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(54) Title: PEPTIDE COMPOUNDS, CONJUGATES THEREOF, AND USES THEREOF

(57) Abstract: Disclosed are peptide compounds of the formula I, drug-reactive linker group conjugates, drug-ligand conjugates, pharmaceutical compositions comprising the compounds and conjugates, and uses thereof. The compounds have useful inhibitory activity against cancer, mitochondrial uncoupling activity, and activity in depolarising membranes, such as mitochondrial membranes.



PEPTIDE COMPOUNDS, CONJUGATES THEREOF, AND USES THEREOF**FIELD OF THE INVENTION**

[0001] The present invention generally relates to peptide compounds of the formula I
5 having useful inhibitory activity against cancer, mitochondrial uncoupling activity, and
activity in depolarising membranes, such as mitochondrial membranes, and to drug-
reactive linker group conjugates, drug-ligand conjugates, pharmaceutical compositions
comprising the compounds and conjugates, and uses thereof.

BACKGROUND TO THE INVENTION

[0002] A number of biologically active peptides are known in the art. Many of these
peptides contain unusual amino acid residues, for example, the rare and synthetically
challenging (2*S*,4*S*,6*R*)-2-amino-6-hydroxy-4-methyl-8-decanoic acid (AHMOD) residue.
One such peptide is culicinin D, which was isolated from *Culicinomyces clavisporus* and
15 found to have useful anti-cancer activity in the MDA-MB-468 cell line.

[0003] Despite their useful activity, a number of biologically active peptides continue to
be limited with regard to their use *in vivo*. One reason for this limitation may be the
difficulty associated with the selective delivery of such therapeutic compounds to an area to
be treated, for example to cancer cells. This lack of selective delivery may in turn lead to
20 problems associated with subtherapeutic or suboptimal dosing and/or an increased
incidence of side effects.

[0004] There is an ongoing need for compounds with useful activity against cancer and
other diseases or conditions. There is also a need for ligand-drug conjugates, such as
antibody-drug conjugates, capable of selectively delivering or targeting compounds to an
25 area to be treated, for example selectively delivering or targeting compounds to cancer cells
and/or cells affected by other diseases or conditions.

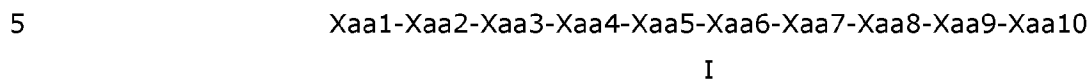
[0005] It is an object of the present invention to go some way to meeting one or more
of these needs; and/or to at least provides the public with a useful choice.

[0006] Other objects of the invention may become apparent from the following
30 description which is given by way of example only.

[0007] In this specification, where reference has been made to external sources of
information, including patent specifications and other documents, this is generally for the
purpose of providing a context for discussing the features of the present invention. Unless
stated otherwise, reference to such sources of information is not to be construed, in any
35 jurisdiction, as an admission that such sources of information are prior art or form part of
the common general knowledge in the art.

SUMMARY OF THE INVENTION

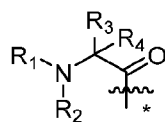
[0008] In a first aspect the present invention provides a compound of formula I or a pharmaceutically acceptable salt or solvate thereof



wherein:

Xaa1 is a group of formula IA

10



IA

wherein

15

R₁ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C(O)C₁-C₁₅alkyl, (CH₂CH₂O)_k-R_d, C(O)(CH₂CH₂O)_k-R_d, C₁-C₁₅alkyl(Q_d), C(O)C₁-C₁₅alkyl(Q_d), C₁-C₁₅alkyl(aryl), C₁-C₁₅alkyl(C₃-C₁₂carbocycle), C₁-C₁₅alkyl(3 to 12 membered heterocycle), or C₁-C₁₅alkyl(heteroaryl);

20

R₂ and R₃ are each independently H, C₁-C₈alkyl, C₁-C₈haloalkyl, or C₃-C₁₂carbocycle; or R₂ and R₃ together form a ring and have the formula -(CR^mRⁿ)_n- wherein n is from 1 to 6, and at each instance of n R^m is independently H, OH, C₁-C₈alkyl, or C₃-C₁₂carbocycle, and Rⁿ is independently H, C₁-C₈alkyl, or C₃-C₁₂carbocycle, or R^m and Rⁿ together represent =O; and

25

R₄ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl; or R₃ and R₄ together with the carbon atom to which they are attached form a C₃-C₈carbocycle or as 3 to 8 membered heterocycle; R_d is H, C₁-C₈alkyl or C₁-C₈haloalkyl; k is an integer from 1 to 10;

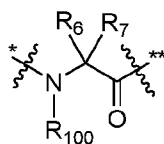
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Q_d is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a; R_a and R_b are each independently H, C₁-C₈alkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, or heteroaryl; and

35

* denotes the bond to Xaa2;

Xaa2 is a group of formula IB



IB

wherein

R₆ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_D)_q), C₁-C₈alkyl(3 to 12 membered heterocycle), C₁-C₈alkyl(heteroaryl), C₁-C₁₅alkyl(Q_a), or C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

Q_a is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a;

Q_b and Q_c are each independently H, OR_c, SR_c, or NR_cR_d;

X is at each instance independently O, S, or NR_e;

R_a, R_b, R_c, R_d, and R_e are each independently H, C₁-C₈alkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, or heteroaryl;

q is from 0 to 3;

R_D at each instance of q is independently OH, OR_a, SR_a, NR_aR_b, halo, C₁-8alkyl, C₁-C₃haloalkyl;

R₇ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl;

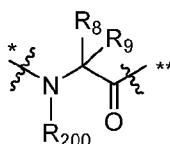
or R₆ and R₇ together with the carbon atom to which they are attached form a 3 to 8 membered heterocycle or C₃-C₈carbocycle;

R₁₀₀ is H or C₁-C₃alkyl;

* denotes the bond to Xaa1; and

** denotes the bond to Xaa3;

Xaa5 is a group of formula IC



IC

wherein

R₈ is C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₁-C₁₅alkyl(aryl), C₁-C₁₅alkyl(C₃-C₁₂carbocycle), C₁-C₁₅alkyl(C₃ to 12 membered heterocycle), or C₁-C₁₅alkyl(heteroaryl);

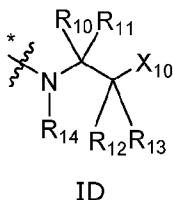
R₉ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl;

R₂₀₀ is H or C₁-C₃alkyl;

* denotes the bond to Xaa4; and

** denotes the bond to Xaa6;

Xaa10 is OH, NR_wR_z or a group of formula ID



wherein

R₁₀ and R₁₂ are each independently H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl);

R₁₁ and R₁₃ is H, C₁-C₃alkyl or C₁-C₃haloalkyl;

or R₁₀ and R₁₁ together with the carbon atom to which they are attached form a C₃-C₈carbocycle or a 3 to 8 membered heterocycle;

or R₁₂ and R₁₃ together with the carbon atom to which they are attached form a C₃-C₈carbocycle or a 3 to 8 membered heterocycle;

R₁₄ is H or C₁-C₃alkyl;

X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;

R₁₅, R₁₆, R₁₇, and R₁₈ are each independently H, C₁-C₈alkyl, C₁-C₈haloalkyl, or C₁-C₈alkyl(Y₁₀);

or R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle or heteroaryl ring;

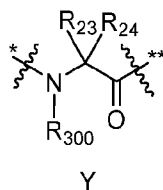
Y₁₀ is OR₁₉, NR₂₀R₂₁ or SR₂₂;

R₁₉, R₂₀, R₂₁, and R₂₂ are each independently H, C₁-C₈alkyl, C₁-C₈haloalkyl;

R_w and R_z are each independently H or C₁-C₃alkyl;

* denotes the bond to Xaa9;

Xaa3, Xaa4, Xaa6, Xaa7 and Xaa8 are each independently a group of formula Y



wherein

R₂₃ is H, C₁-C₈alkyl, C₁-C₈haloalkyl, C₁-C₈alkyl(aryl), C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl);

R₂₄ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl;

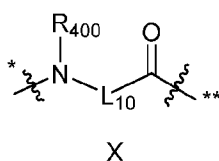
or R₂₃ and R₂₄ together with the carbon atom to which they are attached form a C₃-C₈carbocycle or a 3 to 8 membered heterocycle;

R₃₀₀ is H or C₁-C₃alkyl;

* denotes the bond to Xaa2 in the case of Xaa3, Xaa3 in the case of Xaa4, Xaa5 in the case of Xaa6, Xaa6 in the case of Xaa7, and Xaa7 in the case of Xaa8; and

** denotes the bond to Xaa4 in the case of Xaa3, Xaa5 in the case of Xaa4, Xaa7 in the case of Xaa6, Xaa8 in the case of Xaa7, and Xaa9 in the case of Xaa8; and

Xaa9 is absent, is independently a group of formula Y as defined above, or is a group of the formula X



wherein

R₄₀₀ is H or C₁-C₃alkyl;

L₁₀ is a group of the formula $-(CR^hR^i)_h-$ or $-(CR^hR^i)_i-X^a-(CR^jR^k)_j-$;

R^h and R^j are each independently selected from H, C₁-C₈alkyl, C₁-C₈haloalkyl, C₁-C₈alkyl(aryl), C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl);

Rⁱ and R^k are each independently selected from H, C₁-C₃alkyl, or C₁-C₃haloalkyl; and X^a is selected from O, S, NH, or N(C₁-C₈alkyl);

h, i, and j are each independently selected from 1 to 5, provided that the sum of i and j is 5 or less;

* denotes the bond to Xaa8; and

** denotes the bond to Xaa10;

wherein any alkyl, aryl, carbocycle, heterocycle, or heteroaryl in any of the groups for

R₁, R₂, R₃, R^m, Rⁿ, and R₄ in **Xaa1**,

R₆, Q_a, Q_b, Q_c, X, R_a, R_b, R_c, R_d, R_e, R_D, and R₇ in **Xaa2**,

R₈ and R₉ in **Xaa5**,

R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, X₁₀, R₁₅, R₁₆, R₁₇, R₁₈, Y₁₀, R₁₉, R₂₀, R₂₁, and R₂₂ in **Xaa10**,

R₂₃ and R₂₄ in **Xaa3**, **Xaa4**, **Xaa6**, **Xaa7** and **Xaa8**, and

R₂₃, R₂₄, L₁₀, R^h, Rⁱ, R^j, R^k, and X^a in **Xaa9**

are optionally substituted with one or more optional substituents;

and, preferably, wherein any aryl in any one of the aforementioned groups for Xaa1-Xaa10 is a C₆-C₂₀aryl, preferably C₆-C₁₀aryl, and any heteroaryl in any of the aforementioned groups for Xaa1-Xaa10 is a 5-20 membered heteroaryl, preferably a 5-10 membered heteroaryl;

provided that the compound is not: Culicinin A, Culicinin B, Culicinin C, Culicinin D (R), Culicinin D (S), Leucinostatin A, Leucinostatin A2, Leucinostatin B, Leucinostatin B2, Leucinostatin C, Leucinostatin D, Leucinostatin F, Leucinostatin L, Leucinostatin N, Leucinostatin R, Leucinostatin S, Leucinostatin T, Leucinostatin U, Leucinostatin V,

5 Leucinostatin *I*, Leucinostatin *III*, Leucinostatin *IV*, Leucinostatin *V*, Helioferin A, Helioferin B, Roseoferin A₁, Roseoferin A₂, Roseoferin A₃, Roseoferin B₁, Roseoferin B₂, Roseoferin B₃, Roseoferin C₁, Roseoferin C₂, Roseoferin D₁, Roseoferin D₂, Roseoferin D₃, Roseoferin E₁, Roseoferin E₂, Roseoferin E₃, Roseoferin F, Roseoferin G, Trichoderin A, Trichoderin A1, Trichoderin B, Trichopolyne I, Trichopolyne II, Trichopolyne III, Trichopolyne IV,
10 Trichopolyne V, Trichopolyne VI, or Emericellipsin A (the structures of which compounds are shown in claim 1 below).

[0009] In another aspect the present invention provides a drug-reactive linker conjugate comprising a compound of formula I of the invention or a compound listed in the proviso relating thereto, and a reactive linker group having a reactive site that allows the
15 reactive linker group to be reacted with a ligand.

[0010] In one aspect the present invention provides a pharmaceutical composition comprising:

a compound of formula I of the invention; or
a ligand-drug conjugate of the invention; and
20 a pharmaceutically acceptable carrier.

[0011] In another aspect the present invention provides a compound of formula I, a ligand-drug conjugate of the invention or a pharmaceutical composition of the invention for use in therapy.

[0012] In another aspect the present invention provides a method of treating or
25 preventing a disease or condition susceptible to treatment with a mitochondrial uncoupling agent or complications associated with a disease or condition susceptible to treatment with a mitochondrial uncoupling agent in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention or a
30 pharmaceutical composition of the invention.

[0013] In another aspect the present invention provides a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention, or a pharmaceutical composition of the invention for use in treating or preventing a disease or condition susceptible to treatment with a mitochondrial uncoupling agent or complications
35 associated with a disease or condition susceptible to treatment with a mitochondrial uncoupling agent.

[0014] In another aspect the present invention provides a use of a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention a pharmaceutical composition of the invention in the manufacture of a

medicament for treating or preventing a disease or condition susceptible to treatment with a mitochondrial uncoupling agent or complications associated with a disease or condition susceptible to treatment with a mitochondrial uncoupling agent.

[0015] In another aspect the present invention provides a method of killing a cell, the method comprising administering to the subject an effective amount of a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention or a pharmaceutical composition of the invention.

[0016] In another aspect the present invention provides a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention, or a pharmaceutical composition of the invention for use in killing a cell.

[0017] In another aspect the present invention provides use of a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention or a pharmaceutical composition of the invention the manufacture of a medicament for killing a cell.

[0018] In another aspect the present invention provides a method of killing or inhibiting proliferation of tumour cells or cancer cells, the method comprising administering to the subject an effective amount of a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention or a pharmaceutical composition of the invention.

[0019] In another aspect the present invention provides a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention, or a pharmaceutical composition of the invention for use in killing or inhibiting proliferation of tumour cells or cancer cells.

[0020] In another aspect the present invention provides a use of a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention or a pharmaceutical composition of the invention in the manufacture of a medicament for killing or inhibiting proliferation of tumour cells or cancer cells.

[0021] In another aspect the present invention provides a method of treating or preventing an autoimmune disease, the method comprising administering to the subject an effective amount of a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention or a pharmaceutical composition of the invention.

[0022] In another aspect the present invention provides a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of the invention or a pharmaceutical composition of the invention for use in treating or preventing an autoimmune disease.

[0023] In another aspect the present invention provides a use of a compound of formula I or a compound listed in the proviso relating thereto, a ligand-drug conjugate of

the invention or a pharmaceutical composition of the invention in the manufacture of a medicament for treating or preventing an autoimmune disease.

[0024] In another aspect the present invention provides a method or assay of detecting cancer cells comprising exposing cells to a ligand-drug conjugate of the invention and determining the extent of binding of the ligand-drug conjugate to the cells.

[0025] In another aspect the present invention provides a method of depolarising a membrane, the method comprising contacting the membrane with a compound of formula I of the invention or a compound in the proviso relating thereto, a ligand-drug conjugate of of the invention or a pharmaceutical composition of the invention.

[0026] A compound of formula I of the invention or a compound in the proviso relating thereto, a ligand-drug conjugate of the invention or a pharmaceutical composition of the invention for depolarising a membrane.

[0027] Use a compound of formula I of the invention or a compound in the proviso relating thereto, a ligand-drug conjugate of the invention or a pharmaceutical composition of the invention in the manufacture of a medicament for depolarising a membrane.

[0028] The following embodiments and preferences may relate alone or in combination of any two or more to any of the aspects herein.

[0029] In some embodiments in **Xaa1** R_1 is H, C_1 - C_{15} alkyl, C_1 - C_{15} haloalkyl, and $C(O)C_1$ - C_{15} alkyl, $C(O)C_1$ - C_{15} haloalkyl, $(CH_2CH_2O)_k-R_d$, $C(O)(CH_2CH_2O)_k-R_d$, C_1 - C_{15} alkyl(Q_d), $C(O)C_1$ - C_{15} alkyl(Q_d), preferably H, C_1 - C_{15} alkyl, C_1 - C_{15} haloalkyl, and $C(O)C_1$ - C_{15} alkyl, $C(O)C_1$ - C_{15} haloalkyl, preferably H or $C(O)C_1$ - C_{15} alkyl.

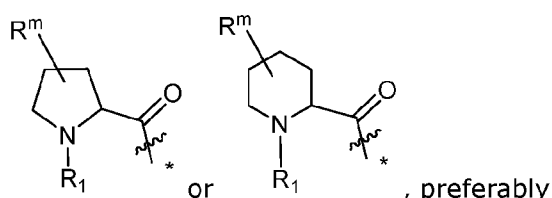
[0030] In some embodiments in **Xaa1** R_2 and R_3 together form a ring.

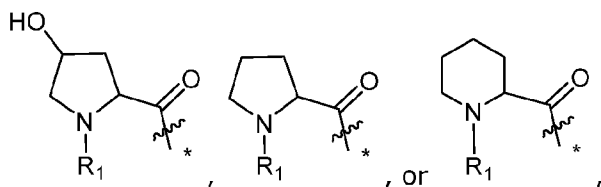
[0031] In some embodiments in **Xaa1** R_2 and R_3 together form a ring and n is an integer selected from 2 to 6, 3 to 6, 3 to 5, or, preferably, 3 to 4.

[0032] In some embodiments in **Xaa1** R_2 and R_3 together form a ring and R^m is independently H, OH, or C_1 - C_8 alkyl, preferably H or OH, and R^n is independently H or C_1 - C_8 alkyl, preferably H, or R^m and R^n together represent =O.

[0033] In some embodiments in **Xaa1** R_2 and R_3 together form a ring and R^m is independently H, OH, or C_1 - C_6 alkyl, preferably H or OH, and R^n is independently H or C_1 - C_6 alkyl, preferably H.

[0034] In some embodiments **Xaa1** is a group of formula:





wherein R^m , R_1 and $*$ are as defined herein.

[0035] In some embodiments in Xaa1 R_1 is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C(O)C₁-C₁₅alkyl, and C(O)C₁-C₁₅haloalkyl, preferably H or C(O)C₁-C₁₅alkyl, preferably H or C(O)C₁-C₁₅alkyl, preferably H or C(O)C₁-C₁₁alkyl.

[0036] In some embodiments in Xaa1 R_2 and R_3 are each independently H, C₁-C₈alkyl, C₁-C₈haloalkyl, or C₃-C₁₂carbocycle, preferably H, C₁-C₈alkyl, or C₁-C₈haloalkyl, preferably H or C₁-C₈alkyl.

[0037] In some embodiments in Xaa1 R_2 is H or C₁-C₆alkyl, preferably H or C₁-C₄alkyl, preferably H.

[0038] In some embodiments in Xaa1 R_3 is C₁-C₈alkyl, preferably C₁-C₆alkyl, preferably C₁-C₄alkyl, preferably CH₃.

[0039] In some embodiments in Xaa1 R_1 is C(O)C₁-C₁₅alkyl, preferably C(O)C₁-C₁₀alkyl, preferably C(O)C₁-C₈alkyl, preferably C(O)C₁-C₅alkyl, preferably C(O)C₁-C₃alkyl.

[0040] In some embodiments in Xaa1 R_1 is (CH₂CH₂O)_k-R_d, C(O)(CH₂CH₂O)_k-R_d, C₁-C₁₅alkyl(Q_d), or C(O)C₁-C₁₅alkyl(Q_d), preferably C₁-C₁₅alkyl(Q_d) or C(O)C₁-C₁₅alkyl(Q_d), preferably C(O)C₁-C₁₅alkyl(Q_d), preferably C(O)C₁-C₁₀alkyl(Q_d), preferably C(O)C₁-C₈alkyl(Q_d), preferably C(O)C₁-C₅alkyl(Q_d).

[0041] In some embodiments in Xaa1 Q_d is OR_a, SR_a, or NR_aR_b, preferably OR_a or NR_aR_b; and optionally R_a and R_b are each independently H or C₁-C₈alkyl, preferably H or CH₃.

[0042] In some embodiments in Xaa1 R_4 is H, C₁-C₃alkyl, or C₁-C₃haloalkyl, preferably H or C₁-C₃alkyl, preferably H.

[0043] In some embodiments Xaa2 is a group of formula IB, wherein R_7 is H, C₁-C₃alkyl, or C₁-C₃haloalkyl, preferably H or C₁-C₃alkyl, preferably H or CH₃.

[0044] In some embodiments Xaa2 is a group of formula IB, wherein

R_6 is C₁-C₁₅alkyl or C₁-C₁₅haloalkyl, preferably C₁-C₁₅alkyl, preferably C₄-C₁₅alkyl, C₆-C₁₅alkyl, C₈-C₁₅alkyl, or C₁₀-C₁₅alkyl.

[0045] In some embodiments Xaa2 is a group of formula IB, wherein R_6 is C₁-C₁₅alkyl(Q_a).

[0046] In some embodiments Xaa2 is a group of formula IB, wherein Q_a is OR_a, SR_a, or NR_aR_b, preferably OR_a or SR_a;

[0047] In some embodiments Xaa2 is a group of formula IB, wherein R_a and R_b are each H or C₁-C₈alkyl.

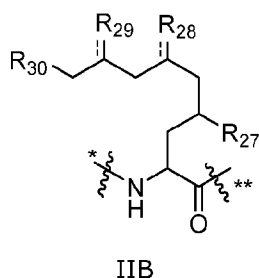
[0048] In some embodiments Xaa2 is a group of formula IB, wherein R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c).

[0049] In some embodiments Xaa2 is a group of formula IB, wherein X is O.

[0050] In some embodiments Xaa2 is a group of formula IB, wherein Q_b and Q_c are each independently H or OR_c, preferably H or OH.

[0051] In some embodiments Xaa2 is a group of formula IB, wherein R_c, R_d, and R_e are each independently H or C₁-C₈alkyl.

[0052] In some embodiments **Xaa2** is a group of formula IIB



wherein

R₂₇ is H or C₁-C₈alkyl, preferably H or CH₃;

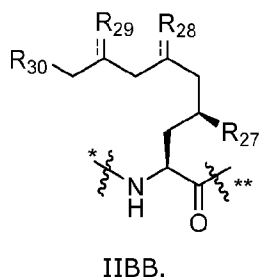
== is a single bond or a double bond;

R₂₈ and R₂₉ are each independently H or OH and == is a single bond or O and == is a double bond; and

R₃₀ is H or C₁-C₈alkyl.

[0053] In some embodiments (i) R₂₈ and R₂₉ are each independently H; (ii) R₂₈ is OH or O and R₂₉ is H; or (iii) R₂₈ is OH and R₂₉ is O.

[0054] In some embodiments **Xaa2** is a group of formula IIBB



[0055] In some embodiments Xaa2 is a group of formula IB, wherein R₆ is C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_d)q), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably C₃-C₁₂carbocycle, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_d)q), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably C₃-C₁₂carbocycle, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_d)q), or C₁-C₈alkyl(heteroaryl).

[0056] In some embodiments Xaa2 is a group of formula IB, wherein R₆ and R₇ together form a 3 to 8 membered heterocycle or C₃-C₈carbocycle, preferably a C₃-C₈carbocycle, preferably a C₃-C₆carbocycle.

[0057] In some embodiments in Xaa5 R8 is C1-C15alkyl or C1-C15haloalkyl, preferably C2-C12alkyl, preferably C2-C9alkyl, C3-C9alkyl, or C4-C9alkyl.

[0058] In some embodiments Xaa5 is a group of formula IC wherein R9 is H or C1-C3alkyl, preferably H.

5 **[0059]** In some embodiments **Xaa10** is a group of formula ID wherein
 R₁₀ and R₁₂ are each independently H, C1-C15alkyl, C1-C15haloalkyl, C3-C12carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C1-C8alkyl(C3-C12carbocycle), C1-C8alkyl(aryl), C1-C8alkyl(3 to 12 membered heterocycle), or C1-C8alkyl(heteroaryl), preferably H, C1-C15alkyl, C3-C12carbocycle, aryl, C1-C8alkyl(C3-C12carbocycle), or C1-C8alkyl(aryl); and
 10 R₁₁ and R₁₃ is H, C1-C3alkyl or C1-C3haloalkyl, preferably H or C1-C3alkyl.

[0060] In some embodiments **Xaa10** is a group of formula ID wherein
 R₁₀ is H, C1-C15alkyl, C1-C15haloalkyl, C3-C12carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C1-C8alkyl(C3-C12carbocycle), C1-C8alkyl(aryl), C1-C8alkyl(3 to 12 membered heterocycle), or C1-C8alkyl(heteroaryl), preferably H, C1-C15alkyl, C3-C12carbocycle, aryl, C1-C8alkyl(C3-C12carbocycle), or C1-C8alkyl(aryl), preferably H, C1-C10alkyl, or C1-C8alkyl(aryl); and
 15 R₁₁, R₁₂, and R₁₃ is H, C1-C3alkyl or C1-C3haloalkyl, preferably H or C1-C3alkyl, preferably H.

20 **[0061]** In some embodiments **Xaa10** is a group of formula ID wherein
 R₁₀ is H, C1-C8alkyl, preferably C1-C6alkyl, preferably C1-C4alkyl, preferably CH₃, or C1-C6alkyl(aryl), preferably C1-C4alkyl(aryl), preferably C1-C3alkyl(aryl).

[0062] In some embodiments Xaa10 is a group of formula ID wherein R₁₁ is H.

[0063] In some embodiments **Xaa10** is a group of formula ID wherein
 25 R₁₂ is H, C1-C15alkyl, C1-C15haloalkyl, C3-C12carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C1-C8alkyl(C3-C12carbocycle), C1-C8alkyl(aryl), C1-C8alkyl(3 to 12 membered heterocycle), or C1-C8alkyl(heteroaryl), preferably H, C1-C15alkyl, C3-C12carbocycle, aryl, C1-C8alkyl(C3-C12carbocycle), or C1-C8alkyl(aryl), preferably H, C1-C10alkyl, or aryl; and
 30 R₁₀, R₁₁, and R₁₃ is H, C1-C3alkyl or C1-C3haloalkyl, preferably H or C1-C3alkyl, preferably H.

[0064] In some embodiments **Xaa10** is a group of formula ID wherein R₁₂ is H or aryl.

[0065] In some embodiments **Xaa10** is a group of formula ID wherein R₁₃ is H.

[0066] In some embodiments **Xaa10** is a group of formula ID wherein R₁₄ is H.

35 **[0067]** In some embodiments **Xaa10** is a group of formula ID wherein
 X₁₀ is OR₁₅ or NR₁₆R₁₇;
 R₁₅ is H, C1-C8alkyl, preferably H;
 R₁₆ is H, C1-C8alkyl, or C1-C8alkyl(Y₁₀), preferably C1-C8alkyl or C1-C8alkyl(Y₁₀);
 R₁₇ is H or C1-C8alkyl, preferably H;

or R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle;

Y₁₀ is OR₁₉, NR₂₀R₂₁ or SR₂₂, preferably OR₁₉, preferably OH;

R₁₉, R₂₀, R₂₁, and R₂₂ are each independently H or C₁-C₈alkyl, preferably H.

5 **[0068]** In some embodiments **Xaa10** is a group of formula ID wherein

X₁₀ is OH or NR₁₆R₁₇;

R₁₆ is H;

R₁₇ is C₁-C₈alkyl, preferably C₁-C₄alkyl, preferably CH₃, or C₁-C₈alkyl(Y₁₀), preferably C₁-C₄alkyl(Y₁₀), preferably C₂-C₄alkyl(Y₁₀), CH₂CH₂(Y₁₀);

10 or R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle; and

Y₁₀ is OH.

[0069] In some embodiments **Xaa10** is a group of formula ID wherein

X₁₀ is OH or NR₁₆R₁₇;

15 R₁₆ is C₁-C₈alkyl, preferably C₁-C₄alkyl, preferably CH₃;

R₁₇ is C₁-C₈alkyl, preferably C₁-C₄alkyl, preferably CH₃, or C₁-C₈alkyl(Y₁₀), preferably C₁-C₄alkyl(Y₁₀), preferably C₂-C₄alkyl(Y₁₀), CH₂CH₂(Y₁₀);

or R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle; and

20 Y₁₀ is OH.

[0070] In some embodiments Xaa10 is a group of formula ID wherein R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle, for example a morpholinyl or piperazinyl ring.

[0071] In some embodiments in the formula Y

25 R₂₃ is H, C₁-C₈alkyl, or C₁-C₈haloalkyl, preferably H or C₁-C₈alkyl, preferably C₁-C₈alkyl;

R₂₄ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl, preferably H or C₁-C₈alkyl;

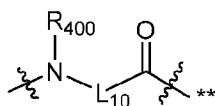
or R₂₃ and R₂₄ together with the carbon atom to which they are attached form a C₃-C₈carbocycle.

[0072] In some embodiments in the formula Y R₂₃ is C₁-C₈alkyl; and R₂₄ is H or C₁-

30 C₈alkyl, preferably R₂₃ is C₁-C₈alkyl and R₂₄ is C₁-C₈alkyl or R₂₃ is C₂-C₈alkyl, preferably C₃-C₈alkyl, and R₂₄ is H.

[0073] In some embodiments **Xaa3**, **Xaa4**, and/or **Xaa7** is a 2-aminoisobutyric acid residue; and/or **Xaa6** and/or **Xaa8** is a leucine residue.

35 **[0074]** In some embodiments **Xaa9** is independently a group of formula Y or is a group of the formula X



X.

[0075] In some embodiments **Xaa9** is a group of the formula X wherein

L_{10} is a group of the formula $-(CR^hR^i)_h-$ or $-(CR^hR^i)_i-X^a-(CR^jR^k)_j-$;

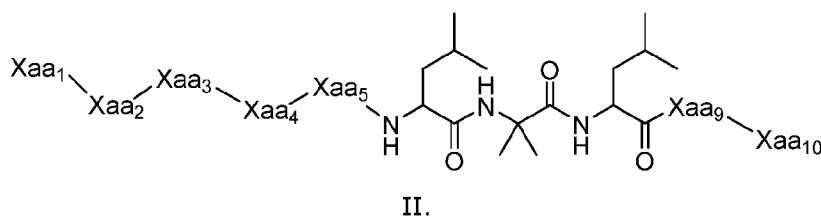
R^h and R^j are each independently selected from H or C_1-C_8 alkyl, preferably H;

R^i and R^k are each independently selected from H or C_1-C_3 alkyl, preferably H; and

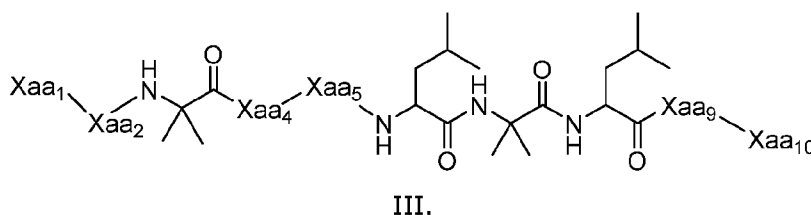
X^a is selected from O, S, NH, or $N(C_1-C_8$ alkyl).

[0076] In some embodiments **Xaa9** is a group of the formula X wherein L_{10} is a group of the formula $-(CR^hR^i)_h-$, preferably wherein h is from 1 to 4, preferably 2 to 4, preferably 2.

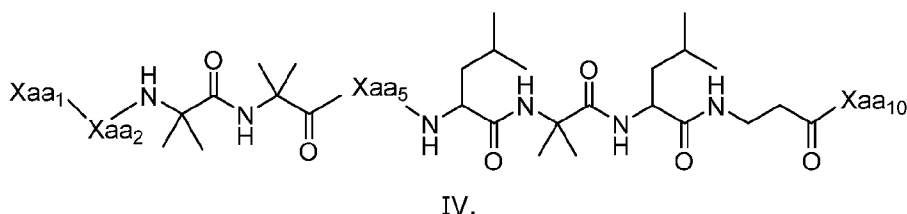
[0077] In some embodiments the compound is of the formula II or a pharmaceutically acceptable salt or solvate thereof



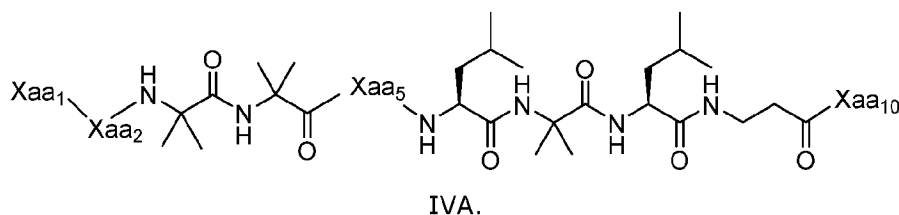
[0078] In some embodiments the compound is of the formula III or a pharmaceutically acceptable salt or solvate thereof



[0079] In some embodiments the compound is of the formula IV or a pharmaceutically acceptable salt or solvate thereof

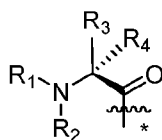


[0080] In some embodiments the compound is a compound of formula IVA or a pharmaceutically acceptable salt or solvate thereof



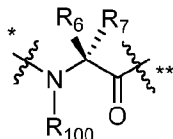
[0081] In some embodiments:

Xaa1 is a group of formula IAA



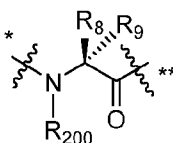
IAA;

and/or

Xaa2 is a group of formula IBB

IBB;

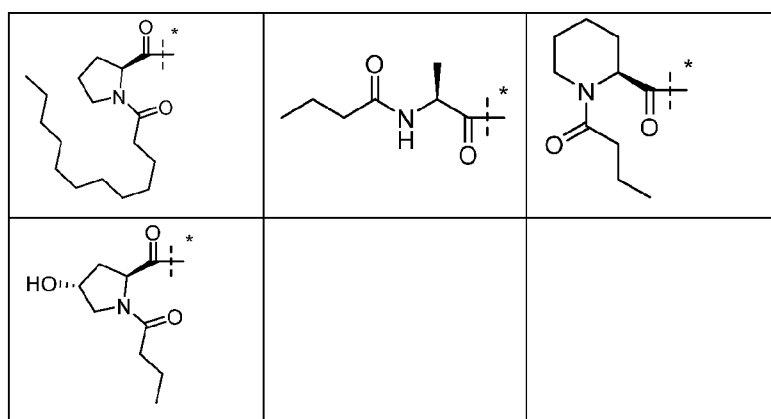
and/or

Xaa5 is a group of formula ICC

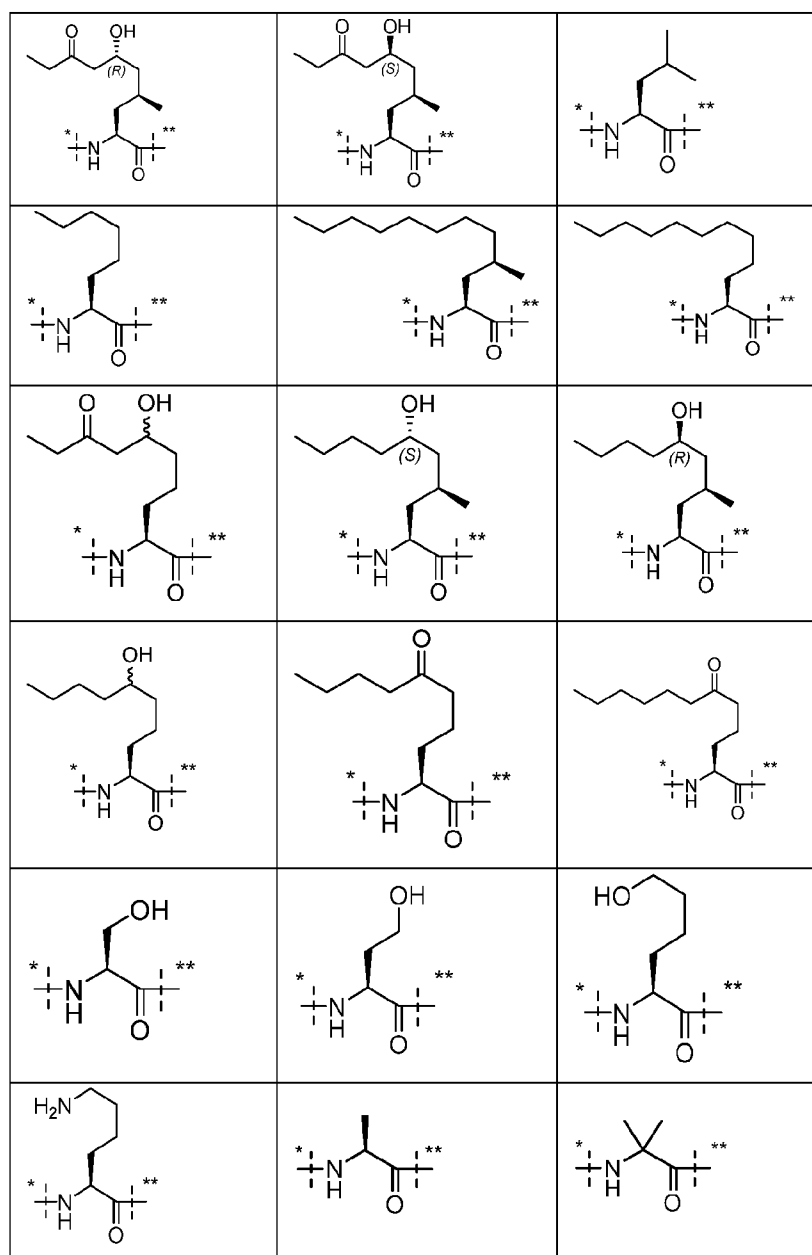
ICC.

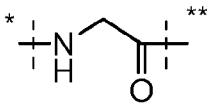
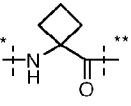
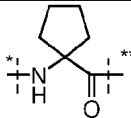
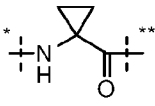
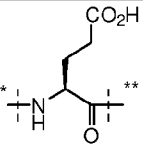
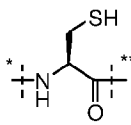
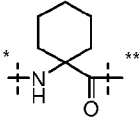
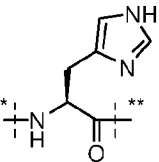
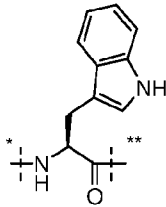
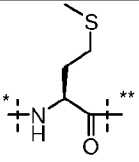
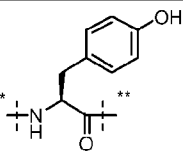
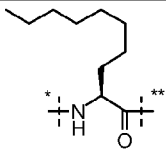
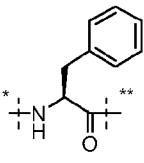
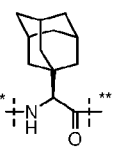
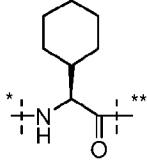
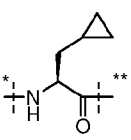
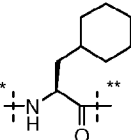
[0082] In some embodiments the one or more optional substituents are selected from the group consisting of halo, N₃, CN, NO₂, OH, NR^xR^y, C₁-C₈haloalkyl, C₁-C₈haloalkoxy, C(O)NR^xR^y, C(O)N(R^x)₂-NHC(O)R^x, SO₂R^x, SO₃R^x, OR^y, SR^x, S(O)R^x, S(O)₂R^x, C(O)R^x, OC(O)R^x, C(O)OR^x, C₁-C₈alkyl and aryl; wherein R^x and R^y are each independently H, aryl or C₁-C₈alkyl.

[0083] In some embodiments **Xaa1** is a group selected from the groups listed in the following table.

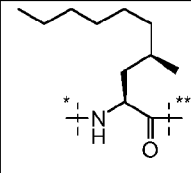
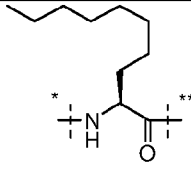
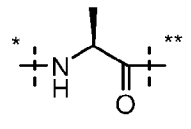
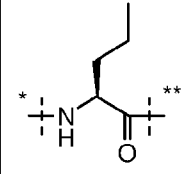
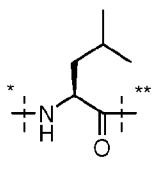
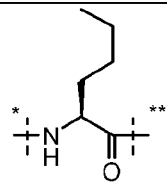
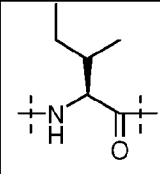


[0084] In some embodiments **Xaa2** is a group selected from the groups listed in the following table.

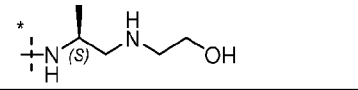
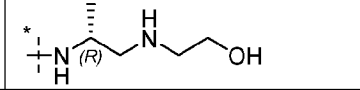
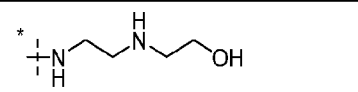
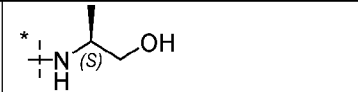
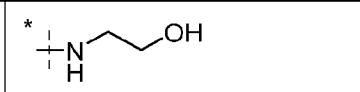
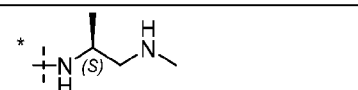
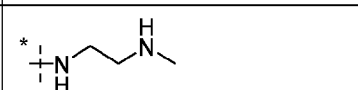
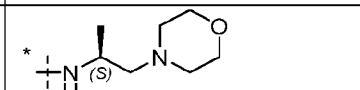
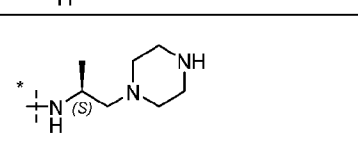
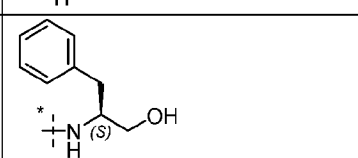
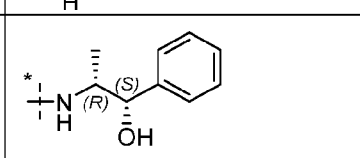
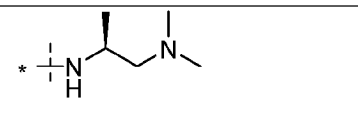
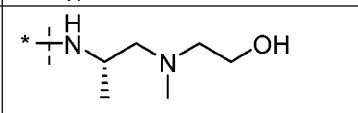


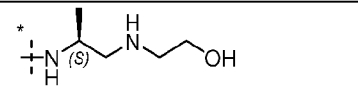
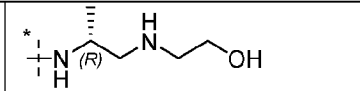
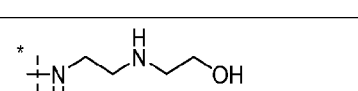
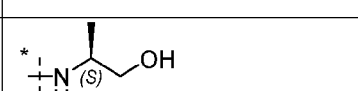
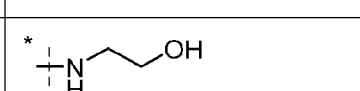
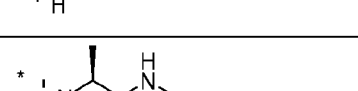
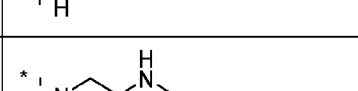
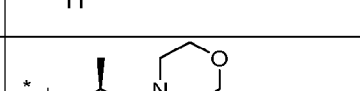
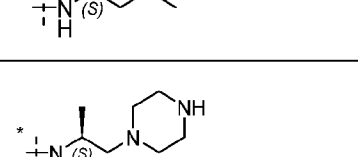
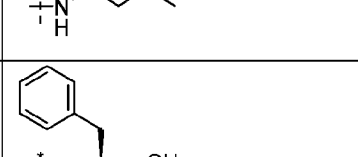
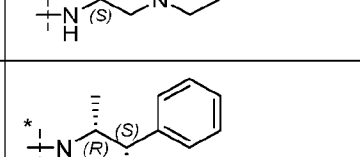
[0085] In some embodiments **Xaa5** is a group selected from the groups listed in the following table.

[0086] In some embodiments **Xaa10** is a group selected from the groups listed in the following table.

[0087] In some embodiments **Xaa10** is a group selected from the groups listed in the following table.

[0088] In some embodiments, the compound is selected from the following compounds of the Examples herein: 520, 1100g, 1200f, 1100d, 170, 500, 1100c, 1200g, 1100e, 450, 240, 51, 480, 510, 50, 420, 1100a, 1200c, 370, 1100h, 1100b, 200c, 490, 230, 200d, 49, 270, 440, 1100f, 54, 410, 320, 380, 390, 1200b, 1100i, 53, 200f, 310, 460, 44, 470, 200g, 260, 200e, 350, 1200h, 45, 360, 290, 250, 340, 46, 52, 210, 200, 42, 48, 430, 43, 220, 1200j, 190, 1200k, 47, 1200d, 200a, 1200e, 200b, 330, 300, 280, 55, 180, 1200a, 1200i, 400 and 200h.

[0089] In some embodiments, the compound is selected from the following compounds of the Examples herein 520, 1100g, 1200f, 1100d, 170, 500, 1100c, 1200g, 1100e, 450,

240, 51, 480, 510, 50, 420, 1100a, 1200c, 370, 1100h, 1100b, 200c, 490, 230, 200d, 49, 270, 440, 1100f, 54, 410, 320, 380, 390, 1200b, 1100i, 53, 200f, 310, 460, 44, 470, 200g, 260, 200e, 350, 1200h, 45, 360, 290, 250, 340, 46, 52, 210, 200, 42, 48, 430, 43, 220, 1200j, 190, 1200k, 47, 1200d, 200a, 1200e, 200b, 330, and 300.

5 **[0090]** In some embodiments, the compound is selected from the following compounds of the Examples herein 520, 1100g, 1200f, 1100d, 170, 500, 1100c, 1100e, 450, 240, 51, 480, 510, 49, and 270.

[0091] In some embodiments, the compound is selected from the following compounds of the Examples herein 1200l, 1200m, 1100j, 1100k, 1100l, 1100m, and 1100n.

10 **[0092]** In some embodiments, the compound has an IC_{50} against a cancer cell line selected from the group consisting of MDA-MB-468, SKBR3, T47D, and NCI-H460 of less than about 5 μ M, for example less than about 4, 3, 2, 1, 0.5, 0.4, 0.3, 0.2, 0.1, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01, 0.009, 0.008, 0.007, 0.006, 0.005, 0.004, or 0.003, or 0.002 μ M, for example as measured by an assays as described in the Examples.

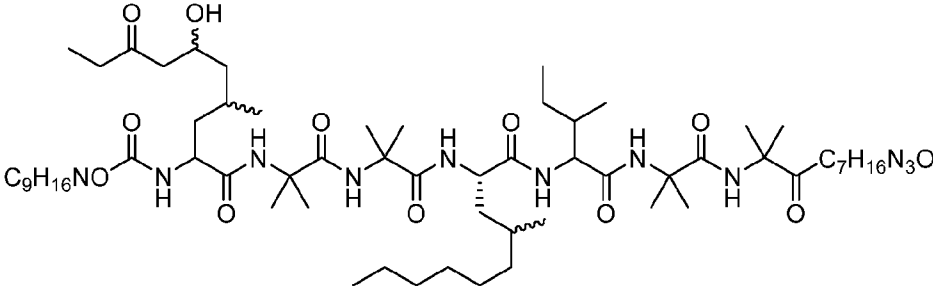
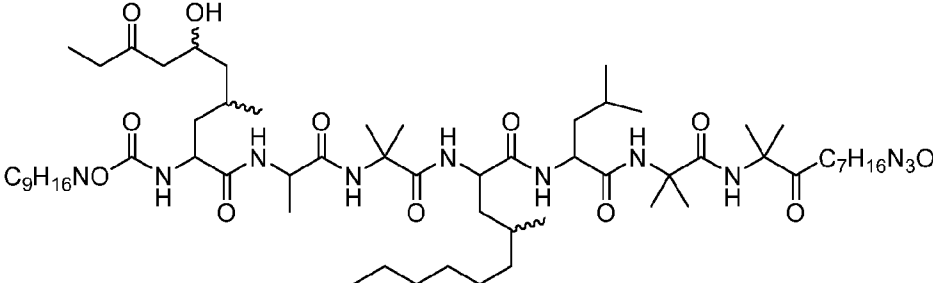
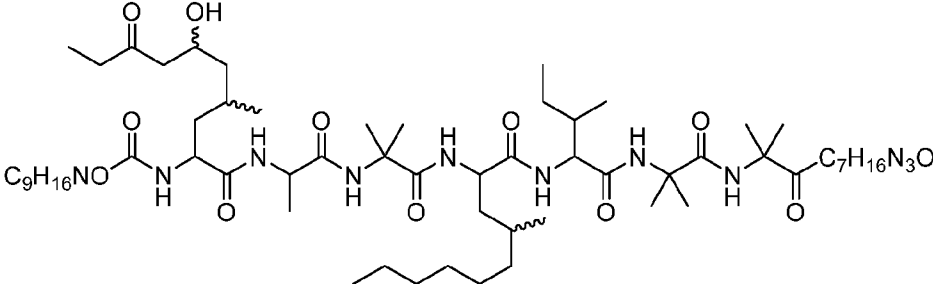
15 **[0093]** In some embodiments the compound has an EC_{50} of less than about 1000 nM, for example less than about 500, 100, 50, 40, 30, 20, or 10 nM, as measured in an assay for collapsing a proton gradient in *E. coli* inverted membrane vesicles, for example as described in Example 12.

[0094] In some embodiments a cell treated with the compound in an oxygen consumption rate assay, for example as described in Example 11, has an oxygen consumption rate at 20 minutes after treating the cell of at least about 110% (for example at least about 120%, 130%, 140%, 150%, 160%, 170%, or 180%, and useful ranges may be selected between any two of these values) of the oxygen consumption rate of an untreated cell.

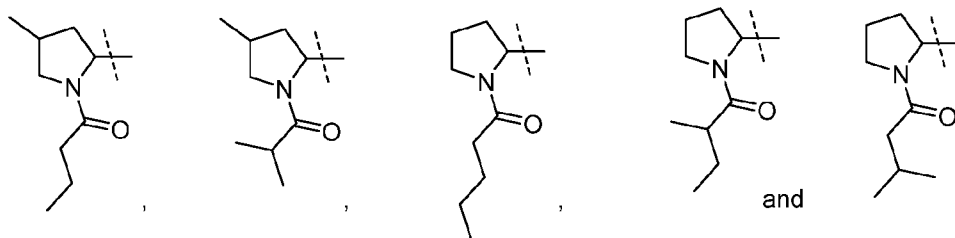
25 **[0095]** In some embodiments a cell treated with the compound in a membrane potential assay using a MITO-ID membrane potential cytotoxicity kit, for example as described in Example 12, has a corrected total cell fluorescence at 590 nm of less than about 60%, for example less than about 50%, 40%, 30%, 20%, 10%, 5%, 1%, 0.5%, or 0.1%, and useful ranges may be selected between any two of these values, of the corrected total cell fluorescence of an untreated cell.

30 **[0096]** In some embodiments the compound is not a compound listed in the table below

Drechmerin A1	
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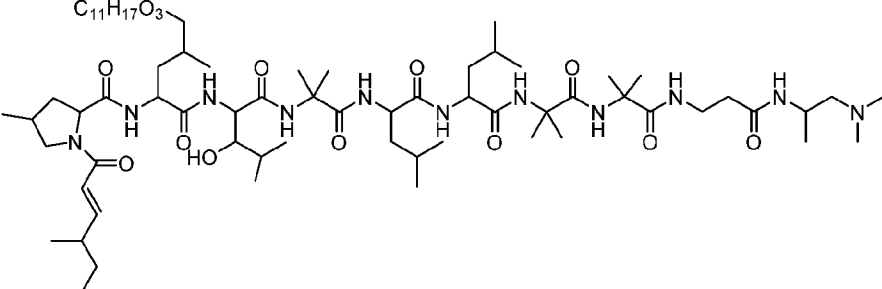
Drechmerin A2	
Drechmerin B1	
Drechmerin B2	

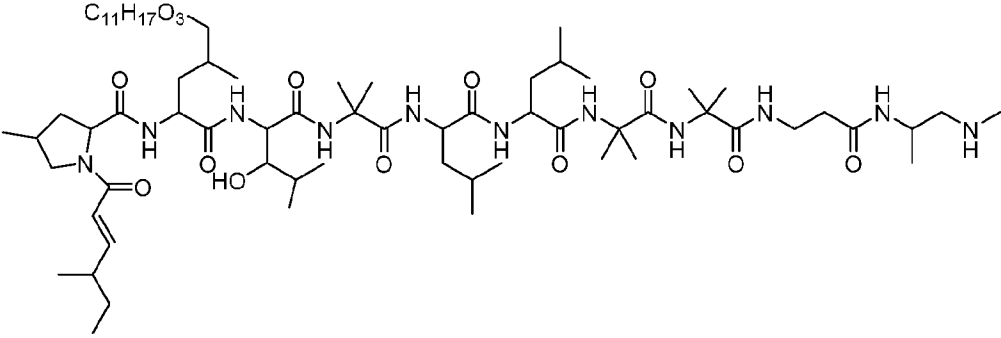
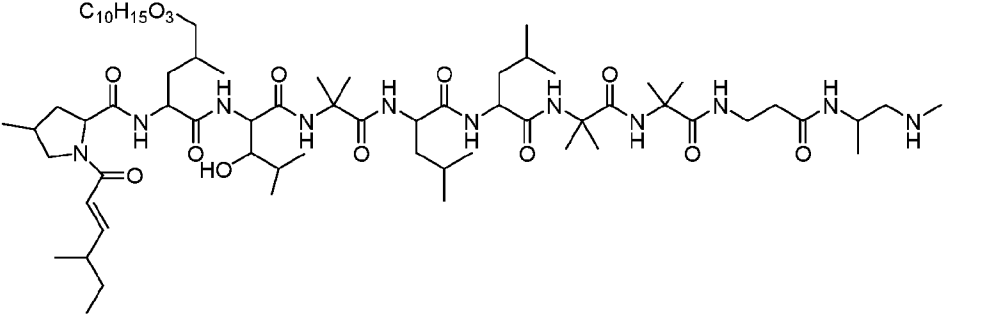
wherein $C_9H_{16}NO$ is selected from the group consisting of



[0097] In some embodiments the compound is not a compound listed in the table below.

5

Leucinoastatin O	
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Leucinostatin Q	
Leucinostatin W	

[0098] In some embodiments the compound is covalently attached to the reactive linker group.

[0099] In some embodiments the compound is covalently attached by the replacement of one or more atom in the compound with the reactive linker group.

[0100] In some embodiments the one or more atom that is replaced with the reactive linker group is a hydrogen atom of an OH, SH, or NH group in the compound or an oxygen atom of a ketone in the compound.

[0101] In some embodiments the reactive linker group is covalently attached to the compound by the replacement of one or more atom in Xaa10 of the compound with the reactive linker group.

[0102] In some embodiments the reactive linker group is covalently attached to the compound by the replacement of one or more atom in X₁₀ of Xaa10 with the reactive linker group.

[0103] In some embodiments:

X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;

R₁₅, R₁₆, and R₁₈ are each H;

and the reactive linker group is covalently attached to the compound by the replacement of R₁₅, R₁₆, or R₁₈ with the reactive linker group.

[0104] In some embodiments

X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;

R₁₅, R₁₆, and R₁₈ are each C₁-C₈alkyl(Y₁₀);

Y₁₀ is OR₁₉, NR₂₀R₂₁, or SR₂₂;

R₁₉, R₂₀, and R₂₂ are each H;

and the reactive linker group is covalently attached to the compound by the replacement of R₁₉, R₂₀, or R₂₂ with the reactive linker group.

[0105] In some embodiments the reactive linker group is covalently attached to the compound by the replacement of one or more atom in Xaa2 of the compound with the reactive linker group.

[0106] In some embodiments

R₆ is C₁-C₁₅alkyl(Q_a);

Q_a is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a;

R_a is H;

and the reactive linker group is covalently attached to the compound by the replacement of R_a with the reactive linker group.

[0107] In some embodiments

R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

X is at each instance independently O, S, or NR_e;

and the reactive linker group is covalently attached to the compound by the replacement of X with the reactive linker group.

[0108] In some embodiments

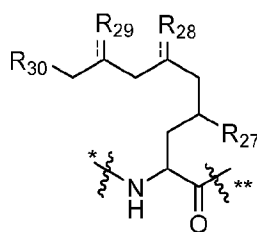
R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

Q_b and Q_c are each independently H, OR_c, SR_c, or NR_cR_d;

at least one of Q_b and Q_c is OH, SH, or NHR_d;

and the reactive linker group is covalently attached to the compound by the replacement of the hydrogen atom of the OH, SH, or NH group of the at least one of Q_b or Q_c with the reactive linker group.

[0109] In some embodiments **Xaa2** is a group of formula IIB



IIB

wherein

R₂₈ and R₂₉ are each independently H or OH and == is a single bond or O and == is a double bond;

at least one of R₂₈ and R₂₉ is OH or O;

and the reactive linker group is covalently attached to the compound by the replacement of the hydrogen atom of the OH or the O of the at least one of R₂₈ or R₂₉ with the reactive linker group.

[0110] In some embodiments the reactive linker group is covalently attached to the compound by the replacement of one or more atom in Xaa1 of the compound with the reactive linker group.

[0111] In some embodiments the reactive linker group is covalently attached to the compound by the replacement of one or more atom in R₁ of Xaa1 with the reactive linker group.

[0112] In some embodiments

R₁ is H;

and the reactive linker group is covalently attached to the compound by the replacement of R₁ with the reactive linker group.

[0113] In some embodiments

(a) R₁ is (CH₂CH₂O)_k-R_d or C(O)(CH₂CH₂O)-R_d,

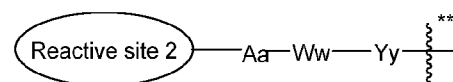
R_d is H, and the reactive linker group is covalently attached to the compound by the replacement of R_d; or

(b) R₁ is C₁-C₁₅alkyl(Q_d) or C(O)C₁-C₁₅alkyl(Q_d),

Q_d is OR_a, SR_a, NR_aR_b, C(O)OR_a

R_a is H, and the reactive linker group is covalently attached to the compound by the replacement of R_a.

[0114] In some embodiments the reactive linker group has the structure



wherein

A is a Stretcher unit;

W is an Amino acid unit (W_w);

Y is a Spacer Unit;

a in the reactive linker group is an integer from 0 to 1;

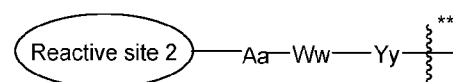
w in the reactive linker group is an integer from 0 to 12;

y in the reactive linker group is an integer from 0 to 2; and

Reactive site 2 is the reactive site that allows the reactive linker group to be reacted with a ligand; and

** denotes a bond to a compound of formula I or a compound listed in the proviso relating thereto.

[0115] In some embodiments the reactive linker group has the structure



wherein

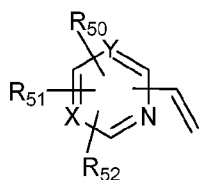
a, w, y are each at least 1, or

a and w are each at least 1 and y is 0, or

w is at least 1 and a and y are each 0, or

a is 1 and w and y are each 0.

[0116] In some embodiments the reactive linker group is a group of formula VI



VI

wherein:

5 X and Y are independently selected from CH or N;

R₅₀ is selected from:

(CH₂)_n—C(O)—*, or,

(CH₂)_m—Z—*, or,

(CH₂)_m—Z—C(O)—*, or,

10 (CH₂)_n—C(O)—Z—*, or,

(CH₂)_m—Z—(CH₂)—C(O)—*, or,

(CH₂)_m—Z—(CH₂)—C(O)—Z—*, or,

(CH₂)_m—Z—C(O)—(CH₂)_n—Z—(CH₂)_n—C(O)—Z—*, or,

(CH₂)_n—CH(CO₂-*)₂, or,

15 (CH₂)_m—Z—(CH₂)₂CH(CO₂-*)₂, or,

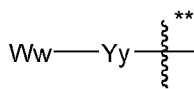
(CH₂)_n—*,

wherein:

Z is independently selected from NH, O or S,

n in the reactive linker group is any integer from 0 to 10,

20 m in the reactive linker group is any integer from 0 to 10, and



* denotes a bond to ; and

R₅₁ and/or R₅₂ are selected from the same groups as R₅₀ or R₅₁ and/or R₅₂ are selected from hydrogen or an electron withdrawing group, such as halogen (F, Cl, or Br), —

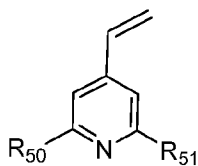
NO₂, —CO₂H, —CO₂R₅₃, COR₅₃, —CHO, —CN, —CF₃, —SO₂NR₅₃R₅₄ where R₅₃ and R₅₄

25 are independently selected from hydrogen or C₁₋₁₀ alkyl; or

R₅₁ and/or R₅₂ are selected from hydrogen, alkyl or phenyl; or

R₅₁ and R₅₂ together with the aromatic nitrogen containing ring to which they are attached form a heteroaryl ring, such as, for example, an indole, indazole, benzimidazole, quinoline, isoquinoline, aziridine or a purine.

30 **[0117]** In some embodiments the reactive linker group is a group of formula VII,



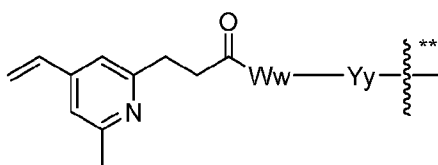
VII

[0118] In some embodiments the reactive linker group is a group of formula VII, wherein

R₅₀ is (CH₂)_n—C(O)—Z-*;

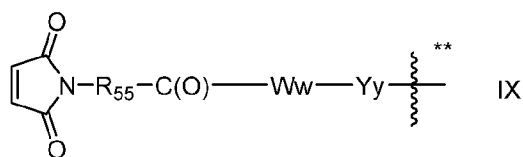
5 R₅₁ is alkyl, preferably C₁–C₈alkyl, preferably C₁–C₃alkyl, preferably CH₃.

[0119] In some embodiments the reactive linker group is a group of formula VIII

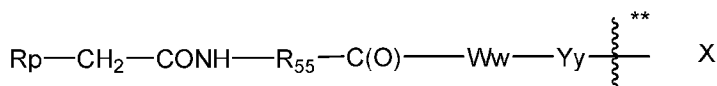


VIII

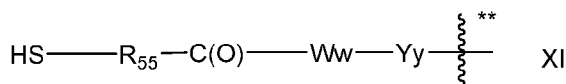
[0120] In some embodiments the reactive linker group is a group of formula IX, X, XI or XA



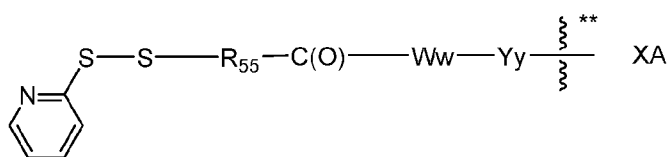
IX



X



XI



XA

wherein

R₅₅ is selected from C₁–C₁₅alkyl, C₃–C₈carbocycle, O(C₁–C₈ alkyl), aryl, C₁–C₁₅alkyl(aryl), arylC₁–C₁₅alkyl, C₁–C₁₅alkyl(C₃–C₈carbocycle), (C₃–C₈carbocycle)–C₁–C₁₅ alkyl, a 3 to 8 membered heterocycle, C₁–C₁₅alkyl-(3 to 8 membered heterocycle), (3 to 8 membered heterocycle)–C₁–C₁₅alkyl, (CH₂CH₂O)_r, and (CH₂CH₂O)_r–CH₂;

R_p is a suitable leaving group; preferably a halogen, preferably I;

r in the reactive linker group is an integer ranging from 1-10.

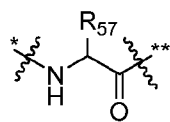
[0121] In some embodiments the reactive linker group is a group of formula IX.

[0122] In some embodiments the reactive linker group is a group of formula IX, wherein R_{55} is a C_1 - C_{15} alkyl, preferably a C_1 - C_5 alkyl.

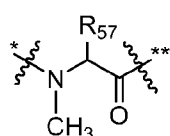
[0123] In some embodiments of the conjugate w and y are both 0.

5 **[0124]** In some embodiments the reactive linker group is a group of formula XA, wherein R_{55} is a C_1 - C_{15} alkyl, preferably a C_1 - C_8 alkyl, preferably a C_1 - C_5 alkyl and w and y are both 0.

[0125] In some embodiments each Amino acid unit is independently a group of formula XII or XIII

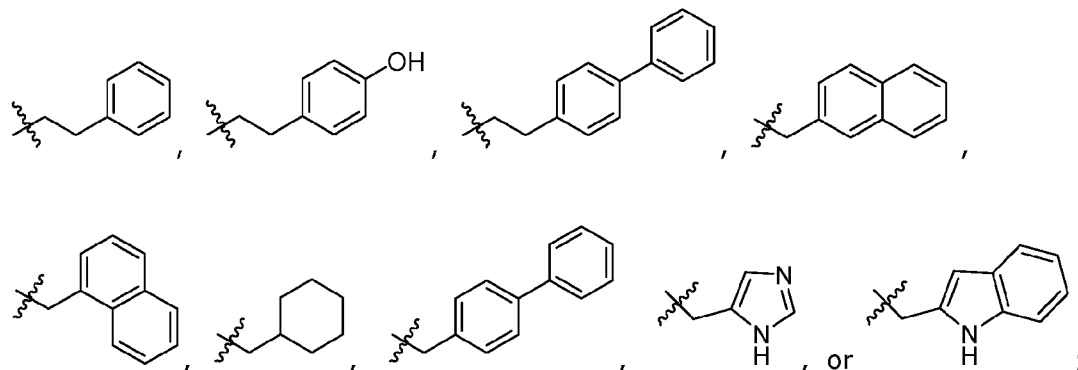


XII



XIII

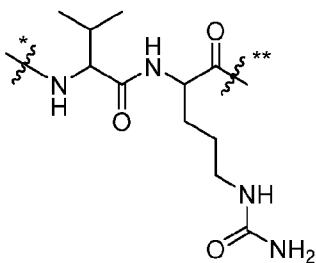
10 wherein R_{57} is hydrogen, methyl, isopropyl, isobutyl, sec-butyl, benzyl, p-hydroxybenzyl, $-\text{CH}_2\text{OH}$, $-\text{CH}(\text{OH})\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{SCH}_3$, $-\text{CH}_2\text{CONH}_2$, $-\text{CH}_2\text{COOH}$, $-\text{CH}_2\text{CH}_2\text{CONH}_2$, $-\text{CH}_2\text{CH}_2\text{COOH}$, $-(\text{CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2$, $-(\text{CH}_2)_3\text{NH}_2$, $-(\text{CH}_2)_3\text{NHCOCH}_3$, $-(\text{CH}_2)_3\text{NHCHO}$, $-(\text{CH}_2)_4\text{NHC}(=\text{NH})\text{NH}_2$, $-(\text{CH}_2)_4\text{NH}_2$, $-(\text{CH}_2)_4\text{NHCOCH}_3$, $-(\text{CH}_2)_4\text{NHCHO}$, $-(\text{CH}_2)_3\text{NHCONH}_2$, $-(\text{CH}_2)_4\text{NHCONH}_2$, $-\text{CH}_2\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{NH}_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl- or phenyl, cyclohexyl,



20 wherein * denotes the bond to Aa; and

** denotes the bond to Yy.

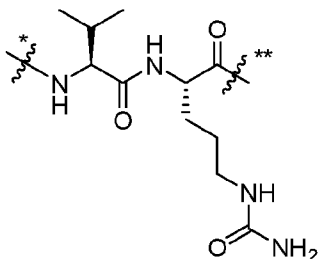
[0126] In some embodiments w is 2 such that Ww is a dipeptide group of formula XIV



XIV

wherein * denotes the bond to the Stretcher unit; and ** denotes the bond to the spacer unit.

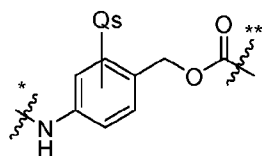
[0127] In some embodiments the Amino acid unit (Ww) is a group of formula XIVi



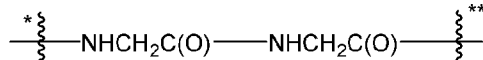
XIVi

5 wherein * denotes the bond to Aa; and ** denotes the bond to Yy.

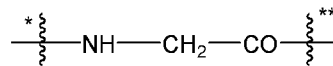
[0128] In some embodiments each Spacer Unit is a group of formula XV, XVI or XVII



XV



XVII



XVI

preferably the Spacer Unit is a group of formula XV;

wherein

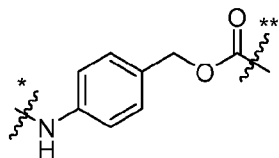
10 Q is $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, -halogen, - nitro or -CN;

s is an integer ranging from 0-4;

* denotes the bond to Ww; and

** denotes the bond to the compound of formula I or a compound listed in the proviso relating thereto.

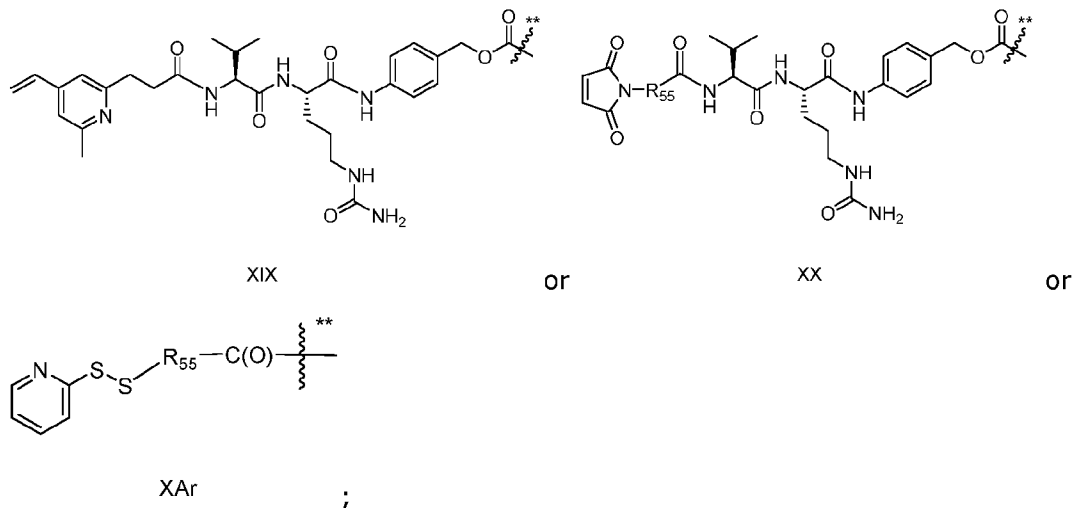
15 **[0129]** In some embodiments y is 1 and the Spacer Unit is a group of formula XVIII



XVIII

wherein * and ** are as defined herein.

[0130] In some embodiments the reactive linker group is a group of formula XIX, XX or XA



wherein R₅₅ is as defined herein, preferably a C₁-C₁₅alkyl, preferably a C₁-C₅alkyl; w and y are each zero; and

5 ** denotes the bond to the compound of formula I or a compound listed in the
proviso relating thereto.

[0131] In some embodiments the ligand-drug conjugate comprising a ligand and one or more compounds of formula I or a compound listed in the proviso relating thereto.

[0132] In some embodiments the compound is attached to the ligand via a linker
10 group.

[0133] In some embodiments the compound is covalently attached to the linker group.

[0134] In some embodiments the compound is covalently attached by the replacement of one or more atom in the compound with the linker group.

[0135] In some embodiments the one or more atom that is replaced with the linker group is a hydrogen atom of an OH, SH, or NH group in the compound or an oxygen atom of a ketone in the compound.

[0136] In some embodiments the linker group is covalently attached to the compound by the replacement of one or more atom in Xaa10 of the compound with the linker group.

[0137] In some embodiments the linker group is covalently attached to the compound by the replacement of one or more atom in X₁₀ of Xaa₁₀ with the linker group.

[0138] In some embodiments:

X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;

R_{15} , R_{16} , and R_{18} are each H;

and the linker group is covalently attached to the compound by the replacement of R₁₅,

25 R₁₆, or R₁₈ with the linker group.

[0139] In some embodiments

X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;

R₁₅, R₁₆, and R₁₈ are each C₁-C₈alkyl(Y₁₀);

Y₁₀ is OR₁₉, NR₂₀R₂₁, or SR₂₂;

30 R_{19} , R_{20} , and R_{22} are each H;

and the linker group is covalently attached to the compound by the replacement of R₁₉, R₂₀, or R₂₂ with the linker group.

[0140] In some the linker group is covalently attached to the compound by the replacement of one or more atom in Xaa2 of the compound with the linker group.

5 **[0141]** In some embodiments

R₆ is C₁-C₁₅alkyl(Q_a);

Q_a is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a;

R_a is H;

and the linker group is covalently attached to the compound by the replacement of
10 R_a with the linker group.

[0142] In some embodiments

R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

X is at each instance independently O, S, or NR_e;

and the linker group is covalently attached to the compound by the replacement of X
15 with the linker group.

[0143] In some embodiments

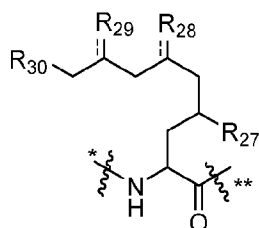
R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

Q_b and Q_c are each independently H, OR_c, SR_c, or NR_cR_d;

at least one of Q_b and Q_c is OH, SH, or NHR_d;

and the linker group is covalently attached to the compound by the replacement of
20 the hydrogen atom of the OH, SH, or NH group of the at least one of Q_b or Q_c with the linker group.

[0144] In some embodiments **Xaa2** is a group of formula IIB



IIB

wherein

R₂₈ and R₂₉ are each independently H or OH and == is a single bond or O and == is a double bond;

at least one of R₂₈ and R₂₉ is OH or O;

and the linker group is covalently attached to the compound by the replacement of
30 the hydrogen atom of the OH or the O of the at least one of R₂₈ or R₂₉ with the linker group.

[0145] In some embodiments the reactive linker group is covalently attached to the compound by the replacement of one or more atom in Xaa1 of the compound with the
35 reactive linker group.

[0146] In some embodiments the reactive linker group is covalently attached to the compound by the replacement of one or more atom in R_1 of Xaa1 with the reactive linker group.

[0147] In some embodiments

5 R_1 is H;
and the reactive linker group is covalently attached to the compound by the replacement of R_1 with the reactive linker group.

[0148] In some embodiments

(a) R_1 is $(CH_2CH_2O)_k-R_d$ or $C(O)(CH_2CH_2O)-R_d$,
10 R_d is H, and the reactive linker group is covalently attached to the compound by the replacement of R_d ; or
(b) R_1 is $C_1-C_{15}alkyl(Q_d)$ or $C(O)C_1-C_{15}alkyl(Q_d)$,
 Q_d is OR_a , SR_a , NR_aR_b , $C(O)OR_a$
 R_a is H, and the reactive linker group is covalently attached to the compound by the
15 replacement of R_a .

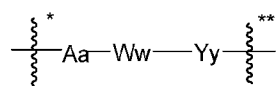
[0149] In some embodiments the linker group is covalently attached to the ligand.

[0150] In some embodiments the linker group is covalently attached to a sulfur atom of the ligand.

[0151] In some embodiments the ligand-drug conjugate has the formula $T-(L-(D)_o)_p$,

20 wherein
 T is a ligand;
 L is a linker group;
 D is a compound of formula I, including the compound in the proviso relating thereto; and
25 o is an integer from 1 to 10, preferably 2 to 6; and p is an integer from 1 to 10.

[0152] In some embodiments the linker group has the structure



wherein

A is a Stretcher unit;

30 W is an Amino acid unit;

Y is a Spacer Unit;

a in the linker group is an integer from 0 to 1;

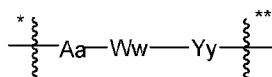
w in the linker group is an integer from 0 to 12;

y in the linker group is an integer from 0 to 2; and

35 $*$ denotes a bond to the ligand; and

$**$ denotes a bond to the compound of formula I or a compound listed in the proviso relating thereto.

[0153] In some embodiments the linker group has the structure



wherein

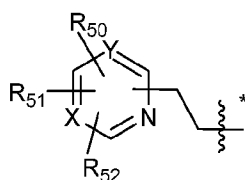
a, w, y are each at least 1, or

a and w are each at least 1 and y is 0, or

5 w is at least 1 and a and y are each 0, or

a is at least 1 and w and y are each 0.

[0154] In some embodiments linker group is a group of formula VIa



VIa

wherein:

10 X and Y are independently selected from CH or N;

R₅₀ is selected from:

(CH₂)_n—C(O)—***, or,

(CH₂)_m—Z—***, or,

(CH₂)_m—Z—C(O)—***, or,

15 (CH₂)_n—C(O)—Z—***, or,

(CH₂)_m—Z—(CH₂)—C(O)—***, or,

(CH₂)_m—Z—(CH₂)—C(O)—Z—***, or,

(CH₂)_m—Z—C(O)—(CH₂)_n—Z—(CH₂)_n—C(O)—Z—***, or,

(CH₂)_n—CH(CO₂-***)₂, or,

20 (CH₂)_m—Z—(CH₂)₂CH(CO₂-***)₂, or,

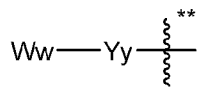
(CH₂)_n—***,

wherein:

Z is independently selected from NH, O or S,

n in the reactive linker group is any integer from 0 to 10,

25 m in the reactive linker group is any integer from 0 to 10, and



*** denotes a bond to

* denotes the bond to the ligand; and

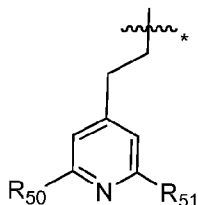
R₅₁ and/or R₅₂ are selected from the same groups as R₅₀ or R₅₁ and/or R₅₂ are selected from hydrogen or an electron withdrawing group, such as halogen (F, Cl, or Br), —

30 NO₂, —CO₂H, —CO₂R₅₃, COR₅₃, —CHO, —CN, —CF₃, —SO₂NR₅₃R₅₄ where R₅₃ and R₅₄ are independently selected from hydrogen or C₁₋₁₀ alkyl; or

R₅₁ and/or R₅₂ are selected from hydrogen, alkyl or phenyl; or

R₅₁ and R₅₂ together with the aromatic nitrogen containing ring to which they are attached form a heteroaryl ring, such as, for example, an indole, indazole, benzimidazole, quinoline, isoquinoline, aziridine or a purine.

[0155] In some embodiments the linker group is a group of formula VIIa

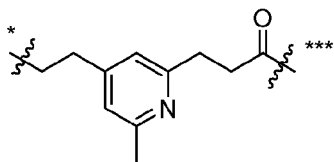


VIIa

[0156] In some embodiments the linker group is a group of formula VIIa, wherein R₅₀ is (CH₂)_n—C(O)—Z—***;

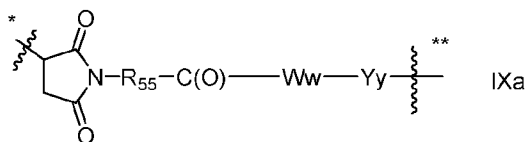
R₅₁ is alkyl, preferably C₁-C₈alkyl, preferably C₁-C₃alkyl, preferably CH₃.

[0157] In some embodiments the linker group is a group of formula VIIIa

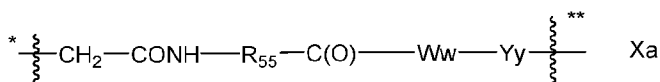


VIIIa

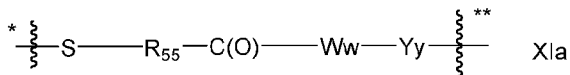
[0158] In some embodiments the linker group is a group of formula IXa, Xa or XIa



IXa



Xa



XIa

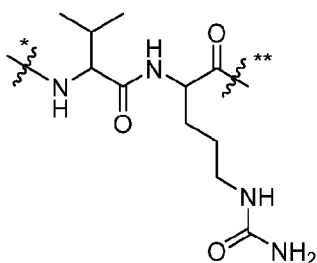
wherein

R₅₅ is selected from C₁-C₁₅alkyl, C₃-C₈carbocycle, O(C₁-C₈ alkyl), aryl, C₁-C₁₅alkyl(aryl), arylC₁-C₁₅alkyl, C₁-C₁₅alkyl(C₃-C₈carbocycle), (C₃-C₈carbocycle)-C₁-C₁₅alkyl, a 3 to 8 membered heterocycle, C₁-C₁₅alkyl-(3 to 8 membered heterocycle), (3 to 8 membered heterocycle)-C₁-C₁₅alkyl, (CH₂CH₂O)_r, and (CH₂CH₂O)_r—CH₂;

r in the linker group is an integer ranging from 1-10;

** denotes the bond to the compound of formula I or a compound listed in the proviso relating thereto; and

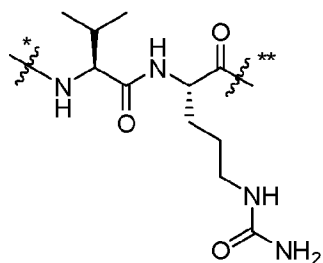
* denotes the bond to the ligand.



XIV

wherein * denotes the bond to Aa; and ** denotes the bond to Yy.

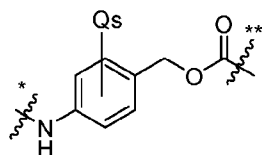
[0165] In some embodiments W is a group of formula XIVi



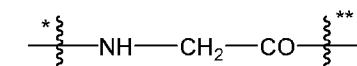
XIVi

5 wherein * denotes the bond to Aa; and ** denotes the bond to Yy.

[0166] In some embodiments each Spacer Unit is a group of formula XV, XVI or XVII

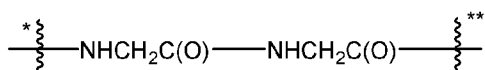


XV



XVI

, or



XVII

preferably the Spacer Unit is a group of formula XV;

10 wherein;

Q when present is $-C_1-C_8$ alkyl, $-O-(C_1-C_8$ alkyl), -halogen, - nitro or -CN;

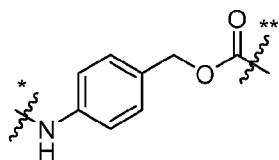
s is an integer ranging from 0-4;

* denotes the bond to Ww; and

** denotes the bond to the compound of formula I or a compound listed in the proviso relating thereto.

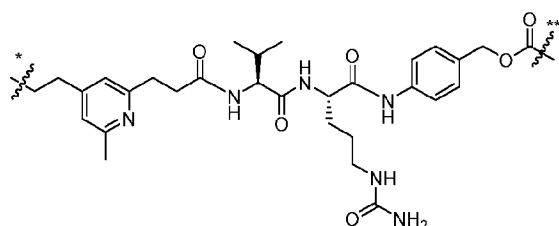
15

[0167] In some embodiments y is 1 and the Spacer Unit is a group of formula XVIII



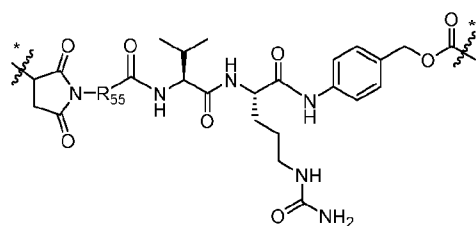
XVIII

[0168] In some embodiments the linker group is a group of formula XIXa, XXa, or XAi



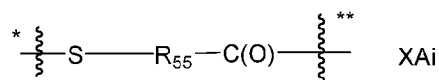
XIXa

or



XXa

or



XAi

- 5 preferably a group of formula XXa or XAi;
wherein R₅₅ is as defined herein, preferably a —C₁-C₁₀alkylene-, preferably a C₁-C₅alkylene;
* denotes the bond to the ligand; and

** denotes the bond to the compound of formula I or a compound listed in the proviso relating thereto.

- 10 **[0169]** In some embodiments the ligand is a globular protein.
[0170] In some embodiments the ligand is an antibody or fragment thereof.
[0171] In some embodiments the ligand is an antibody.
[0172] In some embodiments the antibody is a monoclonal antibody, a bi specific antibody, a chimeric antibody, or a humanized antibody.
- 15 **[0173]** In some embodiments the ligand is an antibody fragment.
[0174] In some embodiments the antibody fragment is a Fab fragment.
[0175] In some embodiments the ligand, for example, the antibody or antibody fragment binds to one or more tumour-associated antigens.
- [0176]** In some embodiments the ligand, for example, the antibody or antibody
- 20 fragment, binds to one or more antigens or cell-surface receptors selected from the group consisting of: BMPR1B (bone morphogenetic protein receptor-type IB); E16 (LAT1, SLC7A5); STEAP1 (six transmembrane epithelial antigen of prostate); 0772P (CA125, MUC16); MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin); Napi2b (NAPI-3B, NPTIIB, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type
- 25 II sodium-dependent phosphate transporter 3b); Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B); PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA

2700050C12, RIKEN cDNA 2700050C12 gene); ETBR (Endothelin type B receptor); MSG783 (RNF124, hypothetical protein FLJ20315); STEAP2 (HGNC—8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein); STEAP4 (HGNC—21923, TNFIAP9, STAMP2, STEAP4, six transmembrane epithelial antigen of prostate 4, six transmembrane prostate protein 2); TrpM4 (BR22450, F1120041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4); CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor); CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792); CD79b (IGb (immunoglobulin-associated beta), B29); FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C); an ErbB receptor; NCA; MDP; IL20R α ; Brevican; Ephb2R; ASLG659; PSCA; GEDA; BAFF-R (B cell-activating factor receptor, BLyS receptor 3, BR3); CD22(B-cell receptor CD22-B isoform); CD79a (CD79A, CD79 α , immunoglobulin-associated alpha); CXCR5 (Burkitt's lymphoma receptor 1); HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+T lymphocytes); P2X5 (Purinergic receptor P2X ligand-gated ion channel 5); CD72 (B-cell differentiation antigen CD72, Lyb-2); LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family); FCRH1 (Fc receptor-like protein 1); FcRH5 (IRTA2, immunoglobulin superfamily receptor translocation associated 2); TENB2 (putative transmembrane proteoglycan); PMEL17 (silver homolog; SILV; D12S53E; PMEL17; SI; SIL); TMEFF1 (transmembrane protein with EGF-like and two follistatin-like domains I; Tomoregulin-1); GDNF-Ra1 (GDNF family receptor alpha 1; GFRA1; GDNFR; GDNFRA; RETL1; TRNR1; RET1L; GDNFR-alpha1; GFR-ALPHA-1); Ly6E (lymphocyte antigen 6 complex, locus E; Ly67, RIG-E, SCA-2, TSA-1); TMEM46 (shisa homolog 2 (*Xenopus laevis*); SHISA2); Ly6G6D (lymphocyte antigen 6 complex, locus G6D; Ly6-D, MEGT1) LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5; GPR49, GPR67); RET (ret proto-oncogene; MEN2A; HSCR1; MEN2B; MTC1; PTC; CDHF12; Hs.168114; RET51; RET-ELE1); LY6K (lymphocyte antigen 6 complex, locus K; LY6K; HSJ001348; FLJ35226); GPR19 (G protein-coupled receptor 19; Mm.4787); GPR54 (KISS1 receptor; KISS1R; GPR54; HOT7T175; AXOR12); ASPHD1 (aspartate beta-hydroxylase domain containing 1; LOC253982); Tyrosinase (TYR; OCAIA; OCA1A; tyrosinase; SHEP3); TMEM118 (ring finger protein, transmembrane 2; RNFT2; FLJ14627); GPR172A (G protein-coupled receptor 172A; GPCR41; FLJ11856; D15Ert747e); CD33; CLL-1; CD30 (tumor necrosis factor receptor SF8, TNFRSF8); CD40; CD70; CanAg; PSMA (prostate specific membrane antigen); CA15-3; CA19-9; L6; Lewis Y; Lewis X; alpha fetoprotein; CA242; placental alkaline phosphatase; prostatic acid phosphatase; epidermal growth factor; MAGE-1; MAGE-2; MAGE-3; MAGE-4; anti-transferrin receptor; p97; MUC1-KLH; CEA; gp100; MART1; Prostate serum antigen (PSA); IL-2 receptor; CD20; CD52; human chorionic gonadotropin; CD38; mucin; P21; MPG; IL-7R; and Neu oncogene product.

[0177] In some embodiments, the ligand, for example, the antibody or antibody fragment, binds to one or more antigens or cell-surface receptors selected from the group consisting of 0772P, MPF, an ErbB receptor, CD22, CD33, CD30, CD40, CD70, CA15-3, epidermal growth factor, IL-2 receptor, CD20, CD52, human chorionic gonadotropin, CD38, and Neu oncogene product.

[0178] In some embodiments the ErbB receptor is selected from the group consisting of epidermal growth factor receptor (EGFR, ErbB1, HER1), HER2 (ErbB2 or p185neu), HER3 (ErbB3) and HER4 (ErbB4 or tyro2).

[0179] In some embodiments the ErbB receptor is HER2.

[0180] In some embodiments, the ligand is selected from the group consisting of Abagovomab, Oregovomab, Amatumumab, Inotuzumab, Epratuzumab, Moxetumomab, Gemtuzumab, Lintuzumab, Brentuximab, Rituximab, Nivolumab, Dacetuzumab, MDX-1411, Vorsetuzumab, Cetuximab, Basiliximab, Daclizumab, Ofatumumab, Tositumomab, Ibritumomab, Obinutuzumab, Alemtuzumab, Smart MI95, Daratumumab, huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7, huMAb4D5-8 (Trastuzumab), pertuzumab, and a combination of any two or more thereof.

[0181] In some embodiments the ligand is selected from the group consisting of huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 huMAb4D5-8 (Trastuzumab), ado-trastuzumab emtansine (Kadcyla), pertuzumab or a combination of any two or more thereof.

[0182] In some embodiments the ligand, for example an antibody or fragment thereof, binds an antigen or cell surface receptor of a cell that produces autoimmune antibodies, for example a receptor or receptor complex expressed on an activated lymphocyte that is associated with an autoimmune disease.

[0183] In some embodiments the antibody binds the IL-7 receptor.

[0184] In some embodiments the ligand is albumin.

[0185] In some embodiments the ligand is a peptide.

[0186] In some embodiments the peptide binds an integrin.

[0187] In some embodiments the integrin is selected from the group comprising $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 6$, $\alpha 5\beta 1$ and $\beta 6$.

[0188] In some embodiments the ligand is attached to the compound via a cysteine residue of the antibody.

[0189] In some embodiments the ligand is attached to the compound via Xaa10 of the compound.

[0190] In some embodiments the cancer is selected from the group comprising breast cancer, colorectal cancer and gastroesophageal cancer.

[0191] In some embodiments the breast cancer is HER2-positive breast cancer.

[0192] In some embodiments the method or use comprises administering one or more additional therapeutic agents.

[0193] In some embodiments the one or more additional therapeutic agents is an anticancer agent.

[0194] In some embodiments the one or more additional therapeutic agents is a ligand-drug conjugate, preferably an antibody-drug conjugate.

5 **[0195]** In some the metabolic disorder is selected from the group comprising diabetes, obesity, fatty liver disease and dyslipidemia.

[0196] In some embodiments the metabolic disorder is type 2 diabetes.

[0197] In some embodiments the method or use comprises treating or preventing complications associated with diabetes or obesity.

10 **[0198]** In some embodiments the mitochondrial uncoupling agent is a metabolic disorder or cancer.

[0199] In some embodiments the disease or disorder susceptible to treatment is cancer.

15 **[0200]** In some embodiments the complications associated with diabetes comprise one or more of cardiovascular disease, neuropathy, nephropathy, retinopathy, and neurodegenerative disorders.

[0201] In some embodiments the subject is a human.

[0202] In some embodiments the extent of binding is determined by immunohistochemistry.

20 **[0203]** In some embodiments, the membrane is a mitochondrial membrane.

[0204] It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7) and, therefore, 25 all sub-ranges of all ranges expressly disclosed herein are hereby expressly disclosed. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner.

[0205] This invention may also be said broadly to consist in the parts, elements and 30 features referred to or indicated in the specification of the application, individually or collectively, and any or all combinations of any two or more said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which this invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

35 **[0206]** Although the present invention is broadly as defined above, those persons skilled in the art will appreciate that the invention is not limited thereto and that the invention also includes embodiments of which the following description gives examples.

BRIEF DESCRIPTION OF THE DRAWINGS

[0207] The invention will now be described with reference to the accompanying Figures in which

[0208] Figure 1 shows growth curves for NZM37 cultures transduced with the GeCKOv2 sgRNA library and challenged with culicinin D (Cul), leucínostatin A and B (Leuc) or maintained in logarithmic growth without drug challenge (Ctrl; all n = 3).

[0209] Figure 2 shows drug concentration time courses used for pharmacological selection of NZM37 GeCKOv2 libraries.

[0210] Figure 3 shows the antiproliferative potency of leucínostatin A and B and culicinin D in treatment-naive NZM37 GeCKOv2 libraries (Ctrl) or libraries selected with leucínostatin (left panel) or culicinin D (right panel). Data are the mean + SEM of IC₅₀ assays performed at screen endpoint on each of three biological replicates for each condition. P-values are from two-tailed t-tests or Mann-Whitney U-tests, with the choice of test depending on the result of a preceding F-test for equivalent variance between groups.

[0211] Figure 4 shows a high-dimensional analysis of the similarity of sgRNA read count distributions by principal component analysis. Samples are plotted in terms of scores for principal components computed from depthnormalised sgRNA read counts. The fraction of total variance in the data explained for the two principal components is denoted in the axis titles.

[0212] Figure 5 shows deconvolution of whole-genome CRISPR-Cas9 knockout screens for genetic modifiers of sensitivity to culicinin D and leucínostatin A and B. Positive selection hits (i.e. CRISPR-induced mutations putatively conferring resistance to the selecting agents) were called using the MAGeCK algorithm from Li et al. (Li, W. *et al.* MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. *Genome Biol.* **15**, 554 (2014)) (a), RIGER algorithm from Luo et al. (Luo, B. *et al.* Highly parallel identification of essential genes in cancer cells. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 20380–5 (2008)) (b), PinAPL-Py algorithm from Spahn et al. (Spahn, P. N. *et al.* PinAPL-Py: A comprehensive web-application for the analysis of CRISPR/Cas9 screens. *Sci. Rep.* **7**, 1–8 (2017)) (c) and CRISPRcloud2⁶ algorithm from Jeong et al. (Jeong, H. H., Kim, S. Y., Rousseaux, M. W. C., Zoghbi, H. Y. & Liu, Z. CRISPRcloud: A secure cloudbased pipeline for CRISPR pooled screen deconvolution. *Bioinformatics* **33**, 2963–2965 (2017)).

[0213] Figures 6A and 6B show changes in oxygen consumption rate (OCR, pmol/min) in permeabilized SKBR3 breast cancer cells exposed to culicinin D, oligomycin A or selected culicinin D analogues; and Figure 6C shows the correlation between OCR (pmol/min) and IC₅₀ values (nmol/L ± SEM).

[0214] Figure 7 shows changes in mitochondrial membrane potential in SKBR3 cells using MITO-ID Cytotoxicity kit on Zeiss LSM710 confocal microscope. Enzo Life Sciences' MITO-ID Membrane Potential Cytotoxicity kit measures mitochondrial membrane potential (MMP) with a cationic dye that fluoresces either orange or green, depending upon MMP

status. **A.** Time-dependent loss of MMP in SKBR3 cells treated with culicinin D or AN-58 (compound **520**) (3 μ M) *versus* controls. **B.** Representative images of SKBR3 cells treated with different analogues of culicinin D and monitored for changes in MMP using MITO-ID Cytotoxicity kit and confocal microscopy (Zeiss LSM710 confocal microscope; scale bar: 100 μ m). **C.** Mean Corrected Total Cell Fluorescence (CTCF) $\pm$ SEM.

[0215] Figure 8 shows the collapse of the proton gradient by AN-58 (compound **520**) in *E. coli* inverted membrane vesicles (IMVs). The proton gradient was measured by the quenching of acridine orange in a chamber that also measured oxygen concentration through a clark-type electrode. Acridine orange fluorescence is plotted as the percentage of the total fluorescence quench (ΔF). Respiration was initiated by the addition of 1mM NADH and various amounts of AN-58 (compound **520**) were added when indicated. **A.** Wild-type *E. coli*, C41. **B.** ATP synthase deletion mutant *E. coli*, C41 Δ atp. **C.** ATP overexpressed in the Δ atp background, C41 Δ atp pBWU13. **D.** The endpoint ΔF from each strain was combined to evaluate the EC₅₀. Error bars indicate standard deviation. The EC₅₀ was 7.5 nM.

DETAILED DESCRIPTION OF THE INVENTION

[0216] The term "comprising" as used in this specification and claims means "consisting at least in part of". When interpreting each statement in this specification and claims that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

[0217] As used herein the term "and/or" means "and" or "or", or both.

[0218] As used herein "(s)" following a noun means the plural and/or singular forms of the noun.

[0219] Asymmetric centers may exist in the compounds described herein. The asymmetric centers may be designated as (*R*) or (*S*), depending on the configuration of substituents in three dimensional space at the chiral carbon atom. All stereochemical isomeric forms of the compounds, including diastereomeric, enantiomeric, and epimeric forms, as well as d-isomers and l-isomers, and mixtures thereof, including enantiomerically enriched and diastereomerically enriched mixtures of stereochemical isomers, are within the scope of the invention.

[0220] Individual enantiomers can be prepared synthetically from commercially available enantiopure starting materials or by preparing enantiomeric mixtures and resolving the mixture into individual enantiomers. Resolution methods include conversion of the enantiomeric mixture into a mixture of diastereomers and separation of the diastereomers by, for example, recrystallization or chromatography, and any other appropriate methods known in the art. Starting materials of defined stereochemistry may be commercially available or made and, if necessary, resolved by techniques well known in the art.

[0221] The compounds described herein may also exist as conformational or geometric isomers, including *cis*, *trans*, *syn*, *anti*, entgegen (*E*), and zusammen (*Z*) isomers. All such isomers and any mixtures thereof are within the scope of the invention.

[0222] Also within the scope of the invention are any tautomeric isomers or mixtures thereof of the compounds described. As would be appreciated by those skilled in the art, a wide variety of functional groups and other structures may exhibit tautomerism. Examples include, but are not limited to, keto/enol, imine/enamine, and thioketone/enethiol tautomerism.

[0223] The compounds described herein may also exist as isotopologues and isotopomers, wherein one or more atoms in the compounds are replaced with different isotopes. Suitable isotopes include, for example, ^1H , ^2H (D), ^3H (T), ^{12}C , ^{13}C , ^{14}C , ^{16}O , and ^{18}O . Procedures for incorporating such isotopes into the compounds described herein will be apparent to those skilled in the art. Isotopologues and isotopomers of the compounds described herein are also within the scope of the invention.

[0224] Also within the scope of the invention are salts of the compounds described herein, including pharmaceutically acceptable salts. Such salts include, acid addition salts, base addition salts, and quaternary salts of basic nitrogen-containing groups. Acid addition salts can be prepared by reacting compounds, in free base form, with inorganic or organic acids. Examples of inorganic acids include, but are not limited to, hydrochloric, hydrobromic, nitric, sulfuric, and phosphoric acid. Examples of organic acids include, but are not limited to, acetic, trifluoroacetic, propionic, succinic, glycolic, lactic, malic, tartaric, citric, ascorbic, maleic, fumaric, pyruvic, aspartic, glutamic, stearic, salicylic, methanesulfonic, benzenesulfonic, isethionic, sulfanilic, adipic, butyric, and pivalic. Base addition salts can be prepared by reacting compounds, in free acid form, with inorganic or organic bases. Examples of inorganic base addition salts include alkali metal salts, alkaline earth metal salts, and other physiologically acceptable metal salts, for example, aluminium, calcium, lithium, magnesium, potassium, sodium, or zinc salts. Examples of organic base addition salts include amine salts, for example, salts of trimethylamine, diethylamine, ethanolamine, diethanolamine, and ethylenediamine. Quaternary salts of basic nitrogen-containing groups in the compounds may be prepared by, for example, reacting the compounds with alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides, dialkyl sulfates such as dimethyl, diethyl, dibutyl, and diamyl sulfates, and the like.

[0225] The compounds described herein may form or exist as solvates with various solvents. If the solvent is water, the solvate may be referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, or a tri-hydrate. All solvated forms and unsolvated forms of the compounds described herein are within the scope of the invention.

[0226] The general chemical terms used herein have their usual meanings.

[0227] The term "alkyl" as used herein alone or in combination with other terms, unless indicated otherwise, refers to a straight-chain or branched saturated or unsaturated acyclic hydrocarbon group. In some embodiments, alkyl groups have from 1 to 15, from 1 to 13, from 1 to 11, from 1 to 10, from 1 to 8, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, from 1 to 2, from 2 to 12, from 2 to 9, from 2 to 8, from 2 to 6, from 2 to 4, from 3 to 9, from 3 to 8, from 4 to 9, from 4 to 15, from 6 to 15, from 8 to 15, from 10 to 15, or 1, or 2, or 3 carbon atoms. In some embodiments, alkyl groups are saturated. Examples of such alkyl groups include but are not limited to -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl, -n-decyl, n-undecyl, n-dodecyl, n-tridecyl, n-tetradecyl, n-pentadecyl, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, -neopentyl, 2-methylbutyl, -isohexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 3,3-dimethylpentyl, 2,3,4-trimethylpentyl, 3-methylhexyl, 2,2-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 3,5-dimethylhexyl, 2,4-dimethylpentyl, 2-methylheptyl, 3-methylheptyl, n-heptyl, isoheptyl, isooctyl, isononyl, isodecyl, isoundecyl, isododecyl, isotridecyl, isotetradecyl, and isopentadecyl and the like. In some embodiments, alkyl groups are unsaturated. Examples of such alkyl groups include but are not limited to -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, 3-hexyl, -acetylenyl, -propynyl, -1-butylnyl, -2-butylnyl, -1-pentylnyl, -2-pentylnyl, -3-methyl-1-butylnyl, and the like. The prefix "C_x-C_y", wherein x and y are each an integer, when used in combination with the term "alkyl" refers to the number of carbon atoms in the alkyl group. In some embodiments "alkyl" groups may be substituted with one or more optional substituents as described herein.

