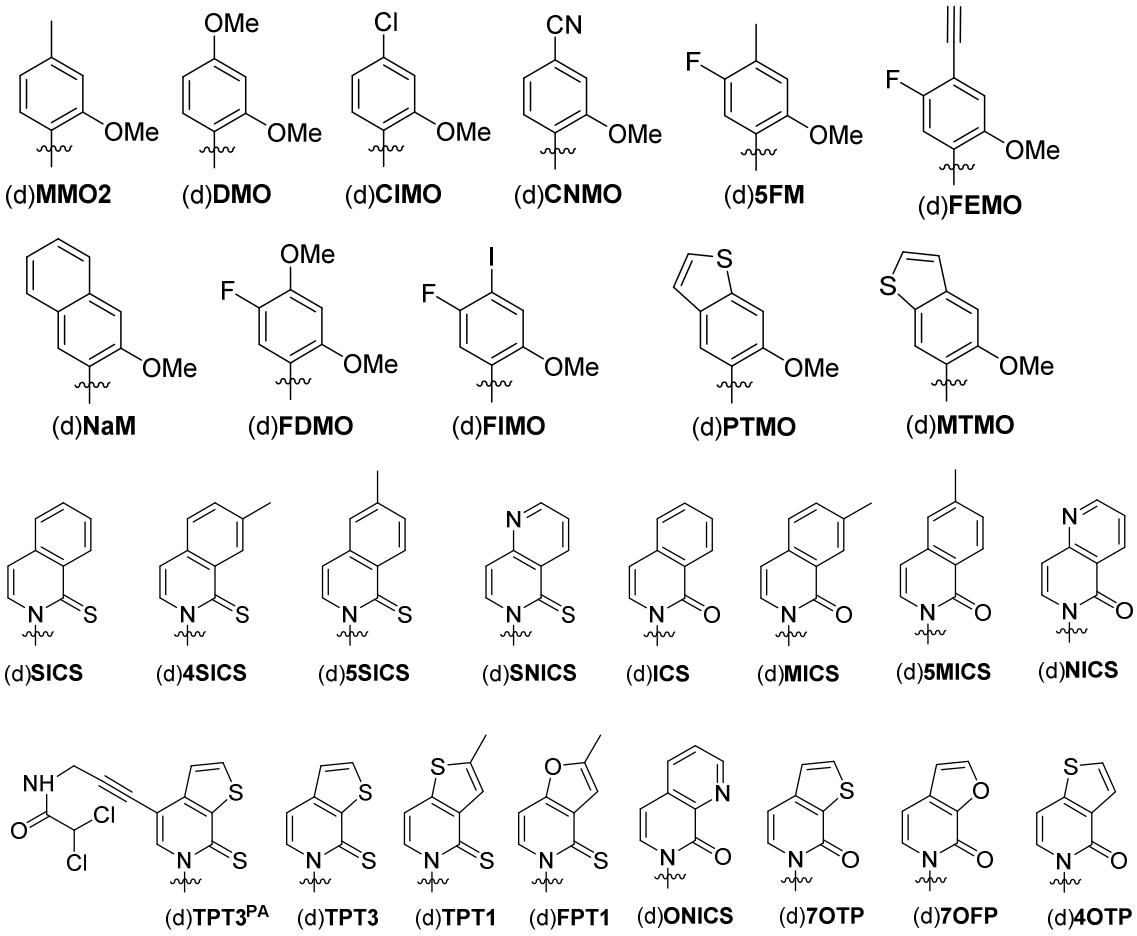
alkynyl, S-alkynyl, N-alkynyl; O-alkyl-O-alkyl, 2'-F, 2'-OCH_3, 2'-$O(CH_2)_2OCH_3$ wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1-C_{10}, alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, -$O[(CH2)_nO]_mCH_3$, -$O(CH_2)_nOCH_3$, -$O(CH_2)_nNH_2$, -$O(CH_2)_nCH_3$, -$O(CH_2)_n$ -ONH_2, and -$O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10; and/or a modification at the 5' position: 5'-vinyl, 5'-methyl (R or S), a modification at the 4' position, 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, and any combination thereof. In some instances, the mutant anticodon or the mutant codon further comprises an unnatural backbone. In some instances, the mutant anticodon and the mutant codon further comprises an unnatural backbone. In some instances, the unnatural nucleotides are recognized by a DNA polymerase, an RNA polymerase, or a reverse transcriptase. In some instances, an unnatural nucleotide is incorporated by the RNA polymerase into the mRNA during transcription to generate a mutant mRNA containing a mutant codon. In some instances, an unnatural nucleotide is incorporated by the RNA polymerase into the tRNA during transcription to generate a mutant tRNA containing a mutant anticodon. In some instances, an unnatural nucleotide is incorporated by the RNA polymerase into the mRNA during transcription to generate a mutant mRNA. In some instances, an unnatural nucleotide is incorporated by the RNA polymerase into the tRNA during transcription to generate a mutant tRNA. In some instances, the mutant tRNA is charged with an unnatural amino acid residue. In some instances, a protein containing an unnatural amino acid is generated during translation utilizing the mutant tRNA and the mutant mRNA.

