

LIQUID PHASE PEPTIDE SUPPORT SYNTHESIS OF PEPTIDES AND PEPTIDOMIMETICS

FIELD OF THE DISCLOSURE

[0001] The present disclosure relates to liquid phase peptide support synthesis of peptides and peptidomimetics, pharmaceutical compositions, and methods of treating GLP1R-associated conditions.

BACKGROUND OF THE DISCLOSURE

[0002] The difference between liquid phase peptide support (LPPS) synthesis and solid phase peptide synthesis (SPPS) is that while the latter is supported on a large solid polymer, the former is supported on well-defined molecules and the difference between LPPS and CSPA is precipitation as an isolation in LPPS vs phase separation in CSPA. SPPS is a well-established and most common and favorite method to prepare polypeptides (Jaradat D., "Thirteen Decades of Peptide Synthesis: Key Developments in Solid Phase Peptide Synthesis and Amide Bond Formation Utilized in Peptide Ligation," *Amino Acids* 50(1):39-68 (2018)). However, its heterogeneity demands large excess molar equivalents of amino acids building blocks to ensure completion of peptide chain elongation. On the other hand, CSPA requires fewer molar equivalents of the building blocks but is tedious and time consuming in isolation and purification. It is demanding to generate suitable chemistry associated with LPPS to remove the need of purification phases and reduce molar equivalents of building blocks, especially unnatural amino acids in synthesis of peptidomimetics that are problematic for scaleup and overall material consumption.

[0003] Initial approaches using known LPPS Tag (Tag1 in Figure 1) (Takahashi et al., "Novel Diphenylmethyl-Derived Amide Protecting Group for Efficient Liquid-Phase Peptide Synthesis: AJIPHASE," *Org Lett.* 14(17):4514-4517 (2012); Takahashi et al., "Novel Diphenylmethyl-Derived Amide Protecting Group for Efficient Liquid-Phase Peptide Synthesis: AJIPHASE," *Org Lett.* 14(17):4514-4517 (2012)) to prepare a 10-mer peptidomimetic (**M1**) composed of five natural and five non-natural amino acid residues were unsuccessful. Peptide chain elongation could only reach the 7th amino acid residue due to poor separation of the tag carrier peptides (TCPs) from reaction mixture.

[0004] Accordingly, there is a need in the art for new tag carriers with better separation of the tag carrier peptides from the reaction mixture. In certain embodiments, the present disclosure meets the needs and provides other advantages.

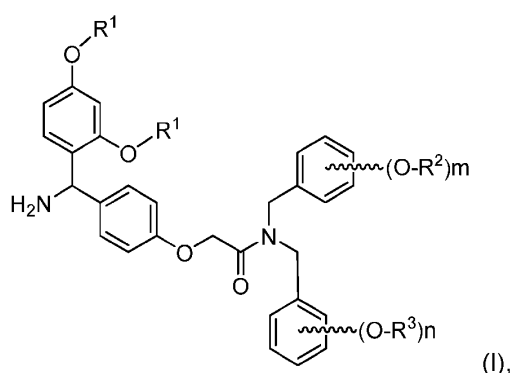
[0005] The foregoing discussion is presented solely to provide a better understanding of the nature of the problems confronting the art and should not be construed in any way as an admission

as to prior art nor should the citation of any reference herein be construed as an admission that such reference constitutes "prior art" to the instant application.

SUMMARY OF THE DISCLOSURE

[0006] Various non-limiting aspects and embodiments of the disclosure are described below.

[0007] In one aspect, the present disclosure provides a compound having a structure of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R¹ is a C₁₋₅ alkyl;

R² is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

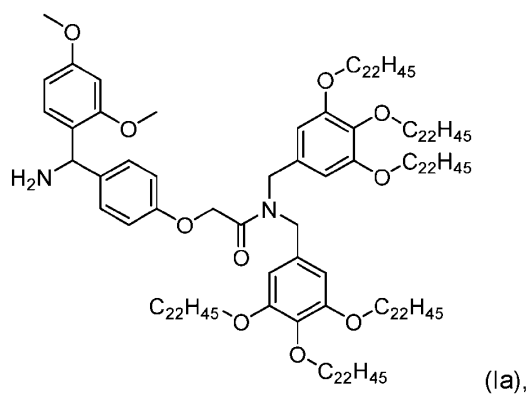
R³ is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof.

[0008] In some embodiments, R¹ is methyl.

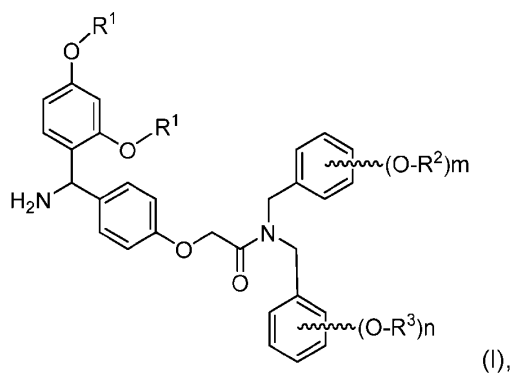
[0009] In some embodiments, R² is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl and R³ is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl. In some embodiments, each R² is independently an optionally substituted C₂₂ alkyl and each R³ is independently an optionally substituted C₂₂ alkyl.

[00010] In some embodiments, the compound has a structure of Formula (Ia):



or a salt thereof.

[00011] In another aspect, the present disclosure provides a process for preparation of a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

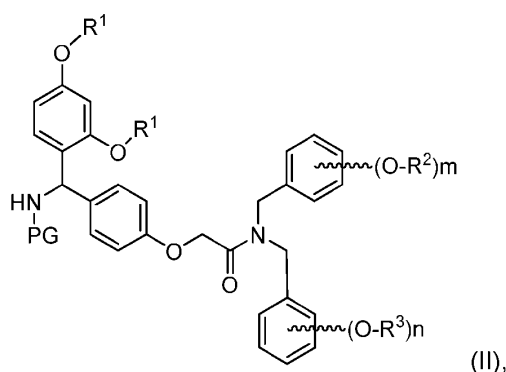
R¹ is a C₁₋₅ alkyl;

R² is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

R³ is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof. This process includes:

(a) providing a compound of Formula (II) having the structure:



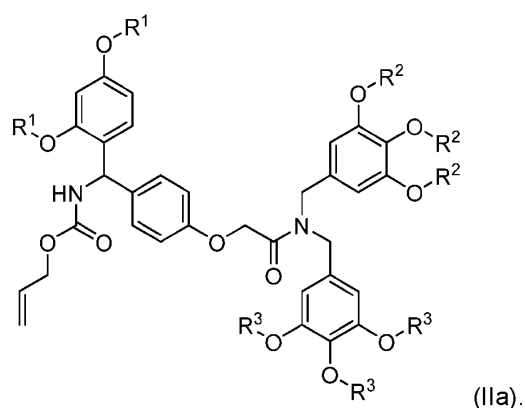
wherein

PG is a suitable protecting group; and

(b) forming the compound of Formula (I) from the compound of Formula (II).

[00012] In some embodiments, PG is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

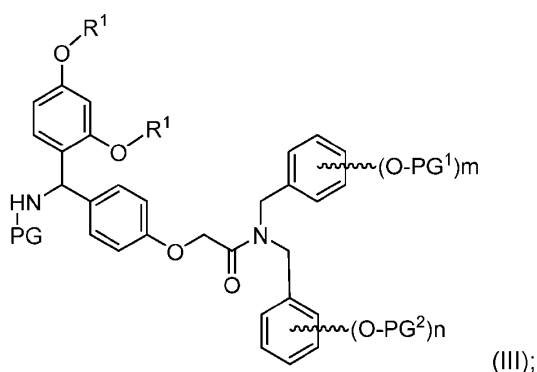
[00013] In some embodiments, the compound of Formula (II) has the structure of Formula (IIa):



[00014] In some embodiments, the step (b) of forming the compound of Formula (I) comprises reacting the compound of Formula (II) with a protecting group removing agent to produce the compound of Formula (I). In some embodiments, the protecting group removing agent is selected from the group consisting of Pd(PPh)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

[00015] In some embodiments, the process for preparation of a compound of Formula (I) further includes a step of

providing a compound of Formula (III) having the structure:



wherein

PG¹ is a suitable protecting group;

PG² is a suitable protecting group; and

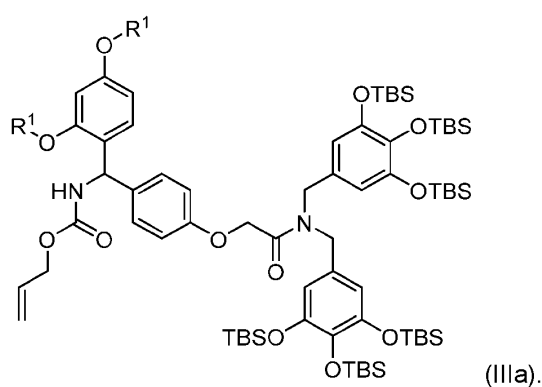
forming the compound of Formula (II) from the compound of Formula (III) prior to the step

[00016] In some embodiments, PG¹ is independently selected from the group consisting of tert-butyldimethylsilyl (TBS), trimethylsilyl (TMS), triethylsilyl (TES), tert-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

[00017] In some embodiments, PG² is independently selected from the group consisting of tert-butyldimethylsilyl (TBS), trimethylsilyl (TMS), triethylsilyl (TES), tert-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

[00018] In some embodiments, each PG¹ and each PG² are the same. In some embodiments, PG¹ and PG² are be different from each other.

[00019] In some embodiments, the compound of Formula (III) has the structure of Formula (IIIa):



[00020] In some embodiments, the step of forming the compound of Formula (II) prior to step (a) comprises:

reacting the compound of Formula (III) with a protecting group removal agent to obtain a deprotected intermediate;

reacting said intermediate with at least one compound of Formula (IV):

R-LG (IV),

wherein

R is an optionally substituted C₁₀₋₃₀ alkyl; and

LG is a suitable leaving group;

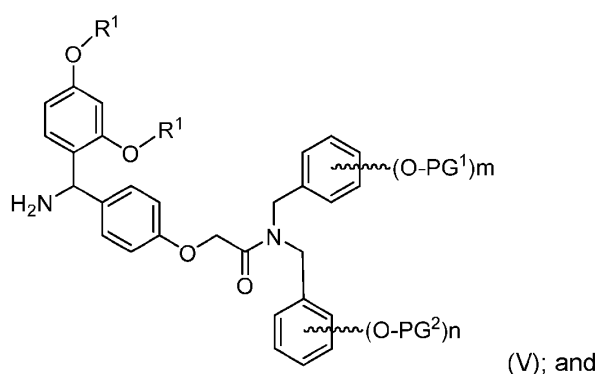
to produce the compound of Formula (II).

[00021] In some embodiments, LG is selected from a group consisting of halogen, OTf, OMs, and OTs.

[00022] In some embodiments, the protecting group removing agent is selected from the group consisting of tetrabutylammonium fluoride (TBAF), NH₄F, SiF₄, AcOH, HCl, LiAlH₄, and K₂CO₃.

[00023] In some embodiments, the process for preparation of a compound of Formula (I) further comprises a step of:

providing a compound of Formula (V) having the structure:



forming the compound of Formula (III) from the compound of Formula (V).

[00024] In some embodiments, the step of forming the compound of Formula (III) comprises: reacting the compound of Formula (V) with a compound of Formula (VIa) or Formula (VIb):

PG-X (VIa) or PG₂O (VIb),

wherein

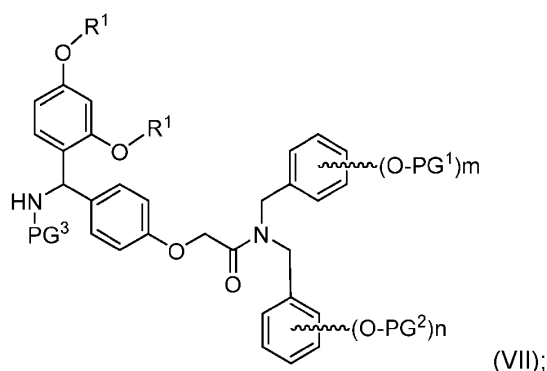
X is Cl or Br;

to produce the compound of Formula (III).

[00025] In some embodiments, the compound of Formula (VIa) or Formula (VIb) is selected from the group consisting of AllocCl, Alloc₂O, Cbz₂O, CbzCl, FmocCl, and Boc₂O.

[00026] In some embodiments, the process for preparation of a compound of Formula (I) further comprises:

providing a compound of Formula (VII) having the structure:

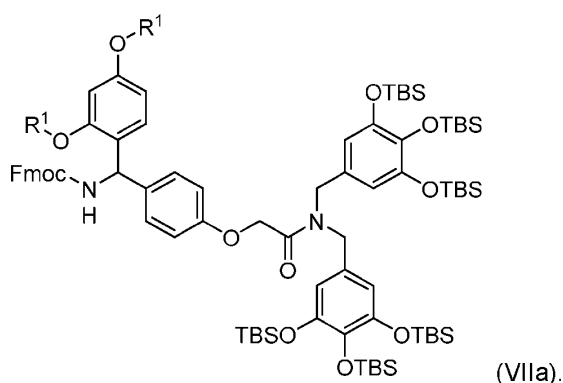


wherein PG³ is a suitable protecting group; and

forming the compound of Formula (V) from the compound of Formula (VII).

[00027] In some embodiments, PG³ is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

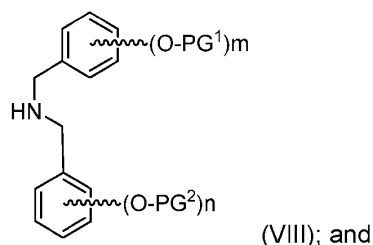
[00028] In some embodiments, the compound of Formula (VII) has the following structure:



[00029] In some embodiments, said step of forming the compound of Formula (V) comprises reacting the compound of Formula (VII) with a protecting group removing agent to produce the compound of Formula (V). In some embodiments, the protecting group removing agent is selected from the group consisting of Pd(PPh)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

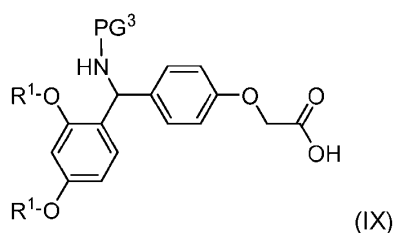
[00030] In some embodiments, the process for preparation of a compound of Formula (I) further comprises:

providing a compound of Formula (VIII) having the structure:



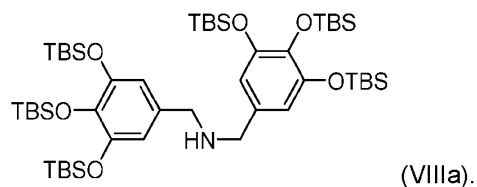
forming the compound of Formula (VII) from the compound of Formula (VIII).

[00031] In some embodiments, the process of forming the compound of Formula (VII) may comprise reacting the compound of Formula (VIII) with a compound of Formula (IX) having the structure:

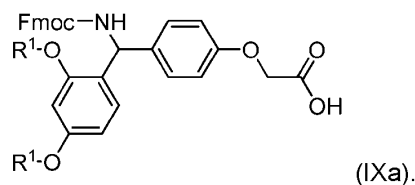


to produce the compound of Formula (VII).

[00032] In some embodiments, the compound of Formula (VIII) has the following structure:

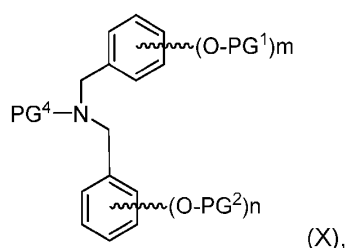


[00033] In some embodiments, the compound of Formula (IX) has the following structure:



[00034] In some embodiments, the process for preparation of a compound of Formula (I) further comprises:

providing a compound of Formula (X) having the structure:

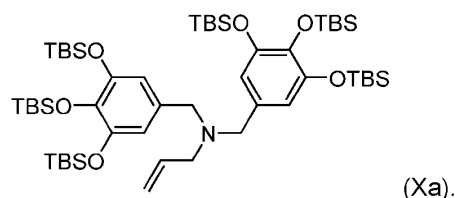


wherein PG⁴ is a suitable protecting group; and

forming the compound of Formula (VIII) from the compound of Formula (X).

[00035] In some embodiments, PG⁴ is selected from the group consisting of allyl, allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

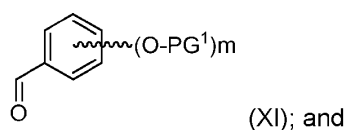
[00036] In some embodiments, the compound of Formula (X) has the following structure:



[00037] In some embodiments, the step of forming the compound of Formula (VIII) comprises reacting the compound of Formula (X) with a protecting group removing agent. In some embodiments, the protecting group removing agent is selected from the group consisting of Pd/K₂CO₃, 1,3-dimethylbarbituric acid/Pd(PPh₃)₄, Pd(PPh₃)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

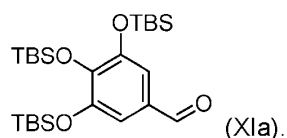
[00038] In some embodiments, the process for preparation of a compound of Formula (I) further comprises:

providing a compound of Formula (XI) having the structure:



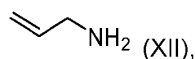
forming the compound of Formula (X) from the compound of Formula (XI).

[00039] In one embodiment, the compound of Formula (XI) has the following structure:



[00040] In some embodiments, the process of forming the compound of Formula (X) comprises:

reacting the compound of Formula (XI) with a compound of Formula (XII):

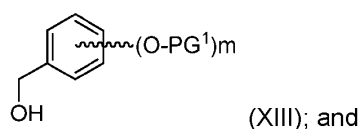


or a salt thereof,

to produce the compound of Formula (X).

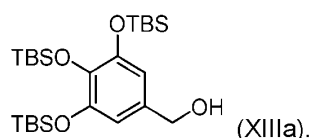
[00041] In some embodiments, the process for preparation of a compound of Formula (I) further comprises:

providing a compound of Formula (XIII) having the structure:



forming the compound of Formula (XI) from the compound of Formula (XIII).

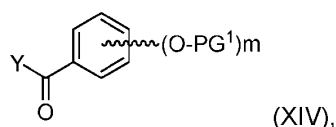
[00042] In some embodiments, the compound of Formula (XIII) has the following structure:



[00043] In some embodiments, the process for forming the compound of Formula (XI) comprises reacting the compound of Formula (XIII) with an oxidizing agent to produce the compound of Formula (XI). In some embodiments, the oxidizing agent may be selected from the group consisting of pyridinium chlorochromate (PCC), pyridinium dichromate (PDC), silver carbonate (Ag_2CO_3), tetra-n-propylammonium perruthenate (TPAP), and Dess-Martin periodinane (DMP).

[00044] In some embodiments, the process for preparation of a compound of Formula (I) further comprises:

providing a compound of Formula (XIV) having the structure:

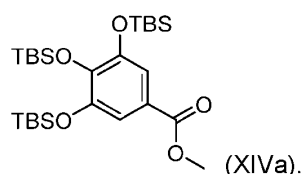


wherein

Y is $-\text{OC}_{1-6}$ alkyl; and

forming the compound of Formula (XIII) from the compound of Formula (XIV).

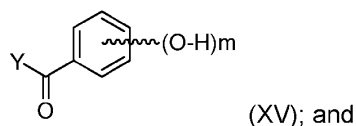
[00045] In one embodiment, the compound of Formula (XIV) has the following structure:



[00046] In some embodiments, the process of forming the compound of Formula (XIII) comprises reacting the compound of Formula (XIV) with a reducing agent. In some embodiments, the reducing agent is selected from the group consisting of LiAlH_4 , DIBALH, and LiBH_4 .

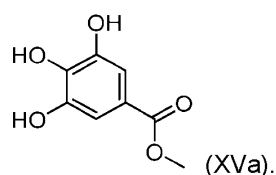
[00047] In some embodiments, the process for preparation of a compound of Formula (I) further comprises:

providing a compound of Formula (XV) having the structure:



forming the compound of Formula (XIV) from the compound of Formula (XV).

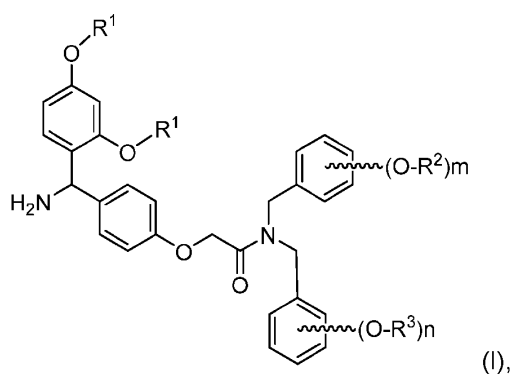
[00048] In some embodiments, the compound of Formula (XV) has the following structure:



[00049] In some embodiments, the process of forming the compound of Formula (XIV) comprises reacting the compound of Formula (XV) with a protecting group introducing agent to produce the compound of Formula (XIV). In some embodiments, the protecting group introducing agent is selected from the group consisting of TBSCl, TBSOTf, TMSCl, TMSOTf, TESCl, TESOTf, TBDPSCl, TBDPSOTf, TIPSCl, and TIPSOTf.

[00050] In yet another aspect, the present disclosure provides a product prepared according to any of the methods described in the present disclosure.

[00051] In some embodiments, the product is a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

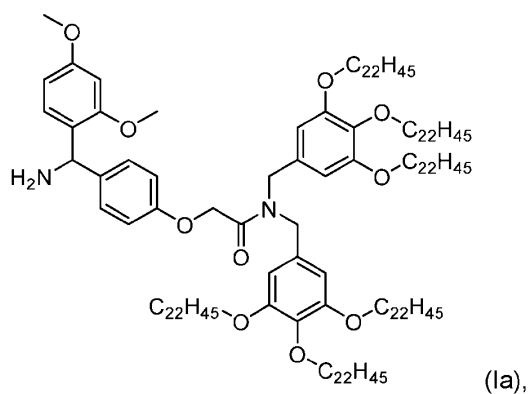
R¹ is a C₁₋₅ alkyl;

R² is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

R³ is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,

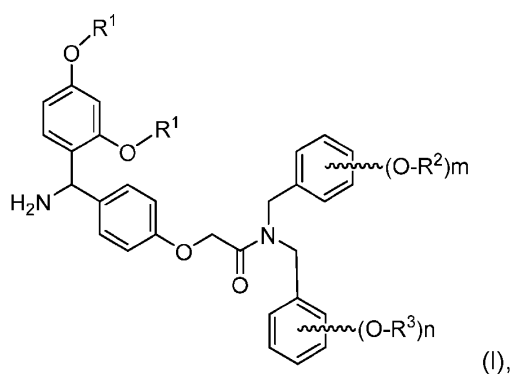
or a salt thereof.

[00052] In one embodiment, the product is a compound of Formula (Ia):



or a salt thereof.

[00053] In a further aspect, the present disclosure provides a method of using a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R¹ is a C₁₋₅ alkyl;

R² is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

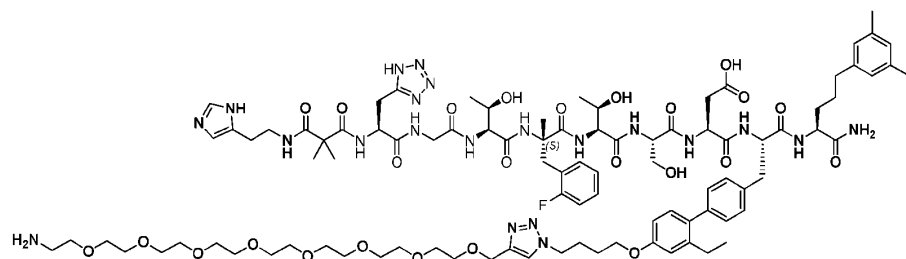
R³ is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof,

for making a peptide or a peptidomimetic.

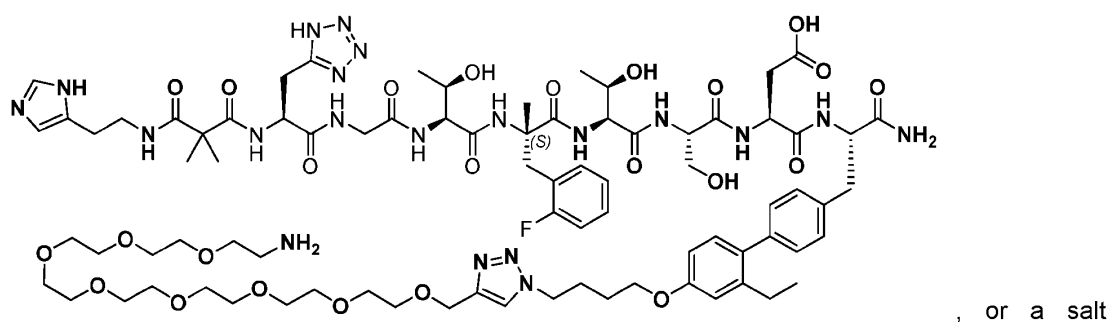
[00054] In some embodiments, the compound of Formula (I) is used as a support or a tag for making a peptide or a peptidomimetic.

[00055] In one embodiment, the peptidomimetic is a compound of Formula :



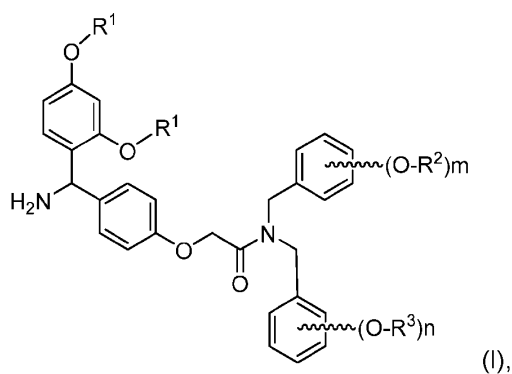
or a salt thereof.

[00056] In one embodiment, the peptidomimetic is a compound of Formula:



thereof.

[00057] In another aspect, the present disclosure provides a compound manufactured using a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

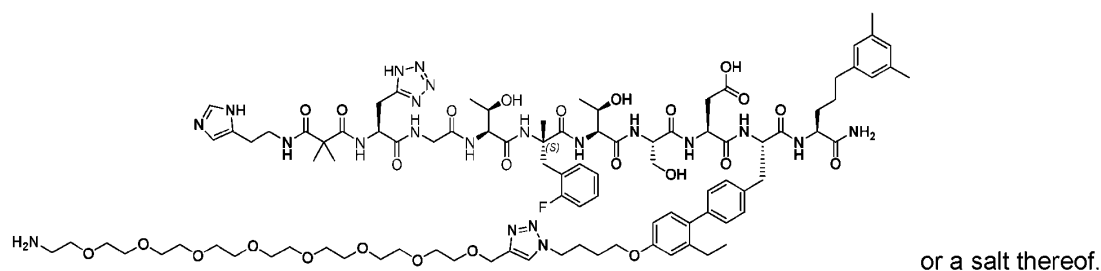
R¹ is C₁₋₅ alkyl;

R² is independently selected at each occurrence thereof from an optionally substituted C₁₀₋₃₀ alkyl; and

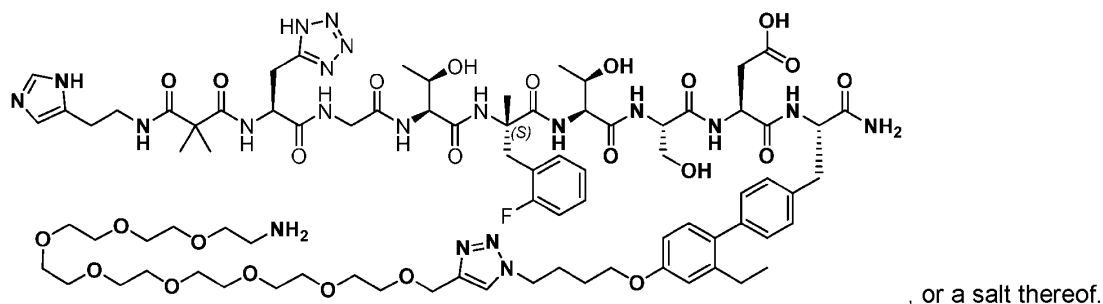
R³ is independently selected at each occurrence thereof from an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof.

[00058] In one embodiment, the compound has a Formula:



[00059] In one embodiment, the compound has a Formula:



[00060] In yet another aspect, provided herein is a pharmaceutical composition comprising the peptidomimetic or the compound of any of the embodiments described herein.

[00061] In a further aspect, provided herein is a pharmaceutical dosage form comprising the peptidomimetic or the compound of any of the embodiments described herein.

[00062] In another aspect, provided herein is a method of selectively targeting GLP1R on a surface of a cell with the compound or the peptidomimetic of any of the embodiments described herein. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a human cell.

[00063] In another aspect, provided herein is a method of enhancing GLP1R activity in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic or the compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein.

[00064] In yet another aspect, provided herein is a method of lowering blood glucose levels in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic or the compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein.

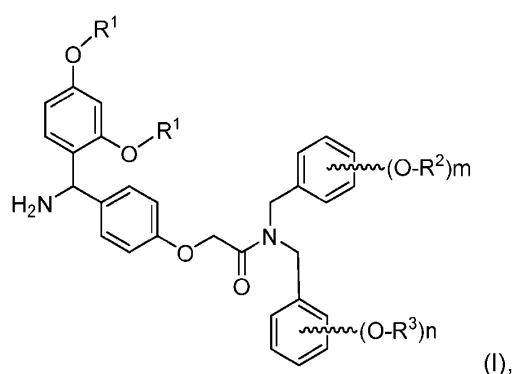
[00065] In a further aspect, provided herein is a method of lowering body weight in an individual in need thereof comprising administering to the individual an effective amount of the

peptidomimetic or the compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein.

[00066] In another aspect, provided herein is a method of treating a GLP1R-associated condition in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic or the compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein. In some embodiments, the GLP1R-associated condition is type II diabetes, obesity, liver disease, coronary artery disease, or kidney disease. In some embodiments, the GLP1R-associated condition is type II diabetes and/or obesity. In some embodiments, the peptidomimetic or the compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein is administered subcutaneously, intravenously, intradermally, intraperitoneally, or intramuscularly.

[00067] In yet another aspect, provided herein is a method of making a peptide or a peptidomimetic, the method comprising the steps of:

(a) providing a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R¹ is C₁₋₅ alkyl;

R² is independently selected at each occurrence thereof from an optionally substituted C₁₀₋₃₀ alkyl; and

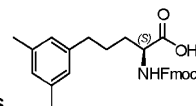
R³ is independently selected at each occurrence thereof from an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof;

(b) providing a first amino acid (AA1); and

(c) coupling the first amino acid (AA1) to the compound of Formula (I) to form a peptide bond between the first amino acid (AA1) and the compound of Formula (I).

[00068] In some embodiments, the first amino acid (AA1) contains a protected amino group.



[00069] In some embodiments, the first amino acid (AA1) is

[00070] In some embodiments, the method of making a peptide or a peptidomimetic may include a step of activating the chemical groups on the first amino acid (AA1) to prepare the first amino acid (AA1) for coupling with the compound of Formula (I) prior to step (c).

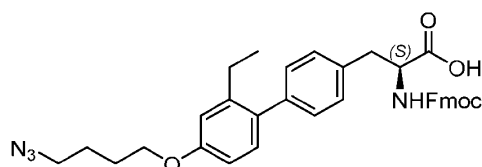
[00071] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

(d) removing the protecting group from the first amino acid (AA1);

(e) providing a second amino acid (AA2); and

(f) coupling the second amino acid (AA2) to the first amino acid (I-AA1) to form a peptide bond between the second amino acid (AA2) and the first amino acid (I-AA1-AA2).

[00072] In some embodiments, the second amino acid (AA2) is

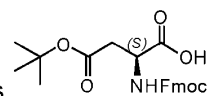


[00073] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

(g) removing the protecting group from the second amino acid (AA2);

(h) providing a third amino acid (AA3); and

(i) coupling the third amino acid (AA3) to the second amino acid to form a peptide bond between the third amino acid (AA3) and the second amino acid (I-AA1-AA2-AA3).



[00074] In some embodiments, the third amino acid (AA3) is

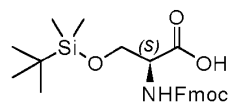
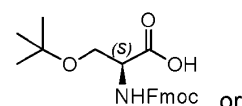
[00075] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

(j) removing the protecting group from the third amino acid (AA3);

(k) providing a fourth amino acid (AA4); and

(l) coupling the fourth amino acid (AA4) to the third amino acid to form a peptide bond between the fourth amino acid (AA4) and the third amino acid (I-AA1-AA2-AA3-AA4).

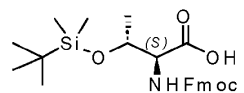
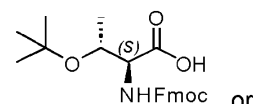
[00076] In some embodiments, the fourth amino acid (AA4) is



[00077] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

- (m) removing the protecting group from the fourth amino acid (AA4);
- (n) providing a fifth amino acid (AA5); and
- (o) coupling the fifth amino acid (AA5) to the fourth amino acid to form a peptide bond between the fifth amino acid (AA5) and fourth amino acid (I-AA1-AA2-AA3-AA4-AA5).

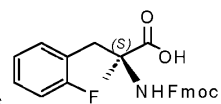
[00078] In some embodiments, the fifth amino acid (AA5) is



[00079] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

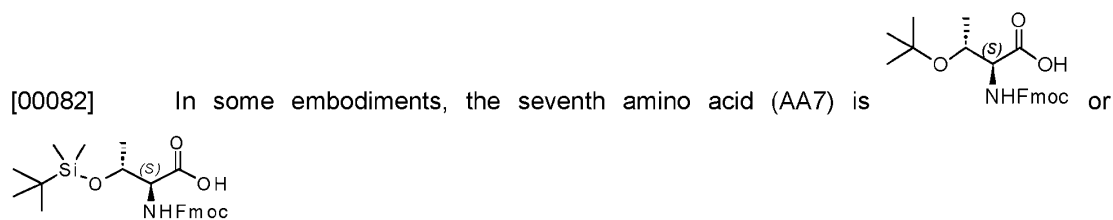
- (p) removing the protecting group from the fifth amino acid (AA5);
- (q) providing a sixth amino acid (AA6); and
- (r) coupling the sixth amino acid (AA6) to the fifth amino acid to form a peptide bond between the sixth amino acid (AA6) and the fifth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6).

[00080] In some embodiments, the sixth amino acid (AA6) is



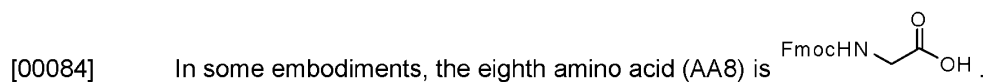
[00081] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

- (s) removing the protecting group from the sixth amino acid (AA6);
- (t) providing a seventh amino acid (AA7); and
- (u) coupling the seventh amino acid (AA7) to the sixth amino acid to form a peptide bond between the seventh amino acid (AA7) and the sixth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6-AA7).



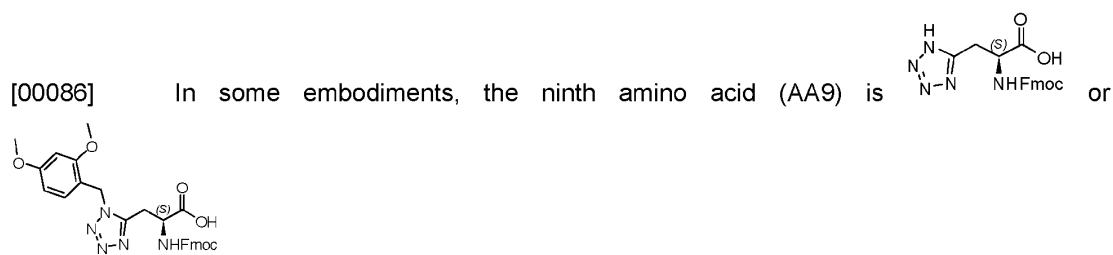
[00083] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

- (v) removing the protecting group from the seventh amino acid (AA7);
- (w) providing a eighth amino acid (AA8); and
- (x) coupling the eighth amino acid (AA8) to the seventh amino acid to form a peptide bond between the eighth amino acid (AA8) and the seventh amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6-AA7-AA8).



[00085] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

- (y) removing the protecting group from the eighth amino acid (AA8);
- (z) providing a ninth amino acid (AA9); and
- (aa) coupling the ninth amino acid (AA9) to the eighth amino acid to form a peptide bond between the ninth amino acid (AA9) and the eighth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6-AA7-AA8-AA9).

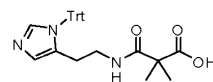


[00087] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

- (bb) removing the protecting group from the ninth amino acid (AA9);
- (cc) providing a tenth amino acid (AA10); and

(dd) coupling the tenth amino acid (AA10) to the ninth amino acid to form a peptide bond between the tenth amino acid (AA10) and the ninth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6-AA7-AA8-AA9-AA10).

[00088] In one embodiment, the tenth amino acid (AA10) is .

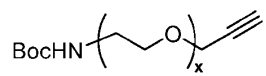


[00089] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

- repeating the steps of (i) removing the protecting group from the amino acid (AA_n);
 - (ii) providing a succeeding amino acid (AA_{n+1}); and
 - (iii) coupling the succeeding amino acid (AA_{n+1}) to the amino acid (AA_n) to form a peptide bond between the succeeding amino acid (AA_{n+1}) and amino acid (AA_n),
- wherein said repeating may be conducted from 1 to 100 times.

[00090] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

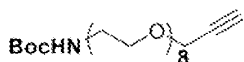
- (ee) removing the protecting group from the tenth amino acid (AA10);
- (ff) providing a compound of Formula (B):



wherein x is an integer from 1 to 15; and

(gg) coupling the compound of Formula (B) to the one of the amino acids in the peptide or the peptidomimetic to form a triazole ring.

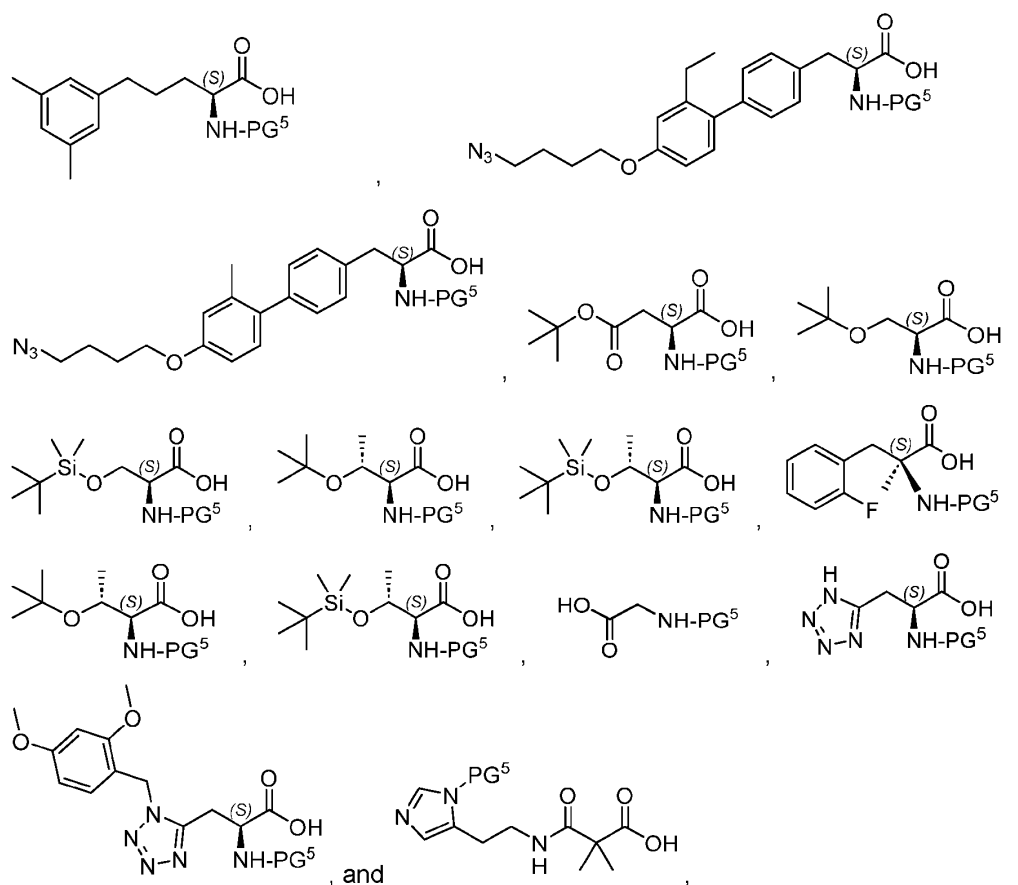
[00091] In some embodiments, the compound of Formula (B) has the following Formula:



[00092] In some embodiments, the method of making a peptide or a peptidomimetic according to any of the above embodiments further comprises:

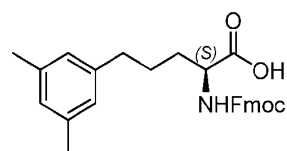
(hh) cleaving the peptide bond between the first amino acid (AA1) and the compound of Formula (I) to obtain the peptide or the peptidomimetic.

[00093] In some embodiments, each one of the first amino acid (AA1), the second amino acid (AA2), the third amino acid (AA3), the fourth amino acid (AA4), the fifth amino acid (AA5), the sixth amino acid (AA6), the seventh amino acid (AA7), the eighth amino acid (AA8), the ninth amino acid (AA9), the tenth amino acid (AA10), and succeeding amino acid (AA_{n+1}) may be independently selected from the group consisting of

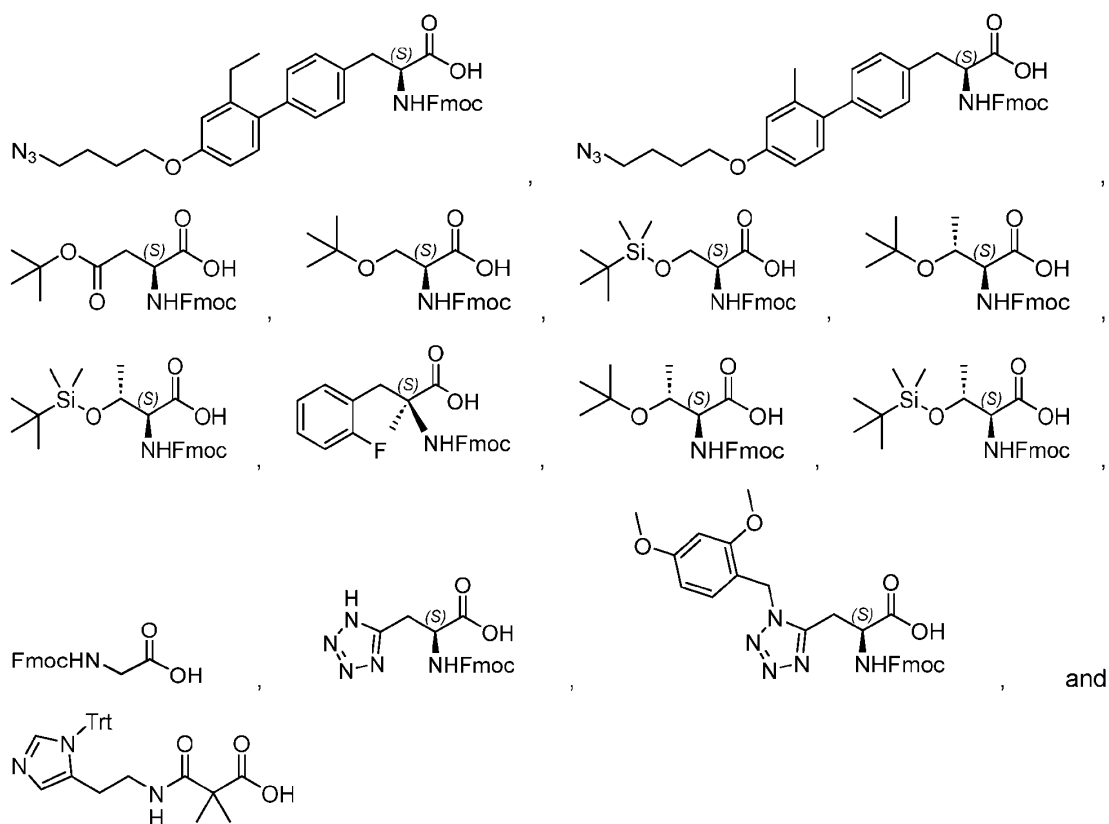


wherein PG⁵ is a suitable protecting group. In some embodiments, PG⁵ is independently selected at each occurrence from the group consisting of triphenylmethyl (Trt), allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

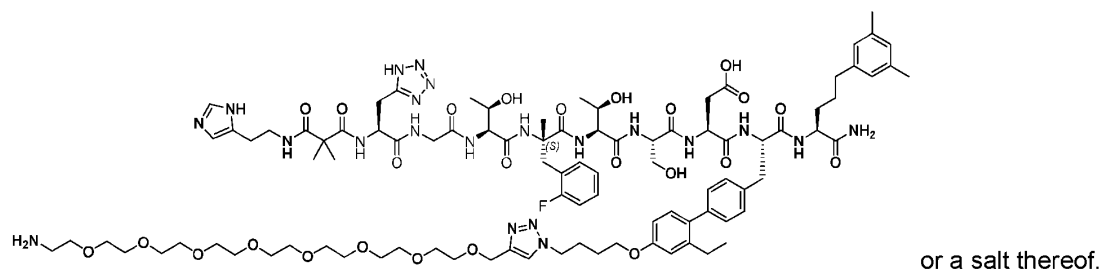
[00094] In some embodiments, each one of the first amino acid (AA1), the second amino acid (AA2), the third amino acid (AA3), the fourth amino acid (AA4), the fifth amino acid (AA5), the sixth amino acid (AA6), the seventh amino acid (AA7), the eighth amino acid (AA8), the ninth amino acid (AA9), the tenth amino acid (AA10), and succeeding amino acid (AA_{n+1}) may be independently



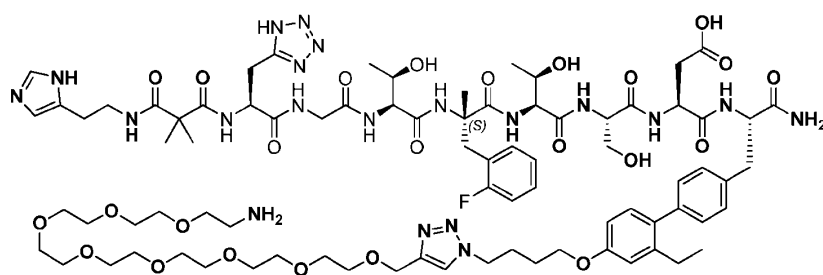
selected from the group consisting of



[00095] In one embodiment, the peptidomimetic has a Formula:

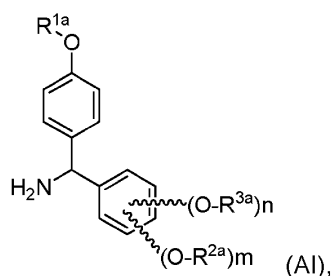


[00096] In one embodiment, the peptidomimetic has a Formula:



, or a salt thereof.

[00097] In another aspect, provided herein is a compound having a structure of Formula (AI):



wherein:

m and n are, independently, an integer from 0 to 5;

R^{1a} is C₁₋₅ alkyl;

R^{2a} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

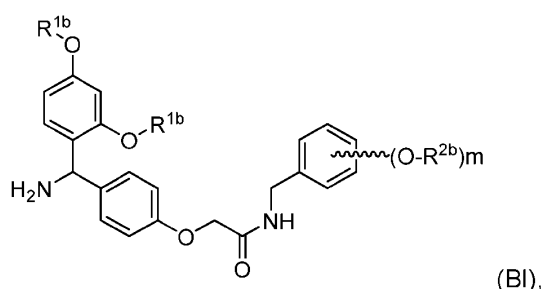
R^{3a} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof.

[00098] In some embodiments, R^{1a} is methyl.

[00099] In some embodiments, R^{2a} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl, and R^{3a} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl. In some embodiments, each R^{2a} is independently an optionally substituted C₂₂ alkyl and each R^{3a} is independently an optionally substituted C₂₂ alkyl.

[000100] In yet another aspect, provided herein is a compound having a structure of Formula (BI):



wherein:

m is an integer from 1 to 5;

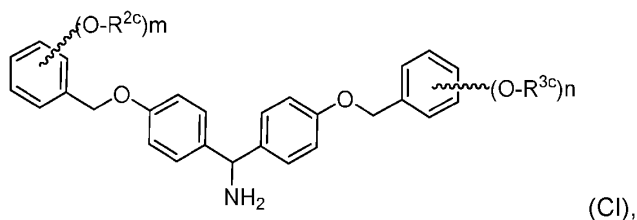
R^{1b} is C₁₋₅ alkyl; and

R^{2b} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl;
or a salt thereof.

[000101] In some embodiments, R^{1b} is methyl.

[000102] In some embodiments, R^{2b} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl. In some embodiments, each R^{2b} is independently an optionally substituted C₂₂ alkyl.

[000103] In yet another aspect, provided herein is a compound having a structure of Formula (CI):



wherein:

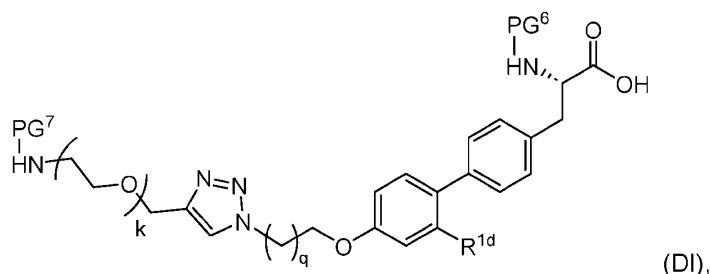
m and n are, independently, an integer from 1 to 5, and (m+n) ≥ 3;

R^{2c} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

R^{3c} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,
or a salt thereof.

[000104] In some embodiments, R^{2c} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl, and R^{3c} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl. In some embodiments, each R^{2c} is independently an optionally substituted C₂₂ alkyl and each R^{3c} is independently an optionally substituted C₂₂ alkyl.

[000105] In yet another aspect, provided herein is a method for preparation of a compound of Formula (DI):



wherein:

k is an integer from 1 to 15;

q is an integer from 0 to 10;

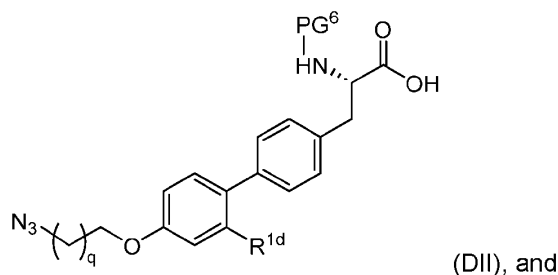
R^{1d} is C₁₋₆ alkyl;

PG⁶ is a suitable protecting group; and

PG⁷ is a suitable protecting group,

or a salt thereof. This method includes:

(a) providing a compound of Formula (DII) having the structure:

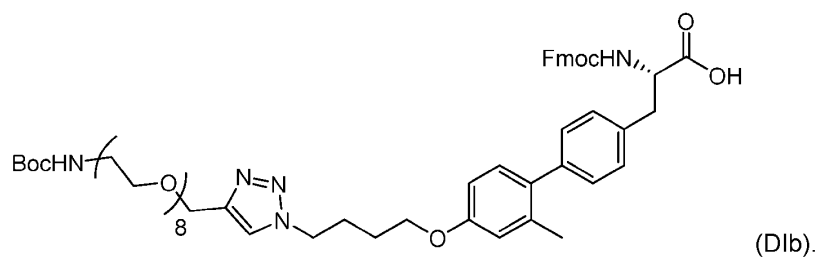
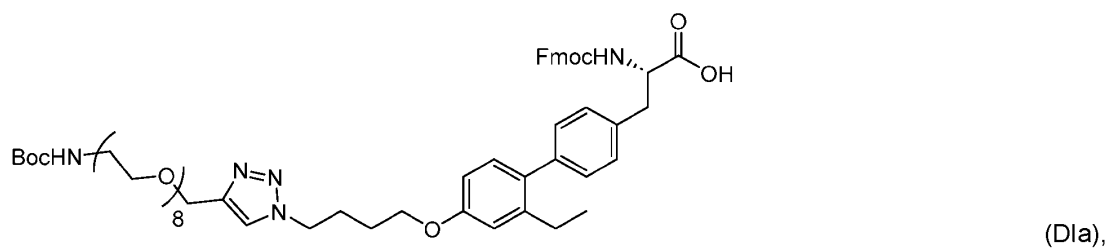


(b) forming the compound of Formula (DI) from the compound of Formula (DII).

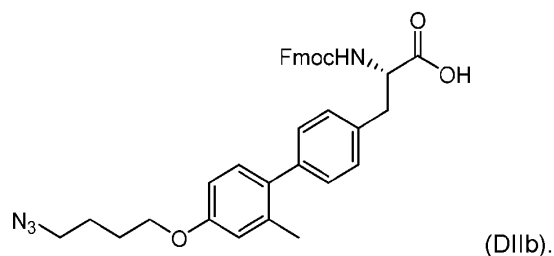
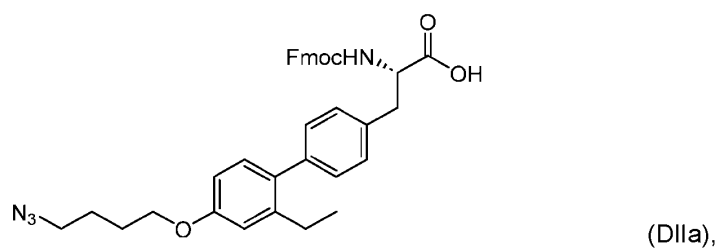
[000106] In some embodiments, PG⁶ is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

[000107] In some embodiments, PG⁷ may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

[000108] In some embodiments, the compound of Formula (DI) has the structure of Formula (DIa) or Formula (DIb):

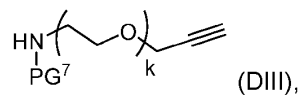


[000109] In some embodiments, the compound of Formula (DII) has the structure of Formula (DIIa) or Formula (DIIb):



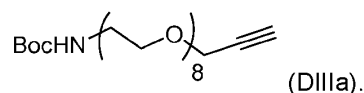
[000110] In some embodiments, the step (b) of forming the compound of Formula (DI) comprises:

reacting the compound of Formula (DII) with a compound of Formula (DIII):



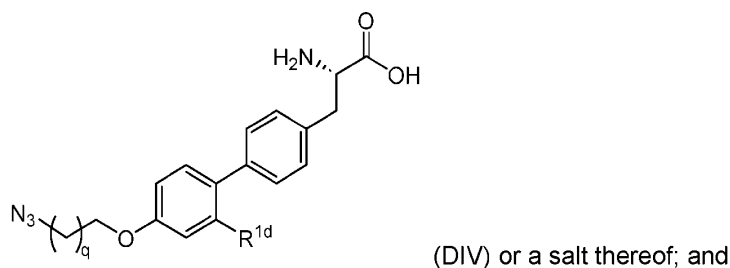
to produce the compound of Formula (II).

[000111] In one embodiment, the compound of Formula (DIII) has the structure of Formula (DIIIa):



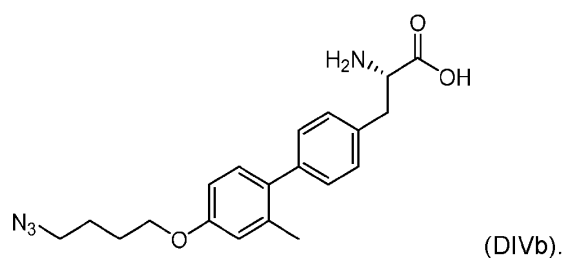
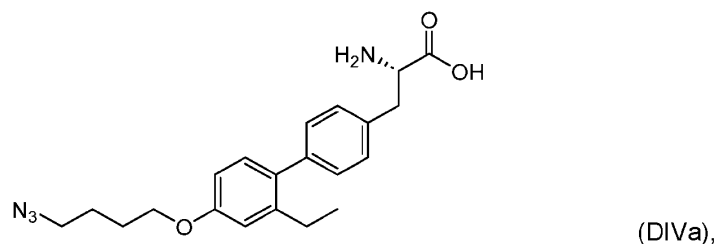
[000112] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

providing a compound of Formula (DIV) having the structure:



forming the compound of Formula (DII) from the compound of Formula (DIV) prior to the step (a).

[000113] In some embodiments, the compound of Formula (DIV) has the structure of Formula (DIVa) or Formula (DIVb):



[000114] In some embodiments, the step of forming the compound of Formula (DII) comprises: reacting the compound of Formula (DIV) with a compound of Formula (DVa) or Formula (DVb):

PG⁶-X (DVa) or PG⁶₂O (DVb),

wherein

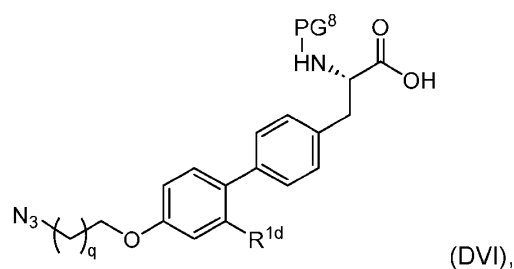
X is OSu, OTf, Cl, or Br;

to produce the compound of Formula (DII).

[000115] In some embodiments, the compound of Formula (DVa) or Formula (DVb) is selected from the group consisting of AllocCl, Alloc₂O, Cbz₂O, CbzCl, FmocCl, FmocOSu, and Boc₂O.

[000116] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

providing a compound of Formula (DVI) having the structure:



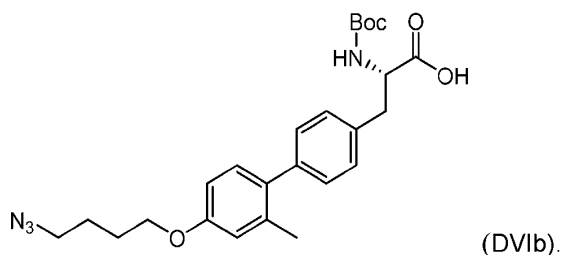
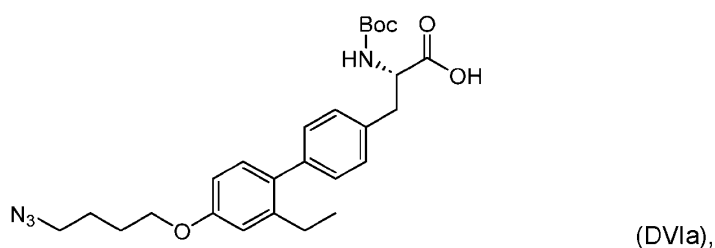
wherein

PG⁸ is a suitable protecting group; and

forming the compound of Formula (DIV) from the compound of Formula (DVI).

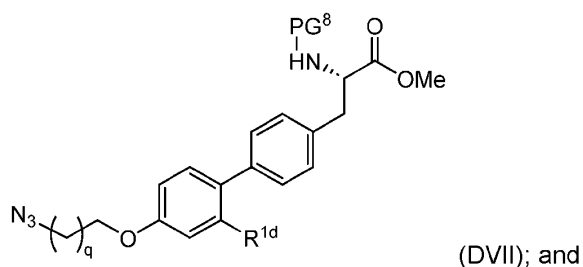
[000117] In some embodiments, the step of forming the compound of Formula (DIV) comprises reacting the compound of Formula (DVI) with a protecting group removal agent to produce the compound of Formula (DIV). In some embodiments, the protecting group removal agent may be selected from the group consisting of Pd(PPh)₃, PhSiH₃, H₂, HCl, piperidine, and trifluoroacetic acid (TFA).

[000118] In some embodiments, the compound of Formula (DVI) has the structure of Formula (DVIa) or Formula (DVIb):



[000119] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

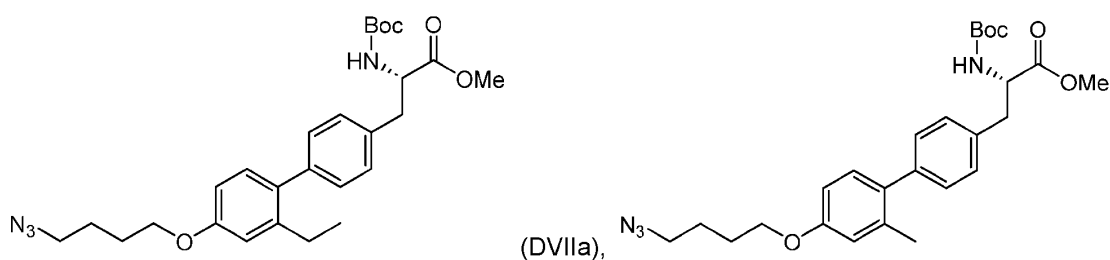
providing a compound of Formula (DVII) having the structure:



forming the compound of Formula (DVI) from the compound of Formula (DVII).

[000120] In some embodiments, the step of forming the compound of Formula (DVI) comprises reacting the compound of Formula (DVII) with a base to produce the compound of Formula (DVI). In some embodiments, the base is selected from the group consisting of LiOH, NaOH, and KOH.

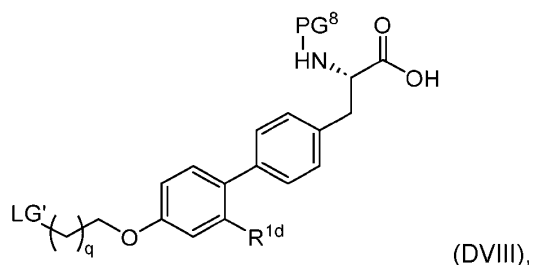
[000121] In some embodiments, the compound of Formula (DVII) has the structure of Formula (DVIIa) or Formula (DVIIb):



(DVIIb).

[000122] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

providing a compound of Formula (DVIII) having the structure:



wherein

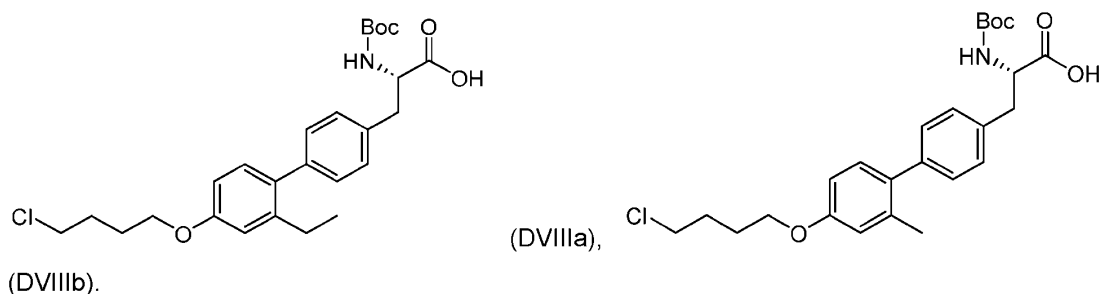
LG' is a suitable living group; and

forming the compound of Formula (DVII) from the compound of Formula (DVIII).

[000123] In some embodiments, the step of forming the compound of Formula (DVII) comprises reacting the compound of Formula (DVIII) with an azido group introducing agent to produce the compound of Formula (DVII). In some embodiments, the azido group introducing agent is selected from the group consisting of NaN₃, TMSN₃, (PhO)₂P(O)N₃, Zn(N₃)₂·2Py, and n-Bu₄NN₃.

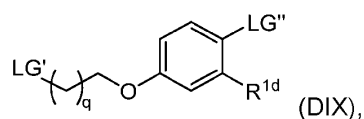
[000124] In some embodiments, LG' is selected from the group consisting of halogen, OTf, OM, and OTs.

[000125] In some embodiments, the compound of Formula (DVIII) has the structure of Formula (DVIIa) or Formula (DVIIb):



[000126] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

providing a compound of Formula (DIX) having the structure:



wherein

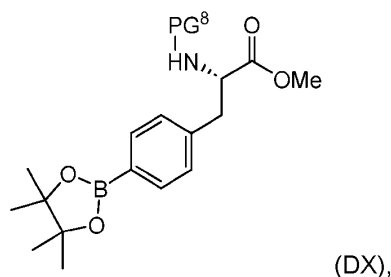
LG'' is a suitable living group; and

forming the compound of Formula (DVIII) from the compound of Formula (DIX).

[000127] In some embodiments, LG'' is selected from the group consisting of halogen, OTf, OM, and OTs.

[000128] In some embodiments, the step of forming the compound of Formula (DVIII) comprises:

reacting the compound of Formula (DIX) with a compound of Formula (DX):



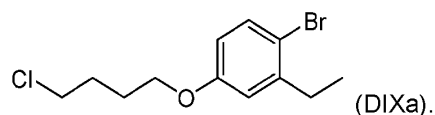
wherein

PG⁸ is a suitable protecting group;

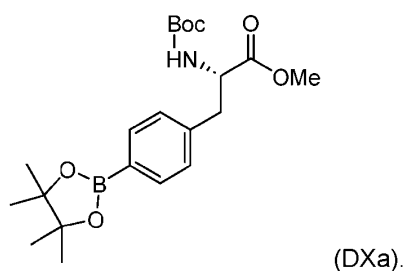
to produce the compound of Formula (DVII).

[000129] In some embodiments, PG⁸ may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc). In some embodiments, PG⁸ is tert-butyloxycarbonyl (Boc).

[000130] In some embodiments, the compound of Formula (DIX) has the structure of Formula (DIXa):

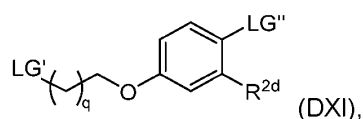


[000131] In some embodiments, the compound of Formula (DX) has the structure of Formula (DXa):



[000132] In some embodiments, the process for preparation of a compound of Formula (DI) may includes the steps of:

providing a compound of Formula (DXI) having the structure:



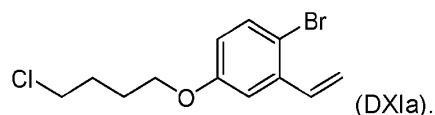
wherein

R^{2d} is C₁₋₆ alkylene; and

forming the compound of Formula (DIX) from the compound of Formula (DXI).

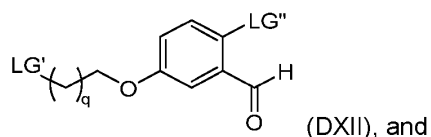
[000133] In some embodiments, the step of forming the compound of Formula (DIX) comprises reacting the compound of Formula (DXI) with a reducing agent to produce the compound of Formula (DIX). In some embodiments, the reducing agent is selected from the group consisting of H₂, NH₄HCO₂, NABH(OAc)₃, and LiAlH₄.

[000134] In some embodiments, the compound of Formula (DXI) has the structure of Formula (DXIa):



[000135] In some embodiments, the process for preparation of a compound of Formula (DI) may includes the steps of:

providing a compound of Formula (DXII) having the structure:



forming the compound of Formula (DXI) from the compound of Formula (DXII).

[000136] In some embodiments, the step of forming the compound of Formula (DXI) comprises:

reacting the compound of Formula (DXII) with a compound of Formula (DXIII):

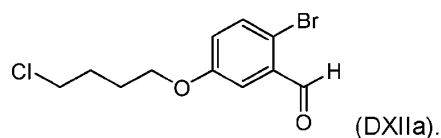
$\text{PPh}_3 \cdot \text{C}_{1-6} \text{ alkyl-Hal}$ (DXIII),

wherein

Hal is halogen,

to produce the compound of Formula (DXI).

[000137] In some embodiments, the compound of Formula (DXII) has the structure of Formula (DXIIa):

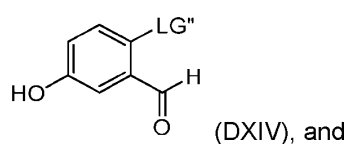


[000138] In some embodiments, the compound of Formula (DXIII) has the structure of Formula (DXIIIa):

$\text{PPh}_3 \cdot \text{MeBr}$ (DXIIIa).

[000139] In some embodiments, the process for preparation of a compound of Formula (DI) may include the steps of:

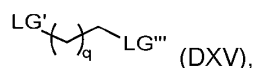
providing a compound of Formula (DXIV) having the structure:



forming the compound of Formula (DXII) from the compound of Formula (DXIV).

[000140] In some embodiments, the step of forming the compound of Formula (DXII) comprises:

reacting the compound of Formula (DXIV) with a compound of Formula (DXV):



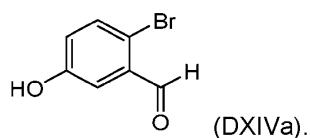
wherein

LG^{'''} is a suitable living group,

to produce the compound of Formula (DXII).

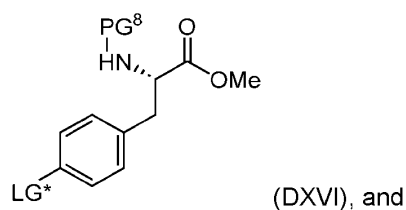
[000141] In some embodiments, the LG^{'''} is selected from the group consisting of halogen, OTf, OMs, and OTs.

[000142] In some embodiments, the compound of Formula (DXIV) has the structure of Formula (DXIVa):



[000143] In some embodiments, the process for preparation of a compound of Formula (DI) may include the steps of:

providing a compound of Formula (DXVI) having the structure:



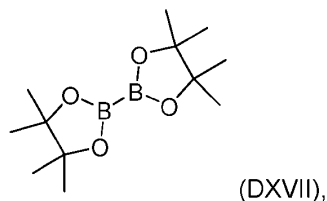
wherein

LG* is a suitable living group,

forming the compound of Formula (DX) from the compound of Formula (DXVI).

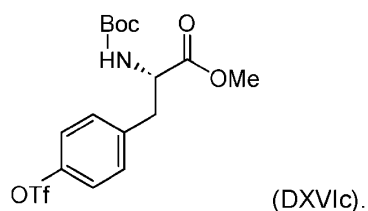
[000144] In some embodiments, LG* is selected from the group consisting of halogen, OTf, OMs, and OTs.

[000145] In some embodiments, the step of forming the compound of Formula (DX) comprises: reacting the compound of Formula (DXVI) with a compound of Formula (DXVII):



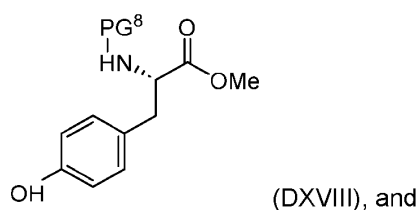
to produce the compound of Formula (DX).

[000146] In some embodiments, the compound of Formula (DXVI) has the structure of Formula (DXVIc):



[000147] In some embodiments, the process for preparation of a compound of Formula (DI) may include the steps of:

providing a compound of Formula (DXVIII) having the structure:



forming the compound of Formula (DXVI) from the compound of Formula (DXVIII).

[000148] In some embodiments, the step of forming the compound of Formula (DXVI) comprises:

reacting the compound of Formula (DXVIII) with a compound of Formula (DXIXa) or Formula (DXIXb):

LG^{*}-X (DXIXa) or LG^{*}₂O (DXIXb),

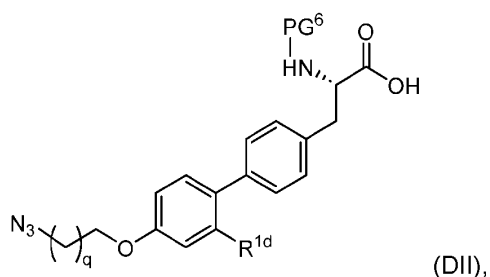
wherein

X is OSu, Cl, or Br;

to produce the compound of Formula (DXVI).

[000149] In some embodiments, the compound of Formula (DXIXa) or Formula (DXIXb) is selected from the group consisting of TfCl, Tf₂O, MsCl, and Ms₂O.

[000150] In a further aspect, provided herein is a method for preparation of a compound of Formula (DII):



wherein:

q is an integer from 0 to 10;

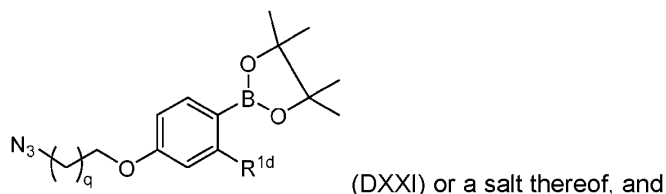
R^{1d} is C₁₋₆ alkyl; and

PG⁶ is a suitable protecting group,

or a salt thereof,

said process comprising:

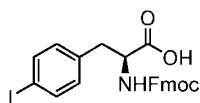
(a) providing a compound of Formula (DXXI) having the structure:



(b) forming the compound of Formula (DII) from the compound of Formula (DXXI).

[000151] In some embodiments, PG⁶ is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

[000152] In some embodiments, the compound of Formula (DXXI) is treated with compound

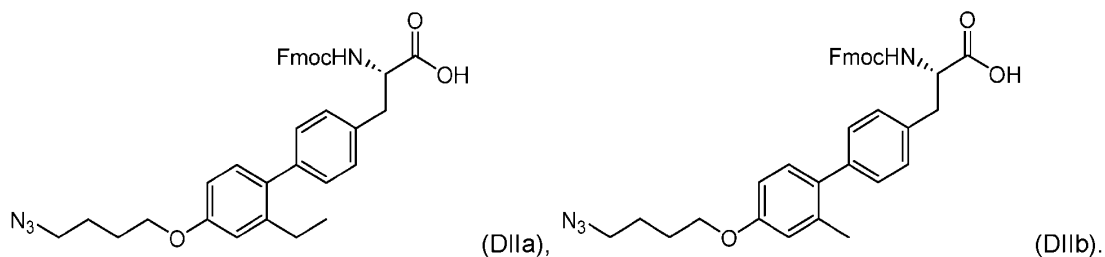


[000153] In some embodiments, the compound of Formula (DXXI) is treated with a catalyst. In some embodiments, the catalyst is Pd(dppf)Cl₂-DCM.

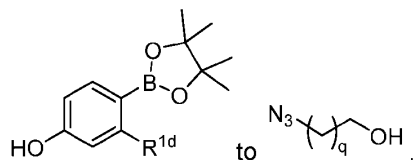
[000154] In some embodiments, the compound of Formula (DXXI) is treated with a base. In some embodiments, the base is K₂CO₃.

[000155] In some embodiments, the preparation is performed in a solvent mixture comprised of H₂O/iPrOH.

[000156] In some embodiments, the compound of Formula (DII) has the structure of Formula (DIIa) or Formula (DIIb):



[000157] In some embodiments, the compound of Formula (DXXI) is formed by exposing

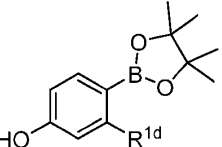
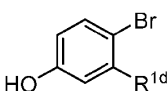


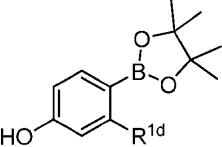
[000158] In some embodiments, the compound of Formula (DXXI) is formed in the presence of PPh_3 .

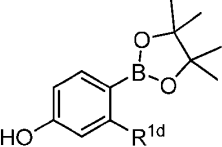
[000159] In some embodiments, the compound of Formula (DXXI) is formed in the presence of DIAD.

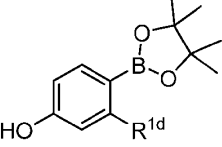
[000160] In some embodiments, the compound of Formula (DXXI) is formed in the presence of molecular sieves of $4\text{\AA}$.

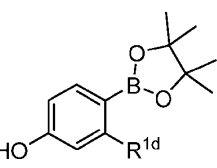
[000161] In some embodiments, the compound of Formula (DXXI) is formed in an organic solvent. In some embodiments, the organic solvent is THF.

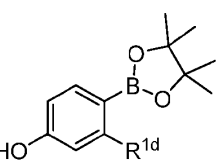
[000162] In some embodiments,  is formed by exposing  to PBin_2 .

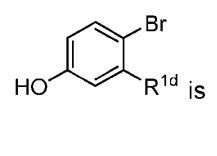
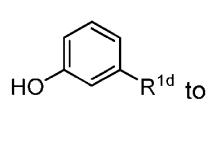
[000163] In some embodiments,  is formed in the presence of $\text{Pd}(\text{OAc})_2$.

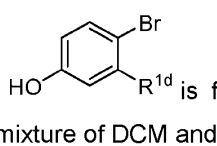
[000164] In some embodiments,  is formed in the presence of PCy_3 .

[000165] In some embodiments,  is formed in the presence of KOAc.

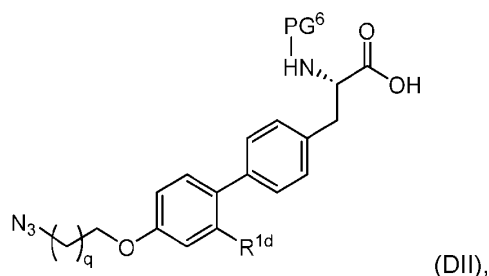
[000166] In some embodiments,  is formed in the presence of dioxane.

[000167] In some embodiments,  is formed at an elevated temperature for a period of time.

[000168] In some embodiments,  is formed by exposing  to $\text{Bu}_4\text{N}^+\text{Br}_3^-$.

[000169] In some embodiments,  is formed in an organic solvent. In some embodiments, the organic solvent is a mixture of DCM and methanol.

[000170] In a further aspect, provided herein is a method for preparation of a compound of Formula (DII):



wherein:

q is an integer from 0 to 10;

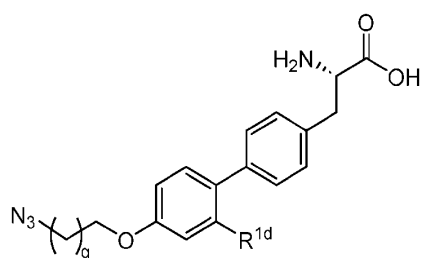
R^{1d} is C_{1-6} alkyl; and

PG^6 is a suitable protecting group,

or a salt thereof,

said process comprising:

(a) providing a compound of Formula (DIV) having the structure:

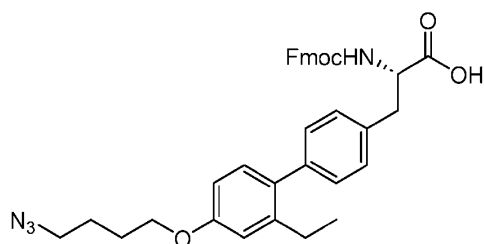


(DIV) or a salt thereof, and

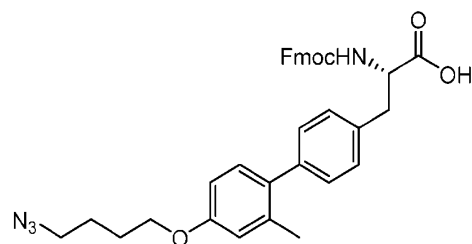
(b) forming the compound of Formula (DII) from the compound of Formula (DIV).

[000171] In some embodiments, PG⁶ may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

[000172] In some embodiments, the compound of Formula (DII) has the structure of Formula (DIIa) or Formula (DIIb):

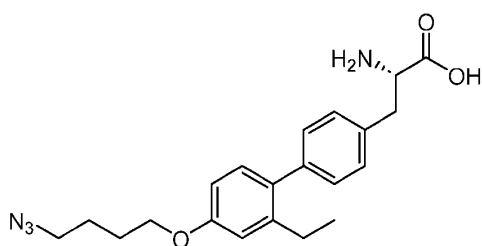


(DIIa),

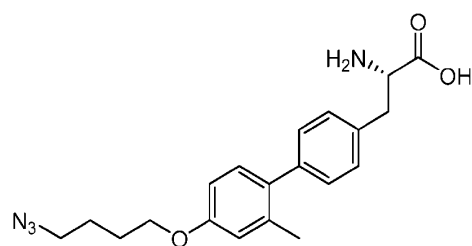


(DIIb).

[000173] In some embodiments, the compound of Formula (DIV) has the structure of Formula (DIVa) or Formula (DIVb):



(DIVa),



(DIVb).

[000174] In some embodiments, the process of forming the compound of Formula (DII) comprises:

reacting the compound of Formula (DIV) with a compound of Formula (DVa) or Formula (DVb):

PG⁶-X (DVa) or PG⁶₂O (DVb),

wherein

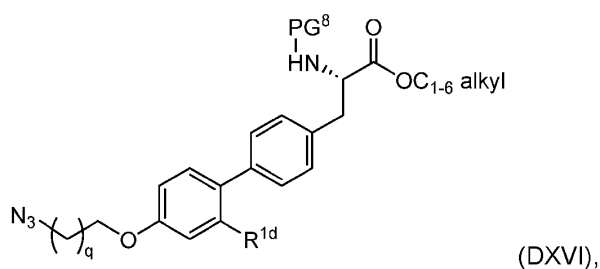
X is OSu, OTf, Cl, or Br;

to produce the compound of Formula (DII).

[000175] In some embodiments, the compound of Formula (DVa) or Formula (DVb) is selected from the group consisting of AllocCl, Alloc₂O, Cbz₂O, CbzCl, FmocCl, FmocOSu, and Boc₂O.

[000176] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

providing a compound of Formula (DXVI) having the structure:



wherein

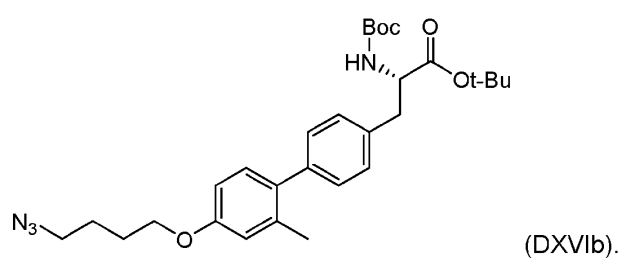
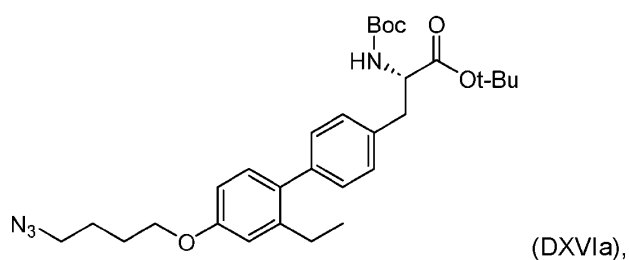
PG⁸ is a suitable protecting group; and

forming the compound of Formula (DIV) from the compound of Formula (DXVI).

[000177] In some embodiments, PG⁸ is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

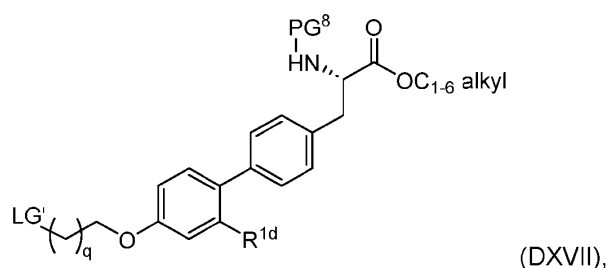
[000178] In some embodiments, the step of forming the compound of Formula (DIV) comprises reacting the compound of Formula (DXVI) with a protecting group removal agent to produce the compound of Formula (DIV). In some embodiments, the protecting group removal agent is selected from the group consisting of HCl, Pd(PPh)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

[000179] In some embodiments, the compound of Formula (DXVI) has the structure of Formula (DXVIa) or Formula (DXVIb):



[000180] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

providing a compound of Formula (DXVII) having the structure:



wherein

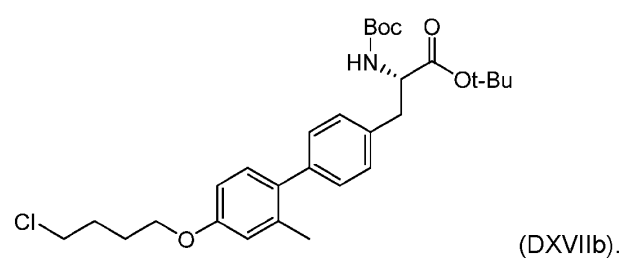
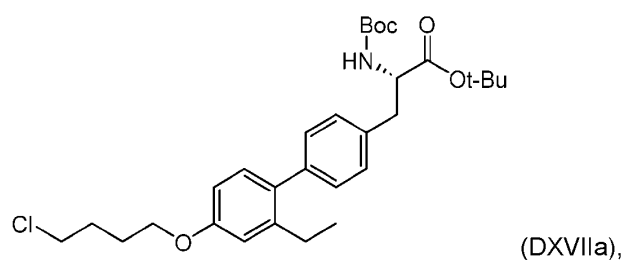
LG' is a suitable living group; and

forming the compound of Formula (DXVI) from the compound of Formula (DXVII).

[000181] In some embodiments, the step of forming the compound of Formula (DXVI) comprises reacting the compound of Formula (DXVII) with an azido group introducing agent to produce the compound of Formula (DXVI). In some embodiments, azido group introducing agent is selected from the group consisting of NaN_3 , TMSN_3 , $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$, $\text{Zn}(\text{N}_3)_2 \cdot 2\text{Py}$, and $n\text{-Bu}_4\text{NN}_3$.

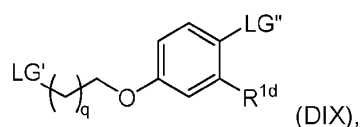
[000182] In some embodiments, LG' is selected from the group consisting of halogen, OTf, OMs, and OTs.

[000183] In some embodiments, the compound of Formula (DXVII) has the structure of Formula (DXVIIa) or Formula (DXVIIb):



[000184] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

providing a compound of Formula (DIX) having the structure:



wherein

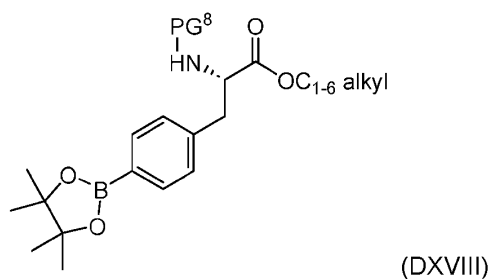
LG'' is a suitable living group; and

forming the compound of Formula (DXVII) from the compound of Formula (DIX).

[000185] In some embodiments, LG'' is selected from the group consisting of halogen, OTf, OMs, and OTs.

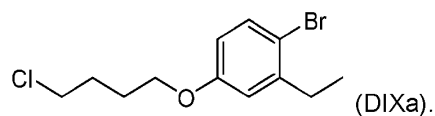
[000186] In some embodiments, the step of forming the compound of Formula (DXVII) comprises:

reacting the compound of Formula (DIX) with a compound of Formula (DXVIII):

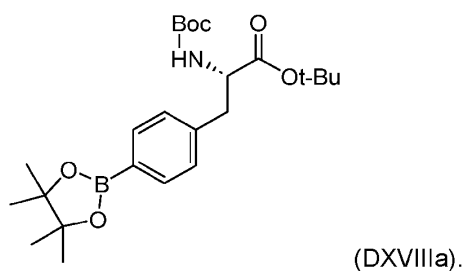


to produce the compound of Formula (DXVII).

[000187] In some embodiment, the compound of Formula (DIX) has the structure of Formula (DIXa):

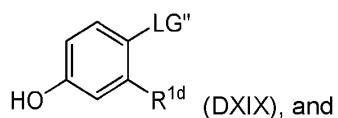


[000188] In some embodiments, the compound of Formula (DXVIII) has the structure of Formula (DXVIIIa):



[000189] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

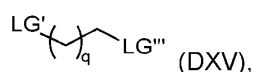
providing a compound of Formula (DXIX) having the structure:



forming the compound of Formula (DIX) from the compound of Formula (DXIX).

[000190] In some embodiments, the step of forming the compound of Formula (DIX) comprises:

reacting the compound of Formula (DXIX) with a compound of Formula (DXV):



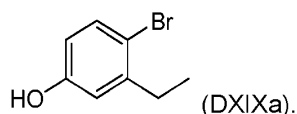
wherein

LG''' is a suitable living group,

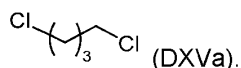
to produce the compound of Formula (DXIX).

[000191] In some embodiments, LG''' is selected from the group consisting of halogen, OTf, OMs, and OTs.

[000192] In some embodiments, the compound of Formula (DXIX) has the structure of Formula (DXIXa):

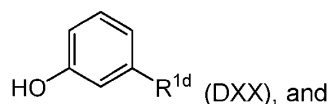


[000193] In some embodiments, the compound of Formula (DXV) has the structure of Formula (DXVa):



[000194] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

providing a compound of Formula (DXX) having the structure:



forming the compound of Formula (DXIX) from the compound of Formula (DXX).

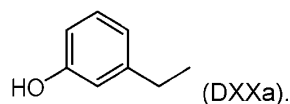
[000195] In some embodiments, the step of

forming the compound of Formula (DXIX) comprises:

reacting the compound of Formula (DXX) with an LG⁺ introducing agent to produce the compound of Formula (DXIX).

[000196] In some embodiments, the LG⁺ introducing agent is NBu₄Br₃.

[000197] In some embodiments, the compound of Formula (DXX) has the structure of Formula (DXXa):



[000198] In another aspect, provided herein is a pharmaceutical composition comprising the peptidomimetic or compound of any of the embodiments described herein.

[000199] In another aspect, provided herein is a pharmaceutical dosage form comprising the peptidomimetic or compound of any of the embodiments described herein.

[000200] In another aspect, provided herein is a method of selectively targeting GLP1R on a surface of a cell with the peptidomimetic or compound of any of the embodiments described herein. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is a pancreatic cell, a brain cell, a heart cell, a vascular tissue cell, a kidney cell, an adipose tissue cell, a liver cell, or a muscle cell.

[000201] In another aspect, provided herein is a method of enhancing GLP1R activity in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic or compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein.

[000202] In another aspect, provided herein is a method of lowering blood glucose levels in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic or compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein.

[000203] In another aspect, provided herein is a method of lowering body weight in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic or compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein.

[000204] In another aspect, provided herein is a method of treating a GLP1R-associated condition in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic or compound of any of the embodiments described herein, the composition described herein, or the dosage form described herein. In some embodiments, the GLP1R-associated condition is type II diabetes, obesity, liver disease, coronary artery disease, or kidney disease. In some embodiments, the GLP1R-associated condition is type II diabetes and/or obesity.

[000205] In various embodiments of any of the method described herein, the compound, the composition, or the dosage form of the present disclosure is administered subcutaneously, intravenously, intradermally, intraperitoneally, or intramuscularly.

[000206] These and other aspects of the present disclosure will become apparent to those skilled in the art after a reading of the following detailed description of the disclosure, including the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[000207] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[000208] Figure 1 shows liquid phase peptide support (LPPS) approaches for making **M1**.

[000209] Figure 2 shows the synthesis of **Tag1-M2**.

[000210] Figure 3 shows the synthesis of **Tag2-M2**.

- [000211] Figure 4 shows the synthesis of **Tag3-M2**.
- [000212] Figure 5 shows the synthesis of **Tag4-M2**.
- [000213] Figure 6 shows the synthesis of **Tag5-M1**.
- [000214] Figure 7 shows the synthesis of **Tag5-M3**.
- [000215] Figure 8 shows some examples of known LPPS Tags.
- [000216] Figure 9 shows SPPS for peptide elongation.
- [000217] Figures 10A-B are tables showing a summary of procedures and conditions used to prepare **M1** using **Tag5**.
- [000218] Figures 11A-B are tables showing elongation conditions and results used for making **M1** using **Tag5**.
- [000219] Figure 12 is a table showing elongation conditions and results used for making **M3** using **Tag5**.
- [000220] Figure 13 is a table showing comparison of five routes used to prepare **AA2** and **AA2+Linker**.
- [000221] Figure 14 shows the synthesis of **AA2** using route 1.
- [000222] Figure 15 shows an alternate process for making **AA2** and **AA2+Linker**.

DETAILED DESCRIPTION

- [000223] The present disclosure provides, in some aspects, compounds useful as a support for liquid phase organic synthesis. Several novel LPPS Tags (**Tag2-Tag5** in Figure 1) were designed by a) increasing hydrophobicity of the Tag for solidification of the TCP constituent to remove residual reactant and reagent as well impurities in solution for easy separation and purification; and b) introducing 2,4-bis(methoxy-benzyl)amine amide moiety (e.g. in **Tag5**) for easy C-N bond cleavage, an GLP1 peptidomimetic was prepared on new Tags following Fmoc/tBu C → N synthesis.
- [000224] New liquid phase peptide support (LPPS) Tags were designed to combine the advantages of solid-phase peptide support (SPPS) synthesis allowing separation of solid supported peptides from elongation mixture and classical solution peptide synthesis (CSPS) using fewer molar equivalents of reactants and reagents growing peptide chain. The increased hydrophobicity of LPPS Tags allowed i) carrying out more steps of peptide elongations on a soluble Tag in elongation solvents and ii) precipitation of tagged carrying/growing peptides in polar solvents. **Tag5** bearing six long alkyl chains allowed LPPS of a hydrophilic GLP1 agonist, using fewer molar equivalents of reactants and reagents compared to SPPS synthesis.
- [000225] Detailed embodiments of the present disclosure are disclosed herein; however, it is to be understood that the disclosed embodiments are merely illustrative of the disclosure that may

be embodied in various forms. In addition, each of the examples given in connection with the various embodiments of the disclosure is intended to be illustrative, and not restrictive. Therefore, specific structural and functional details disclosed herein are not to be interpreted as limiting, but merely as a representative basis for teaching one skilled in the art to variously employ the present disclosure.

Definitions

[000226] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[000227] As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, a reference to “a method” includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

[000228] A “subject” or “patient” or “individual” or “animal”, as used herein, refers to humans, veterinary animals (e.g., cats, dogs, cows, horses, sheep, pigs, etc.) and experimental animal models of diseases (e.g., mice, rats). In a preferred embodiment, the subject is a human.

[000229] The phrase “pharmaceutically acceptable salt”, as used in connection with compositions of the disclosure, refers to any salt suitable for administration to a patient. Suitable salts include, but are not limited to, those disclosed in. Berge *et al.*, “Pharmaceutical Salts”, *J. Pharm. Sci.*, 1977, 66:1, incorporated herein by reference. Examples of salts include, but are not limited to, acid derived, base derived, organic, inorganic, amine, and alkali or alkaline earth metal salts, including but not limited to calcium salts, magnesium salts, potassium salts, sodium salts, salts of hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methane sulfonic acid, ethane sulfonic acid, para-toluene sulfonic acid, salicylic acid, and the like.

[000230] Ranges may be expressed herein as from “about” or “approximately” one particular value and/or to “about” or “approximately” another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value.

[000231] By “comprising” or “containing” or “including” is meant that at least the named compound, element, particle, or method step is present in the composition or article or method, but does not exclude the presence of other compounds, materials, particles, or method steps, even if the other such compounds, material, particles, or method steps have the same function as what is named.

[000232] Compounds of the present disclosure, such as Tags, include those described generally herein, and are further illustrated by the classes, subclasses, and species disclosed herein. As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[000233] As used herein, the term "alkyl" is given its ordinary meaning in the art and may include saturated aliphatic groups, including straight-chain alkyl groups and branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has about 1–30 carbon atoms in its backbone (e.g., C₁–C₃₀ for straight chain, C₂–C₃₀ for branched chain), and alternatively, about 1–10 carbon atoms, or about 1 to 6 carbon atoms. In some embodiments, a cycloalkyl ring has from about 3–10 carbon atoms in their ring structure where such rings are monocyclic or bicyclic, and alternatively about 5, 6 or 7 carbons in the ring structure. In some embodiments, an alkyl group may be a lower alkyl group, wherein a lower alkyl group comprises 1–5 carbon atoms (e.g., C₁–C₅ for straight chain lower alkyls).

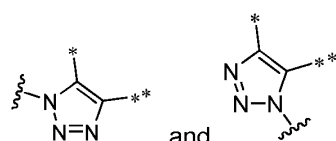
[000234] The term "halogen" means F, Cl, Br, or I; the term "halide" refers to a halogen radical or substituent, namely -F, -Cl, -Br, or -I.

[000235] As described herein, compounds of the disclosure (e.g., Tags) may contain "optionally substituted" moieties. In general, the term "substituted," whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. For example, up to three H atoms in each residue are replaced with alkyl, halogen, haloalkyl, hydroxy, loweralkoxy, carboxy, carboalkoxy (also referred to as alkoxycarbonyl), carboxamido (also referred to as alkylaminocarbonyl), cyano, carbonyl, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, sulfoxide, sulfone, acylamino, amidino, phenyl, benzyl, heteroaryl, phenoxy, benzyloxy, or heteroaryloxy. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this disclosure are preferably those that result in the formation of stable or chemically feasible compounds.

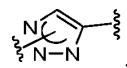
[000236] The term “stable,” as used herein in reference to compounds, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[000237] Unless otherwise stated, structures depicted herein are also meant to include all isomeric (*e.g.*, enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, (Z) and (E) double bond isomers, and (Z) and (E) conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the disclosure.

[000238] Unless otherwise stated, cyclic adducts, *e.g.*, products of a cycloaddition reaction, *e.g.*, an azide-acetylene cycloaddition reaction or a Diels-Alder reaction, depicted herein include all regioisomers, *i.e.*, structural isomers that differ only in the position of a functional group or a substituent. By way of an example, the following structures represent triazole regioisomers, which differ only in the position of the substituent on the triazole ring:



Triazole regioisomers may also be represented by the following structure:



[000239] Unless otherwise stated, all tautomeric forms of the compounds of the disclosure are within the scope of the disclosure.

[000240] Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{11}C - or ^{13}C - or ^{14}C -enriched carbon are within the scope of this disclosure.

[000241] It is also to be understood that the mention of one or more method steps does not preclude the presence of additional method steps or intervening method steps between those steps expressly identified. Similarly, it is also to be understood that the mention of one or more components in a device or system does not preclude the presence of additional components or intervening components between those components expressly identified.

[000242] Unless otherwise stated, all crystalline forms of the compounds of the disclosure and salts thereof are also within the scope of the disclosure. The compounds of the disclosure may be isolated in various amorphous and crystalline forms, including without limitation forms which are anhydrous, hydrated, non-solvated, or solvated. Example hydrates include hemihydrates, monohydrates, dihydrates, and the like. In some embodiments, the compounds of the disclosure are anhydrous and non-solvated. By "anhydrous" is meant that the crystalline form of the compound contains essentially no bound water in the crystal lattice structure, *i.e.*, the compound does not form a crystalline hydrate.

[000243] As used herein, "crystalline form" is meant to refer to a certain lattice configuration of a crystalline substance. Different crystalline forms of the same substance typically have different crystalline lattices (*e.g.*, unit cells) which are attributed to different physical properties that are characteristic of each of the crystalline forms. In some instances, different lattice configurations have different water or solvent content. The different crystalline lattices may be identified by solid state characterization methods such as by X-ray powder diffraction (PXRD). Other characterization methods such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), dynamic vapor sorption (DVS), solid state NMR, and the like further help identify the crystalline form as well as help determine stability and solvent/water content.

[000244] Crystalline forms of a substance include both solvated (*e.g.*, hydrated) and non-solvated (*e.g.*, anhydrous) forms. A hydrated form is a crystalline form that includes water in the crystalline lattice. Hydrated forms may be stoichiometric hydrates, where the water is present in the lattice in a certain water/molecule ratio such as for hemihydrates, monohydrates, dihydrates, *etc.* Hydrated forms may also be non-stoichiometric, where the water content is variable and dependent on external conditions such as humidity.

[000245] In some embodiments, the compounds of the disclosure are substantially isolated. By "substantially isolated" is meant that a particular compound is at least partially isolated from impurities. For example, in some embodiments a compound of the disclosure comprises less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 2.5%, less than about 1%, or less than about 0.5% of impurities. Impurities generally include anything that is not the substantially isolated compound including, for example, other crystalline forms and other substances.

[000246] The term "GLP1R" refers to the glucagon-like peptide 1 receptor and includes recombinant GLP1R protein or a fragment thereof. GLP1R has a sequence of 463 residues. Donnelly, *Br J Pharmacol*, 166(1):27–41 (2011). Glucagon-like peptide 1 (GLP1) is a 31-amino acid peptide hormone released from intestinal L cells following nutrient consumption. The binding of

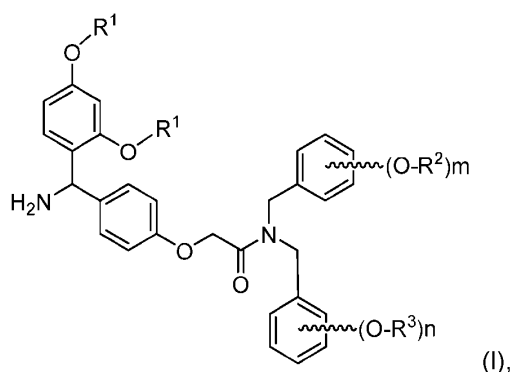
GLP1 to GLP1R potentiates glucose-induced secretion of insulin from pancreatic beta cells, increases insulin expression, inhibits beta-cell apoptosis, promotes beta-cell neogenesis, reduces glucagon secretion, delays gastric emptying, promotes satiety and increases peripheral glucose disposal.

Tags

[000247] According to the foregoing objective and others, the present disclosure provides compounds and precursors and intermediates thereof, that may be used as tags for preparation of GLP1 peptidomimetics. The present disclosure also provides the methods of making these compounds and precursors and intermediates thereof.

[000248] Also provided in the present disclosure are GLP1 peptidomimetics prepared using the tags described herein and pharmaceutical compositions comprising GLP1 peptidomimetics, and methods for treating certain diseases in a subject in need of such treatment.

[000249] In one aspect, the present disclosure provides a compound having a structure of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R^1 is a C_{1-5} alkyl;

R^2 is independently at each occurrence an optionally substituted C_{10-30} alkyl; and

R^3 is independently at each occurrence an optionally substituted C_{10-30} alkyl,
or a salt thereof.

