

Halo-Substituted Protein Kinase C Inhibitors

Protein kinase C (PKC) consists of a family of closely related enzymes that function as serine/threonine kinases. Protein kinase C plays an important role in cell-cell signaling, gene expression, and in the control of cell differentiation and growth. At present, there are currently at least ten known isozymes of PKC that differ in their tissue distribution, enzymatic specificity, and regulation. Nishizuka Y. Annu. Rev. Biochem. 58: 31-44 (1989); Nishizuka Y. Science 258: 607-614 (1992).

Protein kinase C isozymes are single polypeptide chains ranging from 592 to 737 amino acids in length. The isozymes contain a regulatory domain and a catalytic domain connected by a linker peptide. The regulatory and catalytic domains can be further subdivided into constant and variable regions. The catalytic domain of protein kinase C is very similar to that seen in other protein kinases while the regulatory domain is unique to the PKC isozymes. The PKC isozymes demonstrate between 40-80% homology at the amino acid level among the group. However, the homology of a single isozyme between different species is generally greater than 97%.

Protein kinase C is a membrane-associated enzyme that is allosterically regulated by a number of factors, including membrane phospholipids, calcium, and certain membrane lipids such as diacylglycerols that are liberated in response to the activities of phospholipases. Bell, R.M. and Burns, D.J., J. Biol. Chem. 266: 4661-4664 (1991); Nishizuka, Y. Science 258: 607-614 (1992). The protein kinase C isozymes, alpha, beta-1, beta-2 and gamma, require membrane phospholipid, calcium and diacylglycerol/phorbol esters for full activation. The delta, epsilon, eta, and theta forms of PKC are calcium-independent in their mode of activation. The zeta and lambda forms of PKC are

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independent of both calcium and diacylglycerol and are believed to require only membrane phospholipid for their activation.

Only one or two of the protein kinase C isozymes
5 may be involved in a given disease state. For example, the elevated blood glucose levels found in diabetes lead to an isozyme-specific elevation of the beta-2 isozyme in vascular tissues. Inoguchi et al., Proc. Natl. Acad. Sci. USA 89: 11059-11065 (1992). A diabetes-linked
10 elevation of the beta isozyme in human platelets has been correlated with their altered response to agonists. Bastyr III, E.J. and Lu, J. Diabetes 42: (Suppl. 1) 97A (1993). The human vitamin D receptor has been shown to be selectively phosphorylated by protein kinase C beta. This
15 phosphorylation has been linked to alterations in the functioning of the receptor. Hsieh et al., Proc. Natl. Acad. Sci. USA 88: 9315-9319 (1991); Hsieh et al., J. Biol. Chem. 268: 15118-15126 (1993). In addition, recent
20 work has shown that the beta-2 isozyme is responsible for erythroleukemia cell proliferation while the alpha isozyme is involved in megakaryocyte differentiation in these same cells. Murray et al., J. Biol. Chem. 268: 15847-15853 (1993).

The ubiquitous nature of the protein kinase C
25 isozymes and their important roles in physiology provide incentives to produce highly selective PKC inhibitors. Given the evidence demonstrating linkage of certain isozymes to disease states, it is reasonable to assume that inhibitory compounds that are selective to one or two
30 protein kinase C isozymes relative to the other PKC isozymes and other protein kinases are superior therapeutic agents. Such compounds should demonstrate greater efficacy and lower toxicity by virtue of their specificity.

Staurosporine, a microbial indolocarbazole, is a
35 potent inhibitor of protein kinase C that interacts with

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the catalytic domain of the enzyme. Tamaoki et al., Biochem. Biophys. Res. Commun. **135**: 397-402 (1986); Gross et al., Biochem. Pharmacol. **40**: 343-350 (1990). However, the therapeutic usefulness of this molecule and closely related compounds is limited by the lack of specificity for protein kinase C over other protein kinases. Ruegg, U.T. and Burgess, G.M., Trends Pharmacol. Sci. **10**: 218-220 (1989). This lack of selectivity results in unacceptable toxicity in this class of molecules.

An additional class of compounds related to staurosporine, the bisindolemaleimides, has been the focus of recent work. Davis et al., FEBS Lett. **259**: 61-63 (1989); Twoemy et al., Biochem. Biophys. Res. Commun. **171**: 1087-1092 (1990); Toullec et al., J. Biol. Chem. **266**: 15771-15781 (1991); Davis et al., J. Med. Chem. **35**: 994-1001 (1992); Bitetal., J. Med. Chem. **36**: 21-29 (1993). Some of these compounds have demonstrated selectivity for protein kinase C over other protein kinases.

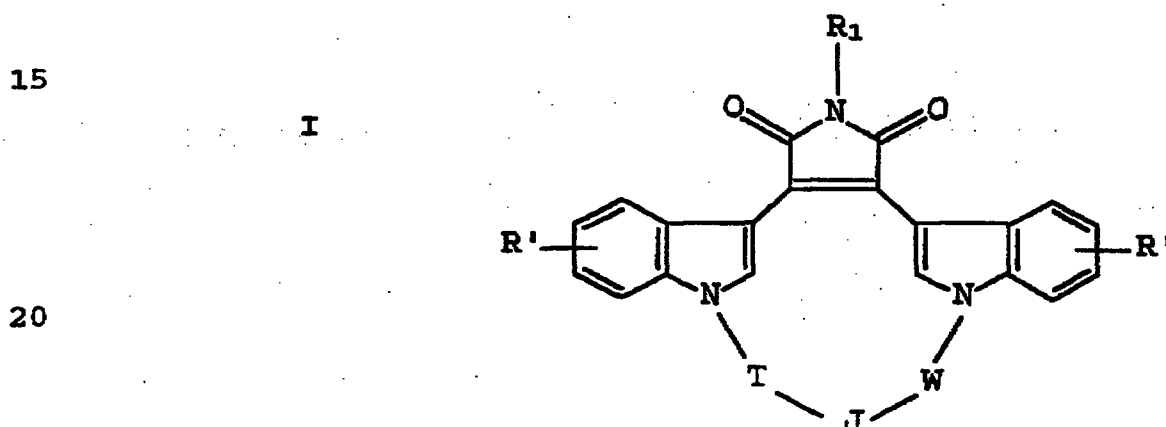
Although compounds that demonstrate specificity to protein kinase C have been discovered, very little is known regarding isozyme selectivity. For example, analysis of the isozyme selectivity of staurosporine, shows little isozyme selectivity with the exception of poor inhibition of the zeta isozyme relative to the other isozymes. McGlynn et al., J. Cell. Biochem. **49**: 239-250 (1992); Ward, N.E., and O'Brian, C.A., Molec. Pharmacol. **41**: 387-392 (1992). Studies of the PKC-selective compound, 3-[1-(3-dimethylaminopropyl)-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione, suggest a slight selectivity for the calcium dependent isozymes. Toullec et al., J. Biol. Chem. **266**: 15771-15781 (1991). Subsequent studies of this compound observed no difference, or possibly slight selectivity, for alpha over beta-1 and beta-2 isozymes. Martiny-Baron et al., J. Biol. Chem. **268**: 9194-9197 (1993); Wilkinson, et al., Biochem. J. **294**: 335-337 (1993).

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Therefore, despite years of research and the identification of classes of compounds that inhibit protein kinase C versus other protein kinases, there remains a need for therapeutically effective isozyme-selective inhibitors.

5. Isozyme-selective inhibitors have utility in treating conditions associated with diabetes mellitus and its complications, in addition to treating ischemia, inflammation, central nervous system disorders, cardiovascular disease, dermatological disease and cancer.

10 One embodiment of the present invention are PKC inhibitors of Formula I:



wherein;

25 R' is independently hydrogen, halo, hydroxy, C₁-C₄ alkyl, C₁-C₄ alkoxy, NR₃R₄ or -NHCO(C₁-C₄ alkyl);

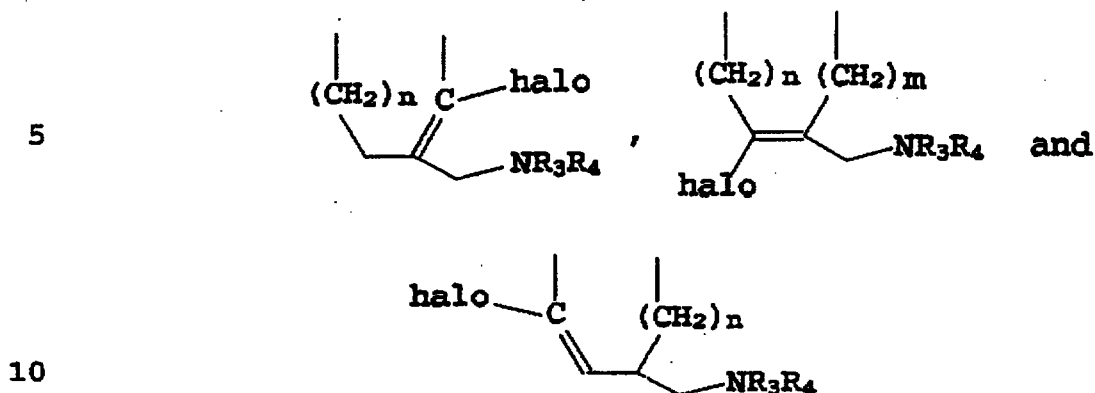
T is C₁-C₄ alkylene optionally substituted with halo or C₁-C₄ alkyl;

W is C₁-C₂ alkylene optionally substituted with halo or
30 C₁-C₄ alkyl;

J is $\begin{array}{c} \text{Y} \\ | \\ -\text{X}-\text{C}- \\ | \\ \text{S} \end{array}$ or when T and W are both methylene, J is

35 selected from the group consisting of

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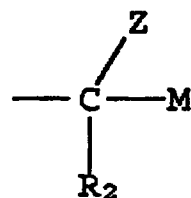
wherein n and m are independently 1 or 2;

X is oxygen, sulfur or a bond between the carbon atoms
15 bridged by X;

Y is halo, C₁-C₄ alkyl or hydrogen;

R₁ is hydrogen or C₁-C₄ alkyl;

S is -CHO or the group



wherein M is hydrogen, $-CH_2OR_3$, $-CH_2NR_3R_4$ or $-NR_3R_4$;

R_2 is hydrogen or halo;

Z is hydrogen or -OR₆;

30 wherein R₃ and R₄ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or taken together with the N atom to which they are bound form a 5 or 6-membered ring; and

R₅ and R₆ are independently hydrogen, C₁-C₄ alkyl,
35 halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or

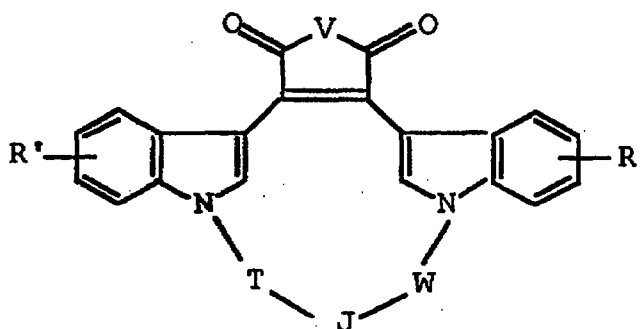
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together form a divalent group selected from the group consisting of $-CR_7R_8-$ wherein R_7 and R_8 are independently hydrogen, C_1-C_4 alkyl or halo(C_1-C_4 alkyl) or R_7 and R_8 taken together with the C atom to which they are bound form a 5 or 6-membered ring

provided that at least one of Y, S, T or W is halo or a halo substituted group, or T and W are both methylene.

In accordance with another embodiment of the present invention novel intermediates of the Formula II are also provided:

II



wherein;

R' is independently hydrogen, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, NR_3R_4 or $-NHCO(C_1-C_4$ alkyl);

V is oxygen or $-N-$;

T is C_1-C_4 alkylene optionally substituted with halo or C_1-C_4 alkyl;

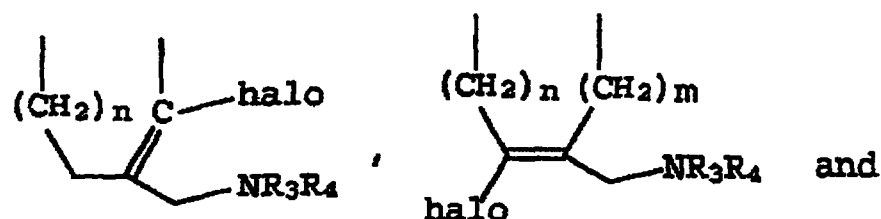
W is C_1-C_2 alkylene optionally substituted with halo or C_1-C_4 alkyl;

J is $\begin{array}{c} Y \\ | \\ -X-C- \\ | \\ S \end{array}$ or when T and W are both methylene, J is

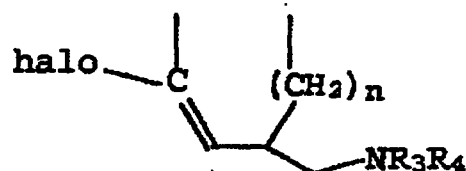
selected from the group consisting of

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wherein n and m are independently 1 or 2;

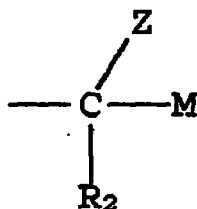
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X is oxygen, sulfur or a bond between the carbon atom bridged by X;

Y is halo, C₁-C₄ alkyl or hydrogen;

S is -CHO or the group

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25

wherein M is hydrogen, -CH₂OR₅, -CH₂NR₃R₄ or -NR₃R₄;

R₂ is hydrogen or halo;

Z is hydrogen or -OR₆;

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wherein R₃ and R₄ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or taken together with the N atom to which they are bound form a 5 or 6-membered ring; and

R₅ and R₆ are independently hydrogen, C₁-C₄ alkyl,

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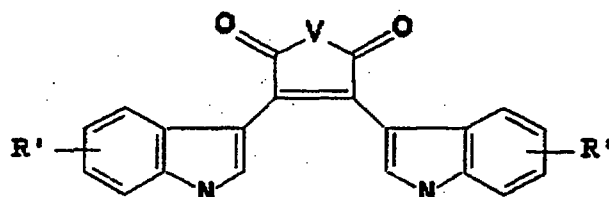
halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or

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together form a divalent group selected from the group consisting of $-CR_7R_8-$ wherein R_7 and R_8 are independently hydrogen, C_1-C_4 alkyl or halo(C_1-C_4 alkyl) or R_7 and R_8 taken together with the C atom to which they are bound form a 5 or 6-membered ring

provided that at least one of Y, S, T or W is halo or a halo substituted group, or T and W are both methylene.

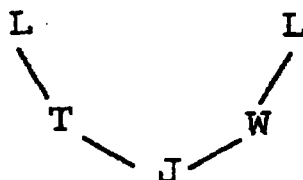
One aspect of the present invention is a method of preparing the compounds of Formula I comprising the steps of combining at a concentration of about 0.001 molar to about 1.5 molar of a compound of the formula:



wherein V is oxygen or NCH_3 ;

R' is independently hydrogen, halo, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy, NR_3R_4 or $-NHCO(C_1-C_4$ alkyl); and

an alkylating agent at a concentration of about 0.001 molar to about 1.5 molar of the formula:



wherein

L is a leaving group;

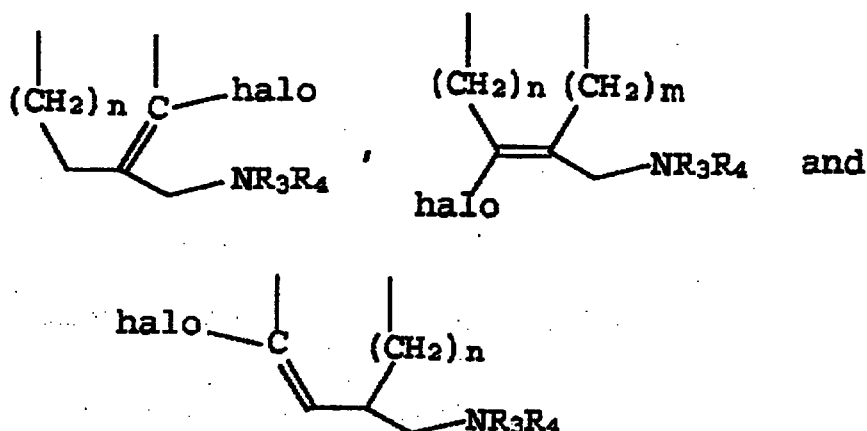
T is C_1-C_4 alkylene optionally substituted with halo or C_1-C_4 alkyl;

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W is C₁-C₂ alkylene optionally substituted with halo or C₁-C₄ alkyl;

J is $\begin{array}{c} Y \\ | \\ -X-C- \\ | \\ S \end{array}$ or when T and W are both methylene, J is

selected from the group consisting of

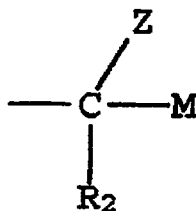


wherein n and m are independently 1 or 2;

X is oxygen, sulfur or a bond between the carbon atom bridged by X;

Y is halo, C₁-C₄ alkyl or hydrogen;

S is -CHO or the group



wherein M is hydrogen, -CH₂OR₅, -CH₂NR₃R₄ or -NR₃R₄;
R₂ is hydrogen or halo; and

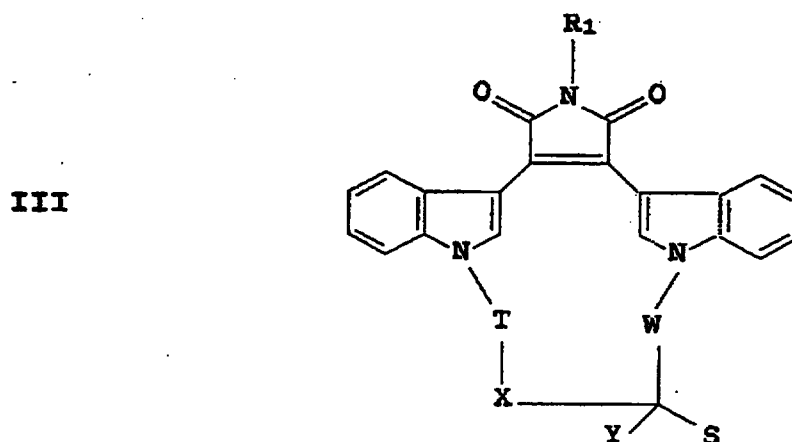
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Z is hydrogen or $-OR_6$;

wherein R_3 and R_4 are independently hydrogen, C_1-C_4 alkyl, halo(C_1-C_4 alkyl), C_1-C_4 alkanoyl, halo(C_1-C_4 alkanoyl) or taken together with the N atom to which they are bound form a 5 or 6-membered ring; and

R_5 and R_6 are independently hydrogen, C_1-C_4 alkyl, halo(C_1-C_4 alkyl), C_1-C_4 alkanoyl, halo(C_1-C_4 alkanoyl) or together form a divalent group selected from the group consisting of $-CR_7R_8-$ wherein R_7 is hydrogen or methyl and R_8 is C_1-C_4 alkyl, halo(C_1-C_4 alkyl) or taken together with the C atom to which they are bound form a 5 or 6-membered ring, and about 0.5 to about 10 equivalents of Cs_2CO_3 at a rate of about 0.1 mL/hour to about 2.0 mL/hour in a polar aprotic solvent.

In accordance with one embodiment of the present invention, a compound of Formula III is provided:



wherein;

R_1 is C_1-C_4 alkyl or hydrogen;

T is C_2-C_4 alkylene optionally substituted with halo or C_1-C_4 alkyl;

W is ethylene optionally substituted with halo or C_1-C_4 alkyl;

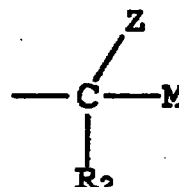
X is oxygen, sulfur or a bond between the carbon atom bridged by X;

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Y is halo, C₁-C₄ alkyl or hydrogen;

S is -CHO or the group

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10 wherein M is hydrogen, -CH₂OR₅, -CH₂NR₃R₄ or -NR₃R₄;R₂ is hydrogen or halo; andZ is hydrogen or -OR₆;

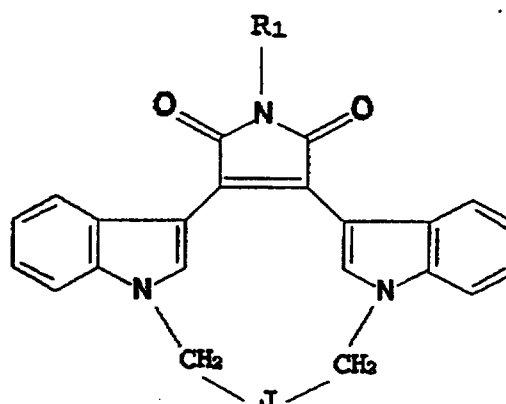
wherein R₃ and R₄ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or taken together with the N atom to which they are bound form a 5 or 6-membered ring; and

R₅ and R₆ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or together form a divalent group selected from the group consisting of -CR₇R₈- wherein R₇ is hydrogen or methyl and R₈ is C₁-C₄ alkyl, halo(C₁-C₄ alkyl) or taken together with the C atom to which they are bound form a 5 or 6-membered ring provided that at least one of Y, S, T or W is halo or a halo substituted group.

25 In another embodiment of the present invention a compound of Formula IV is provided:

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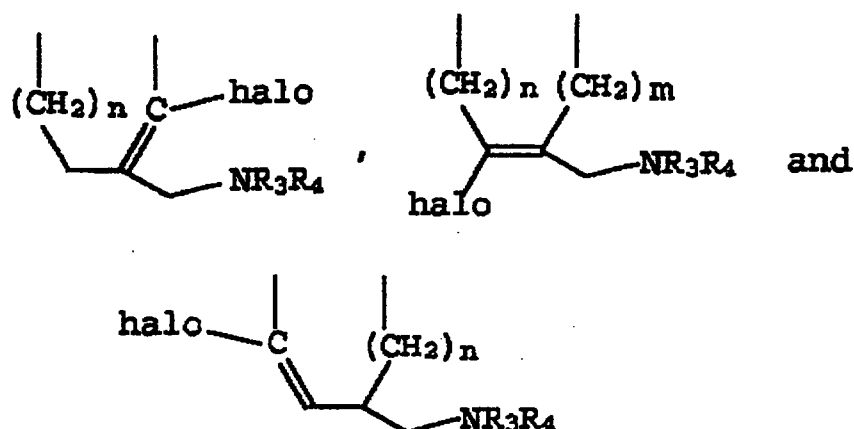
IV



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wherein

J is selected from the group consisting of



wherein R_1 is alkyl or hydrogen;

n and m are independently 1 or 2; and

R_3 and R_4 are independently hydrogen, C_1 - C_4 alkyl, halo(C_1 - C_4 alkyl), C_1 - C_4 alkanoyl, halo(C_1 - C_4 alkanoyl) or

20 taken together with the N atom to which they are bound form a 5 or 6-membered ring.

In accordance with one embodiment of the present invention, there is provided a method of inhibiting PKC activity. The method comprises administering to a mammal in need of such treatment, a pharmaceutically effective amount of a compound of Formula I. The present invention is also directed to a method of selectively inhibiting the beta-1 and beta-2 protein kinase C isozymes, the method comprising administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound of the Formula I.

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The invention further provides methods for treating conditions that protein kinase C has been demonstrated as having a role in the pathology, such as ischemia, inflammation, central nervous system disorders,

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cardiovascular disease, dermatological disease, and cancer. The method comprises administering to a mammal in need of treatment a pharmaceutically effective amount of a compound of the Formula I.

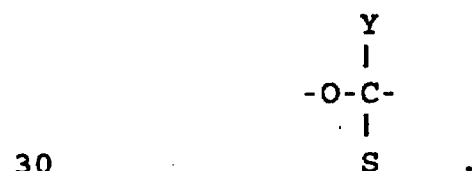
5 This invention is particularly useful in treating diabetic complications. Therefore, the present invention further provides a method for treating diabetes mellitus, which comprises administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound
10 of the Formula I.

One other aspect of this invention is a pharmaceutical formulation comprising a compound of Formula I together with one or more pharmaceutically acceptable excipients, carriers, or diluents.

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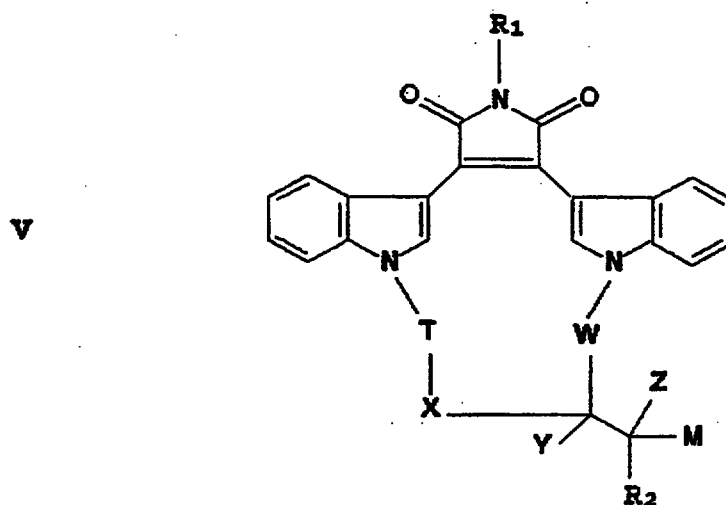
Detailed Description of the Invention

As noted above, the invention provides compounds of Formula I which selectively inhibit protein kinase C. The preferred compounds of this invention are those of
20 Formula I wherein the moieties -T-J-W- contain 4 to 8 atoms, which may be unsubstituted or substituted (with halo or alkyl groups). Most preferably, the moieties -T-J-W- contain 6 atoms. Other preferred compounds of this invention are those compounds of Formula I wherein R₁ is
25 hydrogen, and J is



One preferred group of compounds are the compounds of the Formula V:

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wherein;

15 R_1 is C_1 - C_4 alkyl or hydrogen;

T is C_2 - C_4 alkylene optionally substituted with halo or C_1 - C_4 alkyl;

W is ethylene optionally substituted with halo or C_1 - C_4 alkyl;

20 X is oxygen, sulfur or a bond between the carbon atom bridged by X ;

Y is halo, C_1 - C_4 alkyl or hydrogen;

M is hydrogen, $-CH_2OR_5$, $-CH_2NR_3R_4$ or $-NR_3R_4$;

R_2 is hydrogen or halo;

25 Z is hydrogen or $-OR_6$;

wherein R_3 and R_4 are independently hydrogen, C_1 - C_4 alkyl, halo(C_1 - C_4 alkyl), C_1 - C_4 alkanoyl, halo(C_1 - C_4 alkanoyl) or taken together with the N atom to which they are bound form a 5 or 6-membered ring; and

30 R_5 and R_6 are independently hydrogen, C_1 - C_4 alkyl, halo(C_1 - C_4 alkyl), C_1 - C_4 alkanoyl, halo(C_1 - C_4 alkanoyl) or together form a divalent group selected from the group consisting of $-CR_7R_8-$ wherein R_7 is hydrogen or methyl and R_8 is C_1 - C_4 alkyl, halo(C_1 - C_4 alkyl) or taken together with the
35 C atom to which they are bound form a 5 or 6-membered ring,

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and wherein at least one of Y, R₂, Z, M, T or W is halo or a halo substituted group, more preferably, fluoro or a fluoro substituted group.

