

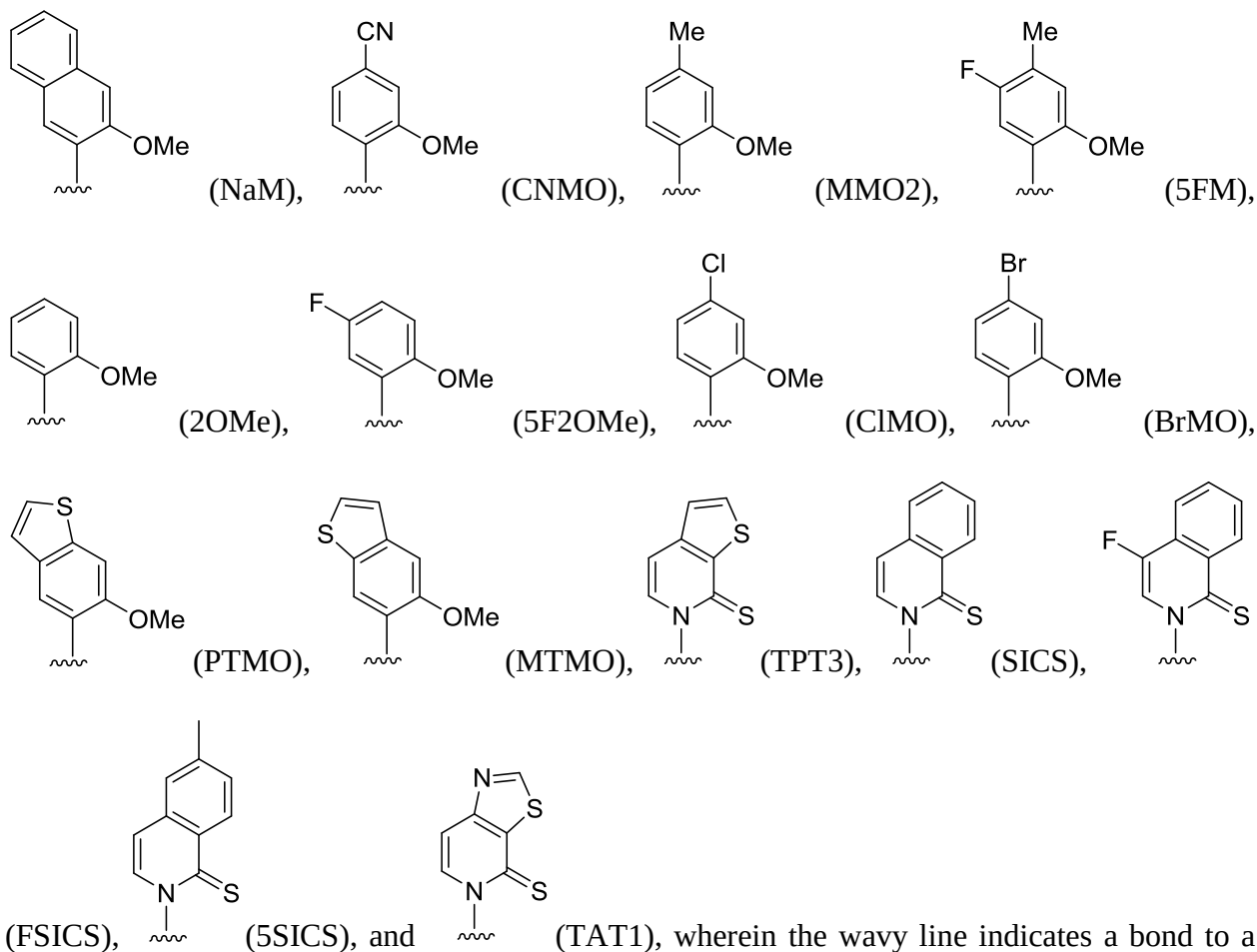
CLAIMS

WHAT IS CLAIMED IS:

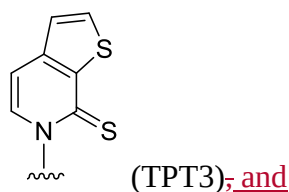
1. A eukaryotic cell comprising:
 - (a) a messenger RNA (mRNA) with a codon comprising a first unnatural base; and
 - (b) a transfer RNA (tRNA) with an anticodon comprising a second unnatural base,wherein the first and second unnatural bases are capable of forming an unnatural base pair (UBP) in the eukaryotic cell, and wherein the mRNA is capable of being translated in the cell to produce a polypeptide comprising at least one unnatural amino acid.
2. The eukaryotic cell of claim 1, wherein the tRNA is charged with an unnatural amino acid.
3. The eukaryotic cell of any one of the preceding claims, further comprising a polypeptide translated from the mRNA, wherein the polypeptide comprises the unnatural amino acid, optionally wherein the polypeptide comprises a eukaryotic glycosylation pattern;and/or
- ~~4. The eukaryotic cell of any one of the preceding claims, further comprising a tRNA synthetase, wherein the tRNA synthetase preferentially aminoacylates the tRNA with the unnatural amino acid;or~~
- ~~54.~~ The eukaryotic cell of any one of the preceding claims, wherein the codon of the mRNA comprises three contiguous nucleobases (N-N-N); and wherein the first unnatural base (X) is located at the first position (X-N-N) in the codon of the mRNA;or
- ~~6. The eukaryotic cell of any one of the preceding claims, wherein the codon of the mRNA comprises three contiguous nucleobases (N-N-N); and~~ wherein the first unnatural base (X) is located at the middle position (N-X-N) in the codon of the mRNA;or

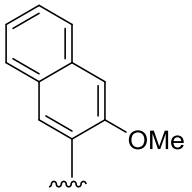
~~7. The eukaryotic cell of any one of the preceding claims, wherein the codon of the mRNA comprises three contiguous nucleobases (N-N-N); and~~ wherein the first unnatural base (X) is located at the last position (N-N-X) in the codon of the mRNA.

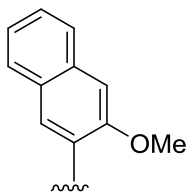
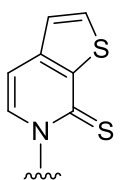
85. The eukaryotic cell of any one of the preceding claims, wherein the first unnatural base and the second unnatural base are each, independently, selected from the group consisting of



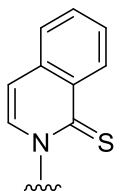
96. The eukaryotic cell of any one of the preceding claims, when the first unnatural base is

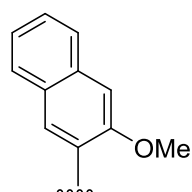


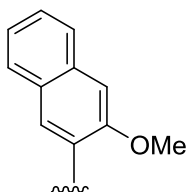
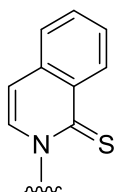
the second unnatural base is  (NaM), ~~and when~~ or the first unnatural base is

 (NaM), ~~and~~ the second unnatural base is  (TPT3), wherein the wavy line indicates a bond to a ribosyl moiety; ~~or wherein~~

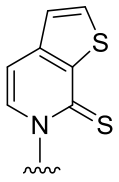
~~10. The eukaryotic cell of any one of the preceding claims, when~~ the first unnatural base is

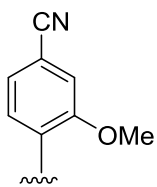
 (5SICS), ~~and~~

the second unnatural base is  (NaM), ~~and when~~ or the first unnatural base is

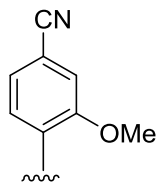
 (NaM), ~~and~~ the second unnatural base is  (5SICS), wherein the wavy line indicates a bond to a ribosyl moiety; ~~or wherein~~

~~11. The eukaryotic cell of any one of the preceding claims, when~~ or wherein the first unnatural

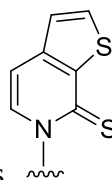
base is  (TPT3), ~~and~~



the second unnatural base is (CNMO), ~~and when or~~ the first unnatural base is



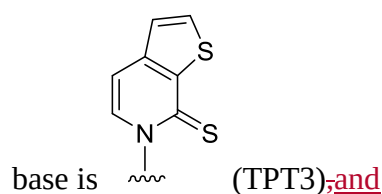
(CNMO), ~~and~~ the second unnatural base is



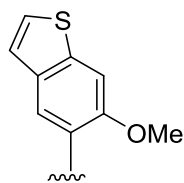
(TPT3), wherein the wavy line

indicates a bond to a ribosyl moiety.:

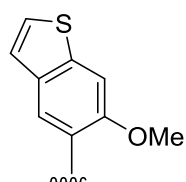
~~12. The eukaryotic cell of any one of the preceding claims, when or wherein~~ the first unnatural



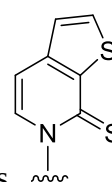
the second unnatural base is



(PTMO), ~~and when or~~ the first unnatural base is



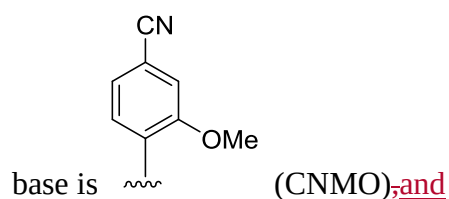
(PTMO), ~~and~~ the second unnatural base is

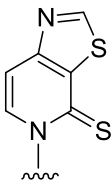
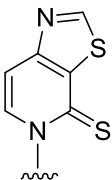


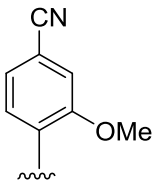
(TPT3), wherein the wavy line

indicates a bond to a ribosyl moiety.:

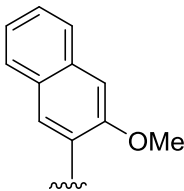
~~13. The eukaryotic cell of any one of the preceding claims, when or wherein~~ the first unnatural

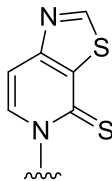
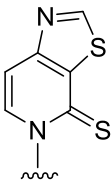


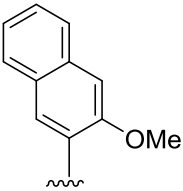
the second unnatural base is  (TAT1), ~~and when or~~ the first unnatural base is 

(TAT1), ~~and~~ the second unnatural base is  (CNMO), wherein the wavy line indicates a bond to a ribosyl moiety.

~~14. The eukaryotic cell of any one of the preceding claims, when or wherein~~ the first unnatural

base is  (NaM), ~~and~~

the second unnatural base is  (TAT1), ~~and when or~~ the first unnatural base is 

(TAT1), the second unnatural base is  (NaM), wherein the wavy line indicates a bond to a ribosyl moiety.