[000250] According to the present disclosure, m may be an integer from 1 to 5 and n may be an integer from 1 to 5, provided that $(m+n) \geq 3$. For example, m may be 1, 2, 3, 4, or 5 and n may be 1, 2, 3, 4, or 5.

[000251] In at least one embodiment, m is 3 and n is 3.

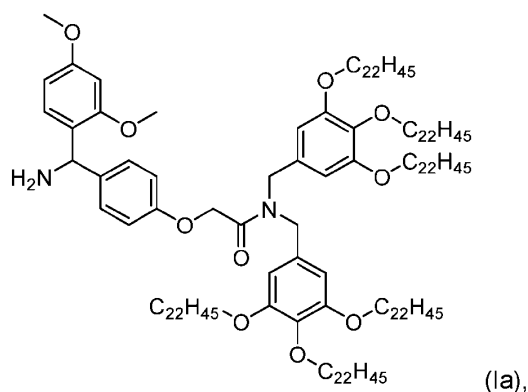
[000252] According to the present disclosure, R^1 may be methyl, ethyl, propyl, butyl, or pentyl. For example, R^1 may be n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, or n-pentyl. In at least one embodiment, R^1 is methyl.

[000253] According to the present disclosure, R^2 is independently at each occurrence an optionally substituted C_{10-30} alkyl. For example, C_{10-30} alkyl may be optionally substituted from 1 to 10 times with a substituent selected from the group consisting of halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy (also referred to as alkoxycarbonyl), carboxamido (also referred to as alkylaminocarbonyl), cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy. For example, C_{10-30} alkyl may be optionally substituted 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times with any of the above-described substituents, wherein each substituent is independently selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy.

[000254] According to the present disclosure, R^3 is independently at each occurrence an optionally substituted C_{10-30} alkyl. For example, C_{10-30} alkyl may be optionally substituted from 1 to 10 times with a substituent selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy (also referred to as alkoxycarbonyl), carboxamido (also referred to as alkylaminocarbonyl), cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy. For example, C_{10-30} alkyl may be optionally substituted 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times with any of the above-described substituents, wherein each substituent is independently selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy.

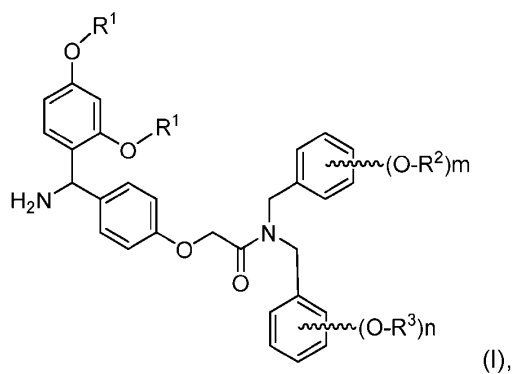
[000255] In at least one embodiment, R^2 is independently at each occurrence an optionally substituted C_{18-26} alkyl, and R^3 is independently at each occurrence an optionally substituted C_{18-26} alkyl. For example, each R^2 is independently an optionally substituted C_{22} alkyl and each R^3 is independently an optionally substituted C_{22} alkyl.

[000256] In one aspect, provided herein is a compound having a structure of Formula (Ia):



or a salt thereof.

[000257] In another aspect, the present disclosure provides a process for preparation of a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and (m+n) ≥ 3;

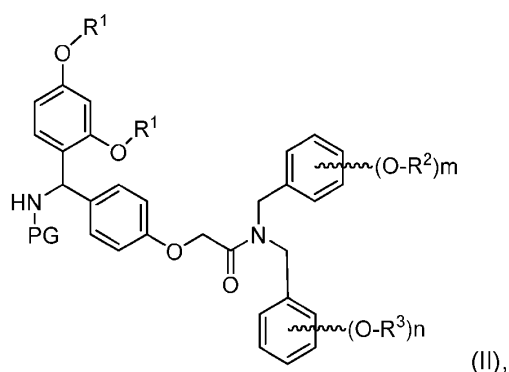
R¹ is a C₁₋₅ alkyl;

R² is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

R³ is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof. This process includes:

(a) providing a compound of Formula (II) having the structure:



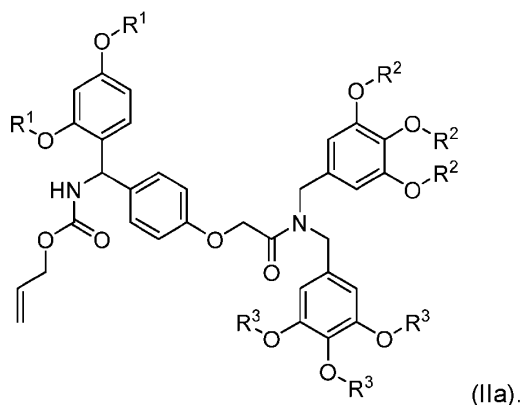
wherein

PG is a suitable protecting group; and

(b) forming the compound of Formula (I) from the compound of Formula (II).

[000258] According to the present disclosure, PG any suitable protecting group that may be used to protect amino group. Suitable protecting groups that may be used are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, PG may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc). In some embodiments, PG is allyloxycarbonyl (Alloc).

[000259] In some embodiments, the compound of Formula (II) has the structure of Formula (IIa):

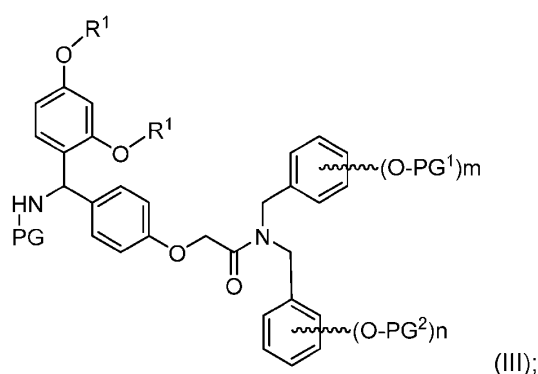


[000260] In some embodiments, the step (b) of forming the compound of Formula (I) may include reacting the compound of Formula (II) with a protecting group removing agent to produce the compound of Formula (I).

[000261] According to the present disclosure, any suitable protecting group removing agent may be used to remove protecting group PG from the amino group of the compound of Formula (II). Suitable protecting group removing agents that may be used to remove a particular protecting group are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinchenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, the protecting group removing agent is selected from the group consisting of Pd(PPh)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

[000262] In some embodiments, the process for preparation of a compound of Formula (I) may include a step of

providing a compound of Formula (III) having the structure:



wherein

PG¹ is a suitable protecting group;

PG² is a suitable protecting group; and

forming the compound of Formula (II) from the compound of Formula (III) prior to the step (a).

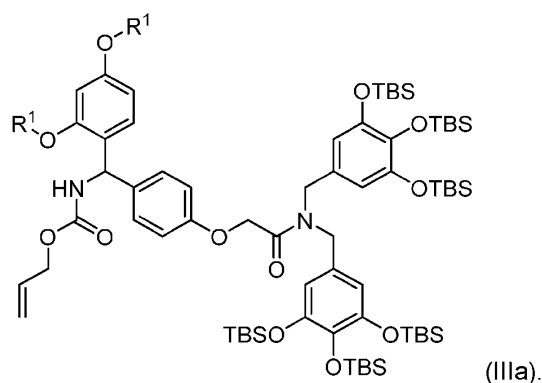
[000263] According to the present disclosure, PG¹ is any suitable protecting group that may be used to protect hydroxy group. Suitable protecting groups that may be used are known to people of skill in the art and may be selected from the ones described in "Handbook of Reagents for Organic Synthesis. Activating Reagents and Protecting Groups," Pearson and Roush, Eds., John Wiley & Sons, 2005, which is hereby incorporated by reference in its entirety. For example, PG¹ may be selected from the group consisting of tert-butyldimethylsilyl (TBS), trimethylsilyl (TMS), triethylsilyl (TES), tert-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

[000264] According to the present disclosure, PG² is any suitable protecting group that may be used to protect hydroxy group. Suitable protecting groups that may be used are known to people of

skill in the art and may be selected from the ones described in "Handbook of Reagents for Organic Synthesis. Activating Reagents and Protecting Groups," Pearson and Roush, Eds., John Wiley & Sons, 2005, which is hereby incorporated by reference in its entirety. For example, PG² may be selected from the group consisting of tert-butyldimethylsilyl (TBS), trimethylsilyl (TMS), triethylsilyl (TES), tert-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

[000265] According to the present disclosure, each PG¹ and each PG² may be the same or different. In some embodiments, PG¹ is the same as PG² group. For example, if m is 3 and n is 3, each of the three PG¹ groups is the same and each of the three PG² groups is the same and PG¹ = PG². In some embodiments, PG¹ is TBS and PG² is TBS. Alternatively, PG¹ and PG² may be different from each other. For example, if m is 3 and n is 3, each of the three PG¹ groups (PG¹¹, PG¹², and PG¹³) are the same (PG¹=PG¹¹=PG¹²=PG¹³) and each of the three PG² groups (PG²¹, PG²², and PG²³) are the same (PG²=PG²¹=PG²²=PG²³), but PG¹ is different from PG². In some embodiments, PG¹ is TBS and PG² is TIPS. Alternatively, each PG¹ and each PG² may be different from each other. For example, if m is 3 and n is 3, each of the three PG¹ groups are the different from each other (PG¹¹ is different from PG¹², PG¹² is different from PG¹³, and PG¹¹ is different from PG¹³) and each of the three PG² groups are the same (PG²¹ is different from PG²², PG²² is different from PG²³, and PG²¹ is different from PG²³), and none of the PG¹¹, PG¹², PG¹³, PG²¹, PG²², and PG²³ are the same.

[000266] In some embodiments, the compound of Formula (III) has the structure of Formula (IIIa):



[000267] In some embodiments, the step of forming the compound of Formula (II) prior to step (a) comprises:

reacting the compound of Formula (III) with a protecting group removal agent to obtain a deprotected intermediate;

reacting said intermediate with at least one compound of Formula (IV):

R-LG (IV),

wherein

R is an optionally substituted C₁₀₋₃₀ alkyl; and

LG is a suitable leaving group;

to produce the compound of Formula (II).

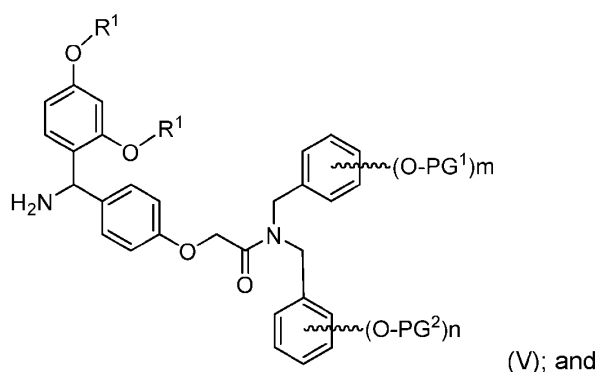
[000268] According to the present disclosure, any suitable protecting group removing agent may be used to remove protecting groups PG¹ and PG² from the hydroxyl groups of the compound of Formula (III). Suitable protecting group removing agents that may be used to remove a particular protecting group are known to people of skill in the art and may be selected from the ones described in "Handbook of Reagents for Organic Synthesis. Activating Reagents and Protecting Groups," Pearson and Roush, Eds., John Wiley & Sons, 2005, which is hereby incorporated by reference in its entirety. For example, the protecting group removing agent may be selected from the group consisting of tetrabutylammonium fluoride (TBAF), NH₄F, SiF₄, AcOH, HCl, LiAlH₄, and K₂CO₃.

[000269] In some embodiments, if PG¹ and PG² are different from each other, different protecting group removing agents may be used. In this case, one protecting group removing agent may be used to selectively remove all of the PG¹ groups present in the compound of Formula (III). Then, the formed intermediate may be reacted with the first compound of Formula (IV), compound of Formula (IVa). After that, another protecting group removing agent may be used to selectively remove all of the PG² groups and then another compound of Formula (IV), compound of Formula (IVb) may be used to modify deprotected hydroxyl groups. In this case, compound of Formula (IVa) may be different from the compound of Formula (IVb).

[000270] According to the present disclosure, LG is a suitable leaving group. Suitable leaving groups may be used are known to people of skill in the art. For example, LG may be selected from a group consisting of halogen, OTf, OMs, and OTs.

[000271] In some embodiments, the process for preparation of a compound of Formula (I) may include a step of:

providing a compound of Formula (V) having the structure:



forming the compound of Formula (III) from the compound of Formula (V).

[000272] In some embodiments, the step of forming the compound of Formula (III) may comprise:

reacting the compound of Formula (V) with a compound of Formula (VIa) or Formula (VIb):

PG-X (VIa) or PG₂O (VIb),

wherein

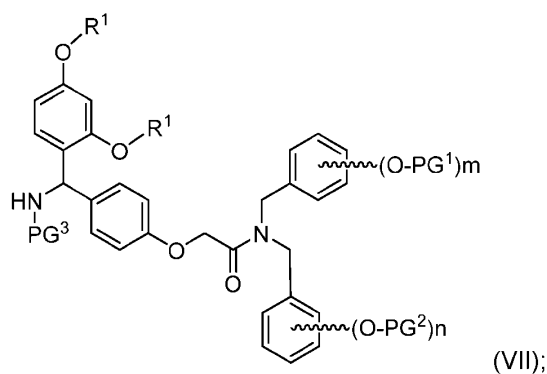
X is Cl or Br;

to produce the compound of Formula (III).

[000273] According to the present disclosure, suitable compounds of Formula (VIa) or Formula (VIb) may be selected from, but are not limited to, the group consisting of AllocCl, Alloc₂O, Cbz₂O, CbzCl, FmocCl, and Boc₂O.

[000274] In some embodiments, the process for preparation of a compound of Formula (I) may include a step of:

providing a compound of Formula (VII) having the structure:

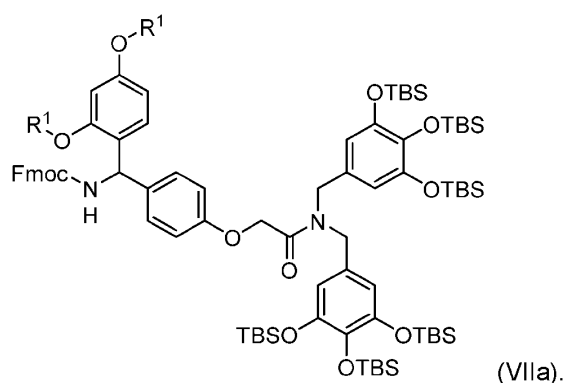


wherein PG³ is a suitable protecting group; and

forming the compound of Formula (V) from the compound of Formula (VII).

[000275] According to the present disclosure, PG³ any suitable protecting group that may be used to protect amino group. Suitable protecting groups that may be used are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, PG³ may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc). In some embodiments, PG³ is 9-fluorenylmethoxycarbonyl (Fmoc).

[000276] In some embodiments, the compound of Formula (VII) has the following structure:

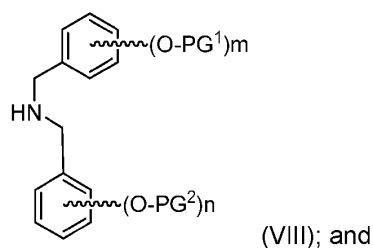


[000277] In some embodiments, the process for preparation of a compound of Formula (V) includes reacting the compound of Formula (VII) with a protecting group removing agent to produce the compound of Formula (V).

[000278] According to the present disclosure, any suitable protecting group removing agent may be used to remove protecting group PG³ from the amino group of the compound of Formula (VII). Suitable protecting group removing agents that may be used to remove a particular protecting group are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, the protecting group removing agent is selected from the group consisting of Pd(PPh)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

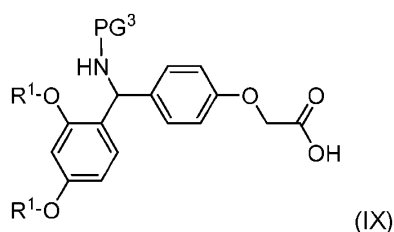
[000279] In some embodiments, the process for preparation of a compound of Formula (I) may include a step of:

providing a compound of Formula (VIII) having the structure:



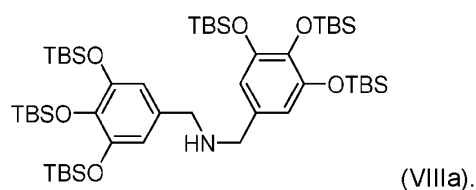
forming the compound of Formula (VII) from the compound of Formula (VIII).

[000280] In some embodiments, the process of forming the compound of Formula (VII) may comprise reacting the compound of Formula (VIII) with a compound of Formula (IX) having the structure:

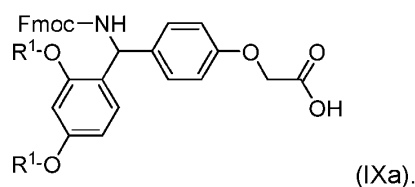


to produce the compound of Formula (VII).

[000281] In one embodiment, the compound of Formula (VIII) has the following structure:

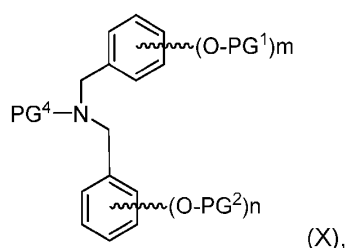


[000282] In another embodiment, the compound of Formula (IX) has the following structure:



[000283] In some embodiments, the process for preparation of a compound of Formula (I) may include a step of:

providing a compound of Formula (X) having the structure:

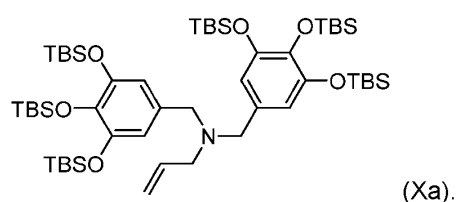


wherein PG^4 is a suitable protecting group; and

forming the compound of Formula (VIII) from the compound of Formula (X).

[000284] According to the present disclosure, PG^4 any suitable protecting group that may be used to protect amino group. Suitable protecting groups that may be used are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, PG^4 may be selected from the group consisting of allyl, allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc). In some embodiments, PG^4 is allyl.

[000285] In one embodiment, the compound of Formula (X) has the following structure:

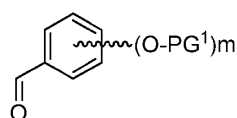


[000286] In some embodiments, the process of forming the compound of Formula (VIII) includes reacting the compound of Formula (X) with a protecting group removing agent.

[000287] According to the present disclosure, any suitable protecting group removing agent may be used to remove protecting group PG^4 from the amino group of the compound of Formula (X). Suitable protecting group removing agents that may be used to remove a particular protecting group are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, the protecting group removing agent is selected from the group consisting of Pd/K_2CO_3 , 1,3-dimethylbarbituric acid/ $Pd(PPh_3)_4$, $Pd(PPh_3)_3$, $PhSiH_3$, H_2 , piperidine, and trifluoroacetic acid (TFA).

[000288] In some embodiments, the process for preparation of a compound of Formula (I) may include a step of:

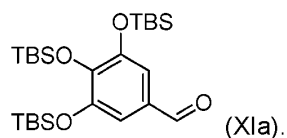
providing a compound of Formula (XI) having the structure:



(XI); and

forming the compound of Formula (X) from the compound of Formula (XI).

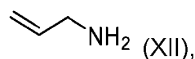
[000289] In one embodiment, the compound of Formula (XI) has the following structure:



(XIa).

[000290] In some embodiments, the process of forming the compound of Formula (X) comprises:

reacting the compound of Formula (XI) with a compound of Formula (XII):



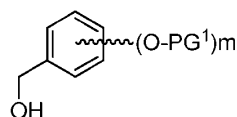
(XII),

or a salt thereof,

to produce the compound of Formula (X).

[000291] In some embodiments, the process for preparation of a compound of Formula (I) may include a step of:

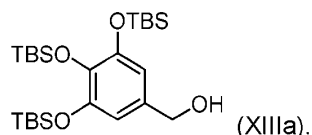
providing a compound of Formula (XIII) having the structure:



(XIII); and

forming the compound of Formula (XI) from the compound of Formula (XIII).

[000292] In one embodiment, the compound of Formula (XIII) has the following structure:



(XIIIa).

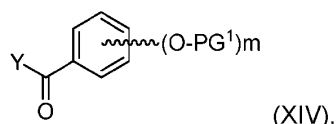
[000293] In some embodiments, the process for forming the compound of Formula (XI) includes reacting the compound of Formula (XIII) with an oxidizing agent to produce the compound of Formula (XI).

[000294] According to the present disclosure, any suitable oxidizing agent that may convert the primary alcohol to the aldehyde group may be used. Suitable oxidizing agents that may be used are known to people of skill in the art. For example, the oxidizing agent may be selected from the group

consisting of pyridinium chlorochromate (PCC), pyridinium dichromate (PDC), silver carbonate (Ag_2CO_3), tetra-n-propylammonium perruthenate (TPAP), and Dess-Martin periodinane (DMP).

[000295] In some embodiments, the process for preparation of a compound of Formula (I) may include a step of:

providing a compound of Formula (XIV) having the structure:

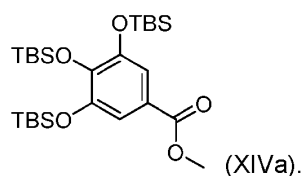


wherein

Y is $-\text{OC}_{1-6}$ alkyl; and

forming the compound of Formula (XIII) from the compound of Formula (XIV).

[000296] In one embodiment, the compound of Formula (XIV) has the following structure:

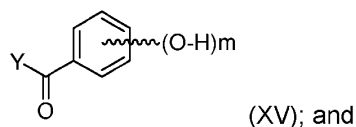


[000297] In some embodiments, the process of forming the compound of Formula (XIII) comprises reacting the compound of Formula (XIV) with a reducing agent.

[000298] According to the present disclosure, any suitable reducing agent that may convert the ester to the primary alcohol group may be used. Suitable reducing agents that may be used are known to people of skill in the art. For example, the reducing agent is selected from the group consisting of LiAlH_4 , DIBALH, and LiBH_4 .

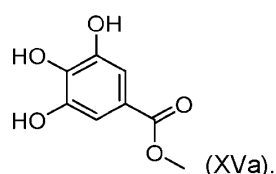
[000299] In some embodiments, the process for preparation of a compound of Formula (I) may include a step of:

providing a compound of Formula (XV) having the structure:



forming the compound of Formula (XIV) from the compound of Formula (XV).

[000300] In one embodiment, the compound of Formula (XV) has the following structure:

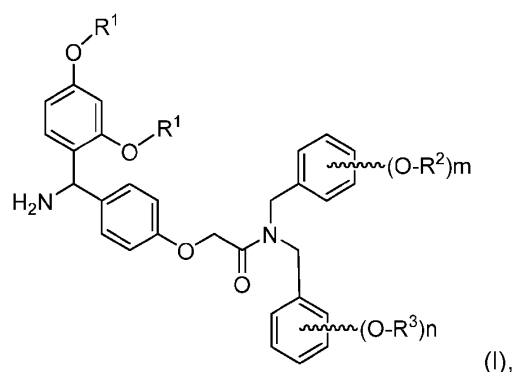


[000301] In some embodiments, the process of forming the compound of Formula (XIV) comprises reacting the compound of Formula (XV) with a protecting group introducing agent to produce the compound of Formula (XIV).

[000302] According to the present disclosure, any suitable protecting group introducing agent may be used to protect the hydroxyl groups in the compound of Formula (XV). Suitable protecting group introducing agents that may be used are known to people of skill in the art. In some embodiments, the protecting group introducing agent is selected from the group consisting of TBSCl, TBSOTf, TMSCl, TMSOTf, TESCl, TESOTf, TBDPSCl, TBDPSOTf, TIPSCl, and TIPSOTf.

[000303] In another aspect, the present disclosure provides a product prepared according to any of the methods described in the present disclosure.

[000304] In one embodiment, the product is a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

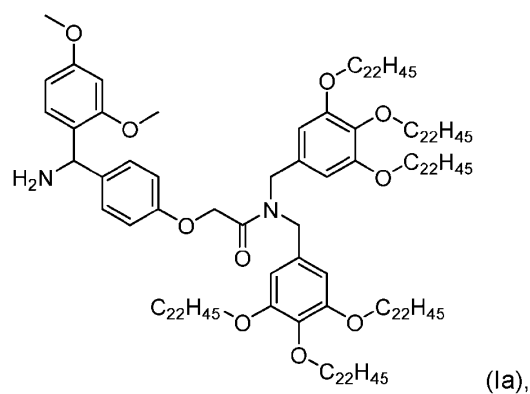
R^1 is a C_{1-5} alkyl;

R^2 is independently at each occurrence an optionally substituted C_{10-30} alkyl; and

R^3 is independently at each occurrence an optionally substituted C_{10-30} alkyl,

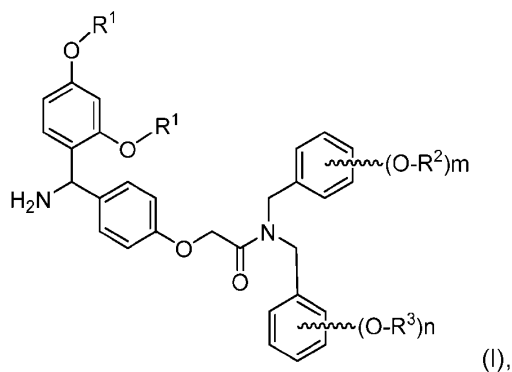
or a salt thereof.

[000305] In another embodiment, the product is a compound of Formula (Ia):



or a salt thereof.

[000306] In another aspect, the present disclosure provides a method of using a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R¹ is a C₁₋₅ alkyl;

R² is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

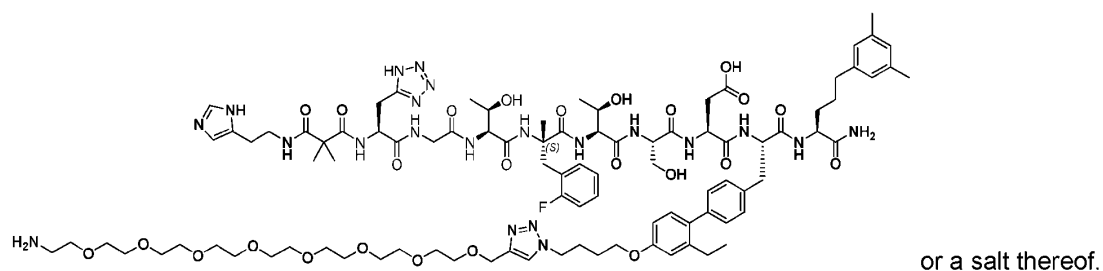
R³ is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof,

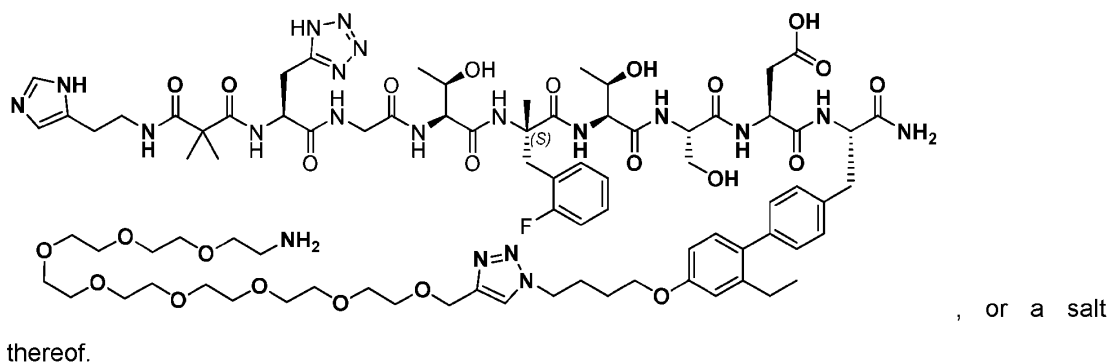
for making a peptide or a peptidomimetic.

[000307] In at least one embodiment, the compound of Formula (I) is used as a support or a tag for making a peptide or a peptidomimetic.

[000308] In one embodiment, the peptidomimetic is a compound of Formula :

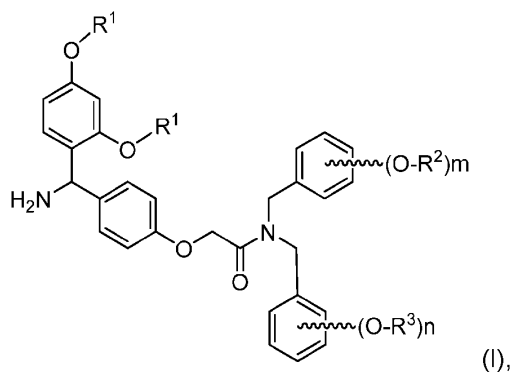


[000309] In one embodiment, the peptidomimetic is a compound of Formula:



thereof.

[000310] In another aspect, the present disclosure provides a compound manufactured using a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

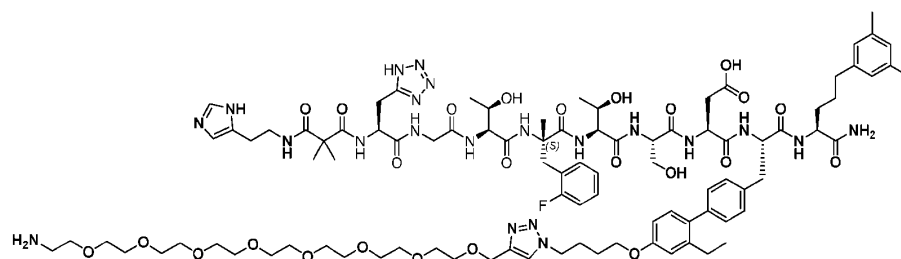
R¹ is C₁₋₅ alkyl;

R² is independently selected at each occurrence thereof from an optionally substituted C₁₀₋₃₀ alkyl; and

R³ is independently selected at each occurrence thereof from an optionally substituted C₁₀₋₃₀ alkyl,

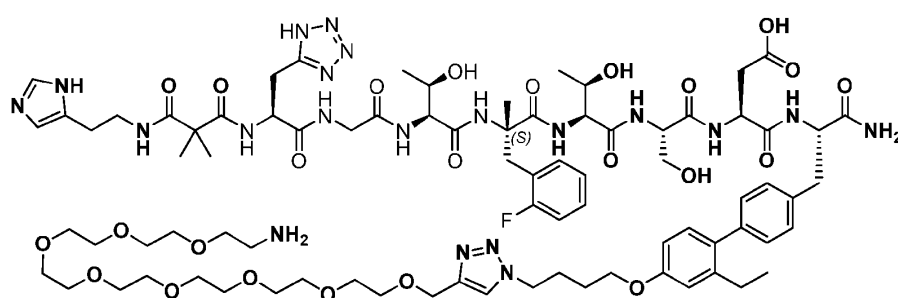
or a salt thereof.

[000311] In one embodiment, the compound has a Formula:



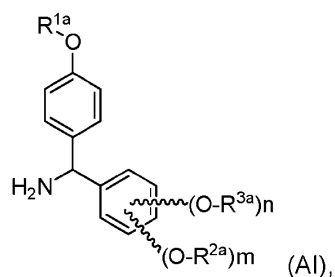
or a salt thereof.

[000312] In one embodiment, the compound has a Formula:



, or a salt thereof.

[000313] The present disclosure provides a compound having a structure of Formula (AI):



wherein:

m and n are, independently, an integer from 0 to 5;

R^{1a} is C₁₋₅ alkyl;

R^{2a} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

R^{3a} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof.

[000314] According to the present disclosure, m may be an integer from 0 to 5 and n may be an integer from 0 to 5. For example, m may be 0, 1, 2, 3, 4, or 5 and n may be 0, 1, 2, 3, 4, or 5.

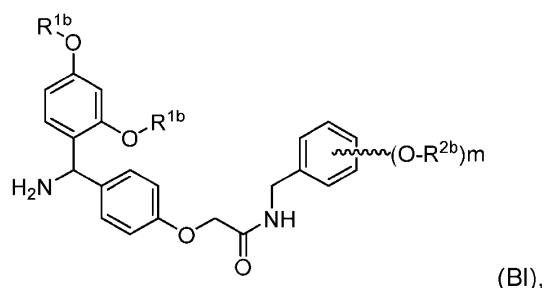
[000315] According to the present disclosure, R^{1a} may be methyl, ethyl, propyl, butyl, or pentyl. For example, R¹ may be n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, or n-pentyl. In at least one embodiment, R^{1a} is methyl.

[000316] According to the present disclosure, R^{2a} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl. For example, C₁₀₋₃₀ alkyl may be optionally substituted from 1 to 10 times with a substituent selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy (also referred to as alkoxycarbonyl), carboxamido (also referred to as alkylaminocarbonyl), cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy. For example, C₁₀₋₃₀ alkyl may be optionally substituted 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times with any of the above-described substituents, wherein each substituent is independently selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy.

[000317] According to the present disclosure, R^{3a} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl. For example, C₁₀₋₃₀ alkyl may be optionally substituted from 1 to 10 times with a substituent selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy (also referred to as alkoxycarbonyl), carboxamido (also referred to as alkylaminocarbonyl), cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy. For example, C₁₀₋₃₀ alkyl may be optionally substituted 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times with any of the above-described substituents, wherein each substituent is independently selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy.

[000318] In at least one embodiment, R^{2a} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl, and R^{3a} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl. For example, each R^{2a} is independently an optionally substituted C₂₂ alkyl and each R^{3a} is independently an optionally substituted C₂₂ alkyl.

[000319] The present disclosure provides a compound having a structure of Formula (BI):



wherein:

m is an integer from 1 to 5;

R^{1b} is C₁₋₅ alkyl; and

R^{2b} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl;
or a salt thereof.

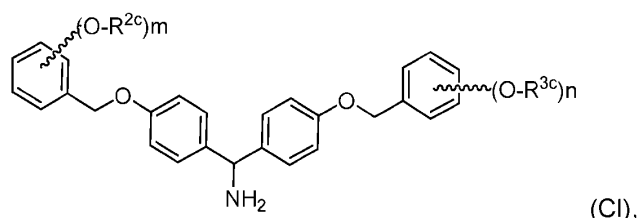
[000320] According to the present disclosure, m may be an integer from 1 to 5. For example, m may be 1, 2, 3, 4, or 5.

[000321] According to the present disclosure, R^{1b} may be methyl, ethyl, propyl, butyl, or pentyl. For example, R^{1b} may be n-propyl, i-propyl, n-butyl, i-butyl, t-butyl, or n-pentyl. In at least one embodiment, R^{1b} is methyl.

[000322] According to the present disclosure, R^{2b} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl. For example, C₁₀₋₃₀ alkyl may be optionally substituted from 1 to 10 times with a substituent selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy (also referred to as alkoxycarbonyl), carboxamido (also referred to as alkylaminocarbonyl), cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy. For example, C₁₀₋₃₀ alkyl may be optionally substituted 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times with any of the above-described substituents, wherein each substituent is independently selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy.

[000323] In at least one embodiment, R^{2b} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl. For example, each R^{2b} is independently an optionally substituted C₂₂ alkyl.

[000324] The present disclosure provides a compound having a structure of Formula (CI):



wherein:

m and n are, independently, an integer from 1 to 5, and (m+n) ≥ 3;

R^{2c} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

R^{3c} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,
or a salt thereof.

[000325] According to the present disclosure, m may be an integer from 1 to 5 and n may be an integer from 1 to 5, provided that $(m+n) \geq 3$. For example, m may be 1, 2, 3, 4, or 5 and n may be 1, 2, 3, 4, or 5.

[000326] According to the present disclosure, R^{2c} is independently at each occurrence an optionally substituted C_{10-30} alkyl. For example, C_{10-30} alkyl may be optionally substituted from 1 to 10 times with a substituent selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy (also referred to as alkoxycarbonyl), carboxamido (also referred to as alkylaminocarbonyl), cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy. For example, C_{10-30} alkyl may be optionally substituted 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times with any of the above-described substituents, wherein each substituent is independently selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy.

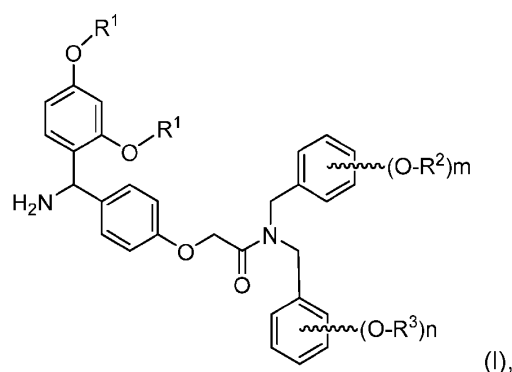
[000327] According to the present disclosure, R^{3c} is independently at each occurrence an optionally substituted C_{10-30} alkyl. For example, C_{10-30} alkyl may be optionally substituted from 1 to 10 times with a substituent selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy (also referred to as alkoxycarbonyl), carboxamido (also referred to as alkylaminocarbonyl), cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy. For example, C_{10-30} alkyl may be optionally substituted 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times with any of the above-described substituents, wherein each substituent is independently selected from halogen, hydroxy, loweralkoxy, carboxy, carboalkoxy, carboxamido, cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, phenyl, benzyl, heteroaryl, phenoxy, or benzyloxy.

[000328] In at least one embodiment, R^{2c} is independently at each occurrence an optionally substituted C_{18-26} alkyl, and R^{3c} is independently at each occurrence an optionally substituted C_{18-26} alkyl. For example, each R^{2c} is independently an optionally substituted C_{22} alkyl and each R^{3c} is independently an optionally substituted C_{22} alkyl.

Methods of Making a Peptide or a Peptidomimetic Using the Tags

[000329] The present disclosure provides a method of making a peptide or a peptidomimetic, the method comprising the steps of:

- (a) providing a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R¹ is C₁₋₅ alkyl;

R² is independently selected at each occurrence thereof from an optionally substituted C₁₀₋₃₀ alkyl; and

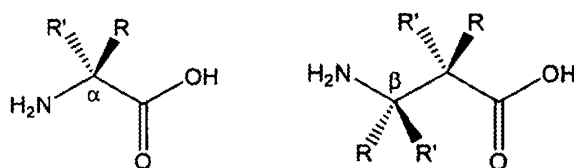
R³ is independently selected at each occurrence thereof from an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof;

(b) providing a first amino acid (AA1); and

(c) coupling the first amino acid (AA1) to the compound of Formula (I) to form a peptide bond between the first amino acid (AA1) and the compound of Formula (I).

[000330] The term “amino acid” refers to a molecule containing both an amino group and a carboxyl group. Amino acids include alpha-amino acids and beta-amino acids, the structures of which are depicted below. In certain embodiments, an amino acid is an alpha amino acid.



alpha-amino acid

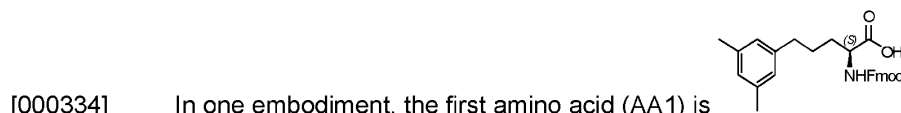
beta-amino acid

[000331] Suitable amino acids that may be used according to the present disclosure include, without limitation, i) natural alpha-amino acids such as D- and L-isomers of the 20 common naturally occurring alpha-amino acids found in peptides and proteins: Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine and derivatives

thereof; ii) unnatural alpha-amino acids; iii) natural beta-amino acids (e.g., beta-alanine), and iv) unnatural beta-amino acids.

[000332] In one embodiment, the step (c) of coupling the AA1 to the compound of Formula (I) comprises coupling the carboxylic acid moiety of the AA1 with the amino moiety of the compound of Formula (I).

[000333] In some embodiments, the first amino acid (AA1) contains a protected amino group.



[000335] In some embodiments, the method of making a peptide or a peptidomimetic may include a step of activating the chemical groups on the first amino acid (AA1) to prepare the first amino acid (AA1) for coupling with the compound of Formula (I) prior to step (c).

[000336] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

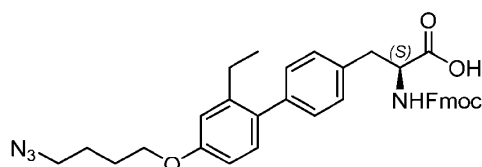
(d) removing the protecting group from the first amino acid (AA1);

(e) providing a second amino acid (AA2); and

(f) coupling the second amino acid (AA2) to the first amino acid (I-AA1) to form a peptide bond between the second amino acid (AA2) and the first amino acid (I-AA1-AA2).

[000337] In one embodiment, the step (f) comprises coupling the carboxylic acid moiety of the AA2 with the amino moiety of the AA1.

[000338] In one embodiment, the second amino acid (AA2) is



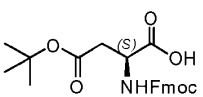
[000339] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

(g) removing the protecting group from the second amino acid (AA2);

(h) providing a third amino acid (AA3); and

(i) coupling the third amino acid (AA3) to the second amino acid to form a peptide bond between the third amino acid (AA3) and the second amino acid (I-AA1-AA2-AA3).

[000340] In one embodiment, the step (i) comprises coupling the carboxylic acid moiety of the AA3 with the amino moiety of the AA2.

[000341] In one embodiment, the third amino acid (AA3) is .

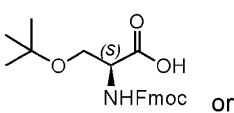
[000342] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

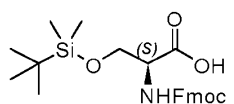
(j) removing the protecting group from the third amino acid (AA3);

(k) providing a fourth amino acid (AA4); and

(l) coupling the fourth amino acid (AA4) to the third amino acid to form a peptide bond between the fourth amino acid (AA4) and the third amino acid (I-AA1-AA2-AA3-AA4).

[000343] In one embodiment, the step (l) comprises coupling the carboxylic acid moiety of the AA4 with the amino moiety of the AA3.

[000344] In one embodiment, the fourth amino acid (AA4) is  or



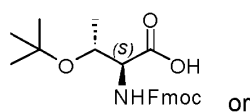
[000345] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

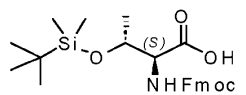
(m) removing the protecting group from the fourth amino acid (AA4);

(n) providing a fifth amino acid (AA5); and

(o) coupling the fifth amino acid (AA5) to the fourth amino acid to form a peptide bond between the fifth amino acid (AA5) and fourth amino acid (I-AA1-AA2-AA3-AA4-AA5).

[000346] In one embodiment, the step (o) comprises coupling the carboxylic acid moiety of the AA5 with the amino moiety of the AA4.

[000347] In one embodiment, the fifth amino acid (AA5) is  or



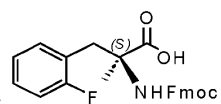
[000348] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

(p) removing the protecting group from the fifth amino acid (AA5);

(q) providing a sixth amino acid (AA6); and

(r) coupling the sixth amino acid (AA6) to the fifth amino acid to form a peptide bond between the sixth amino acid (AA6) and the fifth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6).

[000349] In one embodiment, the step (r) comprises coupling the carboxylic acid moiety of the AA6 with the amino moiety of the AA5.



[000350] In one embodiment, the sixth amino acid (AA6) is

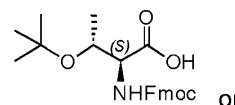
[000351] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

(s) removing the protecting group from the sixth amino acid (AA6);

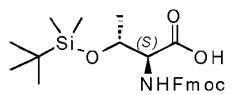
(t) providing a seventh amino acid (AA7); and

(u) coupling the seventh amino acid (AA7) to the sixth amino acid to form a peptide bond between the seventh amino acid (AA7) and the sixth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6-AA7).

[000352] In one embodiment, the step (u) comprises coupling the carboxylic acid moiety of the AA7 with the amino moiety of the AA6.



[000353] In one embodiment, the seventh amino acid (AA7) is



[000354] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

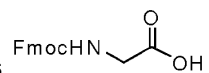
(v) removing the protecting group from the seventh amino acid (AA7);

(w) providing an eighth amino acid (AA8); and

(x) coupling the eighth amino acid (AA8) to the seventh amino acid to form a peptide bond between the eighth amino acid (AA8) and the seventh amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6-AA7-AA8).

[000355] In one embodiment, the step (x) comprises coupling the carboxylic acid moiety of the AA8 with the amino moiety of the AA7.

[000356] In one embodiment, the eighth amino acid (AA8) is



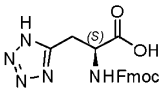
[000357] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

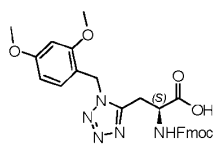
(y) removing the protecting group from the eighth amino acid (AA8);

(z) providing a ninth amino acid (AA9); and

(aa) coupling the ninth amino acid (AA9) to the eighth amino acid to form a peptide bond between the ninth amino acid (AA9) and the eighth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6-AA7-AA8-AA9).

[000358] In one embodiment, the step (aa) comprises coupling the carboxylic acid moiety of the AA9 with the amino moiety of the AA8.

[000359] In one embodiment, the ninth amino acid (AA9) is  or



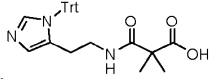
[000360] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

(bb) removing the protecting group from the ninth amino acid (AA9);

(cc) providing a tenth amino acid (AA10); and

(dd) coupling the tenth amino acid (AA10) to the ninth amino acid to form a peptide bond between the tenth amino acid (AA10) and the ninth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6-AA7-AA8-AA9-AA10).

[000361] In one embodiment, the step (dd) comprises coupling the carboxylic acid moiety of the AA10 with the amino moiety of the AA9.

[000362] In one embodiment, the tenth amino acid (AA10) is .

[000363] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

repeating the steps of (i) removing the protecting group from the amino acid (AA_n);

(ii) providing a succeeding amino acid (AA_{n+1}); and

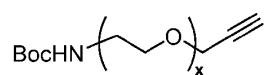
(iii) coupling the succeeding amino acid (AA_{n+1}) to the amino acid (AA_n) to form a peptide bond between the succeeding amino acid (AA_{n+1}) and amino acid (AA_n),

wherein said repeating may be conducted from 1 to 100 times.

[000364] In some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

(ee) removing the protecting group from the tenth amino acid (AA₁₀);

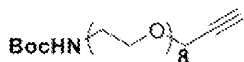
(ff) providing a compound of Formula (B):



wherein x is an integer from 1 to 15; and

(gg) coupling the compound of Formula (B) to the one of the amino acids in the peptide or the peptidomimetic to form a triazole ring.

[000365] In one embodiment, the compound of Formula (B) has the following Formula:



[000366] In some embodiments of the present disclosure, one or more peptides may be attached to the amino acid (AA_n). For example, in some embodiments, the method of making a peptide or a peptidomimetic may include the steps of:

repeating the steps of (i) removing the protecting group from the amino acid (AA_n);

(ii) providing a peptide (AA_{m1}-AA_{mp}), where p is from 2 to 20; and

(iii) coupling the amino on the peptide (AA_{m1}-AA_{mp}) to the amino acid (AA_n) to form a peptide bond between the amino acid on the peptide (AA_{m1}-AA_{mp}) and amino acid (AA_n),

wherein said repeating may be conducted from 1 to 100 times.

[000367] According to the present disclosure, in the peptide (AA_{m1}-AA_{mp}) m may be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, m is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some embodiments, m is 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[000368] According to the present disclosure, the peptide (AA_{m1}-AA_{mp}) may be a dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, or decapeptide.

[000369] The peptides may include all D-amino acids, all L-amino acids, or a mixture of L-amino acids and D-amino acids. In preferred embodiments, the peptide includes only D-amino acids or a mixture of D-amino acids and L-amino acids where the D-amino acid content is greater than 50%, 60%, 70%, 80%, 90%, or 95%.

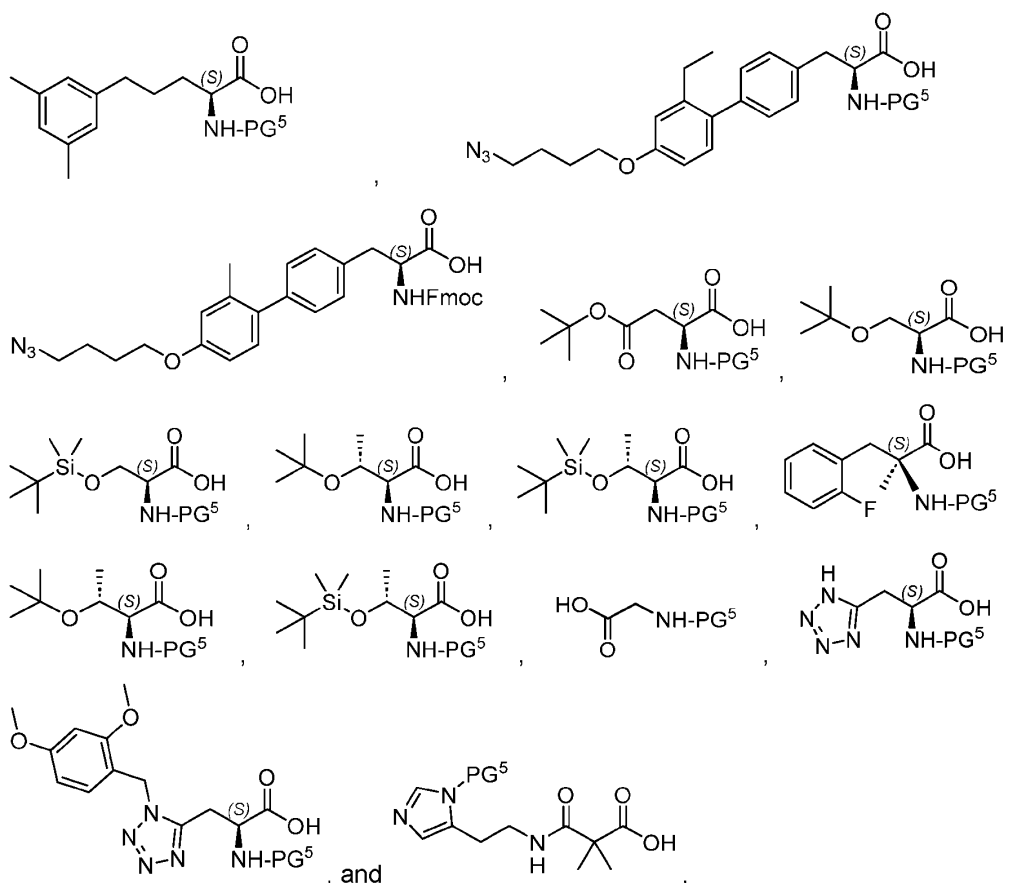
[000370] During the synthesis, the non-participating carboxylic acids or amines on the reacting set of amino acids or peptide fragments may be protected by a suitable protecting group (PG) which may be selectively removed at a later time if desired. A detailed description of these groups and their

selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety.

[000371] In some embodiments, the method of making a peptide or a peptidomimetic according to any of the above embodiments further comprises:

(hh) cleaving the peptide bond between the first amino acid (AA1) and the compound of Formula (I) to obtain the peptide or the peptidomimetic.

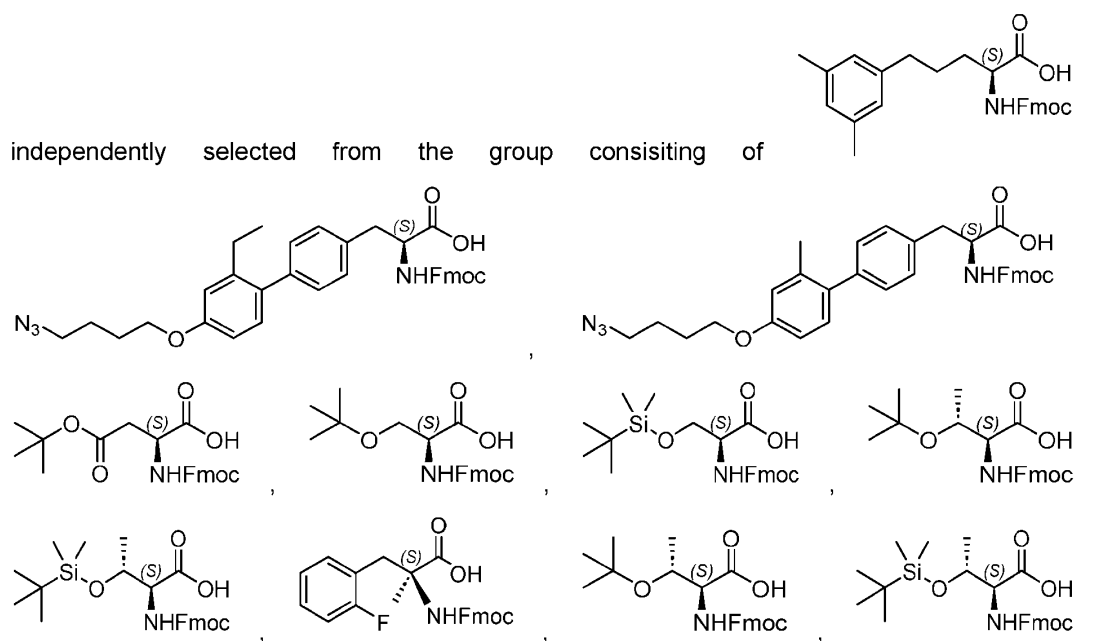
[000372] According to the present disclosure, each one of the first amino acid (AA1), the second amino acid (AA2), the third amino acid (AA3), the fourth amino acid (AA4), the fifth amino acid (AA5), the sixth amino acid (AA6), the seventh amino acid (AA7), the eighth amino acid (AA8), the ninth amino acid (AA9), the tenth amino acid (AA10), and succeeding amino acid (AA_{n+1}) may be independently selected from the group consisting of

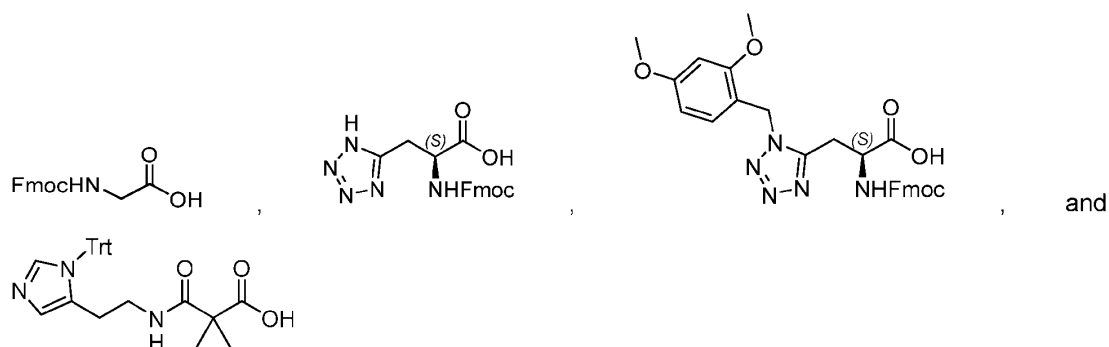


wherein PG⁵ is a suitable protecting group.

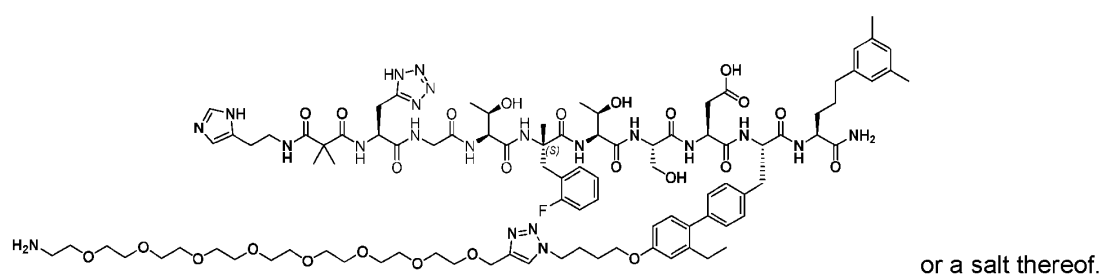
[000373] According to the present disclosure, any suitable protecting group that may be used to protect amino group in amino acids may be used as PG⁵. Suitable protecting groups that may be used are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, PG⁵ may be independently selected at each occurrence from the group consisting of triphenylmethyl (Trt), allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

[000374] According to the present disclosure, each one of the first amino acid (AA1), the second amino acid (AA2), the third amino acid (AA3), the fourth amino acid (AA4), the fifth amino acid (AA5), the sixth amino acid (AA6), the seventh amino acid (AA7), the eighth amino acid (AA8), the ninth amino acid (AA9), the tenth amino acid (AA10), and succeeding amino acid (AA_{n+1}) may be

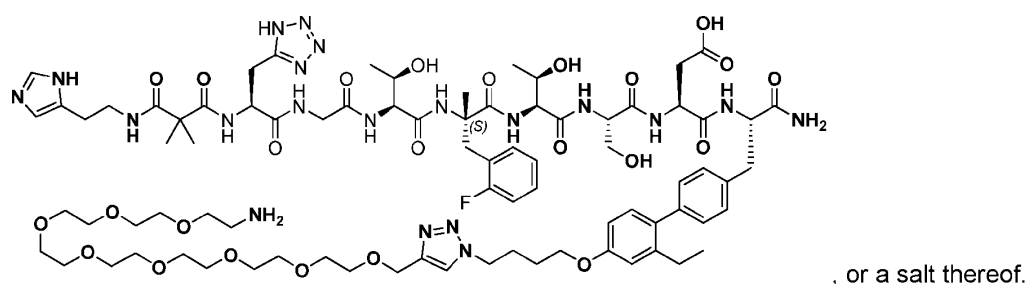




[000375] In one embodiment, the peptidomimetic has a Formula:

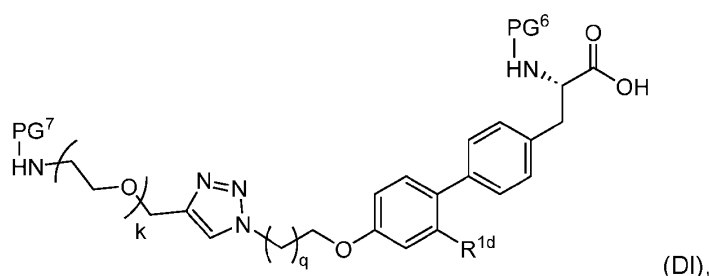


[000376] In one embodiment, the peptidomimetic has a Formula:



[000377] According to the present disclosure, amino acids that may be used to prepare peptides and peptidomimetics may be both natural and unnatural amino acids.

[000378] The present disclosure provides a method for preparation of a compound of Formula (DI):



wherein:

k is an integer from 1 to 15;

q is an integer from 0 to 10;

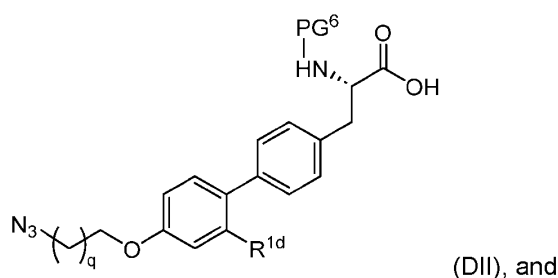
R^{1d} is C₁₋₆ alkyl;

PG⁶ is a suitable protecting group; and

PG⁷ is a suitable protecting group,

or a salt thereof. This method includes:

(a) providing a compound of Formula (DII) having the structure:



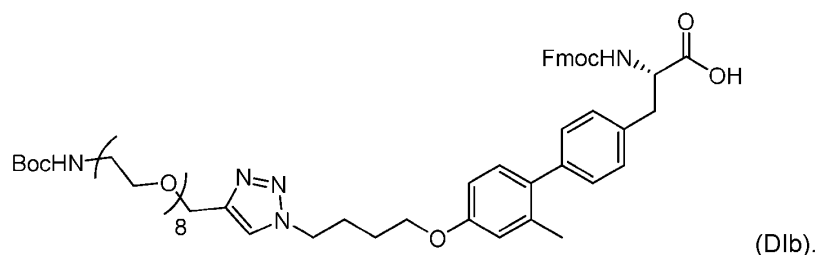
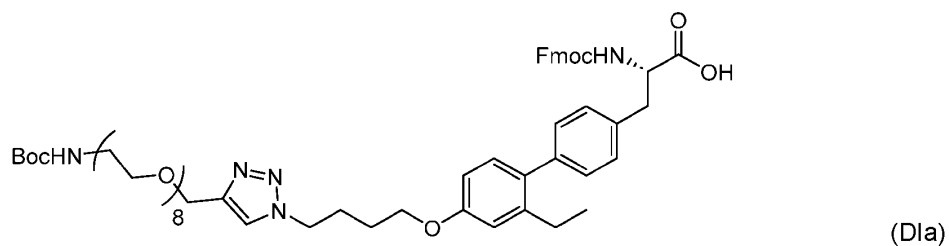
(b) forming the compound of Formula (DI) from the compound of Formula (DII).

[000379] According to the present disclosure, PG⁶ is any suitable protecting group that may be used to protect amino group. Suitable protecting groups that may be used are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, PG⁶ may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc). In some embodiments, PG⁶ is 9-fluorenylmethoxycarbonyl (Fmoc).

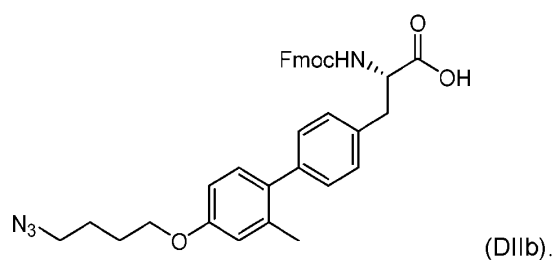
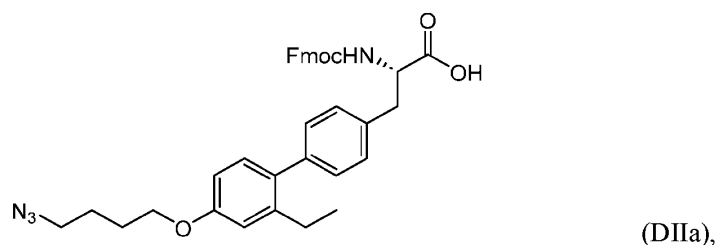
[000380] According to the present disclosure, PG⁷ is any suitable protecting group that may be used to protect amino group. Suitable protecting groups that may be used are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained

in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, PG⁷ may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc). In some embodiments, PG⁷ is tert-butyloxycarbonyl (Boc)

[000381] In one embodiment, the compound of Formula (DI) has the structure of Formula (DIa) or Formula (DIb):

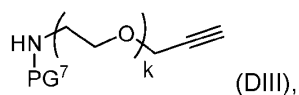


[000382] In another embodiment, the compound of Formula (DII) has the structure of Formula (DIIa) or Formula (DIIb):



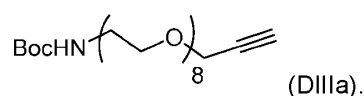
[000383] In some embodiments, the step (b) of forming the compound of Formula (DI) comprises:

reacting the compound of Formula (DII) with a compound of Formula (DIII):



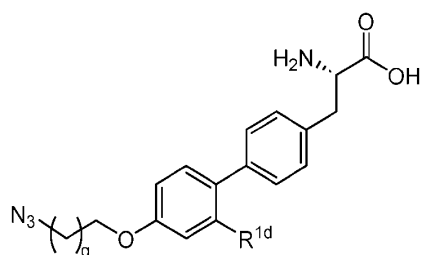
to produce the compound of Formula (II).

[000384] In one embodiment, the compound of Formula (DIII) has the structure of Formula (DIIIa):



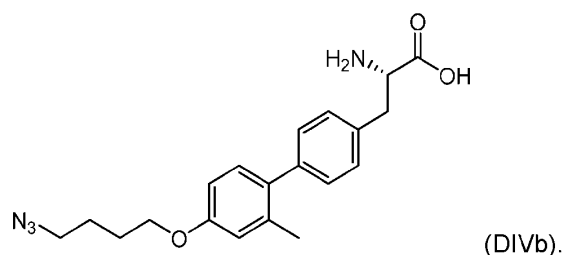
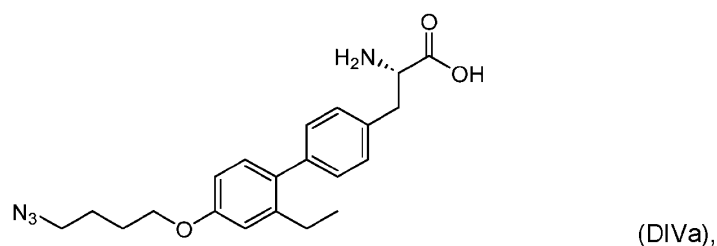
[000385] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

providing a compound of Formula (DIV) having the structure:



forming the compound of Formula (DII) from the compound of Formula (DIV) prior to the step (a).

[000386] In one embodiment, the compound of Formula (DIV) has the structure of Formula (DIVa) or Formula (DIVb):



[000387] In some embodiments, the step of forming the compound of Formula (DII) comprises: reacting the compound of Formula (DIV) with a compound of Formula (DVA) or Formula (DVb):

$\text{PG}^6\text{-X}$ (DVA) or PG^6_2O (DVb),

wherein

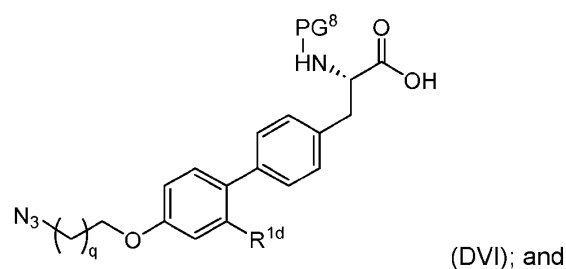
X is OSu, OTf, Cl, or Br;

to produce the compound of Formula (DII).

[000388] According to the present disclosure, the compound of Formula (DVA) or Formula (DVb) may be selected from the group consisting of AllocCl, Alloc₂O, Cbz₂O, CbzCl, FmocCl, FmocOSu, and Boc₂O.

[000389] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

providing a compound of Formula (DVI) having the structure:



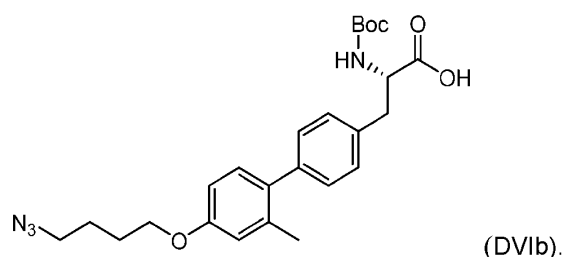
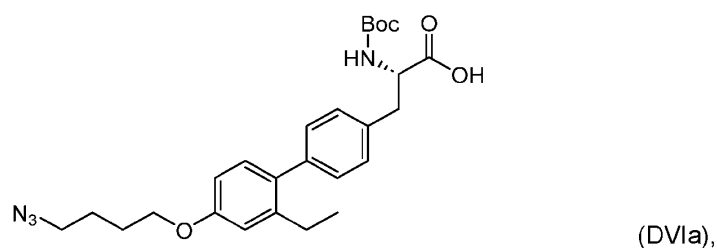
forming the compound of Formula (DIV) from the compound of Formula (DVI).

[000390] In some embodiments, the step of forming the compound of Formula (DIV) comprises:

reacting the compound of Formula (DVI) with a protecting group removal agent to produce the compound of Formula (DIV).

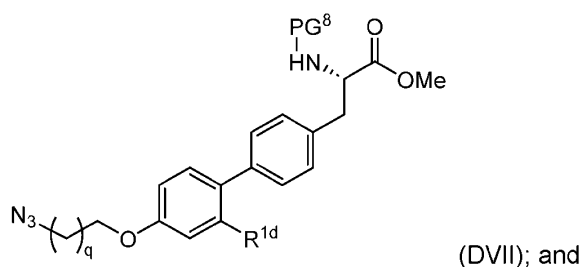
[000391] According to the present disclosure, any suitable protecting group removing agent may be used to remove protecting group PG⁸ from the amino group of the compound of Formula (DVI). Suitable protecting group removing agents that may be used to remove a particular protecting group are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, the protecting group removal agent may be selected from the group consisting of Pd(PPh)₃, PhSiH₃, H₂, HCl, piperidine, and trifluoroacetic acid (TFA).

[000392] In one embodiment, the compound of Formula (DVI) has the structure of Formula (DVIa) or Formula (DVIb):



[000393] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

providing a compound of Formula (DVII) having the structure:



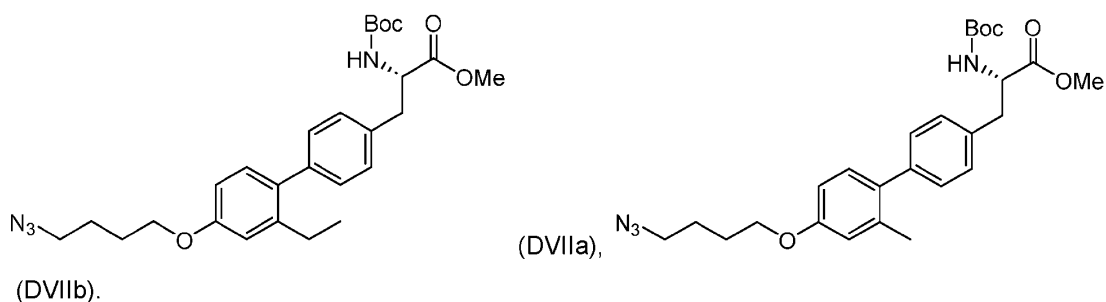
forming the compound of Formula (DVI) from the compound of Formula (DVII).

[000394] In some embodiments, the step of forming the compound of Formula (DVI) comprises:

reacting the compound of Formula (DVII) with a base to produce the compound of Formula (DVI).

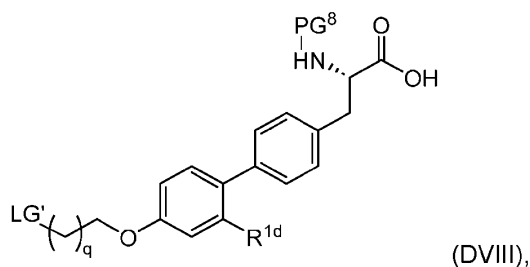
[000395] According to the present disclosure, any suitable base may be used to hydrolyze the methyl ester group in the compound of Formula (DVII). In some embodiments, the base is selected from the group consisting of LiOH, NaOH, and KOH.

[000396] In one embodiment, the compound of Formula (DVII) has the structure of Formula (DVIIa) or Formula (DVIIb):



[000397] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

providing a compound of Formula (DVIII) having the structure:



wherein

LG' is a suitable living group; and

forming the compound of Formula (DVII) from the compound of Formula (DVIII).

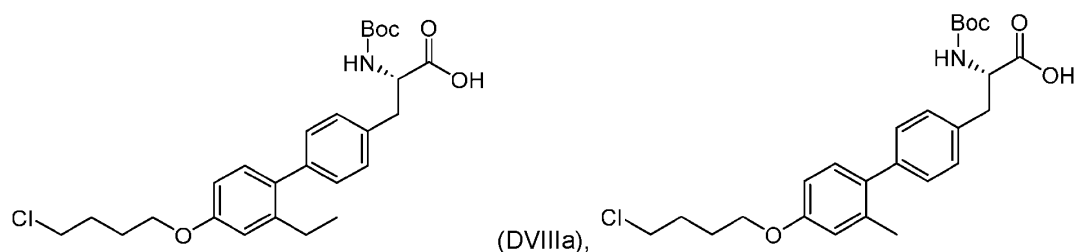
[000398] In some embodiments, the step of forming the compound of Formula (DVII) comprises:

reacting the compound of Formula (DVIII) with an azido group introducing agent to produce the compound of Formula (DVII).

[000399] The azido group introducing agent that may be used to carry out the above embodiment are known to people of skill in the art. In one embodiment, the azido group introducing agent is selected from the group consisting of NaN₃, TMSN₃, (PhO)₂P(O)N₃, Zn(N₃)₂*2Py, and n-Bu₄NN₃.

[000400] In one embodiment, LG' is selected from the group consisting of halogen, OTf, OMs, and OTs.

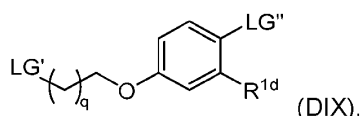
[000401] In another embodiment, the compound of Formula (DVIII) has the structure of Formula (DVIIIa) or Formula (DVIIIb):



(DVIIIb).

[000402] In some embodiments, the process for preparation of a compound of Formula (DI) may include the step of:

providing a compound of Formula (DIX) having the structure:



wherein

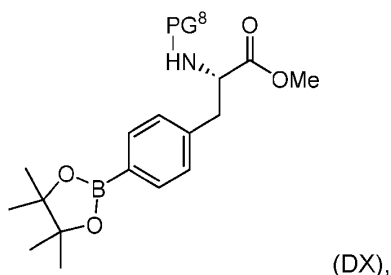
LG'' is a suitable living group; and

forming the compound of Formula (DVIII) from the compound of Formula (DIX).

[000403] In one embodiment, LG'' is selected from the group consisting of halogen, OTf, OMs, and OTs.

[000404] In one embodiment, the step of forming the compound of Formula (DVIII) comprises:

reacting the compound of Formula (DIX) with a compound of Formula (DX):



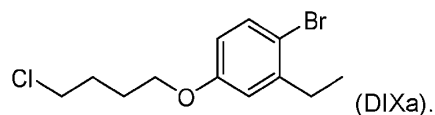
wherein

PG⁸ is a suitable protecting group;

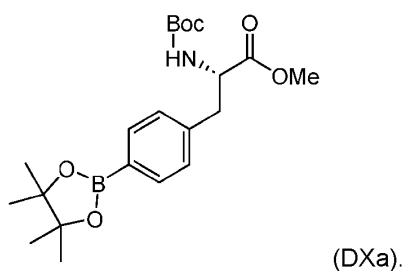
to produce the compound of Formula (DVII).

[000405] According to the present disclosure, PG⁸ is any suitable protecting group that may be used to protect amino group. Suitable protecting groups that may be used are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, PG⁸ may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc). In some embodiments, PG⁸ is tert-butyloxycarbonyl (Boc).

[000406] In another embodiment, the compound of Formula (DIX) has the structure of Formula (DIXa):

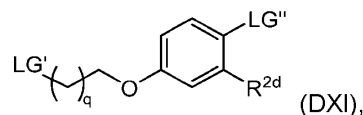


[000407] In yet another embodiment, the compound of Formula (DX) has the structure of Formula (DXa):



[000408] In some embodiments, the process for preparation of a compound of Formula (DI) may include the steps of:

providing a compound of Formula (DXI) having the structure:



wherein

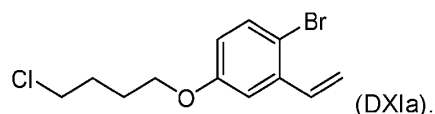
R^{2d} is C_{1-6} alkylene; and

forming the compound of Formula (DIX) from the compound of Formula (DXI).

[000409] In some embodiments, the step of forming the compound of Formula (DIX) comprises reacting the compound of Formula (DXI) with a reducing agent to produce the compound of Formula (DIX).

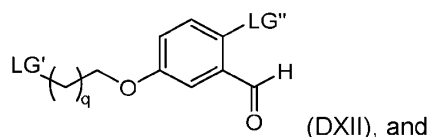
[000410] According to the present disclosure, any reducing agent that is capable of reducing the alkene double bond may be used to prepare the compound of Formula (DIX). In some embodiments, the reducing agent is selected from the group consisting of H_2 , NH_4HCO_2 , $NABH(OAc)_3$, and $LiAlH_4$.

[000411] In one embodiment, the compound of Formula (DXI) has the structure of Formula (DXIa):



[000412] In some embodiments, the process for preparation of a compound of Formula (DI) may include the steps of:

providing a compound of Formula (DXII) having the structure:



forming the compound of Formula (DXI) from the compound of Formula (DXII).

[000413] In some embodiments, the step of forming the compound of Formula (DXI) comprises:

reacting the compound of Formula (DXII) with a compound of Formula (DXIII):

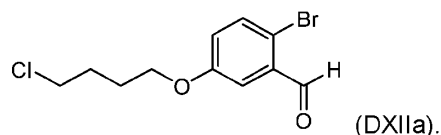
$PPh_3 \cdot C_{1-6} \text{ alkyl-Hal}$ (DXIII),

wherein

Hal is halogen,

to produce the compound of Formula (DXI).

[000414] In one embodiment, the compound of Formula (DXII) has the structure of Formula (DXIIa):

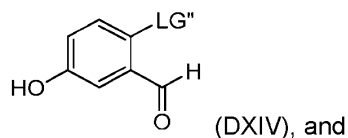


[000415] In another embodiment, the compound of Formula (DXIII) has the structure of Formula (DXIIIa):

$\text{PPh}_3^*\text{MeBr}$ (DXIIIa).

[000416] In some embodiments, the process for preparation of a compound of Formula (DI) may include the steps of:

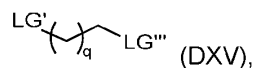
providing a compound of Formula (DXIV) having the structure:



forming the compound of Formula (DXII) from the compound of Formula (DXIV).

[000417] In some embodiments, the step of forming the compound of Formula (DXII) comprises:

reacting the compound of Formula (DXIV) with a compound of Formula (DXV):



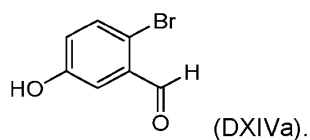
wherein

LG''' is a suitable living group,

to produce the compound of Formula (DXII).

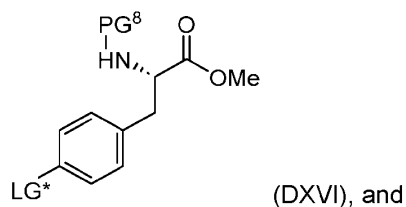
[000418] In one embodiment, the LG''' is selected from the group consisting of halogen, OTf, OM, and OTs.

[000419] In another embodiment, the compound of Formula (DXIV) has the structure of Formula (DXIVa):



[000420] In some embodiments, the process for preparation of a compound of Formula (DI) may include the steps of:

providing a compound of Formula (DXVI) having the structure:



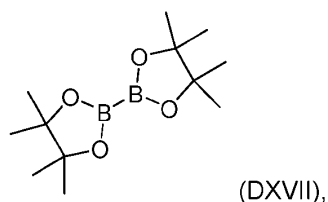
wherein

LG* is a suitable living group,

forming the compound of Formula (DX) from the compound of Formula (DXVI).

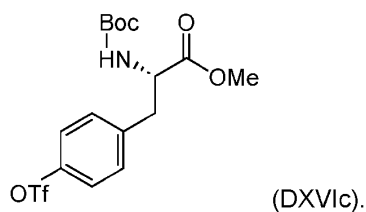
[000421] In one embodiment, LG* is selected from the group consisting of halogen, OTf, OMs, and OTs.

[000422] In some embodiments, the step of forming the compound of Formula (DX) comprises: reacting the compound of Formula (DXVI) with a compound of Formula (DXVII):



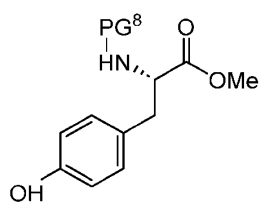
to produce the compound of Formula (DX).

[000423] In one embodiment, the compound of Formula (DXVI) has the structure of Formula (DXVIc):



[000424] In some embodiments, the process for preparation of a compound of Formula (DI) may include the steps of:

providing a compound of Formula (DXVIII) having the structure:



forming the compound of Formula (DXVI) from the compound of Formula (DXVIII).

[000425] In some embodiments, the step of forming the compound of Formula (DXVI) comprises:

reacting the compound of Formula (DXVIII) with a compound of Formula (DXIXa) or Formula (DXIXb):

LG*-X (DXIXa) or LG*₂O (DXIXb),

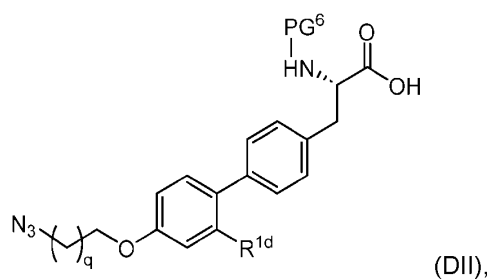
wherein

X is OSu, Cl, or Br;

to produce the compound of Formula (DXVI).

[000426] In one embodiment, the compound of Formula (DXIXa) or Formula (DXIXb) is selected from the group consisting of TfCl, Tf₂O, MsCl, and Ms₂O.

[000427] The present disclosure provides a method for preparation of a compound of Formula (DII):



wherein:

q is an integer from 0 to 10;

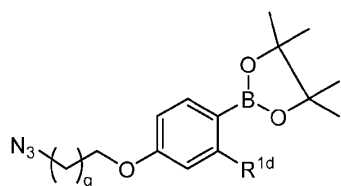
R^{1d} is C₁₋₆ alkyl; and

PG⁶ is a suitable protecting group,

or a salt thereof,

said process comprising:

(a) providing a compound of Formula (DXXI) having the structure:

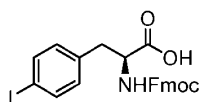


(DXXI) or a salt thereof, and

(b) forming the compound of Formula (DII) from the compound of Formula (DXXI).

[000428] In some embodiments, PG⁶ is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

[000429] In some embodiments, the compound of Formula (DXXI) is treated with compound

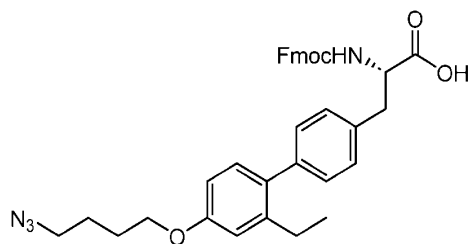


[000430] In some embodiments, the compound of Formula (DXXI) is treated with a catalyst. In some embodiments, the catalyst is Pd(dppf)Cl₂-DCM.

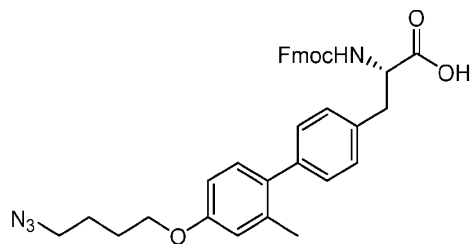
[000431] In some embodiments, the compound of Formula (DXXI) is treated with a base. In some embodiments, the base is K₂CO₃.

[000432] In some embodiments, the preparation is performed in a solvent mixture comprised of H₂O/iPrOH.

[000433] In some embodiments, the compound of Formula (DII) has the structure of Formula (DIIa) or Formula (DIIb):

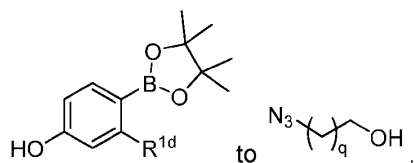


(DIIa),



(DIIb).

[000434] In some embodiments, the compound of Formula (DXXI) is formed by exposing

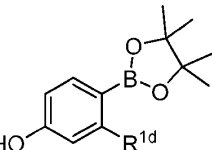
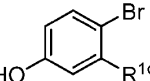


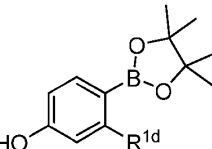
[000435] In some embodiments, the compound of Formula (DXXI) is formed in the presence of PPh_3 .

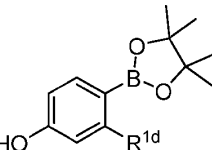
[000436] In some embodiments, the compound of Formula (DXXI) is formed in the presence of DIAD.

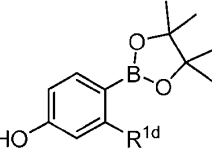
[000437] In some embodiments, the compound of Formula (DXXI) is formed in the presence of molecular sieves of 4Å.

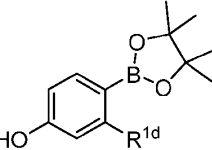
[000438] In some embodiments, the compound of Formula (DXXI) is formed in an organic solvent. In some embodiments, the organic solvent is THF.

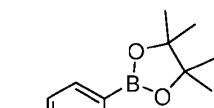
[000439] In some embodiments,  is formed by exposing  to PBin_2 .

[000440] In some embodiments,  is formed in the presence of $\text{Pd}(\text{OAc})_2$.

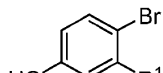
[000441] In some embodiments,  is formed in the presence of PCy_3 .

[000442] In some embodiments,  is formed in the presence of KOAc.

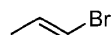
[000443] In some embodiments,  is formed in the presence of dioxane.



[000444] In some embodiments, $\text{HO}-\text{C}_6\text{H}_3(\text{R}^{1d})-\text{B}(\text{OC}(\text{CH}_3)_3)_2$ is formed at an elevated temperature for a period of time.

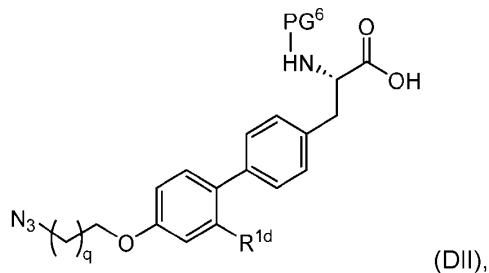


[000445] In some embodiments, $\text{HO}-\text{C}_6\text{H}_3(\text{R}^{1d})-\text{Br}$ is formed by exposing $\text{HO}-\text{C}_6\text{H}_4-\text{R}^{1d}$ to $\text{Bu}_4\text{N}^+\text{Br}_3^-$.



[000446] In some embodiments, $\text{HO}-\text{C}_6\text{H}_3(\text{R}^{1d})-\text{Br}$ is formed in an organic solvent. In some embodiments, the organic solvent is a mixture of DCM and methanol.

[000447] The present disclosure provides a method for preparation of a compound of Formula (DII):



wherein:

q is an integer from 0 to 10;

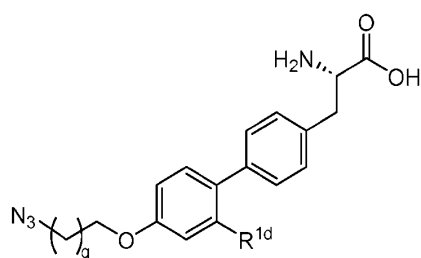
R^{1d} is C_{1-6} alkyl; and

PG^6 is a suitable protecting group,

or a salt thereof,

said process comprising:

(a) providing a compound of Formula (DIV) having the structure:

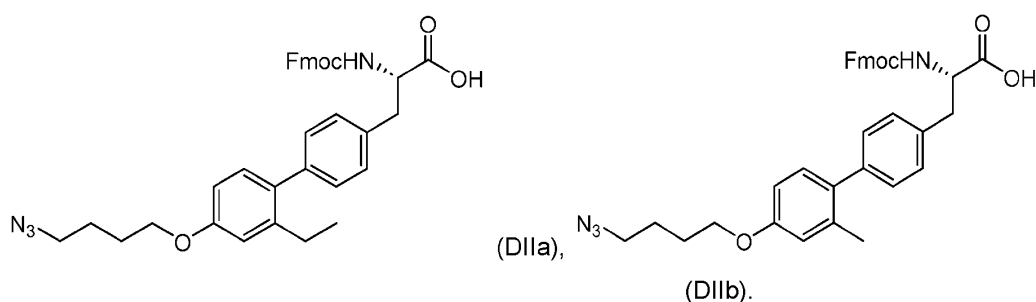


(DIV) or a salt thereof, and

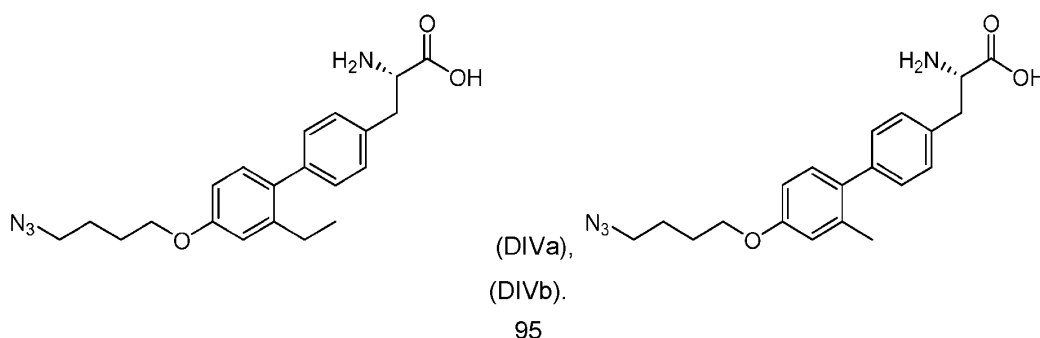
(b) forming the compound of Formula (DII) from the compound of Formula (DIV).

[000448] According to the present disclosure, PG^6 is any suitable protecting group that may be used to protect amino group. Suitable protecting groups that may be used are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, PG^6 may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc). In some embodiments, PG^6 is 9-fluorenylmethoxycarbonyl (Fmoc).

[000449] In one embodiment, the compound of Formula (DII) has the structure of Formula (DIIa) or Formula (DIIb):



[000450] In another embodiment, the compound of Formula (DIV) has the structure of Formula (DIVa) or Formula (DIVb):



[000451] In some embodiments, the process of forming the compound of Formula (DII) comprises:

reacting the compound of Formula (DIV) with a compound of Formula (DVa) or Formula (DVb):

$\text{PG}^6\text{-X}$ (DVa) or PG^6_2O (DVb),

wherein

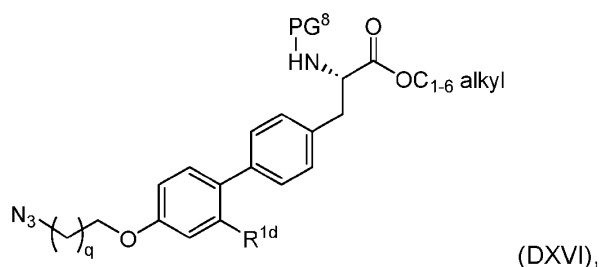
X is OSu, OTf, Cl, or Br;

to produce the compound of Formula (DII).

[000452] According to the present disclosure, the compound of Formula (DVa) or Formula (DVb) is selected from the group consisting of AllocCl, Alloc₂O, Cbz₂O, CbzCl, FmocCl, FmocOSu, and Boc₂O.

[000453] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

providing a compound of Formula (DXVI) having the structure:



wherein

PG^8 is a suitable protecting group; and

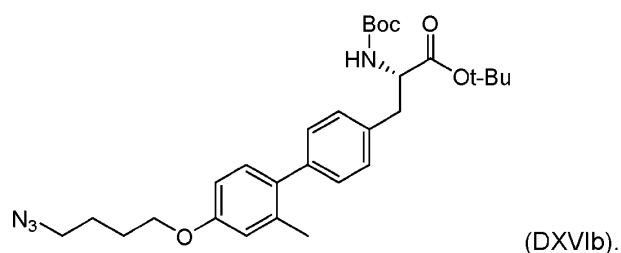
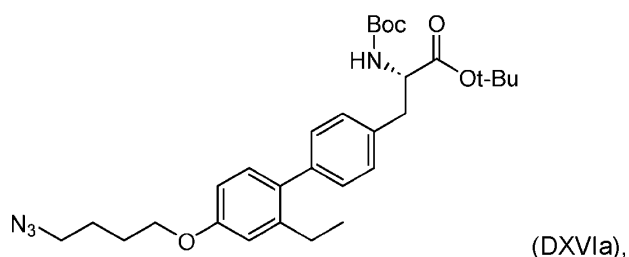
forming the compound of Formula (DIV) from the compound of Formula (DXVI).

[000454] According to the present disclosure, PG^8 is any suitable protecting group that may be used to protect amino group. Suitable protecting groups that may be used are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. For example, PG^8 may be selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc). In some embodiments, PG^8 tert-butyloxycarbonyl (Boc).

[000455] In some embodiments, the step of forming the compound of Formula (DIV) comprises reacting the compound of Formula (DXVI) with a protecting group removal agent to produce the compound of Formula (DIV).

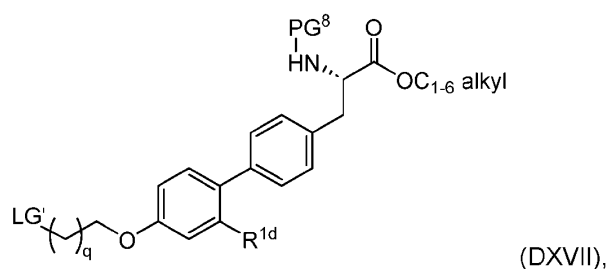
[000456] According to the present disclosure, any suitable protecting group removing agent may be used to remove protecting group PG⁸ from the amino group of the compound of Formula (DVI). Suitable protecting group removing agents that may be used to remove a particular protecting group are known to people of skill in the art. A detailed description of these groups and their selection and chemistry is contained in "The Peptides, Vol. 3", Gross and Meinenhofer, Eds., Academic Press, New York, 1981, which is hereby incorporated by reference in its entirety. In some embodiments, the protecting group removal agent is selected from the group consisting of HCl, Pd(PPh)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

[000457] In one embodiment, the compound of Formula (DXVI) has the structure of Formula (DXVIa) or Formula (DXVIb):



[000458] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

providing a compound of Formula (DXVII) having the structure:



wherein

LG' is a suitable living group; and

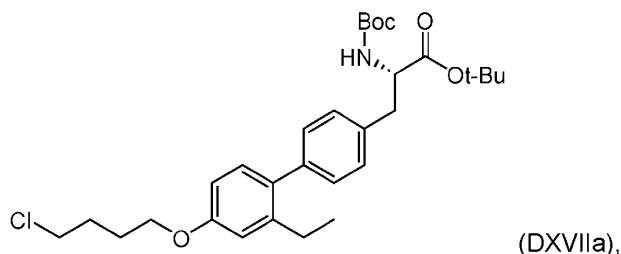
forming the compound of Formula (DXVI) from the compound of Formula (DXVII).

[000459] In some embodiments, the step of forming the compound of Formula (DXVI) comprises reacting the compound of Formula (DXVII) with an azido group introducing agent to produce the compound of Formula (DXVI).

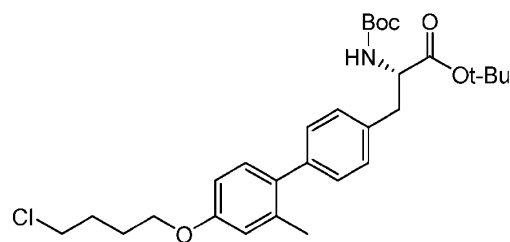
[000460] In some embodiments, azido group introducing agent is selected from the group consisting of NaN_3 , TMSN_3 , $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$, $\text{Zn}(\text{N}_3)_2 \cdot 2\text{Py}$, and $n\text{-Bu}_4\text{NN}_3$.

[000461] In some embodiments, LG' is selected from the group consisting of halogen, OTf, OMs, and OTs.

[000462] In one embodiment, the compound of Formula (DXVII) has the structure of Formula (DXVIIa) or Formula (DXVIIb):



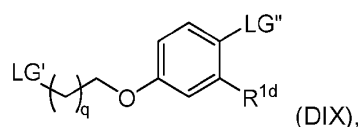
(DXVIIa),



(DXVIIb).

[000463] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

providing a compound of Formula (DIX) having the structure:



(DIX),

wherein

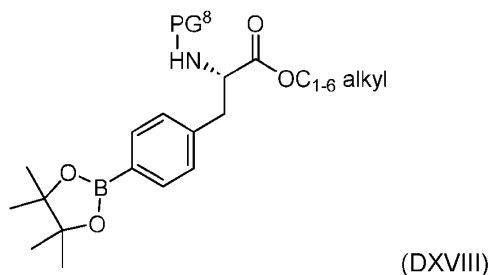
LG'' is a suitable living group; and

forming the compound of Formula (DXVII) from the compound of Formula (DIX).

[000464] In some embodiments, LG'' is selected from the group consisting of halogen, OTf, OMs, and OTs.

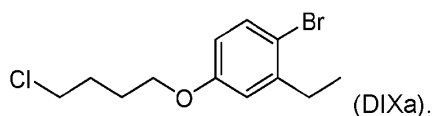
[000465] In some embodiments, the step of forming the compound of Formula (DXVII) comprises:

reacting the compound of Formula (DIX) with a compound of Formula (DXVIII):

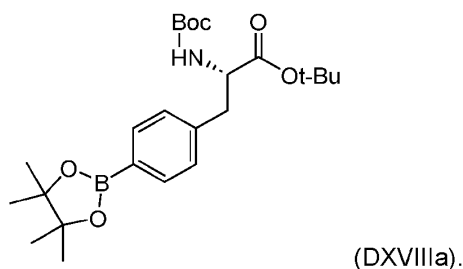


to produce the compound of Formula (DXVII).

[000466] In one embodiment, the compound of Formula (DIX) has the structure of Formula (DIXa):

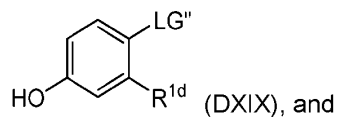


[000467] In another embodiment, the compound of Formula (DXVIII) has the structure of Formula (DXVIIIa):



[000468] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

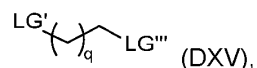
providing a compound of Formula (DXIX) having the structure:



forming the compound of Formula (DIX) from the compound of Formula (DXIX).

[000469] In some embodiments, the step of forming the compound of Formula (DIX) comprises:

reacting the compound of Formula (DXIX) with a compound of Formula (DXV):



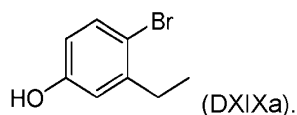
wherein

LG^{viii} is a suitable living group,

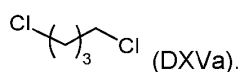
to produce the compound of Formula (DXIX).

[000470] In one embodiment, LG^{viii} is selected from the group consisting of halogen, OTf, OMs, and OTs.

[000471] In another embodiment, the compound of Formula (DXIX) has the structure of Formula (DXIXa):

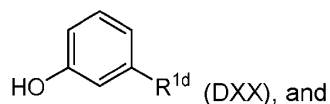


[000472] In another embodiment, the compound of Formula (DXV) has the structure of Formula (DXVa):



[000473] In some embodiments, the process for preparation of a compound of Formula (DII) may include the steps of:

providing a compound of Formula (DXX) having the structure:



forming the compound of Formula (DXIX) from the compound of Formula (DXX).

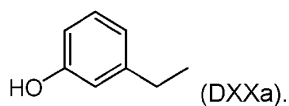
[000474] In some embodiments, the step of

forming the compound of Formula (DXIX) comprises:

reacting the compound of Formula (DXX) with an LG^v introducing agent to produce the compound of Formula (DXIX).

[000475] In one embodiment, the LG^v introducing agent is NBu₄Br₃.

[000476] In another embodiment, the compound of Formula (DXX) has the structure of Formula (DXXa):



Therapeutic Formulations, Administration and Uses

[000477] The present disclosure provides pharmaceutical compositions comprising the peptidomimetic or the compound of the present disclosure.

[000478] The compositions of the disclosure may be formulated with suitable carriers, excipients, and other agents that provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations may be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™, Life Technologies, Carlsbad, CA), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[000479] The dose of a peptidomimetic or a compound administered to a patient may vary depending upon the age and the size of the patient, target disease, conditions, route of administration, and the like. The suitable dose is typically calculated according to body weight or body surface area. When a peptidomimetic or a compound of the present disclosure is used for therapeutic purposes in an adult patient, it may be advantageous to intravenously administer the peptidomimetic or the compound of the present disclosure normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment may be adjusted. Effective dosages and schedules for administering a peptidomimetic or a compound may be determined empirically; for example, patient progress may be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages may be performed using well-known methods in the art (e.g., Mordenti et al., 1991, Pharmaceut. Res. 8:1351).

[000480] Various delivery systems are known and may be used to administer the pharmaceutical composition of the disclosure, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu *et al.*, 1987, J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or

mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration may be systemic or local.

[000481] A pharmaceutical composition of the present disclosure may be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present disclosure. Such a pen delivery device may be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge may readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device may then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[000482] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present disclosure. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present disclosure include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLETTM (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRATM Pen (Abbott Labs, Abbott Park IL), to name only a few.

[000483] In certain situations, the pharmaceutical composition may be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials may be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system may be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984,

in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[000484] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, *etc.* These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, *etc.*, which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [*e.g.*, polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], *etc.* As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, *etc.*, which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, *etc.* The injection thus prepared is preferably filled in an appropriate ampoule.

[000485] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, *etc.* The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Peptidomimetics or Compounds

[000486] In another aspect, the peptidomimetics or compounds (*e.g.*, GLP1 peptidomimetics), disclosed herein are useful, *inter alia*, for the treatment, prevention and/or amelioration of a disease, disorder or condition in need of such treatment.

[000487] In one aspect, the present disclosure provides a method of treating a condition in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a peptidomimetic or compound (*e.g.*, GLP1 peptidomimetic) according to the disclosure, or the composition comprising any compound according to the present disclosure.

[000488] In some embodiments, the peptidomimetics or compounds (*e.g.*, GLP1 peptidomimetics) disclosed herein are useful for treating any disease or disorder in which stimulation, activation and/or targeting of GLP1R would be beneficial. In particular, the compounds

of the present disclosure may be used for the treatment, prevention and/or amelioration of any disease or disorder associated with or mediated by GLP1R expression or activity.

[000489] In some embodiments, the peptidomimetics or compounds (e.g., GLP1 peptidomimetics) disclosed herein are useful for treating a GLP1R-associated condition. In some embodiments, the GLP1R-associated condition is Type 1 or Type 2 diabetes mellitus. The administered peptidomimetic or compound (e.g., GLP1 peptidomimetic) may cause at least one of the following results: induction of insulin secretion, suppression of glucagon release, reduction of blood sugar, improvement of glycemic control, promotion of islet neogenesis, and delay of gastric emptying or potentiation of glucose resistant islets.

