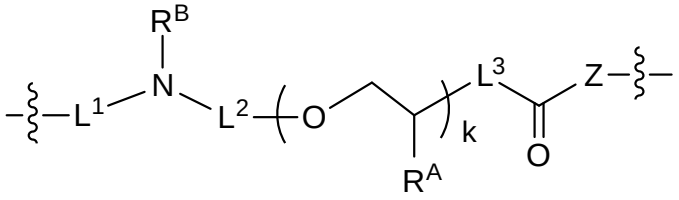
CLAIMS:

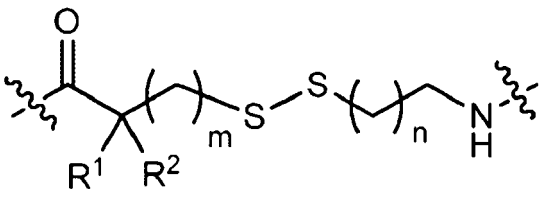
1. A nucleoside or nucleotide covalently attached to a fluorophore through a linker, wherein said linker comprises a structure of formula (III):



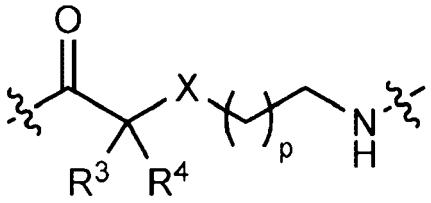
(III)

wherein

L^1 is absent, or comprises a structure of formula (I), (II) ~~or a protecting moiety~~, or combinations thereof;



(I),



(II),

wherein

R^1 is ~~selected from~~ hydrogen or ~~optionally substituted~~ C_{1-6} alkyl;

R^2 is ~~selected from~~ hydrogen or ~~optionally substituted~~ C_{1-6} alkyl;

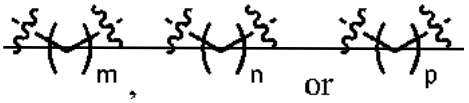
R^3 is ~~selected from optionally substituted~~ C_{1-6} alkyl, -NR^5-C(=O)-R^6, or -NR^7-C(=O)-OR^8;

R^4 is ~~selected from~~ hydrogen or ~~optionally substituted~~ C_{1-6} alkyl;

each of R^5 and R^7 is independently ~~selected from~~ hydrogen, ~~optionally substituted~~ C_{1-6} alkyl, ~~optionally substituted~~ phenyl, or ~~optionally substituted~~ C_{7-12} aralkyl;

each of R^6 and R^8 is independently ~~selected from optionally substituted~~ C_{1-6} alkyl,

~~optionally substituted~~ phenyl, ~~optionally substituted~~ C_{7-12} aralkyl, ~~optionally substituted~~ or C_{3-7} cycloalkyl~~, or optionally substituted 5 to 10 membered heteroaryl~~;

~~each of the methylene repeating unit in~~  ~~is optionally substituted;~~

X is ~~selected from~~ methylene (CH_2), oxygen (O), or sulfur (S);

m is an integer of 0 through 20;

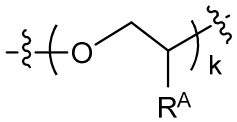
n is an integer of 1 through 20; and

p is an integer of 1 through 20;

L^2 is ~~selected from optionally substituted~~ C_{1-20}10 alkylene, ~~or optionally substituted~~ C_{13-206} heteroalkylene, C_{1-10} alkylene interrupted by a substituted phenyl group, or C_{3-6} heteroalkylene interrupted by a substituted phenyl group, wherein the C_{3-6} heteroalkylene comprising one or more nitrogen atoms and optionally substituted with oxo (=O), and wherein the substituted phenyl group is substituted with one to four substituents selected from the group consisting of nitro, cyano, halo, hydroxy, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and sulfonyl hydroxide;

L^3 is ~~selected from optionally substituted~~ C_{1-206} alkylene, or ~~optionally substituted~~ C_{12-206} heteroalkylene comprising one or more oxygen atoms;