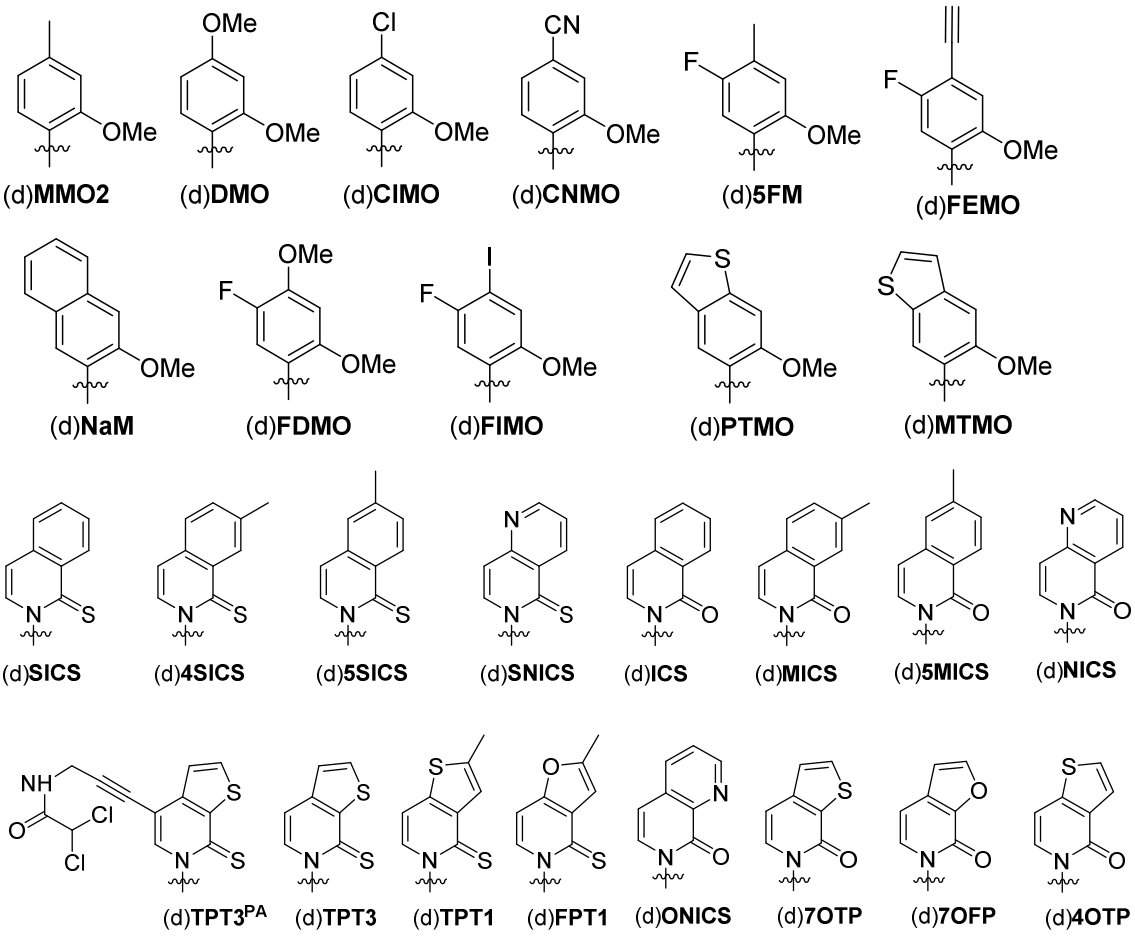
[0007a] In one aspect, provided herein is a method of producing a protein containing an unnatural amino acid, the method comprising:

- preparing a mutant tRNA wherein the mutant tRNA comprises a mutant anticodon sequence selected from Table 1 or 2 that comprises an unnatural nucleotide comprising an unnatural nucleobase selected from



;

- preparing a mutant mRNA wherein the mutant mRNA comprises a mutant codon sequence selected from Table 1 or 2 that comprises an unnatural nucleotide comprising an unnatural nucleobase selected from



;

and

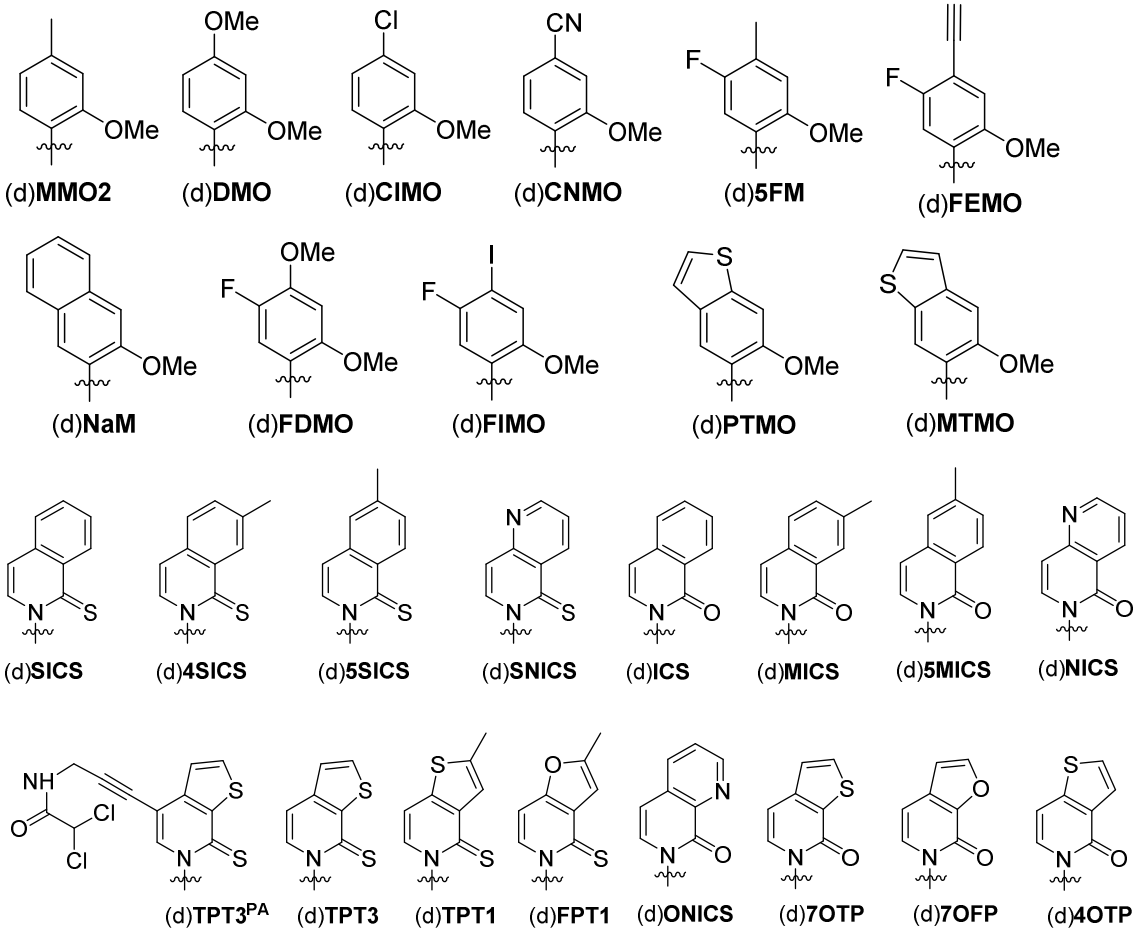
- synthesizing the protein containing an unnatural amino acid utilizing the mutant tRNA and the mutant mRNA;

