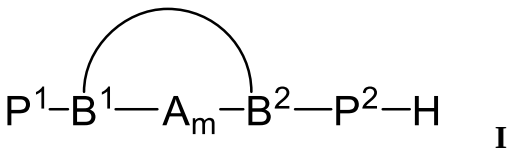
metathesis/hydrogenations of relatively simple substrates that are unlikely to form aggregates on resin. As one of the biggest problems with on-resin peptide synthesis is potential aggregation of the growing peptide within the resin matrix, it remains to be seen whether the conditions will be well-suited for complex, hydrophobic macrocycles. Similarly, transfer hydrogenation using sodium borohydride has been demonstrated in solution phase only.

[0007] It has previously been found that the recommended *in-situ* diimine reductions known in the art to produce saturated stapled peptides are sluggish, impractical and costly. For instance, the attempted hydrogenation of stapled peptide PM2 after 4 x 2 h treatments with 2,4,6-triisopropylbenzenesulfonyl hydrazide only resulted in 50% product conversion (Figure 3). Similar results were obtained for stapled peptides ATSP-7041 and VIP116, with respective yields of 39% and 45% after 3 treatments with the reagents.

[0008] Currently known stapled peptide reduction processes require repeated exposure of the resin-bound alkene to 2,4,6-triisopropylbenzenesulfonyl hydrazide/piperidine until the reaction has proceeded to an acceptable level. Generally, 5 treatments are recommended at a minimum with this scheme. In order to determine the rate of reaction, the reaction mixture is filtered and a small sample of resin beads is treated with TFA to cleave the peptide from the solid support, which is then analysed by HPLC-LCMS. Not only is the entire process cumbersome and impractical, the high cost of reagents such as 2,4,6-triisopropylbenezenesulfonyl hydrazide becomes a major influence in determining how far a reaction should be taken. Taking into account the severe limitations of current technologies, alternative hydrogen transfer strategies to access saturated stapled peptides on resin are needed.

Summary

[0009] The present disclosure provides a process for producing a compound of general Formula I:



I

wherein:

m is an integer between 1 and 8;

wherein R^2 is alkyl;

each n is independently an integer between 0 and 12; and

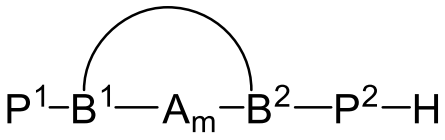
S is a solid state resin;

said reaction occurring between the alkenyl side chain of B^{1a} and the alkenyl side chain of B^{2a} so as to form an intramolecular alkenyl chain;

b) hydrogenating the carbon-carbon double bond formed in step a) so as to produce a saturated alkyl chain; and,

c) cleaving the solid state resin material from P^2 so as to produce a compound of Formula I.

[0009a] In one aspect of the present disclosure there is provided a process for producing a compound of general Formula I:



I

wherein:

m is an integer between 1 and 8;

each A is independently an amino acid residue;

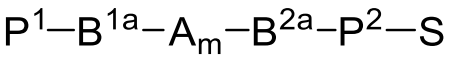
B^1 and B^2 are each substituted amino acid residues covalently coupled together by a saturated alkyl chain;

P^1 and P^2 are each independently one or more amino acid residues, wherein P^1 has a terminal amino group and P^2 has a terminal carboxylic acid group; and,

H is hydrogen;

said process comprising the steps of:

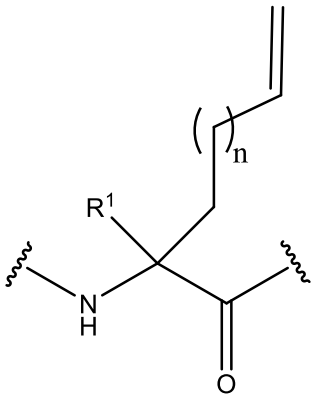
a) performing a metathesis reaction on a compound of general Formula II so as to form an intramolecular alkenyl chain between the B^{1a} and B^{2a} groups



II

wherein:

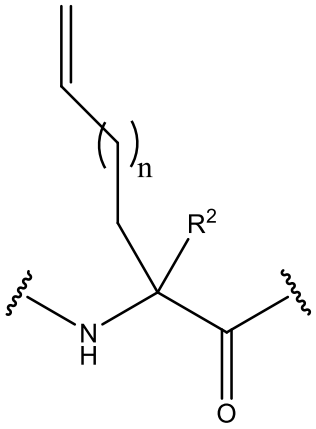
B^{1a} is a group of Formula IIa



IIa

wherein R^1 is alkyl;

