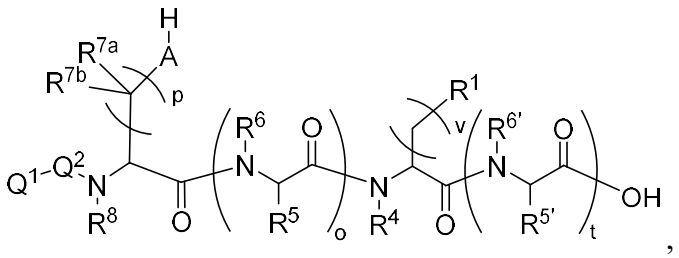
[0006] PapB is a RiPP maturase that catalyzes the insertion of six thioether crosslinks in the PapA polypeptide. PapB catalyzes the insertion of links between the Cys thiol and the b-carbon of the Asp, where the residues being linked are in a CX_3D motif. Prior studies have shown that the enzyme can also accept Glu at the modification site, and that PapB introduces the crosslink to the chemically analogous γ-carbon. In addition, PapB has also been shown to accept a shorter minimal substrate (msPapA), which only has a single pair of crosslinking amino acids in the CX_3D motif. PapB can catalyze both Cß and Cγ thioether linkages, and forms six thioether linkages in the wild type PapA. PapB contains a RS cluster and two ACs (**FIG. 2**). Replacing Asp residue(s) to Glu residue(s) in WT-PapA still results in successful crosslinking. Both Cß and Cy thioether linkages were confirmed by 2D NMR.

[0007] Despite the emergence of various techniques in peptide-based therapeutics, there remains a need in the art for enzymatic systems for rapid and highly specific modification of a broad range of peptide substances to obtain natural products that are unattainable by traditional synthetic chemistry methods. These needs and others are addressed herein.

SUMMARY

[0008] In accordance with the purpose(s) of the disclosure, as embodied and broadly described herein, the disclosure, in one example, relates to methods of chemically modifying a peptide sequence to install one or more thioether linkages. Additionally disclosed are compounds formed using methods of chemically modifying a peptide sequence. Also disclosed are methods of chemically modifying a modified PapA sequence, and compounds formed using methods of chemically modifying a modified PapA sequence.

[0008a] In one aspect, disclosed herein is a method of chemically modifying a compound to install a thioether linkage, the method comprising reacting the compound with PapB, wherein the compound has a structure represented by a formula:



,

wherein o is 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9;

wherein p is 1 or 2;

wherein t is an integer from 0 to 500;

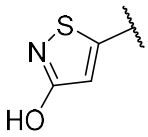
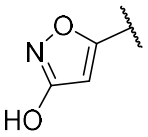
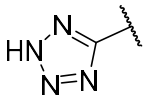
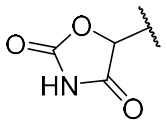
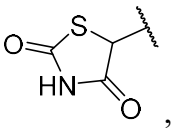
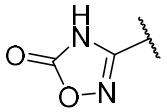
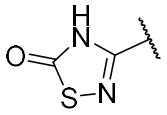
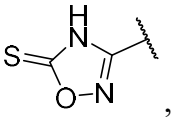
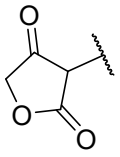
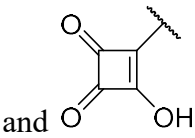
wherein v is 1, 2, 3, 4, or 5;

wherein A is S or Se;

wherein Q^1 is a leader sequence;

wherein Q^2 is a cleavable moiety;

wherein R^1 is selected from $–CO_2H$, –C(O)NHOH, $–SO_2NH_2$, $–SO_2NHC(O)CH_3$, $–SO_3H$, $–NHC(O)NHSO_2CH_3$, $–P(O)(OH)_2$, and a structure selected from:


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and ;

wherein R^4 is selected from hydrogen and methyl;

wherein each occurrence of R^5 and $R^{5'}$, when present, is independently a residue of a side chain of an amino acid;

