METHODS FOR ENHANCING RECOMBINANT ADENO-ASSOCIATED VIRUS YIELD

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/990,099, filed March 16, 2020, the entire content of which is hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates generally to methods for enhancing recombinant adeno-associated virus vector (rAAV) yield, and, more particularly, the invention relates to the use of compounds to enhance rAAV yield.

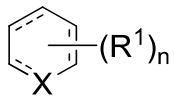
BACKGROUND

[0003] Adeno-associated virus (AAV) is a non-pathogenic, replication-defective parvovirus. Recombinant AAV vectors (rAAV) have many unique features that make them attractive as vectors for gene therapy. In particular, rAAV vectors can deliver therapeutic genes to dividing and non-dividing cells, and these genes can persist for extended periods without integrating into the genome of the targeted cell. Given the widespread therapeutic applications of rAAV, there exists an ongoing need for improved methods of rAAV vector production including methods to achieve high-titer rAAV vector yields. Previous attempts to improve the production of a variety of viral vectors have included the use of cell culture additives such as metals, trace supplements, salts, and others (*see, e.g.*, Williams, J. Gen. Virol., 9(3): 251-5 (1970), Weinbauer *et al.*, Limnology and Oceanography, 54(3): 774-784 (2009), Yang *et al.*, Hepatology, 48(5): 1396-403 (2008), and U.S. Publication No. 20150353899).

SUMMARY ~~OF THE INVENTION~~

[0004] The ~~invention~~disclosure is based, in part, upon the discovery that a host cell used in the production of recombinant adeno-associated virus vectors (rAAV) will produce increased amounts of rAAV, when the host cell is in contact with a solution comprising a compound described herein.

[0005] Thus, in an aspect, provided herein is a method of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising at least one compound of formula (I) or a salt thereof:


(I), wherein:

------- denotes a single bond or a double bond;

n is an integer selected from 1 to 5;

each R^1 is independently, for each occurrence, selected from the group consisting of -OH, C_{1-6}alkyl, C_{1-6}alkyl-OH, C_{1-6}alkyl-O-C_{1-6}alkyl, -O-C_{1-6}alkyl, -C(O)N(R^a)$_2$, and -C(O)OR^b;

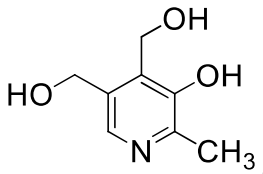
R^a is independently, for each occurrence, hydrogen or C_{1-6}alkyl;

R^b is independently, for each occurrence, hydrogen or C_{1-6}alkyl;

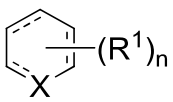
X is C$R^c R^d$ or N;

R^c and R^d are independently, for each occurrence, selected from the group consisting of hydrogen, C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, wherein when R^c is hydrogen, R^d is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, and when R^c is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, R^d is hydrogen; and

wherein the at least one compound is not:


, or a salt thereof.

[0005a] In another aspect, the present disclosure provides a method of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising at least one compound of formula (I) or a salt thereof:


(I), wherein:

------- denotes a single bond or a double bond;

n is an integer selected from 1 to 5;

each R^1 is independently, for each occurrence, selected from the group consisting of -OH, C_{1-6}alkyl, C_{1-6}alkyl-OH, C_{1-6}alkyl-O-C_{1-6}alkyl, -O-C_{1-6}alkyl, -C(O)N(R^a)$_2$, and -C(O)OR^b;

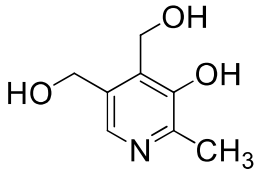
R^a is independently, for each occurrence, hydrogen or C_{1-6}alkyl;

R^b is independently, for each occurrence, hydrogen or C_{1-6}alkyl;

X is CR^cR^d or N;

R^c and R^d are independently, for each occurrence, selected from the group consisting of hydrogen, C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, wherein when R^c is hydrogen, R^d is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, and when R^c is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, R^d is hydrogen; and

wherein the at least one compound is not:



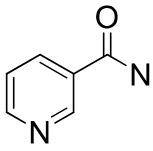
, or a salt thereof.

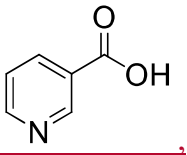
[0005b] In another aspect, the present disclosure provides a composition comprising a host cell and

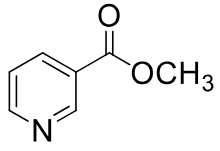
i) a compound of formula (I) or a salt thereof;

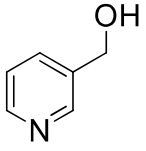
ii) a compound of formula (I-A) or a salt thereof;

iii) a compound of formula (I-B) or a salt thereof;

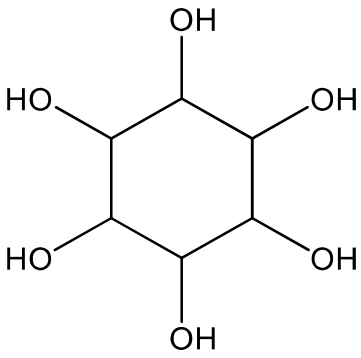
iv) at least one compound selected from the group consisting of: 

, 

, 

, 

, and any combination(s) thereof; or



v) a compound of formula , optionally wherein the host cell further comprises

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats;

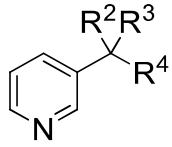
ii) AAV rep and cap genes;

iii) helper genes; or

iv) a combination thereof.