[0228] The term "haloalkyl" as used herein alone or in combination with other terms, unless indicated otherwise, refers to an alkyl group as defined herein substituted with one or more halo. In some embodiments, haloalkyl groups include fluoroalkyl groups. In some embodiments, fluoroalkyl groups include perfluoroalkyl groups. The prefix "C_x-C_y", wherein x and y are each an integer, when used in combination with the term "haloalkyl" refers to the number of carbon atoms in the haloalkyl group. In some embodiments, haloalkyl groups have from 1 to 15, from 1 to 13, from 1 to 11, from 1 to 10, from 1 to 8, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, from 1 to 2, from 2 to 12, from 2 to 9, from 2 to 8, from 2 to 6, from 2 to 4, from 3 to 9, from 3 to 8, from 4 to 9, from 4 to 15, from 6 to 15, from 8 to 15, from 10 to 15, or 1, or 2, or 3 carbon atoms. In some embodiments "haloalkyl" groups may be substituted with one or more optional substituents as described herein.

[0229] The term "carbocycle" as used herein alone or in combination with other terms, unless indicated otherwise, refers to a saturated or unsaturated non-aromatic mono-, bi- or tricyclic alkyl group. In some embodiments, carbocycle groups have from 3 to 12, from 3 to 10, from 3 to 8, from 3 to 6, from 3 to 5 carbon atoms in the ring(s). In some embodiments, carbocycle groups have 5 or 6 ring carbon atoms. Examples of monocyclic

carbocycle groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. In some embodiments, the carbocycle group has from 3 to 8, from 3 to 7, from 3 to 6, from 4 to 6, from 3 to 5, or from 4 to 5 ring carbon atoms. Bi- and tricyclic ring systems include bridged, spiro, and fused carbocycle ring systems. Examples of bi- and tricyclic ring carbocycle systems include, but are not limited to, bicyclo[2.1.1]hexanyl, bicyclo[2.2.1]heptanyl, adamantyl, and decalanyl. In some embodiments, unsaturated carbocycle groups have from 4 to 14, from 5 to 14, from 5 to 10, from 5 to 8, or from 5 to 6 carbon atoms in the ring(s). Examples of unsaturated carbocycle groups include, but are not limited to, cyclohexenyl, cyclopentenyl, cyclohexadienyl, butadienyl, pentadienyl, and hexadienyl. The prefix "C_x-C_y", wherein x and y are each an integer, when used in combination with the term "carbocycle" refers to the number of ring carbon atoms in the carbocycle group. In some embodiments "carbocycle" groups may be substituted with one or more optional substituents as described herein.

[0230] The term "aryl" as used herein alone or in combination with other terms, unless indicated otherwise, refers to a cyclic aromatic hydrocarbon groups that do not contain any ring heteroatoms. Aryl groups include monocyclic, bicyclic and tricyclic ring systems. Examples of aryl groups include, but are not limited to, phenyl, azulenyl, heptalenyl, biphenyl, fluorenyl, phenanthrenyl, anthracenyl, indenyl, indanyl, pentalenyl, and naphthyl. In some embodiments, aryl groups have from 6 to 20, 6 to 14, 6 to 12, or 6 to 10 carbon atoms in the ring(s). In some embodiments, the aryl groups are phenyl or naphthyl. Aryl groups include aromatic-carbocycle fused ring systems. Examples include, but are not limited to, indanyl and tetrahydronaphthyl. The prefix "C_x-C_y", wherein x and y are each an integer, when used in combination with the term "aryl" refers to the number of ring carbon atoms in the aryl group. In some embodiments "aryl" groups may be substituted with one or more optional substituents as described herein.

[0231] The term "heterocycle" as used herein alone or in combination with other terms, unless indicated otherwise, refers to a saturated or unsaturated non-aromatic ring system containing 3 or more ring atoms, of which one or more is a heteroatom. In some embodiments, the heteroatom is nitrogen, oxygen, or sulfur. In some embodiments, the heterocycle group contains one, two, three, or four heteroatoms. In some embodiments, heterocycle groups include mono-, bi- and tricyclic rings having from 3 to 20, 3 to 16, from 3 to 14, from 3 to 12, from 3 to 10, from 3 to 8, or from 3 to 6 ring atoms. Heterocycle groups include partially unsaturated and saturated ring systems, for example, imidazoliny and imidazolidinyl. Heterocycle groups include fused and bridged ring systems containing a heteroatom, for example, quinuclidyl. Heterocycle groups include, but are not limited to, aziridinyl, azetidiny, azepanyl, diazepanyl, 1,3-dioxanyl, 1,3-dioxolanyl, isoxazolidinyl, morpholinyl, piperazinyl, piperidinyl, pyranyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, tetrahydrofuranyl, tetrahydrothienyl, thiadiazolidinyl, and trithianyl. The prefix "x-y membered", wherein x and y are each an integer, when used in combination with the term

"heterocycle" refers to the number of ring atoms in the heterocycle group. In some embodiments "heterocycle" groups may be substituted with one or more optional substituents as described herein.

[0232] The term "heteroaryl" as used herein alone or in combination with other terms,

5 unless indicated otherwise, refers to an aromatic ring system containing 5 or more ring atoms, of which, one or more is a heteroatom. In some embodiments, the heteroatom is nitrogen, oxygen, or sulfur. In some embodiments, heteroaryl groups include mono-, bi- and tricyclic ring systems having from 5 to 20, 5 to 16, from 5 to 14, from 5 to 12, from 5 to 10, from 5 to 8, or from 5 to 6 ring atoms. Heteroaryl groups include, but are not limited to, pyrrolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, thiazolyl, pyridinyl, 10 pyridazinyl, pyrimidinyl, pyrazinyl, thiophenyl, benzothiophenyl, furanyl, benzofuranyl, indolyl, azaindolyl (pyrrolopyridinyl), indazolyl, benzimidazolyl, pyrazolopyridinyl, triazolopyridinyl, benzotriazolyl, benzoxazolyl, benzothiazolyl, imidazopyridinyl, isoxazolopyridinyl, xanthinyl, guaninyl, quinolinyl, isoquinolinyl, tetrahydroquinolinyl, 15 quinoxalinyl, and quinazolinyl. Heteroaryl groups include fused ring systems in which all of the rings are aromatic, for example, indolyl, and fused ring systems in which only one of the rings is aromatic, for example, 2,3-dihydroindolyl. The prefix "x-y membered", wherein x and y are each an integer, when used in combination with the term "heteroaryl" refers to the number of ring atoms in the heteroaryl group. In some embodiments "heteroaryl" groups may be substituted with one or more optional substituents as described herein.

[0233] The terms "alkyl(carbocycle)", "alkyl(heterocycle)", "alkyl(aryl)", and "alkyl(heteroaryl)" as used herein refer to an alkyl group, as defined herein, substituted with a carbocycle, heterocycle, aryl, or heteroaryl group, respectively, each as defined herein, attached to the parent molecular moiety through the alkyl group.

25 **[0234]** The term "halo" or "halogen" is intended to include F, Cl, Br, and I.

[0235] The term "heteroatom" is intended to include oxygen, nitrogen, sulfur, selenium, or phosphorus. In some embodiments, the heteroatom is selected from the group consisting of oxygen, nitrogen, and sulfur.

[0236] As used herein, the term "substituted" is intended to mean that one or more 30 hydrogen atoms in the group indicated is replaced with one or more independently selected suitable substituents, provided that the normal valency of each atom to which the substituent/s are attached is not exceeded, and that the substitution results in a stable compound. In various embodiments, suitable optional substituents in the compounds described herein include but are not limited to halo, N₃, CN, NO₂, OH, NR^xR^y, C₁-C₈haloalkyl, C₁-C₈haloalkoxy, C(O)NR^xR^y, C(O)N(R^x)₂-NHC(O)R^x, SO₂R^x, SO₃R^x, OR^y, SR^x, S(O)R^x, S(O)₂R^x, C(O)R^x, OC(O)R^x, C(O)OR^x, C₁-C₈alkyl, aryl; wherein R^x and R^y are each 35 independently H, aryl or C₁-C₈alkyl. The term "stable" as used herein, unless indicated otherwise, refers to compounds which possess stability sufficient to allow manufacture and

which maintain their integrity for a period of time sufficient to be useful for the purposes described herein.

[0237] The term "carboxyl protecting group" as used herein is means a group that is capable of readily removed to provide the OH group of a carboxyl group and protects the carboxyl group against undesirable reaction during synthetic procedures. Such protecting groups are described in Protective Groups in Organic Synthesis edited by T. W. Greene et al. (John Wiley & Sons, 1999) and 'Amino Acid-Protecting Groups' by Fernando Albericio (with Albert Isidro-Llobet and Mercedes Alvarez) Chemical Reviews 2009 (109) 2455-2504. Examples include, but are not limited to, alkyl and silyl groups, for example methyl, ethyl, *tert*-butyl, methoxymethyl, 2,2,2-trichloroethyl, benzyl, diphenylmethyl, trimethylsilyl, and *tert*-butyldimethylsilyl, and the like.

[0238] The term "amine protecting group" as used herein means a group that is capable of being readily removed to provide the NH₂ group of an amine group and protects the amine group against undesirable reaction during synthetic procedures. Such protecting groups are described in Protective Groups in Organic Synthesis edited by T. W. Greene et al. (John Wiley & Sons, 1999) and 'Amino Acid-Protecting Groups' by Fernando Albericio (with Albert Isidro-Llobet and Mercedes Alvarez) Chemical Reviews 2009 (109) 2455-2504. Examples include, but are not limited to, acyl and acyloxy groups, for example acetyl, chloroacetyl, trichloroacetyl, *o*-nitrophenylacetyl, *o*-nitrophenoxy-acetyl, trifluoroacetyl, acetoacetyl, 4-chlorobutyryl, isobutyryl, picolinoyl, aminocaproyl, benzoyl, methoxy-carbonyl, 9-fluorenylmethoxycarbonyl, 2,2,2-trifluoroethoxycarbonyl, 2-trimethylsilylethoxy-carbonyl, *tert*-butyloxycarbonyl, benzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, 2,4-dichloro-benzyloxycarbonyl, and the like. Further examples include Cbz (carboxybenzyl), Nosyl (*o*- or *p*-nitrophenylsulfonyl), Bpoc (2-(4-biphenyl)isopropoxycarbonyl) and Dde (1-(4,4-dimethyl-2,6-dioxohexylidene)ethyl).

[0239] The term "carboxamide protecting group" as used herein means a group that is capable of being readily removed to provide the NH₂ group of a carboxamide group and protects the carboxamide group against undesirable reaction during synthetic procedures. Such protecting groups are described in Protective Groups in Organic Synthesis edited by T. W. Greene et al. (John Wiley & Sons, 1999) and 'Amino Acid-Protecting Groups' by Fernando Albericio (with Albert Isidro-Llobet and Mercedes Alvarez) Chemical Reviews 2009 (109) 2455-2504. Examples include, but are not limited to, 9-xanthenyl (Xan), trityl (Trt), methyltrityl (Mtt), cyclopropyldimethylcarbonyl (Cpd), and dimethylcyclopropylmethyl (Dmcp).

[0240] The term "pharmaceutically acceptable salt", as used herein, unless indicated otherwise, refers to pharmaceutically acceptable organic or inorganic salts of the compounds described herein. For example, the compounds described herein may contain an amino group, and accordingly acid addition salts can be formed with this amino group. Examples of salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride,

bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-

toluenesulfonate, and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure.

Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

[0241] The terms "pharmaceutically acceptable solvate" or "solvate", as used herein, unless indicated otherwise, refer to an association of one or more solvent molecules and a compound described herein. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, and ethanolamine.

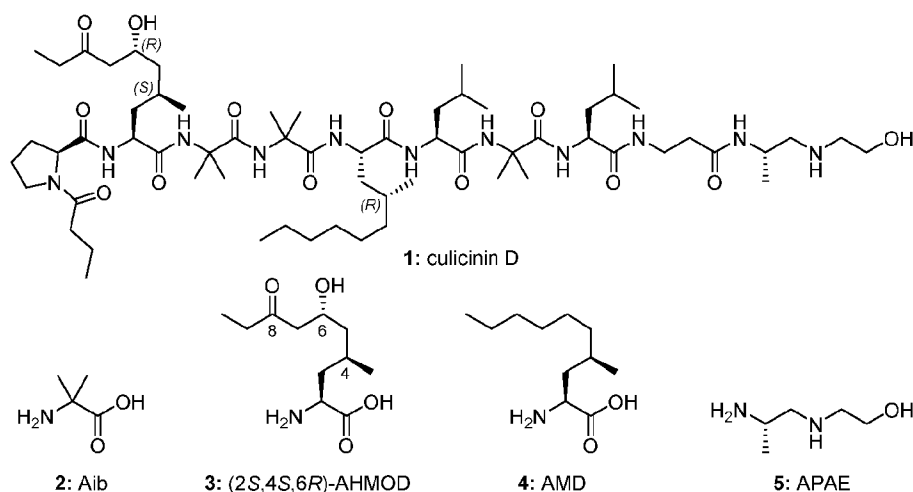
Compounds of formula I

[0242] The present invention relates to compounds of formula I as described herein.

[0243] The inventors have surprisingly found as shown in the Examples that certain compounds of formula I have useful activity in the inhibition of one or more cancer cell lines.

[0244] The compounds of formula I described herein are peptides comprising 8 or 9 amino acid residues (Xaa1 to Xaa8 or Xaa1 to Xaa9, respectively) and an amino building block bound via a peptide bond at the C-terminus (Xaa10).

[0245] In some embodiments, the compounds of formula I are analogues of culicinin D. Culicinin D is a 10 amino acid peptaibol isolated from *Culicinomyces clavisporus* whose structure is shown in Scheme 1.



Scheme 1: Structure of culicinin D **1** and unusual building blocks contained therein.

[0246] Culicinin D **1** comprises several 2-aminoisobutyric acid (Aib, **2**) residues, as well as an acylated proline *N*-terminus. Culicinin D also contains two unusual non-proteinogenic residues, namely (2*S*,4*S*,6*R*)-2-amino-6-hydroxy-4-methyl-8-decanoic acid (AHMOD, **3**),
5 and (2*S*,4*R*)-2-amino-4-methyldecanoic acid (AMD, **4**), as well as the reduced C-terminal diamino alcohol (*S*)-2-(2-aminopropylamino)ethanol (APAE, **5**).

[0247] The compounds of formula I of the invention exclude culicinin D and various other known peptides.

[0248] The term "α-amino acid" or "amino acid", as used herein, unless indicated
10 otherwise, refers to a molecule containing both an amino group and a carboxyl group bound to a carbon which is designated the α-carbon. Suitable amino acids include, without limitation, both the D- and L-isomers of the naturally-occurring amino acids, as well as non-naturally occurring amino acids prepared by organic synthesis or other metabolic routes. Unless the context specifically indicates otherwise, the term amino acid, as used herein, is
15 intended to include amino acid analogs. The term "naturally occurring amino acid" refers to any one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V.

[0249] The terms "polypeptide" and "peptide", as used herein, unless indicated
20 otherwise, and the like are used herein interchangeably to refer to any polymer of amino acid residues of any length. The polymer can be linear or non-linear (e.g., branched), it can comprise modified amino acids or amino acid analogs. The term also encompasses amino acid polymers that have been modified naturally or by intervention, for example, by disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other
25 modification or manipulation, for example conjugation with labeling or bioactive components.

Synthesis of compounds of formula I

[0250] The compounds of formula I may be prepared using the methods and procedures described herein or methods and procedures analogous thereto. Other suitable
30 methods for preparing compounds of formula I will be apparent to those skilled in the art.

[0251] The compounds of formula I may be prepared from readily available starting materials using the methods and procedures described herein. It will be appreciated that where typical or preferred process conditions (for example, reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are indicated, other process conditions
35 can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants used.

[0252] Conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The need for protection and deprotection and the selection of appropriate protecting groups can be readily determined by a person skilled

in the art. Suitable protecting groups for various functional groups as well as suitable conditions for protecting and deprotecting particular functional groups are well known in the art (see, for example, T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Third Edition, Wiley, New York, 1999).

5 **[0253]** The starting materials useful in the methods and reactions are commercially available or can be prepared by known procedures or modifications thereof, for example those described in in standard reference texts such as Fieser and Fieser's *Reagents for Organic Synthesis*, Volumes 1-15 (John Wiley and Sons, 1991), *Organic Reactions*, Volumes 1-40 (John Wiley and Sons, 1991), *March's Advanced Organic Chemistry*, (John Wiley and
10 Sons, 4th Edition), and *Larock's Comprehensive Organic Transformations* (VCH Publishers Inc., 1989).

[0254] The various starting materials, intermediates, and compounds may be isolated and purified where appropriate using conventional techniques such as precipitation, filtration, crystallization, evaporation, distillation, and chromatography. Characterization of
15 the compounds may be performed using conventional methods such as by melting point, mass spectrum, nuclear magnetic resonance, and various other spectroscopic analyses.

[0255] Compounds of formula I comprise a peptide backbone. The backbone may be prepared from suitable amino acids and building blocks, which may be commercially available or prepared synthetically by methods and procedures analogous to those
20 described in the Examples herein or methods and procedures known in the art or analogous thereto. The synthesis of compounds of formula I from such building blocks may be achieved by standard peptide synthetic methods.

[0256] Peptide synthesis typically involves the formation of peptide bonds between two or more amino acids, two or more peptide fragments, or one or more amino acids and one
25 or more peptide fragments. Synthetic peptides may be prepared by solid phase peptide synthesis (SPPS), liquid phase peptide synthesis, or a combination thereof.

Solid phase peptide synthesis (SPPS)

[0257] The basic principle for SPPS is a stepwise addition of amino acids to a growing polypeptide chain anchored via a linker molecule to a solid phase support, typically a resin
30 particle, which allows for cleavage and purification once the polypeptide chain is complete. Briefly, a solid phase resin support and a starting amino acid are attached to one another via a linker molecule. Such resin-linker-acid matrices are commercially available.

[0258] The amino acid to be coupled to the resin is protected at its N α -terminus by a chemical protecting group. The amino acid may also have a side-chain protecting group.
35 Such protecting groups prevent undesired or deleterious reactions from taking place during the process of forming the new peptide bond between the carboxyl group of the amino acid to be coupled and the unprotected N α -amino group of the peptide chain attached to the resin.

[0259] The amino acid to be coupled is reacted with the unprotected Na-amino group of the N-terminal amino acid of the peptide chain, increasing the chain length of the peptide chain by one amino acid. The carboxyl group of the amino acid to be coupled may be activated with a suitable chemical activating agent to promote reaction with the Na-amino group of the peptide chain. The Na-protecting group of N-terminal amino acid of the peptide chain is then removed in preparation for coupling with the next amino acid residue. This technique consists of many repetitive steps making automation attractive whenever possible. Those skilled in the art will appreciate that peptides may be coupled to the Na-amino group of the solid phase bound amino acid or peptide instead of an individual amino acid, for example where a convergent peptide synthesis is desired.

[0260] When the desired sequence of amino acids is achieved, the peptide is cleaved from the solid phase support at the linker molecule.

[0261] SPPS may be carried out using a continuous flow method or a batch flow method. Continuous flow permits real-time monitoring of reaction progress via a spectrophotometer, but has two distinct disadvantages – the reagents in contact with the peptide on the resin are diluted, and scale is more limited due to physical size constraints of the solid phase resin. Batch flow occurs in a filter reaction vessel and is useful because reactants are accessible and can be added manually or automatically.

[0262] Two types of protecting groups are commonly used for protecting the N-alpha-amino terminus: "Boc" (tert-butyloxycarbonyl) and "Fmoc" (9-fluorenylmethyloxycarbonyl). Reagents for the Boc method are relatively inexpensive, but they are highly corrosive and require expensive equipment and more rigorous precautions to be taken. The Fmoc method, which uses less corrosive, although more expensive, reagents is typically preferred.

[0263] For SPPS, a wide variety of solid support phases are available. The solid phase support used for synthesis can be a synthetic resin, a synthetic polymer film or a silicon or silicate surface (e.g. controlled pore glass) suitable for synthesis purposes. Generally, a resin is used, commonly polystyrene suspensions, or polystyrene-polyethyleneglycol, or polymer supports for example polyamide.

[0264] Examples of resins functionalized with linkers suitable for Boc-chemistry include PAM resin, oxime resin SS, phenol resin, brominated Wang resin and brominated PPOA resin. Examples of resins suitable for Fmoc chemistry include amino-methyl polystyrene resins, AMPB-BHA resin, Sieber amide resin, Rink acid resin, Tentagel S AC resin, 2-chlorotrityl chloride resin, 2-chlorotrityl alcohol resin, TentaGel S Trt-OH resin, Knorr-2-chlorotrityl resin, hydrazine-2-chlorotrityl resin, ANP resin, Fmoc photolabile resin, HMBA-MBHA resin, TentaGel S HMB resin, Aromatic Safety Catch resinBAI resin and Fmoc-hydroxylamine 2 chlorotrityl resin. Other resins include PL Cl-Trt resin, PL-Oxime resin and PL-HMBA Resin. Generally resins are interchangeable.

[0265] For each resin appropriate coupling conditions are known in the literature for the attachment of the starting monomer or sub-unit.

[0266] Preparation of the solid phase support includes solvating the support in an appropriate solvent (e.g. dimethylformamide). The solid phase typically increases in volume during solvation, which in turn increases the surface area available to carry out peptide synthesis.

[0267] A linker molecule is then attached to the support for connecting the peptide chain to the solid phase support. Linker molecules are generally designed such that eventual cleavage provides either a free acid or amide at the C-terminus. Linkers are generally not resin-specific. Examples of linkers include peptide acids for example 4-hydroxymethylphenoxyacetyl-4'-methylbenzylhydramine (HMP), or peptide amides for example benzylhydramine derivatives.

[0268] The first amino acid of the peptide sequence may be attached to the linker after the linker is attached to the solid phase support or attached to the solid phase support using a linker that includes the first amino acid of the peptide sequence. Linkers that include amino acids are commercially available.

[0269] The next step is to deprotect the Na-amino group of the first amino acid. For Fmoc SPPS, deprotection of the Na-amino group may be carried out with a mild base treatment (piperazine or piperidine, for example). Side-chain protecting groups may be removed by moderate acidolysis (trifluoroacetic acid (TFA), for example). For Boc SPPS, deprotection of the Na-amino group may be carried out using for example TFA.

[0270] Following deprotection, the amino acid chain extension, or coupling, proceeds by the formation of peptide bonds. This process requires activation of the C- α -carboxyl group of the amino acid to be coupled. This may be accomplished using, for example, in situ reagents, preformed symmetrical anhydrides, active esters, acid halides, or urethane-protected N-carboxyanhydrides. The in situ method allows concurrent activation and coupling. Coupling reagents include carbodiimide derivatives, for example N,N'-dicyclohexylcarbodiimide or N,N-diisopropylcarbodiimide (DIC). Coupling reagents also include uronium or phosphonium salt derivatives of benzotriazole. Examples of such uronium and phosphonium salts include HBTU (O-(1H-benzotriazole-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), BOP (benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate), PyBOP (Benzotriazole-1-yl-oxy-tripyrrolidinophosphonium hexafluorophosphate), PyAOP, HCTU (O-(1H-6-chloro-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), TCTU (O-(1H-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate), HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), TATU (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate), TOTU (O-[cyano(ethoxycarbonyl)methyleneamino]-N,N,N',N''-tetramethyluronium tetrafluoroborate), HAPyU (O-(benzotriazol-1-yl)oxybis-(pyrrolidino)-uronium hexafluorophosphate and COMU

(1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylaminomorpholinomethylene)]-methanaminium hexafluorophosphate). In some embodiments, the coupling reagent is HBTU, HATU, BOP, or PyBOP.

[0271] After the desired amino acid sequence has been synthesized, the peptide is
5 cleaved from the resin. The conditions used in this process depend on the sensitivity of the amino acid composition of the peptide and the side-chain protecting groups. Generally, cleavage is carried out in an environment containing a plurality of scavenging agents to quench the reactive carbonium ions that originate from the protective groups and linkers. Common cleaving agents include, for example, TFA, hydrogen fluoride (HF) and 1,1,1,3,3,3-
10 hexafluoro-2-propanol (HFIP). In some embodiments, where the peptide is bound to the solid phase support via a linker, the peptide chain is cleaved from the solid phase support by cleaving the peptide from the linker.

[0272] The conditions used for cleaving the peptide from the resin may concomitantly remove one or more side-chain protecting groups. In some embodiments, one or more or
15 all protecting groups are removed on cleaving the peptide from the solid phase support.

[0273] The use of protective groups in SPPS is well established. Examples of common protective groups include but are not limited to acetamidomethyl (Acm), acetyl (Ac), adamantyloxy (AdaO), benzoyl (Bz), benzyl (Bzl), 2-bromobenzyl, benzyloxy (BzIO), benzyloxycarbonyl (Z), benzyloxymethyl (Bom), 2-bromobenzyloxycarbonyl (2-Br-Z), tert-butoxy (tBuO), tert-butoxycarbonyl (Boc), tert-butoxymethyl (Bum), tert-butyl (tBu), tert-buthylthio (tButhio), 2-chlorobenzyloxycarbonyl (2-Cl-Z), cyclohexyloxy (cHxO), 2,6-dichlorobenzyl (2,6-DiCl-Bzl), 4,4'-dimethoxybenzhydryl (Mbh), 1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)3-methyl-butyl (ivDde), 4-{N-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)3-methylbutyl]-amino} benzyloxy (ODmab), 2,4-dinitrophenyl (Dnp),
25 fluorenylmethoxycarbonyl (Fmoc), formyl (For), mesitylene-2-sulfonyl (Mts), 4-methoxybenzyl (MeOBzl), 4-methoxy-2,3,6-trimethyl-benzenesulfonyl (Mtr), 4-methoxytrityl (Mmt), 4-methylbenzyl (MeBzl), 4-methyltrityl (Mtt), 3-nitro-2-pyridinesulfonyl (Npys), 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl (Pbf), 2,2,5,7,8-pentamethyl-chromane-6-sulfonyl (Pmc), tosyl (Tos), trifluoroacetyl (Tfa),
30 trimethylacetamidomethyl (Tacm), trityl (Trt) and xanthyl (Xan).

[0274] Where one or more of the side chains of the amino acids of the peptide contains functional groups, such as for example additional carboxylic, amino, hydroxy or thiol groups, additional protective groups may be necessary. For example, if the Fmoc strategy is used, Mtr, Pmc, Pbf may be used for the protection of Arg; Trt, Tmob may be used for the
35 protection of Asn and Gln; Boc may be used for the protection of Trp and Lys; tBu may be used for the protection of Asp, Glu, Ser, Thr and Tyr; and Acm, tBu, tButhio, Trt and Mmt may be used for the protection of Cys. A person skilled in the art will appreciate that there are numerous other suitable combinations.

[0275] The methods for SPPS outlined above are well known in the art. See, for example, Atherton and Sheppard, "Solid Phase Peptide Synthesis: A Practical Approach," New York: IRL Press, 1989; Stewart and Young: "Solid-Phase Peptide Synthesis 2nd Ed.," Rockford, Illinois: Pierce Chemical Co., 1984; Jones, "The Chemical Synthesis of Peptides," Oxford: Clarendon Press, 1994; Merrifield, J. Am. Soc. 85:2146-2149 (1963); Marglin, A. and Merrifield, R.B. Annu. Rev. Biochem. 39:841-66 (1970); and Merrifield R.B. JAMA. 210(7):1247-54 (1969); and "Solid Phase Peptide Synthesis – A Practical Approach" (W.C. Chan and P.D. White, eds. Oxford University Press, 2000). Equipment for automated synthesis of peptides or polypeptides is readily commercially available from suppliers such as Perkin Elmer/Applied Biosystems (Foster City, CA) and may be operated according to the manufacturer's instructions.

[0276] Following cleavage from the resin, the peptide may be separated from the reaction medium, e.g. by centrifugation or filtration. The peptide may then be subsequently purified, e.g. by HPLC using one or more suitable solvents.

[0277] The compounds of formula I may be prepared by a method comprising comprise coupling one or more amino acid and/or one or more peptide by SPPS. Coupling an amino acid or a peptide to another amino acid or peptide as described herein typically comprises forming a peptide bond between the N-terminus of the amino acid or an amino acid of the peptide of one coupling partner and the C-terminus of the amino acid or an amino acid of the peptide of the other coupling partner.

[0278] Confirmation of the identity of the peptides synthesized may be conveniently achieved by, for example, amino acid analysis, mass spectrometry, Edman degradation, and the like.

[0279] The preparation of compounds of formula I may comprise separating the compound from the liquid reaction medium. Any suitable separation methods known in the art may be used, for example, precipitation and filtration. The peptide may be subsequently purified, for example, by HPLC using one or more suitable solvents.

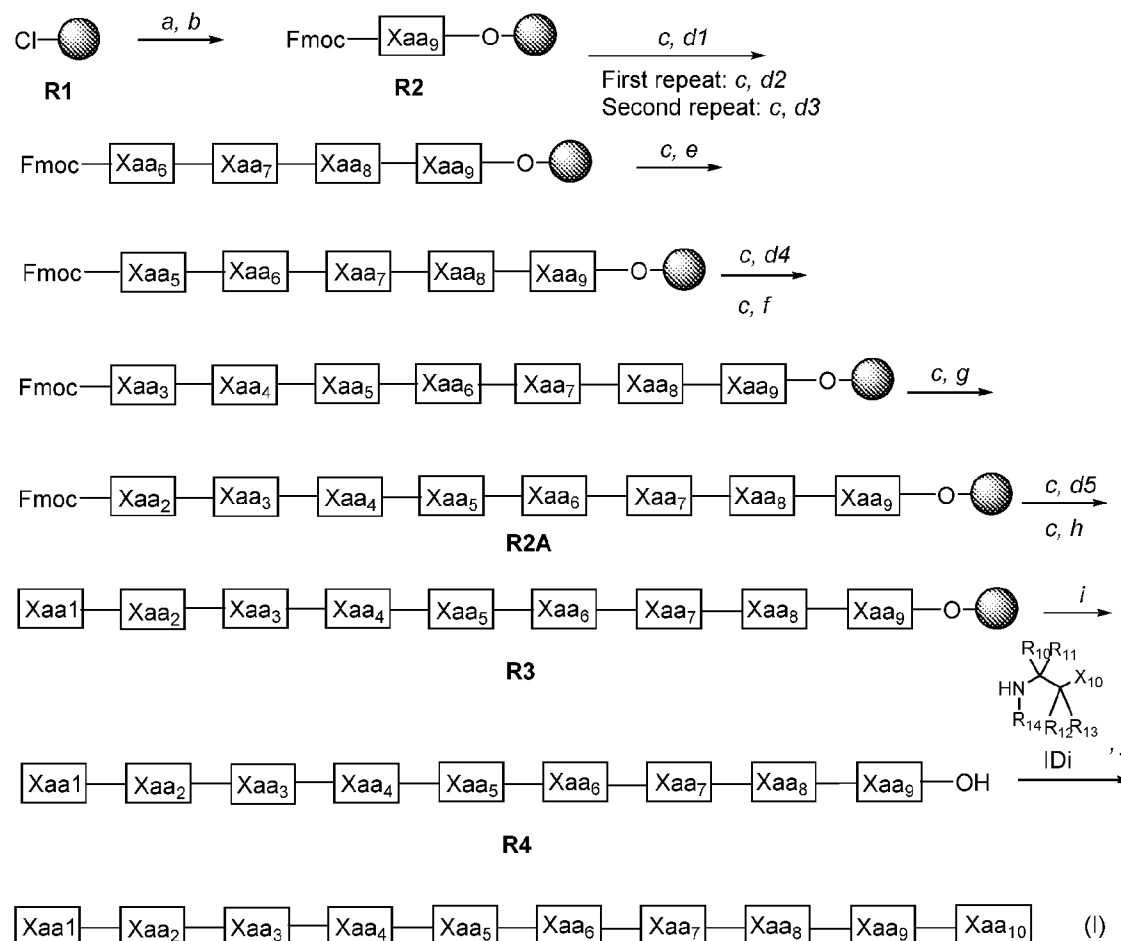
[0280] The peptides, thus, may be pure or purified, or substantially pure or purified. As used herein "purified" does not require absolute purity; rather, it is intended as a relative term where the material in question is more pure than in the environment it was in previously. In practice the material has typically, for example, been subjected to fractionation to remove various other components, and the resultant material has substantially retained its desired biological activity or activities. The term "substantially purified" refers to materials that are at least about 60% free, preferably at least about 75% free, and most preferably at least about 90% free, at least about 95% free, at least about 98% free, or more, from other components with which they may be associated during manufacture.

[0281] The compounds of formula I may be prepared by SPPS by the iterative or sequential addition of requisite amino acids or building blocks, as described in above. For

example, a portion of the compounds of formula I may be synthesised by SPSS and synthesis of the compounds may be completed by traditional solution-phase synthetic techniques. For example, the synthesis of a peptide comprising Xaa1 to Xaa9 may be carried out by SPSS, the peptide may then be cleaved from the resin, and then Xaa10

5 coupled to the C-terminus of the peptide by traditional solution-phase synthetic techniques.

[0282] A general procedure for the synthesis of compounds of formula I is shown in Scheme 2 below.



Scheme 2: Reagents and conditions: a) Fmoc-Xaa9-OH, DIPEA, CH₂Cl₂, 1 × 6 h, coupling repeated with fresh reagents for 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d1)-d5) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, where Fmoc-AA-OH is d1: Fmoc-Xaa8-OH, d2: Fmoc-Xaa7-OH, d3: Fmoc-Xaa6-OH, d4: Fmoc-Xaa4-OH, d5: Fmoc-Xaa1-OH; e) Fmoc-Xaa5-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Xaa3-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Xaa2-OH, HATU, DIPEA, DMF, 2 h; h) C₁-C₁₅alkyl carboxylic acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h or TFA/TIPS/H₂O (95:2.5:2.5, v/v/v), 2 h; j) compound IDi (the amino building block corresponding to Xaa10), DIC, 6-Cl-HOBt, DMF, 12 h.

[0283] With reference to Scheme 2, the synthesis of compounds of formula I may be carried out by SPPS, starting with a suitable resin **R1**, for example 2-chlorotrityl chloride

functionalised polystyrene resin. Amino acid building blocks are then coupled to the resin in a stepwise manner.

[0284] The *N*-terminus (N^c) of the amino acid to be coupled is typically protected using a suitable protecting group, for example Fmoc. Fmoc-protected amino acids described
5 herein are referred to by the nomenclature "Fmoc-AA-OH", where AA denotes the amino acid that has undergone Fmoc protection. Examples of the use of this nomenclature throughout the specification include but are not limited to Fmoc- β -alanine-OH and Fmoc-AMD-OH. Amino acids protected with suitable protecting groups, for example Fmoc-protected amino acids may be commercially available or prepared synthetically by
10 conventional methods known in the art.

[0285] With continued reference to Scheme 2, the first amino acid, Fmoc-Xaa9-OH is reacted with the resin **R1** to attach the unprotected C-terminus of the amino acid to the resin to give resin **R2**. The Fmoc protecting group is then removed, for example using 20% piperidine in the presence of DMF, to allow for the attachment of the next Fmoc-protected
15 amino acid (Fmoc-Xaa8-OH) to the growing chain. This sequence of Fmoc-deprotection and Fmoc-protected amino acid attachment is repeated until a resin comprising a peptide chain made up of Xaa2 to Xaa9 **R2A** is synthesised.

[0286] The conditions for coupling of the various Fmoc protected amino acids of Xaa1 to Xaa9 may vary. For example, HATU may be used as the coupling reagent for Xaa1,
20 Xaa2, Xaa4, Xaa6, Xaa7, and Xaa8, COMU and Oxyma may be used as the coupling reagents for Xaa3 and Xaa5 and DIC may be used as the coupling reagent for Xaa10. Suitable conditions and coupling reagents for coupling the various amino acids or building blocks will be apparent to those skilled in the art.

[0287] Following the formation of resin **R2A**, the Fmoc group at N-terminus of Xaa2 is then removed and the amino acid corresponding to Xaa1 is coupled. The Fmoc protecting
25 group on Xaa1 is removed and the R_1 group of Xaa2 is installed providing the solid phase bound peptide **R3**.

[0288] It will be understood by a person skilled in the art that Scheme 2 shows the synthesis of a compound according to an embodiment of the invention, in which the R_1
30 group of Xaa1 is installed by acylation using a C_1 - C_{15} alkyl carboxylic acid. Other R_1 groups may be installed by reaction of resin **R2A** with a suitable precursor of the R_1 group. Suitable precursors for various R_1 groups described herein will be apparent to a person skilled in the art.

[0289] As shown in Scheme 2, the peptide chain made up of Xaa1 to Xaa9 is cleaved
35 from resin **R3** to give the free peptide **R4**. The free peptide **R4** is then reacted with the compound IDi (the amino building block which corresponds to Xaa10) in the presence of *N,N'*-diisopropylcarbodiimide (DIC) and 6-chloro-1-hydroxy-benzotriazole (6-Cl-HOBt) in dimethylformamide (DMF) to attach the amino group of compound IDi to the C-terminus of the peptide **R4** and give a compound of formula I as defined herein. Alternatively, it will be

understood by a person skilled in the art that in some embodiments a peptide chain attached to the resin made up of Xaa1 to Xaa10 may be prepared and then be cleaved from the resin to give a compound of formula I as defined herein.

[0290] As mentioned above, where one or more of the side chains of the amino acids in the peptide contain functional groups, additional protective groups for these side chains may be necessary. Amino acids comprising side chain protecting groups may be commercially available or prepared by conventional synthetic procedures. Side chain protecting groups are often selected based on their ability to resist cleavage until it is desirable for them to be removed from the growing peptide chain. For example, in some embodiments cleavage from the resin may result in the removal of one or more or all amino acid side chain protecting groups present in the resin bound peptide **R3**. Alternatively, in some embodiments one or more or all of the amino acid side chain protecting groups present in the resin bound peptide **R3** may be retained on cleavage from the resin and removed after coupling the amino building block corresponding to Xaa10.

Drug-reactive linker conjugates

[0291] The present invention also relates to a drug-reactive linker conjugate comprising a compound of formula I as defined herein or a compound listed in the proviso relating thereto (i.e. a compound of formula I as defined herein but excluding the proviso), and a reactive linker group having a reactive site that allows the reactive linker group to be reacted with a ligand, for example an antibody. While the compounds of formula I of the invention exclude certain known peptides, the drug-reactive linker conjugate and the drug-ligand conjugate of the invention include the use of such known peptides. That is, the drug-reactive linker conjugate or drug-ligand conjugate of the invention may comprise a compound of formula I or a compound listed in the proviso relating thereto.

[0292] The compound may be attached to the reactive linker group by covalent or non-covalent bonding. In some embodiments the compound may be attached to the reactive linker group covalently by the replacement of one or more atom, for example a hydrogen atom of an OH, SH or NH group in the compound, or an oxygen atom of a ketone in the compound, with the reactive linker group.

[0293] In various embodiments the reactive linker group may be covalently attached to the compound by the replacement of one or more atoms in Xaa10 of the compound, for example in X10 of Xaa10, with the reactive linker group.

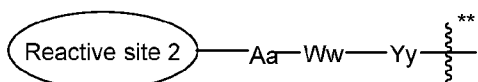
[0294] In various embodiments the reactive linker group may be covalently attached to the compound by the replacement of one or more atoms in Xaa2, for example in R_a of Xaa2, X of Xaa2 or Q_b or Q_c of Xaa2.

[0295] In various embodiments the reactive linker group may be covalently attached to the compound by the replacement of one or more atoms in Xaa2. For example, R_6 in Xaa2 may be $C_1-C_8alkyl(Q_b)-C(X)-C_1-C_8alkyl(Q_c)$; X at each instance may be independently

O, S or NR_e, preferably O; and the reactive linker group may be covalently attached to the compound by the replacement of X with the reactive linker group.

[0296] In various embodiments the reactive linker group may be covalently attached to the compound by the replacement of one or more atoms in Xaa1, for example in R₁ of Xaa1.

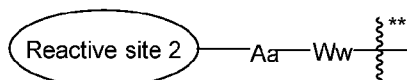
[0297] To prepare a drug-reactive linker conjugate of the present invention, a compound of formula I may be reacted with a reactive site on the reactive linker group. In some embodiments the reactive linker group may have the structure



wherein the reactive linker group comprises a Spacer unit (—Y—), an Amino acid unit (—W—) and a Stretcher unit (—A—) as defined herein, wherein y, w and a are each independently at least 1. Reactive site 2 is the reactive site that allows the reactive linker group to be reacted with a ligand, and ** denotes the bond to the compound.

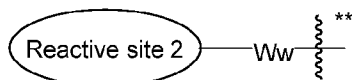
[0298] It will be understood that where a reactive linker group does not comprise a Spacer unit, Ww will be bound to the compound; where a reactive linker group does not comprise a Spacer unit or an Amino acid unit, Aa will be bound to the compound; where a reactive linker group does not comprise a Stretcher unit, Ww is attached to Reactive site 2; where a reactive linker group does not comprise a stretcher unit or an Amino acid unit, Yy is attached to Reactive site 2.

[0299] Alternately, in some embodiments the reactive linker group may have the structure:



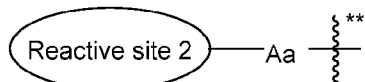
when the Spacer unit (—Y—) is absent and ** is as defined herein.

[0300] In some embodiments the reactive linker group may have the structure:



when both the Stretcher unit (—A—) and the Spacer unit (—Y—) are absent and ** is as defined herein.

[0301] In some embodiments the reactive linker group may also have the structure:



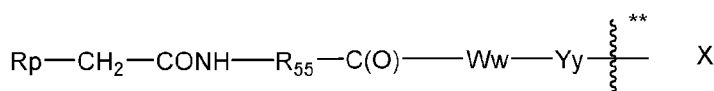
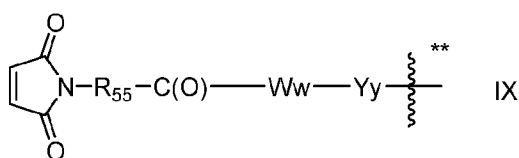
when both the Amino Acid unit (—W—) and the Spacer Unit (—Y—) are absent and ** is as defined herein.

[0302] In general, a suitable reactive linker group has a Stretcher unit linker to an optional Amino Acid unit and an optional Spacer Unit. The various units in the reactive linker group are each described in more detail below.

a. Stretcher unit

[0303] The Stretcher unit (-A-), when present, is capable of linking a ligand for example an antibody, to an Amino acid unit (—W—). In this regard a ligand (T) has a reactive site that allows the ligand, for example an antibody, to be reacted with Reactive site 2 of the Stretcher unit.

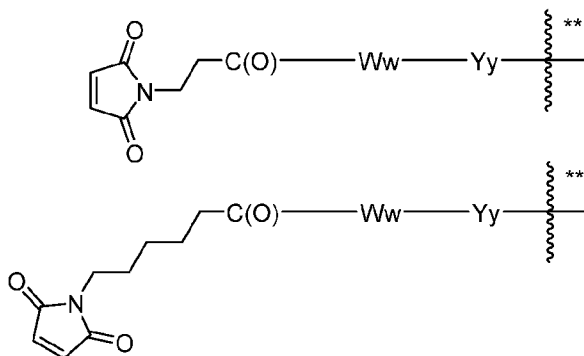
[0304] In one embodiment, the Stretcher unit has a Reactive site 2 that allows the formation of a bond with a sulfur atom of a ligand. The sulfur atom may be derived from a sulfhydryl group of a ligand. Reactive linker groups comprising representative Stretcher units of this embodiment are depicted in formulas IX and X, where ** represents the bond to the compound.



Scheme 3: Reactive linker groups of formula IX and X.

[0305] In the Stretcher unit of the reactive linker of formula IX, the reactive site that allows the reactive linker group to be reacted with a ligand, for example an antibody, may be the carbon-carbon double bond in the maleimide moiety. In such an embodiment, the R₅₅ group may be selected from C₁-C₁₅alkyl, C₃-C₈carbocycle, O(C₁-C₈ alkyl), aryl, C₁-C₁₅alkyl(aryl), arylC₁-C₁₅alkyl, C₁-C₁₅alkyl(C₃-C₈carbocycle), (C₃-C₈carbocycle)-C₁-C₁₅ alkyl, a 3 to 8 membered heterocycle, C₁-C₁₅alkyl-(3 to 8 membered heterocycle), (3 to 8 membered heterocycle)-C₁-C₁₅alkyl, (CH₂CH₂O)_r, and (CH₂CH₂O)_r-CH₂, preferably R₅₅ is a C₁-C₁₅alkyl, preferably a C₁-C₅alkyl.

[0306] Illustrative embodiments of reactive linker groups of formula IX are shown in Scheme 4 below, where R₅₅ is a C₂alkyl or C₅alkyl.

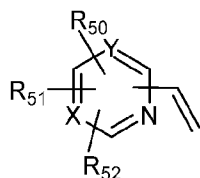


Scheme 4: Illustrative examples of reactive linker groups of formula IX.

[0307] As an alternative to a reactive linker group of formula IX, a reactive linker group of formula X may be used, where R₅₅ is as defined herein. In such an embodiment,

Rp represents a suitable leaving group that is eliminated following reaction with the reactive site on a ligand. Suitable leaving groups are known in the art and include, for example a halogen, preferably Br, Cl or I, preferably I. Leaving groups other than halogens may also be used in reactive linker groups of formula X and suitable non-halogen leaving groups, for example sulfonates, will be apparent to a person skilled in the art.

[0308] Another example of a reactive linker group comprising a Stretcher unit having a reactive site that allows the formation of a bond with a sulfur atom of a ligand is depicted by formula VI below, wherein X, Y, R₅₀, R₅₁ and R₅₂ are as described herein.

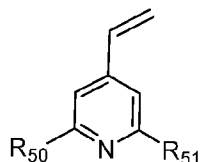


VI

Scheme 5: A reactive linker group of formula VI.

[0309] In the embodiment depicted in Scheme 5, the vinyl group of formula VI acts as the reactive site for reaction with a sulfur atom of a ligand (for example, in a thiolene reaction). The reactive linker group of formula VI may be attached to the compound, through R₅₀, R₅₁ and/or R₅₂ which may be selected from suitable groups described herein.

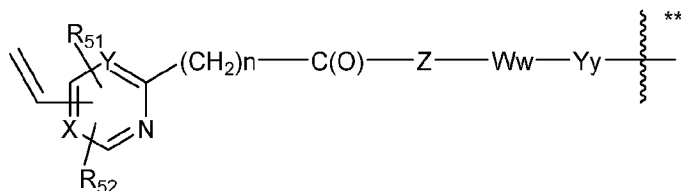
[0310] In some embodiments the reactive linker of formula VI may be a reactive linker of formula VII as shown in Scheme 6, where R₅₀ and R₅₁ are as described herein.



VII

Scheme 6: A reactive linker of formula VII.

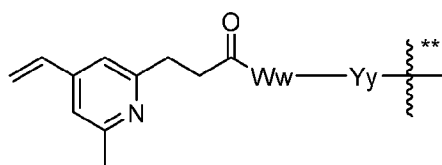
[0311] A representative example of the reactive linker group of formula VI and VII is shown in Scheme 7 below. R₅₀ in this embodiment is a (CH₂)_n-C(O)-Z- group, where n is an integer from 0 to 10 and Z is NH, O or S. The Stretcher unit in this embodiment is attached to the rest of the linker group (Ww-Yy) through Z, and to the compound, through a bond from the Spacer unit (Y) to the compound, denoted by **.



Scheme 7: A representative reactive linker group.

[0312] The reactive linkers described herein may be capable of binding more than one compound of formula I, including a compound in the proviso of claim 1. For example, in the reactive linker group depicted in Scheme 7, R_{51} and R_{52} may also be $(CH_2)_n-C(O)-Z-$ bound to an additional compound of formula I or a compound listed in the proviso relating thereto via Ww-Yy.

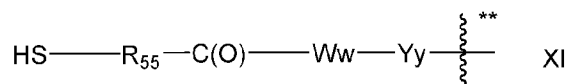
[0313] In a further representative embodiment, the reactive linker of Scheme 7 may be a reactive linker as depicted by formula VIII in Scheme 8 below, where Ww, Yy and ** are as defined herein.



VIII

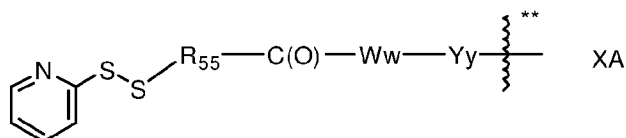
Scheme 8: A representative reactive linker group.

[0314] The above examples all demonstrate reactive linker groups capable of forming a bond with a sulfur atom of a ligand. However, the reactive linker group may be capable of binding to a ligand in other ways. For example, in some embodiments, the reactive linker group may comprise a Stretcher unit that is capable of binding to a ligand via a disulfide bond between a sulfur atom of the ligand and a sulfur atom of the Stretcher unit. A representative reactive linker group of this embodiment is depicted in formula XI below, where R_{55} and ** are as described above.



Scheme 9: Reactive linker group of formula XI.

[0315] Other reactive linker groups will also be apparent to a person skilled in the art. For example, in some embodiments the reactive linker group may have a structure XA as shown in Scheme 10 below, wherein R_{55} , Ww, Yy and ** are as defined herein.



Scheme 10: An example of a reactive linker group.

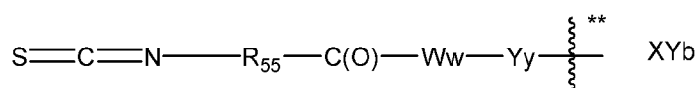
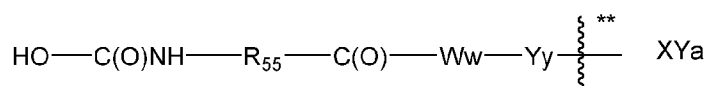
[0316] In some embodiments the drug-reactive linker conjugate of the invention may comprise a reactive linker group of formula XA, in which w and y are both 0 and R_{55} is a C_1 - C_{15} alkyl, preferably a C_1 - C_8 alkyl, preferably a C_1 - C_5 alkyl, preferably a C_1 - C_3 alkyl; preferably a C_2 alkyl.

[0317] In some embodiments in which the reactive linker group is a group of formula XA, the reactive linker group is covalently attached to the compound by the replacement of

one or more atom in Xaa1, preferably by one or more atom in R₁ of Xaa1 with the reactive linker group.

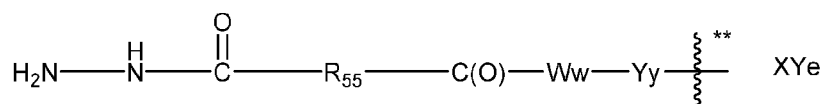
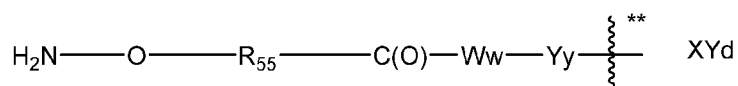
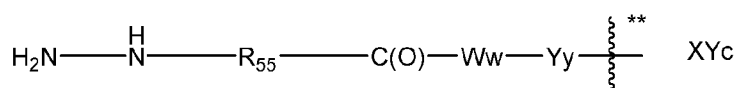
[0318] In some embodiments in which the reactive linker group is a group of formula XA, the reactive linker group is covalently attached by the replacement of one or more atom in Xaa1, preferably by the replacement of R₁ of Xaa1 with the reactive linker group, when R₁ of Xaa1 is H, R₂ and R₃ together form a ring and have the formula $-(CR^mR^n)_n-$ wherein n is from 4 to 6, and at each instance of n, R^m is independently H or OH and Rⁿ is independently H; and R₄ is H.

[0319] In yet another embodiment, the reactive group of the Stretcher may comprise a reactive site that can form a bond with a primary or secondary amino group of a ligand. Examples of such reactive sites include, but are not limited to, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates. Representative linker groups comprising Stretcher units of this embodiment are depicted in Formulas XYa and XYb, wherein R₅₅, Ww, Yy and ** are as defined herein.



Scheme 11: Examples of reactive linker groups.

[0320] In yet another embodiment, the reactive group of the Stretcher may contain a reactive site that is reactive to a modified carbohydrate, an aldehyde or a ketone group that may be present on a ligand. For example, a carbohydrate can be mildly oxidized using a reagent such as sodium periodate and the resulting (CHO) unit of the oxidized carbohydrate can be condensed with a Stretcher unit that contains a functionality such as a hydrazide, an oxime, a primary or secondary amine, a hydrazine, a thiosemicarbazone, a hydrazine carboxylate, and an arylhydrazide such as those described by Kaneko, T. et al. (1991) Bioconjugate Chem 2:133-41. Representative reactive linker groups of this embodiment are depicted by formulas XYc, XYd and XYe, wherein R₅₅, Ww, Yy and ** are as defined herein.



Scheme 12: Examples of reactive linker groups.

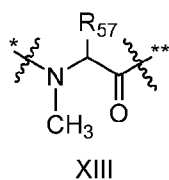
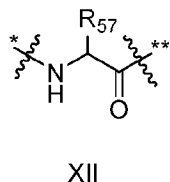
[0321] Alternatively, the reactive linker group, for example the Stretcher unit of the reactive linker group, may comprise an aldehyde or a ketone group that can be reacted with a suitable functionality on the ligand, for example a hydrazide, an oxime, a primary or secondary amine, a hydrazine, a thiosemicarbazone, a hydrazine carboxylate, and an arylhydrazide. It will be understood by a person skilled in the art that in some embodiments the ligand may be modified to introduce into the ligand such a suitable functionality.

[0322] Useful Stretcher units may be incorporated into a reactive linker using commercially available intermediates or by utilizing known techniques of organic synthesis. For example, Stretcher units may be introduced into a reactive linker group by reacting the Stretcher unit with the N-terminus of an Amino Acid unit.

b. Amino acid unit

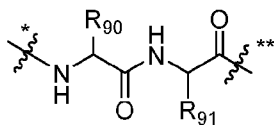
[0323] As described above the reactive linker may comprise an Amino acid unit. The Amino Acid unit (-W-), when present, links the Stretcher unit to the Spacer unit if the Spacer unit is present, links the Stretcher unit to the compound of formula I, or compound in the proviso relating thereto, if the Spacer unit is absent, and is capable of linking the ligand to the compound if the Stretcher unit and Spacer unit are both absent.

[0324] In various embodiments W_w — is a dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit. Each -W- unit is independently a group of formula XII or XIII as shown below, wherein w is an integer ranging from 0 to 12 and R_{57} is as described herein.



Scheme 13: Amino acids units for use in conjugates of the invention.

[0325] In vivo, the Amino Acid unit may be enzymatically cleaved by one or more enzymes, including for example a tumor-associated protease, to liberate the compound of formula I or compound listed in the proviso relating thereto, which in one embodiment is protonated in vivo upon release to provide the compound. Illustrative W_w units are represented by formulas (XYf)-(XYh), wherein * denotes the bond to the Stretcher unit, and ** denotes the bond to the Spacer unit.



Scheme 14: An example of an Amino acid unit for use in conjugates of the invention.

[0326] In the Amino acid unit depicted in Scheme 14, R₉₀ and R₉₁ may be selected from table A1 below.

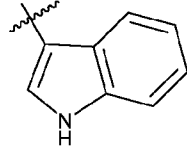
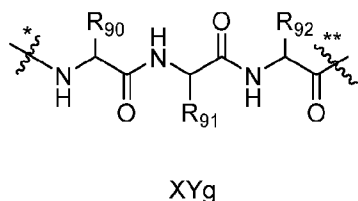
R ₉₀	R ₉₁
benzyl	(CH ₂) ₄ NH ₂
methyl	(CH ₂) ₄ NH ₂
isopropyl	(CH ₂) ₄ NH ₂
isopropyl	(CH ₂) ₃ NHCONH ₂
benzyl	(CH ₂) ₃ NHCONH ₂
isobutyl	(CH ₂) ₃ NHCONH ₂
sec-butyl	(CH ₂) ₃ NHCONH ₂
	(CH ₂) ₃ NHCONH ₂
benzyl	methyl
benzyl	(CH ₂) ₃ NHC(=NH)NH ₂

Table A1: R₉₀ and R₉₁ groups that may be present in Amino acid units of formula XYf.

[0327] Another example of an enzymatically cleavable Amino acid unit is depicted by formula XYg below.



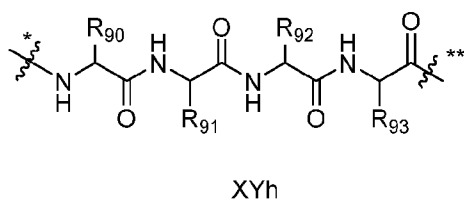
Scheme 15: An Amino acid unit according to an embodiment of the invention.

[0328] In the Amino acid unit depicted in Scheme 15, R₉₀, R₉₁ and R₉₂ may be selected from table A2 below.

R ₉₀	R ₉₁	R ₉₃
benzyl	benzyl	(CH ₂) ₄ NH ₂
isopropyl	benzyl	(CH ₂) ₄ NH ₂
H	benzyl	(CH ₂) ₄ NH ₂

Table A2: R₉₀, R₉₁ and R₉₂ groups that may be present in Amino acid units of formula XYg.

[0329] Another example of an enzymatically cleavable Amino acid unit is depicted by formula XYh below.



Scheme 16: An Amino acid unit according to an embodiment of the invention.

[0330] In the Amino acid unit depicted in Scheme 16, R₉₀, R₉₁, R₉₂ and R₉₃ may be selected from table A3 below.

R ₉₀	R ₉₁	R ₉₃	R ₉₄
H	benzyl	isobutyl	H
methyl	isobutyl	methyl	isobutyl

Table A3: R₉₀, R₉₁, R₉₂ and R₉₃ groups that may be present in Amino acid units of formula XYg.

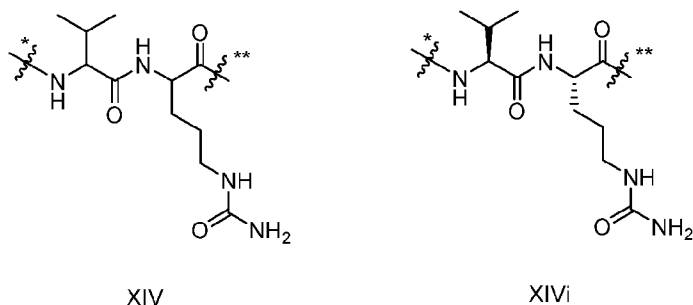
[0331] Exemplary Amino Acid units include, but are not limited to, units of formula (XYf) where: R₉₀ is benzyl and R₉₁ is $-(CH_2)_4NH_2$; R₉₀ isopropyl and R₉₁ is $-(CH_2)_4NH_2$; R₉₀ is isopropyl and R₉₁ is $-(CH_2)_3NHCONH_2$. Another exemplary Amino Acid unit is a unit of formula (XYg) wherein R₉₀ is benzyl, R₉₁ is benzyl, and R₉₁ is $-(CH_2)_4NH_2$.

[0332] Useful $-Ww-$ units can be designed and optimized in their selectivity for enzymatic cleavage by particular enzymes, for example, a tumor-associated protease. In one embodiment, a $-Ww-$ unit is that whose cleavage is catalyzed by cathepsin B, C and D, or a plasmin protease.

[0333] In one embodiment, $-Ww-$ is a dipeptide, tripeptide, tetrapeptide or pentapeptide, preferably a dipeptide.

[0334] When R₅₇, R₉₀, R₉₁, R₉₂ or R₉₃ is other than hydrogen, the carbon atom to which R₅₇, R₉₀, R₉₁, R₉₂ or R₉₃ is attached is chiral. Each carbon atom to which R₅₇, R₉₀, R₉₁, R₉₂ or R₉₃ is attached may be independently in the (S) or (R) configuration.

[0335] In one embodiment, the Amino Acid unit is valine-citrulline as depicted by formulas XIV and XIVi in Scheme 17 below.



Scheme 17: Amino acid units according to embodiments of the invention.

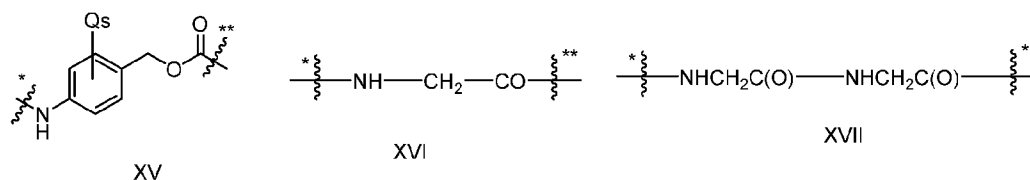
[0336] In another embodiment, the Amino Acid unit is phenylalanine-lysine (i.e. fk). In yet another embodiment of the Amino Acid unit, the Amino Acid unit is N-methylvaline-citrulline. In yet another embodiment, the Amino Acid unit may be selected from the group consisting of 5-aminovaleric acid, homo phenylalanine lysine, tetraisoquinolinecarboxylate lysine, cyclohexylalanine lysine, isonepecotic acid lysine, beta-alanine lysine, glycine serine valine glutamine and isonepecotic acid.

[0337] The Amino Acid unit may comprise natural amino acids. In other embodiments, the Amino Acid unit may comprise non-natural amino acids or a mixture of natural and non-natural amino acids.

c. Spacer

[0338] The Spacer unit ($-Y-$), when present, links an Amino Acid unit to a compound of formula I or compound listed in the proviso relating thereto, when an Amino Acid unit is present. Alternately, the Spacer unit links the Stretcher unit to the compound, when the Amino Acid unit is absent. The Spacer unit is also capable of linking the compound to a ligand, for example an antibody, when both the Amino Acid unit and Stretcher unit are absent.

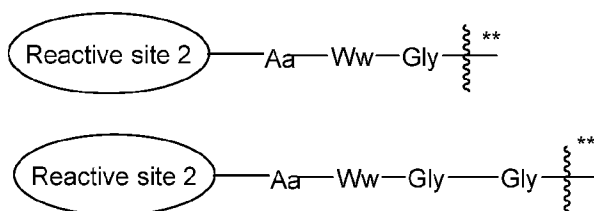
[0339] In some embodiments the Spacer unit may be a group of formula XV, XVI or XVII as shown in Scheme 18 below, wherein Q is C_1 - C_8 alkyl, O -(C_1 - C_8 alkyl), halogen, nitro or CN; s is an integer ranging from 0-4; * denotes the bond to the Amino acid unit and ** denotes the bond to the compound.



Scheme 18: Examples of Spacer units.

[0340] Spacer units may be of two general types: self-immolative and non self-immolative. A non self-immolative Spacer unit is one in which part or all of the Spacer unit remains bound to the compound after cleavage, for example enzymatic cleavage, of an Amino Acid unit from the ligand-drug conjugate. Ligand-drug conjugates of the invention are described in more detail below.

[0341] Examples of a non self-immolative Spacer unit include, but are not limited to a glycine-glycine (Gly-Gly) Spacer unit and a glycine (Gly) Spacer unit (both depicted in Scheme 19 below).



Scheme 19: Examples of non self-immolative Spacer units in reactive linker groups, wherein Aa, Ww, Reactive site 2 and ** are as described herein.

[0342] When a ligand-drug conjugate comprising a glycine-glycine Spacer unit or a glycine Spacer unit undergoes enzymatic cleavage, for example via a tumor-cell associated-protease, a cancer-cell-associated protease or a lymphocyte-associated protease, a glycine-glycine-compound moiety or a glycine-compound moiety is cleaved from the rest of the conjugate. In one embodiment, an independent hydrolysis reaction may take place within

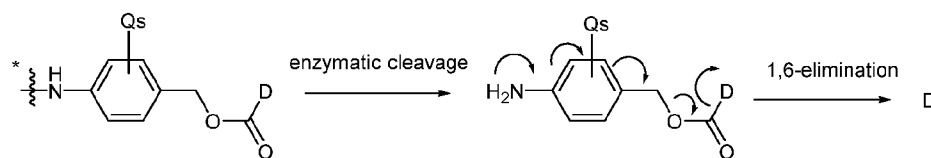
the target cell, cleaving the glycine—compound moiety bond and liberating the compound of formula I or compound listed in the proviso relating thereto.

[0343] In some embodiments, the reactive linker group may comprise a non self-immolative Spacer unit ($-Y-$) is -Gly-Gly-. In some embodiments the reactive linker group may comprise a non self-immolative the Spacer unit ($-Y-$) is -Gly-.

[0344] In one embodiment, the Spacer unit may be absent ($y=0$).

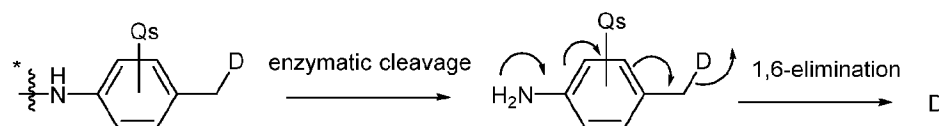
[0345] Alternatively, a drug-reactive linker conjugate or a ligand-drug conjugate may comprise a self-immolative Spacer unit, that can release the compound of formula I or compound listed in the proviso relating thereto, without the need for a separate hydrolysis step. In this embodiment, $-Y-$ is a p-aminobenzyl alcohol (PAB) residue of formula XV, whose phenyl portion is substituted with Q_s as defined herein.

[0346] The PAB residue may be linked to $-Ww-$ via the amine nitrogen atom of the PAB group. The PAB residue may be connected directly to the compound, for example, via a carbonate, carbamate or ether group. Without wishing to be bound by theory a possible mechanism for the release of the compound (D) bound to the PAB residue is depicted in Scheme 20 below.



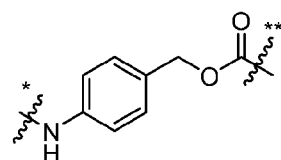
Scheme 20: Proposed mechanism for the release of a compound (D) from a PAB residue, where * denotes the bond to the Amino acid unit.

[0347] Without wishing to be bound by theory scheme 21 depicts a possible mechanism for the release of the compound (D) bound to a PAB group that is attached directly the compound via an ether or amine linkage.



Scheme 21: Proposed mechanism for the release of a compound (D) from a PAB residue, where * denotes the bond to the Amino acid unit.

[0348] In various embodiments the Spacer unit may be a PAB residue of formula XVIII wherein * denotes the bond to the Amino acid unit and ** denotes the bond to the compound.

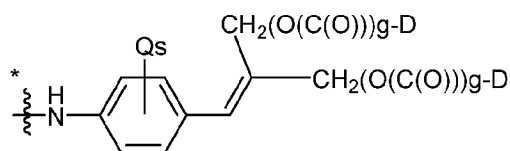


XVIII

Scheme 22: An exemplary Spacer unit XVIII.

[0349] Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group such as 2-aminoimidazol-5-methanol derivatives (Hay et al. (1999) Bioorg. Med. Chem. Lett. 9:2237) and ortho or para-aminobenzylacetals. Spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al., Chemistry Biology, 1995, 2, 223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm, et al., J. Amer. Chem. Soc., 1972, 94, 5815) and 2-aminophenylpropionic acid amides (Amsberry, et al., J. Org. Chem., 1990, 55, 5867). Elimination of amine-containing drugs that are substituted at the α -position of glycine (Kingsbury, et al., J. Med. Chem., 1984, 27, 1447) are also examples of self-immolative spacer units that may be useful.

[0350] In one embodiment, the Spacer unit may be a branched bis(hydroxymethyl)styrene (BHMS) unit as depicted in Scheme 23. Such a Spacer unit may be used to incorporate and release multiple compounds of formula I or compound listed in the proviso relating thereto.



Scheme 23: An example of a branched Spacer unit, wherein Q is C₁-C₈alkyl, O-(C₁-C₈alkyl), halogen, nitro or CN; s is an integer ranging from 0-4; g is 0 or 1, D is a compound of formula I or compound listed in the proviso relating thereto and *denotes the bond to the Amino acid unit.

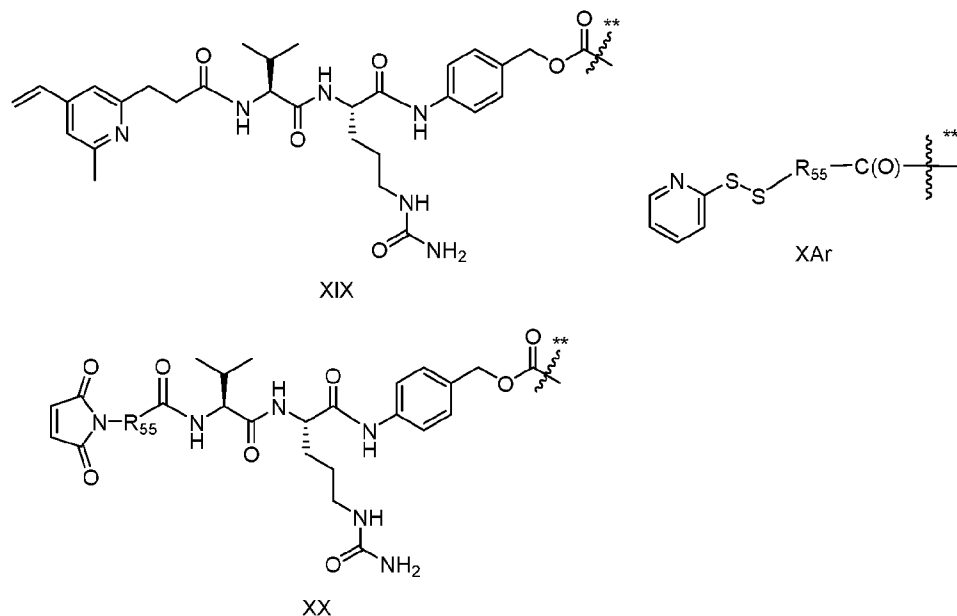
[0351] It will be understood by a person skilled in the art that other reactive linker groups, for example other stretcher units, amino acids units and/or spacer units to those described herein may also be applicable to and/or used in embodiments of the invention. Examples of such linker groups, stretcher units, amino acids units and/or spacer units include but are not limited to those described in US7964566B2 and US20170232108A1, which are incorporated by reference herein in their entirety.

Synthesis of drug-reactive linker conjugates

[0352] Various reactive linker groups have been described herein, including various Stretcher units, Amino acid units and Spacer units that may be used in embodiments of the invention. These Stretcher units, Amino acid units and Spacer units may be assembled in any order to form the reactive linker groups and the drug-reactive linker conjugates comprising the reactive linker groups described herein. For example, the Stretcher unit may be linked to the Amino acid unit first. The Spacer unit may then be reacted with the Amino acid unit to form the reactive linker group. The reactive linker group, preferably the Amino acid unit of the reactive linker group, may then be reacted with a compound of formula I or

compound listed in the proviso relating thereto, to form a drug-reactive linker conjugate of the invention.

[0353] The reactions to link the Stretcher unit, Amino acid unit and Spacer unit together to form the reactive linker group will depend on the nature of these units, in particular, the nature of the functional groups involved in the formation of the bonds between these units. For example, illustrative examples of reactive linker groups are shown in Scheme 24 below.



Scheme 24: Illustrative reactive linker groups, wherein R₅₅ is as described herein and ** denotes the bond to a compound of formula I or compound listed in the proviso relating thereto.

[0354] Although the Stretcher units in reactive linker groups XIX and XX are different, both these reactive linker groups comprise a valine-citrulline residue as the Amino acid unit and a PAB residue as the Spacer unit. As shown in Scheme 24, in both XIX and XX, the Stretcher unit, Amino acid unit and Spacer unit are linked by amide bonds. Suitable conditions for forming such amide bonds are well known and will be apparent to a person skilled in the art.

[0355] In various embodiments the Spacer unit of the reactive linker group may form a bond to the compound. However, when the Spacer unit is absent, the bond between the reactive linker group and the compound may be formed between the Amino acid unit of the reactive linker group, if present.

[0356] In the absence of both a Spacer unit and an Amino acid unit, the bond between the reactive linker group and the compound will be formed between the Stretcher unit and the compound. An example of such an embodiment is depicted in Formula XA of Scheme 24. In such an embodiment the Stretcher unit is capable forming a bond directly with a compound of formula I or compound listed in the proviso relating thereto. Therefore, in such

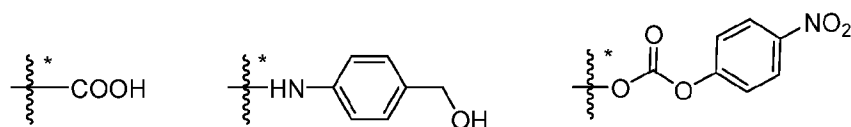
an embodiment the synthesis of a drug-reactive linker conjugate comprises the reaction of a Stretcher unit with the compound.

[0357] In general, to form the drug-reactive linker conjugates of the invention a reactive linker group may be synthesised first, followed by reaction of the reactive linker

group with a compound of formula I or compound listed in the proviso relating thereto. Other methods for the synthesis of drug-reactive linker conjugates are also contemplated herein. For example, a drug may be reacted with a Spacer unit, for example PAB, and the Spacer unit bound to the drug may then be reacted with other parts of the reactive linker, for example the Amino acid unit and then the Stretcher unit to form a drug-reactive linker conjugate. It will be apparent to a person skilled in the art that the Stretcher unit, Amino acid unit, Spacer unit and the compound may be reacted in any order to form a drug-reactive linker conjugate of the invention.

[0358] In various embodiments a reactive linker group comprises a first reactive site (Reactive Site 1), capable of reacting with a compound of formula I or a compound listed in the proviso relating thereto, and a second reactive site (Reactive Site 2), capable of reacting with a ligand, for example an antibody. In various embodiments, the second reactive site is different to the first reactive site to allow for selective reaction.

[0359] The formation of a drug-reactive linker conjugate of the invention may comprise the reaction of the first reactive functional group (Reactive site 1) of a reactive linker group with a reactive functional group on compound of formula I or compound listed in the proviso relating thereto, thereby forming a drug-reactive linker conjugate of the invention. For example, Reactive Site 1 may be reactive to a nitrogen or oxygen atom of the compound of formula I or compound listed in the proviso relating thereto. Illustrative examples of such functional groups are shown in Scheme 25 below, wherein * denotes the bond to the reactive linker group.

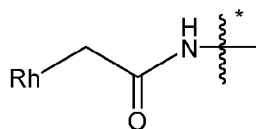


Scheme 25: Illustrative examples of reactive sites of a reactive linker group capable of reacting with a compound of formula I or compound listed in the proviso relating thereto.

[0360] In various embodiments the reactive site of a reactive linker group capable of reacting with a compound of formula I or compound listed in the proviso relating thereto is *-COOH, which reacts with an amine functionality of the compound.

[0361] Following the synthesis of the drug-reactive linker conjugate, the drug-reactive linker conjugate, for example, Reactive Site 2, may then be reacted with a suitable ligand, for example antibody, to produce ligand-drug conjugates. For example, in some embodiments Reactive Site 2 may be reactive to a sulfhydryl group on the ligand, for example on the antibody. As such, Reactive Site 2 may be a thiol accepting group such as a haloacetamide as shown in Scheme 26 below, wherein Rh represents a suitable leaving

group, such as for example O-mesyl, O-tosyl, Cl, Br or I; or a maleimide group, and * denotes the bond to the reactive linker group.



Scheme 26: Illustrative example of a reactive site of a reactive linker group capable of reacting with a ligand, for example an antibody.

[0362] The synthesis of ligand-drug conjugates is described in more detail below.

Synthesis of ligand-drug conjugates of the invention

[0363] The present invention also relates to ligand-drug conjugates (LDCs), for example antibody-drug conjugates (ADCs) comprising one or more compounds of formula I or compounds listed in the proviso relating thereto.

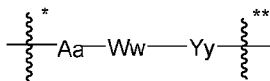
[0364] LDCs, for example ADCs, comprising one or more compounds of formula I or compounds listed in the proviso relating thereto, may have useful activity, for example, in the inhibition of one or more cancer cell lines.

[0365] In various embodiment the ligand-drug conjugate may have the formula T-(L-(D)o)p, wherein T is a ligand; L is a linker group; D is a compound of formula I or compound in the proviso relating thereto; and o is an integer from 1 to 10, preferably 2 to 6; and p is an integer from 1 to 10.

[0366] The synthesis of ligand-drug conjugates of the invention may comprise the reaction of a drug-reactive linker conjugate with a suitable ligand, such as an antibody.

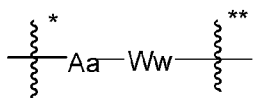
During this reaction, the reactive linker group and ligand react to form the linker group (L), which links the the ligand (T) to the one or more compounds.

[0367] As described in detail herein, a drug-reactive linker conjugate comprising the reactive linker group may comprise a Stretcher unit, an Amino acid unit and/or a Spacer unit. Therefore, the structure of the linker group formed on reaction of a reactive linker group with a suitable ligand will depend on the units present in the reactive linker group. For example, in some embodiments a linker group of a ligand-drug conjugate may have the structure shown in Scheme 27 below, wherein A, Y, W, a, y and w are as defined herein, * denotes a bond to the ligand and ** denotes a bond to the compound of formula I or compound listed in the proviso relating thereto.



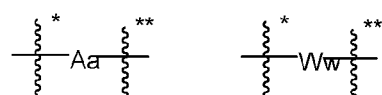
Scheme 27. An example of a linker group according to embodiments of the invention.

[0368] When the reactive linker group does not comprise a Spacer unit, the linker group of a ligand-drug conjugate may have the structure shown in Scheme 28 below, wherein A, W, a, and w are as defined herein, * denotes a bond to the ligand and ** denotes a bond to the compound.



Scheme 28: An example of a linker group according to embodiments of the invention.

[0369] In some embodiments a reactive linker group may comprise a Strecther unit in the absence of an Amino acid unit and a Spacer unit, or may comprise an Amino acid unit in the absence of a Stretcher unit and a Spacer unit. The linker groups of ligand-drug

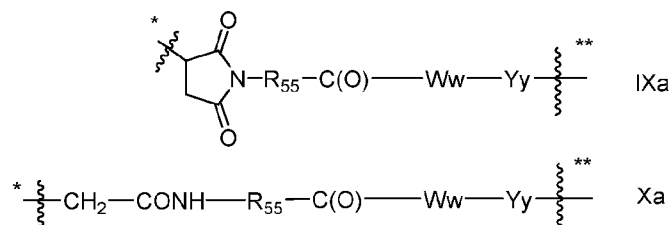


Scheme 29: Examples of linker groups according to embodiments of the invention.

[0370] In various embodiments a reactive linker group may comprise a Stretcher unit, and the ligand, for example the antibody may may form a bond to the Stretcher unit of the reactive linker group. In such an embodiment, the ligand (T) has a reactive site that allows the ligand, for example an antibody, to be reacted with Reactive site 2 of the Stretcher unit as described herein. Useful functional groups that can be present on a ligand, either naturally or via chemical manipulation include, but are not limited to, sulfhydryl ($-\text{SH}$), amino, hydroxyl, carboxy, the anomeric hydroxyl group of a carbohydrate, and carboxyl.

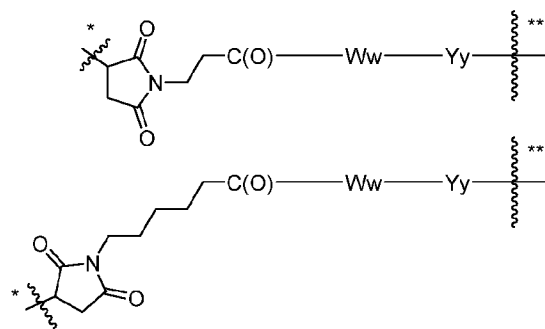
[0371] In some embodiments, the ligand reactive site may be a sulfhydryl or amino group. Sulfhydryl groups may be generated by reduction of an intramolecular disulfide bond of a ligand. Alternatively, sulfhydryl groups may be generated by reaction of an amino group of a lysine moiety of a Ligand using 2-iminothiolane (Traut's reagent) or another sulfhydryl generating reagent. Other functional groups that may be present on a ligand that represent suitable reactive sites to allow the ligand, for example an antibody, to be reacted with Reactive site 2 of the Stretcher unit have already been described in detail herein. Such functional groups include but are not limited to sulfur atoms, primary or secondary amino groups, modified carbohydrates, aldehydes and ketones.

[0372] In some embodiments a ligand, for example an antibody may comprise a sulfur atom, for example from a sulfhydryl group. In such embodiments the linker formed from the reaction of a reactive linker group with the ligand may be a linker group of formula IXa or Xa, wherein R_{55} is as defined herein, ** denotes the bond to the compound, and * denotes the bond to the sulfur atom of the ligand. Linker group IXa is formed from the reaction of reactive linker group IX with a ligand. Linker group Xa is formed from the reaction of reactive linker group X with a ligand.



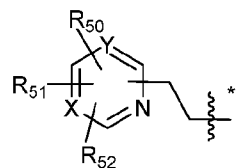
Scheme 30: Linker groups that may be formed from the reaction of a sulfur atom of a ligand with reactive linker groups.

[0373] Illustrative examples of linker groups of formula IXa are also shown in Scheme 31 below.



Scheme 31: Illustrative examples of linker groups of formula IXa.

[0374] Another example of a linker group formed from the reaction of a reactive linker group with a sulfur atom of a ligand, for example an antibody is shown in Scheme 32 below, wherein X, Y, R_{50} , R_{51} and R_{52} are as defined herein and * denotes the bond to the sulfur atom of the ligand. Linker group VIa is formed from the thiolene reaction of reactive linker group VI with a ligand comprising a free thiol.

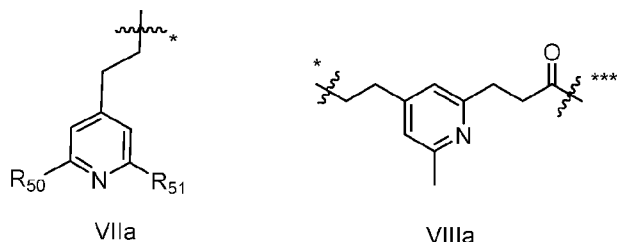


VIa

Scheme 32: Linker group of formula VIa.

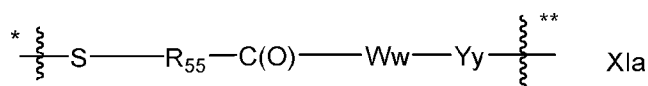
[0375] Illustrative examples of linker group VIa are shown in Scheme 33 below, wherein R_{50} and R_{51} are as defined herein, * denotes the bond to the ligand and ** denotes the bond to Ww-Yy-**, that is, the rest of the linker unit. Linker VIIa is formed from the

reaction of reactive linker VII with a ligand. Linker VIIa is formed from the reaction of reactive linker group VIII with a ligand.



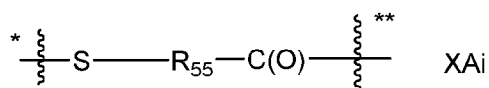
Scheme 33: Illustrative examples of linker groups of formula VIa.

- 5 **[0376]** The formation of a ligand-drug conjugate may comprise the reaction of a reactive linker group comprising a suitable leaving group with a ligand, for example an antibody. Examples of linker groups formed from such a reactive linker groups are shown in Scheme 34 below, wherein R_{55} , W , Y , w , and y are as defined herein, $*$ denotes the bond to the ligand and $**$ denotes the bond to the compound. For example, XIa may be formed
- 10 from reactive linker group XA



Scheme 34: Linker groups according to an embodiment of the invention.

- [0377]** In some embodiments, w and y in the linker groups shown in the Schemes above may both be zero. In such embodiments, the linker groups comprise only a Stretcher unit. An example of such an embodiment is illustrated below in Scheme 35 using the linker group of formula XAi as an example.
- 15



Scheme 35: Illustrative example of a linker group of formula XAi in which w and y of linker group XIa are both zero, and R_{55} , $*$ and $**$ are as defined herein.

- 20 **[0378]** A number of examples have been described above of ligand-drug conjugates formed by the reaction of a reactive site of a Stretcher unit in a drug-reactive linker conjugate with a ligand, for example an antibody. As discussed above, linker groups that do not comprise a Stretcher unit are also contemplated herein. In such embodiments it will be apparent to a person skilled in the art that the ligand will be reacting with a reactive site on
- 25 the Amino acid unit of the reactive linker group to form the ligand-drug conjugate.

- [0379]** In various embodiment the ligand-drug conjugate prepared as described above may have the formula $T-(L-(D)o)_p$, wherein T is a ligand; L is a linker group; D is a compound of formula I or compound in the proviso relating thereto; and o is an integer from 1 to 10, preferably 2 to 6; and p is an integer from 1 to 10. This formula allows for
- 30 multiple compounds to be bound to a single ligand. As indicated in the formula above, the number of compounds bound to the ligand will depend on the number of compounds

capable of binding to the linker group and the number of linker groups capable of binding to the ligand.

[0380] The number of compounds bound to a ligand is known as the drug to ligand ratio (for example, drug to antibody ratio). In some embodiments, the drug to antibody ratio is at least about 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, or 0.9 and useful ranges may be selected from any of these values, for example from about 0.1 to 10, 0.1 to 9, 0.1 to 8, 0.1 to 7, 0.1 to 6, 0.1 to 5, 0.2 to 10, 0.2 to 9, 0.2 to 8, 0.2 to 7, 0.2 to 6, 0.2 to 5, 0.3 to 10, 0.3 to 9, 0.3 to 8, 0.3 to 7, 0.3 to 6, 0.3 to 5, 0.4 to 10, 0.4 to 9, 0.4 to 8, 0.4 to 7, 0.4 to 6, or 0.4 to 5.

[0381] This ratio may be modulated by the use of a linker group capable of binding multiple compounds. Examples of such linker groups include dendritic linkers. A dendritic type linker allows for the attachment, preferably covalent attachment, of more than one compound to a ligand, for example an antibody.

[0382] The drug to ligand ratio may be increased by the use of dendritic linkers, and it will be understood by a person skilled in the art that drug to ligand ratio, that is, drug loading, may be related to the potency of the ligand-drug conjugates described herein.

[0383] Typically, fewer than the theoretical maximum of drug moieties are conjugated to a ligand, for example an antibody during a conjugation reaction. For example, an antibody may contain, many lysine residues that do not react with the drug-reactive linker conjugate or linker reagent. Only the most reactive lysine groups may react with an amine-reactive linker reagent. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds of the invention exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT). Additionally, the antibody must be subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine. The loading (drug/antibody ratio) of an ADC may be controlled in several different manners, including: (i) limiting the molar excess of drug-reactive linker conjugate and/or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

[0384] It is to be understood that where more than one nucleophilic group reacts with a drug-reactive conjugate, or reactive linker followed by drug moiety, then the resulting product may be a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by dual ELISA antibody assay, specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy, and separated by HPLC, e.g., hydrophobic interaction chromatography.

Ligand

[0385] Ligands useful herein include any ligand that binds or reactively associates or complexes with a receptor, antigen or other receptive moiety associated with a given

target-cell population. A ligand is a molecule that binds to, complexes with, or reacts with a moiety of a cell population sought to be therapeutically or otherwise biologically modified. In one aspect, the Ligand acts to deliver the Drug to the particular target cell population with which the Ligand reacts.

5 **[0386]** In some embodiments the conjugation of the compound to a ligand increases the efficacy of the compound in treating or preventing a disease or disorder described herein. In some embodiments the ligand binds to a particular cell population or to cells expressing a particular antigen or cell surface receptor. For example, the ligand may bind to a cell surface receptor or surface protein, which is overexpressed in a diseased cell such as
10 a cancer cell. For example, the ligand may bind to a breast cancer cell, for example, a breast cancer cell expressing the ERB2 receptor, HER2.

[0387] In one embodiment the ligand is a globular protein. In various embodiments the ligand is an antibody, a growth factor, a hormone, a peptide, an aptamer, a small molecule such as a hormone, an imaging agent, or cofactor, or a cytokine.

15 **[0388]** In one exemplary embodiment the ligand binds a specific antigen or cell surface receptor expressed by a cancer cell but not by normal cells or tissues. In various embodiments the ligand binds to two or more types of cancer cells.

[0389] Certain cell surface molecules, including hormone receptors such as human chorionic gonadotropin receptor and gonadotropin releasing hormone receptor are highly
20 expressed in cancer cells. In one embodiment the ligand is a hormone that binds to a hormone receptor. In other embodiments the ligand is a cofactor, a sugar, a drug molecule, an imaging agent, or a fluorescent dye. Some cancer cell types are known to overexpress folate receptors. Accordingly, in various embodiments the ligand is folic acid or other folate derivatives that bind to a folate receptor.

25 **[0390]** In various embodiments the ligand is a cytokine selected from the group comprising IL-1, , IL-2, IL-6, IL-15, CD40L, CD28, granulocyte-colony stimulating factor, macrophage-colony stimulating factor, granulocyte-macrophage colony stimulating factor, leukemia inhibitory factor, tumor necrosis factor, transforming growth factor, epidermal growth factor, insulin-like growth factors, and/or fibroblast growth factor.

30 **[0391]** In various embodiments the ligand is a peptide or cyclic peptide. Examples of cell-and tissue-targeting peptides that may be used for instance, in U.S. Patent Nos.6,232,287; 6,528,481; 7,452,964; 7,671,010; 7,781,565; 8,507,445; and 8,450,278, each of which is incorporated herein by reference.

[0392] Other useful non-immunoreactive protein, polypeptide, or peptide Ligands
35 include, but are not limited to, transferrin, epidennal growth factors ("EGF"), bombesin, gastrin, gastrin-releasing peptide, platelet-derived growth factortransforming growth factors ("TGF"), such as TGF- α and TGF- β , vaccinia growth factor ("VGF"), insulin and insulin-like growth factors I and II, lectins and apoprotein from low density lipoprotein.

[0393] In one embodiment the ligand is a peptide that binds to an integrin selected from the group comprising $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 6$, $\alpha 5\beta 1$ and $\beta 6$. Suitable peptides that bind integrins (integrin-binding peptides or ITPs) are known in the art, for example, peptides based on the RGD (Arg-Gly-Asp) recognition sequence for integrin ligands as described in Arosi et al., 2017. Recent Pat Anticancer Drug Discovery 12(2):148-168, which is incorporated herein by reference. A person of ordinary skill in the art can readily select a suitable peptide to bind a particular target integrin.

Antibodies

[0394] In various embodiments the ligand is an antibody or fragment thereof. In various embodiments the antibody is a monoclonal antibody, a bi specific antibody, a chimeric antibody, or a humanized antibody.

[0395] The term "antibody" as used herein refers to a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. "Antibody" includes intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity. Structurally, an antibody is typically a Y-shaped protein consisting of four amino acid chains, two heavy and two light. Each antibody has primarily two regions: a variable region and a constant region. The variable region, located on the ends of the arms of the Y, binds to and interacts with the target antigen. This variable region includes a complementary determining region (CDR) that recognizes and binds to a specific binding site on a particular antigen. The constant region, located on the tail of the Y, is recognized by and interacts with the immune system (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology*, 5th Ed., Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody.

[0396] The term "antibody" as used herein, also refers to a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, i.e., a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (e.g., IgG, IgE, IgM, IgD, and IgA), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The immunoglobulins can be derived from any species. In one aspect, however, the immunoglobulin is of human, murine, or rabbit origin. In another aspect, the antibodies are polyclonal, monoclonal, bispecific, human, humanized or chimeric antibodies, single chain antibodies, Fv, Fab fragments, F(ab') fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR's,

and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens.

[0397] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al. (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al. (1991) *Nature*, 352:624-628 and Marks et al. (1991) *J. Mol. Biol.*, 222:581-597, for example.

[0398] The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al. (1984) *Proc. Natl. Acad. Sci. USA*, 81:6851-6855).

[0399] Various methods have been employed to produce monoclonal antibodies (MAbs). Hybridoma technology, which refers to a cloned cell line that produces a single type of antibody, uses the cells of various species, including mice (murine), hamsters, rats, and humans. Another method to prepare MAbs uses genetic engineering including recombinant DNA techniques. Monoclonal antibodies made from these techniques include, among others, chimeric antibodies and humanized antibodies. A chimeric antibody combines DNA encoding regions from more than one type of species. For example, a chimeric antibody may derive the variable region from a mouse and the constant region from a human. A humanized antibody comes predominantly from a human, even though it contains nonhuman portions. Like a chimeric antibody, a humanized antibody may contain a completely human constant region. But unlike a chimeric antibody, the variable region may be partially derived from a human. The nonhuman, synthetic portions of a humanized antibody often come from CDRs

in murine antibodies. In any event, these regions are crucial to allow the antibody to recognize and bind to a specific antigen. Methods of producing such antibodies are known in the art, for example, such as those methods described in US2017/0095570 and US7964566, which are herein incorporated by reference.

5 **[0400]** Murine antibodies can be used for diagnostics and short-term therapies.

[0401] Chimeric and humanized antibodies reduce the likelihood of a HAMA (human anti-mouse antibody) response by minimizing the nonhuman portions of administered antibodies. Furthermore, chimeric and humanized antibodies have the additional benefit of activating secondary human immune responses, such as antibody dependent cellular
10 cytotoxicity. Examples of chimeric and humanized antibodies useful herein, and methods for their preparation are described in US7964566, which is herein incorporated by reference.

[0402] A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal and human immunoglobulin constant regions.

15 **[0403]** "Humanized" forms of non-human (e.g., rodent) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or
20 nonhuman primate having the desired specificity, affinity, and capacity.

[0404] "Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

25 **[0405]** The antibody can be a functionally active fragment, derivative or analog of an antibody that immunospecifically binds to cancer cell antigens, viral antigens, or microbial antigens or other antibodies bound to tumor cells or matrix. In this regard, "functionally active" means that the fragment, derivative or analog is able to elicit anti-anti-idiotypic antibodies that recognize the same antigen that the antibody from which the fragment,
30 derivative or analog is derived recognized. Examples of antibody fragments useful herein and methods for their generation are known in the art, for example, those described in US 7964566.

[0406] An "intact" antibody is one which comprises an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2
35 and CH3. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variant thereof.

[0407] The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions

include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc.

[0408] Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes." There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0409] An antibody "which binds" to a cell surface receptor or an antigen of interest is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the cell surface receptor or antigen.

[0410] Antibodies useful herein can be produced using any method known in the art to be useful for the synthesis of antibodies, e.g., by chemical synthesis or by recombinant expression. Suitable methods for the synthesis of antibodies (e.g., recombinant, polyclonal, monoclonal, humanized, human, bispecific antibodies and fragments thereof) include those methods described in US7965466.

[0411] Completely human antibodies are particularly desirable and can be produced using transgenic mice or using "guided selection" as described in US7965466.

[0412] In other embodiments, the antibody is a fusion protein of an antibody, or a functionally active fragment thereof, for example in which the antibody is fused via a covalent bond (e.g., a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the antibody. Preferably, the antibody or fragment thereof is covalently linked to the other protein at the N-terminus of the constant domain.

[0413] Antibodies include analogs and derivatives that are either modified, i.e., by the covalent attachment of any type of molecule as long as such covalent attachment permits the antibody to retain its antigen binding immunospecificity. Antibodies also include antibodies having modifications (e.g., substitutions, deletions or additions) in amino acid residues that interact with Fc receptors.

[0414] Antibodies immunospecific for a cancer cell antigen can be obtained commercially, for example, from Genentech (San Francisco, Calif.) or produced by any method known to one of skill in the art such as, e.g., chemical synthesis or recombinant expression techniques. The nucleotide sequence encoding antibodies immunospecific for a cancer cell antigen can be obtained, e.g., from the GenBank database or a database like it, the literature publications, or by routine cloning and sequencing.

[0415] Examples of antibodies available for the treatment of cancer include, but are not limited to, humanized anti-HER2 monoclonal antibody, HERCEPTIN® (trastuzumab; Genentech) for the treatment of patients with metastatic breast cancer; RITUXAN® (rituximab; Genentech) which is a chimeric anti-CD20 monoclonal antibody for the treatment of patients with non-Hodgkin's lymphoma; OvaRex (AltaRex Corporation, MA) which is a murine antibody for the treatment of ovarian cancer; Panorex (Glaxo Wellcome, N.C.) which is a murine IgG_{2a} antibody for the treatment of colorectal cancer; Cetuximab Erbitux (Imclone Systems Inc., NY) which is an anti-EGFR IgG chimeric antibody for the treatment of epidermal growth factor positive cancers, such as head and neck cancer; Vitaxin (MedImmune, Inc., MD) which is a humanized antibody for the treatment of sarcoma; Campath I/H (Leukosite, MA) which is a humanized IgG₁ antibody for the treatment of chronic lymphocytic leukemia (CLL); Smart MI95 (Protein Design Labs, Inc., CA) which is a humanized anti-CD33 IgG antibody for the treatment of acute myeloid leukemia (AML); LymphoCide (Immunomedics, Inc., NJ) which is a humanized anti-CD22 IgG antibody for the treatment of non-Hodgkin's lymphoma; Smart ID10 (Protein Design Labs, Inc., CA) which is a humanized anti-HLA-DR antibody for the treatment of non-Hodgkin's lymphoma; Oncolym (Techniclone, Inc., CA) which is a radiolabeled murine anti-HLA-Dr10 antibody for the treatment of non-Hodgkin's lymphoma; Allomune (BioTransplant, CA) which is a humanized anti-CD2 mAb for the treatment of Hodgkin's Disease or non-Hodgkin's lymphoma; Avastin (Genentech, Inc., CA) which is an anti-VEGF humanized antibody for the treatment of lung and colorectal cancers; Epratuzamab (Immunomedics, Inc., NJ and Amgen, Calif.) which is an anti-CD22 antibody for the treatment of non-Hodgkin's lymphoma; and CEAcide (Immunomedics, NJ) which is a humanized anti-CEA antibody for the treatment of colorectal cancer.

[0416] Transmembrane or tumor-associated polypeptides are polypeptides that are specifically or more abundantly expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Many such tumor-associated cell surface antigen polypeptides have been identified and are described in literature in the art.

[0417] Antibodies which may be useful in the treatment of cancer include, but are not limited to, antibodies against tumor-associated antigens (TAA). Such tumor-associated antigens are known in the art, and can be prepared for use in generating antibodies using methods and information which are well known in the art. Examples of TAA include the TAA listed below. Information relating to these antigens, all of which are known in the art, including names, alternative names, Genbank accession numbers, sequences and primary reference(s) are described in US7964566 and US20170095570, which are expressly incorporated by reference.

[0418] Antibodies useful in the treatment of cancer include, but are not limited to, antibodies against the following antigens (TAAs): BMPR1B (bone morphogenetic protein

receptor-type IB); E16 (LAT1, SLC7A5); STEAP1 (six transmembrane epithelial antigen of prostate); 0772P (CA125, MUC16); MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin); Napi2b (NAPI-3B, NPTIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b); Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B); PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene); ETBR (Endothelin type B receptor); MSG783 (RNF124, hypothetical protein FLJ20315); STEAP2 (HGNC—8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein); STEAP4 (HGNC—21923, TNFIAP9, STAMP2, STEAP4, six transmembrane epithelial antigen of prostate 4, six transmembrane prostate protein 2); TrpM4 (BR22450, F1120041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4); CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor); CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792); CD79b (IGb (immunoglobulin-associated beta), B29); FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C); an ErbB receptor; NCA; MDP; IL20R α ; Brevican; Ephb2R; ASLG659; PSCA; GEDA; BAFF-R (B cell-activating factor receptor, BLyS receptor 3, BR3); CD22(B-cell receptor CD22-B isoform); CD79a (CD79A, CD79 α , immunoglobulin-associated alpha); CXCR5 (Burkitt's lymphoma receptor 1); HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+T lymphocytes); P2X5 (Purinergic receptor P2X ligand-gated ion channel 5); CD72 (B-cell differentiation antigen CD72, Lyb-2); LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family); FCRH1 (Fc receptor-like protein 1); FcRH5 (IRTA2, immunoglobulin superfamily receptor translocation associated 2); TENB2 (putative transmembrane proteoglycan); PMEL17 (silver homolog; SILV; D12S53E; PMEL17; SI; SIL); TMEFF1 (transmembrane protein with EGF-like and two follistatin-like domains I; Tomoregulin-1); GDNF-Ra1 (GDNF family receptor alpha 1; GFRA1; GDNFR; GDNFRA; RETL1; TRNR1; RET1L; GDNFR-alpha1; GFR-ALPHA-1); Ly6E (lymphocyte antigen 6 complex, locus E; Ly67, RIG-E, SCA-2, TSA-1); TMEM46 (shisa homolog 2 (*Xenopus laevis*); SHISA2); Ly6G6D (lymphocyte antigen 6 complex, locus G6D; Ly6-D, MEGT1); LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5; GPR49, GPR67); RET (ret proto-oncogene; MEN2A; HSCR1; MEN2B; MTC1; PTC; CDHF12; Hs.168114; RET51; RET-ELE1); LY6K (lymphocyte antigen 6 complex, locus K; LY6K; HSJ001348; FLJ35226); GPR19 (G protein-coupled receptor 19; Mm.4787); GPR54 (KISS1 receptor; KISS1R; GPR54; HOT7T175; AXOR12); ASPHD1 (aspartate beta-hydroxylase domain containing 1; LOC253982); Tyrosinase (TYR; OCAIA; OCA1A; tyrosinase; SHEP3); TMEM118 (ring finger

protein, transmembrane 2; RNFT2; FLJ14627); GPR172A (G protein-coupled receptor 172A; GPCR41; FLJ11856; D15Ert747e); CD33; CLL-1; CD30 (tumor necrosis factor receptor SF8, TNFRSF8); CD40; CD70; CanAg; PSMA (prostate specific membrane antigen); CA125 (ovarian), CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), Lewis Y

5 (carcinomas), Lewis X (carcinomas), alpha fetoprotein (carcinomas), CA 242 (colorectal), placental alkaline phosphatase (carcinomas), prostate specific antigen (prostate), prostatic acid phosphatase (prostate), epidermal growth factor (carcinomas), MAGE-1 (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE-4 (carcinomas), anti-transferrin receptor (carcinomas), p97 (melanoma), MUC1-KLH (breast cancer), CEA (colorectal),
 10 gp100 (melanoma), MART1 (melanoma), PSA (prostate), IL-2 receptor (T-cell leukemia and lymphomas), CD20 (non-Hodgkin's lymphoma), CD52 (leukemia), CD33 (leukemia), CD22 (lymphoma), human chorionic gonadotropin (carcinoma), CD38 (multiple myeloma), CD40 (lymphoma), mucin (carcinomas), P21 (carcinomas), MPG (melanoma), and Neu oncogene product (carcinomas). Suitable antibodies to target cancer cells expressing one or more of
 15 these antigens are known in the art, for example, those described in US 7964566, which are herein incorporated by reference.