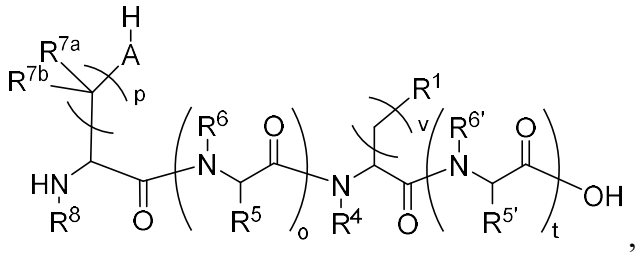
wherein each occurrence of R^6 and $R^{6'}$, when present, is independently selected from hydrogen and methyl, or wherein R^6 or $R^{6'}$ is covalently bonded to R^5 or $R^{5'}$, respectively, and, together with the intermediate atoms, comprise an unsubstituted 5-membered heterocycle;

wherein each of R^{7a} and R^{7b}, when present, is independently selected from hydrogen and C1-C4 alkyl; and

wherein R^8 is selected from hydrogen and methyl,

provided that the compound is not PapA.

[0008b] In another aspect, disclosed herein is a method of chemically modifying a compound to install a thioether linkage, the method comprising reacting the compound with PapB, wherein the compound has a structure represented by a formula:



wherein o is 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9;

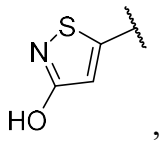
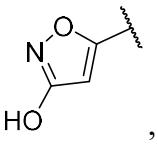
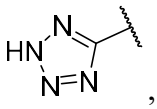
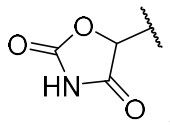
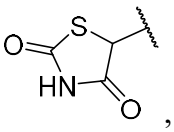
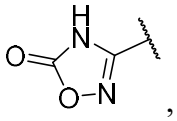
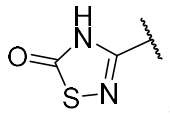
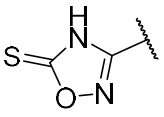
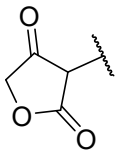
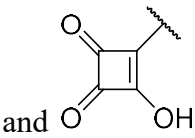
wherein p is 1 or 2;

wherein t is an integer from 0 to 500;

wherein v is 1, 2, 3, 4, or 5;

wherein A is S or Se;

wherein R^1 is selected from $–CO_2H$, –C(O)NHOH, $–SO_2NH_2$, $–SO_2NHC(O)CH_3$, $–SO_3H$, $–NHC(O)NHSO_2CH_3$, $–P(O)(OH)_2$, and a structure selected from:


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and

wherein R^4 is selected from hydrogen and methyl;

wherein each occurrence of R^5 and $R^{5'}$, when present, is independently a residue of a side chain of an amino acid;

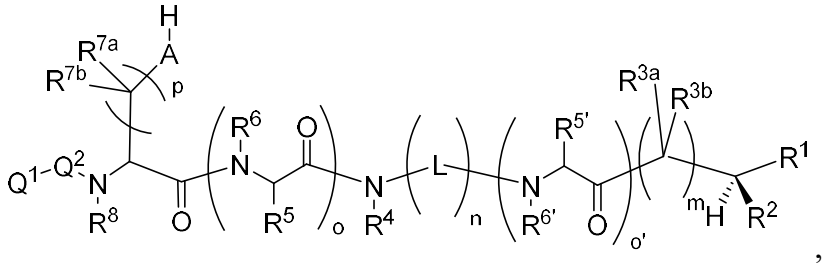
wherein each occurrence of R^6 and $R^{6'}$, when present, is independently selected from hydrogen and methyl, or wherein R^6 or $R^{6'}$ is covalently bonded to R^5 or $R^{5'}$, respectively, and, together with the intermediate atoms, comprise an unsubstituted 5-membered heterocycle;

wherein each of R^{7a} and R^{7b}, when present, is independently selected from hydrogen and C1-C4 alkyl; and

wherein R^8 is selected from hydrogen and methyl,

provided that the compound is not PapA.

