(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2014 (18.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/139008 A1

(51) International Patent Classification:
C07K 5/083 (2006.01) *C07K 5/027* (2006.01)
A61K 38/06 (2006.01) *C07K 5/08* (2006.01)
A61P 3/06 (2006.01)

(21) International Application Number:
PCT/CA2014/050255

(22) International Filing Date:
14 March 2014 (14.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/792,249 15 March 2013 (15.03.2013) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

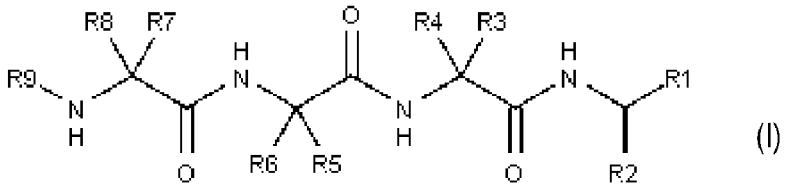
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2014/139008 A1

(54) Title: SMALL MOLECULE MODULATORS OF PCSK9 AND METHODS OF USE THEREOF



(I)

(57) Abstract: A compound of Formula (I): or a pharmaceutically acceptable salt, hydrate, solvate, or racemic mixture or stereoisomer thereof, and methods for preventing or treating an LDL-cholesterol-related disease or disorder using such compound(s), and kits and compositions comprising such compound(s).

TITLE OF THE INVENTION

SMALL MOLECULE MODULATORS OF PCSK9 AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a National Entry Application of PCT application Serial No PCT/CA2014/* filed on March 14, 2014 and published in English under PCT Article 21(2), which itself claims benefit of U.S. provisional application Serial No. 61/792,249, filed on March 15, 2013. All documents above are incorporated herein in their entirety by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

N. A.

FIELD OF THE INVENTION

The present invention relates to small molecule modulators of proprotein convertase subtilisin-kexin type 9 (PCSK9) and methods of use thereof. More specifically, the present invention is concerned with the use of these molecules in the treatment of low-density lipoproteins (LDL)-cholesterol-related diseases or disorders.

BACKGROUND OF THE INVENTION

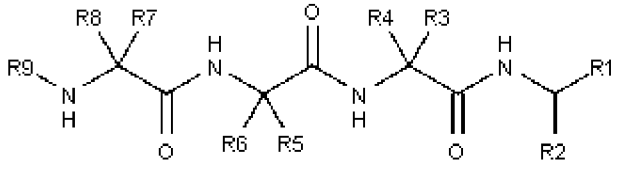
Complications resulting from cardiovascular disorders are the main cause of death worldwide, affecting ~13 million individuals/year, as compared to ~6 million/year due to various forms of cancer. One of the most potent cardiovascular risk factors is elevated levels of low-density lipoprotein (LDL) cholesterol (LDL-C). The incidence of cardiovascular pathologies is expected to increase dramatically in the next two decades. Clinical trial data has demonstrated that reductions in LDL cholesterol levels are related to the rate of coronary events (Law *et al.*, 2003 *BMJ* **326**:1423-1427). Moderate lifelong reduction in plasma LDL cholesterol levels has been shown to be substantially correlated with a significant reduction in the incidence of coronary events (Cohen *et al.*, *N. Engl. J. Med.* **354**:1264-1272), even in populations with a high prevalence of non-lipid-related cardiovascular risk factors. Accordingly, there is great benefit to be gained from the managed control of LDL cholesterol levels. Among important cholesterol-lowering drugs are statins (Briel, M., Nordmann, A. J., and Bucher, H. C. *Curr.Opin.Lipidol.*, **16**: 601-605, 2005). Though well tolerated by the majority of patients, adverse side effects are being compiled (https://www.statineffects.com/info/). The combination of statins with ezetimibe, an intestinal sterol transporter blocker, further reduces LDL-C by ≤ 20%.

Thus, there is a need for the development of alternative strategies to decrease levels of circulating LDL-C (Brown, M. S. and Goldstein, J. L. *Science*, **311**: 1721-1723, 2006; Tall, A. R. *N.Engl.J Med.*, **354**: 1310-1312, 2006).

The low-density lipoprotein receptor (LDLR) is a key player in the regulation of circulating levels of LDL in the bloodstream. Reduced levels of LDLR are associated with elevated plasma cholesterol (LDL-C) which is strongly correlated with an increased risk of atherosclerosis and coronary heart disease – the leading cause of death in industrialized civilizations. Agents that effect an increase of LDLR protein levels at the cell surface would thereby provide a means to normalize LDL-C in an individual affected by plasma hypercholesterolemia. The proprotein convertase PCSK9 decreases LDLR levels by enhancing its degradation in endosomes/lysosomes. It has been established in a number of clinical trials that inhibition of PCSK9-mediated LDLR degradation with monoclonal antibodies dose-dependently decrease LDL-C in healthy human subjects (Lambert et. al., The PCSK9 Decade', *J. Lipid Res*. 2012, *53(12)*, 2515-24 and references cited therein). From a cost and convenience of dosing standpoint, the development of an orally-available, small molecule agent which decreases the amount of secreted PCSK9 would be a desirable alternative to intravenous or subcutaneous delivery of a biologic agent such as an antibody or an oligonucleotide tailored to disrupt the PCSK9 mediated degradation of LDLR.

SUMMARY OF THE INVENTION

More specifically, in accordance with the present invention, there is provided a compound of Formula (I):



or a pharmaceutically acceptable salt, hydrate, solvate, or racemic mixture or stereoisomer thereof,

wherein: R1 is $-CH(OH)R_a$ or $-B(OR_b)(OR_c)$;

R2 is –H, $-CH_2R_d$, $-CHR_d(R_e)$, $-CH_2(CH_2)_mC(O)R_j$, $-CH_2(CH_2)_mC(O)N(R_d)R_e$ or $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$;

R3, R4, R5, R6, R7 and R8 are identical or different, and are independently hydrogen or one of the following groups: a C1-6 alkyl, a C1-6 haloalkyl, a C1-6 thioalkyl, an alkenyl, an alkynyl, an aryl, and an heteroaryl group, wherein said group is optionally substituted with one or more C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, aheteroaryl, –CN, $-C(O)N(R_f)R_g$, $-C(O)OR_f$, $-C(R_f)(R_g)OR_h$, $-OR_f$, $-OC(O)OR_f$, $-OC(O)NR_f(R_g)$, $-SR_f$, $-S(O)_nR_f$, $S(O)_nN(R_f)R_g$, $-S(O)_nN(R_f)C(O)R_g$, $-N(R_f)R_g$, $-N(R_f)C(O)R_g$, $—N(R_f)C(O)OR_g$, $-N(R_f)C(O)N(R_g)(R_h)$,$-N(R_f)S(O)_nR_g$, and $-N(R_f)S(O)N(R_g)R_h$ substituents;

when (R3 and R4) or (R5 and R6) or (R7 and R8) are not hydrogen, the bracketed pairs can also be linked with –C(O) –, $–CO_2–$, –C(O)N(H)–, $–C(O)N(R_x)–$ –O–, –NH–, $–N(R_x)–$, –S–, $–S(O)_n–$, $–S(O)_nN(H)–$, $–S(O)_nN(R_x)–$ radicals to form cyclic structures;

R9 is $R_iC(O)–$, $R_iS(O)_n–$, $R_iOC(O)–$, $R_iNHC(O)$ –, $R_iNHS(O)_n–$, $R_k(R_l)NC(O)–$, $R_l(R_l)NS(O)_n–$; or one or more amino acids residues;

R_a is C1-3 alkyl, C1-2 fluoroalkyl or cyclopropyl;

R_b and R_c are identical or different, and are independently H or C1-6 alkyl, or can be connected together to form a cyclic 5- or 6-membered ring structure, or fused with additional aliphatic or aromatic ring systems, the cyclic 5- or 6-membered ring structure or aliphatic or aromatic ring systems being optionally substituted with one or more C1-6 alkyl and/or C1-6 haloalkyl substituents;

R_d and R_e are identical or different, and are independently H or one of the following groups: a C1-3 alkyl, a C1-3 haloalkyl or a C3-4 cycloalkyl group, or can be connected together directly or with –C(O)–, –C(O)O–, $–C(O)N(R_x)–$ –O–, $–N(R_x)–$, –S–, $–S(O)_n–$,or $–S(O)_nN(R_x)–$ radicals to form cyclic 3-8 membered ring structures;

R_f, R_g, R_h, R_k and R_l are identical or different, and are independently H or one of the following groups: a C1-6 alkyl, a C1-6 haloalkyl or a C3-4 cycloalkyl group, or can be connected together directly or with –C(O)–, –C(O)O–,–C(O)N(R_x)– –O–, $–N(R_x)–$, –S–, $–S(O)_n–$ or $–S(O)_nN(R_x)–$ radicals to form cyclic 3-8 membered ring structures;

R_i is a C1-10 alkyl, a C3-8 cycloalkyl, a C1-10 haloalkyl, a heterocyclyl, an aryl or a heteroaryl group, wherein the group is optionally substituted with one or more of a halogen, C-1-6 aminoalkyl, C1-6 heteroalkyl, C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, heteroaryl and heterocyclyl groups;

R_j is OR_d or $N(R_d)(R_e)$;

R_x is H, C1-6 alkyl, C1-6 haloalkyl, C3-4 cycloalkyl, $–C(O)R_y$, $–C(O)OR_y$, $–C(O)NH_2$, $–C(O)NH(R_y)$ or $–C(O)NHS(O)_nR_y$;

R_y is C1-6 alkyl, C1-6 haloalkyl or C3-4 cycloalkyl;

m is an integer of value 0 or 1; and

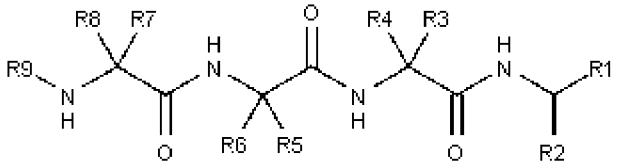
n is an integer of value 1 or 2,

provided that:

when R1 is $–CH(OH)R_a$, R2 is $–CHR_d(R_e)$, $–CH_2(CH_2)_mC(O)R_j$, $–CH_2(CH_2)_mC(O)N(R_d)R_e$ or $–CH_2(CH_2)_mS(O)_nN(R_d)R_e$; and

when R1 is $–B(OR_b)(OR_c)$, R2 is –H, $–CH_3$, $–CHR_d(R_e)$, $–CH_2(CH_2)_mC(O)R_j$, $–CH_2(CH_2)_mC(O)N(R_d)R_e$ or $–CH_2(CH_2)_mS(O)_nN(R_d)R_e$.

More specifically, in accordance with the present invention, there is provided a Compound of Formula (I):



(I)

or a pharmaceutically acceptable salt, hydrate, solvate, or racemic mixture or stereoisomer thereof,

wherein:

R1 is $-CH(OH)R_a$ or $-B(OR_b)(OR_c)$;

R2 is $-H$, $-CH_2R_d$, $-CHR_d(R_e)$, $-CH_2(CH_2)_mC(O)R_j$, $-CH_2(CH_2)_mC(O)N(R_d)R_e$ or $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$;

R3, R4, R5, R6, R7 and R8 are identical or different, and are independently hydrogen or one of the following groups: a C1-6 alkyl, a C1-6 haloalkyl, a C1-6 thioalkyl, a C1-6 aminoalkyl, an alkenyl, an alkynyl, a cycloalkyl, an heterocyclyl, an aryl, and an heteroaryl group, wherein said group is optionally substituted with one or more C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, an heteroaryl, $-CN$, $-C(O)N(R_f)R_g$, $-C(O)OR_f$, $-C(R_f)(R_g)OR_h$, $-OR_f$, $-OC(O)OR_f$, $-OC(O)NR_f(R_g)$, $-SR_f$, $-S(O)_nR_f$, $-S(O)_nN(R_f)R_g$, $-S(O)_nN(R_f)C(O)R_g$, $-N(R_f)R_g$, $-N(R_f)C(O)R_g$, $-N(R_f)C(O)OR_g$, $-N(R_f)C(O)N(R_g)(R_h)$, $-N(R_f)S(O)_nR_g$, and $-N(R_f)S(O)N(R_g)R_h$ substituents;

when (R3 and R4) or (R5 and R6) or (R7 and R8) are not hydrogen, the bracketed pairs can also be linked with $-C(O)-$, $-CO_2-$, $-C(O)N(H)-$, $-C(O)N(R_x)-$, $-O-$, $-NH-$, $-N(R_x)-$, $-S-$, $-S(O)_n-$, $-S(O)_nN(H)-$, $-S(O)_nN(R_x)-$ radicals to form cyclic structures;

R9 is $R_iC(O)-$, $R_iS(O)_n-$, $R_iOC(O)-$, $R_iNHC(O)-$, $R_iNHS(O)_n-$, $R_k(R_l)NC(O)-$, $R_l(R_l)NS(O)_n-$; $R_mOR_iC(O)-$, $R_mC(O)R_iC(O)-$ or one or more amino acids residues;

R_a is C1-3 alkyl, C1-2 fluoroalkyl or cyclopropyl;

R_b and R_c are identical or different, and are independently H or C1-6 alkyl, or can be connected together to form a cyclic 5- or 6-membered ring structure, or fused with additional aliphatic or aromatic ring systems, the cyclic 5- or 6-membered ring structure or aliphatic or aromatic ring systems being optionally substituted with one or more C1-6 alkyl and/or C1-6 haloalkyl substituents;

R_d and R_e are identical or different, and are independently H or one of the following groups: a C1-3 alkyl, a C1-3 haloalkyl or a C3-4 cycloalkyl group, or can be connected together directly or with $-C(O)-$, $-C(O)O-$, $-C(O)N(R_x)-$ $-O-$, $-N(R_x)-$, $-S-$, $-S(O)_n-$,or $-S(O)_nN(R_x)-$ radicals to form cyclic 3-8 membered ring structures;

R_f, R_g, R_h, R_k and R_l are identical or different, and are independently H or one of the following groups: a C1-6 alkyl, a C1-6 haloalkyl or a C3-4 cycloalkyl group, or can be connected together directly or with $-C(O)-$, $-C(O)O-$,$-C(O)N(R_x)-$ $-O-$, $-N(R_x)-$, $-S-$, $-S(O)_n-$ or $-S(O)_nN(R_x)-$ radicals to form cyclic 3-8 membered ring structures;

R_i is a C1-10 alkyl, a C1-10 heteroalkyl, a C3-8 cycloalkyl, a C1-10 haloalkyl, a heterocyclyl, an aryl, a heteroaryl, a C1-10 alkyl-C3-8 cycloalkyl, a C1-10 alkyl-heterocyclyl, a C1-10 alkyl-aryl, a C1-10 alkyl-heteroaryl, a C1-10 heteroalkyl-C3-8 cycloalkyl, a a C1-10 heteroalkyl-heterocyclyl, a C1-10 heteroalkyl-aryl or a C1-10 heteroalkyl-heteroaryl group, wherein the group is optionally substituted with one or more of a halogen, C1-6 aminoalkyl, C1-6 heteroalkyl, C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, heteroaryl and heterocyclyl groups;

R_m is a C1-10 alkyl, a C1-10 heteroalkyl, a C3-8 cycloalkyl, a C1-10 haloalkyl, a heterocyclyl, an aryl or a heteroaryl group, wherein the group is optionally substituted with one or more of a halogen, C1-6 aminoalkyl, C1-6 heteroalkyl, C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, heteroaryl and heterocyclyl groups;

R_j is OR_d or $N(R_d)(R_e)$;

R_x is H, C1-6 alkyl, C1-6 haloalkyl, C3-4 cycloalkyl, $-C(O)R_y$, $-C(O)OR_y$, $-C(O)NH_2$, $-C(O)NH(R_y)$ or $-C(O)NHS(O)_nR_y$;

R_y is C1-6 alkyl, C1-6 haloalkyl or C3-4 cycloalkyl;

m is an integer of value 0 or 1; and

n is an integer of value 1 or 2,

provided that:

1) when R1 is $-CH(OH)R_a$, R2 is $-CHR_d(R_e)$, $-CH_2(CH_2)_mC(O)R_j$, $-CH_2(CH_2)_mC(O)N(R_d)R_e$ or $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$; and

2) when R1 is $-B(OR_b)(OR_c)$, R2 is –H, $-CH_3$, $-CHR_d(R_e)$, $-CH_2(CH_2)_mC(O)R_j$, $-CH_2(CH_2)_mC(O)N(R_d)R_e$ or $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$.

In a specific embodiment of the compound of formula I, (i) when R1 is –CH(OH)-CF3, R9 is not $-C(O)OCH_2$-phenyl or $-C(O)OCH_3$; (ii) when R1 is $B(OH)_2$, R9 is not $-C(O)OCH_3$ or –C(O)-phenyl-phenyl; and/or (iii) when R1 is $CH(OH)CH_2F$, R9 is not $-C(O)(CH_2)_8CH_3$.

In a specific embodiment of the compound of formula I, R1 is $-B(OR_b)(OR_c)$, or $-CH(OH)R_a$; R2 is H or $-CH_2(CH_2)_mC(O)R_j$; R3 is H; R4 is C1-6 alkyl substituted or not, C1-6 thioalkyl substituted or not or aryl substituted or not; R5 is H; R6 is C1-6 alkyl substituted with aryl; R7 is H; R8 is C1-6 alkyl substituted with C1-6 alkyl; and/or R9 is RiOC(O)- or RiC(O)-.

In another specific embodiment of the compound of formula I, R1 is $-B(OR_b)(OR_c)$, or $-CH(OH)R_a$; R2 is H or $-CH_2(CH_2)_mC(O)R_j$; R3 is H; R4 is C1-6 alkyl substituted or not, C1-6 thioalkyl substituted or not or aryl substituted or not; R5 is H; R6 is C1-6 alkyl substituted with aryl; R7 is H; R8 is C1-6 alkyl substituted with C1-6 alkyl; and R9 is RiOC(O)- or RiC(O)-.

In another specific embodiment of the compound of formula I, R1 is $-B(OR_b)(OR_c)$, or $-CH(OH)R_a$; R2 is H or $-CH_2(CH_2)_mC(O)R_j$; R3 is H or C1-6 alkyl substituted or not; R4 is H, C1-6 alkyl substituted or not, C1-6 thioalkyl

substituted or not, aryl substituted or not or cycloalkyl substituted or not; R5 is H; R6 is H, C1-6 alkyl substituted or not, C1-6 thioalkyl substituted or not, aryl substituted or not, or cycloalkane substituted or not ; R7 is H; R8 is H, C1-6 alkyl substituted or not , C1-6 thioalkyl substituted or not, aryl substituted or not , or cycloalkyl substituted or not; and/or R9 is RiOC(O)-, RiC(O)-, $R_mOR_iC(O)$–, $R_mC(O)R_iC(O)$–, or $R_iS(O)_n$–.

In another specific embodiment of the compound of formula I, R1 is $–B(OR_b)(OR_c)$, or $–CH(OH)R_a$; R2 is H or $–CH_2(CH_2)_mC(O)R_j$; R3 is H or C1-6 alkyl substituted or not; R4 is H, C1-6 alkyl substituted or not, C1-6 thioalkyl substituted or not, aryl substituted or not or cycloalkyl substituted or not; R5 is H; R6 is H, C1-6 alkyl substituted or not, C1-6 thioalkyl substituted or not, aryl substituted or not, or cycloalkane substituted or not; R7 is H; R8 is H, C1-6 alkyl substituted or not , C1-6 thioalkyl substituted or not, aryl substituted or not, or cycloalkyl substituted or not; and R9 is RiOC(O)- , RiC(O)-, $R_mOR_iC(O)$–, $R_mC(O)R_iC(O)$–, or $R_iS(O)_n$–.

In a specific embodiment of the compound of formula I, R2; R3 or R4; R5 or R6; and/or R7 R8 can be identical or different and can be the side chain of any natural amino acid except proline in D or L configuration. Other substituents are as defined herein. In a more specific embodiment, (i) R2 is the side chain of glutamine or glycine; (ii) R3 or R4 is the side chain of alanine, phenylalanine, methionine, leucine, valine, glutamine or glycine; (iii) R5 or R6 is the side chain of phenylalanine, glycine, alanine, methionine, or valine; (iv) R7 or R8 is valine, methionine, phenylalanine, glycine or alanine; or (v) any combination of at least two of (i) to (iv).

In another specific embodiment of the compound of formula I, R6 is phenyl-CH_2-; and/or R8 is $(CH_3)_2CH$-. In another specific embodiment, R1 is $–B(OR_b)(OR_c)$. In another specific embodiment, Rb and Rc are H. In another specific embodiment, Rb and Rc are connected to form a cyclic 5 membered ring structure or fused with an aliphatic ring system. In another specific embodiment, R1 is 2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.$0^{2,6}$]decan-4-yl. In another specific embodiment, R1 is $–CH(OH)R_a$. In another specific embodiment, Ra is C1-2 fluoroalkyl. In another specific embodiment, Ra is $-CF_3$. In another specific embodiment, Ra is $–CH_2F$. In another specific embodiment, Ra is C1-3 alkyl. In another specific embodiment, Ra is $–CH_3$. In another specific embodiment, R2 is H. In another specific embodiment, R2 is $–CH_2(CH_2)_mC(O)R_j$. In another specific embodiment, Rj is OR_d. In another specific embodiment, Rd is CH_3. In another specific embodiment, Rj is NH_2.

In another specific embodiment of the compound of formula I, R**3** is H. In another specific embodiment, R3 is C1-6 alkyl substituted or not. In another specific embodiment, R3 is unsubstituted C1-6 alkyl. In another specific embodiment, R3 is CH_3.

In another specific embodiment of the compound of formula I, **R4** is H. In another specific embodiment, R4 is C1-6 alkyl substituted or not. In another specific embodiment, R4 is unsubstituted C1-6 alkyl. In another specific embodiment, R4 is aryl substituted C1-6 alkyl. In another specific embodiment, R4 is $-CH_3$. In another specific embodiment, R4 is CH_3CH_2-. In another specific embodiment, R4 is $-CH_2CH(CH_3)_2$. In another specific embodiment, R4 is aryl substituted C1-6 alkyl. In another specific embodiment, R4 is phenyl-CH_2-. In another specific embodiment, R4 is $-(CH_2)_2C(O)NH2$. In another specific embodiment, R4 is C1-6 thioalkyl substituted or not. In another specific embodiment, R4 is $CH_3S(CH_2)_2$-. In another specific embodiment, R4 is aryl substituted or not. In another specific embodiment, R4 is phenyl-. In another specific embodiment, R4 is cycloalkyl.

In another specific embodiment, **R6** is H. In another specific embodiment, R6 is C1-6 alkyl substituted or not. In another specific embodiment, R6 is substituted C1-6 alkyl. In another specific embodiment, R6 is aryl substituted C1-6 alkyl. In another specific embodiment, R6 is –alkyl-phenyl. In another specific embodiment, R6 is $–CH_2$-phenyl. In another specific embodiment, R6 is $–CH_2$-hydroxyphenyl. In another specific embodiment, R6 is $–(CH_2)_2$-phenyl. In another specific embodiment, R6 is $–CH_2$-indole. In another specific embodiment, R6 is unsubstituted C1-6 alkyl. In another specific embodiment, R6 is unsubstituted C1-6 alkyl. In another specific embodiment, R6 is CH_3. In another

specific embodiment, R6 is -$CH(CH_3)_2$. In another specific embodiment, R6 is C1-6 thioalkyl substituted or not. In another specific embodiment, R6 is $CH_3S(CH_2)_2$-. In another specific embodiment, R6 is aryl substituted or not. In another specific embodiment, R6 is phenyl. In another specific embodiment, R6 is cycloalkyl substituted or not. In another specific embodiment, R6 is benzocyclopentyl.

In another specific embodiment, **R8** is H. In another specific embodiment, R8 is C1-6 alkyl substituted or not. In another specific embodiment, R8 is unsubstituted C1-6 alkyl. In another specific embodiment, R8 is -$CH(CH_3)_2$. In another specific embodiment, R8 is -CH_3. In another specific embodiment, R8 is substituted C1-6 alkyl. In another specific embodiment, R8 is aryl substituted C1-6 alkyl. In another specific embodiment, R8 is –CH_2-phenyl. In another specific embodiment, R8 is C1-6 thioalkyl substituted or not. In another specific embodiment, R8 is $CH_3S(CH_2)_2$-. In another specific embodiment, R8 is aryl substituted or not. In another specific embodiment, R8 is unsubstituted aryl. In another specific embodiment, R8 is phenyl. In another specific embodiment, R8 is cycloalkyl substituted or not. In another specific embodiment, R8 is unsubstituted cycloalkyl. R8 is cyclopentyl. In another specific embodiment, R8 is cyclopropyl.

In another specific embodiment, **R9** is RiOC(O)-. In another specific embodiment, Ri is C1-10 alkyl, substituted or not-. In another specific embodiment, Ri is unsubstituted C1-10 alkyl-. In another specific embodiment, Ri is CH_3-. In another specific embodiment, Ri is substituted C1-10 alkyl-. In another specific embodiment, Ri is $(CH_3)_3C$-. In another specific embodiment, Ri is phenyl-CH_2-.

In another specific embodiment, R9 is RiC(O)-. In another specific embodiment, Ri is aryl substituted or not -. In another specific embodiment, Ri is substituted aryl -. In another specific embodiment, Ri is aryl substituted or not or heteroaryl substituted or not. In another specific embodiment, Ri is aryl substituted or not. In another specific embodiment, Ri is substituted aryl. In another specific embodiment, Ri is aryl-phenyl-. In another specific embodiment, Ri is phenyl-phenyl-. In another specific embodiment, Ri is heteroaryl-phenyl-. In another specific embodiment, Ri is diazirine-phenyl-. In another specific embodiment, Ri is phenyl-phenyl-. In another specific embodiment, Ri is fluorophenyl-. In another specific embodiment, Ri is fluoroalkyl-diazirin-phenyl -. In another specific embodiment, Ri is trifluoromethyl-diazirin-phenyl-. In another specific embodiment, Ri is pyridine-phenyl-. In another specific embodiment, Ri is oxadiazole-phenyl-. In another specific embodiment, Ri is heterocyclyl-phenyl-. In another specific embodiment, Ri is morpholine-phenyl-. In another specific embodiment, Ri is alkyl-phenyl-. In another specific embodiment, Ri is $(CH_3)_2CH$-phenyl-. In another specific embodiment, Ri is $OHCH_2$-phenyl-. In another specific embodiment, Ri is fluorophenyl. In another specific embodiment, Ri is unsubstituted aryl. In another specific embodiment, Ri is phenyl. In another specific embodiment, Ri is heteroaryl substituted or not. In another specific embodiment, Ri is substituted heteroaryl. In another specific embodiment, Ri is aryl–heteroaryl-. In another specific embodiment, Ri is phenyl-heteroaryl-. In another specific embodiment, Ri is phenyl-pyrazole-. In another specific embodiment, Ri is phenyl-methylpyrazole-. In another specific embodiment, Ri is phenyl-thiazole-. In another specific embodiment, Ri is phenyl-pyridine-. In another specific embodiment, Ri is phenyl-furazan-. In another specific embodiment, Ri is heteroaryl-heteroaryl-. In another specific embodiment, Ri is pyridine-isothiazole-. In another specific embodiment, Ri is unsubstituted heteroaryl. In another specific embodiment, Ri is pyridine. In another specific embodiment, Ri is pyrazine. In another specific embodiment, Ri is indole. In another specific embodiment, Ri is 4-[3-trifluoromethyl)-3*H*-diazirin-3-yl]phenyl-. In another specific embodiment, Ri is 3-[3-trifluoromethyl)-3*H*-diazirin-3-yl]phenyl-. In another specific embodiment, Ri is unsubstituted aryl -. In another specific embodiment, Ri is phenyl -. In another specific embodiment, Ri is C1-10 alkyl, substituted or not-. In another specific embodiment, Ri is substituted C1-10 alkyl-. In another specific embodiment, Ri is aryl-C1-10 alkyl-. In another specific embodiment, Ri is phenyl-C1-10 alkyl-. In another specific embodiment, Ri is phenyl-alkyne-. In another specific embodiment, Ri is phenyl-CH_2-.

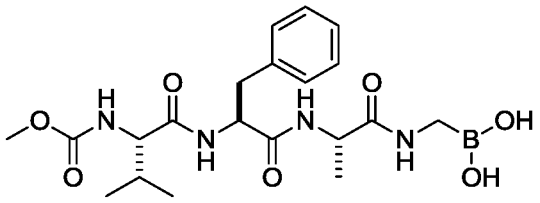
In another specific embodiment, Ri is fluoromethoxy-aryl-(C1-6 alkyl) -. In another specific embodiment, Ri is fluoromethoxy-phenyl-CH_2-. In another specific embodiment, Ri is trifluoromethoxy -phenyl-CH_2 -. In another specific embodiment, Ri is 4-(trifluoromethoxy)phenyl-CH_2 -. In another specific embodiment, Ri is fluoroalkyl-diazirin-phenyl-(C1-10 alkyl). In another specific embodiment, Ri is trifluoromethyl-diazirin-phenyl-CH_2-. In another specific embodiment, Ri is 4-[3-trifluoromethyl)-3*H*-diazirin-3-yl]phenyl-. In another specific embodiment, Ri is 3-[3-trifluoromethyl)-3*H*-diazirin-3-yl]phenyl-. In another specific embodiment, Ri is unsubstituted C1-10 alkyl -. In another specific embodiment, Ri is $CH_3(CH_2)_8$ -.

In another specific embodiment, R9 is $R_mOR_iC(O)$–. In another specific embodiment, R_i is substituted or unsubstituted C1-10 alkyl. In another specific embodiment, R_i is -CH_2-. In another specific embodiment, R_m is substituted or unsubstituted C1-10 alkyl. In another specific embodiment, R_m is -CH_2-. In another specific embodiment, R_m is aryl-CH_2-. In another specific embodiment, R_m is phenyl-CH_2-. In another specific embodiment, R_m is substituted or unsubstituted aryl. In another specific embodiment, R_m is phenyl.

In another specific embodiment, R9 is $R_mC(O)R_iC(O)$–. In another specific embodiment, R_m is heteroaryl. In another specific embodiment, R_m is morpholine. In another specific embodiment, R_i is aryl. In another specific embodiment, R_i is phenyl.

In another specific embodiment, R9 is $R_iS(O)_n$–. In another specific embodiment, R_i is substituted or unsubstituted aryl. In another specific embodiment, R_i is substituted aryl. In another specific embodiment, R_i is substituted phenyl. In another specific embodiment, R_i is phenyl-phenyl.

In another specific embodiment, the compound of formula I is:



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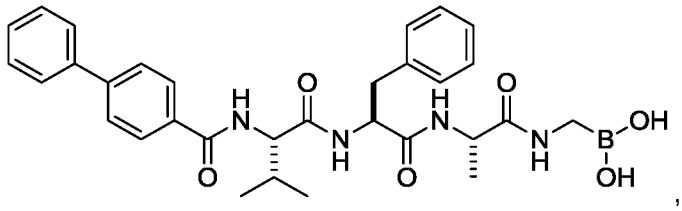

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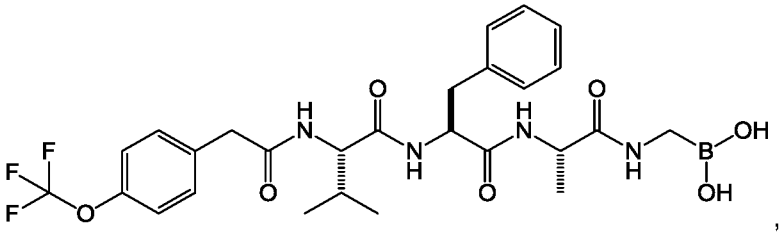

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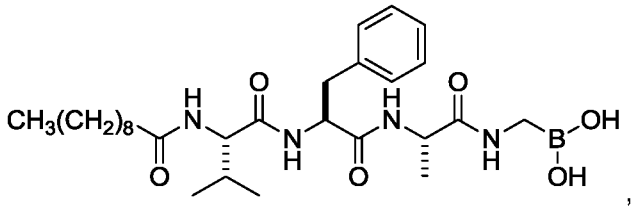

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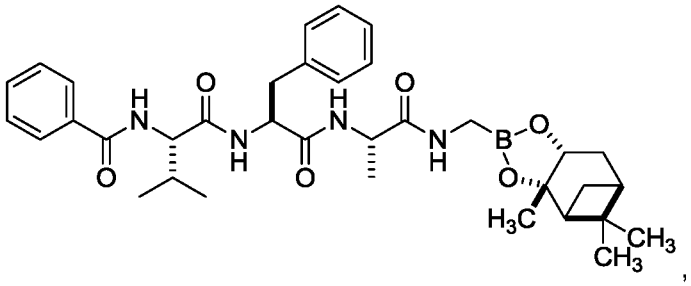

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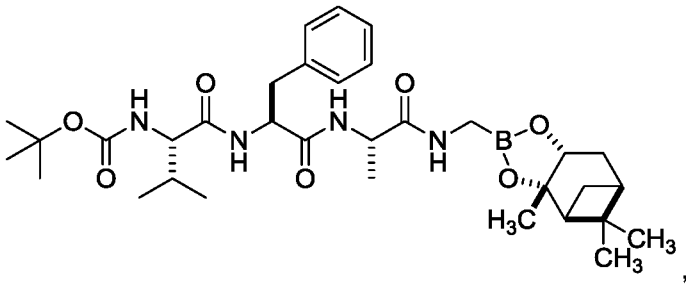

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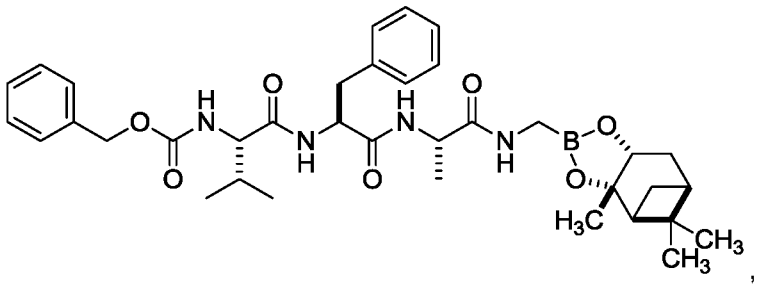

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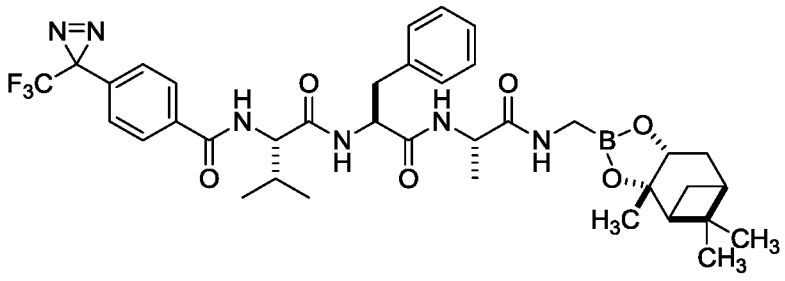

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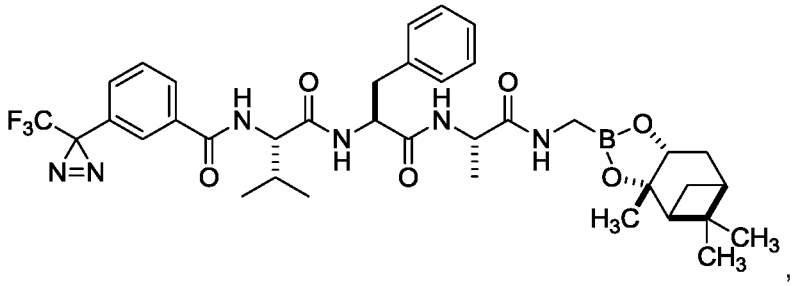

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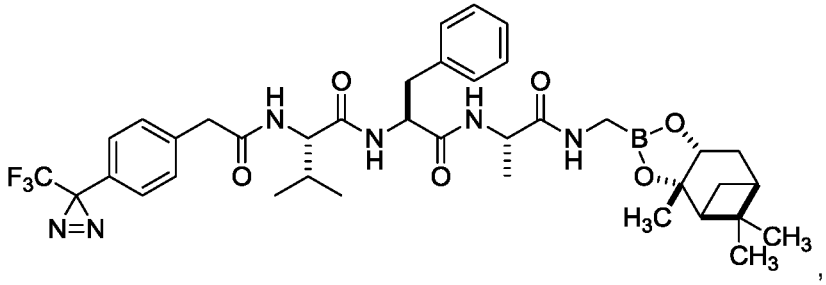

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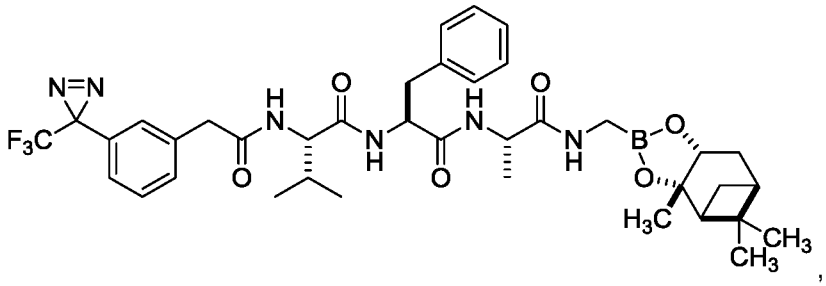

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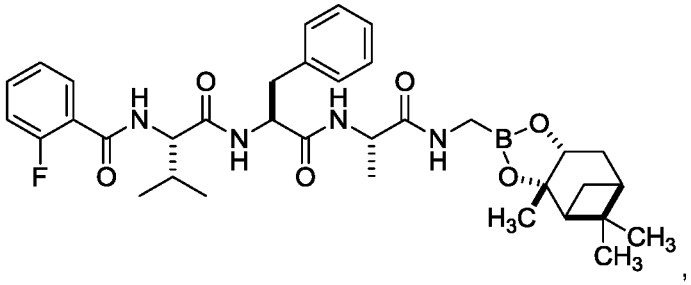

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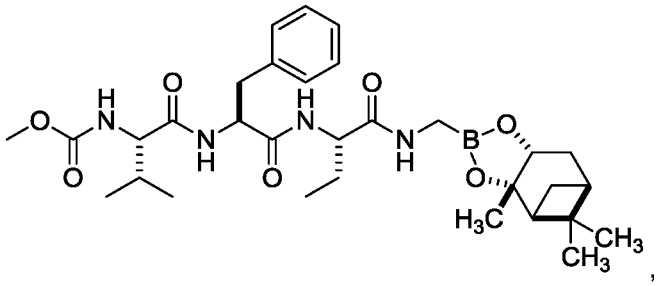

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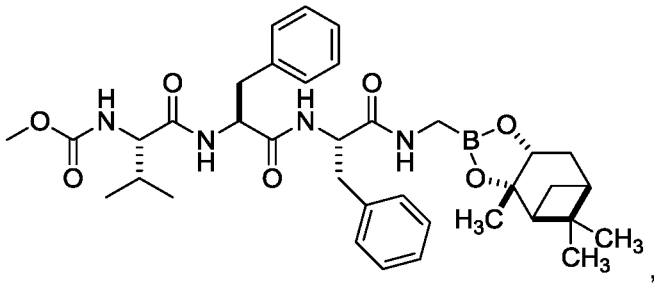

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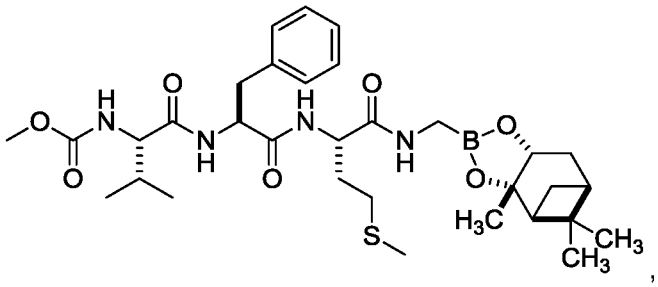

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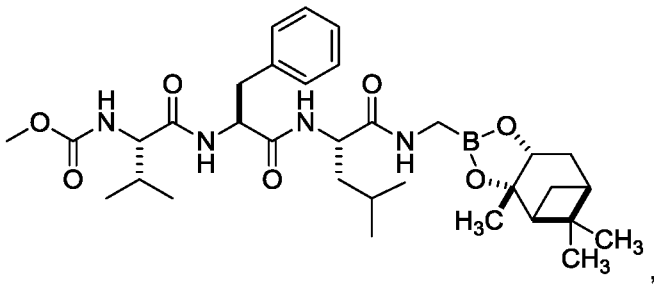

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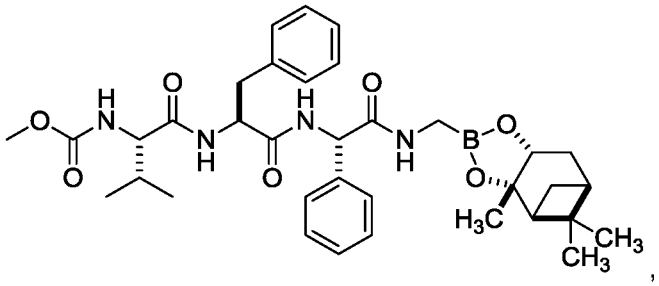

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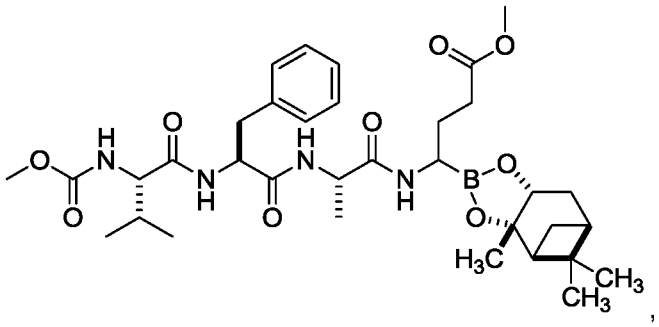

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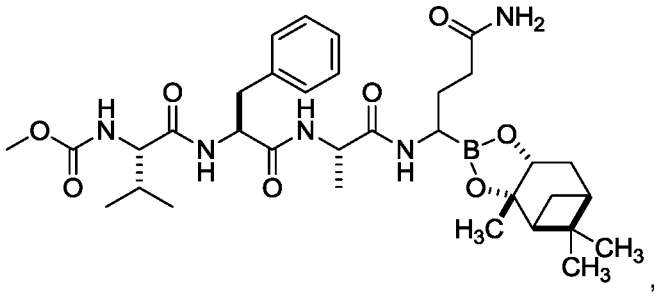

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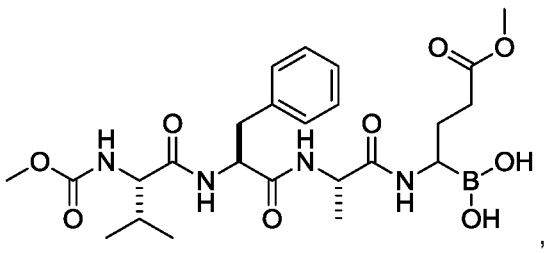

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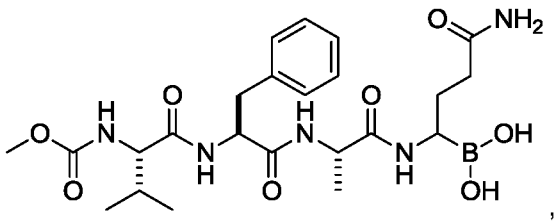

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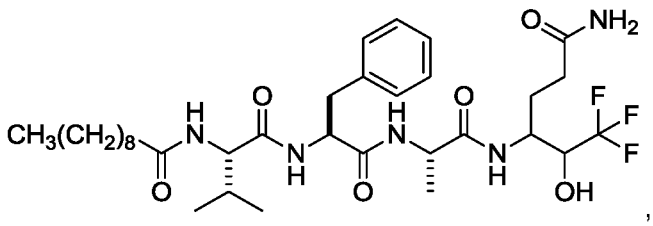

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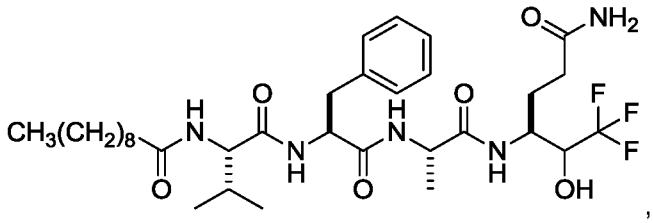

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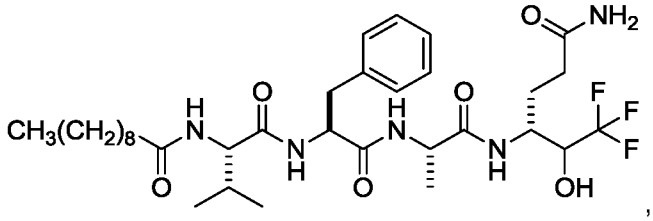

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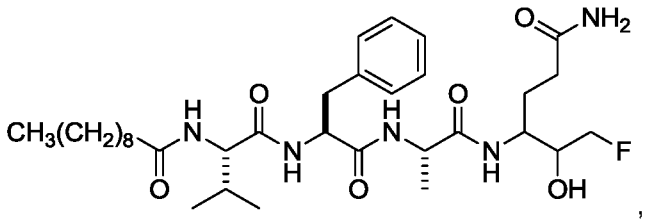

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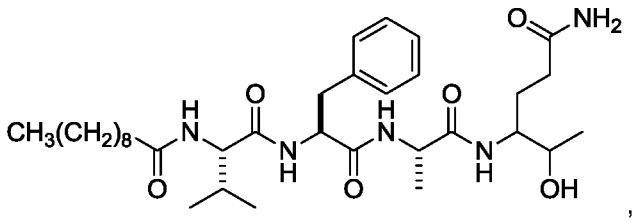

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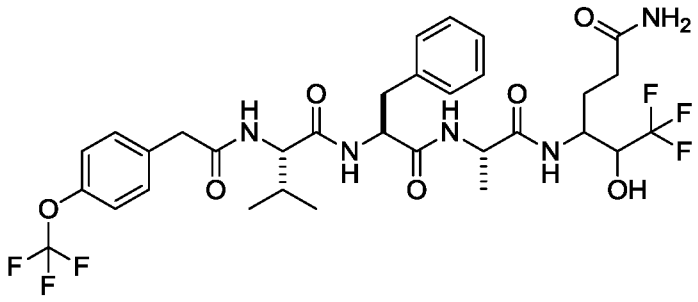

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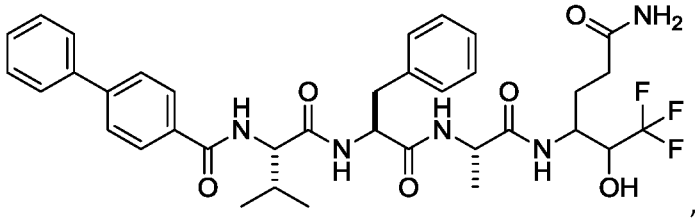

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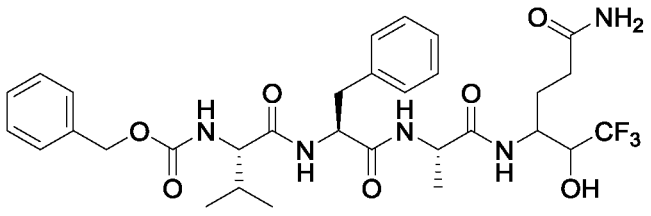

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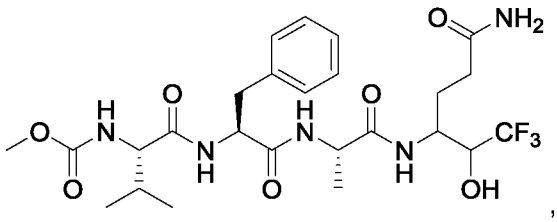

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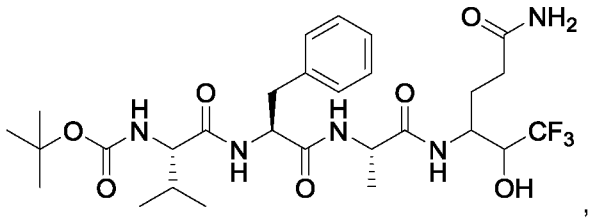

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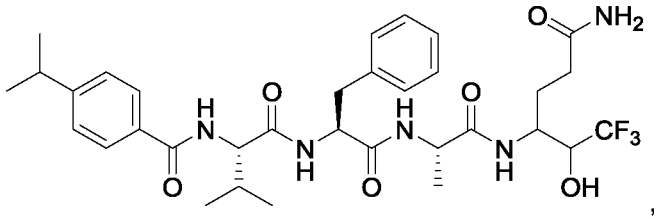

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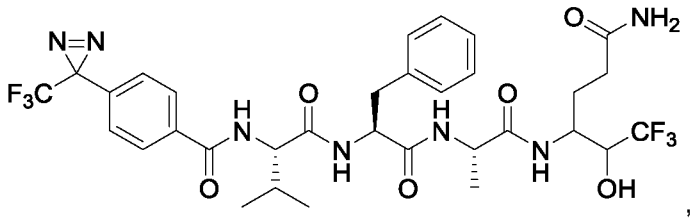

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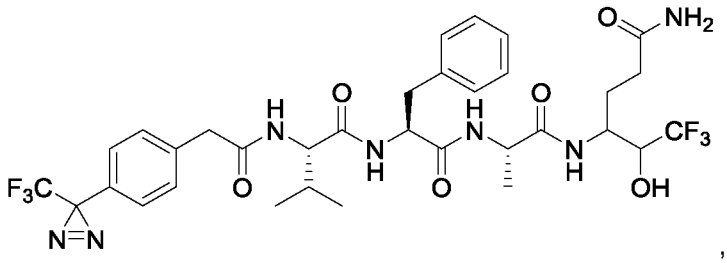

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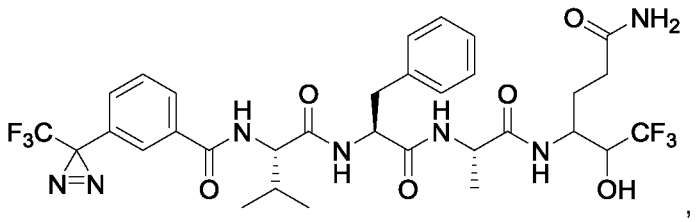

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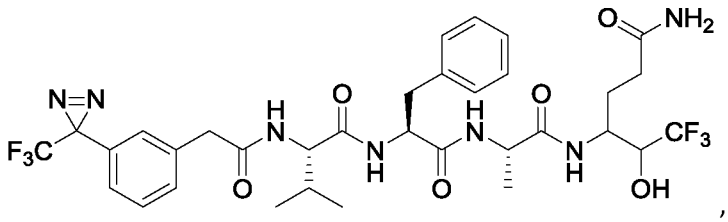

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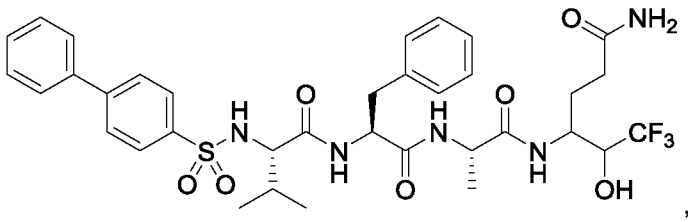

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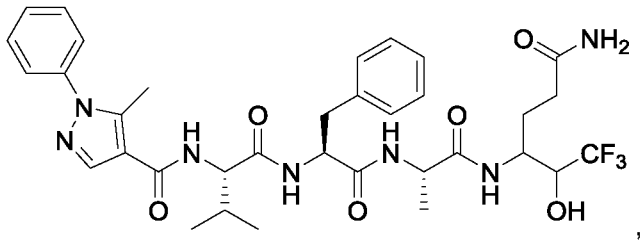

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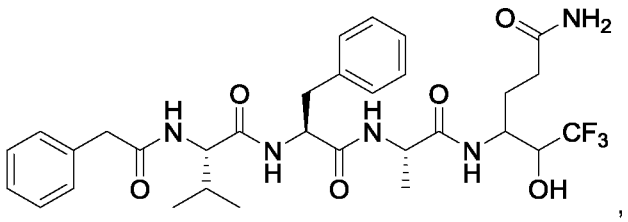

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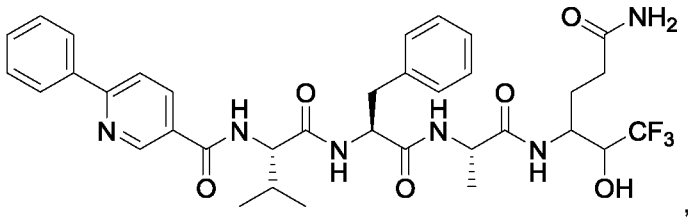

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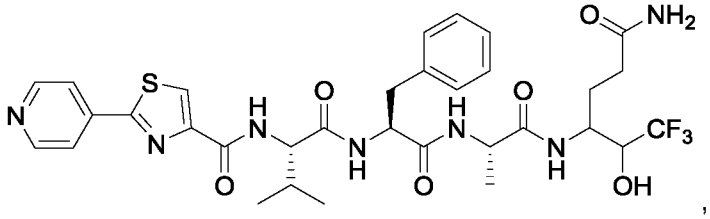

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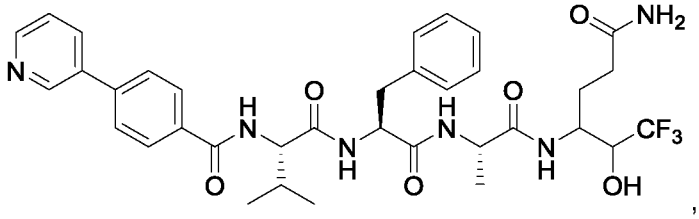

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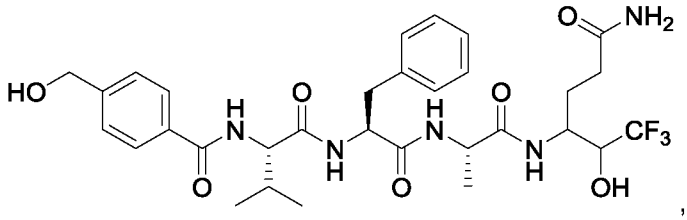

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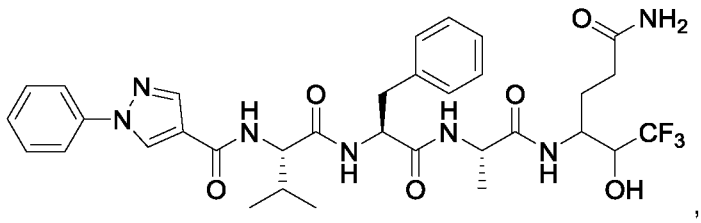

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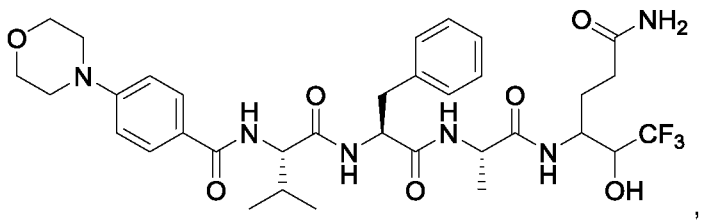

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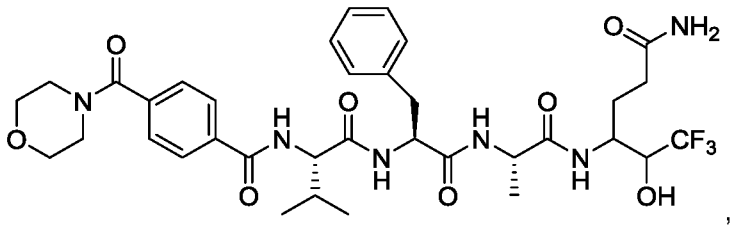

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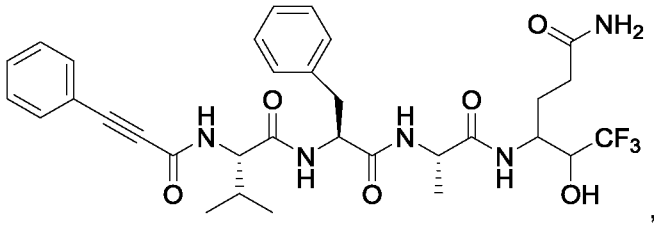

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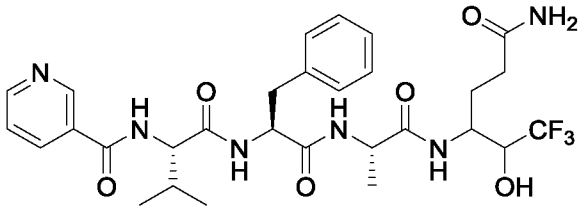

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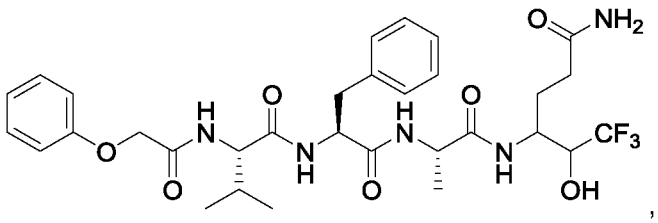

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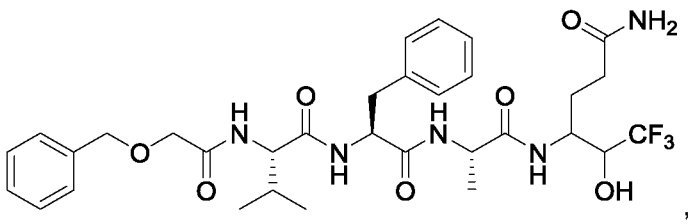

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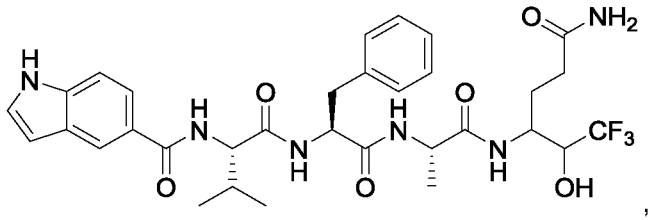

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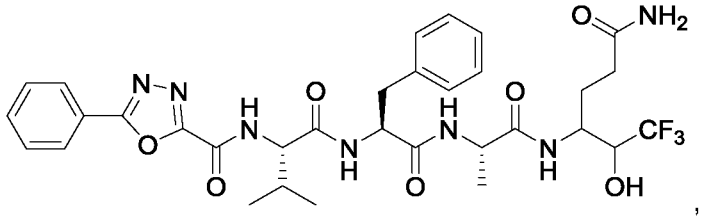

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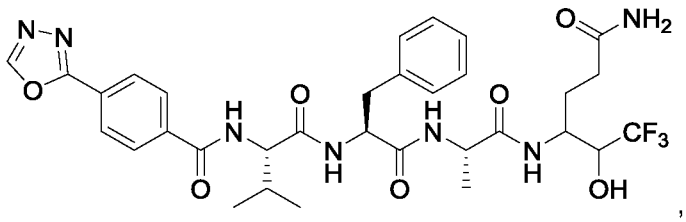

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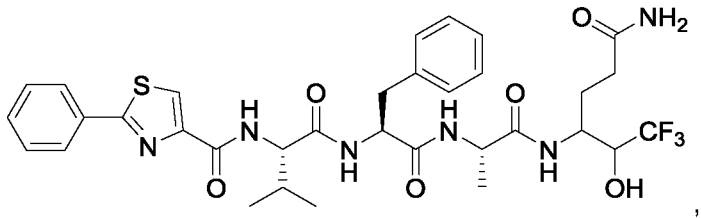

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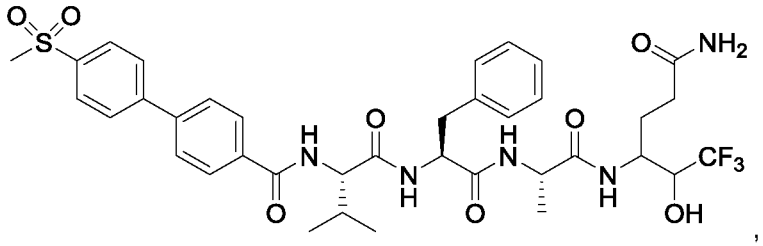

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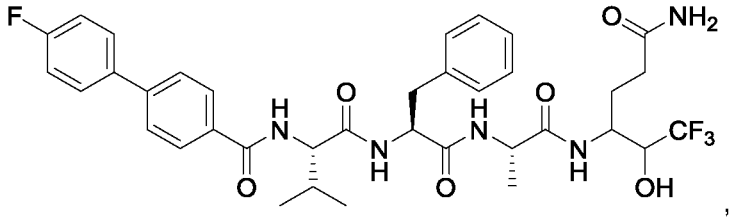

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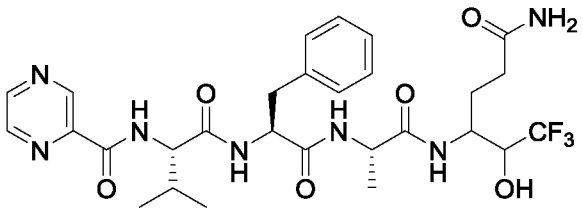

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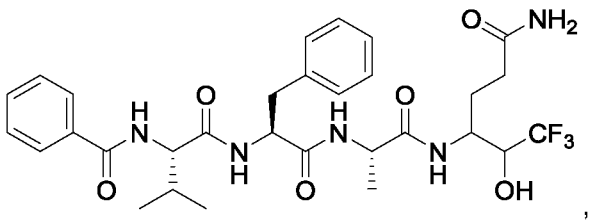

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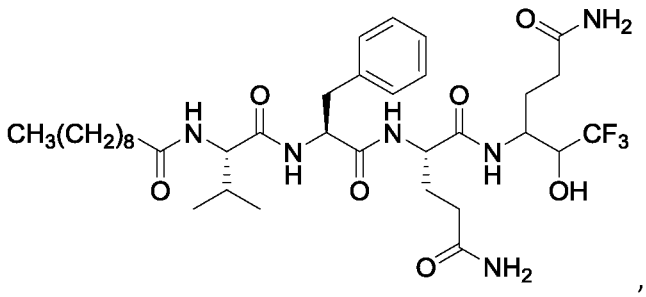

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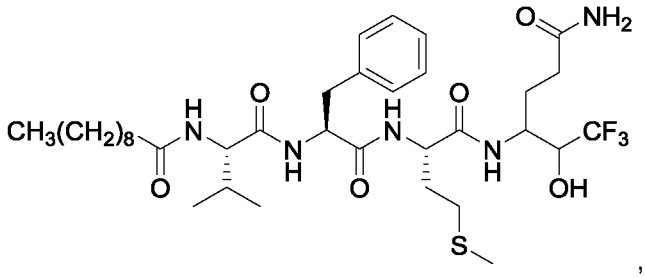

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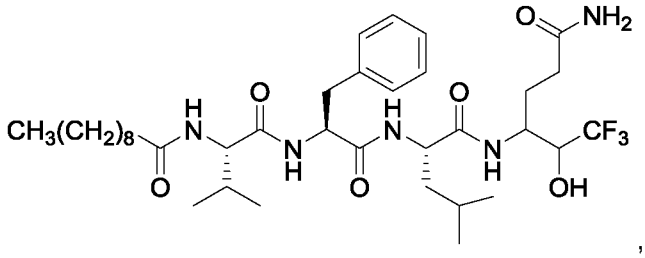

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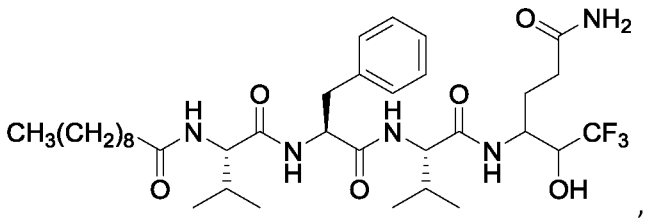

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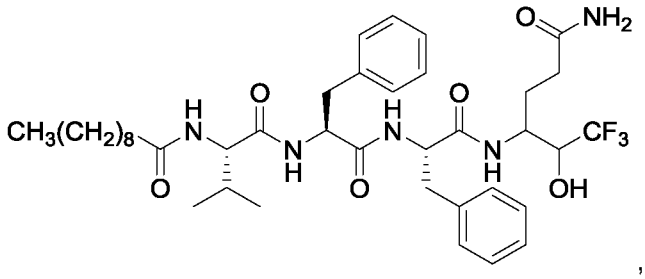

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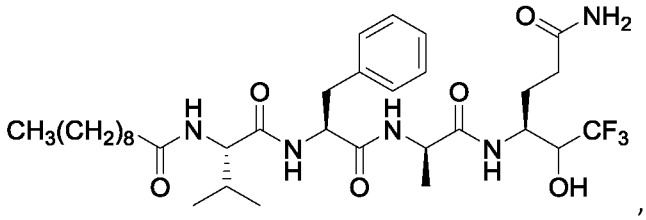

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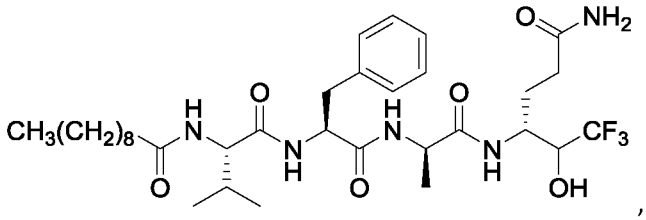

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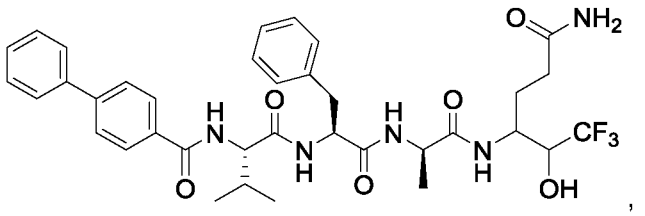

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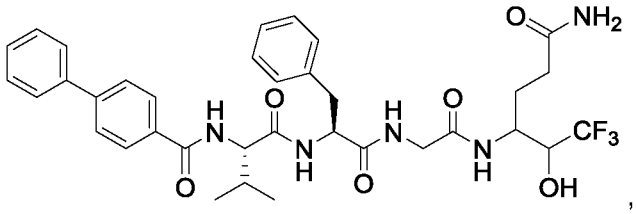

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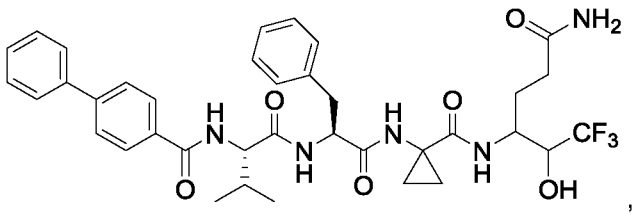

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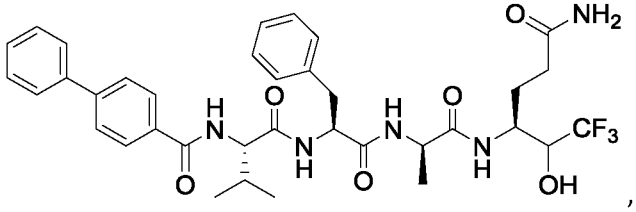

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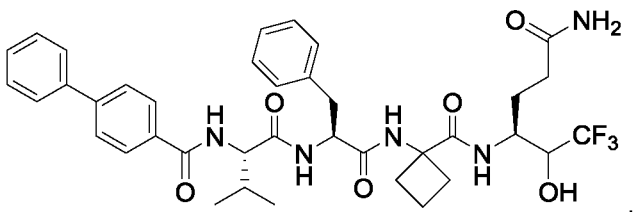

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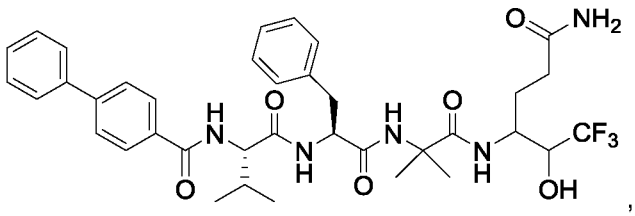

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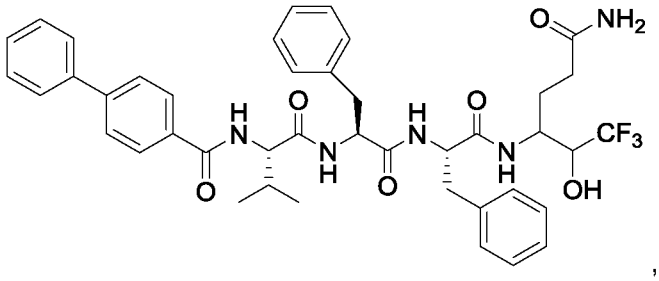

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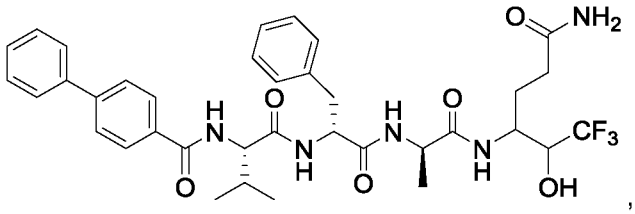

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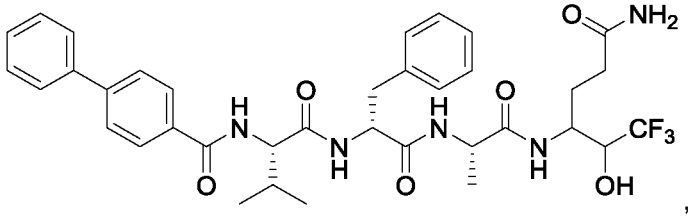

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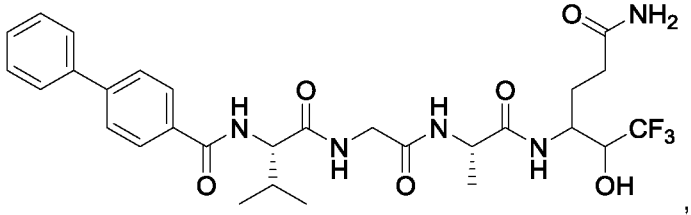

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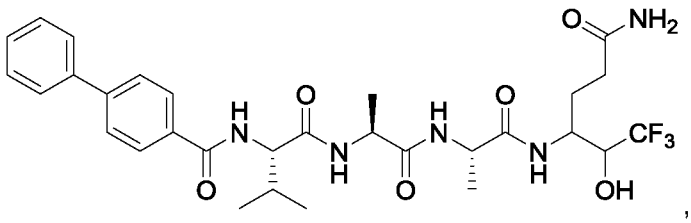

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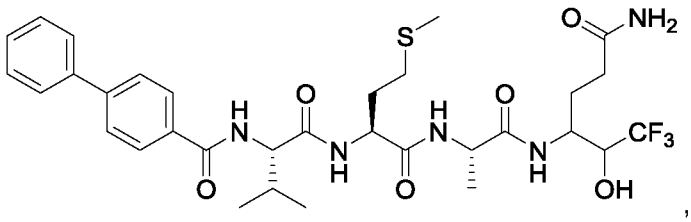

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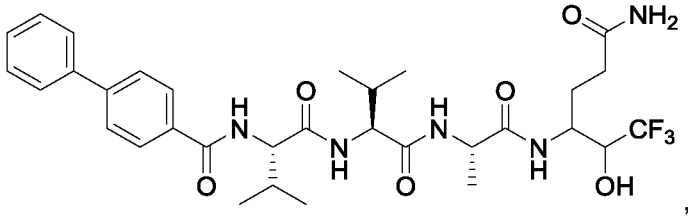

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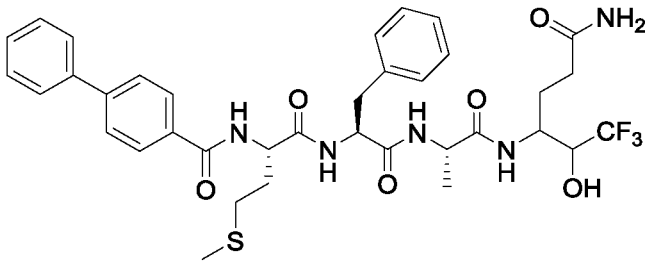

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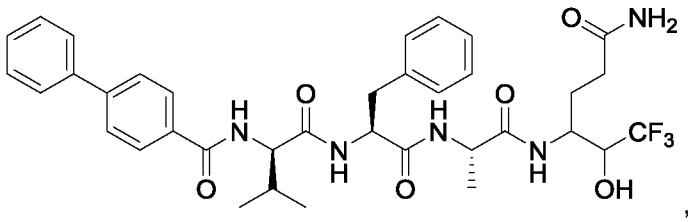

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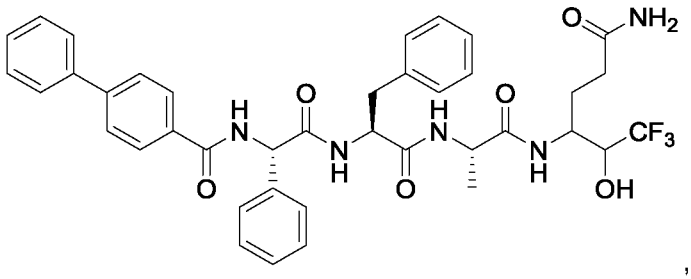

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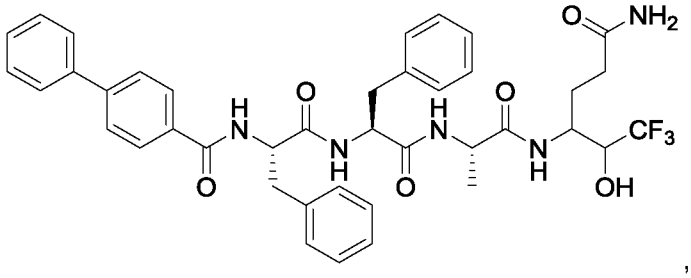

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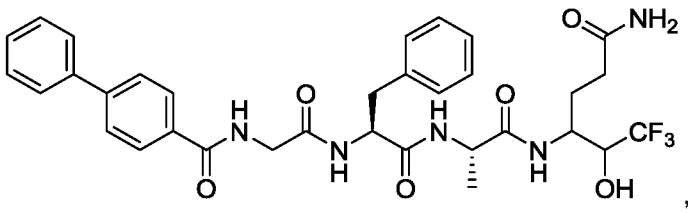

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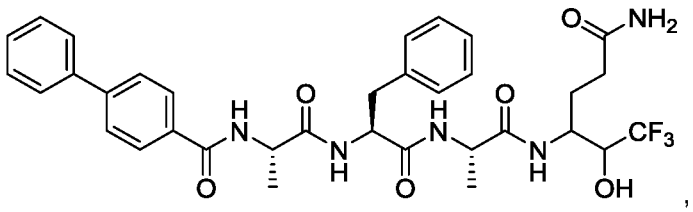

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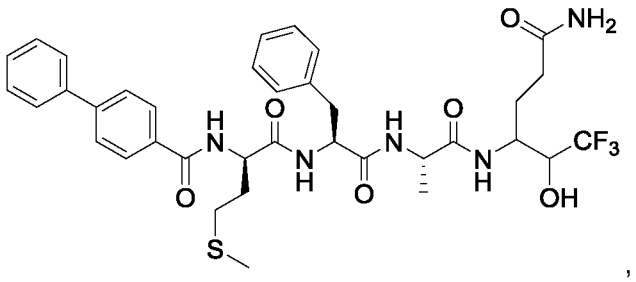

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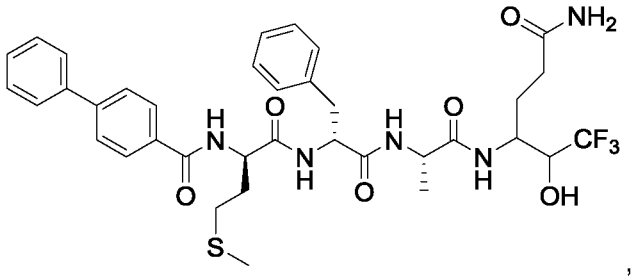

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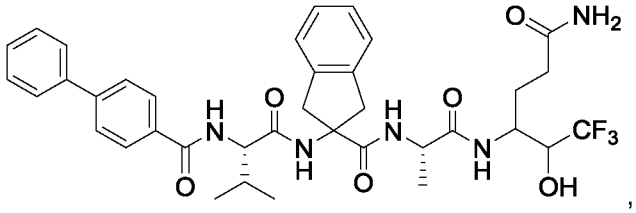

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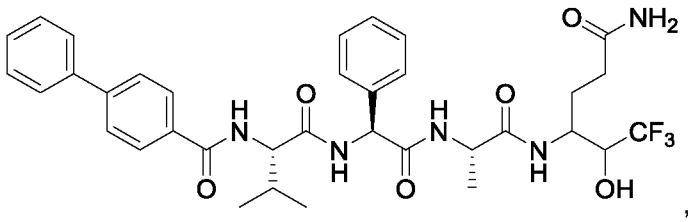

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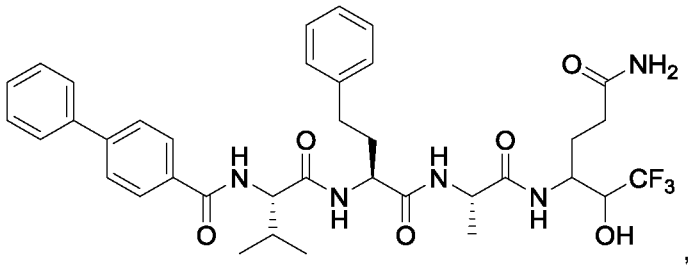

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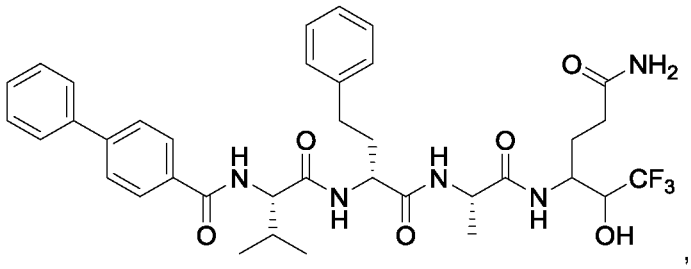

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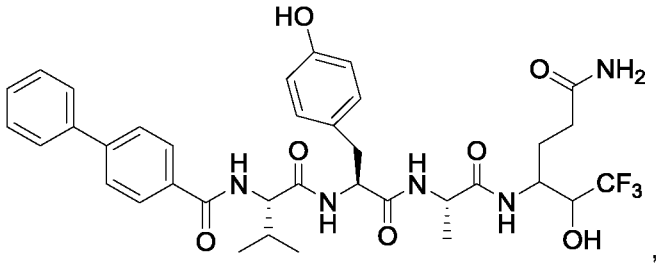

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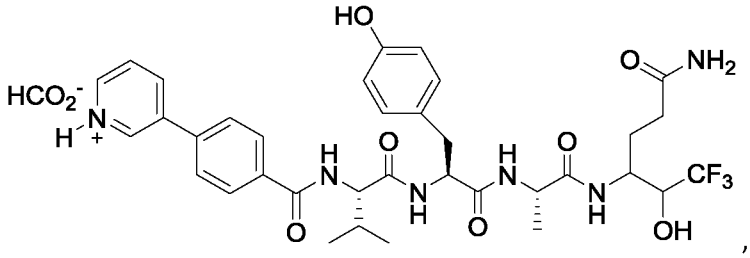

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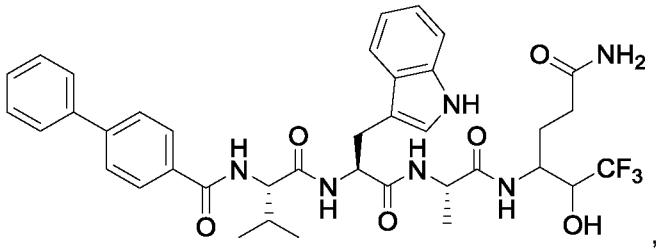

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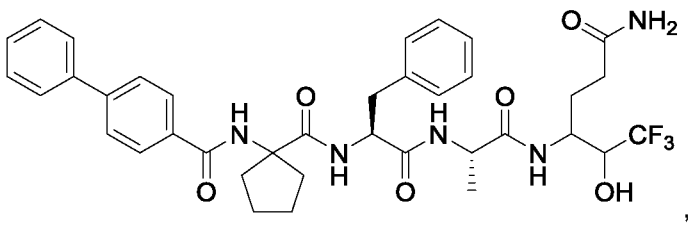

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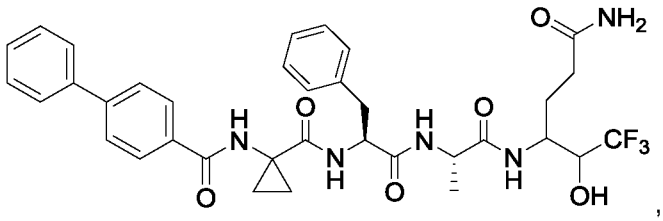
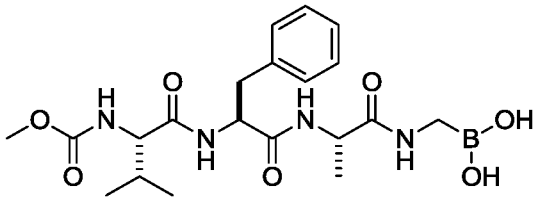


91 .

In a specific embodiment, the compound of the invention is not compound 1; 2; 17; mixture of 21a and 21b; 25 and/or 26.

In accordance with another aspect of the present invention, there is provided a pharmaceutical composition comprising at least one of the compounds of the present invention. In a specific embodiment of the composition, the composition further comprises at least one other compound of the present invention. In another specific embodiment, the composition further comprises at least one other active ingredient which improve a patient's lipid profile. In another specific embodiment, the composition further comprises a pharmaceutical carrier or excipient.

In accordance with another aspect of the present invention, there is provided a compound or a composition of the present invention for use as a medicament. In a specific embodiment, the compound or composition is for the manufacture of a medicament. In another specific embodiment, said medicament is for preventing or treating a low density lipid-cholesterol-related disease or disorder in a subject, with the proviso that the compound is not:



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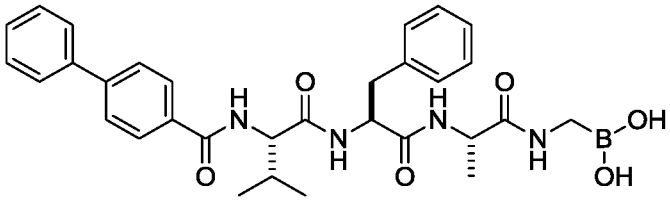

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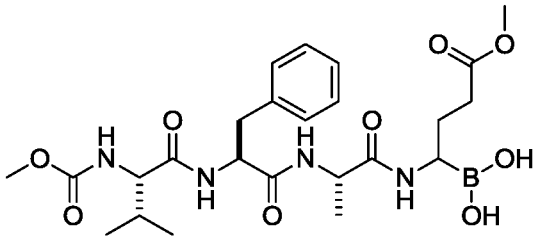

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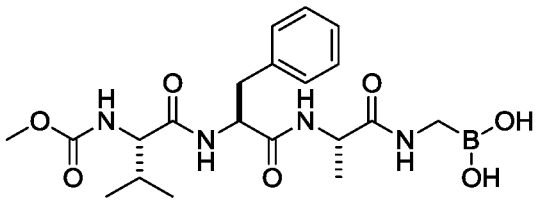
In another specific embodiment of the compound or composition, the low density lipid-cholesterol-related disease or disorder is hypercholesterolemia.

In accordance with another aspect of the present invention, there is provided a method for preventing or treating an LDL-cholesterol-related disease or disorder, comprising administering to a subject in need thereof, a therapeutically effective amount of the compound or the composition of the present invention (*e.g.*, composition comprising the compound of the present invention), with the proviso that the compound is not:



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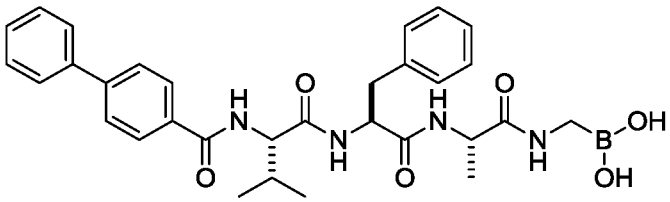

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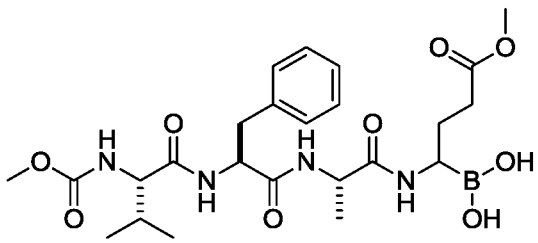

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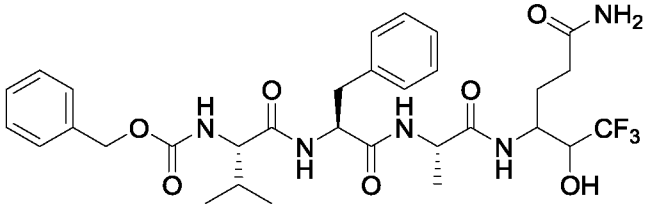

17 25

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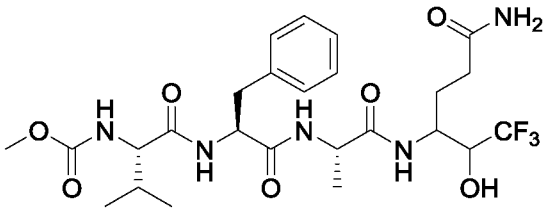
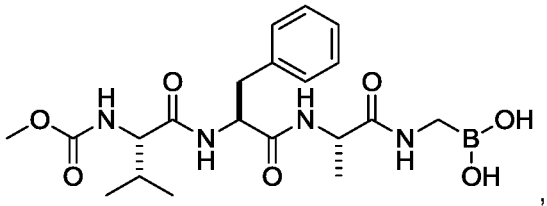


26 .

In a specific embodiment of the method, the low density lipid-cholesterol-related disease or disorder is hypercholesterolemia.

In accordance with another aspect of the present invention, there is provided a use of a compound or composition of the present invention, as a medicament. In a specific embodiment, the use of the compound or composition of the present invention is for preventing or treating a low density lipid-cholesterol-related disease or disorder in a subject, with the proviso that the compound is not:



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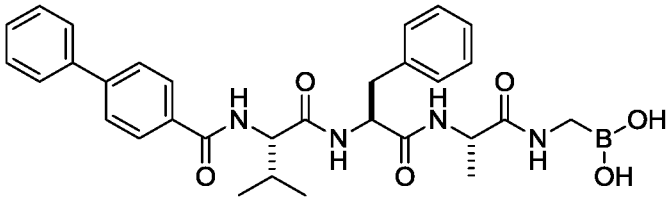

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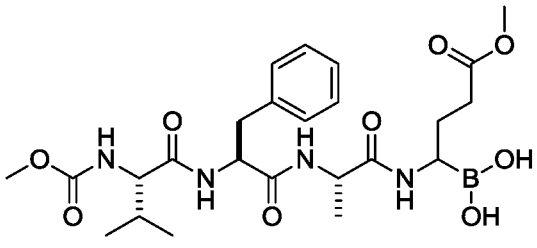

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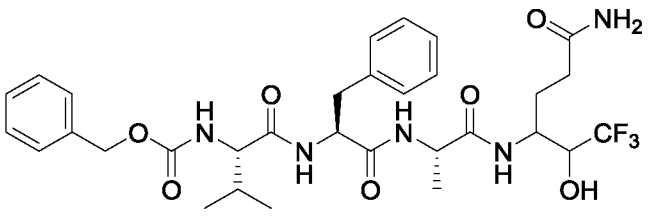

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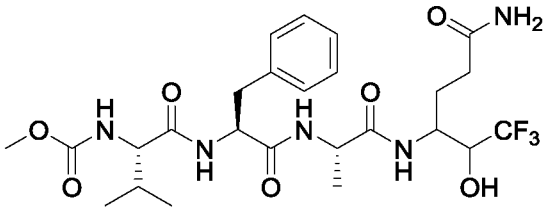
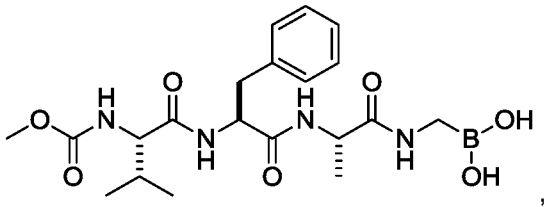


26 .

In a specific embodiment, the use is for the manufacture of a medicament for preventing or treating a low density lipid-cholesterol-related disease in a subject, with the proviso that the compound is not:



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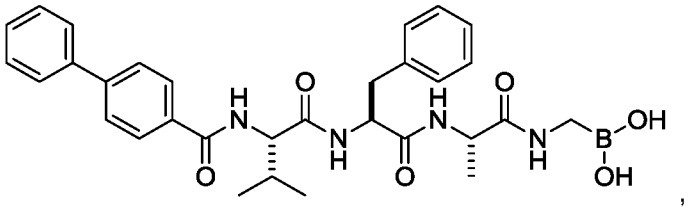

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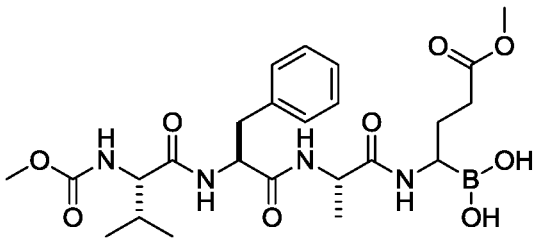

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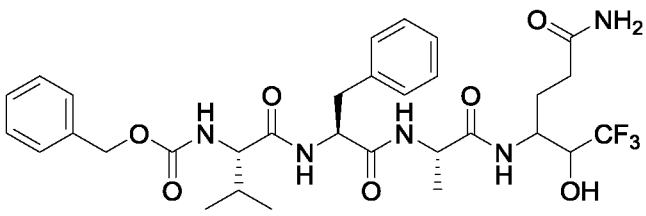

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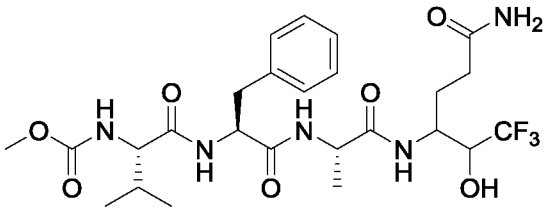
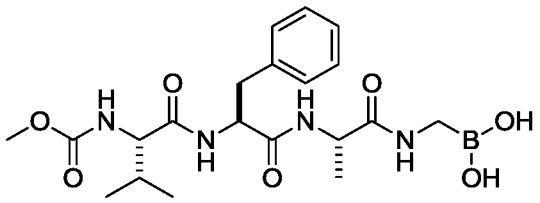


26 .

In another specific embodiment of the use, the low density lipid-cholesterol-related disease or disorder is hypercholesterolemia.

In accordance with another aspect of the present invention, there is provided a kit for preventing or treating a low density lipid-cholesterol-related disease or disorder in a subject comprising (i) at least one of the compounds or composition of the present invention, and (ii) (a) at least one other active ingredient which improve a patient's lipid profile; (b) at least one other compound or composition of the present invention; (c) a container for the compound and/or active ingredients; and/or (d) instructions to use the compound for preventing or treating the low density lipid-cholesterol-related disease or disorder in the subject, with the proviso that the compound is not:



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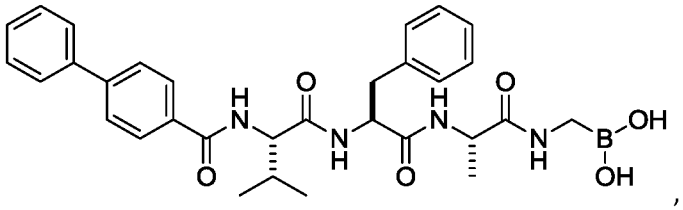

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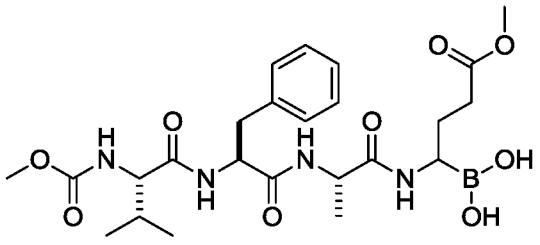

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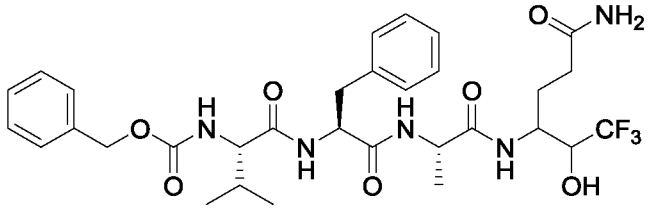

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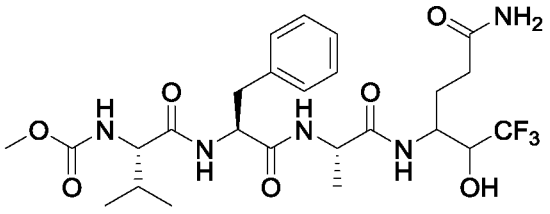


26

In a specific embodiment of the kit, the low density lipid-cholesterol-related disease or disorder is hypercholesterolemia.