[000490] In some embodiments, the GLP1R-associated condition is a neurodegenerative disorder, a cognitive disorder, memory disorder or learning disorder. The neurodegenerative disorder may be, for example, dementia, senile dementia, mild cognitive impairment, Alzheimer-related dementia, Huntington's chorea, tardive dyskinesia, hyperkinesias, mania, Morbus Parkinson, steel-Richard syndrome, Down's syndrome, myasthenia gravis, nerve trauma, brain trauma, vascular amyloidosis, cerebral hemorrhage with amyloidosis, brain inflammation, Friedrich's ataxia, acute confusion disorder, amyotrophic lateral sclerosis, glaucoma and Alzheimer's disease.

[000491] In some embodiments, the GLP1R-associated condition is a liver disease. The liver disease may be, for example, non-alcoholic fatty liver disease (NAFLD), fatty liver, non-alcoholic steatohepatitis (NASH), and cirrhosis.

[000492] In some embodiments, the GLP1R-associated condition is a coronary artery disease. The coronary artery disease may be, for example, cardiomyopathy and myocardial infarction.

[000493] In some embodiments, the GLP1R-associated condition is a kidney disease. The kidney disease may be, for example, hypertension, or chronic kidney failure.

[000494] In some embodiments, the GLP1R-associated condition is an eating disorder. The eating disorder may be, for example, binge eating.

[000495] Without wishing to be bound by theory, the peptidomimetics or compounds (e.g., GLP1 peptidomimetics) disclosed herein may be employed to attenuate the effects of apoptosis-mediated degenerative diseases of the central nervous system such as Alzheimer's Disease, Creutzfeld-Jakob Disease and bovine spongiform encephalopathy, chronic wasting syndrome and other prion mediated apoptotic neural diseases (see, e.g., Perry and Grieg (2004) Current Drug Targets 6:565-571). Administration of a peptidomimetic or compound (e.g., GLP1 peptidomimetic) disclosed herein may also lead to down-modulation of β APP and thereby ameliorate A β mono- or oligomer-mediated pathologies associated with Alzheimer's Disease (see, e.g., Perry et al. (2003) Journal of Neuroscience Research 72:603-612).

[000496] It is also contemplated that the peptidomimetics or compounds (e.g., GLP1 peptidomimetic) disclosed herein may be used to improve learning and memory, for example, by enhancing neuronal plasticity and facilitation of cellular differentiation (see, During et al. (2003) Nature Medicine 9:1173-1179). Further, the compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) disclosed herein may also be used to preserve dopamine neurons and motor function in Morbus Parkinson (see, e.g., Greig et al. (2005) Abstract 897.6, Society for Neuroscience, Washington, D.C.).

[000497] In some embodiments, the peptidomimetics or compounds (e.g., GLP1 peptidomimetics) disclosed herein may also be used to treat a metabolic disorder. The metabolic disorder may be, for example, obesity, dyslipidemia, metabolic syndrome X, and pathologies emanating from islet insufficiency.

[000498] Additional diseases that may be treated by a compound (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) of the present disclosure include autoimmune diseases, in particular, those associated with inflammation, including, but not limited to, autoimmune diabetes, adult onset diabetes, morbid obesity, Metabolic Syndrome X and dyslipidemia. For example, the anti-GLP1R antibody-drug conjugate may be employed as a growth factor for the promotion of islet growth in persons with autoimmune diabetes. The compounds (e.g., an antibody-drug conjugate, a linker-payload and/or a payload) described herein may also be useful in the treatment of congestive heart failure.

[000499] In one aspect, the present disclosure provides a method of selectively targeting an antigen (e.g., GLP1R) on a surface of a cell with a peptidomimetic or compound disclosed herein. In one embodiment, the method of selectively targeting an antigen (e.g., GLP1R) on a surface of a cell with a compound comprises linking the compound to a targeted antibody. In one embodiment, the cell is a mammalian cell. In one embodiment, the cell is a human cell. In one embodiment, the cell is a pancreatic cell or a brain cell.

EXAMPLES

[000500] The following examples illustrate specific aspects of the instant description. The examples should not be construed as limiting, as the examples merely provide specific understanding and practice of the embodiments and their various aspects.

[000501] The abbreviations used in the Examples and throughout the specification are as follows:

Abbreviation	Term
AA1	amino acid 1

Ac	acetyl
ACN	Acetonitrile or CH ₃ CN
Ac ₂ O	acetic anhydride
AcOH	acetic acid
ADC	antibody-drug conjugation
aq.	Aqueous
Ar	argon
BnBr	benzyl bromide
BnOH	benzyl alcohol
Boc	t-butoxycarbonyl
Boc ₂ O	Di-tert-butyl dicarbonate
calcd.	Calculated
CbzCl	benzyl chloroformate
CD	cyclodextrin
CDI	carbonyl diimidazole
CHCl ₃	trichloro-methane
CH ₃ CN	acetonitrile
CH ₃ I	methyl iodide
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
de	diastereomeric excess
DIC	N,N'-Diisopropylcarbodiimide
DIPEA	diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMF-DMA	N,N-dimethyl formamide dimethyl acetal
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
dr	diastereomeric ratio
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee	enantiomeric excess

eq.	equivalent
Et	ethyl
EtOAc	Ethyl acetate
EtOH	ethanol
Et ₃ N	Triethylamine
Fmoc	9-fluorenylmethoxycarbonyl
FmocCl	9-Fluorenylmethyl chloroformate
FmocOSu	N-(9-Fluorenylmethoxycarbonyloxy)succinimide
H ₂	hydrogen
Hv	irradiation with light
HATU	O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
HCl	hydrochloride
HOAt	1-hydroxy-7-azabenzotriazole
HOBt (HOBT)	1-hydroxybenzotriazole
HOSu	N-hydroxysuccinimide
HPLC	high-pressure liquid chromatography
hr	hour
HRMS	High-resolution mass spectrometry
I ₂	iodine
IPA	isopropyl alcohol
K ₂ CO ₃	potassium carbonate
KHSO ₄	potassium bisulfate
KI	potassium iodide
KOAc	potassium acetate
KOH	potassium hydroxide
LCMS	Liquid chromatography–mass spectrometry
LiHMDS	lithium hexamethyldisilazide
LiI	lithium iodide
LiOH·H ₂ O	lithium hydroxide monohydrate
Me	methyl
MPM (PMB)	p-methoxybenzyl

Ms	mesyl (methanesulfonyl)
MS	mass spectrometry
4A MS	4A molecular sieves
MTBE	methyl tert-butyl ether
MW	Molecule weight
MeOH	methanol
MP	Melting Point
N ₂	nitrogen
NaClO	sodium hypochlorite
NaH	sodium hydride
NaOH	sodium hydroxide
NaHCO ₃	sodium bicarbonate
NaN ₃	sodium azide
Na ₂ SO ₄	sodium sulfate
NBS	N-bromosuccinimide
NCS	N-chlorosuccinimide
NH ₄ Cl	ammonium chloride
NH ₄ HCO ₃	ammonium bicarbonate
NIS	N-iodosuccinimide
NMO	N-methylmorpholine oxide
NMP	N-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance
PBr ₃	phosphorus tribromide
Pd/C	palladium on activated carbon
Pd(OH) ₂ /C	palladium hydroxide on activated carbon
Pd(PPh ₃) ₄	tetrakis(triphenylphosphine)palladium
PE	petroleum ether
PEG	polyethylene glycol
Ph	phenyl
Ph ₃ PCH ₃ Br	methyltriphenylphosphonium bromide
PMB-Cl	4-Methoxybenzylchloride
Pr	propyl

Prep-HPLC	preparative high-pressure liquid chromatography
Prep-TLC	preparative thin Layer Chromatography
prep-SFC	preparative supercritical fluid chromatography
psi	pounds per square inch
PTSA (or TsOH)	p-toluenesulfonic acid
Py (pyr)	pyridine
PyBOP	Benzotriazole-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
PE	Petroleum ether
MBHA Resin	MBHA resin (0.3~0.8mmol/g, 100~200 mesh, 1% DVB)
r.t.	room temperature
RT	Retention Time
Rf	retention factor in chromatography
SC CO ₂	supercritical carbon-dioxide
SiO ₂	silicon dioxide
SPPS	solid phase peptide synthesis
t-Bu (tBu)	tert-butyl
t-BuOMe (MTBE, TBME)	Methyl tert-butyl ether
TBAF	tetra-n-butylammonium fluoride
TBAI	tetra-n-butylammonium iodide
TBDMS (TBS)	t-butyldimethylsilyl
TBDPS (BPS)	t-butyldiphenylsilyl
TEA	triethylamine
TES	triethylsilyl
TFA	trifluoroacetic acid
Tfa	trifluoroacetamide
Tf ₂ O	trifluoromethanesulfonic anhydride
THF	tetrahydrofuran
TIPS (TIS)	triisopropylsilane
TLC	thin Layer Chromatography
TMEDA	N,N,N',N'-tetramethylethylenediamine
TMS	trimethylsilyl

TMSA	trimethylsilyl azide
TMSN ₃	trimethylsilyl azide
Tol	p-tolyl
TPP	triphenylphosphine
Tr (Trt)	trityl (triphenylmethyl)
TrtCl	triphenylMethyl chloride
Ts (Tos)	p-toluenesulfonyl
UV	ultraviolet
ZnBr ₂	Zinc Bromide

Example 1. General Methods

[000502] All anhydrous reactions were carried out in oven-dried glassware under an argon atmosphere with agitator or magnetic stirring, unless otherwise stated. Rink Amide Linker was sourced from GL Biochem. All other reagents and anhydrous solvents were purchased from commercial sources and were used without any further purification unless otherwise stated. Reactions (where possible) were monitored using thin layer chromatography (TLC) analysis on plates pre-coated with Silica Gel 60 F254 and were visualized by short wave ultra-violet irradiation (254 nm; where applicable), stained with PMA (5% wt/v) in EtOH or Ninhydrin (5% wt/v) in EtOH, followed by charring at ~200 °C. Unless otherwise specified, column chromatography (silica gel 60, 40 mm) was performed in air and under pressure (0.1-0.3 bar). Bulk solvents (EtOAc, hexane, THF, DCM) for flash chromatography were distilled prior to use. IR spectra were recorded with a FT-IR spectrometer (Thermo NICOLET iS10) using a diamond head sampling and are reported in wavenumbers (cm⁻¹). Residual Solvent report was recorded with GC (SHIMADZU-GC2030 & HS-20). Water contents were recorded on a Mettler toledo C30s Karl Fischer reaction instrument. Melting points (MP) were measured via DSC (Differential Scanning Calorimeter, DSC 214). Inorganic salt report was provided by a Thermo ICS-6000 ion chromatography detector. Purities of synthetic intermediates after chromatographic purification were judged to be >90% by analysis of ¹H NMR spectra. Purities of final compounds were ≥95% (NMR analysis), after trituration with MeOH and MeCN (Honeywell, HPLC grade).

General NMR Methods and Instruments

[000503] ¹H NMR and ¹³C: ¹H NMR spectra were recorded on a Bruker Avance 400 (400 MHz, ¹H, 100 MHz, ¹³C) spectrometer. Chemical shift values (δ) are reported in ppm relative to residual chloroform (δ 7.26 ppm for ¹H; δ 78.0 ppm for ¹³C). Multiplicities are indicated by s (singlet), d

(doublet), t (triplet), q (quartet), p (pentet), h (heptet), dd-doublet of doublets, dt-doublet of triplets, dq-doublet of quartets, m (multiplet), and br (broad). The identification of ^1H and ^{13}C signals was achieved using a combination of ^1H , ^{13}C , DEPT, COSY, HMBC, HMQC, and NOESY experiments. Coupling constants (J) are reported in Hertz (Hz).

Example 2. General LC-MS Methods and Instruments

Method 1

[000504] This method was used for analysis of Intermediates **1**, **2**, **3**, **4**, **5**, **6** and **7** of Tag5. Instrument: Agilent1200+6110MS (Table 1). A Xbridge Protein BEH C4 300A 2.5um 4.6×50mm column was used for chromatography. Diode array (DAD) and evaporative light scattering (ELSD) were used as detection methods. MS mode was positive electrospray ionization. MS range was 50-1500. Mobile phase A was 0.04% TFA in water, and mobile phase B was 0.02% TFA in HPLC grade ACN. The gradient was 50-100% B in 6 min. 50% B in 0.00 min, 50-100% B at 0.00-4.00 min with a hold at 100%B for 1.5min, 100-50% B at 5.51 -6.00min with a hold at 50% B for 0.49 min. The flow rate was 1.50 mL/min.

Table 1. Conditions for LC-MS Method 1

Parameter	Value					
Instrument:	Agilent 1200&6110&SEDEX85					
Column:	Xbridge Protein BEH C4 300 ^a 2.5um 4.6×50mm					
Column temperature:	40°C					
Mobile phase A(MPA)	H ₂ O+0.04 %(v/v) TFA					
Mobile phase B(MPB)	ACN+0.02 %(v/v) TFA					
Flow rate:	1.5 mL/min (0.00-6.00)					
Gradient Ratio:	Time (min)	0	4	5.5	5.51	6
	MPA (%)	50	0	0	50	50
	MPB (%)	50	100	100	50	50
Detection:	ELSD					
MS Mode:	Positive					
MS Range:	50-1500 Da					

Method 2

[000505] This method was used for analysis of Intermediate **8** of Tag5. Instrument: Waters H Class & SQD2 (Table 2). The gradient was 50-100% B in 3.40 min and held at 100%B at 3.40-3.85min, 100-5% B in 0.01min, and then held at 5% for 0.64min. The flow rate was 0.80 ml/min.

Mobile phase A was H₂O+10mM NH₄HCO₃ and mobile phase B was HPLC grade THF. The column used for chromatography was a 2.1*50mm Xbridge Shield RPC18 column (5um particles). Diode array (DAD) detection as well as positive electrospray ionization were used as detection methods. MS range was 100-3000Da.

Table 2. Conditions for LC-MS Method 2

Parameter	Value			
Instrument:	Waters H Class & SQD2			
Column:	Xbridge Shield RP18 2.1*50mm,5um			
Column temperature:	40°C			
Mobile phase A(MPA)	H ₂ O+10mM NH ₄ HCO ₃			
Mobile phase B(MPB)	THF			
Flow rate:	0.8 mL/min			
Gradient Ratio:	Time(min)	0.00	0.40	3.40
	MPA (%)	50	50	0
	MPB (%)	50	50	100
Detection:	383 nm			
MS Mode:	Positive			
MS Range:	100-3000Da			

Method 3

[000506] This method was used for analysis of Intermediate **9** and **Tag5**. Instrument: Waters H Class & SQD2 (Table 3). The gradient was 70-100% B in 16.00 min and held at 100%B in 4.00 min, 100-70% B in 0.01min, and then held at 70% for 3 min, the flow rate was 1.0 ml/min. Mobile phase A was 10mM ammonium bicarbonate and mobile phase B was HPLC grade THF. The column used for chromatography was a Xbridge C18 4.6*150mm column (3.5um particles). Diode array (DAD) as well as positive electrospray ionization were used as detection methods. MS range was 100-3000Da.

Table 3. Conditions for LC-MS Method 3

Parameter	Value
Instrument:	Waters H Class & SQD2
Column:	Xbridge C18 4.6*150mm,3.5um
Column Temperature:	40°C
Mobile phase A(MPA)	H ₂ O+10mM NH ₄ HCO ₃

Mobile phase B(MPB)	THF					
Flow rate:	1.0 mL/min					
Gradient Ratio:	Time(min)	0.00	16.00	20.00	20.01	23.00
	MPA (%)	30	0	0	30	30
	MPB (%)	70	100	100	70	70
Detection:	220 nm					
MS Mode:	Positive					
MS Range:	100-3000					

Method 4

[000507] This method was used for analysis of **Tag1-TCPs (Tag5-1AA)**. LC-MS Conditions: Mobile Phase: 1.5ML/4LTFA in water (solvent A) and 0.75ML/4LTFA in ACN (solvent B), using the elution gradient 50%-100% (solvent B) over 1.35 minutes and holding at 100% for 0.9 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1*30mm, 3um.

Method 5

[000508] This method was used for analysis of **Tag1-2AA, Tag1-3AA, Tag1-4AA, Tag1-5AA, Tag1-6AA, Tag1-7AA, Tag1-10AA**. LC-MS Conditions: Mobile Phase: 1.5ML/4LTFA in water (solvent A) and 0.75ML/4L TFA in ACN (solvent B), using the gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. Column: Xtimate C18 2.1*30mm, 3um.

Method 6

[000509] This method was used for analysis of **M2**. LC-MS conditions: Reverse phase LCMS was carried out using a Chromolith Flash RP-C18 25-3mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Method 7

[000510] This method was used for analysis of **Tag5-2AA, Tag5-3AA, Tag5-4AA, Tag5-5AA, Tag5-6AA, Tag5-7AA, Tag5-8AA, Tag5-9AA, and Tag5-10AA**. LCMS condition (BF): Mobile Phase: 1.5mL/4L TFA in water (solvent A) and 0.75mL/4L TFA in ACN (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: Xtimate C18 2.1*30mm 3um; Wavelength: UV 220nm, 254nm; Column temperature: 50°C; MS ionization: ESI.

Method 8

[000511] This method was used for analysis of **Tag5-2AA**, **Tag5-3AA**, **Tag5-4AA**, **Tag5-5AA**, **Tag5-6AA**, **Tag5-7AA**, **Tag5-8AA**, **Tag5-9AA**, and **Tag5-10AA**. LCMS condition (CR): Mobile Phase: 1.5mL/4L TFA in water (solvent A) and 0.75mL/4L TFA in ACN (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: NanoChrom ChromCore 120 C18 3um, 3.0*30mm; Wavelength: UV 220nm, 254nm; Column temperature: 50°C; MS ionization: ESI.

Method 9

[000512] This method was used for analysis of **Tag5-2AA**, **Tag5-3AA**, **Tag5-4AA**, **Tag5-5AA**, **Tag5-6AA**, **Tag5-7AA**, **Tag5-8AA**, **Tag5-9AA**, and **Tag5-10AA**. LCMS condition (BV): Mobile Phase: 1.5mL/4L TFA in water (solvent A) and 0.75mL/4L TFA in ACN (solvent B), using the elution gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8 ml/min; Column: NanoChrom ChromCore 120 C18 3um, 3.0*30mm; Wavelength: UV 220nm; Column temperature: 50°C; MS ionization: ESI.

Method 10

[000513] This method was used for analysis of **Tag5-2AA**, **Tag5-3AA**, **Tag5-4AA**, **Tag5-5AA**, **Tag5-6AA**, **Tag5-7AA**, **Tag5-8AA**, **Tag5-9AA**, and **Tag5-10AA**. LCMS condition (BK): Instrument & column: Xtimate 3um, C18, 2.1*30mm S/N3U411701820 Mobile Phase: 1.5mL/4L TFA in water (solvent A) and 0.75mL/4L TFA in ACN (solvent B), using the gradient 10%-80% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode; Wavelength 220nm&254nm, Oven Temperature 50°C.

Method 11

[000514] This method was used for analysis of **Tag4-10AA**. HPLC method A: Column: YMC-Pack ODS-A 150*4.6mm, 5um; 2.75ML/4L TFA in water (solvent A) and 2.5ML/4LTFA in ACN (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

Method 12

[000515] This method was used for analysis of **Tag4-10AA**. LCMS condition: Reverse phase LCMS was carried out using a Chromolith Flash RP-C18 25-3mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04%TFA (solvent A).

Method 13

[000516] This method was used for analysis of intermediate **AA10-AA9-AA8-OH**. LC-MS Conditions: Mobile Phase: 1.5mL/4LTFA in water (solvent A) and 0.75mL/4LTFA in ACN (solvent

B), using the elution gradient 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

Method 14

[000517] This method was used for analysis of **DMB-AA9**. Reverse phase HPLC was carried out using a Ultimate XB- C18, 3um, 3.0*50mm column, Mobile phase: 1.0% ACN in water (0.1%TFA) to 5% ACN in water (0.1%TFA) in 1 min; then from 5% ACN in water (0.1%TFA) to 100% ACN (0.1%TFA) in 5 minutes; hold at 100% ACN (0.1%TFA) for 2 minutes; back to 1.0% ACN in water (0.1%TFA) at 8.01min, and hold two minutes. Flow rate: 1.2ml/min.

Method 15

[000518] This method was used for analysis of **M2**. HPLC conditions: Mobile phase: 1.0% ACN in water (0.1%TFA) to 5% ACN in water (0.1%TFA) in 1 minutes; then from 5% ACN in water (0.1%TFA) to 100% ACN (0.1%TFA) in 5 minutes; hold at 100% ACN (0.1%TFA) for 2 minutes; back to 1.0% ACN in water (0.1%TFA) at 8.01 minutes and hold two minutes. Flow rate: 1.2ml/min. Column: Ultimate XB-C18, 3um, 3.0*50mm.

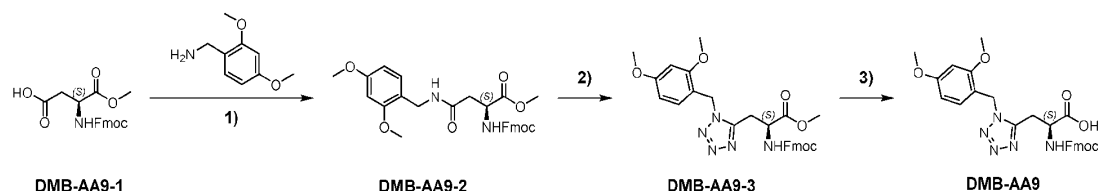
ELSD LCMS Conditions

[000519] Mobile Phase: 10 mM NH₄OAc in water (solvent A) and ACN (solvent B), using the elution gradient 10%-80% (solvent B) over 5 minutes, holding at 80% for 1.95 minutes; changed 80% B to 10% B at 7 min, holding at 10% for 4 minutes at a flow rate of 0.8 mL/min. Column: Shim-pack Scepter C18-120 1.9 μm, 2.1*100 mm.

Example 3. Synthesis of DMB-AA9

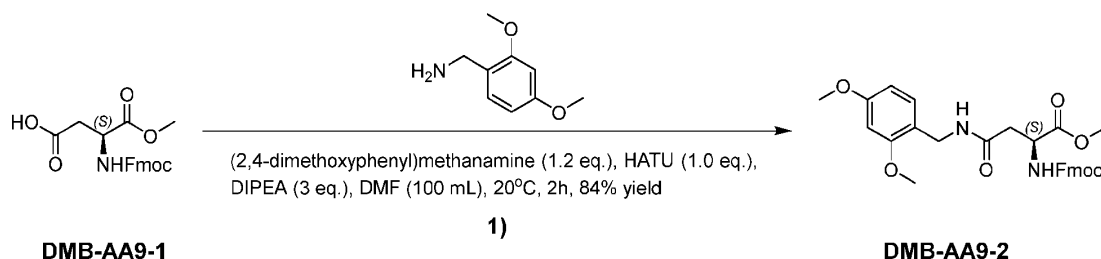
[000520] Scheme 1, below, depicts synthesis of DMB-AA9:

Scheme 1



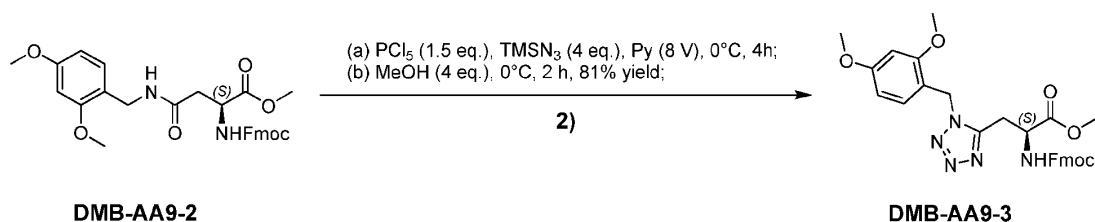
Conditions: 1) (2,4-dimethoxyphenyl)methanamine (1.2 eq.), HATU (1.0 eq.), DIPEA (3 eq.), DMF (100 mL), 20°C, 2h, 84% yield; 2) (a) PCl₅ (1.5 eq.), TMSN₃ (4 eq.), pyridine (8 V), 0°C, 4h; (b) MeOH (4 eq.), 0°C, 2 h, 81% yield; 3) (a) NaOH (3 eq.), THF/H₂O (2:1, 10 mL), 20°C, 2h; (b) FmocOSu (1 eq.), aq. NaHCO₃, 20°C, 2h; 40.5% yield.

Step 1: Synthesis of Methyl (2S)-4-[(2,4-Dimethoxyphenyl)methylamino]-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-oxo-butanoate (DMB-AA9-2)



[000521] To a solution of (3S)-3-(9H-fluoren-9-ylmethoxycarbonylamino)-4-methoxy-4-oxobutanoic acid (**DMB-AA9-1**, 90 g, 243.66 mmol, 1 eq.) in DMF (200 mL) were added HATU (101.91 g, 268.03 mmol, 1.1 eq.), and DIPEA (62.98 g, 487.32 mmol, 84.88 mL, 2 eq.), and the mixture was stirred at 20°C for 30 min. Then (2,4-dimethoxyphenyl)methanamine (44.82 g, 268.03 mmol, 40.37 mL, 1.1 eq.) was added, and the mixture was stirred at 20°C for 1 hr. Most of the reactant was consumed on TLC (PE/EtOAc = 2/1) and the desired compound was detected according to LCMS. The reaction mixture was poured into DCM (1500 mL), washed with H₂O (900 mL) and saturated citric acid (100 mL). The separated organic layer was washed with brine (1000 mL), dried over Na₂SO₄, and filtered. Then it was concentrated under reduced pressure to give the crude product as a light-yellow solid. The crude product was triturated with EtOAc (600 mL) for 12 hr and filtered to give the target compound. The desired compound **DMB-AA9-2** (109 g, 197.59 mmol, 81.09% yield, 94% purity) was obtained as a white solid. ESI-MS (Method 10): RT = 3.896 min, mass calcd. For C₂₉H₃₀O₇N₂H 519.2, m/z found 519.1 [M+H]⁺. ¹H NMR (400MHz, Chloroform-d) δ = 7.76 (d, J=7.5 Hz, 2H), 7.61 (br dd, J=3.6, 6.9 Hz, 2H), 7.44 - 7.37 (m, 2H), 7.36 - 7.28 (m, 2H), 7.15 (d, J=8.3 Hz, 1H), 6.48 - 6.38 (m, 2H), 6.15 (br s, 2H), 4.60 (br s, 1H), 4.44 - 4.26 (m, 4H), 4.26 - 4.19 (m, 1H), 3.84 - 3.72 (m, 9H), 2.97 (br dd, J=4.4, 15.7 Hz, 1H), 2.73 (br dd, J=3.9, 15.7 Hz, 1H).

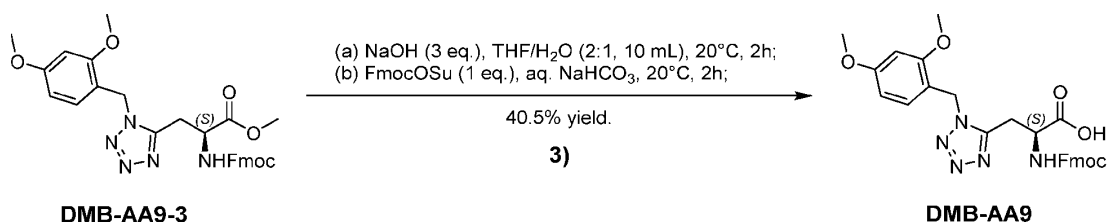
*Step 2: Synthesis of Methyl (2S)-3-[1-[(2,4-Dimethoxyphenyl)methyl]tetrazol-5-yl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoate (**DMB-AA9-3**)*



[000522] To a suspension of compound **DMB-AA9-2** (100 g, 192.84 mmol, 1 eq.) in pyridine (800 mL) was added TMSN₃ (88.87 g, 771.37 mmol, 101.45 mL, 4 eq.), followed by addition of PCl₅ (60.24 g, 289.26 mmol, 1.5 eq.) portion wise. The temperature was kept below 0°C by using ice-brine bath. The reaction mixture was stirred at 0°C for 5hr. LCMS showed that most of the reactant

was consumed. Then MeOH (24.71 g, 771.37 mmol, 31.21 mL, 4 eq.) was added dropwise and the reaction mixture was stirred at 0°C for 1 hr. TLC (PE/EtOAc = 2/1) and LCMS showed that most of the reactant was consumed, and the desired compound was detected as a main peak. The reaction was quenched with MeOH (20 mL) and sat. aq. NaHCO₃ (1000 mL×2), and extracted with EtOAc (1000 mL×3). The aqueous layers were quenched by 8-10% NaClO (1000 mL). The combined organic layers were concentrated under reduced pressure to give a residue, which was re-dissolved in EtOAc (2000 mL) and washed with saturated citric acid (500 mL×2) and brine (1000 mL), dried over Na₂SO₄, and concentrated under reduced pressure to give a residue. The crude product was used directly in the next step without any further purification. The desired compound **DMB-AA9-3** (97 g, 156.86 mmol, 81.34% yield, 87.9% purity) was obtained as a yellow oil. LC-MS: RT= 4.310 min, mass calcd. for C₂₉H₂₉N₅O₆H 544.2[M+H]⁺, m/z found 544.2 [M+H]⁺, Method 10. ¹H NMR (400MHz, Chloroform-d) δ = 7.68 (d, J=7.6 Hz, 2H), 7.48 (br d, J=7.4 Hz, 2H), 7.37 - 7.25 (m, 3H), 7.25 - 7.20 (m, 2H), 7.09 (d, J=8.3 Hz, 1H), 6.43 - 6.31 (m, 2H), 6.09 (br d, J=8.5 Hz, 1H), 4.90 - 4.75 (m, 1H), 4.36 - 4.22 (m, 2H), 4.20 - 4.10 (m, 1H), 3.71 - 3.63 (m, 9H), 3.54 - 3.41 (m, 1H), 3.26 (dd, J=4.6, 16.3 Hz, 1H) ppm.

Step 3: Synthesis of (2S)-3-[1-[(2,4-Dimethoxyphenyl)methyl]tetrazol-5-yl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic Acid (DMB-AA9)



[000523] To a solution of compound **DMB-AA9-3** (95 g, 174.77 mmol, 1 eq) in THF (600 mL) and H₂O (100 mL) were added NaOH (20.97 g, 524.31 mmol, 3 eq) and the reaction mixture was stirred at 20 °C for 2 hr. LCMS showed that the reactant was consumed. The pH of the reaction mixture was adjusted to pH 7-8 using citric acid and the mixture was diluted with H₂O (200 mL), and then extracted with TBME (300 mL×2). To the aqueous layer was added NaHCO₃ (44.05 g, 524.31 mmol, 20.39 mL, 3 eq) and FmocOSu (58.96 g, 174.77 mmol, 1 eq) in THF (600 mL), then the reaction mixture was stirred at 10°C for 12 hr. LCMS showed that the desired compound was detected as a main peak. The reaction was concentrated under reduced pressure to remove THF, then poured into saturated NaHCO₃ (500 mL) and H₂O (300 mL), and extracted with TBME (500 mL×3). The aqueous layer was extracted with EtOAc/MeOH (10/1, 800 mL×3). The combined EtOAc layers were washed with saturated NaHCO₃ (200 mL) and brine (200 mL), then dried over Na₂SO₄

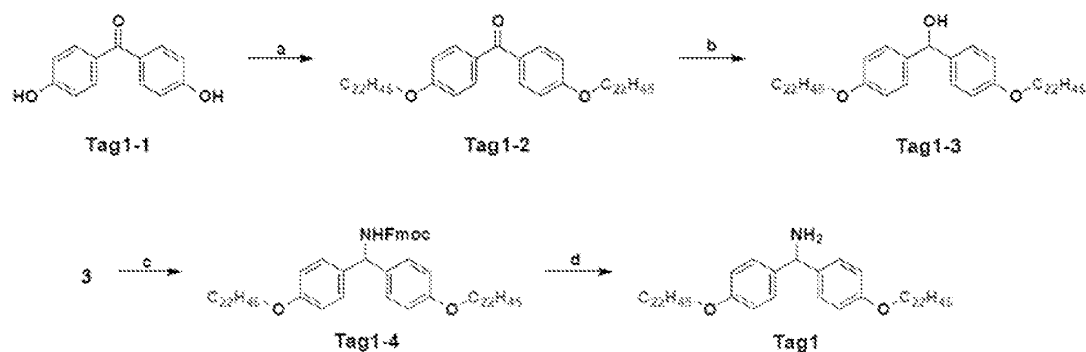
and concentrated under reduced pressure to give a residue. The crude product was triturated with TBME:ACN:EtOAc (10:1:1, 250 mL) for 12 h at 20°C, and filtered to give a light yellow solid. The compound **DMB-AA9** (38 g, 70.90 mmol, 40.57% yield, 98.8% purity) was obtained as a light-yellow solid. LC-MS: RT= 3.207 min, mass calcd. for C₂₈H₂₇N₅O₆H 530.2 [M+H]⁺, m/z found 530.2 [M+H]⁺, Method 7. HPLC purity: 98.84% after trituration (Method 14 was used for the analysis of **DMB-AA9**). ¹H NMR (400MHz, Methanol-d₄) δ = 7.77 (d, J=7.5 Hz, 2H), 7.62 (br d, J=7.3 Hz, 2H), 7.40 - 7.16 (m, 5H), 6.56 - 6.43 (m, 2H), 5.52 - 5.37 (m, 2H), 4.45 (dd, J=5.0, 7.5 Hz, 1H), 4.35 - 4.08 (m, 3H), 3.75 (s, 6H), 3.55 (dd, J=4.9, 15.2 Hz, 1H), 3.47 - 3.35 (m, 1H) ppm. SFC ee = 98.53-1.47 = 97.06%.

Example 4. Synthesis (LPPS) of M2 on Tag1

4.1 Synthesis of Tag 1

[000524] The synthetic route to make Tag1 is shown in Scheme 2.

Scheme 2

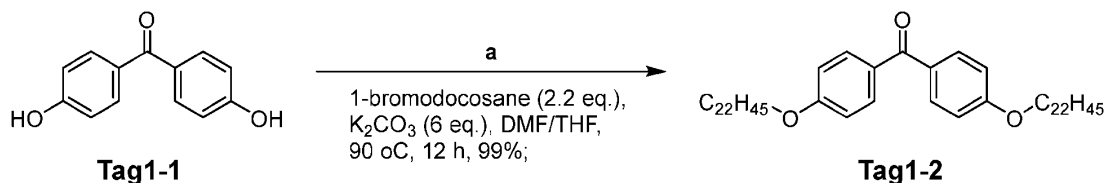


Reagents and conditions: a) 1-bromodocosane (2.2 eq.), K₂CO₃ (6 eq.), DMF/THF, 90 °C, 12 h, 99%; b) NaBH₄ (5 eq.), EtOH (1 V), THF (10 V), 70 °C, 12 h, 96%; c) FmocNH₂ (2 eq.), MeSO₃H (0.3 eq.), toluene/THF, 110 °C, 36 h, 82%; d) 20% piperidine/THF (10 V), 20 °C, 2 h, 92%. Total yield: 71.6% over 4 steps. Total yield: 71.6% for 4 steps.

[000525] The synthesis was reported in U.S. Patent Application Publication No. 2012/0296074 to Hirai et al. Alkylation of **Tag1-1** with 1-bromodocosane yielded **Tag1-2**, which was reduced by sodium borohydride to form alcohol **Tag1-3**. Compound **Tag1-4** was obtained from **Tag1-3** via nucleophilic substitution of 9-fluorenylmethylcarbamate, using methanesulfonic acid as the catalyst. The reaction yield was slightly improved by using co-solvent (toluene/THF) instead of toluene (82% vs 60%). De-protection of Fmoc in **Tag1-4** was carried out using piperidine/THF and afforded **Tag1**.

in total 72% yield. U.S. Patent Application Publication No. 2012/0296074 to Hirai et al. reported 45% yield for Fmoc deprotection of **Tag1-4** using DBU/DCM.

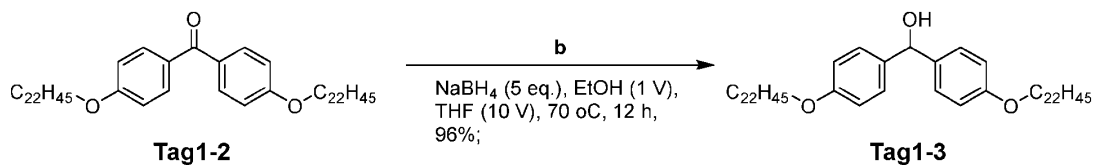
*Step a: Synthesis of Bis(4-docosoxyphenyl)methanone (**Tag1-2**)*



[000526] To a mixture of compound **Tag1-1** (10 g, 46.68 mmol, 1.0 eq.) and 1-bromodocosane (40.00 g, 102.70 mmol, 2.2 eq.) in DMF (200 mL) and THF (40 mL) was added K_2CO_3 (38.71 g, 280.09 mmol, 6 eq.) and the mixture was stirred at 90 °C for 12 hr. The reactant was consumed and a new spot with lower polarity formed on TLC (PE/EtOAc = 2/1). The reaction mixture was poured into water (1000 mL), and the mixture was stirred for 1 h, then the precipitated solid was collected by filtration and slurry-washed with water (200 mL $\times$ 2). The obtained solid was triturated with acetone (500 mL) and collected by filtration of slurry-washed in acetone. After filtration, the obtained solid was dried under reduced pressure to give **Tag1-2** (38.8 g, 46.67 mmol, 99.97% yield) as a white solid.

[000527] Compound **Tag1-2** ($\text{C}_{57}\text{H}_{98}\text{O}_3$, MW: 830.75): ^1H NMR (400MHz, CDCl_3) δ = 7.78 (d, J =8.8 Hz, 4H), 6.95 (d, J =8.8 Hz, 4H), 4.04 (t, J =6.5 Hz, 4H), 1.86 – 1.78 (m, 4H), 1.51 – 1.43 (m, 4H), 1.26 (s, 72H), 0.89 (t, J =6.8 Hz, 6H) ppm.

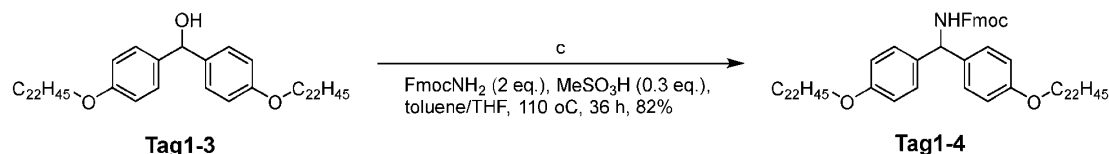
*Step b: Synthesis of Bis(4-docosoxyphenyl)methanol (**Tag1-3**)*



[000528] A solution of compound **Tag1-2** (35 g, 42.10 mmol, 1 eq.) in THF (500 mL) and EtOH (25 mL) was heated to 70°C. Then NaBH_4 (7.96 g, 210.49 mmol, 5.0 eq) was added slowly, and the mixture was stirred at the same temperature for 20 hr. TLC (PE/EtOAc=3/1) showed that the reactant was consumed completely, and the new spot was detected. The reaction mixture was ice-cooled and 1N hydrochloric acid (80 mL) was added dropwise. THF was evaporated, water (450 mL) was added, and 1N hydrochloric acid was added to pH 5 – 7. The slurry was filtered, and the obtained crystals were washed with water and methanol to give the product **Tag1-3** (34 g, 40.80 mmol, 96.91% yield) as a white solid.

[000529] Compound **Tag1-3** ($C_{57}H_{100}O_3$, MW: 832.77): 1H NMR (400MHz, Chloroform- d) δ = 7.29 – 7.25 (m, 4H), 6.88 – 6.84 (m, 4H), 5.77 (d, J =2.8 Hz, 1H), 3.94 (t, J =6.7 Hz, 4H), 2.09 (d, J =3.3 Hz, 1H), 1.81 – 1.73 (m, 4H), 1.48 – 1.41 (m, 4H), 1.35 – 1.26 (m, 72H), 0.91 – 0.87 (m, 6H) ppm.

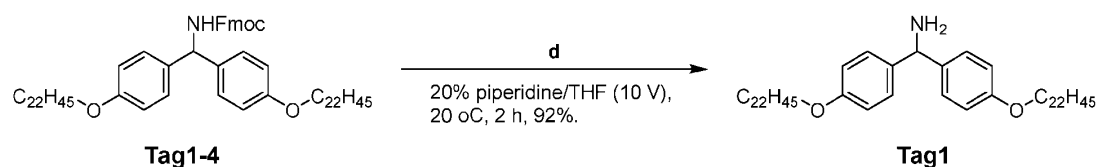
Step c: Synthesis of 9H-Fluoren-9-ylmethyl N-[bis(4-docosoxyphenyl)methyl]carbamate (**Tag1-4**)



[000530] To a mixture of **Tag1-3** (10 g, 12.00 mmol, 1 eq.) and 9H-fluoren-9-ylmethyl carbamate (FmocNH₂, 5.74 g, 24.00 mmol, 2.0 eq.) in toluene (200 mL) was added MsOH (345.97 mg, 3.60 mmol, 256.27 μ L, 0.3 eq.), then the mixture was stirred at 110 °C for 36 hr. Most of the reactant was consumed and new spots formed on TLC (PE/EtOAc = 10/1). The reaction mixture was poured into 5% NaHCO₃ (30 mL), and the mixture was stirred for 1hr, then the precipitated solid was collected by filtration and washed with water (50 mL). The obtained solid was triturated with ACN (200 mL) and the solid was collected by filtration and slurry-washed in methanol. After filtration, the obtained solid was dried under reduced pressure to give **Tag1-4** (11 g, 9.91 mmol, 82.58% yield, 95% purity) as an off-white solid.

[000531] Compound **Tag1-4** ($C_{72}H_{111}NO_4$, MW: 1053.85): 1H NMR (400MHz, Chloroform- d) δ = 9.89 (s, 1H), 7.78 (br d, J =8.4 Hz, 2H), 7.61 (br d, J =7.4 Hz, 2H), 7.44 – 7.31 (m, 4H), 7.14 – 7.10 (m, 1H), 7.12 (d, J =8.6 Hz, 2H), 6.98 (dd, J =8.7, 19.2 Hz, 1H), 6.88 – 6.75 (m, 4H), 5.87 (br s, 1H), 4.43 (d, J =7.0 Hz, 1H), 4.28 – 4.21 (m, 1H), 4.04 (dt, J =2.9, 6.5 Hz, 1H), 3.99 – 3.85 (m, 4H), 1.82 – 1.74 (m, 4H), 1.59 (br s, 4H), 1.27 (s, 72H), 0.91 – 0.89 (m, 6H) ppm.

Step d: Synthesis of Bis(4-docosoxyphenyl)methanamine (**Tag1-5**)



[000532] To a solution of **Tag1-4** (11 g, 10.43 mmol, 1 eq.) in THF (80 mL) was added piperidine (20 mL). Then the mixture was stirred at 20°C for 2 hr. TLC (PE/EtOAc=3/1) showed that the reactant was consumed completely, and one new spot was detected. To the reaction solution was added ACN (400 mL) and the resulting suspension was filtered and washed with ACN (100 mL

× 2) to give an off-white solid. The crude product **Tag1-5** (8 g, 9.61 mmol, 92.14% yield) was obtained as a white solid. It was dried under reduced pressure and used in the synthesis of Tag1-M2 below.

[000533] Compound **Tag1-5** (C₅₇H₁₀₁NO₂, MW: 831.78): ¹H NMR (400MHz, Chloroform-d) δ = 7.29 (s, 4H), 6.86 (d, J=8.6 Hz, 4H), 5.15 (s, 1H), 3.95 (t, J=6.6 Hz, 4H), 1.80 – 1.76 (m, 4H), 1.49 – 1.43 (m, 4H), 1.29 (s, 72H), 0.91 (t, J=6.8 Hz, 6H) ppm.

4.2 Synthesis of Tag1-M2

[000534] Figure 2 and Table 4 outline the synthesis of **M2** using **Tag1**.

Table 4.

Coupling Reaction molar eq to Tag1 (0.04mmol)					de-Fmoc	Product		
Step#	amino acid		DIC	HOAt	Step#	Cpd#	MS (M+H) ⁺	Yield ^c
1	AA1	1.5 eq. ^b	1.5 eq.	1.5 eq.	20V ^d	Tag1-1AA	221.1 [M-Tag1+2H] ⁺	88.2%
2	AA2	1.5 eq.	1.5 eq.	1.5 eq.	20V	Tag1-2AA	585.4 [M-Tag1+2H] ⁺	84.3%
3	AA3	1.5 eq.	1.5 eq.	1.5 eq.	20V	Tag1-3AA	778.5 [M-Tag1+2H+Na] ⁺	82.7%
4	AA4	1.5 eq.	1.5 eq.	1.5 eq.	20V	Tag1-4AA	787.4 [M-Tag1-tBu-TBS+4H] ⁺	93.1%
5	AA5	1.5 eq.	1.5 eq.	1.5 eq.	20V	Tag1-5AA	1002.7 [M-Tag1-tBu-TBS+4H] ⁺	97.2%
6	AA6	1.5 eq.	1.5 eq.	1.5 eq.	20V	Tag1-6AA	1067.7 [M-Tag1-tBu-2TBS+5H] ⁺	84.7%
7	AA7	5 eq.	4.5 eq.	4.5 eq.	20V	Tag1-7AA	1282.7 [M-Tag1-tBu-2TBS+5H] ⁺	60.4%
8	AA10-AA9-AA8-OH ^a	1.05 eq.	HATU ^e (1.2 eq.)	/		Tag1-10AA	786.7 [M-Tag1-tBu-3TBS-DMB-Trt+9H] ²⁺	crude
9	Cleavage					M2	786.8 [M+H] ⁺	18.7%

^a **Tag1-8AA** can't be separated via precipitation in ACN so **Tag1-10AA** was obtained from a coupling reaction of **Tag1-7AA** with **AA10-AA9-AA8** using 1.2eq of HATU (replacing HOAt), following cleavage of **Tag1** to generate **M2**

^b Eq.: molar equivalent to reactant

^c Yield: crude yields

^d V: volume (mL) to Tag (gram)

^e In step 8, the coupling reagent (DIC) was replaced with HATU

General Procedure A for Amide Coupling Reactions to Generate N-Fmoc-Tag1-nAA

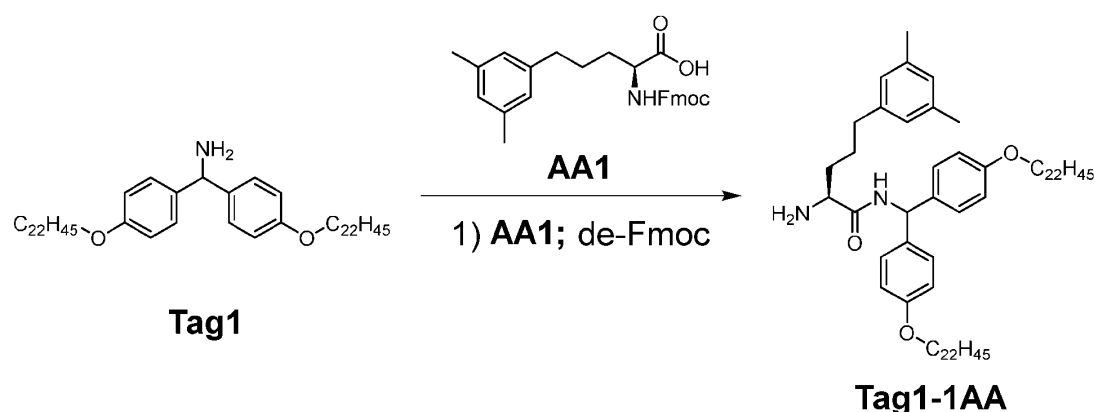
[000535] To a solution of Tag1 or Tag1-(n-1)AA (1 eq.) and AAn (1.5 eq.) in DCM (20V) were added DIC (1.5 eq.) and HOAt (1.5 eq.). The solution was stirred at 20°C for 2 h. TLC (PE/EtOAc = 1/1) showed that the reactant was consumed completely, and new spot was detected, which was further confirmed by LC-MS after cleavage of a small amount. To the reaction solution was added ACN (80V) and the resulting suspension was filtered and washed with MeOH (20V × 2) to give an off-white solid. The crude product was dried under reduced pressure and N-Fmoc-Tag1-(n)AA was obtained as a white solid and used in the next step directly.

General Procedure B for Fmoc Deprotection to Give Tag1-nAA

[000536] A solution of N-Fmoc-Tag1-(n)AA (1 eq.) in 20% piperidine in THF (20V) was stirred at 20°C for 1 hr. TLC (PE/EtOAc=1/1) showed that the reactant was consumed completely, and the desired spot was detected. To the reaction solution was added ACN (80V) and the resulting

suspension was filtered and washed with ACN (20V × 2) to give an off-white solid. The crude product Tag1-nAA was dried under reduced pressure and used in the next step directly.

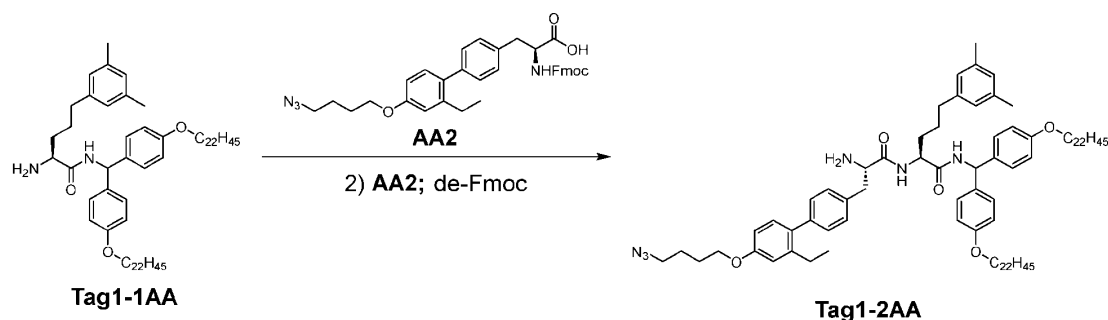
Step 1: Synthesis of (2S)-2-Amino-N-[bis(4-docosoxyphenyl)methyl]-5-(3,5-dimethylphenyl)pentanamide (Tag1-1AA)



[000537] To a solution of **Tag1** (6 g, 7.21 mmol, 1 eq.) and **AA1** (4.80 g, 10.81 mmol, 1.5 eq.) in DCM (100 mL) were added DIC (1.18 g, 9.37 mmol, 1.45 mL, 1.3 eq.) and HOAt (1.28 g, 9.37 mmol, 1.31 mL, 1.3 eq.). The solution was stirred at 20°C for 2 h. TLC (PE/EtOAc=1/1) showed that the reactant was consumed completely, and new spot was detected, which was further confirmed by testing cleavage crude. To the reaction solution was added ACN (400 mL) and the resulting suspension was filtered and washed with MeOH (100 mL × 2) to give an off-white solid. The crude product was dried under reduced pressure and **N-Fmoc-Tag1-1AA** (8 g, 6.36 mmol, 88.23% yield) was obtained as a white solid and used in the next step directly. LCMS (ESI): RT= 4.266 min, mass calcd. for $C_{28}H_{31}N_2O_3^+$ 443.23, m/z found 443.20 [M-TAG-1+H]⁺; (Tag1=C₅₇H₉₉O₂, MS=815.76).

[000538] To a solution of **N-Fmoc-Tag1-1AA** (8 g, 6.36 mmol, 1 eq.) in THF (80 mL) was added piperidine (20 mL). Then the mixture was stirred at 20°C for 1 hr. TLC (PE/EtOAc=1/1) showed that the reactant was consumed completely, and the desired spot was detected. To the reaction solution was added ACN (400 mL) and the resulting suspension was filtered and washed with ACN (100 mL × 2) to give an off-white solid. The crude product **Tag1-1AA** as a white solid was dried under reduced pressure and used in the next step directly.

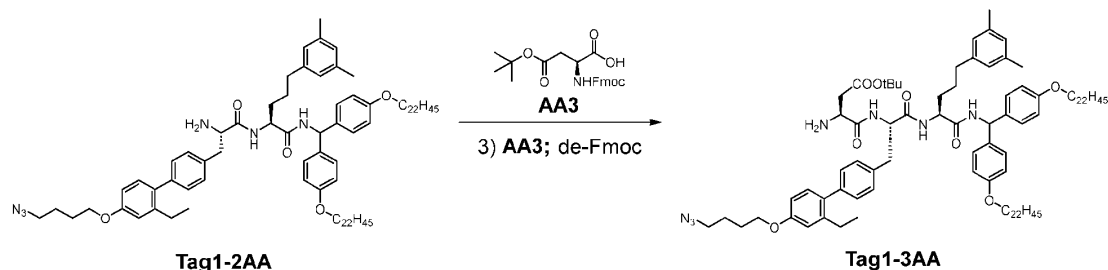
Step 2: Synthesis of (2S)-2-[[[(2S)-2-Amino-3-[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]propanoyl]amino]-N-[bis(4-docosoxyphenyl)methyl]-5-(3,5-dimethylphenyl)pentanamide (Tag1-2AA)



[000539] Reaction was performed according to the general procedure A for amide coupling using **Tag1-1AA** (7 g, 6.76 mmol). The crude product **N-Fmoc Tag1-2AA** (11 g, white solid) was dried and used in the next step directly. LCMS (ESI): RT= 5.759 min, mass calcd. for $C_{49}H_{55}N_6O_5^+$ 807.42, m/z found 807.40 [M- TAG-1+H]⁺; (Tag1 = $C_{57}H_{99}O_2$, MS=815.76).

[000540] Deprotection reaction was performed according to the general procedure B for Fmoc deprotection. **Tag1-2AA** (8 g, 5.71 mmol, 84.27% yield) was obtained as a white solid. LCMS (ESI): RT= 3.568 min, mass calcd. for $C_{34}H_{45}N_6O_3^+$ 585.35, m/z found 585.50 [M- TAG-1+H]⁺; (TAG-1 = $C_{57}H_{99}O_2$, MS=815.76).

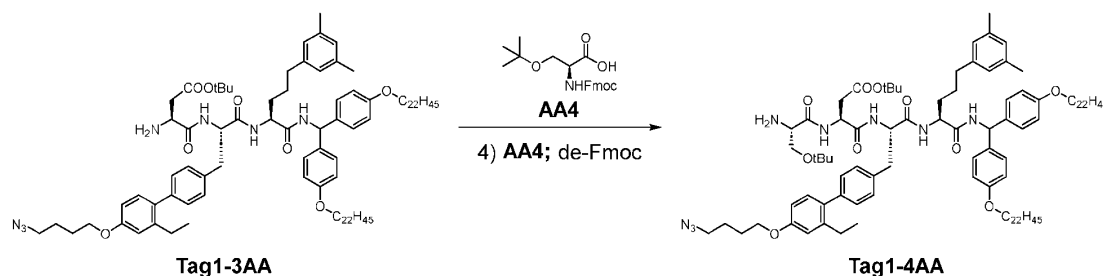
*Step 3: Synthesis of tert-Butyl (3S)-3-Amino-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis(4-docosoxyphenyl)methyl]carbonyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (**Tag1-3AA**)*



[000541] Reaction was performed according to the general procedure A for amide coupling using **Tag1-2AA** (7 g, 5.00 mmol). **N-Fmoc-Tag1-3AA** (8.9 g, 4.96 mmol, 99.25% yield) was obtained as a yellow solid. LCMS (ESI): RT= 5.388 min, mass calcd. for $C_{53}H_{60}N_7O_8^+$ 922.44, m/z found 922.50 [M- TAG-1+H]⁺; (Tag1 = $C_{57}H_{99}O_2$, MS=815.76).

[000542] Deprotection reaction was performed according to the general procedure B for Fmoc deprotection. **Tag1-3AA** (6.5 g, 4.14 mmol, 83.36% yield) was obtained as a white solid. LCMS (ESI): RT= 4.294 min, mass calcd. for $C_{38}H_{50}N_7O_6^+$ 700.37, m/z found 700.50 [M- Tag-1+H]⁺; (TAG-1 = $C_{57}H_{99}O_2$, MS=815.76).

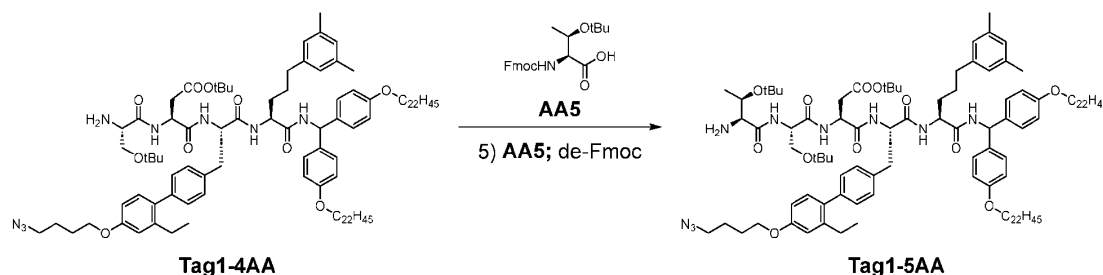
Step 4: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-Amino-3-tert-butoxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis(4-docosoxyphenyl)methylcarbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag1-4AA)



[000543] Reaction was performed according to the general procedure A for amide coupling using **Tag1-3AA** (6.5 g, 4.14 mmol, 1 eq.). **N-Fmoc-Tag1-4AA** (8 g, 4.13 mmol, 99.86% yield) was obtained as a yellow solid. LCMS (ESI): RT= 5.198 min, mass calcd. for $C_{56}H_{65}N_8O_{10}^+$ 1009.47, m/z found 1009.60 [M- TAG-1+H]⁺; (Tag1 = $C_{57}H_{99}O_2$, MS=815.76).

[000544] Deprotection reaction was performed according to the general procedure B for Fmoc deprotection. **Tag1-4AA** (6.6 g, 3.85 mmol, 93.19% yield) was obtained as a white solid. LCMS (ESI): RT= 3.540 min, mass calcd. for $C_{41}H_{55}N_8O_8^+$ 787.41, m/z found 787.50 [M- TAG-1+H]⁺; (Tag1 = $C_{57}H_{99}O_2$, MS=815.76).

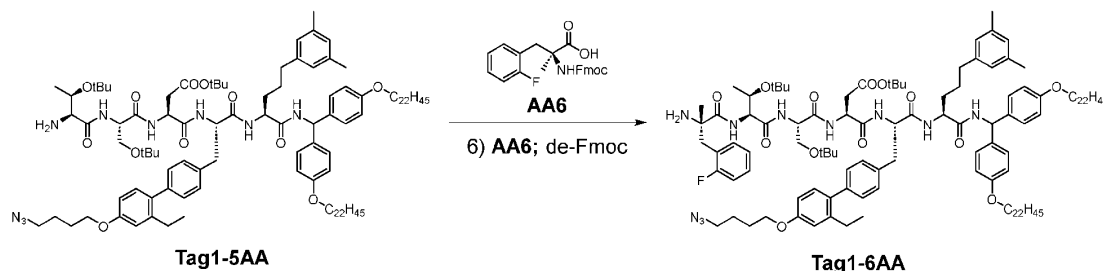
Step 5: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-Amino-3-tert-butoxy-butanoyl] amino]-3-tert-butoxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl] phenyl]methyl]-2-[[[(1S)-1-[bis(4-docosoxyphenyl)methylcarbamoyl]-4-(3,5-dimethylphenyl) butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag1-5AA)



[000545] Reaction was performed according to the general procedure A for amide coupling using **Tag1-4AA** (6.60 g, 3.85 mmol, 1 eq.). **N-Fmoc-Tag1-5AA** (8.0 g, 3.82 mmol, 99.25% yield) was obtained as a white solid. LCMS (ESI): RT= 5.024 min, mass calcd. for $C_{60}H_{72}N_9O_{12}^+$ 1110.52, m/z found 1110.60 [M- TAG-1+H]⁺; (Tag1 = $C_{57}H_{99}O_2$, MS=815.76).

[000546] Deprotection reaction was performed according to the general procedure B for Fmoc deprotection. **Tag1-5AA** (7.0 g, 3.74 mmol, 97.89% yield) was obtained as a white solid which was used directly in the next step. LCMS (ESI): RT= 3.542 min, mass calcd. For $C_{45}H_{62}N_9O_{10}^+$ 888.45, m/z found 888.50 [M-TAG-1+H]⁺; (Tag1 = $C_{57}H_{99}O_2$, MS=815.76).

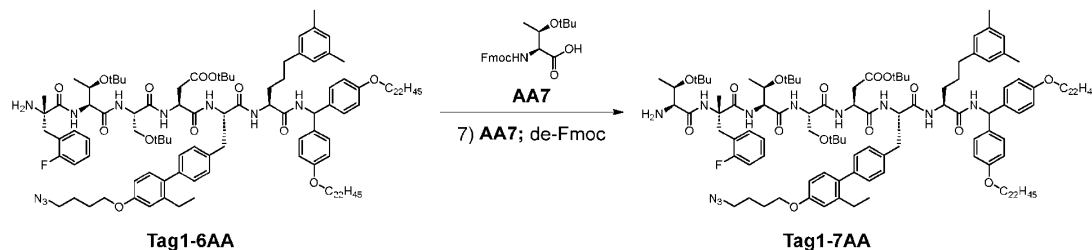
Step 6: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[2-Amino-3-(2-fluorophenyl)-2-methylpropanoyl]amino]-3-tert-butoxy-butanoyl]amino]-3-tert-butoxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis(4-docosoxyphenyl)methylcarbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag1-6AA)



[000547] Reaction was performed according to the general procedure A for amide coupling using **Tag1-5AA** (7.00 g, 3.74 mmol, 1 eq.). **N-Fmoc Tag1-6AA** (7.3 g, 3.21 mmol, 85.87% yield) was obtained as a white solid. LCMS (ESI): RT= 5.024 min, mass calcd. for $C_{70}H_{82}FN_{10}O_{13}^+$ 1289.60, m/z found 1289.60 [M-TAG-1+H]⁺; (Tag1 = $C_{57}H_{99}O_2$, MS=815.76).

[000548] Deprotection reaction was performed according to the general procedure B for Fmoc deprotection. **Tag1-6AA** (6.5 g, 3.17 mmol, 98.69% yield) was obtained as a white solid. LCMS (ESI): RT= 3.699 min, mass calcd. for $C_{55}H_{72}FN_{10}O_{11}^+$ 1067.53, m/z found 1067.60 [M-TAG-1-3'Bu+4H]⁺; (TAG-1 = $C_{57}H_{99}O_2$, MS=815.76).

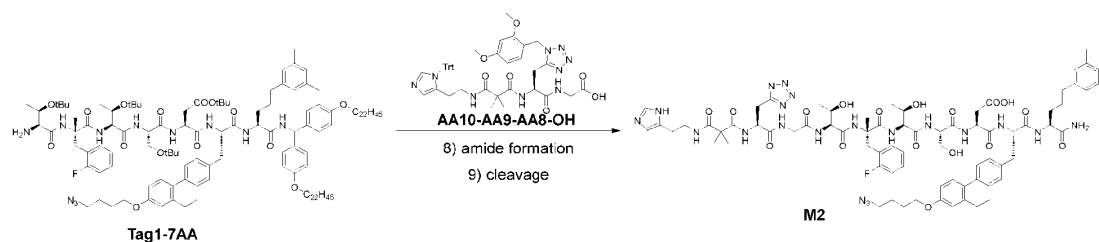
Step 7: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[2-[[[(2S,3R)-2-Amino-3-tert-butoxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-tert-butoxy-butanoyl]amino]-3-tert-butoxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis(4-docosoxyphenyl)methylcarbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag1-7AA)



[000549] To a solution of **Tag1-6AA** (6.5 g, 3.17 mmol, 1 eq.) and **AA7** (6.30 g, 15.85 mmol, 5.0 eq.) in DCM (100 mL) were added DIC (1.80 g, 14.26 mmol, 2.21 mL, 4.5 eq.) and HOAt (1.94 g, 14.26 mmol, 2.00 mL, 4.5 eq.). Then the solution was stirred at 20°C for 2 h. TLC (PE/EtOAc=1/1) showed that the reactant was consumed completely, and a new spot was detected. To the reaction solution was added ACN (400 mL), but only a little solid formed. H₂O (50 mL) was added, and the resulting suspension was filtered and then washed with ACN (200 mL × 2) to give an off-white solid. (Note: the filtration was very slow due to the tiny solid particles.) **N-Fmoc-Tag1-7AA** (6.6 g, 2.72 mmol, 85.69% yield) was obtained as a white solid which was used directly in the next step. LCMS (ESI): RT= 5.437 min, mass calcd. for C₇₄H₈₉FN₁₁O₁₅⁺ 1390.46, m/z found 1390.70 [M- TAG-1+H]⁺; (Tag1 = C₅₇H₉₉O₂, MS=815.76).

[000550] To a solution of **N-Fmoc-Tag1-7AA** (7.80 g, 3.21 mmol, 1 eq.) in THF (80 mL) was added piperidine (20 mL). Then the solution was stirred at 20°C for 2h. TLC (PE/EtOAc=1/1) showed that the reactant was consumed completely, and new spots were detected. The reaction mixture was added to ACN/H₂O=1/8 (600 mL) and the resulting suspension was filtered. (Note: the filtration was very slow due to the tiny solid particles.) The filter cake was triturated with THF/ACN=1/12 (500 mL) and filtered. Then the filter cake was washed with ACN (50 mL×3) to give the crude product. **Tag1-7AA** (5.0 g, 2.26 mmol, 70.54% yield) was obtained as a white solid which was used directly in the next step. LCMS (ESI): RT= 3.550 min, mass calcd. for C₄₅H₆₂N₉O₁₀⁺ 888.45, m/z found 888.50 [M- TAG-1+H]⁺; (Tag1 = C₅₇H₉₉O₂, MS=815.76).

Step 8: Synthesis of (3S)-4-[[[(1S)-1-[[4-[4-(4-Azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-2-[[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[[(2S,3R)-3-hydroxy-2-[[2-[[[(2S)-2-[[3-[2-(1H-imidazol-5-yl) ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoyl]amino]-3-hydroxy-



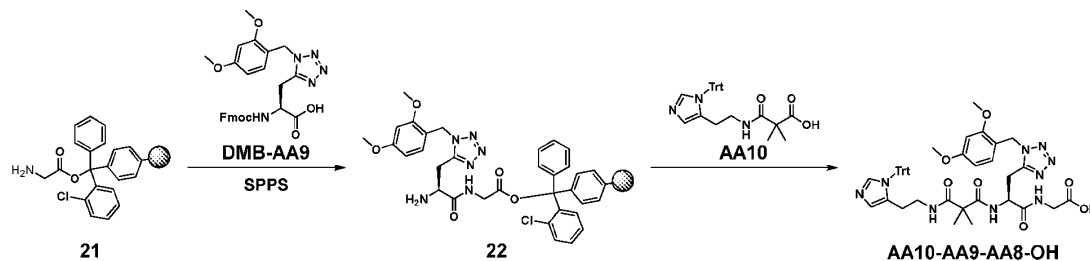
[000551] To a solution of **Tag1-7AA** (300 mg, 135.86 μmol, 1 eq.) and **AA10-AA9-AA8-OH** (116.11 mg, 142.66 μmol, 1.05 eq.) in DMF (0.5 mL) and DCM (1.5 mL) was added HATU (61.99 mg, 163.03 μmol, 1.2 eq.). Then the mixture was stirred at 20°C for 2 h. TLC (PE/EtOAc=1/1) showed

that the reactant was consumed completely, and a new spot was detected. The reaction mixture was quenched by water (50 mL) and extracted with DCM (80 mL*2). The combined organic layers were washed with brine (30 mLx2), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give compound **Tag1-10AA** (408 mg, crude). LCMS (ESI): RT= 3.94 min, m/z calcd. for C₇₅H₁₀₁FN₂₀O₁₇, 786.375 [M-Tag1+H]⁺; m/z found 786.8; (Tag1 = C₅₇H₉₉O₂, MS=815.76). LC-MS Conditions: Method 7.

[000552] The crude **Tag1-10AA** was subjected to acidic cleavage by using TFA cocktail (10 mL, TFA:Tips:H₂O=95:2.5:2.5) for 1.5hr. The reaction mixture was filtered, and the filtrate was concentrated to give a residue, which was purified by prep-HPLC (TFA; mobile phase: [water (TFA)-ACN] B%: 28%-58%, 12min condition). **M2** (40 mg, 25.45 μmol, 18.73% yield) was obtained as a white solid. LCMS: RT= 3.942 min, m/z calcd. for C₇₅H₁₀₁FN₂₀O₁₇, 786.375 [M+H]⁺; m/z found 786.8. LC-MS Conditions: Method 7.

[000553] HPLC: RT=9.66 min, 92.86% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6mm, 5μm; 2.75ML/4LTFA in water (solvent A) and 2.5ML/4LTFA in ACN (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 ml/min.

*Synthesis of 2-[[[(2S)-3-[1-[(2,4-Dimethoxyphenyl)methyl]tetrazol-5-yl]-2-[[2,2-dimethyl-3-oxo-3-[2-(3-tritylimidazol-4-yl)ethylamino]propanoyl]amino]propanoyl]amino] Acetic Acid (**AA10-AA9-AA8-OH**)*



[000554] To a solution of **DMB-AA9** (3.23 g, 6.10 mmol, 2 eq) and DIEA (1.58 g, 12.20 mmol, 2.12 mL, 4 eq) in DMF (20 mL) was added HATU (2.32 g, 6.10 mmol, 2 eq) in one portion at 20°C. The mixture was stirred at 20°C for 0.2 hr. Compound **21** (2.82 g, 3.05 mmol, 37.94% purity, 1 eq) was added and the mixture was stirred at 20°C for 2 hr. After completion, the mixture was filtered, and the collected resin was washed with DMF (25 mL × 3), DCM (25 mL × 3) to give the crude product **Fmoc-22** on chlorotriyl chloride (CTC) resin (4.38 g, 3.05 mmol, 99.93% yield, 60% purity) as a light-yellow solid. LCMS (ESI): RT = 0.942 min, m/z calcd. for C₃₀H₃₀N₆O₇Na, 609.22, [M+Na]⁺; m/z found 609.1; LC-MS Conditions: Method 13.

[000555] To a solution of **Fmoc-22** (4.38 g, 3.05 mmol, 60% purity, 1 eq) in DMF (32 mL) was added piperidine (8 mL) in one portion at 20°C. The mixture was stirred at 20°C for 1 hr. The mixture was filtered, and the collected resin was washed with DMF (25 mL × 3) and DCM (25 mL × 3) to give the crude product **22** on CTC resin (3.7 g, 3.05 mmol, 99.96% yield, 52.7% purity) as a light-yellow solid. LCMS (ESI): RT = 0.716 min, m/z calcd. for C₁₅H₂₀N₆O₅Na, 387.15 [M+Na]⁺; m/z found 387.1; LC-MS Conditions: Method 13.

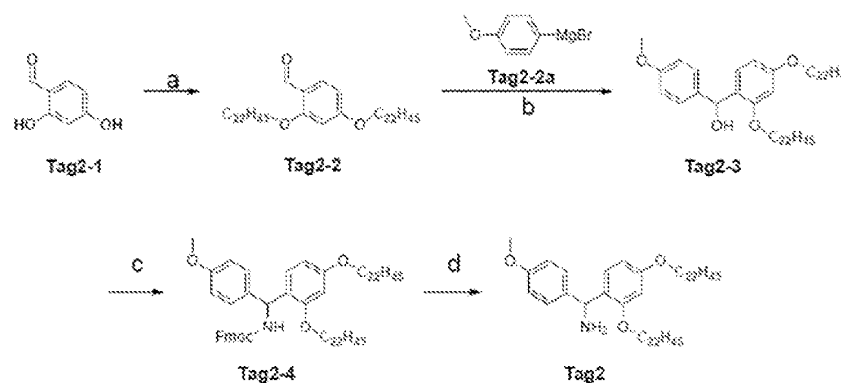
[000556] To a solution of **AA10** (2.85 g, 6.09 mmol, 2 eq) and DIEA (1.57 g, 12.18 mmol, 2.12 mL, 4 eq) in DMF (20 mL) was added HATU (2.32 g, 6.09 mmol, 2 eq) in one portion at 20°C. The mixture was stirred at 20°C for 0.2 hr. Compound **22** (3.7 g, 3.05 mmol, 52.7% purity, 1 eq) was added and the mixture was stirred at 20°C for 2 hr. After completion, the reaction mixture was subjected to acidic cleavage by using AcOH cocktail (20 mL, AcOH:TFE:DCM = 1:1:8) for 2 h. Then the mixture was filtered, and the collected resin was washed with DCM (5 mL × 3). The filtration was washed with H₂O (10 mL × 3) and the combined aqueous layers were extracted with DCM (10 mL × 2). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue, which was purified by C-18 reverse phase chromatography (ISCO®; 40g @C-18 Column, Eluent of 0~60% ACN/H₂O (0.1% AcOH) gradient @ 40 mL/min, 30 min with total volume 1.2 L). After lyophilization, **AA10-AA9-AA8-OH** (260 mg, 287.51 μmol, 9.44% yield, 90% purity) was obtained as an off-white solid. LCMS (ESI): RT = 3.050 min, m/z calcd. for C₄₄H₄₈N₉O₇, 814.36, [M+H]⁺; m/z found 814.4; LC-MS Conditions: Method 13.

Example 5. Synthesis (LPPS) of M2 on Tag2

5.1 Synthesis of Tag 2

[000557] The synthetic route to make **Tag2** is shown in Scheme 3 below.

Scheme 3

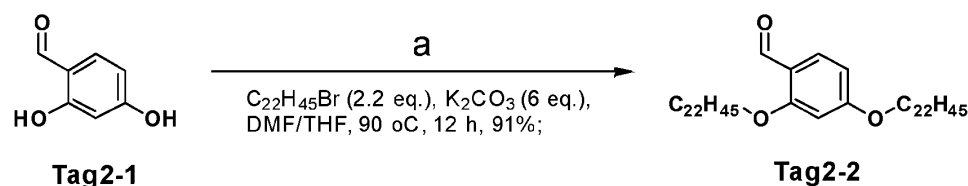


Reagents and conditions: a) $\text{C}_{22}\text{H}_{45}\text{Br}$ (2.2 eq.), K_2CO_3 (6 eq.), DMF/THF, 90 °C, 12 h, 91%; b) (4-methoxyphenyl)magnesium bromide (1 M, 3 eq.), THF, 70 °C, 12 h, 91%; c) FmocNH_2 (2 eq.), MsOH (0.3 eq.), THF, 70 °C, 12 h, 86%; d) 20% piperidine in THF, 20 °C, 2 h, 96%. Total yield: 75.8%

[000558] Treatment of 2,4-dihydroxybenzaldehyde (**Tag2-1**) with 2.2 eq. of 1-bromodocosane in the presence of potassium carbonate in DMF/THF afforded bis C22-alkyl substituted compound (**Tag2-2**). Grignard reaction of **Tag2-2** with Grignard reagent (**Tag2-2a**), followed by nucleophilic substitution of alcohol (**Tag2-3**) with 9-fluorenylmethylcarbamate generated **Tag2-4**. **Tag2** was obtained through a common de Fmoc-protection from **Tag2-4**.

[000559] Both alcohol Tag (**Tag2-3**) and amine Tag (**Tag2**) are new Tags designed to be used to make short peptide acids and amides. The two hydrophobic C22-alkyl chains in **Tag2-3** and **Tag2** retained their TCPs hydrophobicity that may be precipitated out in polar solvents during the synthetic process.

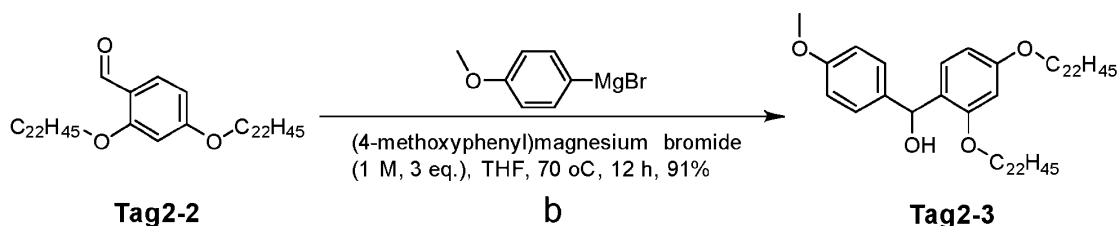
*Step a: Synthesis of 2,4-Di(docosyloxy)benzaldehyde (**Tag2-2**)*



[000560] To a mixture of 2,4-dihydroxybenzaldehyde (**Tag2-1**, 5 g, 36.20 mmol, 1 eq) and 1-bromo-docosane (31.02 g, 79.64 mmol, 2.2 eq) in DMF (100 mL) and THF (20 mL) was added K_2CO_3 (30.02 g, 217.20 mmol, 6 eq) and it was stirred at 90 °C for 12 hr. Most of the reactant was consumed and a new spot formed on TLC (PE/EtOAc = 5/1). The reaction mixture was poured into water (1000

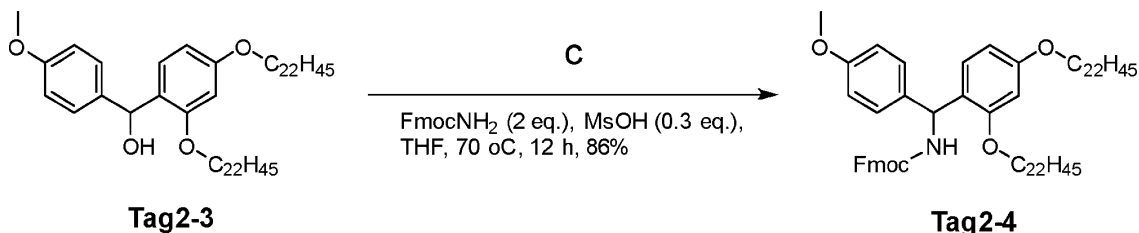
mL), and the mixture was stirred for 1 hr, then the precipitated solid was collected by filtration. The obtained solid was triturated with acetone (1000 mL), collected by filtration and slurry-washed in methanol, and dried under reduced pressure to give the desired compound **Tag2-2** (27 g, crude) as an off-white solid. ¹H NMR (400MHz, Chloroform-d) δ = 10.34 (s, 1H), 7.80 (d, J=8.8 Hz, 1H), 6.52 (dd, J=1.5, 8.8 Hz, 1H), 6.43 (d, J=1.9 Hz, 1H), 4.08 – 3.97 (m, 4H), 1.89 – 1.73 (m, 4H), 1.52 – 1.44 (m, 4H), 1.26 (s, 72H), 0.89 (t, J=6.7 Hz, 6H) ppm.

*Step b: Synthesis of (2,4-Bis(docosyloxy)phenyl)(4-methoxyphenyl)methanol (**Tag2-3**)*



[000561] To a solution of **Tag2-2** (31 g, 41.04 mmol, 1 eq) in THF (300 mL) was added bromo-(4-methoxyphenyl)magnesium (**Tag2-2a**, 1 M, 123.13 mL, 3 eq) and the reaction mixture was stirred at 70° C for 12 hours. TLC (PE:EtOAc = 10:1) showed that the reaction was complete. After cooling to room temperature, 1N hydrochloric acid (100 mL) and THF (100 mL) was added to the reaction. The separated organic layers were washed with brine (50 mLx2), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue, which was triturated with ACN (150 mL) and MeOH (80 mL). The suspension was filtered, thoroughly washed with ACN (100 mL) and methanol (80 mL) to provide the product **Tag2-3** (36 g, 37.53 mmol, 91.43% yield, 90% purity) as a light-yellow solid. ¹H NMR (400MHz, Chloroform-d) δ = 7.30 - 7.27 (m, 2H), 7.17 – 7.00 (m, 1H), 6.90 – 6.77 (m, 2H), 6.49 – 6.32 (m, 2H), 5.98 – 5.49 (m, 1H), 3.99 – 3.85 (m, 4H), 3.85 – 3.70 (m, 4H), 3.53 – 3.35 (m, 2H), 1.80 – 1.68 (m, 4H), 1.43 (br d, J=5.0 Hz, 4H), 1.26 (s, 72H), 0.89 (t, J=6.7 Hz, 6H) ppm.

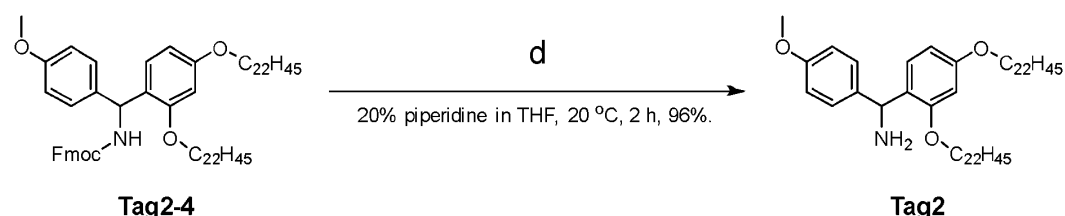
*Step c: Synthesis of (9H-Fluoren-9-yl)methyl((2,4-bis(docosyloxy)phenyl)(4-methoxyphenyl)methyl)carbamate (**Tag2-4**)*



[000562] To a solution of 9H-fluoren-9-ylmethyl carbamate (10.77 g, 45.03 mmol, 1.2 eq) in THF (300 mL) was added **Tag2-3** (36 g, 37.53 mmol, 90% purity, 1.0 eq) and MsOH (1.08 g, 11.26

mmol, 801.41 μ L, 0.3 eq), then the reaction mixture was stirred at 70° C for 12 hours. TLC (PE:EtOAc = 10:1) showed that the reaction was complete. After evaporation to remove most solvent, the residue was triturated with 5% aqueous NaHCO₃ (10 mL), MeOH (80 mL), and ACN (150 mL). The suspension was filtered, and thoroughly washed ACN (120 mL) and methanol (80 mL) sequentially to give **Tag2-4** (39 g, 32.36 mmol, 86.24% yield, 90% purity) as a light-yellow solid. ¹H NMR (400MHz, Chloroform-d) δ = 7.85 – 7.69 (m, 2H), 7.68 – 7.50 (m, 2H), 7.46 – 7.36 (m, 2H), 7.36 – 7.28 (m, 2H), 7.14 (br d, J=8.0 Hz, 2H), 6.81 (br d, J=8.3 Hz, 2H), 6.50 – 6.42 (m, 2H), 6.04 – 5.89 (m, 1H), 4.47 – 4.35 (m, 2H), 4.25 (br t, J=7.0 Hz, 1H), 3.97 – 3.91 (m, 2H), 3.86 – 3.71 (m, 5H), 3.42 – 3.19 (m, 1H), 1.80 – 1.64 (m, 4H), 1.48-1.36 (m, 4H), 1.1.32-1.1 (m, 72H), 0.95-0.82 (m, 6H) ppm.

Step d: Synthesis of (2,4-bis(Docosyloxy)phenyl)(4-methoxyphenyl)methanamine (Tag2)



[000563] To a solution of **Tag2-4** (37 g, 30.70 mmol, 90% purity, 1 eq) in THF (240 mL) was added piperidine (51.73 g, 607.56 mmol, 60 mL, 19.79 eq) and the reaction mixture was stirred at 20° C for 2 hours. TLC (PE:EtOAc = 10:1) showed that the reaction was complete. The mixture was added into ACN (800 mL), the resulting suspension was filtered, and thoroughly washed with ACN (200 mL) and methanol (100 mL) to give **Tag2** (30 g, 29.57 mmol, 96.31% yield, 85% purity) as a light yellow solid. LC-MS (Method 7): RT = 2.038 min, m/z calcd. for C₅₈H₁₀₁O₃ 845.78 [M-NH₃]⁺, found 845.8. ¹H NMR (400MHz, Chloroform-d) δ = 7.29 (d, J=8.5 Hz, 2H), 7.16 – 7.10 (m, 1H), 6.83 (d, J=8.8 Hz, 2H), 6.45 – 6.38 (m, 2H), 5.37 (s, 1H), 3.95 – 3.85 (m, 4H), 3.79 (s, 3H), 1.77 – 1.71 (m, 4H), 1.48 – 1.38 (m, 4H), 1.27 (s, 72H), 0.89 (s, 6H) ppm.

5.2 Synthesis of **Tag2-M2**

[000564] Figure 3 and Table 5 outlines the synthesis of **M2** using **Tag2**.

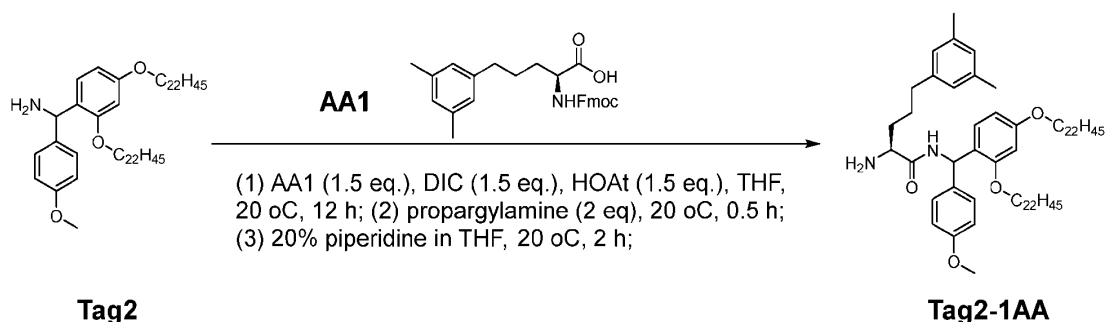
Table 5.

Step #	Amide coupling reaction				Quench	De-Fmoc	Product		
	amino acid		DIC	HOAt	propan-1-amine	20% piperidine in THF	#	MS (M+H) ⁺	Yield (%)
1	AA1	1.5 eq.	1.5 eq.	1.5 eq.	2 eq.	20 V	Tag2-1AA	1065.92, [M+H] ⁺	94.7
2	AA2	1.5 eq.	1.5 eq.	1.5 eq.	2 eq.	20 V	Tag2-2AA	1430.11, [M+H] ⁺	94.7
3	AA3	1.5 eq.	1.5 eq.	1.5 eq.	2 eq.	20 V	Tag2-3AA	700.37 [M-Tag2-tBu+3H] ⁺	91.6
4	AA4	1.5 eq.	1.5 eq.	1.5 eq.	2 eq.	20 V	Tag2-4AA	787.41 [M-Tag2-2tBu+4H] ⁺	89.4
5	AA5	1.5 eq.	1.5 eq.	1.5 eq.	2 eq.	20 V	Tag2-5AA	888.45, [M-Tag2-3tBu+5H] ⁺	79.0
6	AA6	1.5 eq.	1.5 eq.	1.5 eq.	2 eq.	20 V	Tag2-6AA	1067.53, [M-Tag2-3tBu+5H] ⁺	78.5
7	AA7	3 eq.	3 eq.	3 eq.	2 eq.	20 V	Tag2-7AA	1168.58, [M-Tag2-4tBu+6H] ⁺	79.7
8	AA10-AA9-AA8-OH	1.05 eq.	HATU (1.2 eq.)	/	/	/	Tag2-10AA	786.375 [M+2H] ²⁺	/
	cleavage						M2		14.5

General Procedure C for Amide Coupling and Fmoc Deprotection in One-Pot to Give Tag2-nAA

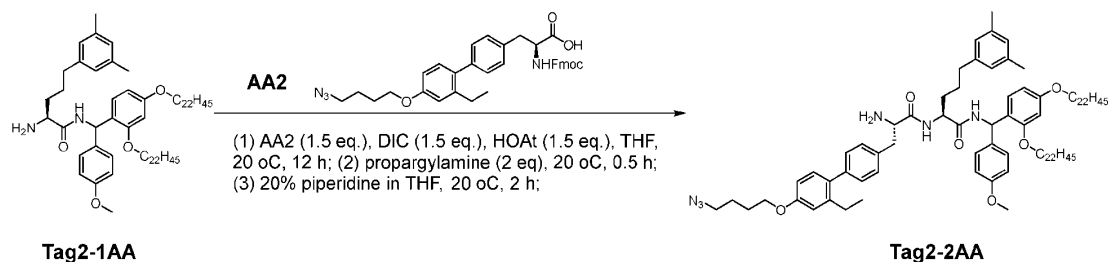
[000565] To a solution of **Tag2** or **Tag2-(n-1)AA** (1 eq.) and **AAn** (1.5 eq.) and HOAt (1.5 eq) in DCM (20V) was added DIC (1.5 eq) in one portion at 20°C. The mixture was stirred at 20°C for 12 hr. After completion, propan-1-amine (2 eq) was added, and the mixture was stirred at 20°C for 0.5 hr. Then 20% piperidine in THF (20V) was added and the mixture was stirred at 20°C for 1 hr. TLC (PE:EtOAc = 3:1) showed that the reaction was complete. The mixture was triturated with ACN/MeOH=3/1 (40V*2) to provide the product **Tag2-nAA** as a light-yellow solid, which was used in the next step directly without purification.

*Step 1: Synthesis of (2S)-2-Amino-N-((2,4-bis(docosyloxy)phenyl)(4-methoxyphenyl)methyl)-5-(3,5-dimethylphenyl)pentanamide (**Tag2-1AA**)*



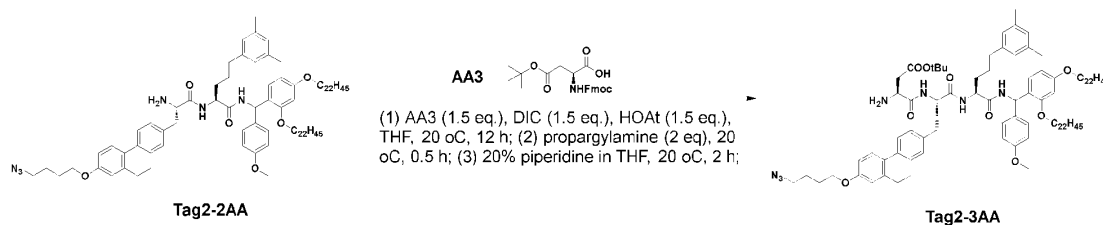
[000566] To a solution of **Tag2** (6.5 g, 7.16 mmol, 95% purity, 1 eq), **AA1** (4.76 g, 10.74 mmol, 1.5 eq), and HOAt (1.46 g, 10.74 mmol, 1.50 mL, 1.5 eq) in DCM (80 mL) was added DIC (1.36 g, 10.74 mmol, 1.66 mL, 1.5 eq) in one portion at 20°C. The mixture was stirred at 20°C for 12 hr. After completion, propan-1-amine (846.45 mg, 14.32 mmol, 1.18 mL, 2 eq) was added and the mixture was stirred at 20°C for 0.5 hr. Then piperidine (16.65 g, 195.52 mmol, 19.31 mL, 27.31 eq) in THF (80 mL) was added and the mixture was stirred at 20°C for 1 hr. TLC (PE:EtOAc = 3:1) showed that the reaction was complete. The mixture was triturated with ACN/MeOH=3/1 (150 mL*2) to provide **Tag2-1AA** (8.5 g, 6.78 mmol, 94.69% yield, 85% purity) as a light-yellow solid, which was used in the next step directly without purification. LCMS (ESI): RT = 2.092 min, m/z calcd. for C₇₁H₁₂₁N₂O₄, 1065.92, [M+H]⁺; m/z found 1065.9; LC-MS Conditions: Method 7.

*Step 2: Synthesis of (2S)-2-((S)-2-Amino-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanamido)-N-((2,4-bis(docosyloxy)phenyl)(4-methoxyphenyl)methyl)-5-(3,5-dimethylphenyl)Pentanamide (**Tag2-2AA**)*



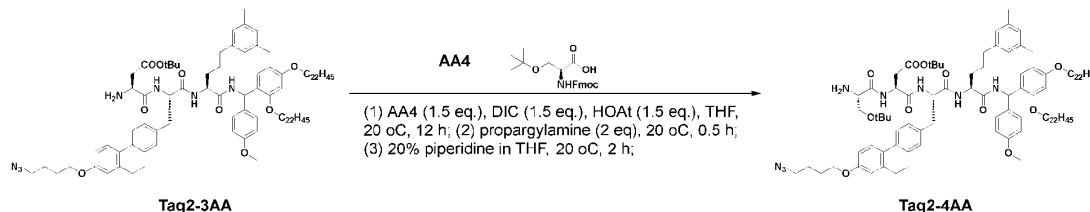
[000567] The reaction was carried out according to the general procedure C for amide coupling and Fmoc deprotection in one-pot using **Tag2-1AA** (3 g, 2.39 mmol, 85% purity, 1 eq) and **AA2** (2.18 g, 3.61 mmol, 1.51 eq). The crude **Tag2-2AA** (3.6 g, 2.27 mmol, 94.68% yield, 90% purity) was obtained as a light-yellow solid. LCMS (ESI): RT = 2.225 min, m/z calcd. for C₉₂H₁₄₄N₆O₆, 1430.11, [M+H]⁺; m/z found 1431.0; LC-MS Conditions: Method 7.

Step 3: Synthesis of (3S)-tert-Butyl 3-amino-4-(((2S)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-(((2,4-bis(docosyloxy)phenyl)(4-methoxyphenyl)methyl)amino)-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoate (Tag2-3AA)



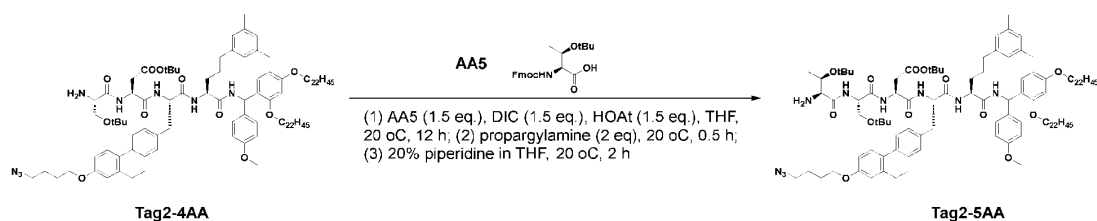
[000568] The reaction was carried out according to the general procedure C for amide coupling and Fmoc deprotection in one-pot using **Tag2-2AA** (3.8 g, 2.39 mmol, 90% purity, 1 eq) and **AA3** (1.48 g, 3.59 mmol, 1.5 eq). The crude **Tag2-3AA** (3.9 g, 2.19 mmol, 91.66% yield, 90% purity) was obtained as a light-yellow solid. LCMS (ESI): RT = 3.660 min, m/z calcd. for C₃₈H₅₀N₇O₆, 700.37 [M-anchor-tBu+3H]⁺; (anchor=C₅₈H₁₀₁O₃, MS=845.8); m/z found 700.4; LC-MS Conditions: Method 7. (anchor = Tag2).

Step 4: Synthesis of (3S)-tert-Butyl 3-((S)-2-amino-3-(tert-butoxy)propanamido)-4-(((2S)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-(((2,4-bis(docosyloxy)phenyl)(4-methoxyphenyl)methyl)amino)-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoate (Tag2-4AA)



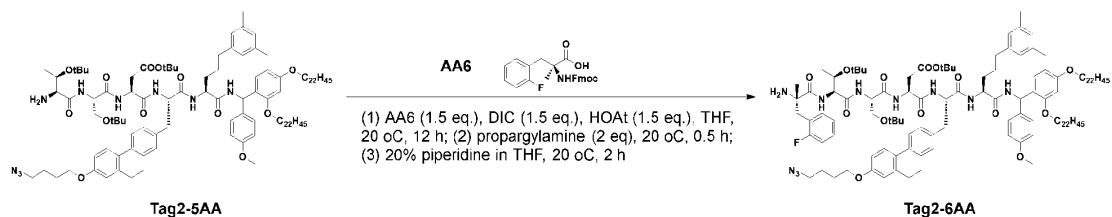
[000569] The reaction was carried out according to the general procedure C for amide coupling and Fmoc deprotection in one-pot using **Tag2-3AA** (3.9 g, 2.19 mmol, 90% purity, 1 eq) and **AA4** (1.26 g, 3.29 mmol, 1.5 eq). The crude **Tag2-4AA** (3.8 g, 1.96 mmol, 89.44% yield, 90% purity) was obtained as a light-yellow solid. LCMS (ESI): RT = 3.540 min, m/z calcd. for C₄₁H₅₅N₈O₈, 787.41 [M-anchor-2tBu+4H]⁺; (anchor=C₅₈H₁₀₁O₃, MS=845.8); m/z found 787.5; LC-MS Conditions: Method 7. (anchor = Tag2).

Step 5: Synthesis of (3S)-tert-Butyl 3-(((S)-2-(((2S,3R)-2-Amino-3-(tert-butoxy)butanoyl)oxy)-3-(tert-butoxy)propanamido)-4-(((2S)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-(((2,4-bis(docosyloxy)phenyl)(4-methoxyphenyl)methyl)amino)-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoate (Tag2-5AA)



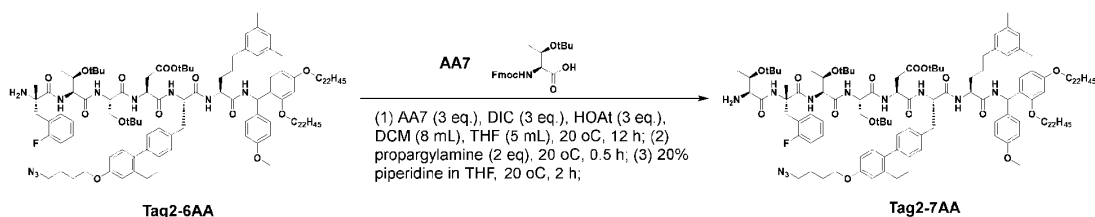
[000570] The reaction was carried out according to the general procedure C for amide coupling and Fmoc deprotection in one-pot using **Tag2-4AA** (3.8 g, 1.96 mmol, 90% purity, 1 eq) and **AA5** (1.17 g, 2.94 mmol, 1.5 eq). The crude **Tag2-5AA** (3.1 g, 1.55 mmol, 78.99% yield, 95% purity) was obtained as a light-yellow solid. LCMS (ESI): RT = 3.510 min, m/z calcd. for C₄₅H₆₂N₉O₁₀, 888.45, [M-anchor-3tBu+5H]⁺; (anchor=C₅₈H₁₀₁O₃, MS=845.8); m/z found 888.5; LC-MS Conditions: Method 7. (anchor = Tag2).

Step 6: Synthesis of (3S)-tert-Butyl 3-((S)-2-(((S)-2-((S)-2-Amino-3-(2-fluorophenyl)-2-methylpropanamido)-3-(tert-butoxy)butanamido)-3-(tert-butoxy)propanamido)-4-(((2S)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-(((2,4-bis(docosyloxy)phenyl)(4-methoxyphenyl)methyl)amino)-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoate (Tag2-6AA**)**



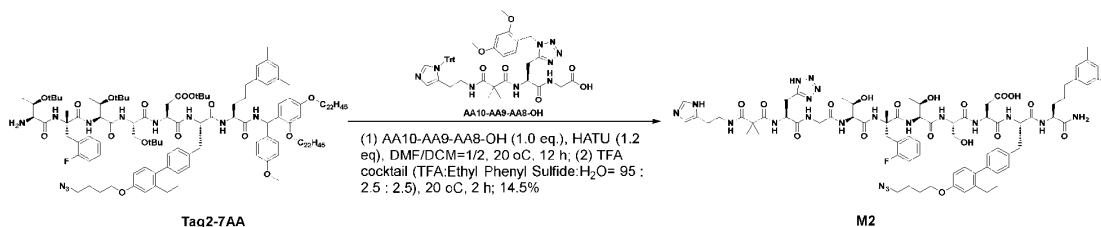
[000571] The reaction was carried out according to the general procedure C for amide coupling and Fmoc deprotection in one-pot using **Tag2-5AA** (1.5 g, 749.31 μmol, 95% purity, 1 eq) and **AA6** (471.44 mg, 1.12 mmol, 1.5 eq). The **Tag2-6AA** (1.36 g, 588.20 μmol, 78.50% yield, 90% purity) was obtained as a light-yellow solid. LCMS (ESI): RT = 3.726 min, m/z calcd. for C₅₅H₇₂FN₁₀O₁₁, 1067.53, [M-anchor-3tBu+5H]⁺; (anchor=C₅₈H₁₀₁O₃, MS=845.8); m/z found 1067.6; LC-MS Conditions: Method 7. (anchor = Tag2).

Step 7: Synthesis of (4R,5S,8S,11S,14S,17S)-tert-Butyl 5-Amino-17-(((2S)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-(((2,4-bis(docosyloxy)phenyl)(4-methoxyphenyl)methyl)amino)-1-oxo-5-(m-tolyl)pentan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-11-(CAN-1-(tert-butoxy)ethyl)-14-(tert-butoxymethyl)-8-(2-fluorobenzyl)-2,2,4,8-tetramethyl-6,9,12,15-tetraoxo-3-oxa-7,10,13,16-tetraazanonadecan-19-oate (Tag2-7AA**)**



[000572] The reaction was carried out according to the general procedure C for amide coupling and Fmoc deprotection in one-pot using **Tag2-6AA** (0.7 g, 302.75 μ mol, 90% purity, 1 eq) and **AA7** (360.99 mg, 908.25 μ mol, 3 eq). The crude **Tag2-7AA** (0.6 g, 241.27 μ mol, 79.69% yield, 90% purity) was obtained as a light-yellow solid. LCMS (ESI): RT = 3.977 min, m/z calcd. for $C_{59}H_{79}FN_{11}O_{13}$, 1168.58, $[M-\text{anchor}-4t\text{Bu}+6H]^+$; (anchor= $C_{58}H_{101}O_3$, MS=845.8); m/z found 1168.5; LC-MS Conditions: Method 7 (anchor = Tag2)(the filtration was very slow and difficult to collect solid particles due to poor precipitate).