wherein the protein is synthesized in a semi-synthetic organism (SSO) and the SSO comprises a nucleoside triphosphate transporter from *Phaeodactylum tricornutum* and a pyrrolysine aminoacyl tRNA synthetase from *Methanosarcina barkeri* or *Methanococcus jannaschii*.

[0007b] In one aspect, provided herein is cell comprising:

a. a nucleoside triphosphate transporter from *Phaeodactylum tricornutum*;

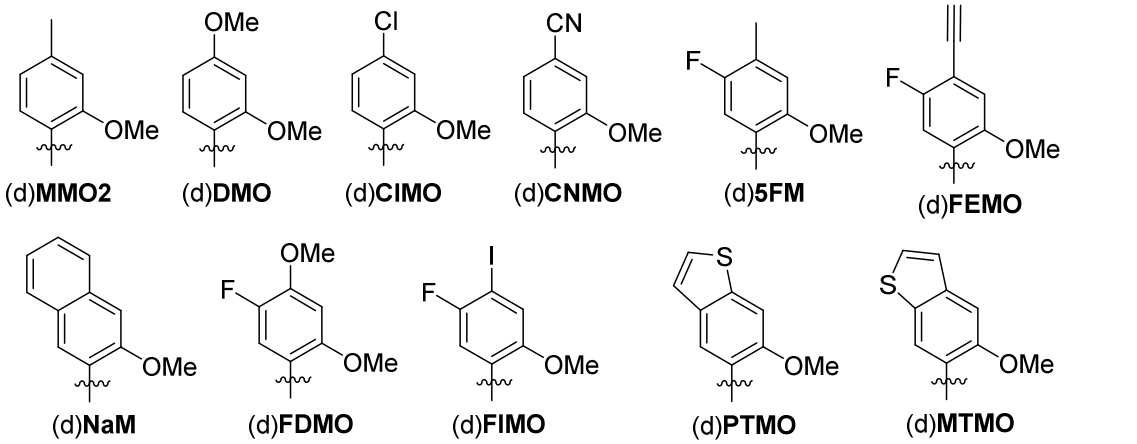
b. a mutant tRNA comprising an unnatural nucleobase selected from

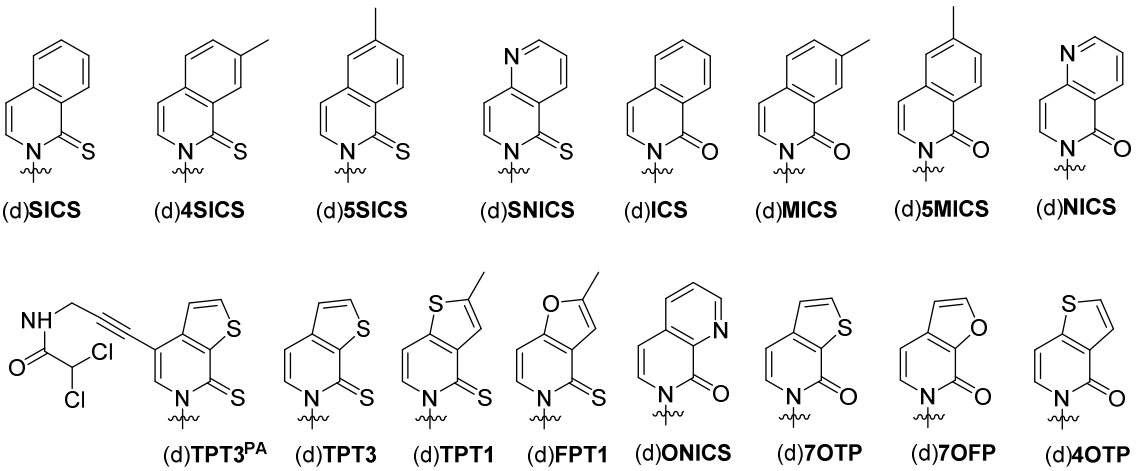


;

c. a pyrrolysine aminoacyl tRNA synthetase from *Methanosarcina barkeri* or *Methanococcus jannaschii*; and