[0008c] In another aspect, disclosed herein is a method of chemically modifying a compound to install a thioether linkage, the method comprising reacting the compound with PapB, wherein the compound has a structure represented by a formula:



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wherein m is 0, 1, 2, 3, or 4;

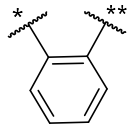
wherein n is 0 or 1;

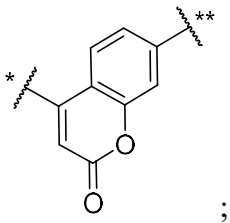
wherein each of o and o' is independently 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9;

wherein p is 1 or 2;

wherein A is S or Se;

wherein L, when present, is selected from C2-C4 alkylene, –(C1-C4 alkylene)$(OCH_2CH_2)_q$, and a structure selected from:





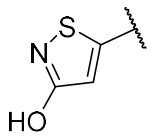
and ;

wherein q is 1, 2, 3, or 4;

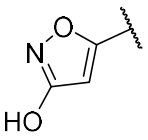
wherein Q^1 is a leader sequence;

wherein Q^2 is a cleavable moiety;

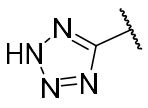
wherein R^1 is selected from $–CO_2H$, –C(O)NHOH, $–SO_2NH_2$, $–SO_2NHC(O)CH_3$, $–SO_3H$, $–NHC(O)NHSO_2CH_3$, $–P(O)(OH)_2$, and a structure selected from:



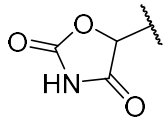
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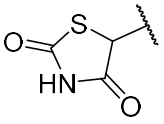
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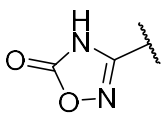
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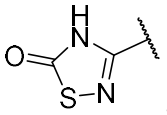
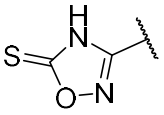
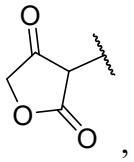
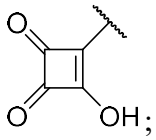
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and ;

wherein R^2 is a residue of a side chain of an amino acid, provided that the amino acid is not isoleucine or threonine;

wherein each of R^{3a} and R^{3b}, when present, is independently selected from C2-C5 alkynyl, C1-C5 azido, and a residue of a side chain of an amino acid;

wherein R^4 is selected from hydrogen and methyl;

wherein each occurrence of R^5 and $R^{5'}$, when present, is independently a residue of a side chain of an amino acid;

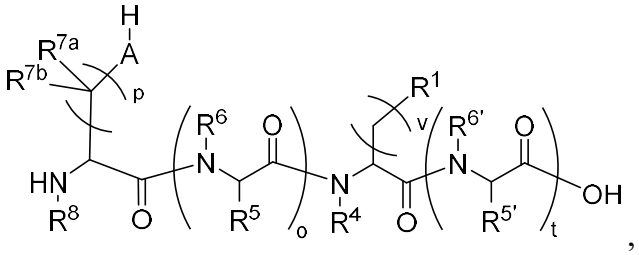
wherein each occurrence of R^6 and $R^{6'}$, when present, is independently selected from hydrogen and methyl, or wherein R^6 or $R^{6'}$ is covalently bonded to R^5 or $R^{5'}$, respectively, and, together with the intermediate atoms, comprise an unsubstituted 5-membered heterocycle;

wherein each of R^{7a} and R^{7b}, when present, is independently selected from hydrogen and C1-C4 alkyl; and

wherein R^8 is selected from hydrogen and methyl,

provided that the compound is not PapA.

[0009] Disclosed are methods of chemically modifying a compound to install a thioether linkage, the method comprising reacting the compound with PapB, wherein the compound has a structure represented by a formula:

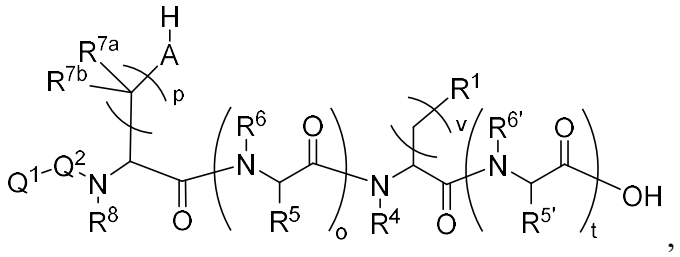

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wherein o is 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9; wherein p is 1 or 2; wherein t is an integer from 0 to 500; wherein v is 1, 2, 3, 4, or 5; wherein A is S or Se; wherein R^1 is selected from $–CO_2H$,