Antibodies to ErbB receptors

[0419] In one embodiment the ligand is an ErbB ligand, for example, an antibody that binds an ErbB receptor. In one embodiment the ErbB receptor is ErbB2 (HER2).

20 **[0420]** The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein described, for example, in Semba et al., *Proc. Natl. Acad. Sci. USA*, 82:6497-6501 (1985) and Yamamoto et al., (1986) *Nature*, 319:230-234 (Genebank accession number X03363). The term "erbB2" refers to the gene encoding human ErbB2 and "neu" refers to the gene encoding rat p185neu. Preferred ErbB2 is native sequence
 25 human ErbB2.

[0421] Antibodies to ErbB receptors are available commercially from a number of sources, including, for example, Santa Cruz Biotechnology, Inc., California, USA.

[0422] Other ErbB ligands include epidermal growth factor (EGF), transforming growth factor alpha (TGF- α), amphiregulin also known as schwannoma or keratinocyte autocrine
 30 growth factor; betacellulin; heparin-binding epidermal growth factor (HB-EGF); a heregulin (such as heregulin- α , heregulin- β 1, heregulin- β 2 and heregulin- β 3); neuregulin-2 (NRG-2); neuregulin-3 (NRG-3); neuregulin-4 (NRG-4) or cripto (CR-1). ErbB ligands which bind EGFR include EGF, TGF- α , amphiregulin, betacellulin, HB-EGF and epiregulin. ErbB ligands which bind ErbB3 include heregulins. ErbB ligands capable of binding ErbB4 include
 35 betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3, NRG-4 and heregulins. The ErbB ligand may also be a synthetic ErbB ligand. The synthetic ligand may be specific for a particular ErbB receptor, or may recognize particular ErbB receptor complexes. An example of a synthetic ligand is the synthetic heregulin/EGF chimera biregulin.

[0423] Humanized anti-ErbB2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®) as described in Table 3 of U.S. Pat. No. 5,821,337 expressly incorporated herein by reference; humanized 520C9 (WO 93/21319) and humanized 2C4 antibodies as described in US7964566.

[0424] An example of a commercially available antibody for the treatment of cancer is humanized anti-HER2 monoclonal antibody is HERCEPTIN® (trastuzumab; Genentech) for the treatment of patients with metastatic breast cancer.

Antibodies for treating autoimmune disease

[0425] In one embodiment, known antibodies for the treatment or prevention of an autoimmune disease are used in accordance with the compositions and methods of the invention. Antibodies immunospecific for an antigen of a cell that is responsible for producing autoimmune antibodies can be obtained commercially or produced by any method known to one of skill in the art such as, e.g., chemical synthesis or recombinant expression techniques. In various embodiments useful antibodies are immunospecific for the treatment of autoimmune diseases include, but are not limited to, Anti-Nuclear Antibody; Anti-ds DNA; Anti-ss DNA, Anti-Cardiolipin Antibody IgM, IgG; Anti-Phospholipid Antibody IgM, IgG; Anti-SM Antibody; Anti-Mitochondrial Antibody; Thyroid Antibody; Microsomal Antibody; Thyroglobulin Antibody; Anti-SCL-70; Anti-Jo; Anti-U₁RNP; Anti-La/SSB; Anti SSA; Anti-SSB; Anti-Perital Cells Antibody; Anti-Histones; Anti-RNP; C-ANCA; P-ANCA; Anti centromere; Anti-Fibrillarin, and Anti-GBM Antibody.

[0426] In certain embodiments, useful antibodies can bind to both a receptor or a receptor complex expressed on an activated lymphocyte. The receptor or receptor complex can comprise an immunoglobulin gene superfamily member, a TNF receptor superfamily member, an integrin, a cytokine receptor, a chemokine receptor, a major histocompatibility protein, a lectin, or a complement control protein. Non-limiting examples of suitable immunoglobulin superfamily members are CD2, CD3, CD4, CD8, CD19, CD22, CD28, CD79, CD90, CD152/CTLA-4, PD-1, and ICOS. Non-limiting examples of suitable TNF receptor superfamily members are CD27, CD40, CD95/Fas, CD134/OX40, CD137/4-1BB, TNF-R1, TNFR-2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, and APO-3. Non-limiting examples of suitable integrins are CD11a, CD11b, CD11c, CD18, CD29, CD41, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD103, and CD104. Non-limiting examples of suitable lectins are C-type, S-type, and I-type lectin.

[0427] In one embodiment, the Ligand binds to an activated lymphocyte that is associated with an autoimmune disease.

Methods and uses

[0428] The inventors have found that certain compounds of formula I have useful anti-proliferative activity against cancer cells. The inventors have also demonstrated that certain compounds of formula I have mitochondrial uncoupling activity. In particular, the inventors

have found that certain compounds of formula I are capable of acting as ionophores and depolarising and/or collapsing the proton gradient across membranes, for example mitochondrial membranes. Without wishing to be bound by theory, it is believed that the compounds of the invention may also have ATP synthase inhibitory activity.

5 **[0429]** Accordingly, the present invention relates to methods of treating or preventing a disease or condition susceptible to treatment with a mitochondrial uncoupling agent or complications associated with a disease or condition susceptible to treatment with a mitochondrial uncoupling agent, killing a cell, including killing or inhibiting proliferation of tumour cells or cancer cells and a method of treating or preventing an autoimmune disease.

10 **[0430]** A "subject" refers to a human or a non-human animal, preferably a vertebrate that is a mammal, preferably a human. Non-human mammals include, but are not limited to, farm animals, such as, cattle, sheep, swine, deer, and goats; sport and companion animals, such as, dogs, cats, and horses; and research animals, such as, mice, rats, rabbits, and guinea pigs. Preferably, the subject is a human.

15 **[0431]** The term "treatment", and related terms such as "treating" and "treat", as used herein, unless indicated otherwise, relates generally to treatment, of a human or a non-human subject, in which some desired therapeutic effect is achieved. The therapeutic effect may, for example, be inhibition, reduction, amelioration, halt, or prevention of the disease or condition.

20 **[0432]** A "therapeutically effective amount" (or "effective amount") is an amount sufficient to effect beneficial or desired results, including clinical results. A therapeutically effective amount can be administered in one or more administrations by various routes of administration. The therapeutically effective amount to be administered to a subject depends on, for example, the purpose for administration, mode of administration, nature
25 and dosage of any co-administered compounds, and characteristics of the subject, such as general health, other diseases, age, sex, genotype, body weight and tolerance to drugs. A person skilled in the art will be able to determine appropriate dosages having regard to these any other relevant factors.

30 **[0433]** The compound of formula I or drug-ligand conjugate is typically administered in the form of a pharmaceutical composition of the invention as described herein. The composition may be administered as a single dose or a multiple dose schedule.

35 **[0434]** The compound of formula I or drug-ligand conjugate can be used or administered as the sole therapeutic agent or in combination with one or more additional therapeutic agents. The compound of formula I or drug-ligand conjugate and one or more additional therapeutic agents may be used or administered simultaneously, sequentially, or separately. The one or more additional therapeutic agents will depend on the disease or condition to be treated or other desired therapeutic benefit. The one or more additional therapeutic agents can be used in therapeutic amounts indicated or approved for the particular agent, as would be known to those skilled in the art.

[0435] In some embodiments, two or more compound of formula I or drug-ligand conjugate are used or administered in combination. The two or more compound of formula I or drug-ligand conjugate may be used or administered simultaneously, sequentially, or separately.

5 **[0436]** The compounds can also be administered in combination with radiation therapy, for example in the treatment of cancer. The terms "radiation therapy" and "radiotherapy" are used interchangeably herein. Radiation therapy is a standard treatment for controlling unresectable or inoperable tumours and/or tumour metastases. The radiation dosage regimen is generally defined in terms of radiation absorbed dose (Gy), time and
10 fractionation, and must be carefully defined by the oncologist. The amount of radiation a patient receives will depend on various considerations, but the two most important are the location of the tumour in relation to other critical structures or organs of the body, and the extent to which the tumour has spread. The type of radiation used may include X-rays, gamma rays, alpha particles, beta particles, photons, electrons, neutrons, radioisotopes,
15 and other forms of ionizing radiation. The compound may be administered simultaneously, sequentially, or separately with the radiation therapy.

[0437] In some embodiments, the one or more additional therapeutic agents are anticancer agents, for example one or more chemotherapeutic agents. Suitable anticancer agents will be apparent to those skilled in the art having regard, for example to the cancer
20 to be treated. Numerous anticancer agents are known in the art. Examples of suitable anticancer agents include those listed in Cancer: Principles and Practice of Oncology, 7th Edition, Devita et al, Lippincott Williams & Wilkins, 2005, which is incorporated herein by reference. Examples of suitable anticancer agents also include those listed in the Merck Index, 14th Edition, 2006, which is incorporated herein by reference. Such anticancer
25 agents include but are not limited to alkaloids and natural products, including camptothecin derivatives for example 9-aminocamptothecin, exatecan, irinotecan, rubitecan and topotecan, podophyllum derivatives for example etoposide and teniposide, taxanes for example docetaxel, paclitaxel and paclitaxel poliglumex, vinca alkaloids for example vinblastine, vincristine, vindesine, vinflunine and vinorelbine, and others for example
30 aplidine, elliptinium acetate, irifulven, ixabepilone, kahalalide F, midostaurin and trabectedin; alkylating agents, including alkyl sulfonates for example busulfan, improsulfan and piposulfan, aziridines for example carboquone, diaziquone and uredepa, ethylenimines and methylmelamines for example altretamine, triethylenemelamine, triethylenephosphoramidate and triethylenethiophosphoramidate, nitrogen mustards for
35 example bendamustine, canfosfamide, chlorambucil, chlornaphazine, cyclophosphamide, estramustine, glufosfamide, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, perfosfamide, prenimustine, trichlormethine, trofosfamide, and uracil mustard, nitrosoureas for example carmustine, chlorozotocin, fotemustine, lomustine, nimustine and ranimustine, and others for example dacarbazine, etoglucid, mitobronitol,

mitolactol, pipobroman, procarbazine and temozolomide; antibiotics and analogs, including actinomycins for example cactinomycin and dactinomycin, anthracyclines for example aclacinomycins, amrubicin, carubicin, daunorubicin, doxorubicin, epirubicin, idarubicin, pirarubicin, valrubicin and zorubicin, and others for example bleomycins, mitomycins, 5 peplomycin, plicamycin, porfiromycin, streptozocin, temsirolimus and zinostatin; antimetabolites, including folic acid analogs and antagonists for example denopterin, edatrexate, methotrexate, nolatrexed, pemetrexed, piritrexi, pteropterin, raltitrexed and trimetrexate, purine analogs for example cladribine, clofarabine, fludarabine, 6-mercaptapurine, nelarabine, thiamiprine, thioguanine and tiazofurine, and pyrimidine 10 analogs for example ancitabine, azacitidine 6-azauridine, capecitabine, carmofur, cytarabine, decitabine, doxifluridine, enocitabine, floxuridine, fluorouracil, gemcitabine, tegafur and troxacitabine; enzymes for example L-asparaginase and ranpirinase; farnesyl transferase inhibitors for example lonafarnib, tipifarnib; immunomodulators for example aldesleukin, interferon- α , interferon- γ , lentinan, mepact, oregovomab, propagermanium, 15 PSK®, roquinimex, sipuleucel-T, sizofiran, teceleukin and ubenimex; immunotoxins for example cintredekin besudotox and denileukin diftotox; monoclonal antibodies for example alemtuzumab, bevacizumab, cetuximab, edrecolomab, epratuzumab, gemtuzumab, ozogamicin, oregovomab, panitumumab, rituximab, tositumomab ¹³¹I and trastuzumab; oligonucleotides for example aprinocarsen and oblimersen sodium; platinum complexes for 20 example carboplatin, cisplatin, lobaplatin, oxaliplatin, picoplatin and satraplatin; retinoids and analogs for example alitretinoin, bexarotene, fenretinide, mofarotene and tarmibarotene; tyrosine kinase inhibitors for example canertinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, sorafenib, sunitinib and vatalanib; and others for example amsacrine, arsenic trioxide, atrasentan, bisantrene, bortezomib, brostallicin, calcitriol, 25 edotecarin, eflornithine, flavopiridol, gallium nitrate, hydroxyurea, liarozole, lonidamine, miltefosine, mitoguazone, mitoxantrone, nitracrine, pentostatin, perifosine, pixantrone, razoxane, seocalcitol, sobuzoxane, spirogermanium, tirapazamine and vorinostat. Such anticancer agents also include without limitation antineoplastic hormonal agents including androgens for example dromostanolone, epitioleone, mepitioleone and testolactone; 30 antiandrogens for example aminoglutethimide, mitotane, trilostane; antiandrogens, bicalutamide, flutamide and nilutamide; antiestrogens for example arzoxifene, droloxifene, fulvestrant, idoxifene, tamoxifen and toremifene; antiprogestins for example onapristone; aromatase inhibitors for example aminoglutethimide, anastrozole, exemestane, fadrozole, formestane, letrozole and vorozole; estrogens for example diethylstilbestrol, fosfestrol, 35 hexestrol and polyestradiol phosphate; LH-RH analogs for example abarelix, buserelin, cetorelix, goserelin, leuprolide and triptorelin; progestogens for example chlormadinone acetate, medroxyprogesterone and megestrol acetate; and somatostatin analogs for example lanreotide. Such anticancer agents also include without limitation antineoplastic

photosensitisers for example 6-aminolevulinic acid, methyl aminolevulinate, motexafin lutetium, porfimer sodium, talaporfin and temoporfin.

[0438] The additional therapeutic agents to be used in combination with the compound of formula (I) or drug-ligand conjugate can be used in therapeutic amounts indicated or approved for the particular agent, for example in the Physicians' Desk Reference (PDR) 47th Edition (1993), as would be known to those skilled in the art.

Mitochondrial uncoupling activity

[0439] Mitochondria are central to cellular metabolism, which provides both energy to sustain biological activities and metabolic intermediates for biosynthesis. In the mitochondrial matrix, acetyl-CoA produced from glucose and lipid metabolism is oxidised through the citric acid (TCA) cycle. The high energy by-products of these oxidation reactions are used in the electron transport chain on the inner membrane of mitochondria to produce energy that is harnessed to generate adenosine triphosphate (ATP). Mitochondrial oxidation of acetyl-CoA and ATP synthesis are coupled in response to cellular energy needs. However, mitochondrial uncouplers can decouple ATP synthesis from mitochondrial oxidation leading to a reduction of mitochondrial energy efficiency, increase of lipid and glucose oxidation, activation of AMPK enzyme, and an alteration of availability of metabolic intermediates for biomass biosynthesis required for cell proliferation.

[0440] Analyses described herein in the examples demonstrate that compounds of formula I have mitochondrial uncoupling activity. Accordingly, in one aspect, the invention relates to a method of treating or preventing a disease or condition susceptible to treatment with a mitochondrial uncoupling agent or complications associated with a disease or condition susceptible to treatment with a mitochondrial uncoupling agent in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of formula I, or a ligand-drug conjugate or pharmaceutical composition described herein. The analyses described in the examples herein show that the compounds are capable of acting as ionophores and collapsing a proton gradient across a mitochondrial membrane. As such, the invention also provides a method of depolarising a membrane, preferably a mitochondrial membrane, the method comprising contacting the membrane with a compound of formula I of the invention or a compound in the proviso relating thereto, a ligand-drug conjugate of of the invention or a pharmaceutical composition of the invention. The method of depolarising a membrane may be useful in treatment of diseases or conditions susceptible to treatment with mitochondrial uncoupling agents or complications associated with such diseases or conditions in subjects in need thereof. In such embodiments, the method comprises administering the compound, conjugate, or pharmaceutical composition to the subject.

[0441] Methods to determine or measure mitochondrial uncoupling activity, such as mitochondrial uncoupling assays, are known in the art, for example, the methods described in WO20160810599, which is incorporated herein by reference. For example, in one

embodiment, cells treated with a compound, conjugate or composition can be stained with a J-aggregate forming dye, such as in the MITO-ID Membrane potential cytotoxicity kit used in the examples described herein. In another embodiment, cells treated with a compound, conjugate or composition can be stained with TMRE (tetramethylrhodamine ethyl ester) and examined by fluorescence microscopy. The proportion of cells that lose mitochondrial TMRE staining following treatment can be used to quantify mitochondrial uncoupling activity. Cellular oxygen consumption analysis in cells may also be used to indicate mitochondrial uncoupling activity. An increase in oxygen consumption in cells following treatment with a compound, conjugate or composition as compared to untreated cells is indicative of mitochondrial uncoupling activity. Suitable methods for performing such an analysis are known to those in the art. For *in vivo* studies, the compound of formula I or drug-ligand conjugate can be administered to an animal (e.g., a mouse), for example by injection, and its effects evaluated. Based on the results, an appropriate dosage range and administration route can be determined.

[0442] In various embodiments the disease or condition susceptible to treatment with a mitochondrial uncoupling agent is a metabolic disorder or cancer.

[0443] The term "metabolic disease" or "metabolic disease" as used herein refers to a group of diseases with symptoms of abnormal glucose and/or lipid metabolism, which share common causal factors of abnormal accumulation of intracellular lipid in cells of various tissues and insulin resistance. Metabolic diseases include, but are not limited to, obesity (excessive fat accumulation in cells of adipose tissue), metabolic syndrome (insulin resistance in peripheral tissues, usually caused by ectopic fat accumulation in cells of liver, muscle, or adipose tissue), type 2 diabetes (insulin resistance in peripheral tissues usually caused by ectopic fat accumulation in cells of liver, muscle, or adipose tissue, and hyperglycemia caused by insulin resistance), complications caused by type 2 diabetes, alcoholic fatty liver disease (ectopic lipid accumulation in liver cells), non-alcoholic liver fatty liver disease (or NAFLD, caused by ectopic lipid accumulation in liver cells, the stages include hepatosteatosis, non-alcoholic steatohepatitis (NASH), cirrhosis, and NAFLD induced hepatocellular carcinoma (HCC)), and dyslipidemia (ectopic accumulation of lipid in cells of liver, muscle, heart, as a result of redistribution of lipid from adipose tissue to other tissues). Agents with mitochondrial uncoupling activity which reduce energy efficiency and boost futile lipid oxidation, would effectively reduce cellular accumulation of lipid, which is the cause of insulin resistance in various metabolic diseases.

[0444] Methods of the invention are useful for the prevention and treatment of metabolic diseases and cancer, including, but not limited to, obesity, metabolic syndrome, type 2 diabetes, alcoholic fatty liver disease, non-alcoholic fatty liver diseases, dyslipidemia, primary cancer, and metastatic cancer.

[0445] Provided herein is a method of treating or ameliorating the symptoms of obesity, pre-type 2 diabetes, type 2 diabetes, non-alcoholic fatty liver diseases or alcoholic

fatty liver disease (characterized by abnormal accumulation of lipid in liver), dyslipidemia (characterized by abnormal lipid deposit in tissue other than adipose), and complications of the above mentioned metabolic diseases, including, but not limited to, hypertension, cardiovascular diseases, nephropathy, and neuropathy. Methods described herein may also be used for preventing metabolic diseases described herein for a subject with risk factors including, but not limited to, dietary, environmental, medical, and genetic predispositions. Methods described herein may also be used for long-term chronic disease management and longevity management by reducing insulin resistance or reducing glucose levels in the blood.

[0446] In various embodiments the metabolic disease is type 2 diabetes, or related diseases leading to insulin resistance or hyperglycemia.

[0447] In one embodiment the metabolic disease is obesity.

[0448] In various embodiments the metabolic disease is non-alcoholic fatty liver disease, (NAFLD), including nonalcoholic steatohepatitis (NASH) and cirrhosis, or alcoholic fatty liver disease (AFLD). In various embodiments, the metabolic disease is hepatic steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis, or NAFLD induced hepatocellular carcinoma (HCC).

[0449] In various embodiments the metabolic disease is complications of type 2 diabetes including but not limited to type 2 diabetes induced hypertension, cardiovascular disease, nephropathy, atherosclerosis, dyslipidemia, retinopathy, neurodegenerative diseases, diabetic heart failure, and neuropathy.

[0450] In one embodiment the metabolic disease is pre-type 2 diabetes. In another embodiment the metabolic disease is dyslipidemia.

[0451] In one embodiment the disease or condition susceptible to treatment with a mitochondrial uncoupling agent is a mitochondrial disease. In various embodiments, the metabolic disease is LHON (leber heredity optic neuropathy), MELAS (mitochondrial myopathy, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes), MERRF (myoclonic epilepsy and ragged red muscle fiber), Leigh Syndrome, MILS (maternally inherited Leigh Syndrome), NARP (neurogenic muscle weakness, ataxia and retinitis pigmentosa), FBSN (familial bilateral striatal necrosis) or KSS (Kearns Sayre Syndrome).

[0452] In one embodiment the disease or condition susceptible to treatment with a mitochondrial uncoupling agent is a heart disease. In various embodiments the heart disease is hypertension or cardiovascular disease

[0453] In one embodiment the disease or condition susceptible to treatment with a mitochondrial uncoupling agent is a central nervous system (CNS) disease. In various embodiments the CNS disease is stroke, Alzheimer's, Parkinson's, Huntington's or ALS (amyotrophic lateral sclerosis).

[0454] In various embodiments the disease or condition susceptible to treatment with a mitochondrial uncoupling agent is a disease associated with increased ROS (reactive oxygen species) production. Increased ROS has been associated with aging, Alzheimer's disease, Parkinson's disease, Huntington's disease, ALS (amyotrophic lateral sclerosis), mitochondrial diseases and cancers.

[0455] In various embodiments the compound, ligand-drug conjugate or composition described herein is suitable for veterinary use and may be used to treat diabetes or a diabetes-associated disease, and the subject is a mammalian animal.

[0456] In various embodiments the compound, ligand-drug conjugate or composition is administered in combination with a second agent indicated for the above-mentioned diseases or diseases, simultaneously or sequentially with administration of the second agent.

[0457] In one embodiment the compound, ligand-drug conjugate or composition is administered in combination with a second anti-diabetic agent. In one embodiment the second anti-diabetic agent is metformin.

[0458] In other embodiments the second anti-diabetic agent is selected from insulin, insulin analogs, sulfonylureas, biguanides, meglitinides, thiazolidinediones, alpha glucosidase inhibitors, GLP-1 agonists, DPP-4 inhibitors, and SGLT2 inhibitors.

[0459] In other embodiments the second anti-diabetic agent is selected from insulin, insulin analogs, sulfonylureas, biguanides, meglitinides, thiazolidinediones, alpha glucosidase inhibitors, GLP-1 agonists, DPP-4 inhibitors, and SGLT2 inhibitors.

[0460] In various embodiments the compound, ligand-drug conjugate or composition is administered in combination with a second anti- non alcoholic fatty liver disease agent, a second anti- alcoholic fatty liver disease agent or a second anti- dyslipidemia agent.

[0461] In one aspect the invention provides a method for long-term disease management of a metabolic disease susceptible to treatment with a mitochondrial uncoupling agent, the method comprising administering to a subject in need thereof an effective amount of a compound, drug-ligand conjugate or a composition described herein.

Cancer

[0462] Compounds of formula I, drug-linker conjugates comprising compounds of formula I and pharmaceutical compositions described herein are useful for treating or preventing cancer.

[0463] In various embodiments the compounds of formula I, drug-linker conjugates comprising compounds of formula I and pharmaceutical compositions are useful for killing or inducing apoptosis of one or more cancerous or tumour cells, inhibiting tumour formation and/or growth, or treating, inhibiting or preventing tumour metastasis.

[0464] In the context of cancer, the term "treating" includes any or all of: preventing growth of tumor cells, cancer cells, or of a tumor; preventing replication or proliferation of

tumor cells or cancer cells, lessening of overall tumor burden or decreasing the number of cancerous cells, and ameliorating one or more symptoms associated with the disease.

[0465] The terms "cancer" and "cancerous" includes the physiological condition in mammals that is typically characterized by unregulated cell growth. A "tumor" comprises one or more cancerous cells.

[0466] Cancers that may be treated using methods described herein include: Solid tumors, including but not limited to: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon cancer, colorectal cancer, kidney cancer, pancreatic cancer, bone cancer, breast cancer, ovarian cancer, prostate cancer, esophageal cancer, stomach cancer, oral cancer, nasal cancer, throat cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, uterine cancer, testicular cancer, small cell lung carcinoma, bladder carcinoma, lung cancer, epithelial carcinoma, glioma, glioblastoma multiforme, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, skin cancer, melanoma, neuroblastoma, retinoblastoma, blood-borne cancers, including but not limited to: acute lymphoblastic leukemia "ALL", acute lymphoblastic B-cell leukemia, acute lymphoblastic T-cell leukemia, acute myeloblastic leukemia "AML", acute promyelocytic leukemia "APL", acute monoblastic leukemia, acute erythroleukemic leukemia, acute megakaryoblastic leukemia, acute myelomonocytic leukemia, acute nonlymphocytic leukemia, acute undifferentiated leukemia, chronic myelocytic leukemia "CML", chronic lymphocytic leukemia "CLL", hairy cell leukemia, multiple myeloma, acute and chronic leukemias:, lymphoblastic, myelogenous, lymphocytic, myelocytic leukemias, Lymphomas:, Hodgkin's disease, non-Hodgkin's Lymphoma, Multiple myeloma, Waldenström's macroglobulinemia, Heavy chain disease, Polycythemia vera.

[0467] In one embodiment the cancer is an ErbB2-expressing cancer or cancer characterised by excessive activation of an ErbB2 receptor.

[0468] An "ErbB2-expressing cancer" is one which produces sufficient levels of ErbB2 at the surface of cells thereof, such that an anti-ErbB2 antibody can bind thereto and have a therapeutic effect with respect to the cancer. A "cancer characterized by excessive activation of an ErbB2 receptor" is one in which the extent of ErbB2 receptor activation in cancer cells significantly exceeds the level of activation of that receptor in non-cancerous cells of the same tissue type.

[0469] In various embodiments the cancer is selected from the group comprising breast cancer, colorectal cancer and gastroesophageal cancer. In one embodiment the breast cancer is HER2-positive breast cancer.

[0470] In one embodiment the method comprises administration compound of formula I, drug-ligand conjugate or pharmaceutical composition separately, simultaneously or sequentially with a chemotherapeutic agent. Examples of chemotherapeutic agents for targeting specific cancers are well known in the art.

[0471] In some embodiments of this aspect, the cancer is metastatic cancer to sites including intraperitoneal cavity, lung or bone.

[0472] The efficacy of a compound of formula I or drug-ligand conjugate can be evaluated both in vitro and in vivo. For example, the compound of formula I or drug-ligand conjugate can be tested in vitro or in vivo for its ability to kill or inhibit proliferation of tumour cells or cancer cell lines, for example using in vitro cell proliferation assays described herein. For in vivo studies, the compound of formula I or drug-ligand conjugate can be administered to an animal (e.g., a mouse), for example by injection, and its effects evaluated. Based on the results, an appropriate dosage range and administration route can be determined. Compounds of formula I, drug-ligand conjugates or pharmaceutical compositions suitable for use in the methods described herein may be screened for utility using transgenic animals or cell lines according to methods well known in the art, such as those described in US 7964566.

Autoimmune disease

[0473] The compound of formula I or drug-ligand conjugate are useful for killing or inhibiting the replication of a cell that produces an autoimmune disease or for treating an autoimmune disease.

[0474] An "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or a co-segregate or manifestation thereof or resulting condition therefrom. Examples of autoimmune diseases or disorders include, but are not limited to arthritis (rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), psoriasis, dermatitis including atopic dermatitis; chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, toxic epidermal necrolysis, systemic scleroderma and sclerosis, responses associated with inflammatory bowel disease (IBD) (Crohn's disease, ulcerative colitis), and IBD with co-segregate of pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, and/or episcleritis), respiratory distress syndrome, including adult respiratory distress syndrome (ARDS), meningitis, IgE-mediated diseases such as anaphylaxis and allergic rhinitis, encephalitis such as Rasmussen's encephalitis, uveitis, colitis such as microscopic colitis and collagenous colitis, glomerulonephritis (GN) such as membranous GN, idiopathic membranous GN, membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN, allergic conditions,

eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) such as cutaneous SLE, lupus (including nephritis, cerebritis, pediatric, non-renal, discoid, alopecia), juvenile onset diabetes, multiple sclerosis

5 (MS) such as spino-optical MS, allergic encephalomyelitis, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including Wegener's granulomatosis, agranulocytosis, vasculitis (including Large Vessel vasculitis (including Polymyalgia Rheumatica and Giant Cell (Takayasu's) Arteritis), Medium Vessel vasculitis (including

10 Kawasaki's Disease and Polyarteritis Nodosa), CNS vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS), aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia, pure red cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia,

15 diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome, myasthenia gravis, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet disease, Castleman's syndrome, Goodpasture's Syndrome, Lambert-Eaton Myasthenic Syndrome, Reynaud's syndrome, Sjorgen's syndrome, Stevens-Johnson

20 syndrome, solid organ transplant rejection (including pretreatment for high panel reactive antibody titers, IgA deposit in tissues, and rejection arising from renal transplantation, liver transplantation, intestinal transplantation, cardiac transplantation, etc.), graft versus host disease (GVHD), pemphigoid bullous, pemphigus (including vulgaris, foliaceus, and pemphigus mucus-membrane pemphigoid), autoimmune polyendocrinopathies, Reiter's

25 disease, stiff-man syndrome, immune complex nephritis, IgM polyneuropathies or IgM mediated neuropathy, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), thrombocytopenia (as developed by myocardial infarction patients, for example), including autoimmune thrombocytopenia, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism;

30 autoimmune endocrine diseases including autoimmune thyroiditis, chronic thyroiditis (Hashimoto's Thyroiditis), subacute thyroiditis, idiopathic hypothyroidism, Addison's disease, Grave's disease, autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), Type I diabetes also referred to as insulin-dependent diabetes mellitus (IDDM), including pediatric IDDM, and Sheehan's syndrome; autoimmune hepatitis,

35 Lymphoid interstitial pneumonitis (HIV), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barré Syndrome, Berger's Disease (IgA nephropathy), primary biliary cirrhosis, celiac sprue (gluten enteropathy), refractory sprue with co-segregate dermatitis herpetiformis, cryoglobulinemia, amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune inner ear disease (AIED), autoimmune hearing loss,

opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory polychondritis, pulmonary alveolar proteinosis, amyloidosis, giant cell hepatitis, scleritis, monoclonal gammopathy of uncertain/unknown significance (MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS; autism, inflammatory myopathy, and focal segmental glomerulosclerosis (FSGS).

[0475] In the context of an autoimmune disease, the term "treating" includes any or all of: preventing replication of cells associated with an autoimmune disease state including, but not limited to, cells that produce an autoimmune antibody, lessening the autoimmune-antibody burden and ameliorating one or more symptoms of an autoimmune disease.

[0476] In one embodiment, the Ligand unit binds to an autoimmune antigen. In one aspect, the antigen is on the surface of a cell involved in an autoimmune condition. In another embodiment, the Ligand unit binds to an autoimmune antigen which is on the surface of a cell. In one embodiment, the Ligand binds to activated lymphocytes that are associated with the autoimmune disease state.

Pharmaceutical compositions

[0477] The present invention further relates to a pharmaceutical composition comprising the compound of formula I or drug-ligand conjugate; and a pharmaceutically acceptable carrier.

[0478] The pharmaceutical composition comprises an effective amount of the compound of formula I or drug-ligand conjugate. The pharmaceutical compositions may comprise two or more compound of formula I or drug-ligand conjugate.

[0479] The term "pharmaceutically acceptable carrier" refers to a carrier (e.g. adjuvant or vehicle) that may be administered to a subject together with the compound of formula I or drug-ligand conjugate, which is generally safe, non-toxic, and neither biologically nor otherwise undesirable, including carriers suitable veterinary as well as human pharmaceutical use.

[0480] Pharmaceutically acceptable carriers that may be used in the compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d- α -tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α -, β -, and γ -cyclodextrin, or chemically modified derivatives such as

hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-3- cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery. Oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents, which are commonly used in the formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions.

[0481] The compositions are formulated to allow for administration to a subject by any chosen route, including but not limited to oral or parenteral (including topical, subcutaneous, intramuscular and intravenous) administration. In some embodiments, the compositions are formulated for administration orally, intravenously, subcutaneously, intramuscularly, transdermally, intraperitoneally, or other pharmacologically acceptable routes. For example, the compositions may be formulated with an appropriate pharmaceutically acceptable carrier (including excipients, diluents, auxiliaries, and combinations thereof) selected with regard to the intended route of administration and standard pharmaceutical practice. For example, the compositions may be administered orally as a powder, liquid, tablet or capsule, or topically as an ointment, cream or lotion. Suitable formulations may contain additional agents as required, including emulsifying, antioxidant, flavouring or colouring agents, and may be adapted for immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release.

[0482] The compositions may be administered via the parenteral route. Examples of parenteral dosage forms include aqueous solutions, isotonic saline or 5% glucose of the active agent, or other well-known pharmaceutically acceptable excipients. Cyclodextrins, for example, or other solubilising agents well-known to those familiar with the art, can be utilized as pharmaceutical excipients for delivery of the therapeutic agent.

[0483] Examples of dosage forms suitable for oral administration include, but are not limited to tablets, capsules, lozenges, or like forms, or any liquid forms such as syrups, aqueous solutions, emulsions and the like, capable of providing a therapeutically effective amount of the composition. Capsules can contain any standard pharmaceutically acceptable materials such as gelatin or cellulose. Tablets can be formulated in accordance with conventional procedures by compressing mixtures of the active ingredients with a solid carrier and a lubricant. Examples of solid carriers include starch and sugar bentonite. Active ingredients can also be administered in a form of a hard shell tablet or a capsule containing a binder, e.g., lactose or mannitol, a conventional filler, and a tableting agent.

[0484] Examples of dosage forms suitable for transdermal administration include, but are not limited, to transdermal patches, transdermal bandages, and the like.

[0485] Examples of dosage forms suitable for topical administration of the compositions include any lotion, stick, spray, ointment, paste, cream, gel, etc., whether applied directly to the skin or via an intermediary such as a pad, patch or the like.

[0486] Examples of dosage forms suitable for suppository administration of the compositions include any solid dosage form inserted into a bodily orifice particularly those inserted rectally, vaginally and urethrally.

[0487] Examples of dosage of forms suitable for injection of the compositions include delivery via bolus such as single or multiple administrations by intravenous injection, subcutaneous, subdermal, and intramuscular administration or oral administration.

[0488] Examples of dosage forms suitable for depot administration of the compositions include pellets or solid forms wherein the active(s) are entrapped in a matrix of biodegradable polymers, microemulsions, liposomes or are microencapsulated.

[0489] Examples of infusion devices for the compositions include infusion pumps for providing a desired number of doses or steady state administration, and include implantable drug pumps. Examples of implantable infusion devices for compositions include any solid form in which the active(s) are encapsulated within or dispersed throughout a biodegradable polymer or synthetic, polymer such as silicone, silicone rubber, silastic or similar polymer.

[0490] Examples of dosage forms suitable for transmucosal delivery of the compositions include depositories solutions for enemas, pessaries, tampons, creams, gels, pastes, foams, nebulised solutions, powders and similar formulations containing in addition to the active ingredients such carriers as are known in the art to be appropriate. Such dosage forms include forms suitable for inhalation or insufflation of the compositions, including compositions comprising solutions and/or suspensions in pharmaceutically acceptable, aqueous, or organic solvents, or mixture thereof and/or powders. Transmucosal administration of the compositions may utilize any mucosal membrane but commonly utilizes the nasal, buccal, vaginal and rectal tissues. Formulations suitable for nasal administration of the compositions may be administered in a liquid form, for example, nasal spray, nasal drops, or by aerosol administration by nebulizer, including aqueous or oily solutions of the polymer particles. Formulations may be prepared as aqueous solutions for example in saline, solutions employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bio-availability, fluorocarbons, and/or other solubilising or dispersing agents known in the art.

[0491] Examples of dosage forms suitable for buccal or sublingual administration of the compositions include lozenges, tablets and the like. Examples of dosage forms suitable for ophthalmic administration of the compositions include inserts and/or compositions comprising solutions and/or suspensions in pharmaceutically acceptable, aqueous, or organic solvents.

[0492] Examples of formulations of compositions may be found in, for example, Sweetman, S. C. (Ed.). Martindale. The Complete Drug Reference, 33rd Edition, Pharmaceutical Press, Chicago, 2002, 2483 pp.; Aulton, M. E. (Ed.) Pharmaceutics. The Science of Dosage Form Design. Churchill Livingstone, Edinburgh, 2000, 734 pp.; and, Ansel, H. C, Allen, L. V. and Popovich, N. G. Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed., Lippincott 1999, 676 pp. Excipients employed in the

manufacture of drug delivery systems are described in various publications known to those skilled in the art including, for example, Kibbe, E. H. Handbook of Pharmaceutical Excipients, 3rd Ed., American Pharmaceutical Association, Washington, 2000, 665 pp. The USP also provides examples of modified-release oral dosage forms, including those formulated as tablets or capsules. See, for example, The United States Pharmacopeia 23/National Formulary 18, The United States Pharmacopeial Convention, Inc., Rockville MD, 1995 (hereinafter "the USP"), which also describes specific tests to determine the drug release capabilities of extended-release and delayed-release tablets and capsules. The USP test for drug release for extended-release and delayed-release articles is based on drug dissolution from the dosage unit against elapsed test time. Descriptions of various test apparatus and procedures may be found in the USP. Further guidance concerning the analysis of extended release dosage forms has been provided by the F.D.A. (See Guidance for Industry. Extended release oral dosage forms: development, evaluation, and application of in vitro/in vivo correlations. Rockville, MD: Center for Drug Evaluation and Research, Food and Drug Administration, 1997).

[0493] The dosage forms described herein can be in the form of physically discrete units suitable for use as unitary dosages for the subjects to be treated, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect.

[0494] Dosage levels of the active ingredients in the pharmaceutical compositions may be varied so as to provide an amount of the active ingredient which is effective to achieve the desired therapeutic effect for a particular patient, composition, and mode of administration, without being toxic to the patient (an effective amount).

[0495] The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions employed, the route of administration, the time of administration, the rate of excretion of the particular compound of formula I or drug-ligand conjugate being employed, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. Generally, the daily amount or regimen should be in the range of about 0.01 mg to about 2000 mg of the compound of formula I or drug-ligand conjugate per kilogram (kg) of body mass.

Kits

[0496] The present invention also provides a kit comprising a compound of formula I or drug-ligand conjugate; and instructions for use.

[0497] The compound of formula I or drug-ligand conjugate is typically in the form of a pharmaceutical composition, and contained within a container. The instructions for use may describe the method(s) of treatment in which the compound of formula I or drug-ligand

conjugate are administered. In various embodiments, the instructions for use describe methods of treating the diseases and conditions indicated herein.

[0498] The container may be any vessel or other sealed or sealable apparatus that can

hold the pharmaceutical composition. Examples include bottles, ampules, divided or multi-chambered holders bottles, wherein each division or chamber comprises a single dose of said composition, a divided foil packet wherein each division comprises a single dose of said composition, or a dispenser that dispenses single doses of said composition. The container can be in any conventional shape or form and is made of a pharmaceutically acceptable material, for example a paper or cardboard box, a glass or plastic bottle or jar, a re-sealable bag, or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. The container employed typically depends on the dosage form involved. More than one container can be used together in a single package for a single dosage form.

[0499] The kits may also comprise a device to administer or to measure out a unit dose of the pharmaceutical composition. The device may include, for example, an inhaler if the composition is an inhalable composition; a syringe and needle if the composition is an injectable composition; a syringe, spoon, pump, or a vessel with or without volume markings if the composition is an oral liquid composition; or any other measuring or delivery device appropriate to the dosage formulation of the composition present in the kit.

[0500] In various embodiments, the kits may comprise, for example in a separate vessel or container, one or more additional therapeutic agent, typically in the form of a pharmaceutical composition comprising the additional therapeutic agent and a pharmaceutically acceptable carrier.

[0501] The following non-limiting examples are provided to illustrate the present invention and in no way limit the scope thereof.

EXAMPLES

[0502] Culicinin D **1** is made up of several building blocks, some of which are shown in Figure 1. The examples that follow show the preparation of various culicinin D analogues and evaluation of their biological activity.

1. Example 1

[0503] This example shows a structure activity relationship (SAR) focusing on the biological activity of culicinin D **1** and peptide analogues **41-55** prepared by replacing the (2*S*,4*S*,6*R*)-2-amino-6-hydroxy-4-methyl-8-decanoic acid (AHMOD) residue with various AHMOD analogues.

1.1 General Methods and Materials

[0504] All reagents were purchased as reagent grade and used without further purification. Solvents for reactions were dried according to standard procedures. *N,N*-Diisopropylethylamine, piperidine, *N,N*-diisopropylcarbodiimide, (1*S*,2*R*)-2-amino-1-phenylpropan-1-ol, ethanolamine, *N*-(2-aminoethyl)ethanolamine, and ninhydrin were purchased from Sigma-Aldrich (St Louis, Missouri). HATU, 2-chlorotrityl chloride functionalised polystyrene resin, and Oxyma were purchased from Novabiochem (Merck, Germany). COMU and 6-Cl-HOBt were purchased from Aapptec (Louisville, KY). Fmoc-Aib-OH was purchased from CS Bio (Shanghai, China). Fmoc-AD-OH was purchased from Global Science. *N*-Methylethylenediamine and L-phenylalaninol were purchased from Merck. Fmoc-Cha-OH, Boc-D-alaninol and Boc-L-alaninol were purchased from AK Scientific. Unless stated otherwise, all Fmoc-protected amino acids were purchased from CS Bio. Unless stated otherwise all other amino acids used in the syntheses described herein may be purchased from commercial sources, for example CS Bio.

[0505] Semi-preparative RP-HPLC was performed on a Thermo Scientific (Waltham, MA) Dionex Ultimate 3000 HPLC equipped with a four channel UV detector at 210, 225, 254, and 280 nm using either an analytical column [Waters (Milford, MA) XTerra® MS C18, 4.6 × 150 mm, 5 µm] at a flow rate of 1 mLmin⁻¹ or a semi-preparative column (XTerra® MS C18, 10 × 250 mm, 5 µm) at a flow rate of 5 mLmin⁻¹. A suitably adjusted gradient of 5% B to 95% B was used, where solvent A was 0.1% formic acid in H₂O and B was 0.1% formic acid in acetonitrile for AHMOD containing peptides; and A was 0.1% TFA in H₂O and B was 0.1% TFA in acetonitrile for the remaining analogues.

[0506] LC-MS spectra were acquired on either an Agilent Technologies (Santa Clara, CA) 1120 Compact LC equipped with a Hewlett-Packard (Palo Alto, CA) 1100 MSD mass spectrometer or an Agilent Technologies 1260 Infinity LC equipped with an Agilent Technologies 6120 quadrupole mass spectrometer. An analytical column (Agilent C3, 150 mm × 3.0 mm, 3.5 µm) was used at a flow rate of 0.3 mLmin⁻¹ using a linear gradient of 5% B to 95% B over 30 min, where solvent A was 0.1% formic acid in H₂O and B was 0.1% formic acid in acetonitrile.

[0507] Solvents were dried by passage over alumina. Reactions were monitored by thin layer chromatography (tlc) on silica gel plates, and visualised using UV light and/or staining with ninhydrin. Flash column chromatography was carried out using Davisil 40-60 micron silica. The optical rotation of pure compounds was obtained using an Autopol IV automatic polarimeter using a 100 mm path length cell.

[0508] NMR spectra were recorded on a Bruker 400 or 500 MHz instrument at room temperature, and chemical shifts are reported in parts per million (ppm) and calibrated to tetramethylsilane (0 ppm) as an internal standard in ¹H spectra, and residual solvent (77 ppm) in ¹³C spectra. Multiplicities are reported as follows: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of

triplets, ddd = doublet of doublet of doublets. High-resolution mass spectra (HRMS) were obtained using a Bruker microTOF-Q II mass spectrometer operating at a nominal accelerating voltage of 70 eV in electron-spray ionisation MS (ESIMS) mode.

[0509] Unless alternative general methods and materials are given, the above general materials and methods are applicable to all of the examples below.

1.2 Abbreviations

[0510] 6-Cl-HOBt, 6-chloro-1-hydroxy-benzotriazole; 2-CTC, 2-chlorotrityl chloride functionalised polystyrene resin; AA, amino acid; Acc-OH, 1-aminocyclopropane-1-carboxylic acid; Acb-OH, 1-aminocyclobutane-1-carboxylic acid; Acp-OH, 1-aminocyclopropane-1-carboxylic acid; Ahc-OH, 1-aminocyclohexane-1-carboxylic acid; AD-OH, 2-aminodecanoic acid; Adg-OH, 1-adamantylglycine; alpha-MEM, minimum essential medium Eagle alpha modification; Boc, *tert*-butyl carbamate; Cha-OH, cyclohexylalanine; Chg-OH, cyclohexylglycine; COMU, 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylaminomorpholinomethylene)]-methanaminium hexafluorophosphate; Cpa-OH, cyclopropylalanine; DIC, *N,N'*-diisopropylcarbodiimide; DIPEA, *N,N*-diisopropylamine; DMEM, Dulbecco's modified Eagle's medium; DMF, *N,N*-dimethylformamide; eq, equivalents; ESI, electron spray ionisation; FCS, fetal calf serum; Fmoc, 9-fluorenylmethyl carbamate; Fmoc-OSu, Fmoc-succinimide; HATU, 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium hexafluorophosphate 3-oxide; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; HMDS, hexamethyldisilazane; HRMS, high-resolution mass spectrometry; Hyp-OH, 4-hydroxyproline; IBX, 2-iodoxybenzoic acid; IC₅₀, concentration causing half-maximal inhibition; LRMS, low-resolution mass spectrometry; MS, mass spectrometry; Nle-OH, norleucine; Nle(6OH)-OH, 6-hydroxynorleucine; NMR, nuclear magnetic resonance spectroscopy; Oxyma, ethyl 2-cyano-2-(hydroxyimino)acetate; pet. ether, petroleum ether; Pip-OH, pipecolic acid; RP-HPLC, reverse-phase high performance liquid chromatography; RPMI, Roswell Park Memorial Institute; rt, room temperature; SPPS, solid-phase peptide synthesis; STR, short tandem repeat; TFA, trifluoroacetic acid; TIPS, triisopropylsilane; tlc, thin layer chromatography; Trt, trityl, triphenylmethyl; UV, ultra-violet; DMA, *N,N*-dimethylacetamide; DTT, DL-dithiothreitol; MC, 6-maleimidocaproyl; NMP, *N*-methyl-2-pyrrolidone; PABA, para-4-Aminobenzyl alcohol; PABC, *para*-aminobenzoyloxycarbonyl; PABOH = *para*-aminobenzyl alcohol; PBS = phosphate buffered saline; PNP, Bis(4-nitrophenyl) carbonate; PySSPA, 3-(2-pyridyldithio)propionic acid; SMCC, 4-(*N*-Maleimidomethyl)cyclohexanecarboxylic acid *N*-hydroxysuccinimide ester; TCEP, tris(2-carboxyethyl)phosphine hydrochloride; EEDQ, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline; Gly, glycine; Glu, glutamic acid; Gln, glutamine; Asp, aspartic acid; Lys, lysine; Tyr, tyrosine; Trp, tryptophan; Phe, phenylalanine; Ala, alanine; Cys, cysteine; His, histidine; Iva, isovaline; Nle, norleucine; Nva, norvaline; Met, methionine; Pro, proline; Arg, arginine; Ser, serine; Hse, homoserine;

Hyp, hydroxyproline; HyLeu, hydroxyleucine; Thr, threonine; Val, valine; Cit, citrulline; Leu, leucine.

[0511] Unless stated otherwise, these abbreviations are applicable to all of the examples below.

5

1.3 General procedures for preparation of culicinin D building blocks and AHMOD analogues

1.3.1 General procedure A for Wittig olefination:

10 **[0512]** Lithium bis(trimethylsilyl)amide (LiHMDS) (1.0 M in THF, 1.8 eq) was added slowly to a suspension of the Wittig salt (2 eq.) in THF (60mL/mmol aldehyde), at -40 °C, and the mixture allowed to stir at -40 °C for 1 h. A solution of the aldehyde (1 eq) in THF (20 mL/mmol) was added slowly, and the mixture allowed to stir at -40 °C for 2 h, then allowed to warm to rt and stirred until the reaction was complete by tlc analysis. Sat. aq.

15 NH₄Cl was added, and the mixture extracted three times with ethyl acetate. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure to give the crude products which were purified *via* flash column chromatography using the eluent indicated.

1.3.2 General procedure B for hydrogenation:

20 **[0513]** A solution of the alkene (1 eq.) in methanol (10 mL/mmol) was stirred in the presence of catalytic Pd(OH)₂/C under an atmosphere of H₂ for 18 h. The mixture was filtered through Celite and concentrated under reduced pressure to give the crude products which were purified *via* flash column chromatography using the eluent indicated.

25

1.3.3 General procedure C for Grignard addition:

[0514] The Grignard reagent (2 eq.) was added slowly to a solution of the aldehyde (1 eq.) in diethyl ether (15 mL/mmol) at 0 °C, and the mixture allowed to stir for 2 h. An equal volume of sat. aq. NH₄Cl was added, and the organic layer removed. The aqueous layer was

30 further extracted twice with ethyl acetate and the combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure to give the crude material. The crude product was adsorbed onto Celite and purified twice *via* automated flash column chromatography (silica gel, ethyl acetate/pet. ether 1:9 to 1:3 gradient over 40 min).

1.3.4 General procedure D for Boc-deprotection/Fmoc-protection of amines:

[0515] The Boc-protected amine (1 eq.) was stirred in a solution of trifluoroacetic acid/dichloromethane (1:4, v/v) at rt for 45 min, then the volatiles removed under reduced pressure. The crude unprotected amine was then diluted with 1,4-dioxane/sat. aq. NaHCO₃ (1:1, v/v) and Fmoc-OSu (1.05 eq.) added. The mixture was allowed to stir at rt for 6-18 h,

then the 1,4-dioxane removed under reduced pressure. The aqueous residue was extracted three times with ethyl acetate and the combined organic extracts were dried over MgSO_4 and concentrated under reduced pressure to give the crude Fmoc-protected amines which were purified *via* flash column chromatography using the eluent indicated.

5

1.3.5 General procedure E for hydrolysis of the methyl ester:

[0516] The ester (1 eq.) was dissolved in isopropyl alcohol/0.8 M aq. CaCl_2 (7:3, v/v), and an aqueous solution of NaOH (1 M, 2 eq.) added dropwise. The mixture was allowed to stir at rt for 6-12 h, then the iPrOH removed under reduced pressure. The aqueous residue was extracted three times with ethyl acetate, and the combined organic extracts dried over MgSO_4 and concentrated under reduced pressure to give the crude amino acid, which was purified *via* flash column chromatography using the eluent indicated.

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1.3.6 General procedure F for oxidation of alcohols 27 and 28:

[0517] IBX (2 eq.) was added to a solution of the alcohol (1 eq.) in DMSO (10 mL/mmol) and the mixture allowed to stir at rt for 18 h. An equal volume of ice was added, and the mixture extracted three times with diethyl ether. The combined organic extracts were washed twice with water, dried over MgSO_4 , and concentrated under reduced pressure. The crude ketone was purified *via* flash column chromatography using the eluent indicated.

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1.4 General Procedure for Peptide Synthesis

[0518] The peptides were assembled manually by Fmoc solid phase peptide synthesis (SPPS) using a fritted glass reaction vessel.

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1.4.1 Method 1. General procedure for the attachment of Fmoc- β -alanine-OH to resin:

[0519] To 2-chlorotrityl chloride functionalised polystyrene resin (0.1 mmol) pre-swollen in anhydrous CH_2Cl_2 (5 mL, 15 min), was added a solution of Fmoc- β -Ala-OH (2 eq, 62 mg, 0.2 mmol) and DIPEA (5 eq, 87 μL , 0.5 mmol) in anhydrous CH_2Cl_2 (1 mL). The reaction mixture was gently agitated at room temperature for 6 h, filtered and repeated once for a further 12 h with fresh reagents. The reaction mixture was removed by filtration and the resin washed with CH_2Cl_2 (3 $\times$ 5 mL). A mixture of CH_2Cl_2 /MeOH/DIPEA (8:1.5:0.5, v/v/v, 2 mL) was added and the reaction agitated for 10 min to cap unreacted resin.

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1.4.2 Method 2. General procedure for removal of N^α -Fmoc-protecting group:

[0520] The peptidyl resin was treated with a solution of 20% piperidine in DMF (v/v, 2 mL) and the mixture agitated at room temperature for 5 min, filtered and repeated once for a further 15 min. The resin was filtered and washed DMF (3 $\times$ 3 mL).

1.4.3 Method 3. General procedure for SPPS:

[0521] To the peptidyl resin (0.1 mmol) was added a mixture of appropriate Fmoc-protected amino acid (5 eq, 0.5 mmol), HATU (4.9 eq, 186 mg, 0.49 mmol) and DIPEA (10 eq, 172 μ L, 1 mmol) in DMF (1 mL). The reaction mixture was agitated at room temperature for 1 h, filtered and repeated once for a further 1 h with fresh reagents. The resin was washed with DMF (3 $\times$ 3 mL), and CH₂Cl₂ (3 $\times$ 3 mL).

1.4.4 Method 4. General procedure for the difficult coupling reaction between resin-bound Aib and Fmoc-Aib-OH:

[0522] To the peptidyl resin (0.1 mmol) was added a mixture of Fmoc-Aib-OH (5 eq, 163 mg, 0.5 mmol), COMU (5 eq, 214 mg, 0.5 mmol), Oxyma (5 eq, 71 mg, 0.5 mmol) and DIPEA (10 eq, 172 μ L, 1 mmol) in DMF (1 mL). The reaction mixture was agitated at room temperature for 2 h, filtered and repeated once for a further 2 h with fresh reagents. The resin was filtered and washed with DMF (2 $\times$ 3 mL) and CH₂Cl₂ (2 $\times$ 3 mL).

1.4.5 Method 5. General procedure for the attachment of Fmoc-AMD-OH or Fmoc-AD-OH:

[0523] To the peptidyl resin (0.1 mmol) was added a mixture of Fmoc-AMD-OH (2 eq, 85 mg, 0.2 mmol) or or Fmoc-AD-OH (2 eq, 82 mg, 0.2 mmol), COMU (2 eq, 85 mg, 0.2 mmol), Oxyma (2 eq, 29 mg, 0.2 mmol) and DIPEA (4 eq, 70 μ L, 0.4 mmol) in DMF (1 mL). The reaction mixture was agitated at room temperature for 3 h, after which the resin was filtered and washed with DMF (3 $\times$ 3 mL).

1.4.6 Method 6. General procedure for the attachment of Fmoc-AHMOD-OH:

[0524] To the peptidyl resin (0.1 mmol) was added a mixture of Fmoc-AHMOD-OH (2 eq, 90 mg, 0.2 mmol), HATU (1.9 eq, 72mg, 0.19 mmol) and DIPEA (4 eq, 70 μ L, 0.4 mmol) in DMF (1 mL). The reaction mixture was agitated at room temperature for 2 h. The resin was washed with DMF (3 $\times$ 3 mL), and CH₂Cl₂ (3 $\times$ 3 mL).

1.4.7 Method 7. General procedure for the attachment of *N*-terminal butyric acid/fatty acid:

[0525] To the peptidyl resin (0.1 mmol) was added a mixture of butyric acid (5 eq, 47 μ L, 0.5 mmol), HATU (4.9 eq, 186 mg, 0.49 mmol) and DIPEA (10 eq, 172 μ L, 1 mmol) in DMF (1 mL). The reaction mixture was agitated at room temperature for 1 h, filtered and repeated once for a further 1 h with fresh reagents. The resin was washed with DMF (3 $\times$ 3 mL), and CH₂Cl₂ (3 $\times$ 3 mL).

1.4.8 Method 8. General procedure for HFIP-mediated resin cleavage:

[0526] Resin-bound peptide was cleaved from the resin by gentle agitation in a mixture of CH₂Cl₂/HFIP (4:1, v/v, 5 mL) for 30 min. The filtrate was partially concentrated under a gentle stream of N₂, diluted with H₂O/CH₃CN (1:1, v/v, 10 mL) and lyophilised.

1.4.9 Method 9. General procedure for TFA-mediated resin cleavage:

[0527] Resin-bound protected peptide was cleaved from the resin by gentle agitation in a mixture of TFA/TIPS/H₂O (95:2.5:2.5, v/v/v, 5 mL) for 1 h. The resin was drained, then treated with a second portion of the cleavage cocktail and the combined filtrates partially concentrated under a gentle stream of N₂. The residue was diluted with H₂O/MeCN (1:1, 10 mL) and lyophilised.

1.4.10 Method 10. General procedure for late-stage solution phase C-terminal coupling of APAE:

[0528] To the purified peptide dissolved in DMF was added a mixture of DIC (6 eq), 6-Cl-HOBt (6 eq), APAE-2TFA salt (3 eq) and DIPEA (6 eq). The reaction mixture was agitated at room temperature for 12-48 h.

1.4.11 Method 11. General procedure for the attachment of Fmoc-A6OD-OH:

[0529] To the peptidyl resin (0.1 mmol) was added a mixture of Fmoc-A6OD-OH (1 eq, 42 mg, 0.1 mmol), COMU (1 eq, 43 mg, 0.1 mmol), Oxyma (1 eq, 14 mg, 0.1 mmol), and DIPEA (2 eq, 34 µL, 0.2 mmol) in DMF (1 mL). The reaction mixture was agitated at room temperature for 2 h. The resin was washed with DMF (3 × 3 mL), and CH₂Cl₂ (3 × 3 mL).

1.4.12 Method 12. General procedure for late-stage solution phase C-terminal coupling of L-alaninol:

[0530] To the purified peptide (1 eq) dissolved in DMF was added a mixture of DIC (6 eq), 6-Cl-HOBt (6 eq), and L-alaninol (3 eq). The reaction mixture was agitated at room temperature for 12 h.

1.4.13 Method 13. General procedure for capping:

[0531] The peptidyl resin was treated with a solution of 20% Ac₂O in DMF (v/v, 2 mL) and the mixture agitated at rt for 15 min, filtered, and repeated once for a further 15 min. The resin was filtered and washed with DMF (3 × 3 mL).

1.5 Cell lines and general procedure for antiproliferative assay:

[0532] Three breast cancer cell lines (MDA-MB-468, SKBR3 and T47D) and a non-small cell lung cancer cell line (NCI-H460) were obtained from American Type Culture Collection (ATCC; Rockville, MD). STR phenotyping confirmed authenticity. Cells were maintained in

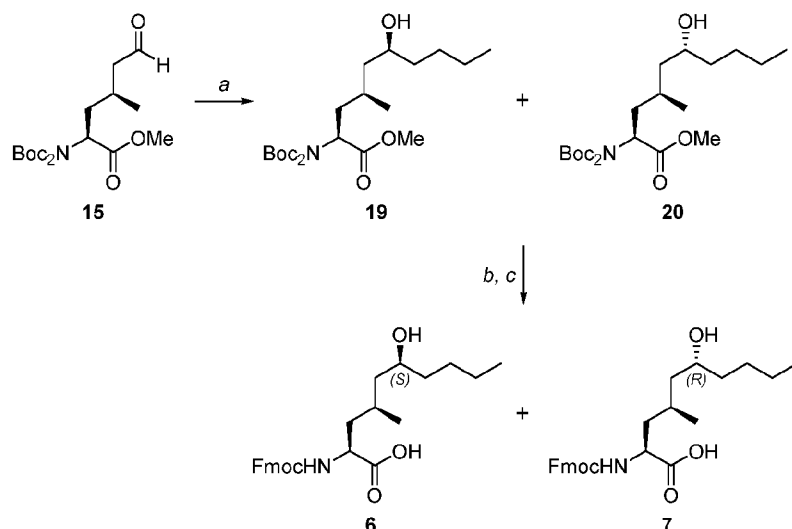
culture under humidified atmospheric conditions with 5% CO₂ at 37 °C, with <3 months cumulative passage from authenticated stocks. MDA-MB-468 and SKBR3 cells were cultured in DMEM medium containing 10% fetal calf serum (FCS), while T47D cells were grown in RPMI medium + 10% FCS. Alpha MEM medium supplemented with 5% FCS was used to culture NCI-H460 cells. Testing of cell cultures for mycoplasma contamination was carried out using Plasmotest Mycoplasma Detection kit (InvivoGen). The sensitivity of four cell lines to culicinin D and culicinin D analogues was examined under aerobic conditions using an antiproliferative assay. Cells were harvested, counted and seeded at a density of 2000 (MDA-MB-468 and SKBR3), 1,500 (T47D) or 300 (NCI-H460) cells/well in 96 well tissue culture plates (Nunc). Cells were incubated over-night to allow attachment, then exposed to peptides using 3-fold serial dilutions in duplicate, and incubated for 5 days. Subsequently medium was aspirated and replaced with fresh medium. Cultures were then stained with sulphorhodamine B to measure total cells as described in Vichai, V.; Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat. Protoc.* **2006**, *1*, 1112-1116. The absorbance-concentration plots were fitted using 4-parameter logistic regression, and IC₅₀ was determined by interpolation as the drug concentration that reduced staining to 50% versus peptide-free controls on the same plate. Values are means and errors are SEM for multiple independent experiments (n=2 to 3 replicates).

1.6 Procedure for the preparation of culicinin D building blocks and AHMOD analogues

1.6.1 Synthesis of culicinin D building blocks

[0533] (2*S*,4*S*,6*R*)- and (2*S*,4*S*,6*S*)-Fmoc-AHMOD, (2*S*,4*R*)-Fmoc-AMD, APAE, and Aldehyde **15** were prepared as detailed previously in Ko *et al.*, and Kavianinia *et al.* (Ko, K.; Wagner, S.; Yang, S.; Furkert, D. P.; Brimble, M. A. Improved Synthesis of the Unnatural Amino Acids AHMOD and AMD, Components of the Anticancer Peptaibol Culicinin D. *J. Org. Chem.* **2015**, *80*, 8631-8636 and Kavianinia, I.; Kunalingam, L.; Harris, P. W. R.; Cook, G. M.; Brimble, M. A. Total Synthesis and Stereochemical Revision of the Anti-Tuberculosis Peptaibol Trichoderin A. *Org. Lett.* **2016**, *18*, 3878-3881).

1.6.2 Synthesis of AHMOD analogues 6 and 7



Scheme 36. Synthesis of Fmoc-protected 8-deoxy analogues **6** and **7**. **Reagents and**

conditions: a) *n*-BuMgBr, Et₂O, -78 °C, 1 h, **19** 18%, **20** 21%; b) (i) TFA/CH₂Cl₂, rt, 0.5-1 h; (ii) Fmoc-OSu, aq. NaHCO₃/1,4-dioxane (1:1), rt, 18 h; c) NaOH, 0.8 M CaCl₂/iPrOH (3:7), rt, 6-8 h, **6** 65%, **7** 66% over 2 steps.

1.6.2.1 Synthesis of alcohol 19 and 20

[0534] (S)-Alcohol **19** and (R)-alcohol **20** were prepared according to general procedure **C** from aldehyde **15** in 18% and 21% yield, respectively, as a separable mixture of colourless oils. **19**: $\alpha_D^{26} = -33.7$ (c 3.33, CHCl₃); **¹H NMR** (400 MHz, CDCl₃): δ 4.97 (dd, *J* = 4.4, 10.6 Hz, 1H), 3.72 (s, 3H), 3.71-3.65 (m, 1H), 2.17 (ddd, *J* = 2.6, 11.0, 13.6 Hz, 1H), 1.77-1.63 (m, 2H), 1.50 (s, 18H), 1.44-1.26 (m, 8H), 0.96 (d, *J* = 6.3 Hz, 3H), 0.90 (t, *J* = 6.8 Hz, 3H); **HRMS** (ESI⁺): *m/z* 454.2779 [M + Na]⁺ (calcd for C₂₂H₄₁NO₇Na⁺ 454.2775); **20**: $\alpha_D^{21} = -25.7$ (c 0.9, CHCl₃); **¹H NMR** (400 MHz, CDCl₃): δ 4.96 (dd, *J* = 5.3, 9.1 Hz, 1H), 3.71 (s, 3H), 3.70-3.64 (m, 1H), 2.00-1.88 (m, 2H), 1.74-1.65 (m, 1H), 1.50 (s, 18H), 1.46-1.38 (m, 4H), 1.36-1.24 (m, 4H), 0.96 (d, *J* = 6.5 Hz, 3H), 0.90 (t, *J* = 6.9 Hz, 3H); **HRMS** (ESI⁺): *m/z* 454.2786 [M + Na]⁺ (calcd for C₂₂H₄₁NO₇Na⁺ 454.2775).

1.6.2.2 Synthesis of Fmoc-amino acid 6

[0535] Fmoc-amino acid **6** was prepared in two steps according to general procedure **D** to give ester **59** (not shown in scheme 36 above) in 85% yield as a colourless oil (ethyl acetate/pet. ether 3:7 as eluent). $\alpha_D^{21} = -9.4$ (c 0.74, CHCl₃); **¹H NMR** (400 MHz, CDCl₃): δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.62-7.58 (m, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.33-7.28 (m, 2H), 5.34 (br d, *J* = 8.4 Hz, 1H), 4.44-4.34 (m, 3H), 4.23 (t, *J* = 7.1 Hz, 1H), 3.75 (s, 3H), 3.74-3.65 (m, 1H), 1.88-1.73 (m, 2H), 1.61-1.53 (m, 1H), 1.48-1.28 (m, 8H), 1.00 (d, *J* = 6.4 Hz, 3H), 0.91 (t, *J* = 6.8 Hz, 3H); **HRMS** (ESI⁺): *m/z* 476.2423 [M + Na]⁺ (calcd for C₂₇H₃₅NO₅Na⁺ 476.2407); followed by hydrolysis according to general procedure **E** to give **6**

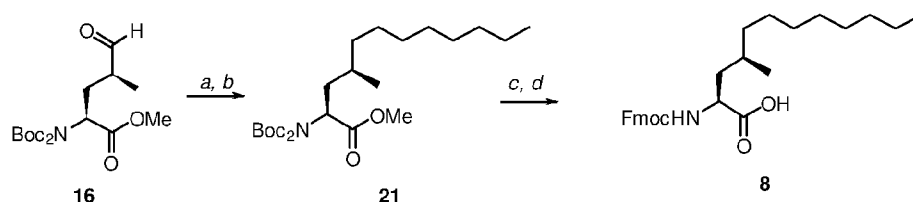
in 76% yield as a colourless solid (ethyl acetate/pet. ether 1:1 + 0.5% AcOH as eluent).

$\alpha_D^{21} = -10.8$ (c 0.59, CHCl_3); **$^1\text{H NMR}$** (400 MHz, CDCl_3): δ 7.74-7.70 (m, 2H), 7.59-7.53 (m, 2H), 7.37-7.33 (m, 2H), 7.29-7.24 (m, 2H), 6.50-6.10 (br s, 1H), 5.89 (d, $J = 8.2$ Hz, 1H), 4.45-4.29 (m, 3H), 4.18 (t, $J = 7.0$ Hz, 1H), 3.73-3.63 (m, 1H), 1.90-1.80 (m, 2H), 1.63-1.56 (m, 1H), 1.49-1.20 (m, 8H), 0.97 (d, $J = 6.3$ Hz, 3H), 0.91-0.82 (m, 3H); **HRMS** m/z (ESI+) 462.2245 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{26}\text{H}_{33}\text{NO}_5\text{Na}^+$ 462.2251).

1.6.2.3 Synthesis of Fmoc-amino acid 7

[0536] Fmoc-amino acid **7** was prepared in two steps according to general procedure **D** to give ester **60** (not shown in scheme 36 above) in 93% yield as a colourless oil (ethyl acetate/pet. ether 3:7 as eluent). $\alpha_D^{21} = +6.1$ (c 0.83, CHCl_3); **$^1\text{H NMR}$** (400 MHz, CDCl_3): δ 7.76 (d, $J = 7.6$ Hz, 2H), 7.60 (dd, $J = 3.4, 7.2$ Hz, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.33-7.29 (m, 2H), 5.25 (br d, $J = 8.6$ Hz, 1H), 4.46-4.38 (m, 3H), 4.23 (t, $J = 6.8$ Hz, 1H), 3.75 (s, 3H), 3.74-3.68 (m, 1H), 1.90-1.79 (m, 1H), 1.65 (t, $J = 7.4$ Hz, 2H), 1.46-1.23 (m, 8H), 0.98 (d, $J = 6.5$ Hz, 3H), 0.91 (t, $J = 6.9$ Hz, 3H); **HRMS** (ESI+): m/z 476.2405 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{27}\text{H}_{35}\text{NO}_5\text{Na}^+$ 476.2407); followed by hydrolysis according to general procedure **E** to give **7** in 71% yield as a colourless solid (ethyl acetate/pet. ether 1:1 + 0.5% AcOH as eluent). $\alpha_D^{21} = +7.1$ (c 1.19, CHCl_3); **$^1\text{H NMR}$** (400 MHz, CDCl_3): δ 7.71-7.68 (m, 2H), 7.57-7.53 (m, 2H), 7.36-7.30 (m, 2H), 7.27-7.21 (m, 2H), 5.81 (d, $J = 8.5$ Hz, 1H), 4.45-4.39 (m, 1H), 4.37-4.29 (m, 2H), 4.16 (t, $J = 7.1$ Hz, 1H), 3.71-3.64 (m, 1H), 1.90-1.78 (m, 1H), 1.73-1.64 (m, 2H), 1.48-1.20 (m, 8H), 0.93 (d, $J = 6.4$ Hz, 3H), 0.86 (t, $J = 6.8$ Hz, 3H); **HRMS** m/z (ESI+) 462.2239 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{26}\text{H}_{33}\text{NO}_5\text{Na}^+$ 462.2251).

1.6.3 Synthesis of AHMOD analogue 8



Scheme 37. Synthesis of Fmoc-protected (4R)-methyl alkyl analogue **8**. **Reagents and conditions:** a) $n\text{-C}_7\text{H}_{15}\text{PPh}_3\text{Br}$, LiHMDS, THF, -40°C to rt, 6-18 h; b) $\text{Pd}(\text{OH})_2/\text{C}$, H_2 , MeOH, rt, 18 h, 46% over 2 steps; c) (i) TFA/ CH_2Cl_2 , rt, 0.5-1 h; (ii) Fmoc-OSu, aq. NaHCO_3 /1,4-dioxane (1:1), rt, 18 h; d) NaOH, 0.8 M CaCl_2 /PrOH (3:7), rt, 6-8 h, 70% over 2 steps.

1.6.3.1 Synthesis of aldehyde 16

[0537] Aldehyde **16** was prepared from L-glutamic acid or L-2-aminoadipic acid according to the method described in the literature by Martín *et al.* (Padrón, J. M.; Kokotos, G.; Martín, T.; Markidis, T.; Gibbons, W. A.; Martín, V. S. Enantiospecific synthesis of α -

amino acid semialdehydes: a key step for the synthesis of unnatural unsaturated and saturated α -amino acids. *Tetrahedron: Asymmetry* **1998**, 9, 3381-3394).

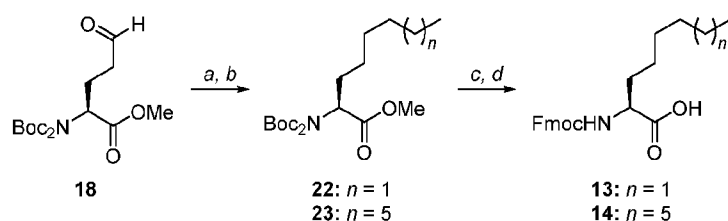
1.6.3.2 Synthesis of ester **21**

- 5 **[0538]** Ester **21** was prepared in two steps according to general procedure **A** to give the alkene **56** (not shown in scheme 37 above) in 51% yield as a colourless oil (ethyl acetate/pet. ether 1:19 as eluent). $\alpha_D^{23} = -38.9$ (c 0.054, MeOH); **¹H NMR** (400 MHz, CDCl₃): δ 5.33 (dt, $J = 8.1, 15.2$ Hz, 1H), 5.17-4.99 (m, 1H), 4.84 (t, $J = 6.9$ Hz, 1H), 3.70 (s, 3H), 2.73-2.62 (m, 1H), 2.21-2.14 (m, 1H), 2.07-1.87 (m, 2H), 1.66-1.58 (m, 1H), 1.49 (s, 18H), 1.34-1.21 (m, 8H), 0.98 (d, $J = 6.6$ Hz, 3H), 0.87 (t, $J = 6.8$ Hz, 3H); **HRMS** (ESI⁺): m/z 464.2978 [M + Na]⁺ (calcd for C₂₄H₄₃NO₆Na⁺ 464.2983); followed by
- 10 hydrogenation according to general procedure **B** to give **21** in 90% yield as a colourless oil (ethyl acetate/pet. ether 1:19 as eluent). $\alpha_D^{24} = -31$ (c 0.1, MeOH); **¹H NMR** (400 MHz, CDCl₃): δ 4.95 (dd, $J = 4.4, 10.5$ Hz, 1H), 3.71 (s, 3H), 2.01 (ddd, $J = 3.5, 10.4, 14.1$ Hz, 1H), 1.78 (ddd, $J = 4.5, 10.1, 14.5$ Hz, 1H), 1.50 (s, 18H), 1.43-1.33 (m, 1H), 1.25 (br s, 14H), 0.91 (d, $J = 6.5$ Hz, 3H), 0.88 (t, $J = 6.8$ Hz, 3H); **HRMS** (ESI⁺): m/z 466.3124 [M + Na]⁺ (calcd for C₂₄H₄₅NO₆Na⁺ 466.3139).
- 15

1.6.3.3 Synthesis of Fmoc-amino acid **8**

- 20 **[0539]** Fmoc-amino acid **8** was prepared in two steps according to general procedure **D** to give ester **61** (not shown in scheme 37 above) in 97% yield as a colourless oil (ethyl acetate/pet. ether 1:9 as eluent). $\alpha_D^{21} = -4.4$ (c 0.16, CHCl₃); **¹H NMR** (400 MHz, CDCl₃): δ 7.78-7.75 (m, 2H), 7.61-7.58 (m, 2H), 7.42-7.38 (m, 2H), 7.33-7.29 (m, 2H), 5.10 (d, $J = 8.8$ Hz, 1H), 4.49-4.39 (m, 3H), 4.24 (t, $J = 7.0$ Hz, 1H), 3.74 (s, 3H), 1.63-1.48 (m, 3H), 1.32-1.18 (m, 14H), 0.94 (d, $J = 5.9$ Hz, 3H), 0.88 (t, $J = 6.6$ Hz, 3H); **HRMS** (ESI⁺): m/z 488.2778 [M + Na]⁺ (calcd for C₂₉H₃₉NO₄Na⁺ 488.2771); followed by hydrolysis
- 25 according to general procedure **E** to give **8** in 72% yield as a colourless oil (ethyl acetate/pet. ether 1:9 + 0.5% AcOH as eluent). $\alpha_D^{21} = -5.0$ (c 0.87, CHCl₃); **¹H NMR** (400 MHz, CDCl₃): δ 10.17 (br s, 1H), 7.74 (d, $J = 7.5$ Hz, 2H), 7.60-7.57 (m, 2H), 7.38 (t, $J = 7.4$ Hz, 2H), 7.29 (t, $J = 7.4$ Hz, 2H), 5.14 (d, $J = 8.7$ Hz, 1H), 4.47-4.38 (m, 3H), 4.22 (t, $J = 6.9$ Hz, 1H), 1.65-1.49 (m, 3H), 1.32-1.21 (m, 14H), 0.94 (d, $J = 6.2$ Hz, 3H), 0.88 (t, $J = 6.5$ Hz, 3H); **HRMS** m/z (ESI⁺) 474.2611 [M + H]⁺ (calcd for C₂₈H₃₇NO₄Na⁺ 474.2615).
- 30

1.6.4 Synthesis of AHMOD analogues **13** and **14**



Scheme 38. Synthesis of Fmoc-protected 4-desmethyl alkyl analogues **13** and **14**.

Reagents and conditions a) *n*-C₃H₇PPh₃I, or *n*-C₇H₁₅PPh₃Br, LiHMDS, THF, -40 °C, 6-18 h; b) Pd(OH)₂/C, H₂, MeOH, rt, 18 h, **22** 59%, **23** 42% over 2 steps; c) (i) TFA/CH₂Cl₂ (1:1), rt, 0.5-1 h; (ii) Fmoc-OSu, aq. NaHCO₃/1,4-dioxane (1:1), rt, 18 h; d) NaOH, 0.8 M

5 CaCl₂/*i*PrOH (3:7), rt, 6-8 h, **13** 34%, **14** 38% over 2 steps.

1.6.4.1 Synthesis of aldehyde 18

[0540] Aldehyde **18** was prepared from L-glutamic acid according to the method described in the literature by Martín *et al.* (Padrón, J. M.; Kokotos, G.; Martín, T.; Markidis, T.; Gibbons, W. A.; Martín, V. S. Enantiospecific synthesis of α-amino acid semialdehydes: a key step for the synthesis of unnatural unsaturated and saturated α-amino acids. *Tetrahedron: Asymmetry* **1998**, 9, 3381-3394).

1.6.4.2 Synthesis of ester 22

15 **[0541]** Ester **22** was prepared in two steps according to general procedure **A** to give the alkene **57** (not shown in scheme 38 above) in 69% yield as a pale yellow oil (ethyl acetate/pet. ether 1:19 as eluent). $\alpha_D^{24} = -30.4$ (c 0.023, MeOH); **¹H NMR** (400 MHz, CDCl₃): δ 5.45-5.29 (m, 2H), 4.87 (dd, *J* = 4.8, 8.9 Hz, 1H), 3.71 (s, 3H), 2.22-1.86 (m, 6H), 1.50 (s, 18H), 0.95 (t, *J* = 7.5 Hz, 3H); **HRMS** (ESI⁺): *m/z* 394.2195 [M + Na]⁺ (calcd for C₁₉H₃₃NO₆Na⁺ 394.2200); followed by hydrogenation according to general procedure **B** to give **22** in 85% yield as a colourless oil (ethyl acetate/pet. ether 1:19 as eluent). $\alpha_D^{22} = -32.2$ (c 0.73, CHCl₃); **¹H NMR** (400 MHz, CDCl₃): δ 4.85 (dd, *J* = 5.1, 9.6 Hz, 1H), 3.71 (s, 3H), 2.13-2.04 (m, 1H), 1.93-1.83 (m, 1H), 1.50 (s, 18H), 1.36-1.24 (m, 8H), 0.88 (t, *J* = 6.8 Hz, 3H); **HRMS** (ESI⁺): *m/z* 396.2354 [M + Na]⁺ (calcd for C₁₉H₃₅NO₆Na⁺ 396.2357).

25

1.6.4.3 Synthesis of ester 23

[0542] Ester **23** was prepared in two steps according to general procedure **A** to give the alkene **58** (not shown in scheme 38 above) in 63% yield as a pale yellow oil (ethyl acetate/pet. ether 1:19 as eluent). **¹H NMR** (400 MHz, CDCl₃): δ 5.44-5.32 (m, 2H), 4.89-4.85 (dd, *J* = 4.8, 8.9 Hz, 1H), 3.71 (s, 3H), 2.22-2.04 (m, 3H), 2.00 (q, *J* = 6.6 Hz, 2H), 1.95-1.87 (m, 1H), 1.50 (s, 18H), 1.34-1.26 (m, 8H), 0.88 (t, *J* = 6.8 Hz, 3H); **HRMS** *m/z* (ESI⁺): 450.2814 [M + Na]⁺ (calcd for C₂₃H₄₁NO₆Na⁺ 450.2826); followed by hydrogenation according to general procedure **B** to give **23** in 66% yield as a colourless oil

35 (ethyl acetate/pet. ether 1:19 as eluent). $\alpha_D^{24} = -29.4$ (c 0.085, MeOH); **¹H NMR** (400 MHz, CDCl₃): δ 4.85 (dd, *J* = 5.1, 9.6 Hz, 1H), 3.71 (s, 3H), 2.13-2.04 (m, 1H), 1.92-1.83 (m, 1H), 1.50 (s, 18H), 1.34-1.25 (m, 16H), 0.88 (t, *J* = 6.8 Hz, 3H); **HRMS** (ESI⁺): *m/z* 452.2981 (calcd for C₂₃H₄₃NO₆Na⁺ 452.2983).

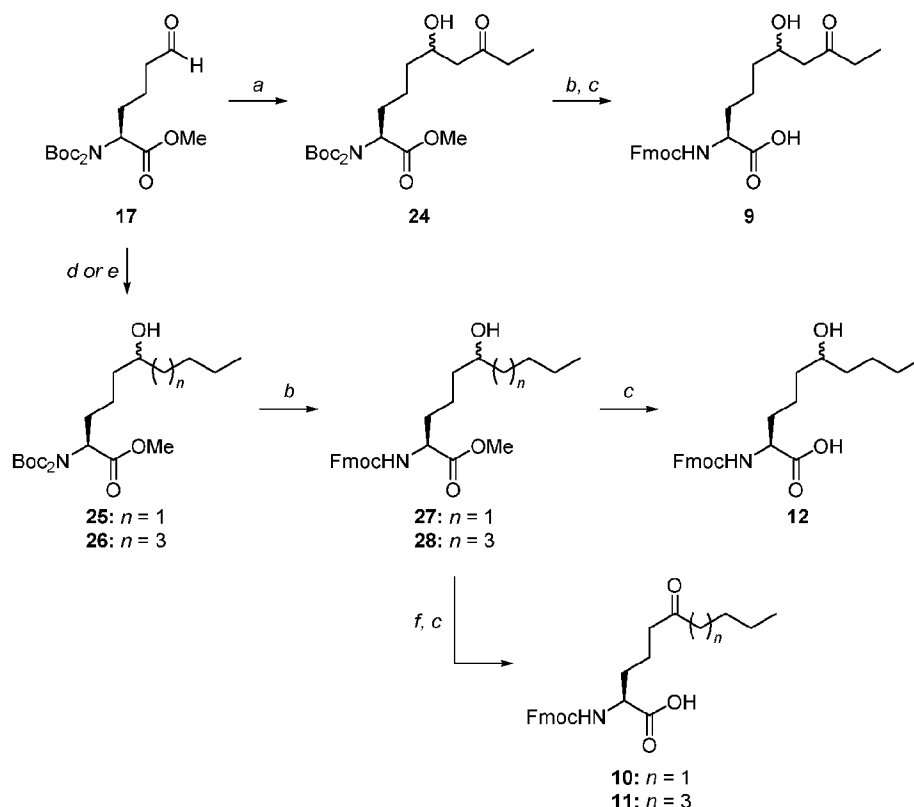
1.6.4.4 Synthesis of Fmoc-amino acid **13**

[0543] Fmoc-amino acid **13** was prepared in two steps according to general procedure **D** to give ester **62** (not shown in scheme 38 above) in 85% yield as a colourless solid (ethyl acetate/pet. ether 1:19 as eluent). $\alpha_D^{22} = +5.4$ (c 4.55, CHCl₃); **¹H NMR** (400 MHz, CDCl₃): δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (br dd, *J* = 4.2, 7.0 Hz, 2H), 7.40 (br t, *J* = 7.5 Hz, 2H), 7.32 (br t, *J* = 7.4 Hz, 2H), 5.25 (br d, *J* = 8.2 Hz, 1H), 4.49-4.35 (m, 3H), 4.23 (t, *J* = 7.0 Hz, 1H), 3.75 (s, 3H), 1.86-1.81 (m, 1H), 1.70-1.63 (m, 1H), 1.37-1.23 (br m, 8H), 0.88 (t, *J* = 6.8 Hz, 3H); **HRMS** (ESI+): *m/z* 418.1993 [M + Na]⁺ (calcd for C₂₄H₂₉NO₄Na⁺ 418.1989); followed by hydrolysis according to general procedure **E** to give **13** in 40% yield as a colourless solid (ethyl acetate/pet. ether 1:9 + 0.5% AcOH as eluent). $\alpha_D^{21} = +2.2$ (c 1.19, CHCl₃); **¹H NMR** (400 MHz, CDCl₃): δ 10.38 (br s, 1H), 7.74 (d, *J* = 7.5 Hz, 2H), 7.60-7.53 (m, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 2H), 5.32 (d, *J* = 8.3 Hz, 1H), 4.48-4.37 (m, 3H), 4.22 (t, *J* = 7.0 Hz, 1H), 1.94-1.84 (m, 1H), 1.77-1.65 (m, 1H), 1.41-1.22 (m, 8H), 0.87 (t, *J* = 6.5 Hz, 3H); Data were consistent with previously reported values.

1.6.4.5 Synthesis of Fmoc-amino acid **14**

[0544] Fmoc-amino acid **14** was prepared in two steps according to general procedure **D** to give ester **63** (not shown in scheme 38 above) in 90% yield as a colourless solid (ethyl acetate/pet. ether 1:9 as eluent). $\alpha_D^{22} = +11.1$ (c 0.11, CHCl₃); **¹H NMR** (400 MHz, CDCl₃): δ 7.77-7.74 (m, 2H), 7.62-7.59 (m, 2H), 7.42-7.38 (m, 2H), 7.33-7.29 (m, 2H), 5.25 (d, *J* = 8.5 Hz, 1H), 4.49-4.35 (m, 3H), 4.23 (t, *J* = 7.0 Hz, 1H), 3.76 (s, 3H), 1.88-1.79 (m, 1H), 1.71-1.61 (m, 1H), 1.35-1.22 (m, 16H), 0.88 (t, *J* = 6.6 Hz, 3H); **HRMS** (ESI+): *m/z* 452.2780 [M + H]⁺ (calcd for C₂₈H₃₈NO₄⁺ 452.2795); 474.2601 [M + Na]⁺ (calcd for C₂₈H₃₇NO₄Na⁺ 474.2615); followed by hydrolysis according to general procedure **E** to give **14** in 42% yield as a colourless solid (ethyl acetate/pet. ether 1:9 + 0.5% AcOH as eluent). $\alpha_D^{19} = -0.8$ (c 0.48, MeOH); **¹H NMR** (400 MHz, CD₃OD): δ 7.78 (d, *J* = 7.2 Hz, 2H), 7.67 (t, *J* = 5.8 Hz, 2H), 7.38 (t, *J* = 6.7 Hz, 2H), 7.30 (t, *J* = 7.0 Hz, 2H), 4.35-4.31 (m, 2H), 4.24-4.21 (m, 1H), 4.17-4.12 (m, 1H), 1.90-1.77 (m, 1H), 1.74-1.62 (m, 1H), 1.44-1.23 (m, 16H), 0.89-0.87 (m, 3H); Data were consistent with previously reported values.

1.6.5 Synthesis of AHMOD analogues 9-12



Scheme 39. Synthesis of Fmoc-protected 4-desmethyl analogues **9** – **12**. **Reagents and conditions:** a) 2-butanone, *n*-BuLi, THF, -78 °C, 3.5 h, 68%; b) (i) TFA/CH₂Cl₂ (1:4), rt, 0.5-1 h; (ii) Fmoc-OSu, 10% aq. NaHCO₃/1,4-dioxane (1:1), rt, 18 h; c) NaOH, 0.8M CaCl₂, *i*PrOH/H₂O (7:3), rt, 6-8 h, **9** 47% over 2 steps; **10** 86%, **11** 75%, **12** 87%; d) *n*-BuMgBr, Et₂O, -78 °C, 3 h, 40%; e) *n*-C₆H₁₃MgBr, Et₂O, -78 °C, 3 h, 35%; f) IBX, DMSO, rt, 18 h, 90%.

1.6.5.1 Synthesis of aldehyde **17**

[0545] Aldehyde **17** was prepared from L-2-aminoadipic acid according to the method described in the literature by Martín *et al.* (Padrón, J. M.; Kokotos, G.; Martín, T.; Markidis, T.; Gibbons, W. A.; Martín, V. S. Enantiospecific synthesis of α-amino acid semialdehydes: a key step for the synthesis of unnatural unsaturated and saturated α-amino acids. *Tetrahedron: Asymmetry* **1998**, 9, 3381-3394).

1.6.5.2 Synthesis of β-hydroxyketone **24**

[0546] *n*-BuLi (1.4 M in cyclohexane, 1.26 mL, 1.77 mmol) was added dropwise to a solution of diisopropylamine (250 μL, 1.77 mmol) in THF (30 mL) at -78 °C. The mixture was allowed to warm to 0 °C for 20 min, then recooled to -78 °C. 2-Butanone (145 μL, 1.61 mmol) was added dropwise, followed by a solution of the aldehyde **17** (579 mg, 1.61 mmol) in THF (10 mL). The mixture was allowed to stir at -78 °C for 3.5 h, then quenched with

sat. aq. NH_4Cl (40 mL) and allowed to warm to rt. The mixture was extracted with ethyl acetate (3 $\times$ 30 mL), and the combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give the crude as a pale yellow oil. The crude was purified via flash column chromatography (silica gel, ethyl acetate/pet. ether 1:4 as eluent) to give **24** (476 mg, 68%) as a pale yellow oil and a 1:1 mixture of diastereomers. **^1H NMR** (400 MHz, CDCl_3 , * denotes diastereomer peaks): δ 4.85 (dd, J = 5.2, 9.4 Hz, 1H), 4.07-4.00 (m, 1H), 3.71 (s, 3H), 3.05 (br s, 0.5H), 3.00* (br s, 0.5H), 2.62-2.42 (m, 4H), 2.16-2.06 (m, 1H), 1.96-1.84 (m, 1H), 1.60-1.36 (m, 22H), 1.06 (t, J = 7.4 Hz, 3H); **HRMS** (ESI+): m/z 454.2416 [$\text{M} + \text{Na}$] $^+$ (calcd for $\text{C}_{21}\text{H}_{37}\text{NO}_8\text{Na}^+$ 454.2411).

1.6.5.3 Synthesis of Fmoc-amino acid 9

[0547] Fmoc-amino acid **9** was prepared in two steps according to general procedure **D** to give the ester **64** (not shown in scheme 39 above) in 76% yield as a colourless foam and a 1:1 mixture of diastereomers (ethyl acetate/pet. ether 3:7 as eluent). **^1H NMR** (400 MHz, CDCl_3): δ 7.76 (d, J = 7.6 Hz, 2H), 7.61-7.58 (m, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.31 (td, J = 1.0, 7.4 Hz, 2H), 5.43 (br t, J = 7.7 Hz, 1H), 4.43-4.34 (m, 3H), 4.22 (t, J = 7.0 Hz, 1H), 4.07-3.99 (m, 1H), 3.75 (s, 3H), 2.91-2.65 (br s, 1H), 2.61-2.47 (m, 2H), 2.43 (q, J = 7.4 Hz, 2H), 1.92-1.81 (m, 1H), 1.76-1.64 (m, 1H), 1.57-1.34 (m, 4H), 1.05 (t, J = 7.2 Hz, 3H); **HRMS** (ESI+): m/z 476.2040 [$\text{M} + \text{Na}$] $^+$ (calcd for $\text{C}_{26}\text{H}_{31}\text{NO}_6\text{Na}^+$ 476.2044); followed by hydrolysis according to general procedure **E** to give **9** in 62% yield as a colourless foam and a 1:1 mixture of diastereomers (methanol/dichloromethane 1:19 as eluent). **^1H NMR** (400 MHz, CDCl_3): δ 7.74 (d, J = 7.5 Hz, 2H), 7.58 (t, J = 6.4 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.29 (t, J = 7.4 Hz, 2H), 6.05 (br s, 2H), 5.64 (m, 1H), 4.49-4.32 (m, 3H), 4.20 (t, J = 6.9 Hz, 1H), 4.12-4.02 (m, 1H), 2.59-2.48 (m, 2H), 2.41 (q, J = 7.3 Hz, 2H), 1.95-1.83 (m, 1H), 1.82-1.65 (m, 1H), 1.61-1.38 (m, 4H), 1.12 (t, J = 7.2 Hz, 3H); **HRMS** (ESI+): m/z 462.1888 [$\text{M} + \text{Na}$] $^+$ (calcd for $\text{C}_{25}\text{H}_{29}\text{NO}_6\text{Na}^+$ 462.1887).

1.6.5.4 Synthesis of alcohol 25

[0548] Alcohol **25** was prepared according to general procedure **C** in 40% yield as a colourless oil and a 1:1 mixture of diastereomers. **^1H NMR** (400 MHz, CDCl_3): δ 4.86 (dd, J = 5.2, 9.4 Hz, 1H), 3.71 (s, 3H), 3.63-3.56 (m, 1H), 2.19-2.07 (m, 1H), 1.98-1.84 (m, 1H), 1.54-1.27 (m, 28H), 0.90 (t, J = 7.0 Hz, 3H); **HRMS** m/z (ESI+) 440.2620 [$\text{M} + \text{Na}$] $^+$ (calcd for $\text{C}_{21}\text{H}_{39}\text{NO}_7\text{Na}^+$ 440.2619).

1.6.5.5 Synthesis of alcohol 26

[0549] Alcohol **26** was prepared according to general procedure **C** in 35% yield as a colourless oil and a 1:1 mixture of diastereomers. **^1H NMR** (CDCl_3 , 400 MHz): δ 4.86 (dd, J = 5.2, 9.4 Hz, 1H), 3.71 (s, 3H), 3.62-3.56 (m, 1H), 2.20-2.06 (m, 1H), 1.97-1.84 (m, 1H),

1.55-1.37 (m, 26H), 1.34-1.24 (m, 6H), 0.88 (t, $J = 6.4$ Hz, 3H); **HRMS** m/z (ESI+) 468.2928 $[M + Na]^+$ (calcd for $C_{23}H_{43}NO_7Na^+$ 468.2932).

1.6.5.6 Synthesis of ester 27

5 **[0550]** Ester **27** was prepared according to general procedure **D** in 86% yield as a colourless solid and as a 1:1 mixture of diastereomers (ethyl acetate/pet. ether 3:7 as eluent). **¹H NMR** (400 MHz, $CDCl_3$): δ 7.78-7.76 (m, 2H), 7.61-7.59 (m, 2H), 7.43-7.38 (m, 2H), 7.33-7.29 (m, 2H), 5.34-5.31 (m, 1H), 4.45-4.35 (m, 3H), 4.23 (t, $J = 7.0$ Hz, 1H), 3.76 (s, 3H), 3.58 (br s, 1H), 1.92-1.81 (m, 1H), 1.77-1.64 (m, 1H), 1.54-1.25 (m, 10H),
10 0.90 (t, $J = 6.9$ Hz, 3H); **HRMS** m/z (ESI+) 462.2247 $[M + Na]^+$ (calcd for $C_{26}H_{33}NO_5Na^+$ 462.2251).

1.6.5.7 Synthesis of ester 28

15 **[0551]** Ester **28** was prepared according to general procedure **D** in 61% yield as a colourless foam and as a 1:1 mixture of diastereomers (ethyl acetate/pet. ether 3:7 as eluent). **¹H NMR** ($CDCl_3$, 500 MHz): δ 7.76 (d, $J = 7.4$ Hz, 2H), 7.61-7.58 (m, 2H), 7.39 (t, $J = 7.6$ Hz, 2H), 7.32-7.29 (m, 2H), 5.40 (t, $J = 8.7$ Hz, 1H), 4.43-4.36 (m, 3H), 4.22 (t, $J = 7.1$ Hz, 1H), 3.75 (s, 3H), 3.60-3.54 (m, 1H), 1.90-1.81 (m, 1H), 1.75-1.63 (m, 1H), 1.59-1.35 (m, 6H), 1.33-1.24 (m, 8H), 0.88 (t, $J = 7.1$ Hz, 3H); **HRMS** m/z (ESI+) 490.2558 $[M + Na]^+$ (calcd for $C_{28}H_{37}NO_5Na^+$ 490.2564).

1.6.5.8 Synthesis of Fmoc-amino acid 12

25 **[0552]** Fmoc-amino acid **12** was prepared according to general procedure **E** in 87% yield as a colourless foam and as a 1:1 mixture of diastereomers (ethyl acetate/pet. ether 1:1+ 0.5% AcOH as eluent). **¹H NMR** (400 MHz, $CDCl_3$, * denotes diastereomer peaks): δ 7.73 (d, $J = 7.5$ Hz, 2H), 7.57 (t, $J = 6.5$ Hz, 2H), 7.37 (t, $J = 7.4$ Hz, 2H), 7.30-7.26 (m, 2H), 6.24 (br s, 1H), 5.66 (br d, $J = 7.8$ Hz, 1H), 4.48-4.33 (m, 3H), 4.19 (t, $J = 6.9$ Hz, 1H), 3.62-3.53 (br m, 1H), 1.94-1.62 (m, 2H), 1.57-1.21 (m, 10H), 0.87 (t, $J = 6.7$ Hz, 3H); **HRMS** m/z (ESI+) 448.2083 $[M + Na]^+$ (calcd for $C_{25}H_{31}NO_5Na^+$ 448.2094).

1.6.5.9 Synthesis of Fmoc-amino acid 10

35 **[0553]** Fmoc-amino acid **10** was prepared in two steps according to general procedure **F** to give ketone **65** (not shown in scheme 39 above) in 91% yield as a colourless foam (ethyl acetate/pet. ether 1:3 as eluent). $\alpha_D^{20} = +3.8$ (c 1.81, $CHCl_3$); **¹H NMR** ($CDCl_3$, 400 MHz): δ 7.76 (d, $J = 7.5$ Hz, 2H), 7.62-7.59 (m, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.32 (t, $J = 7.4$ Hz, 2H), 5.40 (d, $J = 8.0$ Hz, 1H), 4.43-4.35 (m, 3H), 4.23 (t, $J = 7.0$ Hz, 1H), 3.76 (s, 3H), 2.46-2.42 (m, 2H), 2.38 (t, $J = 7.4$ Hz, 2H), 1.89-1.79 (m, 1H), 1.73-1.51 (m, 5H), 1.35-1.25 (m, 2H), 0.90 (t, $J = 7.3$ Hz, 3H); **HRMS** m/z (ESI+) 460.2092 $[M + Na]^+$ (calcd for $C_{26}H_{31}NO_5Na^+$ 460.2094); followed by hydrolysis according to general procedure **E** to

give **10** in 86% yield as a colourless solid (ethyl acetate/pet. ether 1:1 + 0.5% AcOH as eluent). $\alpha_D^{22} = +11.5$ (c 1.02, CHCl₃); **¹H NMR** (CDCl₃, 400 MHz): δ 7.75 (d, *J* = 7.3 Hz, 2H), 7.61-7.58 (m, 2H), 7.39 (t, *J* = 7.3 Hz, 2H), 7.30 (t, *J* = 7.3 Hz, 2H), 5.51 (d, *J* = 7.6 Hz, 1H), 4.41-4.35 (m, 3H), 4.22 (t, *J* = 7.0 Hz, 1H), 2.50-2.43 (m, 2H), 2.41-2.35 (m, 2H), 1.92-1.84 (m, 1H), 1.78-1.63 (m, 3H), 1.58-1.51 (m, 2H), 1.34-1.25 (m, 2H), 0.89 (t, *J* = 7.3 Hz, 3H); **HRMS** *m/z* (ESI+) 446.1922 [M + Na]⁺ (calcd for C₂₅H₂₉NO₅Na⁺ 446.1938).

1.6.5.10 Synthesis of Fmoc-amino acid **11**

[0554] Fmoc-amino acid **11** was prepared in two steps according to general procedure **F** to give ketone **66** (not shown in scheme 39 above) in 90% yield as a colourless foam (ethyl acetate/pet. ether 3:7 as eluent). $\alpha_D^{21} = +9.2$ (c 1.22, CHCl₃); **¹H NMR** (CDCl₃, 400 MHz): δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.61-7.58 (m, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.32-7.28 (m, 2H), 5.45 (br d, *J* = 8.2 Hz, 1H), 4.43-4.35 (m, 3H), 4.22 (t, *J* = 7.1 Hz, 1H), 3.74 (s, 3H), 2.45-2.41 (m, 2H), 2.36 (t, *J* = 7.4 Hz, 2H), 1.88-1.78 (m, 1H), 1.72-1.51 (m, 5H), 1.31-1.25 (m, 6H), 0.87 (t, *J* = 7.0 Hz, 3H); **HRMS** *m/z* (ESI+) 488.2404 [M + Na]⁺ (calcd for C₂₈H₃₅NO₅Na⁺ 488.2407); 466.2580 [M + H]⁺ (calcd for C₂₈H₃₆NO₅⁺ 466.2588); followed by hydrolysis according to general procedure **E** to give **11** in 75% yield as a colourless foam (ethyl acetate/pet. ether 3:7 + 0.5% AcOH as eluent). $\alpha_D^{24} = +10.2$ (c 1.05, CHCl₃); **¹H NMR** (CDCl₃, 400 MHz): δ 9.18 (br s, 1H), 7.73 (d, *J* = 7.5 Hz, 2H), 7.60-7.57 (m, 2H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.31-7.27 (m, 2H), 5.60 (br d, *J* = 8.0 Hz, 1H), 4.52-4.34 (m, 3H), 4.20 (t, *J* = 7.0 Hz, 1H), 2.46-2.32 (m, 4H), 1.92-1.83 (m, 1H), 1.76-1.61 (m, 3H), 1.58-1.49 (m, 2H), 1.30-1.23 (m, 6H), 0.86 (t, *J* = 6.6 Hz, 3H); **HRMS** *m/z* (ESI+) 474.2243 [M + Na]⁺ (calcd for C₂₇H₃₃NO₅Na⁺ 474.2251).

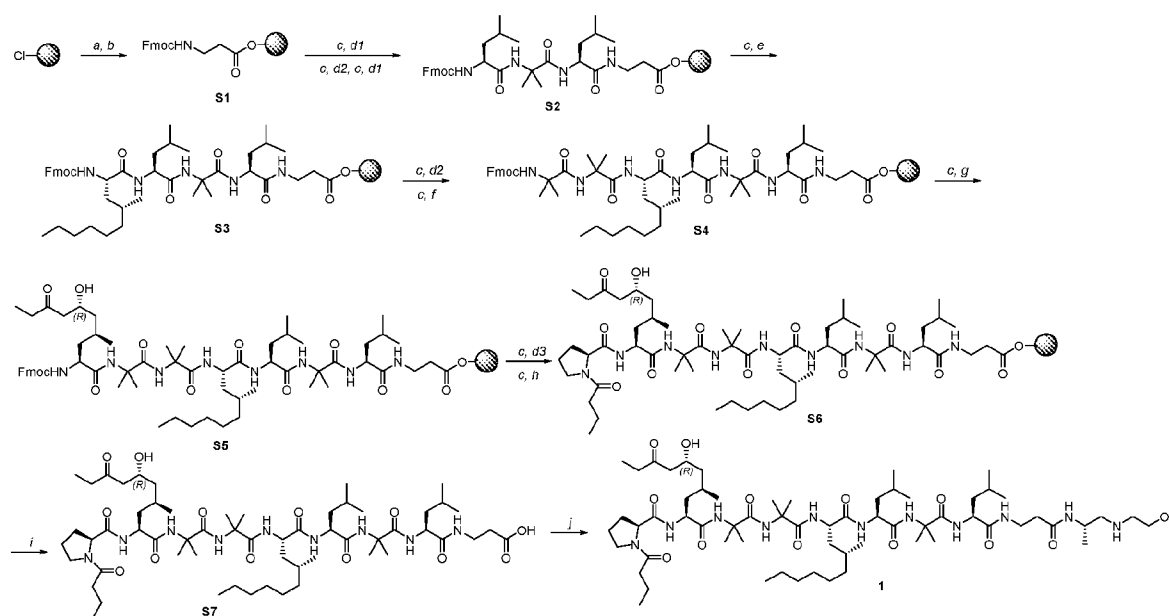
1.7 Procedure for the synthesis of culicinin D and culicinin D analogues

[0555] Having prepared the culicinin D **1** building blocks and AHMOD analogues as described in section 1.6 above, culicinin D **1** and a series of culicinin D analogues **41-55** were prepared as follows. The general procedures section 1.4 includes the methods referred to in this section.

1.7.1 Total Synthesis of culicinin D **1**

The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form

peptidyl resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-(2*S*,4*S*,6*R*)-AHMOD was then coupled using **Method 6** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S6**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S7** as white fluffy flakes (42 mg, 37% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions identified with the correct *m/z* were combined and lyophilised to afford the culicinin D **1** as a white amorphous solid (26 mg, 21% overall yield, > 98% purity); *R*_t 23.6 min; LRMS: *m/z* (ESI-MS) 1234.7 ([*M*+*H*]⁺ requires 1234.8).



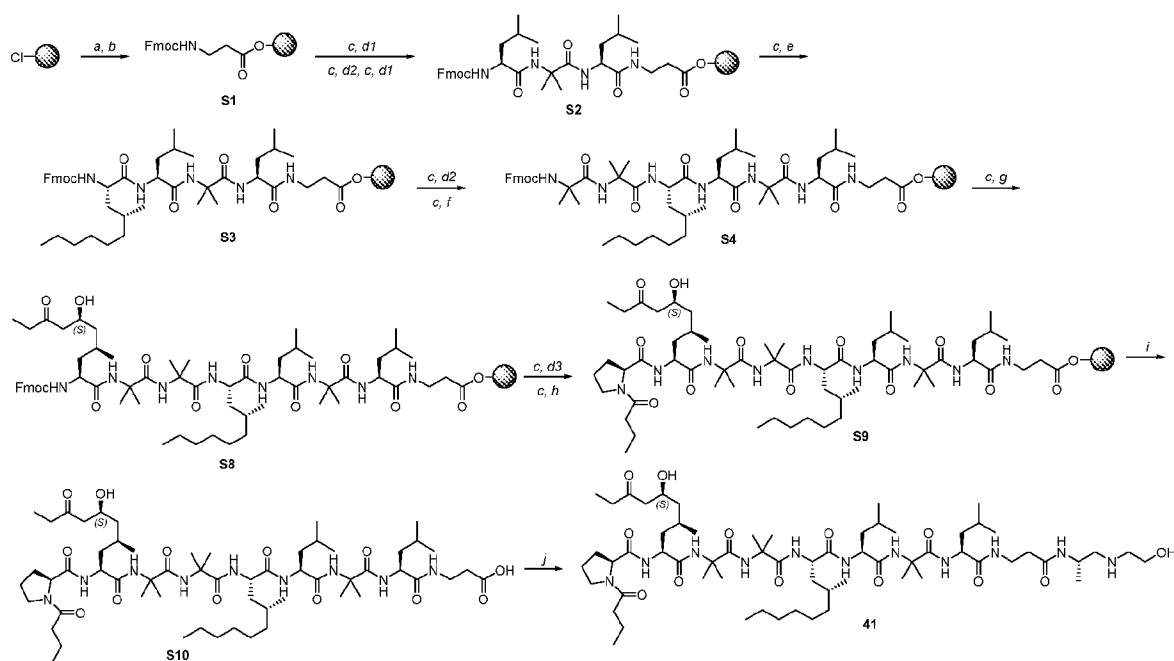
Scheme 40. Synthesis of culicinin D **1**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-(6*R*)-AHMOD-OH, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.2 Synthesis of culicinin D analogue **41**

[0556] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**.

The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to **Method 5**.

The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-(2S,4S,6S)-AHMOD was then coupled using **Method 6** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S9**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S10** as white fluffy flakes (30 mg, 26% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide **41** was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the culicinin D analogue **41** as a white amorphous solid (15 mg, 12% overall yield, > 98% purity); R_t 23.4 min; LRMS: *m/z* (ESI-MS) 1235.0 ([M+H]⁺ requires 1234.8).

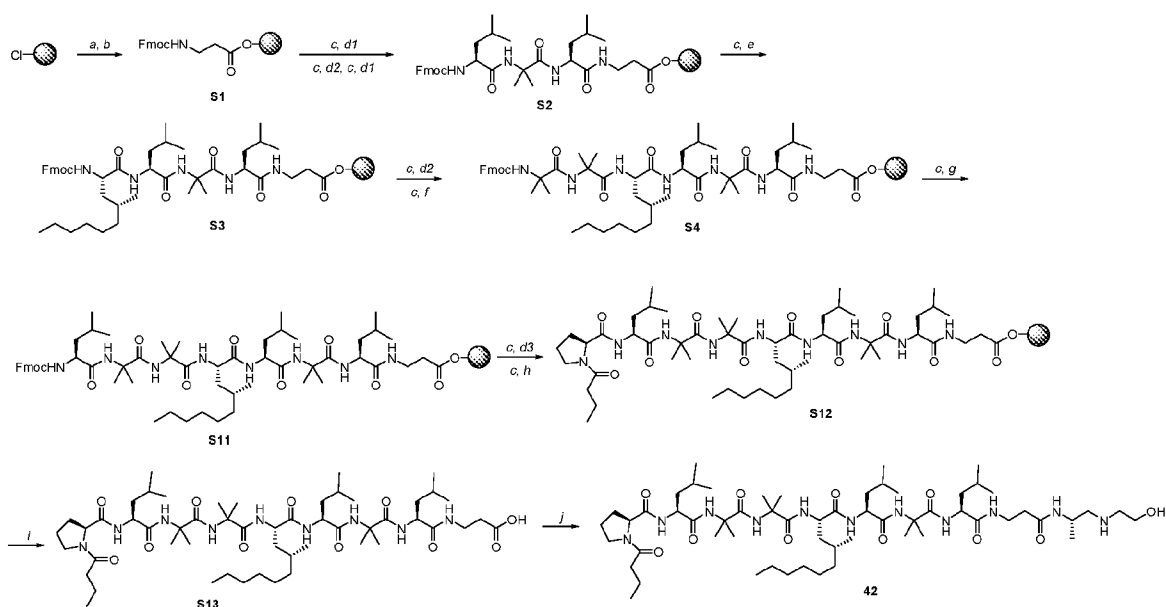


Scheme 41. Synthesis of culicinin D analogue **41**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-(6S)-

AHMOD-OH, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.3 Synthesis of culicinin D analogue 42

5 **[0557]** The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified
10 **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Leu-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric
15 acid via **Method 7** to complete the linear peptide sequence of **S12**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S13** as white fluffy flakes (8 mg, 8%
20 yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford culicinin D analogue **42** as a white
25 amorphous solid (6 mg, 5% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq AMD building block was used); R_t 23.6 min; LRMS: *m/z* (ESI-MS) 1134.7([M+H]⁺ requires 1133.8).

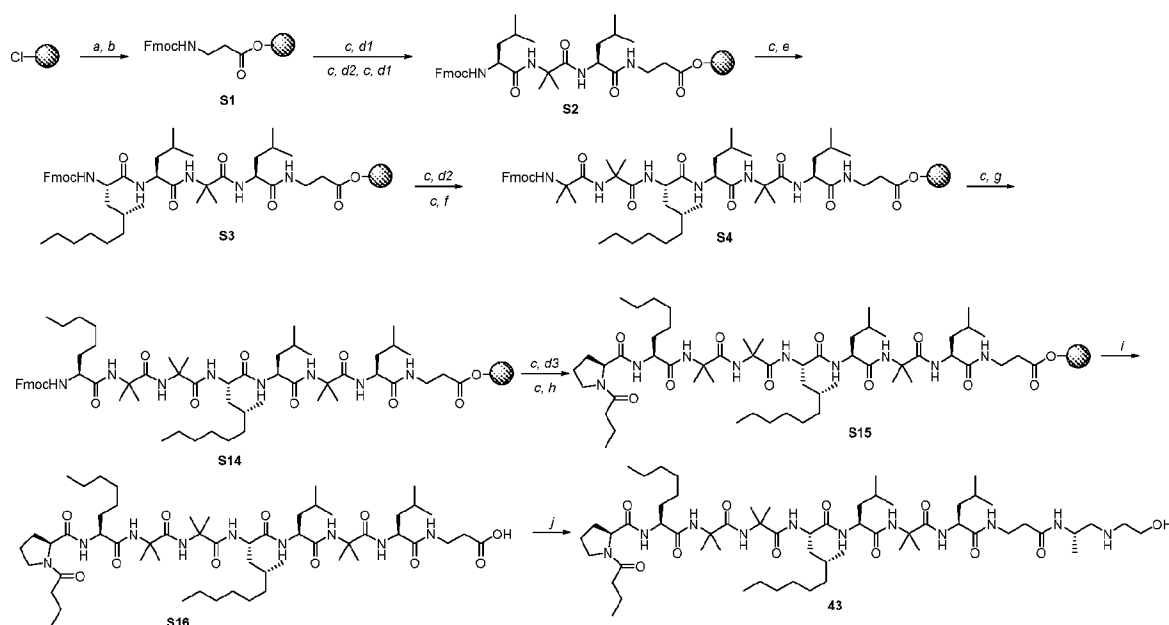


Scheme 42. Synthesis of culicinin D analogue **42**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Leu-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.4 Synthesis of culicinin D analogue 43

[0558] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, AHMOD analogue building block **13** was then coupled using modified **Method 5** (1 eq. of AHMOD analogue building block was used), followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S15**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S16** as white fluffy flakes (10 mg, 9% yield). Late-stage solution phase C-terminal

coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford culicinin D analogue **43** as a white amorphous solid (5 mg, 4% overall yield, > 95% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD and 1 eq. AHMOD analogue building blocks were used); R_t 24.6 min; LRMS: m/z (ESI-MS) 1162.8 ($[M+H]^+$ requires 1162.8).

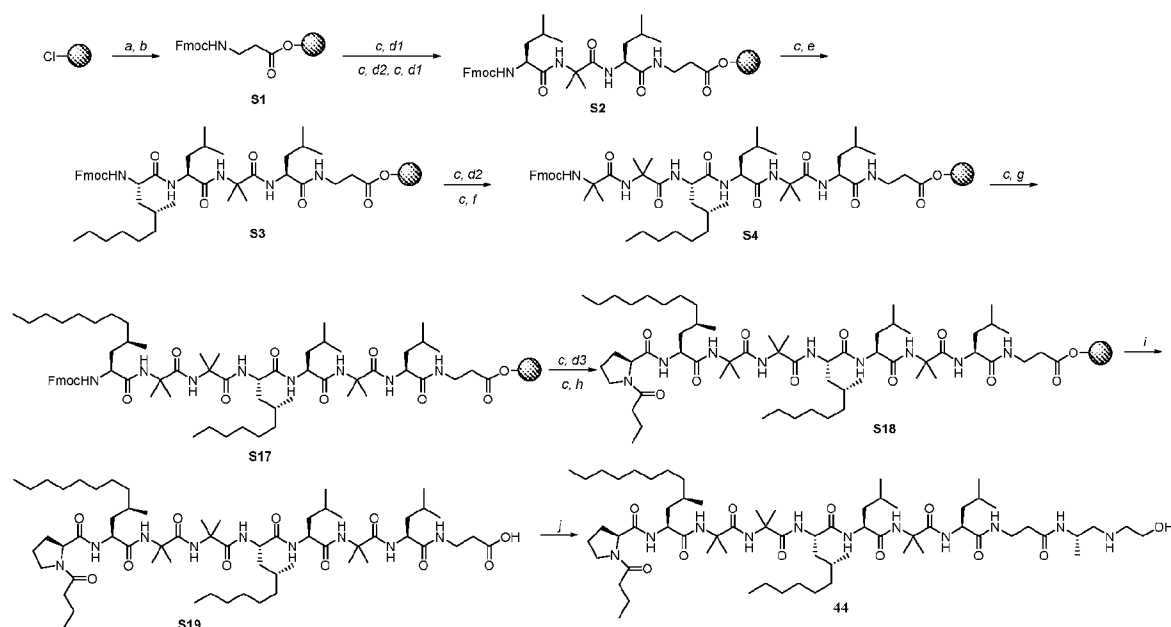


Scheme 43. Synthesis of culicinin D analogue **43**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) AHMOD analogue **13**, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.5 Synthesis of culicinin D analogue 44

[0559] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^{α} -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^{α} -Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^{α} -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the

coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^o-Fmoc-protecting group using **Method 2**, AHMOD analogue building block **8** was then coupled using modified **Method 5** (1 eq. of AHMOD analogue building block was used), followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S18**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S19** as white fluffy flakes (8mg, 7% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford culicinin D analogue **44** as a white amorphous solid (4 mg, 3% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD and 1 eq. AHMOD analogue building blocks were used); R_t 26.5 min; LRMS: *m/z* (ESI-MS) 1232.6 ([M+H]⁺ requires 1232.9).

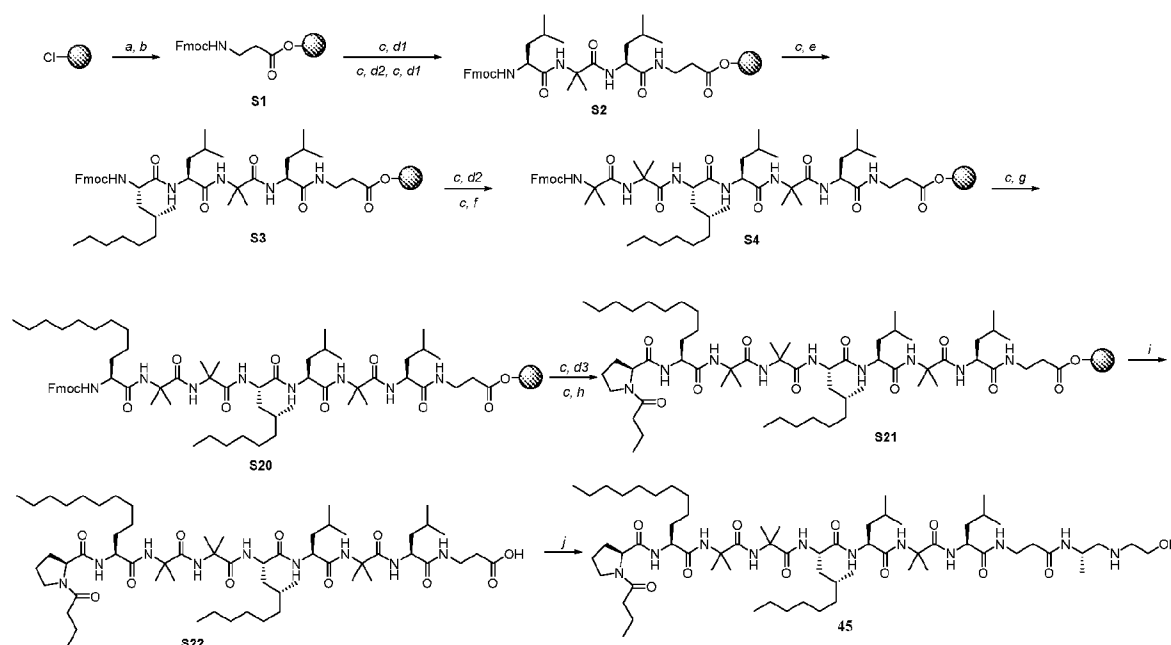


Scheme 44. Synthesis of culicinin D analogue **44**. **Reagents and conditions:** a) Fmoc-

β Ala-OH, DIPEA, CH₂Cl₂, 1 $\times$ 6 h, 1 $\times$ 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) AHMOD analogue **8**, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.6 Synthesis of culicinin D analogue 45

[0560] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^o-Fmoc-protecting group using **Method 2**, AHMOD analogue building block **14** was then coupled using modified **Method 5** (1 eq. of AHMOD analogue building block was used), followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S21**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S22** as white fluffy flakes (13 mg, 11% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford culicinin D analogue **45** as a white amorphous solid (7 mg, 6% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD and 1 eq. AHMOD analogue building blocks were used); R_t 20.9 min; LRMS: m/z (ESI-MS) 1218.9 ([M+H]⁺ requires 1218.9).

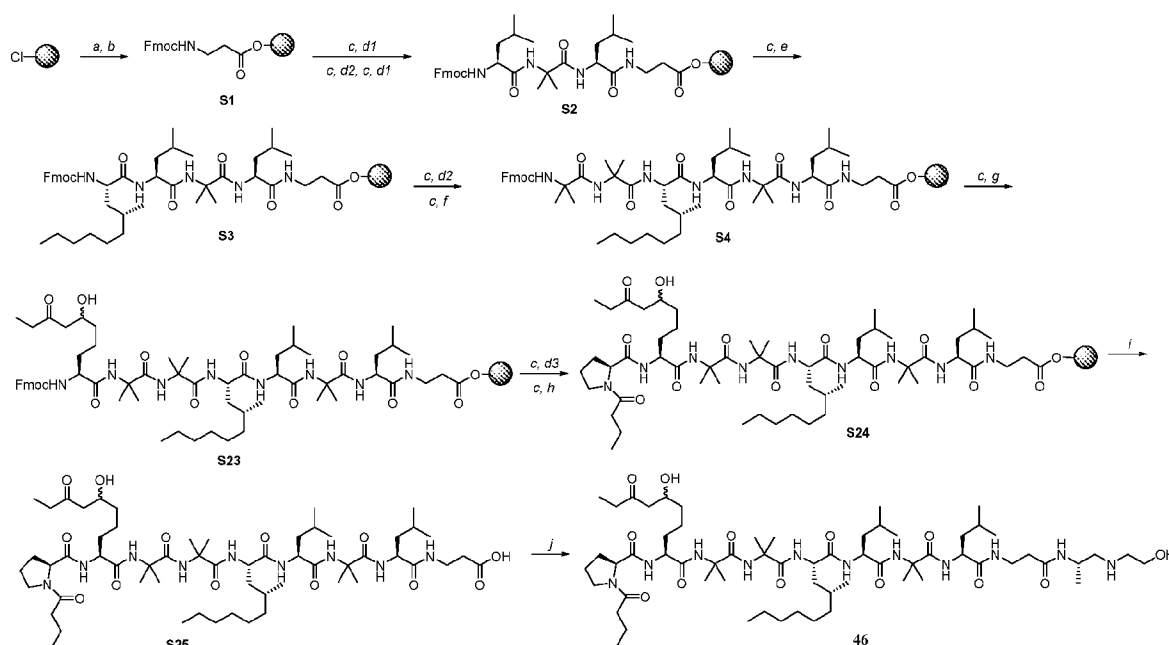


Scheme 45. Synthesis of culicinin D analogue **45**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) AHMOD analogue **14**, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.7 Synthesis of culicinin D analogue 46

[0561] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^o-Fmoc-protecting group using **Method 2**, AHMOD analogue building block **9** was then coupled using modified **Method 5** (1 eq. of AHMOD analogue building block was used), followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S24**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S25** as white fluffy flakes (7 mg, 6% yield). Late-stage solution phase C-terminal

coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford culicinin D analogue **46** as a white amorphous solid (3 mg, 2% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD and 1 eq. AHMOD analogue building blocks were used); R_t 21.9 min; LRMS: m/z (ESI-MS) 1220.8 ($[M+H]^+$ requires 1220.8).

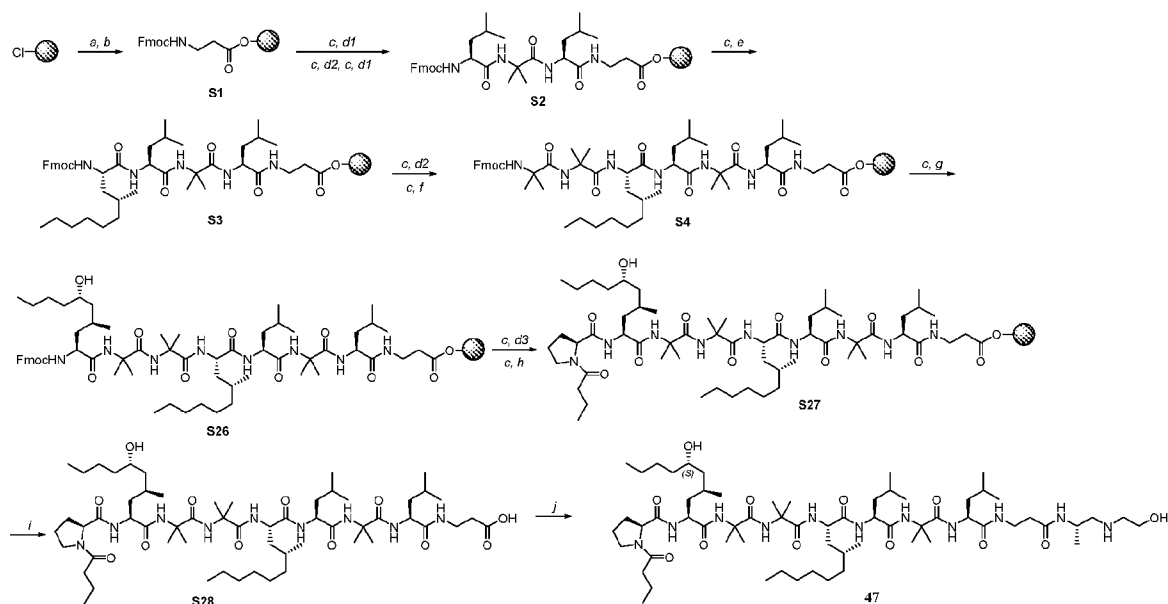


Scheme 46. Synthesis of culicinin D analogue **46**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) AHMOD analogue **9**, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.8 Synthesis of culicinin D analogue **47**

[0562] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^α -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α -Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^α -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the

coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, AHMOD analogue building block **6** was then coupled using modified **Method 5** (1 eq. of AHMOD analogue building block was used), followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S27**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S28** as white fluffy flakes (8 mg, 6% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford culicinin D analogue **47** as a white amorphous solid (4 mg, 3% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD and 1 eq. AHMOD analogue building blocks were used); R_t 23.7 min; LRMS: *m/z* (ESI-MS) 1220.8 ([M+H]⁺ requires 1220.8).

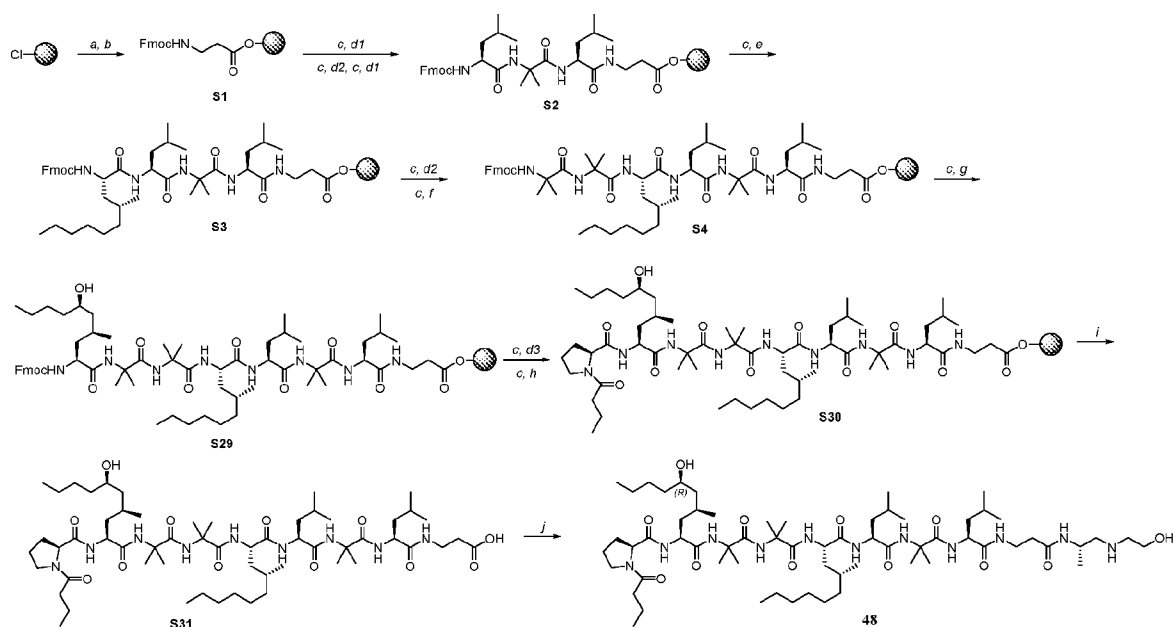


Scheme 47. Synthesis of culicinin D analogue **47**. **Reagents and conditions:** a) Fmoc-

βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) AHMOD analogue **6**, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.9 Synthesis of culicinin D analogue 48

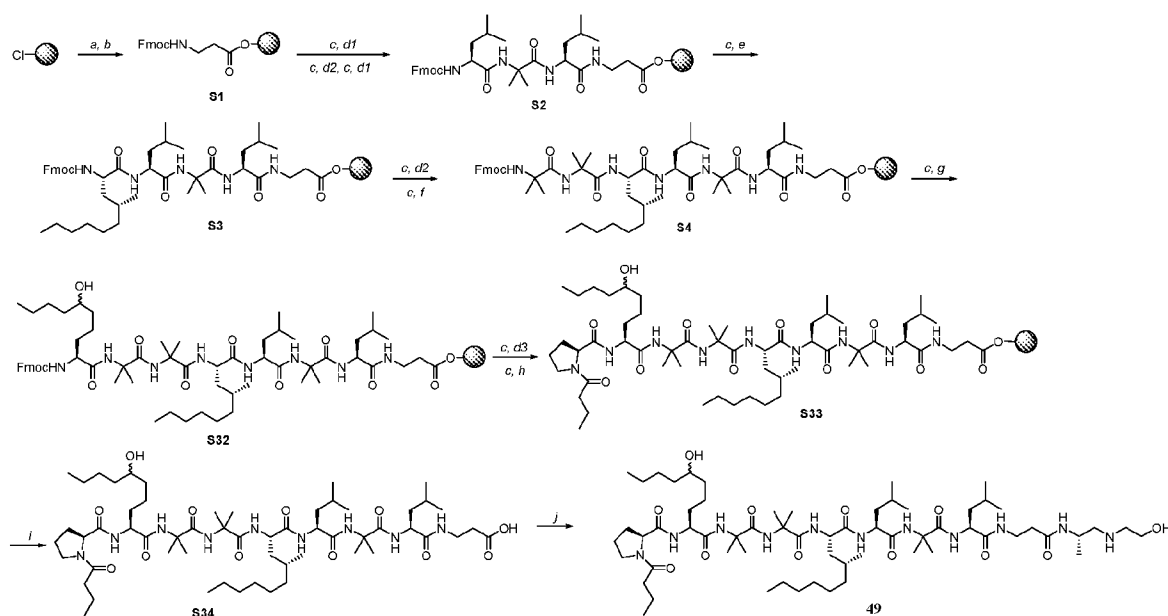
[0563] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^o-Fmoc-protecting group using **Method 2**, AHMOD analogue building block **7** was then coupled using modified **Method 5** (1 eq. of AHMOD analogue building block was used), followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S30**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S31** as white fluffy flakes (10 mg, 9% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford culicinin D analogue **48** as a white amorphous solid (5 mg, 4% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD and 1 eq. AHMOD analogue building blocks were used); R_t 23.5 min; LRMS: m/z (ESI-MS) 1220.8 ([M+H]⁺ requires 1220.8).



Scheme 48. Synthesis of culicinin D analogue **48**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH₂Cl₂, 1 $\times$ 6 h, 1 $\times$ 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) AHMOD analogue **7**, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.10 Synthesis of culicinin D analogue **49**

[0564] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^o-Fmoc-protecting group using **Method 2**, AHMOD analogue building block **12** was then coupled using modified **Method 5** (1 eq. of AHMOD analogue building block was used), followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S33**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S34** as white fluffy flakes (9 mg, 8% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford culicinin D analogue **49** as a white amorphous solid (4 mg, 3% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD and 1 eq. AHMOD analogue building blocks were used); R_t 23.0 min; LRMS: *m/z* (ESI-MS) 1206.8 ([M+H]⁺ requires 1206.8).

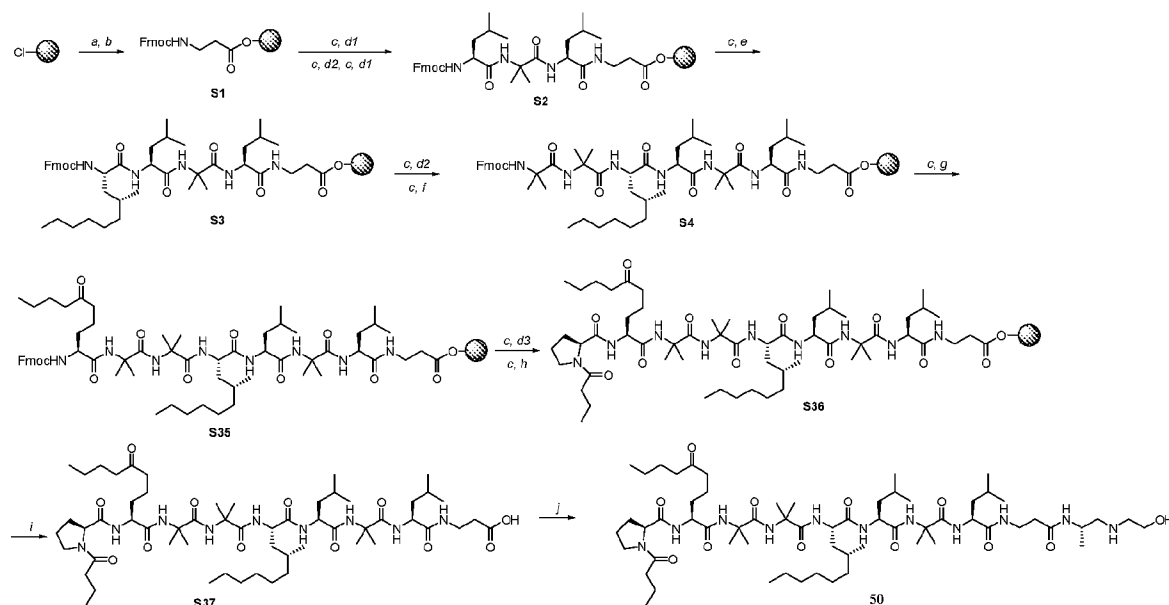


Scheme 49. Synthesis of culicinin D analogue **49**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) AHMOD analogue **12**, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

10 1.7.11 Synthesis of culicinin D analogue **50**

[0565] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to **Method 5**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, AHMOD analogue building block **10** was then coupled using **Method 5**, followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S35**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S37** as white fluffy flakes (39 mg, 35% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was

purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford culicinin D analogue **50** as a white amorphous solid (25 mg, 20% overall yield, > 98% purity); R_t 24.0 min; LRMS: m/z (ESI-MS) 1204.8 ($[M+H]^+$ requires 1204.8).

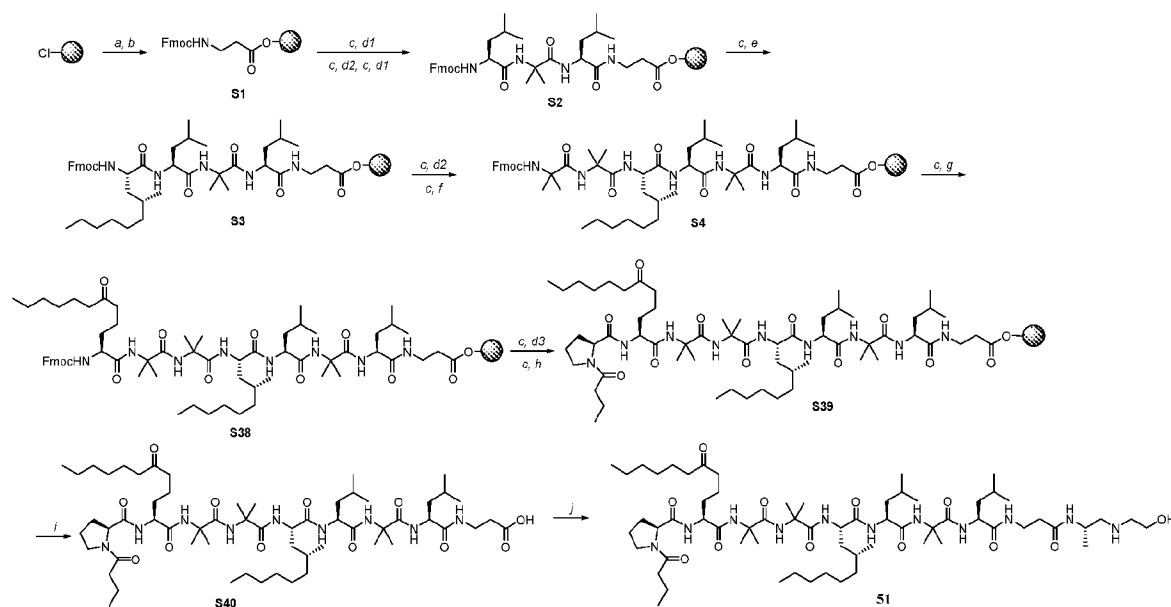


Scheme 50. Synthesis of culicinin D analogue **50**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) AHMOD analogue **10**, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.12 Synthesis of culicinin D analogue **51**

[0566] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^α -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α -Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^α -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^α -Fmoc-protecting group using **Method 2**, AHMOD analogue building block **11** was then coupled using modified **Method 5** (1 eq. of AHMOD analogue building block was used), followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the

linear peptide sequence of **S38**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S40** as white fluffy flakes (7 mg, 6% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford culicinin D analogue **51** as a white amorphous solid (4 mg, 2% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD and 1 eq. AHMOD analogue building blocks were used); R_t 25.7 min; LRMS: m/z (ESI-MS) 1232.8 ($[M+H]^+$ requires 1232.8).



Scheme 51. Synthesis of culicinin D analogue **51**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) AHMOD analogue **11**, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.13 Synthesis of culicinin D analogue **52**

[0567] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH,

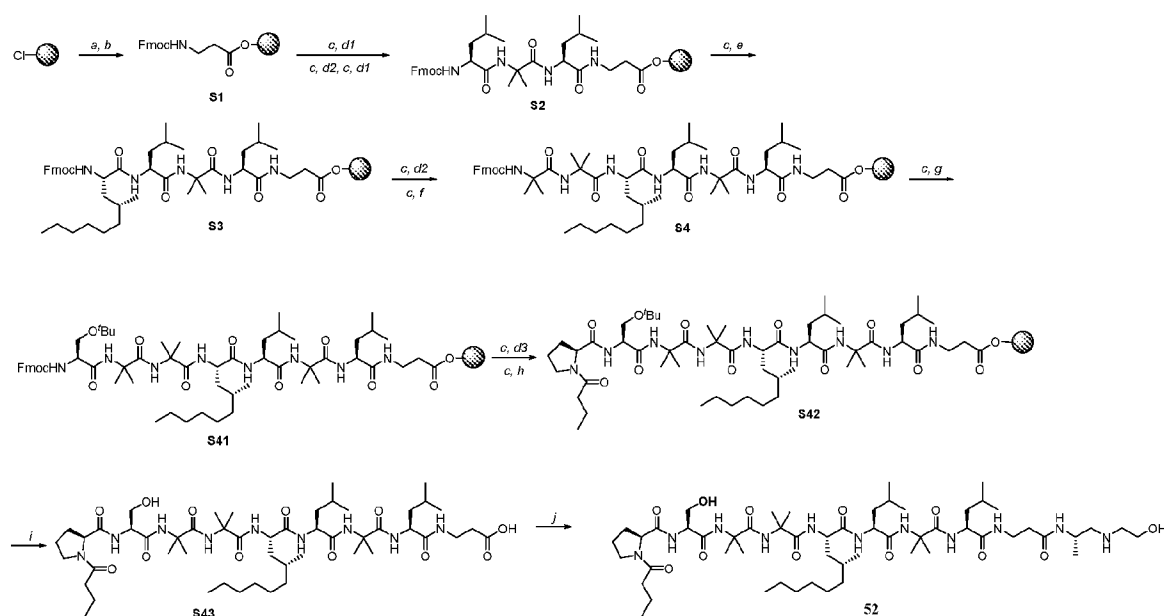
Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the

5 coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Ser(OtBu)-OH was then coupled using **Method 3**, followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S42**. Resin-bound peptide was cleaved using

10 **Method 9**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S43** as white fluffy flakes (38 mg, 38% yield). Late-stage

15 solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford culicinin D analogue **52** as a white amorphous solid (17 mg, 15% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD building block was used); R_t 22.9 min; LRMS: *m/z* (ESI-MS) 1108.0 ([M+H]⁺

20 requires 1108.7).

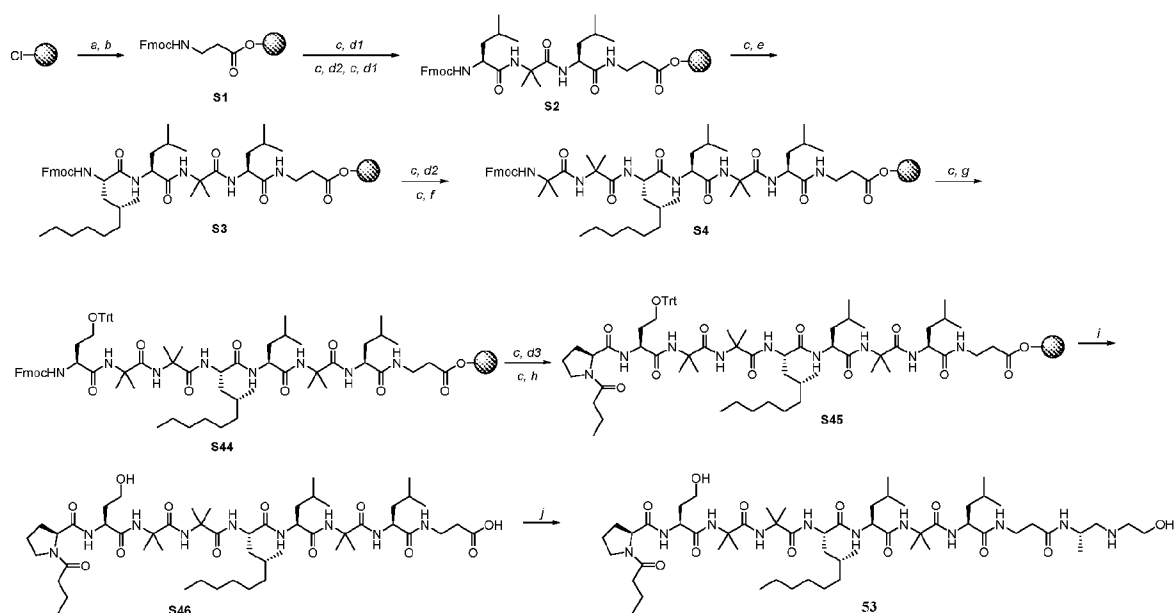


Scheme 52. Synthesis of culicinin D analogue **52**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-

Ser(OtBu)-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) TFA/TIPS/H₂O (95:2.5:2.5, v/v/v), 2h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.14 Synthesis of culicinin D analogue 53

5 **[0568]** The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised resin according to **Method 1** to form resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5**
10 (0.5 eq. of Fmoc-AMD-OH was used). The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Hse(OTrt)-OH was then coupled using **Method 3**, followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to
15 complete the linear peptide sequence of **S45**. Resin-bound peptide was cleaved using **Method 9**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S46** as white fluffy flakes (19 mg, 19 % yield). Late-stage
20 solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford culicinin D analogue **53** as a white amorphous solid (11
25 mg, 10% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD building block was used); R_t 22.0 min; LRMS: *m/z* (ESI-MS) 1122.8 ([M+H]⁺ requires 1122.7).

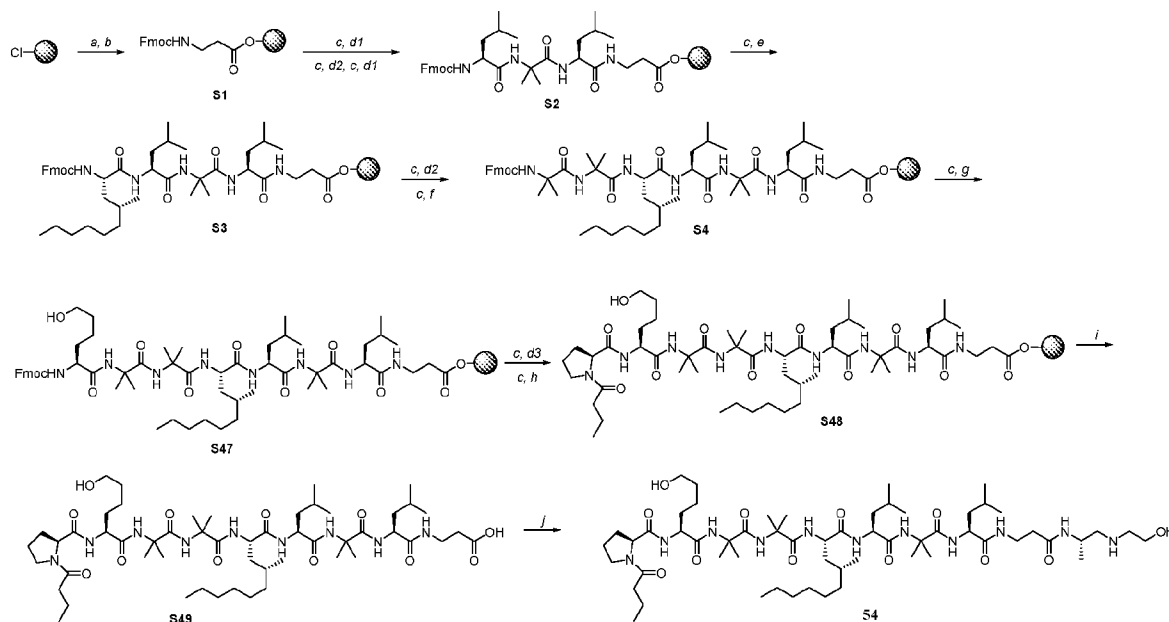


Scheme 53. Synthesis of culicinin D analogue **53**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Hse(OTrt)-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) TFA/TIPS/H₂O (95:2.5:2.5, v/v/v), 2h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.15 Synthesis of culicinin D analogue **54**

[0569] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised resin according to **Method 1** to form resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Nle(6OH)-OH was then coupled using **Method 3**, followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S48**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S49** as white fluffy flakes (14 mg, 13 % yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol

moiety using **Method 8**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford culicinin D analogue **54** as a white amorphous solid (9 mg, 8 % overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD building block was used); R_t 23.5 min; LRMS: m/z (ESI-MS) 1150.6 ($[M+H]^+$ requires 1150.8).



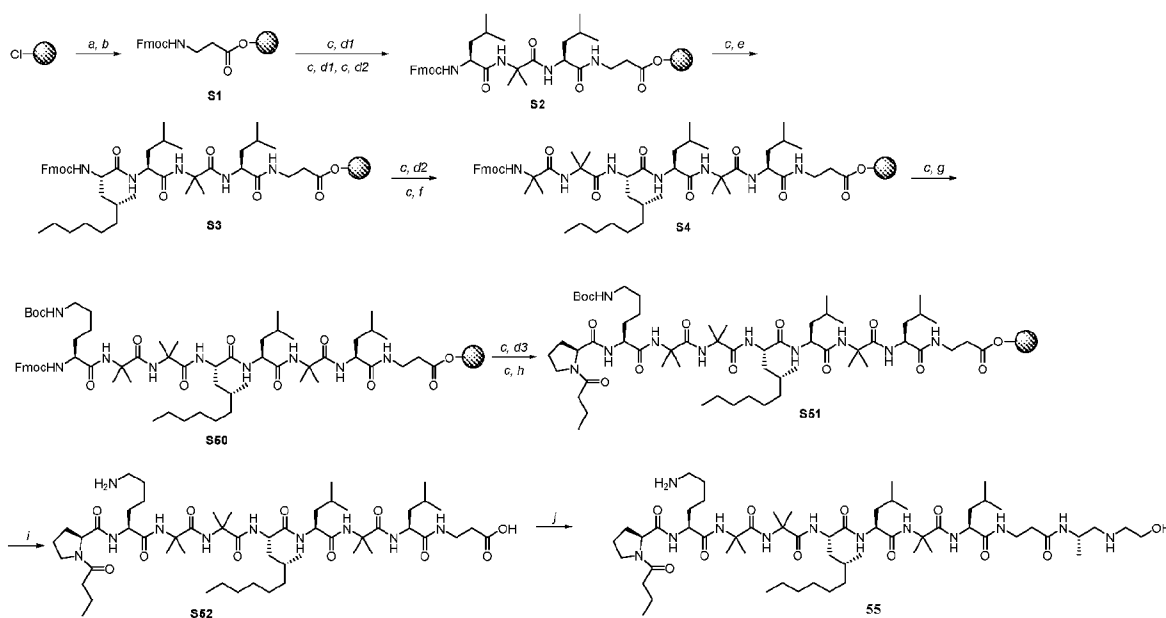
Scheme 54. Synthesis of culicinin D analogue **54**. **Reagents and conditions:** a) Fmoc-

βAla-OH, DIPEA, CH_2Cl_2 , 1 × 6 h, 1 × 12 h; b) CH_2Cl_2 /MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Nle(6OH)-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h, 2h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

1.7.16 Synthesis of culicinin D analogue 55

[0570] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^q -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^q -Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to modified **Method 5** (0.5 eq. of Fmoc-AMD-OH was used). The N^q -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S4**. After removal of N^q -Fmoc-protecting group using **Method 2**, Fmoc-Lys(Boc)-OH was then coupled using **Method 3**,

followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S51**. Resin-bound peptide was cleaved using **Method 9**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S52** as white fluffy flakes (23 mg, 23 % yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford culicinin D analogue **55** as a white amorphous solid (13 mg, 11 % overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq. AMD building block was used); R_t 18.4 min; LRMS: m/z (ESI-MS) 1149.9 ($[M+H]^+$ requires 1149.8).



Scheme 55. Synthesis of culicinin D analogue **55**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH₂Cl₂, 1 $\times$ 6 h, 1 $\times$ 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Lys(Boc)-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) TFA/TIPS/H₂O (95:2.5:2.5, v/v/v), 2h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

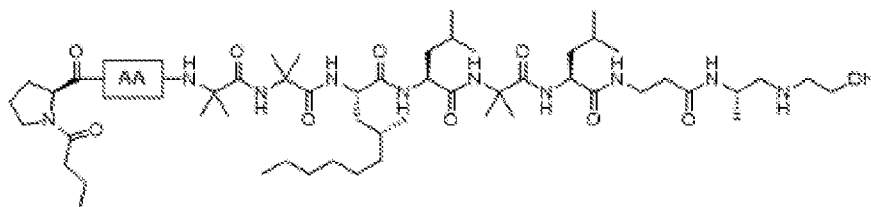
1.8 Biological evaluation of culicinin D **1** and culicinin D analogues **41-55**

[0571] Culicinin D **1** and the peptide analogues **41** – **55** were assessed for antiproliferative activity against three breast cancer cell lines (MDA-MB-468, SKBR3, and

T47D), as well as a non-small cell lung cancer line NCI-H460 (Table 1). Cells were treated with 3-fold serial dilutions of the peptides and maintained under drug-exposure for 5 days. Monitoring of cell proliferation and cell viability was performed by sulphorhodamine B-based assay as previously described in Hay *et al.* (Hay, M. P.; Gamage, S. A.; Kovacs, M. S.; Pruijn, F. B.; Anderson, R. F.; Patterson, A. V.; Wilson, W. R.; Brown, J. M.; Denny, W. A. Structure-activity relationships of 1,2,4-benzotriazine 1,4-dioxides as hypoxia-selective analogues of tirapazamine. *J. Med. Chem.* **2003**, 46, 169-182). The IC₅₀ was determined by interpolation as the drug concentration reducing staining to 50% of controls on the same plate.

- 10 **[0572]** **Table 1.** Antiproliferative activity of culicinin D (**1**) and analogues **41** – **55** against cancer cell lines MDA-MB-468, SKBR3, T47D, and NCI-H460.

Analogues of culicinin D with general structure:



Entry	Compound/ analogue number	Activity against cancer cell line IC ₅₀ (μM) ± SEM			
		MDA-MB-468	SKBR3	T47D	NCI-H460
1	1 (culicinin D)	0.008 ± 0.0005	0.032 ± 0.0025	0.005 ± 0.0006	0.005 ± 0.0007
2	41	0.011 ± 0.001	0.035 ± 0.0008	0.007 ± 0.0001	0.008 ± 0.001
3	42	0.09 ± 0.04	0.28 ± 0.06	0.07 ± 0.01	0.13 ± 0.12
4	43	0.14 ± 0.01	0.24 ± 0.06	0.17 ± 0.02	0.19 ± 0.09
5	44	0.040 ± 0.022	0.236 ± 0.125	0.031 ± 0.005	0.037 ± 0.005
6	45	0.059 ± 0.008	0.280 ± 0.011	0.232 ± 0.107	0.382 ± 0.259
7	46	0.07 ± 0.02	0.50 ± 0.05	0.08 ± 0.02	0.19 ± 0.01
8	47	0.297 ± 0.145	>0.5	0.264 ± 0.112	0.234 ± 0.081
9	48	0.111 ± 0.045	0.327 ± 0.013	0.197 ± 0.061	0.126 ± 0.036
10	49	0.02 ± 0.01	0.10 ± 0.01	0.008 ± 0.007	0.008 ± 0.003
11	50	0.010 ± 0.001	0.03 ± 0.01	0.010 ± 0.002	0.010 ± 0.001

12	51	0.008 ± 0.0007	0.018 ± 0.0022	0.006 ± 0.0004	0.005 ± 0.0005
13	52	0.074 ± 0.009	>0.5	0.066 ± 0.010	0.127 ± 0.011
14	53	0.03 ± 0.01	0.108 ± 0.03	0.02 ± 0.01	0.03 ± 0.01
15	54	0.025 ± 0.004	0.059 ± 0.014	0.014 ± 0.005	0.028 ± 0.002
16	55	>1.0	>1.0	>1.0	>1.0

[0573] Table 1 shows that all compounds had antiproliferative activity against the cancer cell lines tested.

5 **2. Example 2**

[0574] The previous example shows analogues of the (2*S*,4*S*,6*R*)-AHMOD building block of culicinin D **1**.

[0575] This example builds on the structure-activity relationship (SAR) from example 1 above. In this example, further analogues of culicinin D were prepared and investigated to further evaluate the SAR.

[0576] In particular, this example shows the role of the other unusual amino acid residue (2*S*,4*R*)-AMD (**4**) and helix-inducing residue Aib (**2**) to the biological activity of culicinin D **1**. Therefore a series of analogues of culicinin D **1** were prepared where **4** was replaced with an assortment of commercially available amino acids, aimed at further simplifying the synthesis of culicinin D analogues by avoiding the need for custom amino acid synthesis. An alanine scan of a simplified analogue was then carried out to determine the effect of each residue on antiproliferative activity.

2.1 General methods, materials and abbreviations

[0577] The general methods, materials and abbreviations used in this example are the same as those outlined in sections 1.1 to 1.5 above.

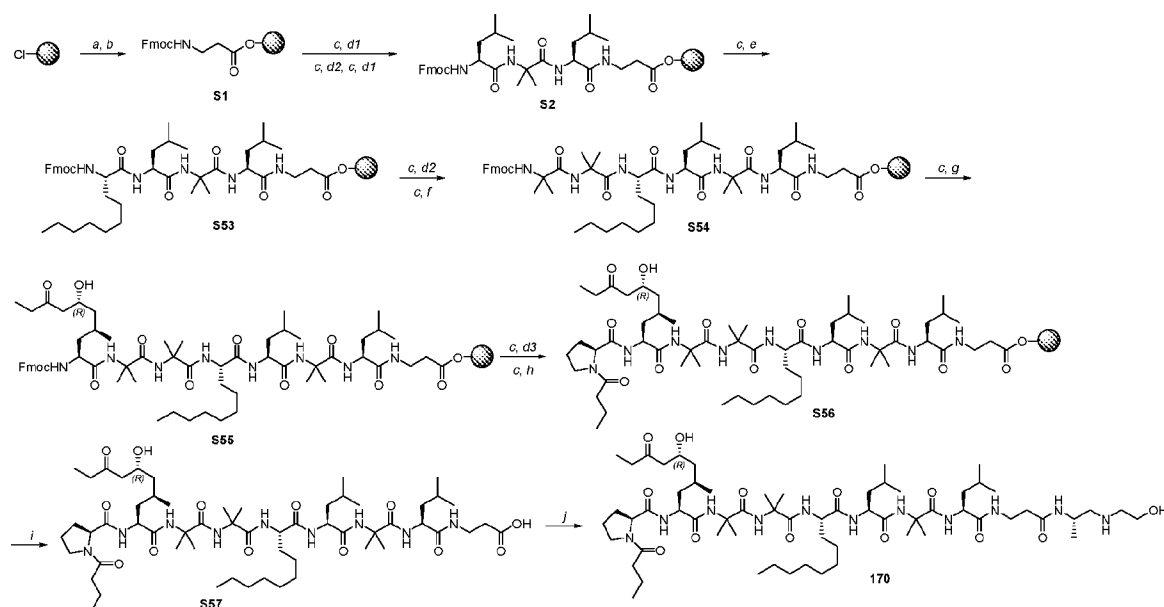
[0578] A series of culicinin D analogues were prepared as follows. The general procedures section 1.4 includes the methods referred to in this section.

2.4.1 Peptide 170: ButyrylPro-AHMOD-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0580] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**,

Fmoc-(2*S*,4*S*,6*R*)-AHMOD was then coupled using **Method 6** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S56**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method.

Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S57** as white fluffy flakes (26 mg, 23% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the **peptide 170** as a white amorphous solid (17 mg, 14% overall yield, > 97% purity); R_t 23.3 min; LRMS: m/z (ESI-MS) 1220.7 ($[M+H]^+$ requires 1220.8).

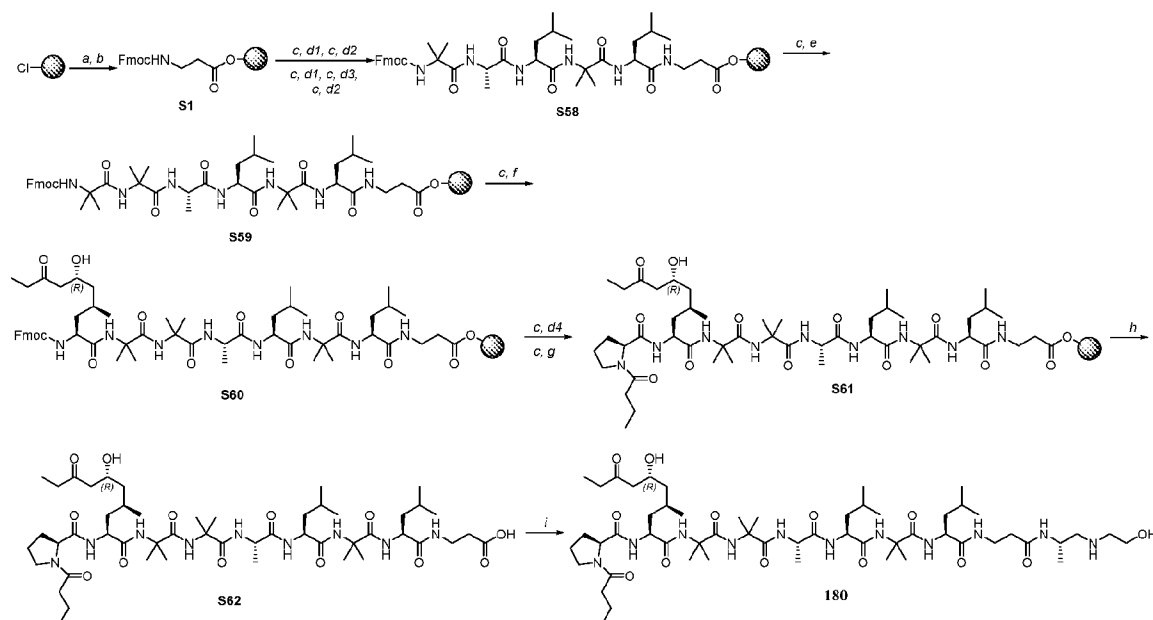


Scheme 56. Synthesis of peptide analogue **170**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-(6*R*)-AHMOD-OH, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBT, DMF, 12 h.

2.4.2 Peptide 180: ButyrylPro-AHMOD-Aib-Aib-Ala-Leu-Aib-Leu-βAla-APAE

[0581] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was

then removed using **Method 2** and Fmoc-Ala-OH was coupled according to modified **Method 3**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S59**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-(2*S*,4*S*,6*R*)-AHMOD (0.5 eq. of Fmoc-AHMOD-OH was used) was then coupled using **Method 6** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S61**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S62** as white fluffy flakes (10 mg, 10% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford **peptide 180** as a white amorphous solid (6 mg, 5% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq AHMOD building block was used); R_t 19.1 min; LRMS: *m/z* (ESI-MS) 1122.7 ([M+H]⁺ requires 1122.7).

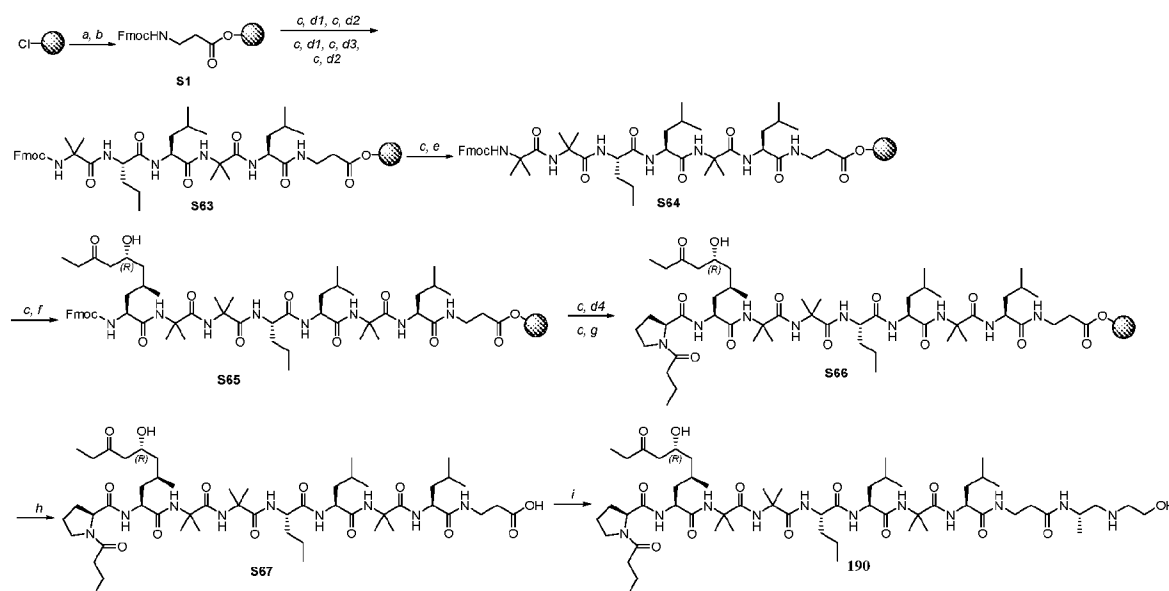


Scheme 57. Synthesis of peptide analogue **180**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Ala-OH, d4: Fmoc-Pro-OH; e) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; f) Fmoc-(6*R*)-AHMOD-OH, HATU, DIPEA, DMF, 2 h; g)

butyric acid, HATU, DIPEA, DMF, 2 × 1 h; h) HFIP/CH₂Cl₂ (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.3 Peptide 190: ButyrylPro-AHMOD-Aib-Aib-Nva-Leu-Aib-Leu-βAla-APAE

5 [0582] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotriyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-Nva-OH was coupled according to modified
10 **Method 3**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S64**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-(2*S*,4*S*,6*R*)-AHMOD (0.5 eq. of Fmoc-AHMOD-OH was used) was then coupled using **Method 6** followed by coupling of Fmoc-Pro-OH using **Method 3** and
15 butyric acid via **Method 7** to complete the linear peptide sequence of **S66**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S67** as white fluffy flakes
20 (13 mg, 12% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford **peptide 190** as a
25 white amorphous solid (8 mg, 7% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq AHMOD building block was used); R_t 20.3 min; LRMS: *m/z* (ESI-MS) 1150.9 ([M+H]⁺ requires 1150.8).

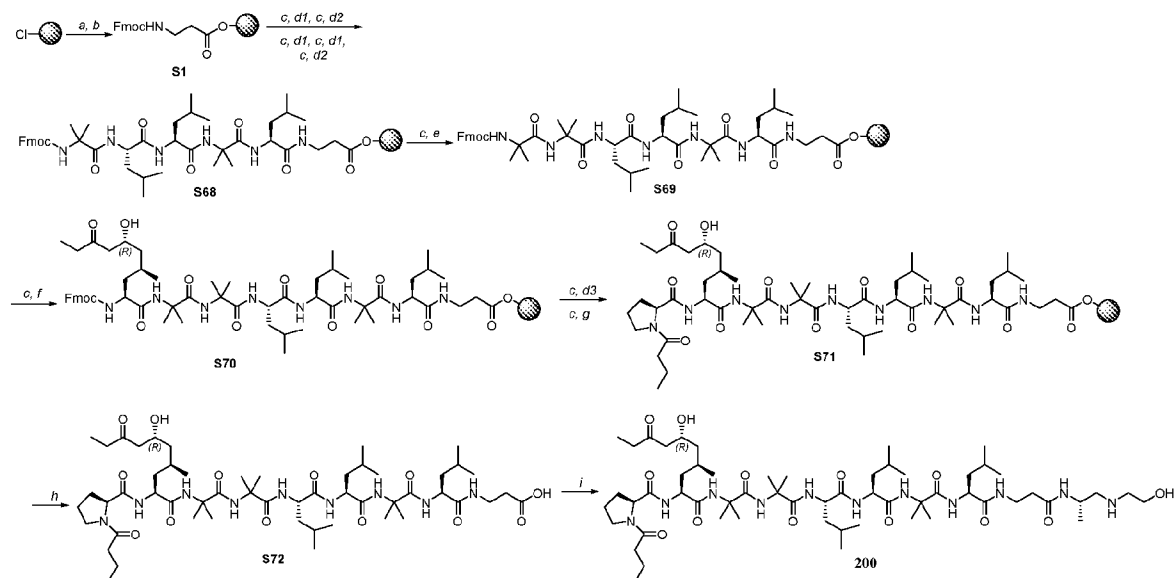


Scheme 58. Synthesis of peptide analogue **190**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Nva-OH, d4: Fmoc-Pro-OH; e) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; f) Fmoc-(6R)-AHMOD-OH, HATU, DIPEA, DMF, 2 h; g) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; h) HFIP/CH₂Cl₂ (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.4 Peptide 200: ButyrylPro-AHMOD-Aib-Aib-Leu-Leu-Aib-Leu-βAla-APAE

[0583] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-Leu-OH was coupled according to modified **Method 3**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S69**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-(2S,4S,6R)-AHMOD was then coupled using **Method 6** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S71**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S72** as white fluffy flakes (73 mg, 68% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in

the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford **peptide 200** as a white amorphous solid (43 mg, 37% overall yield, > 97% purity); R_t 20.7 min; LRMS: m/z (ESI-MS) 1164.9 ($[M+H]^+$ requires 1164.8).

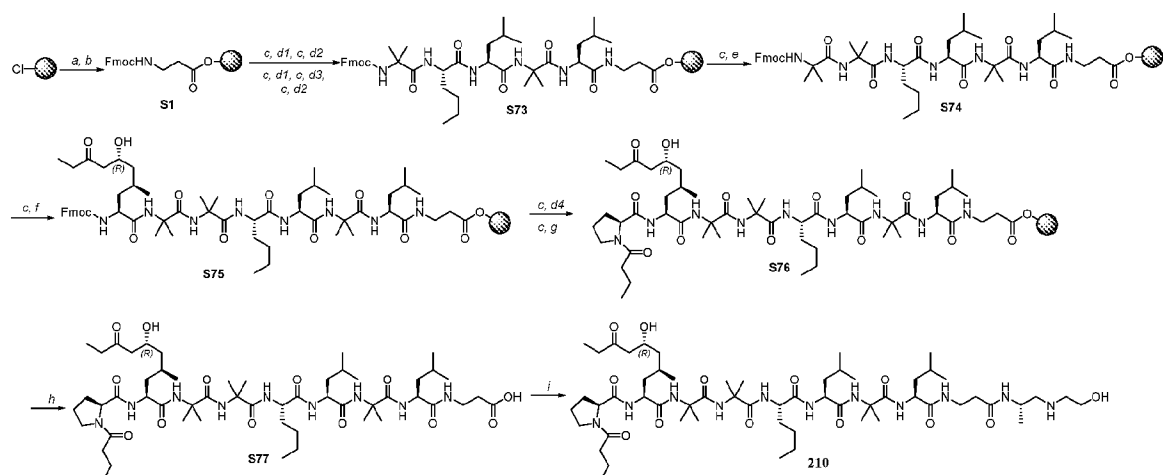


Scheme 59. Synthesis of peptide analogue **200**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; f) Fmoc-(6R)-AHMOD-OH, HATU, DIPEA, DMF, 2 h; g) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; h) HFIP/CH₂Cl₂ (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.5 Peptide 210: ButyrylPro-AHMOD-Aib-Aib-Nle-Leu-Aib-Leu-βAla-APAE

[0584] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-Nle-OH was coupled according to modified **Method 3**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S74**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-(2S,4S,6R)-AHMOD was then coupled using **Method 6** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S76**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and

RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S77** as white fluffy flakes (60 mg, 56% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **210** as a white amorphous solid (32 mg, 27% overall yield, > 98% purity); R_t 20.9 min; m/z (ESI-MS) 1164.8 ($[M+H]^+$ requires 1164.8).

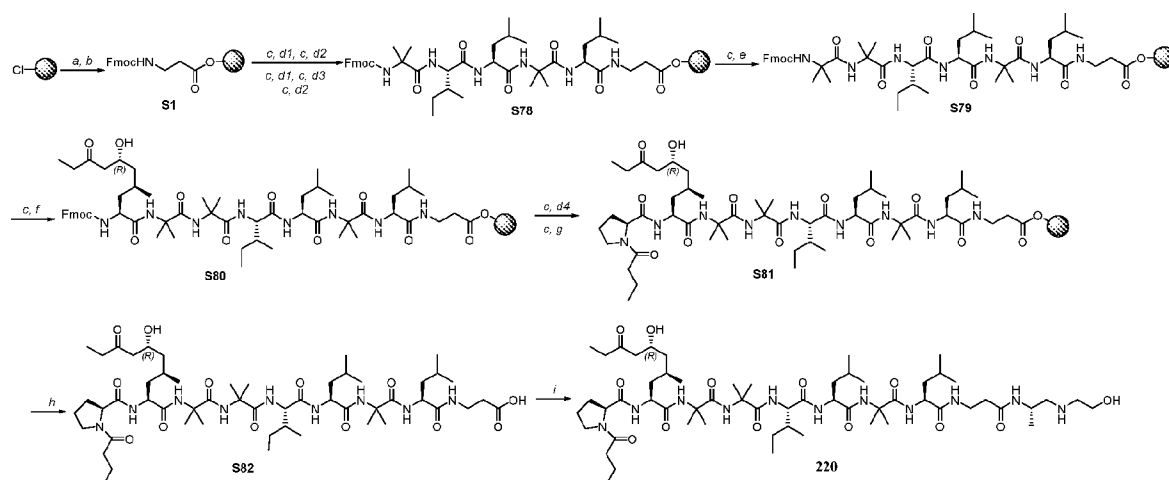


- Scheme 60.** Synthesis of peptide analogue **210**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Nle-OH, d4: Fmoc-Pro-OH; e) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; f) Fmoc-(6R)-AHMOD-OH, HATU, DIPEA, DMF, 2 h; g) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; h) HFIP/ CH_2Cl_2 (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.6 Peptide 220: ButyrylPro-AHMOD-Aib-Aib-Ile-Leu-Aib-Leu- β Ala-APAE

[0585] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotriyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^{α} -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^{α} -Fmoc-protecting group was then removed using **Method 2** and Fmoc-Ile-OH was coupled according to modified **Method 3**. The N^{α} -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S79**. After removal of N^{α} -Fmoc-protecting group using **Method 2**, Fmoc-(2S,4S,6R)-AHMOD (0.5 eq. of Fmoc-AHMOD-OH was used) was then coupled using **Method 6** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S81**. Resin-bound

peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S82** as white fluffy flakes (18 mg, 75% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford **peptide 220** as a white amorphous solid (8mg, 7% overall yield, > 98% purity, low yield attributed to deletion peptides, due to 0.5 eq AHMOD building block was); R_t 20.6 min; LRMS: m/z (ESI-MS) 1164.9 ($[M+H]^+$ requires 1164.8).



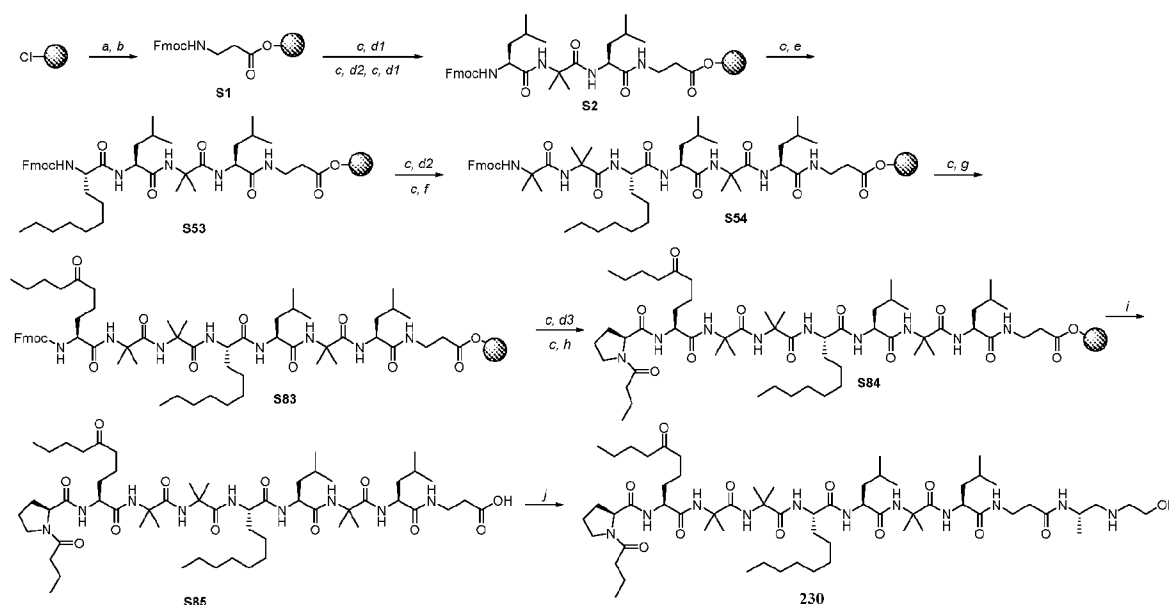
Scheme 61. Synthesis of peptide analogue **220**. **Reagents and conditions:** a) Fmoc-

β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Ile-OH, d4: Fmoc-Pro-OH; e) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; f) Fmoc-(6R)-AHMOD-OH, HATU, DIPEA, DMF, 2 h; g) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; h) HFIP/ CH_2Cl_2 (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.7 Peptide 230: ButyrylPro-A6OD-Aib-Aib-AD-Leu-Aib-Leu- β Ala-APAE

[0586] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotriyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to

form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH (0.5 eq. of Fmoc-A6OD-OH was used) was then coupled using **Method 11** followed by coupling of Fmoc-Ala-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S84**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S85** as white fluffy flakes (27 mg, 24% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **230** as a white amorphous solid (13 mg, 11% overall yield, >98% purity); *R*_t 24.8 min; LRMS: *m/z* (ESI-MS) 1190.9 ([*M*+*H*]⁺ requires 1190.8).

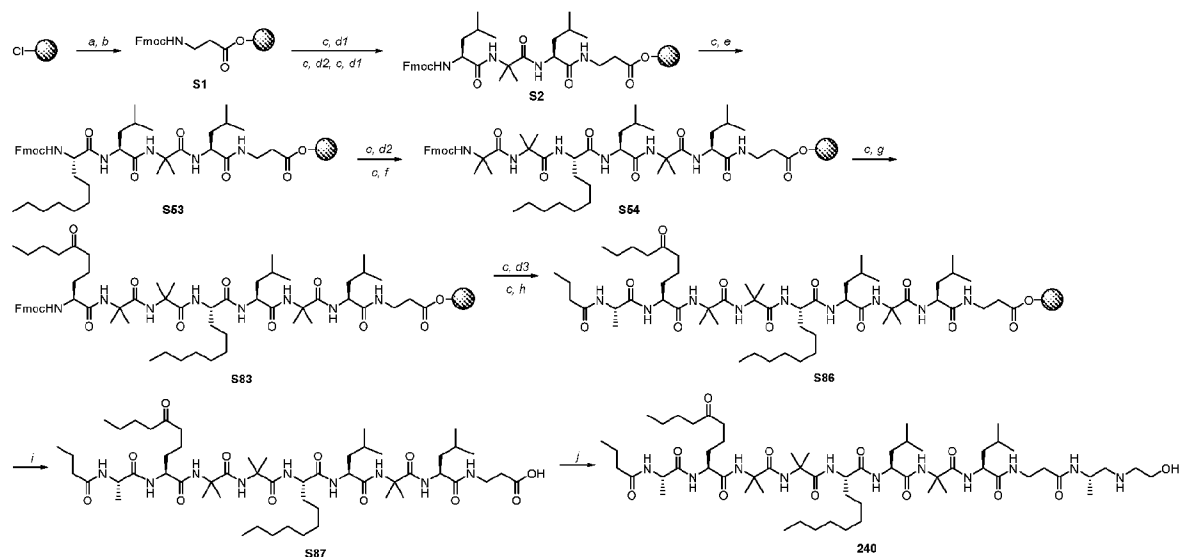


Scheme 62. Synthesis of peptide analogue **230**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.8 Peptide 240: ButyrylAla-A6OD-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0587] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**.

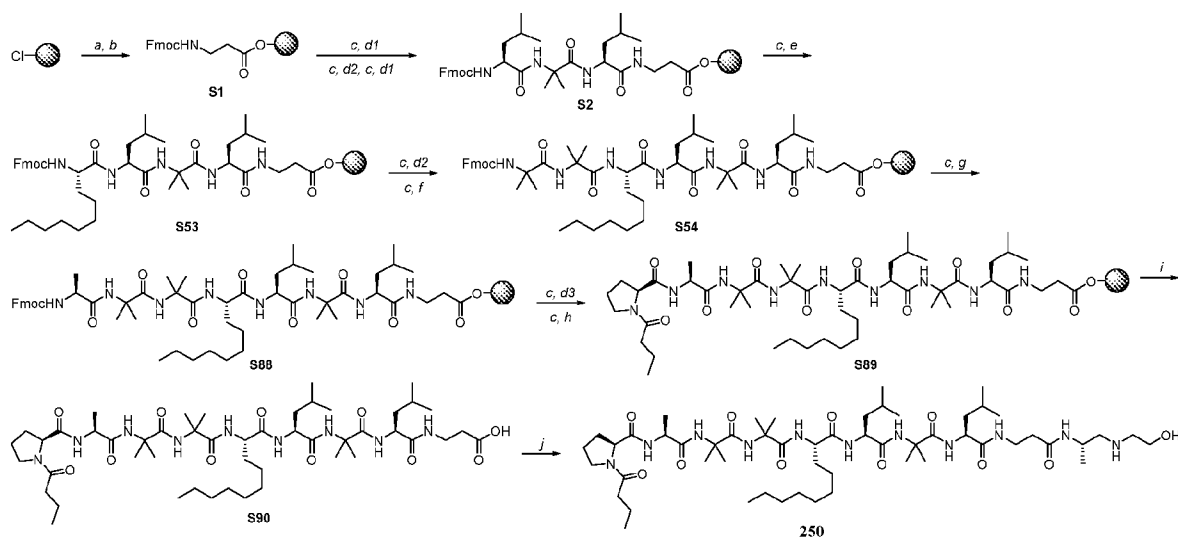
The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Ala-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S86**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S87** as white fluffy flakes (31 mg, 29% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **240** as a white amorphous solid (25 mg, 21% overall yield, >98% purity); R_t 18.5 min; LRMS: *m/z* (ESI-MS) 1164.6 ([M+H]⁺ requires 1164.8).



Scheme 63. Synthesis of peptide analogue **240**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Ala-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.9 Peptide 250: ButyrylPro-Ala-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0588] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to **Method 5**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Ala-OH and Fmoc-Pro-OH were then coupled using **Method 3** followed by butyric acid via **Method 7** to complete the linear peptide sequence of **S89**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S90** as white fluffy flakes (84 mg, 86% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide analogue **250** as a white amorphous solid (46 mg, 47% overall yield, > 98% purity); R_t 17.7 min; LRMS: *m/z* (ESI-MS) 1078.4 ([M+H]⁺ requires 1078.8).

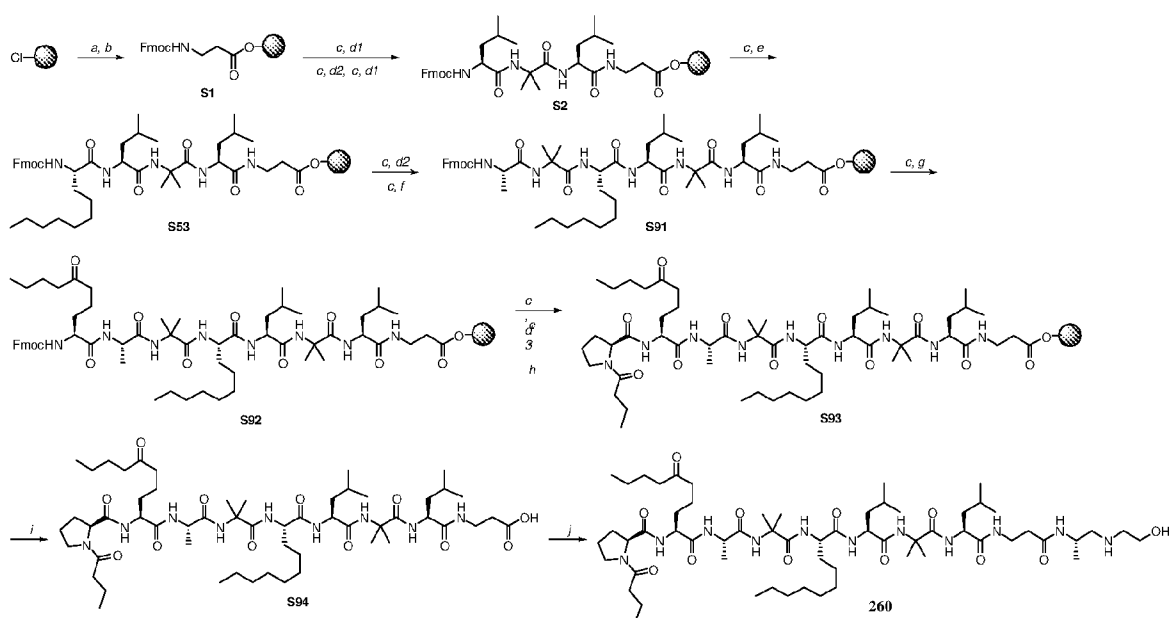


Scheme 64. Synthesis of peptide analogue **250**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Ala-OH,

HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.10 Peptide 260: ButyrylPro-A6OD-Ala-Aib-AD-Leu-Aib-Leu-βAla-APAE

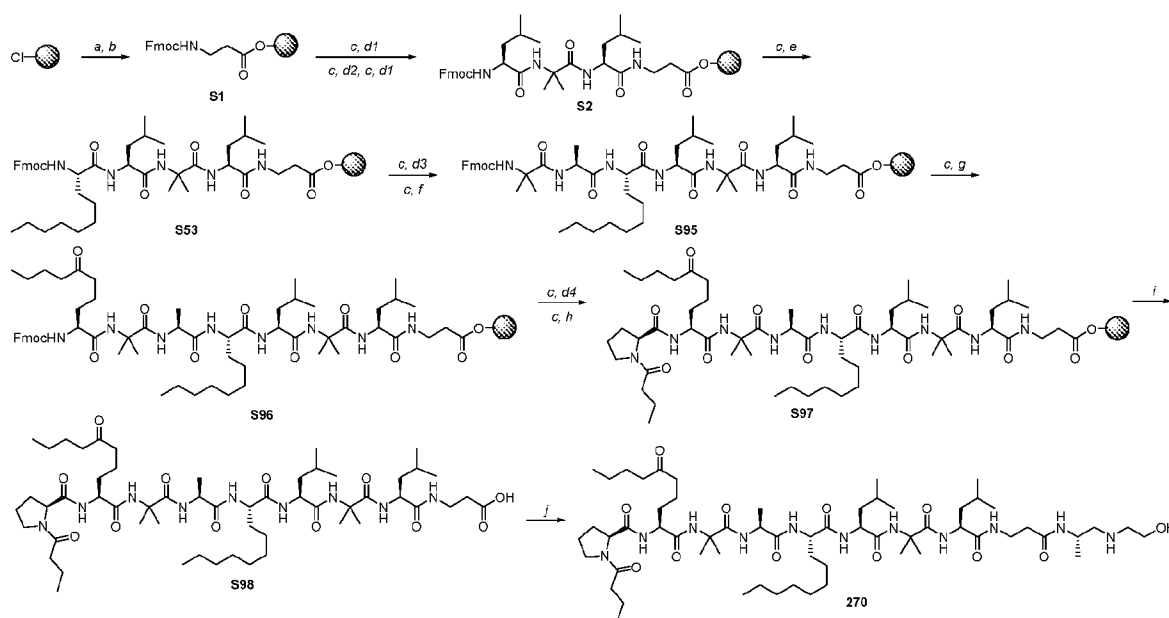
- 5 **[0589]** The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The
- 10 N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of Fmoc-Ala-OH via **Method 4** to form peptidyl resin **S91**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S93**.
- 15 Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S94** as white fluffy flakes (51 mg, 47% yield). Late-stage solution phase C-terminal coupling was employed to
- 20 introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **260** as a white amorphous solid (19 mg, 16% overall yield, > 98% purity); R_t 19.7 min; LRMS: *m/z* (ESI-MS) 1176.6 ([M+H]⁺ requires 1176.8).
- 25



Scheme 65. Synthesis of peptide analogue **260**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH₂Cl₂, 1 $\times$ 6 h, 1 $\times$ 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Ala-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.11 Peptide 270: ButyrylPro-A6OD-Aib-Ala-AD-Leu-Aib-Leu- β Ala-APAE

- 10 **[0590]** The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotriptyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin S1. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The
- 15 N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Ala-OH via **Method 3**, and subsequently the coupling of Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S95**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S97**.
- 20 Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S98** as white fluffy flakes (24.7 mg, 23% yield). Late-stage solution phase C-terminal coupling was employed to
- 25 introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **270** as a white amorphous solid (10 mg, 9% overall yield, > 98% purity); R_t 24.0 min;
- 30 LRMS: *m/z* (ESI-MS) 1176.7 ([M+H]⁺ requires 1176.8).

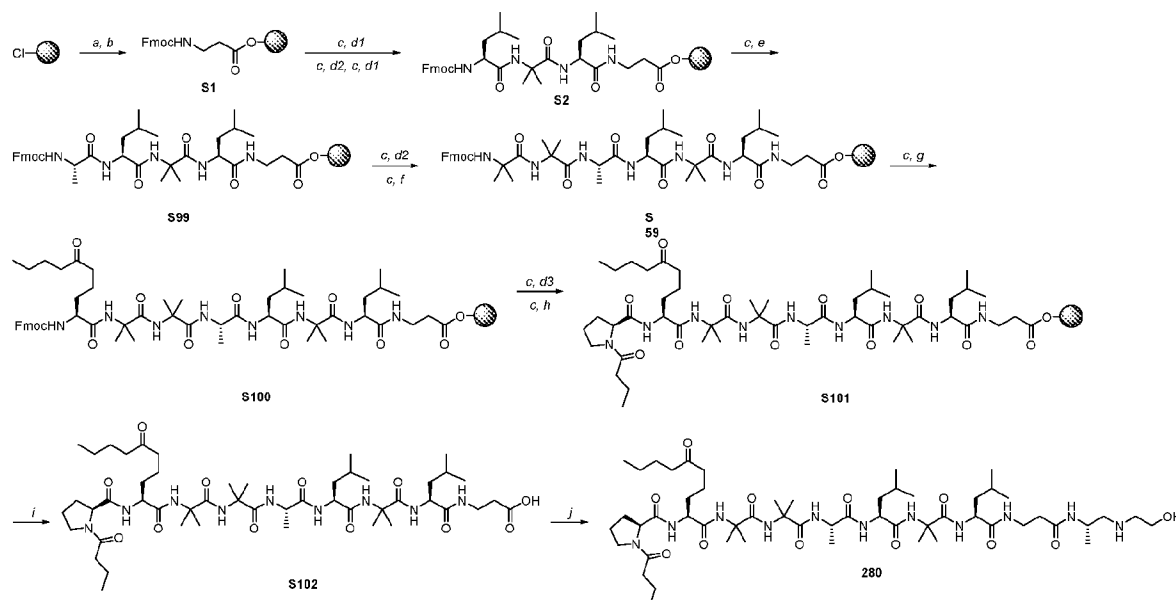


Scheme 66. Synthesis of peptide analogue **270**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Ala-OH d4: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.12 Peptide **280**: ButyrylPro-A6OD-Aib-Aib-Ala-Leu-Aib-Leu-βAla-APAE

[0591] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-Ala-OH was coupled according to **Method 3**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S59**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S101**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S102** as white fluffy flakes (39 mg, 39% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude

peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **280** as a white amorphous solid (22 mg, 20% overall yield, >98% purity); R_t 16.9 min; LRMS: m/z (ESI-MS) 1092.6 ($[M+H]^+$ requires 1092.7).

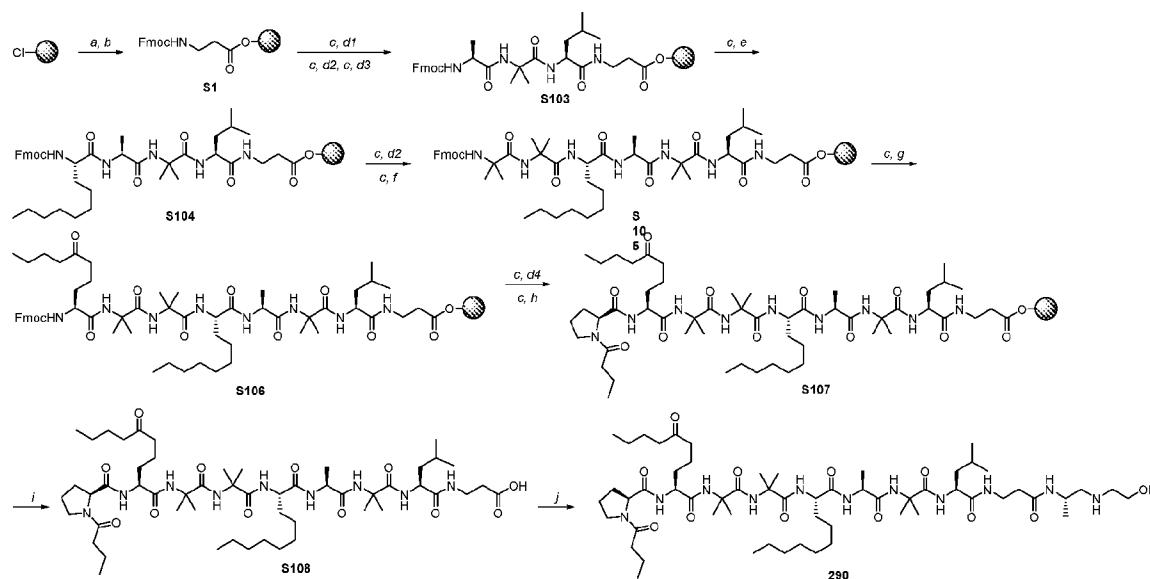


Scheme 67. Synthesis of peptide analogue **280**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-Ala-OH, HATU, DIPEA, DMF, 2 × 1 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.13 Peptide 290: ButyrylPro-A6OD-Aib-Aib-AD-Ala-Aib-Leu-βAla-APAE

[0592] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Ala-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S105**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S107**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method.

Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S108** as white fluffy flakes (16.5 mg, 32% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **290** as a white amorphous solid (15 mg, 26% overall yield, 83% purity); R_t 18.0 min; LRMS: m/z (ESI-MS) 1148.6 ($[M+H]^+$ requires 1148.8).

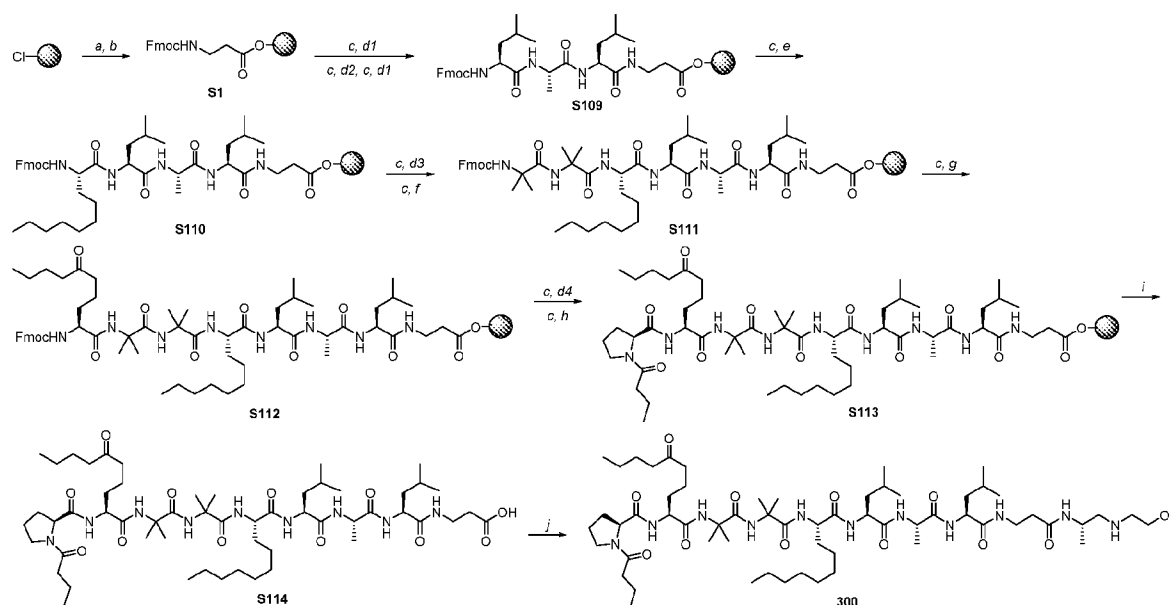


Scheme 68. Synthesis of peptide analogue **290**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Ala-OH, d4: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBT, DMF, 12 h.

2.4.14 Peptide 300: ButyrylPro-A6OD-Aib-Aib-AD-Leu-Ala-Leu- β Ala-APAE

[0593] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Ala-OH and Fmoc-Leu-OH via **Method 3**. The N^α -Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^α -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S111**. After removal of N^α -Fmoc-protecting group using **Method 2**,

Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S113**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S114** as white fluffy flakes (26 mg, 24% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **300** as a white amorphous solid (8 mg, 7% overall yield, 95% purity); R_t 23.6 min; LRMS: m/z (ESI-MS) 1176.7 ($[M+H]^+$ requires 1176.8).

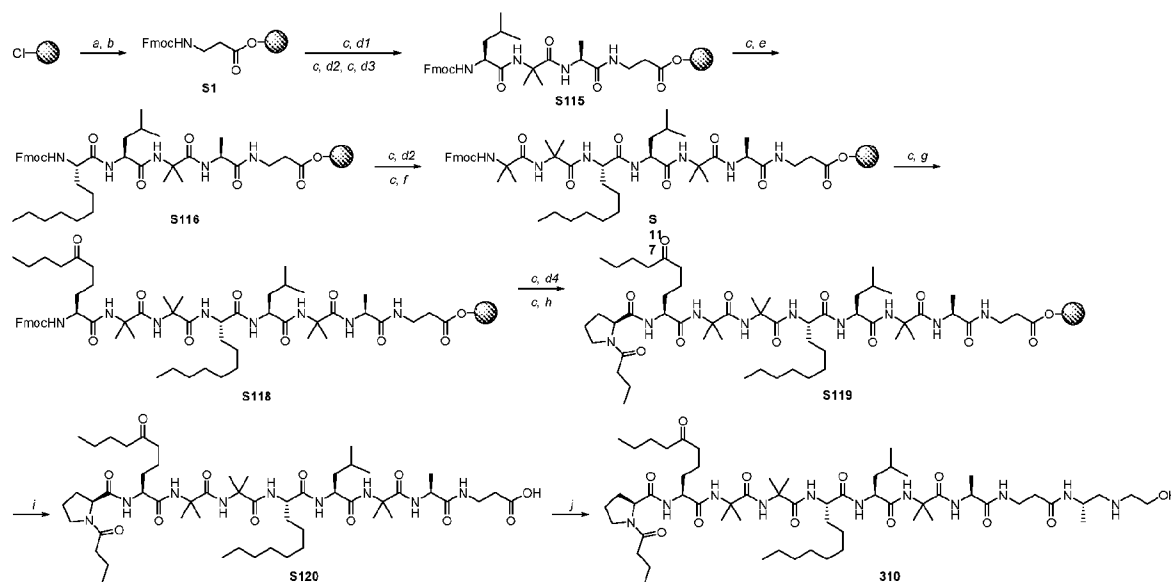


Scheme 69. Synthesis of peptide analogue **300**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Ala-OH, d3: Fmoc-Aib-OH, d4: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-A6OD-OH, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBT, DMF, 12 h.

2.4.15 Peptide 310: ButyrylPro-A6OD-Aib-Aib-AD-Leu-Aib-Ala-APAE

[0594] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Ala-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was

then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S117**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S119**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S120** as white fluffy flakes (23.4 mg, 22% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **310** as a white amorphous solid (15 mg, 13% overall yield, 96% purity); R_t 21.9 min; LRMS: *m/z* (ESI-MS) 1148.7 ([M+H]⁺ requires 1148.8).

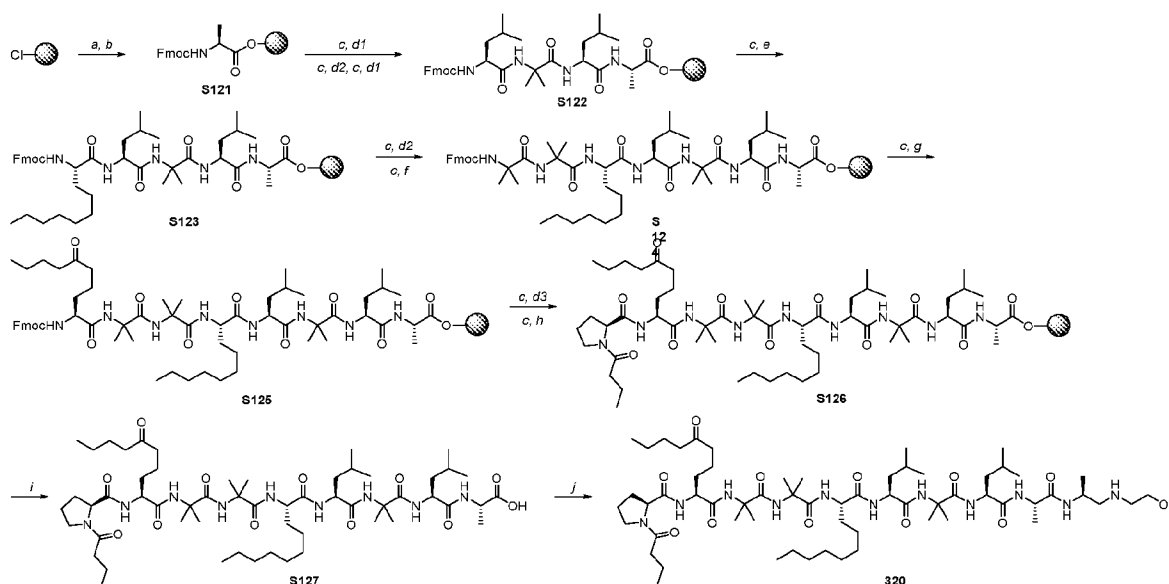


Scheme 70. Synthesis of peptide analogue **310**. **Reagents and conditions:** a) Fmoc-

β Ala-OH, DIPEA, CH₂Cl₂, 1 $\times$ 6 h, 1 $\times$ 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Ala-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Leu-OH, d4: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.16 Peptide 320: ButyrylPro-A6OD-Aib-Aib-AD-Leu-Aib-Leu-Ala-APAE

[0595] The C-terminal residue Fmoc-Ala-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S121**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S124**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S126**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S127** as white fluffy flakes (24.6 mg, 23% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **320** as a white amorphous solid (11 mg, 9% overall yield, > 98% purity); *R*_t 20.3 min; LRMS: *m/z* (ESI-MS) 1190.6 ([*M*+*H*]⁺ requires 1190.9).

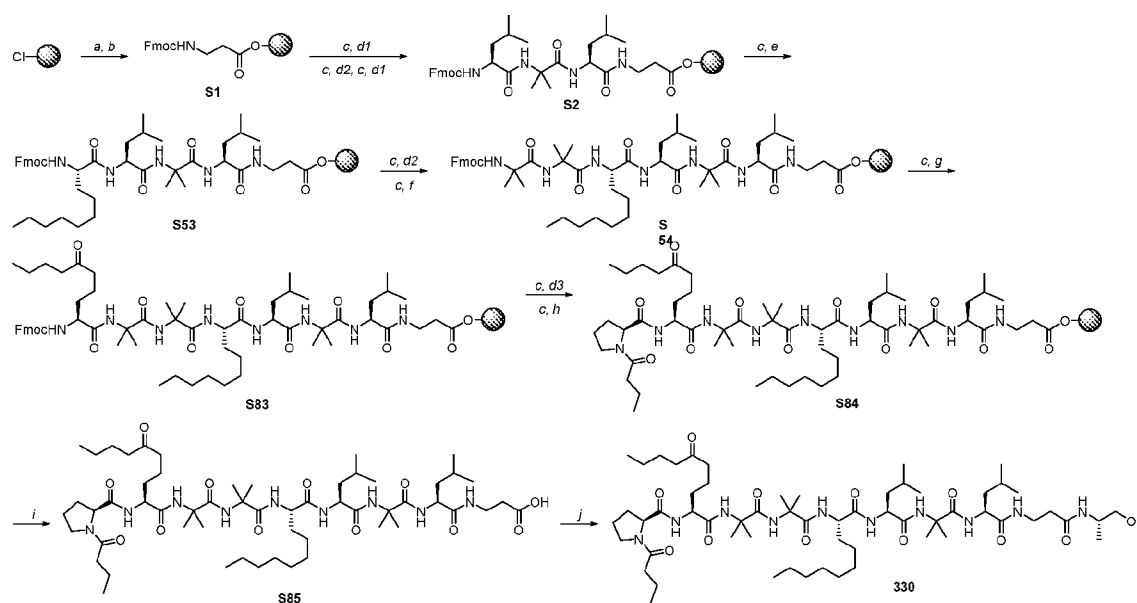


Scheme 71. Synthesis of peptide analogue **320**. **Reagents and conditions:** a) Fmoc-Ala-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma,

DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.17 Peptide 330: ButyrylPro-A6OD-Aib-Aib-AD-Leu-Aib-Leu-βAla-Alaol

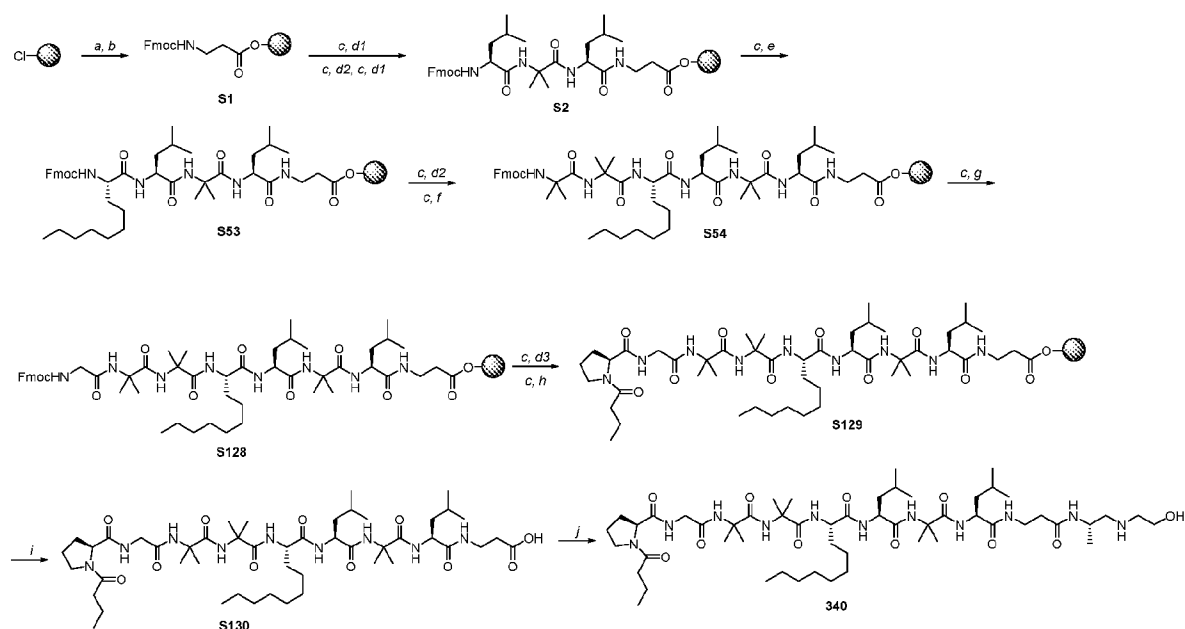
[0596] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S84**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S85** as white fluffy flakes (22 mg, 20% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal L-alaninol moiety using **Method 12**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **330** as a white amorphous solid (10.9 mg, 10% overall yield, >95% purity); R_t 21.3 min; LRMS: *m/z* (ESI-MS) 1147.6 ([M+H]⁺ requires 1147.8).



