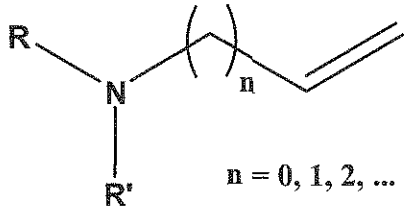
Furthermore, nucleophilic substitution with an alkyl halide or the equivalent arizidine compound (Scheme IIa) cannot be used to selectively alkylate the thiol under conditions that are acceptable for a protein, so a different alkylation method must be provided.

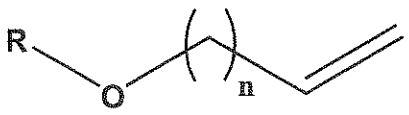
Accordingly, there is a need to provide a method for alkylating the thiol group of Cys to obtain a Lys(Ac) mimic that overcomes or ameliorates one or more disadvantages disclosed above. Additionally, such a method will also be useful for installing other modifications (e.g., pegylation and ubiquitination) onto a thiol-containing compound such as a peptide or a protein.

SUMMARY

~~In a first aspect, t~~There is provided a method of alkylating a thiol group (R-S-H) or seleno group (R-Se-H) in a target molecule wherein the method comprises: reacting a target molecule comprising at least one thiol group with a compound of formula (I) or (II):

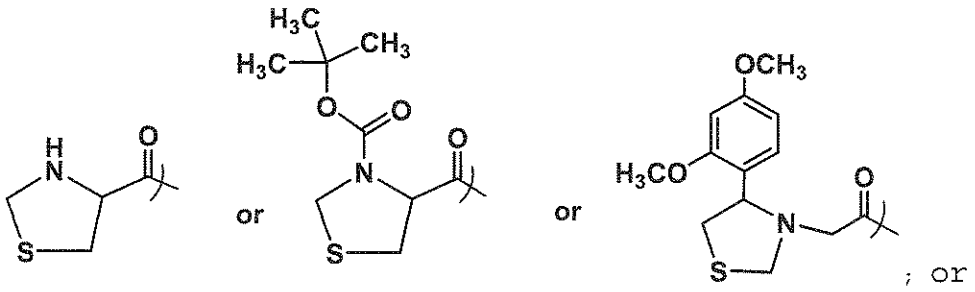


(I)



(II)

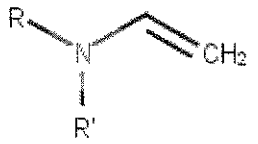
wherein R is an acetyl group or any other acyl group or is a group comprising any one of:



; or

wherein R in formula (II) can also be an alkyl group; and wherein R' is selected from a group consisting of a hydrogen, a methyl group and an ethyl group.

In a first aspect, there is provided a method of alkylating a thiol group or a seleno group in a target molecule, the method comprising: a thiol-ene reaction comprising reacting said thiol or seleno group with a compound of formula (I) or (II):



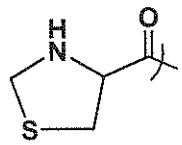
(I)



(II)

Wherein: R is an acetyl group, an acyl group (other than acetyl), a poly(ethylene glycol)-containing acyl group ("PEG-containing acyl group") or is selected from the group consisting of:

7a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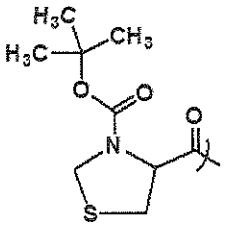
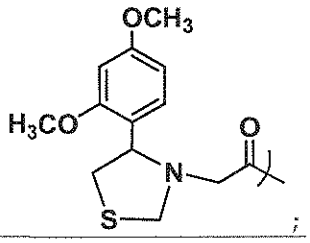

, and

;

R1 is an alkyl group, an acyl group, PEG, or a PEG-containing acyl group; and R′ is selected from the group consisting of a hydrogen, a methyl group and an ethyl group.

In one embodiment, the target molecule is an organic compound. In one embodiment, the organic compound is a peptide or a protein molecule. In still another embodiment, the peptide is selected from an oligopeptide, a polypeptide or a synthetic peptide. In yet another embodiment, the protein molecule is selected from the group consisting of a recombinant protein and a protein complex. One important aspect of the disclosed method resides in its ability to be performed on protein molecules under conditions which would not denature the protein or compromise its structural integrity.

