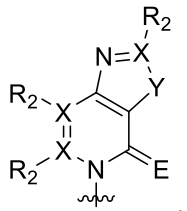
CLAIMS

WHAT IS CLAIMED IS:

1. A nucleobase of the structure:



,

wherein:

each X is independently carbon or nitrogen;

R_2 is present when X is carbon and is independently hydrogen, alkyl, alkenyl, alkynyl, methoxy, methanethiol, methaneseleno, halogen, cyano, or azide group;

Y is sulfur, oxygen, or selenium; and

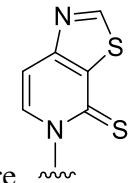
E is oxygen, sulfur, or selenium;

wherein the wavy line represents attachment of the nucleobase to a ribosyl, deoxyribosyl, or dideoxyribosyl moiety, wherein the ribosyl, deoxyribosyl, or dideoxyribosyl moiety is in free form, is connected to a mono-phosphate, diphosphate, triphosphate, α-thiotriphosphate, β-thiotriphosphate, or γ-thiotriphosphate group, or is included in a polynucleotide.

2. The nucleobase of claim 1, wherein X is carbon.

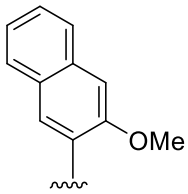
3. The nucleobase of claim 1 or 2, wherein E is sulfur.

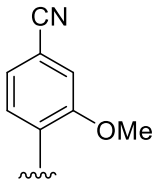
4. The nucleobase of any one of claims 1 to 3, wherein Y is sulfur.

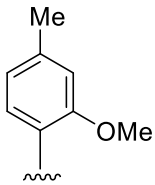
5. The nucleobase of claim 1, which has the structure .

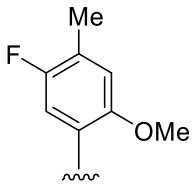
6. An unnatural base pair comprising the nucleobase of any one of claims 1 to 5.

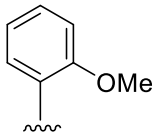
7. The unnatural base pair of claim 6, further comprising a complementary base-pairing nucleobase selected from:

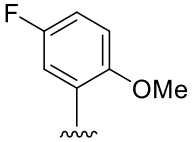


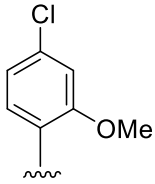
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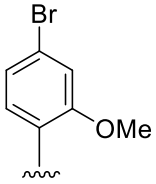
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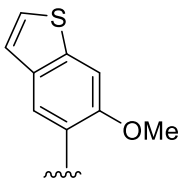
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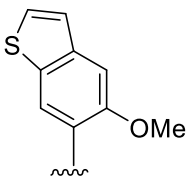
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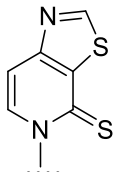
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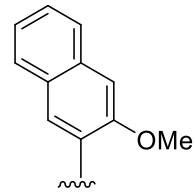
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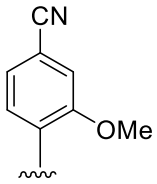
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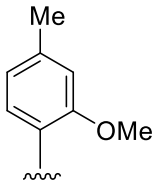
8. A double stranded oligonucleotide comprising a first oligonucleotide strand that comprises the nucleobase of any one of claims 1 to 5, and a second oligonucleotide strand that is complementary to the first oligonucleotide strand, wherein the second oligonucleotide strand comprises a complementary base-pairing nucleobase in a complementary base-pairing site thereof.