Scheme 72. Synthesis of peptide analogue **330**. *Reagents and conditions:* a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) L-alaninol, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.18 Peptide 340: ButyrylPro-Gly-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

- [0597]** The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Gly-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S129**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S130** as white fluffy flakes (62 mg, 64% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 12**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **340** as a white amorphous solid (22 mg, 20% overall yield, >98% purity); R_t 23.4 min; LRMS: *m/z* (ESI-MS) 1064.8 ([M+H]⁺ requires 1064.7).

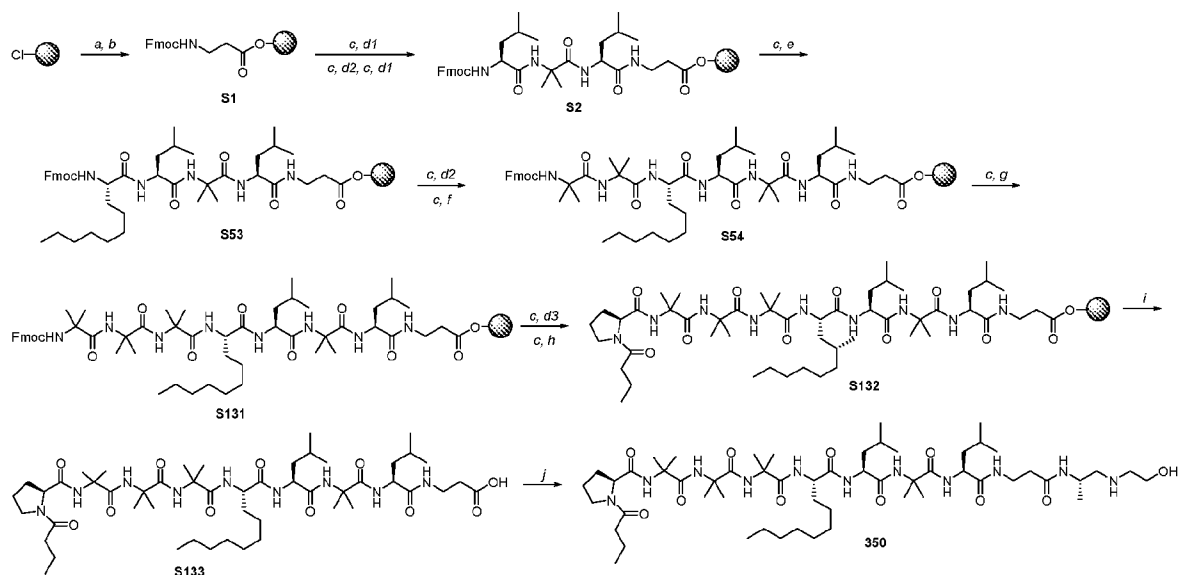


Scheme 73. Synthesis of peptide analogue **340**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Gly-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.19 Peptide 350: ButyrylPro-Aib-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0598] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Aib-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S132**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S133** as white fluffy flakes (60 mg, 56% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 12**. The crude peptide was

purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **350** as a white amorphous solid (34 mg, 29% overall yield, >98% purity); R_t 20.9 min; LRMS: m/z (ESI-MS) 1164.8 ($[M+H]^+$ requires 1164.7).

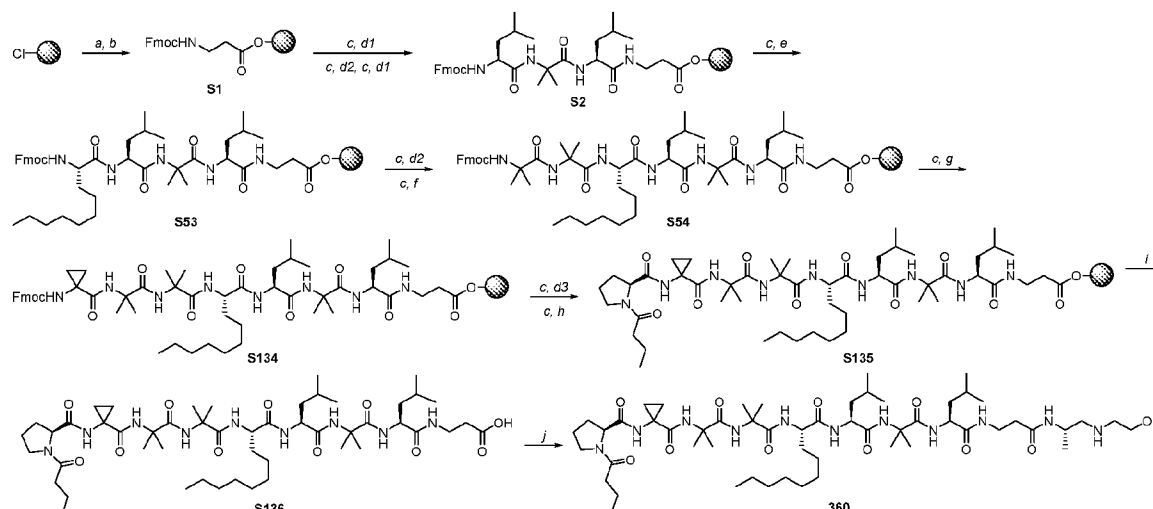


Scheme 74. Synthesis of peptide analogue **350**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Aib-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.20 Peptide 360: ButyrylPro-Acc-Aib-Aib-AD-Leu-Aib-Leu- β Ala-APAE

[0599] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Acc-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S135**. Resin-bound peptide was cleaved using **Method 8**, and the crude peptide **S136** was isolated after lyophilisation as white fluffy flakes (120 mg, 93% purity). Crude **S136** was

carried forward without further purification. Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **360** as a white amorphous solid (81 mg, 74% overall yield, >97% purity); R_t 17.7 min; LRMS: m/z (ESI-MS) 1090.5 ($[M+H]^+$ requires 1090.8).



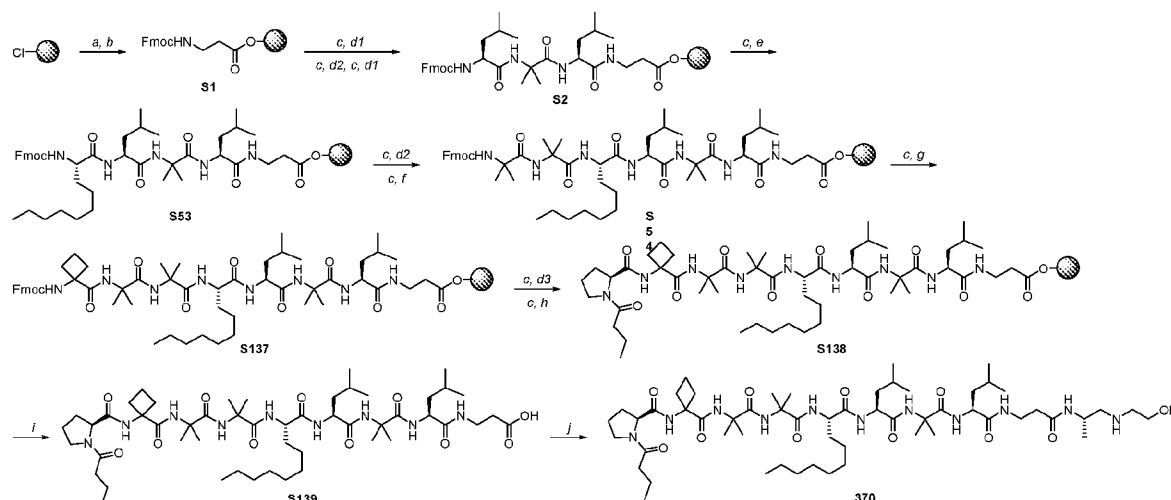
Scheme 75. Synthesis of peptide analogue **360**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Aib-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.21 Peptide 370: ButyrylPro-Acb-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0600] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Acb-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S138**. Resin-bound peptide was cleaved using **Method 8**, and the crude peptide **S139** was isolated after lyophilisation as white fluffy flakes (100 mg, crude). Crude **S139** was carried

forward without further purification. Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC.

5 Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **370** as a white amorphous solid (19.6 mg, 18% overall yield, > 98% purity); R_t 18.8 min; LRMS: m/z (ESI-MS) 1104.5 ($[M+H]^+$ requires 1104.8).



Scheme 76. Synthesis of peptide analogue **370**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Acb-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

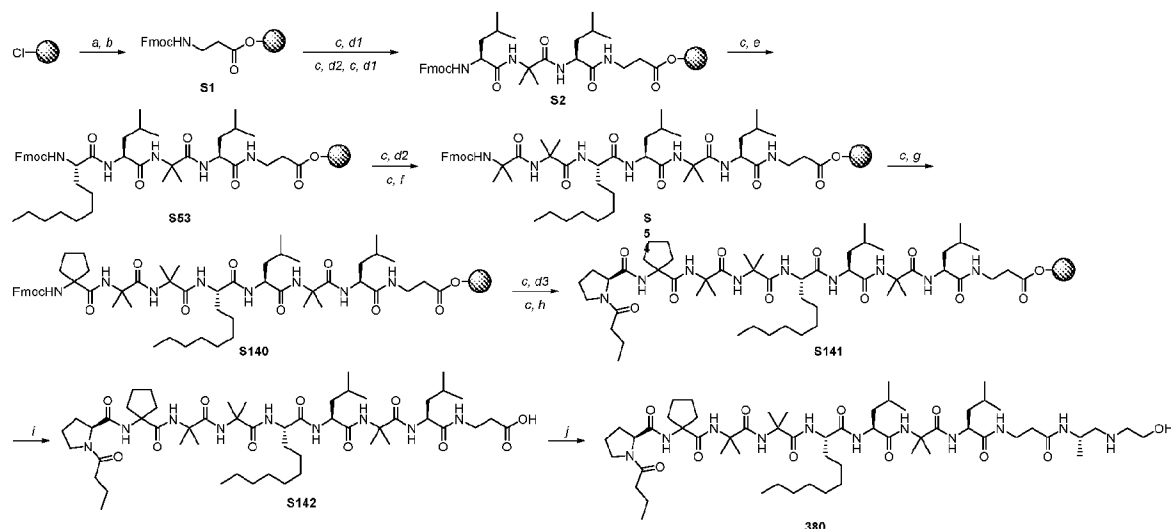
2.4.22 Peptide 380: ButyrylPro-Acp-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0601] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**.

20 The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to

25 form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Acp-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S141**. Resin-bound peptide was cleaved using **Method 8**, and the crude peptide **S142** was isolated after lyophilisation as white fluffy flakes (100 mg, crude). Crude **S142** was carried

forward without further purification. Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **380** as a white amorphous solid (21.3 mg, 19% overall yield, 92% purity); R_t 19.1 min; LRMS: m/z (ESI-MS) 1118.6 ($[M+H]^+$ requires 1118.8).

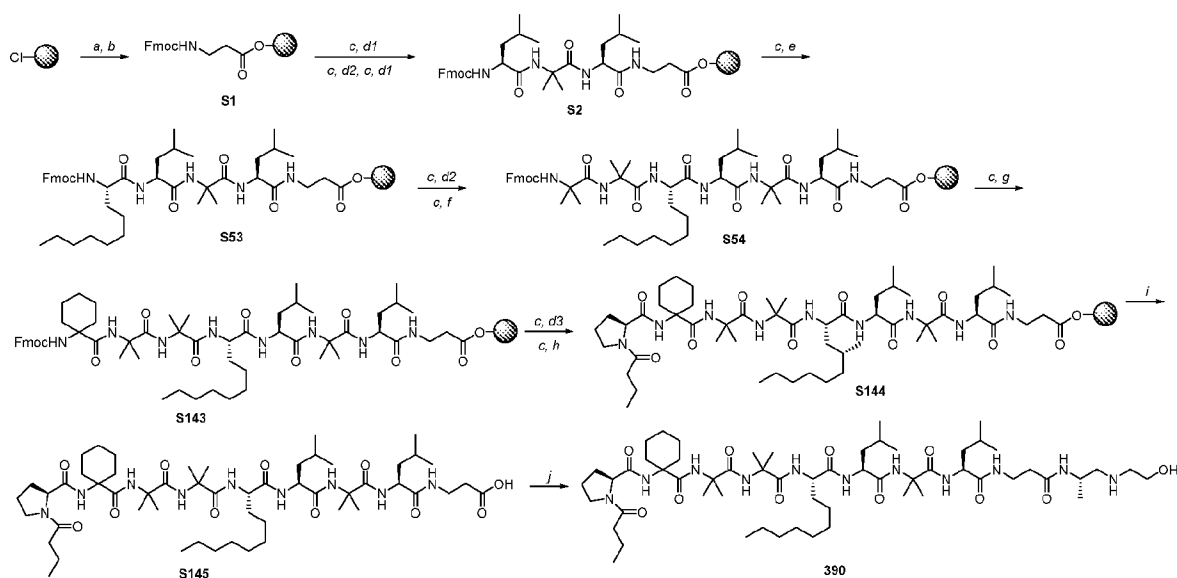


Scheme 77. Synthesis of peptide analogue **380**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Acp-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.23 Peptide 390: ButyrylPro-Ahc-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0602] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Ahc-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S144**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by

semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S145** as white fluffy flakes (58 mg, 55% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 12**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **390** as a white amorphous solid (34mg, 30% overall yield, >97% purity); R_t 26.4 min; LRMS: m/z (ESI-MS) 1132.9 ($[M+H]^+$ requires 1132.8).

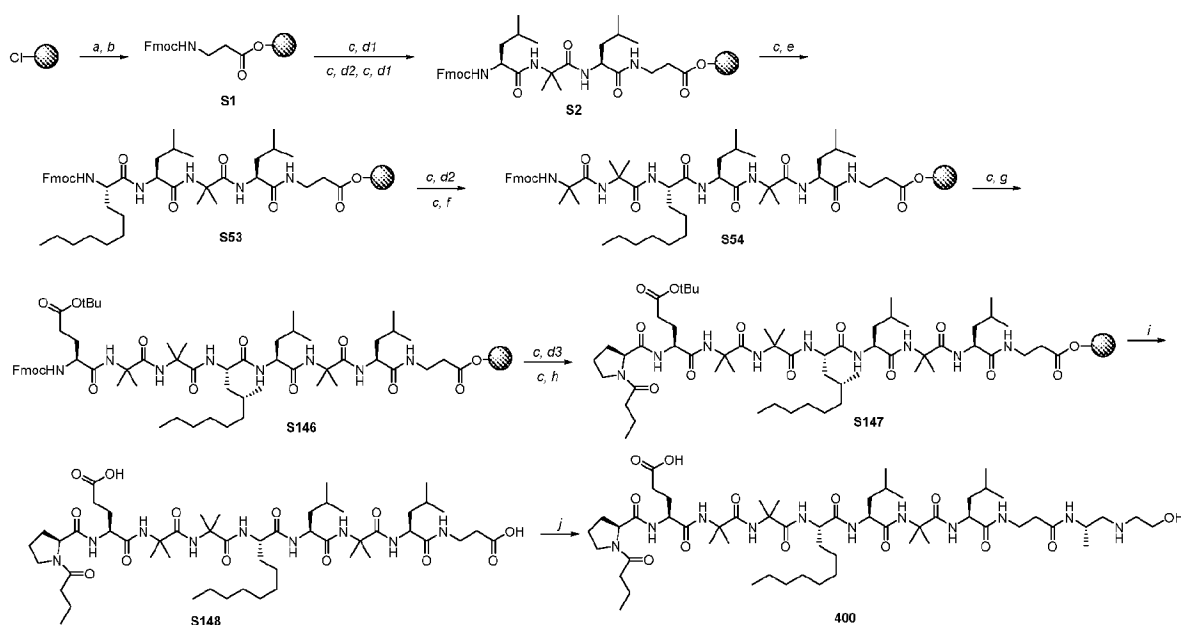


Scheme 78. Synthesis of peptide analogue **390**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1×6 h, 1×12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1×5 min, 1×15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2×1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2×2 h; g) Fmoc-Ahc-OH, HATU, DIPEA, DMF, 2×1 h; h) butyric acid, HATU, DIPEA, DMF, 2×1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.24 Peptide 400: ButyrylPro-Glu-Aib-Aib-AD-Leu-Aib-Leu- β Ala-APAE

[0603] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotriyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to

form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Glu(OtBu)-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S147**. Resin-bound peptide was cleaved using **Method 9**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S148** as white fluffy flakes (51 mg, 49% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **400** as a white amorphous solid (25 mg, 22% overall yield, >98% purity); *R*_t 21.2 min; LRMS: *m/z* (ESI-MS) 1136.8 ([M+H]⁺ requires 1136.7).

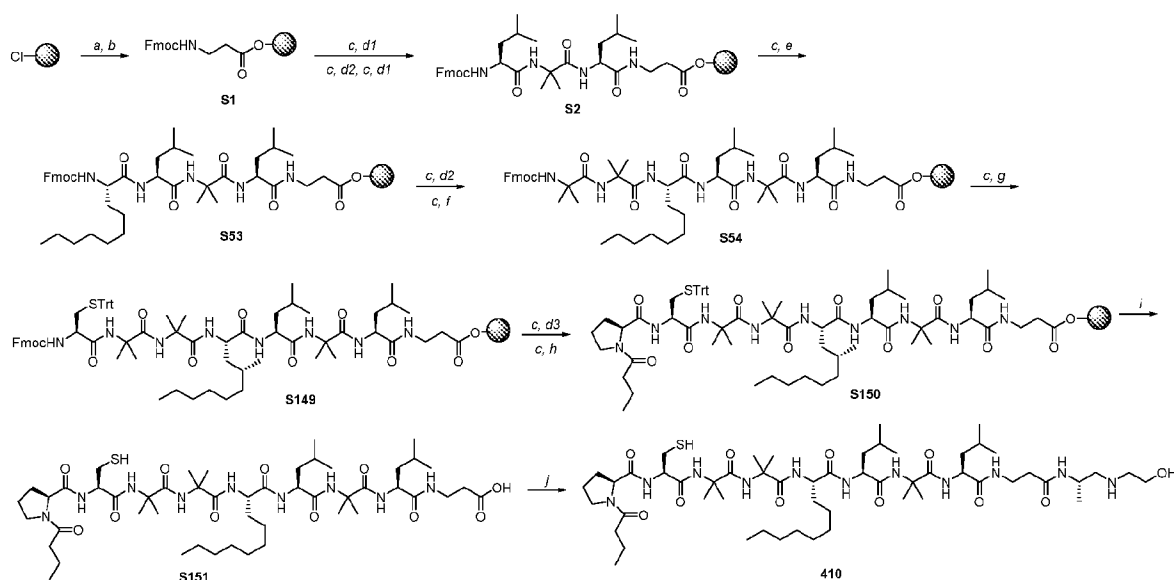


Scheme 79. Synthesis of peptide analogue **400**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Glu(OtBu)-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) TFA/TIPS/H₂O (95:2.5:2.5, v/v/v) for 2 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.25 Peptide 410: ButyrylPro-Cys-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0604] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-

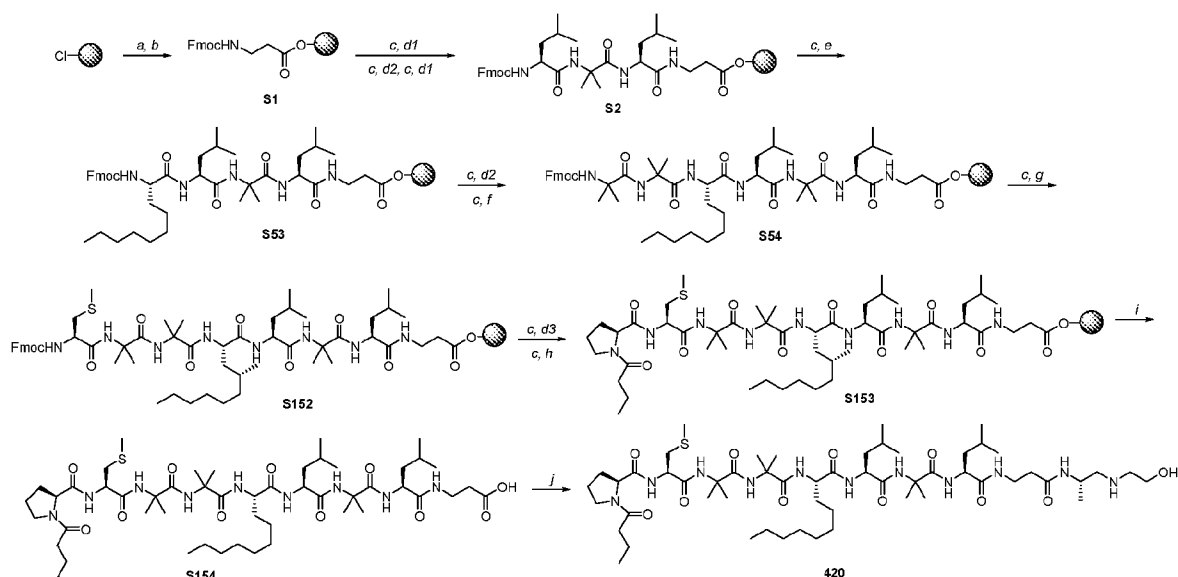
Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Cys(Trt)-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S150**. Resin-bound peptide was cleaved using **Method 9**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S151** as white fluffy flakes (61 mg, 55% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **410** as a white amorphous solid (42 mg, 38% overall yield, >98% purity); R_t 22.6 min; LRMS: *m/z* (ESI-MS) 1110.5 ([M+H]⁺ requires 1110.7).



Scheme 80. Synthesis of peptide analogue **410**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH₂Cl₂, 1 $\times$ 6 h, 1 $\times$ 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Cys(Trt)-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) TFA/TIPS/H₂O (95:2.5:2.5, v/v/v) for 2 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.26 Peptide 420: ButyrylPro-Met-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0605] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Met-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S153**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S154** as white fluffy flakes (49 mg, 47% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **420** as a white amorphous solid (28 mg, 24% overall yield, >98% purity); *R*_t 23.2 min; LRMS: *m/z* (ESI-MS) 1138.5 ([*M*+*H*]⁺ requires 1138.7).



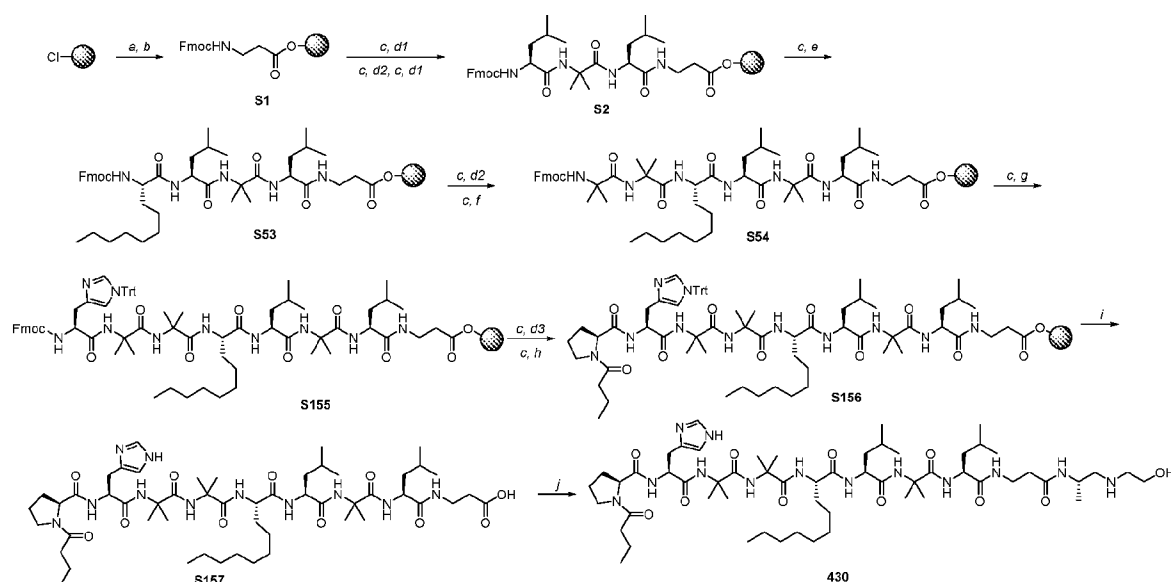
Scheme 81. Synthesis of peptide analogue **420**. **Reagents and conditions:** a) Fmoc-

βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma,

DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Met-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

5 2.4.27 Peptide 430: ButyrylPro-His-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

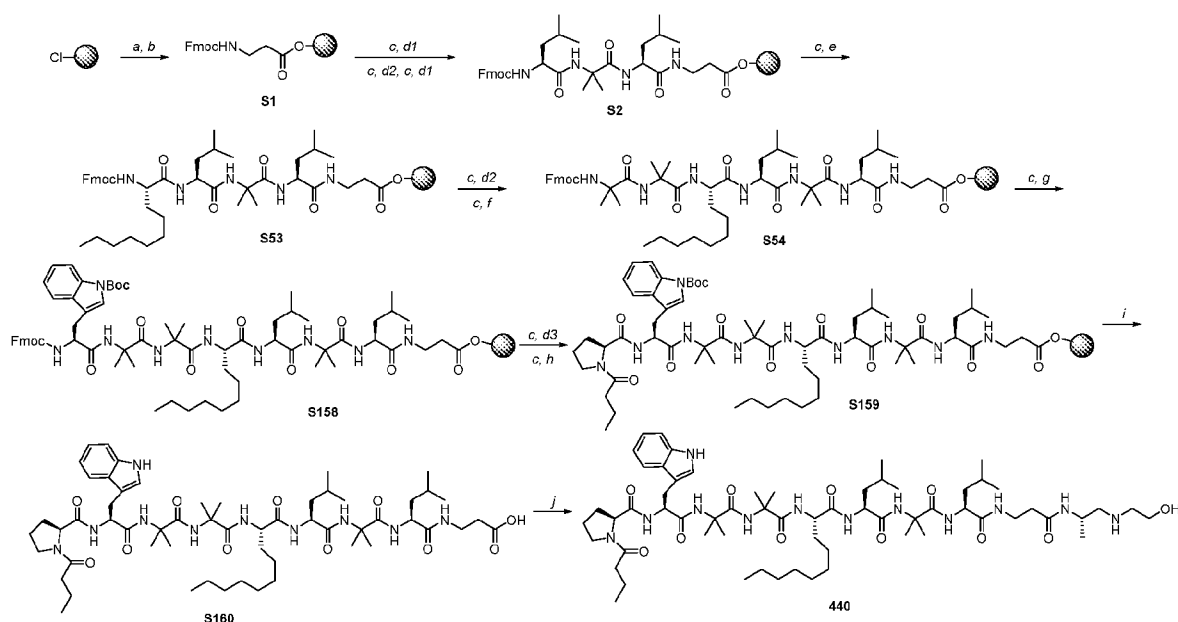
[0606] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-His(Trt)-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S156**. Resin-bound peptide was cleaved using **Method 9**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S157** as white fluffy flakes (43 mg, 41% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **430** as a white amorphous solid (21mg, 18% overall yield, >98% purity); R_t 18.6 min; LRMS: *m/z* (ESI-MS) 1144.6 ([M+H]⁺ requires 1144.7).



Scheme 82. Synthesis of peptide analogue **430**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH₂Cl₂, 1 $\times$ 6 h, 1 $\times$ 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-His(Trt)-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) TFA/TIPS/H₂O (95:2.5:2.5, v/v/v) for 2 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.28 Peptide **440**: ButyrylPro-Trp-Aib-Aib-AD-Leu-Aib-Leu- β Ala-APAE

[0607] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Trp(Boc)-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S159**. Resin-bound peptide was cleaved using **Method 9**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S160** as white fluffy flakes (48 mg, 44% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **440** as a white amorphous solid (25 mg, 20% overall yield, >98% purity); R_t 26.7 min; LRMS: m/z (ESI-MS) 1193.8 ([M+H]⁺ requires 1193.8).

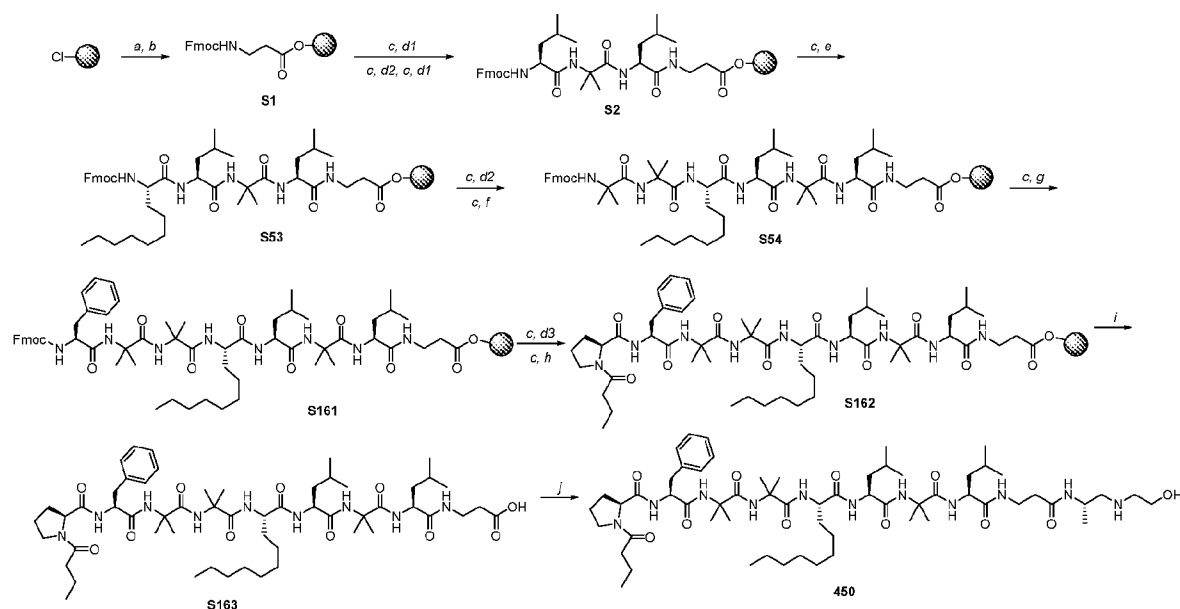


Scheme 83. Synthesis of peptide analogue **440**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Trp(Boc)-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) TFA/TIPS/H₂O (95:2.5:2.5, v/v/v) for 2 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.29 Peptide 450: ButyrylPro-Phe-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0608] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotriyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Phe-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S162**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S163** as white fluffy flakes (51 mg, 48% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was

purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **450** as a white amorphous solid (28 mg, 24% overall yield, >97% purity); R_t 24.1 min; LRMS: m/z (ESI-MS) 1154.5 ($[M+H]^+$ requires 1154.8).

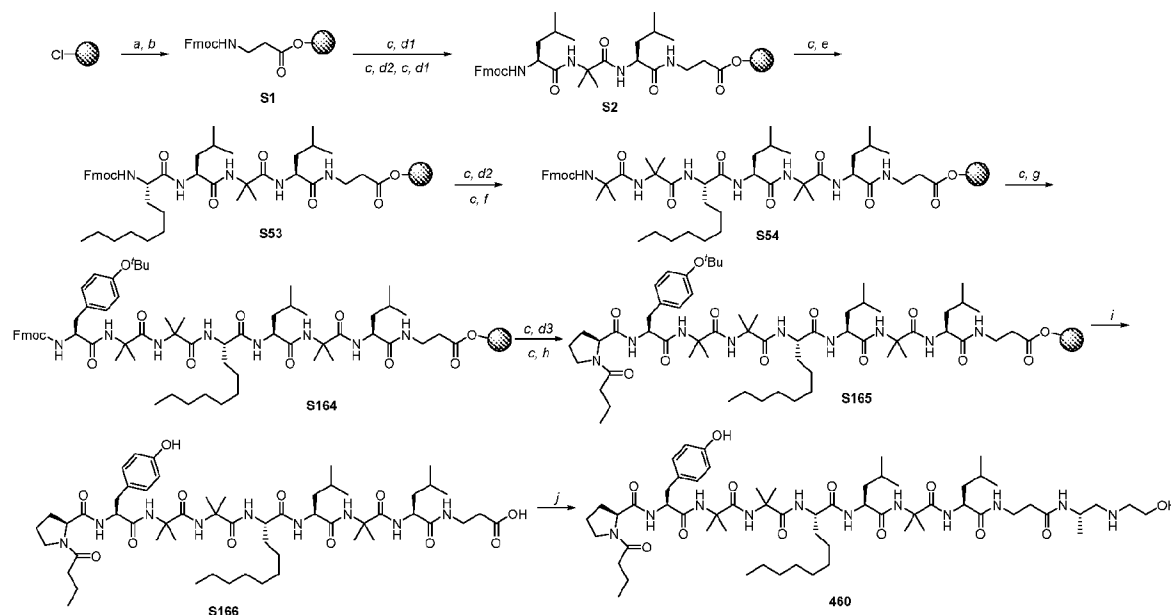


Scheme 84. Synthesis of peptide analogue **450**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Phe-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.30 Peptide **460**: ButyrylPro-Tyr-Aib-Aib-AD-Leu-Aib-Leu- β Ala-APAE

[0609] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N $^\alpha$ -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N $^\alpha$ -Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N $^\alpha$ -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N $^\alpha$ -Fmoc-protecting group using **Method 2**, Fmoc-Tyr(tBu)-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S165**. Resin-bound peptide was cleaved using **Method 9**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method.

Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S166** as white fluffy flakes (47 mg, 43% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **460** as a white amorphous solid (20 mg, 17% overall yield, >97% purity); R_t 23.2 min; LRMS: m/z (ESI-MS) 1170.6 ($[M+H]^+$ requires 1170.8).

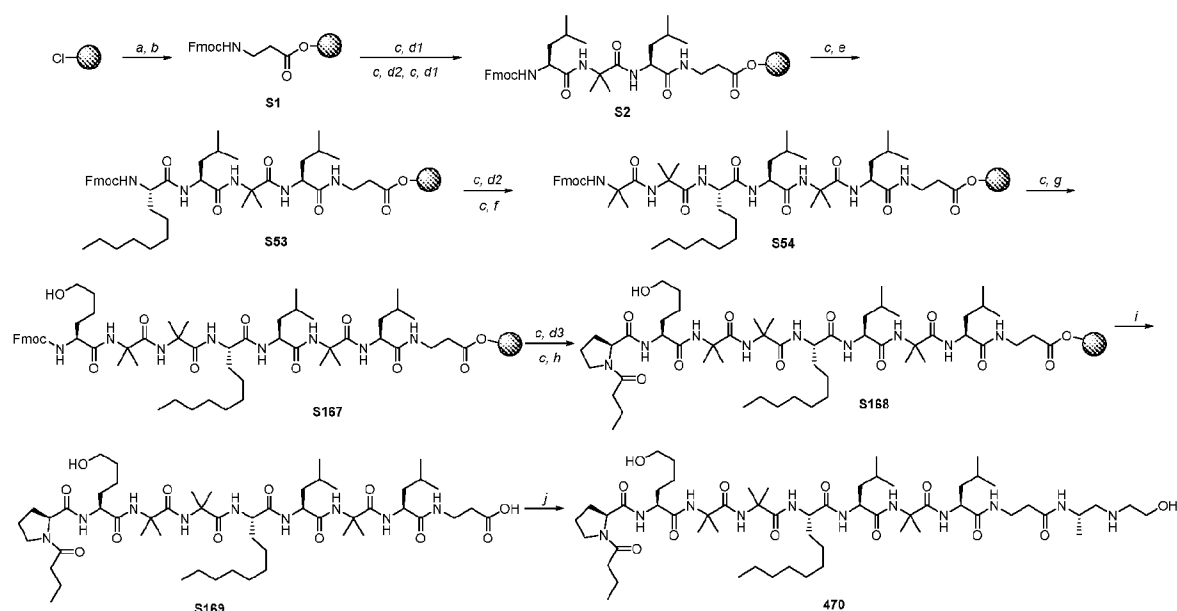


Scheme 85. Synthesis of peptide analogue 460. Reagents and conditions: a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1×6 h, 1×12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1×5 min, 1×15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2×1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2×2 h; g) Fmoc-Tyr(tBu)-OH, HATU, DIPEA, DMF, 2×1 h; h) butyric acid, HATU, DIPEA, DMF, 2×1 h; i) TFA/TIPS/ H_2O (95:2.5:2.5, v/v/v) for 2 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.31 Peptide 470: ButyrylPro-Nle(6OH)-Aib-Aib-AD-Leu-Aib-Leu- β Ala-APAE

[0610] The C-terminal residue Fmoc- β -alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to

form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-L-Nle(6-OH)-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S168**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S169** as white fluffy flakes (21 mg, 20% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 12**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **470** as a white amorphous solid (12 mg, 10% overall yield, >97% purity); *R*_t 21.1 min; LRMS: *m/z* (ESI-MS) 1136.9 ([*M*+*H*]⁺ requires 1136.8).

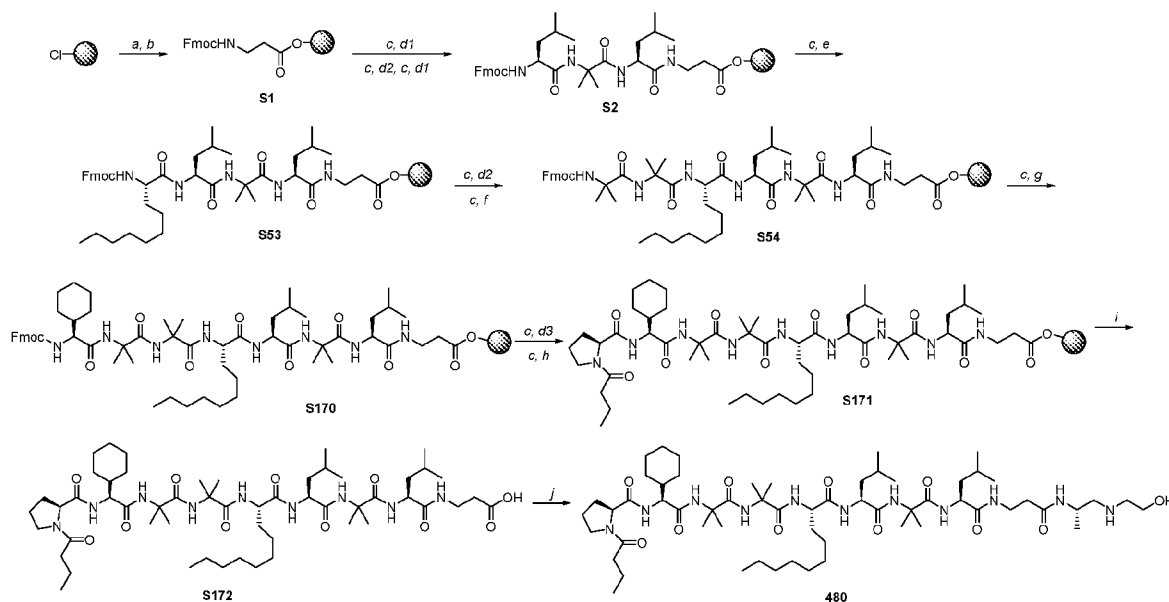


Scheme 86. Synthesis of peptide analogue **470**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-L-Nle(6-OH)-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.32 Peptide 480: ButyrylPro-Chg-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0611] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**.

The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-L-Chg-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S171**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S172** as white fluffy flakes (58 mg, 55% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **480** as a white amorphous solid (24 mg, 20% overall yield, >97% purity); *R*_t 26.9 min; LRMS: *m/z* (ESI-MS) 1146.9 ([*M*+*H*]⁺ requires 1146.8).

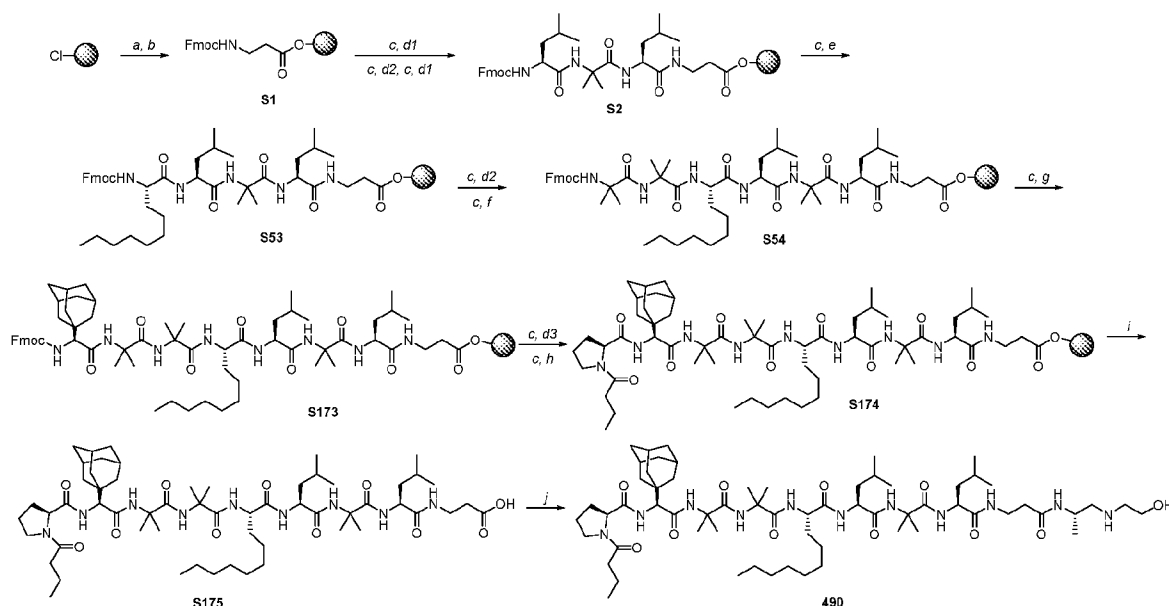


Scheme 87. Synthesis of peptide analogue **480**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-L-Chg-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.33 Peptide 490: ButyrylPro-Adg-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0612] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**.

The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Adg-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S174**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S175** as white fluffy flakes (72 mg, 65% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **490** as a white amorphous solid (31 mg, 25% overall yield, >96% purity); *R*_t 25.5 min; LRMS: *m/z* (ESI-MS) 1198.6 ([*M*+*H*]⁺ requires 1198.7).



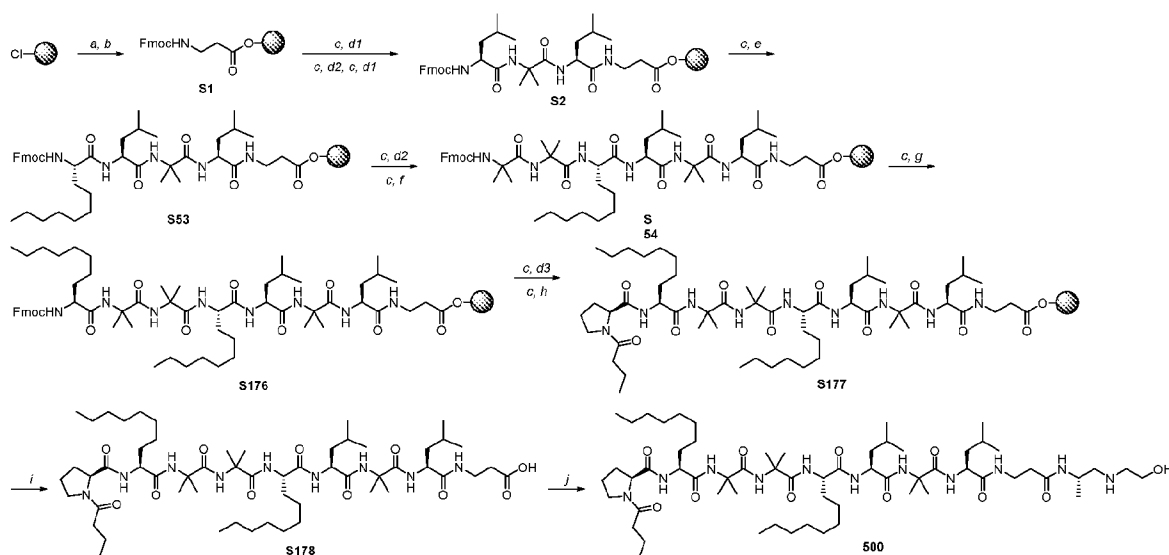
Scheme 88. Synthesis of peptide analogue **490**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h,

d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Adg-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

5

2.4.34 Peptide 500: ButyrylPro-AD-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0613] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotriptyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-AD-OH was then coupled using **Method 5** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S177**. Resin-bound peptide was cleaved using **Method 8**, and the crude peptide **S178** was isolated after lyophilisation as white fluffy flakes (120 mg, 94% purity). Crude **S178** was carried forward without further purification. Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **500** as a white amorphous solid (48 mg, 41% overall yield, > 97% purity); R_t 20.7 min; LRMS: *m/z* (ESI-MS) 1176.7 ([M+H]⁺ requires 1176.9).



Scheme 89. Synthesis of peptide analogue **500**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c)

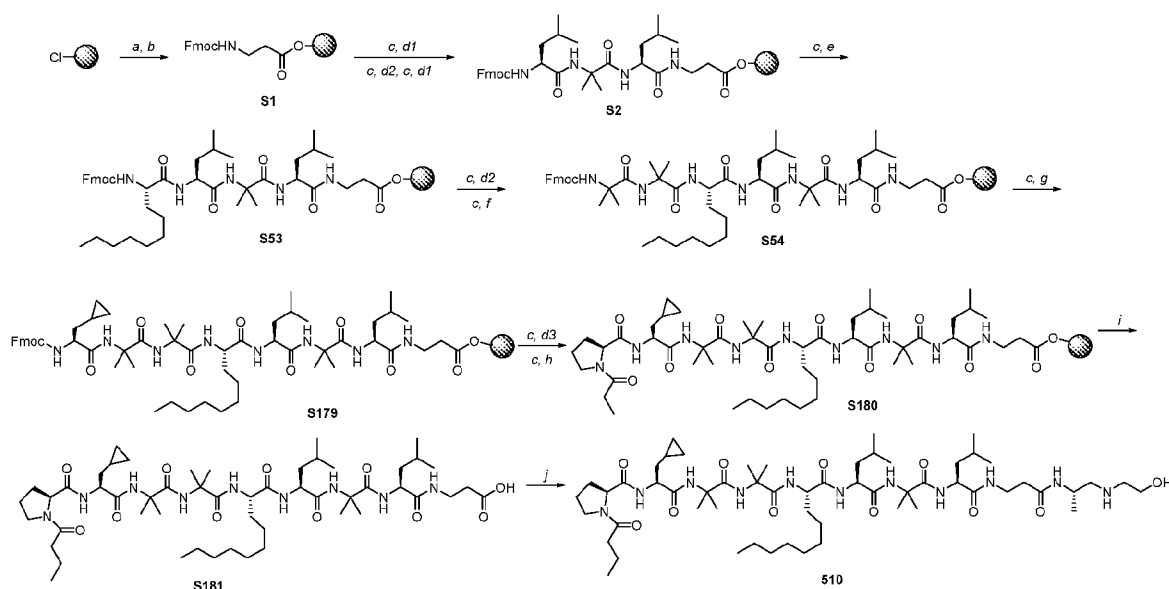
20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i)

5 HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.35 Peptide 510: ButyrylPro-Cpa-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0614] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**.

10 The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to
15 form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Cpa-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S180**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method.
20 Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S181** as white fluffy flakes (51 mg, 50% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general
25 method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the peptide analogue **510** as a white amorphous solid (27 mg, 23% overall yield, >97% purity); R_t 25.9 min; LRMS: *m/z* (ESI-MS) 1118.9 ([M+H]⁺ requires 1118.8).

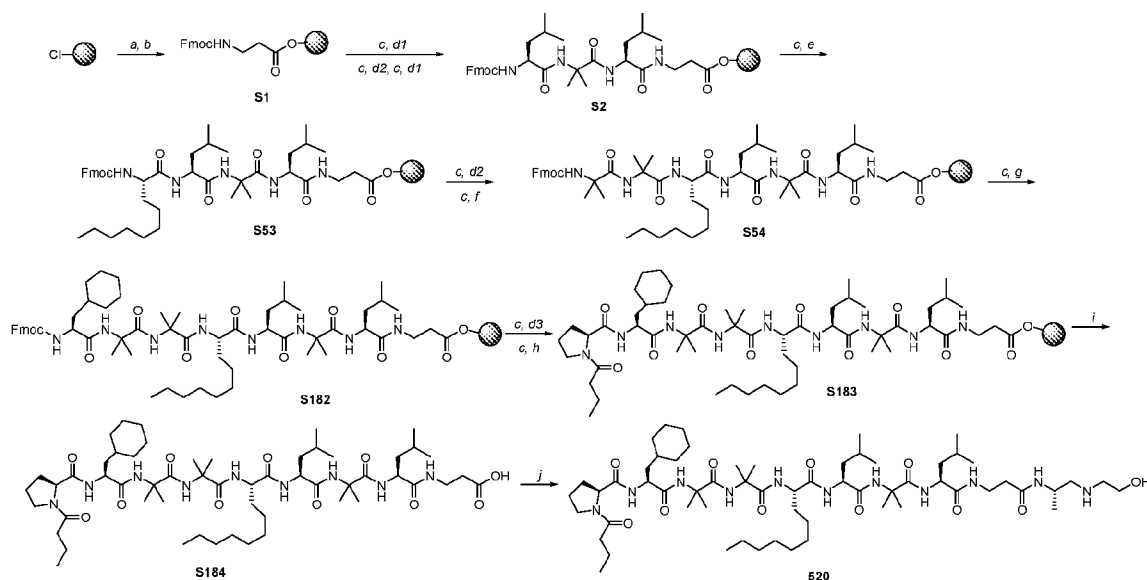


Scheme 90. Synthesis of peptide analogue **510**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cpa-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.4.36 Peptide 520: ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE

[0615] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotriyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S183**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S184** as white fluffy flakes (68 mg, 64% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general

method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford the peptide analogue **520** as a white amorphous solid (43 mg, 37% overall yield, >97% purity); R_t 24.6 min; LRMS: m/z (ESI-MS) 1160.6 ($[M+H]^+$ requires 1160.8).



Scheme 91. Synthesis of peptide analogue **520**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

2.5 Biological evaluation of peptide analogues:

[0616] Analogues were assessed for antiproliferative activity against three breast cancer cell lines (MDA-MB-468, SKBR3, and T47D), as well as a non-small cell lung cancer line (NCI-H460).

[0617] Cells were treated with 3-fold serial dilutions of the peptides and maintained under drug exposure for 5 days. Monitoring of cell proliferation and cell viability was performed by using sulphorhodamine B-based assay as previously described. The IC_{50} was determined by interpolation as the drug concentration reducing staining to 50% of controls on the same plate.

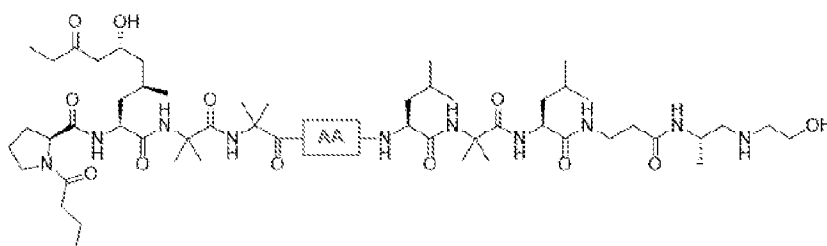
Replacement of the AMD residue:

[0618] To assess the effect that the AMD-5 residue has on antiproliferative activity, analogues **170** – **220** were prepared (Table 2). Based on the results of the antiproliferative

testing shown in Table 2, 2-aminodecanoic acid was chosen as a replacement for AMD in the synthesis of further analogues.

[0619] Table 2. Antiproliferative activity of analogues **170** – **220**, modified at AMD-5. Note the same cutoffs of >0.5, >1, >10 μM are used in this table as for Table 1.

Analogues of structure:



Entry	Compound/ analogue number	Activity against cell line ($\text{IC}_{50} \pm \text{SEM}$)			
		MDA-MB-468	SKBR3	T47D	NCI-H460
1	(culicinin D) 1	0.008 ± 0.001	0.032 ± 0.003	0.005 ± 0.001	0.005 ± 0.001
2	170	0.004 ± 0.001	0.025 ± 0.003	0.003 ± 0.001	0.008 ± 0.001
3	180	>1	>30	>1	>10
4	190	0.274 ± 0.053	>1	0.420 ± 0.174	>1
5	200	0.090 ± 0.0004	>1	0.130 ± 0.023	0.496 ± 0.116
6	210	0.090 ± 0.020	>1	0.124 ± 0.028	>0.5
7	220	0.142 ± 0.015	>1	0.251 ± 0.027	>0.5

5

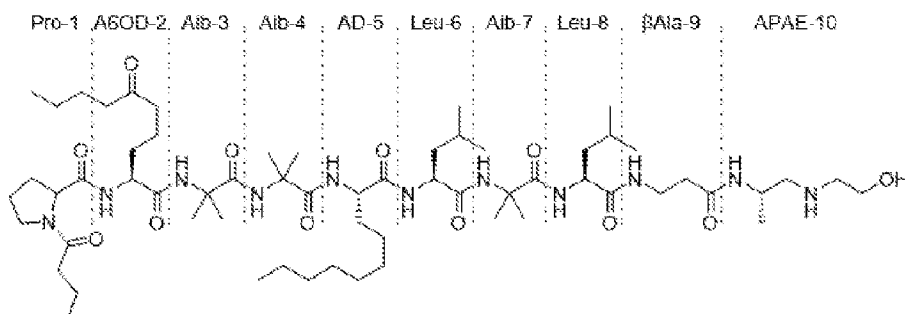
Alanine scan:

[0620] To facilitate rapid synthesis of useful quantities of analogues, a simplified culicinin D analogue **230** was prepared using (2S)-amino-6-oxo-decanoic acid (A6OD-2) as AHMOD replacement and 2-aminodecanoic acid to replace the AMD residue. A systematic alanine scan of all 10 residues in **230** was then undertaken to assess the effect of the other residues on antiproliferative activity (Table 3).

10

Table 3. Antiproliferative activity of alanine-scan analogues **230** – **330**.

Parent structure (**230**):



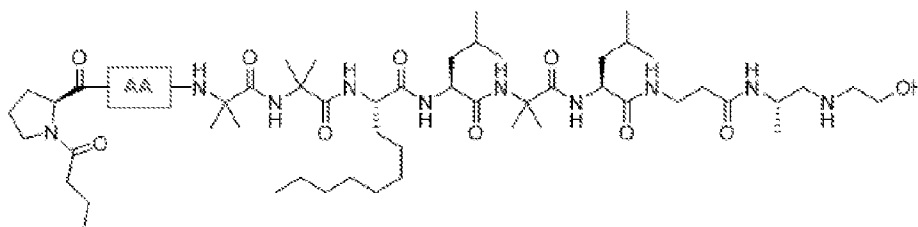
Entry	Analogue	Activity against cell line (μM , $\text{IC}_{50} \pm \text{SEM}$)			
		MDA-MB-468	SKBR3	T47D	H460
1	Parent, 230	0.02 ± 0.001	0.047 ± 0.001	0.011 ± 0.002	0.018 ± 0.003
2	Ala-1, 240	0.007 ± 0.0001	0.018 ± 0.0042	0.006 ± 0.001	0.005 ± 0.002
3	Ala-2, 250	0.068 ± 0.008	0.343 ± 0.034	0.061 ± 0.009	0.071 ± 0.001
4	Ala-3, 260	0.042 ± 0.002	0.075 ± 0.019	0.031 ± 0.002	0.044 ± 0.019
5	Ala-4, 270	0.021 ± 0.003	0.024 ± 0.010	0.027 ± 0.003	0.020 ± 0.004
6	Ala-5, 280	>1	>1	>1	>1
7	Ala-6, 290	0.066 ± 0.001	0.130 ± 0.007	0.051 ± 0.005	0.103 ± 0.029
8	Ala-7, 300	>1	>1	>1	0.266 ± 0.074
9	Ala-8, 310	0.314 ± 0.069	>1	0.101 ± 0.007	0.096 ± 0.064
10	Ala-9, 320	0.026 ± 0.002	0.036 ± 0.003	0.021 ± 0.0003	0.016 ± 0.006
11	Alaol-10, 330	>0.5	>1	>0.5	0.291 ± 0.021

[0621] APAE-10 was replaced with L-alaninol (analogue **330**), rather than alanine, to better mimic the parent aminoalcohol C-terminal residue.

[0622] Next, analogues where AHMOD/A6OD was replaced with a variety of amino acids were examined for activity (Table 4).

Table 4. Antiproliferative activity of analogues **340** – **520**.

Analogues of structure:



Entry	Analogue number	Activity against cell line ($\text{IC}_{50} \pm \text{SEM}$)			
		MDA-MB-468	SKBR3	T47D	H460
1	170	0.004 ± 0.001	0.025 ± 0.003	0.003 ± 0.001	0.008 ± 0.001
2	230	0.02 ± 0.001	0.047 ± 0.001	0.011 ± 0.002	0.018 ± 0.003
3	340	0.076 ± 0.010	0.310 ± 0.034	0.087 ± 0.008	0.133 ± 0.015
4	250	0.068 ± 0.008	0.343 ± 0.034	0.061 ± 0.009	0.071 ± 0.001
5	350	0.048 ± 0.003	0.150 ± 0.0002	0.029 ± 0.001	0.057 ± 0.002
6	360	0.064 ± 0.007	0.236 ± 0.011	0.057 ± 0.009	0.087 ± 0.001
7	370	0.016 ± 0.002	0.068 ± 0.007	0.017 ± 0.002	0.019 ± 0.0005

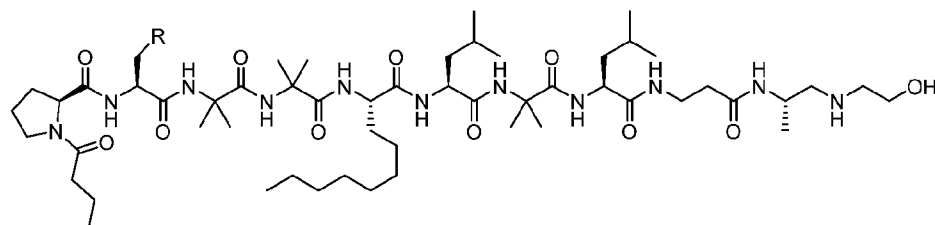
8	380	0.029 ± 0.003	0.118 ± 0.011	0.029 ± 0.002	0.036 ± 0.003
9	390	0.031 ± 0.002	0.096 ± 0.018	0.025 ± 0.003	0.039 ± 0.003
10	400	>10	>10	>10	>10
11	410	0.262 ± 0.047	>1	0.166 ± 0.001	0.249 ± 0.050
12	420	0.011 ± 0.001	0.043 ± 0.009	0.008 ± 0.001	0.010 ± 0.001
13	430	0.136 ± 0.007	>1	0.151 ± 0.016	0.456 ± 0.245
14	440	0.021 ± 0.002	0.060 ± 0.004	0.015 ± 0.001	0.023 ± 0.0004
15	450	0.007 ± 0.0002	0.025 ± 0.002	0.008 ± 0.0002	0.006 ± 0.003
16	460	0.035 ± 0.001	0.155 ± 0.010	0.032 ± 0.005	0.071 ± 0.037
17	470	0.039 ± 0.01	0.27 ± 0.001	0.049 ± 0.004	0.045 ± 0.0002
18	480	0.010 ± 0.001	0.021 ± 0.006	0.008 ± 0.001	0.006 ± 0.001
19	490	0.021 ± 0.0004	0.072 ± 0.002	0.016 ± 0.0004	0.017 ± 0.0002
20	500	0.005 ± 0.001	0.015 ± 0.002	0.007 ± 0.001	0.005 ± 0.001
21	510	0.010 ± 0.002	0.029 ± 0.007	0.010 ± 0.002	0.008 ± 0.001
22	520	0.003 ± 0.0002	0.012 ± 0.005	0.004 ± 0.0001	0.002 ± 0.0004

[0623] Tables 2 to 4 show that all analogues had antiproliferative activity against the cell lines tested.

5 3. Example 3

[0624] This example builds on the SAR from examples 1 and 2 above by investigating the effect of the *N*-terminal lipid tail and *C*-terminal amino alcohol of culicinin D **1** on anticancer activity.

[0625] A collection of analogues based on culicinin D analogue **520** (see Scheme 92 below) were therefore prepared.



520: R = cyclohexyl

Scheme 92: Culicinin analogue 520.

3.1 General methods and materials

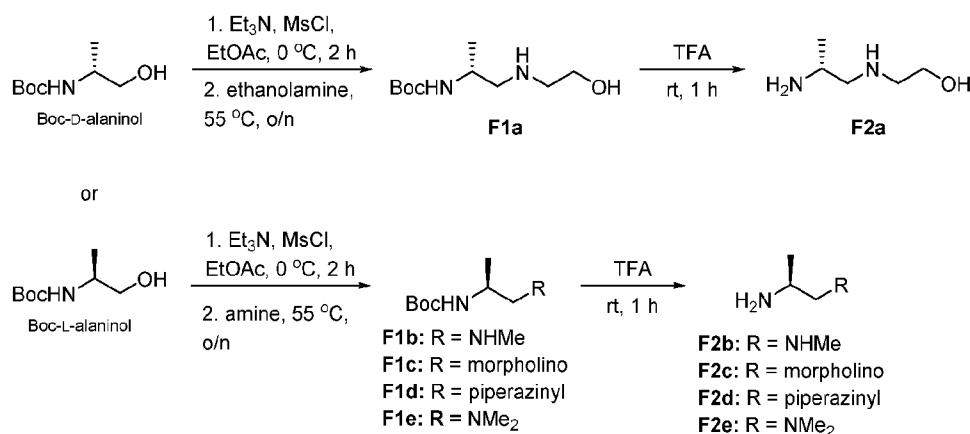
[0626] The general methods, materials and abbreviations used in this example are the same as those outlined in sections 1.1 to 1.5 above.

3.2 Synthesis of APAE

[0627] (R)- and (S)-APAE **5** were prepared from Boc-D-alaninol and Boc-L-alaninol as described in Kavianinia *et al.* (Kavianinia, I.; Kunaligam, L.; Harris, P. W. R.; Cook, G. M.; Brimble, M. A. Total Synthesis and Stereochemical Revision of the Anti-Tuberculosis

5 Peptaibol Trichoderin A. *Org. Lett.* 2016, 18 (5), 3878–3881).

3.3 Synthesis of C-terminal amine building blocks **F2a-F2e** for culicinin D analogues **1200b**, **1200f**, **1200h**, **1200i**, and **1200l**



10 **Scheme 93:** Synthesis of C-terminal amine building blocks **F2a-F2e**

[0628] As shown in Scheme 93 above and described in detail below, compounds **F2a** – **F2e** were prepared over 3 steps from either Boc-L-alaninol or Boc-D-alaninol according to the previously published procedure in Hansen *et al.* (Hansen, M. M.; Kallman, N. J.; Koenig, T. M.; Linder, R. J.; Richey, R. N.; Rizzo, J. R.; Ward, J. A.; Yu, H.; Zhang, T. Y.; Mitchell, D. Double Heck Route to a Dibenzoepine and Convergent Suzuki Cross-Coupling Strategy for the Synthesis of an MR Antagonist. *Org. Process Res. Dev.* **2017**, 21 (2), 208–217).

[0629] Triethylamine (2 eq.) was added dropwise to a solution of Boc-L-alaninol (1 eq.) in ethyl acetate (10 mL) at 0 °C, followed by methanesulfonyl chloride (1.2 eq.) dropwise. The thick white suspension was allowed to stir at 0 °C for 2 h, then sat. aq. NaHCO₃ (20 mL) added. The organic layer was washed with brine (30 mL) and dried over Na₂SO₄. The requisite amine (6 eq.) was added, and the mixture heated at 50–70 °C for 20 h. The mixture was allowed to cool to rt and washed with brine (2 × 50 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give the crude material. The crude was purified *via* flash column chromatography (silica gel) using the eluent indicated to give the Boc-protected building blocks **F1a** – **F1e**.

[0630] The Boc-protected building blocks **F1a** – **F1e** were treated with excess neat TFA at rt for 1 h, then the volatiles removed under reduced pressure. The resulting gum was triturated with cold diethyl ether and the resulting white precipitate collected *via* filtration and dried under high vacuum to give the TFA salt of the deprotected building blocks **F2a** –

F2e.

3.1.1 *tert*-Butyl (*R*)-(1-((2-hydroxyethyl)amino)propan-2-yl)carbamate, F1a

[0631] Obtained as a colourless solid in 24% yield from Boc-D-alaninol and ethanolamine. $\alpha_D^{22} = +2.6$ (c 1.54, CHCl₃); lit.² (for enantiomer) $\alpha_D^{21} = -2.6$ (c 1.5, CHCl₃); **¹H NMR** (CDCl₃, 400 MHz): δ 4.70 (br d, 1H, *J* = 7.8 Hz), 3.81-3.76 (m, 1H), 3.64 (t, 2H, *J* = 5.2 Hz), 2.84-2.74 (m, 2H), 2.66-2.59 (m, 2H), 2.31 (br s, 2H), 1.44 (s, 9H), 1.14 (d, 3H, *J* = 6.6 Hz); Spectra were consistent with literature values.

3.1.2 (*R*)-2-((2-Aminopropyl)amino)ethan-1-ol, F2a ("*R*)-APAE")

[0632] Obtained as the TFA salt in 98% yield from **F1a**. An analytical sample was purified *via* flash column chromatography (silica gel, dichloromethane/methanol/NH₄OH 6:4:1 as eluent) to give F2a as a yellow oil. $\alpha_D^{23} = -38.2$ (c 1.23, MeOH); **¹H NMR** (CD₃OD, 500 MHz): δ 3.65 (t, 2H, *J* = 5.5 Hz), 3.12-3.05 (m, 1H), 2.78-2.67 (m, 3H), 2.52 (dd, 1H, *J* = 8.4, 12.3 Hz), 1.15 (d, 3H, *J* = 6.6 Hz); **HRMS** *m/z* (ESI+) 119.1177 [M + H]⁺ (calcd for C₅H₁₅N₂O⁺ 119.1179).

3.1.3 *tert*-Butyl (*S*)-(1-(methylamino)propan-2-yl)carbamate, F1b

[0633] Obtained as a pale yellow oil in 21% yield from Boc-L-alaninol and methylamine. $\alpha_D^{19} = +5.4$ (c 0.74, CHCl₃); **¹H NMR** (CDCl₃, 400 MHz): δ 4.68 (br s, 1H), 3.78-3.72 (m, 1H), 2.58 (d, 2H, *J* = 5.9 Hz), 2.43 (s, 3H), 1.44 (s, 9H), 1.14 (d, 3H, *J* = 6.6 Hz); **HRMS** *m/z* (ESI+) 145.1338 [M + H]⁺ (calcd for C₇H₁₇N₂O⁺ 145.1335). Spectra were consistent with literature values.

3.1.4 (*S*)-*N*¹-Methylpropane-1,2-diamine, F2b ("*MPD*")

[0634] Obtained as the TFA salt in 96% yield from **F1b**. An analytical sample was purified *via* flash column chromatography (silica gel, dichloromethane/methanol/NH₄OH 6:4:1 as eluent) to give **F2b** as a yellow oil. $\alpha_D^{23} = +6.1$ (c 1.25, MeOH); **¹H NMR** (CD₃OD, 500 MHz): δ 3.36-3.30 (m, 1H), 2.90 (dd, 1H, *J* = 5.1, 12.8 Hz), 2.79 (dd, 1H, *J* = 8.4, 12.8 Hz), 2.57 (s, 3H), 1.27 (d, 3H, *J* = 6.7 Hz); **HRMS** *m/z* (ESI+) 89.1077 [M + H]⁺ (calcd for C₄H₁₃N₂⁺ 89.1073).

3.1.5 *tert*-Butyl (*S*)-(1-morpholinopropan-2-yl)carbamate, F1c

[0635] Obtained as a pale yellow solid in 33% yield from Boc-L-alaninol and morpholine. $\alpha_D^{22} = +9.5$ (c 6.13, CHCl₃); **¹H NMR** (CDCl₃, 500 MHz): δ 4.75 (br s, 1H), 3.76-3.65 (m, 5H), 2.53-2.50 (m, 2H), 2.41-2.37 (m, 2H), 2.33-2.29 (m, 1H), 2.24-2.21 (m, 1H), 1.45 (s, 9H), 1.15 (d, 3H, *J* = 6.6 Hz); **HRMS** *m/z* (ESI+) 245.1853 [M + H]⁺ (calcd for C₁₂H₂₅N₂O₃⁺ 245.1860). Spectra were consistent with literature values.

3.1.6 (S)-1-morpholinopropan-2-amine, F2c ("MPA")

[0636] Obtained as the TFA salt in 98% yield from **F1c**. An analytical sample was purified via flash column chromatography (silica gel, dichloromethane/methanol/NH₄OH 12:4:1 as eluent) to give **F2c** as a yellow oil. $\alpha_D^{23} = +6.0$ (c 0.435, MeOH); **¹H NMR** (CD₃OD, 500 MHz): δ 3.72-3.66 (m, 4H), 3.11-3.05 (m, 1H), 2.57-2.53 (m, 2H), 2.38-2.34 (m, 2H), 2.25-2.17 (m, 2H), 1.06 (d, 3H, $J = 6.3$ Hz); **HRMS** m/z (ESI+) 145.1338 [M + H]⁺ (calcd for C₇H₁₇N₂O⁺ 145.1335).

3.1.7 tert-Butyl (S)-(1-(piperazin-1-yl)propan-2-yl)carbamate, F1d

[0637] Obtained as a pale yellow oil in 64% yield from Boc-L-alaninol and piperazine. $\alpha_D^{20} = +11.9$ (c 1.41, CHCl₃); **¹H NMR** (CDCl₃, 400 MHz): δ 4.73 (br s, 1H), 3.75-3.66 (m, 1H), 2.91-2.82 (m, 4H), 2.49-2.44 (m, 2H), 2.37-2.32 (m, 2H), 2.31-2.18 (m, 2H), 1.45 (s, 9H), 1.15 (d, 3H, $J = 6.4$ Hz); **HRMS** m/z (ESI+) 244.2019 [M + H]⁺ (calcd for C₁₂H₂₆N₃O₂⁺ 244.2020); 266.1837 [M + Na]⁺ (calcd for C₁₂H₂₅N₃O₂Na⁺ 266.1839).

3.1.8 (S)-1-(piperazin-1-yl)propan-2-amine, F2d ("PAPA")

[0638] Obtained as the TFA salt in 97% yield from **F1d**. An analytical sample was purified via flash column chromatography (silica gel, dichloromethane/methanol/NH₄OH 6:4:1 as eluent) to give **F2d** as a yellow oil. $\alpha_D^{23} = +41.8$ (c 8.82, MeOH); **¹H NMR** (CD₃OD, 500 MHz): δ 3.39-3.33 (m, 1H), 3.00-2.95 (m, 4H), 2.69-2.65 (m, 2H), 2.49-2.45 (m, 2H), 2.43-2.37 (m, 2H), 1.22 (d, 3H, $J = 6.5$ Hz); **HRMS** m/z (ESI+) 144.1497 [M + H]⁺ (calcd for C₇H₁₈N₃⁺ 144.1495).

3.1.9 tert-Butyl (S)-(1-(dimethylamino)propan-2-yl)carbamate, F1e

[0639] Obtained as a colourless solid in 38% yield from Boc-L-alaninol and dimethylamine. **¹H NMR** (CDCl₃, 400 MHz): δ 4.76 (br s, 1H), 3.70-3.60 (m, 1H), 2.29 (dd, 1H, $J = 8.6, 12.2$ Hz), 2.22 (s, 6H), 2.12 (dd, 1H, $J = 6.0, 12.3$ Hz), 1.45 (s, 9H), 1.16 (d, 3H, $J = 6.4$ Hz); **HRMS** m/z (ESI+) 203.1748 [M + H]⁺ (calcd for C₁₀H₂₂N₂O₂⁺ 203.1754).

3.1.10 (S)-N¹,N¹-Dimethylpropane-1,2-diamine trifluoroacetic acid salt, F2e ("DMPD")

[0640] Obtained as the TFA salt in 92% yield from **F1e**. **¹H NMR** (CD₃OD, 400 MHz): δ 3.91-3.83 (m, 1H), 3.41 (dd, 1H, $J = 7.0, 13.7$ Hz), 3.33 (dd, 1H, $J = 5.6, 13.7$ Hz), 2.96 (s, 6H), 1.43 (d, 3H, $J = 6.8$ Hz); Spectra were consistent with literature values.

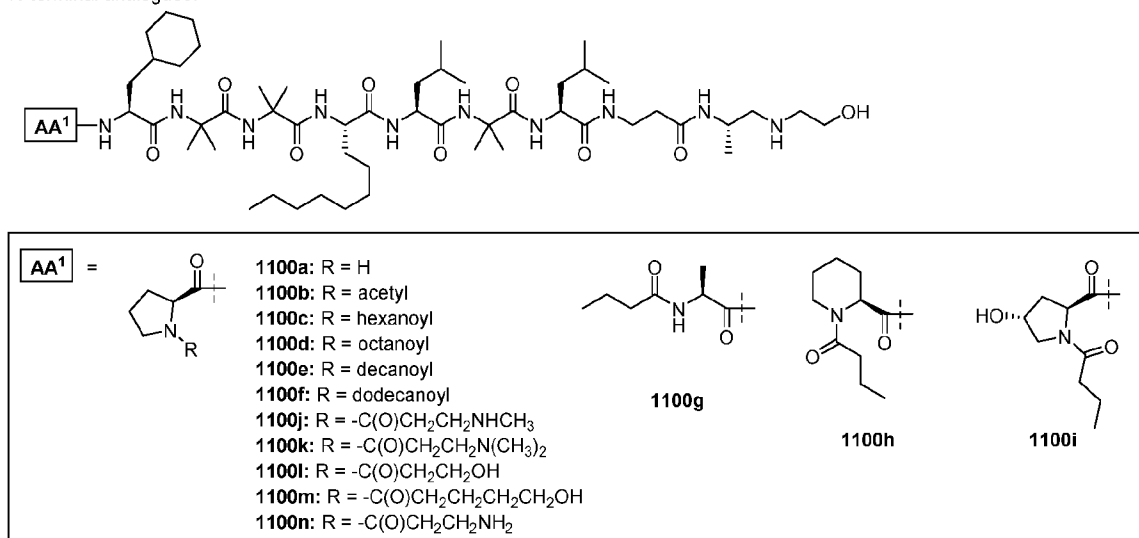
3.2 General procedure for peptide synthesis

[0641] A series of culicinin D analogues were prepared as follows. The general procedures section 1.4 includes the methods referred to in this section.

3.3 Synthesis of *N*-terminal culicinin D analogues 1100a to 1100n

[0642] Scheme 94 shows the *N*-terminal analogues **1100a-n** prepared in this example based on culicinin D-derived **520**.

N-terminal analogues:

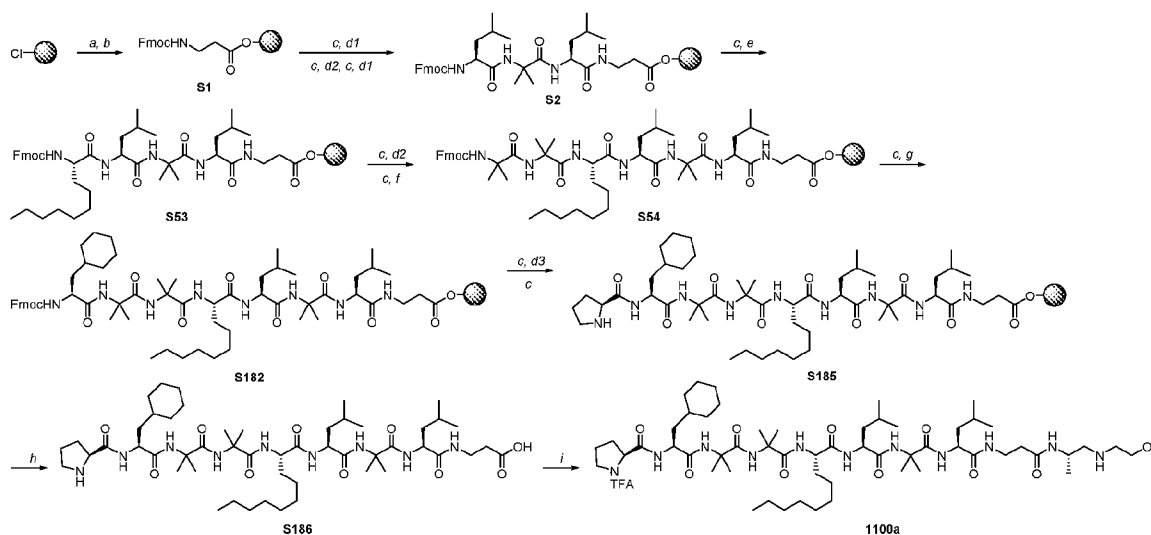


5 **Scheme 94:** The structure of *N*-terminal analogues **1100a-n** based upon culicinin D-derived **520**.

3.3.1 Pro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-(S)-APAE, **1100a**

[0643] The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH and Fmoc-Pro-OH were then coupled using **Method 3** to complete the linear peptide sequence of **S185**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford **S186** as a white amorphous solid (51.5 mg, 47% yield, > 98% purity). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford TFA amide peptide analogue **1100a** as a white amorphous solid

(45 mg, 38% overall yield, > 98% purity); R_t 25.3 min; LRMS: m/z (ESI-MS) 1186.7 ($[M+H]^+$ requires 1186.8).

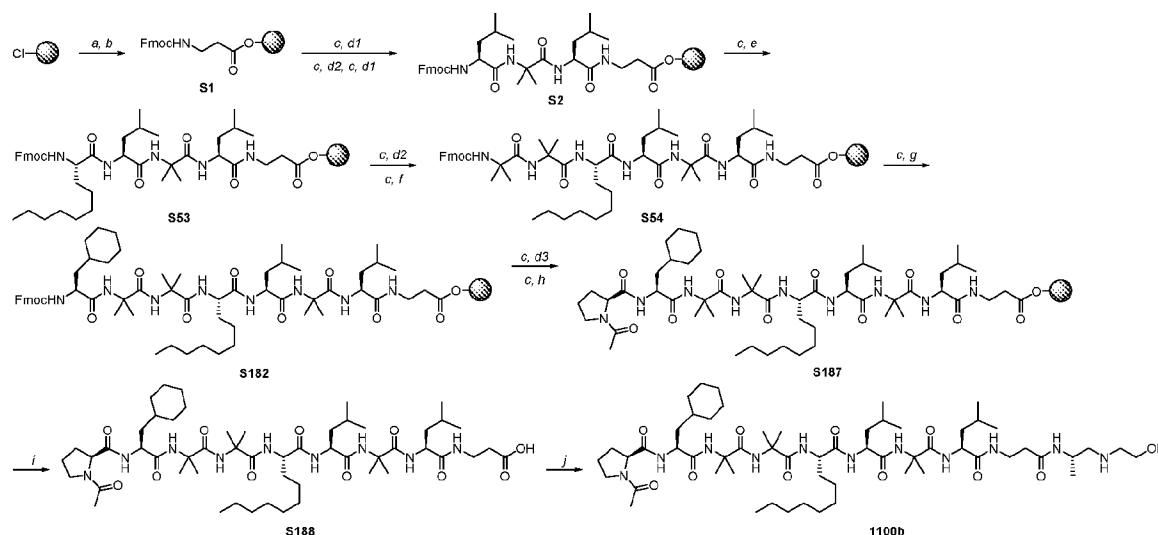


Scheme 95. Synthesis of peptide analogue **1100a**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) HFIP/ CH_2Cl_2 (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBt, DMF, 16 h.

3.3.2 AcetylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-(S)-APAE, **1100b**

[0644] The C-terminal residue Fmoc- β -alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH and Fmoc-Pro-OH were then coupled using **Method 3** followed by acetic acid via **Method 7** to complete the linear peptide sequence of **S187**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford **S188** as a white amorphous solid (56 mg, 54% yield, > 98% purity). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were

collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1100b** as a white amorphous solid (47 mg, 42% overall yield, > 98% purity); R_t 24.7 min; LRMS: m/z (ESI-MS) 1132.7 ($[M+H]^+$ requires 1132.8).

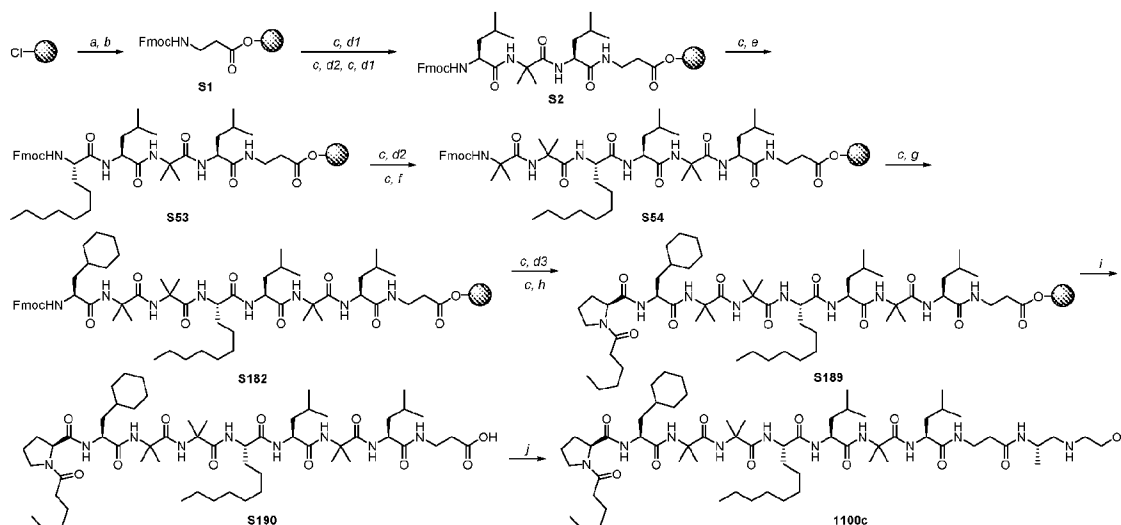


Scheme 96. Synthesis of peptide analogue **1100b**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) acetic acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.3 HexanoylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-(S)-APAE, **1100c**

[0645] The C-terminal residue Fmoc- β -alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^0 -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^0 -Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^0 -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^0 -Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH and Fmoc-Pro-OH were then coupled using **Method 3** followed by hexanoic acid via **Method 7** to complete the linear peptide sequence of **S189**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford **S190** as a white amorphous solid (52 mg, 48%

yield, > 98% purity). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1100c** as a white amorphous solid (37 mg, 31% overall yield, > 98% purity); R_t 25.8 min; LRMS: m/z (ESI-MS) 1188.8 ($[M+H]^+$ requires 1188.9).

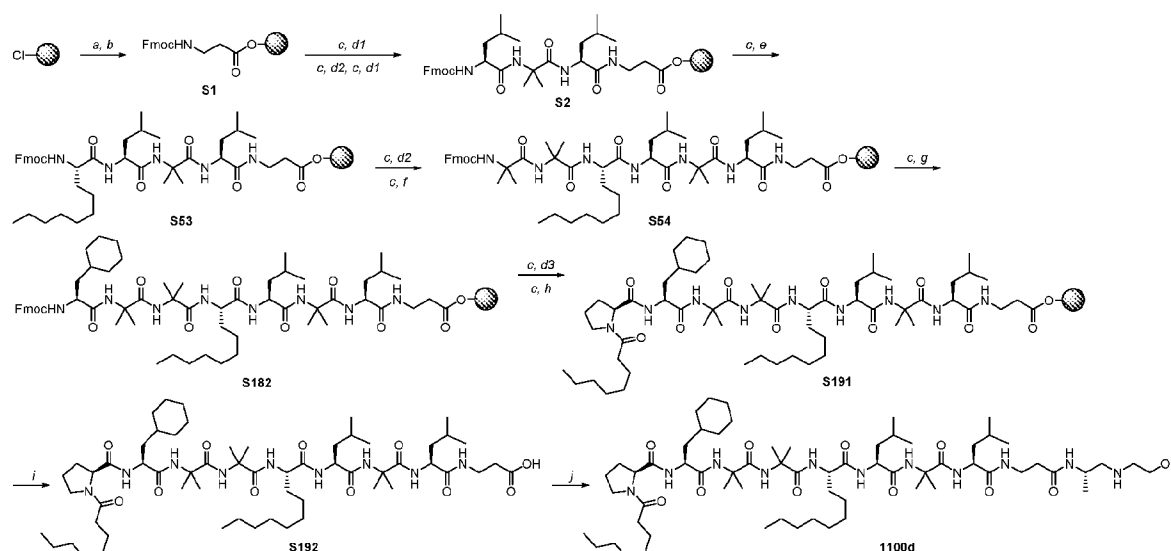


Scheme 97. Synthesis of peptide analogue **1100c**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) hexanoic acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.4 OctanoylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-(S)-APAE, **1100d**

[0646] The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH and Fmoc-Pro-OH were then coupled using **Method 3** followed by octanoic acid via **Method 7** to complete the linear peptide sequence of **S191**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-

HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford **S192** as a white amorphous solid (50 mg, 45% yield, > 98% purity). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1100d** as a white amorphous solid (40 mg, 33% overall yield, > 98% purity); R_t 31.6 min; LRMS: m/z (ESI-MS) 1216.6 ($[M+H]^+$ requires 1216.9).

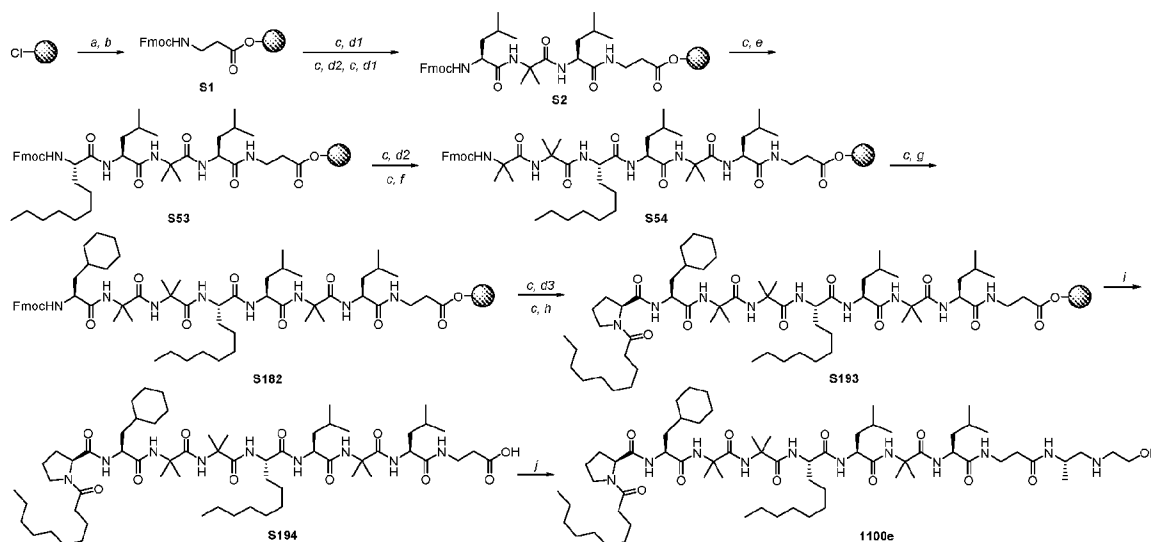


Scheme 98. Synthesis of peptide analogue **1100d**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) octanoic acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.5 DecanoylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-(S)-APAE, **1100e**

[0647] The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**,

Fmoc-Cha-OH and Fmoc-Pro-OH were then coupled using **Method 3** followed by decanoic acid via **Method 7** to complete the linear peptide sequence of **S195**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford **S194** as a white amorphous solid (51 mg, 45% yield, > 98% purity). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1100e** as a white amorphous solid (50 mg, 40% overall yield, > 98% purity); R_t 31.5 min; LRMS: m/z (ESI-MS) 1244.8 ($[M+H]^+$ requires 1244.9).

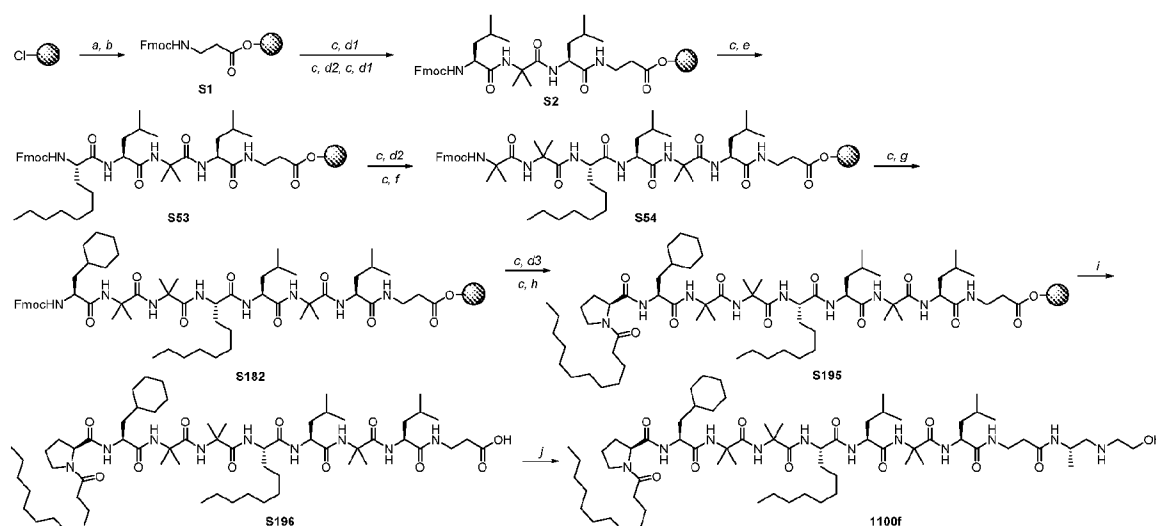


Scheme 99. Synthesis of peptide analogue **1100e**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) decanoic acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.6 DodecanoylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-(S)-APAE, **1100f**

[0648] The C-terminal residue Fmoc- β -alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α -Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The

N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH and Fmoc-Pro-OH were then coupled using **Method 3** followed by dodecanoic acid via **Method 7** to complete the linear peptide sequence of **S195**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford **S196** as a white amorphous solid (55 mg, 47% yield, > 98% purity). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide analogue **1100f** as a white amorphous solid (51 mg, 40% overall yield, > 98% purity); R_t 34.4 min; LRMS: *m/z* (ESI-MS) 1272.6 ([M+H]⁺ requires 1273.0).

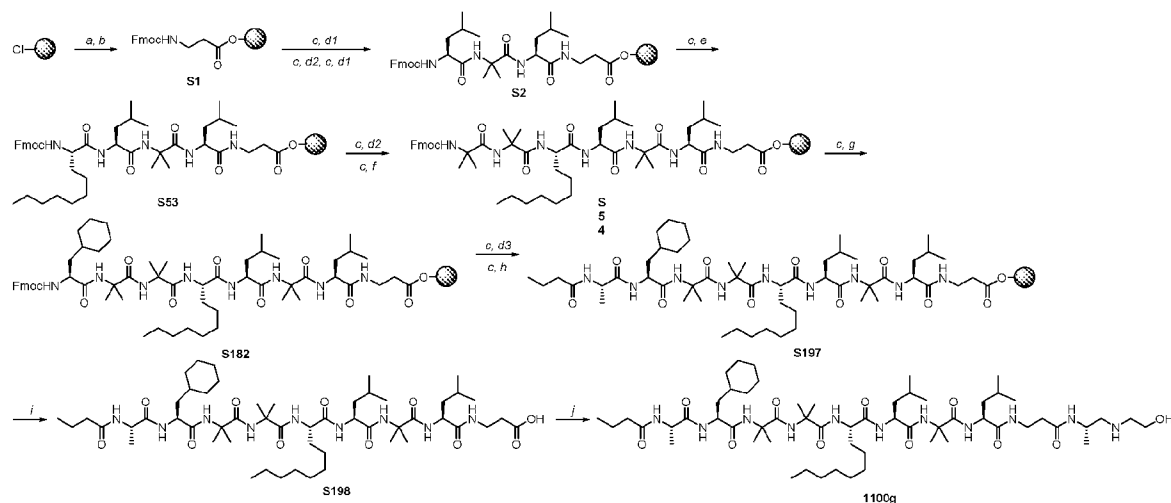


Scheme 100. Synthesis of peptide analogue **1100f**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) dodecanoic acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.7 ButyrylAla-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-(S)-APAE, **1100g**

[0649] The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**.

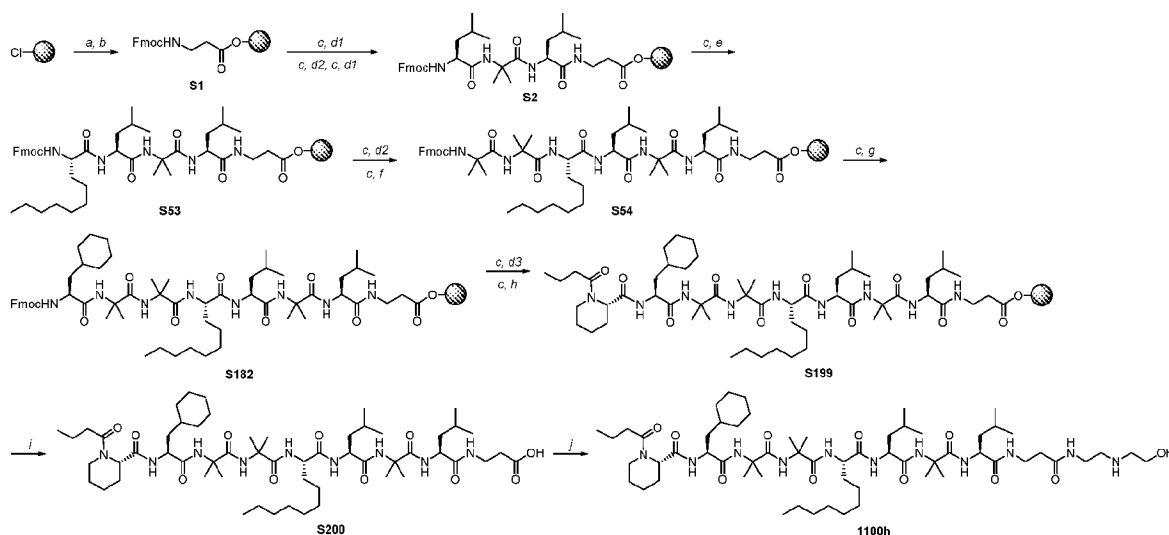
The N^o-Fmoc-protecting group was removed using Method 2 followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH and Fmoc-Ala-OH were then coupled using **Method 3** followed by butyric acid via **Method 7** to complete the linear peptide sequence of **S197**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford **S198** as a white amorphous solid (37.6 mg, 36% yield, > 98% purity). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide analogue **1100g** as a white amorphous solid (35 mg, 31% overall yield, > 98% purity); R_t 25.4 min; LRMS: *m/z* (ESI-MS) 1134.8 ([M+H]⁺ requires 1134.8).



Scheme 101. Synthesis of peptide analogue **1100g**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.8 ButyrylPip-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-(S)-APAE, 1100h

[0650] The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH and Fmoc-Pip-OH were then coupled using **Method 3** followed by butyric acid via **Method 7** to complete the linear peptide sequence of **S199**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford **S200** as a white amorphous solid (50 mg, 47% yield, > 98% purity). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide analogue **1100h** as a white amorphous solid (39 mg, 33% overall yield, > 98% purity); *R*_t 26.8 min; LRMS: *m/z* (ESI-MS) 1174.8 ([*M*+*H*]⁺ requires 1174.9).

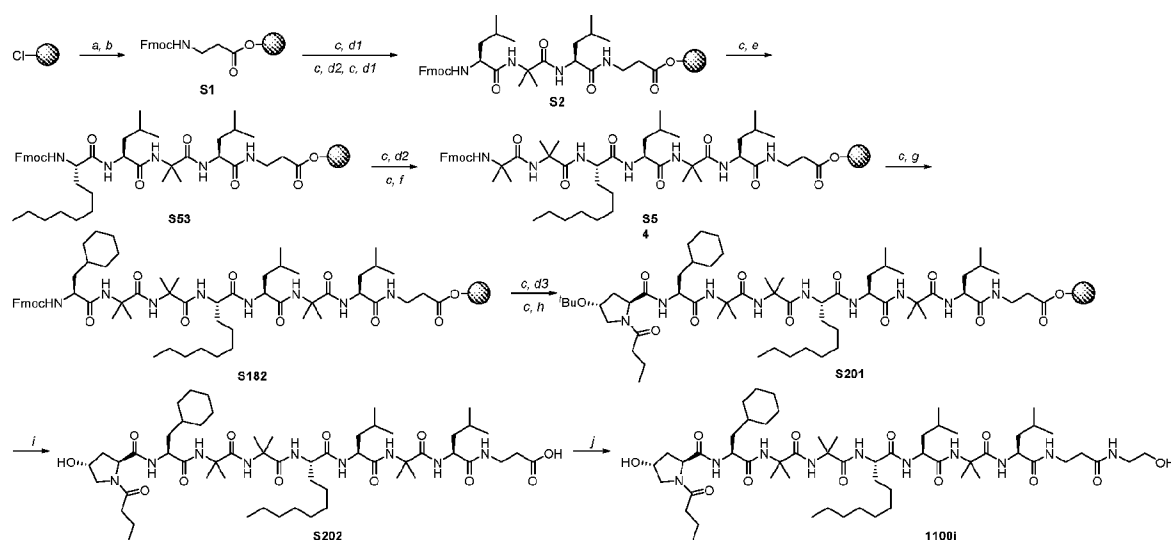


Scheme 102. Synthesis of peptide analogue **1100h**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH,

HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.9 ButyrylHyp-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-(S)-APAE, 1100i

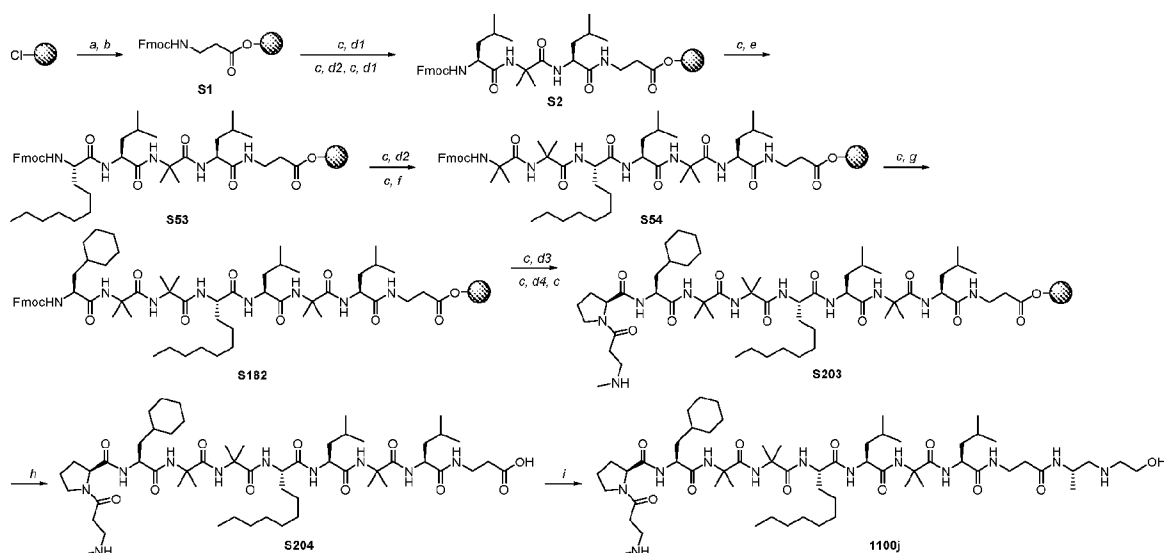
- 5 **[0651]** The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The
- 10 N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH and Fmoc-Hyp(tBu)-OH were then coupled using **Method 3** followed by
- 15 butyric acid via **Method 7** to complete the linear peptide sequence of **S201**. Resin-bound peptide was cleaved using **Method 9**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford **S202** as a white amorphous solid (35.5 mg, 33% yield, > 98% purity). Late-stage solution phase C-terminal coupling was
- 20 employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide analogue **1100i** as a white amorphous solid (33 mg, 28% overall yield, > 98% purity); R_t
- 25 23.9 min; LRMS: *m/z* (ESI-MS) 1176.7 ([M+H]⁺ requires 1176.8).



