Claims

1. A method of peptide synthesis comprising a compound as set forth in claim 14 or 15, or a resin as set forth in claim 11.

2. A process for the production of a peptide comprising a C-terminal tertiary *N,N*-bis(2-mercaptoethyl)-amide group; *N*-(2-mercaptoethyl)-*N*-(3-aminopropyl)-amide group or *N,N*-bis(3-mercaptopropyl)-amide, the process comprising the step of providing a dialkylamine resin as set forth in claim 11.

3. A method according to claim 1 or a process according to claim 2 wherein the method or process respectively comprises native chemical ligation (NCL).

4. A method or process according to claim 3 wherein the method or process respectively further comprises: (i) converting the ligation product to a thioester and performing NCL between the thioester and a cys-peptide; or (ii) allowing the ligation product to directly react with a cys-peptide *in situ;*

and wherein one or more further ligation steps may optionally follow.

5. A method or process according to any one of the preceding claims wherein the process comprises the addition of a thiol additive and wherein the thiol additive is optionally selected from the group consisting of: alkyl thiol, benzylmercaptan, MESNa and methyl mercaptoacetate.

6. A method or process according to any one of the preceding claims wherein the method or process comprises Fmoc solid-phase peptide synthesis.

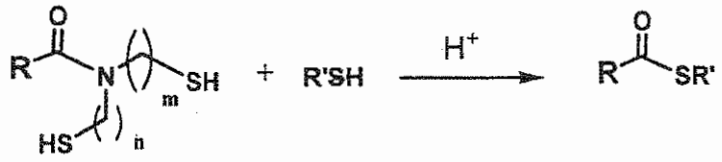
7. A method or process according to any one of the preceding claims wherein the method or process comprises synthesizing a polypeptide or protein.

8. A method or process of synthesizing a peptide, polypeptide or protein according to any one of the preceding claims, wherein the method or process comprises:

(i) converting one or more cysteine residues at one or more of the ligation junctions to other amino acid residues and/or folding the peptide, polypeptide or protein; and/or

(ii) purifying and obtaining the peptide, polypeptide or protein.

9. A method of thioester peptide synthesis as shown in the reaction schematic below:

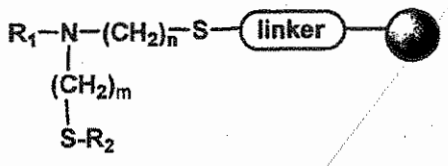


in which, independently, n = 2 or 3 and m = 2 or 3, and in which RCO is an amino acyl or peptidyl group, or in which R is one or more groups independently selected from hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphonyl and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$, and in which R'SH is a thiol-containing compound.

10. A method according to claim 9 wherein the thiol-containing compound is 3-mercaptopropionic acid or benzylmercaptan or methyl mercaptoacetate or MESNa.

11. A dialkylamine resin having the following formula (i) or (ii):

(i)



in which:

 (ie. the circular shape in the formula) represents a resin or solid support

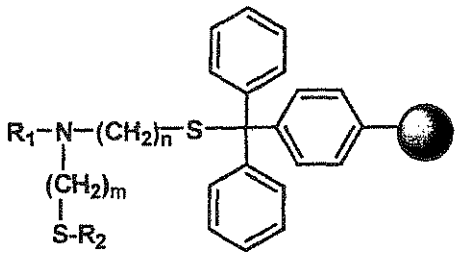
n= independently 2,3; m= independently 2,3

R_1 is H, Fmoc

R_2 is H or a protecting group such as Trt (trityl or triphenylmethyl);

and "linker" is any linker compatible with Fmoc chemistry, including a benzyl derivative or Trt.

(ii)

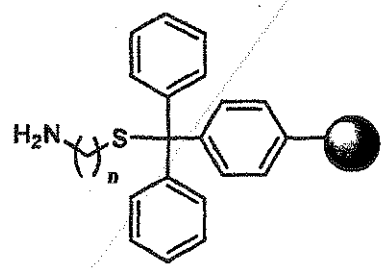


n = 2, 3; m = 2, 3
R_1 = H, Fmoc
R_2 = Trt

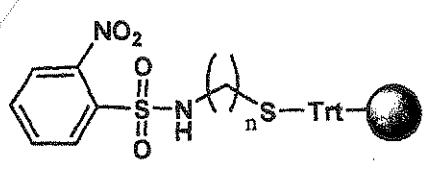
and in which ● (ie. the circular shape in the formula) represents a resin or solid support.

12. A process for the production of a dialkylamine resin according to claim 11, comprising loading the dialkylamine, $HS(CH_2)_mNH(CH_2)_nSH$, to a trityl resin, or comprising the following steps:

(i) reaction of formula (A) below with *o*-NO_2PhSO_2-Cl, DIEA, and CH_2Cl_2-DMF to obtain formula (B)

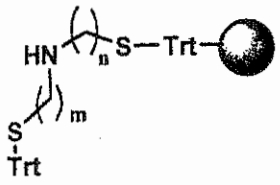


(A)



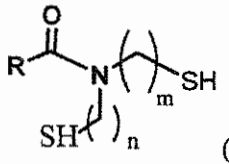
(B)

(ii) alkylation of formula (B) and thiolytic removal of the sulfonyl group to obtain formula (C)



(C)

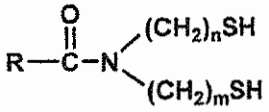
13. A process according to claim 12 wherein the process further comprises the step of Fmoc SSPS followed by cleavage to obtain formula (D):



(D)

in which n = 2 or 3 and m = 2 or 3; and in which RCO is an amino acyl or peptidyl group, or in which R is one or more groups independently selected from hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphonyl and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$

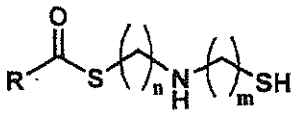
14. A carboxyamide compound having the following structural formula, in which, independently, n = 2 or 3 and m = 2 or 3



and in which RCO is an amino acyl or peptidyl group, or in which R is one or more groups independently selected from hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine,

alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphonyl and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$.

15. A compound having the following structural formula, in which, independently, n = 2 or 3 and m = 2 or 3



and in which RCO is an amino acyl or peptidyl group, or in which R is one or more groups independently selected from hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphonyl and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$.

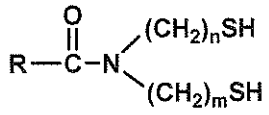
16. The method of claim 9, the resin of claim 11, the process of claim 13, or the compound of claim 14 or 15, wherein: (i) both n and m = 2; (ii) n = 2, and m=3; or n = 3 and m = 2.

17. A method, process, resin or compound as set forth in any one of the preceding claims wherein in place of a BMEA moiety a BMEA variant is used in which cysteine replaces a mercaptoethylamine.

18. The use of methyl mercaptoacetate as a thiol additive for native chemical ligation.

Claims

1. A method of peptide synthesis comprising a step of reacting a compound to form a polypeptide or protein, the compound having the following structural formula:



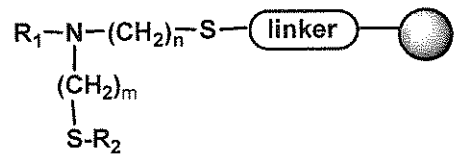
wherein

n is 2 or 3;

m is 2 or 3;

and RC(O)- is an amino acyl or peptidyl group, or R is selected from the group consisting of hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphonyl and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$.

2. A method of peptide synthesis comprising the step of reacting a resin of formula:



wherein



represents a resin or solid support;

n is 2 or 3;

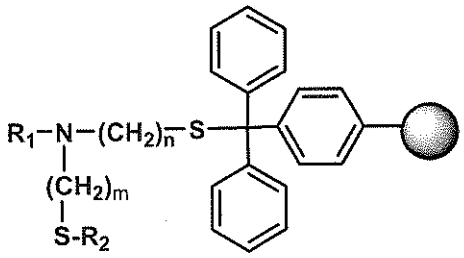
m is 2 or 3;

R_1 is H or Fmoc;

R_2 is H or a protecting group; and

"linker" is any linker compatible with Fmoc chemistry.

3. The method of claim 2, wherein the resin is of formula:



wherein R_2 is trityl.

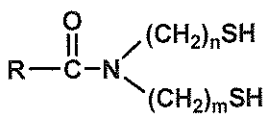
4. The method of claim 2, wherein the peptide comprises a C-terminal tertiary *N*,*N*-bis(2-mercaptoethyl)-amide group; *N*-(2-mercaptoethyl)-*N*-(3-aminopropyl)-amide group or *N*,*N*-bis(3-mercaptopropyl)-amide.

5. The method of claim 2, further comprising a step of reacting a thiol additive selected from the group consisting of an alkyl thiol, benzyl mercaptan, MESNa, and methyl mercaptoacetate.

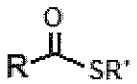
6. The method of claim 2, comprising an Fmoc solid-phase peptide synthesis.

7. The method of claim 2, wherein the product of the method of peptide synthesis is a polypeptide or protein.

8. A method of thioester peptide synthesis comprising the steps of reacting a compound of formula:



with a thiol-containing compound R'SH in the presence of a protic acid, thereby forming a thioester compound of formula:



;

wherein

n is 2 or 3;

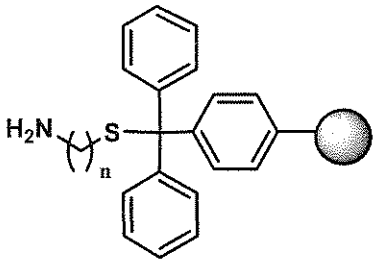
m is 2 or 3;

and RC(O)- is an amino acyl or peptidyl group, or R is selected from the group consisting of hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphonyl and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$.

9. The method of claim 8, wherein the thiol-containing compound R'SH is selected from the group consisting of 3-mercaptopropionic acid, benzylmercaptan, methyl mercaptoacetate, and MESNa.

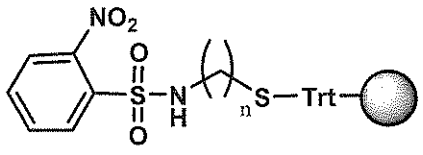
10. A method comprising steps of:

(i) reacting a resin of formula (A):



(A)

with *o*-NO_2PhSO_2-Cl, DIEA, and CH_2Cl_2-DMF to obtain a resin of formula (B):

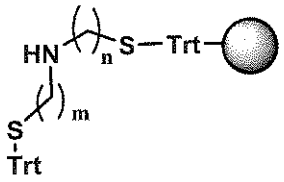


(B);

(ii) alkylating the resin of of formula (B); and

(iii) removing the sulfonyl group thiolytically;

thereby producing a resin of formula (C):



(C)

wherein:

Trt is trityl;

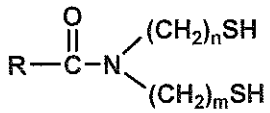
n is 2 or 3; and

m is 2 or 3.

11. The method of claim 10, further comprising steps of:

(iv) performing Fmoc solid phase peptide synthesis on the resin of formula (C); and

(v) cleaving from the resin to produce a compound of formula (D):



wherein

n is 2 or 3;

m is 2 or 3; and

and RC(O)- is an amino acyl or peptidyl group, or R is selected from the group consisting of hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphonyl and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$.

12. The method of claim 8, wherein:

i) n is 2, and m is 2; or

ii) n is 2, and m is 3; or

iii) n is 3, and m is 2.

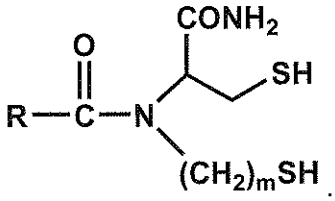
13. The method of claim 11, wherein:

i) n is 2, and m is 2; or

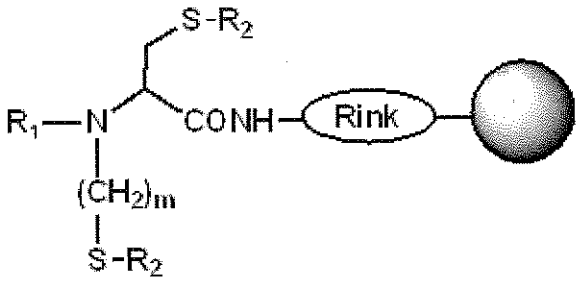
ii) n is 2, and m is 3; or

iii) n is 3, and m is 2.

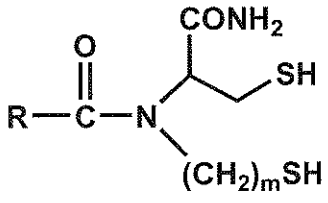
14. The method of claim 1, wherein in place of a N,N-bis(2-mercaptoethy) (BMEA) moiety a BMEA variant is used in which cysteine replaces a mercaptoethylamine, wherein the structure of the BMEA variant is



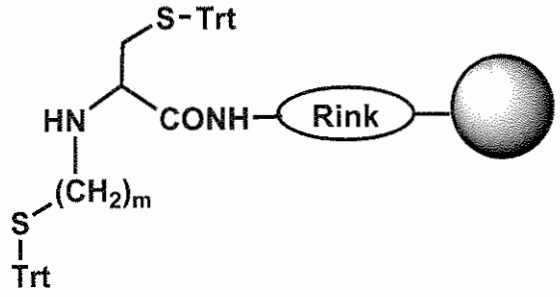
15. The method of claim 2, wherein in place of a N,N-bis(2-mercaptoethyl)-amide (BMEA) moiety a BMEA variant is used in which cysteine replaces a mercaptoethylamine wherein the structure of the BMEA variant is



16. The method of claim 8, wherein in place of a N,N-bis(2-mercaptoethyl)-amide (BMEA) moiety a BMEA variant is used in which cysteine replaces a mercaptoethylamine wherein the structure of the BMEA variant is



17. The method of claim 10, wherein in place of a N,N-bis(2-mercaptoethyl)-amide (BMEA) moiety a BMEA variant is used in which cysteine replaces a mercaptoethylamine wherein the structure of the BMEA variant is



18. The method of claim 1, wherein the method is conducted at a pH of between 4 and 6.

19. The method of claim 1, wherein the method comprises the step of applying microwave radiation.

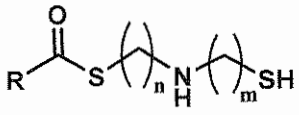
20. The method of claim 2, wherein the method is conducted at a pH of between 4 and 6.

21. The method of claim 2, wherein the method comprises the step of applying microwave radiation.

22. The method of claim 8, wherein the method is conducted at a pH of between 4 and 6.

23. The method of claim 8, wherein the method comprises the step of applying microwave radiation.

24. A method of peptide synthesis comprising a step of reacting a compound to form a polypeptide or protein, the compound having the following structural formula:



wherein

n is 2 or 3;

m is 2 or 3;

and RC(O)- is an amino acyl or peptidyl group, or R is selected from the group consisting of hydrogen, oxygen, sulfur, alkyl, alkenyl, alkynyl, thioalkyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, halo, carboxyl, haloalkyl, haloalkynyl, hydroxyl, alkoxy, thioalkoxy, alkenyloxy, haloalkoxy, haloalkenyloxy, nitro, amino, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroheterocyclyl, alkylamino, dialkylamino, alkenylamine, alkynylamino, acyl, alkenoyl, alkynoyl, acylamino, diacylamino, acyloxy, alkylsulfonyloxy, heterocycloxy, heterocycloamino, haloheterocycloalkyl, alkylsulfenyl, alkylcarbonyloxy, alkylthio, acylthio, phosphorus-containing groups such as phosphonyl and phosphinyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(alkyl), and -C(O)N(alkyl)$_2$.

25. The method of claim 24, wherein the method is conducted at a pH of between 4 and 6.

26. The method of claim 24, wherein the method comprises the step of applying microwave radiation.