*Step 8: Synthesis of (3S)-4-[[[(1S)-1-[[4-[4-(4-Azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-2-[[[(2S,3R)-2-[[[(2S)-3-(2-fluorophenyl)-2-[[[(2S,3R)-3-hydroxy-2-[[2-[[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic Acid (**M2**)*



[000573] To a solution of reactant **AA10-AA9-AA8-OH** (216.01 mg, 238.86 μ mol, 90% purity, 1.1 eq), and **Tag2-7AA** (540 mg, 217.14 μ mol, 90% purity, 1 eq) in DMF (2 mL) and DCM (6 mL) was added HATU (99.08 mg, 260.57 μ mol, 1.2 eq) in one portion at 20°C. The mixture was stirred at 20°C for 2 hr. LCMS showed the reaction converted completely. The mixture was subjected to acidic cleavage by using TFA cocktail (14 mL, TFA:Ethyl Phenyl Sulfide: H_2O = 95:2.5:2.5) for 50 min. The reaction mixture was filtered, and the filtrate was quenched by water/ACN=4/1 (150 mL) and extracted with PE (20 mL*2). The combined aqueous layers were lyophilized to give a residue, which was purified by prep-HPLC (column: Welch Xtimate C18 100*40mm*3 μ m; mobile phase: [water (TFA)-ACN]; B%: 28%-58%, 12min condition) to afford the product **M2** (50 mg, 31.58 μ mol, 14.54% yield, 99.27% purity) as a white solid. LCMS (ESI): RT = 3.910 min, m/z calcd. for $C_{75}H_{101}FN_{20}O_{17}$,

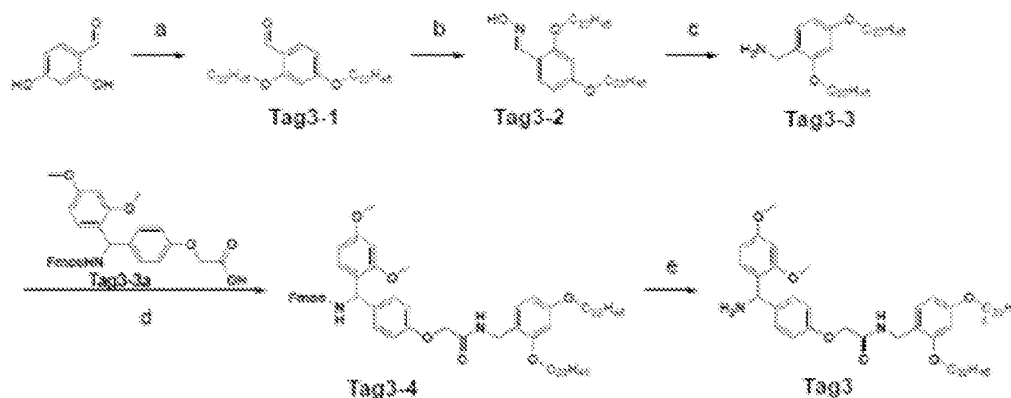
786.375 [M+H]⁺; m/z found 786.8; LC-MS Conditions: Method 7. HPLC: RT = 4.86 min. HPLC conditions: Mobile phase: 1.0% ACN in water (0.1%TFA) to 5% ACN in water (0.1%TFA) in 1 minutes; then from 5% ACN in water (0.1%TFA) to 100% ACN (0.1%TFA) in 5 minutes; hold at 100% ACN (0.1%TFA) for 2 minutes; back to 1.0% ACN in water (0.1%TFA) at 8.01 minutes and hold two minutes. Flow rate: 1.2ml/min. Column: Ultimate XB-C18, 3um, 3.0*50mm.

Example 6. Synthesis (LPPS) of M2 on Tag3

6.1 Synthesis of Tag3

[000574] The synthetic route to make Tag3 is shown in Scheme 4.

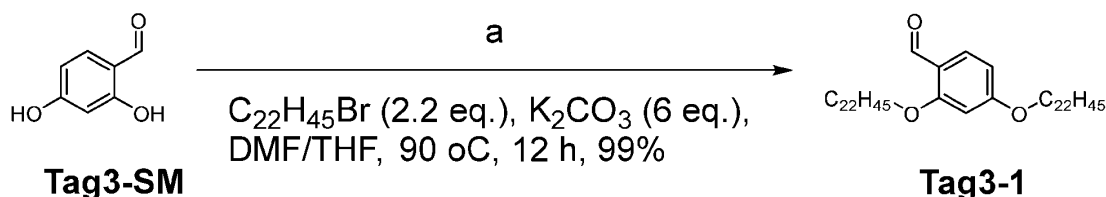
Scheme 4



Reagents and conditions: a) C₂₂H₄₅Br (2.2 eq.), K₂CO₃ (6 eq.), DMF/THF, 90 °C, 12 h, 99%; b) NH₂OH.HCl (3 eq.), Et₃N (5 eq.), toluene (20 mL), 100 °C, 12 h, 93%; c) DIBALH (3.0 eq.), toluene (30 mL), 100 °C, 12 h, 91%; d) 2-[4-[(2,4-dimethoxyphenyl)-(9H-fluoren-9-ylmethoxycarbonylamino) methyl] phenoxy]acetic acid (1.5 eq.), DIC (1.5 eq.), HOAt (1.5 eq.), DCM (2 mL), 20 °C, 12 h, 68%; e) 20% piperidine in THF, 20 °C, 2 h, 99%. Total yield: 56.4%.

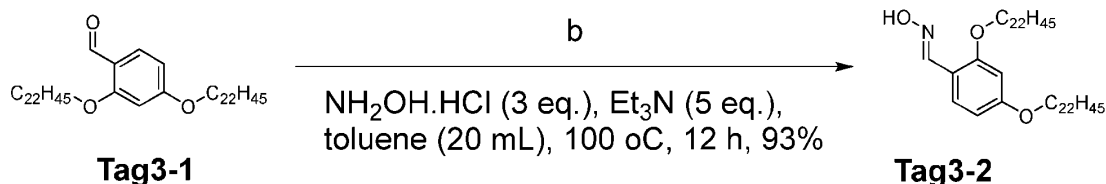
[000575] Bis-alkylation of 2,4-dihydroxybenzaldehyde with C₂₂-alkyl bromide, followed by treatment of **Tag3-1** with same equivalent of hydroxylamine hydrochloride in the presence of TEA in toluene provided the oxime product **Tag3-2**. DIBAL-reduction of **Tag3-2** to amine **Tag3-3** and subsequent coupling with acid **Tag3-3a** provided amide **Tag3-4**. Reagent **Tag3-3a** (CAS: 145069-56-3, Rink Amide Linker.), 4-[(2,4-dimethoxyphenyl)(fmoc-amino)me] phenoxy acetic acid is commonly used in solid phase synthesis for preparation of C- terminal primary amide-peptide. Finally, Fmoc deprotection of **Tag3-4** provided **Tag3**.

Step a: Synthesis of 2,4-Di(docosoxy)benzaldehyde (Tag3-1)



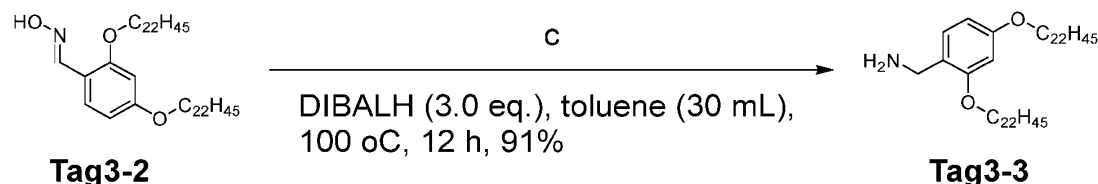
[000576] To a mixture of 2,4-dihydroxybenzaldehyde (2 g, 14.48 mmol, 1 eq) and 1-bromodocosane (12 g, 30.81 mmol, 2.13 eq) in DMF (50 mL) was added K_2CO_3 (12.01 g, 86.88 mmol, 6 eq) and it was stirred at 90 °C for 12 hr. Most of the reactant was consumed and a new spot formed on TLC (PE/EtOAc = 5/1). After completion, the reaction mixture was poured into water (500 mL), the mixture was stirred for 1 hr, and the precipitated solid was collected by filtration. The obtained solid was triturated with acetone (200 mL) and the solid was collected by filtration and slurry-washed in methanol. After filtration, the obtained solid was dried under reduced pressure to give the title compound as an off-white solid. The obtained solid was triturated again with PE (100 mL) and the solid was collected by filtration. The desired compound **Tag3-1** (10.9 g, 14.43 mmol, 99.67% yield, n/a purity) was obtained as an off-white solid. ^1H NMR (400MHz, Chloroform-d) δ = 10.33 (s, 1H), 7.80 (d, J =8.6 Hz, 1H), 6.52 (d, J =8.8 Hz, 1H), 6.42 (d, J =2.1 Hz, 1H), 4.08 – 3.97 (m, 4H), 1.89 – 1.69 (m, 4H), 1.52 – 1.42 (m, 4H), 1.26 (s, 72H), 0.89 (t, J =6.8 Hz, 6H) ppm.

Step b: Synthesis of (1E)-2,4-Di(docosoxy)benzaldehyde Oxime (Tag3-2)



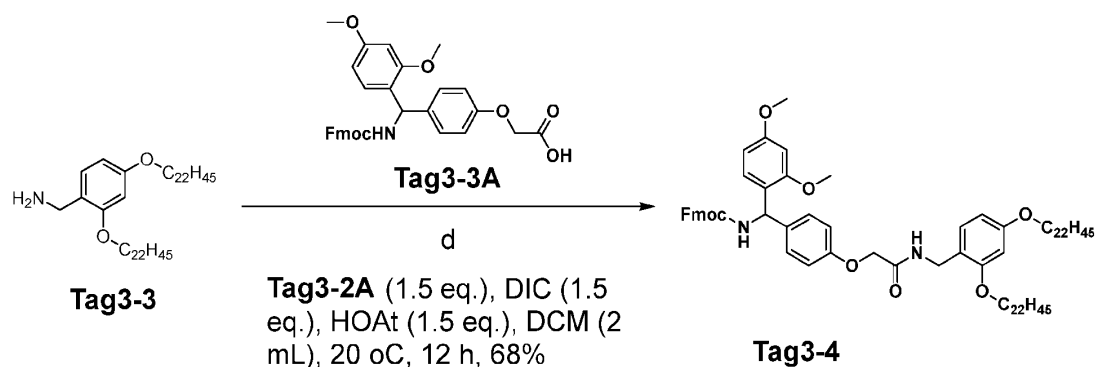
[000577] To a mixture of **Tag3-1** (6.5 g, 8.61 mmol, 1 eq) in toluene (90 mL) was added $\text{NH}_2\text{OH}\cdot\text{HCl}$ (5.98 g, 86.06 mmol, 10 eq) and Et_3N (8.71 g, 86.06 mmol, 11.98 mL, 10 eq), and reaction mixture was stirred at 100 °C for 12 hr. Most of the reactant was consumed and a new spot with larger polarity was formed on TLC (PE/EtOAc = 10/1). After completion, the reaction mixture was cooled down to 20 °C and poured into ACN (300 mL), then the precipitated solid was collected by filtration and slurry-washed in methanol. The obtained solid was triturated with ACN (300 mL) and the solid was collected by filtration. After filtration, the obtained solid was dried under reduced pressure to give the title compound as a brown solid. The desired compound **Tag3-2** (6.2 g, 8.05 mmol, 93.53% yield) was obtained as a brown solid. ^1H NMR (400MHz, Chloroform-d) δ = 8.46 (s, 1H), 7.65 (d, J =8.5 Hz, 1H), 6.51 – 6.41 (m, 2H), 4.03 – 3.91 (m, 4H), 1.87 – 1.71 (m, 4H), 1.50 – 1.42 (m, 4H), 1.27 (s, 72H), 0.89 (t, J =6.8 Hz, 6H) ppm.

Step 3: Synthesis of [2,4-Di(docosoxy)phenyl]methanamine (Tag3-3)



[000578] To a mixture of **Tag3-2** (5 g, 6.49 mmol, 1 eq) in THF (50 mL) was added DIBALH (1 M, 19.47 mL, 3 eq) dropwise at 0°C and the reaction mixture was stirred at 90°C for 12 hr. Most of the reactant was consumed and a new spot with small polarity was formed on TLC (PE/EtOAc = 10/1). The reaction mixture was poured into saturated sodium tartrate tetrahydrate (100 mL), then the precipitated solid was collected by filtration and slurry-washed in methanol. The obtained solid was triturated with ACN (30 mL) and the solid was collected by filtration. After filtration, the obtained solid was dried under reduced pressure to give the title compound **Tag3-3** (4.5 g, 5.95 mmol, 91.66% yield) as an off-white solid. ¹H NMR (400MHz, Chloroform-d) δ = 6.61 – 6.29 (m, 3H), 4.14 – 3.65 (m, 6H), 2.84 (br s, 2H), 1.83 – 1.73 (m, 4H), 1.4 – 1.5 (m, 4H), 1.27 (br s, 72H), 0.89 (br t, J=6.5 Hz, 6H) ppm.

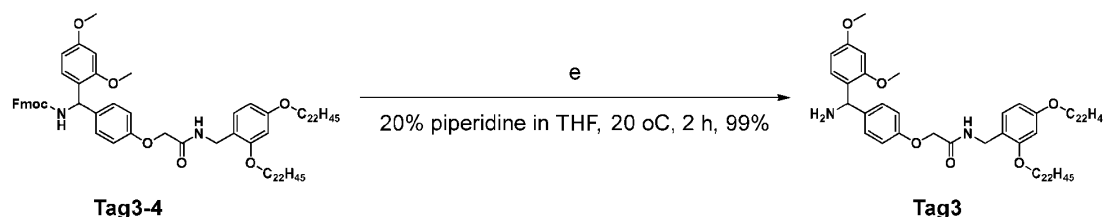
Step 4: Synthesis of 9H-Fluoren-9-ylmethyl N-[[4-[2-[[2,4-di(docosoxy)phenyl]methylamino]-2-oxoethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamate (Tag3-4)



[000579] To a solution of **Tag3-3** (2 g, 2.64 mmol, 1 eq) and 2-[4-[(2,4-dimethoxyphenyl)-(9H-fluoren-9-ylmethoxycarbonylamino)methyl]phenoxy]acetic acid **Tag3-3a** (2.14 g, 3.97 mmol, 1.5 eq) in DCM (20 mL) was added DIC (500.58 mg, 3.97 mmol, 614.21 μ L, 1.5 eq) and HOAt (539.89 mg, 3.97 mmol, 554.87 μ L, 1.5 eq) and the resulting mixture was stirred at 20°C for 2 hr. Most of the reactant was consumed and new spots with large polarity were formed on TLC (PE/EtOAc = 3/1). ACN (100 mL) was added to the reaction mixture and the resulting suspension was filtered and washed with MeOH (30 mL $\times$ 2) to give an off-white solid. The obtained solid was triturated with ACN

(30 mL) and the solid was collected by filtration. After filtration, the obtained solid was dried under reduced pressure to give the title compound **Tag3-4** (2.3 g, 1.80 mmol, 68.06% yield) as an off-white solid. ¹H NMR (400MHz, Chloroform-d) δ = 7.77 (br d, J =7.1 Hz, 2H), 7.60 (br d, J =6.8 Hz, 1H), 7.49 – 7.27 (m, 5H), 7.22 – 7.02 (m, 4H), 6.95 – 6.69 (m, 2H), 6.57 – 6.36 (m, 4H), 6.02 (br d, J =8.6 Hz, 1H), 5.75 (br d, J =7.6 Hz, 1H), 4.55 – 4.14 (m, 5H), 4.08 – 3.66 (m, 10H), 4.08 – 3.63 (m, 1H), 1.95 – 1.67 (m, 4H), 1.54 – 1.41 (m, 4H), 1.27 (s, 72H), 0.89 (t, J =6.8 Hz, 6H) ppm.

Step 5: Synthesis of 2-[4-[Amino-(2,4-dimethoxyphenyl)methyl]phenoxy]-N-[[2,4-di(docosoxy)phenyl]methyl]acetamide (Tag3)



[000580] To a solution of **Tag3-4** (2.2 g, 1.72 mmol, 1 eq) in THF (20 mL) was added piperidine (6.90 g, 81.01 mmol, 8 mL, 47.05 eq) and the resulting mixture was stirred at 20 °C for 2 hr. Most of the reactant was consumed and new spots with large polarity formed on TLC (PE/EtOAc = 9/1). LCMS showed that the desired mass was detected. To the reaction solution was added ACN (100 mL) and the resulting suspension was filtered and washed with MeOH (30 mL $\times$ 2) to give an off-white solid. The obtained solid was triturated with ACN (20 mL) and the solid was collected by filtration. The obtained solid was dried under reduced pressure to give the title compound **Tag3** (1.8 g, 1.71 mmol, 99.04% yield) as an off-white solid. LCMS (ESI): RT= 2.055 min, mass calcd. for $\text{C}_{68}\text{H}_{114}\text{N}_2\text{O}_6\text{H}$ 1055.9 $[\text{M}+\text{H}]^+$, $\text{C}_{68}\text{H}_{112}\text{NO}_6^+$ 1038.8 $[\text{M}-\text{NH}_2]^+$, m/z found 1038.8 $[\text{M}-\text{NH}_2]^+$; LCMS condition: Method 12. ¹H NMR (400MHz, Chloroform-d) δ = 7.22 (br d, J =8.5 Hz, 1H), 7.16 – 7.04 (m, 2H), 6.84 – 6.67 (m, 2H), 6.52 (d, J =2.3 Hz, 1H), 6.49 – 6.29 (m, 4H), 5.36 (s, 1H), 4.43 – 4.24 (m, 1H), 4.05 – 3.85 (m, 5H), 3.82 – 3.70 (m, 6H), 3.19 (s, 2H), 1.83 – 1.75 (m, 4H), 1.26 (s, 76H), 0.90 – 0.87 (m, 6H) ppm.

6.2 Synthesis of Tag3-nAA

[000581] Figure 4 and Table 6 below outline the synthesis of **M2** using **Tag3**.

Table 6

Coupling reaction						de-Fmoc		Product		
#	amino acid		DIC	HOAt	yield	Step#	20% piperidine in THF	yield		Yield %
f	AA1	1.5 eq.	1.5 eq.	1.5 eq.	78.0%	g	30 V	Tag3-1AA	1259.0 [M+H] ⁺	95.0
h	AA2	1.5 eq.	1.5 eq.	1.5 eq.	63.0%	i	30 V	Tag3-2AA	585.3 [M-Tag3+2H] ⁺	97.0
j	AA3	1.5 eq.	1.5 eq.	1.5 eq.	74.0%	k	20 V	Tag3-3AA	700.37 [M-Tag3-tBu+3H] ⁺	89.0
l	AA4	1.5 eq.	1.5 eq.	1.5 eq.	89.0%	m	20 V	Tag3-4AA	787.41, [M-Tag3-2tBu+4H] ⁺	91.0
n	AA5	1.5 eq.	1.5 eq.	1.5 eq.	90.0%			N-FmocTag3-5AA	1110.52, [M-Tag3-3tBu+5H] ⁺	
total yield to make Tag3-5AA										21.7

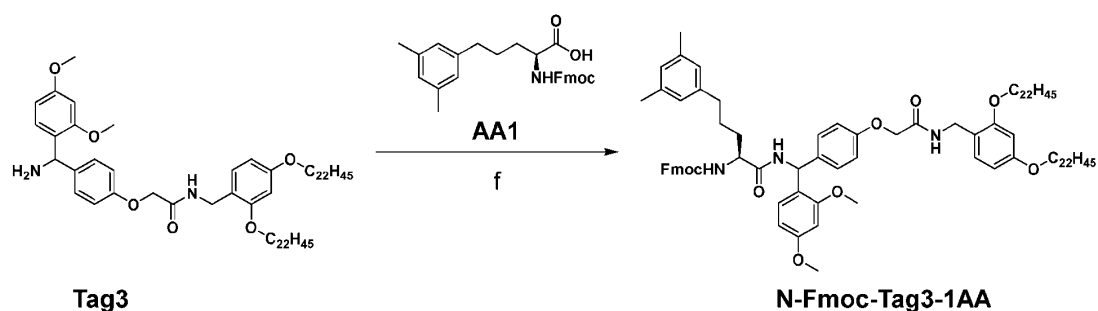
General Procedure D for Amide Coupling to Generate N-Fmoc-Tag3-nAA

[000582] To a solution of **Tag3** or **Tag3-(n-1)AA** (1 eq.) and AAn (1.5 eq.) in DCM (20V) were added DIC (1.5 eq.) and HOAt (1.5 eq.), then the resulting mixture was stirred at 20 °C for 2 hr. Most of the reactant was consumed and new spots with large polarity formed on TLC (PE/EtOAc = 3/1). A small sample was taken and piperidine was added to the small sample to remove Fmoc group. The sample was checked by LCMS, which showed that the desired mass was detected. To the reaction solution was added ACN (80V) and the resulting suspension was filtered and washed with MeOH (30V × 2) to give an off-white solid. The obtained solid was triturated with ACN (20 V) and the solid was collected by filtration. After filtration, the obtained solid was dried under reduced pressure to give the title compound **N-Fmoc-Tag3-nAA** as an off-white solid.

General Procedure E for Fmoc Deprotection to Generate Tag3-nAA:

[000583] A solution of **N-Fmoc-Tag3-nAA** (1 eq.) in 20% piperidine in THF (10V) was stirred at 20°C for 1 hr. Most of the reactant was consumed and new spots formed on TLC (PE/EtOAc = 9/1). LCMS showed the desired mass was detected. To the reaction solution was added ACN (100 V) and the resulting suspension was filtered and washed with MeOH (30V × 2) to give the product. The obtained solid was triturated with ACN (20V) and the solid was collected by filtration. The title compound **Tag3-nAA** was obtained as an off-white solid.

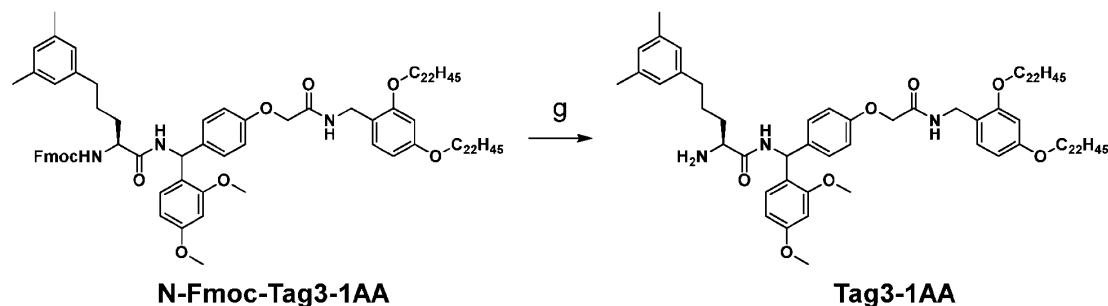
*Step f: Synthesis of 9H-Fluoren-9-ylmethyl N-[(1S)-1-[[[4-[2-[[2,4-di(docosoxy)phenyl] methylamino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbonyl]-4-(3,5-dimethylphenyl)butyl]carbamate (**N-Fmoc-Tag3-1AA**)*



[000584] The reaction was performed using the general procedure D for amide coupling using **Tag3** (1 g, 947.29 μmol , 1 eq) and **AA1** (630.23 mg, 1.42 mmol, 1.5 eq) in DCM (20 mL). **N-Fmoc-Tag3-1AA** (1.1 g, 742.66 μmol , 78.40% yield) was obtained as an off-white solid. LCMS (ESI): RT= 2.252 min, mass calcd. for $\text{C}_{81}\text{H}_{131}\text{N}_3\text{O}_7\text{H}$ 1259.9, m/z found 1260.0 $[\text{M-Fmoc}+\text{H}]^+$; LCMS condition: Method 12.

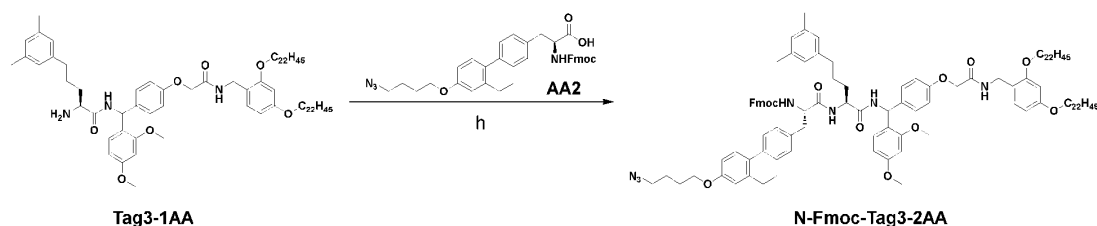
[000585] ^1H NMR (400MHz, Chloroform- d) δ = 7.83 – 7.50 (m, 4H), 7.45 – 7.26 (m, 5H), 7.12 – 6.61 (m, 9H), 6.55 – 6.17 (m, 4H), 5.55 – 5.27 (m, 1H), 4.54 – 4.09 (m, 6H), 4.06 – 3.70 (m, 8H), 3.69 – 3.55 (m, 3H), 3.49 (br s, 6H), 3.17 (br s, 2H), 2.50 (br s, 2H), 2.27 (br s, 6H), 2.01 (s, 2H), 1.27 (br s, 76H), 0.91 – 0.87 (m, 6H) ppm.

Step g: Synthesis of (2S)-2-Amino-N-[[4-[2-[[2,4-di(docosoxy)phenyl]methylamino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]-5-(3,5-dimethylphenyl)pentanamide (**Tag3-1AA**)



[000586] The reaction was performed using general procedure E for Fmoc deprotection using **N-Fmoc-Tag3-1AA** (1 g, 675.15 μmol , 1 eq) in THF (10 mL). **Tag3-1AA** (0.81 g, 643.41 μmol , 95.30% yield) was obtained as an off-white solid. LCMS (ESI): RT= 6.848 min, mass calcd. for $\text{C}_{81}\text{H}_{131}\text{N}_3\text{O}_7\text{H}$ 1258.9 $[\text{M}+\text{H}]^+$, m/z found 1259.0 $[\text{M}+\text{H}]^+$; LCMS condition: Method 12.

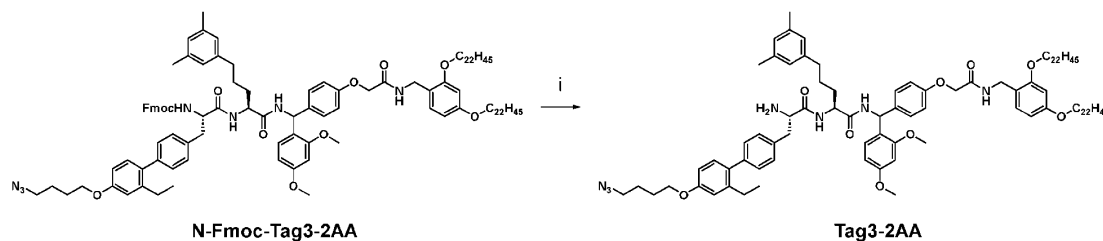
Step h: Synthesis of 9H-Fluoren-9-ylmethyl N-[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-[[2,4-di(docosoxy)phenyl]methylamino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]carbamate (**N-Fmoc-Tag3-2AA**)



[000587] The reaction was performed using the general procedure D for amide coupling using **Tag3-1AA** (0.6 g, 476.60 μmol , 1 eq) and **AA2** (432.30 mg, 714.90 μmol , 1.5 eq). **N-Fmoc-Tag3-2AA 5** (0.56 g, 303.43 μmol , 63.66% yield) was obtained as an off-white solid. LCMS (ESI): RT= 6.048 min, mass calcd. for $\text{C}_{49}\text{H}_{54}\text{N}_6\text{O}_5\text{Na}$ 829.4, m/z found 829.4 $[\text{M-Rink amide-Ancor}+2\text{H}]^+$; Rink amide = $\text{C}_{17}\text{H}_{18}\text{O}_4$, anchor = $\text{C}_{51}\text{H}_{97}\text{NO}_2$; LC-MS Conditions: Method 7.

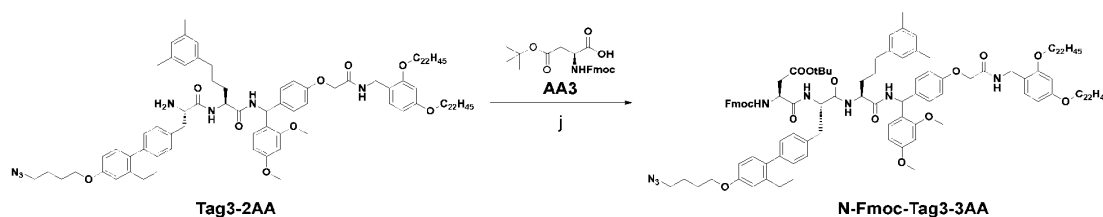
[000588] ^1H NMR (400MHz, Chloroform- d) δ = 7.75 (br d, J =6.3 Hz, 2H), 7.57 – 7.28 (m, 5H), 7.24 – 6.89 (m, 10H), 6.86 – 6.62 (m, 5H), 6.54 – 6.31 (m, 5H), 6.20 (br s, 1H), 5.27 (br s, 1H), 4.53 – 4.07 (m, 7H), 4.06 – 3.53 (m, 12H), 3.40 (br t, J =6.3 Hz, 2H), 3.17 (br d, J =7.3 Hz, 2H), 2.64 – 2.45 (m, 4H), 2.22 (br d, J =3.3 Hz, 6H), 1.95 – 1.71 (m, 8H), 1.27 (s, 82H), 1.06 (br t, J =7.2 Hz, 3H), 0.90 – 0.87 (m, 6H) ppm.

Step i: Synthesis of (2S)-2-((S)-2-Amino-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanamido)-N-((4-(2-((2,4-bis(docosyloxy)benzyl)amino)-2-oxoethoxy)phenyl)(2,4-dimethoxyphenyl)methyl)-5-(3,5-dimethylphenyl)pentanamide (**Tag3-2AA**)



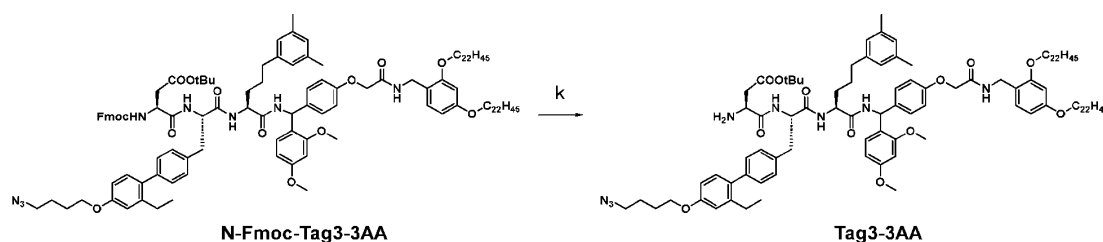
[000589] The reaction was performed using general procedure E for Fmoc deprotection using **N-Fmoc-Tag3-2AA** (400 mg, 216.73 μmol , 1 eq), **Tag3-2AA** (380 mg, 210.67 μmol , 97.20% yield, 90% purity) as a light-yellow solid was obtained. LC-MS Conditions: Method 7.

Step j: Synthesis of (3S)-tert-Butyl 3-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-4-(((2S)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-((4-(2-((2,4-bis(docosyloxy)benzyl)amino)-2-oxoethoxy)phenyl)(2,4-dimethoxyphenyl)methyl)amino)-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoate (**N-Fmoc-Tag3-3AA**)



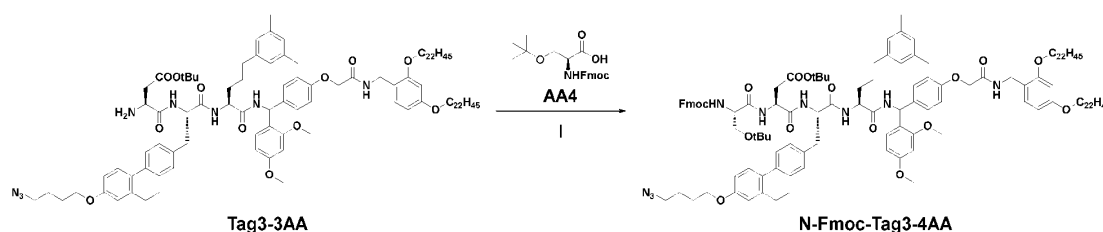
[000590] The reaction was performed using the general procedure D for amide coupling using **Tag3-2AA** (380 mg, 210.67 μmol , 90% purity, 1 eq) and **AA3** (130.02 mg, 316.01 μmol , 1.5 eq). **N-Fmoc-Tag3-3AA** (350 mg, 156.19 μmol , 74.14% yield, 90% purity) was obtained as a light-yellow solid. LCMS (ESI): RT = 5.542 min, m/z calcd. For $\text{C}_{53}\text{H}_{60}\text{N}_7\text{O}_8$, 922.44, [M-Rink amide-AnchortBu+3H]⁺; Rink amide= $\text{C}_{17}\text{H}_{18}\text{O}_4$, anchor= $\text{C}_{51}\text{H}_{97}\text{NO}_2$; m/z found 922.3; LC-MS Conditions: Method 7.

*Step k: Synthesis of (3S)-tert-Butyl 3-Amino-4-(((2S)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-(((4-(2-((2,4-bis(docosyloxy)benzyl)amino)-2-oxoethoxy)phenyl)(2,4-dimethoxyphenyl) methyl)amino)-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoate (**Tag3-3AA**)*



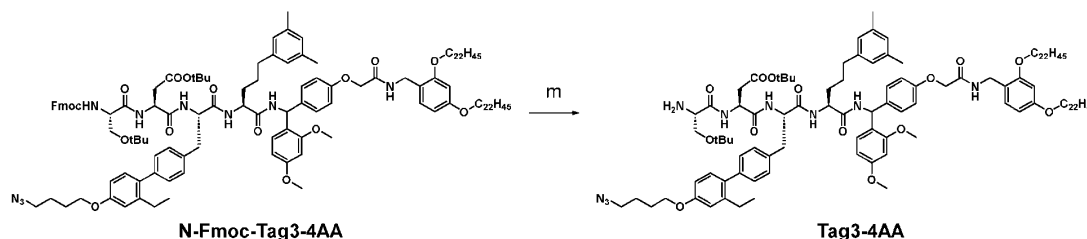
[000591] The reaction was performed using general procedure E for Fmoc deprotection using **N-Fmoc-Tag3-3AA** (350 mg, 156.19 μmol , 90% purity, 1 eq). **Tag3-3AA** (280 mg, 140.43 μmol , 89.91% yield, 90% purity) was obtained as a light-yellow solid. LCMS (ESI): RT = 3.810 min, m/z calcd. For $\text{C}_{38}\text{H}_{50}\text{N}_7\text{O}_6$, 700.37, [M-Rink amide-AnchortBu+3H]⁺; Rink amide= $\text{C}_{17}\text{H}_{18}\text{O}_4$, anchor= $\text{C}_{51}\text{H}_{97}\text{NO}_2$; m/z found 700.3; LC-MS Conditions: Method 7.

*Step l: Synthesis of (3S)-tert-Butyl 3-(((S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)propanamido)-4-(((2S)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-(((4-(2-((2,4-bis(docosyloxy)benzyl)amino)-2-oxoethoxy)phenyl)(2,4-dimethoxyphenyl) methyl)amino)-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoate (**N-Fmoc-Tag3-4AA**)*



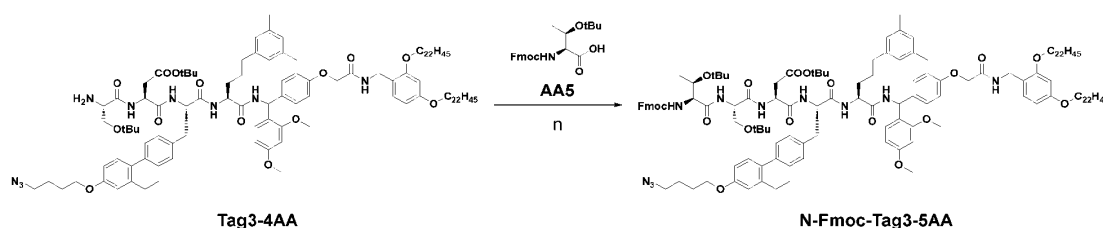
[000592] The reaction was performed using the general procedure D for amide coupling using **Tag3-3AA** (280 mg, 140.35 μmol , 90% purity, 1 eq) and **AA4** (80.72 mg, 210.52 μmol , 1.5 eq). **N-Fmoc-Tag3-4AA** (300 mg, 124.94 μmol , 89.02% yield, 90% purity) was obtained as a light-yellow solid. LCMS (ESI): RT = 5.337 min, m/z calcd. for $\text{C}_{56}\text{H}_{65}\text{N}_8\text{O}_{10}$, 1009.47, $[\text{M-Rink amide-Anchor-2tBu+4H}]^+$; Rink amide= $\text{C}_{17}\text{H}_{18}\text{O}_4$, anchor= $\text{C}_{51}\text{H}_{97}\text{NO}_2$; m/z found 1009.4; LC-MS Conditions: Method 7.

*Step m: Synthesis of (3S)-tert-Butyl 3-((S)-2-Amino-3-(tert-butoxy)propanamido)-4-(((2S)-3-(4'-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-(((4-(2-((2,4-bis(docosyloxy)benzyl)amino)-2-oxoethoxy)phenyl)(2,4-dimethoxyphenyl)methyl)amino)-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoate (**Tag3-4AA**)*



[000593] The reaction was performed using general procedure E for Fmoc deprotection using **N-Fmoc-Tag3-4AA** (280 mg, 116.67 μmol , 90% purity, 1 eq). **NTag3-4AA** (230 mg, 106.83 μmol , 91.56% yield, 90% purity) was obtained as a light-yellow solid. LC-MS Conditions: Method 7.

*Step n: Synthesis of (5S,8S,11S)-tert-Butyl 11-(((2S)-3-(4'-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-1-(((2S)-1-(((4-(2-((2,4-bis(docosyloxy)benzyl)amino)-2-oxoethoxy)phenyl)(2,4-dimethoxyphenyl)methyl)amino)-5-(3,5-dimethylphenyl)-1-oxopentan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-5-(CAN-1-(tert-butoxy)ethyl)-8-(tert-butoxymethyl)-1-(9H-fluoren-9-yl)-3,6,9-trioxo-2-oxa-4,7,10-triazatridecan-13-oate (**N-Fmoc-Tag3-5AA**)*



[000594] To a solution of **Tag3-4AA** (230 mg, 106.83 μ mol, 90% purity, 1 eq), **AA5** (63.69 mg, 160.24 μ mol, 1.5 eq) and HOAt (21.81 mg, 160.24 μ mol, 22.42 μ L, 1.5 eq) in DCM (4 mL) was added DIC (20.22 mg, 160.24 μ mol, 24.81 μ L, 1.5 eq) in one portion at 20°C under N₂. The mixture was stirred at 20°C for 2 hr. To monitor the reaction, a small amount of residue was subjected to acidic cleavage by using TFA cocktail (0.1 mL, TFA:TIPS:H₂O:anisole= 95:2.5:2.5:4.5) for 1 h. LCMS showed the reaction converted completely. The mixture was triturated with ACN (20 mL*3) to give the crude product **N-Fmoc-Tag3-4AA** (250 mg, 97.10 μ mol, 90.90% yield, 90% purity) as a light-yellow solid.

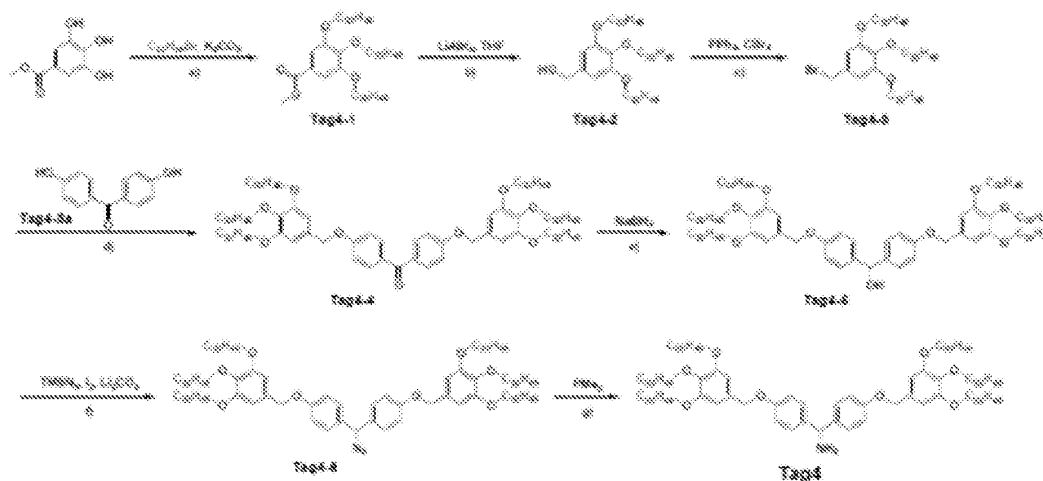
[000595] LCMS (ESI): RT = 5.170 min, m/z calcd. for $C_{60}H_{72}N_9O_{12}$, 1110.52, [M-Rink amide-Anchor-3tBu+5H]⁺; Rink amide= $C_{17}H_{18}O_4$, anchor= $C_{51}H_{97}NO_2$; m/z found 1110.4; LC-MS Conditions: Method 7.

Example 7. Synthesis (LPPS) of M2 on Tag4

7.1 Synthesis of Tag 4

[000596] The synthetic route for the preparation of Tag4 is shown in Scheme 5 below.

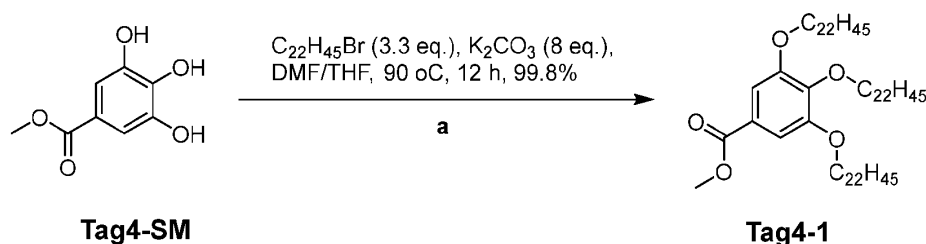
Scheme 5



Reagents and conditions: a) $C_{22}H_{45}Br$ (3.3 eq.), K_2CO_3 (8 eq.), DMF/THF, 90 °C, 12 h, 99.8%; b) $LiAlH_4$ (3 eq.), THF, 70 °C, 3 h, 92.3%; c) PBr_3 (3 eq.), DCM, 20 °C, 3h, 63%; d) 55A (1 eq.), K_2CO_3 (6 eq.), NMP, 90 °C, 16h, 90.5%; e) $NaBH_4$ (5 eq.), EtOH (1 V), THF (10 V), 70 °C, 12 h, 89.9%; f) I_2 (1.8 eq.), Li_2CO_3 (0.02 eq.), $TMSN_3$ (4.5 eq.), DCM, 12 h, 37°C, 80.9%; g) Pme_3 (3 eq.), H_2O , THF/DCM, 20 °C, 12 h, 66.8%. Total yield: 25.5%

[000597] Tris-alkylation of **3,4,5-trihydroxy benzoyl ester** with C22-alkyl bromide, followed by LAH reduction of ester **Tag4-1** produced alcohol **Tag4-2** (Matsuda et al., "Solution-Phase Synthesis of Oligodeoxyribonucleotides Using the H-Phosphonate Method with N-Unprotected 5'-Phosphite Monomers," *RSC Adv.* 11:38094-38107 (2021), which is hereby incorporated by reference in its entirety). Conversion of alcohol **Tag4-2** to bromo analog **Tag4-3** with triphenylphosphine and carbon tetra bromide, followed by alkylation on **Tag4-3a**, generated hex-alkylated derivative **Tag4-4**. Conversion of ketone **Tag4-4** to alcohol **Tag4-5** with sodium borohydride and then conversion of alcohol **Tag4-5** to azide **Tag4-6** with trimethylsilylazide as nucleophile in the presence of lithium carbonate as base, with final reduction of azide **Tag4-6** with trimethylphosphine produced **Tag4**.

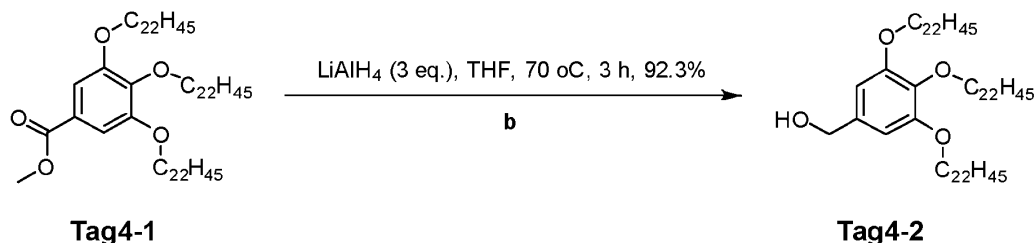
Step a: Synthesis of Methyl 3,4,5-tri(Docosyloxy)benzoate (Tag4-1)



[000598] To a mixture of methyl 3,4,5-trihydroxybenzoate (5 g, 27.15 mmol, 1 eq.) and 1-bromodocosane (34.90 g, 89.60 mmol, 3.3 eq.) in DMF (100 mL) and THF (20 mL) was added K_2CO_3 (30.02 g, 217.22 mmol, 8 eq.) and the reaction mixture was stirred at 90 °C for 12 hr. Most of the reactant was consumed and a new spot with smaller polarity formed on TLC (PE/EtOAc = 3/1). After completion of the reaction, the reaction mixture was poured into purified water (1000 mL), the mixture was stirred for 1 hr, and the precipitated solid was collected by filtration. The obtained solid was triturated with acetone (300 mL) and the solid was collected by filtration and slurry-washed in methanol. After filtration, the obtained solid was dried under reduced pressure to give the title compound as an off-white solid. Compound **Tag4-1** (30.1 g, 27.12 mmol, 99.88% yield) was obtained as a gray solid. 1H NMR (400MHz, Chloroform- d) δ = 7.29 (s, 2H), 4.03 (dt, J =2.5, 6.4 Hz, 6H), 3.91

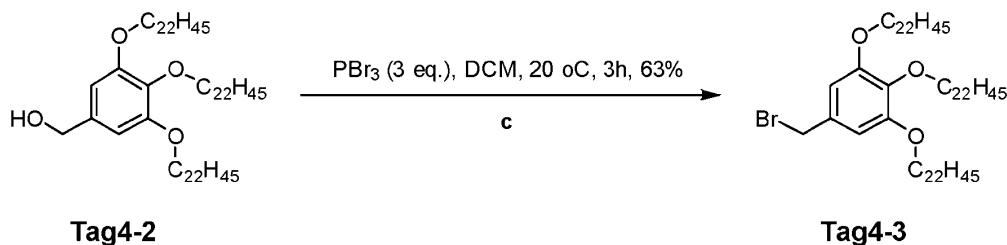
(s, 2H), 3.93 – 3.87 (m, 1H), 1.85 – 1.71 (m, 6H), 1.54 – 1.45 (m, 6H), 1.28 (s, 108H), 0.90 (t, J=6.8 Hz, 9H) ppm.

Step b: Synthesis of [3,4,5-tri(Docosoxy)phenyl]methanol (Tag4-2)



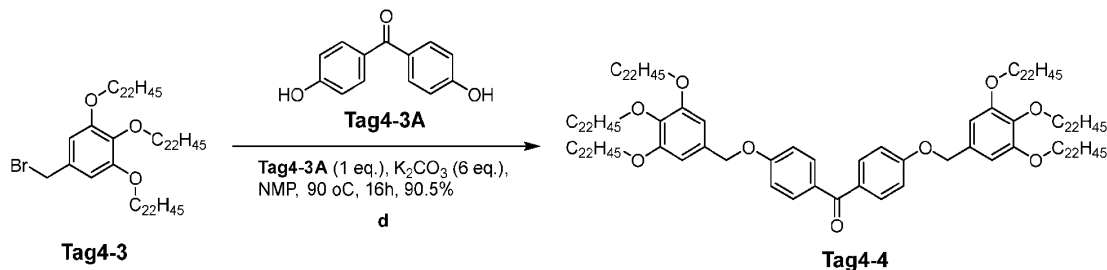
[000599] To a solution of compound **Tag4-1** (1 g, 900.99 μmol , 1 eq.) in THF (10 mL) was added LiAlH_4 (102.59 mg, 2.70 mmol, 2.70 mL, 3.0 eq.) at 0°C. Then the mixture was heated to 70°C for 3 hr. TLC (PE/EA=20/1) showed that most of the reactant was consumed completely and one main new spot was detected. The reaction mixture was quenched with 1N HCl and the mixture was stirred at 20°C for 1h. Then filtered and the filter cake was concentrated to give the product. Compound **Tag4-2** (0.9 g, 831.88 μmol , 92.33% yield) was obtained as a gray solid. ^1H NMR (400 MHz, Chloroform- d) δ ppm 6.58 (s, 2 H) 4.62 (s, 2 H) 3.93 - 4.04 (m, 6 H) 1.72 - 1.87 (m, 6 H) 1.43 - 1.55 (m, 6 H) 1.28 (s, 108 H) 0.90 (t, J=6.79 Hz, 9 H).

Step c: Synthesis of 5-(Bromomethyl)-1,2,3-tri(docosoxy)benzene (Tag4-3)



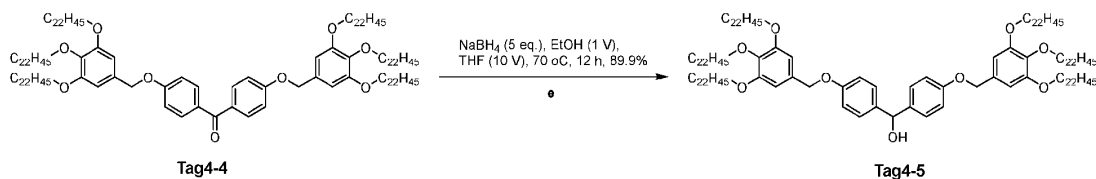
[000600] To a solution of compound **Tag4-2** (0.9 g, 831.88 μmol , 1 eq.) in DCM (10 mL) was added a solution of PBr_3 (675.54 mg, 2.50 mmol, 3.0 eq.) in DCM (10 mL). Then the solution was stirred at 20°C for 3 h. TLC (PE/EA=10/1) showed that the reactant was consumed completely, and one small polarity spot was detected. The reaction mixture was quenched with H_2O (50 mL) and ACN (50 mL) and the mixture was stirred at 20°C for 1h. Then filtered and the filter cake was dried by evaporation of the solvent to give the product. Compound **Tag4-3** (0.6 g, 524.12 μmol , 63.00% yield) was obtained as a white solid. ^1H NMR (400 MHz, Chloroform- d) δ ppm 6.58 (s, 2 H) 4.44 (s, 2 H) 3.92 – 4.00 (m, 6 H) 1.86 (dt, J=6.53, 3.26 Hz, 7 H) 1.42 – 1.52 (m, 8 H) 1.26 (s, 125 H) 0.86 – 0.93 (m, 12 H).

Step d: Synthesis of Bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methanone (Tag4-4)



[000601] A solution of Compound **Tag4-3a** (500 mg, 2.33 mmol, 1.0 eq.) and K_2CO_3 (1.94 g, 14.00 mmol, 6.0 eq.) in NMP (150 mL) was degassed and purged with N_2 for 10 times about 10 min. Then the mixture was heated to 90°C , then a solution of **Tag4-3** (5.37 g, 4.69 mmol, 2.01 eq.) in NMP (350 mL) was added under N_2 . Then the mixture was degassed and purged with N_2 for 10 times about 10 min, stirred at 90°C for 16 hr. TLC (PE/EA=20/1) showed that starting material was consumed completely and the desired spot was detected. The reaction solution was added slowly to the stirring ACN (1000 ml), then the reaction mixture was filtered; and the filter cake was washed with MeOH (250 mL*3) and ACN (250 mL*3). The filter cake was dried by evaporation of the residual solvent to give the product. Compound **Tag4-4** (5.5 g, 2.11 mmol, 90.55% yield, 90% purity) was obtained as a white solid. ^1H NMR (400 MHz, Chloroform- d) δ ppm 7.80 (d, $J=8.75$ Hz, 4 H) 6.99 – 7.09 (m, 4 H) 6.63 (s, 4 H) 5.03 (s, 4 H) 3.95 – 4.01 (m, 12 H) 1.80 (br dd, $J=14.07$, 6.44 Hz, 12 H) 1.43 – 1.54 (m, 12 H) 1.26 (s, 226 H) 0.89 (t, $J=6.69$ Hz, 18 H).

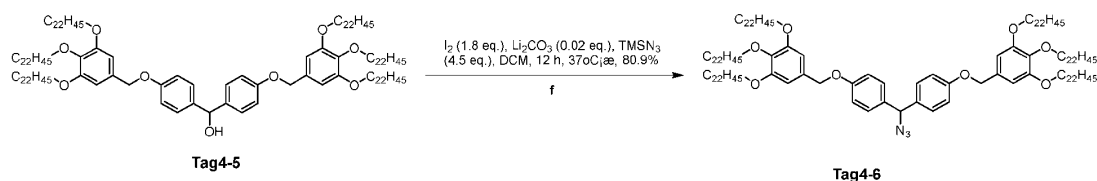
Step e: Synthesis of Bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methanol (Tag4-5)



[000602] To a solution of **Tag4-4** (5.5 g, 2.35 mmol, 1 eq.) in THF (100 mL) and EtOH (25 mL) was added NaBH_4 (444.21 mg, 11.74 mmol, 5.0 eq.) slowly, and the mixture was heated to 70°C and stirred at the 77°C for 5 hr. TLC (PE/EA=10/1) showed that starting material was consumed completely and the new spot was detected. The reaction mixture slowly was added to sat. NH_4Cl /ACN (550 mL/750 mL). The slurry was filtered, and the obtained crystals were washed with ACN (250 mL*3) and methanol (250 mL*3) to give the

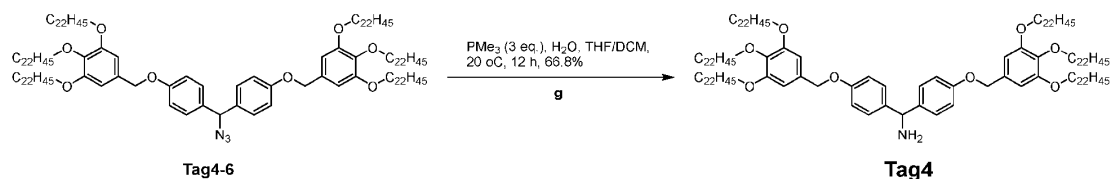
product. Compound **Tag4-5** (5.5 g, 2.11 mmol, 89.92% yield, 90% purity) was obtained as a white solid. ^1H NMR (400 MHz, Chloroform- d) δ ppm 7.30 (d, $J=8.63$ Hz, 4 H) 6.96 (d, $J=8.63$ Hz, 4 H) 6.62 (s, 4 H) 5.81 (br s, 1 H) 4.94 (s, 4 H) 3.95 – 4.01 (m, 12 H) 1.74 – 1.84 (m, 12 H) 1.48 (br s, 12 H) 1.28 (s, 226 H) 0.90 (t, $J=6.75$ Hz, 18 H).

Step f: Synthesis of 5-[[4-[Azido-[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methyl]phenoxy]methyl]phenoxy]methyl]-1,2,3-tri(docosoxy)benzene (Tag4-6**)**



[000603] To a mixture of compound **Tag4-5** (5.5 g, 2.35 mmol, 1 eq.) and I_2 (1.07 g, 4.22 mmol, 850.79 μL , 1.8 eq.) in DCM (105 mL) were added Li_2CO_3 (3.47 mg, 46.93 μmol , 31.67 μL , 0.02 eq.) and TMSN_3 (1.22 g, 10.56 mmol, 1.39 mL, 4.5 eq.), then the reaction mixture was stirred at 37 °C for 12 hr. The reactant was consumed, and the desired spot formed on TLC (PE/EtOAc = 20/1). The reaction solution was added slowly to the stirring ACN (750 mL) and sat. Na_2SO_3 (250 mL), then the reaction mixture was filtered; and the filter cake was washed with methanol (250 mL*3) and ACN (250 mL*3). The filter cake was dried by evaporation of the residual solvent to give the product. **Tag4-6** (5.0 g, 1.90 mmol, 80.95% yield, 90% purity) was obtained as a white solid. ^1H NMR (400 MHz, Chloroform- d) δ ppm 7.23 (br d, $J=8.63$ Hz, 4H) 6.96 (br d, $J=8.63$ Hz, 4H) 6.61 (s, 4 H) 5.62 (s, 1H) 5.65 (s, 1H) 4.93 (s, 4H) 3.94 – 3.99 (m, 12H) 1.76 – 1.84 (m, 12H) 1.43 – 1.50 (m, 12H) 1.27 (s, 226H) 0.87 – 0.90 (m, 18H).

Step g: Synthesis of Bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methanamine (Tag4**)**



[000604] To a solution of **Tag4-6** (4.5 g, 1.90 mmol, 1 eq.) in THF (50 mL), DCM (50 mL), and H_2O (2.5 mL) was added PMe_3 (1 M, 5.70 mL, 3.0 eq.). Then the reaction mixture was stirred at 20°C for 12 hr. TLC (PE/EA=20/1) showed that the reactant was consumed completely, and TLC (PE/EA=1/1) showed that the desired spot was detected. The reaction

solution was added slowly to the stirring ACN (800 mL) and H₂O (200 mL), then the reaction mixture was filtered; the filter cake was triturated with ACN (800 mL) again and then the filter cake was washed with MeOH (250 mL*3) and ACN (250 mL*3). The filter cake was dried by evaporation of the residual solvent to give the product **Tag4** (3.5 g, 1.27 mmol, 66.84% yield, 85% purity) was obtained as a white solid. ¹H NMR (400 MHz, Chloroform-d) δ ppm 7.28 – 7.33 (m, 4H) 6.90 – 6.99 (m, 4H) 6.60 – 6.66 (m, 4H) 5.16 (s, 1H) 4.93 (s, 4H) 3.95 – 4.01 (m, 12H) 1.76 – 1.82 (m, 12H) 1.48 (br s, 12H) 1.28 (s, 226H) 0.88 – 0.92 (m, 18H).

7.2 Synthesis of **Tag4-M2**

[000605] Figure 5 and Table 7 below outline the synthesis of **M2** using **Tag4**.

Table 7.

Step#	coupling						de-Fmoc		Product	
	#	amino acid		DIC	HOAt	yield	#	20% piperidine in THF	yield	# MS (M+H) ⁺
1	a	AA1	1.5 eq.	1.5 eq.	1.5 eq.	70.4%	b	20 V	100.0%	Tag4-1AA
2	c	AA2	1.5 eq.	1.5 eq.	1.5 eq.	67.6%	d	20 V	96.3%	Tag4-2AA
3	e	AA3	1.5 eq.	1.5 eq.	1.5 eq.	59.1%	f	20 V	84.6%	Tag4-3AA
4	g	AA4	1.5 eq.	1.5 eq.	1.5 eq.	86.4%	h	20 V	99.2%	Tag4-4AA
5	i	AA5	1.5 eq.	1.5 eq.	1.5 eq.	85.7%	j	20 V	82.0%	Tag4-5AA
6	k	AA6	1.5 eq.	1.5 eq.	1.5 eq.	71.5%	l	20 V	93.0%	Tag4-6AA
7	m	AA7	5 eq.	5 eq.	5 eq.	90.4%	n	20 V	87.8%	Tag4-7AA
8	o	AA8	1.5 eq.	1.5 eq.	1.5 eq.	86.8%	p	20 V	94.2%	Tag4-8AA
9	q	DMB-AA9	1.5 eq.	1.5 eq.	1.5 eq.	88.1%	r	20 V	100.0%	Tag4-9AA
10	s	AA10	1.5 eq.	1.5 eq.	1.5 eq.	90.0%				Tag4-10AA
11	t	cleavage				0.7%*				M2

*: low yield is due to non-efficient cleavage of **M2** from **Tag4**.

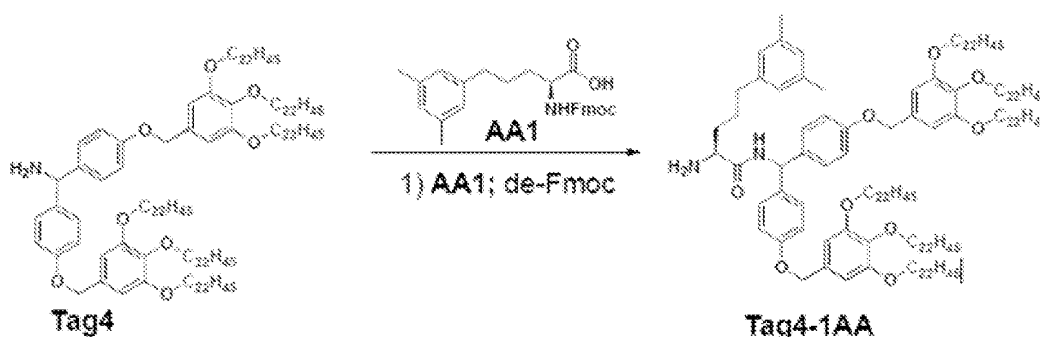
General Procedure F for Amide Coupling to Prepare N-Fmoc-Tag4-nAA

[000606] To a solution of **Tag4** or **Tag4-(n-1)AA** (1 eq.) and **AA_n** (1.5 eq.) and HOAt (1.5 eq) in DCM (20V) were added DIC (1.5 eq.) and HOAt (1.5 eq). The solution was stirred at 20°C for 12 h. A small sample was taken and subjected to acidic cleavage by using a TFA cocktail (TFA: TIPS: H₂O = 95:2.5:2.5). The sample was filtered and analyzed by LCMS which showed that the reactant was consumed, and the desired mass was detected. To the reaction solution was added ACN (80V) and the resulting suspension was filtered and washed with ACN (20V × 2) to give an off-white solid. The solvent was removed by filtration and the filter cake was dried by evaporation of the residual solvent to give the product **N-Fmoc-Tag4-nAA** as an off-white solid, which was used directly in the next step.

General Procedure G for Fmoc Deprotection to Give Tag4-nAA

[000607] A solution of compound **N-Fmoc-Tag4-nAA** (1 eq.) in 20% piperidine in THF and DCM (20V) was stirred at 20°C for 2 hr. TLC (PE/EA=1/1) showed that the reactant was consumed completely, and the desired spot was detected. To the reaction solution was added ACN (200V) and the resulting suspension was filtered and washed with ACN (40V × 2) to give Tag4-nAA as an off-white solid. The crude product was dried under reduced pressure and used in the next step directly without further purification.

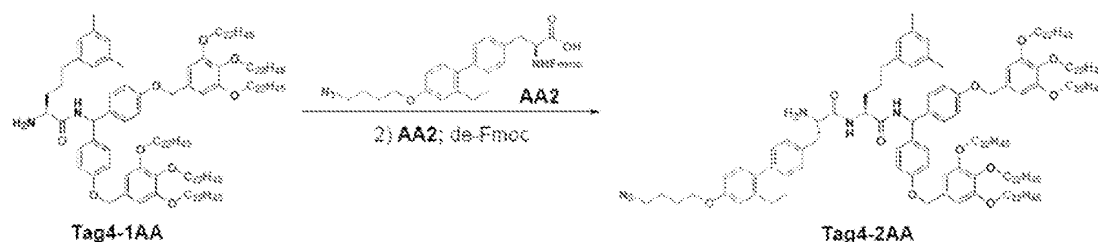
Step 1: Synthesis of (2S)-2-Amino-N-[bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methyl]-5-(3,5-dimethylphenyl)pentanamide (Tag4-1AA)



[000608] The reaction was carried out following the general procedure F for amide coupling using **Tag4** (2.5 g, 1.07 mmol, 1 eq.) and **AA1** (709.88 mg, 1.60 mmol, 1.5 eq.). **N-Fmoc-Tag4-1AA** (2.6 g, 751.31 μmol, 70.41% yield, 80% purity) was obtained as an off-white solid. LC-MS: RT= 4.061 min, mass calcd. for $C_{28}H_{31}N_2O_3^+$ 443.23, m/z found 443.20 [M – anchor + H]⁺; (anchor= $C_{159}H_{286}O_8$, MS=2325.21). LCMS condition: Method 12. anchor = Tag4.

[000609] The reaction was carried out following the general procedure G for Fmoc deprotection using **N-Fmoc-Tag4-1AA** (2.0 g, 722.41 μmol, 1 eq.). **Tag4-1AA** (1.6 g, crude) was obtained as a white solid which was used directly in the next step. LC-MS: 443.2 (M+H)⁺.

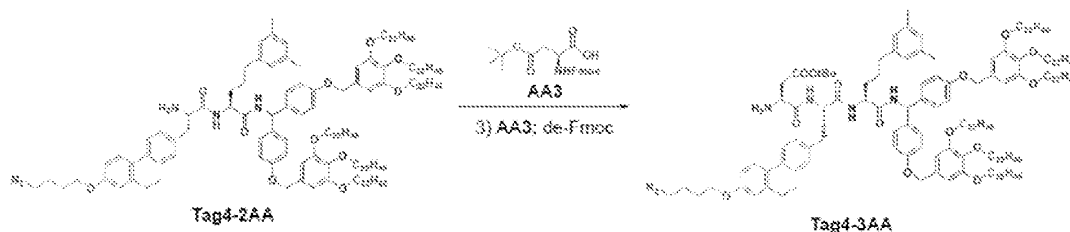
Step 2: Synthesis of (2R)-2-[[[(2S)-2-Amino-3-[4-[4-(4-azidobutoxy)-2-ethylphenyl]phenyl]propanoyl] amino]-N-[bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methyl]-5-(3,5-dimethylphenyl) pentanamide (Tag4-2AA)



[000610] The reaction was carried out following the general procedure F for amide coupling using **Tag4-1AA** (1.6 g, 628.37 μmol , 1 eq.), **N-Fmoc-Tag4-2AA** (1.9 g, 424.52 μmol , 67.56% yield, 70% purity) was obtained as a white solid which was used directly in the next step. LC-MS: RT= 5.525 min, mass calcd. for $\text{C}_{49}\text{H}_{55}\text{N}_6\text{O}_5^+$ 807.42, m/z found 807.40 [M-anchor + H]⁺; (anchor = $\text{C}_{159}\text{H}_{286}\text{O}_8$, MS=2325.21). LCMS condition: Method 12. (anchor = Tag4).

[000611] The reaction was carried out following the general procedure G for Fmoc deprotection using **N-Fmoc-Tag4-2AA** (1.9 g, 606.46 μmol , 1 eq.). **Tag4-2AA** (1.7 g, 584.05 μmol , 96.31% yield) was obtained as an off-white solid. LC-MS: RT= 3.534 min, mass calcd. for $\text{C}_{47}\text{H}_{55}\text{N}_6\text{O}_5^+$ 783.42, m/z found 783.40 [M-anchor + $\text{C}_{13}\text{H}_{11}\text{O}_2 + \text{H}$]⁺; (anchor = $\text{C}_{159}\text{H}_{286}\text{O}_8$, MS=2325.21); mass calcd. for $\text{C}_{34}\text{H}_{45}\text{N}_6\text{O}_3^+$ 585.35, m/z found 585.40 [M-anchor + H]⁺; (anchor = $\text{C}_{159}\text{H}_{286}\text{O}_8$, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

Step 3: Synthesis of tert-Butyl (3S)-3-Amino-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag4-3AA**)**

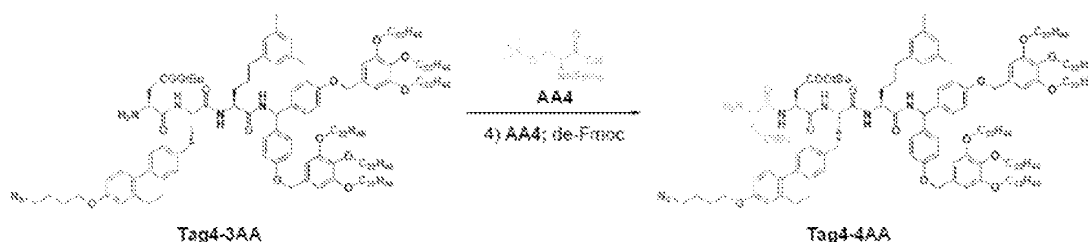


[000612] The reaction was carried out following the general procedure F for amide coupling using **Tag4-2AA** (1.7, 584.05 μmol , 1 eq.). **N-Fmoc-Tag4-3AA** (1.9 g, 345.02 μmol , 59.07% yield, 60% purity) was obtained as an off-white solid. LC-MS: RT= 5.159 min, mass calcd. for $\text{C}_{53}\text{H}_{60}\text{N}_7\text{O}_8^+$ 922.41, m/z found 922.40 [M-anchor + H]⁺; (anchor = $\text{C}_{159}\text{H}_{286}\text{O}_8$, MS=2325.21). LCMS condition: Method 12. (anchor = Tag4)

[000613] The reaction was carried out following the general procedure G for Fmoc deprotection using **N-Fmc-Tag4-3AA** (1.9 g, 575.04 μmol , 1 eq.), **Tag4-3AA** (1.50 g, 486.71 μmol , 84.64% yield)

was obtained as an off-white solid. LC-MS: RT= 3.633 min, mass calcd. for $C_{51}H_{60}N_7O_8^+$ 898.44, m/z found 898.50 [M-tBu-anchor+ $C_{13}H_{11}O_2+H$] $^+$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21); mass calcd. for $C_{38}H_{50}N_7O_6^+$ 700.37, m/z found 700.50 [M-tBu-anchor+H] $^+$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21); LCMS condition :Method 12. (anchor = Tag4)

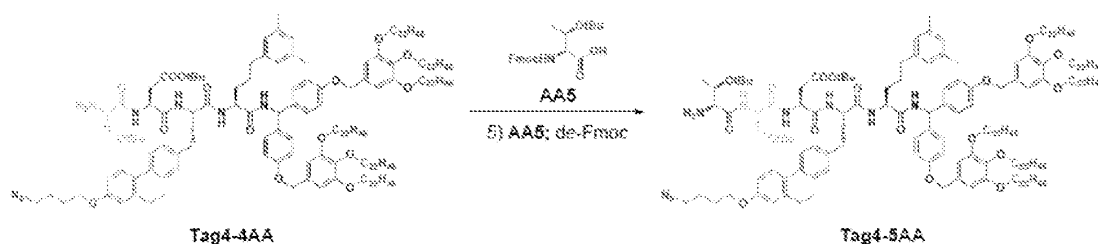
Step 4: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-Amino-3-tert-butoxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy] phenyl]methylcarbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag4-4AA)



[000614] The reaction was carried out following the general procedure F for amide coupling using **Tag4-3AA** (1.5 g, 486.71 μmol , 1 eq.) in DCM (50 mL). **N-Fmoc-Tag4-4AA** (1.45 g, 420.62 μmol , 86.42% yield) was obtained as an off-white solid. LC-MS: RT= 5.019 min, mass calcd. for $C_{56}H_{65}N_8O_{10}^+$ 1009.40, m/z found 1009.40 [M-anchor+H] $^+$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21). LCMS condition: Method 12. (anchor = Tag4).

[000615] The reaction was carried out following the general procedure G for Fmoc deprotection using **N-Fmoc-Tag4-4AA** (1.4 g, 406.11 μmol , 1 eq.). **Tag4-4AA** (1.30 g, 403.09 μmol , 99.26% yield) was obtained as an off-white solid. LC-MS: RT= 3.527 min, mass calcd. For: $C_{54}H_{65}N_8O_{10}^+$ 985.47, m/z found 985.50 [M-tBu-anchor +H] $^+$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21); mass calcd. for $C_{41}H_{55}N_8O_8^+$ 787.41, m/z found 787.50 [M-tBu-anchor+H] $^+$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

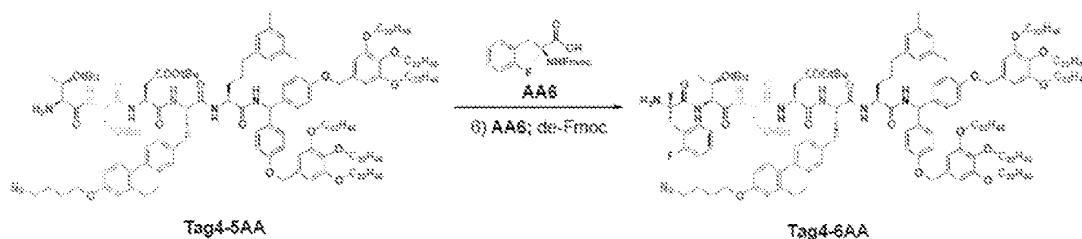
Step 5: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-Amino-3-tert-butoxy-butanoyl]amino]-3-tert-butoxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methylcarbamoyl]-4-(3,5-dimethylphenyl)butyl] amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag4-5AA)



[000616] The reaction was carried out following the general procedure F for amide coupling using **Tag4-4AA** (1.45 g, 420.62 μmol). **N-Fmoc-Tag4-5AA** (1.3 g, 360.66 μmol , 85.74% yield) was obtained as an off-white solid. LC-MS: RT=4.894 min, mass calcd. for $\text{C}_{60}\text{H}_{72}\text{N}_9\text{O}_{12}^+$ 1110.52, m/z found 1110.60 [M-tBu-anchor +H]⁺; (anchor= $\text{C}_{159}\text{H}_{286}\text{O}_8$, MS=2325.21); mass calcd. For $\text{C}_{73}\text{H}_{82}\text{N}_9\text{O}_{14}^+$ 1308.57, m/z found 1308.60 [M-tBu-anchor+H]⁺; (anchor= $\text{C}_{159}\text{H}_{286}\text{O}_8$, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

[000617] The reaction was carried out following the general procedure G for Fmoc deprotection using **N-Fmoc-Tag4-5AA** (1.30 g, 360.66 μmol , 1 eq.). **Tag4-5AA** (1.0 g, 295.66 μmol , 81.98% yield) was obtained as an off-white solid which was used directly in the next step. LC-MS: RT= 3.557 min, mass calcd. for: $\text{C}_{58}\text{H}_{72}\text{N}_9\text{O}_{12}^+$ 1086.52, m/z found 1086.30 [M-3tBu-anchor+ $\text{C}_{13}\text{H}_{11}\text{O}_2$ +4H]⁺; (anchor= $\text{C}_{159}\text{H}_{286}\text{O}_8$, MS=2325.21); mass calcd. for $\text{C}_{45}\text{H}_{62}\text{N}_9\text{O}_{10}^+$ 888.45, m/z found 888.50 [M-3tBu-anchor+4H]⁺; (anchor= $\text{C}_{159}\text{H}_{286}\text{O}_8$, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

Step 6: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[2-Amino-3-(2-fluorophenyl)-2-methylpropanoyl]amino]-3-tert-butoxy-butanoyl]amino]-3-tert-butoxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methylcarbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag4-6AA)

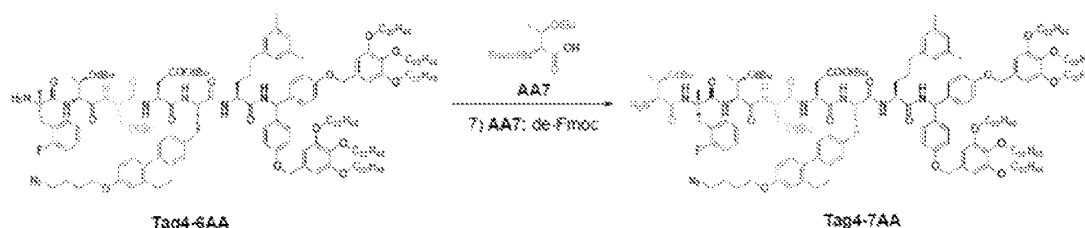


[000618] The reaction was carried out following the general procedure F for amide coupling using **Tag4-5AA** (1.0 g, 295.66 μmol , 1 eq.). **N-Fmoc-Tag4-6AA** (0.8 g, 211.43 μmol , 71.51% yield) was obtained as an off-white solid. LC-MS: RT=5.208 min, mass calcd. for $\text{C}_{70}\text{H}_{82}\text{FN}_{10}\text{O}_{13}^+$ 1289.60,

m/z found 1289.60 [M-3tBu-anchor+4H]⁺; (anchor=C₁₅₉H₂₈₆O₈, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

[000619] The reaction was carried out following the general procedure G for Fmoc deprotection using **N-Fmoc-Tag4-6AA** (0.8 g, 211.43 μmol, 1 eq.). **Tag4-6AA** (0.7 g, 196.55 μmol, 92.96% yield) was obtained as an off-white solid. LC-MS: RT= 3.697 min, mass calcd. for C₆₈H₈₂FN₁₀O₁₃⁺ 1265.60, m/z found 1265.70 [M-3tBu-anchor+ C₁₃H₁₁O₂+4H]⁺, (anchor=C₁₅₉H₂₈₆O₈, MS=2325.21); RT= 3.630 min, mass calcd. for C₅₅H₇₂FN₁₀O₁₁⁺ 1067.53, m/z found 1067.60 [M-3tBu-anchor+4H]⁺; (anchor=C₁₅₉H₂₈₆O₈, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

*Step 7: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[2-[[[(2S,3R)-2-Amino-3-tert-butoxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-tert-butoxy-butanoyl]amino]-3-tert-butoxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methylcarbamoyl]-4-(3,5-dimethylphenyl) butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (**Tag4-7AA**)*

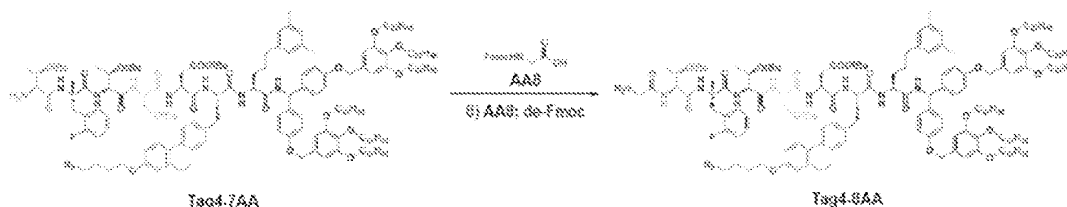


[000620] The reaction was carried out following the general procedure F for amide coupling using **Tag4-6AA** (0.7 g, 196.55 μmol, 1 eq.). **N-Fmoc-Tag4-7AA** (0.7 g, 177.62 μmol, 90.37% yield) was obtained as an off-white solid. LC-MS: RT=5.224 min, mass calcd. for C₇₄H₈₉FN₁₁O₁₅⁺ 1390.64, m/z found 1390.60 [M-3tBu-anchor+4H]⁺; (anchor=C₁₅₉H₂₈₆O₈, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

[000621] The reaction was carried out following the general procedure G for Fmoc deprotection using **N-Fmoc-Tag4-7AA** (0.7 g, 177.62 μmol, 1 eq.). **Tag4-7AA** (0.58 g, 155.97 μmol, 87.81% yield) was obtained as an off-white solid which was used directly in the next step. LC-MS: RT= 3.869 min, mass calcd. for C₇₂H₈₉FN₁₁O₁₅⁺ 1366.65, m/z found 1366.70 [M-3tBu-anchor+ C₁₃H₁₁O₂+4H]⁺, (anchor=C₁₅₉H₂₈₆O₈, MS=2325.21); mass calcd. for C₅₉H₇₉FN₁₁O₁₃⁺ 1168.58, m/z found 1168.60 [M-3tBu-anchor+4H]⁺; (anchor=C₁₅₉H₂₈₆O₈, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

Step 8: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[2-[[[(2S,3R)-2-[(2-Aminoacetyl)amino]-3-tert-butoxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-tert-butoxy-butanoyl]amino]-3-tert-butoxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]

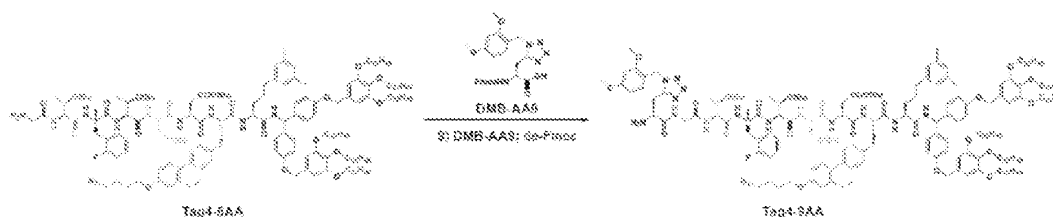
methyl]-2-[[[(1S)-1-[bis[4-[[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methylcarbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (**Tag4-8AA**)



[000622] The reaction was carried out following the general procedure F for amide coupling using **Tag4-7AA** (0.6 g, 161.35 μmol , 1 eq.). **N-Fmoc-Tag4-8AA** (0.56 g, 140.07 μmol , 86.81% yield) was obtained as an off-white solid. LC-MS: RT= 5.092 min, mass calcd. For $\text{C}_{76}\text{H}_{92}\text{FN}_{12}\text{O}_{16}^{+}$ 1447.67, $[\text{M}-3\text{tBu-anch}+4\text{H}]^{+}$; (anch=C₁₅₉H₂₈₆O₈, MS=2325.21); LCMS condition: Method 12. (anch = Tag4)

[000623] The reaction was carried out following the general procedure G for Fmoc deprotection using **N-Fmoc-Tag4-8AA** (0.56 g, 140.07 μmol , 1 eq.). **Tag4-8AA** (0.5 g, 132.42 μmol) was obtained as an off-white solid which was used directly in the next step. LC-MS: RT= 3.848 min, mass calcd. for $\text{C}_{61}\text{H}_{82}\text{FN}_{12}\text{O}_{14}^{+}$ 1225.60, m/z found 1225.70 $[\text{M}-3\text{tBu-anch}+4\text{H}]^{+}$, (anch=C₁₅₉H₂₈₆O₈, MS=2325.21); mass calcd. for $\text{C}_{74}\text{H}_{92}\text{FN}_{12}\text{O}_{16}^{+}$ 1423.67, m/z found 1423.70 $[\text{M}-3\text{tBu-anch}+4\text{H}]^{+}$; (anch=C₁₅₉H₂₈₆O₈, MS=2325.21); LCMS condition: Method 12. (anch = Tag4)

Step 9: Synthesis of *tert*-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[[2-[[[(2S,3R)-2-[[[2-[[[(2S)-2-Amino-3-[1-[[[2,4-dimethoxyphenyl]methyl]tetrazol-5-yl]propanoyl]amino]acetyl]amino]-3-*tert*-butoxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-*tert*-butoxy-butanoyl]amino]-3-*tert*-butoxy-propanoyl]amino]-4-[[[(1S)-1-[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[bis[4-[[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methylcarbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (**Tag4-9AA**)

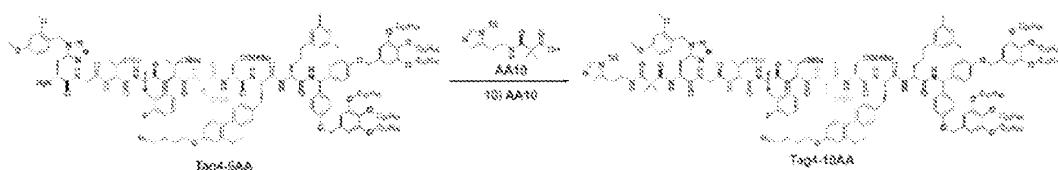


[000624] The reaction was carried out following the general procedure F for amide coupling using **Tag4-8AA** (105.19 mg, 198.64 μmol , 1.5 eq.), **DMB-AA9** (105.19 mg, 198.64 μmol , 1.5 eq.), DIC (25.07 mg, 198.64 μmol , 30.76 μL , 1.5 eq.) and HOAt (27.04 mg, 198.64 μmol , 27.79 μL , 1.5 eq.). **N-Fmoc-Tag4-9AA** (0.5 g, 116.62 μmol , 88.07% yield) was obtained as an off-white solid. LC-

MS: RT=4.817 min, mass calcd. for $C_{80}H_{98}FN_{17}O_{17}^{+}$ 793.86., found 794.30 [M-3tBu-PMB-anchor+7H] $^{2+}$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21); mass calcd. for $C_{95}H_{106}FN_{17}O_{19}$ 892.89, found 893.50 [M-3tBu-PMB-anchor+7H] $^{+}$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

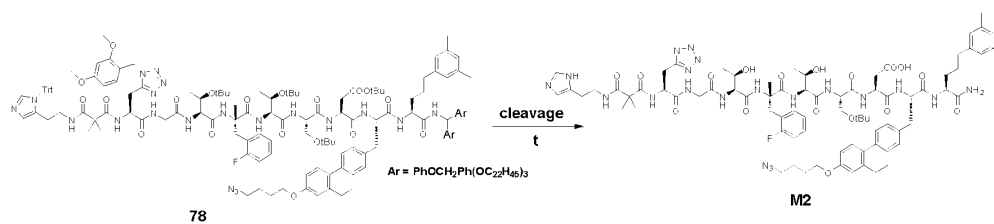
[000625] The reaction was carried out following the general procedure G for Fmoc deprotection using **N-Fmoc-Tag4-9AA** (0.5 g, 116.62 μ mol, 1 eq). **Tag4-9AA** (0.5 g, crude) was obtained as an off-white solid. LC-MS: RT= 3.838 min, mass calcd. for $C_{64}H_{86}FN_{17}O_{16}^{+}$ 683.80, m/z found 683.20 [M-4tBu-DMB-anchor+7H] $^{2+}$, (anchor= $C_{159}H_{286}O_8$, MS=2325.21); mass calcd. for $C_{77}H_{96}FN_{17}O_{18}^{2+}$ 782.85, m/z found 782.30 [M-4tBu-DMB-anchor+7H] $^{+}$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21); LCMS condition: Method 12. (anchor = Tag4)

*Step 10: Synthesis of tert-Butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-Azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methylcarbamoyl]-4-(3,5-dimethylphenyl) butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-3-tert-butoxy-2-[[[(2S,3R)-3-tert-butoxy-2-[[2-[[[(2S,3R)-3-tert-butoxy-2-[[2-[[[(2S)-3-[1-[(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]-2-[[2,2-dimethyl-3-oxo-3-[2-(3-tritylimidazol-4-yl)ethylamino]propanoyl]amino]propanoyl]amino]acetyl]amino]butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (**Tag4-10AA**)*



[000626] The reaction was carried out following the general procedure F for amide coupling using **Tag4-9AA** (0.5 g, 123.00 μ mol, 1 eq.) in DCM (15 mL) and THF (15 mL), **AA10** (86.26 mg, 184.50 μ mol, 1.5 eq.), DIC (23.28 mg, 184.50 μ mol, 28.57 μ L, 1.5 eq.), and HOAt (25.11 mg, 184.50 μ mol, 25.81 μ L, 1.5 eq.). **Tag4-10AA** (0.5 g, 110.75 μ mol, 90.04% yield) was obtained as an off-white solid which was used directly in the next step. LC-MS: RT= 3.874 min, mass calcd. for $C_{75}H_{101}FN_{20}O_{172}^{+}$ 786.37, found 787.00 [M-4tBu-DMB-Trt-anchor+8H] $^{2+}$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21); mass calcd. for $C_{88}H_{111}FN_{20}O_{19}$ 885.41, found 886.00 [M-4tBu-DMB-Trt-anchor+8H] $^{+}$; (anchor= $C_{159}H_{286}O_8$, MS=2325.21); LCMS condition: Method 12. Note: (anchor = Tag4)

*Step 11: Synthesis of Bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methanamine (**M2**)*



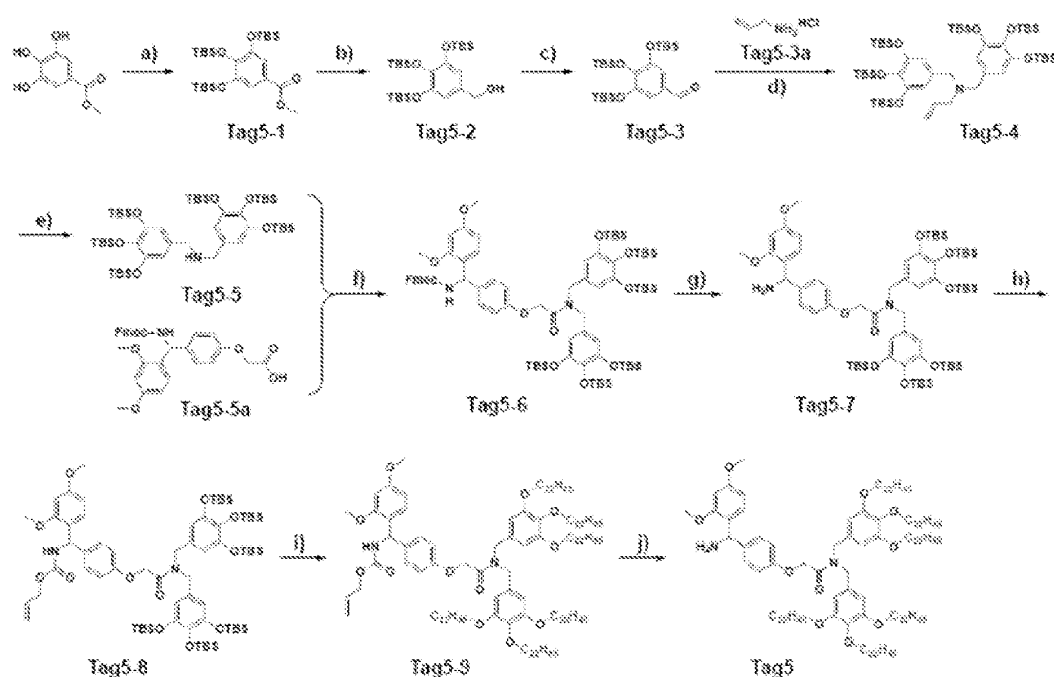
[000627] To a mixture of **Tag4-10AA** (432.77 mg, 95.86 μmol , 1 eq.) in TFA (4.5 mL) and H₂O (0.25 mL) was added triisopropylsilane (143.92 mg, 908.82 μmol , 186.67 μL , 9.48 eq.) in one portion at 20°C under N₂. The mixture was stirred at 20°C for 1.5 hr. LCMS showed that the desired mass was detected. LC-MS: RT = 3.822 min, m/z calcd. for C₇₅H₁₀₀FN₂₀O₁₇, 1570.75 [M-Boc-4tBu+2H]²⁺, m/z found 786.20, LCMS condition: Method 12. The mixture was filtered and the filtrate was diluted with t-BuOMe (1000 mL) to give precipitate, which was centrifuged for 10 min (5000 R) to give the crude product. The residue was purified by Prep-HPLC (column: Welch Xtimate C18 100*40mm*3 μm ; mobile phase: [water (TFA)-ACN]; B%: 30%-60%, 15min). Compound **M2** (1 mg, 5.98e-1 μmol , 94% purity) was obtained as a white solid. LCMS (ESI): RT = 3.748 min, m/z calcd. for C₇₅H₁₀₀FN₂₀O₁₇ 1570.75 [M-Boc-4tBu+2H]²⁺, m/z found 786.20, LCMS condition: Method 12. HPLC RT=9.411 min, 94% purity.

Example 8. Synthesis (LPPS) of M1 and M3 on Tag5

8.1 Synthesis of Tag 5

[000628] **Tag5** was prepared via 10 steps as shown in Scheme 6 below.

Scheme 6

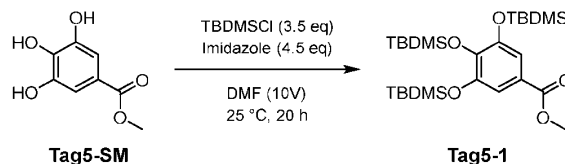


[000629] **Tag5-3** was prepared from methyl 3,4,5-trihydroxybenzoate as described in Horenstein et al., "Synthesis of Unprotected ($\pm$)-Tunichrome An-1, a Tunicate Blood Pigment," *J. Am. Chem. Soc.* 111(16):6242–6246 (1989), which is hereby incorporated by reference in its entirety. Reductive amination of **Tag5-3a** with 2 eq. of **Tag5-3** gave **Tag5-4** in 70% yield. Removing *N*-allyl group of **Tag5-4** with 1,3-dimethylbarbituric acid in the presence of Pd(PPh₃)₄ catalyst provided **Tag5-5**, which was coupled with **Tag5-5a** to form **Tag5-6**. Fmoc deprotection of **Tag5-6** in piperidine, provided **Tag5-7**. *N*-Alloc protection of **Tag5-7** and subsequent removal of TBS in **Tag5-8** and alkylation with C₂₂-alkyl bromide in one pot generated **Tag5-9**. Removal of *N*-alloc group from **Tag5-9** provided final product, **Tag5**. Replacing *N*-Fmoc of **Tag5-5a** with *N*-alloc was attempted for further improvement; however, **Tag5-5a** without *N*-Fmoc was poorly soluble in organic solvents. The amounts and yields for each step are summarized in Table 8 below.

Table 8. The Amounts and Yields for the Intermediates and Tag5

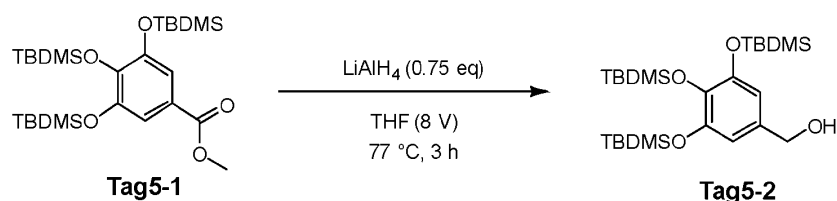
#	structure	MF	MW	Am t (kg)	LC purit y	Yiel d	Physical property		IUPAC name
							Purificati on	MP °C	
1		C26H50O5Si3	526.9	7.9	99%	90%	Trituration ; white solid	70.7 2	methyl 3,4,5-tris((tert-butyl)dimethylsilyloxy)benzoate
2		C25H50O4Si3	498.9	3.4 8	99%	90%	Crude Off-white solid	69.7 0	(3,4,5-tris((tert-butyl)dimethylsilyloxy)phenyl)methanol
3		C25H48O4Si3	496.9	3.1 3	99%	90%	Crude Yellow solid	73.3 3	3,4,5-tris((tert-butyl)dimethylsilyloxy)benzaldehyde
4		C53H103NO6Si6	1018.9	1.9	95%	70%	Column purificatio n Yellow Semisolid	80.4 9	N,N-bis(3,4,5-tris((tert-butyl)dimethylsilyloxy)benzyl)prop-2-en-1-amine
5		C50H99NO6Si6	978.8	1.6 9	95%	92%	Trituration Yellow oil		bis(3,4,5-tris((tert-butyl)dimethylsilyloxy)benzyl)amine
6		C82H126N2O12Si6	1500.4	2.2	85%	83%	Crude Yellow oil		((9H-fluoren-9-yl)methyl ((4-(2-bis(3,4,5-tris((tert-butyl)dimethylsilyloxy)benzyl)amino)-2-oxoethoxy)phenyl)(2,4-dimethoxyphenyl)methyl)carbamate
7		C67H116N2O10Si6	1278.2	1.8	84%	94%	Crude Yellow oil		2-(4-(amino(2,4-dimethoxyphenyl)methyl)phenoxy)-N,N-bis(3,4,5-tris((tert-butyl)dimethylsilyloxy)benzyl)acetamide
8		C71H120N2O12Si6	1362.2	1.6	98%	95%	Column purificatio n Pale- yellow gum		allyl ((4-(2-bis(3,4,5-tris((tert-butyl)dimethylsilyloxy)benzyl)amino)-2-oxoethoxy)phenyl)(2,4-dimethoxyphenyl)methyl)carbamate
9		C167H300N2O12	2528.2	0.4 0	95%	80%	Column purificatio n Off- white solid	74.2 8	allyl tris(docosyloxy)benzyl)amino)-2-oxoethoxy)phenyl)(2,4-dimethoxyphenyl)methyl)carbamate
10		C163H296N2O10	2444.2	0.3 7	95%	95	Trituration Off-white solid	75.9	2-(4-(amino(2,4-dimethoxyphenyl)methyl)phenoxy)-N,N-bis(3,4,5-tris(docosyloxy)benzyl)acetamide

Step a: Synthesis of Methyl 3,4,5-Tris((tert-butyldimethylsilyl)oxy)benzoate (Tag5-1)



[000630] To a clear solution of methyl 3,4,5-trihydroxybenzoate (3 kg, 16.29 mol, 1 eq) in DMF (24 L) was added imidazole (4.99 kg, 73.31 mol, 4.5 eq) in one portion to give a clear solution. TBDMSCl (8.59 kg, 57.02 mol, 6.99 L, 3.5 eq) was added in four portions (2.148 kg x 4) over a period of 2 hours at a rate sufficient to maintain internal temperature between 25 °C and 30 °C under N₂. After the addition, a yellow solution was obtained. The reaction was stirred at 25 °C for 16 h under N₂ until the thick suspension was formed. TLC results indicated that the starting material was completely consumed and one major new spot (petroleum ether:EtOAc = 9:1, R_f=0.58) was detected. The reaction mixture was pumped into transit barrels (2* 20 L). sat. NaHCO₃ (15 L) solution was charged to 50 L of jacket flask. 20 L (1/2 volume) of the reaction suspension was added to the above sat. NaHCO₃ (15 L) solution with intensive stirring and extracted with PE (6 L x 2). The other (1/2 volume) of the reaction mixture was treated as above. All of the organic layers were combined, washed with brine (10 L), and dried over Na₂SO₄. The organic layer was filtered and concentrated under reduced pressure (~ 45°C, -0.8 atm) to give ~10 kg of pale-green oil. The pale-green oil was dissolved in MeOH (28 L) and stirred at 0~4 °C (internal temperature) for 12 h. The white solid has precipitated out. The product **Tag5-1** (7.8 kg, 14.80 mol, 90.86% yield) was obtained as a white solid via filtration and dried under reduced pressure. ¹HNMR: (400 MHz, CDCl₃) δ = 7.23 (s, 2H), 3.85 (s, 3H), 1.00 (s, 9H), 0.99 – 0.86 (m, 18H), 0.24 (s, 12H), 0.15 (s, 6H); LC-MS: (analyzed by Method 1) Rt= 3.183 min, m/z=527.3 [M+H]⁺.

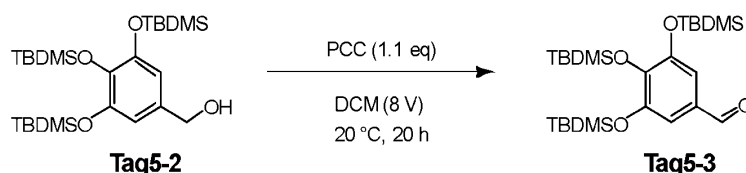
Step b: Synthesis of [3,4,5-Tris[[tert-butyl(dimethyl)silyl]oxy]phenyl]methanol (Tag5-2)



[000631] A solution of **Tag5-1** (4 kg, 7.59 mol, 1 eq) in THF (15 L) was added to a suspension of LAH (216.09 g, 5.69 mol, 0.75 eq) in THF (16 L) at a rate sufficient to maintain a gentle reflux (internal temperature: 66 °C). The reaction mixture was stirred at internal temperature: 66°C (oil bath temperature: 77 °C) for 3 hr. TLC indicated that **Tag5-1** was completely consumed and one new

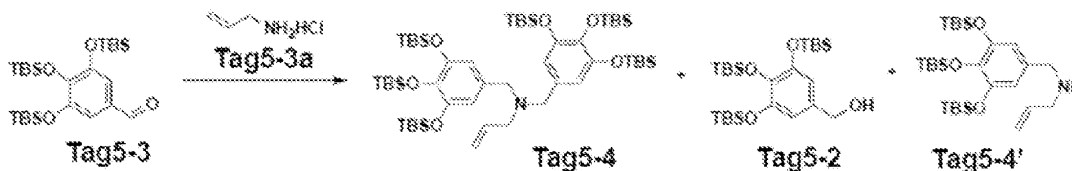
spot (petroleum ether:ethyl acetate= 10:1, R_f = 0.41) was formed. The reaction mixture was cooled to (internal temperature: 0 °C) and diluted with 12 L of methyl *t*-butyl ether (MTBE). The reaction mixture was stirred rapidly, and 216 mL of water was added dropwise until the color turned from grey to white, then 216 mL of NaOH solution (wt%, 15%) was added. The resulting mixture was stirred for 10 mins. Water (620 mL) and silica gel (500 g) were sequentially added to the reaction mixture. The heterogeneous mixture was filtered through Celite and the resulting filtrate was concentrated *in vacuo* to give a colorless oil. It was further vacuumed by oil pump to remove the solvent residue and give **Tag5-2** (3.48 kg, 6.84 mol, 90.1% yield, 98% purity) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ = 6.50 (s, 2H), 4.49 (s, 2H), 1.49 (br s, 1H), 1.00 (s, 9H), 0.95 (s, 18H), 0.21 (s, 12H), 0.13 (s, 6H); LC-MS: (analyzed by method 1) R_t = 3.25 min, m/z =481.3 $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$.

Step c: Synthesis of 3,4,5-Tris[[tert-butyl(dimethyl)silyl]oxy]benzaldehyde (Tag5-3)

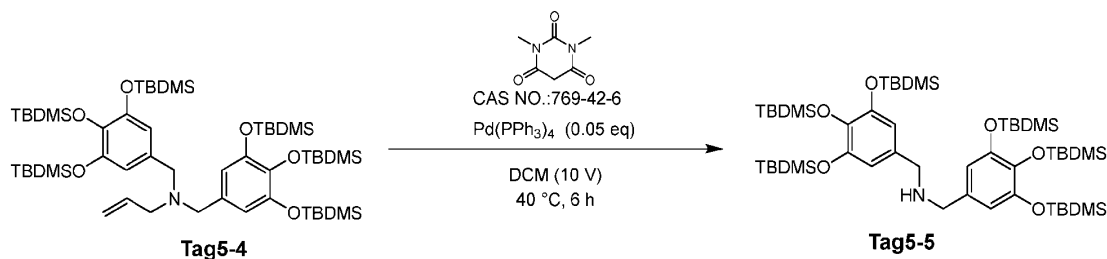


[000632] To a suspension of PCC (1.58 kg, 7.32 mol, 1.05 eq) in DCM (12 L) was added a solution of **Tag5-2** (3.48 kg, 6.98 mol, 1 eq) in DCM (16 L) at 0 °C. The reaction mixture was stirred at 20 °C for 20 h. TLC indicated that reactant **Tag5-2** was consumed completely, and one major new spot was detected (petroleum ether:ethyl acetate= 10:1, PMA, R_f = 0.61). MTBE (12 L) and silica gel (800 g) were added sequentially to the reaction mixture, which was then stirred for 10 min and filtered through Celite. The filtrate was concentrated *in vacuo*; the resulting dark brown oil was diluted with petroleum ether (15 L) and passed through a short pad of silica/celite (800 g/400 g) and concentrated to afford **Tag5-3** (3.13 kg, 6.30 mol, 90.34% yield) as a pale-yellow solid. ^1H NMR (400 MHz, CDCl_3) δ = 9.74 (s, 1H), 7.04 (s, 2H), 1.00 (s, 9H), 0.96 (s, 18H), 0.26 (s, 12H), 0.16 (s, 6H). LC-MS: (analyzed by method 1) R_t = 3.550 min, m/z = 497.4 $[\text{M}+\text{H}]^+$. **Tag5-3** was directly used for next step without purification.

Step d: Synthesis of N,N-Bis[[3,4,5-tris[[tert-butyl(dimethyl)silyl]oxy]phenyl]methyl]prop-2-en-1-amine (Tag5-4)



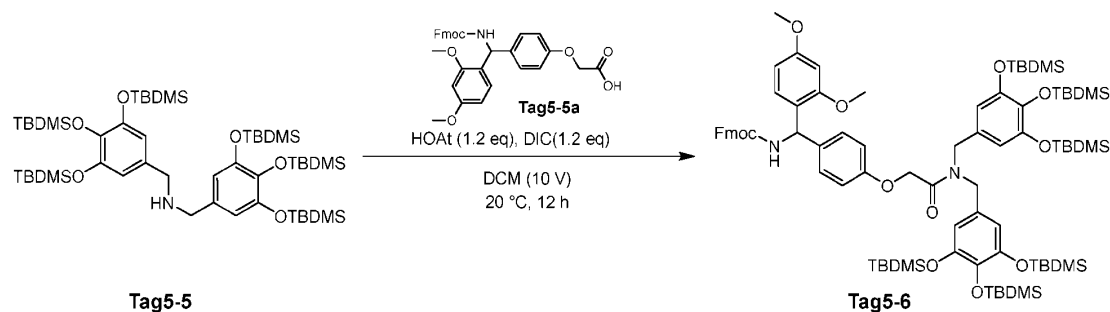
[000633] To a solution of **Tag5-3a** (240 g, 2.51 mol, 98% purity, 1 eq) was added NaOAc (721.82 g, 8.80 mol, 3.5 eq) in THF (8 L) and MeOH (12.5 L), and the mixture was stirred for 30 min under N₂, then a solution of **Tag5-3** (1.40 kg, 2.77 mol, 98% purity, 1.1 eq) in THF (1.5 L) and HOAc (3.02 kg, 50.28 mol, 2.88 L, 20 eq) were added at 20 °C sequentially. The reaction mixture was stirred for 30 min. A solution of NaBH₃CN (157.98 g, 2.51 mol, 1 eq) in THF (1.5 L) was added to the reaction mixture dropwise over a period for 30 min, then the reaction was stirred at 25 °C for 30 min. Additional solution of **Tag5-3** (1.66 kg, 3.27 mol, 98% purity, 1.3 eq) in THF (1.5 L) was added, and the reaction was stirred for 30 min. Additional solution of NaBH₃CN (236.98 g, 3.77 mol, 1.5 eq) in THF (1.5 L) was dropwise added to the reaction mixture over a period for 1 h, then the reaction was stirred at 25 °C for 12 hr. TLC indicated that the reactant **Tag5-3** was consumed, and one major spot has appeared (petroleum ether/ethyl acetate=20/1, staining reagent: PMA, R_f=0.66). Two impurities **Tag5-2** and **Tag5-4'** were detected as well. The reaction was quenched by addition of sat. aq. NaHCO₃ (18 L, 3.6 kg of NaHCO₃ was suspended in 16 L of H₂O) and the separated aq. solution was then extracted with MTBE (12 L * 2). The combined organic layers were washed with brine (8 L), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product (~3.8 kg) as colorless oil, which was identified by HNMR (ratio of **4:2:4'** = 1.0:0.57:0.28). The crude material was mixed with silica gel (4.5 kg) and was purified by column chromatography using petroleum ether/ethyl acetate = 1/0 to 100/10 to give **Tag5-4** (1.88 kg, 1.73 mol, 71.2% yield based on **3a**). ¹H NMR (400 MHz, CDCl₃) δ = 6.50 (s, 4H), 6.02 – 5.71 (m, 1H), 5.21 (d, *J* = 17.6 Hz, 1H), 5.12 (d, *J* = 10.0 Hz, 1H), 3.37 (br s, 4H), 2.98 (br d, *J* = 4.8 Hz, 2H), 0.99 (s, 18H), 0.93 (s, 36H), 0.20 (s, 24H), 0.11 (s, 12H). LC-MS: (analyzed by Method 1) Rt= 4.313 min, m/z=1018.6 [M+H]⁺.
Step e: Synthesis of 1-[3,4,5-Tris[[tert-butyl(dimethyl)silyl]oxy]phenyl]-N-[[3,4,5-tris[[tert-butyl(dimethyl)silyl]oxy]phenyl]methyl]methanamine (Tag5-5)



[000634] To a solution of 1,3-dimethylbarbituric acid (CAS No.: 769-42-6, 885.13 g, 5.67 mol, 3.2 eq) in DCM (12.5 L) was added a solution of **Tag5-4** (1.9 kg, 1.77 mol, 95% purity, 1 eq) in DCM (4 L). The reaction was degassed and purged 3 times with N₂ and then Pd(PPh₃)₄ (61.41 g, 53.15 mmol, 0.03 eq) was added. The reaction mixture was degassed and purged 3 times with N₂ and

stirred at inner temperature 39 °C (oil batch temperature: 45 °C) for 4 hr under N₂ atmosphere. TLC indicated that reactant **Tag5-4** was consumed completely and one new spot (petroleum ether: ethyl acetate= 10:1, NH₃.H₂O(0.5%,v/v), Ninhydrin, R_f= 0.62) was formed. The reaction mixture was cooled to 10°C and quenched by addition NH₃.H₂O (400 mL, wt%: 25%). A white solid has precipitated out. Sulfhydryl silica gel (350 g), silica gel (700 g), and Na₂SO₄ (1000 g) were added to the reaction, and the reaction mixture was stirred for 30 min. The reaction mixture was filtered through a Celite (500 g) plug to give **Tag5-5** (1.69 kg, 1.64 mol, 92.6% yield, 95% purity) as yellow oil, which was dissolved in DCM (10 L) and used directly without purification. ¹H NMR (400 MHz, CDCl₃) δ (ppm) = 6.46 (s, 4H), 3.59 (br s, 4H), 0.99 (s, 18H), 0.96 – 0.87 (m, 36H), 0.20 (s, 24H), 0.12 (s, 12H). LC-MS: (analyzed by method 1) Rt = 4.21 min, m/z=978.5 [M+H]⁺.

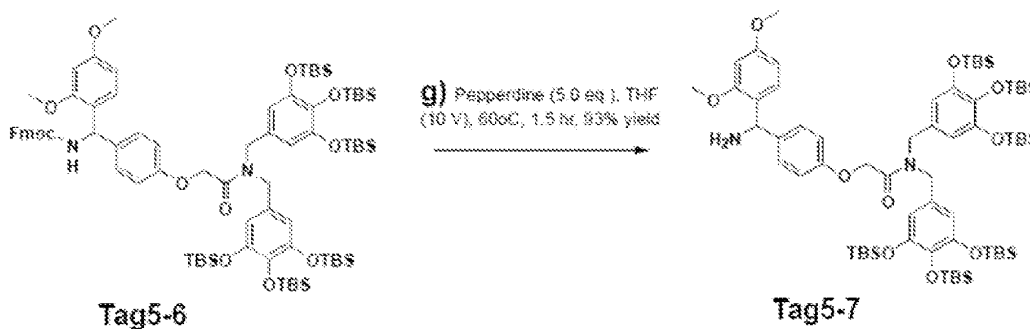
*Step f: Synthesis of 9H-Fluoren-9-ylmethyl N-[[4-[2-[Bis[[3,4,5-tris[[tert-butyl(dimethyl)silyl]oxy]phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamate (**Tag5-6**)*



[000635] To a solution of **Tag5-5a** (913.17 g, 1.69 mol, 1.13 eq) in DCM (6 L) was added HOAt (245.71 g, 1.81 mol, 252.53 mL, 1.2 eq) and DIC (227.82 g, 1.81 mol, 279.53 mL, 1.2 eq), and the reaction mixture was stirred at 25°C for 30 min. Then a solution of **Tag5-5** (1.55 kg, 1.50 mol, 95% purity, 1 eq) in DCM (10 L) was added, and the reaction mixture was stirred at 25°C for 8 hr. TLC indicated that reactant **Tag5-5** was consumed, and one new major spot was detected (petroleum ether:ethyl acetate= 5:1, 4% DCM to improve the solubility, R_f= 0.39). The reaction mixture was quenched by addition sat. NaHCO₃ (5 L*3) and then extracted with DCM (3 L * 2). The combined organic layers were washed with brine (2.5 L), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate=20/1 to 1/1) to provide **Tag5-6** as a white solid (2.2 kg, 1.25 mol, 82.85% yield, 85% purity). ¹H NMR (400 MHz, CDCl₃) δ (ppm) = 7.77 (br d, J = 7.3 Hz, 2H), 7.59 (br d, J = 6.4 Hz, 2H), 7.39 (br d, J = 6.6 Hz, 2H), 7.31 (br d, J = 6.4 Hz, 2H), 7.24 – 7.14 (m, 1H), 7.11 – 7.08 (m, 2H), 6.79 (br d, J = 8.5 Hz, 2H), 6.50 – 6.47 (m, 2H), 6.38 (m, 2H), 6.31 (s, 2H), 6.06 – 5.99 (m, 1H), 5.86 – 5.72 (m, 1H), 4.66 (s, 2H), 4.46 – 4.38 (m, 4H), 4.29 – 4.19 (m, 3H), 3.81 (s,

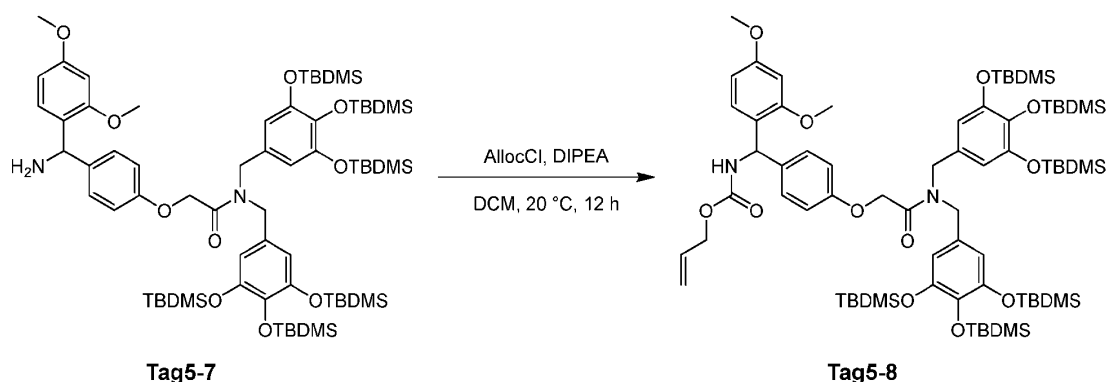
3H), 3.74 (s, 3H), 1.00 (s, 18H), 0.92 (s, 36H), 0.19 (s, 24H), 0.13 (s, 12H). LC-MS: (analyzed by Method 1) R_t = 5.39 min, m/z = 251.0 $[M+6H]^{6+}$.

Step g: Synthesis of 2-[4-[Amino-(2,4-dimethoxyphenyl)methyl]phenoxy]-N,N-bis[[3,4,5-tris[[tert-butyl(dimethyl)silyl]oxy]phenyl]methyl]acetamide (Tag5-7)



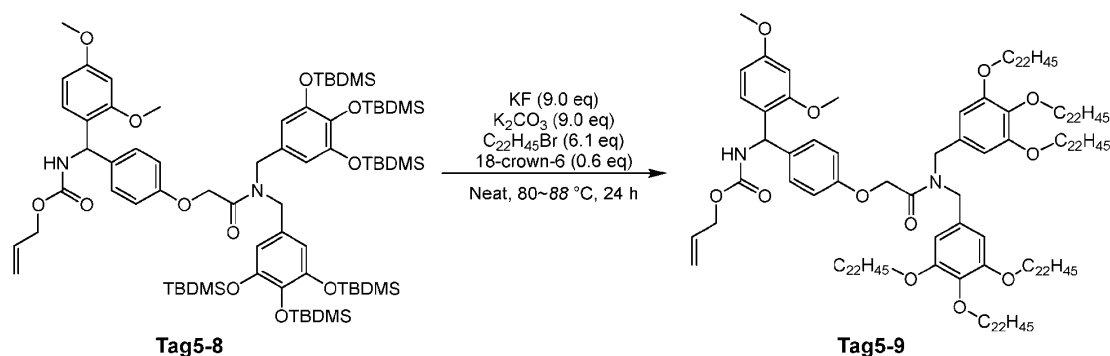
[000636] To the of **Tag5-6** (2.2 kg, 1.25 mol, 85% purity, 1 eq) in THF (16 L) was added piperidine (530.63 g, 6.23 mol, 615.44 mL, 5 eq). The reaction mixture was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 60 °C for 2 hr under N_2 atmosphere. TLC showed formation of a one new major spot (petroleum ether:ethyl acetate = 1:1, R_f = 0.16). The reaction mixture was concentrated to afford a crude (1.8 kg, 1.18 mol, 94.91% yield, 84% purity) as yellow oil, which was dissolved in DCM (12 L), washed with saturated ammonium chloride (10 L *2), water (10 L), and brine (10 L). It was then dried over Na_2SO_4 and filtered to give a solution, which was concentrated to give **Tag5-7** (1.8 kg, 94.9% yield in 84% purity). **Tag5-7** was used directly in the next step without purification. 1NMR (400 MHz, $CDCl_3$) δ (ppm) 7.27 (br d, J = 8.6 Hz, 2H), 7.12 (d, J = 8.0 Hz, 1H), 6.81 (d, J = 8.6 Hz, 2H), 6.44 (s, 2H), 6.39 (s, 2H), 6.31 (s, 2H), 5.38 (s, 1H), 4.66 (s, 2H), 4.43 (s, 2H), 4.29 (s, 2H), 3.79 – 3.76 (m, 6H), 1.00 (s, 18H), 0.92 (s, 36H), 0.25 – 0.16 (m, 24H), 0.13 (s, 12H). LC-MS: (analyzed by Method 1) R_t = 4.30 min, m/z = 1260.7 $[M+H-NH_3]^+$.

Step h: Synthesis of Allyl N-[[4-[2-[Bis[[3,4,5-tris[[tert-butyl(dimethyl)silyl]oxy]phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamate (Tag5-8)



[000637] To a solution of **Tag5-7** (1.8 kg, 1.18 mol, 84% purity, 1 eq) in DCM (20 L) was added DIEA (305.78 g, 2.37 mol, 412.10 mL, 2 eq) and allyl carbonochloride (285.17 g, 2.37 mol, 250.15 mL, 2 eq). The reaction mixture was stirred at 25 °C for 12 hr. TLC showed that the starting material was consumed completely, and one major new spot (petroleum ether:ethyl acetate = 5:1, R_f = 0.45) was detected. The reaction mixture was quenched by addition of sat. NH_4Cl (5 L) and then extracted with DCM (5 L * 2). The combined organic layers were washed with sat. NH_4Cl (5 L * 2), brine (2 L), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give crude product as a yellow oil, which was purified by column chromatography (SiO_2 , petroleum ether/ethyl acetate = 20:1~3:1, 2% of DCM was added into the eluent solvent to promote the solubility) to provide **Tag5-8** in two fractions in total 1.59 kg (97% yield in >98% purity). Fraction 1 (1.35 kg, 991.02 mmol, 99% purity) 83.78% yield as yellow oil. Fraction 2 (380 g, ~80% purity) was further purified to afford 236 g, 160.02 mmol, 98.1% purity as a pale-yellow oil. ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 7.10 (br d, J = 8.8 Hz, 3H, 26, 28, 37), 6.77 (d, J = 8.6 Hz, 2H), 6.52 - 6.42 (m, 2H), 6.37 (s, 2H), 6.30 (s, 2H), 6.01 - 5.78 (m, 3H), 5.30 (br dd, J = 1.6, 17.2 Hz, 1H), 5.21 (br dd, J = 1.3, 10.4 Hz, 1H), 4.64 (s, 2H), 4.61 - 4.58 (m, 2H), 4.42 (s, 2H), 4.26 (s, 2H), 3.80 (s, 3H), 3.71 (s, 3H), 0.99 (s, 18H), 0.95 - 0.88 (m, 36H), 0.18 (s, 24H), 0.12 (s, 12H). LC-MS: (Method 2) R_t = 2.22 min, m/z = 1379.74 $[\text{M} + \text{H}_3\text{O}]^+$; m/z = 2741.2 $[\text{2M} + \text{H}_3\text{O}]^+$

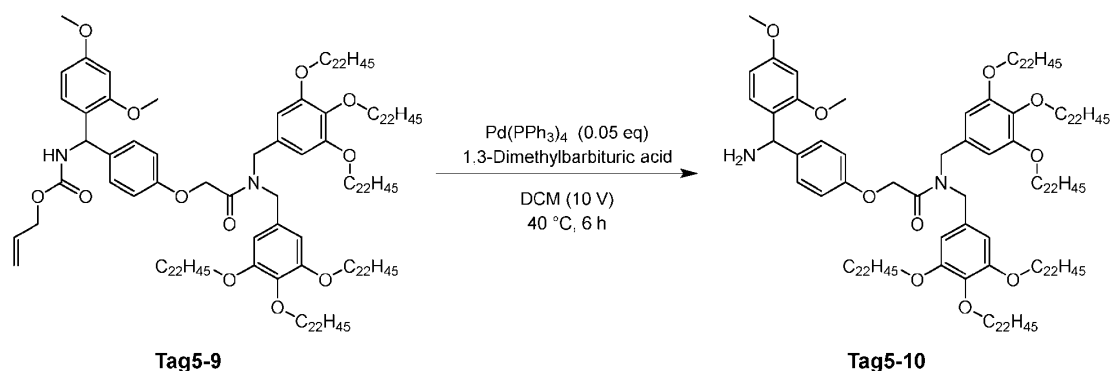
*Step i: Synthesis of Allyl N-[[4-[2-[Bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamate (**Tag5-9**)*



[000638] The 5 batches of reactions were conducted in parallel on 50 g scale. To a mixture of **Tag5-8** (50.0 g, 33.80 mmol, 1 eq) and 1-bromodocosane (86.2 g, 206.19 mmol, 6.1 eq) was added K_2CO_3 (42.1 g, 304.2 mmol, 9 eq), 18-crown-6 (5.4 g, 20.3 mmol, 0.6 eq), and KF (17.67 g, 304.21 mmol, 9 eq). The reaction mixture was degassed and purged 3 times with N_2 , and then the mixture was stirred at 85 °C for 24 hr under N_2 atmosphere. LCMS indicated that **Tag5-8** was consumed. TLC showed one new major spot (THF/petroleum ether = 1:5, R_f = 0.35). The reaction mixture was suspended in THF (400 mL), filtered through a silica gel plug to remove the inorganic salt, and the filtrate was concentrated to afford a residue. The residue was purified by flash silica gel chromatography (ISCO®; 330 g SepaFlash® Silica Flash Column, Eluent of 0~50% THF/petroleum ether gradient: 120 mL/min) to give **Tag5-9** (76 g, 30.06 mmol, 88.94% yield).

[000639] Additional four reactions were conducted and purified as above. Then the 5 batches of purified **Tag5-9** were combined and triturated with MeOH (200 mL, 70 °C, 2 hr), then cooled to 20 °C, and filtered to give 386 g of **Tag5-9** (purity: ~97.7%) totally. ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 7.12 (br d, J = 8.5 Hz, 3H), 6.81 (br d, J = 8.6 Hz, 2H), 6.50 – 6.43 (m, 2H), 6.40 (s, 2H), 6.30 (s, 2H), 6.00 (br d, J = 8.8 Hz, 1H), 5.97 – 5.87 (m, 1H), 5.86 – 5.72 (br d, J = 8.6 Hz, 1H), 5.38 – 5.26 (m, 1H), 5.25 – 5.08 (m, 1H), 4.71 (s, 2H), 4.59 (br d, J = 5.4 Hz, 2H), 4.50 (s, 2H), 4.40 (s, 2H), 3.93 (br t, J = 5.7 Hz, 4H), 3.90 – 3.81 (m, 8H), 3.80 (s, 3H), 3.72 (s, 3H), 1.82 – 1.68 (m, 12H), 1.53 – 1.20 (m, 228H), 0.89 (t, J = 6.8 Hz). LC-MS (Method 3): R_t = 11.33 min, m/z = 2544.7/2545.99 $[\text{M} + \text{H}_3\text{O}]^+$. The NMR data was matched to the targeted structure using HSQC. The chemical Shift of C55 was 69.30 ppm and HMBC: H55 was correlated with C13.

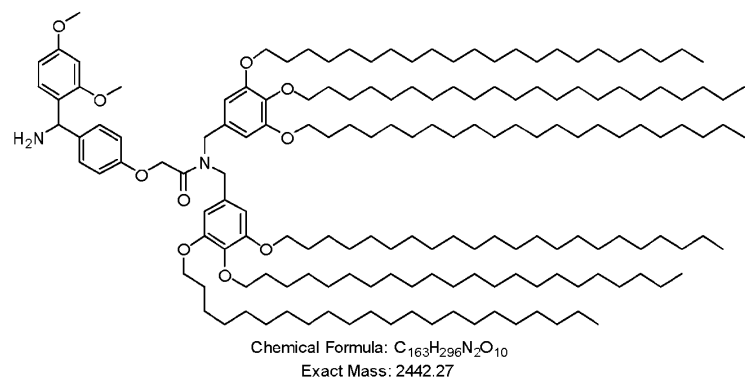
Step j: Synthesis of 2-[4-[Amino-(2,4-dimethoxyphenyl)methyl]phenoxy]-N,N-bis[[3,4,5-tri(docosoxy)phenyl]methyl]acetamide (Tag5)



[000640] To a solution of 1,3-dimethylhexahydropyrimidine-2,4,6-trione (15.3 g, 97.7 mmol, 3.2 eq) in DCM (560 mL) was added **Tag5-9** (80 g, 30.54 mmol, 1 eq), the reaction was degassed and purged 3 times with N₂, and then Pd(PPh₃)₄ (1.41 g, 1.22 mmol, 0.04 eq) was added. The mixture was degassed and purged 3 times with N₂ and stirred at 45 °C for 6 hr under N₂ atmosphere. TLC indicated **Tag5-9** was consumed, and one major new spot (petroleum ether:THF = 1:1, R_f = 0.14, PMA) was detected. The reaction was concentrated, and the residue was triturated in MeOH (1L) at 70 °C for 1 hr, and then cooled to 20 °C. The mixture was filtrated to provide a crude product, which was further triturated in ACN (1L, 70 °C, 1 hr), cooled to 20°C, filtrated, and washed with ACN (400 mL), MeOH (400mL), EtOAc (400 mL), and petroleum ether (400 mL) sequentially, then dried under vacuum to give **Tag5** (74.8 g, 29.32 mmol, 95.99% yield, 95.79% purity) as an off-white solid. Additional four repeating reactions were conducted as described above to generate total **Tag5** (303.9 g in 95.5% purity). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.14 - 7.25 (m, 2 H), 7.03 (br d, J=7.75 Hz, 1 H), 6.76 (br d, J=8.25 Hz, 2 H), 6.34 (br d, J=12.38 Hz, 4 H), 6.22 (br s, 2 H), 5.30 (br s, 1 H), 4.65 (s, 2 H), 4.23 - 4.50 (m, 4 H), 3.63 - 3.96 (m, 18 H), 1.67 (br d, J=5.50 Hz, 12 H), 1.37 (br s, 12 H), 1.18 (s, 216 H), 0.81 (br t, J=6.44 Hz, 18 H) ppm. ¹³C NMR (101 MHz, CDCl₃) δ = 168.42, 159.83, 157.67, 156.74, 153.62, 153.27, 137.65 – 137.58, 137.58 – 137.50, 131.79, 131.09, 128.17, 128.07, 114.29, 107.04, 105.04, 104.00, 98.74, 77.25, 73.48, 73.43, 69.18, 69.14, 67.75, 55.37, 55.34, 52.88, 49.67, 48.50, 31.97, 30.42, 29.77, 29.71, 29.57, 29.51, 29.42, 26.23, 22.74, 14.16. LC-MS (Method 3): Rt = 11.20 min, m/z=2444.02 [M+H]⁺.

8.2 Characterization of Tag5

[000641] Characterization of Tag5 is shown in Table 9.



TAG-5

Table 9. Characterization of Tag5

Compound ID	Tag5
Exact Mass	2442.27
State	Off-white Solid
Stability	Stability under different pH
Overall yield	25% yield for 10 steps synthesis with >95% HPLC purity for 400g production, using 3 chromatography purifications on steps d, f, h
IUPAC	2-(4-(amino(2,4-dimethoxyphenyl)methyl)phenoxy)-N,N-bis(3,4,5-tris(docosyloxy)benzyl)acetamide
SMIL	O=C(COC1=CC=C(C(N)C2=C(OC)C=C(OC)C=C2)C=C1)N(CC3=CC(OCC CCCCCCCCCCCCCCCCCCCC)=C(OCCCCCCCCCCCCCCCCCCCCCCC)C(OCCCCCCCCCCCCCCCCCCCCCCC)=C3)CC4=CC(OCCCCCCCCCCCC CCCCCCCCCCCC)=C(OCCCCCCCCCCCCCCCCCCCCCCCCCCC)C(OCCCCCCCC CCCCCCCCCCCCCCCCCC)=C4

IR Data

[000642] The powder sample of Tag5 was placed directly onto the surface of ATR, while the crystal (Znse) surface of ATR was fully covered by the sample. Sample compression bar was used to compress the sample to evenly distribute the sample and fully contact it with the crystal surface of ATR. IR conditions used to collect IR data are shown in Table 10.

Table 10. IR Conditions

Parameter	Value
Instrument Manufacturer	Thermo
Instrument model	NICOLET iS10
Sample preparation mode	KBr disc
Method Parameters	40°C

Detector:	DTGS KBr	Resolution:	4
Beamsplitter:	KBr	Sample gain:	1
Source:	IR	Optical velocity:	0.4747
Number of sample scans:	16	Aperture:	150
Number of background scans:		16	

[000643] $\lambda = 2915.54 \text{ cm}^{-1}$ indicated C-H (CH_3) bond; $\lambda = 2848.94 \text{ cm}^{-1}$ indicated C-H (CH_2) bond; $\lambda = 1647.99 \text{ cm}^{-1}$ indicated C=O (C=ONR_2) bond; $\lambda = 1505.99 \text{ cm}^{-1}$ indicated C-N bond; $\lambda = 1466.52 \text{ cm}^{-1}$ indicated C=C (Ph) bond; $\lambda = 1379.34 \text{ cm}^{-1}$ indicated C-H (CH_3) bond; and $\lambda = 1174.13 \text{ cm}^{-1}$ indicated C-O-C bond.

Determination of Moisture Content

[000644] The sample was weighted into a dry and clean vial. The vial was sealed. After the or the instrument reached the preset temperature (150°C), the vial was placed in the furnace. After the Karl Fischer reaction ended, the water content of the sample was obtained (Table 11). The moisture content was determined to be 0.26%.

Table 11. Conditions Used to Determine Moisture Content in the Sample

Parameter	Value
Extraction time	30s
Start drift	25 $\mu\text{g}/\text{min}$
Stopping criteria	Drift Relative Stop 5 $\mu\text{g}/\text{min}$
Anode solution	HYDRANAL /Coulomat AK
Cathode solution	HYDRANAL /Coulomat CG-K
Stirring speed	45%
Atmosphere	Dry Air
Gas flow rate	80mL/min
Vial Volume	10 L

Ion Chromatography

[000645] Chromatography conditions that were used are shown in Tables 12-13. The following content of ions were determined: Cl⁻: 0.01%; Br⁻: 0.01%; F⁻: <0.01; CO₃²⁻: 0.02%.

Table 12. F- Ion Chromatography Conditions

Instrument name	Thermo ICS-6000	
Mobile phase	KOH	
Flow rate	1.0mL/min	
Guard column	Dionex IonPac AG18, 4*50mm	
Column	Dionex IonPac AS18, 4*250mm	
Column temp	30	
Cell Temperature	35	
Detection Mode	Conductivity detection	
Suppressor current	99mA	
Injection volume	25μL	
Analysis time	16 min	
KOH Conc.	Time	KOH Conc.
	0	5
	6	5
	8	40
	12	40
	12.1	5
	16	Stop

Table 13. Cl⁻, Br⁻, CO₃²⁻ Ion Chromatography Conditions

Instrument name	Thermo ICS-6000
Mobile phase	Isocratic 25mM KOH
Flow rate	1.0mL/min
Guard column	Dionex IonPac AG11-HC, 4*50mm
Column	Dionex IonPac AS11-HC, 4*250mm
Column temp	30°C
Cell Temperature	35°C
Detection Mode	Conductivity detection
suppressor current	62mA

Injection volume	25µL
Analysis time	10 in

Differential Scanning Calorimeter

[000646] Sample (1-8 mg) was placed into a high-pressure crucible and sealed with the matching tool. The prepared sample was placed into the corresponding position of instrument, the parameters in the software were selected and the sequence run started (Table 14).

[000647] The first peak (melting point, endothermic) was observed at 76.3°C. Second peak (C22 should be volatile, endothermic) was at ~371°C.

Table 14. Differential Scanning Calorimeter (DSC) Conditions

Parameter	Value	
Instrument	Differential Scanning Calorimeter	
Instrument Model	DSC 214	
Manufacturers	NETZSCH	
Crucible Type	High voltage gold plated crucible	
Gas	Nitrogen	
Flow rate	100mL/min	
Temperature program		
Starting Temp/°C	Heating Rate/K /min	Final Temp/°C
30	10	400

Determination of the Residue Solvent (RS)

[000648] Residual Solvent (GC and HS conditions) are shown in Table 15. Instrument: SHIMADZU-GC2030 & HS-20. The column used for chromatography was DB-624 (25 m × 0.2 mm, ID 1.12 µm). P/N: 128-1324. Split Ratio: 30. Control Mode: linear Velocity. Flow: 1.2 mL/min. Carrier Gas : N₂. Detector: Flame Ionization Detector (FID). All solvents residues were found to be less than the limit of quantitation (LOQ).

Table 15. Residue Solvent Conditions

Parameter	Value			
Detector Temperature:	260 °C			
Makeup (N ₂) Flow:	30 mL/min			
H ₂ Flow:	40 mL/min			
Air Flow:	400 mL/min			
Column Temperature	Rate	Temperature	Hold Time	

		45 °C	0.2 min	
	2 °C /min	50 °C	0.0 min	
	35 °C /min	250 °C	3.3 min	
Run Time:	11.7 min			
Head-space Method:	Oven Temp	Sample Line Temp		TR.Line Temp
	110 °C	150 °C		180 °C
	Equilibrating Time: 10 min			
Sample Concentration:	100 mg/mL			
Diluent:	1,3-Dimethyl-2 imidazolidinone			

Solubility Testing

[000649] **Tag5** (10 mg) was added into solvent (100 μ L) and shaken at different temperatures (Table 16). It was found that Tag5 is soluble in DCM and THF, PE/DCM, and PE/THF in any ratio, not in DMF and DMF/DCM, not in DMSO and DMSO/DCM (See Table 16).

Table 16. Solubility of Tag5 in Organic Solvents

NO.	solvent	Ratio (%)	solubility	
			25°C	40°C
1	DCM	100%	clear	clear
2	THF/DCM	10%	clear	clear
3	THF/DCM	20%	unclear	clear
4	THF/DCM	30%	unclear	clear
5	THF/DCM	40%	unclear	clear
6	THF/DCM	50%	unclear	clear
7	THF/DCM	60%	unclear	clear
8	THF/DCM	70%	unclear	clear
9	THF/DCM	80%	unclear	clear
10	THF/DCM	90%	unclear	clear
11	THF	100%	clear	clear
12	DMF/DCM	10%	unclear	unclear
13	DMF/DCM	20%	unclear	unclear
14	DMF/DCM	30%	unclear	unclear
15	DMF/DCM	40%	unclear	unclear
16	DMF/DCM	50%	unclear	unclear

17	DMF	100%	unclear	unclear
18	DMSO/DCM	10%	unclear	unclear
19	DMSO/DCM	20%	unclear	unclear
20	DMSO/DCM	30%	unclear	unclear
21	DMSO/DCM	40%	unclear	unclear
22	DMSO/DCM	50%	unclear	unclear
23	DMSO	100%	unclear	unclear
24	PE/DCM	10%	clear	clear
25	PE/THF	10%	clear	clear

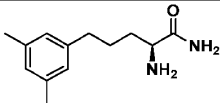
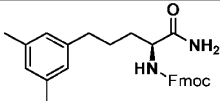
Stability Testing

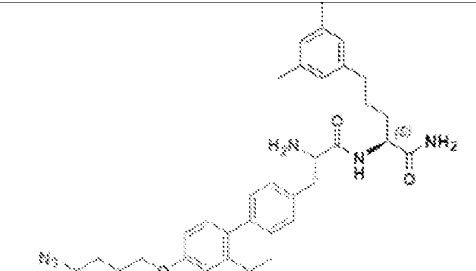
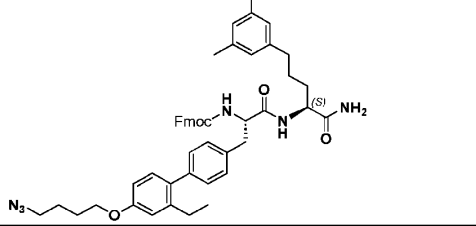
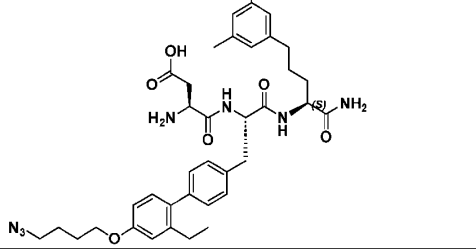
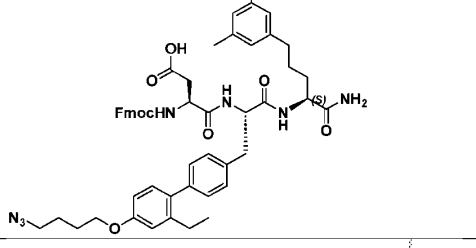
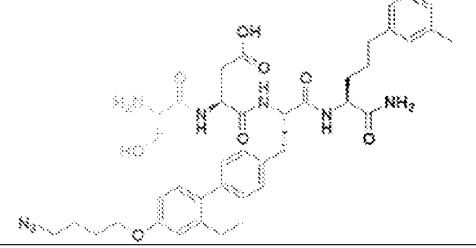
[000650] Stability Testing for Tag5 was performed at pH = 2 for 2 hrs using the following procedure. **Tag5** (1 g, 98.01% purity) was added to a 50 mL flask with a magnetic stir bar in THF (20 mL) and the mixture was stirred for 5 min to dissolve the material. A solution of TFA in DCM (v/v= 1: 5, 2 mL) was added dropwise to the solution containing Tag5 to reach pH2 and the resulting solution was continuously stirred for 2 hr. The reaction was monitored by LCMS, which showed more than five peaks. It was concluded that **Tag5** is not very stable in acidic solution (pH = 2).

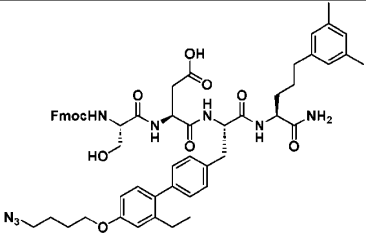
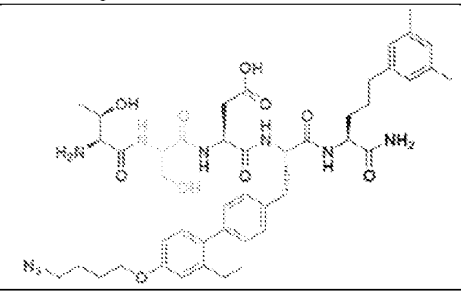
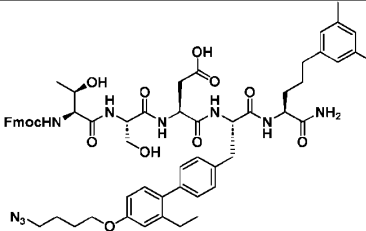
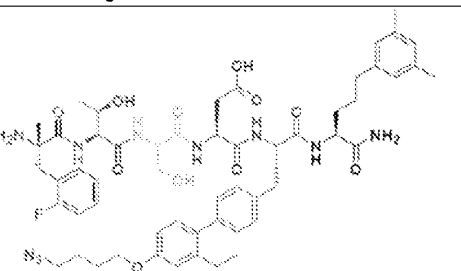
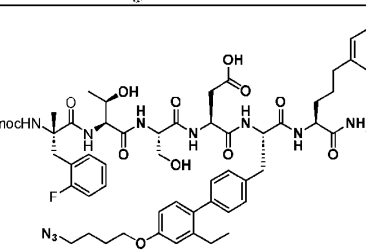
8.3 Synthesis (LPPS) of an GLP1 Peptidomimetic on Tag5

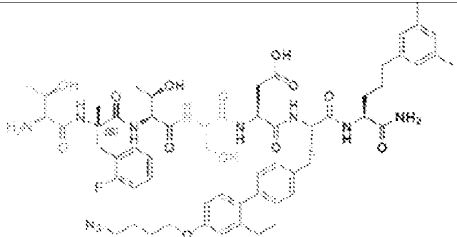
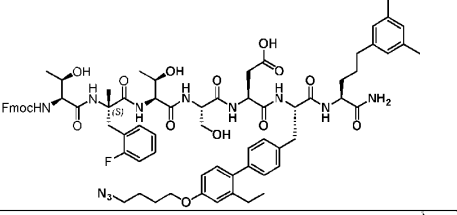
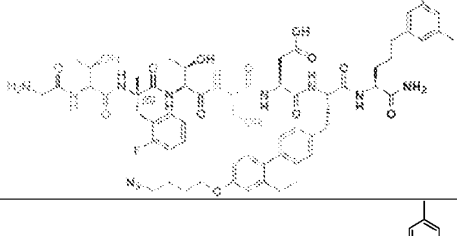
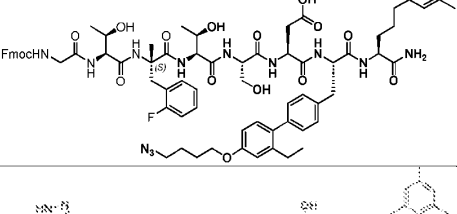
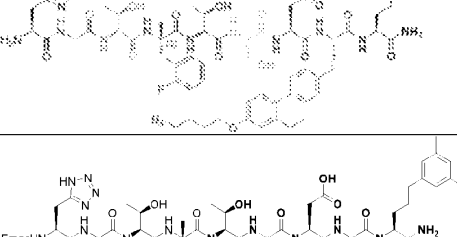
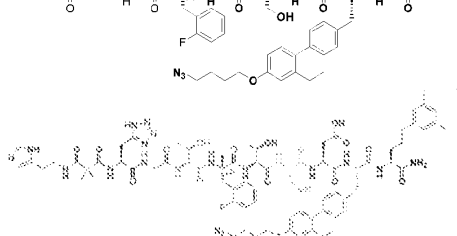
[000651] The synthesis of an GLP1 peptidomimetic (**M1**) on Tag5 is shown in Figures 6 and 10A-B and Table 17. The amino-acids used to prepare this peptidomimetic are shown in Table 18.

Table 17

<i>Product</i>			
#	Structure	Found MS (M+H) ⁺	Calcu. MF / MW after cleavage of Tag5
1AA		221.1 [M-TAG+2H] ⁺	C ₁₃ H ₂₀ N ₂ OH / 221.17
NFmoc-1AA		443.2 [M-TAG+2H] ⁺	C ₂₈ H ₃₀ N ₂ O ₃ H 443.24

2AA		585.4 [M-TAG+2H] ⁺	C ₃₄ H ₄₄ N ₆ O ₃ H / 585.36
NFmoc-2AA		807.4 [M-TAG+2H] ⁺	C ₄₉ H ₅₄ N ₆ O ₅ H 807.43
3AA		700.4 [M-TAG-tBu+3H] ⁺	C ₃₈ H ₄₉ N ₇ O ₆ H / 700.38
NFmoc-3AA		922.5 [M-TAG-tBu+3H] ⁺	C ₅₃ H ₅₉ N ₇ O ₈ H 922.45
4AA		787.4 [M-TAG-tBu-TBS+4H] ⁺	C ₄₁ H ₅₄ N ₈ O ₈ H / 787.4

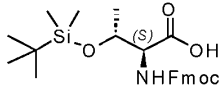
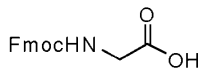
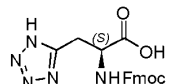
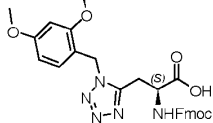
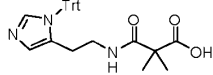
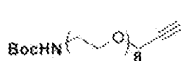
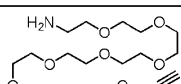
NFmoc-4AA		1009.4 [M-TAG-tBu-TBS+4H] ⁻	C ₅₆ H ₆₄ N ₈ O ₁₀ H 1009.48
5AA		1002.7 [M-TAG-tBu-TBS+4H] ⁺	C ₅₁ H ₇₅ N ₉ O ₁₀ SiH / 1002.55
NFmoc-5AA		1110.5 [M-TAG-tBu-2TBS+5H] ⁺	C ₆₀ H ₇₁ N ₉ O ₁₂ H 1110.53
6AA		1067.7 [M-TAG-tBu-2TBS+5H]	C ₅₅ H ₇₁ FN ₁₀ O ₁₁ H / 1067.54
NFmoc-6AA		1290.8 [M-TAG-tBu-2TBS+5H] ⁺	C ₇₀ H ₈₁ FN ₁₀ O ₁₃ H 1289.61

7AA		1282.7 [M-TAG-tBu-2TBS+5H] ⁺	C ₆₅ H ₉₂ FN ₁₁ O ₁₃ SiH / 1282.67
NFmoc-7AA		1390.5 [M-TAG-tBu-3TBS+6H] ⁺	C ₇₄ H ₈₈ FN ₁₁ O ₁₅ H 1390.65
8AA		1225.6 [M-TAG-tBu-3TBS+6H] ⁺	C ₆₁ H ₈₁ FN ₁₂ O ₁₄ H / 1225.61
NFmoc-8AA		1447.6 [M-TAG-tBu-3TBS+6H] ⁺	C ₇₆ H ₉₁ FN ₁₂ O ₁₆ H 1447.68
9AA		1364.7 [M-TAG-tBu-3TBS-DMB+7H] ⁺	C ₆₅ H ₈₆ FN ₁₇ O ₁₅ H / 1364.66
NFmoc-9AA		794.2 [M-TAG-tBu-3TBS-DMB+8H] ²⁺	C ₈₀ H ₉₆ FN ₁₇ O ₁₇ H, 1586.74 [M-TAG-tBu-3TBS-DMB+7H] ⁺ , C ₈₀ H ₉₆ FN ₁₇ O ₁₇ H ₂ , 793.87 [M-TAG-tBu-3TBS-DMB+8H] ²⁺
10AA		786.7 [M-TAG-tBu-3TBS-DMB-Trt+9H] ²⁺	C ₇₅ H ₉₉ FN ₂₀ O ₁₇ H ₂

Tag5-M1		990.4, [M-Tag5-tBu-3TBS-DMB-Trt-Boc+10H] ²⁺	C ₉₄ H ₁₃₆ N ₂₁ O ₂₅ H ₂ , 990.01, [M-Tag5-tBu-3TBS-DMB-Trt-Boc+10H] ²⁺
M1		990.5, [M+2H] ²⁺	C ₉₄ H ₁₃₆ N ₂₁ O ₂₅ FH ₂ , 990.01, [M+2H] ²⁺
AA10-AA9-AA8		814.4 [M+H] ⁺	C ₁₆ H ₂₃ N ₉ O ₅ , 421.18

Table 18

Cpd # No.	structure	Source*	MF/MW	ESI-MS M/Z	IUPAC Name
AA1		CAS# 2104031-58-3 ^{a, b}	C ₂₈ H ₂₉ NO ₄ 443.2	466.1 [M+Na] ⁺	(2S)-5-(3,5-dimethylphenyl)-2-(9H-fluoren-9-ylmethoxycarbonylamino)pentanoic acid
AA2			C ₃₆ H ₃₆ N ₄ O ₅ 604.2	627.3 [M+Na] ⁺	(2S)-3-[4-[4-(4-azidobutoxy)-2-ethylphenyl]phenyl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic acid
AA3			C ₂₃ H ₂₅ NO ₆ 411.1	412.1 [M+H] ⁺	(2S)-4-tert-butoxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-oxo-butanoic acid
AA4			C ₂₂ H ₂₅ NO ₅ 383.1	384.1 [M+H] ⁺	(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic acid
TBS-AA4			C ₂₄ H ₃₁ NO ₅ Si 441.20	442.2 [M+H] ⁺	(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic acid
AA5			C ₂₃ H ₂₇ NO ₅ 397.1	398.1 [M+H] ⁺	N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-(tert-butyl)-L-threonine
TBS-AA5			C ₂₅ H ₃₃ NO ₅ Si 455.21	456.22 [M+H] ⁺	(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoic acid
AA6			C ₂₅ H ₂₂ FNO 419.1	420.1 [M+H] ⁺	(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(2-fluorophenyl)-2-methyl-propanoic acid
AA7			C ₂₃ H ₂₇ NO ₅ 397.1	398.1 [M+H] ⁺	(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoic acid

TBS-AA7			C25H33NO5 Si 455.21	456.22 [M+H] ⁺	N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-(tert-butyl dimethylsilyl)-L-threonine
AA8			C17H15NO4 297.1	298.1 [M+H] ⁺	2-(9H-fluoren-9-ylmethoxycarbonylamino)acetic acid
AA9		CAS# 954147-35-4a	C19H17N5O4 379.13	380.0 [M+H] ⁺	(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(1H-triazol-5-yl)propanoic acid
DMB-AA9		CAS# 2765065-45-8b	C28H27N5O6 529.20	530.4 [M+H] ⁺	(2S)-3-[1-[(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic acid
AA10		CAS# 2131830-60-7b	C29H30N3O3 468.2	468.1 [M+H] ⁺	2,2-dimethyl-3-oxo-3-[2-(3-tritylimidazol-4-yl)ethylamino]propanoic acid
N-Boc-NH-PEG8-alkyne		CAS# 1196732-52-1d	C24H45NO10 507.30	508.3 [M+H] ⁺	tert-butyl N-[2-[2-[2-[2-[2-[2-(2-prop-2-ynoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl] carbamate
NH2-PEG8-alkyne		CAS# 1196732-52-1	C19H37NO8 407.30	408.3 [M+H] ⁺	3,6,9,12,15,18,21,24-octaooxaheptacos-26-yn-1-amine

^a GB2551945 to Andrews et al., which is hereby incorporated by reference in its entirety

^b WO2022/056494 to Han et al., which is hereby incorporated by reference in its entirety

^c Sureshbabu et al., "Synthesis of Tetrazole Analogues of Amino Acids Using Fmoc Chemistry: Isolation of Amino Free Tetrazoles and Their Incorporation into Peptides," *Tetrahedron Lett.* 48(39):7038–7041 (2007), which is hereby incorporated by reference in its entirety

^d Murelli et al., "Chemical Control Over Immune Recognition: A Class of Antibody-Recruiting Small Molecules That Target Prostate Cancer," *J. Am. Chem. Soc.* 131(47):17090-17092 (2009), which is hereby incorporated by reference in its entirety

General Procedure H for Amide Coupling to Give N-Fmoc-Tag5-nAA

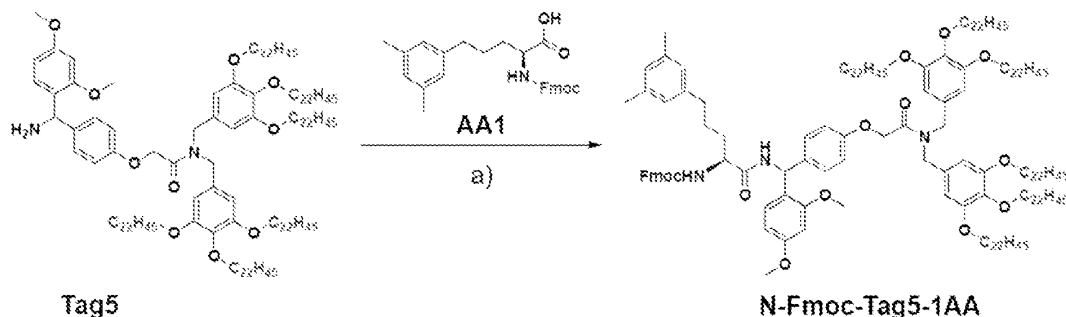
[000652] To a mixture of **Tag5** or **Tag5-(n-1)AA** (1 eq.) in DCM (50 V) were added **AA**n (1.5 eq.), HOAt (1.5 eq.), and DIC (1.5 eq.). The resulting suspension was stirred at 20 °C for 2 h. A small sample of the reaction mixture was taken, suspended with ACN, and centrifuged 2 times (3 min at 6000 rpm). The formed precipitate was dissolved in 1 drop of DCM, cleaved using TFA/TIPS/H₂O

(95/2.5/2.5), diluted with MeOH/H₂O (2/1), and filtered. The filtrate was checked by LCMS to confirm the desired mass. The reaction was concentrated under reduced pressure to give a residue, which was added into ACN/MeOH (2/1, 60V) and stirred for 1 h. The suspension was filtered and washed 2 times with ACN (10 V). The precipitate was collected and used directly in the next step without any further purification. The desired compound **N-Fmoc-Tag5-nAA** was obtained as a white solid.

General Procedure I for Fmoc Deprotection to Prepare Tag5-nAA

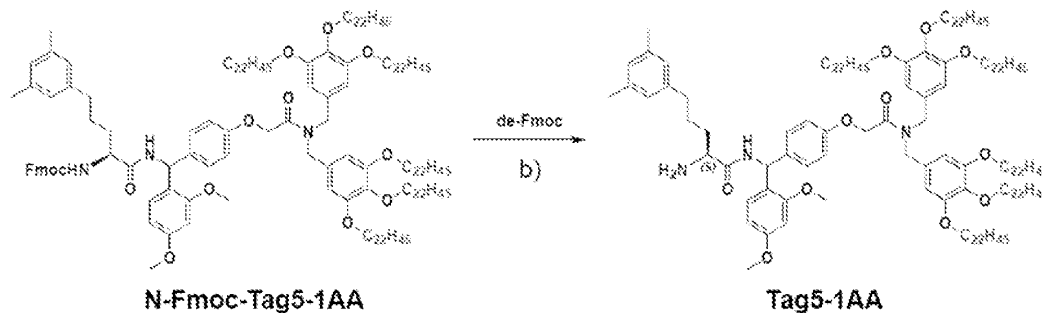
[000653] A clear solution of **N-Fmoc-Tag5-nAA** (1 eq.) in 20% piperidine/THF (20V) was stirred at 50 °C for 0.5 hr. A small amount of the sample was suspended with CAN and centrifuged 2 times (3 min at 6000 rpm). The precipitate was collected, redissolved in 1 drop of DCM, cleaved using TFA/TIPS/H₂O (95/2.5/2.5), diluted with MeOH/H₂O (2/1), and filtered. The filtrate was checked by LCMS to confirm the completion of the reaction. The reaction was concentrated under reduced pressure to give a residue, which was added into ACN/MeOH (2/1, 60V), and stirred for 1 h. The suspension was filtered and washed 2 times with ACN (10 V). The precipitate was collected and was used directly in the next step without any further purification. The desired compound **Tag5-nAA** was obtained as a white solid.

*Step a: Synthesis of 9H-Fluoren-9-ylmethyl N-[(1S)-1-[[[4-[2-[Bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl] carbamoyl]-4-(3,5-dimethylphenyl)butyl]carbamate (**N-Fmoc-Tag5-1AA**)*



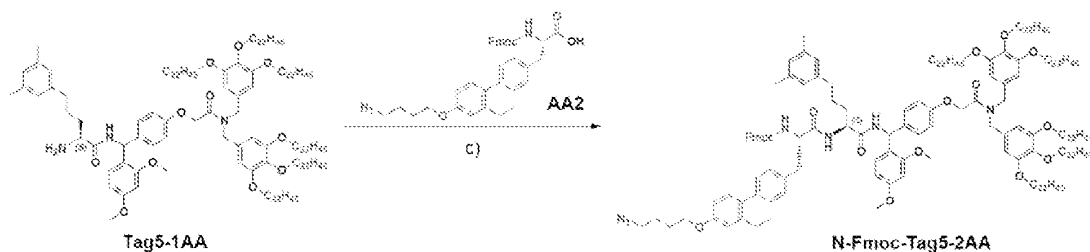
[000654] The reaction was carried out using the general procedure H for amide coupling using **Tag5** (1 g, 409.15 μmol, 1 eq), **AA1** (272 mg, 0.62 mmol, 1.5 eq), HOAt (84 mg, 0.62 mmol, 1.5 eq), and DIC (77 mg, 0.62 mmol, 95.1 μL, 1.5 eq). The reaction was monitored by LC-MS. **N-Fmoc-Tag5-1AA** (1.18 g, 344.59 μmol, 84.22% yield) was obtained as a white solid. LC-MS: RT = 5.17 min, mass calcd. for C₂₈H₃₀N₂O₃H 443.24, m/z found 443.2 [M-TAG+2H]⁺.

*Step b: Synthesis of (2S)-2-Amino-N-[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]-5-(3,5-dimethylphenyl) Pentanamide (**Tag5-1AA**)*



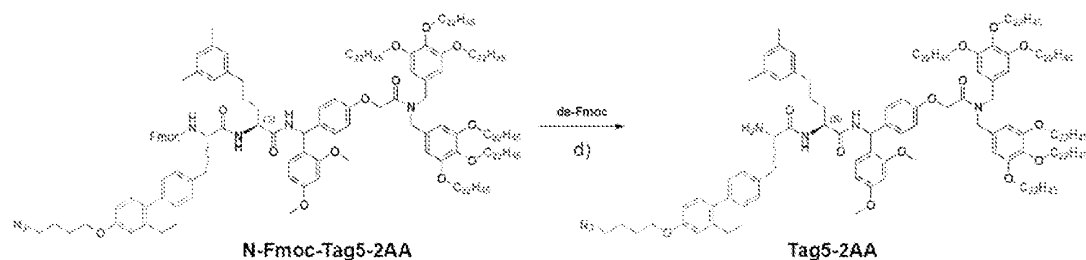
[000655] The reaction was carried out using the general procedure I for Fmoc deprotection. A clear solution of compound **N-Fmoc-Tag5-1AA** (1.16 g, 404.24 μmol , 1 eq) in 20% piperidine/THF (20 mL) was stirred at 50 °C for 0.5 hr. The reaction was monitored by LC-MS. **Tag5-1AA** (1.06 g, 400.40 μmol , 99.05% yield) was obtained as a white solid. LC-MS: RT = 2.10 min, mass calcd. for $\text{C}_{13}\text{H}_{20}\text{N}_2\text{O}_5\text{H}$ 221.17, m/z found 221.1 [M-TAG+2H]⁺.

*Step c: Synthesis of 9H-Fluoren-9-ylmethyl N-[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl) butyl]amino]-2-oxo-ethyl]carbamate (**N-Fmoc-Tag5-2AA**)*



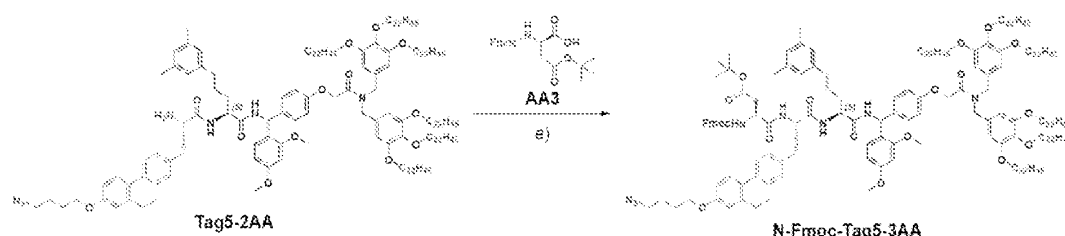
[000656] The reaction was carried out using the general procedure H for amide coupling. To a mixture of compound **Tag5-1AA** (1.06 g, 400.40 μmol , 1 eq) in DCM (40 mL) were added **AA2** (363 mg, 600.6 μmol , 1.5 eq), HOAt (81.7 mg, 0.6 mmol, 84 μL , 1.5 eq), and DIC (76 mg, 0.6 mmol, 93 μL , 1.5 eq). The resulting suspension was stirred at 20 °C for 2 h. **N-Fmoc-Tag5-2AA** (1.25 g, 386.51 μmol , 96.53% yield) was obtained as a white solid. LC-(ESI):MS RT = 6.72 min, mass calcd. for $\text{C}_{49}\text{H}_{54}\text{N}_6\text{O}_5\text{H}$ 807.43, m/z found 807.4 [M-TAG+2H]⁺.

Step d: Synthesis of (2S)-2-[[[(2S)-2-Amino-3-[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]propanoyl]amino]-N-[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]-5-(3,5-dimethylphenyl)pentanamide (**Tag5-2AA**)



[000657] The reaction was carried out using the general procedure I for Fmoc deprotection. A clear solution of compound **N-Fmoc-Tag5-2AA** (1.25 g, 386.51 μmol , 1 eq) in 20% piperidine/THF (25 mL) was stirred at 50 °C for 0.5 hr. **Tag5-2AA** (1.15 g, crude) was obtained as a white solid. LC-MS: RT = 4.46 min, mass calcd. for $\text{C}_{34}\text{H}_{44}\text{N}_6\text{O}_3\text{H}$ 585.36, m/z found 585.4 $[\text{M-TAG}+2\text{H}]^+$.

Step e: Synthesis of tert-Butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-(9H-fluoren-9-ylmethoxycarbonylamino)-4-oxo-butanoate (**N-Fmoc-Tag5-3AA**)



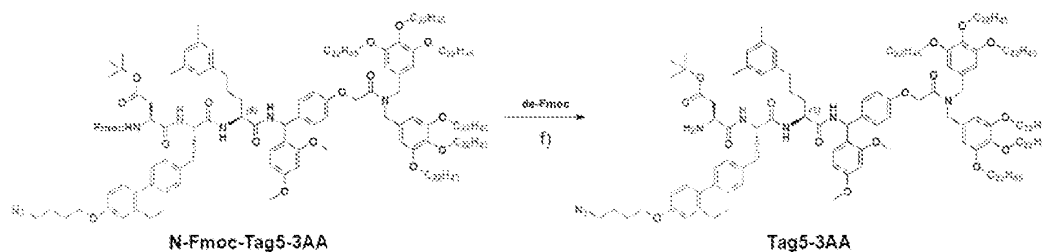
[000658] The reaction was carried out using the general procedure H for amide coupling. To a mixture of compound **Tag5-2AA** (1.15 g, 381.83 μmol , 1 eq) in DCM (60 mL) were added **AA3** (235.5 mg, 0.57 mmol, 1.5 eq), HOAt (78 mg, 0.57 mmol, 80.1 μL , 1.5 eq), and DIC (72.3 mg, 0.57 mmol, 88.7 μL , 1.5 eq). The resulting suspension was stirred at 20 °C for 2 h. **N-Fmoc-Tag5-3AA** (1.3 g, 381.76 μmol , 99.98% yield) was obtained as a white solid.

[000659] LCMS condition (CR): Reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3 μm , 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000660] LC-MS: RT = 6.25 min, mass calcd. for $C_{53}H_{59}N_7O_8H$ 922.45, m/z found 922.4 [M-TAG-tBu+3H]⁺.

[000661] LCMS condition (BF): Reverse phase LCMS was carried out using a Xtimate C18 2.1*30mm 3um column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

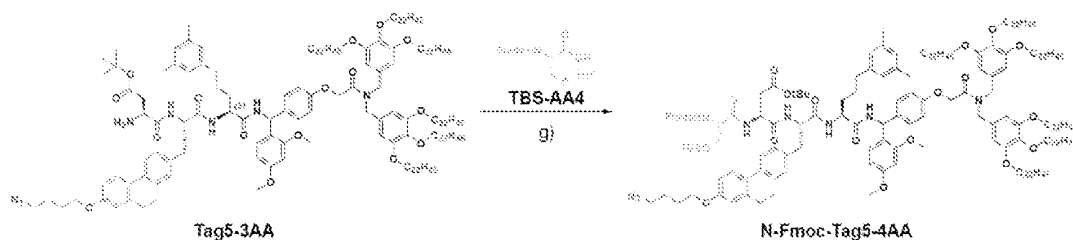
Step f: Synthesis of tert-Butyl (3S)-3-amino-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-bis[[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl) butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag5-3AA)



[000662] The reaction was carried out using the general procedure I for Fmoc deprotection. A clear solution of compound **N-Fmoc-Tag5-3AA** (1.30 g, 381.76 μ mol, 1 eq) in 20% piperidine/THF (26 mL) was stirred at 50 °C for 0.5 hr. Compound **Tag5-3AA** (1.2 g, 377.00 μ mol, 98.75% yield) was obtained as a white solid.

[000663] LC-MS: RT = 4.646 min, mass calcd. for $C_{38}H_{49}N_7O_6H$ 700.38, m/z found 700.5 [M-TAG-tBu+3H]⁺; RT = 5.065 min, mass calcd. for $C_{42}H_{57}N_7O_6Na$ 778.43, m/z found 778.5 [M-TAG+2H+Na]⁺.

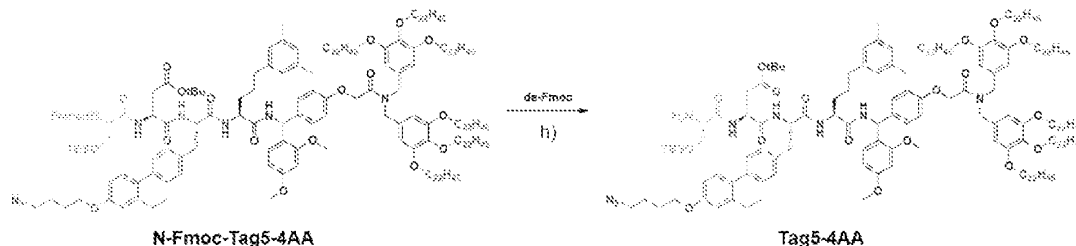
Step g: Synthesis of tert-Butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-bis[[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino) propanoyl]amino]-4-oxo-butanoate (N-Fmoc-Tag5-4AA)



[000664] The reaction was carried out using the general procedure H for amide coupling. To a mixture of compound **Tag5-3AA** (1.1 g, 345.59 μmol , 1 eq) in DCM (50 mL) were added **TBS-AA4** (229 mg, 0.52 mmol, 1.5 eq), HOAt (70.5 mg, 0.52 mmol, 1.5 eq), and DIC (65.4 mg, 0.52 mmol, 80.3 μL , 1.5 eq). The resulting suspension was stirred at 20 °C for 2 h. The reaction was monitored by LC-MS. Compound **N-Fmoc-Tag5-4AA** (1.06 g, 293.91 μmol , 85.05% yield) was obtained as a white solid.

[000665] LC-MS: RT = 6.01 min, mass calcd. for $\text{C}_{56}\text{H}_{64}\text{N}_8\text{O}_{10}\text{H}$ 1009.48, m/z found 1009.4 [M-TAG-tBu-TBS+4H]⁺. LCMS condition (BF): Reverse phase LCMS was carried out using a Xtimate C18 2.1*30mm 3 μm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

*Step h: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-amino-3-[tert-butyl(dimethyl)silyl]oxypropanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[[4-[2-bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxoethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbonyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxoethyl]amino]-4-oxo-butanoate (**Tag5-4AA**)*

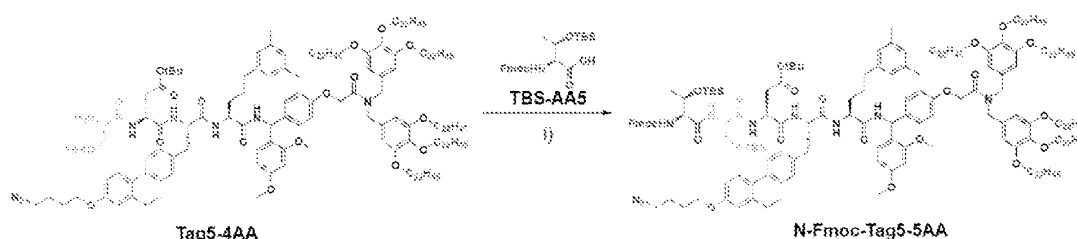


[000666] A clear solution of compound **N-Fmoc-Tag5-4AA** (1.05 g, 291.13 μmol , 1 eq) in 20% piperidine/THF (20 mL) was stirred at 50 °C for 0.5 hr. Following generic precipitation procedures the desired compound **Tag5-4AA** (985 mg, 291.05 μmol , 99.97% yield) was obtained as a white solid.

[000667] LC-MS: RT = 4.24 min, mass calcd. for $\text{C}_{41}\text{H}_{54}\text{N}_8\text{O}_8\text{H}$ 787.42, m/z found 787.4 [M-TAG-tBu-TBS+4H]⁺; RT = 5.10 min, mass calcd. for $\text{C}_{47}\text{H}_{68}\text{N}_8\text{O}_8\text{SiH}$ 901.50, m/z found 901.5 [M-

TAG-tBu+3H]⁺. LCMS condition (BF): Reverse phase LCMS was carried out using a Xtimate C18 2.1*30mm 3um column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

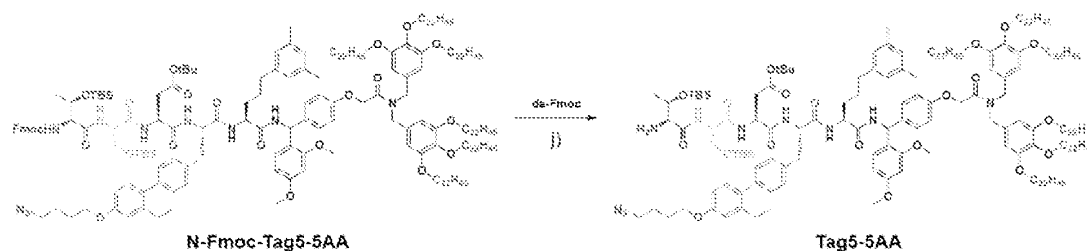
Step i: Synthesis of tert-Butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]ox-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (N-Fmoc-Tag5-5AA)



[000668] To a mixture of compound **Tag5-4AA** (985 mg, 291.05 μ mol, 1 eq) in DCM (50 mL) were added **TBS-AA5** (2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoic acid (199 mg, 0.44 mmol, 1.5 eq), HOAt (59.4 mg, 0.44 mmol, 1.5 eq), and DIC (55 mg, 0.44 mmol, 67.6 μ L, 1.5 eq). The resulting suspension was stirred at 20 °C for 2 h. Following generic precipitation procedures the compound **N-Fmoc-Tag5-5AA** (1.05 g, 274.73 μ mol, 94.39% yield) was obtained as a white solid.

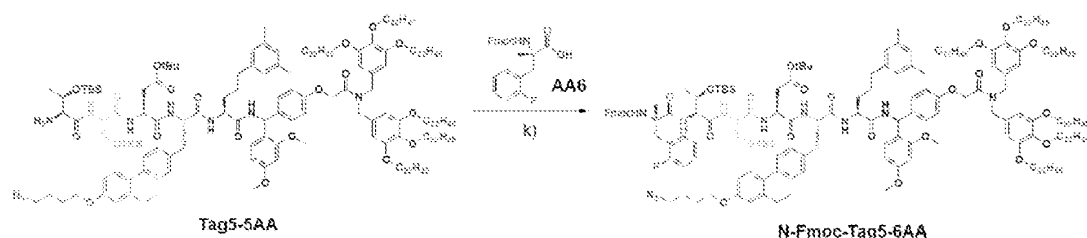
[000669] LC-MS: RT = 5.91 min, mass calcd. for C₆₀H₇₁N₉O₁₂H 1110.53, m/z found 1110.6 [M-TAG-tBu-2TBS+5H]⁺. LCMS condition (BF): Reverse phase LCMS was carried out using a Xtimate C18 2.1*30mm 3um column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step j: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-amino-3-[tert-butyl(dimethyl)silyl]oxy-propanoyl] amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag5-5AA)



[000670] A clear solution of compound **N-Fmoc-Tag5-5AA** (1.05 g, 291.13 μmol , 1 eq) in 20% piperidine/THF (20 mL) was stirred at 50 °C for 0.5 hr. Following generic precipitation procedures to get desired compound **Tag5-5AA** (0.83 g, 245.25 μmol , 84.24% yield) as a white solid. LC-MS: RT = 5.107 min, mass calcd. for $\text{C}_{51}\text{H}_{75}\text{N}_9\text{O}_{10}\text{SiH}$ 1002.55, m/z found 1002.7 $[\text{M-TAG-tBu-TBS}+4\text{H}]^+$. LCMS condition (CR): Reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3 μm , 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step k: Synthesis of tert-Butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(2-fluorophenyl)-2-methyl-propanoyl] amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (N-Fmoc-Tag5-6AA)

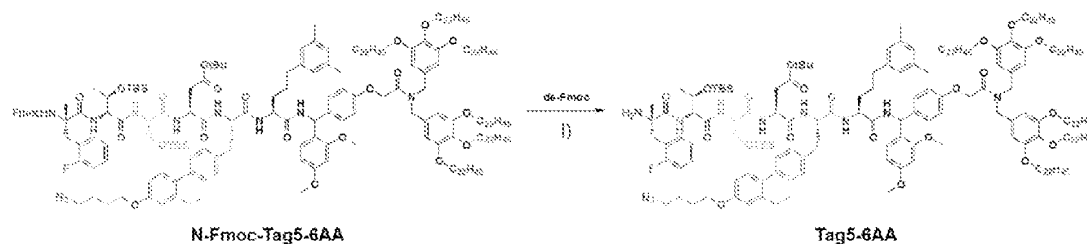


[000671] To a mixture of compound **Tag5-5AA** (0.83 g, 245.25 μmol , 1 eq) in DCM (50 mL) were added **AA6** (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(2-fluorophenyl)-2-methyl-propanoic acid (154.3 mg, 367.9 μmol , 1.5 eq), HOAt (84 mg, 0.62 mmol, 1.5 eq), and DIC (78 mg, 0.62 mmol, 96 μL , 1.5 eq). The resulting suspension was stirred at 20 °C for 2 h. Following generic precipitation procedures the desired compound **N-Fmoc-Tag5-6AA** (0.95 g, crude) was obtained as a white solid.

[000672] LC-MS: RT = 6.46 min, mass calcd. for $C_{70}H_{81}FN_{10}O_{13}H$ 1289.61, m/z found 1290.8 [M-TAG-tBu-2TBS+5H]⁺. LCMS condition (BV): Reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3um, 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000673] LC-MS: RT = 6.25 min, mass calcd. for $C_{70}H_{81}FN_{10}O_{13}H$ 1289.61, m/z found 1289.5 [M-TAG-tBu-2TBS+5H]⁺. LCMS condition (BF): Reverse phase LCMS was carried out using aXtimate C18 2.1*30mm 3um column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step l: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[[(2S)-2-amino-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[[4-[2-bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag5-6AA)

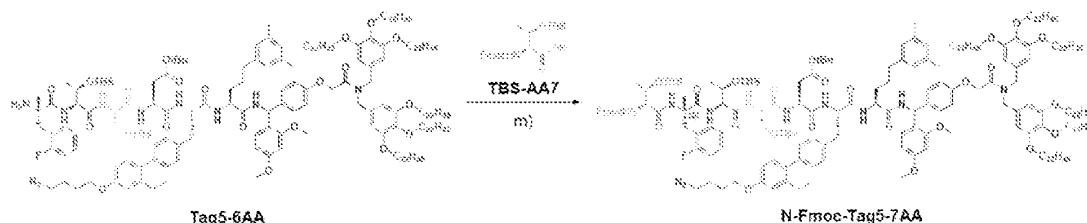


[000674] A clear solution of compound **N-Fmoc-Tag5-6AA** (0.95 g, 237.43 μmol, 1 eq) in 20% piperidine/THF (20 mL) was stirred at 50 °C for 0.5 hr. Following generic precipitation procedures the compound **Tag5-6AA** (0.85 g, 224.93 μmol, 94.74% yield) was obtained as a white solid.

[000675] LC-MS: RT = 4.64 min, mass calcd. for $C_{55}H_{71}FN_{10}O_{11}H$ 1067.54, m/z found 1067.7 [M-TAG-tBu-2TBS+5H]⁺. LCMS condition (CR): reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3um, 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step m: Synthesis of tert-Butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[[4-[2-bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-

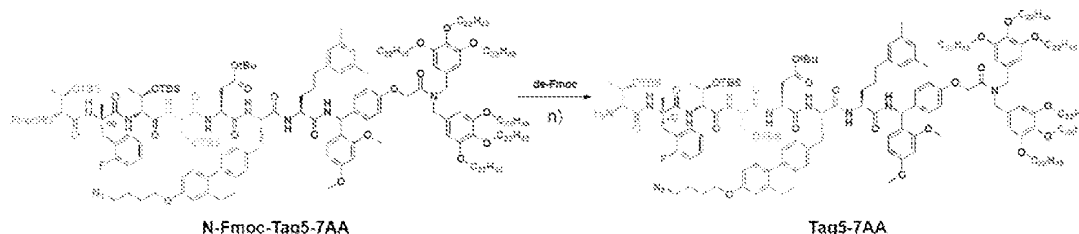
dimethoxyphenyl)methyl]carbonyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-
 [[[2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-
 ylmethoxycarbonylamino)butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (**N-Fmoc-Tag5-7AA**)



[000676] To a mixture of compound **Tag5-6AA** (0.85 g, 224.93 μmol , 1 eq) in DCM (50 mL) were added **TBS-AA7** (2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoic acid (1.02 g, 2.25 mmol, 10 eq), HOAt (306.16 mg, 2.25 mmol, 314.65 μL , 10 eq), and DIC (283.87 mg, 2.25 mmol, 348.30 μL , 10 eq). The resulting suspension was stirred at 40 °C for 12 hrs. Following generic precipitation procedures the compound **N-Fmoc-Tag5-7AA** (0.91 g, crude) was obtained as a white solid.

[000677] LC-MS: RT = 6.24 min, mass calcd. for $\text{C}_{74}\text{H}_{88}\text{FN}_{11}\text{O}_{15}\text{H}$ 1390.65, m/z found 1390.5 $[\text{M-TAG-tBu-3TBS}+6\text{H}]^+$. LCMS condition (BF): reverse phase LCMS was carried out using aXtimate C18 2.1*30mm 3 μm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

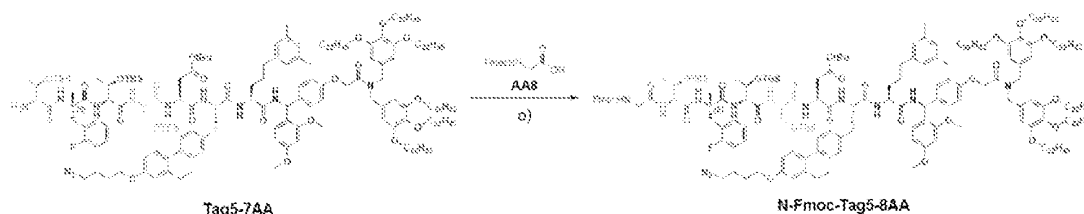
Step n: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-amino-3-[tert-butyl(dimethyl)silyl]oxybutanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbonyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate;molecular hydrogen (**Tag5-7AA**)



[000678] A clear solution of compound **N-Fmoc-Tag5-7AA** (0.91 g, 238.10 μmol , 1 eq) in 20% piperidine/THF (3 mL) was stirred at 50 °C for 0.5 hr. Following generic precipitation procedures the compound **Tag5-7AA** (0.85 g, 236.13 μmol , 99.17% yield) was prepared as a white solid.

[000679] LC-MS: RT = 5.72 min, mass calcd. for $\text{C}_{66}\text{H}_{92}\text{FN}_{11}\text{O}_{13}\text{SiH}$ 1282.67, m/z found 1282.7 [M-TAG-tBu-2TBS+5H]⁺. LCMS condition (CR): Reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3 μm , 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

*Step o: Synthesis of tert-Butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[[4-[2-bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-(9H-fluoren-9-ylmethoxycarbonylamino)acetyl]amino]butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (**N-Fmoc-Tag5-8AA**)*



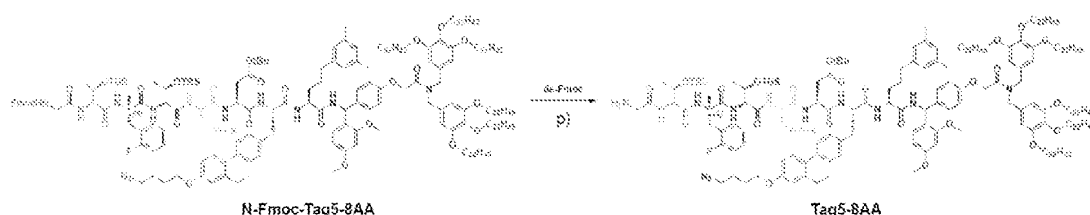
[000680] To a mixture of compound **Tag5-7AA** (0.85 g, 236.13 μmol , 1 eq) in DCM (50 mL) were added **AA8** 2-(9H-fluoren-9-ylmethoxycarbonylamino)acetic acid (105.3 mg, 354 μmol , 1.5 eq), HOAt (48.2 mg, 354 μmol , 1.5 eq), and DIC (44.7 mg, 354 μmol , 54.8 μL , 1.5 eq). The resulting suspension was stirred at 20 °C for 2 h. Following generic precipitation procedures the desired compound **N-Fmoc-Tag5-8AA** (0.79 g, 184.86 μmol , 78.29% yield) was obtained as a white solid.

[000681] LC-MS: RT = 6.32 min, mass calcd. for $\text{C}_{76}\text{H}_{91}\text{FN}_{12}\text{O}_{16}\text{H}$ 1447.68, m/z found 1448.6 [M-TAG-tBu-3TBS+6H]⁺. LCMS condition (BV): reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3 μm , 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000682] LC-MS: RT = 6.11 min, mass calcd. for $\text{C}_{76}\text{H}_{91}\text{FN}_{12}\text{O}_{16}\text{H}$ 1447.68, m/z found 1447.6 [M-TAG-tBu-3TBS+6H]⁺. LCMS condition (BF): reverse phase LCMS was carried out using a

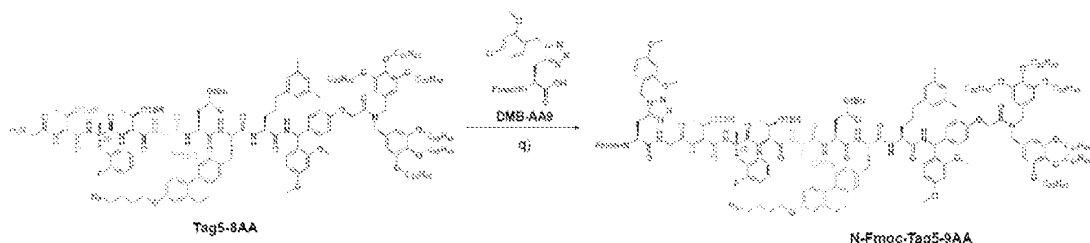
Xtimate C18 2.1*30mm 3um column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step p: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[2-[[[(2S,3R)-2-[(2-aminoacetyl)amino]-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbonyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (Tag5-8AA)



[000683] A clear solution of compound **N-Fmoc-Tag5-8AA** (0.79 g, 184.86 μmol , 1 eq.) in 20% piperidine/THF (20 mL) was stirred at 50 °C for 0.5 hr. Following generic precipitation procedures the compound **Tag5-8AA** (0.74 g, 182.66 μmol , 98.81% yield) was obtained as a white solid. LC-MS: RT = 4.47 min, mass calcd. for $\text{C}_{61}\text{H}_{81}\text{FN}_{12}\text{O}_{14}\text{H}$ 1225.61, m/z found 1225.6 [M-TAG-tBu-3TBS+6H]⁺.

Step q: Synthesis of tert-Butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-[bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbonyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-[[[(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(1H-triazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (N-Fmoc-Tag5-9AA)



[000684] **Using protected AA9.** To a mixture of compound **Tag5-8AA** (50 mg, 12.34 μ mol, 1 eq) in DCM (5 mL) were added **AA9** (7 mg, 18.51 μ mol, 1.5 eq), HOAt (2.52 mg, 18.51 μ mol, 1.5 eq), and DIC (2.34 mg, 18.51 μ mol, 2.87 μ L, 1.5 eq). The resulting suspension was stirred at 20 °C for 2 h. No desired mass was detected (Table 19).

Table 19. Reaction Between Tag5-8AA and AA9

Tag5-8AA	AA		DIC	HOAt	Condition	Crude by LC-MS
1 eq	AA9	1.5 eq.	1.5 eq.	1.5 eq.	20 °C for 2 h	No coupling product
1 eq	DMB-AA9	1.5 eq.	1.5 eq.	1.5 eq.	20 °C for 2 h	Reaction works

[000685] LCMS condition (CR): Reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3 μ m, 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000686] LCMS condition (BF): Reverse phase LCMS was carried out using a Xtimate C18 2.1*30mm 3 μ m column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

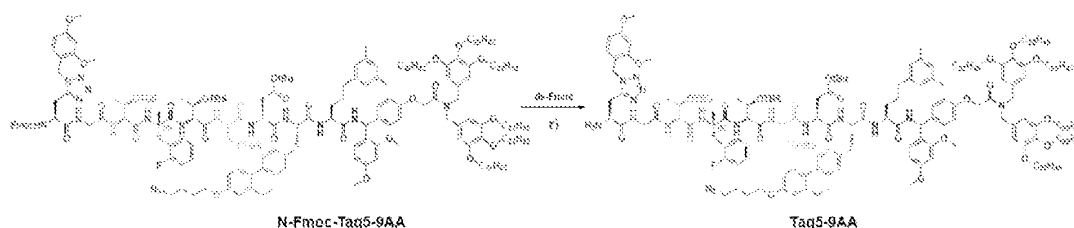
[000687] **Using protected DMB-AA9.** To a mixture of compound **Tag5-8AA** (0.5 g, 123.42 μ mol, 1 eq) in DCM (5 mL) were added **DMB-AA9** (98 mg, 185 μ mol, 1.5 eq), HOAt (25.2 mg, 185 μ mol, 1.5 eq), and DIC (23.36 mg, 185 μ mol, 28.6 μ L, 1.5 eq). The resulting suspension was stirred at 20 °C for 2 h. Following generic precipitation procedures the desired compound **N-Fmoc-Tag5-9AA** (510 mg, 111.77 μ mol, 90.57% yield) was obtained as a white solid (Table 19).

[000688] LC-MS: RT = 5.91 min, mass calcd. for C₈₀H₉₆FN₁₇O₁₇H, 1586.74 [M-TAG-tBu-3TBS-DMB+7H]⁺, C₈₀H₉₆FN₁₇O₁₇H₂, 793.87 [M-TAG-tBu-3TBS-DMB+8H]²⁺, m/z found 794.3 [M-TAG-tBu-3TBS-DMB+8H]²⁺. LCMS condition (BV): Reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3 μ m, 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000689] LC-MS: RT = 5.68 min, mass calcd. for C₈₀H₉₆FN₁₇O₁₇H, 1586.74 [M-TAG-tBu-3TBS-DMB+7H]⁺, C₈₀H₉₆FN₁₇O₁₇H₂, 793.87 [M-TAG-tBu-3TBS-DMB+8H]²⁺, m/z found 794.2 [M-TAG-tBu-3TBS-DMB+8H]²⁺. LCMS condition (BF): Reverse phase LCMS was carried out using a Xtimate C18 2.1*30mm 3 μ m column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step r: Synthesis of tert-Butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[2-[[[(2S,3R)-2-[[2-[[[(2S)-2-amino-3-[1-[(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]propanoyl]amino]acetyl]amino]-3-tert-

butyl(dimethyl)silyl]oxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (**Tag5-9AA**)



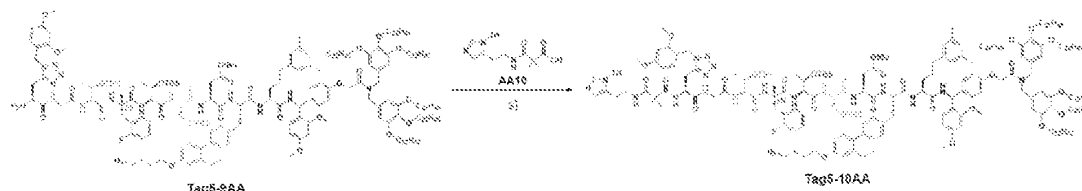
[000690] A clear solution of compound **N-Fmoc-Tag5-9AA** (500 mg, 109.58 μmol , 1 eq.) in 20% piperidine/THF (20 mL) was stirred at 50 °C for 0.5 hr. Following generic precipitation procedures the desired compound **Tag5-9AA** (0.47 g, 108.28 μmol , 98.81% yield) was obtained as a white solid.

[000691] LC-MS: RT = 4.78 min, mass calcd. For $\text{C}_{65}\text{H}_{86}\text{FN}_{17}\text{O}_{15}\text{H}$ 1364.66, m/z found 1364.7 [M-TAG-tBu-3TBS-DMB+7H]⁺. LCMS condition (CR): Reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3 μm , 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000692] LC-MS: RT = 4.47 min, mass calcd. For $\text{C}_{65}\text{H}_{86}\text{FN}_{17}\text{O}_{15}\text{H}$ 1364.66, m/z found 1364.7 [M-TAG-tBu-3TBS-DMB+7H]⁺. LCMS condition (BF): Reverse phase LCMS was carried out using a Xtimate C18 2.1*30mm 3 μm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

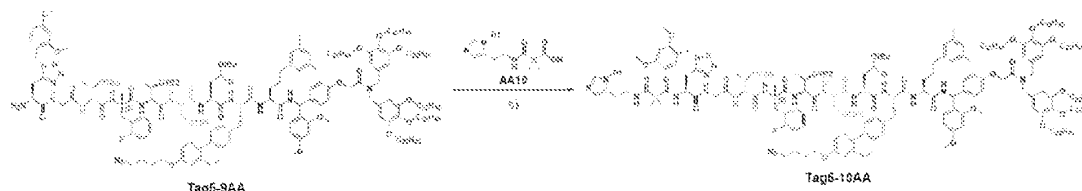
Step s: Synthesis of tert-Butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-Azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-[[4-[2-bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecyloxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbamoyl]-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S)-3-[1-[(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]-2-[[2,2-dimethyl-3-oxo-3-[2-(3-tritylimidazol-4-yl)ethylamino]propanoyl]amino]

propanoyl]amino]acetyl]amino]butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (Tag5-10AA)



[000693] To a mixture of compound **Tag5-9AA** (450 mg, 103.67 μmol , 1 eq) in DCM (20 mL) were added **AA10** (72.73 mg, 155.5 μmol , 1.5 eq), HOAt (21.2 mg, 155.5 μmol , 1.5 eq), and DIC (19.6 mg, 155.5 μmol , 24.1 μL , 1.5 eq). The resulting suspension was stirred at 20 °C for 2 h. Following generic precipitation procedures the desired compound **Tag5-10AA** (480 mg, 100.21 μmol , 96.66% yield) was obtained as a white solid. LC-MS (Method 4): RT = 4.456 min, mass calcd. for $\text{C}_{75}\text{H}_{99}\text{FN}_{20}\text{O}_{17}\text{H}$ 1571.76 $[\text{M-TAG-tBu-3TBS-DMB-Trt}+8\text{H}]^+$, $\text{C}_{75}\text{H}_{99}\text{FN}_{20}\text{O}_{17}\text{H}_2$, 786.38 $[\text{M-TAG-tBu-3TBS-DMB-Trt}+9\text{H}]^{2+}$, m/z found 786.7 $[\text{M-TAG-tBu-3TBS-DMB-Trt}+9\text{H}]^{2+}$; LC-MS (Method 4): RT = 4.85 min, mass calcd. for $\text{C}_{75}\text{H}_{99}\text{FN}_{20}\text{O}_{17}\text{H}$ 1571.76 $[\text{M-TAG-tBu-3TBS-DMB-Trt}+8\text{H}]^+$, $\text{C}_{75}\text{H}_{99}\text{FN}_{20}\text{O}_{17}\text{H}_2$, 786.38 $[\text{M-TAG-tBu-3TBS-DMB-Trt}+9\text{H}]^{2+}$, m/z found 786.8 $[\text{M-TAG-tBu-3TBS-DMB-Trt}+9\text{H}]^{2+}$.

Step t: Synthesis of tert-Butyl (3S)-4-[[[(1S)-2-[[[(1S)-1-[[[4-[2-Bis[[3,4,5-tris(docosa-1,3,5,7,9,11,13,15,17,19,21-undecaynoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]carbonyl]-4-(3,5-dimethylphenyl)butyl]amino]-1-[[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-(tert-butoxycarbonylamino)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[[(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S)-3-[1-[(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]-2-[[2,2-dimethyl-3-oxo-3-[2-(3-tritylimidazol-4-yl)ethylamino]propanoyl]amino]propanoyl]amino]acetyl]amino]butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (Tag5-M1)

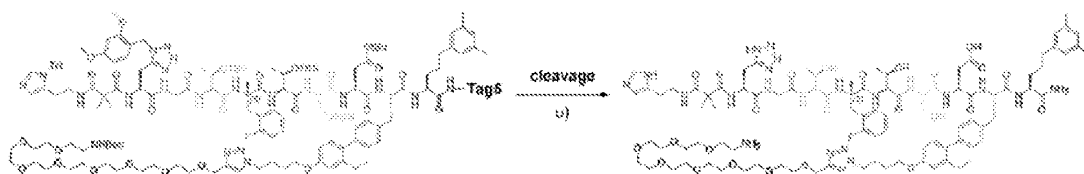


[000694] The compound **Tag5-10AA** (0.3 g, 62.63 μ mol, 1 eq) was dissolved in H₂O (1 mL) and THF (4 mL), then a solution of CuSO₄·5H₂O (23.46 mg, 93.94 μ mol, 1.5 eq) and sodium ascorbate (18.61 mg, 93.94 μ mol, 1.5 eq) in H₂O (1 mL), a solution of TBTA (16.62 mg, 31.31 μ mol, 0.5 eq) in DMSO (0.5 mL), and a solution of tert-butyl **PEG8-Linker** (95.37 mg, 187.89 μ mol, 3 eq) in THF (4 mL) were added. The mixture was stirred at 40 °C for 12 hr. Following generic precipitation procedures the desired compound **Tag5-M1** (330 mg, 62.29 μ mol, 99.46% yield) was obtained as a white solid.

[000695] LC-MS: RT = 3.72 min, mass calcd. for C₉₄H₁₃₆FN₂₁O₂₅H₂, 990.01 [M-TAG-tBu-3TBS-DMB-Trt-Boc+10H]²⁺, m/z found 990.6 [M-TAG-tBu-3TBS-DMB-Trt-Boc+10H]²⁺. LCMS condition (CR): Reverse phase LCMS was carried out using a NanoChrom ChromCore 120 C18 3 μ m, 3.0*30mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000696] LC-MS: RT = 3.39 min, mass calcd. for C₉₄H₁₃₆FN₂₁O₂₅H₂, 990.01, m/z found 990.4 [M-TAG-tBu-3TBS-DMB-Trt-Boc+10H]²⁺; RT = 3.99 min, mass calcd. For C₁₁₃H₁₅₀FN₂₁O₂₅H₂, 1111.06, m/z found 1111.5 [M-TAG-tBu-3TBS-DMB-Boc+9H]²⁺. LCMS condition (BF): Reverse phase LCMS was carried out using a Xtimate C18 2.1*30mm 3 μ m column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% ACN containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

*Step u: Synthesis of (3S)-4-[[[(1S)-1-[[4-[4-(4-Azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[(1S)-1-carbamoyl-4-(3,5-dimethylphenyl)butyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-2-[[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[[(2S,3R)-3-hydroxy-2-[[2-[[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic Acid (**M1**)*



[000697] To a mixture of **Tag5-M1** with all side-chain protective groups (430 mg, 81.17 μ mol, 1 eq) in DCM (10 mL) was added a TFA cocktail (TFA/TIPS/H₂O = 95/2.5/2.5) (30 mL). The resulting suspension was stirred at 20 °C for 2 hr. A sample was taken, suspended with PE/MTBE (3/1, 1 mL), and centrifuged to give a solid. The solid was diluted with MeOH and H₂O, filtered, and the filtrate was checked by LCMS, which showed the desired mass for **M1**. The reaction was

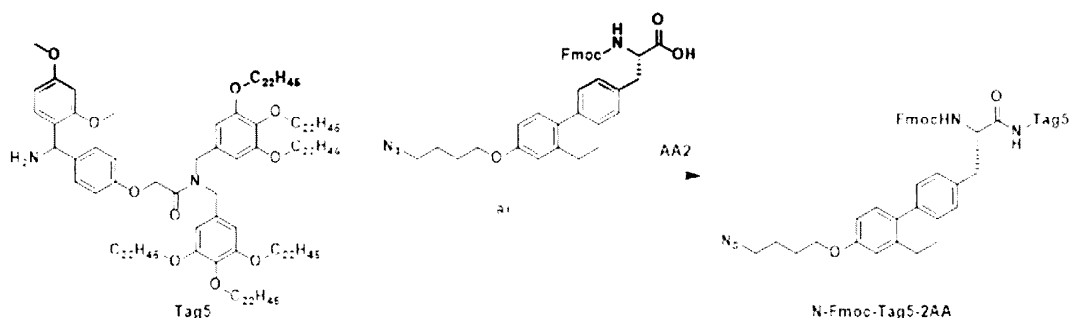
concentrated under reduced pressure to give a residue, which was added into PE/TBME (3/1, 20V) and stirred for 1 h. The suspension was filtered to give a white solid. The residue was purified by prep-HPLC (basic condition): column: Boston Prime C18 150*30mm*5um; mobile phase: [water (ammonia hydroxide v/v)-ACN]; gradient: 25%-45% B over 11 min. The desired compound **M1** (5.1 mg, 2.45 μ mol, 3.02% yield, 95% purity) was obtained as a white solid. LC-MS: RT = 3.399 min, mass calcd. for $C_{94}H_{136}N_{21}O_{25}FH$ 1979.01 $[M+H]^+$, $C_{94}H_{136}N_{21}O_{25}FH_2$ 990.01 $[M+2H]^{2+}$, m/z found 990.5 $[M+2H]^{2+}$; LCMS condition: Method 7.

[000698] **M1** was characterized using 1H NMR, ^{19}F NMR (non-TFA salt) and **M1**(TFA Salt), F NMR, ^{13}C NMR, and UPLC. UPLC of **M1** showed the impurity at RT=6.5min, which was the system impurity. LCMS of **M1** showed that the main peak at retention time of 1.564 min corresponds to the desired product **M1** with the observed mass of $[M+H]^+=1980.3$, $[M/2+H]^+=990.3$, $[M/3+H]^+=660.7$, $[M/4+H]^+=495.8$.

[000699] **M1**-HCl salt identification by IC showed that the HCl quality content was 2.08% and a molar content was 1.15 mol eq.

8.4 Synthesis (LPPS) of **M3** on Tag5

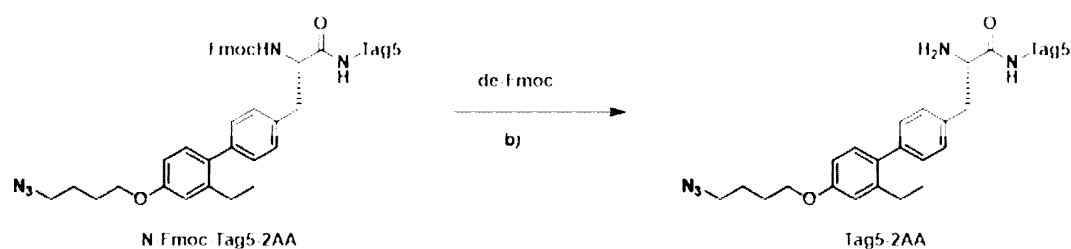
[000700] The synthesis of **M3** on Tag5 is shown herein. Figure 7 shows the synthesis of **Tag5-M3** and Figure 12 shows the elongation conditions and results to make **M3** using **Tag5**. *Step a: Synthesis of 9H-fluoren-9-ylmethyl N-[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[4-[2-[bis[4-[[3,4,5-tri(docosoxy)phenyl]methoxy]phenyl]methylamino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]amino]-2-oxo-ethyl]carbamate (N-Fmoc-Tag5-2AA)*



[000701] To a mixture of **Tag5** (2 g, 757 μ mol, 1 eq.) in DCM (50 mL) were added **AA2** (687 mg, 1.14 mmol, 1.5 eq) in THF (40 mL) and were added HOAt (155 mg, 1.14 mmol, 1.5 eq.) and DIC (143.28 mg, 1.14 mmol, 175.81 μ L, 1.5 eq.). Then the solution was stirred at 50 $^{\circ}C$ for 12 hrs. monitored by LC-MS trace. This reaction was carried out using the general procedure H for amide

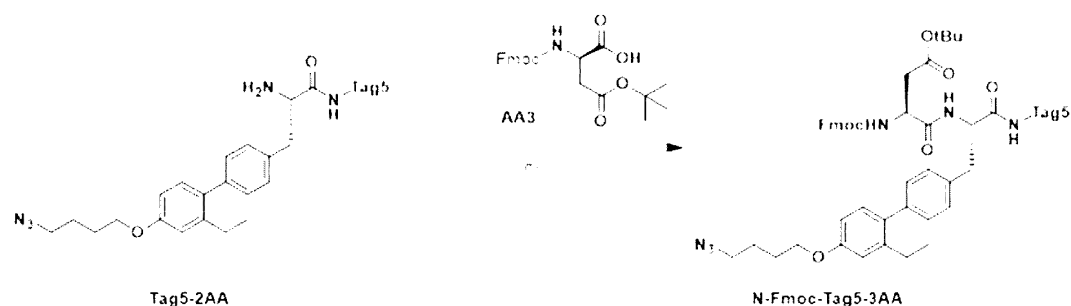
coupling to give **N-Fmoc-Tag5-2AA** (2.3 g, 712.30 μmol , 94.11% yield) as a white solid. LC-MS: RT = 5.506 min, mass calcd. for $\text{C}_{36}\text{H}_{37}\text{N}_5\text{O}_4\text{Na}$ 626.03, m/z found 626.70 $[\text{M-TAG}+\text{H}+\text{Na}]^+$.

[000702] Step b: Synthesis of rac-(2S)-2-amino-3-[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]-N,N-bis[3,4,5-tri(docosoxy)phenyl]methyl]propanamide (**Tag5-2AA**)



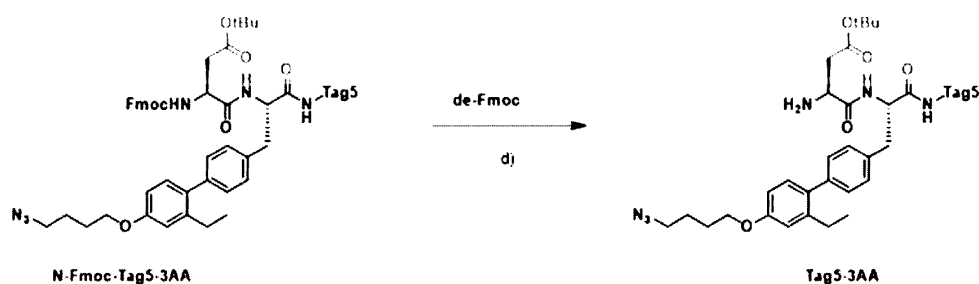
[000703] A clear solution of compound **N-Fmoc-Tag5-2AA** (1 g, 366.11 μmol , 1 eq) in 20% piperidine/THF (20 mL) was stirred at 50 $^{\circ}\text{C}$ for 2 hr without monitoring. Generic precipitation procedures were followed to get **Tag5-2AA** (0.9 g, 358.68 μmol , 97.97% yield) as a white solid.