~~157.~~ The eukaryotic cell of any one of claims 3 to ~~146~~, wherein the at least one unnatural amino acid:

- is a lysine analogue;
- comprises an aromatic side chain;
- comprises an azido group;
- comprises an alkyne group; or

comprises an aldehyde or ketone group, or optionally wherein

~~16. The eukaryotic cell of claim 15, wherein~~ the at least one unnatural amino acid is selected from the group consisting of N6-((azidoethoxy)-carbonyl)-L-lysine (AzK), N6-((propargylethoxy)-carbonyl)-L-lysine (PraK), BCN-L-lysine, norbornene lysine, TCO-lysine, methyltetrazine lysine, allyloxycarbonyllysine, 2-amino-8-oxononanoic acid, 2-amino-8-oxooctanoic acid, p-acetyl-L-phenylalanine, p-azidomethyl-L-phenylalanine (pAMF), p-iodo-L-phenylalanine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, p-propargyloxyphenylalanine, p-propargyl-phenylalanine, 3-methyl-phenylalanine, L-Dopa, fluorinated phenylalanine, isopropyl-L-phenylalanine, p-azido-L-phenylalanine, p-acyl-L-phenylalanine, p-benzoyl-L-phenylalanine, p-bromophenylalanine, p-amino-L-phenylalanine, isopropyl-L-phenylalanine, O-allyltyrosine, O-methyl-L-tyrosine, O-4-allyl-L-tyrosine, 4-propyl-L-tyrosine, phosphotyrosine, tri-O-acetyl-GlcNAc-serine, L-phosphoserine, phosphoserine, L-3-(2-naphthyl)alanine, 2-amino-3-((2-((3-(benzyloxy)-3-oxopropyl)amino)ethyl)sulfonyl)propanoic acid, 2-amino-3-(phenylsulfonyl)propanoic, selenocysteine, N6-(((2-azidobenzyl)oxy)carbonyl)-L-lysine, N6-(((3-azidobenzyl)oxy)carbonyl)-L-lysine, or N6-(((4-azidobenzyl)oxy)carbonyl)-L-lysine.

~~178.~~ The eukaryotic cell of claim ~~167~~, wherein the at least one unnatural amino acid is N6-((azidoethoxy)-carbonyl)-L-lysine (AzK).

~~189.~~ The eukaryotic cell of any one of the preceding claims, wherein the eukaryotic cell is a human cell, optionally

~~19. The eukaryotic cell of the immediately preceding claim,~~ wherein the human cell is a HEK293T cell.

~~20~~10. The eukaryotic cell of any one of claims 1 to ~~189~~, wherein the cell is a mammalian cell, optionally wherein the cell is a hamster cell, further optionally.

~~21. The eukaryotic cell of the immediately preceding claim, wherein~~ the mammalian cell is a Chinese hamster ovary (CHO) cell.

~~22~~11. The eukaryotic cell of any one of claims ~~18-219-10~~, further comprising a polypeptide translated from the mRNA, wherein the polypeptide comprises the unnatural amino acid and a mammalian glycosylation pattern.

~~23~~12. The eukaryotic cell of any one of the preceding claims, wherein the cell is isolated.

~~24~~13. A semi-synthetic organism comprising the eukaryotic cell of any one of the preceding claims.

~~25~~14. A eukaryotic cell culture comprising a plurality of eukaryotic cells of any one of claims 1-
~~24~~13.

~~26~~15. A method of delivering a cell to an organism, comprising contacting the organism with the cell of any one of claims 1-~~23~~12.

~~27~~16. The method of claim ~~26~~15, wherein the organism is a mammal, optionally wherein the mammal is a human.

~~28~~17. A method of producing a polypeptide comprising at least one unnatural amino acid in a eukaryotic cell, comprising:

(a) introducing into the cell:

(i) a messenger RNA (mRNA) with a codon comprising a first unnatural base; and
(ii) a transfer RNA (tRNA) with an anticodon comprising a second unnatural base
in the eukaryotic cell, wherein the first and second unnatural bases are capable of
forming an unnatural base pair (UBP) in the eukaryotic cell; and
(b) translating the polypeptide comprising the at least one unnatural amino acid from
the mRNA using the tRNA.

2918. The method of the preceding claim, wherein the tRNA is charged with an unnatural amino acid.

3019. A method of producing a polypeptide comprising at least one unnatural amino acid in a eukaryotic cell, comprising:

(a) providing a eukaryotic cell comprising:
(i) a messenger RNA (mRNA) with a codon comprising a first unnatural base;
(ii) a transfer RNA (tRNA) with an anticodon comprising a second unnatural base, wherein the first and second unnatural bases are capable of forming an unnatural base pair (UBP) in the eukaryotic cell; and
(b) translating the polypeptide comprising the at least one unnatural amino acid from the mRNA using the tRNA by a ribosome that is endogenous to the eukaryotic cell.

3120. A method of producing a polypeptide in a eukaryotic cell, wherein the polypeptide comprises at least one unnatural amino acid, the method comprising:

(a) providing a eukaryotic cell, the eukaryotic cell comprising:
(i) an mRNA comprising a codon, wherein the codon comprises a first unnatural base;

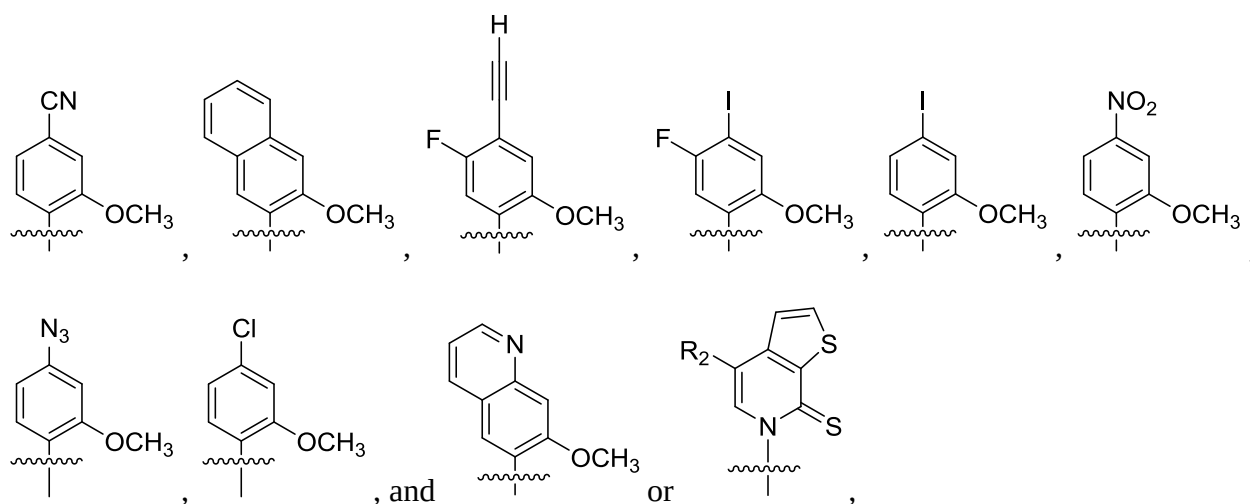
- (ii) a tRNA comprising an anti-codon, wherein the anti-codon comprises a second unnatural base, and wherein the first and second unnatural bases are capable of forming a complimentary base pair; and
 - (iii) a tRNA synthetase, wherein the tRNA synthetase preferentially aminoacylates the tRNA with the at least one unnatural amino acid compared to a natural amino acid; and
- (b) providing the one more unnatural amino acids to the eukaryotic cell, wherein the eukaryotic cell produces the polypeptide comprising the at least one unnatural amino acid.

~~32.21.~~ The method of any one of claims ~~26 to 31~~17-20, wherein the codon of the mRNA comprises three contiguous nucleobases (N-N-N); and wherein the first unnatural base (X) is located at the first position (X-N-N) in the codon of the mRNA., or

~~33.—The method of any one of claims 26 to 31, wherein the codon of the mRNA comprises three contiguous nucleobases (N-N-N); and wherein~~ the first unnatural base (X) is located at the middle position (N-X-N) in the codon of the mRNA., or

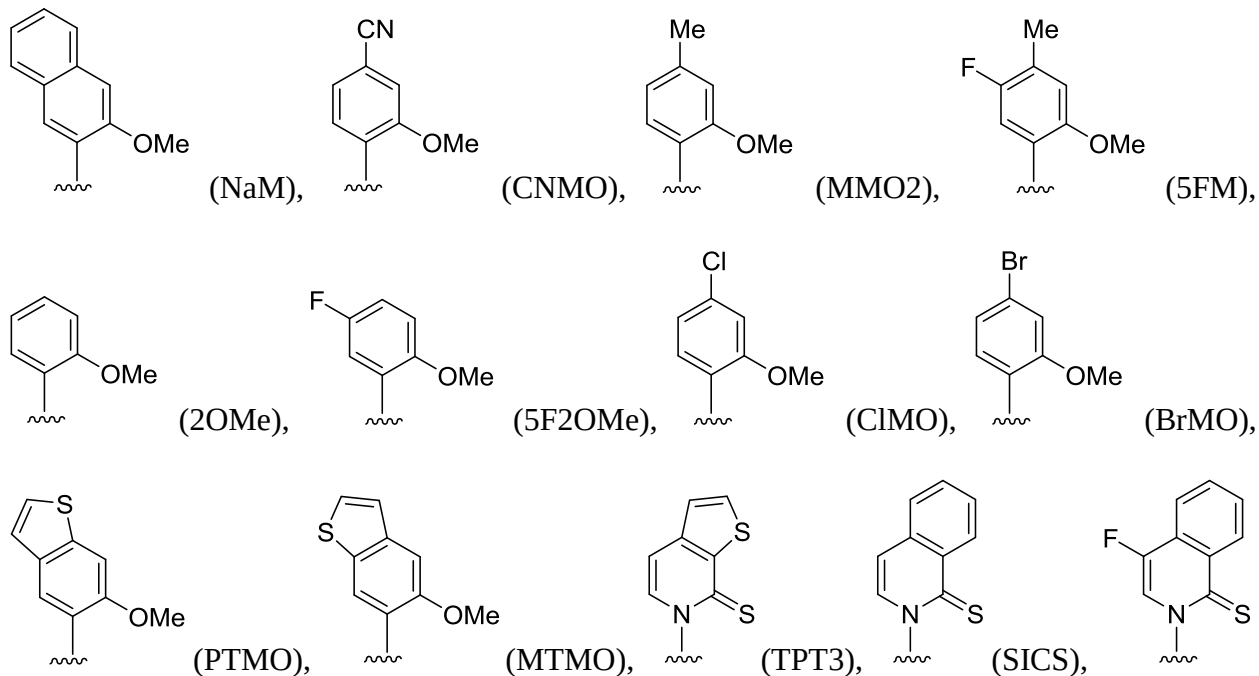
~~34.—The method of any one of claims 26 to 31, wherein the codon of the mRNA comprises three contiguous nucleobases (N-N-N); and wherein~~ the first unnatural base (X) is located at the last position (N-N-X) in the codon of the mRNA.

