74}N_{10})^{+4} (Cl^{-})_4 - 3CH_2Cl_2 - 3/2 (C_6C_{14})$, Mr = 1557.29, had a triclinic, space group $P\bar{1}$ (no. 2), with $a = 14.221(6)$, $b = 15.374(3)$, $c = 20.531(5) \text{ \AA}$, $\alpha = 100.03(2)$, $\beta = 101.71(3)$, $\gamma = 106.23(3)^\circ$, $V = 4091(2) \text{ \AA}^3$, $\rho_{calc} = 1.26 \text{ g-cm}^{-3}$ for $Z = 2$, $F(000) = 1642$, $m(\text{Mo K}\alpha) = 3.888 \text{ cm}^{-3}$, $\lambda = 0.71073 \text{ \AA}$. One molecule of n-hexane and one of methylene chloride are disordered within the lattice. A total of 853 parameters were refined in blocks to a final $R = 0.0824$, $wR = 0.100$ and a goodness-of-fit = 2.607 using 4869 reflections having $F > 4s(F)$.

In accord with what was found in the solution phase NMR experiments, the tetraprotonated form of turcasarin 11 is found to adopt a nearly C_2 symmetric twisted "figure-eight" conformation in the solid state, and not an open circular conformation. This twist conformation serves to define two enantiomers (both of which are seen in the X-ray structure) such that the molecular geometry of 11 in the solid state is closer to that of the "twisted ribbons" in FIG. 4, rather than that of the more open circle-like "loop."

As can be seen from an inspection of FIG. 4, the twist observed in the solid state also serves to define two pentaphyrin-like "hemi-macrocycles" as well as found smaller "pockets." Occupying each of these latter pockets are two chloride anions held within hydrogen bonding distance to the nitrogens. These hydrogen bonding interactions, as implied above, are currently considered to account for the slow conformational interconversion observed at room temperature in solution

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on the NMR time scale. However, it is of interest to note that at the point of "ribbon crossing" the two pyrrole-containing π systems are separated from one another by a van der Waals distance of 3.268 Å.

5

Turcasarin 11 may be considered as being a 40 π -electron annulene. Thus, turcasarin should not be aromatic as predicted by the $(4n + 2)$ rule for aromatic systems. However, it displays a Soret-like absorption in the visible portion of the electronic spectrum that is considerably red-shifted compared to that of the porphyrins. On the other hand, no evidence of ring current effects are seen for the pyrrolic proton resonances in the proton NMR spectrum. Thus, 11 is considered as being π delocalized, but not aromatic.

NMR studies were also used to obtain solution state structural information. The ^{13}C NMR spectrum shows, for instance, apparent C_2 symmetry in the macrocycle frame as judged by the number of observable peaks. The ^1H NMR spectrum is also consistent with C symmetry as judged by the number of signals in the interior NH and exterior *b*-pyrrolic and meso-proton region. The signals in the alkyl region, on the other hand, proved more complex, with no clear assignment of symmetry or structure being possible from immediate inspection. In fact, complex splitting patterns for the methylene protons of the alkyl sidechains were observed.

The complex splitting patterns mentioned above lead to the suggestion that the macrocycle may exist in two limiting conformations. These two conformations result from a twist along a long axis of the molecule as shown in FIG. 4. Turcasarin 11 could therefore exist as a pair of enantiomers. It would thus be dissymmetric even though it possesses no formal stereogenic centers. Such a postulate of conformational chirality, even though

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unexpected for a fully conjugated macrocycle, would, of course, account for the observed complex splitting patterns which would arise as the result of an imposed diastereotopicity on the alkyl side chains.

5

Definitive ^1H NMR peak assignments were made by COSY (FIG. 5) and NOESY. The NOESY spectrum also showed significant exchange crosspeaks in all regions indicating that the two enantiomers can readily interconvert, at least on the time scale of such experiments. However, variable temperature ^1H NMR (CDCl_3 , 50°C to -50°C), by virtue of very little change in the chemical shifts, indicated that this interconversion is slow on the NMR time scale at room temperature. This slow interconversion could reflect conformational stabilization imposed by the presumably strong hydrogen bonding interactions between the macrocycle and the chloride anions observed in the solid state.

20 Additionally, the data indicates that the rate of interconversion between the two limiting enantiomeric figure-eight conformations is slow on the NMR time scale at room temperature. This slow interconversion is considered to reflect the presumably strong hydrogen bonding interactions found in the solid state, where the four chloride counter ions are observed to be in intimate hydrogen bonding contact with certain pyrrole NH groups of the protonated with four hydrogens macrocycle.

30 The present data demonstrate that conjugated, polypyrrolic macrocycles need not be flat (or near-flat) in their overall three dimensional structure but rather can adopt conformations that make them potentially chiral.

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EXAMPLE 4

SYNTHESIS OF THE BIS-URANYL CHELATE OF TURCASARIN 11

In a 25 mL round-bottomed flask equipped with an argon inlet and a magnetic stirrer, turcasarin 11 hydrochloride (6.2 mg, 5.25×10^{-6} mol) was dissolved in 10 mL 2-propanol. To the resulting blue solution, pyridine (5 mL) was added followed by $\text{UO}_2(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ (20 mg, excess). The blue mixture was stirred at reflux under an argon blanket for three hours. The solvent was removed *in vacuo*. The residue was taken up in CH_2Cl_2 and purified chromatographically (silica gel, 3 x 15 cm, CH_2Cl_2 as eluent). The only band (green) to elute was collected, and the solvent was removed *in vacuo* to afford a green solid. This solid can be recrystallized from diffusion of isopropyl ether into a CH_2Cl_2 solution of 2 (or from hexanes layered on a CHCl_3 solution of 2) to afford fine green needles. Yield: 5.7 mg (69%). M.p.: dec. > 250°C; H NMR (300 MHz, CD_2Cl_2) δ 0.38 (t, $J = 6.4$ Hz, 6H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.33 (t, $J = 7.3$ Hz, 6H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.51 (s, H_2O), 1.66 (t, $J = 7.4$ Hz, 6H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.86 (t, $J = 7.4$ Hz, 6H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.09 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.26 (m, 8H, $\text{CH}_2\text{CH}_2\text{CH}_3$ & $\text{CH}_2\text{CH}_2\text{CH}_3$), 2.80 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 3.35 (s, 6H, CH_3), 3.47 (m, 4H, CH_2CH_3), 3.59 (s, 6H, CH_3), 3.84 (m, 4H, CH_2CH_3), 4.36 (s, 2H, pyrrole-CH), 4.70 (d, $J = 4$ Hz., 2H, pyrrole-CH), 7.34 (d, $J = 4.2$ Hz., 2H, pyrrole-CH), 8.15 (s, 2H, pyrrole-CH), 8.21 (s, 2H, pyrrole-CH), 9.29 (s, 2H, NH); UV/vis (CH_2Cl_2) λ_{max} 445.5, 681.5 nm; MS (FAB) m/e 1570; HRMS (FAB) m/e calc'd. for $\text{C}_{68}\text{H}_{72}\text{N}_{10}\text{O}_4^{238}\text{U}_2$; 1568.6754, found: 1568.6754. The H NMR spectrum of complex appears similar to that of the parent compound 11, including diastereotopic splitting patterns in the alkyl region. This may suggest a coordination mode in which the "figure-eight" macrocyclic frame observed in the metal-free ligand is retained more or less unchanged in the case of this particular metallo-adduct. Characterization data: UV/vis

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(CH₂Cl₂): λ_{\max} [nm] = 445, 682; LRMS (FAB): Calculated for C₆₈H₇₄N₁₀U₂O₄; m/z = 1570 Found: 1570; ¹H NMR spectrum: Similar to that of the parent compound 11, including diastereoetopic splitting patterns in the alkyl region.

5 This example demonstrates the use of turcasarins as metal ion chelating agents and particularly as a chelating agent of uranyl cations. It is contemplated that further chelates may be used as photodynamic agents, for example, chelates with diamagnetic lanthanide cations,

10 such as La(III), Lu(III) or In(III).

EXAMPLE 5
SYNTHESIS OF TURCASARIN HAVING
DIFFERENT SUBSTITUENTS THAN TURCASARIN 11

15

15,20,37,42-Tetraethyl-2,11,16,19,24,33,38,41-octamethylturcasarin has also been prepared. 4,4'-Diethyl-5,5'-diformyl-3,3'-dimethyl-2,2'-bipyrrole (71.6 mg., 0.00026 mol) was dissolved in 20 mL absolute ethanol

20 with the aide of a heat gun. CHCl₃ was added (500 mL), followed by 2,5-bis(4-methyl-2-pyrrolyl)pyrrole (59.2 mg., 0.000263 mol). HCl gas was bubbled into the stirred reaction mixture for five minutes. The reaction was then stirred under an argon blanket for four hours. The CHCl₃

25 was removed using a rotary evaporator. The ethanolic residue was treated with concentrated aqueous NaHCO₃ until effervescence ceased and the extracted into chloroform in a separatory funnel. The organic layer was separated off, dried over Na₂SO₄ and taken to dryness in

30 vacuo. The residue was taken up in a minimum amount of methylene chloride and ammonia gas was bubbled into the solution for one minute. The resulting solution was purified chromatographically on a pre-basified 2 x 20 centimeter silica gel column (achieved by bubbling

35 ammonia through a methylene chloride-silica slurry before packing). The first major purple band using methylene chloride as the eluent was collected, placed in a

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separatory funnel, and washed first with water (2 x 25 mL) and then 1N HCl (3 x 25 mL). The organic layer was separated off, dried over Na₂SO₄, and taken to dryness in vacuo. Yield: 16.1 mg (11.5%). The ¹H NMR spectrum of this newer system, while retaining spectral features characteristic of the NH, *b*-pyrrolic and meso proton resonances, remained essentially unchanged relative to 11, and proved far less complex in the alkyl region. Characterization data: ¹H NMR (300 MHz, CDCl₃): δ = 1.03 (t, $J_{\text{HH}} = 7$ Hz, 6H, CH₂CH₃), 1.09 (t, $J_{\text{HH}} = 7$ Hz, 6H, CH₂CH₃), 1.67 (s, H₂O), 1.90 (s, 6H, CH₃), 2.09 (s, 6H, CH₃), 2.33 (q, $J_{\text{HH}} = 7$ Hz, 4H, CH₂CH₃), 2.39 (q, $J_{\text{HH}} = 7$ Hz, 4H, CH₂CH₃), 2.45 (s, 6H, CH₃), 2.80 (s, 6H, CH₃), 6.76 (s, 2H, meso-H), 6.95 (s, 2H, meso-H), 7.04 (s, 2H, pyrrole CH), 7.62 (d, 2H, pyrrole CH), 8.60 (s, 2H, pyrrole CH), 9.68 (d, 2H, pyrrole CH), 14.23 (s, 2H, NH), 14.44 (s, 2H, NH), 14.67 (s, 2H, NH), 15.21 (s, 2H, NH), 15.58 (s, 2H, NH); UV/vis (CH₂Cl₂): $\lambda_{\text{max}} = 644$ nm; HRMS (FAB): Calculated for C₆₀H₆₃N₁₀: $m/z = 923.5237$. Found: 923.5252 ((M + 1)⁺ - 4 HCl).

Using the procedure of this example, it is contemplated that compounds of formula I may be prepared where R¹, R² or R³ is hydroxy substituted methyl, ethyl, or propyl; carboxy or ester substituted methyl, ethyl or propyl; polyglycol substituted methyl, ethyl, or propyl, by employing the appropriate substituted bipyrrroles and terpyrrroles.

30

EXAMPLE 6**TURCASARINS AS BINDING AGENTS**

The turcasarins may be used as receptors for anions, cations or neutral molecules provided the turcasarin is of appropriate charge. For instance, anionic turcasarin may be prepared for use as a metal cation chelant by removing protons from the secondary amine groups in the

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- 40 -

macrocycle in organic solution using strong bases such as butyl lithium.

5 Due to the presence of four chloride anion binding pockets in the tetraprotonated form of 11, it is expected that turcasarin, in analogy to several other expanded porphyrins, could serve as an anion binding and transport agent under solution phase conditions.

10

EXAMPLE 7
SYNTHESIS OF SUBSTITUTED TURCASARINS
AND FURTHER ANALOGUES

15 It will also be apparent to one of skill in the art that many turcasarins may be obtained within the context of this synthetic methodology. For example, it is also envisioned that different combinations of bipyrrroles and terpyrrroles may be employed in the condensation step to produce various analogues of 11.

20

A preliminary example concerns the use of 5,5'-diformyl-4,4'-dipropyl-2,2'-bipyrrrole as a starting material for use in reaction sequences in accordance with the present invention. This synthetic process involves 25 reaction with compound 9 in a condensation which will produce an analogue of compound 11, corresponding to the turcasarin of Formula I wherein R¹ and R² are propyl, and R³ and R⁴ is hydrogen.

30

Accordingly, it is contemplated that a variety of approaches may be employed, in accordance with the present invention, to prepare systems bearing a wide variety of alkyl and/or aryl substituents in the *meso* and/or β -pyrrolic positions.

35

For example, *meso* substituted compounds may be prepared to test the extent to which the presence of

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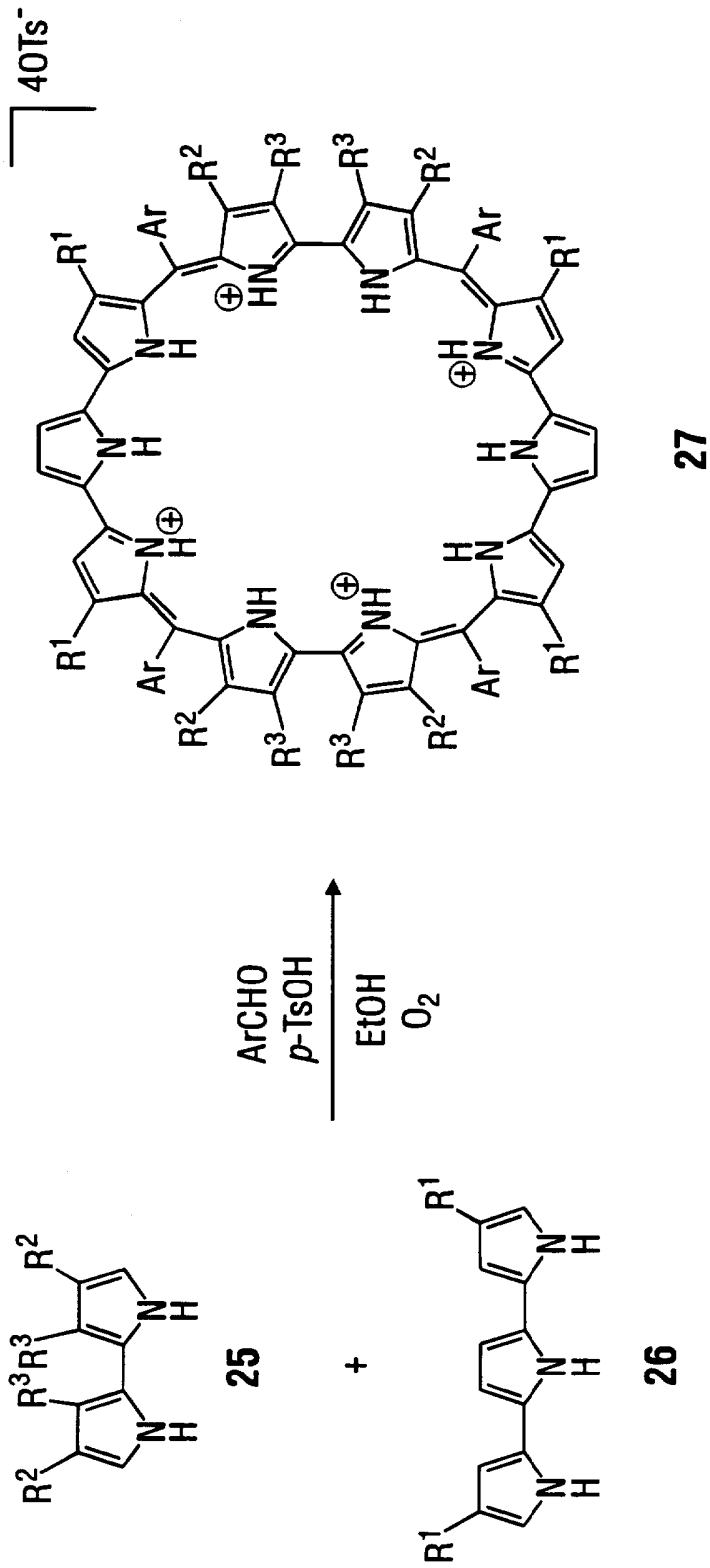
different groups, such as, for example, 4'-phenyl and/or 2'-phenyl donating groups may augment effective nitrogen lone pair basicity and/or enhance higher pH phosphate transport capability. Extensions to systems bearing two (or more) *meso* substituents are also contemplated within the scope of the invention. In any event, it is important to appreciate that adding further *meso* substituents will, in all likelihood, induce substantial distortions of the macrocycle. Thus, these syntheses should provide compounds that will allow steric effects to be assessed. It is contemplated that transport and pK_a' tests will be conducted to determine the effects of the various substitutions.

It is contemplated that phenyl-bearing turcasarins may be prepared by a "direct insertion" procedure. This procedure (a specific example of which is shown in Scheme A) involves the condensation of a bis- α -free bipyrrrole (e.g., 25) with an α , ω -free bis(pyrrolyl)-pyrrole (e.g., 26) in the presence of benzaldehyde or substituted benzaldehyde under turcasarin forming conditions, as described herein, which will afford bisarylturcasarin 27. In Scheme A, a range of other macrocyclic and non-macrocyclic products may also be obtained.