[000704] Step c: Synthesis of tert-butyl rac-(3S)-3-(9H-fluoren-9-ylmethoxycarbonylamino)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[[[4-[2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethoxy]phenyl]-(2,4-dimethoxyphenyl)methyl]amino]-2-oxo-ethyl]amino]butanoate (**N-Fmoc-Tag5-3AA**)



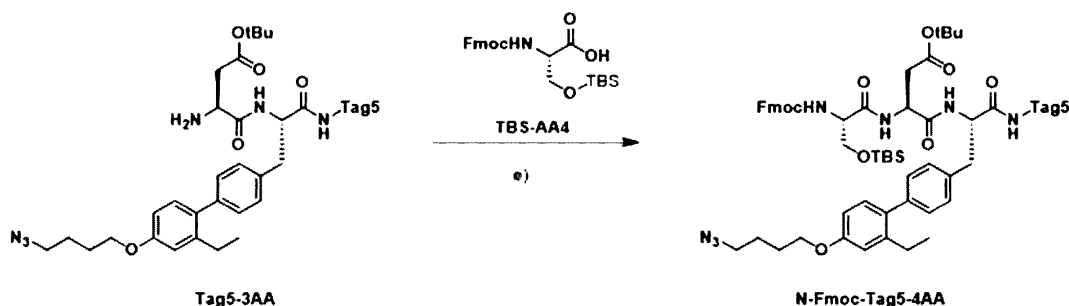
[000705] To a mixture of compound **Tag5-2AA** (0.9 g, 320.45 μmol , 1 eq) in DCM (40 mL) were added AA3 (395.55 mg, 961.36 μmol , 3.0 eq), HOAt (130.85 mg, 961.36 μmol , 134.48 μL , 3.0 eq) and DIC (121.32 mg, 961.36 μmol , 148.86 μL , 3.0 eq). The resulting suspension was stirred at 20 °C for 2 h. Generic precipitation procedures were followed to get desired compound **N-Fmoc-Tag5-3AA** (1.2 g, crude) as a white solid. LC-MS: RT = 5.038 min, mass calcd. for $\text{C}_{40}\text{H}_{43}\text{N}_6\text{O}_7$ 719.31, m/z found 719.30 $[\text{M}-\text{TAG}-\text{tBu}+2\text{H}]^+$.

[000706] Step d: Synthesis of tert-butyl rac-(3S)-3-amino-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]butanoate (**Tag5-3AA**)



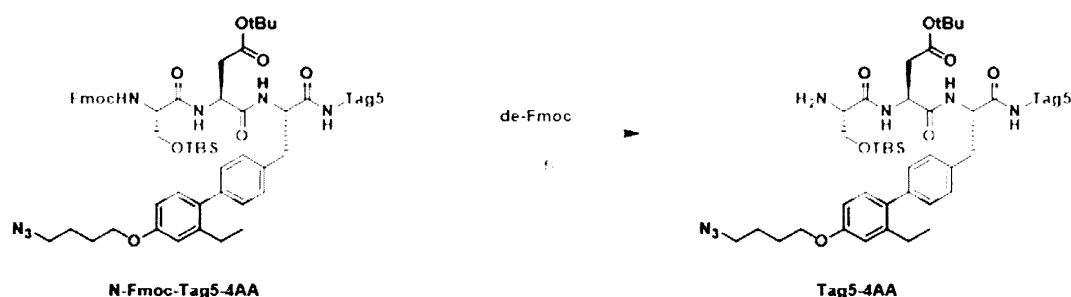
[000707] A clear solution of compound **N-Fmoc-Tag5-3AA** (1.2 g, crude) in 20% piperidine/THF (25 mL) was stirred at 50 °C for 2 hrs without monitoring. Generic precipitation procedures were followed to get desired compound **Tag5-3AA** (1.11 g, 414.12 μ mol, 100.00% yield) as a white solid.

[000708] *Step e: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoyl]amino]butanoate (**N-Fmoc-Tag5-4AA**)*



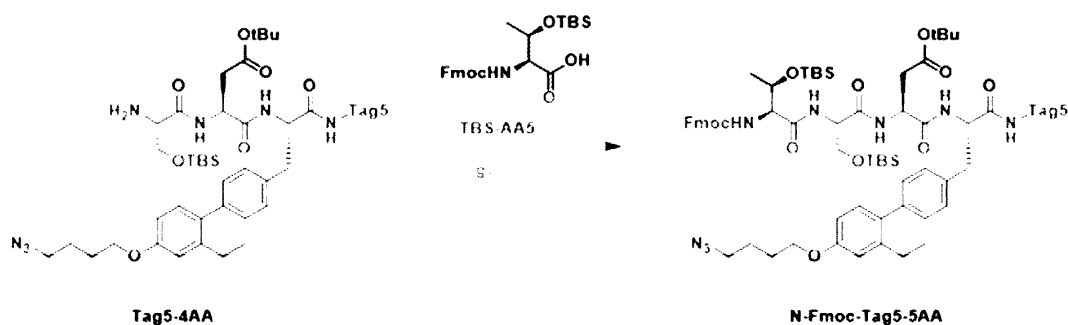
[000709] To a mixture of compound **Tag5-3AA** (1.11 g, 414.12 μ mol, 1 eq.) in DCM (60 mL) were added TBS-AA4 (548.61 mg, 1.24 mmol, 3.0 eq.), DIC (156.78 mg, 1.24 mmol, 192.37 μ L, 3.0 eq.) and HOAt (169.10 mg, 1.24 mmol, 173.79 μ L, 3.0 eq.). Generic precipitation procedures were followed to get desired compound **N-Fmoc-Tag5-4AA** (1.2 g, 386.60 μ mol, 93.36% yield) as a white solid. LC-MS: RT = 4.791 min, mass calcd. for $C_{43}H_{47}N_7O_9Na_2$ 851.34, m/z found 851.70 [M-TAG-tBu-OTBS+4H]⁺.

[000710] *Step f: Synthesis of tert-butyl rac-(3S)-4-oxo-3-[[rac-(2S)-2-amino-3-[tert-butyl(dimethyl)silyl]oxy-propanoyl]amino]-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]butanoate (**Tag5-4AA**)*



[000711] A clear solution of compound **N-Fmoc-Tag5-4AA** (1.2 g, crude) in 20% piperidine/THF (25 mL) was stirred at 50 °C for 2 hr without monitoring. Generic precipitation procedures were followed to get desired compound **Tag5-4AA** (1.1 g, 381.71 μ mol, 98.74% yield) as a white solid.

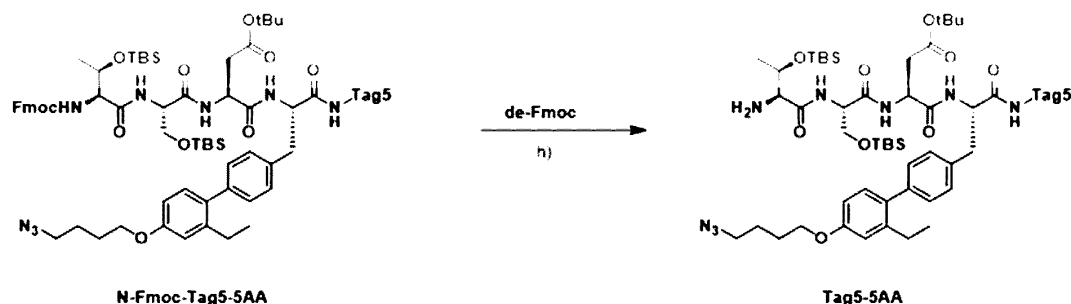
[000712] *Step g: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoyl]amino]propanoyl]amino]butanoate* (**N-Fmoc-Tag5-5AA**)



[000713] To a mixture of compound **Tag5-4AA** (1.1 g, 381.71 μ mol, 1 eq.) in THF (40 mL) were added TBS- AA5 (521.75 mg, 1.15 mmol, 3.0 eq.), DIC (144.52 mg, 1.15 mmol, 177.32 μ L, 3.0 eq.) and HOAt (155.87 mg, 1.15 mmol, 160.19 μ L, 3.0 eq.). The resulting suspension was stirred at 20 °C for 2 h, monitored by LC-MS. Generic precipitation procedures were followed to get desired compound **N-Fmoc-Tag5-5AA** (1.25 g, 376.58 μ mol, 98.66% yield) was obtained as a white solid. LC-MS: RT = 4.670 min, mass calcd. for $C_{47}H_{55}N_8O_{11}$ 907.39, m/z found 907.50 [M-TAG-tBu-2OTBS+5H]⁺.

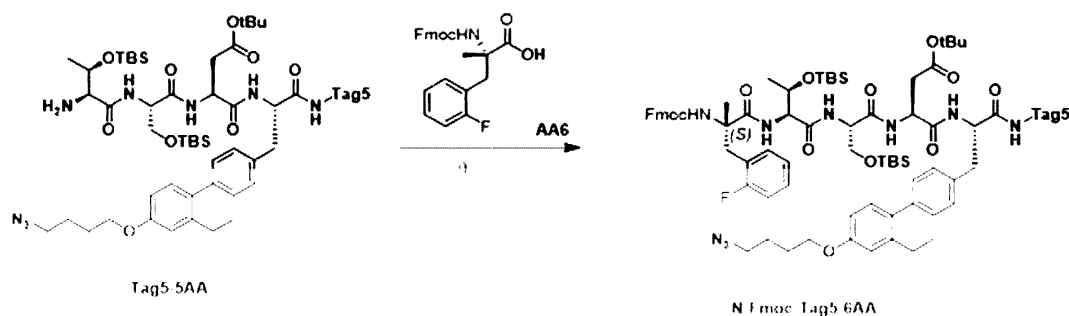
[000714] *Step h: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-*

[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[rac-(2S,3R)-2-amino-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]propanoyl]amino]butanoate (**Tag5-5AA**)



[000715] A clear solution of compound **N-Fmoc-Tag5-5AA** (1.25 g, 376.58 μmol , 1 eq.) in 20% Piperidine/THF (20 mL) was stirred at 50 °C for 2 hr. Generic precipitation procedures were followed to get desired compound **Tag5-5AA** (1.1 g, crude) as a white solid.

[000716] *Step i: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(2-fluorophenyl)-2-methylpropanoyl]amino]butanoyl]amino]propanoyl]amino]butanoate (**N-Fmoc-Tag5-6AA**)*

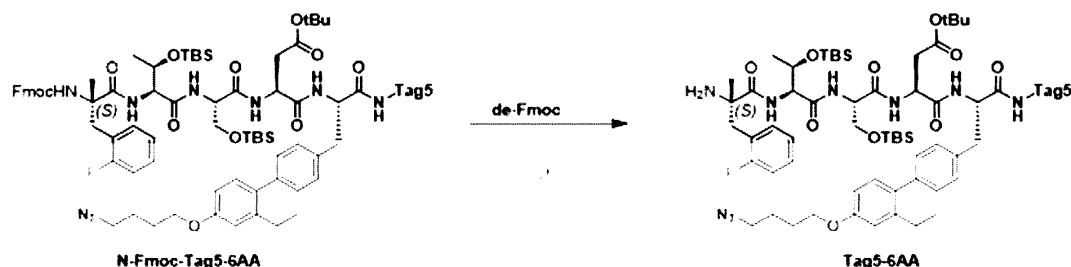


[000717] To a mixture of compound **Tag5-5AA** (1.1 g, 355.17 μmol , 1 eq.) in THF (50 mL) were added **AA6** (rac-(2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(2-fluorophenyl)-2-methylpropanoic acid (297.95 mg, 710.34 μmol , 2.0 eq), HOAt (96.68 mg, 710.34 μmol , 99.37 μL , 2.0 eq) and DIC (89.65 mg, 710.34 μmol , 109.99 μL , 2.0 eq). The resulting suspension was stirred at 20 °C for 2 h. Generic precipitation procedures were followed to give the desired compound **N-Fmoc-Tag5-6AA** (1.2 g, 343.00 μmol , 96.57% yield) as a white solid. LC-MS: RT = 5.821 min, mass calcd. for $\text{C}_{57}\text{H}_{65}\text{FN}_9\text{O}_{12}$ 1086.47, m/z found 1086.20 [M-TAG-tBu-2OTBS+5H]⁺.

[000718] *Step j: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-*

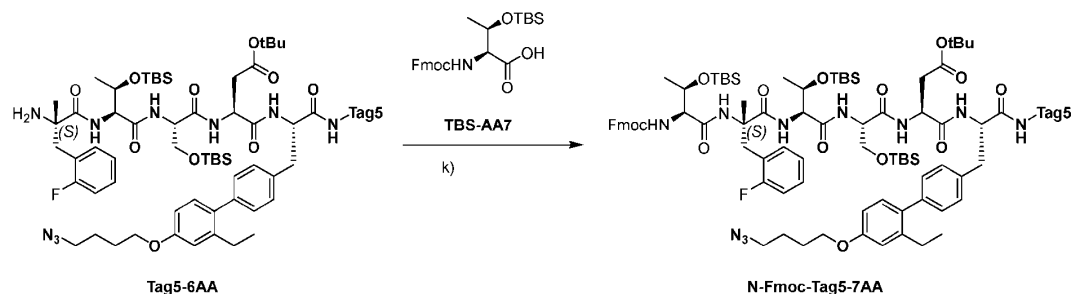
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[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[rac-(2S,3R)-2-[[2-amino-3-(2-fluorophenyl)-2-methylpropanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]propanoyl]amino]butanoate (**Tag5-6AA**)



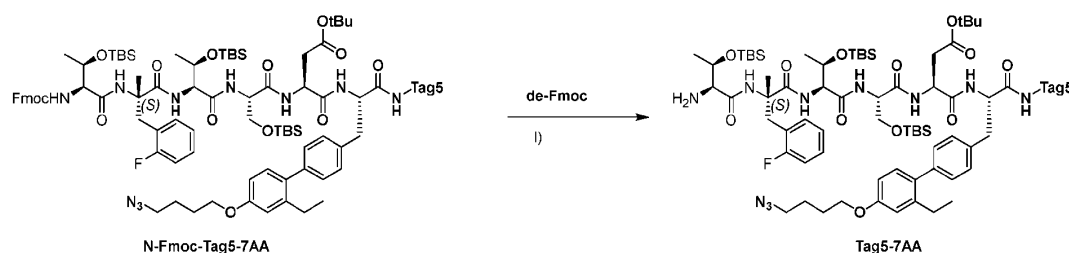
[000719] A clear solution of compound **N-Fmoc-Tag5-6AA** (1.2 g, 343.00 μmol , 1 eq.) in 20% piperidine/THF (20 mL) was stirred at 50 °C for 2 hr. Generic precipitation procedures were followed to get desired compound **Tag5-6AA** (1.1 g, 335.75 μmol , 97.88% yield) as a white solid.

[000720] *Step k: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[3-(2-fluorophenyl)-2-methyl-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoyl]amino]propanoyl]amino]butanoyl]amino]propanoyl]amino]butanoate (**N-Fmoc-Tag5-7AA**)*



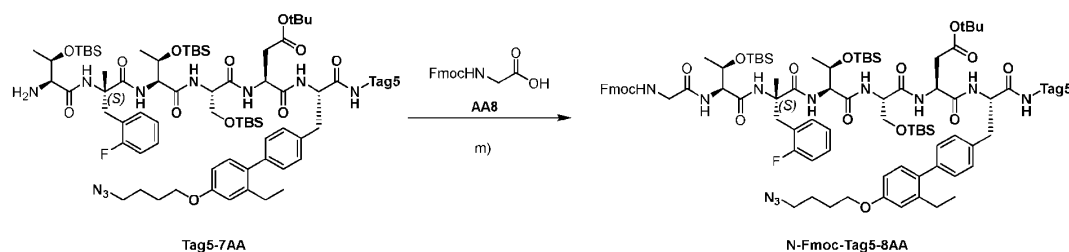
[000721] To a mixture of compound **Tag5-6AA** (1.1 g, 335.75 μmol , 1 eq) in THF (40 mL) were added **TBS-AA7** (rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoic acid (1.53 g, 3.36 mmol, 10.0 eq), HOAt (456.98 mg, 3.36 mmol, 469.66 μL , 10.0 eq) and DIC (423.71 mg, 3.36 mmol, 519.89 μL , 10.0 eq). The resulting suspension was stirred at 20 °C for 2 h. Generic precipitation procedures were followed to get desired compound **N-Fmoc-Tag5-7AA** (1.2 g, 323.11 μmol , 96.24% yield) as a white solid. LC-MS: RT = 5.487min, mass calcd. for $\text{C}_{61}\text{H}_{71}\text{FN}_{10}\text{O}_{14}\text{H}$ 1187.51, m/z found 1087.40 $[\text{M-TAG-tBu-3OTBS}+6\text{H}]^+$.

[000722] *Step l: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[3-(2-fluorophenyl)-2-methyl-2-[[rac-(2S,3R)-2-amino-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]propanoyl]amino]butanoyl]amino]propanoyl]amino]butanoate (Tag5-7AA)*



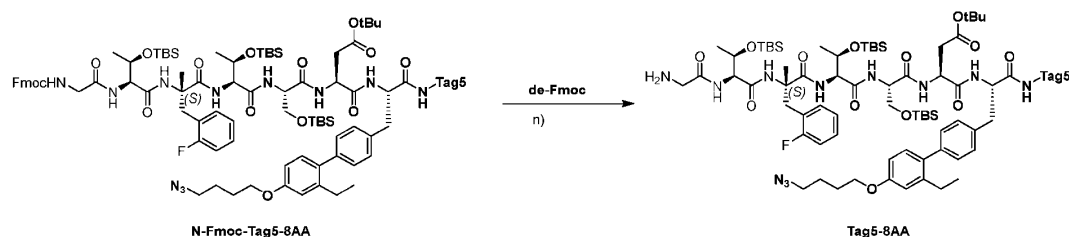
[000723] A clear solution of compound **N-Fmoc-Tag5-7AA** (1.1 g, 296.18 μmol , 1 eq.) in 20% piperidine/THF (20 mL) was stirred at 50 °C for 2 hr. Generic precipitation procedures were followed to get desired compound **Tag5-7AA** (1.0 g, 286.40 μmol , 96.70% yield) as a white solid.

[000724] *Step m: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[3-(2-fluorophenyl)-2-methyl-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-(9H-fluoren-9-ylmethoxycarbonylamino)acetyl]amino]butanoyl]amino]propanoyl]amino]butanoyl]amino]propanoyl]amino]butanoate (N-Fmoc-Tag5-8AA)*



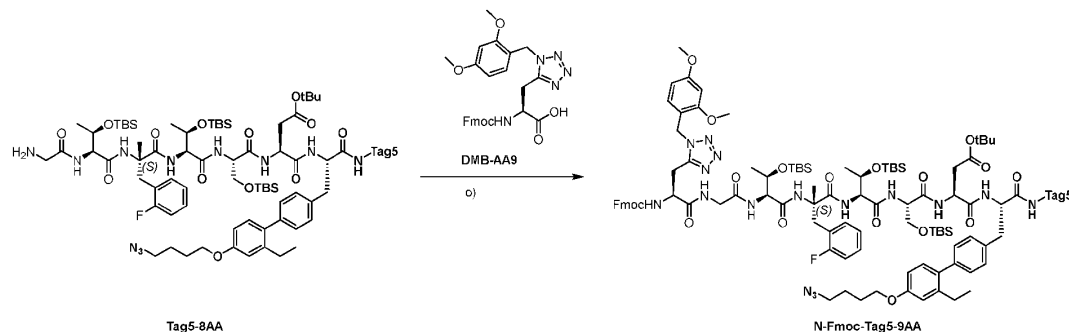
[000725] To a mixture of compound **Tag5-7AA** (1.0 g, 286.40 μmol , 1 eq) in THF (40 mL) were added **AA8** (2-(9H-fluoren-9-ylmethoxycarbonylamino)acetic acid (851.47 mg, 2.86 mmol, 10.0 eq), **DIC** (361.43 mg, 2.86 mmol, 443.48 μL , 10.0 eq) and **HOAt** (389.81 mg, 2.86 mmol, 400.63 μL , 10.0 eq). The resulting suspension was stirred at 50 °C for 12 hr. Generic precipitation procedures were followed to get desired compound **N-Fmoc-Tag5-8AA** (0.91 g, crude) as a white solid. LC-MS: RT = 5.597 min, mass calcd. for $\text{C}_{63}\text{H}_{74}\text{FN}_{11}\text{O}_{15}\text{H}$ 1244.53, m/z found 1244.57 [**M-TAG-tBu-3OTBS+7H**]⁺.

[000726] Step n: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[3-(2-fluorophenyl)-2-methyl-2-[[rac-(2S,3R)-2-[(2-aminoacetyl)amino]-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]propanoyl]amino]butanoyl]amino]propanoyl]amino]butanoate (**Tag5-8AA**)



[000727] A clear solution of compound **N-Fmoc-Tag5-8AA** (1.0 g, 265.19 μmol , 1 eq.) in 20% piperidine/THF (30 mL) was stirred at 50 °C for 2 hr. Generic precipitation procedures were followed to get desired compound **Tag5-8AA** (0.9 g, 253.61 μmol , 95.64% yield) as a white solid.

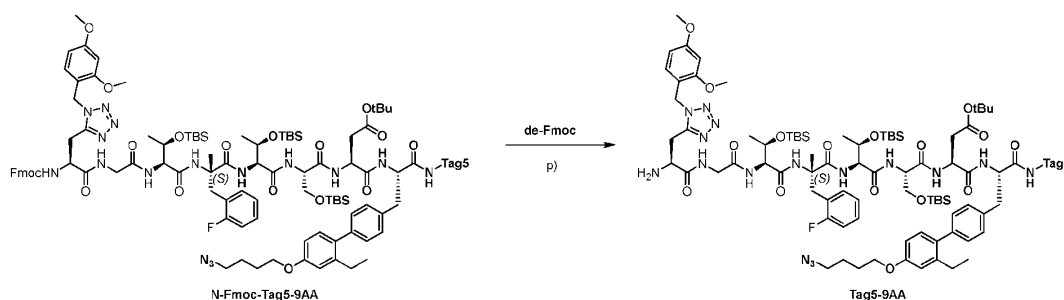
[000728] Step o: Synthesis of tert-butyl rac-(3S)-4-oxo-4-[[rac-(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-[[rac-(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[3-(2-fluorophenyl)-2-methyl-2-[[rac-(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-[[rac-(2S)-3-[1-(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoyl]amino]acetyl]amino]butanoyl]amino]propanoyl]amino]butanoyl]amino]propanoyl]amino]butanoate (**N-Fmoc-Tag5-9AA**)



[000729] To a mixture of compound **Tag5-8AA** (0.9 g, 253.61 μmol , 1 eq.) in THF (40 mL) were added **DMB-AA9** (rac-(2S)-3-[1-[(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]-2-(9H-fluoren-9-ylmethoxycarbonyl amino)propanoic acid (402.90 mg, 760.84 μmol , 3.0 eq.), HOAt (103.56 mg, 760.84 μmol , 106.43 μL , 3.0 eq) and DIC (96.02 mg, 760.84 μmol , 117.81 μL , 3.0 eq.). The resulting suspension was stirred at 20 °C for 2 h. Generic precipitation procedures were followed to get

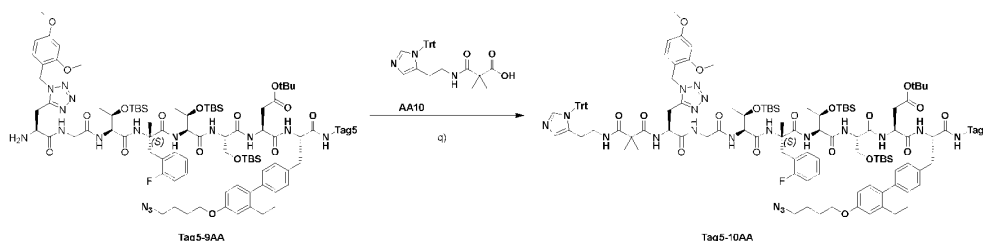
desired compound **N-Fmoc-Tag5-9AA** (0.95 g, 233.98 μmol , 92.26% yield) as a white solid. LC-MS: RT = 6.248 min, mass calcd. for $\text{C}_{71}\text{H}_{87}\text{FN}_{16}\text{O}_{16}\text{H}$ 1439.65, m/z found 1439.30 $[\text{M-TAG-PMB-3OTBS}+7\text{H}]^+$.

[000730] Step p: Synthesis of tert-butyl (3S)-3-[[[(2S)-2-[[[(2S,3R)-2-[[2-[[[(2S)-2-amino-3-[1-[(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]propanoyl]amino]acetyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-butanoyl]amino]-3-[tert-butyl(dimethyl)silyl]oxy-propanoyl]amino]-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-4-oxo-butanoate (**Tag5-9AA**)



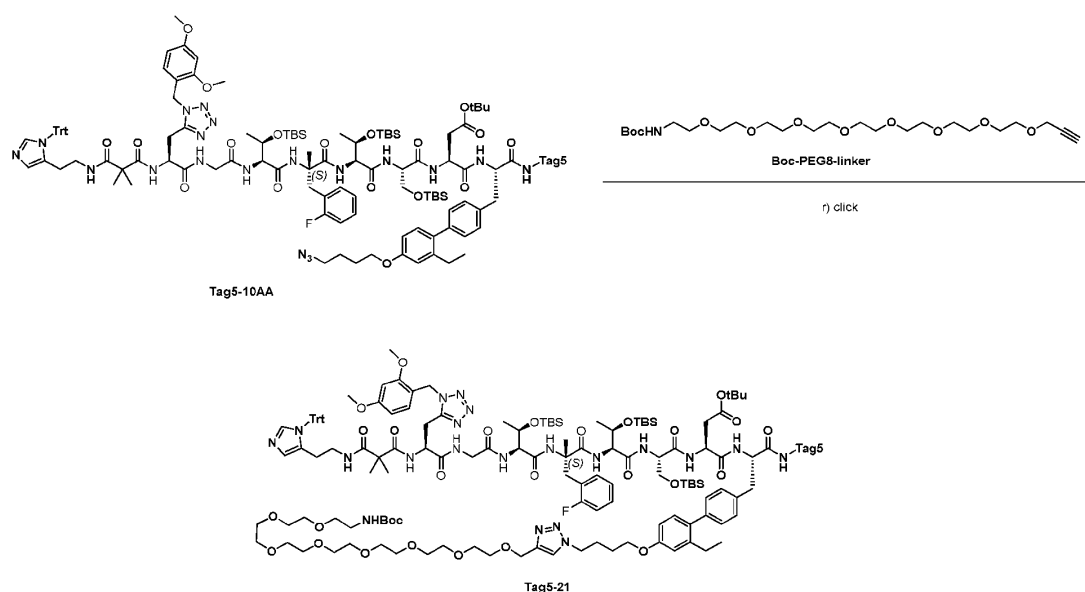
[000731] A clear solution of compound **N-Fmoc-Tag5-9AA** (0.95 g, 233.98 μmol , 1 eq.) in 20% Piperidine/THF (20 mL) was stirred at 50 °C for 2 hr. It was a clear solution. Generic precipitation procedures were followed to get desired compound **Tag5-9AA** (850 mg, 221.47 μmol , 94.65% yield) as a white solid.

[000732] Step q: Synthesis of tert-butyl (3S)-4-[[[(1S)-1-[[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]methyl]-2-bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-2-oxo-ethyl]amino]-3-[[[(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-[[[(2S)-3-[1-[(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]-2-[[2,2-dimethyl-3-oxo-3-[2-(3-tritylimidazol-4-yl)ethylamino]propanoyl]amino]propanoyl]amino]acetyl]amino]butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanoate (**Tag5-10AA**)



[000733] To a mixture of compound **Tag5-9AA** (550 mg, 143.30 μmol , 1 eq.) in THF (20 mL) were added **AA10** (2,2-dimethyl-3-oxo-3-[2-(3-tritylimidazol-4-yl)ethylamino]propanoic acid (201.01 mg, 429.91 μmol , 3.0 eq.), HOAt (58.52 mg, 429.91 μmol , 60.14 μL , 3.0 eq.) and DIC (54.25 mg, 429.91 μmol , 66.57 μL , 3.0 eq.). The resulting suspension was stirred at 20 °C for 12 h. Generic precipitation procedures were followed to get desired compound **Tag5-10AA** (590 mg, 137.61 μmol , 96.03% yield) as a white solid. LC-MS: RT = 3.887 min, mass calcd. for $\text{C}_{62}\text{H}_{82}\text{FN}_{19}\text{O}_{16}\text{H}$ 1368.62, m/z found 1368.20 [M-TAG-PMB-3OTBS+7H]⁺.

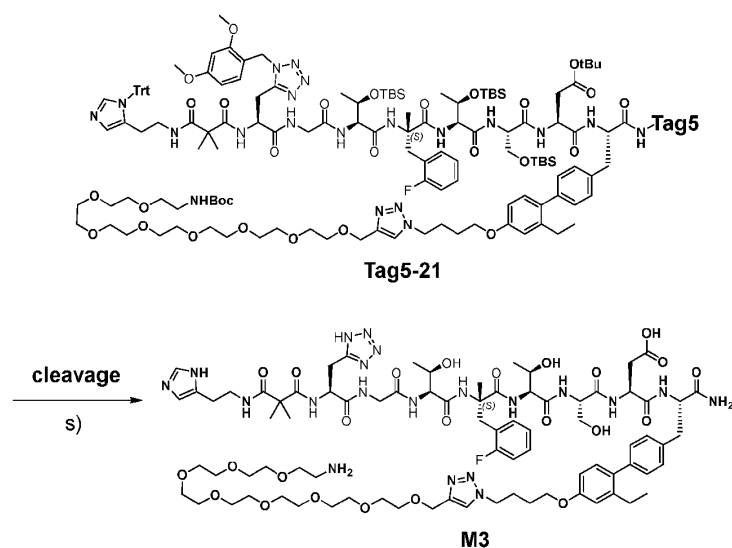
[000734] Step r: Synthesis of tert-butyl (3S)-4-[[[(1S)-2-[bis[[3,4,5-tri(docosoxy)phenyl]methyl]amino]-1-[[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-[2-(tert-butoxycarbonylamino)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[[(2S)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-[[[(2S,3R)-3-[tert-butyl(dimethyl)silyl]oxy-2-[[2-[[[(2S)-3-[1-[(2,4-dimethoxyphenyl)methyl]tetrazol-5-yl]-2-[[2,2-dimethyl-3-oxo-3-[2-(3-tritylimidazol-4-yl)ethylamino]propanoyl]amino]propanoyl]amino]acetyl]amino]butanoyl]amino]-3-(2-fluorophenyl)-2-methyl-propanoyl]amino]butanoyl]amino]propanoyl]amino]-4-oxo-butanooate (**Tag5-21**)



[000735] The compound **Tag5-10AA** (0.55 g, 128.28 μ mol, 1 eq) in THF (18 mL) H₂O (2 mL) t-BuOH (2 mL) were added Boc-PEG8-Linker (tert-butyl N-[2-[2-[2-[2-[2-[2-(2-prop-2-ynoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]carbamate (130.23 mg, 256.56 μ mol, 2.0 eq), HOVc (40.67 mg, 230.90 μ mol, 42.81 μ L, 1.8 eq) and CuSO₄.5H₂O (41.00 mg, 164.20 μ mol, 1.28 eq). The mixture was stirred at 100 °C for 60 min in microwave. Generic precipitation

procedures were followed to give the desired compound **Tag5-21** (0.56 g, crude) as a white solid. LC-MS: RT = 3.004 min, mass calcd. for $C_{81}H_{119}FN_{20}O_{24}H_2$ 888.44, m/z found 888.60 $[M-TAG-Trt-PMB-3TBS-Boc-tBu+10H]^+$.

[000736] *Step s: Synthesis of (3S)-4-[[[(1S)-2-amino-1-[[4-[4-[4-[2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]methyl]-2-oxo-ethyl]amino]-3-[[[(2S)-2-[[[(2S,3R)-2-[[3-(2-fluorophenyl)-2-[[[(2S,3R)-3-hydroxy-2-[[2-[[[(2S)-2-[[3-[2-(1H-imidazol-5-yl)ethylamino]-2,2-dimethyl-3-oxo-propanoyl]amino]-3-(1H-tetrazol-5-yl)propanoyl]amino]acetyl]amino]butanoyl]amino]-2-methyl-propanoyl]amino]-3-hydroxy-butanoyl]amino]-3-hydroxy-propanoyl]amino]-4-oxo-butanoic acid (**M3**)*



[000737] To a mixture of compound **Tag5-21** (0.56 g, 116.78 μmol , 1 eq.) in DCM (5 mL) was added TFA cocktail (TFA/TIPS/ H_2O = 95/2.5/2.5) (40 mL). The resulting suspension was stirred at 20 °C for 2 hr. A sample was taken and suspended with PE/MTBE (3/1, 1 mL), centrifuged to give a solid, which was diluted with MeOH and H_2O , filtered, and the filtration was checked by LCMS, which showed the desired mass for **M3**. The reaction was concentrated under reduced pressure to give a residue, which was added into PE/TBME (3/1, 20V) and stirred for 1 h. The suspension was filtered to give a white solid, which was purified by prep-HPLC (TFA condition): column: C18 100×40mm; mobile phase: [water (TFA)-ACN]; gradient: 10%-40% B over 8 min. The product **M3** (33 mg, 18.29 μmol , 15.66% yield, 98.41% purity) was obtained as a white solid which was checked by LCMS ES10784-2734-P1A6 and HPLC ES10784-2734-P1C6. LCMS (ESI): RT = 2.995 min, mass calcd. for $C_{81}H_{119}FN_{20}O_{24}H_2$ 888.44, m/z found 888.60 $[M+2H]^{2+}$. LCMS condition: Mobile Phase: 1.5mL/4L TFA in water (solvent A) and acetonitrile (solvent B), using the elution gradient

10%-80% (solvent B) over 6 minutes and holding at 80% for 2 minutes at a flow rate of 1.2mL/min; Column: Ultimate XB-C18 3*50mm 3um; Wave length: UV 220nm, 215nm, 254nm; Column temperature: 50°C. HPLC AC RT=7.757 min, 98.41% purity. HPLC method A: Column: YMC-Pack ODS-A 150*4.6mm, 5um; 2.75mL/4L TFA in water (solvent A) and 2.5mL/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 10 minutes and holding at 80% for 5 minutes at a flow rate of 1.5 mL/min.

Example 9. Results and Discussion of Examples 1-8

9.1 Known Liquid-Phase Peptide Support Tag

[000738] To overcome the challenges in SPPS of certain peptides using large excess of building blocks, especially difficult to be synthesized, LPPS were investigated to facilitate solution chemistry on a soluble Tag to reduce reactants and agents and workup after each step to grow peptide chain. Numerous soluble SPPS tags instead of insoluble resins have evolved (Liu et al., "Total Synthesis of Semaglutide Based on a Soluble Hydrophobic-Support-Assisted Liquid-Phase Synthetic Method," *ACS Comb Sci.* 22(12):821-825 (2020), which is hereby incorporated by reference in its entirety), including, shown in Figure 8, chloromethylated polystyrene polymer (CPS) **1** (Shemyakin et al., "Synthesis of Peptides in Solution on a Polymeric Support I. Synthesis of Glycylglycyl-L-Leucylglycine," *Tetrahedron Lett.* 6:2323-2327 (1965), which is hereby incorporated by reference in its entirety), poly(ethylene glycol) (PEG) **2** (Mutter et al., "Rapid Procedure for Liquid-Phase Peptide Synthesis: The Crystallization Method," *Angew. Chem., Int. Ed. Engl.* 13:88-89 (1974), which is hereby incorporated by reference in its entirety), hydrophobic benzyl alcohols (HBAs) **3-8** (Okada et al., "Improved Tag-Assisted Liquid-Phase Peptide Synthesis: Application to the Synthesis of the Bradykinin Receptor Antagonist Icatibant Acetate," *Org. Process Res. Dev.* 23:2576-2581 (2019); Aihara et al., "Liquid-Phase Synthesis of Bridged Peptides Using Olefin Metathesis of a Protected Peptide with a Long Aliphatic Chain Anchor," *Org. Lett.* 17:696-699 (2015); Sanguliya et al., "Syntheses of Melanotan II and YSL Amide by Ajiphase Methodology," *Pharm. Chem. J.* 53:462-466 (2019), which are hereby incorporated by reference in their entirety), silylated tag (STag) **9** (Yano et al., "Silylated Tag-Assisted Peptide Synthesis: Continuous One-Pot Elongation for the Production of Difficult Peptides under Environmentally Friendly Conditions," *Molecules* 26:3497 (2021), which is hereby incorporated by reference in its entirety). Recently, a soluble and hydrophobic-tags-assisted liquid phase peptide synthetic method was reported (Okada et al., "Tag-Assisted Liquid-Phase Peptide Synthesis Using Hydrophobic Benzyl Alcohols as Supports," *J. Org. Chem.* 78(2):320-327 (2013); Tana et al., "A Practical Solution-Phase Synthesis of an Antagonistic Peptide of TNF- α Based on Hydrophobic Tag Strategy," *Chem. Commun.*

46(43):8219–8221 (2009), which are hereby incorporated by reference in their entirety). Since all coupling and de-protecting reactions were carried out in homogeneous solutions, the required amounts of amino acids (1.05–1.5 eq), coupling agents (1.05–1.5 eq), and solvents may be minimized. Additionally, the reaction process and the characterization of the TCPs may be monitored through common analytical techniques, such as by NMR and MS analysis (Fujita et al., “Soluble Tag-Assisted Peptide Head-to-Tail Cyclization: Total Synthesis of Mahafacyclin B,” *Org. Lett.* 15(6):1155–1157 (2013); Kitada et al., “Soluble-Support-Assisted Electrochemical Reactions: Application to Anodic Disulfide Bond Formation,” *Org. Lett.* 14(23):5960–5963 (2012); Kitada et al., “Hydrophobic Tag-Assisted Liquid-Phase Synthesis of a Growth Hormone-Inhibiting Peptide Somatostatin,” *Bioorg. Med. Chem. Lett.* 21(15):4476–4479 (2011), which are hereby incorporated by reference in their entirety). However, known LPPS Tags are not always robust when applied to a wide range of peptides synthesis, especially synthesis of longer and/or more aqueous soluble peptides. It seems challenging to predict the TCPs using those chemical tags to be contradictory to enhance solubility in one solvent while simultaneously diminishing solubility in another solvent (Sharma et al., “Liquid-Phase Peptide Synthesis (LPPS): A Third Wave for the Preparation of Peptides,” *Chem. Rev.* 122:13516–13546 (2022), which is hereby incorporated by reference in its entirety).

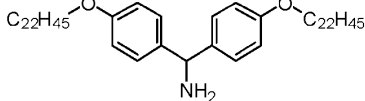
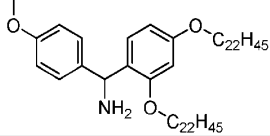
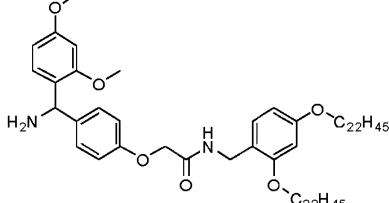
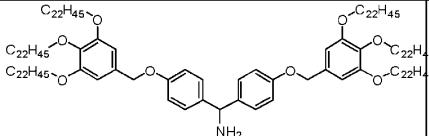
9.2 Novel Liquid-Phase Peptide Synthesis Tag

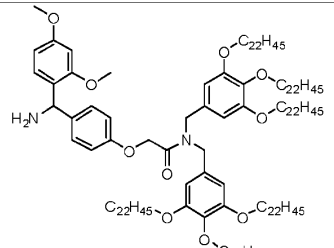
[000739] The basic criterion for the LPPS Tags is soluble in peptide elongations but insoluble in quenched solutions to facilitate their precipitation and filtration. The precipitated product may be collected and the undesirable impurities such as excess amino acids, catalysts, and coupling agents may be rinsed away. Additionally, the properties of LPPS Tags must be stable under the de-Fmoc condition and differ from the properties of the peptides to be purified. Therefore, ideal LPPS should combine the advantages of CSPA and SPPS for peptide elongations, including (1) carry out in solution on a soluble tag using few molar equivalents of building blocks to grow peptide chain, (2) introduce natural / unnatural amino-acid building blocks under various reaction conditions, (3) allow small to large-scale production of various peptides, and (4) optionally may be automated.

[000740] **M1** has a primary amine-PEG 8 moiety, significantly increasing the hydrophilicity (aq. solubility ~ 60mM), and new LPPS Tags were required to increase **M1**-production yields and reduce time of separation in LPPS synthesis. Considering the C terminal of amide in **M1** structure, four different hydrophobic tags were designed (**Tag2-Tag5** in Figure 1 and Table 20). **Tag2** and **Tag3** have similar hydrophobicity as **Tag1** with two C22-alkyl chains, and the peptide elongations on all three Tags were found only up to 7-mers with limited amount precipitates in polar solvents, although **Tag3** is relatively easier to be cleaved from its TCPs, compared to other two Tags. **Tag4-TCPs** were

relatively not easy to be cleaved to release the peptides due to lack of 2,4-dimeoxyphenyl)-methylene amine-amide structure, which is commonly used in solid phase synthesis for preparation of C- terminal primary amide. **Tag5** was designed to have six hydrophobic C22-alkyl chains for increasing its TCP hydrophobicity to be participated in polar solvents. **Tag5** was also designed to incorporate (2,4-dimeoxyphenyl)-methyl amine-amide to release C-term amide peptides for easily monitoring the elongation process. Among five tags, **Tag5** was used to complete 10 circles of mono-peptide elongation to generate **Tag5-10AA** and then clicked with a PEG8 linker via [3+2] cycloaddition to generate **Tag5-M1**. **Tag5** achieved sustainable peptide chain elongation of bioactive peptides bearing varieties of unnatural amino acids, including **M1**, since its hydrophobically tagged peptides may be separated as precipitates at each step by addition of polar organic solvents (ACN/MeOH). This approach may be applied chain elongation efficiently for not only peptides but also other bioactive entities.

Table 20. New Tags

TAG #	Name	Structure	Cleavage	Advantages and disadvantages
Tag1	Symmetric Ar ₂ CHNH ₂ Tag (AJM)		TFA:TIPS:H ₂ O = 95:2.5:2.5	Difficult filtration after 6-mer peptide
Tag2	Diarylmethanamine Tag		TFA:Ethyl Phenyl Sulfide:H ₂ O = 95:2.5:2.5	Difficult filtration after 6-mer peptide
Tag3	Rink amide Tag		TFA:TIPS:H ₂ O = 95:2.5:2.5	Difficult filtration after 6-mer peptide.
Tag4	Symmetric double gallate Tag		TFA:TIPS:H ₂ O = 95:2.5:2.5	Non-completed cleavage

Tag5	Rink amide dibenzylamine Tag		TFA:TIPS:H ₂ O = 95:2.5:2.5	Successful synthesis of 10-mer on Tag5
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[000741] **Tag5** was prepared via 10 steps shown in Figure 6. Preparation of **Tag5-3** from methyl 3,4,5-trihydroxybenzoate was reported (Horenstein et al., "Synthesis of Unprotected ($\pm$)-Tunicchrome An-1, a Tunicate Blood Pigment," *J. Am. Chem. Soc.* 111(16):6242–6246 (1989), which is hereby incorporated by reference in its entirety). Reductive amination of **Tag5-3a** with 2 eq. of **Tag5-3** gave **Tag5-4** in 70% yield. Removing *N*-allyl group on **Tag5-4** with 1,3-dimethylbarbituric acid in the presence of Pd(PPh₃)₄ catalyst provided **Tag5-5**, which was carried in a coupling reaction with **Tag5-5a**, followed by de-Fmoc protection of **Tag5-6** in piperidine to provide **Tag5-7**. *N*-Alloc protection of **Tag5-7** for removing TBS in **Tag5-8** and subsequent alkylation with C22-alkyl bromide in one pot generated **Tag5-9**. Removing *N*-alloc in **Tag5-9** provided final **Tag5**. Replacing *N*-Fmoc of **Tag5-5a** with *N*-alloc was tried but **Tag5-5a** without *N*-Fmoc was poorly soluble in organic solvents.

[000742] The LPPS process per mono-peptide elongation includes 4 steps shown in Figure 9. First, condensation reaction on Tag from **Tag-(n-1)AA-NH₂** with *N*-Fmoc-amino acid (**AA-NHFmoc**) in the presence of amide coupling reagent generates crude **Tag-nAA-NHFmoc**. Second, precipitate newly formed **Tag-nAA-NHFmoc** by adding ACN or mixed ACN/MeOH and wash away soluble unreacted reactants and reagents; alternatively quench unreacted residual activated carboxylic acid (**AA-NHFmoc** or byproduct) with propan-1-amine (2 eq) at 20°C for 0.5 hr. Third, de-protect of **Tag-nAA-NHFmoc** with piperidine. Forth, repeat step 2 to precipitate **Tag-nAA-NH₂** for next step of condensation. Finally cleave the TCP from the Tag and simultaneously de-protection of *N*-, *O*-, and/or *S*-protective groups under acidic condition to generate the desired peptides (**n-AA-NH₂**).

[000743] The comparison of TCP elongations on five tags to make **M1**, respectively, is summarized in Table 21, including molar equivalents of the reagents and total yields per Tag-route. The generic procedures for making **M1** are as follows. Stepwise peptide elongation with 1.5 eq of **AA1**, **AA2**, **AA3**, **AA4**, **AA5**, **AA6**, **AA8**, **AA9**, and **AA10**, in 50V solvent (V stands volume (mL) of

solvent per gram of AA#) at room temperature for 2 hrs., but 5-10 eq of AA7 for 12 hrs, following either with or without quenching with propan-1-amine (2 eq) at 20°C for 0.5 hr. After removal of most solvent via concentration, the newly formed *N*-Fmoc protected Tag5-peptides were precipitated from the reaction mixture by addition of 60V of ACN/MeOH (1 to 1) and purified by washing away soluble reactants and reagents. Redissolving the precipitate with 20% piperidine in DCM/THF (any ratio) for deprotection of *N*-Fmoc generated Tag5-peptides for further peptide elongation. Continue sequential steps of coupling and quenching/removal of residual reactant / de-Fmoc until the peptide had been synthesized. Reaction mixture or crude product was subjected to acidic cleavage by using a TFA cocktail (TFA:TIPS:H₂O = 95:2.5:2.5) for 2 hrs and filtrated. All reactions were monitored by LCMS operated following. A small portion of crude was dissolved in DMF and cleaved by same TFA cocktail, and the filtration was checked by LCMS to confirm if the reactant was consumed, and the desired product was detected.

Table 21. Comparison of Using Tag1-Tag5 to Prepare M1

Step #	Tag1-TCP	Tag2-TCP	Tag3-TCP	Tag4-TCP	Tag5-TCP
1	1.5 eq. AA1	1.5 eq. AA1	1.5 eq. AA1	1.5 eq. AA1	1.5 eq. AA1
2	1.5 eq. AA2	1.5 eq. AA2	1.5 eq. AA2	1.5 eq. AA2	1.5 eq. AA2
3	1.5 eq. AA3	1.5 eq. AA3	1.5 eq. AA3	1.5 eq. AA3	1.5 eq. AA3
4	1.5 eq. AA4	1.5 eq. AA4	1.5 eq. AA4	1.5 eq. AA4	1.5 eq. TBS-AA4
5	1.5 eq. AA5	1.5 eq. AA5	1.5 eq. AA5	1.5 eq. AA5	1.5 eq. TBS-AA5
6	1.5 eq. AA6	1.5 eq. AA6	/	1.5 eq. AA6	1.5 eq. AA6
7	5 eq. AA7	5 eq. AA7	/	5 eq. AA7	10 eq. TBS-AA7
8	/	/	/	1.5 eq. AA8	1.5 eq. AA8
9	/	/	/	1.5 eq. DMB-AA9	1.5 eq. DMB-AA9
10	/	/	/	1.5 eq. AA10	1.5 eq. AA10
11	1.05 eq. AA10-AA9-AA8-OH	1.05 eq. AA10-AA9-AA8-OH	/	/	3 eq. PEG8-Linker
Product	Tag1-10AA	Tag2-10AA	Tag3-5AA	Tag4-10AA	Tag5-10AA-Linker
Total Yield	28.4%	36.3%	21.7%	4.7%	45% (>98% purity) from one-pot process

[000744] Figure 6 illustrates the LPPS of **M1** on **Tag5** via 10 amide coupling reactions, followed by deprotection of N-Fmoc after each condensation, one [3+2] cycloaddition with a PEG8-alkyne catalyzed by CuSO₄ and sodium ascorbate, and final cleavage of **Tag5** and protective groups to produce **M1**. The conditions and results were summarized in Figures 11A-B.

[000745] The approach I for the TCP elongation on Tag5 to make **M1** included: (1) coupling reaction with amino acid (1.5eq), DIC (1.5 eq), HOAt (1.5 eq), in DCM (40-50 V) at 20 °C for 2 h; (2) removing DCM and then addition of ACN or ACN/MeOH (2/1, 60V) to precipitate newly formed **Tag5-TCP-N-Fmoc**; (3) deprotecting N-Fmoc with 20% piperidine in THF, at 50 °C for 0.5 h.; (4) removing all solvents and participating newly formed **Tag5-TCP** with addition of ACN or ACN/MeOH to collect dry **Tag5-TCP** for next step of peptide elongation; (5) repeating steps 1 to 4 to get final Tag5-10AA, followed by clicking with PEG8-Linker (3 eq.) in THF catalyzed by CuSO₄·H₂O (1.5 eq) and sodium ascorbate (1.5 eq.), TBTA (0.5 eq.) in H₂O +/- DMSO at 40°C for 12hr; (6) repeating step 2 to collect newly formed **Tag5-M1**; (7) removing **Tag5** and protective groups using TFA cocktail (TFA/TIPS/H₂O = 95/2.5/2.5) in DCM (3/1, 100 V), 20°C, 2hrs.; and (8) purifying crude **M1** by RF-HPLC.

[000746] The approach II for the TCP elongation on Tag5 to make **M1** included: 1) coupling reaction with amino acid (1.5 eq), DIC (1.5 eq), HOAt (1.5 eq), in DCM (40-50 V) at 20 °C for 2 h; (2) quenching unreacted N-Fmoc AAs with propan-1-amine (2 eq) at 20°C for 0.5 hr.; (3) deprotecting N-Fmoc with 20% piperidine in THF, at 50 °C for 0.5 h.; (4) removing all solvents and then addition of ACN or ACN/MeOH (2/1, 60V) to precipitate newly formed **Tag5-TCP**; and following above steps 5 to 8 to get pure **M1**.

[000747] Both approaches were carried out in solution, thereby avoiding the use of excesses of reagents (except AA7) for completion of each reaction step, reduction of isomerization (99%ee for **M1**) and other byproducts. The process confers the chain special properties that facilitate solution chemistry and workup and produced high purity (>98% HPLC purity for **M1**) products without the use of chromatography.

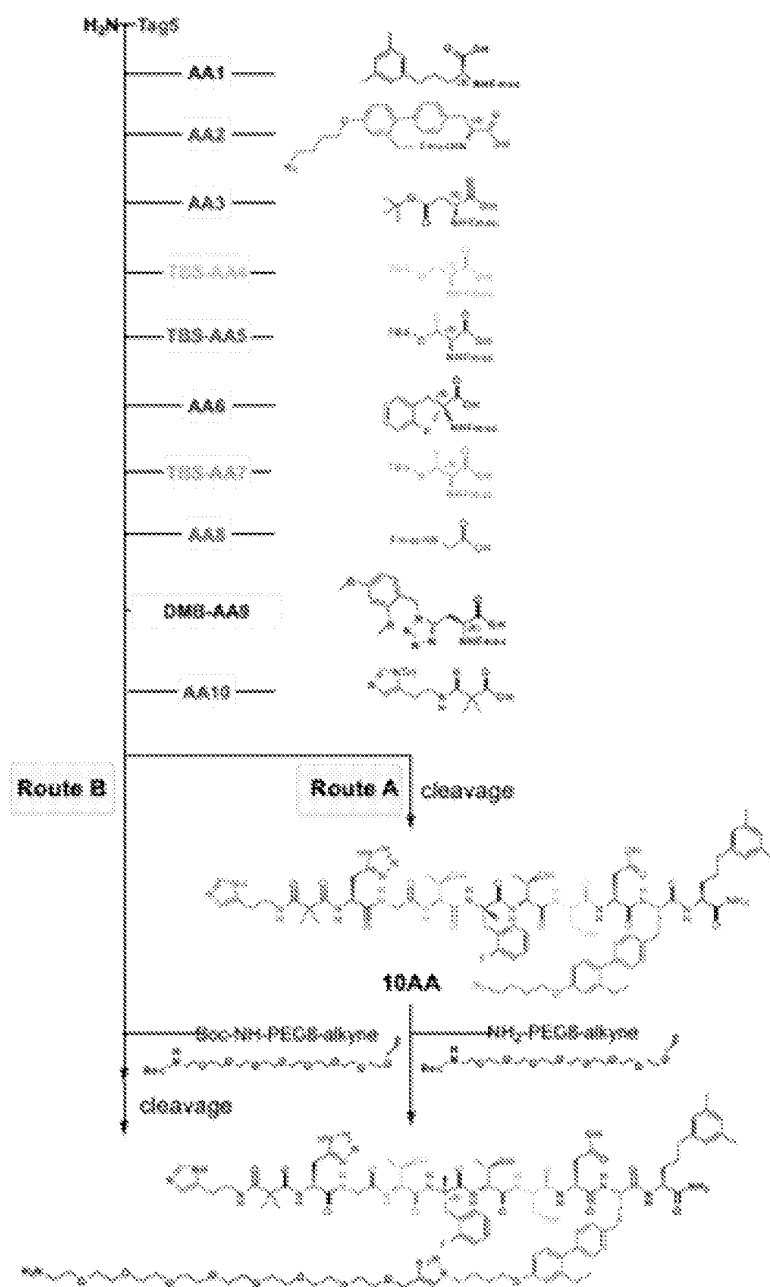
9.3 Conclusion

[000748] By combining the advantages of solid-phase and liquid-phase techniques, **Tag5** was found to be efficient in the LPPS strategy as the purification by precipitation and filtration to achieve sustainable peptide chain elongation of bioactive peptides bearing varieties of unnatural amino acids was achieved. **Tag5** was designed with six C22-alkyl chains and used to synthesize a GLP1R agonist (**M1**) using 1.5 molar equivalents of most reactants and reagents, which significantly reduced the amounts of the unnatural amino-acid building blocks compared to SPPS synthesize and reduced overall cost for synthesis of **M1**. **Tag5** was also designed with (2,4-dimeoxyphenyl)-methyl amine-amide structure that may be cleaved in pH2-3 TFA cocktail to release elongated peptides. The

synthesis of **Tag5** was scalable and **Tag5** was produced in total 25% yields for 400 grams in 10-steps. This approach may be applied chain elongation efficiently for not only peptides but also other bioactive entities.

[000749] An optimized process for the synthesis of the GLP1R agonist (**M1**) is shown in Scheme 6A below. Sequential peptide elongation used 1.5 eq. of AA1, AA2, AA3, AA4, AA5, AA6, AA8, AA9, AA10 in 50 V solvent at room temperature for 2 hr, but 10 eq. of AA7 for 12 hr to drive the reaction to completion. Repetition of similar procedure provided final **Tag5-10AA**, which was converted to **M1** in two routes. In Route A, **Tag5** and protective groups were removed using TFA cocktail ((TFA:TIPS:H₂O = 95:2.5:2.5), 2 hr) to produce peptide **10AA**, which was clicked with the PEG8-alkyne catalyzed by CuSO₄ and sodium ascorbate to furnish the desired compound **M1**. In Route B, the [2+3] click reaction was carried on **Tag5** followed by the acidic cleavage of compound **M1** from the TCP under the same conditions. All steps were carried out in solution phase, thereby avoiding the use of excesses of reagents (except **AA7**) for completion of each reaction step, and the reduction of isomerization and other byproducts to produce **M1** in high purity (99% ee on chiral HPLC and >98% purity on uHPLC). The total yield achieved was >45% for **M1** using one-pot pathway, comparable to the SPPS method. The desired compound **M1** was obtained as a white solid. LC-MS: RT = 3.748 min, mass calcd. for C₉₄H₁₃₆FN₂O₂₅H₂, 990.01 [M+2H]²⁺, m/z found 990.7 [M+2H]²⁺. HRMS: (ESI): m/z calcd. for C₉₄H₁₃₆FN₂O₂₅H 1979.01 [M+H]⁺, C₉₄H₁₃₆FN₂O₂₅H₂ 990.01 [M+2H]²⁺, m/z found 1978.9949 [M+H]⁺, 990.0101 [M+2H]²⁺. ¹H NMR (400MHz, Methanol-d₄) δ = 8.80 (s, 1H), 8.05 (s, 1H), 7.35 (s, 1H), 7.25 (d, J=7.8 Hz, 2H), 7.20 (br t, J=7.8 Hz, 1H), 7.05 - 7.00 (m, 4H), 6.97 - 6.92 (m, 1H), 6.90 - 6.84 (m, 2H), 6.82 - 6.76 (m, 4H), 4.67 (s, 2H), 4.58 - 4.49 (m, 4H), 4.46 - 4.35 (m, 3H), 4.27 - 4.22 (m, 1H), 4.17 - 3.99 (m, 7H), 3.88 - 3.74 (m, 3H), 3.72 - 3.61 (m, 29H), 3.60 - 3.43 (m, 4H), 3.32 - 3.24 (m, 3H), 3.17 (br t, J=4.8 Hz, 2H), 3.03 - 2.91 (m, 3H), 2.84 - 2.36 (m, 7H), 2.26 (s, 6H), 2.16 (quin, J=7.4 Hz, 2H), 1.90 - 1.77 (m, 4H), 1.67 - 1.31 (m, 10H), 1.30 - 1.21 (m, 6H), 1.04 (t, J=7.4 Hz, 3H) ppm.

Scheme 6A. An optimized process for the synthesis of GLP1R agonist **M1**.



Example 10. Synthesis of (S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic Acid (AA2) and (S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic Acid (AA2) and (S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic Acid (AA2)

yl)methoxy)carbonyl)amino)-3-(4'-(4-(4-(29,29-dimethyl-27-oxo-2,5,8,11,14,17,20,23,28-nona-26-azatriacetyl)-1H-1,2,3-triazol-1-yl)butoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic Acid (AA2+Linker)

10.1 Instruments and Conditions

Table 22. Instruments and Conditions

Instrument names	Conditions	
LCMS (SHIMADZU LCMS-2020)	Column	Kinetex® EVO C18 2.1x30mm 5um
	Detector	PDA (220nm&254nm)
HPLC (SHIMADZU LC-20AB)	Column	Kinetex C18 LC Column 4.6X50mm,5um
	Detector	PDA (220nm&254nm)
SFC (SHIMADZU LC-30ADsf)	Column	Chiralcel OD-3 50x4.6mm I.D., 3um
	Detector	PDA
NMR (Bruker_400MHz)	HNMR	

Table 23. Abbreviations for Example 10

THF	Tetrahydrofuran
DMF	N,N-dimethylformamide
ACN	Acetonitrile
MeOH	Methanol
DCM	Dichloromethane
KOAc	Potassium acetate
B ₂ pin ₂	bis(pinacolato)diboron
PCy ₃	Tricyclohexylphosphine
FmocOSu	9-Fluorenylmethyl Succinimidyl Carbonate
EDTA-2Na	Ethylene diamine tetraacetic acid, disodium salt
TBTA	1-(1-benzyltriazol-4-yl)-N, N-bis[(1-benzyltriazol-4-yl)methyl]methanamine

10.2 Synthesis of AA2 and AA2+Linker

Table 24. Properties of AA2 and AA2+Linker

	AA2	AA2+Linker
MF	C ₃₆ H ₃₆ N ₄ O ₅	C ₆₀ H ₈₁ N ₅ O ₁₅
MW	604.27	1111.57
Appearance	colorless oil White solid	light brown oil White solid
Purity by HPLC / LCMS	>98%	>98%
Storage recommendation	Dry and <-20°C	Dry and <-20°C
Solubility	Insoluble in H ₂ O	> 8 g/mL in CH ₃ CN/H ₂ O, 1:1
Stability	Stable	Stable

[000750] Several methods were used to prepare AA2 and AA2+Linker (Figures 13-14).

Step a: Synthesis of 2-Bromo-5-((4-methoxybenzyl)oxy)benzaldehyde (AA2-2)

[000751] To a mixture of **AA2-1** (10 g, 49.75 mmol, 1.0 eq.) and K₂CO₃ (10.31 g, 74.62 mmol, 1.5 eq.) in DMF (100 mL) was added PMB-Cl (11.69 g, 74.62 mmol, 10.16 mL, 1.5 eq.). The mixture was stirred at 25 °C for 12 hr. The reaction mixture was concentrated under reduced pressure. The residue was diluted with H₂O (100 mL) and extracted with EtOAc (100 mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. Compound **AA2-2** (21 g, crude) was obtained as a white solid which was used directly in the next step without any further purification.

[000752] ¹H NMR (400 MHz, DMSO-d₆) δ = 10.16 (s, 1H), 7.69 (d, J=8.8 Hz, 1H), 7.43 - 7.34 (m, 3H), 7.28 (dd, J=3.3, 8.8 Hz, 1H), 6.99 - 6.90 (m, 2H), 5.10 (s, 2H), 3.75 (s, 3H).

Step b: Synthesis of 1-Bromo-4-((4-methoxybenzyl)oxy)-2-vinylbenzene (AA2-3)

[000753] To a solution of Ph₃PCH₃Br (4.56 g, 12.77 mmol, 1.0 eq.) in THF (25 mL) was added *t*-BuOK (7.16 g, 63.83 mmol, 5.0 eq.) under nitrogen. The mixture was stirred for 1 hr at 0 °C. Then **AA2-2** (4.1 g, 12.77 mmol, 1.0 eq.) in THF (25 mL) was added dropwise. The mixture was stirred for 3 hr at 25 °C. The reaction mixture was diluted with H₂O (100 mL) and extracted with EtOAc (100 mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 5% ethyl acetate/petroleum ether gradient @ 30 mL/min) for 24 min with total volume 0.9 L. Compound **AA2-3** (2.93 g, 9.18 mmol, 71.91% yield) was obtained as a white solid.

[000754] ¹H NMR (400 MHz, Chloroform-d) δ = 7.46 - 7.29 (m, 3H), 7.18 - 7.09 (m, 1H), 7.06 - 6.96 (m, 1H), 6.95 - 6.86 (m, 2H), 6.81 - 6.70 (m, 1H), 5.72 - 5.59 (m, 1H), 5.40 - 5.29 (m, 1H), 5.03 - 4.90 (m, 2H), 3.86 - 3.74 (m, 3H).

Step c: Synthesis of 2-(4-((4-Methoxybenzyl)oxy)-2-vinylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (AA2-4)

[000755] A solution of **AA2-3** (200 mg, 626.58 μmol , 1 eq.) in 1,4-dioxane (3 mL) was treated with $\text{Pd}(\text{PPh}_3)_4$ (72.41 mg, 62.66 μmol , 0.1 eq.) and KOAc (122.99 mg, 1.25 mmol, 2 eq.), and then 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (477.34 mg, 1.88 mmol, 3 eq.) was added. The mixture was stirred at 80 °C for 12 hr under nitrogen. The reaction mixture was diluted with brine (30 mL) and extracted with EtOAc (30 mL $\times$ 2). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 4 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 10% ethyl acetate/petroleum ether gradient @ 18 mL/min). Compound **AA2-4** (190 mg, 518.76 μmol , 82.79% yield) was obtained as a white solid.

[000756] ^1H NMR (400 MHz, Chloroform- d) δ = 7.75 (d, J =8.4 Hz, 1H), 7.55 (dd, J =10.9, 17.5 Hz, 1H), 7.36 (d, J =8.6 Hz, 2H), 7.22 (d, J =2.4 Hz, 1H), 6.97 - 6.89 (m, 2H), 6.87 (dd, J =2.4, 8.4 Hz, 1H), 5.67 (d, J =17.4 Hz, 1H), 5.26 (d, J =11.0 Hz, 1H), 5.03 (s, 2H), 3.82 (s, 3H), 1.34 (s, 12H).

Step d & e: Synthesis of (S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4'-((4-methoxybenzyl)oxy)-2'-vinyl-[1,1'-biphenyl]-4-yl)propanoate (AA2-6)

[000757] A solution of **AA2-4** (100 mg, 273.03 μmol , 1 eq.) in 1,4-dioxane (3 mL) and H_2O (1 mL) was treated with K_2CO_3 (56.60 mg, 409.55 μmol , 1.5 eq.) and $\text{Pd}(\text{PPh}_3)_4$ (31.55 mg, 27.30 μmol , 0.1 eq.), and then **AA2-5** (116.69 mg, 273.03 μmol , 1 eq.) was added. The mixture was stirred at 80 °C for 2.5 hr under nitrogen. The reaction mixture was diluted with brine (30 mL) and extracted with EtOAc (30 mL $\times$ 2). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 4 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 10% Ethyl acetate/Petroleum ether gradient @ 18 mL/min) for 14 min with total volume 0.3 L. Compound **AA2-6** (100 mg, 193.20 μmol , 70.76% yield) was obtained as a yellow oil.

[000758] LCMS (ESI): RT = 0.950 min, mass calcd. for $\text{C}_{31}\text{H}_{35}\text{NO}_6\text{Na}$ 540.24, $[\text{M}+\text{Na}]^+$, m/z found 540.1 $[\text{M}+\text{Na}]^+$. Reverse phase LC-MS was carried out using method A.

[000759] ^1H NMR (400 MHz, Chloroform- d) δ = 7.39 (d, J = 8.6 Hz, 2H), 7.27 - 7.25 (m, 2H), 7.23 (s, 2H), 7.17 (dd, J = 8.3, 16.2 Hz, 3H), 6.97 - 6.91 (m, 3H), 6.67 (dd, J = 11.0, 17.4 Hz, 1H), 5.67 (dd, J = 1.1, 17.4 Hz, 1H), 5.22 - 5.15 (m, 1H), 5.05 (s, 2H), 3.83 (s, 3H), 3.74 (s, 3H), 1.47 - 1.35 (m, 1H), 1.43 (s, 8H).

Step f: Synthesis of (S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(2'-ethyl-4'-hydroxy-[1,1'-biphenyl]-4-yl)propanoate (AA2-7)

[000760] To a solution of **AA2-6** (2.8 g, 5.41 mmol, 1.0 eq.) in MeOH (25 mL) was added Pd/C (300 mg, 10% palladium on activated carbon). The reaction mixture was stirred for 48 hr at 25 °C under hydrogen (15 psi), filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 30% ethyl acetate/petroleum ether, gradient @ 35 mL/min) for 22 min with total volume 0.9 L. Compound **AA2-7** (1.85 g, 4.60 mmol, 85.01% yield, 99.3% purity) was obtained as a colorless oil.

[000761] LCMS (ESI): RT = 0.935 min, mass calcd. for C₂₃H₂₉NO₅Na 422.20 [M+Na]⁺, m/z found 422.1 [M+Na]⁺. Reverse phase LC-MS was carried out using method A.

[000762] ¹H NMR (400 MHz, Chloroform-d) δ = 7.22 - 7.11 (m, 4H), 7.04 (d, J = 8.2 Hz, 1H), 6.78 (d, J = 2.6 Hz, 1H), 6.69 (dd, J = 2.6, 8.2 Hz, 1H), 5.24 (s, 1H), 5.05 (br d, J = 8.4 Hz, 1H), 4.70 - 4.58 (m, 1H), 3.73 (s, 3H), 3.20 - 3.11 (m, 1H), 3.11 - 3.02 (m, 1H), 2.53 (q, J = 7.6 Hz, 2H), 1.42 (s, 9H), 1.08 (t, J = 7.5 Hz, 3H).

Step g: Synthesis of (S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(4'-(4-chlorobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoate (AA2-8)

[000763] To a solution of **AA2-7** (350 mg, 876.14 μmol, 1 eq.) and K₂CO₃ (242.18 mg, 1.75 mmol, 2.0 eq.) in DMF (5 mL) was added 1-chloro-4-iodo-butane (287.11 mg, 1.31 mmol, 1.5 eq.) at 25 °C. The mixture was stirred at 50 °C for 12 hr. The residue was diluted with brine (50 mL) and extracted with EtOAc (50 mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 12 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 30% ethyl acetate/petroleum ether gradient @ 35 mL/min) for 14 min with total volume 0.4 L. Compound **AA2-8** (340 mg, 79.5% yield) was obtained as a yellow oil.

[000764] LCMS (ESI): RT = 1.145 min, mass calcd. for C₂₇H₃₆ClNO₅Na 512.22 [M+Na]⁺, m/z found 512.2 [M+Na]⁺. Reverse phase LC-MS was carried out using method A.

Step h: Synthesis of (S)-Methyl 3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-2-((tert-butoxycarbonyl)amino)propanoate (AA2-9)

[000765] To a solution of **AA2-8** (1.6 g, 3.27 mmol, 1.0 eq.) in DMF (15 mL) was added K₂CO₃ (902.51 mg, 6.53 mmol, 2.0 eq.), KI (54.20 mg, 326.51 μmol, 0.1 eq.), and NaN₃ (460 mg, 7.08 mmol, 2.1 eq.). The mixture was stirred at 50 °C for 7 hr. The reaction mixture was diluted with brine 50 mL and extracted with EtOAc (50 mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The water layers were

quenched by addition of aqueous NaClO (1.0 M, 100 mL). Compound **AA2-9** (1.7 g, crude) was obtained as a yellow oil.

[000766] LCMS (ESI): RT = 1.140 min, mass calcd. for $C_{27}H_{36}N_4O_5Na$ 519.27, m/z found 519.3 [M+Na]⁺. Reverse phase LC-MS was carried out using method A.

Step i: Synthesis of (S)-3-(4'-(4-Azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-2-((tert-butoxycarbonyl)amino)propanoic Acid (AA2-10)

[000767] To a solution of **AA2-9** (1.7 g, 3.42 mmol, 1.0 eq.) in THF (12 mL) was added LiOH·H₂O (287.31 mg, 6.85 mmol, 2.0 eq.) in H₂O (6 mL) at 0 °C, and then the mixture was allowed to gradually warm to 25 °C and was stirred for 2 hr. The mixture was treated with EtOAc (30 mL) and extracted with water (25 mL × 2). The combined aqueous layers were acidified (1 M aqueous HCl) and extracted with EtOAc (50 mL × 3). The combined organic layer was dried by anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. Compound **AA2-10** (2.01 g, crude) was obtained as a yellow oil which was used directly in the next step without any further purification.

Step j: Synthesis of (S)-2-Amino-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic Acid Hydrochloride (AA2-11)

[000768] Compound **AA2-10** (2.01 g, 4.17 mmol, 1.0 eq.) was dissolved in 4.0 M HCl/EtOAc (20 mL). The mixture was stirred at 25 °C for 1 hr. The reaction mixture was filtered. The filter cake was washed with EtOAc (30 mL) and dried under vacuum. Compound **AA2-11** (1 g, crude) was obtained as a white solid which was used directly in the next step without any further purification.

[000769] LCMS (ESI): RT = 1.121 min, mass calcd. for $C_{21}H_{27}N_4O_3$ 383.21 [M+H]⁺, m/z found 383.2 [M+H]⁺. Reverse phase LC-MS was carried out using method A.

Step k: Synthesis of (S)-2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)propanoic Acid (AA2)

[000770] To a solution of **AA2-11** (1 g, 2.39 mmol, 1.0 eq.) in THF (15 mL) was added NaHCO₃ (401.07 mg, 4.77 mmol, 2.0 eq.) in H₂O (8 mL), and then (2,5-dioxopyrrolidin-1-yl) 9H-fluoren-9-ylmethyl carbonate (805.23 mg, 2.39 mmol, 1.0 eq.) was added at 0 °C. The mixture was stirred for 12 hr at 25 °C. The reaction mixture was diluted with brine (100 mL) and acidified (1 M aqueous HCl) to pH = 2~3. The reaction mixture was extracted with EtOAc (30 mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0 ~ 10% Methanol/Dichloromethane @ 30 mL/min) for 16 min with total volume 0.6 L. Product **AA2** (1.3 g, 2.14 mmol, 89.52% yield, 99.4% purity) was obtained as a white foam.

[000771] LCMS (ESI): RT = 1.113 min, mass calcd. for $C_{36}H_{36}N_4O_5Na$ 627.26 [M+Na]⁺, m/z found 627.3 [M+Na]⁺. Reverse phase LC-MS was carried out using method A.

[000772] ^1H NMR (400MHz, DMSO- d_6) δ = 7.88 (d, J = 7.5 Hz, 2H), 7.81 (d, J = 8.6 Hz, 1H), 7.66 (t, J = 6.9 Hz, 2H), 7.40 (dt, J = 2.3, 7.3 Hz, 2H), 7.34 - 7.25 (m, 4H), 7.14 (d, J = 8.2 Hz, 2H), 6.97 (d, J = 8.4 Hz, 1H), 6.83 (d, J = 2.4 Hz, 1H), 6.76 (dd, J = 2.5, 8.5 Hz, 1H), 4.27 - 4.17 (m, 3H), 4.17 - 4.13 (m, 1H), 4.03 - 3.98 (m, 2H), 3.42 (t, J = 6.7 Hz, 2H), 3.13 (br dd, J = 3.9, 13.8 Hz, 1H), 2.96 - 2.87 (m, 1H), 2.52 (d, J = 1.8 Hz, 2H), 2.43 (q, J = 7.4 Hz, 2H), 1.82 - 1.74 (m, 2H), 1.73 - 1.66 (m, 2H), 0.98 - 0.90 (m, 3H) ppm.

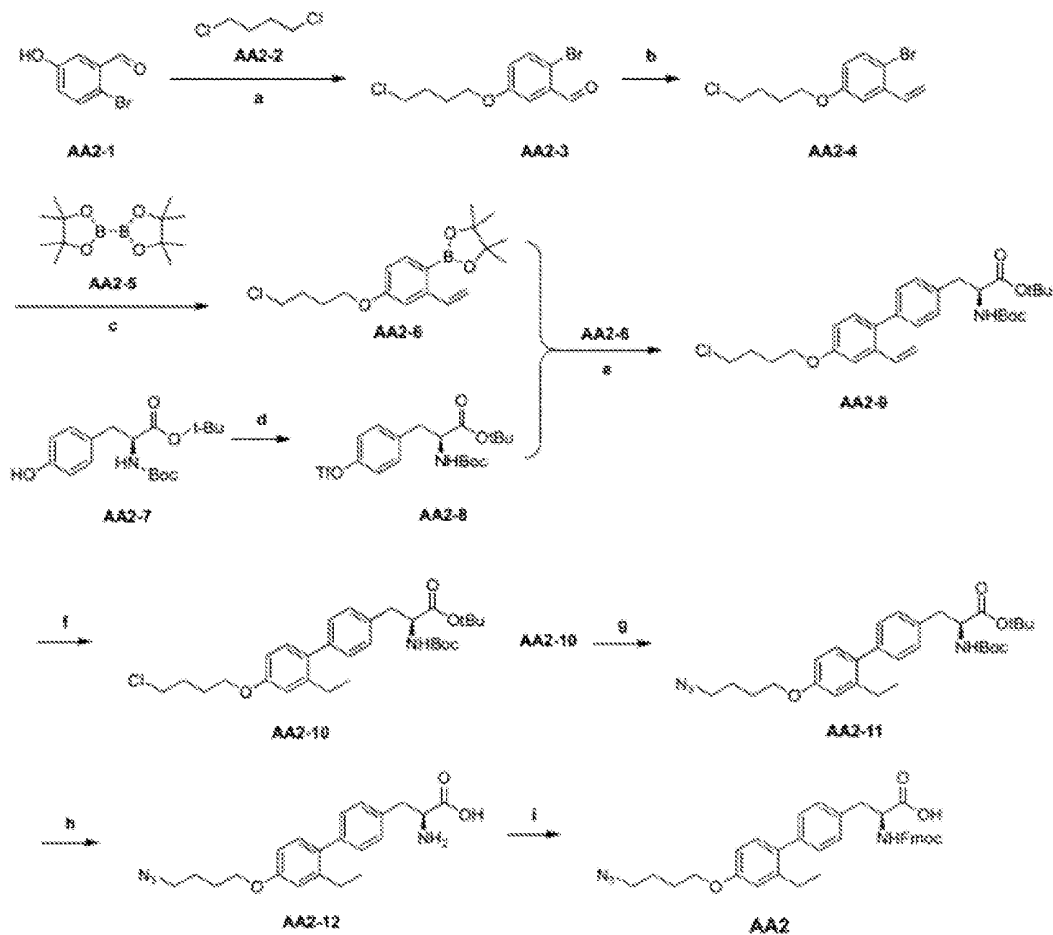
Step I: Synthesis of Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(((trifluoromethyl)sulfonyl)oxy)phenyl)propanoate (AA2-5)

[000773] To a solution of **AA2-5a** (10 g, 33.86 mmol, 1 eq.) and pyridine (16.07 g, 203.16 mmol, 16.40 mL, 6 eq.) in DCM (100 mL) was added TiCl_4 (14.33 g, 50.79 mmol, 8.38 mL, 1.5 eq.) at 0 °C. The mixture was stirred at 25 °C for 2 hr. TLC indicated that the starting material was consumed completely, and one new spot formed. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with EA 300mL and extracted with 1 M HCl (aq) 100mL. The organic layers were washed with brine 100 mL, dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give a residue.

[000774] **AA2-5** (16 g, 31.71 mmol, 93.65% yield, 84.7% purity) was obtained as a yellow oil which was confirmed by LCMS: LCMS (ESI): RT = 0.980 min, m/z calcd. for $\text{C}_{11}\text{H}_{13}\text{F}_3\text{NO}_5\text{S}$, 328.04, $[\text{M}-\text{Boc}+\text{H}]^+$, m/z found 327.9 $[\text{M}-\text{Boc}+\text{H}]^+$. LCMS conditions: 1.5ML/4LTFA in water (solvent A) and 0.75ML/4LTFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 ml/min; Column: MERCK, RP-18e 25-2mm; Wavelength: UV 220nm, 254nm; Column temperature: 50 °C; MS ionization: ESI.

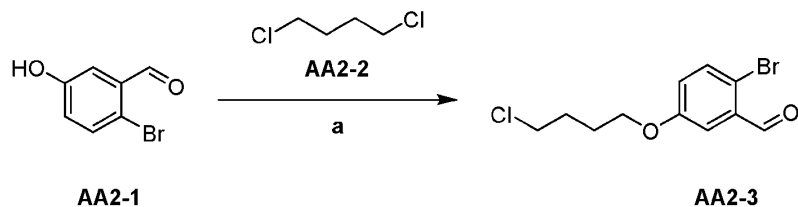
[000775] The synthesis of AA2 using route 2 is shown in Scheme 7 below.

Scheme 7



Reagents and Conditions: (a) AA2-2 (5.0 eq.), K_2CO_3 (2.0 eq.), DMF, 60°C, 12 h (b) Ph_3PMel (1.2 eq.), DBU (2.0 eq.), THF, 0~20°C, 48 h (c) AA2-5 (1.0 eq.), $Pd(dppf)Cl_2$ (0.05 eq.), KOAc (3.0 eq.), dioxane, 80°C, 12 h, 40.52%; (d) Tf_2O (1.5 eq.), Py (6.0 eq.), CH_2Cl_2 , 0~25°C, 2 h (e) AA2-6 (0.95 eq.), $Pd(dppf)Cl_2$ (0.1 eq.), K_2CO_3 (1.5 eq.), dioxane/ H_2O , 80°C, 12 h (f) H_2 , Pd/C (10%), MeOH, 25°C, 12 h, 72.23%; (g) $TMSN_3$, (1.5 eq.) TBAF (3.0 eq.), THF, 0~50°C, 12 h (h) $HCl/EtOAc$ (10 V), 0~25°C, 1 h, 86.02%; (i) Fmoc-Osu (1.0 eq.), $NaHCO_3$ (2.0 eq.), THF/ H_2O (2:1), 25°C, 12 h; 60.77%. Total yield was 10.4%.

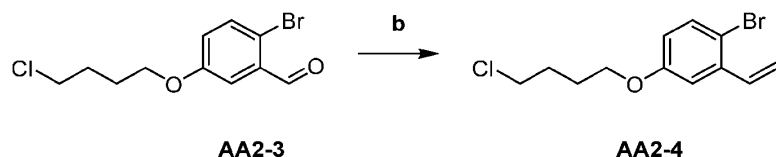
Step a: Synthesis of 2-Bromo-5-(4-chlorobutoxy)benzaldehyde (AA2-3)



[000776] To a solution of **AA2-1** (157.96 g, 1.24 mol, 138.56 mL, 5 eq) in DMF (100 mL) was added K_2CO_3 (68.76 g, 497.47 mmol, 2 eq), and the reaction was heated 60 °C. Then **AA2-2** (50 g, 248.73 mmol, 1 eq) in DMF (200 mL) was added dropwise at 60 °C. The mixture was stirred at 60 °C for 12 h. The residue was diluted with brine (250 mL) and extracted with EtOAc (250 mL $\times$ 2). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The colorless oil was stirred in PE (600 mL) for 10 minute and filtered. The filter cake was washed three times with PE. The organic layers were concentrated under vacuum to give the colorless oil. Compound **AA2-3** (130 g, crude) (130 g, crude) was obtained as a colorless oil which were confirmed by ^1H NMR.

[000777] ^1H NMR (400MHz, Chloroform- d) δ = 10.26 - 10.20 (m, 1H), 7.46 (d, J =8.8 Hz, 1H), 7.32 (d, J =3.3 Hz, 1H), 6.97 (dd, J =3.3, 8.8 Hz, 1H), 3.98 (t, J =5.7 Hz, 2H), 3.58 (br t, J =6.1 Hz, 2H), 1.97 - 1.90 (m, 4H).

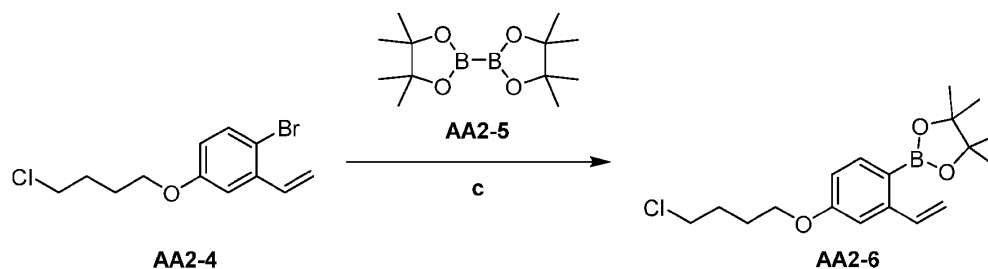
Step d: Synthesis of 1-Bromo-4-((4-methoxybenzyl)oxy)-2-vinylbenzene (AA2-4)



[000778] To a solution of $\text{Ph}_3\text{PCH}_2\text{I}$ (66.55 g, 164.63 mmol, 1.2 eq) in THF (280 mL) was added DBU (41.77 g, 274.38 mmol, 41.36 mL, 2 eq) under nitrogen. The mixture was stirred for 30 minute at 0 °C. Then **AA2-3** (4.1 g, 12.77 mmol, 1.0 eq.) in THF (160 mL) was added dropwise. The mixture was stirred for 48 h at 25 °C. The reaction mixture was diluted with H_2O (400 mL) and extracted with EtOAc (400 mL $\times$ 2). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The colorless oil was stirred in PE (600 mL) for 10 minute and filtered. The filter cake was washed with PE for three times. The organic layers were concentrated under vacuum to give the colorless oil. Compound **AA2-4** (32 g, crude) was obtained as colorless oil which were confirmed by ^1H NMR.

[000779] ^1H NMR (400MHz, Chloroform- d) δ = 7.34 (d, J =8.9 Hz, 1H), 7.01 (d, J =3.0 Hz, 1H), 6.95 (dd, J =10.9, 17.4 Hz, 1H), 6.64 - 6.57 (m, 1H), 5.63 (dd, J =0.8, 17.4 Hz, 1H), 5.29 (dd, J =0.8, 10.9 Hz, 1H), 3.95 - 3.86 (m, 2H), 3.54 (t, J =6.2 Hz, 2H), 1.93 - 1.84 (m, 4H).

Step c: Synthesis of 2-(4-((4-Methoxybenzyl)oxy)-2-vinylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**AA2-6**)

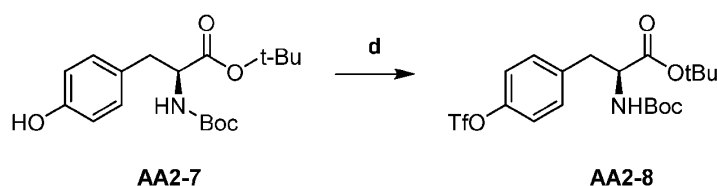


[000780] A solution of **AA2-4** (26.5 g, 91.51 mmol, 1 eq) in dioxane (300 mL) was treated with **AA2-5** (6.70 g, 9.15 mmol, 0.1 eq) and KOAc (26.94 g, 274.52 mmol, 3 eq), then Pd(dppf)Cl₂ (23.24 g, 91.51 mmol, 1 eq) was added. The mixture was stirred at 80 °C for 12 h under nitrogen. The reaction mixture was diluted with brine (300 mL) and extracted with EtOAc (300 mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by flash silica gel chromatography (ISCO®; 330g SepaFlash® Silica Flash Column, Eluent of 0~20 % ethyl acetate/petroleum ether gradient @ 100 mL/min, 40 min with total volume 4000 mL). Compound **AA2-6** (15.8 g, 37.08 mmol, 40.52% yield, 79% purity) was obtained as a colorless oil which was checked by LCMS and ^1H NMR.

[000781] LCMS (ESI): RT = 3.185 min, m/z calcd. for C₁₈H₂₇BClO₃ [M+H]⁺ 337.17, m/z found 337.2 [M+H]⁺; Mobile Phase: 1.5ML/4LTFA in water (solvent A) and 0.75ML/4L TFA in acetonitrile (solvent B), using the gradient 30%-90% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode.

[000782] ^1H NMR (400MHz, Chloroform- d) δ = 7.77 (d, J =8.3 Hz, 1H), 7.58 (dd, J =10.9, 17.5 Hz, 1H), 7.15 (d, J =2.4 Hz, 1H), 6.79 (dd, J =2.4, 8.3 Hz, 1H), 5.70 (dd, J =1.3, 17.4 Hz, 1H), 5.31 - 5.25 (m, 1H), 4.03 (t, J =5.7 Hz, 2H), 3.61 (t, J =6.2 Hz, 2H), 1.99 - 1.93 (m, 4H), 1.36 - 1.32 (m, 12H).

Step d: Synthesis of tert-Butyl (2S)-2-(tert-butoxycarbonylamino)-3-[4-(trifluoromethylsulfonyloxy)phenyl]propanoate (**AA2-8**)

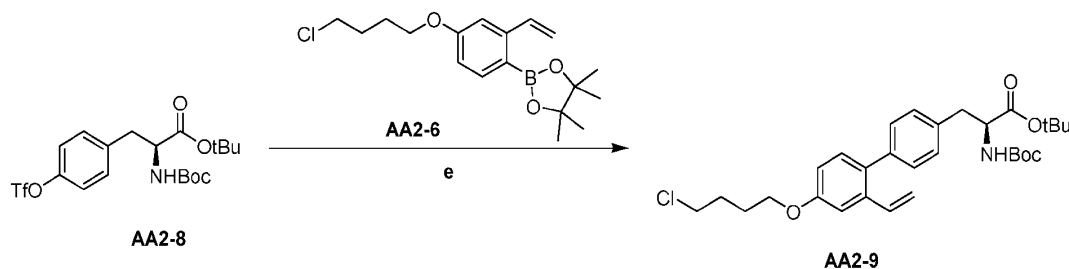


[000783] To a solution of **AA2-7** (44 g, 130.41 mmol, 1 eq) and pyridine (61.89 g, 782.43 mmol, 63.15 mL, 6 eq) in DCM (400 mL) was added TiF_2O (55.19 g, 195.61 mmol, 32.27 mL, 1.5 eq) at 0 °C. The mixture was stirred at 25 °C for 2 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with EtOAc (400mL) and extracted with citric acid (aq.) (400 mL). The organic layers were washed with NaHCO_3 (400mL) and brine (400 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 330 g SepaFlash® Silica Flash Column, Eluent of 0 ~30% ethyl acetate/petroleum ether, gradient @ 100 mL/min) for 30 min with total volume 3 L. Compound **AA2-8** (115 g, crude) was obtained as a colorless oil which was checked by LCMS and ^1H NMR.

[000784] LCMS (ESI): RT = 4.307 min, mass calcd. for $\text{C}_{19}\text{H}_{26}\text{F}_3\text{NO}_7\text{S}$ 469.14 $[\text{M}+\text{H}]^+$, m/z found 939.4 $[2\text{M}+\text{H}]^+$. Reverse phase LC-MS was carried out using a XBridge C18 3.5 um 21*20 mm, with a flow rate of 0.8 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02%TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000785] ^1H NMR (400MHz, Chloroform- d) δ = 7.31 - 7.22 (m, 2H), 7.21 - 7.13 (m, 2H), 5.16 (br d, $J=7.8$ Hz, 1H), 4.51 - 4.19 (m, 1H), 3.16 - 2.92 (m, 2H), 1.37 (d, $J=14.1$ Hz, 18H).

[000786] **Step e: Synthesis of (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(2'-ethyl-4'-hydroxy-[1,1'-biphenyl]-4-yl)propanoate (AA2-9)**



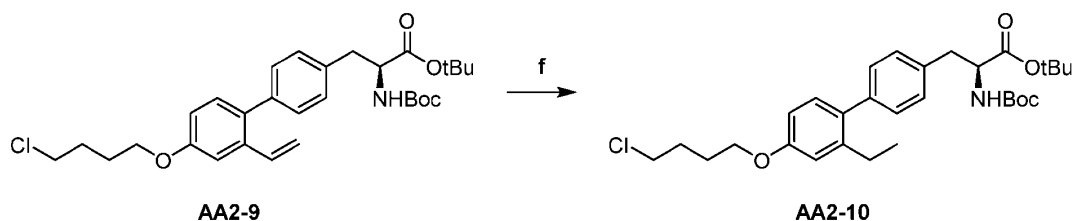
[000787] A solution of **AA2-8** (20.93 g, 44.59 mmol, 0.95 eq) in dioxane (160 mL) and H_2O (50mL) was treated with **AA2-6** (3.43 g, 4.69 mmol, 0.1 eq) and K_2CO_3 (9.73 g, 70.40 mmol, 1.5 eq), and then $\text{Pd}(\text{dppf})\text{Cl}_2$ (15.8 g, 46.93 mmol, 1 eq) was added. The mixture was stirred at 80°C for 12 h under nitrogen. The reaction mixture was diluted with brine (250 mL) and extracted with EtOAc (250 mL $\times$ 2). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and

concentrated under reduced pressure. The crude product was purified by flash silica gel chromatography (ISCO®; 330g SepaFlash® Silica Flash Column, Eluent of 0~25 % Ethyl acetate/Petroleum ether gradient @ 100 mL/min, 30 min with total volume 3000 mL). Compound **AA2-9** (17 g, crude) was obtained as a colorless oil which was checked by LCMS and ¹H NMR.

[000788] LCMS (ESI): RT = 4.003min, m/z calcd. for C₃₀H₄₀NaClNO₅ [M+Na]⁺ 552.26, m/z found 552.3 [M+Na]⁺; Mobile Phase: 1.5ML/4LTFA in water (solvent A) and 0.75ML/4L TFA in acetonitrile (solvent B), using the gradient 30%-90% (solvent B) over 6 minutes and holding at 80% for 0.5 minutes at a flow rate of 0.8ml/min. ESI source, Positive ion mode.

[000789] ¹H NMR (400MHz, Chloroform-d) δ = 7.32 - 7.18 (m, 8H), 7.16 (d, J=2.6 Hz, 1H), 6.89 (dd, J=2.6, 8.5 Hz, 1H), 6.69 (dd, J=10.9, 17.5 Hz, 1H), 5.70 (dd, J=1.0, 17.4 Hz, 1H), 5.23 - 5.17 (m, 1H), 5.09 (br d, J=7.6 Hz, 1H), 4.55 - 4.44 (m, 1H), 4.09 (t, J=5.7 Hz, 2H), 3.66 (t, J=6.2 Hz, 2H), 3.11 (br d, J=6.1 Hz, 3H), 2.04 - 1.95 (m, 4H), 1.47 - 1.42 (m, 25H), 1.40 (s, 5H).

Step f: Synthesis of tert-Butyl (2S)-2-(tert-butoxycarbonylamino)-3-[4-[4-(4-chlorobutoxy)-2-ethyl-phenyl]phenyl]propanoate (AA2-10)

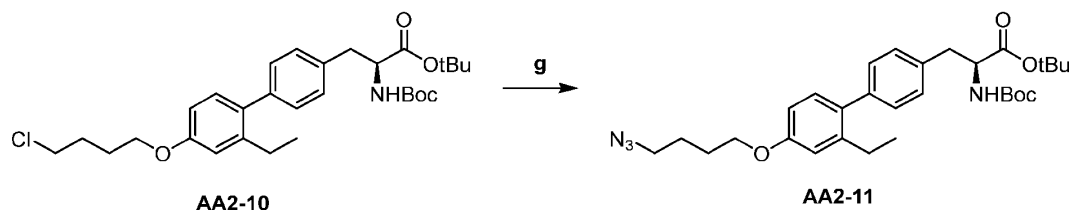


[000790] To a solution of **AA2-9** (17 g, 32.07 mmol, 1 eq) in MeOH (150 mL) was added Pd/C (2.5 g, 3.21 mmol, 10% purity, 0.1 eq). The mixture was stirred at 25 °C under H₂ (376.47 mg, 186.76 mmol) (15 psi) for 12 h. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was used in the next step directly without purification. Compound **AA2-10** (14.5 g, 23.16 mmol, 72.23% yield, 85% purity) was obtained as a colorless oil which was checked by LCMS and ¹H NMR.

[000791] LCMS: (ESI): Rt = 5.343 min, mass calcd. for C₃₀H₄₃ClNO₅ [M+H]⁺ 532.28, m/z found 1087.5 [2M+Na]⁺; Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000792] ¹H NMR (400MHz, Chloroform-d) δ = 7.28 - 7.20 (m, 5H), 7.14 - 7.06 (m, 1H), 6.86 (br s, 1H), 6.78 (br dd, J=2.3, 8.1 Hz, 1H), 5.14 - 4.99 (m, 1H), 4.55 - 4.41 (m, 1H), 4.09 - 4.02 (m, 2H), 3.66 (br t, J=5.9 Hz, 2H), 3.14 - 3.06 (m, 2H), 2.58 (q, J=7.6 Hz, 2H), 2.01 (br d, J=4.1 Hz, 4H), 1.46 - 1.41 (m, 18H), 1.15 - 1.06 (m, 3H).

Step g: Synthesis of *tert*-Butyl (2*S*)-3-[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]-2-(*tert*-butoxycarbonylamino)propanoate (**AA2-11**)

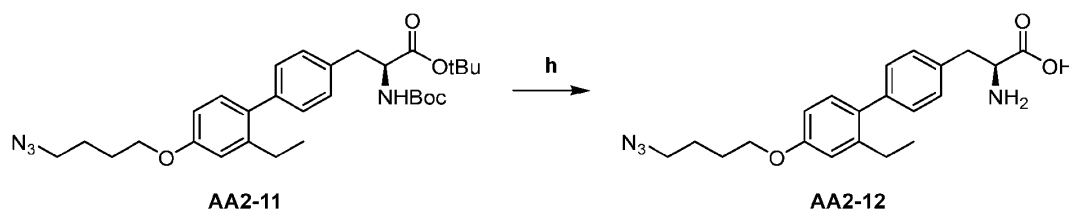


[000793] To a solution of **AA2-10** (14.5 g, 27.25 mmol, 1 eq) in THF (110 mL) was added TMSN₃ (9.42 g, 81.75 mmol, 10.75 mL, 3 eq), followed by portion wise addition of TBAF (1 M, 81.75 mL, 3 eq) (the temperature was kept below 0°C (ice-brine bath)). The reaction mixture was stirred at 50°C for 12hr. The reaction mixture was diluted with H₂O (120mL) and extracted with EtOAc (120mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was used in the next step directly without purification. Compound **AA2-11** (14 g, crude) was obtained as a colorless oil which was checked by LCMS and HPLC.

[000794] LCMS: (ESI): Rt =5.304 min, mass calcd. for C₃₀H₄₂NaN₄O₅ [M+Na]⁺ 561.32, m/z found 561.3 [M+Na]⁺. Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3mm, with a flow rate of 0.8ml/min, eluting with a gradient of 30% to 90% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000795] HPLC: purity 80% (Retention time = 9.96min). Mobile Phase: 2.75ML/4L TFA in water (solvent A) and 2.5ML/4LTFA in acetonitrile (solvent B), using the elution gradient 50%-100% (solvent B) over 10 minutes and holding at 100% for 5 minutes at a flow rate of 1.5 ml/min; Column: YMC-Pack ODS-A150*4.6mm, 5um.

Step h: Synthesis of (2*S*)-2-Amino-3-[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]propanoic Acid (**AA2-12**)

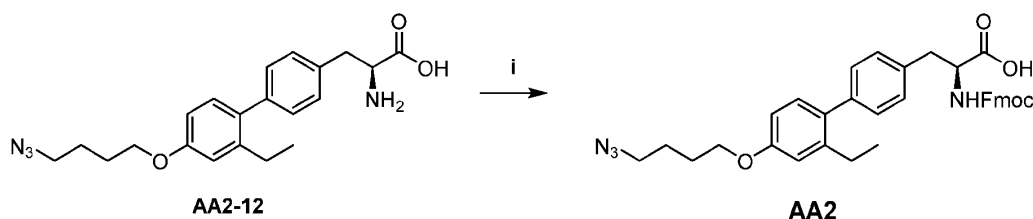


[000796] To **AA2-12** (14 g, 25.99 mmol, 1 eq) was added 4M HCl/EtOAc (120 mL). The mixture was stirred at 25 °C for 12 h. The suspension mixture was concentrated under vacuum to give a

white solid. No further purification, used directly in next step. Compound **AA2-12** (9 g, 22.36 mmol, 86.02% yield, 95% purity) was obtained as a white solid which was confirmed by LCMS.

[000797] LCMS (ESI): RT = 1.587 min, mass calcd. for $C_{21}H_{27}N_4O_3$ 383.20 $[M+H]^+$, m/z found 383.1 $[M+H]^+$. Reverse phase LC-MS was carried out using a XBridge C18 3.5 μm 21*20 mm, with a flow rate of 0.8 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step i: Synthesis of (2R)-3-[4-[4-(4-Azidobutoxy)-2-ethyl-phenyl]phenyl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic Acid (AA2)

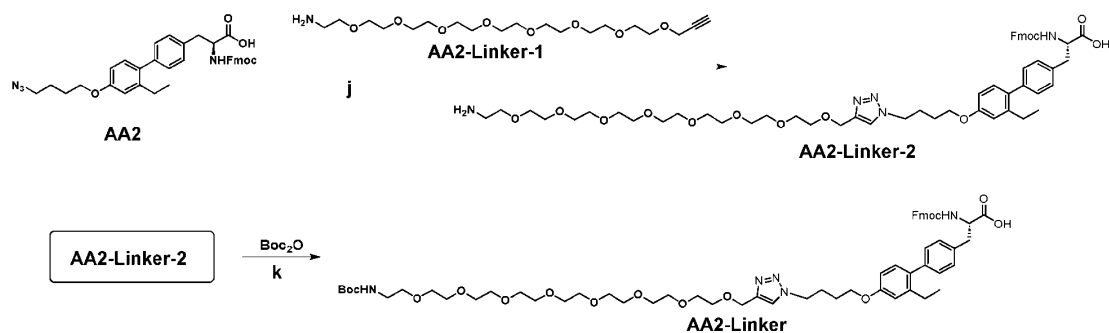
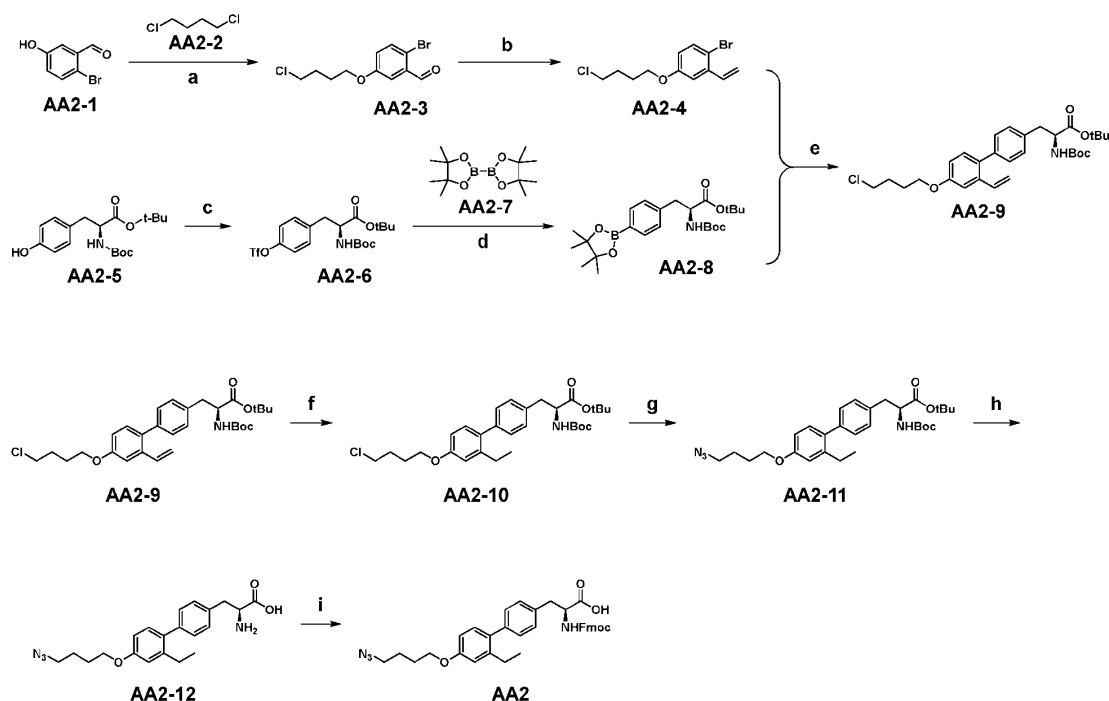


[000798] To compound **AA2-12** (9 g, 23.53 mmol, 1 eq) in THF (90 mL) was added $NaHCO_3$ (5.93 g, 70.60 mmol, 2.75 mL, 3 eq) in H_2O (45 mL), and then FmocOSu (8.73 g, 25.89 mmol, 1.1 eq) was added at 0 °C. The mixture was stirred for 12 h at 25 °C. The reaction mixture was diluted with brine (100 mL) and acidified (1 M aqueous HCl) to pH = 2~3. The reaction mixture was extracted with EtOAc (120 mL $\times$ 2). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 220 g SepaFlash® Silica Flash Column, Eluent of 0 ~20% ethyl acetate/dichloromethane, gradient @ 100 mL/min) for 40 min. Compound **AA2** (9.2 g, 14.30 mmol, 60.77%, 94% purity) was obtained as a colorless foam which was checked by LCMS and 1H NMR.