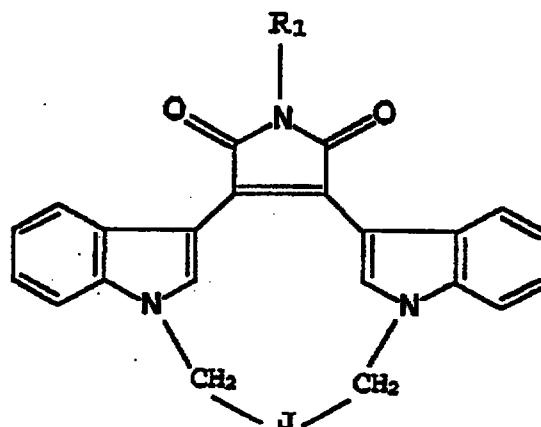
Preferred compounds of Formula V include
 5 compounds wherein X is oxygen, R₁ is hydrogen, T is C₂-C₃ alkylene, optionally substituted and W is ethylene, optionally substituted. Preferably the T and/or W are substituted with one or more fluoro groups.

In another embodiment of the present invention a
 10 compound of Formula VI is provided:

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VI

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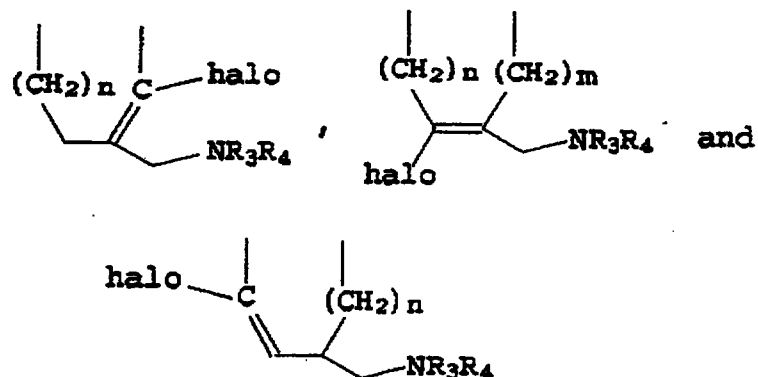


wherein

J is selected from the group consisting of

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wherein R_1 is alkyl or hydrogen;

n and m are independently 1 or 2; and

R_3 and R_4 are independently hydrogen, C_1 - C_4 alkyl, halo(C_1 - C_4 alkyl), C_1 - C_4 alkanoyl, halo(C_1 - C_4 alkanoyl) or
5 taken together with the N atom to which they are bound form a 5 or 6-membered ring.

The term "halo" represents fluoro, chloro, bromo, or iodo.

The term " C_1 - C_4 alkyl" refers to a cyclo, straight
10 or branched chain alkyl group having from one to four carbon atoms such as methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl, t-butyl and the like. Similarly, a " C_2 - C_4 alkyl" represents a cyclo, straight or branched chain alkyl group having two
15 to four carbon atoms. A haloalkyl group is an alkyl group substituted with one or more halo atoms, preferably one to three halo atoms. One example of a haloalkyl group is trifluoromethyl. A C_1 - C_4 alkoxy is a C_1 - C_4 alkyl group covalently bonded through an -O- linkage.

20 The term " C_1 - C_4 alkylene" is a straight chain alkylene moiety of the formula $-(CH_2)_r-$, wherein r is one to four. Examples of C_1 - C_4 alkylene include methylene, ethylene, trimethylene, tetramethylene and the like. Similarly, a " C_2 - C_4 alkylene" represents a two to four
25 carbon, straight alkylene moiety.

The term " C_1 - C_4 alkenylene" represent a one to four carbon straight chain hydrocarbon containing one or more double bonds, typically one or two double bonds. Examples of C_2 - C_4 alkenylene groups include ethenylene,
30 propenylene, and 1,3-butadieneyl.

The term " C_1 - C_4 alkanoyl" is the acyl residue of a C_1 - C_4 carboxylic acid. Examples of C_1 - C_4 alkanoyl groups are acetyl, propanoyl, butanoyl and the like. A haloalkanoyl group is an alkanoyl group substituted with one or more

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halo atoms, typically one to three halo atoms. An example of a haloalkanoyl group is trifluoroacetyl.

The term "leaving group" as used in the specification is understood by those skilled in the art.

5 Generally, a leaving group is any group or atom that enhances the electrophilicity of the atom to which it is attached for displacement. Preferred leaving groups are triflate, mesylate, tosylate, imidate, chloride, bromide, and iodide.

10 The term "carboxy protecting group" (P) as used in the specification refers to one of the ester derivatives of the carboxylic acid group commonly employed to block or protect the carboxylic acid group while reactions are carried out on other functional groups on the compound.

15 The species of carboxy-protecting group employed is not critical so long as the derivatized carboxylic acid is stable to the condition of subsequent reactions and can be removed at the appropriate point without disrupting the remainder of the molecule. T.W. Greene and P. Wuts,

20 Protective Groups in Organic Synthesis, John Wiley and Sons, New York, N.Y., 1991, Chapter 5, provide a list of commonly employed protecting groups. See also E. Haslam, Protective Groups in Organic Chemistry, J.G.W. McOmie, Ed., Plenum Press, New York, N.Y., 1973. A related term is
25 "protected carboxy," which refers to a carboxy group protected with a carboxy protecting group.

The term "hydroxy protecting group" (P) as used in the specification refers to one of the ether or ester derivatives of the hydroxy group commonly employed to block
30 or protect the hydroxy group while reactions are carried out on other functional groups on the compound. The species of hydroxy protecting group employed is not critical so long as the derivatized hydroxy group is stable to the condition of subsequent reactions and can be removed
35 at the appropriate point without disrupting the remainder

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of the molecule. T.W. Greene and P. Wuts, Protective Groups in Organic Synthesis, John Wiley and Sons, New York, N.Y., 1991, provide a list of commonly employed protecting groups. Preferred hydroxy protecting groups are tert-butyl-
5 butyldiphenylsilyloxy (TBDPS), tert-butyltrimethylsilyloxy (TBDMS), triphenylmethyl (trityl), methoxytrityl, or an alkyl or aryl ester. A related term is "protected hydroxy," which refers to a hydroxy group protected with a hydroxy protecting group.

10 The term "amino protecting group" (P) as used in the specification refers to substituents of the amino group commonly employed to block or protect the amino functionality while reacting other functional groups on the compound. The species of amino-protecting group employed
15 is not critical so long as the derivatized amino group is stable to the condition of subsequent reactions and can be removed at the appropriate point without disrupting the remainder of the molecule. T. W. Greene and P. Wuts, Protective Groups in Organic Synthesis, Chapter 7, provide
20 a list of commonly employed protecting groups. See also J. W. Barton, Protective Groups in Organic Chemistry, Chapter 2. Preferred amino-protecting groups are t-butoxycarbonyl, phthalide, a cyclic alkyl, and benzyloxycarbonyl. The related term "protected amino" defines an amino group
25 substituted with an amino protecting group as defined.

 The term "-NH protective groups" as used in the specification refers to sub-class of amino protecting groups that are commonly employed to block or protect the
-NH functionality while reacting other functional groups on
30 the compound. The species of protecting group employed is not critical so long as the derivatized amino group is stable to the condition of subsequent reactions and can be removed at the appropriate point without disrupting the remainder of the molecule. T. W. Greene and P. Wuts,
35 Protective Groups in Organic Synthesis, Chapter 7, page

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362-385, provide a list of commonly employed protecting groups. Preferred -NH protecting groups are carbamate, amide, alkyl or aryl sulfonamide. The related term "protected -NH" defines a group substituted with an -NH protecting group as defined.

The term "pharmaceutically effective amount", as used herein, represents an amount of a compound of the invention that is capable of inhibiting PKC activity in mammals. The particular dose of the compound administered according to this invention will, of course, be determined by the particular circumstances surrounding the case, including the compound administered, the route of administration, the particular condition being treated, and similar considerations. The compounds can be administered by a variety of routes including the oral, rectal, transdermal, subcutaneous, topical, intravenous, intramuscular or intranasal routes. For all indications, a typical daily dose will contain from about 0.01 mg/kg to about 20 mg/kg of the active compound of this invention. Preferred daily doses will be about 0.05 to about 10 mg/kg, ideally about 0.1 to about 5 mg/kg. However, for topical administration a typical dosage is about 1 to about 500 μg compound per cm^2 of an affected tissue. Preferably, the applied amount of compound will range from about 30 to about 300 $\mu\text{g}/\text{cm}^2$, more preferably, from about 50 to about 200 $\mu\text{g}/\text{cm}^2$, and, most preferably, from about 60 to about 100 $\mu\text{g}/\text{cm}^2$.

The term "treating," as used herein, describes the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of a compound of present invention to prevent the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder.

-20-

The term "isozyme selective" means the preferential inhibition of one protein kinase C isozyme (or subgroup) over the other isozymes. For example a compound may selectively inhibit the beta-1 or beta-2 isozymes over protein kinase C isozymes alpha, gamma, delta, epsilon, zeta, and eta. In general, isozyme selective compounds demonstrate a minimum of a eightfold differential (preferably a ten fold differential) in the dosage required to inhibit one subtype compared to the dosage required for equal inhibition of a different subtype (for example, inhibition of PKC beta-1 or beta-2 isozyme as compared to the alpha protein kinase C isozyme) as measured in the PKC assay. The compounds demonstrate this differential across the range of inhibition and are exemplified at the IC_{50} , i.e., a 50% inhibition. Thus, for example, a beta-1 and beta-2 isozyme-selective compound inhibits the beta-1 and beta-2 isozymes of protein kinase C at much lower concentrations with lower toxicity by virtue of their minimal inhibition of the other PKC isozymes.

The compounds of Formula I having a basic moiety, eg NR_3R_4 , can also be in the form of their pharmaceutically acceptable acid addition salts thereof. Acids commonly employed to form such salts include inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, as well as organic acids such as para-toluenesulfonic, methanesulfonic, oxalic, para-bromophenylsulfonic, carbonic, succinic, citric, benzoic, acetic acid, and related inorganic and organic acids. Such pharmaceutically acceptable salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, 2-butyne-

-21-

1,4 dioate, 3-hexyne-2, 5-dioate, benzoate, chlorobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, hippurate, β -
5 hydroxybutyrate, glycollate, maleate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and like salts.

In addition to pharmaceutically-acceptable salts,
10 other salts can be used in accordance with the present invention. They may serve as intermediates in the purification of the compounds, in the preparation of other salts, or in the identification and characterization of the compounds or intermediates.

15 The pharmaceutically acceptable salts of compounds of Formula I can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, ethyl acetate and the like. Mixtures of such solvates can also be prepared. The source of such solvates can be from
20 the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent. Such solvates are within the scope of the present invention.

Various stereoisomeric forms of the compounds of
25 Formula I may exist; for example, T or W may include a chiral carbon atom in the substituted alkylene moiety. The compounds of Formula I are typically prepared as racemates and can conveniently be used as such, but individual enantiomers can be isolated or synthesized by conventional
30 techniques if so desired. Such racemates and individual enantiomers and mixtures thereof form part of the present invention.

The present invention also encompasses the pharmaceutically acceptable prodrugs of the compounds of
35 Formula I. A prodrug is a drug which has been chemically

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modified and may be biologically inactive (at the enzyme's site of action), but can be degraded or modified by one or more enzymatic or other *in vivo* processes to produce the parent bioactive form. The prodrug preferably has a

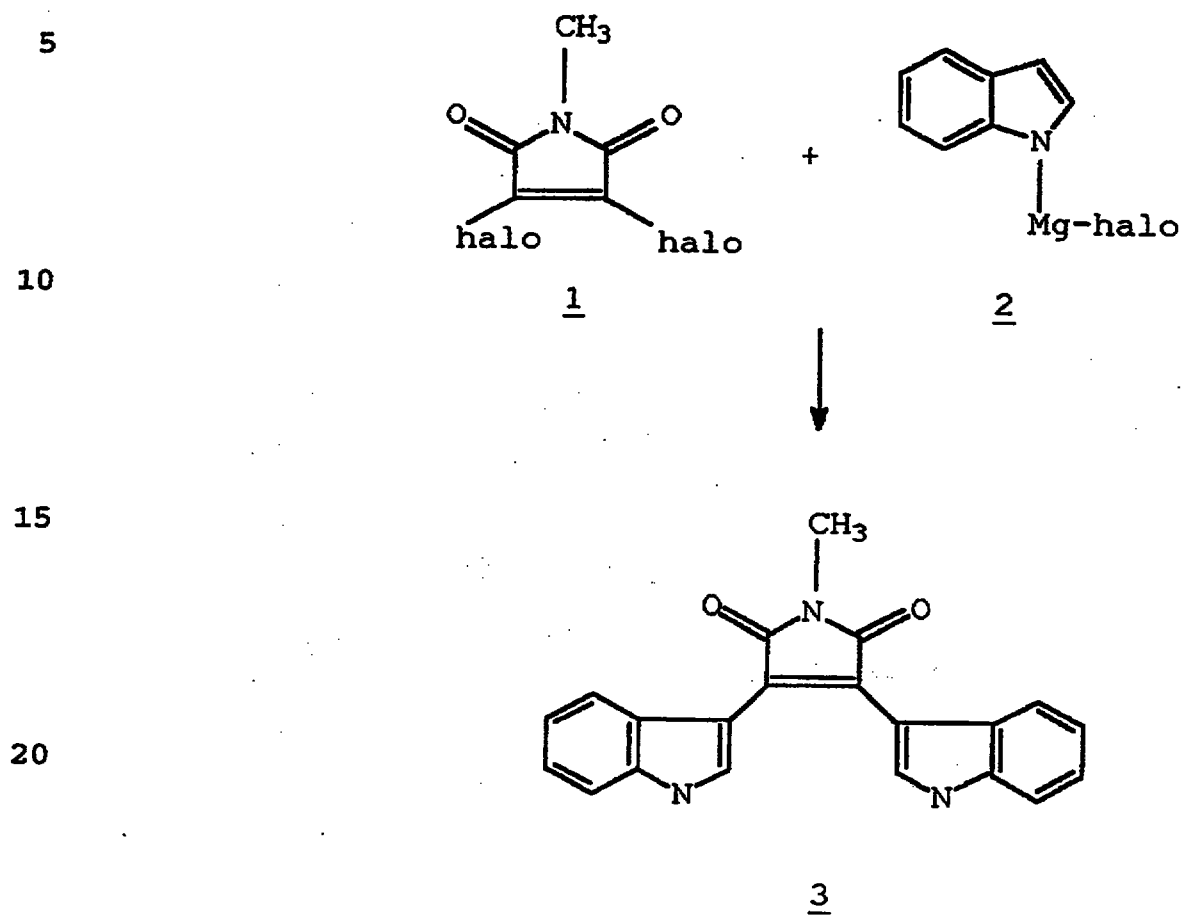
5 different pharmacokinetic profile than the parent, enabling easier absorption across the mucosal epithelium, better salt formation or solubility, and/or improved systemic stability (an increase in plasma half-life, for example). Conventional procedures for the selection and preparation
10 of suitable prodrug derivatives are described, (for example, in H. Bundgaard, Design of Prodrugs, (1985). Typically, such chemical modifications include the following:

- 1) ester or amide derivatives which may be cleaved by esterases or lipases;
- 15 2) peptides which may be recognized by specific or nonspecific proteases; or
- 3) derivatives that accumulate at a site of action through membrane selection of a prodrug form; or a modified prodrug form, or any combination of 1
20 to 3, supra.

The synthesis of certain bis-indole-N-maleimide derivatives is described in Davis et al., U.S. Patent 5,057,614, the disclosure of which is incorporated herein
25 by reference. Generally, the compounds of the present invention may be prepared as follows:

-23-

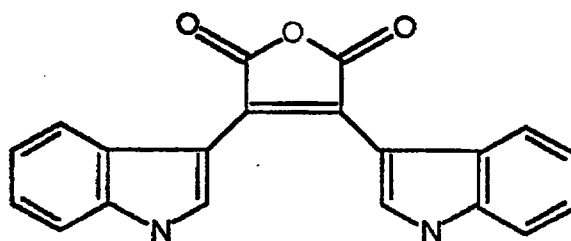
Scheme 1



-24-

The halo group in accordance with scheme 1 is preferably fluoro, chloro, bromo, or iodo. Compound 1 is preferably 2,3-dichloro N-methylmaleimide. The reaction between Compound 1 and the indole, Compound 2, is commonly known as a Grignard reaction. The reaction is carried out in an inert organic solvent, such as toluene, at a temperature between room temperature and the reflux temperature of the reaction mixture. Most significantly, the reaction depicted in Scheme I is dependent on solvent conditions. When carried out in a toluene:THF:ether solvent system, the reaction provides Compound 3 in greater than 80 percent yield and greater than 95 percent purity. The product is precipitated from the reaction mixture with ammonium chloride, NH_4Cl . The resulting intermediate, Compound 3, may be isolated by standard techniques.

Bis-3,4(3'-indolyl)-1N-methyl-pyrrole-2,5-dione, Compound 3, may then be converted by alkaline hydrolysis to the corresponding anhydride of the Formula 4 by techniques known in the art and described in Brenner et al., Tetrahedron 44: 2887-2892 (1988). Preferably, Compound 3 is reacted with 5 N KOH in ethanol at a temperature ranging from 25°C to reflux to produce the compound of Formula 4:

4

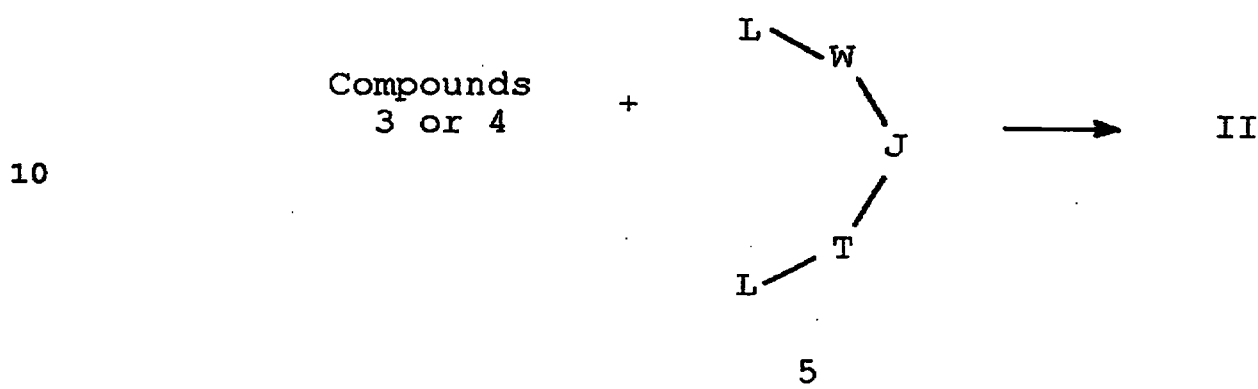
Compounds of Formula 3 are generally more stable than the compounds of the Formula 4. Therefore, it is preferred that Compounds 3 are reacted in accordance with Scheme 2 to produce the compounds of Formula I. However,

-25-

one skilled in the art would recognize that the compounds of the Formula 4, may also be reacted according to Scheme 2.

5

Scheme 2



15

T, J, and W are the same as previously defined. L is a good leaving group such as chloro, bromo, iodo, mesyl, tosyl, and the like. L may also be a hydroxy or other precursor that may be readily converted to a good leaving group by techniques known in the art. For example, the hydroxy may be readily converted to a sulfonic ester such as mesyl by reacting the hydroxy with methanesulfonyl chloride to produce the mesylate leaving group.

The reaction represented by Scheme 2 is accomplished by any of the known methods of preparing N-substituted indoles. This reaction usually involves approximately equimolar amounts of the two reagents, although other ratios, especially those wherein the alkylating reagent is in excess, are operative. The reaction is best carried out in a polar aprotic solvent employing an alkali metal salt or other such alkylation conditions as are appreciated in the art. When the leaving group is bromo or chloro, a catalytic amount of iodide salt, such as potassium iodide may be added to speed the reaction. Reaction conditions include the following:

-26-

Potassium hexamethyldisilazide in dimethylformamide or tetrahydrofuran, sodium hydride in dimethylformamide.

Preferably, the reaction is carried out under slow reverse addition with cesium carbonate in either
5 acetonitrile, dimethylformamide (DMF), or tetrahydrofuran (THF). The temperature of the reaction is preferably from about ambient temperature to about the reflux temperature of the reaction mixture.

One skilled in the art would recognize that the
10 reaction described in Scheme 2 may be employed with the compounds L-T' and L-W', wherein T' and W' are a protected carboxy, protected hydroxy, or a protected amine. After the alkylation of Scheme 2, T' and W' may be converted to moieties capable of coupling to form J. The coupling of T'
15 and W' to form the various ether or thioether derivatives is known in the art and described in, for example Ito et al., Chem. Pharm. Bull. 41(6): 1066-1073 (1993); Kato, et al., J. Chem. Pharm. Bull. 34: 486 (1986); Goodrow, et al., Synthesis 1981: 457; Harpp, et al., J. Am. Chem. Soc.
20 93: 2437 (1971); and Evans, et al., J. Org. Chem. 50: 1830 (1985).

One skilled in the art would also recognize that compound 3 may be converted to the compounds of Formula I in a two step synthesis as described in Scheme 3.

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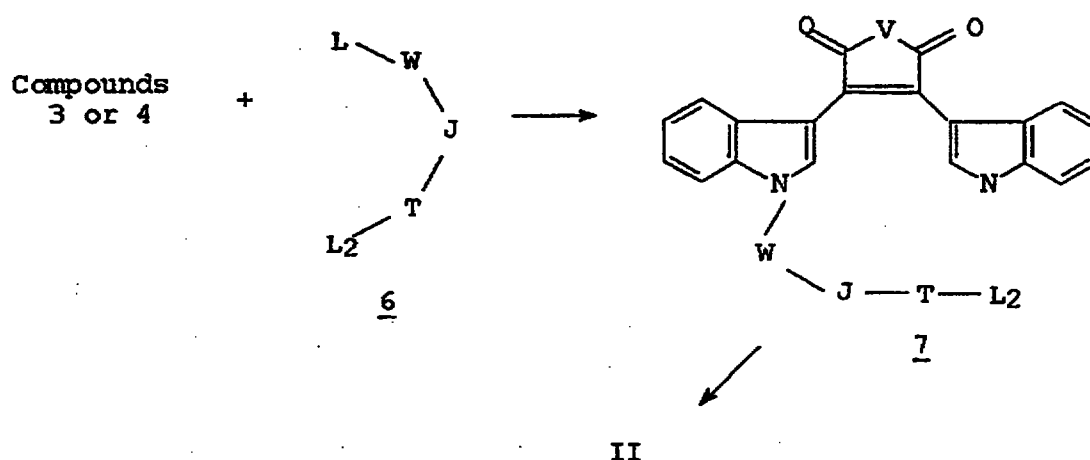
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Scheme 3



T, J, W, V and L are as previously defined. L₂ is a
 25 protected hydroxy or other group that may be readily
 converted to a good leaving group by techniques known in
 the art. The coupling between Compound 3 or 4 and Compound
 6 is an alkylation as previously discussed. The
 monoalkylated intermediate, 7, is deprotected, and L₂ is
 30 converted to a leaving group. For example, if the hydroxy
 is protected with t-butyldimethylsilyl (TBDMS), TBDMS is
 selectively removed using acidic methanol. The resulting
 free hydroxy is then converted to a leaving group, such as
 an alkyl halide, preferably an alkyl iodide or bromide (CBr,
 35 in triphenylphosphine) or sulfonate (mesyl chloride in

-28-

triethylamine). The macrolide is then formed by alkylating under slow reverse addition to a solution of base, such as potassium hexamethyldisilazide, or sodium hydride but preferably Cs_2CO_3 in a polar aprotic solvent such as acetonitrile, DMF, THF at temperatures ranging from ambient to reflux.

The compounds of Formula I may be prepared in substantially higher yield when the alkylation is carried out under slow reverse addition to Cs_2CO_3 in a polar aprotic solvent. Slow reverse addition involves combining a mixture of compound and alkylating agent (Scheme 2) or the compound (Scheme 3) with the base at a rate from about 0.1 mL/hour to about 2.0 mL/hour. The concentration of each reagent in the mixture is about 1.5 molar to about 0.001 molar. When carried out with the monoalkylated compound (Scheme 3) the concentration is from about 3 molar to about 0.001 molar. The slow addition results in a concentration of reagents in the reaction vessel of about 0.01 molar to 1.5 molar. One skilled in the art would recognize that at a higher rate of addition a lower concentration of reagents could be used in the reaction. Likewise, at a slower rate of addition, a higher concentration of reagents could be used in the reaction. Preferably, the compound is added at about 0.14 mL/hour with the compound and the alkylating agent at 0.37 molar. It is preferred that the Cs_2CO_3 be added in excess -- most preferably a 4:1 ratio Cs_2CO_3 to alkylating agent. Preferred polar aprotic solvents are acetonitrile, dimethylformamide (DMF), acetone, dimethylsulfoxide (DMSO), dioxane, diethylene glycol methyl ether (diglyme), tetrahydrofuran (THF), or other polar aprotic solvents in which the reagents are soluble. The reaction is carried out at temperatures ranging from about 0°C to reflux. One skilled in the art would recognize that the ratio of the mixture of the compound and alkylating agent is not critical. However, it is preferred that the

-29-

reagents are mixed in a ratio of 0.5 to 3 equivalents of each other. Most preferably, the reagents are mixed 1:1.

When V is N-CH₃, Compound II is converted to the corresponding anhydride (V is O) by alkaline hydrolysis.

- 5 Alkaline hydrolysis involves reacting the compound with a base (such as sodium hydroxide or potassium hydroxide), in either C₁-C₄ alcohol (preferably ethanol), DMSO/water, dioxane/water, or acetonitrile/water at a temperature ranging from about 25°C to preferably about reflux. The
10 concentration of the reactants is not critical.

- The anhydride (V is O) is converted to the maleimide of Formula I by ammonolysis. Ammonolysis involves reacting the anhydride with an excess of hexamethyldisilazane or an ammonium salt (ammonium acetate,
15 bromide, or chloride) and C₁-C₄ alcohol (preferably methanol) in an polar aprotic solvent such as DMF at room temperature. Preferably, the hexamethyldisilazane or an ammonium salt is reacted at a ratio greater than about 5:1 equivalents of anhydride.

- 20 Scheme 4 depicts the preparation of an intermediate useful in accordance with the present invention for preparing the compounds of Formula III, wherein X is O.

