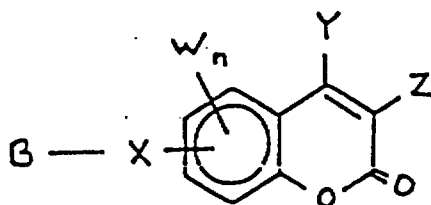


SUMMARY OF THE INVENTION

The current invention provides non-nucleosidic, stable, photoactive compounds that can be used as photo-crosslinking reagents in nucleic acid hybridization assays and therapeutic applications, as well as techniques and intermediates that can be used to prepare the final products.

The compounds comprise coumarinyl derivatives prepared by linking the phenyl ring of a coumarin molecule or derivative to a hydroxy or polyhydroxy hydrocarbon molecule, such as one of the terminal hydroxy groups of a glycerol molecule. The (poly)hydroxy hydrocarbon moiety of the resulting compound is equivalent to the sugar of a nucleoside, while the coumarin moiety occupies the position of a base. Accordingly, the compounds can be inserted into growing polynucleotide chains using automated (or manual) techniques of polynucleotide synthesis. The double bond between the 3 and 4 positions of the coumarin ring system is a photoactive group that covalently crosslinks to nucleosides in the complementary strand when an oligonucleotide containing this non-nucleoside analogue (the "probe") is used in a hybridization assay and/or therapeutic application.

For the most part, the photoactive compound has the formula



in which the substituents and linking groups are described below in more detail.

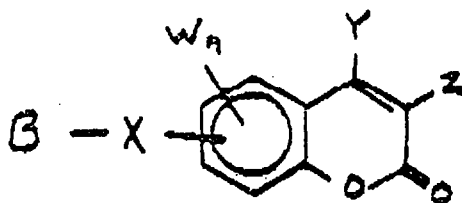
The (poly)hydroxy hydrocarbon backbones give maximum flexibility and stability to the oligosaccharide structure in which they are located as well as good solubility in aqueous and organic media.

In accordance with a first aspect of the present invention, ^{there is provided} ^{Insert claim 1}
 In accordance with a second aspect of the present invention, ^{there is provided} ^{Insert claim 2}

In accordance with a third aspect of the present invention, ^{there is provided} ^{Insert claim 3}
 In accordance with a fourth aspect of the present invention, ^{there is provided} ^{Insert claim 4}

CLAIMS

1. A compound having the formula:



wherein

B represents (1) a linear, branched, or cyclic hydrocarbon group containing from 2 to 10 carbon atoms and, if cyclic, containing a 5- or 6-membered ring or (2) a heterocyclic aromatic ring system containing a 5- or 6-membered ring, said B(1) or B(2) being substituted with 1, 2, or 3 groups of the formula OR_1 , wherein R_1 independently represent H or a hydroxy-protecting or hydroxy-coupling group capable of protecting or coupling a hydroxy group during synthesis of a polynucleotide, or OR_1 represent a nucleotide or a polynucleotide connected to the remainder of said formula, and wherein one to three carbon atoms of the hydrocarbon group are replaced by an oxygen, nitrogen, or sulfur atom, with the proviso that B does not represent a deoxyribose or a ribose moiety;

X represents (1) a bond, (2) a linear, branched, or cyclic hydrocarbon group containing 1 to 10 carbon atoms or (3) the X(2) group in which one to three carbon atoms of the hydrocarbon group are replaced by an oxygen, sulfur, or nitrogen atom and wherein the shortest linking chain of atoms in X between atoms in other parts of said formula attached to X is 1 to 10 atoms, wherein X is optionally substituted with 1-3 substituents selected from the group consisting of hydroxy, halogen, amino, amido, azido, carboxy, oxo, perfluoromethyl, and cyano functional groups; and wherein X is attached to the benzo ring of said formula directly or through W;

n is 0, 1, 2, or 3;

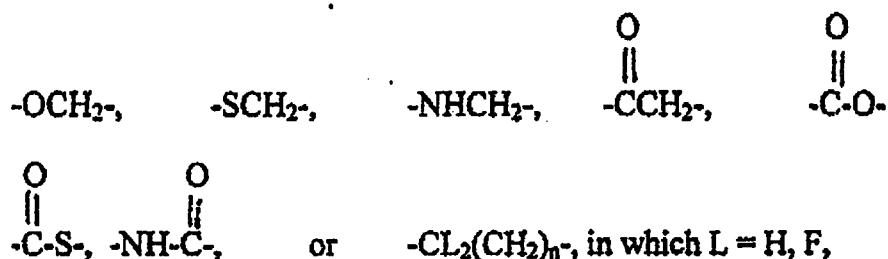
each W independently represents a hydroxy, halogen, amino, amido, azido, nitro, thio, carboxy, carbonyl, perfluoromethyl, or cyano functional group; an unsubstituted hydrocarbyl

group of 10 or fewer carbon atoms; or said hydrocarbyl group substituted with 1-3 of said functional groups or in which one carbon atom is replaced by an oxygen, sulfur, or nitrogen atom and wherein two Ws together can represent a ring fused to the benzo ring of said formula;

with the provisos that (1) when X or W is a substituted hydrocarbon, the total number of substituents in X or W is less than the total number of carbon atoms in said X or W and no more than one substituent or heteroatom is attached to a given carbon, unless said substituents are halogen atoms on said given carbon and (2) the total carbon atoms in all W substituents is 15 or fewer; and

Y and Z independently represent H or lower alkyl or F.