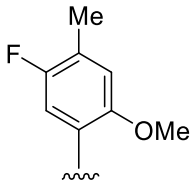
9. The double stranded oligonucleotide of claim 8, wherein the first oligonucleotide strand comprises 

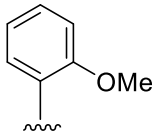
; and the second oligonucleotide strand comprises a complementary base pairing nucleobase selected from:

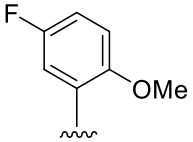


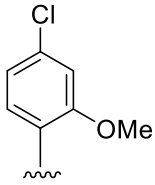
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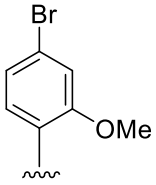
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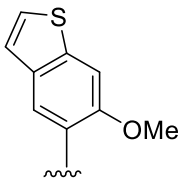
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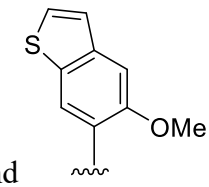
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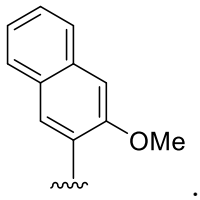
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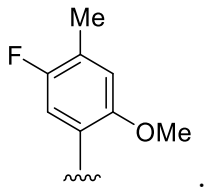
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, and


in a complementary base-pairing site thereof.

10. The double stranded oligonucleotide of claim 9, wherein the second oligonucleotide strand comprises the complementary base pairing nucleobase 

.

11. The double stranded oligonucleotide of claim 9, wherein the second oligonucleotide strand comprises the complementary base pairing nucleobase 

.

12. A plasmid comprising a gene encoding a transfer RNA (tRNA) and/or a gene encoding a protein of interest, wherein the gene comprises the unnatural base pair of claim 7.

13. An mRNA comprising the nucleobase of any one of claims 1 to 5, or comprising a codon, the codon comprising the nucleobase of any one of claims 1 to 5.

14. A tRNA comprising the nucleobase of any one of claims 1 to 5.

15. A transfer RNA (tRNA) comprising:

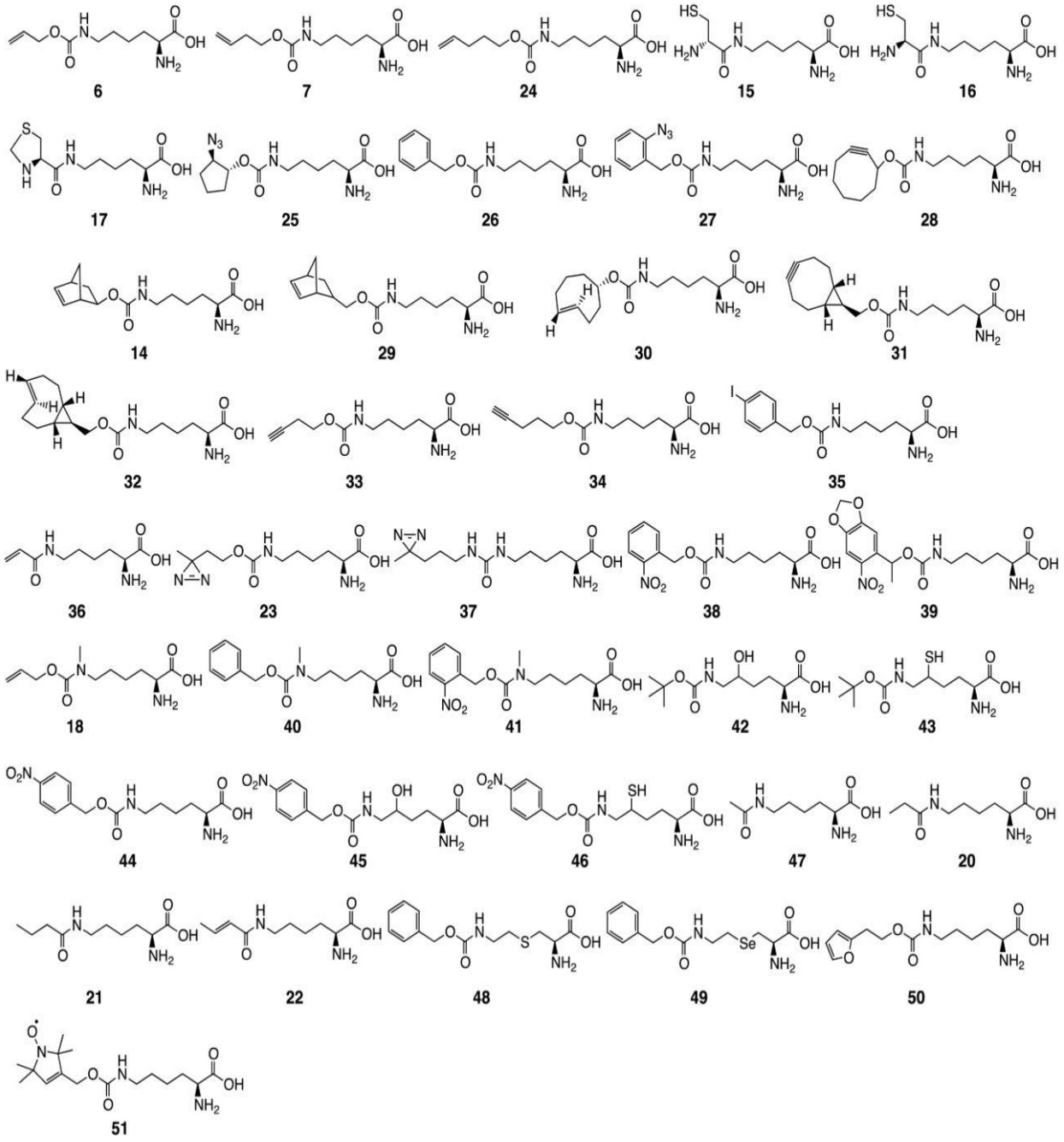
an anticodon comprising the nucleobase of any one of claims 1 to 5, wherein the anticodon is capable of pairing with an unnatural codon that encodes an unnatural amino acid; and