[0006] In another ~~aspect~~example, provided herein is a method of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising a compound of formula (I) or a salt thereof.

[0007] In certain ~~embodiment~~examples, the solution comprises at least one compound of formula (I-A) or a salt thereof:



(I-A), wherein:

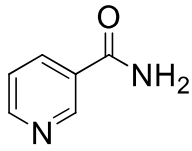
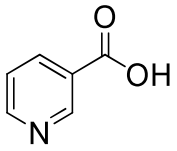
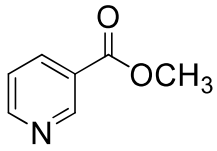
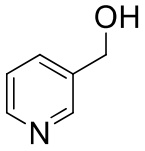
R^2 and R^3 are independently, for each occurrence, hydrogen or -OH,

or R^2 and R^3 can be taken together to form oxo;

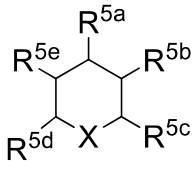
R^4 is selected from the group consisting of hydrogen, -OH, -O-C_{1-6}alkyl, and -$N(R^e)_2$; and

R^e is independently, for each occurrence, hydrogen or C_{1-6}alkyl.

[0008] In some ~~embodiment~~examples, the solution comprises at least one compound selected from the group consisting of:


, 
, 
, 
, and any combination(s) thereof.

[0009] In certain ~~embodiment~~examples, the solution comprises at least one compound of formula (I-B) or a salt thereof:

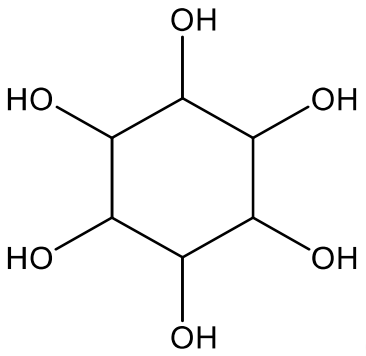

(I-B), wherein:

X is CR^cR^d; and

R^{5a}, R^{5b}, R^{5c}, R^{5d}, and R^{5e} are independently, for each occurrence, -OH, -O-C_{1-6}alkyl, C_{1-6}alkyl, C_{1-6}alkyl-OH, and C_{1-6}alkyl-O-C_{1-6}alkyl; and

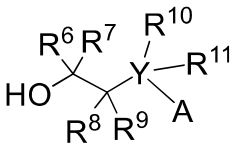
R^c and R^d are independently, for each occurrence, selected from the group consisting of hydrogen, C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, wherein when R^c is hydrogen, R^d is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, and when R^c is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, R^d is hydrogen.

[0010] In some ~~embodiment~~examples, the solution comprises the compound of formula (III):



(III).

[0011] In another ~~aspect~~example, provided herein is a method of producing rAAV, the method comprising contacting a host cell with a solution comprising at least one compound of formula (II) or a salt thereof:



(II), wherein:

R^6 and R^7 are independently, for each occurrence, selected from the group consisting of hydrogen, -OH, -CN, halogen, and C_{1-6}alkyl;

R^8 and R^9 are independently, for each occurrence, selected from the group consisting of hydrogen, -OH, -CN, halogen, and C_{1-6}alkyl;

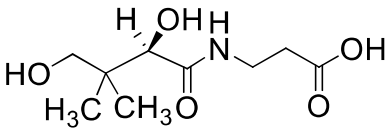
Y is C or N;

R^{10} and R^{11} are independently, for each occurrence, selected from the group consisting of hydrogen, -OH, and C_{1-6}alkyl;

A is selected from the group consisting of hydrogen, C_{1-6}alkyl, and $-C(O)N(R^f)_2$; and

R^f is independently, for each occurrence, selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{1-6}alkyl-C(O)OH, and C_{1-6}alkyl-OH;

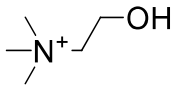
wherein the at least one compound is not:



or a salt thereof.

[0012] In another ~~aspect~~example, provided herein is a method of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising a compound of formula (II) or a salt thereof.

[0013] In certain ~~embodiment~~examples, the at least one compound of formula (II) is a compound of formula (IV):



(IV).

[0014] In another ~~aspect~~example, provided herein is a method of producing rAAV or increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising a B vitamin selected from vitamin B_2, vitamin B_7, vitamin B_9, and vitamin B_{12}.

[0015] In some ~~embodiment~~examples, the methods as described herein may further comprise the steps of harvesting and purifying the rAAV.

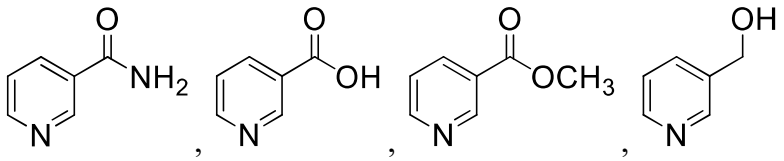
[0016] It is contemplated that the concentration of a compound described herein (*e.g.*, a compound of formula (I), (I-A), (I-B), (II), (III), or (IV), or a B vitamin (*e.g.*, vitamin B_2, vitamin B_7, vitamin B_9, or vitamin B_{12})) in solution may be greater than or equal to 10 mM, greater than or equal to 12 mM, greater than or equal to 15 mM, greater than or equal to 1 mM, greater than or equal to 0.5 mM, greater than 0.5 mM, between 0.5 mM and 15 mM, or between 1 mM and 10 mM. In one ~~embodiment~~example, the concentration of the at least one compound (*e.g.*, a compound of formula (I), (I-A), (I-B), (II), (III), or (IV), or a B vitamin (*e.g.*, vitamin B_2, vitamin B_7, vitamin B_9, or vitamin B_{12})) in the solution is sufficient to produce at least 1.2-fold greater quantities of secreted rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound. In another ~~embodiment~~example, the concentration of the at least one compound (*e.g.*, a compound of formula (I), (I-A), (I-B), (II), (III), or (IV), or a B vitamin (*e.g.*, vitamin B_2, vitamin B_7, vitamin B_9, or vitamin B_{12})) in solution is sufficient to produce at least 1.2-fold greater quantities of total rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound. In one ~~embodiment~~example, the host cell is contacted with the solution comprising the at least one compound for at least 2 days.