-30-

Scheme 4

5

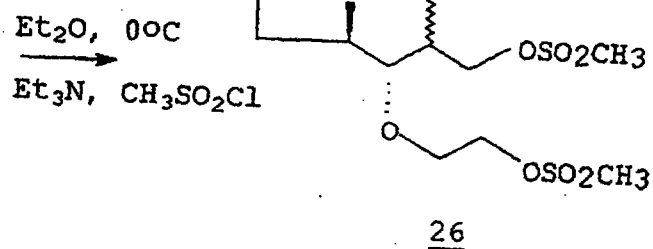
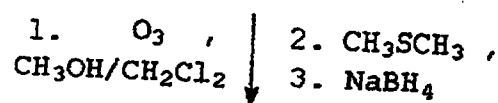
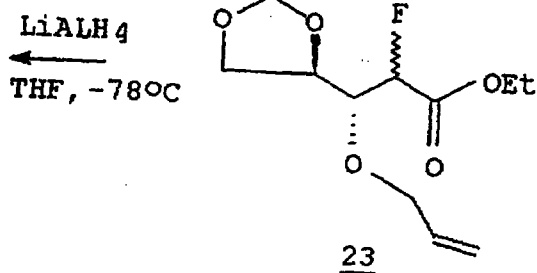
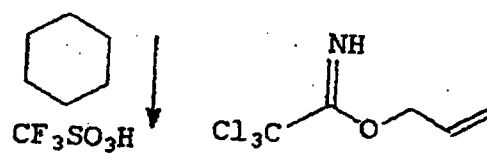
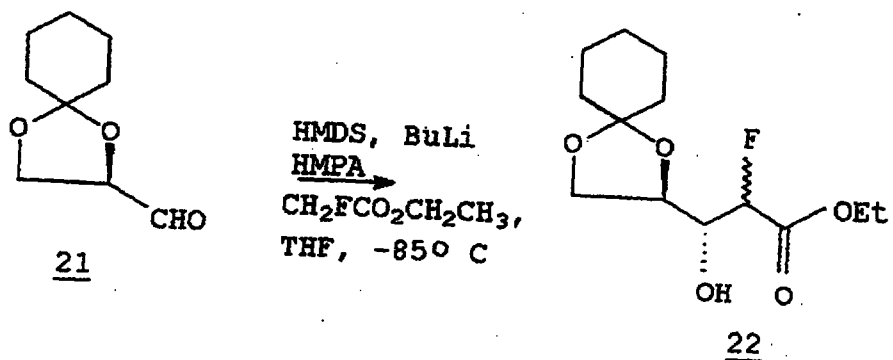
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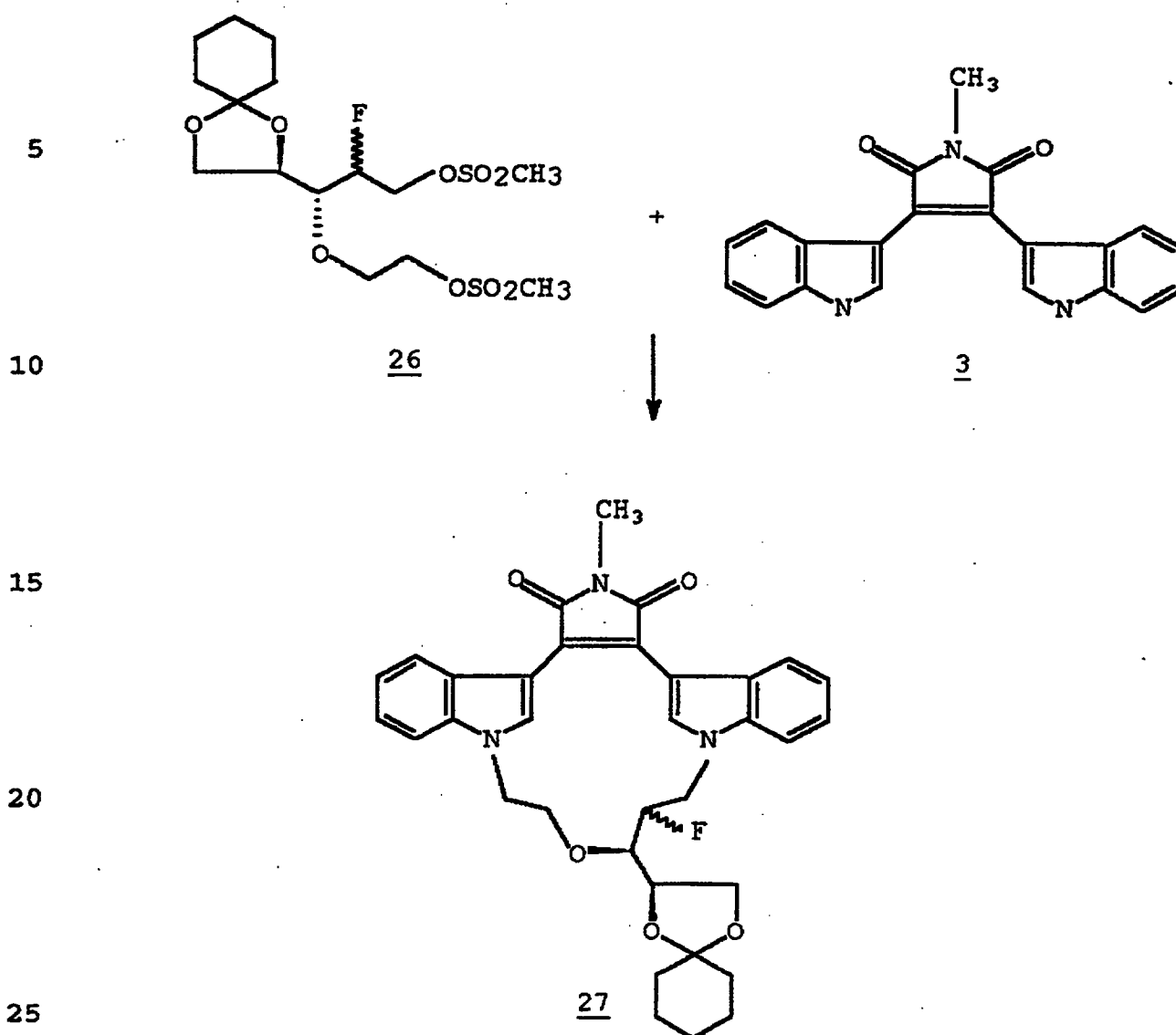
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-31-



The following examples and preparations are provided merely to further illustrate the invention and not to limit it.

-32-

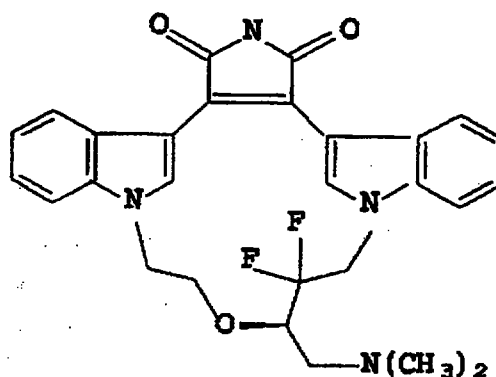
Example 1

Synthesis of Bisfluorinated Six Atom Bridged
Bisindolylmaleimides

5 A compound of formula VII:

10

VII

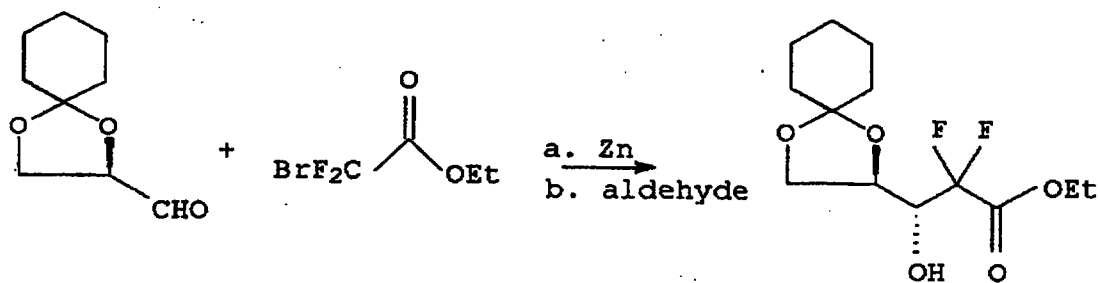


15

is produced as follows:

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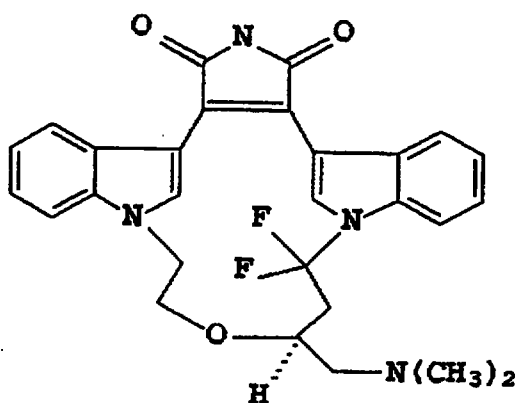
30

Compound 8a is converted into the compound of
35 Formula VII using the steps outlined in scheme 4.

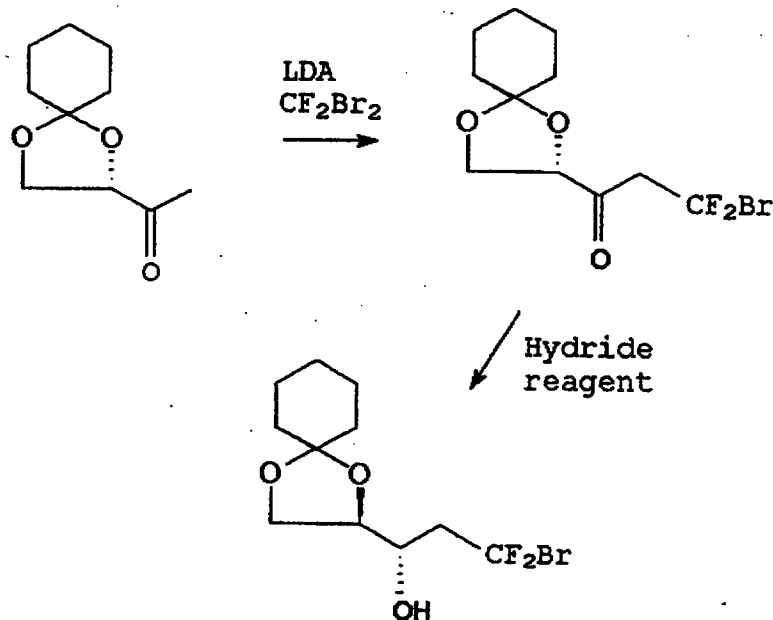
- 33 -

A compound of formula VIII:

VIII



is produced as follows:

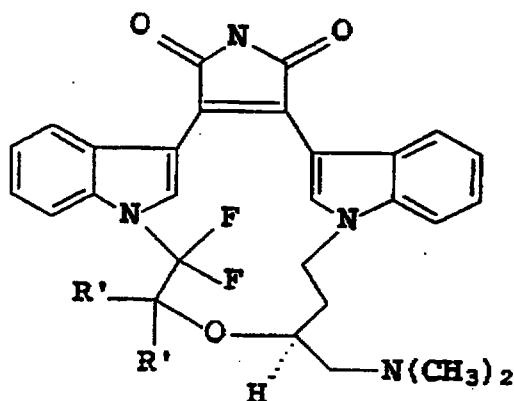


This compound is converted into the compound of Formula VIII using the steps outlined in scheme 4.

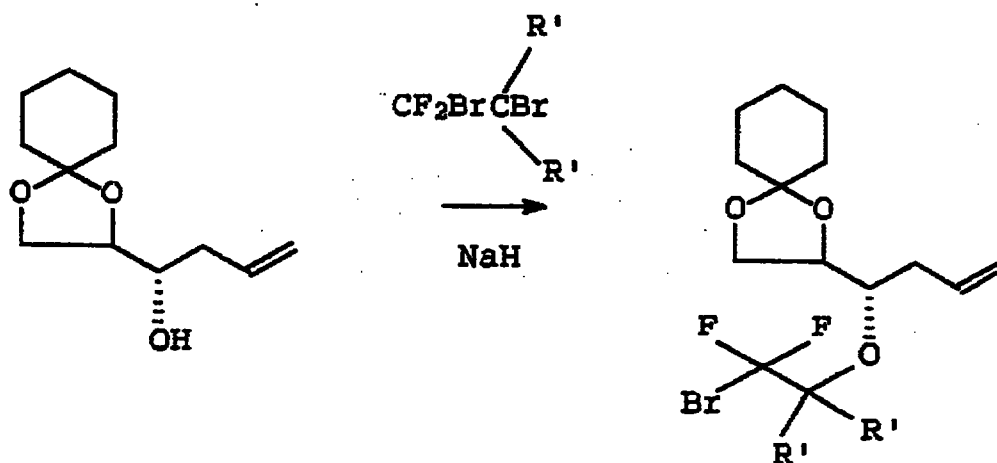
-34-

A compound of formula IX:

IX



is produced as follows:



This compound, wherein R' is H or F, is converted into the compound of Formula IX using the steps outlined in scheme

35 4.

-35-

Example 2

Synthesis of C2-Monofluorinated Seven Atom Bridged
Bisindolylmaleimides

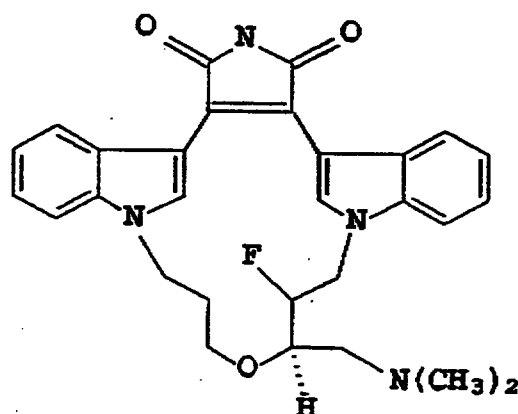
5

A compound of formula X:

10

X

15

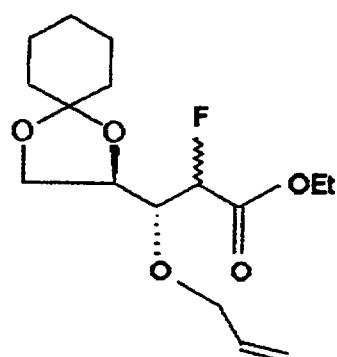


is produced as follows:

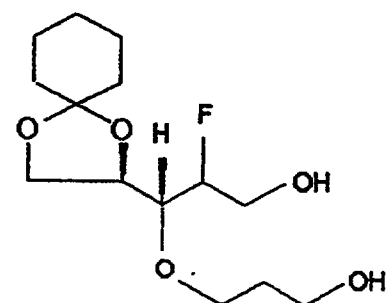
20

25

30



a. LiAlH_4
b. BH_3 THF
c. H_2O_2 NaOH



This compound is converted into the compound of Formula X
using the steps outlined in scheme 4.

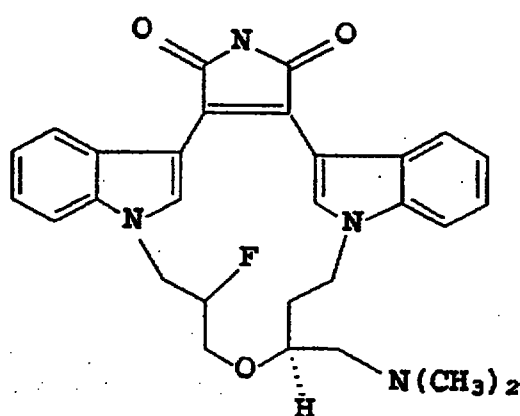
-36-

Example 3

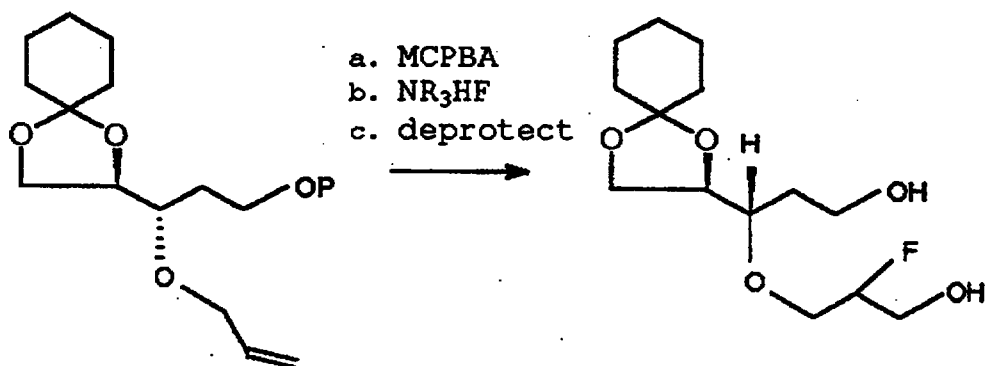
Synthesis of C6-Monofluorinated Seven Atom Bridged
Bisindolylmaleimides

A compound of formula XI:

XI



is produced as follows:



This compound is converted into the compound of Formula XI
using the steps outlined in scheme 4.

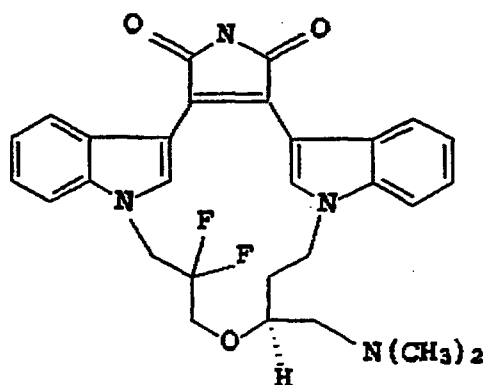
-37-

Example 4

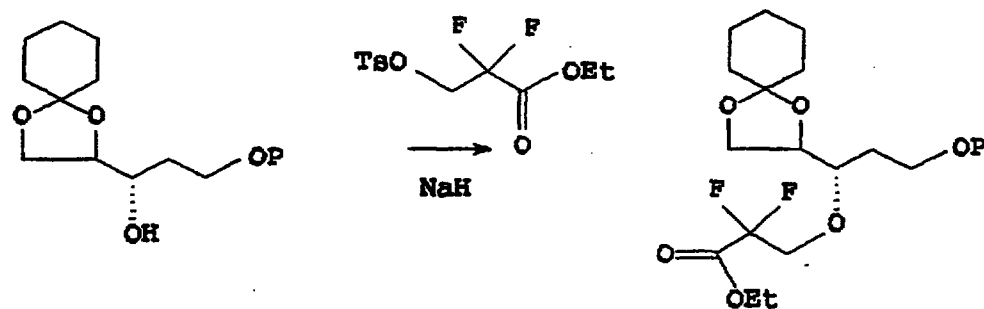
Synthesis of C6-Difluorinated Seven Atom Bridged
Bisindolylmaleimides

A compound of formula XII:

XII



is produced as follows:



This compound is converted into the compound of Formula XII
using the steps outlined in scheme 4.

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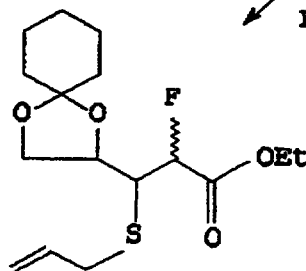
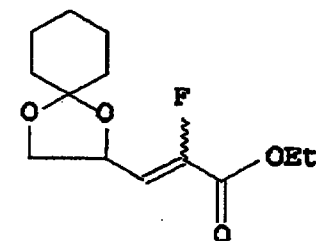
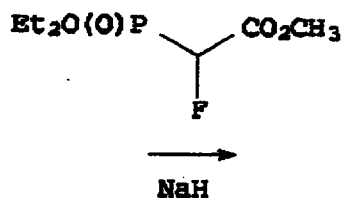
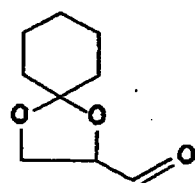
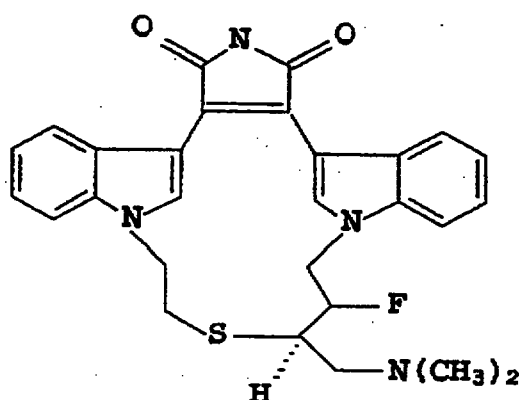
Example 5

Synthesis of Fluorinated Six Atom Thio-Bridged
Bisindolylmaleimides

A compound of formula XIII:

XIII

is produced as follows:



This compound is converted into the compound of Formula XIII using the steps outlined in scheme 4.

-39-

Example 6

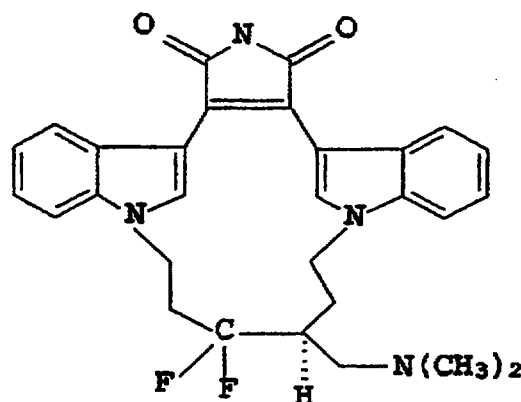
**Synthesis of C4-Sidechain derivatives of Fluorinated Six
Atom Bridged Bisindolylmaleimides**

5

A compound of formula XIV:

10

XIV



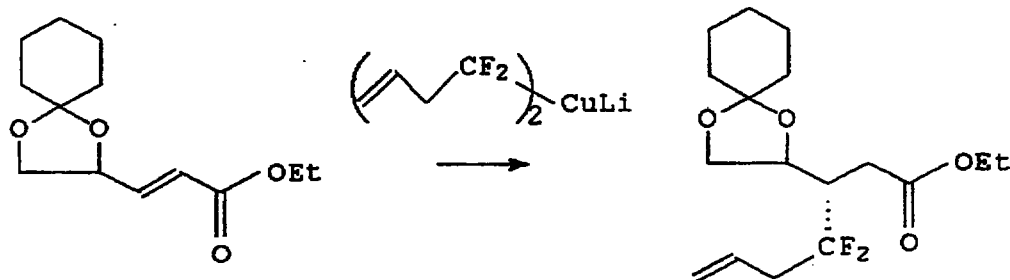
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is produced as follows:

20

25

30



This compound is converted into the compound of Formula XIV
35 using the steps outlined in scheme 4.

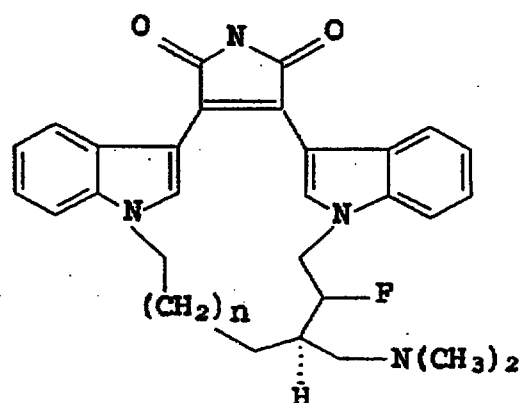
-40-

Example 7

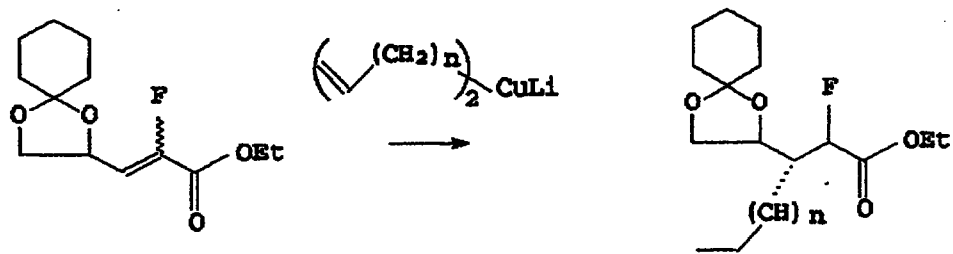
Synthesis of Fluoroalkene Six Atom Bridged
Bisindolylmaleimides

A compound of formula XV:

XV



is produced as follows:

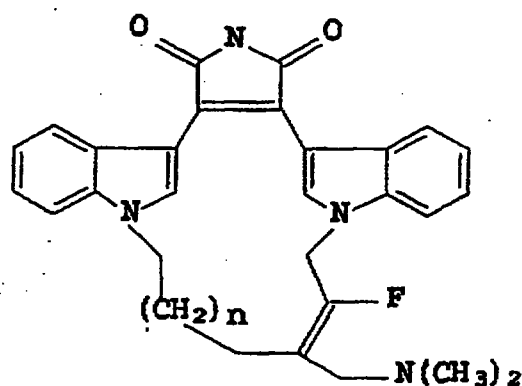


This compound is converted into the compound of Formula XV
using the steps outlined in scheme 4.

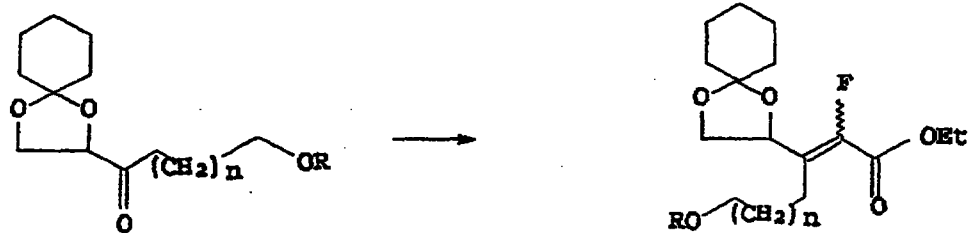
Example 8

Synthesis of Fluoroalkene Seven Atom Bridged
Bisindolylmaleimides

A compound of formula XVI:

XVI
(E and Z forms)

is produced as follows:

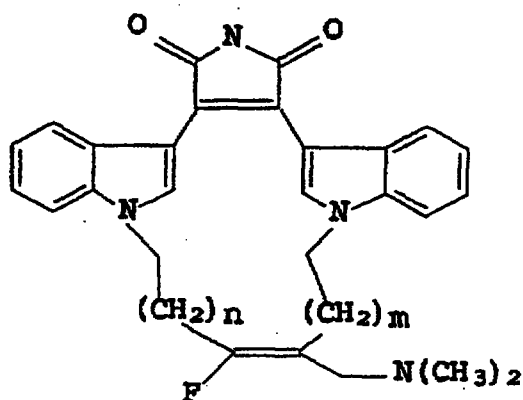


This compound is converted into the compound of Formula XVI
using the steps outlined in scheme 4.

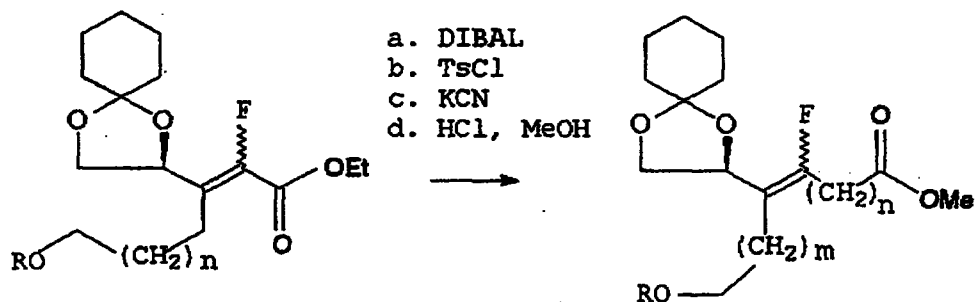
-42-

A compound of formula XVII:

XVII
(E and Z forms)



is produced as follows:

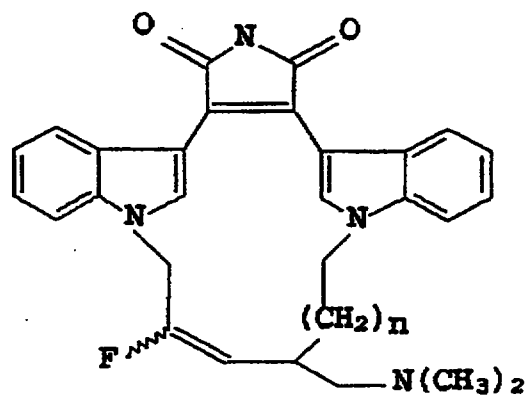


This compound is converted into the compound of Formula
XVII using the steps outlined in scheme 4.

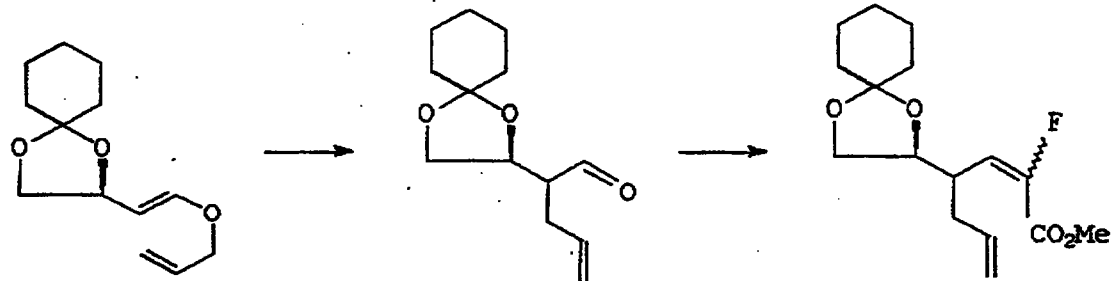
-43-

A compound of formula XVIII:

XVIII
(E and Z forms)



is produced as follows:



This compound is converted into the compound of Formula XVIII using the steps outlined in scheme 4.

-44-

Example 9

Synthesis of C2-methylated Fluorinated Six Atom Bridged
Bisindolylmaleimides

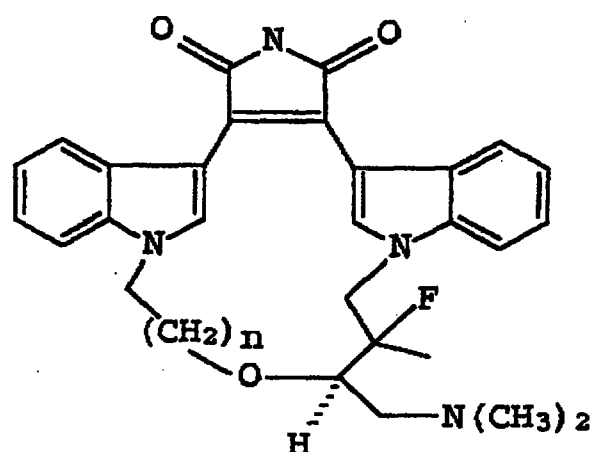
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A compound of
formula XIX:

10

XIX

15

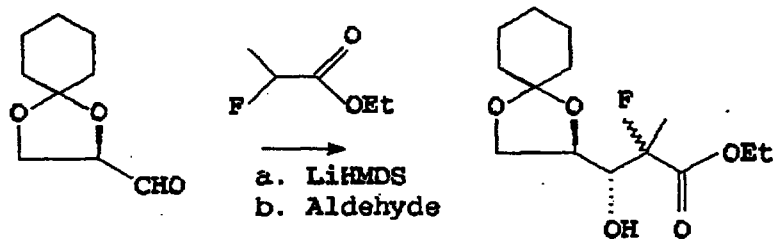


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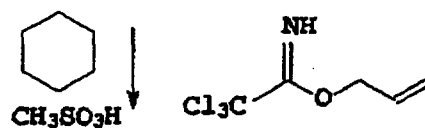
is produced as follows:

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-45-

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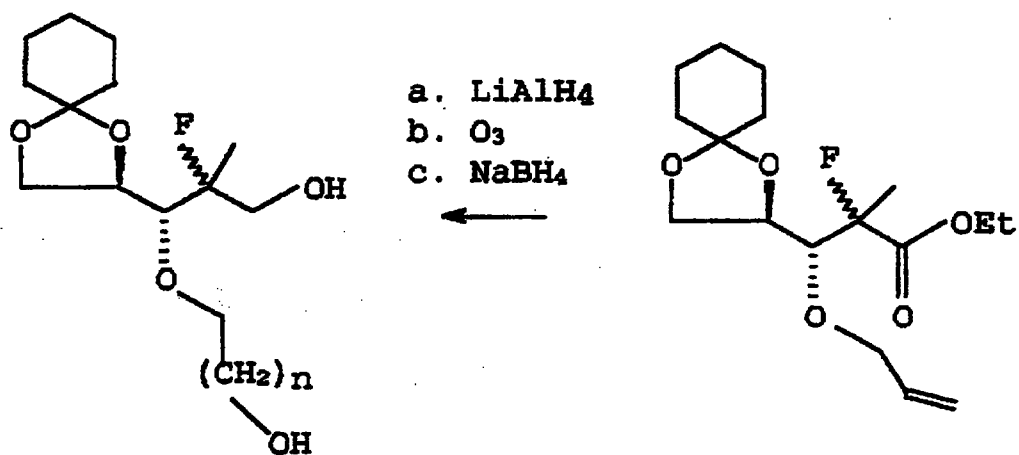
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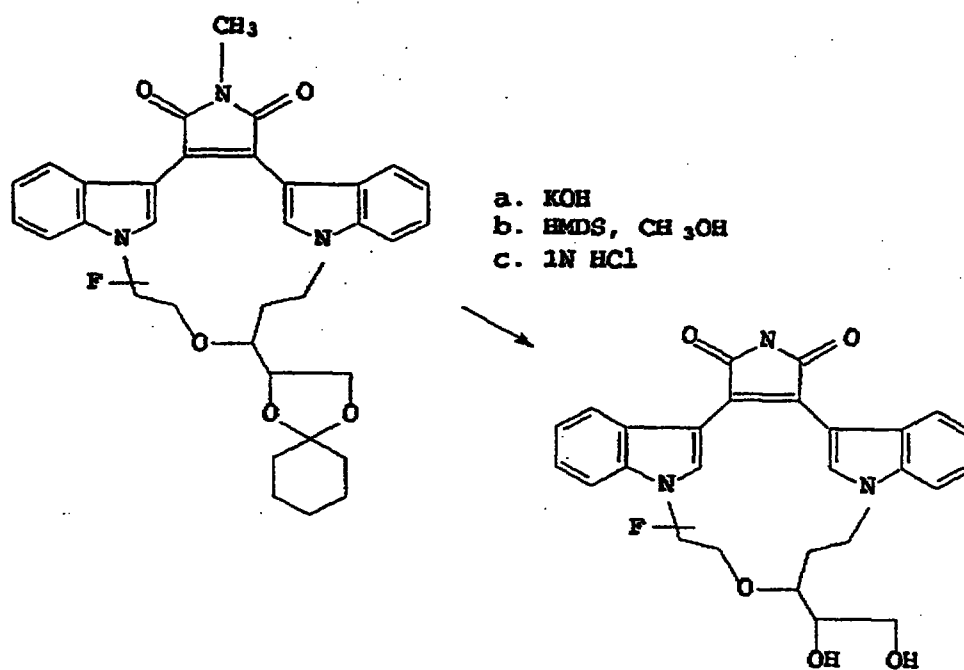
30



XIX

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Example 10

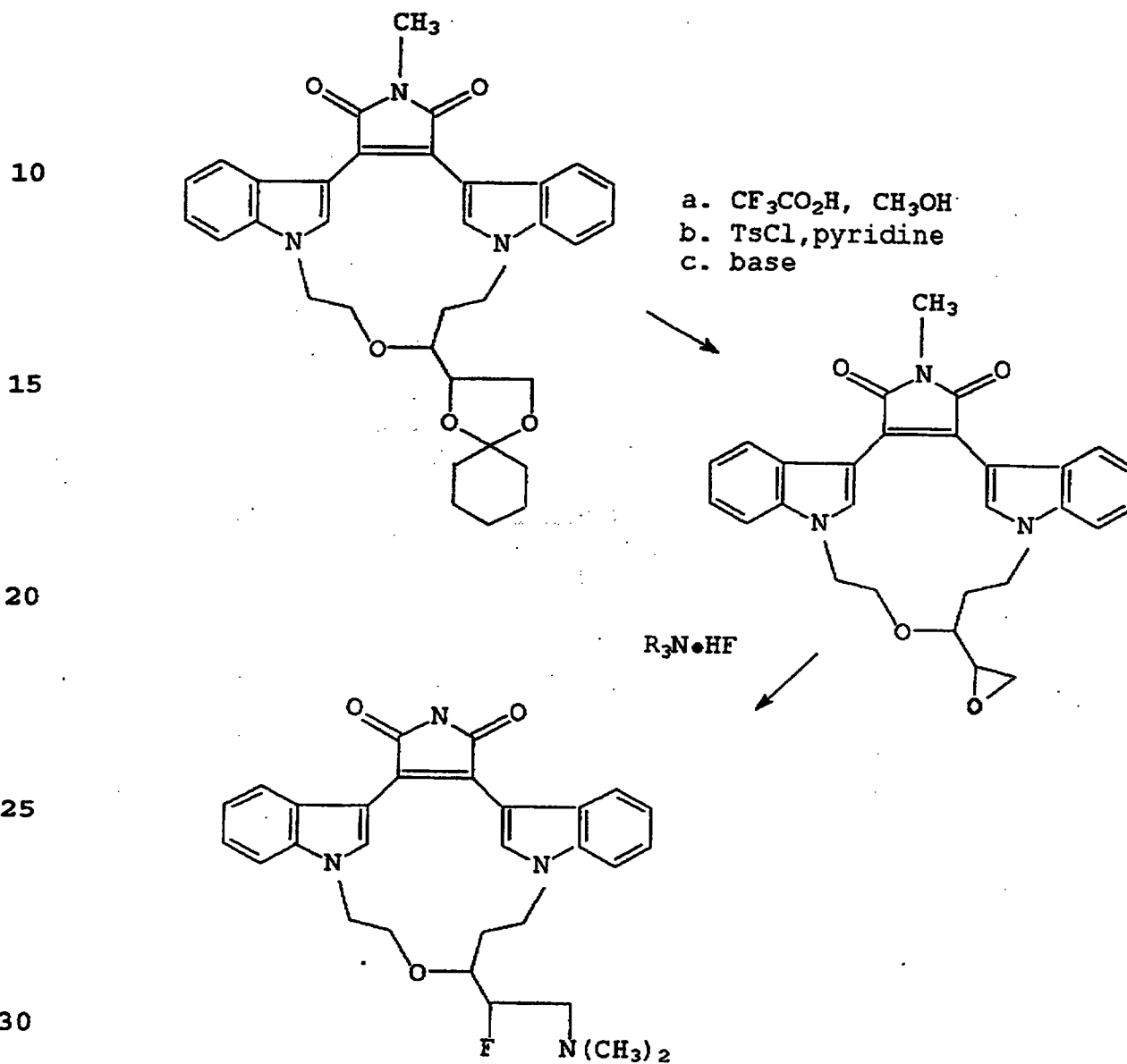
**Synthesis of C3-Derivative Fluorinated Six Atom Bridged
Bisindolylmaleimides**

-47-

Example 11

Synthesis of C3-Derivative Fluorinated Six Atom Bridged
Bisindolylmaleimides

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Example 12

**Synthesis of C3-Derivative Fluorinated Six Atom Bridged
Bisindolylmaleimides**

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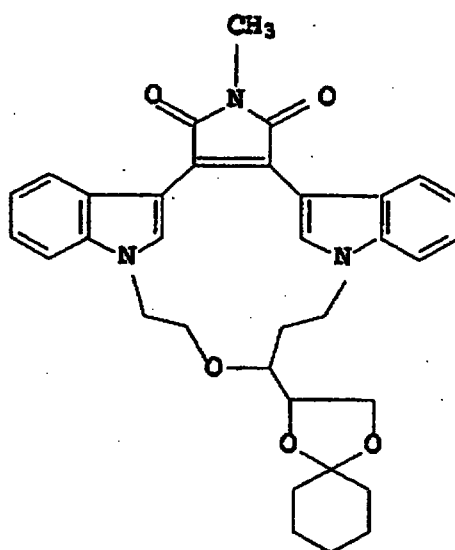
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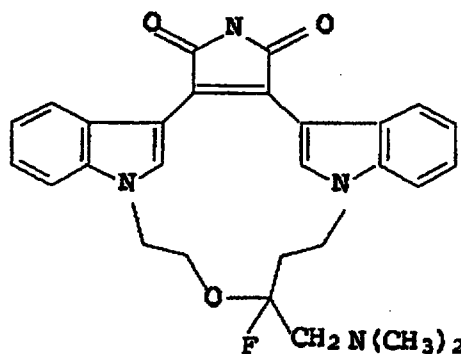
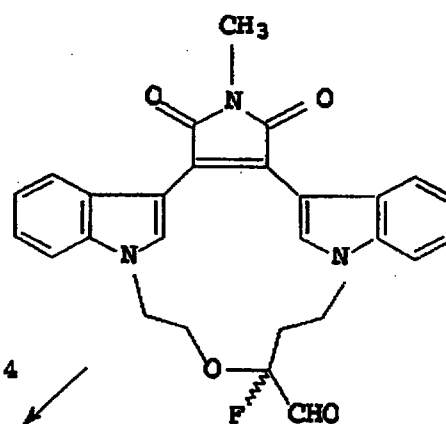
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- a. $\text{CF}_3\text{CO}_2\text{H}$, CH_3OH
b. NaIO_4
c. TMSCl , NEt_3
d. Electrophilic
fluorinating agent

scheme 4

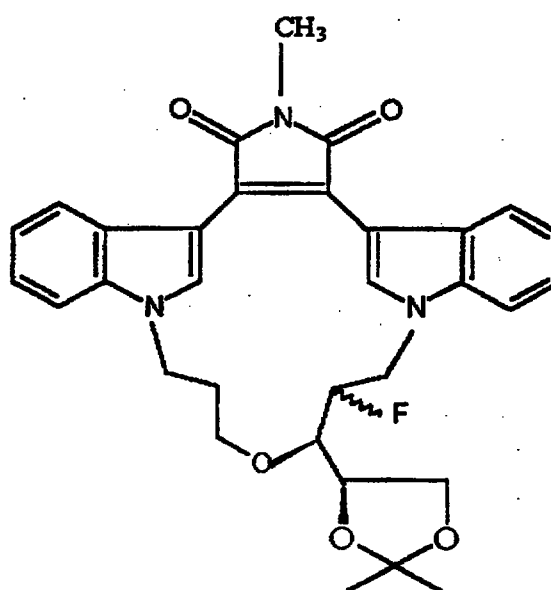


-49-

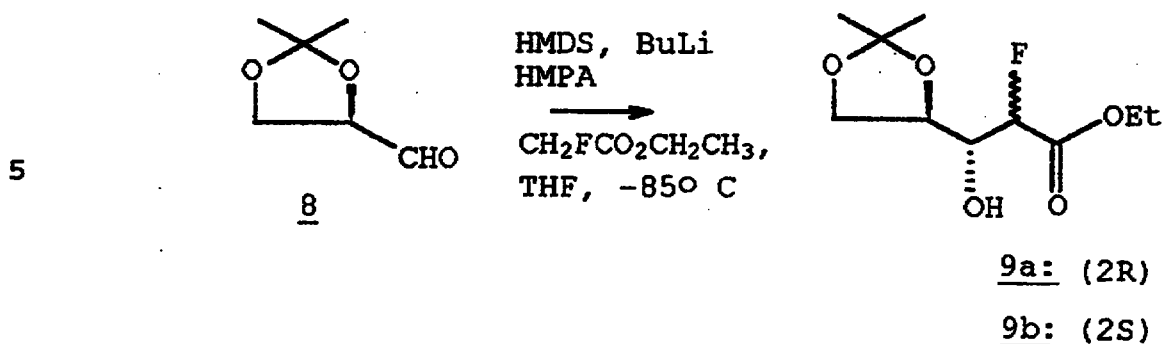
In the following examples and preparations, melting point, nuclear magnetic resonance spectra, mass spectra, high pressure liquid chromatography over silica gel, N,N-dimethylformamide, palladium on charcoal, tetrahydrofuran, and ethyl acetate are abbreviated M.Pt., NMR, MS, HPLC, DMF, Pd/C, THF, and EtOAc, respectively. The terms "NMR" and "MS" indicate that the spectrum was consistent with the desired structure.

10 Example 13

Preparation of the bisindolylmaleimide of the formula:

14

-50-



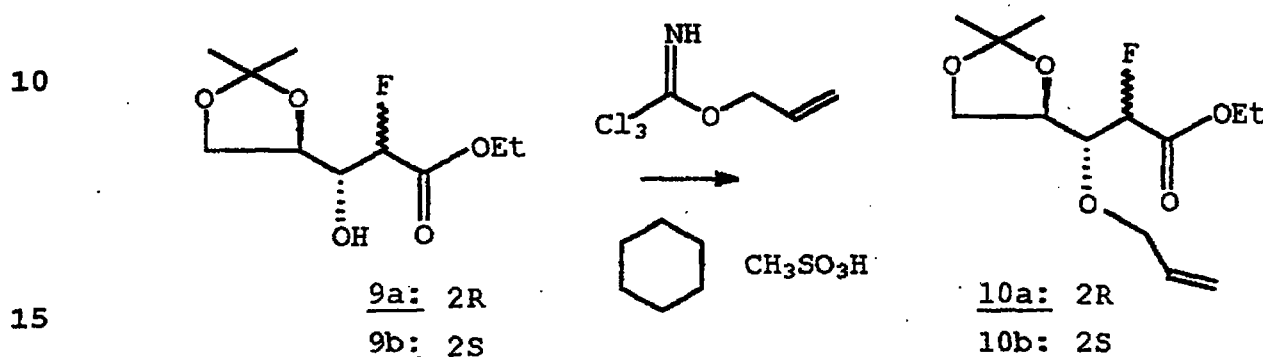
10 Ethyl (3S,4R)-2-fluoro-3-hydroxy-4,5-O-isopropylidene pentanoate.

To a stirred round-bottom flask containing 50 mL of anhydrous THF and 6.1 mL (0.03 mol) of hexamethyl disilazane (HMDS) at 0°C, was added 12 mL (0.03 mol) of a 2.5M solution of butyllithium in hexane. The resulting mixture was allowed to return to room temperature and stirred for 10 min. Then it was lowered to -85°C. A mixture of 1.80g (0.010 mol) of hexamethyl phosphoric triamide (HMPA) and 1.00mL (0.010 mol) of ethyl fluoroacetate was added dropwise as rapidly as possible while not allowing the temperature to rise above -85°C. After 5 additional min. 880 mg (0.00677 mol) of 2,3-O-isopropylidene D-glyceraldehyde (8) was added. The mixture was stirred for 10 min and then quenched at -85°C with 5 mL saturated ammonium chloride. On warming to room temperature the mixture was diluted with 80mL CH₂Cl₂ and washed with water (60mLx3). The CH₂Cl₂ layer was separated and dried over Na₂SO₄. After evaporation of the volatiles in vacuo, the residue was loaded on a short silica gel column. It was flushed with CH₂Cl₂, then 30% CH₃CN/CCl₄ (or ethyl acetate), yielding crude product.

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The crude product was chromatographed on a silica gel column using 15% (v/v) CH₃CN/CCl₄ as eluent, yielding two fractions with partial separation of two diastereomers: fraction 1, 177mg, (9a, 34%, 9b, 66% based on ¹HNMR)

5 fraction 2, 377mg (9a, 62%, 9b, 38%). Overall yield 34.7%, in which 9a, was 52.7%, and 9b, was 47.3%



Ethyl (3S,4R) 3-allyloxy-2-fluoro-4,5-O-isopropylidene pentanoate.

20

Alcohol 9, 327 mg (1.39 mmol, which is a mixture of 9a, 62% and 9b, 38%), was evaporated with toluene and 327 mg (1.39 mmol), was dissolved in 6 mL cyclohexane. Under N₂ atmosphere and stirring, allyl trichloroacetimidate (423 μL, 561 mg, 2.78 mmol) was added, followed by trifluoromethanesulfonic acid (20 μL) in 5 μL portions over 20 min. A dark brown oily ppt. began to form instantly. The reaction mixture was stirred at room temperature for 60h. A white ppt. was formed, and that ppt. was filtered and washed with cyclohexane twice. The filtrate was evaporated to a oily liquid. It was chromatographed on a silica gel column using 10% (v/v) ethyl acetate-hexane as eluent, affording two fractions. The first fraction contained mainly 10a and the second fraction 10b. The fractions were purified on a silica column using 25% (v/v)

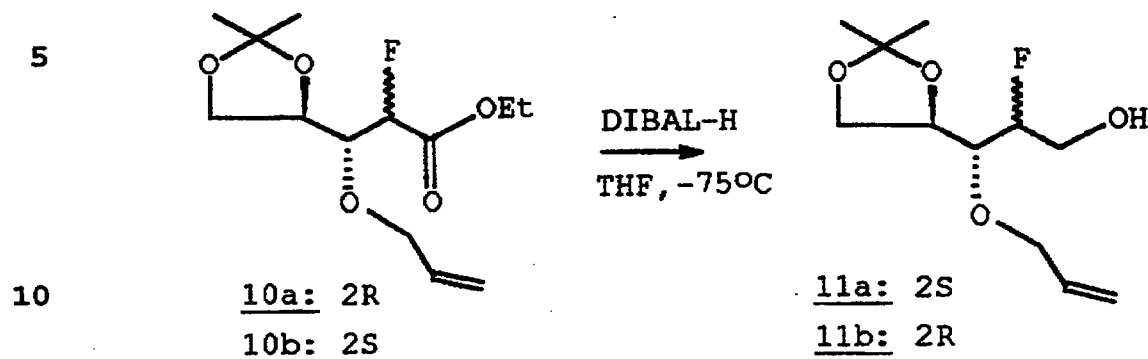
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hexane/CH₂Cl₂ as eluent, yielding 10a, 133mg (34.8%) and 10b, 83 mg (21.7%). Total yield 56.5%.



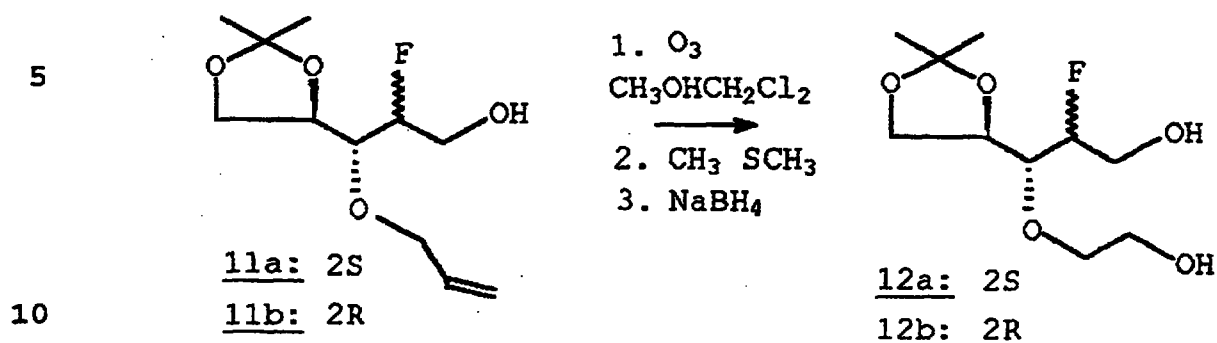
(3S,4R) 3-Allyloxy-2-fluoro-4,5-O-isopropylidene pentanol
(11).

15 The ester 10a, 60 mg (0.217 mmol) was evaporated with toluene twice. It was dissolved in 3.0 mL dry THF and cooled down to -75°C. Under N₂ with stirring, DIBAL-H toluene solution 0.68 mL (1.29 M, 0.877 mmol) was added dropwise over 20 min. The resulting solution was stirred at -75°C for another 1.5h. Then it was allowed to warm up to -5°C and quenched with 4 mL ethyl acetate. After stirring for 10 min. wet Na₂SO₄ (2g) was added. The mixture was stirred at -5°C for 2 h. The solid was filtered away and washed with ethyl acetate twice. The filtrate was

20

25 evaporated under reduced pressure. The residue was chromatographed on a silica gel column using 30% (v/v) ethyl acetate-hexane as eluent, yielding pure 11a, 32 mg (63.4%). 11b is obtained the same way from 11b.

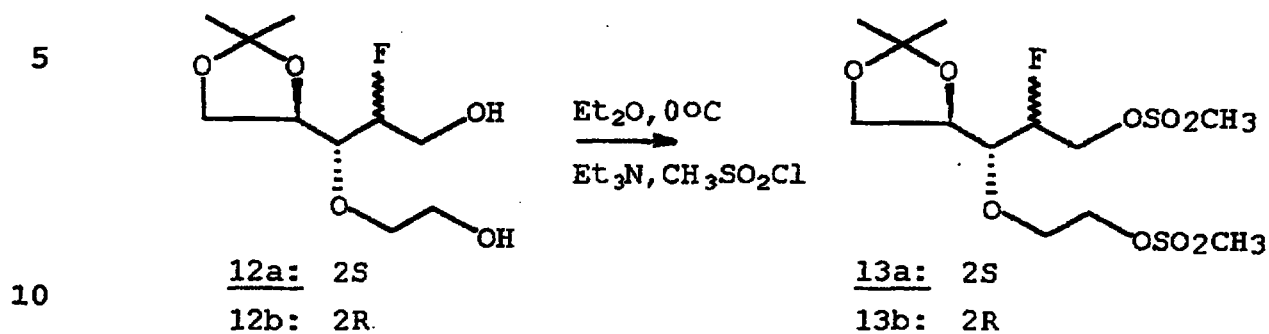
-53-

Diol (12):

Compound 11 (a mixture of 11a and 11b), 23 mg (0.099 mmol) was dissolved in 3 mL CH₃OH and 1 mL CH₂Cl₂. At -78°C, it was bubbled with O₃ until the solution turns bright blue. The solution was then bubbled with argon for 5 min. A drop of dimethyl sulfide was added, and the mixture was stirred for 10 min. Sodium borohydride 30 mg. (0.794 mmol, 8 eq) was added and stirred for 5 min. The reaction mixture was allowed to return to ambient temperature and was stirred for another 1 h. After adding 3 drops of saturated NH₄Cl aqueous solution, the mixture was stirred for another 1 h. The volatiles were removed *in vacuo*. The residue was dissolved in methanol, and ethyl acetate was added to replace methanol by co-evaporation. The white ppt. was filtered away and the filtrate was evaporated. The residue was chromatographed on a silica gel column using ethyl acetate. After removal of the trace unknown components, 13 mg (55.3%) of 12a and 7 mg (29.8%) of 12b were obtained.

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Mesylation and Coupling Reactions



15 The diol (12a), 12 mg (0.050 mmol) was dissolved
in 5 mL anhydrous ethyl ether and cooled to 0°C under N₂
atmosphere. Under stirring 35 μ L (0.250 mmol, 5 eq.) of
Et₃N was added, followed by 20 μ L (0.25 mmol, 5 eq.) of
mesyl chloride. The resulting mixture was stirred at 0°C
20 for 5 h. The ppt. was filtered away and washed with ethyl
ether. The filtrate was washed with water (2x) and brine
(2x) and dried over Na₂SO₄. After evaporation of the
solvents in vacuo, a yellowish oil 13a, 9 mg (43.3%) was
obtained.

25 The precipitate was dissolved in water and
extracted with ethyl acetate. The ethyl acetate layer was
washed with NaHCO₃ aqueous solution twice and dried over
Na₂SO₄. After evaporation of the solvent in vacuo, 11 mg
(55.3%) of 13a was obtained. Totally 20 mg of 13a was
30 obtained, a 100% yield.