In one embodiment, the disclosed method advantageously provides a simple, one-step process for modifying one or more amino acid residues present on a target molecule, such as biotin, histone or ubiquitin. In one embodiment, the disclosed method is capable of modifying specific amino acid residues to form ideal mimics of acetylated lysine. In one embodiment, the disclosed method selectively modifies one or more cysteine residues on the target molecule to form ideal mimics of acetylated lysine. More particularly,

the disclosed method is directed to the alkylation of one or more cysteine residues via reaction with an appropriate alkylation agent to form acetylated lysine analogs. The type of acetylated lysine analog formed depends on the specific alkylating agent used in the reaction.

In one embodiment, the alkylation agent is a compound of formula (I) or (II) as described above. Advantageously, the inventors have found that the alkylation agent is capable of reacting with the thiol group present on the cysteine residue to form N-acetyl-4-thialysine, in which the thioether linkage is a close isosteric replacement of the 4-methylene group in natural acetylated lysine. As the position of this substitution is sterically distant from the acetamide nitrogen (by two carbon atoms), little differences are detected between the mimic and the natural acetylated lysine in physicochemical and biochemical properties.

Advantageously, the disclosed method overcomes technical problems of feasibility, low yield and low selectivity which plague the prior art methods. In contrast, the disclosed method is very robust and provides near 100% yield of the Lys-Ac mimics (e.g., N-acetyl-4-thialysine) in considerably short reaction times of from 30 minutes to 2 hours or lesser.

~~In another aspect, t~~There is also provided the use of a compound of Formula (I) as defined above as an alkylating agent for alkylation of a target molecule.

~~In yet another aspect, t~~There is also provided the use of a compound of Formula (II) as defined above as an alkylating agent for alkylation of a target molecule comprising a thiol-group (R-S-H) or a seleno group (R-Se-H).

~~In still another aspect, t~~There is also provided the use of a compound of Formula (I) for installing an acetylated lysine residue analog in a target molecule.

~~In another aspect, t~~There is also provided a pharmaceutical composition comprising a target molecule modified according to the method as defined above.

In another aspect, there is provided a method of alkylating a target molecule comprising a thiol group or a seleno group, the method comprising: reacting the compound of Formula (I) as defined herein with the thiol or the seleno group of said target molecule to alkylate the target molecule via a thiol-ene reaction.

In another aspect, there is provided a method, comprising: reacting the compound of Formula (I) as defined herein with a target molecule having a thiol group or a seleno group to install an acetylated lysine residue analog in the target molecule via a thiol-ene reaction.

Definitions

The following words and terms used herein shall have the meaning indicated:

The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

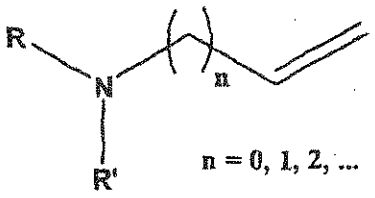
Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically

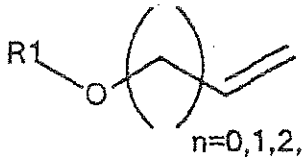
Claims

1. A method of alkylating a thiol group or a seleno group in a target molecule, the method comprising:

a thiol-ene reaction comprising reacting said thiol or seleno group with a compound of formula (I) or (II):



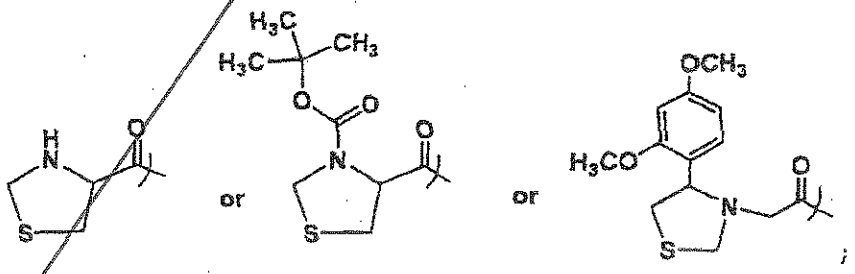
(I)



(II)

Wherein:

R is an acetyl group, an acyl group (other than acetyl), a poly(ethylene glycol)-containing acyl group ("PEG-containing acyl group") or is selected from a group consisting of:



;

R1 is an alkyl group, an acyl group, PEG, or a PEG-containing acyl group;

and

R' is selected from a group consisting of a hydrogen, a methyl group and an ethyl group, and wherein said reacting step is performed in a buffer.