[0017] It is contemplated that the host cell may be a mammalian cell, for example, a HeLa, HEK293, COS, A549, BHK, or Vero cell. It is also contemplated that the host cell may be an insect cell, for example, a Sf9, Sf-21, Tn-368, or BTI-Tn-5B1-4 (High-Five) cell. In one ~~embodiment~~example, the host cell is a HeLa cell. In another ~~embodiment~~example, the host cell is a HEK293 cell.

It is contemplated that a host cell may comprise recombinant nucleic acid construct comprising a heterologous nucleotide sequence flanked by AAV inverted terminal repeats. In one ~~embodiment~~example, the host cell may comprise rep and cap genes (*e.g.*, AAV rep and cap genes). In one ~~embodiment~~example, the host cell may comprise helper virus genes, *e.g.*, supplied *via* a helper plasmid or *via* infection with adenovirus. In one ~~embodiment~~example, the host cell comprises i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) rep and cap genes, and iii) helper virus genes.

[0018] In one ~~embodiment~~example, the host cell produces at least 1.2-fold greater quantities of secreted rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound (*e.g.*, a compound of formula (I), (I-A), (I-B), (II), (III), or (IV), or a B vitamin (*e.g.*, vitamin B_2, vitamin B_7, vitamin B_9, or vitamin B_{12})). In another ~~embodiment~~example, the host cell produces at least 1.2-fold greater quantities of total rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound (*e.g.*, a compound of formula (I), (I-A), (I-B), (II), (III), or (IV), or a B vitamin (*e.g.*, vitamin B_2, vitamin B_7, vitamin B_9, or vitamin B_{12})).

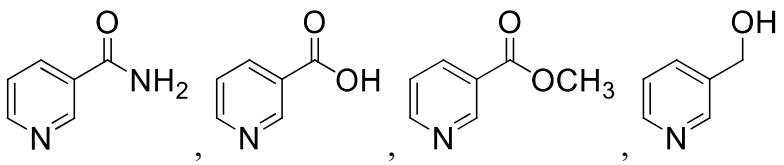
[0019] In some ~~embodiment~~examples, the ~~invention~~disclosure provides methods of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising at least one compound selected from the group consisting of:



, and any combination(s) thereof,

wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

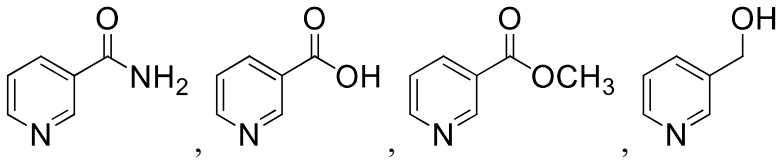
[0020] In some ~~embodiment~~examples, the ~~invention~~disclosure provides methods of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising at least one compound selected from the group consisting of:



, and any combination(s) thereof,

wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

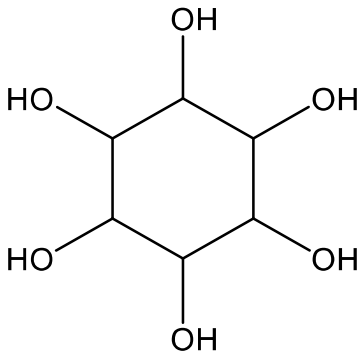
[0021] In some ~~embodiment~~examples, the ~~invention~~disclosure provides compositions comprising a host cell and at least one compound selected from the group consisting of:



, and any combination(s) thereof,

wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

[0022] In some ~~embodiment~~examples, the ~~invention~~disclosure provides methods of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising



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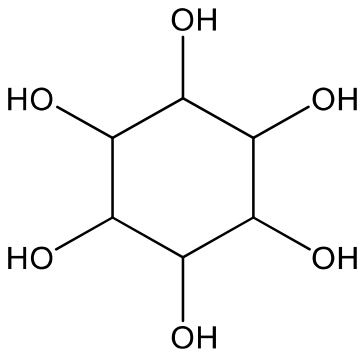
wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

[0023] In some ~~embodiment~~examples, the ~~invention~~disclosure provides methods of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising



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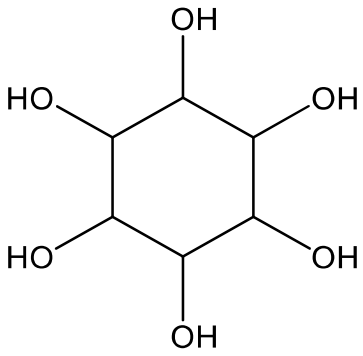
wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

[0024] In some ~~embodiment~~examples, the ~~invention~~disclosure provides compositions comprising a host cell and



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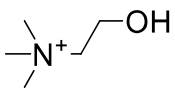
wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