B^{2a} is a group of Formula IIb



IIb

wherein R^2 is alkyl;

each n is independently an integer between 0 and 12; and

S is a solid state resin;

said reaction occurring between the alkenyl side chain of B^{1a} and the alkenyl side chain of B^{2a} so as to form an intramolecular alkenyl chain;

b) hydrogenating the carbon-carbon double bond formed in step a) so as to produce a saturated alkyl chain,

wherein the hydrogenating is conducted with a hydrosilane reagent, wherein the hydrosilane reagent is triethylsilane; and,

c) cleaving the solid state resin material from P^2 so as to produce a compound of Formula I.

[00010] The following options may be used in conjunction with the above, either individually or in any suitable combination.

[00011] The m variable may be an integer between 1 and 6. Each A may independently be a naturally occurring L-α-amino acid. At least one A may be an unnaturally occurring amino acid. R^1 and R^2 may each be ethyl. S may comprise a polymeric material.

[00012] Step a) of the process above may be a metathesis reaction. It may be conducted in the presence of a catalyst. The catalyst may comprise ruthenium. The catalyst added to a suspension of a compound of Formula II may be fresh. The fresh catalyst may be added in separate aliquots, for example fresh catalyst may be added in 2, 3, 4 or 5aliquots during the reaction of step b). Fresh catalyst may be added to a compound of Formula II twice before conducting step b). The temperature that the metathesis reaction of step a) is conducted at may be between 15 °C and 60 °C.

[00013] Step b) of the process above may be a hydrogenation reaction. The hydrogenation reaction of step b) may use a hydrosilane reagent of Formula SiH_nR_{4-n}, where n = 1, 2, 3 or 4 and R is an organic group. The hydrosilane reagent may be a trialkylhydrosilane reagent of general Formula [R_3SiH], wherein R is alkyl. Each R may be independently selected from the group consisting of methyl, ethyl, propyl, butyl and pentyl. Each R may be ethyl. When each R is ethyl, the trialkylhydrosilane reagent is triethylhydrosilane.

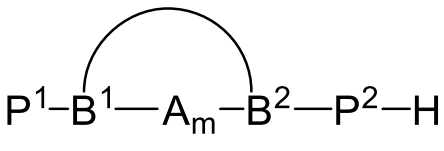
[00014] The hydrogenation reaction of step b) may occur after the completion of step a), or it may occur simultaneously. When the reactions of step a) and step b) are conducted at the same

time, the hydrosilane reagent may be added to the reaction mixture concomitantly with the catalyst. The hydrosilane reagent may be added to the reaction mixture at the end of step a) without performing an isolation step. The hydrogenation reaction may occur at a temperature between about 40 °C and about 60 °C. To achieve this reaction temperature, the reaction mixture may be heated using a non-microwave heating source.

[00015] To conduct the reaction of step b), fresh hydrosilane reagent may be added to the reaction mixture in separate aliquots. The addition may occur 2, 3, 4 or 5 times. Aliquots of the fresh hydrosilane reagent may be added to the reaction mixture three times. When multiple separate aliquots are added to the reaction mixture, the aliquots may be added at 2 hour intervals. Following the final addition of hydrosilane reagent, the reaction mixture may be left overnight before proceeding to step c). The steps a) and b) may occur in the same vessel without isolating the products of step a) before performing the reaction of step b).

[00016] Once the hydrogenation reaction of step b) has completed, the cleavage step of step c) may be conducted. The cleavage reaction of step c) may use an acid capable of cleaving peptides from a solid support resin. Once cleaved, the product formed is a compound of Formula I. The compound of Formula I produced may be between 5 and 20 residues in length. The compound may be produced by solid-phase synthesis.

[00017] In one example, the process of the present disclosure involves producing a compound of Formula I



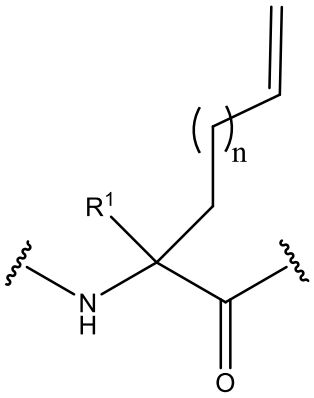
I

wherein: m is 3; each A is independently a naturally occurring amino acid residue; B^1 and B^2 are each substituted amino acid residues covalently coupled together by a saturated alkyl chain; P^1 and P^2 are each independently one or more amino acid residues, wherein P^1 has a terminal amino group and P^2 has a terminal carboxylic acid group; and H is hydrogen. The process comprises the steps of:

a) performing a metathesis reaction on a compound of general Formula II so as to form an intramolecular alkenyl chain between the B^{1a} and B^{2a} groups

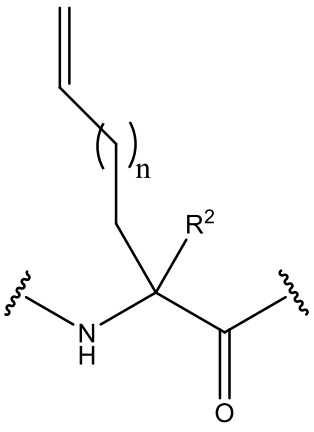
$$P^1–B^{1a}–A_m–B^{2a}–P^2–S \quad \textbf{II}$$

wherein: B^{1a} is a group of Formula IIa



IIa

wherein R^1 is ethyl; B^{2a} is a group of Formula IIb



IIb

wherein R^2 is ethyl; the n of Formula IIa is 8 and the n of Formula IIb is 5; and S is a solid state resin; said reaction occurring between the alkenyl side chain of B^{1a} and the alkenyl side chain of B^{2a} so as to form an intramolecular alkenyl chain;

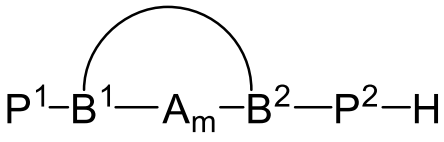
b) hydrogenating the carbon-carbon double bond formed in step a) so as to produce a saturated alkyl chain; and,

c) cleaving the solid state resin material from P^2 so as to produce a compound of Formula I.

[00018] In the process of this example, the process of step a) includes adding two aliquots of a ruthenium-based alkylidene catalyst to a suspension of the compound of Formula II, wherein the compound of Formula II is suspended in a halogenated alkane solvent, such as dichloroethane. The reaction mixture comprising the halogenated alkane solvent, the compound of Formula II and the ruthenium-based alkylidene catalyst is held at a temperature between 15 °C and 60 °C. Following completion of the reaction of step a), between 2 and 3 aliquots of a neat

trialkylhydrosilane reagent of general Formula [R_3SiH] is added, with 2 hours elapsing between each addition of the trialkylhydrosilane reagent. During step b), the temperature is held between 40 °C and 60 °C, with the heating supplied by a source that is a non-microwave heating source. After the final addition of the trialkylhydrosilane reagent, an acid capable of cleaving the peptide from the solid state resin is added, resulting in the production of a compound of Formula I.

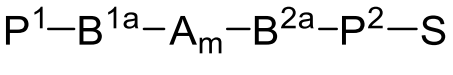
[00019] In another example, the process of the present disclosure involves producing a compound of Formula I



I

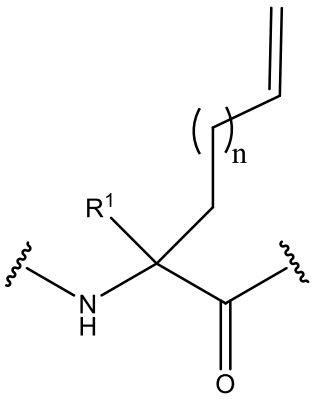
wherein: m is 3; each A is independently a naturally occurring amino acid residue; B^1 and B^2 are each substituted amino acid residues covalently coupled together by a saturated alkyl chain; P^1 and P^2 are each independently one or more amino acid residues, wherein P^1 has a terminal amino group and P^2 has a terminal carboxylic acid group; and H is hydrogen. The process comprising the steps of:

a) performing a metathesis reaction on a compound of general Formula II so as to form an intramolecular alkenyl chain between the B^{1a} and B^{2a} groups



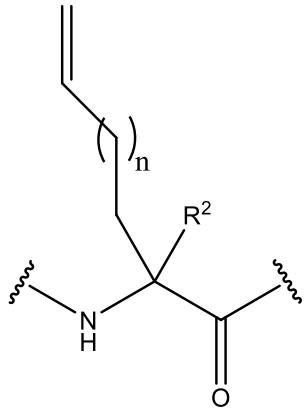
II

wherein: B^{1a} is a group of Formula IIa



IIa

wherein R^1 is alkyl; B^{2a} is a group of Formula IIb



IIb

wherein R^2 is alkyl; the n of Formula IIa is between 3 and 8 and the n of Formula IIb is between 2 and 5; and S is polystyrene; said reaction occurring between the alkenyl side chain of B^{1a} and the alkenyl side chain of B^{2a} so as to form an intramolecular alkenyl chain;

b) hydrogenating the carbon-carbon double bond formed in step a) so as to produce a saturated alkyl chain; and,

c) cleaving the solid state resin material from P^2 so as to produce a compound of Formula I.

[00020] In the process of this example, the process of step a) may include adding 4 aliquots of a Grubbs II catalyst to a suspension of the compound of Formula II, wherein the compound of Formula II may be suspended in dichloroethane. The reaction mixture comprising the halogenated alkane solvent, the compound of Formula II and the ruthenium-based alkylidene catalyst may be held at a temperature between 15 °C and 60 °C. After the fourth aliquot of catalyst is added and the reaction of step a) may be complete or substantially complete, step b) begins with the addition of the first aliquot of triethylhydrosilane. A total of up to 3 aliquots of neat fresh triethylhydrosilane reagent may be added during this step, with each aliquot of triethylhydrosilane added at 2 hour intervals. During step b), the temperature may be held between 40 °C and 60 °C, with the heating supplied by a source that is a non-microwave heating source. After the final addition of the trialkylhydrosilane reagent, trifluoroacetic acid may be added to cleave the peptide from the solid state resin, resulting in the production of a compound of Formula I. The resulting saturated stapled peptide may be between 10 and 15 residues in length.

[00021] In the present disclosure, there is also provided a compound of Formula I that is obtained by the process above.

[00022] The following options may be used in conjunction with the above, either alone or in any suitable combination.

[00023] The product that is obtained by the process above may be a saturated stapled peptide. The saturated stapled peptide may be stabilised in an α-helical conformation, or at least a portion of the saturated stapled peptide may be stabilized in an α-helical conformation. A saturated stapled peptide in this context is one in which the linker which staples the peptide has no double or triple bonds. It will be understood that the stapled peptide may contain other unsaturation.

Brief Description of Drawings

[00024] Figure 1. Representative schemes of known methods for the preparation of stapled peptides.

[00025] Figure 2. Alternative tandem metathesis/reduction strategies using solid-state reagents and/or non-silane reagents.

[00026] Figure 3. Results of the attempted hydrogenation of stapled peptide PM2 after 4 x 2 h treatments with 2,4,6-triisopropylbenzenesulfonyl hydrazide as determined by HPLC.

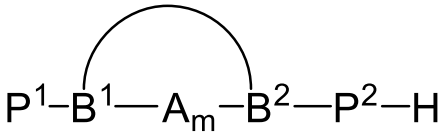
[00027] Figure 4. Results of an *in vitro* study to determine the effect of saturating the intramolecular linker on the biological response to a range of stapled peptides, wherein the study comprised the titration of stapled peptide geometric isomers and reduced (i.e. saturated) stapled peptides onto T22 p53 reporter cell lines in the presence of 2% FCS.

Definitions

[00028] The term “stapled peptides” as used herein refer to peptide or peptide-like chains respectively that incorporate two or more modified amino acids, such that when the peptide or peptide-like chain becomes a stapled peptide, the alkenyl chains of the substituted amino acids are covalently joined to produce an intramolecular alkenyl linker, which is then hydrogenated in the present invention, whereby the alkyl intramolecular linker constricts at least a portion of the peptide chain in at least one conformation.

CLAIMS

1. A process for producing a compound of general Formula I:



I

wherein:

m is an integer between 1 and 8;

each A is independently an amino acid residue;

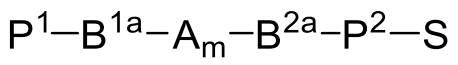
B^1 and B^2 are each substituted amino acid residues covalently coupled together by a saturated alkyl chain;

P^1 and P^2 are each independently one or more amino acid residues, wherein P^1 has a terminal amino group and P^2 has a terminal carboxylic acid group; and,

H is hydrogen;

said process comprising the steps of:

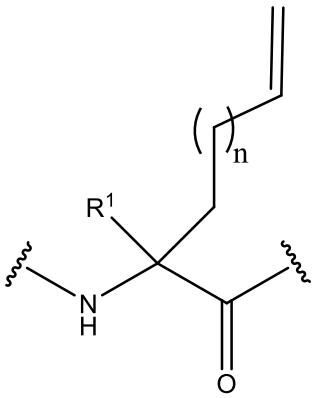
a) performing a metathesis reaction on a compound of general Formula II so as to form an intramolecular alkenyl chain between the B^{1a} and B^{2a} groups



II

wherein:

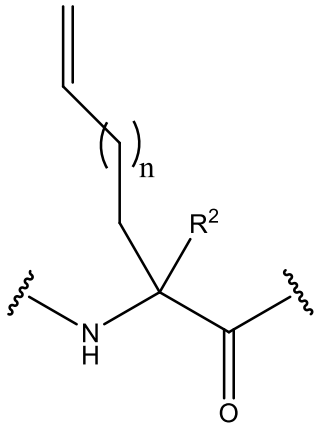
B^{1a} is a group of Formula IIa



IIa

wherein R^1 is alkyl;

B^{2a} is a group of Formula IIb



IIb

wherein R^2 is alkyl;

each n is independently an integer between 0 and 12; and

S is a solid state resin;

said reaction occurring between the alkenyl side chain of B^{1a} and the alkenyl side chain of B^{2a} so as to form an intramolecular alkenyl chain;

b) hydrogenating the carbon-carbon double bond formed in step a) so as to produce a saturated alkyl chain,

wherein the hydrogenating is conducted with a hydrosilane reagent, wherein the hydrosilane reagent is triethylsilane; and,

c) cleaving the solid state resin material from P^2 so as to produce a compound of Formula I.

2. The process of claim 1 wherein m is an integer between 1 and 6.

3. The process of claim 1 or claim 2 wherein each A is independently a naturally occurring L-α-amino acid.

4. The process of claim 1 or claim 2 wherein at least one A is an unnatural amino acid.

5. The process of any one of claims 1 to 4 wherein R^1 is methyl.

6. The process of any one of claims 1 to 5 wherein R^2 is methyl.

7. The process of any one of claims 1 to 6 wherein S comprises a polymeric material.

8. The process of any one of claims 1 to 7 wherein step a) is conducted in the presence of a catalyst.

9. The process of claim 8 wherein the catalyst comprises ruthenium.

10. The process of claim 8 or claim 9 wherein fresh catalyst is added to the compound of Formula II in separate aliquots, said addition occurring 2, 3, 4 or 5 times.

11. The process of claim 10 wherein fresh catalyst is added to a compound of Formula II twice before conducting step b).

12. The process of any one of claims 8 to 11 wherein the metathesis reaction occurs at a temperature between 15 °C and 60 °C.

13. The process of any one of claims 1 to 12 wherein the hydrosilane reagent is added to the reaction mixture concomitantly with the catalyst.

14. The process of any one of claims 1 to 13 wherein the hydrogenation reaction of step b) is conducted at a temperature between 40 °C and 60 °C.

15. The process of claim 14 comprising heating the reaction mixture to said temperature using a non-microwave heating source.

16. The process of any one of claims 1 to 15 wherein fresh hydrosilane reagent is added to the reaction mixture in separate aliquots, said addition occurring 2, 3, 4 or 5 times.

17. The process of claim 16 wherein aliquots of fresh hydrosilane reagent are added to the reaction mixture three times.

18. The process of claim 17 wherein the aliquots are added at 2 hour intervals.

19. The process of claim 17 or claim 18 wherein the reaction mixture is left overnight after the third aliquot addition before conducting step c).

20. The process of any one of claims 1 to 19 wherein steps a) and b) occur in the same vessel without isolating the products of step a) before performing the reaction of step b).

21. The process of any one of claims 1 to 20 wherein step c) uses an acid capable of cleaving peptides from a solid support resin.

22. The process of any one of claims 1 to 21 wherein the compound of Formula I produced is between 5 and 20 residues in length.

23. The process of any one of claims 1 to 22 wherein a compound of Formula II is produced by solid-phase peptide synthesis.