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(54) Title: ENHANCED SENSITIVITY POLYMERASE CHAIN REACTIONS

FIGURE 1

(57) Abstract: The present invention is directed to tri-phenyl compounds that enhance the amplification of a nucleic acid of interest in a polymerase chain reaction (PCR) assay, kits comprising the same and applications thereof.

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ENHANCED SENSITIVITY POLYMERASE CHAIN REACTIONS

FIELD OF THE INVENTION

[001] The present invention describes novel materials, kits and methods for enhancing the sensitivity of polymerase chain reactions (PCRs).

BACKGROUND OF THE INVENTION

[002] Nucleic acid polymerases are enzymes, whose primary function is to polymerize new nucleic acids such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) using an existing DNA or RNA template. Polymerases typically are involved in the processes of replication and transcription.

[003] "Polymerase chain reaction" or "PCR" refers to a procedure or technique in which amounts of a pre-selected fragment of nucleic acid, RNA and/or DNA, are amplified. Generally, sequence information from the ends of the region of interest or beyond is employed to design oligonucleotide primers. These primers will be identical or similar in sequence to opposite strands of the template to be amplified. PCR can be used to amplify specific RNA sequences and cDNA transcribed from total cellular RNA. The PCR process is disclosed in U.S. Pat. Nos. 4,683,195; 4,683,202; and 4,965,188, each of which is incorporated herein by reference.

[004] The practical benefits of PCR nucleic acid amplification have been rapidly appreciated in the fields of genetics, molecular biology, cellular biology, clinical chemistry, forensic science, and analytical biochemistry, as described in the following review volumes and articles: Erlich (ed.), 1989, PCR Technology, Stockton Press (New York); Erlich et al. (eds.), 1989, Polymerase Chain Reaction, Cold Spring Harbor Press (Cold Spring Harbor, N.Y.); Innis et al., 1990, PCR Protocols, Academic Press (New York); and White et al., 1989, Trends in Genetics 5/6:185-189. PCR can replace a large fraction of molecular cloning and mutagenesis operations commonly performed in bacteria, having advantages of speed, simplicity, lower cost, and increased safety. Furthermore, PCR permits the rapid and highly sensitive qualitative and even quantitative analysis of nucleic acid sequences, often with greatly increased safety because so much PCR product is made that non-isotopic detection modes suffice.

[005] PCR is conducted in an automated cycler, which heats and cools the reaction mixture in a short time. PCR typically involves denaturation of double stranded DNA to single stranded; primer annealing to the single stranded DNA, and primer extension by polymerase incorporation of dNTPs. Cyclic conductance of these steps results in amplification of the DNA.

[006] After PCR amplification, common practice is to detect amplified nucleic acid by reacting the amplified nucleic acid with a reagent that carries an analytical signal generator or a reagent that facilitates separation of amplified nucleic acid from other components of the PCR reaction mixture.

[007] Polymerases are useful in genetic engineering, nucleotide sequencing, DNA labeling, site-directed mutagenesis, and the like. DNA polymerases find application in polymerase chain reactions (PCR), and various DNA polymerases suitable for the PCR method have been developed and commercialized.

[008] Polymerase activity can be modulated, in part, by other molecules which bind to the polymerase. Such modulation may comprise enhancing polymerase activity or diminishing such activity, which in turn modulates multiple cellular processes, and other applications. Compounds which bind to polymerases and thereby modulate its activity thus will have a wide array of important applications.

SUMMARY OF THE INVENTION

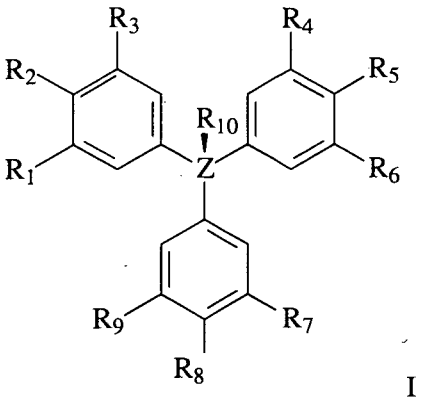
[009] In one embodiment, this invention provides a method of enhancing amplification of a nucleic acid of interest in a polymerase chain reaction (PCR) assay, said method comprising:

a. preparing a reaction mixture comprising:

i. a nucleic acid of interest;

ii. reagents for nucleic acid amplification in a PCR assay; and

iii. a compound represented by the structure of formula I:



I

wherein

Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R_1 to R_9 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_3, R_4, or R_7, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof; and

b. conducting a PCR assay on the reaction mixture in (a).

[0010] In one embodiment, this invention provides a method of rapid diagnosis of a disease or condition in a subject, said method comprising:

a. isolating nucleic acid from a sample;

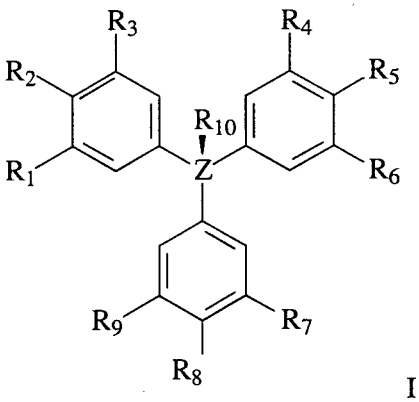
b. preparing a reaction mixture comprising:

i. the nucleic acid in (a);

ii. reagents for nucleic acid amplification in a PCR assay;

iii. oligonucleotide primers specifically interacting with a sequence associated with a disease or condition; and

iv. a compound represented by the structure of formula I:



I

wherein

Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R_1 to R_9 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_3, R_4, or R_7, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino,

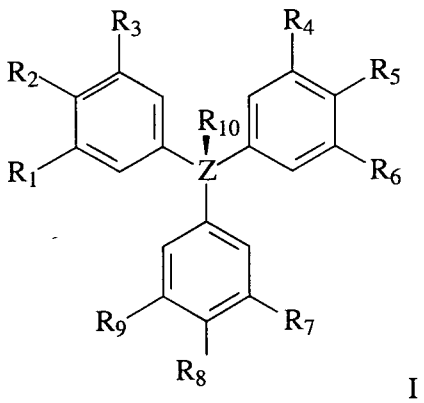
alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof;

c. conducting a PCR assay on the reaction mixture in (b);

d. correlating the presence or quantity of an amplified product in (c) with a disease or condition in a subject;

whereby qualitative or quantitative detection of said amplified product is achieved more rapidly than in samples assayed in the absence of said compound represented by the structure of formula I.

[0011] In one embodiment, this invention provides a kit comprising reagents for conducting a PCR assay, and a compound represented by the structure of formula I:



I

wherein

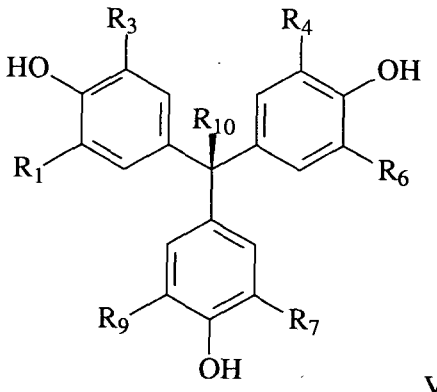
Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R_1 to R_9 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_3, R_4,

or R_7, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

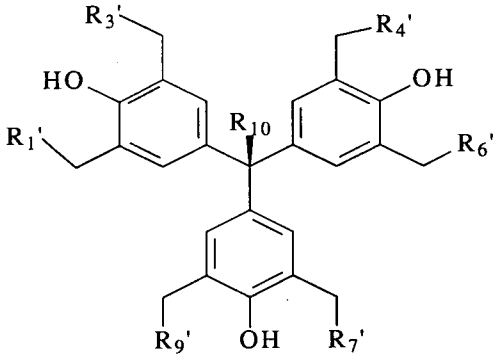
[0012] In one embodiment the structure of formula I is represented by the structure of formula V:



V

wherein R_1, R_3, R_4, R_6, R_7, R_9 and R_{10} are as described above.

[0013] In another embodiment the structure of formula I is represented by the structure of formula VII:

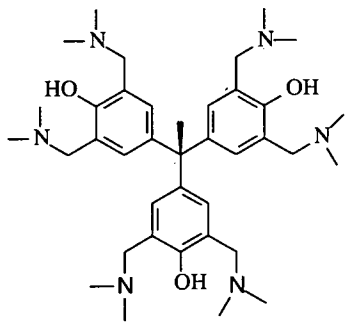


VII

wherein R_1', R_3', R_4', R_6' R_7', and R_9' are the same or different comprising halogen, aryl, alkyl, cycloalkyl, heterocycloalkyl, alkoxy, monoalkylamino, dialkylamino or arylamino; and

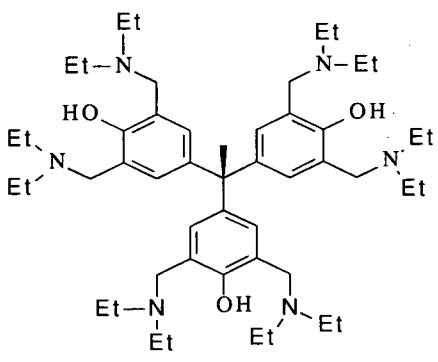
R_{10} is as described above.

[0014] In another embodiment the structure of formula I is represented by the structure of formula VIII:



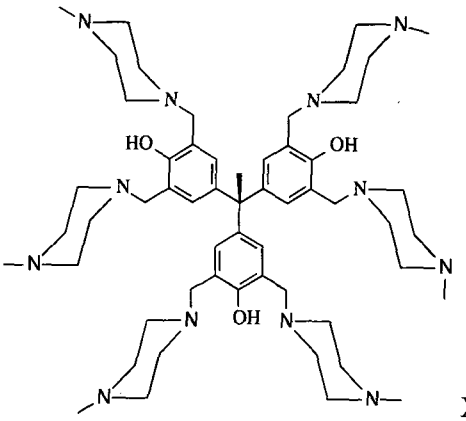
VIII

[0015] In another embodiment the structure of formula I is represented by the structure of formula IX:



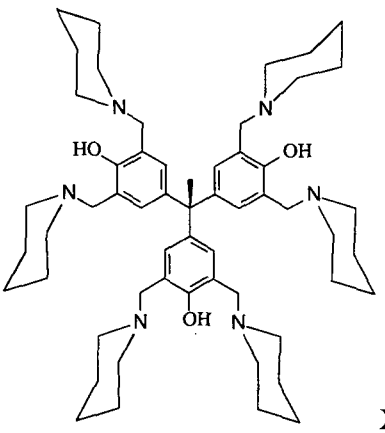
IX

[0016] In another embodiment the structure of formula I is represented by the structure of formula X:



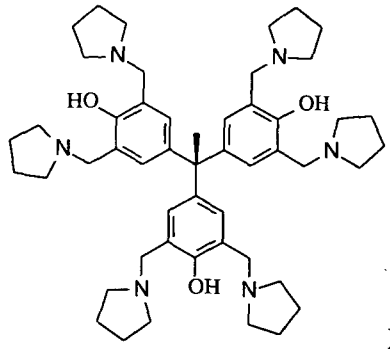
X

[0017] In another embodiment the structure of formula I is represented by the structure of formula XI:



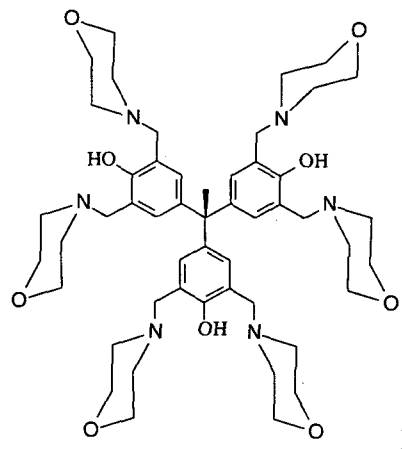
XI

[0018] In another embodiment the structure of formula I is represented by the structure of formula XII:



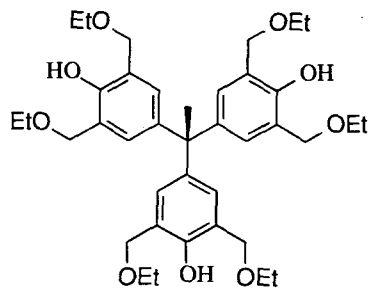
XII

[0019] In another embodiment the structure of formula I is represented by the structure of formula XIII:



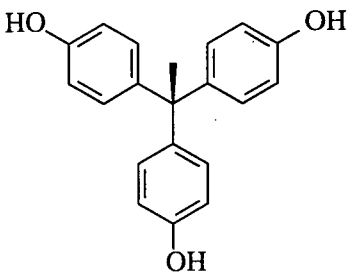
XIII

[0020] In another embodiment the structure of formula I is represented by the structure of formula XIV:



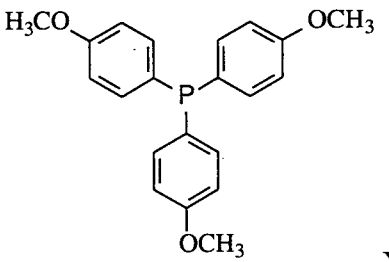
XIV

[0021] In another embodiment the structure of formula I is represented by the structure of formula XV:



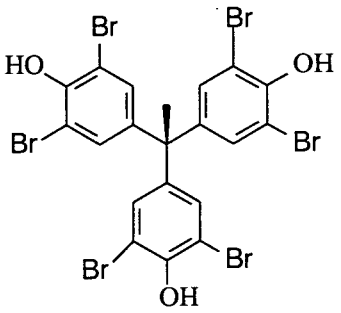
XV

[0022] In another embodiment the structure of formula I is represented by the structure of formula XVI:



XVI

[0023] In another embodiment the structure of formula I is represented by the structure of formula XVII:



XVII

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The subject matter regarded as the invention is particularly pointed out and distinctly claimed in the concluding portion of the specification. The invention, however, both as to

organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings in which:

[0025] Fig. 1 is a PCR Autoradiograph of Compounds XIV-XVII at 125 nM.

[0026] Fig. 2 is a PCR Autoradiograph of Compounds XIV-XVII at 62.5 nM (Fig 2A), and the PCR activation at 62.5 nM as fold of control (Fig. 2B).

[0027] Fig 3 depicts an agarose gel development of a GAPDH PCR product, conducted with compound XVI at different concentrations of 0.0625, 0.125, 0.25 and 0.5 μM. ST refers to the control.

[0028] Fig 4 depicts an agarose gel development of a GAPDH PCR product, conducted with compound XV and XVII at different concentrations of 60 and 120 nM.

[0029] Fig 5 depicts an agarose gel development of an HTLV-I PCR product, conducted with compound XVII and 0.015, 0.05 and 0.15 μg of cDNA. Symbols: ST- refers to control+DMSO, pos- refers to cDNA from uninfected cells, neg-refers to a sample without cDNA.