Scheme 103. Synthesis of peptide analogue **1100i**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH₂Cl₂, 1 $\times$ 6 h, 1 $\times$ 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Hyp(tBu)-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) TFA/TIPS/H₂O (95:2.5:2.5), 2 $\times$ 1 h; j) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.10 *N*-Me- β Ala-Pro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-APAE, **1100j**

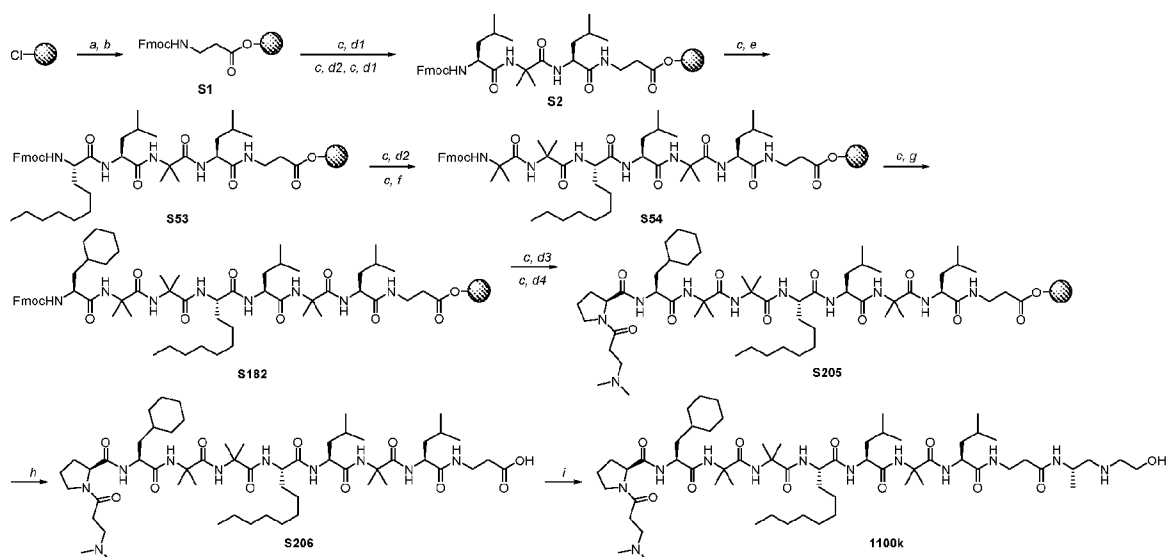
- [0652]** The C-terminal residue Fmoc- β -alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The *N*^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The *N*^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The *N*^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of *N*^o-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH and Fmoc-*N*-Me- β Ala-OH using **Method 3**, followed by removal of the *N*-terminal Fmoc protecting group via **Method 2** to complete the linear peptide sequence of **S203**. Resin bound peptide was cleaved using **Method 8** and lyophilised to afford crude peptide **S204** as a white solid (62 mg). Late-stage solution phase C-terminal coupling was employed to introduce the C terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford analogue **1100j** as a white amorphous solid (3 mg, 5% overall yield, 95% purity); *R*_t 25.6 min; LRMS: *m/z* (ESI-MS) 1175.8 ([M+H]⁺requires 1175.8).



Scheme 1. Synthesis of peptide analogue **1100j**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH, d4: Fmoc-N-Me-βAla-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) HFIP/CH₂Cl₂ (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.11 *N,N*-Dimethyl-βAla-Pro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE, **1100k**

[0653] The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotriethyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The *N*^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The *N*^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The *N*^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of *N*^o-Fmoc-protecting group using **Method 2**, Fmoc-AD-OH was then coupled using **Method 5** followed by coupling of Fmoc-Pro-OH and *N,N*-dimethyl-βAla-OH using **Method 3** to complete the linear peptide sequence of **S205**. Resin bound peptide was cleaved using **Method 8**, and lyophilised to afford crude peptide **S206** as a white solid (54 mg). Late-stage solution phase C-terminal coupling was employed to introduce the C terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford analogue **1100k** as a white amorphous solid (5 mg, 6% overall yield, 95% purity); *R*_t 20.4 min; LRMS: *m/z* (ESI-MS) 1189.8 ([*M*+*H*]⁺ requires 1189.9).

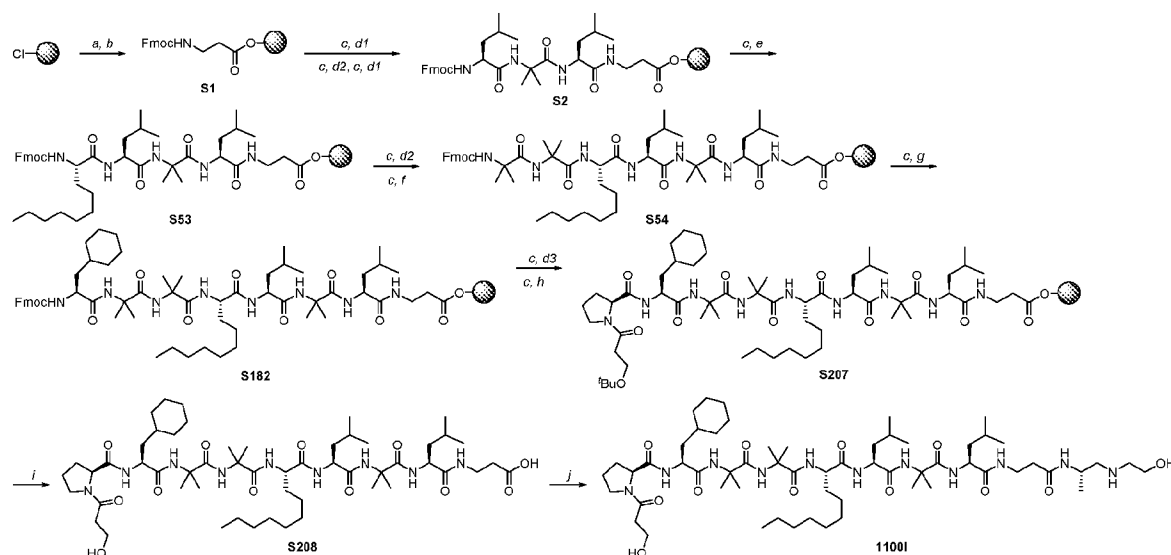


Scheme 2. Synthesis of peptide analogue **1100k**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH, d4: *N,N*-dimethyl-βAla-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) HFIP/CH₂Cl₂ (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.12 3-Hydroxypropanoyl-Pro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE, **1100I** [**0654**]

The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The *N*^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The *N*^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The *N*^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of *N*^α-Fmoc-protecting group using **Method 2**, Fmoc-AD-OH was then coupled using **Method 5** followed by coupling of Fmoc-Pro-OH and 3-(*tert*-butoxy)propionic acid via **Method 3** to complete the linear peptide sequence of **S207**. Resin bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S208** as white fluffy flakes (15 mg, 13% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC.

Fractions identified with the correct m/z were combined and lyophilised to afford analogue **1100I** as a white amorphous solid (7 mg, 6% overall yield, > 98% purity); R_t 25.3 min; LRMS: m/z (ESI-MS) 1162.7 ($[M+H]^+$ requires 1162.8).

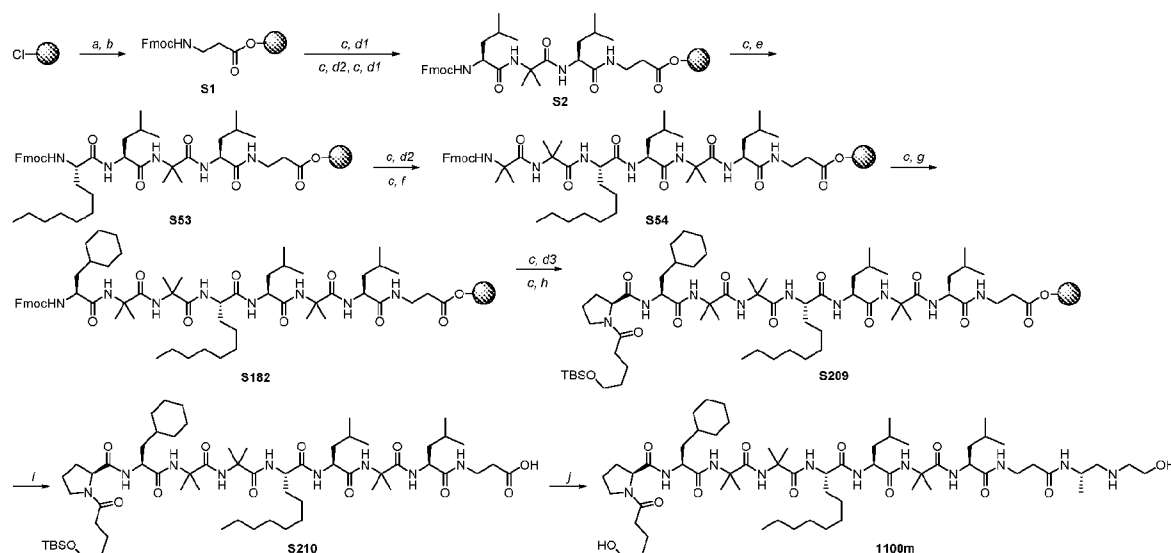


Scheme 3. Synthesis of peptide analogue **1100I**. **Reagents and conditions:** a) Fmoc- β Ala-OH, DIPEA, CH_2Cl_2 , 1 $\times$ 6 h, 1 $\times$ 12 h; b) $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{DIPEA}$ (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) 3-(*tert*-butoxy)propionic acid, HATU, DIPEA, DMF, 2 $\times$ 1 h; i) HFIP/ CH_2Cl_2 (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, rt, 16 h.

3.3.13 5-Hydroxypentanoyl-Pro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-APAE, **1100m**

[0655] The C-terminal residue Fmoc- β -alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^{α} -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^{α} -Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^{α} -Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^{α} -Fmoc-protecting group using **Method 2**, Fmoc-AD-OH was then coupled using **Method 5** followed by coupling of Fmoc-Pro-OH and 5-(*tert*-butyldimethylsilyloxy)pentanoic acid via **Method 3** to complete the linear peptide sequence of **S209**. Resin bound peptide was cleaved using **Method 8**, and lyophilised to afford crude peptide **S210** as a white solid (49 mg). Late-stage solution phase C-terminal coupling was employed to introduce the C terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified with concomitant removal of the TBS protecting group by

semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford analogue **1100m** as a white amorphous solid (4 mg, 5% overall yield, 95% purity); R_t 23.8 min; LRMS: m/z (ESI-MS) 1190.8 ($[M+H]^+$ requires 1190.8).



Scheme 4. Synthesis of peptide analogue **1100m**. **Reagents and conditions:** a) Fmoc-

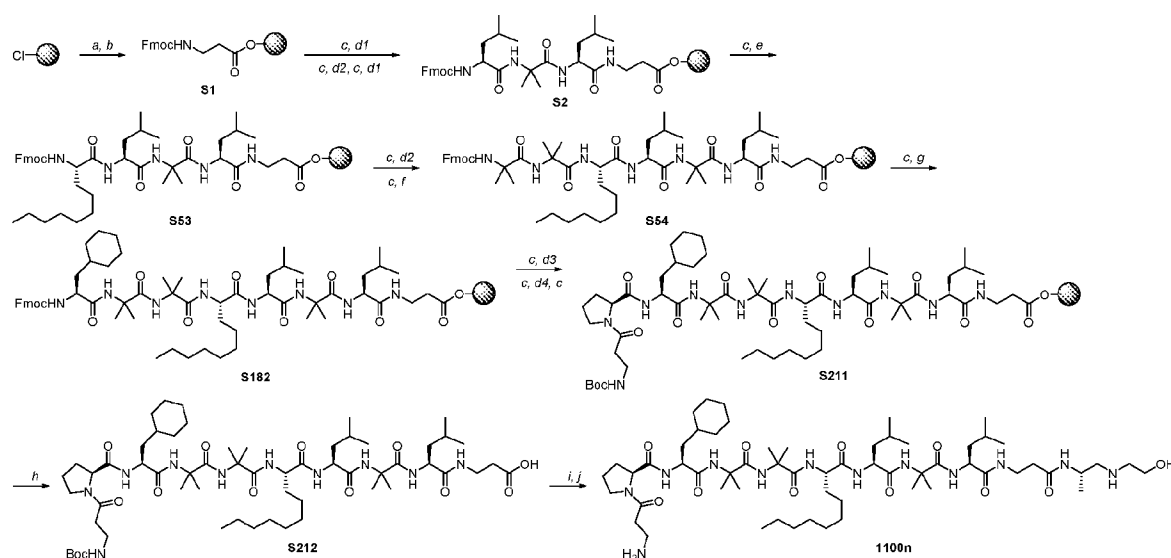
βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) 5-(*tert*-butyldimethylsilyloxy)pentanoic acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBT, DMF, rt, 16 h. TBS protecting group removed upon exposure to eluent during RP-HPLC purification.

3.3.14 βAla-Pro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-APAE, 1100n

[0656] The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**.

The *N*^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The *N*^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The *N*^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of *N*^α-Fmoc-protecting group using **Method 2**, Fmoc-AD-OH was then coupled using **Method 5** followed by coupling of Fmoc-Pro-OH and Boc-βAla-OH via **Method 3** to complete the linear peptide sequence of **S211**. Resin bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-

preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S212** as white fluffy flakes (12 mg, 10% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C terminal aminoalcohol moiety using **Method 10**. The crude peptide was treated with TFA/CH₂Cl₂ (1:1, v/v) for 1 h to remove the *N*-terminal Boc protecting group, then purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford analogue **1100n** as a white amorphous solid (8 mg, 7% overall yield, > 98% purity); R_t 20.8 min; LRMS: m/z (ESI-MS) 1161.7 ([M+H]⁺requires 1161.8).



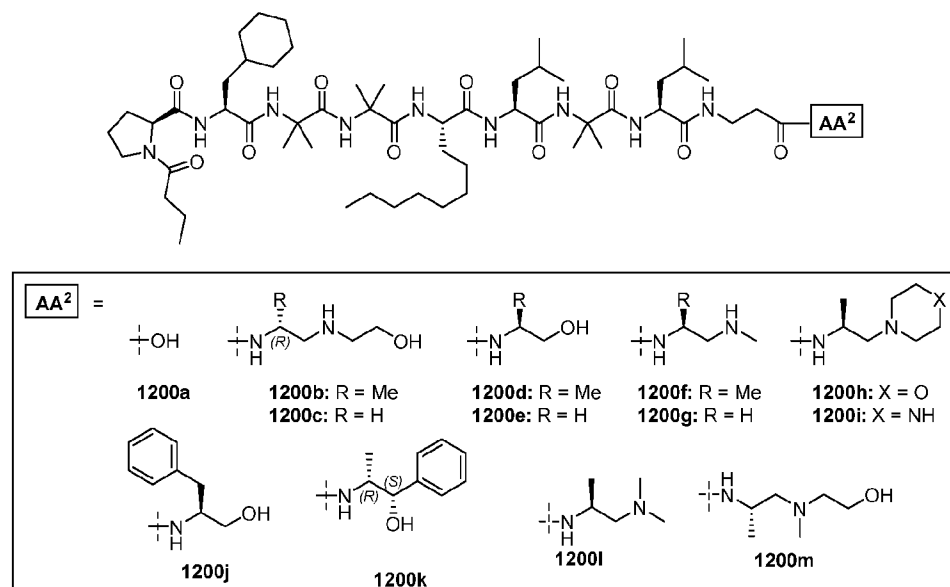
Scheme 5. Synthesis of peptide analogue **1100n**. **Reagents and conditions:** a) Fmoc-

βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH, d4: Boc-βAla-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 2 h; h) HFIP/CH₂Cl₂ (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBT, DMF, rt, 16 h; j) TFA/CH₂Cl₂ (1:1, v/v), 1 h.

3.4 Synthesis of C-terminal culicinin D analogues 1200a-1200m

[0657] Scheme 104 shows the C-terminal analogues **1200a-m** prepared in this example based on culicinin-D derived **520**.

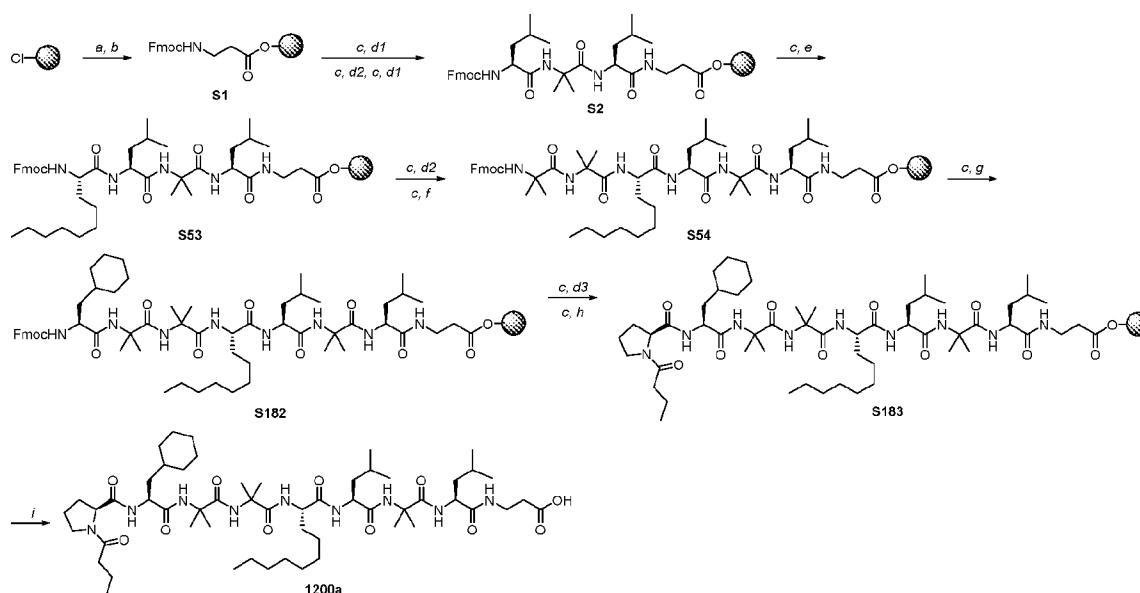
C-terminal analogues:



5 Scheme 104: The structure of C-terminal analogues **1200a-m** based upon culicinin D-derived **520**.

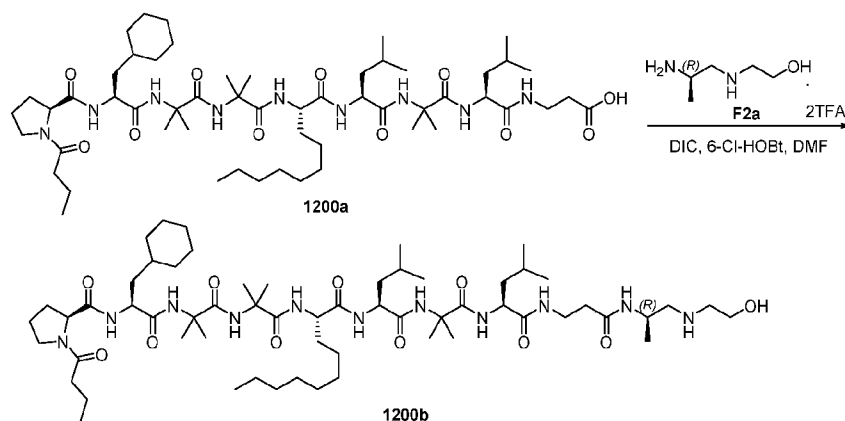
3.4.1 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla, **1200a**

[0658] The C-terminal residue Fmoc-β-alanine-OH was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH was coupled using **Method A** and Fmoc-Pro-OH was then coupled using **Method 3** followed by butyric acid via **Method 7** to complete the linear peptide sequence of **S183**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford the title peptide **1200a** as a white amorphous solid (50 mg, 47% overall yield, > 98% purity); R_t 26.7 min; LRMS: *m/z* (ESI-MS) 1060.7 ([M+H]⁺ requires 1060.7).



Scheme 105. Synthesis of peptide analogue **1200a**. **Reagents and conditions:** a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Cha-OH, HATU, DIPEA, DMF, 3 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h.

3.4.2 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-(R)-APAE, **1200b**

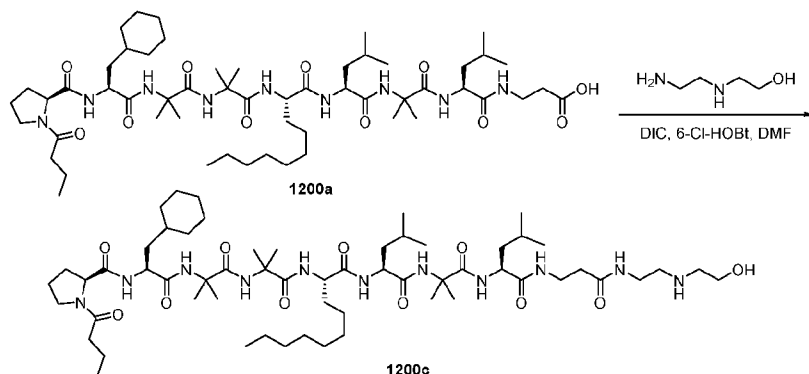


Scheme 106. Synthesis of peptide analogue **1200b**.

[0659] DIPEA (7 μL, 38.2 mmol) was added to a solution of (R)-APAE TFA salt **F2a** (12 mg, 38.2 mmol) in DMF (300 μL) and the mixture allowed to stand for 10 min. The solution was then added to a mixture of the peptide **1200a** (13.5 mg, 12.7 μmol), DIC (12 μL, 76.4 μmol), and 6-Cl-HOBt (13 mg, 76.4 μmol) in DMF (300 μL) and the reaction mixture agitated overnight at rt. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined

and lyophilised to afford peptide analogue **1200b** as a white amorphous solid (12 mg, 81% yield, 38% overall yield, > 98% purity); R_t 25.1 min; LRMS: m/z (ESI-MS) 1160.6 ($[M+H]^+$ requires 1160.8).

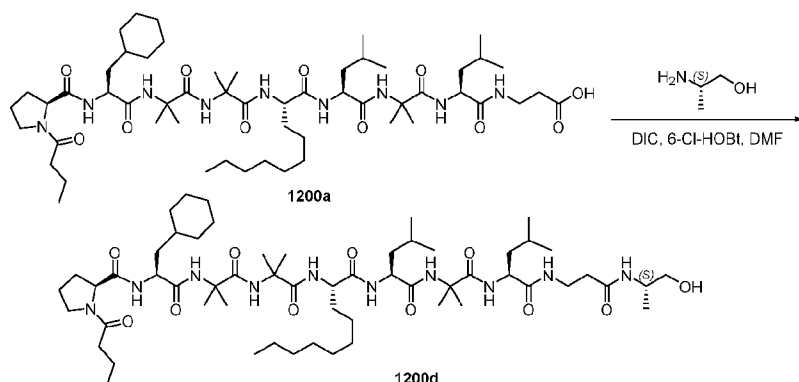
5 3.4.3 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-AEAE, **1200c**



Scheme 107. Synthesis of peptide analogue **1200c**.

[0660] DIPEA (7.2 μ L, 41.0 μ mol), *N*-(2-aminoethyl)ethanolamine (4.2 μ L, 41.0 μ mol), DIC (13 μ L, 82.0 μ mol), and 6-Cl-HOBT (14 mg, 82.0 μ mol) were added to a solution of peptide **1200a** (14.5 mg, 13.7 μ mol) in DMF (600 μ L) and the mixture agitated at rt overnight. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200c** as a white amorphous solid (12 mg, 80% yield, 38% overall yield, > 98% purity); R_t 25.4 min; LRMS: m/z (ESI-MS) 1146.7 ($[M+H]^+$ requires 1146.8).

3.4.4 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-Alaninol, **1200d**



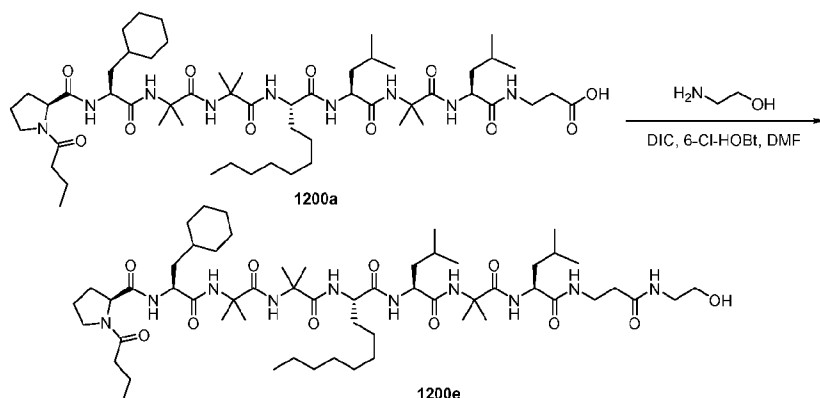
Scheme 108. Synthesis of peptide analogue **1200d**.

[0661] DIPEA (6.7 μ L, 38.2 μ mol), L-alaninol (3 μ L, 38.2 μ mol), DIC (12 μ L, 76.4 μ mol), and 6-Cl-HOBT (13 mg, 76.4 μ mol) were added to a solution of peptide **1200a** (13.5 mg, 12.7 μ mol) in DMF (600 μ L) and the mixture agitated at rt overnight. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method.

Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200d** as a white amorphous solid (10 mg, 70% yield, 33% overall yield, > 98% purity); R_t 27.3 min; LRMS: m/z (ESI-MS) 1117.7 ($[M+H]^+$ requires 1117.8).

5

3.4.5 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-Aminoethanol, **1200e**

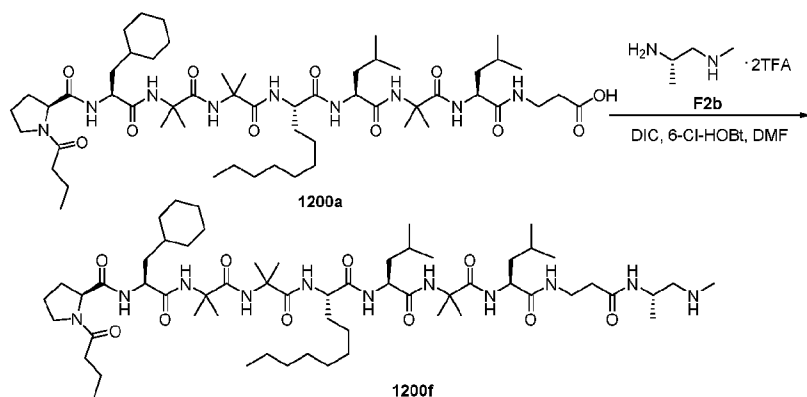


Scheme 109. Synthesis of peptide analogue **1200e**.

[0662] DIPEA (7.2 μ L, 41.0 μ mol), ethanolamine (2.5 μ L, 41.0 μ mol), DIC (12.7 μ L, 82.0 μ mol), and 6-Cl-HOBt (13.9 mg, 82.0 μ mol) were added to a solution of peptide **1200a** (14.5 mg, 13.7 μ mol) in DMF (600 μ L) and the mixture agitated at rt overnight. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200e** as a white amorphous solid (10 mg, 66% yield, 31% overall yield, > 98% purity); R_t 26.6 min; LRMS: m/z (ESI-MS) 1103.7 ($[M+H]^+$ requires 1103.8).

15

3.4.6 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-MPD, **1200f**



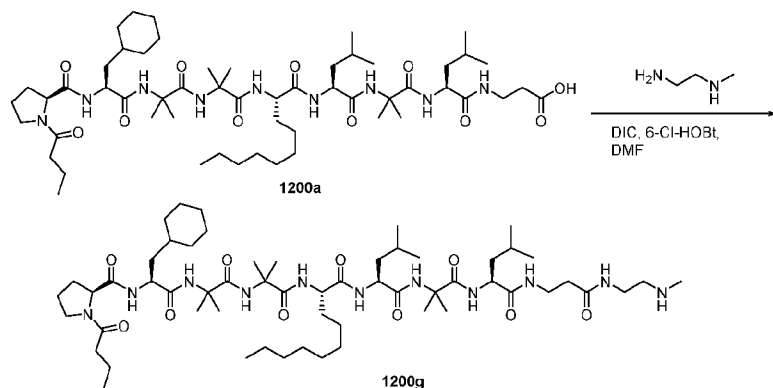
Scheme 110. Synthesis of peptide analogue **1200f**.

20

[0663] DIPEA (6 μ L, 32.6 μ mol) was added to a solution of MPD TFA salt **F2b** (9.3 mg, 32.6 μ mol) in DMF (300 μ L) and the mixture allowed to stand for 10 min. The solution was then added to a mixture of the peptide **1200a** (11.5 mg, 10.9 μ mol), DIC (10 μ L, 65.1

μmol), and 6-Cl-HOBt (11.0 mg, 65.1 μmol) in DMF (300 μL) and the reaction mixture agitated overnight at rt. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200f** as a white amorphous solid (5 mg, 41% yield, 19% overall yield, >98% purity); R_t 27.5 min; LRMS: m/z (ESI-MS) 1130.7 ($[M+H]^+$ requires 1130.8).

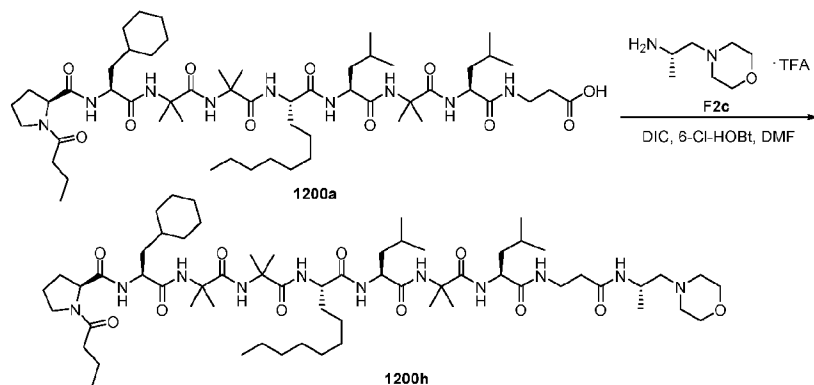
3.4.7 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-MED, 1200g



Scheme 111. Synthesis of peptide analogue **1200g**.

[0664] N^1 -Methylethane-1,2-diamine (2.6 mg, 35.4 μmol) was added to a mixture of the peptide **1200a** (12.5 mg, 11.8 μmol), DIC (11 μL, 70.7 μmol), and 6-Cl-HOBt (12 mg, 70.7 μmol) in DMF (300 μL) and the reaction mixture agitated overnight at rt. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200g** as a white amorphous solid (11 mg, 84% yield, 39% overall yield, >98% purity); R_t 26.0 min; LRMS: m/z (ESI-MS) 1116.7 ($[M+H]^+$ requires 1116.8).

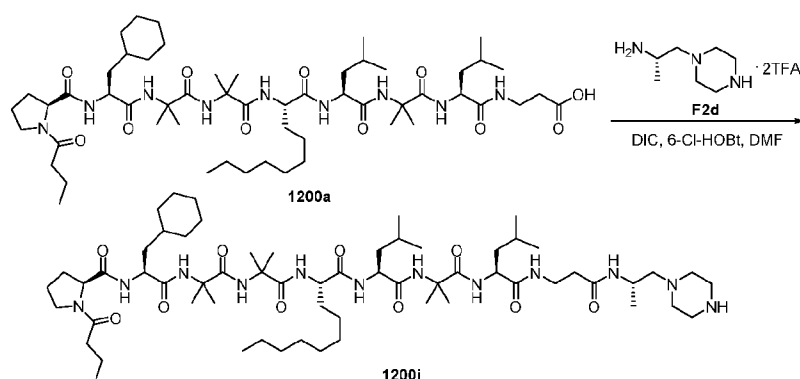
3.4.8 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-MPA, 1200h



Scheme 112. Synthesis of peptide analogue **1200h**.

[0665] DIPEA (6.2 μ L, 35.4 μ mol) was added to a solution of MPA TFA salt **F2c** (13.2 mg, 35.4 μ mol) in DMF (300 μ L) and the mixture allowed to stand for 10 min. The solution was then added to a mixture of the peptide **1200a** (12.5 mg, 11.8 μ mol), DIC (11 μ L, 70.7 μ mol), and 6-Cl-HOBt (12 mg, 70.7 μ mol) in DMF (300 μ L) and the reaction mixture agitated overnight at rt. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200h** as a white amorphous solid (8 mg, 57% yield, 27% overall yield, > 98% purity); R_t 29.7 min; LRMS: m/z (ESI-MS) 1186.6 ($[M+H]^+$ requires 1186.9).

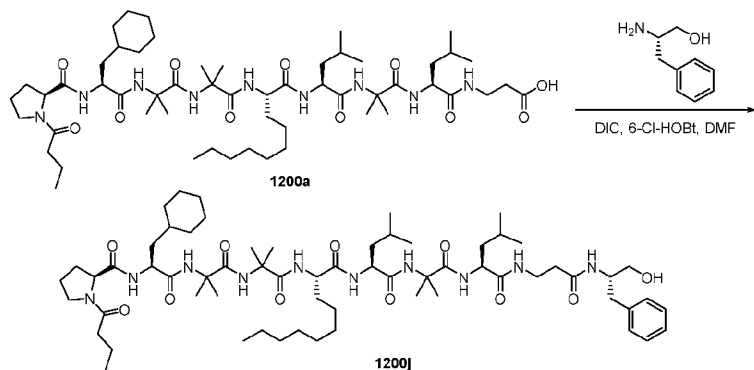
3.4.9 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu- β Ala-PAPA, **1200i**



Scheme 113. Synthesis of peptide analogue **1200i**.

[0666] DIPEA (21.5 μ L, 123 μ mol) was added to a solution of PAPA TFA salt **F2d** (19.9 mg, 32.6 μ mol) in DMF (300 μ L) and the mixture allowed to stand for 10 min. The solution was then added to a mixture of the peptide **1200a** (14.5 mg, 13.7 μ mol), DIC (12.7 μ L, 82.0 μ mol), and 6-Cl-HOBt (13.9 mg, 82.0 μ mol) in DMF (300 μ L) and the reaction mixture agitated overnight at rt. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200i** as a white amorphous solid (9 mg, 56% yield, 26% overall yield, >98% purity); R_t 22.7 min; LRMS: m/z (ESI-MS) 1185.8 ($[M+H]^+$ requires 1185.9).

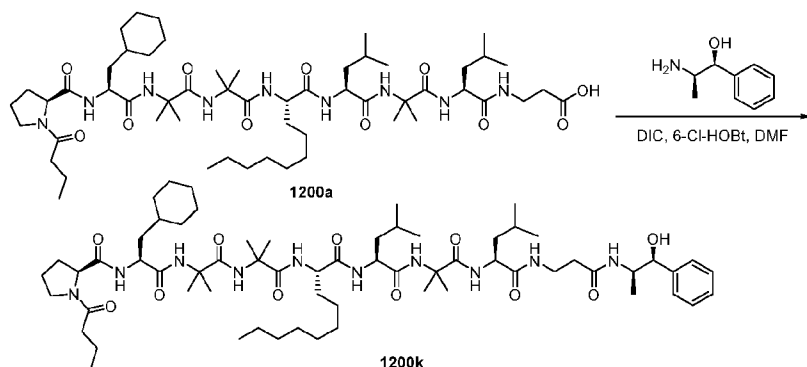
3.4.10 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-Pheol, 1200j



Scheme 114. Synthesis of peptide analogue **1200j**.

[0667] DIPEA (6.7 μL , 38.2 μmol), L-phenylalaninol (5.8 mg, 38.2 μmol), DIC (12 μL , 76.4 μmol), and 6-Cl-HOBt (13 mg, 76.4 μmol) were added to a solution of peptide **1200a** (13.5 mg, 12.7 μmol) in DMF (600 μL) and the mixture agitated at rt overnight. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200j** as a white amorphous solid (10 mg, 66% yield, 31% overall yield, >98% purity); R_t 29.2 min; LRMS: m/z (ESI-MS) 1193.8 ($[\text{M}+\text{H}]^+$ requires 1193.8).

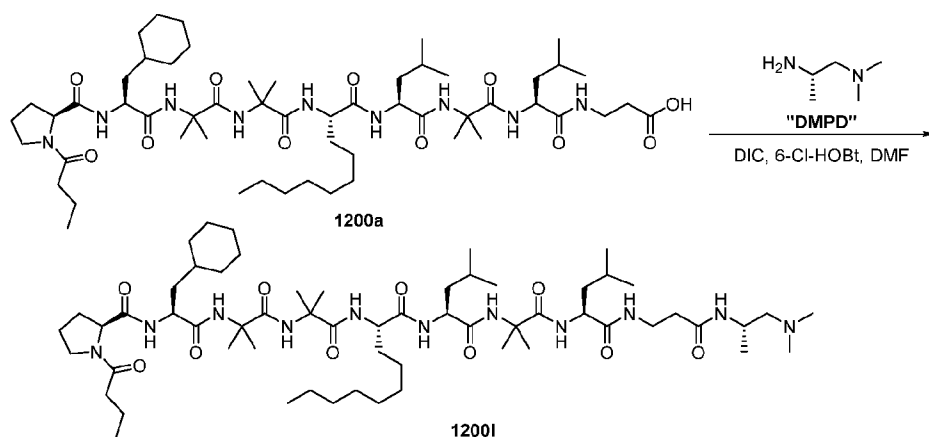
3.4.11 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-(1S,2R)-norephedrine, 1200k



Scheme 115. Synthesis of peptide analogue **1200k**.

[0668] DIPEA (7.2 μL , 41.0 μmol), (1S,2R)-2-amino-1-phenylpropan-1-ol (6.2 mg, 41.0 μmol), DIC (13 μL , 82.0 μmol), and 6-Cl-HOBt (14 mg, 82.0 μmol) were added to a solution of peptide **1200a** (14.5 mg, 13.7 μmol) in DMF (600 μL) and the mixture agitated at rt overnight. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200k** as a white amorphous solid (13 mg, 80% yield, 37% overall yield, >98% purity); R_t 28.9 min; LRMS: m/z (ESI-MS) 1193.7 ($[\text{M}+\text{H}]^+$ requires 1193.8).

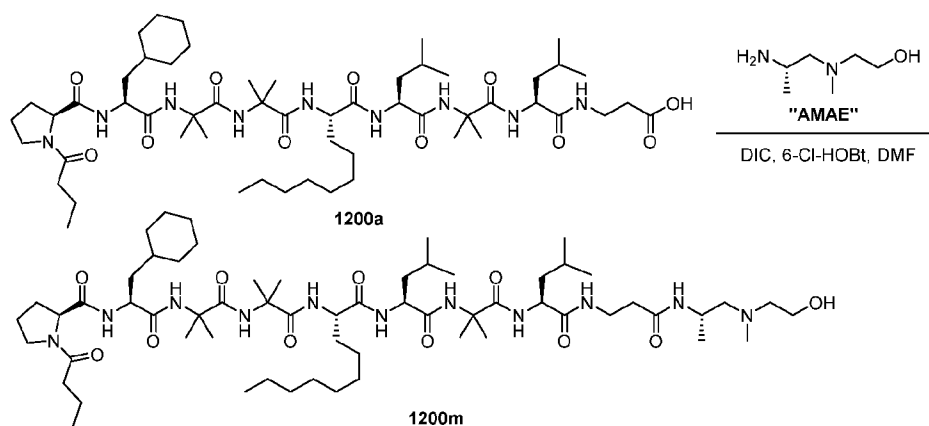
3.4.12 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-DMPD, 1200l



Scheme 115A. Synthesis of peptide analogue **1200l**.

- 5 **[0669]** DIPEA (39 μ L, 223 μ mol), **DMPD-TFA (F2e)** (48 mg, 223 μ mol), DIC (69 μ L, 447 μ mol), and 6-Cl-HOBt (76 mg, 447 μ mol) were added to a solution of crude peptide **1200a** (79 mg, 74.0 μ mol) in DMF (1 mL) and the mixture agitated at rt overnight. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and
- 10 RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200l** as a white amorphous solid (22 mg, 26% overall yield, 95% purity); R_t 29.8 min; LRMS: m/z (ESI-MS) 1144.8 ($[M+H]^+$ requires 1144.8).

3.4.13 ButyrylPro-Cha-Aib-Aib-AD-Leu-Aib-Leu-βAla-AMAE, 1200m



15

Scheme 115B. Synthesis of peptide analogue **1200m**.

- [0670]** **AMAE** was prepared according to a published procedure (Kavianinia, I. *et al.* Total Synthesis and Stereochemical Revision of the Anti-Tuberculosis Peptaibol Trichoderin A. *Org. Lett.* **2016**, 18 (15), 3878–3881).
- 20 **[0671]** DIPEA (5 μ L, 28.3 μ mol), **AMAE** (4.0 mg, 28.3 μ mol), DIC (9 μ L, 56.6 μ mol), and 6-Cl-HOBt (12 mg, 56.6 μ mol) were added to a solution of crude peptide **1200a** (10.0 mg, 9.43 μ mol) in DMF (1 mL) and the mixture agitated at rt overnight. The crude peptide

was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide analogue **1200m** as a white amorphous solid (4 mg, 36% yield, 95% purity); R_t 25.4 min; LRMS: m/z (ESI-MS) 1174.8 ($[M+H]^+$ requires 1174.9).

3.5 Biological evaluation of analogues:

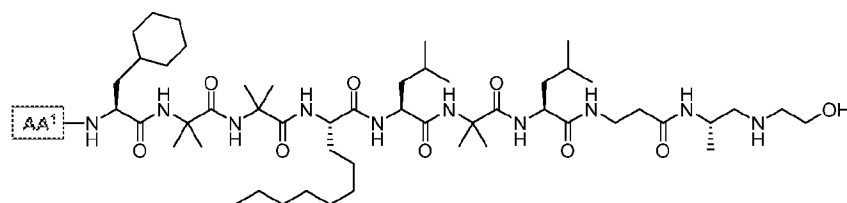
[0672] Analogues were assessed for antiproliferative activity against three breast cancer cell lines (MDA-MB-468, SKBR3, and T47D), as well as a non-small cell lung cancer line (NCI-H460).

[0673] Cells were treated with 3-fold serial dilutions of the peptides and maintained under drug exposure for 5 days. Monitoring of cell proliferation and cell viability was performed by using sulphorhodamine B-based assay as previously described. The IC_{50} was determined by interpolation as the drug concentration reducing staining to 50% of controls on the same plate.

3.6 N-Terminal analogues:

Table 5. Antiproliferative activity of *N*-terminal analogues **1100a** – **1100n**; data for **520** shown for comparison.

Analogues of structure:



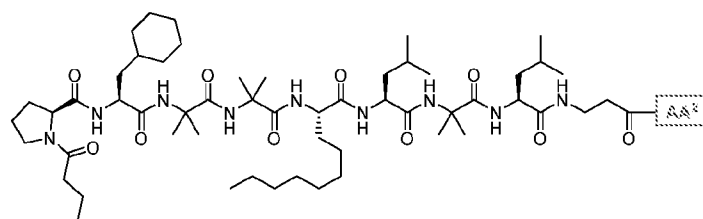
Entry	Analogue number	Activity against cell line (μM , $IC_{50} \pm SEM$)			
		MDA-MB-468	SKBR3	T47D	H460
1	520	0.003 ± 0.0002	0.012 ± 0.005	0.004 ± 0.0001	0.002 ± 0.0004
2	1100a	0.010 ± 0.001	0.057 ± 0.017	0.013 ± 0.003	0.009 ± 0.002
3	1100b	0.013 ± 0.001	0.052 ± 0.012	0.015 ± 0.002	0.011 ± 0.001
4	1100c	0.005 ± 0.001	0.018 ± 0.004	0.007 ± 0.001	0.004 ± 0.0005
5	1100d	0.004 ± 0.001	0.015 ± 0.002	0.007 ± 0.001	0.004 ± 0.0004
6	1100e	0.006 ± 0.001	0.020 ± 0.003	0.013 ± 0.001	0.009 ± 0.001
7	1100f	0.023 ± 0.002	0.073 ± 0.012	0.063 ± 0.009	0.037 ± 0.002
8	1100g	0.003 ± 0.0005	0.010 ± 0.002	0.005 ± 0.001	0.002 ± 0.0002

9	1100h	0.011 ± 0.001	0.034 ± 0.005	0.011 ± 0.001	0.009 ± 0.001
10	1100i	0.037 ± 0.004	0.124 ± 0.024	0.038 ± 0.006	0.040 ± 0.004
11	1100j	0.059 ± 0.0045	0.093 ± 0.0145	0.087 ± 0.0162	0.085 ± 0.0068
12	1100k	0.038 ± 0.0045	0.087 ± 0.0147	0.068 ± 0.0117	0.060 ± 0.0047
13	1100l	0.014 ± 0.002	0.041 ± 0.009	0.017 ± 0.003	0.014 ± 0.005
14	1100m	0.014 ± 0.0013	0.056 ± 0.011	0.016 ± 0.0026	0.012 ± 0.0008
15	1100n	0.164 ± 0.015	0.310 ± 0.016	0.164 ± 0.007	0.182 ± 0.0096

3.7 C-Terminal analogues

Table 6. Antiproliferative activity of C-terminal analogues **1200a** – **1200m**; data for **520** shown for comparison.

Analogues of structure:



Entry	Analogue number	Activity against cell line (μM, IC ₅₀ ± SEM)			
		MDA-MB-468	SKBR3	T47D	H460
1	520	0.003 ± 0.0002	0.012 ± 0.005	0.004 ± 0.0001	0.002 ± 0.0004
2	1200a	>1	>1	>1	>1
3	1200b	0.031 ± 0.005	0.205 ± 0.098	0.031 ± 0.004	0.050 ± 0.010
4	1200c	0.010 ± 0.002	0.067 ± 0.002	0.015 ± 0.004	0.017 ± 0.004
5	1200d	0.336 ± 0.032	0.509 ± 0.054	0.490 ± 0.020	0.130 ± 0.004
6	1200e	0.363 ± 0.013	0.512 ± 0.050	0.767 ± 0.123	0.223 ± 0.014
7	1200f	0.003 ± 0.0005	0.013 ± 0.002	0.005 ± 0.001	0.003 ± 0.0004
8	1200g	0.005 ± 0.001	0.027 ± 0.002	0.008 ± 0.001	0.010 ± 0.001
9	1200h	0.059 ± 0.005	0.099 ± 0.010	0.036 ± 0.003	0.034 ± 0.001
10	1200i	>1	>1	>1	>1
11	1200j	0.144 ± 0.011	0.178 ± 0.012	0.247 ± 0.048	0.045 ± 0.002
12	1200k	0.242 ± 0.023	0.344 ± 0.015	0.471 ± 0.081	0.089 ± 0.005
13	1200l	0.004 ±	0.010 ±	0.006 ±	0.004 ±

		0.0003	0.0013	0.0009	0.0006
14	1200m	0.002 ±	0.008 ±	0.003 ±	0.002 ±
		0.0003	0.0013	0.0002	0.0002

[0674] As shown in Tables 5 and 6 above, all of the analogues had antiproliferative activity against the cell lines tested.

5 **4. Example 4**

[0675] This example builds on the SAR from examples 1 to 3 above.

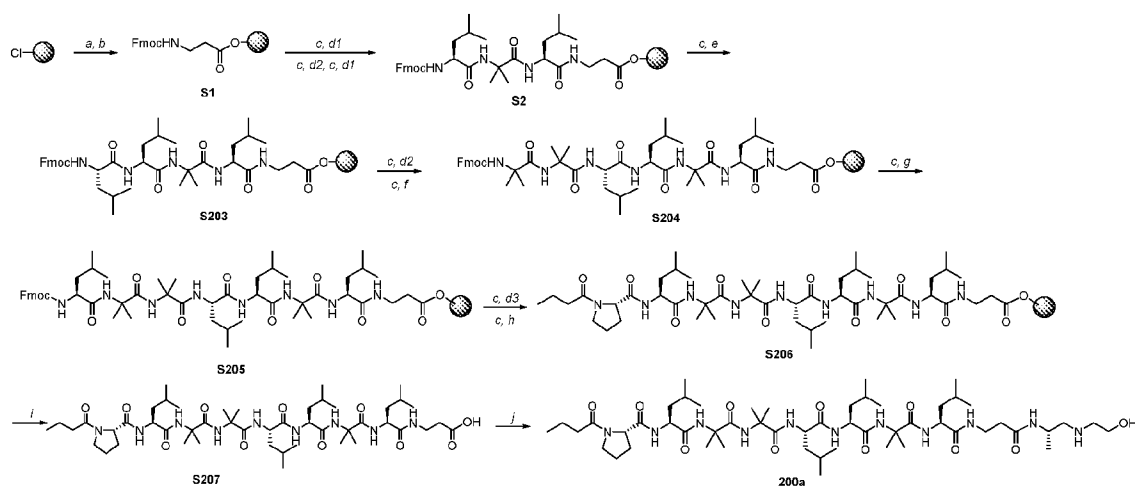
4.1 General methods and materials

[0676] The general methods, materials and abbreviations used in this example are the same as those outlined in sections 1.1 to 1.5 above.

4.2 Synthesis of analogues

4.2.1 Synthesis of analogue 200a

[0677] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-Leu-OH was coupled according to **Method 3**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S204**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Leu-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S206**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S207** as white fluffy flakes (64 mg, 66% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford analogue **200a** as a white amorphous solid (25 mg, 23 % overall yield, > 98% purity); R_t 20.9 min; LRMS: *m/z* (ESI-MS) 1064.7 ([M+H]⁺ requires 1064.7).

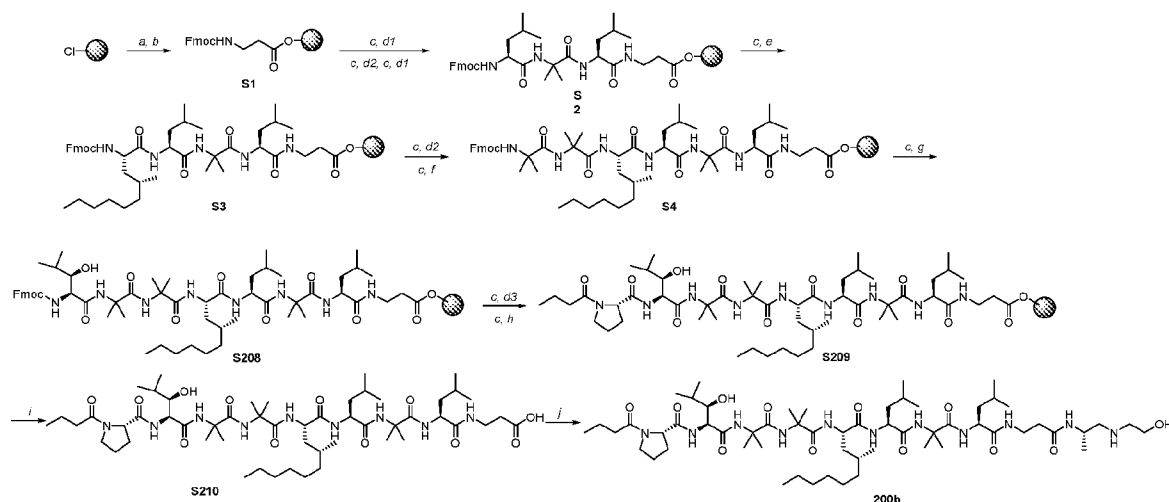


Scheme 116: Reagents and conditions: a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-Leu-OH, HATU, DIPEA, DMF, 2 × 1 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Leu-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

4.2.2 Synthesis of analogue 200b

[0678] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S4**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-(2S,3R)HyLeu-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S209**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S210** as white fluffy flakes (15 mg, 14% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to

afford analogue **200b** as a white amorphous solid (3 mg, 3% overall yield, > 97% purity); R_t 22.8 min; LRMS: m/z (ESI-MS) 1150.8 ($[M+H]^+$ requires 1150.8).

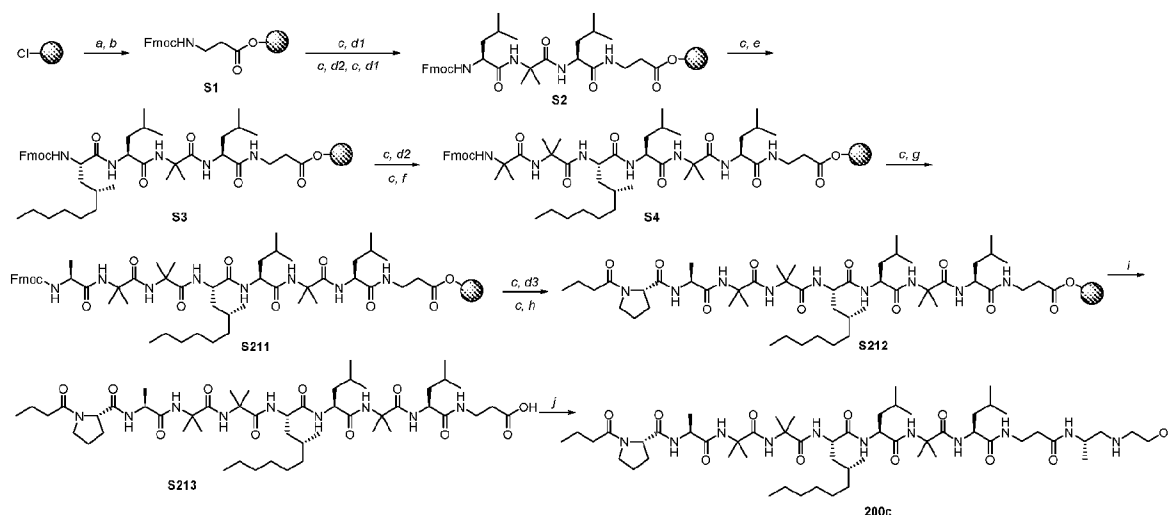


Scheme 117: Reagents and conditions: a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-(2*S*,3*R*)HyLeu-OH, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

4.2.3 Synthesis of analogue 200c

[0679] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to **Method 5**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-Ala-OH was then coupled using **Method 3** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S212**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S213** as white fluffy flakes (57 mg, 62% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method.

Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford analogue **200c** as a white amorphous solid (37 mg, 34% overall yield, > 98% purity); R_t 23.1 min; LRMS: m/z (ESI-MS) 1092.7 ($[M+H]^+$ requires 1092.7).

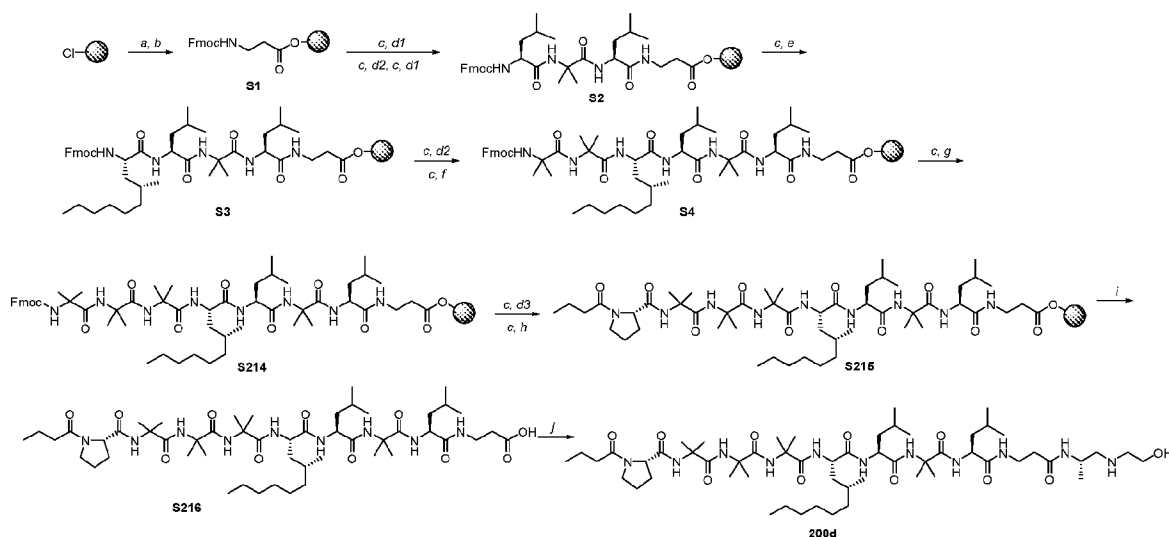


Scheme 118: Reagents and conditions: a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Ala-OH, HATU, DIPEA, DMF, 2 × 1 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

4.2.4 Synthesis of analogue 200d

[0680] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S4**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Aib-OH was then coupled using **Method 4** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S215**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S216** as white fluffy flakes (49 mg, 48% yield). Late-stage solution phase C-terminal coupling was employed to

introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford analogue **200d** as a white amorphous solid (22 mg, 20% overall yield, > 98% purity); R_t 25.3 min; LRMS: m/z (ESI-MS) 1106.7 ($[M+H]^+$ requires 1106.8).

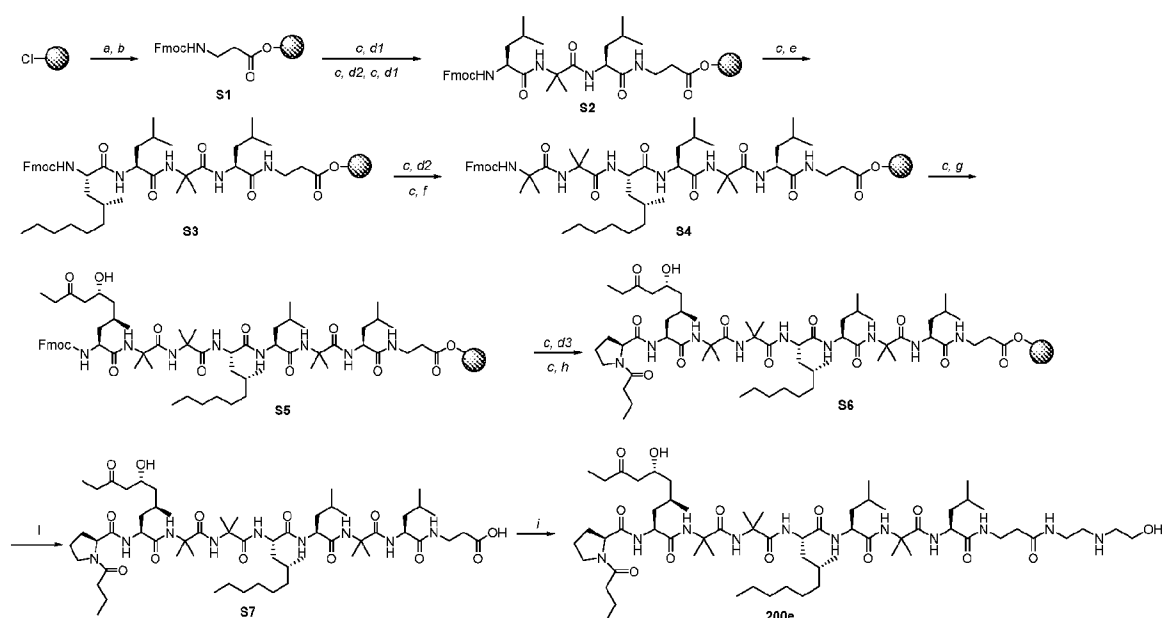


Scheme 119: Reagents and conditions: a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; HFIP/CH₂Cl₂ (1:4), 0.5 h; j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

4.2.5 Synthesis of analogue 200e

[0681] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotriyl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^α-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AMD-OH was coupled according to **Method 5**. The N^α-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S4**. After removal of N^α-Fmoc-protecting group using **Method 2**, Fmoc-(2S,4S,6R)-AHMOD was then coupled using **Method 6** followed by coupling of Fmoc-Pro-OH using **Method 3** and butyric acid via **Method 7** to complete the linear peptide sequence of **S6**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method.

Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford peptide **S7** as white fluffy flakes (25 mg, 22% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct m/z were combined and lyophilised to afford analogue **200e** as a white amorphous solid (8 mg, 6% overall yield, > 97% purity); R_t 23.2 min; LRMS: m/z (ESI-MS) 1220.6 ($[M+H]^+$ requires 1220.8).

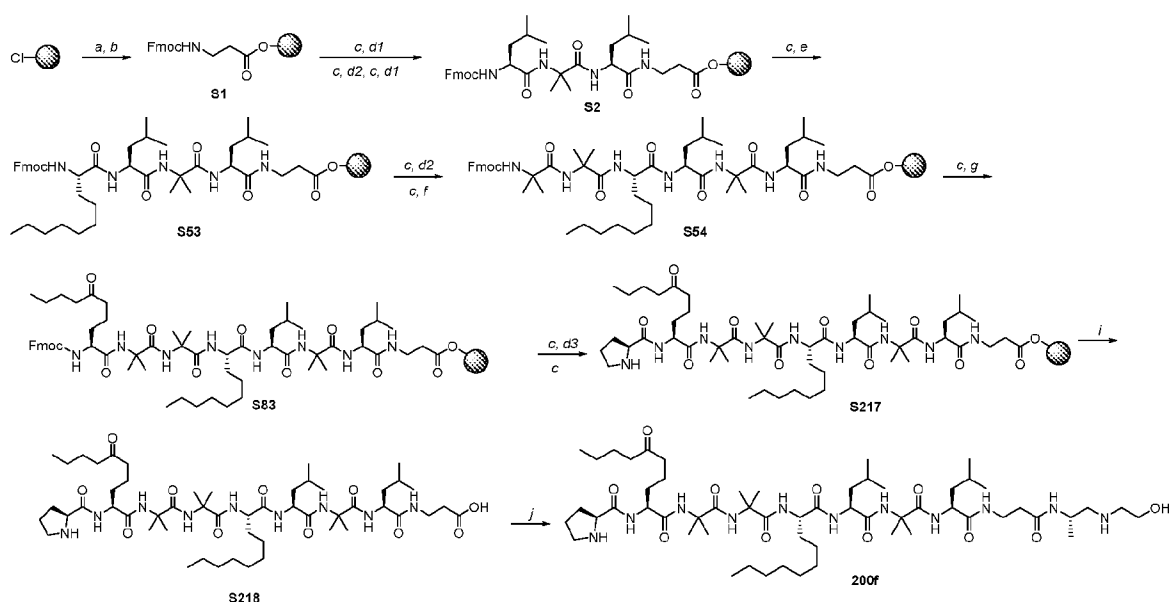


Scheme 120: Reagents and conditions: a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AMD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-(6R)-AHMOD-OH, HATU, DIPEA, DMF, 2 h; h) butyric acid, HATU, DIPEA, DMF, 2 × 1 h; i) AEAE, DIC, 6-Cl-HOBt, DMF, 12 h.

4.2.6 Synthesis of analogue 200f

[0682] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to

form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** to complete the linear peptide sequence of **S217**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S218** as white fluffy flakes (14 mg, 12% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford analogue **200f** as a white amorphous solid (7 mg, 5 % overall yield, > 98% purity); R_t 25.8 min; LRMS: *m/z* (ESI-MS) 1216.8 ([M+TFA]⁺ requires 1216.8).

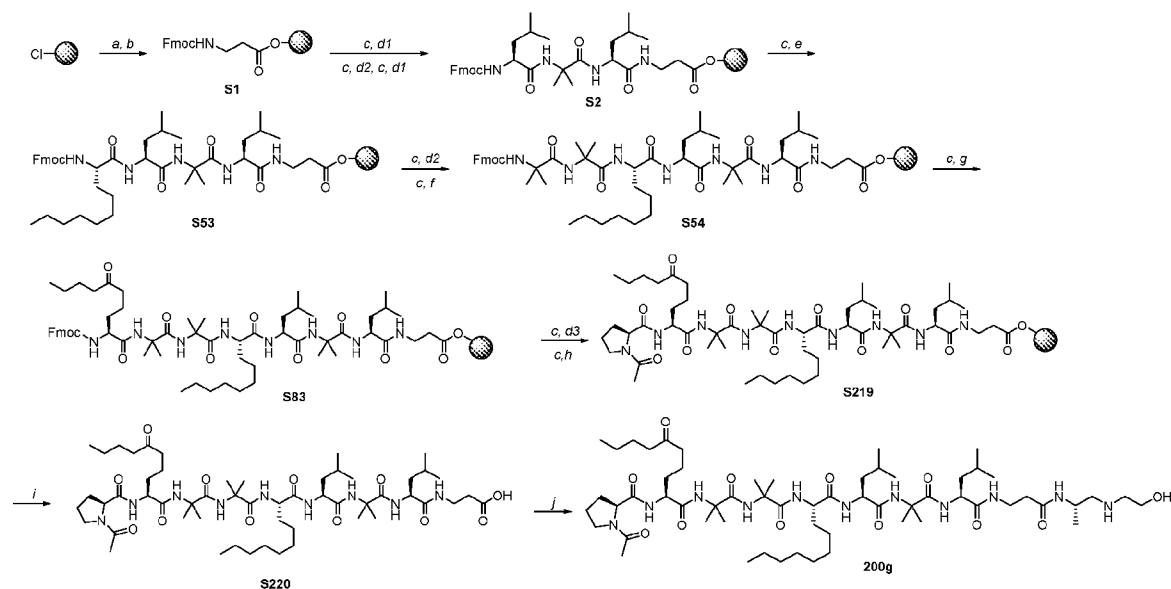


Scheme 121 : Reagents and conditions: a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-A6OD-OH, COMU, Oxyma, DIPEA, DMF, 2 h; h) HFIP/CH₂Cl₂ (1:4), 0.5 h; i) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

4.2.7 Synthesis of analogue 200g

[0683] The C-terminal residue Fmoc-β-alanine-OH, was attached to 2-chlorotrityl chloride functionalised polystyrene resin according to **Method 1** to form peptidyl resin **S1**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group was

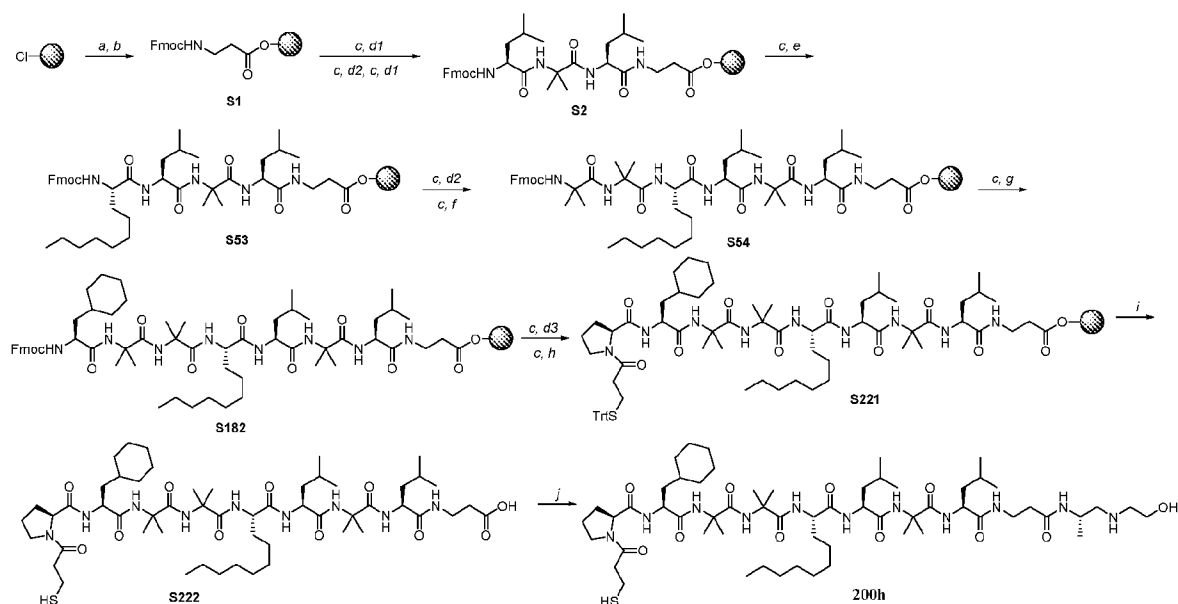
then removed using **Method 2** and Fmoc-AD-OH was coupled according to **Method 5**. The N^o-Fmoc-protecting group was removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form peptidyl resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-A6OD-OH was then coupled using **Method 11** followed by coupling of Fmoc-Pro-OH using **Method 3** followed by capping via **Method 13** to complete the linear peptide sequence of **S219**. Resin-bound peptide was cleaved using **Method 8**, and the crude residue was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford peptide **S220** as white fluffy flakes (22 mg, 19% yield). Late-stage solution phase C-terminal coupling was employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide was purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions were collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* were combined and lyophilised to afford analogue **200g** as a white amorphous solid (11 mg, 9 % overall yield, > 98% purity); R_t 24.6 min; LRMS: *m/z* (ESI-MS) 1162.9 ([M+H]⁺ requires 1162.8).



Scheme 122: Reagents and conditions: a) Fmoc-βAla-OH, DIPEA, CH₂Cl₂, 1 × 6 h, 1 × 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 × 5 min, 1 × 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 × 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 × 2 h; g) Fmoc-A6OD-OH, HATU, DIPEA, DMF, 2 h; h) 20% Ac₂O/DMF, 2 × 15 min; i) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

4.2.8 Synthesis of analogue 200h

[0684] The C-terminal residue Fmoc- β -alanine-OH, is attached to 2-chlorotriethyl chloride functionalised polystyrene resin according to **Method 1** to form resin **S1**. The N^o-Fmoc-protecting group is removed using **Method 2** followed by coupling of Fmoc-Leu-OH, Fmoc-Aib-OH and Fmoc-Leu-OH via **Method 3**. The N^o-Fmoc-protecting group is then removed using **Method 2** and Fmoc-AD-OH is coupled according to **Method 5**. The N^o-Fmoc-protecting group is removed using **Method 2** followed by coupling of Fmoc-Aib-OH via **Method 3**, and subsequently the coupling of second Fmoc-Aib-OH via **Method 4** to form resin **S54**. After removal of N^o-Fmoc-protecting group using **Method 2**, Fmoc-Cha-OH is then coupled using **Method 3**, followed by coupling of Fmoc-Pro-OH using **Method 3** and 3-(tritylthio)propionic acid via **Method 3** to complete the linear peptide sequence of **S221**. Resin-bound peptide is cleaved using **Method 9**, and the crude residue is purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions are collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* are combined and lyophilised to afford peptide **S222**. Late-stage solution phase C-terminal coupling is employed to introduce the C-terminal aminoalcohol moiety using **Method 10**. The crude peptide is purified by semi-preparative RP-HPLC using conditions outlined in the general method. Fractions are collected at 1 min intervals and analysed by ESI-MS and RP-HPLC. Fractions identified with the correct *m/z* are combined and lyophilised to afford culicinin D analogue **200 h**.



Scheme 123. Synthesis of culicinin D analogue **200h**. **Reagents and conditions:** a)

Fmoc- β Ala-OH, DIPEA, CH₂Cl₂, 1 $\times$ 6 h, 1 $\times$ 12 h; b) CH₂Cl₂/MeOH/DIPEA (8:1.5:0.5, v/v); c) 20% piperidine/DMF, 1 $\times$ 5 min, 1 $\times$ 15 min; d) Fmoc-AA-OH, HATU, DIPEA, DMF, 2 $\times$ 1 h, d1: Fmoc-Leu-OH, d2: Fmoc-Aib-OH, d3: Fmoc-Pro-OH; e) Fmoc-AD-OH, COMU, Oxyma, DIPEA, DMF, 3 h; f) Fmoc-Aib-OH, COMU, Oxyma, DIPEA, DMF, 2 $\times$ 2 h; g) Fmoc-Cha-OH,

HATU, DIPEA, DMF, 2 h; h) 3-(tritylthio)propionic acid, HATU, DIPEA, DMF, 2 × 1 h; i) TFA/TIPS/H₂O (95:2.5:2.5, v/v/v); j) APAE, DIC, 6-Cl-HOBt, DMF, 12 h.

4.3 Biological evaluation of analogues

5 **[0685]** Analogues were assessed for antiproliferative activity against three breast cancer cell lines (MDA-MB-468, SKBR3, and T47D), as well as a non-small cell lung cancer line (NCI-H460).

10 **[0686]** Cells were treated with 3-fold serial dilutions of the peptides and maintained under drug exposure for 5 days. Monitoring of cell proliferation and cell viability was performed by using sulphorhodamine B-based assay as previously described. The IC₅₀ was determined by interpolation as the drug concentration reducing staining to 50% of controls on the same plate.

[0687] **Table 7.** Antiproliferative activity of analogues **200a to 200g**.

Entry	Analogue	Activity against cell line (μM, IC ₅₀ ± SEM)			
		MDA-MB-468	SKBR3	T47D	H460
1	200a	0.32 ± 0.026	> 1	0.33 ± 0.05	> 0.5
2	200b	0.37 ± 0.14	> 0.5	> 0.5	> 0.5
3	200c	0.017 ± 0.002	0.067 ± 0.011	0.014 ± 0.001	0.016 ± 0.004
4	200d	0.020 ± 0.002	0.044 ± 0.006	0.009 ± 0.002	0.024 ± 0.007
5	200e	0.044 ± 0.013	0.189 ± 0.034	0.027 ± 0.007	0.084 (n = 1)
6	200f	0.032 ± 0.006	0.088 ± 0.027	0.022 ± 0.003	0.040 ± 0.002
7	200g	0.041 ± 0.007	0.107 ± 0.011	0.024 ± 0.005	0.048 ± 0.002

15 5. Example 5

[0688] This example investigated the mechanism of action of culicinin D.

20 **[0689]** A whole-genome CRISPR knockout screen was performed in the NZM37 cutaneous melanoma cell line (*TERT* mutant, *BRAF* wild type, *NRAS* wild type). NZM37 cells previously transduced with the GeCKOv2 single guide RNA (sgRNA) library as described in Sanjana *et al.* (Sanjana, N. E., Shalem, O. & Zhang, F. Improved vectors and genome-wide libraries for CRISPR screening. *Nat. Methods* 11, 783–784 (2014)) (lentiGuide-Puro vector) were exposed to culicinin D in an on-off, escalating schedule that inhibited total growth by a factor of approximately 10⁷ relative to nontreated control cultures (Fig. 1 and 2).

25 **[0690]** Separate treatments with the known mitochondrial-uncoupling peptides leucinostatins A and B, as described in Shima *et al.* (Shima, a, Fukushima, K., Arai, T. & Terada, H. Dual inhibitory effects of the peptide antibiotics leucinostatins on oxidative phosphorylation in mitochondria. *Cell Struct. Funct.* 15, 53–8 (1990)), were included as a parallel reference article.

30 **[0691]** Assessment of the antiproliferative activity of culicinin D and leucinostatins A and B at the conclusion of the screen identified that cultures challenged with either agent

had developed resistance to both relative to drug-naïve cultures (Fig. 3). Characterisation of sgRNA distributions in drug-treated and control cultures (all $n = 3$) by principal component analysis identified highly consistent distributions in control cultures, with divergence in drug-challenged libraries consistent with selection effects (Fig. 4). Deconvolution of the screens and calling of positively selected hits (i.e. CRISPR-induced loss-of-function mutations enriched following drug treatment) using the MAGeCK, RIGER, PinAPL-Py and CRISPRcloud2 statistical algorithms identified genes putatively involved in cellular response to these agents. Notably, high-confidence hits were shared by the two articles and included *KEAP1*, *OMA1*, *MRRF* and *SLC25A37*, all factors intimately involved in mitochondrial biology.

[0692] The evolution of cross-resistance to culicinin D and leucinoastatins in NZM37 culture challenged with either agent, the consistency in sgRNA enrichment observed for both articles and known functions of highly selected genes in mitochondrial biology is in keeping with a shared mechanism of action for culicinin D and leucinoastatins A and B, likely involving mitochondrial uncoupling.

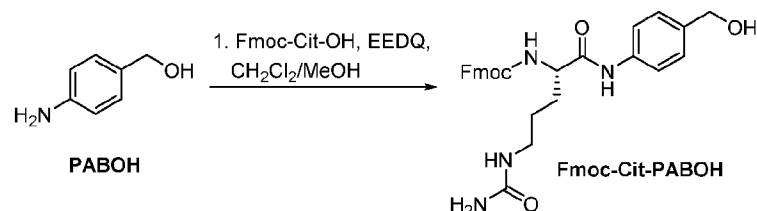
6. Example 6

[0693] This example describes the preparation of a drug-ligand conjugate of the invention.

6.1 Synthesis of Trastuzumab-MC-Val-Cit-PABC-520

6.1.1 Synthesis of Fmoc-Cit-PABOH

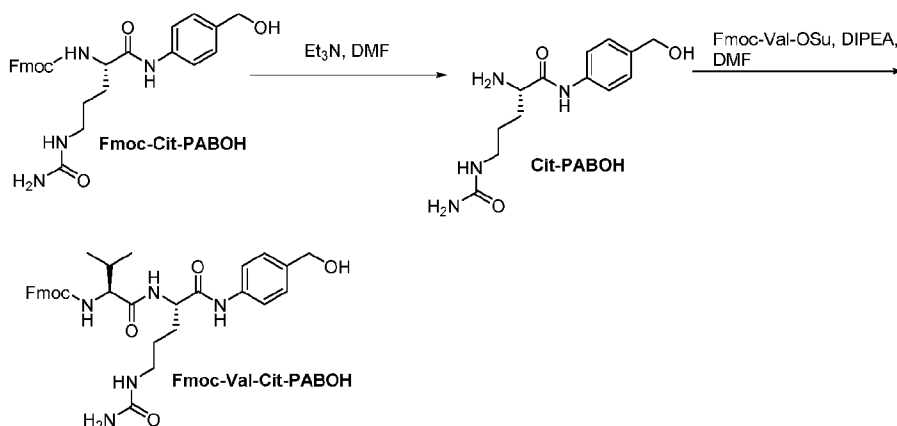
[0694] A solution of Fmoc-Cit-OH (500 g, 1.2 mmol), 4-aminobenzyl alcohol (154 mg, 1.2 mmol) and EEDQ (296 mg, 1.2 mmol) in THF (50 mL) was stirred at room temperature overnight, and the solvent was then removed *in vacuo*. Subsequent trituration (Et₂O) and collection by filtration (washing with Et₂O) afforded *Fmoc-Cit-PABOH* which was used without further purification (390 mg, 64% yield based on crude purity, 85% purity by analytical LCMS as judged by peak area of RP-HPLC at 210 nm). Analytical LC-MS: Agilent Zorbax-C3, (150 mm x 3.0 mm, 3.5 μ m), linear gradient of 95% A:5% B to 5% A:95% B, where solvent A was 0.1% formic acid in H₂O and B was 0.1% formic acid in acetonitrile, (ca. 3% B/min), 0.3 mL/min; R_t 17.7 min; m/z (ESI-MS) $[M+H]^+$ calcd: 503.2; found: 503.3.



Scheme 124A: Synthesis of Fmoc-Cit-PABOH

6.1.2 Synthesis of Fmoc-Val-Cit-PABOH

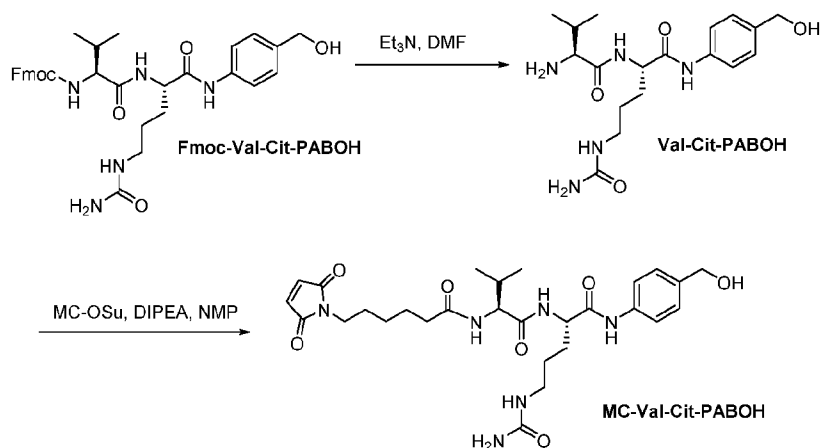
[0695] A solution of Fmoc-Cit-PABOH (390 mg, 0.77 mmol) in 40% triethylamine/DMF (v/v) (10 mL) was stirred at room temperature for 24 h, and the solvent then removed *in vacuo* to afford Cit-PABOH, which was used without further purification. A solution of crude Cit-PABOH (from Fmoc-Cit-PABOH, 0.77 mmol), Fmoc-Val-OSu (335 mg, 0.77 mmol) and DIPEA (133 μ L, 0.77 mmol) in DMF (15 mL) was stirred at room temperature overnight, and the solvent was then removed *in vacuo*. Subsequent trituration (Et₂O) and collection by filtration (washing with Et₂O) afforded *Fmoc-Val-Cit-PABOH* which was used without further purification (285 mg, 61% yield based on crude purity, 95% purity by analytical LCMS as judged by peak area of RP-HPLC at 210 nm). Analytical LC-MS: Agilent Zorbax-C3, (150 mm x 3.0 mm, 3.5 μ m), linear gradient of 95% A:5% B to 5% A:95% B, where solvent A was 0.1% formic acid in H₂O and B was 0.1% formic acid in acetonitrile, (ca. 3% B/min), 0.3 mL/min; R_t 18.8 min; *m/z* (ESI-MS) [M+H]⁺ calcd: 602.3; found: 602.3.



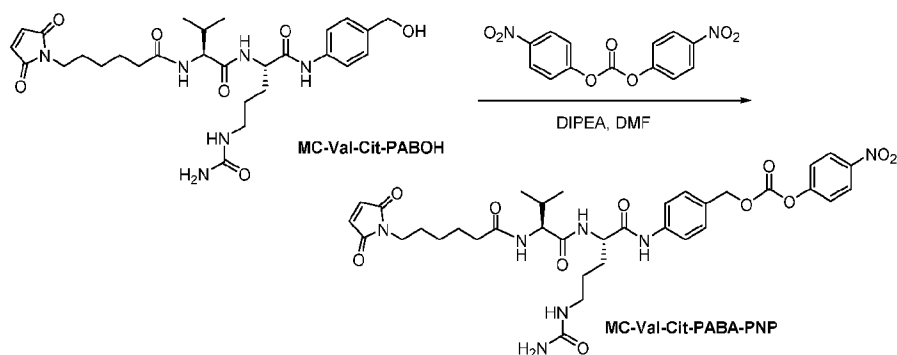
Scheme 125A: Synthesis of Fmoc-Val-Cit-PABOH

6.1.3 Synthesis of MC-Val-Cit-PABOH

[0696] A solution of Fmoc-Val-Cit-PABOH (285 mg, 0.47 mmol) in 40% triethylamine/DMF (v/v) (10 mL) was stirred at room temperature for 24 h, and the solvent was then removed *in vacuo* to afford Val-Cit-PABOH, which was used without further purification. A solution of crude Val-Cit-PABOH (from Fmoc-Cit-PABOH, 0.47 mmol), 6-Maleimidohexanoic acid *N*-hydroxysuccinimide ester (144 mg, 0.47 mmol) and DIPEA (80 μ L, 0.47 mmol) in DMF (5 mL) was stirred at room temperature overnight, and the solvent was then removed *in vacuo*. Subsequent trituration (Et₂O) and collection by filtration (washing with Et₂O) afforded *MC-Val-Cit-PABOH* which was used without further purification (115 mg, 42% yield based on crude purity, 85% purity by analytical LCMS as judged by peak area of RP-HPLC at 210 nm). Analytical LC-MS: Agilent Zorbax-C3, (150 mm x 3.0 mm, 3.5 μ m), linear gradient of 95% A:5% B to 5% A:95% B, where solvent A was 0.1% formic acid in H₂O and B was 0.1% formic acid in acetonitrile, (ca. 3% B/min), 0.3 mL/min; R_t 14.2 min; *m/z* (ESI-MS) [M+H]⁺ calcd: 573.3; found: 573.3.

**Scheme 126A:** Synthesis of MC-Val-Cit-PABOH**6.1.4 Synthesis of MC-Val-Cit-PABC-PNP**

- 5 **[0697]** A solution of MC-Val-Cit-PABOH (115 mg, 0.2 mmol), bis(4-nitrophenyl) carbonate (0.60 mg, 0.2 mmol) and DIPEA (34 μ L 0.2 mmol) in DMF (5 mL) was stirred at room temperature for 40 h, and the solvent was then removed *in vacuo*. The crude MC-Val-Cit-PABC-PNP was purified by semi-preparative RP-HPLC using a Dionex UltiMate® 3000 on a Agilent C18 column (Zorbax 300SB-C18, 9.4 x 250 mm 5 μ m) using a gradient of
- 10 95%A:5%B to 5%A:95%B over 90 min, where solvent A was 0.1% TFA in H₂O and B was 0.1% TFA in acetonitrile, (ca.1% B/min). Fractions identified with the correct *m/z* were combined and lyophilised to afford *MC-Val-Cit-PABC-PNP* as a white amorphous solid (20 mg, 14% yield, 90% purity by analytical LCMS as judged by peak area of RP-HPLC at 210 nm). Analytical LC-MS: Agilent Zorbax-C3, (150 mm x 3.0 mm, 3.5 μ m), linear gradient of
- 15 95% A:5% B to 5% A:95% B, where solvent A was 0.1% formic acid in H₂O and B was 0.1% formic acid in acetonitrile, (ca. 3% B/min), 0.3 mL/min; *R_t* 21.2 min; *m/z* (ESI-MS) [M+H]⁺ calcd: 738.5; found: 738.5.

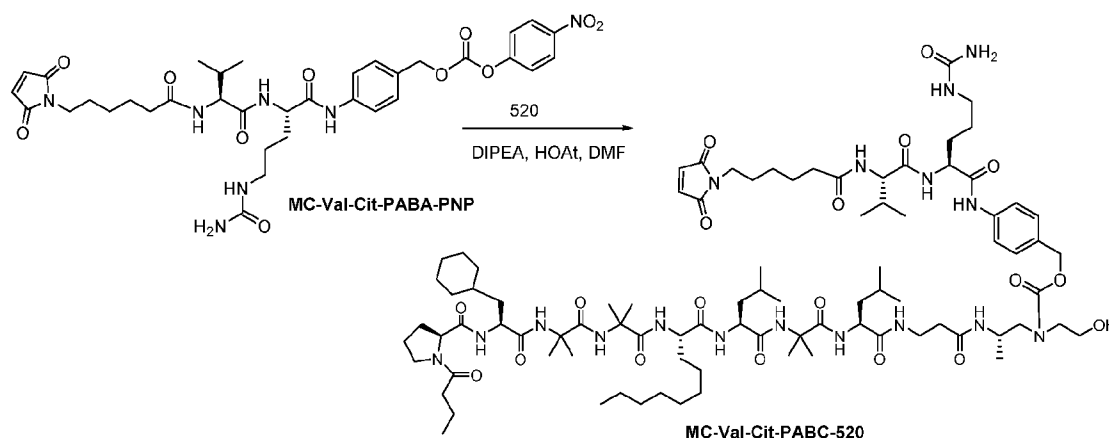
**Scheme 127A:** Synthesis of MC-Val-Cit-PABA-PNP

20

6.1.5 Synthesis of MC-Val-Cit-PABC-520

- [0698]** A solution of MC-Val-Cit-PABC-PNP (20 mg, 0.027 mmol), compound **520** (31 mg, 0.027 mmol), 1-hydroxy-7-azabenzotriazole (HOAt) (4 mg, 0.027 mmol), and DIPEA

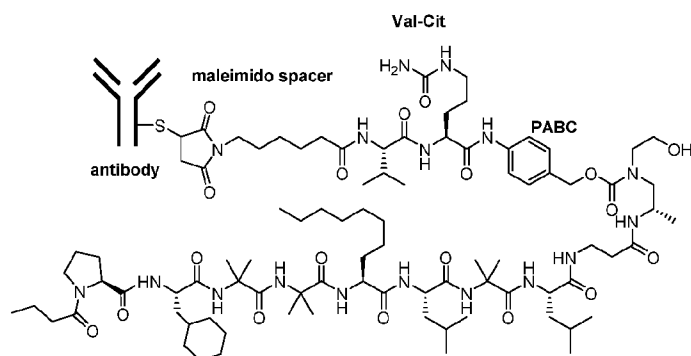
(93 μ L, 0.54 mmol) in DMF (5 mL) was stirred at room temperature for 22 h, and the solvent was then removed *in vacuo*. The crude MC-Val-Cit-PABC-520 was purified by semi-preparative RP-HPLC using a Dionex UltiMate® 3000 on a Agilent C18 column (Zorbax 300SB-C18, 9.4 x 250 mm 5 μ m) using a gradient of 95%A:5%B to 5%:95%B over 90 min, where solvent A was 0.1% TFA in H₂O and B was 0.1% TFA in acetonitrile, (ca.1% B/min). Fractions identified with the correct *m/z* were combined and lyophilised to afford MC-Val-Cit-PABC-520 as a white amorphous solid (19 mg, 40% yield, 85% purity by analytical LCMS as judged by peak area of RP-HPLC at 210 nm). Analytical LC-MS: Agilent Zorbax-C3, (150 mm x 3.0 mm, 3.5 μ m), linear gradient of 95% A:5% B to 5% A:95% B, where solvent A was 0.1% formic acid in H₂O and B was 0.1% formic acid in acetonitrile, (ca. 3% B/min), 0.3 mL/min; *R_t* 26.1 min; *m/z* (ESI-MS) calcd: 1759.2; found:1759.1.



Scheme 128A: Synthesis of MC-Val-Cit-PABC-520

6.1.6 Synthesis of Trastuzumab-MC-Val-Cit-PABC-520

[0699] Trastuzumab antibody (5 mg/mL) in PBS buffer containing 4 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4, was partially reduced with TCEP (3 molar excess) at 25 °C for 2 h. The reductant was then removed by buffer exchange into fresh PBS buffer containing 4 mM EDTA, pH 7.4, by gel filtration (Nap-5 column, GE Healthcare). MC-Val-Cit-PABC-520 (5 eq. per thiol) in DMA (final concentration 10% v/v) was added and the resulting mixture was incubated at 25 °C. After 2 h, the resulting antibody-drug conjugate (ADC) mixture was concentrated by centrifugal ultrafiltration to remove unreacted linker-cytotoxin. Drug to antibody ratio (DAR) was calculated to be 0.9 using hydrophobic interaction chromatography (HIC-HPLC); TSKgel Butyl-NPR column (0.46 x 3.5 cm; Tosoh Bioscience, South San Francisco, CA, USA), linear gradient of 100% A:0% B to 0% A:100% B, where solvent A was 25 mM phosphate buffer, pH 7.0, containing 1.5 M ammonium sulphate and B was 25 mM potassium phosphate, pH 7.0, containing 25% isopropanol.



Scheme 129A: Structure of Trastuzumab-MC-Val-Cit-PABC-520

7. Example 7

- 5 **[0700]** This example describes alternative methods to those set forth in Example 6 that may be used to prepare drug-ligand conjugates of the invention.

7.1 Synthesis of Trastuzumab-MC-Val-Cit-PABC-520

7.1.1 Synthesis of Fmoc-Cit-PABOH

- 10 **[0701]** A solution of Fmoc-Cit-OH (1 eq.), 4-aminobenzyl alcohol (1.5 eq.) and EEDQ (2 eq.) in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (2:1, v/v) is stirred at room temperature overnight, and the solvents are then removed *in vacuo*. Subsequent trituration (Et_2O) and collection by filtration (washing with Et_2O) affords the *Fmoc-Cit-PABOH* which is used without further purification.

7.1.2 Synthesis of Cit-PABOH

- 15 **[0702]** A solution of Fmoc-Cit-PABOH in 20% diethylamine/DMF (v/v) is stirred at room temperature for 3 h, and the solvent is then removed *in vacuo* to afford the Cit-PABOH, which is used without further purification.

7.1.3 Synthesis of Fmoc-Val-Cit-PABOH

- 20 **[0703]** A solution of crude Cit-PABOH (1 eq.), Fmoc-Val-OH (1 eq.), HBTU (1 eq.) and DIPEA (4 eq.) in DMF is stirred at room temperature overnight, and the solvent is then removed *in vacuo*. The crude is purified, such as by flash chromatography on silica gel (MeOH/ CH_2Cl_2 , gradient elution from 0 - 10% MeOH), to give the Fmoc-Val-Cit-PABOH.

7.1.4 Synthesis of Val-Cit-PABOH

- 30 **[0704]** A solution of partially purified Fmoc-Val-Cit-PABOH in 20% diethylamine/DMF (v/v) is stirred at room temperature for 3 h, and the solvent then is removed *in vacuo*. The crude is purified, such as by flash chromatography on silica gel (MeOH/ CH_2Cl_2 , gradient elution from 0 - 10% MeOH), to give the Val-Cit-PABOH.

7.1.5 Synthesis of MC-Val-Cit-PABOH

[0705] A solution of Val-Cit-PABOH (1 eq.) is dissolved in NMP and 6-maleimidocaproic acid *N*-succinimidyl ester (MC-OSu) (1.5 equiv.) is added. The reaction is allowed to stir for 18 h. The volatiles are evaporated at reduced pressure and then the residue is triturated
5 with Et₂O to afford MC-Val-Cit-PABOH.

7.1.6 Synthesis of MC-Val-Cit-PABC-PNP

[0706] MC-Val-Cit-PABOH (1 eq.) is dissolved in DMF and bis(4-nitrophenyl) carbonate (4 eq.) and DIPEA (4 eq.) is added. The reaction mixture is allowed to stir for 3 days. The
10 DMF is removed and the crude is purified, such as by flash chromatography on silica gel (MeOH/CH₂Cl₂, gradient elution from 0 - 10% MeOH), to give the MC-Val-Cit-PABC-PNP.

7.1.7 Synthesis of MC-Val-Cit-PABC-520

[0707] Compound 520 (1 eq.), MC-Val-Cit-PABC-PNP (2 eq.), DIPEA (20 eq.), and
15 HOAt (1.5 eq.) is stirred in DMF for 22 h. The crude peptide is purified by semi-preparative RP-HPLC and lyophilised to afford MC-Val-Cit-PABC-520.

7.1.8 Synthesis of Trastuzumab-MC-Val-Cit-PABC-520

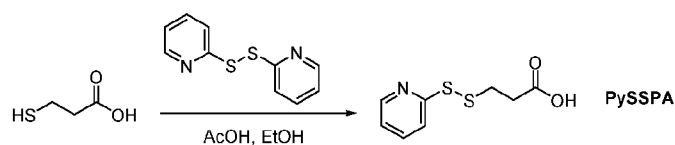
[0708] Antibody drug conjugates (ADC) with an average of four drugs per antibody are
20 prepared by partial reduction of the antibody with an excess of a reducing reagent such as TCEP or DTT at 25 °C in PBS buffer for 2 h. The reductant is then removed by buffer exchange into fresh phosphate buffer by gel filtration (Nap-5 column, GE Healthcare). Determination of the number of free thiols present is carried out using 5,5'-dithiobis(2-nitrobenzoic acid). MC-Val-Cit-PABC-520 (5 eq. per thiol) in DMA (final concentration 5%
25 v/v) is added and the resulting mixture is incubated at 30 °C overnight. The resulting ADC mixture may be purified by gel filtration (Nap-5, PBS) to remove unreacted linker-cytotoxin conjugate, desalted if desired, and purified by size-exclusion chromatography.

8. Example 8

[0709] This example describes methods that may be used to prepare drug-ligand
30 conjugates of the invention.

8.1.1 Synthesis of 3-(2-pyridyldithio)propionic acid (PySSPA)

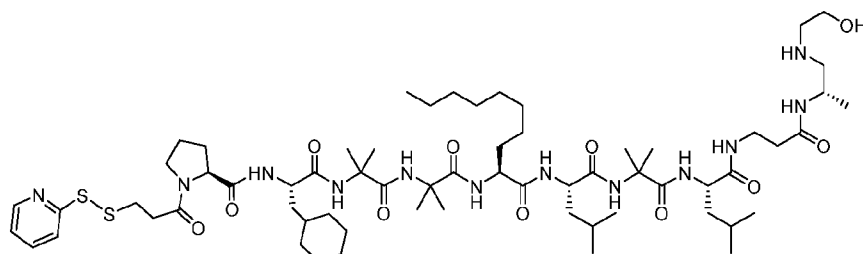
[0710] To a solution of 2,2'-dipyridyl disulphide (1 eq.) and AcOH (0.02 eq.) in ethanol
35 (EtOH) is added slowly a solution of 3-mercaptopropionic acid (0.05 eq.) in EtOH, and the mixture is stirred at room temperature overnight. The solvent then is removed in vacuo, and the crude is purified, such as by flash chromatography on silica gel (pet. ether/ethyl acetate (EtOAc), 1:1), to give the 3-(2-pyridyldithio)propionic acid.



Scheme 132: Synthesis of PySSPA

8.1.2 Synthesis of PySSPA-200h

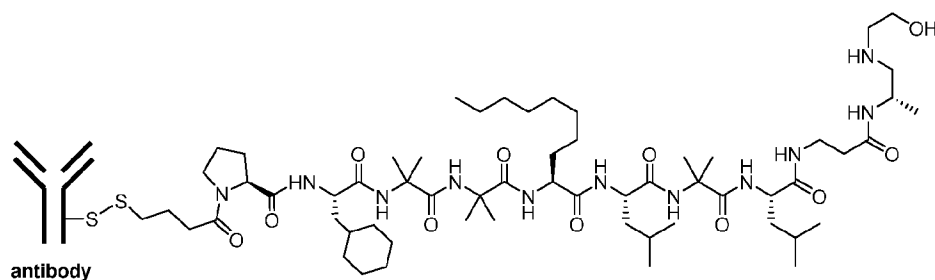
- 5 **[0711]** To a solution of compound 200h (1 eq.) in CH_2Cl_2 is added 3-(2-pyridyldithio)propionic acid (2 eq.), HOBt (1 eq.) and triethylamine (Et_3N) (1 eq.). After stirring for 72 h at room temperature, the mixture is evaporated and the crude is purified by semi-preparative RP-HPLC and lyophilised to afford PySSPA-200h.



10 **Scheme 133:** Structure of PySSPA-200h

8.1.3 Synthesis of Trastuzumab-PySSPA-200h

- [0712]** Antibody drug conjugates with an average of four drugs per antibody are prepared by partial reduction of the antibody with an excess of a reducing reagent such as TCEP or DTT at 25 °C in PBS buffer for 2 h. The reductant is then removed by buffer exchange into fresh phosphate buffer by gel filtration (Nap-5 column, GE Healthcare).
 15 Determination of the number of free thiols present is carried out using 5,5'-dithiobis(2-nitrobenzoic acid). PySSPA-AN-XX (5 eq. per thiol) in DMA (final concentration 5% v/v) is added and the resulting mixture is incubated at 30 °C overnight. The resulting ADC mixture
 20 may be purified by gel filtration (Nap-5, PBS) to remove unreacted linker-cytotoxin conjugate, desalted if desired, and purified by size-exclusion chromatography.



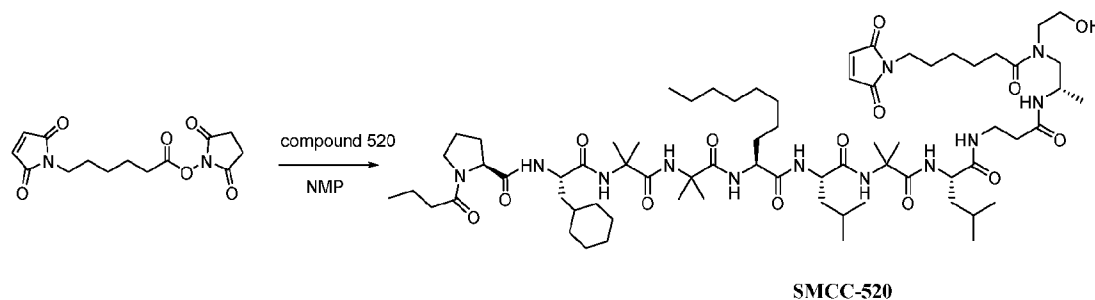
Scheme 134: Structure of Trastuzumab-PySSPA-200h

9. Example 9

[0713] This example describes the preparation of a drug-ligand conjugate of the invention.

9.1.1 Synthesis of SMCC-520

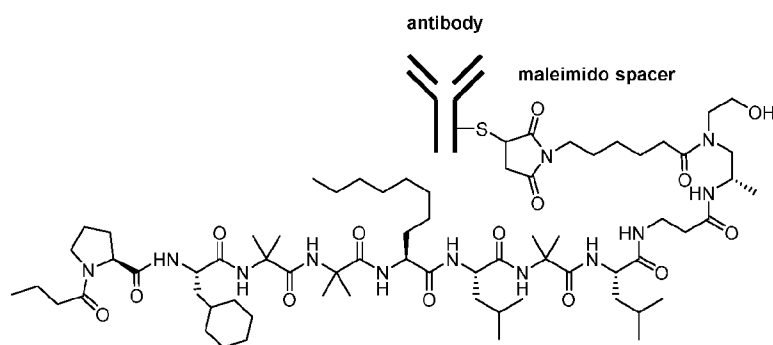
[0714] To the solution of compound **520** (10 mg, 0.009 mmol) in NMP (5 mL) was added MC-OSu (3 mg mg, 0.009 mmol). The reaction mixture was allowed to stir for 18 h. Solvent was then removed *in vacuo*. The crude **SMCC-520** was purified by semi-preparative RP-HPLC using a Dionex UltiMate® 3000 on a Agilent C18 column (Zorbax 300SB-C18, 9.4 x 250 mm 5 µm) using a gradient of 95%A:5%B to 5%A:95%B over 90 min, where solvent A was 0.1% TFA in H₂O and B was 0.1% TFA in acetonitrile, (*ca.* 1% B/min). Fractions identified with the correct *m/z* were combined and lyophilised to afford **SMCC-520** as a white amorphous solid (5 mg, 41% yield, 90% purity by analytical LCMS as judged by peak area of RP-HPLC at 210 nm). Analytical LC-MS: Agilent Zorbax-C3, (150 mm x 3.0 mm, 3.5 µm), linear gradient of 95% A:5% B to 5% A:95% B, where solvent A was 0.1% formic acid in H₂O and B was 0.1% formic acid in acetonitrile, (*ca.* 3% B/min), 0.3 mL/min; *R_t* 29.8 min; *m/z* (ESI-MS) 1353.8; found:1353.8.