d. an mRNA comprising an unnatural nucleobase selected from





[0007c] In one aspect, provided herein is a method of producing a protein in a cell, wherein the protein comprises an unnatural amino acid, the method comprising:

transporting an unnatural nucleotide into the cell as described herein using the nucleoside triphosphate transporter; and

providing the unnatural amino acid to the cell; wherein

the unnatural nucleotide is incorporated by an RNA polymerase during transcription of a double-stranded oligonucleotide comprising an unnatural oligonucleotide to generate the mutant mRNA and the mutant tRNA; and

the protein containing the unnatural amino acid is generated during translation of the mutant mRNA utilizing the mutant tRNA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Various aspects of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0009] Fig. 1A illustrates the chemical structure of the d**NaM**-d**TPT3** UBP and a natural dA-dT base pair.

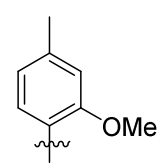
[0010] Fig. 1B illustrates the gene cassette used to express sfGFP(A**X**C)^{151} and tRNA(G**Y**T)^{Ser}. P_{T7} and T_{T7} denote the T7 RNAP promoter and terminator, respectively. In controls where sfGFP is expressed in the absence of *serT*, the sequence following the sfGFP T7 terminator is absent.

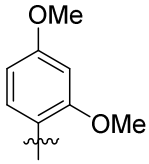
CLAIMS

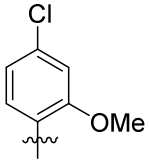
What is claimed is:

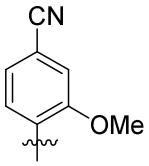
1. A method of producing a protein containing an unnatural amino acid, the method comprising:

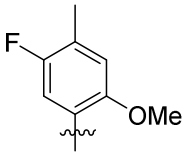
- preparing a mutant tRNA wherein the mutant tRNA comprises a mutant anticodon sequence selected from Table 1 or 2 that comprises an unnatural nucleotide comprising an unnatural nucleobase selected from

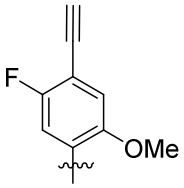

(d)**MMO2**

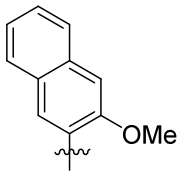

(d)**DMO**

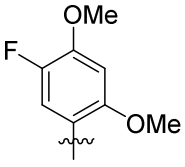

(d)**ClMO**

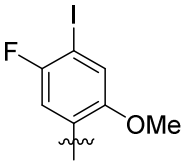

(d)**CNMO**

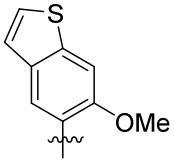

(d)**5FM**

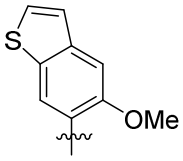

(d)**FEMO**

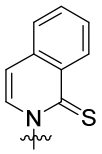

(d)**NaM**

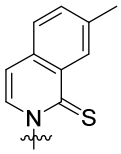

(d)**FDMO**

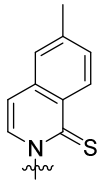

(d)**FIMO**

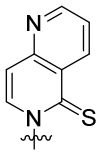

(d)**PTMO**

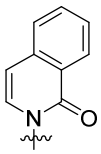

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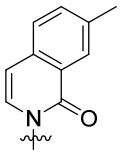

(d)**SICS**

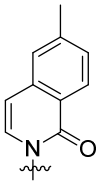

(d)**4SICS**

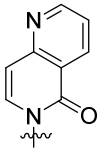

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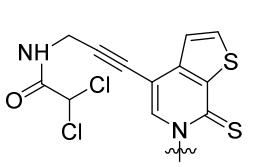

(d)**SNICS**

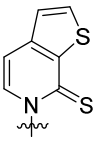

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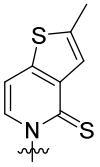

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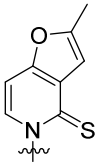

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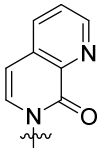

(d)**NICS**

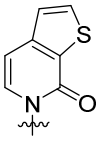

(d)**TPT3^{PA}**

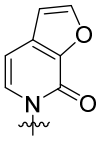

(d)**TPT3**

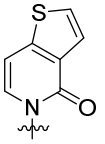

(d)**TPT1**


(d)**FPT1**

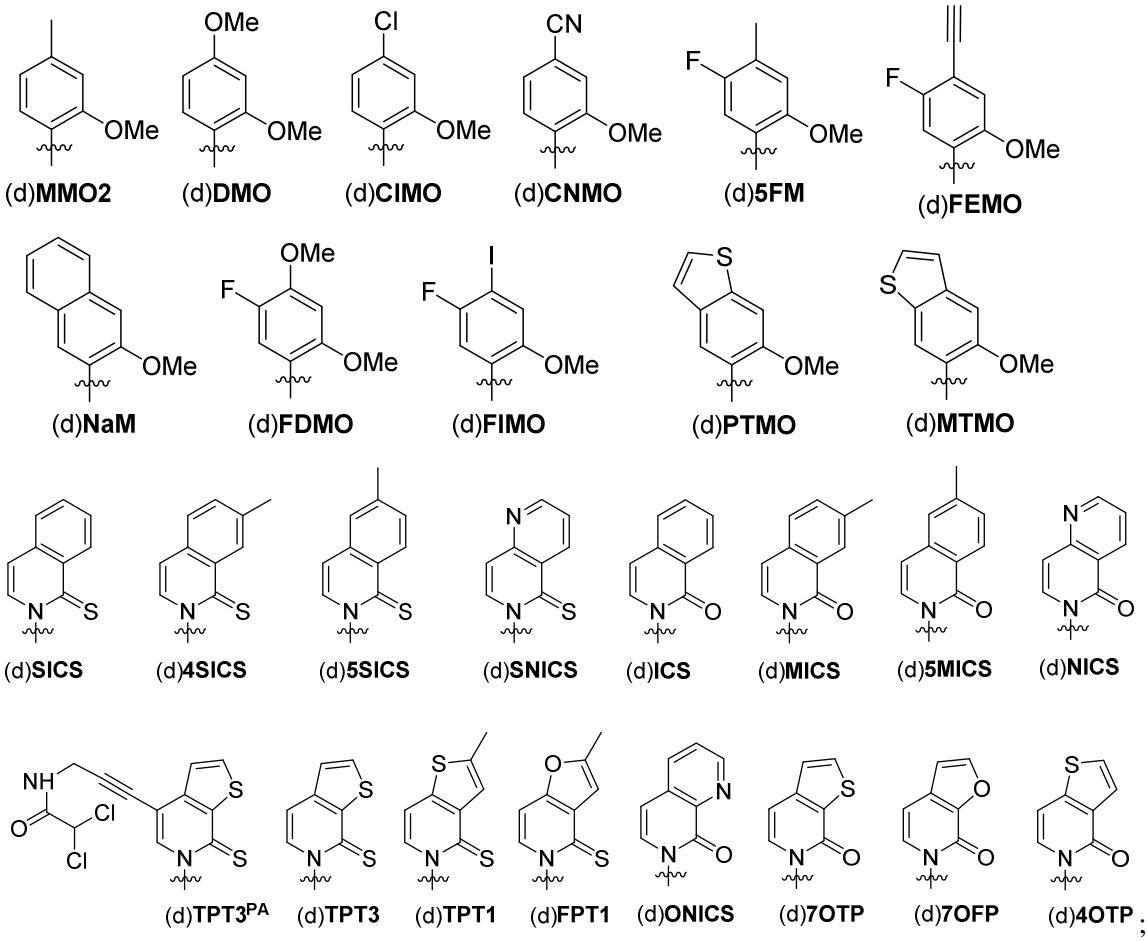

(d)**ONICS**


(d)**7OTP**


(d)**7OFP**


(d)**4OTP**;

- preparing a mutant mRNA wherein the mutant mRNA comprises a mutant codon sequence selected from Table 1 or 2 that comprises an unnatural nucleotide comprising an unnatural nucleobase selected from



and

- synthesizing the protein containing an unnatural amino acid utilizing the mutant tRNA and the mutant mRNA;

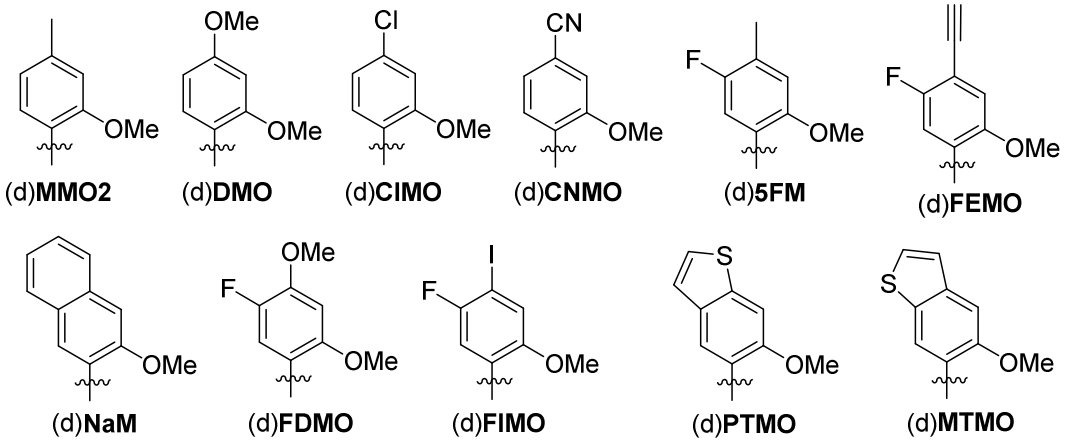
wherein the protein is synthesized in a semi-synthetic organism (SSO) and the SSO comprises a nucleoside triphosphate transporter from *Phaeodactylum tricornutum* and a pyrrolysine aminoacyl tRNA synthetase from *Methanosarcina barkeri* or *Methanococcus jannaschii*.

