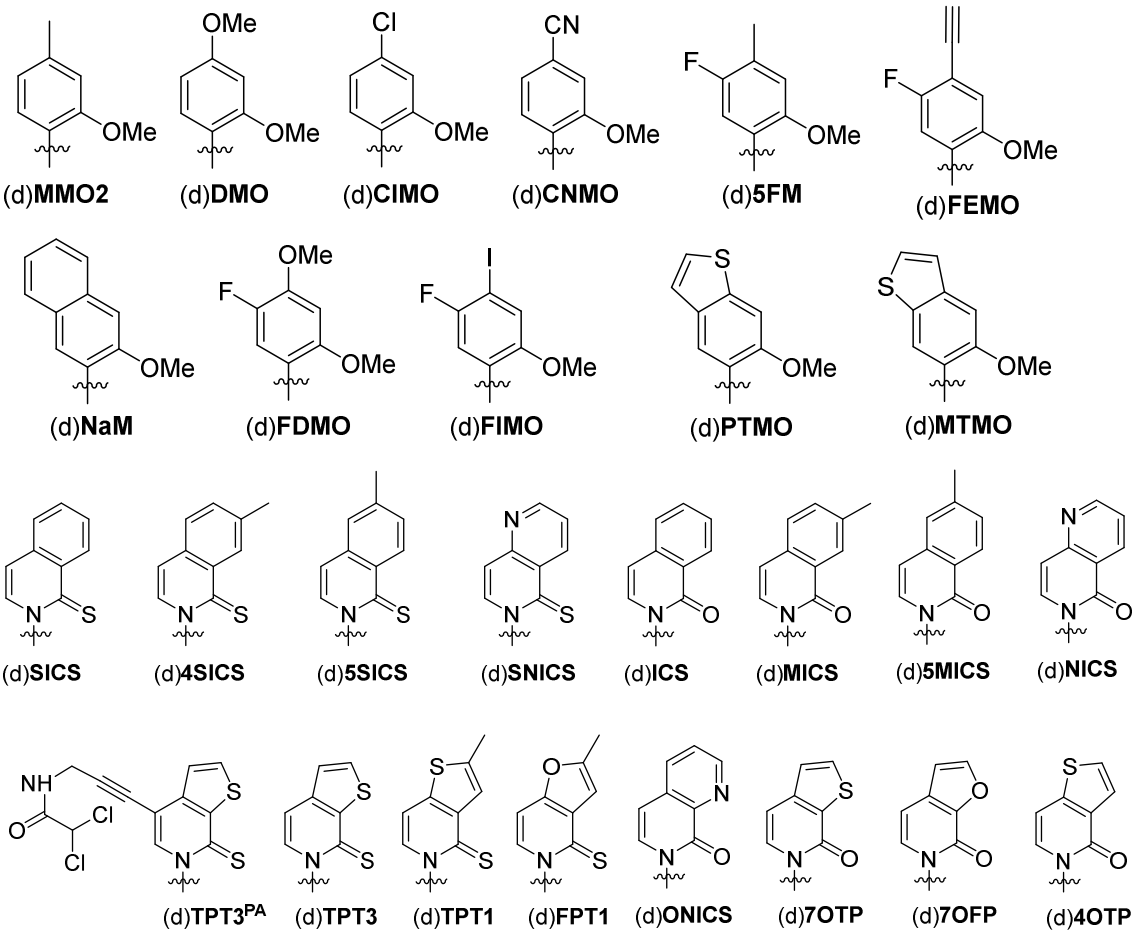
alkynyl, S-alkynyl, N-alkynyl; O-alkyl-O-alkyl, 2'-F, 2'-OCH_3, 2'-$O(CH_2)_2OCH_3$ wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1-C_{10}, alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, -$O[(CH2)_nO]_mCH_3$, -$O(CH_2)_nOCH_3$, -$O(CH_2)_nNH_2$, -$O(CH_2)_nCH_3$, -$O(CH_2)_n$ -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.