BRIEF DESCRIPTION OF THE DRAWINGS

In the appended drawings:

Figure 1 shows (A) the effect of 19 compounds on PCSK9 secretion, as part of the initial screening. HepG2 cells stably expressing WT PCSK9(+V5) were incubated for 24h in culture media alone (-), or containing either DMSO control (DMSO) or 100 µM of the indicated compounds. The PCSK9 levels in the recovered media and cell fractions were quantified by ELISA and expressed as ng PCSK9/ml fraction. The inhibitory effect of the indicated compounds on PCSK9 secretion was estimated from the decrease of the media/cell ratio relative to DMSO control. The stars identify compounds having an inhibitory activity; and (B) the structure of compounds a to j;

Figure 2 shows the dose dependent effect of 3 compounds on PCSK9 secretion. HepG2 cells stably expressing WT PCSK9(+V5) were incubated for 24h in the absence (DMSO) or presence of increasing concentrations of each indicated compound. The culture media and cells were recovered and the level of PCSK9 in each fraction was quantified by ELISA. The level of PCSK9 in cells was corrected for the amount of total protein, which was measured by the Bio-Rad DC protein assay. At each concentration, the inhibitory effect of the indicated compounds on PCSK9 secretion was estimated from the decrease of the media/cell ratio relative to DMSO control;

Figure 3 shows the cell toxicity assay for 5 compounds relative to DMSO control. HepG2 cells stably expressing WT PCSK9(+V5) were incubated for 24h in the absence (DMSO) or presence of increasing concentrations of each indicated compound. The level of toxicity was measured by a MTT cell toxicity assay;

Figure 4 shows the effect of a PCSK9 inhibitory compound on the level of LDLR at the surface of HepG2 naïve cells. Cells were incubated for 20h in the absence (DMSO) or presence of 33.3 µM of compound 19. Non-permeabilized cells were analyzed by immunofluorescence. LDLR was stained using a human LDLR Ab (green labeling). Three different fields are shown for each DMSO control and compound 19 condition. Note the increase of LDLR at the cell surface induced by compound 19;

Figure 5 shows the effect of PCSK9 inhibitory compounds on the activity of cell surface LDLR, as measured by Dil-LDL uptake in HepG2 naive cells **(A)** and HEK293 naïve cells **(B)**, or on total LDLR levels, as measured by Western blot analysis in HepG2 naïve cells **(C)**. The negative control is compound i. HepG2 naïve cells, which express endogenous PCSK9 **(A)** or HEK293 naïve cells, which lack PCSK9 expression **(B)**, were incubated for 6h in the absence (DMSO) or presence of 33.3 µM of the indicated compounds prior to the addition of Dil-LDL that was followed by an additional 18h-incubation. For each condition, Dil-LDL uptake (fluorescence of Dil fluorescent probe) was corrected for the total number of cells (fluorescence of CyQuant™ GR dye) and expressed as % activity of the DMSO control. Data represents an average of 2 to 5 **(A)** or 2 to 3 **(B)** independent experiments performed in triplicate. **(C)** Western blot analysis of total LDLR in HepG2 naïve cells incubated for 24h in the absence (Cnt_0) or presence of increasing concentrations of compound 19. Total LDLR levels normalized to β-actin are plotted for each condition as % control (Cnt). Quantification of protein bands was obtained using Image J™ software; and

Figure 6 shows the *in vivo* inhibition of PCSK9. Selected inhibitors are tested in heterozygote $Ldlr^{+/-}$ mice that exhibit "humanized" LDLc profiles (LDLc x 2-3) and generated by intercrossing mice having WT and $Ldlr^{-/-}$ backgrounds. These mice express either normal levels of mouse PCSK9 ($Pcsk9^{+/+}$), no PCSK9 ($Pcsk9^{-/-}$) and/or one or five copies of transgene encoding human PCSK9-D374Y from its own human promoter (TgDY). The human WT form of PCSK9 is also tested.

DETAILED DESCRIPTION OF THE INVENTION

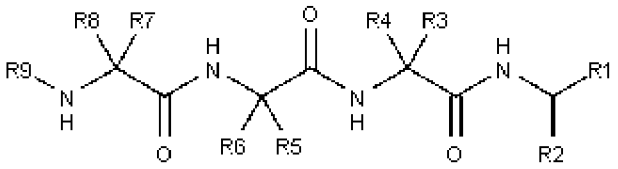
PCSK9 also known as neural apoptosis- regulated convertase 1 (NARC-1), is a proteinase K-Hke subtilase of 692 amino acids in human (NP_777596.2), and comprises a signal peptide (1-30) followed by a prosegment (residues 31-152), a catalytic domain (residues 153-454) and a C-terminal Cys-His-rich domain (CHRD; residues 455-692). PCSK9 is expressed in cells capable of proliferation and differentiation such as hepatocytes, kidney mesenchymal cells, intestinal ileum, colon epithelia and embryonic brain telencephalic neurons (Seidah et al., 2003, Proc. Natl. Acad. Sci. USA 100:928-933).

Following translocation in the ER, the prosegment of PCSK9 is autocatalytically cleaved at the $VFAQ_{152}\downarrow SIP$ site. In PCs, the prosegment (pro) is an intramolecular chaperone/inhibitor that is usually removed intracellularly to yield a fully active protease. Different from other PCs, PCSK9 is secreted as a stable non-covalent complex [pro≡PCSK9]. Accordingly, enhanced degradation of the LDLR induced by PCSK9 does not require the catalytic activity of the mature PCSK9 form. In human and mouse plasma, both full-length PCSK9 (153-692) and a truncated form PCSK9-ΔN218 (219-692) can be detected. The latter, which has no activity on LDLR, is likely generated by Furin and/or PC5, since they cleave PCSK9 *ex vivo* at RFHR218↓.

In the studies disclosed herein, the present inventors have generated and identified inhibitory compounds directed against human PCSK9 and shown that these compounds inhibit PCSK9 activity (*e.g.*, PCSK9 secretion, induced LDLR degradation in the human hepatocarcinoma-derived cell lines such as HepG2).

Compounds

The present invention relates to compounds of Formula (I):



(I)

or a pharmaceutically acceptable salt, hydrate, solvate, or racemic mixture or stereoisomer thereof,

wherein:

R1 is $-CH(OH)R_a$ or $-B(OR_b)(OR_c)$;

R2 is $-H$, $-CH_2R_d$, $-CHR_d(R_e)$, $-CH_2(CH_2)_mC(O)R_j$, $-CH_2(CH_2)_mC(O)N(R_d)R_e$ or $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$;

R3, R4, R5, R6, R7 and R8 are identical or different, and are independently hydrogen or one of the following groups: a C1-6 alkyl, a C1-6 haloalkyl, a C1-6 thioalkyl, a C1-6 aminoalkyl, an alkenyl, an alkynyl, a cycloalkyl, an heterocyclyl, an aryl, and an heteroaryl group, wherein said group is optionally substituted with one or more C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, an heteroaryl, $-CN$, $-C(O)N(R_f)R_g$, $-C(O)OR_f$, $-C(R_f)(R_g)OR_h$, $-OR_f$, $-OC(O)OR_f$, $-OC(O)NR_f(R_g)$, $-SR_f$, $-S(O)_nR_f$, $-S(O)_nN(R_f)R_g$, $-S(O)_nN(R_f)C(O)R_g$, $-N(R_f)R_g$, $-N(R_f)C(O)R_g$, $-N(R_f)C(O)OR_g$, $-N(R_f)C(O)N(R_g)(R_h)$, $-N(R_f)S(O)_nR_g$, and $-N(R_f)S(O)N(R_g)R_h$ substituents;

when (R3 and R4) or (R5 and R6) or (R7 and R8) are not hydrogen, the bracketed pairs can also be linked with $-C(O)-$, $-CO_2-$, $-C(O)N(H)-$, $-C(O)N(R_x)-$, $-O-$, $-NH-$, $-N(R_x)-$, $-S-$, $-S(O)_n-$, $-S(O)_nN(H)-$, $-S(O)_nN(R_x)-$ radicals to form cyclic structures;

R9 is $R_iC(O)-$, $R_iS(O)_n-$, $R_iOC(O)-$, $R_iNHC(O)-$, $R_iNHS(O)_n-$, $R_k(R_l)NC(O)-$, $R_l(R_l)NS(O)_n-$; $R_mOR_iC(O)-$, $R_mC(O)R_iC(O)-$ or one or more amino acids residues;

R_a is C1-3 alkyl, C1-2 fluoroalkyl or cyclopropyl;

R_b and R_c are identical or different, and are independently H or C1-6 alkyl, or can be connected together to form a cyclic 5- or 6-membered ring structure, or fused with additional aliphatic or aromatic ring systems, the cyclic 5- or 6-membered ring structure or aliphatic or aromatic ring systems being optionally substituted with one or more C1-6 alkyl and/or C1-6 haloalkyl substituents;

R_d and R_e are identical or different, and are independently H or one of the following groups: a C1-3 alkyl, a C1-3 haloalkyl or a C3-4 cycloalkyl group, or can be connected together directly or with $-C(O)-$, $-C(O)O-$, $-C(O)N(R_x)-$ $-O-$, $-N(R_x)-$, $-S-$, $-S(O)_n-$,or $-S(O)_nN(R_x)-$ radicals to form cyclic 3-8 membered ring structures;

R_f, R_g, R_h, R_k and R_l are identical or different, and are independently H or one of the following groups: a C1-6 alkyl, a C1-6 haloalkyl or a C3-4 cycloalkyl group, or can be connected together directly or with –C(O)–, –C(O)O–,–C(O)N(R_x)– –O–, –N(R_x)–, –S–, –S(O)$_n$– or –S(O)$_n$N(R_x)– radicals to form cyclic 3-8 membered ring structures;

R_i is a C1-10 alkyl, a C1-10 heteroalkyl, a C3-8 cycloalkyl, a C1-10 haloalkyl, a heterocyclyl, an aryl, a heteroaryl, a C1-10 alkyl-C3-8 cycloalkyl, a C1-10 alkyl-heterocyclyl, a C1-10 alkyl-aryl, a C1-10 alkyl-heteroaryl, a C1-10 heteroalkyl-C3-8 cycloalkyl, a a C1-10 heteroalkyl-heterocyclyl, a C1-10 heteroalkyl-aryl or a C1-10 heteroalkyl-heteroaryl group, wherein the group is optionally substituted with one or more of a halogen, C1-6 aminoalkyl, C1-6 heteroalkyl, C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, heteroaryl and heterocyclyl groups;

R_m is a C1-10 alkyl, a C1-10 heteroalkyl, a C3-8 cycloalkyl, a C1-10 haloalkyl, a heterocyclyl, an aryl or a heteroaryl group, wherein the group is optionally substituted with one or more of a halogen, C1-6 aminoalkyl, C1-6 heteroalkyl, C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, heteroaryl and heterocyclyl groups;

R_j is OR_d or N(R_d)(R_e);

R_x is H, C1-6 alkyl, C1-6 haloalkyl, C3-4 cycloalkyl, –C(O)R_y, –C(O)OR_y, –C(O)NH_2, –C(O)NH(R_y) or –C(O)NHS(O)$_n$$R_y$;

R_y is C1-6 alkyl, C1-6 haloalkyl or C3-4 cycloalkyl;

m is an integer of value 0 or 1; and

n is an integer of value 1 or 2,

provided that:

1) when R1 is –CH(OH)R_a, R2 is –CHR_d(R_e), –CH_2(CH_2)$_m$C(O)R_j, –CH_2(CH_2)$_m$C(O)N(R_d)R_e or –CH_2(CH_2)$_m$S(O)$_n$N(R_d)R_e; and

2) when R1 is –B(OR_b)(OR_c), R2 is –H, –CH_3, –CHR_d(R_e), –CH_2(CH_2)$_m$C(O)R_j, –CH_2(CH_2)$_m$C(O)N(R_d)R_e or –CH_2(CH_2)$_m$S(O)$_n$N(R_d)R_e.

As recited above, the present invention encompasses additional amino acid residues in R9. One or more amino acid residues in R9 can facilitate active transport of the compounds of the present invention across cell membranes (see Koren, E.; Torchilin, V. P. Cell Penetrating Peptides: Breaking Through to the Other Side. *Trends Mol. Med.* **2012**, *18(7)*, 385-93 and references therein.

Specific embodiments of the current invention include, but are not limited to the following:

(2*S*)-3-Methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-(phenylformamido)butanamide; **5**.

benzyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate; **7**.

tert-Butyl*N*-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate; **6**.

(2*S*)-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-({4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}formamido)butanamide; **8a**.

(2*S*)-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-({3-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}formamido)butanamide; **8b**.

(2*S*)-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-(2-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}acetamido)butanamide; **8c**.

(2*S*)-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-(2-{3-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}acetamido)butanamide; **8d**.

(2*S*)-2-[(2-fluorophenyl)formamido]-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]butanamide; **9**.

{[(2*S*)-2-[(2*S*)-2-[(2*S*)-3-Methyl-2-{2-[4-(trifluoromethoxy)phenyl]acetamido}butanamido]-3-phenylpropanamido]propanamido]methyl}boronic acid; **3**.

{[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-Decanamido-3-methylbutanamido]-3-phenylpropanamido]propanamido]methyl}boronic acid; **4**.

{[(2*S*)-2-[(2*S*)-2-[(2*S*)-3-Methyl-2-[(4-phenylphenyl)formamido]butanamido]-3-phenylpropanamido]propanamido]methyl}boronic acid; **2**.

{[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(Methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]propanamido]methyl}boronic acid; **1**.

[(1*R/S*)-3-Carbamoyl-1-[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]propanamido]propyl]boronic acid; **18**.

[(1*R/S*)-4-Methoxy-1-[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]propanamido]-4-oxobutyl]boronic acid; **17**.

Methyl *N*-[(1*S*)-1-{[(1*R/S*)-1-{[(1*S*)-1-{[(1*S*)-3-carbamoyl-1-[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]propyl]carbamoyl}ethyl]carbamoyl}-2-phenylethyl]carbamoyl}-2-methylpropyl]carbamate; **16**.

Methyl (4*R/S*)-4-[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(Methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]-2-phenylacetamido]-4-[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]butanoate; **15**.

Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(*S*)-phenyl({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)methyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate; **14**.

Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-1-{[(1*S*)-3-methyl-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)butyl]carbamoyl}-2-phenylethyl]carbamoyl}propyl]carbamate; **13**.

Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-1-{[(1*S*)-3-(methylsulfanyl)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)propyl]carbamoyl}-2-phenylethyl]carbamoyl}propyl]carbamate; **12**.

Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(1*S*)-2-phenyl-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate; **11**.

Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)propyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate; **10**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **19**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-(((3*S*)-6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **20a**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-(((3*R*)-6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **20b**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1-fluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **21a** and **21b**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **22a** and **22b**.

6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-(4-(trifluoromethoxy)phenyl)acetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide; **23**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **24**.

Benzyl ((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate; **25**.

Methyl ((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate; **26**.

tert-Butyl ((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate; **27**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-isopropylbenzamide; **28**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzamide; **29**.

6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)acetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide; **30**.

N-((2S)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzamide; **31**.

6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-(3-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)acetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide; **32**.

4-((*S*)-2-((*S*)-2-((*S*)-2-([1,1'-Biphenyl]-4-sulfonamido)-3-methylbutanamido)-3-phenylpropanamido)propanamido)-6,6,6-trifluoro-5-hydroxyhexanamide; **33**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide; **34**.

6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-phenylacetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide; **35**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-6-phenylnicotinamide; **36**)

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-2-(pyridin-4-yl)thiazole-4-carboxamide; **37**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(pyridin-3-yl)benzamide; **38**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(hydroxymethyl)benzamide; **39**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-1-phenyl-1*H*-pyrazole-4-carboxamide; **40**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-morpholinobenzamide; **41**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(morpholine-4-carbonyl)benzamide; **42**.

6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(3-phenylpropiolamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide; **43**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)nicotinamide; **44**.

6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-phenoxyacetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide; **45**.

4-((6*S*,9*S*,12*S*)-9-Benzyl-6-isopropyl-12-methyl-4,7,10-trioxo-1-phenyl-2-oxa-5,8,11-triazatridecan-13-amido)-6,6,6-trifluoro-5-hydroxyhexanamide; **46**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-1*H*-indole-5-carboxamide; **47**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-5-phenyl-1,3,4-oxadiazole-2-carboxamide; **48**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(1,3,4-oxadiazol-2-yl)benzamide; **49**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-2-phenylthiazole-4-carboxamide; **50**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4'-(methylsulfonyl)-[1,1'-biphenyl]-4-carboxamide; **51**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4'-fluoro-[1,1'-biphenyl]-4-carboxamide; **52**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)pyrazine-2-carboxamide; **53**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)benzamide; **54**.

; 2*S*)-*N*¹-(6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)-2-((*S*)-2-((*S*)-2-decanamido-3-methylbutanamido)-3-phenylpropanamido)pentanediamide; **55**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-4-(methylthio)-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **56**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **57**)

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **58**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **59**.

N-((2*S*)-1-(((2*S*)-1-(((2*R*)-1-(((3*S*)-6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **60**.

N-((2*S*)-1-(((2*S*)-1-(((2*R*)-1-(((3*R*)-6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide; **61**.

N-((2*S*)-1-(((2*S*)-1-(((2*R*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **62**.

N-((2*S*)-1-(((2*S*)-1-((2-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-2-oxoethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **63**.

N-((2*S*)-1-(((2*S*)-1-((1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)carbamoyl)cyclopropyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **64**.

N-((2*S*)-1-(((2*S*)-1-(((2*R*)-1-(((3*S*)-6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **65**.

N-((2*S*)-1-(((2*S*)-1-((1-(((3*S*)-6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)carbamoyl)cyclobutyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **66**.

N-((2*S*)-1-(((2*S*)-1-((1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **67**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **68**.

N-((2*S*)-1-(((2*R*)-1-(((2*R*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **69**.

N-((2*S*)-1-(((2*R*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **70**.

N-((2*S*)-1-((2-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **71**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **72**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-4-(methylthio)-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **73**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **74**.

N-((5*S*,8*S*,11*S*)-17-amino-8-benzyl-11-methyl-6,9,12,17-tetraoxo-14-(2,2,2-trifluoro-1-hydroxyethyl)-2-thia-7,10,13-triazaheptadecan-5-yl)-[1,1'-biphenyl]-4-carboxamide; **75**.

N-((2*R*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **76**.

N-((1*S*)-2-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxo-1-phenylethyl)-[1,1'-biphenyl]-4-carboxamide; **77**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **78**.

N-(2-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-[1,1'-biphenyl]-4-carboxamide; **79**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **80**.

N-((5*R*,8*S*,11*S*)-17-amino-8-benzyl-11-methyl-6,9,12,17-tetraoxo-14-(2,2,2-trifluoro-1-hydroxyethyl)-2-thia-7,10,13-triazaheptadecan-5-yl)-[1,1'-biphenyl]-4-carboxamide; **81**.

N-((5*R*,8*R*,11*S*)-17-Amino-8-benzyl-11-methyl-6,9,12,17-tetraoxo-14-(2,2,2-trifluoro-1-hydroxyethyl)-2-thia-7,10,13-triazaheptadecan-5-yl)-[1,1'-biphenyl]-4-carboxamide; **82**.

2-((*S*)-2-([1,1'-Biphenyl]-4-carboxamido)-3-methylbutanamido)-*N*-((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)-2,3-dihydro-1*H*-indene-2-carboxamide; **83**.

N-((2*S*)-1-(((1*S*)-2-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-2-oxo-1-phenylethyl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **84**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-4-phenylbutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **85**.

N-((2*S*)-1-(((2*R*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-4-phenylbutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **86**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **87**.

3-(4-(((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamoyl)phenyl)pyridin-1-ium formate; **88**.

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide; **89**.

N-(1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)cyclopentyl)-[1,1'-biphenyl]-4-carboxamide; **90**.

N-(1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)cyclopropyl)-[1,1'-biphenyl]-4-carboxamide; **91**.

Compounds listed above are identified by numbers (in bold). These numbers also refer to the specific examples where each of the above listed compounds is further described. If there were discrepancies between structures and names used herein for compounds of the present invention, structures will prevail.

Definitions:

Chemical groups

As used herein, the term "**alkyl**" refers to a monovalent straight or branched chain, saturated or unsaturated aliphatic hydrocarbon radical having a number of carbon atoms in the specified range. Thus, for example, "C1-6 alkyl" (or "C1-C6 alkyl") refers to any of the hexyl alkyl and pentyl alkyl isomers as well as n-, iso-, sec- and t-butyl, n- and iso- propyl, ethyl and methyl. As another example, "C1-4 alkyl" refers to n-, iso-, sec- and t-butyl, n- and isopropyl, ethyl and methyl. As another example, "C1-3 alkyl" refers to n-propyl, isopropyl, ethyl and methyl. Alkyl include unsaturated aliphatic hydrocarbon including alkyne (R-C≡C-R); and/or alkene (R-C=C-R).

The term "**halogen**" (or "halo") refers to fluorine, chlorine, bromine and iodine (alternatively referred to as fluoro, chloro, bromo, and iodo). The term "**haloalkyl**" refers to an alkyl group as defined above in which one or more of the hydrogen atoms have been replaced with a halogen (i.e., F, Cl, Br and/or I). Thus, for example, "**C1-6 haloalkyl**" (or "**C1-C6 haloalkyl**") refers to a C1 to C6 linear or branched alkyl group as defined above with one or more halogen substituents. The term "**fluoroalkyl**" has an analogous meaning except that the halogen substituents are restricted to fluoro. Suitable fluoroalkyls include the series $(CH2)_{0-4}CF_3$ (i.e., trifluoromethyl, 2,2,2-trifluoroethyl, 3,3,3-trifluoro-n-propyl, etc.).

The term "**heteroalkyl**" is given its ordinary meaning in the art and refers to alkyl groups as described herein in which one or more carbon atoms is replaced with a heteroatom (*e.g.*, oxygen, nitrogen, sulfur, or derivatives thereof, and the like). Examples of heteroalkyl groups include, but are not limited to, alkoxy, alkyl-substituted amino, thiol such as methionine side group. Up to two heteroatoms may be consecutive. When a prefix such as C2-6 is used to refer to a heteroalkyl group, the number of carbons (2-6, in this example) is meant to include the heteroatoms as well.

The term "**aminoalkyl**" refers to an alkyl group as defined above in which one or more of the hydrogen or carbon atoms has been replaced with an nitrogen or amino derivative. Thus, for example, "**C1-6 aminoalkyl**" (or "**C1-C6 aminoalkyl**") refers to a C1 to C6 linear or branched alkyl group as defined above with one or more amino derivatives (*e.g.*, NH, amide, diazirin, etc.).

The term "**thioalkyl**" refers to an alkyl group as defined above in which one or more of the hydrogen or carbon atoms has been replaced with a sulfur atom or thiol derivative. Thus, for example, "**C1-6 aminoalkyl**" (or "**C1-C6 aminoalkyl**") refers to a C1 to C6 linear or branched alkyl group as defined above with one or more sulfur atoms or thiol derivatives (*e.g.*, S, SH, etc.).

Aminoalkyl and thioalkyls are specific embodiments of and encompassed by the term "**heteroalkyl**" or substituted alkyl depending on the heteroatom replaces a carbon atom or an hydrogen atom.

The term "**cycloalkyl**" refers to saturated alicyclic hydrocarbon consisting of saturated 3-8 membered rings optionally fused with additional (1-3) aliphatic (cycloalkyl) or aromatic ring systems, each additional ring consisting of a 3-8 membered ring. It includes without being so limited cyclopropane, cyclobutane,cyclopentane, and cyclohexane.

The term "**heterocyclyl**" refers to (i) a 4- to 7-membered saturated heterocyclic ring containing from 1 to 3 heteroatoms independently selected from N, O and S, or (ii) is a heterobicyclic ring (*e.g.,* benzocyclopentyl). Examples of **4- to 7-membered, saturated heterocyclic rings** within the scope of this invention include, for example, azetidinyl, piperidinyl, morpholinyl, thiomorpholinyl, thiazolidinyl, isothiazolidinyl, oxazolidinyl, isoxazolidinyl, pyrrolidinyl, imidazolidinyl, piperazinyl, tetrahydrofuranyl, tetrahydrothienyl, pyrazolidinyl, hexahydropyrimidinyl, thiazinanyl, thiazepanyl, azepanyl, diazepanyl, tetrahydropyranyl, tetrahydrothiopyranyl, and dioxanyl. Examples of 4- to 7-membered, unsaturated heterocyclic rings within the scope of this invention include mono-unsaturated heterocyclic rings corresponding to the saturated heterocyclic rings listed in the preceding sentence in which a single bond is replaced with a double bond (e.g., a carbon-carbon single bond is replaced with a carbon-carbon double bond).

The term "**C(O)**" refers to carbonyl. The terms "$S(O)_2$" and "SO_2" each refer to sulfonyl. The term "S(O)" refers to sulfinyl.

The term "**aryl**" refers to aromatic (unsaturated) compounds consisting of 3-8 membered rings, optionally fused with additional (1-3) aliphatic (cycloalkyl) or aromatic ring systems, each additional ring consisting of 3-8 membered ring. In a specific embodiment, it refers to phenyl, benzocyclopentyl, or naphthyl. The aryl of particular interest is phenyl. The term "**heteroaryl**" refers to (i) a 3-, 4-, 5- or 6-membered heteroaromatic ring containing from 1 to 3 heteroatoms independently selected from N, O and S, or (ii) is a heterobicyclic ring selected from quinolinyl, isoquinolinyl, and quinoxalinyl. Suitable 3-, 4-, 5- and 6-membered heteroaromatic rings include, for example, diazirin, pyridyl (also referred to as pyridinyl), pyrrolyl, diazine (*e.g.i,* pyrazinyl, pyrimidinyl, pyridazinyl), triazinyl, thienyl, furanyl, imidazolyl, pyrazolyl, triazolyl, oxazolyl, iso-oxazolyl, oxadiazolyl, oxatriazolyl, thiazolyl, isothiazolyl, and thiadiazolyl. Heteroaryls of particular interest are pyrrolyl, imidazolyl, pyridyl, pyrazinyl, quinolinyl (or quinolyl), isoquinolinyl (or isoquinolyl), and quinoxalinyl. Suitable heterobicyclic ring include indolyl.

As used herein, and unless otherwise specified, the terms "**alkyl**", "**haloalkyl**", "**aminoalkyl**", "**cycloalkyl**", "**heterocyclyl**", "**aryl**", "**heteroalkyl**" and "**heteroaryl**" and the terms designating their specific embodiments (*e.g.*, butyl, fluoropropyl, aminobutyl, cyclopropane, morpholine, phenyl, pyrazole, etc.) encompass the substituted (i.e. in the case of haloalkyl and aminoalkyl, in addition to their halogen and nitrogen substituents, respectively) and unsubstituted embodiments of these groups . Hence for example, the term "phenyl" encompasses unsubstituted phenyl as well as fluorophenyl, hydroxyphenyl, methylsulfonyl phenyl (or biphenyl), trifluoromethyl-diazirin-phenyl,

isopropyl-phenyl, trifluorohydroxy-phenyl. Similarly, the term pyrazole, encompass unsubstituted pyrazole as well as methylpyrazole. The one or more substituents may be an amine, halogen, hydroxyl, C1-6 aminoalkyl, C1-6 heteroalkyl, C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, heteroaryl and heterocyclyl groups (etc.).

It is understood that the specific rings listed above are not a limitation on the rings which can be used in the present invention. These rings are merely representative.

Unless expressly stated to the contrary in a particular context, any of the various cyclic rings and ring systems described herein may be attached to the rest of the compound at any ring atom (i.e., any carbon atom or any heteroatom) provided that a stable compound results.

Unless expressly stated to the contrary, all ranges cited herein are inclusive. For example, a heteroaromatic ring described as containing from "1 to 4 heteroatoms" means the ring can contain 1, 2, 3 or 4 heteroatoms. It is also understood that any range cited herein includes within its scope all of the sub-ranges within that range. Thus, for example, a heterocyclic ring described as containing from "1 to 4 heteroatoms" is intended to include as aspects thereof, heterocyclic rings containing 2 to 4 heteroatoms, 3 or 4 heteroatoms, 1 to 3 heteroatoms, 2 or 3 heteroatoms, 1 or 2 heteroatoms, 1 heteroatom, 2 heteroatoms, 3 heteroatoms, and 4 heteroatoms.

As another example, an aryl or heteroaryl described as optionally substituted with "from 1 to 4 substituents" is intended to include as aspects thereof, an aryl or heteroaryl substituted with 1 to 4 substituents, 2 to 4 substituents, 3 to 4 substituents, 4 substituents, 1 to 3 substituents, 2 to 3 substituents, 3 substituents, 1 to 2 substituents, 2 substituents, and 1 substituent.

When any variable (e.g., XA or XB) occurs more than one time in any constituent or in Formula I or in any other formula depicting and describing compounds of the present invention, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

Unless expressly stated to the contrary, substitution by a named substituent is permitted on any atom in a ring (e.g., cycloalkyl, heterocyclyl, aryl, or heteroaryl) provided such ring substitution is chemically allowed and results in a stable compound.

Salts, esters, hydrates and solvates

The compounds of the present invention include pharmacologically acceptable salts and ester derivatives thereof as well as hydrates or solvates thereof and all stereoisomeric forms of the referenced compounds. The compounds and pharmacologically acceptable esters thereof of the present invention can form pharmacologically acceptable salts if necessary.

Salts

The terms "**pharmacologically acceptable salt**" refer to a salt to which the compounds of the present invention can be converted that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see *e.g.*, Berge, S.M. *et al.*, 1977 *J. Pharm. Sci.* **66**:1-19). Examples of such

salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and di-carboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like. Preferred examples of such a salt include alkali metal salts such as a sodium salt, a potassium salt, a lithium salt, magnesium or calcium salts; alkaline earth metal salts such as a calcium salt and a magnesium salt; metal salts such as an aluminum salt, an iron salt, a zinc salt, a copper salt, a nickel salt and a cobalt salt; amine salts such as inorganic salts including an ammonium salt; organic salts or ammonium salts such as a t-octylamine salt, a dibenzylamine salt, a morpholine salt, a glucosamine salt, a phenylglycine alkyl ester salt, an ethylenediamine salt, an N-methylglucamine salt, a guanidine salt, a diethylamine salt, a triethylamine salt, a dicyclohexylamine salt, an N,N'-dibenzylethylenediamine salt, a chloroprocaine salt, a procaine salt, a diethanolamine salt, an N-benzyl-phenethylamine salt, a piperazine salt, a tetramethylammonium salt and a tris(hydroxymethyl)aminomethane salt; inorganic acid salts such as hydrohalic acid salts such as a hydrofluoride, a hydrochloride, a hydrobromide or a hydroiodide, a nitrate, a perchlorate, a sulfate or a phosphate; lower alkanesulfonates such as a methanesulfonate, trifluoromethanesulfonate or an ethanesulfonate; arylsulfonates such as a benzenesulfonate or a p-toluenesulfonate and the like, which are non-toxic to living organisms; organic acid salts such as an acetate, a malate, adipate, a fumarate, a succinate, a citrate, alginate, ascorbate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, cinnamate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, itaconate, lactate, maleate, mandelate, sulfonate, methanesulfonate, trifluoromethanesulfonates, ethanesulfonates 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, tartrate, thiocyanate, tosylate, trifluoroacetic acid, undecanoate, a tartrate, an oxalate or a maleate; and amino acid salts such as a glycine salt, a lysine salt, an arginine salt, an ornithine salt, histidine, a glutamate or an aspartate salt. Additionally, basic nitrogen containing groups may be quaternized with such agents as lower alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates including dimethyl, diethyl, and dibutyl sulfate; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and strearyl chlorides, bromides and iodides, aralkyl halides including benzyl and phenethyl bromides, and others. For further example, see S. M. Berge, et al. "Pharmaceutical Salts," J. Pharm. Sci. 1977, 66, 1-19. Such salts can be formed quite readily by those skilled in the art using standard techniques.

Preferred examples of the salts formed with an acidic group present in the compounds of the present invention include metal salts such as alkali metal salts (*e.g.*, sodium salts, potassium salts and lithium salts), alkali

earth metal salts (*e.g.*, calcium salts and magnesium salts), aluminum salts and iron salts; amine salts such as inorganic amine salts (*e.g.*, ammonium salts) and organic amine salts (*e.g.*, t-octylamine salts, dibenzylamine salts, morpholine salts, glucosamine salts, phenylglycinealkyl ester salts, ethylenediamine salts, N-methylglucamine salts, guanidine salts, diethylamine salts, triethylamine salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts, chloroprocaine salts, procaine salts, diethanolamine salts. N-benzylphenethylamine salts, piperazine salts, tetramethylammonium salts and tris(hydroxymethyl)aminomethane salts; and amino acid salts such as glycine salts, lysine salts, arginine salts, ornithine salts, glutamates and aspartates.

All salts are intended to be pharmaceutically acceptable salts within the scope of the invention and all salts are considered equivalent to the free forms of the corresponding compounds for purposes of the invention.

Esters

Physiologically/pharmaceutically acceptable esters are also useful as active medicaments. The term "**pharmaceutically acceptable esters**" embraces esters of the compounds of the present invention, in which hydroxy groups (*e.g.*, in carboxylic acid) have been converted to the corresponding esters and may act as a prodrug which, when absorbed into the bloodstream of a warm-blooded animal, may cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy. Such esters can be formed with inorganic or organic acids such as nitric acid, sulphuric acid, phosphoric acid, citric acid, formic acid, maleic acid, acetic acid, succinic acid, tartaric acid, methanesulphonic acid, p-toluenesulphonic acid and the like, which are non-toxic to living organisms. Further examples are the esters with aliphatic or aromatic acids such as acetic acid or with aliphatic alcohol (*e.g.*, alkyl esters, including methyl, ethyl, propyl, isopropyl, butyl, isobutyl or pentyl esters, and the like) or aromatic alcohols (*e.g.*, benzyl ester).

Esters can be prepared from their corresponding acids or salts by a variety of methods known to those skilled in the art, such as, for example, by first transforming the acid to the acid chloride and then reacting the acid chloride with a suitable alcohol. Other suitable methods for making esters are described in Kemp and Vellaccio, 1980.

Where esters of the invention have a basic group, such as an amino group, the compound can be converted to a salt by reacting it with an acid, and in the case where the esters have an acidic group, such as a sulfonamide group, the compound can be converted to a salt by reacting it with a base. The compounds of the present invention encompass such salts.

Salts and esters of the compounds of the present invention may be prepared by known method by employing appropriate starting materials or intermediate compounds that are readily available and/or are described herein.

Generally, a desired salt of a compound of this invention can be prepared *in situ* during the final isolation and purification of a compound by means well known in the art. For example, a desired salt can be prepared by separately reacting the purified compound in its free base or free acid form with a suitable organic or inorganic acid, or suitable organic or inorganic base, respectively, and isolating the salt thus formed. In the case of basic compounds, for example, the free base is treated with anhydrous HCl in a suitable solvent such as THF, and the salt

isolated as a hydrochloride salt. In the case of acidic compounds, the salts may be obtained, for example, by treatment of the free acid with anhydrous ammonia in a suitable solvent such as ether and subsequent isolation of the ammonium salt. These methods are conventional and would be readily apparent to one skilled in the art.

The compounds of this invention may be esterified by a variety of conventional procedures including reacting the appropriate anhydride, carboxylic acid or acid chloride with the alcohol group of a compound of this invention. The appropriate anhydride is reacted with the alcohol in the presence of a base to facilitate acylation such as 1,8-bis[dimethylamino]naphthalene or N,N-dimethylaminopyridine. Or, an appropriate carboxylic acid can be reacted with the alcohol in the presence of a dehydrating agent such as dicyclohexylcarbodiimide, 1-[3-dimethylaminopropyl]-3-ethylcarbodiimide or other water soluble dehydrating agents which are used to drive the reaction by the removal of water, and, optionally, an acylation catalyst. Esterification can also be effected using the appropriate carboxylic acid in the presence of trifluoroacetic anhydride and, optionally, pyridine, or in the presence of N,N-carbonyldiimidazole with pyridine. Reaction of an acid chloride with the alcohol can be carried out with an acylation catalyst such as 4-DMAP or pyridine.

One skilled in the art would readily know how to successfully carry out these as well as other known methods of etherification of alcohols.

Prodrugs and solvates

Prodrugs and solvates of the compounds of the invention are also contemplated herein. A discussion of prodrugs is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems (1987) 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, (1987) Edward B. Roche, ed., American Pharmaceutical Association and Pergamon Press. The term "prodrug" means a compound (*e.g.*, a drug precursor) that is transformed *in vivo* to yield a compound of the present invention or a pharmaceutically acceptable salt, hydrate or solvate of the compound. The transformation may occur by various mechanisms (*e.g.*, by metabolic or chemical processes), such as, for example, through hydrolysis in blood. A discussion of the use of prodrugs is provided by T. Higuchi and W. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987.

For example, if a compound of the present invention or a pharmaceutically acceptable salt, hydrate or solvate of the compound contains a carboxylic acid functional group, a prodrug can comprise an ester formed by the replacement of the hydrogen atom of the acid group with a group such as, for example, (C1–C8)alkyl, (C2–C12)alkanoyloxymethyl, 1-(alkanoyloxy)ethyl having from 4 to 9 carbon atoms, 1-methyl-1-(alkanoyloxy)-ethyl having from 5 to 10 carbon atoms, alkoxycarbonyloxymethyl having from 3 to 6 carbon atoms, 1-(alkoxycarbonyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1-(alkoxycarbonyloxy)ethyl having from 5 to 8 carbon atoms, N-(alkoxycarbonyl)aminomethyl having from 3 to 9 carbon atoms, 1-(N-(alkoxycarbonyl)amino)ethyl having from 4 to 10 carbon atoms, 3-phthalidyl, 4-crotonolactonyl, gamma-butyrolacton-4-yl, di-N,N-(C1–C2)alkylamino(C2–C3)alkyl

(such as β-dimethylaminoethyl), carbamoyl-(C_1–C_2)alkyl, N,N-di(C_1–C_2)alkylcarbamoyl-(C_1–C_2)alkyl and piperidino-, pyrrolidino- or morpholino(C_2–C_3)alkyl, and the like.

Similarly, if a compound of the present invention contains an alcohol functional group, a prodrug can be formed by the replacement of the hydrogen atom of the alcohol group with a group such as, for example, (C1–C6)alkanoyloxymethyl, 1-((C_1–C_6)alkanoyloxy)ethyl, 1-methyl-1-((C_1–C_6)alkanoyloxy)ethyl, (C_1–C_6)alkoxycarbonyloxymethyl, N-(C_1–C_6)alkoxycarbonylaminomethyl, succinoyl, (C_1–C_6)alkanoyl, α-amino(C_1–C_4)alkanyl, arylacyl and α-aminoacyl, or α-aminoacyl-α-aminoacyl, where each α-aminoacyl group is independently selected from the naturally occurring L-amino acids, $P(O)(OH)_2$, —$P(O)(O(C_1$–$C_6)alkyl)_2$ or glycosyl (the radical resulting from the removal of a hydroxyl group of the hemiacetal form of a carbohydrate), and the like.

If a compound of the present invention incorporates an amine functional group, a prodrug can be formed by the replacement of a hydrogen atom in the amine group with a group such as, for example, R-carbonyl, RO-carbonyl, NRR'-carbonyl where R and R' are each independently (C_1–C_{10})alkyl, (C_3–C_7)cycloalkyl, benzyl, or R-carbonyl is a natural α-aminoacyl or natural α-aminoacyl, —C(OH)C(O)OY1 wherein Y1 is H, (C_1–C_6)alkyl or benzyl, —$C(OY_2)Y_3$ wherein Y_2 is (C_1–C_4) alkyl and Y_3 is (C_1–C_6)alkyl, carboxy (C_1–C_6)alkyl, amino(C_1–C_4)alkyl or mono-N— or di-N,N-(C_1–C_6)alkylaminoalkyl, —C(Y4)Y5 wherein Y_4 is H or methyl and Y_5 is mono-N— or di-N,N-(C_1–C_6)alkylamino morpholino, piperidin-1-yl or pyrrolidin-1-yl, and the like.

One or more compounds of the invention may exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like, and it is intended that the invention embrace both solvated and unsolvated forms. “Solvate” means a physical association of a compound of this invention with one or more solvent molecules. This physical association involves varying degrees of ionic and covalent bonding, including hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. “Solvate” encompasses both solution-phase and isolatable solvates. Non-limiting examples of suitable solvates include ethanolates, methanolates, and the like. A “Hydrate” is a solvate wherein the solvent molecule is H_2O.

Preparation of solvates is generally known. Thus, for example, M. Caira et al, J. Pharmaceutical Sci., 93(3), 601–611 (2004) describe the preparation of the solvates of the antifungal fluconazole in ethyl acetate as well as from water. Similar preparations of solvates, hemisolvate, hydrates and the like are described by E. C. van Tonder et al, AAPS Pharm Sci Tech., 5(1), article 12 (2004); and A. L. Bingham et al, Chem. Commun., 603–604 (2001). A typical, non-limiting, process involves dissolving the inventive compound in desired amounts of the desired solvent (organic or water or mixtures thereof) at a higher than ambient temperature, and cooling the solution at a rate sufficient to form crystals which are then isolated by standard methods. Analytical techniques such as, for example I. R. spectroscopy, show the presence of the solvent (or water) in the crystals as a solvate (or hydrate).

Hydrates

As used herein the terms, "**pharmaceutically acceptable hydrate**" refer to the compounds of the instant

invention crystallized with one or more molecules of water to form a hydrated form.

Stereoisomers, diastereomers, enantiomers, racemates, tautomers

The compounds of the present invention have asymmetric carbon atoms/chiral centers and, as a result of the selection of substituents and substituent patterns, can contain additional chiral centers, and thus can occur as mixtures of stereoisomers (racemates), or as individual diastereomers, or enantiomers. The present invention embraces all of these forms.

Diastereomers (sometimes called diastereoisomers) are stereoisomers that are not enantiomers. Diastereomerism occurs when two or more stereoisomers of a compound have different configurations at one or more (but not all) of the equivalent (related) stereocenters and are not mirror images of each other. When two diastereoisomers differ from each other at only one stereocenter they are epimers. Each stereocenter gives rise to two different configurations and thus to two different stereoisomers.

Diastereomers differ from enantiomers in that the latter are pairs of stereoisomers which differ in all stereocenters and are therefore mirror images of one another. Enantiomers of a compound with more than one stereocenter are also diastereomers of the other stereoisomers of that compound that are not their mirror image. Diastereomers have different physical properties and different reactivity, unlike enantiomers. Diastereomers of the present invention include tomatidine and 3 alpha-hydroxy-tomatidine for exemple.

To the extent substituents and substituent patterns provide for the existence of tautomers (e.g., keto-enol tautomers) in the compounds of the invention, all tautomeric forms of these compounds, whether present individually or in mixtures, are within the scope of the present invention. Compounds of the present invention having a hydroxy substituent on a carbon atom of a heteroaromatic ring are understood to include compounds in which only the hydroxy is present, compounds in which only the tautomeric keto form (i.e., an oxo substitutent) is present, and compounds in which the keto and enol forms are both present.

For purposes of this Specification, "**pharmaceutically acceptable tautomer**" means any tautomeric form of any compound of the present invention.

The purification of enantiomers and the separation of isomeric mixtures of a compound of the present invention may be accomplished by standard techniques known in the art.

A "**stable**" compound is a compound which can be prepared and isolated and whose structure and properties remain or can be caused to remain essentially unchanged for a period of time sufficient to allow use of the compound for the purposes described herein (e.g., therapeutic or prophylactic administration to a subject). The compounds of the present invention are limited to stable compounds embraced by Formula I.

Synthetic methods for preparing the compounds of the present invention are illustrated in the following general procedures, schemes, and examples. Starting materials are commercially available or may be prepared according to procedures known in the art or as illustrated herein. The compounds of the invention are illustrated by means of the specific examples shown below. However, these specific examples are not to be construed as forming

the only genus that is considered as the invention. These examples further illustrate details for the preparation of the compounds of the present invention. Those skilled in the art will readily understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare these compounds.

All temperatures are in degrees Celsius. Mass spectra (MS) were measured by electrospray ion-mass spectroscopy (ESI) on an Agilent 6120 Quadrapole™ MS coupled to an Agilent 1100™ series HPLC instrument. NMR spectra were recorded on a Varian Mercury spectrometer at 400 MHz for ^{1}H and 376 MHz for ^{19}F.

For the purposes of this specification, the following abbreviations have the indicated meanings:

AcOH	=	acetic acid
Alk	=	alkyl
Ar	=	aryl
atm	=	atmosphere
BINAP	=	2,2'-bis(diphenylphosphino)-1,1'-binaphthalene
Boc	=	*tert*-butoxycarbonyl
n-BuLi	=	*n*-butyllithium
Cbz	=	carboxybenzyl
CH_2Cl_2	=	dichloromethane
DBU	=	1,8-diazabicyclo[5.4.0]undec-7-ene
DEAD	=	diethyl azodicarboxylate
DIPEA	=	*N*,*N*-diisopropylethylamine
DMAP	=	4-(dimethylamino)pyridine
DMF	=	*N*,*N*-dimethylformamide
DMSO	=	dimethyl sulfoxide
ESI	=	electrospray ionization
Et_3N	=	triethylamine
Et_2O	=	diethylether
EtOAc or EA	=	ethyl acetate
EtOH	=	ethyl alcohol
h	=	hour(s)
H_2	=	hydrogen
HATU	=	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HCl	=	hydrochloric acid
HPLC	=	High Pressure Liquid Chromatography
iPrOH	=	2-propanol
KF	=	potassium fluoride
LC-MS	=	Liquid Chromatography Mass Spectrometry
LiOH	=	lithium hydroxide
MeCN	=	acetonitrile
MeMgBr	=	methylmagnesium bromide
MeOH	=	methyl alcohol
MeTHF	=	2-methyltetrahydrofuran
$MgSO_4$	=	magnesium sulfate

min	=	minute(s)
MS	=	mass spectroscopy
MTBE	=	methyl *tert*-butyl ether
N_2	=	nitrogen
$NaBH_4$	=	sodium borohydride
$NaHCO_3$	=	sodium bicarbonate
NaOH	=	sodium hydroxide
Na_2SO_4	=	sodium sulfate
NH_3	=	ammonia
NH_4Cl	=	ammonium chloride
NH_4OH	=	ammonium hydroxide
NMP	=	*N*-methyl 2-pyrrolidinone
NMR	=	nuclear magnetic resonance spectroscopy
Moc	=	methoxycarbonyl
P	=	pressure
Pd/C	=	palladium on charcoal
PG	=	protecting group
Ph	=	phenyl
Pyr	=	pyridine
rbf	=	round bottom flask
Rf	=	retention factor on silica gel
rt	=	room temperature
TBDMS	=	*tert*-butyldimethylsilyl
Ts	=	toluene-4-sulfonyl
TFA	=	trifluoroacetic acid
TFAA	=	trifluoroacetic anhydride
TFA-NHS	=	trifluoroacetic *N*-hydroxysuccinimide
THF	=	tetrahydrofuran
TLC	=	thin layer chromatography
TMEDA	=	*N*,*N*,*N*',*N*'-tetramethylethylenediamine
T3P	=	Propylphosphonic anhydride

The preparation of trifluoroacetic *N*-hydroxysuccinimide (TFA-NHS) is described in the literature: Sohn, C. H.; Lee, J. E.; Sweredoski, M. J.; Graham, R. L. J.; Smith, G. T.; Hess, S.; Czerwieniec, G.; Loo, J. A.; Deshaies, R. J.; Beauchamp, J. L. *J. Am. Chem.Soc.* **2012**, *134*, 2672−2680.

The preparation of (*S*)-3-*tert*-butoxycarbonyl-4-(2-benzyloxycarbonylethyl)oxazolidin-5-one is described in the literature: Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B.; Sleebs, B. E. *Aust. J. Chem.* **2000**, *53*, 425-433. (R)-3-*tert*-butoxycarbonyl-4-(2-benzyloxycarbonylethyl)oxazolidin-5-one can also be prepared in an analogous manner starting from *N*-Boc-D-glutamic acid 5-benzyl ester.

Pharmaceutical compositions

In another aspect, the present invention provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of compounds of the present invention, formulated together with a pharmaceutically acceptable carrier and/or excipient.

Pharmaceutical compositions of the invention also can be administered in combination therapy, i.e., combined with other agents. For example, the combination therapy can include at least one other cholesterol-reducing agent. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below.

As used herein, "**pharmaceutically acceptable carrier**" or "**pharmaceutically acceptable excipient**" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier should be suitable for oral, intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

Carrier/Excipients

A pharmaceutical composition of the invention may include a pharmaceutically acceptable antioxidant. Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as, aluminum monostearate and gelatin.

Pharmaceutically acceptable carriers or excipients include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent

is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, one can include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition.

Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Examples of pharmaceutical preparations for oral administration include tablets for internal use (such as uncoated tablets, sugar-coated tablets, coating tablets, enteric coated tablets and chewable tablets), tablets administered to oral cavity (such as buccal preparations, sublingual tablets, troches and adhesive tablets), powders, capsules (such as hard capsules and soft capsules), granules (such as coated granules, pills, troches, liquids preparations or pharmaceutically acceptable sustained release pharmaceutical preparations). Specific examples of liquid preparations capable of being orally administered are solutions for internal use, shake mixtures, suspensions, emulsions, syrups, dry syrups, elixirs, infusion and decoction and lemonades.

The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredient, from about 0.1 per cent to about 70 per cent, or from about 1 percent to about 30 percent of active ingredient in combination with a pharmaceutically acceptable carrier.

Doses and Dosage regimen

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

For administration of the compounds, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per day, week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months.

In some methods, two or more compounds with different activity are administered simultaneously, in which case the dosage of each compound administered falls within the ranges indicated. The compound is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of compound in the patient. In some methods, dosage is adjusted to achieve a plasma concentration of the compound of about 1-1000 µg/ml and in some methods about 25-300 µg/ml.

Alternatively, a compound can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the compound in the patient. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated or until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response (*e.g.*, decreased plasma LDL/cholesterol levels) for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the

ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A "**therapeutically effective amount**" or "**effective amount**" or "**therapeutically effective dosage**" of compounds of the invention can result in a lowering of LDL-C level in a subject, a decrease in severity of at least one disease symptom (*e.g.*, a decrease in plasma LDL-cholesterol, or a decrease in a symptom of a LDL-cholesterol-related disorder), an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction in the subject.

Routes of administration

A composition of the present invention can be administered by one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Routes of administration for compounds of the invention include oral, intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal injection and infusion. Alternatively, a compound of the invention can be administered by a nonparenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. Therapeutic compositions can be administered with medical devices known in the art. In certain embodiments, the compound of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. The liposomes may comprise one or more moieties which are selectively transported into specific cells, tissues or organs, thus enhance targeted drug delivery (see, *e.g.*, V. V. Ranade, 1989 *J. Clin. Pharmacol.* **29**:685). In an embodiment, the compound of the invention can be formulated to be delivered to the liver (i.e., to hepatocytes).

Functional characterization of the compounds

The functional characteristics of the compounds of the present invention can be tested *in vitro* and *in vivo*. For example, compounds can be tested for the ability to inhibit PCSK9 proteolytic activity, PCSK9-dependent effects on LDLR (*e.g.*, LDLR mediated uptake of LDL-C), PCSK9-dependent LDLR degradation including the interaction of PCSK9 to LDLR, and to decrease LDL-C *in vivo*.

PCSK9 binding to LDLR can be detected by surface plasmon resonance (SPR) (using BIAcore®) by immobilizing LDLR to a solid support and detecting soluble PCSK9 binding to the LDLR. Alternatively, PCSK9 can be immobilized, and LDLR binding can be detected. PCSK-9/LDLR binding can also be analyzed by ELISA (*e.g.*, by detecting PCSK9 binding to immobilized LDLR), by fluorescence resonance energy transfer (FRET), or phage display. To perform FRET, fluorophore-labeled PCSK9 binding to LDLR in solution can be detected (see, for example, U.S. Pat. No. 5,631,169). PCSK9 binding to LDLR has been detected by coimmunoprecipitation (Lagace *et al.*, 2006 *J. Clin. Inv.* **116**(11):2995-3005). For example, to examine PCSK9-LDLR binding in this manner, HepG2 cells are cultured in sterol-depleted medium for 18 hours. Purified PCSK9 is added to the medium in the presence of 0.1 mM chloroquine and the cells are incubated for one hour. Cells are lysed in mild detergent (1% digitonin w/vol). PCSK9 or LDLR is immunoprecipitated from cell lysates, separated by SDS-PAGE, and immunoblotted to detect the presence of coimmunoprecipitated LDLR or PCSK9, respectively (Lagace *et al.*, 2006, *supra*).

These assays may be conducted with a mutant form of PCSK9 that binds to LDLR with a higher affinity (*e.g.*, hPCSK9 D374Y, Lagace *et al.*, 2006, *supra*). Hepatocytes express LDLR on the cell surface. Addition of purified PCSK9 to cultured hepatocyte cells (*e.g.*, HepG2 cells, ATCC HB-8065, HuH7 cells, primary human or mouse hepatocytes) produces a decrease in LDLR expression in a dose- and time-dependent manner (Lagace *et al.*, 2006 *supra*). Compounds of the invention can be tested for the ability to increase LDLR levels in hepatocytes. For example, HepG2 cells are cultured in sterol-depleted medium (DMEM supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin sulfate, and 1 g/1 glucose, 5% (vol/vol) newborn calf lipoprotein-deficient serum (NCLPDS), 10 µM sodium compactin, and 50 µM sodium mevalonate) for 18 hours to induce LDLR expression. Purified PCSK9 (5 µg/ml) is added to the medium. LDLR levels in cells harvested at 0, 0.5, 1, 2, and 4 hours after addition of PCSK9 are determined (Lagace *et al.*, 2006, *supra*). LDLR levels can be determined by flow cytometry, FRET, immunoblotting, or other means. LDL-C uptake by cells (*e.g.*, HepG2 cells, HuH7 cells) can be measured using fluorescently-labeled LDL-C, Dil-LDL (3,3'-dioctadecylindocarbocyanine-low density lipoprotein) as described by Stephan and Yurachek (1993, *J. Lipid Res.* **34**: 325-330). Briefly, cells are incubated in culture with Dil-LDL (20-100 µg protein/ml) at 37°C for 2 hours. Cells are washed, lysed, and the concentration of internalized Dil-LDL is quantitated using a spectrofluorometer. LDL-C uptake can be measured in cells contacted with a PCSK9 inhibitory compound (prior to, and/or during the period in which Dil-LDL is present in the cell culture).

Transgenic mice overexpressing human PCSK9 in liver have increased levels of plasma LDL-C relative to non-transgenic mice (Lagace *et al*., 2006 *supra*). See also Maxwell and Breslow, 2004 *Proc. Natl. Acad. Sci. USA*, **101**:7100, describing overexpression of PCSK9 using an adenovirus vector in mice. PCSK9$^{-/-}$ mice have been produced (Rashid *et al*., 2005 *Proc. Natl. Acad. Sci.* **102**(5):5374-5379). These mice can be genetically modified to express a mouse or a human PCSK9 transgene. Compounds can be tested in any of these models, or in animals which are not genetically modified, for the ability to clear or reduce total cholesterol and/or LDL-C.

The kinetics of LDL clearance from plasma can be determined by injecting animals with [^{125}I]-labelled LDL, obtaining blood samples at 0, 5, 10, 15, and 30 minutes after injection, and quantitating [^{125}I]-LDL in the samples (Rashid *et al*., 2005 *supra*). The rate of LDL clearance is increased in PCSK9$^{-/-}$ mice relative to wild type mice (Rashid *et al*., 2005 *supra*). Increased LDL clearance in animals administered a compound indicates that the agent inhibits PCSK9 activity *in vivo*.

Decreases in total plasma cholesterol, plasma triglycerides, and/or LDL-C in response to treatment with a compound are indicative of therapeutic efficacy against PCKS9 activity. Cholesterol and lipid profiles can be determined by colorimetric, gas-liquid chromatographic, or enzymatic means using commercially available kits.

Methods/assays to determine PCSK9 activity are described below. As used herein the terms "**PCSK9 activity**" and "**PCSK9 function**" refer to detectable (direct or indirect) enzymatic, biochemical or cellular activity attributable to PCSK9. Without being so limited, such activities include the effect of PCSK9 on reducing the level of LDLR at the cell surface, on reducing plasma LDL-C and/or the PCSK9 proteinase activity itself (*e.g.*, PCSK9 secretion).

***In vitro* analysis of PCSK9 secretion and processing.**

The secretion and/or the proPCSK9 to PCSK9 processing are tested using ELISA assay or a biosynthetic approach using the measurement of PCSK9 in cells and media by Western blot. A decrease in PCSK9 secretion or response in the presence of compound is indicative that the compound inhibits a PCSK9 activity. For statistical significance each experiment is performed in triplicate. "Dose-dependent" responses of compound(s) are performed.

***In vitro* analysis of PCSK9-dependant LDLR degradation.**

Compound is also tested for its ability to inhibit the LDLR enhanced degradation by PCSK9 on mouse or human hepatocyte cell lines such as HepG2 or HuH7. The assay consists in the addition of wild type (WT), mutants or chimeric PCSK9, either following transfection or directly to the culture supernatants in the presence or absence of the tested compound. "Dose-dependent" responses of compound(s) are performed. For statistical significance each "dose-responses" experiment is done in duplicate or triplicate for 4 to 6 different dosages.

The inhibition of the PCSK9 activity is evidence by an increase of the LDLR protein expression and/or at the cell surface, as evidenced by:

- Western blot analysis of cell lysates for the total LDLR;
- FACS analysis for cell surface for LDLR;

• Fluorescent Dil-LDL incorporation monitoring the cell surface activity of LDLR.

The Dil-LDL fluorescent uptake assay consists in the fluorescence measurement of the Dil-LDL cellular incorporation via LDLR internalization (a measurement of cell surface LDLR activity). The cells are incubated in a 96-well format in the presence or absence of different doses of tested compound for 2h, and then Dil-LDL was added for an additional 2h. The inhibition of the PCSK9 activity is detected by an increase in the Dil-LDL fluorescence.

a. WT PCSK9. The assay consists in the addition of wild type (WT) PCSK9, either as conditioned media from transfected cells or purified, and added to the culture supernatants, in the presence or absence of the tested compound. The dose routinely chosen for PCSK9 added extracellularly is 1 ug/ml.

b. Mutants PCSK9 (gain of function). In order to further characterize whether the tested compound can inhibit the function of a gain of function mutation, the cells are incubated with purified mutant proteins, in the presence or absence of different doses of tested compound. Purified PCSK9 mutants are PCSK9-D374Y, for example (exhibiting a ~25-fold higher affinity towards LDLR) or S127R (showing an increase stability of PCSK9). The dose routinely chosen for PCSK9 and its gain-of-function natural mutant D374Y, added extracellularly are 1 µg/ml and 0.2 µg/ml. Others PCSK9 mutants are similarly used. The assay could also be conducted using culture medium harvested from cells transfected with gain of function PCSK9 mutants.

c. Chimeric PCSK9. Chimeric protein fusing the PCSK9 with the transmembrane and cytosolic domains of the cell surface angiotensin converting enzyme 2 (PCSK9-ACE2) is tested for measuring the activity of the tested compound on the PCSK9 extracellular pathway activity. Alternatively, chimeric protein fusing PCSK9 with the transmembrane and cytosolic domains of the Lamp-1 which directly traffic the protein to the endosomes/lysosomes (PCSK9-Lamp1) is tested for measuring the activity of the tested compound on the PCSK9 intracellular pathway activity. The stable cells expressing the chimeric PCSK9-ACE2 or PCSK9-Lamp1 are available and are be incubated in the presence or absence of different doses of tested compound. Chimeric protein also included PCSK9 with a V5 tag.

d. Primary human hepatocytes. The PCSK9 inhibitory compounds are tested on mouse and human primary hepatocytes in order to measure their effect on cell surface LDLR. The advantage of using the mouse primary hepatocytes is that it also measures the specificity of the compound in the context of a wild type or knockout mouse expressing or lacking PCSK9, respectively. HepG2 and HuH7 cells that no longer express PCSK9 endogenously (*e.g.*, under a shRNA knockdown) are also used for similar drug specificity purpose.

PCSK9 contained in conditioned media from transfected cells. Human wild type PCSK9 (PCSK9-WT) and gain-of-function (PCSK9-D374Y) proteins are produced by over-expression in HEK293 cells or Huh7 cells. Briefly, HEK293 or Huh7 cell lines are grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (Invitrogen) and maintained at 37 ºC under 5% CO2. HEK293 cells are transfected with jetPRIME™ (Polyplus transfection), and Huh7 cells are transfected with Lipofectamine™ 2000 (Invitrogen), according to the manufacturer's protocol. Twenty-four hours post-transfection, cells are washed and incubated in serum-free medium. Conditioned

media containing secreted human PCSK9-D374Y or PCSK9-WT proteins are collected 24 hours later. The level of PCSK9 proteins in conditioned media is quantified by enzyme-linked immunosorbent assay (ELISA), as described previously (Dubuc G, Tremblay M, Paré G, Jacques H, Hamelin J, Benjannet S, Boulet L, Genest J, Bernier L, Seidah NG, Davignon J. 2010. A new method for measurement of total plasma PCSK9: clinical applications. J Lipid Res. 51:140-149.).

Human HepG2 cells stably expressing PCSK9. Human HepG2 cells (ATCC, HB-8065) were transfected with the plasmid construct (human PCSK9 tagged at the C-terminus with V5) using the Fugene™ HD transfection reagent (Roche). Namely, HepG2 cells were transfected in a 35mm culture dish using Fugene™ HD optimised protocol for HepG2 cells (2ug DNA for 7ul Fugene™ HD, following the manufacturer's instructions). 72 hours post transfection, the cells are trypsinised and transferred onto a 100mm dish containing the selection medium: DMEM (high glucose + sodium pyruvate) (Wisent)/10%FBS and 600ug/ml G418 (Wisent). After 10 days of selection, a cell pool expressing the gene of interest is obtained. See also Seidah, NG, Benjannet, S, Wickham, L, Marcinkiewicz, J, Jasmin, SB, Stifani, S, Baska, A, Prat, A and Chretien, M. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc. Natl. Acad. Sci. U.S.A*, *100*:928-933, **2003**; Benjannet, S., Rhainds, D., Essalmani, R., Mayne, J., Wickham, L., Jin, W., Asselin, M.C., Hamelin, J., varret, M., Allard, D., Trillard, M., Abifadel, M., Tebon, A., Attie, A.D., Rader, D.J., Boileau, C., Brissette, L., Chretien, M., Prat, A., and Seidah, N.G. NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol. *J. Biol. Chem.*, *279*: 48865-48875, **2004**; and Benjannet, S., Rhainds, D., Hamelin, J., Nassoury, N., and Seidah, N.G. The proprotein convertase PCSK9 is inactivated by furin and/or PC5/6A: Functional consequences of natural mutations and post-translational modifications. *J. Biol. Chem.*, *281*:30561-30572, **2006**.

PCSK9 Secretion assay: detection of secreted PCSK9 by ELISA. In 12-well plates (Greiner BioOne™), HepG2 cells stably expressing PCSK9(+V5) were seeded at a density of 1 x 10^6/well in complete media and incubated for 20 hours, at 37 °C. Cells were rinsed once with 2 ml/well of D-PBS (+ Calcium, + Magnesium) (Wisent) followed by addition of 0.5 ml/well incubation media (+0.07% BSA) containing the inhibitory compounds at various concentrations (11, 33, 100 µM) or DMSO control (0.4% final) and overnight (24 hours) incubation. 24 hours-conditioned media was collected, centrifuged at 130 x g for 5 minutes to remove cell debris, and the supernatant removed for PCSK9 quantification by ELISA (100 µl of 1:30 dilution). Cells were washed once with ice-cold D-PBS (+ Calcium, + Magnesium) (Wisent), then lysed with 250 ul/well of radioimmunoprecipitation assay buffer (RIPA) (50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 1% Nonident P-40, 0.5% sodium deoxycholate, 0.1% SDS) containing a mixture of protease inhibitors (Roche Applied Science) for 30 min on ice, and pelleted at 11,300 x g for 5 minutes. Supernatant was removed for cellular PCSK9 quantification by ELISA (100 µl of 1:20 dilution) and total protein determination by the Bio-Rad™ DC Protein assay (Bio-Rad) (4 µl cell lysate in duplicate) (Dubuc G, Tremblay M, Paré G, Jacques H, Hamelin J, Benjannet S, Boulet L, Genest J, Bernier L, Seidah NG, Davignon J. 2010. A new

method for measurement of total plasma PCSK9: clinical applications. J Lipid Res. 51:140-149). The ratio of PCSK9 concentration of media (M) over cells (C) was determined and compounds which decreased M/C by > 30% were considered active.

Detection by Western blot. Cells are washed 3x in phosphate-buffered saline (PBS) and lysed in complete RIPA buffer (50 mM Tris/HCl, pH 8.0, 1% (v/v) Nonidet P40, 0.5% sodium deoxycholate, 150 mM NaCl and 0.1% (v/v) SDS) supplemented with 1x Complete Protease Inhibitor Mixture (Roche Applied Science). Proteins are separated by 8% SDS-polyacrylamide gel electrophoresis and blotted on polyvinylidene difluoride (PVDF, Perkin Elmer) membranes (GE Healthcare), which were blocked for 1 h in TBS-T (50mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20) containing 5% nonfat dry milk. Membranes are then incubated 3h in 5% nonfat milk with either a polyclonal hPCSK9 antibody (1:2500), a human LDLR antibody (1:1000, R&D Systems), or a rabbit β-Actin antibody (1:2500, Sigma). Appropriate horseradish peroxidase-conjugated secondary antibody (1:10,000, Sigma) is used for detection with enhanced chemiluminescence using the ECL plus kit (GE Healthcare).

Fluorescence-Activated cell sorting (FACS) quantification of cell surface LDLR levels. HuH7 cells are incubated for 4h or 18h at 37°C with various PCSK9 constructs in the presence or absence of the added compound(s), and then washed 3x with calcium/magnesium free Dulbecco's phosphate-buffered saline (DPBS) containing 0.5% bovine serum albumin (Sigma) and 1 g/l glucose (solution A). Cells are then incubated 5 min at 37□C with 500 µl of 1x Versene™ solution (Invitrogen) and layered on 5 ml of solution A. Cells are then centrifuged for 5 min at 1000 rpm and re-suspended in 1 ml of solution A containing 1:100 of monoclonal LDLR antibody C7 directed against human LDLR (mAb-C7, Santa Cruz Biotechnology) for 40 min. Cells are washed once with 5 ml of solution A, centrifuged and re-suspended for 20 min in 1 ml of PBS containing 1:250 of Alexa Fluor 647 donkey anti-mouse (Molecular Probes). Cells are washed and re-suspended in 300 µl of PBS 0.2% of propidium iodide (PI). Viable cells (PI-negative) are then analyzed by FACS for both PI and Alexa Fluor 647 using the FACS BD LSR (BD Biosciences).

Immunofluorescence of LDLR in human Huh7 cells. HepG2 naïve cells were plated on Poly-L-Lysine-coated (50 ug/ml) round microscope cover slips 1.12 mm thickness (Fisherbrand 12CIR #1) that were placed in a 24-well cell culture plate. Seeding was performed in DMEM complete media (10% FBS) and 24 hours later the media was swapped for media (+0.07% BSA) containing 33.3 µM of the different inhibitor compounds (300 µl/well). A volume of DMSO equivalent to the volume of inhibitor compound was also diluted in 0.07% BSA containing media and used as a negative control. After a 20 hour incubation period, the cells were washed 3x with PBS and then fixed for 10 min with 3.7% paraformaldehyde. Immunofluorescence of human LDLR (green labeling) was performed under non-permeabilizing conditions. After an additional 3 washes with PBS, cells were blocked for 30 min with 1% BSA, followed by overnight incubation at 4°C with primary antibody (1:200 goat polyclonal anti-hLDLR in 1% BSA, R&D Systems). Following a final 3 washes with PBS, antigen-antibody complexes were revealed by 1-hour incubation at room temperature with Alexa fluor-tagged secondary antibody and mounted in ProLong Gold Antifade Reagent

(Molecular Probes, Invitrogen). Immunofluorescence analyses were performed with a confocal miscroscope (Zeiss LSM-710).

Dil-LDL uptake cell-based assay for PCSK9 activity. HepG2 naive cells and HEK293 naive cells were plated in 96-well plates (CellBind black plate with clear bottom (Corning; Cat # 3340)) at a density of 25,000 cells/well, in complete media (DMEM high glucose (+ sodium pyruvate for HepG2) (Wisent) + 10% FBS). After 20 hours, cells were washed for 30 min with serum free DMEM media (100 µl/well), the wash media was removed and replaced with 100 µl/well of incubation media (DMEM high glucose (+ sodium pyruvate for HepG2) (Wisent) + 0.07% BSA (Sigma-Aldrich)) containing compound at various concentrations (11, 33, 100 µM) or DMSO control (0.4% final). Each condition was prepared in triplicates. After 6 hours incubation at 37 °C, Dil-LDL (Biomedical Technologies (Cat #BT-904)) was added to the cell media (5 µl) at 5 µg/ml final concentration, and cells returned to tissue culture incubator for another 18 hours (a total of 24 hours incubation with the compounds). After 2 washes (200 µl/well) with ice-cold D-PBS (Wisent) and aspiration of the final wash, plates were scanned (bottom read) on a SpectraMax GeminiEM™ plate reader (Molecular Devices). For each well, raw Dil-LDL uptake was measured as the average fluorescence intensity (RFU) (ex: 520 nm/ em: 575 nm, cutoff: 550 nm) of 9 readings in 3 different points in the well. Dil-LDL uptake in each well was corrected for total number of cells by performing a CyQuant™ cell assay (Invitrogen; Cat # C7026). Namely, after recording the Dil fluorescence, the plate was frozen overnight at -80°C. The next day, the plates were thawed at room temperature, the cells lysed according to the manufacturer's protocol, and the number of cells/well was determined by measuring the fluorescence (RFU) of the CyQuant green fluorescent dye bound to the cellular nucleic acids (ex: 485 nm/ em: 538 nm, cutoff: 515 nm). For each condition, Dil-RFU was divided by CyQuant-RFU. Corrected dil-LDL uptake is reported as % DMSO control and was obtained from triplicate wells.

Transfections, biosynthetic analyses, immunoprecipitations of PCSK9. Transfections are done with 3 x 10^5 HEK293 cells using Effectene™ (Qiagen) and a total of 0.5 µg of cDNAs. Alternatively, 5 x 10^5 HuH7 or 6 x 10^5 HepG2 cells are transfected with a total of 4 µg of cDNAs in Lipofectamine™ 2000 (Invitrogen). Two days post-transfection, HEK293 cells are washed and then incubated for various times with either 250 µCi/ml [35S]Met/Cys (PerkinElmer Life Sciences). The cells are lysed in modified RIPA buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5), 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and a protease inhibitor mixture (Roche Applied Science), after which the lysates and media are prepared for immunoprecipitation. The antibodies used are the anti-V5 mAb (Invitrogen, 1:500), and proprietary rabbit anti-PCSK9 31-454 (A-03). Immunoprecipitates are resolved by SDS-PAGE on 8% Tricine gels and autoradiographed. These experiments are repeated at least three times. Quantitation is performed on a Storm Imager™ (Amersham Biosciences) by using the ImageQuant™ version 5.2 software. Biosynthesis is a sensitive method that allows for testing whether the compounds affect proPCSK9 to PCSK9 activation in the endoplasmic reticulum and hence the secretion of active PCSK9 complexed with its prosegment into the medium. Cells engineered to express endogenously and stably PCSK9 (e.g. PCSK9-V5) WT or mutated variants

are incubated with the compound(s) and the different PCSK9 forms present in the media and cell lysates are analysed by Western blot using sensitive human PCSK9 antibodies.

MTT Toxicity Assay. HepG2 stable cells over-expressing hPCSK9(+V5) were seeded in complete DMEM (high glucose + sodium pyruvate) (Wisent) + 10% FBS media in 96-well plates (Greiner BioOne) at a density of 1 x 10^5 cells/well (100 µl) and incubated for 20 hours. Cells were rinsed once with 100 ul/well of serum free DMEM media, followed by addition of 100 µl/well of DMEM (high glucose + sodium pyruvate) (Wisent) + 0.07% BSA (Sigma-Aldrich) media containing compound at various concentrations (11, 33, 100 µM) or DMSO control (0.4% final) followed by overnight (24 hours) incubation. The MTT assay (Promega) consisted of addition of 20 µl/well MTT reagent and incubation for 45 minutes at 37°C, following the manufacturer's instructions. Absorbance was recorded at 490 nm and corrected for background due to nonspecific absorbance (690 nm).

Methods of using the compounds

PCSK9 has been implicated in cholesterol homeostasis, as it appears to have a specific role in cholesterol biosynthesis or uptake. In a study of cholesterol-fed rats, it was reported that PCSK9 was downregulated in a similar manner to other genes involved in cholesterol biosynthesis, (Maxwell *et al.*, 2003 *J Lipid Res.* **44**:2109-2119). PCSK9 expression has been found to be upregulated by statins in a manner attributed to the cholesterol-lowering effects of the drugs (Dubuc *et al.*, 2004, *Arterioscler Thromb Vasc Biol.* **24**: 1454-1459). Adenoviral expression of PCSK9 results in a time-dependent increase in circulating low density lipoprotein (LDL) cholesterol (LDL-C) (Benjannet *et al.*, 2004 *J. Biol. Chem.* **279**:48865-48875), and mice with PCSK9 gene deletions have increased levels of hepatic LDL receptors (LDLR) and clear LDL-C from the plasma more rapidly (Rashid *et al.*, 2005 *supra*). Medium from HepG2 cells which are transiently transfected with PCSK9 is found to reduce the amount of cell surface LDLRs and internalization of LDL-C when transferred to untransfected HepG2 cells (Cameron *et al.*, 2006 *Human Mol. Genet.* **15**:1551-1558). Additionally, purified PCSK9 added to the medium of HepG2 cells reduced the number of cell-surface LDLRs in a dose- and time-dependent manner (Lagace *et al.*, 2006, *supra*).

A number of mutations in the gene PCSK9 have been associated with autosomal dominant hypercholesterolemia (ADH), an inherited metabolism disorder which is characterized by marked elevations of LDL-C particles in the plasma that can lead to premature cardiovascular failure (*e.g.*, Abifadel *et al*, 2003 *Nat. Genetics* **34**:154-156; Tirnms *et al*, 2004 *Hum. Genetics* **114**:349-353; Leren, 2004 *Clin. Genet.* **65**:419-422).

Increased expression or upregulation of PCSK9 is associated with increased plasma levels of LDL-C, and inhibition or lack of expression of PCSK9 is associated with low LDL-C plasma levels. Lower levels of LDL-C associated with sequence variations in PCSK9 confer protection against coronary heart disease (Cohen, *et al*, 2006 *N. Engl. J. Med.* **354**:1264-1272).

The compounds described herein have *in vitro* and *in vivo* therapeutic utilities. For example, these molecules can be administered to cells in culture, *e.g.*, *in vitro* or *ex vivo*, or in a subject in need thereof, *e.g.*, *in vivo*, to treat, prevent or diagnose a variety of disorders associated with PCSK9 activity/function.

Compounds of the present invention are particularly suitable for treating human patients having, or at risk for, elevated cholesterol or a condition associated with elevated cholesterol (*e.g.,* LDL cholesterol), including a lipid disorder (*e.g.*, hyperlipidemia, hypercholesterolemia, xanthomatosis). Compounds of the present invention may also be suitable for treating human patients having ateriosclerotic conditions (*e.g.*, atherosclerosis), coronary artery disease, cardiovascular disease, stroke, ischemia, peripheral vascular diseases, and prophylactically for patients at risk for these disorders, *e.g.*, due to the presence of one or more risk factors (*e.g.*, hypertension, cigarette smoking, diabetes, obesity, or hyperhomocysteinemia).

As used herein the terms "**LDL-cholesterol-related disease or disorder**" refer to a disease or condition resulting in part from a high level of circulating LDL-cholesterol in the blood stream. Without being so limited, LDL-cholesterol-related diseases or disorders include hyperlipidemia, hypercholesterolemia, xanthomatosis and cardiovascular diseases such as ateriosclerotic conditions (*e.g.*, atherosclerosis), coronary artery disease, stroke, ischemia and peripheral vascular diseases.

As used herein, the terms "treat/treating/treatment" and "prevent/preventing/prevention", refer to eliciting the desired biological response, i.e. a therapeutic and prophylactic effect, respectively. In accordance with the subject invention, the therapeutic effect comprises a decrease/reduction in the progress of the LDL-cholesterol-related disease or disorder or in the severity of associated symptoms or a complete cure of the LDL-cholesterol-related disease or disorder and/or associated symptoms. In accordance with the invention, a prophylactic effect may comprise a delay or decrease in the onset of, progression of, or the severity of LDL-cholesterol-related disease or disorder and associated symptoms (*e.g.*, decreased plasma LDL/cholesterol levels), following administration of a compound or composition of the present invention. In an embodiment, the compound or composition of the present invention, comprising a compound of Formula (1) treats or prevent the LDL-cholesterol-related disease or disorder in a subject. The methods, compositions formulations and uses described herein are suitable for both humans and animals (including birds), preferably mammals.

Combination therapy

When compounds are administered together with another agent, the two can be administered sequentially in either order or simultaneously (in the same composition or in different compositions). In some embodiments, a compound is administered to a subject who is also receiving therapy with a second agent useful for treating the disease/condition (*e.g.*, a second cholesterol-reducing agent). Examples of active ingredients that may be administered in combination with of the present invention include, but are not limited to, other compounds which improve a patient's lipid profile, such as (a) HMG-CoA reductase inhibitors, (*e.g.*, statins, including lovastatin, simvastatin, fluvastatin, rosuvastatin, pravastatin, rivastatin, atorvastatin, itavastatin, pitavastatin, cerivastatin and

other statins). Statins inhibit cholesterol synthesis by blocking HMGCoA, a key enzyme in cholesterol biosynthesis; (b) cholesterol absorption inhibitors, such as stanol esters, beta-sitosterol, sterol glycosides such as tiqueside; and azetidinones, such as ezetimibe; (c) inhibitors of cholesterol ester transport protein (CETP) (*e.g.*, anacetrapib or dalcetrapib) which are now in clinical trials to increase HDL and decrease LDL cholesterol; (d) niacin and related compounds, such as nicotinyl alcohol, nicotinamide, and nicotinic acid or a salt thereof; (e) bile acid sequestrants (cholestyramine, colestipol (*e.g.*, colestipol hydrochloride), dialkylaminoalkyl derivatives of a cross-linked dextran, Colestid®, LoCholest®. Bile acid sequestrants interrupt the recycling of bile acids from the intestine to the liver; (f) acyl CoA:cholesterol acyltransferase (ACAT) inhibitors, such as avasimibe and melinamide, and including selective ACAT-1 and ACAT-2 inhibitors and dual inhibitors; (g) PPARy agonists, such as gemfibrozil and fenofibric acid derivatives (fibrates), including clofibrate, fenofibrate, bezafibrate, ciprofibrate, and etofibrate; (h) microsomal triglyceride transfer protein (MTP)/ApoB secretion inhibitors; (i) anti-oxidant vitamins, such as vitamins C and E and beta carotene; (j) thyromimetics; (k) LDL receptor inducers, (l) platelet aggregation inhibitors, for example glycoprotein IIb/IIIa fibrinogen receptor antagonists and aspirin; (m) vitamin B 12 (also known as cyanocobalamin); (n) folic acid or a pharmaceutically acceptable salt or ester thereof, such as the sodium salt and the methylglucamine salt; (o) FXR and LXR ligands, including both inhibitors and agonists; (p) agents that enhance ABCA1 gene expression; (q) ileal bile acid transporters and ® long chain alpha, omego-dicarboxylic acids.

A combination therapy regimen may be additive, or it may produce synergistic results (*e.g.*, reductions in cholesterol greater than expected for the combined use of the two agents). In some embodiments, combination therapy with a compound and a cholesterol-reducing agent (*e.g.*, a statin, fibrates, ezetimibe or a combination thereof) produces synergistic results (*e.g.*, synergistic reductions in cholesterol). In some subjects, this can allow reduction in the dosage of the cholesterol-reducing agent to achieve the desired cholesterol levels. Compounds may be useful for subjects who are intolerant to therapy with another cholesterol-reducing agent, or for whom therapy with another cholesterol-reducing agent has produced inadequate results (*e.g.*, subjects who experience insufficient LDL-C reduction on statin therapy).

A compound described herein can be administered to a subject with elevated cholesterol (*e.g.*, LDL-cholesterol) (*e.g.*, a human subject with total plasma cholesterol levels of 200 mg/dl or greater, a human subject with LDL-C levels of 160 mg/dl or greater).

Kits

The present invention also encompasses kits comprising at least one compound of the present invention. For example, the kit can comprise one or more compounds preventing and/or treating LDL-cholesterol-related disease or disorder. The kit may optionally include one or more control samples and a device (*e.g.*, syringe, etc.). The kit may optionally include one or more other active ingredient which improves a patient's lipid profile. The compounds or ingredients can be packaged in a suitable container. The kit can further comprise instructions for using the kit (*e.g.*, to use the compound for preventing or treating the low density lipid-cholesterol-related disease or disorder in the

subject).

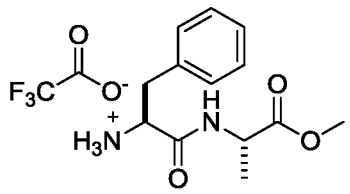
The invention having been fully described, it is further illustrated by the following examples and claims, which are illustrative and are not meant to be further limiting. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are within the scope of the present invention and claims. The contents of all references, including issued patents and published patent applications, cited throughout this application are hereby incorporated by reference.

MODE(S) FOR CARRYING OUT THE INVENTION

The present invention is illustrated in further details by the following non-limiting examples.

Intermediate 1

(*S*)-Methyl 2-((*S*)-2-amino-3-phenylpropanamido)propanoate trifluoroacetate

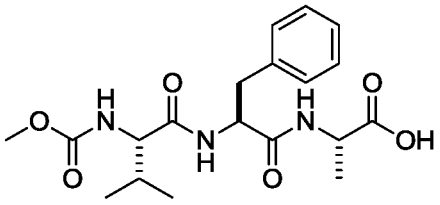


Step 1: DIPEA (46 mL, 260 mmol, 3.5 eq.) was added dropwise over 10 min to a stirred solution of *N*-Boc-L-phenylalanine (20 g, 74 mmol), L-alanine methyl ester hydrochloride (12.6 g, 90.5 mmol, 1.2 eq.) and HATU (42.9 g, 113 mmol, 1.5 eq.) in DMF (250 mL) at 0°C followed by warming to rt. After 18 h of stirring, the reaction mixture was poured into a cold saturated solution of $NaHCO_3$ and extracted with 1:1 EtOAc:hexanes (2x). The combined organic fractions were washed with water and brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified on silica gel eluting with an increasing proportion of EtOAc in hexanes.

Step 2: A stirred solution of the product from Step 1 (14.8 g, 42.2 mmol) in CH_2Cl_2 (141 mL) at 0°C was treated with TFA (32.5 mL, 422 mmol, 10 eq.) dropwise. The reaction mixture was subsequently warmed to rt and stirred for an additional 3 h before being concentrated to dryness. The residue was triturated with Et_2O and the solid was collected by filtration and dried under high vacuum to afford the title compound.

Intermediate 2

(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(Methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]propanoic acid

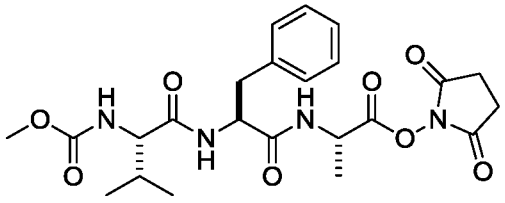


Step 1: Collidine (3.0 mL, 23 mmol, 2.5 eq.) was added dropwise over 10 min to a stirred solution of *N*-methoxycarbonyl-L-valine (1.60 g, 9.13 mmol), Intermediate 1 (3.49 g, 9.59 mmol, 1.05 eq.) and HATU (3.82 g, 10.0 mmol, 1.1 eq.) in DMF (30 mL) at 0°C with stirring at this temperature for 1 h and then at rt for 18 h. The mixture was then recooled to 0°C and a saturated aqueous solution of $NaHCO_3$ (50 mL) was added slowly followed by the addition of Et_2O (50 mL). The mixture was stirred vigorously for 20 min and the solid product was collected by filtration, washed with separate portions of water and Et_2O, and dried under suction and high vacuum.

Step 2: 1 M LiOH (10.65 mL, 10.65 mmol, 1.25 eq.) was added dropwise to a stirred suspension of the product from Step 1 (3.47 g, 8.52 mmol) in a mixture of THF (40 mL) and MeOH (20 mL) followed by slow warming to rt over several hours. The vessel contents were then recooled to 0°C, additional 1 M LiOH (3.4 mL, 3.4 mmol, 0.4 eq.) was added dropwise and the mixture was stirred at rt for an additional 2 h prior to acidification to pH 4 with 1 M HCl at 0°C and extraction with ethyl acetate (2x). The combined organic fractions were washed with water, half saturated brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the title compound which was used without further purification.

Intermediate 3

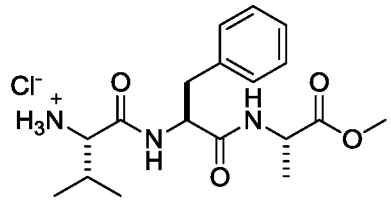
2,5-Dioxopyrrolidin-1-yl (2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]propanoate



DIPEA (0.33 mL, 1.9 mmol, 3.0 eq.) was added to a solution of Intermediate 2 (0.25 g, 0.64 mmol) and TFA-NHS (0.40 g, 1.9 mmol, 3.0 eq.) in DMF (4.5 mL) at -78°C. The reaction mixture was then warmed slowly to 0°C and stirred for 2 h at this temperature before being partitioned between water and EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (2x). The combined organic layers were washed with half saturated brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the title compound that was used in subsequent reactions without further purification.

Intermediate 4

Methyl (2*S*)-2-[(2*S*)-2-[(2*S*)-2-amino-3-methylbutanamido]-3-phenylpropanamido]propanoate hydrochloride



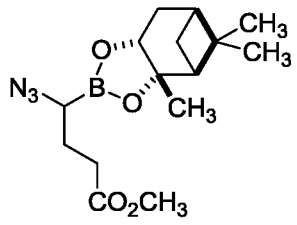
Step 1: DIPEA (6.7 mL, 38 mmol, 3.5 eq.) was added dropwise over 10 min to a stirred solution of Intermediate 1 (4.0 g, 11 mmol), *N*-Boc-L-Valine (2.62 g, 12.0 mmol, 1.1 eq.) and HATU (5.0 g, 13 mmol, 1.2 eq.) in DMF (35 mL) at 0°C. After stirring overnight at rt, the vessel contents were poured into a mixture of ice (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and Et_2O (200 mL) and the mixture was stirred for 30 min. The solid product was collected by filtration, washed with water and 1:1 Et_2O/ hexanes and was dried under suction and high vacuum.

Step 2: TFA (7.3 mL, 95 mmol, 10 eq.) was added dropwise to a stirred solution of the product from Step 1 (4.28 g, 9.52 mmol) in CH_2Cl_2 (30 mL) at 0°C. The reaction vessel contents were then held at rt for 5 h before being concentrated to dryness. The residue was triturated with Et_2O and the solid product was collected by filtration and dried under high vacuum.

Step 3: 4 M HCl in dioxane (14 mL, 56 mmol, 5.9 eq.) was added to a stirred suspension of the product from Step 2 (4.40 g, 9.52 mmol) in MeOH (20 mL) with stirring overnight at rt. After concentrating the mixture to dryness, Et_2O was added with stirring for 20 min and the solid product was collected by filtration and dried under high vacuum to afford the title compound.

Intermediate 5

Methyl (4*R/S*)-4-azido-4-[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.$0^{2,6}$]decan-4-yl]butanoate



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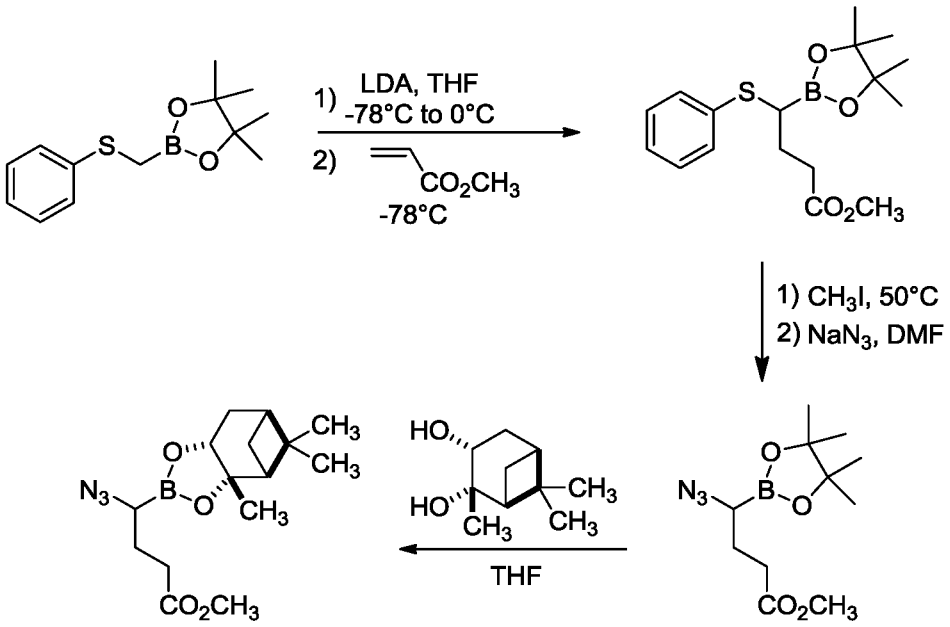


Step 1: DIPEA (6.2 mL, 44 mmol, 1.1 eq.) and THF (40 mL) were placed in a flame-dried rbf flushed with N_2. The temperature was cooled to -78°C and *n*-BuLi (2.5 M in hexanes, 17 mL, 42 mmol, 1.0 eq.) was added dropwise to the reaction vessel over a period of 2 min. The reaction mixture was then warmed to 0°C and stirred for 5 min before being cooled back to -78°C. A solution of 4,4,5,5,-tetramethyl-2-phenylsulfanylmethyl-1,3,2-dioxaborolane (10 mL, 42 mmol) in THF (10 mL) was added dropwise to the reaction mixture followed by stirring at 0°C. After 1 h at this temperature, the reaction vessel contents were recooled to -78°C and methyl acrylate (7.6 mL, 85 mmol, 2.0 eq.) was introduced dropwise. After 6 h at -78°C, the reaction was quenched by pouring into 200 mL of 10% citric acid aqueous solution. The layers were separated and the aqueous layer was extracted with Et_2O (3x). The organics were combined, washed with half saturated brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The product was isolated by flash chromatography on silica gel eluting with an increasing proportion of EtOAc in hexanes.

Step 2: The product from Step 1 (1.80 g, 5.35 mmol), iodomethane (6.6 mL, 110 mmol, 20 eq.) and acetonitrile (8 mL) were heated together in a N_2 flushed sealed tube at 50 °C. After 24 h at this temperature, the vessel contents were concentrated under reduced pressure. The residue was used directly in the next step.

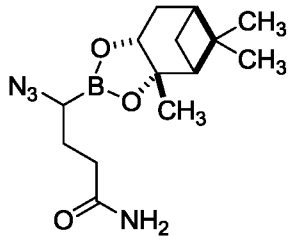
Step 3: The product from Step 2 (1.89 g, 5.35 mmol) was dissolved in DMF (11 mL) and treated with sodium azide (0.70 g, 11 mmol, 2.0 eq.) with stirring overnight at rt. The reaction mixture was then partitioned between EtOAc and water. The layers were separated and the aqueous layer extracted with EtOAc (2x). The combined

organics were washed with half saturated brine (3x), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was used directly in the next step.

Step 4: A solution of the product from Step 3 (1.43 g, 5.35 mmol) and (1*S*,2*S*,3*R*,5*S*)-(+)-pinanediol (1.00 g, 5.90 mmol, 1.1 eq.) in THF (21 mL) was stirred at rt for 18 h. The reaction vessel contents were concentrated to dryness and the residue was purified by flash chromatography on silica gel eluting with an increasing proportion of EtOAc in hexanes to afford the title compound.

Intermediate 6

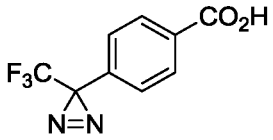
(4*R/S*)-4-Azido-4-[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.$0^{2,6}$]decan-4-yl]butanamide



Intermediate 5 (0.30 g, 0.94 mmol) was stirred with 7.0 M NH_3 in MeOH (4 mL, 30 mmol, 30 eq.) for 42 h at rt in a N_2 flushed sealed tube. The vessel contents were then concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel eluting with an increasing proportion of MeOH in EtOAc to afford the title compound.