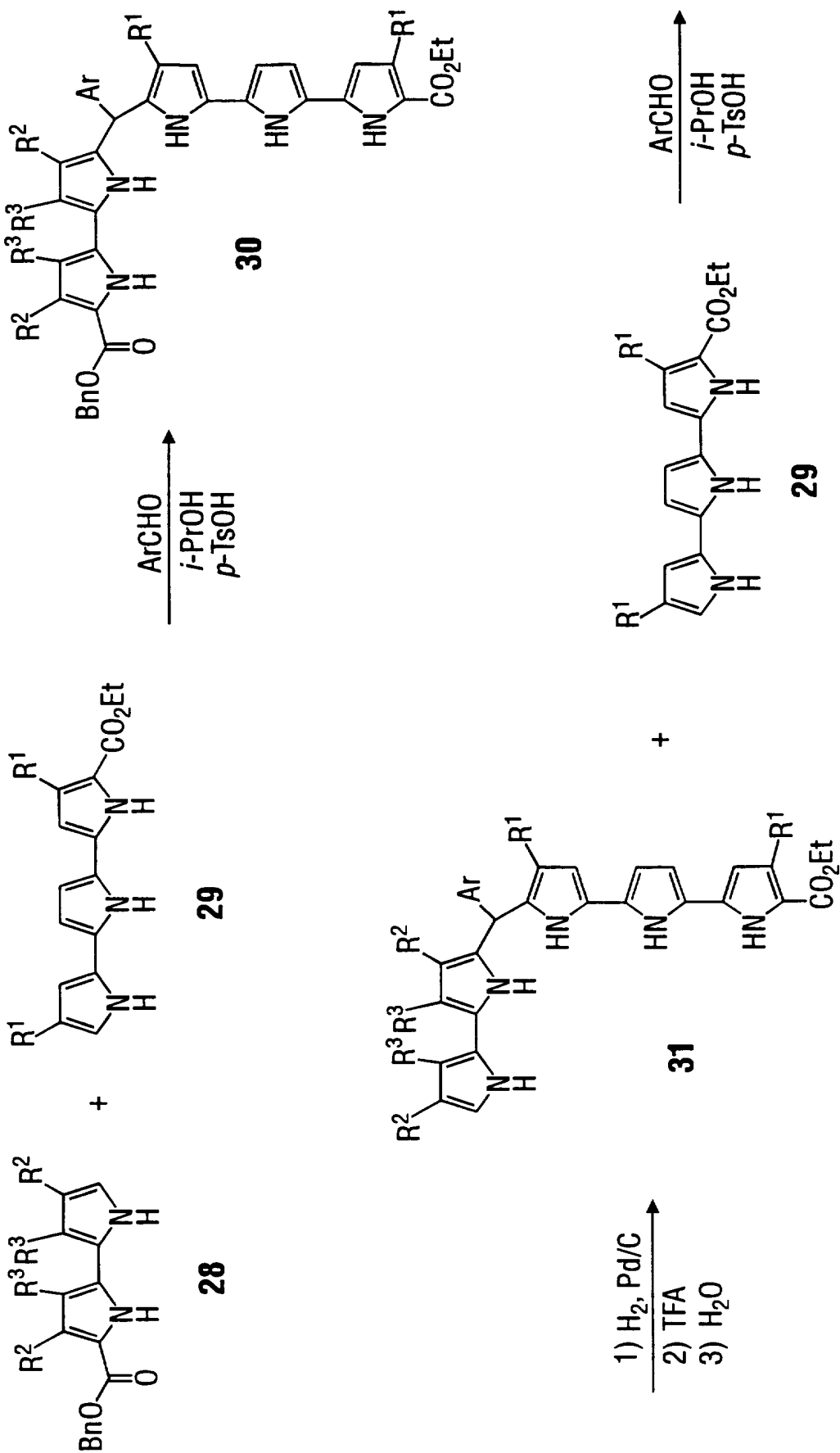
Other syntheses can be envisioned such as, for instance, the synthesis shown in Scheme B in which an α -free, ω -protected bipyrrrole (e.g., structure 28 in Scheme B), is co-condensed with an α -free terpyrrrole (e.g., 29) in the presence of an aromatic aldehyde. The benzyl ester of the resulting terpyrrolyl-bipyrrrole 30 can then be selectively cleaved under standard debenzoylation conditions and decarboxylated with trifluoroacetic acid to give the α -free terpyrrolyl-bipyrrrole, structure 31. Acid catalyzed condensation of terpyrrolyl-bipyrrrole 31 with another molecule of α -free terpyrrrole 29 in the presence of an aromatic aldehyde

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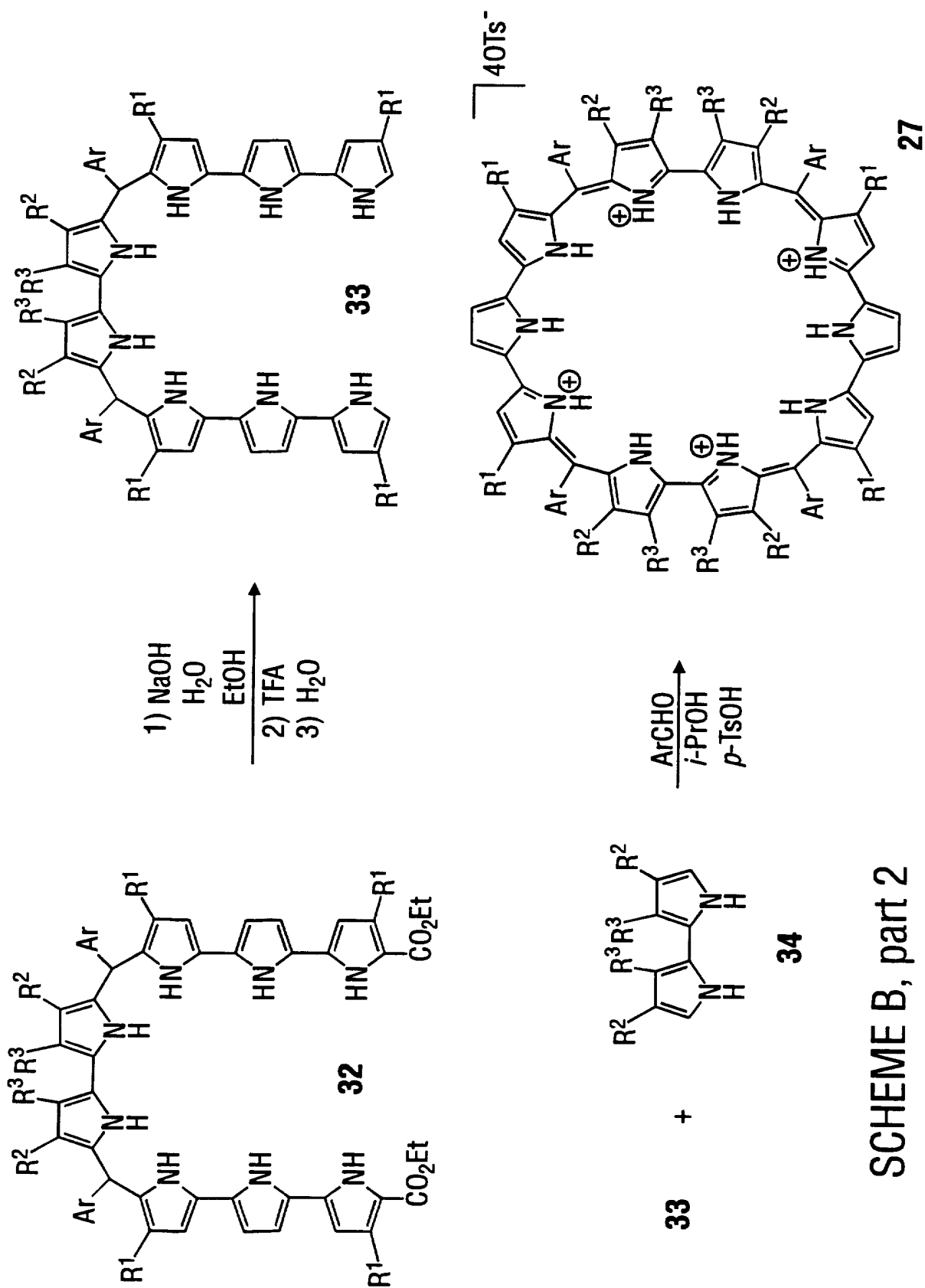
yields an ethyl ester-protected diaryl, bis(terpyrrolyl)-bipyrrole 32, which can be deprotected to form the α , ω -free bis(terpyrrolyl)-bipyrrole 33. Acid catalyzed co-condensation of 33 with an α , ω -free bipyrrole (e.g., 34) in the presence of an aromatic aldehyde, under turcasarin-forming conditions such as those described in Example 3, affords a turcasarin with each of its four "meso" positions substituted with aryl groups (e.g., 27).



SCHEME A



SCHEME B, part 1



SCHEME B, part 2

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It will, of course, be understood that in syntheses such as those exemplified in Schemes A and B, substituents in the starting compounds, such as those groups represented by R^{1-3} may be varied as desired. Thus, by using the appropriately substituted starting units, a turcasarin product may be produced in which groups R^{1-3} (as represented by structure 27) may be substituted with any of the R^{1-3} groups listed above.

Extensions of known methods may also be used that would allow one of skill in the art, in light of the present disclosure, to prepare functionalized turcasarins bearing one or more non-alkyl substituents in the β positions. One procedure for entry into such systems involves the preparation of carboxyalkyl substituted systems, for example, compounds bearing substituents such as $-(CH_2)_n-CO_2H$, and then further elaborating these to form the corresponding hydroxyalkyl, aminoalkyl, thiol, sulfonate, ether, amide, ester, or formylalkyl derivatives.

In addition to the direct conversions described above, it is important to appreciate that carboxyalkyl substituted turcasarins may also be used as the basis for obtaining other, more complex, functionalized systems. For instance, the carboxylic acid group of the carboxyalkyl-bearing turcasarins may be activated using standard reagents (such as, for example, thionyl chloride or DCC) and used to prepare either ester- or amide-linked conjugates.

Said conjugates could include compounds that contain one or more nucleic acid base ("nucleobase") or sugar ("saccharide") subunits, as is described in detail in the following Examples. For example, in such syntheses, one may condense a protected aminoalkyl nucleobase, for instance, the known material, 1-(2-aminoethyl)-4-

- 47 -

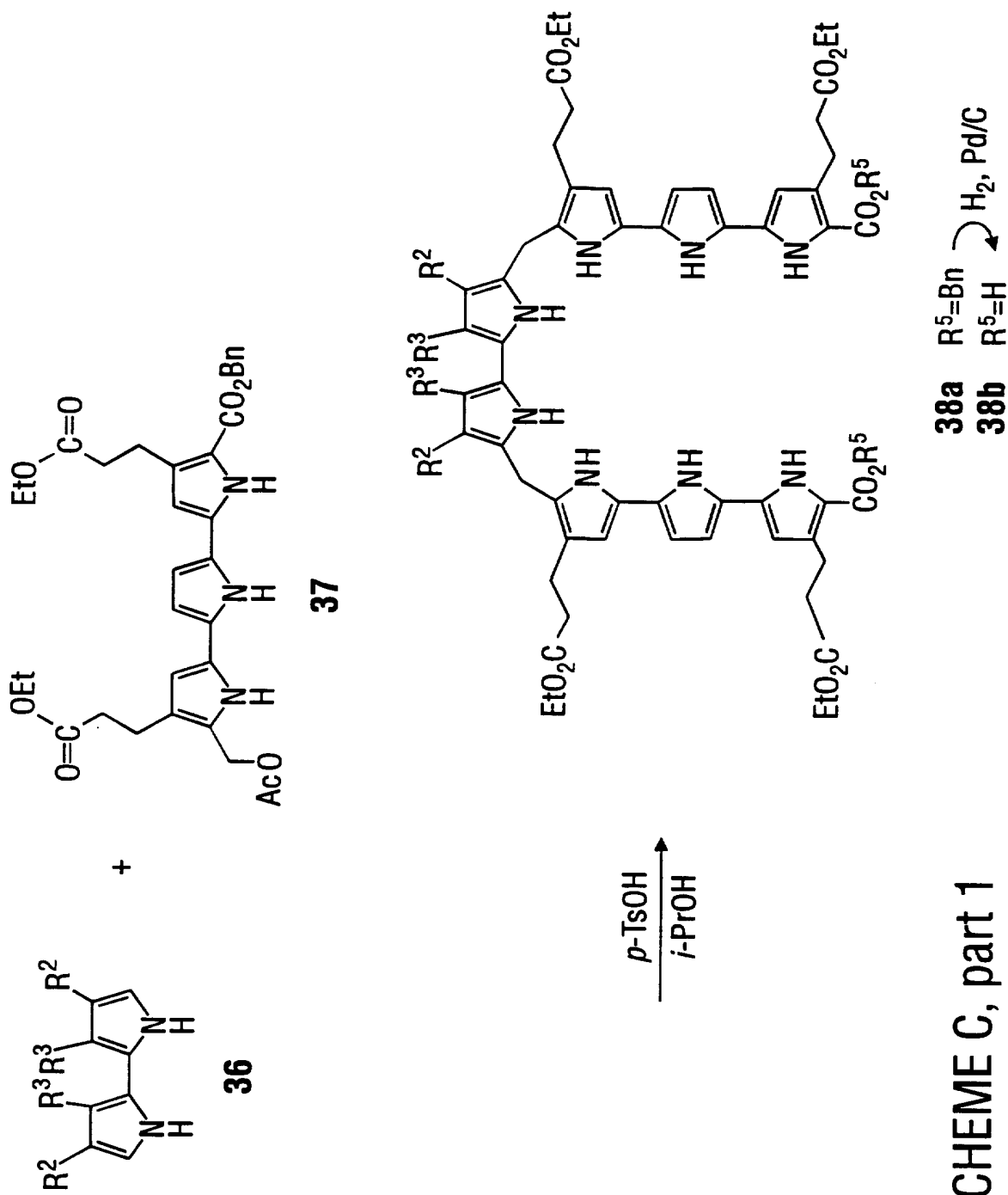
triphenylmethyl)amino]-pyrimidine-2-one, with the activated turcasarin carboxylic acid and subsequently effect nucleobase deprotection. Similarly, one may employ an amino-bearing protected sugar, such as per-O-acetylated glucosamine, and subsequent deprotection. In addition, one may use such a conjugation approach to prepare complex ethers, esters, or amides where the ether, ester, or amide linkages are used to append a wide variety of polyfunctional substituted alcohol and/or amine fragments on to the turcasarin periphery.

The carboxyalkyl substituted turcasarins may be prepared by several routes. For example, as shown in Scheme C, condensation of bipyrrrole 36 with terpyrrrole 37 may be used to obtain, following debenzylation, bis(dicarboxyterpyrrolyl)bipyrrrole 38b. Following condensation of compound 38b with bipyrrrole 39, under turcasarin-forming conditions such as those described in Example 3, there will be provided turcasarin 40 bearing four carboxyalkyl substituents.

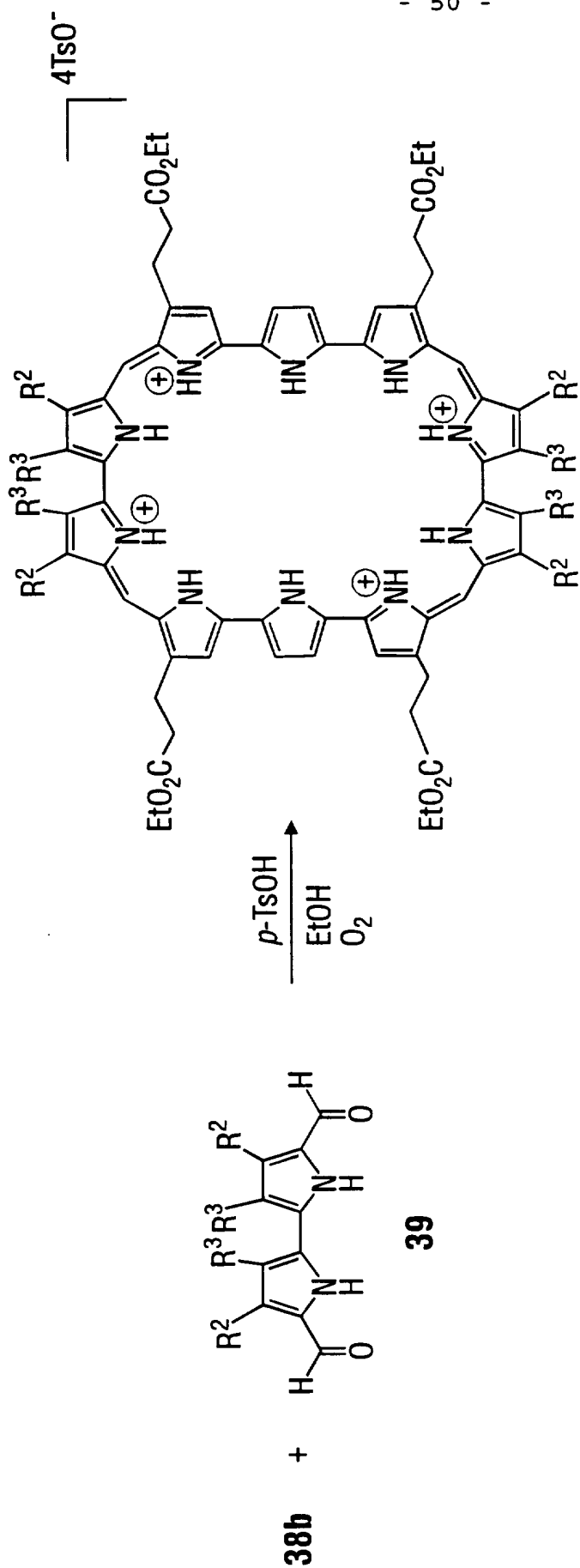
Secondly, in retrosynthetic analogy to the preparation of 3,8,17,22-tetraethyl-12-(carboxyethyl)-2,7,13,18,23-pentamethylsapphyrin, benzyl 3,5-dimethyl-4-(methoxycarbonylethyl)-pyrrole-2-carboxylate 41 may be converted, as shown in reaction Scheme D, to its corresponding bipyrrrole 44a (3,3'-bis(methoxycarbonylethyl)-5,5'-bis(benzyloxycarbonyl)-4,4'-dimethyl-2,2'-bipyrrrole) via sulfuric acid oxidation to acid 42, followed by standard iodination (43), and copper bronze mediated Ullman coupling. Following standard debenzylation to produce 44b and Clezy formulation with trifluoroacetic acid (TFA) and triethylorthoformate, condensation of the resulting bipyrrrole 45 (3,3'-bis(methoxycarbonylethyl)-5,5'-diformyl-4,4'-dimethyl-2,2'-bipyrrrole) with the terpyrrrole 46 of reaction Scheme E under turcasarin-