2. The method of claim 1, wherein the semi-synthetic organism comprises a microorganism.

3. The method of any one of the claims 1 or 2, wherein the semi-synthetic organism comprises a bacterium.

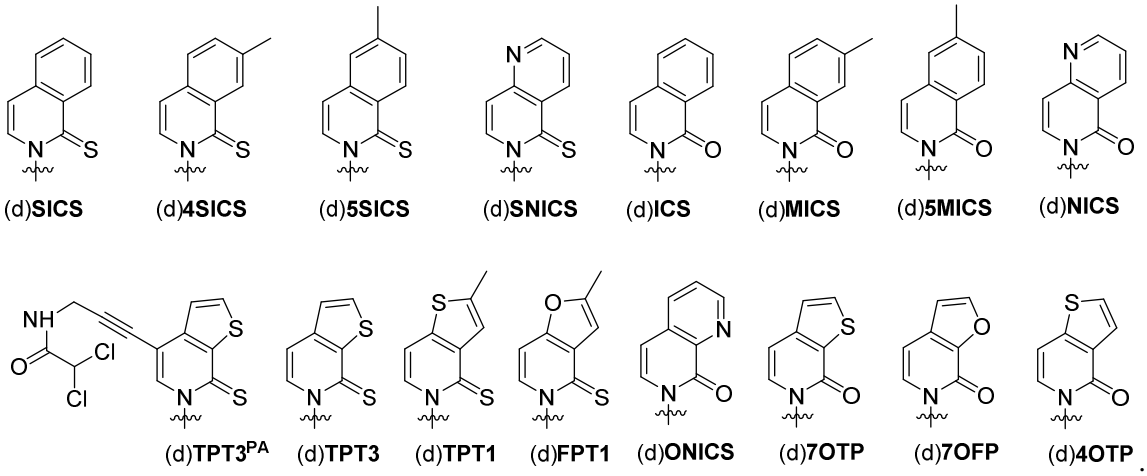
4. The method of any one of the claims 1-3, wherein the semi-synthetic organism comprises an *Escherichia coli*.

5. The method of any one of the claims 1-4, wherein the unnatural nucleobase is selected from

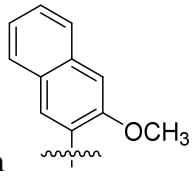
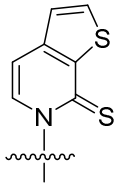


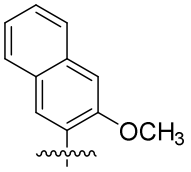
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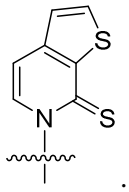
6. The method of any one of the claims 1-4, wherein the unnatural nucleobase is selected from



.

7. The method of any one of the claims 1-4, wherein the unnatural nucleobase in the mutant mRNA and/or the mutant tRNA is selected from  and  .

8. The method of any one of the claims 1-7, wherein the unnatural nucleobase in the mutant mRNA is  .

9. The method of any one of the claims 1-8, wherein the unnatural nucleobase in the mutant tRNA is .

10. The method of any one of the claims 1-9, wherein the unnatural nucleotide further comprises an unnatural sugar moiety.

11. The method of claim 10, wherein the unnatural sugar moiety of the unnatural nucleotide is selected from the group consisting of a modification at the 2' position: OH; substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, $SO_2\,CH_3$, ONO_2, NO_2, N_3, NH_2F; O-alkyl, S-alkyl, N-alkyl; O-alkenyl, S- alkenyl, N-alkenyl; O-alkynyl, S-alkynyl, N-alkynyl; O-alkyl-O-alkyl, 2'-F, 2'-OCH_3, 2'-$O(CH_2)_2OCH_3$ wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1-C_{10}, alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, -O[(CH2)n O]mCH_3, -$O(CH_2)$nOCH_3, -$O(CH_2)$n NH_2, -$O(CH_2)$n CH_3, -$O(CH_2)$n -ONH_2, and -$O(CH_2)$n$ON[(CH_2)$n $CH_3)]_2$, where n and m are from 1 to about 10; and/or a modification at the 5' position: 5'-vinyl, 5'-methyl (R or S), a modification at the 4' position, 4'-S, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, and any combination thereof.

12. The method of any one of the claims 1-11, wherein the mutant anticodon or the mutant codon further comprises an unnatural backbone.

13. The method of any one of the claims 1-11, wherein the mutant anticodon and the mutant codon further comprise an unnatural backbone.

14. The method of any one of the claims 1-13, wherein an unnatural nucleotide is incorporated by an RNA polymerase into the mRNA during transcription to generate the mutant mRNA containing the mutant codon.

15. The method of any one of the claims 1-14, wherein an unnatural nucleotide is incorporated by an RNA polymerase into the tRNA during transcription to generate the mutant tRNA containing the mutant anticodon.

16. The method of any one of the claims 1-15, wherein the mutant tRNA is charged with an unnatural amino acid residue.

17. The method of any one of claims 1-16, wherein the unnatural amino acid is *p*-azido-phenylalanine (*p*AzF).

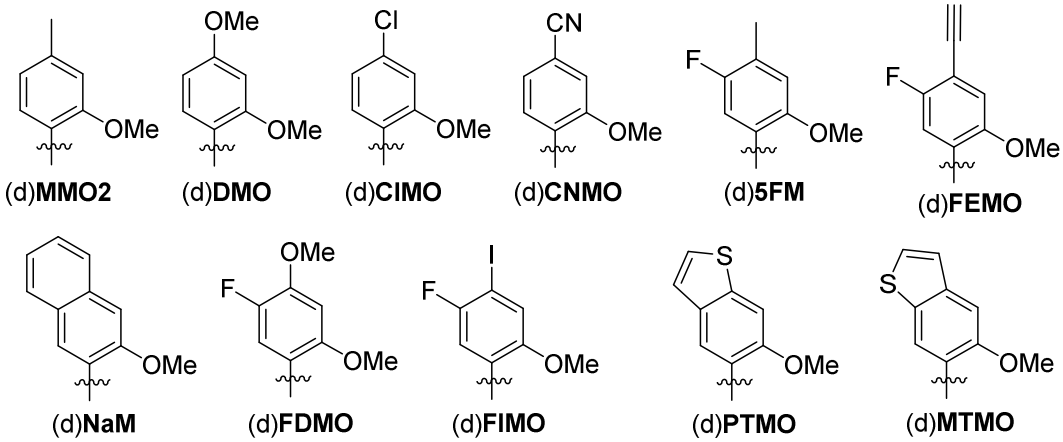
18. The method of any one of claims 1-16, wherein the unnatural amino acid is N^6-[(2-propynyloxy)carbonyl]-L-lysine (PrK).