R^A is ~~selected from~~ hydrogen, cyano, hydroxy, halogen, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, or azido, and wherein at least one of the repeating units



of comprises an azido group;

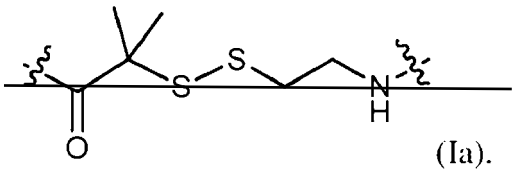
Z is ~~selected from~~ oxygen (O) or NR^C;

each of R^B and R^C is independently ~~selected from~~ hydrogen or ~~optionally substituted~~ C_{1-6} alkyl; and

k is an integer of 1 through ~~50~~10.

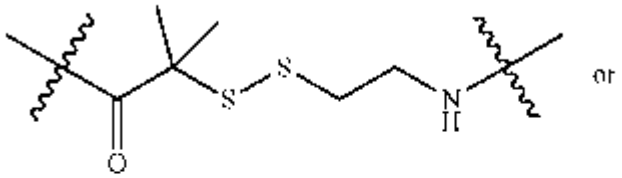
2. The nucleoside or nucleotide of Claim 1, wherein L^1 is absent.

3. The nucleoside or nucleotide of Claim 1, wherein L^1 comprises the ~~structural~~ structure of formula (Ia) or (Ib):



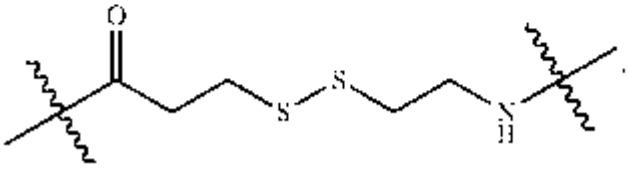
(Ia).

(Ia)



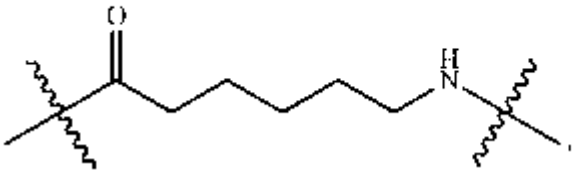
or

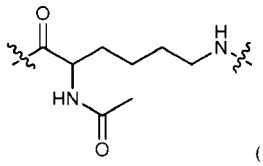
(Ib)



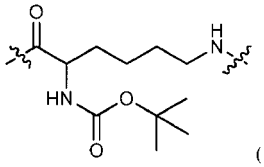
4. The nucleoside or nucleotide of Claim 1, wherein L^1 comprises the ~~structural~~ structure of formula (IIa), (IIb), (IIc), (IId), (IIe), or (IIf):

(IIa)

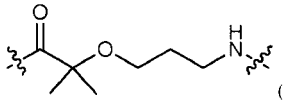




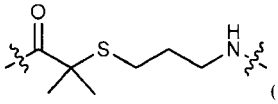
(IIb).



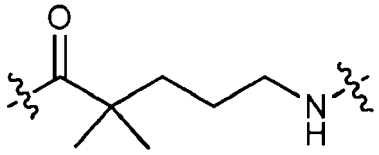
(IIc).



(IId).



(IIe).



(IIf).

5. The nucleoside or nucleotide of Claim 1, wherein L^2 is heptylene.

6. The nucleoside or nucleotide of Claim 1, wherein L^2 is ~~optionally substituted~~ C_{1}~~3~~-$_{20}$~~6~~ heteroalkylene comprising one or more nitrogen atoms.

7. The nucleoside or nucleotide of Claim 6, wherein at least one of the carbon atom of said C_{1}~~3~~-$_{20}$~~6~~ heteroalkylene is substituted with oxo (=O).

8. The nucleoside or nucleotide of Claim ~~5~~7, wherein L^2 is interrupted by a substituted phenyl group.

~~9. The nucleoside or nucleotide of Claim 8, wherein the phenyl group is substituted with one or more substituents selected from nitro, cyano, halo, hydroxy, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy or sulfonyl hydroxide.~~

~~10.~~9. The nucleoside or nucleotide of Claim 1, wherein k is 2.