- 48 -

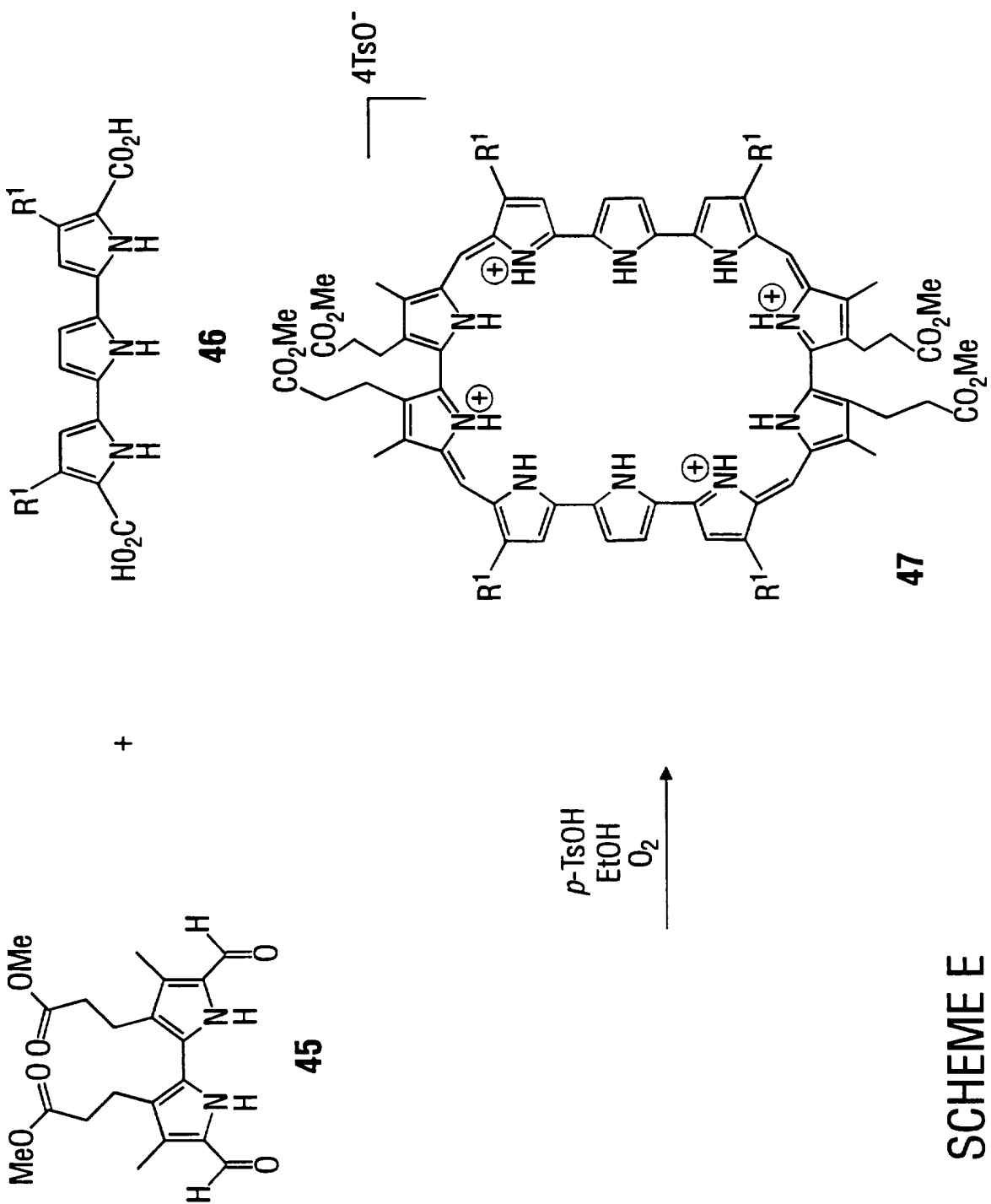
forming conditions will provide a turcasarin containing at least four carboxyalkyl substituents protected as their corresponding methyl esters, structure 47.



SCHEME C, part 1



SCHEME C, part 2



SCHEME E

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EXAMPLE 8

SYNTHESIS OF TURCASARIN-NUCLEOBASE CONJUGATES

It is contemplated that turcasarin-based dibasic
5 phosphate chelators may be modified so as to obtain
ditopic binding systems that display high inherent
specificity for a given purine or pyrimidine derived
nucleotide. This may be achieved by adding a synthetic
appendage of a nucleobase moiety. For example, the known
10 aminobutyl cytosine derivative may be employed with an
acid-catalyzed detritylation procedure.

Alternatively, the mode of base attachment may be
varied, for example, at the level of coupling, protecting
15 group, precursors, and the length, nature, and
orientation of any linking groups. The effects of such
variations on yield, selective binding, and other
properties of the resultant turcasarin-based molecule,
such as, for example, effective through-membrane
20 transport capability may then be determined and any
adjustments made accordingly. For example, the nature of
R groups may be changed, or secondary amides converted to
tertiary amides.

It is contemplated nucleobase "chelation" may be
25 achieved via complementary Watson-Crick type base-pairing
interactions. These compounds should display base-selec-
tive transport capability. This may be specifically
examined by various structural, static binding, and
30 dynamic transport analyses. In particular, it will be
determined whether the cytosine-for-guanine selectivity
holds in the case of suitably designed synthetic
conjugates. In addition, it will be tested whether this
same base-pairing approach suffices to engender
35 nucleobase selectivity in the case of adenine-thymine
pairing.

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As an extension of the above analyses, doubly functionalized systems may be synthesized for use in the selective binding of dinucleotides, some of which have interesting antiviral properties, as well as for the recognition and transport of mononucleotides. In the latter case, the possible combination of both Watson-Crick and Hoogsteen type base-pairing interactions could confer a degree of specificity not available using simpler systems. To the extent this proves true, it is possible that the doubly functionalized turcasarin system could serve as a viable antiviral adjuvant, capable not only of binding and solubilizing the phosphate portion of a nucleotide monophosphate, but also of effecting its selective through-membrane transport at or near physiologic pH.

In view of the successful studies carried out in the sapphyrin series, (for example, it was found that the attachment of a nucleobase to a sapphyrin core greatly enhanced the nucleotide recognition selectivity for transport), it is expected that the functionalization of turcasarins which because of their larger core size and increased basicity are inherently much better for anion recognition and phosphate anion chelation than sapphyrins, will lead to superior systems relative to any produced to date.

Reaction Schemes F through I represent examples of the synthesis of turcasarin nucleobase conjugates. The synthetic methodology represented in these reaction schemes may be straightforwardly adapted for the synthesis of any given turcasarin nucleobase conjugate by employing the desired starting materials. Groups R^{1-3} are as defined hereinabove in Schemes F through I.

An example of the preparation of a turcasarin nucleobase conjugate is shown in reaction Scheme F.

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Here, a tetracarboxyalkyl bearing turcasarin, tetra(ethoxycarbonylethyl)-turcasarin **40** (as prepared hereinabove in Scheme C) is saponified to its diacid form **49** by treatment with a 1:1 mixture of HCl and

5 trifluoroacetic acid. DCC coupling of turcasarin diacid **49** with trityl protected aminoethyl cytosine **50** in methylene chloride at 0°C followed by deprotection with TFA affords the amide linked bis(aminoethyl)cytosine turcasarin conjugate **51**.

10

Similarly, as shown in Scheme G, the amide linked bis(aminoethyl)guanosine turcasarin conjugate **55** is prepared by DCC coupling of turcasarin diacid **49** with benzoyl-protected aminoethyl guanosine **54** in DMF at 0°C

15 followed by deprotection with TFA.

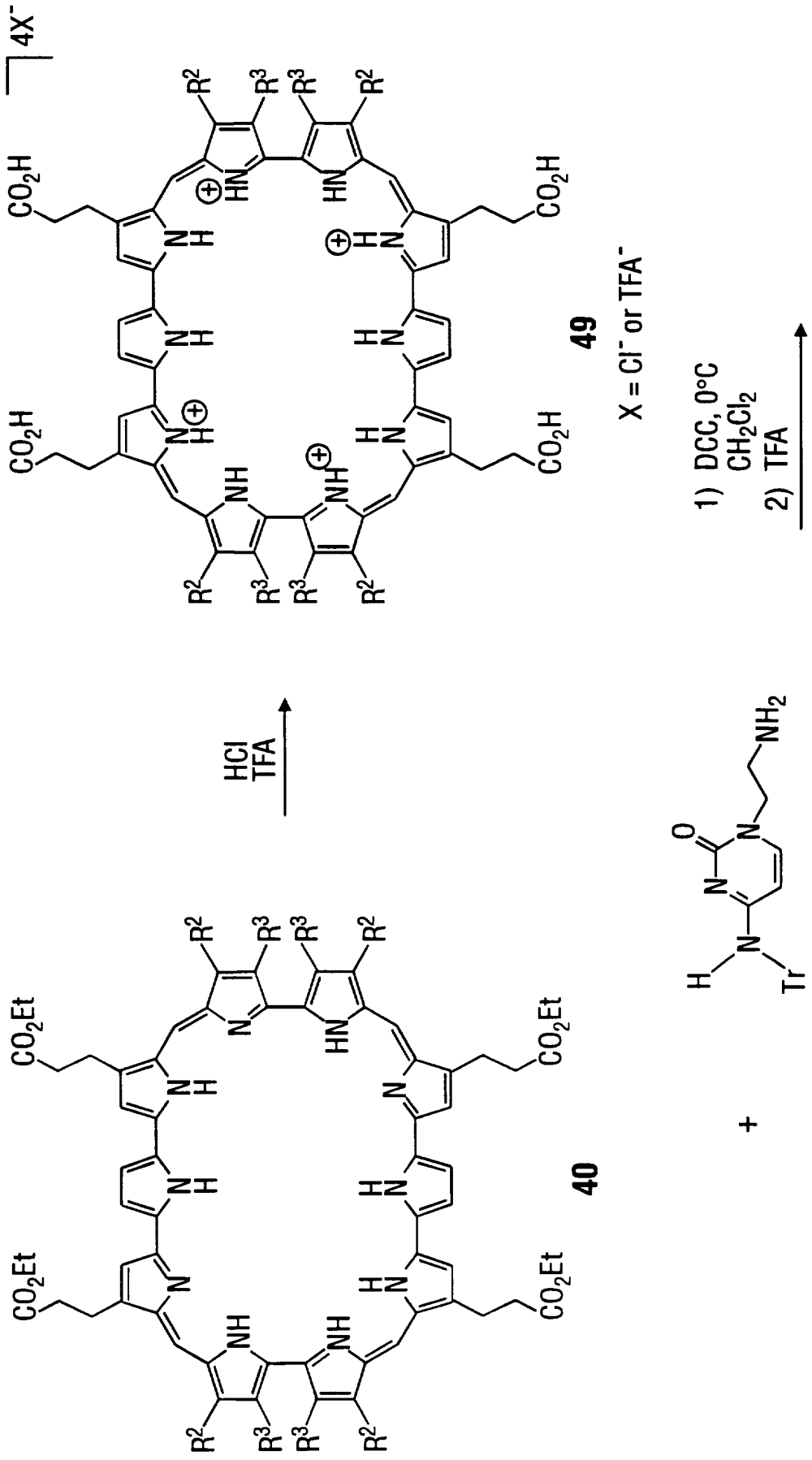
In another example of a dicarboxyalkyl bearing turcasarin, as shown in Scheme H, the tetra(methoxycarbonylethyl)-turcasarin **47** is saponified

20 to diacid **57** by treatment with a 1:1 mixture of HCl and trifluoroacetic acid. DCC coupling of turcasarin diacid **57** with trityl-protected aminoethyl cytosine **58** in methylene chloride at 0°C followed by deprotection with TFA affords the amide linked bis(aminoethyl)cytosine

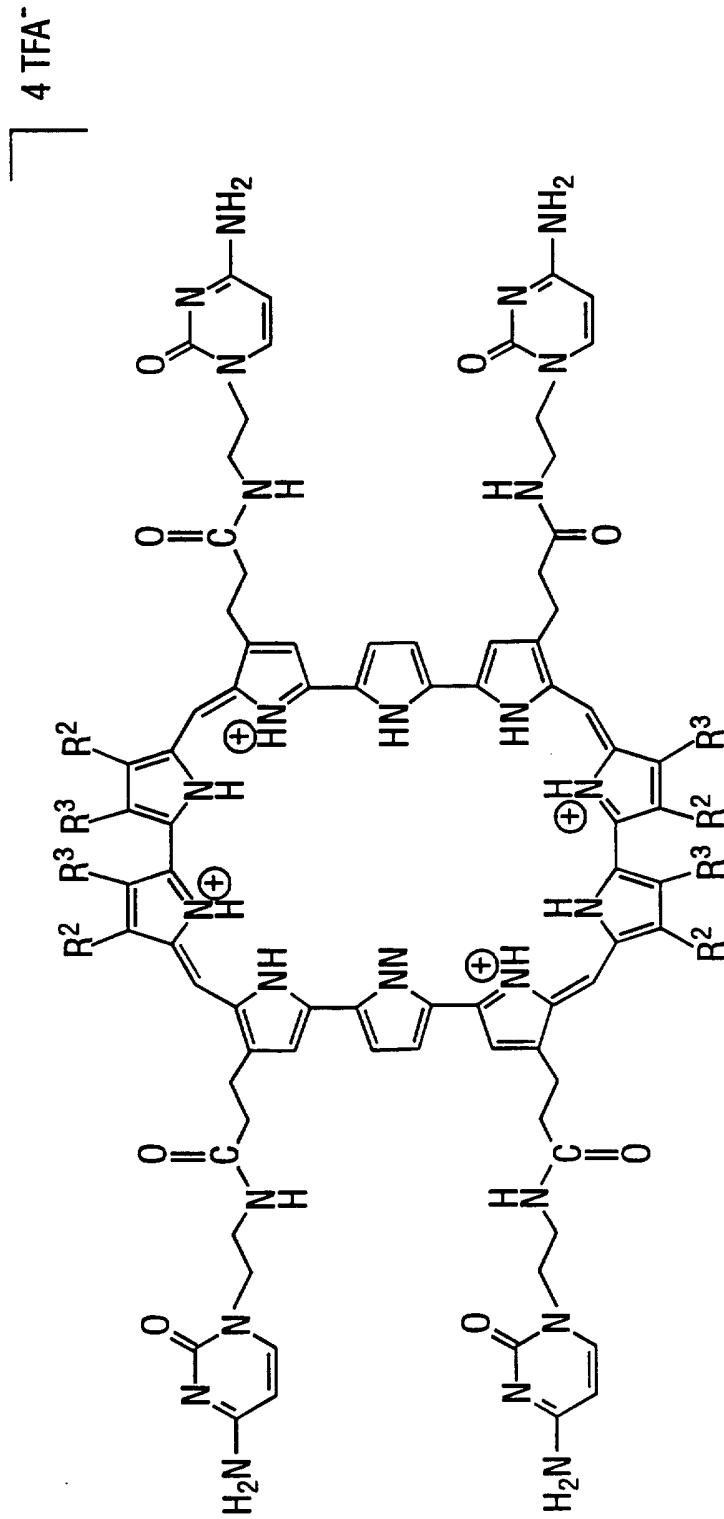
25 turcasarin conjugate **59**.

The amide linked bis(aminoethyl)guanosine turcasarin conjugate **63** of Scheme I is prepared by DCC coupling of turcasarin diacid **61** with benzoyl-protected aminoethyl

30 guanosine **62** in DMF at 0°C followed by deprotection with TFA.