[0030] Fig 6 depicts an agarose gel development of HTLV-I PCR product, conducted with compound XVII or VIII at 15 or 30 nM with 8 ng of cDNA from Jurkat uninfected cells (Fig. 6A) and SLBI-HTLV-I infected cells (Fig. 6B).

[0031] Fig 7 depicts an agarose gel development of HTLV-I PCR product, conducted with a mixture of Jurkat uninfected cells with SLB-I infected cells at 1:1 infected/uninfected ratio (Fig. 7A) and 1:100 ratio, respectively (Fig. 7B), and compound XVII or VIII at 15 nM

DETAILED DESCRIPTION OF THE PRESENT INVENTION

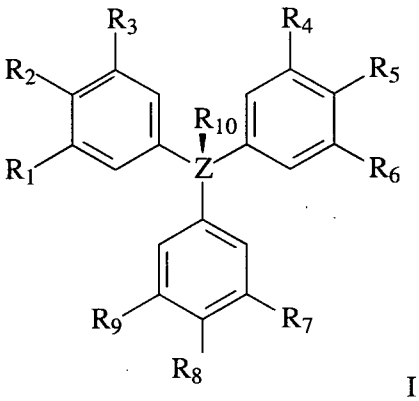
[0032] In the following detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. However, it will be understood by those skilled in the art that the present invention may be practiced without these specific details. In other instances, well-known methods, procedures, and components have not been described in detail so as not to obscure the present invention.

[0033] The present invention relates, in some embodiments, to the use of a novel class of tri-phenyl compounds and kits comprising the same for, *inter alia,* increasing the sensitivity of the PCR reaction, enhancing amplification of a nucleic acid of interest in a polymerase chain reaction (PCR) assay and for rapid diagnosis of a disease or condition in a subject These compounds may interact with the polymerase enzyme and are useful in increasing the amount of the PCR product, and thereby its sensitivity and the speed of reaction.

[0034] The methods and/or kits of this invention make use of a series of compounds, as described herein, referred to herein as "compounds of the invention". Any embodiment as described hereinbelow should be understood to encompass an embodiment thereof, as to being a component of a kit of method of this invention.

Compounds for Use in the Methods and/or Kits of the Invention:

[0035] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula I:



I

wherein

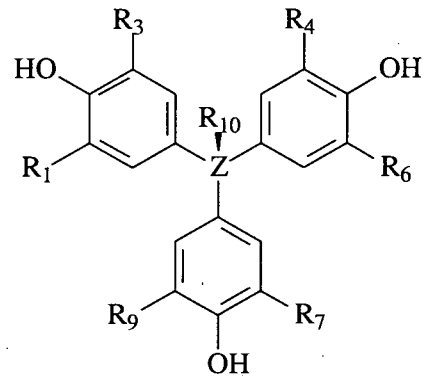
Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R_1 to R_9 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_3, R_4,

or R_7, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0036] one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula II:



II

wherein

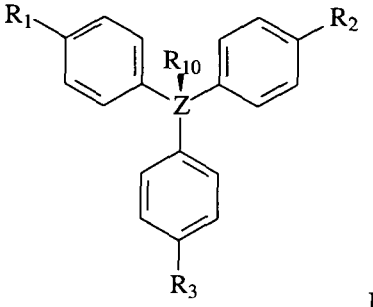
Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R_1, R_3, R_4, R_6, R_7 and R_9 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_3, R_4, or R_7, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0037] In another embodiment Z is carbon. In another embodiment R_{10} is a methyl group. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are - $(CH_2)_n$-heterocycloalkyl group, wherein n is between 1-6. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are - $(CH_2)_n$-aminoalkyl group, wherein n is between 1-6. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are -$(CH_2)_n$-dialkylamino group, wherein n is between 1-6 In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are -(CH_2)n-$N(CH_3)_2$ group, wherein n is between 1-6. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are -$(CH_2)_n$-$N(Et)_2$ group, wherein n is between 1-6. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are -$(CH_2)_n$-aryl group, wherein n is between 1-6. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are -$(CH_2)_n$-heteroaryl group, wherein n is between 1-6. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are -$(CH_2)_n$-haloalkyl group, wherein n is between 1-6. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are -$(CH_2)_n$-alkoxy group, wherein n is between 1-6. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are -$(CH_2)_n$-ethoxy group, wherein n is between 1-6. In another embodiment R_1, R_3, R_4, R_6, R_7, and R_9 are -$(CH_2)_n$-cycloalkyl group, wherein n is between 1-6.

[0038] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula III:



III

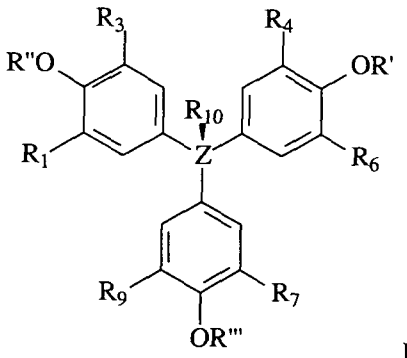
wherein

Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R_1, R_2, and R_3 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_1, R_2, or R_3, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0039] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula IV:



IV

wherein

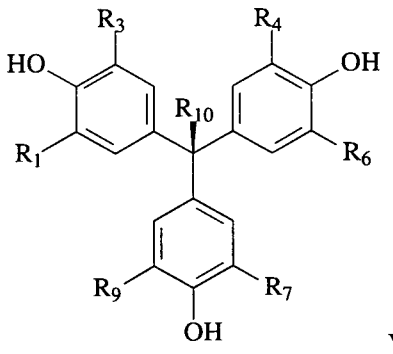
Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R', R'' and R''' are independently the same or different comprising hydrogen, alkyl, haloalkyl, alkylamino, alkylester, phenyl, benzyl, alkanyloyl, acetyl or benzoyl;

R_1, R_3, R_4, R_6, R_7 and R_9 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_3, R_4, or R_7, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

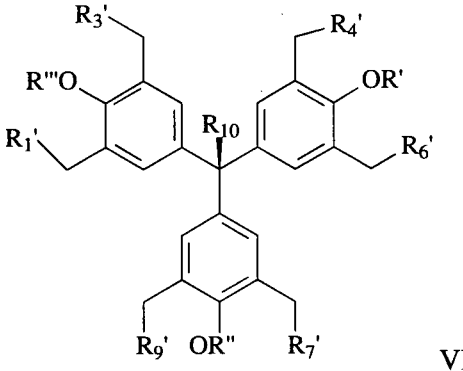
[0040] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula V:



V

wherein R_1, R_3, R_4, R_6, R_7 , R_9 and R_{10} are as defined above; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0041] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula V:



[0042] VI

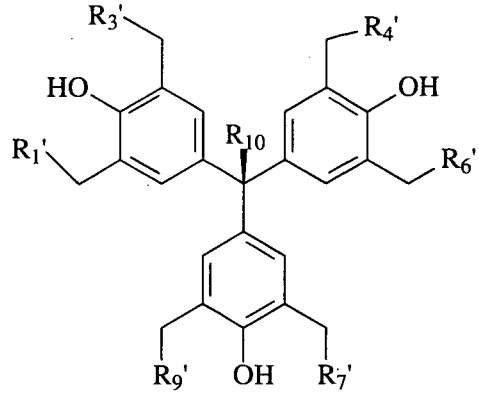
wherein

R', R'', R''' are independently the same or different comprising hydrogen, alkyl, haloalkyl, alkylester, phenyl, benzyl, alkanyloyl, acetyl or benzoyl;

R_1', R_3', R_4', R_6' R_7', and R_9' are the same or different comprising halogen, aryl, alkyl, cycloalkyl, heterocycloalkyl, alkoxy, amino, monoalkylamino, dialkylamino or arylamino group; and R_7 is as described above; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0043] In another embodiment, R_1', R_3', R_4', R_6' R_7', and R_9' are dialkylamino group. In another embodiment, R_1', R_3', R_4', R_6' R_7', and R_9' are dimethylamino group. In another embodiment, R_1', R_3', R_4', R_6' R_7', and R_9' are diethylamino group. In another embodiment, R_1', R_3', R_4', R_6' R_7', and R_9' are N-piperidine group. In another embodiment, R_1', R_3', R_4', R_6' R_7', and R_9' are N-pyrolidine group. In another embodiment, R_1', R_3', R_4', R_6' R_7', and R_9' are N-piperazine group. In another embodiment, R_1', R_3', R_4', R_6' R_7', and R_9' are N-piperazine-4-methyl group. In another embodiment, R_1', R_3', R_4', R_6' R_7', and R_9' are N-morpholine group. In another embodiment, R_1', R_3', R_4', R_6' R_7', and R_9' are ethoxy group.

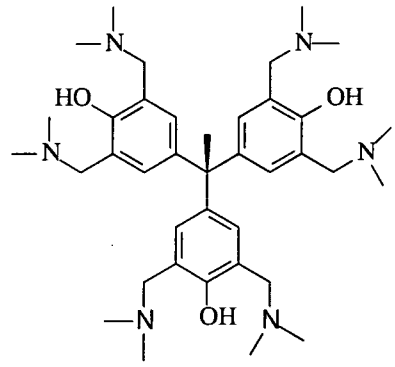
[0044] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula VII:



VII

wherein R_1', R_3', R_4', R_6' R_7', and R_9' are the same or different comprising halogen, aryl, alkyl, cycloalkyl, heterocycloalkyl, alkoxy, amino, monoalkylamino, dialkylamino or arylamino group; and R_{10} is as described above; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

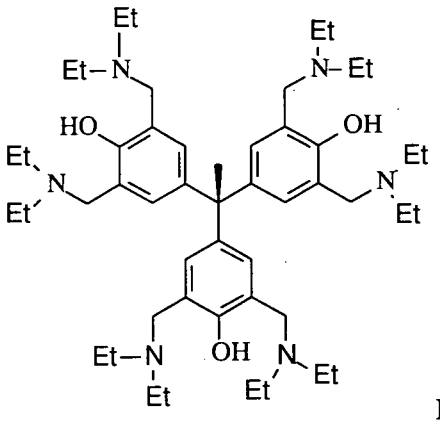
[0045] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula VIII:



VIII

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

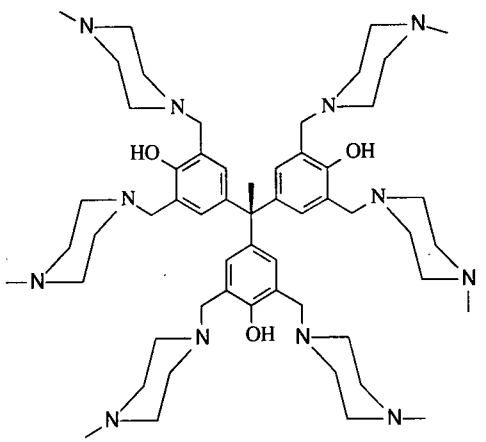
[0046] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula IX:



IX

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

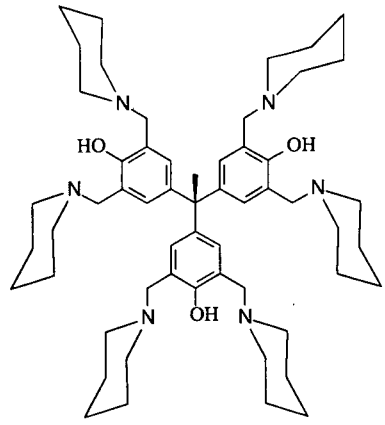
[0047] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula X:



X

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0048] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XI:

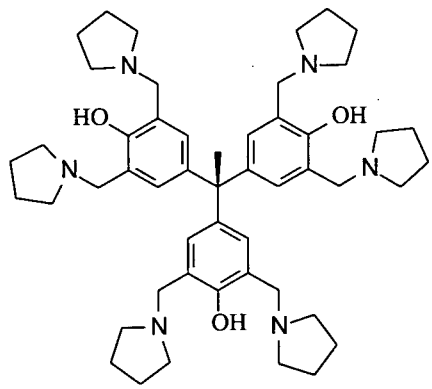


XI

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0049] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XII:

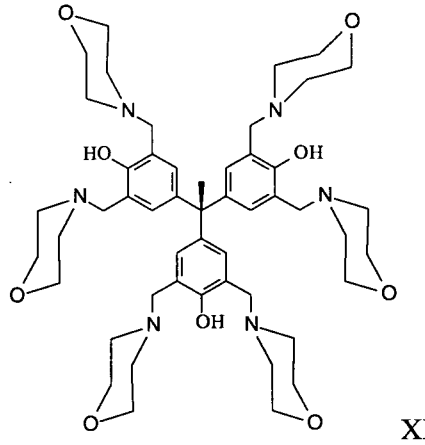
[0050]



XII

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

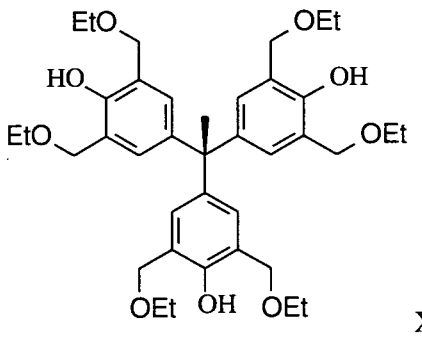
[0051] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XIII:



XIII

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

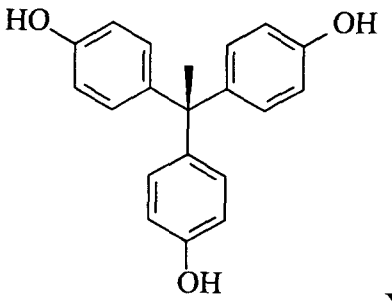
[0052] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XIV:



XIV

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

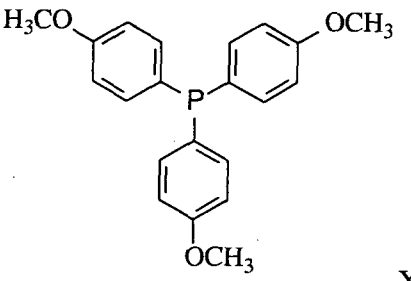
[0053] In another embodiment the structure of formula I is represented by the structure of formula XV:



XV

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

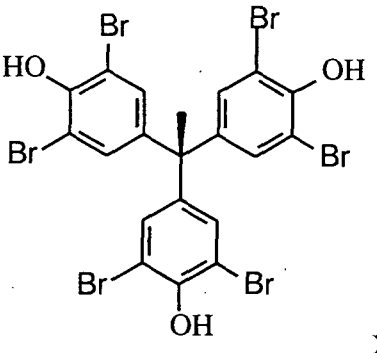
[0054] In another embodiment the structure of formula I is represented by the structure of formula XVI:



XVI

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

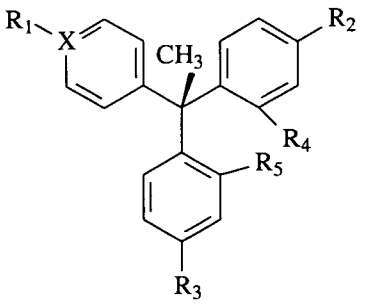
[0055] In another embodiment the structure of formula I is represented by the structure of formula XVII:



XVII

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0056] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XVIII:



XVIII

wherein

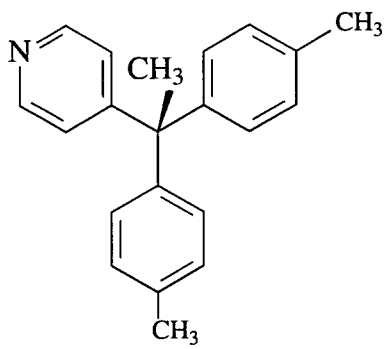
X is carbon or nitrogen;

R_1 is nothing, $OCH_2COOAlk$, alkoxy or halogen

R_2 and R_3 are independently hydrogen, alkyl, alkoxy, hydroxyl, or $OCH_2COOAlk$; and

R_4 and R_5 are hydrogens or form together a saturated or unsaturated of 5-7 carbocyclic ring; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

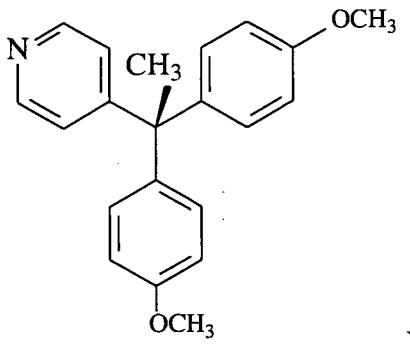
[0057] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XIX:



XIX

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

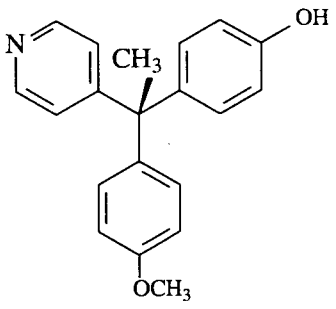
[0058] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XX:



XX

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

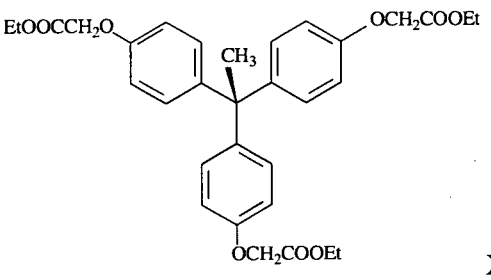
[0059] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XXI:



XXI

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

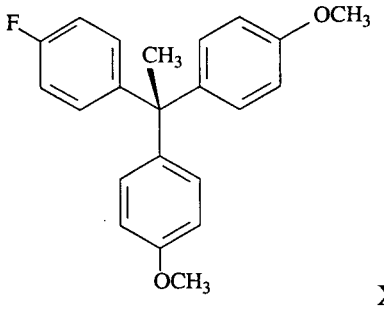
[0060] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XXII:



XXII

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

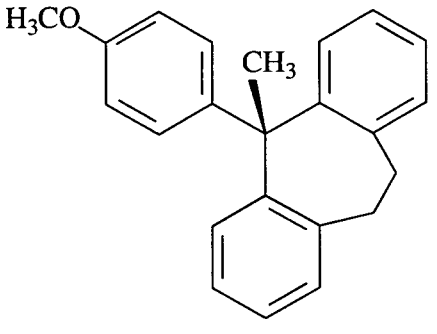
[0061] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XXIII:



XXIII

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

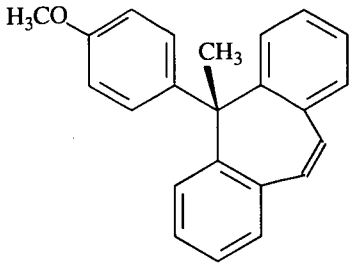
[0062] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XXIV:



XXIV

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

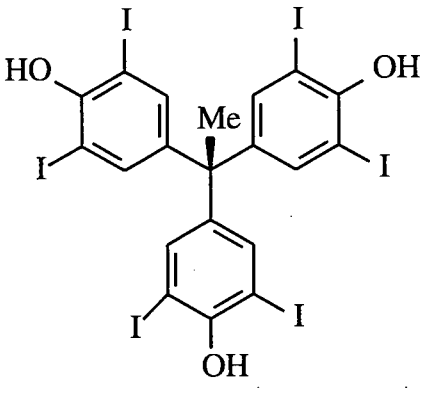
[0063] In one embodiment, the methods of this invention comprise the use of a compound represented by the structure of formula XXV:



XXV

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0064] In one embodiment, this invention provides a compound represented by the structure of formula XXVI:



XXVI

or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0065] The term "alkyl" refers, in one embodiment, to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain and cyclic alkyl groups. In one embodiment, the alkyl group has 1-12 carbons. In another embodiment, the alkyl group has 1-7 carbons. In another embodiment, the alkyl group has 1-6 carbons. In another embodiment, the alkyl group has 1-7 carbons. In another embodiment, the alkyl group has 2-6 carbons. In another embodiment, the alkyl group has 1-7 carbons. In another embodiment, the alkyl group has 2-8 carbons. In another embodiment, the alkyl group has 3-6 carbons. In another embodiment, the alkyl group has 3-7 carbons. In another embodiment, the alkyl group has 1-4 carbons. In another embodiment, the branched alkyl is an alkyl substituted by alkyl side chains of 1 to 5 carbons. In another

embodiment, the branched alkyl is an alkyl substituted by haloalkyl side chains of 1 to 5 carbons. The alkyl group may be unsubstituted or substituted by a halogen, haloalkyl, hydroxyl, alkoxy, carbonyl, amido, alkylamido, dialkylamido, nitro, cyano, amino, monoalkylamino, dialkylamino, carboxyl, thio and/or thioalkyl.

[0066] An "alkenyl" group refers, in one embodiment, to an unsaturated hydrocarbon, including straight chain, branched chain and cyclic groups having one or more double bonds. The alkenyl group may have one double bond, two double bonds, three double bonds, etc. In another embodiment, the alkenyl group has 2-12 carbons. In another embodiment, the alkenyl group has 2-6 carbons. In another embodiment, the alkenyl group has 2-4 carbons. In another embodiment the alkenyl group is ethenyl ($-CH=CH_2$) Examples of alkenyl groups are ethenyl, propenyl, butenyl, cyclohexenyl, etc. The alkenyl group may be unsubstituted or substituted by a halogen, hydroxy, alkoxy, carbonyl, amido, alkylamido, dialkylamido, nitro, cyano, amino, monoalkylamino, dialkylamino, carboxyl, thio and/or thioalkyl.

[0067] An alkynyl" group refers, in one embodiment, to an unsaturated hydrocarbon, including straight chain, branched chain and cyclic groups having one or more triple bonds. The alkynyl group may have one triple bond, two triple bonds, triple double bonds, etc. In another embodiment, the alkynyl group has 2-12 carbons. In another embodiment, the alkynyl group has 2-6 carbons. In another embodiment, the alkenyl group has 2-4 carbons. In another embodiment the alkynyl group is ethynyl ($-CH\equiv CH_2$). Examples of alkynyl groups are ethynyl, propynyl, butynyl, cyclohexynyl, etc. The alkynyl group may be unsubstituted or substituted by a halogen, hydroxy, alkoxy, carbonyl, amido, alkylamido, dialkylamido, nitro, cyano, amino, monoalkylamino, dialkylamino, carboxyl, thio and/or thioalkyl.

[0068] An "alkoxy" group refers, in another embodiment is an alkyl group as defined above, which is linked to oxygen. Examples of alkoxy groups are ethoxy, propoxy, tert-butoxy etc..

[0069] A "haloalkyl" group refers, in one embodiment, to an alkyl group as defined above, which is substituted by one or more halogen atoms, e.g. by F, Cl, Br or I.

[0070] An "aryl" group refers, in another embodiment, to an aromatic group having at least one carbocyclic aromatic group or heterocyclic aromatic group, which may be unsubstituted or substituted by one or more groups selected from halogen, haloalkyl, hydroxy, alkoxy, carbonyl,

amido, alkylamido, dialkylamido, nitro, cyano, amino, monoalkylamino, dialkylamino, carboxy or thio or thioalkyl. In another embodiment, the aryl group is between 4-12 membered ring(s). In another embodiment, the aryl group is between 6-18 membered ring(s). In another embodiment, the aryl group is between 4-8 membered ring(s). In another embodiment, the aryl group is a 6 membered ring. In another embodiment, the aryl group is a fused ring system comprising of between 2-3 rings. Nonlimiting examples of aryl rings are phenyl, naphthyl, pyranyl, pyrrolyl, pyrazinyl, pyrimidinyl, pyrazolyl, pyridinyl, furanyl, thiophenyl, thiazolyl, imidazolyl, isoxazolyl, and the like.

[0071] A "heteroaryl" group refers, in another embodiment, to an aromatic group having at least one heterocyclic aromatic group, which may be unsubstituted or substituted by one or more groups selected from halogen, haloalkyl, hydroxy, alkoxy, carbonyl, amido, alkylamido, dialkylamido, nitro, cyano, amino, monoalkylamino, dialkylamino, carboxy or thio or thioalkyl. In another embodiment, the heteroaryl group is between 4-12 membered ring(s). In another embodiment, the heteroaryl group is between 6-18 membered ring(s). In another embodiment, the heteroaryl group is between 4-8 membered ring(s). In another embodiment, the heteroaryl group is a 6 membered ring. In another embodiment, the heteroaryl group is a fused ring system comprising of between 2-3 rings. Nonlimiting examples of heteroaryl rings are pyrrolyl, thienyl, thiazolyl, benzothienyl, naphthothienyl, purinyl, isothiazolyl, furyl, furazanyl, isobenznzofuranyl, pyranyl, chromenyl, xanthenyl, phenoxyxanthiinyl, indolyl, isoindolyl, indolizinyl, isoindolyzinyl, benzothienyl, oxazolyl, isoxazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, and the like.

[0072] A "hydroxyl" group refers, in one embodiment, to an OH group. In some embodiments, when R_1, R_2 or R_3 of the compounds of the present invention is OR, then R is not OH.

[0073] In one embodiment, the term "halo" refers to a halogen, such as F, Cl, Br or I.

[0074] In another embodiment, the phrase "phenol" refers to an alcohol (OH) derivative of benzene.

[0075] An "amino" group refers to, in one embodiment, to a nitrogen atom attached by single bonds to hydrogen atoms, alkyl groups, alkenyl groups or aryl groups as described above, as

described above, or a combination thereof. Nonlimiting examples of amino groups are NH_2, $N(Me)_2$, $N(Et)_2$, $N(Ph)_2$ and the like.

[0076] A "cycloalkyl" group refers, in one embodiment, to a non-aromatic, monocyclic or polycyclic ring comprising carbon and hydrogen atoms. A cycloalkyl group can have one or more carbon-carbon double bonds in the ring so long as the ring is not rendered aromatic by their presence. Examples of cycloalkyl groups include, but are not limited to, (C_3-C_7)cycloalkyl groups, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl, and saturated cyclic and bicyclic terpenes and (C_3-C_7)cycloalkenyl groups, such as cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, and cycloheptenyl, and unsaturated cyclic and bicyclic terpenes. Preferably, the cycloalkyl group is a monocyclic ring or bicyclic to a ring structure comprising in addition to carbon atoms, sulfur, oxygen, nitrogen or any combination thereof, as part of the ring. In another embodiment the cycloalkyl is a 3-12 membered ring. In another embodiment the cycloalkyl is a 6 membered ring. In another embodiment the cycloalkyl is a 5-7 membered ring. In another embodiment the cycloalkyl is a 4-8 membered ring. In another embodiment, the cycloalkyl group may be unsubstituted or substituted by a halogen, haloalkyl, hydroxyl, alkoxy, carbonyl, amido, alkylamido, dialkylamido, cyano, nitro, CO_2H, amino, monoalkylamino, dialkylamino, carboxyl, thio and/or thioalkyl.

[0077] A "heterocycloalkyl" group refers, in one embodiment, to a non-aromatic, monocyclic or polycyclic ring comprising carbon and in addition to carbon, sulfur, phosphor, oxygen or nitrogen, as part of the ring. A heterocycloalkyl group can have one or more double bonds in the ring so long as the ring is not rendered aromatic by their presence. Examples of heterocycloalkyl groups include, but are not limited to, piperidine, piperazine, pyrane, morpholine. Preferably, the heterocycloalkyl group is a monocyclic ring or bicyclic to a ring structure comprising in addition to carbon atoms, sulfur, oxygen, nitrogen or any combination thereof, as part of the ring. In another embodiment the heterocycloalkyl is a 3-12 membered ring. In another embodiment the heterocycloalkyl is a 6 membered ring. In another embodiment the heterocycloalkyl is a 5-7 membered ring. In another embodiment the heterocycloalkyl is a 4-8 membered ring. In another embodiment, the heterocycloalkyl group may be unsubstituted or substituted by a halogen, haloalkyl, hydroxyl, alkoxy, carbonyl, amido, alkylamido, dialkylamido, cyano, nitro, CO_2H, amino, monoalkylamino, dialkylamino, carboxyl, thio and/or thioalkyl. In another embodiment the heterocycloalkyl is a cyclic urea, imidazolinyl, imidazolidinyl, pyrrolinyl, pyrrolidinyl,

oxazolinyl, isoxazolinyl, oxazolidinyl, oxazolidonyl, isoxazolidonyl, pyrazolinyl, pyrazolidinyl, piperidyl, piperazine, morpholinyl.

[0078] The terms "alkylalkoxy", "alkylhaloalkyl", "alkylaryl", "alkylcycloalkyl", "alkylheterocycloalkyl", "alkylheteroaryl" and "alkylamino" refer, in one embodiment, to an alkyl group, as defined above, linked to alkoxy, haloalkyl, aryl, cycloalkyl, heterocycloalkyl, heteroaryl or amino group, respectively. The alkoxy, haloalkyl, aryl, cycloalkyl, heterocycloalkyl, heteroaryl or amino groups are as defined hereinabove. Examples include, but are not limited to, CH_2-OEt, CH_2-N-piperidine, CH_2-N-piperazine, CH_2-$N(Me)_2$ etc.

[0079] In another embodiment, the fused heterocycloalkyl of formula I-V with the main aromatic ring forms a phenylpyrrolidone group. In another embodiment, the fused aryl of formula I-V, with the main aromatic ring forms a naphthalene group. In another embodiment, the fused heteroaryl of formula I-V, with the main aromatic ring forms a quinoline or isoquinoline group.

[0080] In one embodiment, this invention provides for the use of a compound as herein described and/or, its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[0081] In one embodiment, the term "isomer" includes, but is not limited to, optical isomers and analogs, structural isomers and analogs, conformational isomers and analogs, and the like.