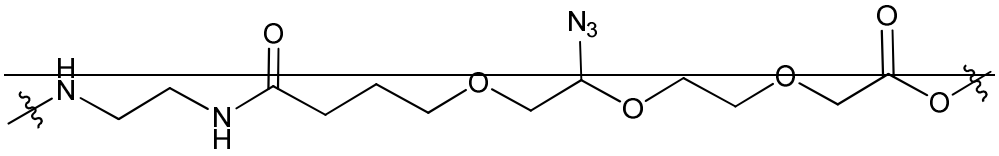
~~11.~~10. The nucleoside or nucleotide of Claim 1, wherein L^3 is ethylene.

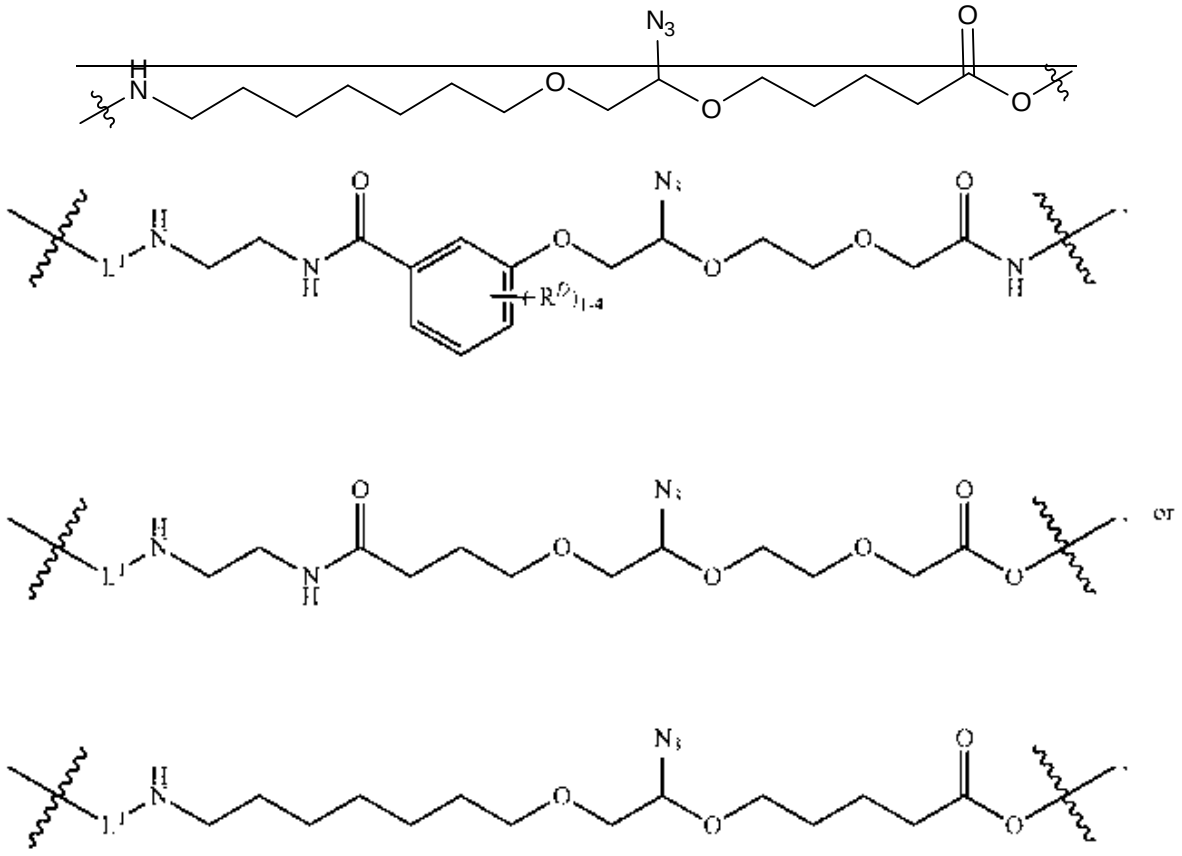
~~12.~~11. The nucleoside or nucleotide of Claim 1, wherein L^3 is optionally substituted C_{1}~~2~~-$_{20}$~~6~~ heteroalkylene comprising one or more oxygen atoms.

~~13. The nucleoside or nucleotide of Claim 1, wherein said protecting moiety comprises a molecule that protects against DNA damage.~~

~~14.~~12. The nucleoside or nucleotide of Claim 1, wherein each of R^B and R^C is hydrogen.

~~15.~~13. The nucleoside or nucleotide of Claim 1, wherein the structure of formula (III) is also represented by ~~formula~~:



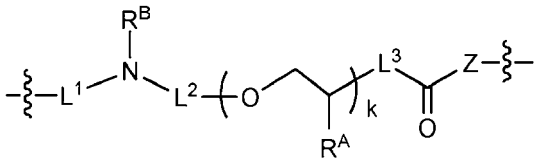


wherein each R^D is independently nitro, cyano, halo, hydroxy, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy or sulfonyl hydroxide.

14. An oligonucleotide comprising a nucleotide according to claim 1.

~~16.~~15. A kit comprising a nucleoside or nucleotide according to Claim 1.

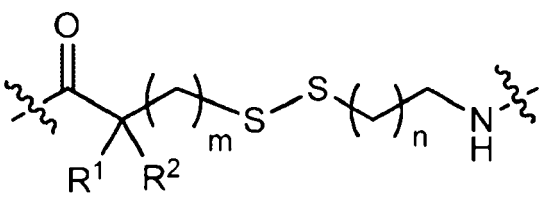
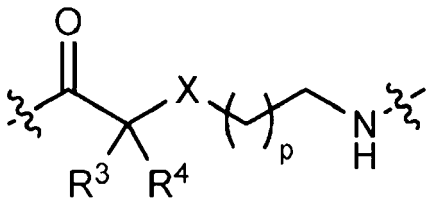
~~17.~~16. A reagent for modifying a nucleoside or a nucleotide, comprising a fluorophore and a linker, said linker comprises a structure of formula (III):



(III)

wherein

L^1 is absent, or comprises a structure of formula (I), (II) ~~or a protecting moiety~~, or combinations thereof;


(I),

(II),

wherein

R^1 is ~~selected from~~ hydrogen or ~~optionally substituted~~ C_{1-6} alkyl;

R^2 is ~~selected from~~ hydrogen or ~~optionally substituted~~ C_{1-6} alkyl;

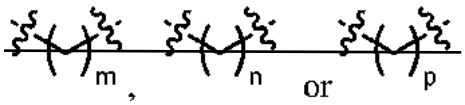
R^3 is ~~selected from optionally substituted~~ C_{1-6} alkyl, -NR^5-C(=O)-R^6, or -NR^7-C(=O)-OR^8;

R^4 is ~~selected from~~ hydrogen or ~~optionally substituted~~ C_{1-6} alkyl;

each of R^5 and R^7 is independently ~~selected from~~ hydrogen, ~~optionally substituted~~ C_{1-6} alkyl, ~~optionally substituted~~ phenyl, or ~~optionally substituted~~ C_{7-12} aralkyl;

each of R^6 and R^8 is independently ~~selected from optionally substituted~~ C_{1-6} alkyl,

~~optionally substituted~~ phenyl, ~~optionally substituted~~ C_{7-12} aralkyl, ~~optionally substituted~~ or C_{3-7} cycloalkyl~~, or optionally substituted 5 to 10 membered heteroaryl~~;

~~each of the methylene repeating unit in~~  ~~is optionally substituted;~~

X is ~~selected from~~ methylene (CH_2), oxygen (O), or sulfur (S);

m is an integer of 0 through 20;

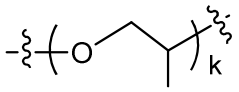
n is an integer of 1 through 20; and

p is an integer of 1 through 20;

L^2 is ~~selected from optionally substituted~~ C_{1-20}10 alkylene, ~~or optionally substituted~~ C_{13-20}6 heteroalkylene, C_{1-10} alkylene interrupted by a substituted phenyl group, or C_{3-6} heteroalkylene interrupted by a substituted phenyl group, wherein the C_{3-6} heteroalkylene comprising one or more nitrogen atoms and optionally substituted with oxo (=O), and wherein the substituted phenyl group is substituted with one to four substituents selected from the group consisting of nitro, cyano, halo, hydroxy, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy and sulfonyl hydroxide;

L^3 is ~~selected from optionally substituted~~ C_{1-20} alkylene, or ~~optionally substituted~~ C_{1-20} heteroalkylene;

R^A is ~~selected from~~ hydrogen, cyano, hydroxy, halogen, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, or azido, and wherein at least one of the repeating units



of R^A comprises an azido group;

Z is ~~selected from~~ oxygen (O) or NR^C;

each of R^B and R^C is independently ~~selected from~~ hydrogen or ~~optionally substituted~~ C_{1-6} alkyl; and

k is an integer of 1 through ~~50~~10.

17. A method for detecting a nucleotide that has been incorporated into a polynucleotide, comprising:

(a) incorporating a nucleotide according to claim 1 into a polynucleotide; and

(b) detecting a fluorescent signal from said nucleotide that was incorporated in step (a).

18. The method of claim 17, further comprising providing a template nucleic acid strand and a partially hybridized nucleic acid strand, wherein step (a) incorporates into the hybridized strand at least one nucleotide that is complementary to a nucleotide at the corresponding position of the template strand, and wherein step (b) identifies the base of the incorporated nucleotide, thereby indicating the identity of the complementary nucleotide of the template strand.

19. A method of sequencing a template nucleic acid molecule, comprising:

incorporating one or more labeled nucleotides into a strand of nucleic acid complementary to the template nucleic acid;

determining the identity of the base present in one or more incorporated labeled nucleotides in order to determine the sequence of the template nucleic acid molecule;

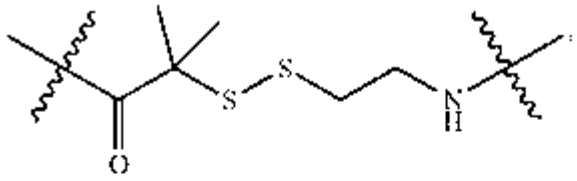
wherein the identity of the base present in said one or more labeled nucleotides is determined by detecting a fluorescent signal produced by said labeled nucleotides; and

wherein at least one incorporated labeled nucleotide is a nucleotide according to claim 1.

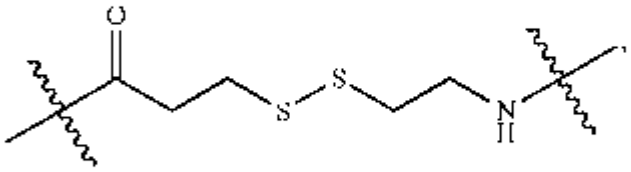
20. The method of claim 19, wherein the identity of the base present in said one or more nucleotides is determined after each nucleotide incorporation step.

21. The nucleoside or nucleotide of claim 13, wherein L^1 comprises the structure of formula (Ia), (Ib), (IIa), (IIb), (IIc), (IId), (IIe), or (IIf):

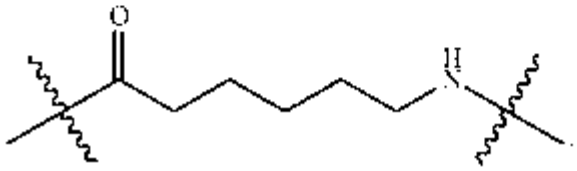
(Ia)



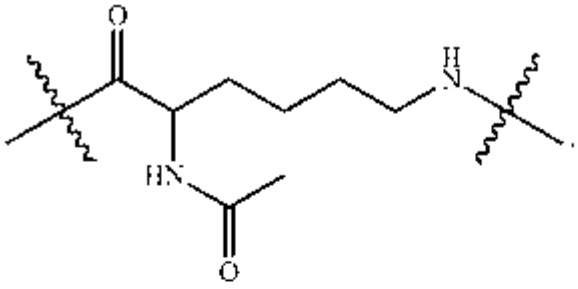
(Ib)



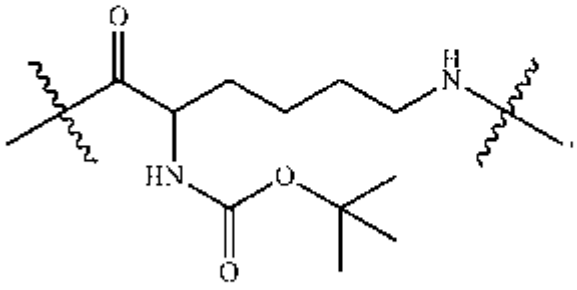
(IIa)



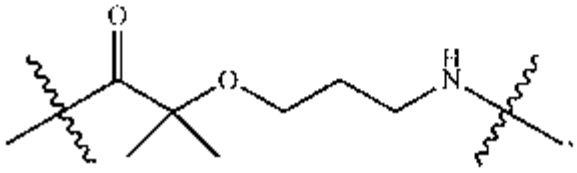
(IIb)



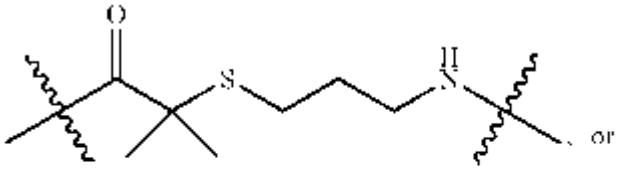
(IIc)



(IId)

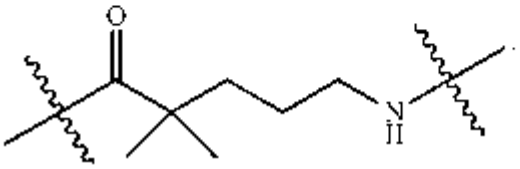


(IIe)

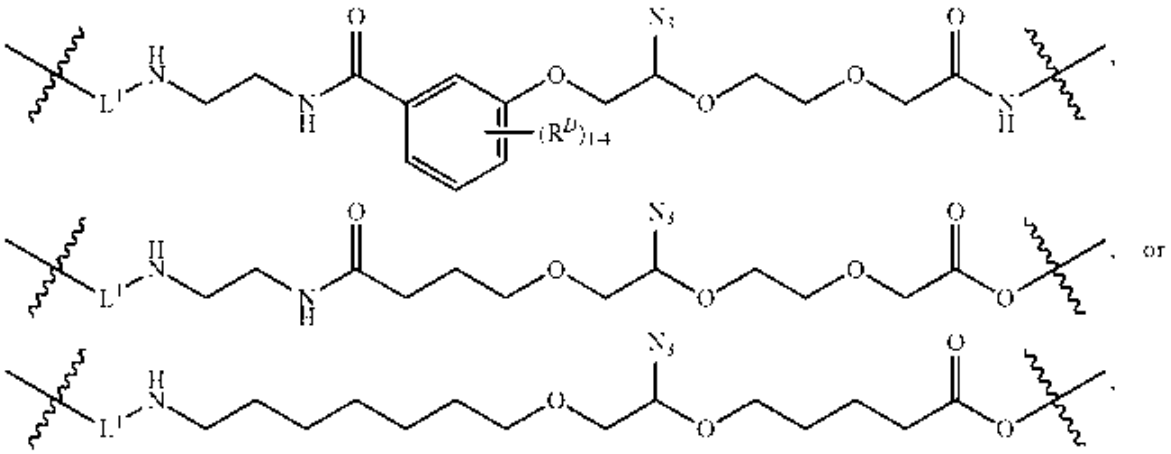


or

(IIf)



22. The reagent of claim 16, wherein the structure of formula (III) is also represented by:

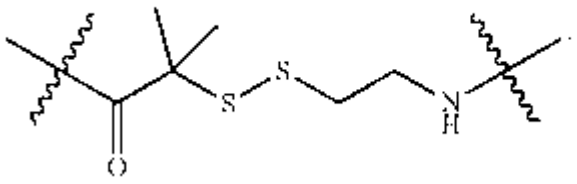


or

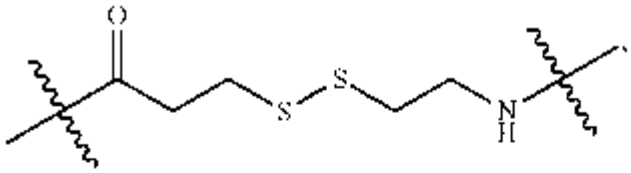
wherein each R^D is independently nitro, cyano, halo, hydroxy, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} haloalkyl, C_{1-6} haloalkoxy or sulfonyl hydroxide.

23. The reagent of claim 22, wherein L^1 comprises the structure of formula (Ia), (Ib), (IIa), (IIb), (IIc), (IId), (IIe), or (IIf):

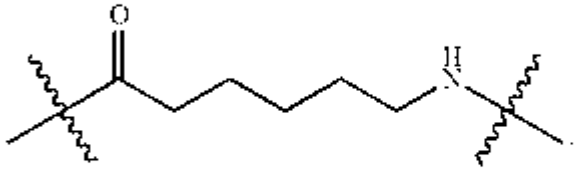
(Ia)



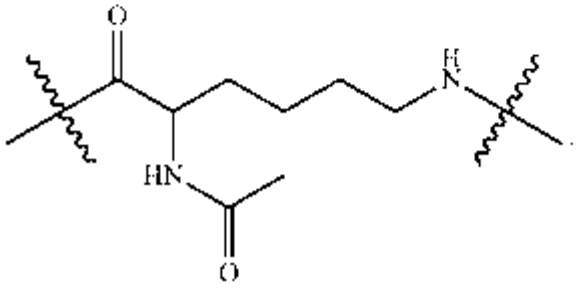
(Ib)



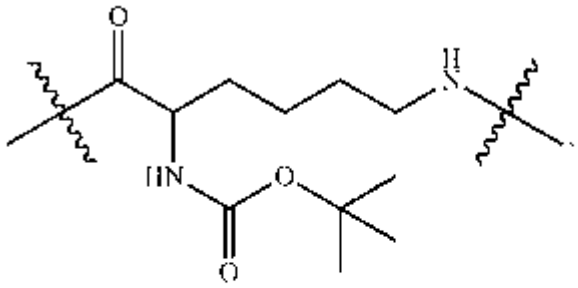
(IIa)



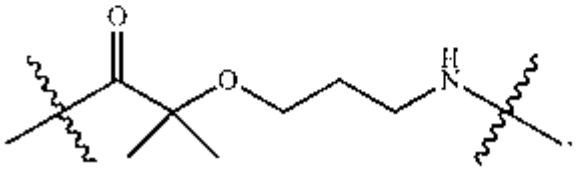
(IIb)



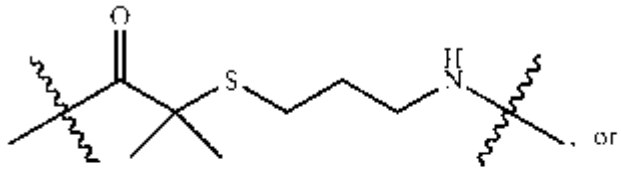
(IIc)



(IId)

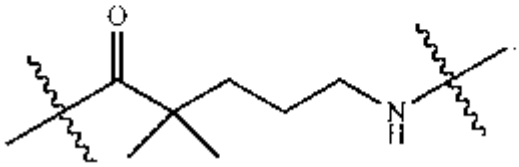


(IIe)



, or

(IIf)



~~18. The nucleoside or nucleotide of Claim 4, wherein the structure of formula (III) is also represented by:~~

