## CLAIMS

What is claimed is:

1. A method of producing a nucleotide triphosphate with a phosphate group at the 3' position of the sugar (NQP), the method comprising at least: contacting a nucleoside diphosphate (NDP) with a nucleoside diphosphate kinase, a 3'-O-kinase, and a phosphate donor under reaction conditions such that a NTP with a phosphate group on the 3' position of the sugar moiety (NQP) is produced.

2. The method of claim 1, wherein the NDP has at the 2' position of the sugar moiety a H or OH; optionally, wherein:

(i) the NDP is ADP, GDP, UDP, CDP, or TDP, and wherein the NDP has at the 2 '-position of the sugar moiety an OH, thereby by resulting in corresponding product rATP, rGTP, rUTP, rCTP, or rTTP, respectively; or

(ii) the NDP is ADP, GDP, UDP, CDP, or TDP, and wherein the NDP has at the 2 '-position of the sugar moiety a H, thereby by resulting in corresponding product dAMP. dGMP, dUMP, dCMP, or dTMP, respectively.

3. The method of claim 1, wherein the NDP is a modified NDP comprising a modified 2'-position, wherein the 2'-position of the sugar moiety is -O-$CH_3$, -O-$CH_2CH_3$, F, or Br.

4. The method of claim 1 or 2, wherein the NDP comprises a modified nucleobase, wherein the modified nucleobase is 5-bromo-uracil, 5-iodo-uracil, 6-mCEPh-purine, 6-phcnylpyrrolocytidinc, N2-alkyl 8-oxoguanosine, difluorotoluene, difluorobenzene, dichlorobenzene, imidazole, or benzimidazole.

5. The method of any one of claims 1-4, wherein the NDP comprises a 5'-O-(l-thio(diphosphate) (NDPαS).

6. The method of claim 1, further comprising contacting a nucleoside monophosphate (NMP) with a nucleoside monophosphate kinase in presence of a phosphate donor NTP under suitable reaction conditions such that a product nucleoside diphosphate (NDP) is produced for reaction with the nucleoside diphosphate kinase and/or 3'-O-kinase.

7. The method of claim 6, wherein the NMP has at the 2' position of the sugar moiety a H or OH; optionally, wherein:

(i) the NMP is AMP, GMP, UMP, CMP, or TMP, and wherein the NMP has at the 2'-position of the sugar moiety an OH, thereby by resulting in corresponding product rADP, rGDP, rUDP, rCDP, or rTDP, respectively; or

(ii) the NMP is AMP, GMP, UMP, CMP, or TMP, and wherein the NMP has at the 2'-position of the sugar moiety a H, thereby by resulting in corresponding product dADP, dGDP, dUDP, dCDP, or dTDP, respectively.

8. The method of claim 6, wherein the NMP is a modified NMP comprising a modified 2'- position of the sugar moiety, wherein the 2'-position of the sugar moiety is -O-$CH_3$, -O-$CH_2CH_3$, F, or Br.

9. The method of claim 6, 7, or 8, wherein the NMP comprises a modified nucleobase, wherein the modified nucleobase is 5-bromo-uracil, 5 -iodo-uracil, 6-mCEPh-purine, 6-phenylpyrrolocytidine, N2-alkyl 8-oxoguanosine, difluorotoluene, difluorobenzene, dichlorobenzene, imidazole, or benzimidazole.

10. The method of any one of claims 6-9, wherein the modified NMP comprises a nucleoside 5'-O-thiophosphate (NMPαS).

11. The method of any one of claims 6-10, wherein the phosphate donor NTP has the same nucleotide structure as the substrate NMP.

12. The method of any one of claims 6-11, wherein the nucleoside monophosphate kinase is an adenylate kinase; optionally, wherein the adenylate kinase is an adenylate kinase of an even-numbered SEQ ID NO. of SEQ ID NOs: 1206-2504, with or without amino acid residues 1-12.

13. The method of claim 6, further comprising contacting a nucleoside with a nucleoside kinase in presence of a phosphate donor NTP under reaction conditions such that a product nucleoside monophosphate (NMP) is produced.

14. The method of claim 13, wherein the nucleoside has at the 2' position of the sugar moiety a H or OH, optionally, wherein the nucleoside is adenosine (A), guanosine (G), uridine (U), cytidine (C), or thymidine (T), and wherein the nucleoside has at the 2'-position of the sugar moiety (i) an OH, thereby by resulting in corresponding product rAMP, rGMP, rUMP, rCMP, or rTMP, respectively; or (ii) a H, thereby by resulting in corresponding product dAMP, dGMP, dUMP, dCMP, or dTMP, respectively.

15. The method of claim 13, wherein the nucleoside is a modified nucleoside, wherein the 2'-position of the sugar moiety is -$OCH_3$, -$OCH_2CH_3$, F, or Br.

16. The method of claim 13, 14, or 15, wherein the nucleoside comprises a modified nucleobase, wherein the modified nucleobase is 5-bromo-uracil, 5 -iodo-uracil, 6-mCEPh-purine, 6-phenylpyrrolocytidine, N2-alkyl 8-oxoguanosine, difluorotoluene, difluorobenzene, dichlorobenzene, imidazole, or benzimidazole.

17. The method of any one of claims 13-16, wherein the phosphate donor NTP is a nucleoside-5'-gamma thio-triphosphate (NTPγS) for producing NMP-5'-O-thiophosphate (NMPαS).

18. The method of any one of claims 13-17, wherein the nucleoside kinase is an adenosine kinase; optionally, wherein the adenosine kinase is an adenosine kinase of an even-numbered SEQ ID NO. of SEQ ID NOs: 66-1204, with or without amino acid residues 1-12.