[0082] In one embodiment, the term "isomer" is meant to encompass optical isomers of the tri-phenyl compound. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereroisomeric form, or mixtures thereof, which form possesses properties useful in the treatment of telomerase activity conditions described herein. In one embodiment, the tri-phenyl compounds are the pure (*R*)-isomers. In another embodiment, the tri-phenyl compounds are the pure (*S*)-isomers. In another embodiment, the tri-phenyl compounds are a mixture of the (*R*) and the (*S*) isomers. In another embodiment, the tri-phenyl compounds are a racemic mixture comprising an equal amount of the (*R*) and the (*S*) isomers. It is well known in the art how to prepare optically-active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).

[0083] The invention includes "salts" of the compounds of this invention, which may be produced, in one embodiment, to form alkali metal salts and to form addition salts of free acids

or free bases. Suitable acid addition salts of compounds of formula I-XXVI may be prepared from an inorganic acid or from an organic acid. In one embodiment, examples of inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. In one embodiment, organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucoronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, oxalic, p-toluenesulphonic, mesylic, salicylic, p-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethylsulfonic, benzenesulfonic, sulfanilic, stearic, cyclohexylaminosulfonic, algenic, galacturonic acid. In one embodiment, suitable pharmaceutically-acceptable base addition salts of compounds of formula I-XXVI include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N′-dibenzylethyleneldiamine, choline, chloroprocaine, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procain. All of these salts may be prepared by conventional means from the corresponding compounds.

[0084] Salts can be prepared, from the phenolic compounds, in other embodiments, by treatment with inorganic bases, for example, sodium hydroxide. In another embodiment, esters of the phenolic compounds can be made with aliphatic and aromatic carboxylic acids, for example, acetic acid and benzoic acid esters.

[0085] The invention also includes use of *N*-oxides of the amino substituents of the compounds described herein.

Methods of Enhancing Amplification of a Nucleic Acid of Interest in a Polymerase Chain Reaction (PCR) Assay.

[0086] In one embodiment, this invention provides a method of enhancing amplification of a nucleic acid of interest in a polymerase chain reaction (PCR) assay, said method comprising:

a. preparing a reaction mixture comprising:

i. a nucleic acid of interest;

ii. reagents and enzyme for nucleic acid amplification in a PCR assay; and

iii. a compound of this invention; and

b. conducting a PCR assay on the reaction mixture in (a).

[0087] In some embodiments, this invention provides methods and kits which are used for enhancing amplification of a nucleic acid of interest in a PCR assay, comprising adding a compound of this invention to a reaction mixture used in PCR assays.

[0088] In one embodiment, such methods increase the efficiency of PCR reaction, the sensitivity of detection, the rapidity of detection and/or isolation, and other applications of PCR technology as will be understood by one skilled in the art.

[0089] In one embodiment, the efficiency is determined by increasing the sensitivity of detecting nucleic acids; and/or by rapid nucleic-acid diagnosis; and/or increasing the yield of the PCR product; and/or reducing the quantity of the nucleic acid sample.

[0090] In one embodiment, this invention provides a method of rapid diagnosis of a disease or condition in a subject, said method comprising:

a. isolating nucleic acid from a sample;

b. preparing a reaction mixture comprising:

i. the nucleic acid in (a);

ii. reagents for nucleic acid amplification in a PCR assay

iii. oligonucleotide primers specifically interacting with a sequence associated with a disease or condition; and

iv. a compound of this invention

c. conducting a PCR assay on the reaction mixture in (b);

d. correlating the presence or quantity of an amplified product in (c) with a disease or condition in a subject;

whereby qualitative or quantitative detection of said amplified product is achieved more rapidly than in samples assayed in the absence of said compound of this invention.

[0091] In another embodiment, the methods and kits are used for early detection of diseases. In another embodiment, the methods and kits are used in various applications in the fields of genetics, molecular biology, cellular biology, analytical biochemistry, clinical chemistry, and forensic science. In another embodiment, the methods and kits are used for parental diagnosis.

[0092] In one embodiment, the methods of this invention make use of the described compounds of this invention in contacting or binding a polymerase enzyme in an amount effective to increase polymerase activity and thereby mediating the PCR product.

[0093] In one embodiment the methods and kits of this invention make use of the PCR reagents for nucleic acid amplification in a PCR assay comprising nucleoside triphosphates, a polymerase, and single-stranded oligonucleotide primers designed to amplify a selected target nucleic acid sequence in said nucleic acid of interest.

[0094] In another embodiment the PCR reagents further comprise a buffer comprising KCl, Tris and $MgCl_2$. In another embodiment the buffer is at pH 8-9.

[0095] In another embodiment, a magnesium compound is added to the PCR reagents. In another embodiment the magnesium compound is in Mg^{2+}. The Mg^{2+} increases the PCR efficiency by maintaining the highest activity of the Taq enzyme. In another embodiment the magnesium compound is $MgCl_2$. In another embodiment, the PCR reaction can be performed without Mg^{2+}, however, with reduced efficiency.

[0096] In another embodiment the nucleoside triphosphate is a deoxynucleotide triphosphate (dNTP) mixture. In another embodiment the dNTP mixture comprises dATP (2'-deoxyadenosine 5'-triphosphate), dTTP (2'-deoxythymidine 5'-triphosphate), dCTP (2'-deoxycytidine 5'-triphosphate), and dGTP (2'-deoxyguanosine 5'-triphosphate). In another embodiment, the polynucleotide polymerase is a thermostable polymerase (Taq enzyme).

[0097] In one embodiment, the methods and kits of this invention make use of a nucleic acid. In another embodiment, the nucleic acid of interest is deoxyribonucleic acid (DNA). In another embodiment, the nucleic acid of interest is ribonucleic acid (RNA) and said reaction mixture further comprises a reverse transcriptase enzyme. In another embodiment, the methods and kits of this invention are utilized to detect the presence of a nucleic acid of interest in a sample. In

another embodiment, the methods and kits of this invention are utilized to isolate a nucleic acid of interest.

[0098] In one embodiment, the present invention allows improving the efficacy of DNA polymerase reactions, such as primer extension reaction, DNA and/or RNA sequencing, PCR, real time reverse transcription-PCR (RT-PCR) and other reactions, which can be catalyzed by a DNA polymerase and/or reverse transcriptase (RT).

[0099] In one embodiment polymerases for use in PCR applications include E. Coli DNA polymerase. In another embodiment, a DNA polymerase includes thermally stable DNA polymerases. In one embodiment, thermally stable DNA polymerases may include but are not limited to *Vent* and *Pfu*.

[00100] In another embodiment, DNA polymerases for use in PCR applications are DNA polymerases that are obtained from *Thermus aquaticus* and are called "Taq." Taq polymerase is widely used in PCR practice. Saiki *et al.*, Science 239, 487 (1989); Gelfand *et al.*, U.S. Pat. No. 4,889,818. In addition to its DNA polymerase activity, Taq DNA polymerase also possesses 5'-3' polymerization-dependent exonuclease activity, but it lacks 3'-5' exonuclease activity. (Longley *et al.*, Nuc. Acids Res. 18, 7317-7322 (1990); Blanco *et al.*, Gene 100, 27-38 (1991); Bernad et al., Cell 59, 219-228 (1989); Lawyer *et al.*, supra; Holland *et al.*, Proc. Natl Acad. Sci. 88, 7276-7280 (1991); and Kelly and Joyce, J. Mol. Biol. 164, 529-560 (1983)). Studies have identified the 5'-3' exonuclease activity as being an intrinsic part of Taq DNA polymerase. (Longely *et al.*, supra; and Barnes *et al.*, Gene 112, 29-35 (1992).

[00101] In another embodiment, DNA polymerases for use in PCR applications comprise of *Pwo* or *Pfu,* obtained from *Archaea,* which have *proofreading mechanisms* (mechanisms that check for errors) and can significantly reduce the number of mutations that occur in the copied DNA sequence. Combinations of both *Taq* and *Pfu* provide both high processivity (fast polymerisation) and high fidelity (accurate duplication of DNA).

[00102] Other forms of Taq DNA polymerase are available. AmpliTaq® is a commercially available genetically engineered version of Taq DNA polymerase and is substantially equivalent to the native form. Also commercially available is a truncated gene product, the Stoffel fragment, Stoffel Fragment is a modified form of AmpliTaq® DNA Polymerase from which the N-terminal 289 amino acids are deleted. Stoffel Fragment differs from AmpliTaq® DNA Polymerase in that it

is more thermostable (by approximately 2-fold), exhibits optimal activity over a broader range of magnesium ion concentration (2 mM-10 mM) and lacks intrinsic 5' to 3' exonuclease activity. The unique properties of Stoffel Fragment make it especially useful for Arbitrarily Primed PCR (AP-PCR) or Random Amplified Polymorphic DNA (RAPD) amplification methods, whereby genomic DNA is amplified with a set of short primers of arbitrary sequence.

[00103] In one embodiment, primers for use in PCR applications comprise a single-stranded oligonucleotide primer. In some embodiments, the term "primer" refers to a single stranded DNA or RNA molecule that can hybridize to a polynucleotide template and prime enzymatic synthesis of a second polynucleotide strand. A primer useful in the methods/kits of the invention is, in some embodiments, between 10 to 100 nucleotides in length, or in another embodiment 17-50 nucleotides in length, or in another embodiment 30-70 nucleotides in length.

[00104] In one embodiment, this invention provides methods and kits for the significant improvement of the processivity of DNA polymerases, which in one embodiment is effected by the formation of a covalent linkage between the polymerase domain and a compound of this invention.

[00105] As used herein, "processivity" refers to the ability of a polynucleotide modifying enzyme, for example a polymerase, to remain attached to the template or substrate and perform multiple modification reactions. "Modification reactions" include but are not limited to polymerization, and exonucleolytic cleavage. "Processivity" also refers to the ability of a polynucleotide modifying enzyme, for example a polymerase, to modify relatively long (for example 0.5-1 kb, 1-5 kb or 5 kb or more) tracts of nucleotides. "Processivity" also refers to the ability of a polynucleotide modifying enzyme, for example a DNA polymerase, to perform a sequence of polymerization steps without intervening dissociation of the enzyme from the growing DNA chains. "Processivity" can depend on the nature of the polymerase, the sequence of a DNA template, the compounds of this invention, and reaction conditions, for example, salt concentration, temperature or the presence of specific proteins.

[00106] Methods for measuring processivity of a DNA polymerase are generally known in the art, e.g., as described in Sambrook et al. 1989, In Molecular Cloning, 2nd Edition, CSH Press, 7.79-7.83 and 13.8, and as described in U.S. patent application with Ser. No. 2002/0119467, hereby incorporated by reference. Processivity and increased processivity can be measured

according the methods defined herein and in Pavlov et al., supra and WO 01/92501 A1. Processivity can also be measured by any known method in the art, e.g., as described in U.S. Pat. No. 5,972,603, the entirety of which is incorporated herein by reference.

[00107] In one embodiment, determining effects on DNA processivity is accomplished by heat denaturation of a DNA template containing a target sequence, annealing of a primer to the DNA strand and extension of the annealed primer with a DNA polymerase. Effects on net DNA processivity is determined via obtaining the ratio of DNA synthesis activity versus 3'-5' exonuclease activity (for reviews, see, e.g., Kelman et al., 1998 *Processivity of DNA polymerases: two mechanisms, one goal.* Structure 6(2):121-5; Wyman and Botchan, 1995, *DNA replication. A familiar ring to DNA polymerase processivity.* Curr Biol. 5(4):334-7; and Von Hippel et al., 1994, *On the processivity of polymerases.* Ann N Y Acad Sci. 726:118-31).

[00108] Methods/kits to enhance polymerase processivity can be used in a variety of applications, including genetic engineering, recombinant protein production, diagnostic assays, PCR cloning, PCR assay for gene expression studies, forensic applications, and others, as will be appreciated by one skilled in the art.

[00109] In one embodiment a PCR test sample comprises one or more targeted nucleic acid sequences in a total volume of usually about 20-200 µl with the following reagents: an aqueous buffer, pH 8-9 at room temperature, usually also containing approximately 0.05M KCl; all four common nucleoside triphosphates (e.g., for DNA polymerase, the four common dNTPs: dATP, dTTP, dCTP, and dGTP) at concentrations of approximately 10^{-5} M-10^{-3} M; a magnesium compound, usually $MgCl_2$, usually at a concentration of about 1 to 5 mM; a polynucleotide polymerase, preferably a thermostable DNA polymerase, usually at a concentration of 10^{-10} to 10^{-8} M; single-stranded oligonucleotide primers, usually 15 to 30 nucleotides long and usually composed of deoxyribonucleotides, containing base sequences which are Watson-Crick complementary to sequences on both strands of the target nucleic acid sequence(s). Each primer usually is present at a concentration of 10^{-7} to 10^{-5} M; primers are synthesized by solid-phase methods well known in the art of nucleic acid chemistry; and a compound of this invention.

[00110] In another embodiment, the methods and kits of this invention may make use of other known components including but not limited to: solvents; buffers; detergents (e. g., Triton X-100, Nonidet P-40 (NP-40), Tween-20) and agents that disrupt mismatching of nucleotide pairs,

such as dimethylsulfoxide (DMSO), and tetramethylammonium chloride (TMAC); empty syringes; tubing, gauze, pads, disinfectant solution, etc. (In some embodiments, the solvents used for PCR contain a buffering agent, (e. g., Tris-HCl) and non-buffering salts (e.g., KCl). The buffering agent may be any known buffers in the art, and may be varied to optimize PCR results by routine experimentation. One skilled in the art will readily be able to determine optimal buffering conditions. Some PCR buffers may be optimized depending on the enzyme used. For example, AmpliTaq Gold® DNA polymerase has an optimum KCl concentration of 50 mM; AmpliTaq© DNA Polymerase, Stoffel fragment has an optimum KCl concentration of 10 mM; and rTth DNA Polymerase and rTth DNA Polymerase XL have an optimum KCl concentration of 75-100 mM.

[00111] In one embodiment, the amplification of a sequence of interest increases in a range of between about 1.2-1.5-fold by using a compound of this invention as compared to a controlled sample. In another embodiment, the amplification of a sequence of interest increases in a range of between about 1.5-2.5 folds as compared to a control sample. In another embodiment, the amplification of a sequence of interest increases in a range of between about 2.5-3 folds as compared to a control sample. In another embodiment, the amplification of a sequence of interest increases in a range of between about 1.2-3.5 folds as compared to a control sample. In another embodiment, 62.5 nM of compound XIV enhanced the PCR activity by 1.4 compared to the control solution, according to Example 10 and Figure 2B. In another embodiment, 62.5 nM of compound XV enhanced the PCR activity by 2.3 compared to the control solution, according to Example 10 and Figure 2B. In another embodiment, 62.5 nM of compound XVI enhanced the PCR activity by 1.7 compared to the control solution, according to Example 10 and Figure 2B. In another embodiment, 62.5 nM of compound XVII enhanced the PCR activity by 2.8 compared to the control solution, according to Example 10 and Figure 2B. In another embodiment, the amplification of a sequence of interest depends on the concentration of the compound of this invention.