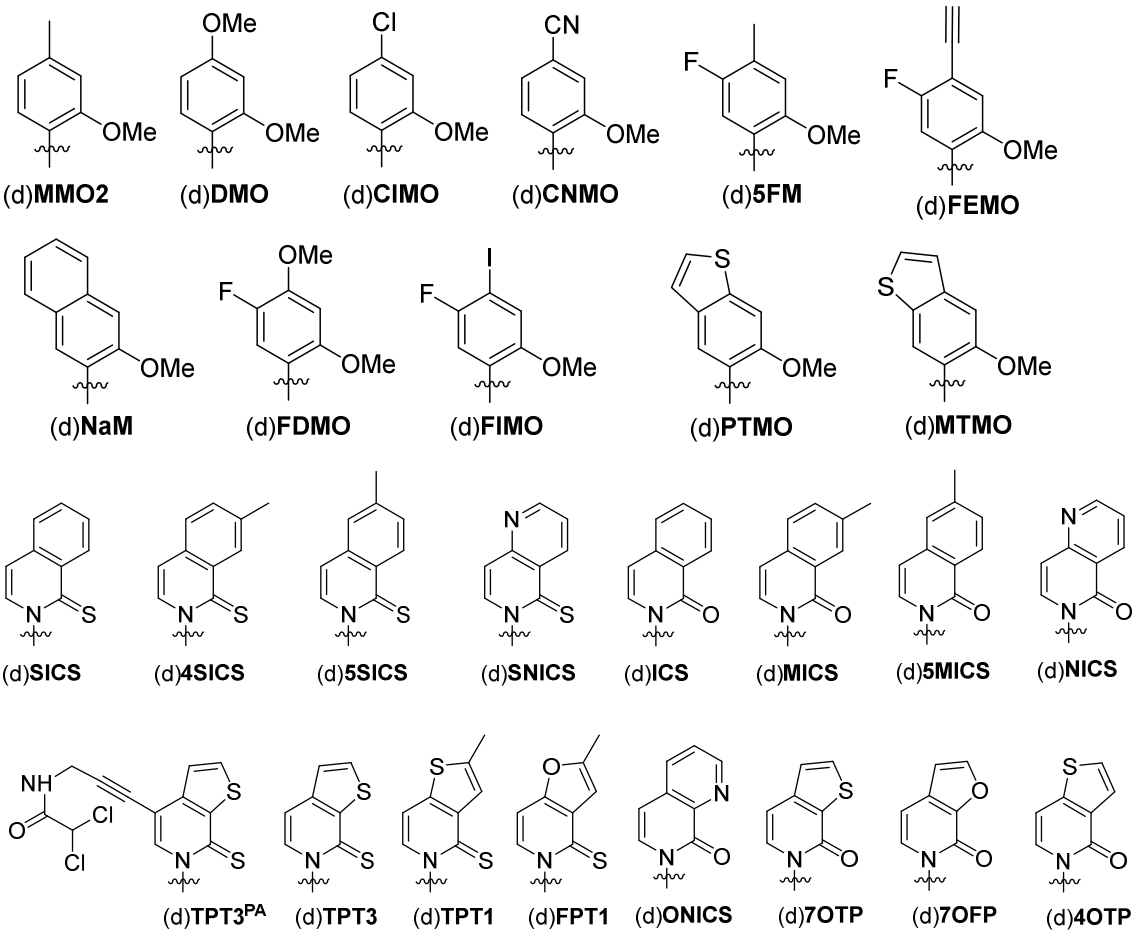
[0007a] In one aspect, provided herein is a method of producing a protein containing an unnatural amino acid, the method comprising:

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



, wherein the X or Y in the mutant anticodon sequence selected from Table 1 or 2 represents the first unnatural nucleotide;

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



, wherein the X or Y in the mutant codon sequence selected from Table 1 or 2 represents the second unnatural nucleotide;