[000799] LCMS (ESI): RT = 5.574 min, mass calcd. for $C_{36}H_{37}N_4O_5$ 605.26 $[M+H]^+$, m/z found 605.2 $[M+H]^+$. Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3mm, with a flow rate of 0.8 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

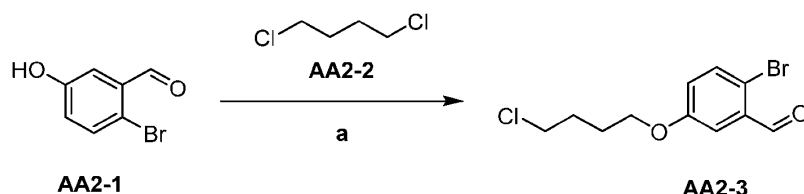
[000800] 1H NMR (400 MHz, Chloroform- d) δ = 10.73 (br s, 1H), 7.79 (br d, J =7.5 Hz, 2H), 7.65 - 7.52 (m, 2H), 7.42 (t, J =7.4 Hz, 2H), 7.37 - 7.30 (m, 2H), 7.29 - 7.18 (m, 4H), 7.16 - 7.04 (m, 1H), 6.88 (d, J =2.0 Hz, 1H), 6.77 (dd, J =2.1, 8.4 Hz, 1H), 6.27 (br d, J =7.5 Hz, 1H), 5.52 (br d, J =8.3 Hz, 1H), 4.86 - 4.76 (m, 1H), 4.54 - 4.36 (m, 2H), 4.24 (br t, J =6.9 Hz, 1H), 4.09 - 4.00 (m, 2H), 3.41 (t, J =6.5 Hz, 2H), 3.36 - 3.15 (m, 2H), 2.58 (q, J =7.4 Hz, 2H), 1.97 - 1.81 (m, 4H), 1.11 (t, J =7.5 Hz, 3H).

[000801] The synthesis of AA2 and AA2-Linker using route3 is shown in Schemes 8-9 below.



Reagents and Conditions: (j) AA2-Linker-1 (1.2 eq.), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.0 eq.), sodium L-ascorbate (1.0 eq.), TBTA (0.5 eq.), DMSO/ H_2O =1/4, 25 °C, 5 hr; k) Boc_2O (2 eq.), NaHCO_3 (5 eq.), THF (5 mL), H_2O (2 mL), 25 °C, 12 hr.

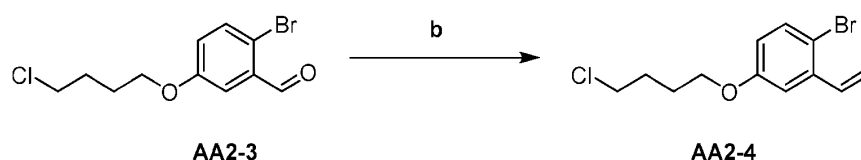
Step a: Synthesis of 2-Bromo-5-(4-chlorobutoxy)benzaldehyde (AA2-3)



[000802] To a solution of **AA2-2** (157.96 g, 1.24 mol, 138.56 mL, 5.0 eq.) in DMF (280 mL) was added K_2CO_3 (68.76 g, 497.47 mmol, 2.0 eq.), then the reaction was heated to 60 °C. Then **AA2-1** (50 g, 248.73 mmol, 1.0 eq.) in DMF (200 mL) was added dropwise at 60 °C. The mixture was stirred at 60 °C for 12 hr. The residue was diluted with brine (500 mL) and extracted with EtOAc (500mL $\times$ 2). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (Ethyl acetate/Petroleum ether = 0:1 to 1:20). Compound **AA2-3** (320 g, crude) was obtained as a colorless oil.

[000803] ^1H NMR (400MHz, Chloroform- d) δ = 10.25 (s, 1H), 7.48 (d, J =8.8 Hz, 1H), 7.35 (d, J =3.3 Hz, 1H), 6.99 (dd, J =3.1, 8.7 Hz, 1H), 4.02 - 3.97 (m, 2H), 3.61 - 3.57 (m, 2H), 3.57 - 3.52 (m, 8H), 1.97 - 1.93 (m, 4H), 1.93 - 1.87 (m, 8H).

Step b: Synthesis of 1-Bromo-4-(4-chlorobutoxy)-2-vinyl-benzene (AA2-4)

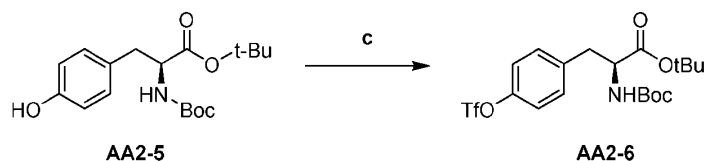


[000804] To a solution of iodo-methyl-triphenyl- λ^5 -phosphane (99.82 g, 246.94 mmol, 1.2 eq.) in THF (350 mL) was added DBU (62.66 g, 411.57 mmol, 62.04 mL, 2 eq) under nitrogen. The mixture was stirred for 1h at 0 °C. Then Compound **AA2-3** (60 g, 205.78 mmol, 1.0 eq.) in THF (250 mL) was added dropwise. The mixture was stirred at 25 °C for 48 h. The reaction mixture was diluted with ice- H_2O (600 mL) and extracted with EtOAc (600 mL $\times$ 2). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The colorless oil was stirred in petroleum ether (PE, 1000 mL) for 10 minute and filtered. The filter cake was washed

with PE for three times. The organic layers were concentrated under vacuum to give the colorless oil. Compound **AA2-4** (160 g, crude) was obtained as a colorless oil.

[000805] ^1H NMR (400MHz, Chloroform- d) δ = 7.41 - 7.34 (m, 1H), 7.33 (d, J =8.8 Hz, 1H), 6.99 (d, J =3.0 Hz, 1H), 6.92 (dd, J =10.9, 17.4 Hz, 1H), 6.63 - 6.58 (m, 1H), 5.60 (dd, J =0.9, 17.4 Hz, 1H), 5.27 (dd, J =0.8, 10.9 Hz, 1H), 3.90 (t, J =5.8 Hz, 2H), 3.56 - 3.51 (m, 2H), 1.91 - 1.87 (m, 4H).

Step c: Synthesis of *tert*-Butyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-(trifluoromethylsulfonyloxy)phenyl]propanoate (**AA2-6**)

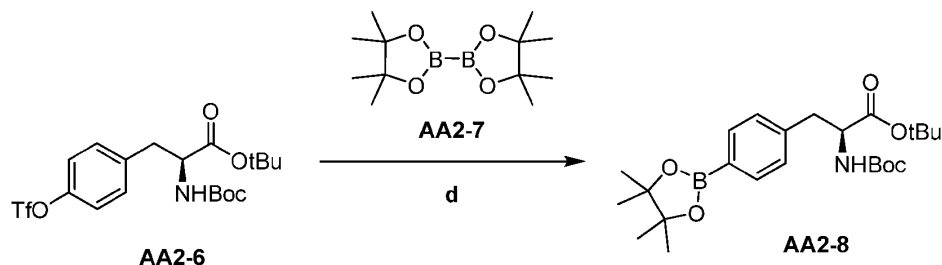


[000806] To a solution of **AA2-5** (50.00 g, 148.19 mmol, 1.0 eq.) and pyridine (70.33 g, 889.16 mmol, 71.77 mL, 6.0 eq.) in DCM (500 mL) was added TiF_2O (62.72 g, 222.29 mmol, 36.68 mL, 1.5 eq.) at 0 °C. The mixture was stirred at 25 °C for 2 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with EtOAc (500mL) and extracted with citric acid (aq.) (200 mL). The organic layers were washed with NaHCO_3 (200ml) and brine (200 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (Ethyl acetate/Petroleum ether = 0:1 - 1:5). Compound **AA2-6** (270 g, crude) was obtained as a colorless oil.

[000807] LCMS (ESI): RT = 4.251 min, mass calcd. for $\text{C}_{19}\text{H}_{26}\text{F}_3\text{NO}_7\text{SNa}$ 492.14 $[\text{M}+\text{Na}]^+$, m/z found 492.2 $[\text{M}+\text{Na}]^+$. Reverse phase LC-MS was carried out using a X Bridge C18 3.5 μm 21*20 mm, with a flow rate of 0.8 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

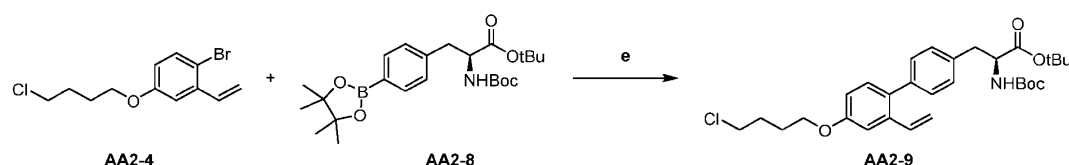
[000808] ^1H NMR (400MHz, Chloroform- d) δ = 7.30 - 7.25 (m, 2H), 7.21 - 7.17 (m, 2H), 5.13 (br d, J =7.8 Hz, 1H), 4.45 (q, J =6.5 Hz, 1H), 3.13 - 3.01 (m, 2H), 1.39 (d, J =14.6 Hz, 18H).

Step d: Synthesis of *tert*-Butyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]propanoate (**AA2-8**)



[000809] A stirred mixture of **AA2-6** (60 g, 127.80 mmol, 1.0 eq.), KOAc (37.63 g, 383.41 mmol, 3.0 eq.) and **AA2-7** (32.45 g, 127.80 mmol, 1.0 eq.) in DMSO (300 mL) was warmed to 40 °C and de-gassed. The Pd(dppf)Cl₂ (4.68 g, 6.39 mmol, 0.05 eq) was charged, then the mixture was de-gassed again, and heated to 100 °C. Then the mixture was stirred at 100°C for 4 hr. The reaction mixture was diluted with brine (300 mL) and extracted with EtOAc (300 mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was used in the next step directly without purification. Compound **AA2-8** (57 g, crude) was obtained as a colorless oil.

[000810] ¹H NMR (400MHz, Chloroform-d) δ = 7.69 (d, J=7.8 Hz, 2H), 7.13 (br d, J=7.5 Hz, 2H), 5.02 (br d, J=8.0 Hz, 1H), 4.44 - 4.35 (m, 1H), 3.09 - 2.95 (m, 2H), 1.37 (s, 18H), 1.30 (s, 12H).
Step e: Synthesis of (S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-(2'-ethyl-4'-hydroxy-[1,1'-biphenyl]-4-yl)propanoate (AA2-9)



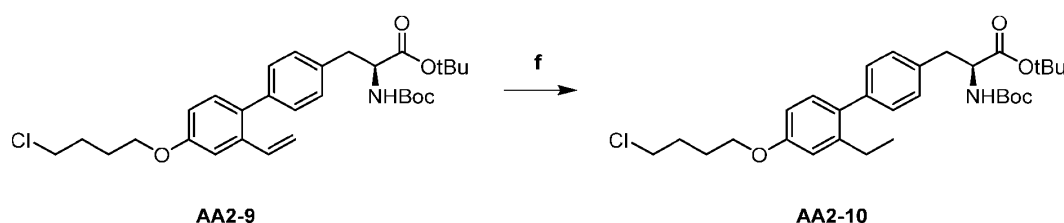
[000811] A solution of **AA2-8** (57 g, 127.41 mmol, 1.0 *eq.*), **AA2-3** (61.50 g, 127.41 mmol, 60% purity, 1.0 *eq.*), K₂CO₃ (52.83 g, 382.23 mmol, 3.0 *eq.*) and Pd(dppf)Cl₂ (9.32 g, 12.74 mmol, 0.1 *eq.*) in dioxane (500 mL) and H₂O (120 mL) was de-gassed several times with N₂. Then the mixture was stirred at 80°C for 3 h. The reaction mixture was quenched by addition of H₂O (500 mL), diluted with ethyl acetate (200 mL), and extracted with ethyl acetate (500 mL × 2). The combined organic layers were washed with sat. NaHCO₃ (250 mL × 2), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue. The crude product was purified by flash silica gel chromatography (ISCO®; 330g SepaFlash® Silica Flash Column, Eluent of 0~25 % Ethyl acetate/Petroleum ether gradient @ 100 mL/min, 30 min with total volume 3000 mL). Compound **AA2-9** (46 g, 78.10 mmol, 61.30% yield, 90% purity) was obtained as a colorless oil.

[000812] LCMS (ESI): RT = 6.403min, m/z calcd. for $C_{30}H_{40}ClNa_2NO_5$ $[M+2Na]^+$ 575.26, m/z found 575.3 $[M+2Na]^+$; LC-MS Conditions: Mobile Phase: 7 mL/4L TFA in water (solvent A) and 0.75mL/4L TFA in acetonitrile (solvent B), using the elution gradient 10%-80% (solvent B) over 0.7minutes and holding at 80% for 0.4 minutes at a flow rate of 1.5 mL/min; Column: Agilent Pursult 5 C18 20*2.0mm.

[000813] ¹H NMR (400MHz, Chloroform-d) δ = 7.27 - 7.19 (m, 5H), 7.16 (d, J=2.6 Hz, 1H), 6.89 (dd, J=2.6, 8.5 Hz, 1H), 6.70 (dd, J=10.9, 17.5 Hz, 1H), 5.70 (dd, J=1.1, 17.5 Hz, 1H), 5.20 (dd,

J=1.0, 11.0 Hz, 1H), 5.10 (br d, J=7.9 Hz, 1H), 4.56 - 4.47 (m, 1H), 4.09 (t, J=5.7 Hz, 2H), 3.69 - 3.63 (m, 2H), 3.11 (br d, J=5.9 Hz, 2H), 2.05 - 1.97 (m, 4H), 1.46 - 1.43 (m, 18H).

Step f: Synthesis of tert-Butyl (2S)-2-(tert-butoxycarbonylamino)-3-[4-[4-(4-chlorobutoxy)-2-ethyl-phenyl]phenyl]propanoate (AA2-10)

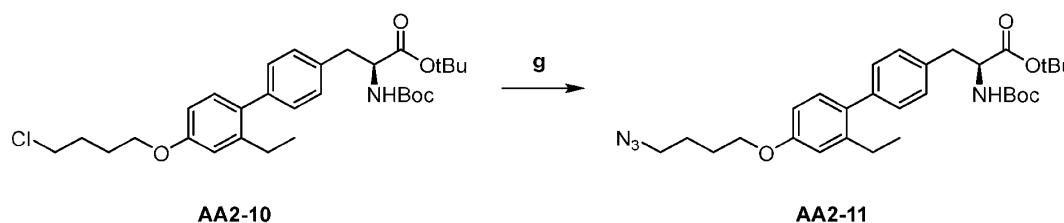


[000814] To a solution of **AA2-9** (46 g, 86.78 mmol, 1.0 eq.) in MeOH (200 mL) was added Pd/C (5 g, 8.68 mmol, 10% purity, 0.1 eq.). The mixture was stirred at 25 °C under H₂ (376.47 mg, 186.76 mmol) (15 psi) for 12 h. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was used in the next step directly without purification. Compound **AA2-10** (46 g, crude) was obtained as a colorless oil.

[000815] LCMS: (ESI): Rt = 5.356 min, mass calcd. for C₃₀H₄₂ClNO₅ [M+H]⁺ 531.28, m/z found 1085.4 [2M+Na]⁺. Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000816] ¹H NMR (400MHz, Chloroform-d) δ = 7.28 - 7.15 (m, 5H), 7.10 (d, J=8.3 Hz, 1H), 6.85 (d, J=2.4 Hz, 1H), 6.76 (dd, J=2.6, 8.4 Hz, 1H), 5.10 (br d, J=8.1 Hz, 1H), 4.55 - 4.43 (m, 1H), 4.04 (t, J=5.7 Hz, 2H), 3.64 (t, J=6.2 Hz, 2H), 3.09 (br d, J=6.0 Hz, 2H), 2.57 (q, J=7.5 Hz, 2H), 2.03 - 1.93 (m, 4H), 1.45 - 1.41 (m, 18H), 1.10 (t, J=7.5 Hz, 3H).

Step g: Synthesis of (S)-Methyl 3-(4'-(4-azidobutoxy)-2'-ethyl-[1,1'-biphenyl]-4-yl)-2-((tert-butoxycarbonyl)amino)propanoate (AA2-11)

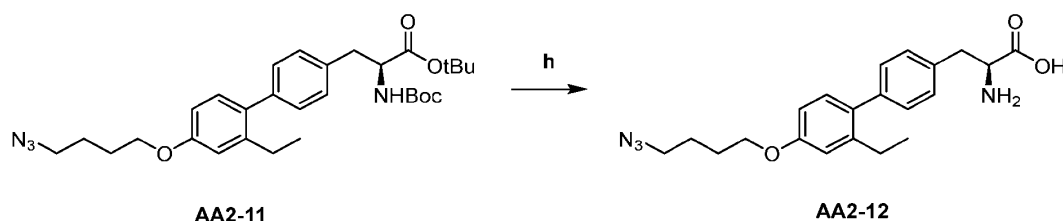


[000817] To a solution of **AA2-10** (54 g, 101.48 mmol, 1.0 eq.) in THF (400 mL) were added TMSN₃ (35.08 g, 304.45 mmol, 40.04 mL, 3.0 eq.) and potassium fluoride (17.69 g, 304.45 mmol, 7.13 mL, 3.0 eq.), followed by portion wise addition of TBAF (1 M, 30.44 mL, 0.3 eq.) and the reaction mixture was kept at the temperature below 0°C (ice-brine bath). The reaction mixture was stirred at

50°C for 12hr. The reaction mixture was diluted with H₂O (420 mL) and extracted with EtOAc (420 mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was used in the next step directly without purification. Compound **AA2-11** (31.6 g, 58.08 mmol, 57.23% yield, 99% purity) was obtained as a colorless oil. [000818] LCMS: (ESI): Rt =4.936 min, mass calcd. for C₃₀H₄₂Na₂N₄O₅ [M+2Na]⁺ 584.32, m/z found 584.3 [M+2Na]⁺; Reverse phase. LCMS was carried out using Chromolith Flash RP-C18 25-3mm, with a flow rate of 0.8ml/min, eluting with a gradient of 30% to 90% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000819] ¹H NMR (400MHz, Chloroform-d) δ = 7.24 - 7.17 (m, 4H), 7.10 (d, J=8.3 Hz, 1H), 6.85 (d, J=2.6 Hz, 1H), 6.77 (dd, J=2.6, 8.4 Hz, 1H), 5.09 (br d, J=8.2 Hz, 1H), 4.54 - 4.48 (m, 1H), 4.04 (t, J=5.9 Hz, 2H), 3.39 (t, J=6.6 Hz, 2H), 3.09 (br d, J=6.2 Hz, 2H), 2.57 (q, J=7.6 Hz, 2H), 1.95 - 1.79 (m, 4H), 1.45 - 1.40 (m, 17H), 1.10 (t, J=7.6 Hz, 3H).

Step h: Synthesis of (2S)-2-Amino-3-[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]propanoic Acid (AA2-12)

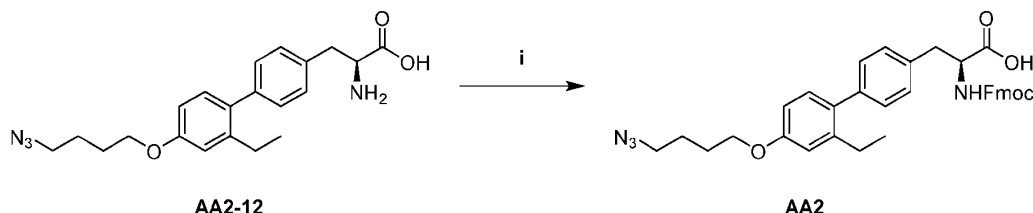


[000820] To **AA2-11** (31.5 g, 58.48 mmol, 1.0 eq.) was added HCl/EtOAc (210 mL). The mixture was stirred at 25 °C for 12 h. The suspension mixture was concentrated under vacuum to give a white solid. Compound **AA2-12** (37 g, crude) was obtained as a white solid.

[000821] LCMS (ESI): RT = 2.962 min, mass calcd. for C₂₁H₂₇N₄O₃ 383.20 [M+H]⁺, m/z found 383.1 [M+H]⁺. Reverse phase LC-MS was carried out using a X Bridge C18 3.5 um 21*20 mm, with a flow rate of 0.8 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000822] ¹H NMR (400MHz, DMSO-d₆) δ = 8.58 (br s, 3H), 7.34 (d, J=8.0 Hz, 2H), 7.22 (d, J=7.8 Hz, 2H), 7.04 (d, J=8.3 Hz, 1H), 6.88 (d, J=2.3 Hz, 1H), 6.81 (dd, J=2.4, 8.4 Hz, 1H), 4.18 (br s, 1H), 4.02 (br t, J=5.9 Hz, 2H), 3.42 (t, J=6.7 Hz, 2H), 3.20 (br d, J=6.0 Hz, 2H), 2.57 - 2.51 (m, 2H), 1.84 - 1.66 (m, 4H), 1.05 (t, J=7.4 Hz, 3H).

Step i: Synthesis of (2R)-3-[4-[4-(4-Azidobutoxy)-2-ethyl-phenyl]phenyl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic Acid (**AA2**)

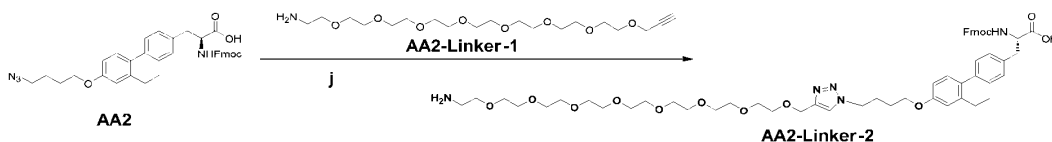


[000823] To a solution of compound **AA2-12** (37 g, 96.74 mmol, 1.0 eq.) in THF (400 mL) was added NaHCO₃ (24.38 g, 290.23 mmol, 11.29 mL, 3.0 eq.) in H₂O (200 mL), and then FmocOSu (35.90 g, 106.42 mmol, 1.1 eq.) was added at 0 °C. The reaction mixture was diluted with brine (200mL) and acidified with 1 M aqueous HCl to pH = 2~3. The reaction mixture was extracted with EtOAc (200 mL × 2). The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 330 g SepaFlash® Silica Flash Column, Eluent of 0 ~20% Ethyl acetate/Dichloromethane, gradient @ 100 mL/min) for 40 min with total volume 4 L. Compound **AA2** (45 g, 65.49 mmol, 67.69% yield, 88% purity) was obtained as a colorless oil foam.

[000824] LCMS (ESI): RT =5.482 min, mass calcd. for C₃₆H₃₇N₄O₅ 605.26 [M+H]⁺, m/z found 605.2 [M+H]⁺. Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000825] ¹H NMR (400MHz, Chloroform-d) δ = 10.00 (br s, 1H), 7.78 (d, J=7.5 Hz, 2H), 7.64 - 7.49 (m, 2H), 7.41 (t, J=7.4 Hz, 2H), 7.36 - 7.28 (m, 2H), 7.26 - 7.12 (m, 4H), 7.11 - 7.02 (m, 1H), 6.86 (d, J=2.0 Hz, 1H), 6.76 (br dd, J=2.3, 8.3 Hz, 1H), 6.27 (br d, J = 7.5 Hz, 1H), 5.47 (br d, J=8.0 Hz, 1H), 4.85 - 4.75 (m, 1H), 4.54 - 4.34 (m, 2H), 4.23 (br t, J=6.9 Hz, 1H), 4.08 - 3.99 (m, 2H), 3.40 (t, J=6.7 Hz, 2H), 3.36 - 3.26 (m, 1H), 3.24 - 3.14 (m, 1H), 2.56 (q, J=7.5 Hz, 2H), 1.97 - 1.80 (m, 4H), 1.09 (t, J=7.4 Hz, 3H).

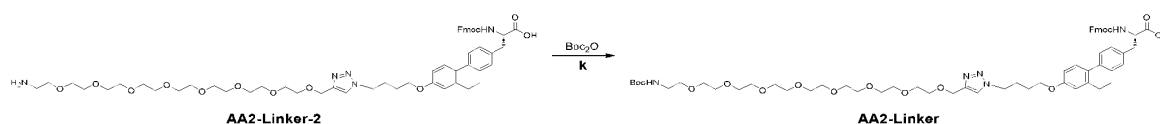
Step j: Synthesis of (2S)-3-[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-(2-Aminoethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic Acid (**AA2-Linker-2**)



[000826] To a solution of compound **AA2** (100 mg, 165.37 μmol , 1.0 *eq.*), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (41.29 mg, 165.37 μmol , 1.0 *eq.*), and sodium L-ascorbate (32.76 mg, 165.37 μmol , 1.0 *eq.*) in H_2O (0.8 mL) was added a solution of TBTA (43.88 mg, 82.69 μmol , 0.5 *eq.*) and **AA2-Linker-1** (134.78 mg, 330.75 μmol , 2.0 *eq.*) in DMSO (0.2 mL). The mixture was stirred at 30 °C for 2 hr. LCMS showed that **AA2** was consumed completely and one main peak with desired mass was detected. The reaction mixture was diluted with brine (10mL) and extracted with EtOAc/MeOH=5/1 (15 mL $\times$ 2). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. Compound **AA2-Linker-2** (0.2 g, crude) was obtained as a yellow oil.

[000827] LCMS (ESI): RT = 3.389 min, m/z calcd. for $C_{55}H_{74}N_5O_{13}$ 1012.52 $[M+H]^+$, m/z found 1012.5 $[M+H]^+$. LC-MS method A: a MERCK, RP-18e 25-2mm column, with a flow rate of 1.5 mL/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

Step k: Synthesis of (2S)-3-[4-[4-[4-[4-[2-[2-[2-[2-[2-[2-(tert-Butoxycarbonylamino)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethoxymethyl]triazol-1-yl]butoxy]-2-ethyl-phenyl]phenyl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic Acid (B)



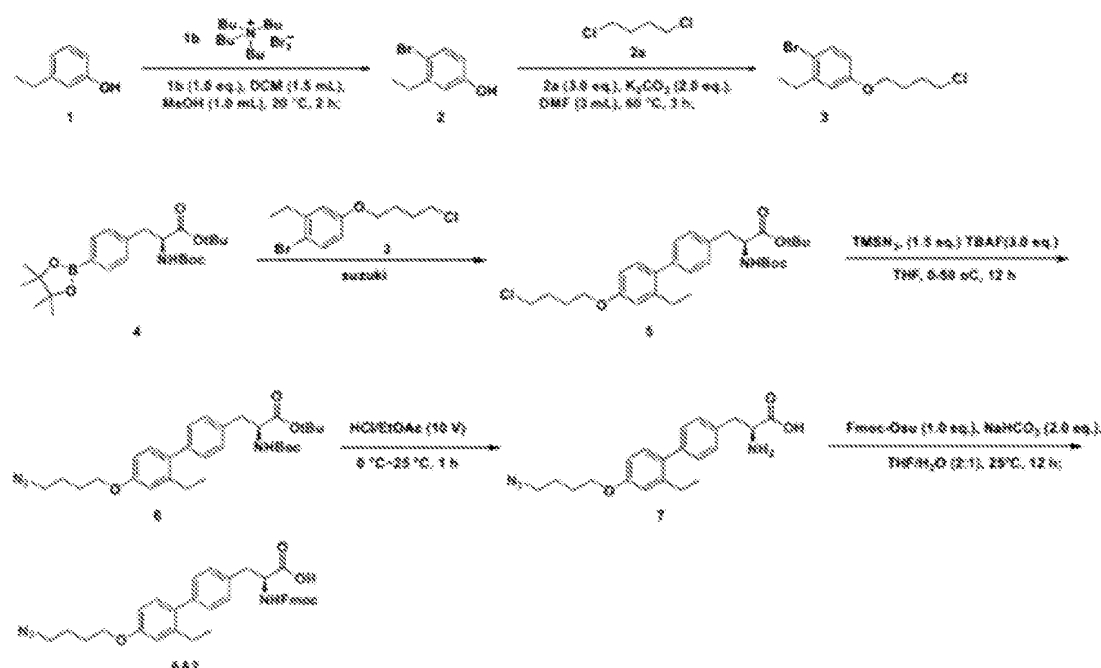
[000828] To a solution of compound **AA2-Linker-2** (0.2 g, 197.59 μ mol, 1.0 eq.) in THF (1 mL) and NaHCO_3 (1 M, 1 mL, 5.06 eq.) was added $(\text{Boc})_2\text{O}$ (43.12 mg, 197.59 μ mol, 45.39 μ L, 1.0 eq.). Then the mixture was stirred at 20°C for 2 h. LCMS showed that compound **AA2-Linker-2** was consumed completely, and the desired MS was detected. The reaction mixture was quenched by addition of H_2O (10 mL), and then diluted with $\text{DCM}/\text{MeOH} = 10/1$ (10 mL) and extracted with $\text{DCM}/\text{MeOH} = 10/1$ (20 mL $\times$ 2). The combined organic layers were washed with sat. NaCl (10 mL $\times$ 2), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 12 g Sepa Flash® Silica Flash Column, Eluent of 0~30% MeOH/DCM @ 30 mL/min). The desired compound **AA2-Linker** (70 mg, 56.64 μ mol, 28.66% yield, 90% purity) was obtained as a white solid.

[000829] LCMS: (ESI): Rt = 3.319 min, mass calcd. for $C_{60}H_{82}N_5O_{15}$ $[M+H]^+$ 1112.57, m/z found 1112.20 $[M+H]^+$. Reverse phase LCMS was carried out using Chromolith Flash RP-C18 25-3mm, with a flow rate of 0.8ml/min, eluting with a gradient of 10% to 80% acetonitrile containing 0.02% TFA (solvent B) and water containing 0.04% TFA (solvent A).

[000830] $^1\text{H NMR}$ (CDCl_3 , 400MHz) δ 7.75 (d, J = 8.0 Hz, 2H), 7.63 - 7.52 (m, 3H), 7.38 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 7.2 Hz, 2H), 7.17 (s, 4H), 7.05 (d, J = 8.4 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.71 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 5.44 (d, J = 7.6 Hz, 1H), 5.11 (s, 1H), 4.78 - 4.65 (m, 3H), 4.52 - 4.42 (m, 3H), 4.37 - 4.29 (m, 1H), 4.21 (t, J = 6.8 Hz, 1H), 4.01 (t, J = 5.6 Hz, 2H), 3.73 - 3.58 (m, 28H), 3.52 (t, J = 4.8 Hz, 2H), 3.37 - 3.12 (m, 4H), 2.52 (q, J = 7.6 Hz, 2H), 2.20 - 2.10 (m, 2H), 1.87 - 1.77 (m, 2H), 1.44 (s, 9H), 1.06 (t, J = 7.6 Hz, 3H).

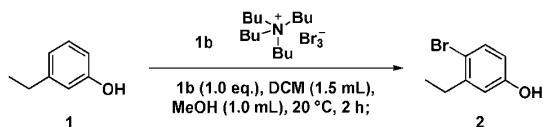
[000831] The synthesis of AA2 using route 4 is shown in Scheme 10 below.

Scheme 10



Reagents and Conditions: (a) 1b (1.0 eq.), DCM (1.5 mL), MeOH (1.0 mL), 20 °C, 2 h; (b) 2a (3.0 eq.), K_2CO_3 (2.0 eq.), DMF (3 mL), 60 °C, 3 h, 70.5%; (c) $\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{CH}_2\text{Cl}_2$ (0.05 eq.), K_2CO_3 (3.0 eq.), Dioxane/ H_2O , 80 °C, 3 h, 29.5%. Steps from compound 4 to AA2 are same as in Route 5.

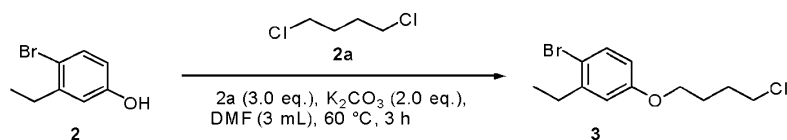
Step 1: Synthesis of 4-Bromo-3-ethylphenol (2)



[000832] To a solution of 3-ethylphenol (500 mg, 4.09 mmol, 495.05 μ L, 1 eq) in DCM (7.5 mL) and MeOH (5 mL) was added tetrabutyl ammonium tribromide (1.97 g, 4.09 mmol, 1 eq) at 20°C. The mixture was stirred at 20°C for 2 hr. The reaction was monitored by TLC (Petroleum ether: Ethyl acetate = 3:1) which indicated that reactant was consumed and one new spot with lower polarity was observed. The reaction mixture was quenched by H₂O (40 mL) and extracted with EtOAc (30 mLx2). The combined organic layers were washed with 1 M HCl (20 mL) and brine (20 mLx2), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The product 4-bromo-3-ethyl-phenol (800 mg, crude) was obtained as a colorless oil, which was checked by ¹H NMR.

[000833] ¹H NMR (400MHz, Chloroform-d) δ = 7.21 (d, J=8.8 Hz, 1H), 6.93 (d, J=3.0 Hz, 1H), 6.81 (dd, J=3.0, 8.5 Hz, 1H), 2.60 (q, J=7.4 Hz, 2H), 1.14 (t, J=7.5 Hz, 3H).

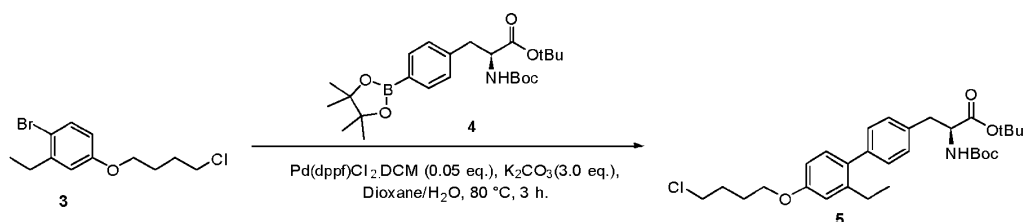
Step 2: Synthesis of 1-bromo-4-(4-chlorobutoxy)-2-ethylbenzene (3)



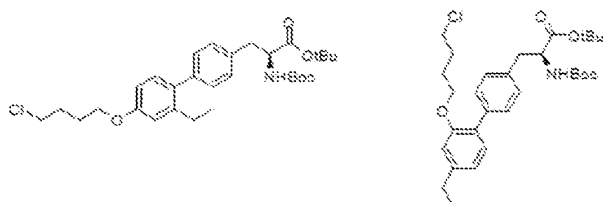
[000834] To a solution of 4-bromo-3-ethyl-phenol (2) (800 mg, 3.98 mmol, 1 eq) and K₂CO₃ (1.10 g, 7.96 mmol, 2 eq) in DMF (15 mL) was added 1,4-dichlorobutane (1.52 g, 11.94 mmol, 1.33 mL, 3 eq) at 20 °C. The mixture was stirred at 60 °C for 12 h. The reaction was monitored by TLC (Petroleum ether: Ethyl acetate = 3:1) which indicated that reactant was consumed and one new spot with lower polarity was observed. The reaction mixture was quenched by H₂O (40 mL) and extracted with EtOAc (40 mLx2). The combined organic layers were washed with 1 M HCl (20 mL) and brine (30 mLx2), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The product 3 1-bromo-4-(4-chlorobutoxy)-2-ethyl-benzene (910 mg, 2.81 mmol, 70.59% yield, 90% purity) was obtained as a colorless oil, which was checked by ¹H NMR and 2D NMR. The compound was used in next step without purification.

[000835] ¹H NMR (400MHz, Chloroform-d) δ = 7.40 (d, J=8.8 Hz, 1H), 6.80 (d, J=3.0 Hz, 1H), 6.64 - 6.58 (m, 1H), 3.98 (t, J=5.8 Hz, 2H), 3.66 - 3.61 (m, 2H), 2.72 (q, J=7.5 Hz, 2H), 2.00 - 1.95 (m, 4H), 1.27 - 1.19 (m, 3H).

Step 3: Synthesis of *tert*-Butyl (2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[4-(4-chlorobutoxy)-2-ethyl-phenyl]phenyl]propanoate (3)



[000836] To a solution of compound **3** (1 g, 3.43 mmol, 1 eq) and compound **4** (1.53 g, 3.43 mmol, 1 eq) in dioxane (10 mL) and H₂O (2 mL) were added Pd(dppf)Cl₂, CH₂Cl₂ (140.02 mg, 171.46 μmol, 0.05 eq), and K₂CO₃ (1.42 g, 10.29 mmol, 3 eq), the mixture was stirred at 80 °C for 3 hr. TLC (Petroleum ether : Ethyl acetate=10:1) indicated one new spot formed. LCMS showed that the reaction was complete. The reaction mixture was quenched by H₂O (20 mL) and extracted with EtOAc (30 mLx2). The combined organic layers were washed with brine (20 mLx2), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0~10% EtOAc/PE gradient @ 20 mL/min) for 35 min with total volume 1.2 L. the product **5** (0.6 g, 1.01 mmol, 29.59% yield, 90% purity) was obtained as a colorless oil. The Regio isomer impurity may be isolated by chromatography.

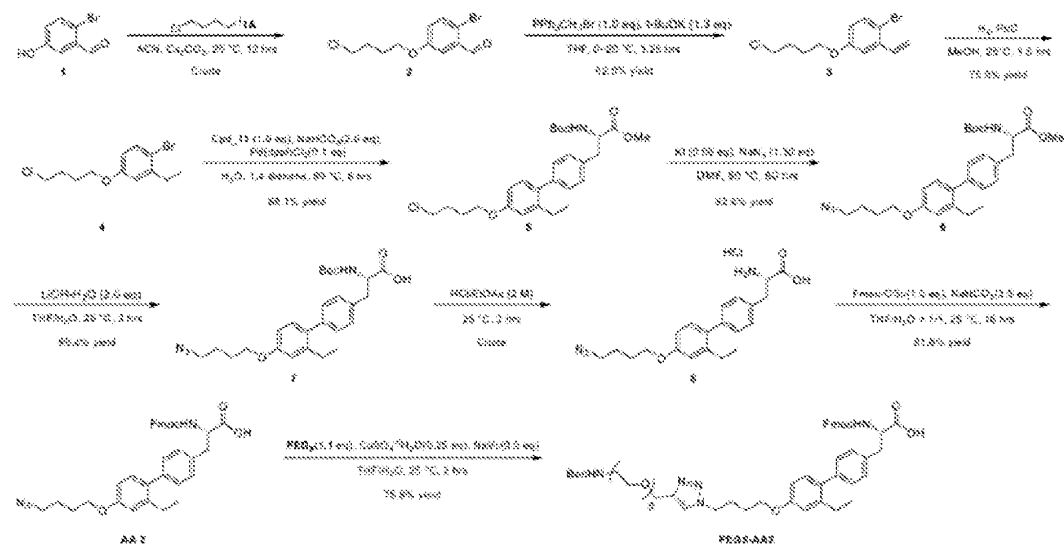


[000837] For product **5**, LCMS (ESI): RT = 1.217 min, m/z calcd. For C₄₂H₄₉NO₇Na 554.28; found 554.1 [M+Na]⁺; LC-MS Conditions: Mobile Phase: 1.5mL/4LTFA in water (solvent A) and 0.75mL/4LTFA in acetonitrile (solvent B), using the elution gradient 5%-95% (solvent B) over 0.7 minutes and holding at 95% for 0.4 minutes at a flow rate of 1.5 mL/min.

[000838] ¹H NMR (400 MHz, Chloroform-d) δ = 7.21 (s, 4H), 7.10 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 2.5 Hz, 1H), 6.76 (dd, J = 2.6, 8.3 Hz, 1H), 5.14 - 5.01 (m, 1H), 4.56 - 4.45 (m, 1H), 4.05 (t, J = 5.7 Hz, 2H), 3.65 (t, J = 6.2 Hz, 2H), 3.09 (br d, J = 6.3 Hz, 2H), 2.57 (q, J = 7.5 Hz, 2H), 2.04 - 1.94 (m, 4H), 1.44 (s, 9H), 1.41 (s, 9H), 1.10 (t, J = 7.5 Hz, 3H).

[000839] The synthesis of AA2 and AA2-Linker using route 5 is shown in Schemes 11-12 and Table 25 below.

Scheme 11



Scheme 12

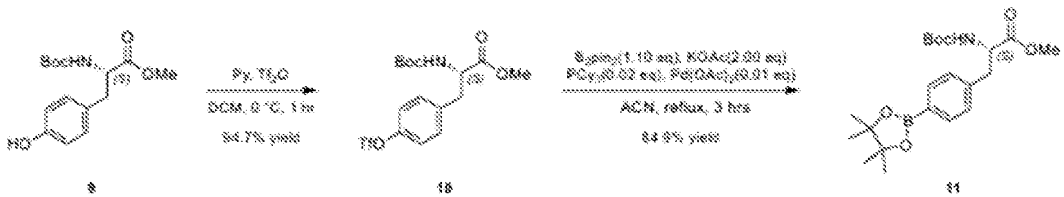


Table 25

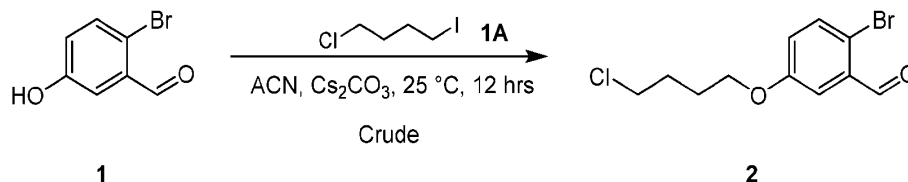
Step	ELN No.	Raw material (Equivalent /Volume)				Condition	Product
		Reactant 1	Reactant 2	Reagent	Solvent		
1'-1	EC1652-28	Cpd_1: 500 g, (1.00 eq)	Cpd_1A: 597 g, (1.10 eq)	Cs ₂ CO ₃ : 1.13 kg, (1.40 eq)	ACN: 3.00 L	25 °C / 12 h	736 g (crude), brown oil
1'-2	EC1652-131	Cpd_1: 2.00 kg (1.00 eq)	Cpd_1A: 2.39 kg (1.10 eq)	Cs ₂ CO ₃ : 4.54 kg (1.40 eq)	ACN: 12.0 L	25 °C / 12 h	2930 g (crude), brown oil
1'-3	EC1652-144 (2 batches)	Cpd_1: 2.00 kg (1.00 eq)	Cpd_1A: 2.39 kg (1.10 eq)	Cs ₂ CO ₃ : 4.54 kg (1.40 eq)	ACN: 12.0 L	25 °C / 12 h	5720 g (crude), brown oil
2'-1	EC1652-22	Cpd_2: 71.6 g (1.00 eq)	PPh ₃ CH ₂ Br: 86.1 g, (1.10 eq)	tBuOK: 35.1 g, (1.50 eq)	THF: 700 mL	0 - 20 °C / 3.25 h	45.2 g (96.6% purity), colorless oil
2'-2	EC1652-29 (2 batches)	Cpd_2: 368 g, (1.00 eq)	PPh ₃ CH ₂ Br: 445 g, (1.10 eq)	tBuOK: 181 g, (1.50 eq)	THF: 3.70 L	0 - 20 °C / 4 h	274 g (99.4% purity), colorless oil

2'-3	EC1652-154 (3 batches)	Cpd_2: 300 g, (1.00 eq)	PPh ₃ CH ₃ Br: 358 g (1.00 eq)	tBuOK: 146 g, (1.30 eq)	THF: 3.00 L	0 - 20 °C / 3.25 h	632 g (99.3% purity) & 589 g (91.6% purity), colorless oil
2'-4	EC1652-157 (3 batches)	Cpd_2: 333 g, (1.00 eq)	PPh ₃ CH ₃ Br: 398 g, (1.00 eq)	tBuOK: 162 g, (1.30 eq)	THF: 3.00 L	0 - 20 °C / 3.25 h	
2'-5	EC1652-161 (3 batches)	Cpd_2: 333 g, (1.00 eq)	PPh ₃ CH ₃ Br: 403 g, (1.00 eq)	tBuOK: 162 g, (1.30 eq)	THF: 3.00 L	0 - 20 °C / 3.25 h	504 g (99.4% purity) & 770 g (94.1% purity), colorless oil
2'-6	EC1652-162 (3 batches)	Cpd_2: 333 g, (1.00 eq)	PPh ₃ CH ₃ Br: 403 g, (1.00 eq)	tBuOK: 162 g, (1.30 eq)	THF: 3.00 L	0 - 20 °C / 3.25 h	
2'-7	EC1652-168 (3 batches)	Cpd_2: 333 g, (1.00 eq)	PPh ₃ CH ₃ Br: 403 g, (1.00 eq)	tBuOK: 162 g, (1.30 eq)	THF: 3.00 L	0 - 20 °C / 3.25 h	
2'-8	EC1652-170 (3 batches)	Cpd_2: 333 g, (1.00 eq)	PPh ₃ CH ₃ Br: 403 g, (1.00 eq)	tBuOK: 162 g, (1.30 eq)	THF: 3.00 L	0 - 20 °C / 3.25 h	1250 g (99.8% purity) & 550 g (crude), colorless oil
2'-9	EC1652-175 (2 batches)	Cpd_2: 350 g, (1.00 eq)	PPh ₃ CH ₃ Br: 423 g, (1.00 eq)	tBuOK: 173 g, (1.30 eq)	THF: 3.50 L	0 - 20 °C / 3.25 h	
3'-1	EC1652-24	Cpd_3: 60.0 g, (1.00 eq)	H ₂ (50 Psi)	Pt/C: 6.00 g, (0.10 eq)	MeOH: 300 mL	25 °C / 4 h	50.2 g (94.6% purity), light brown oil
3'-2	EC1652-40 (2 batches)	Cpd_3: 137 g, (1.00 eq)	H ₂ (50 Psi)	Pt/C: 13.7 g, (0.10 eq)	MeOH: 700 mL	25 °C / 2.5 h	251 g (92.6% purity), light brown oil
3'-3	EC1652-163 (3 batches)	Cpd_3: 210 g, (1.00 eq)	H ₂ (50 Psi)	Pt/C: 21.0 g, (0.10 eq)	MeOH: 1050 mL	25 °C / 1.5 h	540 g (92.5% purity), colorless oil
3'-4	EC1652-174 (3 batches)	Cpd_3: 168 g, (1.00 eq)	H ₂ (50 Psi)	Pt/C: 16.8 g, (0.10 eq)	MeOH: 840 mL	25 °C / 1.5 h	425 g (90.9% purity), colorless oil
3'-5	EC1652-180 (7 batches)	Cpd_3: 178 g, (1.00 eq)	H ₂ (50 Psi)	Pt/C: 17.8 g, (0.10 eq)	MeOH: 900 mL	25 °C / 1.5 h	1030 g (92.2% purity), colorless oil
3'-6	EC1652-186 (5 batches)	Cpd_3: 204 g, (1.00 eq)	H ₂ (50 Psi)	Pt/C: 20.4 g, (0.10 eq)	MeOH: 1000 mL	25 °C / 1.5 h	835 g (92.3% purity), colorless oil
3'-7	EC5661-2 (3 batches)	Cpd_3: 172 g, (1.00 eq)	H ₂ (50 Psi)	Pt/C: 17.2 g, (0.10 eq)	MeOH: 860 mL	25 °C / 1.5 h	460 g (92.3% purity), yellow oil
4'-1	EC481-505	Cpd_9: 100 g, (1.00 eq)	Tf ₂ O: 114 g, (1.20 eq)	Pyridine: 66.9 g, (2.50 eq)	DCM: 1.00 L	0 °C / 1 h	144 g (100% purity), yellow solid

4'-2	EC481-737	Cpd_9: 500 g, (1.00 eq)		Tf ₂ O: 573 g, (1.20 eq)	Pyridine: 334 g, (2.50 eq)	DCM: 2.50 L	0 °C / 1 h	712 g (98.1% purity), yellow solid
4'-3	EC481-745	Cpd_9: 4.50 kg, (1.00 eq)		Tf ₂ O: 5.16 kg, (1.20 eq)	Pyridine: 3.01 kg, (2.50 eq)	DCM: 22.5 L	0 °C / 1 h	6.34 kg (97.3% purity), yellow solid
5'-1	EC481-525	Cpd_10: 300 g, (1.00 eq)		PCy ₃ : 3.94 g, (0.02 eq) B ₂ Pin ₂ : 187 g, (1.05 eq)	Pd(OAc) ₂ : 1.58 g, (0.01 eq) KOAc: 137 g, (2.00 eq)	ACN: 3.00 L	81 °C / 12 h	276 g (97.7% purity), yellow oil
5'-2	EC481-743	Cpd_10: 350 g, (1.00 eq)		PCy ₃ : 4.51 g, (0.02 eq) B ₂ Pin ₂ : 214 g, (1.05 eq)	Pd(OAc) ₂ : 1.80 g, (0.01 eq) KOAc: 157 g, (2.00 eq)	ACN: 3.00 L	81.6 °C / 3 h	313 g (92.4% purity), yellow oil
5'-3	EC481-759 (2 batches)	Cpd_10: 3.07 kg, (1.00 eq)		PCy ₃ : 39.5 g, (0.02 eq) B ₂ Pin ₂ : 1.88 kg, (1.05 eq)	Pd(OAc) ₂ : 15.8 g, (0.01 eq) KOAc: 1.38 kg, (2.00 eq)	ACN: 26.5 L	81.6 °C / 3 h	1764 g (96.2% purity) & 3256 g (96.9% purity), yellow oil
6'-1	EC1652-41	Cpd_11: 70.6 g, (1.05 eq)	Cpd_4: 50.0 g, (1.00 eq)	Pd(dppf)Cl ₂ : 11.8 g, (0.10 eq)	NaHCO ₃ : 27.2 g, (2.0 eq)	Dioxane/H ₂ O: 300 mL/100 mL	80 °C / 6.5 h	67.0 g (97.5% purity), light yellow oil
6'-2	EC5661-1	Cpd_11: 500 g, (1.05 eq)	Cpd_4: 362 g, (1.00 eq)	Pd(dppf)Cl ₂ : 82.7 g, (0.10 eq)	NaHCO ₃ : 189 g, (2.0 eq)	Dioxane/H ₂ O: 2.10 L/0.70 L	80 °C / 6 h	465 g (97.2% purity), light yellow oil
6'-3	EC5661-4	Cpd_11: 4.04 kg, (1.05 eq)	Cpd_4: 2.90 kg, (1.00 eq)	Pd(dppf)Cl ₂ : 671 g, (0.10 eq)	NaHCO ₃ : 1.54 kg, (2.0 eq)	Dioxane/H ₂ O: 18.0 L/6.00 L	80 °C / 8 h	4.12 kg (96.2% purity), yellow oil
7'-1	EC1652-59	Cpd_5: 62.0 g, (1.00 eq)		KI: 10.3 g, (0.50 eq) + 1.94 g, (0.10 eq)	NaN ₃ : 9.72 g, (1.20 eq) + 1.52 g, (0.20 eq)	DMF: 600 mL	50 °C / 16 h + 16 h	53.5 g (98.4% purity), light yellow oil
7'-2	EC5661-3 (2 batches)	Cpd_5: 225 g, (1.00 eq)		KI: 44.4 g, (0.60 eq)	NaN ₃ : 37.7 g, (1.30 eq)	DMF: 2.20 L	50 °C / 30 h	440 g (98.7% purity), light yellow oil
7'-3	EC5661-6	Cpd_5: 225 g, (1.00 eq)		KI: 43.9 g, (0.60 eq)	NaN ₃ : 37.3 g, (1.30 eq)	DMF: 2.20 L	50 °C / 30 h	1140 g (98.0% purity), light yellow oil
7'-4	EC5661-9 + EC5661-10	Cpd_5: 1000 g, (1.00 eq)		KI: 195 g, (0.60 eq) + 32.5 g, (0.10 eq)	NaN ₃ : 165 g, (1.20 eq) + 19.1 g, (0.15 eq)	DMF: 10.0 L	50 °C / 64 h	
7'-5	EC5661-11 (2 batches)	Cpd_5: 1440 g, (1.00 eq)		KI: 281 g, (0.60 eq)	NaN ₃ : 238 g, (1.30 eq)	DMF: 14.4 L	50 °C / 64 h	2660 g (97.8% purity), yellow oil
8'-1	EC1652-72	Cpd_6: 52.5 g, (1.00 eq)		N/A	LiOH·H ₂ O: 8.73 g (2.00 eq)	THF/H ₂ O: 350 mL/150 mL	25 °C / 3 h	48.3 g (98.8% purity), colorless oil
8'-2	EC5661-5	Cpd_6: 430 g, (1.00 eq)		N/A	LiOH·H ₂ O: 71.7 g (2.00 eq)	THF/H ₂ O: 2.80 L/1.20 L	25 °C / 3 h	385 g (98.0% purity), light yellow oil
8'-3	EC5661-13 (2 batches)	Cpd_6: 1880 g, (1.00 eq)		N/A	LiOH·H ₂ O: 310 g (2.00 eq)	THF/H ₂ O: 13.2 L/5.60 L	25 °C / 3 h	3650 g (97.4% purity), yellow oil

9'-1	EC1652-76	Cpd_7: 47.3 g, (1.00 eq)		N/A	N/A	HCl/EA: 470 mL	25 °C / 2 h	41.0 g (97.2% purity), white solid
9'-2	EC5661-7	Cpd_7: 380 g, (1.00 eq)		N/A	N/A	HCl/EA: 3.80 L	25 °C / 2 h	340 g (98.8% purity), white solid
9'-3	EC5661-17 (12 batches)	Cpd_7: 380 g, (1.00 eq)		N/A	N/A	HCl/EA: 3.00 L	25 °C / 2 h	3.09 kg (97.8% purity), white solid
10'-1	EC1652-78	Cpd_8: 40.0 g, (1.00 eq)		Fmoc-OSu: 31.3 g, (1.00 eq)	NaHCO ₃ : 27.2 g, (3.50 eq)	THF/H ₂ O: 200 mL/200 mL	25 °C / 16 h	47.0 g (99.4% purity), light yellow foam
10'-2	EC5661-8	Cpd_8: 335 g, (1.00 eq)		Fmoc-OSu: 266 g, (1.00 eq)	NaHCO ₃ : 232 g, (3.50 eq)	THF/H ₂ O: 1.60 L/1.60 L	25 °C / 16 h	341 g (99.8% purity), colorless oil
10'-3	EC5661-19 (2 batches)	Cpd_8: 1.52 kg, (1.00 eq)		Fmoc-OSu: 1.20 kg, (1.00 eq)	NaHCO ₃ : 1.04 kg, (3.50 eq)	THF/H ₂ O: 7.60 L/7.60 L	25 °C / 16 h	3620 g (96.8% purity), light yellow oil
11'-1	EC5661-20	AA2: 30.0 g, (1.00 eq)	PEG8: 27.6 g, (1.10 eq)	NaVc: 4.90 g, (0.50 eq)	CuSO ₄ .5H ₂ O: 3.09 g, (0.25 eq)	THF/H ₂ O: 300 mL/90.0 mL	25 °C / 2 h	47.2 g (99.4% purity), light yellow oil
11'-2	EC5661-24	AA2: 240 g, (1.00 eq)	PEG8: 214 g, (1.05 eq)	NaVc: 38.0 g, (0.50 eq)	CuSO ₄ .5H ₂ O: 23.9 g, (0.25 eq)	THF/H ₂ O: 2.40 L/0.72 L	25 °C / 2 h	341 g (98.8% purity), yellow oil
11'-3	EC5661-28 (2 batches)	AA2: 1300 g, (1.00 eq)	PEG8: 1140 g, (1.05 eq)	NaVc: 206 g, (0.50 eq)	CuSO ₄ .5H ₂ O: 130 g, (0.25 eq)	THF/H ₂ O: 13.0 L/3.90 L	25 °C / 2 h	3550 g (98.9% purity), light brown oil

Synthesis of Compound (2)



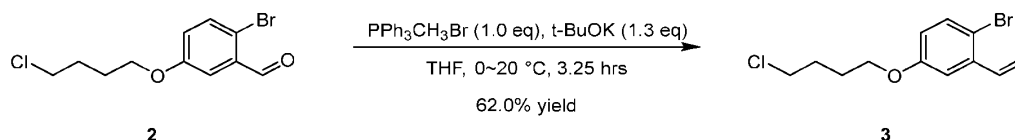
[000840] A general procedure that was used is as follows. To a solution of compound (1) (2.00 kg, 9.95 mol, 1.00 eq) in ACN (12.0 L) was added Cs₂CO₃ (4.54 kg, 13.9 mol, 1.40 eq) and compound (1A) (2.39 kg, 10.9 mol, 1.10 eq). The mixture was stirred at 25 °C for 12 hrs. The reaction was monitored by LCMS, which showed that compound (1) was consumed completely, and desired mass (Rt = 0.654 min) was observed.

[000841] Two batches were combined to work up. The reaction mixture was filtered, and the filter liquor was concentrated under reduced pressure to give a residue. The residue was dissolved in ethyl acetate (5.00 L) and filtered to remove salt. The filter liquor was concentrated in vacuo to give the crude product. Compound (2) (5720 g, 19.3 mol, 98.8% purity) was obtained as brown oil.

The structure and purity were confirmed by HNMR and HPLC (HPLC purity: 98.8% (220 nm)). The crude product was used in next step without any purification.

[000842] HNMR (CDCl₃, 400MHz): δ 10.2 (s, 1H), 7.51 (d, J = 8.4 Hz, 1H), 7.39 (d, J = 3.2 Hz, 1H), 7.02 (dd, J₁ = 8.8 Hz, J₂ = 3.2 Hz, 1H), 4.04 - 4.02 (m, 2H), 3.63 - 3.60 (m, 2H), 1.99 - 1.91 (m, 4H).

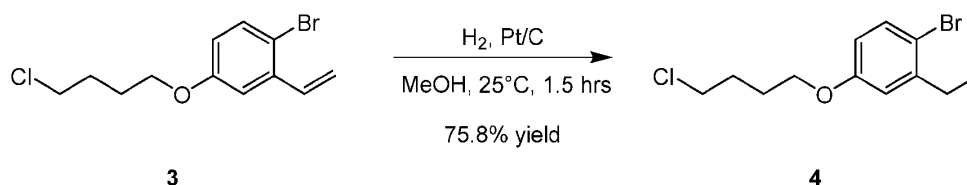
Synthesis of Compound (3)



[000843] A general procedure that was used is as follows. To a solution of PPh₃CH₃Br (403 g, 1.13 mol, 1.00 eq) in THF (1.80 L) was added tBuOK (164 g, 1.31 mol, 1.30 eq) at 0 - 5 °C under N₂. The mixture was stirred at 0 - 5 °C for 0.25 hr. A solution of compound (2) (333 g, 1.13 mol, 1.00 eq) in THF (1.20 L) was added dropwise to above solution. The mixture was stirred at 20 °C for 3 hrs. The reaction was monitored by LCMS, which showed that compound (2) was consumed completely. Three batches were combined to work up. The reaction mixture was partitioned between MTBE (8.00 L) and H₂O (8.00 L). The organic phase was separated, washed with brine (3.00 L * 2), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate = 1/0, petroleum ether/ethyl acetate = 1/0, R_f = 0.60). Compound (3) (504 g, 1.73 mol, 99.4% purity) was obtained as colorless oil. The structure and purity were confirmed by HNMR and HPLC. Compound (3) (770 g, 2.50 mol, 94.1% purity) was obtained as colorless oil.

[000844] HNMR (CDCl₃, 400MHz): δ 7.40 (d, J = 8.4 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 6.61 (dd, J₁ = 8.4 Hz, J₂ = 2.4 Hz, 1H), 3.97 (t, J = 5.6 Hz, 2H), 3.63 (t, J = 5.2 Hz, 2H), 2.72 (q, J = 7.6 Hz, 2H), 2.00 - 1.93 (m, 4H), 1.23 (t, J = 7.2 Hz, 3H).

Synthesis of Compound (4)



[000845] A general procedure that was used is as follows. To a solution of compound (3) (178 g, 613 mmol, 1.00 eq) in MeOH (900 mL) was added Pt/C (17.8 g, 4.24 mmol, 5% purity) under N₂. The mixture was degassed, purged 3 times with H₂, and stirred at 25 °C for 1.5 hrs under H₂.

atmosphere (50 Psi). The reaction was monitored by LCMS, which showed that compound (**3**) was consumed completely and 7.23% of byproduct (Rt = 0.680 min) and 92.7% of desired product (Rt = 0.729 min) were formed. Seven batches were combined to work-up. The reaction mixture was filtered, and the filter was concentrated to give the crude product. The crude product was used in the next step without further purification. Compound (**4**) (1030 g, 3.26 mol, 75.8% yield, 92.2% purity) was obtained as yellow oil. The structure and purity were confirmed by HNMR and HPLC.

[000846] HNMR (CDCl₃, 400MHz): δ 7.40 (d, J = 8.8 Hz, 1H), 6.79 (d, J = 3.2 Hz, 1H), 6.61 (dd, J₁ = 8.4 Hz, J₂ = 2.8 Hz, 1H), 3.97 (t, J = 6.0 Hz, 2H), 3.63 (t, J = 6.0 Hz, 2H), 2.72 (q, J = 7.6 Hz, 2H), 2.00 - 1.94 (m, 4H), 1.23 (t, J = 7.6 Hz, 3H)

[000847] The reaction conditions used to prepare compound (**4**) are shown in Scheme 13 and Table 26 below.

Scheme 13

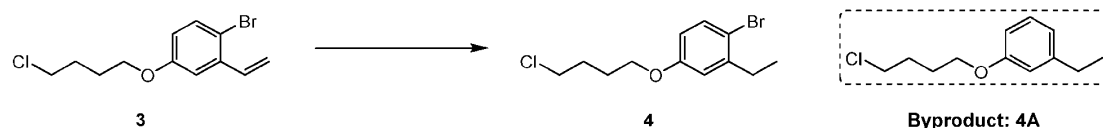
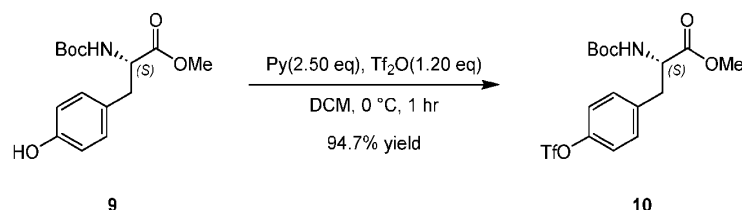


Table 26.

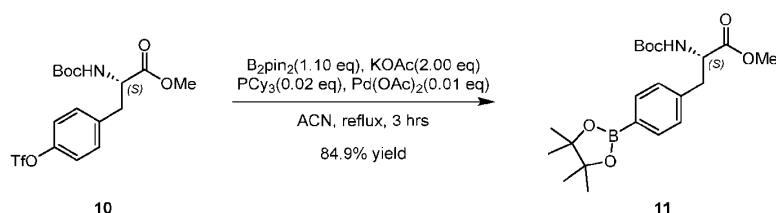
Condition	catalyst	Reagent	Solvent/ Vol	Temp. / Time	IPC / LCMS
3'-1	Pd/C	N/A	MeOH/ 20V	25 °C / 12 h	Cpd_4A via LCMS
3'-2	Pd/C	HCl/0.1 eq	MeOH/ 20V	25 °C / 12 h	Cpd_4A via LCMS
3'-3	Pd/C	N/A	MeOH/ 20V	25 °C / 1 h	14.0% Cpd_4 and 83.9% Cpd_4A via LCMS
3'-4	Pd(OH) ₂	HCl/0.1 eq	MeOH/ 20V	25 °C / 1 h	48.3% Cpd_4, 3.23% Cpd_3 and 47.9% Cpd_4A via LCMS
3'-5	Pd/C	N/A	THF/ 20V	25 °C / 1 h	67.5% Cpd_4 and 26.5 % Cpd_4A via LCMS
3'-6	Pt/C	N/A	MeOH/ 20V	25 °C / 1 h	94.8% Cpd_4 and 4.43% Cpd_4A via LCMS
3'-7	PtO ₂	N/A	MeOH/ 20V	25 °C / 1 h	78.3% Cpd_4 and 20.7% Cpd_4A via LCMS
3'-8	Pt/C	N/A	MeOH/ 20V	25 °C / 4 h	92.9% Cpd_4 and 4.01% Cpd_4A via HPLC
3'-9	Pt/C	PPh ₃ , 0.2 eq	MeOH/ 5V	25 °C / 4 h	91.5% Cpd_4 and 5.71% Cpd_4A via HPLC
3'-10	Pt/C	(2-(2- pyridyl)pyridine, 0.2 eq	MeOH/ 5V	25 °C / 4 h	94.5% Cpd_4 and 1.53% By- product via HPLC
3'-11	Pt/C	N/A	MeOH/ 5V	25 °C / 4 h	94.6% Cpd_4 and 2.40% Cpd_4A via HPLC

Synthesis of Compound (10)

[000848] A general procedure that was used is as follows. To a solution of compound (9) (4.50 kg, 15.2 mol, 1.00 eq) in dry DCM (22.5 L) was added Tf_2O (5.16 kg, 18.2 mol, 3.02 L, 1.20 eq) at 0°C , then Py (3.01 kg, 38.0 mol, 3.07 L, 2.50 eq) was added dropwise at 0°C under N_2 . The mixture was stirred at 0°C for 1 hr. The reaction was monitored using LCMS, which showed that the desired m/z peak ($R_t = 0.617$ min) has formed. The reaction mixture was quenched by adding water 4.50 L at 0°C , then the cooling bath was removed. The saturated citric acid solution was added to the mixture to adjust pH to 2 – 3. After separation, the organic layer was washed with 5% citric acid solution (4.50 L * 3), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give the product. Compound (10) (6.34 kg, 14.4 mol, 94.7% yield, 97.3% purity) was obtained as a yellow solid. The structure and purity were confirmed by HNMR and HPLC. The crude product was used in the next step without further purification.

[000849] MS cal.: 427.09, MS observed: $[\text{M}+\text{Na}]^+ = 450.0$. HPLC purity: 97.3% (220 nm).

[000850] HNMR (CDCl_3 , 400MHz): δ 7.24 - 7.19 (m, 4H), 5.03 (d, $J = 7.2$ Hz, 1H), 4.60 (d, $J = 6.8$ Hz, 1H), 3.71 (s, 3H), 3.20 - 3.01 (m, 2H), 1.41 (s, 9H).

Synthesis of Compound (11)

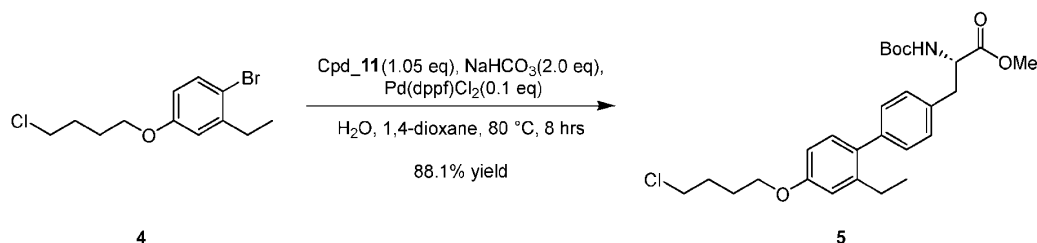
[000851] A general procedure that was used is as follows. To a mixture of compound (10) (3.07 kg, 7.05 mol, 1.00 eq) in ACN (26.5 L) was added B_2pin_2 (bis(pinacolato)diboron) (1.88 kg, 7.40 mol, 1.05 eq), KOAc (1.38 kg, 14.0 mol, 2.00 eq), and PCy_3 (39.5 g, 140 mmol, 0.02 eq). The mixture was degassed and purged 3 times with N_2 . Then $\text{Pd}(\text{OAc})_2$ (15.8 g, 70.4 mmol, 0.01 eq) was added to the mixture under N_2 . The reaction mixture was heated to reflux (81.6°C) for 3 hrs under N_2 . The reaction was monitored by HPLC, which showed one main peak ($R_t = 3.905$ min). The reaction

mixture was filtered, and the filter cake was washed with ACN (1.00 L). The combined filtrate was concentrated and diluted with MTBE (14.0 L), then washed with water (4.50 L * 2), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate=10/1 to 5/1, R_f = 0.48) to give two fractions of compound (11): fraction 1 (1.76 kg, 4.19 mol, 29.7% yield, 96.2% purity) (was obtained as yellow oil) and fraction 2 (3.26 kg, 7.78 mol, 55.2% yield, 96.9% purity) (was obtained as yellow oil). The structure and purity were confirmed by HNMR, LCMS, and HPLC.

[000852] MS cal.: 405.23, MS observed: [M+Na]⁺ = 428.2.

[000853] HNMR (CDCl₃, 400MHz): δ 7.74 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.0 Hz, 2H), 4.95 (d, J = 7.2 Hz, 1H), 4.68 - 4.37 (m, 1H), 3.70 (s, 3H), 3.16 - 3.05 (m, 2H), 1.42 (s, 9H), 1.34 (s, 12H).

Synthesis of Compound (5)



[000854] A general procedure that was used is as follows. To a solution of compound (11) (4.04 kg, 9.64 mol, 1.05 eq) and compound (4) (2.90 kg, 9.18 mol, 1.00 eq) in dioxane (18.0 L) and H₂O (6.00 L) was added NaHCO₃ (1.54 kg, 18.3 mol, 2.00 eq), Pd(dppf)Cl₂ (671 g, 917 mmol, 0.10 eq) under N₂, and then the mixture was stirred at 80 °C for 8 hrs under N₂. The reaction was monitored by LCMS, which showed that compound (4) was consumed completely, and desired mass (R_t = 0.744 min) was formed. The reaction solution was work-up in three batches. The reaction mixture was diluted with H₂O (12.0 L) and extracted with EtOAc (8.00 L * 2). The combined organic layers were washed with brine (5.00 L * 2), dried over Na₂SO₄, and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate = 10/1 to 3/1, petroleum ether/ethyl acetate = 5/1, R_f = 0.40). Compound (5) (4.12 kg, 8.09 mol, 88.1% yield, 96.2% purity) was obtained as yellow oil. The structure and purity were confirmed by HNMR, LCMS, HPLC, Special LCMS, Special HPLC, and SFC.

[000855] MS cal.: 489.23, MS observed: [M+Na]⁺ = 512.1. Special LCMS of compound (5): MS cal.: 489.23, MS observed: [M-Boc+H]⁺ = 390.1. HPLC purity: 96.2% (220 nm). Special HPLC purity: 96.5% (220 nm). SFC data for compound (5): e.e. value %: 100%.

[000856] ¹H NMR (CDCl₃, 400MHz): δ 7.22 (d, J = 7.6 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 7.10 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 2.0 Hz, 1H), 6.76 (dd, J₁ = 8.4 Hz, J₂ = 2.4 Hz, 1H), 5.04 (d, J = 8.0 Hz, 1H), 4.64 (d, J = 7.2 Hz, 1H), 4.04 (t, J = 6.0 Hz, 2H), 3.74 (s, 3H), 3.65 (t, J = 6.0 Hz, 2H), 3.19 - 3.05 (m, 2H), 2.56 (q, J = 7.6 Hz, 2H), 2.03 - 1.96 (m, 4H), 1.43 (s, 9H), 1.10 (t, J = 7.6 Hz, 3H).

[000857] The reaction conditions used to prepare compound (5) are shown in Scheme 14 and Table 27 below.

Scheme 14

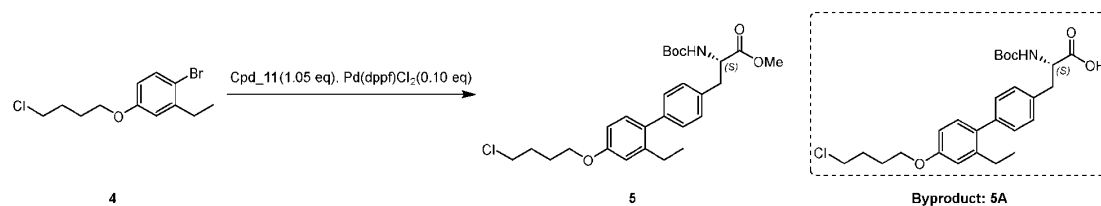
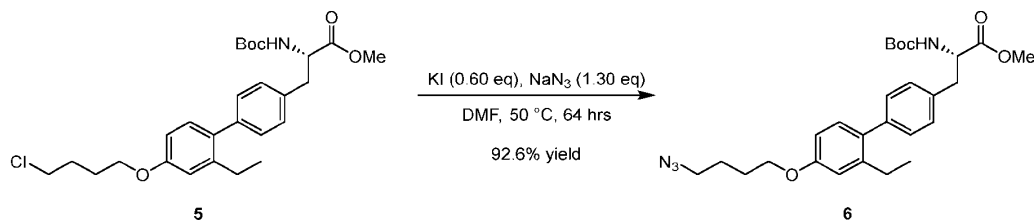


Table 27.

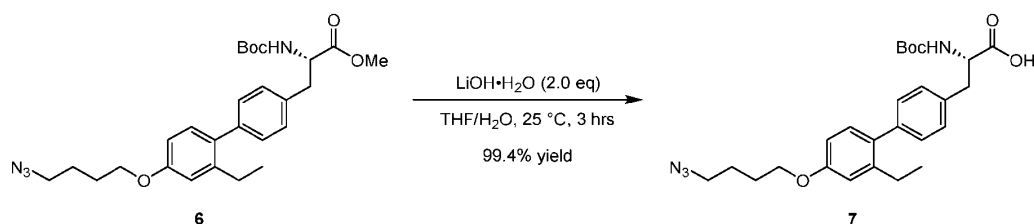
Condition	Base	Solvent	Temp. / Time	IPC / LCMS
6'-1	K ₂ CO ₃	Dioxane / H ₂ O	80 °C / 18 h	After 2 h: 69.5% of Cpd_5 and 12.3% of Cpd_5A via LCMS After 18 h: 77.8% of Cpd_5A via LCMS,
6'-2	Na ₂ CO ₃	Dioxane / H ₂ O	80 °C / 18 h	After 2 h: 68.5% of Cpd_5 and 2.75% of Cpd_5A via LCMS After 4.5 h: 49.4% of Cpd_5 and 23.5% of Cpd_5A via LCMS
6'-3	Na ₂ CO ₃	Dioxane	80 °C / 4.5 h	38.1% Cpd_4, 2.42% Cpd_11 and 10.2% Cpd_5 via LCMS
6'-4	K ₂ HPO ₄	Dioxane/H ₂ O	80 °C / 4.5 h	23.0% Cpd_4, 2.99% Cpd_11 and % 56.8Cpd_5 via LCMS
6'-5	KOAc	Dioxane/H ₂ O	80 °C / 4.5 h	25.2% Cpd_4, 9.42% Cpd_11 and 34.8% Cpd_5 via LCMS
6'-6	K ₂ HPO ₄	Dioxane/H ₂ O	80 °C / 16 h	7.15% Cpd_4, 12.5% Cpd_11 and 67.9% Cpd_5 via LCMS
6'-7	NaHCO ₃	Dioxane/H ₂ O	80 °C / 4.5 h	77.5% Cpd_5 via LCMS

Synthesis of Compound (6)

[000858] A general procedure that was used is as follows. To a solution of compound (5) (1440 g, 2.83 mol, 1.00 eq) in DMF (14.4 L) was added KI (281 g, 1.70 mol, 0.60 eq), then added NaN₃ (238 g, 3.68 mol, 1.30 eq). The mixture was stirred at 50 °C for 64 hrs. The reaction was monitored by special LCMS, which showed that compound (5) was consumed completely, and desired mass (Rt = 11.176 min) was detected. The reaction solution was cooled to 20 °C. Work-up was performed in two batches. To the reaction solution was added Na₂CO₃ (5%, 15.0 L) (pH was confirmed to be > 9). The mixture was extracted with EtOAc (8.00 L* 2). Organic phases were combined, washed with Na₂CO₃ (5%, 6.00 L* 2) and brine (6.00 L), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuum to removed EtOAc (bath temperature <30°C). The crude product was purified by column chromatography (SiO₂, petroleum ether/ethyl acetate = 10/1 to 3/1, petroleum ether/ethyl acetate = 5/1, R_f = 0.40). Compound (6) (2660 g, 5.24 mol, 92.6% yield, 97.8% purity) was obtained as yellow oil. The structure and purity were confirmed by HNMR, LCMS, HPLC, and special HPLC.

[000859] LCMS of compound (6): MS cal.: 496.27, MS observed: [M+Na]⁺ =519.2. Special LCMS of compound (6): MS cal.: 496.27, MS observed: [M-Boc+H]⁺ =397.1. HPLC of compound (6): HPLC purity: 96.2% (220 nm). Special HPLC purity: 97.8 % (220 nm)

[000860] HNMR (CDCl₃, 400MHz): δ 7.21 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 7.10 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 2.8 Hz, 1H), 6.76 (dd, J₁ = 8.4 Hz, J₂ = 2.4 Hz, 1H), 5.04 (d, J = 8.0 Hz, 1H), 4.68 - 4.56 (m, 1H), 4.04 (t, J = 5.6 Hz, 2H), 3.74 (s, 3H), 3.39 (t, J = 6.4 Hz, 2H), 3.21 - 3.00 (m, 2H), 2.56 (q, J = 7.6 Hz, 2H), 1.96 - 1.79 (m, 4H), 1.43 (s, 9H), 1.10 (t, J = 7.6 Hz, 3H).

Synthesis of Compound (7)

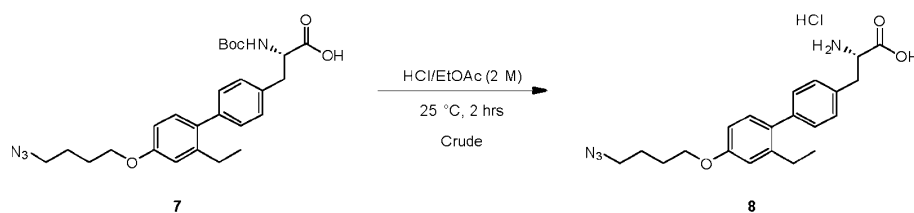
[000861] A general procedure that was used is as follows. To a solution of compound (6) (1880 g, 3.70 mol, 1.00 eq) in THF (13.2 L) and H₂O (5.60 L) was added LiOH·H₂O (310 g, 1.71 mol, 2.00

eq). The mixture was stirred at 25 °C for 3 hrs. The reaction was monitored by LCMS, which showed that compound (6) was consumed completely, and desired mass ($R_t = 0.689$ min) was formed. To the reaction was added ice-water (10.0 L), then 1M HCl was added to adjusted pH to 4 ~ 5. The mixture was extracted with EtOAc (8.00 L * 2), the combined organic phase was washed with H₂O (5.00 L) and brine (5.00 L), dried over anhydrous Na₂SO₄, filtered, and concentrated to give the crude product. The crude product was used into the next step without further purification. Compound (7) (3650 g, 7.37 mol, 99.4% yield, 97.4% purity) was obtained as yellow oil. The structure and purity were confirmed by HNMR, LCMS, and HPLC.

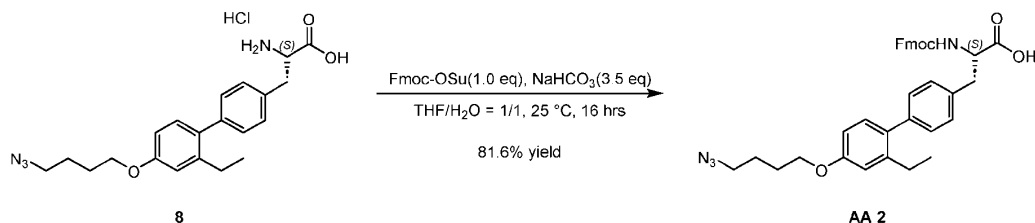
[000862] LCMS for compound (7): MS cal.: 482.25, MS observed: $[M+Na]^+ = 505.2$. HPLC purity: 97.4% (220 nm).

[000863] HNMR (CDCl₃, 400MHz): δ 7.22 (s, 4H), 7.09 (d, $J = 8.4$ Hz, 1H) 6.84 (d, $J = 2.4$ Hz, 1H), 6.75 (dd, $J_1 = 8.4$ Hz, $J_2 = 2.4$ Hz, 1H), 5.02 (d, $J = 6.8$ Hz, 1H), 4.65 - 4.46 (m, 1H), 4.03 (t, $J = 5.6$ Hz, 2H), 3.39 (d, $J = 6.4$ Hz, 2H), 3.26 (dd, $J_1 = 14.0$ Hz, $J_2 = 4.8$ Hz, 1H), 3.17 - 2.88 (m, 1H), 2.56 (q, $J = 7.2$ Hz, 2H), 1.97 - 1.76 (m, 4H), 1.47 - 1.31 (m, 9H), 1.09 (t, $J = 7.2$ Hz, 3H).

Synthesis of Compound (8)



Synthesis of Compound (AA2)

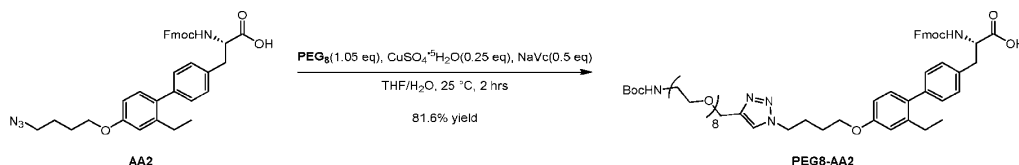


[000867] A general procedure that was used is as follows. To a solution of compound (**8**) (1520 g, 3.55 mol, 97.8% purity, 1.00 eq, HCl) in THF (7.60 L) and H₂O (7.60 L) was added NaHCO₃ (1.04 kg, 12.4 mol, 3.50 eq) and FmocOSu (1.20 kg, 3.55 mol, 1.00 eq). The mixture was stirred at 25 °C for 16 hrs. The reaction was monitored by LCMS which showed that compound (**8**) was consumed completely and the desired mass (Rt = 0.778 min) was formed. The reaction was quenched with H₂O (8.00 L) and 1 M HCl was added to adjust pH to 4 ~ 5. The mixture was extracted with EtOAc (8.00 L * 2) and combined organic phase was washed with brine (4.00 L * 2), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to give the crude product (5.04 kg). The crude product was purified by column chromatography (SiO₂, dichloromethane/ethyl acetate = 1/0 to 1/1, dichloromethane/ethyl acetate = 5/1, R_f = 0.20). **AA2** (3620 g, 5.79 mol, 81.6% yield, 96.8% purity) was obtained as yellow oil. The structure and purity were confirmed by HNMR, Special LCMS, HPLC, special HPLC, and SFC.

[000868] LCMS for **AA2**: MS cal.: 604.27, MS observed: [M+Na]⁺ = 627.1. Special LCMS of **AA2**: MS cal.: 604.27, MS observed: [M-Fmoc+H]⁺ = 383.1. HPLC purity: 96.8% (220 nm). Special HPLC purity: 96.8% (220 nm). SFC of **AA2**: e.e. value %: 100%.

[000869] HNMR (CDCl₃, 400MHz): δ 7.75 (d, J = 7.2 Hz, 2H), 7.62 - 7.50 (m, 2H), 7.38 (t, J = 6.8 Hz, 2H), 7.32 - 7.25 (m, 2H), 7.24 - 6.98 (m, 5H), 6.82 (s, 1H), 6.72 (dd, J₁ = 8.0 Hz, J₂ = 2.0 Hz, 1H), 5.34 (s, 1H), 4.76 (s, 1H), 4.56 - 4.28 (m, 2H), 4.19 (t, J = 7.2 Hz, 1H), 4.02 (t, J = 5.6 Hz, 2H), 3.39 (t, J = 6.4 Hz, 2H), 3.34 - 2.83 (m, 2H), 2.52 (q, J = 7.2 Hz, 2H), 1.99 - 1.78 (m, 4H), 1.06 (t, J = 7.2 Hz, 3H).

Synthesis of Compound (PEG8-AA2)



[000870] A general procedure that was used is as follows. To a solution of **AA2** (1300, 2.08 mol, 1.00 eq), **PEG8** (1140 g, 2.19 mol, 1.05 eq), and NaVc (206 g, 1.04 mol, 0.50 eq) in THF (13.0

L) was added a solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (130 g, 520 mmol, 0.25 eq) in H_2O (3.90 L). The mixture was stirred at 25 °C for 2 hrs. The reaction was monitored by LCMS, which showed that **AA2** was consumed completely, and desired mass ($R_t = 0.705$ min) was formed. Water (15.0 L) was added to the reaction mixture, and it was then extracted with EtOAc (10.0 L * 2). The combined organic phase was washed with HCl (1 M, 6.00 L), EDTA-2Na (5%, 6.00 L * 3), H_2O (6.00 L), and brine (6.00 L), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (SiO_2 , dichloromethane/methanol = 1/0 to 8/1, dichloromethane/methanol = 15/1, $R_f = 0.25$). **PEG8-AA2** (3550 g, 3.16 mol, 75.8% yield, 98.9% purity) was obtained as light brown oil. The structure and purity were confirmed by HNMR, LCMS, special LCMS, HPLC, Special HPLC, SFC, ROI, and KF.

[000871] LCMS for **PEG8-AA2**: MS cal.: 1111.57, MS observed: $[(\text{M-Boc+H})/2]^+ = 506.8$. LCMS of **PEG8-AA2**: MS cal.: 1111.57, MS observed: $[(\text{M-Boc+H})/2]^+ = 506.7$. Special LCMS of **PEG8-AA2**: MS cal.: 1111.57, MS observed: $[(\text{M-Boc+H})/2]^+ = 507.0$. HPLC purity: 100% (220 nm). Special HPLC purity: 98.9% (220 nm). Chiral SFC Report for **PEG8-AA2**: e.e. value %: 100%.

[000872] HNMR (CDCl_3 , 400MHz): δ 7.75 (d, $J = 8.0$ Hz, 2H), 7.63 - 7.52 (m, 3H), 7.38 (t, $J = 7.2$ Hz, 2H), 7.30 (t, $J = 7.2$ Hz, 2H), 7.17 (s, 4H), 7.05 (d, $J = 8.4$ Hz, 1H), 6.80 (d, $J = 2.0$ Hz, 1H), 6.71 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.6$ Hz, 1H), 5.44 (d, $J = 7.6$ Hz, 1H), 5.11 (s, 1H), 4.78 - 4.65 (m, 3H), 4.52 - 4.42 (m, 3H), 4.37 - 4.29 (m, 1H), 4.21 (t, $J = 6.8$ Hz, 1H), 4.01 (t, $J = 5.6$ Hz, 2H), 3.73 - 3.58 (m, 28H), 3.52 (t, $J = 4.8$ Hz, 2H), 3.37 - 3.12 (m, 4H), 2.52 (q, $J = 7.6$ Hz, 2H), 2.20 - 2.10 (m, 2H), 1.87 - 1.77 (m, 2H), 1.44 (s, 9H), 1.06 (t, $J = 7.6$ Hz, 3H).

[000873] The reaction conditions used to prepare **PEG8-AA2** are shown in Scheme 15 and Table 28 below.

Scheme 15

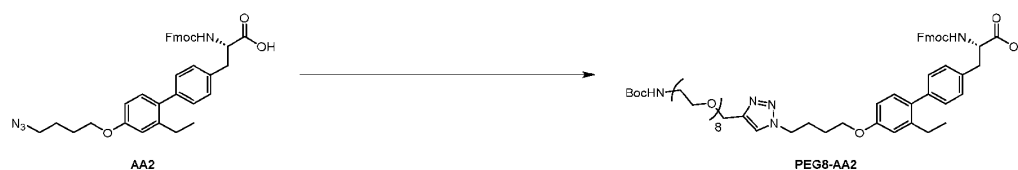


Table 28.

Batch	Eq. t of PEG8	Eq. of NaVc	Eq. of CuSO ₄ .5H ₂ O	Eq. of TBTA	Solvent V/V	Temp. / Time	IPC / LCMS
11'-1	1.10	1.00	1.00	0.50	DMSO/H ₂ O 2/1	25 °C / 40 h	87.2% PEG8-AA2 via LCMS
11'-2	1.10	1.00	1.00	/	DMSO/H ₂ O 1/4	25 °C / 16 h	17.7% AA2 and 70.7% PEG8-AA2 via LCMS
11'-3	1.10	0.50	0.25	/	MeOH/H ₂ O 5/1	25 °C / 16 h	1.45% AA2 and 94.2% PEG8-AA2 via LCMS
11'-4	1.10	0.50	0.25	/	ACN/H ₂ O 3/1	25 °C / 12 h	73.9% AA2 and 24.8% PEG8-AA2 via LCMS
11'-5	1.10	0.50	0.25	/	Acetone/H ₂ O 3/1	25 °C / 12 h	1.64% AA2 and 91.1% PEG8-AA2 via LCMS
11'-6	1.10	0.50	0.25	/	tBuOH/H ₂ O 3/1	25 °C / 12 h	88.0% PEG8-AA2 via LCMS
11'-7	1.10	0.50	0.25	/	THF/H ₂ O 3/1	25 °C / 3 h	97.8% PEG8-AA2 via LCMS

[000874] The synthesis of PEG-Linker is shown in Scheme 16 and Table 29 below

Scheme 16

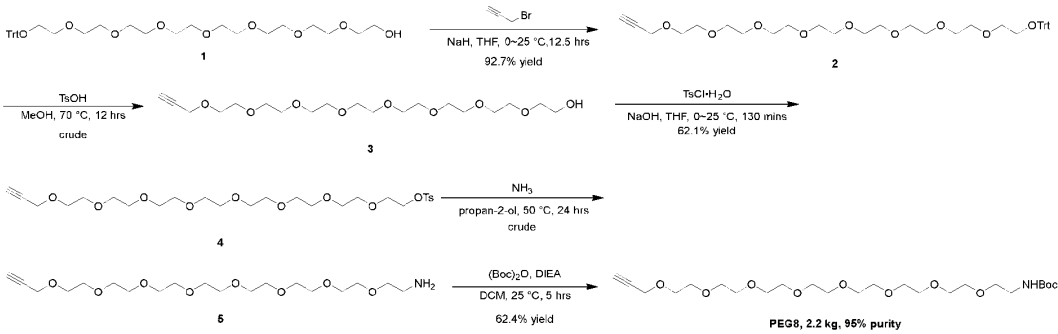
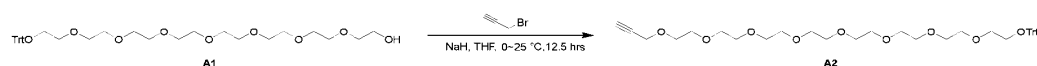


Table 29.

Step	Raw material (Equivalent /Volume)				Condition	IPC	Product
	Reactant 1	Reactant 2	Reagent	Solvent	Temp. / Time	TLC	H NMR
1'	Cpd_1:380 g (1.00 eq)	3-bromoprop- yne:129.1 g (1.4 eq)	NaH: 29.76g (1.2 eq)	THF : 1.9 L	0-25 °C/ 12.5 hrs	DCM: MeOH = 10: 1, I ₂ , Rf = 0.7	368 g (crude), brown oil,
1'-1	Cpd_1:2.00 kg (1.00 eq)	3-bromoprop- yne:679.50 g (1.4 eq)	NaH: 156.66 g (1.2 eq)	THF : 10 L	0-25 °C/ 12.5 hrs	DCM: MeOH = 10: 1, I ₂ , Rf = 0.7	2.0 kg (crude), brown oil,
1'-2	Cpd_1: 3.5 kg (1.00 eq)	3-bromoprop- yne:1.19 Kg (1.4 eq)	NaH: 274.15 g (1.2 eq)	THF : 17.5 L	0-25 °C/ 12.5 hrs	DCM: MeOH = 10: 1, I ₂ , Rf = 0.7	3.5 kg (crude), brown oil,
1'-3	Cpd_1: 3.5 kg (1.00 eq)	3-bromoprop- yne:1.19 Kg (1.4 eq)	NaH: 274.15 g (1.2 eq)	THF : 17.5 L	0-25 °C/ 12.5 hrs	DCM: MeOH = 10: 1, I ₂ , Rf = 0.7	3.5 kg (crude), brown oil,
2'	Cpd_2:346 g (1.00 eq)	TsOH.H ₂ O: 50.57 g (0.5 eq)	N/A	MeOH: 1.73 L	70 °C/ 12 hrs	DCM: MeOH = 10: 1, I ₂ , Rf = 0.5	374 g, (crude), off-white solid

2'-1	Cpd_2: 2.0 kg (1.00 eq)	TsOH.H ₂ O: 288.19 g (0.5 eq)	N/A	MeOH : 10.0 L	70 °C/ 12 hrs	DCM: MeOH = 10: 1, l ₂ , Rf = 0.5	2.0 kg (crude), off-white oil
2'-2-1	Cpd_2: 3.5 kg (1.00 eq)	TsOH.H ₂ O: 503.28 g (0.5 eq)	N/A	MeOH : 17.5 L	70 °C/ 12 hrs	DCM: MeOH = 10: 1, l ₂ , Rf = 0.5	2.5 kg (crude), black brown oil
2'-2-2	Cpd_2: 3.5 kg (1.00 eq)	TsOH.H ₂ O: 503.28 g (0.5 eq)	N/A	MeOH : 17.5 L	70 °C/ 12 hrs	DCM: MeOH = 10: 1, l ₂ , Rf = 0.5	2.5 kg (crude), black brown oil
3'	Cpd_3: 240 g (1.00eq)	NaOH: 94 g (4.0 eq)	TsCl 280 g (2.5 eq)	THF: 2.5 L	0~20 °C/ 130 min	DCM: MeOH = 10: 1, PMA, Rf = 0.43	230 g (98.64% purity)
3'-1	Cpd_3: 1240 g (1.00eq)	NaOH: 485.67 g (4.0 eq)	TsCl 1.45 kg (2.5 eq)	THF: 12.957 L	0~20 °C/ 130 min	DCM: MeOH = 10: 1, PMA, Rf = 0.6	1210 g (98.8% purity) brown oil
3'-2-1	Cpd_3: 2170 g (1.00eq)	NaOH: 847.96 g (4.0 eq)	TsCl 2.53 kg (2.5 eq)	THF: 22.670 L	0~25 °C/ 130 min	DCM: MeOH = 10: 1, PMA, Rf = 0.6	1880 g (98.6% purity) Black brown oil
3'-2-2	Cpd_3: 2170 g (1.00eq)	NaOH: 847.96 g (4.0 eq)	TsCl 2.53 kg (2.5 eq)	THF: 22.670 L	0~25 °C/ 130 min	DCM: MeOH = 10: 1, PMA, Rf = 0.6	1880 g (98.6% purity) Black brown oil
4'	Cpd_4: 230 g (1.00eq)	NH ₃ /MeOH 1150 MI (7 M, 20 eq)	N/A	N/A	50 °C/ 24 hrs	DCM: MeOH = 10: 1, l ₂ , Rf = 0.1	250 g (crude) yellow oil
4'-1	Cpd_4: 1.21 kg (1.00eq)	NH ₃ /MeOH 1150 MI (7 M, 19.75 eq)	N/A	N/A	50 °C/ 24 hrs	DCM: MeOH = 10: 1, l ₂ , Rf = 0.1	1.2 kg (crude) brown oil
4'-2-1	Cpd_4: 1200 g (1.00eq)	NH ₃ : 409 g (11.42eq)	N/A	Propan-2-ol: 1600 mL	50 °C/ 24 hrs	DCM: MeOH = 10: 1, l ₂ , Rf = 0.1	856.6 g (crude) black brown oil
4'-2-2	Cpd_4: 1200 g (1.00eq)	NH ₃ : 409 g (11.42eq)	N/A	Propan-2-ol: 1600 mL	50 °C/ 24 hrs	DCM: MeOH = 10: 1, l ₂ , Rf = 0.1	856.6 g (crude) black brown oil
4'-2-3	Cpd_4: 1200 g (1.00eq)	NH ₃ : 409 g (11.42eq)	N/A	Propan-2-ol: 1600 mL	50 °C/ 24 hrs	DCM: MeOH = 10: 1, l ₂ , Rf = 0.1	856.6 g (crude) black brown oil
5'-1	Cpd_5: 700 g (1.00eq)	N/A	N/A	N/A	N/A	DCM: MeOH = 10: 1, l ₂ , Rf = 0.43	230 g (99.1% purity)
5'-2	Cpd_5: 2570 g (1.00eq)	Boc ₂ O: 4.13 kg (3.0 eq)	DIEA: 3.26 g (4.00eq)	DCM: 11.8 L	25 °C/ 5 hrs	DCM: MeOH = 10: 1, l ₂ , Rf = 0.50	2.0 kg (98.2% purity)

Synthesis of Compound (A2)

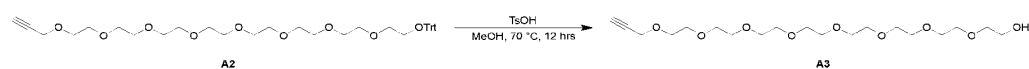


[000875] A general procedure that was used is as follows. To a mixture of compound **A1** (3.50 kg, 5.71 mol, 1.00 eq) in THF (17.5 L) was added NaH (274 g, 6.85 mol, 60% purity, 1.20 eq) in portions at 0 °C. The mixture stirred at 0 °C for 0.5 h and then 3-bromoprop-1-yne (1.19 kg, 8.00 mol, 861 mL, 80% purity, 1.40 eq) was added to the reaction at 0 °C and the mixture stirred at 25 °C for 12 hrs. The reaction was monitored by TLC (dichloromethane: methanol = 10/ 1). TLC indicated that compound **A1** (R_f = 0.60) was consumed completely and one new spot (R_f = 0.70) formed. The reaction mixture was quenched with ice water (10.0 L) at 0 °C, and then extracted with EtOAc (5.00 L * 3). The combined organic layers were washed with brine (5.00 L * 2), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. Two batches were combined after work up. The crude product was used to next step without any purification (according to the HNMR, the

product contained some mineral oil which did not affect the next reaction). Compound **A2** (7.00 kg, 10.5 mol, 98.5% purity) was obtained as black brown oil. The structure and purity were confirmed by LCMS and HPLC.

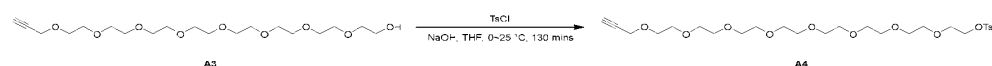
[000876] LCMS for compound **A2**: MS cal.: 650.35, MS observed: $[M+H_2O]^+ = 668.3$. HPLC purity: 98.5%.

Synthesis of Compound (A3)



[000877] A general procedure that was used is as follows. To a solution of compound **A2** (3.50 kg, 5.30 mol, 98.5% purity, 1.00 eq) in MeOH (17.5 L) was added TsOH•H₂O (503 g, 2.65 mol, 0.50 eq). The mixture was stirred at 70 °C for 12 hrs. The reaction was monitored by TLC (dichloromethane: methanol = 10/ 1). TLC indicated that compound **A2** (R_f = 0.60) was consumed completely and new spots (R_{f1} = 0.50, R_{f2} = 0.80) were observed. K₂CO₃ (500 g) was added to the reaction mixture, and it was concentrated under reduced pressure to give a residue. The residue was dissolved in DCM (10.0 L), the mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. Two batches were combined after work up. The crude product was used to next step without any purification. Compound **A3** (5.00 kg, crude) was obtained as black brown oil.

Synthesis of Compound (A4)

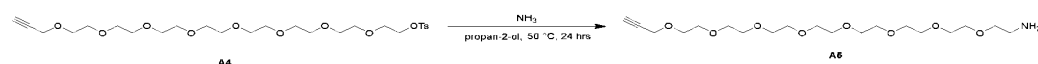


[000878] A general procedure that was used is as follows. To a solution of compound **A3** (2.17 kg, 5.30 mol, 1.00 eq) in THF (18.3 L) was added NaOH (847 g, 21.2 mol, 4.00 eq) at 0 °C over 10 min, then 4-methylbenzenesulfonyl chloride (2.53 kg, 13.2 mol, 2.50 eq) in THF (4.37 L) was added the reaction mixture at 0 °C. The mixture was stirred at 25 °C for 2 hrs. The reaction was monitored by LC-MS, which showed that compound **A3** was consumed completely and one main peak with desired m/z (R_t = 0.532 min) was observed. The reaction mixture was quenched by addition of H₂O (5.00 L) at 25°C and extracted with EtOAc (5.00 mL * 2). The combined organic layers were washed with brine (5.00 L * 2), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. Two batches were combined after work up. The residue was purified by column chromatography (SiO₂, dichloromethane: methanol = 1/ 0 to 10/ 1, dichloromethane: methanol = 10/ 1, R_f = 0.60). Compound **A4** (3.76 kg, 6.59 mol, 98.6% purity) was obtained as black brown oil. The structure and purity were confirmed by H NMR, HPLC, and LCMS.

[000879] LCMS: MS cal.: 562.24, MS observed: $[M+H]^+ = 563.2$. HPLC: 98.69% purity (220 nm).

[000880] HNMR (CDCl₃, 400 MHz): δ 7.77 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 4.17 (d, J = 8.0 Hz, 2H), 4.10 (t, J_1 = 4.0 Hz, J_2 = 8.0 Hz, 2H), 3.67 - 3.59 (m, 30H), 2.42 (s, 4H).