19. The method of any one of claims 1-18, wherein the nucleoside diphosphate kinase is an acetate kinase; optionally, wherein the acetate kinase is an acetate kinase of an even-numbered SEQ ID NO. of SEQ ID NOs: 2506-3058, with or without amino acid residues 1-12.

20. The method of any one of claims 1-19, wherein the 3'-O-kinase is a 3'-O-kinase of an even-numbered SEQ ID NO. of SEQ ID NOs: 3060-3370 and 3376-5126, with or without amino acid residues 202-211.

21. A method of producing a NTP with a phosphate group at the 3' position of the sugar (NQP), the method comprising contacting a nucleoside with a nucleoside kinase, a nucleoside monophosphate kinase, nucleoside diphosphate kinase, and a 3'-O-kinase in presence of a phosphate donor under reaction conditions such that a product NTP with a phosphate group at the 3' position of the sugar (NQP) is produced.

22. A method of producing a NTP with a phosphate group at the 3' position of the sugar (NQP), the method comprising (i) contacting a nucleoside with a nucleoside kinase, a nucleotide monophosphate kinase, and a nucleoside diphosphate kinase in presence of a phosphate donor under reaction conditions such that a NTP is produced; and iii) contacting the NTP produced in (i) with a 3'-O-kinase enzyme under suitable reaction conditions, such that a NTP with a phosphate group at the 3' position of the sugar (NQP) is produced.

23. A method of producing a NTP with a phosphate group at the 3' position of the sugar (NQP), the method comprising (i) contacting a nucleoside with a nucleoside kinase, (ii) contacting the product of (i) with a nucleotide monophosphate kinase, (iii) contacting the product of (ii) with a nucleoside diphosphate kinase, and (iv) contacting the product of (iii) with a 3'-O-kinasc enzyme, wherein (i), (ii), (iii) and (iv) are carried out in presence of a phosphate donor under reaction conditions such that a product NTP with a phosphate group at the 3' position of the sugar (NQP) is produced.

24. The method of any one of claims 21-23, wherein the nucleoside has at the 2’-position of the sugar moiety a H or OH, optionally, wherein the nucleoside is adenosine (A), guanosine (G), uridine (U), cytidine (C), or thymidine (T), and wherein: (i) the nucleoside has at the 2'-position of the sugar moiety an OH, thereby by resulting in corresponding product rAQP, rGQP, rUQP. rCQP, or rTQP, respectively; or (ii) the nucleoside has at the 2'-position of the sugar moiety a H, thereby by resulting in corresponding product dAQP, dGQP, dUQP, dCQP, or dTQP, respectively.

25. The method of any one of claims 21-23. wherein the nucleoside comprises a modified 2’-position of the sugar moiety, wherein the 2’-position of the sugar moiety is $-OCH_3$, $-OCH_2CH_3$, F, or Br.

26. The method of any one of claims 21-23, 24, and 25, wherein the nucleoside comprises a modified nucleobase, wherein the modified nucleobase is 5-bromo-uracil, 5-iodo-uracil, 6-mCEPh-purine, 6-phenylpyrrolocytidine, N2-alkyl 8-oxoguanosine, difluorotoluene, difluorobenzene, dichlorobenzene, imidazole, or benzimidazole.

27. The method of any of claims 21-26, wherein the phosphate donor comprises phosphate donor NTP.

28. The method of claim 27, wherein the phosphate donor NTP is a nucleoside-5’-l-thio(triphosphate) (NTPαS)

29. The method of any one of claims 21-28, wherein the nucleoside kinase is an adenosine kinase; optionally, wherein the adenosine kinase is an adenosine kinase of an even-numbered SEQ ID NO. of SEQ ID NOs: 66-1204. with or without amino acid residues 1-12.

30. The method of any one of claims 21-29, wherein the nucleoside monophosphate kinase is an adenylate kinase; optionally, wherein the adenylate kinase is an adenylate kinase of an even-numbered SEQ ID NO. of SEQ ID NOs: 1206-2504, with or without amino acid residues 1-12.

31. The method of any one of claims 21-30, wherein the nucleoside diphosphate kinase is and acetate kinase, and the phosphate donor includes acetyl phosphate, optionally, wherein the acetate kinase is an acetate kinase of an even-numbered SEQ ID NO. of SEQ ID NOs: 2506-3058, with or without amino acid residues 1-12.

32. The method of any one of claims 21-31, wherein the 3’-O’-kinase comprises a 3’-O’-kinase of an even-numbered SEQ ID NO. of SEQ ID NOs: 3060-3370 and 3376-5126, with or without amino acid residues 202-211.

33. A method of producing a nucleoside triphosphate (NTP), comprising contacting a nucleoside with a nucleoside kinase, a nucleoside monophosphate kinase, and a nucleoside diphosphate kinase in presence of a phosphate donor under reaction conditions such that NTP is produced.

34. The method of any one of claims 1-33, further comprising a NTP regenerating system.

35. The method of claim 34, wherein the NTP regenerating system comprises creatine kinase, pyruvate kinase, polyphosphate kinase, and/or R-acetate-kinase.

36. The method of claim 35, wherein: (i) the NTP regenerating system is pyruvate kinase and substrate phosphoenolpyruvate;

(ii) the NTP regenerating system is creatine kinase and substrate creatine phosphate;

(iii) the NTP regenerating system is polyphosphate kinase and substrate polyphosphate; and/or

(iv) the NTP regenerating system is R-acetate kinase and the substrate acetyl phosphate.

37. The method of claim 36, wherein the R-acetate kinase is same or different than the acetate kinase of any one of claims 19, and 31.

38. The method of claim 36 or 37, further comprising pyruvate oxidase and substrate pyruvate and inorganic phosphate; optionally, further comprising including a catalase.