2. The compound of claim 1, wherein X, in either orientation, is



Cl, I, or Br and $n = 0, 1, \text{ or } 2$.

3. The compound of Claim 1, wherein X is a cyclic structure with a 5- or 6-membered ring or a 5- or 6-membered heteroring containing one O, S, or N atom.

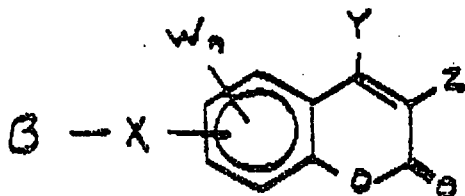
4. The compound of Claim 1, wherein all of said formula to the right of X represents coumarin or a derivative thereof within said formula.

5. The compound of Claim 1, wherein X is covalently connected to the 7 position of a coumarin moiety.

12. The compound of Claim 1, wherein said nucleotide or polynucleotide is connected to said compound via a phosphorous-containing linking group.

13. The compound of Claim 12, wherein said phosphorous-containing group is a phosphate group.

18. The compound of Claim 1, wherein at least one R_1 represents a trityl, pixyl, dimethoxytrityl, monomethoxytrityl, phosphite, phosphoramidite, phosphate, H-phosphonate, phosphorothioate, methylphosphonate, phosphodithioate or phosphotriester group.
19. The compound of Claim 1, wherein B contains a heterocyclic ring selected from the group consisting of furan, pyran, pyrrole, pyrazole, imidazole, piperidine, pyridine, pyrazine, pyrimidine, pyridazine, thiophene, acridine, indole, quinoline, isoquinoline, quinazoline, quinoxaline, xanthene, and 1,2-benzopyran.
20. A compound of Claim 1, wherein said compound is a polynucleotide of the formula $(N_{m1}Q_{m2}N_{m3})_{m3}$ in which each N represents a nucleotide of a desired polynucleotide sequence, Q represents the nucleotide-replacing molecule in the formula of claim 1, and $m1$, $m2$, and $m3$ are integers, and $m3$ is from 1 to 10.
21. A compound of Claim 20 wherein $m1$ and $m2$ are less than 100.
22. A compound of Claim 21 wherein at least one Q is at a terminal position and at least one Q is at an interior position of said polynucleotide sequence.
23. A compound of Claim 21 wherein at least one Q is at a interior position of said polynucleotide sequence.
24. A compound of Claim 21 wherein Q is at a terminal position of said polynucleotide sequence.
25. A compound of Claim 1 wherein B-X- is $R_1-O-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH(OR_1)-CH_2-O-$.
27. A compound having the formula:



wherein

B represents (1) a branched or cyclic hydrocarbon group containing from 3 to 10 carbon atoms and, if cyclic, containing a 5- or 6-membered ring or (2) a heterocyclic aromatic ring system containing a 5- or 6-membered ring, said B(1) or B(2) being substituted with 1, 2, or 3 groups of the formula OR_1 , wherein R_1 represent H or a hydroxy-protecting or hydroxy-coupling group capable of protecting or coupling a hydroxy group during synthesis of a polynucleotide, or 2 groups when OR_1 represents a nucleotide or a polynucleotide connected to the remainder of said formula, and wherein one to three carbon atoms of the hydrocarbon group are replaced by an oxygen, nitrogen or sulfur atom, with the proviso that B does not represent a ribose or deoxyribose:

X represents (1) a bond, (2) a linear, branched, or cyclic hydrocarbon group containing 1 to 10 carbon atoms or (3) said X(2) group in which one to three carbon atoms of the hydrocarbon group are replaced by an oxygen, sulfur, or nitrogen atom, and wherein the shortest linking chain of atoms in X between atoms in other parts of said formula attached to X is 1 to 10 atoms, wherein X is optionally substituted with 1-3 substituents selected from the group consisting of hydroxy, halogen, amino, amido, azido, carboxy, oxo, perfluoromethyl, and cyano functional groups; and wherein X is attached to the benzo ring of said formula directly or through W;

n is 1, 2, or 3;