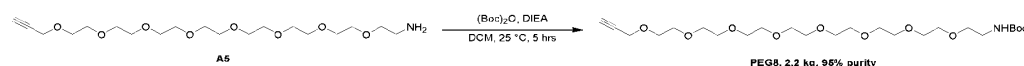
Synthesis of Compound (A5)



[000881] A general procedure that was used is as follows. A solution of compound **A4** (1.20 kg, 2.10 mol, 98.6% purity, 1.00 eq) in propan-2-ol (6.00 L) saturated with NH₃ (409 g, 24.0 mol, 11.4 eq) (gas: NH₃) was stirred under 15 Psi at 50 °C for 24 hrs in a 10.0 L of sealed tube or autoclave. The reaction was monitored by TLC (dichloromethane: methanol = 10/ 1). TLC indicated that compound **A4** (R_f = 0.50) was consumed completely and new spots (R_{f1} = 0.30, R_{f2} = 0) were formed. The reaction mixture was concentrated under reduced pressure to give a residue. Three batches were combined after work up. The crude product was used to next step without any purification. Compound **A5** (2.57 g, crude) was obtained as black brown oil.

[000882] NH₃/isopropanol was used instead of NH₃/MeOH to avoid the formation of a byproduct in which OTs group was methoxylated. The crude product which contained TsOH was used for next step without further purification.

Synthesis of PEG8



[000883] A general procedure that was used is as follows. To a solution of compound **A5** (2.57 kg, 6.31 mol, 1.00 eq) in DCM (11.8 L) was added DIEA (3.26 kg, 25.2 mol, 4.39 L, 4.00 eq) and (Boc)₂O (4.13 kg, 18.9 mol, 4.35 L, 3.00 eq). The mixture was stirred at 25 °C for 5 hrs. The reaction was monitored by TLC (dichloromethane: methanol = 10/ 1). TLC indicated that compound **A5** (R_f = 0.20) was consumed completely and new spots (R_{f1} = 0.50, R_{f2} = 0.40) were formed. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, dichloromethane: methanol = 1/ 0 to 80/ 1, dichloromethane: methanol = 10/ 1, R_f = 0.50). Compound **PEG8** (2.00 kg, 3.94 mol) was obtained in 62.4% yield. The structure and purity were confirmed by HNMR and LCMS-CAD.

[000884] MS cal.: 507.30, MS observed: $[M+H_2O]^+ = 525.4$. LCMS-CAD: 98.22% purity (220 nm).

[000885] HNMR (CDCl₃, 400 MHz): δ : 5.03 (s, 1H), 4.18 (d, J = 2.4 Hz, 2H), 3.69 - 3.60 (m, 28H), 3.52 (t, J = 5.2 Hz, 2H), 3.31- 3.28 (m, 2H), 2.42 (t, J = 2.4 Hz, 1H), 1.42 (s, 9H).

[000886] Figure 15 shows an alternate process for making **AA2** and **AA2+Linker**. The following paragraphs provide the detailed synthetic procedure and characterization of each intermediate that together form the product AA2-Linker:

[000887] **Step a:** To a solution of 3-ethylphenol (50.0 g, 409 mmol, 1.0 eq.) in DCM (150 mL) and MeOH (100 mL) was added tetrabutylammonium (205 g, 426 mmol, 1.04 eq.) portions by portions. The mixture was stirred at 20 °C for 2 hrs. TLC (PE/ EtOAc = 5/1) showed new spots were detected. The reaction mixture was concentrated under reduced pressure, then quenched by H₂O (1 L) and extracted with EtOAc (1 L x 2). The combined organic layers were washed with HCl (1 M, 1 L x 2) and brine (1 L x 2), and dried over Na₂SO₄. The organic layer was filtered and concentrated under reduced pressure to give (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(4-iodophenyl)propanoic acid (76.0 g, 92.4% yield) as yellow oil. (76.0 g used for next step directly). H NMR:ES27762-110-P1A (CDCl₃, 400 MHz); δ: 7.37 (d, *J* = 8.8 Hz, 1H), 6.74 (d, 1H, *J* = 3.2 Hz), 6.57 (dd, *J* = 3.2 Hz, 1H), 4.76 (s, 1 H), 2.69 (q, *J* = 8.0 Hz, 2H), 1.22 (t, *J* = 8.0 Hz, 3H).

[000888] **Step b:** The mixture of 4-bromo-3-ethyl-phenol (10 g, 49.7 mmol, 1.0 eq.), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (14.5 g, 57.2 mmol, 1.15 eq.), KOAc (9.76 g, 99.5 mmol, 2.0 eq.), PCy₃ (558 mg, 1.99 mmol, 0.04 eq.), Pd(OAc)₂ (223 mg, 1.00 mmol, 0.02 eq.) in dioxane (200 mL) was stirred at 90 °C for 4 hrs under N₂ atmosphere. LCMS showed desired mass was detected. TLC (PE/EtOAc = 10/1) showed new spots were detected. The mixture was filtered and washed with EtOAc (100 mL X 2) to give the organic layer. The organic layer was concentrated to give a residue. The residue was purified by flash silica gel chromatography (ISCO®; 330 g SepaFlash® Silica Flash Column, Eluent of 0~10% Ethyl acetate/Petroleum ether gradient @ 100 mL/min) to give 3-ethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (11.0 g, 37.7 mmol, 85% purity, 75.8% yield) as brown oil. HNMR:ES27762-102-P1A (CDCl₃, 400 MHz): δ 7.69 (d, 1H, *J* = 8.0 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H), 6.62-6.65 (m, 1H), 4.98 (s, 1H), 2.88 (q, *J* = 7.2 Hz, 2H), 1.34 (s, 12H), 1.19 (t, *J* = 7.2 Hz, 3H); LCMS: *m/z* = 249.1 [M+H]⁺.

Batch	Cpd 2	Solvent	Pd/Ln	Time (h)	Temp. (°C)	Conv.%
1	500 mg	THF (2.5 mL)	Pd(OAc) ₂ (0.01 eq.), PCy ₃ (0.02 eq.)	3	45	20
2	200 mg	Dioxane (2 mL)	Pd(OAc) ₂ (0.02 eq.), PCy ₃ (0.04 eq.)	3	90	100

3	200 mg	Dioxane (2 mL)	Pd ₂ dba ₃ (0.02 eq.) PCy ₃ (0.04 eq.)	3	90	82
4	200 mg	Dioxane (2 mL)	Pd(PPh ₃) ₂ Cl ₂	3	90	57.8
5	10 g	Dioxane (200 mL)	Pd(OAc) ₂ (0.02 eq.), PCy ₃ (0.04 eq.)	4	90	100

[000889] **Step c:** To a mixture of 3-ethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (10.3 g, 41.7 mmol, 1.2 eq.), 4-azidobutan-1-ol (4.00 g, 34.7 mmol, 1 eq.), Molecular sieve 4A (3.83 g, 8.69 mmol, 0.25 eq.) in THF (20 mL) was added DIAD (8.43 g, 41.69 mmol, 8.08 mL, 1.2 eq.) at 20 °C. The mixture was stirred at 20 °C for 0.5 h. The solution of PPh₃ (10.93 g, 41.69 mmol, 1.2 eq.) was added dropwise at 0-5 °C. The mixture was stirred at 20 °C for 3 hrs. LCMS showed desired mass was detected. To the mixture was added H₂O (50 mL), and the mixture was extracted with EtOAc (50 mL X 3) to give the organic layer. The organic layer was washed with brine (20 mL), dried over Na₂SO₄, filtered, and concentrated to afford the residue. The residue was purified by flash silica gel chromatography (ISCO®; 330 g SepaFlash® Silica Flash Column, Eluent of 0~10% Ethyl acetate/Petroleum ether gradient @ 100 mL/min) to give the 2-(4-(4-azidobutoxy)-2-ethylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (4.31 g, 12.5 mmol, 35.9% yield) as yellow oil. H NMR: ES27762-108-P1B (CDCl₃, 400 MHz) δ 7.73 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 2.4 Hz, 1H), 6.70 (dd, J = 2.4, 8.4 Hz, 1H), 4.02 (t, J = 5.6 Hz, 2H), 3.37 (t, J = 6.8 Hz, 2H), 2.90 (q, J = 7.2 Hz, 2H), 1.79-1.90 (m, 4H), 1.34 (s, 12H), 1.20 (t, J = 7.2 Hz, 3H). LCMS: ES27762-108-P1A m/z = 318.1 [M-N₂+H]⁺. 2D NMR was obtained to confirm the identity of the product as the correct regio-isomer.

Batch	4-azidobutan-1-ol	THF (mL)	Reagent	Temp. (°C)	Yield %	Note
1	1 g	8	Na ₂ SO ₄ (8 eq.)	20	30	The addition of PPh ₃ solid makes the reaction temp. difficult to be controlled.

2	500 mg	10	Na ₂ SO ₄ (8 eq.)	0-20	N/A	The solution of PPh ₃ in THF was added.
3	500 mg	10	molecular sieves, 4Å (0.25 eq.)	0-20	48.8	The solution of PPh ₃ in THF was added.
4	4 g	40	molecular sieves, 4Å (0.25 eq.)	0-20	35.9	The solution of PPh ₃ in THF was added.

[000890] **Step d:** To a mixture of 2-[4-(4-azidobutoxy)-2-ethyl-phenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (200 mg, 579 μ mol, 1.0 eq.), (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(4-iodophenyl)propanoic acid (327.11 mg, 637.23 μ mol, 1.1 eq.), K₂CO₃ (160 mg, 1.16 mmol, 2.0 eq.) in H₂O /isopropyl alcohol (2 mL, 1/4) was added Pd(dppf)Cl₂-CH₂Cl₂ (47.31 mg, 57.93 μ mol, 0.1 eq) under N₂ atmosphere. The mixture was stirred at 50 °C for 12 hrs. LCMS showed desired mass was detected. TLC (DCM/MeOH = 10/1) showed new spots were detected. The mixture was added to H₂O (2 mL), adjusted with sat. citric acid to pH ~5, extracted with EtOAc (5 mL X 3) to give the organic layer. The organic layer was washed with brine (2 mL), dried over Na₂SO₄, filtered, and concentrated to give the residue. The residue was purified by prep-TLC (SiO₂, DCM/MeOH = 10/1) to give (2S)-3-[4-[4-(4-azidobutoxy)-2-ethyl-phenyl]phenyl]-2-(9H-fluoren-9-ylmethoxycarbonylamino)propanoic acid (80 mg, 132.30 μ mol, 22.84% yield) as yellow solid. H NMR: ES27762-104-P1H (CD₃OD, 400 MHz) δ 7.76 (d, J = 7.2 Hz, 2H), 7.60-7.70 (m, 2H), 7.30-7.48 (m, 2H), 7.25-7.28 (m, 4H), 7.10 (d, J = 7.6 Hz, 2H), 6.90 (d, J = 8.0 Hz, 1H), 6.78 (d, J = 4.0 Hz, 1H), 6.69 (dd, J = 2.4, 8.0 Hz, 1H), 4.42-4.50 (m, 1H), 4.30-4.40 (m, 1H), 4.10-4.20 (m, 2H), 3.99 (t, J = 6.0 Hz, 2H), 3.37 (t, J = 6.6 Hz, 2H), 3.20-3.30 (m, 1H), 2.90-3.00 (m, 1H), 2.44 (q, J = 1.8, 7.4 Hz, 2H), 1.70-1.90 (m, 4H), 0.94 (t, J = 7.6 Hz, 3H).

[000891] **Step e:** **5a** (2.0 eq), HOVc (1.8 eq.), CuSO₄.5H₂O (1.28 eq.), and THF (30 V) are combined at 100 °C for 1 h, with microwave. The general procedure that was used is the same as described above. To a solution of **AA2** (1300 g, 2.08 mol, 1.00 eq), **PEG8** (1140 g, 2.19 mol, 1.05 eq), and NaVc (206 g, 1.04 mol, 0.50 eq) in THF (13.0 L) was added a solution of CuSO₄.5H₂O (130 g, 520 mmol, 0.25 eq) in H₂O (3.90 L). The mixture was stirred at 25 °C for 2 hrs. The reaction was monitored by LCMS, which showed that **AA2** was consumed completely, and desired mass (Rt = 0.705 min) was formed. Water (15.0 L) was added to the reaction mixture, and it was then extracted with EtOAc (10.0 L * 2). The combined organic phase was washed with HCl (1 M, 6.00 L), EDTA-

2Na (5%, 6.00 L * 3), H₂O (6.00 L), and brine (6.00 L), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (SiO₂, dichloromethane/methanol = 1/0 to 8/1, dichloromethane/methanol = 15/1, R_f = 0.25). **PEG8-AA2** (3550 g, 3.16 mol, 75.8% yield, 98.9% purity) was obtained as light brown oil. The structure and purity were confirmed by HNMR, LCMS, special LCMS, HPLC, Special HPLC, SFC, ROI, and KF.

[000892] LCMS for **PEG8-AA2**: MS cal.: 1111.57, MS observed: [(M-Boc+H)/2]⁺ = 506.8. LCMS of **PEG8-AA2**: MS cal.: 1111.57, MS observed: [(M-Boc+H)/2]⁺ = 506.7. Special LCMS of **PEG8-AA2**: MS cal.: 1111.57, MS observed: [(M-Boc+H)/2]⁺ = 507.0. HPLC purity: 100% (220 nm). Special HPLC purity: 98.9% (220 nm). Chiral SFC Report for **PEG8-AA2**: e.e. value %: 100%.

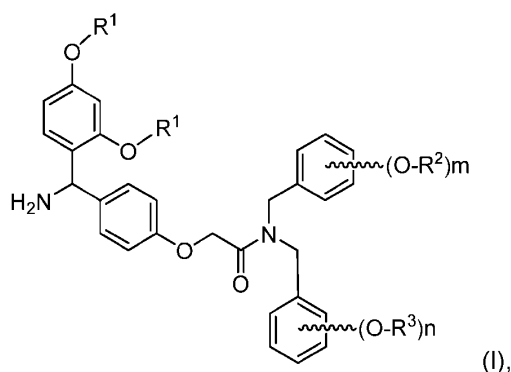
[000893] HNMR (CDCl₃, 400MHz): δ 7.75 (d, J = 8.0 Hz, 2H), 7.63 - 7.52 (m, 3H), 7.38 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 7.2 Hz, 2H), 7.17 (s, 4H), 7.05 (d, J = 8.4 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.71 (dd, J₁ = 8.4 Hz, J₂ = 1.6 Hz, 1H), 5.44 (d, J = 7.6 Hz, 1H), 5.11 (s, 1H), 4.78 - 4.65 (m, 3H), 4.52 - 4.42 (m, 3H), 4.37 - 4.29 (m, 1H), 4.21 (t, J = 6.8 Hz, 1H), 4.01 (t, J = 5.6 Hz, 2H), 3.73 - 3.58 (m, 28H), 3.52 (t, J = 4.8 Hz, 2H), 3.37 - 3.12 (m, 4H), 2.52 (q, J = 7.6 Hz, 2H), 2.20 - 2.10 (m, 2H), 1.87 - 1.77 (m, 2H), 1.44 (s, 9H), 1.06 (t, J = 7.6 Hz, 3H).

[000894] As various changes may be made in the above-described subject matter without departing from the scope and spirit of the present disclosure, it is intended that all subject matter contained in the above description, or defined in the appended claims, be interpreted as descriptive and illustrative of the present disclosure. Many modifications and variations of the present disclosure are possible in light of the above teachings. Accordingly, the present description is intended to embrace all such alternatives, modifications, and variances which fall within the scope of the appended claims.

[000895] All patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference in their entirety as if physically present in this specification.

WHAT IS CLAIMED IS:

1. A compound having a structure of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R¹ is a C₁₋₅ alkyl;

R² is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

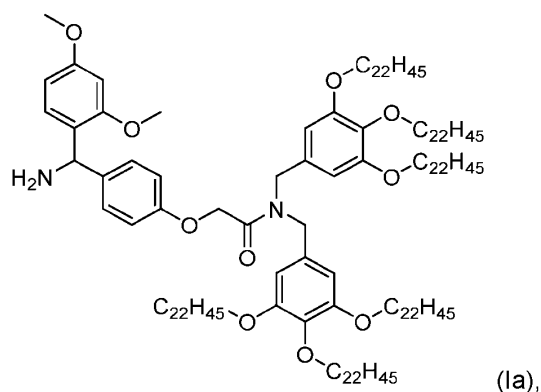
R³ is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,
or a salt thereof.

2. The compound of claim 1, wherein R¹ is methyl.

3. The compound of claim 1, wherein R² is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl, and R³ is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl.

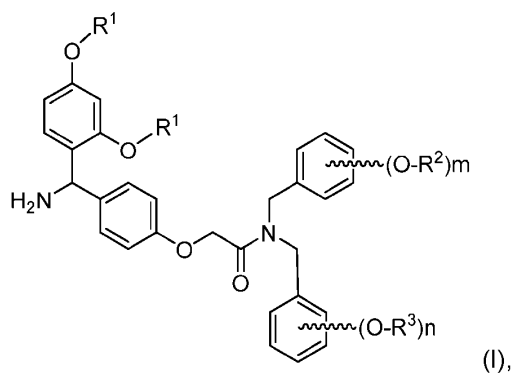
4. The compound of claim 1, wherein each R² is independently an optionally substituted C₂₂ alkyl and each R³ is independently an optionally substituted C₂₂ alkyl.

5. The compound of claim 1, wherein the compound has the structure of Formula (Ia):



or a salt thereof.

6. A process for preparation of a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R^1 is a C_{1-5} alkyl;

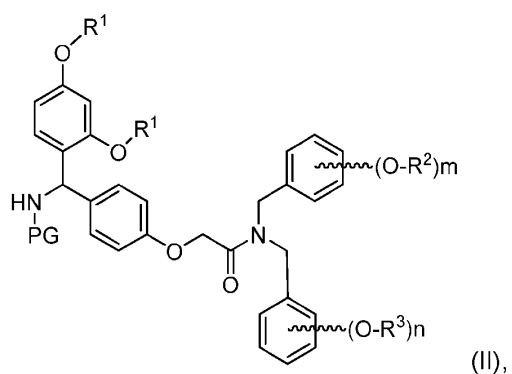
R^2 is independently at each occurrence an optionally substituted C_{10-30} alkyl; and

R^3 is independently at each occurrence an optionally substituted C_{10-30} alkyl,

or a salt thereof,

said process comprising:

(a) providing a compound of Formula (II) having the structure:



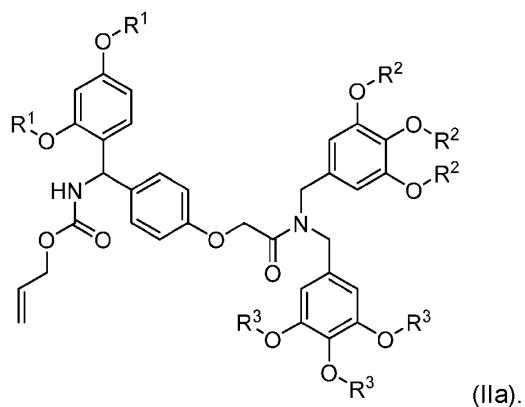
wherein

PG is a suitable protecting group; and

(b) forming the compound of Formula (I) from the compound of Formula (II).

7. The process according to claim 6, wherein PG is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

8. The process according to claim 6, wherein the compound of Formula (II) has the structure of Formula (IIa):

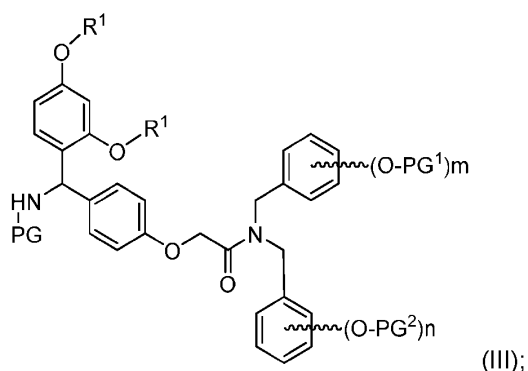


9. The process according to claim 6, wherein said step (b) of forming the compound of Formula (I) comprises:

reacting the compound of Formula (II) with a protecting group removing agent to produce the compound of Formula (I).

10. The process according to claim 9, wherein the protecting group removing agent is selected from the group consisting of Pd(PPh)_3 , PhSiH_3 , H_2 , piperidine, and trifluoroacetic acid (TFA).

11. The process according to claim 6 further comprising the steps of:
providing a compound of Formula (III) having the structure:



wherein

PG^1 is a suitable protecting group;

PG^2 is a suitable protecting group; and

forming the compound of Formula (II) from the compound of Formula (III) prior to the step (a).

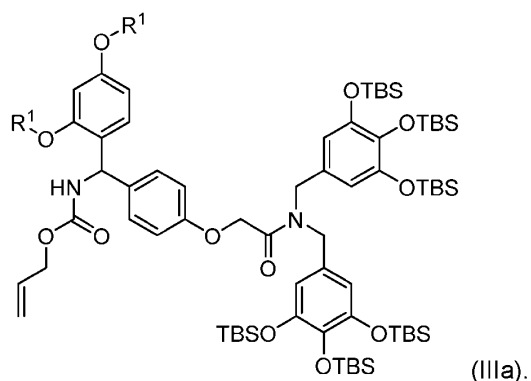
12. The process according to claim 11, wherein PG^1 is independently selected at each occurrence from the group consisting of tert-butyldimethylsilyl (TBS), trimethylsilyl (TMS), triethylsilyl (TES), tert-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

13. The process according to claim 11, wherein PG^2 is independently selected at each occurrence from the group consisting of tert-butyldimethylsilyl (TBS), trimethylsilyl (TMS), triethylsilyl (TES), tert-butyldiphenylsilyl (TBDPS), and triisopropylsilyl (TIPS).

14. The process according to claim 11, wherein each PG^1 and each PG^2 are the same.

15. The process according to claim 11, wherein PG^1 and PG^2 are different from each other.

16. The process according to claim 11, wherein the compound of Formula (III) has the structure of Formula (IIIa):



17. The process according to claim 11, wherein said step of forming the compound of Formula (II) prior to step (a) comprises:

reacting the compound of Formula (III) with a protecting group removal agent to obtain a deprotected intermediate;

reacting said intermediate with at least one compound of Formula (IV):

R-LG (IV),

wherein

R is an optionally substituted C_{10-30} alkyl; and

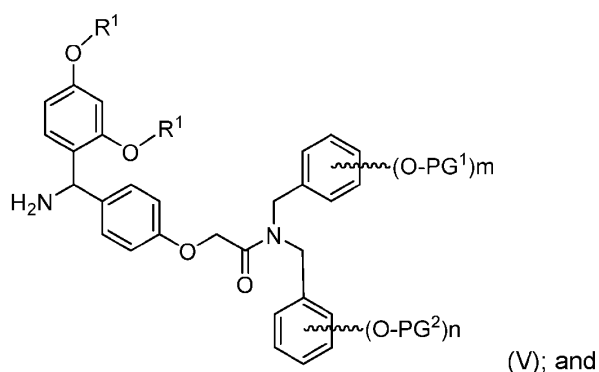
LG is a suitable leaving group;

to produce the compound of Formula (II).

18. The process according to claim 17, wherein LG is selected from a group consisting of halogen, OTf , OMs , and OTs .

19. The process according to claim 17, wherein the protecting group removal agent is selected from the group consisting of tetrabutylammonium fluoride (TBAF), NH_4F , SiF_4 , AcOH , HCl , LiAlH_4 , and K_2CO_3 .

20. The process according to claim 11 further comprising the steps of:
providing a compound of Formula (V) having the structure:



forming the compound of Formula (III) from the compound of Formula (V).

21. The process according to claim 20, wherein said step of forming the compound of Formula (III) comprises:

reacting the compound of Formula (V) with a compound of Formula (VIa) or Formula (VIb):
PG-X (VIa) or PG₂O (VIb),

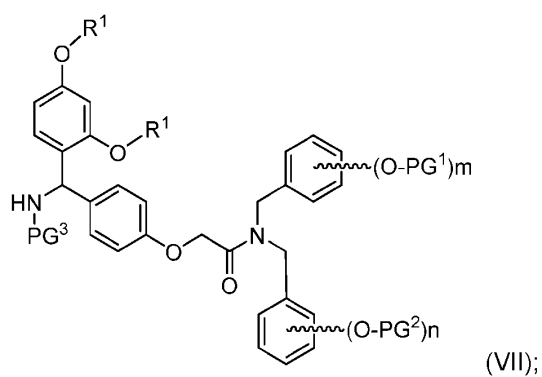
wherein

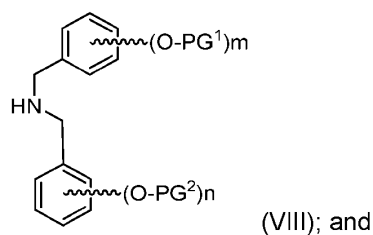
X is Cl or Br;

to produce the compound of Formula (III).

22. The process according to claim 21, wherein the compound of Formula (VIa) or Formula (VIb) is selected from the group consisting of AllocCl, Alloc₂O, Cbz₂O, CbzCl, FmocCl, and Boc₂O.

23. The process according to claim 20 further comprising:
providing a compound of Formula (VII) having the structure:

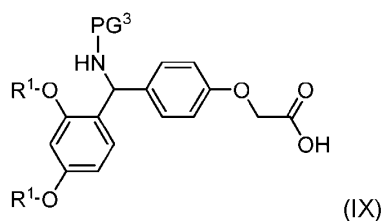




forming the compound of Formula (VII) from the compound of Formula (VIII).

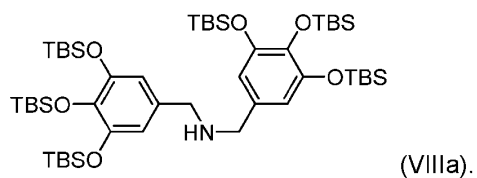
29. The process according to claim 28, wherein said step of forming the compound of Formula (VII) comprises:

reacting the compound of Formula (VIII) with a compound of Formula (IX) having the structure:

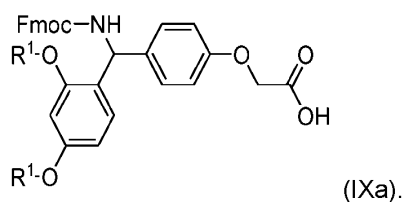


to produce the compound of Formula (VII).

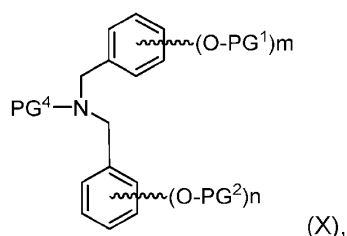
30. The process according to claim 28, wherein the compound of Formula (VIII) has the following structure:



31. The process according to claim 29, wherein the compound of Formula (IX) has the following structure:



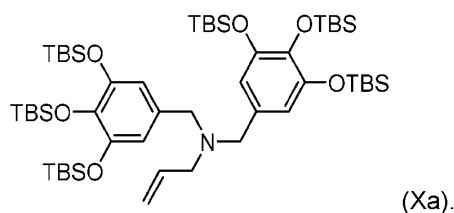
32. The process according to claim 28 further comprising:
providing a compound of Formula (X) having the structure:



wherein PG⁴ is a suitable protecting group; and
forming the compound of Formula (VIII) from the compound of Formula (X).

33. The process according to claim 32, wherein PG⁴ is selected from the group consisting of allyl, allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

34. The process according to claim 32, wherein the compound of Formula (X) has the following structure:

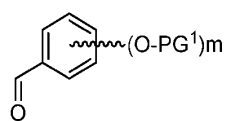


35. The process according to claim 32, wherein said step of forming the compound of Formula (VIII) comprises:

reacting the compound of Formula (X) with a protecting group removing agent.

36. The process according to claim 35, wherein the protecting group removing agent is selected from the group consisting of Pd/K₂CO₃, 3-dimethylbarbituric acid/Pd(PPh₃)₄, Pd(PPh₃)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

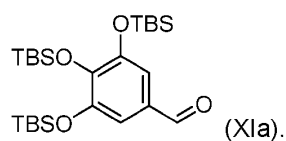
37. The process according to claim 32 further comprising:
providing a compound of Formula (XI) having the structure:



(XI); and

forming the compound of Formula (X) from the compound of Formula (XI).

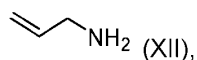
38. The process according to claim 37, wherein the compound of Formula (XI) has the following structure:



(XIa).

39. The process according to claim 37, wherein said step of forming the compound of Formula (X) comprises:

reacting the compound of Formula (XI) with a compound of Formula (XII):

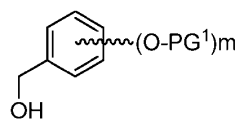


(XII),

or a salt thereof,

to produce the compound of Formula (X).

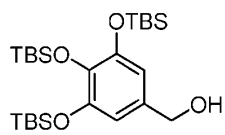
40. The process according to claim 37 further comprising:
providing a compound of Formula (XIII) having the structure:



(XIII); and

forming the compound of Formula (XI) from the compound of Formula (XIII).

41. The process according to claim 40, wherein the compound of Formula (XIII) has the following structure:



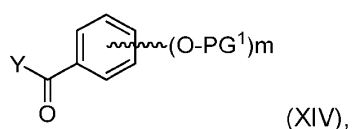
(XIIIa).

42. The process according to claim 40, wherein said step of forming the compound of Formula (XI) comprises:

reacting the compound of Formula (XIII) with an oxidizing agent to produce the compound of Formula (XI).

43. The process according to claim 42, wherein the oxidizing agent is selected from the group consisting of pyridinium chlorochromate (PCC), pyridinium dichromate (PDC), silver carbonate (Ag_2CO_3), tetra-n-propylammonium perruthenate (TPAP), and Dess-Martin periodinane (DMP).

44. The process according to claim 40 further comprising:
providing a compound of Formula (XIV) having the structure:

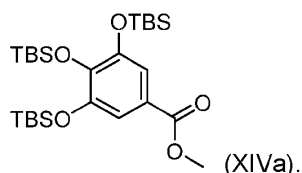


wherein

Y is $-\text{OC}_{1-6}$ alkyl; and

forming the compound of Formula (XIII) from the compound of Formula (XIV).

45. The process according to claim 44, wherein the compound of Formula (XIV) has the following structure:



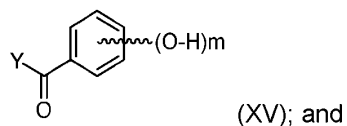
46. The process according to claim 44, wherein said step of forming the compound of Formula (XIII) comprises:

reacting the compound of Formula (XIV) with a reducing agent.

47. The process according to claim 46, wherein the reducing agent is selected from the group consisting of LiAlH_4 , DIBALH, and LiBH_4 .

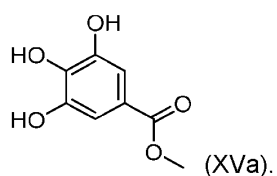
48. The process according to claim 44 further comprising:

providing a compound of Formula (XV) having the structure:



forming the compound of Formula (XIV) from the compound of Formula (XV).

49. The process according to claim 48, wherein the compound of Formula (XV) has the following structure:



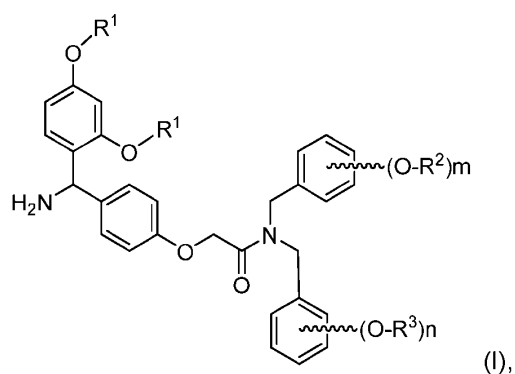
50. The process according to claim 48, wherein said step of forming the compound of Formula (XIV) comprises:

reacting the compound of Formula (XV) with a protecting group introducing agent to produce the compound of Formula (XIV).

51. The process according to claim 50, wherein the protecting group introducing agent is selected from the group consisting of TBSCl, TBSOTf, TMSCl, TMSOTf, TESCl, TESOTf, TBDPSCl, TBDPSOTf, TIPSCl, and TIPSOTf.

52. A product prepared by the method according to any one of claims 6-51.

53. The product of claim 52, wherein the product is a compound of Formula (I):



wherein:

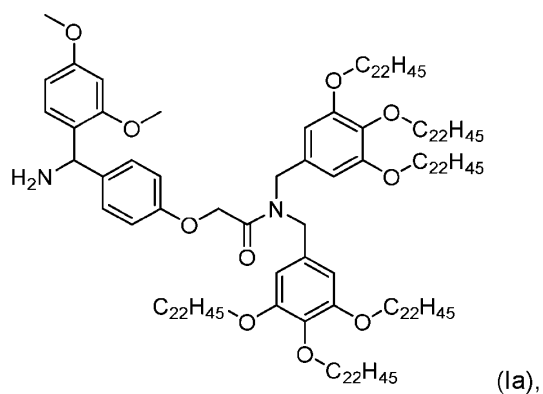
m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R^1 is a C_{1-5} alkyl;

R^2 is independently at each occurrence an optionally substituted C_{10-30} alkyl; and

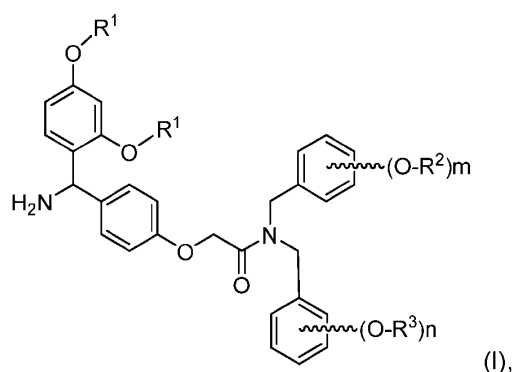
R^3 is independently at each occurrence an optionally substituted C_{10-30} alkyl,
or a salt thereof.

54. The product of claim 53, wherein the product is a compound of Formula (Ia):



or a salt thereof.

55. A method of using a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R^1 is a C_{1-5} alkyl;

R^2 is independently at each occurrence an optionally substituted C_{10-30} alkyl; and

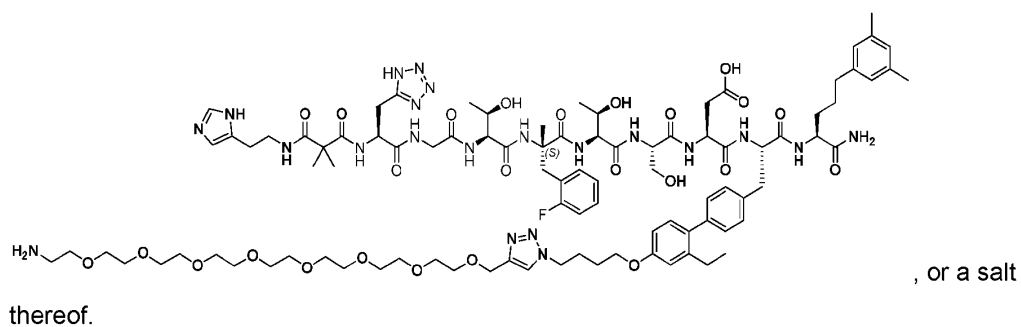
R^3 is independently at each occurrence an optionally substituted C_{10-30} alkyl,

or a salt thereof,

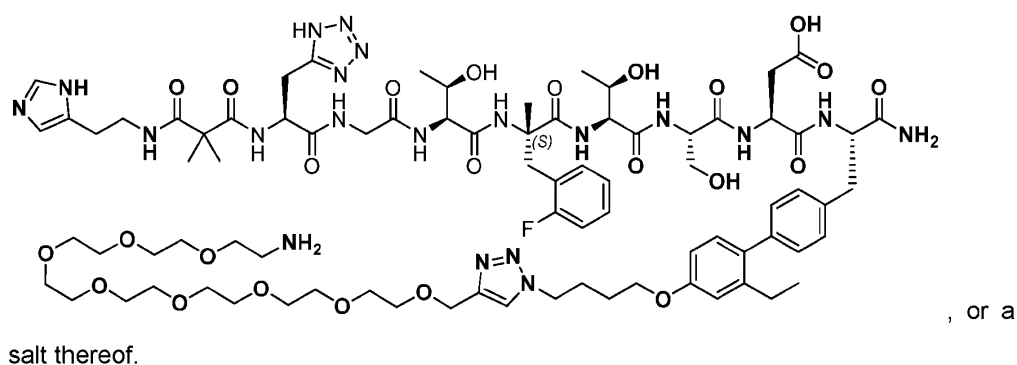
for making a peptide or a peptidomimetic.

56. The method of claim 55, wherein the compound of Formula (I) is used as a support for making a peptide or a peptidomimetic.

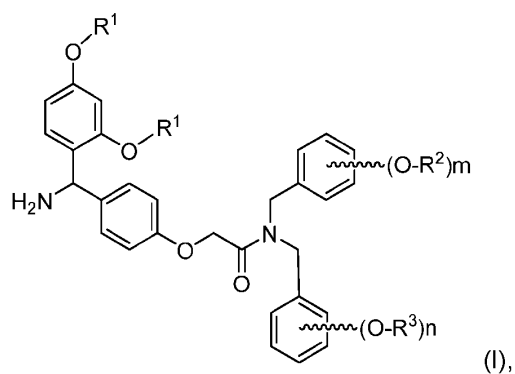
57. The method of claim 55, wherein the peptidomimetic is a compound of Formula:



58. The method of claim 55, wherein the peptidomimetic is a compound of Formula:



59. A compound manufactured using a compound of Formula (I):



wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

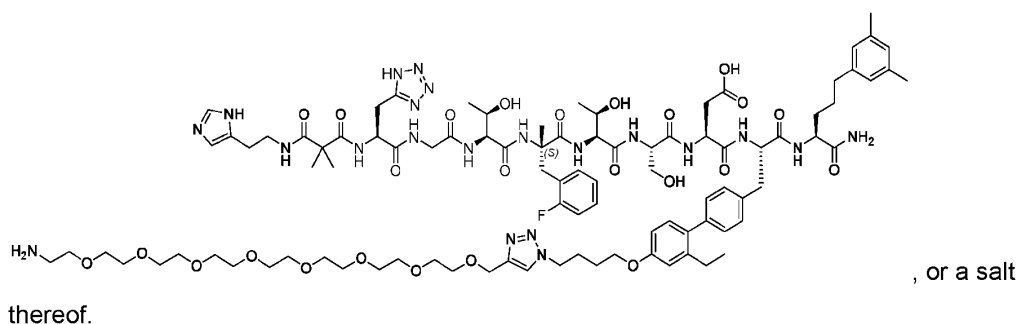
R^1 is C_{1-5} alkyl;

R^2 is independently selected at each occurrence thereof from an optionally substituted C_{10-30} alkyl; and

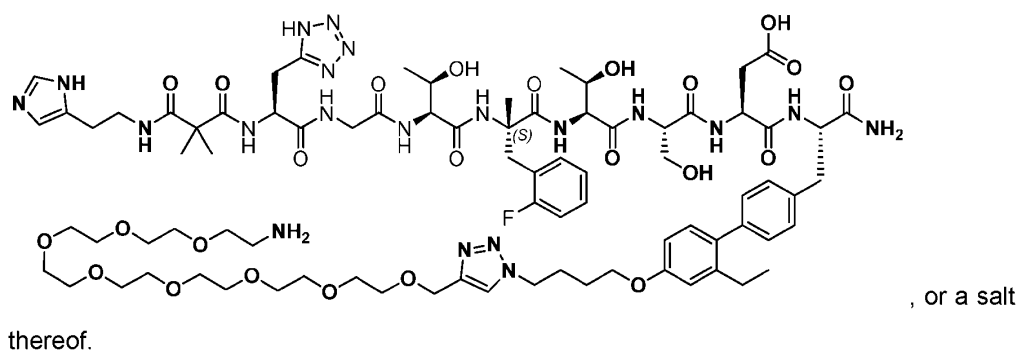
R^3 is independently selected at each occurrence thereof from an optionally substituted C_{10-30} alkyl,

or a salt thereof.

60. The compound of claim 59, wherein the compound has a Formula:



61. The compound of claim 59, wherein the compound has a Formula:



62. A pharmaceutical composition comprising the peptidomimetic of claim 57 or the compound according to any of claims 59-60.

63. A pharmaceutical dosage form comprising the peptidomimetic of claim 57 or the compound according to any of claims 59-60.

64. A method of selectively targeting GLP1R on a surface of a cell with the peptidomimetic of claim 57 or the compound according to any of claims 59-60.

65. The method of claim 64, wherein the cell is a mammalian cell.

66. The method of claim 64 or 65, wherein the cell is a human cell.

67. A method of enhancing GLP1R activity in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic of claim 57 or the

compound according to any of claims 59-60, the composition of claim 62, or the dosage form of claim 63.

68. A method of lowering blood glucose levels in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic of claim 57 or the compound according to any of claims 59-60, the composition of claim 62, or the dosage form of claim 63.

69. A method of lowering body weight in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic of claim 57 or the compound according to any of claims 59-60, the composition of claim 62, or the dosage form of claim 63.

70. A method of treating a GLP1R-associated condition in an individual in need thereof comprising administering to the individual an effective amount of the peptidomimetic of claim 57 or the compound according to any of claims 59-60, the composition of claim 62, or the dosage form of claim 63.

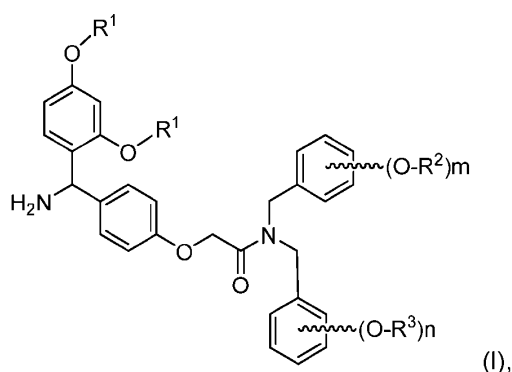
71. The method of claim 70, wherein the GLP1R-associated condition is type II diabetes, obesity, liver disease, coronary artery disease, or kidney disease.

72. The method of claim 70, wherein the GLP1R-associated condition is type II diabetes and/or obesity.

73. The method of any of claims 67-72, wherein the peptidomimetic of claim 57 or the compound according to any of claims 59-60, the composition of claim 62, or the dosage form of claim 63 is administered subcutaneously, intravenously, intradermally, intraperitoneally, or intramuscularly.

74. A method of making a peptide or a peptidomimetic, the method comprising the steps of:

(a) providing a compound of Formula (I):



(I),

wherein:

m and n are, independently, an integer from 1 to 5, and $(m+n) \geq 3$;

R^1 is C_{1-5} alkyl;

R^2 is independently selected at each occurrence thereof from an optionally substituted C_{10-30} alkyl; and

R^3 is independently selected at each occurrence thereof from an optionally substituted C_{10-30} alkyl,

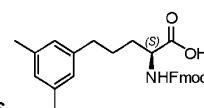
or a salt thereof;

(b) providing a first amino acid (AA1); and

(c) coupling the first amino acid (AA1) to the compound of Formula (I) to form a peptide bond between the first amino acid (AA1) and the compound of Formula (I).

75. The method of claim 74, wherein the first amino acid (AA1) contains a protected amino group.

76. The method of claim 74, wherein the first amino acid (AA1) is



77. The method of claim 74 further comprising the step of:

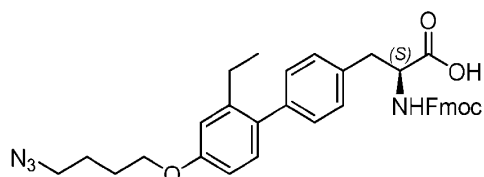
activating the chemical groups on the first amino acid (AA1) to prepare the first amino acid (AA1) for coupling with the compound of Formula (I) prior to step (c).

78. The method of claim 74 further comprising the steps of:

(d) removing the protecting group from the first amino acid (AA1);

- (e) providing a second amino acid (AA2); and
- (f) coupling the second amino acid (AA2) to the first amino acid (I-AA1) to form a peptide bond between the second amino acid (AA2) and the first amino acid (I-AA1-AA2).

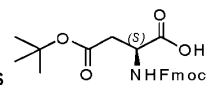
79. The method of claim 78, wherein the second amino acid (AA2) is



80. The method of claim 78 further comprising the steps of:

- (g) removing the protecting group from the second amino acid (AA2);
- (h) providing a third amino acid (AA3); and
- (i) coupling the third amino acid (AA3) to the second amino acid to form a peptide bond between the third amino acid (AA3) and the second amino acid (I-AA1-AA2-AA3).

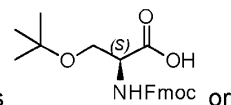
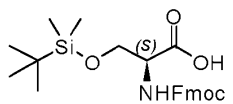
81. The method of claim 80, wherein the third amino acid (AA3) is



82. The method of claim 80 further comprising the steps of:

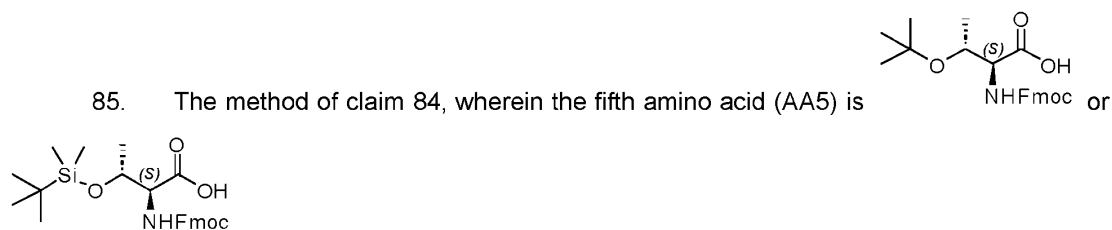
- (j) removing the protecting group from the third amino acid (AA3);
- (k) providing a fourth amino acid (AA4); and
- (l) coupling the fourth amino acid (AA4) to the third amino acid to form a peptide bond between the fourth amino acid (AA4) and the third amino acid (I-AA1-AA2-AA3-AA4).

83. The method of claim 82, wherein the fourth amino acid (AA4) is

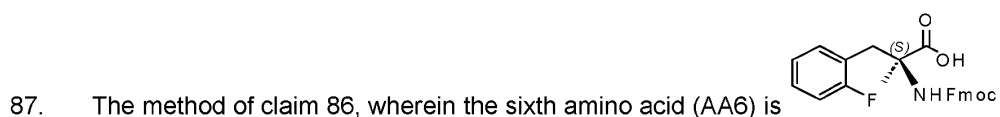


84. The method of claim 82 further comprising:

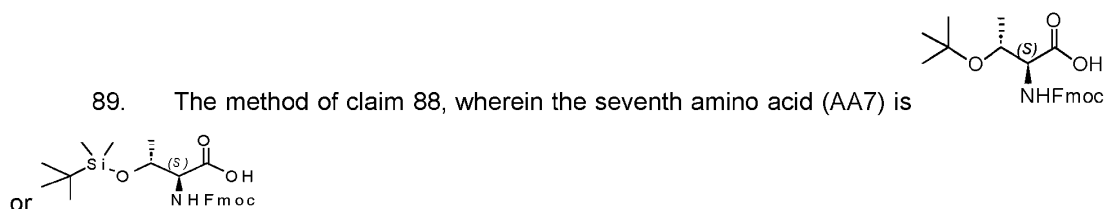
- (m) removing the protecting group from the fourth amino acid (AA4);
- (n) providing a fifth amino acid (AA5); and
- (o) coupling the fifth amino acid (AA5) to the fourth amino acid to form a peptide bond between the fifth amino acid (AA5) and fourth amino acid (I-AA1-AA2-AA3-AA4-AA5).



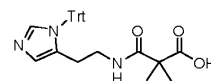
86. The method of claim 84 further comprising:
- (p) removing the protecting group from the fifth amino acid (AA5);
 - (q) providing a sixth amino acid (AA6); and
 - (r) coupling the sixth amino acid (AA6) to the fifth amino acid to form a peptide bond between the sixth amino acid (AA6) and the fifth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6).



88. The method of claim 86 further comprising:
- (s) removing the protecting group from the sixth amino acid (AA6);
 - (t) providing a seventh amino acid (AA7); and
 - (u) coupling the seventh amino acid (AA7) to the sixth amino acid to form a peptide bond between the seventh amino acid (AA7) and the sixth amino acid (I-AA1-AA2-AA3-AA4-AA5-AA6-AA7).

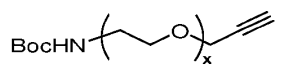


95. The method of claim 94, wherein the tenth amino acid (AA10) is .



96. The method of claim 94 further comprising:
 repeating the steps of (i) removing the protecting group from the amino acid (AA_n);
 (ii) providing a succeeding amino acid (AA_{n+1}); and
 (iii) coupling the succeeding amino acid (AA_{n+1}) to the amino acid (AA_n) to form a peptide bond between the succeeding amino acid (AA_{n+1}) and amino acid (AA_n),
 wherein said repeating is conducted from 1 to 100 times.

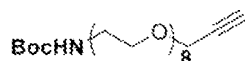
97. The method of claim 94 further comprising:
 (ee) removing the protecting group from the tenth amino acid (AA10);
 (ff) providing a compound of Formula (B):



wherein x is an integer from 1 to 15; and

- (gg) coupling the compound of Formula (B) to the one of the amino acids in the peptide or the peptidomimetic to form a triazole ring.

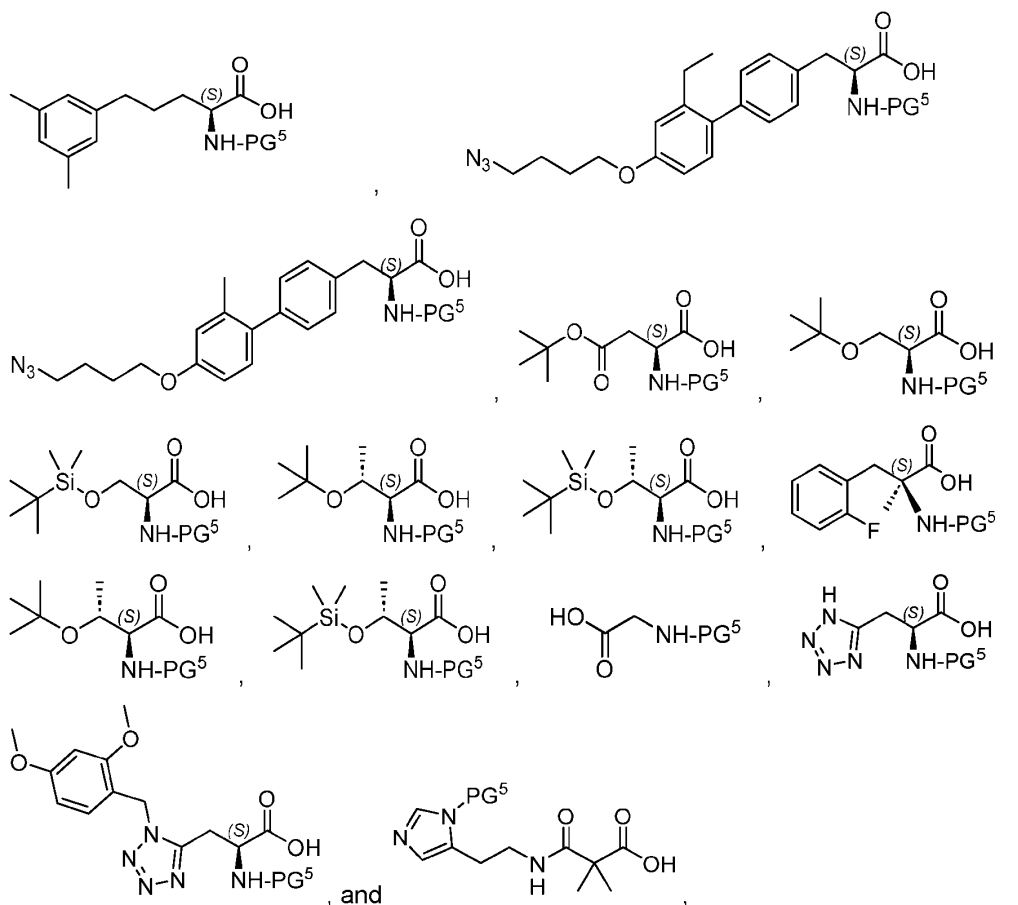
98. The method of claim 97, wherein the compound of Formula (B) has the following Formula:



99. The method according to any one of claims 74-98 further comprising:
 (hh) cleaving the peptide bond between the first amino acid (AA1) and the compound of Formula (I) to obtain the peptide or the peptidomimetic.

100. The method according to any one of claims 74-98, wherein each one of the first amino acid (AA1), the second amino acid (AA2), the third amino acid (AA3), the fourth amino acid (AA4), the fifth amino acid (AA5), the sixth amino acid (AA6), the seventh amino acid (AA7), the eighth

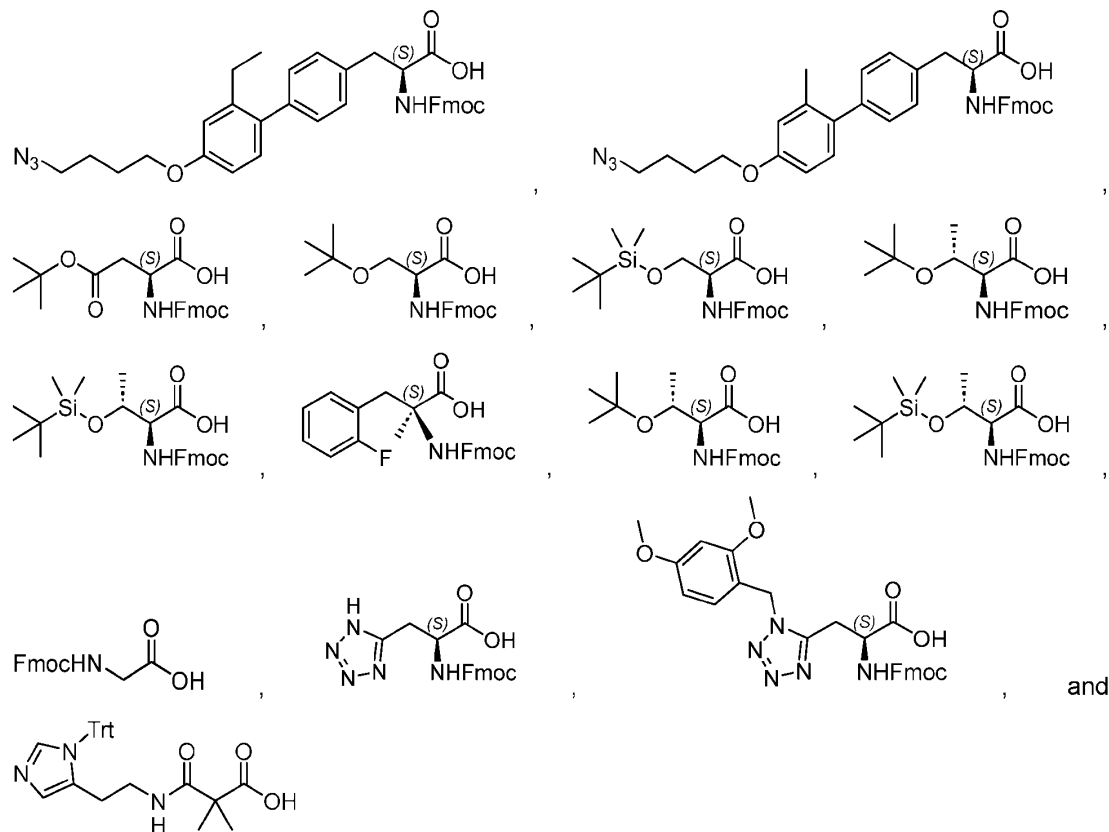
amino acid (AA8), the ninth amino acid (AA9), the tenth amino acid (AA10), and succeeding amino acid (AA_{n+1}) are independently selected from the group consisting of



wherein PG⁵ is a suitable protecting group.

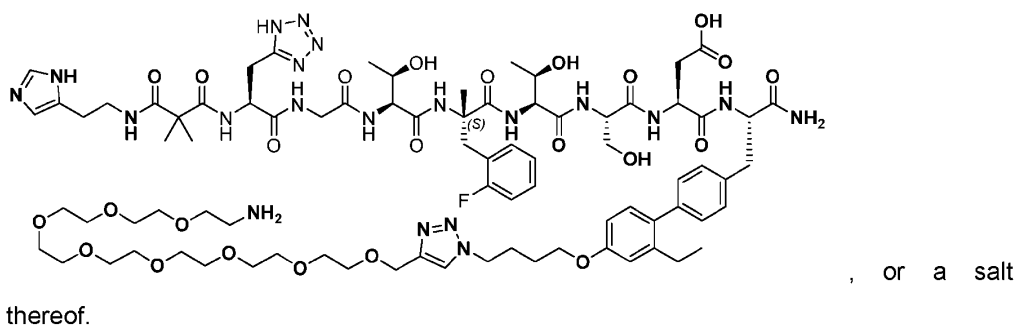
101. The method according to claim 100, wherein PG⁵ is independently selected at each occurrence from the group consisting of triphenylmethyl (Trt), allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

102. The method according to any one of claims 74-98, wherein each one of the first amino acid (AA1), the second amino acid (AA2), the third amino acid (AA3), the fourth amino acid (AA4), the fifth amino acid (AA5), the sixth amino acid (AA6), the seventh amino acid (AA7), the eighth amino acid (AA8), the ninth amino acid (AA9), the tenth amino acid (AA10), and succeeding amino

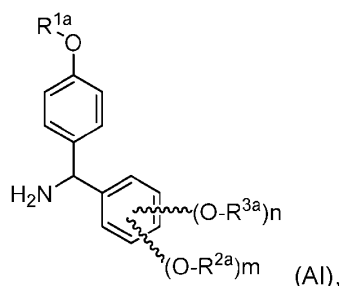
Cc1ccc(cc1)CCCC[C@H](C(=O)O)NHFmoc[illegible]

thereof.

104. The method of claim 99, wherein the peptidomimetic has a Formula:



105. A compound having a structure of Formula (AI):



wherein:

m and n are, independently, an integer from 0 to 5;

R^{1a} is C₁₋₅ alkyl;

R^{2a} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

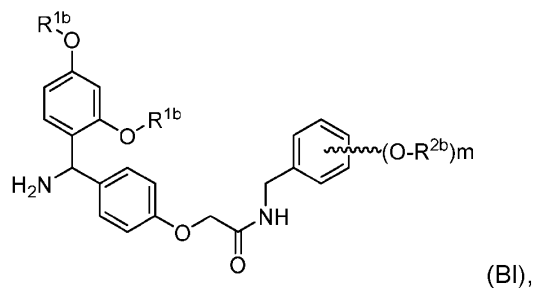
R^{3a} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,
or a salt thereof.

106. The compound of claim 105, wherein R^{1a} is methyl.

107. The compound of claim 105, wherein R^{2a} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl and R^{3a} is independently at each occurrence an optionally substituted C₁₈₋₂₆ alkyl.

108. The compound of claim 105, wherein each R^{2a} is independently an optionally substituted C₂₂ alkyl and each R^{3a} is independently an optionally substituted C₂₂ alkyl.

109. A compound having a structure of Formula (BI):



wherein:

m is an integer from 1 to 5;

R^{1b} is C₁₋₅ alkyl; and

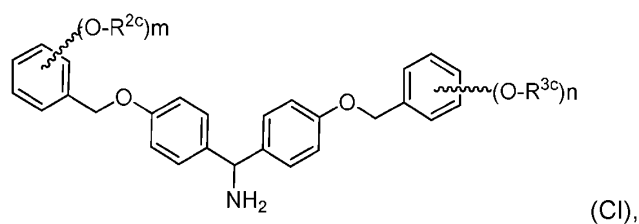
R^{2b} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl;
or a salt thereof.

110. The compound of claim 109, wherein R^{1b} is methyl.

111. The compound of claim 109, wherein each R^{2b} is independently an optionally substituted C₁₈₋₂₆ alkyl.

112. The compound of claim 109, wherein each R^{2b} is independently an optionally substituted C₂₂ alkyl.

113. A compound having a structure of Formula (CI):



wherein:

m and n are, independently, an integer from 1 to 5, and (m+n) ≥ 3;

R^{2c} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl; and

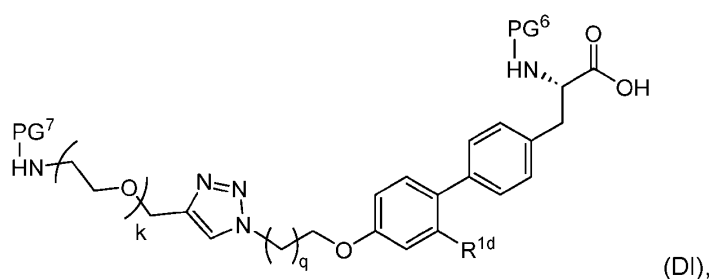
R^{3c} is independently at each occurrence an optionally substituted C₁₀₋₃₀ alkyl,

or a salt thereof.

114. The compound of claim 113, wherein R^{2c} is independently at each occurrence an optionally substituted C_{18-26} alkyl and R^{3c} is independently at each occurrence an optionally substituted C_{18-26} alkyl.

115. The compound of claim 113, wherein each R^{2c} is independently an optionally substituted C_{22} alkyl and each R^{3c} is independently an optionally substituted C_{22} alkyl.

116. A process for preparation of a compound of Formula (DI):



wherein:

k is an integer from 1 to 15;

q is an integer from 0 to 10;

R^{1d} is C_{1-6} alkyl;

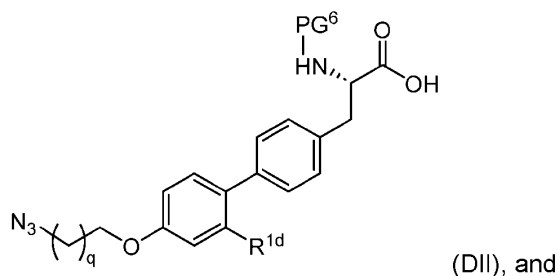
PG^6 is a suitable protecting group; and

PG^7 is a suitable protecting group,

or a salt thereof,

said process comprising:

(a) providing a compound of Formula (DII) having the structure:

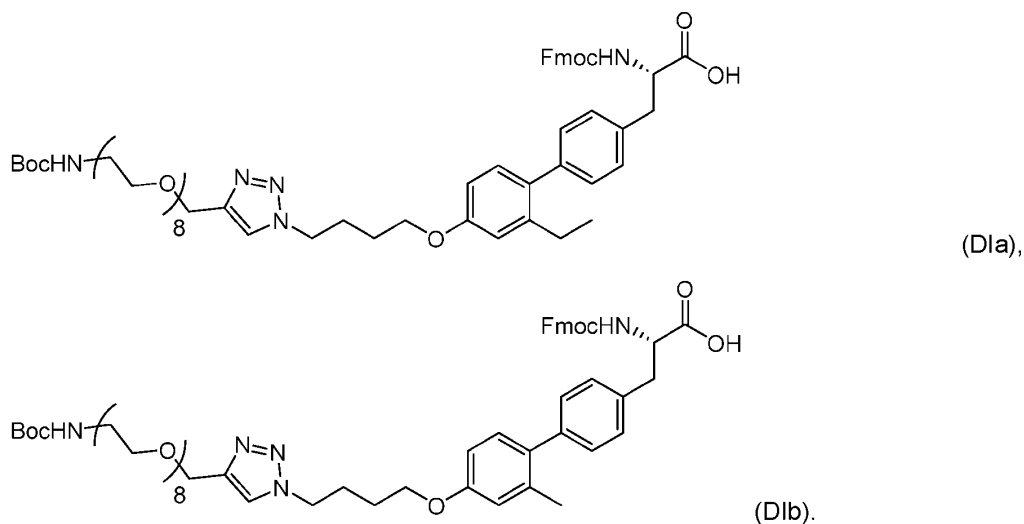


(b) forming the compound of Formula (DI) from the compound of Formula (DII).

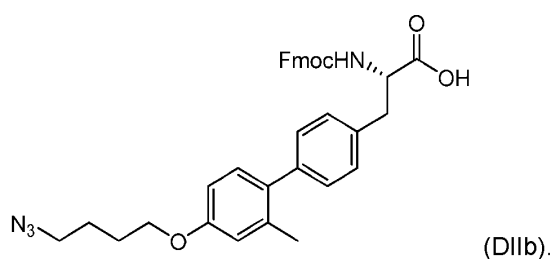
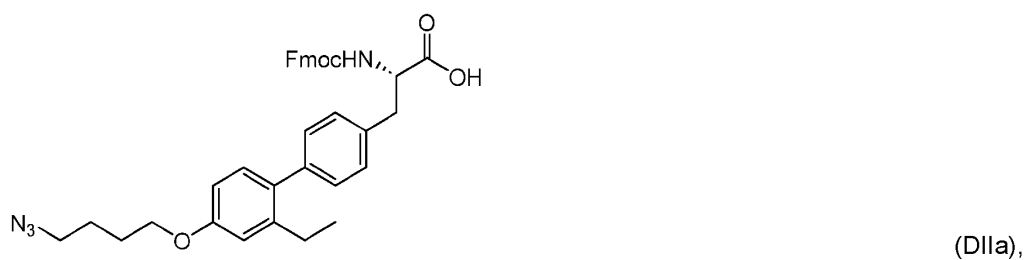
117. The process according to claim 116, wherein PG⁶ is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

118. The process according to claim 116, wherein PG⁷ is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

119. The process according to claim 116, wherein the compound of Formula (DI) has the structure of Formula (DIa) or Formula (DIb):

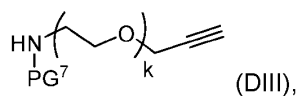


120. The process according to claim 116, wherein the compound of Formula (DII) has the structure of Formula (DIIa) or Formula (DIIb):



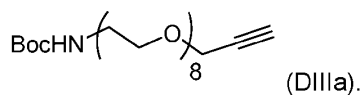
121. The process according to claim 116, wherein said step (b) of forming the compound of Formula (DI) comprises:

reacting the compound of Formula (DII) with a compound of Formula (DIII):

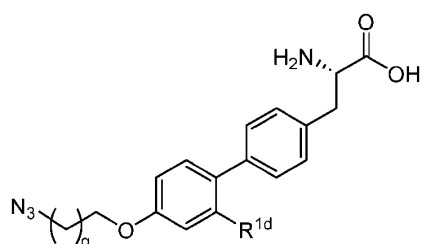


to produce the compound of Formula (II).

122. The process according to claim 121, wherein the compound of Formula (DIII) has the structure of Formula (DIIIa):



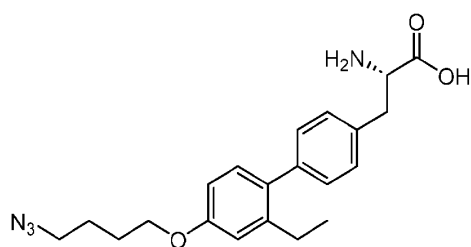
123. The process according to claim 116 further comprising the steps of:
providing a compound of Formula (DIV) having the structure:



(DIV) or a salt thereof; and

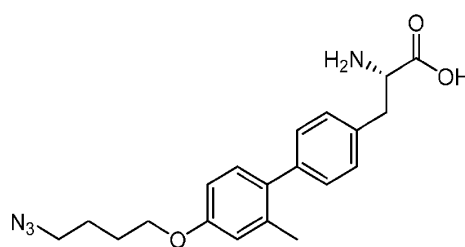
forming the compound of Formula (DII) from the compound of Formula (DIV) prior to the step (a).

124. The process according to claim 123, wherein the compound of Formula (DIV) has the structure of Formula (DIVa) or Formula (DIVb):



(DIVa),

(DIVb).



125. The process according to claim 123, wherein said step of forming the compound of Formula (DII) comprises:

reacting the compound of Formula (DIV) with a compound of Formula (DVa) or Formula (DVb):

$\text{PG}^6\text{-X}$ (DVa) or PG^6_2O (DVb),

wherein

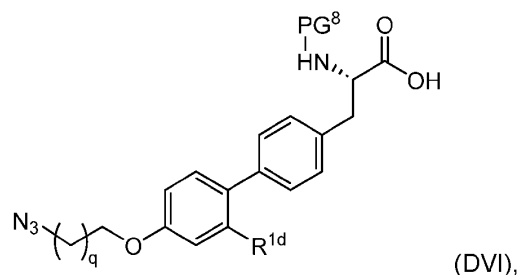
X is OSu, OTf, Cl, or Br;

to produce the compound of Formula (DII).

126. The process according to claim 125, wherein the compound of Formula (DVa) or Formula (DVb) is selected from the group consisting of AllocCl, Alloc₂O, Cbz₂O, CbzCl, FmocCl, FmocOSu, and Boc₂O.

127. The process according to claim 123 further comprising the steps of:

providing a compound of Formula (DVI) having the structure:



wherein

PG⁸ is a suitable protecting group; and

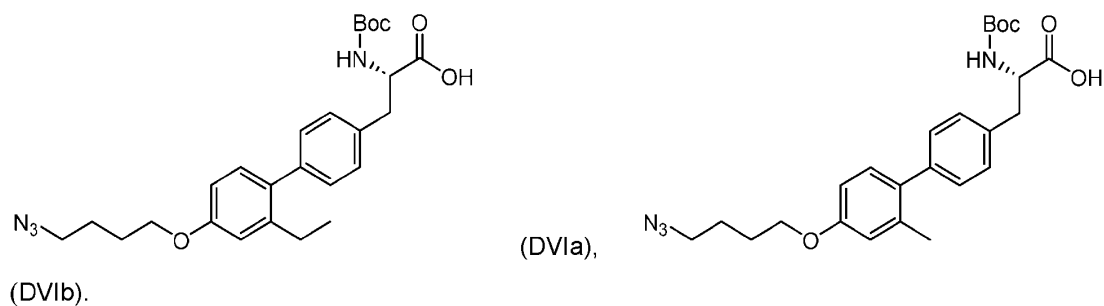
forming the compound of Formula (DIV) from the compound of Formula (DVI).

128. The process according to claim 127, wherein said step of forming the compound of Formula (DIV) comprises:

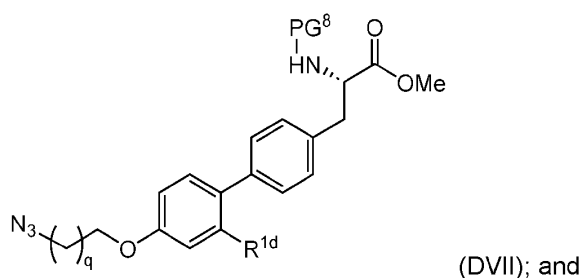
reacting the compound of Formula (DVI) with a protecting group removal agent to produce the compound of Formula (DIV).

129. The process according to claim 128, wherein the protecting group removal agent is selected from the group consisting of Pd(PPh)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

130. The process according to claim 127, wherein the compound of Formula (DVI) has the structure of Formula (DVla) or Formula (DVlb):



131. The process according to claim 127 further comprising the steps of:
providing a compound of Formula (DVII) having the structure:



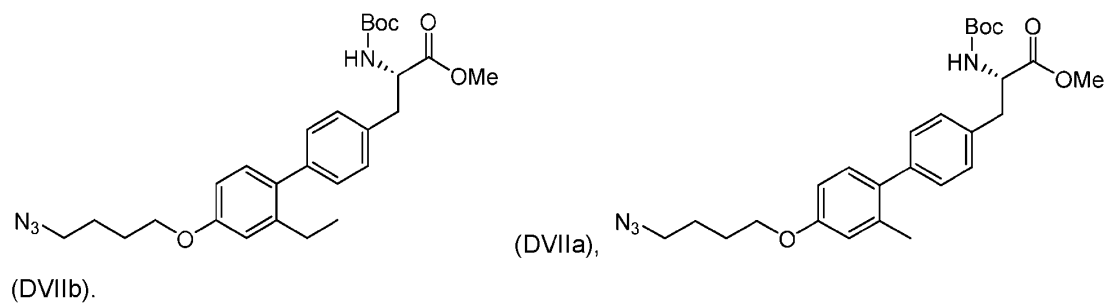
forming the compound of Formula (DVI) from the compound of Formula (DVII).

132. The process according to claim 131, wherein said step of forming the compound of Formula (DVI) comprises:

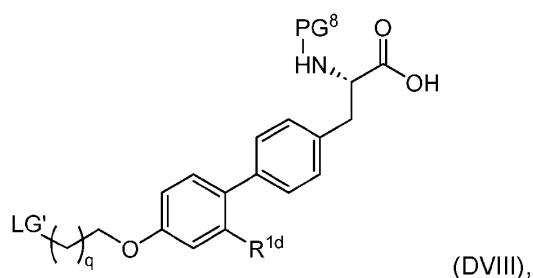
reacting the compound of Formula (DVII) with a base to produce the compound of Formula (DVI).

133. The process according to claim 128, wherein the base is selected from the group consisting of LiOH, NaOH, and KOH.

134. The process according to claim 131, wherein the compound of Formula (DVII) has the structure of Formula (DVIIa) or Formula (DVIIb):



135. The process according to claim 131 further comprising the steps of:
providing a compound of Formula (DVIII) having the structure:



wherein

LG' is a suitable living group; and

forming the compound of Formula (DVII) from the compound of Formula (DVIII).

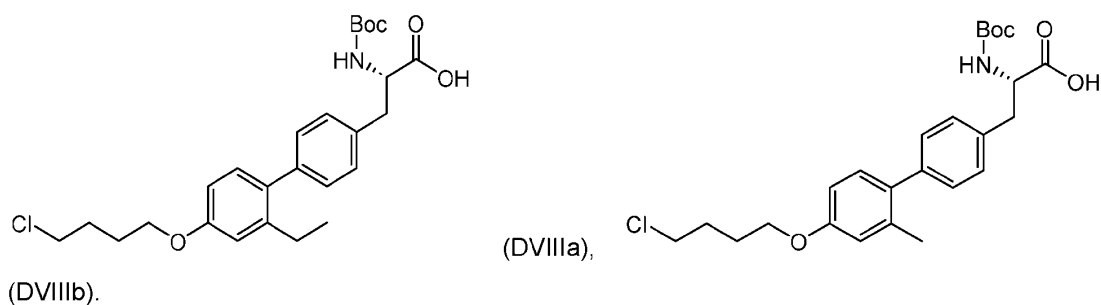
136. The process according to claim 135, wherein said step of forming the compound of Formula (DVII) comprises:

reacting the compound of Formula (DVIII) with an azido group introducing agent to produce the compound of Formula (DVII).

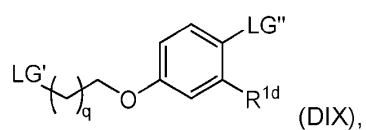
137. The process according to claim 136, wherein the azido group introducing agent is selected from the group consisting of NaN_3 , TMSN_3 , $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$, $\text{Zn}(\text{N}_3)_2 \cdot 2\text{Py}$, and $n\text{-Bu}_4\text{NN}_3$.

138. The process according to claim 135, wherein the LG' is selected from the group consisting of halogen, OTf, OMs, and OTs.

139. The process according to claim 135, wherein the compound of Formula (DVIII) has the structure of Formula (DVIIIa) or Formula (DVIIIb):



140. The process according to claim 135 further comprising the steps of:
providing a compound of Formula (DIX) having the structure:



wherein

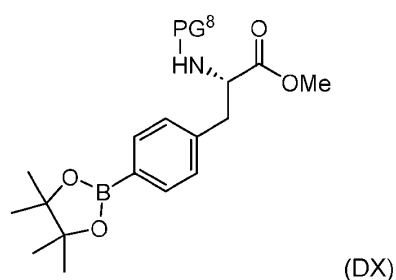
LG'' is a suitable living group; and

forming the compound of Formula (DVIII) from the compound of Formula (DIX).

141. The process according to claim 140, wherein the LG'' is selected from the group consisting of halogen, OTf, OMs, and OTs.

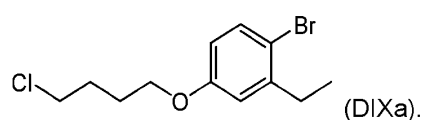
142. The process according to claim 140, wherein said step of forming the compound of Formula (DVIII) comprises:

reacting the compound of Formula (DIX) with a compound of Formula (DX):

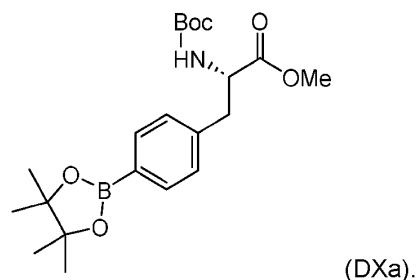


to produce the compound of Formula (DVII).

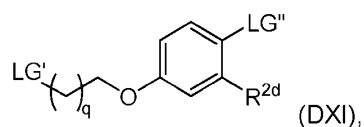
143. The process according to claim 140, wherein the compound of Formula (DIX) has the structure of Formula (DIXa):



144. The process according to claim 142, wherein the compound of Formula (DX) has the structure of Formula (Dxa):



145. The process according to claim 140 further comprising the steps of:
providing a compound of Formula (DXI) having the structure:



wherein

R^{2d} is C_{1-6} alkylene; and

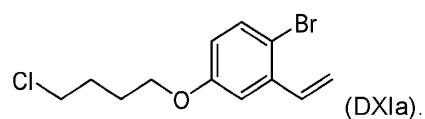
forming the compound of Formula (DIX) from the compound of Formula (DXI).

146. The process according to claim 145, wherein said step of forming the compound of Formula (DIX) comprises:

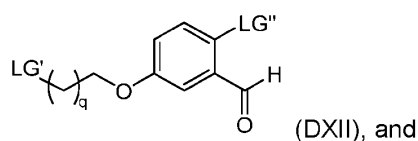
reacting the compound of Formula (DXI) with a reducing agent to produce the compound of Formula (DIX).

147. The process according to claim 145, wherein the reducing agent is selected from the group consisting of H_2 , NH_4HCO_2 , $NABH(OAc)_3$, and $LiAlH_4$.

148. The process according to claim 141, wherein the compound of Formula (DXI) has the structure of Formula (DXIa):



149. The process according to claim 145 further comprising the steps of:
providing a compound of Formula (DXII) having the structure:



forming the compound of Formula (DXI) from the compound of Formula (DXII).

150. The process according to claim 149, wherein said step of forming the compound of Formula (DXI) comprises:

reacting the compound of Formula (DXII) with a compound of Formula (DXIII):

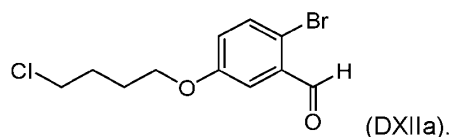
$\text{PPh}_3^*\text{C}_{1-6}\text{ alkyl-Hal}$ (DXIII),

wherein

Hal is halogen,

to produce the compound of Formula (DXI).

151. The process according to claim 149, wherein the compound of Formula (DXII) has the structure of Formula (DXIIa):

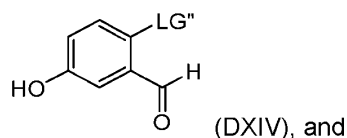


152. The process according to claim 150, wherein the compound of Formula (DXIII) has the structure of Formula (DXIIIa):

$\text{PPh}_3^*\text{MeBr}$ (DXIIIa).

153. The process according to claim 149 further comprising the steps of:

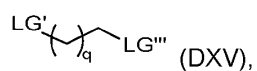
providing a compound of Formula (DXIV) having the structure:



forming the compound of Formula (DXII) from the compound of Formula (DXIV).

154. The process according to claim 153, wherein said step of forming the compound of Formula (DXII) comprises:

reacting the compound of Formula (DXIV) with a compound of Formula (DXV):



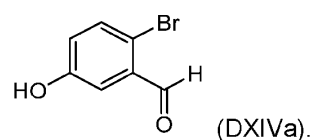
wherein

LG''' is a suitable living group,

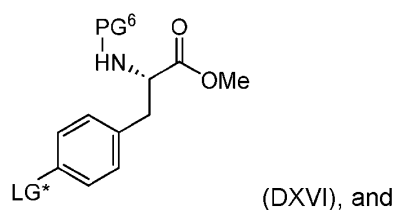
to produce the compound of Formula (DXII).

155. The process according to claim 154, wherein the LG''' is selected from the group consisting of halogen, OTf, OMs, and OTs.

156. The process according to claim 153, wherein the compound of Formula (DXIV) has the structure of Formula (DXIVa):



157. The process according to claim 142 further comprising the steps of:
providing a compound of Formula (DXVI) having the structure:



wherein

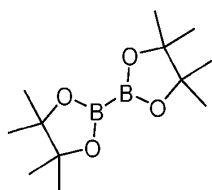
LG* is a suitable living group,

forming the compound of Formula (DX) from the compound of Formula (DXVI).

158. The process according to claim 157, wherein the LG* is selected from the group consisting of halogen, OTf, OMs, and OTs.

159. The process according to claim 157, wherein said step of forming the compound of Formula (DX) comprises:

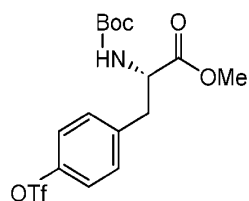
reacting the compound of Formula (DXVI) with a compound of Formula (DXVII):



(DXVII),

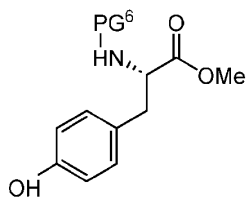
to produce the compound of Formula (DX).

160. The process according to claim 157, wherein the compound of Formula (DXVI) has the structure of Formula (DXVIc):



(DXVIc).

161. The process according to claim 157 further comprising the steps of:
providing a compound of Formula (DXVIII) having the structure:



(DXVIII), and

forming the compound of Formula (DXVI) from the compound of Formula (DXVIII).

162. The process according to claim 161, wherein said step of forming the compound of Formula (DXVI) comprises:

reacting the compound of Formula (DXVIII) with a compound of Formula (DXIXa) or Formula (DXIXb):

LG*-X (DXIXa) or LG*₂O (DXIXb),

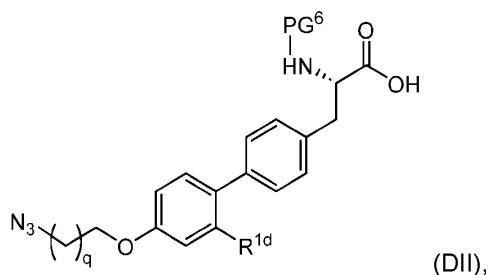
wherein

X is OSu, Cl, or Br;

to produce the compound of Formula (DXVI).

163. The process according to claim 162, wherein the compound of Formula (DXIXa) or Formula (DXIXb) is selected from the group consisting of TfCl, Tf₂O, MsCl, and Ms₂O.

164. A process for preparation of a compound of Formula (DII):



wherein:

q is an integer from 0 to 10;

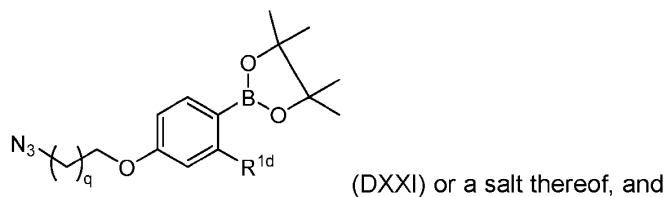
R^{1d} is C₁₋₆ alkyl; and

PG⁶ is a suitable protecting group,

or a salt thereof,

said process comprising:

(a) providing a compound of Formula (DXXI) having the structure:



(b) forming the compound of Formula (DII) from the compound of Formula (DXXI).

165. The process according to claim 164, wherein PG⁶ is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

166. The process according to claim 164, wherein the compound of Formula (DXXI) is



167. The process according to claim 164, wherein the compound of Formula (DXXI) is treated with a catalyst.

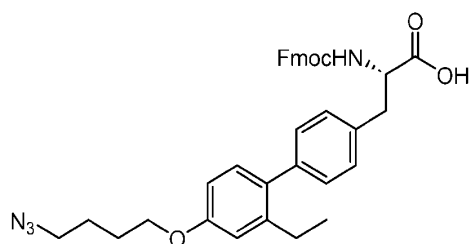
168. The process according to claim 167, wherein the catalyst is Pd(dppf)Cl₂-DCM.

169. The process according to claim 164, wherein the compound of Formula (DXXI) is treated with a base.

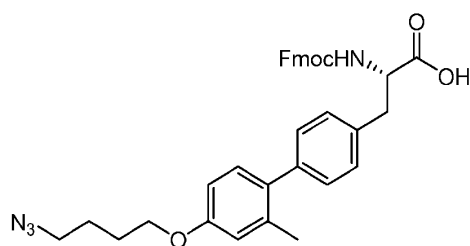
170. The process according to claim 169, wherein the base is K₂CO₃.

171. The process according to claim 164, wherein the preparation is performed in a solvent mixture comprised of H₂O/iPrOH.

172. The process according to claim 164, wherein the compound of Formula (DII) has the structure of Formula (DIIa) or Formula (DIIb):

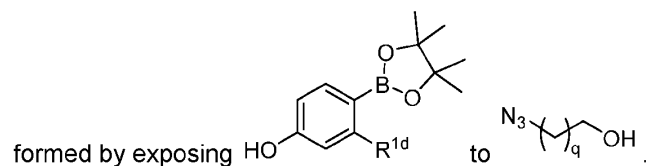


(DIIa),



(DIIb).

173. The process according to claim 164, wherein the compound of Formula (DXXI) is



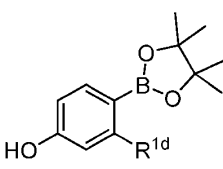
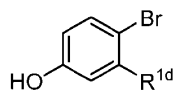
174. The process according to claim 173, wherein the compound of Formula (DXXI) is formed in the presence of PPh_3 .

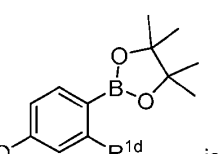
175. The process according to claim 173, wherein the compound of Formula (DXXI) is formed in the presence of DIAD.

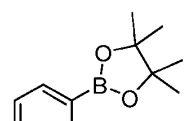
176. The process according to claim 173, wherein the compound of Formula (DXXI) is formed in the presence of molecular sieves of 4Å.

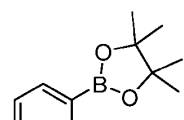
177. The process according to claim 173, wherein the compound of Formula (DXXI) is formed in an organic solvent.

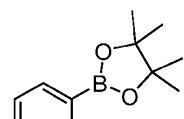
178. The process according to claim 177, wherein the organic solvent is THF.

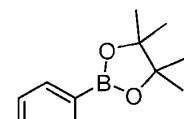
179. The process according to claim 173, wherein  is formed by exposing  to PBin_2 .

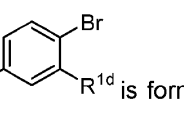
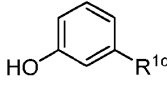
180. The process according to claim 179, wherein  is formed in the presence of $\text{Pd}(\text{OAc})_2$.

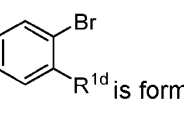
181. The process according to claim 179, wherein  is formed in the presence of PCy₃.

182. The process according to claim 179, wherein  is formed in the presence of KOAc.

183. The process according to claim 179, wherein  is formed in the presence of dioxane.

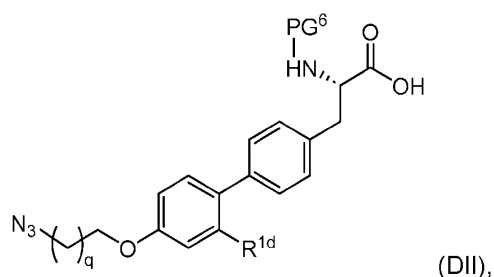
184. The process according to claim 179, wherein  is formed at an elevated temperature for a period of time.

185. The process according to claim 179, wherein  is formed by exposing  to Bu₄N⁺Br₃⁻.

186. The process according to claim 185, wherein  is formed in an organic solvent.

187. The process according to claim 186, wherein the organic solvent is a mixture of DCM and methanol.

188. A process for preparation of a compound of Formula (DII):



wherein:

q is an integer from 0 to 10;

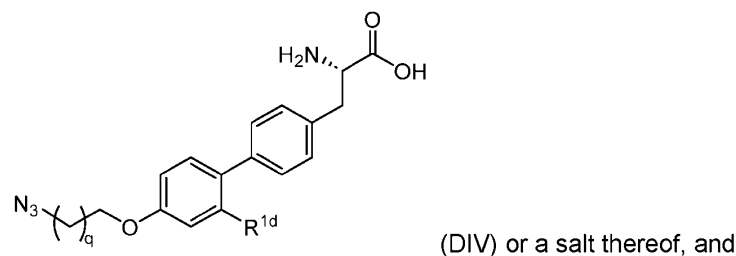
R^{1d} is C₁₋₆ alkyl; and

PG⁶ is a suitable protecting group,

or a salt thereof,

said process comprising:

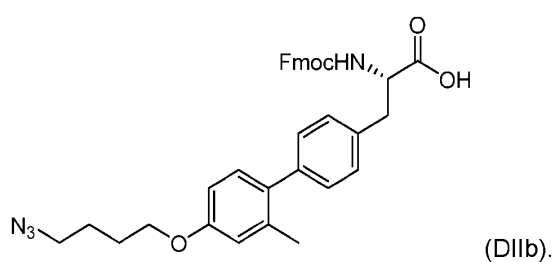
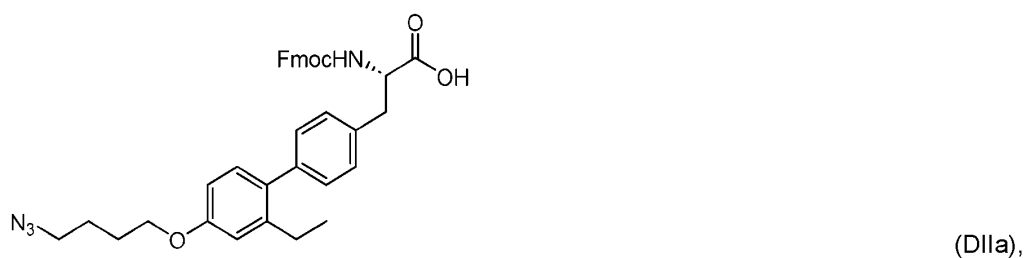
(a) providing a compound of Formula (DIV) having the structure:



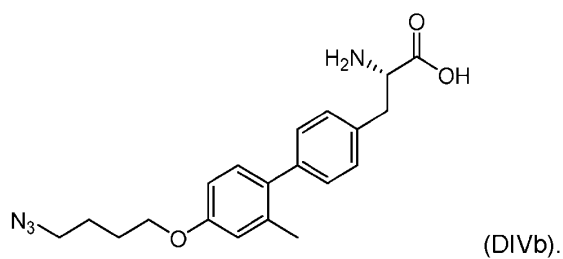
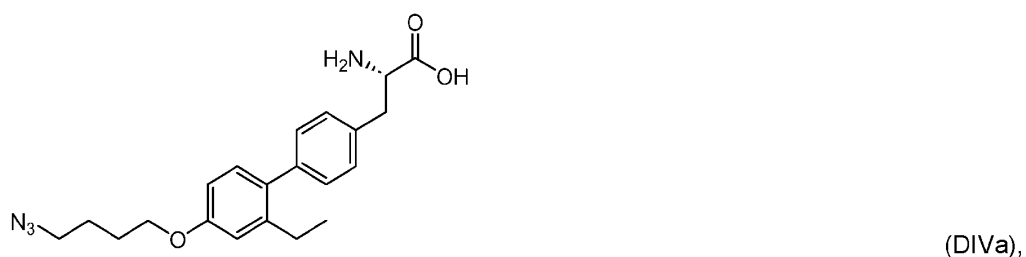
(b) forming the compound of Formula (DII) from the compound of Formula (DIV).

189. The process according to claim 188, wherein PG⁶ is selected from the group consisting of allyloxycarbonyl (Alloc), benzyloxycarbonyl (Cbz), tert-butyloxycarbonyl (Boc), and 9-fluorenylmethoxycarbonyl (Fmoc).

190. The process according to claim 188, wherein the compound of Formula (DII) has the structure of Formula (DIIa) or Formula (DIIb):



191. The process according to claim 188, wherein the compound of Formula (DIV) has the structure of Formula (DIVa) or Formula (DIVb):



192. The process according to claim 188, wherein said step of forming the compound of Formula (DII) comprises:

reacting the compound of Formula (DIV) with a compound of Formula (DVa) or Formula (DVb):

$\text{PG}^6\text{-X}$ (DVa) or PG^6O (DVb),

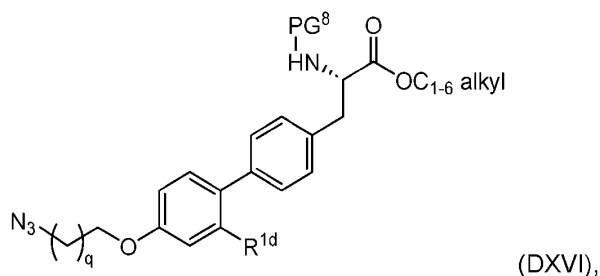
wherein

X is OSu, Otf, Cl, or Br;

to produce the compound of Formula (DII).

193. The process according to claim 192, wherein the compound of Formula (DVa) or Formula (DVb) is selected from the group consisting of AllocCl, Alloc₂O, Cbz₂O, CbzCl, FmocCl, FmocOSu, and Boc₂O.

194. The process according to claim 188 further comprising the steps of:
providing a compound of Formula (DXVI) having the structure:



wherein

PG⁸ is a suitable protecting group; and

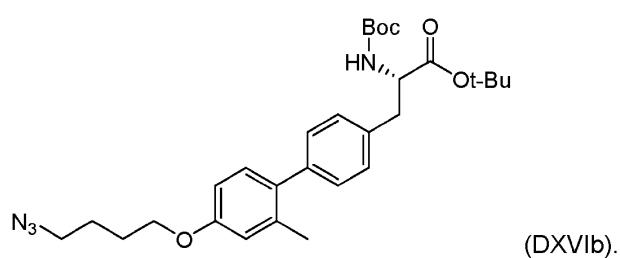
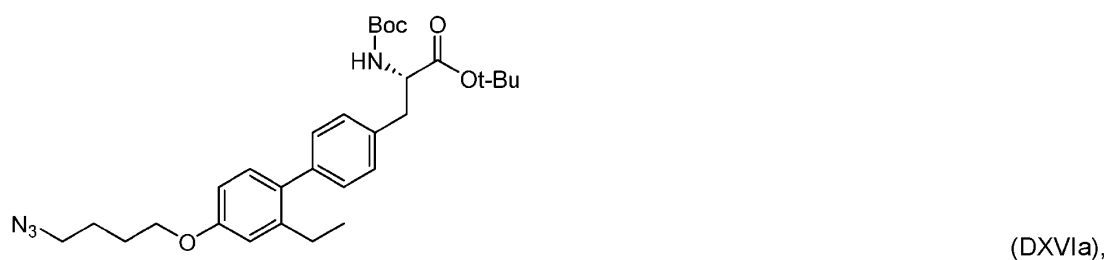
forming the compound of Formula (DIV) from the compound of Formula (DXVI).

195. The process according to claim 194, wherein said step of forming the compound of Formula (DIV) comprises:

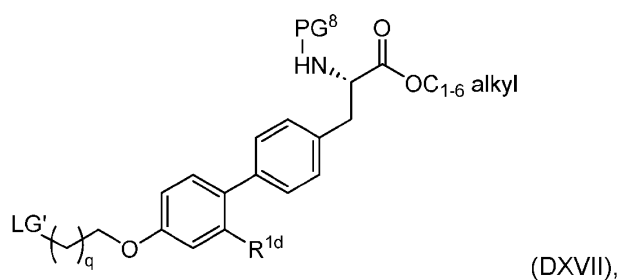
reacting the compound of Formula (DXVI) with a protecting group removal agent to produce the compound of Formula (DIV).

196. The process according to claim 195, wherein the protecting group removal agent is selected from the group consisting of HCl, Pd(PPh)₃, PhSiH₃, H₂, piperidine, and trifluoroacetic acid (TFA).

197. The process according to claim 194, wherein the compound of Formula (DXVI) has the structure of Formula (DXVIa) or Formula (DXVIb):



198. The process according to claim 194 further comprising the steps of:
providing a compound of Formula (DXVII) having the structure:



wherein

LG' is a suitable living group; and

forming the compound of Formula (DXVI) from the compound of Formula (DXVII).

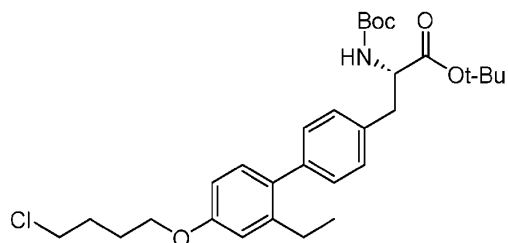
199. The process according to claim 198, wherein said step of forming the compound of Formula (DXVI) comprises:

reacting the compound of Formula (DXVII) with an azido group introducing agent to produce the compound of Formula (DXVI).

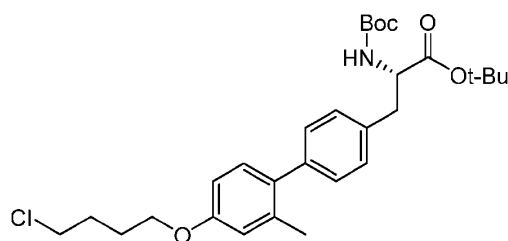
200. The process according to claim 199, wherein the azido group introducing agent is selected from the group consisting of NaN_3 , $TMSN_3$, $(PhO)_2P(O)N_3$, $Zn(N_3)_2 \cdot 2Py$, and $n-Bu_4NN_3$.

201. The process according to claim 198, wherein the LG' is selected from the group consisting of halogen, OTf, OMs, and OTs.

202. The process according to claim 198, wherein the compound of Formula (DXVII) has the structure of Formula (DXVIIa) or Formula (DXVIIb):

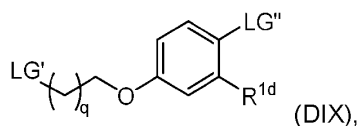


(DXVIIa),



(DXVIIb).

203. The process according to claim 198 further comprising the steps of:
providing a compound of Formula (DIX) having the structure:



(DIX),

wherein

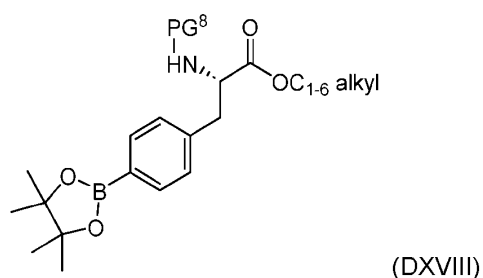
LG'' is a suitable living group; and

forming the compound of Formula (DXVII) from the compound of Formula (DIX).

204. The process according to claim 203, wherein the LG'' is selected from the group consisting of halogen, OTf, OMs, and OTs.

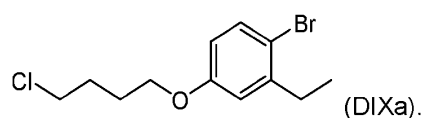
205. The process according to claim 203, wherein said step of forming the compound of Formula (DXVII) comprises:

reacting the compound of Formula (DIX) with a compound of Formula (DXVIII):

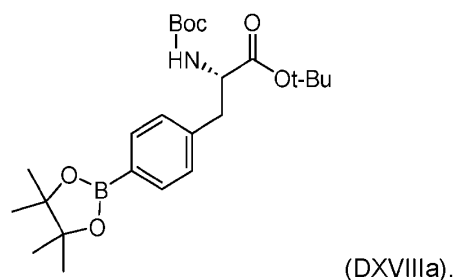


to produce the compound of Formula (DXVII).

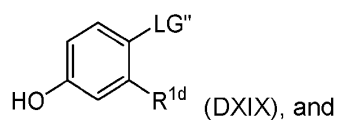
206. The process according to claim 203, wherein the compound of Formula (DIX) has the structure of Formula (DIXa):



207. The process according to claim 205, wherein the compound of Formula (DXVIII) has the structure of Formula (DXVIIIa):



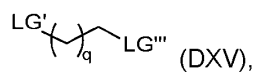
208. The process according to claim 203 further comprising the steps of:
providing a compound of Formula (DXIX) having the structure:



forming the compound of Formula (DIX) from the compound of Formula (DXIX).

209. The process according to claim 208, wherein said step of forming the compound of Formula (DIX) comprises:

reacting the compound of Formula (DXIX) with a compound of Formula (DXV):



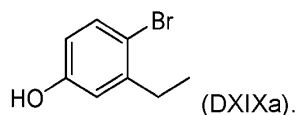
wherein

LG''' is a suitable living group,

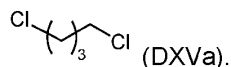
to produce the compound of Formula (DXIX).

210. The process according to claim 209, wherein the LG''' is selected from the group consisting of halogen, OTf, OMs, and OTs.

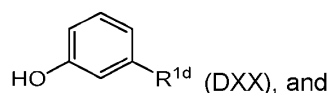
211. The process according to claim 208, wherein the compound of Formula (DXIX) has the structure of Formula (DXIXa):



212. The process according to claim 209, wherein the compound of Formula (DXV) has the structure of Formula (DXVa):



213. The process according to claim 208 further comprising the steps of:
providing a compound of Formula (DXX) having the structure:



forming the compound of Formula (DXIX) from the compound of Formula (DXX).

214. The process according to claim 213, wherein said step of forming the compound of Formula (DXIX) comprises:

reacting the compound of Formula (DXX) with an LG'' introducing agent to produce the compound of Formula (DXIX).

215. The process according to claim 214, wherein the LG'' introducing agent is NBu₄Br₃.

216. The process according to claim 213 wherein the compound of Formula (DXX) has the structure of Formula (DXXa):

