CLAIMS

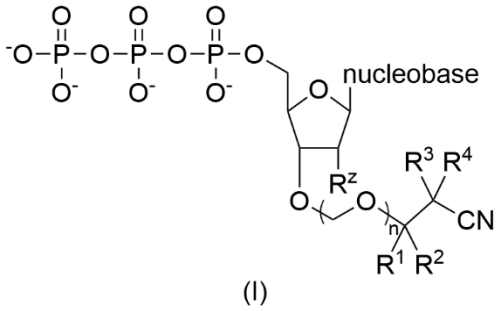
1. A method of synthesizing a single-stranded nucleotide sequence, the method comprising:

   (i) adding a blocked nucleoside triphosphate to an initiator nucleotide sequence to incorporate a corresponding blocked nucleotide thereto in the presence of a template independent polymerase; and

   (ii) removing a removable terminating group from the incorporated blocked nucleotide in the presence of one or more base(s) to obtain a corresponding nucleotide that is unblocked at the 3'-O position,

   wherein step (ii) is adapted to be carried out in aqueous conditions, and

   wherein the blocked nucleoside triphosphate has general formula (I):



(I)

   wherein

   n = 0 or 1;

   R^1, R^2, R^3, R^4 are each independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl; and

   R^z is H or OH.

2. The method of claim 1, further comprising

   (iii) adding a blocked nucleoside triphosphate of the general formula (I) 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.

3. The method of any one of the preceding claims, wherein the polymerase comprises terminal deoxynucleotidyl transferase (TdT) and/or polymerase theta (POLQ).

4. The method of any one of the preceding claims, wherein each step of the method is adapted to be carried out in aqueous conditions.

5. 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-(carboxyhydroxylmethyl)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, methoxyarninomethyl-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.

6. 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.

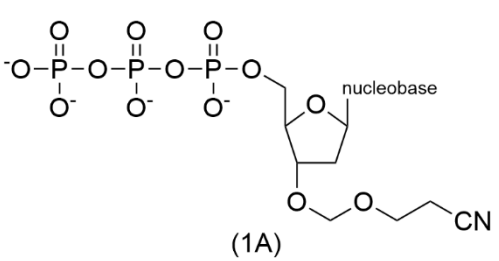
7. The method of any one of the preceding claims, wherein the single-stranded nucleotide sequence comprises a single-stranded deoxynucleotide sequence.

8. The method of any one of the preceding claims, wherein the initiator nucleotide sequence comprises a single-stranded deoxynucleotide sequence or part thereof.

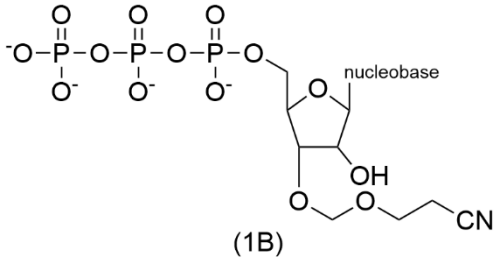
9. The method of any one of the preceding claims, wherein the blocked nucleoside triphosphate comprises a deoxyribonucleoside triphosphate.

10. 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.

11. The method according to any one of the preceding claims, wherein the blocked nucleoside triphosphate is selected from the following:



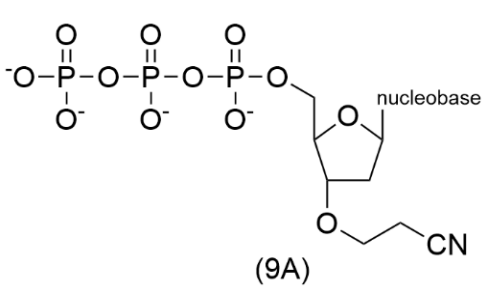
(1A)



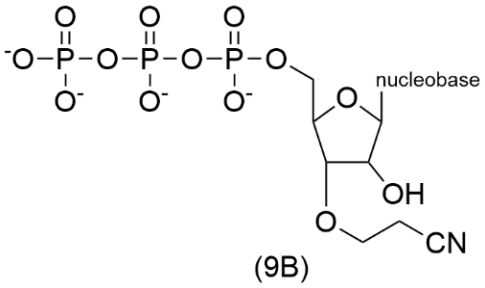
(1B),

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

12. The method according to any one of the preceding claims, wherein the blocked nucleoside triphosphate is selected from the following:



(9A)



(9B),

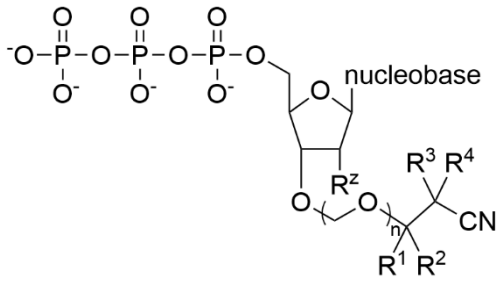
wherein nucleobase is selected from the group consisting of adenine (A), cytosine (C), guanine (G), uracil (U) and thymine (T).

13. The method of claim 11 or claim 12, wherein the nucleobase is thymine (T).

14. The method of claim 1 or 2, wherein step (i) and/or (iii) comprises forming a phosphodiester linkage between the initiator nucleotide sequence and the blocked nucleoside triphosphate.

15. The method of claim 1 or 2, wherein step (i) and/or (iii) comprises release of a pyrophosphate.

16. A method of preparing a blocked nucleoside triphosphate of formula (I):



(I)

wherein

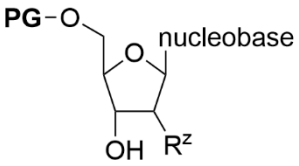
n = 0 or 1;

R^1, R^2, R^3, R^4 are each independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl; and

R^z is H or OH,

the method comprising:

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

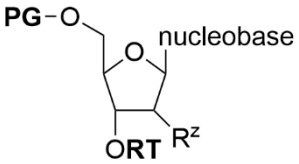


(VII)

wherein

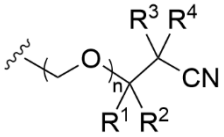
R^z is H or OH and **PG** is a protecting group;

(ii) introducing a removable terminating group (RT) to the nucleoside having general formula (VII), by substituting H of the OH group at the 3' position of formula (VII) with **RT** in one step or in two steps, to obtain a nucleoside having general formula (IX):



(IX)

wherein R^z is H or OH and **RT** has the general formula (VIIIa):



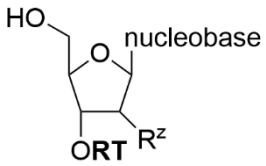
(VIIIa)

wherein

n = 0 or 1;

R^1, R^2, R^3, and R^4 are each independently selected from hydrogen, alkyl, alkenyl, aryl and heteroaryl;

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



(X) ; and

(iv) converting the hydroxy group in the nucleotide having general formula (X) to triphosphate group to obtain the blocked nucleoside triphosphate.

17. The method of claim 16, 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).

18. The method of any one of claims 16 to 17, wherein (ii) is carried out in one step and wherein the step is carried out in the presence of acrylonitrile and caesium carbonate (Cs_2CO_3).

19. The method of any one of claims 16 to 17, wherein (ii) is carried out in two steps, and wherein first step is carried out in the presence of acetic anhydride (Ac_2O), dimethylsulfoxide (DMSO) and acetic acid (AcOH), and second step is carried out in the presence of 3-hydroxypropanenitrile, N-iodosuccinimide (NIS), trifluoromethanesulfonic acid (TfOH) and 1,2-dichloroethane (1,2-DCE).