CLAIMS

What is claimed is:

1. A method of chemically modifying a compound to install a thioether linkage, the method comprising reacting the compound with PapB, wherein the compound has a structure represented by a formula:



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wherein o is 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9;

wherein p is 1 or 2;

wherein t is an integer from 0 to 500;

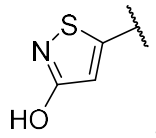
wherein v is 1, 2, 3, 4, or 5;

wherein A is S or Se;

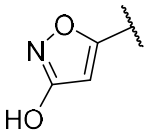
wherein Q^1 is a leader sequence;

wherein Q^2 is a cleavable moiety;

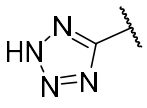
wherein R^1 is selected from $–CO_2H$, $–C(O)NHOH$, $–SO_2NH_2$, $–SO_2NHC(O)CH_3$, $–SO_3H$, $–NHC(O)NHSO_2CH_3$, $–P(O)(OH)_2$, and a structure selected from:



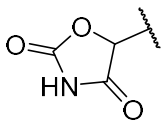
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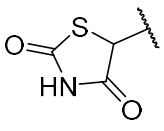
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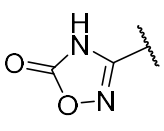
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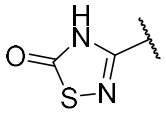
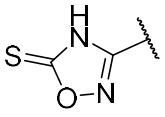
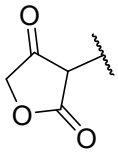
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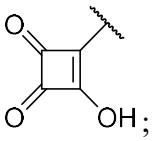


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and ;

wherein R^4 is selected from hydrogen and methyl;

wherein each occurrence of R^5 and $R^{5'}$, when present, is independently a residue of a side chain of an amino acid;

wherein each occurrence of R^6 and $R^{6'}$, when present, is independently selected from hydrogen and methyl, or wherein R^6 or $R^{6'}$ is covalently bonded to R^5 or $R^{5'}$, respectively, and, together with the intermediate atoms, comprise an unsubstituted 5-membered heterocycle;

wherein each of R^{7a} and R^{7b}, when present, is independently selected from hydrogen and C1-C4 alkyl; and

wherein R^8 is selected from hydrogen and methyl,

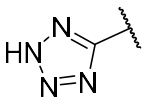
provided that the compound is not PapA.

2. The method of claim 1, wherein o is 0, 1, 2, 3, 4, 5, 6, or 7.

3. The method of claim 1 or claim 2, wherein t is 0.

4. The method of any one of claims 1 to 3, wherein v is 1 or 2.

5. The method of any one of claims 1 to 4, wherein R^1 is $–CO_2H$ or a structure:


.

6. The method of any one of claims 1 to 4, wherein R^1 is $–CO_2H$.

7. The method of any one of claims 1 to 6, wherein the cleavable moiety is $–CO_2–$(C4-

C8 alkylene)–OC(O)–.

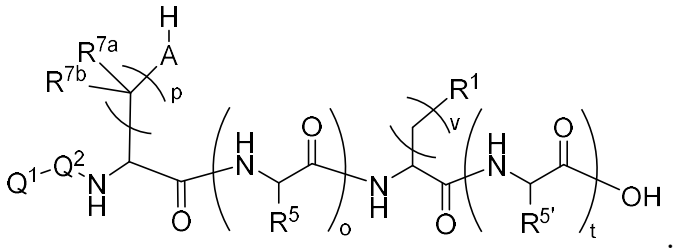
8. The method of any one of claims 1 to 6, wherein the cleavable moiety is –CO_2CH_2CH=$CHCH_2OC(O)$–.