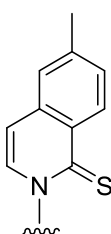
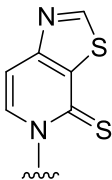
~~35.22.~~ The method of any one of claims ~~26 to 34~~17-21, wherein the one or more unnatural bases ~~comprising of~~ the codon of the mRNA is of the formula

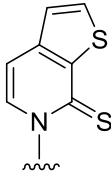


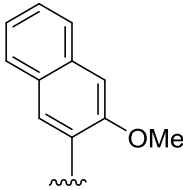
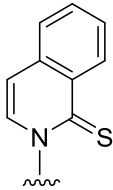
wherein R₂ is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, methoxy, methanethiol, methaneseleno, halogen, cyano, and azido, and the wavy line indicates a bond to a ribosyl moiety.

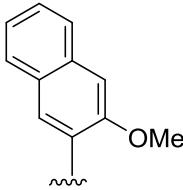
3623. The method of any one of claims ~~26 to 35~~ 17-22, wherein the first unnatural base or the second unnatural base is selected from the group consisting of

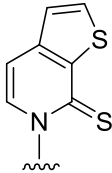


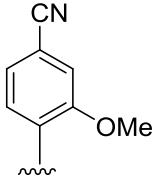
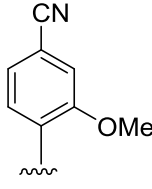
 (FSICS),  (5SICS), and  (TAT1), wherein the wavy line indicates a bond to a ribosyl moiety.

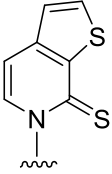
~~3724.~~ The method of claim ~~3623~~, wherein the first unnatural base is  (TPT3) and

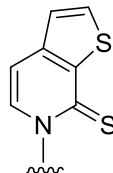
the second unnatural base is  (NaM), or the first unnatural base is  (5SICS)

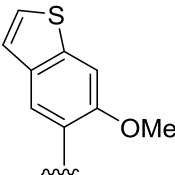
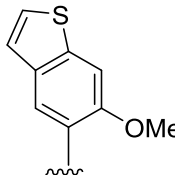
and the second unnatural base is  (NaM), wherein the wavy line indicates a bond to a ribosyl moiety. ~~or~~

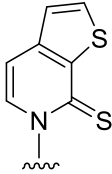
~~38.~~ ~~The method of claim 36, wherein~~ the first unnatural base is  (TPT3)

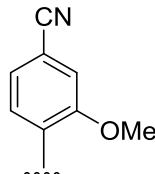
and the second unnatural base is  (CNMO), or the first unnatural base is 

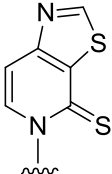
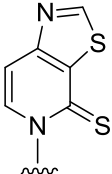
(CNMO) and the second unnatural base is  (TPT3), wherein the wavy line indicates a bond to a ribosyl moiety. ~~or~~

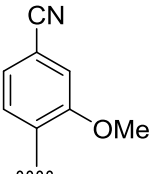
39. ~~The method of claim 36, wherein~~ the first unnatural base is  (TPT3) and

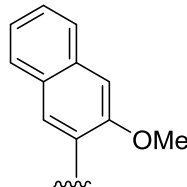
the second unnatural base is  (PTMO), or the first unnatural base is 

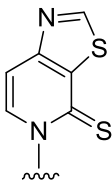
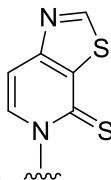
(PTMO) and the second unnatural base is  (TPT3), wherein the wavy line indicates a bond to a ribosyl moiety; or

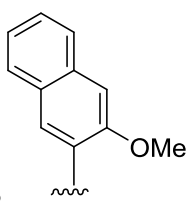
40. ~~The method of claim 36, wherein~~ the first unnatural base is  (CNMO) and

the second unnatural base is  (TAT1), or the first unnatural base is  (TAT1) and

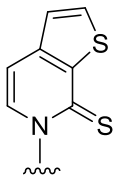
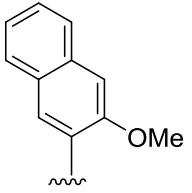
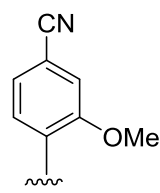
the second unnatural base is  (CNMO), wherein the wavy line indicates a bond to a ribosyl moiety; or

41. ~~The method of claim 36, wherein~~ the first unnatural base is  (NaM) and

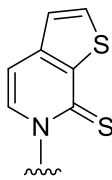
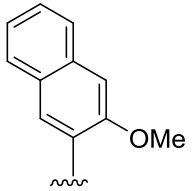
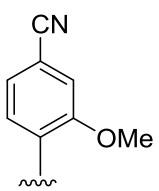
the second unnatural base is  (TAT1), or the first unnatural base is  (TAT1) and

the second unnatural base is  (NaM), wherein the wavy line indicates a bond to a ribosyl moiety.

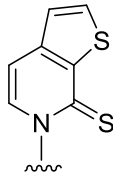
~~4225.~~ The method of any one of claims ~~26 to 36~~ 17-24, wherein the codon of the mRNA comprises three contiguous nucleobases (N-N-N), wherein the first unnatural base (X) is located at the first position (X-N-N) in the codon of the mRNA, wherein the first unnatural base is selected

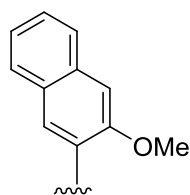
from  (TPT3),  (NaM), and  (CNMO), and wherein the wavy line indicates a bond to a ribosyl moiety ~~;~~ or

~~43. — The method of any one of claims 26 to 36, wherein~~ the codon of the mRNA comprises three contiguous nucleobases (N-N-N), wherein the first unnatural base (X) is located at the middle position (N-X-N) in the codon of the mRNA, wherein the first unnatural base is selected from

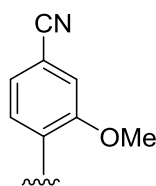
 (TPT3),  (NaM), and  (CNMO), and wherein the wavy line indicates a bond to a ribosyl moiety ~~;~~ or

~~44. — The method of any one of claims 26 to 36,~~ wherein the codon of the mRNA comprises three contiguous nucleobases (N-N-N), wherein the first unnatural base (X) is located at the last position

(N-N-X) in the codon of the mRNA, wherein the unnatural base is selected from  (TPT3),



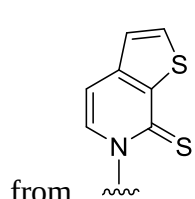
(NaM), and



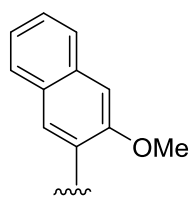
(CNMO), and wherein the wavy line indicates a bond to

a ribosyl moiety; or

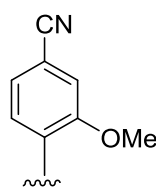
~~45. The method of any one of claims 26 to 36,~~ wherein the anticodon of the tRNA comprises three contiguous nucleobases (N-N-N); and wherein the second unnatural base (X) is located at the first position (X-N-N) in the anticodon of the tRNA, wherein the second unnatural base is selected



(TPT3),



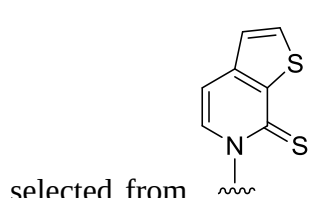
(NaM), and



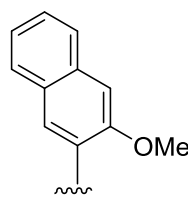
(CNMO), and wherein the wavy

line indicates a bond to a ribosyl moiety; or

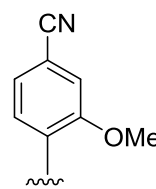
~~46. The method of any one of claims 26 to 36,~~ wherein the anticodon of the tRNA comprises three contiguous nucleobases (N-N-N); and wherein the second unnatural base (X) is located at the middle position (N-X-N) in the anticodon of the tRNA, wherein the second unnatural base is



(TPT3),



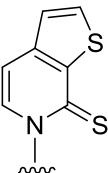
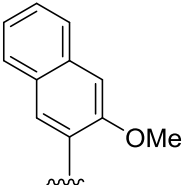
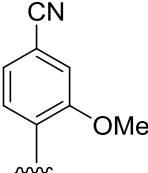
(NaM), and



(CNMO), and wherein

the wavy line indicates a bond to a ribosyl moiety; or

~~47. The method of any one of claims 26 to 36,~~ wherein the anticodon of the tRNA comprises three contiguous nucleobases (N-N-N); and wherein the second unnatural base (X) is located at the last position (N-N-X) in the anticodon of the tRNA, wherein the second unnatural base is selected

from  (TPT3),  (NaM), and  (CNMO), and wherein the wavy line indicates a bond to a ribosyl moiety.

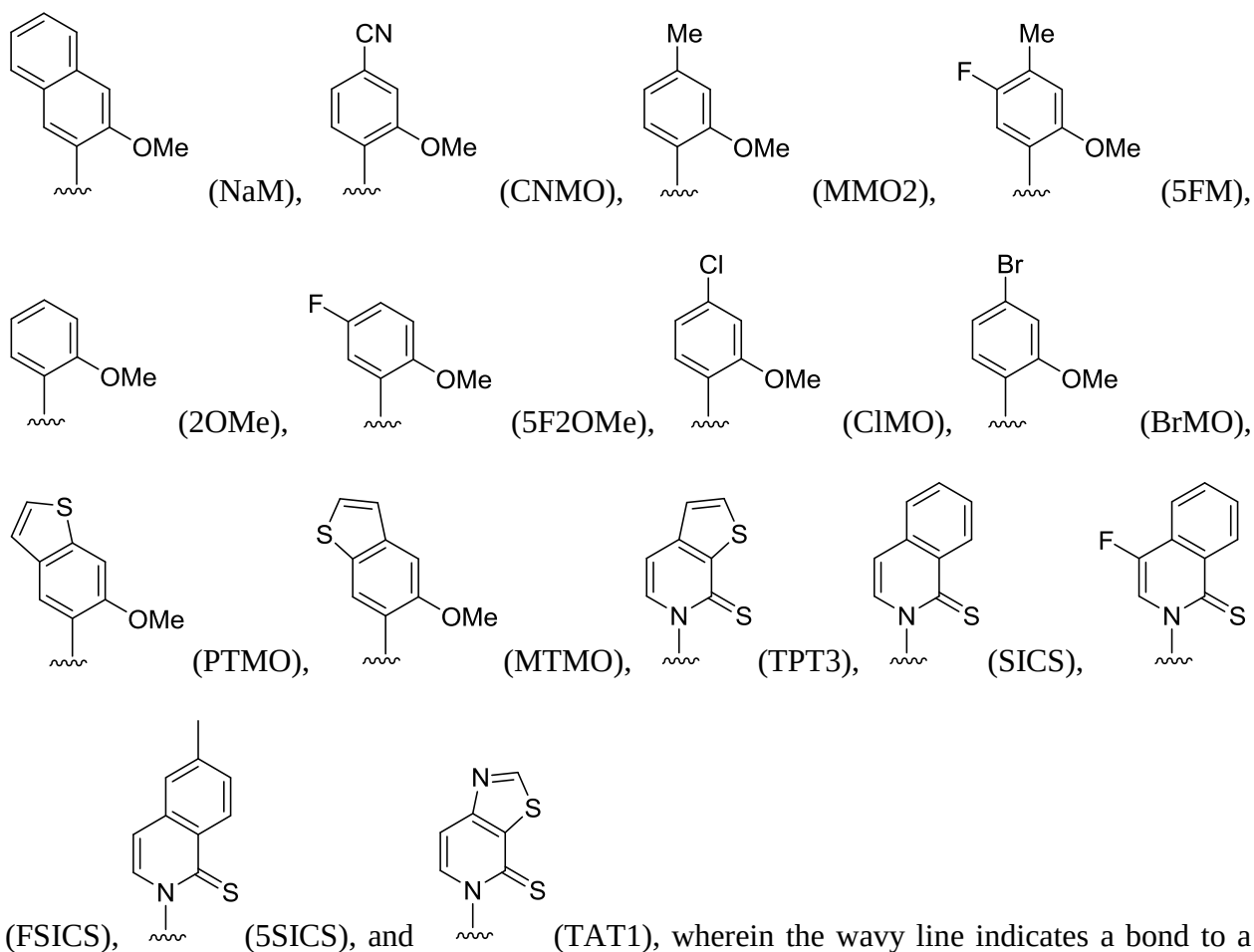
~~48~~26. The method of any one of claims ~~26 to 36~~ 17-25, wherein the codon and the anticodon each comprise three contiguous nucleobases (N-N-N), wherein the first unnatural base (X) of the codon in the mRNA is located at a first position (X-N-N) of the codon, and the second unnatural base (Y) of the anticodon of the tRNA is located at the last position (N-N-Y) of the anticodon-; or

~~49.~~ ~~The method of any one of claims 26 to 36,~~ wherein the codon and the anticodon each comprise three contiguous nucleobases (N-N-N), wherein the codon in the mRNA comprises a first unnatural base (X) located at the middle position (N-X-N) of the codon, and the anticodon in the tRNA comprises a second unnatural base (Y) located at the middle position (N-Y-N) of the anticodon-; or

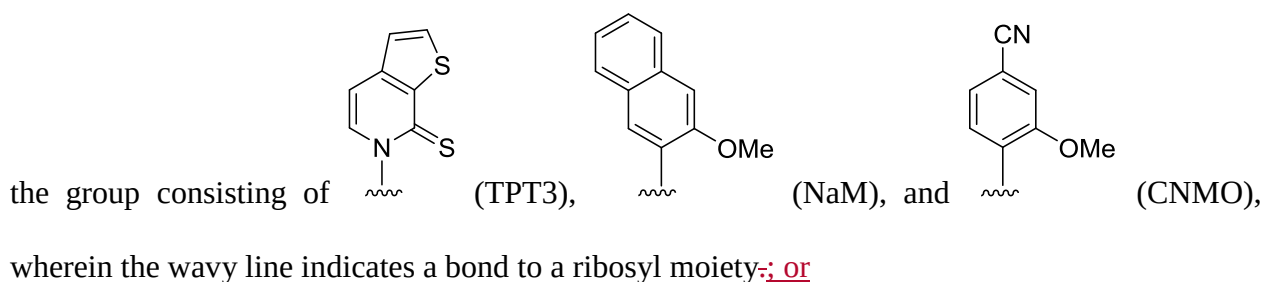
~~50.~~ ~~The method of any one of claims 26 to 36,~~ wherein the codon and the anticodon each comprise three contiguous nucleobases (N-N-N), wherein the codon in the mRNA comprises a first unnatural base (X) located at the last position (N-N-X) of the codon, and the anticodon in the tRNA comprises a second unnatural base (Y) located at the first position (Y-N-N) of the anticodon.

~~51~~27. The method of ~~any one of claims 48 to 50~~ claim 26, wherein the first unnatural base (X) located in the codon of the mRNA and the second unnatural base (Y) located in the anticodon of the tRNA are the same or are different.

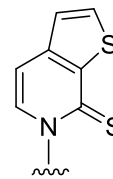
5228. The method of ~~any one of claims 48 to 51~~ claim 26 or 27, wherein the first unnatural base (X) located in the codon of the mRNA and the second unnatural base (Y) located in the anticodon of the tRNA are selected from the group consisting of



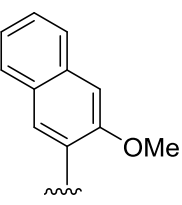
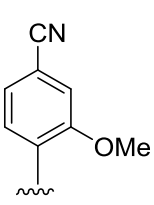
5329. The method of claim **5228**, wherein the first unnatural base (X) located in the codon of the mRNA and the second unnatural base (Y) located in the anticodon of the tRNA are selected from



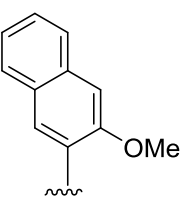
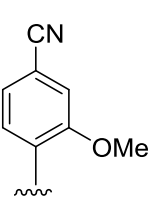
~~54. The method of claim 53,~~ wherein the first unnatural base (X) located in the codon of the

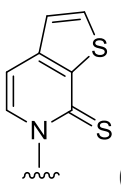


mRNA and the second unnatural base (Y) located in the anticodon of the tRNA are both

(TPT3),  (NaM), or  (CNMO), wherein the wavy line indicates a bond to a ribosyl moiety. or

~~55. The method of claim 53,~~ wherein the first unnatural base (X) located in the codon of the

mRNA is selected from  (NaM) and  (CNMO), and the second

unnatural base (Y) located in the anticodon of the tRNA is  (TPT3), wherein in each case the wavy line indicates a bond to a ribosyl moiety.

5630. The method of any one of claims ~~26-29, 31, 33, 35 to 41, 43, 46, and 49~~ 15-29, wherein the codon in the mRNA is selected from AXC, GXC or GXU, wherein X is the first unnatural base. optionally

~~57. The method of the immediately preceding claim,~~ wherein the anticodon in the tRNA is selected from GYU, GYC, and AYC, and Y is a second unnatural base.

~~58~~31. The method of claim ~~57~~30, wherein the codon in the mRNA is AXC and the anticodon in the tRNA is GYU;or

~~59.~~—~~The method of claim 57, wherein~~ the codon in the mRNA is GXC and the anticodon in the tRNA is GYC;; or

~~60.~~—~~The method of claim 57, wherein~~ the codon in the mRNA is GXU and the anticodon is AYC.

~~61~~32. The method of any one of claims ~~26 to 60~~17-31, wherein the first unnatural base or the second unnatural base comprise a modified sugar moiety selected from the group consisting of:

a modification at the 2' position comprising:

OH, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂F, or a combination thereof;

O-alkyl, S-alkyl, N-alkyl, or a combination thereof;

O-alkenyl, S-alkenyl, N-alkenyl, or a combination thereof;

O-alkynyl, S-alkynyl, N-alkynyl, or a combination thereof;

O-alkyl-O-alkyl, 2'-F, 2'-OCH₃, 2'-O(CH₂)₂OCH₃, or a combination thereof, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C₁-C₁₀, alkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl, -O[(CH₂)_nO]_mCH₃, -O(CH₂)_nOCH₃, -O(CH₂)_nNH₂, -O(CH₂)_nCH₃, -O(CH₂)_n-NH₂, and -O(CH₂)_nON[(CH₂)_nCH₃]₂, wherein n and m are from 1 to about 10;

a modification at the 5' position comprising:

5'-vinyl, 5'-methyl (R or S), or a combination thereof;

a modification at the 4' position comprising:

4'-S, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic

properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, or a combination thereof;

or a combination thereof; and/or

~~62. The method of any one of claims 26 to 61, wherein~~ the at least one unnatural amino acid:

is a lysine analogue;

comprises an aromatic side chain;

comprises an azido group;

comprises an alkyne group; or

comprises an aldehyde or ketone group; optionally

~~63. The method of any one of claims 26 to 61,~~ wherein at least one unnatural amino acid is

selected from the group consisting of N6-((azidoethoxy)-carbonyl)-L-lysine (AzK), N6-((propargylethoxy)-carbonyl)-L-lysine (PraK), BCN-L-lysine, norbornene lysine, TCO-lysine, methyltetrazine lysine, allyloxycarbonyllysine, 2-amino-8-oxononanoic acid, 2-amino-8-oxooctanoic acid, p-acetyl-L-phenylalanine, p-azidomethyl-L-phenylalanine (pAMF), p-iodo-L-phenylalanine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, p-propargyloxyphenylalanine, p-propargyl-phenylalanine, 3-methyl-phenylalanine, L-Dopa, fluorinated phenylalanine, isopropyl-L-phenylalanine, p-azido-L-phenylalanine, p-acyl-L-phenylalanine, p-benzoyl-L-phenylalanine, p-bromophenylalanine, p-amino-L-phenylalanine, isopropyl-L-phenylalanine, O-allyltyrosine, O-methyl-L-tyrosine, O-4-allyl-L-tyrosine, 4-propyl-L-tyrosine, phosphotyrosine, tri-O-acetyl-GlcNAc-serine, L-phosphoserine, phosphoserine, L-3-(2-naphthyl)alanine, 2-amino-3-((2-((3-(benzyloxy)-3-oxopropyl)amino)ethyl)selanyl)propanoic acid, 2-amino-3-(phenylselanyl)propanoic, selenocysteine, N6-(((2-azidobenzyl)oxy)carbonyl)-L-lysine, N6-(((3-azidobenzyl)oxy)carbonyl)-L-lysine, and N6-(((4-azidobenzyl)oxy)carbonyl)-L-lysine.

~~64~~33. The method of claim ~~63~~32, wherein the unnatural amino acid is N6-((azidoethoxy)-carbonyl)-L-lysine (AzK).

~~65~~34. The method of any one of claims ~~26 to 64~~ 17-33, wherein the cell is a human cell, optionally

~~66. The method of claim 65,~~ wherein the human cell is a HEK293T cell, or

~~67. The method of any one of claims 26 to 64,~~ wherein the cell is a hamster cell, optionally

~~68. The method of claim 67,~~ wherein the hamster cell is a Chinese hamster ovary (CHO) cell.

~~69~~35. The method of any one of claims ~~26 to 68~~ 17-34, wherein the tRNA is derived from *Methanococcus jannaschii*, *Methanosarcina barkeri*, *Methanosarcina mazei*, or *Methanosarcina acetivorans*, and/or

~~70. The method of any one of claims 26 to 69,~~ wherein the cell comprises a tRNA synthetase derived from *Methanococcus jannaschii*, *Methanosarcina barkeri*, *Methanosarcina mazei*, or *Methanosarcina acetivorans*.

~~71~~36. A system for expression of an unnatural polypeptide comprising:

(a) at least one unnatural amino acid;

(b) an mRNA encoding the unnatural polypeptide, said mRNA comprising at least one codon comprising one or more first unnatural bases;

(c) a tRNA comprising at least one anti-codon comprising one or more second unnatural bases wherein the one or more first unnatural bases and the one or more second unnatural bases are capable of forming one or more complementary base pairs; and

(d) a eukaryotic ribosome capable of translating the mRNA into a polypeptide comprising the unnatural amino acid using the tRNA and tRNA synthetase,

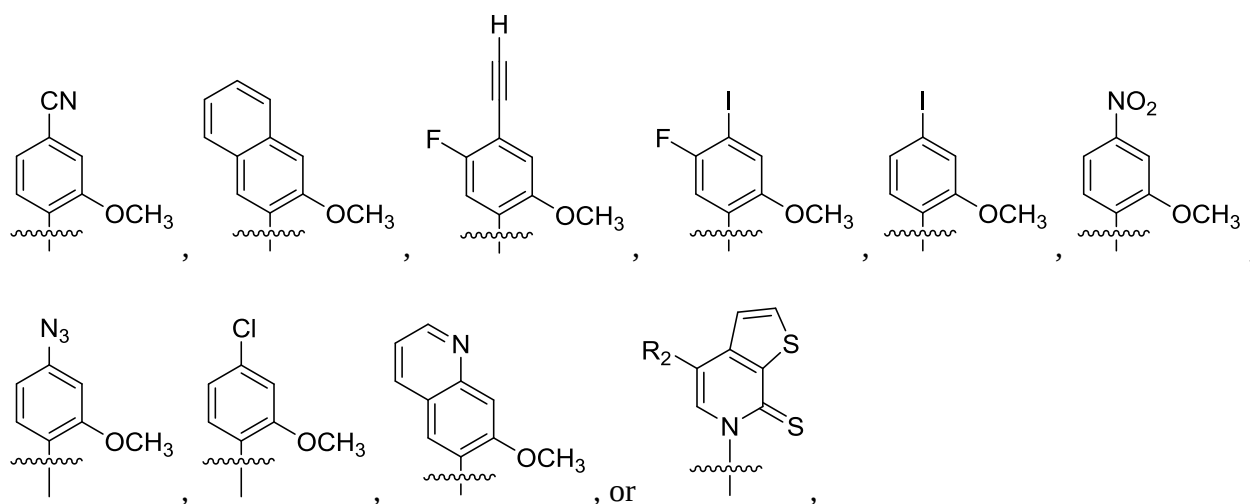
wherein the tRNA is charged with the unnatural amino acid, or the system further comprises a tRNA synthetase or one or more nucleic acid constructs comprising a nucleic acid sequence encoding a tRNA synthetase, wherein the tRNA synthetase preferentially aminoacylates the tRNA with the at least one unnatural amino acid.

7237. The system of claim 7136, wherein the at least one codon of the mRNA comprises three contiguous nucleobases (N-N-N); and wherein the one or more first unnatural bases (X) is located at the first position (X-N-N) in the at least one codon of the mRNA, or

~~73. The system of claim 71, wherein the at least one codon of the mRNA comprises three contiguous nucleobases (N-N-N); and wherein the one or more first unnatural bases (X) is located at the middle position (N-X-N) in the codon of the mRNA, or~~

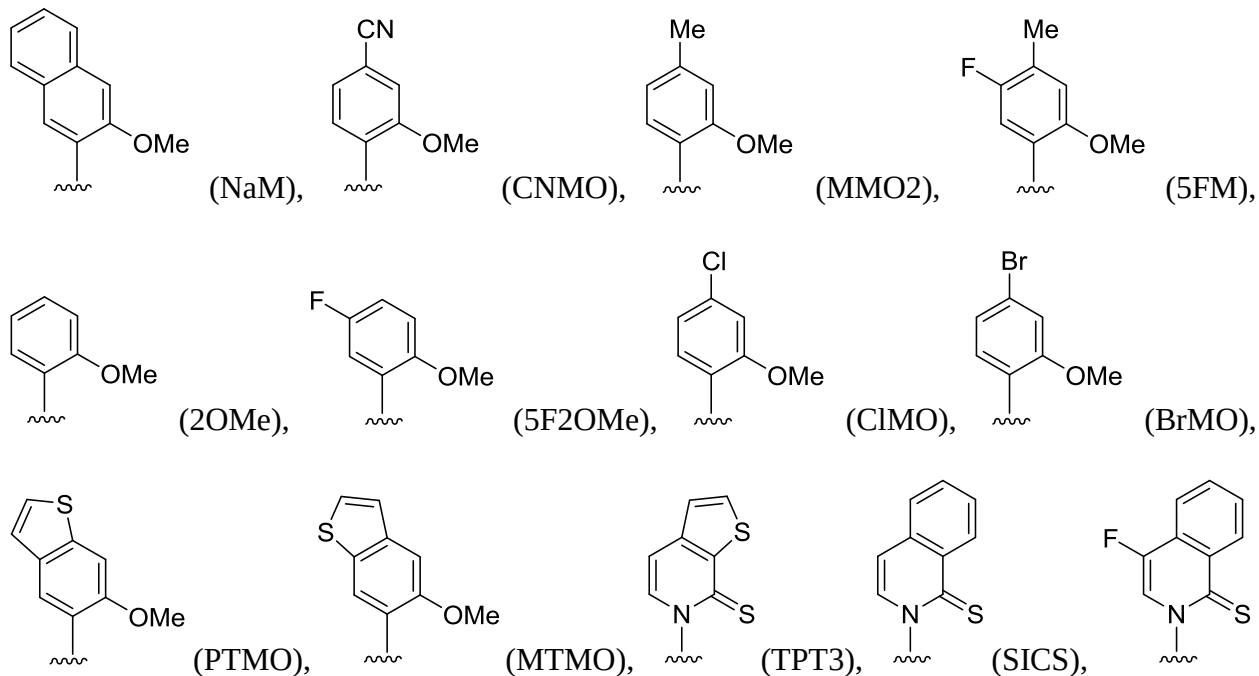
~~74. The system of claim 71, wherein the at least one codon of the mRNA comprises three contiguous nucleobases (N-N-N); and wherein the one or more first unnatural bases (X) is located at the last position (N-N-X) in the at least one codon of the mRNA.~~

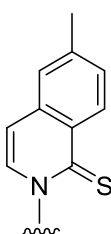
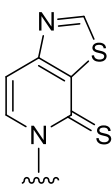
7538. The system of any one of claims ~~71 to 7436-37~~, wherein the one or more unnatural bases is of the formula

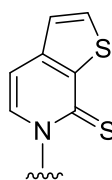


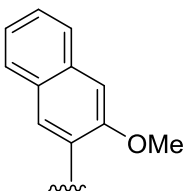
wherein R₂ is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, methoxy, methanethiol, methaneseleno, halogen, cyano, and azido, and the wavy line indicates a bond to a ribosyl moiety.

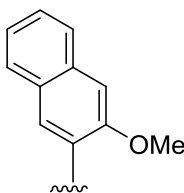
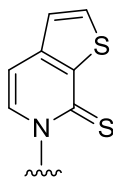
7639. The system of any one of claims ~~71 to 74~~**36-38**, wherein the one or more first unnatural bases or the one or more second unnatural bases is selected from the group consisting of

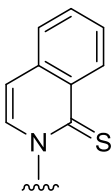


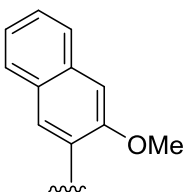
(FSICS),  (5SICS), and  (TAT1), wherein the wavy line indicates a bond to a ribosyl moiety.

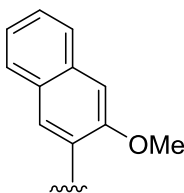
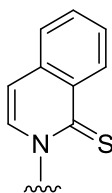
~~7740.~~ The system of claim ~~7639~~, ~~when~~wherein the one or more first unnatural bases is  (TPT3),

and the one or more second unnatural bases is  (NaM), ~~and when or~~ the one or more

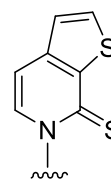
first unnatural bases is  (NaM), and the second unnatural base is  (TPT3), wherein the wavy line indicates a bond to a ribosyl moiety ~~;~~ or

~~78.~~ ~~The system of claim 76, when~~ the one or more first unnatural bases is  (5SICS),

and the one or more second unnatural bases is  (NaM), ~~and or~~ when the one or more

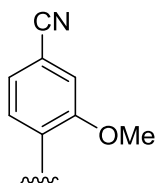
first unnatural base is  (NaM), the one or more second unnatural bases is  (5SICS), wherein the wavy line indicates a bond to a ribosyl moiety ~~;~~ or

79. ~~The system of claim 76, when~~ the one or more first unnatural bases is



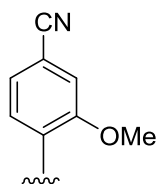
(TPT3),

and the one or more second unnatural bases is

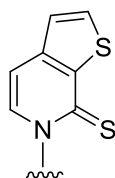


(CNMO), ~~and when or~~ the one or more

first unnatural bases is

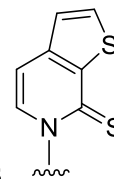


(CNMO), and the one or more second unnatural bases is



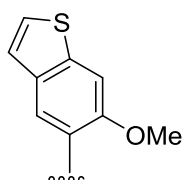
(TPT3), wherein the wavy line indicates a bond to a ribosyl moiety; or

80. ~~The system of claim 76, when~~ the one or more first unnatural bases is



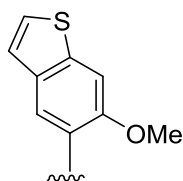
(TPT3), and

the one or more second unnatural bases is

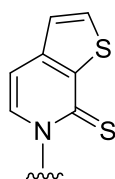


(PTMO), ~~and when or~~ the one or more

first unnatural bases is

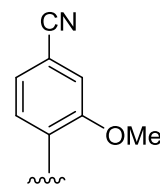


(PTMO), and the one or more second unnatural bases is

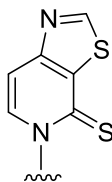


(TPT3), wherein the wavy line indicates a bond to a ribosyl moiety; or

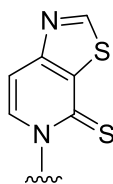
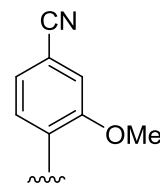
81. ~~The system of claim 76, when~~ the one or more first unnatural bases is (CNMO); and



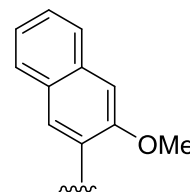
the one or more second unnatural bases is (TAT1); ~~and when or~~ the one or more first



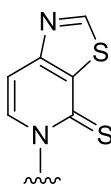
unnatural bases is (TAT1); and the one or more second unnatural bases is (CNMO), wherein the wavy line indicates a bond to a ribosyl moiety; or



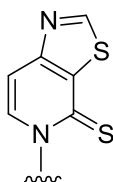
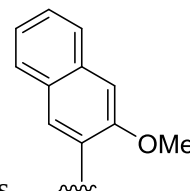
82. ~~The system of claim 76, when~~ the one or more first unnatural bases is (NaM); and



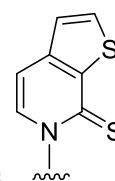
the one or more second unnatural bases is (TAT1); ~~and when or~~ the one or more first

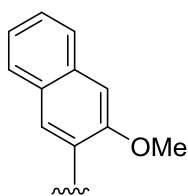


unnatural bases is (TAT1); and the one or more second unnatural bases is (NaM), wherein the wavy line indicates a bond to a ribosyl moiety; or



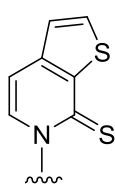
83. ~~The system of claim 76, when~~ the one or more first unnatural bases is (TPT3) and



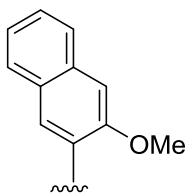


the one or more second unnatural bases is (NaM), wherein the wavy line indicates a bond to a ribosyl moiety-; or

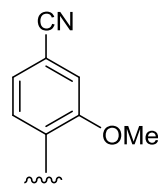
~~84. The system of any one of claims 71 to 74,~~ wherein the one or more first unnatural bases is



(TPT3),

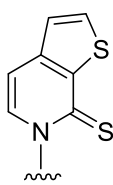


(NaM), and

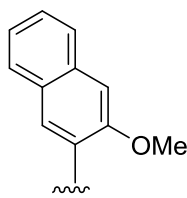


(CNMO), wherein the wavy line indicates a bond to a ribosyl moiety.

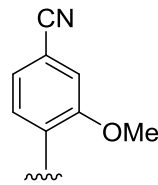
~~8541.~~ The system of claim ~~71~~36, wherein the at least one codon of the mRNA comprises three contiguous nucleobases (N-N-N), wherein the one or more first unnatural bases (X) is located at the first position (X-N-N) in the codon of the mRNA, wherein the one or more first unnatural bases



(TPT3),

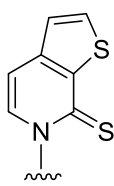


(NaM), and

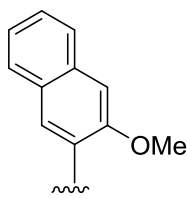


(CNMO), and wherein the wavy line indicates a bond to a ribosyl moiety-; or

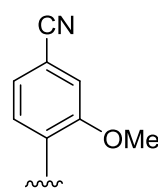
~~86. The system of claim 71,~~ wherein the at least one codon of the mRNA comprises three contiguous nucleobases (N-N-N), wherein the one or more first unnatural bases (X) is located at the middle position (N-X-N) in the codon of the mRNA, wherein the one or more first unnatural



(TPT3),

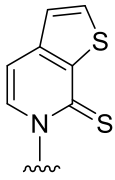
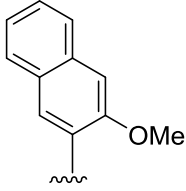
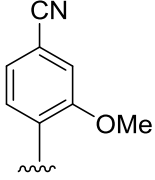


(NaM), and

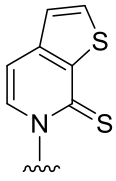
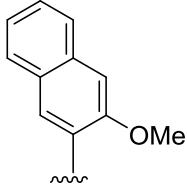
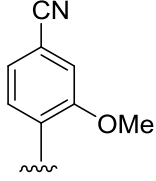


(CNMO), and wherein the wavy line indicates a bond to a ribosyl moiety-; or

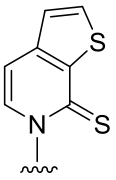
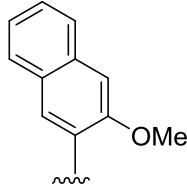
87. ~~The system of claim 71,~~ wherein the at least one codon of the mRNA comprises three contiguous nucleobases (N-N-N), wherein the one or more first unnatural base (X) is located at the last position (N-N-X) in the codon of the mRNA, wherein the one or more first unnatural base is

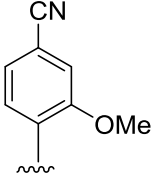
selected from  (TPT3),  (NaM), and  (CNMO), and wherein the wavy line indicates a bond to a ribosyl moiety; or

88. ~~The system of claim 71,~~ wherein the at least one anticodon of the tRNA comprises three contiguous nucleobases (N-N-N); and wherein the one or more second unnatural base (X) is located at the first position (X-N-N) in the anticodon of the tRNA, wherein the one or more second

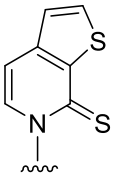
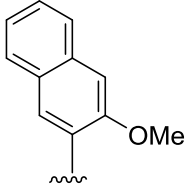
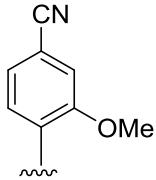
unnatural bases is selected from  (TPT3),  (NaM), and  (CNMO), and wherein the wavy line indicates a bond to a ribosyl moiety; or

89. ~~The system of claim 71,~~ wherein the at least one anticodon of the tRNA comprises three contiguous nucleobases (N-N-N); and wherein the one or more second unnatural bases (X) is located at the middle position (N-X-N) in the anticodon of the tRNA, wherein the one or more

second unnatural bases is selected from  (TPT3),  (NaM), and

 (CNMO), and wherein the wavy line indicates a bond to a ribosyl moiety; or

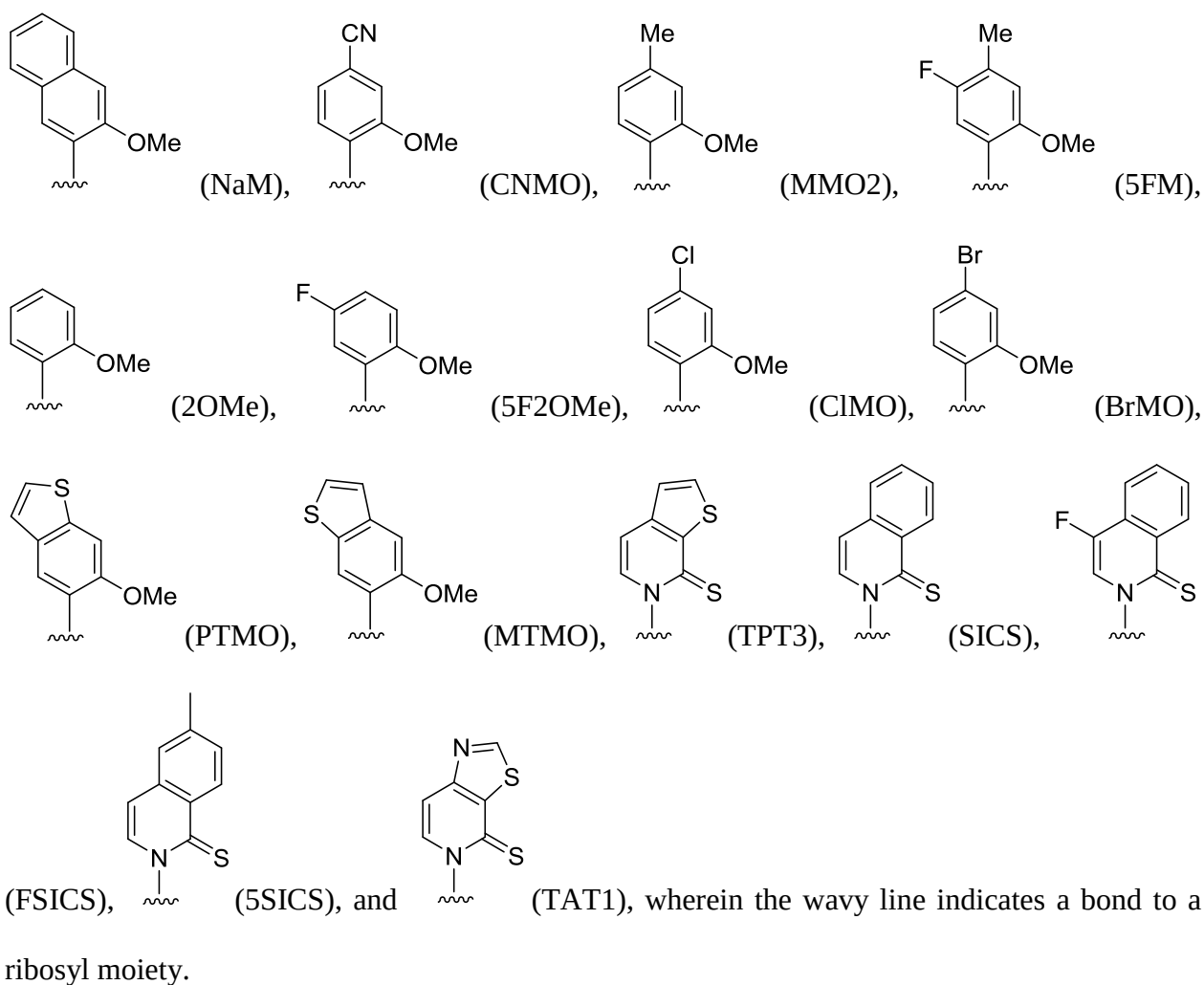
~~90. — The system of claim 71,~~ wherein the at least one anticodon of the tRNA comprises three contiguous nucleobases (N-N-N); and wherein the one or more second unnatural bases (X) is located at the last position (N-N-X) in the anticodon of the tRNA, wherein the one or more second

unnatural base is selected from  (TPT3),  (NaM), and  (CNMO), and wherein the wavy line indicates a bond to a ribosyl moiety; or

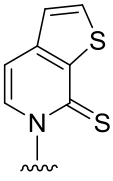
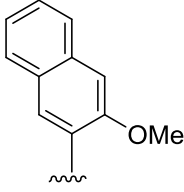
~~91. — The system of claim 71,~~ wherein the at least one codon and the at least one anticodon each, independently, comprise three contiguous nucleobases (N-N-N), and wherein the at least one codon comprises one or more first unnatural bases (X) located at the first position (X-N-N) of the codon, and the at least one anticodons in the tRNA comprises the one or more second unnatural bases (Y) located at the last position (N-N-Y) of the anticodon; optionally

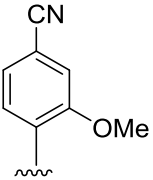
~~92. — The system of claim 91,~~ wherein the one or more first unnatural bases (X) located in the codon of the mRNA and the one or more second unnatural bases (Y) located in the anticodon of the tRNA are the same or are different; and/or

~~93. — The system of any one of claims 91 to 92, wherein~~ the one or more first unnatural bases (X) located in the codon of the mRNA and the one or more second unnatural bases (Y) located in the anticodon of the tRNA are selected from the group consisting of

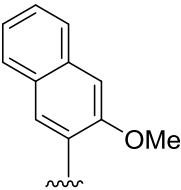
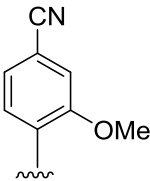


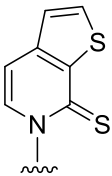
9442. The system of claim 9341, wherein the one or more first unnatural bases (X) located in the codon of the mRNA and the one or more second unnatural bases (Y) located in the anticodon of

the tRNA are selected from the group consisting of  (TPT3),  (NaM), and

 (CNMO), wherein the wavy line indicates a bond to a ribosyl moiety-, further optionally

~~95. The system of claim 94,~~ wherein the one or more first unnatural base (X) located in the

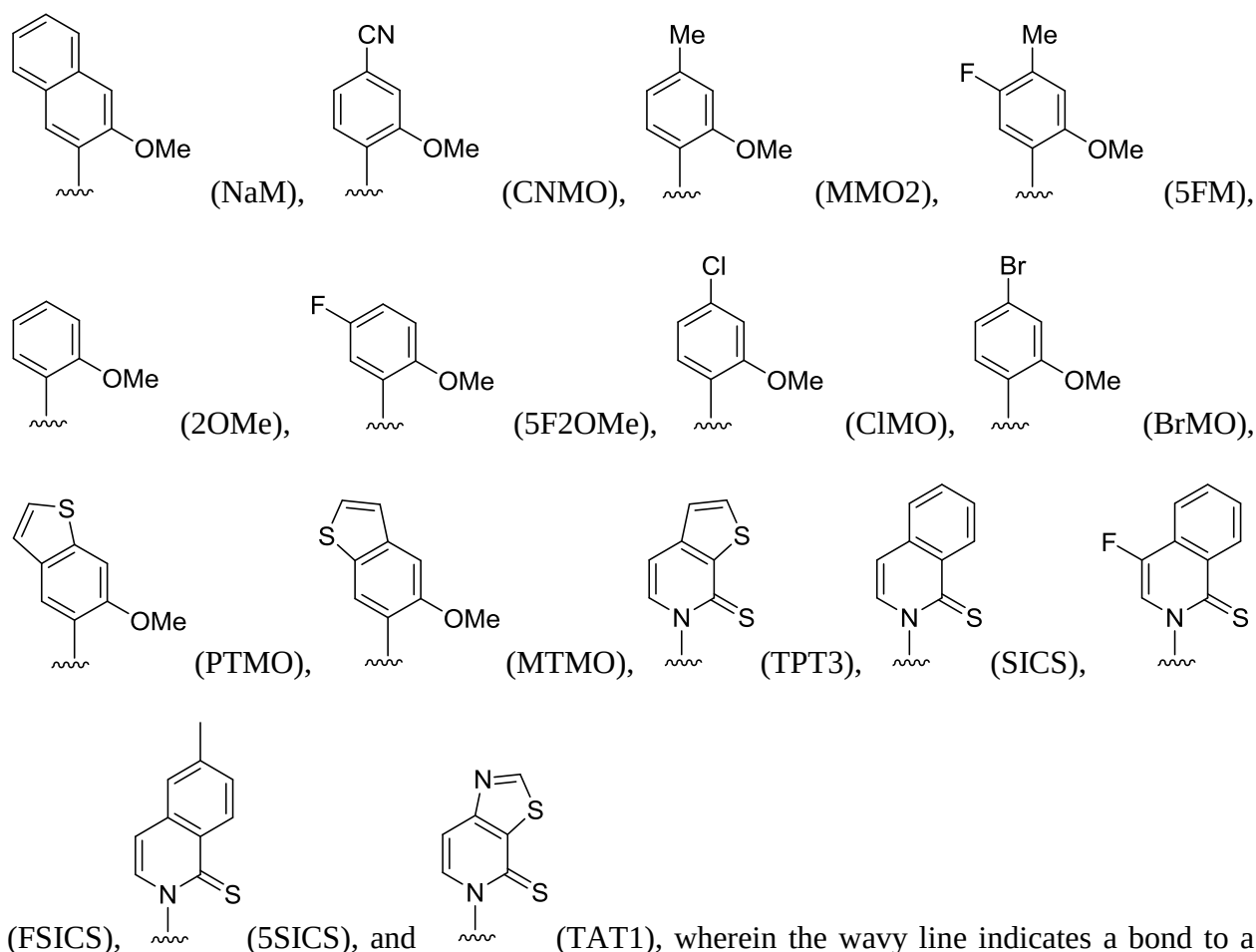
codon of the mRNA is selected from  (NaM) and  (CNMO), and the

one or more second unnatural bases (Y) located in the anticodon of the tRNA is  (TPT3), wherein in each case the wavy line indicates a bond to a ribosyl moiety.

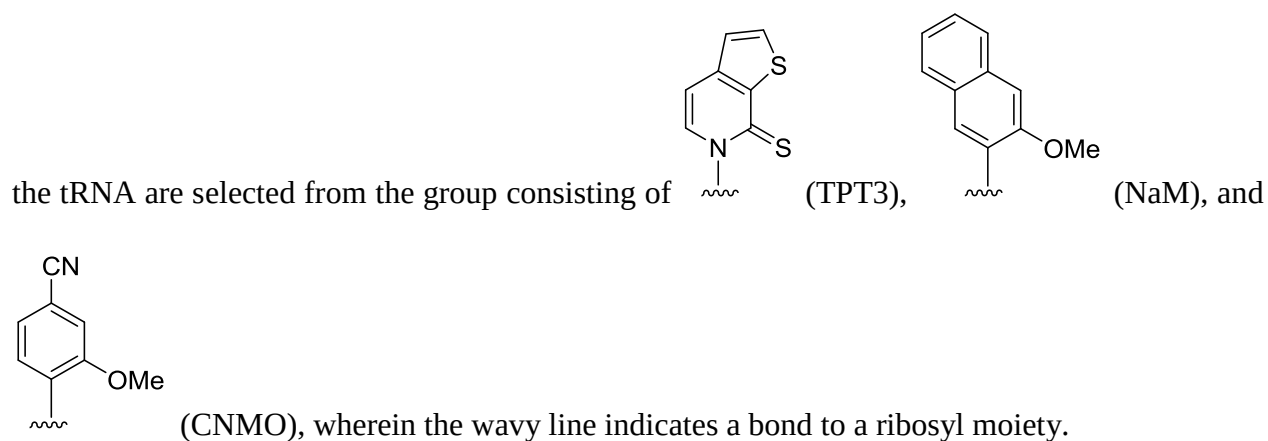
~~9643.~~ The system of claim ~~7136~~, wherein the at least one codon and the at least one anticodon each, independently, comprise three contiguous nucleobases (N-N-N), and wherein the at least one codon in the mRNA comprises the one or more first unnatural bases (X) located at a middle position (N-X-N) of the at least one codon, and the at least one anticodon in the tRNA comprises the one or more second unnatural bases (Y) located at a middle position (N-Y-N) of the anticodon. optionally

~~97. The system of claim 96,~~ wherein the one or more first unnatural bases (X) located in the codon of the mRNA and the one or more second unnatural bases (Y) located in the anticodon of the tRNA are the same or are different. and/or

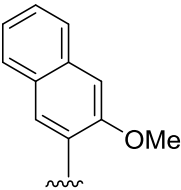
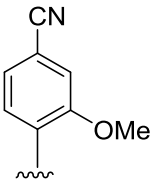
~~98. The system of any one of claims 96 to 97, wherein~~ the one or more first unnatural bases (X) located in the codon of the mRNA and the one or more second unnatural bases (Y) located in the anticodon of the tRNA are selected from the group consisting of

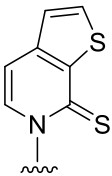


9944. The system of claim **9843**, wherein the one or more first unnatural bases (X) located in the codon of the mRNA and the one or more second unnatural bases (Y) located in the anticodon of



~~100~~45. The system of claim 9944, wherein the one or more first unnatural bases (X) located in the