a recognition element, wherein the recognition element promotes selective charging of the tRNA with the unnatural amino acid by an aminoacyl tRNA synthetase.

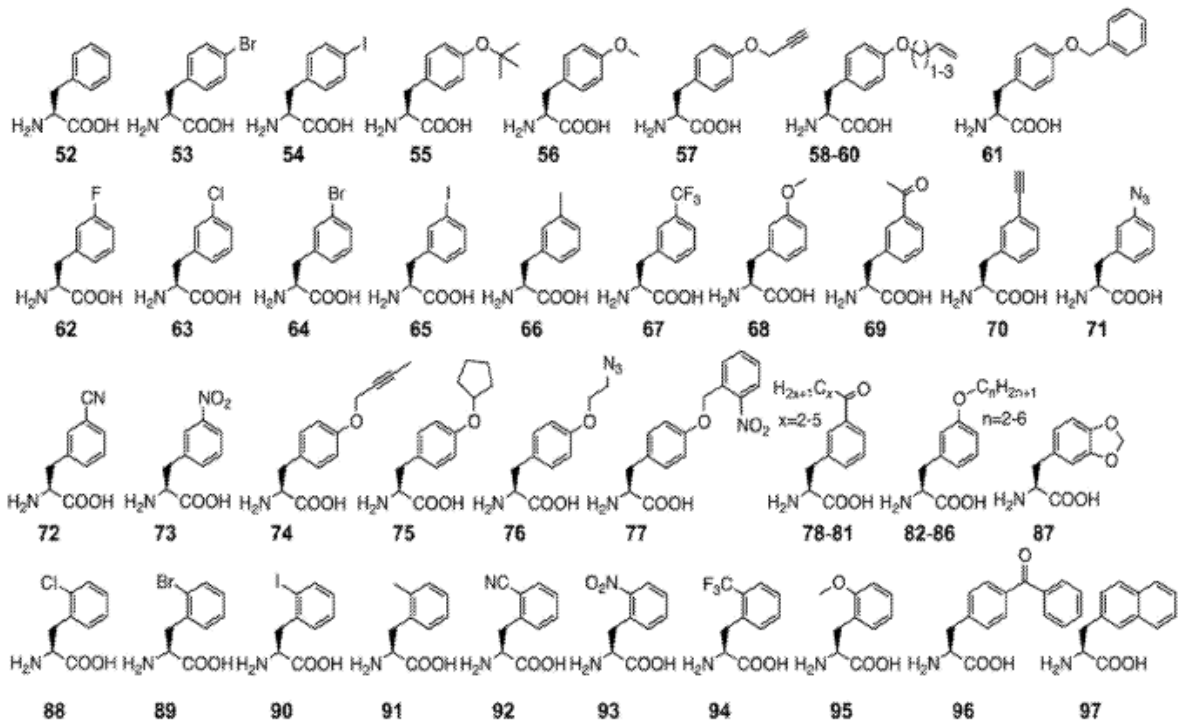
16. The tRNA of claim 15, wherein the aminoacyl tRNA synthetase is derived from *Methanosarcina*, or a variant thereof, or *Methanococcus* or a variant thereof.