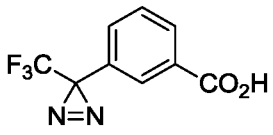
Intermediate 7a

4-(3-(Trifluoromethyl)-3*H*-diazirin-3-yl)benzoic acid



Intermediate 7b

3-(3-(Trifluoromethyl)-3*H*-diazirin-3-yl)benzoic acid



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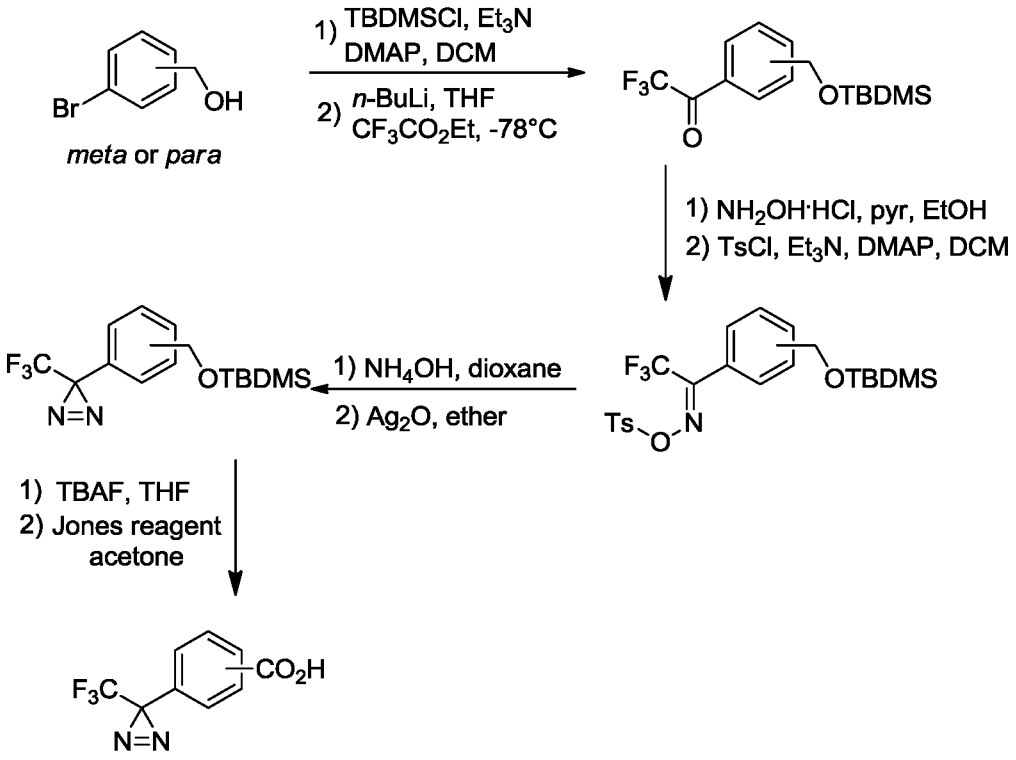


Step 1: Et_3N (11 mL, 80 mmol, 1.5 eq.) and DMAP (0.65 g, 5.3 mmol, 0.1 eq.) were added to a stirred solution of 4-bromobenzyl alcohol (to prepare 7a) or 3-bromobenzyl alcohol (to prepare 7b) (10.00 g, 53.46 mmol) in CH_2Cl_2 (60 mL) at 0°C followed by *tert*-butyldimethylsilyl chloride (8.86 g, 58.8 mmol, 1.1 eq.) in a portion-wise manner. The reaction vessel contents were then warmed to rt for 3 h. Water was added and the mixture was stirred until all solids had dissolved. The mixture was transferred to a separatory funnel and extracted with CH_2Cl_2 (3x). The combined organic fractions were washed with a saturated aqueous solution of NH_4Cl and water, and then were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was used directly in the next step.

Step 2: *n*-BuLi (2.5 M in hexanes, 24 mL, 60 mmol, 1.1 eq.) was added dropwise over 30 min to a stirred solution of the product from Step 1 (15.89 g, 52.74 mmol) in THF (175 mL) at -78°C. The reaction was stirred at this temperature for 30 min prior to the dropwise introduction of a solution of ethyl trifluoroacetate (7.6 mL, 63 mmol, 1.2 eq.) in THF (30 mL) over 30 min. The reaction was stirred for an additional 90 min at -78°C before being partitioned between EtOAc and a saturated solution of NH_4Cl. The phases were separated and the aqueous layer was extracted with EtOAc (2x). The combined organic fractions were washed with a saturated solution of brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by distillation under high vacuum (P = 10 mmHg) collecting the fraction boiling between 160-170 °C.

Step 3: The product from Step 2 (8.0 g, 25 mmol) and hydroxylamine hydrochloride (1.92 g, 27.6 mmol, 1.1 eq.) were heated together at reflux in a mixture of pyridine (12 mL) and EtOH (6 mL). After 3 h, the solvents were removed under reduced pressure and the residue was partitioned between EtOAc and water. The phases were separated and the aqueous layer was extracted with EtOAc (2x). The combined organic fractions were washed with a saturated solution of brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified on silica gel with an increasing proportion of EtOAc in hexanes.

Step 4: *p*-Toluenesulfonyl chloride (3.29 g, 17.2 mmol, 1.15 eq.) was added in a portion-wise manner to a stirred solution of the product from Step 3 (5.0 g, 15 mmol), Et_3N (2.5 mL, 18 mmol, 1.2 eq.) and DMAP (0.183 g, 1.5 mmol, 0.10 eq.) in CH_2Cl_2 (26 mL) at 0°C. Following completion of the reaction as judged by TLC, the mixture was concentrated under reduced pressure and the residue was partitioned between EtOAc and water. The layers were separated and the organic phase was washed with additional water and then with brine before being dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was used without additional purification in the next step.

Step 5: Concentrated NH_4OH (68 mL) was added to a stirred solution of the product from Step 4 (6.85 g, 14.1 mmol) in dioxane (68 mL) at 10 °C in a thick-walled glass tube. The tube was capped and the contents were stirred at rt for 48 h. Water and EtOAc were then added and the layers were separated. The aqueous layer was extracted with EtOAc (2x) and the combined organic fractions were washed with a saturated solution of brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified on silica gel with an increasing proportion of EtOAc in hexanes.

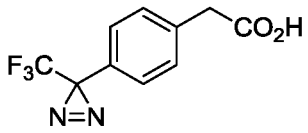
Step 6: A solution of the product from Step 5 (1.8 g, 5.4 mmol) in Et_2O (54 mL) was stirred with Ag_2O (2.50 g, 10.9 mmol, 2 eq.) at rt. After 4 h, a second portion of Ag_2O was added (2.50 g, 10.9 mmol, 2 eq.) and the mixture was stirred at rt overnight. Hexanes (100 mL) were then added and the mixture filtered through a silica gel pad. The pad was washed with 9:1 hexanes/EtOAc and the filtrate was concentrated under reduced pressure. The residue was used directly in the next step.

Step 7: TBAF (1.0 M in THF, 6.2 mL, 6.2 mmol, 1.2 eq.) was added dropwise to a solution of the product from Step 6 (1.70 g, 5.15 mmol) in THF (25 mL) at 0°C followed by stirring at rt for 1 h. The mixture was then partitioned between EtOAc and water and the phases were separated. The aqueous phase was extracted with EtOAc (2x) and the combined organics were washed with a saturated solution of brine, dried (MgSO4), filtered and concentrated under reduced pressure. The residue was purified on silica gel with an increasing proportion of EtOAc in hexanes.

Step 8: Jones reagent (2.5 M, 0.56 mL, 1.4 mmol, 2.0 eq.) was added to a solution of the product from Step 6 (0.15 g, 0.69 mmol) in acetone (7.0 mL) with stirring at 0°C for 1 h. The mixture was then diluted with EtOAc and washed with water in a separatory funnel until no color was visible in the aqueous layer. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the title compound.

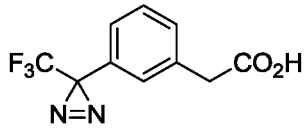
Intermediate 7c

2-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)acetic acid



Intermediate 7d

2-(3-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)acetic acid



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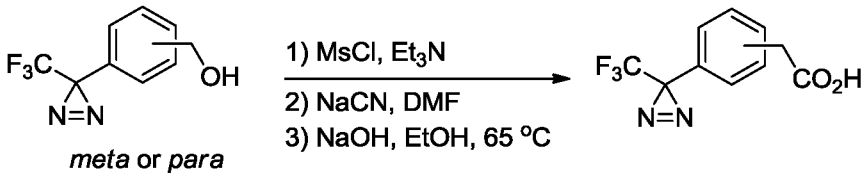


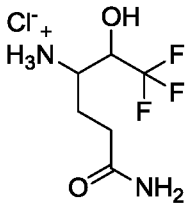
Step 1: MsCl (0.22 mL, 2.8 mmol, 1.5 eq.) was added dropwise to a solution of the *para*- or *meta*-product (400 mg, 1.85 mmol) from step 7 in the preparation of Intermediate 7a or 7b and Et_3N (0.52 mL, 3.7 mmol, 2 eq.) in Et_2O (19 mL) at 0 °C with stirring at this temperature for 15 min and then at rt. After 2h, the mixture was filtered, the solid material was washed with ether, and the filtrate was concentrated in vacuo. The residue was used in the next step without further purification.

Step 2: The product from Step 1 (535 mg, 1.82 mmol) and NaCN (134 mg, 2.70 mmol, 1.5 eq.) were stirred together in DMF (10 mL) at rt overnight. The mixture was subsequently partitioned between water and Et_2O and the layers were separated. The organic phase was washed with water, then with brine, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography on silica gel eluting with 1/4 EtOAc/hexanes.

Step 3: The product from Step 2 (200 mg, 0.89 mmol) was stirred with 1 M NaOH (4.5 mL, 5 eq.) in EtOH (4.5 mL) at 65 °C overnight. After cooling to rt, the reaction was quenched by the addition of 1 M HCl (5 mL) and the mixture was partitioned between water and Et_2O. The layers were separated and the aqueous phase was extracted with additional Et_2O (2x). The organics were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography on silica gel eluting with 1/9 EtOH/hexanes containing 1% AcOH to afford the title compound as a pale yellow solid.

Intermediate 8

4-Amino-6,6,6-trifluoro-5-hydroxyhexanamide hydrochloride



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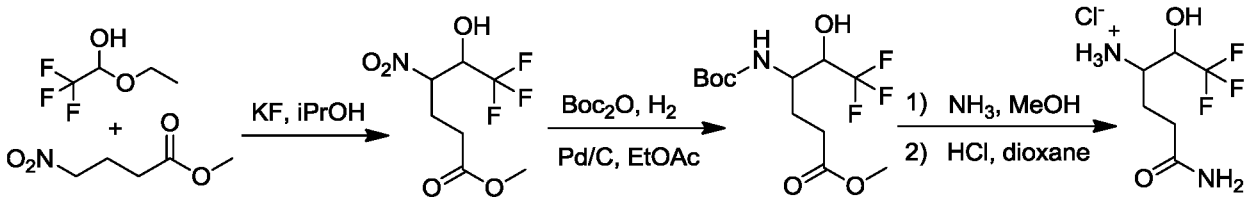


Step 1: Trifluoroacetaldehyde ethyl hemiacetal (90%, 15 mL, 110 mmol, 1.8 eq.), methyl 4-nitrobutyrate (8.0 mL, 62 mmol), potassium fluoride (3.65 g, 62.8 mmol) and *i*-PrOH (20 mL) were placed in a sealed reaction vessel and the mixture was stirred at rt for 20 h. The mixture was then partitioned between EtOAc (200 mL) and a 1:1 mixture of 5% (w/w) aqueous citric acid and brine (300 mL) and the layers were separated. The organic phase was washed with additional brine (200 mL) and the combined aqueous layers were extracted with EtOAc (200 mL). The

organic layers were combined, dried over $MgSO_4$, filtered and concentrated by rotary evaporation under reduced pressure while keeping the bath temperature at 20 °C. The residue was purified by column chromatography on silica gel by eluting with 20% EtOAc/hexanes.

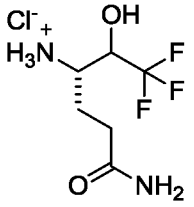
Step 2: A mixture of the product from Step 1 (13.0 g, 52.9 mmol), di-*tert*-butyl dicarbonate (15.0 g, 68.7 mmol, 1.3 eq.) and 10% Pd/C (4.00 g) was placed in a septum-capped rbf under vacuum and degassed EtOAc (100 mL) was introduced by syringe. The mixture was then placed under an atmosphere of H_2 via a latex balloon and the reaction vessel was sonicated in an ultrasonic bath for 2 min followed by stirring of the contents at rt for 24 h. Following completion of the reaction as judged by TLC, the vessel was flushed with N_2 and CH_2Cl_2 was added. The suspension was filtered through a Celite pad and the pad was washed thoroughly with a mixture of EtOAc and CH_2Cl_2. The filtrate was concentrated in vacuo and the residue was treated with 1:4 mixture of Et_2O/heptane. After standing for 4 h, the solid product was collected by suction filtration and washed with a small amount of 1:4 Et_2O/heptane to afford the desired diasteromer in >10:1 diastereomeric purity.

Step 3: A solution of NH_3 in MeOH (7.0 M, 30 mL, 200 mmol, 20 eq.) was added to the product from Step 2 (3.0 g, 9.5 mmol) at 0 °C under N_2 in a thick walled glass tube and the entrance was sealed with a Teflon screw cap followed by heating of the contents at 35 °C for 5 days. The mixture was then concentrated under reduced pressure and the residue was stirred with Et_2O overnight. The solid product was collected by suction filtration and dried under high vacuum.

Step 4: A solution of HCl in 1,4-dioxane (4 M, 1.2 mL, 5 mmol, 5 eq.) was added dropwise to a suspension of the product from Step 3 (0.30 g, 1.0 mmol) in CH_2Cl_2 (2 mL) at 0 °C followed by stirring at rt until the reaction was determined to be complete by LCMS (3 h). The reaction mixture was then concentrated to dryness under reduced pressure to afford the title compound. The material was stored under a N_2 atmosphere at -15 °C and was used in subsequent reactions without further purification.

Intermediate 9

(4*S*)-4-Amino-6,6,6-trifluoro-5-hydroxyhexanamide hydrochloride



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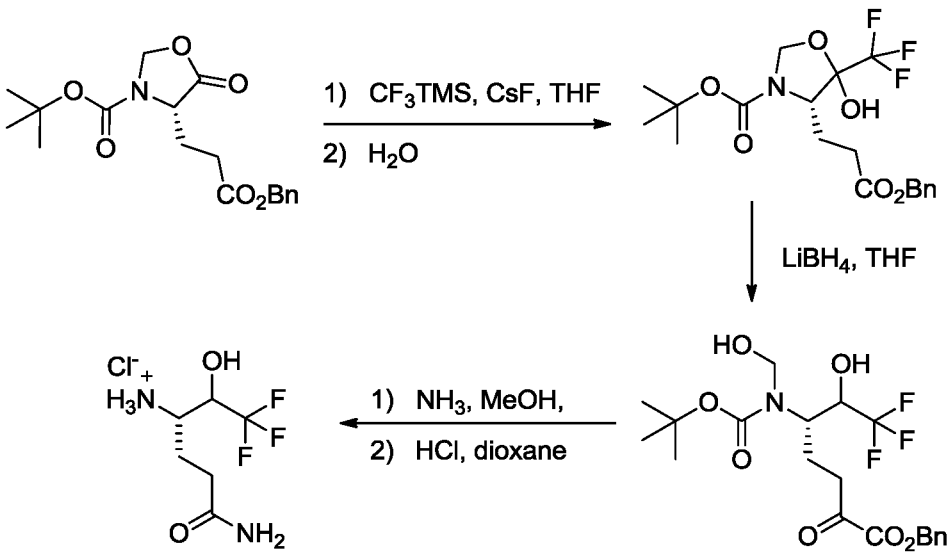


Step 1: A solution of (*S*)-3-*tert*-butoxycarbonyl-4-(2-benzyloxycarbonylethyl)oxazolidin-5-one (4.97 g, 14.2 mmol) in dry THF (30 mL) was treated with CF_3TMS (2.5 mL, 17 mmol, 1.2 eq.) and CsF (0.34 g, 2.2 mmol, 0.15 eq.) under an atmosphere of N_2. The reaction vessel was then sonicated in an ultrasonic bath and the progress of the reaction was monitored by TLC. After 1h, water (2.5 mL) was added and sonication was continued until hydrolysis of the intermediate silyl ether was judged to be complete by TLC (30 min). EtOAc was added and the mixture was washed with water and brine. The aqueous phases were extracted with EtOAc and the combined organic layers were dried over $Na_2SO_4/MgSO_4$, filtered and concentrated under vacuum and the residue was used directly in the next step.

Step 2: A solution of $LiBH_4$ in THF (2.0 M, 6.5 mL, 13 mmol, 1.05 eq.) was added dropwise to a solution of the product from Step 1 (5.28 g, 12.6 mmol) in THF (50 mL) at a rate sufficient to maintain internal temperature below 30 °C. Following completion of the reaction as judged by TLC (5 min), the reaction vessel was cooled to 0 °C and the reaction was quenched by the addition of ice water. The reaction vessel contents were partitioned between EtOAc and water and the layers were separated. The aqueous phase was extracted with additional EtOAc (2x) and the combined organics were washed with brine, dried over $MgSO_4/Na_2SO_4$, filtered and concentrated under vacuum to afford the product as a 2.2:1 mixture of diastereomers. These were separated by repeated flash chromatography on silica gel eluting with an increasing proportion of EtOAc (0-30%) in toluene. The desired diastereomer corresponded to the more polar compound and was the minor isomer produced in the reaction.

Step 3: A solution of NH_3 in MeOH (7.0 M, 15 mL, 100 mmol, 110 eq.) was added to the product from Step 2 (0.38 g, 0.90 mmol) at 0 °C under N_2 in a thick walled glass tube and the entrance was sealed with a Teflon screw

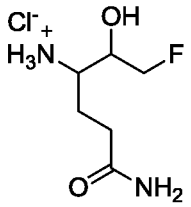
cap followed by heating of the contents at 45 °C for 48 h. The mixture was then concentrated under reduced pressure and the residue was azeotroped with ether/heptane (2 x) prior to trituration with Et_2O for 1 h. The solid product was collected by suction filtration and dried under high vacuum.

Step 4: A solution of HCl in 1,4-dioxane (4 M, 0.70 mL, 2.8 mmol, 28 eq.) was added dropwise to a suspension of the product from Step 3 (30 mg, 0.10 mmol) in CH_2Cl_2 (0.7 mL) at 0 °C followed by stirring at rt until the reaction was determined to be complete by LCMS (2 h). The reaction mixture was then concentrated to dryness under reduced pressure to afford the title compound. The material was utilized immediately in subsequent reactions without additional purification.

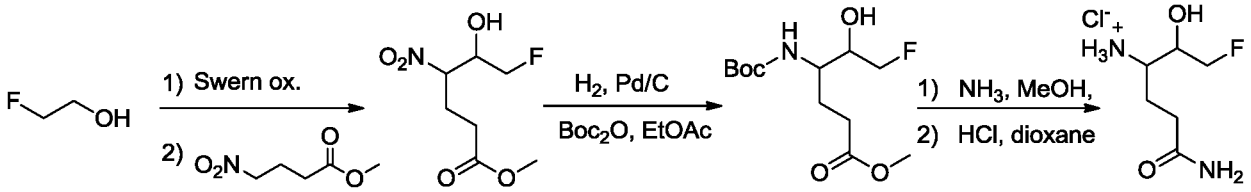
The above sequence can also be utilized to prepare (4R, 5S)- and (*4R, 5R*)-4-amino-6,6,6-trifluoro-5-hydroxyhexanamide hydrochloride from (*R*)-3-*tert*-butoxycarbonyl-4-(2-benzyloxycarbonylethyl)oxazolidin-5-one.

Intermediate 10

4-Amino-6-fluoro-5-hydroxyhexanamide hydrochloride



Reaction sequence:



Step 1: A solution of DMSO (8.7 mL, 120 mmol, 3 eq.) in CH_2Cl_2 (15 mL) was added slowly to a solution of oxalyl chloride (4.2 mL, 48 mmol, 1.2 eq.) in CH_2Cl_2 (50 mL) at -78 °C followed by stirring at this temperature for 30 min. A solution of 2-fluoroethanol (2.4 mL, 41 mmol) in CH_2Cl_2 (20 mL) was then introduced dropwise and the mixture was warmed slowly to -40°C over 1 h followed by recooling to -78 °C prior to the dropwise addition of Et_3N (28.5 mL, 204 mmol, 5 eq.). The formation of insoluble material necessitated shaking to the reaction vessel by hand to ensure homogeneity was maintained over the course of the addition. The suspension was then warmed to rt and

stirred for an additional 1.5 h, after which methyl 4-nitrobutyrate (4 mL, 31.2 mmol, 0.77 eq.) was added to the reaction vessel while cooling in a water bath at rt. The mixture was stored at -15 °C overnight and then stirred at rt for 2.5 h prior to partitioning of the mixture between EtOAc (100 mL) and an aqueous solution prepared from 10% citric acid and brine. The aqueous phase was extracted with EtOAc (100 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated by rotary evaporation under reduced pressure at a bath temperature of 20 °C. The residue was subjected to flash chromatography on silica gel eluting with an increasing proportion of EtOAc (0-50%) in hexanes to afford the product as a mixture of diastereomers.

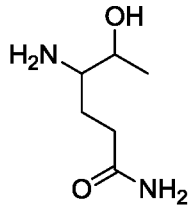
Step 2: The product from Step 1 (4.01 g, 20.0 mmol), di-*tert*-butyl dicarbonate (5.23 g, 24.0 mmol, 1.25 eq.) and 10% Pd/C (1.00 g) were placed under vacuum in a septum capped rbf and degassed EtOAc (50 mL) was introduced via syringe. The mixture was then placed under an atmosphere of H_2 via a latex balloon and the reaction vessel contents were sonicated for 2 min followed by stirring of the contents at rt for 24 h. Sonication of the reaction vessel contents was then repeated for another 2 min period and the mixture was stirred for an additional 24 h at rt. Following completion of the reaction as judged by TLC, the vessel was flushed with N_2 and CH_2Cl_2 was added. The suspension was filtered through a Celite pad and the pad was washed thoroughly with a mixture of EtOAc and CH_2Cl_2. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography on silica gel eluting with an increasing proportion of EtOAc (10-50%) in hexanes. The major diastereomer eluted first (less polar) and the minor diastereomer eluted second (more polar). Both diastereomers were purified further by trituration with Et_2O/hexanes to afford the products in >10:1 diastereomeric purity as determined by ^1H NMR.

Step 3: A solution of NH_3 in MeOH (7.0 M, 6.0 mL, 42 mmol, 30 eq.) was added to the products isolated in Step 2 (400 mg, 1.43 mmol) at 0 °C under N_2 in a thick walled glass tube and the entrance was sealed with a Teflon screw cap followed by heating of the contents at 40 °C for 3 d. The mixture was then concentrated to dryness under reduced pressure and the residue was triturated with Et_2O. The solid products were collected by suction filtration and dried under vacuum.

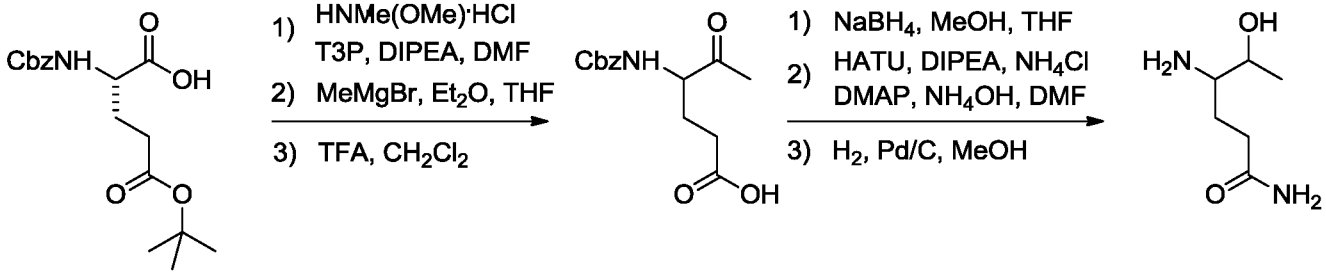
Step 4: A solution of HCl in 1,4-dioxane (4.0 M, 1.0 mL, 4.0 mmol, 14 eq.) was added dropwise to a suspension of the products from Step 3 (75 mg, 0.28 mmol) in CH_2Cl_2 (1 mL) at 0 °C followed by stirring at rt until the reaction was determined to be complete by LCMS (2 h). The reaction mixture was then concentrated to dryness under reduced pressure to afford the title compound which was used immediately in subsequent reactions without further purification.

Intermediate 11

4-Amino-5-hydroxyhexanamide



Reaction sequence:



Step 1: T3P (50 wt.% in EtOAc, 21 mL, 35 mmol, 1.2 eq.) and DIPEA (12.5 mL, 71.8 mmol, 2.4 eq.) were added in succession to a mixture of *N*-Cbz-L-glutamic acid (10.0 g, 29.6 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (3.46 g, 35.5 mmol, 1.2 eq.) in DMF (15 mL) at 0 °C followed by stirring at rt for 1 h. The reaction vessel contents were then poured into a cold aqueous 0.5 M HCl and extracted with EtOAc (2x). The combined organics were washed with a cold 0.5 M HCl and then with brine. The combined organics were dried over $MgSO_4$ and filtered through a pad of silica gel. The pad was washed with additional EtOAc and the filtrate was concentrated to dryness. The residue was used directly in the next step.

Step 2: A solution of 3.0 M MeMgBr in Et_2O (34 mL, 100 mmol, 3.0 eq.) was added dropwise to a solution of the product from Step 1 (12.9 g, 34.0 mmol) in THF (100 mL) at -78 °C. Following completion of the addition, the mixture was warmed to rt and stirred at this temperature until the reaction was determined to be complete (2 h) by LCMS. The reaction vessel contents were then poured into a cold aqueous 0.5 M HCl and extracted with EtOAc (2x). The combined organics were washed with cold aqueous 0.5 M HCl and brine, and then were dried over $MgSO_4$, filtered and concentrated under vacuum to dryness. The ketone product was isolated by flash chromatography on silica gel eluting with an increasing proportion of EtOAc (25-50%) in hexanes.

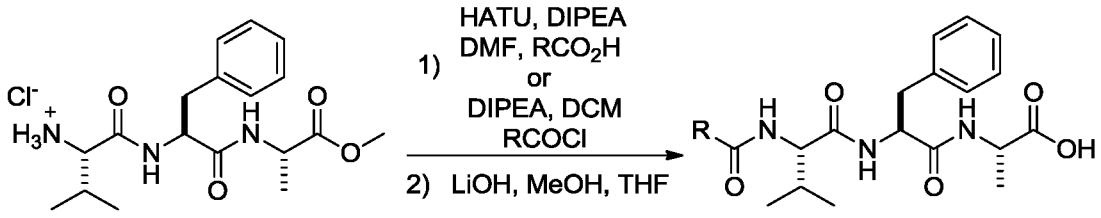
Step 3: TFA (10 mL, 130 mmol, 5.4 eq.) was added dropwise to a stirred solution of the product from Step 2 (8.08 g, 24.1 mmol) in CH_2Cl_2 (2 mL) at -30 °C. The reaction vessel contents were then warmed slowly to rt and stirred at this temperature for 3 h before being concentrated to dryness. The residue containing the product was azeotroped with toluene (3x), dried under high vacuum and used directly in the next step.

Step 4: $NaBH_4$ (170 mg, 4.44 mmol, 2.5 eq.) and MeOH (1 mL) were added in succession to a solution of the product from Step 3 (500 mg, 1.79 mmol) in THF (4 mL) at -78 °C followed by warming to 0 °C with stirring at this temperature for 45 min. The reaction vessel contents were then partitioned between 10% (w/w) citric acid aqueous solution and EtOAc, and the layers were separated. The aqueous phase was extracted with additional EtOAc and the combined organics were washed with brine, dried over Na_2SO_4, filtered, treated with DIPEA (0.62 mL) and concentrated by rotary evaporation under reduced pressure at a bath temperature of 20 °C. The residue was dissolved in DMF (5 mL) at 0 °C and treated with DMAP (22 mg, 0.18 mmol, 0.1 eq.), HATU (817 mg, 2.15 mmol, 1.2 eq.), DIPEA (1.87 mL, 10.7 mmol, 6 eq.) and NH_4Cl (481 mg, 9.0 mmol, 5 eq.) followed by warming to rt and stirring overnight. Concentrated aqueous NH_4OH (1.8 mL, 25 mmol, 14 eq.) and DMF (5 mL) were then added with continued stirring at rt for 3 d. The reaction vessel contents were then poured into brine (50 mL) and extracted with EtOAc (50 mL x 2). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to dryness, and the residue was triturated with a mixture of Et_2O/EtOAc. The solid product was collected by suction filtration and dried under high vacuum.

Step 5: A mixture of the product from Step 4 (0.27 g, 0.95 mmol), 10% Pd/C (100 mg) was placed in a rubber septum sealed flask under high vacuum. Degassed MeOH (5 mL) was then added via syringe and the mixture was placed under a N_2 atmosphere supplied by a latex balloon. The reaction vessel was immersed into an ultrasonic bath and sonicated for 2 min. The N_2 atmosphere was purged by delivery of H_2 by latex balloon and the mixture was stirred at rt. After 5 h the reaction was judged to be complete by TLC analysis. The H_2 atmosphere was replaced with N_2, CH_2Cl_2 was added and the suspension was filtered through a Celite pad which was subsequently washed with a mixture of MeOH/CH_2Cl_2. Concentration of the filtrate in vacuo and trituration of the residue with MeOH in EtOAc afforded the title compound.

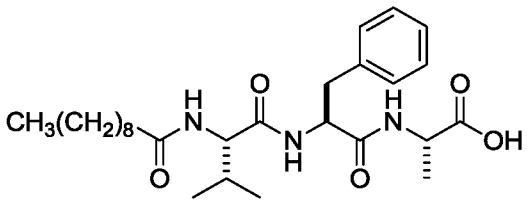
Preparation of Intermediates 12-14

Reaction Sequence:



Intermediate 12

(*S*)-2-((*S*)-2-((*S*)-2-Decanamido-3-methylbutanamido)-3-phenylpropanamido)propanoic acid

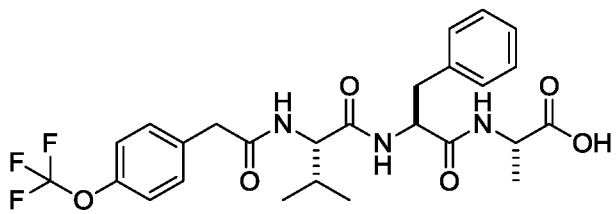


Step 1: Decanoyl chloride (0.73 mL, 3.6 mmol, 1.1 eq.) and Et_3N (1.6 mL, 12 mmol, 3.5 eq.) were added dropwise in succession to a stirred suspension of Intermediate 4 (1.25 g, 3.24 mmol) in THF (15 mL) at 0 °C followed by slow warming to rt. After stirring overnight, additional THF (70-80 mL) was added to facilitate stirring of the mixture and a second portion of decanoyl chloride (0.10 mL, 0.50 mmol, 0.15 eq.) was added with continued stirring at rt until the reaction was judged to be complete (24 h) by LCMS. The reaction vessel contents were then poured into a cold mixture of aqueous 0.5 M HCl, EtOAc and hexanes. The solid product was collected by gravity filtration, washed with water and 1:1 Et_2O/hexanes before being dried under high vacuum.

Step 2: An aqueous solution of 1 M LiOH (9 mL, 9 mmol, 3 eq.) was added to a stirred suspension of the product from Step 1 (1.47 g, 2.92 mmol) in MeOH (9 mL) and THF (18 mL) at 0 °C followed by warming to rt. After 4 h, additional MeOH (18 mL) was added and the suspension was stirred overnight at rt. LCMS analysis indicated that the reaction had not reached completion after 24 h. The mixture was recooled to 0 °C and an additional portion of 1 M LiOH (3 mL, 3 mmol, 1 eq.) was added followed by stirring at rt for a second 24 h period. At this point, the reaction still had not yet reached completion due to the low solubility of the starting ester in the reaction media. The addition of a third portion of MeOH and 1 M LiOH (3 mL, 3 mmol, 1 eq.) was necessary to push the reaction to completion. The mixture was subsequently filtered to remove solids and the filtrate was acidified to pH 1 with 1 M HCl followed by concentration to dryness. The solid residue was suspended in water, collected by suction filtration and washed with water and Et_2O. Drying of the solid under suction and high vacuum provided the title compound.

Intermediate 13

(*S*)-2-((*S*)-2-((*S*)-3-Methyl-2-(2-(4-(trifluoromethoxy)phenyl)acetamido)butanamido)-3-phenylpropanamido)propanoic acid

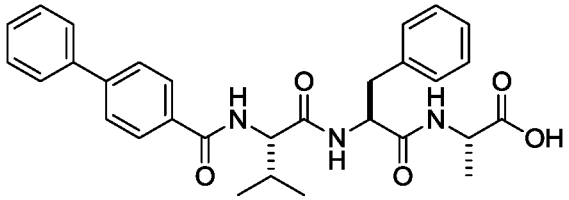


Step 1: DIPEA (0.51 mL, 2.9 mmol, 3.5 eq.) was added dropwise over 10 min to a solution of Intermediate 4 (0.32 g, 0.83 mmol), 4-(trifluoromethoxy)phenylacetic acid (0.20 g, 0.91 mmol, 1.1 eq.) and HATU (0.38 g, 1.0 mmol, 1.2 eq.) in DMF (3 mL) at 0 °C followed by slow warming to rt with stirring overnight. The reaction vessel contents were then diluted with Et_2O and poured into a mixture of ice and saturated aqueous $NaHCO_3$. This mixture was stirred for 30 min until the ice had melted and the solid product was collected by suction filtration, washed with water and Et_2O and dried under high vacuum.

Step 2: An aqueous solution of 1 M LiOH (3 mL, 3 mmol, 4 eq.) was added to a rapidly stirred suspension of the product from Step 1 (0.40 g, 0.73 mmol) in MeOH (3 mL) and THF (6 mL) at 0 °C. After 15 min at this temperature, the reaction vessel was warmed to rt and the progress of the reaction was monitored by LC-MS. After 2 h, the mixture was cooled to 0 °C and acidified to pH 1 by the addition of an aqueous solution of 0.05 M HCl (100 mL) with stirring for an additional 1 h at rt. The solid product was collected by suction filtration, washed with water and dried under suction and high vacuum to afford the title compound.

Intermediate 14

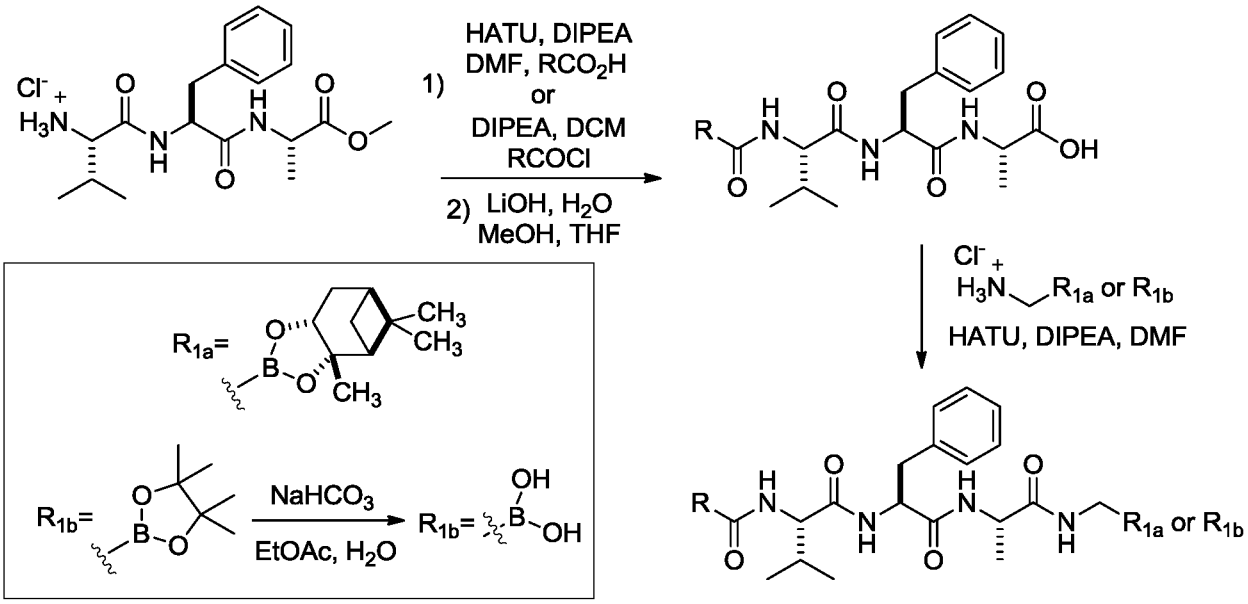
(*S*)-2-((*S*)-2-((*S*)-2-([1,1'-Biphenyl]-4-ylcarboxamido)-3-methylbutanamido)-3-phenylpropanamido)propanoic acid



Step 1: DIPEA (0.42 mL, 2.4 mmol, 3.5 eq.) was added dropwise over 10 min to a solution of Intermediate 4 (0.32 g, 0.83 mmol, 1.2 eq.), biphenyl-4-carboxylic acid (145 mg, 0.732 mmol) and HATU (316 mg, 0.832 mmol, 1.2 eq.) in DMF (3 mL) at 0 °C followed by stirring at rt for 3 d. The mixture was then diluted with EtOAc and poured into a mixture of ice and a saturated aqueous solution of $NaHCO_3$ with stirring at rt until the ice melted (ca. 30 min). The solid product was collected by suction filtration, washed successively with water and EtOAc before being dried under suction and high vacuum.

Step 2: An aqueous solution of 1 M LiOH (3 mL, 3 mmol, 5 eq.) was added to a suspension of the product from Step 1 (0.33 g, 0.62 mmol) in MeOH (3 mL) and THF (6 mL) at 0 °C followed by rapid stirring at rt until the reaction was judged to be complete (3 h) by LCMS. The reaction vessel contents were then recooled to 0 °C and acidified by the addition of an aqueous solution of 0.05 M HCl (100 mL) followed by stirring at rt for 1 h. The solid product was collected by suction filtration, washed with water and Et_2O, and dried under suction and high vacuum to afford the title compound.

General Sequence 1:



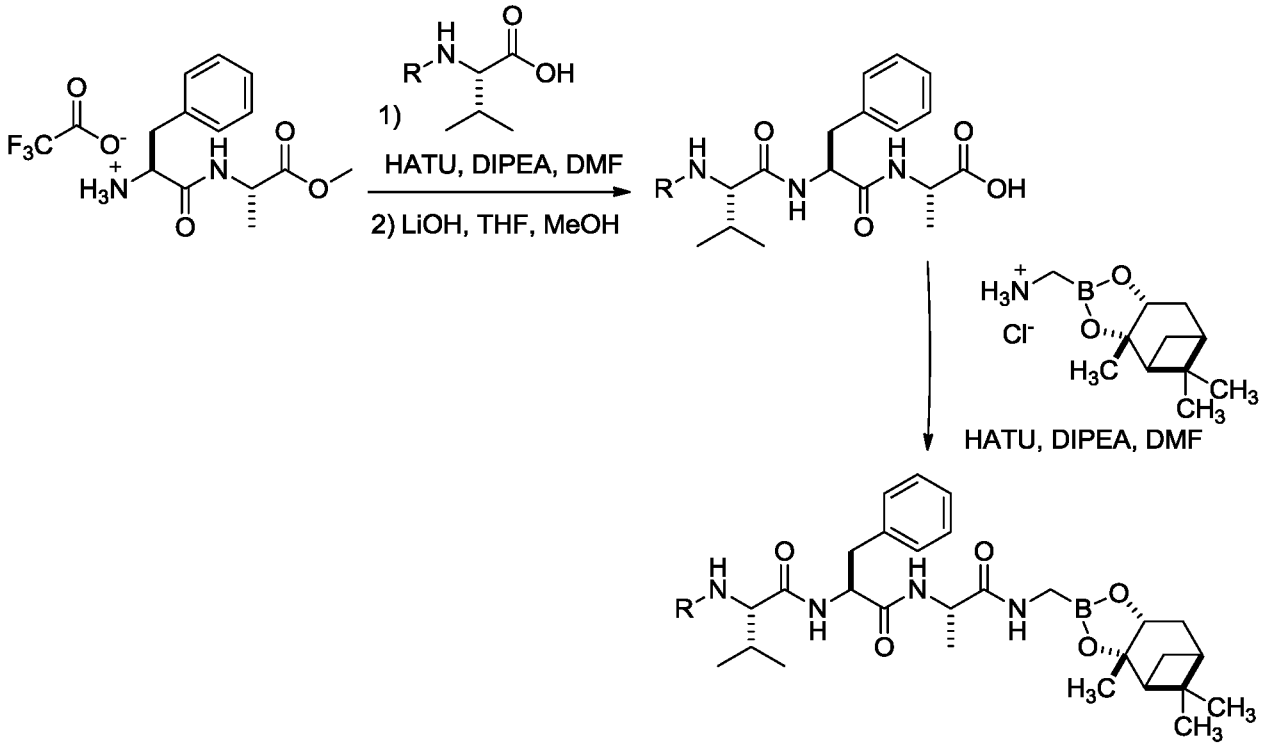
Step 1 (for RCO_2H): DIPEA (3.5 eq) was added to a stirred suspension of Intermediate 4 (0.83 mmol), HATU (1.2 eq.) and the appropriate acid (1.1 eq.) in DMF (3.0 mL) at 0°C with slow warming to rt and stirring overnight. Saturated $NaHCO_3$ aqueous solution and EtOAc were then added and the solid product was collected by suction filtration, washed with water and EtOAc and under high vacuum.

Step 1 (for RC(O)Cl): The appropriate acid chloride (1.1 eq.) was added portionwise to a stirred suspension of Intermediate 4 (1.3 mmol) and DIPEA (2.2 eq.) in CH_2Cl_2 (13 mL) at 0°C followed by slow warming to rt and stirring overnight. Additional CH_2Cl_2 and water were added to the reaction vessel and the solid product was isolated by suction filtration, washed with CH_2Cl_2 and water and dried under high vacuum.

Step 2: 1 M Aqueous LiOH (4 eq.) was added dropwise to a stirred suspension of the product from Step 1 (0.73 mmol) in MeOH (3 mL) and THF (6 mL) at 0°C. After 15 min, the reaction was warmed to rt and stirred for an additional 3 h. Crushed ice was then added and the mixture was acidified to pH 3 with 1 M HCl. After stirring for an additional 1 h, the solid product was isolated by suction filtration and dried under high vacuum. Analogs that failed to precipitate under these conditions were isolated by an extractive workup with EtOAc (3x). The combined organics were washed with brine, dried (Na_2SO_4), filtered and concentrated and the residue was used directly in the next step.

Step 3: DIPEA (2.5 eq.) was added to a stirred suspension of the product from Step 2 (0.14 mmol), HATU (1.1 eq.) and aminomethylboronic acid pinacolester hydrochloride (1.1 eq.) or boro-Gly-(+)-pinanediol hydrochloride (1.1 eq.) in DMF (1 mL) at 0°C followed by slow warming to rt with stirring overnight. Half-saturated $NaHCO_3$ aqueous solution and EtOAc were then added and the mixture was agitated in an ultrasonic bath for 1 h. The solid product was isolated by suction filtration and dried under high vacuum.

General Sequence 2:

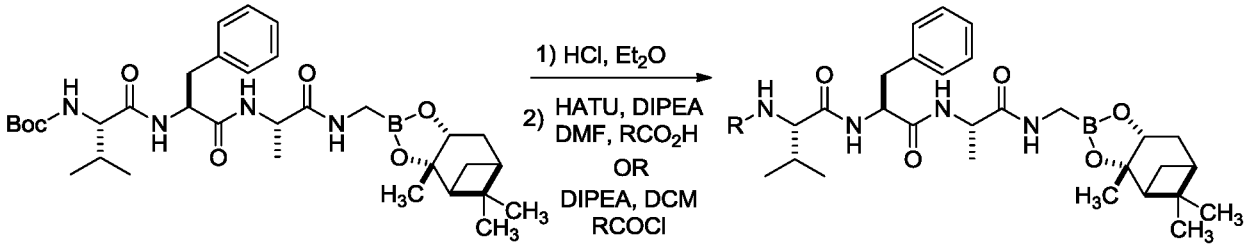


Step 1: DIPEA (38 mmol, 3.5 eq.) was added dropwise over 10 min to a solution of Intermediate 1 (11 mmol), an L-valine derivative (12 mmol, 1.1 eq.) and HATU (13 mmol, 1.2 eq.) in DMF (35 mL) at 0°C followed by slow warming to rt with stirring overnight. The mixture was then poured into a mixture of crushed ice (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and Et_2O (200 mL) and stirred for 30 min until the ice melted. The solid product was collected by suction filtration, washed with water and 1:1 Et_2O/ hexanes, and dried under high vacuum.

Step 2: The product from Step 1 (3.33 mmol) was suspended in a mixture of THF (20 mL) and MeOH (10 mL) and treated with 1 M LiOH (4.3 mL, 4.3 mmol, 1.3 eq.) at 0°C followed by slow warming to rt with stirring overnight. The mixture was then recooled to 0°C, acidified to pH 4 with 1 M HCl, and extracted with EtOAc (3x). The combined organics were dried ($MgSO_4$), filtered and concentrated and the residue was used directly in the next step.

Step 3: DIPEA (2.0 mmol, 2.5 eq.) was added dropwise to a stirred suspension of the product from Step 2 (0.90 mmol), boro-Gly-(+)-pinanediol hydrochloride (1.1 mmol, 1.2 eq.) and HATU (1.03 mmol, 1.1 eq.) in DMF (4.5 mL) at 0°C followed by slow warming to rt. After stirring overnight, the mixture was diluted with half saturated $NaHCO_3$ aqueous solution and extracted with EtOAc (3x). The combined organics were washed with half saturated brine (2x), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was stirred with Et_2O and the solid material was collected by filtration and dried under high vacuum to afford the final product.

General Sequence 3

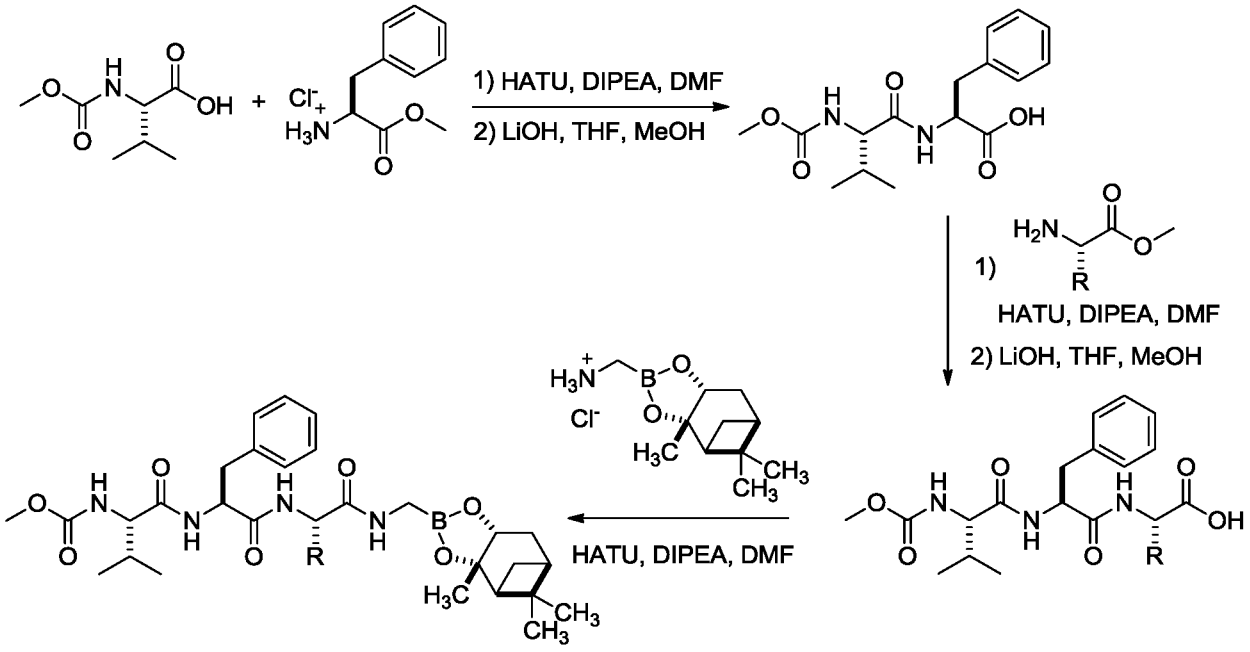


Step 1: The final product from General Sequence 2 with R = Boc (0.51 mmol) was treated at 0 °C with a solution of 2 M HCl in Et_2O (20 mmol, 40 eq.) with stirring for 18 h at this temperature followed by concentration under reduced pressure. The residue was used directly in the next step.

Step 2 (for RCO_2H): DIPEA (0.27 mmol, 3.0 eq.) was added dropwise to a stirred suspension of the product from Step 1 (0.089 mmol), HATU (0.11 mmol,1.2 eq.) and the appropriate carboxylic acid (1.1 eq.) in DMF (0.89 mL) at 0°C followed by slow warming to rt. After stirring overnight, EtOAc and half saturated $NaHCO_3$ aqueous solution were added and the solid product was collected by suction filtration, washed with water and dried under high vacuum.

Step 2 (for RCOCl): DIPEA (0.13 mmol, 3.0 eq.) was added to a stirred suspension of the product from Step 1 (0.042 mmol) in CH_2Cl_2 (0.4 mL) at 0°C followed by the portion-wise introduction of the appropriate acid chloride (0.064 mmol, 1.5 eq.) The reaction was warmed slowly to rt and stirred overnight. Thereafter, the reaction was diluted with EtOAc and half saturated $NaHCO_3$ and the layers were separated. The aqueous layer was extracted with EtOAc (2x) and the combined organic layers were washed with half saturated brine (2x), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was triturated with Et_2O and the solid product was isolated by filtration and dried under high vacuum.

General Sequence 4



Step 1: HATU (11.9 g, 31.4 mmol, 1.1 eq.) and DIPEA (14.9 mL, 85.5 mmol, 3.0 eq.) were added to a solution of (*S*)-2-((methoxycarbonyl)amino)-3-methylbutanoic acid (5.00 g, 28.5 mmol) and L-phenylalanine methyl ester hydrochloride (6.15 g, 28.5 mmol, 1.0 eq.) in DMF (100 mL) at 0°C followed by slow warming to rt with stirring overnight. The mixture was subsequently diluted with EtOAc and washed with aqueous solutions of 10% HCl, saturated $NaHCO_3$ and brine. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated under reduced pressure and the residue was used directly in next step.

Step 2: Aqueous 1 M LiOH (60 mL) was added to a solution of the product from Step 1 (9.58 g, 28.5 mmol) in THF (120 mL) and MeOH (60 mL) at 0°C. After stirring at rt for 3 h, the mixture was acidified to pH 2-3 with 1 M HCl and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was triturated with a mixture EtOAc and hexanes and the solid product was collected by suction filtration and dried under high vacuum.

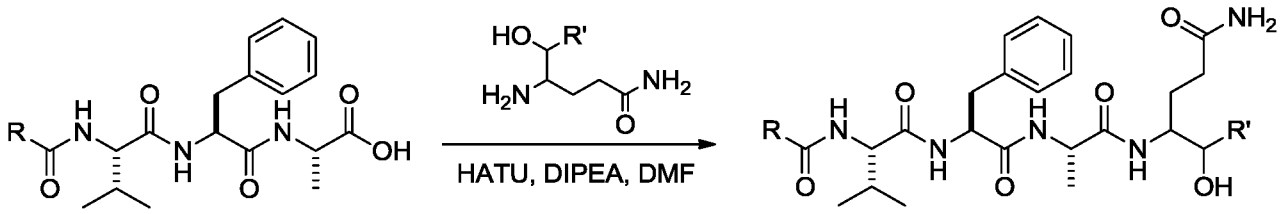
Step 3: HATU (1.12 mmol, 1.2 eq.) and DIPEA (3.7 mmol, 4.0 eq.) were added sequentially to a stirred mixture of the product from Step 2 (0.93 mmol) and the appropriate protected amino acid (0.93 mmol) in DMF (5 mL) at 0°C followed by slow warming to rt and stirring at this temperature for 3 h. The mixture was then partitioned between EtOAc and 10% HCl aqueous solution. The organic phase was separated and washed with saturated

$NaHCO_3$ and brine, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel eluting with an increasing proportion of MeOH in CH_2Cl_2.

Step 4: A stirred suspension of the product from Step 3 (0.47 mmol) in THF (2 mL) and MeOH (1 mL) at 0°C was treated with aqueous 1 M LiOH (0.95 mmol, 2.0 eq.), warmed slowly to rt and stirred for 3 h. The mixture was then acidified with 1 M HCl to pH 2-3 and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was triturated with a mixture of EtOAc and Et_2O and the solid product was isolated by suction filtration and dried under high vacuum.

Step 5: A stirred suspension of the product from Step 4 (0.11 mmol) and boro-Gly-(+)-pinanediol hydrochloride (0.12 mmol, 1.1 eq.) in DMF (1 mL) at 0°C was treated with HATU (0.13 mmol, 1.2 eq.) and DIPEA (0.44 mmol, 4.0 eq.) followed by slow warming to rt and stirring overnight. The reaction vessel contents were subsequently partitioned between EtOAc and half saturated $NaHCO_3$ aqueous solution. The EtOAc layer was separated and the aqueous layer extracted with additional EtOAc (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was triturated with water and the solid product was isolated by suction filtration and dried under high vacuum.

General Sequence 5

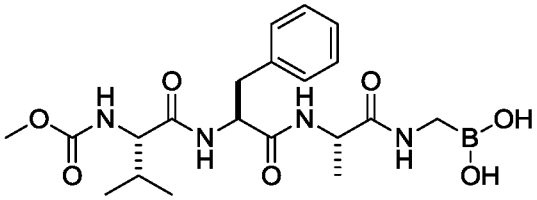


DIPEA (3.5 eq.) was added dropwise to a stirred suspension of the appropriate carboxylic acid (Intermediate 12,13 or 14), the appropriate amine (Intermediate 8, 9, 10 or 11, 1.2-1.4 eq.) and HATU (1.2-1.3 eq.) in DMF (0.1 M) at 0 °C followed by slow warming to rt and stirring overnight. The reaction mixture was then diluted with EtOAc and stirred with an ice-cold, half-saturated $NaHCO_3$ aqueous solution. The solid amide product was isolated by filtration, washed with EtOAc and water, followed by drying under suction and high vacuum. When the product was soluble in EtOAc, the layers were separated and the aqueous phase was extracted with additional EtOAc. The combined organics were washed with an aqueous solution of 5% LiCl and saturated brine, and then were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was triturated with Et_2O and the solid product was collected by filtration and dried under high vacuum.

Compounds described in Examples 1 to 24 are identified by the example numbers. Hence the compound disclosed in Example 1 is designated compound 1.

Example 1 (1)

{[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(Methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]propanamido]methyl}boronic acid



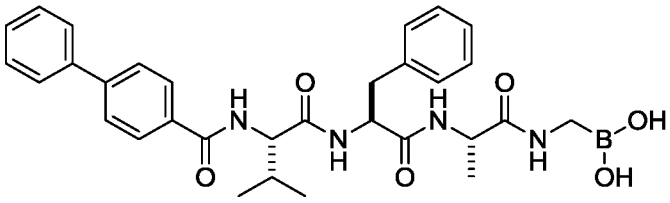
2,4,6-Collidine (0.14 mL, 1.1 mmol, 2.1 eq.) was added dropwise to a suspension of Intermediate 2 (0.20 g, 0.51 mmol), HATU (0.194 g, 0.510 mmol) and aminomethylboronic acid pinacol ester hydrochloride (0.099 g, 0.51 mmol) in DMF (2.9 mL) at 0°C with slow warming to rt and stirring overnight. The vessel contents were subsequently partitioned between half-saturated $NaHCO_3$ aqueous solution and EtOAc. The organic layer was separated and the aqueous phase was extracted with EtOAc (2x). The combined organics were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was triturated with mixture of EtOAc/Et_2O/water with agitation in an ultrasonic bath for 1 h. The solid material was collected by suction filtration and dried under high vacuum to afford the title compound.

^{1}H NMR (methanol-d4): δ 7.31-7.18 (5 H), 4.63 (1 H), 4.50 (1 H), 3.80 (1 H, d), 3.64 (3 H), 3.20 (1 H), 2.94 (1 H), 2.34 (2 H), 1.92 (1 H), 1.42 (3 H), 0.83-0.75 (6 H). MS ESI: 473.3 $[M+Na]^+$

Examples 2-5 were prepared using General Sequence 1:

Example 2 (2)

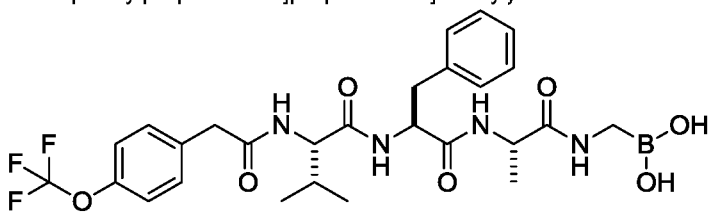
{[(2*S*)-2-[(2*S*)-2-[(2*S*)-3-Methyl-2-[(4-phenylphenyl)formamido]butanamido]-3-phenylpropanamido]propanamido]methyl}boronic acid



^{1}H NMR (methanol-d4): δ 7.89 (2 H), 7.73 (2 H), 7.68 (2 H), 7.47 (2 H), 7.38 (1 H), 7.28-7.22 (2 H), 7.21-7.13 (2 H), 7.09 (1 H), 4.69 (1 H), 4.39 (1 H), 4.32 (1 H), 3.23 (1 H), 2.93 (1 H), 2.35-2.22 (2 H), 2.13 (1 H), 1.41-1.31 (3 H), 1.00-0.88 (6 H). MS ESI: 595.4 $[M+Na]^+$

Example 3 (3)

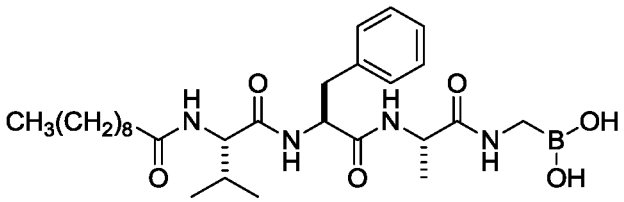
{[(2*S*)-2-[(2*S*)-2-[(2*S*)-3-Methyl-2-{2-[4-(trifluoromethoxy)phenyl]acetamido}butanamido]-3-phenylpropanamido]propanamido]methyl}boronic acid



^{1}H NMR (DMSO-d_6): δ 8.26 (1 H), 8.16 (1 H), 8.20-8.12 (2 H) 7.38-7.32 (2 H), 7.30-7.12 (7 H), 4.53 (1 H), 4.30 (1 H), 4.10 (1 H), 3.55 (1 H), 3.45 (1 H), 3.04 (1 H), 2.77 (1 H), 2.27 (1 H), 1.89 (1 H), 1.22-1.18 (3 H), 0.74-0.72 (6 H). MS ESI: 617.3 $[M+Na]^+$

Example 4 (4)

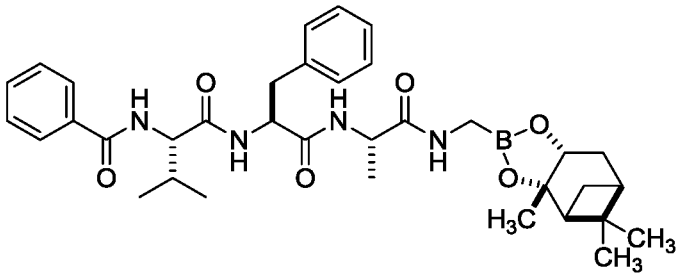
{[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-Decanamido-3-methylbutanamido]-3-phenylpropanamido]propanamido]methyl}boronic acid



^{1}H NMR (methanol-d4): δ 7.29-7.16 (5 H), 4.59 (1 H), 4.48 (1 H), 4.04 (1 H), 3.15 (1 H), 2.94 (1 H), 2.33 (2 H), 2.26-2.14 (2 H), 1.95 (1 H), 1.56 (2 H), 1.40 (3 H), 1.35-1.20 (12 H), 0.91-0.81 (9 H). MS ESI: 569.4 $[M+Na]^+$

Example 5 (5)

(2*S*)-3-Methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-(phenylformamido)butanamide

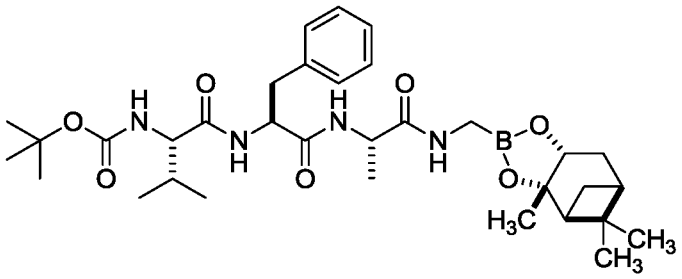


^{1}H NMR (DMSO-d_6): δ 8.22-8.16 (2 H), 8.11-8.03 (2 H), 7.84 (2 H), 7.55 (1 H), 7.50-7.45 (2 H), 7.26-7.09 (5 H), 4.57 (1 H), 4.29 (1 H), 4.20 (2 H), 3.05 (1 H), 2.77 (1 H), 2.37 (2 H), 2.22 (1 H), 2.10-1.99 (2 H), 1.86 (1 H), 1.80 (1 H), 1.67 (1 H), 1.28-1.17 (10 H), 0.83 (3 H), 0.79 (3 H), 0.75 (3 H). MS ESI: 653.5 $[M+Na]^+$

Examples 6 and 7 were prepared using General Sequence 2:

Example 6 (6)

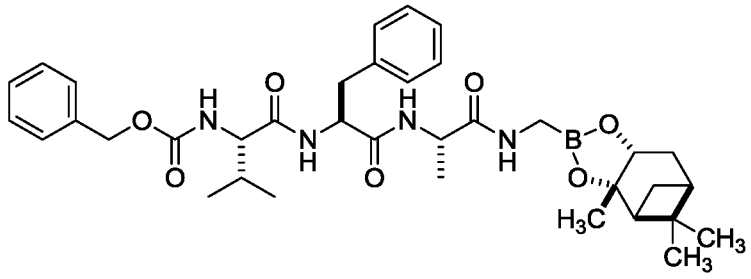
tert-Butyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate



^{1}H NMR (DMSO-d_6): δ 8.26 (1 H), 8.08 (1 H), 7.87 (1 H), 7.19-7.16 (5 H), 6.64 (1 H), 4.58 (1 H), 4.29 (1 H), 4.19 (1 H), 3.68 (1 H), 3.02 (1 H), 2.74 (1 H), 2.38 (2 H), 2.22 (1 H), 2.06 (1 H), 1.87 (1 H), 1.84-1.73 (2 H), 1.68 (1 H), 1.36 (9 H), 1.31-1.14 (10 H), 0.79 (3 H), 0.73-0.61 (6 H). MS ESI: 627.5 $[M+H]^+$

Example 7 (7)

benzyl N-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate

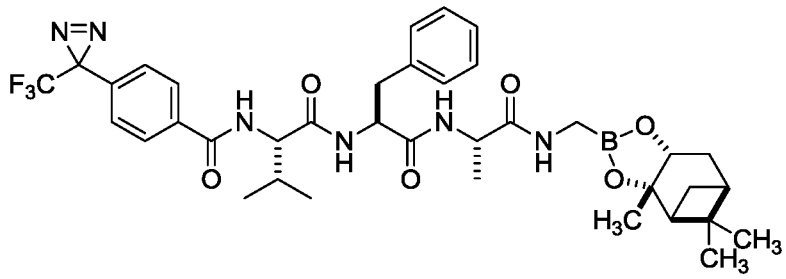


^{1}H NMR (DMSO-d6): δ 8.21 (1 H), 8.09 (1 H), 7.98 (1 H), 7.40-7.29 (5 H), 7.27-7.12 (6 H), 5.02 (2 H), 4.56 (1 H), 4.29 (1 H), 4.19 (1 H), 3.80 (1 H), 3.04 (1 H), 2.76 (1 H), 2.38 (2 H), 2.22 (1 H), 2.06 (1 H), 1.89-1.76 (3 H), 1.68 (1 H), 1.30-1.15 (10 H), 0.79 (3 H), 0.72 (6 H). MS ESI: 683.4 $[M+Na]^+$

Examples 8a, 8b, 8c, 8d and 9 were prepared using General Sequence 3:

Example 8a (8a)

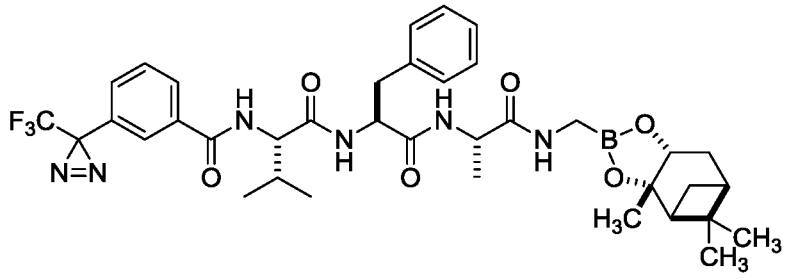
(2*S*)-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-({4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}formamido)butanamide



^{1}H NMR (DMSO-d$_6$): δ 8.36 (1 H), 8.16 (1 H), 8.10-8.04 (2 H), 7.93 (2 H), 7.38 (2 H), 7.24-7.05 (5 H), 4.55 (1 H), 4.32-4.14 (3 H), 3.03 (1 H), 2.76 (1 H), 2.36 (2 H), 2.20 (1 H), 2.08-1.96 (2 H), 1.86 (1 H), 1.78 (1 H), 1.66 (1 H), 1.29-1.13 (10 H), 0.84-0.72 (9 H). MS ESI: 739.4 $[M+H]^+$

Example 8b (8b)

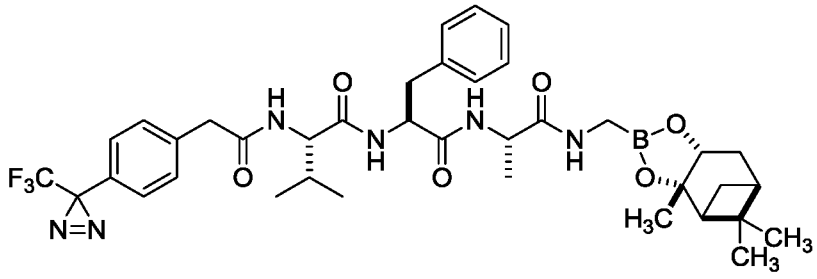
(2*S*)-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-({3-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}formamido)butanamide



^{1}H NMR (DMSO-d_6): δ 8.46 (1 H), 8.16-8.05 (3 H), 8.00 (1 H), 7.65-7.60 (2 H), 7.55 (1 H), 7.24-7.19 (2 H), 7.17-7.05 (3 H), 4.54 (1 H), 4.31-4.15 (3 H), 3.04 (1 H), 2.76 (1 H), 2.39-2.34 (2 H), 2.20 (1 H), 2.09-1.97 (2 H), 1.86 (1 H), 1.79 (1 H), 1.67 (1H), 1.30-1.11 (10 H), 0.86-0.74 (9 H). MS ESI: 739.4 $[M+H]^+$

Example 8c (8c)

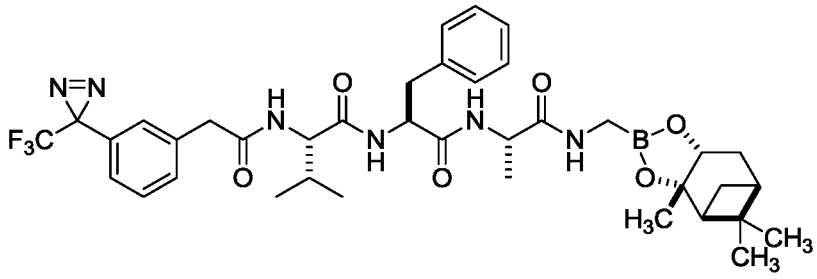
(2*S*)-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-(2-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}acetamido)butanamide



^{1}H NMR (DMSO-d_6): δ 8.14-8.04 (3 H), 7.39-7.33 (2 H,) 7.24-7.10 (8 H), 4.51 (1 H), 4.28 (1 H), 4.18 (1 H) 4.09 (1 H), 3.60-3.44 (2 H), 3.01 (1 H), 2.75 (1 H), 2.38-2.34 (2 H), 2.21 (1 H), 2.05 (1 H), 1.92-1.83 (2 H), 1.79 (1 H), 1.66 (1 H), 1.29-1.09 (10 H), 0.81-0.65 (9 H). MS ESI: 753.5 $[M+H]^+$

Example 8d (8d)

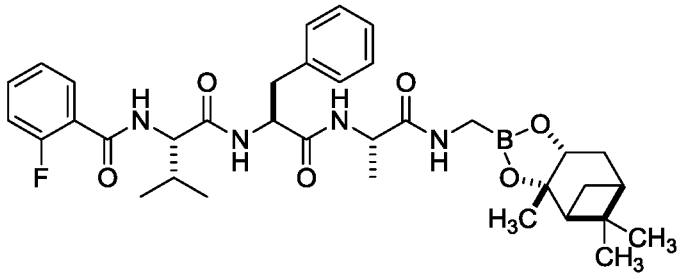
(2*S*)-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]-2-(2-{3-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}acetamido)butanamide



^{1}H NMR (DMSO-d_6): δ 8.15-8.03 (3 H), 7.44-7.36 (2 H), 7.23-7.09 (8 H), 4.56-4.42 (2 H), 4.30-4.07 (2 H), 3.63-3.39 (2 H), 3.02 (1 H), 2.76 (1 H), 2.38-2.33 (2 H), 2.20 (1 H), 2.04 (1 H), 1.93-1.82 (2 H), 1.78 (1 H), 1.66 (1 H), 1.28-1.09 (10 H), 0.80-0.75 (3 H), 0.75-0.67 (6 H). MS ESI: 753.4 $[M+H]^+$

Example 9 (9)

(2*S*)-2-[(2-fluorophenyl)formamido]-3-methyl-*N*-[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]butanamide

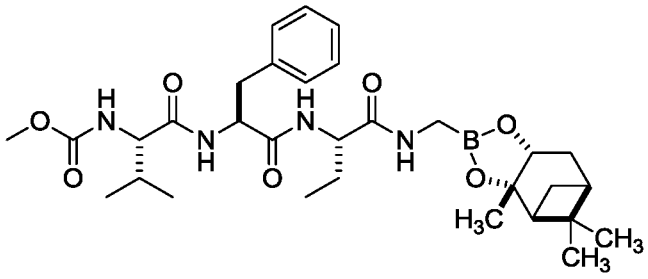


^{1}H NMR (DMSO-d_6): δ 8.25 (1 H), 8.14-8.08 (2 H), 8.03 (1 H), 7.64-7.51 (2 H), 7.33-7.11 (7 H), 4.60 (1 H), 4.30 (2 H), 4.18 (1 H), 3.05 (1 H), 2.75 (1 H), 2.38 (2 H), 2.20 (1 H), 2.10-1.93 (2 H), 1.87 (1 H), 1.79 (1 H), 1.67 (1 H), 1.29-1.18 (10 H), 0.82-0.74 (9 H). MS ESI: 649.4 $[M+H]^+$

Examples 10-14 were prepared using General Sequence 4:

Example 10 (10)

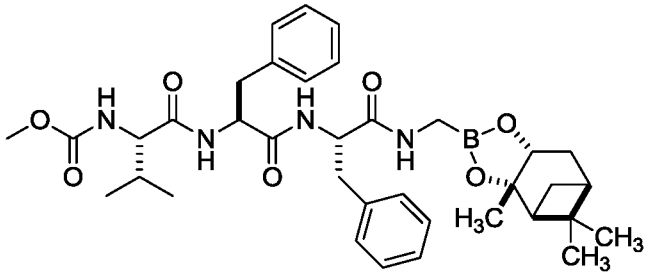
Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(1*S*)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)propyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate



^{1}H NMR (DMSO-d_6): δ 8.11-8.04 (2 H), 7.98 (1 H), 7.27-7.21 (4 H), 7.17 (1 H), 7.05 (1 H), 4.58 (1 H), 4.22-4.14 (2 H), 3.77 (1 H), 3.52 (3 H), 3.02 (1 H), 2.78 (1 H), 2.37 (2 H), 2.22 (1 H), 2.06 (1 H), 1.89-1.77 (3 H), 1.71-1.61 (2 H), 1.52 (1 H), 1.31-1.24 (4 H), 1.23-1.20 (3 H), 0.85-0.77 (6 H), 0.74-0.69 (6 H). MS ESI: 599.4 $[M+H]^+$

Example 11 (11)

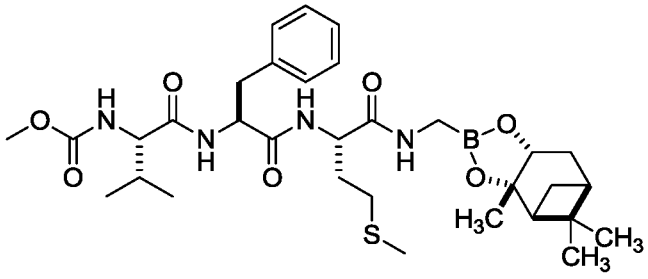
Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(1*S*)-2-phenyl-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)ethyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate



^{1}H NMR (DMSO-d_6): δ 8.23 (1 H), 8.09 (1 H), 7.88 (1 H), 7.29-7.12 (10 H), 7.03 (1 H), 4.59-4.45 (2 H), 4.24 (1 H), 3.76 (1 H), 3.53 (3 H), 3.00-2.68 (4 H), 2.39 (2 H), 2.24 (1 H), 2.09 (1 H), 1.90 (1 H), 1.86-1.78 (2 H), 1.71 (1 H), 1.34-1.26 (4 H), 1.25-1.20 (3 H), 0.81 (3 H), 0.77-0.62 (6 H). MS ESI: 661.5 $[M+H]^+$

Example 12 (12)

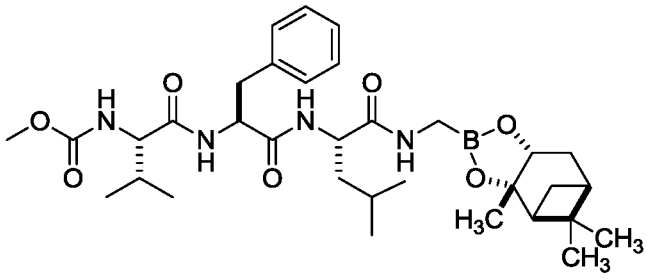
Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-1-{[(1*S*)-3-(methylsulfanyl)-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)propyl]carbamoyl}-2-phenylethyl]carbamoyl}propyl]carbamate



^{1}H NMR (DMSO-d_6): δ 8.13 (1 H), 8.03 (1 H), 7.95 (1 H), 7.27-7.15 (5 H), 7.06 (1 H), 4.54 (1 H), 4.34 (1 H), 4.22 (1 H), 3.77 (1 H), 3.52 (3 H), 3.02 (1 H), 2.79 (1 H), 2.47-2.32 (4 H), 2.22 (1 H), 2.06 (1 H), 2.01 (3 H), 1.93-1.74 (5 H), 1.69 (1 H), 1.30-1.21 (7 H), 0.79 (3 H), 0.72 (6 H). MS ESI: 645.4 $[M+H]^+$

Example 13 (13)

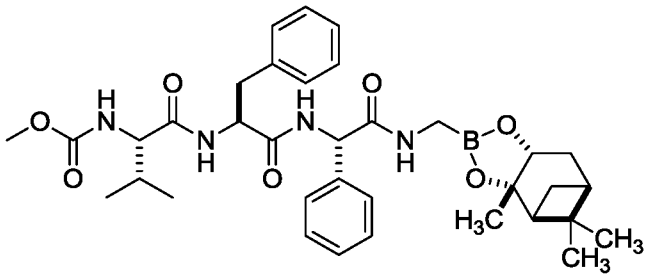
Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-1-{[(1*S*)-3-methyl-1-({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)butyl]carbamoyl}-2-phenylethyl]carbamoyl}propyl]carbamate



^{1}H NMR (DMSO-d_6): δ 8.12 (1 H), 8.07 (1 H, s), 8.00 (1 H), 7.26-7.14 (5 H), 7.04 (1 H), 4.56 (1 H), 4.31 (1 H), 4.18 (1 H), 3.79 (1 H), 3.52 (3 H), 3.01 (1 H), 2.78 (1 H), 2.30-2.36 (2 H), 2.21 (1 H), 2.05 (1 H), 1.89-1.76 (3 H), 1.68 (1 H), 1.56 (1 H), 1.48-1.39 (2 H), 1.30-1.24 (4 H), 1.22 (3 H), 0.86 (3 H), 0.85 (3H), 0.76 (3 H), 0.72 (6 H). MS ESI: 627.5 $[M+H]^+$

Example 14 (14)

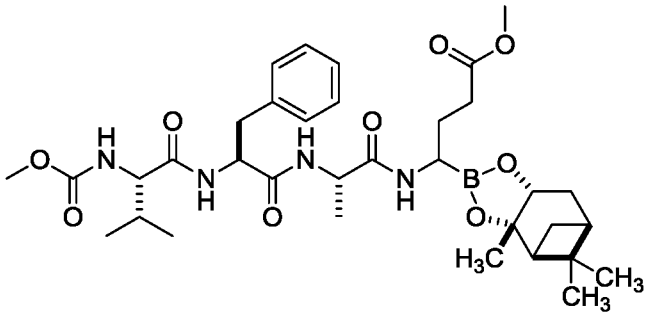
Methyl *N*-[(1*S*)-2-methyl-1-{[(1*S*)-2-phenyl-1-{[(*S*)-phenyl({[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]methyl}carbamoyl)methyl]carbamoyl}ethyl]carbamoyl}propyl]carbamate



^{1}H NMR (DMSO-d_6): δ 8.66 (0.5 H), 8.53 (0.5 H), 8.44 (0.5 H), 8.32 (0.5 H), 8.05 (0.5 H H), 7.97 (0.5 H), 7.92 (1 H), 7.42-7.00 (10 H), 5.47 (1 H), 4.68 (1 H), 4.27 (1 H), 3.77 (1 H), 3.52 (3 H), 2.95 (1 H), 2.76 (1 H), 2.58-2.42 (2 H overlapped), 2.23 (1 H), 2.07 (1 H), 1.94-1.77 (3 H), 1.65 (1 H), 1.32-1.19 (7 H), 0.79 (3 H), 0.75-0.63 (6 H). MS ESI: 647.4 $[M+H]^+$

Example 15 (15)

Methyl (4*R/S*)-4-[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(Methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]-2-phenylacetamido]-4-[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]butanoate

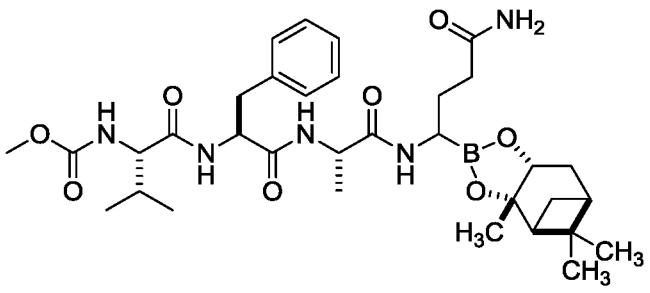


Intermediate 5 (0.027 g, 0.084 mmol), Intermediate 3 (0.062 g, 0.13 mmol, 1.5 eq) and platinum oxide (0.002 g, 0.008 mmol, 0.1 eq.) were placed in an rbf and sealed with a septum. The flask was purged (vacuum, N_2) and degassed THF (2.5 mL) was added. The mixture was then stirred under a H_2 atmosphere (P = 1 atm) provided via a latex balloon. After 18 h, EtOAc and a saturated aqueous solution of $NaHCO_3$ were added. The layers were separated and the aqueous layer extracted with additional EtOAc (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel eluting with an increasing proportion of EtOAc in hexanes to afford the title compound as a mixture of diastereomers.

^{1}H NMR (Acetone-d_6): δ 7.89 (0.5 H), 7.77 (0.5 H), 7.61-7.51 (2 H), 7.31-7.17 (5 H), 6.52 (0.5 H), 6.48 (0.5 H), 4.58 (1 H), 4.45 (1 H), 4.17 (1 H), 3.87 (1 H), 3.61-3.55 (6 H), 3.21 (2 H), 2.97 (1 H), 2.68 (1 H), 2.56-2.32 (2 H), 2.31-2.17 (2 H), 1.94-1.69 (5 H), 1.44 (1 H), 1.34-1.23 (9 H), 0.86-0.81 (9 H). MS ESI: 671.5 $[M+H]^+$

Example 16 (16)

Methyl *N*-[(1*S*)-1-{[(1*R/S*)-1-{[(1*S*)-1-{[(1*S*)-3-carbamoyl-1-[(1*S*,2*S*,6*R*,8*S*)-2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.0^{2,6}]decan-4-yl]propyl]carbamoyl}ethyl]carbamoyl}-2-phenylethyl]carbamoyl}-2-methylpropyl]carbamate

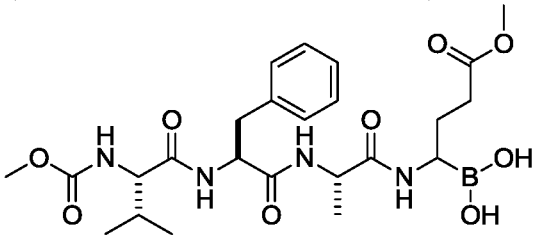


Intermediate 6 (0.12 g, 0.39 mmol), Intermediate 3 (0.30 g, 0.61 mmol, 1.6 eq) and platinum oxide (0.009 g, 0.04 mmol, 0.1 eq.) were placed in an rbf sealed with a septum. The flask was purged (vacuum then N_2) and degassed THF (2.5 mL) was added. The mixture was then stirred under a H_2 atmosphere (P = 1 atm) delivered via a latex balloon. After 18 h, methanol and silica gel were added to the reaction vessel and the mixture was concentrated. The residue was poured onto the top of a silica gel column pre-packed with EtOAc. Flash chromatography on silica gel eluting with an increasing proportion of MeOH in EtOAc, concentration of the fractions containing the desired material, trituration of the residue with EtOAc and collection of the solid product by suction filtration followed by drying under high vacuum gave the title compound as a mixture of diastereomers.

^{1}H NMR (methanol-d4): δ 7.30-7.18 (5 H), 4.59 (1 H), 4.49 (1 H), 4.18 (1 H), 3.77 (1 H), 3.63 (3 H), 3.20 (1 H), 2.95 (1 H), 2.64 (1 H), 2.37-2.10 (4 H), 1.97-1.70 (6 H), 1.42 (3 H), 1.37-1.31 (4 H), 1.27 (3 H), 0.86 (3 H), 0.82-0.77 (6 H). MS ESI: 656.5 $[M+H]^+$

Example 17 (17)

[(1*R/S*)-4-Methoxy-1-[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]propanamido]-4-oxobutyl]boronic acid

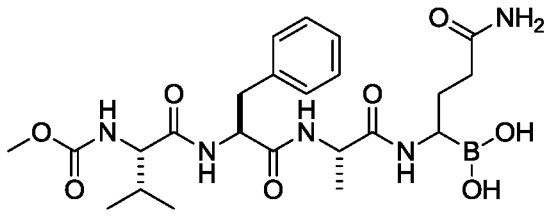


Example 15 (0.010 g, 0.015 mmol) was stirred with polymer-supported benzeneboronic acid (3 mmol/g loading, 0.058 g) in 1 mL of 1:1 acetonitrile/water for 18 h at rt. The resin was removed by filtration and was washed with water and acetonitrile. The filtrate was concentrated and additional portions of polymer-supported benzeneboronic acid (3 mmol/g, 0.058 g) and acetonitrile (5 mL) were added with stirring at rt for 18 h. The second portion of resin was removed by filtration and was washed with water and acetonitrile. Concentration of the filtrate in vacuo to remove acetonitrile, freeze drying of the residual aqueous solution, and trituration of the residue with Et_2O afforded the title compound.

^{1}H NMR (MeOH-d_4): δ 7.31-7.17 (5 H), 4.58 (1 H), 4.47 (1 H), 3.80 (1 H), 3.67-3.61 (6 H), 3.19 (1 H), 2.95 (1 H), 2.58 (1 H), 2.46-2.39 (2 H), 1.93 (1 H), 1.86-1.65 (2 H), 1.43 (3 H), 0.84-0.76 (6 H). MS ESI: 559.3 $[M+Na]^+$

Example 18 (18)

[(1*R/S*)-3-Carbamoyl-1-[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-[(methoxycarbonyl)amino]-3-methylbutanamido]-3-phenylpropanamido]propanamido]propyl]boronic acid



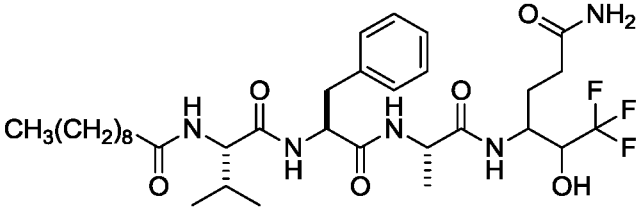
A sample of the final product from example 16 (0.020g, 0.030 mmol) was suspended in a mixture of Et_2O (2.5 mL), water (1 mL) and EtOAc (0.5 mL). Phenylboronic acid (0.036g, 0.30 mmol, 10 eq.) was added and the mixture was stirred at rt for 18 h. The phases were separated and the aqueous layer was washed with EtOAc (5x) and the collected organics were discarded. The aqueous layer was freeze dried and the residue was triturated with EtOAc to afford the title compound.

^{1}H NMR (DMSO-d_6 + 5% H_2O): δ 8.08-7.96 (2 H), 7.65 (1 H), 7.37 (1 H), 7.28-7.12 (5 H), 7.04 (1 H), 6.69 (1 H), 4.55 (1 H), 4.30 (1 H), 3.75 (1 H), 3.51 (3 H), 2.99 (1 H), 2.75 (1 H), 2.56 (1 H), 2.02-1.95 (2 H), 1.79 (1 H), 1.69-1.40 (2 H), 1.20-1.12 (3 H), 0.68 (6 H). MS ESI: 544.3 $[M+Na]^+$

The following examples were prepared according to General Sequence 5:

Example 19 (19)

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide

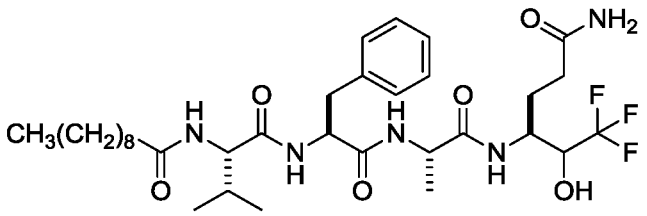


Prepared as a mixture of diastereomers from Intermediate 12 (0.10 g, 0.20 mmol), Intermediate 8 (57 mg, 0.24 mmol, 1.2 eq.), HATU (91 mg, 0.24 mmol, 1.2 eq.) and DIPEA (0.12 mL, 0.69 mmol, 3.5 eq.) in DMF (4 mL).

^{1}H NMR (DMSO-d_6): δ 8.02-7.87 (3 H), 7.71 (1 H), 7.25-7.12 (6 H), 6.71 (1 H), 4.52 (1 H), 4.21 (1 H), 4.07 (1 H), 3.97-3.77 (2 H), 3.03 (1 H), 2.78 (1 H), 2.20-1.93 (4 H), 1.93-1.81 (2 H), 1.64 (1 H), 1.49-1.38 (2 H), 1.29-1.15 (15 H), 0.85 (3 H), 0.73 (6 H). ^{19}F NMR (DMSO-d_6): δ -74.65, -74.72. ESI-MS: 672.5 $[M+H^+]$

Example 20a (20a)

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-(((3*S*)-6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide

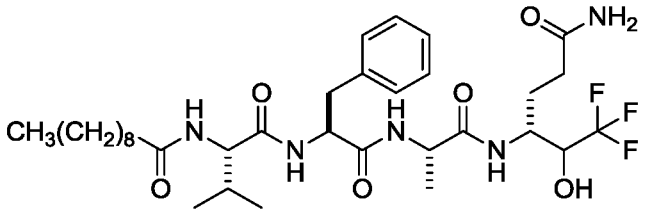


Prepared from Intermediate 12 (40 mg, 0.082 mmol), Intermediate 9 (23 mg, 0.1 mmol, 1.2 eq.), HATU (38 mg, 0.1 mmol, 1.2 eq.) and DIPEA (50 µL, 0.29 mmol, 3.5 eq.) in DMF (2 mL). The solid product was purified by dissolution in hot EtOH, treatment with activated charcoal and filtration through a Celite pad. The pad was washed with additional hot EtOH and the filtrate was concentrated under reduced pressure to afford the title compound as a solid following trituration with EtOAc containing a few drops of MeOH.

^{1}H NMR (DMSO-d_6): δ 8.01 (1 H), 7.98 (1 H), 7.92 (1 H), 7.72 (1 H), 7.25-7.12 (6 H), 6.71 (1 H), 6.53 (1 H), 4.52 (1 H), 4.21 (1 H), 4.07 (1 H), 3.93 (1 H), 3.82 (1 H), 3.03 (1 H), 2.78 (1 H), 2.19-1.97 (4 H), 1.97-1.81 (2 H), 1.60 (1 H), 1.49-1.39 (2 H), 1.29-1.16 (12 H), 1.18 (3 H), 0.85 (3 H), 0.73 (6 H). ^{19}F NMR (DMSO-d_6): δ -74.65. ESI-MS: 672.5 [M+H$^+$].

Example 20b (20b)

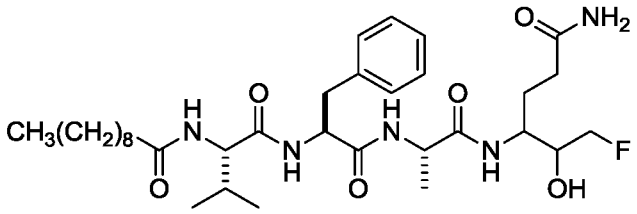
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-(((3*R*)-6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide



Prepared as for 20a from Intermediate 12 and *ent*-Intermediate 9.

Examples 21a (21a), 21b (21b)

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1-fluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide



Compound 21a was prepared from Intermediate 12 (100 mg, 0.20 mmol), the minor diastereomer of Intermediate 10 (56 mg, 0.28 mmol, 1.4 eq.), HATU (100 mg, 0.26 mmol, 1.3 eq.) and DIPEA (0.12 mL, 0.69 mmol, 3.5 eq.) in DMF (3 mL). The title compound was isolated as a mixture of two diastereomers by flash chromatography on silica gel eluting with an increasing proportion of MeOH (5% to 10%) in CH_2Cl_2. Compound 21b was prepared in an analogous manner utilizing the major diastereomer of Intermediate 10.

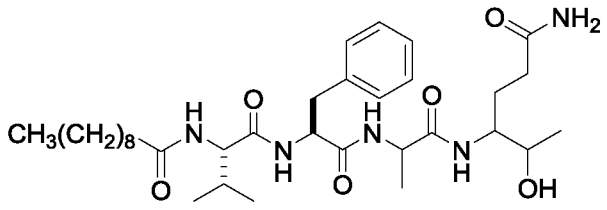
Compound b (see figure 1) is a mixture of compounds 21a and 21b.

21a: ^{1}H NMR (DMSO-d_6): δ 8.01-7.93 (2 H), 7.74-7.62 (2 H), 7.24-7.13 (6 H), 6.69 (1 H), 5.28 (1 H), 4.52 (1 H, m), 4.44-4.03 (4 H), 3.66-3.46 (2 H), 3.03 (1 H), 2.79 (1 H), 2.19-1.82 (6 H), 1.59-1.38 (3 H), 1.30-1.16 (15 H), 0.85 (3 H), 0.74 (6 H). ^{19}F NMR (DMSO-d6): δ 3.84, 3.18. ESI-MS: 636.5 $[M+H]^+$

21b: ^{1}H NMR (DMSO-d_6): δ 8.04-7.95 (2 H), 7.74-7.67 (1 H), 7.55 (0.5 H), 7.48 (0.5 H), 7.26-7.12 (6 H), 6.71 (1 H), 4.52 (1 H), 4.37-4.04 (4 H), 3.80-3.67 (2 H), 3.03 (1 H), 2.78 (1 H), 2.20-1.92 (4 H), 1.87 (1 H), 1.76-1.58 (2 H), 1.50-1.40 (2 H), 1.29-1.17 (15 H), 0.85 (3 H), 0.77-0.70 (6 H). ESI-MS: 636.5 $[M+H]^+$

Examples 22a (22a) and 22b (2b)

N-((2*S*)-1-(((2*S*)-1-((-1-((6-Amino-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide



Prepared from Intermediate 12 (100 mg, 0.204 mmol), Intermediate 11 (40 mg, 0.27 mmol, 1.3 eq.), HATU (91 mg, 0.24 mmol, 1.2 eq.) and DIPEA (0.12 mL, 0.69 mmol, 3.5 eq.) in DMF (3 mL). The two diastereomeric products were separable into by flash chromatography on silica gel eluting with an increasing proportion of MeOH

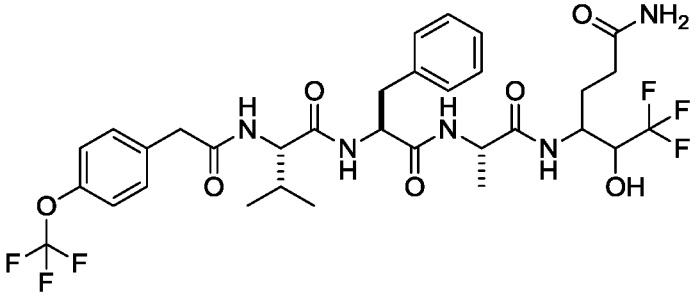
(5% to 10%) in CH_2Cl_2. Compound 22a was the first eluting (less polar) diastereomer and Compound 22b was the second eluting (more polar) diastereomer. Both products were isolated as solids following concentration of the appropriate fractions and trituration of the residue with water and Et_2O.