Dimesylate 13a, 20 mg (0.0507 mmol) and
bisindolyl-maleimide 17.3 mg (0.0507 mmol) were combined
and dissolved in 2.5 mL anhydrous DMF (dried over molecular
sieves) and added via syringe pump addition to a suspension

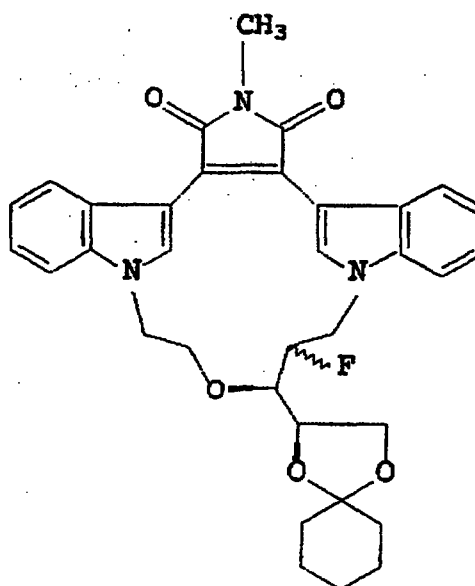
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-55-

of cesium carbonate (66 mg, 0.203 mmol in 3 mL anhydrous DMF at 50°C under N₂ over 40 h. The reaction mixture was stirred at 50°C for another 10 h. It was evaporated in vacuo to remove the volatiles. The residue was dissolved in CHCl₃, and washed with saturated NaHCO₃ aqueous solution and brine. The chloroform layer was separated and dried over Na₂SO₄. After evaporation, the red solid residue was chromatographed on silica gel column with elution of 10% (v/v) acetone/CHCl₃. The desired compound 14a, 7 mg (25.4%) was eluted out first, followed by one of the starting material, bisindoyl-maleimide 13 mg (75%).

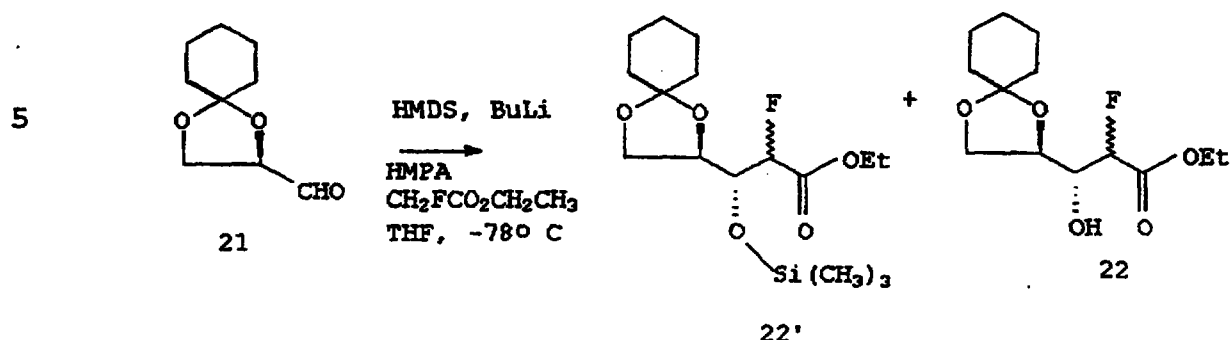
Example 14

Preparation of the bisindolyl maleimide of the formula:



Compounds 21a and 21b are stereoisomers, 21a is in the 2R conformation; 21b is in the 2S conformation. The nomenclature of the chiral center changes when the ester is reduced to the alcohol (i.e. compound 24a becomes 2S).

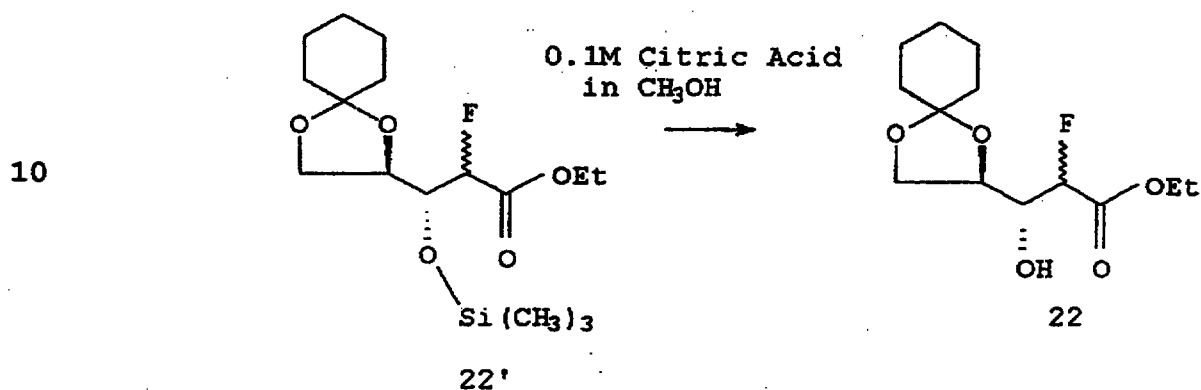
-56-



To a stirred round-bottomed flask containing 40 mL of anhydrous THF and 5.0 mL (0.0255 mol.) of hexamethyl disilazane 9.0 mL (0.0225 mol) of a 2.5M solution of butyl lithium in hexane was added and reacted in an ice bath under N₂. After stirring at room temperature for 10 min, it was cooled to -78°C, and 3.6g (0.020 mol) of HMPA and 2.0 mL (0.020 mol) of ethyl fluoroacetate were added dropwise in 5 min. After stirring for 5 additional min. 760 mg (4.46 mmol) of cyclohexylidene-glyceraldehyde (21, prepared as Ref. JOC, 1992, 57 page 648, and JOC, 1995, 60, pages 585-587, distilled at 60°C/0.5 mmHg) was added quickly. The mixture was allowed to stir for 10 additional min. and then was quenched at -78°C with 5 mL of saturated ammonium chloride. On warming to room temperature the mixture was diluted with 60 mL hexane. The hexane layer was separated. The remaining layer was extracted with 30 mL hexane. The hexane solutions are combined and washed with saturated ammonium chloride (100 mL x 3) and dried over Na₂SO₄. After evaporation under reduced pressure, the residue was chromatographed on a silica gel column using 30% ethyl acetate-hexane, yielding the main product 22' 740mg (47.6%), which is a mixture of two isomers with the major isomer 22'a (74%) and the minor 22'b (26%) based on ¹HNMR. The aqueous washing was extracted with CHCl₃. After

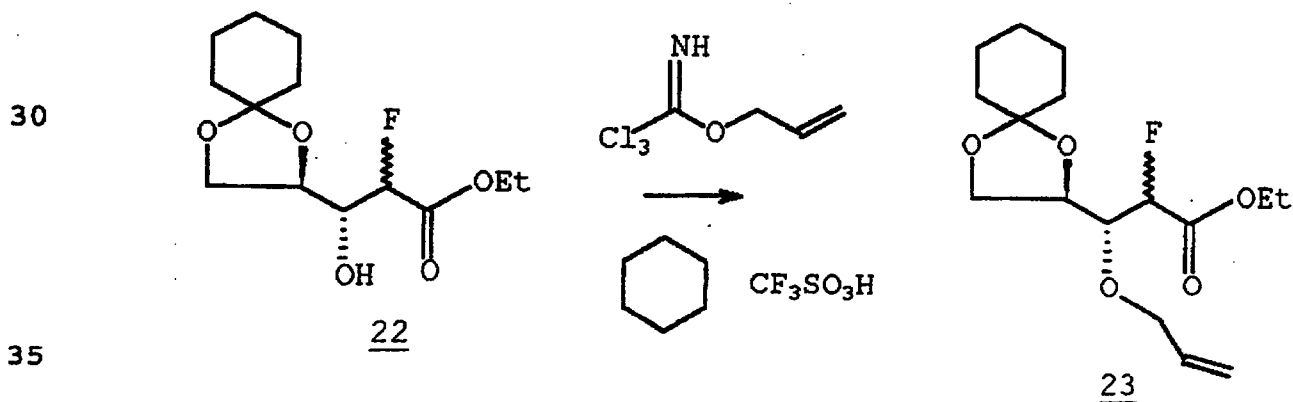
-57-

evaporation and chromatographing, 92 mg (7.5%) of 21 was obtained from the chloroform layer, which is also a mixture of two isomers. Pure isomer 22'a was obtained from the mixture 22' by chromatography on silica gel column eluted with CHCl_3 .



15

The starting material 22', 740 mg (2.1 mmol) was dissolved in 60ml CH_3OH . 1.2 g citric acid was added. The mixture was stirred at ambient temperature for 4 h. The solvent was removed on rotary evaporator. The residue was taken up with ethyl acetate (100 mL) and washed with saturated NaHCO_3 aqueous solution (100 mL x 2) and water. The ethyl acetate layer was dried over Na_2SO_4 and evaporated, yielding 22, 600 mg (100%), which is a mixture of isomer 22a (69%) and 22b (31%) based on ^1H NMR. Pure 22a was obtained by hydrolysis of 22'a under the same condition producing a 100% yield.

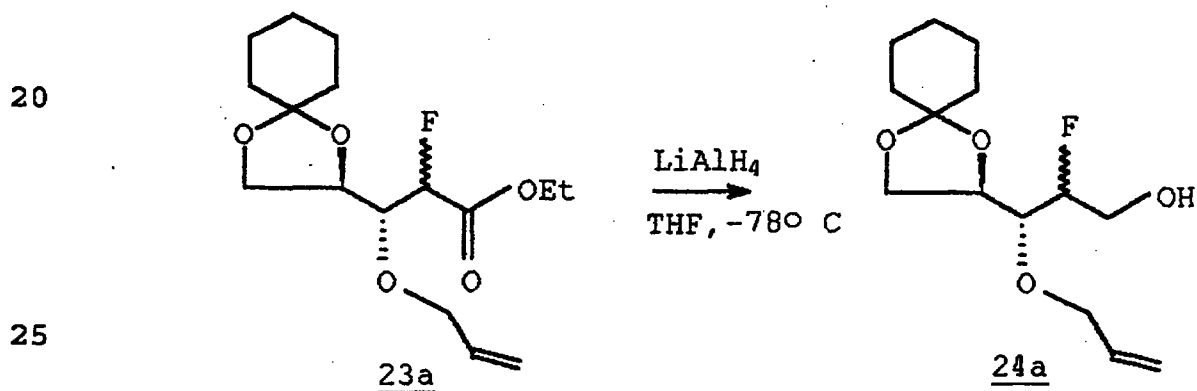


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-58-

Alcohol 22; 655 mg (2.37 mmol, a mixture of isomer 21a (69%) and 21b (31%)) was dissolved in 30 mL cyclohexane and 1.50 mL (9.8 mmol) allyl trichloroacetimidate was added. Then 100 μ L of $\text{CF}_3\text{SO}_3\text{H}$ was added dropwise over 30 min. The reaction mixture was stirred at ambient temperature under N_2 for 46 h. TLC showed that there was about 20% starting material left. Additional 60 μ L $\text{CF}_3\text{SO}_3\text{H}$ was added and the reaction mixture was stirred for another 24 h. The ppt. was filtered and washed with cyclohexane. The filtrate was evaporated and the residue was chromatographed on silica gel column using 10% ethyl acetate-hexane as eluent, yielding 23a, 511 mg (68.1%) and 23b, 160 mg (21.3%).

Pure 23a is also obtained from pure 21a using the same condition above for comparison.

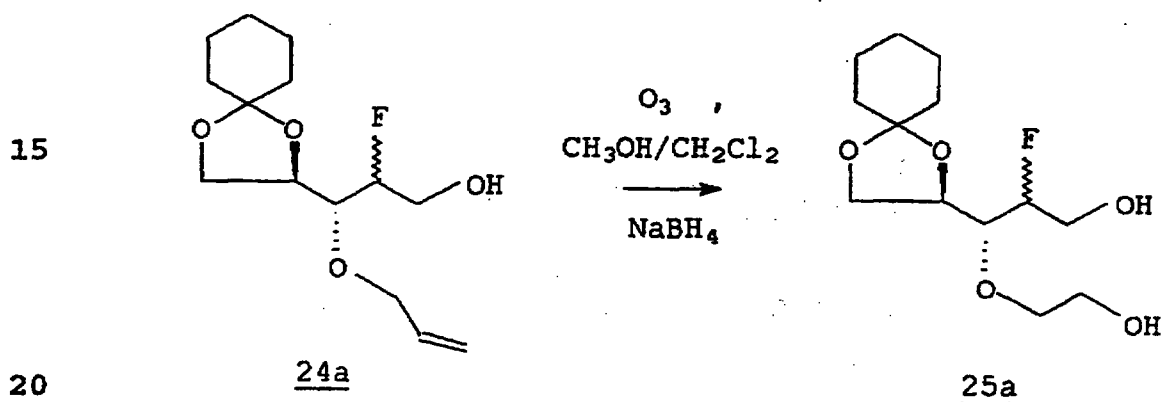


The ester 23a, 635 mg (2.00 mmol) was evaporated with toluene twice and dissolved in 10 mL anhydrous THF. It was dropwise added to a suspension of 200 mg (5.27 mmol, 2.5 eq.) LiAlH_4 in 40 mL anhydrous THF at -78°C under N_2 with stirring. After adding the sample, the reaction mixture was stirred for 20 min. It was warmed up to 0°C and stirred at 0°C for 20 min. Then 5 mL ethyl acetate was added. After stirring for 5 min., 4 g wet sodium sulfate

-59-

was added. The mixture was stirred for 30 min. The solid was filtered away and washed with ethyl acetate twice. The filtrate was evaporated on rotary evaporator. The residue was chromatographed on a silica gel column using 30% ethyl acetate/hexane as eluent. After removing some starting material 23a, 19 mg, the main product was eluted out, as 24a, 413 mg (75.0%), followed by some 24b, 30 mg (5.5%).

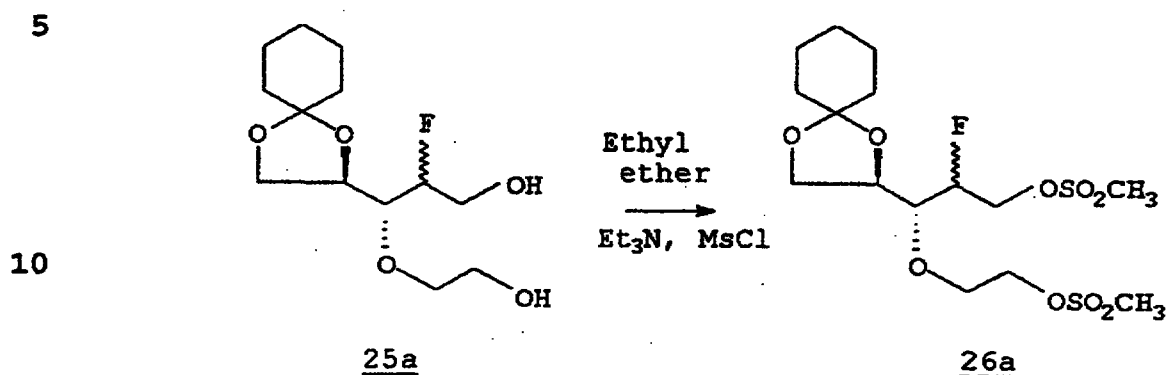
Compound 24b (44 mg) was obtained by reduction of 23b (112 mg) using DIBAL-H in THF using a similar procedure as above, with 45% yield.



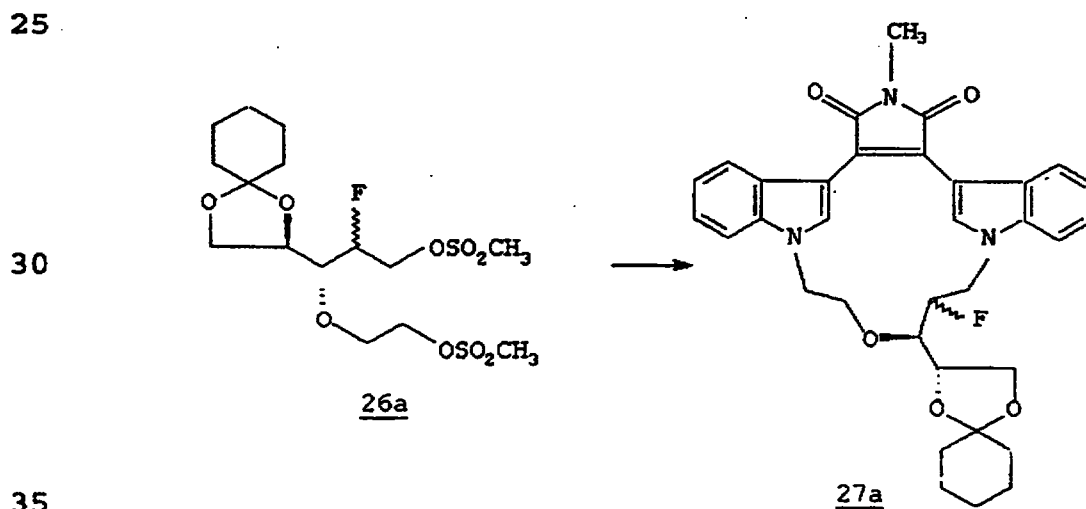
The alcohol 24a, 295 mg (1.08 mmol) was dissolved in 20 mL mixture solvent of $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ (1:1). It was cooled down to -78°C . Ozone was bubbled through until a blue color began to appear. Argon then was bubbled in to exclude excess O_3 . Several drops of CH_3SCH_3 was added and the solution was stirred for 5 min. Then 245 mg (6.48 mmol) of NaBH_4 was added at -78°C . After stirring for 5 min., the reaction mixture was allowed to return to ambient temperature and stirred for 1 h. The volatiles were removed in vacuo. The residue was chromatographed on silica gel column using ethyl acetate as eluent, yielding 25a, 232 mg (77.5%).

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The diol 25b was obtained following the same procedure above. The reaction was initiated with 230 mg of 24b, and 158 mg of 25b was obtained with a yield of 67.7%.



Diol 25a, 195 mg (0.70 mmol) was dissolved in 50 mL diethyl
15 ether. Triethyl amine 583 μ L (4.2 mmol) was added and
followed by 342 μ L (4.2 mmol) of methanesulfonyl chloride.
The mixture was stirred at ambient temperature under N₂ for
3 h. 50 mLs of water, was added to dissolve the ppt. The
ether layer was separated and washed with water (50 mL x
20 2). After drying over anhydrous Na₂SO₄, it was evaporated
at reduced pressure to yield a yellowish liquid 26a, 308 mg
(100%).

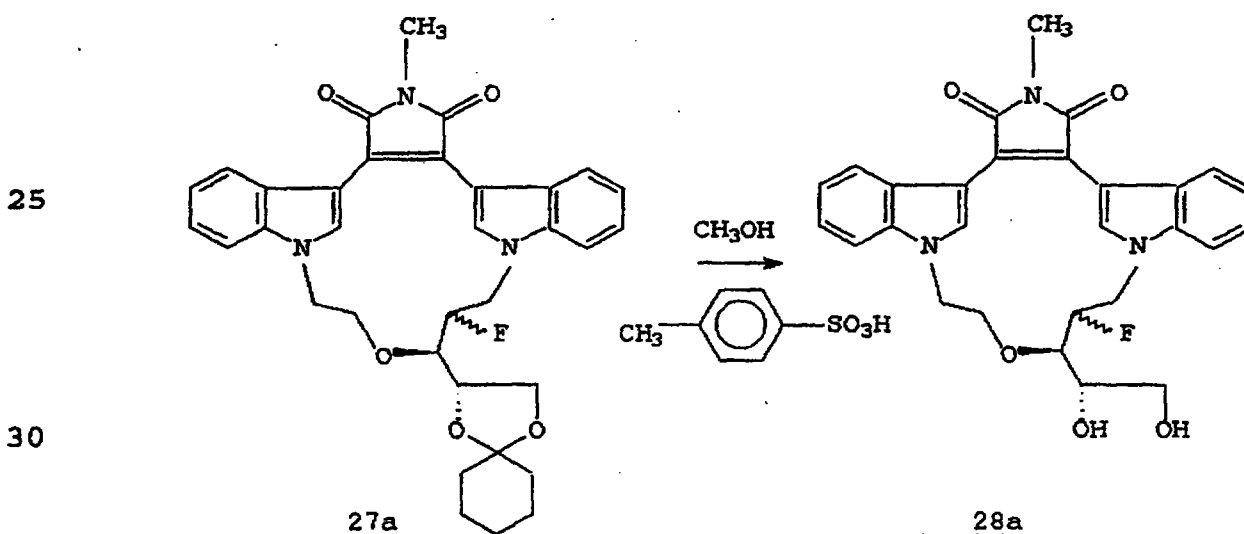


-61-

To a 100 mL round-bottomed flask containing 768 mg (2.36 mmol) cesium carbonate in 40 mL anhydrous DMF at 50°C under N₂, 10 mL of DMF solution containing 26a, 257 mg (0.59 mmol) and bisindolylmaleimide, 202 mg (0.59 mmol) was added dropwise via a syringe pump over a period of 48 h. After stirred at 50°C for additional 24 h, the reaction mixture was diluted with 100 mL CHCl₃, and washed with brine (50 mL x 2), then water (50 mL x 2). The chloroform layer was dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The residue was dissolved in CHCl₃, and chromatographed on silica gel column using 5% acetone/CHCl₃ as eluent. The first component eluted out is the desired product 27a, 185 mg. It was recrystallized from CHCl₃/CH₃OH, yielding 130 mg (37.7%) and a filtrate.

Compound 27b was obtained in the same way as above, except that the addition time of the mesylate and bisindolyl maleimide via a syringe pump was 80 h. The reaction was initiated with 97 mg of the diol 25b, and 64 mg of 27b was obtained with an overall yield of 26.1%.

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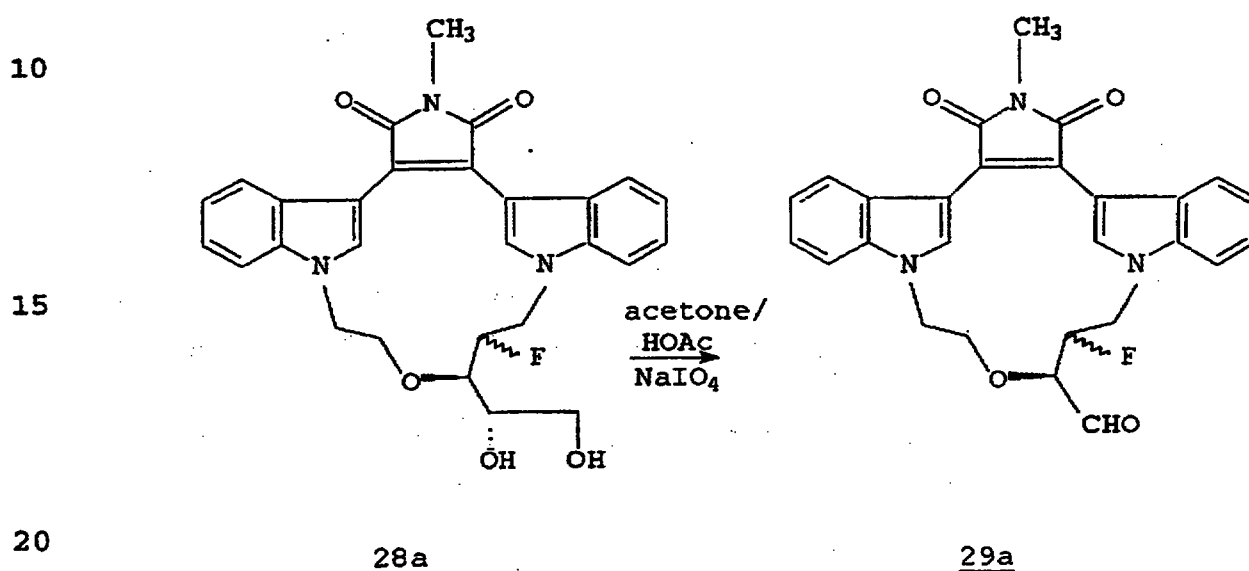


The starting material 27a, 50 mg (0.099 mmol) was dissolved in 50 mL methanol. To it 1 mL water and 200 mg

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of p-toluene-sulfonic acid monohydrate (1.05 mmol) were added. The reaction mixture was stirred at 50°C for 4 h. It was evaporated on rotary evaporator and the residue was chromatographed on silica gel column using 5% CH₃OH/CHCl₃ as
5 eluent. The main component 28a was recrystallized from acetone/CH₃OH, yielding crystalline 28a, 23 mg and a filtrate which contains 18 mg of 28a (95.0%).



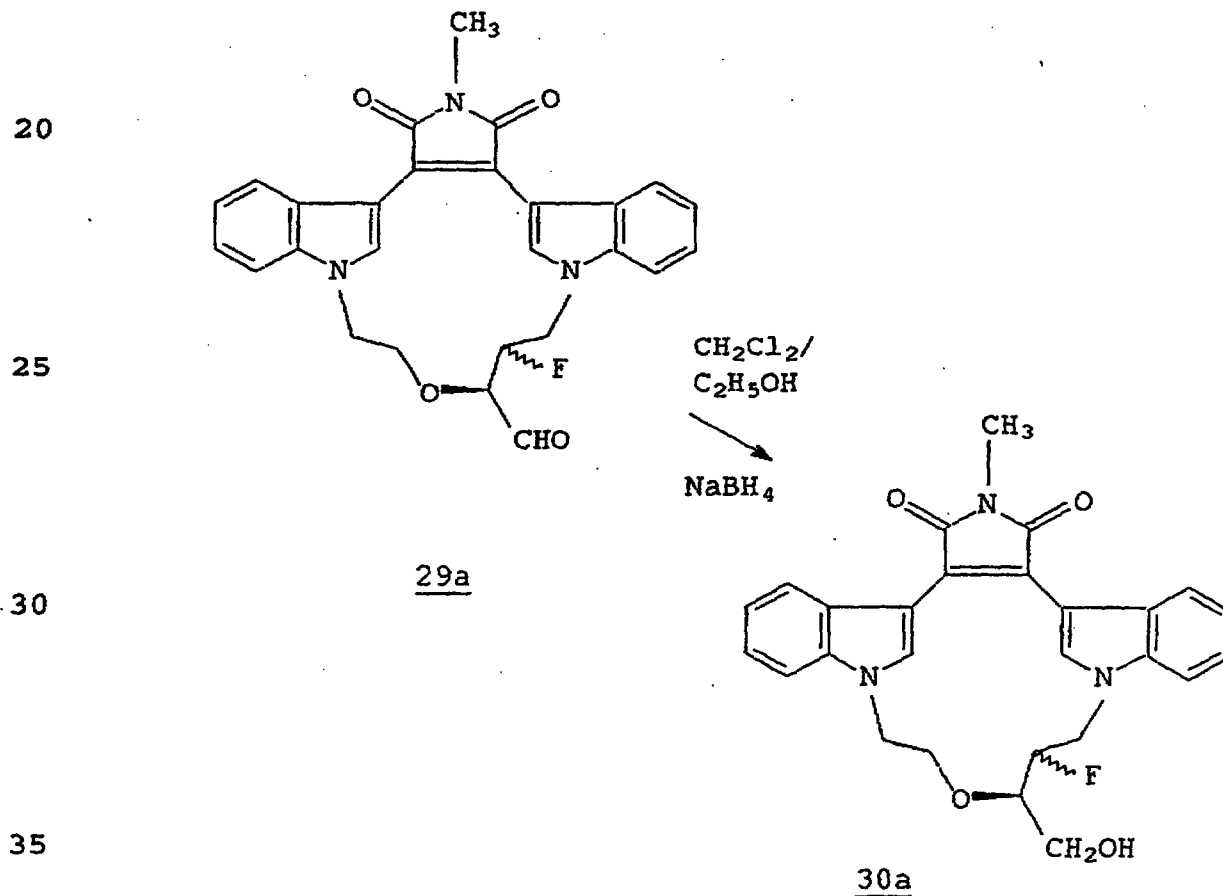
Diol 28a, 55 mg (0.109 mmol) was dissolved in 20 ml of acetone-HOAc mixture (1:1). To the solution, 100 mg of NaIO₄·3H₂O (0.373 mmol) in 2.5 ml water was added. The
25 mixture was stirred at ambient temperature for 2.5 h. It was evaporated on a rotoevaporator to reduce solution volume to half, and then diluted with 30 ml CH₂Cl₂. The mixture was then washed with water twice, aqueous NaHCO₃, and water again. The CH₂Cl₂ layer was separated, dried over
30 Na₂SO₄ and evaporated to dryness, yielding crude aldehyde 29a.

The crude aldehyde 29a was dissolved in 14 ml CH₂Cl₂ and cooled to -78°C. Sodium borohydride, 30 mg (0.79 mmol) was dissolved in 6 ml of SP reagent alcohol and added
35 to the solution. The mixture was stirred under N₂ for 40

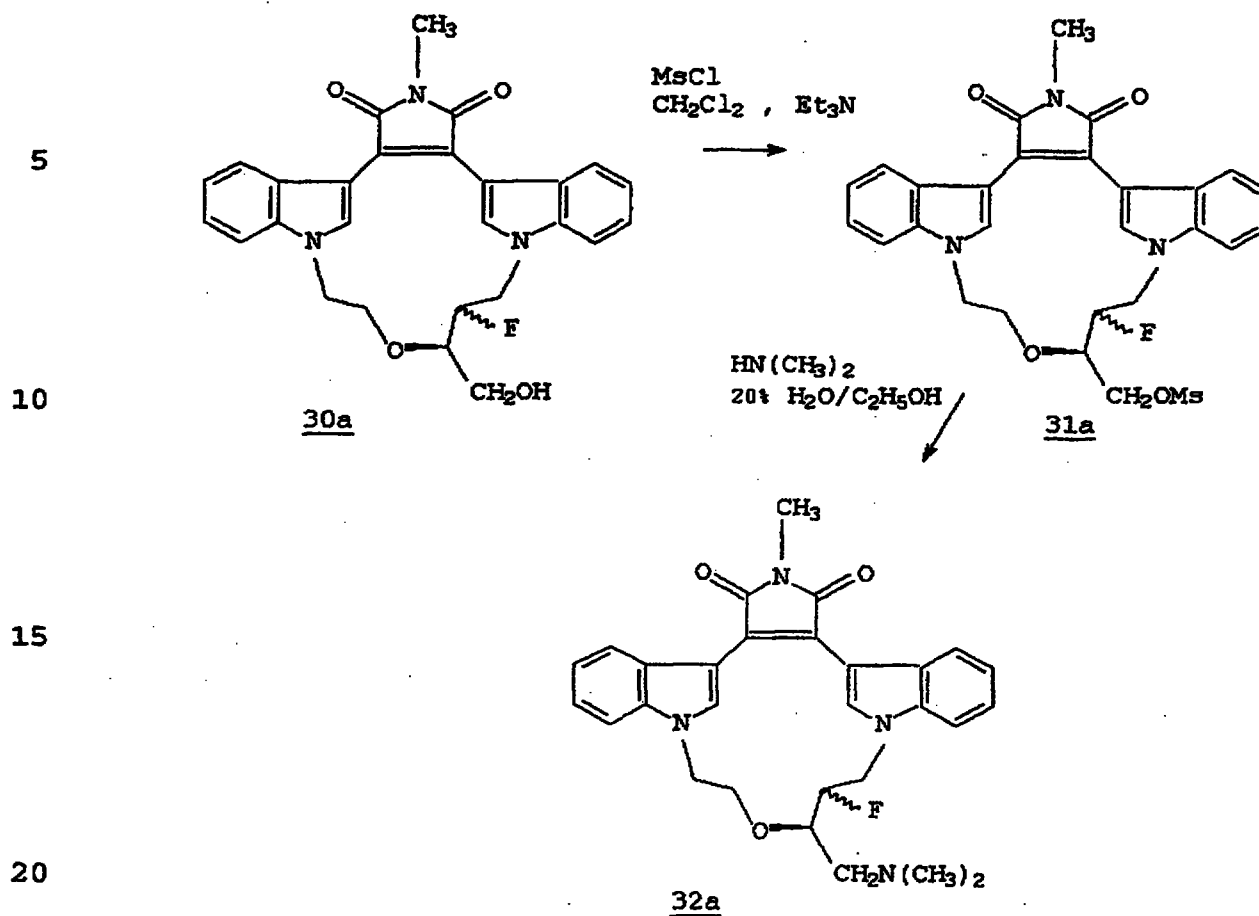
-63-

min. It was quenched with 500 μ l CH_3CHO and then allowed to return room temperature. The mixture was evaporated to reduce the solution volume to half and then mixed with 10 ml $\text{C}_2\text{H}_5\text{OH}$, 2 ml of Potassium Sodium tartrate saturated aqueous solution. The mixture was stirred for 5 h. It was evaporated and taken up with 30 ml CH_2Cl_2 . The CH_2Cl_2 layer was separated, washed with water three times and dried over Na_2SO_4 . After evaporation, the residue was chromatographed on a silica gel column using 10% ethyl acetate in CH_2Cl_2 , yielding compound 30'a, 7mg (14.1%). The main component was eluted with ethyl acetate, yielding the desired product 30a, 41 mg (79.3%).

Compound 30b was obtained from the corresponding diol 28b (with a yield of 63.4%) following the same procedure as above except that the solvent for the cleavage of the diol was a mixture of $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ (2:1).



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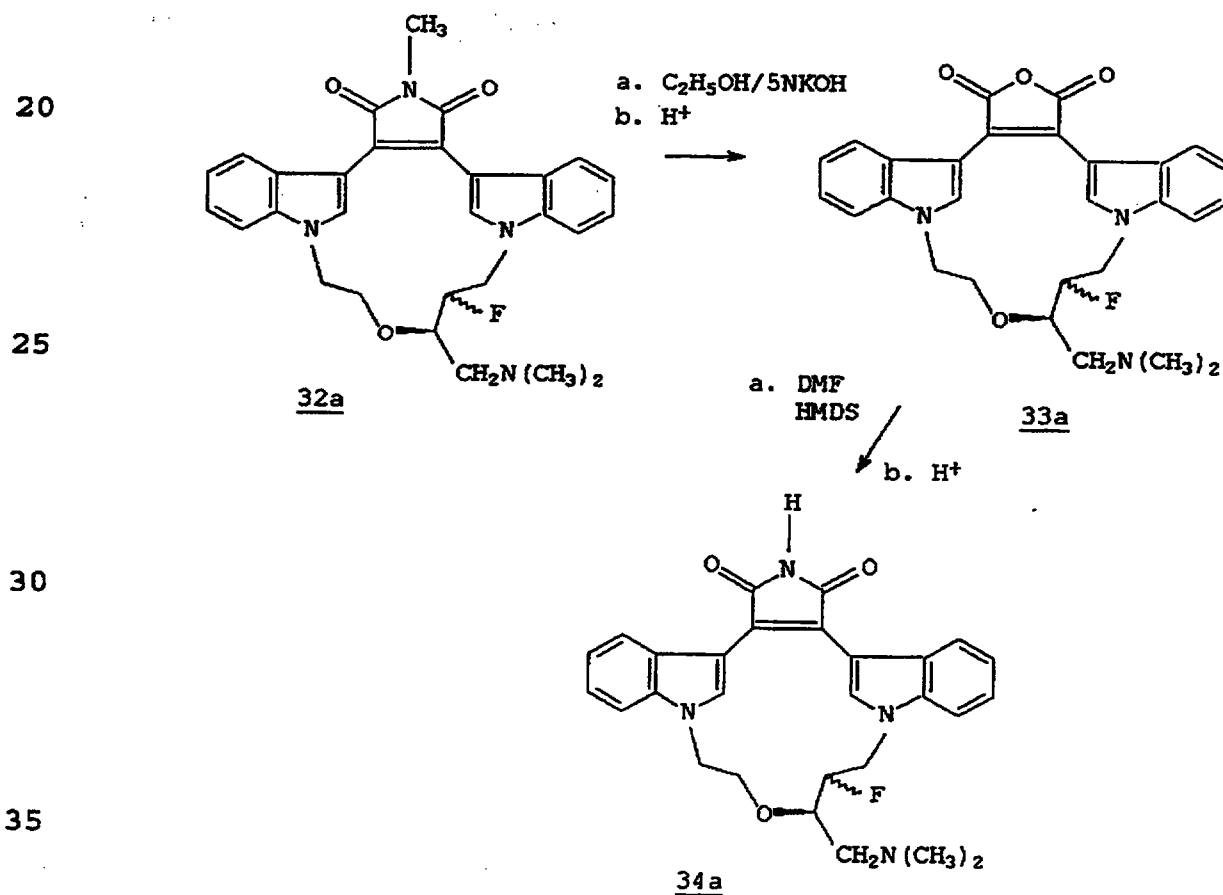


The alcohol 30a, 16 mg (0.0338 mmol) was dissolved in 10 mL CH₂Cl₂. To it 70 μ L (0.52 mmol) of triethylamine and 28 μ L (0.34 mmol) of methanesulfonyl chloride were added. The reaction mixture was stirred at ambient temperature under N₂ for 3 h. It was transferred to a separatory funnel and washed with water three times.

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After evaporation it yields crude 31a, 23 mg. TLC shows only one spot.

The crude mesylate 31a, 23 mg (~0.0338 mmol) was placed in a 25 mL-round-bottomed flask and 12 mL C_2H_5OH was added. The flask was cooled down using dry ice/acetone bath. Into it 1.2 g $HN(CH_3)_2$ and 3 mL water were transferred via cannula. The flask was then sealed with a teflon stopper and tied using copper wire. It was stirred at 100°C for 8 h. After the flask cooled to room temperature, the volatiles were removed on rotary evaporator. The residue was dissolved in 20 mL ethyl acetate and washed with aqueous $NaHCO_3$ (20 mL x 2), water (20 mL x 2). After removing solvent it yields 24 mg crude produce 32a which was recrystallized from methanol, obtaining pure 32a.



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The imide 32a, 24 mg (0.048 mmol) was dissolved in a mixture of 3 mL C_2H_5OH and 3 mL 5N KOH. It was stirred at 80°C for 24 h. The ethanol was removed on rotary evaporator and the aqueous suspension was cooled to 0°C and acidified with 5 N HCl. A violet precipitate appears. After stirring for 10 min., the aqueous mixture was neutralized with dilute KOH and extracted with ethyl acetate. The ethyl acetate layer was washed with aqueous $NaHCO_3$ twice and water. After drying over K_2CO_3 and evaporation, it yields 24 mg crude 33a.

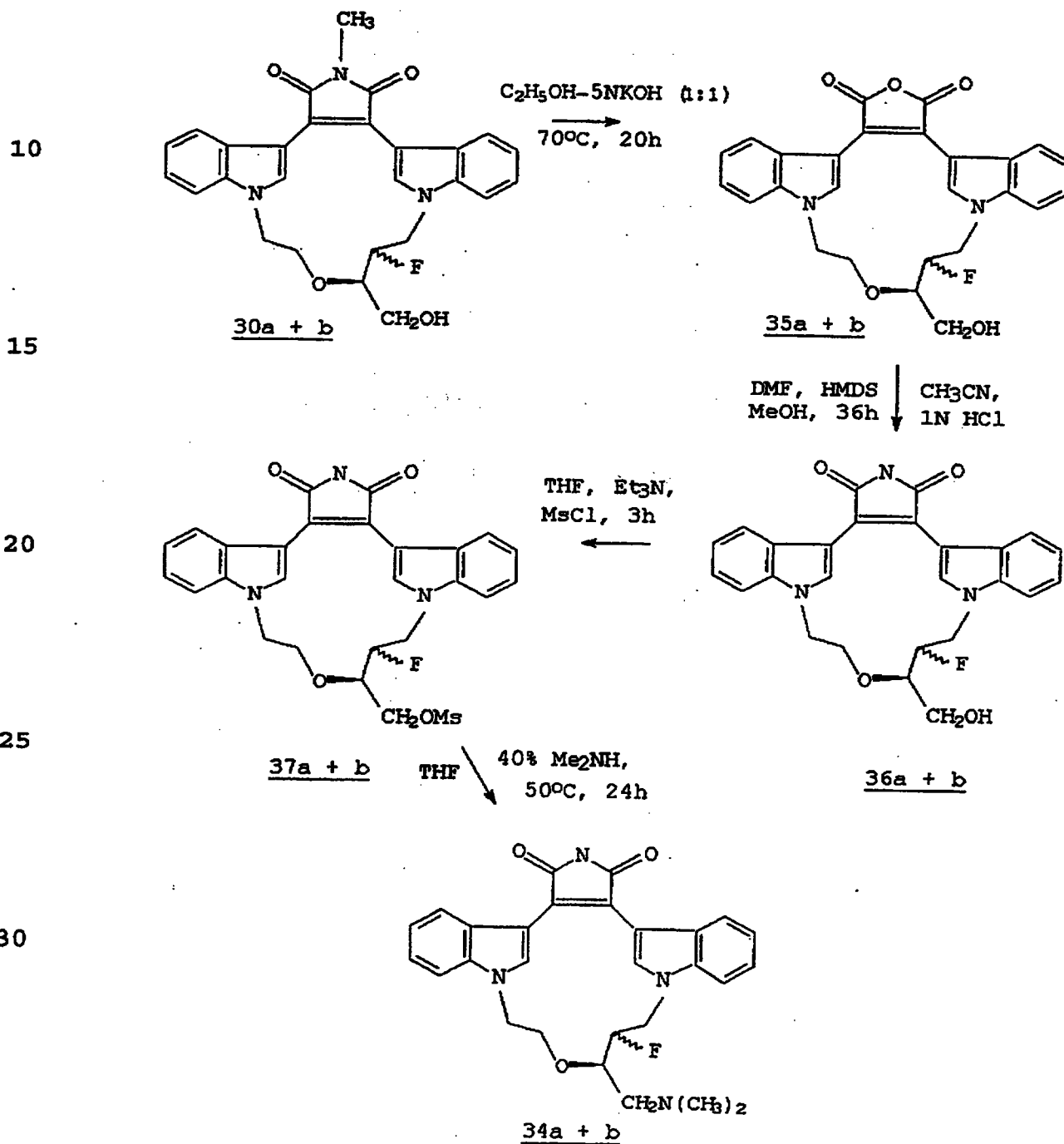
The anhydride 33a, 24 mg was dissolved in 5 mL anhydrous DMF. To it 250 μ L (1.19 mmol 1,1,1,3,3,3 - hexamethyl disilazane was added, followed by adding 25 μ L CH_3OH (0.62 mmol). The resulting mixture was stirred at ambient temperature under N_2 for 38 h. The volatiles was removed in vacuo. The residue was dissolved in 6 mL mixture solution of CH_3CN - 1 N HCl (2:1) and stirred at ambient temperature for 1 h. The organic solvent was removed and the aqueous suspension was neutralized with 1 N KOH and extracted with ethyl acetate. The ethyl acetate layer was washed with 0.1 N KOH and water twice and evaporated to dryness. The residue was separated on silica gel column eluted with ethyl acetate. The second band eluted out is the desired product 14a which was recrystallized from CH_2Cl_2 - hexane, yielding 5 mg (overall yield from 30a, 20%).

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Example 15

Alternative Scheme for Producing Compounds 34(a and b)
from Compounds 30 (a and b).

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Alcohol 30a, 50mg (0.106 mmol) was mixed with 20 ml of C_2H_5OH - 5N KOH (1:1). It was stirred at 70°C under N_2 for 20 h. It then was cooled to 0°C and acidified with 5N HCl. Red ppt immediately appeared. Methylene chloride, 40 ml, was added. The organic layer was separated and washed with water (30 ml X 4), and dried over Na_2SO_4 . After evaporation, the residue was chromatographed on a silica gel column using 5% ethyl acetate in CH_2Cl_2 , yielding 35a, 33 mg (68%).

The anhydride 35a, 54mg (0.117 mmol) was dissolved in 5 ml anhydrous DMF. HMDS (1, 1, 1, 3, 3, 3-hexamethyl disilazane). 500 μ l (2.36 mmol) and methanol, 48 μ l (2.36 mmol) were added. The mixture was stirred under N_2 at ambient temperature for 36 h. The volatiles were removed in vacuo and the residue was stirred with 10 ml CH_3CN and 5 ml 1N HCl for 1 h. It was then concentrated and extracted with CH_2Cl_2 . The methylene chloride layer was washed with water, brine, dried over Na_2SO_4 , and evaporated. The residue was separated on a silica gel column using CH_2Cl_2 - CH_3CN (9:1), yielding the first component which was the starting material (20 mg, 37.0%), then the desired product at 36a, 31 mg (57.4%).

Compound 36b, 3.4 mg (39%) was obtained from corresponding alcohol 30b, 9 mg (0.019 mmol) following the same procedure as above except that far more excess of HMDS (250 μ l, 1.18 mmol) and methanol (24 μ l, 1.18 mmol) was used than that for 36a.

Amination:

Compound 36a, 31 mg (0.068 mmol) was dissolved in 15 ml anhydrous THF. To it 240 μ l (1.56 mmol) of triethylamine and 84 μ l (1.02 mmol) of methane sulfonyl chloride were added under nitrogen atmosphere. The mixture was stirred at ambient temperature for 3 h. The volatiles were removed in vacuo. The residue was dissolved in 30 ml CH_2Cl_2 and washed with 1N HCl, brine twice, dried over Na_2SO_4 ,

-69-

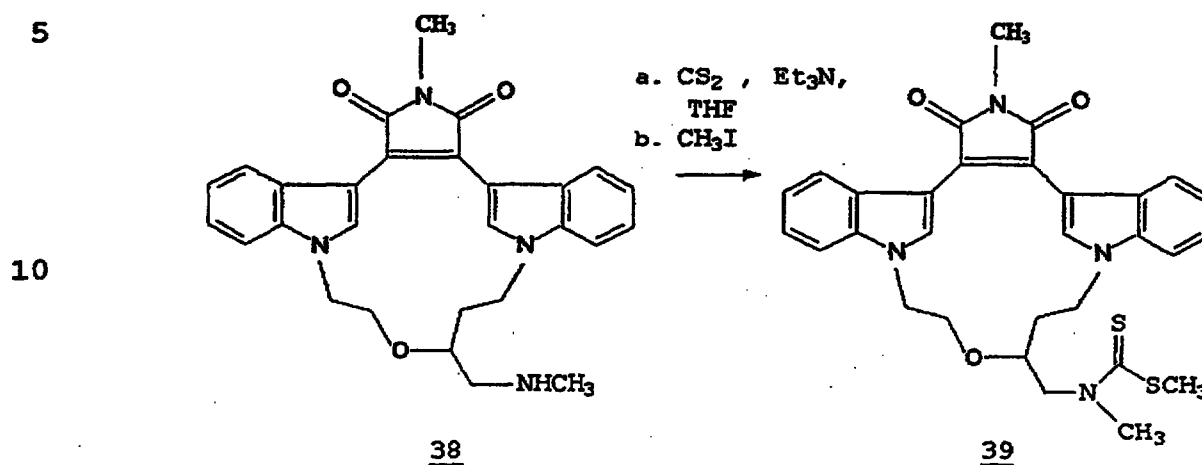
and evaporated. The residue was dissolved in 6 ml of distilled THF and 1 ml of 40% dimethylamine in water. The flask was sealed with a Teflon stopper and stirred at 50° for 24 h. The mixture was cooled to 0°C and evaporated to
5 remove the volatiles. The residue was purified on silica gel column using 0-10% Et₃N in ethyl acetate, yielding the desired compound 34a, 13.2 mg (40.2%).

Compound 34b (1.9 mg) was obtained from 36b (3.4 mg) using the same procedure as for 34a with a yield of
10 52%.

- 70 -

Example 16

Synthesis of a Dithiocarbamate Derivative:

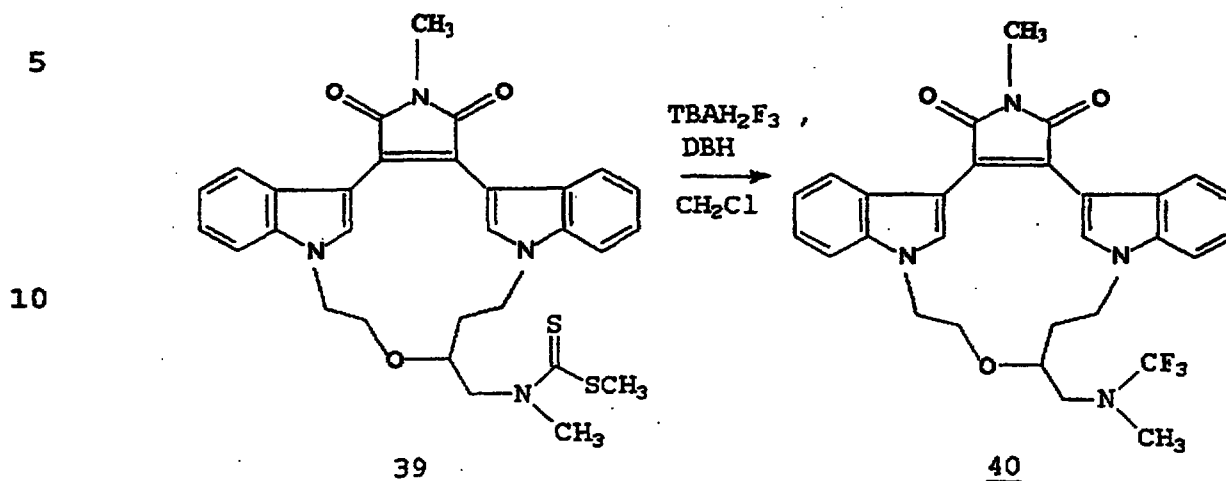


20 mg of Compound 38 (0.044mmol, 1eq.) was transferred to an oven dried 25 mL round bottom flask equipped with stir bar, septum, and N_2 balloon. 10 μl of THF was added via canula followed by 4.45mg of triethylamine (0.044mmol, 1.1eq.) and then 3.8 mg carbon disulfide (0.05 mmol, 1.2eq.) via syringe. This red solution was allowed to stir for ~ 15 min. and then 7.1mg of methyl iodide (0.05 mmol, 1.2eq.) was added via syringe. The reaction was allowed to stir at room temp overnight.

The clear red solution turned cloudy after approximately two hours. TLC (10% MeOH in CH_2Cl_2) showed total loss of starting material. The reaction was transferred to a separatory funnel with EtOAc and washed with 40 mL of H_2O followed by 40 mL of brine. The organic layer was collected and passed through MgSO_4 in a sintered glass funnel to dry it. The solvent was removed to give a purple solid (compound 39). A sample was analyzed by IS/MS. The IS/MS detected a MH^+ peak at 559, total yield was 23mg.

-71-

Conversion of the Dithiocarbamate to Trifluoromethyl Group



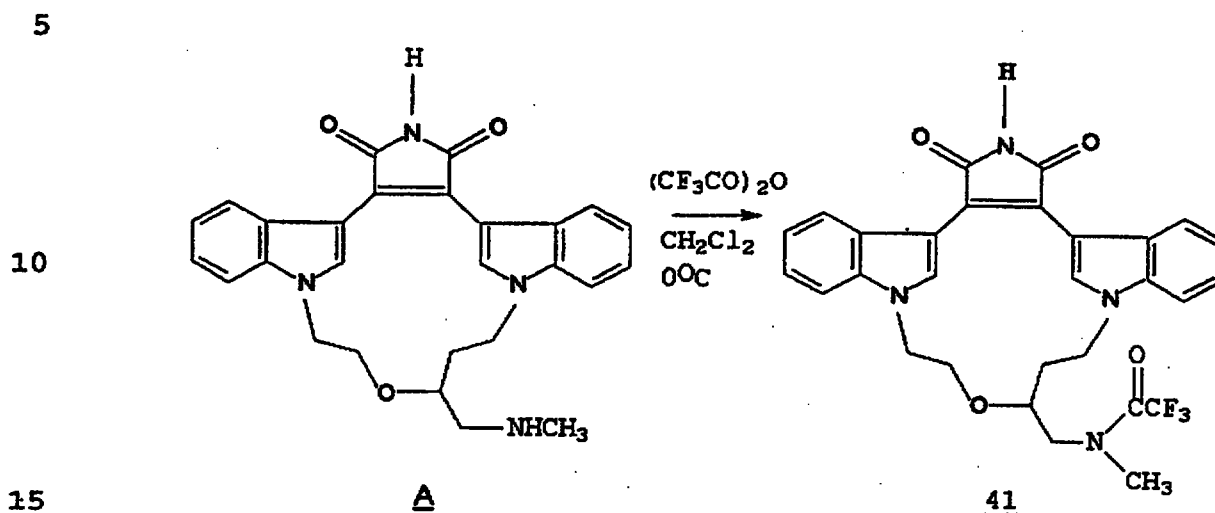
The 23mg (0.041 mmol, 1eq.) of dithiocarbamate 39 was dissolved in anhydrous CH_2Cl_2 in a dry 25 ml round bottom flask equipped with stir bar, septum, and N_2 balloon. The solution was cooled in an ice bath for about 15 min. 0.047g 1,3-dibromo 5,5 - dimethylhydantoin (0.164 mmol, 4eq.) was then added quickly as a solid followed by 0.06g of tetrabutylammonium dihydrogentrifluoride (0.205 mmol, 5 eq.) via syringe. (The solution went from reddish/purple to an orange/brown color).

The reaction was stirred at 0°C for 1.5 hrs. It was then dumped into a separatory funnel filled with 30 mL H_2O . The CH_2Cl_2 layer was washed again with 25 ml H_2O , collected, and derived over MgSO_4 . The solvent was removed to give a dark brown/orange oil. The product was purified through the use of a silica gel, starting with CH_2Cl_2 as the mobile phase and gradually adding methanol. Three separate spots were obtained, the third sample was collected, and the solvent was removed to yield 16.5 mg (75%) of product 40.

- 72 -

Example 17

Acylation of the amine of Compound A



7 mg (0.0154 mmol) of Compound A was dissolved in 1 mL dry CH₂Cl₂. It was cooled to 0°C under N₂, and stirred while 3.7 μL (0.046mmol, 3eq) of pyridine was added, followed by 2.06 μL (0.018 mmol, 1.2eq.) of trifluoroacetic anhydride (Aldrich). The reaction mixture was stirred at 0°C from 2:17 p.m. to 4:20 p.m. (TLC 1) and continued until 7:00 p.m. (TCL 2). No more progress of the reaction. Another batch of 3.7 μL pyridine and 2.6 μL trifluoroacetic anhydride were added. After stirring for 4 h, TLC shows no significant progress of the reaction. The reaction mixture was allowed to warm up to room temperature and stirred for 3h. Again, TLC shows no significant progress of the reaction. A third batch of 3.7 μL pyridine and 2.0mL of trifluoro acetic anhydride was added the mixture was stirred for 2h, TLC shows the conversion is improved.

The reaction was stopped and the volatiles were evaporated under reduced pressure. The residue was dissolved in CHCl₃ and washed with saturated NaHCO₃ aq. solution and water, twice, and the CHCl₃ layer was

-73-

evaporated. The residue was chromatographed on a silica gel column eluted with 10% acetone-CHCl₃ (v/v) obtaining two pure components, 208.1 and 208.2. The starting material remaining in the column was eluted with 10% CH₃OH-CHCl₃,
5 containing 2% Et₃N, labeled as 208.3.

208.1 ~ 1mgRf= 0.67

208.2 ~ 3mgRf=0.45

208.3 ~ 4mgRf=0

10

¹HNMR in CDCl₃

208.2 file: GZW.013 (Dept. 3): -N-CH₃, δ 3.10 ppm.

208.3 file: GZW.014 (Dept.3): -N-CH₃, δ 2.40 ppm.

15 In CDCl₃, the methyl-group signal shows a significant low field shift from δ 2.40 ppm to δ 3.10 ppm. Therefore the desired product is the product 2 (compound 41).

The experiment was repeated as described below:

20

A collection of recovered material 208.1, ~ 5 mg. (0.011 mmol) was co-evaporated with toluene twice and dissolved in 2 mL dry CH₂Cl₂ (over molecular sieve). 40 μL pyridine (0.497 mmol, 45 eq.) was added at 0°C, followed by 10 μL
25 (0.071 mmol, 6.5 eq.) of (CF₃CO)₂O. The mixture was stirred under N₂ for 2 hr TLC 1 showed reaction was complete. The volatiles were evaporated in vacuo and the residue was passed through a tiny silicon gel column using 10% acetone -CHCl₃ as eluent, yielding 5 mg product labeled
30 as 209-2.

-74-

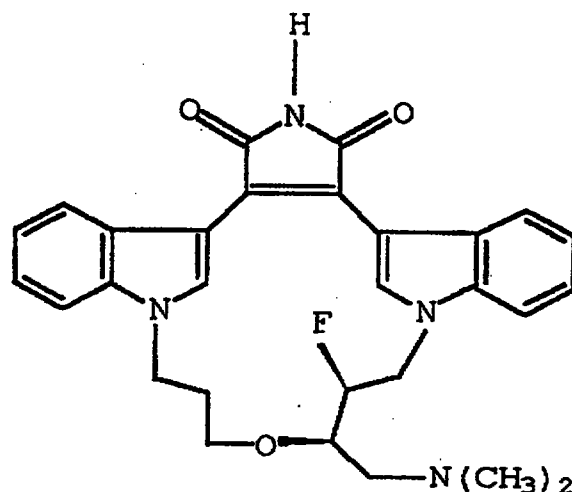
Example 18

Preparation of the bisindolyl maleimide of the formula:

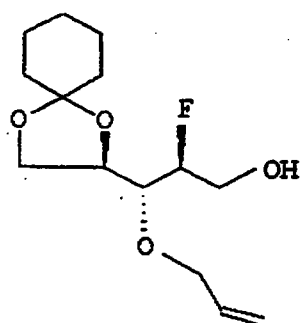
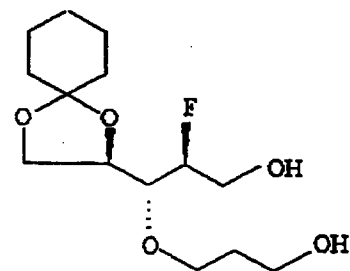
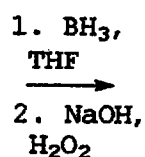
5

10

15



20

24a42a

25

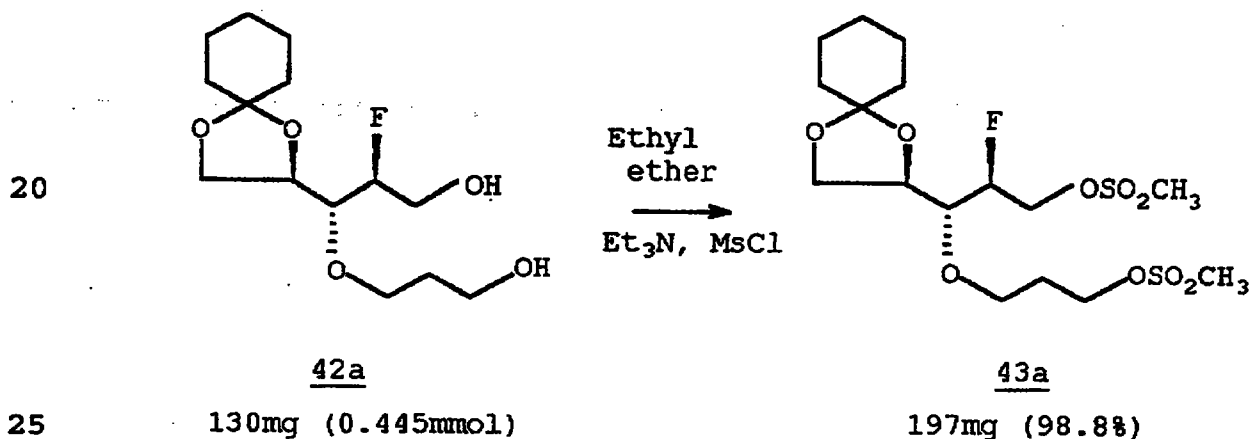
Compound 24a, 140 mg (0.511 mmol) was evaporated with toluene twice and dissolved in 5.0 ml of freshly distilled anhydrous THF. It was cooled to 0°C (ice bath) and stirred under N_2 . To it 5.0 ml (5.0 mmol) of 1.0 M solution of BH_3 .THF in THF was added via a syringe. The resulting mixture was allowed to warm up slowly to room temperature and stirred under N_2 for 15 h. It was cooled with an ice-bath and then 10 ml of 10% NaOH was added, followed by addition of 10 ml of 50% H_2O_2 . The resulting

-75-

white cloudy mixture was stirred at room temperature for 5 h. It was evaporated on a rotoevaporator to remove THF and the residue was diluted with 50 ml water. It was extracted with ethyl acetate (40 ml. x 3). The ethyl acetate layer was washed with brine (50 ml) and dried over Na_2SO_4 . After evaporation the residue was separated on a silica gel column (1 cm X 12.5 cm, flash chromatography) using toluene (20 ml), 30% - 100% ethyl acetate in hexane (145 ml). The fourth component was identified to be the desired produce 42a, 69 mg (46%).

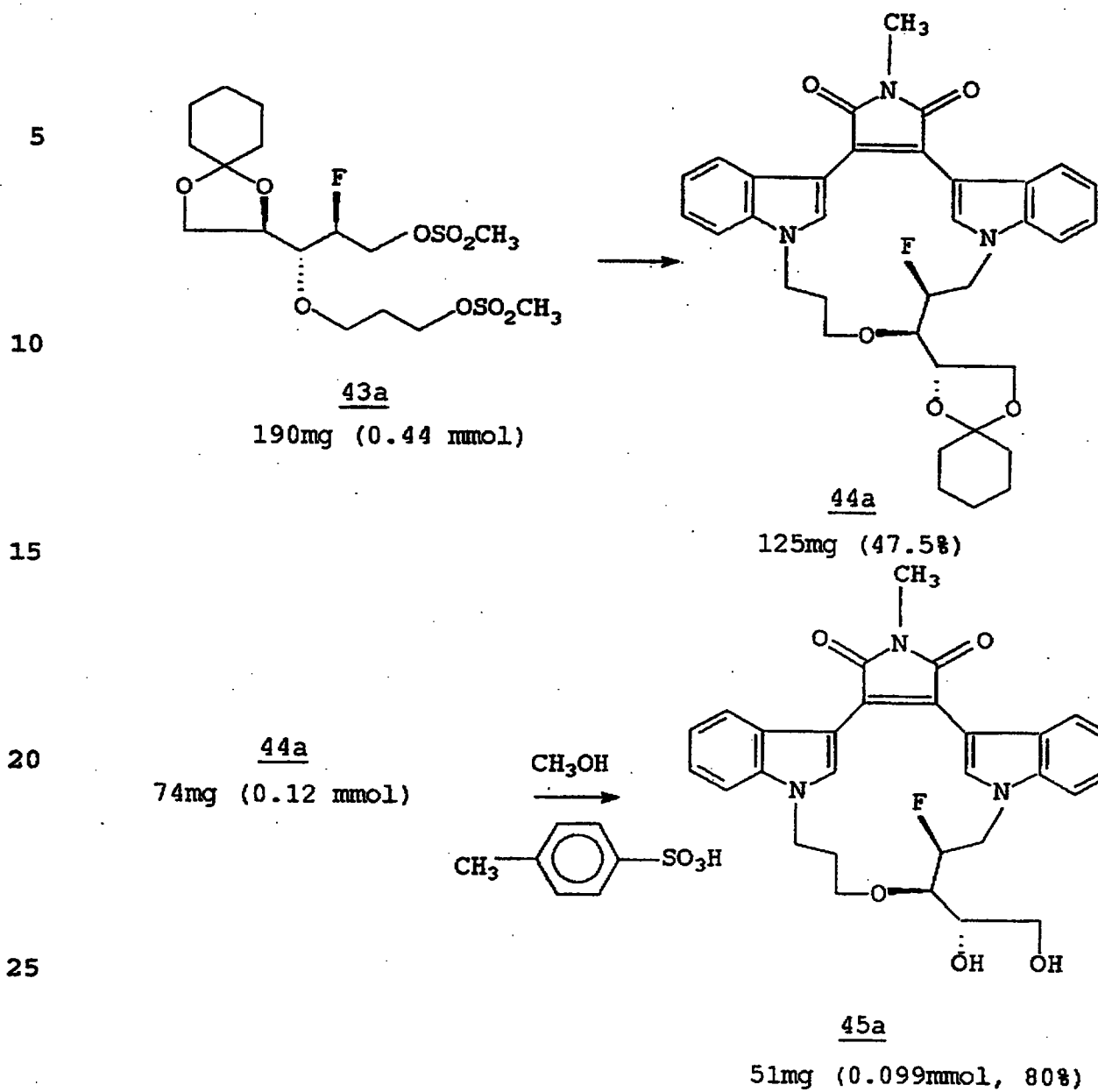
Compound 49 was synthesized using the following steps and the same general procedure as discussed in detail in Example 14.

15



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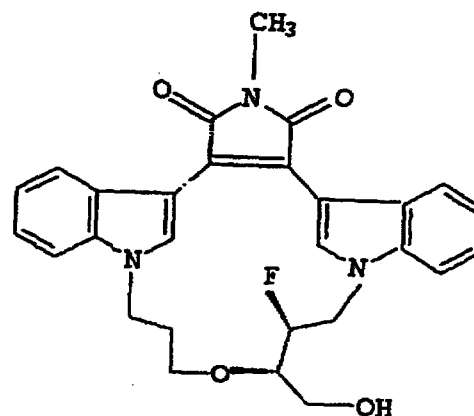
5

10

45a
51mg
(0.099 mmol)

acetone/
HOAc
NaIO₄

CH₂Cl₂/
C₂H₅OH
NaBH₄

46a

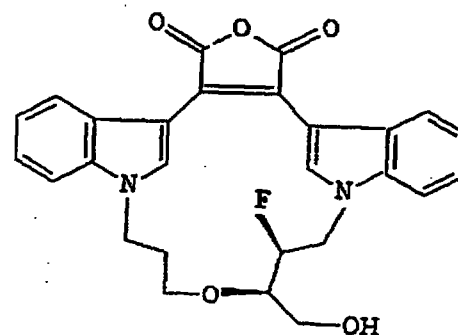
46mg (0.094 mmol, 95%)

15

a. C₂H₅OH/5NKOH
b. H⁺

46a
46mg (0.094 mmol)

20

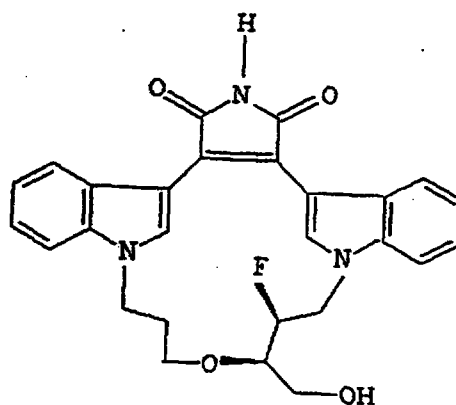
47a

36mg (0.076 mmol, 81%)

25

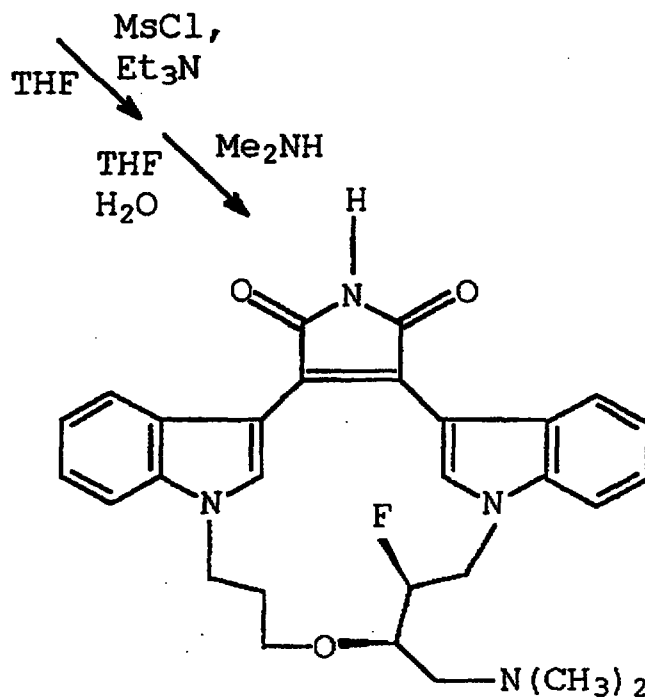
a. DMF
HMDS
CH₃OH
b. H⁺

30

48a

20mg (0.043 mmol, 56%)

-78-

48a49a**Example 19****Protein Kinase C Inhibition In Vitro Assay****Reaction Mixture:**

- 10 μ l Ca²⁺⁺ (9.4mM stock)
- 55 μ l lipids (PS 5 μ g/well, DG 0.6 μ g/well) or HEPES
- 5 μ l Compound or DMSO (Test compounds initial concentration at 5000nM)
- 10 μ l Myelin Basal Protein (MBP) Substrate (MBP 3 mg/ml, lot# 451-026)
- 10 μ l ATP (300 μ M ATP, 0.25 μ Ci/well AT³²P, and 10 mM MgCl₂)
- 10 μ l Enzyme (PKC α 1:80 in HEPES, β_{II} 1:30, in HEPES)

HEPES buffer stock is 100 mM, pH 7.5.

The total Reaction Mixture equaled 100 μ l and was incubated for 10 minutes at 30°C. The reaction was stopped with the addition of 100 μ l 25% TCA. 25 μ l of a 1 mg/ml BSA solution was added and 200 μ l of the reaction mixture

-79-

was transferred to a 96-well glass fiber filtration plate (Millipore Cat.# MAFCNOB50). The supernatant was filtered and washed three times with 10% TCA. The bottom of the filtration plate and the installed carrier was removed.

- 5 100 μ l of Microscint-20 (Packard Cat.# 6013621) was added and the samples were loaded onto a Packard topcounter.

Lipid Preparation

- 10 Lipids (Avanti Polar Lipids) were added to a borosilicate glass culture tube (25 x 150mm). The lipids were dried under nitrogen until the chloroform was evaporated. The lipids were resuspended in HEPES buffer, and sonicated for approximately 30 seconds, then vortexed to mix well. Lipids were kept on wet ice until the
15 addition to the assay.

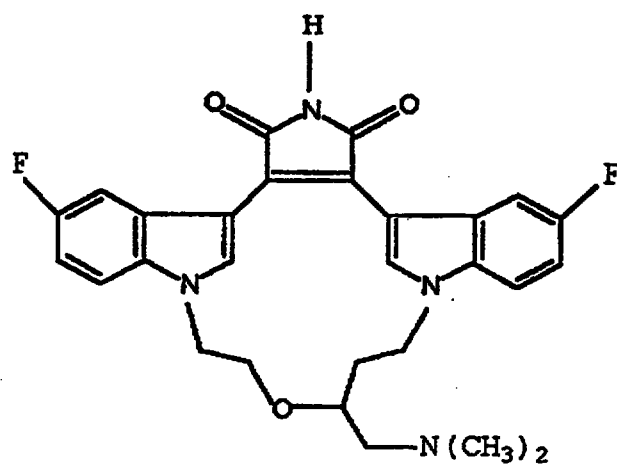
Results

- The IC_{50} values were determined for each of the following compounds, using the above *in vitro* assay. Five
20 concentrations of each compound were tested against PKC alpha (PKC α) and PKC beta II (PKC β_{II}). The concentrations of the tested compounds were 5000 nM, 500 nM, 50 nM, 5 nM, 1nM and zero (no compound, DMSO only). The sample without any added compound was used to determine 100% activity of
25 the PKC enzyme in the assay. The IC_{50} values were estimated from the inhibition curves employing these concentrations.

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5

10

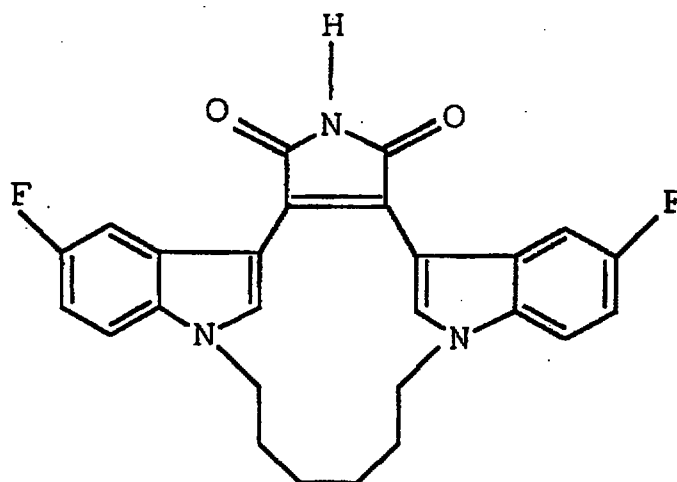
IC₅₀ Values in nanoMolar (nM)

	PKC α	PKC β_{II}
15	57	5.0

20

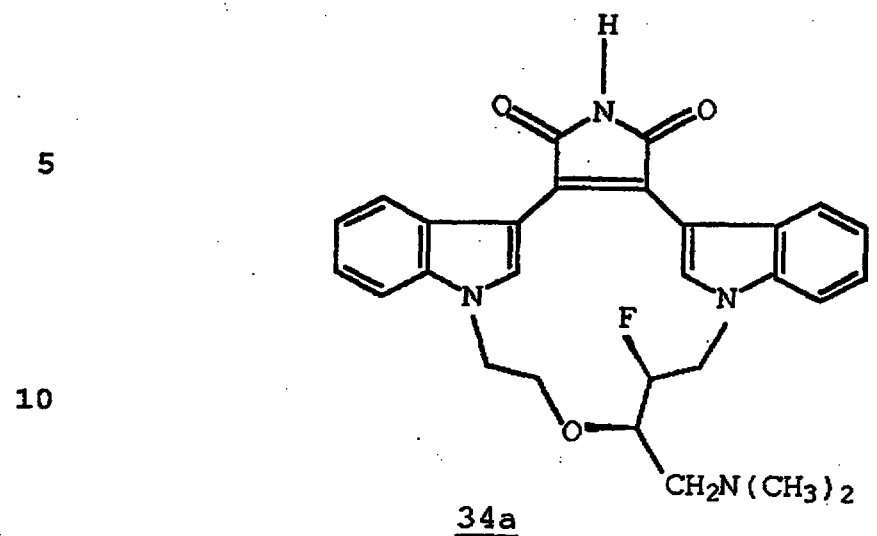
25

30

IC₅₀ Values in nanoMolar (nM)

	PKC α	PKC β_{II}
35	79	5.5

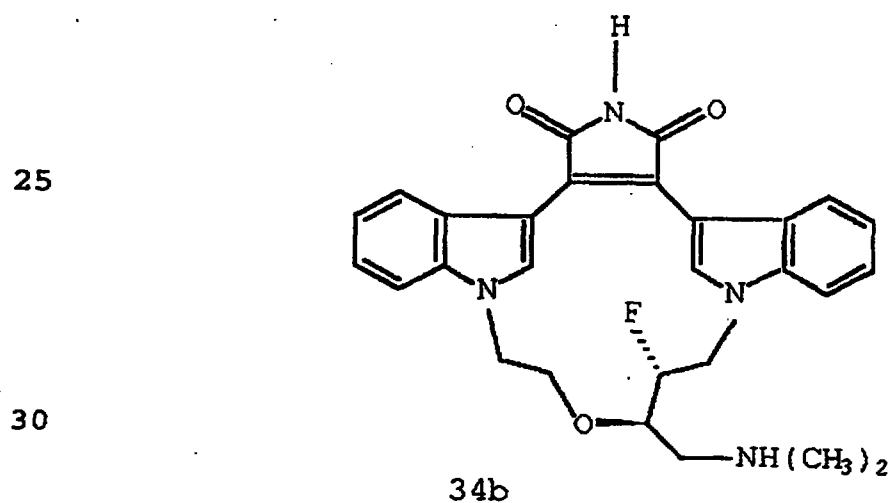
- 81 -

IC₅₀ Values in nanoMolar (nM)

15

PKC α	PKC β_{II}
140	3.5

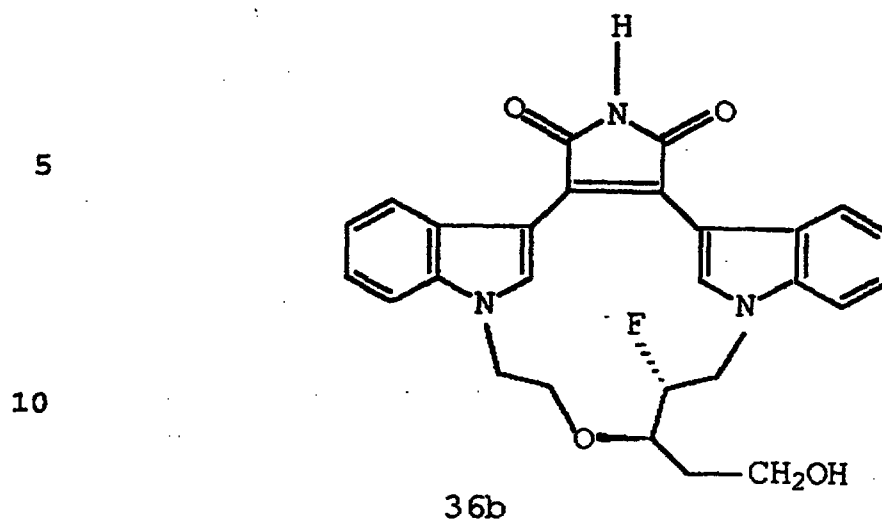
20

IC₅₀ Values in nanoMolar (nM)

35

PKC α	PKC β_{II}
26	2.4

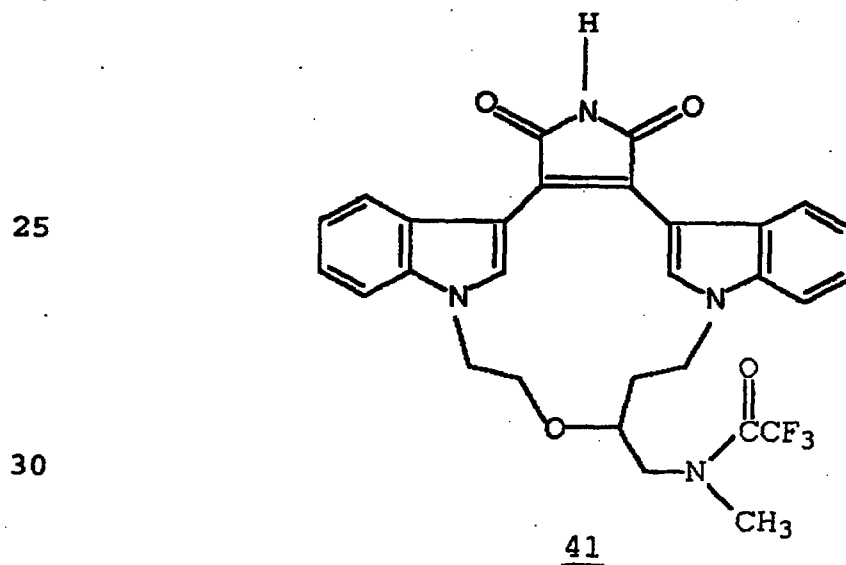
- 82 -

IC₅₀ Values in nanoMolar (nM)

15

	PKC α	PKC β_{II}
	2.6	2.2

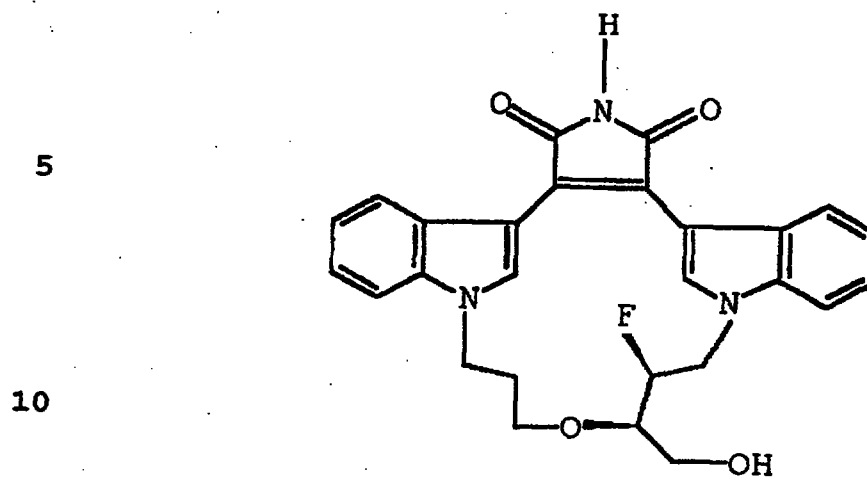
20

IC₅₀ Values in nanoMolar (nM)

35

	PKC α	PKC β_{II}
	2700	100

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48aIC₅₀ Values in nanoMolar (nM)

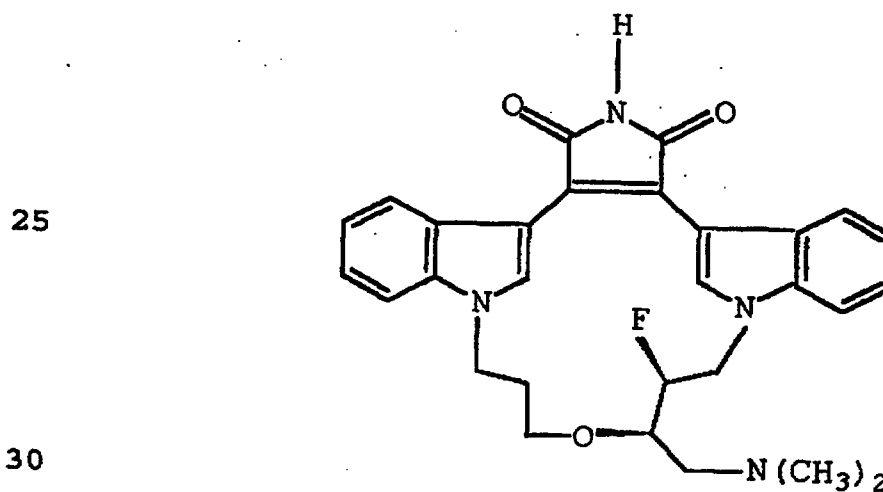
15

PKC α PKC β_{II}

130

13

20

49aIC₅₀ Values in nanoMolar (nM)

35

PKC α PKC β_{II}

1300

90

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As an inhibitor of protein kinase C, the compounds disclosed herein are useful in the treatment of conditions in which protein kinase C has demonstrated a role in pathology. Conditions recognized in the art include: diabetes mellitus and its complications, ischemia, inflammation, central nervous system disorders, cardiovascular disease, Alzheimer's disease, dermatological disease and cancer.