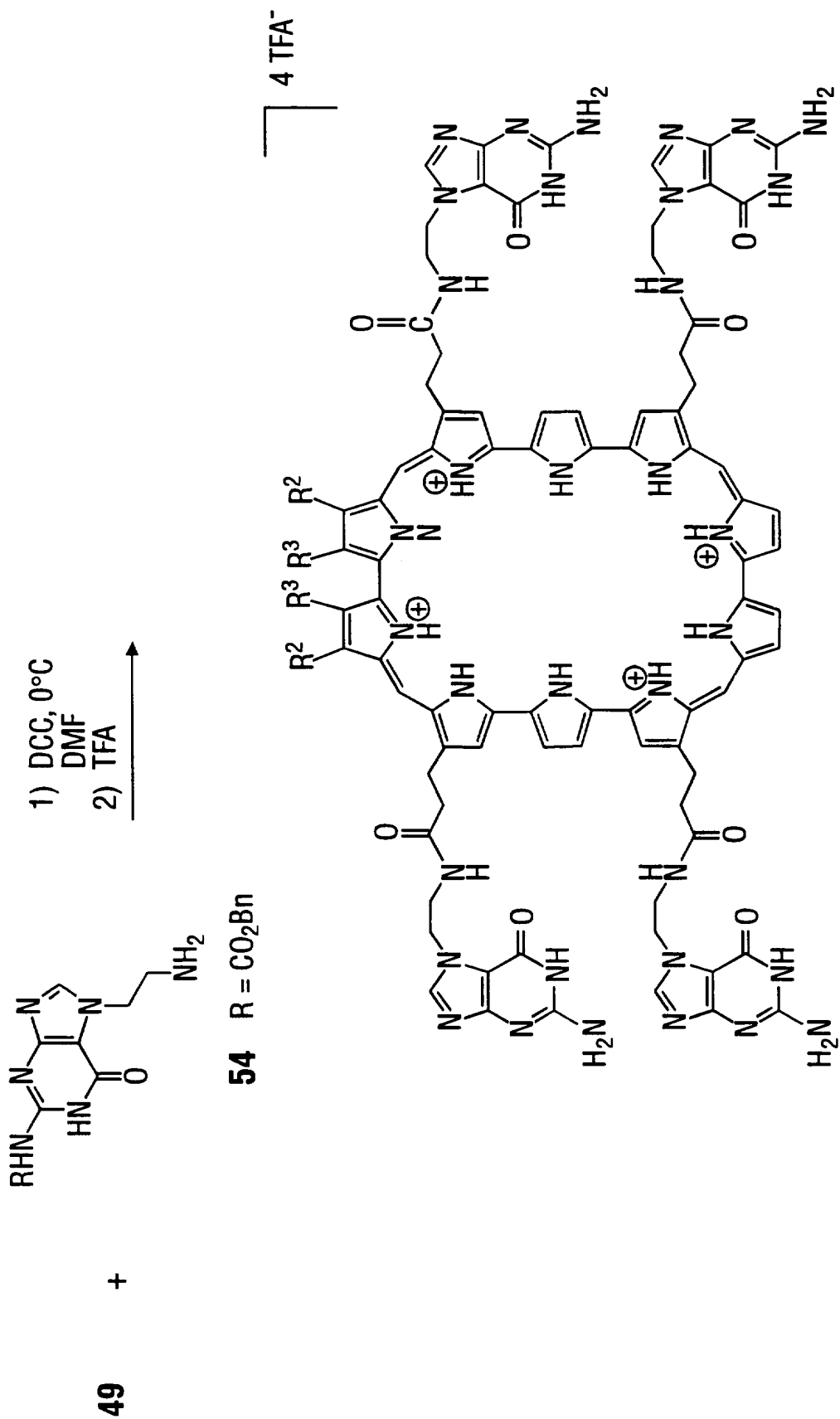


SCHEME F, part 1



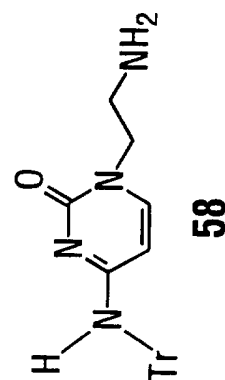
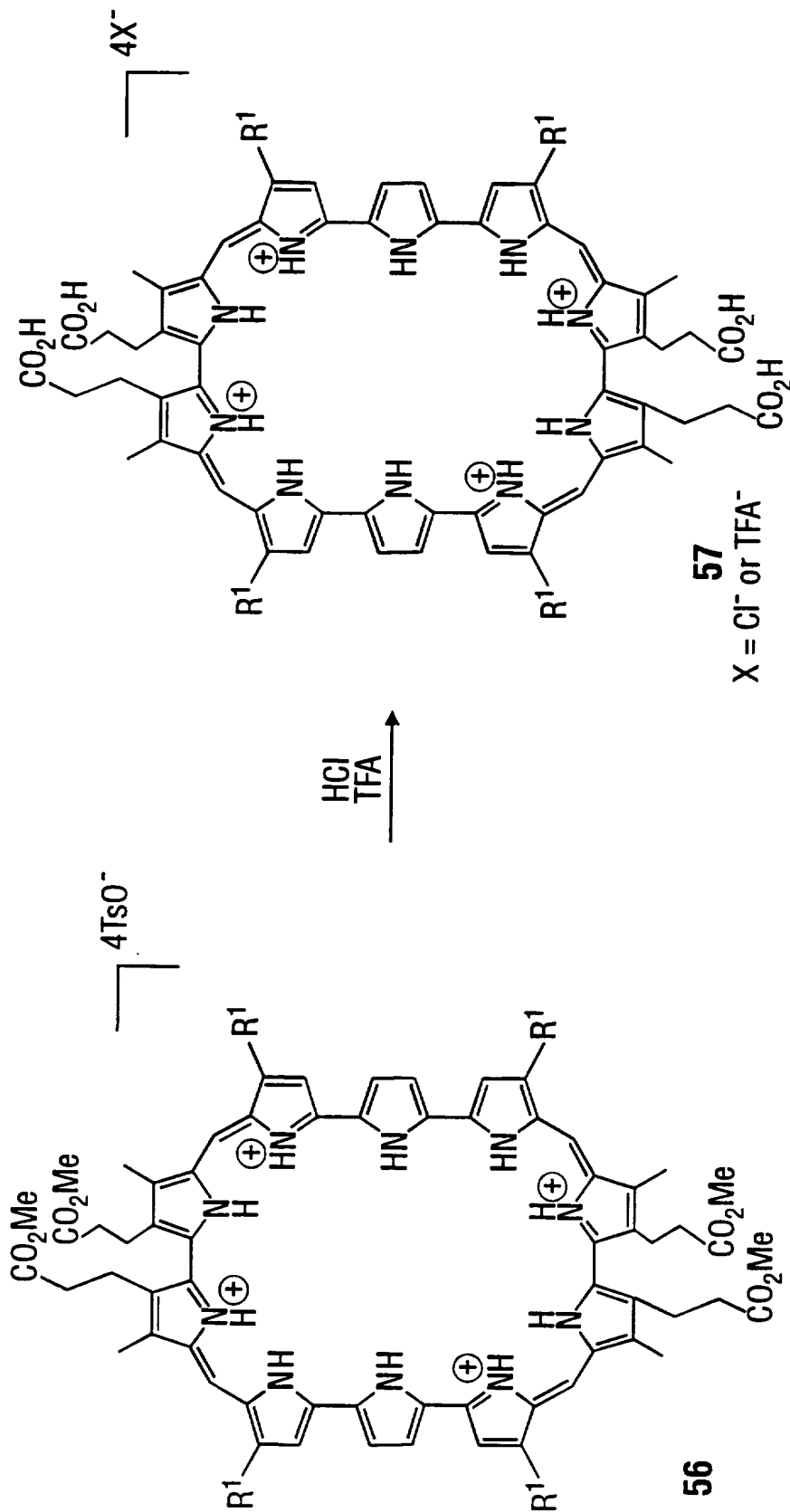
51

SCHEME F, part 2



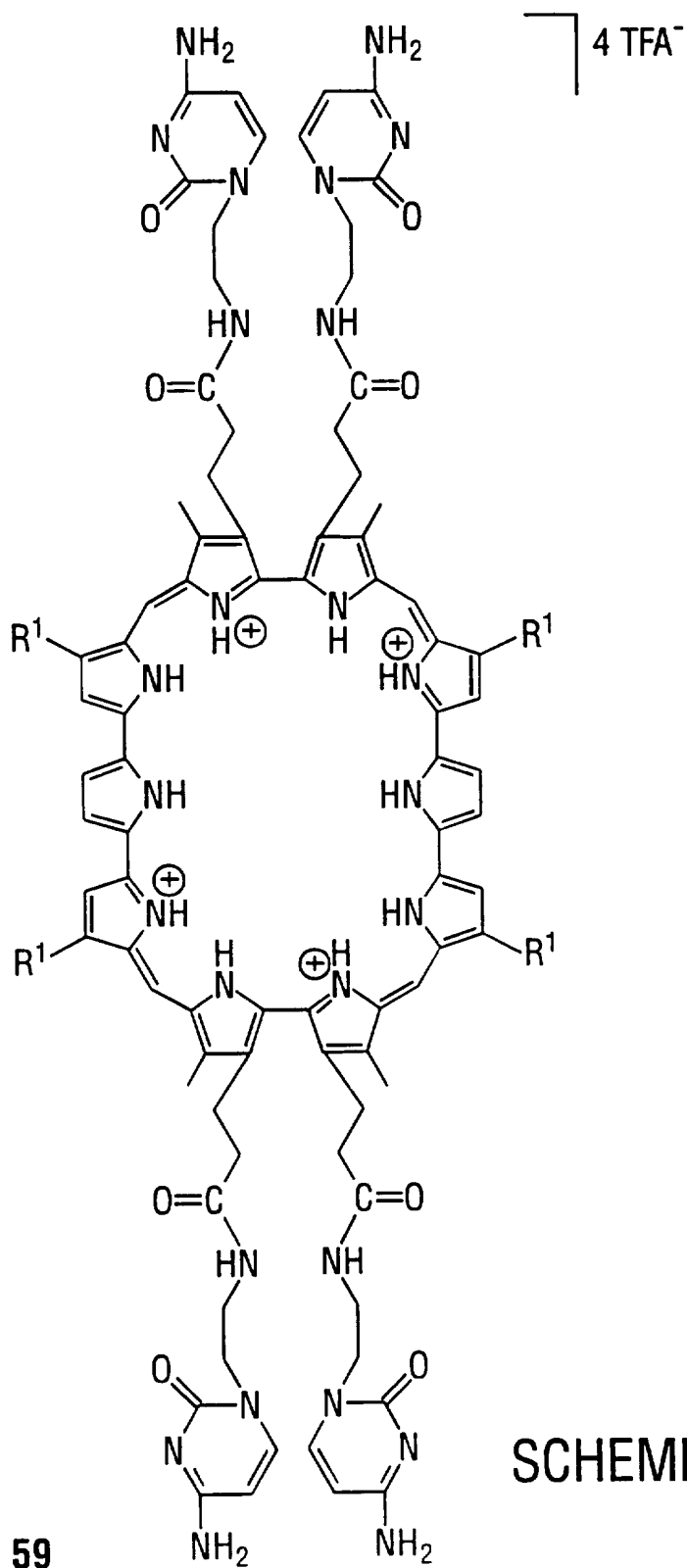
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SCHEME G



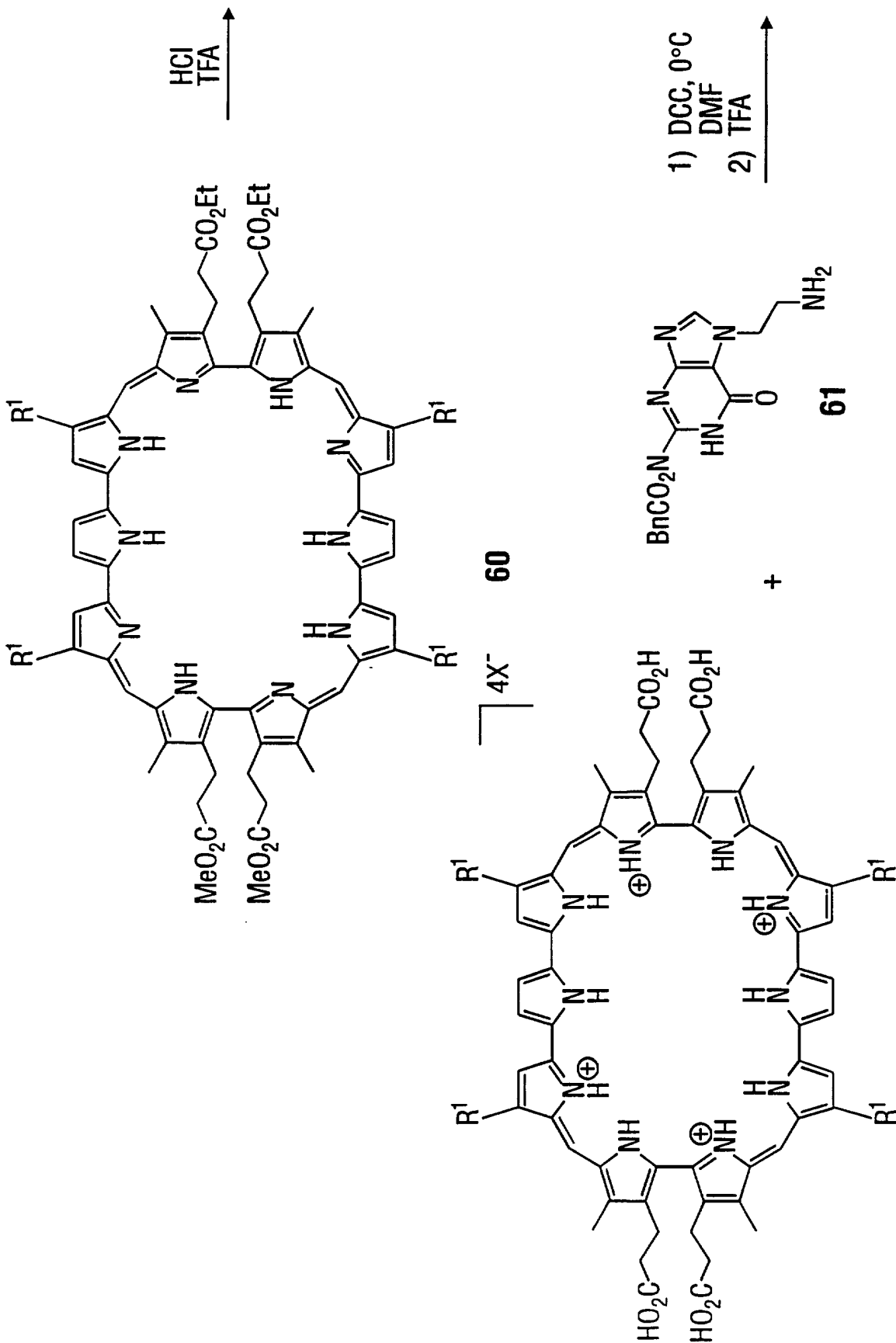
1) DCC, 0°C
 CH_2Cl_2
 2) TFA

SCHEME H, part 1

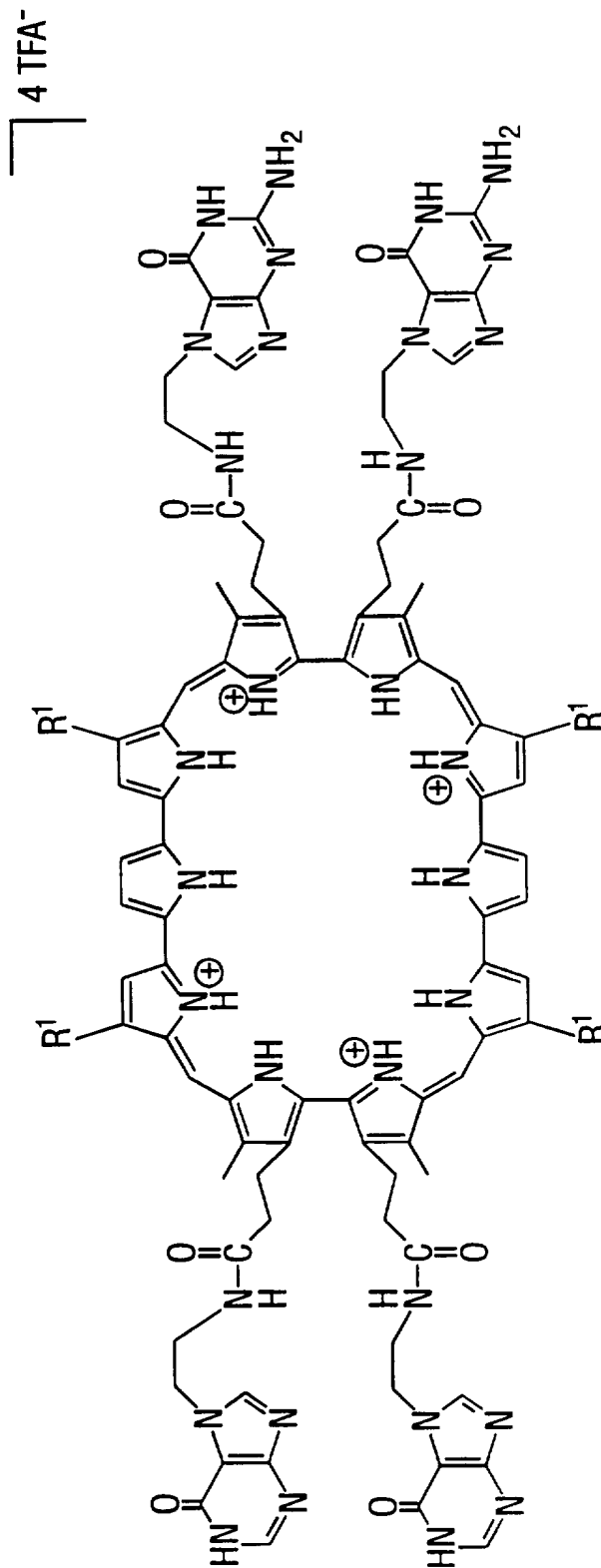


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SCHEME H, part 2



SCHEME I, part 1



63

SCHEME I, part 2

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EXAMPLE 9

SYNTHESIS OF TURCASARIN-SACCHARIDE CONJUGATES

Turcasarin derivatives or conjugates including one
5 or more saccharide units may be prepared according to the
synthetic methodology described hereinbelow. The sugar
units (represented originally by structures 65 and 75) in
Schemes J through O are intended to represent any
individual sugar or sugar derivative, such as those set
10 forth in Table 2, or polymers thereof, and include
modified sugars, such as methyl, amino, and phosphate
sugars, and D-, L-, α and β forms of said sugars.

The synthetic methodology represented in the
15 following reaction schemes may be straightforwardly
adapted for the synthesis of any turcasarin saccharide
compound as desired. Groups R¹⁻³ in Schemes J through O
are as defined hereinabove.

20 Referring now to Scheme J, amide-linked turcasarin
saccharide conjugates such as those represented by
structure 66b, are prepared by coupling diacid chloride
substituted turcasarin 64 (prepared by treating its
respective diacid turcasarin with thionyl chloride) with
25 the acetoxy protected HBr salt of amino saccharide 65 in
methylene chloride and pyridine, and deprotecting with
KOH in methanol.