[00112] In one embodiment, the methods and kits of this invention make use of the PCR assay for PCR cloning, which is used to clone a PCR product.

[00113] DNA cloning vectors, are an essential tool in DNA cloning. The term "vector" is commonly known in the art and defines a cosmid, plasmid, phage, virus, bacmid, phagemid, shuttle vector and the like, which can serve as a DNA vehicle into which DNA can be cloned.

[00114] There are at least three strategies for cloning PCR products. 1) T/A cloning. T/A cloning takes advantage of the terminal transferase activity of *Taq* polymerase and other non-proofreading DNA polymerases which adds a single 3'-A overhang to each end of the PCR product. The resulting PCR product is then ligated into a linear vector with a 3´ terminal 'T' or 'U' at both ends. 2) Restriction stick end cloning. A restriction enzyme target site is introduced into each of the PCR primers. The resulting PCR product and cloning vector are digested with the restriction enzymes to generate complementary ends at the PCR product and the vector which are then ligated. 3) Blunt-end cloning. Blunt-end PCR product generated by proof-reading polymerase such as the *Pfu* DNA Polymerase can also be cloned into a blunt-end vector.

[00115] In one embodiment, the methods and kits of this invention make use of the PCR assay for trace gene expression.

[00116] In one embodiment, the methods and kits of this invention make use of the PCR assay for forensic applications.

[00117] Forensic applications includes forensic analysis, including microbial, DNA and toxicology screens. Forensic applications include but not limited to testing samples collected from crime victims and crime scenes and/or testing contagion sampling on tissues or organs that may be used for transplantation, as well as testing diseased tissue and surgical instrumentalities, and/or testing parenthood identification, and/or testing samples collected from bioterrorism acts, state- sponsored bioweapons programs, industrial scale accidents and analyses of natural anthrax cases.

[00118] In one embodiment, the methods and kits of this invention make use of a PCR assay wherein the PCR is a reverse transcriptase PCR (RT-PCR) assay.

[00119] RT-PCR is a method used to amplify, isolate or identify a known sequence from a cellular or tissue RNA. The PCR reaction is preceded by a reaction using reverse transcriptase to convert RNA to cDNA. RT-PCR is widely used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites and, if the genomic DNA sequence of a gene is known, to map the location of exons and introns in the gene. The 5' end of a gene (corresponding to the transcription start site) is typically identified by a RT-PCR method, named RACE-PCR, short for *Rapid Amplification of cDNA Ends*.

[00120] In one embodiment, the methods and kits of this invention make use of a PCR assay, wherein the assay is a real time- PCR (rt-PCR) assay.

[00121] Real time PCR, also called quantitative real time polymerase assay, is used to measure the quantity of a PCR product. The procedure follows the general pattern of polymerase chain reaction, but the DNA is quantified after each round of amplification; this is the "real-time" aspect of it. Two common methods of quantification are the use of fluorescent dyes that intercalate with double-strand DNA, and modified DNA oligonucleotide probes that fluoresce when hybridized with a complementary DNA. Real time PCR methods use fluorescent dyes, such as Sybr Green, or fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time. Frequently, real-time polymerase chain reaction is combined with reverse transcription polymerase chain reaction to quantify low abundance messenger RNA (mRNA), enabling a researcher to quantify relative gene expression at a particular time, or in a particular cell or tissue type. Real time PCR is used also for the detection of viral and bacterial pathogens in clinical samples and for the detection of cancer cells in patients with a history of leukemias (and other cancers such as those that arise in the breast, lung, colon, esophagus and skin).

[00122] In one embodiment, the methods and kits of this invention make use of a PCR assay, which is a Quantitative-PCR (Q-PCR) assay.

[00123] Quantitative PCR (Q-PCR) is used to measure the quantity of a PCR product (preferably real-time). It is the method of choice to quantitatively measure starting amounts of DNA, cDNA or RNA. Q-PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample.

[00124] A quantitative reverse transcriptase polymerase chain reaction (QRT-PCR) method is provided for rapidly and accurately detecting low abundance RNA species in a population of RNA molecules (for example, and without limitation, total RNA or mRNA), including the steps of: a) incubating an RNA sample with a reverse transcriptase and a high concentration of a target sequence-specific reverse transcriptase primer under conditions suitable to generate cDNA; b) subsequently adding suitable polymerase chain reaction (PCR) reagents to the reverse transcriptase reaction, including a high concentration of a PCR primer set specific to the cDNA and a thermostable DNA polymerase to the reverse transcriptase reaction, and c) cycling the

PCR reaction for a desired number of cycles and under suitable conditions to generate PCR product ("amplicons") specific to the cDNA.

[00125] In one embodiment the methods and kits of this invention make use of the PCR reagents and the compounds of this invention. In another embodiment, the concentration of the compounds of this invention in said PCR reagents mixture is between about 1×10^{-9} to 10×10^{-9} M. In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 5×10^{-9} to 130×10^{-9} In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 1×10^{-9} to 100×10^{-9} M. In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 10×10^{-9} to 20×10^{-9} M. In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 1×10^{-9} to 30×10^{-9} M. In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 20×10^{-9} to 50×10^{-9} M. In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 30×10^{-9} to 70×10^{-9} M. In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 70×10^{-9} to 100×10^{-9} M. In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 60×10^{-9} to 130×10^{-9} M. In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 60×10^{-9} to 70×10^{-9} M. In another embodiment, the concentration of a compound of this invention in said PCR reagents is between about 120×10^{-9} to 130×10^{-9} M.

[00126] In one embodiment, the effects of the compounds of this invention on the polymerase chain reaction (PCR) is investigated via quantitative PCR product measurements, scanning electron microscopy (SEM), high-resolution transmission electron microscopy (TEM) and x-ray photoelectron spectroscopy (XPS). In another embodiment, the reaction products are analyzed on agarose gel wherein the gel is photographed.

[00127] In one embodiment, the compounds of this invention have a chemical interaction with the PCR components, and thereby may increase the PCR efficiency. In another embodiment, the compounds of this invention have the potential to act as catalysts in a variety of biochemical reactions.

PCR Kits

[00128] In some embodiments, this invention provides kits which may comprise at least one compound of this invention, in any form or embodiment as described herein. In some embodiments, the term "a" is to be understood to encompass a single or multiple of the indicated material. In some embodiments, the term "a" or "an" refers to at least one.

[00129] In some embodiments, any of the kits of this invention will consist of a compound of this invention, in any form or embodiment as described herein. In some embodiments, of the kits of this invention will consist essentially of a compound of this invention, in any form or embodiment as described herein.

[00130] In some embodiments, the term "comprise" refers to the inclusion of the indicated active agent, such as the compounds of this invention, as well as inclusion of other active agents, and PCR reagents as are known in the art. In some embodiments, any of the kits of this invention will comprise a compound of formula I -XXVI, in any form or embodiment as described herein. In some embodiments, any of the kits of this invention will consist of a compound of formula I - XXVI in any form or embodiment as described herein. In some embodiments, of the kits of this invention will consist essentially of a compound of I-XXVI, in any form or embodiment as described herein. In some embodiments, the term "consisting essentially of" refers to a kit whose only active ingredient is the indicated active ingredient, however, other compounds may be included which are for stabilizing, preserving, etc. In some embodiments, the term "consisting essentially of" refers to a composition, whose only active ingredient with a comparable mode of action, or comparable molecular target is the indicated active ingredient, however, other active ingredients may be incorporated, with such secondary active ingredients acting on different targets, or in a palliative capacity. In some embodiments, the term "consisting essentially of" may refer to components which facilitate the release of the active ingredient. In some embodiments, the term "consisting" refers to a composition, which contains the active ingredient and a pharmaceutically acceptable carrier or excipient.

[00131] In another embodiment, the invention provides a kit comprising a compound of this invention, as herein described, or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

[00132] In some embodiments, this invention provides a kit which is used for increasing the efficacy of polymerase chain reactions, comprising a compound of this invention. In one embodiment, this invention provides a kit useful in the fields of genetics, molecular biology, cellular biology, analytical biochemistry, clinical chemistry, and forensic sciences for simple, rapid, safe, sensitive, and accurate diagnosis of diseases and/or conditions.

[00133] In one embodiment, the kit may comprise a first container containing a compound of this invention and at least a second container having one or more components suitable for performing a polymerase chain reaction. In another embodiment the second container may contain one of more of (a) dNTPs; (b) a DNA polymerase or reverse transcriptase (RT) enzyme; (c) reaction buffer(s) and (d) a primer. In another embodiment, the kit may comprise two or more, e.g. three, four or five separate containers with these or other components packaged separately or in combinations thereof.

[00134] In one embodiment, the amounts and proportions of reagents provided in the kit may be selected so as to provide optimum results for a particular application.

[00135] In one embodiment, the kits of this invention may be supplied in all manner of containers such that the activities of the different components are substantially preserved, while the components themselves are not substantially adsorbed or altered by the materials of the container. Suitable containers include but are not limited to ampoules, bottles, test tubes, vials, flasks, syringes, bags and envelopes (e.g., foil-lined), and the like. The containers may be formed of any suitable material including but not limited to glass, organic polymers (e.g., polycarbonate, polystyrene, polyethylene, etc.), ceramic, metal (e.g., aluminum), metal alloys (e.g., steel), cork, and the like. In another embodiment, the containers may contain one or more sterile access ports (e.g., for access via a needle), such as may be provided by a septum. Preferred materials for septa include rubber and polymers including but not limited to, for example, polytetrafluoroethylene of the type sold under the trade name TEFLON by DuPont (Wilmington, DE). In addition, the containers may contain two or more compartments separated by partitions or membranes that can be removed to allow mixing of the components.

[00136] In one embodiment, the kits of this invention may also be supplied with other items known in the art and/or which may be desirable from a commercial and user standpoint, such as instructions for performing PCR.

[00137] In one embodiment, instructional materials provided with kits of this invention may be printed (e.g., on paper) and/or supplied in an electronic-readable medium (e.g., floppy disc, CD-ROM, DVD-ROM, zip disc, videotape, audio tape, etc.). Alternatively, instructions may be provided by directing a user to an Internet web site (e.g., specified by the manufacturer or distributor of the kit) and/or via electronic mail.

[00138] The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

[00139] **EXAMPLES**

EXAMPLE 1

Synthesis of compound of formula VIII

[00140] 1,1,1-tris(4-hydroxyphenyl)ethane (4 g, 13 mM), formaldehyde (3.6 g,120 mM) and a 40% solution of dimethylamine in water (15 ml) were added to a solution of 50 ml water and 60 ml EtOH. The solution was refluxed for 2.5 hours. Partial evaporation of the solvent precipitated a white solid, which was filtered, washed with water and dried to give 7.85 g white solid of compound of formula VII, 93% yield, mp. =169°.

[00141] NMR $CDCl_3$ ™ 6.64(6H, s, ArH), 3.40(12H, s, CH_2), 2.22(36H, s,N- CH_3), 2.06(3H, s, C-CH_3).

EXAMPLE 2

Synthesis of compound of formula IX

[00142] Compound of formula IX was synthesized by a process comparable to that described in Example 1.

[00143] NMR $CDCl_3$ ™ 6.71(6H,s, ArH),3.58(12H,s,CH_2),2.54(24H,q,J=7.0 Hz),1.04(24H,t,J=7.0 Hz).

EXAMPLE 3

Synthesis of compound of formula X

[00144] 1,1,1-tris(4-hydroxyphenyl)ethane (1.53 gr,5 mM), formaldehyde (1.35 gr, 45 mM) and 1-methyl piperazine (2.5 ml,50 mM) in 20 ml water and 25 ml EtOH were refluxed for 3 hours. Evaporation provided a solid that by TLC and NMR contained 2 products, which was not the starting material. Formaldehyde (0.75 gr,25 mM) and 1-methyl piperazine (1.5 ml, 30 mM) were added to 5 ml water and 10 ml EtOH and the reaction was refluxed for 4 hours. Evaporation and workup gave 3.3 gr light yellow- white solid, 67% yield, mp. -63°. Soluble in ethanol, and very good solubility in water.

[00145] NMR $CDCl_3$ ™ 6.67(6H,s,ArH), 3.53(12H, s, CH_2), 2.44(48H,br.m,ring piperazine),2.26(18H,s,N- CH_3), 2.00(3H,s,C-CH_3).

EXAMPLE 4

Synthesis of compound of formula XI

[00146] A compound of formula XI was synthesized by a process comparable to that described in Example 1. A white solid was obtained. mp. =178°.

[00147] NMR $CDCl_3$ ™ 6.68(6H,s, ArH),3.55(12H,s,CH_2),2.51(24H,br.t,N- CH_2 ring),2.03(3H,s,C-CH_3), 1.55(24H,br.t,N- CH_2 ring),1.42(12H,br.s).

[00148] **EXAMPLE 5**

Synthesis of compound of formula XII

[00149] A compound of formula XII was synthesized by a process comparable to that described in Example 1. A white solid was obtained. m.p. =135°.

[00150] NMR $CDCl_3$ □ 6.68(6H,s, ArH),3.61(12H,s,CH_2),2.51(24H,br.t,N- CH_2 ring),2.03(3H,s,C-CH_3), 1.76(24H,br.t,N- CH_2 ring).

EXAMPLE 6

Synthesis of compound of formula XIII

[00151] A compound of formula XIII was synthesized by a process comparable to that described in Example 1. White solid was obtained. mp. =212°.

[00152] NMR $CDCl_3$ □ 6.68(6H,s, ArH),3.69(24H,t,J=4.5 Hz ,N- CH_2 ring),3.52 (12H,s,CH_2),2.45(24H,br.t,O- CH_2 ring),2.03(3H,s,C-CH_3).

EXAMPLE 7

Synthesis of compound of formula XIV

[00153] **Step 1:** Compound of formula VII (2.98 g, 4.6 mM,), prepared by a process as described in Example 1 was added to 20 ml acetic anhydride, and heated to 100° for 4 hours. The mixture was cooled and water was added. The mixture was stirred overnight at room temperature, and then extracted with CH_2Cl_2. The solvent was evaporated to give a nona-acetate derivative as yellow oil and was further purified by chromatography (silica gel; 1%MeOH/CH_2Cl_2) to give 3.2 gr of viscous yellow oil, 80% yield..

[00154] **Step 2:** A KOH (4g) solution in water was added to a solution of the nona-acetate of step 1 (2.5 g) in 20 ml EtOH. The mixture was stirred for 20 hours at room temperature. The mixture was acidified with HCl, and extracted with CH_2Cl_2. The solvent was evaporated and gave 2.2 g of a yellow oil that and was further purified by column chromatography (silica gel; 2% MeOH/CH_2Cl_2) and recrystallyzed from toluene-hexane to give 1 gr of compound XII, 53% yield, white solid, mp-78°. TLC – Rf=0.55 in 5%MeOH/CH_2Cl_2.

[00155] NMR $CDCl_3$ ™ 7.93(3H,s,OH),6.79(6H,s,Ar-H),4.54(12H,s,Ar-CH_2),3.55(12H,q,J=7.0 Hz,CH_2),2.05(3H,s,C-CH_3),1.22(18H,t,J=7.0 Hz,CH_3).

EXAMPLE 8

Synthesis of 1,1,1-tris(4-hydroxy-3, 5 –dibromo-phenyl)-ethane (compound XVII)

[00156] **Step 1:** A solution of NaOH (1 g, 25 mM) in 10 ml water and dimethyl sulphate (5.1 gr, 40 mM)(1:8 molar ratio) was added during 1 hour and simultaneously in portions to a solution of 1,1,1-tris(4-hydroxyphenyl)-ethane (1.53 g, 5 mM) in 20 ml ethanol and 10 ml water. The solution was then refluxed for 1 hour, and stirred 70 hours at RT. The white precipitate was

filtered, washed with water and dried to give 1.74 g of 1,1,1-tris(4-methoxyphenyl)-ethane. Recrystalization twice from 50 ml ethanol gave 1.15 gr white crystals, 66% yield, m.p.-160°. TLC Rf=0.85 in CH_2Cl_2.

[00157] NMR $CDCl_3$ ™ 6.99,6.79(12H, AB_q, J_{AB}=8.8 Hz), 3.78(9H,s,OCH_3), 2.11(3H, s,CH_3).

[00158] **Step 2:** To a solution of 1,1,1-tris(4-methoxyphenyl)-ethane (0.49 gr,1.4 mM,),from step 1, in 22 ml 1,2-dichloroethane, a solution of bromine (1.65 gr, 10.2) (7.3:1 ratio) in 5 ml 1,2-dichloroethane was added in portions. The solution was stirred at RT overnight and heated for 3 hours to 70°, and worked up (sodium thiosulphate) to give 1.0 gr crude product. TLC shows no starting material, but NMR showed mixtures, indicating that the bromination was not complete (m at 6.90 ppm, and 4 methoxy). The solid was brominated again with 1 gr bromine and refluxed 18 hours. The mixture was worked up as above and triturated with hot ethanol to give 0.27 gr white solid, 23% yield, mp= 160°. TLC Rf=0.95 in CH_2Cl_2.

[00159] NMR $CDCl_3$ ™ 7.16(6H,s,ArH), 3.92,3.91(6:4 ratio)(9H,2s,OCH_3),2.04,2.03 (4:6 ratio)(3H,s,CH_3).

EXAMPLE 9

Synthesis of 1,1,1-tris(4-hydroxy-3, 5 –diiodo-phenyl)-ethane

[00160] To 1,1,1-tris(4-hydroxyphenyl)-ethane (1.53 gr, 5 mM) in 40 ml ethanol and 40 ml water cooled in ice, KOH (2.2 gr,39.2 mM) followed by KI (5.8 gr,34.8 mM) and iodine (8.8 gr,34.7 mM) were added. The color turns from violet to brown. The reaction was stirred at room temperature for 3 hours. The mixture was added to crushed ice. Concentrated HCl was added to obtain acidic pH and was treated with thiosulphate solution and worked up. Evaporation gave 5.1 gr light brown solid, hexa iodo product followed by trituration in ethanol gave 3 gr white solid, 61% yield, mp=230°. RF=0.8 (in 5%MeOH- CH_2Cl_2).

[00161] NMR $CDCl_3$ ™ 7.3(6H,s),5.77(br.s,OH),1.97(3H,s,CH_3).

EXAMPLE 10

Enhancement of PCR products by Compounds of This Invention

Methods:

[00162] Solutions of 62.5 nM and 125 nM of a compounds XIV, XV, XVI and XVII were prepared and added to a PCR reaction containing radioactive nucleotide (2 μci [αP^{32}]dCTP) and the following primers: 5` AATCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT3` (10^{-16}mol) and 5` ATCGCTTCTCGGCCTTTT3`(0.1 μg) and 2 units of thermostable DNA polymerase (Taq pol.) 28 cycles of 30s at 94 °C, 30s at 59°C, 90s at 72°C. The reaction products were analyzed on 12.5% non-denaturing polyacrylamide gel (PAG) followed by autoradiography.

Results:

[00163] Compounds XIV, XV, XVI and XVII enhanced the PCR activity by using 62.5 nM or 125 nM solutions (Figures1A, 2A and 2B). Compounds XIV, XV, XVI and XVII enhanced the PCR activity by 1.4, 2.3, 1.7 and 2.8 folds respectively, compared to a control solution (Figure 2B).

EXAMPLE 11

PCR Assay For Identification of Specific Gene (GAPDH)

Methods:

[00164] Total RNA was extracted from cells using the Tri-reagent kit. RT-PCR for GAPDH was performed using PCR reaction mixture (Sigma), GAPDH primers:

Fw: 5'-TTCACCACCATGGAGAAGGC-3' and

Rv: 5'-GGCATGGACTGTGGTCATGA-3'.

The amount of cDNA in the PCR assay was 150 ng. Compounds XIV-XVII were added to the PCR reaction mixture at various concentrations in DMSO. DMSO, 0.8% final concentration was added as a control. Thirty five cycles of (94°C, 55°C, 72°C of 30s each). Before the first cycle the samples were subjected to 94°C for 5 min. After the last cycle the samples were incubated at 72°C for 7 min. The reaction products were analyzed on 1% agarose gel in the presence of EtBr and the gel was photographed by ChemiImager ™ 5500 equipment using a short wavelength UV lamp.

Results:

[00165] The GAPDH reaction product using compound XVI was enhanced in a concentration dependant manner from 0.0625 μM to 0.5μM. (Figure 3).

[00166] Similar results were obtained using compound XV at 60 nM and 120 nM, while compound XVII at 120 nM decreased the enhancement of GAPDH compared to 60nM. (Figure 4).

EXAMPLE 12

PCR assay for identification of Human T cell leukemia type I

(HTLV-I) infection

Methods:

[00167] **Infected Cells**: Total RNA was isolated from SLB-I cells which are chronically infected with HTLV-I and constantly produce the virus. RT-PCR was performed using specific HTLV-I primers directed to the 3` LTR of the virus.

Fw: 5'-ATCCACGCCGGTTGAGTCGC-3';

Rv: 5'-CACTCAGTCGTGAATGAAAG-3' .

Decreasing amounts of cDNA were used in the absence or presence of Compound XVII at 15 nM. Control samples were: -cDNA or cDNA derived from uninfected Jurkat cells (T lymphoma cells). Thirty five cycles of (94°C, 55°C, 72°C of 30s each). Before the first cycle the samples were subjected to 94°C for 5 min. After the last cycle the samples were incubated at 72°C for 7 min. The reaction products were analyzed on 1% agarose gel in the presence of EtBr and the gel was photographed by ChemiImager ™ 5500 equipment using a short wavelength UV lamp.

Results:

[00168] The HTLV-I reaction product using compound XVII was enhanced compared to the control (ST) and increased with higher concentration of cDNA (from 0.015 to 0.15 μg. (Figure 5).

[00169] PCR assay was performed in the absence or presence of compounds XVII and VIII (15 or 30 nM) and 8 ng of cDNA from SLBI infected and Jurkat uninfected cells. HTLV-I was not detected in the uninfected cells, and was detected in the SLBI-HTLV-I cells. (Figure 6A and 6B).

[00170] Mixtures of Jurkat uninfected cells with SLB-I infected cells at different ratios

Infected:uninfected- 1:1, 1:10, 1:100, 1:1000, 1:10,000 were prepared. Total RNA was extracted and RT-PCR was performed. 15 nM of compound XVII or VII was added to the PCR reaction mixture containing 4 ng of cDNA in a ratio of Infected/uninfected of 1:1 respectively (Figure 7A) and 8ng cDNA in a ratio of Infected/uninfected of 1:100 respectively (Figure 7B). In both experiments the HTLV-I was identified and detected.

[00171] While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

CLAIMS

What is claimed is:

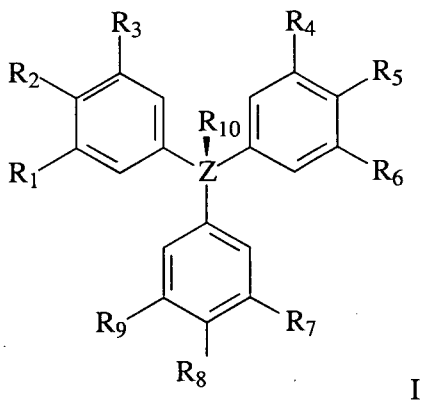
1. A method of enhancing amplification of a nucleic acid of interest in a polymerase chain reaction (PCR) assay, said method comprising:

a. preparing a reaction mixture comprising:

i. a nucleic acid of interest;

ii. reagents for nucleic acid amplification in a PCR assay; and

iii. a compound represented by the structure of formula I:



I

wherein

Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R_1 to R_9 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_3, R_4, or R_7, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

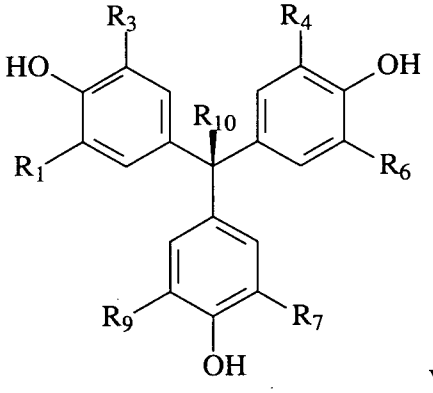
R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof; and

b. conducting a PCR assay on the reaction mixture in (a).

2. The method of claim 1, wherein said compound of formula 1, is at a concentration ranging from between about 5×10^{-9} to 130×10^{-9} M .

3. The method of claim 1, wherein said reagents for nucleic acid amplification in a PCR assay comprise nucleoside triphosphates, a thermostable processive polymerase, and single-stranded oligonucleotide primers designed to amplify a selected target nucleic acid sequence in said nucleic acid of interest.

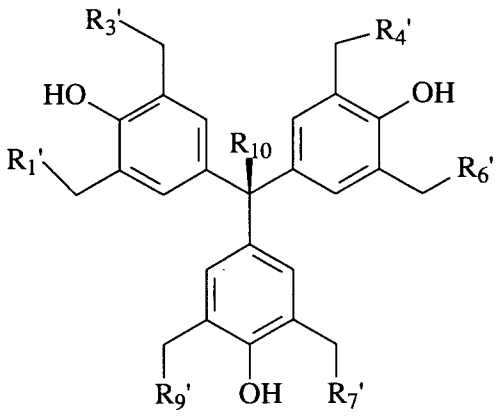
4. The method of claim 1, wherein said compound is represented by the structure of formula V:



V

wherein R_1, R_3, R_4, R_6, R_7, R_9 and R_{10} are as described above.

5. The method of claim 4, wherein said compound is represented by the structure of formula VII:

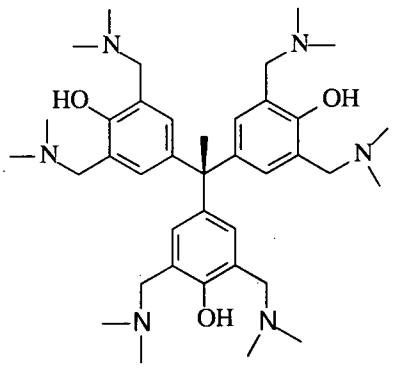


VII

wherein R_1', R_3', R_4', R_6' R_7', and R_9' are the same or different comprising halogen, aryl, alkyl, cycloalkyl, heterocycloalkyl, alkoxy, monoalkylamino, dialkylamino or arylamino; and

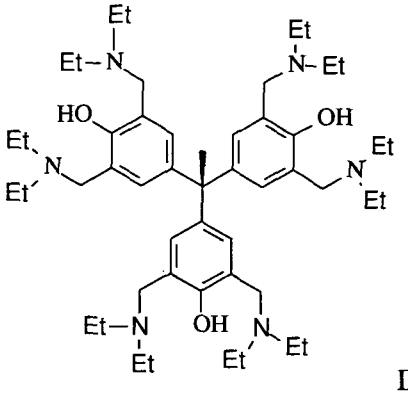
R_{10} is as described above.

6. The method of claim 5, wherein said compound is represented by the structure of formula VIII:



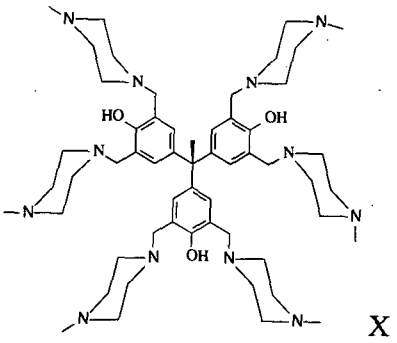
VIII

7. The method of claim 5, wherein said compound is represented by the structure of formula IX:



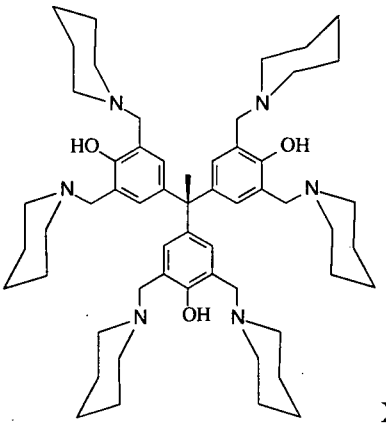
IX

8. The method of claim 5 wherein said compound is represented by the structure of formula X:



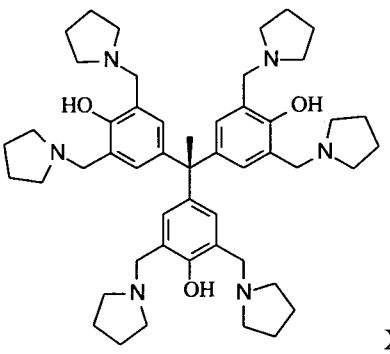
X

9. The method of claim 5, wherein said compound is represented by the structure of formula XI:



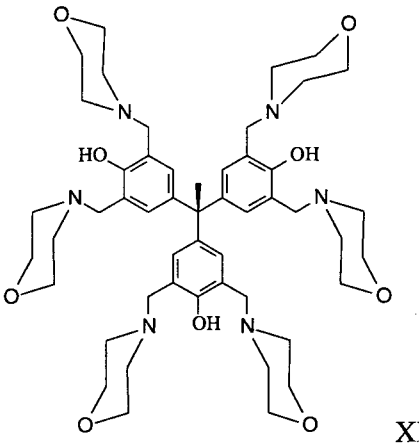
XI

10. The method of claim 5, wherein said compound is represented by the structure of formula XII:



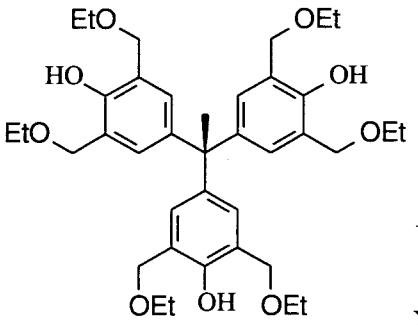
XII

11. The method of claim 5, wherein said compound is represented by the structure of formula XIII:



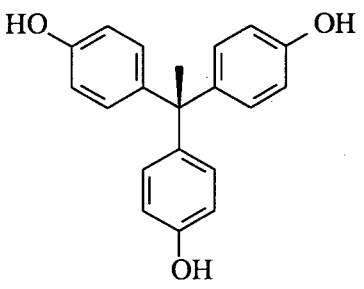
XIII

12. The method of claim 5, wherein said compound is represented by the structure of formula XIV:



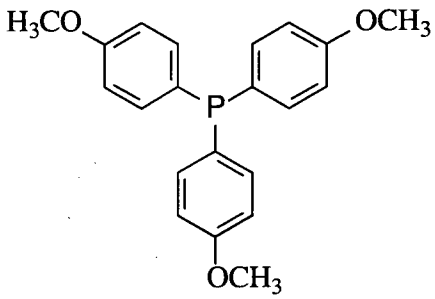
XIV

13. The method of claim 1, wherein said compound is represented by the structure of formula XV:



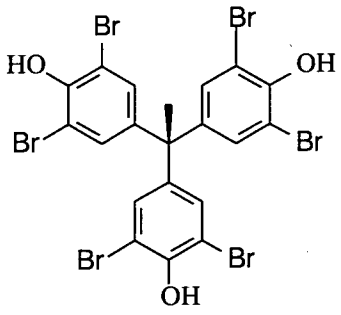
XV

14. The method of claim 1, wherein said compound is represented by the structure of formula XVI:



XVI

15. The method of claim 1, wherein said compound is represented by the structure of formula XVII:



XVII

16. The method of claim 1, wherein said nucleic acid of interest is deoxyribonucleic acid (DNA).

17. The method of claim 1, wherein said nucleic acid of interest is ribonucleic acid (RNA) and said reaction mixture further comprises a reverse transcriptase enzyme.