Protein kinase C inhibitors have been shown to block inflammatory responses such as neutrophil oxidative burst, CD3 down-regulation in T-lymphocytes, and phorbol-induced paw edema. Twoemy, B. et al. Biochem. Biophys. Res. Commun. 171: 1087-1092 (1990); Mulqueen, M.J. et al. Agents Actions 37: 85-89 (1992). Accordingly, as inhibitors of PKC, the present compounds are useful in treating inflammation.

Protein kinase C activity plays a central role in the functioning of the central nervous system. Huang, K.P. Trends Neurosci. 12: 425-432 (1989). In addition, protein kinase C inhibitors have been shown to prevent the damage seen in focal and central ischemic brain injury and brain edema. Hara, H. et al. J. Cereb. Blood Flow Metab. 10: 646-653 (1990); Shibata, S. et al. Brain Res. 594: 290-294 (1992). Recently, protein kinase C has been determined to be implicated in Alzheimer's disease. Shimohama, S. et al., Neurology 43: 1407-1413 (1993). Accordingly, the compounds of the present invention are useful in treating Alzheimer's disease and ischemic brain injury.

Protein kinase C activity has long been associated with cell growth, tumor promotion and cancer. Rotenberg, S.A. and Weinstein, I.B. Biochem. Mol. Aspects Sel. Cancer 1: 25-73 (1991). Ahmad et al., Molecular Pharmacology: 43 858-862 (1993). It is known that protein kinase C inhibitors are effective in preventing tumor growth in animals. Meyer, T. et al. Int. J. Cancer 43:

-85-

851-856 (1989); Akinagaka, S. et al. Cancer Res. 51: 4888-4892 (1991). The compounds of the present invention also act as multidrug reversal (MDR) agents making them effective compounds when administered in conjunction with other chemotherapeutic agents.

Protein kinase C activity also plays an important role in cardiovascular disease. Increased protein kinase C activity in the vasculature has been shown to cause increased vasoconstriction and hypertension. A known protein kinase C inhibitor prevented this increase. Bilder, G.E. et al. J. Pharmacol. Exp. Ther. 252: 526-530 (1990). Because protein kinase C inhibitors demonstrate inhibition of the neutrophil oxidative burst, protein kinase C inhibitors are also useful in treating cardiovascular ischemia and improving cardiac function following ischemia. Muid, R.E. et al. FEBS Lett. 293: 169-172 (1990); Sonoki, H. et al. Kokyu-To Junkan 37: 669-674 (1989). The role of protein kinase C in platelet function has also been investigated and as shown elevated protein kinase C levels being correlated with increased response to agonists. Bastyr III, E.J. and Lu, J. Diabetes 42: (Suppl. 1) 97A (1993). PKC has been implicated in the biochemical pathway in the platelet-activity factor modulation of microvascular permeability. Kobayashi et al., Amer. Phys. Soc. H1214-H1220 (1994). Potent protein kinase C inhibitors have been demonstrated to affect agonist-induced aggregation in platelets. Toullec, D. et al. J. Biol. Chem. 266: 15771-15781 (1991). Protein kinase C inhibitors also block agonist-induced smooth muscle cell proliferation. Matsumoto, H. and Sasaki, Y. Biochem. Biophys. Res. Commun. 158: 105-109 (1989). Therefore, the present compounds are useful in treating cardiovascular disease, atherosclerosis and restenosis.

Abnormal activity of protein kinase C has also been linked to dermatological disorders such as psoriasis.

-86-

Horn, F. et al. J. Invest Dermatol. 88: 220-222 (1987);
Raynaud, F. and Evain-Brion, D. Br. J. Dermatol. 124: 542-
546 (1991). Psoriasis is characterized by abnormal
proliferation of keratinocytes. Known protein kinase C
5 inhibitors have been shown to inhibit keratinocyte
proliferation in a manner that parallels their potency as
PKC inhibitors. Hegemann, L. et al. Arch. Dermatol. Res.
283: 456-460 (1991); Bollag, W.B. et al. J. Invest.
Dermatol. 100: 240-246 (1993). Accordingly, the compounds
10 as inhibitors of PKC are useful in treating psoriasis.

Protein kinase C has been linked to several
different aspects of diabetes. Excessive activity of
protein kinase C has been linked to insulin signaling
defects and therefore to the insulin resistance seen in
15 Type II diabetes. Karasik, A. et al. J. Biol. Chem. 265:
10226-10231 (1990); Chen, K.S. et al. Trans. Assoc. Am.
Physicians 104: 206-212 (1991); Chin, J.E. et al. J. Biol.
Chem. 268: 6338-6347 (1993). In addition, studies have
demonstrated a marked increase in protein kinase C activity
20 in tissues known to be susceptible to diabetic compli-
cations when exposed to hyperglycemic conditions. Lee, T.-
S. et al. J. Clin. Invest. 83: 90-94 (1989); Lee, T. S.
et al. Proc. Natl. Acad. Sci. USA 86: 5141-5145
(1989); Craven, P.A. and DeRubertis, F.R. J. Clin. Invest.
25 83: 1667-1675 (1989); Wolf, B.A. et al. J. Clin. Invest.
87: 31-38 (1991); Tesfamariam, B. et al. J. Clin. Invest.
87: 1643-1648 (1991).

The compounds of Formula I are preferably
formulated prior to administration. Therefore, yet another
30 embodiment of the present invention is a pharmaceutical
formulation comprising a compound of Formula I and one or
more pharmaceutically acceptable carriers, diluents or
excipients.

The present pharmaceutical formulations are
35 prepared by known procedures using well known and readily

-87-

available ingredients. In making the compositions of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosol (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 1 to about 500 mg, more typically about 5 to about 300 mg, of the active ingredient. However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances including the condition to be treated, the choice of compound to be administered and the chosen route of administration. Therefore the above dosage ranges are not

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intended to limit the scope of the invention in any way. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined
5 quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

In addition to the above formulations, the compounds of the present invention may be administered
10 topically. Topical formulations are ointments, creams, and gels. Ointments generally are prepared using either (1) an oleaginous base, i.e., one consisting of fixed oils or hydrocarbons, such as white petrolatum or mineral oil, or (2) an absorbent base, i.e., one consisting of an anhydrous
15 substance or substances which can absorb water, for example anhydrous lanolin. Customarily, following formation of the base, whether oleaginous or absorbent, the active ingredient (compound) is added to an amount affording the desired concentration.

20 Creams are oil/water emulsions. They consist of an oil phase (internal phase), comprising typically fixed oils, hydrocarbons, and the like, such as waxes, petrolatum, mineral oil, and the like, and an aqueous phase (continuous phase), comprising water and any water-soluble
25 substances, such as added salts. The two phases are stabilized by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryl sulfate; hydrophilic colloids, such as acacia colloidal clays, veegum, and the like. Upon formation of the emulsion, the
30 active ingredient (compound) customarily is added to an amount to achieve the desired concentration.

Gels comprise a base selected from an oleaginous base, water, or an emulsion-suspension base. To the base is added a gelling agent which forms a matrix in the base,
35 increasing its viscosity. Examples of gelling agents are

-89-

hydroxypropyl cellulose, acrylic acid polymers, and the like. Customarily, the active ingredient (compounds) is added to the formulation at the desired concentration at a point preceding addition of the gelling agent.

5 The amount of compound incorporated into a topical formulation is not critical; the concentration should only be a range sufficient to permit ready application of the formulation to the an affected tissue area in an amount which will deliver the desired amount of
10 compound.

 The customary amount of a topical formulation to be applied to an affected tissue will depend upon an affected tissue size and concentration of compound in the formulation. Generally, the formulation will be applied to
15 the effected tissue in an amount affording from about 1 to about 500 μg compound per cm^2 of an affected tissue. Preferably, the applied amount of compound will range from about 30 to about 300 $\mu\text{g}/\text{cm}^2$, more preferably, from about 50 to about 200 $\mu\text{g}/\text{cm}^2$, and, most preferably, from about 60 to
20 about 100 $\mu\text{g}/\text{cm}^2$.

 The following formulation examples are illustrative only and are not intended to limit the scope of the invention in anyway.

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Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

5

10

	Quantity (mg/capsule)
Active agent	250
starch, dried	200
magnesium stearate	10
Total	460 mg

15

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

Formulation 2

20

A tablet is prepared using the ingredients below:

25

30

	Quantity (mg/capsule)
Active agent	250
cellulose, microcrystalline	400
silicon dioxide, fumed	10
stearic acid	5
Total	665 mg

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The components are blended and compressed to form tablets each weighing 665 mg.

5 Formulation 3

An aerosol solution is prepared containing the following components:

10		Quantity (mg/capsule)
	Active agent	.025
	ethanol	29.75
15	Propellant 22 (chlorodifluoromethane)	70.00
	Total	100.00

20

The active compound is mixed with ethanol. The mixture is added to a portion of the Propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with
25 the remainder of the propellant. The valve units are then fitted to the container.

Formulation 4

30 Tablets each containing 60 mg of active ingredient are made as follows:

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	Quantity (mg/capsule)
Active agent	60 mg
starch	45 mg
microcrystalline cellulose	35 mg
polyvinylpyrrolidone (as 10% solution in water)	4 mg
sodium carboxymethyl starch	4.5 mg
magnesium stearate	0.5 mg
talc	1 mg
Total	150 mg

20 The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 25 50°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each 30 weighing 150 mg.

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Formulation 5

Capsules each containing 80 mg of medicament are made as follows:

5

10

15

	Quantity (mg/capsule)
Active agent	80 mg
starch	59 mg
microcrystalline cellulose	59 mg
magnesium stearate	2 mg
Total	200 mg

The active ingredient, cellulose, starch and magnesium stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6

25

Suppositories each containing 225 mg of active ingredient may be made as follows:

- 94 -

	Quantity (mg/capsule)
Active agent	225 mg
saturated fatty acid glycerides	2,000 mg
Total	2,225 mg

10 The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

15. Formulation 7

Suspensions each containing 50 mg of medicament per 5 mL dose are made as follows:

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	Quantity (mg/capsule)
Active agent	50 mg
sodium carboxymethyl cellulose	50 mg
5 syrup	1.25 mg
benzoic acid solution	0.10 mg
10 flavor	q.v.
color	q.v.
15 purified water to total	5 mL

The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

Formulation 8

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An intravenous formulation may be prepared as follows:

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	Quantity (mg/capsule)
Active agent	250 mg
isotonic saline	1,000 mg

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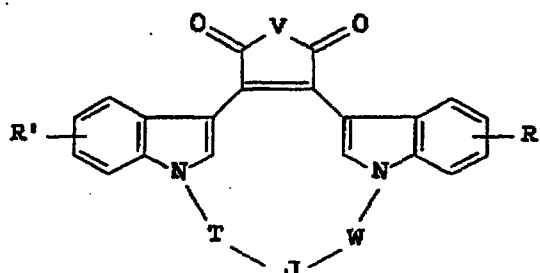
The solution of the above ingredients is administered intravenously at a rate of 1 mL per minute to a subject in need of treatment.



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Claims:

1. A compound of the formula:



wherein;

R' is independently hydrogen, halo, hydroxy, C₁-C₄ alkyl, C₁-C₄ alkoxy, NR₃R₄ or -NHCO(C₁-C₄ alkyl);

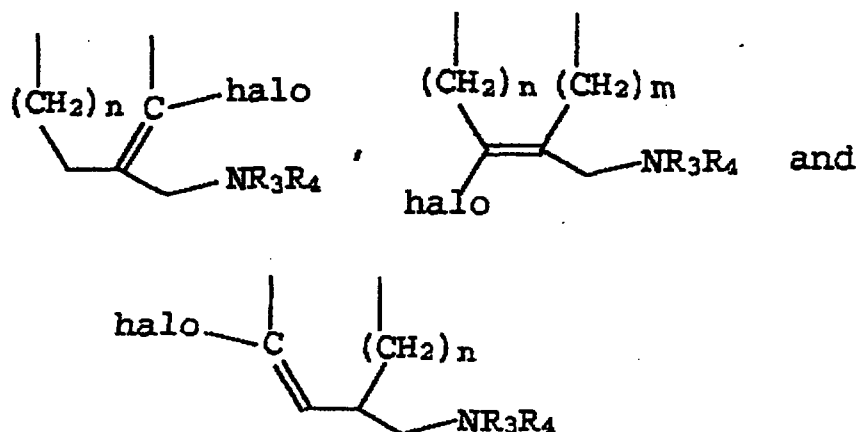
V is -O-, -NH- or -NC₁-C₄ alkyl-;

T is C₁-C₄ alkylene optionally substituted with halo or C₁-C₄ alkyl;

W is C₁-C₂ alkylene optionally substituted with halo or C₁-C₄ alkyl;

J is $\begin{array}{c} Y \\ | \\ -X-C- \\ | \\ S \end{array}$ or when T and W are both methylene, J is

selected from the group consisting of





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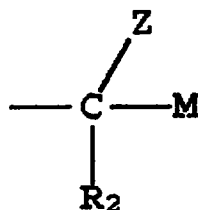
wherein n and m are independently 1 or 2;

X is oxygen, sulfur or a bond between the carbon atom bridged by X;

Y is halo, C₁-C₄ alkyl or hydrogen;

5 S is -CHO or the group

10



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wherein M is hydrogen, -CH₂OR₅, -CH₂NR₃R₄ or -NR₃R₄;

R₂ is hydrogen or halo; and

Z is hydrogen or -OR₆;

wherein R₃ and R₄ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or R₃ and R₄ taken together with the N atom to

20 which they are bound form a 5 or 6-membered ring; and

R₅ and R₆ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or together form a divalent group of the formula -CR₇R₈-

25 wherein R₇ and R₈ are independently hydrogen, C₁-C₄ alkyl or halo(C₁-C₄ alkyl) or R₇ and R₈ taken together with the C atom to which they are bound form a 5 or 6-membered ring

provided that at least one of Y, S, T or W is halo or a halo substituted group, or T and W are both methylene.

2. The compound of claim 1 wherein at least one of
30 Y, S, T or W is fluoro or a fluoro substituted group.



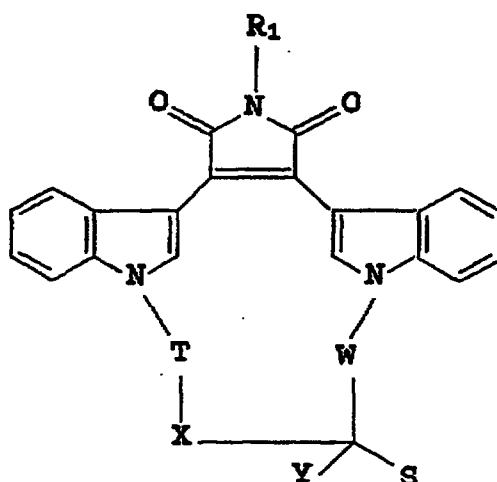
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3. A compound of the formula:

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wherein;

R_1 is C_1 - C_4 alkyl or hydrogen;

20 T is C_2 - C_4 alkylene optionally substituted with halo or C_1 - C_4 alkyl;

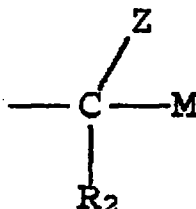
W is ethylene optionally substituted with halo or C_1 - C_4 alkyl;

X is oxygen, sulfur or a bond between the carbon atom bridged by X ;

25 Y is halo, C_1 - C_4 alkyl or hydrogen;

S is $-CHO$ or the group

30



35

wherein M is hydrogen, $-CH_2OR_3$, $-CH_2NR_3R_4$ or $-NR_3R_4$;



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R_2 is hydrogen or halo; and

Z is hydrogen or $-OR_6$;

wherein R_3 and R_4 are independently hydrogen, C_1 - C_4 alkyl, halo(C_1 - C_4 alkyl), C_1 - C_4 alkanoyl, halo(C_1 - C_4 alkanoyl) or R_3 and R_4 taken together with the N atom to which they are bound form a 5 or 6-membered ring; and

R_5 and R_6 are independently hydrogen, C_1 - C_4 alkyl, halo(C_1 - C_4 alkyl), C_1 - C_4 alkanoyl, halo(C_1 - C_4 alkanoyl) or together form a divalent group of the formula $-CR_7R_8-$ wherein R_7 and R_8 are independently hydrogen C_1 - C_4 alkyl, or halo(C_1 - C_4 alkyl) or R_7 and R_8 taken together with the C atom to which they are bound form a 5 or 6-membered ring

provided that at least one of Y, S, T or W is halo or a halo substituted group.

4. The compound of claim 3 wherein at least one of Y, S, T or W is fluoro or a fluoro substituted group.

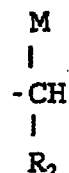
5. The compound of claim 3 wherein W is fluoro substituted ethylene.

6. The compound of claim 3 wherein T is fluoro substituted ethylene.

7. The compound of claim 3 wherein T is fluoro substituted trimethylene.

8. The compound of claim 3 wherein R_1 and Y are hydrogen and X is oxygen.

9. The compound of claim 5 wherein R_1 and Y are hydrogen, X is oxygen and S is



10. The compound of claim 9 wherein T is ethylene.

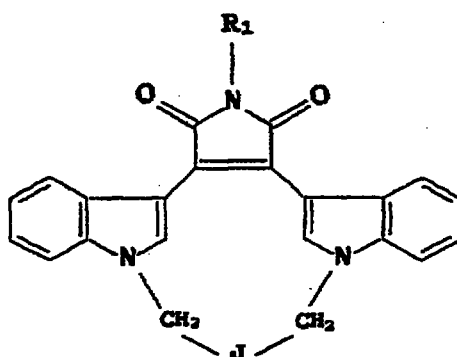


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11. A compound of the formula:

5

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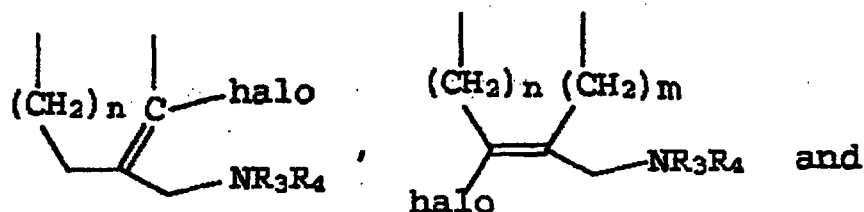


wherein

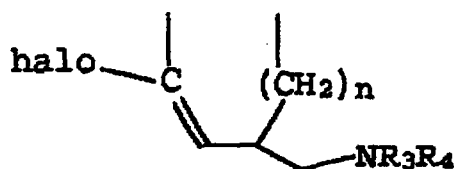
J is selected from the group consisting of

15

20



25



wherein R_1 is C_1 - C_4 alkyl or hydrogen;

n and m are independently 1 or 2; and

30

R_3 and R_4 are independently hydrogen, C_1 - C_4 alkyl, halo(C_1 - C_4 alkyl), C_1 - C_4 alkanoyl, halo(C_1 - C_4 alkanoyl) or R_3 and R_4 taken together with the N atom to which they are bound form a 5 or 6-membered ring.

12. The compound of claim 9 wherein R_1 is hydrogen.



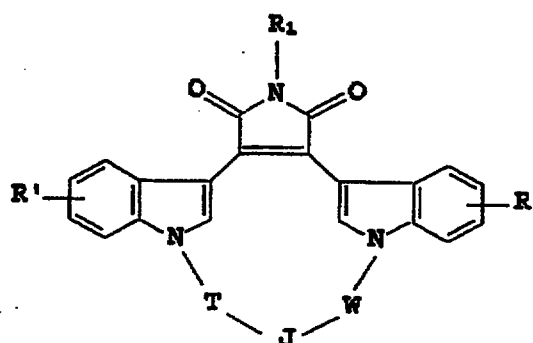
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13. The compound of claim 9 wherein the halo substituent of J is fluoro.

14. A pharmaceutical composition comprising a compound of the formula:

5

10



15 wherein;

R' is independently hydrogen, halo, hydroxy, C₁-C₄ alkyl, C₁-C₄ alkoxy, NR₃R₄ or -NHCO(C₁-C₄ alkyl);

R₁ is C₁-C₄ alkyl or hydrogen;

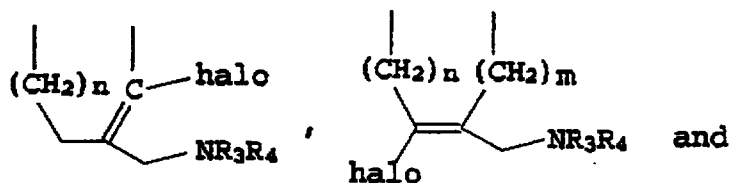
20 T is C₁-C₄ alkylene optionally substituted with halo or C₁-C₄ alkyl;

W is C₁-C₂ alkylene optionally substituted with halo or C₁-C₄ alkyl;

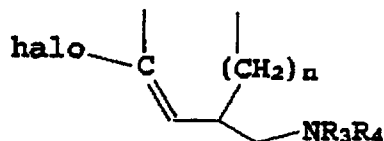
25 J is $\begin{array}{c} Y \\ | \\ -X-C- \\ | \\ S \end{array}$, or when T and W are both methylene, J is

selected from the group consisting of

30



35





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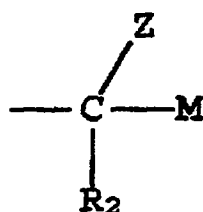
wherein n and m are independently 1 or 2;

X is oxygen, sulfur or a bond between the carbon atom bridged by X;

Y is halo, C₁-C₄ alkyl or hydrogen;

5 S is -CHO or the group

10



15

wherein M is hydrogen, -CH₂OR₃, -CH₂NR₃R₄ or -NR₃R₄;

R₂ is hydrogen or halo; and

Z is hydrogen or -OR₆;

20

wherein R₃ and R₄ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or R₃ and R₄ taken together with the N atom to which they are bound form a 5 or 6-membered ring; and

R₅ and R₆ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or together form a divalent group of the formula -CR₇R₈-

25

wherein R₇ and R₈ are independently hydrogen, C₁-C₄ alkyl or halo(C₁-C₄ alkyl) or R₇ and R₈ taken together with the C atom to which they are bound form a 5 or 6-membered ring

provided that at least one of Y, S, T or W is halo or a halo substituted group, or T and W are both methylene;

30

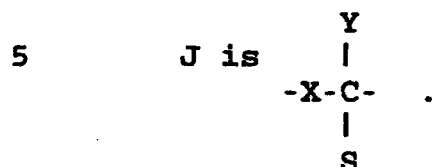
and

a pharmaceutically acceptable excipient, carrier, or diluent.



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15. The pharmaceutical composition of claim 14
wherein



16. The pharmaceutical composition of claim 15
10 wherein
S is



X is oxygen; and

R₁ is hydrogen.

20 17. The pharmaceutical composition of claim 16
wherein at least one of Y, S, T or W is fluoro or a fluoro
substituted group.

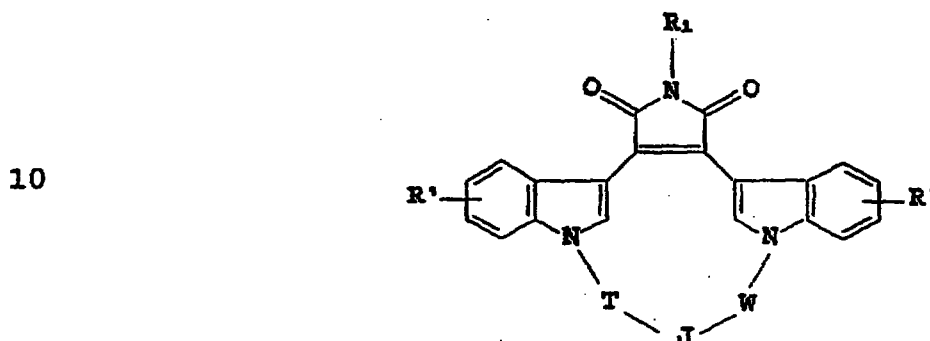
18. The pharmaceutical composition of claim 14
wherein

25 T and W are methylene and the halo substituent is
fluoro.

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19. A method of treating a mammal having a disease or condition associated with abnormal protein kinase C activity, said method comprising administering to said mammal a pharmaceutically effective amount of compound of the formula:



15 wherein;

R' is independently hydrogen, halo, hydroxy, C₁-C₄ alkyl, C₁-C₄ alkoxy, NR₃R₄ or -NHCO(C₁-C₄ alkyl);

R₁ is C₁-C₄ alkyl or hydrogen;

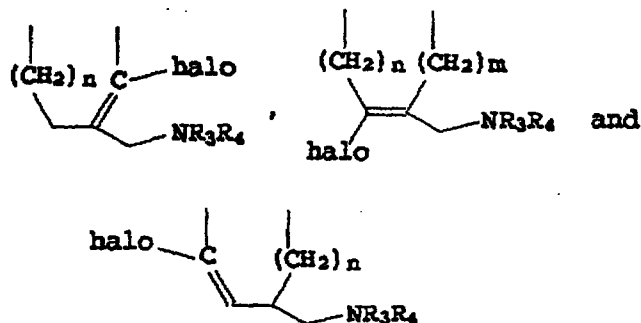
20 T is C₁-C₄ alkylene optionally substituted with halo or C₁-C₄ alkyl;

W is C₁-C₂ alkylene optionally substituted with halo or C₁-C₄ alkyl;

25 J is $\begin{array}{c} Y \\ | \\ -X-C- \\ | \\ S \end{array}$ or when T and W are both methylene, J is

selected from the group consisting of

30



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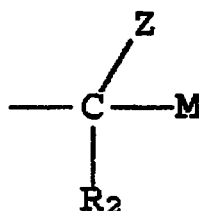
wherein n and m are independently 1 or 2;

X is oxygen, sulfur or a bond between the carbon atom bridged by X;

Y is halo, C₁-C₄ alkyl or hydrogen;

5 S is -CHO or the group

10



15 wherein M is hydrogen, -CH₂OR₃, -CH₂NR₃R₄ or -NR₃R₄;

R₂ is hydrogen or halo; and

Z is hydrogen or -OR₆;

wherein R₃ and R₄ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or R₃ and R₄ taken together with the N atom to which they are bound form a 5 or 6-membered ring; and

20 R₅ and R₆ are independently hydrogen, C₁-C₄ alkyl, halo(C₁-C₄ alkyl), C₁-C₄ alkanoyl, halo(C₁-C₄ alkanoyl) or together form a divalent group of the formula -CR₇R₈- wherein R₇ and R₈ are independently hydrogen, C₁-C₄ alkyl or halo(C₁-C₄ alkyl) or R₇ and R₈ taken together with the C atom to which they are bound form a 5 or 6-membered ring

provided that at least one of Y, S, T or W is halo or a halo substituted group, or T and W are both methylene; and

30 and a pharmaceutically acceptable excipient, carrier, or diluent.

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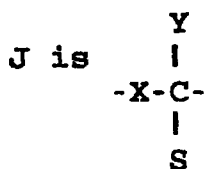


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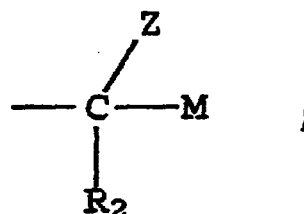
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20. The method of claim 19 wherein



21. The method of claim 20
wherein

S is



X is oxygen; and

R₁ is hydrogen.

22. The method of claim 20 wherein at least one of Y,
S, T or W is fluoro or a fluoro substituted group.

23. The method of claim 19
wherein

T and W are methylene and the halo substituent is
fluoro.

24. The method of claim 19 wherein W is fluoro
substituted ethylene.

25. The method of claim 19 wherein T is fluoro
substituted ethylene.

26. The method of claim 19 wherein T is fluoro
substituted trimethylene.

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