Scheme 135: Synthesis of SMCC-520

9.1.2 Synthesis of Trastuzumab-SMCC-520 (non-cleavable)

[0715] Trastuzumab antibody (5 mg/mL) in PBS buffer containing 4 mM EDTA, pH 7.4, was partially reduced with TCEP (3 molar excess) at 25 °C for 2 h. The reductant was then removed by buffer exchange into fresh PBS buffer containing 4 mM EDTA, pH 7.4, by gel filtration (Nap-5 column, GE Healthcare). **SMCC-520** (5 eq. per thiol) in DMA (final concentration 10% v/v) was added and the resulting mixture was incubated at 25 °C. After 2 h, the resulting ADC mixture was concentrated by centrifugal ultrafiltration to remove unreacted linker-cytotoxin. The drug to antibody ratio (DAR) was calculated to be 0.5 using hydrophobic interaction chromatography (HIC-HPLC); TSKgel Butyl-NPR column (0.46 x 3.5 cm; Tosoh Bioscience, South San Francisco, CA, USA), linear gradient of 100% A:0% B to 0% A:100% B, where solvent A was 25 mM phosphate buffer, pH 7.0, containing 1.5 M ammonium sulphate and B was 25 mM potassium phosphate, pH 7.0, containing 25% isopropanol.



Scheme 136: Structure of Trastuzumab-SMCC-520

10. Example 10

- 5 **[0716]** This example describes alternative methods to those set forth in Example 9 that may be used to prepare drug-ligand conjugates of the invention.

10.1.1 Synthesis of SMCC-520

- 10 **[0717]** A solution of compound 520 (1 eq.) is dissolved in NMP and MC-OSu (1.5 eq.) is added. The reaction is allowed to stir for 18 h. The volatiles are evaporated at reduced pressure and then the remaining crude residue is purified by semi-preparative RP-HPLC and lyophilised to afford SMCC-520.

10.1.2 Synthesis of Trastuzumab-SMCC-520 (non-cleavable)

- 15 **[0718]** Antibody drug conjugates with average of four drugs per antibody are prepared by partial reduction of the antibody with an excess of a reducing reagent such as TCEP or DTT at 25 °C in PBS buffer for 2 h. The reductant is then removed by buffer exchange into fresh phosphate buffer by gel filtration (Nap-5 column, GE Healthcare). Determination of the number of free thiols present is carried out using 5,5'-dithiobis(2-nitrobenzoic acid).
- 20 SMCC-520 (5 eq. per thiol) in DMA (final concentration 5% v/v) is added and the resulting mixture is incubated at 30 °C overnight. The resulting ADC mixture may be purified by gel filtration (Nap-5, PBS) to remove unreacted linker-cytotoxin conjugate, desalted if desired, and purified by size-exclusion chromatography.

11. Example 11

- [0719]** This example investigated the antiproliferative activity and effect on extracellular flux of the culicinin D and analogues.

11.1 Methods and materials

- 30 **[0720]** Culicinin D (compound **1**) and the peptide analogues AN-58, AN-95, and AN-103 (compounds **520**, **1100i**, and **1200e**, respectively) were assessed for antiproliferative activity against 3 breast cancer cell lines (MDA-MB-468, SKBR3 and T47D), as well as a

non-small cell lung cancer line NCI-H460. Cells were treated with 3-fold serial dilutions of the peptides and maintained under drug-exposure for 5 days. Monitoring of cell proliferation and cell viability was performed by sulphorhodamine B-based assay and the IC₅₀ determined by interpolation as the drug concentration reducing staining to 50% of controls on the same plate.

[0721] *In vitro* studies were performed to investigate the effects of culicinin D and the peptide analogues on oxidative phosphorylation, uncoupling of mitochondrial membrane potential. Measurements of mitochondrial energetics in SKBR3 permeabilized cells exposed to culicinin D, the selected analogues or oligomycin A (positive control) were acquired using a Seahorse extracellular flux (XF) analysis.

11.2 Results

[0722] The culicinin D analogues tested showed a broad range of antiproliferative activity against the panel of neoplastic cell lines. Anticancer activity was greater relative to culicinin D with the analogues.

[0723] XF assays demonstrated that culicinin D reversed a decrease in oxygen consumption upon injection of the ATP synthase inhibitor oligomycin A. Culicinin D also prevented suppression of oxygen consumption when administered prior to oligomycin A (Figure 6A). These observations are consistent with disruption of the mitochondrial membrane potential ($\Delta\Psi_m$).

[0724] Changes in oxygen consumption rate (OCR, pmol/min) in permeabilized SKBR3 breast cancer cells exposed to culicinin D or the analogues tested were significantly different (Figure 6B) and also showed a good correlation ($R^2 = 0.861$; Figure 6C) with anti-proliferative activities as measured by IC₅₀ values (nmol/L $\pm$ SEM). These observations are consistent with disruption of the mitochondrial membrane potential ($\Delta\Psi_m$) being correlated with the anti-neoplastic activity of culicinin D and the analogues.

12. Example 12

[0725] This example investigated the effect of culicinin D and analogues on the mitochondrial membrane potential.

12.1 Methods and materials

[0726] **MITO-ID membrane potential cytotoxicity kit:** Enzo Life Sciences' MITO-ID Membrane Potential Cytotoxicity kit was used to measure mitochondrial membrane potential with a cationic dye that fluoresces either green or orange depending upon status of the mitochondrial membrane potential. In energized cells with intact membrane potentials, the MITO-ID dye accumulates as orange fluorescence 'J-aggregates' (Ex/Em=480/590nm) in the mitochondria in addition to persisting as a green fluorescent monomer (Ex/Em = 480/530nm) in the cytosol. If the mitochondrial membrane potential is disrupted in cells,

the MITO-ID dye exists primarily as green fluorescent monomers throughout the cytosol and no longer exhibits orange fluorescence in the mitochondria.

[0727] Fluorescence microplate reader: A fluorescence microplate reader with a filter set with Ex=490nm/Em=570-590nm was used.

5 **[0728] Cell preparation:** SKBR3 cells were seeded in 96-well black wall and clear bottom plates (BD Falcon, #353219) the day before test compound/probe addition. Cells were seeded in culture medium (Dulbecco's modified Eagle's medium (DMEM) +10% fetal calf serum (FCS)) at a density of 1.5×10^4 cells per well and at a seeding volume of 100 μ l per well. The microplate was kept in a humidified CO₂ incubator overnight. On the following
10 day the growth media was aspirated and 100 μ l of phenol red-free media + 10% FCS was added to each well.

[0729] Positive control: Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) is a proton ionophore and uncoupler of oxidative phosphorylation in mitochondria. Addition of CCCP causes a dose-dependent reduction in mitochondrial orange fluorescence. CCCP was
15 used as positive control for mitochondrial membrane potential loss. The CCCP Control (supplied as a 2mM stock solution in dimethyl sulfoxide (DMSO)) was diluted in medium to a final concentration of 2 μ M.

[0730] Test compounds: Culicinin D (AN-05, compound **1**) and the analogues AN-58, AN-95 and AN-103 (compounds **520**, **1100i**, and **1200e**, respectively) were tested at 3 μ M
20 final concentration and imaged after a total of 30 minutes exposure.

[0731] Membrane potential assay: Microplates containing live cells were prepared as described in the Cell preparation section above. The cells were treated with 20 μ l of selected compounds for 30 minutes. For the positive control, 20 μ l of the provided CCCP control was dispensed to a final concentration of 2 μ M for 30 minutes. 100 μ l of the prepared MITO-ID MP
25 Dye Loading Solution was dispensed into each well. Plates were incubated for 30 minutes at 37°C, protected from light. Plates were observed with a fluorescence microplate reader using an excitation setting of 490nm and an emission setting of 590nm. In live and healthy cells, the mitochondria will fluorescence orange following aggregation of the MITO-ID MP dye. The orange J-aggregates emit at 590 nm. The fluorescence value in blank wells with
30 added growth medium were subtracted from the values for those wells with cells treated with the test compounds.

[0732] Zeiss LSM710 confocal microscopy: A Zeiss LSM710 confocal microscope with an AlexaFluor488/Tetramethyl rhodamine (TRITC) filter set was employed to image the control and drug-treated cells.

35 **[0733] Inverted membrane vesicles (IMVs):** The ability of AN-58 (compound **520**) to collapse a proton gradient in *E. coli* C41 (wild-type parent strain), *E. coli* Δ -atp (ATP synthase deletion mutant), and *E. coli* Δ -atp + pBWU13 (ATP synthase deletion mutant with ATP synthase restored via an overexpression plasmid) was tested. Two measurements were performed for each *E. coli* strain – a measurement of O₂ (measuring the activity of the

electron transport chain), and a measurement of acridine orange fluorescence (measuring the protons pumped by the electron transport chain, with more fluorescence quenching corresponding to more protons that have been pumped).

12.2 Results

[0734] A preliminary fluorescence microplate screen assay showed a time-dependent reduction of mitochondrial membrane potential (MMP) observed in SKBR3 cells exposed to culicinin D (AN-05; Figure 7A). A 30 minute-exposure time was elected for further analysis of culicinin D (AN-05) and its analogues AN-58, AN-95, AN-103. An initial 1 μ M concentration of the positive control CCCP was optimized to 2 μ M for the cells being used.

[0735] A significant reduction ($P < 0.005$) in MMP, measured as total cell orange fluorescence corrected for the fluorescence background (corrected total cell fluorescence (CTCF)) was found in positive control (CCCP), AN-05, AN-58 and AN-95-treated cells compared to control cells. The difference between the control and AN-103-treated cells was found to be not significant at a $p < 0.05$ (Figure 7B-C and Table 8). This shows that culicinin D (AN-05, compound **1**), AN-58 and AN-95 are mitochondrial membrane uncoupling/depolarisation agents.

[0736] **Table 8.** Mean Corrected Total Cell Fluorescence (CTCF) $\pm$ SEM.

	Corrected Total Cell Fluorescence (CTCF)	MEAN CTCF	SDEV	SEM
Control IMAGE 1	27595352	33923019	10529665	6079305
Control IMAGE 2	28095505			
Control IMAGE 3	46078199			
AN103 3 μ M IMAGE 1	19816868	18372062	2394322	1382363
AN103 3 μ M IMAGE 2	15608292			
AN103 3 μ M IMAGE 3	19691026			
AN95 3 μ M IMAGE 1	1364971	1109340	370448.5	213879
AN95 3 μ M IMAGE 2	684503			
AN95 3 μ M IMAGE 3	1278546			
AN05 3 μ M IMAGE 1	280708	237811	183937	106196
AN05 3 μ M IMAGE 2	36216			
AN05 3 μ M IMAGE 3	396509			
AN58 3 μ M IMAGE 1	427698	240159	201885	116559
AN58 3 μ M IMAGE 2	26475			
AN58 3 μ M IMAGE 3	266303			
CCCP 2 μ M IMAGE 1	-46821	-13213	33398.43	19283
CCCP 2 μ M IMAGE 2	-12789			
CCCP 2 μ M IMAGE 3	19972			

[0737] The reduced MMP observed in drug-treated cells by confocal microscopy is consistent with IC₅₀ and Oxygen Consumption Rate (OCR) data (Example 11 and Figures 6A-C).

[0738] These findings are consistent with OCR and proton gradient data in *E. coli* inverted membrane vesicles (IMVs). Changes in oxygen consumption and proton gradient were monitored in an *E. coli* ATP synthase deletion mutant (versus wild-type parent). It was found that the culicinin D analogue AN-58 (compound **520**) was a potent ionophore, being able to collapse the proton gradient. However, this ability is independent of the presence of functional ATP synthase (Figure 8).

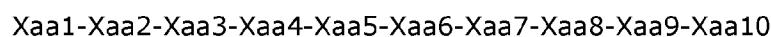
INDUSTRIAL APPLICATION

[0739] The compounds of formula I described herein have useful inhibitory activity against various cancers. As such, these compounds and drug-ligand conjugates comprising such compounds are useful for treating such cancers. The compounds of formula I and drug-ligand conjugates are also useful for the treatment or prevention of diseases or conditions susceptible to treatment with a mitochondrial uncoupling agent or complications associated with such diseases or conditions.

[0740] Drug-reactive linker group conjugates comprising such compounds are useful as intermediates in the preparation of such drug-ligand conjugates.

[0741] The following numbered paragraphs define certain aspects and embodiments of the invention described herein.

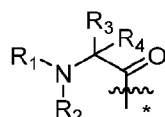
1. A compound of formula I or a pharmaceutically acceptable salt or solvate thereof



I

wherein:

Xaa1 is a group of formula IA



IA

wherein

R₁ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C(O)C₁-C₁₅alkyl, (CH₂CH₂O)_k-R_d, C(O)(CH₂CH₂O)_k-R_d, C₁-C₁₅alkyl(Q_d), C(O)C₁-C₁₅alkyl(Q_d), C₁-C₁₅alkyl(aryl), C₁-

C₁₅alkyl(C₃-C₁₂carbocycle), C₁-C₁₅alkyl(3 to 12 membered heterocycle), or C₁-C₁₅alkyl(heteroaryl);

R₂ and R₃ are each independently H, C₁-C₈alkyl, C₁-C₈haloalkyl, or C₃-C₁₂carbocycle; or R₂ and R₃ together form a ring and have the formula $-(CR^mR^n)_n-$ wherein

n is from 1 to 6, and at each instance of n

R^m is independently H, OH, C₁-C₈alkyl, or C₃-C₁₂carbocycle, and

Rⁿ is independently H, C₁-C₈alkyl, or C₃-C₁₂carbocycle,

or R^m and Rⁿ together represent =O; and

R₄ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl;

or R₃ and R₄ together with the carbon atom to which they are attached form a C₃-C₈carbocycle or as 3 to 8 membered heterocycle;

R_d is H, C₁-C₈alkyl or C₁-C₈haloalkyl;

k is an integer from 1 to 10;

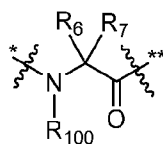
Q_d is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a;

R_a and R_b are each independently H, C₁-C₈alkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, or heteroaryl;

and

* denotes the bond to Xaa2;

Xaa2 is a group of formula IB



IB

wherein

R₆ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_D)_q), C₁-C₈alkyl(3 to 12 membered heterocycle), C₁-C₈alkyl(heteroaryl), C₁-C₁₅alkyl(Q_a), or C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

Q_a is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a;

Q_b and Q_c are each independently H, OR_c, SR_c, or NR_cR_d;

X is at each instance independently O, S, or NR_e;

R_a, R_b, R_c, R_d, and R_e are each independently H, C₁-C₈alkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, or heteroaryl;

q is from 0 to 3;

R_D at each instance of q is independently OH, OR_a, SR_a, NR_aR_b, halo, C₁-C₈alkyl, C₁-C₈haloalkyl;

R₇ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl;

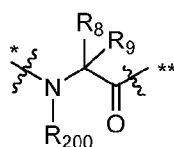
or R₆ and R₇ together with the carbon atom to which they are attached form a 3 to 8 membered heterocycle or C₃-C₈carbocycle;

R₁₀₀ is H or C₁-C₃alkyl;

* denotes the bond to Xaa1; and

** denotes the bond to Xaa3;

Xaa5 is a group of formula IC



IC

wherein

R₈ is C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₁-C₁₅alkyl(aryl), C₁-C₁₅alkyl(C₃-C₁₂carbocycle), C₁-C₁₅alkyl(C₃ to 12 membered heterocycle), or C₁-C₁₅alkyl(heteroaryl);

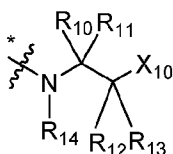
R₉ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl;

R₂₀₀ is H or C₁-C₃alkyl;

* denotes the bond to Xaa4; and

** denotes the bond to Xaa6;

Xaa10 is OH, NR_wR_z or a group of formula ID



ID

wherein

R₁₀ and R₁₂ are each independently H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl);

R₁₁ and R₁₃ is H, C₁-C₃alkyl or C₁-C₃haloalkyl;

or R₁₀ and R₁₁ together with the carbon atom to which they are attached form a C₃-C₈carbocycle or a 3 to 8 membered heterocycle;

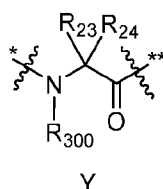
or R₁₂ and R₁₃ together with the carbon atom to which they are attached form a C₃-C₈carbocycle or a 3 to 8 membered heterocycle;

R₁₄ is H or C₁-C₃alkyl;

X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;

R₁₅, R₁₆, R₁₇, and R₁₈ are each independently H, C₁-C₈alkyl, C₁-C₈haloalkyl, or C₁-C₈alkyl(Y₁₀);
 or R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle or heteroaryl ring;
 Y₁₀ is OR₁₉, NR₂₀R₂₁ or SR₂₂;
 R₁₉, R₂₀, R₂₁, and R₂₂ are each independently H, C₁-C₈alkyl, C₁-C₈haloalkyl;
 R_w and R_z are each independently H or C₁-C₃alkyl;
 * denotes the bond to Xaa9;

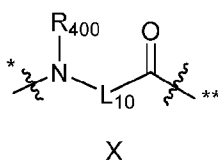
Xaa3, Xaa4, Xaa6, Xaa7 and Xaa8 are each independently a group of formula Y



wherein

R₂₃ is H, C₁-C₈alkyl, C₁-C₈haloalkyl, C₁-C₈alkyl(aryl), C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl);
 R₂₄ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl;
 or R₂₃ and R₂₄ together with the carbon atom to which they are attached form a C₃-C₈carbocycle or a 3 to 8 membered heterocycle;
 R₃₀₀ is H or C₁-C₃alkyl;
 * denotes the bond to Xaa2 in the case of Xaa3, Xaa3 in the case of Xaa4, Xaa5 in the case of Xaa6, Xaa6 in the case of Xaa7, and Xaa7 in the case of Xaa8; and
 ** denotes the bond to Xaa4 in the case of Xaa3, Xaa5 in the case of Xaa4, Xaa7 in the case of Xaa6, Xaa8 in the case of Xaa7, and Xaa9 in the case of Xaa8; and

Xaa9 is absent, is independently a group of formula Y as defined above, or is a group of the formula X



wherein

R₄₀₀ is H or C₁-C₃alkyl;
 L₁₀ is a group of the formula -(CR^hRⁱ)_i- or -(CR^hRⁱ)_i-X^a-(CR^jR^k)_j-;
 R^h and R^j are each independently selected from H, C₁-C₈alkyl, C₁-C₈haloalkyl, C₁-C₈alkyl(aryl), C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl);
 Rⁱ and R^k are each independently selected from H, C₁-C₃alkyl, or C₁-C₃haloalkyl; and

X^a is selected from O, S, NH, or N(C₁-C₈alkyl);

h, i, and j are each independently selected from 1 to 5, provided that the sum of i and j is 5 or less;

* denotes the bond to Xaa8; and

** denotes the bond to Xaa10;

wherein any alkyl, aryl, carbocycle, heterocycle, or heteroaryl in any of the groups for

R₁, R₂, R₃, R^m, Rⁿ, and R₄ in **Xaa1**,

R₆, Q_a, Q_b, Q_c, X, R_a, R_b, R_c, R_d, R_e, R_D, and R₇ in **Xaa2**,

R₈ and R₉ in **Xaa5**,

R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, X₁₀, R₁₅, R₁₆, R₁₇, R₁₈, Y₁₀, R₁₉, R₂₀, R₂₁, and R₂₂ in **Xaa10**,

R₂₃ and R₂₄ in **Xaa3**, **Xaa4**, **Xaa6**, **Xaa7** and **Xaa8**, and

R₂₃, R₂₄, L₁₀, R^h, Rⁱ, R^j, R^k, and X^a in **Xaa9**

are optionally substituted with one or more optional substituents;

and, preferably, wherein any aryl in any one of the aforementioned groups for Xaa1-Xaa10 is a C₆-C₂₀aryl, preferably C₆-C₁₀aryl, and any heteroaryl in any of the aforementioned groups for Xaa1-Xaa10 is a 5-20 membered heteroaryl, preferably a 5-10 membered heteroaryl;

provided that the compound is not: Culicinin A, Culicinin B, Culicinin C, Culicinin D (R), Culicinin D (S), Leucinostatin A, Leucinostatin A2, Leucinostatin B, Leucinostatin B2, Leucinostatin C, Leucinostatin D, Leucinostatin F, Leucinostatin L, Leucinostatin N, Leucinostatin R, Leucinostatin S, Leucinostatin T, Leucinostatin U, Leucinostatin V, Leucinostatin I, Leucinostatin III, Leucinostatin IV, Leucinostatin V, Helioferin A, Helioferin B, Roseoferin A₁, Roseoferin A₂, Roseoferin A₃, Roseoferin B₁, Roseoferin B₂, Roseoferin B₃, Roseoferin C₁, Roseoferin C₂, Roseoferin D₁, Roseoferin D₂, Roseoferin D₃, Roseoferin E₁, Roseoferin E₂, Roseoferin E₃, Roseoferin F, Roseoferin G, Trichoderin A, Trichoderin A1, Trichoderin B, Trichopolyne I, Trichopolyne II, Trichopolyne III, Trichopolyne IV, Trichopolyne V, Trichopolyne VI, or Emericellipsin A (the structures of which compounds are shown in claim 1 below).

2. A compound of paragraph 1, wherein in **Xaa1** R₁ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, and C(O)C₁-C₁₅alkyl, C(O)C₁-C₁₅haloalkyl, (CH₂CH₂O)_k-R_d, C(O)(CH₂CH₂O)_k-R_d, C₁-C₁₅alkyl(Q_d), C(O)C₁-C₁₅alkyl(Q_d), preferably H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, and C(O)C₁-C₁₅alkyl, C(O)C₁-C₁₅haloalkyl, preferably H or C(O)C₁-C₁₅alkyl.
3. A compound of paragraph 1, wherein in **Xaa1** R₂ and R₃ together form a ring.
4. A compound of paragraph 1 or paragraph 3, wherein in **Xaa1** R₂ and R₃ together form a ring and n is an integer selected from 2 to 6, 3 to 6, 3 to 5, or, preferably, 3 to 4.

5. A compound of paragraph 1, 3 or 4, wherein in **Xaa1** R_2 and R_3 together form a ring and

R^m is independently H, OH, or C_1 - C_8 alkyl, preferably H or OH, and

R^n is independently H or C_1 - C_8 alkyl, preferably H,

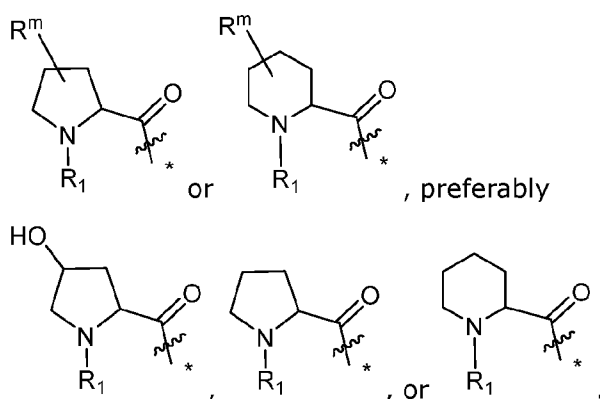
- 5 or R^m and R^n together represent =O.

6. A compound of paragraph 1, 3 or 4, wherein in **Xaa1** R_2 and R_3 together form a ring and

R^m is independently H, OH, or C_1 - C_6 alkyl, preferably H or OH, and

R^n is independently H or C_1 - C_6 alkyl, preferably H.

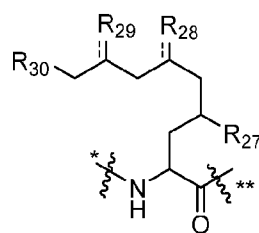
- 10 7. A compound of any one of paragraphs 1 or 3 to 6, wherein **Xaa1** is a group of formula:



wherein R^m , R_1 and * are as defined in any one of the preceding paragraphs.

- 15 8. A compound of any one of paragraphs 1 or 3 to 7, wherein in **Xaa1** R_1 is H, C_1 - C_{15} alkyl, C_1 - C_{15} haloalkyl, $C(O)C_1$ - C_{15} alkyl, and $C(O)C_1$ - C_{15} haloalkyl, preferably H or $C(O)C_1$ - C_{15} alkyl, preferably H or $C(O)C_1$ - C_{13} alkyl, preferably H or $C(O)C_1$ - C_{11} alkyl.
9. A compound of paragraph 1 or 2, wherein in **Xaa1** R_2 and R_3 are each independently H, C_1 - C_8 alkyl, C_1 - C_8 haloalkyl, or C_3 - C_{12} carbocycle, preferably H, C_1 - C_8 alkyl, or C_1 - C_8 haloalkyl, preferably H or C_1 - C_8 alkyl.
- 20 10. A compound of paragraph 1 or 2 or 9, wherein in **Xaa1** R_2 is H or C_1 - C_6 alkyl, preferably H or C_1 - C_4 alkyl, preferably H.
11. A compound of paragraph 1, 2, 9 or 10, wherein in **Xaa1** R_3 is C_1 - C_8 alkyl, preferably C_1 - C_6 alkyl, preferably C_1 - C_4 alkyl, preferably CH_3 .
- 25 12. A compound of any one of paragraphs 9 to 11, wherein in **Xaa1** R_1 is $C(O)C_1$ - C_{15} alkyl, preferably $C(O)C_1$ - C_{10} alkyl, preferably $C(O)C_1$ - C_8 alkyl, preferably $C(O)C_1$ - C_5 alkyl, preferably $C(O)C_1$ - C_3 alkyl.
13. A compound of any one of paragraphs 1 to 11, wherein in **Xaa1** R_1 is $(CH_2CH_2O)_k-R_d$, $C(O)(CH_2CH_2O)_k-R_d$, C_1 - C_{15} alkyl(Q_d), or $C(O)C_1$ - C_{15} alkyl(Q_d), preferably C_1 - C_{15} alkyl(Q_d) or $C(O)C_1$ - C_{15} alkyl(Q_d), preferably $C(O)C_1$ - C_{15} alkyl(Q_d), preferably $C(O)C_1$ - C_{10} alkyl(Q_d), preferably $C(O)C_1$ - C_8 alkyl(Q_d), preferably $C(O)C_1$ - C_5 alkyl(Q_d).
- 30

14. A compound of any one of paragraphs 1 to 7, 9 to 11 and 13, wherein in **Xaa1** Q_d is OR_a, SR_a, or NR_aR_b, preferably OR_a or NR_aR_b; and optionally R_a and R_b are each independently H or C₁₋₈alkyl, preferably H or CH₃.
15. A compound of any one of paragraphs 1 to 14, wherein in **Xaa1** R₄ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl, preferably H or C₁-C₃alkyl, preferably H.
16. A compound of any one of paragraphs 1 to 15, wherein **Xaa2** is a group of formula IB, wherein R₇ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl, preferably H or C₁-C₃alkyl, preferably H or CH₃.
17. A compound of any one of paragraphs 1 to 16, wherein **Xaa2** is a group of formula IB, wherein
R₆ is C₁-C₁₅alkyl or C₁-C₁₅haloalkyl, preferably C₁-C₁₅alkyl, preferably C₄-C₁₅alkyl, C₆-C₁₅alkyl, C₈-C₁₅alkyl, or C₁₀-C₁₅alkyl.
18. A compound of any one of paragraphs 1 to 16, wherein **Xaa2** is a group of formula IB, wherein R₆ is C₁-C₁₅alkyl(Q_a).
19. A compound of any one of paragraphs 1 to 16 or 18, wherein **Xaa2** is a group of formula IB, wherein Q_a is OR_a, SR_a, or NR_aR_b, preferably OR_a or SR_a;
20. A compound of any one of paragraphs 1 to 16, 18 or 19, wherein **Xaa2** is a group of formula IB, wherein R_a and R_b are each H or C₁-C₈alkyl.
21. A compound of any one of paragraphs 1 to 16, wherein **Xaa2** is a group of formula IB, wherein R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c).
22. A compound of any one of paragraphs 1 to 16 or 21, wherein **Xaa2** is a group of formula IB, wherein X is O.
23. A compound of any one of paragraphs 1 to 16, 21 or 22, wherein **Xaa2** is a group of formula IB, wherein Q_b and Q_c are each independently H or OR_c, preferably H or OH.
24. A compound of any one of paragraphs 1 to 16 or 21 to 23, wherein **Xaa2** is a group of formula IB, wherein R_c, R_d, and R_e are each independently H or C₁-C₈alkyl.
25. A compound of any one of paragraphs 1 to 16 or 21 to 24, wherein **Xaa2** is a group of formula IIB



IIB

wherein

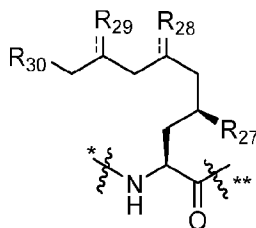
R₂₇ is H or C₁-C₈alkyl, preferably H or CH₃;

== is a single bond or a double bond;

R₂₈ and R₂₉ are each independently H or OH and --- is a single bond or O and = is a double bond; and

R₃₀ is H or C₁-C₈alkyl.

26. A compound of paragraph 25, wherein (i) R₂₈ and R₂₉ are each independently H; (ii) R₂₈ is OH or O and R₂₉ is H; or (iii) R₂₈ is OH and R₂₉ is O.
27. A compound of paragraph 25 or 26, wherein **Xaa2** is a group of formula IIBB



IIBB.

28. A compound of any one of paragraphs 1 to 16, wherein **Xaa2** is a group of formula IB, wherein R₆ is C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_d)_q), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably C₃-C₁₂carbocycle, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_d)_q), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably C₃-C₁₂carbocycle, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_d)_q), or C₁-C₈alkyl(heteroaryl).
29. A compound of any one of paragraphs 1 to 15, wherein **Xaa2** is a group of formula IB, wherein R₆ and R₇ together form a 3 to 8 membered heterocycle or C₃-C₈carbocycle, preferably a C₃-C₈carbocycle, preferably a C₃-C₆carbocycle.
30. A compound of any one of paragraphs 1 to 29, wherein in **Xaa5** R₈ is C₁-C₁₅alkyl or C₁-C₁₅haloalkyl, preferably C₂-C₁₂alkyl, preferably C₂-C₉alkyl, C₃-C₉alkyl, or C₄-C₉alkyl.
31. A compound of any one of paragraphs 1 to 30, wherein **Xaa5** is a group of formula IC wherein R₉ is H or C₁-C₃alkyl, preferably H.
32. A compound of any one of paragraphs 1 to 31, wherein **Xaa10** is a group of formula ID wherein
R₁₀ and R₁₂ are each independently H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably H, C₁-C₁₅alkyl, C₃-C₁₂carbocycle, aryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), or C₁-C₈alkyl(aryl); and
R₁₁ and R₁₃ is H, C₁-C₃alkyl or C₁-C₃haloalkyl, preferably H or C₁-C₃alkyl.
33. A compound of any one of paragraphs 1 to 32, wherein **Xaa10** is a group of formula ID wherein
R₁₀ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably H, C₁-C₁₅alkyl, C₃-C₁₂carbocycle, aryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), or C₁-C₈alkyl(aryl), preferably H, C₁-C₁₀alkyl, or C₁-C₈alkyl(aryl); and

R₁₁, R₁₂, and R₁₃ is H, C₁-C₃alkyl or C₁-C₃haloalkyl, preferably H or C₁-C₃alkyl, preferably H.

34. A compound of any one of paragraphs 1 to 33, wherein **Xaa10** is a group of formula ID wherein

R₁₀ is H, C₁-C₈alkyl, preferably C₁-C₆alkyl, preferably C₁-C₄alkyl, preferably CH₃, or C₁-C₆alkyl(aryl), preferably C₁-C₄alkyl(aryl), preferably C₁-C₃alkyl(aryl).

35. A compound of any one of paragraphs 1 to 34, wherein **Xaa10** is a group of formula ID wherein R₁₁ is H.

36. A compound of any one of paragraphs 1 to 32, wherein **Xaa10** is a group of formula ID wherein

R₁₂ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably H, C₁-C₁₅alkyl, C₃-C₁₂carbocycle, aryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), or C₁-C₈alkyl(aryl), preferably H, C₁-C₁₀alkyl, or aryl; and

R₁₀, R₁₁, and R₁₃ is H, C₁-C₃alkyl or C₁-C₃haloalkyl, preferably H or C₁-C₃alkyl, preferably H.

37. A compound of paragraph 36, wherein **Xaa10** is a group of formula ID wherein R₁₂ is H or aryl;

38. A compound of paragraph 36 or 37, wherein **Xaa10** is a group of formula ID wherein R₁₃ is H.

39. A compound of any one of paragraphs 36 to 38, wherein **Xaa10** is a group of formula ID wherein R₁₄ is H.

40. A compound of any one of paragraphs 1 to 39, wherein **Xaa10** is a group of formula ID wherein

X₁₀ is OR₁₅ or NR₁₆R₁₇;

R₁₅ is H, C₁-C₈alkyl, preferably H;

R₁₆ is H, C₁-C₈alkyl, or C₁-C₈alkyl(Y₁₀), preferably C₁-C₈alkyl or C₁-C₈alkyl(Y₁₀);

R₁₇ is H or C₁-C₈alkyl, preferably H;

or R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle;

Y₁₀ is OR₁₉, NR₂₀R₂₁ or SR₂₂, preferably OR₁₉, preferably OH;

R₁₉, R₂₀, R₂₁, and R₂₂ are each independently H or C₁-C₈alkyl, preferably H.

41. A compound of any one of paragraphs 1 to 40, wherein **Xaa10** is a group of formula ID wherein

X₁₀ is OH or NR₁₆R₁₇;

R₁₆ is H;

R₁₇ is C₁-C₈alkyl, preferably C₁-C₄alkyl, preferably CH₃, or C₁-C₈alkyl(Y₁₀), preferably C₁-C₄alkyl(Y₁₀), preferably C₂-C₄alkyl(Y₁₀), CH₂CH₂(Y₁₀);

or R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle; and

Y₁₀ is OH.

42. A compound of any one of paragraphs 1 to 40, wherein **Xaa10** is a group of formula ID wherein

X₁₀ is OH or NR₁₆R₁₇;

R₁₆ is C₁-C₈alkyl, preferably C₁-C₄alkyl, preferably CH₃;

R₁₇ is C₁-C₈alkyl, preferably C₁-C₄alkyl, preferably CH₃, or C₁-C₈alkyl(Y₁₀), preferably C₁-C₄alkyl(Y₁₀), preferably C₂-C₄alkyl(Y₁₀), CH₂CH₂(Y₁₀);

or R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle; and

Y₁₀ is OH.

43. A compound of any one of paragraphs 1 to 40, wherein **Xaa10** is a group of formula ID wherein R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle, for example a morpholinyl or piperazinyl ring.

44. A compound of any one of paragraphs 1 to 43, wherein in the formula Y

R₂₃ is H, C₁-C₈alkyl, or C₁-C₈haloalkyl, preferably H or C₁-C₈alkyl, preferably C₁-C₈alkyl;

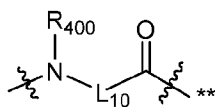
R₂₄ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl, preferably H or C₁-C₈alkyl;

or R₂₃ and R₂₄ together with the carbon atom to which they are attached form a C₃-C₈carbocycle.

45. A compound of any one of paragraphs 1 to 44, wherein in the formula Y R₂₃ is C₁-C₈alkyl; and R₂₄ is H or C₁-C₈alkyl, preferably R₂₃ is C₁-C₈alkyl and R₂₄ is C₁-C₈alkyl or R₂₃ is C₂-C₈alkyl, preferably C₃-C₈alkyl, and R₂₄ is H.

46. A compound of any one of paragraphs 1 to 45, wherein **Xaa3**, **Xaa4**, and/or **Xaa7** is a 2-aminoisobutyric acid residue; and/or **Xaa6** and/or **Xaa8** is a leucine residue.

47. A compound of any one of paragraphs 1 to 46, wherein **Xaa9** is independently a group of formula Y as defined in any one of paragraphs 1 to 46, or is a group of the formula X



X.

48. A compound of any one of paragraphs 1 to 47, wherein **Xaa9** is a group of the formula X wherein

L₁₀ is a group of the formula $-(CR^hR^i)_h-$ or $-(CR^hR^i)_i-X^a-(CR^jR^k)_j-$;

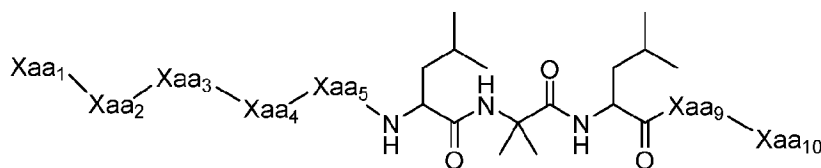
R^h and R^j are each independently selected from H or C₁-C₈alkyl, preferably H;

Rⁱ and R^k are each independently selected from H or C₁-C₃alkyl, preferably H; and

X^a is selected from O, S, NH, or N(C₁-C₈alkyl).

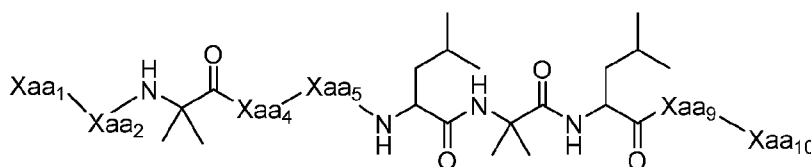
49. A compound of any one of paragraphs 1 to 48, wherein **Xaa9** is a group of the formula X wherein L_{10} is a group of the formula $-(CR^hR^i)_h-$, preferably wherein h is from 1 to 4, preferably 2 to 4, preferably 2.

50. A compound of any one of paragraphs 1 to 49, wherein the compound is of the formula II or a pharmaceutically acceptable salt or solvate thereof



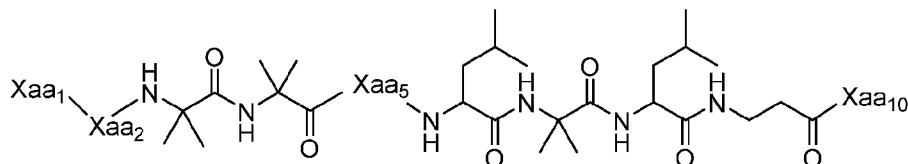
II.

51. A compound of any one of paragraphs 1 to 50, wherein the compound is of the formula III or a pharmaceutically acceptable salt or solvate thereof



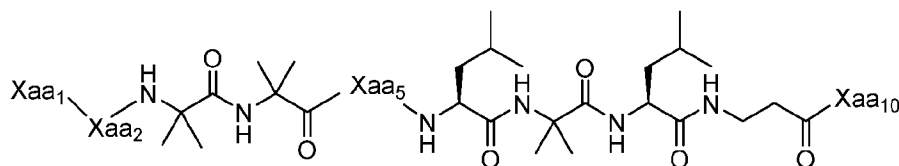
III.

52. A compound of paragraph 1 to 51, wherein the compound is of the formula IV or a pharmaceutically acceptable salt or solvate thereof



IV.

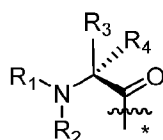
53. A compound of paragraph 1 to 52, wherein the compound is a compound of formula IVA or a pharmaceutically acceptable salt or solvate thereof



IVA.

54. A compound of any one of paragraphs 1 to 53, wherein:

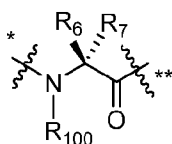
Xaa1 is a group of formula IAA



IAA;

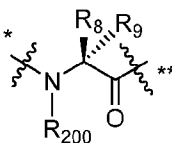
and/or

Xaa2 is a group of formula IBB



IBB;

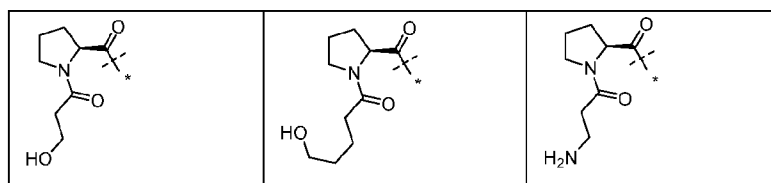
and/or

Xaa5 is a group of formula ICC

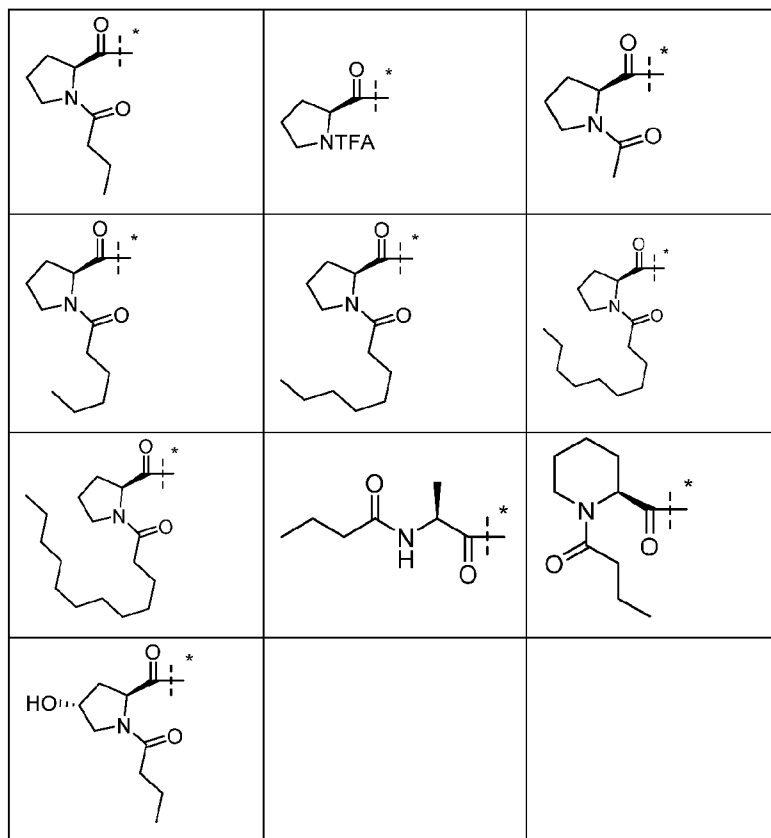
ICC.

55. A compound of any one of the preceding paragraphs, wherein the one or more optional substituents are selected from the group consisting of halo, N₃, CN, NO₂, OH, NR^xR^y, C₁-C₈haloalkyl, C₁-C₈haloalkoxy, C(O)NR^xR^y, C(O)N(R^x)₂-NHC(O)R^x, SO₂R^x, SO₃R^x, OR^y, SR^x, S(O)R^x, S(O)₂R^x, C(O)R^x, OC(O)R^x, C(O)OR^x, C₁-C₈alkyl and aryl; wherein R^x and R^y are each independently H, aryl or C₁-C₈alkyl.

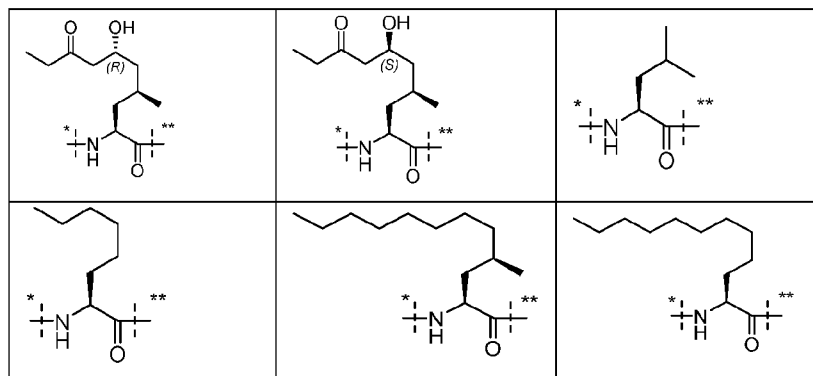
56. A compound of any one of the preceding paragraphs, wherein **Xaa1** is a group selected from the groups listed in the following table.

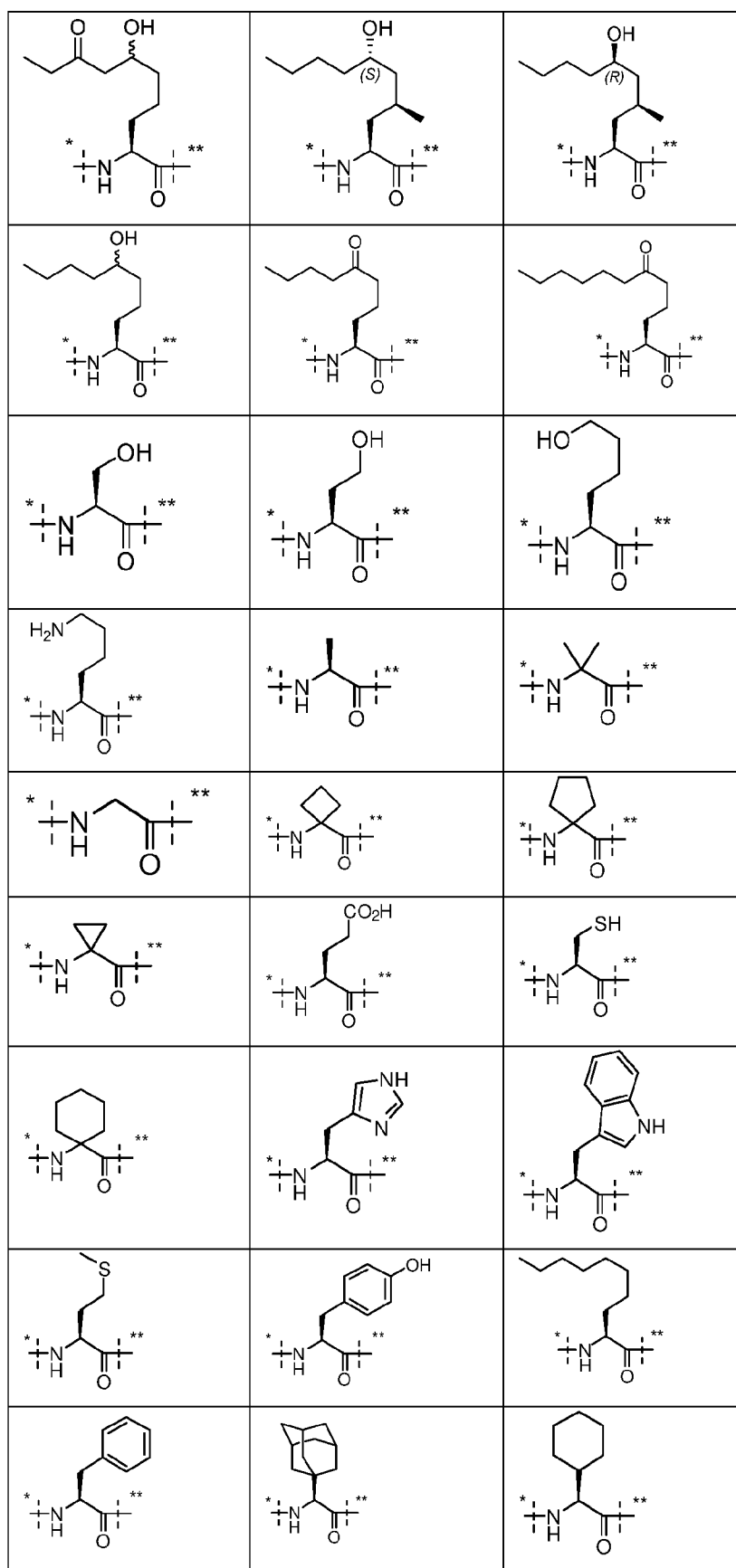


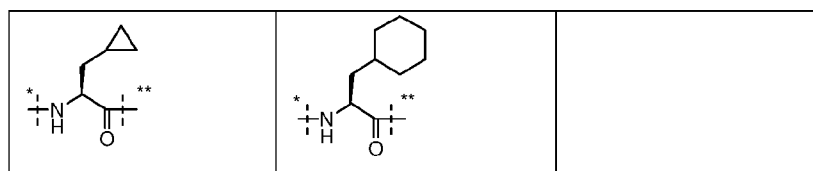
57. A compound of any one of the preceding paragraphs, wherein **Xaa1** is a group selected from the groups listed in the following table.



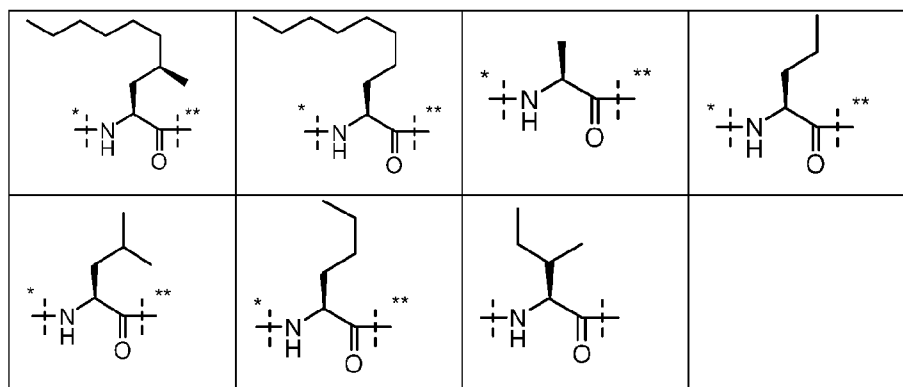
5 58. A compound of any one of the preceding paragraphs, wherein **Xaa2** is a group selected from the groups listed in the following table.



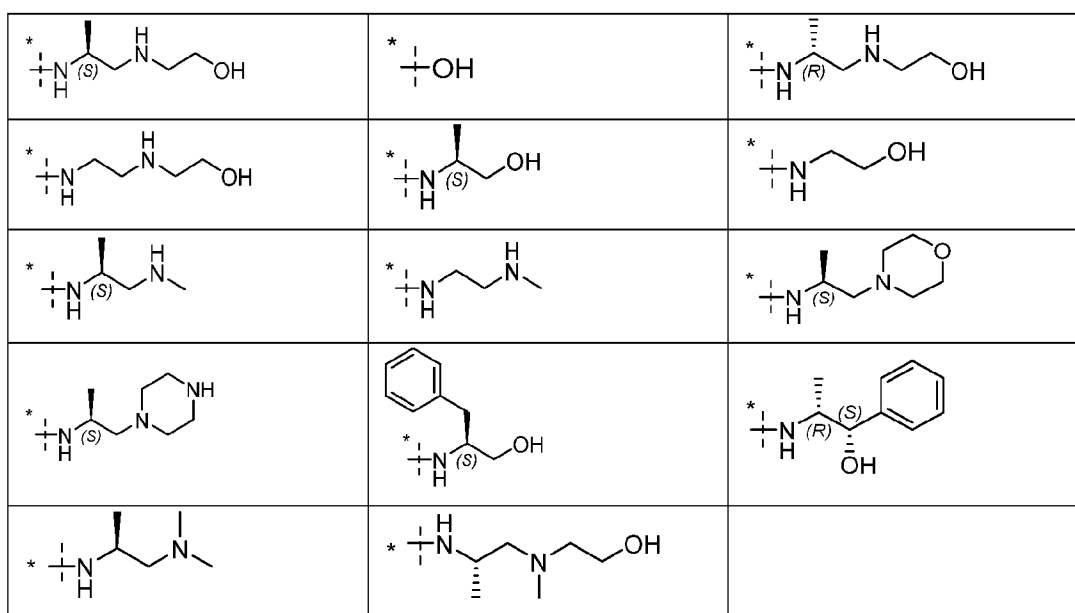




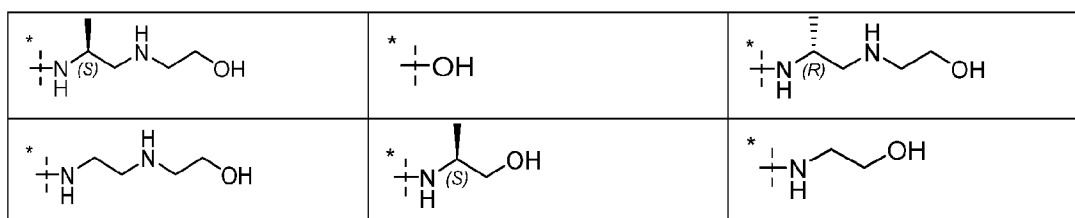
59. A compound of any one of the preceding paragraphs, wherein **Xaa5** is a group selected from the groups listed in the following table.

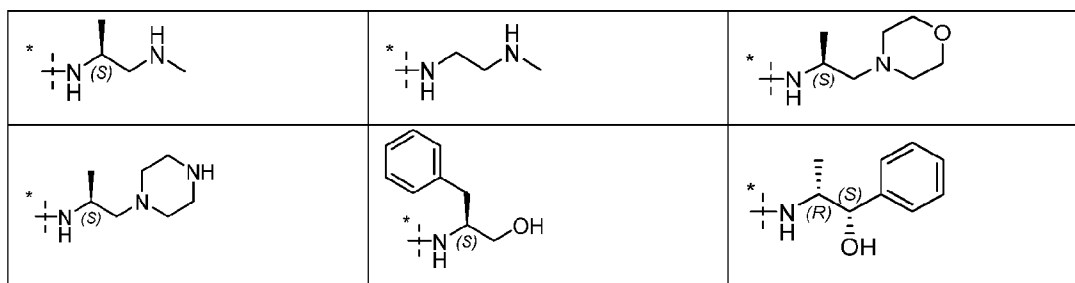


5 60. A compound of any one of the preceding paragraphs, wherein **Xaa10** is a group selected from the groups listed in the following table.



61. A compound of any one of the preceding paragraphs, wherein **Xaa10** is a group selected from the groups listed in the following table.





62. A compound of any one of the preceding paragraphs, wherein the compound is selected from the following compounds of the Examples herein: 520, 1100g, 1200f, 1100d, 170, 500, 1100c, 1200g, 1100e, 450, 240, 51, 480, 510, 50, 420, 1100a, 1200c, 370, 1100h, 1100b, 200c, 490, 230, 200d, 49, 270, 440, 1100f, 54, 410, 320, 380, 390, 1200b, 1100i, 53, 200f, 310, 460, 44, 470, 200g, 260, 200e, 350, 1200h, 45, 360, 290, 250, 340, 46, 52, 210, 200, 42, 48, 430, 43, 220, 1200j, 190, 1200k, 47, 1200d, 200a, 1200e, 200b, 330, 300, 280, 55, 180, 1200a, 1200i, 400 and 200h.
63. A compound according to paragraph 62, wherein the compound is selected from the following compounds of the Examples herein 520, 1100g, 1200f, 1100d, 170, 500, 1100c, 1200g, 1100e, 450, 240, 51, 480, 510, 50, 420, 1100a, 1200c, 370, 1100h, 1100b, 200c, 490, 230, 200d, 49, 270, 440, 1100f, 54, 410, 320, 380, 390, 1200b, 1100i, 53, 200f, 310, 460, 44, 470, 200g, 260, 200e, 350, 1200h, 45, 360, 290, 250, 340, 46, 52, 210, 200, 42, 48, 430, 43, 220, 1200j, 190, 1200k, 47, 1200d, 200a, 1200e, 200b, 330, and 300.
64. A compound according to paragraph 62, wherein the compound is selected from the following compounds of the Examples herein 520, 1100g, 1200f, 1100d, 170, 500, 1100c, 1100e, 450, 240, 51, 480, 510, 49, and 270.
65. A compound according to paragraph 1, wherein the compound is selected from the following compounds of the Examples herein 1200l, 1200m, 1100j, 1100k, 1100l, 1100m, and 1100n.
66. The compound of any one of the preceding paragraphs, wherein the compound has an IC_{50} against a cancer cell line selected from the group consisting of MDA-MB-468, SKBR3, T47D, and NCI-H460 of less than about 5 μ M, for example less than about 4, 3, 2, 1, 0.5, 0.4, 0.3, 0.2, 0.1, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01, 0.009, 0.008, 0.007, 0.006, 0.005, 0.004, or 0.003, or 0.002 μ M, for example as measured by an assays as described in the Examples.
67. The compound of any one of the preceding paragraphs, wherein the compound has an EC_{50} of less than about 1000 nM, for example less than about 500, 100, 50, 40, 30, 20, or 10 nM, as measured in an assay for collapsing a proton gradient in *E. coli* inverted membrane vesicles, for example as described in Example 12.

68. A drug-reactive linker conjugate comprising a compound of any one of paragraphs 1 to 67 or a compound listed in the proviso of paragraph 1, and a reactive linker group having a reactive site that allows the reactive linker group to be reacted with a ligand.
69. The conjugate of paragraph 68, wherein the compound is covalently attached to the reactive linker group.
70. The conjugate of paragraph 68 or 69 wherein the compound is covalently attached by the replacement of one or more atom in the compound with the reactive linker group.
71. The conjugate of any one of paragraphs 68 to 70, wherein the one or more atom that is replaced with the reactive linker group is a hydrogen atom of an OH, SH, or NH group in the compound or an oxygen atom of a ketone in the compound.
72. The conjugate of any one of paragraphs 68 to 71, wherein the reactive linker group is covalently attached to the compound by the replacement of one or more atom in Xaa10 of the compound with the reactive linker group.
73. The conjugate of any one of paragraphs 68 to 72, wherein the reactive linker group is covalently attached to the compound by the replacement of one or more atom in X₁₀ of Xaa10 with the reactive linker group.
74. The conjugate of paragraph 73, wherein:
X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;
R₁₅, R₁₆, and R₁₈ are each H;
and the reactive linker group is covalently attached to the compound by the replacement of R₁₅, R₁₆, or R₁₈ with the reactive linker group.
75. The conjugate of paragraph 73, wherein
X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;
R₁₅, R₁₆, and R₁₈ are each C₁-C₈alkyl(Y₁₀);
Y₁₀ is OR₁₉, NR₂₀R₂₁, or SR₂₂;
R₁₉, R₂₀, and R₂₂ are each H;
and the reactive linker group is covalently attached to the compound by the replacement of R₁₉, R₂₀, or R₂₂ with the reactive linker group.
76. The conjugate of any one of paragraphs 68 to 71, wherein the reactive linker group is covalently attached to the compound by the replacement of one or more atom in Xaa2 of the compound with the reactive linker group.
77. The conjugate of paragraph 76, wherein
R₆ is C₁-C₁₅alkyl(Q_a);
Q_a is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a;
R_a is H;
and the reactive linker group is covalently attached to the compound by the replacement of R_a with the reactive linker group.
78. The conjugate of paragraph 76, wherein
R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

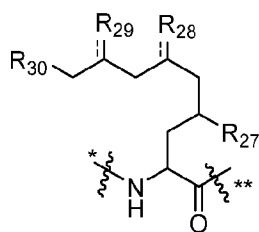
X is at each instance independently O, S, or NR_e;

and the reactive linker group is covalently attached to the compound by the replacement of X with the reactive linker group.

79. The conjugate of paragraph 76, wherein

- 5 R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);
 Q_b and Q_c are each independently H, OR_c, SR_c, or NR_cR_d;
 at least one of Q_b and Q_c is OH, SH, or NHR_d;
 and the reactive linker group is covalently attached to the compound by the
 replacement of the hydrogen atom of the OH, SH, or NH group of the at least one of
 10 Q_b or Q_c with the reactive linker group.

80. The conjugate of any one of paragraphs 68 to 71 or 76, wherein **Xaa2** is a group of formula IIB



IIB

15 wherein

R₂₈ and R₂₉ are each independently H or OH and == is a single bond or O and === is a double bond;

at least one of R₂₈ and R₂₉ is OH or O;

and the reactive linker group is covalently attached to the compound by the

20 replacement of the hydrogen atom of the OH or the O of the at least one of R₂₈ or R₂₉ with the reactive linker group.

81. The conjugate of any one of paragraphs 68 to 71, wherein the reactive linker group is covalently attached to the compound by the replacement of one or more atom in Xaa1 of the compound with the reactive linker group.

25 82. The conjugate of any one of paragraphs 81, wherein the reactive linker group is covalently attached to the compound by the replacement of one or more atom in R₁ of Xaa1 with the reactive linker group.

83. The conjugate of any one of paragraphs 81 or 82, wherein

R₁ is H;

30 and the reactive linker group is covalently attached to the compound by the replacement of R₁ with the reactive linker group.

84. The conjugate of any one of paragraphs 81 to 83, wherein

(c) R₁ is (CH₂CH₂O)_k-R_d or C(O)(CH₂CH₂O)-R_d,

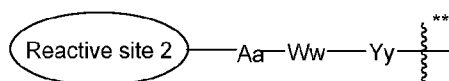
R_d is H, and the reactive linker group is covalently attached to the compound by the
 35 replacement of R_d; or

(d) R_1 is C_1 - C_{15} alkyl(Q_d) or $C(O)C_1$ - C_{15} alkyl(Q_d),

Q_d is OR_a , SR_a , NR_aR_b , $C(O)OR_a$

R_a is H, and the reactive linker group is covalently attached to the compound by the replacement of R_a .

- 5 85. The conjugate of any one of paragraphs 68 to 84, wherein the reactive linker group has the structure



wherein

A is a Stretcher unit;

10 W is an Amino acid unit (Ww);

Y is a Spacer Unit;

a in the reactive linker group is an integer from 0 to 1;

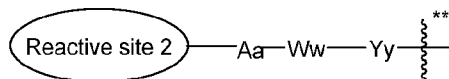
w in the reactive linker group is an integer from 0 to 12;

y in the reactive linker group is an integer from 0 to 2; and

15 Reactive site 2 is the reactive site that allows the reactive linker group to be reacted with a ligand; and

** denotes a bond to a compound of any one of paragraphs 1 to 67 or a compound listed in the proviso of paragraph 1.

- 20 86. The conjugate of any one of paragraphs 68 to 85, wherein the reactive linker group has the structure



wherein

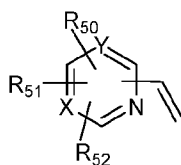
a, w, y are each at least 1, or

a and w are each at least 1 and y is 0, or

25 w is at least 1 and a and y are each 0, or

a is 1 and w and y are each 0.

87. The conjugate of any one of paragraphs 68 to 86, wherein the reactive linker group is a group of formula VI



VI

30 wherein:

X and Y are independently selected from CH or N;

R_{50} is selected from:

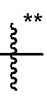
$(\text{CH}_2)_n\text{—C(O)—*}$, or,
 $(\text{CH}_2)_m\text{—Z—*}$, or,
 $(\text{CH}_2)_m\text{—Z—C(O)—*}$, or,
 $(\text{CH}_2)_n\text{—C(O)—Z—*}$, or,
5 $(\text{CH}_2)_m\text{—Z—(CH}_2\text{)—C(O)—*}$, or,
 $(\text{CH}_2)_m\text{—Z—(CH}_2\text{)—C(O)—Z—*}$, or,
 $(\text{CH}_2)_m\text{—Z—C(O)—(CH}_2\text{)}_n\text{—Z—(CH}_2\text{)}_n\text{—C(O)—Z—*}$, or,
 $(\text{CH}_2)_n\text{—CH(CO}_2\text{—*})_2$, or,
 $(\text{CH}_2)_m\text{—Z—(CH}_2\text{)}_2\text{CH(CO}_2\text{—*})_2$, or,
10 $(\text{CH}_2)_n\text{—*}$,

wherein:

Z is independently selected from NH, O or S,

n in the reactive linker group is any integer from 0 to 10,

m in the reactive linker group is any integer from 0 to 10, and

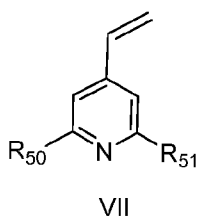
15 * denotes a bond to Ww—Yy— ; and

R_{51} and/or R_{52} are selected from the same groups as R_{50} or R_{51} and/or R_{52} are selected from hydrogen or an electron withdrawing group, such as halogen (F, Cl, or Br), —
 NO_2 , — CO_2H , — CO_2R_{53} , COR_{53} , —CHO, —CN, — CF_3 , — $\text{SO}_2\text{NR}_{53}\text{R}_{54}$ where R_{53} and R_{54}
are independently selected from hydrogen or C_{1-10} alkyl; or

20 R_{51} and/or R_{52} are selected from hydrogen, alkyl or phenyl; or

R_{51} and R_{52} together with the aromatic nitrogen containing ring to which they are attached form a heteroaryl ring, such as, for example, an indole, indazole, benzimidazole, quinoline, isoquinoline, aziridine or a purine.

88. The conjugate of any one of paragraphs 68 to 87, wherein the reactive linker group is a group of formula VII,
25

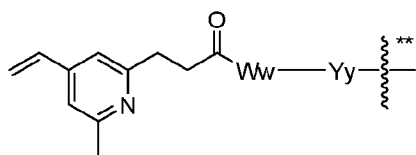


89. The conjugate of paragraph 88, wherein the reactive linker group is a group of formula VII, wherein

R_{50} is $(\text{CH}_2)_n\text{—C(O)—Z—*}$;

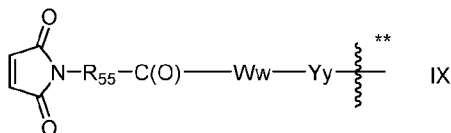
30 R_{51} is alkyl, preferably $\text{C}_1\text{—C}_8$ alkyl, preferably $\text{C}_1\text{—C}_3$ alkyl, preferably CH_3 .

90. The conjugate of any one of paragraphs 68 to 89, wherein the reactive linker group is a group of formula VIII

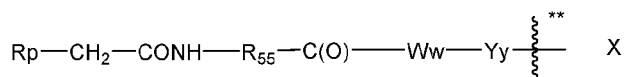


VIII

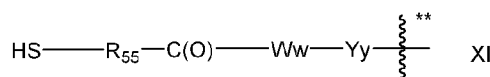
91. The conjugate of any one of paragraphs 68 to 86, wherein the reactive linker group is a group of formula IX, X, XI or XA



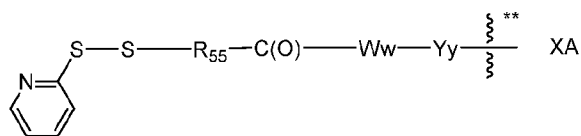
IX



X



XI



XA

5 wherein

R₅₅ is selected from C₁-C₁₅alkyl, C₃-C₈carbocycle, O(C₁-C₈ alkyl), aryl, C₁-C₁₅alkyl(aryl), arylC₁-C₁₅alkyl, C₁-C₁₅alkyl(C₃-C₈carbocycle), (C₃-C₈carbocycle)-C₁-C₁₅ alkyl, a 3 to 8 membered heterocycle, C₁-C₁₅alkyl-(3 to 8 membered heterocycle), (3 to 8 membered heterocycle)-C₁-C₁₅alkyl, (CH₂CH₂O)_r, and (CH₂CH₂O)_r-CH₂;

10 R_p is a suitable leaving group; preferably a halogen, preferably I;

r in the reactive linker group is an integer ranging from 1-10.

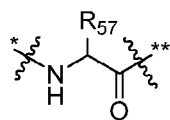
92. The conjugate of paragraph 91, wherein the reactive linker group is a group of formula IX.

15 93. The conjugate of paragraph 91 or 92, wherein the reactive linker group is a group of formula IX, wherein R₅₅ is a C₁-C₁₅alkyl, preferably a C₁-C₅alkyl.

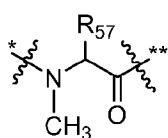
94. The conjugate of any one of paragraphs 91 to 93, wherein w and y are both 0.

95. The conjugate of paragraph 91, wherein the reactive linker group is a group of formula XA, wherein R₅₅ is a C₁-C₁₅alkyl, preferably a C₁-C₈alkyl, preferably a C₁-C₅alkyl and w and y are both 0.

- 20 96. The conjugate of any one of paragraphs 68 to 95, wherein each Amino acid unit is independently a group of formula XII or XIII

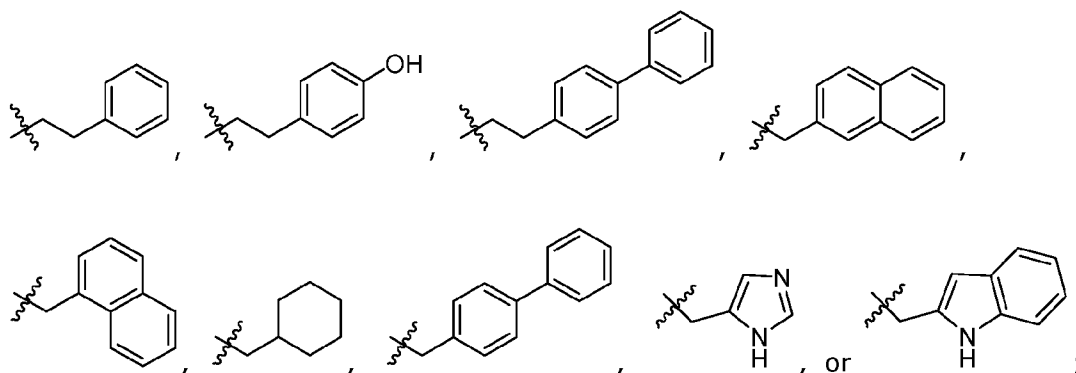


XIII



XIII

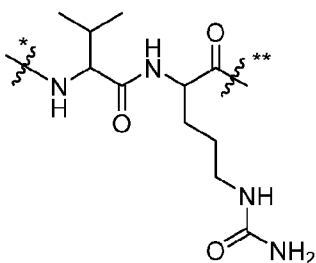
wherein R₅₇ is hydrogen, methyl, isopropyl, isobutyl, sec-butyl, benzyl, p-hydroxybenzyl, —CH₂OH, —CH(OH)CH₃, —CH₂CH₂SCH₃, —CH₂CONH₂, —CH₂COOH, —CH₂CH₂CONH₂, —CH₂CH₂COOH, —(CH₂)₃NHC(=NH)NH₂, —(CH₂)₃NH₂, —(CH₂)₃NHCOCH₃, —(CH₂)₃NHCHO, —(CH₂)₄NHC(=NH)NH₂, —(CH₂)₄NH₂, —(CH₂)₄NHCOCH₃, —(CH₂)₄NHCHO, —(CH₂)₃NHCONH₂, —(CH₂)₄NHCONH₂, —CH₂CH₂CH(OH)CH₂NH₂, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl- or phenyl, cyclohexyl,



wherein * denotes the bond to Aa; and

** denotes the bond to Yy.

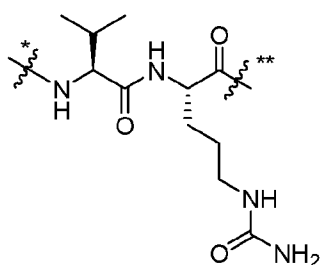
97. The conjugate of paragraph 96, wherein w is 2 such that Ww is a dipeptide group of formula XIV



XIV

wherein * denotes the bond to the Stretcher unit; and ** denotes the bond to the spacer unit.

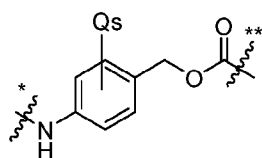
98. The conjugate of paragraph 96 or 97, wherein the Amino acid unit (Ww) is a group of formula XIVi



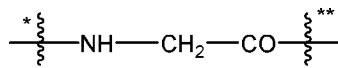
XIVi

wherein * denotes the bond to Aa; and ** denotes the bond to Yy.

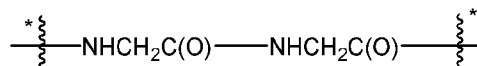
99. The conjugate of any one of paragraphs 68 to 98, wherein each Spacer Unit is a group of formula XV, XVI or XVII



XV



XVI



XVII

5

preferably the Spacer Unit is a group of formula XV;

wherein

Q is $-C_1-C_8$ alkyl, $-O-(C_1-C_8$ alkyl), -halogen, - nitro or -CN;

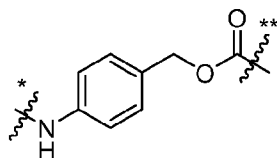
s is an integer ranging from 0-4;

10

* denotes the bond to Ww; and

** denotes the bond to the compound of any one of paragraphs 1 to 67 or a compound listed in the proviso of paragraph 1.

100. The conjugate of any one of paragraphs 68 to 99, wherein y is 1 and the Spacer Unit is a group of formula XVIII

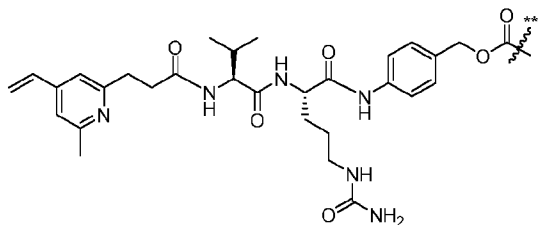


XVIII

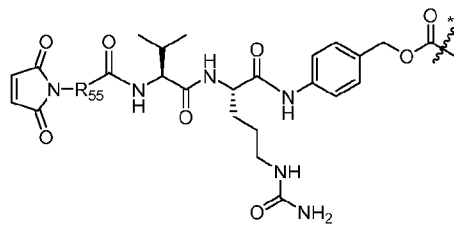
15

wherein * and ** are as defined in paragraph 99.

101. The conjugate of any one of paragraphs 68 to 100, wherein the reactive linker group is a group of formula XIX, XX or XA



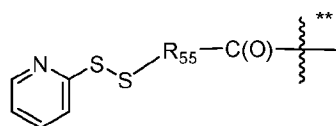
XIX



XX

or

or



XAr

;

wherein R₅₅ is as defined in any one of the preceding paragraphs, preferably a C₁-C₁₅alkyl, preferably a C₁-C₅alkyl;

w and y are each zero; and

- 5 ** denotes the bond to the compound of any one of paragraphs 1 to 67 or a compound listed in the proviso of paragraph 1.

102. A ligand-drug conjugate comprising a ligand and one or more compounds of any one of paragraphs 1 to 67 or a compound listed in the proviso of paragraph 1.

103. The ligand-drug conjugate of paragraph 102, wherein the compound is attached to the
10 ligand via a linker group.

104. The ligand-drug conjugate of paragraph 102 or 103, wherein the compound is covalently attached to the linker group.

105. The ligand-drug conjugate of any one of paragraphs 102 to 104, wherein the compound is covalently attached by the replacement of one or more atom in the
15 compound with the linker group.

106. The ligand-drug conjugate of paragraph 105, wherein the one or more atom that is replaced with the linker group is a hydrogen atom of an OH, SH, or NH group in the compound or an oxygen atom of a ketone in the compound.

107. The ligand-drug conjugate of any one of paragraphs 102 to 106, wherein the linker
20 group is covalently attached to the compound by the replacement of one or more atom in Xaa10 of the compound with the linker group.

108. The ligand-drug conjugate of any one of paragraphs 102 to 107, wherein the linker group is covalently attached to the compound by the replacement of one or more atom in X₁₀ of Xaa10 with the linker group.

- 25 109. The ligand-drug conjugate of paragraph 108, wherein:

X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;

R₁₅, R₁₆, and R₁₈ are each H;

and the linker group is covalently attached to the compound by the replacement of R₁₅, R₁₆, or R₁₈ with the linker group.

- 30 110. The ligand-drug conjugate of paragraph 108, wherein

X₁₀ is OR₁₅, NR₁₆R₁₇, or SR₁₈;

R₁₅, R₁₆, and R₁₈ are each C₁-C₈alkyl(Y₁₀);

Y₁₀ is OR₁₉, NR₂₀R₂₁, or SR₂₂;

R₁₉, R₂₀, and R₂₂ are each H;

and the linker group is covalently attached to the compound by the replacement of R₁₉, R₂₀, or R₂₂ with the linker group.

111. The ligand-drug conjugate of any one of paragraphs 102 to 106, wherein the linker group is covalently attached to the compound by the replacement of one or more atom in Xaa2 of the compound with the linker group.

112. The ligand-drug conjugate of paragraph 111, wherein

R₆ is C₁-C₁₅alkyl(Q_a);

Q_a is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a;

R_a is H ;

and the linker group is covalently attached to the compound by the replacement of R_a with the linker group.

113. The ligand-drug conjugate of paragraph 111, wherein

R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

X is at each instance independently O, S, or NR_e;

and the linker group is covalently attached to the compound by the replacement of X with the linker group.

114. The ligand-drug conjugate of paragraph 111, wherein

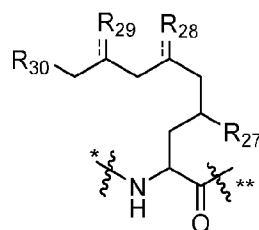
R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

Q_b and Q_c are each independently H, OR_c, SR_c, or NR_cR_d;

at least one of Q_b and Q_c is OH, SH, or NHR_d ;

and the linker group is covalently attached to the compound by the replacement of the hydrogen atom of the OH, SH, or NH group of the at least one of Q_b or Q_c with the linker group.

115. The ligand-drug conjugate of any one of paragraphs 102 to 106, wherein **Xaa2** is a group of formula IIB



IIB

wherein

R₂₈ and R₂₉ are each independently H or OH and --- is a single bond or O and = is a double bond;

at least one of R₂₈ and R₂₉ is OH or O;

and the linker group is covalently attached to the compound by the replacement of the hydrogen atom of the OH or the O of the at least one of R₂₈ or R₂₉ with the linker group.

116. The conjugate of any one of paragraphs 102 to 106, wherein the reactive linker group is covalently attached to the compound by the replacement of one or more atom in Xaa1 of the compound with the reactive linker group.

117. The conjugate of any one of paragraphs 116, wherein the reactive linker group is covalently attached to the compound by the replacement of one or more in R₁ of Xaa1 with the reactive linker group.

118. The conjugate of paragraphs 116 or 117, wherein

R₁ is H;

and the reactive linker group is covalently attached to the compound by the replacement of R₁ with the reactive linker group.

119. The conjugate of paragraphs 116 to 118, wherein

(a) R₁ is (CH₂CH₂O)_k-R_d or C(O)(CH₂CH₂O)-R_d,

R_d is H, and the reactive linker group is covalently attached to the compound by the replacement of R_d; or

(b) R₁ is C₁-C₁₅alkyl(Q_d) or C(O)C₁-C₁₅alkyl(Q_d),

Q_d is OR_a, SR_a, NR_aR_b, C(O)OR_a

R_a is H, and the reactive linker group is covalently attached to the compound by the replacement of R_a.

120. The ligand-drug conjugate of any one of paragraphs 102 to 119, wherein the linker group is covalently attached to the ligand.

121. The ligand-drug conjugate of any one of paragraphs 102 to 120, wherein the linker group is covalently attached to a sulfur atom of the ligand.

122. The ligand-drug conjugate of any one of paragraphs 102 to 121, the ligand-drug conjugate having the formula T-(L-(D)_o)_p,

wherein

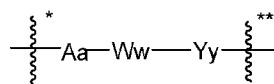
T is a ligand;

L is a linker group;

D is a compound of any one of paragraphs 1 to 67 or a compound in the proviso relating thereto; and

o is an integer from 1 to 10, preferably 2 to 6; and p is an integer from 1 to 10.

123. The ligand-drug conjugate of paragraph 122, wherein the linker group has the structure



wherein

A is a Stretcher unit;

W is an Amino acid unit;

Y is a Spacer Unit;

a in the linker group is an integer from 0 to 1;

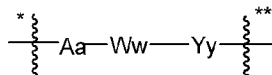
w in the linker group is an integer from 0 to 12;

y in the linker group is an integer from 0 to 2; and

* denotes a bond to the ligand; and

** denotes a bond to the compound of any one of paragraphs 1 to 67 or a compound listed in the proviso of paragraph 1.

124. The ligand-drug conjugate of any paragraph 123, wherein the linker group has the structure



wherein

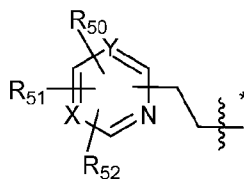
a, w, y are each at least 1, or

a and w are each at least 1 and y is 0, or

w is at least 1 and a and y are each 0, or

a is at least 1 and w and y are each 0.

125. The ligand-drug conjugate of any one of paragraphs 123 or 124, wherein the linker group is a group of formula VIa



VIa

wherein:

X and Y are independently selected from CH or N;

R₅₀ is selected from:

(CH₂)_n-C(O)-***, or,

(CH₂)_m-Z-***, or,

(CH₂)_m-Z-C(O)-***, or,

(CH₂)_n-C(O)-Z-***, or,

(CH₂)_m-Z-(CH₂)-C(O)-***, or,

(CH₂)_m-Z-(CH₂)-C(O)-Z-***, or,

(CH₂)_m-Z-C(O)-(CH₂)_n-Z-(CH₂)_n-C(O)-Z-***, or,

(CH₂)_n-CH(CO₂-***)₂, or,

(CH₂)_m-Z-(CH₂)₂CH(CO₂-***)₂, or,

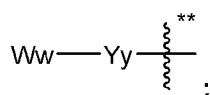
(CH₂)_n-***,

wherein:

Z is independently selected from NH, O or S,

n in the reactive linker group is any integer from 0 to 10,

m in the reactive linker group is any integer from 0 to 10, and



*** denotes a bond to

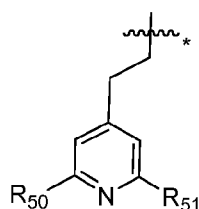
* denotes the bond to the ligand; and

R₅₁ and/or R₅₂ are selected from the same groups as R₅₀ or R₅₁ and/or R₅₂ are selected from hydrogen or an electron withdrawing group, such as halogen (F, Cl, or Br), —NO₂, —CO₂H, —CO₂R₅₃, COR₅₃, —CHO, —CN, —CF₃, —SO₂NR₅₃R₅₄ where R₅₃ and R₅₄ are independently selected from hydrogen or C₁₋₁₀ alkyl; or

R₅₁ and/or R₅₂ are selected from hydrogen, alkyl or phenyl; or

R₅₁ and R₅₂ together with the aromatic nitrogen containing ring to which they are attached form a heteroaryl ring, such as, for example, an indole, indazole, benzimidazole, quinoline, isoquinoline, aziridine or a purine.

126. The ligand-drug conjugate of any one of paragraphs 123 to 125, wherein the linker group is a group of formula VIIa



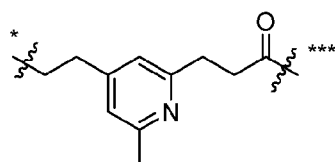
VIIa

127. The ligand-drug conjugate of paragraph 126, wherein the linker group is a group of formula VIIa, wherein

R₅₀ is (CH₂)_n—C(O)—Z-***;

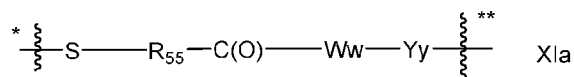
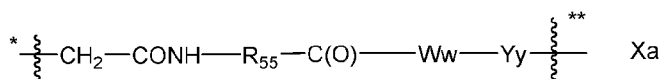
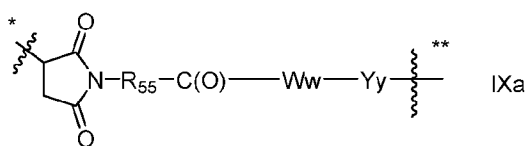
R₅₁ is alkyl, preferably C₁-C₈alkyl, preferably C₁-C₃alkyl, preferably CH₃.

128. The ligand-drug conjugate of any one of paragraphs 123 to 127, wherein the linker group is a group of formula VIIIa



VIIIa

129. The ligand drug conjugate of any one of paragraphs 123 or 124, wherein the linker group is a group of formula IXa, Xa or XIa



wherein

R₅₅ is selected from C₁-C₁₅alkyl, C₃-C₈carbocycle, O(C₁-C₈ alkyl), aryl, C₁-C₁₅alkyl(aryl), arylC₁-C₁₅alkyl, C₁-C₁₅alkyl(C₃-C₈carbocycle), (C₃-C₈carbocycle)-C₁-C₁₅ alkyl, a 3 to 8 membered heterocycle, C₁-C₁₅alkyl-(3 to 8 membered heterocycle), (3 to 8 membered heterocycle)-C₁-C₁₅alkyl, (CH₂CH₂O)_r, and (CH₂CH₂O)_r-CH₂;

r in the linker group is an integer ranging from 1-10;

** denotes the bond to the compound of any one of paragraphs 1 to 67 or a compound listed in the proviso of paragraph 1; and

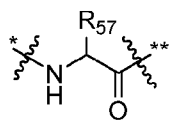
* denotes the bond to the ligand.

130. The ligand-drug conjugate of paragraph 129, wherein the linker group is a group of formula IXa.

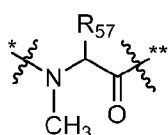
131. The ligand-drug conjugate of paragraph 129 or 130, wherein the linker group is a group of formula IXa, wherein R₅₅ is a C₁-C₁₅alkyl, preferably a C₁-C₅alkyl.

132. The conjugate of paragraph 129, wherein the reactive linker group is a group of formula XA, wherein R₅₅ is a C₁-C₁₅alkyl, preferably a C₁-C₈alkyl, preferably a C₁-C₅alkyl and w and y are both 0.

133. The ligand-drug conjugate of any one of paragraphs 123 to 131, wherein each Amino acid unit is independently a group of formula XII or XIII

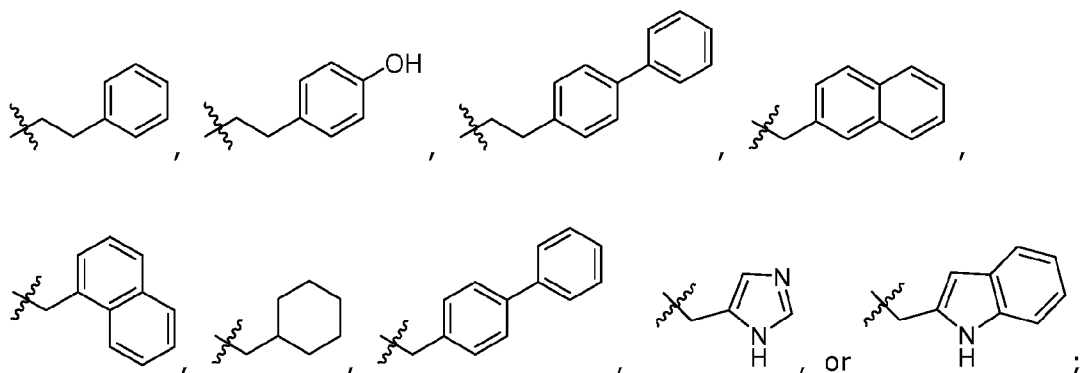


XIII



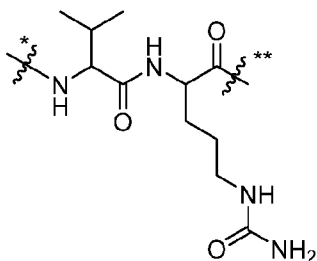
XIII

wherein R₅₇ is hydrogen, methyl, isopropyl, isobutyl, sec-butyl, benzyl, p-hydroxybenzyl, —CH₂OH, —CH(OH)CH₃, —CH₂CH₂SCH₃, —CH₂CONH₂, —CH₂COOH, —CH₂CH₂CONH₂, —CH₂CH₂COOH, —(CH₂)₃NHC(=NH)NH₂, —(CH₂)₃NH₂, —(CH₂)₃NHCOCH₃, —(CH₂)₃NHCHO, —(CH₂)₄NHC(=NH)NH₂, —(CH₂)₄NH₂, —(CH₂)₄NHCOCH₃, —(CH₂)₄NHCHO, —(CH₂)₃NHCONH₂, —(CH₂)₄NHCONH₂, —CH₂CH₂CH(OH)CH₂NH₂, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl- or phenyl, cyclohexyl,



5 wherein * denotes the bond to Aa; and
 ** denotes the bond to Yy.

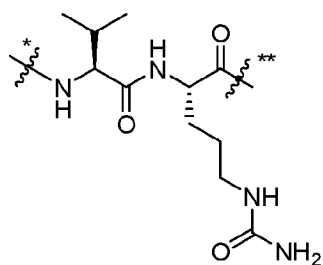
134. The ligand-drug conjugate of any one of paragraphs 123 to 133, wherein w is 2
 such that Ww is a dipeptide group consisting of a first and a second W, each of the first
 and second W having the formula XII, preferably R₃₁ of the first W is isopropyl and/or
 10 R₃₁ of the second W is $-(CH_2)_3NHCONH_2$, preferably R₃₁ of the first W is isopropyl and
 R₃₁ of the second W is $-(CH_2)_3NHCONH_2$.
135. The ligand-drug conjugate of any one of paragraphs 123 to 134, wherein w is 2
 such that the Ww is a dipeptide group of formula XIV



15 XIV

wherein * denotes the bond to Aa; and ** denotes the bond to Yy.

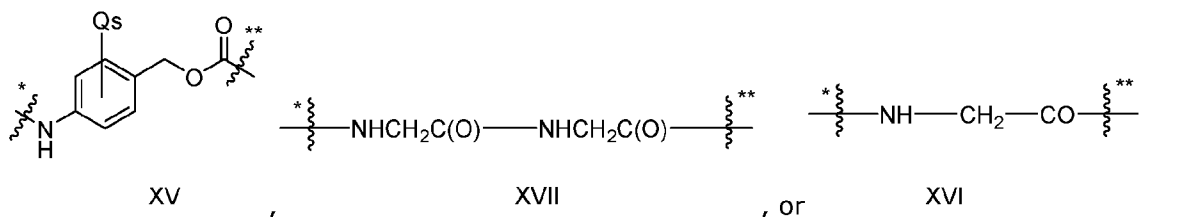
136. The ligand-drug conjugate of any one of paragraphs 123 to 135, wherein W is
 a group of formula XVI



XVI

20 wherein * denotes the bond to Aa; and ** denotes the bond to Yy.

137. The ligand-drug conjugate of any one of paragraphs 123 to 136, wherein each Spacer Unit is a group of formula XV, XVI or XVII



preferably the Spacer Unit is a group of formula XV;

wherein;

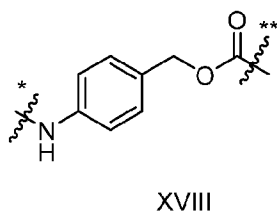
Q when present is $-C_1-C_8$ alkyl, $-O-(C_1-C_8$ alkyl), -halogen, -nitro or -CN;

s is an integer ranging from 0-4;

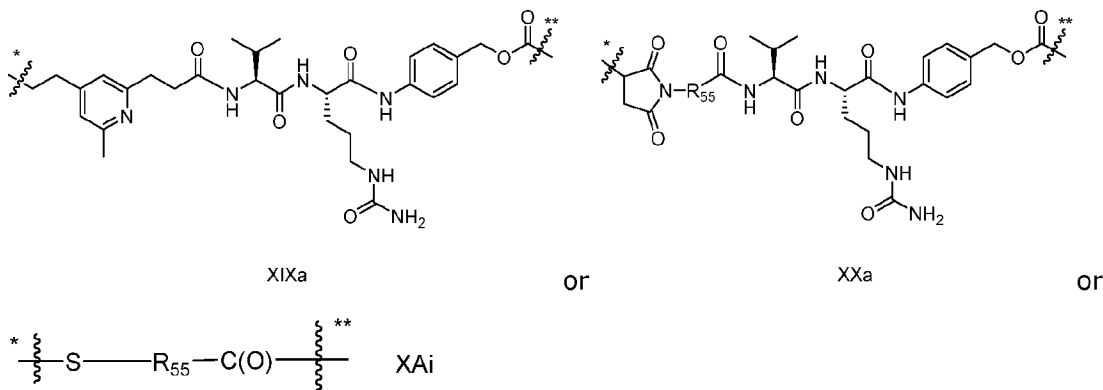
* denotes the bond to Ww; and

** denotes the bond to the compound of any one of paragraphs 1 to 67 or a compound listed in the proviso of paragraph 1.

138. The ligand-drug conjugate of any one of paragraphs 123 to 137, wherein y is 1 and the Spacer Unit is a group of formula XVIII



139. The ligand-drug conjugate of any one of paragraphs 122 to 138, wherein the linker group is a group of formula XIXa, XXa, or XAi



preferably a group of formula XXa;

wherein R₅₅ is as defined in any one of the preceding paragraphs, preferably a —C₁—C₁₀ alkylene-, preferably a C₁–C₅alkylene;

* denotes the bond to the ligand; and

** denotes the bond to the compound of any one of paragraphs 1 to 67 or a compound listed in the proviso of paragraph 1.

140. The ligand-drug conjugate of any one of paragraphs 102 to 139, wherein the ligand is a globular protein.
141. The ligand-drug conjugate of any one of paragraphs 102 to 140, wherein the ligand is an antibody or fragment thereof.
- 5 142. The ligand-drug conjugate of any one of paragraphs 102 to 141, wherein the ligand is an antibody.
143. The ligand-drug conjugate of paragraph 141 or 142, wherein the antibody is a monoclonal antibody, a bi specific antibody, a chimeric antibody, or a humanized antibody.
- 10 144. The ligand-drug of any one of paragraphs 102 to 143, wherein the ligand is an antibody fragment.
145. The ligand-drug conjugate of paragraph 141 or 144, wherein the antibody fragment is a Fab fragment.
- 15 146. The ligand-drug conjugate of any one of paragraphs 102 to 145, wherein the ligand, for example, the antibody or antibody fragment binds to one or more tumour-associated antigens.
147. The ligand-drug conjugate of any one of paragraphs 102 to 146, wherein the ligand, for example, the antibody or antibody fragment, binds to one or more antigens or cell-surface receptors selected from the group consisting of: BMPR1B (bone morphogenetic protein receptor-type IB); E16 (LAT1, SLC7A5); STEAP1 (six transmembrane epithelial antigen of prostate); 0772P (CA125, MUC16); MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin); Napi2b (NAPI-3B, NPTIIB, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b); Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B); PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene); ETBR (Endothelin type B receptor); MSG783 (RNF124, hypothetical protein FLJ20315); STEAP2 (HGNC—8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein); STEAP4 (HGNC—21923, TNFIAP9, STAMP2, STEAP4, six transmembrane epithelial antigen of prostate 4, six transmembrane prostate protein 2); TrpM4 (BR22450, F1120041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4); CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor); CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792); CD79b (IGb (immunoglobulin-associated beta), B29); FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C); an ErbB receptor; NCA; MDP; IL20R α ; Brevican;
- 20
- 25
- 30
- 35

Ephb2R; ASLG659; PSCA; GEDA; BAFF-R (B cell-activating factor receptor, BLyS receptor 3, BR3); CD22(B-cell receptor CD22-B isoform); CD79a (CD79A, CD79 α , immunoglobulin-associated alpha); CXCR5 (Burkitt's lymphoma receptor 1); HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+T lymphocytes); P2X5 (Purinergic receptor P2X ligand-gated ion channel 5); CD72 (B-cell differentiation antigen CD72, Lyb-2); LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family); FCRH1 (Fc receptor-like protein 1); FcRH5 (IRTA2, immunoglobulin superfamily receptor translocation associated 2); TENB2 (putative transmembrane proteoglycan); PMEL17 (silver homolog; SILV; D12S53E; PMEL17; SI; SIL); TMEFF1 (transmembrane protein with EGF-like and two follistatin-like domains I; Tomoregulin-1); GDNF-Ra1 (GDNF family receptor alpha 1; GFRA1; GDNFR; GDNFRA; RETL1; TRNR1; RET1L; GDNFR-alpha1; GFR-ALPHA-1); Ly6E (lymphocyte antigen 6 complex, locus E; Ly67, RIG-E, SCA-2, TSA-1); TMEM46 (shisa homolog 2 (*Xenopus laevis*); SHISA2); Ly6G6D (lymphocyte antigen 6 complex, locus G6D; Ly6-D, MEGT1); LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5; GPR49, GPR67); RET (ret proto-oncogene; MEN2A; HSCR1; MEN2B; MTC1; PTC; CDHF12; Hs.168114; RET51; RET-ELE1); LY6K (lymphocyte antigen 6 complex, locus K; LY6K; HSJ001348; FLJ35226); GPR19 (G protein-coupled receptor 19; Mm.4787); GPR54 (KISS1 receptor; KISS1R; GPR54; HOT7T175; AXOR12); ASPHD1 (aspartate beta-hydroxylase domain containing 1; LOC253982); Tyrosinase (TYR; OCA1A; OCA1A; tyrosinase; SHEP3); TMEM118 (ring finger protein, transmembrane 2; RNFT2; FLJ14627); GPR172A (G protein-coupled receptor 172A; GPCR41; FLJ11856; D15Ert4747e); CD33; CLL-1; CD30 (tumor necrosis factor receptor SF8, TNFRSF8); CD40; CD70; CanAg; PSMA (prostate specific membrane antigen); CA15-3; CA19-9; L6; Lewis Y; Lewis X; alpha fetoprotein; CA242; placental alkaline phosphatase; prostatic acid phosphatase; epidermal growth factor; MAGE-1; MAGE-2; MAGE-3; MAGE-4; anti-transferrin receptor; p97; MUC1-KLH; CEA; gp100; MART1; Prostate serum antigen (PSA); IL-2 receptor; CD20; CD52; human chorionic gonadotropin; CD38; mucin; P21; MPG; IL-7R; and Neu oncogene product.

148. The ligand-drug conjugate of any one of paragraphs 102-147, wherein the ligand, for example, the antibody or antibody fragment, binds to one or more antigens or cell-surface receptors selected from the group consisting of 0772P, MPF, an ErbB receptor, CD22, CD33, CD30, CD40, CD70, CA15-3, epidermal growth factor, IL-2 receptor, CD20, CD52, human chorionic gonadotropin, CD38, and Neu oncogene product.

149. The ligand-drug conjugate of paragraph 147 or 148, wherein the ErbB receptor is selected from the group consisting of epidermal growth factor receptor (EGFR, ErbB1, HER1), HER2 (ErbB2 or p185^{neu}), HER3 (ErbB3) and HER4 (ErbB4 or tyro2).

150. The ligand-drug conjugate of any one of paragraphs 147 to 149, wherein the ErbB receptor is HER2.

151. The ligand-drug conjugate of any one of paragraphs 102-150 wherein the ligand is selected from the group consisting of Abagovomab, Oregovomab, Amatuximab, Inotuzumab, Epratuzumab, Moxetumomab, Gemtuzumab, Lintuzumab, Brentuximab, Rituximab, Nivolumab, Dacetuzumab, MDX-1411, Vorsetuzumab, Cetuximab, Basiliximab, Daclizumab, Ofatumumab, Tositumomab, Ibritumomab, Obinutuzumab, Alemtuzumab, Smart MI95, Daratumumab, huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7, huMAb4D5-8 (Trastuzumab), pertuzumab, and a combination of any two or more thereof.
152. The ligand-drug conjugate of any one of paragraphs 102 to 151 wherein the ligand is selected from the group consisting of huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 huMAb4D5-8 (Trastuzumab), ado-trastuzumab emtansine (Kadcyla), pertuzumab or a combination of any two or more thereof.
153. The ligand-drug conjugate of any one of paragraphs 102 to 152 wherein the ligand, for example an antibody or fragment thereof, binds an antigen or cell surface receptor of a cell that produces autoimmune antibodies, for example a receptor or receptor complex expressed on an activated lymphocyte that is associated with an autoimmune disease.
154. The ligand-drug conjugate of paragraph 153 wherein the antibody binds the IL-7 receptor.
155. The ligand-drug conjugate of any one of paragraphs 102 to 140, wherein the ligand is albumin.
156. The ligand-drug conjugate of any one of paragraphs 102 to 140, wherein the ligand is a peptide.
157. The ligand-drug conjugate of paragraph 156 wherein the peptide binds an integrin.
158. The ligand -drug conjugate of paragraph 157 wherein the integrin is selected from the group comprising $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 6$, $\alpha 5\beta 1$ and $\beta 6$.
159. The ligand-drug conjugate of any one of paragraphs 102 to 158 wherein the ligand is attached to the compound via a cysteine residue of the antibody.
160. The ligand-drug conjugate of any one of paragraphs 102 to 159 wherein the ligand is attached to the compound via Xaa10 of the compound.
161. A pharmaceutical composition comprising:
a compound of formula I as defined in any one paragraphs 1 to 67; or
a ligand-drug conjugate of any one of paragraphs 102 to 160; and
a pharmaceutically acceptable carrier.
162. A compound of formula I as defined in any one paragraphs 1 to 67, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161 for use in therapy.
163. A method of treating or preventing a disease or condition susceptible to treatment with a mitochondrial uncoupling agent or complications associated with a disease or

condition susceptible to treatment with a mitochondrial uncoupling agent in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161.

164. A compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160, or a pharmaceutical composition of paragraph 161 for use in treating or preventing a disease or condition susceptible to treatment with a mitochondrial uncoupling agent or complications associated with a disease or condition susceptible to treatment with a mitochondrial uncoupling agent.

165. Use of a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161 in the manufacture of a medicament for treating or preventing a disease or condition susceptible to treatment with a mitochondrial uncoupling agent or complications associated with a disease or condition susceptible to treatment with a mitochondrial uncoupling agent.

166. A method of killing a cell, the method comprising administering to the subject an effective amount of a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161.

167. A compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160, or a pharmaceutical composition of paragraph 161 for use in killing a cell.

168. Use of a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161 in the manufacture of a medicament for killing a cell.

169. A method of killing or inhibiting proliferation of tumour cells or cancer cells, the method comprising administering to the subject an effective amount of a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161.

170. A compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160, or a pharmaceutical composition of paragraph 161 for use in killing or inhibiting proliferation of tumour cells or cancer cells.