17. The tRNA of claim 15, wherein the unnatural amino acid comprises an aromatic moiety.

18. The tRNA of claim 15, wherein the unnatural amino acid is a lysine derivative selected from the following structures:



or a phenylalanine derivative selected from the following structures:



19. A structure comprising the formula:

N1 - Zx - N2

wherein:

each Z is independently a nucleobase of any one of claims 1 to 5, which is bonded to a ribosyl or deoxyribosyl;

N1 is one or more nucleotides, or a terminal phosphate group attached at the 5’ end of the ribosyl or deoxyribosyl of Z;

N2 is one or more nucleotides, or a terminal hydroxyl group attached to the 3’ end of the ribosyl or deoxyribosyl of Z; and

x is an integer from 1 to 20.

20. The structure of claim 19, wherein the structure encodes a gene.

21. The structure of claim 20, wherein Zx is located in a translated region of the gene.

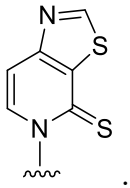
22. The structure of claim 20, wherein Zx is located in an untranslated region of the gene.

23. The structure of any one of claims 19-22, wherein x is 1.

24. A polynucleotide library, wherein the library comprises at least 100 unique polynucleotides, and wherein each polynucleotide comprises at least one nucleobase of any one of claims 1 to 5.

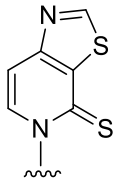
25. The polynucleotide library of claim 24, wherein the library comprises at least 5000 unique polynucleotides, and wherein each polynucleotide comprises at least one nucleobase of any one of claims 1 to 5.

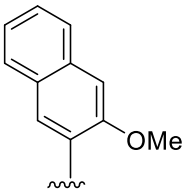
26. A nucleoside triphosphate comprising a nucleobase, wherein the nucleobase is selected from a nucleobase of any one of claims 1 to 4.



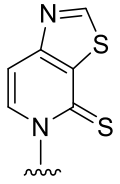
27. The nucleoside triphosphate of claim 26, wherein the nucleobase is .

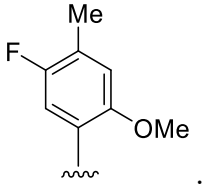
28. The nucleoside triphosphate of claim 26 or 27, wherein the nucleoside of the nucleoside triphosphate comprises ribose or deoxyribose.

29. A DNA comprising an unnatural base pair comprising (i) a first nucleobase having the structure 

, and (ii) a second nucleobase having the structure 

.

30. A DNA comprising an unnatural base pair comprising a first nucleobase having the structure 

, and a second nucleobase having the structure 

.

31. The nucleobase of any one of claims 1-5, wherein the polynucleotide is a bicyclic nucleic acid, a linked nucleic acid, a peptide nucleic acid (PNA), a locked nucleic acid (LNA), or a phosphorothioate-containing nucleic acid.

32. The nucleobase of any one of claims 1-5, wherein the polynucleotide is an RNA.

33. The nucleobase of any one of claims 1-5, wherein the polynucleotide is a DNA.