9. The method of any one of claims 1 to 6, wherein the cleavable moiety is a protease recognition sequence.

10. The method of any one of claims 1 to 9, wherein PapB installs a single thioether linkage in the compound.

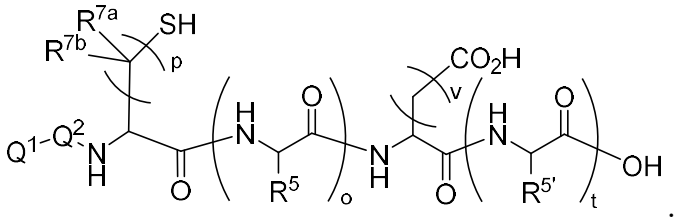
11. The method of any one of claims 1 to 9, wherein PapB installs two or more thioether linkages in the compound.

12. The method of any one of claims 1 to 11, wherein the compound has a structure represented by a formula:



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13. The method of any one of claims 1 to 12, wherein the compound has a structure represented by a formula:



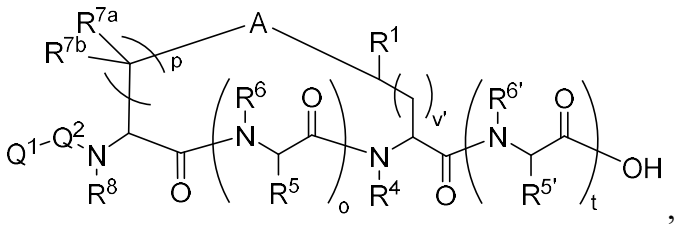
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14. The method of any one of claims 1 to 13, wherein the method further comprises addition of a reducing agent.

15. The method of any one of claims 1 to 14, wherein the method further comprises addition of a protease.

16. The method of any one of claims 1 to 15, wherein the method produces a thioether

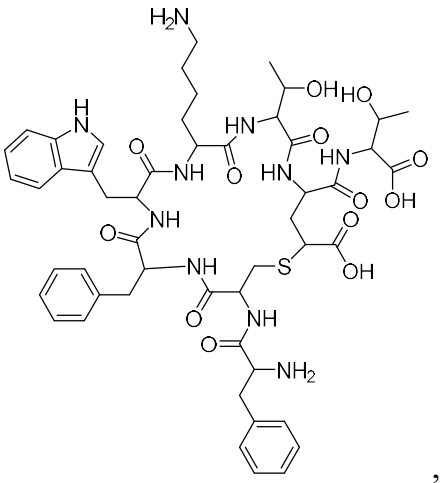
compound having a structure represented by a formula:



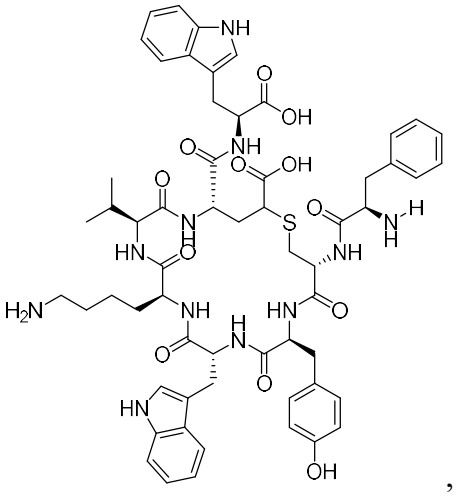
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wherein v' is 0, 1, 2, or 3.

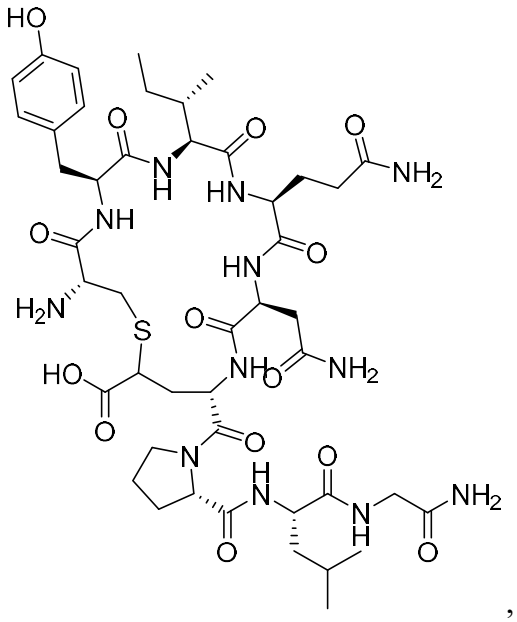
17. The method of claim 16, wherein the thioether compound is selected from:



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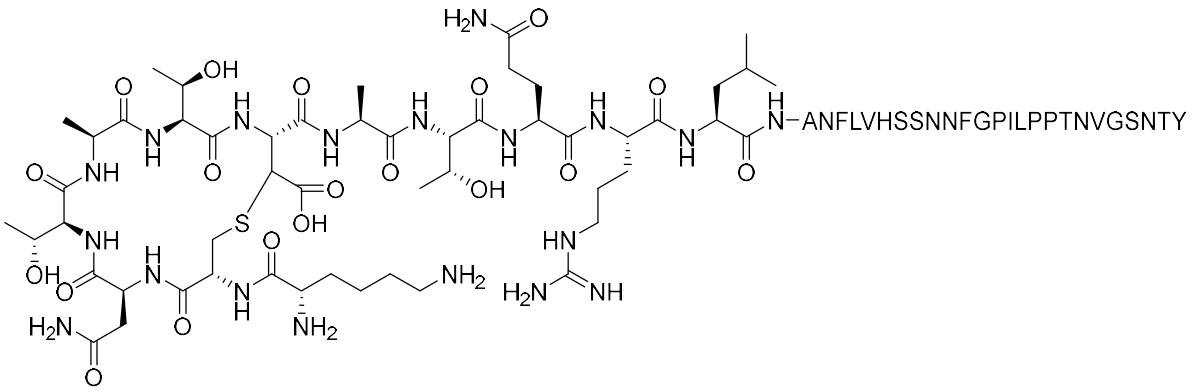


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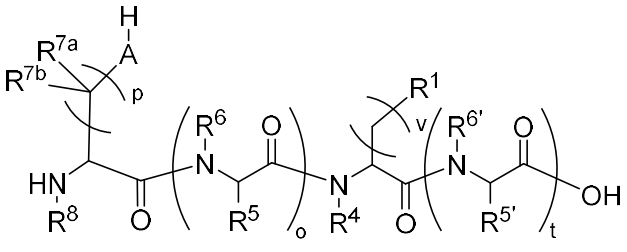
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and



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18. A method of chemically modifying a compound to install a thioether linkage, the method comprising reacting the compound with PapB, wherein the compound has a structure represented by a formula:



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wherein o is 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9;

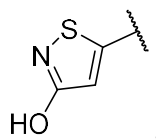
wherein p is 1 or 2;

wherein t is an integer from 0 to 500;

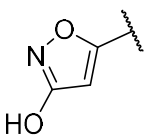
wherein v is 1, 2, 3, 4, or 5;

wherein A is S or Se;

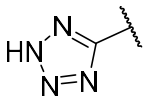
wherein R^1 is selected from $–CO_2H$, –C(O)NHOH, $–SO_2NH_2$, $–SO_2NHC(O)CH_3$, $–SO_3H$, $–NHC(O)NHSO_2CH_3$, $–P(O)(OH)_2$, and a structure selected from:



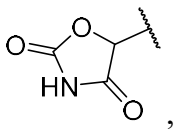
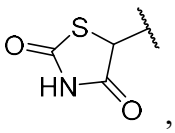
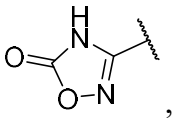
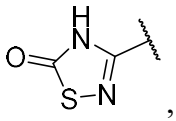
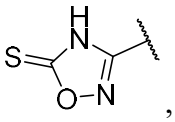
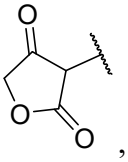
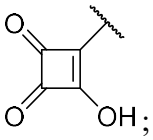
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wherein R^4 is selected from hydrogen and methyl;

wherein each occurrence of R^5 and $R^{5'}$, when present, is independently a residue of a side chain of an amino acid;