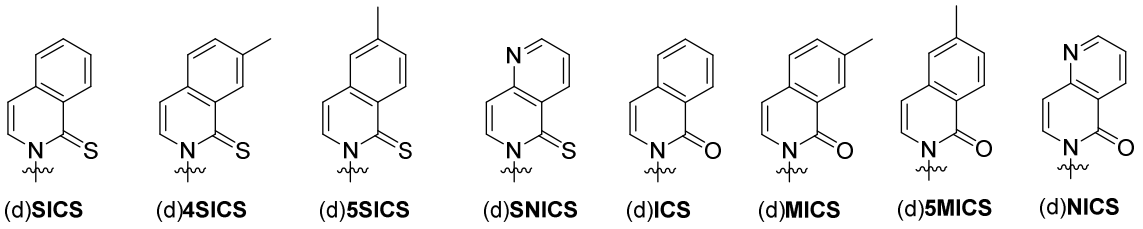
19. The method of any one of claims 1-18, wherein the mutant tRNA is a mutant of a tRNA from *Methanosarcina mazei*.

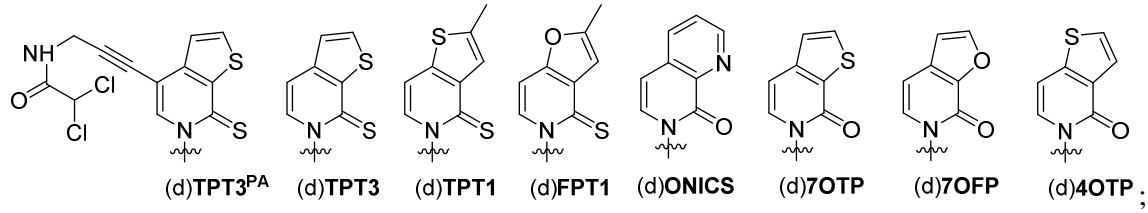
20. A cell comprising:

a. a nucleoside triphosphate transporter from *Phaeodactylum tricornutum*;

b. a mutant tRNA comprising an unnatural nucleobase selected from





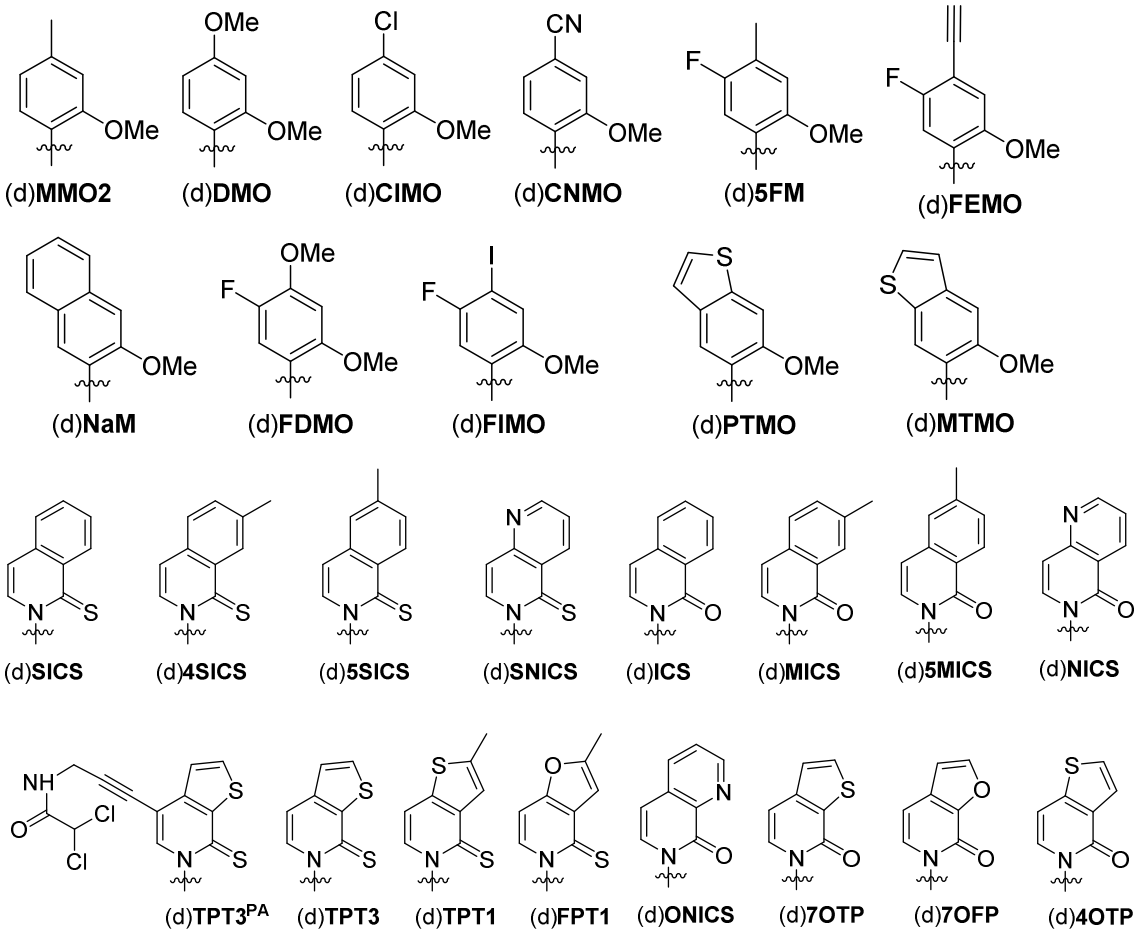


;

;

c. a pyrrolysine aminoacyl tRNA synthetase from *Methanosarcina barkeri* or *Methanococcus jannaschii*; and

d. an mRNA comprising an unnatural nucleobase selected from



.