22a: ^{1}H NMR (DMSO-d_6): δ 8.15 (1 H), 7.96 (1 H), 7.73 (1 H), 7.45 (1 H), 7.26-7.14 (6 H), 6.69 (1 H), 4.56 (1 H), 4.40 (1 H), 4.16 (1 H), 4.10 (1 H), 3.50-3.41 (2 H), 2.95 (1 H), 2.84 (1 H), 2.20-2.02 (2 H), 2.02-1.82 (4 H), 1.56-1.40 (3 H), 1.30-1.17 (12 H), 1.07 (3 H), 0.99 (3 H), 0.85 (3 H), 0.764 (3 H), 0.748 (3 H). ESI-MS: 618.5 $[M+H]^+$.

22b: ^{1}H NMR (DMSO-d_6): δ 8.00 (1 H), 7.93 (1 H), 7.71 (1 H), 7.52 (1 H), 7.25-7.12 (6 H), 6.68 (1 H), 4.63 (1 H), 4.53 (1 H), 4.21 (1 H), 4.07 (1 H), 3.52-3.39 (2 H), 3.03 (1 H), 2.78 (1 H), 2.19-1.81 (6 H), 1.51-1.39 (3 H), 1.29-1.18 (15 H), 0.98 (3 H), 0.85 (3 H), 0.74 (6 H). ESI-MS: 618.5 $[M+H]^+$.

Example 23 (23)

6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-(4-(trifluoromethoxy)phenyl)acetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide

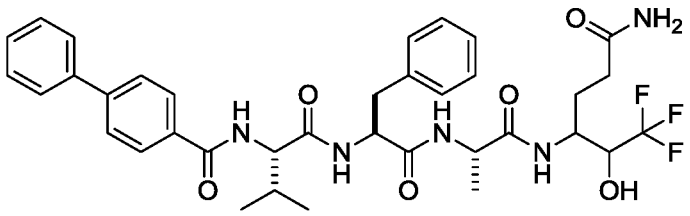


Prepared from Intermediate 13 (108 mg, 0.20 mmol), Intermediate 8 (57 mg, 0.24 mmol, 1.2 eq.), HATU (91 mg, 0.24 mmol, 1.2 eq.), DIPEA (0.12 mL, 0.69 mmol, 3.5 eq.) in DMF (2 mL). The title product was isolated as a mixture of diastereomers by flash chromatography on silica gel eluting with an increasing proportion of MeOH (5% to 10%) in CH_2Cl_2 followed by trituration with water and Et_2O.

^{1}H NMR (DMSO-d_6): δ 8.11-8.05 (2 H), 7.99 (0.4 H), 7.92-7.90 (1.6 H), 7.36 (2 H), 7.27 (2 H), 7.24-7.11 (6 H), 6.72 (1 H), 6.50 (1 H), 4.53 (1 H), 4.22 (1 H), 4.10 (1 H), 3.96-3.78 (2 H), 3.57 (1 H), 3.46 (1 H), 3.02 (1 H), 2.77 (1 H), 2.10-1.82 (4 H), 1.64 (1 H), 1.20 (1.8 H), 1.16 (1.2 H), 0.73 (6 H). ^{19}F NMR (DMSO-d_6): δ -57.2 (both diastereomers), -74.6 (one diastereomer), -74.71 (other diastereomer). ESI-MS: 720.3 $[M+H^+]$.

Example 24 (24)

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



Prepared as a mixture of diastereomers from Intermediate 14 (70 mg, 0.14 mmol), Intermediate 8 (46 mg, 0.18 mmol, 1.3 eq.), HATU (62 mg, 0.16 mmol, 1.2 eq.), DIPEA (85 µL, 0.49 mmol, 3.5 eq.) in DMF (1.4 mL).

^{1}H NMR (DMSO-d_6): δ 8.27 (1 H), 8.11-8.00 (2 H), 7.97-7.86 (3 H), 7.78 (2 H), 7.74 (2 H), 7.50 (2 H), 7.42 (1 H), 7.26-7.09 (6 H), 6.71 (1 H), 6.52 (1 H), 4.60 (1 H), 4.26-4.19 (2 H), 3.98-3.77 (2 H), 3.05 (1 H), 2.79 (1 H), 2.12-1.82 (4 H), 1.63 (1 H), 1.23 (1.5 H), 1.19 (1.5 H), 0.84 (3 H, d), 0.762 (1.5 H), 0.752 (1.5 H). ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69. ESI-MS: 698.4 [M+H$^+$].

Schemes 1 through 9 and the following text describe the general experimental procedures that were utilized to prepare compound examples 25-91. All reactions sensitive to moisture, atmospheric oxygen and/or carbon dioxide were performed under an anhydrous nitrogen atmosphere in solvents that were pre-dried over molecular sieves and degassed by several freeze-thaw cycles under hi-vacuum. Modifications to the described procedures, where necessary, are included with the description of the specific examples to which they apply. Final compounds were isolated as beige to colorless powders. Compounds prepared from intermediate 8 were generally isolated as 1:1 mixture of diastereomers, unless indicated otherwise.

General procedure for amine to carboxylic acid coupling with HATU, DIPEA:

DIPEA (3.5 eq.) was added dropwise to a stirred mixture of the appropriate amine (1.2 eq.), HATU (1.2 eq.) and the appropriate carboxylic acid at a concentration of 0.2 M in DMF at -20 °C followed by slow warming to 0 °C and stirring at rt overnight. The reaction vessel contents were then diluted with EtOAc and stirred with an ice-cold, half-saturated $NaHCO_3$ aqueous solution. The solid amide product was isolated by filtration, washed with water and EtOAc, and dried under suction and hi-vacuum. In cases where the product was soluble in EtOAc, the layers were separated and the aqueous phase was extracted with additional EtOAc. The combined organics were washed with aqueous solutions of 5-10% LiCl or 1:1 saturated brine/water, followed by drying over $MgSO_4$. The organics were filtered and concentrated under reduced pressure and the residue was triturated with ether containing 0-50% EtOAc. The solid product was collected by filtration and dried under high vacuum. In cases where purification by trituration was insufficient to reach a final purity of >90-95%, the isolated material was subjected to recrystallization or by flash chromatography on silica gel eluting with an increasing proportion of EtOAc or MeOH in CH_2Cl_2.

General procedure for amine to carboxylic acid coupling using isobutyl chloroformate:

(Shieh, Wen-Chung; Carlson, John A.; Shore, Michael E.; *Tetrahedron Lett.* **1999**, *40*, 7167-70.)

A 0.35 M solution of isobutyl chloroformate (1.1 eq.) in CH_2Cl_2 or THF was added slowly over a period of 1 h to a mixture of an appropriate amine hydrochloride (1.1 eq.), *N*-methylmorpholine (2.2 eq.) and an appropriate carboxylic acid at a concentration of 0.1 M in CH_2Cl_2 or THF at 0°C. Stirring at this temperature was continued for an additional 15 min prior to quenching of the reaction by the addition of saturated aqueous sodium bicarbonate solution and EtOAc. The solid amide product was collected by suction filtration and washed sequentially with water, EtOAc and ether. Drying under suction and hi-vacuum typically afforded the desired compound as a colorless to beige powder. Soluble products were isolated by an extractive workup procedure (EtOAc (2x), saturated aqueous $NaHCO_3$ and NaCl washes, Na_2SO_4 to dry the organic layer, filtration, concentration in vacuo). In cases where purification by trituration (typically with ether or with 1:1 EtOAc/ether) was insufficient to reach a final purity of >90-95%, the product was purified by recrystallization (in the specified solvent mixture) or by flash chromatography on silica gel eluting with an increasing proportion of EtOAc or MeOH in CH_2Cl_2, or EtOAc in hexanes.

General procedure for ester saponification with lithium hydroxide:

Aqueous LiOH (1 M, 1.5-2 eq.) was added dropwise to the appropriate methyl or ethyl ester at a concentration of 0.1 M in a 2:1 mixture of MeOH and THF at 0°C. After 15 min at this temperature, the reaction was warmed to rt and stirred until the reaction was judged complete by LCMS or TLC analysis. Crushed ice was then added and the mixture was acidified to pH 3-4 with 1 M HCl. After stirring for an additional 1 h, the solid product was isolated by suction filtration and dried under high vacuum. Products that failed to precipitate under these conditions were isolated by an extraction with EtOAc (2x), after which the combined organics were washed with brine, dried ($MgSO_4$), filtered and concentrated, and the residue was used directly in the next step.

General procedure for Boc deprotection with 4 M HCl in dioxane:

A 4 M solution of HCl in 1,4-dioxane (10-20 eq.) was added slowly to the appropriate Boc-protected amine at a concentration of 0.5 M in CH_2Cl_2 at 0 °C. The resulting mixture was then stirred at rt and the reaction progress was monitored by LCMS or TLC. When necessary, additional HCl in dioxane was added to drive the reaction to completion. The reaction mixture was then concentrated to dryness under reduced pressure to afford the desired deprotected amine hydrochloride salt which was used directly in the next reaction without further purification.

General procedure for Benzyl ester or Cbz-deprotection via hydrogenolysis:

A measured volume of deoxygenated MeOH to achieve a substrate concentration of 0.1-0.2 M was added via cannula to the appropriate Cbz-protected amine or benzyl ester and 10% Pd/C (30 wt%) in a septum-capped rbf under vacuum. The reaction vessel contents were then placed under an atmosphere of H_2 via a latex balloon and the reaction vessel was agitated in an ultrasonic bath for 2 min. The mixture was stirred at rt until the reaction was judged to be complete by LCMS or TLC analysis whereupon the vessel was flushed with N_2 and the reaction mixture was diluted with an equal volume of CH_2Cl_2. The suspension was filtered through a Celite pad and the pad was washed thoroughly with a 1:1 mixture of MeOH and CH_2Cl_2. The filtrate was concentrated and dried under hi-vacuum and the residue was used directly in the next step.

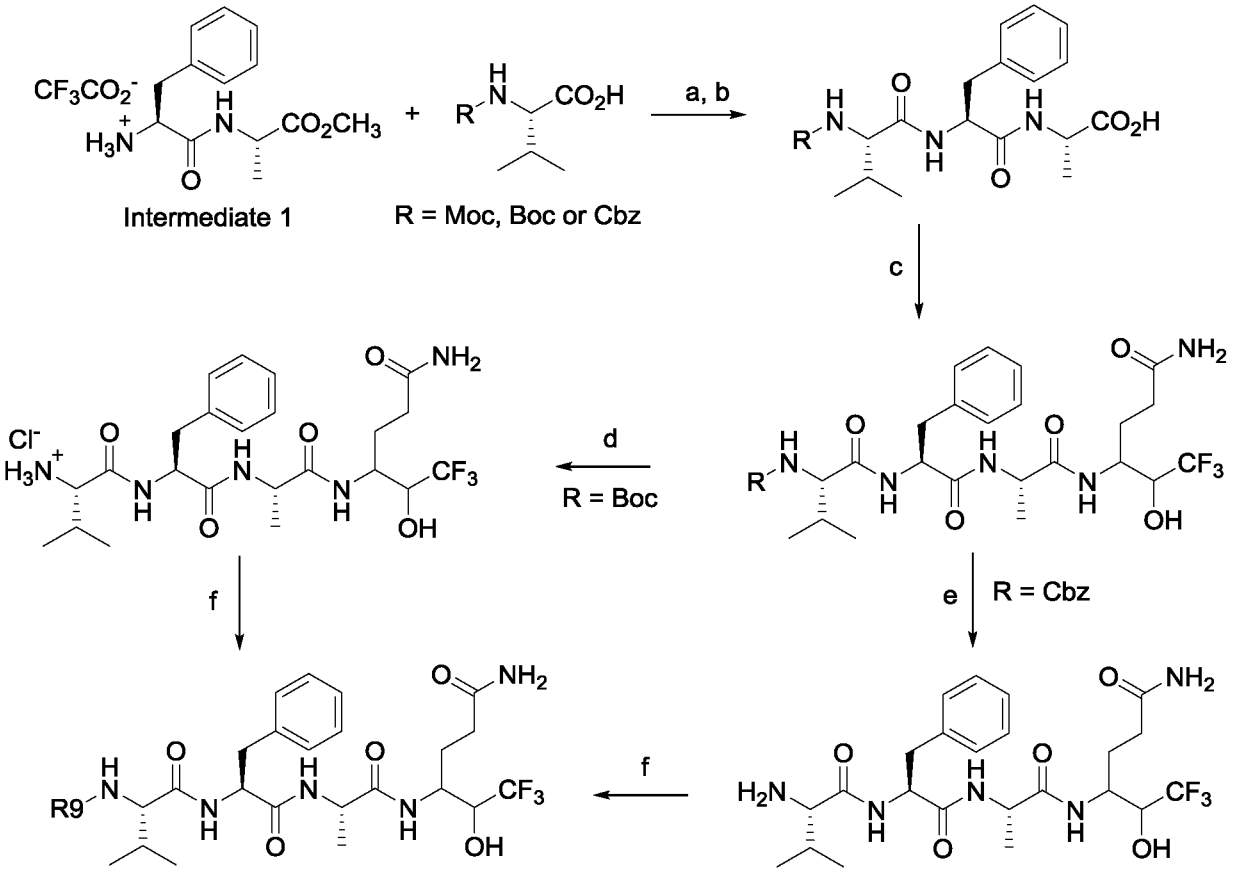
General procedure for R9 introduction using acyl or sulfonyl chlorides:

The appropriate acyl or sulfonyl chloride (1.1 eq.) was added portion-wise to a stirred mixture of DIPEA (2.2 eq.) or Et_3N (2.2 eq.) and the appropriate amine or amine hydrochloride at a concentration of 0.1-0.2 M in CH_2Cl_2 at 0°C followed by slow warming to rt with stirring until the reaction was judged complete by LCMS or TLC analysis. Additional CH_2Cl_2 and dilute aqueous HCl were added to the reaction vessel and the solid product was isolated by suction filtration, washed with CH_2Cl_2 and water and dried under high vacuum. Non-solid or soluble products were isolated by an extractive workup with CH_2Cl_2 or EtOAc followed by washing of the combined organic extracts with saturated solutions of $NaHCO_3$ and brine, drying over $MgSO_4$ and concentration under reduced pressure. Trituration of the residue with Et_2O typically generated a solid product that was isolated by filtration and dried under high vacuum.

General procedure for preparation of *N*-acyl amino acids:

A suitable acyl chloride (1 eq.) was added in one portion at rt to a 3:2:6 mixture of aqueous NaOH (1 M, 2 eq.), THF and Et_2O containing an appropriate amino acid followed by rapid stirring overnight. The acylated product was isolated by extraction with EtOAc (2x) following adjustment of the mixture to pH 2-3 with 10% aqueous HCl. The combined organics were washed with brine, dried over Na_2SO_4 and concentrated to dryness. Recrystallization of the residue from CH_2Cl_2/Et_2O afforded the desired *N*-acyl amino acid in suitable purity for use in the subsequent reaction.

Scheme 1[a]

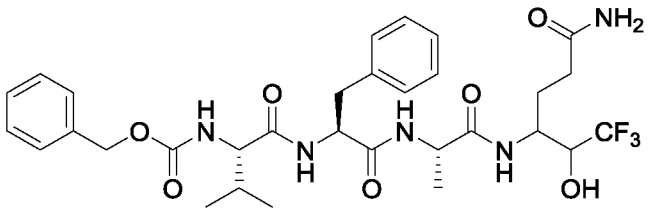


[a]Reagents and conditions: a) HATU, DIPEA, DMF, -20°C to rt; b) LiOH, H_2O, THF, MeOH, 0°C to rt; c) intermediate 8, 9 or *ent*-9, HATU, DIPEA, DMF, -20°C to rt; d) 4 M HCl in dioxane or 2 M HCl in ether, 0°C to rt; e) H_2 (1 atm), 10% Pd/C, MeOH; f) appropriate acyl or sulfonyl chloride, Et_3N or DIPEA, THF or DMF, 0°C to rt or appropriate carboxylic acid, HATU, DIPEA, DMF, 0°C to rt.

Compound examples prepared according to Scheme 1:

Example 25

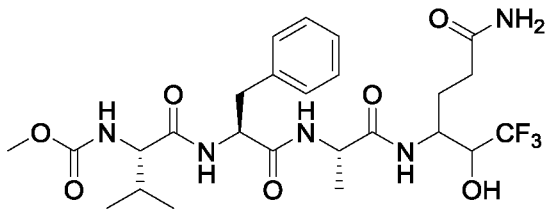
Benzyl ((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate



^{1}H NMR (DMSO-d_6): δ 8.10 (0.5 H), 8.00 (1.5 H), 7.90 (1 H), 7.40-7.08 (12 H), 6.72 (1 H), 6.50 (1 H), 5.00 (2 H), 4.55 (1 H), 4.20 (1 H), 3.99-2.65 (3 H), 3.00 (1 H), 2.72 (1 H), 2.12-1.75 (4 H), 1.60 (1 H), 1.18 (3 H), 0.70 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.65, -74.69; MS ESI: 652.4 $[M+H]^+$

Example 26

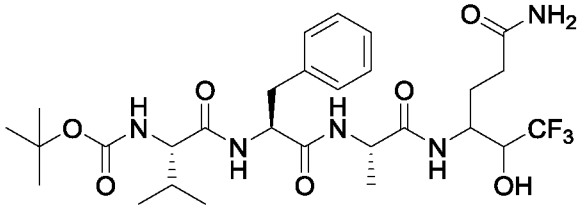
Methyl ((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate



^{1}H NMR (DMSO-d_6): δ 8.12 (0.5 H), 8.05 (0.5 H); 7.94 (1 H); 7.63 (0.5 H), 7.51 (0.5 H); 7.35-7.12 (6 H), 7.03 (1 H), 6.72 (1 H), 6.56 (0.5 H), 6.52 (0.5 H), 4.56 (1 H), 4.28 (1 H), 4.15-3.98 (2 H), 3.73 (1 H), 3.50 (3 H), 3.00 (1 H), 2.75 (1 H), 2.04 (2 H), 1.82 (1 H), 1.85-1.62 (2 H), 1.20 (1.5 H), 1.17 (1.5 H), 0.72 (6 H); ^{19}F NMR (DMSO-d_6): δ -75.25, -75.37; MS ESI: 576.3 $[M+H]^+$

Example 27

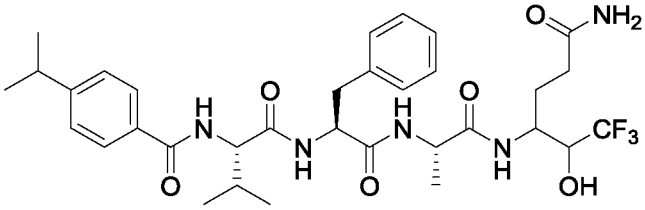
tert-Butyl ((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate



^{1}H NMR (DMSO-d_6): δ 8.12 (0.5 H), 8.04 (0.5 H), 7.86 (2 H), 7.24-7.08 (6 H), 6.69 (1 H), 6.62 (1 H), 6.47 (1 H), 4.58 (1 H), 4.20 (1 H), 3.96-3.75 (2 H), 3.66 (1 H), 3.00 (1 H), 2.73 (1 H), 2.10-1.80 (3 H), 1.77 (1 H), 1.61 (1 H), 1.38 (9 H), 1.18 (3 H), 0.64 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.66, -74.69; MS ESI: 618.4 $[M+H]^+$

Example 28

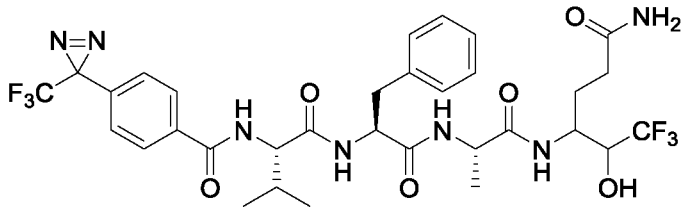
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-isopropylbenzamide



^{1}H NMR (DMSO-d_6): δ 8.14-7.93 (3 H), 7.84 (1 H), 7.75 (2 H), 7.30 (2 H), 7.25-7.01 (6 H), 6.70 (1 H), 6.46 (1 H), 4.58 (1 H), 4.25-4.12 (2 H), 3.98-3.72 (2 H), 3.02 (1 H), 2.93 (1 H), 2.74 (1 H), 2.11-1.78 (4 H), 1.60 (1 H), 1.20 (9 H), 0.80 (3 H), 0.70 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.65, -74.70; MS ESI: 664.4 $[M+H]^+$.

Example 29

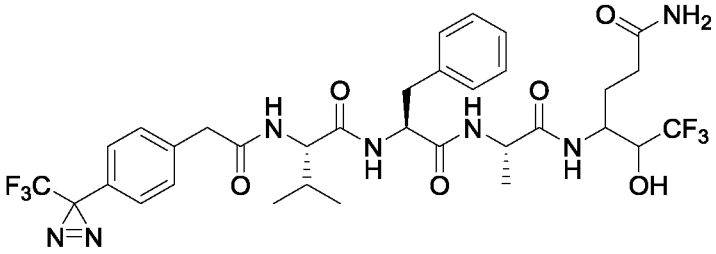
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzamide



^{1}H NMR (DMSO-d_6): δ 8.38 (1 H), 8.10-7.80 (5 H), 7.37 (2 H), 7.24-7.02 (6 H), 6.67 (1 H), 6.45 (1 H), 4.55 (1 H), 4.20 (2 H), 3.97-3.72 (2 H), 3.01 (1 H), 2.77 (1 H), 2.12-1.80 (4 H), 1.60 (1 H), 1.20 (3 H), 0.80 (3 H), 0.75 (3 H); ^{19}F NMR (DMSO-d_6): δ -64.90, -74.66, -74.70; MS ESI: 730.4 $[M+H]^+$

Example 30

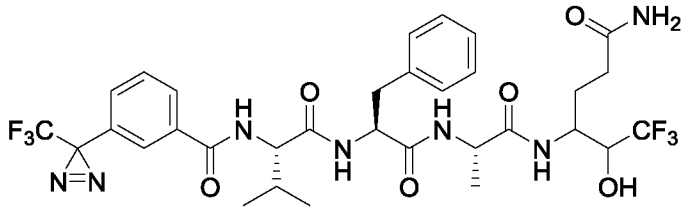
6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-(4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)acetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide



^{1}H NMR (DMSO-d_6): δ 8.08 (2 H), 7.86 (2 H), 7.37 (2 H), 7.30-7.02 (8 H), 6.68 (1 H), 6.44 (1 H), 4.50 (1 H), 4.20 (1 H), 4.10 (1 H), 3.98-3.75 (2 H), 3.36 (1 H), 3.24 (1 H), 3.00 (1 H), 2.73 (1 H), 2.12-1.80 (4 H), 1.60 (1 H), 1.18 (3 H), 0.68 (6 H); MS ESI: 744.4 $[M+H]^+$

Example 31

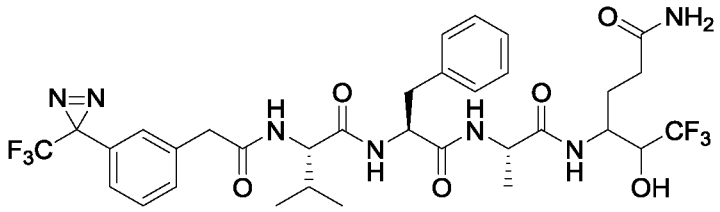
N-((2S)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-3-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzamide



^{1}H NMR (DMSO-d_6): δ 8.46 (1 H), 8.16 (1 H), 8.00 (1 H), 7.98-7.84 (2 H), 7.60 (2 H), 7.52 (1 H), 7.27-7.02 (6 H), 6.69 (1 H), 6.47 (1 H), 4.55 (1 H), 4.21 (2 H), 3.97-3.75 (2 H), 3.01 (1 H), 2.77 (1 H), 2.12-1.80 (4 H), 1.64 (1 H), 1.18 (3 H), 0.80 (6 H); MS ESI: 730.3 $[M+H]^+$

Example 32

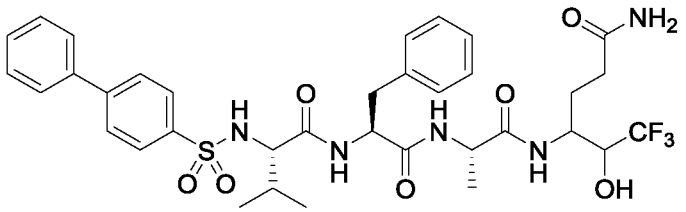
6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-(3-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)phenyl)acetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide



^{1}H NMR (DMSO-d_6): δ 8.20-8.05 (2 H), 8.00-7.90 (2 H), 7.40 (2 H), 7.26-7.08 (8 H), 6.71 (1 H), 6.59 (1 H), 4.51 (1 H), 4.20 (1 H), 4.09 (1 H), 3.98-3.75 (2 H), 3.60 (1 H), 3.42 (1 H), 3.00 (1 H), 2.75 (1 H), 2.10-1.80 (4 H), 1.60 (1 H), 1.18 (3 H), 0.70 (6 H); MS ESI: 744.4 $[M+H]^+$

Example 33

4-((*S*)-2-((*S*)-2-((*S*)-2-([1,1'-Biphenyl]-4-sulfonamido)-3-methylbutanamido)-3-phenylpropanamido)propanamido)-6,6,6-trifluoro-5-hydroxyhexanamide

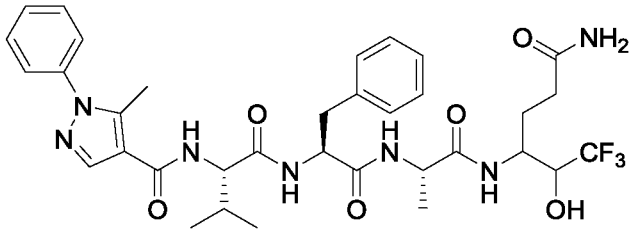


Extractive workup in the final step was performed with 9:1 CH_2Cl_2:MeOH as the organic phase; final purification was accomplished by flash chromatography on silica gel eluting with 2-6% MeOH/CH_2Cl_2.

^{1}H NMR (DMSO-d_6): δ 8.10 (1 H), 7.95 (1 H), 7.90-7.75 (2 H), 7.72-7.60 (6 H), 7.47 (2 H), 7.40 (1 H), 7.23-7.07 (6 H), 6.70 (1 H), 6.46 (1 H), 4.30 (1 H), 4.18 (1 H), 3.95-3.75 (2 H), 3.53 (1 H), 2.89 (1 H), 2.63 (1 H), 2.06-1.71 (4 H), 1.58 (1 H), 1.13 (3 H), 0.70 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.66, -74.71; MS ESI: 734.4 $[M+H]^+$

Example 34

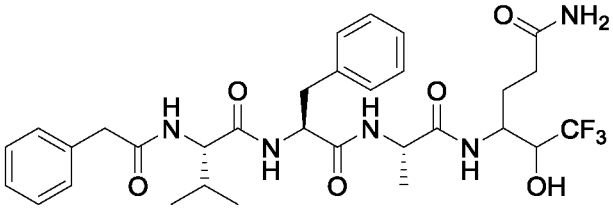
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.20 (1 H), 8.10-7.95 (2 H), 7.90 (1 H), 7.82 (1 H), 7.60-7.40 (5 H), 7.24-7.05 (6 H), 6.70 (1 H), 6.54 (1 H), 4.57 (1 H), 4.20 (2 H), 3.98-3.76 (2 H), 3.02 (1 H), 2.80 (1 H), 2.47 (3 H), 2.13-1.80 (4 H), 1.62 (1 H), 1.20 (3 H), 0.80 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69; MS ESI: 702.4 $[M+H]^+$

Example 35

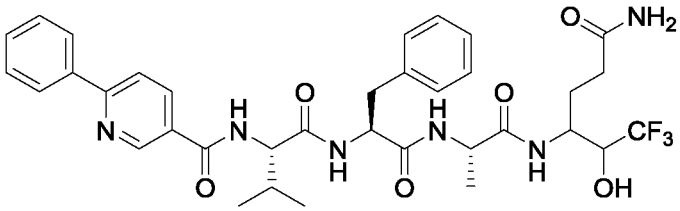
6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-phenylacetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide



^{1}H NMR (DMSO-d_6): δ 8.05 (1 H), 8.00-7.82 (3 H), 7.30-7.08 (11 H), 6.70 (1 H), 6.50 (1 H), 4.53 (1 H), 4.22 (1 H), 4.10 (1 H), 3.97-3.76 (2 H), 3.53 (1 H), 3.40 (1 H), 3.01 (1 H), 2.77 (1 H), 2.10-1.80 (4 H), 1.62 (1 H), 1.20 (3 H), 0.72 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69; MS ESI: 636.4 $[M+H]^+$

Example 36

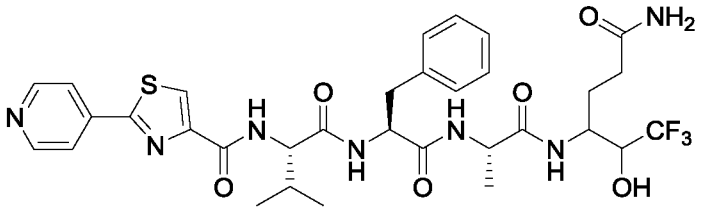
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-6-phenylnicotinamide



^{1}H NMR (DMSO-d_6): δ 9.07 (1 H), 8.62 (1 H), 8.26 (1 H), 8.20-7.97 (5 H), 7.90 (1 H), 7.58-7.42 (3 H), 7.25-7.02 (6 H), 6.70 (1 H), 6.60 (1 H), 4.57 (1 H), 4.22 (2 H), 3.99-3.75 (2 H), 3.03 (1 H), 2.80 (1 H), 2.12-1.80 (4 H), 1.62 (1 H), 1.20 (3 H), 0.80 (6 H); MS ESI: 699.4 $[M+H]^+$

Example 37

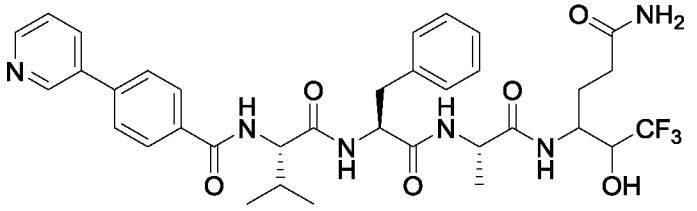
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-2-(pyridin-4-yl)thiazole-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.76 (2 H), 8.49 (1 H), 8.38 (1 H), 8.15-7.82 (5 H), 7.11-7.00 (6 H), 6.70 (1 H), 6.50 (1 H), 4.59 (1 H), 4.33 (1 H), 4.22 (1 H), 3.97-3.74 (2 H), 3.02 (1 H), 2.77 (1 H), 2.15-1.80 (4 H), 1.62 (1 H), 1.20 (3 H), 0.80 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.66; MS ESI: 706.3 $[M+H]^+$

Example 38

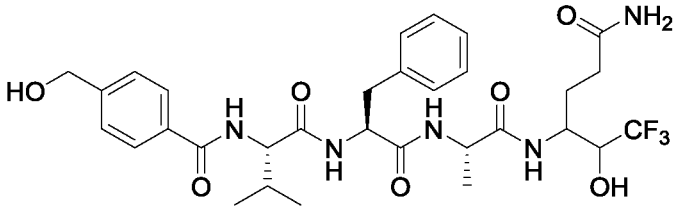
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(pyridin-3-yl)benzamide



^{1}H NMR (DMSO-d_6): δ 8.97 (1 H), 8.60 (1 H), 8.31 (1 H), 8.20-7.70 (8 H), 7.50 (1 H), 7.12-7.00 (6 H), 6.70 (1 H), 6.50 (1 H), 4.60 (1 H), 4.22 (2 H), 4.07-3.71 (2 H), 3.01 (1 H), 2.79 (1 H), 2.17-1.78 (4 H), 1.61 (1 H), 1.18 (3 H), 0.79 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.65; MS ESI: 699.4 $[M+H]^+$

Example 39

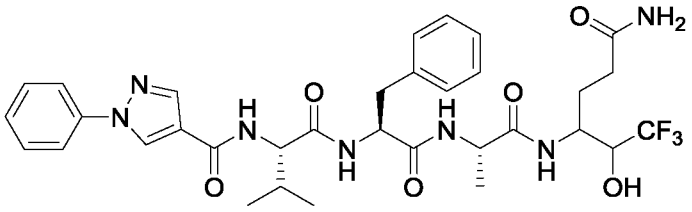
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(hydroxymethyl)benzamide



^{1}H NMR (DMSO-d_6): δ 8.12 (1 H), 8.08 (1.5 H), 8.0 (0.5 H), 7.86 (1 H), 7.80 (2 H), 7.40 (2 H), 7.24-7.04 (6 H), 6.70 (1 H), 6.50 (1 H), 5.30 (1 H), 4.59 (1 H), 4.56 (2 H), 4.20 (2 H), 3.97-3.76 (2 H), 3.03 (1 H), 2.75 (1 H), 2.10-1.80 (4 H), 1.60 (1 H), 1.19 (3 H), 0.80 (3 H), 0.70 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69; MS ESI: 652.3 $[M+H]^+$

Example 40

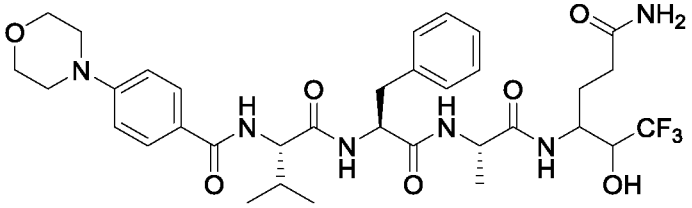
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-1-phenyl-1*H*-pyrazole-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 9.04 (1 H), 8.19 (1 H), 8.16 (1 H), 8.00-7.83 (3 H), 7.82 (2 H), 7.54 (2 H), 7.38 (1 H), 7.23-7.02 (6 H), 6.71 (1 H), 6.51 (1 H), 4.55 (1 H), 4.23-4.18 (2 H), 3.98-3.77 (2 H), 3.04 (1 H), 2.80 (1 H), 2.14-1.80 (4 H), 1.62 (1 H), 1.20 (3 H), 0.80 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.65, -74.70; MS ESI: 688.3 $[M+H]^+$

Example 41

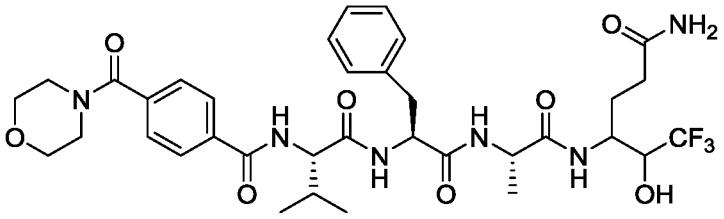
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-morpholinobenzamide



^{1}H NMR (DMSO-d_6): δ 8.08-7.98 (2 H), 7.98-7.82 (2 H), 7.76 (2 H), 7.26-7.06 (6 H), 6.97 (2 H), 6.70 (1 H), 6.55 (1 H), 4.56 (1 H), 4.25-4.08 (2 H), 3.98-3.78 (2 H), 3.72 (4 H), 3.20 (4 H), 3.02 (1 H), 2.77 (1 H), 2.14-1.80 (4 H), 1.60 (1 H), 1.20 (3 H), 0.80 (3 H), 0.70 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.65; MS ESI: 707.4 $[M+H]^+$

Example 42

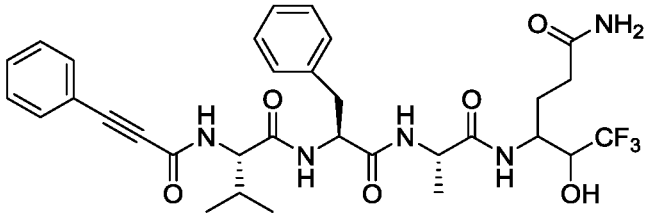
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(morpholine-4-carbonyl)benzamide



^{1}H NMR (DMSO-d_6): δ 8.31 (1 H), 8.15-8.10 (1.5 H), 8.01 (0.5 H), 7.97-7.82 (3 H), 7.50 (2 H), 7.27-7.04 (6 H), 6.70 (1 H), 6.52 (1 H), 4.60 (1 H), 4.20 (2 H), 4.00-3.77 (2 H), 3.75-3.42 (8 H), 3.03 (1 H), 2.78 (1 H), 2.15-1.80 (4 H), 1.63 (1 H), 1.20 (3 H), 0.83 (3 H), 0.77 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69; MS ESI: 735.4 $[M+H]^+$

Example 43

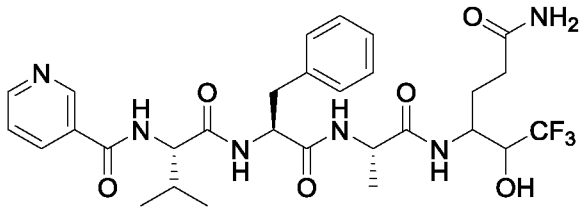
6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(3-phenylpropiolamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide



^{1}H NMR (DMSO-d_6): δ 8.74 (1 H), 8.14 (1 H), 8.06 (0.5 H), 8.00 (0.5 H), 7.92 (1 H), 7.59 (2 H), 7.54-7.40 (3 H), 7.27-7.17 (6 H), 6.70 (1 H), 6.52 (1 H), 4.66 (1 H), 4.31-4.11 (2 H), 3.98-3.72 (2 H), 3.02 (1 H), 2.78 (1 H), 2.12-1.80 (4 H), 1.62 (1 H), 1.18 (3 H), 0.78 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69; MS ESI: 646.4 $[M+H]^+$

Example 44

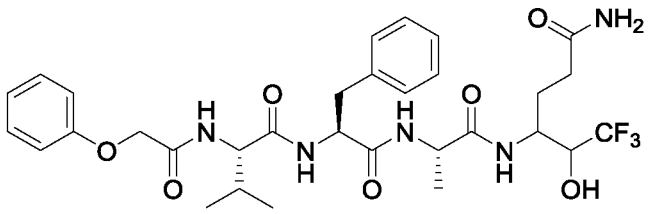
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)nicotinamide



^{1}H NMR (DMSO-d_6): δ 8.99 (1 H), 8.70 (1 H), 8.47 (1 H), 8.17 (1 H), 8.10 (1 H), 8.01 (0.5 H), 7.98 (0.5 H), 7.90 (1 H), 7.50 (1 H), 7.24-7.02 (6 H), 6.70 (1 H), 6.48 (1 H), 4.58 (1 H), 4.20 (2 H), 3.97-3.75 (2 H), 3.03 (1 H), 2.78 (1 H), 2.12-1.80 (4 H), 1.63 (1 H), 1.20 (3 H), 0.85 (3 H), 0.77 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.68; MS ESI: 623.3 $[M+H]^+$

Example 45

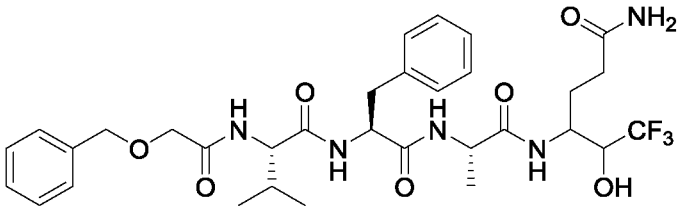
6,6,6-Trifluoro-5-hydroxy-4-((*S*)-2-((*S*)-2-((*S*)-3-methyl-2-(2-phenoxyacetamido)butanamido)-3-phenylpropanamido)propanamido)hexanamide



^{1}H NMR (DMSO-d_6): δ 8.20 (1 H), 8.03 (0.5 H), 7.98 (0.5 H), 7.92 (1 H), 7.72 (1 H), 7.30-7.06 (8 H), 6.99-6.84 (3 H), 6.68 (1 H), 6.53 (1 H), 4.60-4.45 (3 H), 4.27-4.13 (2 H), 3.98-3.76 (2 H), 3.03 (1 H), 2.76 (1 H), 2.15-1.80 (4 H), 1.62 (1 H), 1.20 (3 H), 0.70 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.60, -74.66; MS ESI: 652.4 $[M+H]^+$

Example 46

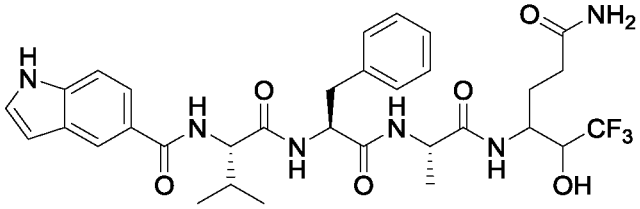
4-((6*S*,9*S*,12*S*)-9-Benzyl-6-isopropyl-12-methyl-4,7,10-trioxo-1-phenyl-2-oxa-5,8,11-triazatridecan-13-amido)-6,6,6-trifluoro-5-hydroxyhexanamide



^{1}H NMR (DMSO-d_6): δ 8.23 (1 H), 8.07 (0.5 H), 8.00 (0.5 H), 7.90 (1 H), 7.42-7.02 (12 H), 6.70 (1 H), 6.50 (1 H), 4.56 (1 H), 4.50 (2 H), 4.26-4.11 (2 H), 3.99-3.72 (4 H), 3.03 (1 H), 2.72 (1 H), 2.12-1.90 (4 H), 1.61 (1 H), 1.20 (3 H), 0.72 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69; MS ESI:666.4 $[M+H]^+$

Example 47

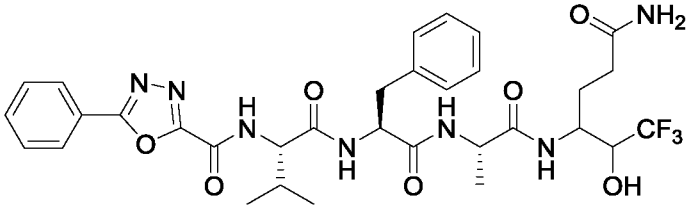
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-1*H*-indole-5-carboxamide



^{1}H NMR (DMSO-d_6): δ 11.31 (1 H), 8.17-7.95 (4 H), 7.84 (1 H), 7.60 (1 H), 7.40 (2 H), 7.14-7.01 (6 H), 6.70 (1 H), 6.58-6.40 (2 H), 4.60 (1 H), 4.24-4.10 (2 H), 3.98-3.72 (2 H), 3.02 (1 H), 2.68 (1 H), 2.15-1.80 (4 H), 1.60 (1 H), 1.20 (3 H), 0.82 (3 H), 0.70 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.69; MS ESI:661.3 $[M+H]^+$

Example 48

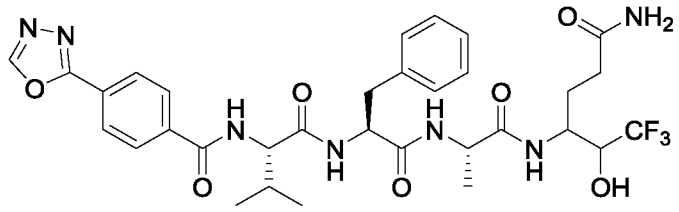
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-5-phenyl-1,3,4-oxadiazole-2-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.87 (1 H), 8.31 (1 H), 8.15-8.00 (3 H), 7.90 (1 H), 7.70-7.52 (3 H), 7.27-6.98 (6 H), 6.70 (1 H), 6.46 (1 H), 4.60 (1 H), 4.23 (2 H), 3.98-3.72 (2 H), 3.04 (1 H), 2.73 (1 H), 2.14-1.80 (4 H), 1.60 (1 H), 1,20 (3 H), 0.82 (3 H), 0.73 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.67, -74.70; MS ESI:690.3 $[M+H]^+$

Example 49

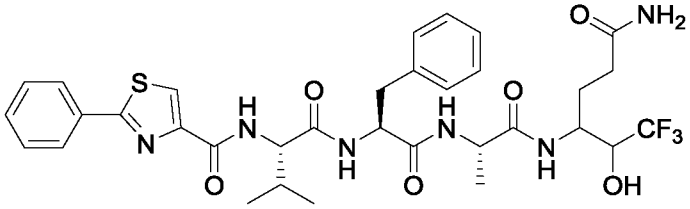
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-(1,3,4-oxadiazol-2-yl)benzamide



^{1}H NMR (DMSO-d_6): δ 9.40 (1 H), 8.43 (1 H), 8.18-7.95 (5.5 H), 7.96-7.80 (1.5 H), 7.27-7.00 (6 H), 6.70 (1 H), 6.50 (1 H), 4.58 (1 H), 4.27-4.14 (2 H), 3.98-3.75 (2 H), 3.02 (1 H), 2.79 (1 H), 2.14-1.80 (4 H), 1.60 (1 H), 1.18 (3 H), 0.82 (3 H), 0.76 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.63, -74.68; MS ESI:690.4 $[M+H]^+$

Example 50

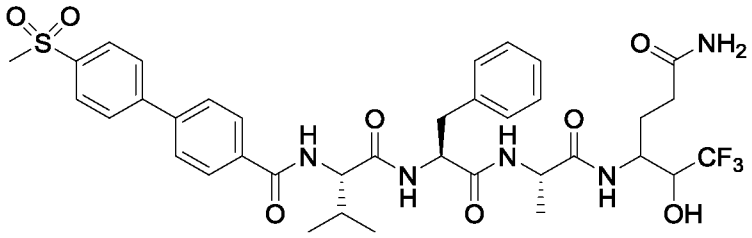
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-2-phenylthiazole-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.38 (1 H), 8.32 (1 H), 8.10 (0.5 H), 8.05 (0.5 H), 8.00 (3 H), 7.92 (1 H), 7.60-7.50 (3 H), 7.23 (2 H), 7.15 (3 H), 7.05 (1 H), 6.69 (1 H), 6.50 (1 H), 4.60 (1 H), 4,53 (1 H), 4.21 (1 H), 3.97-3.72 (2 H), 3.04 (1 H), 2.77 (1 H), 2.14-1.80 (4 H), 1.60 (1 H), 1.20 (3 H), 0.80 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.68; MS ESI:705.3 $[M+H]^+$

Example 51

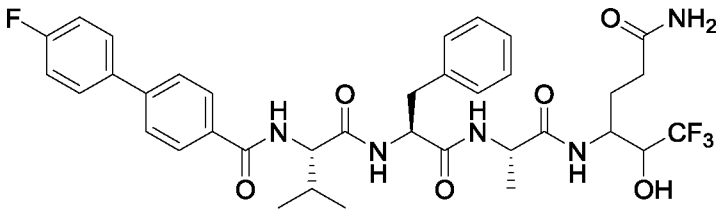
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4'-(methylsulfonyl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.33 (1 H), 8.13-7.93 (8 H), 7.92-7.80 (3 H), 7.27-7.04 (6 H), 6.70 (1 H), 6.48 (1 H), 4.60 (1 H), 4.22 (2 H), 3.98-3.77 (2 H), 3.25 (3 H), 3.04 (1 H), 2.77 (1 H), 2.12-1.80 (4 H), 1.61 (1 H), 1.20 (3 H), 0.82 (3 H), 0.77 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69; MS ESI:776.3 $[M+H]^+$

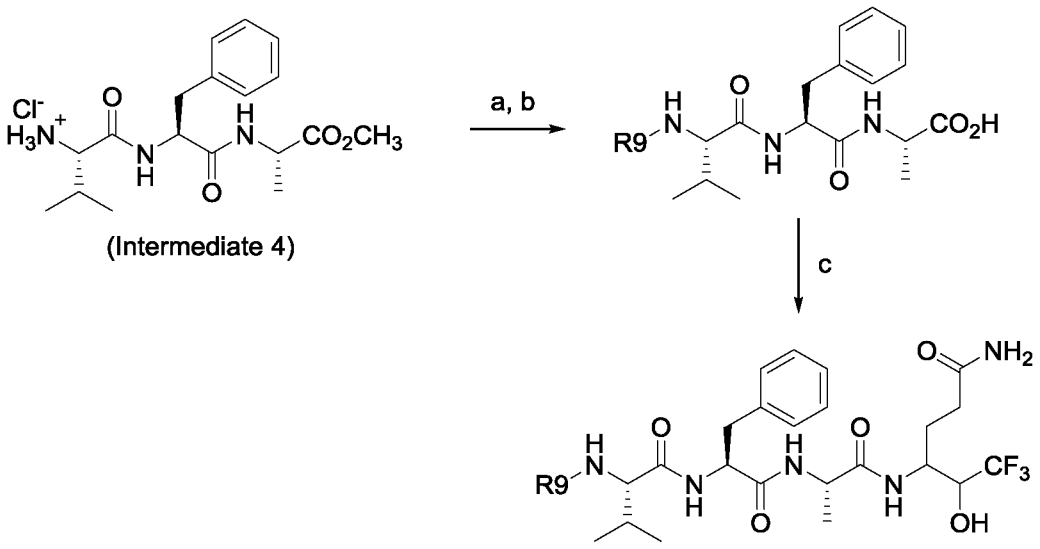
Example 52

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-4'-fluoro-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.26 (1 H), 8.14-8.00 (2 H), 7.99-7.82 (3 H), 7.80-7.68 (4 H), 7.30 (2 H), 7.26-7.02 (6 H), 6.70 (1 H), 6.50 (1 H), 4.60 (1 H), 4.30-4.10 (2 H), 3.99-3.72 (2 H), 3.03 (1 H), 2.78 (1 H), 2.16-1.77 (4 H), 1.63 (1 H), 1.20 (3 H), 0.80 (3 H), 0.70 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69, -115.0; MS ESI:716.3 $[M+H]^+$

Scheme 2[a]

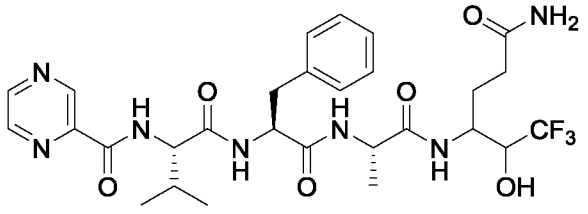


[a]Reagents and conditions: a) appropriate acyl or sulfonyl chloride, Et_3N, THF, 0°C to rt or appropriate carboxylic acid, HATU, DIPEA, DMF, -20°C to rt; b) LiOH, H_2O, THF, MeOH, 0°C to rt; c) intermediate 8, 9 or *ent*-9, HATU, DIPEA, DMF, -20°C to rt.

Compound examples prepared according to Scheme 2:

Example 53

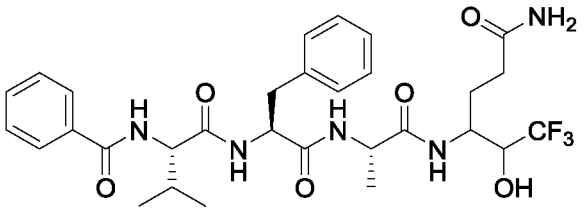
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)pyrazine-2-carboxamide



^{1}H NMR (DMSO-d_6): δ 9.19 (1 H), 8.90 (1 H), 8.73 (1 H), 8.45-8.35 (2 H), 8.13 (0.5 H), 8.03 (0.5 H), 7.90 (1 H), 7.27-7.10 (5 H), 7.05 (1 H), 6.70 (1 H), 6.50 (1 H), 4.60 (1 H), 4.35 (1 H), 4.20 (1 H), 3.98-3.72 (2 H), 3.02 (1 H), 2.71 (1 H), 2.14-1.80 (4 H), 1.60 (1 H), 1.20 (3 H), 0.77 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.68; MS ESI:624.4 $[M+H]^+$

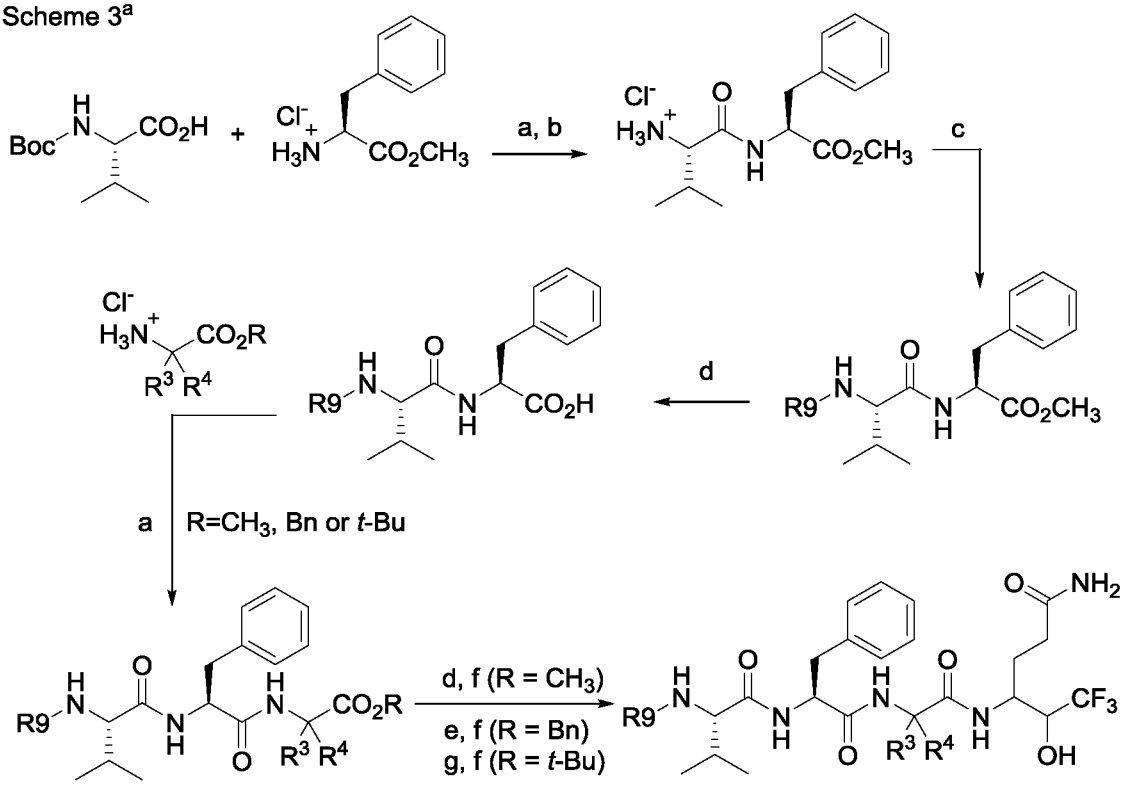
Example 54

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)benzamide



^{1}H NMR (DMSO-d_6): δ 8.20 (1 H), 8.07 (1.5 H), 8.00 (0.5 H), 7.88 (1 H), 7.80 (2 H), 7.50 (1 H), 7.45 (2 H), 7.23-7.02 (6 H), 6.70 (1 H), 6.50 (1 H), 4.56 (1 H), 4.24-4.12 (2 H), 3.96-3.76 (2 H), 3.01 (1 H), 2.78 (1 H), 2.10-1.80 (4 H), 1.61 (1 H), 1.18 (3 H), 0.80 (3 H), 0.72 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.65, -74.70; MS ESI:622.4 $[M+H]^+$

Scheme 3[a]

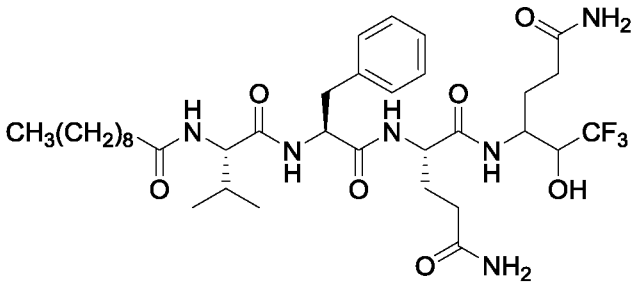


[a]Reagents and conditions: a) HATU, DIPEA, DMF, 0°C to rt; b) 4 M HCl in dioxane, CH_2Cl_2, 0°C to rt; c) decanoyl chloride or 4-phenylbenzoyl chloride, Et_3N, THF, 0°C to rt; d) LiOH, H_2O, THF, MeOH, 0°C to rt; e) H_2 (1 atm), 10% Pd/C, MeOH; f) intermediate 8, 9 or *ent*-9, HATU, DIPEA, DMF, -20°C to rt; g) TFA/CH_2Cl_2 (1:1), 0°C to rt.

Compound examples prepared according to Scheme 3:

Example 55

(2*S*)-*N*[1]-(6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)-2-((*S*)-2-((*S*)-2-decanamido-3-methylbutanamido)-3-phenylpropanamido)pentanediamide

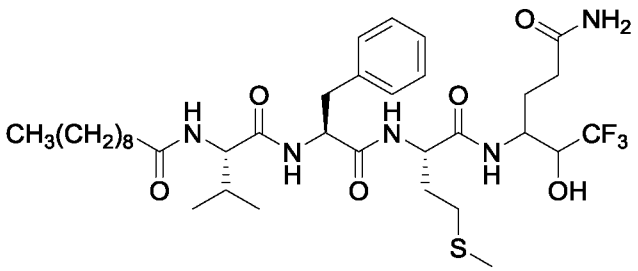


Compound was purified by recrystallization from ethanol/water with decolourization using activated charcoal.

^{1}H NMR (DMSO-d_6): δ 8.01-7.86 (3 H), 7.71 (1 H), 7.26-7.05 (7 H), 6.80-6.64 (2 H), 6.44 (1 H), 4.55 (1 H), 4.20 (1 H), 4.03 (1 H), 3.98-3.80 (2 H), 3.01 (1 H), 2.80 (1 H), 2.20-1.94 (6 H), 1.94-1.55 (5 H), 1.50-1.30 (2 H), 1.30-1.10 (12 H), 0.82 (3 H), 0.75 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.61, -74.70; MS ESI: 729.5 $[M+H]^+$

Example 56

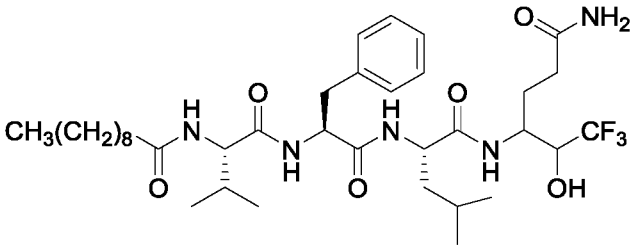
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-4-(methylthio)-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide



^{1}H NMR (DMSO-d_6): δ 8.10-7.82 (3 H), 7.75 (1 H), 7.23-7.08 (6 H), 6.70 (1 H), 6.50 (1 H), 4.52 (1 H), 4.30 (1 H), 4.04 (1 H), 3.98-3.80 (2 H), 3.00 (1 H), 2.80 (1 H), 2.44-2.30 (2 H), 2.20-1.55 (9 H), 2.00 (3 H), 1.52-1.30 (2 H), 1.30-1.10 (12 H), 0.81 (3 H), 0.73 (6 H); MS ESI: 732.5 $[M+H]^+$

Example 57

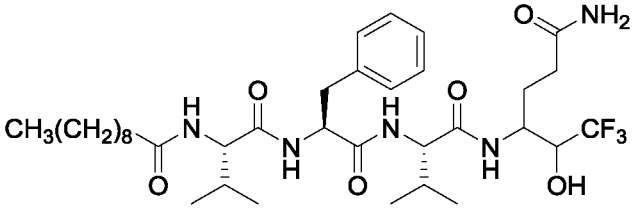
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide



^{1}H NMR (DMSO-d_6): δ 7.98 (1 H), 7.95 (0.5 H), 7.83 (1.5 H), 7.73 (1 H), 7.23-7.06 (6 H), 6.70 (1 H), 6.47 (1 H), 4.53 (1 H), 4.44 (1 H), 4.05 (1 H), 3.96-3.78 (2 H), 3.00 (1 H), 2.80 (1 H), 2.20-1.80 (6 H), 1.70-1.33 (6 H), 1.30-1.10 (12 H), 0.83 (9 H), 0.73 (6 H); MS ESI: 714.5 $[M+H]^+$

Example 58

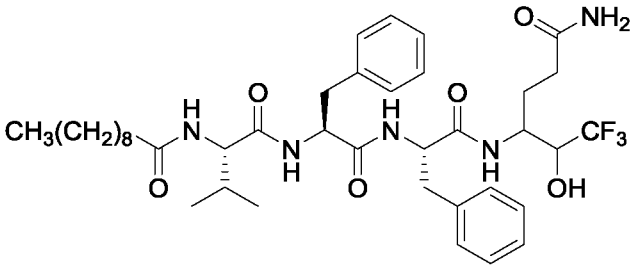
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide



^{1}H NMR (DMSO-d_6): δ 8.04-7.87 (2 H), 7.79-7.70 (2 H), 7.23-7.04 (6 H), 6.70 (1 H), 6.44 (1 H), 4.55 (1 H), 4.19-4.01 (2 H), 3.90 (1 H), 3.80 (1 H), 2.98 (1 H), 2.78 (1 H), 2.20-1.76 (7 H), 1.63 (1 H), 1.50-1.32 (2 H), 1.30-1.13 (12 H), 0.80 (9 H), 0.75 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.61, -74.69; MS ESI: 700.5 $[M+H]^+$

Example 59

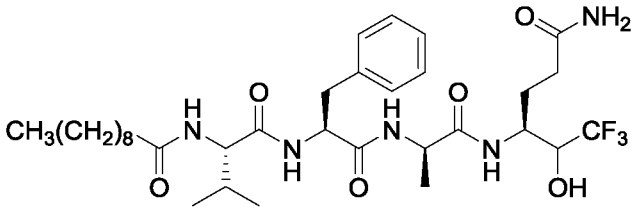
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide



^{1}H NMR (DMSO-d_6): δ 8.03 (1 H), 8.00 (1 H), 7.90 (1 H), 7.70 (1 H), 7.30-7.07 (11 H), 6.72 (1 H), 6.47 (1 H), 4.60-4.42 (2 H), 4.05 (1 H), 4.00-3.74 (2 H), 3.00-2.82 (2 H), 2.80-2.62 (2 H), 2.20-1.97 (3 H), 1.97-1.73 (3 H), 1.62 (1 H), 1.52-1.32 (2 H), 1.30-1.15 (12 H), 0.82 (3 H), 0.70 (6 H); MS ESI: 748.5 $[M+H]^+$

Example 60

N-((2*S*)-1-(((2*S*)-1-(((2*R*)-1-(((3*S*)-6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide

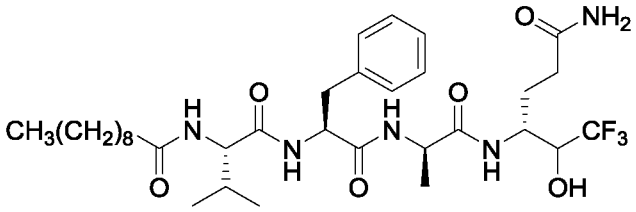


Single diastereomer; prepared using intermediate 9.

^{1}H NMR (DMSO-d_6): δ 8.07 (1 H), 7.95 (1 H), 7.78 (1 H), 7.70 (1 H), 7.25-7.10 (6 H), 6.70 (1 H), 6.40 (1 H), 4.43 (1 H), 4.17 (1 H), 4.09 (1 H), 3.96-3.79 (2 H), 2.94 (1 H), 2.81 (1 H), 2.20-1.80 (6 H), 1.68 (1 H), 1.51-1.38 (2 H), 1.30-1.14 (12 H), 1.05 (3 H), 0.83 (3 H), 0.74 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.67; MS ESI: 672.5 $[M+H]^+$

Example 61

N-((2*S*)-1-(((2*S*)-1-(((2*R*)-1-(((3*R*)-6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)decanamide

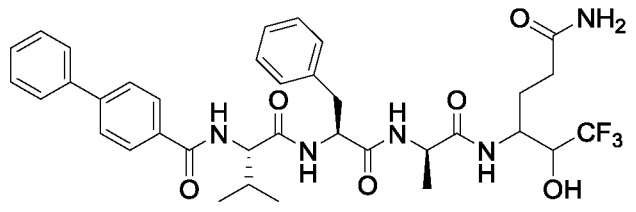


Single diastereomer; prepared using intermediate *ent*-9; purified by slow recrystallization from EtOAc/EtOH.

^{1}H NMR (DMSO-d_6): δ 8.10 (1 H), 7.97 (1 H), 7.81 (1 H), 7.64 (1 H), 7.25-7.10 (6 H), 6.66 (1 H), 6.43 (1 H), 4.42 (1 H), 4.18 (1 H), 4.10 (1 H), 3.96-3.77 (2 H), 2.95 (1 H), 2.80 (1 H), 2.20-1.80 (6 H), 1.62 (1 H), 1.44-1.36 (2 H), 1.30-1.14 (12 H), 1.02 (3 H), 0.82 (3 H), 0.74 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.62; MS ESI: 672.5 $[M+H]^+$

Example 62

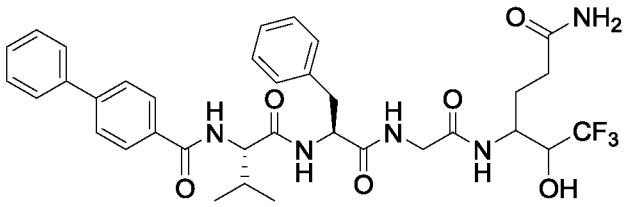
N-((2*S*)-1-(((2*S*)-1-(((2*R*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.32-8.16 (2 H), 8.03 (1 H), 7.99-7.89 (2 H), 7.83-7.62 (5 H), 7.55-7.43 (2 H), 7.40 (1 H), 7.24-7.01 (6 H), 6.70 (1 H), 6.42 (1 H), 4.50 (1 H), 4.32-4.12 (2 H), 3.99-3.77 (2 H), 2.96 (1 H), 2.81 (1 H), 2.27-1.78 (4 H), 1.62 (1 H), 1.03 (3 H), 0.81 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.66, -74.69; MS ESI: 698.4 $[M+H]^+$

Example 63

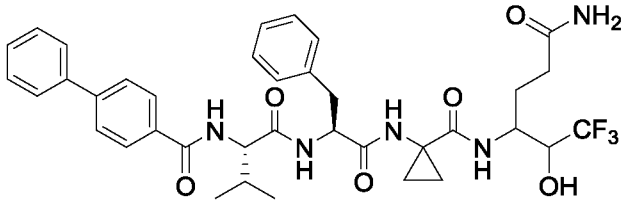
N-((2*S*)-1-(((2*S*)-1-((2-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-2-oxoethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.30-8.20 (2 H), 8.10 (1 H), 7.91 (2 H), 7.84 (1 H), 7.80-7.64 (4 H), 7.47 (2 H), 7.40 (1 H), 7.25-7.01 (6 H), 6.70 (1 H), 6.50 (1 H), 4.60 (1 H), 4.23 (1 H), 4.00-3.80 (2 H), 3.79-3.59 (2 H), 3.01 (1 H), 2.81 (1 H), 2.16-1.79 (4 H), 1.62 (1 H), 0.83 (3 H), 0.78 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.63, -74.68; MS ESI: 684.4 $[M+H]^+$

Example 64

N-((2*S*)-1-(((2*S*)-1-((1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)carbamoyl)cyclopropyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide

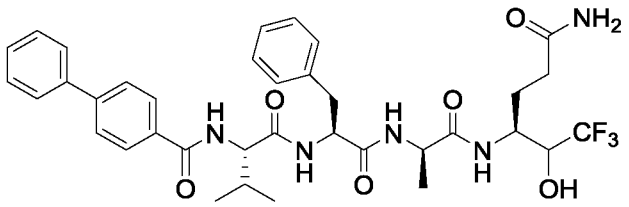


Isolated as a 2.4:1 mix of diastereomers.

^{1}H NMR (DMSO-d_6): δ 8.21 (0.7 H), 8.15 (0.3 H), 8.10 (0.3 H), 8.00 (0.7 H), 7.98 (0.3 H), 7.90 (0.7 H), 7.70-7.58 (2 H), 7.50-7.36 (4 H), 7.18 (2 H), 7.10 (2 H), 6.97-6.75 (6 H), 6.40 (0.3 H), 6.38 (0.7 H), 6.00 (1 H), 4.00 (1 H), 3.90 (1 H), 3.67-3.50 (2 H), 2.70-2.50 (2 H), 1.86-1.30 (5 H), 0.94-0.72 (1.7 H), 0.72-0.48 (6.3 H), 0.30 (0.7 H), 0.13 (0.3 H), 0.02 (1 H); ^{19}F NMR (DMSO-d_6): δ -74.62 (minor diastereomer), -74.84 (major diastereomer); MS ESI: 710.4 $[M+H]^+$

Example 65

N-((2*S*)-1-(((2*S*)-1-(((2*R*)-1-(((3*S*)-6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide

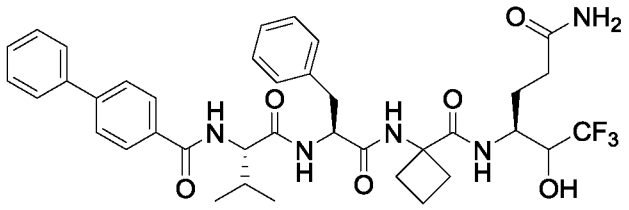


Single diastereomer; prepared using intermediate 9.

^{1}H NMR (DMSO-d_6): δ 8.30 (1 H), 8.20 (1 H), 8.03 (1 H), 7.97 (2 H), 7.81 (1 H), 7.76-7.68 (4 H), 7.50 (2 H), 7.40 (1 H), 7.24-7.05 (6 H), 6.70 (1 H), 6.40 (1 H), 4.50 (1 H), 4.33-4.16 (2 H), 3.97-3.79 (2 H), 2,95 (1 H), 2.82 (1 H), 2.10-1.78 (4 H), 1.70 (1 H), 1.05 (3 H), 0.82 (3 H), 0.78 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.66; MS ESI: 698.4 $[M+H]^+$

Example 66

N-((2*S*)-1-(((2*S*)-1-((1-(((3*S*)-6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)carbamoyl)cyclobutyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide

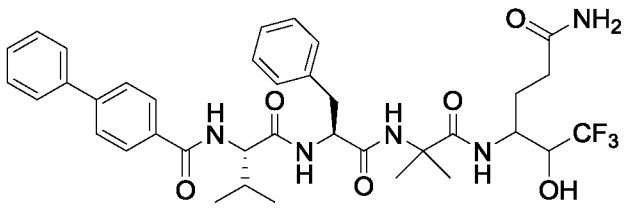


Single diastereomer; prepared using intermediate 9.

^{1}H NMR (DMSO-d_6): δ 8.40 (1 H), 8.35 (2 H), 7.97 (2 H), 7.73 (4 H), 7.50 (2 H), 7.40 (1 H), 7.30-7.12 (6 H), 7.06 (1 H), 6.70 (1 H), 6.36 (1 H), 4.43 (1 H), 4.23 (1 H), 3.92 (1 H), 3.81 (1 H), 3.06-2.88 (2 H), 2.52 (1 H), 2.24 (1 H), 2.13-1.53 (9 H), 0.90 (3 H), 0.84 (3 H) ; ^{19}F NMR (DMSO-d_6): δ -74.62; MS ESI: 724.4 $[M+H]^+$

Example 67

N-((2*S*)-1-(((2*S*)-1-((1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide

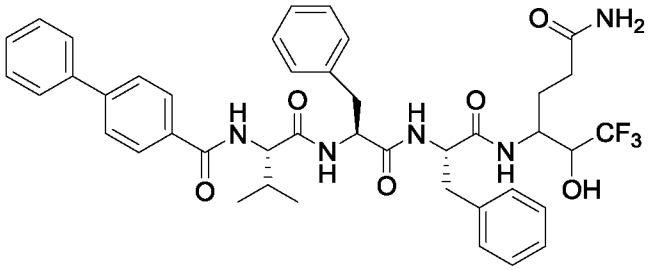


Purified by flash chromatography on silica gel eluting 0-4% MeOH in EtOAc.

^{1}H NMR (DMSO-d_6): δ 8.31-8.18 (2 H), 7.99-7.88 (3 H), 7.80-7.64 (4 H), 7.47 (2 H), 7.42-7.27 (2 H), 7.25-7.00 (6 H), 6.70 (1 H), 6.36 (1 H), 4.46 (1 H), 4.24 (1 H), 3.98-3.80 (2 H), 3.01 (1 H), 2.82 (1 H), 2.18-1.80 (4 H), 1.62 (1 H), 1.32-1.20 (6 H), 0.95-0.75 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.57, -74.67; MS ESI: 712.4 $[M+H]^+$

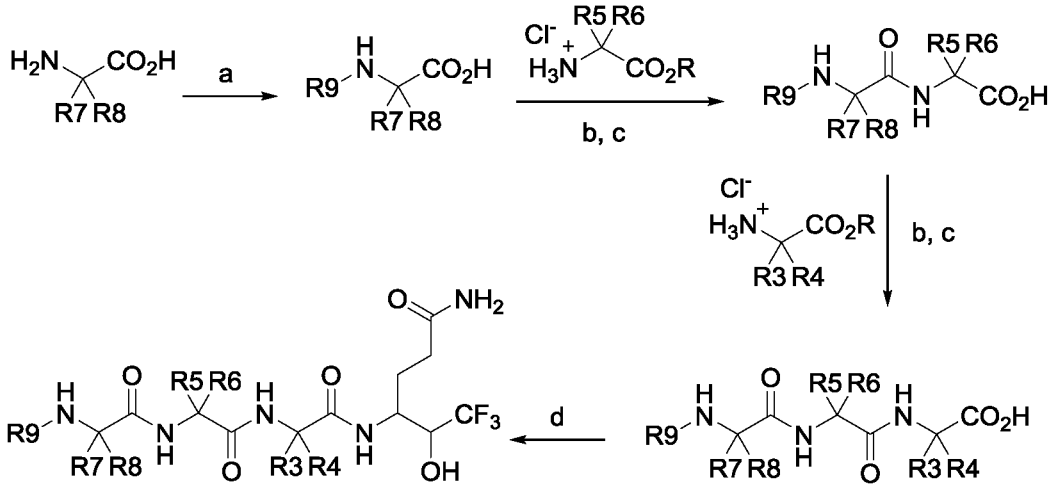
Example 68

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.27 (1 H), 8.13 (2 H), 8.00 (1 H), 7.94 (2 H), 7.76 (4 H), 7.50 (2 H), 7.42 (1 H), 7.28-7.02 (11 H), 6.72 (1 H), 6.55 (1 H), 4.62-4.46 (2 H), 4.23 (1 H), 3.99-3.77 (2 H), 3.05-2.88 (2 H), 2.86-2.63 (2 H), 2.13-1.74 (4 H), 1.65 (1 H), 0.83 (3 H), 0.74 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.69, -74.72; MS ESI: 774.4 $[M+H]^+$

Scheme 4[a]

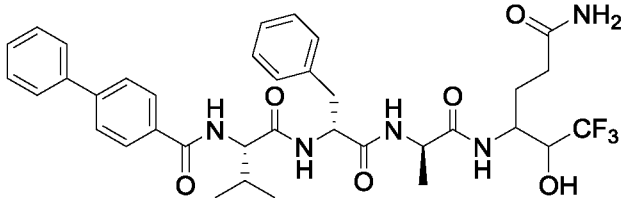


[a]Reagents and conditions: a) appropriate acyl chloride, NaOH, H_2O, THF, 0°C to rt; b) *N*-methylmorpholine, *i*-BuOCOCl, CH_2Cl_2, 0°C or HATU, DIPEA, DMF, 0°C to rt; c) LiOH, H_2O, THF, MeOH, 0°C to rt; d) intermediate 8, 9 or *ent*-9, HATU, DIPEA, DMF, -20°C to rt.

Compound examples prepared according to Scheme 4:

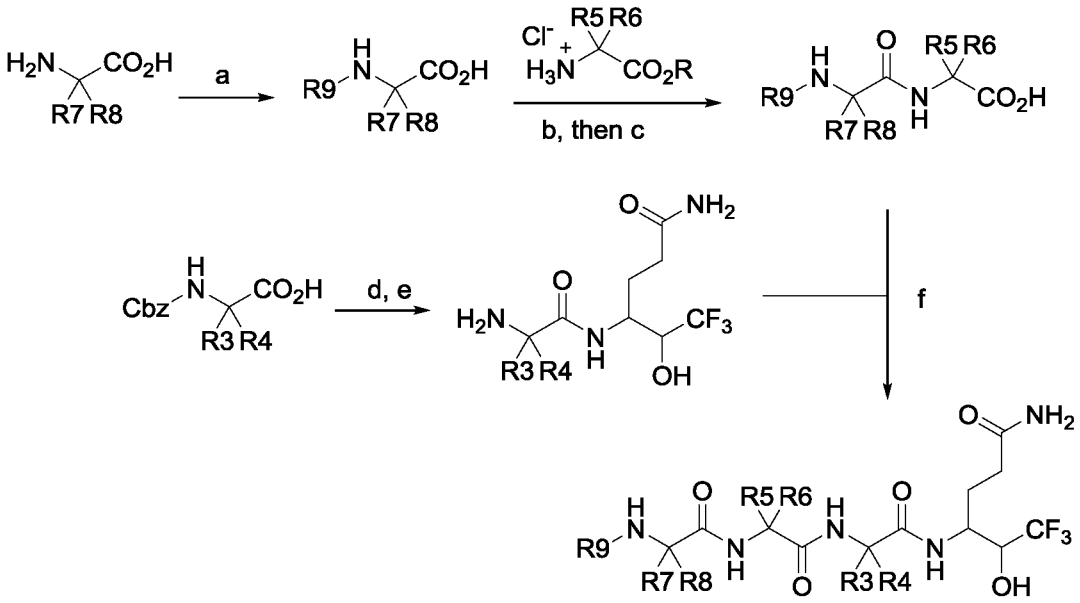
Example 69

N-((2*S*)-1-(((2*R*)-1-(((2*R*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.42 (1 H), 8.30 (1 H), 8.14 (1 H), 7.95 (2 H), 7.80 (0.5 H), 7.73 (4.5 H), 7.46 (2 H), 7.40 (1 H), 7.24 (2 H), 7.20-7.05 (4 H), 6.70 (1 H), 6.45 (1 H), 4.56 (1 H), 4.22 (1 H), 4.17 (1 H), 3.97-3.74 (2 H), 3.12 (1 H), 2.68 (1 H), 2.10-1.80 (4 H), 1.60 (1 H), 1.35-1.20 (3 H), 0.75 (3 H), 0.58 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.63, -74.75; MS ESI: 698.4 $[M+H]^+$

Scheme 5[a]

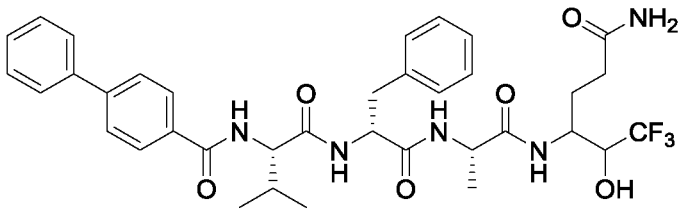


[a]Reagents and conditions: a) appropriate acyl chloride, NaOH, H_2O, THF, 0°C to rt; b) *N*-methylmorpholine, *i*-BuOCOCl, CH_2Cl_2, 0°C or HATU, DIPEA, DMF, 0°C to rt; c) LiOH, H_2O, THF, MeOH, 0°C to rt; d) intermediate 8, 9 or *ent*-9, HATU, DIPEA, DMF, -20°C to rt; e) H_2 (1 atm), 10% Pd/C, MeOH; f) HATU, DIPEA, DMF, 0°C to rt.

Compound examples prepared according to Scheme 5:

Example 70

N-((2*S*)-1-(((2*R*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



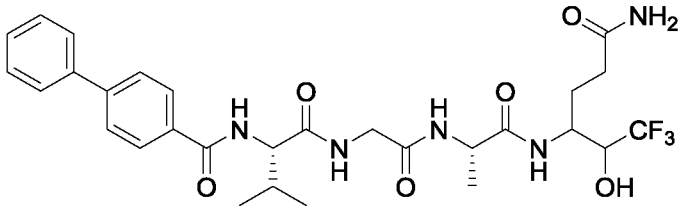
Isolated as a 4:1 mixture of diastereomers.

^{1}H NMR (DMSO-d_6): δ 8.48 (1 H), 8.36 (1 H), 8.05 (1 H), 7.97 (2 H), 7.88 (1 H), 7.76 (4 H), 7.50 (2 H), 7.40 (1 H), 7.23 (2 H), 7.20-7.06 (4 H), 6.70 (1 H), 6.53 (1 H), 4.52 (1 H), 4.22 (1 H), 4.17 (1 H), 3.96 (1 H), 3.83 (1 H), 3.05 (1

H), 2.76 (1 H), 2.18-1.82 (4 H), 1.61 (1 H), 1.18 (3 H), 0.78 (3 H), 0.57 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.63; MS ESI: 698.4 $[M+H]^+$

Example 71

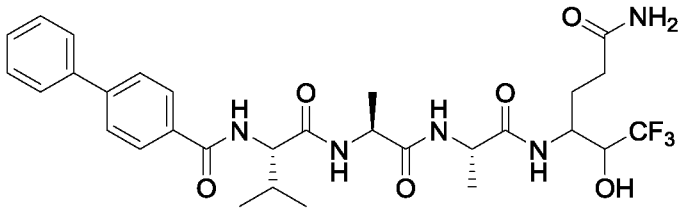
N-((2*S*)-1-((2-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.44-8.30 (2 H), 8.00 (2 H), 7.90 (2 H), 7.76 (4 H), 7.48 (2 H), 7.40 (1 H), 7.18 (1 H), 6.68 (1 H), 6.53 (1 H), 4.23 (2 H), 3.99-3.80 (2 H), 3.76 (2 H), 2.20-1.80 (4 H), 1.62 (1 H), 1.20 (3 H), 0.97 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64. -74.66; MS ESI: 608.4 $[M+H]^+$

Example 72

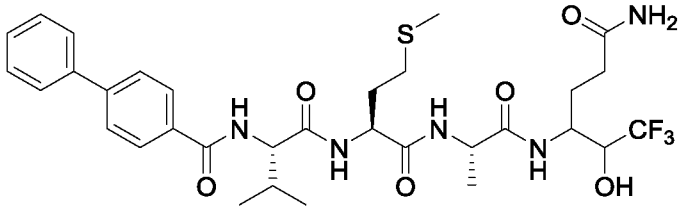
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.32 (1 H), 8.18 (1 H), 8.00-7.80 (4 H), 7.72 (4 H), 7.48 (2 H), 7.40 (1 H), 7.17 (1 H), 6.68 (1 H), 6.56 (1 H), 4.30 (2 H), 4.20 (1 H), 3.98-3.73 (2 H), 2.20-1.80 (4 H), 1.60 (1 H), 1.25-1.10 (6 H), 0.93 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.53, -74.54; MS ESI: 622.4 $[M+H]^+$

Example 73

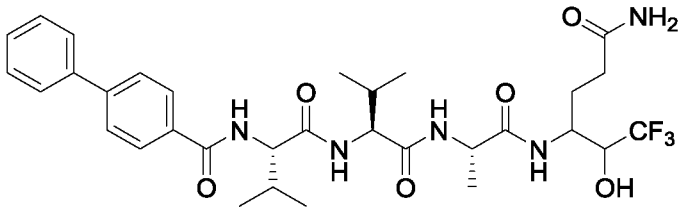
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-4-(methylthio)-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.39 (1 H), 8.16 (1 H), 8.00-7.85 (4 H), 7.75 (4 H), 7.47 (2 H), 7.40 (1 H), 7.19 (0.5 H), 7.11 (0.5 H), 6.70 (1 H), 4.38 (1 H), 4.30-4.14 (2 H), 3.96-3.76 (2 H), 2.42 (2 H), 2.20-1.72 (6 H), 2.00 (3 H), 1.60 (1 H), 1.20 (3 H), 0.94 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.63, -74.69; MS ESI: 682.4 $[M+H]^+$

Example 74

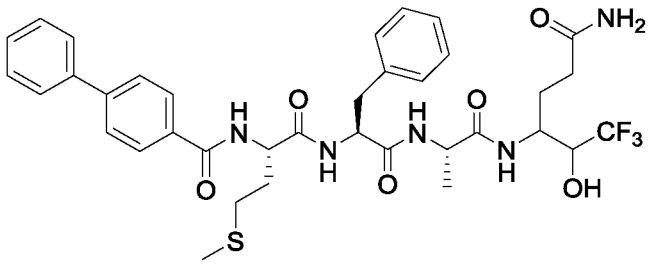
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.40 (1 H), 8.03-7.80 (5 H), 7.74 (4 H), 7.46 (2 H), 7.40 (1 H), 7.20 (0.5 H), 7.06 (0.5 H), 6.65 (1 H), 6.47 (1 H), 4.33 (1 H), 4.30-4.10 (2 H), 3.95-3.72 (2 H), 2.21-1.79 (5 H), 1.60 (1 H), 1.23-1.10 (3 H), 0.92 (6 H), 0.83 (6 H) ; ^{19}F NMR (DMSO-d_6): δ -74.66, -74.70; MS ESI: 650.4 $[M+H]^+$

Example 75

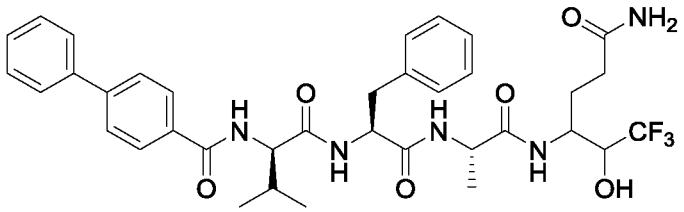
N-((5*S*,8*S*,11*S*)-17-amino-8-benzyl-11-methyl-6,9,12,17-tetraoxo-14-(2,2,2-trifluoro-1-hydroxyethyl)-2-thia-7,10,13-triazaheptadecan-5-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.54 (1 H), 8.03 (2 H), 7.98 (2 H), 7.89 (1 H), 7.75 (4 H), 7.48 (2 H), 7.40 (1 H), 7.23-7.05 (6 H), 6.70 (1 H), 6.48 (1 H), 4.58 (1 H), 4.53 (1 H), 4.20 (1 H), 4.00-3.76 (2 H), 3.03 (1 H), 2.80 (1 H), 2.40 (2 H), 2.15-1.80 (5 H), 2.02 (3 H), 1.62 (1 H), 1.21 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.66; MS ESI: 730.4 $[M+H]^+$

Example 76

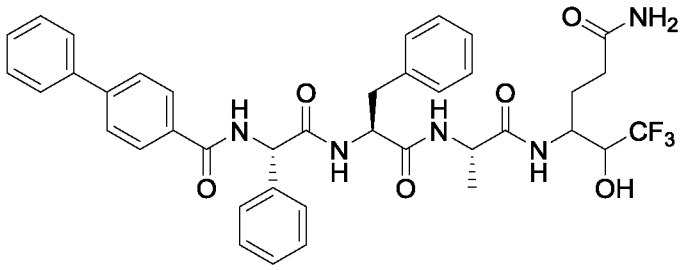
N-((2*R*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.44 (1 H), 8.31 (1 H), 8.17 (1 H), 7.95 (2 H), 7.87 (0.5 H), 7.80 (0.5 H), 7.77 (4 H), 7.44 (2 H), 7.40 (1 H), 7.24 (2 H), 7.20-7.07 (4 H), 6.70 (1 H), 4.57 (1 H), 4.22 (1 H), 4.18 (1 H), 3.96-3.75 (2 H), 3.13 (1 H), 2.73 (1 H), 2.10-1.80 (4 H), 1.60 (1 H), 1.25 (3 H), 0.77 (3 H), 0.58 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.63, -74.75; MS ESI: 698.4 $[M+H]^+$

Example 77

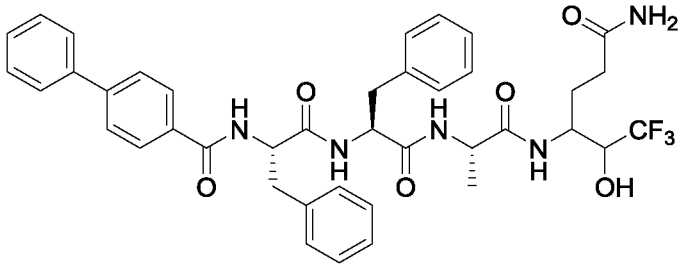
N-((1*S*)-2-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxo-1-phenylethyl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.81 (1 H), 8.41 (1 H), 8.10-7.92 (3 H), 7.86 (1 H), 7.75 (4 H), 7.50 (2 H), 7.40 (1 H), 7.38-7.00 (11 H), 6.70 (1 H), 6.48 (1 H), 5.63 (1 H), 4.60 (1 H), 4.20 (1 H), 3.98-3.78 (2 H), 3.09 (1 H), 2.82 (1 H), 2.17-1.80 (3 H), 1.61 (1 H), 1.20 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.67; MS ESI: 732.4 $[M+H]^+$

Example 78

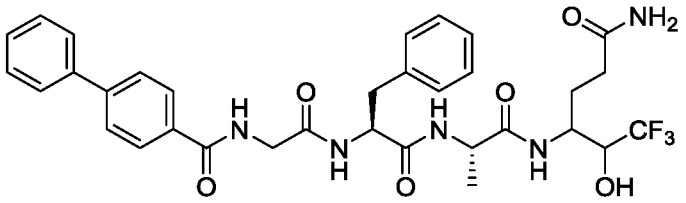
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.60 (1 H), 8.17 (2 H), 7.90 (1 H), 7.84 (2 H), 7.72 (4 H), 7.50 (2 H), 7.40 (1 H), 7.33-7.09 (11 H), 6.70 (1 H), 6.49 (1 H), 4.63 (1 H), 4.58 (1 H), 4.22 (1 H), 4.00-3.78 (2 H), 3.03 (2 H), 2.95 (1 H), 2.82 (1 H), 2.18-1.80 (3 H), 1.61 (1 H), 1.21 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.65; MS ESI: 746.4 $[M+H]^+$

Example 79

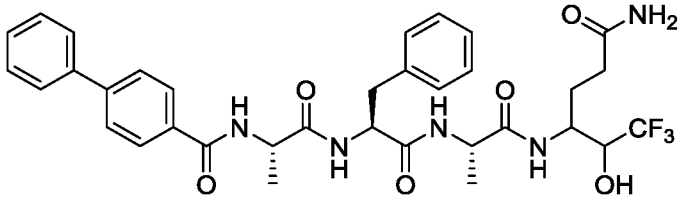
N-(2-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.79 (1 H), 8.21-8.12 (2 H), 7.94 (2 H), 7.87-7.63 (5 H), 7.45 (2 H), 7.40 (1 H), 7.26-7.07 (6 H), 6.70 (1 H), 6.47 (1 H), 4.57 (1 H), 4.21 (1 H), 4.00-3.70 (3 H), 3.02 (1 H), 2.80 (1 H), 2.15-1.80 (4 H), 1.62 (1 H), 1.21 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.68; MS ESI: 656.4 $[M+H]^+$

Example 80

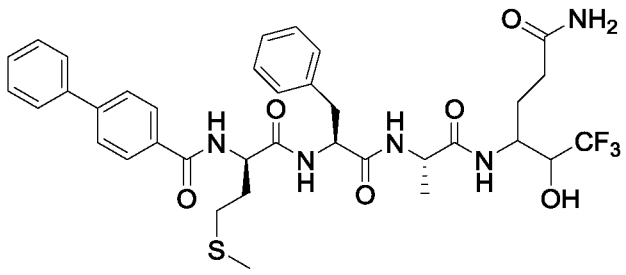
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.57 (1 H), 8.03-7.95 (4 H), 7.82 (1 H), 7.77 (4 H), 7.50 (2 H), 7.40 (1 H), 7.22-7.05 (6 H), 6.70 (1 H), 6.50 (1 H), 4.50 (1 H), 4.40 (1 H), 4.20 (1 H), 4.00-3.77 (2 H), 3.02 (1 H), 2.81 (1 H), 2.16-1.80 (3 H), 1.61 (1 H), 2.22 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.65, -74.68; MS ESI: 670.4 $[M+H]^+$

Example 81

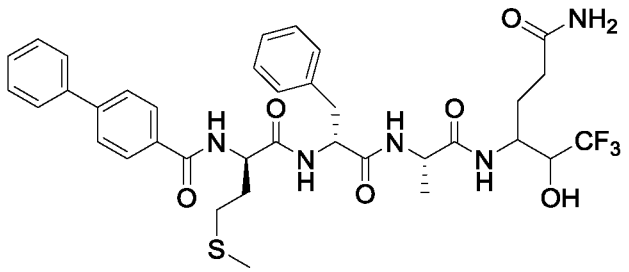
N-((5*R*,8*S*,11*S*)-17-amino-8-benzyl-11-methyl-6,9,12,17-tetraoxo-14-(2,2,2-trifluoro-1-hydroxyethyl)-2-thia-7,10,13-triazaheptadecan-5-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.52 (1 H), 8.35 (1 H), 8.17 (1 H), 7.96 (2 H), 7.90 (0.5 H), 7.72 (4.5 H), 7.48 (2 H), 7.40 (1 H), 7.24-7.04 (6 H), 6.70 (1 H), 6.50 (1 H), 4.59 (1 H), 4.51 (1 H), 4.21 (1 H), 3.98-3.78 (2 H), 3.10 (1 H), 2.73 (1 H), 2.38-2.15 (2 H), 2.12-1.80 (3 H), 2,00 (3 H), 1.80-1.48 (3 H), 1.24 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.72; MS ESI: 730.4 $[M+H]^+$

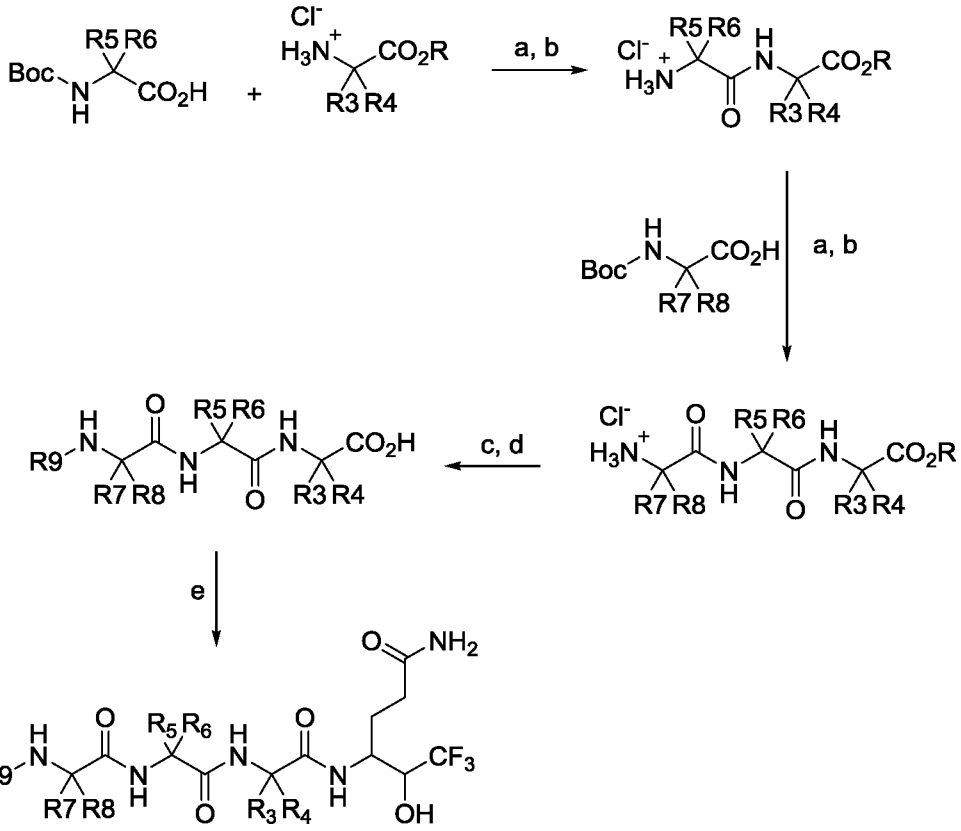
Example 82

N-((5*R*,8*R*,11*S*)-17-Amino-8-benzyl-11-methyl-6,9,12,17-tetraoxo-14-(2,2,2-trifluoro-1-hydroxyethyl)-2-thia-7,10,13-triazaheptadecan-5-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.60 (0.5 H), 8.56 (0.5 H), 8.18-8.05 (2 H), 7.98 (2 H), 7.85 (0.5 H), 7.75 (4.5 H), 7.48 (2 H), 7.40 (1 H), 7.23-7.11 (6 H), 6.77 (0.5 H), 6.67 (0.5 H), 6.45 (1 H), 4.50 (2 H), 4.20 (1 H), 3.99-3.78 (2 H), 2.98 (1 H), 2.84 (1 H), 2.44 (2 H), 2.15-1.52 (6 H), 2.00 (3 H), 1.11 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.65; MS ESI: 730.3 $[M+H]^+$

Scheme 6[a]

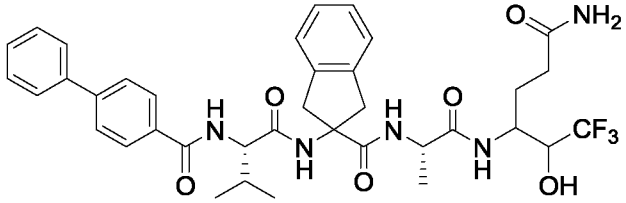


[a]Reagents and conditions: a) HATU, DIPEA, DMF, -20°C to rt; b) 4 M HCl in dioxane, CH_2Cl_2, 0°C to rt; c) appropriate acyl or sulfonyl chloride, Et_3N, THF, 0°C to rt, or appropriate carboxylic acid, HATU, DIPEA, DMF, 0°C to rt; d) LiOH, H_2O, THF, MeOH, 0°C to rt; e) intermediate 8, 9 or *ent*-9, HATU, DIPEA, DMF, -20°C to rt.

Compound examples prepared according to Scheme 6:

Example 83

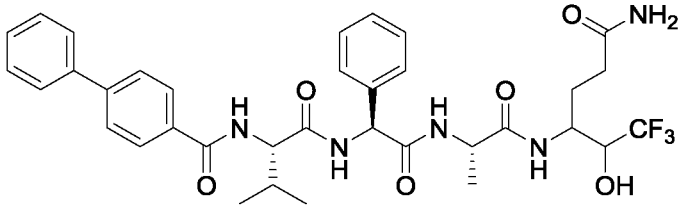
2-((*S*)-2-([1,1'-Biphenyl]-4-carboxamido)-3-methylbutanamido)-*N*-((2*S*)-1-((6-amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)-2,3-dihydro-1*H*-indene-2-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.72 (1 H), 8.48 (1 H), 7.99 (2 H), 7.75 (5 H), 7.50 (3 H), 7.40 (1 H), 7.22-7.00 (5 H), 6.70 (0.5 H), 6.63 (0.5 H), 6.45 (1 H), 4.16 (1 H), 4.01 (1 H), 3.98-3.73 (2 H), 3.58 (1 H), 3.39 (1 H), 3.24 (2 H), 2.18-1.80 (4 H), 1.62 (1 H), 1.18 (3 H), 0.94 (3 H), 0.77 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.60, -74.66; MS ESI: 710.4 $[M+H]^+$

Example 84

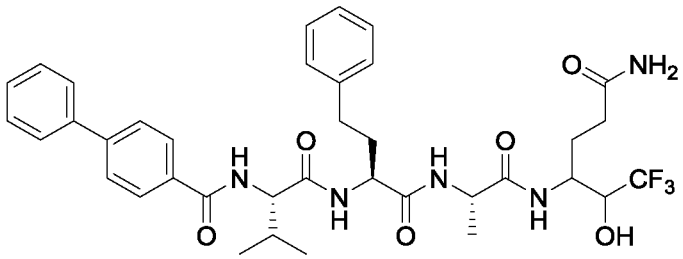
N-((2*S*)-1-(((1*S*)-2-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-2-oxo-1-phenylethyl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.42 (3 H), 7.96 (2 H), 7.85 (0.5 H), 7.76 (4.5 H), 7.50 (2 H), 7.40 (3 H), 7.36-7.20 (3 H), 7.15 (0.5 H), 7.04 (0.5 H), 6.69 (0.5 H), 6.63 (0.5 H), 6.48 (1 H), 5.58 (1 H), 4.40 (1 H), 4.22 (1 H), 3.85 (1 H), 3.75 (1 H), 2.17 (1 H), 2.05-1.75 (3 H), 1.57 (1 H), 1.20 (3 H), 0.90 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.67, -74.70; MS ESI: 684.3 $[M+H]^+$

Example 85

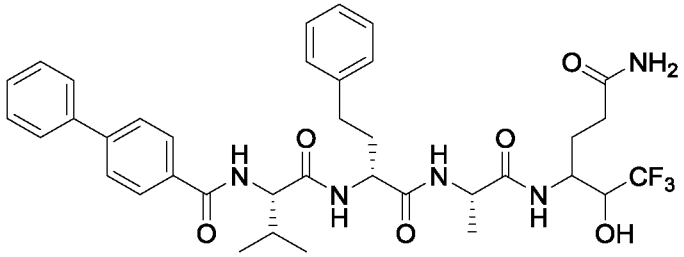
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-4-phenylbutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.60 (1 H), 8.22 (1 H), 8.03-7.86 (4 H), 7.76 (4 H), 7.50 (2 H), 7.40 (1 H), 7.28-7.07 (6 H), 6.70 (1 H), 6.60 (1 H), 4.40-4.15 (3 H), 4.06-3.72 (2 H), 2.60 (2 H), 2.20 (1 H), 2.15-1.76 (5 H), 1.61 (1 H), 1.20 (3 H), 0.97 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.71; MS ESI: 712.4 $[M+H]^+$

Example 86

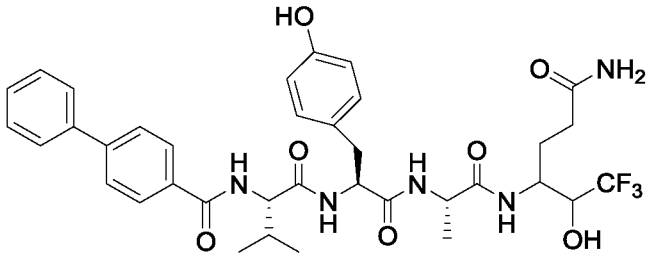
N-((2*S*)-1-(((2*R*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-4-phenylbutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.57-8.42 (2 H), 8.00 (2 H), 7.93 (1 H), 7.84 (1 H), 7.74 (4 H), 7.49 (2 H), 7.40 (1 H), 7.25 (2 H), 7.16 (4 H), 6.70 (1 H), 6.48 (1 H), 4.22 (3 H), 3.98-3.75 (2 H), 2.61 (1 H), 2.51 (1 H), 2.21-1.72 (6 H), 1.61 (1 H), 1.20 (3 H), 1.00 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.63, -74.65; MS ESI: 712.4 $[M+H]^+$

Example 87

N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide

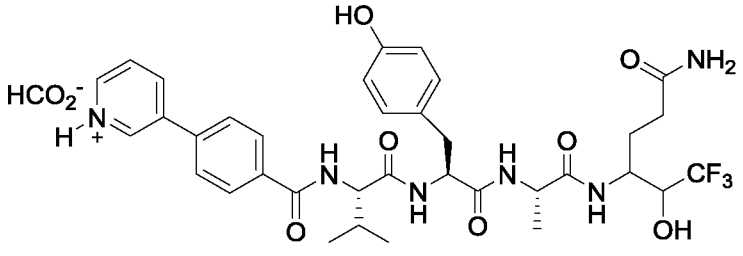


Purified by RP-HPLC (stationary phase: Phenomenex C12 Synergi 4u Max-RP; mobile phase A: 0.1% formic acid in H_2O; mobile phase B: 0.1% formic acid in CH_3CN; gradient 18-58 % B in A; run time = 20 min.

^{1}H NMR (DMSO-d_6): δ 9.12 (1 H), 8.29 (1 H), 8.06-7.82 (5 H), 7.75 (4 H), 7.50 (2 H), 7.40 (1 H), 7.20 (1 H), 7.00 (2 H), 6.69 (1 H), 6.62-6.44 (3 H), 4.50 (1 H), 4.20 (2 H), 4.00-3.78 (2 H), 2.92 (1 H), 2.67 (1 H), 2.18-1.80 (4 H), 1.62 (1 H), 1.20 (3 H), 0.80 (6 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.69; MS ESI: 714.4 $[M+H]^+$

Example 88

3-(4-(((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamoyl)phenyl)pyridin-1-ium formate

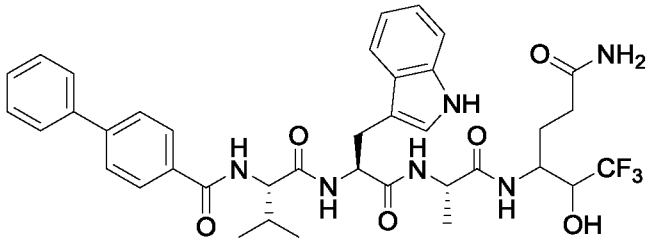


Purified by RP-HPLC (stationary phase: Phenomenex C12 Synergi 4u Max-RP 8A 50x20 mm; mobile phase A: 0.1% formic acid in H_2O; mobile phase B: 0.1% formic acid in CH_3CN; gradient 12-52 % B in A; run time = 20 min.

^1H NMR (DMSO-d_6): δ 9.20 (1 H), 8.94 (1 H), 8.60 (1 H), 8.50 (1 H), 8.38 (1 H), 8.14 (1 H), 8.08-7.87 (5 H), 7.83 (2 H), 7.51 (1 H), 7.19 (0.5 H), 7.15 (0.5 H), 7.00 (2 H), 6.70 (1 H), 6.62 (1 H), 6.66 (2 H), 4.48 (1 H), 4.20 (2 H), 3.96-3.74 (2 H), 2.91 (1 H), 2.57 (1 H), 2.11-1.80 (4 H), 1.61 (1 H), 1.19 (3 H), 0.85 (3 H), 0.77 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.64, -74.70; MS ESI: 715.4 $[M+H]^+$

Example 89

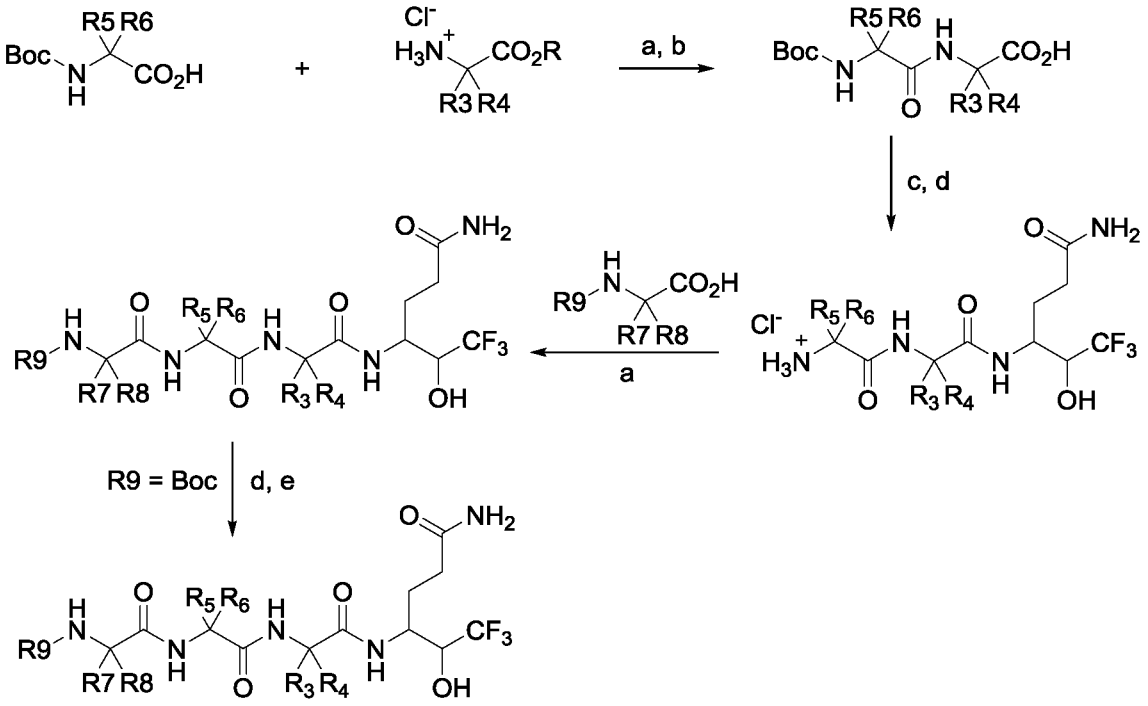
N-((2*S*)-1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-[1,1'-biphenyl]-4-carboxamide



^1H NMR (DMSO-d_6): δ 10.79 (1 H), 8.28 (1 H), 8.13 (1 H), 8.05-7.80 (4 H), 7.75 (4 H), 7.58 (1 H), 7.50 (2 H), 7.41 (1 H), 7.30 (1 H), 7.18 (2 H), 7.04 (1 H), 6.95 (1 H), 6.73 (1 H), 6.50 (1 H), 4.61 (1 H), 4.30-4.15 (2 H), 3.99-3.77 (2 H), 3.14 (1 H), 2.98 (1 H), 2.15-1.80 (4 H), 1.65 (1 H), 1.30-1.10 (3 H), 0.87 (3 H), 0.80 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.65; MS ESI: 737.4 $[M+H]^+$

Purified by RP-HPLC (stationary phase: Phenomenex C12 Synergi 4u Max-RP 8A 50x20 mm; mobile phase A: 0.1% formic acid in H_2O; mobile phase B: 0.1% formic acid in CH_3CN; gradient 20-60 % B in A; run time = 20 min.

Scheme 7[a]

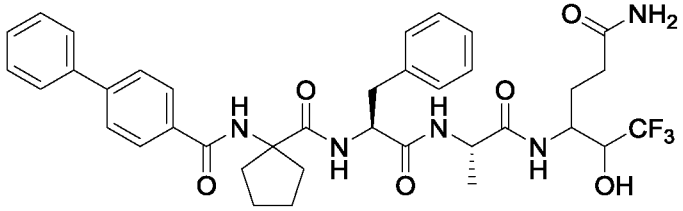


[a]Reagents and conditions: a) HATU, DIPEA, DMF, -20°C to rt; b) LiOH, H_2O, THF, MeOH, 0°C to rt; c) intermediate 8, 9 or *ent*-9, HATU, DIPEA, DMF, 0°C to rt; d) 4 M HCl in dioxane, CH_2Cl_2, 0°C to rt; e) appropriate acyl or sulfonyl chloride, Et_3N, THF, 0°C to rt, or appropriate carboxylic acid, HATU, DIPEA, DMF, 0°C to rt.

Compound examples prepared according to Scheme 7:

Example 90

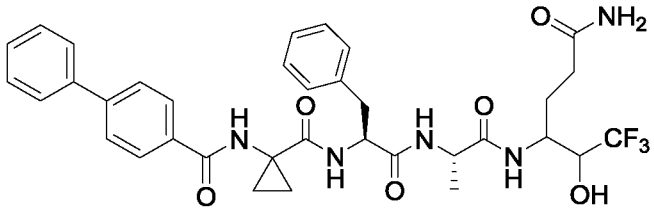
N-(1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)cyclopentyl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 8.66 (0.5 H), 8.61 (0.5 H), 8.00 (2 H), 7.86-7.60 (6 H), 7.58-7.36 (4 H), 7.13 (6 H), 6.68 (1 H), 6.45 (1 H), 4.44 (1 H), 4.15 (1 H), 3.99-3.78 (2 H), 3.10 (1 H), 2.87 (1 H), 2.25 (1 H), 2.17-1.74 (5 H), 1.73-1.40 (6 H), 1.23 (3 H); ^{19}F NMR (DMSO-d_6): δ -74.62, -74.67; MS ESI: 710.4 $[M+H]^+$

Example 91

N-(1-(((2*S*)-1-(((2*S*)-1-((6-Amino-1,1,1-trifluoro-2-hydroxy-6-oxohexan-3-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)cyclopropyl)-[1,1'-biphenyl]-4-carboxamide



^{1}H NMR (DMSO-d_6): δ 9.10 (1 H), 8.02-7.90 (3 H), 7.85-7.66 (6 H), 7.50 (2 H), 7.40 (1 H), 7.12 (6 H), 6.70 (1 H), 6.50 (1 H), 4.53 (1 H), 4.20 (1 H), 4.00-3.78 (2 H), 3.02 (1 H), 2.90 (1 H), 2.18-1.79 (3 H), 1.61 (1 H), 1.40-1.15 (5 H), 1.03 (1 H), 0.93 (1 H); ^{19}F NMR (DMSO-d_6): δ -74.63, -74.71; MS ESI: 682.3 $[M+H]^+$.

Compounds of the invention were then screened using biological PCSK9-based assays.

Example 92

Initial screening

HepG2 cells stably expressing WT PCSK9(+V5) were incubated overnight (24 hours) in the absence (DMSO) or presence of 10 µM, 100 µM or increasing concentrations (11, 33, 100 µM) of a tested compound. 24 hours-conditioned media was collected, centrifuged, and the supernatant removed for PCSK9 quantification by ELISA (100 µl of 1:30 dilution). Cells were lysed with 250 ul/well of radioimmunoprecipitation assay buffer (RIPA) (50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 1% Nonident P-40, 0.5% sodium deoxycholate, 0.1% SDS) containing a mixture of protease inhibitors (Roche Applied Science), and pelleted at 11,300 x g for 5 minutes. Supernatant was removed for cellular PCSK9 quantification by ELISA (100 µl of 1:20 dilution) and total protein determination by the Bio-Rad DC Protein assay (Bio-Rad) (Dubuc G, Tremblay M, Paré G, Jacques H, Hamelin J, Benjannet S, Boulet L, Genest J, Bernier L, Seidah NG, Davignon J. 2010. A new method for measurement of total plasma PCSK9: clinical applications. J Lipid Res. 51:140-149). Over 165 compounds were subjected to a first screen at 100 µM by PCSK9 secretion assay.

Certain compounds identified to reduce the PCSK9-media/cell ratio by at least 30% versus DMSO control (30% inhibition) in this assay were further tested for their ability to inhibit PCSK9 secretion in a dose dependent manner. At least 24 compounds were identified to inhibit PCSK9 secretion in a dose-dependent fashion (compounds described in examples 1 to 24).

Figure 1 and Table I present a selection of the tested compounds. **Figure 2** illustrate the dose-dependent inhibition of PCSK9 secretion in HepG2 cells stably expressing PCSK9(+V5) for a selection of the tested compounds, namely 19, 22a and 23, which reduce the PCSK9-media/cell between 30% and 50% versus DMSO control, at a dose of 33.3 µM.

Table I. PCSK9 secretion for selected compounds tested at a concentration of 10 µM

Example #	Secretion Inhibition (% Activity)[a]
24	88
29	83
52	79
58	81
59	82
60	73
66	92
70	74
75	100

The % activity was calculated using the following formula:

$$\%\ \text{Activity} = \left\{ 1 - \frac{([PCSK9]_{media}/[PCSK9]_{cells})_{inhibitor}}{([PCSK9]_{media}/[PCSK9]_{cells})_{DMSO}} \right\} \times 100\ \%$$

Example 93

Cytotoxicity Assays

Following the initial screening, the cytotoxicity of compounds was tested using either a MTT toxicity assay (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) according to manufacturer's protocol (Promega) (for see Figure 3) or using a cell viability assay (See Table II).

For the MTT toxicity assay, HepG2 stable cells over-expressing WT PCSK9(+V5) were seeded in 96-well plates (Greiner BioOne) and incubated for 20 hours. Following a brief wash, cells were incubated overnight (24 hours) in the presence of increasing concentrations (11, 33, 100 µM) of compounds or in the presence of DMSO (control). The MTT assay (Promega) consisted of addition of 20 µl/well MTT reagent and incubation for 45 minutes at 37 °C, following the manufacturer's instructions. Absorbance was recorded at 490 nm and corrected for background due to nonspecific absorbance (690 nm). Compounds with a low toxicity at 33.3 µM or less were selected as exemplified in Figure 3 for compounds 19, 22a, 23, 4 and 17.

For the cell viability assay, the cell density of HepG2 cells was measured in the presence of 33 µM of compounds or in the presence of DMSO (control) during the PCSK9 secretion assay described in Example 92. Table II below presents compounds with a low toxicity at 33.3 µM or less.

Table II : Cell viability in the presence of selected compounds at a concentration of 33 µM presented as a percentage of that obtained in the absence thereof (DMSO)

Example #	Cell viability (% DMSO Cnt)
59	83
58	93
29	88
60	80

The activity of selected PCSK9 inhibitory compounds on PCSK9 was further characterized, using a variety of assays including Western blot analyses and Biosynthetic analysis for the effect on PCSK9 autoprocessing and secretion. The effect on LDLR degradation and activity of the selected PCSK9 inhibitory compounds was characterized using a series of assays, including immunofluorescence assay, Dil-LDL uptake assay and FACS analysis for cell-surface LDLR, and Western blot analyses for total LDLR.

Example 94

Immunofluorescence assay

The PCSK9 inhibitory compounds were characterized by immunofluorescence for cell-surface LDLR and for human transferrin receptor, as control, in HepG2 naïve cells. Cells were plated on Poly-L-Lysine-coated (50 ug/ml) round microscope cover slips 1.12 mm thickness (Fisherbrand 12CIR #1) that were placed in a 24-well cell culture plate. At 24 h following seeding, cells were incubated in the absence (DMSO) or in the presence of 33.3 µM of PCSK9 inhibitor. After a 20 hour incubation period, the cells were fixed with 3.7% paraformaldehyde. Immunofluorescence of human LDLR (green labeling) was performed under non-permeabilizing conditions. Cells were blocked with 1% BSA, followed by overnight incubation at 4°C with primary antibody (1:200 goat polyclonal anti-hLDLR in 1% BSA, R&D Systems). Antigen-antibody complexes were revealed by 1-hour incubation at room temperature with Alexa fluor-tagged secondary antibody and mounted in ProLong Gold Antifade Reagent (Molecular Probes, Invitrogen). Immunofluorescence analyses were performed with a confocal miscroscope (Zeiss LSM-710). Cell nuclei were stained with DAPI (blue labeling). LDLR was stained with anti-LDLR Abs (green labeling). Inhibition of PCSK9 activity on LDLR degradation was detected by an increase of LDLR at the cell surface, as exemplified in **Figure 4** for compound 19.

Example 95

Dil-LDL assay

The PCSK9 inhibitory compounds were also characterized using a Dil-LDL fluorescent uptake assay as described in Poirier *et al.*, *J. Biol. Chem.* **284**: 28856-28864, 2009. The method entails the fluorescence measurement of the Dil-LDL cellular incorporation via LDLR internalization (a measurement of cell surface LDLR activity) in human hepatocyte derived HuH7 or HepG2 cell lines, or human embryonic HEK293 cells, in the presence or absence of compounds.

HepG2 naïve cells and HEK293 naïve cells were plated in 96-well plates (CellBind™ black plate with clear bottom (Corning; Cat # 3340)). At 20h post-seeding, cells were incubated in the absence (DMSO or negative control) or in the presence of different concentrations of compounds. Each condition was prepared in triplicates. After 6 hours incubation, Dil-LDL (Biomedical Technologies (Cat #BT-904)) was added to the cell media, and cells returned to tissue culture incubator for another 18 hours. Plates were scanned (bottom read) on a SpectraMax GeminiEM™ plate reader (Molecular Devices). For each well, raw Dil-LDL uptake was measured as the average fluorescence intensity (RFU) (ex: 520 nm/ em: 575 nm, cutoff: 550 nm) of 9 readings in 3 different points in the well. Dil-LDL uptake in each well was corrected for total number of cells by performing a CyQuant™ cell assay (Invitrogen, Cat #C7026) according to the manufacturer's instructions. Corrected Dil-LDL uptake is reported as a % of DMSO control and was obtained from triplicate wells. The inhibition of the PCSK9 activity on LDLR degradation is detected by an increase in the Dil-LDL uptake (exemplified in **Figure 5A** for compounds 19, 22a, 23, 24, 21a, 21b in HepG2 naïve cells). Compound i was selected as a negative control and compounds 1, 17 and 2 display no measurable activity in this assay in HepG2 cells. The specificity of the compounds for PCSK9 is exemplified in **Figure 5B**, which shows that in HEK293 cells, known to lack PCSK9 expression, the same set of compounds have no effect on Dil-LDL uptake.

Table III : Dil-LDL uptake in HepG2 cells in the presence of selected compounds at a concentration of 10 µM presented as a percentage of that obtained in the absence thereof (DMSO)

Example #	Dil-LDL uptake (% DMSO Cnt)
24	224 ± 33
29	207 ± 21
52	254 ± 71
58	261 ± 48
59	253 ± 75
60	224 ± 43
66	237 ± 59
70	224 ± 47
75	172 ± 48

In order to further test whether these compounds can also inhibit the function of a gain of function mutant D374Y of PCSK9, the cells expressing PCSK9-D374Y are analyzed by using a Dil-LDL fluorescent uptake assay. The inhibitory effect of compounds is also characterized using a liver-derived mouse cell line FL-83B (ATCC, CRL-2390) in the Dil-LDL uptake assay.

Example 96

Western blot

Compounds were tested for their ability to inhibit the LDLR enhanced degradation of PCSK9 in human hepatocyte cell lines HepG2. Cells were incubated for 24h in the absence or presence of tested PCSK9 inhibitory

compounds. Following incubation, cells were lysed in RIPA. Proteins were separated by SDS-polyacrylamide gel electrophoresis and blotted on polyvinylidene difluoride (PVDF, Perkin Elmer) membranes (GE Healthcare). Membranes were then incubated in the presence human LDLR antibody (1:1000, R&D Systems) and β-actin antibody (1:2500, Sigma). Appropriate horseradish peroxidase-conjugated secondary antibody (1:10,000, Sigma) was used for detection with enhanced chemiluminescence using the ECL plus kit (GE Healthcare). The inhibition of the PCSK9 function in these assays was detected by an increased ratio of total LDLR/β-Actin, as measured by Western blot analyses. Quantification of protein bands was obtained using Image J™ software. **Figure 5C** exemplifies the Western blot analysis of total LDLR in HepG2 naïve cells incubated for 24h in the absence (Cnt_0) or presence of increasing concentrations of compound 19. Total LDLR levels normalized to β-actin are plotted for each condition as % control (Cnt).

Example 97

FACS assay

The ability of PCSK9 inhibitory compounds to prevent the activity of PCSK9 on LDLR at the cell surface was also measured by using flow cytometry analyses as described in Benjannet S. *et al. J. Biol. Chem.* **285**: 40965-40978, 2010. The level of LDLR at the HepG2 cell surface was measured by using anti human LDLR antibody (1: 100, mAb-C7, Santa Cruz Biotechnology) and a secondary Alexa Fluor 647 donkey anti-mouse (Molecular Probes) antibody. Viable cells (PI-negative) were then analyzed by FACS for both PI and Alexa Fluor 647 using the FACS BD LSR (BD Biosciences). Cell surface LDLR was reported relative to control (untreated cells). The inhibition of the PCSK9 function in these assays was detected by an increased number of positive cells which correlated with an increased expression of LDLR at the cell surface.

More particularly, Human hepatoblastoma HepG2 cells (4 x 10^5 cells/well) were seeded in 12 well plates (Greiner BioOne) in complete media and cultured for 20 hours, at 37 °C, followed by a 1 hour wash in serum free media at 37 °C. Next, the wash media was removed and the cells were incubated overnight (24 hours) with 0.9 ml/well incubation media containing compounds at 10 uM or DMSO control (0.4% final). After one rinse with buffer A followed by another rinse with EDTA solution (2.5mM EDTA-2Na), the cells were detached by incubation in the presence of fresh EDTA solution (1 ml) at 37 °C for 20 minutes. All the steps following cell detachment were conducted on ice or 4 °C. To terminate the detachment, the cells were transferred in a 15 ml tube containing 3 ml of buffer A and centrifuged immediately for 5 minutes at 900 rpm (100 g) (4 °C). The cells were then resuspended in 500 ul buffer A together with 1:250 human LDLR antibody and incubated on ice for 10 minutes. Cells were washed once with 5 ml buffer A, centrifuged for 5 minutes, resuspended in 500 ul buffer A containing 1:500 Alexa Fluor 647 secondary antibody and incubated on ice for 5 min. After the staining, cells were washed once with 5 ml buffer A, centrifuged for 5 minutes and resuspended in 300 ul buffer A containing 1.67 ug/ml propidium iodide. A fixed number (2,000 cells) of viable cells (propidium iodide negative) were then analyzed by FACS for Alexa Fluor 647 (cell surface

LDLR) using a FACS-CyAn ADP flow cytometer (Beckman Coulter (DAKO)) and Summit software (Summit Software Inc.).

Materials:

Human HepG2 cell line: ATCC, HB-8065

Seeding media: complete media - EMEM (high glucose + sodium pyruvate) (Wisent) + 10% FBS

Wash media: serum free media - EMEM (high glucose + sodium pyruvate) (Wisent)

Incubation media with compounds: EMEM (high glucose + sodium pyruvate) (Wisent) + 0.07% BSA (Sigma-Aldrich)

Buffer A: 1X D-PBS without calcium and magnesium (Wisent 311-425-CL) + 0.5 %BSA (Sigma A7409-50ml) + 1 ug/L Glucose (Wisent 609-036-EL).

Primary antibody: human LDLR antibody, monoclonal Mouse IgG_1 clone #472413 (MAB2148, R&D Systems)

Secondary antibody: Alexa Fluor® 647 goat anti-mouse IgG (H+L) *2 mg/mL* (A21235, Molecular Probes)

Propidium iodide: 1mg/ml solution (P4864, Sigma)

Results are presented in Table IV.

Table IV - FACS-LDLR expressed as a percentage of LDLR positive cells in the presence and absence (DMSO) of selected compounds at a concentration of 10 µM

Example #	FACS-LDLR (% DMSO Cnt)
24	116 ± 5
29	233 ± 50
52	138 ± 20
58	166 ± 28
59	265 ± 35
60	138 ± 8
66	122 ± 11
70	118 ± 18
75	174 ± 41

The PCSK9 inhibitory compounds are also tested on mouse and human primary hepatocytes in order to measure their effect on cell surface LDLR. The advantage of using the mouse primary hepatocytes is that it allows for measurement of the specificity of the compound in the context of a wild type or knockout mouse expressing or lacking PCSK9, respectively.

Example 98

Characterisation of the inhibition of PCSK9-induced LDLR degradation in animal models

Candidate PCSK9 inhibitory compounds are then used in mice models expressing human PCSK9 to assess their ability in lowering plasma cholesterol levels *in vivo*.

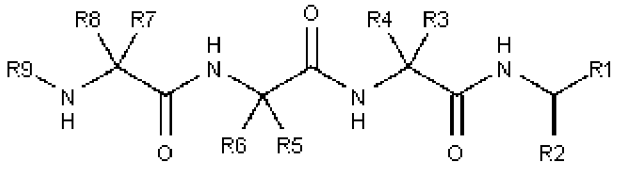
The compounds are tested in mice overexpressing human PCSK9. Transgenic lines (Herbert, B., *et al.* (2010). *Arteriosclerosis, Thrombosis, and Vascular Biology*, **30** (7):1333-1339) that carry a ~190 kb of human genomic DNA expressing human PCSK9 (WT, D374Y low or D374Y high) from its own promoter are constructed. Further crosses generate a mouse strain expressing the human PCSK9 transgene in a *Pcsk9*$^{-/-}$ *Ldlr*$^{+/-}$ background (*Ldlr* heterozygote). This eliminates interference through the endogenous expression of the mouse PCSK9 genes and increases their LDLc levels (**Figure 6**). By multiple backcrosses, these model mice are obtained in a pure C57BL/6 background for the homogeneity and reproducibility of the analyses. As control for specificity, the effect of the transgenes in an *Ldlr*$^{-/-}$ background is tested.

Mouse injections: The compounds are injected intravenously to WT, *Pcsk9*$^{-/-}$, *Ldlr*$^{-/-}$ and *Pcsk9*-Tg mice, in 6 mice/genotype (**Figure 6**). Total cholesterol (TC), LDLc and PCSK9 levels are measured every day during the first week and every third day for the next 2 weeks. The level of remaining uncomplexed PCSK9 in plasma is also measured by immunoprecipitation using a previously described antibody (Zaid, A., et al. (2008). *Hepatology*, **48**(2): 646-65). In another set of experiments, 6 mice at the time point of lowest LDLc (4-7 days post-injection) are sacrificed and their FPLC plasma lipid profiles, as well as liver LDLR protein, are analyzed. Any overt toxicity and/or morbidity effect is carefully monitored. The controls of *Pcsk9*$^{-/-}$, *Ldlr*$^{-/-}$ mice permit to verify that the effect observed is PCSK9-dependent.

Effect of statin + PCSK9 inhibitory compounds: The combination of atorvastatin and inhibitory compound is evaluated, as statins increase PCSK9 expression while lowering that of the LDLR (Dubuc, G., *et al.* (2004). Arteriosclerosis, Thrombosis, and Vascular Biology, **24**(8): 1454-1459; Lakoski, S.G., *et al.* (2009). *The Journal of Clinical Endocrinology and Metabolism*, **94**(7): 2537-2543), and it was shown that statins decrease even further LDLc of *Pcsk9*$^{-/-}$ mice (Rashid, S., *et al.* (2005). *Proc Natl Acad Sci USA* **102**(15):5374-5379).

CLAIMS:

1. Compound of Formula (I):



(I)

or a pharmaceutically acceptable salt, hydrate, solvate, or racemic mixture or stereoisomer thereof,

wherein:

R1 is $–CH(OH)R_a$ or $–B(OR_b)(OR_c)$;

R2 is –H, $–CH_2R_d$, $–CHR_d(R_e)$, $–CH_2(CH_2)_mC(O)R_j$, $–CH_2(CH_2)_mC(O)N(R_d)R_e$ or $–CH_2(CH_2)_mS(O)_nN(R_d)R_e$;

R3, R4, R5, R6, R7 and R8 are identical or different, and are independently hydrogen or one of the following groups: a C1-6 alkyl, a C1-6 haloalkyl, a C1-6 thioalkyl, a C1-6 aminoalkyl, an alkenyl, an alkynyl, a cycloalkyl, an heterocyclyl, an aryl, and an heteroaryl group, wherein said group is optionally substituted with one or more C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, an heteroaryl, –CN, $–C(O)N(R_f)R_g$, $–C(O)OR_f$, $–C(R_f)(R_g)OR_h$, $–OR_f$, $–OC(O)OR_f$, $–OC(O)NR_f(R_g)$, $–SR_f$, $–S(O)_nR_f$, $-S(O)_nN(R_f)R_g$, $–S(O)_nN(R_f)C(O)R_g$, $–N(R_f)R_g$, $–N(R_f)C(O)R_g$, $—N(R_f)C(O)OR_g$, $–N(R_f)C(O)N(R_g)(R_h)$, $–N(R_f)S(O)_nR_g$, and $–N(R_f)S(O)N(R_g)R_h$ substituents;

when (R3 and R4) or (R5 and R6) or (R7 and R8) are not hydrogen, the bracketed pairs can also be linked with $–C(O)–$, $–CO_2–$, $–C(O)N(H)–$, $–C(O)N(R_x)–$, –O–, –NH–, $–N(R_x)–$, –S–, $–S(O)_n–$, $–S(O)_nN(H)–$, $–S(O)_nN(R_x)–$ radicals to form cyclic structures;

R9 is $R_iC(O)–$, $R_iS(O)_n–$, $R_iOC(O)–$, $R_iNHC(O)–$, $R_iNHS(O)_n–$, $R_k(R_l)NC(O)–$, $R_l(R_l)NS(O)_n–$; $R_mOR_iC(O)–$, $R_mC(O)R_iC(O)–$ or one or more amino acids residues;

R_a is C1-3 alkyl, C1-2 fluoroalkyl or cyclopropyl;

R_b and R_c are identical or different, and are independently H or C1-6 alkyl, or can be connected together to form a cyclic 5- or 6-membered ring structure, or fused with additional aliphatic or aromatic ring systems, the cyclic 5- or 6-membered ring structure or aliphatic or aromatic ring systems being optionally substituted with one or more C1-6 alkyl and/or C1-6 haloalkyl substituents;

R_d and R_e are identical or different, and are independently H or one of the following groups: a C1-3 alkyl, a C1-3 haloalkyl or a C3-4 cycloalkyl group, or can be connected together directly or with $–C(O)–$, $–C(O)O–$, $–C(O)N(R_x)–$ –O–, $–N(R_x)–$, –S–, $–S(O)_n–$,or $–S(O)_nN(R_x)–$ radicals to form cyclic 3-8 membered ring structures;

R_f, R_g, R_h, R_k and R_l are identical or different, and are independently H or one of the following groups: a C1-6 alkyl, a C1-6 haloalkyl or a C3-4 cycloalkyl group, or can be connected together directly or with $–C(O)–$, $–C(O)O–$,$–C(O)N(R_x)–$ –O–, $–N(R_x)–$, –S–, $–S(O)_n–$ or $–S(O)_nN(R_x)–$ radicals to form cyclic 3-8 membered ring structures;

R_i is a C1-10 alkyl, a C1-10 heteroalkyl, a C3-8 cycloalkyl, a C1-10 haloalkyl, a heterocyclyl, an aryl, a heteroaryl, a C1-10 alkyl-C3-8 cycloalkyl, a C1-10 alkyl-heterocyclyl, a C1-10 alkyl-aryl, a C1-10 alkyl-heteroaryl, a C1-10 heteroalkyl-C3-8 cycloalkyl, a a C1-10 heteroalkyl-heterocyclyl, a C1-10 heteroalkyl-aryl or a C1-10 heteroalkyl-heteroaryl group, wherein the group is optionally substituted with one or more of a halogen, C1-6 aminoalkyl, C1-6 heteroalkyl, C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, heteroaryl and heterocyclyl groups;

R_m is a C1-10 alkyl, a C1-10 heteroalkyl, a C3-8 cycloalkyl, a C1-10 haloalkyl, a heterocyclyl, an aryl or a heteroaryl group, wherein the group is optionally substituted with one or more of a halogen, C1-6 aminoalkyl, C1-6 heteroalkyl, C1-6 alkyl, C3-8 cycloalkyl, C1-6 haloalkyl, aryl, heteroaryl and heterocyclyl groups;

R_j is OR_d or $N(R_d)(R_e)$;

R_x is H, C1-6 alkyl, C1-6 haloalkyl, C3-4 cycloalkyl, $-C(O)R_y$, $-C(O)OR_y$, $-C(O)NH_2$, $-C(O)NH(R_y)$ or $-C(O)NHS(O)_nR_y$;

R_y is C1-6 alkyl, C1-6 haloalkyl or C3-4 cycloalkyl;

m is an integer of value 0 or 1; and

n is an integer of value 1 or 2,

provided that:

1) when R1 is $-CH(OH)R_a$, R2 is $-CHR_d(R_e)$, $-CH_2(CH_2)_mC(O)R_j$, $-CH_2(CH_2)_mC(O)N(R_d)R_e$ or $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$; and
2) when R1 is $-B(OR_b)(OR_c)$, R2 is –H, $-CH_3$, $-CHR_d(R_e)$, $-CH_2(CH_2)_mC(O)R_j$, $-CH_2(CH_2)_mC(O)N(R_d)R_e$ or $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$.

2. The compound of claim 1, wherein:
   a. R1 is $-B(OR_b)(OR_c)$, or $-CH(OH)R_a$;
   b. R2 is H or $-CH_2(CH_2)_mC(O)R_j$;
   c. R3 is H or C1-6 alkyl substituted or not;
   d. R4 is H, C1-6 alkyl substituted or not, C1-6 thioalkyl substituted or not, aryl substituted or not or cycloalkyl substituted or not;
   e. R5 is H;
   f. R6 is H, C1-6 alkyl substituted or not, C1-6 thioalkyl substituted or not, aryl substituted or not, or cycloalkane substituted or not ;
   g. R7 is H;
   h. R8 is H, C1-6 alkyl substituted or not , C1-6 thioalkyl substituted or not, aryl substituted or not , or cycloalkyl substituted or not; and/or
   i. R9 is $RiOC(O)$-, $RiC(O)$-, $R_mOR_iC(O)$–, $R_mC(O)R_iC(O)$– or $R_iS(O)_n$–.

,

3. The compound of claim 1 or 2, wherein:

a. R1 is $-B(OR_b)(OR_c)$, or $-CH(OH)R_a$;
b. R2 is H or $-CH_2(CH_2)_mC(O)R_j$;
c. R3 is H or C1-6 alkyl substituted or not;
d. R4 is H, C1-6 alkyl substituted or not, C1-6 thioalkyl substituted or not, aryl substituted or not or cycloalkyl substituted or not;
e. R5 is H;
f. R6 is H, C1-6 alkyl substituted or not, C1-6 thioalkyl substituted or not, aryl substituted or not, or cycloalkane substituted or not;
g. R7 is H;
h. R8 is H, C1-6 alkyl substituted or not , C1-6 thioalkyl substituted or not, aryl substituted or not, or cycloalkyl substituted or not; and
i. R9 is $RiOC(O)-$, $RiC(O)-$, $R_mOR_iC(O)-$, $R_mC(O)R_iC(O)-$, or $R_iS(O)_n-$.

4. The compound of any one of claims 1 to 3, wherein:
   a. **R1** is $-B(OR_b)(OR_c)$.

5. The compound of claim 4, wherein:
   a. Rb and Rc are H.

6. The compound of claim 4, wherein:
   a. Rb and Rc are connected to form a cyclic 5 membered ring structure or fused with an aliphatic ring system.

7. The compound of claim 6, wherein:
   a. R1 is 2,9,9-trimethyl-3,5-dioxa-4-boratricyclo[6.1.1.$0^{2,6}$]decan-4-yl.

8. The compound of any one of claims 1 to 3, wherein:
   a. R1 is $-CH(OH)R_a$.

9. The compound of any one of claims 1 to 3 or 8, wherein:
   a. Ra is C1-2 fluoroalkyl.

10. The compound of claim 9, wherein:
    a. Ra is $-CF_3$.

11. The compound of claim 9, wherein:
    a. Ra is $-CH_2F$.

12. The compound of claim 8, wherein:
    a. Ra is C1-3 alkyl.

13. The compound of claim 12, wherein:
    a. Ra is $-CH_3$.

14. The compound of any one of claims 1 to 13, wherein:
    a. **R2** is H.

15. The compound of any one of claims 1 to 13, wherein:
    a. R2 is $-CH_2(CH_2)_mC(O)R_j$.

16. The compound of claim 15, wherein:
    a. Rj is OR_d.

17. The compound of claim 16, wherein:
    a. Rd is CH_3.

18. The compound of claim 15, wherein:
    a. Rj is NH_2.

19. The compound of any one of claims 1 to 18, wherein:
    a. R**3** is H.

20. The compound of any one of claims 1 to 18, wherein:
    a. R3 is C1-6 alkyl substituted or not.

21. The compound of claim 20, wherein:
    a. R3 is unsubstituted C1-6 alkyl.

22. The compound of claim 21, wherein:
    a. R3 is CH_3.

23. The compound of any one of claims 1 to 22, wherein:
    a. **R4** is H.

24. The compound of any one of claims 1 to 22, wherein:
    a. R4 is C1-6 alkyl substituted or not.

25. The compound of claim 24, wherein:
    a. R4 is unsubstituted C1-6 alkyl.

26. The compound of claim 25, wherein:
    a. R4 is $-CH_3$.

27. The compound of claim 25, wherein:
    a. R4 is CH_3CH_2-.

28. The compound of claim 25, wherein:
    a. R4 is $-CH_2CH(CH_3)_2$.

29. The compound of claim 24, wherein:
    a. R4 is substituted C1-6 alkyl.

30. The compound of claim 29, wherein:
    a. R4 is aryl substituted C1-6 alkyl.

31. The compound of claim 30, wherein:
    a. R4 is phenyl-CH_2-.

32. The compound of claim 29, wherein:
    a. R4 is -$(CH_2)_2C(O)NH_2$.

33. The compound of any one of claims 1 to 22, wherein:
    a. R4 is C1-6 thioalkyl substituted or not.

34. The compound of claim 33, wherein:
    a. R4 is $CH_3S(CH_2)_2$-.

35. The compound of any one of claims 1 to 22, wherein:
    a. R4 is aryl substituted or not.

36. The compound of claim 35, wherein:
    a. R4 is phenyl.

37. The compound of any one of claims 1 to 22, wherein:
    a. R4 is cycloalkyl.

38. The compound of any one of claims 1 to 37, wherein:
    a. **R6** is H.

39. The compound of any one of claims 1 to 37, wherein:
    a. R6 is C1-6 alkyl substituted or not.

40. The compound of claim 39, wherein:
    a. R6 is substituted C1-6 alkyl.

41. The compound of claim 40, wherein:
    a. R6 is aryl substituted C1-6 alkyl.

42. The compound of claim 41, wherein:
    a. R6 is –alkyl-phenyl.

43. The compound of claim 42, wherein:

a. R6 is $-CH_2$-phenyl.

44. The compound of claim 42 or 43, wherein:
    a. R6 is $-CH_2$-hydroxyphenyl.

45. The compound of claim 42, wherein:
    a. R6 is $-(CH_2)_2$-phenyl.

46. The compound of claim 41, wherein:
    a. R6 is $-CH_2$-indole.

47. The compound of claim 39, wherein:
    a. R6 is unsubstituted C1-6 alkyl.

48. The compound of claim 47, wherein:
    a. R6 is unsubstituted C1-6 alkyl.

49. The compound of claim 48, wherein:
    a. R6 is CH_3.

50. The compound of claim 48, wherein:
    a. R6 is $-CH(CH_3)_2$.

51. The compound of any one of claims 1 to 37, wherein:
    a. R6 is C1-6 thioalkyl substituted or not.

52. The compound of claim 51, wherein:
    a. R6 is $CH_3S(CH_2)_2$-.

53. The compound of any one of claims 1 to 37, wherein:
    a. R6 is aryl substituted or not.

54. The compound of claim 53, wherein:
    a. R6 is phenyl.

55. The compound of any one of claims 1 to 37, wherein:
    a. R6 is cycloalkyl substituted or not.

56. The compound of claim 55, wherein:
    a. R6 is benzocyclopentyl.

57. The compound of any one of claims 1 to 56, wherein:
    a. **R8** is H.

58. The compound of any one of claims 1 to 56, wherein:
    a. R8 is C1-6 alkyl substituted or not.

59. The compound of claim 58, wherein:
    a. R8 is unsubstituted C1-6 alkyl.

60. The compound of claim 59, wherein:
    a. R8 is $-CH(CH_3)_2$.

61. The compound of claim 59, wherein:
    a. R8 is $-CH_3$.

62. The compound of claim 58, wherein:
    a. R8 is substituted C1-6 alkyl.

63. The compound of claim 62, wherein:
    a. R8 is aryl substituted C1-6 alkyl.

64. The compound of claim 63, wherein:
    a. R8 is $–CH_2$-phenyl.

65. The compound of any one of claims 1 to 56, wherein:
    a. R8 is C1-6 thioalkyl substituted or not.

66. The compound of claim 65, wherein:
    a. R8 is $CH_3S(CH_2)_2$-.

67. The compound of any one of claims 1 to 56, wherein:
    a. R8 is aryl substituted or not.

68. The compound of claim 67, wherein:
    a. R8 is unsubstituted aryl.

69. The compound of claim 68, wherein:
    a. R8 is phenyl.

70. The compound of any one of claims 1 to 56, wherein:
    a. R8 is cycloalkyl substituted or not.

71. The compound of claim 70, wherein:
    a. R8 is unsubstituted cycloalkyl.

72. The compound of claim 71, wherein:
    a. R8 is cyclopentyl.

73. The compound of claim 71, wherein:
    a. R8 is cyclopropyl.

74. The compound of any one of claims 1 to 73, wherein:
    a. **R9** is RiOC(O)-.

75. The compound of claim 74, wherein:
    a. Ri is C1-10 alkyl, substituted or not.

76. The compound of claim 75, wherein:
    a. Ri is unsubstituted C1-10 alkyl-.

77. The compound of claim 76, wherein:
    a. Ri is CH_3-.

78. The compound of claim 75, wherein:
    a. Ri is substituted C1-10 alkyl-.

79. The compound of claim 78, wherein:
    a. Ri is phenyl-CH_2-.

80. The compound of any one of claims 1 to 73, wherein:
    a. R9 is RiC(O)-.

81. The compound of claim 80, wherein:
    a. Ri is aryl substituted or not or heteroaryl substituted or not.

82. The compound of claim 81, wherein:
    a. Ri is aryl substituted or not.

83. The compound of claim 82, wherein:
    a. Ri is substituted aryl.

84. The compound of claim 83, wherein:
    a. Ri is aryl-phenyl-.

85. The compound of claim 84, wherein:
    a. Ri is phenyl-phenyl-.

86. The compound of claim 84, wherein:
    a. Ri is heteroaryl-phenyl-.

87. The compound of claim 86, wherein:
    a. Ri is diazirine-phenyl-.

88. The compound of claim 87, wherein:
    a. Ri is trifluoromethyl-diazirin-phenyl-.

89. The compound of claim 86, wherein:
    a. Ri is pyridine-phenyl-.

90. The compound of claim 86, wherein:
    a. Ri is oxadiazole-phenyl-.

91. The compound of claim 83, wherein:
    a. Ri is heterocyclyl-phenyl-.

92. The compound of claim 91, wherein:
    a. Ri is morpholine-phenyl-.

93. The compound of claim 83, wherein:
    a. Ri is alkyl-phenyl-.

94. The compound of claim 93, wherein:
    a. Ri is $(CH_3)_2CH$-phenyl-.

95. The compound of claim 93, wherein:
    a. Ri is $OHCH_2$-phenyl-.

96. The compound of claim 83, wherein:
    a. Ri is fluorophenyl.

97. The compound of claim 82, wherein:
    a. Ri is unsubstituted aryl.

98. The compound of claim 97, wherein:
    a. Ri is phenyl.

99. The compound of claim 81, wherein:
    a. Ri is heteroaryl substituted or not.

100. The compound of claim 99, wherein:
    a. Ri is substituted heteroaryl.

101. The compound of claim 100, wherein:
    a. Ri is aryl–heteroaryl-.

102. The compound of claim 101, wherein:
   a. Ri is phenyl-heteroaryl-.

103. The compound of claim 102, wherein:
   a. Ri is phenyl-pyrazole-.

104. The compound of claim 102, wherein:
   a. Ri is phenyl-methylpyrazole-.

105. The compound of claim 102, wherein:
   a. Ri is phenyl-thiazole-.

106. The compound of claim 102, wherein:
   a. Ri is phenyl-pyridine-.

107. The compound of claim 102, wherein:
   a. Ri is phenyl-furazan-.

108. The compound of claim 100, wherein:
   a. Ri is heteroaryl-heteroaryl-.

109. The compound of claim 108, wherein:
   a. Ri is pyridine-isothiazole-.

110. The compound of claim 99, wherein:
   a. Ri is unsubstituted heteroaryl.

111. The compound of claim 110, wherein:
   a. Ri is pyridine.

112. The compound of claim 111, wherein:
   a. Ri is pyrazine.

113. The compound of claim 112, wherein:
   a. Ri is indole.

114. The compound of claim 80, wherein:
   a. Ri is C1-10 alkyl, substituted or not-.

115. The compound of claim 114, wherein:
   a. Ri is substituted C1-10 alkyl-.

116. The compound of claim 115, wherein:

a. Ri is aryl-C1-10 alkyl-.

117. The compound of claim 116, wherein:
a. Ri is phenyl-C1-10 alkyl-.

118. The compound of claim 117, wherein:
a. Ri is phenyl-alkyne-.

119. The compound of claim 117, wherein:
a. Ri is phenyl-CH_2-.

120. The compound of claim 119, wherein:
a. Ri is fluoromethoxy-phenyl-CH_2-.

121. The compound of claim 120, wherein:
a. Ri is trifluoromethoxy-phenyl-CH_2-.

122. The compound of claim 121, wherein:
a. Ri is 4-(trifluoromethoxy)phenyl-CH_2-.

123. The compound of claim 117, wherein:
a. Ri is fluoroalkyl-diazirin-phenyl-(C1-10 alkyl)-.

124. The compound of claim 123, wherein:
a. Ri is trifluoromethyl-diazirin-phenyl-CH_2-.

125. The compound of claim 124, wherein:
a. Ri is 4-[3-trifluoromethyl)-3*H*-diazirin-3-yl]phenyl-.

126. The compound of claim 125, wherein:
a. Ri is 3-[3-trifluoromethyl)-3*H*-diazirin-3-yl]phenyl-.

127. The compound of claim 114, wherein:
a. Ri is unsubstituted C1-10 alkyl -.

128. The compound of claim 127, wherein:
a. Ri is $CH_3(CH_2)_8$-.

129. The compound of any one of claims 1 to 73, wherein:
a. R9 is $R_mOR_iC(O)$–.

130. The compound of claim 129, wherein:
a. R_i is substituted or unsubstituted C1-10 alkyl.

131. The compound of claim 130, wherein:
   a. R_i is -CH_2-.

132. The compound of any one of claims 129 to 131, wherein:
   a. R_m is substituted or unsubstituted C1-10 alkyl.

133. The compound of claim 132, wherein:
   a. R_m is -CH_2-.

134. The compound of claim 133, wherein:
   a. R_m is aryl-CH_2-.

135. The compound of claim 134, wherein:
   a. R_m is phenyl-CH_2-.

136. The compound of any one of claims 129 to 131, wherein:
   a. R_m is substituted or unsubstituted aryl.

137. The compound of claim 136, wherein:
   a. R_m is phenyl.

138. The compound of any one of claims 1 to 73, wherein:
   a. R9 is $R_mC(O)R_iC(O)$–.

139. The compound of claim 138, wherein:
   a. R_m is heteroaryl.

140. The compound of claim 139, wherein:
   a. R_m is morpholine.

141. The compound of any one of claims 138 to 140, wherein:
   a. R_i is aryl.

142. The compound of claim 141, wherein:
   a. R_i is phenyl.

143. The compound of any one of claims 1 to 73, wherein:
   a. R9 is $R_iS(O)_n$–.

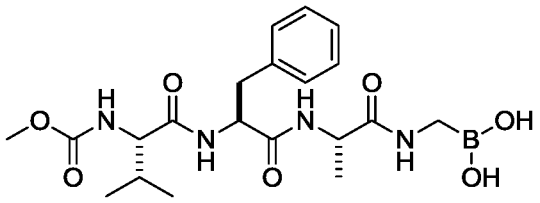
144. The compound of claim 143, wherein:
   a. R_i is substituted or unsubstituted aryl.

145. The compound of claim 144, wherein:
   a. R_i is substituted aryl.

146. The compound of claim 145, wherein:
   a. R_i is substituted phenyl.

147. The compound of claim 146, wherein:
   a. R_i is phenyl-phenyl.

148. The compound of claim 1, which is:



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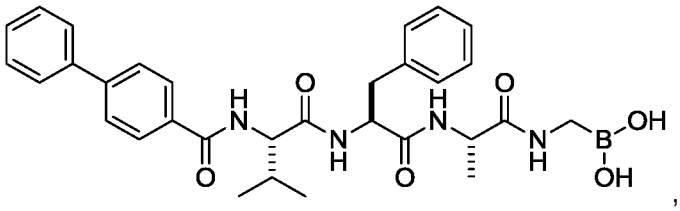

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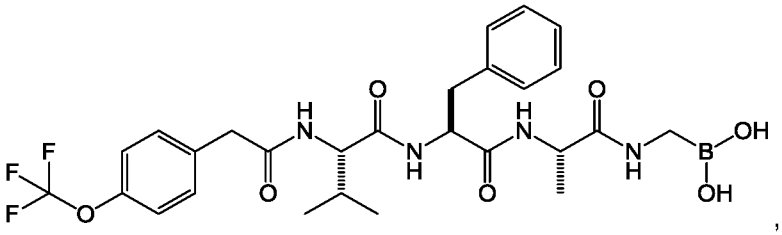

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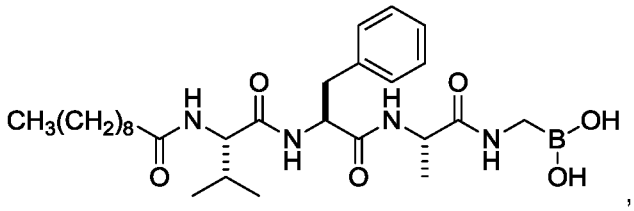

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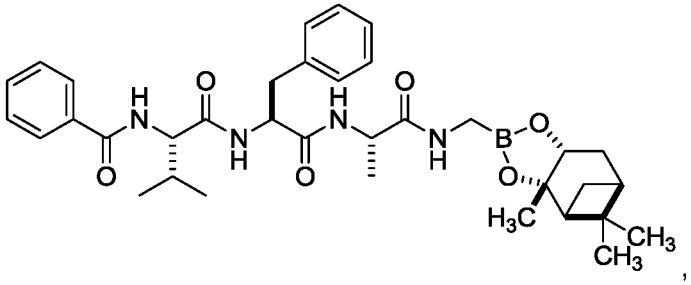

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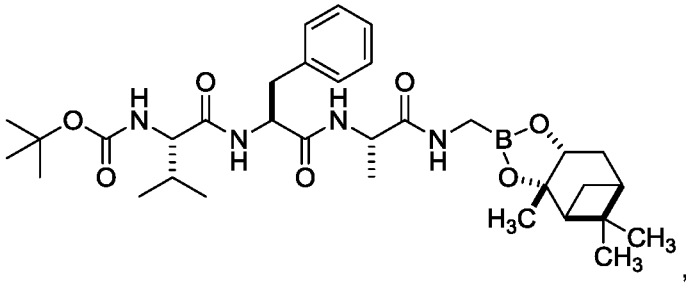

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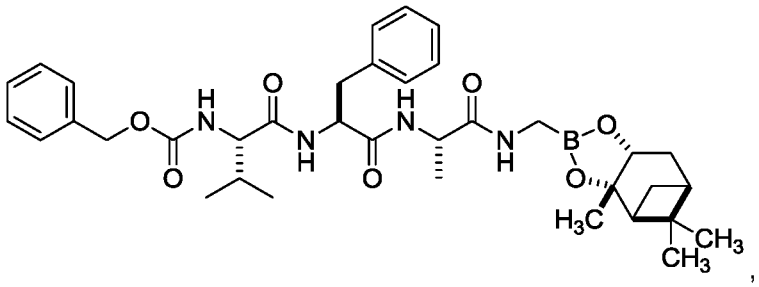

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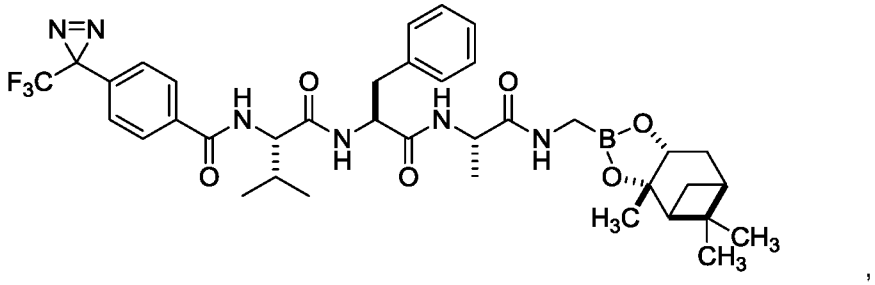

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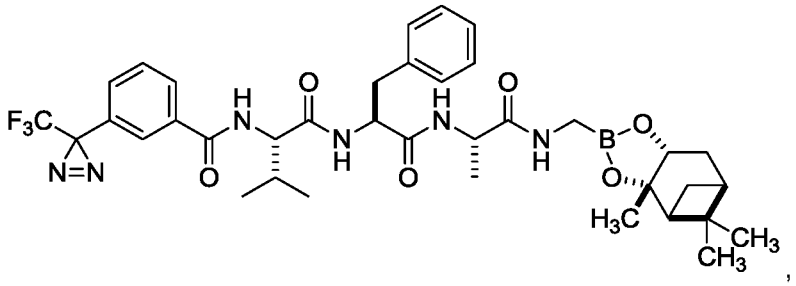

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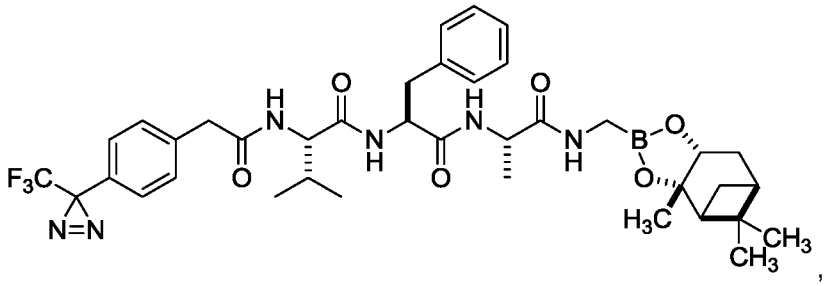

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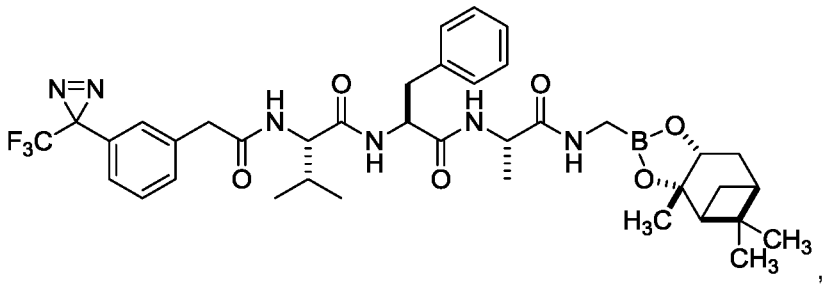

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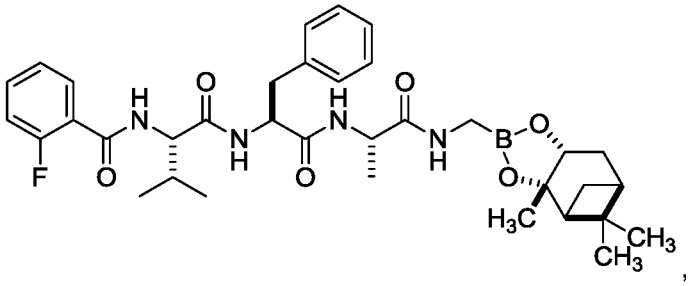

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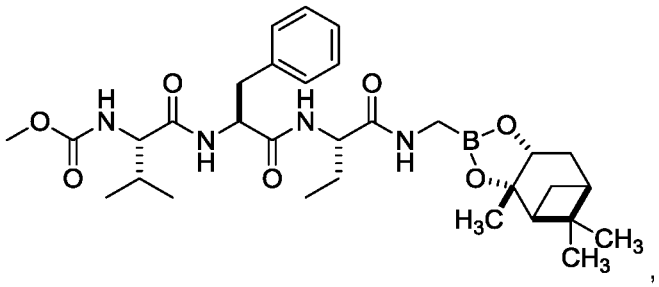

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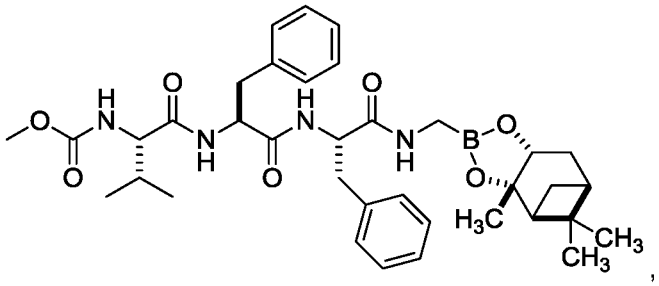

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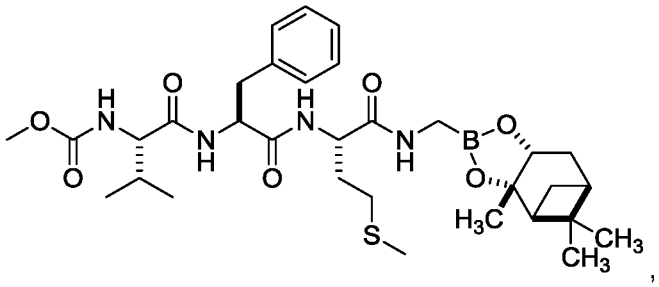

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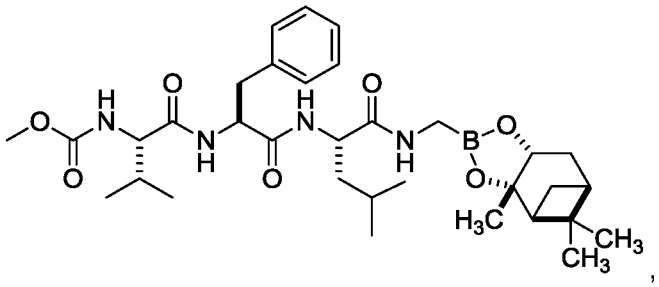

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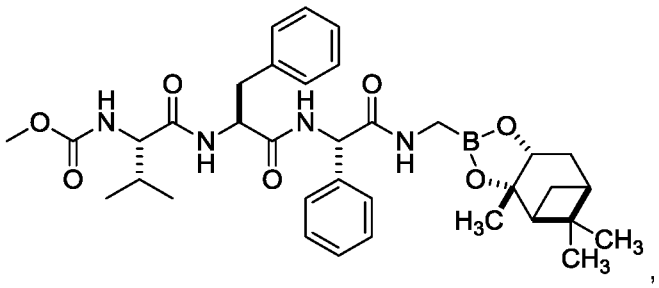

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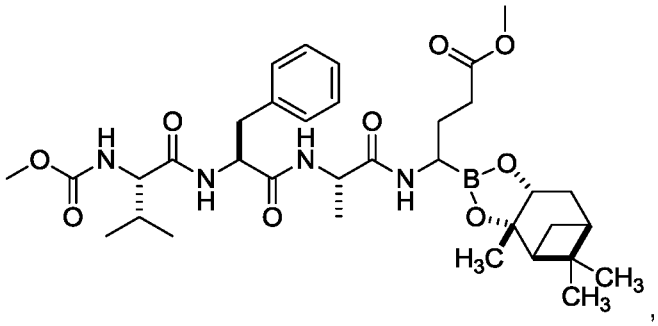

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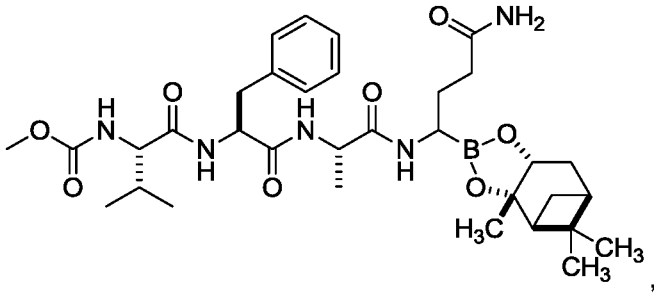

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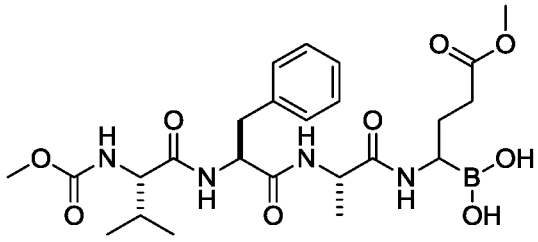

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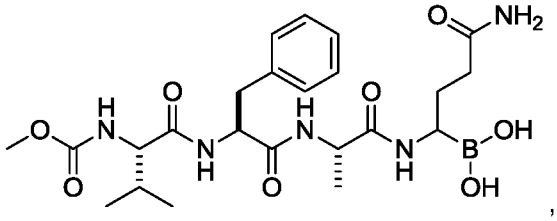

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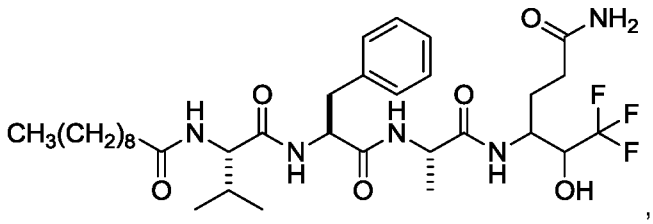

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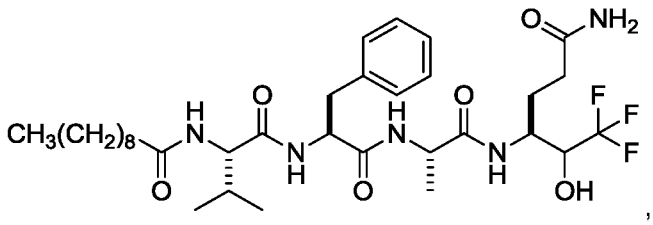

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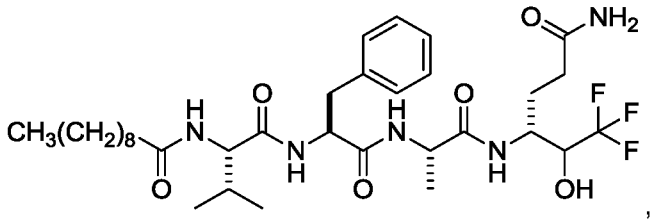

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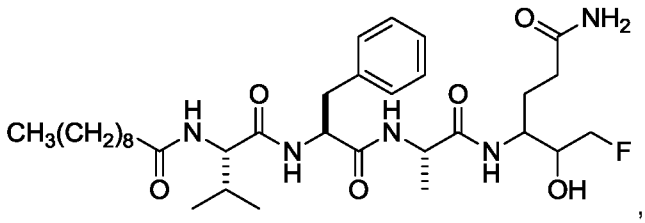

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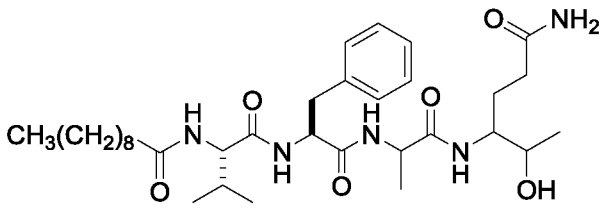

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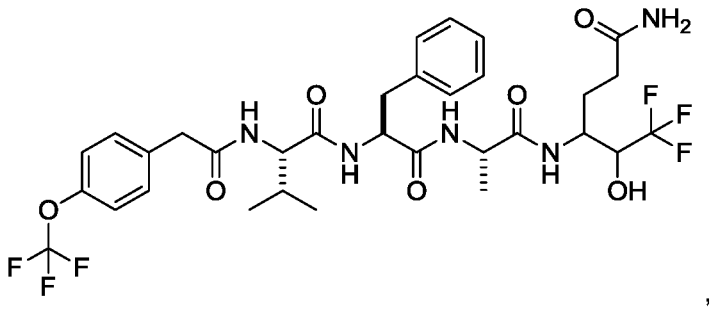

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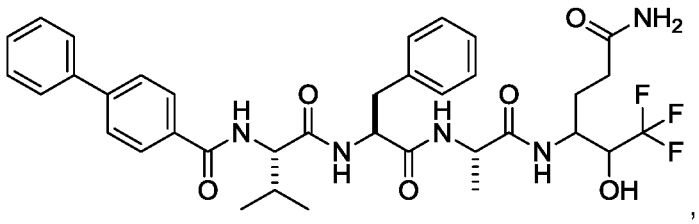

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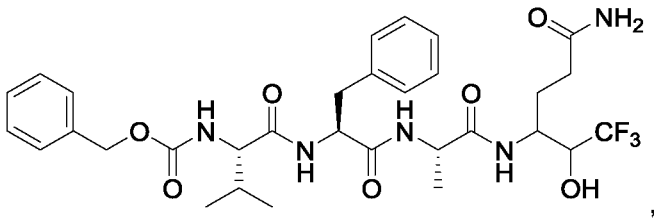

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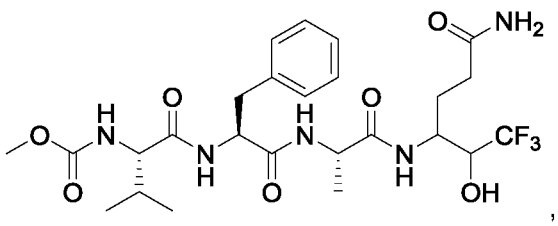

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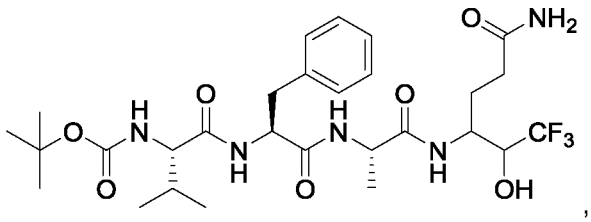

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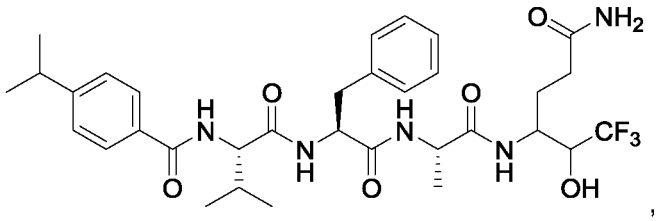

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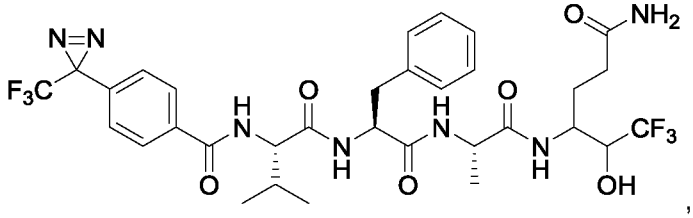

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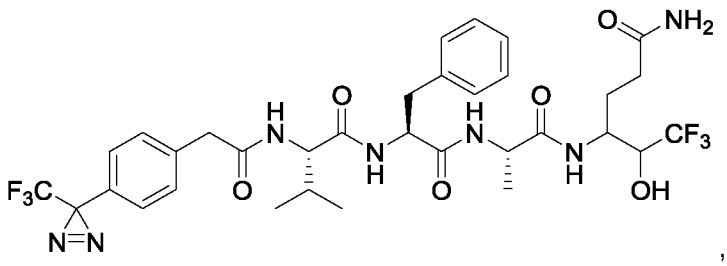

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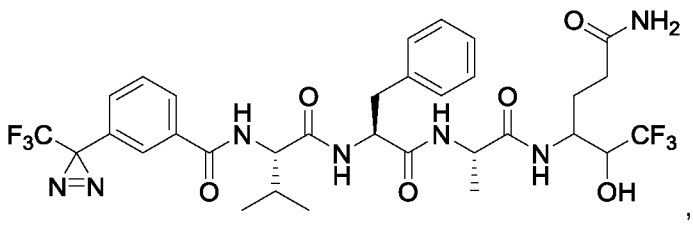

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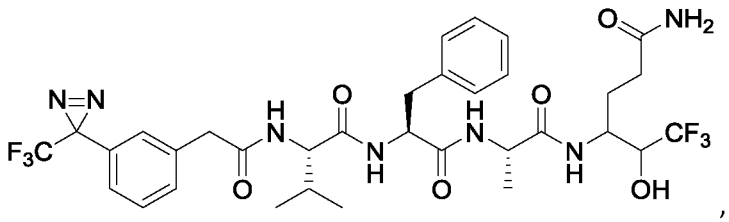

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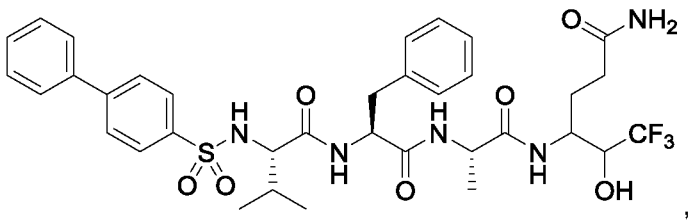

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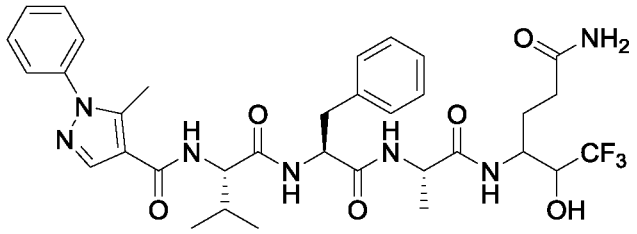

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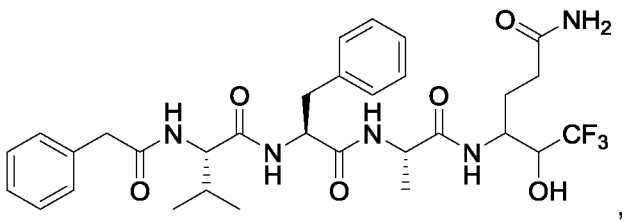

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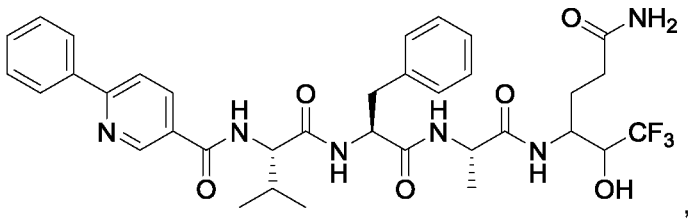

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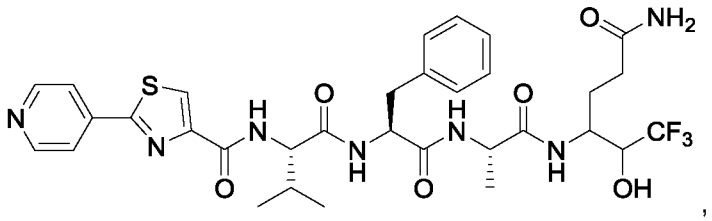

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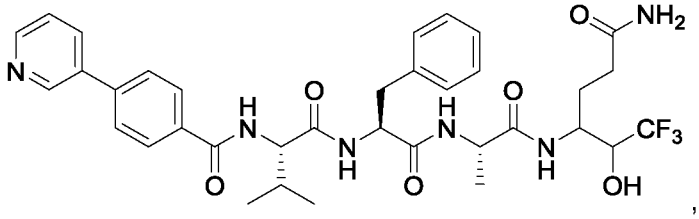

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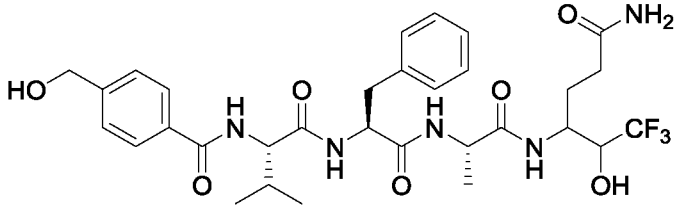

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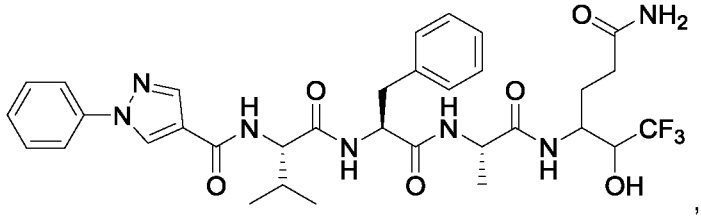

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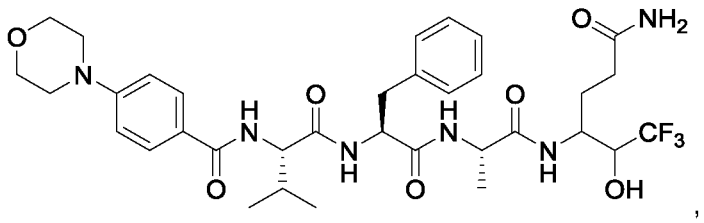

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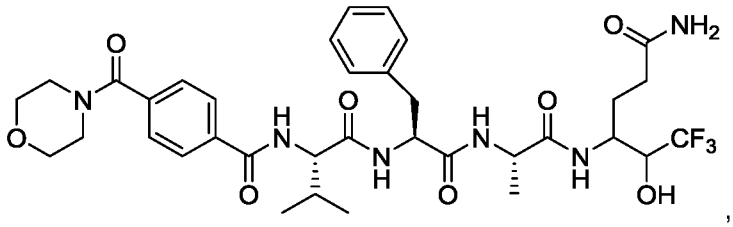

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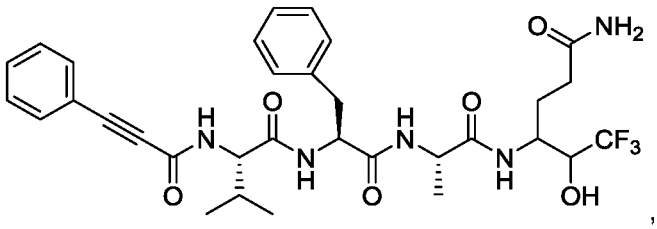

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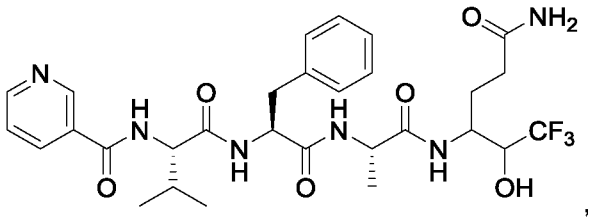

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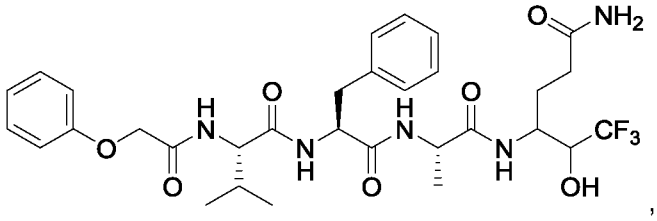

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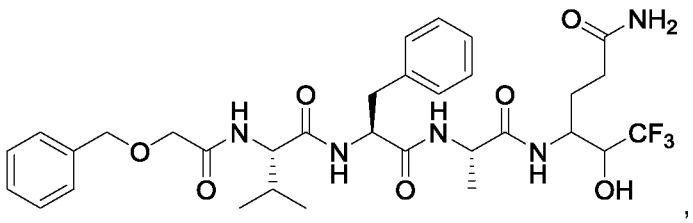

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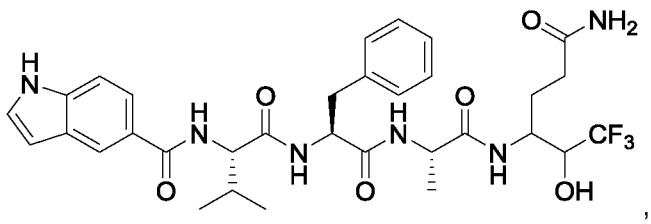

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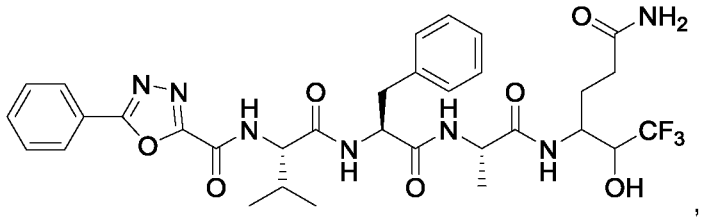

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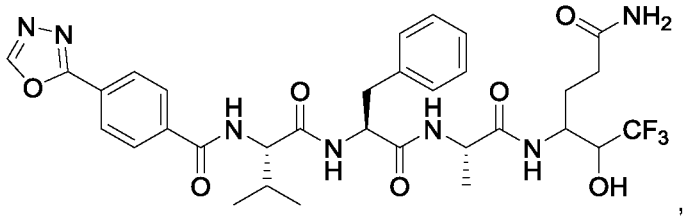

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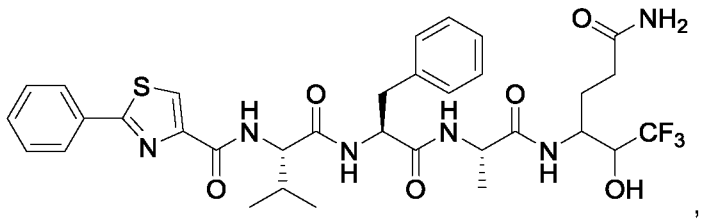

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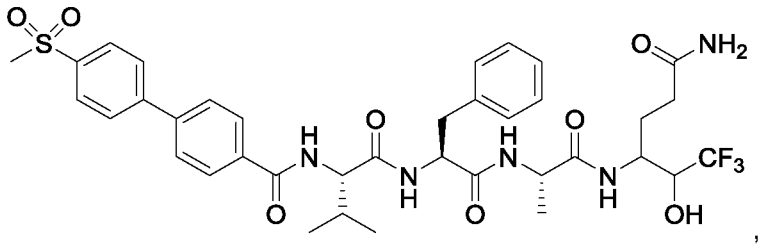

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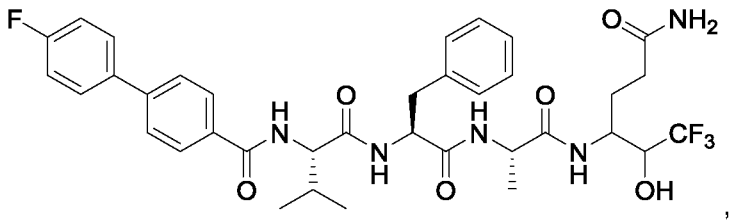

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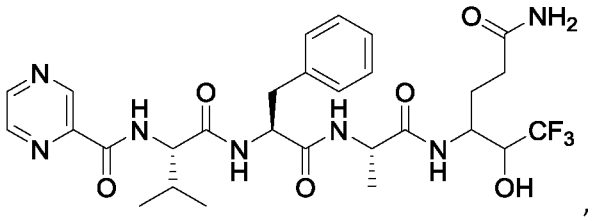

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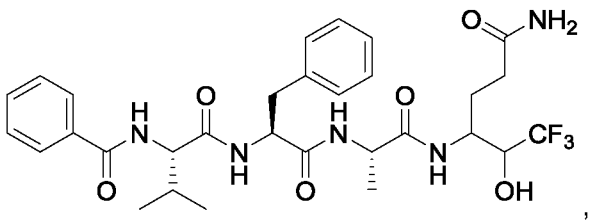

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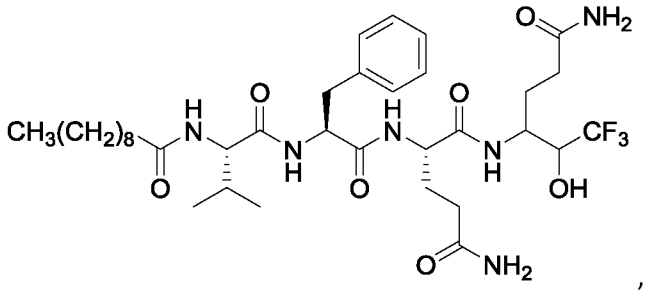

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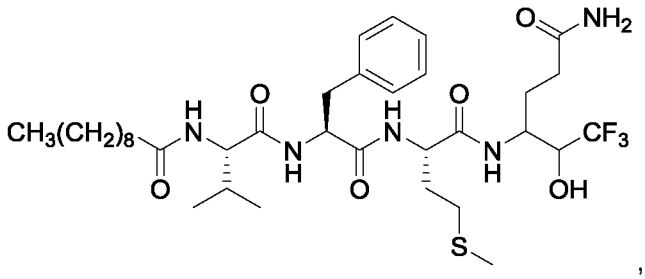

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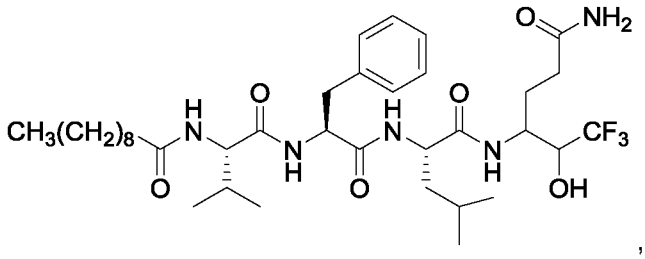

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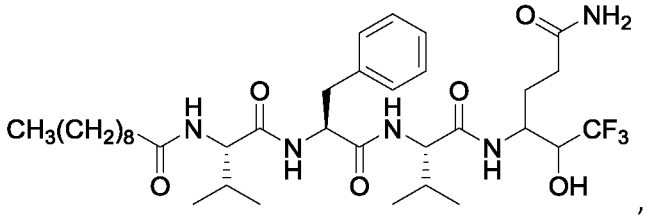

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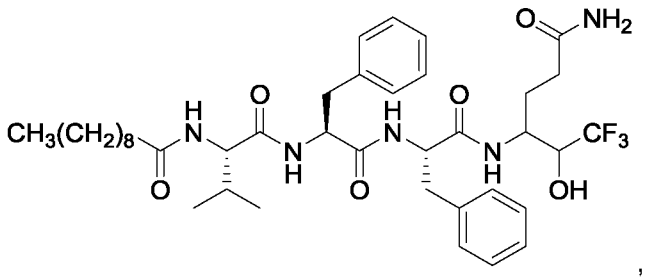

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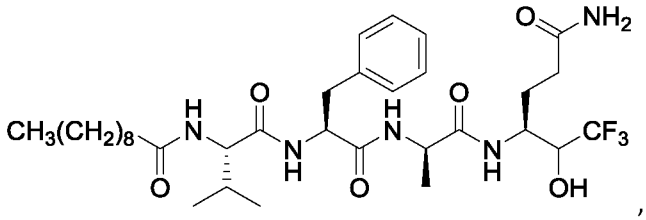

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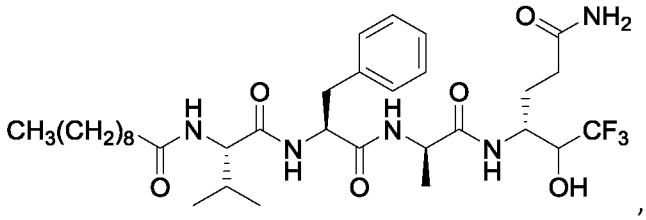

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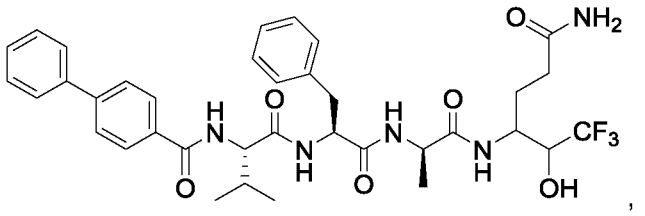

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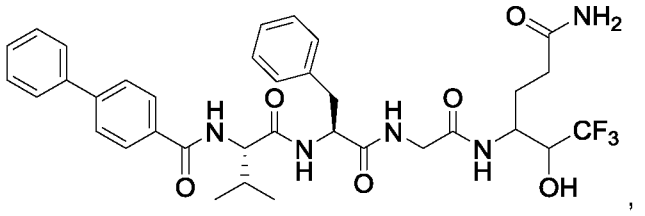

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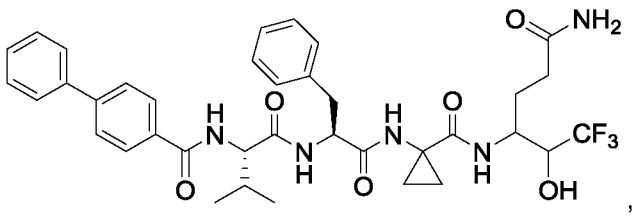

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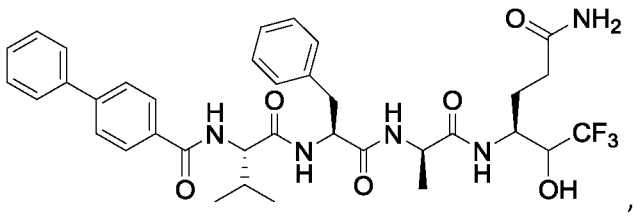

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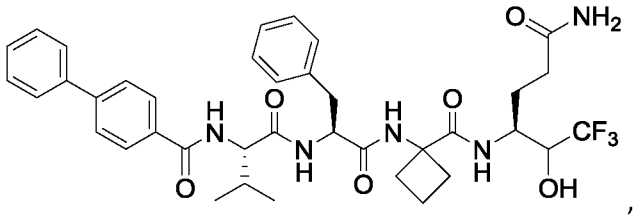

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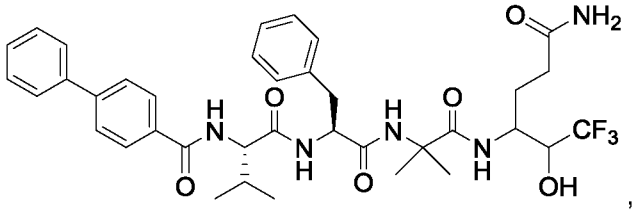

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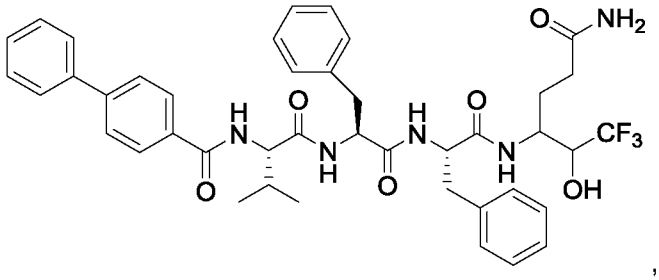

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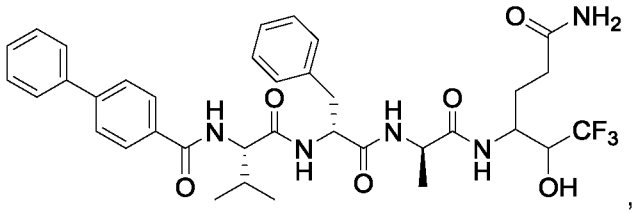

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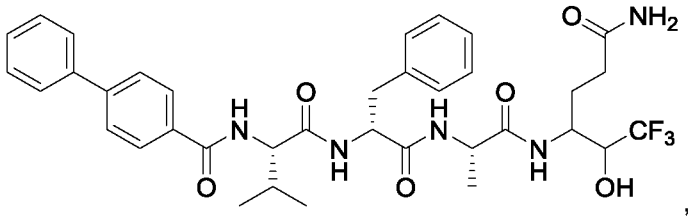

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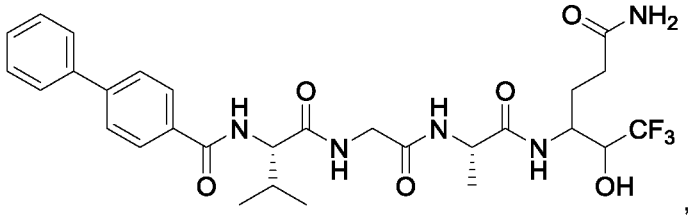

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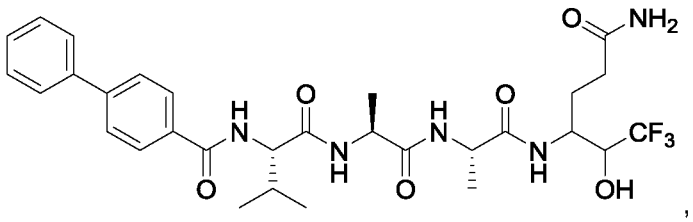

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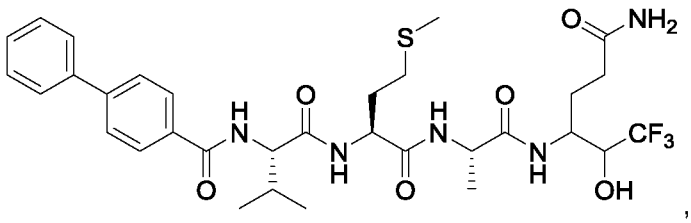

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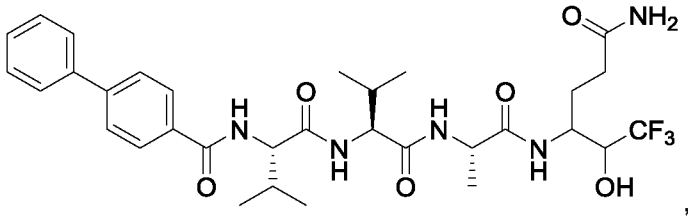

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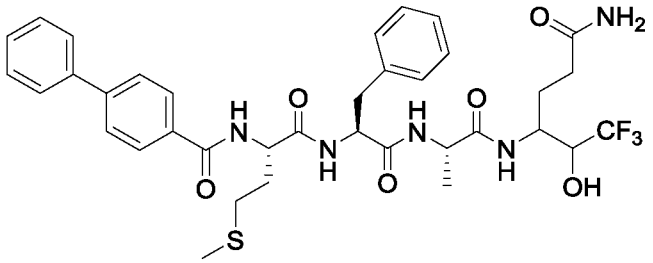

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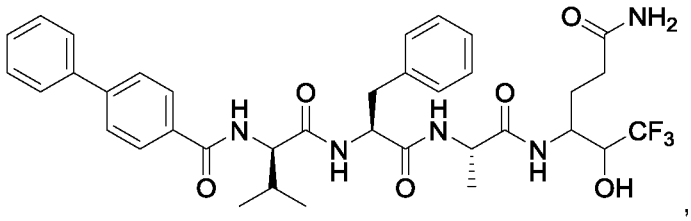

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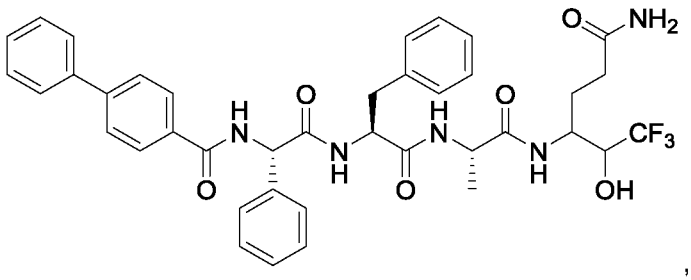

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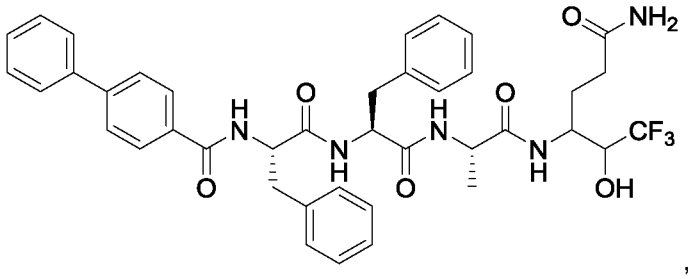

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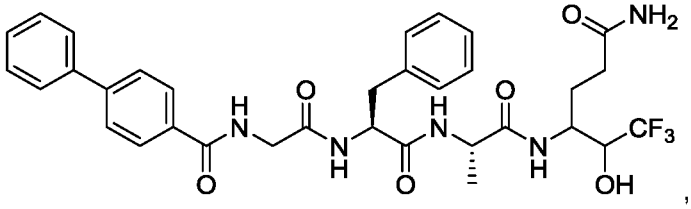

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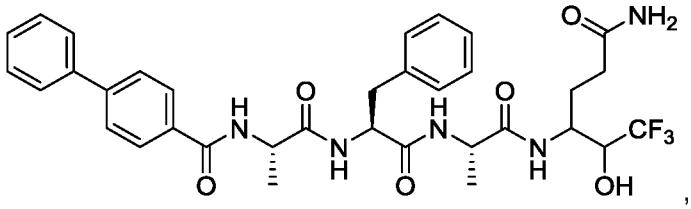

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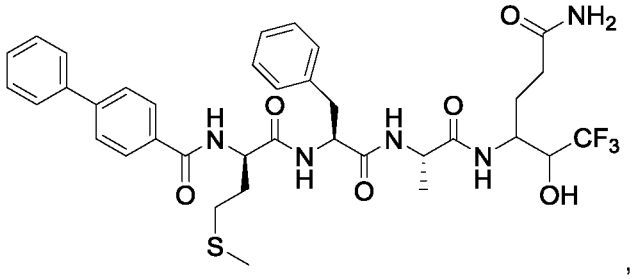

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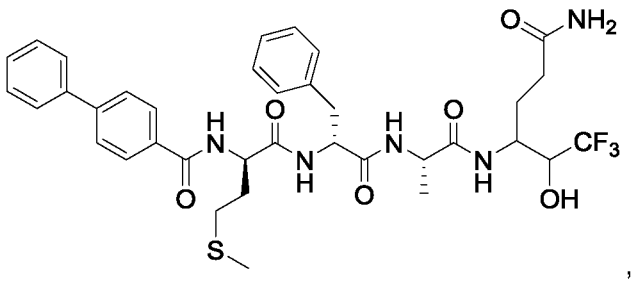

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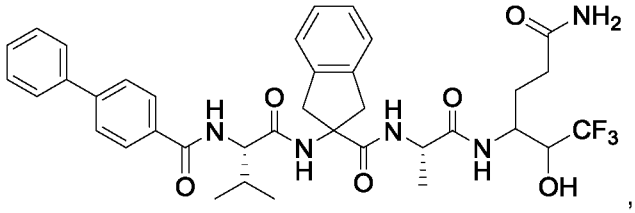

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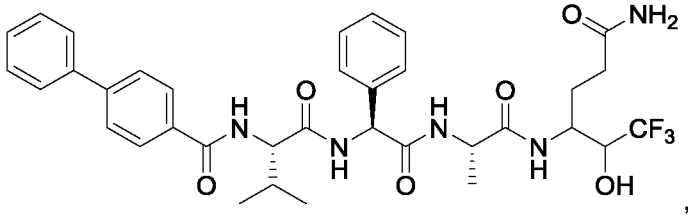

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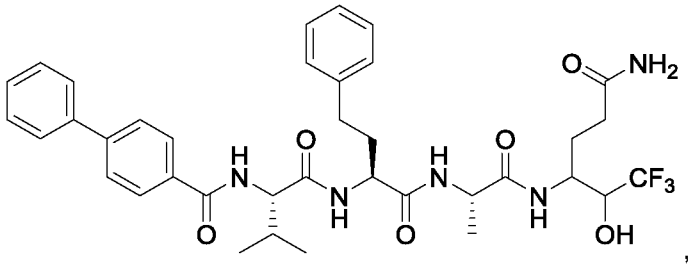

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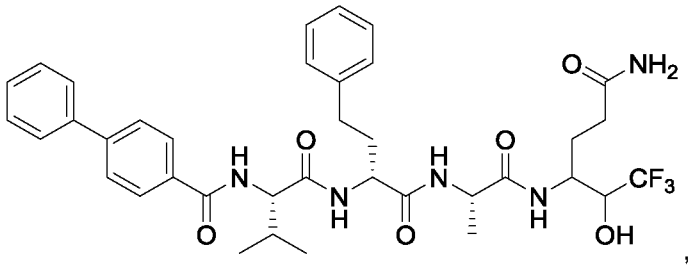

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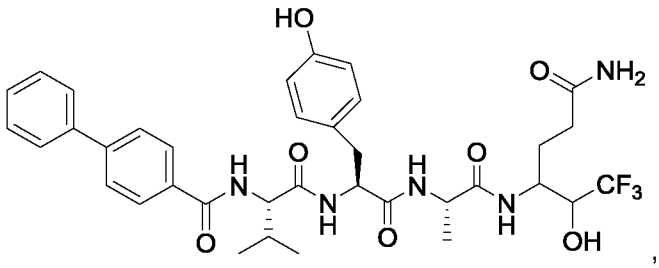

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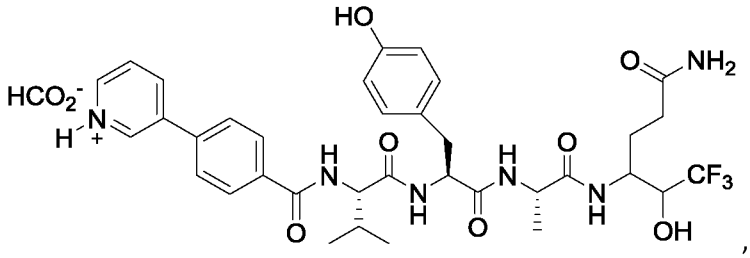

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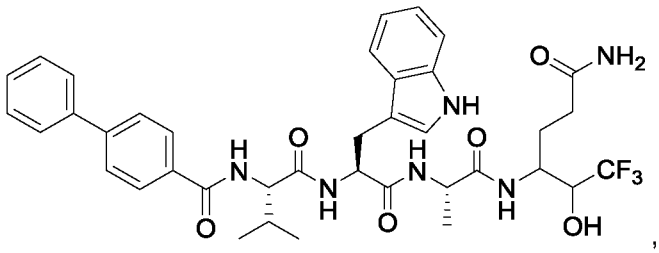

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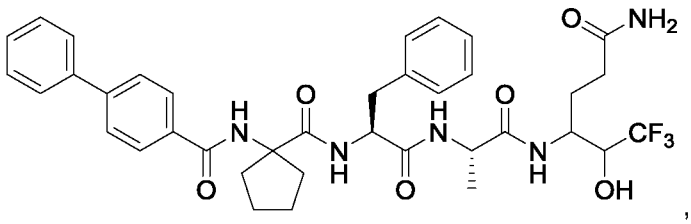

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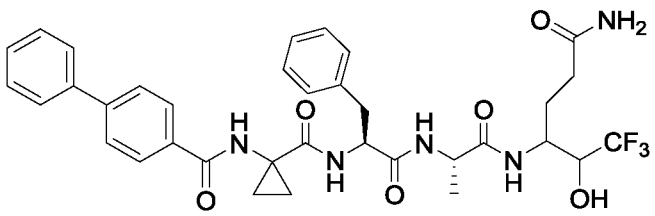


91 .

149. A pharmaceutical composition comprising at least one of the compounds as defined any one of claims 1 to 148.

150. The pharmaceutical composition of claim 61, further comprising at least one other compound of any one of claims 1 to 148.

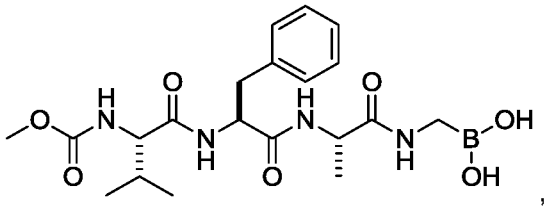
151. The pharmaceutical composition of claims 149 or 150, further comprising at least one other active ingredient which improve a patient's lipid profile.

152. The pharmaceutical composition of any one of claims 150 to 151, further comprising a pharmaceutical carrier or excipient.

153. The compound of any one of claims 1 to 148 or the composition of any one of claims 149 to 152 for use as a medicament.

154. The compound of any one of claims 1 to 148 or the composition of any one of claims 149 to 152 for the manufacture of a medicament.

155. The compound or composition of claim 153 or 154, wherein said medicament is for preventing or treating a low density lipid-cholesterol-related disease or disorder in a subject, with the proviso that the compound is not:



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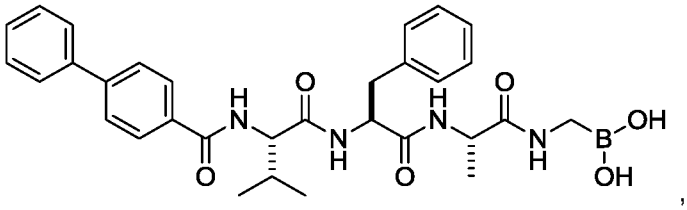

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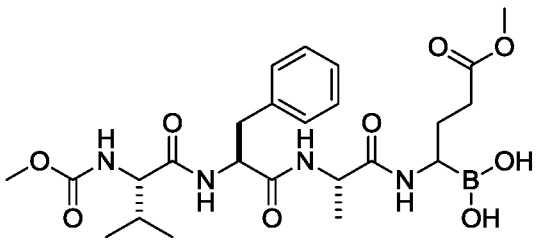

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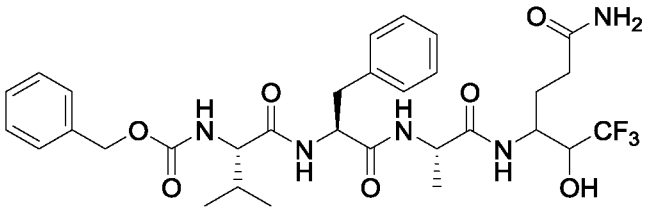
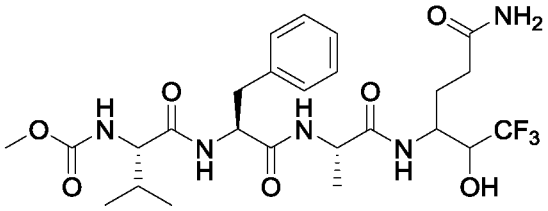


25

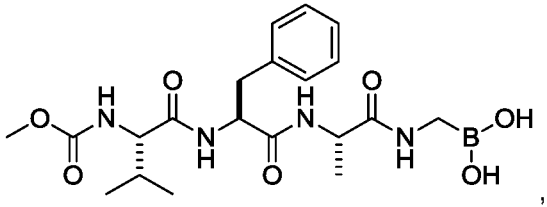
or



26

156. The compound or composition of claim 155, wherein the low density lipid-cholesterol-related disease or disorder is hypercholesterolemia.

157. A method for preventing or treating an LDL-cholesterol-related disease or disorder, comprising administering to a subject in need thereof a therapeutically effective amount of the compound as defined in any one of claims 1 to 148, or of the composition of any one of claims 149 to 152, with the proviso that the compound is not:



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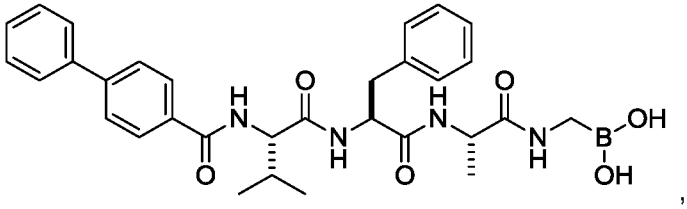

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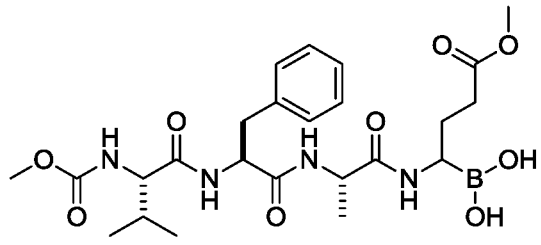

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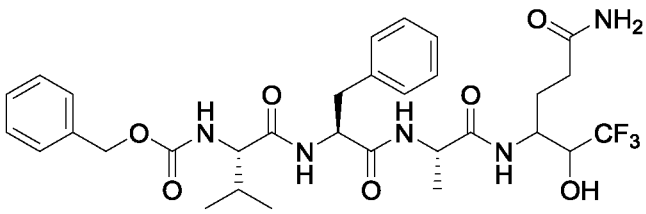
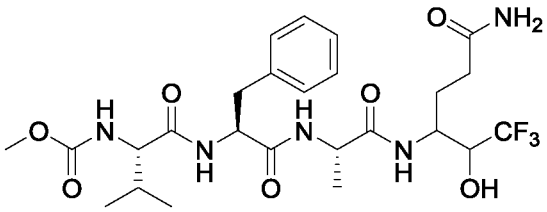


25

or

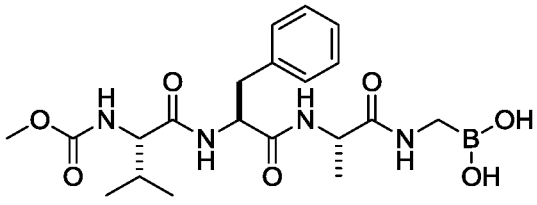


26 .

158. The method of claim 157, wherein the low density lipid-cholesterol-related disease or disorder is hypercholesterolemia.

159. Use of the compound of formula I as defined in any one of claims 1 to 148, or of the composition as defined in any one of claims 149 to 152, as a medicament.

160. Use of the compound as defined in any one of claims 1 to 148, or of the composition as defined in any one of claims 149 to 152, for preventing or treating a low density lipid-cholesterol-related disease or disorder in a subject, with the proviso that the compound is not:



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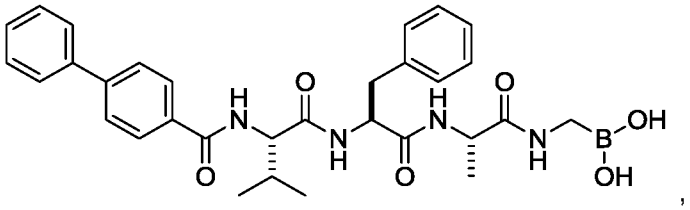

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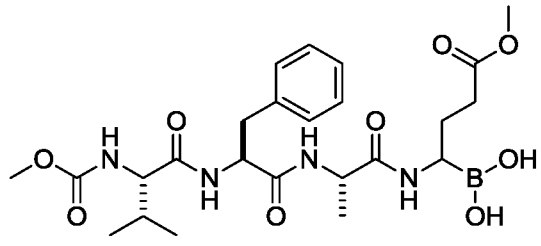
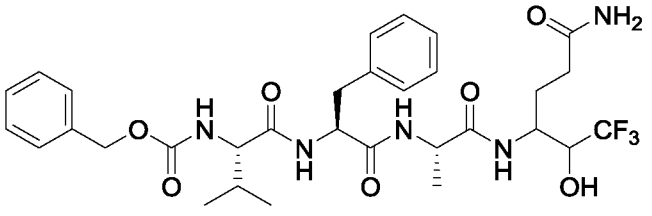
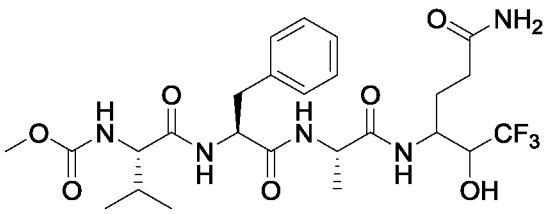




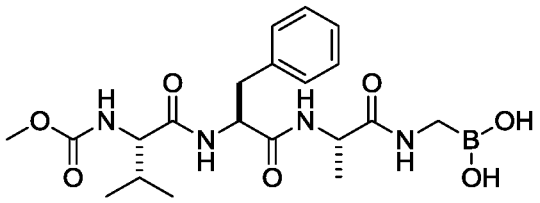
17 25

or



26 .

161. Use of the compound as defined in any one of claims 1 to 148, or of the composition as defined in any one of claims 149 to 152, for the manufacture of a medicament for preventing or treating a low density lipid-cholesterol-related disease in a subject, with the proviso that the compound is not:



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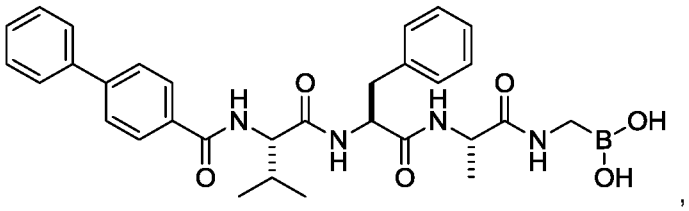

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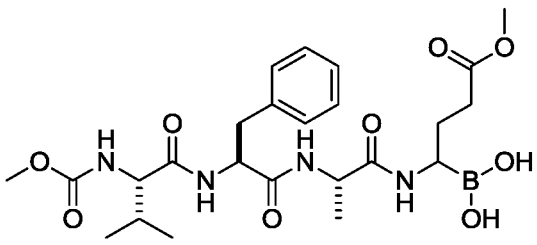
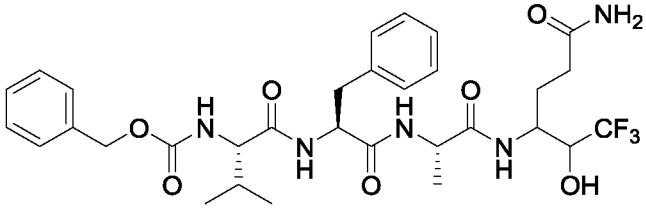
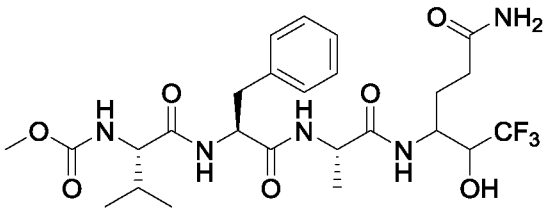


17 ,



25

or



26 .

162. The use of any one of claims 159 to 161, wherein the low density lipid-cholesterol-related disease or disorder is hypercholesterolemia.

163. A kit for preventing or treating a low density lipid-cholesterol-related disease or disorder in a subject comprising

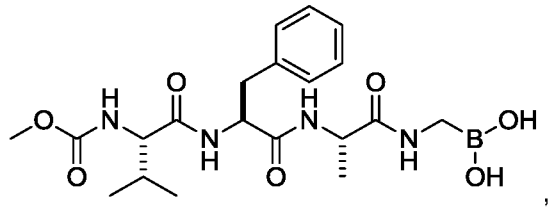
(i) at least one of the compounds as defined in any one of claims 1 to 148 or of the composition as defined in any one of claims 149 to 152, and

(i) (a) at least one other active ingredient which improve a patient's lipid profile;

(b) at least one other compound as defined in any one of claims 1 to 148 or composition as defined in any one of claims 149 to 152;

(c) a container for the compound and/or active ingredients; and/or

(d) instructions to use the compound for preventing or treating the low density lipid-cholesterol-related disease or disorder in the subject, with the proviso that the compound is not:



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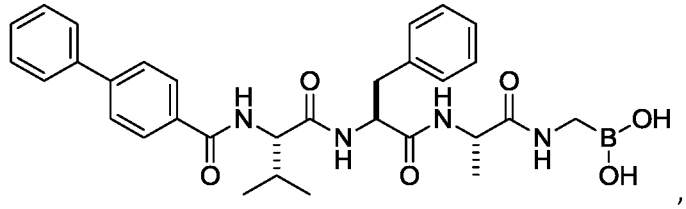

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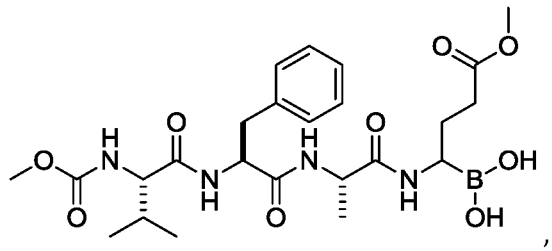

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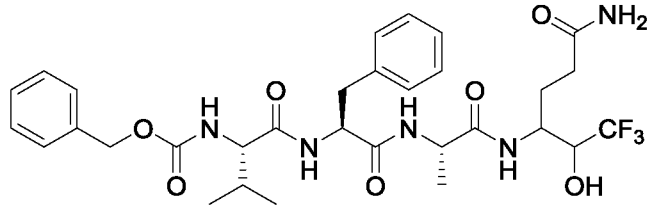
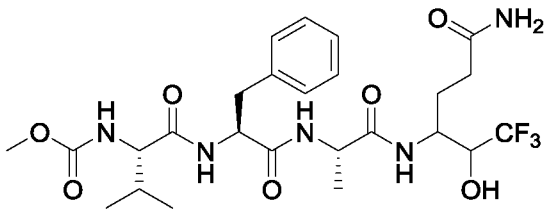


25

or



26 .

164. The kit of claim 163, wherein the low density lipid-cholesterol-related disease or disorder is hypercholesterolemia.

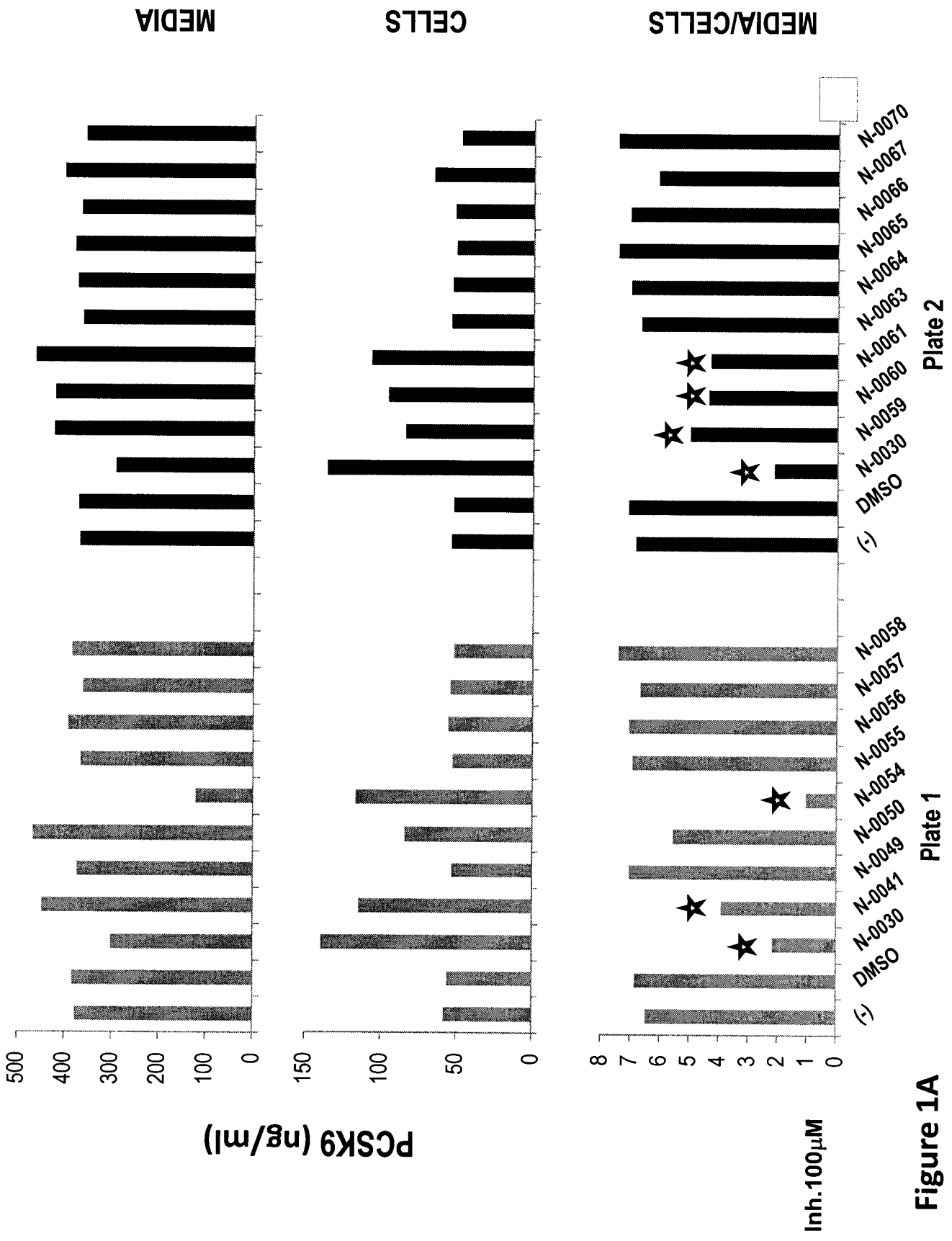
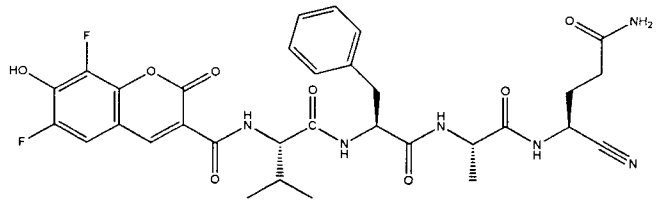
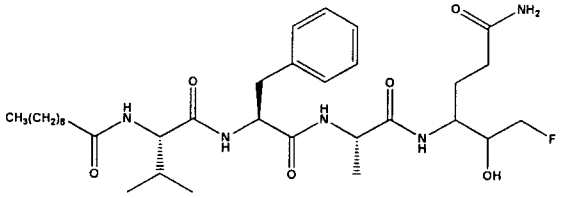

MEDIA
CELLS
MEDIA/CELLS
PCSK9 (ng/ml)
Inh.100μM
Plate 1
Plate 2
(-)
DMSO
N-0030
N-0041
N-0049
N-0050
N-0054
N-0055
N-0056
N-0057
N-0058
(-)
DMSO
N-0030
N-0059
N-0060
N-0061
N-0063
N-0064
N-0065
N-0066
N-0067
N-0070

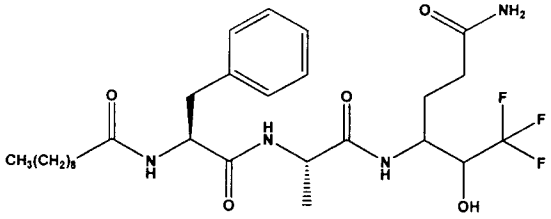
Figure 1A



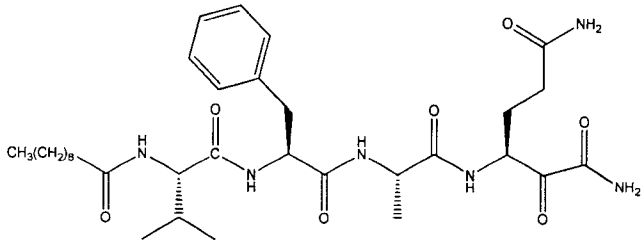
a



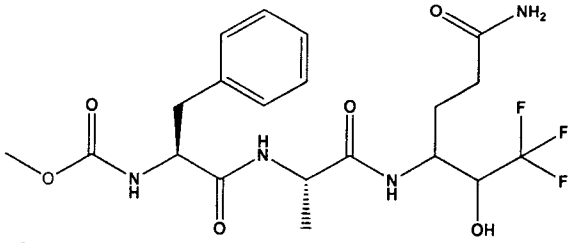
b



c or d*

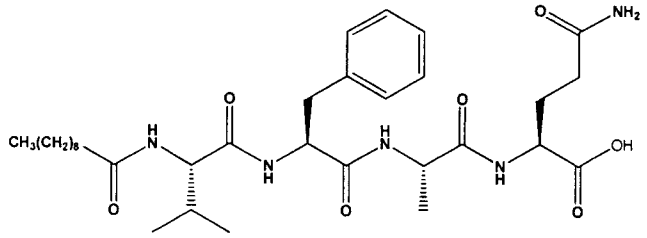


e

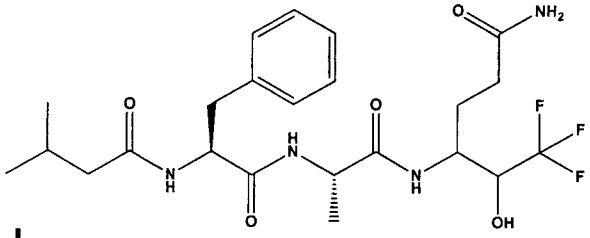


f

Figure 1B



g



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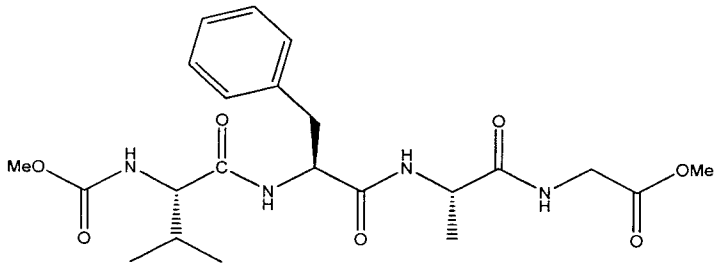

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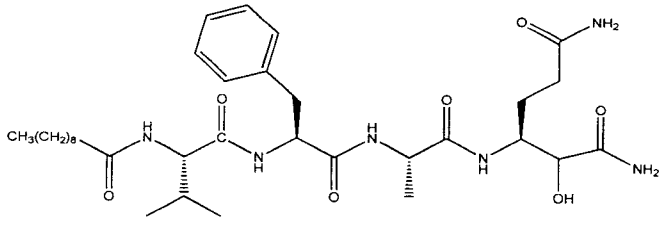

j

Figure 1B (Continued)

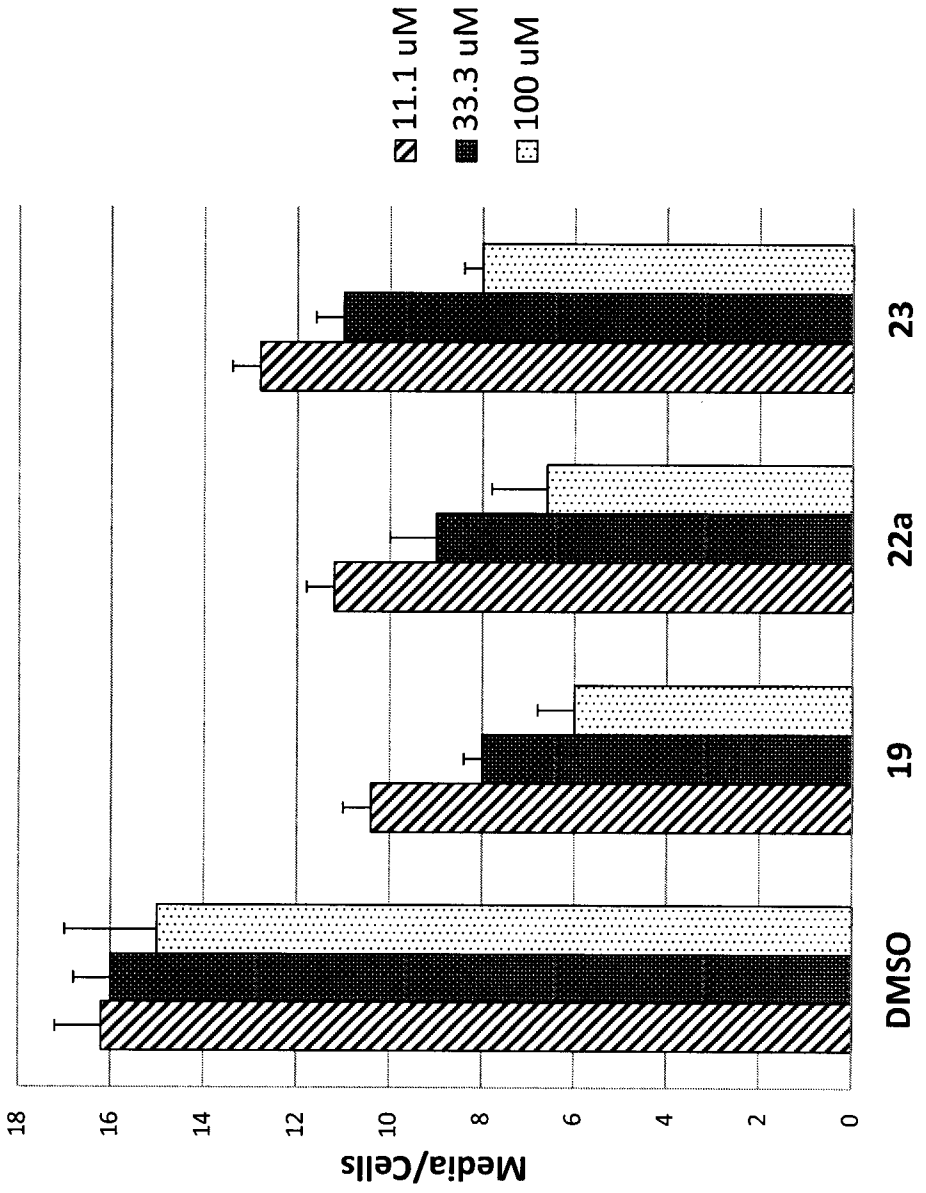
11.1 uM
33.3 uM
100 uM
Media/Cells
0
2
4
6
8
10
12
14
16
18
DMSO
19
22a
23

Figure 2

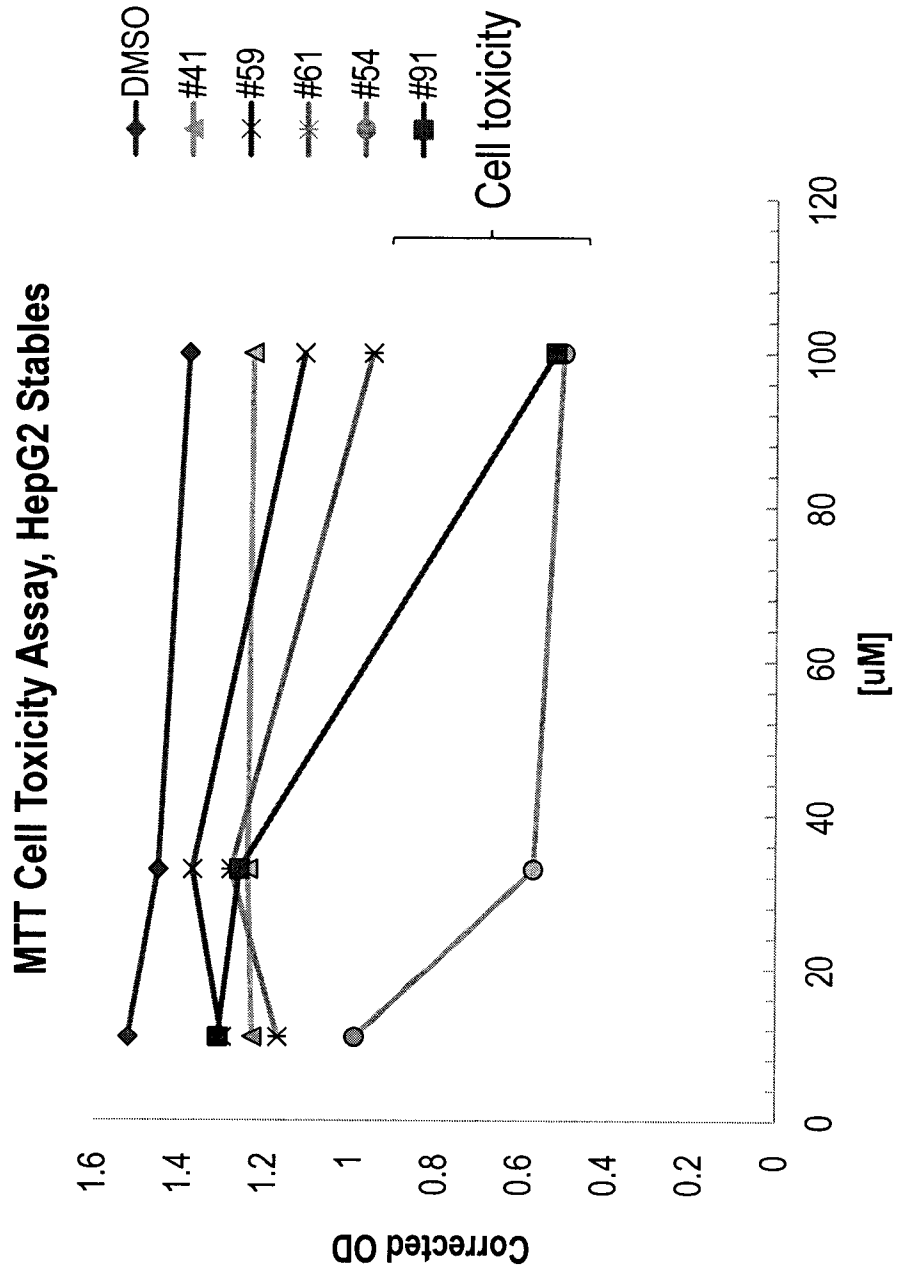
MTT Cell Toxicity Assay, HepG2 Stables
DMSO
#41
#59
#61
#54
#91
Cell toxicity
Corrected OD
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
[uM]

Figure 3

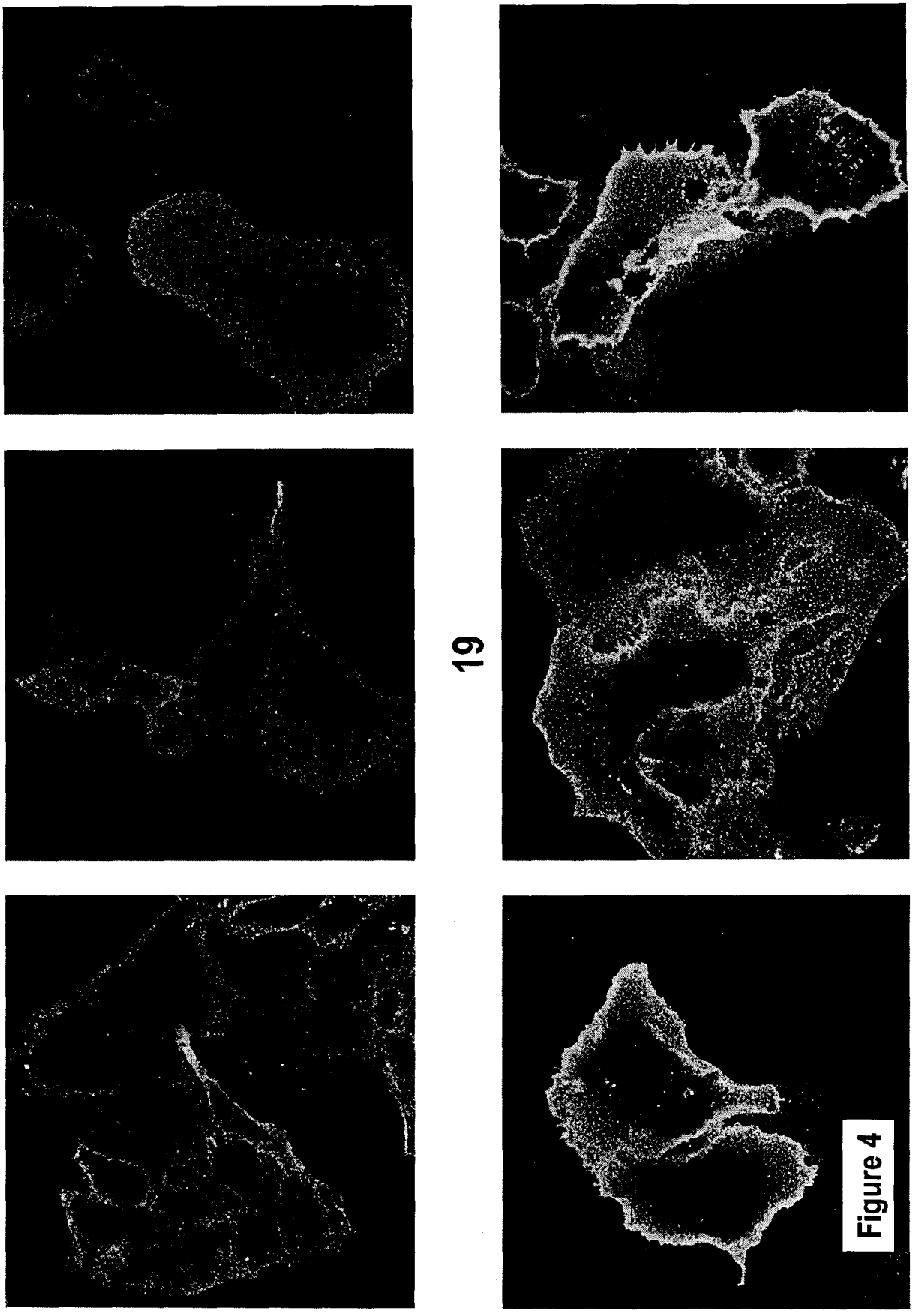

Figure 4

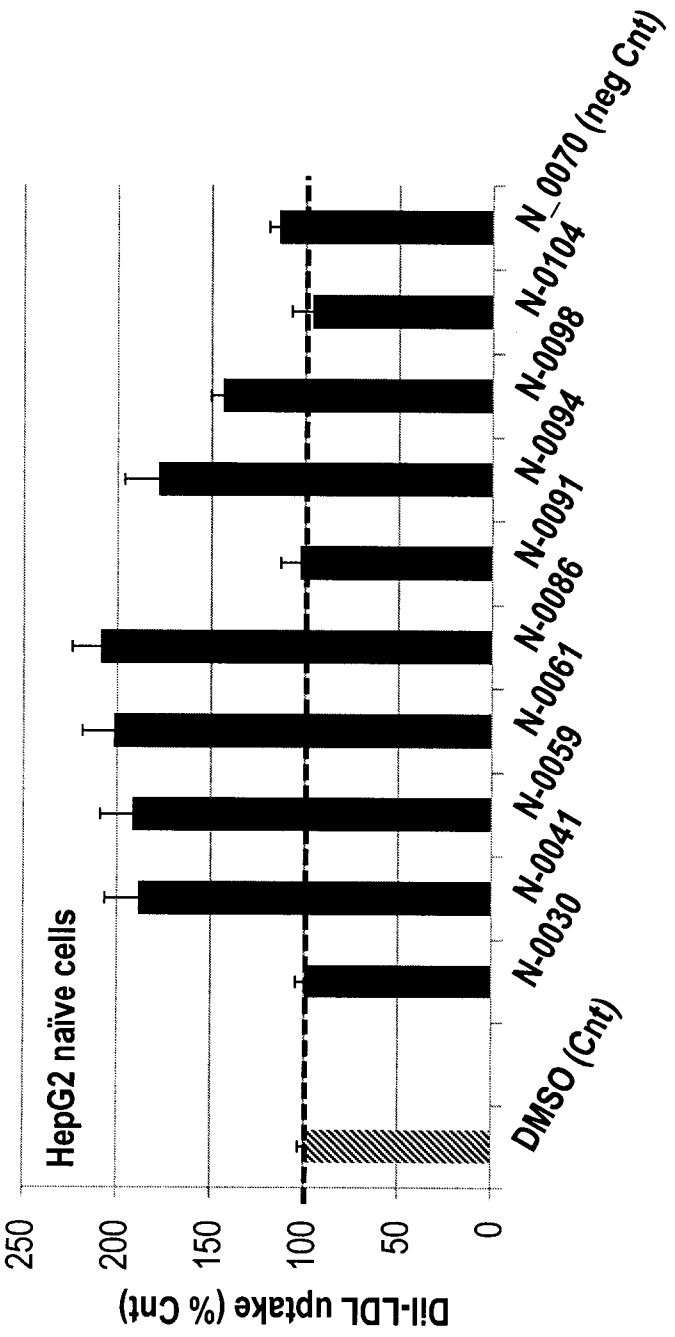
HepG2 naïve cells
Dil-LDL uptake (% Cnt)
250
200
150
100
50
0
DMSO (Cnt)
N-0030
N-0041
N-0059
N-0061
N-0086
N-0091
N-0094
N-0098
N-0104
N_0070 (neg Cnt)

Figure 5A

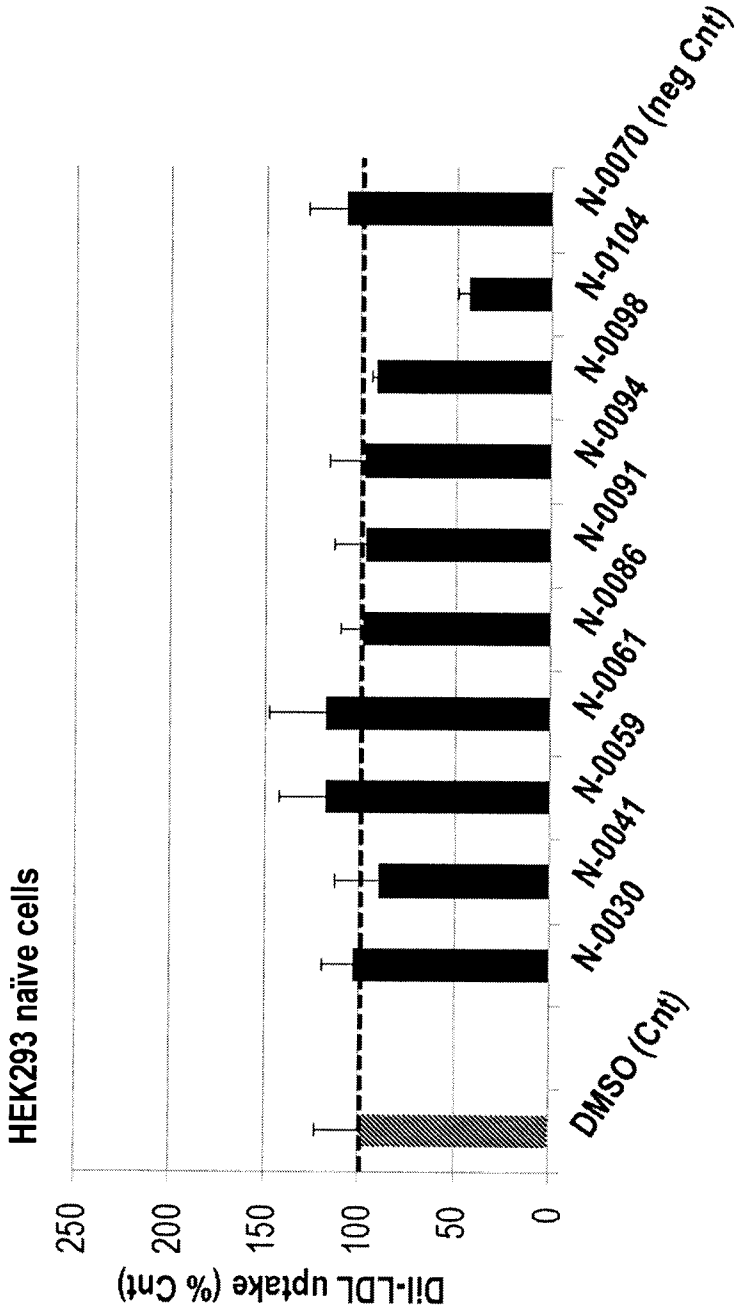
HEK293 naïve cells
Dil-LDL uptake (% Cnt)
250
200
150
100
50
0
DMSO (Cnt)
N-0030
N-0041
N-0059
N-0061
N-0086
N-0091
N-0094
N-0098
N-0104
N-0070 (neg Cnt)

Figure 5B

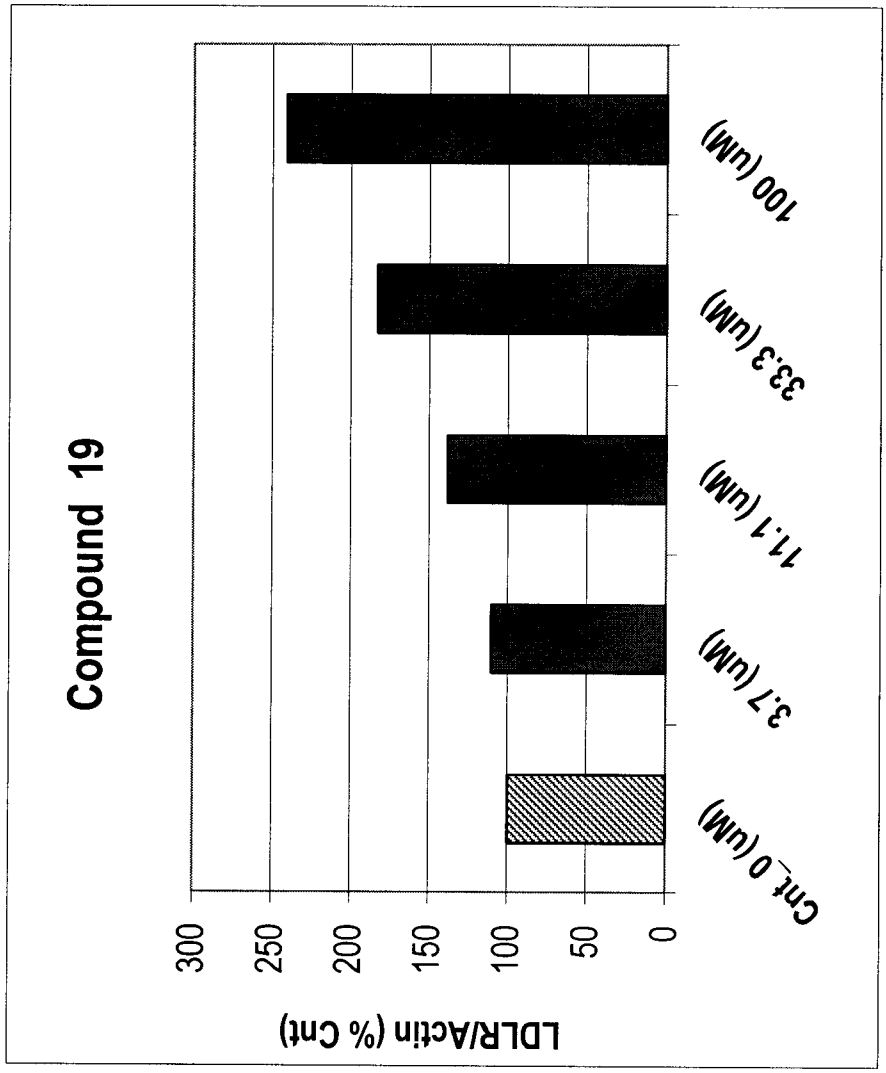
Compound 19
LDLR/Actin (% Cnt)
300
250
200
150
100
50
0
Cnt_0 (uM)
3.7 (uM)
11.1 (uM)
33.3 (uM)
100 (uM)

Figure 5C

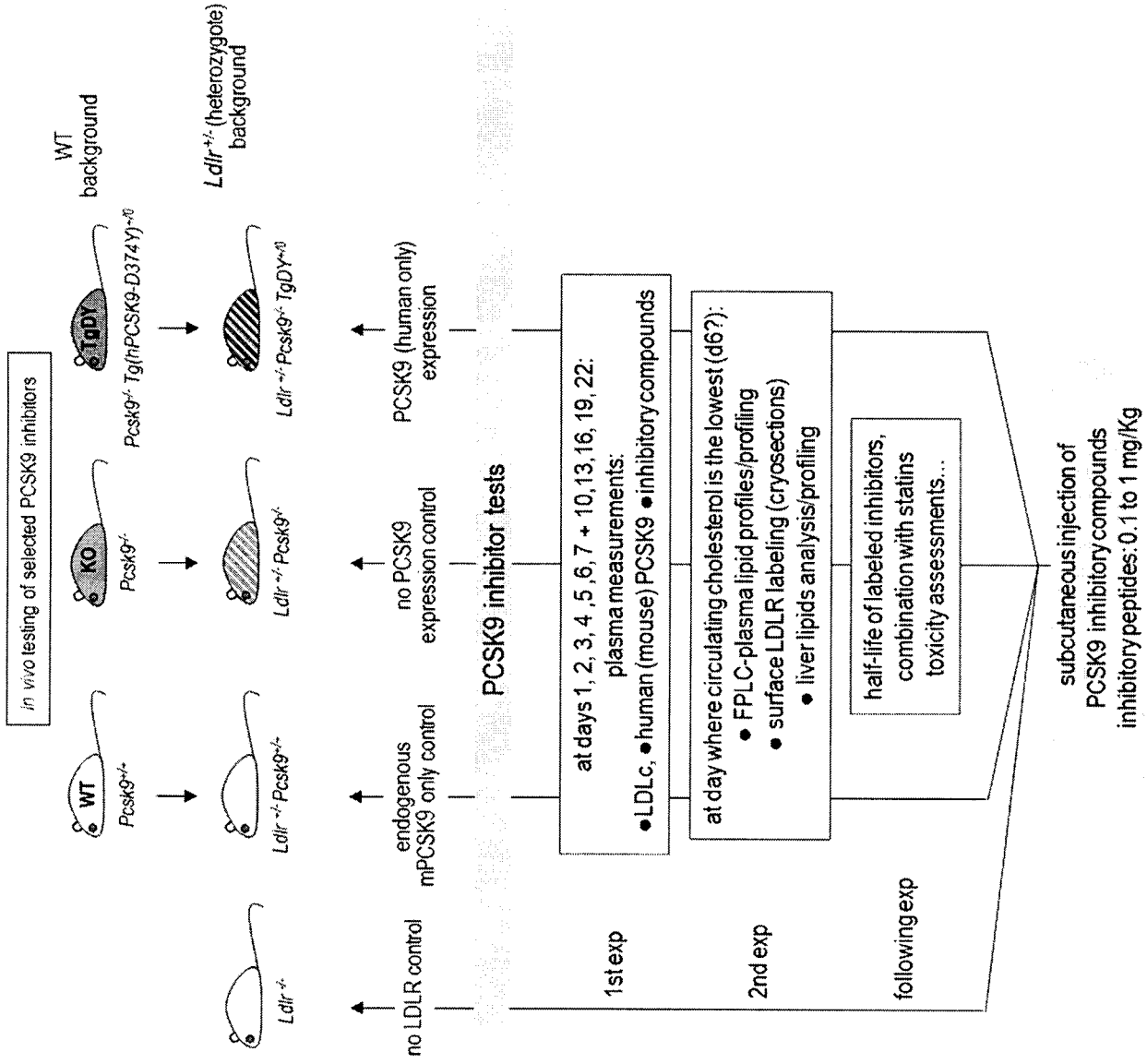
in vivo testing of selected PCSK9 inhibitors
WT
Pcsk9+/+
Ldlr-/- Pcsk9+/+
Ldlr+/-
KO
Pcsk9-/-
Ldlr+/- Pcsk9-/-
TgDY
Pcsk9-/- Tg(hPCSK9-D374Y)+/0
Ldlr-/- Pcsk9-/- TgDY+/0
WT background
Ldlr+/- (heterozygote) background
no LDLR control
endogenous mPCSK9 only control
no PCSK9 expression control
PCSK9 (human only) expression
PCSK9 inhibitor tests
1st exp
at days 1, 2, 3, 4 ,5 ,6, 7 + 10,13,16, 19, 22:
plasma measurements:
●LDLc, ●human (mouse) PCSK9 ●inhibitory compounds
2nd exp
at day where circulating cholesterol is the lowest (d6?):
● FPLC-plasma lipid profiles/profiling
● surface LDLR labeling (cryosections)
● liver lipids analysis/profiling
following exp
half-life of labeled inhibitors, combination with statins toxicity assessments...
subcutaneous injection of PCSK9 inhibitory compounds
inhibitory peptides: 0.1 to 1 mg/Kg

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2014/050255

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 5/083*** (2006.01), ***A61K 38/06*** (2006.01), ***A61P 3/06*** (2006.01), ***C07K 5/027*** (2006.01), ***C07K 5/08*** (2006.01)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: C07K 5/083 (2006.01), A61K 38/06 (2006.01), A61P 3/06 (2006.01), C07K 5/027 (2006.01), C07K 5/08 (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
STN CASRegistry and MARPAT, TotalPatent, Scopus, Canadian patent database
Keywords: hypercholesterolemia, proprotein convertase subtilisin-kexin 9, (PCSK9), inhibitor, neural apoptosis-regulated convertase 1 (NARC-1)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/22496 A2 (ATTWOOD, MR. et al) 28 May 1998 (28-05-1998). See whole document.	1, 4, 5, 19-32, 35-43, 45-55, 57-67, 70-73, 149, 150, 152-156, 159, 163 and 164
X	WO 2011/109355 A1 (SHENK, KD. et al) 09 September 2011 (09-09-2011). See whole document.	1, 4-7, 19, 20, 23, 24, 29-31, 35, 38-43, 45-50, 53, 54, 57, 58, 62-64, 80, 114, 115, 149, 150, 152-156, 159, 163 and 164
X	WO 2003/092605 A2 (BACHOVCHIN, WW.) 13 November 2003 (13-11-2003). See whole document especially compounds 12 and 14 (pages 73 and 74).	1, 4, 5, 19, 20, 23, 24, 29-31, 38-40, 57, 58, 62-64, 74, 75, 78, 79, 138, 149, 150, 152-156, 159, 163 and 164

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent but published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 June 2014 (02-06-2014)	Date of mailing of the international search report 18 June 2014 (18-06-2014)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Nathalie Chartrand (819) 994-2341

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEBEAU, AM. et al., Optimization of peptide-based inhibitors of prostate-specific antigen (PSA) as targeted imaging agents for prostate cancer. Bioorganic & Medicinal Chemistry, 2009, Vol. 17, No. 14, pages 4888-4893, ISSN 0968-0896. See whole document especially compounds 1, 30, 45 and 47.	1, 4, 5, 19-21, 23-25, 38-40, 57, 58, 62, 138, 140, 149, 150 and 152
X	MORRIS, TS. et al., In vitro and ex vivo inhibition of hepatitis A virus 3C proteinase by a peptidyl monofluoromethyl ketone. Bioorganic & Medicinal Chemistry, 1997, Vol. 5, No. 5, pages 797-807, ISSN 0968-0896. See whole document especially compound 12a.	1-3, 8, 9, 11, 15, 19-26, 38, 39, 47-49, 57-59, 80, 114, 127, 149, 150 and 152
X	WO 98/13461 A1 (MCIVER, JM. et al) 02 April 1998 (02-04-1998). See pages 2-3.	1, 8-10, 19-26, 38, 57, 58, 62-64, 74-77 and 80
P,X	WO 2013/123456 A1 (HIGUCHI, RI. et al) 22 August 2013 (22-08-2013). See whole document.	1-7, 14, 19, 20, 23, 24, 29, 38, 39, 47-50, 55, 57-64, 67-73, 138, 149, 150, 152-156, 159, 163 and 164
A	SEIDAH, NG. et al., Annexin A2 is a natural extrahepatic inhibitor of the PCSK9-induced LDL receptor degradation. PLoS ONE, July 2012 (07-2012), Vol. 7, No. 7, e41865 (pages 1-13), ISSN 1932-6203. See whole document.	1-164
A	SEIDAH, NG. and Prat, A., The biology and therapeutic targeting of the proprotein convertases. Nature Reviews Drug Discovery, May 2012 (05-2012), Vol. 11, pages 367-383, ISSN 1474-1784. See whole document.	1-164

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claim Nos.: 157 and 158
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 157 and 158 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under Rule 39.1(iv) of the PCT. However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claims 1-148.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2014/050255

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO9822496A2	28 May 1998 (28-05-1998)	WO9822496A2	28 May 1998 (28-05-1998)
		WO9822496A3	16 July 1998 (16-07-1998)
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		TW201341403A	16 October 2013 (16-10-2013)
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(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 105431447 A
(43)申请公布日 2016.03.23

(21)申请号 201480026888.4
(22)申请日 2014.03.14
(30)优先权数据
61/792,249 2013.03.15 US
(85)PCT国际申请进入国家阶段日
2015.11.11
(86)PCT国际申请的申请数据
PCT/CA2014/050255 2014.03.14
(87)PCT国际申请的公布数据
WO2014/139008 EN 2014.09.18
(71)申请人 阿达拉塔合伙有限公司
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(74)专利代理机构 北京集佳知识产权代理有限公司 11227
代理人 郑斌 彭鲲鹏
(51)Int.Cl.
C07K 5/083(2006.01)
A61K 38/06(2006.01)
A61P 3/06(2006.01)
C07K 5/027(2006.01)
C07K 5/08(2006.01)

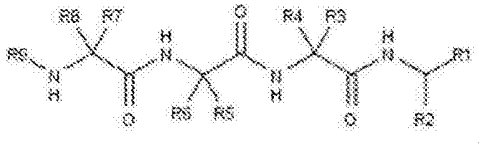
权利要求书36页 说明书125页 附图10页

(54)发明名称

PCSK9的小分子调节剂和其使用方法

(57)摘要

一种式(I)化合物：

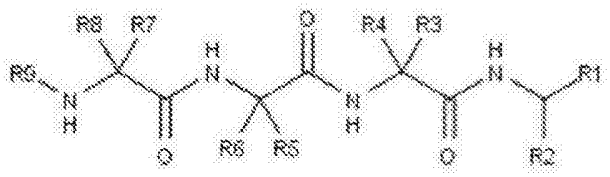


(I)

或其药学上可接受的盐、水合物、溶剂合物或外消旋混合物或立体异构体，和使用所述化合物预防或治疗LDL-胆固醇相关疾病或病症的方法，以及包含所述化合物的试剂盒和组合物。

CN 105431447 A

1. 一种式 (I) 化合物 :



(I)

或其药学上可接受的盐、水合物、溶剂合物或外消旋混合物或立体异构体,

其中 :

R1 为 -CH(OH)R_a或 -B(OR $_b$) (OR$_c$) ;

R2 为 -H、-CH_2R_d、-$CHR_d(R_e)$、-$CH_2(CH_2)_mC(O)R_j$、-$CH_2(CH_2)_mC(O)N(R_d)R_e$或 -CH $_2(CH_2)_mS(O)_nN(R_d)R_e$;

R3、R4、R5、R6、R7 和 R8 是相同或不同的且独立地为氢或以下基团中的一者 :C1-6 烷基、C1-6 卤代烷基、C1-6 硫代烷基、C1-6 氨基烷基、烯基、炔基、环烷基、杂环基、芳基和杂芳基,其中所述基团任选地经一个或多个 C1-6 烷基、C3-8 环烷基、C1-6 卤代烷基、芳基、杂芳基、-CN、-C(O)N(R_f)R_g、-C(O)OR$_f$、-C(R_f) (R_g)OR$_h$、-OR$_f$、-OC(O)OR$_f$、-OC(O)NR_f(R_g)、-SR_f、-S(O)$_n$R$_f$、-S(O)$_n$N(R_f)R_g、-S(O)$_n$N(R_f)C(O)R_g、-N(R_f)R_g、-N(R_f)C(O)R_g、--N(R_f)C(O)OR$_g$、-N(R_f)C(O)N(R_g) (R_h)、-N(R_f)S(O)$_n$R$_g$和 -N(R $_f$)S(O)N(R_g)R$_h$取代基取代 ;

当 (R3 和 R4) 或 (R5 和 R6) 或 (R7 和 R8) 不是氢时,括号内的对也可以与 -C(O)-、-CO_2-、-C(O)N(H)-、-C(O)N(R_x)-、-O-、-NH-、-N(R_x)-、-S-、-S(O)$_n$-、-S(O)$_n$N(H)-、-S(O)$_n$N(R_x)- 基团连接以形成环状结构 ;

R9 为 R_iC(O)-、R_iS(O)$_n$-、RiOC(O)-、R_iNHC(O)-、R_iNHS(O)$_n$-、R_k(R_1)NC(O)-、R_1(R_1)NS(O)$_n$-、R_mOR_iC(O)-、R_mC(O)R_iC(O)- 或一个或多个氨基酸残基 ;

R_a为 C1-3 烷基、C1-2 氟烷基或环丙基 ;

R_b和 R $_c$是相同或不同的且独立地为 H 或 C1-6 烷基或可以连接在一起以形成环状 5- 或 6- 员环结构,或与另外的脂族或芳族环体系稠合,所述环状 5- 或 6- 员环结构或脂族或芳族环体系任选地经一个或多个 C1-6 烷基和 / 或 C1-6 卤代烷基取代基取代 ;

R_d和 R $_e$是相同或不同的且独立地为 H 或以下基团中的一者 :C1-3 烷基、C1-3 卤代烷基或 C3-4 环烷基,或可以直接或与 -C(O)-、-C(O)O-、-C(O)N(R_x)--O-、-N(R_x)-、-S-、-S(O)$_n$- 或 -S(O)$_n$N(R_x)- 基团连接在一起以形成环状 3-8 员环结构 ;

R_f、R_g、R_h、R_k和 R $_1$是相同或不同的且独立地为 H 或以下基团中的一者 :C1-6 烷基、C1-6 卤代烷基或 C3-4 环烷基,或可以直接或与 -C(O)-、-C(O)O-、-C(O)N(R_x)-、-O-、-N(R_x)-、-S-、-S(O)$_n$- 或 -S(O)$_n$N(R_x)- 基团连接在一起以形成环状 3-8 员环结构 ;

R_i为 C1-10 烷基、C1-10 杂烷基、C3-8 环烷基、C1-10 卤代烷基、杂环基、芳基、杂芳基、C1-10 烷基 -C3-8 环烷基、C1-10 烷基 - 杂环基、C1-10 烷基 - 芳基、C1-10 烷基 - 杂芳基、C1-10 杂烷基 -C3-8 环烷基、C1-10 杂烷基 - 杂环基、C1-10 杂烷基 - 芳基或 C1-10 杂烷基 - 杂芳基,其中所述基团任选地经一个或多个卤素、C1-6 氨基烷基、C1-6 杂烷基、C1-6 烷基、C3-8 环烷基、C1-6 卤代烷基、芳基、杂芳基和杂环基取代 ;

R_m为 C1-10 烷基、C1-10 杂烷基、C3-8 环烷基、C1-10 卤代烷基、杂环基、芳基或杂芳基,

其中所述基团任选地经一个或多个卤素、C1-6 氨基烷基、C1-6 杂烷基、C1-6 烷基、C3-8 环烷基、C1-6 卤代烷基、芳基、杂芳基和杂环基取代；

R_j为 OR_d或 $N(R_d)(R_e)$；

R_x为 H、C1-6 烷基、C1-6 卤代烷基、C3-4 环烷基、$-C(O)R_y$、$-C(O)OR_y$、$-C(O)NH_2$、$-C(O)NH(R_y)$ 或 $-C(O)NHS(O)_nR_y$；

R_y为 C1-6 烷基、C1-6 卤代烷基或 C3-4 环烷基；

m 为值 0 或 1 的整数；且

n 为值 1 或 2 的整数，

限制条件为：

1) 当 R1 为 $-CH(OH)R_a$时，R2 为 $-CHR_d(R_e)$、$-CH_2(CH_2)_mC(O)R_j$、$-CH_2(CH_2)_mC(O)N(R_d)R_e$ 或 $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$；且

2) 当 R1 为 $-B(OR_b)(OR_c)$ 时，R2 为 -H、$-CH_3$、$-CHR_d(R_e)$、$-CH_2(CH_2)_mC(O)R_j$、$-CH_2(CH_2)_mC(O)N(R_d)R_e$或 $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$。

2. 根据权利要求 1 所述的化合物，其中：

a. R1 为 $-B(OR_b)(OR_c)$ 或 $-CH(OH)R_a$；

b. R2 为 H 或 $-CH_2(CH_2)_mC(O)R_j$；

c. R3 为 H 或取代的或未取代的 C1-6 烷基；

d. R4 为 H、取代的或未取代的 C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷基；

e. R5 为 H；

f. R6 为 H、取代的或未取代的 C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷烃；

g. R7 为 H；

h. R8 为 H、取代的或未取代的 C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷基；且 / 或

i. R9 为 $RiOC(O)-$、$RiC(O)-$、$R_mOR_iC(O)-$、$R_mC(O)R_iC(O)-$ 或 $R_iS(O)_n-$。

3. 根据权利要求 1 或 2 所述的化合物，其中：

a. R1 为 $-B(OR_b)(OR_c)$ 或 $-CH(OH)R_a$；

b. R2 为 H 或 $-CH_2(CH_2)_mC(O)R_j$；

c. R3 为 H 或取代的或未取代的 C1-6 烷基；

d. R4 为 H、取代的或未取代的 C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷基；

e. R5 为 H；

f. R6 为 H、取代的或未取代的 C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷烃；

g. R7 为 H；

h. R8 为 H、取代的或未取代的 C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷基；且

i. R9 为 $RiOC(O)-$、$RiC(O)-$、$R_mOR_iC(O)-$、$R_mC(O)R_iC(O)-$ 或 $R_iS(O)_n-$。

4. 根据权利要求 1 至 3 中任一权利要求所述的化合物，其中：

a. R1 为 $-B(OR_b)(OR_c)$。

5. 根据权利要求 4 所述的化合物，其中：

a. Rb 和 Rc 为 H。

6. 根据权利要求 4 所述的化合物，其中：

a. Rb 和 Rc 连接以形成环状 5 员环结构或与脂族环体系稠合。

7. 根据权利要求 6 所述的化合物，其中：

a. R1 为 2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 $[6.1.1.0^{2,6}]$ 癸 -4- 基。

8. 根据权利要求 1 至 3 中任一权利要求所述的化合物，其中：

a. R1 为 $-CH(OH)R_a$。

9. 根据权利要求 1 至 3 或 8 中任一权利要求所述的化合物，其中：

a. Ra 为 C1-2 氟烷基。

10. 根据权利要求 9 所述的化合物，其中：

a. Ra 为 $-CF_3$。

11. 根据权利要求 9 所述的化合物，其中：

a. Ra 为 $-CH_2F$。

12. 根据权利要求 8 所述的化合物，其中：

a. Ra 为 C1-3 烷基。

13. 根据权利要求 12 所述的化合物，其中：

a. Ra 为 $-CH_3$。

14. 根据权利要求 1 至 13 中任一权利要求所述的化合物，其中：

a. R2 为 H。

15. 根据权利要求 1 至 13 中任一权利要求所述的化合物，其中：

a. R2 为 $-CH_2(CH_2)_mC(O)R_j$。

16. 根据权利要求 15 所述的化合物，其中：

a. R_j 为 OR_d。

17. 根据权利要求 16 所述的化合物，其中：

a. Rd 为 CH_3。

18. 根据权利要求 15 所述的化合物，其中：

a. Rj 为 NH_2。

19. 根据权利要求 1 至 18 中任一权利要求所述的化合物，其中：

a. R3 为 H。

20. 根据权利要求 1 至 18 中任一权利要求所述的化合物，其中：

a. R3 为取代的或未取代的 C1-6 烷基。

21. 根据权利要求 20 所述的化合物，其中：

a. R3 为未取代的 C1-6 烷基。

22. 根据权利要求 21 所述的化合物，其中：

a. R3 为 CH_3。

23. 根据权利要求 1 至 22 中任一权利要求所述的化合物，其中：

a. R4 为 H。

24. 根据权利要求 1 至 22 中任一权利要求所述的化合物，其中：

a. R4 为取代的或未取代的 C1-6 烷基。

25. 根据权利要求 24 所述的化合物，其中：

a. R4 为未取代的 C1-6 烷基。

26. 根据权利要求 25 所述的化合物，其中：

a. R4 为 $-CH_3$。

27. 根据权利要求 25 所述的化合物，其中：

a. R4 为 CH_3CH_2-。

28. 根据权利要求 25 所述的化合物，其中：

a. R4 为 $-CH_2CH(CH_3)_2$。

29. 根据权利要求 24 所述的化合物，其中：

a. R4 为取代的 C1-6 烷基。

30. 根据权利要求 29 所述的化合物，其中：

a. R4 为经芳基取代的 C1-6 烷基。

31. 根据权利要求 30 所述的化合物，其中：

a. R4 为苯基 $-CH_2-$。

32. 根据权利要求 29 所述的化合物，其中：

a. R4 为 $-(CH_2)_2C(O)NH_2$。

33. 根据权利要求 1 至 22 中任一权利要求所述的化合物，其中：

a. R4 为取代的或未取代的 C1-6 硫代烷基。

34. 根据权利要求 33 所述的化合物，其中：

a. R4 为 $CH_3S(CH_2)_2-$。

35. 根据权利要求 1 至 22 中任一权利要求所述的化合物，其中：

a. R4 为取代的或未取代的芳基。

36. 根据权利要求 35 所述的化合物，其中：

a. R4 为苯基。

37. 根据权利要求 1 至 22 中任一权利要求所述的化合物，其中：

a. R4 为环烷基。

38. 根据权利要求 1 至 37 中任一权利要求所述的化合物，其中：

a. R6 为 H。

39. 根据权利要求 1 至 37 中任一权利要求所述的化合物，其中：

a. R6 为取代的或未取代的 C1-6 烷基。

40. 根据权利要求 39 所述的化合物，其中：

a. R6 为取代的 C1-6 烷基。

41. 根据权利要求 40 所述的化合物，其中：

a. R6 为经芳基取代的 C1-6 烷基。

42. 根据权利要求 41 所述的化合物，其中：

a. R6 为 - 烷基 - 苯基。

43. 根据权利要求 42 所述的化合物，其中：

a. R6 为 $-CH_2-$ 苯基。

44. 根据权利要求 42 或 43 所述的化合物，其中：

a. R6 为 $-CH_2-$ 羟苯基。

45. 根据权利要求 42 所述的化合物，其中：

a. R6 为 $-(CH_2)_2-$ 苯基。

46. 根据权利要求 41 所述的化合物，其中：

a. R6 为 $-CH_2-$ 吲哚。

47. 根据权利要求 39 所述的化合物，其中：

a. R6 为未取代的 C1-6 烷基。

48. 根据权利要求 47 所述的化合物，其中：

a. R6 为未取代的 C1-6 烷基。

49. 根据权利要求 48 所述的化合物，其中：

a. R6 为 CH_3。

50. 根据权利要求 48 所述的化合物，其中：

a. R6 为 $-CH(CH_3)_2$。

51. 根据权利要求 1 至 37 中任一权利要求所述的化合物，其中：

a. R6 为取代的或未取代的 C1-6 硫代烷基。

52. 根据权利要求 51 所述的化合物，其中：

a. R6 为 $CH_3S(CH_2)_2-$。

53. 根据权利要求 1 至 37 中任一权利要求所述的化合物，其中：

a. R6 为取代的或未取代的芳基。

54. 根据权利要求 53 所述的化合物，其中：

a. R6 为苯基。

55. 根据权利要求 1 至 37 中任一权利要求所述的化合物，其中：

a. R6 为取代的或未取代的环烷基。

56. 根据权利要求 55 所述的化合物，其中：

a. R6 为苯并环戊基。

57. 根据权利要求 1 至 56 中任一权利要求所述的化合物，其中：

a. R8 为 H。

58. 根据权利要求 1 至 56 中任一权利要求所述的化合物，其中：

a. R8 为取代的或未取代的 C1-6 烷基。

59. 根据权利要求 58 所述的化合物，其中：

a. R8 为未取代的 C1-6 烷基。

60. 根据权利要求 59 所述的化合物，其中：

a. R8 为 $-CH(CH_3)_2$。

61. 根据权利要求 59 所述的化合物，其中：

a. R8 为 $-CH_3$。

62. 根据权利要求 58 所述的化合物，其中：

a. R8 为取代的 C1-6 烷基。

63. 根据权利要求 62 所述的化合物，其中：

a. R8 为经芳基取代的 C1-6 烷基。

64. 根据权利要求 63 所述的化合物，其中：

a. R8 为 $-CH_2-$ 苯基。

65. 根据权利要求 1 至 56 中任一权利要求所述的化合物，其中：

a. R8 为取代的或未取代的 C1-6 硫代烷基。

66. 根据权利要求 65 所述的化合物，其中：

a. R8 为 $CH_3S(CH_2)_2-$。

67. 根据权利要求 1 至 56 中任一权利要求所述的化合物，其中：

a. R8 为取代的或未取代的芳基。

68. 根据权利要求 67 所述的化合物，其中：

a. R8 为未取代的芳基。

69. 根据权利要求 68 所述的化合物，其中：

a. R8 为苯基。

70. 根据权利要求 1 至 56 中任一权利要求所述的化合物，其中：

a. R8 为取代的或未取代的环烷基。

71. 根据权利要求 70 所述的化合物，其中：

a. R8 为未取代的环烷基。

72. 根据权利要求 71 所述的化合物，其中：

a. R8 为环戊基。

73. 根据权利要求 71 所述的化合物，其中：

a. R8 为环丙基。

74. 根据权利要求 1 至 73 中任一权利要求所述的化合物，其中：

a. R9 为 RiOC(O)-。

75. 根据权利要求 74 所述的化合物，其中：

a. Ri 为取代的或未取代的 C1-10 烷基。

76. 根据权利要求 75 所述的化合物，其中：

a. Ri 为未取代的 C1-10 烷基 -。

77. 根据权利要求 76 所述的化合物，其中：

a. Ri 为 CH_3-。

78. 根据权利要求 75 所述的化合物，其中：

a. Ri 为取代的 C1-10 烷基 -。

79. 根据权利要求 78 所述的化合物，其中：

a. Ri 为苯基 $-CH_2-$。

80. 根据权利要求 1 至 73 中任一权利要求所述的化合物，其中：

a. R9 为 RiC(O)-。

81. 根据权利要求 80 所述的化合物，其中：

a. Ri 为取代的或未取代的芳基或取代的或未取代的杂芳基。

82. 根据权利要求 81 所述的化合物，其中：

a. Ri 为取代的或未取代的芳基。

83. 根据权利要求 82 所述的化合物，其中：

a. Ri 为取代的芳基。

84. 根据权利要求 83 所述的化合物，其中：

a. Ri 为芳基 - 苯基 -。

85. 根据权利要求 84 所述的化合物，其中：

a. Ri 为苯基 - 苯基 -。

86. 根据权利要求 84 所述的化合物，其中：

a. Ri 为杂芳基 - 苯基 -。

87. 根据权利要求 86 所述的化合物，其中：

a. Ri 为双吖丙啶 - 苯基 -。

88. 根据权利要求 87 所述的化合物，其中：

a. Ri 为三氟甲基 - 双吖丙啶 - 苯基 -。

89. 根据权利要求 86 所述的化合物，其中：

a. Ri 为吡啶 - 苯基 -。

90. 根据权利要求 86 所述的化合物，其中：

a. Ri 为噁二唑 - 苯基 -。

91. 根据权利要求 83 所述的化合物，其中：

a. Ri 为杂环基 - 苯基 -。

92. 根据权利要求 91 所述的化合物，其中：

a. Ri 为吗啉 - 苯基 -。

93. 根据权利要求 83 所述的化合物，其中：

a. Ri 为烷基 - 苯基 -。

94. 根据权利要求 93 所述的化合物，其中：

a. Ri 为 $(CH_3)_2CH$- 苯基 -。

95. 根据权利要求 93 所述的化合物，其中：

a. Ri 为 $OHCH_2$- 苯基 -。

96. 根据权利要求 83 所述的化合物，其中：

a. Ri 为氟苯基。

97. 根据权利要求 82 所述的化合物，其中：

a. Ri 为未取代的芳基。

98. 根据权利要求 97 所述的化合物，其中：

a. Ri 为苯基。

99. 根据权利要求 81 所述的化合物，其中：

a. Ri 为取代的或未取代的杂芳基。

100. 根据权利要求 99 所述的化合物，其中：

a. Ri 为取代的杂芳基。

101. 根据权利要求 100 所述的化合物，其中：

a. Ri 为芳基 - 杂芳基 -。

102. 根据权利要求 101 所述的化合物，其中：

a. Ri 为苯基 - 杂芳基 -。

103. 根据权利要求 102 所述的化合物，其中：

a. Ri 为苯基 - 吡唑 -。

104. 根据权利要求 102 所述的化合物，其中：

a. Ri 为苯基 - 甲基吡唑 -。

105. 根据权利要求 102 所述的化合物，其中：

a. Ri 为苯基 - 噻唑 -。

106. 根据权利要求 102 所述的化合物，其中：

a. Ri 为苯基 - 吡啶 -。

107. 根据权利要求 102 所述的化合物，其中：

a. Ri 为苯基 - 呋咕 -。

108. 根据权利要求 100 所述的化合物，其中：

a. Ri 为杂芳基 - 杂芳基 -。

109. 根据权利要求 108 所述的化合物，其中：

a. Ri 为吡啶 - 异噻唑 -。

110. 根据权利要求 99 所述的化合物，其中：

a. Ri 为未取代的杂芳基。

111. 根据权利要求 110 所述的化合物，其中：

a. Ri 为吡啶。

112. 根据权利要求 111 所述的化合物，其中：

a. Ri 为吡嗪。

113. 根据权利要求 112 所述的化合物，其中：

a. Ri 为吲哚。

114. 根据权利要求 80 所述的化合物，其中：

a. Ri 为取代的或未取代的 C1-10 烷基 -。

115. 根据权利要求 114 所述的化合物，其中：

a. Ri 为取代的 C1-10 烷基 -。

116. 根据权利要求 115 所述的化合物，其中：

a. Ri 为芳基 -C1-10 烷基 -。

117. 根据权利要求 116 所述的化合物，其中：

a. Ri 为苯基 -C1-10 烷基 -。

118. 根据权利要求 117 所述的化合物，其中：

a. Ri 为苯基 - 炔 -。

119. 根据权利要求 117 所述的化合物，其中：

a. Ri 为苯基 $-CH_2-$。

120. 根据权利要求 119 所述的化合物，其中：

a. Ri 为氟甲氧基 - 苯基 $-CH_2-$。

121. 根据权利要求 120 所述的化合物，其中：

a. Ri 为三氟甲氧基 - 苯基 $-CH_2-$。

122. 根据权利要求 121 所述的化合物，其中：

a. Ri 为 4-(三氟甲氧基) 苯基 $-CH_2-$。

123. 根据权利要求 117 所述的化合物，其中：

a. Ri 为氟烷基 - 双吖丙啶 - 苯基 -(C1-10 烷基)-。

124. 根据权利要求 123 所述的化合物，其中：

a. Ri 为三氟甲基 - 双吖丙啶 - 苯基 $-CH_2-$。

125. 根据权利要求 124 所述的化合物，其中：

a. Ri 为 4-[3- 三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 -。

126. 根据权利要求 125 所述的化合物，其中：

a. Ri 为 3-[3- 三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 -。

127. 根据权利要求 114 所述的化合物，其中：

a. Ri 为未取代的 C1-10 烷基 -。

128. 根据权利要求 127 所述的化合物，其中：

a. Ri 为 $CH_3(CH_2)_8-$。

129. 根据权利要求 1 至 73 中任一权利要求所述的化合物，其中：

a. R9 为 $R_mOR_iC(O)-$。

130. 根据权利要求 129 所述的化合物，其中：

a. R_i 为取代的或未取代的 C1-10 烷基。

131. 根据权利要求 130 所述的化合物，其中：

a. R_i 为 $-CH_2-$。

132. 根据权利要求 129 至 131 中任一权利要求所述的化合物，其中：

a. R_m 为取代的或未取代的 C1-10 烷基。

133. 根据权利要求 132 所述的化合物，其中：

a. R_m 为 $-CH_2-$。

134. 根据权利要求 133 所述的化合物，其中：

a. R_m 为芳基 $-CH_2-$。

135. 根据权利要求 134 所述的化合物，其中：

a. R_m 为苯基 $-CH_2-$。

136. 根据权利要求 129 至 131 中任一权利要求所述的化合物，其中：

a. R_m 为取代的或未取代的芳基。

137. 根据权利要求 136 所述的化合物，其中：

a. R_m 为苯基。

138. 根据权利要求 1 至 73 中任一权利要求所述的化合物，其中：

a. R9 为 $R_mC(O)R_iC(O)-$。

139. 根据权利要求 138 所述的化合物，其中：

a. R_m 为杂芳基。

140. 根据权利要求 139 所述的化合物，其中：

a. R_m为吗啉。

141. 根据权利要求 138 至 140 中任一权利要求所述的化合物，其中：

a. R_i为芳基。

142. 根据权利要求 141 所述的化合物，其中：

a. R_i为苯基。

143. 根据权利要求 1 至 73 中任一权利要求所述的化合物，其中：

a. R9 为 $R_iS(O)_n$-。

144. 根据权利要求 143 所述的化合物，其中：

a. R_i为取代的或未取代的芳基。

145. 根据权利要求 144 所述的化合物，其中：

a. R_i为取代的芳基。

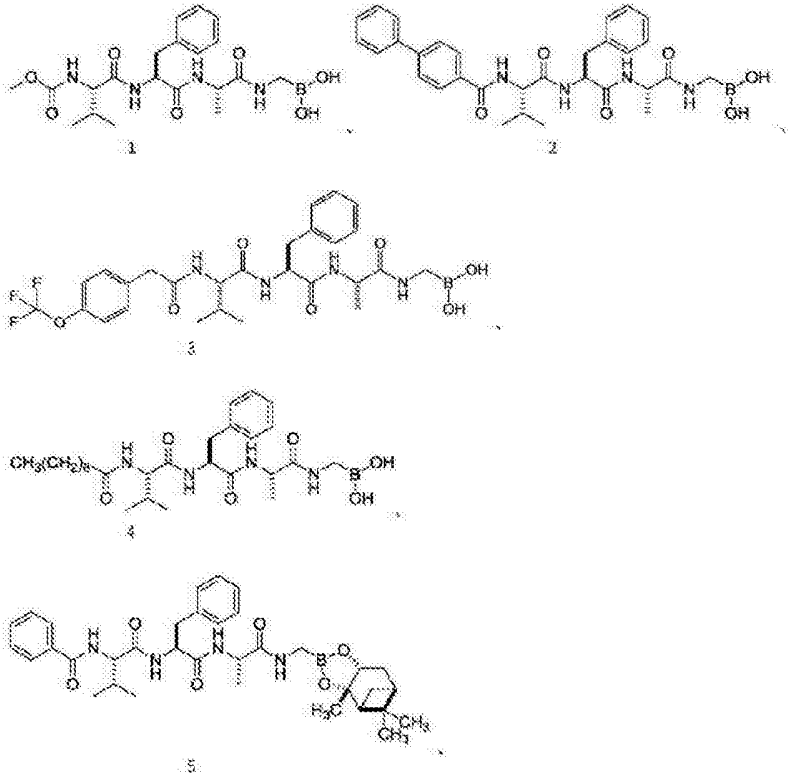
146. 根据权利要求 145 所述的化合物，其中：

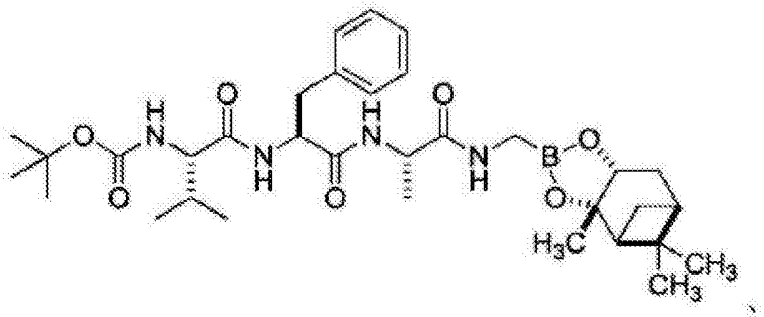
a. R_i为取代的苯基。

147. 根据权利要求 146 所述的化合物，其中：

a. R_i为苯基 - 苯基。

148. 根据权利要求 1 所述的化合物，其为：





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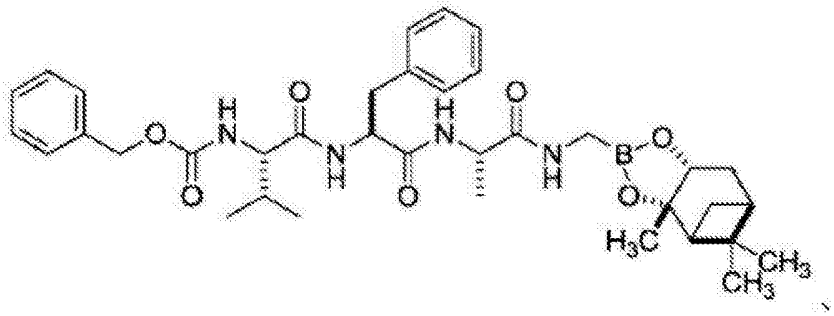

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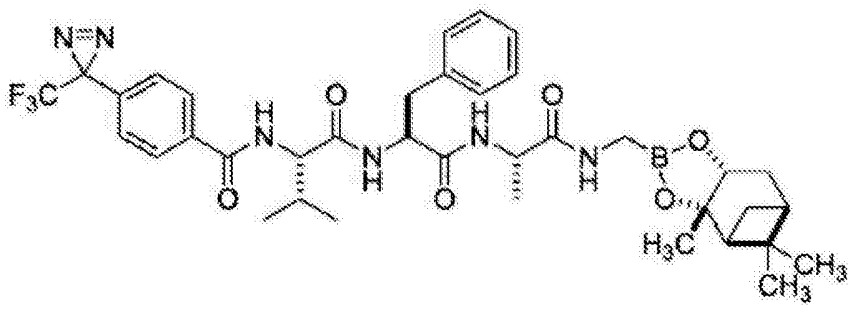

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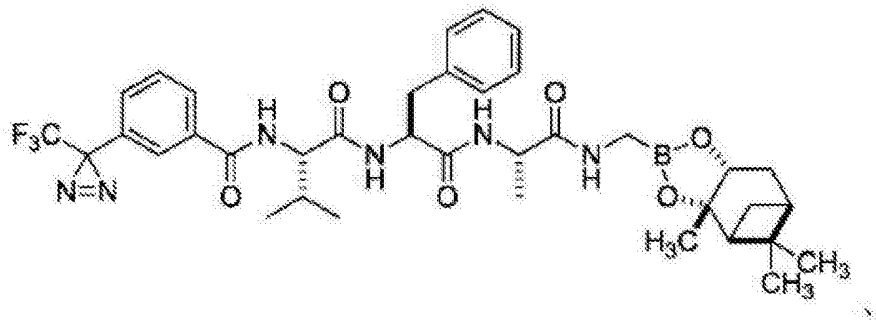

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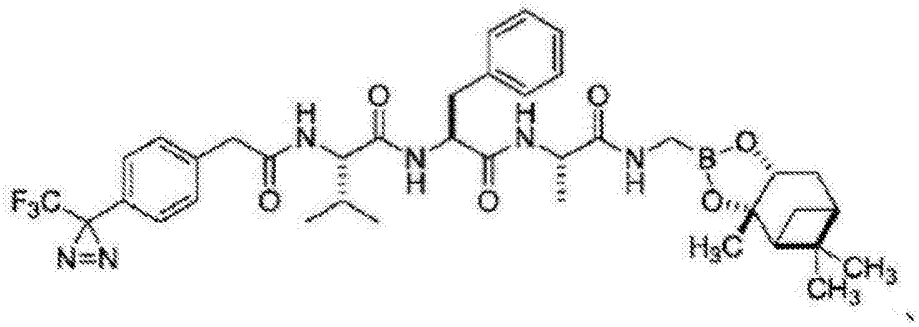

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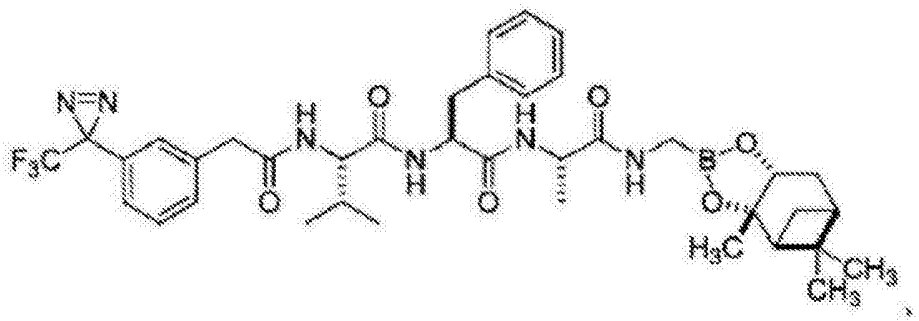

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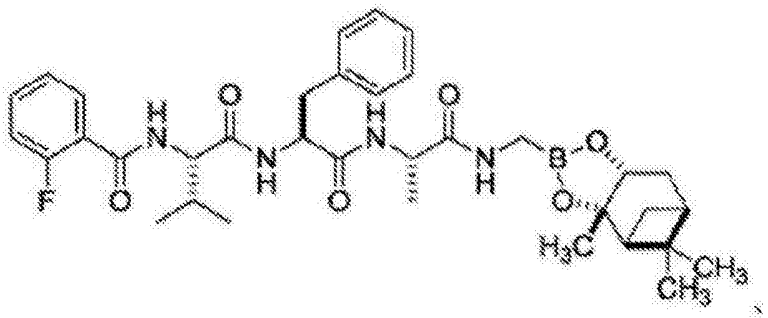

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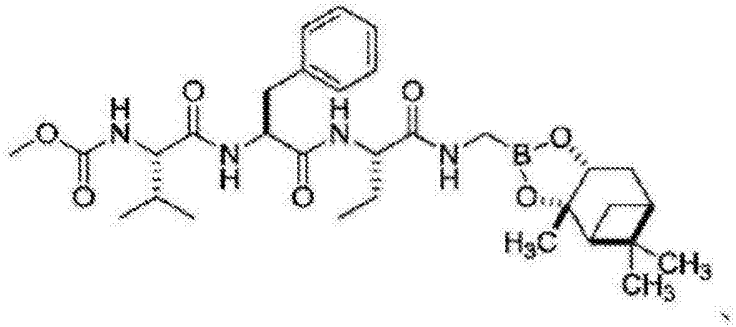

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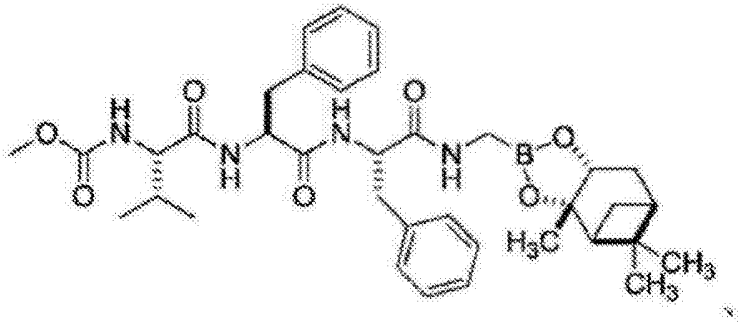

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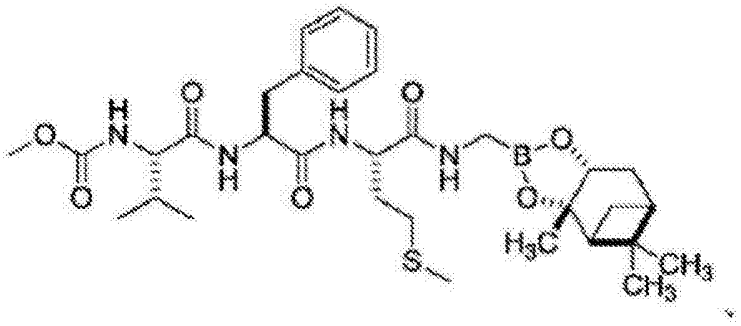

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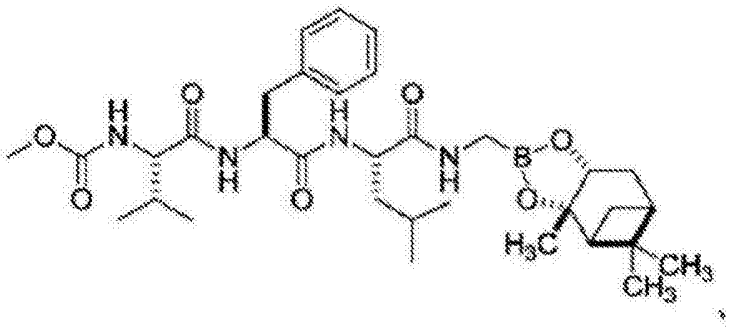

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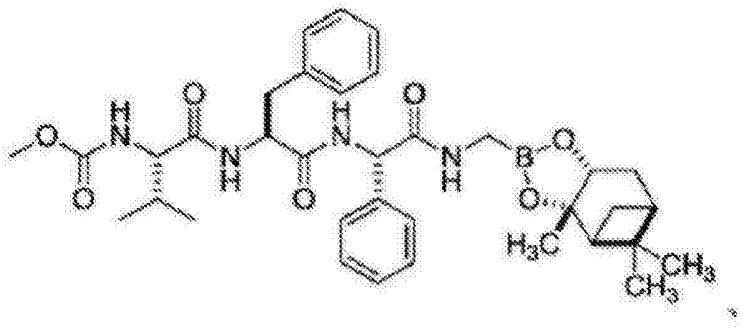

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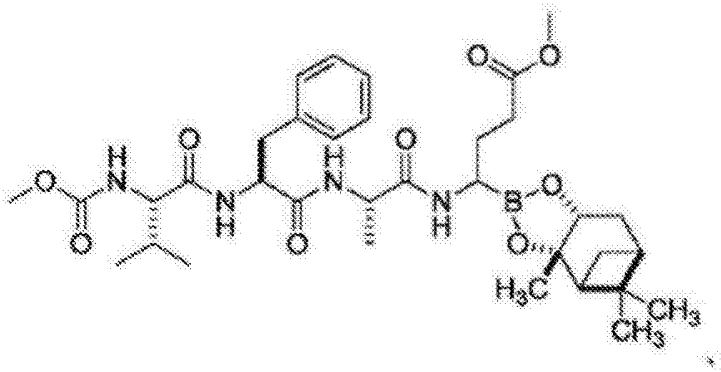

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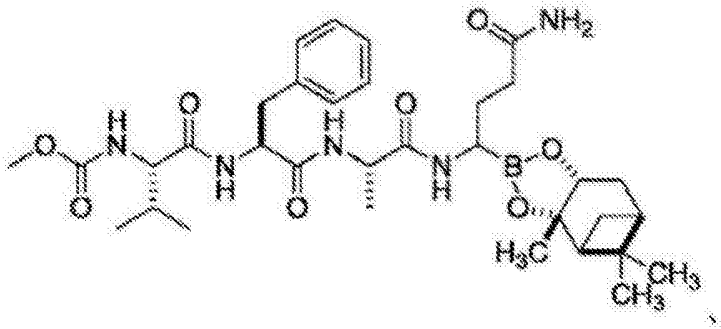

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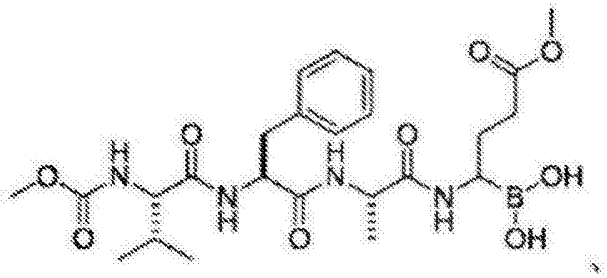

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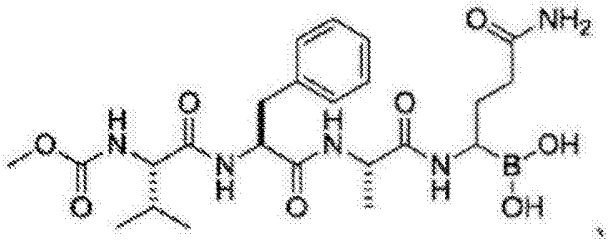

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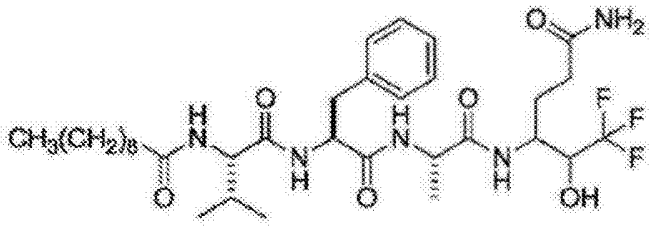

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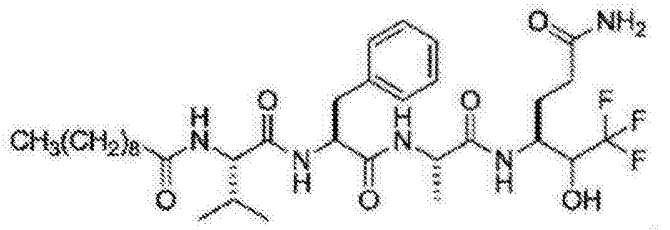

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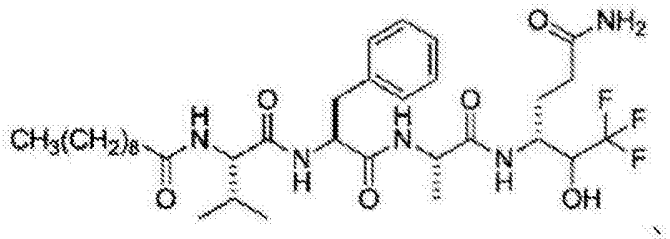

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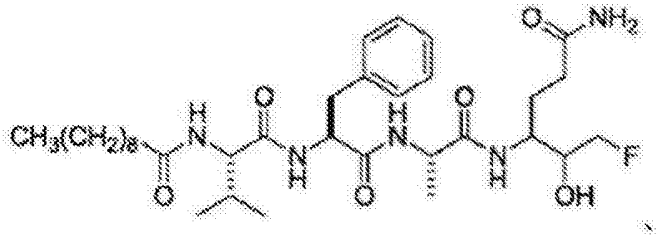

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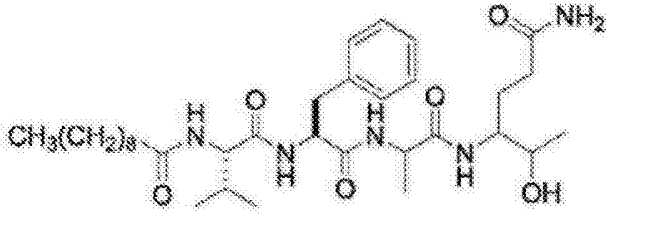

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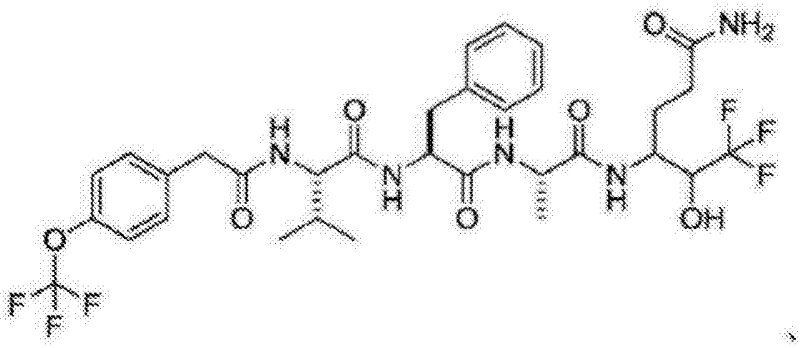

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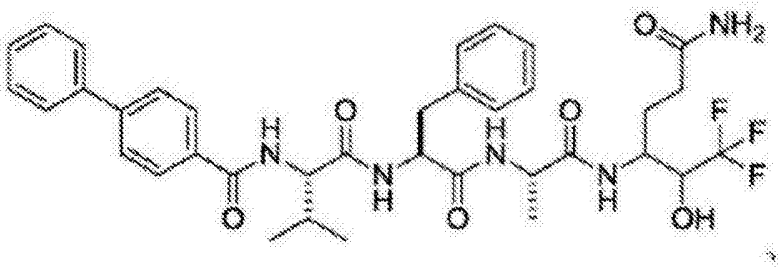

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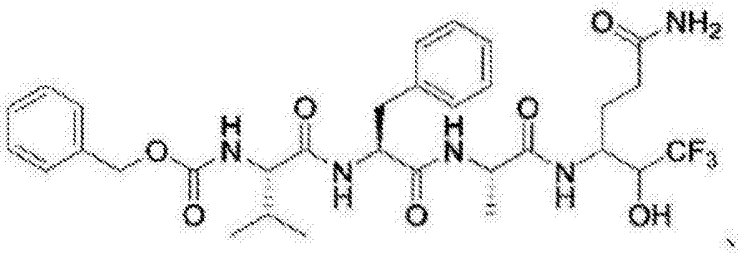

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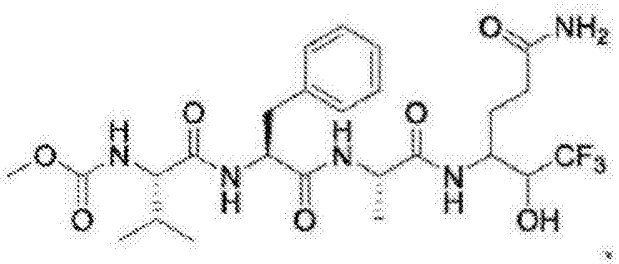

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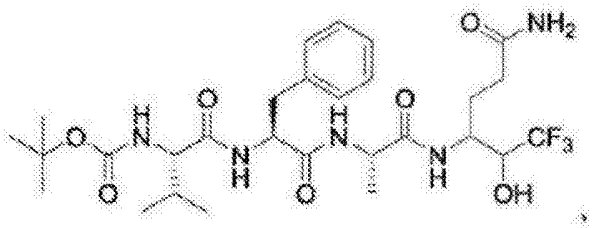

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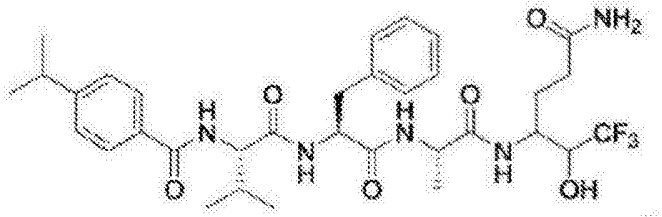

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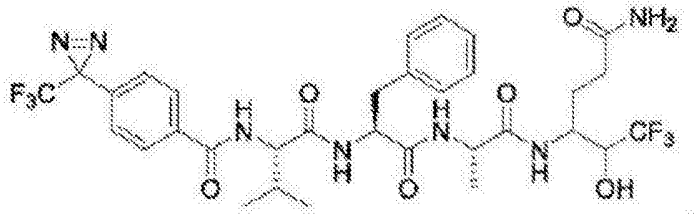

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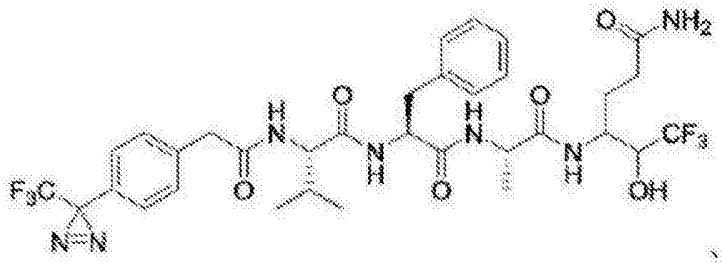

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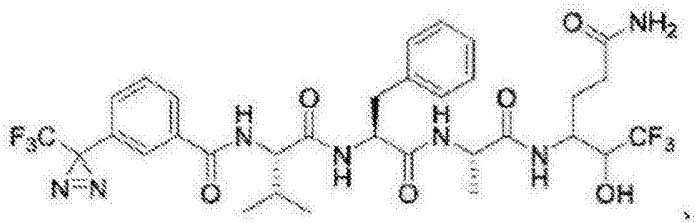

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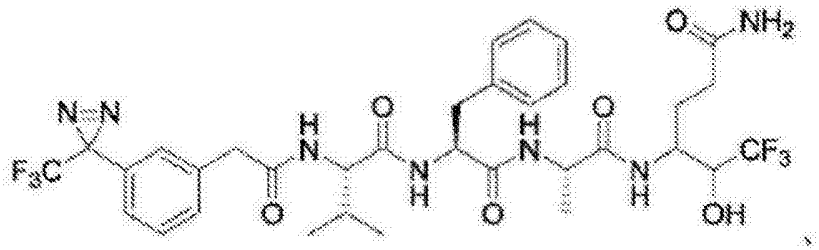

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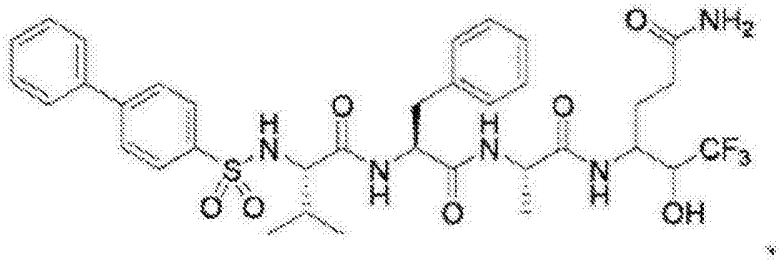

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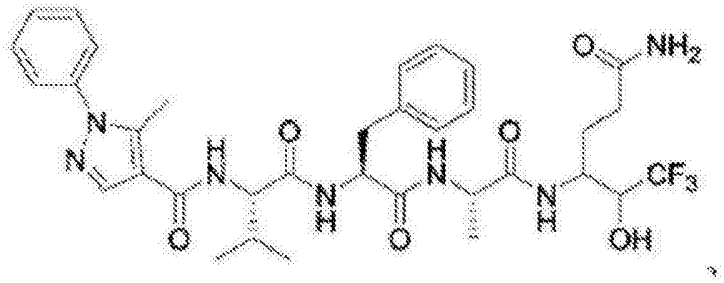

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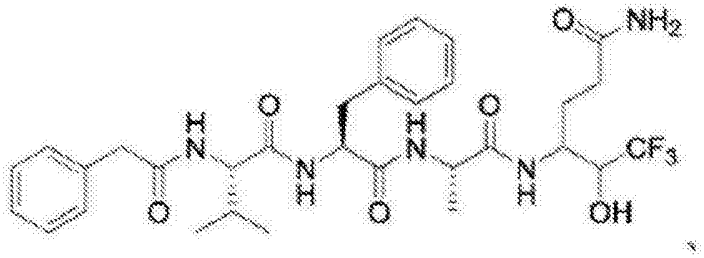

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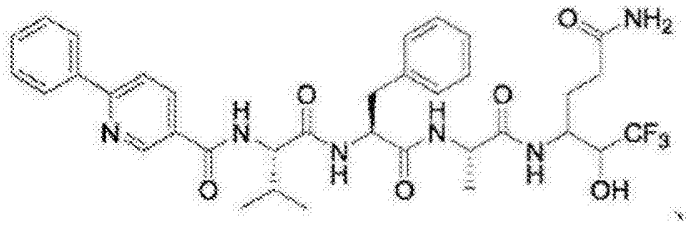

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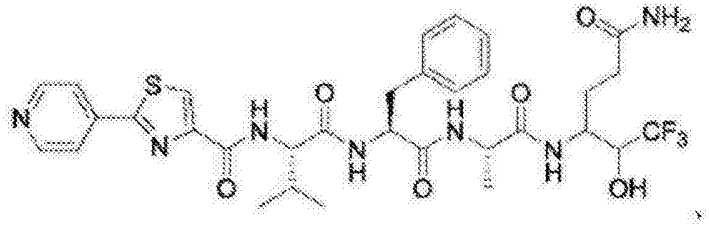

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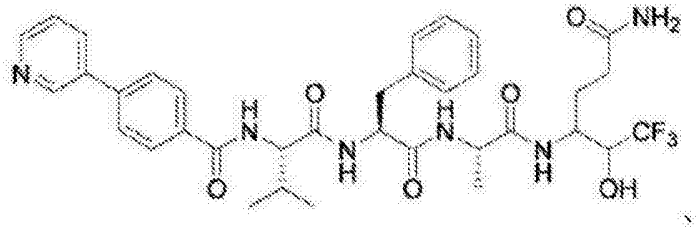

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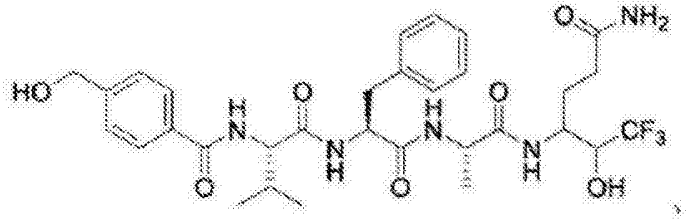

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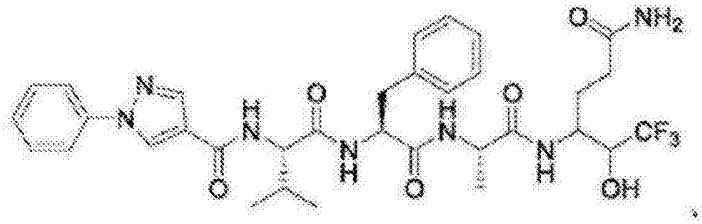

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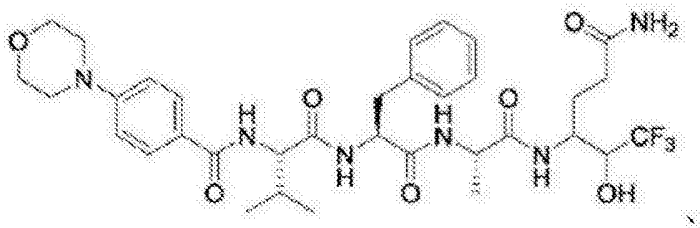

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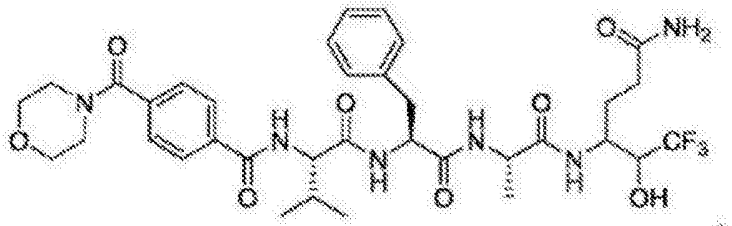

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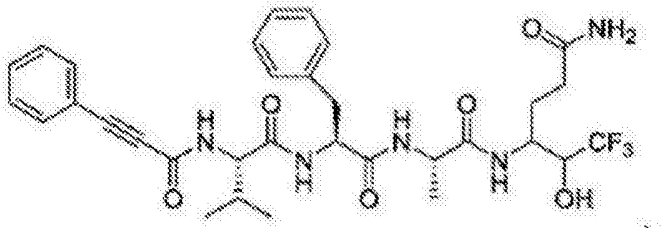

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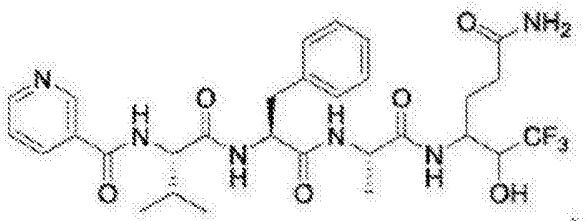

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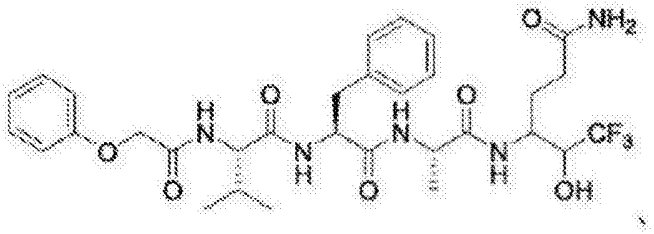

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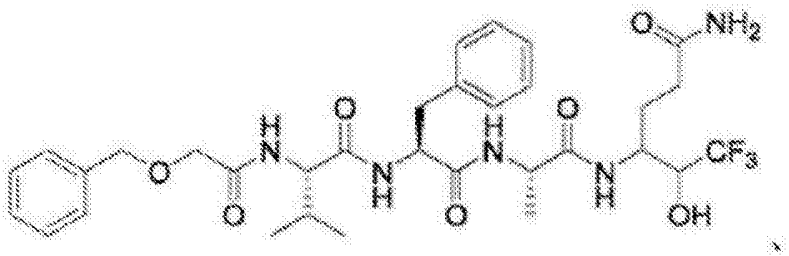

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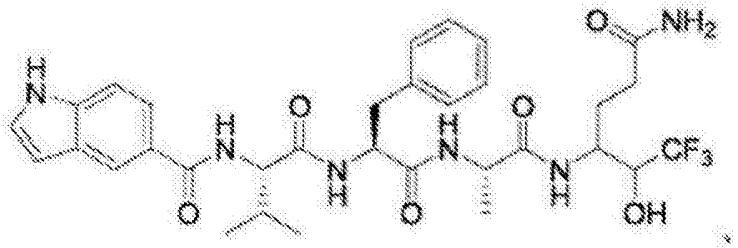

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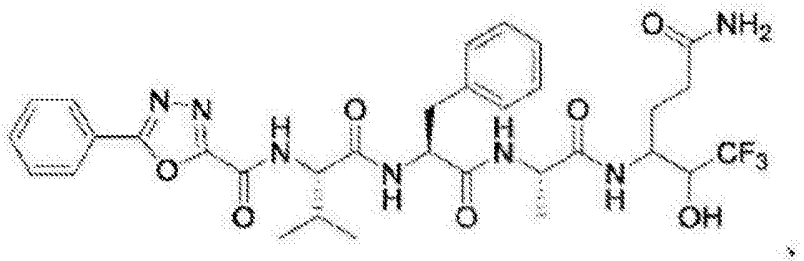

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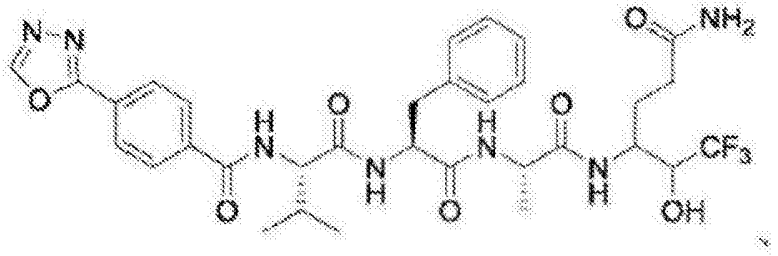

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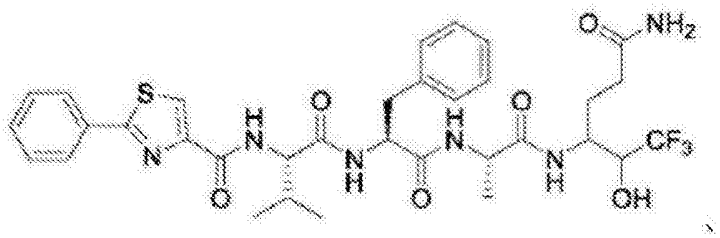

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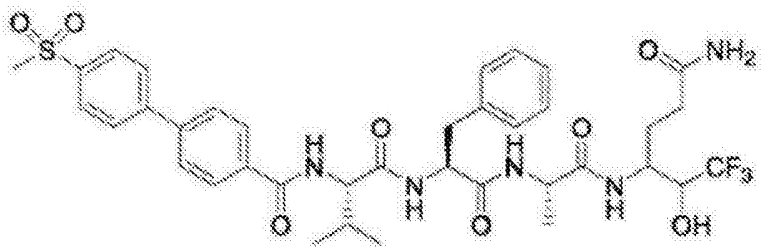

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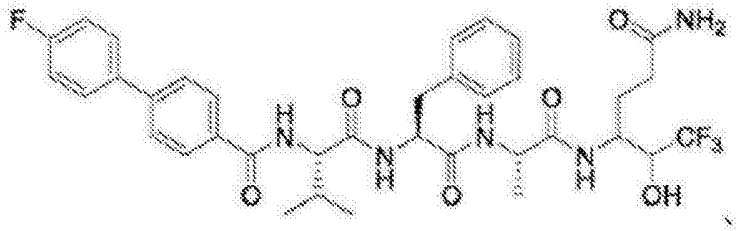

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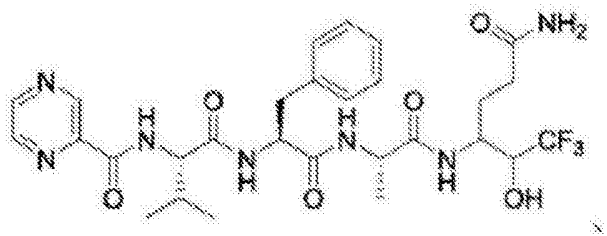

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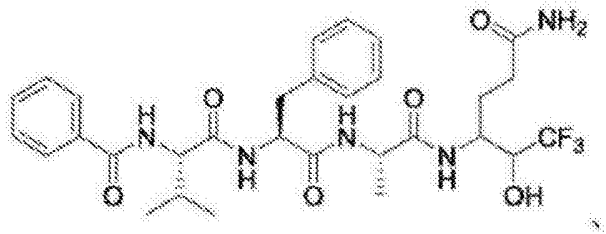

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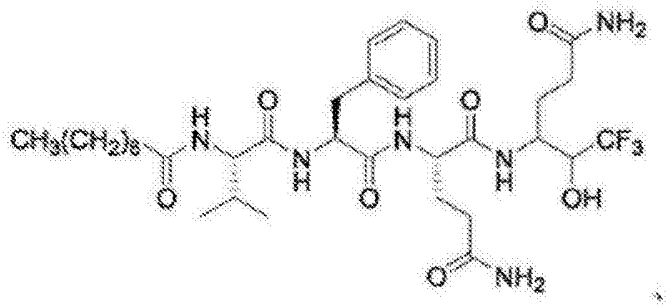

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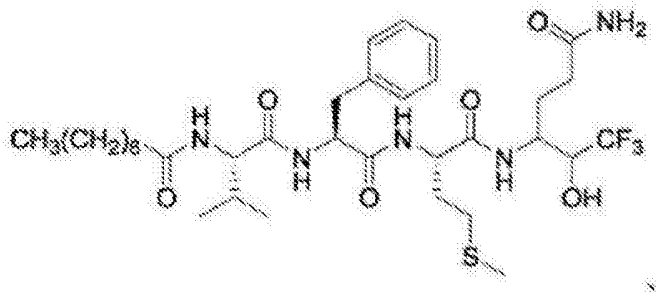

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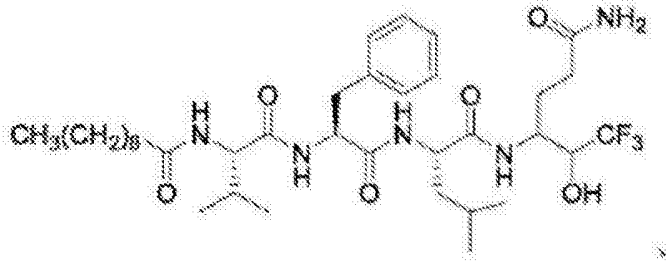

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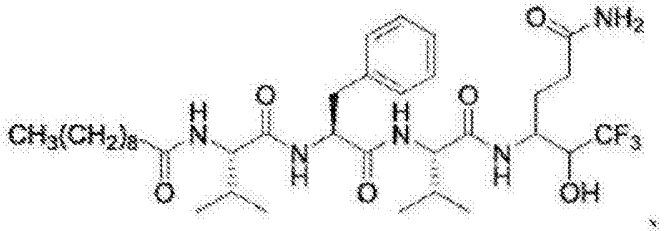

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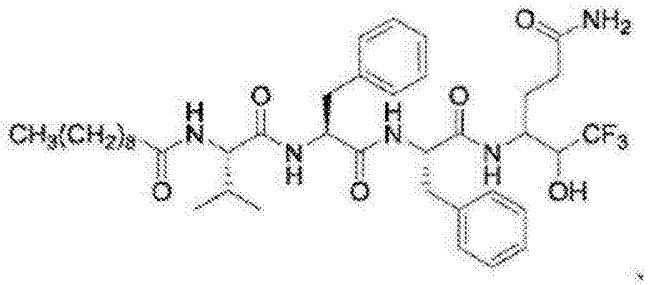

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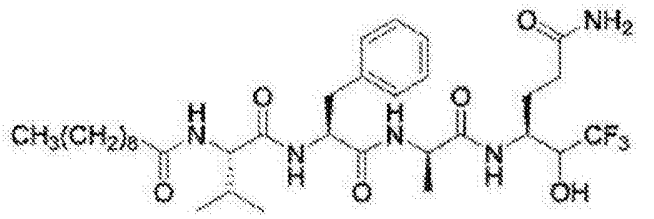

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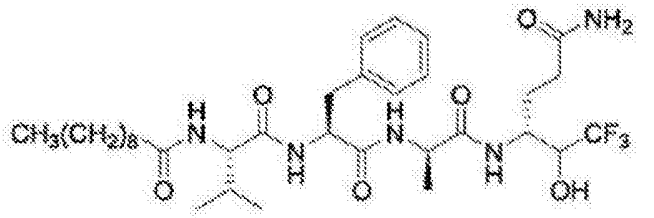

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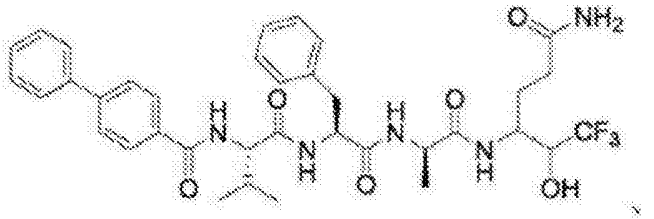

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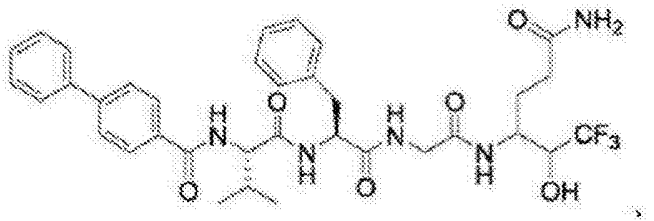

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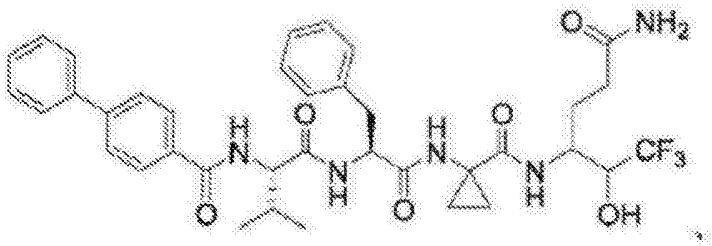

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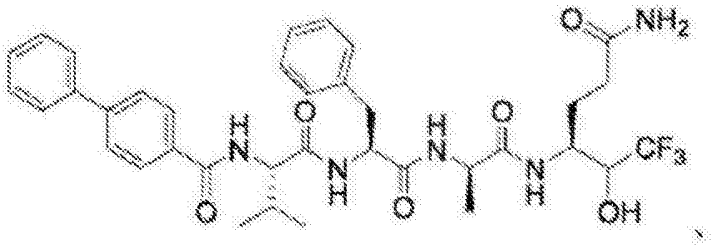

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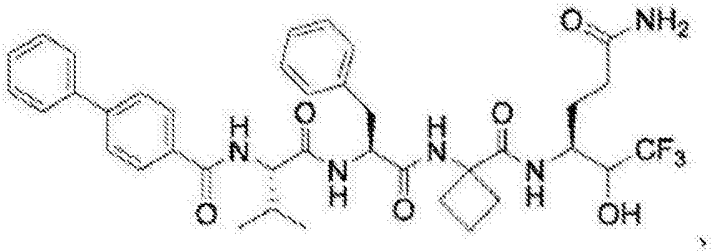

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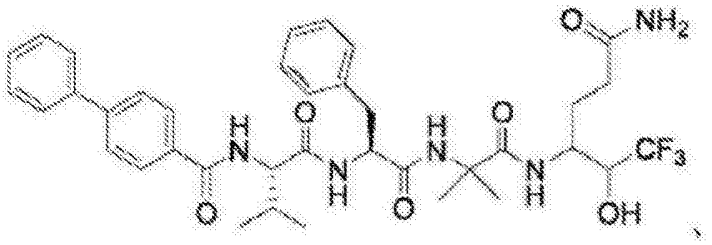

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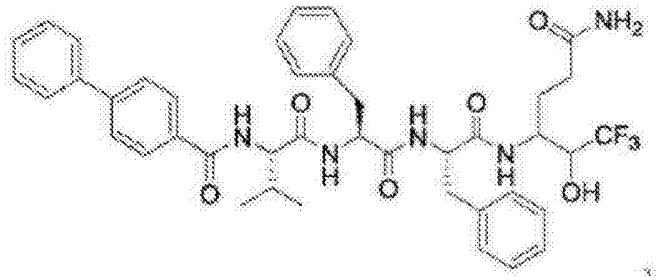

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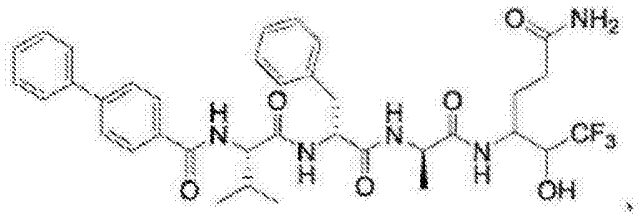

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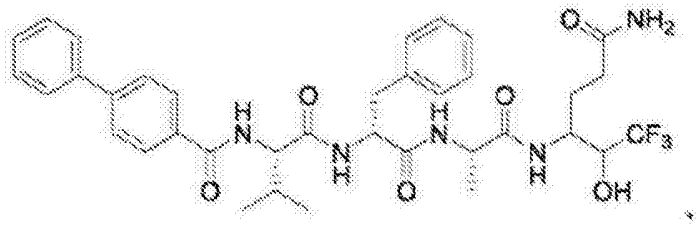

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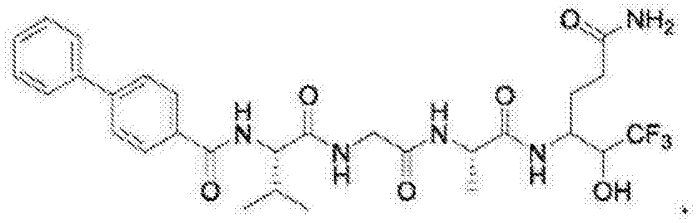

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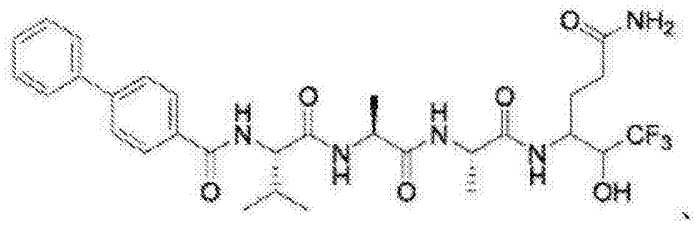

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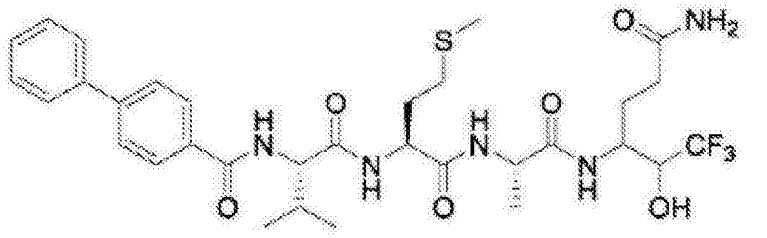

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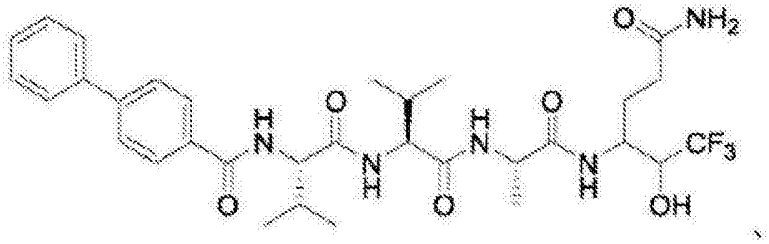

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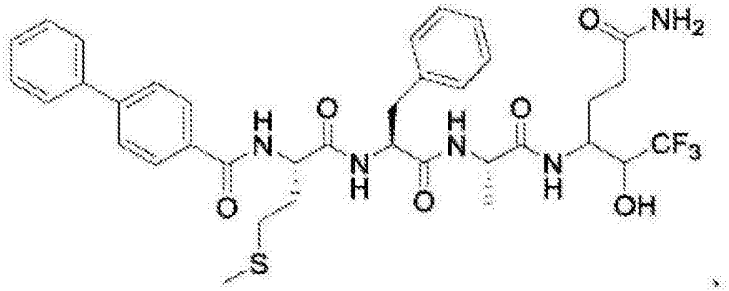

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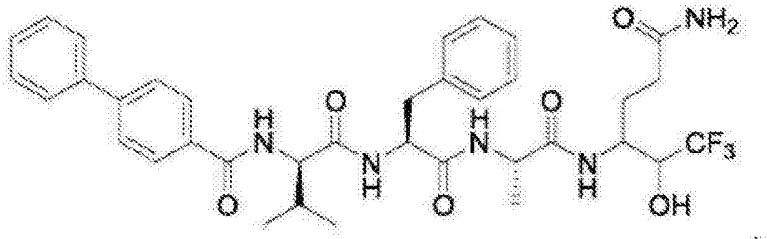

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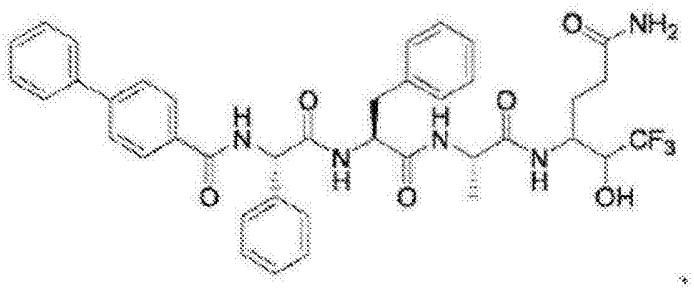

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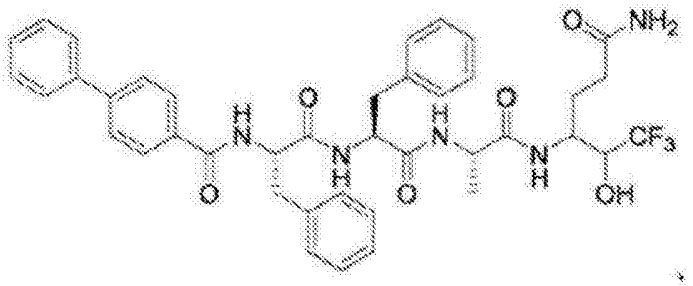

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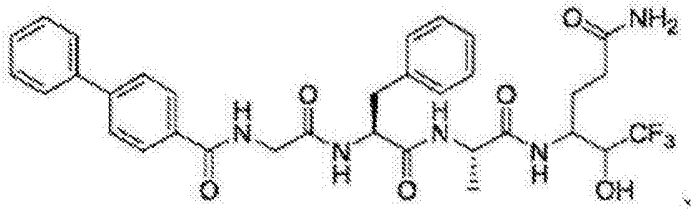

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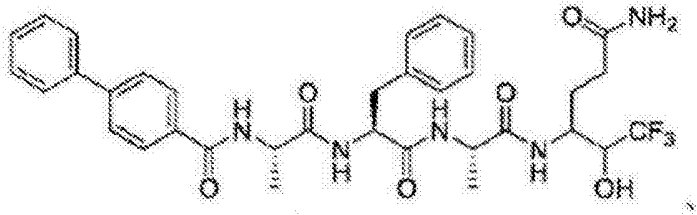

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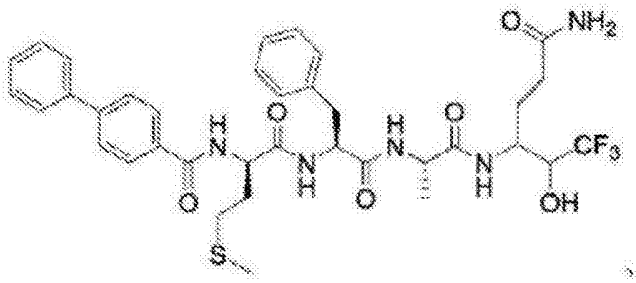

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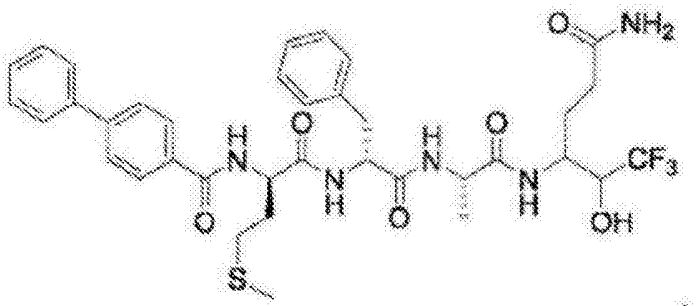

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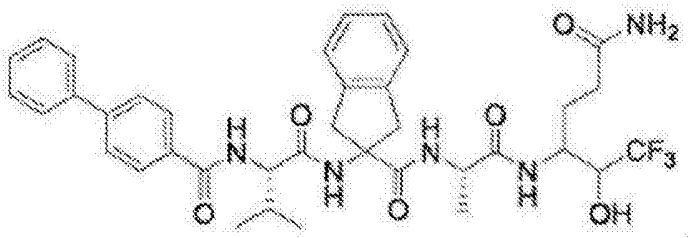

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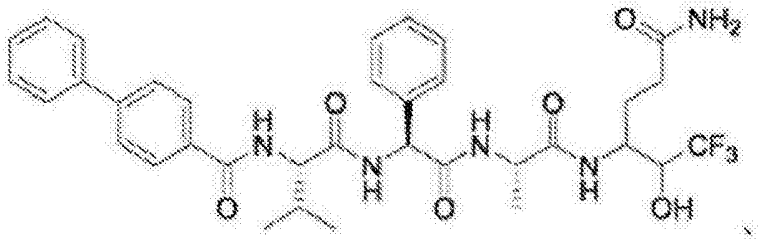

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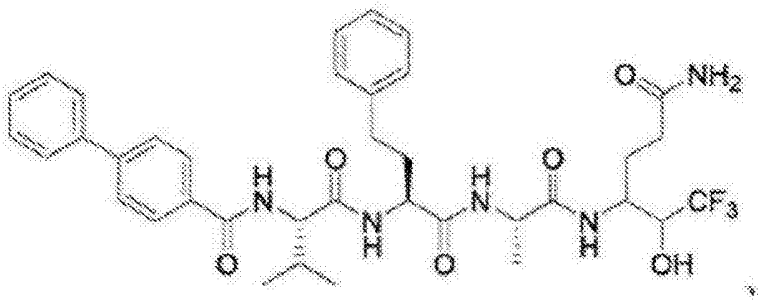

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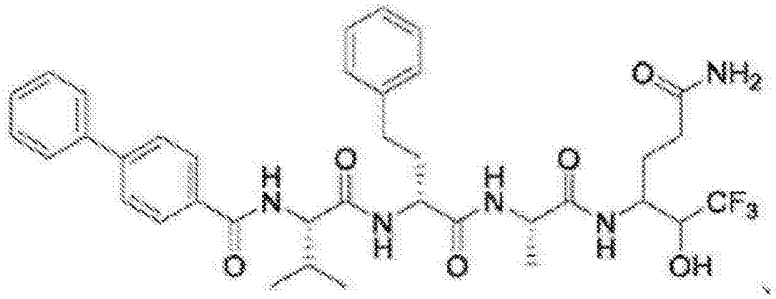

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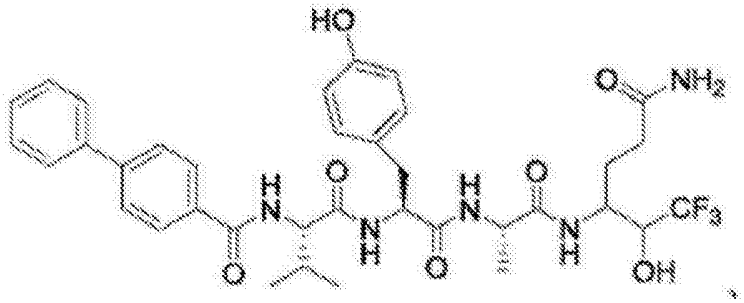

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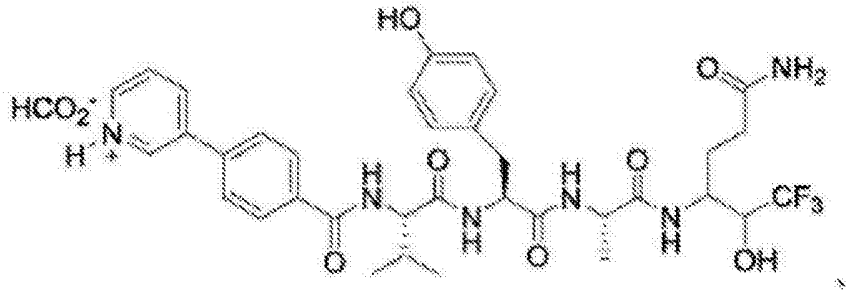

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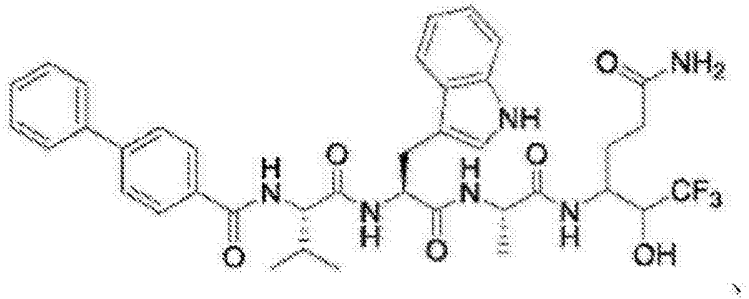

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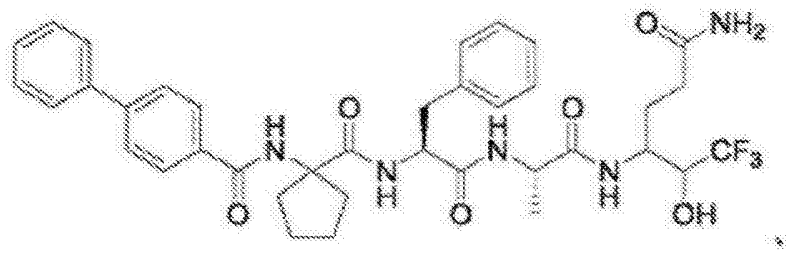

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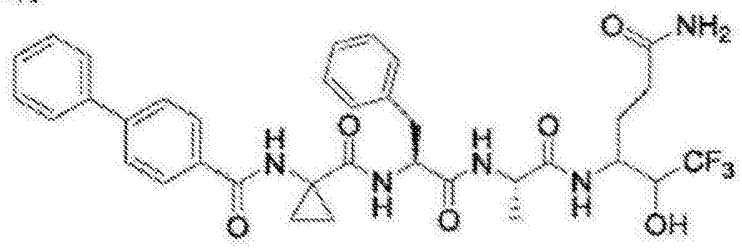
或



91

149. 一种药物组合物，其包含至少一种如权利要求 1 至 148 中任一权利要求所定义的化合物。

150. 根据权利要求 61 所述的药物组合物，其进一步包含至少一种如权利要求 1 至 148 中任一权利要求所述的其它化合物。

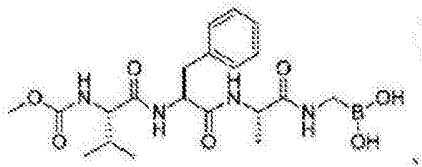
151. 根据权利要求 149 或 150 所述的药物组合物，其进一步包含至少一种改善患者的血脂谱的其它活性成分。

152. 根据权利要求 150 至 151 中任一权利要求所述的药物组合物，其进一步包含药物载体或赋形剂。

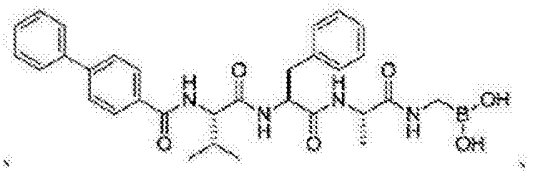
153. 根据权利要求 1 至 148 中任一权利要求所述的化合物或根据权利要求 149 至 152 中任一权利要求所述的组合物，其用作药剂。

154. 根据权利要求 1 至 148 中任一权利要求所述的化合物或根据权利要求 149 至 152 中任一权利要求所述的组合物，其用于制造药剂。

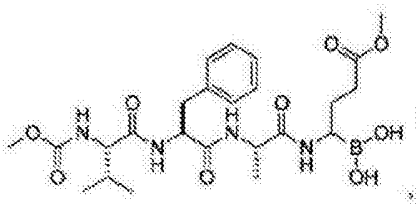
155. 根据权利要求 153 或 154 所述的化合物或组合物，其中所述药剂用于预防或治疗受试者的低密度脂蛋白 - 胆固醇相关疾病或病症，限制条件为所述化合物不是以下 ：



1、



2、



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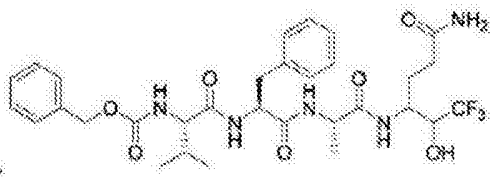

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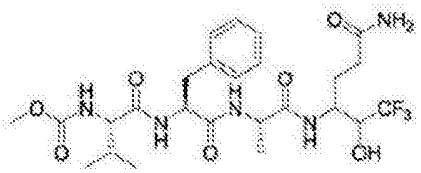
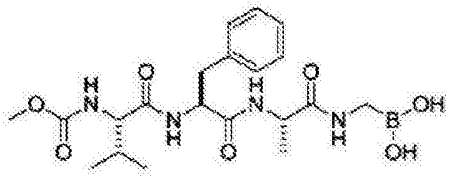


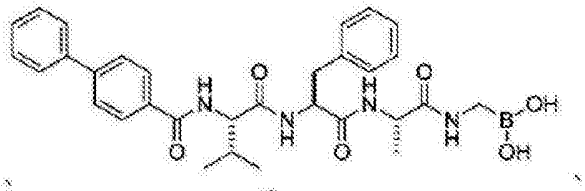
26、

156.根据权利要求 155 所述的化合物或组合物，其中所述低密度脂蛋白－胆固醇相关疾病或病症是高胆固醇血症。

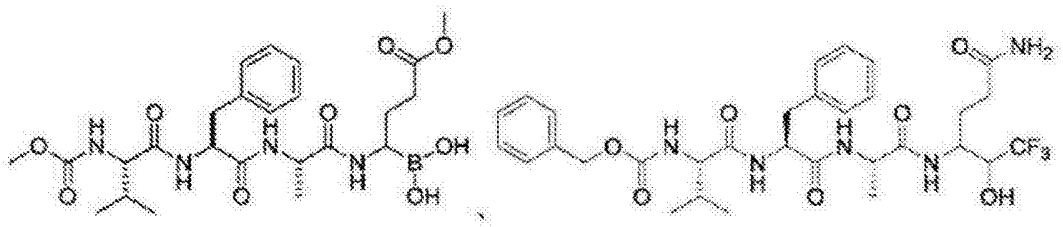
157.一种预防或治疗 LDL-胆固醇相关疾病或病症的方法，其包括对有此需要的受试者施用治疗上有效量的根据权利要求 1 至 148 中任一权利要求所定义的化合物或根据权利要求 149 至 152 中任一权利要求所述的组合物，限制条件为所述化合物不是以下：



1、

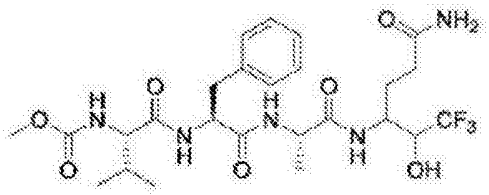


2、



17 25

或

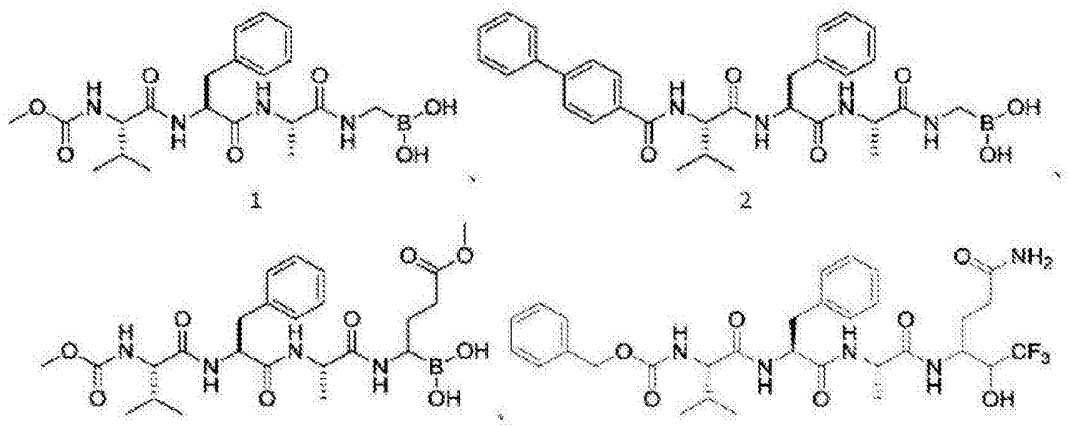


26

158. 根据权利要求 157 所述的方法，其中所述低密度脂蛋白 - 胆固醇相关疾病或病症是高胆固醇血症。

159. 根据权利要求 1 至 148 中任一权利要求所定义的式 I 化合物或根据权利要求 149 至 152 中任一权利要求所定义的组合物的用途，其用作药剂。

160. 根据权利要求 1 至 148 中任一权利要求所定义的化合物或根据权利要求 149 至 152 中任一权利要求所定义的组合物的用途，其用于预防或治疗受试者的低密度脂蛋白 - 胆固醇相关疾病或病症，限制条件为所述化合物不是以下 ：



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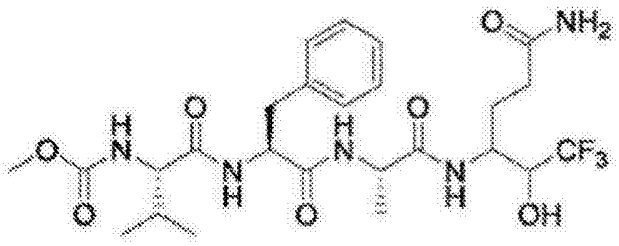
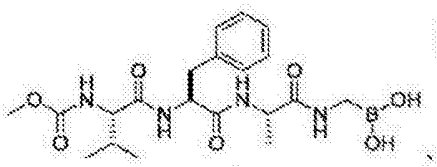
或

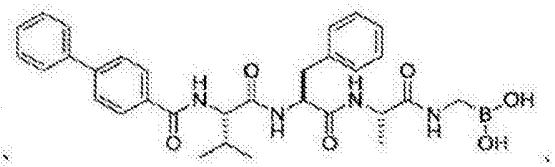


26 。

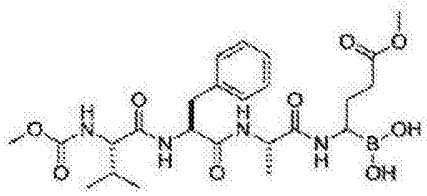
161. 根据权利要求 1 至 148 中任一权利要求所定义的化合物或根据权利要求 149 至 152 中任一权利要求所定义的组合物的用途，其用于制造用于预防或治疗受试者的低密度脂蛋白 - 胆固醇相关疾病的药剂，限制条件为所述化合物不是以下：



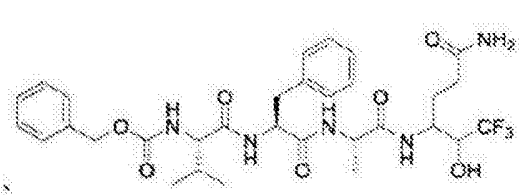
1 、



2 、



17 、



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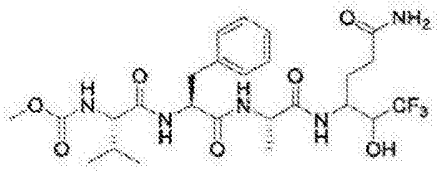
或



26 。

162. 根据权利要求 159 至 161 中任一权利要求所述的用途，其中所述低密度脂蛋白 - 胆固醇相关疾病或病症为高胆固醇血症。

163. 一种用于预防或治疗受试者的低密度脂蛋白 - 胆固醇相关疾病或病症的试剂盒，其包括

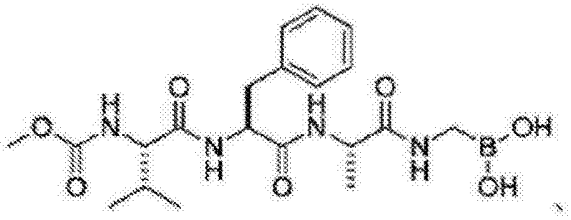
(i) 至少一种根据权利要求 1 至 148 中任一权利要求所定义的化合物或根据权利要求 149 至 152 中任一权利要求所定义的组合物，和

(i)(a) 至少一种改善患者的血脂谱的其它活性成分；

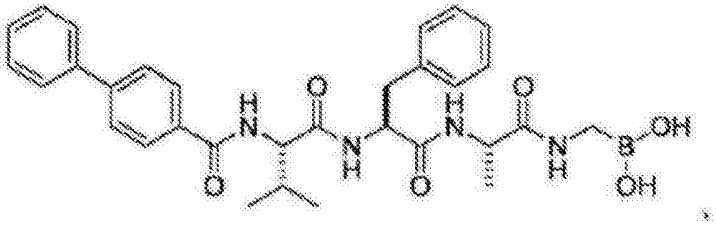
(b) 至少一种其它的根据权利要求 1 至 148 中任一权利要求所定义的化合物或根据权利要求 149 至 152 中任一权利要求所定义的组合物；

(c) 用于所述化合物和 / 或活性成分的容器 ;和 / 或

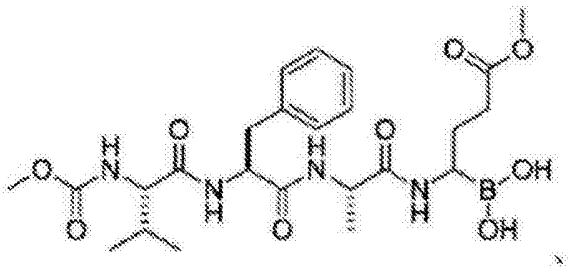
(d) 关于使用所述化合物来预防或治疗所述受试者的低密度脂蛋白 - 胆固醇相关疾病或病症的说明书,限制条件为所述化合物不是以下 :



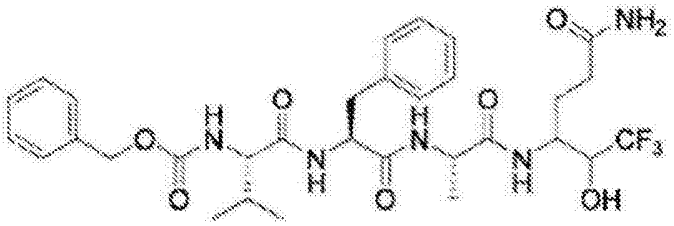
1、



2、

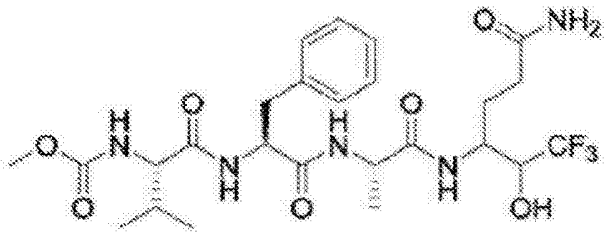


17、



25

或



26。

164. 根据权利要求 163 所述的试剂盒，其中所述低密度脂蛋白 - 胆固醇相关疾病或病症是高胆固醇血症。

PCSK9 的小分子调节剂和其使用方法

[0001] 相关申请案

[0002] 本申请是提交于 2014 年 3 月 14 日且根据 PCT 第 21(2) 条以英文公开的 PCT 申请序列号 PCT/CA2014/* 的进入国家阶段申请，所述申请本身就要求提交于 2013 年 3 月 15 日的美国临时申请序列号 61/792,249 的权益。以上所有文献在此整体并入作为参考。

技术领域

[0003] 关于联邦资助的研究或开发的声明

[0004] N.A.

[0005] 本发明涉及前蛋白转化酶枯草杆菌蛋白酶 - 科信 9 型 (PCSK9) 的小分子调节剂和其使用方法。更具体地，本发明涉及这些分子在治疗低密度脂蛋白 (LDL)- 胆固醇相关的疾病或病症中的用途。

[0006] 发明背景

[0007] 由心血管疾病导致的并发症是世界范围内死亡的主要原因，影响～ 1300 万人 / 年，较之以由于各种形式的癌症影响～ 600 万人 / 年。其中最强效的心血管危险因素是升高的低密度脂蛋白 (LDL) 胆固醇 (LDL-C) 水平。预计心血管病变的发生率在未来二十年里将大幅增加。临床试验数据表明，降低 LDL 胆固醇水平都与冠状动脉事件的发生率有关 (Law 等人，2003 BMJ 326 :1423-1427)。中度终身降低血浆 LDL 胆固醇水平已被证明是基本上与冠状动脉事件的发生率显著降低相关 (Cohen 等人，N.Engl.J.Med.354 :1264-1272)，即使在非脂质相关的心血管危险因素发病率较高的人群中。因此，有很大的好处将从 LDL 胆固醇水平的管理控制得到。其中重要的降胆固醇药物是他汀类药物 (Briel，M.、Nordmann，A.J. 和 Bucher，H.C.Curr.Opin.Lipidol.，16 :601-605，2005)。尽管大多数患者耐受性良好，但是不良副作用正在整理中 (https ://www.statineffects.com/info/)。他汀类药物与依泽替米贝（一种肠固醇转运阻滞剂）的组合进一步降低了 LDL-C ≤ 20％。

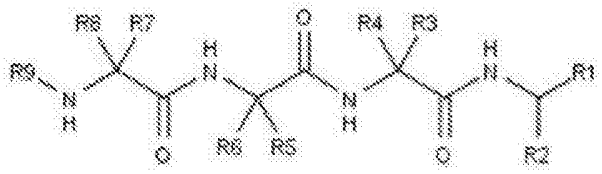
[0008] 因此，有必要开发可降低循环 LDL-C 的替代战略 (Brown，M.S. 和 Goldstein，J.L.Science，311 :1721-1723，2006 ;Tall，A.R.N.Engl.JMed.，354 :1310-1312，2006)。

[0009] 低密度脂蛋白受体 (LDLR) 是调节血液中循环 LDL 水平的关键调节者。降低的 LDLR 水平是与升高的血浆胆固醇 (LDL-C) 相关联，升高的血浆胆固醇强烈地与动脉粥样硬化和冠心病（工业化文明中死亡的首要原因）的风险增加相关。在细胞表面达成增加 LDLR 蛋白水平的试剂将因此提供受血浆高胆固醇血症影响的个体正常化 LDL-C 的方法。前蛋白转化酶 PCSK9 通过加强其在核内体 / 溶酶体中的降解来降低 LDLR 水平。已经确定了一些临床试验，以单克隆抗体抑制 PCSK9- 介导的 LDLR 降解会剂量依赖性地降低健康人受试者的 LDL-C(Lambert 等人，The PCSK9Decade'，J.Lipid Res.2012，53(12)，2515-24 和其中引用的文献）。从给药的成本和便利的观点来看，一种会降低分泌的 PCSK9 的量的口服用小分子试剂的开发将是静脉内或皮下递送生物药剂（如定制来破坏 LDLR 的 PCSK9 介导降解的抗体或寡核苷酸）的理想替代方案。

发明概要

[0010] 更具体来说，根据本发明，提供一种式（I）化合物：

[0011]



[0012] 或其药学上可接受的盐、水合物、溶剂合物或外消旋混合物或立体异构体，

[0013] 其中：R1 为 $-CH(OH)R_a$ 或 $-B(OR_b)(OR_c)$；

[0014] R2 为 -H、$-CH_2R_d$、$-CHR_d(R_e)$、$-CH_2(CH_2)_mC(O)R_j$、$-CH_2(CH_2)_mC(O)N(R_d)R_e$ 或 $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$；

[0015] R3、R4、R5、R6、R7 和 R8 是相同或不同的且独立地为氢或以下基团中的一者：C1-6 烷基、C1-6 卤代烷基、C1-6 硫代烷基、烯基、炔基、芳基和杂芳基，其中所述基团任选地经一个或多个 C1-6 烷基、C3-8 环烷基、C1-6 卤代烷基、芳基、杂芳基、-CN、$-C(O)N(R_f)R_g$、$-C(O)OR_f$、$-C(R_f)(R_g)OR_h$、$-OR_f$、$-OC(O)OR_f$、$-OC(O)NR_f(R_g)$、$-SR_f$、$-S(O)_nR_f$、$-S(O)_nN(R_f)R_g$、$-S(O)_nN(R_f)C(O)R_g$、$-N(R_f)R_g$、$-N(R_f)C(O)R_g$、$--N(R_f)C(O)OR_g$、$-N(R_f)C(O)N(R_g)(R_h)$、$-N(R_f)S(O)_nR_g$ 和 $-N(R_f)S(O)N(R_g)R_h$ 取代基取代；

[0016] 当（R3 和 R4）或（R5 和 R6）或（R7 和 R8）不是氢时，括号内的对也可以与 $-C(O)-$、$-CO_2-$、$-C(O)N(H)-$、$-C(O)N(R_x)--O-$、-NH-、$-N(R_x)-$、-S-、$-S(O)_n-$、$-S(O)_nN(H)-$、$-S(O)_nN(R_x)-$ 基团连接以形成环状结构；

[0017] R9 为 $R_iC(O)-$、$R_iS(O)_n-$、$R_iOC(O)-$、$R_iNHC(O)-$、$R_iNHS(O)_n-$、$R_k(R_l)NC(O)-$、$R_l(R_l)NS(O)_n-$；或一个或多个氨基酸残基；

[0018] R_a 为 C1-3 烷基、C1-2 氟烷基或环丙基；

[0019] R_b 和 R_c 是相同或不同的且独立地为 H 或 C1-6 烷基或可以连接在一起以形成环状 5- 或 6- 员环结构，或与另外的脂族或芳族环体系稠合，所述环状 5- 或 6- 员环结构或脂族或芳族环体系任选地经一个或多个 C1-6 烷基和 / 或 C1-6 卤代烷基取代基取代；

[0020] R_d 和 R_e 是相同或不同的且独立地为 H 或以下基团中的一者：C1-3 烷基、C1-3 卤代烷基或 C3-4 环烷基，或可以直接或与 $-C(O)-$、$-C(O)O-$、$-C(O)N(R_x)--O-$、$-N(R_x)-$、-S-、$-S(O)_n-$ 或 $-S(O)_nN(R_x)-$ 基团连接在一起以形成环状 3-8 员环结构；

[0021] R_f、R_g、R_h、R_k 和 R_l 是相同或不同的且独立地为 H 或以下基团中的一者：C1-6 烷基、C1-6 卤代烷基或 C3-4 环烷基，或可以直接或与 $-C(O)-$、$-C(O)O-$、$-C(O)N(R_x)--O-$、$-N(R_x)-$、-S-、$-S(O)_n-$ 或 $-S(O)_nN(R_x)-$ 基团连接在一起以形成环状 3-8 员环结构；

[0022] R_i 为 C1-10 烷基、C3-8 环烷基、C1-10 卤代烷基、杂环基、芳基或杂芳基，其中所述基团任选地经一个或多个卤素、C1-6 氨基烷基、C1-6 杂烷基、C1-6 烷基、C3-8 环烷基、C1-6 卤代烷基、芳基、杂芳基和杂环基取代；

[0023] R_j 为 OR_d 或 $N(R_d)(R_e)$；

[0024] R_x 为 H、C1-6 烷基、C1-6 卤代烷基、C3-4 环烷基、$-C(O)R_y$、$-C(O)OR_y$、$-C(O)$

NH_2、-C(O)NH(R_y) 或 -C(O)NHS(O)$_n$$R_y$；

[0025] R_y为 C1-6 烷基、C1-6 卤代烷基或 C3-4 环烷基；

[0026] m 为值 0 或 1 的整数；且

[0027] n 为值 1 或 2 的整数，

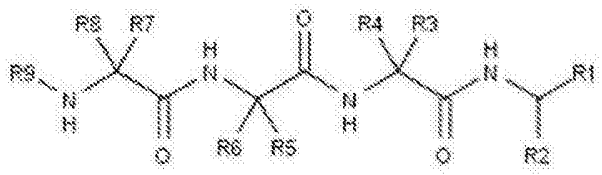
[0028] 限制条件为：

[0029] 当 R1 为 -CH(OH)R_a时，R2 为 -CHR$_d$(R_e)、-CH_2(CH_2)$_m$C(O)R_j、-CH_2(CH_2)$_m$C(O)N(R_d)R_e或 -CH_2(CH_2)$_m$S(O)$_n$N(R_d)R_e；且

[0030] 当 R1 为 -B(OR$_b$)(OR$_c$) 时，R2 为 -H、-CH_3、-CHR$_d$(R_e)、-CH_2(CH_2)$_m$C(O)R_j、-CH_2(CH_2)$_m$C(O)N(R_d)R_e或 -CH_2(CH_2)$_m$S(O)$_n$N(R_d)R_e。

[0031] 更具体来说，根据本发明，提供一种式 (I) 化合物：

[0032]



(I)

[0033] 或其药学上可接受的盐、水合物、溶剂合物或外消旋混合物或立体异构体，

[0034] 其中：

[0035] R1 为 -CH(OH)R_a或 -B(OR$_b$)(OR$_c$)；

[0036] R2 为 -H、-CH_2R_d、-CHR$_d$(R_e)、-CH_2(CH_2)$_m$C(O)R_j、-CH_2(CH_2)$_m$C(O)N(R_d)R_e或 -CH_2(CH_2)$_m$S(O)$_n$N(R_d)R_e；

[0037] R3、R4、R5、R6、R7 和 R8 是相同或不同的且独立地为氢或以下基团中的一者：C1-6 烷基、C1-6 卤代烷基、C1-6 硫代烷基、C1-6 氨基烷基、烯基、炔基、环烷基、杂环基、芳基和杂芳基，其中所述基团任选地经一个或多个 C1-6 烷基、C3-8 环烷基、C1-6 卤代烷基、芳基、杂芳基、-CN、-C(O)N(R_f)R_g、-C(O)OR_f、-C(R_f)(R_g)OR_h、-OR_f、-OC(O)OR_f、-OC(O)NR_f(R_g)、-SR_f、-S(O)$_n$$R_f$、-S(O)$_n$N($R_f$)$R_g$、-S(O)$_n$N($R_f$)C(O)$R_g$、-N($R_f$)$R_g$、-N($R_f$)C(O)$R_g$、--N($R_f$)C(O)O$R_g$、-N($R_f$)C(O)N($R_g$)($R_h$)、-N($R_f$)S(O)$_n$$R_g$和 -N($R_f$)S(O)N($R_g$)$R_h$取代基取代；

[0038] 当 (R3 和 R4) 或 (R5 和 R6) 或 (R7 和 R8) 不是氢时，括号内的对也可以与 -C(O)-、-CO_2-、-C(O)N(H)-、-C(O)N(R_x)-、-O-、-NH-、-N(R_x)-、-S-、-S(O)$_n$-、-S(O)$_n$N(H)-、-S(O)$_n$N(R_x)- 基团连接以形成环状结构；

[0039] R9 为 R_iC(O)-、R_iS(O)$_n$-、R_iOC(O)-、R_iNHC(O)-、R_iNHS(O)$_n$-、R_k(R_l)NC(O)-、R_l(R_l)NS(O)$_n$-、R_mOR_iC(O)-、R_mC(O)R_iC(O)- 或一个或多个氨基酸残基；

[0040] R_a为 C1-3 烷基、C1-2 氟烷基或环丙基；

[0041] R_b和 R_c是相同或不同的且独立地为 H 或 C1-6 烷基或可以连接在一起以形成环状 5- 或 6- 员环结构，或与另外的脂族或芳族环体系稠合，所述环状 5- 或 6- 员环结构或脂族或芳族环体系任选地经一个或多个 C1-6 烷基和 / 或 C1-6 卤代烷基取代基取代；

[0042] R_d和 R_e是相同或不同的且独立地为 H 或以下基团中的一者：C1-3 烷基、C1-3 卤代烷基或 C3-4 环烷基，或可以直接或与 -C(O)-、-C(O)O-、-C(O)N(R_x)--O-、-N(R_x)-、-S-、-S(O)$_n$- 或 -S(O)$_n$N(R_x)- 基团连接在一起以形成环状 3-8 员环结构；

[0043] R_f、R_g、R_h、R_k和R_1是相同或不同的且独立地为H或以下基团中的一者：C1-6烷基、C1-6卤代烷基或C3-4环烷基，或可以直接或与-C(O)-、-C(O)O-、-C(O)N(R_x)--O-、-N(R_x)-、-S-、-S(O)$_n$-或-S(O)$_n$N(R_x)-基团连接在一起以形成环状3-8员环结构；

[0044] R_i为C1-10烷基、C1-10杂烷基、C3-8环烷基、C1-10卤代烷基、杂环基、芳基、杂芳基、C1-10烷基-C3-8环烷基、C1-10烷基-杂环基、C1-10烷基-芳基、C1-10烷基-杂芳基、C1-10杂烷基-C3-8环烷基、C1-10杂烷基-杂环基、C1-10杂烷基-芳基或C1-10杂烷基-杂芳基，其中所述基团任选地经一个或多个卤素、C1-6氨基烷基、C1-6杂烷基、C1-6烷基、C3-8环烷基、C1-6卤代烷基、芳基、杂芳基和杂环基取代；

[0045] R_m为C1-10烷基、C1-10杂烷基、C3-8环烷基、C1-10卤代烷基、杂环基、芳基或杂芳基，其中所述基团任选地经一个或多个卤素、C1-6氨基烷基、C1-6杂烷基、C1-6烷基、C3-8环烷基、C1-6卤代烷基、芳基、杂芳基和杂环基取代；

[0046] R_j为OR_d或N(R_d)(R_e)；

[0047] R_x为H、C1-6烷基、C1-6卤代烷基、C3-4环烷基、-C(O)R_y、-C(O)OR_y、-C(O)NH_2、-C(O)NH(R_y)或-C(O)NHS(O)$_n$$R_y$；

[0048] R_y为C1-6烷基、C1-6卤代烷基或C3-4环烷基；

[0049] m为值0或1的整数；且

[0050] n为值1或2的整数，

[0051] 限制条件为：

[0052] 1)当R1为-CH(OH)R_a时，R2为-CHR_d(R_e)、-CH_2(CH_2)$_m$C(O)R_j、-CH_2(CH_2)$_m$C(O)N(R_d)R_e或-CH_2(CH_2)$_m$S(O)$_n$N(R_d)R_e；且

[0053] 2)当R1为-B(OR_b)(OR_c)时，R2为-H、-CH_3、-CHR_d(R_e)、-CH_2(CH_2)$_m$C(O)R_j、-CH_2(CH_2)$_m$C(O)N(R_d)R_e或-CH_2(CH_2)$_m$S(O)$_n$N(R_d)R_e。

[0054] 在式I化合物的一特定实施方案中，(i)当R1为-CH(OH)-CF3时，R9不是-C(O)OCH_2-苯基或-C(O)OCH_3；(ii)当R1为B(OH)$_2$时，R9不是-C(O)OCH_3或-C(O)-苯基-苯基；和/或(iii)当R1为CH(OH)CH_2F时，R9不是-C(O)(CH_2)$_8$$CH_3$。

[0055] 在式I化合物的一特定实施方案中，R1为-B(OR_b)(OR_c)或-CH(OH)R_a；R2为H或-CH_2(CH_2)$_m$C(O)R_j；R3为H；R4为取代的或未取代的C1-6烷基、取代的或未取代的C1-6硫代烷基或取代的或未取代的芳基；R5为H；R6为经芳基取代的C1-6烷基；R7为H；R8为经C1-6烷基取代的C1-6烷基；和/或R9为RiOC(O)-或RiC(O)-。

[0056] 在式I化合物的另一特定实施方案中，R1为-B(OR_b)(OR_c)或-CH(OH)R_a；R2为H或-CH_2(CH_2)$_m$C(O)R_j；R3为H；R4为取代的或未取代的C1-6烷基、取代的或未取代的C1-6硫代烷基或取代的或未取代的芳基；R5为H；R6为经芳基取代的C1-6烷基；R7为H；R8为经C1-6烷基取代的C1-6烷基；且R9为RiOC(O)-或RiC(O)-。

[0057] 在式I化合物的另一特定实施方案中，R1为-B(OR_b)(OR_c)或-CH(OH)R_a；R2为H或-CH_2(CH_2)$_m$C(O)R_j；R3为H或取代的或未取代的C1-6烷基；R4为H、取代的或未取代的C1-6烷基、取代的或未取代的C1-6硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷基；R5为H；R6为H、取代的或未取代的C1-6烷基、取代的或未取代的C1-6硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷烃；R7为H；R8为H、取代的或未取代的

C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷基；和 / 或 R9 为 RiOC(O)-、RiC(O)-、$R_mOR_iC(O)$-、$R_mC(O)R_iC(O)$- 或 $R_iS(O)_n$-。

[0058] 在式 I 化合物的另一特定实施方案中，R1 为 $-B(OR_b)(OR_c)$ 或 $-CH(OH)R_a$；R2 为 H 或 $-CH_2(CH_2)_mC(O)R_j$；R3 为 H 或取代的或未取代的 C1-6 烷基；R4 为 H、取代的或未取代的 C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷基；R5 为 H；R6 为 H、取代的或未取代的 C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷烃；R7 为 H；R8 为 H、取代的或未取代的 C1-6 烷基、取代的或未取代的 C1-6 硫代烷基、取代的或未取代的芳基或取代的或未取代的环烷基；且 R9 为 RiOC(O)-、RiC(O)-、$R_mOR_iC(O)$-、$R_mC(O)R_iC(O)$- 或 $R_iS(O)_n$-。

[0059] 在式 I 化合物的一特定实施方案中，R2；R3 或 R4；R5 或 R6；和 / 或 R7R8 可为相同或不同的且可为除了以 D 或 L 构型的脯氨酸以外的任何天然氨基酸的侧链。其它取代基是如本文所定义。在一个更具体的实施方案中，(i)R2 为谷氨酰胺或甘氨酸的侧链；(ii)R3 或 R4 为丙氨酸、苯丙氨酸、蛋氨酸、亮氨酸、缬氨酸、谷氨酰胺或甘氨酸的侧链；(iii)R5 或 R6 为苯丙氨酸、甘氨酸、丙氨酸、蛋氨酸或缬氨酸的侧链；(iv)R7 或 R8 为缬氨酸、蛋氨酸、苯丙氨酸、甘氨酸或丙氨酸；或 (v)(i) 至 (iv) 中至少两者的任何组合。

[0060] 在式 I 化合物的另一特定实施方案中，R6 为苯基 $-CH_2-$；和 / 或 R8 为 $(CH_3)_2CH$-。在另一特定实施方案中，R1 为 $-B(OR_b)(OR_c)$。在另一特定实施方案中，Rb 和 Rc 为 H。在另一特定实施方案中，Rb 和 Rc 连接以形成环状 5 员环结构或与脂族环体系稠合。在另一特定实施方案中，R1 为 2,9,9- 三甲基 -3,5-- 二氧杂 -4- 硼杂三环 $[6.1.1.0^{2,6}]$ 癸 -4- 基。在另一特定实施方案中，R1 为 $-CH(OH)R_a$。在另一特定实施方案中，Ra 为 C1-2 氟烷基。在另一特定实施方案中，Ra 为 $-CF_3$。在另一特定实施方案中，Ra 为 $-CH_2F$。在另一特定实施方案中，Ra 为 C1-3 烷基。在另一特定实施方案中，Ra 为 $-CH_3$。在另一特定实施方案中，R2 为 H。在另一特定实施方案中，R2 为 $-CH_2(CH_2)_mC(O)R_j$。在另一特定实施方案中，R_j 为 OR_d。在另一特定实施方案中，Rd 为 CH_3。在另一特定实施方案中，Rj 为 NH_2。

[0061] 在式 I 化合物的另一特定实施方案中，R3 为 H。在另一特定实施方案中，R3 为取代的或未取代的 C1-6 烷基。在另一特定实施方案中，R3 为未取代的 C1-6 烷基。在另一特定实施方案中，R3 为 CH_3。

[0062] 在式 I 化合物的另一特定实施方案中，R4 为 H。在另一特定实施方案中，R4 为取代的或未取代的 C1-6 烷基。在另一特定实施方案中，R4 为未取代的 C1-6 烷基。在另一特定实施方案中，R4 为芳基取代的 C1-6 烷基。在另一特定实施方案中，R4 为 $-CH_3$。在另一特定实施方案中，R4 为 CH_3CH_2-。在另一特定实施方案中，R4 为 $-CH_2CH(CH_3)_2$。在另一特定实施方案中，R4 为芳基取代的 C1-6 烷基。在另一特定实施方案中，R4 为苯基 $-CH_2-$。在另一特定实施方案中，R4 为 $-(CH_2)_2C(O)NH2$。在另一特定实施方案中，R4 为取代的或未取代的 C1-6 硫代烷基。在另一特定实施方案中，R4 为 $CH_3S(CH_2)_2$-。在另一特定实施方案中，R4 为取代的或未取代的芳基。在另一特定实施方案中，R4 为苯基 -。在另一特定实施方案中，R4 为环烷基。

[0063] 在另一特定实施方案中，R6 为 H。在另一特定实施方案中，R6 为取代的或未取代的 C1-6 烷基。在另一特定实施方案中，R6 为取代的 C1-6 烷基。在另一特定实施方案中，R6 为芳基取代的 C1-6 烷基。在另一特定实施方案中，R6 为 - 烷基 - 苯基。在另一特定实施方

案中，R6 为 $-CH_2-$ 苯基。在另一特定实施方案中，R6 为 $-CH_2-$ 羟苯基。在另一特定实施方案中，R6 为 $-(CH_2)_2-$ 苯基。在另一特定实施方案中，R6 为 $-CH_2-$ 吲哚。在另一特定实施方案中，R6 为未取代的 C1-6 烷基。在另一特定实施方案中，R6 为未取代的 C1-6 烷基。在另一特定实施方案中，R6 为 CH_3。在另一特定实施方案中，R6 为 $-CH(CH_3)_2$。在另一特定实施方案中，R6 为取代的或未取代的 C1-6 硫代烷基。在另一特定实施方案中，R6 为 $CH_3S(CH_2)_2-$。在另一特定实施方案中，R6 为取代的或未取代的芳基。在另一特定实施方案中，R6 为苯基。在另一特定实施方案中，R6 为取代的或未取代的环烷基。在另一特定实施方案中，R6 为苯并环戊基。

[0064] 在另一特定实施方案中，R8 为 H。在另一特定实施方案中，R8 为取代的或未取代的 C1-6 烷基。在另一特定实施方案中，R8 为未取代的 C1-6 烷基。在另一特定实施方案中，R8 为 $-CH(CH_3)_2$。在另一特定实施方案中，R8 为 $-CH_3$。在另一特定实施方案中，R8 为取代的 C1-6 烷基。在另一特定实施方案中，R8 为芳基取代的 C1-6 烷基。在另一特定实施方案中，R8 为 $-CH_2-$ 苯基。在另一特定实施方案中，R8 为取代的或未取代的 C1-6 硫代烷基。在另一特定实施方案中，R8 为 $CH_3S(CH_2)_2-$。在另一特定实施方案中，R8 为取代的或未取代的芳基。在另一特定实施方案中，R8 为未取代的芳基。在另一特定实施方案中，R8 为苯基。在另一特定实施方案中，R8 为取代的或未取代的环烷基。在另一特定实施方案中，R8 为未取代的环烷基。R8 为环戊基。在另一特定实施方案中，R8 为环丙基。