18. The method of claim 1, wherein said method is utilized to detect the presence of a nucleic acid of interest in a sample.

19. The method of claim 1, wherein said method is utilized to isolate a nucleic acid of interest.

20. A method of rapid diagnosis of a disease or condition in a subject, said method comprising:

a. isolating nucleic acid from a sample;

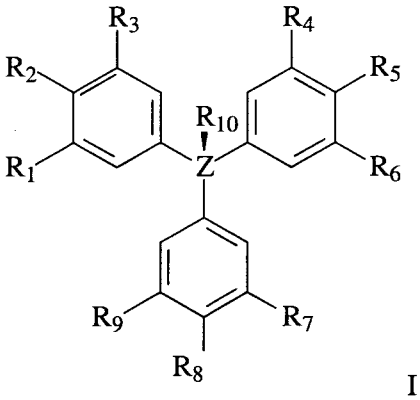
b. preparing a reaction mixture comprising:

i. the nucleic acid in (a);

ii. reagents for nucleic acid amplification in a PCR assay;

iii. oligonucleotide primers specifically interacting with a sequence associated with a disease or condition; and

iv. a compound represented by the structure of formula I:



I

wherein

Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R_1 to R_9 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_3, R_4, or R_7, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino,

alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof;

c. conducting a PCR assay on the reaction mixture in (b);

d. correlating the presence or quantity of an amplified product in (c) with a disease or condition in a subject;

whereby qualitative or quantitative detection of said amplified product is achieved more rapidly than in samples assayed in the absence of said compound represented by the structure of formula I.

21. The method of claim 20, wherein said method indicates the severity of the disease or condition.

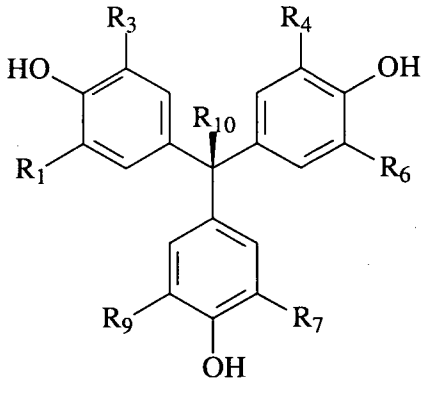
22. The method of claim 20, wherein said method indicates responsiveness of the subject to therapy.

23. The method of claim 20, wherein said compound is at a concentration ranging from between about 5×10^{-9} to 130×10^{-9} M .

24. The method of claim 20, wherein said reagents for nucleic acid amplification in a PCR assay comprise nucleoside triphosphates, a thermostable processive polymerase, and single-stranded oligonucleotide primers designed to amplify a selected target nucleic acid sequence in said nucleic acid of interest.

25. The method of claim 24 wherein said nucleoside triphosphates for DNA polymerase are dNTPs: dATP, dTTP, dCTP, and dGTP).

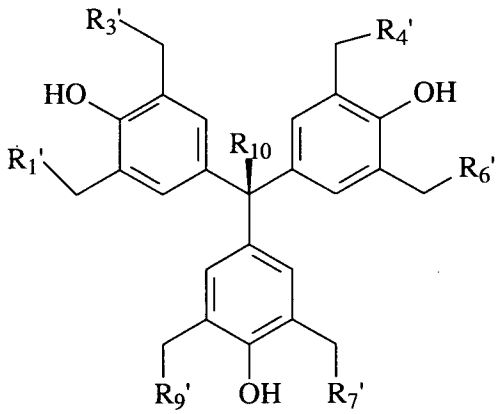
26. The method of claim 20 wherein said compound is represented by the structure of formula V:



V

wherein R_1, R_3, R_4, R_6, R_7, R_9 and R_{10} are as described above.

27. The method of claim 26, wherein said compound is represented by the structure of formula VII:

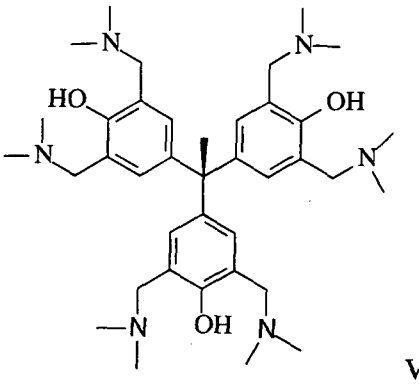


VII

wherein R_1', R_3', R_4', R_6' R_7', and R_9' are the same or different comprising halogen, aryl, alkyl, cycloalkyl, heterocycloalkyl, alkoxy, monoalkylamino, dialkylamino or arylamino; and

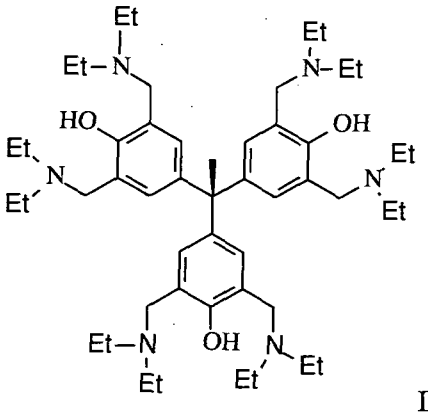
R_{10} is as described above.

28. The method of claim 27, wherein said compound is represented by the structure of formula VIII:



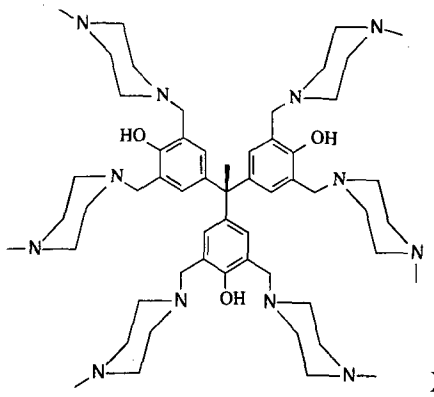
VIII

29. The method of claim 27, wherein said compound is represented by the structure of formula IX:



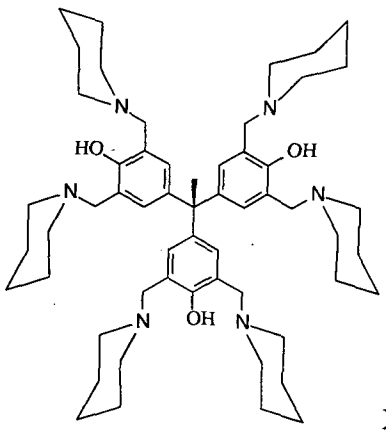
IX

30. The method of claim 27, wherein said compound is represented by the structure of formula X:



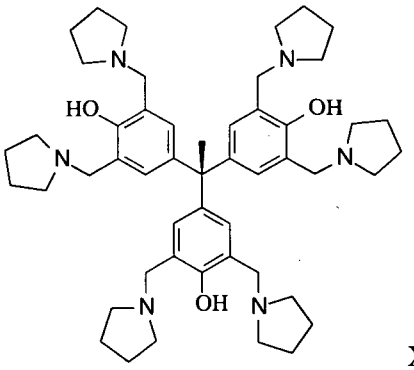
X

31. The method of claim 27, wherein said compound is represented by the structure of formula XI:



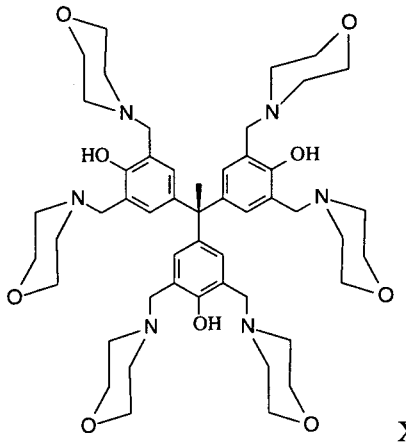
XI

32. The method of claim 27, wherein said compound is represented by the structure of formula XII:



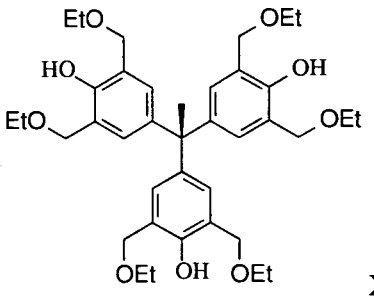
XII

33. The method of claim 27, wherein said compound is represented by the structure of formula XIII:



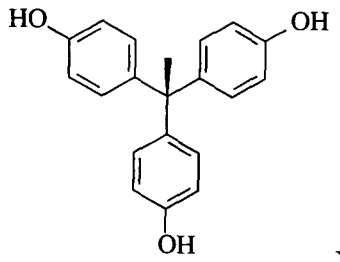
XIII

34. The method of claim 27, wherein said compound is represented by the structure of formula XIV:



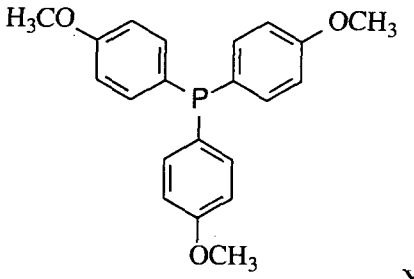
XIV

35. The method of claim 26, wherein said compound is represented by the structure of formula XV:



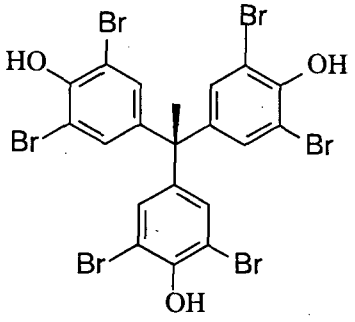
XV

36. The method of claim 20, wherein said compound is represented by the structure of formula XVI:



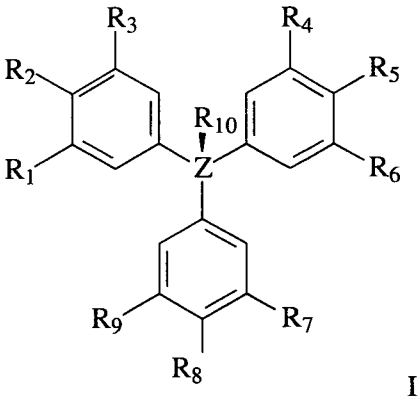
XVI

37. The method of claim 26, wherein said compound is represented by the structure of formula XVII:



XVII

38. A kit comprising reagents for conducting a PCR assay, and a compound represented by the structure of formula I:



I

wherein

Z is carbon, nitrogen, phosphor, arsine, silicon or germanium;

R_1 to R_9 are the same or different, H, D, OH, halogen, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, alkylalkoxy, haloalkyl, alkylhaloalkyl, haloaryl, aryloxy, amino, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido. alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or R_3, R_4, or R_7, forms a fused cycloalkyl, heterocycloalkyl, aromatic or heteroaromatic ring with the main aromatic ring; and

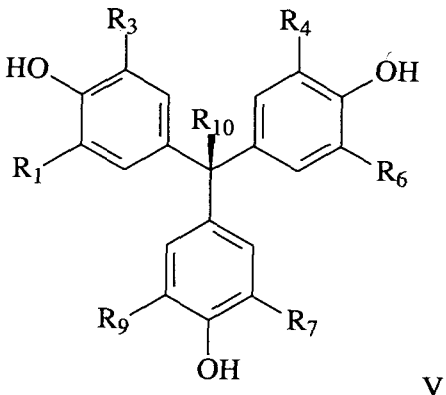
R_{10} is nothing, H, D, OH, halogen, oxo, nitro, CN, nitrileamido, amidosulfide, amino, aldehyde, substituted ketone, -COOH, ester, trifluoromethyl, amide, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, arylalkyl, alkylaryl, arylsulfonyl, arylalkylenesulfonyl, alkoxy, haloalkyl, haloaryl, cycloalkyl, alkylcycloalkyl, aryloxy, monoalkylamino, dialkylamino, alkylamido, arylamino, arylamido, alkylthio, arylthio, heterocycloalkyl, alkylheterocycloalkyl, heterocycloalkylalkyl, heteroaryl, hetroarylalkyl, alkylheteroaryl; or its isomer, salt, hydrate, N-oxide, crystal or any combination thereof.

39. The kit of claim 38, wherein said compound of formula I, is at a concentration range of between about 5×10^{-9} to 130×10^{-9} M.

40. The kit of claim 38, wherein said reagents comprise nucleoside triphosphates, a thermostable processive polymerase, and single-stranded oligonucleotide primers designed to amplify a nucleic acid sequence of interest.

41. The kit of claim 40, further comprising a reverse transcriptase.

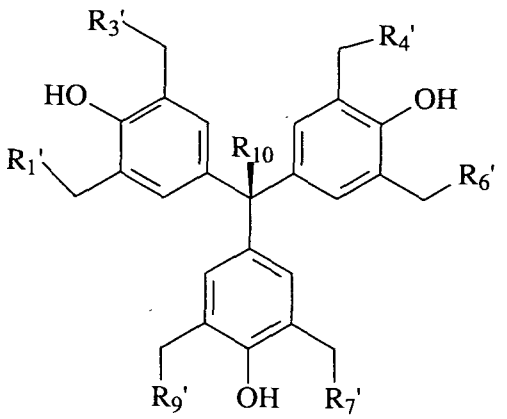
42. The kit of claim 38 wherein said compound is represented by the structure of formula V:



V

wherein R_1, R_3, R_4, R_6, R_7, R_9 and R_{10} are as described above.