2. The method of claim 1, wherein said buffer is at a pH between 4 to 7.

3. The method of claim 2, wherein the target molecule is a peptide or protein.

4. The method of claim 3, wherein the peptide is selected from the group consisting of an oligopeptide, a polypeptide and a synthetic peptide.

5. The method of claim 3, wherein the protein is selected from the group consisting of a recombinant protein and a protein complex.

6. The method of claim 5, wherein the target molecule is histone or ubiquitin.

7. The method of any one of the preceding claims, wherein the acyl group is an aliphatic acyl group, or an alicyclic acyl group, or an aromatic acyl group, or an amino acyl or a peptidyl or a proteinyl group.

8. The method of claim 7, wherein the aliphatic acyl group is selected from the group consisting of a linear aliphatic acyl group, a branched aliphatic acyl group, a tert-butyloxycarbonyl group, a derivative of tert-butyloxycarbonyl group, a benzyloxycarbonyl group and a derivative of benzyloxycarbonyl group.

9. The method of claim 7, wherein the acyl group is selected from the group consisting of linear aliphatic acyl groups having 1 to 20 or 1 to 7 carbon atoms and branched aliphatic acyl groups having 1 to 7 carbon atoms.

10. The method of claim 7, wherein the acyl group is selected from the group consisting of formyl group, acetyl group, propionyl group, 2-propionyl group, butyryl group, isobutyryl group, pentanoyl group, hexanoyl group, allylcarbonyl group, cyclohexylmethylcarbonyl group, and C_3-C_6 cycloalkylcarbonyl groups, such as cyclopropylcarbonyl group, cyclopentylcarbonyl group, cyclohexylcarbonyl group, and 1-cyclohexenylcarbonyl group.

11. The method of claim 7, wherein the aromatic acyl group is selected from the group consisting of benzoyl group, 4-methylbenzoyl group, and 4-methoxybenzoyl group.

12. The method of any one of the preceding claims, wherein the alkyl group is selected from the group consisting of methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, sec-butyl, pentyl, hexyl, octyl, nonyl, decyl, undecyl, dodecyl, trideyl, tetradecyl, pentadecyl, hexadecyl, octadecyl, and wherein said poly(ethylene glycol) in said PEG-containing acyl group is poly(ethylene.glycol)-ethyl or poly(ethylene.glycol)-propyl.

13. Use of a compound of Formula (I) as defined in any one of claims 1 to 12 as an alkylating agent for alkylating a target molecule in the presence of a buffer.

14. Use of a compound of Formula (II) as defined in any one of claims 1 to 12 as alkylating agent for alkylating a target molecule in the presence of a buffer.

15. Use of a compound of Formula (I) for installing an acetylated lysine residue analog in a target molecule.

16. The use of any one of claims 13 to 15, wherein the target molecule is an organic molecule.

17. The use of claim 16, wherein the organic molecule is a peptide or protein.

18. The use of claim 17, wherein the peptide is selected from the group consisting of an oligopeptide, a polypeptide and a synthetic peptide.

19. The use of claim 17, wherein the protein is selected from the group consisting of a recombinant protein and a protein complex.

20. A pharmaceutical composition comprising a target molecule modified according to the method of any one of claims 1 to 12.

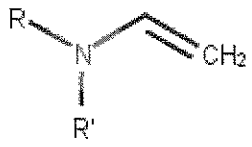
21. The method of any one of claims 1 to 12 wherein the compound of formula (I) is N-vinylacetamide.

22. The method of any one of claims 1 to 12, wherein the reaction step is performed in the presence of an additional thiol compound.

CLAIMS

1. A method of alkylating a thiol group or a seleno group in a target molecule, the method comprising:

a thiol-ene reaction comprising reacting said thiol or seleno group with a compound of formula (I) or (II):



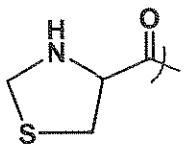
(I)



(II)

Wherein:

R is an acetyl group, an acyl group (other than acetyl), a poly(ethylene glycol)-containing acyl group ("PEG-containing acyl group") or is selected from the group consisting of:



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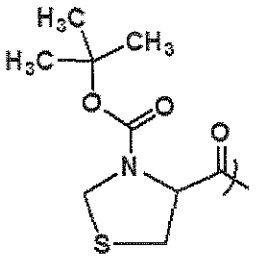
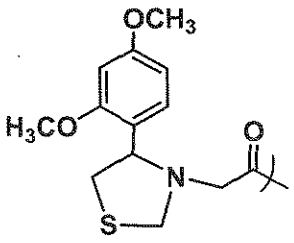


, and



;

R1 is an alkyl group, an acyl group, PEG, or a PEG-containing acyl group;

and

R' is selected from the group consisting of a hydrogen, a methyl group and an ethyl group.

2. The method of claim 1, wherein said reacting step is performed in an acetate buffer.

3. The method of claim 2, wherein the target molecule is a peptide or protein.

4. The method of claim 3, wherein the peptide is selected from the group consisting of an oligopeptide, a polypeptide and a synthetic peptide.

5. The method of claim 3, wherein the protein is selected from the group consisting of a recombinant protein, a protein complex, a histone and a ubiquitin.

6. The method of claim 1, wherein the acyl group is an aliphatic acyl group, an alicyclic acyl group, an aromatic acyl group, an amino acyl group, a peptidyl group, or a proteinyl group.

7. The method of claim 1, wherein the acyl group is selected from the group consisting of a linear aliphatic acyl group, a branched aliphatic acyl group, a tert-butyloxycarbonyl group, a derivative of a tert-butyloxycarbonyl group, a benzyloxycarbonyl group and a derivative of a benzyloxycarbonyl group.

8. The method of claim 6, wherein the acyl group is selected from the group consisting of linear aliphatic acyl groups having 1 to 20 carbon atoms and branched aliphatic acyl groups having 1 to 7 carbon atoms.

9. The method of claim 6, wherein the acyl group is selected from the group consisting of a formyl group, a propionyl group, a 2-propionyl group, a butyryl group, an isobutyryl group, a pentanoyl group, a hexanoyl group, an

allylcarbonyl group, a cyclohexylmethylcarbonyl group, and a C_3-C_6 cycloalkylcarbonyl group.

10. The method of claim 6, wherein the aromatic acyl group is selected from the group consisting of a benzoyl group, a 4-methylbenzoyl group, and a 4-methoxybenzoyl group.

11. The method of claim 1, wherein the alkyl group is selected from the group consisting of methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, sec-butyl, pentyl, hexyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, and octadecyl, and wherein said poly(ethylene glycol) in said PEG-containing acyl group is poly(ethylene glycol)-ethyl or poly(ethylene glycol)-propyl.

12. A method of alkylating a target molecule comprising a thiol group or a seleno group, the method comprising: reacting the compound of Formula (I) as defined in claim 1 with the thiol or the seleno group of said target molecule to alkylate the target molecule via a thiol-ene reaction.

13. A method, comprising: reacting the compound of Formula (I) as defined in claim 1 with a target molecule having a thiol group or a seleno group to install an acetylated lysine residue analog in the target molecule via a thiol-ene reaction.

14. The method of claim 12, wherein the target molecule is an organic molecule selected from the group consisting of a peptide, an oligopeptide, a polypeptide, a synthetic peptide, a protein, a recombinant protein and a protein complex.

15. The method of claim 1 wherein the compound of formula (I) is N-vinylacetamide.

16. The method of claim 1, wherein the reaction step is performed in the presence of an additional thiol compound.

17. The method of claim 13, wherein the target molecule is an organic molecule selected from the group consisting of a peptide, an oligopeptide, a polypeptide, a synthetic peptide, a protein, a recombinant protein and a protein complex.

18. The method of claim 2, wherein the buffer is at a pH of 4.