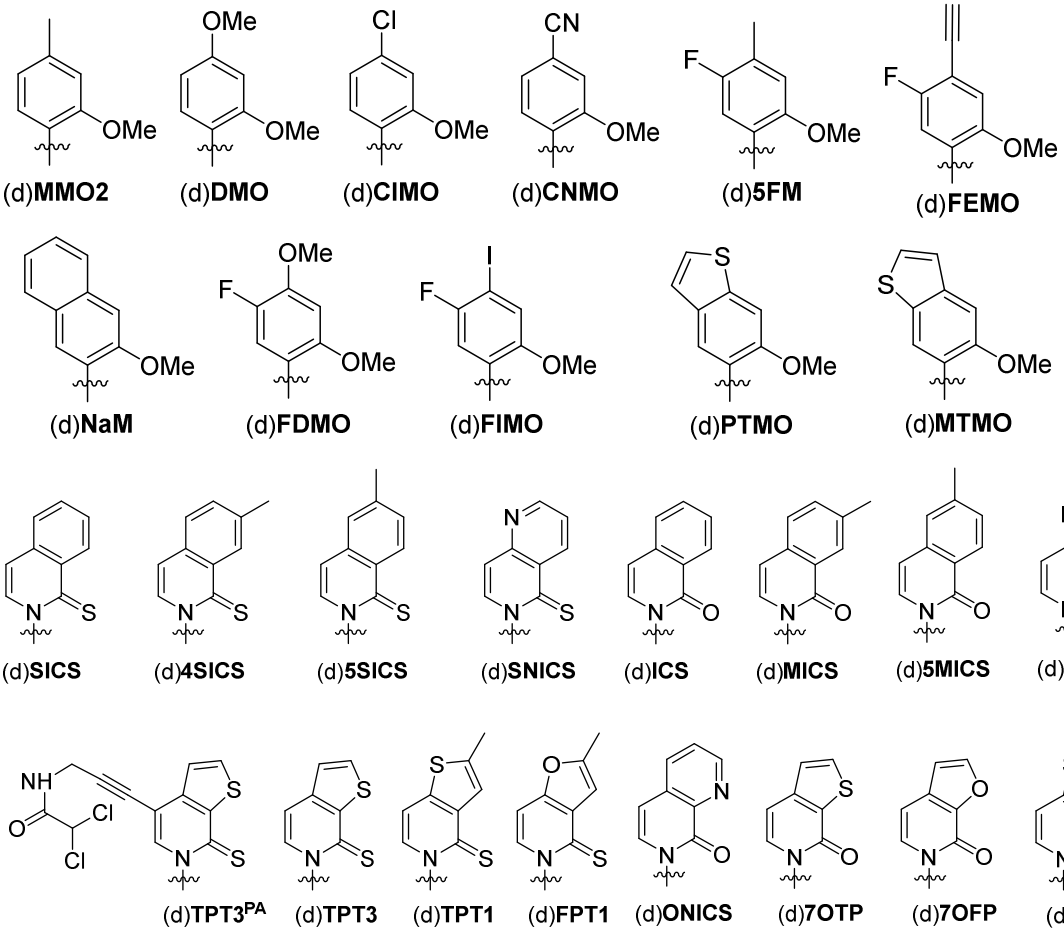
and

- 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), wherein the SSO comprises a bacterium.

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

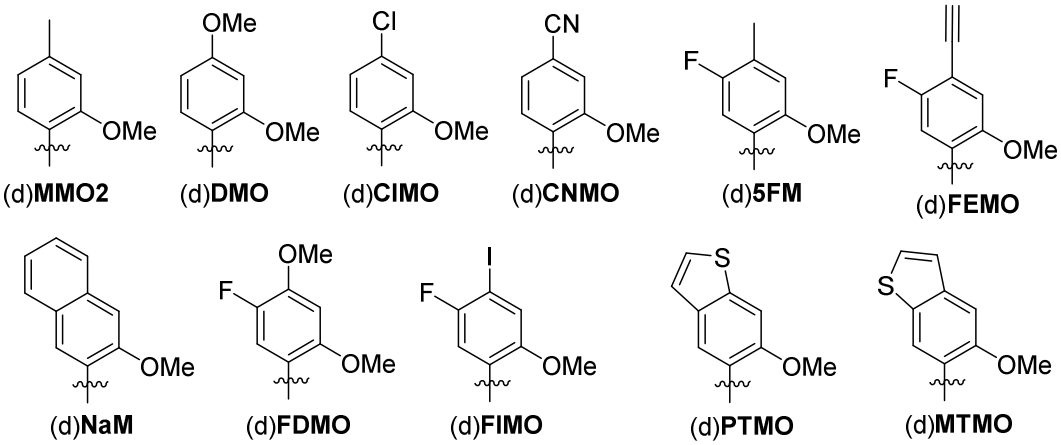
a. a mutant tRNA comprising a mutant anticodon sequence, wherein the anticodon sequence comprises a first unnatural nucleobase selected from

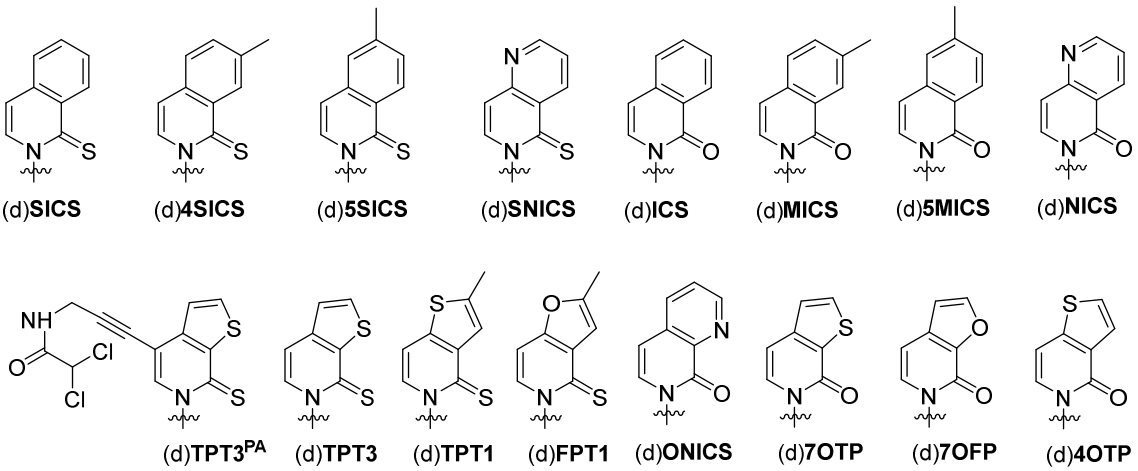


;

and

b. a mutant mRNA comprising a mutant codon, into which an unnatural nucleotide has been incorporated, wherein the unnatural nucleotide comprises a second unnatural nucleobase selected from





, wherein the cell is a bacterium.