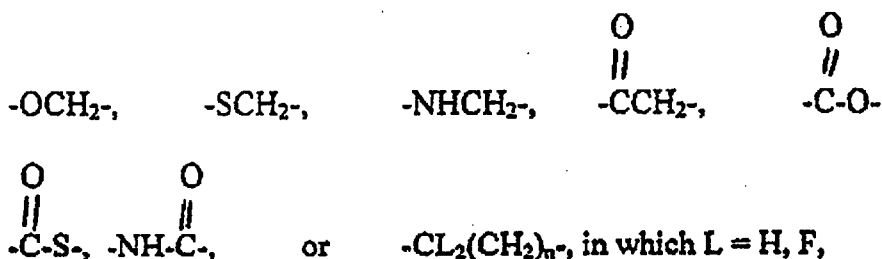
each W independently represents a hydroxy, halogen, amino, amido, azido, nitro, thio, carboxy, carbonyl, perfluoromethyl, or cyano functional group; an unsubstituted hydrocarbyl group of 10 or fewer carbon atoms; or said hydrocarbyl group substituted with 1-3 of said functional groups or in which one carbon atoms replaced by an oxygen, sulfur, or nitrogen atom and wherein two Ws together can represent a ring fused to the benzo ring of said formula;

with the provisos that (1) when X or W is a substituted hydrocarbon, the total number of substituents in X or W is less than the total number of carbon atoms in said X or W and no more than one substituent or heteroatom is attached to a given carbon, unless said substituents are

halogen atoms on said given carbon, and (2) the total carbon atoms in all W substituents is 15 or fewer; and

Y and Z independently represent H or lower alkyl.

28. The compound of claim 27, wherein X, in either orientation, is



Cl, I, or Br and n = 0, 1, or 2.

29. The compound of Claim 27, wherein X is a cyclic structure with a 5- or 6-membered ring or a 5- or 6-membered heteroring containing one O, S, or N atom.

30. The compound of Claim 27, wherein W is a pyrone or furan ring fused to the phenyl ring of said formula.

31. The compound of Claim 27, wherein all of said formula to the right of X represents psoralen, cis-benzodipyrone, or trans-benzodipyrone or a derivative thereof within said formula.

32. The compound of Claim 27, wherein X is covalently connected to the 4 position of a furan ring of a psoralen moiety.

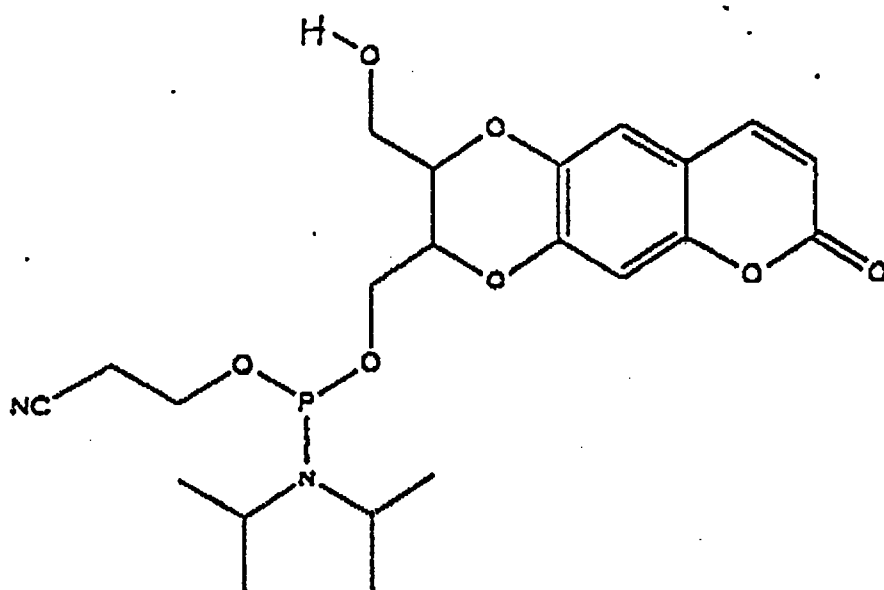
39. The compound of Claim 27, wherein at least one R₁ represents a trityl, pixyl, dimethoxytrityl, monomethoxytrityl, phosphite, phosphoramidite, phosphate, H-phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate or phosphotriester group.

40. The compound of Claim 27, wherein B contains a heterocyclic ring selected from the group consisting of furan, pyran, pyrrole, pyrazole, imidazole, piperidine, pyridine, pyrazine, pyrimidine, pyridazine, thiophene, acridine, indole, quinoline, isoquinoline, quinazoline, quinoxaline, xanthene, and 1,2-benzopyran.

41. A compound of Claim 27, wherein said compound is a polynucleotide of the formula $(N_{m1}Q_{m2}N_{m3})_{m3}$ in which each N represents a nucleotide of a desired polynucleotide sequence, Q represents the nucleotide-replacing molecule in the formula of claim 1, and $m1$, $m2$, and $m3$ are integers, and $m3$ is from 1 to 10.

43. The compound of Claim 1, wherein said formula contains one photoactive bond and said bond is located between the carbons to which Y and Z are attached in said formula.

44. A compound having the formula:



45. A compound having the formula:

