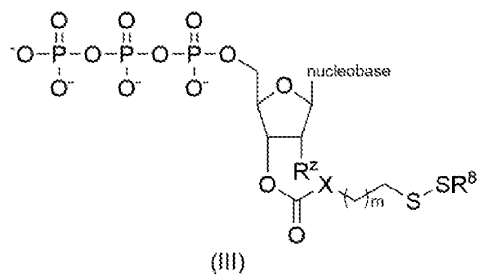
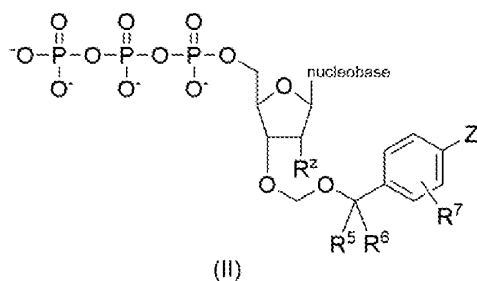
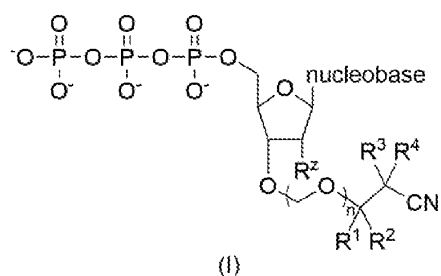


CLAIMS

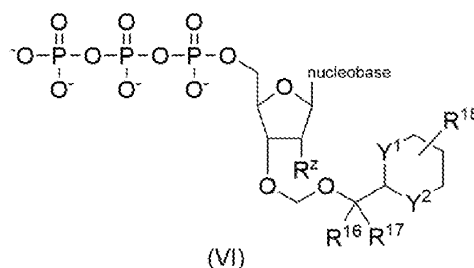
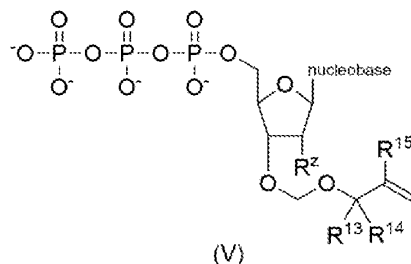
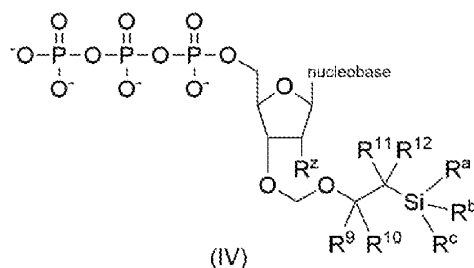
1. A method of synthesizing a single-stranded nucleotide sequence, the
5 method comprising:

- (i) adding a blocked nucleoside triphosphate to an initiator nucleotide sequence to incorporate a corresponding blocked nucleotide thereto in the presence of a polymerase,

10 wherein the blocked nucleoside triphosphate has one of the general formulae (I), (II), (III), (IV), (V) and (VI):



66



wherein

5

$n = 0$ or 1 ;

$m = 0$ to 20 ;

R^Z is H or OH;

$R^1, R^2, R^3, R^4, R^5, R^6, R^a, R^b$ and R^c are each independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

10

R^7 is selected from hydrogen, alkyl, halogen, $-OR^{19}$, $-NR^{20}R^{21}$ and $-SR^{22}$, wherein R^{19}, R^{20}, R^{21} and R^{22} are independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

$R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}$ and R^{18} are independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

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X is a heteroatom selected from O, S and NH;

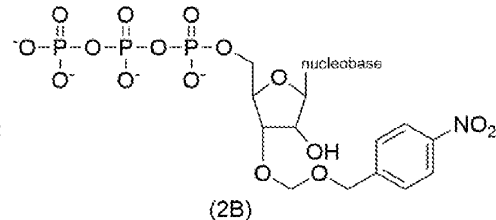
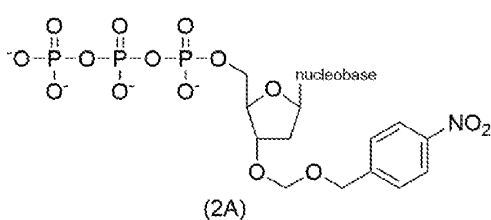
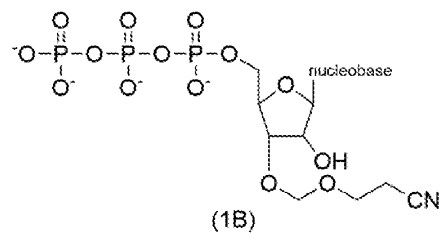
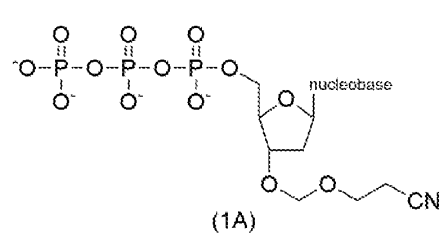
Y^1 and Y^2 are independently selected from S and Se; and

Z is a chemical moiety that is capable of being released under suitable conditions to trigger removal of the adjacent benzyl linker.

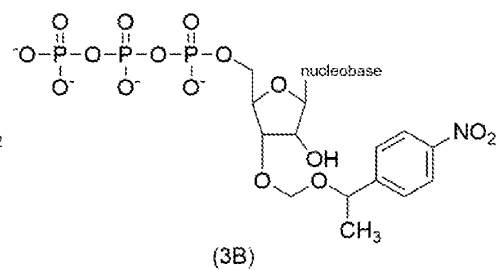
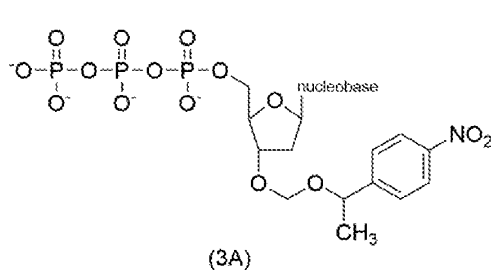
2. The method of claim 1, wherein Z is NO₂.
3. The method of claim 1, wherein R^a, R^b and R^c are each methyl.
4. The method of claim 1, further comprising
 - (ii) removing a removable terminating group from the incorporated blocked nucleotide to obtain a corresponding nucleotide that is unblocked at the 3'-O position.
5. The method of claim 4, further comprising
 - (iii) adding a blocked nucleoside triphosphate of any one of the general formulae (I), (II), (III), (IV), (V) and (VI) to the 3'-O position of the unblocked nucleotide obtained in step (ii) in the presence of a polymerase; and
 - (iv) optionally repeating step (ii) and/or (iii) one or more times until a single-stranded nucleotide sequence of a desired length is obtained.
6. The method of any one of the preceding claims, wherein the polymerase is a template independent polymerase.
7. The method of any one of the preceding claims, wherein the polymerase comprises terminal deoxynucleotidyl transferase (TdT) and/or polymerase theta (POLQ).
8. The method of claim 4, wherein the step (ii) of removing the removable terminating group is adapted to be carried out in aqueous conditions.

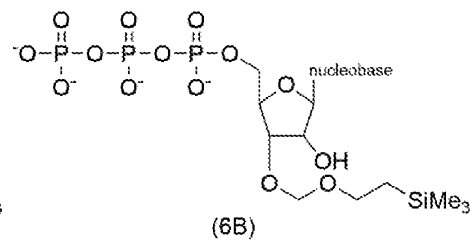
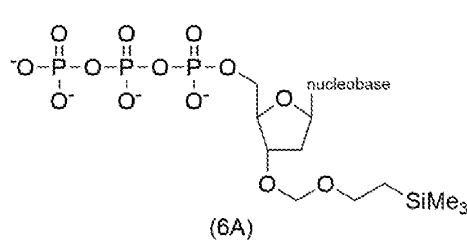
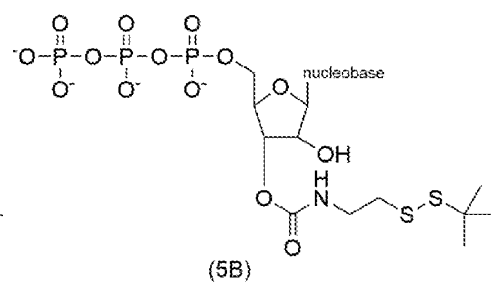
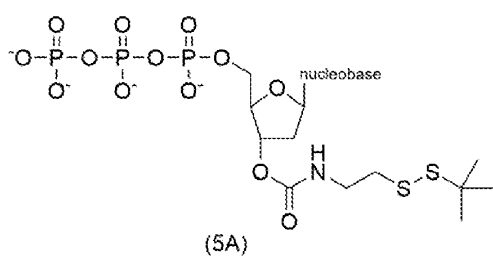
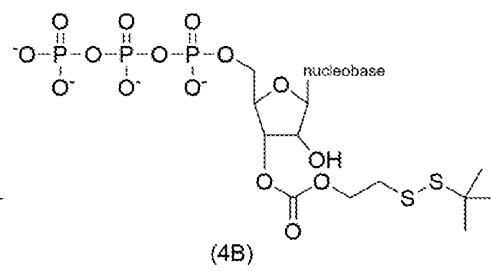
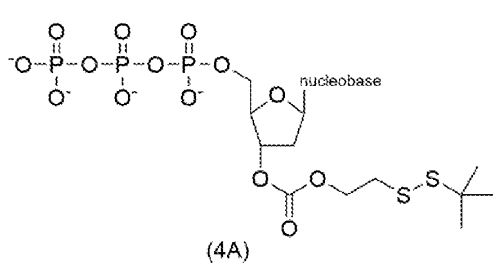
9. The method of any one of the preceding claims, wherein each step of the method is adapted to be carried out in aqueous conditions.
10. The method of any one of the preceding claims, wherein the nucleobase is selected from the group consisting of adenine (A), cytosine (C), guanine (G), thymine (T), uracil (U), uric acid, isocytosine, isoguanine, 2-aminopurine, 2,6-diaminopurine, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N-6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N-6-isopentenyladenine, uracil-5-oxy acetic acid, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-S-oxyacetic acid, 5-methyl-2-thiouracil and 3-(3-amino-3-N-2-carboxypropyl) uracil.
11. The method of any one of the preceding claims, wherein the method is substantially devoid of the formation of side products that are reactive to the nucleobases of the nucleotide sequence.
12. The method of any one of the preceding claims, wherein the single-stranded nucleotide sequence comprises a single-stranded deoxynucleotide sequence.
13. The method of any one of the preceding claims, wherein the initiator nucleotide sequence comprises a single-stranded deoxynucleotide sequence or part thereof.

14. The method of any one of the preceding claims, wherein the blocked nucleoside triphosphate comprises a deoxyribonucleoside triphosphate.
- 5 15. The method of any one of the preceding claims, wherein the blocked nucleoside triphosphate comprises a deoxyribonucleoside triphosphate selected from the group consisting of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate and deoxythymidine triphosphate.
- 10 16. The method according to any one of the preceding claims, wherein the blocked nucleoside triphosphate is selected from the following:

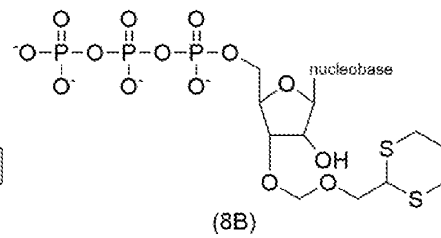
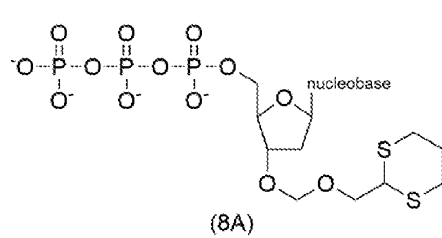
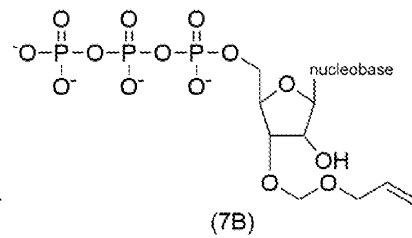
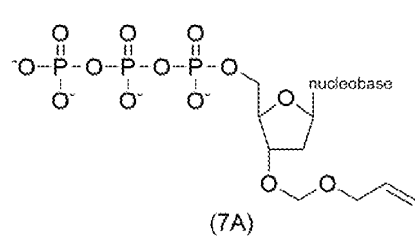


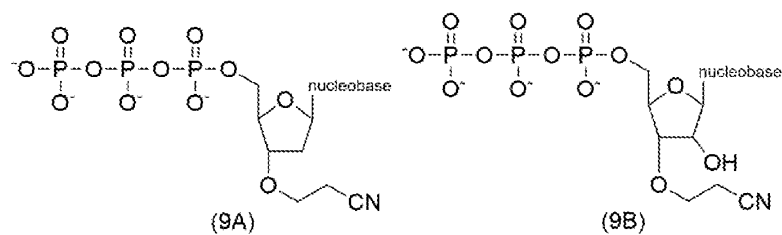
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, wherein nucleobase is selected from the group consisting of adenine (A), cytosine (C), guanine (G), uracil (U) and thymine (T).

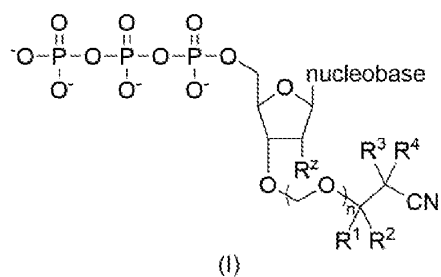
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17. The method of claim 1 or 5, wherein step (i) and/or (iii) comprises forming a phosphodiester linkage between the initiator nucleotide sequence and the blocked nucleoside triphosphate.

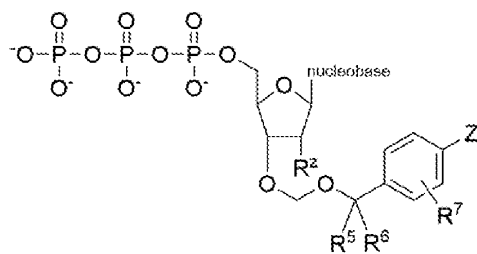
10 18. The method of claim 1 or 5, wherein step (i) and/or (iii) comprises release of a pyrophosphate.

19. A blocked nucleoside triphosphate for the method of any one of claims 1 to 18, the blocked nucleoside triphosphate having any one of the general formulae (I), (II), (III), (IV), (V) and (VI):

15

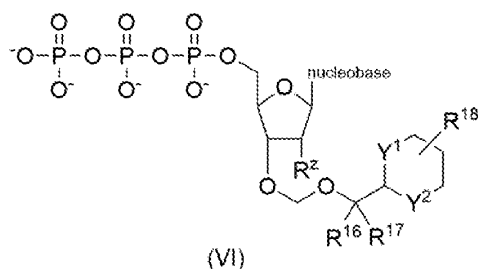
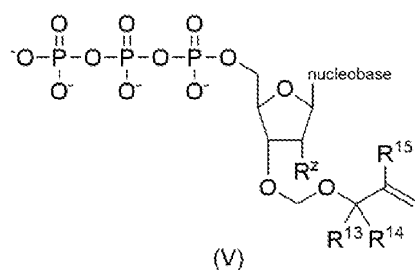
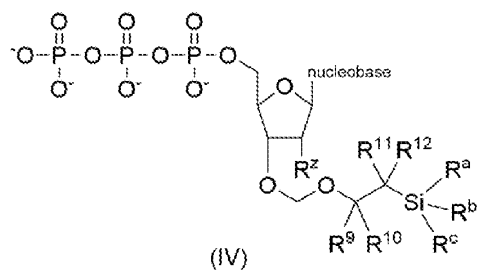
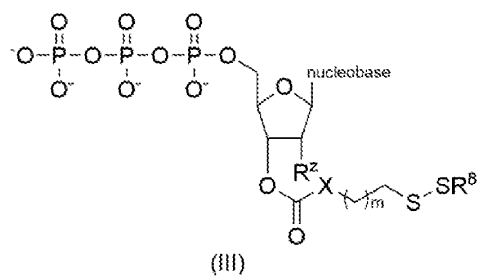


(I)



(II)

72



5

wherein

n = 0 or 1;

m = 0 to 20;

R^Z is H or OH;R¹, R², R³, R⁴, R⁵, R⁶, R^a, R^b and R^c are each independently selected

10

from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

R^7 is selected from hydrogen, alkyl, halogen, $-OR^{19}$, $-NR^{20}R^{21}$ and $-SR^{22}$, wherein R^{19} , R^{20} , R^{21} and R^{22} are independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} and R^{18} are independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

X is a heteroatom selected from O, S and NH;

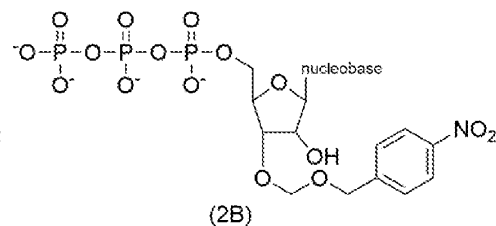
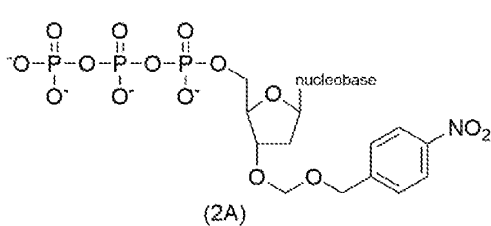
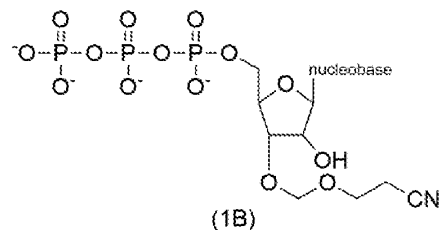
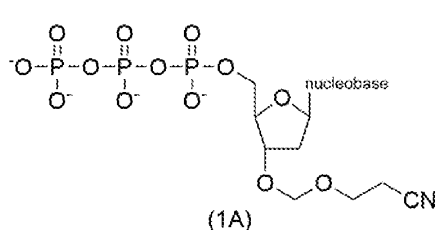
Y^1 and Y^2 are independently selected from S and Se;

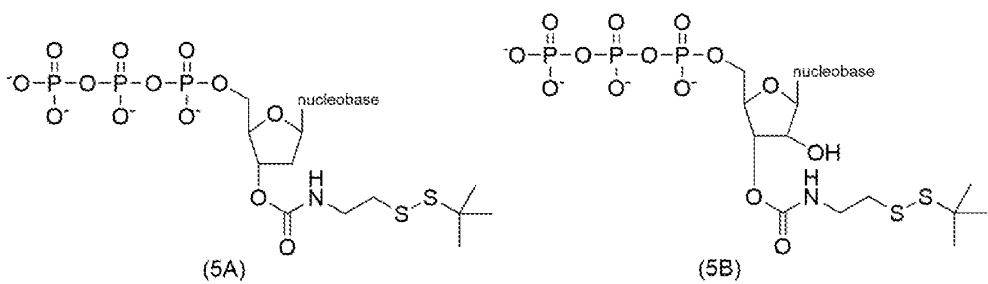
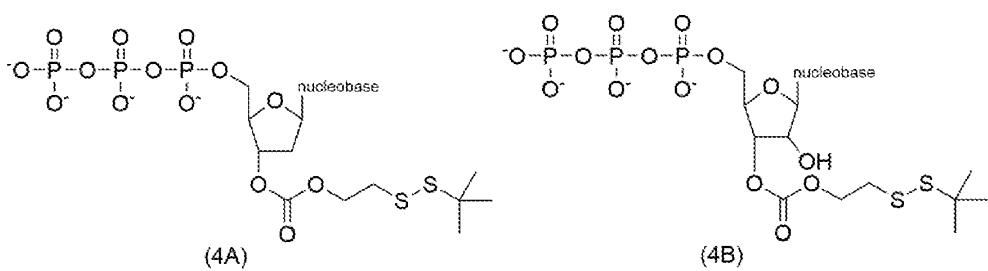
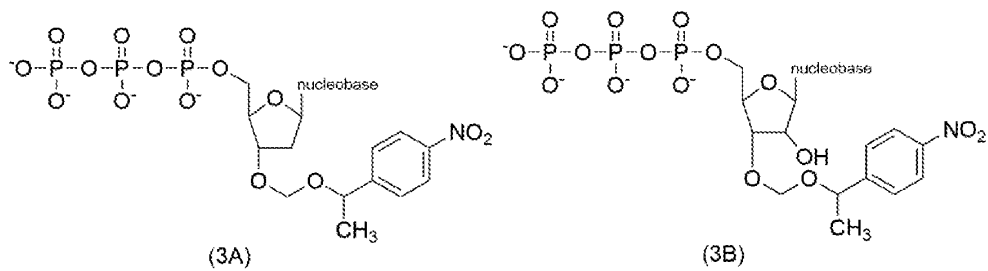
Z is a chemical moiety that is capable of being released under suitable conditions to trigger removal of the adjacent benzyl linker, and with the proviso that when $n = 0$, R^1 and R^2 are both not hydrogen.

20. The blocked nucleoside triphosphate of claim 19, wherein Z is NO_2 .

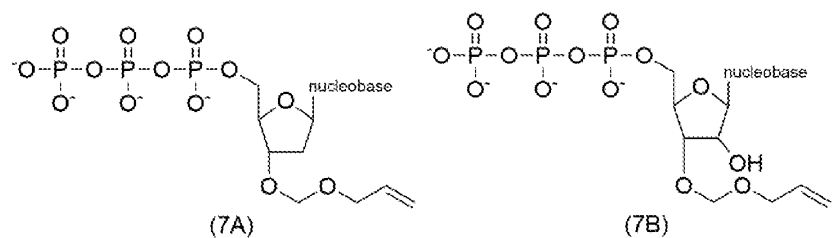
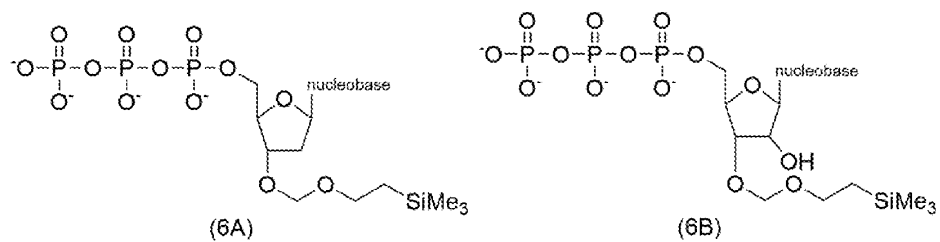
21. The blocked nucleoside triphosphate of claim 19, wherein R^a , R^b and R^c are each methyl.

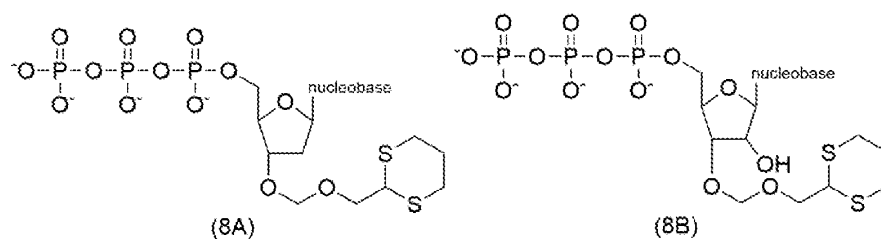
22. The blocked nucleoside triphosphate according to claim 19 selected from the following:





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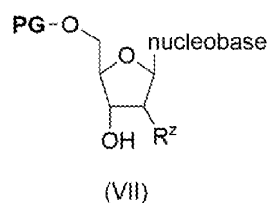


wherein nucleobase is selected from the group consisting of adenine (A), cytosine (C), guanine (G), uracil (U) and thymine (T).

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23. A method of preparing a blocked nucleoside triphosphate of any one of claims 19 to 22, the method comprising:

(i) providing a nucleoside having general formula (VII):



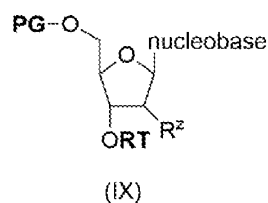
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wherein

R^z is H or OH and **PG** is a protecting group;

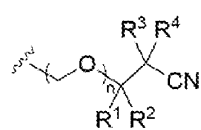
(ii) introducing a removable terminating group (RT) to the nucleoside having general formula (VII) to obtain a nucleoside having general formula (IX):

15

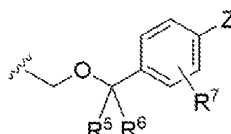


wherein R^z is H or OH and **RT** is selected from any one of the general formulae (VIIIa), (VIIIb), (VIIIc), (VIId), (VIIf) and (VIIf):

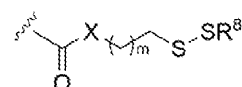
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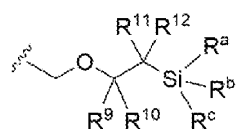
(VIIIa)



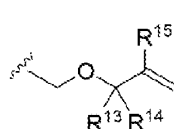
(VIIIb)



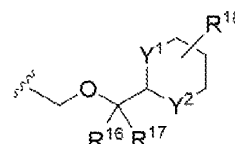
(VIIIc)



(VIIIId)



(VIIIe)



(VIIIf)

wherein

$n = 0$ or 1 ;

$m = 0$ to 20 ;

R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^a , R^b and R^c are each independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

R^7 is selected from hydrogen, alkyl, halogen, $-OR^{19}$, $-NR^{20}R^{21}$ and $-SR^{22}$, wherein R^{19} , R^{20} , R^{21} and R^{22} are independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} and R^{18} are independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

X is a heteroatom selected from O, S and NH;

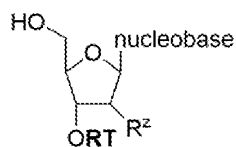
Y^1 and Y^2 are independently selected from S and Se;

Z is a chemical moiety that is capable of being released under suitable conditions to trigger removal of the adjacent benzyl linker, and

with the proviso that when $n = 0$, R^1 and R^2 are both not hydrogen;

- (iii) removing the protecting group (PG) from the nucleoside having general formula (IX) to obtain a nucleoside having general formula (X):

77



(X)

; and

- (iv) converting the hydroxy group in the nucleotide having general formula (X) to triphosphate group to obtain a blocked nucleoside triphosphate of any one of claims 19 to 22.

5

24. The method of claim 23, wherein Z is NO₂.

25. The method of claim 23, wherein R^a, R^b and R^c are each methyl.

- 10 26. The method of claim 23, wherein the protecting group is selected from the group consisting of dimethoxytrityl (DMT), *tert*-butyldimethylsilyl (TBS), 9-phenylxanthen-9-yl and 9-(*p*-tolyl)-xanthen-9-yl, tri-*iso*-propylsilyloxy-methyl (TOM), trimethylsilyl (TMS) and tri-*iso*propylsilyl (TIPS).

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