[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.

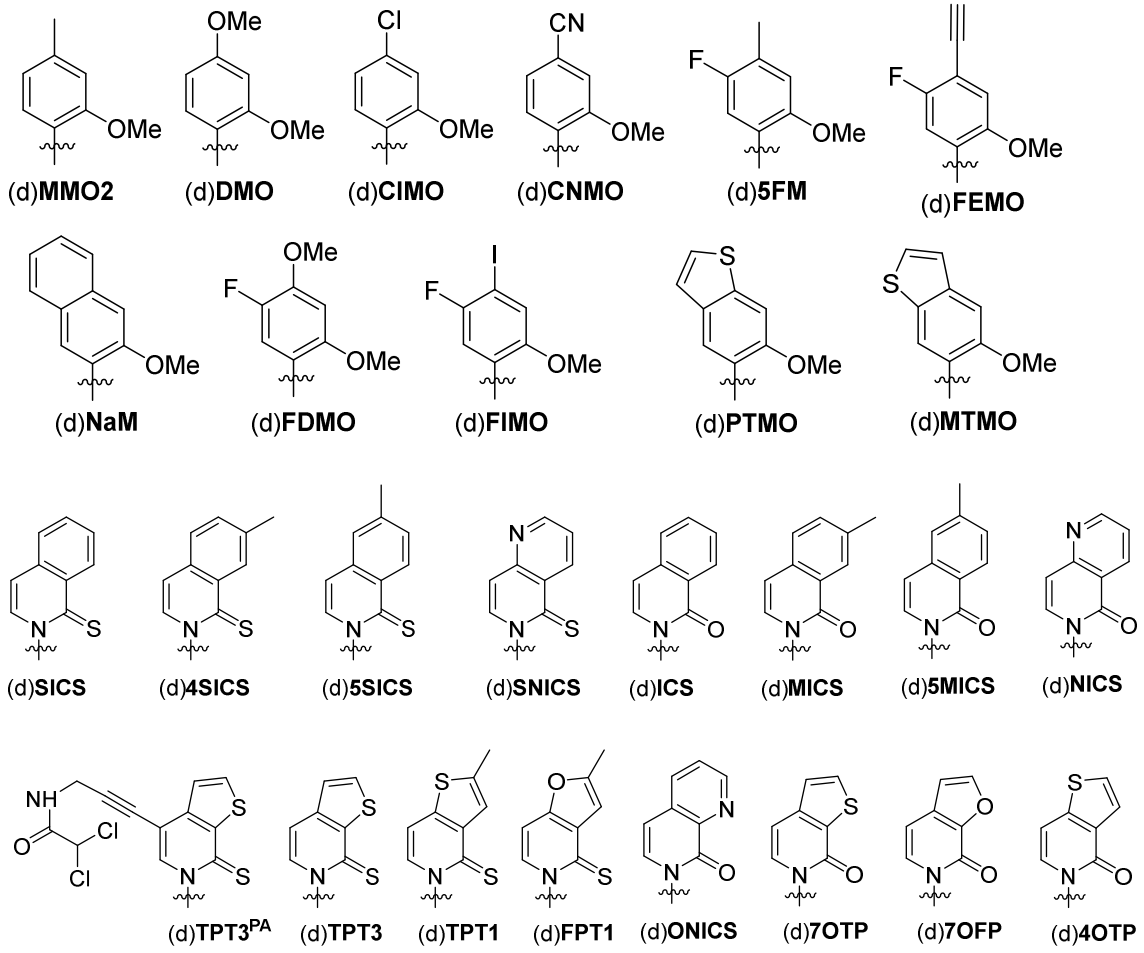
[0010] Fig. 1B illustrates the gene cassette used to express sfGFP(A**X**C)^{151} and tRNA(G**Y**T)^{Ser}. P_{T7} and T_{T7} denote the T7 RNAP promoter and terminator, respectively. In controls where sfGFP is expressed in the absence of *serT*, the sequence following the sfGFP T7 terminator is absent.