In the same fashion, the bis(acid
30 chloride)turcasarin 67 as shown in Scheme K is prepared
by treating its respective diacid turcasarin, formed as
shown in Scheme H, with thionyl chloride. The amide-
linked bis(saccharide) turcasarin 68b may be prepared by
coupling turcasarin diacid chloride 67 with the acetoxy
35 protected HBr salt of amino saccharide 65 in methylene
chloride and pyridine, followed by deprotection with KOH
in methanol.

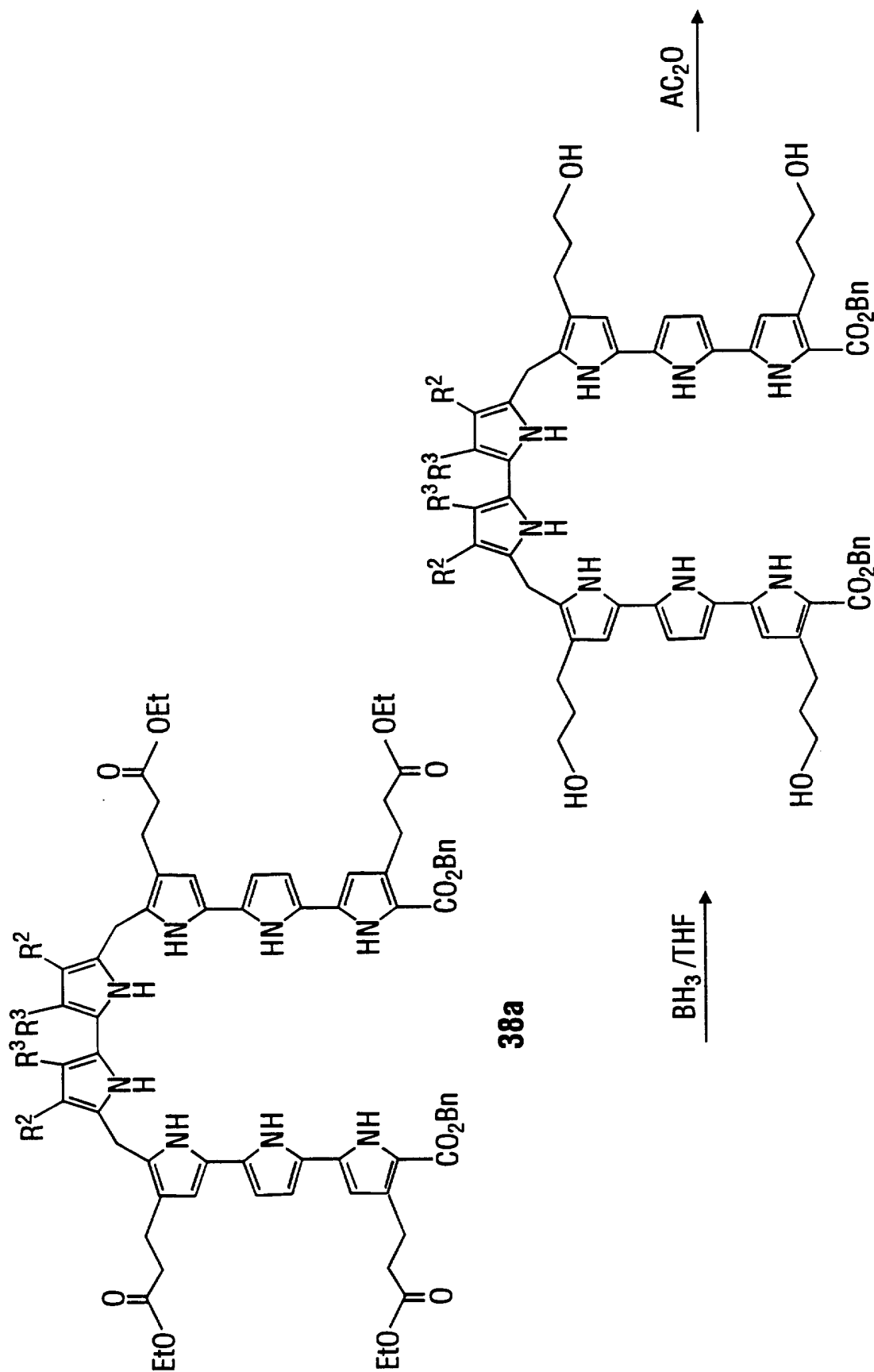
- 64 -

To prepare ether-linked bis(saccharide)turcasarin conjugates, the dihydroxy functionalized turcasarins, such as those represented by structure **73b** in Scheme L, may be prepared. For example, reduction of the methyl esters of macrocycle **69** with borane-THF to the corresponding alcohols followed by protection with acetic anhydride will provide the acetoxy-protected diol **71a**. Standard debenzylation to afford **71b** followed by condensation with diformyl bipyrrrole **72**, under turcasarin-forming conditions such as those described in Example 1, will provide the acetoxy-protected dihydroxy turcasarin **73a**. Deprotection of the hydroxyl groups is achieved by treatment with HCl in methanol to afford dihydroxyturcasarin **73b**.

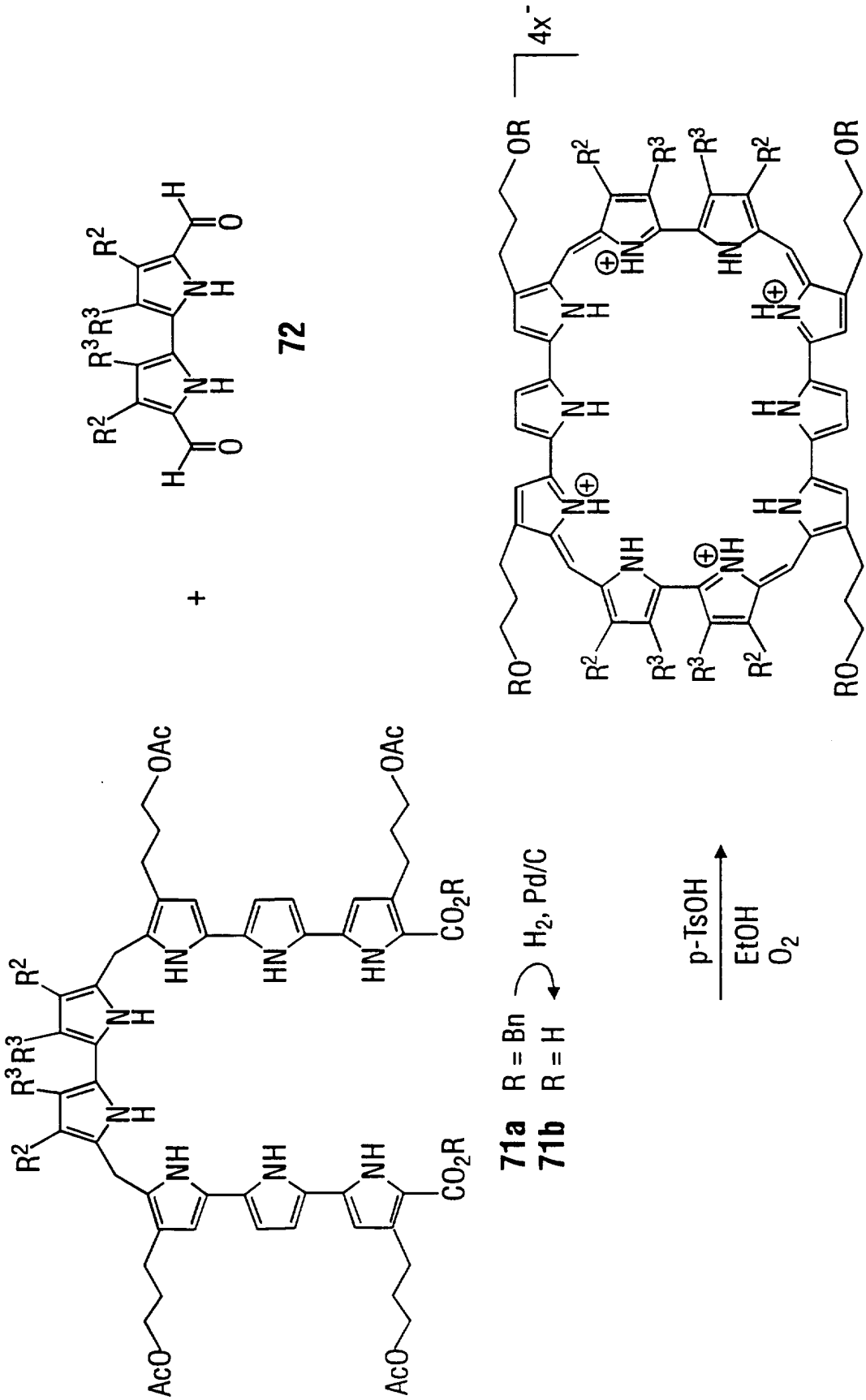
15

Dihydroxyturcasarins, formed as described above (Scheme L), can be coupled with acetoxy- and/or benzoyl-protected bromo-substituted saccharide units, such as structure **75** as shown in Scheme M, in methylene chloride with a catalyst such as silver triflate and barium carbonate. This results in the production of acetoxy- and/or benzoyl-protected bis(saccharide)turcasarin conjugates such as **76a** in Scheme M. Treatment of the acetoxy and/or benzoyl protected bis(saccharide)turcasarin **76a** with KOH in methanol yields the corresponding deprotected bis(saccharide)turcasarin conjugate **76b** (Scheme M).

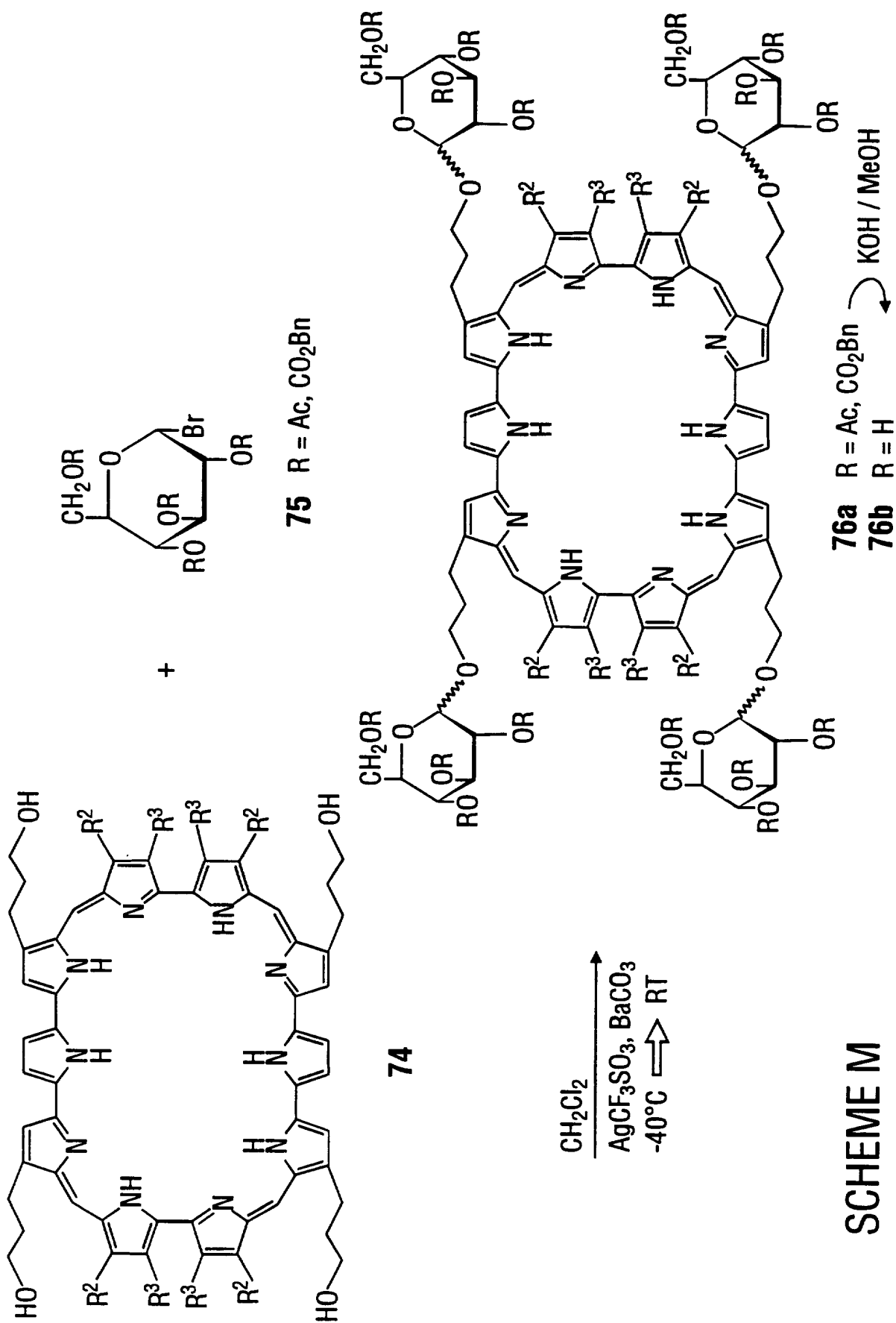
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SCHEME L, part 1



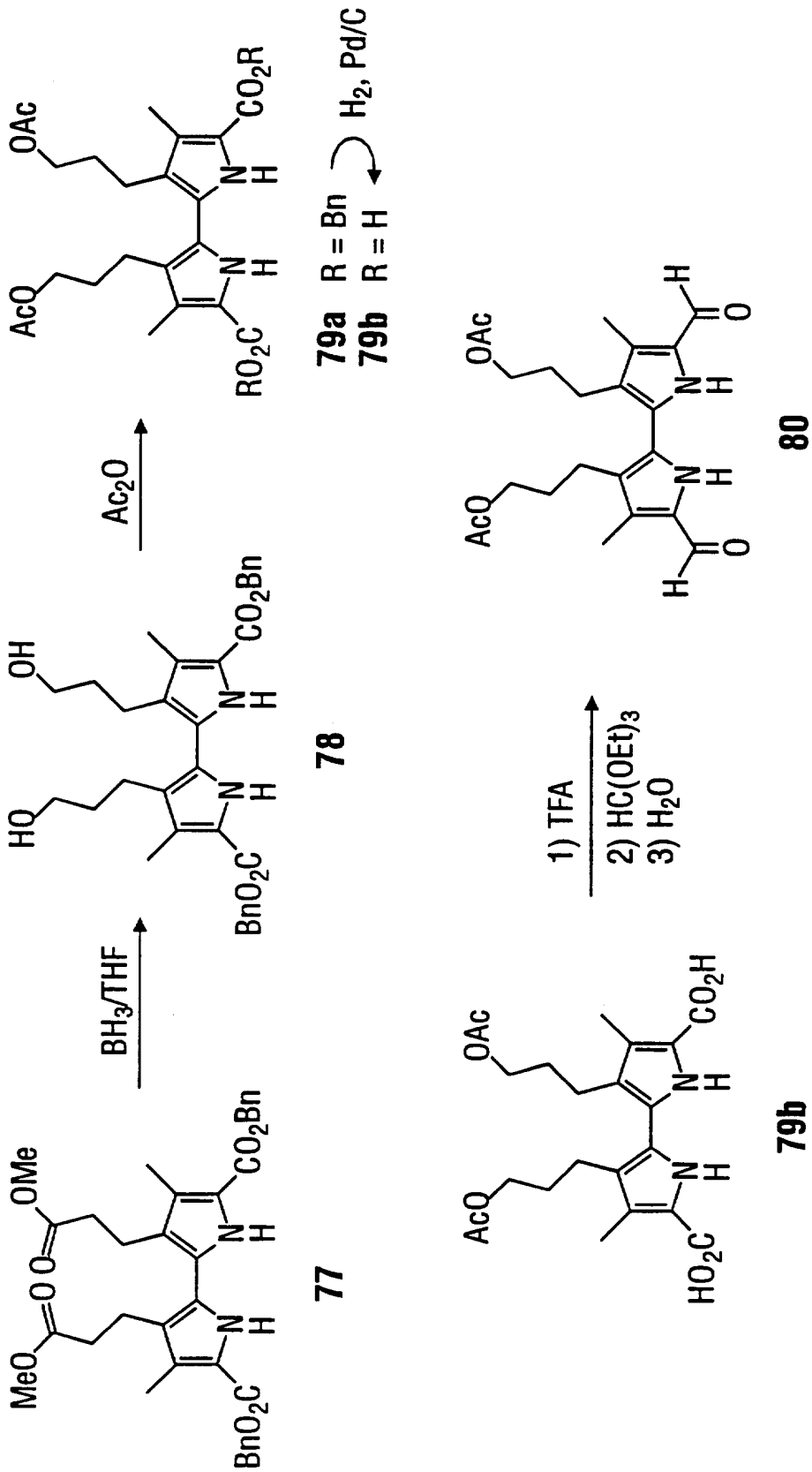
SCHEME L, part 2



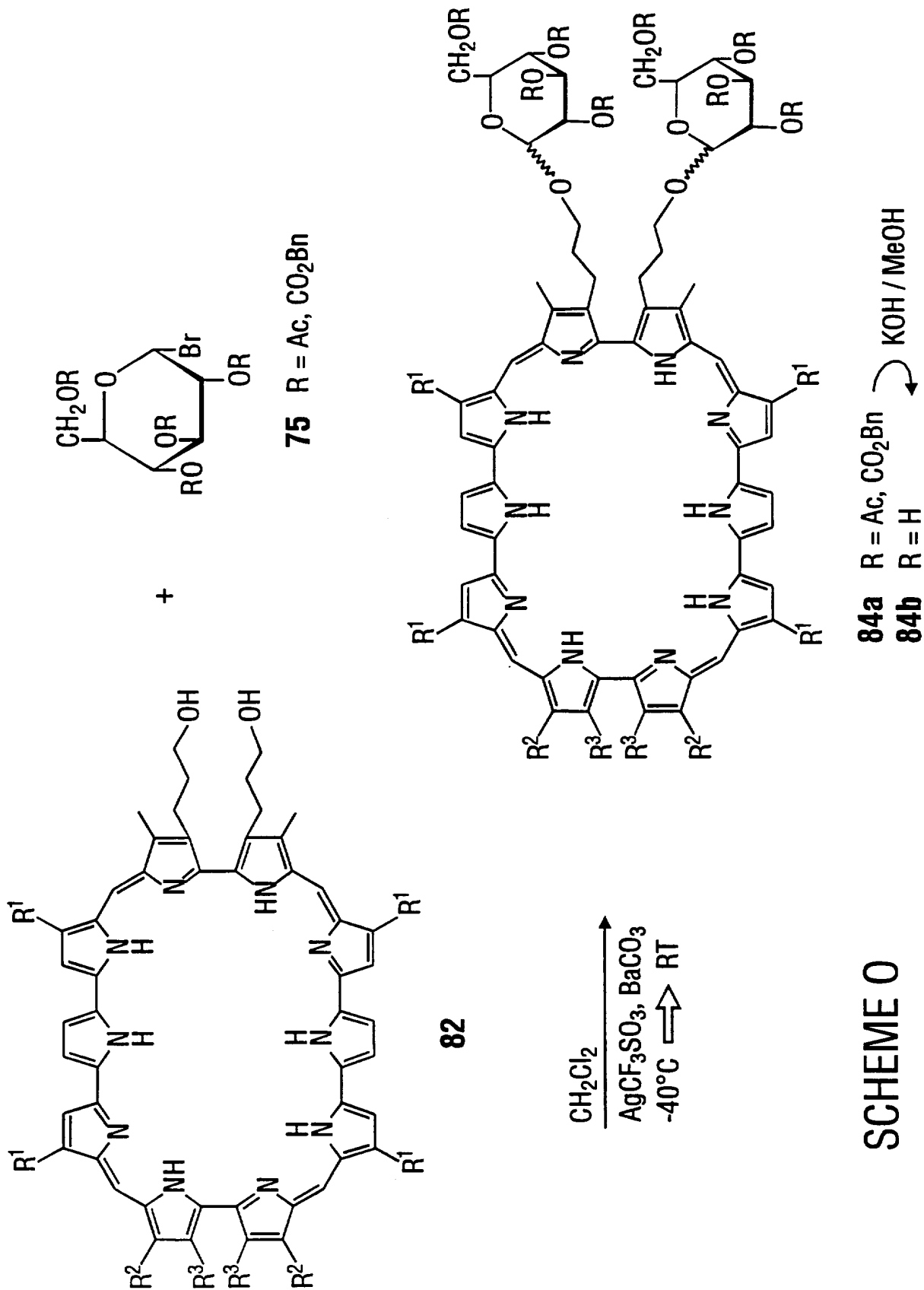
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To prepare an ether linked bis(saccharide)turcasarin conjugate with the ether linkages in the beta positions of the residual bipyrrrole units on the turcasarin periphery, the appropriate acetoxy-protected diformyl bipyrrrole is prepared as shown in Scheme N. Reduction of the methyl esters of bipyrrrole 77 with borane-THF to the corresponding alcohols followed by protection with acetic anhydride provides the acetoxy-protected diol 79a. Standard debenzylation affords diacid 79b, followed by Clezy formulation with TFA and triethylorthoformate to yield the acetoxy-protected bipyrrrole 80. Condensation of bipyrrrole 80 with diacid 81 (prepared in accordance with the procedure shown in Scheme C) under turcasarin-forming conditions will provide the acetoxy-protected dihydroxy turcasarin 82a. Deprotection of the hydroxyl groups is achieved by treatment with HCl in methanol to afford dihydroxyturcasarin 82b.

Referring now to Scheme O, the dihydroxyturcasarin thus formed can be coupled with acetoxy- and/or benzoyl-protected bromo-substituted saccharide units, such as structure 75, in methylene chloride with silver triflate and barium carbonate to afford the acetoxy- and/or benzoyl-protected bis(saccharide)turcasarin conjugate 84a. Treatment of the acetoxy and/or benzoyl protected bis(saccharide)turcasarin 84a with KOH in methanol will yield the corresponding deprotected bis(saccharide)turcasarin conjugate 84b.



SCHEME N, part 1



SCHEME O

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EXAMPLE 10
TURCASARIN AND TURCASARIN ANALOGUES AS CHELATING
RECEPTORS

5 It is contemplated that the diprotonated turcasarin systems will act as effective receptors for a variety of anions. For instance, it is contemplated that diprotonated turcasarin may bind both fluoride and phosphate anions in a strong and non-labile manner.

10

 Furthermore, it is believed that the monoprotonated form of turcasarin will act as an effective carrier for the through-membrane transport of guanosine-5'-monophosphate in Pressman type model system. Thus, protonated turcasarins are proposed to be of use in molecular recognition.

15

 Once a range of turcasarin analogues have been generated, as described herein in the foregoing detailed examples, the thermodynamics and kinetics of anion binding under a range of conditions and with an array of different anions may be determined. The structure and function of the most promising turcasarin compounds may then be optimized such that they bind either phosphate-bearing nucleotides, or chloride ions, with high affinity and selectivity at neutral pH.

20

25

 As a complement to structural studies, quantitative analyses may also be conducted. For example, the relevant pK_a' values for various turcasarins may be determined by employing the methods previously used to determine the pK_a' values for sapphyrin and anthraphyrin. One should beware of possible "artifacts" arising from anion chelation, which can be avoided by using non-chelating buffers and solvents. Such initial studies should be followed by studies in which the various pK_a' values are recorded in different solvents and in the

30

35

- 75 -

presence of different anions such as hydrosulfate, bicarbonate, azide, cyanide, fluoride, bromide, and iodide, that are of biological relevance. Other qualitative tests (e.g. UV/vis, FABMS) may also be used to reconfirm that the turcasarins do not form complexes under physiological conditions with Na^+ , K^+ , Ca^{2+} , or other bio-cations. The reason for these latter studies is that such cation complex formation could preclude efficient anion binding and transport.

10

Once pK_a' values are recorded, a second set of quantitative analyses may be performed to determine the actual affinity constants for each and every relevant receptor-to-anionic interaction. Thus, for instance, K_s for $[\text{H}_6\text{Tur}^{2+}\cdot\text{GMP}^{2-}]$ formation in a variety of solvent systems may be measured in a similar manner to that for the hydrohalide salts of sapphyrin and anthrephyrin. Standard methods as quantitative UV/vis titrations and concentration dependent NMR chemical shift analyses may be used, along with more sophisticated techniques such as those involving static and time-resolved fluorescence. The latter methods offer considerable advantages and are particularly useful for measuring high affinity constants (i.e. those in the $K_s \geq 10^6 \text{ M}^{-1}$ range). These fluorescence-based methods require highly colored materials with good singlet state emission characteristics, but these criteria are clearly met by the turcasarins.

30

Quantitative kinetic studies may also be carried out and used to determine whether the rate limiting step in GMP (or chloride) transport involves initial receptor-anion complex formation, through-membrane carrier-complex diffusion, product release, and/or rate of carrier back-diffusion. On- and off-rates for complex formation may be measured, for example, by dynamic NMR,

35

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UV/vis, or time-resolved fluorescence, in, e.g., simple water-saturated dichloromethane solutions. More precise analyses of receptor-mediated transport may also be made, again with a mind to determining the dynamics of
5 complexation and decomplexation. The U-tube model system may be employed, and when appropriate, more sophisticated membrane analogues such as mixed phosphatidylcholine-cholesterol liposomes may be used.

10 For the latter studies, it may prove most convenient to prepare nucleotide or halide encapsulating liposomes and then determine the kinetics of anion extrusion as a function of carrier concentration and/or external solution pH. Here again, either UV/vis or time-resolved
15 fluorescence analyses may be used. Here, it might prove necessary to add a specific fluorophore, such as 6-methoxy-N-(3-sulphopropyl)quinolinium (a halide selective reagent) so as to be able to detect small quantities of any escaped anions.

20

EXAMPLE 11

TURCASARIN AND ANALOGUES AS CELLULAR ANION TRANSPORTERS

Using a H₂O-CH₂Cl₂-H₂O Pressman-type U-tube model
25 system, it is believed turcasarin will be found to be capable of effecting through-membrane transport of GMP and other nucleotides at near-neutral pH (i.e. in the pH 6.0 to 6.5 regime).

30 The effective transport by turcasarin, a larger, more basic system, may derive from lower in-core NH⁺-to-NH⁺ repulsions. In any event, these properties make turcasarin an ideal candidate for use in the delivery of phosphorylated compounds such as antivirals.
35 The ability of turcasarin to effect nucleotide transport in a manner that is free of any chloride (or other halide) anion inhibition (even though this same material

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binds chloride anion quite effectively at very low pH and in the solid state) is particularly important.

Furthermore, it is contemplated that transport
5 effected by this latter turcasarin carrier could be made somewhat nucleobase selective by adding the appropriate complementary TIPS derivative to the organic membrane phase. For example, in the presence of C-Tips
10 (triisopropylsilyl-protected cytidine), the rate of turcasarin-mediated GMP transport was observed to be substantially enhanced. It is also contemplated that it should be possible to effect base-specific phosphate (or phosphonate) entity transport under physiological
15 conditions and also through synthetic selection and thereby design turcasarin anion receptors that are selective for either phosphate-derived antivirals, or chloride anion, or both.

In further studies, those kinetic and thermodynamic
20 factors that militate both for and against rapid, selective transport of phosphorylated nucleotides and nucleotide analogues can be precisely determined. Both the use of simple U-tube and more elaborate liposomal
test systems is contemplated.

25

EXAMPLE 12

USE OF TURCASARIN AND ANALOGUES AS THERAPEUTIC AGENTS

30 Turcasarin compounds of the present invention are contemplated to be of use as anion transporters in various embodiments relating to human treatment. The turcasarins are particularly contemplated for use as delivery agents for antiviral compounds and may thus be
35 employed to combat a variety of diseases including AIDS, herpes, hepatitis and measles. Turcasarin compounds

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optimized for chloride transport are also contemplated for use in the treatment of cystic fibrosis.

In developing the turcasarin compounds of the present invention for therapeutic use as anti-viral transporters, *in vitro* tests will first be conducted. These will follow protocols similar to those used earlier to screen the photodynamic antiviral activity of sapphyrin and several other expanded porphyrins. In brief, a monolayer of Vero cells will be infected with HSV-1, coated with an overlay culture medium, and then exposed to various relative and absolute concentrations of both putative carrier and known active antiviral. Then, following incubation at 37°C, adjuvant efficacy will be determined by counting the number of plaque forming units (PFU) obtained in the presence and absence of a given carrier.

Following such *in vitro* tests, the activity of promising turcasarin receptors will be followed-up, for example, in anti-HIV screens, and then in *in vivo* animal studies. These studies will be conducted according to the standard practice for such animal trials, the execution of which will be known to those of skill in the art.

During the animal trial stage, the turcasarin compounds, whether used in antiviral delivery, or for chloride transport in cystic fibrosis treatment, may be modified further if required. They might, for instance, be modified to overcome poor water solubility or susceptibility to *in vivo* degradation. Alternatively, if such problems occur, the turcasarins could be enveloped within a bio-compatible liposome (made, e.g. from Cremophor) and then administered intravenously. Such an approach has previously resulted in good *in vivo* murine adenocarcinoma photodynamic tumor killing with a

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water-insoluble texaphyrin-type expanded porphyrin. The "Trojan Horse" method could also be employed to deliver the turcasarin antiviral carrier *in vivo* to the desired locus of biological activity. Here, the idea would be to
5 use non-infectious viral membrane material to produce liposomes and then use these in turn as transport vehicles to get the putative carrier to the site of cellular infectivity.

10 Toxicity studies will also be carried out at this stage. The methods for determining both acute and chronic toxicity will be known to those of skill in the art. Available evidence indicates that turcasarins, like sapphyrins, will be relatively nontoxic. Toxicity can be
15 investigated in relation to solubility, net charge at physiologic pH, and changes in appended β -pyrrolic and/or meso substituents.

Furthermore, the phosphate-binding turcasarin
20 compounds of the present invention may act as receptors and transporters for other biologically important molecules with negative charges, particularly, polynucleic acids such as DNA, RNA and oligonucleotides. Another dimension to the invention, therefore, concerns
25 the possibility of using turcasarins in the transport of DNA molecules, such as antisense DNA constructs, into cells for use in so-called gene therapy programs.

Normal cellular uptake of negatively-charged DNA is
30 known to be limited. Current *in vitro* methods rely on severe cellular modifications which often cause excessive cell damage, and as a result, are not viable *in vivo*. The "coating" of nucleic acid phosphate groups with turcasarins, thus rendering them suitable for diffusional
35 uptake *in vivo*, is therefore very attractive and even has implications for chromosomal gene therapy.

EXAMPLE 13

ANION EXCHANGE PROCEDURES5 Tetrahydrobromide salt of Turcasarin

In a 125 ml separatory funnel, turcasarin 11 hydrochloride (20.1 mg, 1.70×10^{-5} mol) was dissolved in 50 ml dichloromethane. The deep green solution was washed with 10% aqueous NaOH (3 x 50 ml). Washing the
10 resulting deep purple organic phase with 1N HBr afforded a deep green solution that was separated off and dried over anhydrous sodium sulfate. The solvent was removed with the aid of a rotary evaporator to afford the tetrahydrobromide salt of turcasarin (23.1 mg, 1.70×10^{-5}
15 mol, 100%) as a gold colored solid with a metallic luster. UV/vis λ_{\max} (CH_2Cl_2): 646.6 nm.

Tetraperchlorate salt of Turcasarin

In a 125 ml separatory funnel, turcasarin 11
20 hydrochloride (18.2 mg, 1.54×10^{-5} mol) was dissolved in 50 ml dichloromethane. The deep green solution was washed with 10% aqueous NaOH (3 x 50 ml). Washing the resulting deep purple organic phase with 1N HClO_4
25 afforded a deep green solution that was separated off and dried over anhydrous sodium sulfate. The solvent was removed with the aid of a rotary evaporator to afford the tetraperchlorate salt of turcasarin (22.1 mg, 1.54×10^{-5}
mol, 100%) as a gold colored solid with a metallic luster. UV/vis λ_{\max} (CH_2Cl_2): 636.8 nm.

30

Tetratrifluoroacetate salt of Turcasarin

In a 125 ml separatory funnel, turcasarin 11 hydrochloride (19.6 mg, 1.65×10^{-5} mold) was dissolved in 50 ml dichloromethane. The deep green solution was
35 washed with 10% aqueous NaOH (3 x 50 ml). The resulting deep purple organic phase was separated off and dried

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over anhydrous sodium sulfate. One drop of trifluoroacetic acid (TFA) was added and the solution changed to a deep green color. The solvent was removed with the aid of a rotary evaporator to afford the
5 tetratrifluoroacetate salt of turcasarin (24.6 mg, 1.65×10^{-5} mol, 100%) as a gold colored solid with a metallic luster. UV/vis λ_{\max} (CH_2Cl_2): 564.5 nm and 628.5 nm.

The UV/vis λ_{\max} peak shifts of these examples
10 demonstrate that the anion(s) bound by turcasarin may be replaced by a different anion(s), thereby providing a method for the production of a variety of turcasarin anion complexes and demonstrating a property of "exchanging" of anions.

15

EXAMPLE 14**ZINC CHELATION PROCEDURE**

In a 50 ml round bottomed flask equipped with a
20 magnetic stirrer and an argon inlet, turcasarin 11 hydrochloride (5.0 mg, 4.23×10^{-6} mol) was dissolved in 10 ml anhydrous isopropyl alcohol. Pyridine (5 ml) was then added to the blue solution. (UV/vis λ_{\max} of the resulting purple (free base) solution in CH_2Cl_2 : 603.5 nm.)
25 ZnCl_2 (10 mg, excess) was dissolved in 5 ml isopropyl alcohol and added to the stirred reaction mixture. The reaction was allowed to stir at room temperature overnight. The resulting precipitate was collected via vacuum filtration and washed with isopropyl alcohol. The
30 product was recrystallized from CH_2Cl_2 layered with n-hexane to afford gold needles. Yield: 3.3 mg.
UV/vis λ_{\max} (CH_2Cl_2): 585.5 nm.

This example demonstrates that the turcasarins are
35 useful as chelants for a variety of metals such as zinc.

* * *

While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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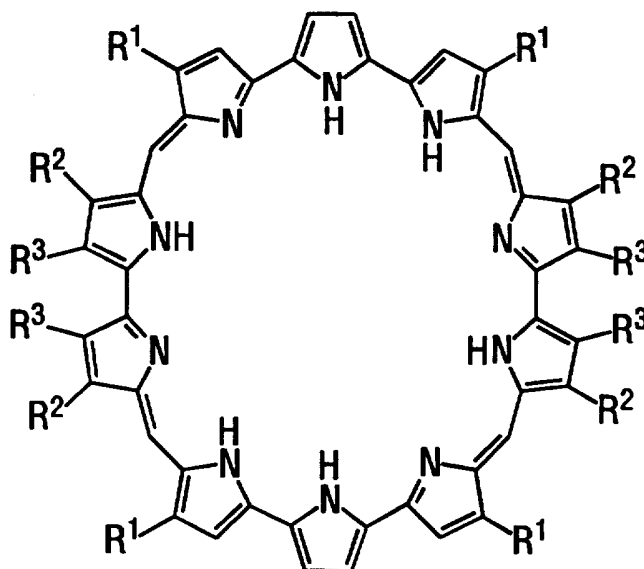
CLAIMS:

1. A compound of formula I:

5

10

15



20 wherein, individually in each occurrence, each of R^{1-3} is
 a monoradical moiety of hydrogen, alkyl, alkyl halide,
 alkene, alkyne, aryl, amino, hydroxyl, alkoxy, carboxy,
 carboxamide, ester, amide, sulfonato, hydroxy substituted
 25 alkyl, glycol, polyglycol, alkoxy substituted alkyl,
 carboxy substituted alkyl, amino substituted alkyl,
 sulfonato substituted alkyl, ester substituted alkyl,
 amide substituted alkyl, substituted aryl, substituted
 alkyl, substituted ester, substituted ether, substituted
 amide, thiol, alkyl thiol, alkoxy carbonyl,
 30 aryloxy carbonyl, aldehyde, ketone, carboxylic acid,
 phosphate, phosphate substituted alkyl, phosphonate,
 phosphonate substituted alkyl, sulfate, sulfate
 substituted alkyl, combination thereof, or is of the
 formula $(CH_2)_m-A-(CH_2)_n-B$ wherein A is CH_2 , O, S, NH or
 35 NR^4 or wherein A is a diradical moiety selected from any
 of the R^{1-3} groups, oxy, sulfide, carbonyl, alkene,
 alkyne, alkyl halide, hydroxyalkyl, glycol, polyglycol,

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alkyl thiol, substituted alkyl, phosphate, phosphonate, sulfate, phosphate substituted alkyl, phosphonate substituted alkyl, sulfate substituted alkyl, carboxy, carboxamide, thiol-substituted carboxamide, derivatized carboxamide of formula CONR^4 where R^4 may be alkyl, 5 alkyl halide, hydroxyalkyl, glycol, polyglycol, or alkyl thiol; wherein B independently in each occurrence is a nucleobase, saccharide, nucleotide, expanded porphyrin, steroid, amino acid, peptide, polypeptide, turcasarin or 10 polymeric or solid support matrix, wherein n is 0-10, wherein m is 0-10, and wherein the total number of carbon atoms in each of R^{1-4} is less than or equal to 20, or salt thereof.

15

2. The compound of claim 1, wherein each R^1 is of the formula $(\text{CH}_2)_m\text{-A-(CH}_2)_n\text{-B}$ wherein A is CH_2 , and B is a nucleobase.

20

3. The compound of claim 2, wherein the nucleobase is AraC, erythrohydroxynonyladenine, AraAMP, Floxuridine, Azaribine, Fluorouracil (5-FU), Azathioprine, Idoxuridine, Azauridine, LOMPD, AZT, Mercaptopurine, 25 Bromodeoxyuridine, PFA, Chlorodeoxyuridine, Thioguanine, Cytarabine, Trifluoromethylde-oxyuridine, Deoxyuridine, Xylo-GMP, DideoxyInosine DDI.

30

4. The compound of claim 2, wherein the nucleobase is any of the nucleobases shown in FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E and 7F.

35

5. The compound of claim 1, wherein each R^1 is of the formula $(\text{CH}_2)_m\text{-A-(CH}_2)_n\text{-B}$ wherein A is CH_2 , and B is an nucleotide.

6. The compound of claim 5, wherein the nucleotide is an oligonucleotide or a polynucleotide.

5

7. The compound of claim 1, wherein each R¹ is hydroxy substituted alkyl.

10

8. The compound of claim 1, wherein each R¹ is carboxy substituted alkyl.

15

9. The compound of claim 1, wherein each R¹ is a saccharide.

20

10. The compound of claim 1, further defined as being the salt of hydrogen chloride.

25

11. The compound of claim 1, further defined as having structure 11 of FIG. 1.

30

12. The compound of claim 1, wherein each R² is of the formula (CH₂)_m-A-(CH₂)_n-B wherein A is CH₂, and B is a nucleobase.

35

13. The compound of claim 12, wherein the nucleobase is AraC, erythrohydroxynonyladenine, AraAMP, Floxuridine, Azaribine, Fluorouracil (5-FU), Azathioprine, Idoxuridine, Azauridine, LOMPD, AZT, Mercaptopurine, Bromodeoxyuridine, PFA, Chlorodeoxyuridine, Thioguanine, Cytarabine, Trifluoromethylde-oxyuridine, Deoxyuridine, Xylo-GMP, DideoxyInosine DDI.

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14. The compound of claim 12, wherein the nucleobase is any of the nucleobases shown in FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E and FIG. 7F.

5

15. The compound of claim 1, wherein each R^2 is of the formula $(CH_2)_m-A-(CH_2)_n-B$ wherein A is CH_2 , and B is a nucleotide.

10

16. The compound of claim 15, wherein the nucleotide is an oligonucleotide or a polynucleotide.

15

17. The compound of claim 1, wherein each R^2 is hydroxy substituted alkyl.

20

18. The compound of claim 1, wherein each R^2 is carboxy substituted alkyl.

25

19. The compound of claim 1, wherein each R^2 is a saccharide.

30

20. The compound of claim 1, wherein each R^3 is of the formula $(CH_2)_m-A-(CH_2)_n-B$ wherein A is CH_2 , and B is a nucleobase.

35

21. The compound of claim 20, wherein the nucleobase is AraC, erythrohydroxynonyladenine, AraAMP, Floxuridine, Azaribine, Fluorouracil (5-FU), Azathioprine, Idoxuridine, Azauridine, LOMPD, AZT, Mercaptopurine, Bromodeoxyuridine, PFA, Chlorodeoxyuridine, Thioguanine,

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Cytarabine, Trifluoromethylde-oxyuridine, Deoxyuridine, Xylo-GMP, DideoxyInosine DDI.

- 5 22. The compound of claim 20, wherein the nucleobase is any of the nucleobases shown in FIG. 7A, FIG. 7B, FIG. 7C, FIG. 7D, FIG. 7E and FIG. 7F.
- 10 23. The compound of claim 1, wherein each R^3 is of the formula $(CH_2)_m-A-(CH_2)_n-B$ wherein A is CH_2 , and B is an nucleotide.
- 15 24. The compound of claim 23, wherein the nucleotide is an oligonucleotide or a polynucleotide.
- 20 25. The compound of claim 1, wherein each R^3 is hydroxy substituted alkyl.
- 25 26. The compound of claim 1, wherein each R^3 is carboxy substituted alkyl.
- 30 27. The compound of claim 1, wherein each R^3 is a saccharide.
- 35 28. The compound of claim 1, wherein R^{1-3} are each alkyl.
29. The compound of claim 1, wherein the total number of carbon atoms in each of R^{1-4} is less than or equal to 10.

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30. A metal chelate of the compound of claim 1.

31. The metal chelate of claim 30, further defined as
5 being a uranyl chelate.

32. The use of the metal chelate of claim 30 in the
preparation of a pharmaceutical composition for use as a
10 radiation sensitizer.

33. The use of the metal chelate of claim 31 in the
preparation of a pharmaceutical composition for use as a
15 radiation sensitizer.

34. The use of the compound of claim 1 in the
preparation of a pharmaceutical composition for use as a
20 radiation sensitizer.

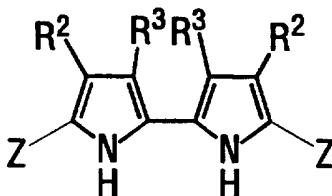
35. The use of the compound of claim 1 as a metal
chelating agent.
25

36. A process for the production of turcasarins,
comprising: condensing a bipyrrrole and a terpyrrrole in
the presence of an acid under conditions such that a
30 turcasarin is formed.

- 91 -

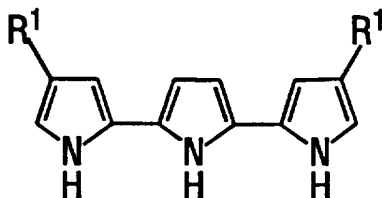
37. The process of claim 36, wherein the bipyrrole is of formula II:

5



wherein Z is H or -CHO and the terpyrrole is of formula III:

10



15

wherein for both bipyrrole and terpyrrole, individually in each occurrence, each of R^{1-3} is a monoradical moiety of hydrogen, alkyl, alkyl halide, alkene, alkyne, aryl, amino, hydroxyl, alkoxy, carboxy, carboxamide, ester, amide, sulfonato, hydroxy substituted alkyl, glycol, polyglycol, alkoxy substituted alkyl, carboxy substituted alkyl, amino substituted alkyl, sulfonato substituted alkyl, ester substituted alkyl, amide substituted alkyl, substituted aryl, substituted alkyl, substituted ester, substituted ether, substituted amide, thiol, alkyl thiol, alkoxy carbonyl, aryloxy carbonyl, aldehyde, ketone, carboxylic acid, phosphate, phosphate substituted alkyl, phosphonate, phosphonate substituted alkyl, sulfate, sulfate substituted alkyl, combination thereof or is of the formula $(CH_2)_m-A-(CH_2)_n-B$ wherein A is CH_2 , O, S, NH or NR^4 or wherein A is a diradical moiety selected from any of the R^{1-3} groups, oxy, sulfide, carbonyl, alkene, alkyne, alkyl halide, hydroxyalkyl, glycol, polyglycol, alkyl thiol, substituted alkyl, phosphate, phosphonate, sulfate, phosphate substituted alkyl, phosphonate substituted alkyl, sulfate substituted alkyl, carboxy,

35

- 92 -

carboxyamide, thiol-substituted carboxyamide, derivatized
carboxyamide of formula CONR^4 where R^4 may be alkyl,
alkyl halide, hydroxyalkyl, glycol, polyglycol, or alkyl
thiol; wherein B independently in each occurrence is a
5 nucleobase, saccharide, nucleotide, expanded porphyrin,
steroid, amino acid, peptide, polypeptide, turcasarin or
polymeric or solid support matrix, wherein n is 0-10,
wherein m is 0-10, and wherein the total number of carbon
atoms in each of R^{1-4} is less than or equal to 20, or salt
10 thereof.

38. The process of claim 36, wherein the acid is
hydrogen chloride.
15

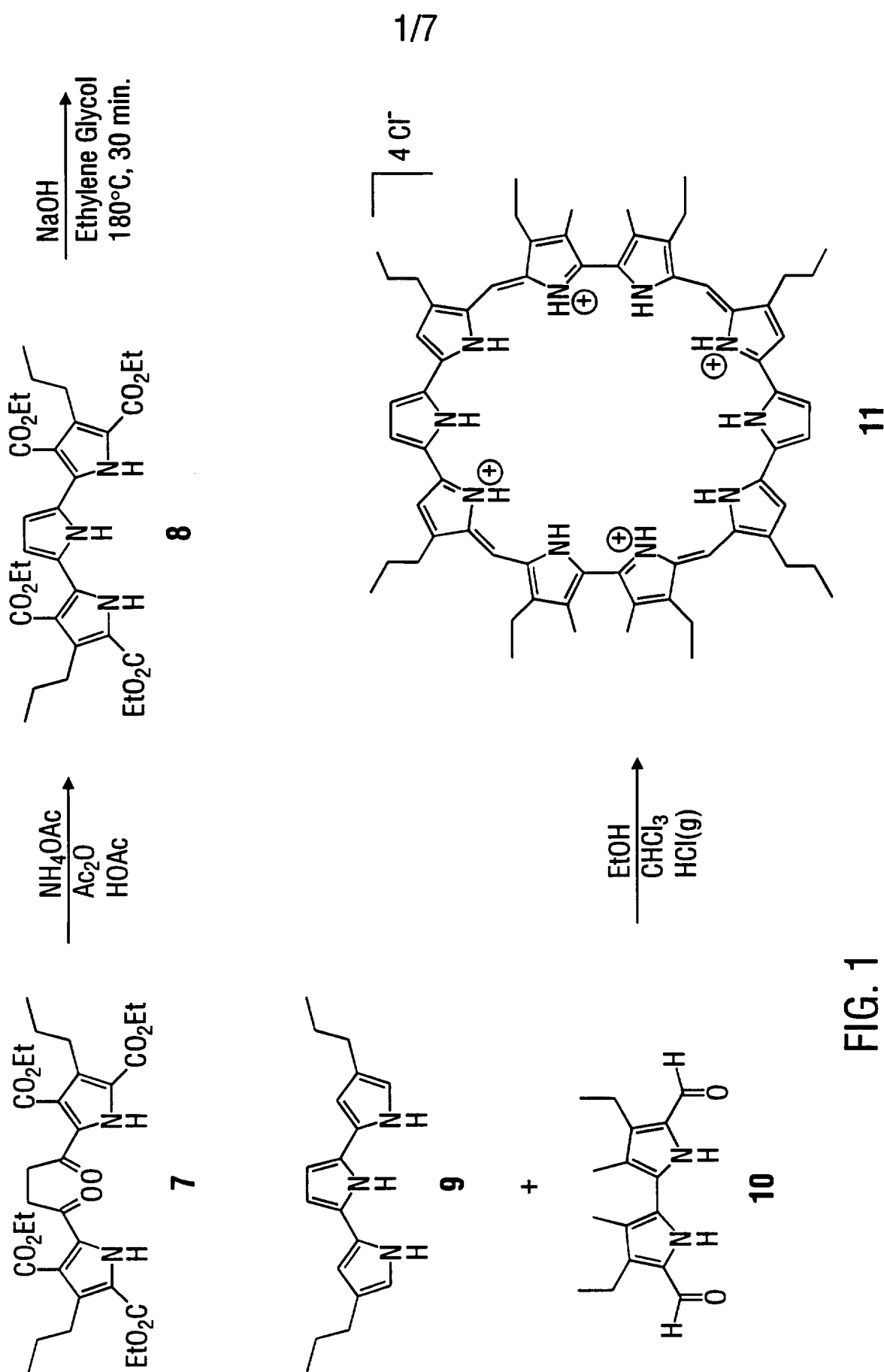
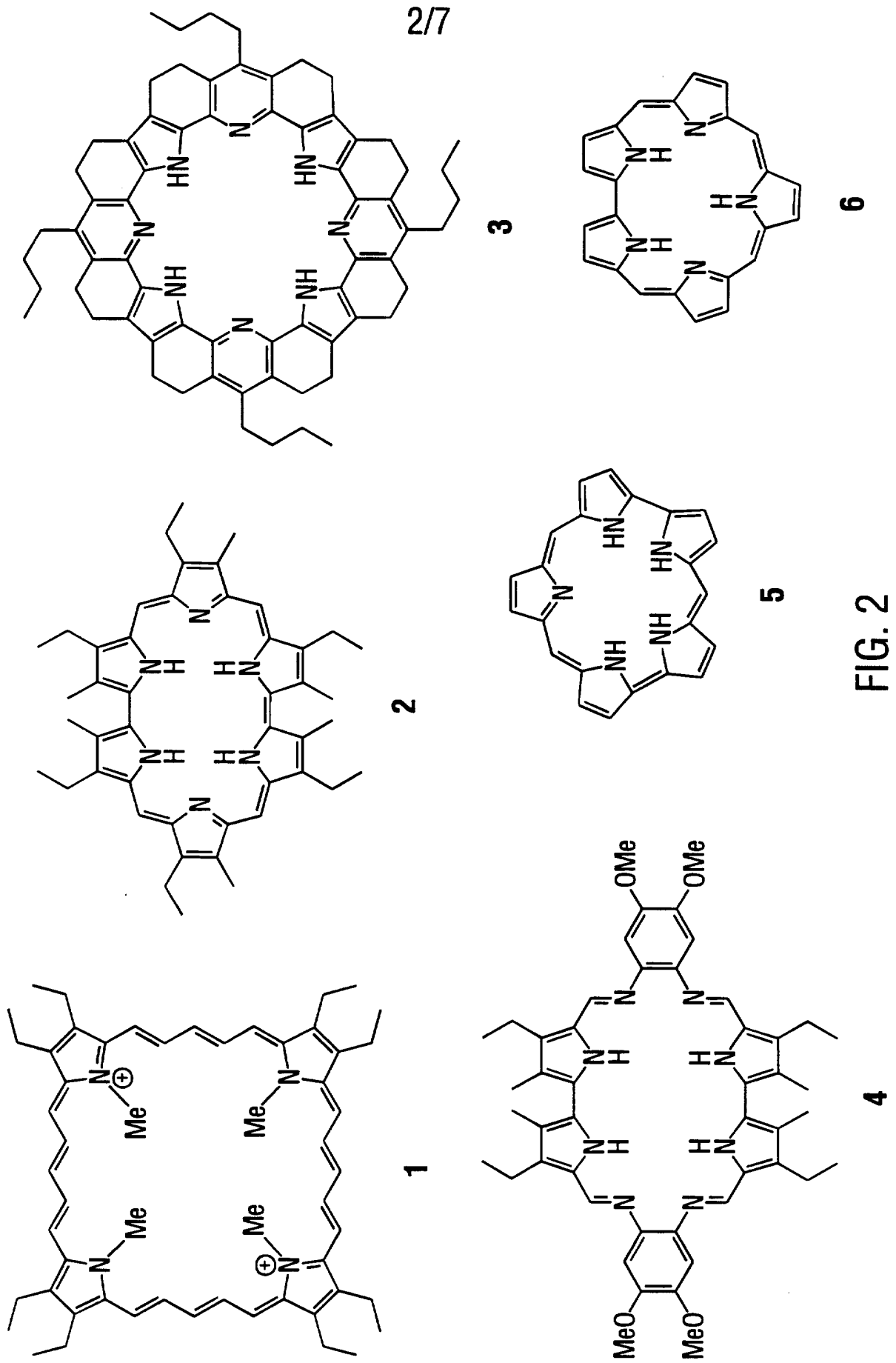