43. The kit of claim 42, wherein said compound is represented by the structure of formula VII:

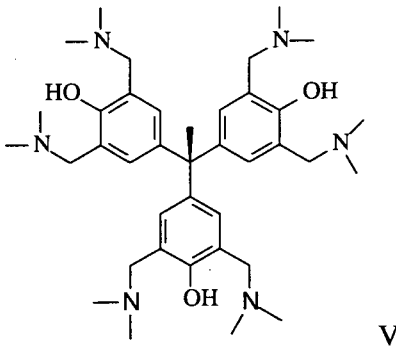


VII

wherein R_1', R_3', R_4', R_6' R_7', and R_9' are the same or different comprising halogen, aryl, alkyl, cycloalkyl, heterocycloalkyl, alkoxy, monoalkylamino, dialkylamino or arylamino; and

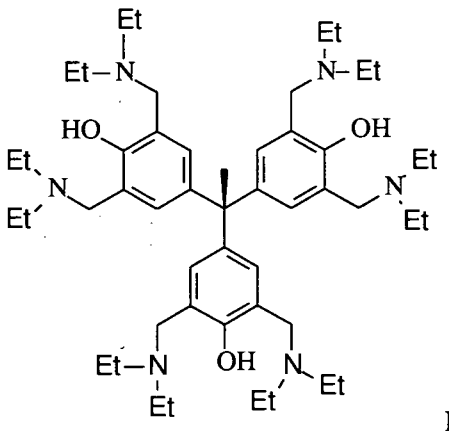
R_{10} is as described above.

44. The kit of claim 43, wherein said compound is represented by the structure of formula VIII:



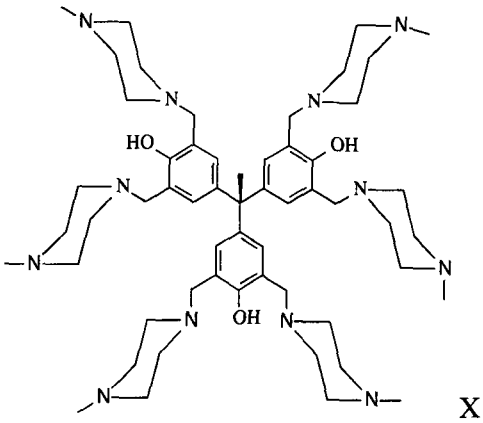
VIII

45. The kit of claim 43, wherein said compound is represented by the structure of formula IX:



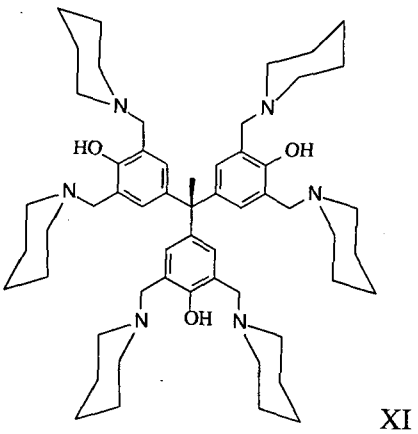
IX

46. The kit of claim 43, wherein said compound is represented by the structure of formula X:



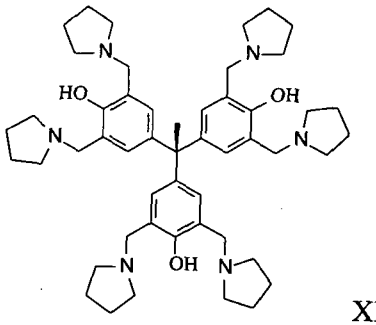
X

47. The kit of claim 43, wherein said compound is represented by the structure of formula XI:



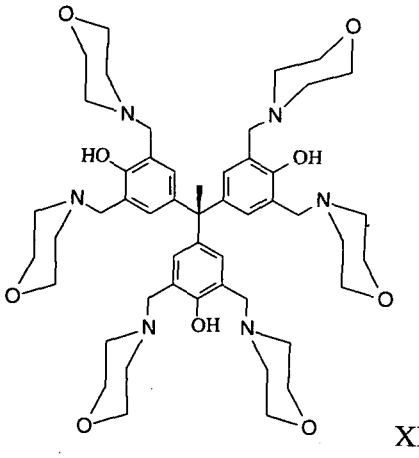
XI

48. The kit of claim 43, wherein said compound is represented by the structure of formula XII:



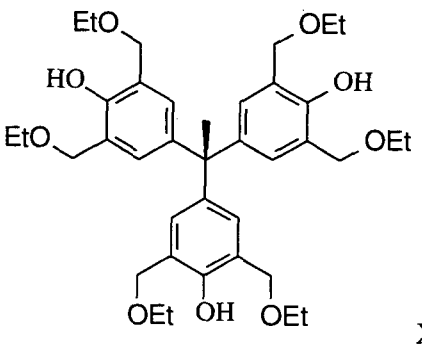
XII

49. The kit of claim 43, wherein said compound is represented by the structure of formula XIII:



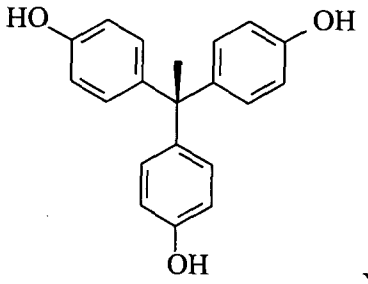
XIII

50. The kit of claim 43, wherein said compound is represented by the structure of formula XIV:



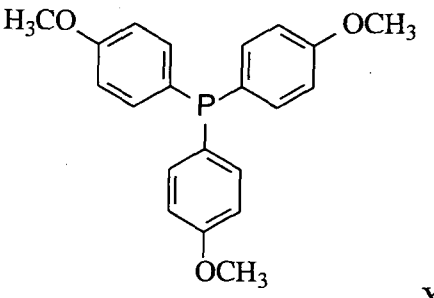
XIV

51. The kit of claim 42, wherein said compound is represented by the structure of formula XV:



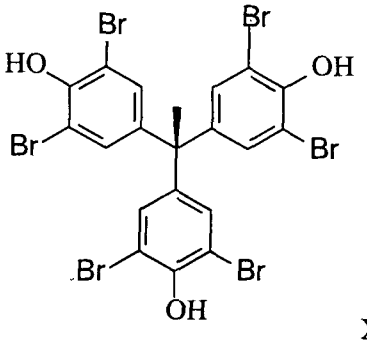
XV

52. The kit of claim 42, wherein said compound is represented by the structure of formula XVI:



XVI

53. The kit of claim 42 wherein said compound is represented by the structure of formula XVII:



XVII

FIGURE 1

FIGURE 2A

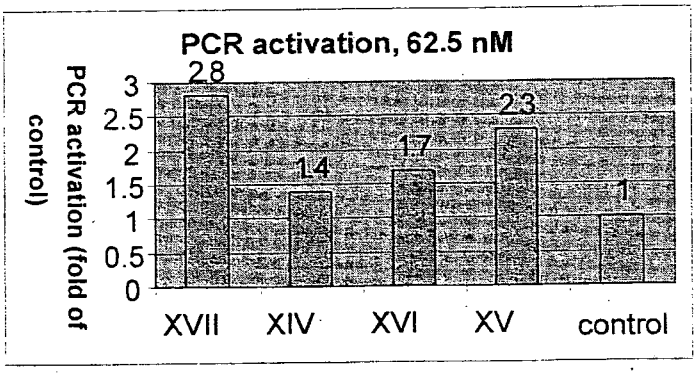


FIGURE 2B

FIGURE 3

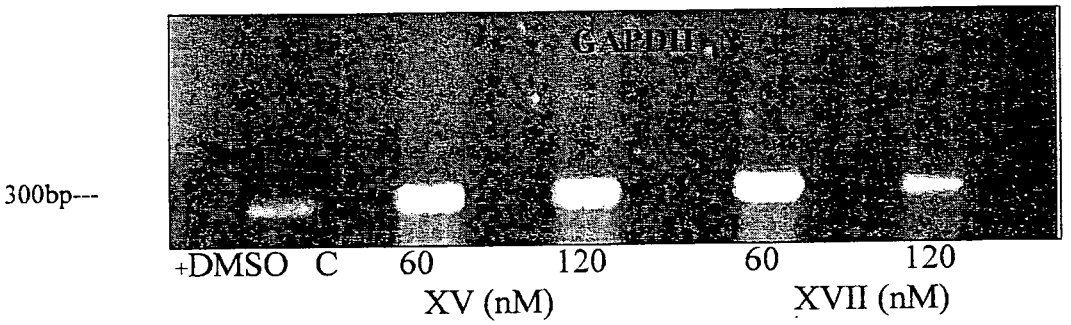


FIGURE 4

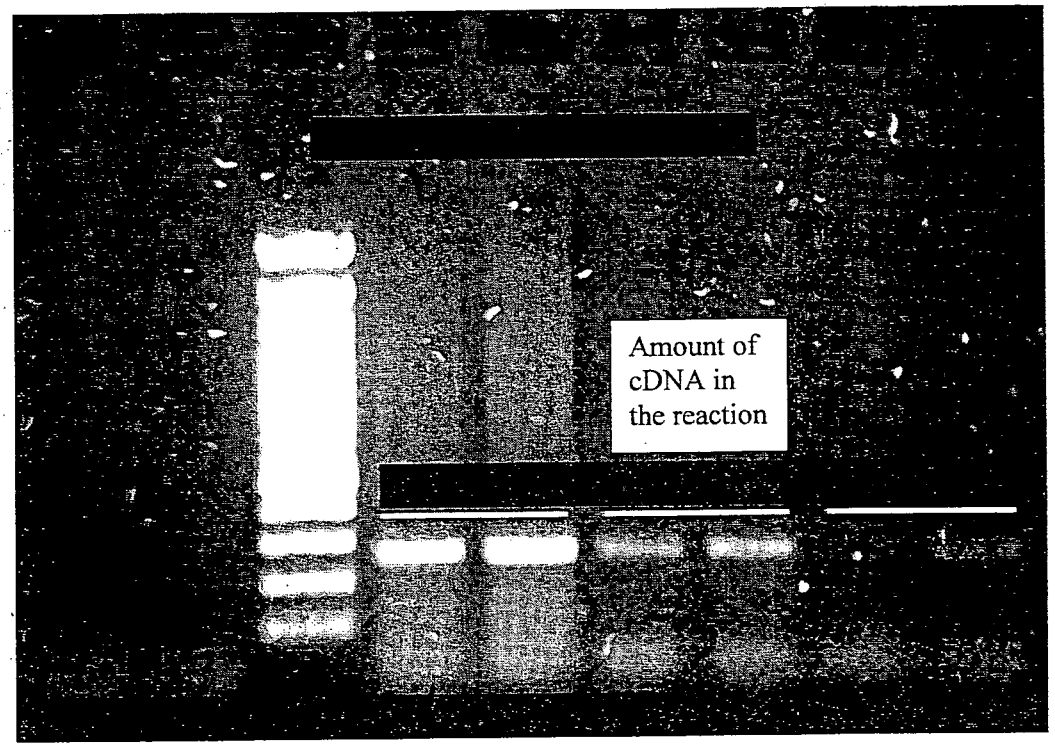


FIGURE 5

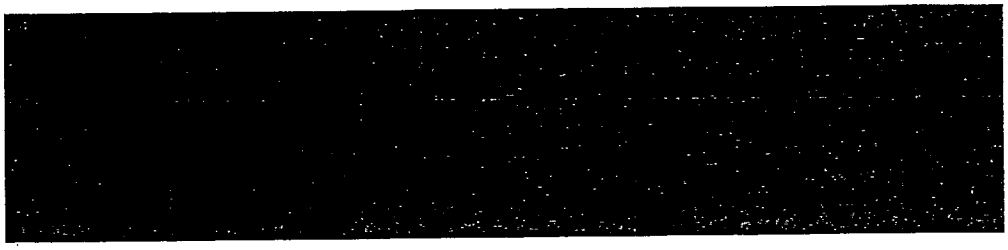


FIGURE 6A

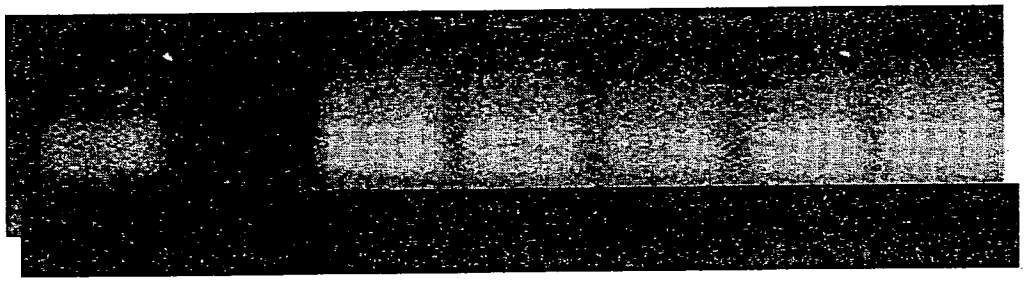


FIGURE 6B

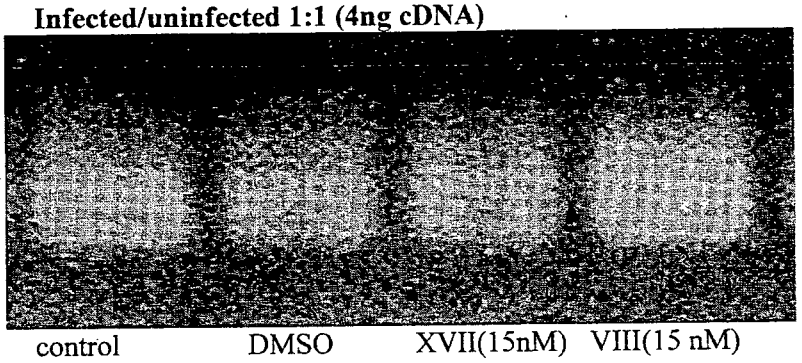


FIGURE 7A

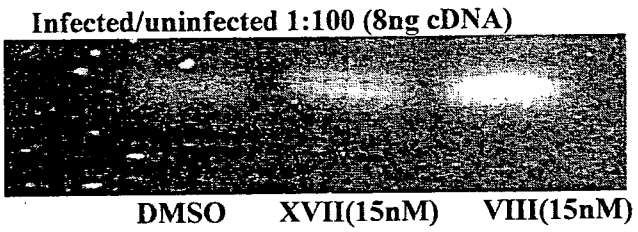


FIGURE 7B