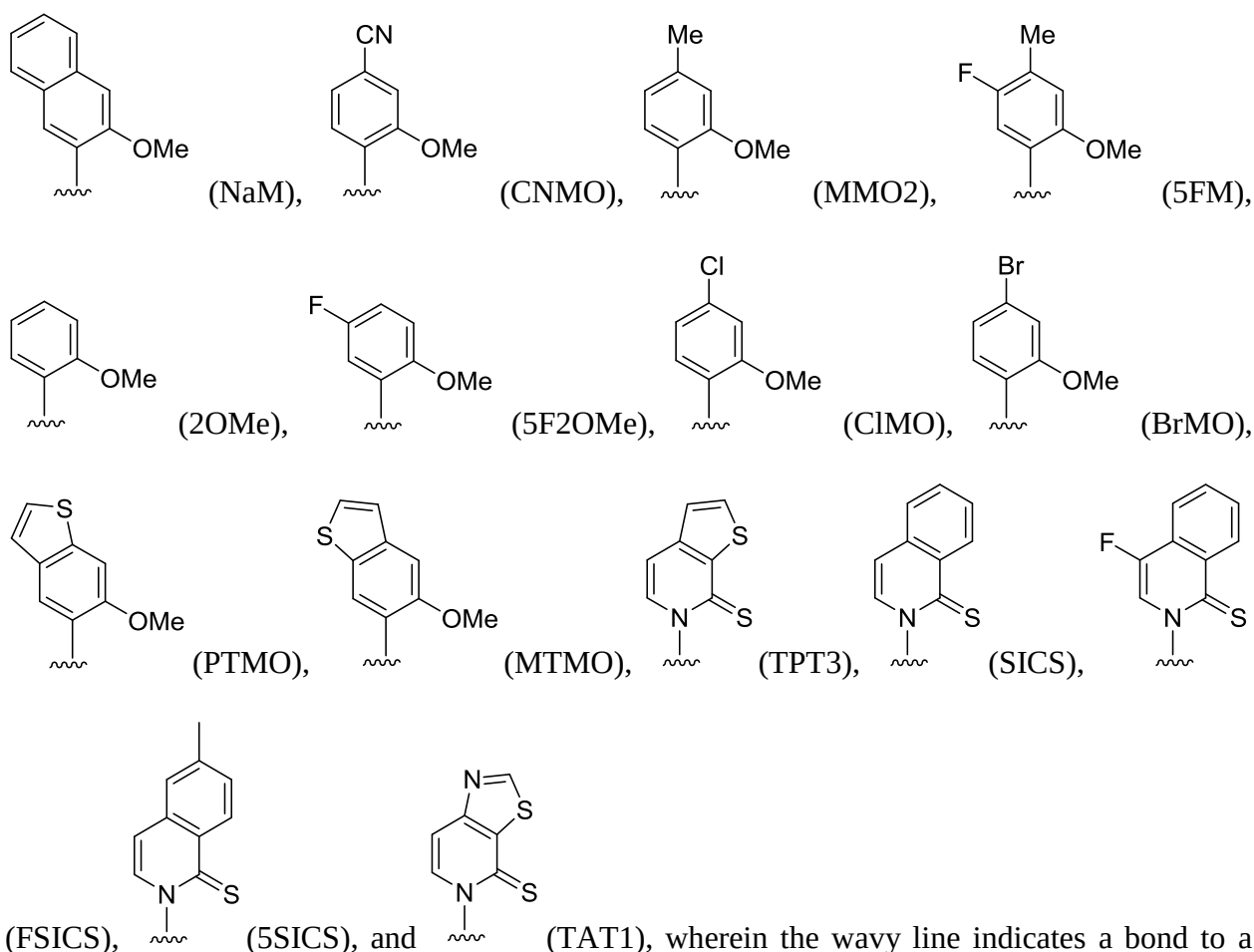
codon of the mRNA is selected from  (NaM) and  (CNMO), and the

one or more second unnatural bases (Y) located in the anticodon of the tRNA is  (TPT3), wherein in each case the wavy line indicates a bond to a ribosyl moiety.

~~101~~46. The system of claim 7136, wherein the at least one codon and the at least one anticodon each, independently, comprise three contiguous nucleobases (N-N-N), and wherein the at least one codon in the mRNA comprises the one or more first unnatural bases (X) located at the last position (N-N-X) of the at least one codon, and the at least one anticodon in the tRNA comprises the one or more second unnatural bases (Y) located at the first position (Y-N-N) of the anticodon-, optionally

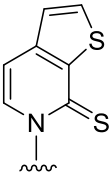
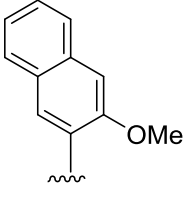
~~102.—The system of claim 101,—~~ wherein the one or more first unnatural bases (X) located in the codon of the mRNA and the one or more second unnatural bases (Y) located in the anticodon of the tRNA are the same or are different-and/or

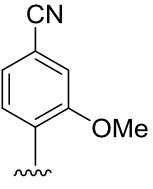
~~103.—The system of any one of claims 101 to 102, wherein~~ the one or more first unnatural bases (X) located in the codon of the mRNA and the one or more second unnatural bases (Y) located in the anticodon of the tRNA are selected from the group consisting of



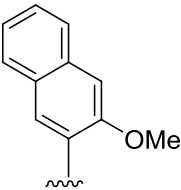
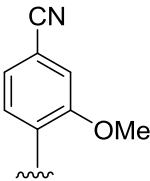
ribosyl moiety.

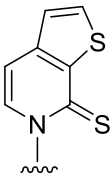
~~10447~~. The system of claim ~~10346~~, wherein the one or more first unnatural bases (X) located in the codon of the mRNA and the one or more second unnatural bases (Y) located in the anticodon

of the tRNA are selected from the group consisting of
  (TPT3),
  (NaM),

and
  (CNMO), wherein the wavy line indicates a bond to a ribosyl moiety-, optionally

~~105. The system of claim 104,~~ wherein the one or more first unnatural bases (X) located in the

codon of the mRNA is selected from  (NaM) and  (CNMO), and the

one or more second unnatural bases (Y) located in the anticodon of the tRNA is  (TPT3), wherein in each case the wavy line indicates a bond to a ribosyl moiety.

~~106~~48. The system of any one of claims ~~71 to 105~~36-47, wherein the at least one codon in the mRNA is selected from AXC, GXC or GXU, wherein X is the one or more first unnatural bases,~~1~~
optionally

~~107. The system of the immediately preceding claim,~~ wherein the at least one anticodon in the tRNA is selected from GYU, GYC, and AYC, and Y is the one or more second unnatural bases,~~1~~
further optionally

~~108. The system of claim 107,~~ wherein the at least one codon in the mRNA is AXC and the at least one anticodon in the tRNA is GYU~~1~~.

~~109. The system of claim 107, wherein~~ the at least one codon in the mRNA is GXC and the at least one anticodon in the tRNA is GYC~~1~~, or

~~110. The system of claim 107, wherein~~ the at least one codon in the mRNA is GXU and the at least one anticodon is AYC.

~~11149.~~ The system of any one of claims ~~71 to 110~~36-48, wherein the tRNA is derived from *Methanococcus jannaschii*, *Methanosarcina barkeri*, *Methanosarcina mazei*, or *Methanosarcina acetivorans*;₁

~~112.~~ The system of any one of claims ~~71 to 111~~, wherein the tRNA synthetase is derived from *Methanococcus jannaschii*, *Methanosarcina barkeri*, *Methanosarcina mazei*, or *Methanosarcina acetivorans*;₁

~~113.~~ The system of any one of claims ~~71 to 112~~, which the system is in vitro or cell-free;₁

~~114.~~ The system of any one of claims ~~71 to 113~~, comprising the system comprises a cell lysate;₁

~~115.~~ The system of any one of claims ~~71 to 113~~, which the system is a reconstituted system of purified components;₁ and/or

~~116.~~ The system of any one of claims ~~71 to 112~~, which the system is in a eukaryotic cell;₁
optionally

~~117.~~ The system of claim ~~116~~, wherein the eukaryotic cell is a human cell;₂

~~118.~~ The system of claim ~~116~~, wherein the eukaryotic cell is a HEK293T cell;₁

~~119.~~ The system of claim ~~116~~, wherein the eukaryotic cell is a hamster cell;₁

~~120.~~ The system of claim ~~119~~, wherein the hamster cell is or a Chinese hamster ovary (CHO) cell.

~~121~~50. The system of any one of claims ~~71 to 120~~36-49, wherein the unnatural amino acid:
is a lysine analogue;
comprises an aromatic side chain;
comprises an azido group;
comprises an alkyne group; or
comprises an aldehyde or ketone group, optionally

~~122. The system of any one of claims 71 to 121,~~ wherein the unnatural amino acid is selected from the group consisting of N6-((azidoethoxy)-carbonyl)-L-lysine (AzK), N6-((propargylethoxy)-carbonyl)-L-lysine (PraK), BCN-L-lysine, norbornene lysine, TCO-lysine, methyltetrazine lysine, allyloxycarbonyllysine, 2-amino-8-oxononanoic acid, 2-amino-8-oxooctanoic acid, p-acetyl-L-phenylalanine, p-azidomethyl-L-phenylalanine (pAMF), p-iodo-L-phenylalanine, m-acetylphenylalanine, 2-amino-8-oxononanoic acid, p-propargyloxyphenylalanine, p-propargyl-phenylalanine, 3-methyl-phenylalanine, L-Dopa, fluorinated phenylalanine, isopropyl-L-phenylalanine, p-azido-L-phenylalanine, p-acyl-L-phenylalanine, p-benzoyl-L-phenylalanine, p-bromophenylalanine, p-amino-L-phenylalanine, isopropyl-L-phenylalanine, O-allyltyrosine, O-methyl-L-tyrosine, O-4-allyl-L-tyrosine, 4-propyl-L-tyrosine, phosphotyrosine, tri-O-acetyl-GlcNAcp-serine, L-phosphoserine, phosphoserine, L-3-(2-naphthyl)alanine, 2-amino-3-((2-((3-(benzyloxy)-3-oxopropyl)amino)ethyl)selenyl)propanoic acid, 2-amino-3-(phenylselenyl)propanoic, selenocysteine, N6-(((2-azidobenzyl)oxy)carbonyl)-L-lysine, N6-(((3-azidobenzyl)oxy)carbonyl)-L-lysine, and N6-(((4-azidobenzyl)oxy)carbonyl)-L-lysine, further optionally.

~~123. The system of any one of claims 71 to 122,~~ wherein the unnatural amino acid is N6-((azidoethoxy)-carbonyl)-L-lysine (AzK).

~~124~~51. The system of any one of claims ~~71 to 123~~36-50, wherein the tRNA is charged with the unnatural amino acid.