FIG. 1



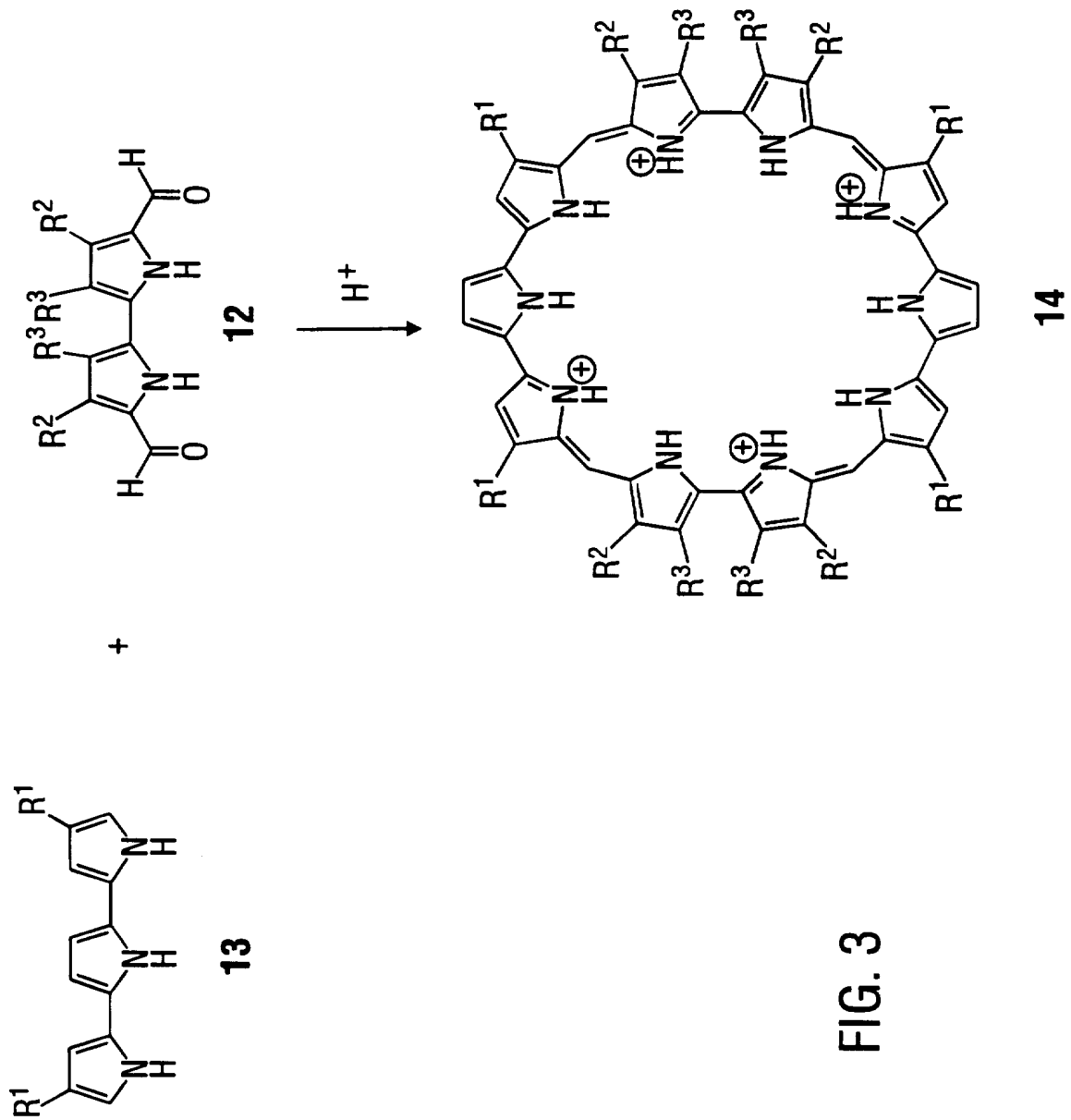


FIG. 3

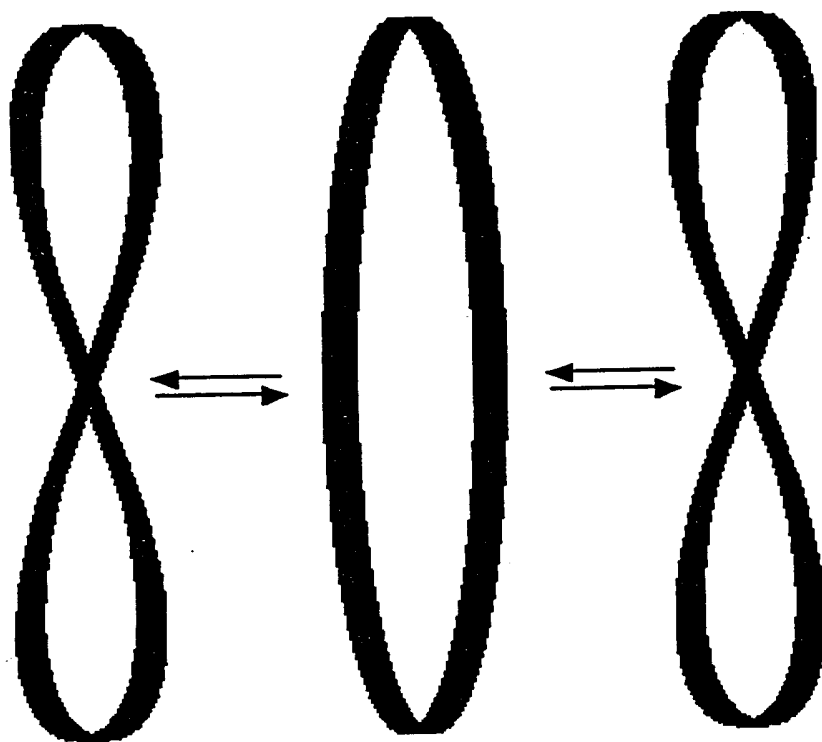


FIG. 4

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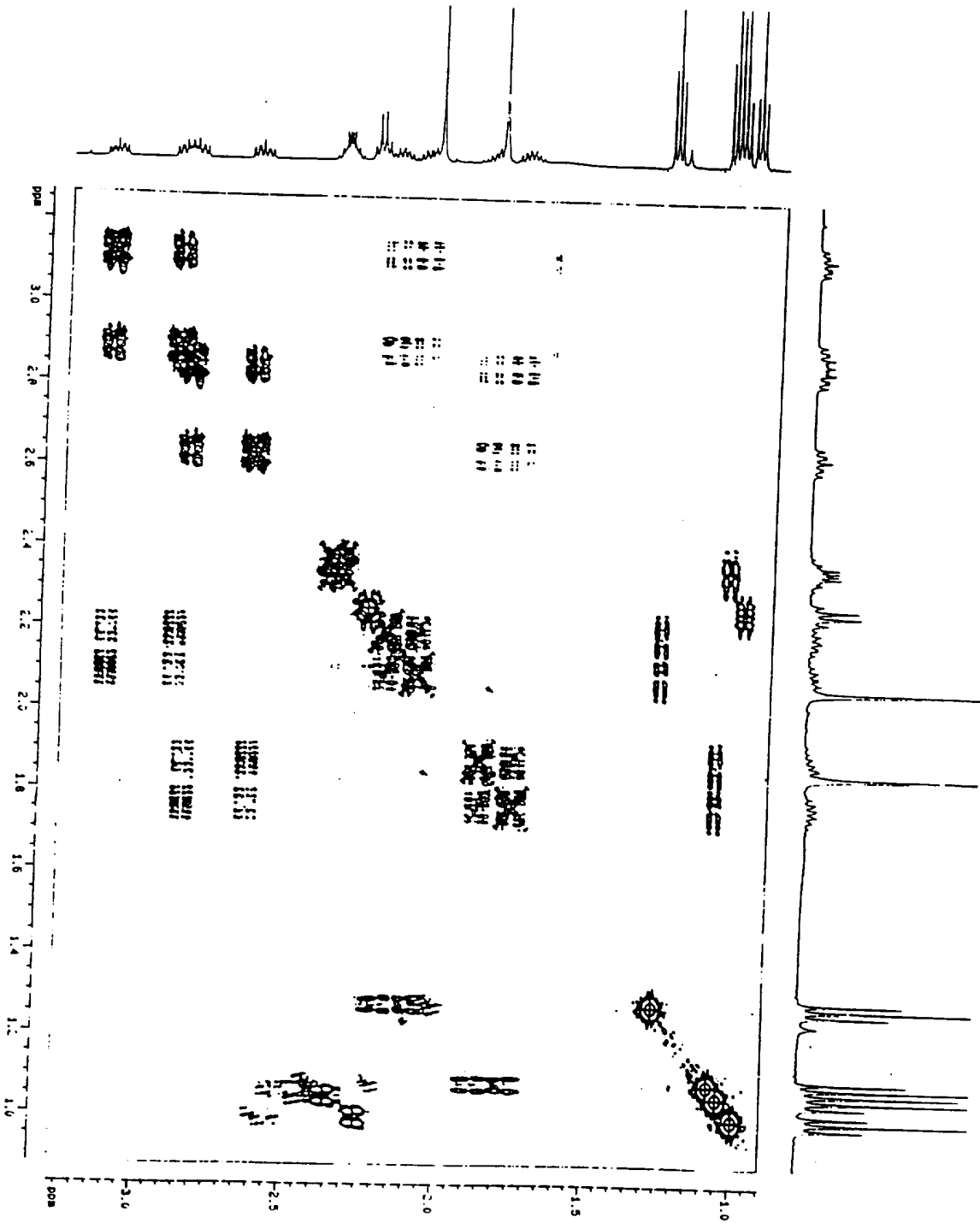


FIG. 5

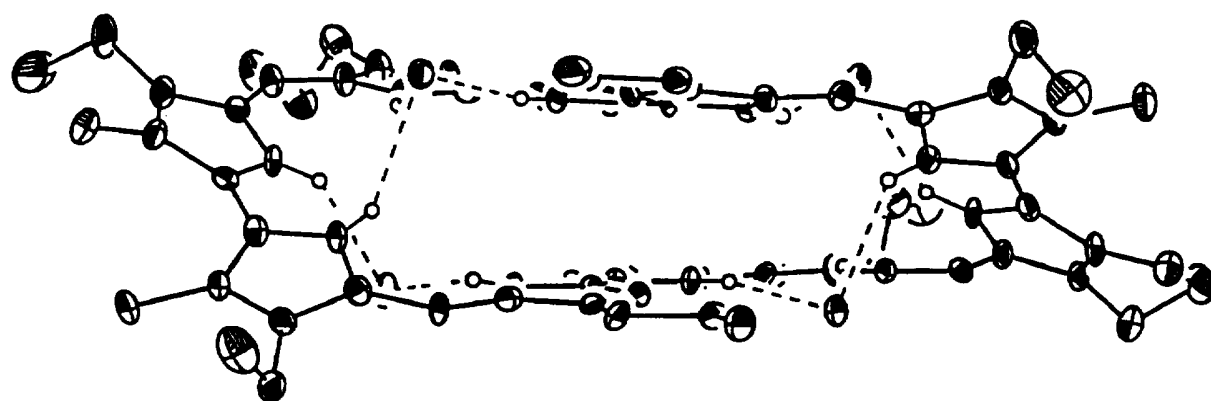
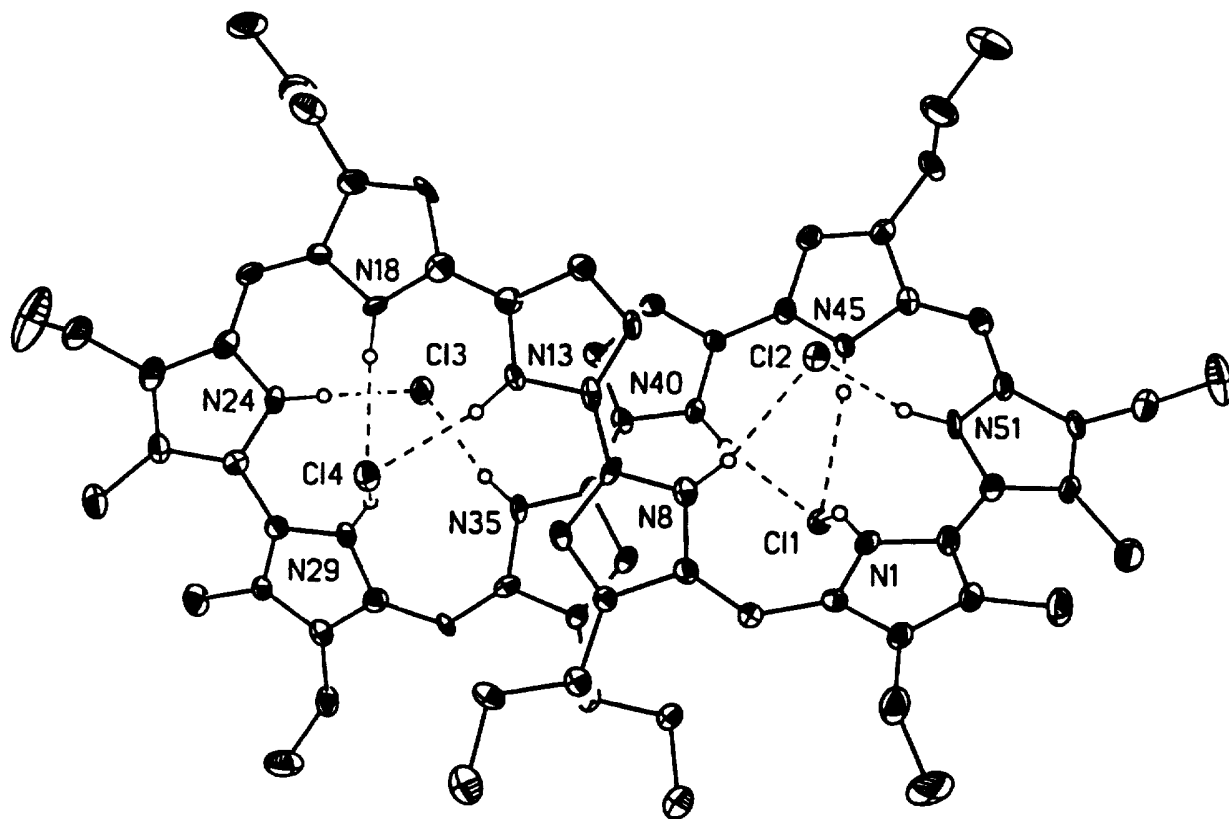
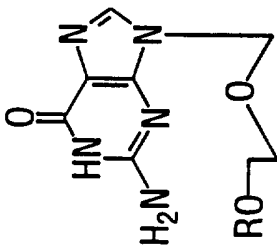
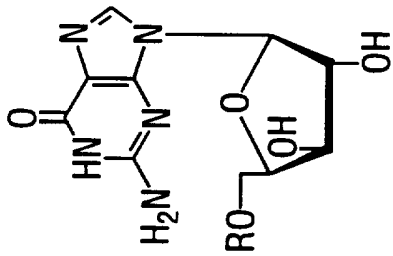


FIG. 6



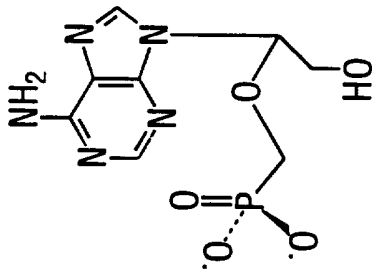
- a. $\text{R} = \text{H}$
- b. $\text{R} = \text{PO}_3^{2-}$
- c. $\text{R} = \text{PO}_3\text{PO}_3\text{PO}_3^{2-}$

FIG. 7A



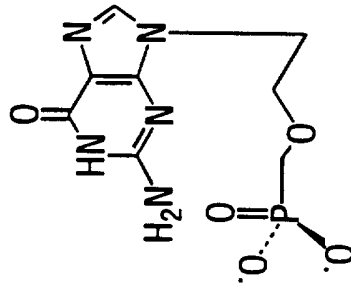
- d. $\text{R} = \text{H}$
- e. $\text{R} = \text{PO}_3^{2-}$

FIG. 7B



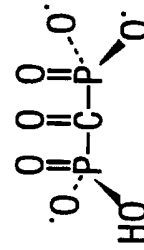
f.

FIG. 7C



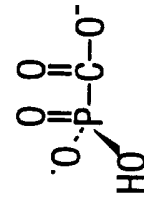
g.

FIG. 7D



i.

FIG. 7E



h.

FIG. 7F

INTERNATIONAL SEARCH REPORT

 Intern al Application No
 PCT/US 95/00530

A. CLASSIFICATION OF SUBJECT MATTER

 IPC 6 C07D487/22 A61K31/40 C07D519/00 C07H13/10 C07H15/26
 C07F9/6561 //(C07D487/22, 259:00, 209:00, 209:00, 209:00, 209:00,
 209:00, 209:00, 209:00), (C07D519/00, 487:00, 473:00, 473:00,

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 C07D C07H 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	ANGEWANDTE CHEMIE INTERNATIONAL EDITION., vol.33, no.14, , WEINHEIM DE pages 1509 - 1512 J.L. SESSLER ET AL. 'Turcasarin, the largest expanded porphyrin to date' see compound 9 and page 1512, reference 14 ---	1, 30, 31
X	CHEMTRACTS : ORGANIC CHEMISTRY, vol.7, , NEW YORK, US pages 409 - 412 C. BRÜCKNER ET AL. 'Turcasarin, the largest expanded porphyrin to date' see pages 409 and 410, background and compound 3 ---	1, 34, 35
A	WO,A,92 05109 (UNIVERSITY OF TEXAS) 2 April 1992 see page 6 -----	34

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Date of the actual completion of the international search

15 September 1995

Date of mailing of the international search report

22. 09. 1995

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Authorized officer

ALFARO FAUS I.

INTERNATIONAL SEARCH REPORT

Internat'l Application No
PCT/US 95/00530

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 473:00)

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)

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)

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4

Date of the actual completion of the international search 15 September 1995	Date of mailing of the international search report 22.09.96
Name and mailing address of the ISA 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	Authorized officer ALFARO FAUS I.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 95/00530

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		AT-T- 127098	15-09-95
		AU-A- 7072391	15-04-92
		EP-A- 0515404	02-12-92