171. Use of a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161 in the manufacture of a medicament for killing or inhibiting proliferation of tumour cells or cancer cells.
172. A method of treating or preventing an automimmune disease, the method comprising administering to the subject an effective amount of a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161.
173. A compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160, or a pharmaceutical composition of paragraph 161 for use in treating or preventing an autoimmune disease.
174. Use of a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161 in the manufacture of a medicament for treating or preventing an autoimmune disease.
175. A method, a compound, ligand-drug conjugate, pharmaceutical composition or use of any one of paragraphs 163 to 165 wherein the disease or condition susceptible to treatment with a mitochondrial uncoupling agent is a metabolic disorder or cancer.
176. A method, a compound, ligand-drug conjugate, pharmaceutical composition or use of any one of paragraphs 163 to 165 wherein the disease or condition susceptible to treatment is cancer.
177. A method, compound, ligand-drug conjugate, pharmaceutical composition or use of any one of paragraphs 169 to 171, 175, or 176 wherein the cancer is selected from the group comprising breast cancer, colorectal cancer and gastroesophageal cancer.
178. A method compound, ligand-drug conjugate, pharmaceutical composition or use of paragraph 177 wherein the breast cancer is HER2-positive breast cancer.
179. A method, compound, ligand-drug conjugate or pharmaceutical composition or use of any one of paragraphs 162 to 178 comprising administering one or more additional therapeutic agents.
180. A method, compound, ligand-drug conjugate or pharmaceutical composition or use of paragraph 179 wherein the one or more additional therapeutic agents is an anticancer agent.
181. A method, compound, ligand-drug conjugate or pharmaceutical composition or use of paragraph 179 or 180 wherein the one or more additional therapeutic agents is a ligand-drug conjugate, preferably an antibody-drug conjugate.

182. A method, compound, ligand-drug conjugate or pharmaceutical composition or use of paragraph 175 wherein the metabolic disorder is selected from the group comprising diabetes, obesity, fatty liver disease and dyslipidemia.
183. A method, compound, ligand-drug conjugate or pharmaceutical composition or use of paragraph 175 wherein the metabolic disorder is type 2 diabetes.
184. A method, compound, ligand-drug conjugate or pharmaceutical composition or use of any one of paragraphs 163-165 comprising treating or preventing complications associated with diabetes or obesity.
185. A method, compound, ligand-drug conjugate or pharmaceutical composition or use of paragraph 184 wherein the complications associated with diabetes comprise one or more of cardiovascular disease, neuropathy, nephropathy, retinopathy, and neurodegenerative disorders.
186. A method, compound, ligand-drug conjugate or pharmaceutical composition or use of any one of paragraphs 162 to 185 wherein the subject is a human.
187. A method or assay of detecting cancer cells comprising exposing cells to a ligand-drug conjugate of any one of paragraphs 102 to 160 and determining the extent of binding of the ligand-drug conjugate to the cells.
188. A method or assay of paragraph 187 wherein the extent of binding is determined by immunohistochemistry.
189. A method of depolarising a membrane, the method comprising contacting the membrane with a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161.
190. A compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161 for depolarising a membrane.
191. Use a compound of formula I as defined in any one paragraphs 1 to 67 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of paragraphs 102 to 160 or a pharmaceutical composition of paragraph 161 in the manufacture of a medicament for depolarising a membrane.
192. The method, compound, or use of any one of paragraphs 189 to 191, wherein the membrane is a mitochondrial membrane.
193. The method, compound, or use of any one of paragraphs 189 to 192, for treating a disease or condition as defined in any one of paragraphs 153 to 186.

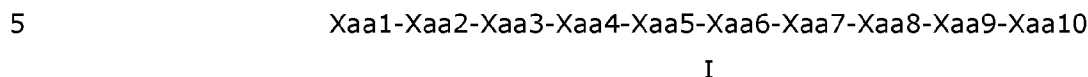
[0742] Where in the foregoing description reference has been made to elements or integers having known equivalents, then such equivalents are included as if they were individually set forth.

[0743] Any documents referred to herein including, but not limited to, patents, patent applications, journal articles, books, and the like, are incorporated herein by reference in their entirety. Any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

- 5 **[0744]** Although the invention has been described by way of example and with reference to particular embodiments, it is to be understood that modifications and/or improvements may be made without departing from the scope or spirit of the invention.

CLAIMS

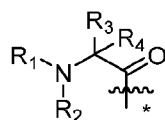
1. A compound of formula I or a pharmaceutically acceptable salt or solvate thereof



wherein:

Xaa1 is a group of formula IA

10



IA

wherein

15

R₁ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C(O)C₁-C₁₅alkyl, (CH₂CH₂O)_k-R_d, C(O)(CH₂CH₂O)_k-R_d, C₁-C₁₅alkyl(Q_d), C(O)C₁-C₁₅alkyl(Q_d), C₁-C₁₅alkyl(aryl), C₁-C₁₅alkyl(C₃-C₁₂carbocycle), C₁-C₁₅alkyl(3 to 12 membered heterocycle), or C₁-C₁₅alkyl(heteroaryl);

20

R₂ and R₃ are each independently H, C₁-C₈alkyl, C₁-C₈haloalkyl, or C₃-C₁₂carbocycle; or R₂ and R₃ together form a ring and have the formula -(CR^mRⁿ)_n- wherein n is from 1 to 6, and at each instance of n R^m is independently H, OH, C₁-C₈alkyl, or C₃-C₁₂carbocycle, and Rⁿ is independently H, C₁-C₈alkyl, or C₃-C₁₂carbocycle, or R^m and Rⁿ together represent =O; and

25

R₄ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl; or R₃ and R₄ together with the carbon atom to which they are attached form a C₃-C₈carbocycle or as 3 to 8 membered heterocycle; R_d is H, C₁-C₈alkyl or C₁-C₈haloalkyl; k is an integer from 1 to 10;

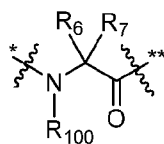
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Q_d is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a; R_a and R_b are each independently H, C₁-C₈alkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, or heteroaryl; and

35

* denotes the bond to Xaa2;

Xaa2 is a group of formula IB



IB

wherein

R₆ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_D)_q), C₁-C₈alkyl(3 to 12 membered heterocycle), C₁-C₈alkyl(heteroaryl), C₁-C₁₅alkyl(Q_a), or C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c);

Q_a is OR_a, SR_a, NR_aR_b, C(O)OR_a, OC(O)R_a;

Q_b and Q_c are each independently H, OR_c, SR_c, or NR_cR_d;

X is at each instance independently O, S, or NR_e;

R_a, R_b, R_c, R_d, and R_e are each independently H, C₁-C₈alkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, or heteroaryl;

q is from 0 to 3;

R_D at each instance of q is independently OH, OR_a, SR_a, NR_aR_b, halo, C₁-8alkyl, C₁-C₈haloalkyl;

R₇ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl;

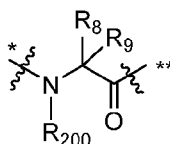
or R₆ and R₇ together with the carbon atom to which they are attached form a 3 to 8 membered heterocycle or C₃-C₈carbocycle;

R₁₀₀ is H or C₁-C₃alkyl;

* denotes the bond to Xaa1; and

** denotes the bond to Xaa3;

Xaa5 is a group of formula IC



IC

wherein

R₈ is C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₁-C₁₅alkyl(aryl), C₁-C₁₅alkyl(C₃-C₁₂carbocycle), C₁-C₁₅alkyl(C₃ to 12 membered heterocycle), or C₁-C₁₅alkyl(heteroaryl);

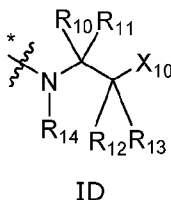
R₉ is H, C₁-C₃alkyl, or C₁-C₃haloalkyl;

R₂₀₀ is H or C₁-C₃alkyl;

* denotes the bond to Xaa4; and

** denotes the bond to Xaa6;

Xaa10 is OH, NR_wR_z or a group of formula ID



wherein

R_{10} and R_{12} are each independently H, $\text{C}_1\text{-C}_{15}$ alkyl, $\text{C}_1\text{-C}_{15}$ haloalkyl, $\text{C}_3\text{-C}_{12}$ carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, $\text{C}_1\text{-C}_8$ alkyl($\text{C}_3\text{-C}_{12}$ carbocycle), $\text{C}_1\text{-C}_8$ alkyl(aryl), $\text{C}_1\text{-C}_8$ alkyl(3 to 12 membered heterocycle), or $\text{C}_1\text{-C}_8$ alkyl(heteroaryl);

R_{11} and R_{13} is H, $\text{C}_1\text{-C}_3$ alkyl or $\text{C}_1\text{-C}_3$ haloalkyl;

or R_{10} and R_{11} together with the carbon atom to which they are attached form a $\text{C}_3\text{-C}_8$ carbocycle or a 3 to 8 membered heterocycle;

or R_{12} and R_{13} together with the carbon atom to which they are attached form a $\text{C}_3\text{-C}_8$ carbocycle or a 3 to 8 membered heterocycle;

R_{14} is H or $\text{C}_1\text{-C}_3$ alkyl;

X_{10} is OR_{15} , $\text{NR}_{16}\text{R}_{17}$, or SR_{18} ;

R_{15} , R_{16} , R_{17} , and R_{18} are each independently H, $\text{C}_1\text{-C}_8$ alkyl, $\text{C}_1\text{-C}_8$ haloalkyl, or $\text{C}_1\text{-C}_8$ alkyl(Y_{10});

or R_{16} and R_{17} together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle or heteroaryl ring;

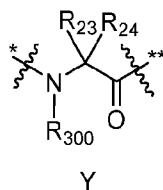
Y_{10} is OR_{19} , $\text{NR}_{20}\text{R}_{21}$ or SR_{22} ;

R_{19} , R_{20} , R_{21} , and R_{22} are each independently H, $\text{C}_1\text{-C}_8$ alkyl, $\text{C}_1\text{-C}_8$ haloalkyl;

R_w and R_z are each independently H or $\text{C}_1\text{-C}_3$ alkyl;

* denotes the bond to Xaa9;

Xaa3, **Xaa4**, **Xaa6**, **Xaa7** and **Xaa8** are each independently a group of formula Y



wherein

R_{23} is H, $\text{C}_1\text{-C}_8$ alkyl, $\text{C}_1\text{-C}_8$ haloalkyl, $\text{C}_1\text{-C}_8$ alkyl(aryl), $\text{C}_1\text{-C}_8$ alkyl($\text{C}_3\text{-C}_{12}$ carbocycle), $\text{C}_1\text{-C}_8$ alkyl(3 to 12 membered heterocycle), or $\text{C}_1\text{-C}_8$ alkyl(heteroaryl);

R_{24} is H, $\text{C}_1\text{-C}_3$ alkyl, or $\text{C}_1\text{-C}_3$ haloalkyl;

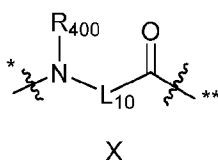
or R_{23} and R_{24} together with the carbon atom to which they are attached form a $\text{C}_3\text{-C}_8$ carbocycle or a 3 to 8 membered heterocycle;

R₃₀₀ is H or C₁-C₃alkyl;

* denotes the bond to Xaa2 in the case of Xaa3, Xaa3 in the case of Xaa4, Xaa5 in the case of Xaa6, Xaa6 in the case of Xaa7, and Xaa7 in the case of Xaa8; and

** denotes the bond to Xaa4 in the case of Xaa3, Xaa5 in the case of Xaa4, Xaa7 in the case of Xaa6, Xaa8 in the case of Xaa7, and Xaa9 in the case of Xaa8; and

Xaa9 is absent, is independently a group of formula Y as defined above, or is a group of the formula X



wherein

R₄₀₀ is H or C₁-C₃alkyl;

L₁₀ is a group of the formula $-(CR^hR^i)_h-$ or $-(CR^hR^i)_i-X^a-(CR^jR^k)_j-$;

R^h and R^j are each independently selected from H, C₁-C₈alkyl, C₁-C₈haloalkyl, C₁-C₈alkyl(aryl), C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl);

Rⁱ and R^k are each independently selected from H, C₁-C₃alkyl, or C₁-C₃haloalkyl; and X^a is selected from O, S, NH, or N(C₁-C₈alkyl);

h, i, and j are each independently selected from 1 to 5, provided that the sum of i and j is 5 or less;

* denotes the bond to Xaa8; and

** denotes the bond to Xaa10;

wherein any alkyl, aryl, carbocycle, heterocycle, or heteroaryl in any of the groups for

R₁, R₂, R₃, R^m, Rⁿ, and R₄ in **Xaa1**,

R₆, Q_a, Q_b, Q_c, X, R_a, R_b, R_c, R_d, R_e, R_D, and R₇ in **Xaa2**,

R₈ and R₉ in **Xaa5**,

R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, X₁₀, R₁₅, R₁₆, R₁₇, R₁₈, Y₁₀, R₁₉, R₂₀, R₂₁, and R₂₂ in **Xaa10**,

R₂₃ and R₂₄ in **Xaa3**, **Xaa4**, **Xaa6**, **Xaa7** and **Xaa8**, and

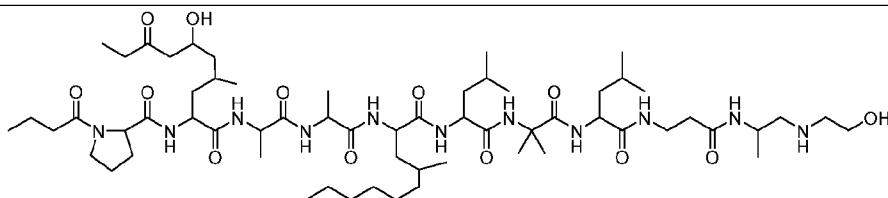
R₂₃, R₂₄, L₁₀, R^h, Rⁱ, R^j, R^k, and X^a in **Xaa9**

are optionally substituted with one or more optional substituents;

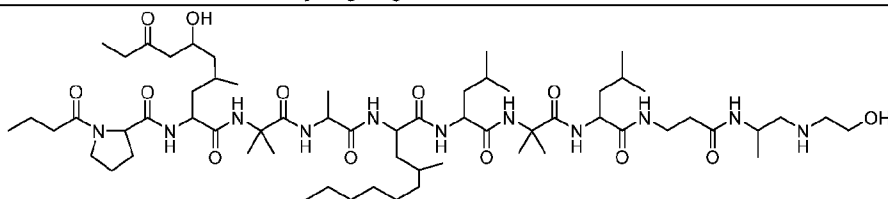
and, preferably, wherein any aryl in any one of the aforementioned groups for Xaa1-Xaa10 is a C₆-C₂₀aryl, preferably C₆-C₁₀aryl, and any heteroaryl in any of the aforementioned groups for Xaa1-Xaa10 is a 5-20 membered heteroaryl, preferably a 5-10 membered heteroaryl;

provided that the compound is not:

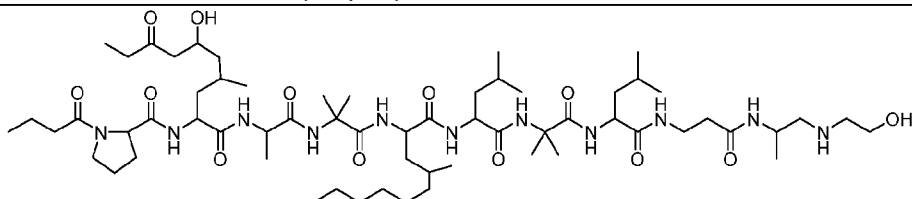
Culicinin A



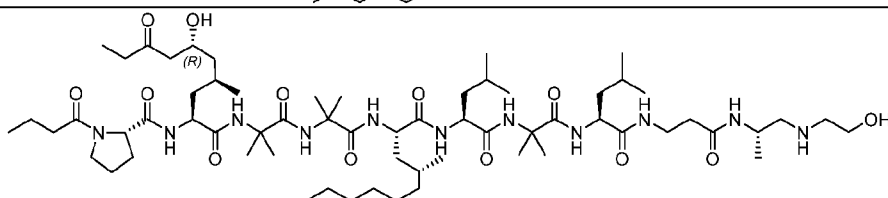
Culicinin B



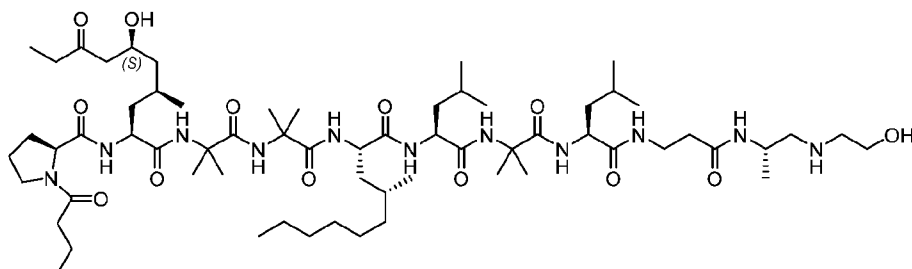
Culicinin C



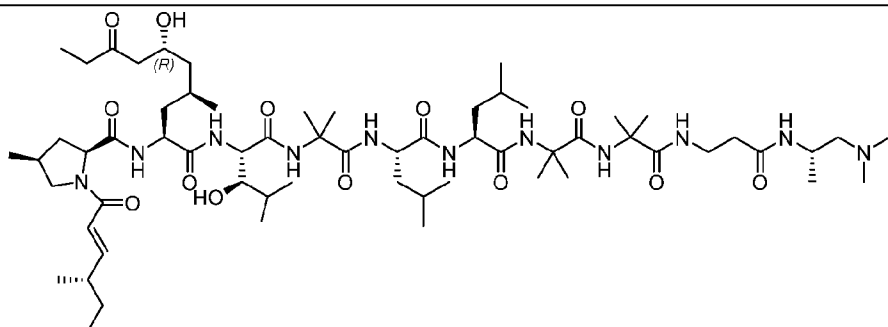
Culicinin D (R)



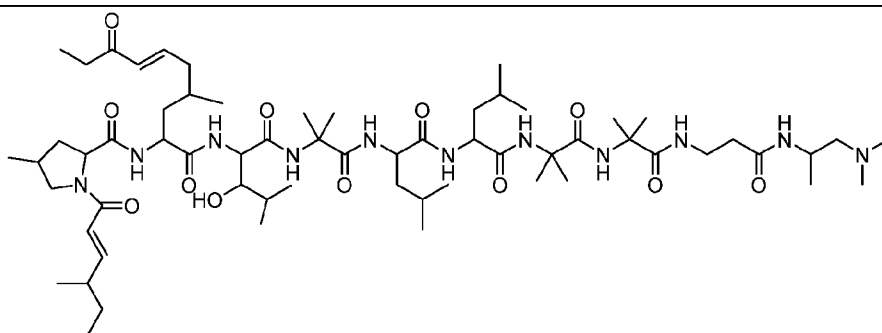
Culicinin D (S)



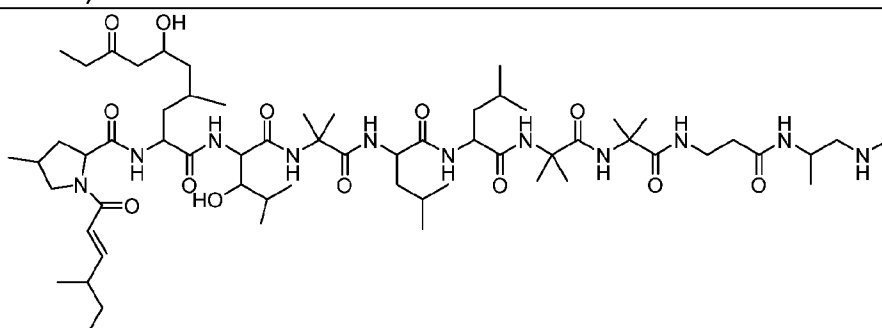
Leucinostatin A



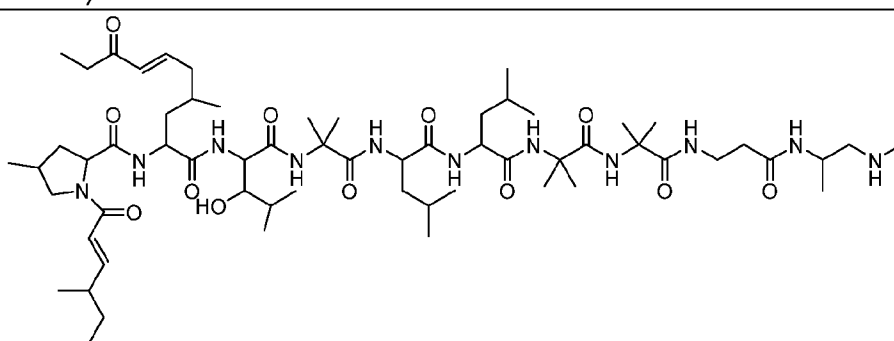
Leucinostatin
A2



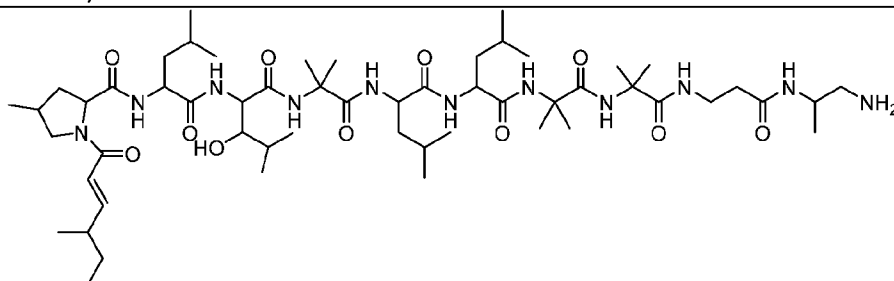
Leucinostatin B



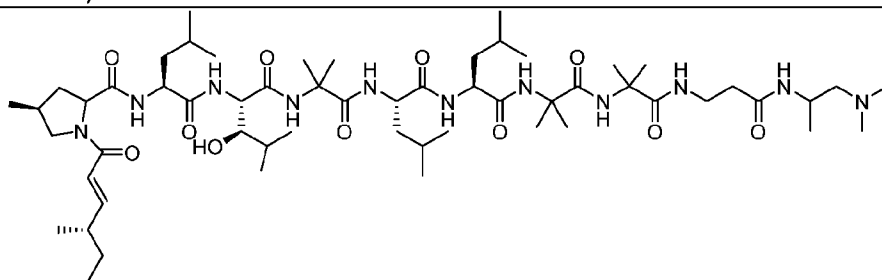
Leucinostatin
B2



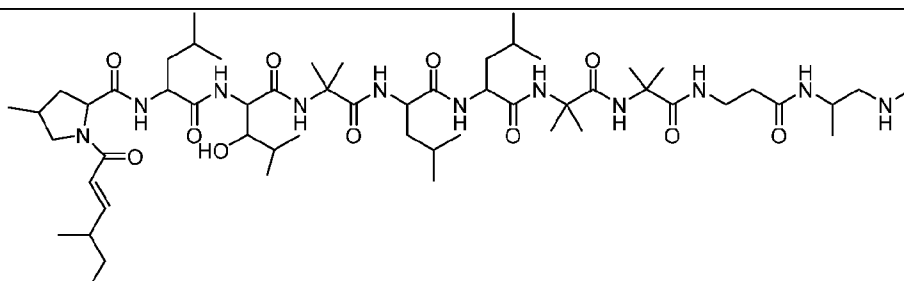
Leucinostatin C



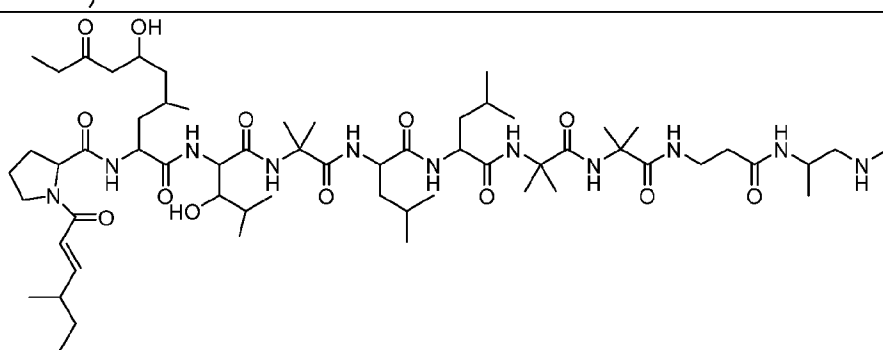
Leucinostatin D



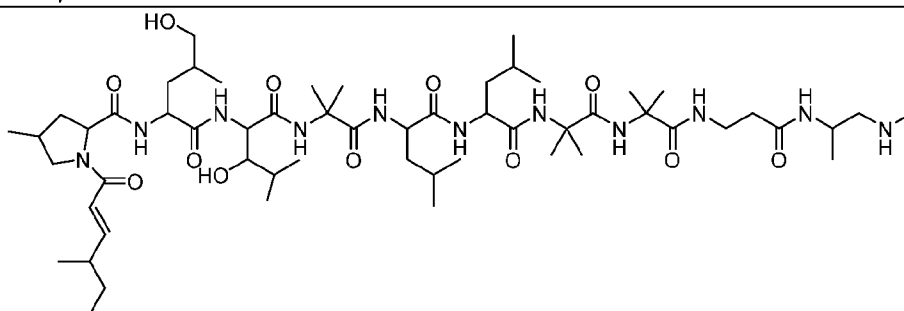
Leucinostatin F



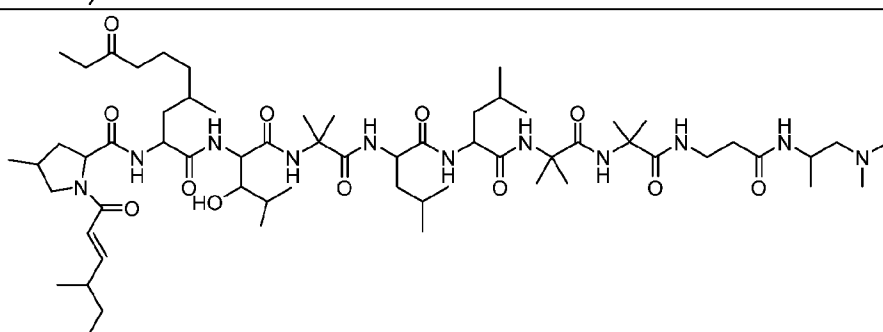
Leucinostatin L



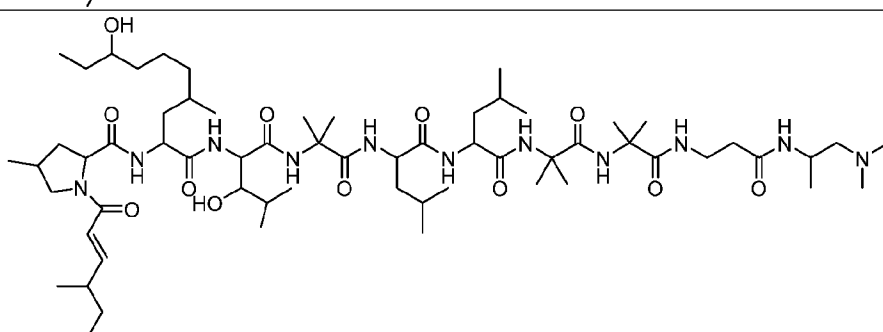
Leucinostatin N



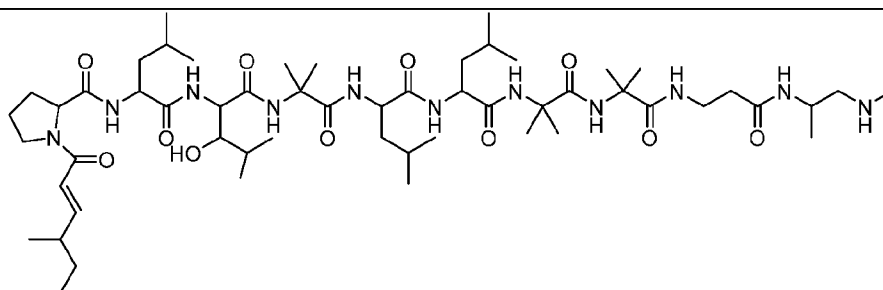
Leucinostatin R



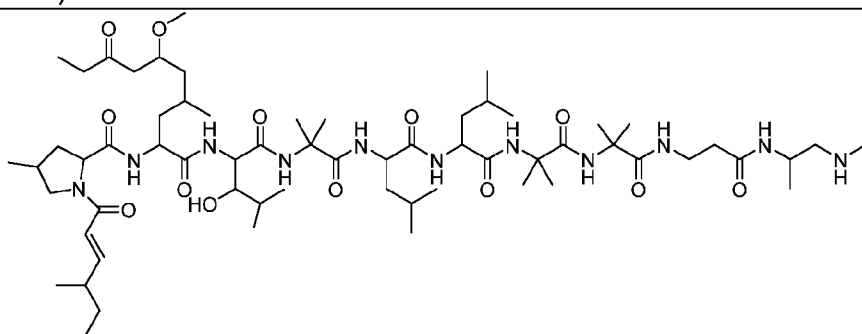
Leucinostatin S



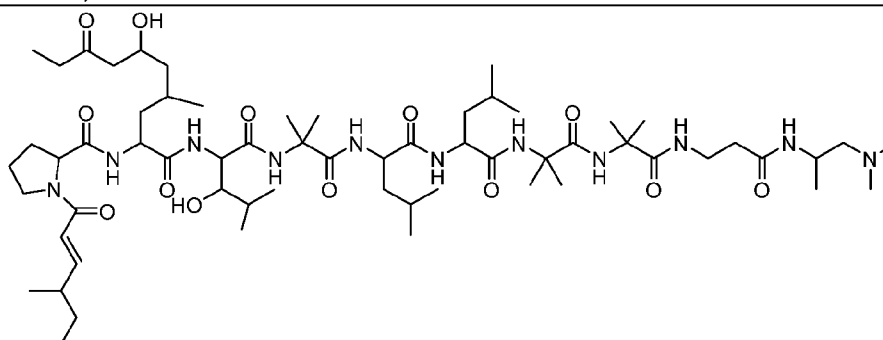
Leucinostatin T



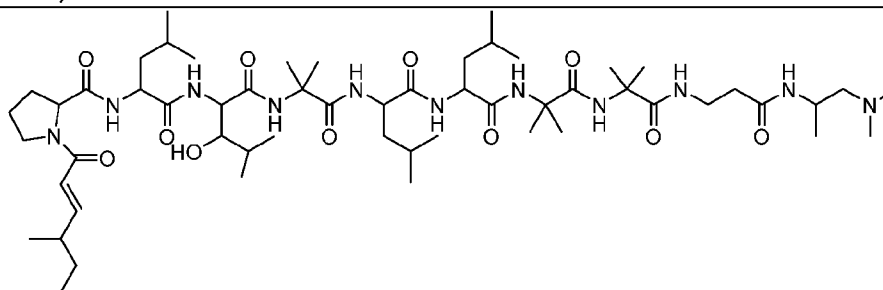
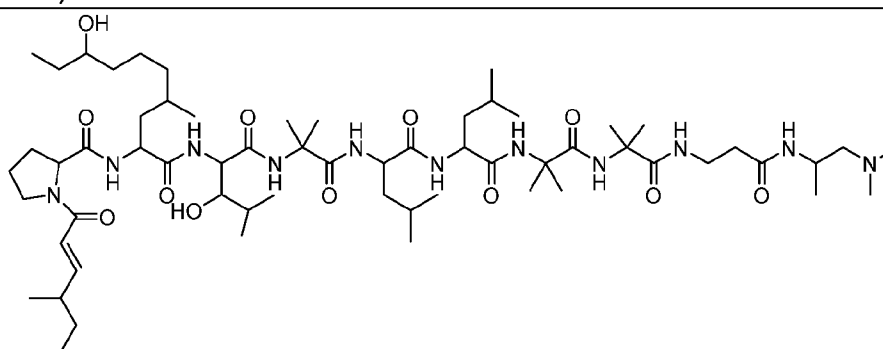
Leucinostatin U

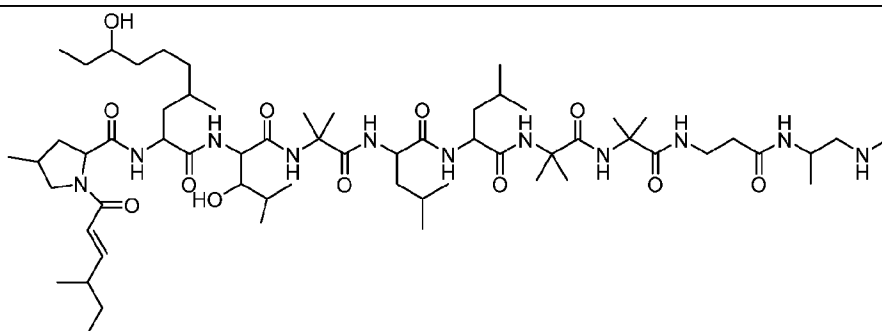


Leucinostatin V

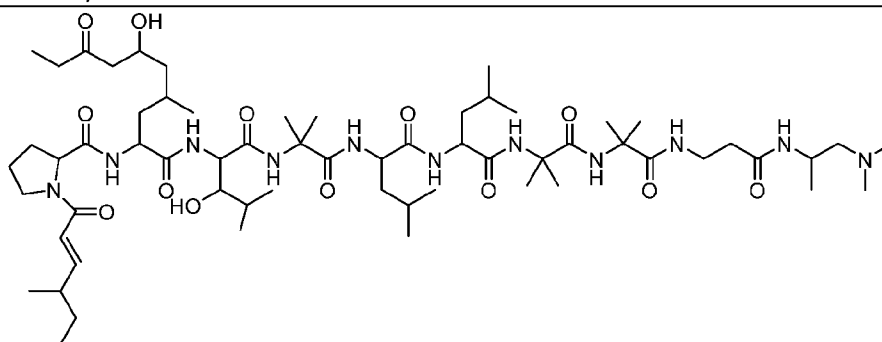


Leucinostatin I

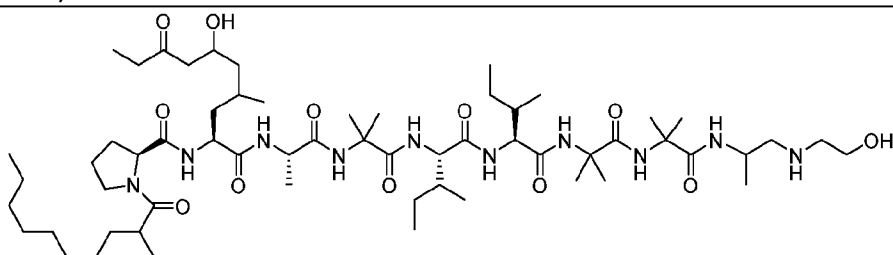
Leucinostatin
III

Leucinostatin
IV

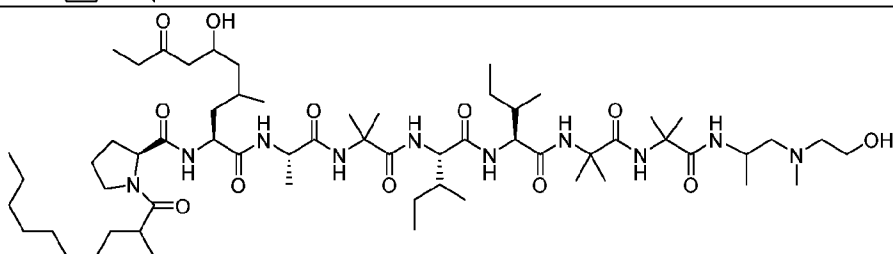
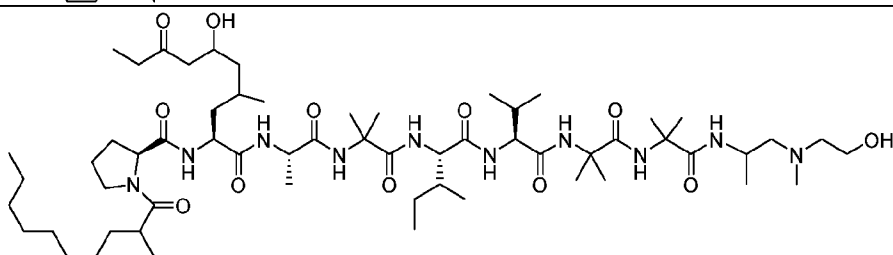
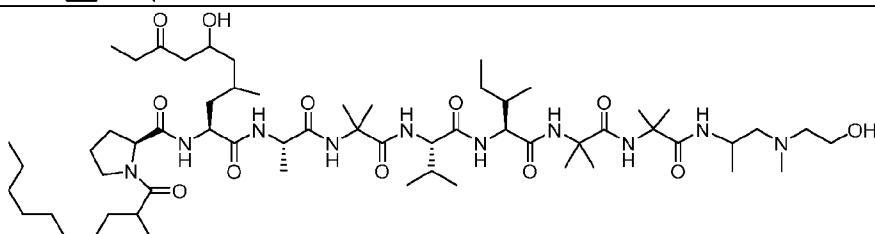
Leucinostatin V

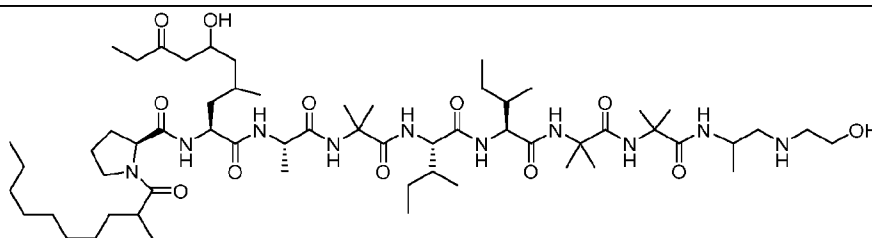
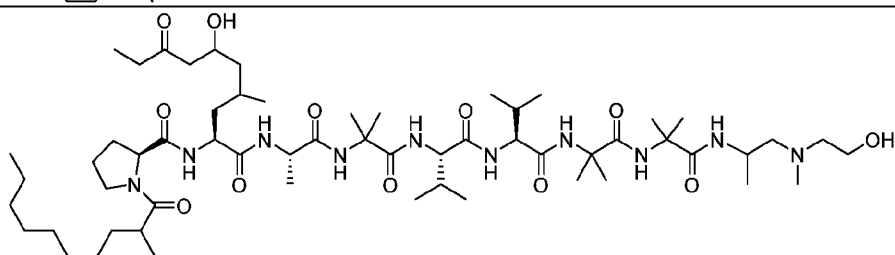
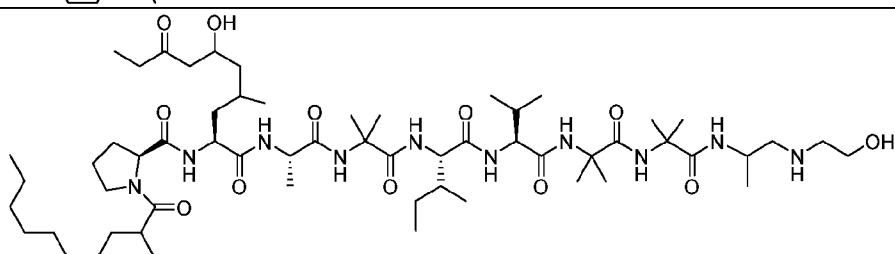
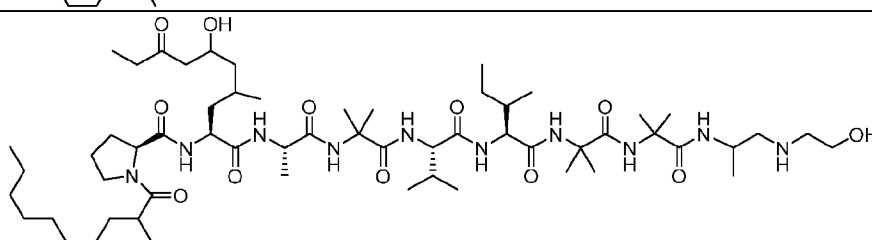
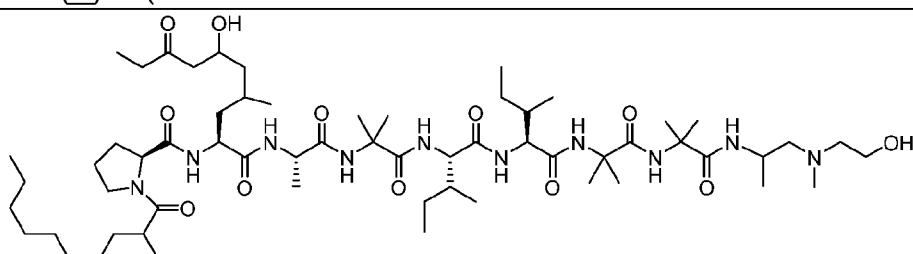
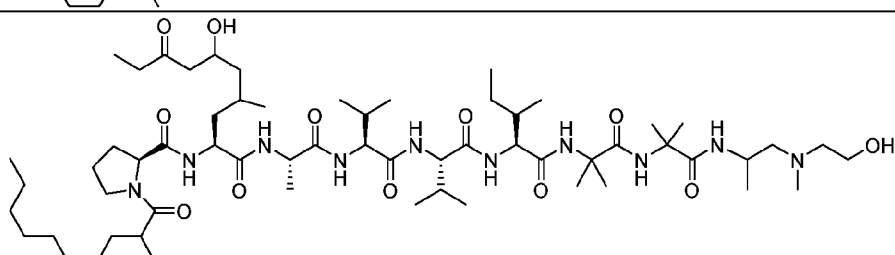
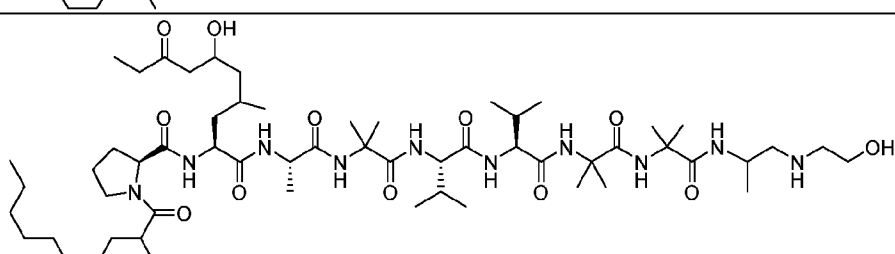


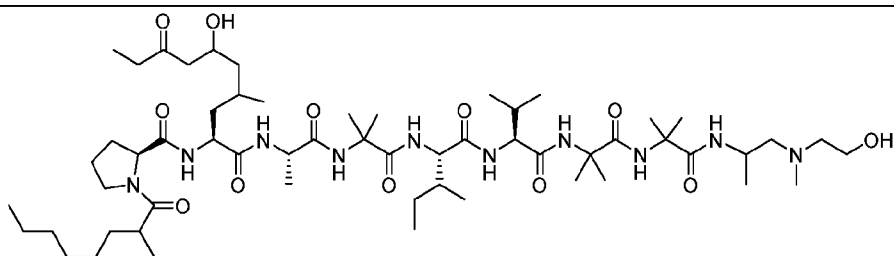
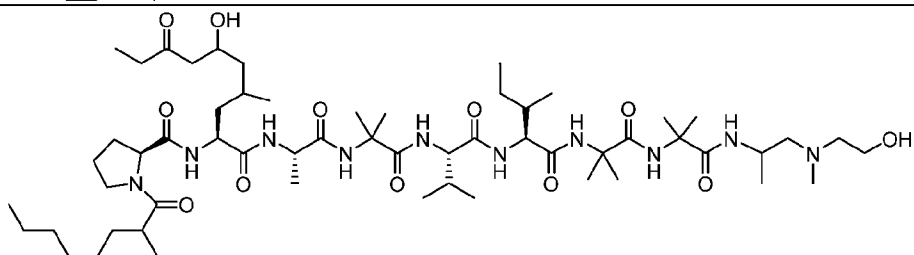
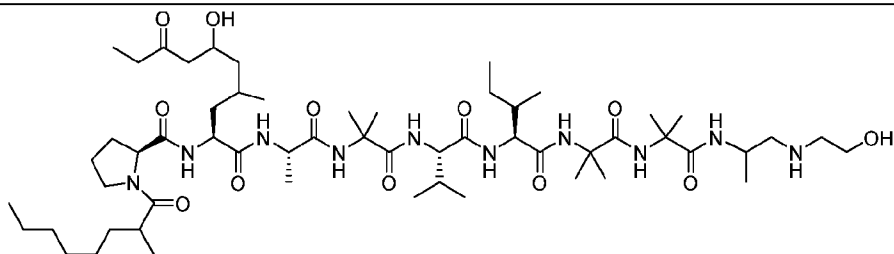
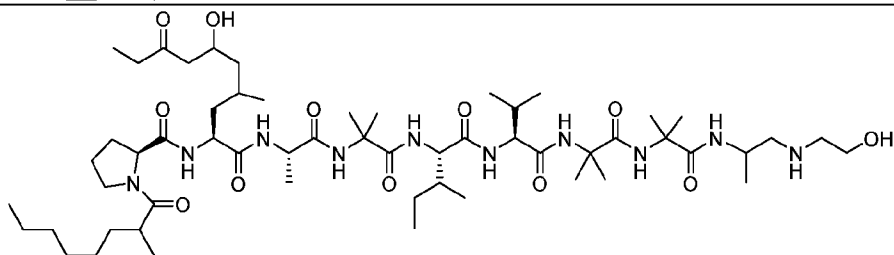
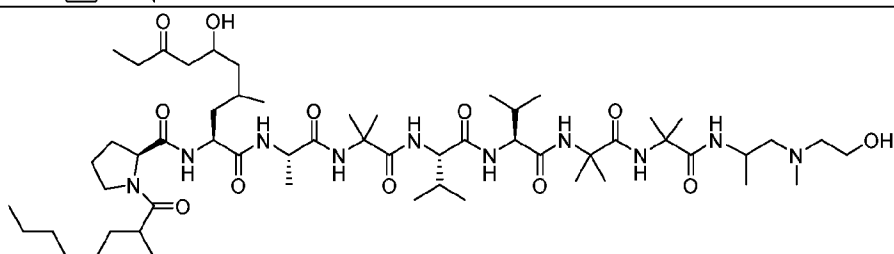
Helioferin A



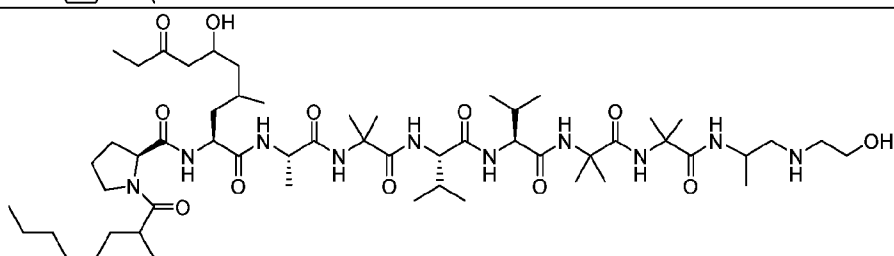
Helioferin B

Roseoferin A₁Roseoferin A₂

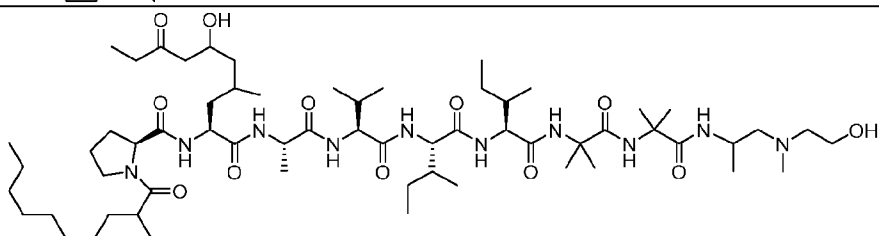
Roseoferin A₃Roseoferin B₁Roseoferin B₂Roseoferin B₃Roseoferin C₁Roseoferin C₂Roseoferin D₁

Roseoferin D₂Roseoferin D₃Roseoferin E₁Roseoferin E₂Roseoferin E₃

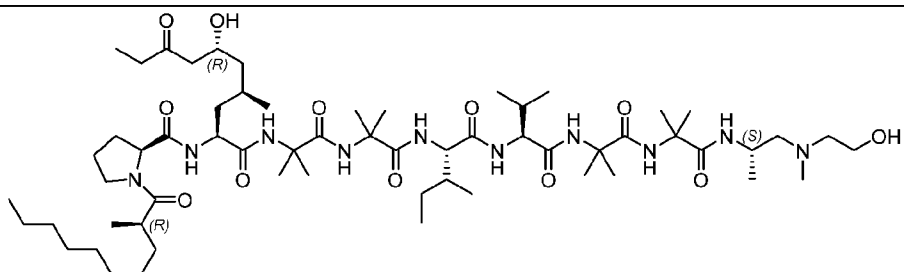
Roseoferin F



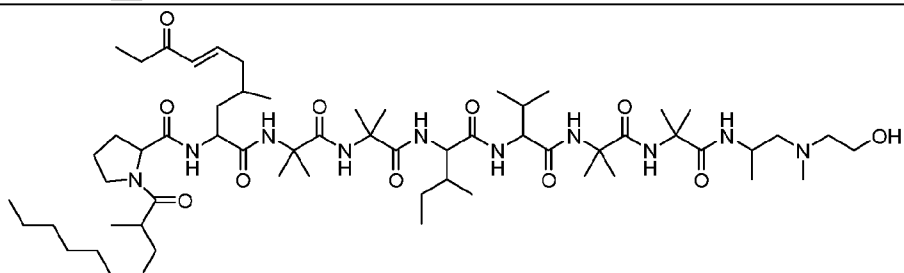
Roseoferin G



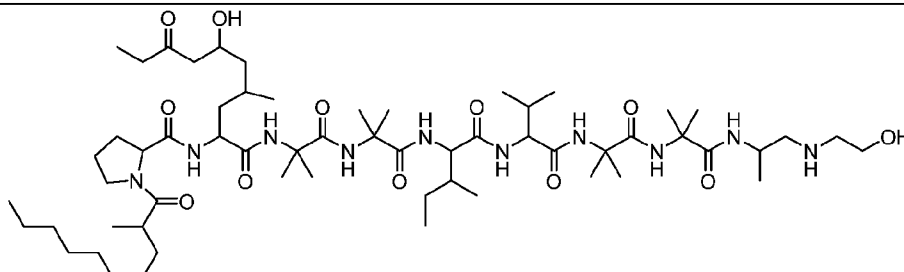
Trichoderin A



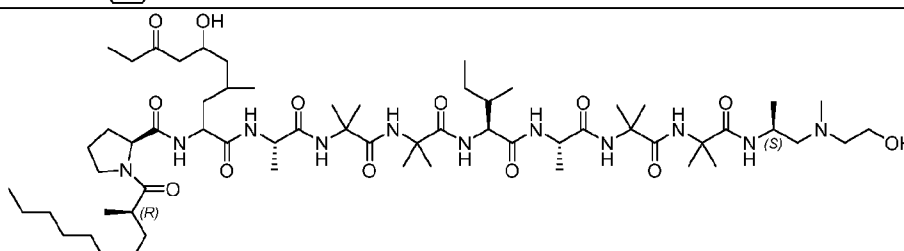
Trichoderin A1



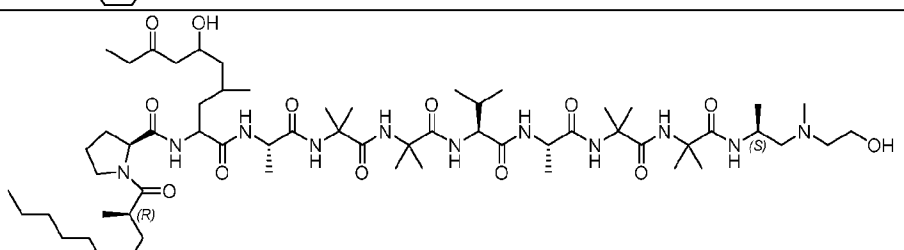
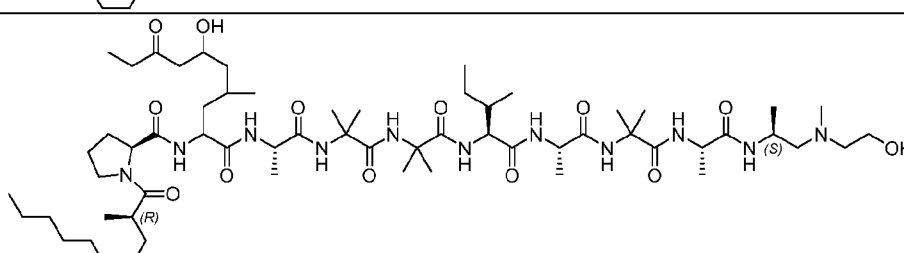
Trichoderin B



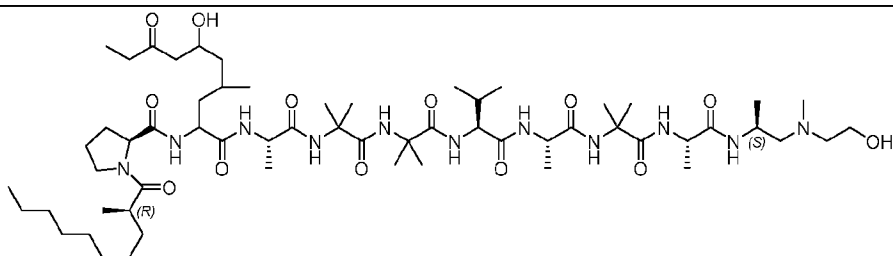
Trichopolyne I



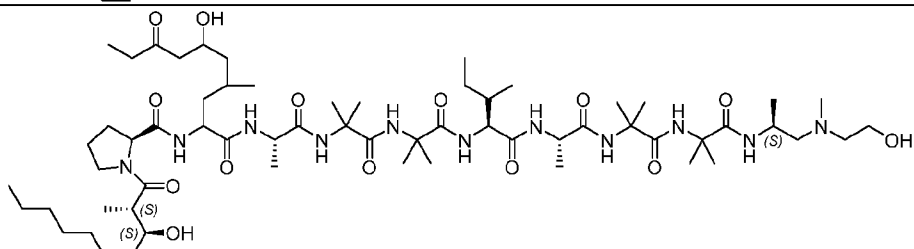
Trichopolyne II

Trichopolyne
III

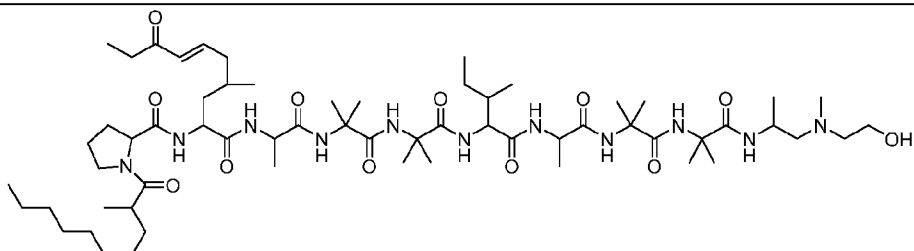
Trichopolyne IV



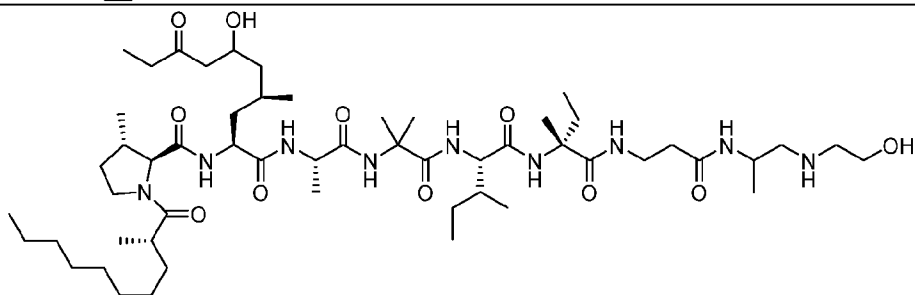
Trichopolyne V



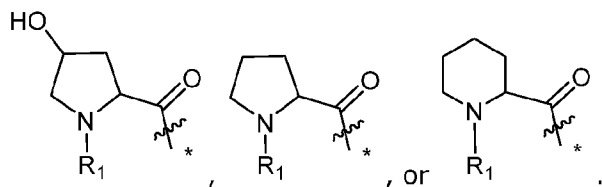
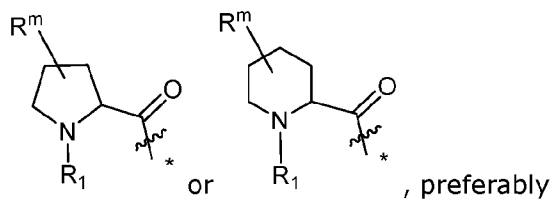
Trichopolyne VI



Emericellipsin A



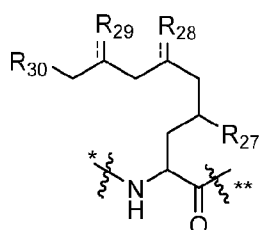
2. A compound of claim 1, wherein in **Xaa1** R₂ and R₃ together form a ring and n is an integer selected from 2 to 6, 3 to 6, 3 to 5, or, preferably, 3 to 4.
3. A compound of claim 1 or 2, wherein **Xaa1** is a group of formula:



5

wherein R^m, R₁ and * are as defined in any one of the preceding claims.

4. A compound of any one of claims 1 to 3, wherein in **Xaa1** R₁ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C(O)C₁-C₁₅alkyl, and C(O)C₁-C₁₅haloalkyl, preferably H or C(O)C₁-C₁₅alkyl, preferably H or C(O)C₁-C₁₃alkyl, preferably H or C(O)C₁-C₁₁alkyl.
5. A compound of claim 1, wherein in **Xaa1** R₂ is H or C₁-C₆alkyl, preferably H or C₁-C₄alkyl, preferably H; and/or R₃ is C₁-C₈alkyl, preferably C₁-C₆alkyl, preferably C₁-C₄alkyl, preferably CH₃.
6. A compound of claim 5, wherein in **Xaa1** R₁ is C(O)C₁-C₁₅alkyl, preferably C(O)C₁-C₁₀alkyl, preferably C(O)C₁-C₈alkyl, preferably C(O)C₁-C₅alkyl, preferably C(O)C₁-C₃alkyl.
7. A compound of any one of claims 1 to 3 and 5, wherein in **Xaa1** R₁ is (CH₂CH₂O)_k-R_d, C(O)(CH₂CH₂O)_k-R_d, C₁-C₁₅alkyl(Q_d), or C(O)C₁-C₁₅alkyl(Q_d), preferably C₁-C₁₅alkyl(Q_d) or C(O)C₁-C₁₅alkyl(Q_d), preferably C(O)C₁-C₁₅alkyl(Q_d), preferably C(O)C₁-C₁₀alkyl(Q_d), preferably C(O)C₁-C₈alkyl(Q_d), preferably C(O)C₁-C₅alkyl(Q_d).
8. A compound of any one of claims 1 to 3, 5, and 7, wherein in **Xaa1** Q_d is OR_a, SR_a, or NR_aR_b, preferably OR_a or NR_aR_b; and optionally R_a and R_b are each independently H or C₁-8alkyl, preferably H or CH₃.
9. A compound of any one of claims 1 to 8, wherein **Xaa2** is a group of formula IB, wherein
R₆ is C₁-C₁₅alkyl or C₁-C₁₅haloalkyl, preferably C₁-C₁₅alkyl, preferably C₄-C₁₅alkyl, C₆-C₁₅alkyl, C₈-C₁₅alkyl, or C₁₀-C₁₅alkyl; or
R₆ is C₁-C₁₅alkyl(Q_a); or
R₆ is C₁-C₈alkyl(Q_b)-C(X)-C₁-C₈alkyl(Q_c).
10. A compound of any one of claims 1 to 9, wherein **Xaa2** is a group of formula IB, wherein Q_a is OR_a, SR_a, or NR_aR_b, preferably OR_a or SR_a; and optionally R_a and R_b are each H or C₁-C₈alkyl.
11. A compound of any one of claims 1 to 9, wherein **Xaa2** is a group of formula IB, wherein X is O; and/or Q_b and Q_c are each independently H or OR_c, preferably H or OH; and/or R_c, R_d, and R_e are each independently H or C₁-C₈alkyl.
12. A compound of any one of claims 1 to 9 and 11, wherein **Xaa2** is a group of formula IIB



IIB

wherein

R₂₇ is H or C₁-C₈alkyl, preferably H or CH₃;

== is a single bond or a double bond;

R₂₈ and R₂₉ are each independently H or OH and $\equiv$ is a single bond or O and $\equiv$ is a double bond; and

R₃₀ is H or C₁-C₈alkyl.

13. A compound of claim 12, wherein (i) R₂₈ and R₂₉ are each independently H; (ii) R₂₈ is OH or O and R₂₉ is H; or (iii) R₂₈ is OH and R₂₉ is O.
14. A compound of any one of claims 1 to 8, wherein **Xaa2** is a group of formula IB, wherein R₆ is C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_d)_q), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably C₃-C₁₂carbocycle, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_d)_q), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably C₃-C₁₂carbocycle, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl(R_d)_q), or C₁-C₈alkyl(heteroaryl); or R₆ and R₇ together form a 3 to 8 membered heterocycle or C₃-C₈carbocycle, preferably a C₃-C₈carbocycle, preferably a C₃-C₆carbocycle.
15. A compound of any one of claims 1 to 14, wherein in **Xaa5** R₈ is C₁-C₁₅alkyl or C₁-C₁₅haloalkyl, preferably C₂-C₁₂alkyl, preferably C₂-C₉alkyl, C₃-C₉alkyl, or C₄-C₉alkyl.
16. A compound of any one of claims 1 to 15, wherein **Xaa10** is a group of formula ID wherein
R₁₀ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably H, C₁-C₁₅alkyl, C₃-C₁₂carbocycle, aryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), or C₁-C₈alkyl(aryl), preferably H, C₁-C₁₀alkyl, or C₁-C₈alkyl(aryl); and
R₁₁, R₁₂, and R₁₃ is H, C₁-C₃alkyl or C₁-C₃haloalkyl, preferably H or C₁-C₃alkyl, preferably H.
17. A compound of any one of claims 1 to 16, wherein **Xaa10** is a group of formula ID wherein
R₁₀ is H, C₁-C₈alkyl, preferably C₁-C₆alkyl, preferably C₁-C₄alkyl, preferably CH₃, or C₁-C₆alkyl(aryl), preferably C₁-C₄alkyl(aryl), preferably C₁-C₃alkyl(aryl); and/or R₁₁ is H.
18. A compound of any one of claims 1 to 15, wherein **Xaa10** is a group of formula ID wherein
R₁₂ is H, C₁-C₁₅alkyl, C₁-C₁₅haloalkyl, C₃-C₁₂carbocycle, aryl, 3 to 12 membered heterocycle, heteroaryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), C₁-C₈alkyl(aryl), C₁-C₈alkyl(3 to 12 membered heterocycle), or C₁-C₈alkyl(heteroaryl), preferably H, C₁-C₁₅alkyl, C₃-C₁₂carbocycle, aryl, C₁-C₈alkyl(C₃-C₁₂carbocycle), or C₁-C₈alkyl(aryl), preferably H, C₁-C₁₀alkyl, or aryl; and
R₁₀, R₁₁, and R₁₃ is H, C₁-C₃alkyl or C₁-C₃haloalkyl, preferably H or C₁-C₃alkyl, preferably H.
19. A compound of claim 18, wherein **Xaa10** is a group of formula ID wherein

R₁₂ is H or aryl; and optionally R₁₃ is H, R₁₄ is H, or R₁₃ and R₁₄ are each H.

20. A compound of any one of claims 1 to 19, wherein **Xaa10** is a group of formula ID wherein

X₁₀ is OR₁₅ or NR₁₆R₁₇;

5 R₁₅ is H, C₁-C₈alkyl, preferably H;

R₁₆ is H, C₁-C₈alkyl, or C₁-C₈alkyl(Y₁₀), preferably C₁-C₈alkyl or C₁-C₈alkyl(Y₁₀);

R₁₇ is H or C₁-C₈alkyl, preferably H;

or R₁₆ and R₁₇ together with the nitrogen atom to which they are attached form a 3 to 8 membered heterocycle;

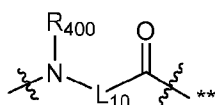
10 Y₁₀ is OR₁₉, NR₂₀R₂₁ or SR₂₂, preferably OR₁₉, preferably OH;

R₁₉, R₂₀, R₂₁, and R₂₂ are each independently H or C₁-C₈alkyl, preferably H.

21. A compound of any one of claims 1 to 20, wherein in the formula Y R₂₃ is C₁-C₈alkyl; and R₂₄ is H or C₁-C₈alkyl, preferably R₂₃ is C₁-C₈alkyl and R₂₄ is C₁-C₈alkyl or R₂₃ is C₂-C₈alkyl, preferably C₃-C₈alkyl, and R₂₄ is H.

- 15 22. A compound of any one of claims 1 to 21, wherein **Xaa3**, **Xaa4**, and/or **Xaa7** is a 2-aminoisobutyric acid residue; and/or **Xaa6** and/or **Xaa8** is a leucine residue.

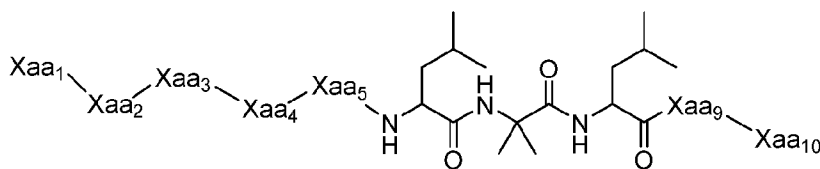
23. A compound of any one of claims 1 to 22, wherein **Xaa9** is independently a group of formula Y as defined in any one of claims 1 to 46, or is a group of the formula X



- 20 X; and

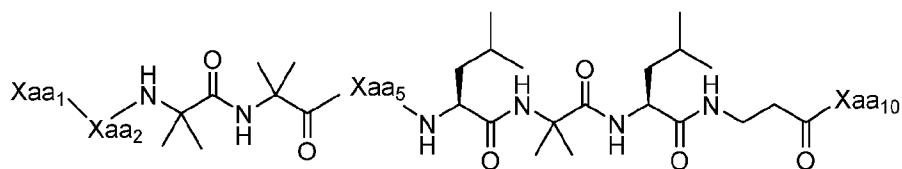
optionally L₁₀ is a group of the formula $-(CR^hR^i)_h-$, preferably wherein h is from 1 to 4, preferably 2 to 4, preferably 2.

24. A compound of any one of claims 1 to 23, wherein the compound is of the formula II or a pharmaceutically acceptable salt or solvate thereof



- 25 II.

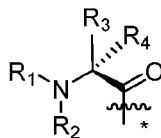
25. A compound of claim 1 to 24, wherein the compound is a compound of formula IVA or a pharmaceutically acceptable salt or solvate thereof



- 30 IVA.

26. A compound of any one of claims 1 to 25, wherein:

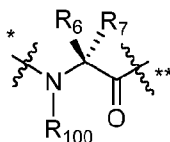
Xaa1 is a group of formula IAA



IAA;

and/or

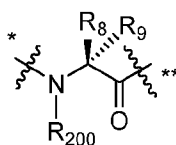
5 **Xaa2** is a group of formula IBB



IBB;

and/or

Xaa5 is a group of formula ICC



ICC.

27. A drug-reactive linker conjugate comprising a compound of any one of claims 1 to 26 or a compound listed in the proviso of claim 1, and a reactive linker group having a reactive site that allows the reactive linker group to be reacted with a ligand.

15 28. A ligand-drug conjugate comprising a ligand and one or more compounds of any one of claims 1 to 26 or a compound listed in the proviso of claim 1.

29. The ligand-drug conjugate of claim 28, the ligand-drug conjugate having the formula T-(L-(D)o)p,

wherein

20 T is a ligand;

L is a linker group;

D is a compound of any one of claims 1 to 26 or a compound in the proviso relating thereto; and

o is an integer from 1 to 10, preferably 2 to 6; and p is an integer from 1 to 10.

25 30. The ligand-drug conjugate of claim 29, wherein the ligand is a globular protein or a peptide.

31. The ligand-drug conjugate of claim 29 or 30, wherein the ligand is an antibody or fragment thereof.

30 32. The ligand-drug conjugate of any one of claims 29 to 31, wherein the ligand, for example, the antibody or antibody fragment binds to one or more tumour-associated antigens; or the ligand, for example an antibody or fragment thereof, binds an antigen

or cell surface receptor of a cell that produces autoimmune antibodies, for example a receptor or receptor complex expressed on an activated lymphocyte that is associated with an autoimmune disease.

33. A pharmaceutical composition comprising:

- 5 a compound of formula I as defined in any one of claims 1 to 26; or
 a ligand-drug conjugate of any one of claims 28 to 32; and
 a pharmaceutically acceptable carrier.

34. A method of (a) treating or preventing a disease or condition susceptible to treatment with a mitochondrial uncoupling agent or complications associated with a disease or
10 condition susceptible to treatment with a mitochondrial uncoupling agent in a subject in need thereof; (b) killing a cell; (c) killing or inhibiting proliferation of tumour cells or cancer cells; or (d) treating or preventing an autoimmune disease, the method comprising administering to the subject an effective amount of a compound of formula I as defined in any one claims 1 to 26 or a compound in the proviso relating thereto, a
15 ligand-drug conjugate of any one of claims 28 to 32 or a pharmaceutical composition of claim 33.

35. A method of depolarising a membrane, the method comprising contacting the membrane with a compound of formula I as defined in any one claims 1 to 26 or a compound in the proviso relating thereto, a ligand-drug conjugate of any one of claims
20 28 to 32 or a pharmaceutical composition of claim 33.

36. The method of claim 35, wherein the membrane is a mitochondrial membrane.

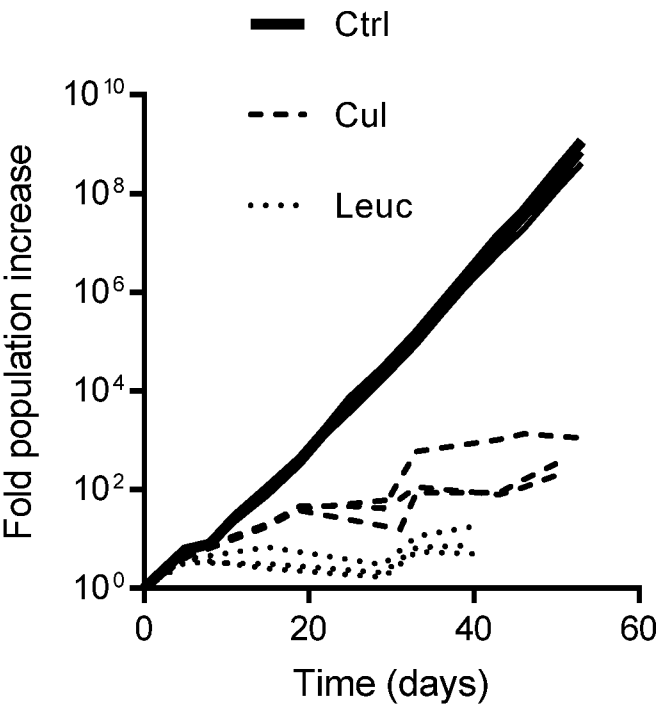


FIGURE 1

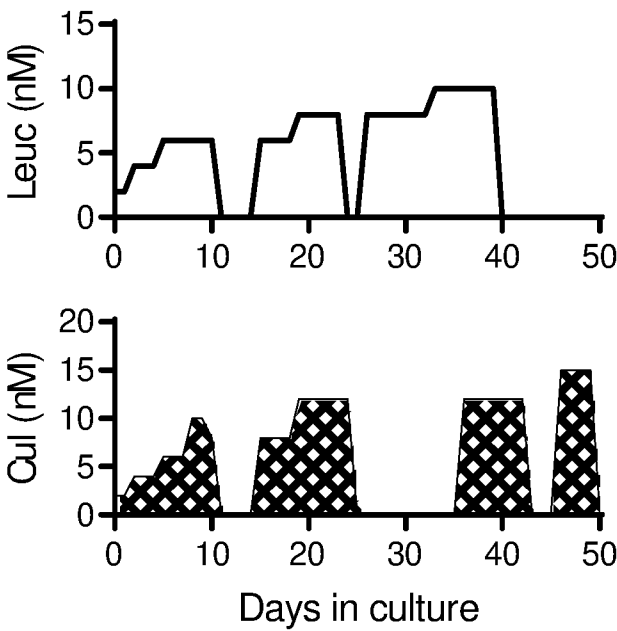


FIGURE 2

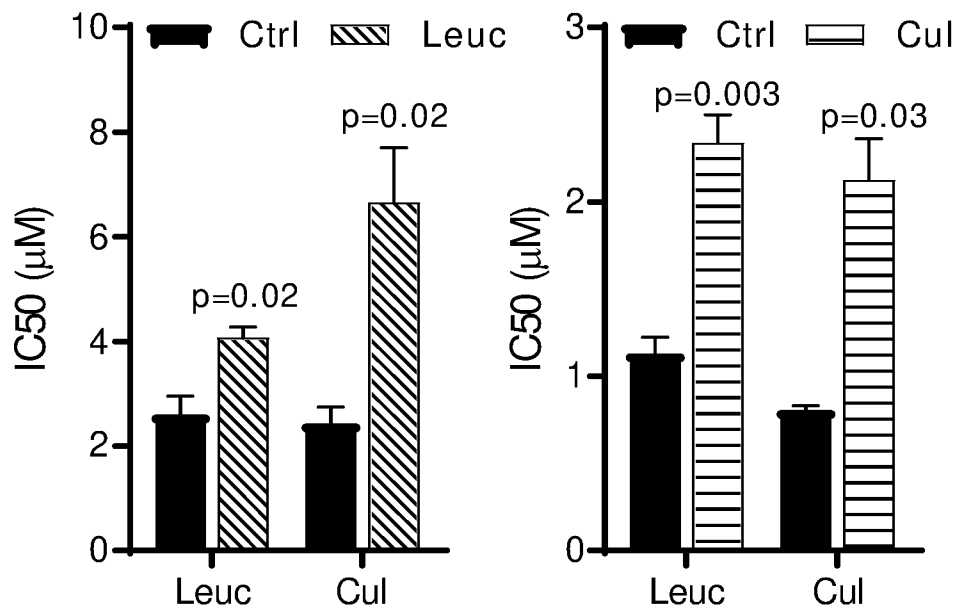


FIGURE 3

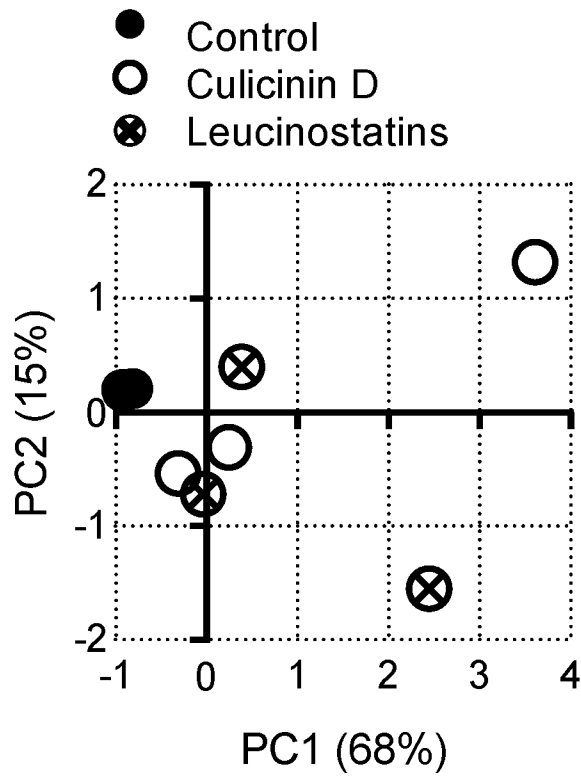


FIGURE 4

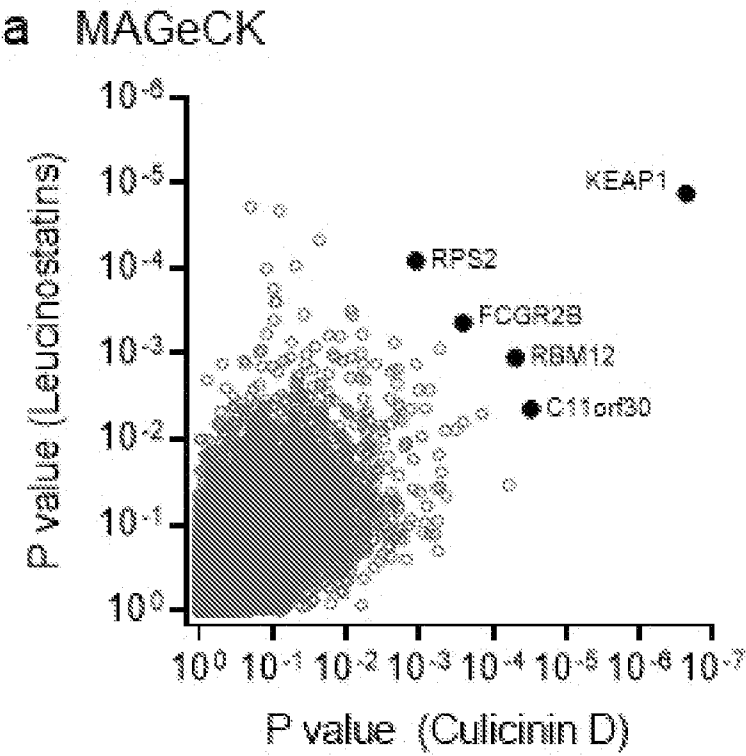


FIGURE 5A

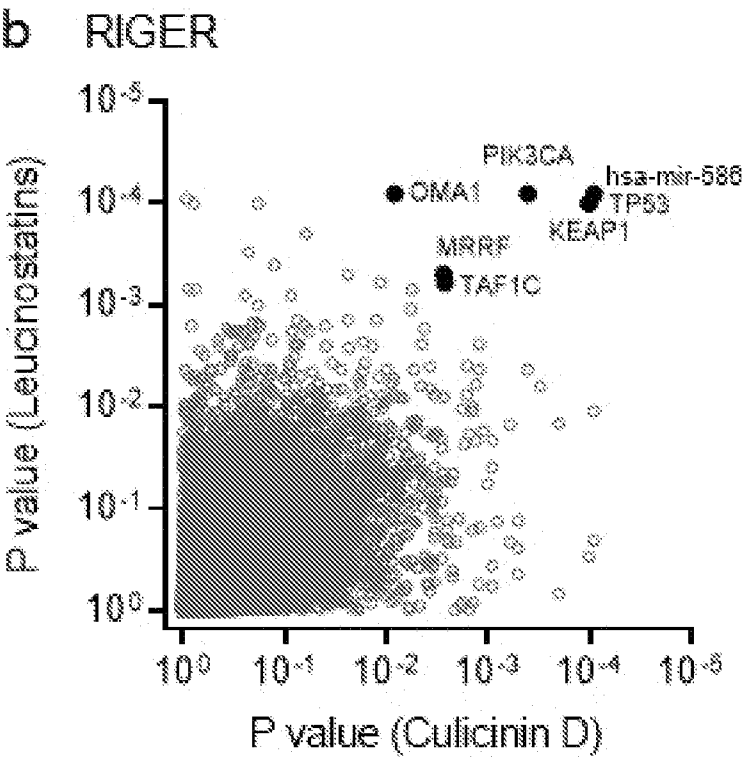


FIGURE 5B

c PinAPL-Py

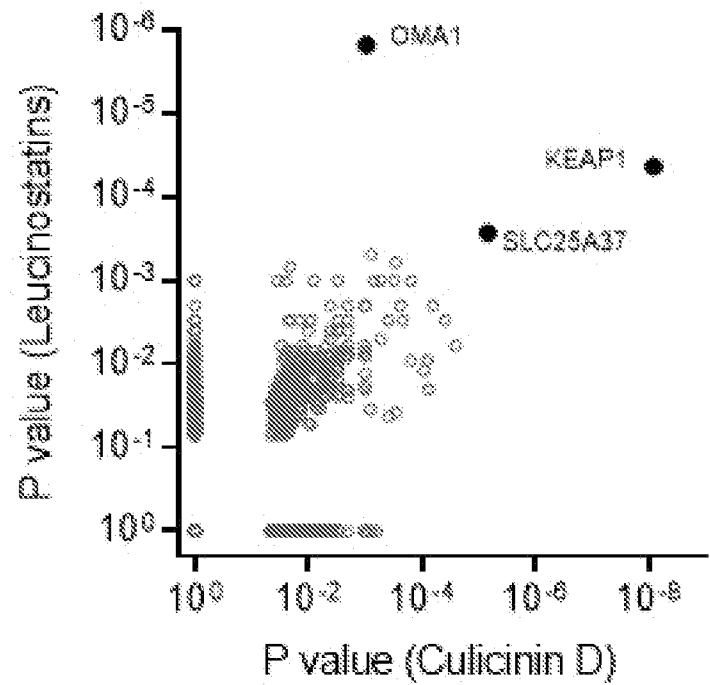


FIGURE 5C

d CRISPRcloud2

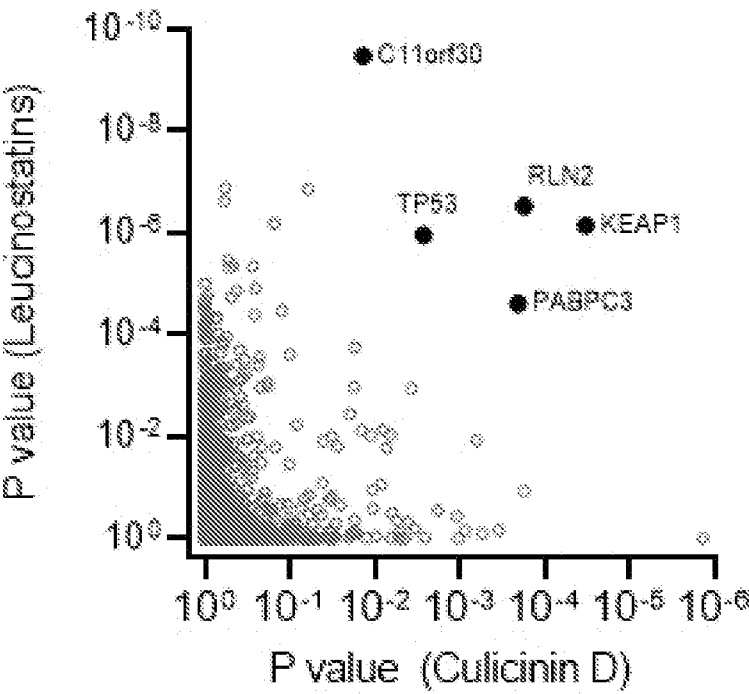


FIGURE 5D

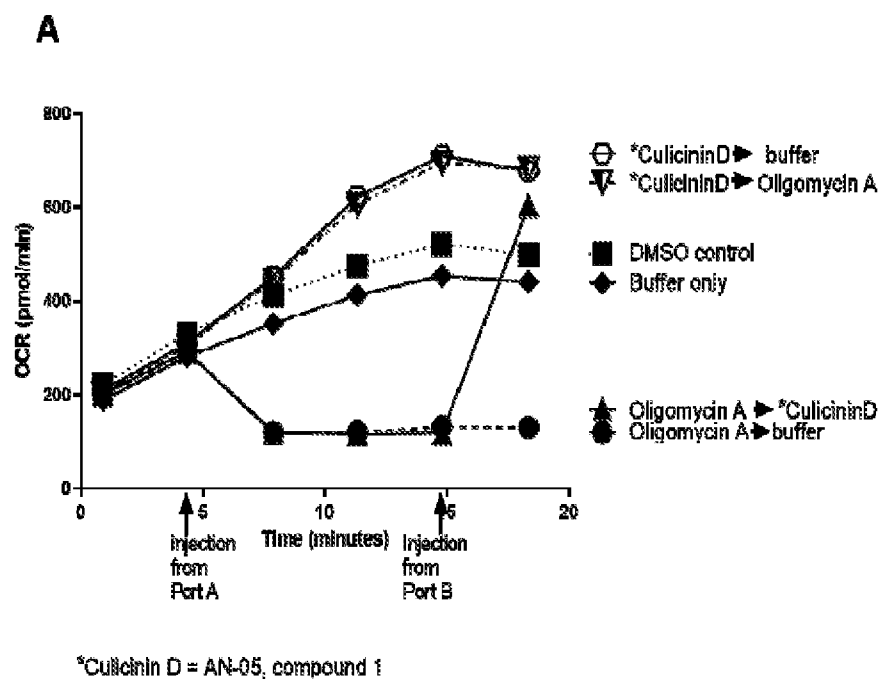
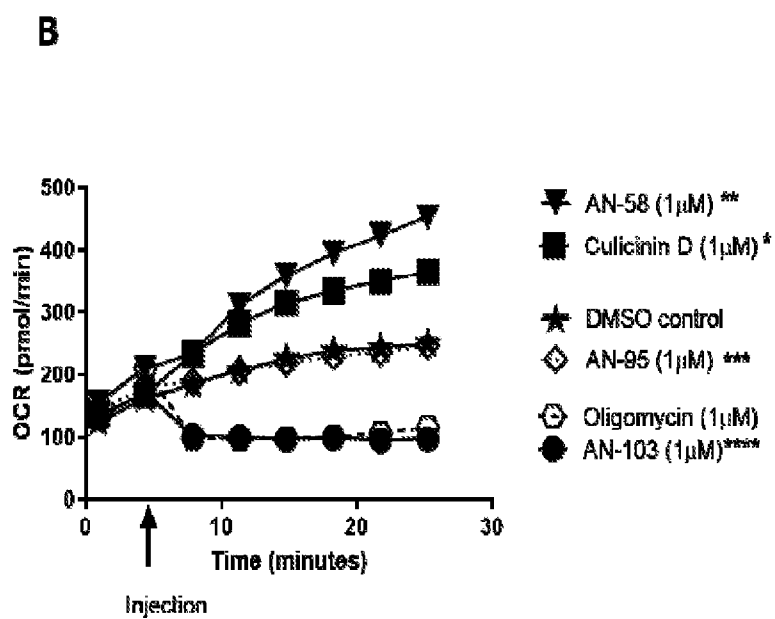


Figure 6A



* culicinin D = AN-05, compound 1

** AN-58 = compound 520

*** AN-95 = compound 1100i

****AN-103 =compound 1200e

Figure 6B

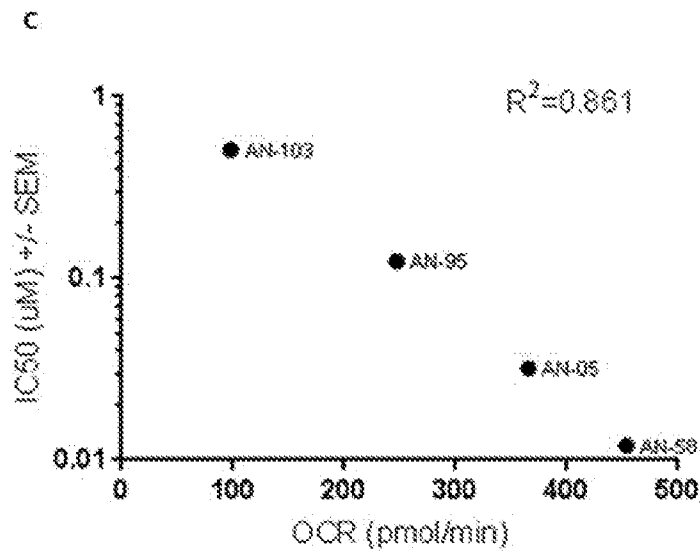


Figure 6C

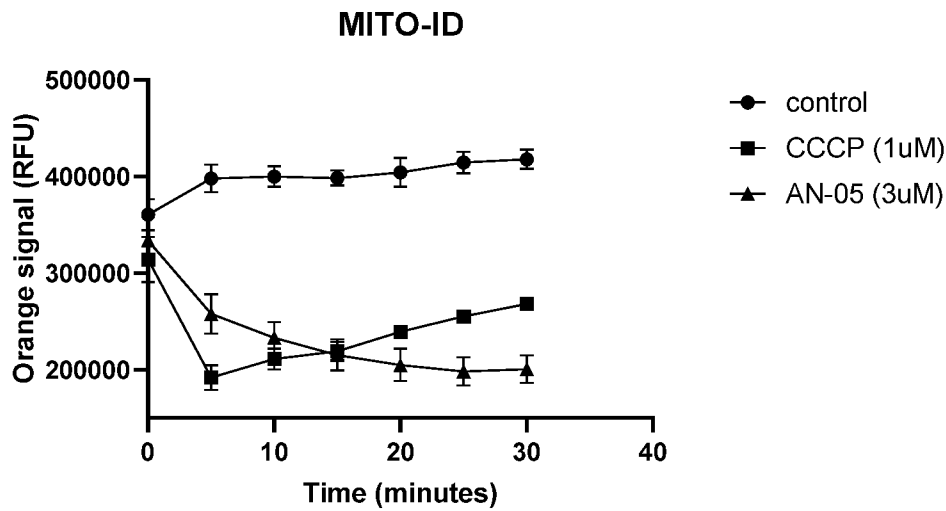


Figure 7A

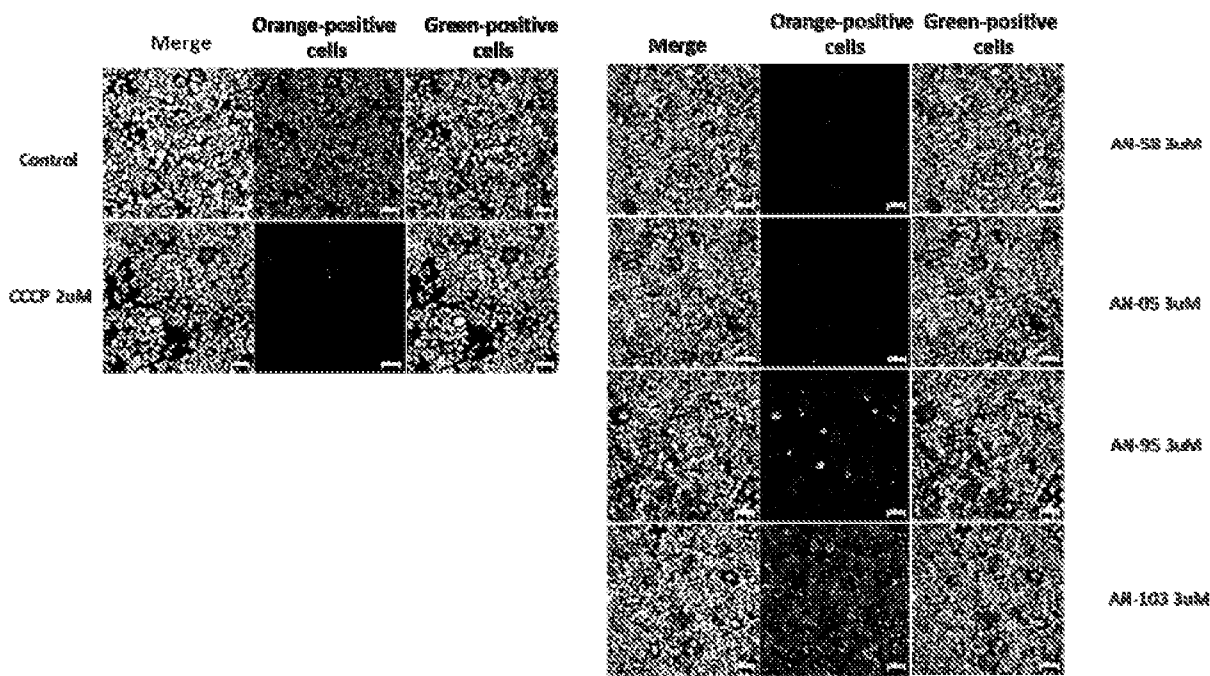


Figure 7B

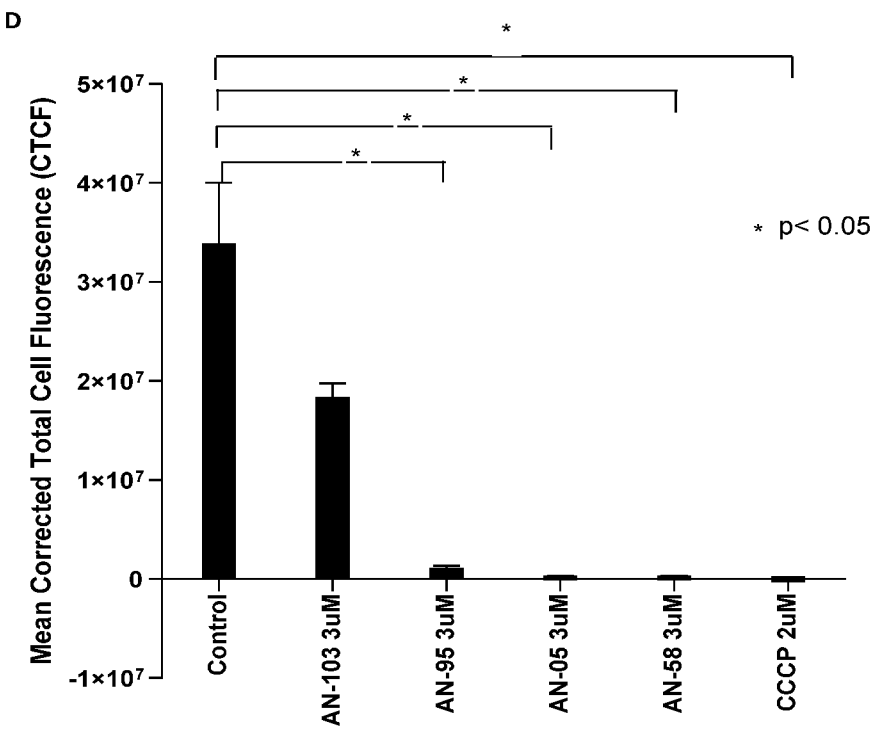


Figure 7C

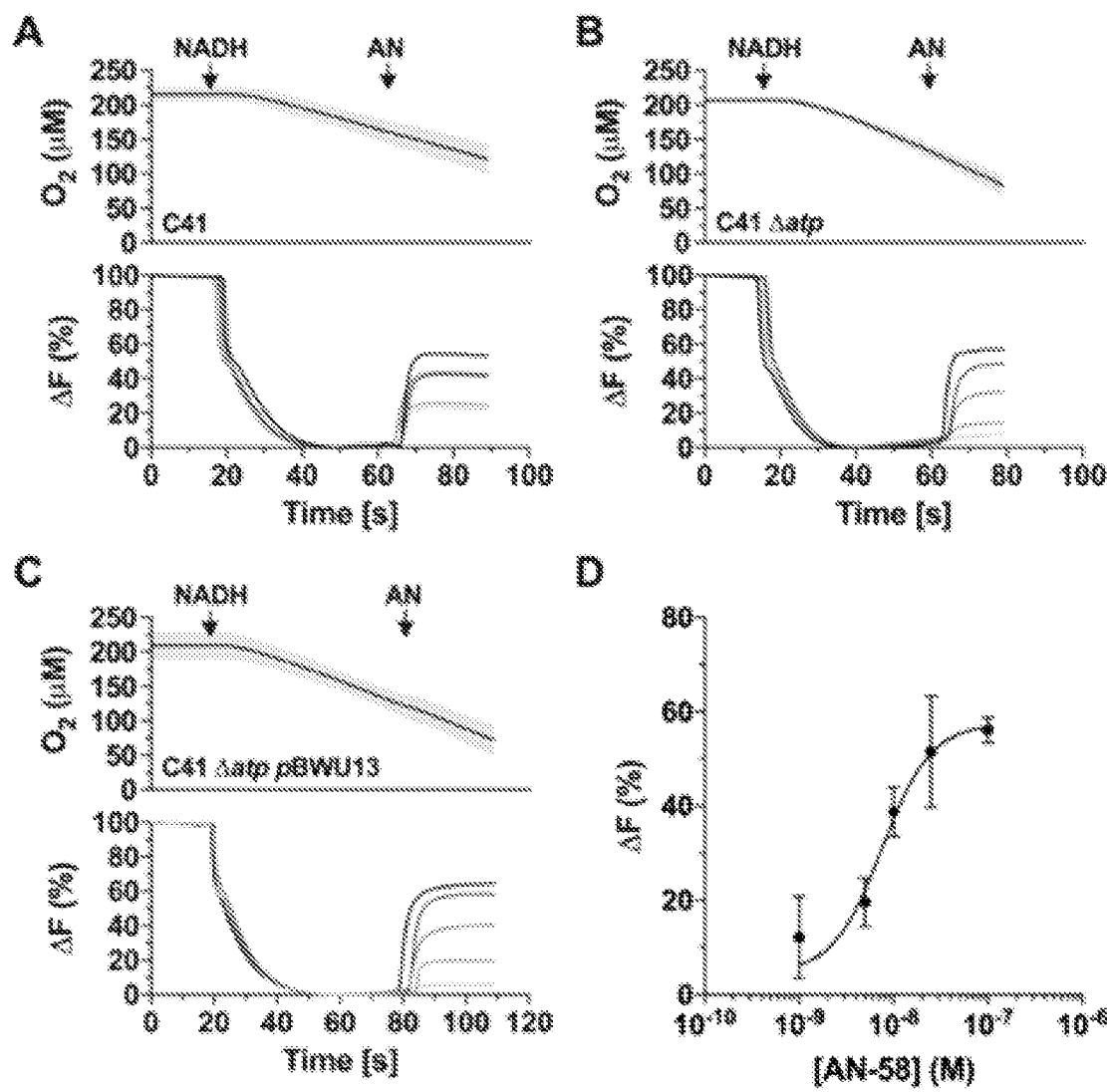


Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2019/059597

A. CLASSIFICATION OF SUBJECT MATTER

**C07K 7/06 (2006.01) A61K 39/395 (2006.01) A61K 36/062 (2006.01) A61K 38/00 (2006.01) A61K 47/68 (2017.01)
A61P 35/00 (2006.01)**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CHEMICAL ABSTRACTS REGISTRY, CAPLUS: Structure search based on structure of claim 1.

Esp@cenet: AUCKLAND UNISERVICES as Applicant and keyword PEPTIDE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"D" document cited by the applicant in the international application	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
17 January 2020

Date of mailing of the international search report
17 January 2020

Name and mailing address of the ISA/AU

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INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/IB2019/059597
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Stubbing, Louise A. et al, "Synthesis and antiproliferative activity of culicinin D analogues containing simplified AHMOD-based residues", European Journal of Medicinal Chemistry (2019), 177, 235-246, CODEN: EJMCA5; ISSN: 0223-5234; DOI 10.1016/j.ejmech.2019.05.052 Table 1	1-36
X	EP 3345917 A1 (BACOPA AG) 11 July 2018 pages 73-151; compounds 5488, 5527, 5942, 6067	1-6, 8-23, 26, 33-36
X	Stach, Michaela et al, "Improved Strategy for the Synthesis of the Anticancer Agent Culicinin D", European Journal of Organic Chemistry (2015), 2015(28), 6341-6350, CODEN: EJOCHF; ISSN: 1099-0690; DOI 10.1002/ejoc.201500872 Scheme 1, Table 1, compounds 7b-7d; page 6346	1-4, 8-11, 15-36
X	Hung, Kuo-yuan et al, "Synthesis of the peptaibol framework of the anticancer agent culicinin D: Stereochemical assignment of the AHMOD moiety", Organic Letters (2012), 14(22), 5784-5787, CODEN: ORLEF7; ISSN: 1523-7052; DOI 10.1021/ol302852q Figure 2	1-4, 8-11, 15-26
A	Ko, Kwang-Yoon et al, "Improved Synthesis of the Unnatural Amino Acids AHMOD and AMD, Components of the Anticancer Peptaibol Culicinin D", Journal of Organic Chemistry (2015), 80(17), 8631-8636, CODEN: JOCEAH; ISSN: 0022-3263; DOI 10.1021/acs.joc.5b01265 whole document	1-36
A	Zhang, Wei et al, "Synthesis of protected (2S,4R)-2-amino-4-methyldecanoic acid, a proposed component of culicinins" Chinese Chemical Letters (2008), 19(9), 1068-1070, CODEN: CCLEE7; ISSN: 1001-8417; DOI 10.1016/j.cclet.2008.06.027 whole document	1-36
A	He, Haiyin et al, "Culicinin D, an antitumor peptaibol produced by the fungus Culicinomyces clavissporus, strain LL-121252", Journal of Natural Products (2006), 69(5), 736-741, CODEN: JNPRDF; ISSN: 0163-3864; DOI 10.1021/np058133r whole document	1-36
A	Zhang, Wei et al, "An improved synthesis of (2S,4S)- and (2S,4R)-2-amino-4-methyldecanoic acids: assignment of the stereochemistry of culicinins", Journal of Peptide Science (2011), 17(8), 576-580, CODEN: JPSIEI; ISSN: 1075-2617; DOI 10.1002/psc.1376 whole document	1-36
A	Zhang, Wei et al, "Synthesis of the C-terminal pentapeptide of the peptaibol culicinins", Journal of Peptide Science (2009), 15(5), 366-368, CODEN: JPSIEI; ISSN: 1075-2617; DOI 10.1002/psc.1124 whole document	1-36
Form PCT/ISA/210 (fifth sheet) (July 2019)		

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-26 (all in full) and claims 27-36 (all in part) relating to compounds of formula I excluding those in the proviso

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

1. Claims 1-26 (all in full) and claims 27-36 (all in part) are directed to compounds of formula I excluding those in the proviso, conjugates thereof, compositions thereof and the use of any of the compounds, conjugates or compositions in medical treatment. The feature of compounds of formula I excluding those in the proviso is specific to this group of claims.
2. Claims 27-36 (all in part) are directed to conjugates of compounds of the proviso in claim 1, compositions thereof and the use of any of the conjugates or compositions in medical treatment. The feature of compounds of the proviso in claim 1 is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions is the general formula I of claim 1. However it is considered that this feature is known in this particular art. Compounds of the proviso conform to the general formula I and at least some of the compounds of the proviso are known, as is acknowledged in the present description.

Therefore this common feature cannot be a special technical feature. Hence there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/IB2019/059597	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
EP 3345917 A1	11 July 2018		
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2019)			