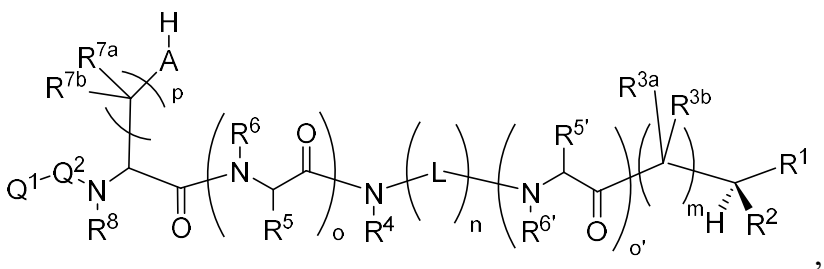
wherein each occurrence of R^6 and $R^{6'}$, when present, is independently selected from hydrogen and methyl, or wherein R^6 or $R^{6'}$ is covalently bonded to R^5 or $R^{5'}$, respectively, and, together with the intermediate atoms, comprise an unsubstituted 5-membered heterocycle;

wherein each of R^{7a} and R^{7b}, when present, is independently selected from hydrogen and C1-C4 alkyl; and

wherein R^8 is selected from hydrogen and methyl,

provided that the compound is not PapA.

19. A method of chemically modifying a compound to install a thioether linkage, the method comprising reacting the compound with PapB, wherein the compound has a structure represented by a formula:


,

wherein m is 0, 1, 2, 3, or 4;

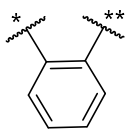
wherein n is 0 or 1;

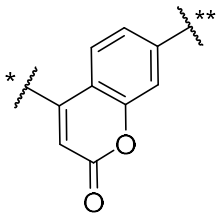
wherein each of o and o' is independently 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9;

wherein p is 1 or 2;

wherein A is S or Se;

wherein L, when present, is selected from C2-C4 alkylene, –(C1-C4 alkylene)$(OCH_2CH_2)_q$, and a structure selected from:



and 

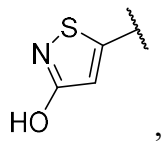
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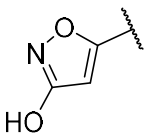
wherein q is 1, 2, 3, or 4;

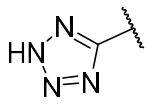
wherein Q^1 is a leader sequence;

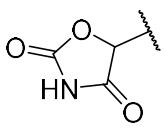
wherein Q^2 is a cleavable moiety;

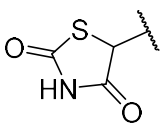
wherein R^1 is selected from $–CO_2H$, –C(O)NHOH, $–SO_2NH_2$, $–SO_2NHC(O)CH_3$, $–SO_3H$, $–NHC(O)NHSO_2CH_3$, $–P(O)(OH)_2$, and a structure selected from:

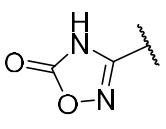


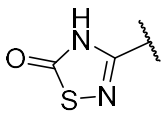
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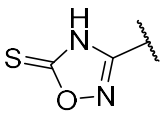
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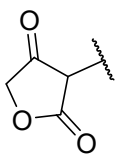
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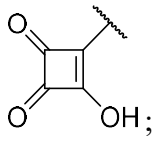
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and ;

wherein R^2 is a residue of a side chain of an amino acid, provided that the amino acid is not isoleucine or threonine;

wherein each of R^{3a} and R^{3b}, when present, is independently selected from C2-C5 alkynyl, C1-C5 azido, and a residue of a side chain of an amino acid;

wherein R^4 is selected from hydrogen and methyl;

wherein each occurrence of R^5 and $R^{5'}$, when present, is independently a residue of a side chain of an amino acid;

wherein each occurrence of R^6 and $R^{6'}$, when present, is independently selected from hydrogen and methyl, or wherein R^6 or $R^{6'}$ is covalently bonded to R^5 or $R^{5'}$, respectively, and, together with the intermediate atoms, comprise an unsubstituted 5-membered heterocycle;

wherein each of R^{7a} and R^{7b}, when present, is independently selected from hydrogen and C1-C4 alkyl; and

wherein R^8 is selected from hydrogen and methyl,

provided that the compound is not PapA.

20. The method of claim 19, wherein PapB installs a single thioether linkage in the compound.

21. The method of claim 19, wherein PapB installs two or more thioether linkages in the compound.

22. The method of claim 19 or claim 20, wherein the method produces a thioether compound having a structure represented by a formula:

