

Scheme 2. *N*-to-*S* acyl transfer using an *N,N*-di-(2-mercaptoethyl)-substituted tertiary amide to generate thioester peptides for NCL.

Summary

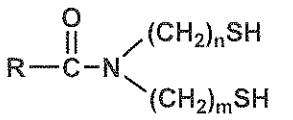
Peptide thioesters are essential building blocks for protein synthesis. The technology we have developed provides easy access to peptide thioesters which are convenient to prepare by Fmoc solid phase peptide synthesis and are also relatively efficient for use in NCL with a cysteinyl-peptide.

Our design is based on a C-terminal tertiary amide, namely N,N-bis(2-mercaptoethy)-amide or BMEA of which the nitrogen is substituted with two beta-mercaptoethyl or HS-et groups.

The presence of two such HS-Et *N*-substitutions makes this amide always ready to undergo an intramolecular thiolysis reaction (i.e. *N*→*S* acyl transfer) to convert itself to a thioester. Once formed, the thioester product can be used directly for NCL with a cysteinyl peptide, leading to formation of the ligation. In contrast, other thioester precursors do not allow both thioester and thio-thioester exchange to occur in one step and require the additional step of generating the thioester before NCL can proceed.

We have also devised a straightforward method for preparing the BMEA linker-derived resin onto which a peptide chain can be assembled easily using standard Fmoc solid phase peptide synthesis protocols. Model studies with a panel of selected BMEA peptides have validated our hypothesis and demonstrated the scope of these peptides for NCL as well as their utility in protein synthesis (e.g. histone H3 synthesis).

In one aspect, there is provided 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:



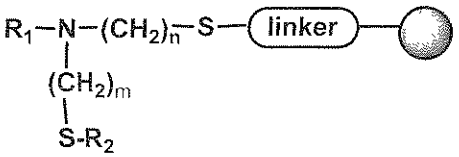
wherein

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)$_2$.

In one aspect, there is provided 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;

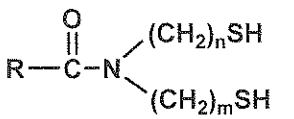
m is 2 or 3;

R_1 is H or Fmoc;

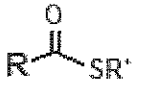
R_2 is H or a protecting group; and

"linker" is any linker compatible with Fmoc chemistry.

In one aspect, there is provided 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

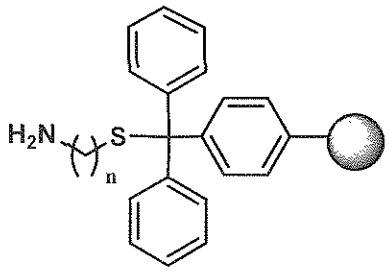
n is 2 or 3;

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)$_2$.

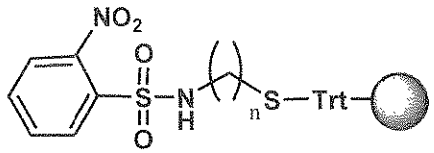
In one aspect, there is provided a method comprising steps of:

(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):

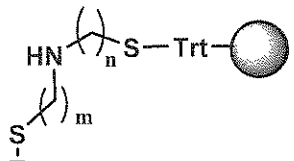


(B);

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

(iii) removing the sulfonyl group thiolytically;

thereby producing a resin of formula (C):



(C)

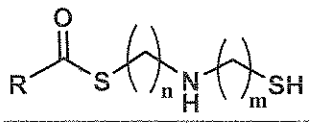
wherein:

Trt is trityl;

n is 2 or 3; and

m is 2 or 3.

In one aspect, there is provided 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)$_2$.

These and other aspects of the ~~invention~~disclosure will be apparent from the more detailed description of the ~~invention~~disclosure below and the Examples section of this specification.

It should be understood that whilst various references are made herein to BMEA / BMEA moieties, the ~~invention~~disclosure is not limited to such moieties and, as such, references herein to

BMEA / BMEA moieties apply *mutatis mutandis* to variants of BMEA / BMEA moieties as taught herein, unless the context indicates otherwise. In this regard, we have demonstrated that variants of BMEA work in which one N-substitution is 2-mercaptoethyl and one is 3-mercaptopropyl (see Example 4). Further, the teachings herein in relation to BMEA / BMEA moieties may be modified by the use of a cysteine in place of a mercaptoethylamine of the