[0025] In some ~~embodiment~~examples, the ~~invention~~disclosure provides methods of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising:



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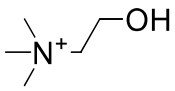
wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

[0026] In some ~~embodiment~~examples, the ~~invention~~disclosure provides methods of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising:



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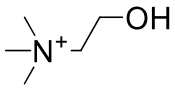
wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

[0027] In some ~~embodiment~~examples, the ~~invention~~disclosure provides compositions comprising a host cell and



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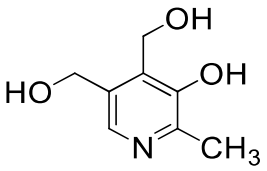
wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

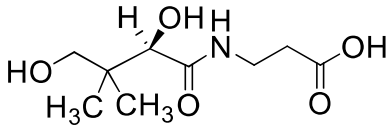
[0028] In some ~~embodiment~~examples, the ~~invention~~disclosure provides methods of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising at least one compound selected from the group consisting of vitamin B_2, vitamin B_7, vitamin B_9, vitamin B_{12}, and any combination(s) thereof, wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

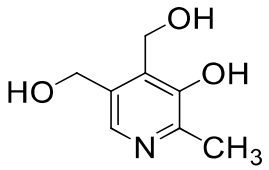
[0029] In some ~~embodiment~~examples, the ~~invention~~disclosure provides methods of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising at least one compound selected from the group consisting of vitamin B_2, vitamin B_7, vitamin B_9, vitamin B_{12}, and any combination(s) thereof, wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

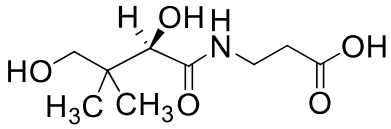
[0030] In some ~~embodiment~~examples of provided methods, the solution does not comprise



, or a salt thereof.

[0031] In some ~~embodiment~~examples of provided methods, the solution does not comprise  or a salt thereof.

[0032] In some ~~embodiment~~examples, provided compositions do not comprise , or a salt thereof.

[0033] In some ~~embodiment~~examples, provided compositions do not comprise  or a salt thereof.

[0034] In some ~~embodiment~~examples, the ~~invention~~disclosure provides compositions comprising a host cell and at least one compound selected from the group consisting of vitamin B_2, vitamin B_7, vitamin B_9, vitamin B_{12}, and any combination(s) thereof, wherein the host cell comprises: i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, and iii) optionally, helper virus genes.

[0035] In other ~~aspect~~examples, the ~~invention~~disclosure provides a rAAV produced by any of the contemplated methods, a composition comprising a rAAV produced by any of the contemplated methods, or a composition comprising a host cell and at least one compound described herein (*e.g.*, a compound of formula (I), (I-A), (I-B), (II), (III), or (IV), or a B vitamin (*e.g.*, vitamin B_2, vitamin B_7, vitamin B_9, vitamin B_{12}, or any combination(s) thereof), or any combination(s) thereof).

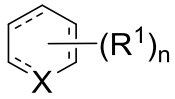
[0036] These and other ~~aspect~~examples and features of the ~~invention~~disclosure are described in the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] The foregoing and other objects, features and advantages of the invention will become apparent from the following description of preferred embodiments, as illustrated in the accompanying drawings. Like referenced elements identify common features in the corresponding drawings.

WHAT IS CLAIMED IS:

1. A method of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising at least one compound of formula (I) or a salt thereof:


(I), wherein:

-------- denotes a single bond or a double bond;

n is an integer selected from 1 to 5;

each R^1 is independently, for each occurrence, selected from the group consisting of -OH, C_{1-6}alkyl, C_{1-6}alkyl-OH, C_{1-6}alkyl-O-C_{1-6}alkyl, -O-C_{1-6}alkyl, -C(O)N(R^a)$_2$, and -C(O)OR^b;

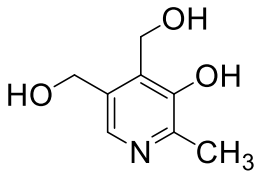
R^a is independently, for each occurrence, hydrogen or C_{1-6}alkyl;

R^b is independently, for each occurrence, hydrogen or C_{1-6}alkyl;

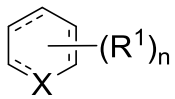
X is C$R^c R^d$ or N;

R^c and R^d are independently, for each occurrence, selected from the group consisting of hydrogen, C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, wherein when R^c is hydrogen, R^d is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, and when R^c is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, R^d is hydrogen; and

wherein the at least one compound is not:


, or a salt thereof.

2. A method of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising at least one compound of formula (I) or a salt thereof:


(I), wherein:

-------- denotes a single bond or a double bond;

n is an integer selected from 1 to 5;

each R^1 is independently, for each occurrence, selected from the group consisting of -OH, C_{1-6}alkyl, C_{1-6}alkyl-OH, C_{1-6}alkyl-O-C_{1-6}alkyl, -O-C_{1-6}alkyl, -C(O)N(R^a)$_2$, and -C(O)OR^b;

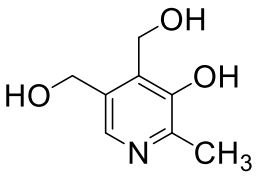
R^a is independently, for each occurrence, hydrogen or C_{1-6}alkyl;

R^b is independently, for each occurrence, hydrogen or C_{1-6}alkyl;

X is C$R^c R^d$ or N;

R^c and R^d are independently, for each occurrence, selected from the group consisting of hydrogen, C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, wherein when R^c is hydrogen, R^d is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, and when R^c is selected from C_{1-6}alkyl, -OH, and -O-C_{1-6}alkyl, R^d is hydrogen; and

wherein the at least one compound is not:



, or a salt thereof.

3. The method of claim 1 or 2, wherein X is N.

4. The method of ~~any one of claims 1-3~~claim 1 or 2, wherein n is 1 or 5.

5. The method of ~~any one of claims 1-4~~claim 1 or 2, wherein:

i) each R^1 is independently, for each occurrence, selected from the group consisting of C_{1-6}alkyl-OH, -C(O)N(R^a)$_2$, and -C(O)OR^b;

ii) each R^1 is independently, for each occurrence, selected from the group consisting of CH_2OH, -C(O)NH_2, -C(O)OH, and -C(O)OCH_3;

iii) X is C$R^c R^d$;

iv) R^c is hydrogen and R^d is -OH; or

v) R^1 is -OH.

~~6. The method of any one of claims 1-5, wherein each R^1 is independently, for each occurrence, selected from the group consisting of CH_2OH, -C(O)NH_2, -C(O)OH, and -C(O)OCH_3.~~

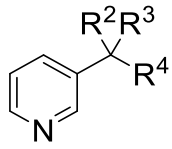
~~7. The method of claim 1 or 2, wherein X is C$R^c R^d$.~~

~~8. The method of any one of claims 1, 2, and 7, wherein R^e is hydrogen and R^d is -OH.~~

~~9. The method of any one of claims 1, 2, 3, and 7-8, wherein n is 5.~~

~~10. The method of any one of claims 1, 2, 3, 4, and 7-9, wherein R^1 is -OH.~~

~~11~~6. The method of claim 1 or 2, wherein the solution comprises at least one compound of formula (I-A) or a salt thereof:



(I-A), wherein:

R^2 and R^3 are independently, for each occurrence, hydrogen or -OH,

or R^2 and R^3 can be taken together to form oxo;

R^4 is selected from the group consisting of hydrogen, -OH, -O-C_{1-6}alkyl, and -$N(R^e)_2$; and

R^e is independently, for each occurrence, hydrogen or C_{1-6}alkyl.

~~12~~7. The method of claim ~~11~~6, wherein:

i) R^2 and R^3 are taken together to form oxo;

ii) R^2 and R^3 are both hydrogen;

iii) R^4 is selected from the group consisting of -NH_2, -OH, and -OCH_3; or

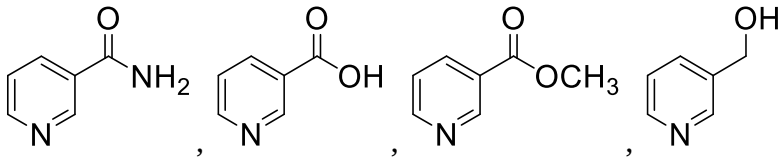
iv) a combination thereof.

~~13. The method of claim 11 or 12, wherein R^4 is selected from the group consisting of -NH_2, -OH, and -OCH_3.~~

~~14. The method of claim 11, wherein R^2 and R^3 are both hydrogen.~~

~~15. The method of claim 11 or 14, wherein R^4 is -OH.~~

~~16~~8. The method of any one of claims 1, 2, and ~~11~~6, wherein the solution comprises at least one compound selected from the group consisting of:



, and any combination(s) thereof;

optionally wherein the host cell further comprises

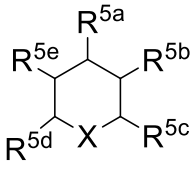
i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats;

ii) AAV rep and cap genes;

iii) helper genes; or

iv) a combination thereof.

~~17~~9. The method of claim 1 or 2, wherein the solution comprises at least one compound of formula (I-B) or a salt thereof:



(I-B), wherein:

X is CR^cR^d; and

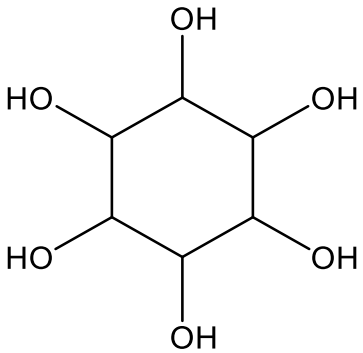
R^{5a}, R^{5b}, R^{5c}, R^{5d}, and R^{5e} are independently, for each occurrence, -OH, -O-C_{1-6}alkyl, C_{1-6}alkyl, C_{1-6}alkyl-OH, and C_{1-6}alkyl-O-C_{1-6}alkyl,

wherein R^c and R^d are as defined in claim 1.

~~18~~10. The method of claim ~~17~~9, wherein:

i) R^c is hydrogen and R^d is -OH;

ii) R^{5a}, R^{5b}, R^{5c}, R^{5d}, and R^{5e} are -OH; or



iii) the solution comprises the compound ;

optionally wherein the host cell further comprises

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats;

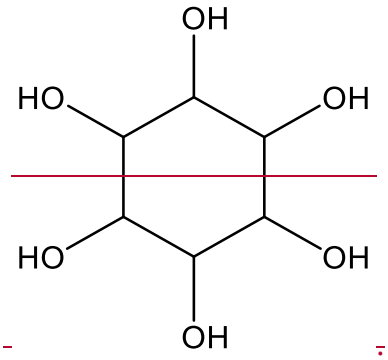
ii) AAV rep and cap genes;

iii) helper genes; or

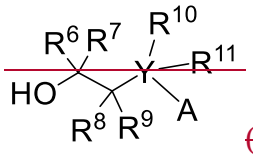
iv) a combination thereof.

~~19. The method of claim 17 or 18, wherein R^{5a}, R^{5b}, R^{5c}, R^{5d}, and R^{5e} are -OH.~~

~~20. The method of any one of claims 17-19, wherein the solution comprises the compound~~



~~21. A method of producing rAAV, the method comprising contacting a host cell with a solution comprising at least one compound of formula (II) or a salt thereof:~~



~~(II), wherein:~~

~~R^6 and R^7 are independently, for each occurrence, selected from the group consisting of hydrogen, -OH, -CN, halogen, and C_{1-6}alkyl;~~

~~R^8 and R^9 are independently, for each occurrence, selected from the group consisting of hydrogen, -OH, -CN, halogen, and C_{1-6}alkyl;~~

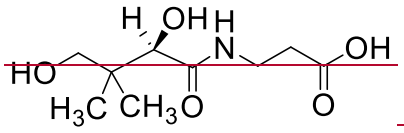
~~Y is C or N;~~

~~R^{10} and R^{11} are independently, for each occurrence, selected from the group consisting of hydrogen, -OH, and C_{1-6}alkyl;~~

~~A is selected from the group consisting of hydrogen, C_{1-6}alkyl, and $-C(O)N(R^f)_2$;~~ ~~and~~

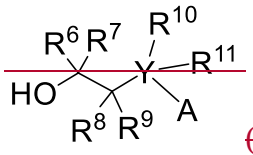
R^f is independently, for each occurrence, selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{1-6}alkyl-C(O)OH, and C_{1-6}alkyl-OH;

wherein the at least one compound is not:



or a salt thereof.

22. A method of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising at least one compound of formula (II) or a salt thereof:



(II), wherein:

R^6 and R^7 are independently, for each occurrence, selected from the group consisting of hydrogen, -OH, -CN, halogen, and C_{1-6}alkyl;

R^8 and R^9 are independently, for each occurrence, selected from the group consisting of hydrogen, -OH, -CN, halogen, and C_{1-6}alkyl;

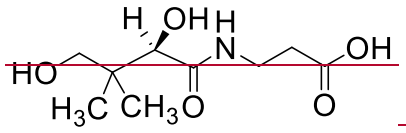
Y is C or N;

R^{10} and R^{11} are independently, for each occurrence, selected from the group consisting of hydrogen, -OH, and C_{1-6}alkyl;

A is selected from the group consisting of hydrogen, C_{1-6}alkyl, and $-C(O)N(R^f)_2$; and

R^f is independently, for each occurrence, selected from the group consisting of hydrogen, C_{1-6}alkyl, C_{1-6}alkyl-C(O)OH, and C_{1-6}alkyl-OH;

wherein the at least one compound is not:



or a salt thereof.

23. The method of claim 21 or 22, wherein R^6 and R^7 are hydrogen.

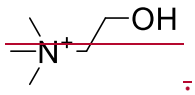
24. The method of any one of claims 21-23, wherein R^8 and R^9 are hydrogen.

~~25. The method of any one of claims 21-24, wherein Y is N.~~

~~26. The method of any one of claims 21-25, wherein R^{10} and R^{11} are CH_3.~~

~~27. The method of any one of claims 21-26, wherein A is CH_3.~~

~~28. The method of any one of claims 21-27, wherein the solution comprises the compound~~



~~.~~

~~29. A method of producing rAAV or increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising at least one B vitamin selected from the group consisting of vitamin B_2, vitamin B_7, vitamin B_9, vitamin B_{12}, and any combination(s) thereof.~~

~~30~~11. The method of any one of claims 1-~~29~~10, wherein the final concentration of the at least one compound ~~or B vitamin~~ in the solution is:

i) greater than or equal to 0.5 mM; or

ii) between 0.5 mM and 10 mM.

~~31. The method of any one of claims 1-29, wherein the final concentration of the at least one compound or B vitamin in the solution is greater than or equal to 0.5 mM.~~

~~32. The method of any one of claims 1-29, wherein the final concentration of the at least one compound or B vitamin in the solution is between 0.5 mM and 10 mM.~~

~~33. The method of any one of claims 1-29, wherein the final concentration of the at least one compound or B vitamin in the solution is between 1 mM and 10 mM.~~

~~34~~12. The method of any one of claims 1-~~33~~11, wherein the final concentration of the at least one compound ~~or B vitamin~~ in the solution is sufficient to produce at least 1.2-fold greater quantities of secreted rAAV or total rAAV, compared to that produced by a host cell not contacted with a solution comprising the at least one compound ~~or B vitamin~~.

~~35. The method of any one of claims 1-33, wherein the final concentration of the at least one compound in the solution is sufficient to produce at least 1.2-fold greater quantities of total~~

~~rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound or B vitamin.~~

~~36. The method of any one of claims 1-33, wherein the final concentration of the at least one compound in the solution is sufficient to produce 1.2 to 2.5-fold greater quantities of secreted rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound or B vitamin.~~

~~37. The method of any one of claims 1-33, wherein the final concentration of the at least one compound in the solution is sufficient to produce 1.2 to 2.5-fold greater quantities of total rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound or B vitamin.~~

~~38~~13. The method of any one of claims 1-~~37~~12, wherein the host cell is contacted with the solution comprising the at least one compound ~~or B vitamin~~ for at least 2 days.

~~39~~14. The method of any one of claims 1-~~38~~13, further comprising the steps of harvesting and purifying the rAAV.

~~40~~15. The method of any one of claims 1-~~39~~14, wherein the host cell is a mammalian cell or an insect cell.

~~41~~16. The method of claim ~~40~~15, wherein:

i) the host cell is a mammalian cell selected from the group consisting of HeLa, HEK293, COS, A549, BHK, and Vero cells; or

ii) the host cell is an insect cell selected from the group consisting of Sf9, Sf-21, Tn-368, and BTI-Tn-5B1-4 (High-Five) cells.

~~42. The method of claim 41, wherein the host cell is a HeLa cell.~~

~~43. The method of claim 41, wherein the host cell is a HEK293 cell.~~

~~44. The method of any one of claims 1-39, wherein the host cell is an insect cell.~~

~~45. The method of claim 44, wherein the host cell is selected from the group consisting of Sf9, Sf-21, Tn-368, and BTI-Tn-5B1-4 (High-Five) cells.~~

~~46. The method of any one of claims 1-45, wherein the host cell comprises a heterologous nucleotide sequence flanked by AAV inverted terminal repeats.~~

~~47. The method of any one of claims 1-46, wherein the host cell comprises rep and cap genes.~~

~~48. The method of any one of claims 1-47, wherein the host cell comprises helper virus genes.~~

~~49~~17. The method of any one of claims 1-~~48~~7, 9, and 11-16, wherein the host cell comprises i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, ii) AAV rep and cap genes, ~~and~~ iii) helper virus genes, or iv) a combination thereof.

~~50~~18. The method of any one of claims 1-~~49~~17, wherein the host cell produces at least 1.2-fold greater quantities of secreted rAAV or total rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound.

~~51. The method of any one of claims 1-49, wherein the host cell produces at least 1.2-fold greater quantities of total rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound or B vitamin.~~

~~52. The method of any one of claims 1-49, wherein the host cell produces 1.2 to 2.5-fold greater quantities of secreted rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound or B vitamin.~~

~~53. The method of any one of claims 1-49, wherein the host cell produces 1.2 to 2.5-fold greater quantities of total rAAV compared to that produced by a host cell not contacted with a solution comprising the at least one compound or B vitamin.~~

~~54~~19. The method of any one of claims 1-~~53~~18, wherein:

i) the solution comprises cell culture medium or production medium;

ii) the method comprises culturing of the host cell in a suspension culture or adherent culture; or

iii) the method comprises culturing of the host cell in a 1L to 2000L bioreactor.

~~55. The method of any one of claims 1-53, wherein the solution comprises production medium.~~

~~56. The method of any one of claims 1-55 comprising culturing the host cell in a suspension culture.~~

~~57. The method of any one of claims 1-55 comprising culturing the host cell in an adherent culture.~~

~~58. The method of any one of claims 1-56 comprising culturing the host cell in a 1L bioreactor.~~

~~59. The method of any one of claims 1-56 comprising culturing the host cell in a 2L bioreactor.~~

~~60. The method of any one of claims 1-56 comprising culturing the host cell in a 3L bioreactor.~~

~~61. The method of any one of claims 1-56 comprising culturing the host cell in a 250L bioreactor.~~

~~62. The method of any one of claims 1-56 comprising culturing the host cell in a 500L bioreactor.~~

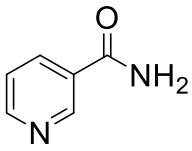
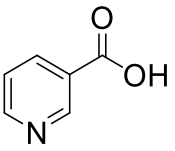
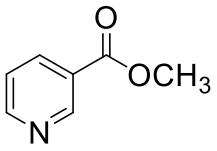
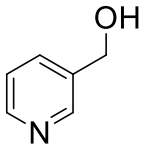
~~63. The method of any one of claims 1-56 comprising culturing the host cell in a 2,000L bioreactor.~~

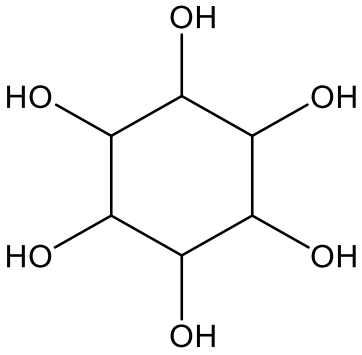
~~64~~20. A composition comprising a host cell and

i) a compound of formula (I) or a salt thereof;

ii) a compound of formula (I-A) or a salt thereof;

iii) a compound of formula (I-B) or a salt thereof;

iv) at least one compound selected from the group consisting of: , , , , and any combination(s) thereof; or



v) a compound of formula ,

optionally wherein the host cell further comprises

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats;

ii) AAV rep and cap genes;

iii) helper genes; or

iv) a combination thereof.

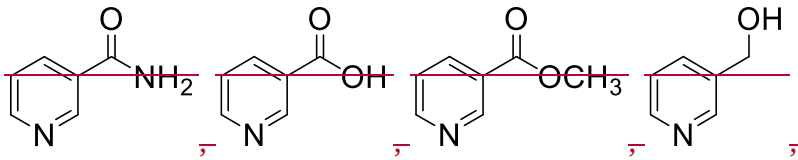
~~65. A composition comprising a host cell and a compound of formula (I-A) or a salt thereof.~~

~~66. A composition comprising a host cell and a compound of formula (I-B) or a salt thereof.~~

~~67. A composition comprising a host cell and a compound of formula (II) or a salt thereof.~~

~~68. A composition comprising a host cell and at least one B vitamin selected from the group consisting of vitamin B_2, vitamin B_7, vitamin B_9, and vitamin B_{12}, and any combination(s) thereof.~~

69. A method of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising at least one compound selected from the group consisting of:



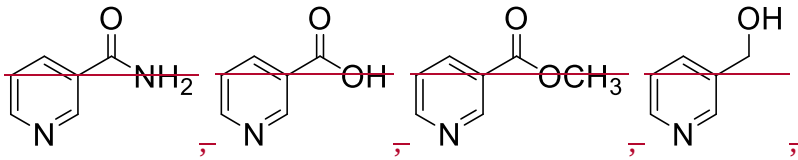
, and any combination(s) thereof

wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

70. A method of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising at least one compound selected from the group consisting of:



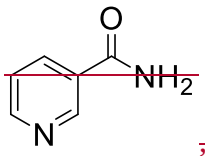
, and any combination(s) thereof

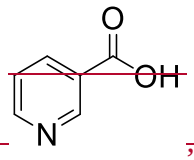
wherein the host cell comprises:

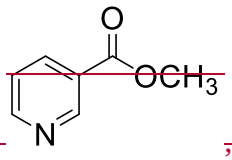
i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

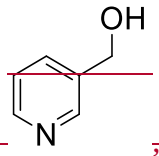
ii) AAV rep and cap genes.

71. A composition comprising a host cell and at least one compound selected from the group consisting of:



, 

, 

, 

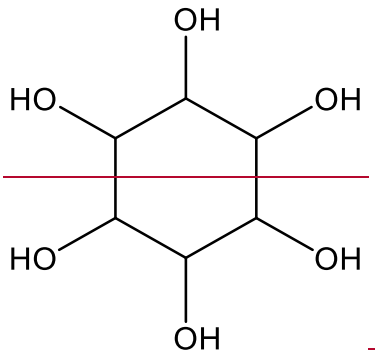
, and any combination(s) thereof

wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

72. A method of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising



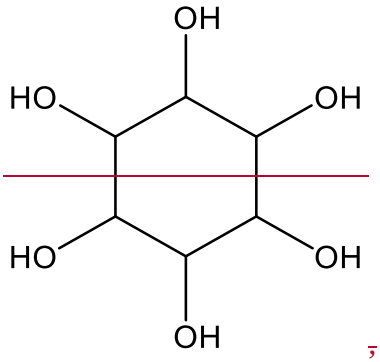
,

wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

73. A method of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising



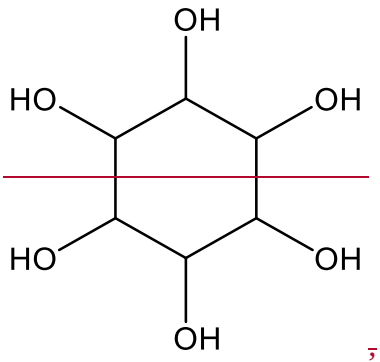
,

wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

74. A composition comprising a host cell and



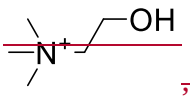
,

wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

75. A method of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising:



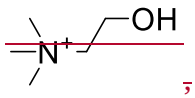
,

wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

76. A method of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising:



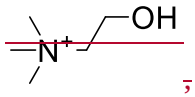
,

wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

77. A composition comprising a host cell and



,

wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

78. A method of producing recombinant adeno-associated virus (rAAV), the method comprising contacting a host cell with a solution comprising at least one B vitamin selected from the group consisting of vitamin B_2, vitamin B_7, vitamin B_9, and vitamin B_{12}, and any combination(s) thereof, wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

79. A method of increasing rAAV titer yield, the method comprising contacting a host cell with a solution comprising at least one B vitamin selected from the group consisting of vitamin B_2, vitamin B_7, vitamin B_9, and vitamin B_{12}, and any combination(s) thereof, wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

80. The method of any one of claims 69, 70, 72, 73, 75, 76, 78, or 79, wherein the host cell further comprises helper genes.

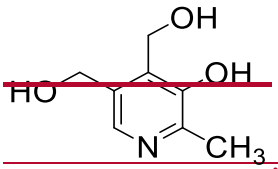
81. A composition comprising a host cell and at least one B vitamin selected from the group consisting of vitamin B_2, vitamin B_7, vitamin B_9, vitamin B_{12}, and any combination(s) thereof, wherein the host cell comprises:

i) a heterologous nucleotide sequence flanked by AAV inverted terminal repeats, and

ii) AAV rep and cap genes.

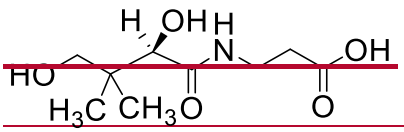
82. The composition of any one of claims 71, 74, 77, or 81, wherein the host cell further comprises helper genes.

83. The method of any one of claims 1-20, wherein the solution does not comprise

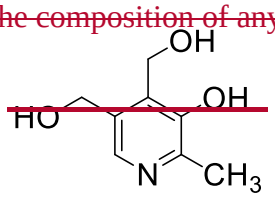


, or a salt thereof.

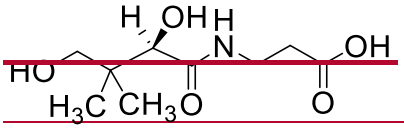
84. The method of any one of claims 21-28, wherein the solution does not comprise



or a salt thereof.

85. The composition of any one of claims 64-66, 71, or 74, wherein the composition does not comprise 

, or a salt thereof.

86. The composition of claim 67 or 77, wherein the composition does not comprise 

or a salt thereof.