CLAIMS

What is claimed is:

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

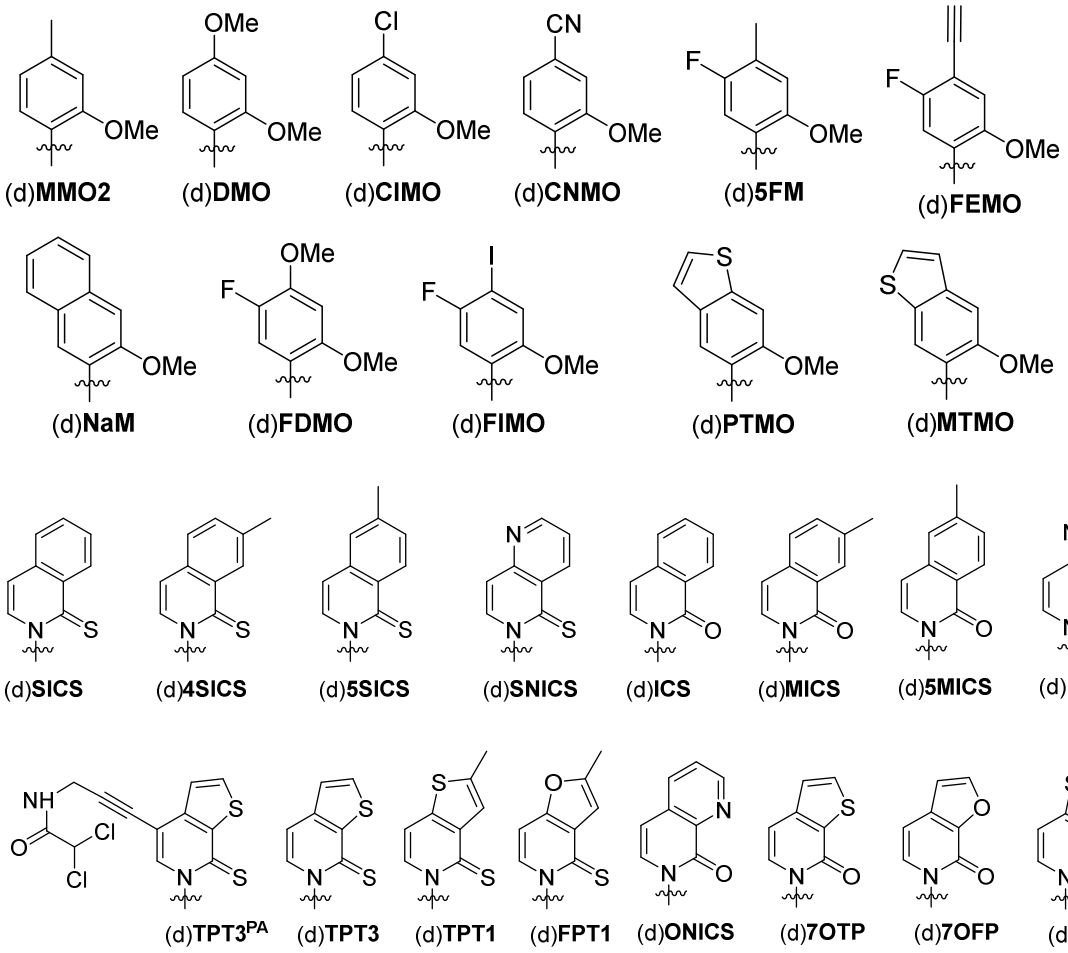
    - preparing a mutant tRNA wherein the mutant tRNA comprises a mutant anticodon sequence selected from Table 1 or 2 that comprises a first unnatural nucleotide comprising a first unnatural nucleobase selected from



,

wherein the X or Y in the mutant anticodon sequence selected from Table 1 or 2 represents the first unnatural nucleotide;

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



,

wherein the X or Y in the mutant codon sequence selected from Table 1 or 2 represents the second unnatural nucleotide;

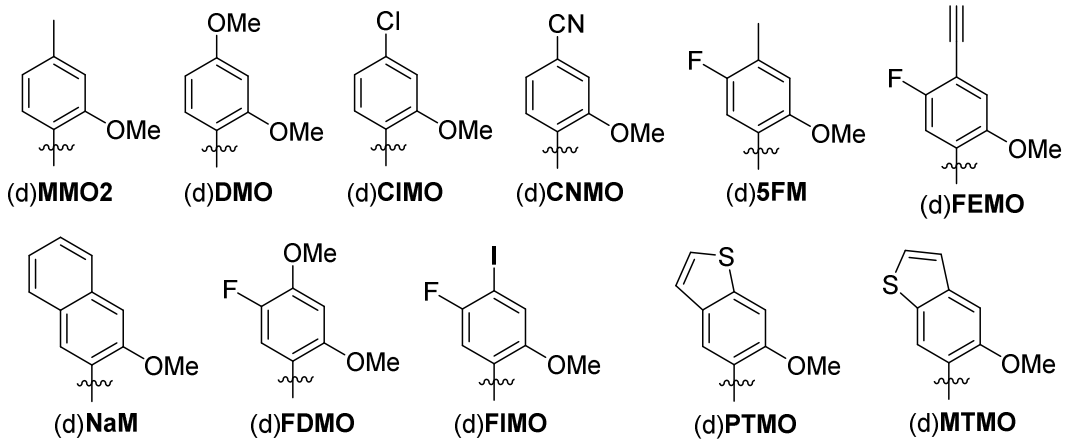
and

- 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), wherein the SSO comprises a bacterium.

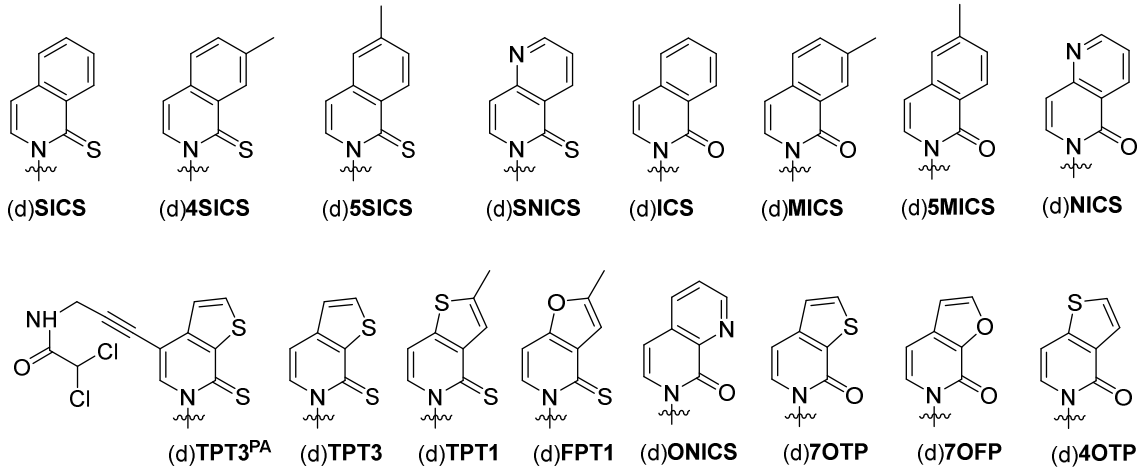
2. The method of claim 1, wherein the semi-synthetic organism comprises an *Escherichia coli*.

3. The method of any one of the claims 1-2, wherein the first or second unnatural nucleobase is selected from



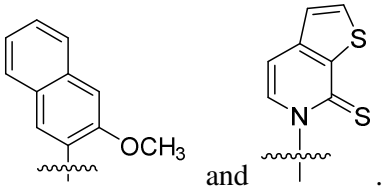
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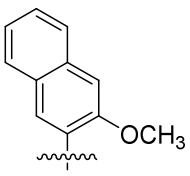
4. The method of any one of the claims 1-2, wherein the first or second unnatural nucleobase is selected from

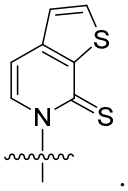


.

5. The method of any one of the claims 1-3, wherein the second unnatural nucleobase in the mutant mRNA and/or the first unnatural nucleobase in the mutant tRNA is selected from

 and .

6. The method of any one of the claims 1-5, wherein the second unnatural nucleobase in the mutant mRNA is .

7. The method of any one of the claims 1-6, wherein the first unnatural nucleobase in the mutant tRNA is  .

8. The method of any one of the claims 1-7, wherein the first or second unnatural nucleotide further comprises an unnatural sugar moiety.

9. The method of claim 8, wherein the unnatural sugar moiety of the first or second 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_2CH_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 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.

10. The method of any one of the claims 1-9, wherein the mutant anticodon or the mutant codon further comprises an unnatural backbone.

11. The method of any one of the claims 1-9, wherein the mutant anticodon and the mutant codon further comprise an unnatural backbone.

12. The method of any one of the claims 1-11, wherein a second unnatural nucleotide is incorporated by an RNA polymerase into the mRNA during transcription to generate the mutant mRNA containing the mutant codon.

13. The method of any one of the claims 1-12, wherein a first unnatural nucleotide is incorporated by an RNA polymerase into the tRNA during transcription to generate the mutant tRNA containing the mutant anticodon.

14. The method of any one of the claims 1-13, wherein the mutant tRNA is charged with an unnatural amino acid residue.

15. The method of any one of claims 1-14, wherein the unnatural amino acid is *p*-azido-phenylalanine (*p*AzF).

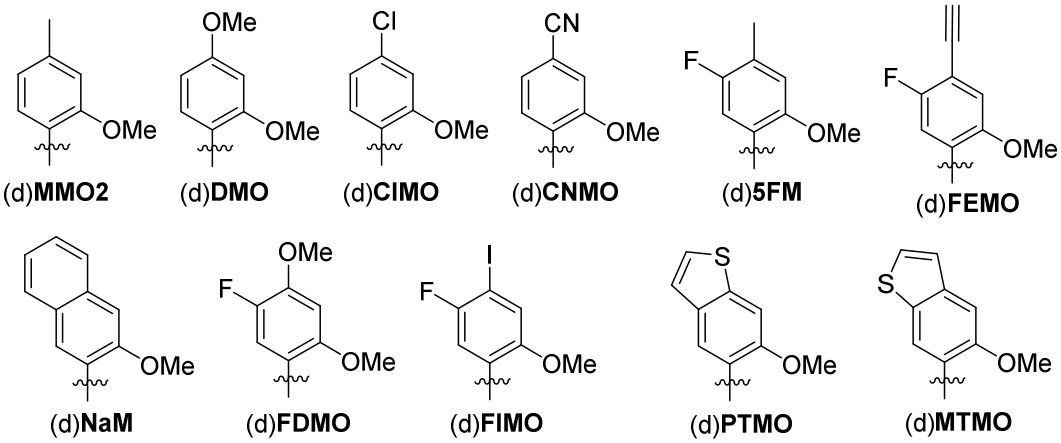
16. The method of any one of claims 1-14, wherein the unnatural amino acid is N^6-[(2-