21. The cell of claim 20, wherein the cell further comprises an oligonucleotide encoding the tRNA.

22. The cell of claim 20 or 21, wherein the cell further comprises an oligonucleotide encoding the pyrrolysine aminoacyl tRNA synthetase.

23. The cell of any one of claims 20-22, wherein the cell further comprises an oligonucleotide encoding the mRNA.

24. The cell of any one of claims 20-23, wherein the cell further comprises an oligonucleotide that encodes the tRNA and the mRNA.

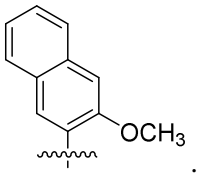
25. The cell of any one of claims 20-24, wherein the cell further comprises an oligonucleotide that encodes the tRNA, the mRNA, and the pyrrolysine aminoacyl tRNA synthetase.

26. The cell of any one of claims 20-25, wherein the tRNA comprises one or more anti-codons selected from GYT and GYC, wherein Y is the unnatural nucleobase.

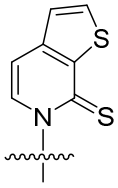
27. The cell of claim 26, wherein the tRNA comprises the anti-codon GYT.

28. The cell of claim 26, wherein the tRNA comprises the anti-codon GYC.

29. The cell of any one of claims 26-28, wherein the unnatural nucleobase Y is



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30. The cell of any one of claims 26-28, wherein the unnatural nucleobase Y is 

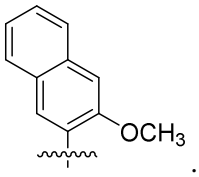
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31. The cell of any one of claims 20-30, wherein the mRNA comprises one or more codons selected from AXC and GXC, wherein X is the unnatural nucleobase.

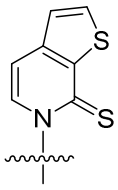
32. The cell of claim 31, wherein the mRNA comprises the codon AXC.

33. The cell of claim 31, wherein the mRNA comprises the codon GXC.

34. The cell of any one of claims 31-33, wherein the unnatural nucleobase X is

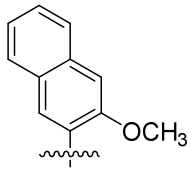
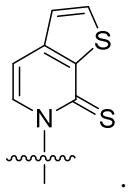


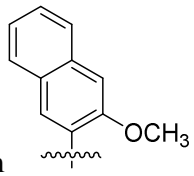
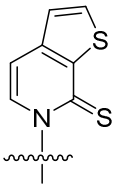
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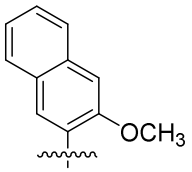
35. The cell of any one of claims 31-33, wherein the unnatural nucleobase X is 

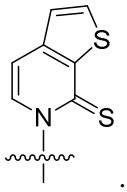
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36. The cell of any one of claims 20-28 and 30-34, wherein the mRNA comprises the

unnatural nucleobase , and the tRNA comprises the unnatural nucleobase .

37. The cell of any one of claims 20-25, wherein the unnatural nucleobase in the mutant mRNA and/or the mutant tRNA is selected from  and .

38. The cell of claim 37, wherein the unnatural nucleobase in the mutant mRNA is .

39. The cell of claim 37 or 38, wherein the unnatural nucleobase in the mutant tRNA is .

40. The cell of any one of claims 20-39, wherein the pyrrolysine aminoacyl tRNA synthetase is from *Methanosarcina barkeri*.

41. The cell of any one of claims 20-39, wherein the pyrrolysine aminoacyl tRNA synthetase is from *Methanococcus jannaschii*.

42. The cell of any one of claims 20-41, wherein the mutant tRNA is a mutant of a tRNA from *Methanosarcina mazei*.

43. The cell of any one of claims 20-42, wherein the cell is a microorganism.

44. The cell of any one of claims 20-42, wherein the cell is a bacterium.

45. The cell of any one of claims 20-42, wherein the cell is an *Escherichia coli*.

46. A method of producing a protein in a cell, wherein the protein comprises an unnatural

amino acid, the method comprising:

transporting an unnatural nucleotide into the cell of any one of claims 20-45 using the nucleoside triphosphate transporter; and

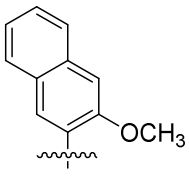
providing the unnatural amino acid to the cell; wherein

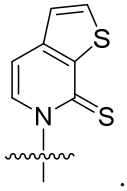
the unnatural nucleotide is incorporated by an RNA polymerase during transcription of a double-stranded oligonucleotide comprising an unnatural oligonucleotide to generate the mutant mRNA and the mutant tRNA; and

the protein containing the unnatural amino acid is generated during translation of the mutant mRNA utilizing the mutant tRNA.

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47. The method of claim 46, wherein the unnatural nucleotide transported into the cell comprises an unnatural nucleobase.

48. The method of claim 47, wherein the unnatural nucleobase is 

or 

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49. The method of any one of claims 46-48, wherein the unnatural amino acid is *p*-azido-phenylalanine (*p*AzF).

50. The method of any one of claims 46-48, wherein the unnatural amino acid is N^6-[(2-propynyloxy)carbonyl]-L-lysine (PrK).