propynyloxy)carbonyl]-L-lysine (PrK).

17. The method of any one of claims 1-16, wherein the mutant tRNA is a mutant of a tRNA from *Methanosarcina mazei*.

18. A cell comprising:

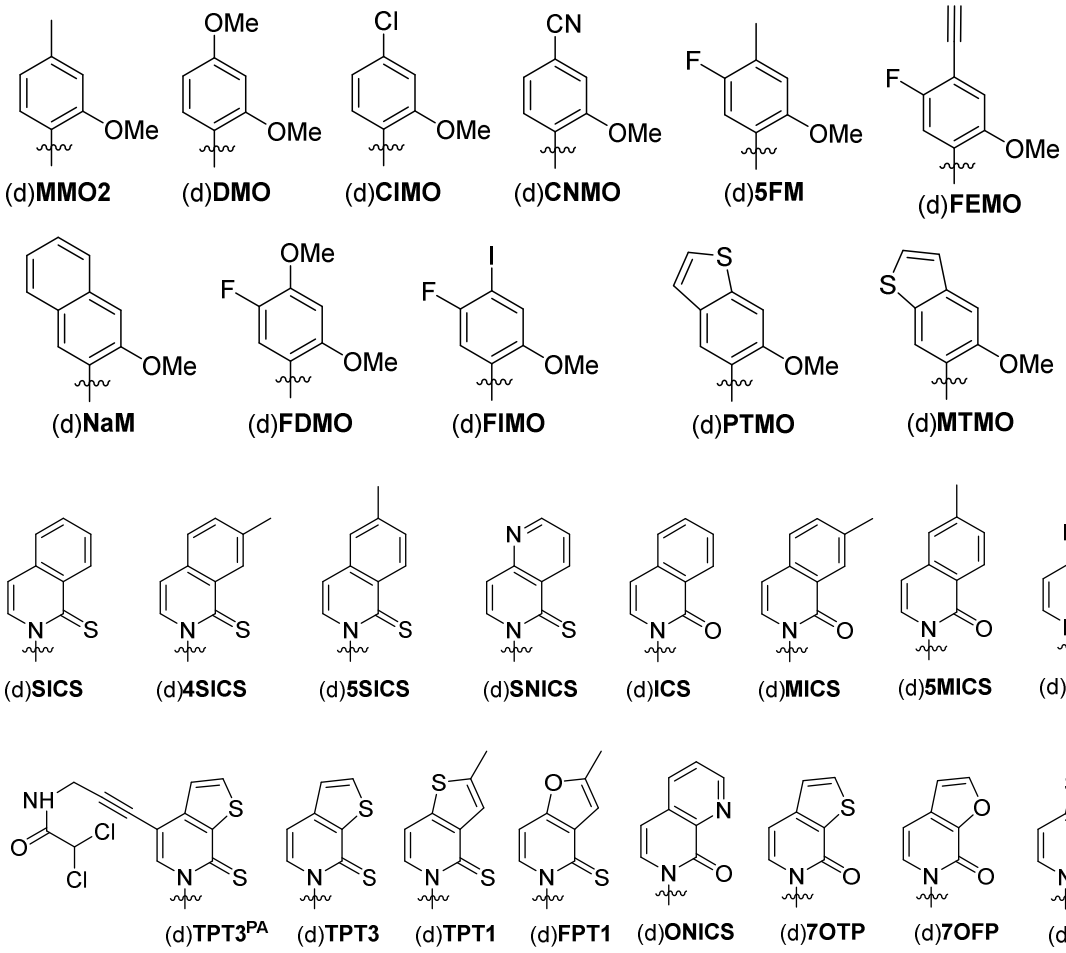
a. a mutant tRNA comprising a mutant anticodon sequence, wherein the anticodon sequence comprises a first unnatural nucleobase selected from



;

and

b. a mutant mRNA comprising a mutant codon, into which an unnatural nucleotide has been incorporated, wherein the unnatural nucleotide comprises a second unnatural nucleobase selected from



,

wherein the cell is a bacterium.

19. The cell of claim 18, wherein the cell further comprises an oligonucleotide encoding the tRNA.

20. The cell of claim 18 or 19, wherein the cell further comprises an oligonucleotide encoding a pyrrolysine aminoacyl tRNA synthetase.

21. The cell of any one of claims 18-20, wherein the cell further comprises an oligonucleotide encoding the mRNA.

22. The cell of claim 18 or 20, wherein the cell further comprises an oligonucleotide that encodes the tRNA and the mRNA.

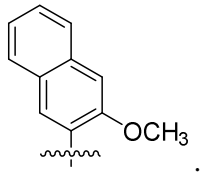
23. The cell of claim 18, wherein the cell further comprises an oligonucleotide that encodes the tRNA, the mRNA, and a pyrrolysine aminoacyl tRNA synthetase.

24. The cell of any one of claims 18-23, wherein the tRNA comprises one or more anti-codons selected from GYT and GYC, wherein Y is the first unnatural nucleobase.

25. The cell of claim 24, wherein the tRNA comprises the anti-codon GYT.

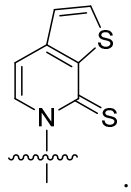
26. The cell of claim 24, wherein the tRNA comprises the anti-codon GYC.

27. The cell of any one of claims 24-26, wherein the first unnatural nucleobase Y is



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28. The cell of any one of claims 24-26, wherein the first unnatural nucleobase Y is



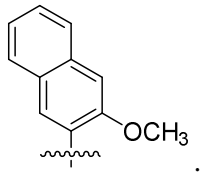
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29. The cell of any one of claims 18-28, wherein the mRNA comprises one or more codons selected from AXC and GXC, wherein X is the second unnatural nucleobase.

30. The cell of claim 29, wherein the mRNA comprises the codon AXC.

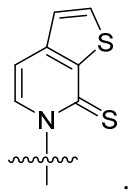
31. The cell of claim 29, wherein the mRNA comprises the codon GXC.

32. The cell of any one of claims 29-31, wherein the second unnatural nucleobase X is

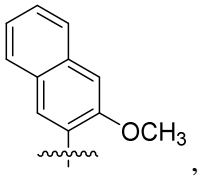
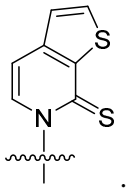


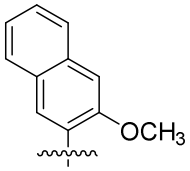
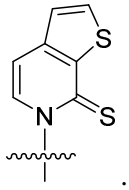
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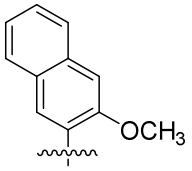
33. The cell of any one of claims 29-31, wherein the second unnatural nucleobase X is

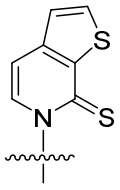


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34. The cell of any one of claims 18-26 and 28-32, wherein the mRNA comprises the second unnatural nucleobase , and the tRNA comprises the first unnatural nucleobase .

35. The cell of any one of claims 18-23, wherein the second unnatural nucleobase in the mutant mRNA and/or the first unnatural nucleobase in the mutant tRNA is selected from  and .

36. The cell of claim 35, wherein the second unnatural nucleobase in the mutant mRNA is .

37. The cell of claim 35 or 36, wherein the first unnatural nucleobase in the mutant tRNA is .

38. The cell of any one of claims 18-37, wherein the pyrrolysine aminoacyl tRNA synthetase is from *Methanosarcina barkeri*.

39. The cell of any one of claims 18-37, wherein the pyrrolysine aminoacyl tRNA synthetase is from *Methanococcus jannaschii*.

40. The cell of any one of claims 18-39, wherein the mutant tRNA is a mutant of a tRNA from *Methanosarcina mazei*.

41. The cell of any one of claims 18-40, wherein the cell is a microorganism.

42. The cell of any one of claims 18-40, wherein the cell is an *Escherichia coli*.

43. A method of producing a protein in a cell, wherein the protein comprises an unnatural amino acid, the method comprising:

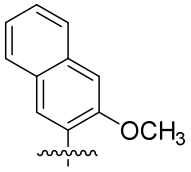
transporting an unnatural nucleotide into the cell of any one of claims 18-42 using a nucleoside triphosphate transporter; and

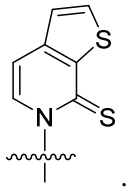
providing the unnatural amino acid to the cell; wherein

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.

44. The method of claim 43, wherein the unnatural nucleotide transported into the cell comprises an unnatural nucleobase.

45. The method of claim 44, wherein the unnatural nucleobase is 

or 

.

46. The method of any one of claims 43-45, wherein the unnatural amino acid is *p*-azido-phenylalanine (*p*AzF).

47. The method of any one of claims 43-45, wherein the unnatural amino acid is N^6-[(2-propynyloxy)carbonyl]-L-lysine (PrK).