[0065] 在另一特定实施方案中，R9 为 RiOC(O)-。在另一特定实施方案中，Ri 为取代的或未取代的 C1-10 烷基 -。在另一特定实施方案中，Ri 为未取代的 C1-10 烷基 -。在另一特定实施方案中，Ri 为 CH_3-。在另一特定实施方案中，Ri 为取代的 C1-10 烷基 -。在另一特定实施方案中，Ri 为 $(CH_3)_3C-$。在另一特定实施方案中，Ri 为苯基 $-CH_2-$。

[0066] 在另一特定实施方案中，R9 为 RiC(O)-。在另一特定实施方案中，Ri 为取代的或未取代的芳基 -。在另一特定实施方案中，Ri 为取代的芳基 -。在另一特定实施方案中，Ri 为取代的或未取代的芳基或取代的或未取代的杂芳基。在另一特定实施方案中，Ri 为取代的或未取代的芳基。在另一特定实施方案中，Ri 为取代的芳基。在另一特定实施方案中，Ri 为芳基 - 苯基 -。在另一特定实施方案中，Ri 为苯基 - 苯基 -。在另一特定实施方案中，Ri 为杂芳基 - 苯基 -。在另一特定实施方案中，Ri 为双吖丙啶 - 苯基 -。在另一特定实施方案中，Ri 为苯基 - 苯基 -。在另一特定实施方案中，Ri 为氟苯基 -。在另一特定实施方案中，Ri 为氟烷基 - 双吖丙啶 - 苯基 -。在另一特定实施方案中，Ri 为三氟甲基 - 双吖丙啶 - 苯基 -。在另一特定实施方案中，Ri 为吡啶 - 苯基 -。在另一特定实施方案中，Ri 为噁二唑 - 苯基 -。在另一特定实施方案中，Ri 为杂环基 - 苯基 -。在另一特定实施方案中，Ri 为吗啉 - 苯基 -。在另一特定实施方案中，Ri 为烷基 - 苯基 -。在另一特定实施方案中，Ri 为 $(CH_3)_2CH-$ 苯基 -。在另一特定实施方案中，Ri 为 $OHCH_2-$ 苯基 -。在另一特定实施方案中，Ri 为氟苯基。在另一特定实施方案中，Ri 为未取代的芳基。在另一特定实施方案中，Ri 为苯基。在另一特定实施方案中，Ri 为取代的或未取代的杂芳基。在另一特定实施方案中，Ri 为取代的杂芳基。在另一特定实施方案中，Ri 为芳基 - 杂芳基 -。在另一特定实施方案中，Ri 为苯基 - 杂芳基 -。在另一特定实施方案中，Ri 为苯基 - 吡唑 -。在另一特定实施方案中，Ri 为苯基 - 甲基吡唑 -。在另一特定实施方案中，Ri 为苯基 - 噻唑 -。在另一特定实施方案中，Ri 为苯基 - 吡啶 -。在另一特定实施方案中，Ri 为苯基 - 呋喃 -。在另一

特定实施方案中，Ri 为杂芳基 - 杂芳基 -。在另一特定实施方案中，Ri 为吡啶 - 异噻唑 -。在另一特定实施方案中，Ri 为未取代的杂芳基。在另一特定实施方案中，Ri 为吡啶。在另一特定实施方案中，Ri 为吡嗪。在另一特定实施方案中，Ri 为吲哚。在另一特定实施方案中，Ri 为 4-[3- 三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 -。在另一特定实施方案中，Ri 为 3-[3- 三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 -。在另一特定实施方案中，Ri 为未取代的芳基 -。在另一特定实施方案中，Ri 为苯基 -。在另一特定实施方案中，Ri 为取代的或未取代的 C1-10 烷基 -。在另一特定实施方案中，Ri 为取代的 C1-10 烷基 -。在另一特定实施方案中，Ri 为芳基 -C1-10 烷基 -。在另一特定实施方案中，Ri 为苯基 -C1-10 烷基 -。在另一特定实施方案中，Ri 为苯基 - 炔 -。在另一特定实施方案中，Ri 为苯基 $-CH_2-$。

[0067] 在另一特定实施方案中，Ri 为氟甲氧基 - 芳基 -(C1-6 烷基)-。在另一特定实施方案中，Ri 为氟甲氧基 - 苯基 $-CH_2-$。在另一特定实施方案中，Ri 为三氟甲氧基 - 苯基 $-CH_2-$。在另一特定实施方案中，Ri 为 4-(三氟甲氧基) 苯基 $-CH_2-$。在另一特定实施方案中，Ri 为氟烷基 - 双吖丙啶 - 苯基 -(C1-10 烷基)。在另一特定实施方案中，Ri 为三氟甲基 - 双吖丙啶 - 苯基 $-CH_2-$。在另一特定实施方案中，Ri 为 4-[3- 三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 -。在另一特定实施方案中，Ri 为 3-[3- 三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 -。在另一特定实施方案中，Ri 为未取代的 C1-10 烷基 -。在另一特定实施方案中，Ri 为 $CH_3(CH_2)_8-$。

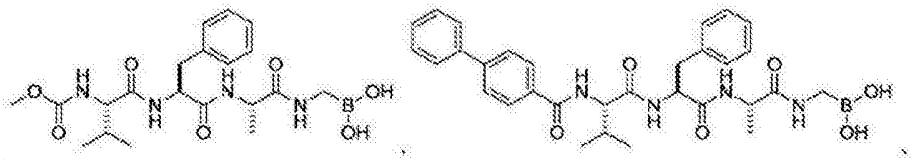
[0068] 在另一特定实施方案中，R9 为 $R_mOR_iC(O)-$。在另一特定实施方案中，R_i 为取代的或未取代的 C1-10 烷基。在另一特定实施方案中，R_i 为 $-CH_2-$。在另一特定实施方案中，R_m 为取代的或未取代的 C1-10 烷基。在另一特定实施方案中，R_m 为 $-CH_2-$。在另一特定实施方案中，R_m 为芳基 $-CH_2-$。在另一特定实施方案中，R_m 为苯基 $-CH_2-$。在另一特定实施方案中，R_m 为取代的或未取代的芳基。在另一特定实施方案中，R_m 为苯基。

[0069] 在另一特定实施方案中，R9 为 $R_mC(O)R_iC(O)-$。在另一特定实施方案中，R_m 为杂芳基。在另一特定实施方案中，R_m 为吗啉。在另一特定实施方案中，R_i 为芳基。在另一特定实施方案中，R_i 为苯基。

[0070] 在另一特定实施方案中，R9 为 $R_iS(O)_n-$。在另一特定实施方案中，R_i 为取代的或未取代的芳基。在另一特定实施方案中，R_i 为取代的芳基。在另一特定实施方案中，R_i 为取代的苯基。在另一特定实施方案中，R_i 为苯基 - 苯基。

[0071] 在另一特定实施方案中，所述式 I 化合物为 :

[0072]



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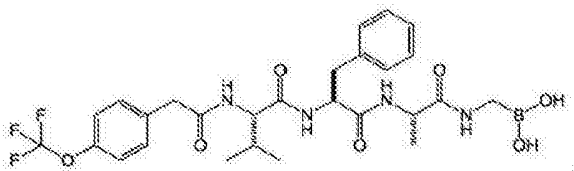

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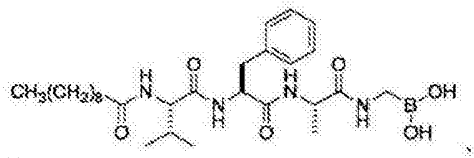

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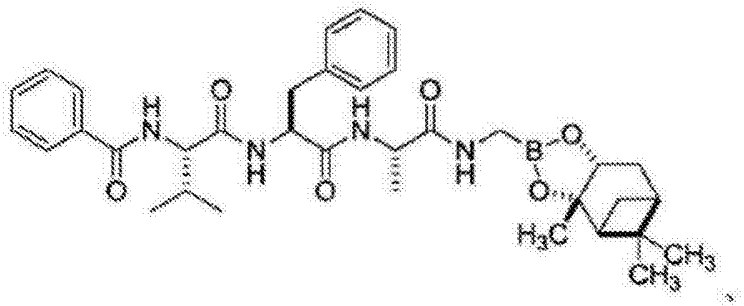

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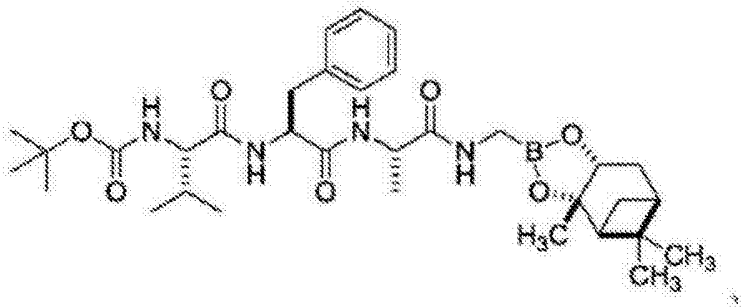

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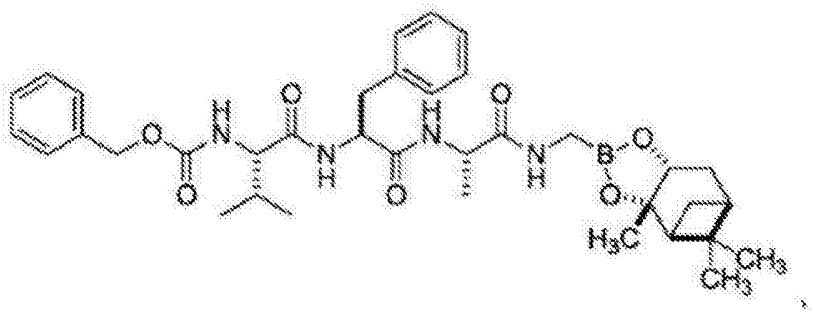

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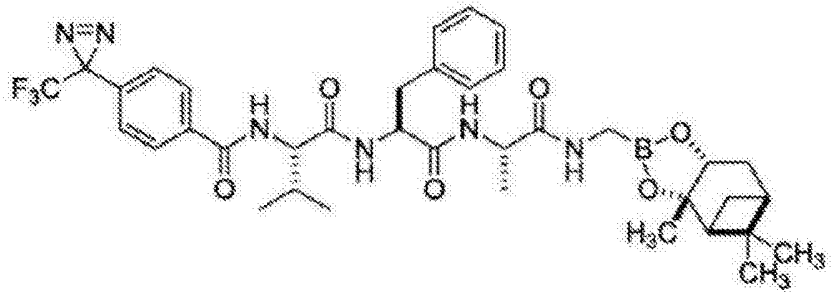

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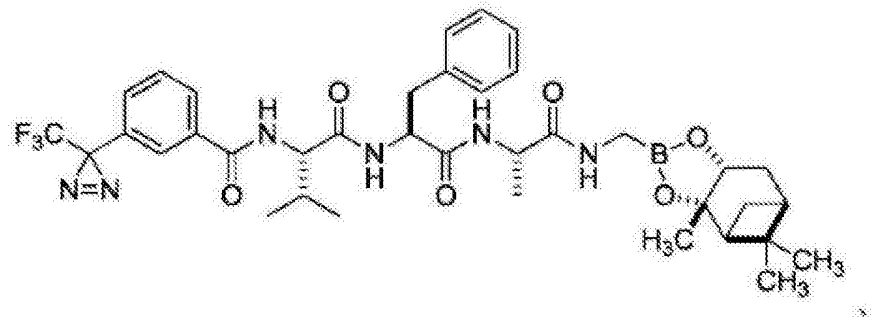
[0074]



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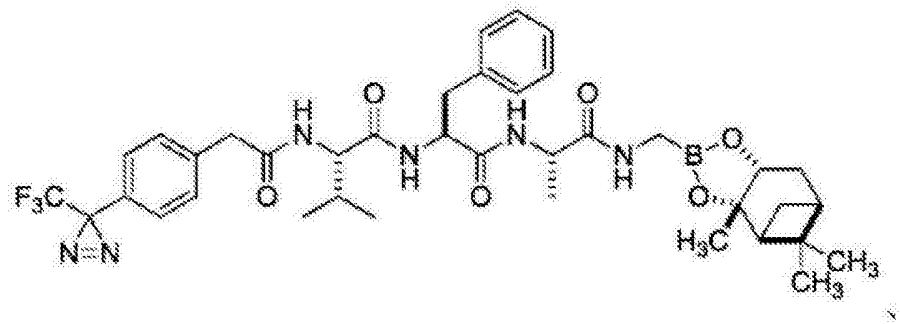

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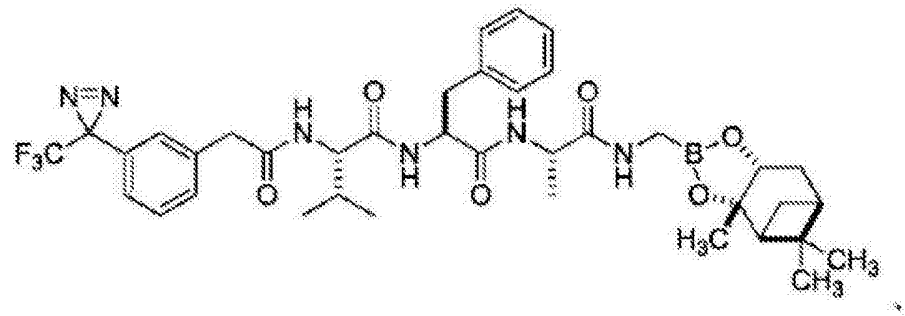

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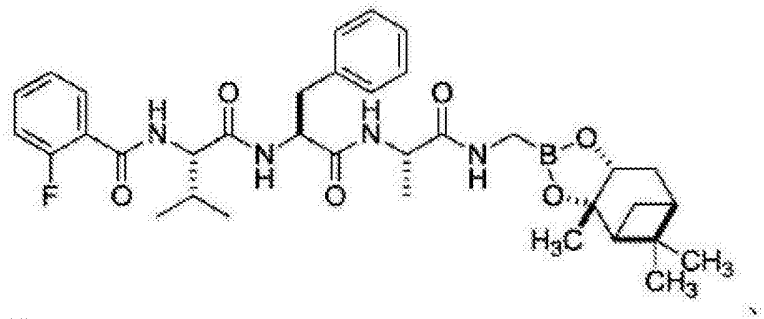

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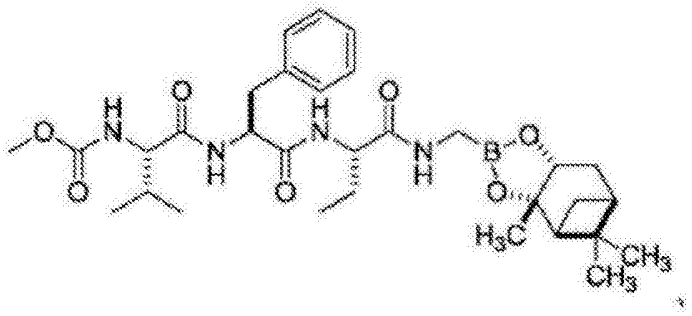
[0075]



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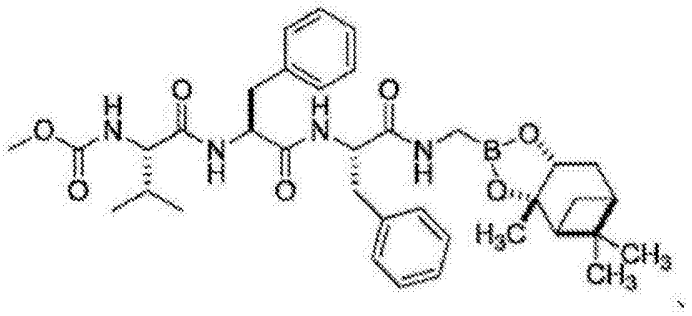

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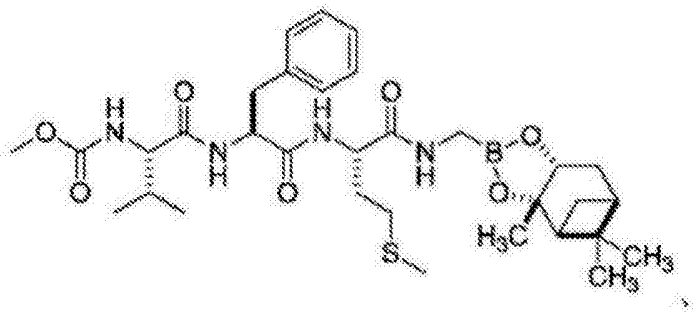

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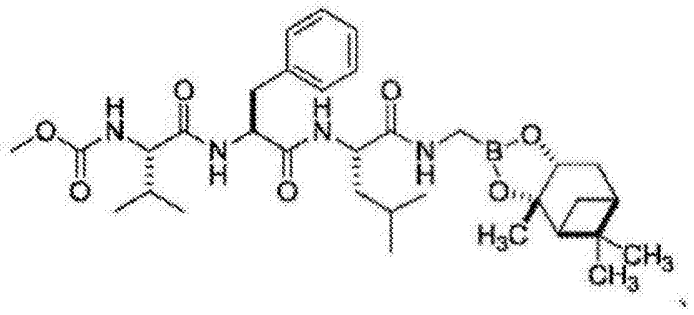

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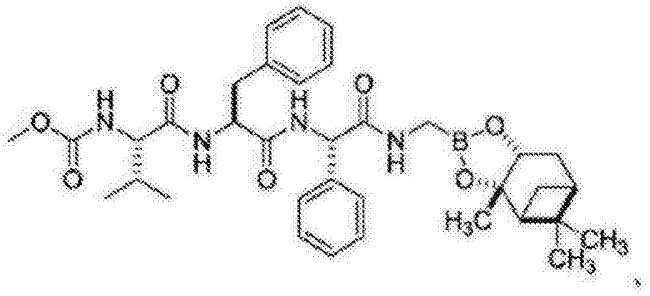

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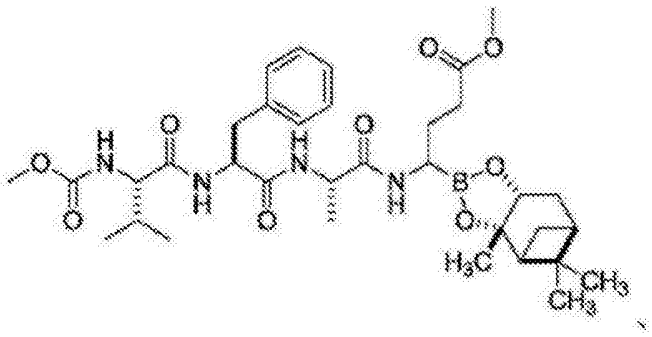

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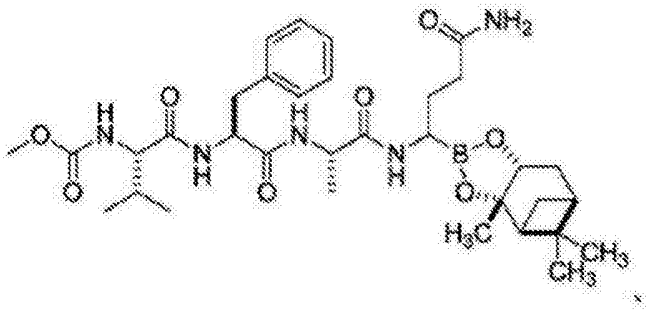

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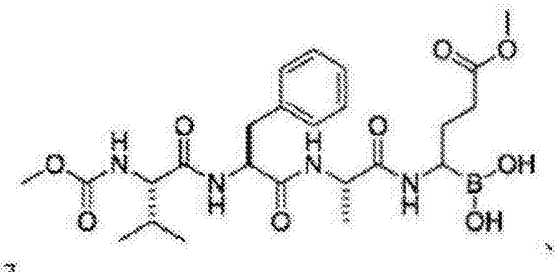

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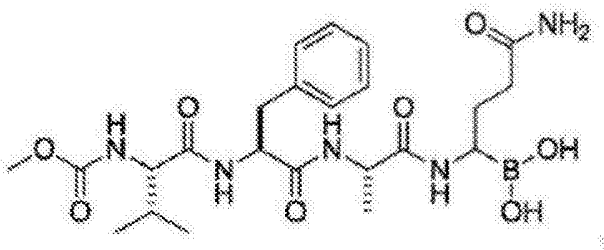

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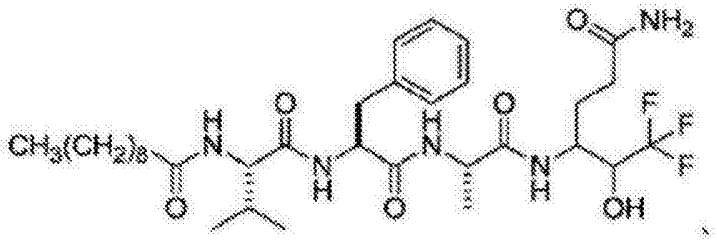

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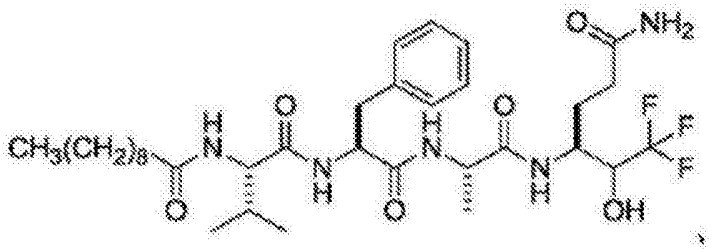

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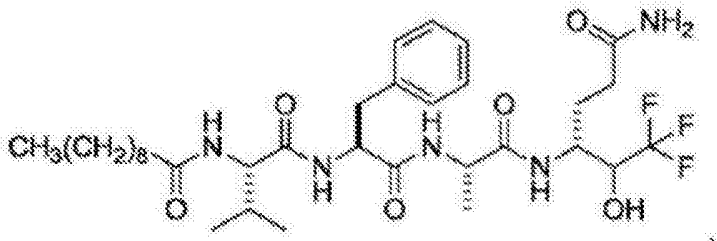

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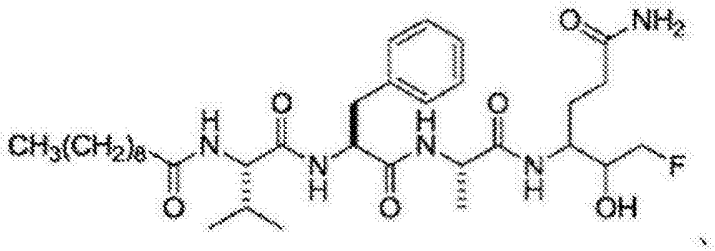
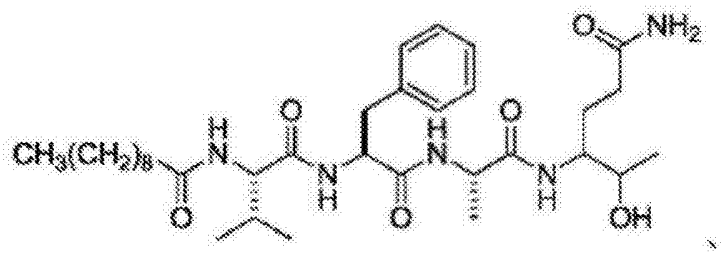
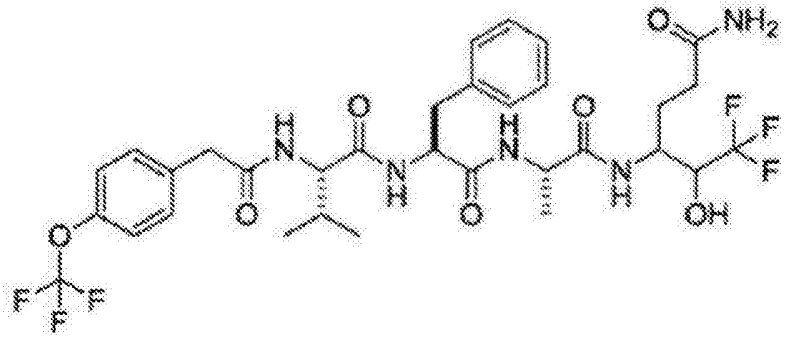


21a 和 21b

[0078]



22a 和 22b



23

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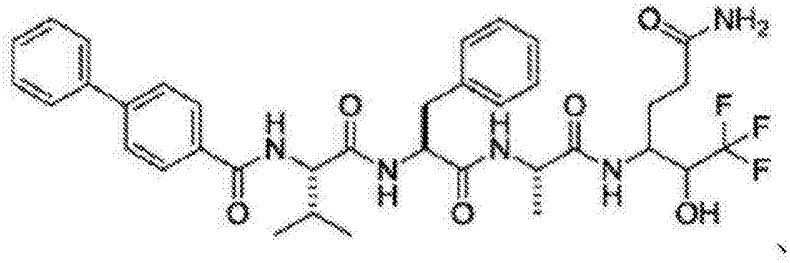

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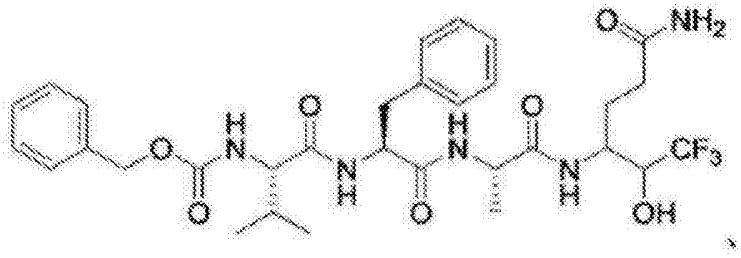

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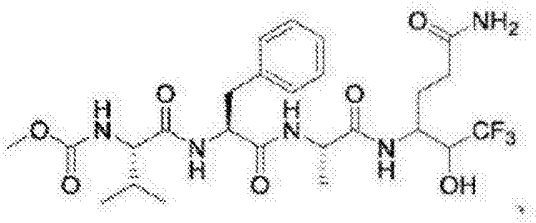

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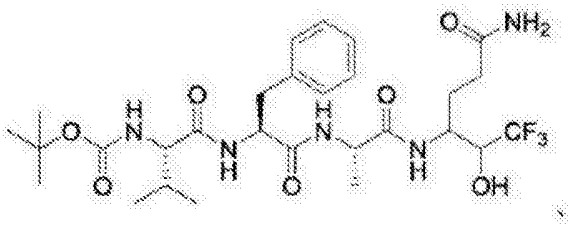

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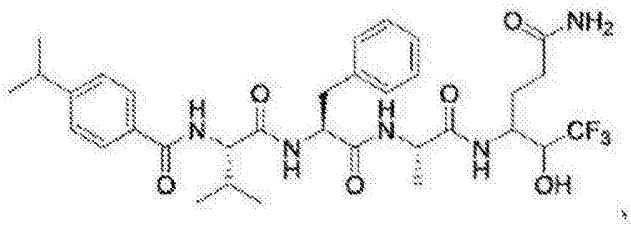

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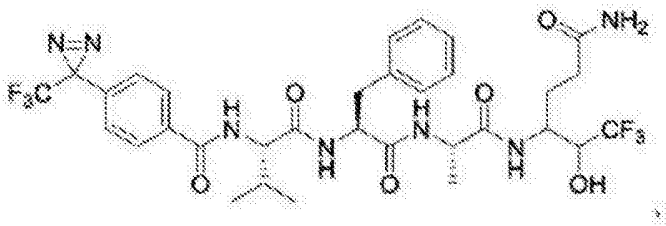

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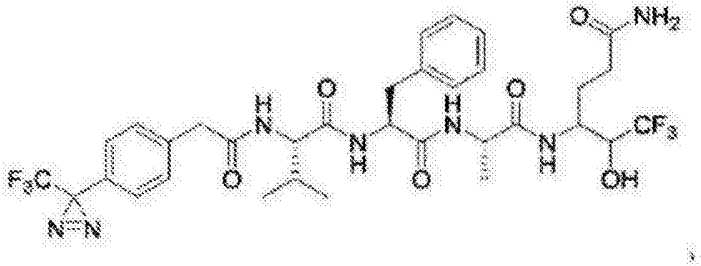

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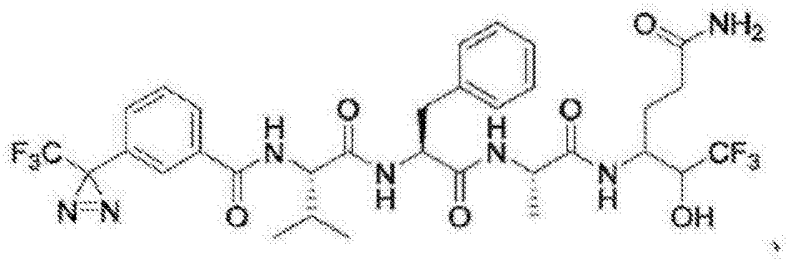

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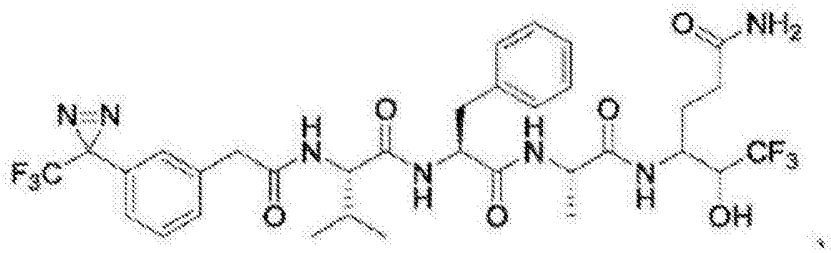

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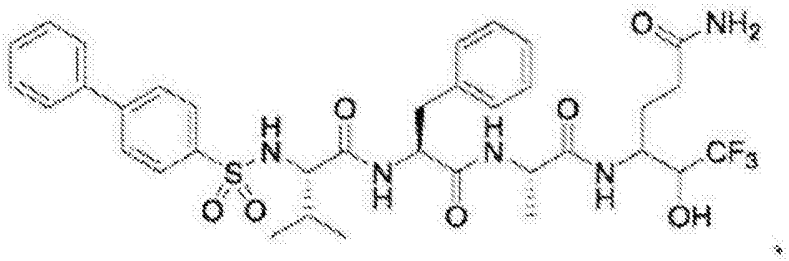

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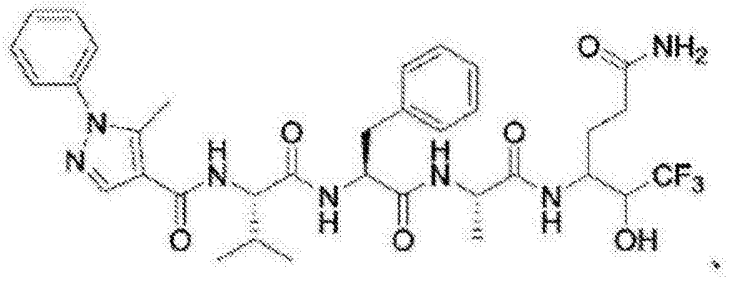

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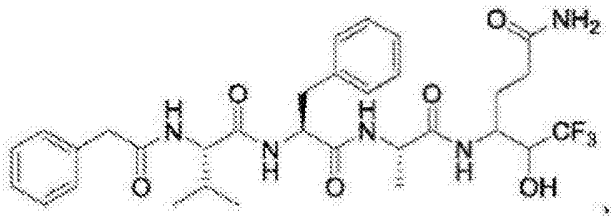
[0081]



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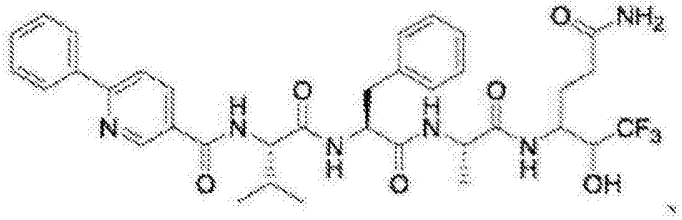

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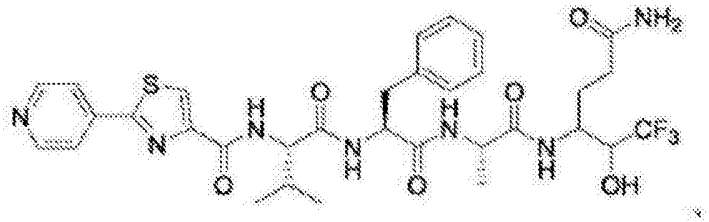

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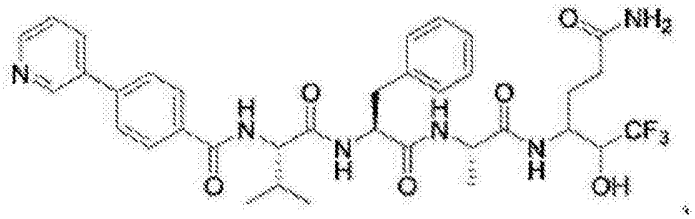

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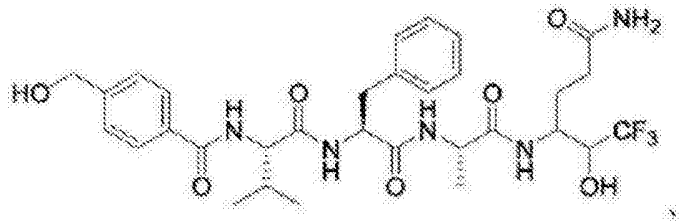

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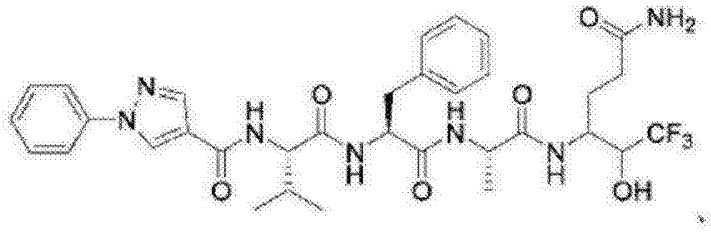

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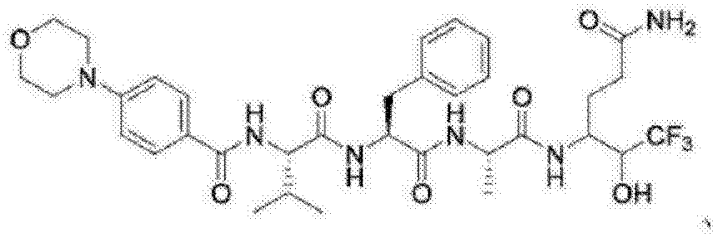

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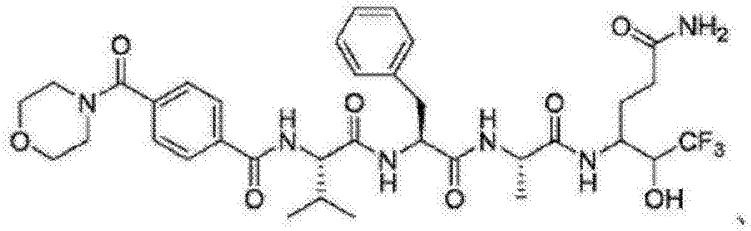

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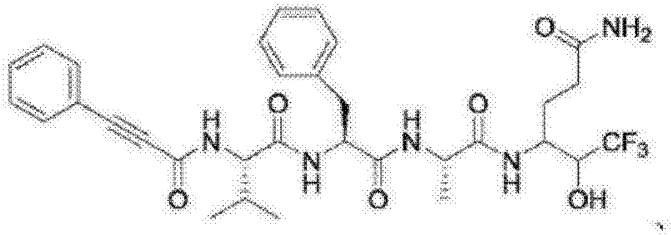

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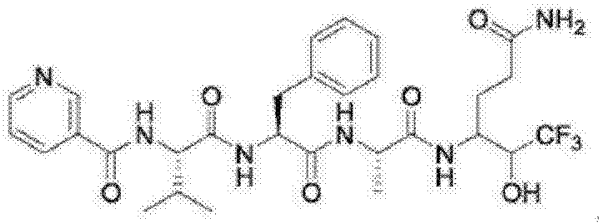

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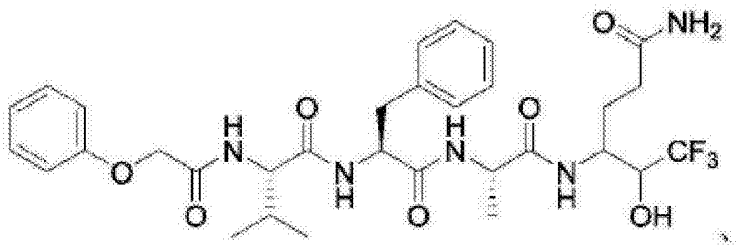

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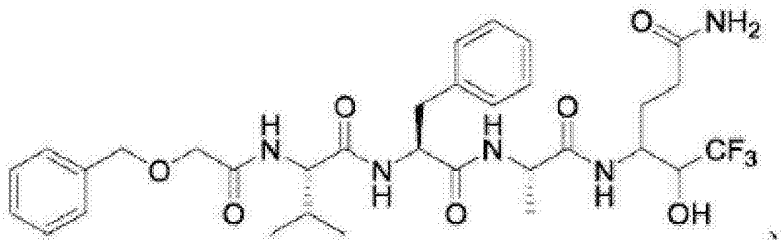

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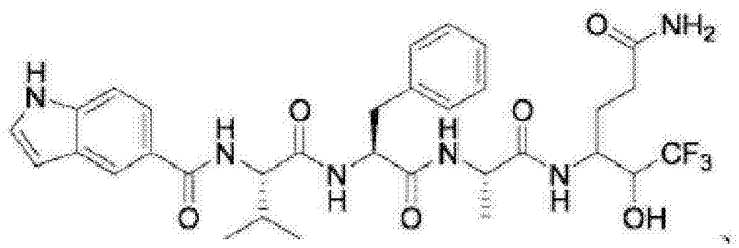

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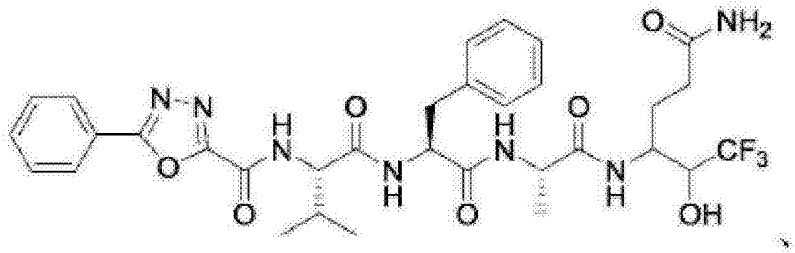

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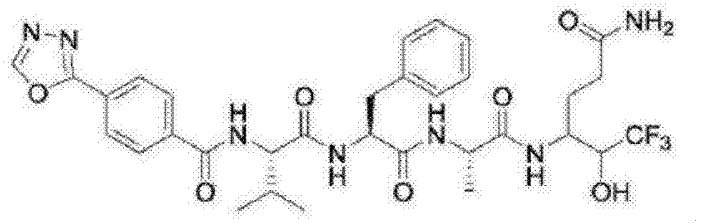

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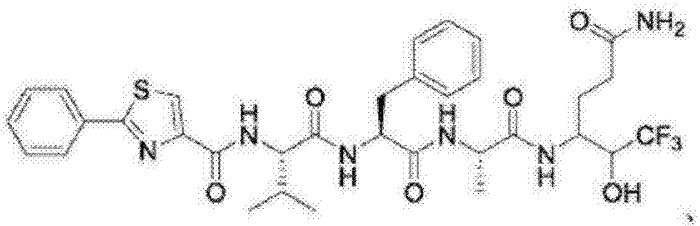

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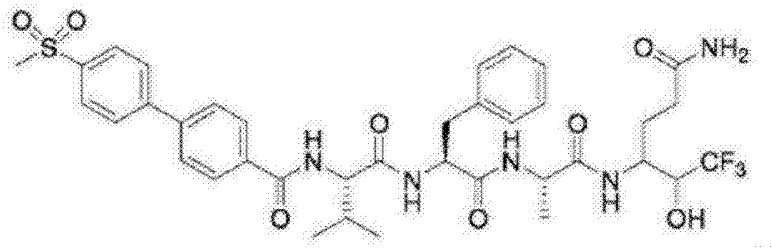

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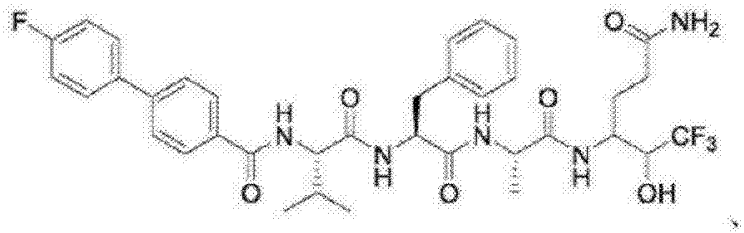

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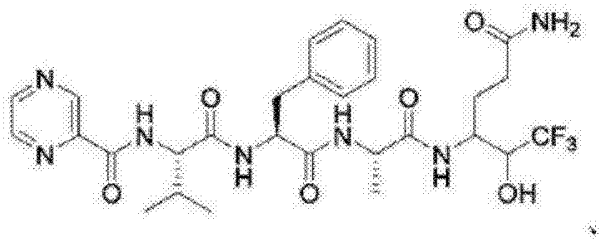

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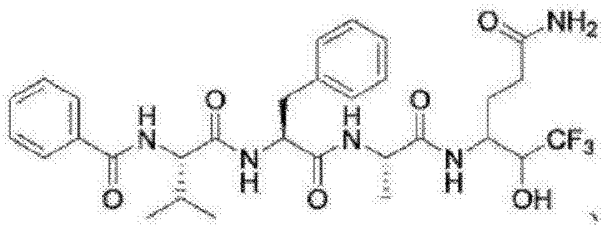

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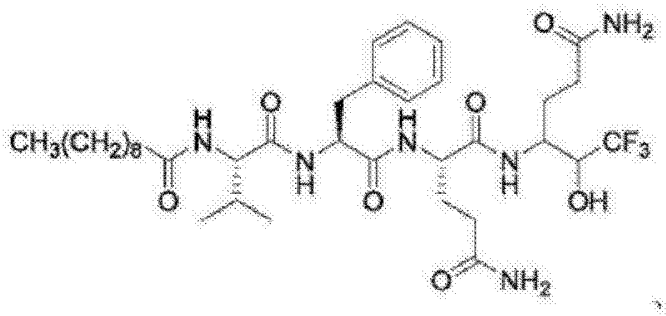

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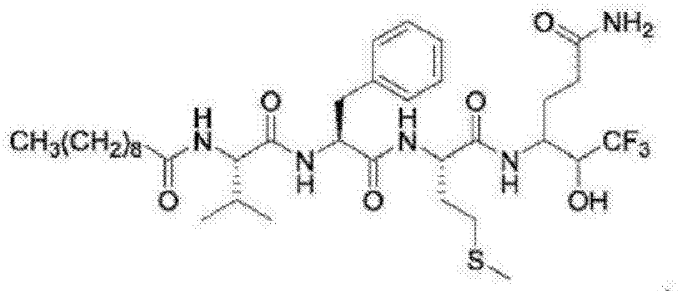

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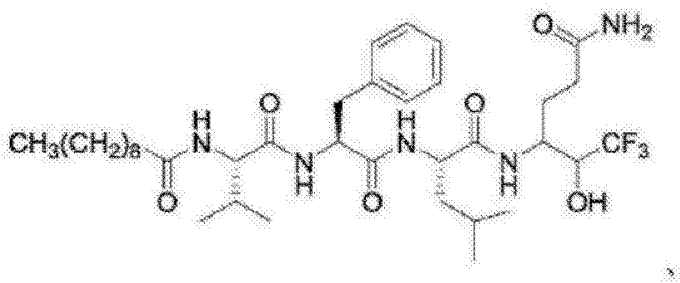

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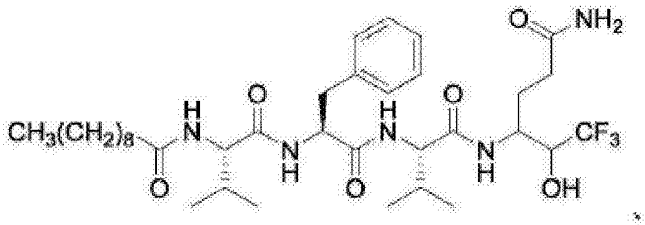

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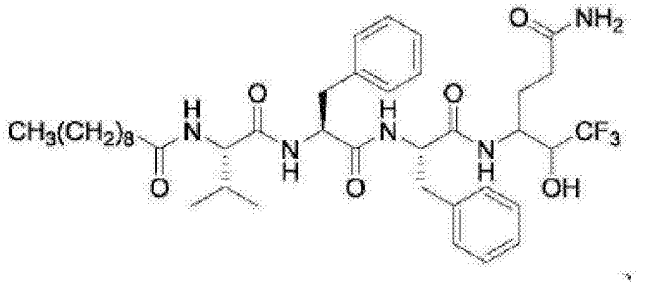

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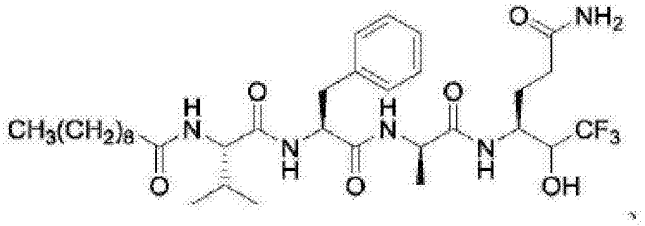

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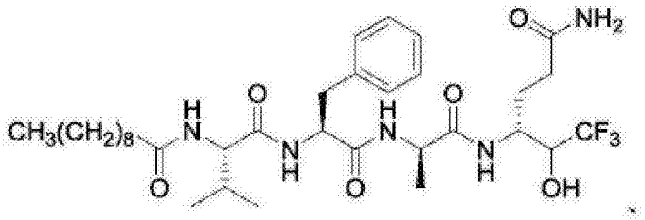

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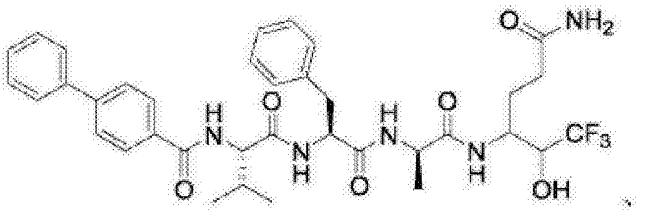

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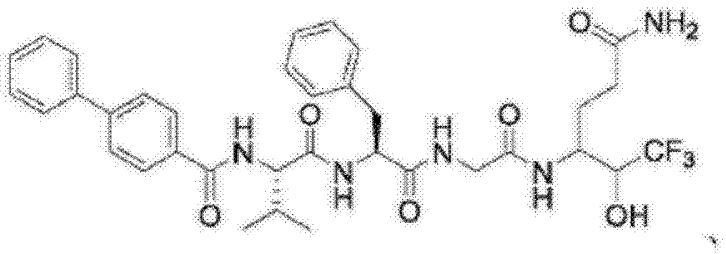

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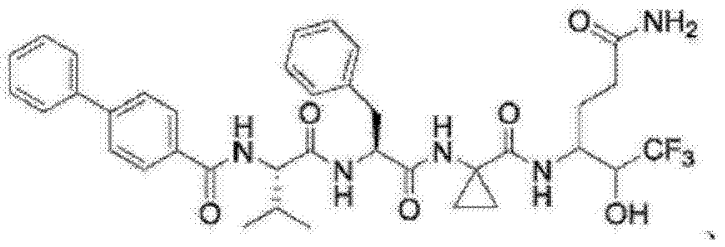

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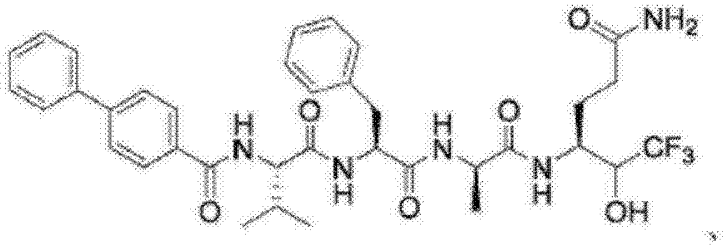

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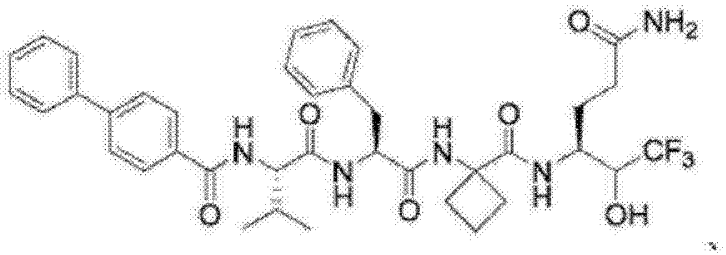

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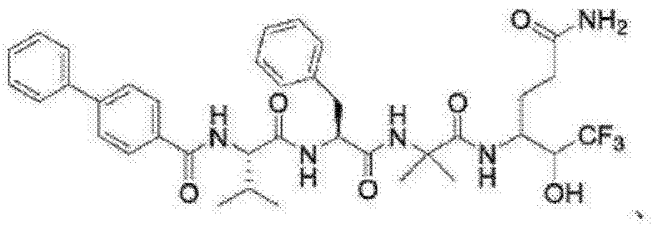

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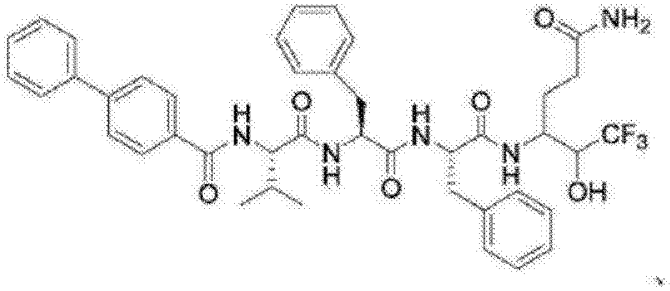

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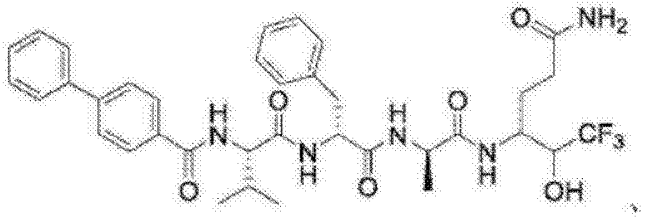

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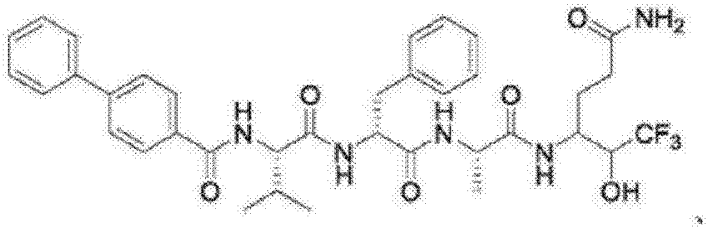

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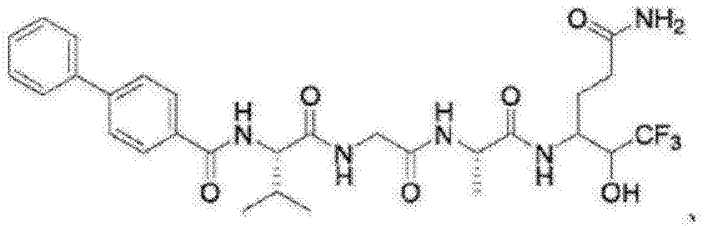

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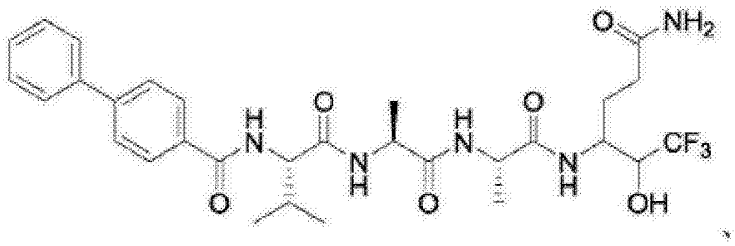

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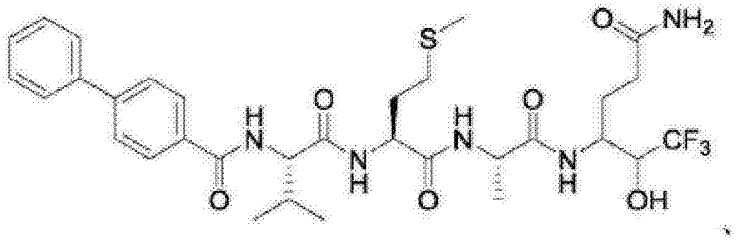

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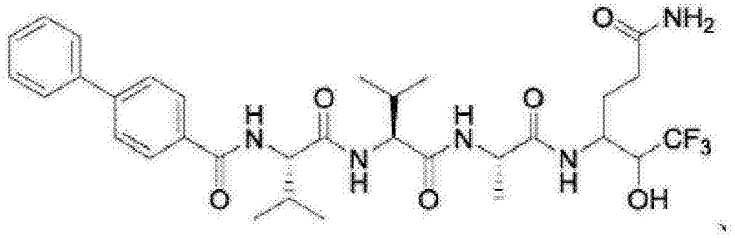

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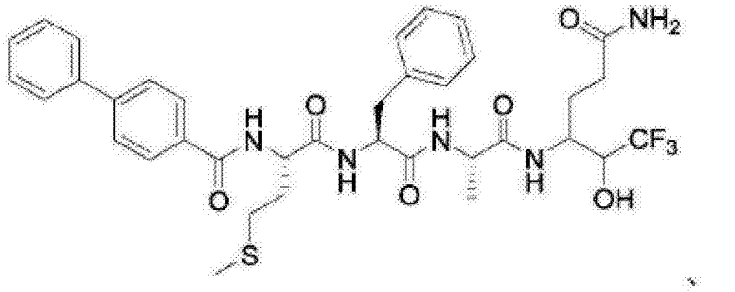

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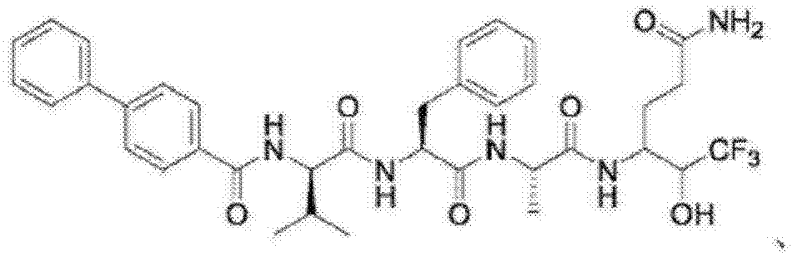

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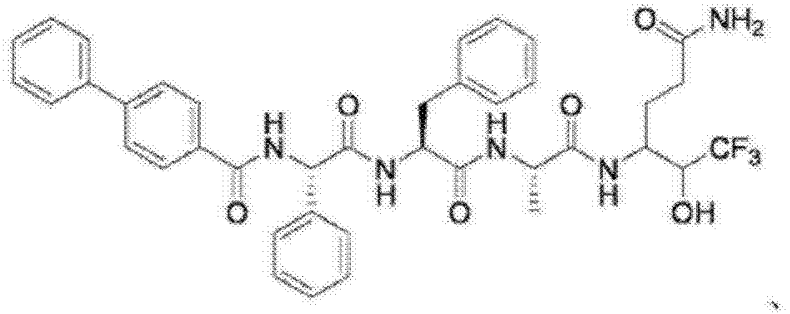

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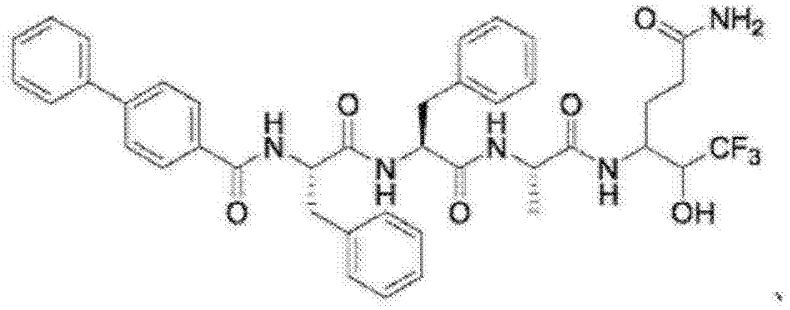

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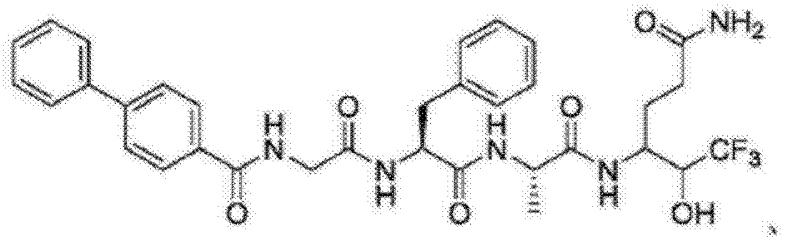

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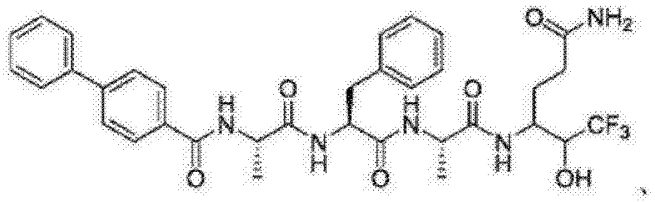

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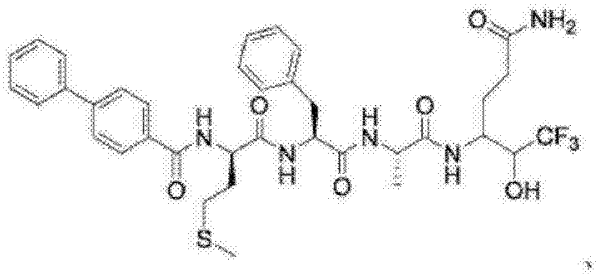

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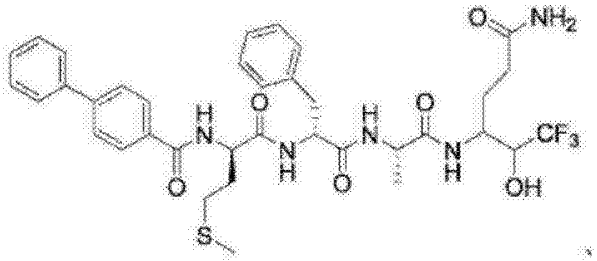

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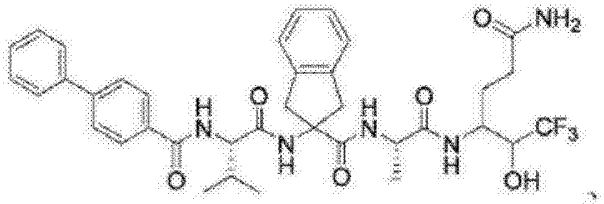

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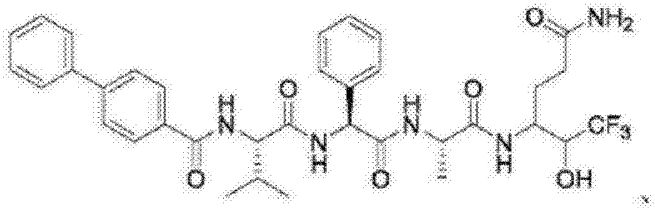

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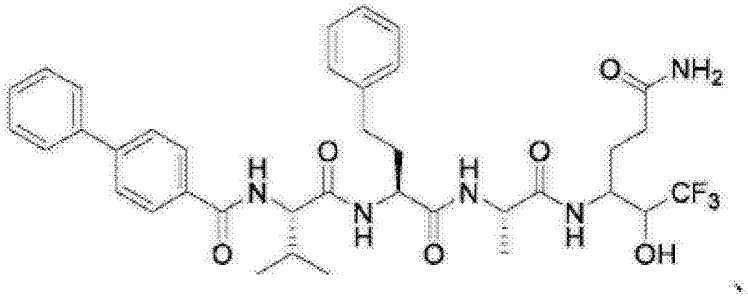

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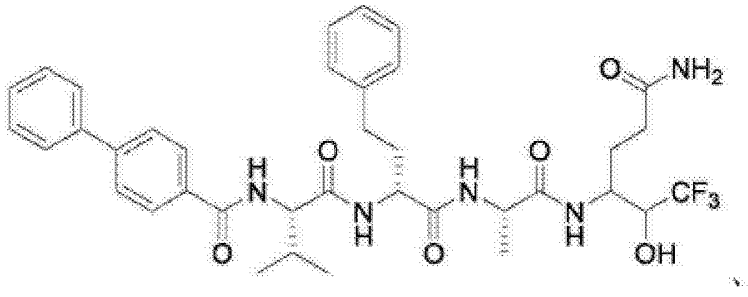

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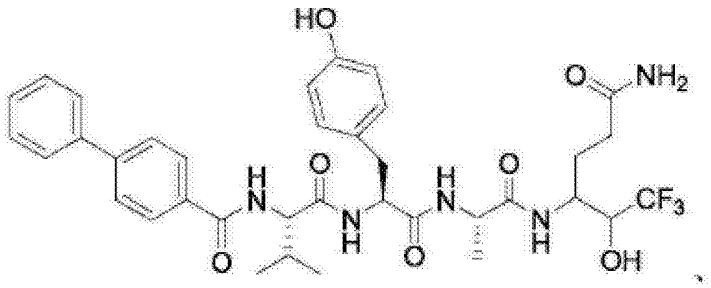

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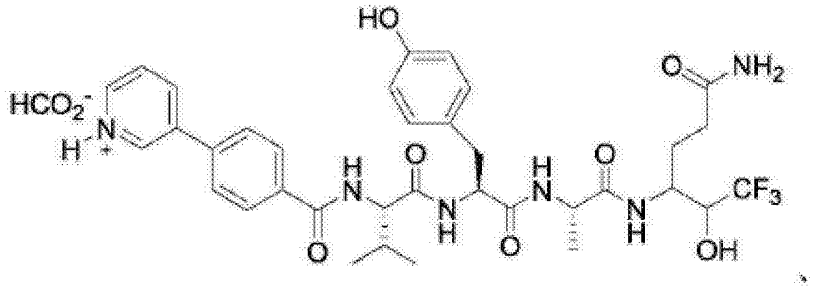

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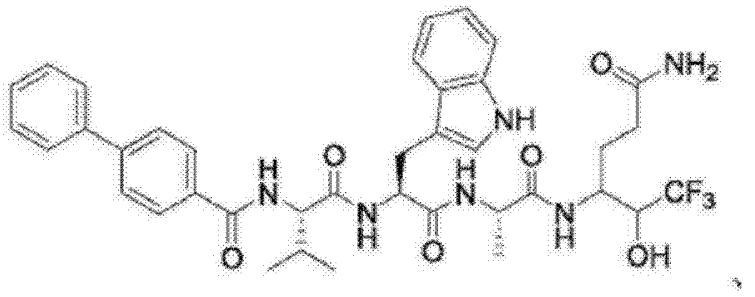

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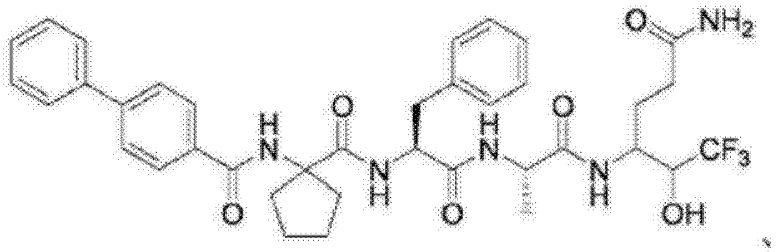

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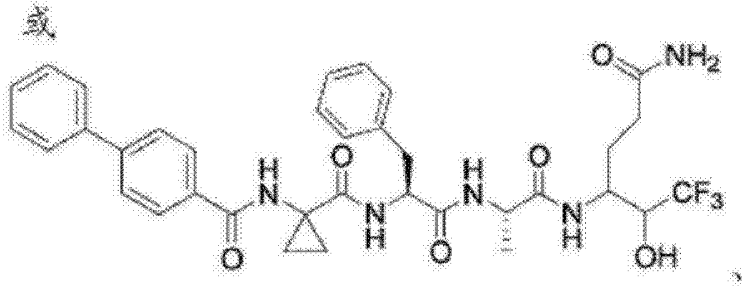


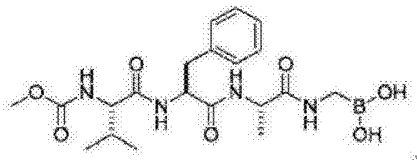
91

[0094] 在一个具体实施方案中，本发明化合物不是化合物 1 ；2 ；17 ；21a 和 21b 的混合物；25 和 / 或 26。

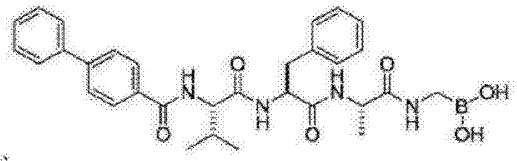
[0095] 根据本发明的另一方面，提供一种药物组合物，其包含至少一种本发明化合物。在所述组合物的一具体实施方案中，所述组合物进一步包含至少一种其它本发明化合物。在另一特定实施方案中，所述组合物进一步包含至少一种会改善患者的血脂谱的其它活性成分。在另一特定实施方案中，所述组合物进一步包含药物载体或赋形剂。

[0096] 根据本发明的另一方面，提供一种本发明化合物或组合物，其用作药剂。在一个具体实施方案中，所述化合物或组合物是用于制造药剂。在另一特定实施方案中，所述药剂是用于预防或治疗受试者的低密度脂蛋白 - 胆固醇相关疾病或病症，其限制条件为所述化合物不是以下：

[0097]

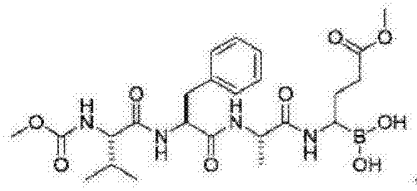


1



2

或

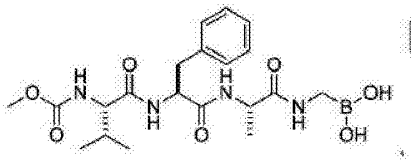


17

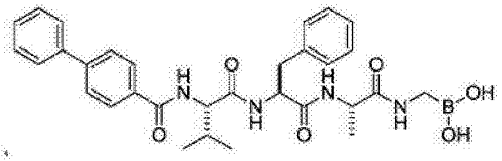
[0098] 在所述化合物或组合物的另一具体实施方案中，所述低密度脂蛋白－胆固醇相关疾病或病症为高胆固醇血症。

[0099] 根据本发明的另一方面，提供一种预防或治疗 LDL- 胆固醇相关疾病或病症的方法，其包括对有此需要的受试者施用治疗上有效量的本发明化合物或组合物（例如，包含本发明化合物的组合物），其限制条件为所述化合物不是以下：

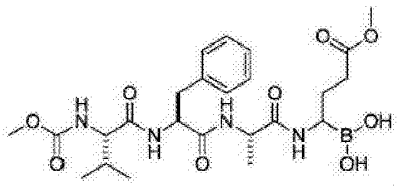
[0100]

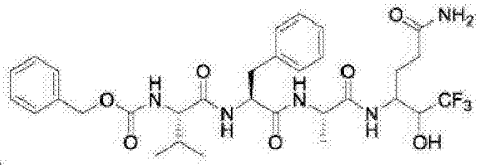


1



2

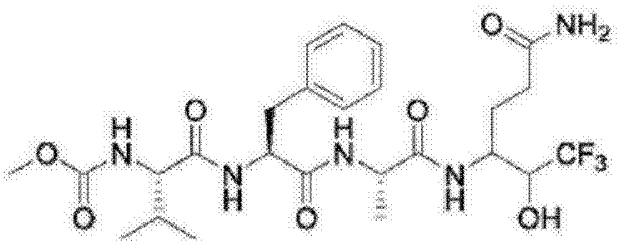




[0101]

17 25

或

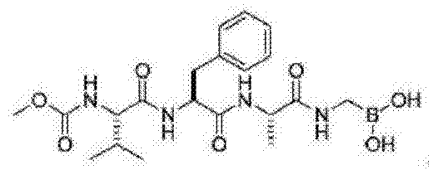


26

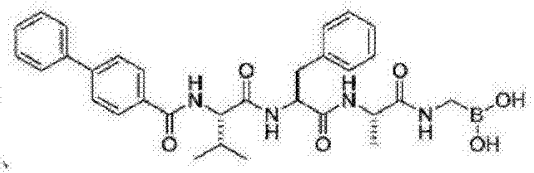
[0102] 在所述方法的一具体实施方案中，所述低密度脂蛋白－胆固醇相关疾病或病症为高胆固醇血症。

[0103] 根据本发明的另一方面，提供一种本发明化合物或组合物的用途，其用作药剂。在一个具体实施方案中，本发明化合物或组合物的用途是用于预防或治疗受试者的低密度脂蛋白－胆固醇相关疾病或病症，其限制条件为所述化合物不是以下：

[0104]



1、



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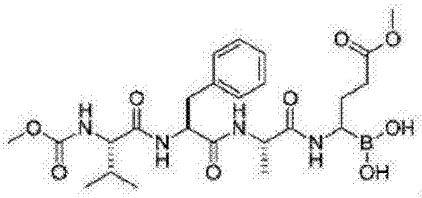
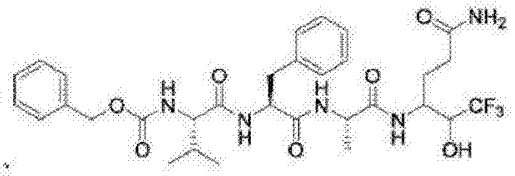


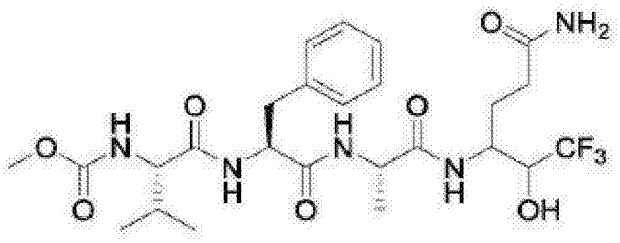
17

或



25、

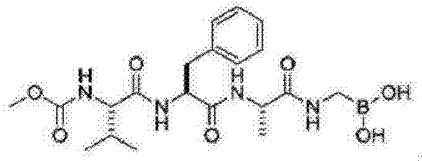
[0105]



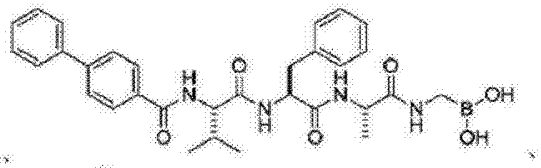
26。

[0106] 在一个具体实施方案中，所述用途是用于制造用于预防或治疗受试者的低密度脂蛋白－胆固醇相关疾病的药剂，其限制条件为所述化合物不是以下：

[0107]



1



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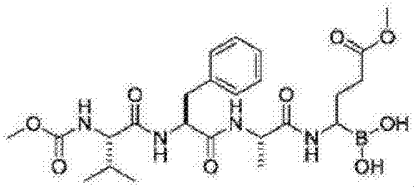

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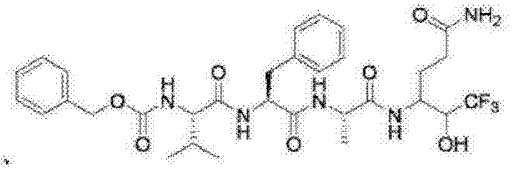
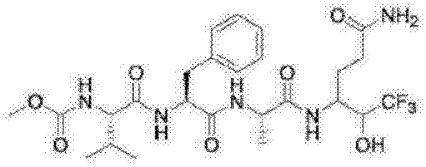


25

或

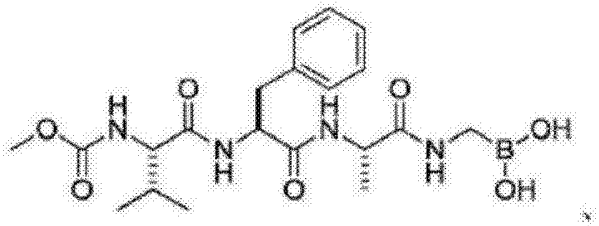


26

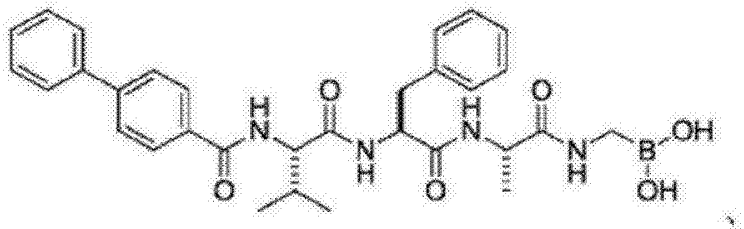
[0108] 在所述用途的另一个具体实施方案中，所述低密度脂蛋白－胆固醇相关疾病或病症为高胆固醇血症。

[0109] 根据本发明的另一方面，提供一种用于预防或治疗受试者的低密度脂蛋白－胆固醇相关疾病或病症的试剂盒，所述试剂盒包括 (i) 至少一种本发明化合物或组合物，和 (ii)(a) 至少一种改善患者的血脂谱的其它活性成分；(b) 至少一种其它本发明化合物或组合物；(c) 用于所述化合物和 / 或活性成分的容器；和 / 或 (d) 关于使用所述化合物来预防或治疗所述受试者的低密度脂蛋白－胆固醇相关疾病或病症的说明书，其限制条件为所述化合物不是以下：

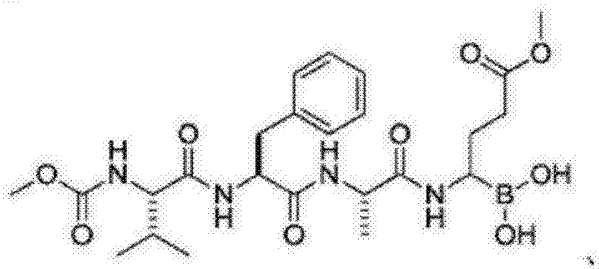
[0110]



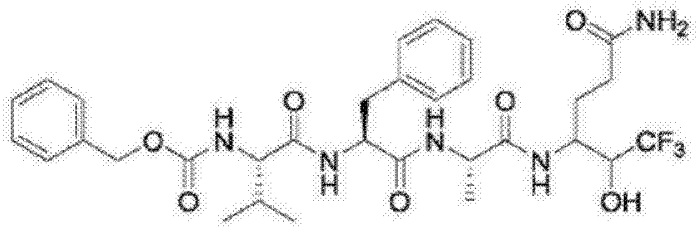
1



2



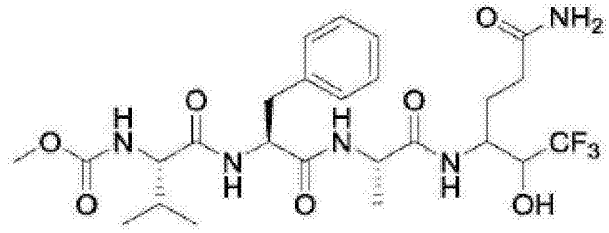
17



25

或

[0111]



26

[0112] 在所述试剂盒的一个具体实施方案中，所述低密度脂蛋白－胆固醇相关疾病或病

症为高胆固醇血症。

[0113] 附图简述

[0114] 在附图中：

[0115] 图 1 示出了 (A)19 种化合物对 PCSK9 分泌的效应，作为初始筛选的一部分。将稳定表达 WT PCSK9(+V5) 的 HepG2 细胞在只有培养基 (-) 或含有 DMSO 对照 (DMSO) 或 100 μM 标明化合物的培养基中培养 24h。在回收的培养基和细胞级分中的 PCSK9 水平是通过 ELISA 量化且表示为 ng PCSK9/ml 级分。标明化合物对 PCSK9 分泌的抑制效应是从相对于 DMSO 对照降低培养基 / 细胞比来评估。星号识别具有抑制活性的化合物；以及 (B) 化合物 a 至 j 的结构；

[0116] 图 2 示出了 3 种化合物对 PCSK9 分泌的剂量依赖性效果。在不存在 (DMSO) 或存在渐增浓度的每种标明化合物下培养稳定表达 WT PCSK9(+V5) 的 HepG2 细胞 24h。回收培养基和细胞并通过 ELISA 量化每种级分中的 PCSK9 水平。细胞中的 PCSK9 水平针对总蛋白质量校正，其是通过 Bio-Rad DC 蛋白测定法测量。在每种浓度下，标明化合物对 PCSK9 分泌的抑制效应是从相对于 DMSO 对照的培养基 / 细胞比降低来评估；

[0117] 图 3 示出了 5 种化合物相对于 DMSO 对照的细胞毒性测定法。在不存在 (DMSO) 或存在渐增浓度的每种标明化合物下培养稳定表达 WT PCSK9(+V5) 的 HepG2 细胞 24h。通过 MTT 细胞毒性测定法测量毒性水平；

[0118] 图 4 示出了 PCSK9 抑制化合物对 HepG2 初始细胞的表面上的 LDLR 水平的效应。在不存在 (DMSO) 或存在 33.3 μM 化合物 19 下培养细胞 20h。通过免疫荧光分析非透化细胞。使用人类 LDLRAb 染色 LDLR(绿色标记)。显示每种 DMSO 对照和化合物 19 条件的三种不同场。注意，在细胞表面的 LDLR 的增加由化合物 19 诱导；

[0119] 图 5 示出了 PCSK9 抑制化合物对细胞表面 LDLR 的活性的效应，如通过在 HepG2 初始细胞 (A) 和 HEK293 初始细胞 (B) 中的 DiI-LDL 摄取所测得，或对总 LDLR 水平的效应，如通过在 HepG2 初始细胞 (C) 中的 Western 印迹分析所测得。阴性对照是化合物 i。在不存在 (DMSO) 或存在 33.3 μM 标明化合物下培养表达内源性 PCSK9 的 HepG2 初始细胞 (A) 或缺乏 PCSK9 表达的 HEK293 初始细胞 (B)6h，然后添加 DiI-LDL，接着再进行 18h- 培养。对于每种条件，Dil-LDL 摄取 (Dil 荧光探针的荧光) 是针对细胞总数 (CyQuant™ GR 染料的荧光) 校正且表示为 DMSO 对照的活性％。数据呈现以一式三份进行的 2 至 5 个 (A) 或 2 至 3 个 (B) 独立实验的平均值。(C) 在不存在 (Cnt_0) 或存在渐增浓度的化合物 19 下培养 24h 的 HepG2 初始细胞中的总 LDLR 的 Western 印迹分析。将标准化为 β- 肌动蛋白的总 LDLR 水平针对每种条件以对照 (Cnt)％绘图。使用图像 J™软件获得蛋白质带的量化；和

[0120] 图 6 示出了 PCSK9 的体内抑制。在展现" 人源化" LDLc 谱 (LDLc x 2-3) 并通过具有 WT 和 $Ldlr^{-/-}$ 背景的相互杂交小鼠产生的杂合子 $Ldlr^{+/-}$ 小鼠中测试选定的抑制剂。这些小鼠表现正常水平的小鼠 PCSK9($Pcsk9^{+/+}$)、不表现 PCSK9($Pcsk9^{-/-}$) 和 / 或从其自身的人类启动子 (TgDY) 编码人类 PCSK9-D374Y 的转基因的一或五种副本。还测试 PCSK9 的人类 WT 形式。

具体实施方式

[0121] PCSK9 也称为神经细胞凋亡 - 调节的转化酶 1(NARC-1)，是一种人的 692 个氨基

酸的蛋白酶 K-Hke 枯草杆菌酶 (NP_777596.2)，并包括信号肽 (1-30)，接着前片段 (残基 31-152)、催化结构域 (残基 153-454) 和 C- 端 Cys-His- 富结构域 (CHRD ;残基 455-692)。PCSK9 是在能够增殖和分化的细胞，诸如肝细胞、肾间质细胞、肠回肠、结肠上皮细胞和胚胎脑端脑的神经元中表达 (Seidah 等人，2003，Proc. Natl. Acad. Sci. USA 100 :928-933)。

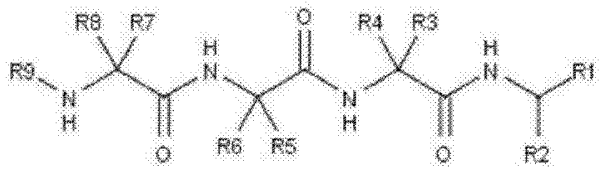
[0122] 继在 ER 中易位后，PCSK9 的前片段在 $VFAQ_{152}\downarrow$ SIP 位点自动催化裂解。在 PC 中，前片段 (pro) 是分子内分子伴侣 / 抑制剂，其通常在细胞内移除以产生完全活性的蛋白酶。不同于其它 PC，PCSK9 是分泌为稳定的非共价络合物 [pro ≡ PCSK9]。因此，由 PCSK9 诱导的 LDLR 的降解增强不需要成熟 PCSK9 形式的催化活性。在人类和小鼠血浆中，可以检测到两种全长的 PCSK9(153-692) 和截短形式的 PCSK9- ΔN218(219-692)。后者对 LDLR 没有活性，它很可能由弗林蛋白酶和 / 或 PC5 产生，因为它们在 RFHR218 ↓ 体外裂解 PCSK9。

[0123] 在本文所公开的研究中，本发明者已产生并识别针对人类 PCSK9 的抑制化合物，并不显示，这些化合物抑制 PCSK9 活性 (例如，在人肝癌来源的细胞系如 HepG2 中，PCSK9 分泌、诱导的 LDLR 降解)。

[0124] 化合物

[0125] 本发明涉及式 (I) 化合物 :

[0126]



(I)

[0127] 或其药学上可接受的盐、水合物、溶剂合物或外消旋混合物或立体异构体，

[0128] 其中 :

[0129] R1 为 $-CH(OH)R_a$ 或 $-B(OR_b)(OR_c)$;

[0130] R2 为 -H、$-CH_2R_d$、$-CHR_d(R_e)$、$-CH_2(CH_2)_mC(O)R_j$、$-CH_2(CH_2)_mC(O)N(R_d)R_e$ 或 $-CH_2(CH_2)_mS(O)_nN(R_d)R_e$;

[0131] R3、R4、R5、R6、R7 和 R8 是相同或不同的且独立地为氢或以下基团中的一者 : C1-6 烷基、C1-6 卤代烷基、C1-6 硫代烷基、C1-6 氨基烷基、烯基、炔基、环烷基、杂环基、芳基和杂芳基，其中所述基团任选地经一个或多个 C1-6 烷基、C3-8 环烷基、C1-6 卤代烷基、芳基、杂芳基、-CN、$-C(O)N(R_f)R_g$、$-C(O)OR_f$、$-C(R_f)(R_g)OR_h$、$-OR_f$、$-OC(O)OR_f$、$-OC(O)NR_f(R_g)$、$-SR_f$、$-S(O)_nR_f$、$-S(O)_nN(R_f)R_g$、$-S(O)_nN(R_f)C(O)R_g$、$-N(R_f)R_g$、$-N(R_f)C(O)R_g$、$--N(R_f)C(O)OR_g$、$-N(R_f)C(O)N(R_g)(R_h)$、$-N(R_f)S(O)_nR_g$ 和 $-N(R_f)S(O)N(R_g)R_h$ 取代基取代 ;

[0132] 当 (R3 和 R4) 或 (R5 和 R6) 或 (R7 和 R8) 不是氢时，括号内的对也可以与 $-C(O)-$、$-CO_2-$、$-C(O)N(H)-$、$-C(O)N(R_x)-$、-O-、-NH-、$-N(R_x)-$、-S-、$-S(O)_n-$、$-S(O)_nN(H)-$、$-S(O)_nN(R_x)-$ 基团连接以形成环状结构 ;

[0133] R9 为 $R_iC(O)-$、$R_iS(O)_n-$、$R_iOC(O)-$、$R_iNHC(O)-$、$R_iNHS(O)_n-$、$R_k(R_l)NC(O)-$、$R_l(R_l)NS(O)_n-$、$R_mOR_iC(O)-$、$R_mC(O)R_iC(O)-$ 或一个或多个氨基酸残基 ;

[0134] R_a 为 C1-3 烷基、C1-2 氟烷基或环丙基 ;

[0135] R_b 和 R_c 是相同或不同的且独立地为 H 或 C1-6 烷基或可以连接在一起以形成环状 5- 或 6- 员环结构，或与另外的脂族或芳族环体系稠合，所述环状 5- 或 6- 员环结构或脂族

或芳族环体系任选地经一个或多个C1-6烷基和/或C1-6卤代烷基取代基取代；

[0136] R_d和R_e是相同或不同的且独立地为H或以下基团中的一者：C1-3烷基、C1-3卤代烷基或C3-4环烷基，或可以直接或与-C(O)-、-C(O)O-、-C(O)$N(R_x)$--O-、-$N(R_x)$-、-S-、-$S(O)_n$-或-$S(O)_nN(R_x)$-基团连接在一起以形成环状3-8员环结构；

[0137] R_f、R_g、R_h、R_k和R_1是相同或不同的且独立地为H或以下基团中的一者：C1-6烷基、C1-6卤代烷基或C3-4环烷基，或可以直接或与-C(O)-、-C(O)O-、-C(O)$N(R_x)$--O-、-$N(R_x)$-、-S-、-$S(O)_n$-或-$S(O)_nN(R_x)$-基团连接在一起以形成环状3-8员环结构；

[0138] R_i为C1-10烷基、C1-10杂烷基、C3-8环烷基、C1-10卤代烷基、杂环基、芳基、杂芳基、C1-10烷基-C3-8环烷基、C1-10烷基-杂环基、C1-10烷基-芳基、C1-10烷基-杂芳基、C1-10杂烷基-C3-8环烷基、C1-10杂烷基-杂环基、C1-10杂烷基-芳基或C1-10杂烷基-杂芳基，其中所述基团任选地经一个或多个卤素、C1-6氨基烷基、C1-6杂烷基、C1-6烷基、C3-8环烷基、C1-6卤代烷基、芳基、杂芳基和杂环基取代；

[0139] R_m为C1-10烷基、C1-10杂烷基、C3-8环烷基、C1-10卤代烷基、杂环基、芳基或杂芳基，其中所述基团任选地经一个或多个卤素、C1-6氨基烷基、C1-6杂烷基、C1-6烷基、C3-8环烷基、C1-6卤代烷基、芳基、杂芳基和杂环基取代；

[0140] R_j为OR_d或$N(R_d)(R_e)$；

[0141] R_x为H、C1-6烷基、C1-6卤代烷基、C3-4环烷基、-C(O)R_y、-C(O)OR_y、-C(O)NH_2、-C(O)$NH(R_y)$或-C(O)$NHS(O)_nR_y$；

[0142] R_y为C1-6烷基、C1-6卤代烷基或C3-4环烷基；

[0143] m为值0或1的整数；且

[0144] n为值1或2的整数，

[0145] 限制条件为：

[0146] 1)当R1为-CH(OH)R_a时，R2为-$CHR_d(R_e)$、-$CH_2(CH_2)_mC(O)R_j$、-$CH_2(CH_2)_mC(O)N(R_d)R_e$或-$CH_2(CH_2)_mS(O)_nN(R_d)R_e$；且

[0147] 2)当R1为-$B(OR_b)(OR_c)$时，R2为-H、-CH_3、-$CHR_d(R_e)$、-$CH_2(CH_2)_mC(O)R_j$、-$CH_2(CH_2)_mC(O)N(R_d)R_e$或-$CH_2(CH_2)_mS(O)_nN(R_d)R_e$。

[0148] 如上所引用，本发明包括在R9中的另外的氨基酸残基。在R9中的一个或多个氨基酸残基可促进本发明化合物跨细胞膜的活性传输(参见Koren,E.;Torchilin,V.P.Cell Penetrating Peptides:Breaking Through to the Other Side.Trends Mol.Med.2012,18(7),385-93和其中的参考文献)。

[0149] 本发明的具体实施方案包括(但不限于)以下各者：

[0150] (2S)-3-甲基-N-[(1S)-2-苯基-1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9-三甲基-3,5-二氧杂-4-硼杂三环[6.1.1.$0^{2,6}$]癸-4-基]甲基}氨基甲酰基)乙基]氨基甲酰基}乙基]-2-(苯基甲酰胺基)丁酰胺；5。

[0151] N-[(1S)-2-甲基-1-{[(1S)-2-苯基-1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9-三甲基-3,5-二氧杂-4-硼杂三环[6.1.1.$0^{2,6}$]癸-4-基]甲基}氨基甲酰基)乙基]氨基甲酰基}乙基]氨基甲酰基}丙基]氨基甲酸苄酯；7。

[0152] N-[(1S)-2- 甲基 -1-{[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基] 氨基甲酰基 } 丙基] 氨基甲酸叔丁酯 ;6。

[0153] (2S)-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基]-2-({4-[3-(三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 } 甲酰胺基) 丁酰胺 ;8a。

[0154] (2S)-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基]-2-({3-[3-(三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 } 甲酰胺基) 丁酰胺 ;8b。

[0155] (2S)-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基]-2-(2-{4-[3-(三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 } 乙酰胺基) 丁酰胺 ;8c。

[0156] (2S)-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基]-2-(2-{3-[3-(三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 } 乙酰胺基) 丁酰胺 ;8d。

[0157] (2S)-2-[(2- 氟苯基) 甲酰胺基]-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基] 丁酰胺 ;9。

[0158] {[(2S)-2-[(2S)-2-[(2S)-3- 甲基 -2-{2-[4-(三氟甲氧基) 苯基] 乙酰胺基 } 丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 甲基 } 硼酸 ;3。

[0159] {[(2S)-2-[(2S)-2-[(2S)-2- 癸酰胺基 -3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 甲基 } 硼酸 ;4。

[0160] {[(2S)-2-[(2S)-2-[(2S)-3- 甲基 -2-[(4- 苯基苯基) 甲酰胺基] 丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 甲基 } 硼酸 ;2。

[0161] {[(2S)-2-[(2S)-2-[(2S)-2-[(甲氧基羰基) 氨基]-3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 甲基 } 硼酸 ;1。

[0162] [(1R/S)-3- 氨基甲酰基 -1-[(2S)-2-[(2S)-2-[(2S)-2-[(甲氧基羰基) 氨基]-3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 丙基] 硼酸 ;18。

[0163] [(1R/S)-4- 甲氧基 -1-[(2S)-2-[(2S)-2-[(2S)-2-[(甲氧基羰基) 氨基]-3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基]-4- 氧代丁基] 硼酸 ;17。

[0164] N-[(1S)-1-{[(1R/S)-1-{[(1S)-1-{[(1S)-3- 氨基甲酰基 -1-[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 丙基] 氨基甲酰基 } 乙基] 氨基甲酰基 }-2- 苯基乙基] 氨基甲酰基 }-2- 甲基丙基] 氨基甲酸甲基酯 ;16。

[0165] (4R/S)-4-[(2S)-2-[(2S)-2-[(2S)-2-[(甲氧基羰基) 氨基]-3- 甲基丁酰胺基]-3- 苯基丙酰胺基]-2- 苯基乙酰胺基]-4-[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 丁酸甲酯 ;15。

[0166] N-[(1S)-2- 甲基 -1-{[(1S)-2- 苯基 -1-{[(S)- 苯基 ({[(1S,2S,6R,8S)-2,9,

9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 甲基] 氨基甲酰基 } 乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯 ;14。

[0167] N-[(1S)-2- 甲基 -1-{[(1S)-1-{[(1S)-3- 甲基 -1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 丁基] 氨基甲酰基 }-2- 苯基乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯 ;13。

[0168] N-[(1S)-2- 甲基 -1-{[(1S)-1-{[(1S)-3-(甲基磺酰基)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 丙基] 氨基甲酰基 }-2- 苯基乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯 ;12。

[0169] N-[(1S)-2- 甲基 -1-{[(1S)-2- 苯基 -1-{[(1S)-2- 苯基 -1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯 ;11。

[0170] N-[(1S)-2- 甲基 -1-{[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 丙基] 氨基甲酰基 } 乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯 ;10。

[0171] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺 ;19。

[0172] N-((2S)-1-(((2S)-1-(((2S)-1-(((3S)-6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺 ;20a。

[0173] N-((2S)-1-(((2S)-1-(((2S)-1-(((3R)-6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺 ;20b。

[0174] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1- 氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺 ;21a 和 21b。

[0175] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺 ;22a 和 22b。

[0176] 6,6,6- 三氟 -5- 羟基 -4-((S)-2-((S)-2-((S)-3- 甲基 -2-(2-(4-(三氟甲氧基) 苯基) 乙酰胺基) 丁酰胺基)-3- 苯基丙酰胺基) 丙酰胺基) 己酰胺 ;23。

[0177] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;24。

[0178] ((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 氨基甲酸苄酯 ;25。

[0179] ((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲

基-1-氧代丁-2-基)氨基甲酸甲酯;26。

[0180] ((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)氨基甲酸叔丁酯;27。

[0181] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-4-异丙基苯甲酰胺;28。

[0182] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-4-(3-(三氟甲基)-3H-双吖丙啶-3-基)苯甲酰胺;29。

[0183] 6,6,6-三氟-5-羟基-4-((S)-2-((S)-2-((S)-3-甲基-2-(2-(4-(3-(三氟甲基)-3H-双吖丙啶-3-基)苯基)乙酰胺基)丁酰胺基)-3-苯基丙酰胺基)丙酰胺基)己酰胺;30。

[0184] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-3-(3-(三氟甲基)-3H-双吖丙啶-3-基)苯甲酰胺;31。

[0185] 6,6,6-三氟-5-羟基-4-((S)-2-((S)-2-((S)-3-甲基-2-(2-(3-(3-(三氟甲基)-3H-双吖丙啶-3-基)苯基)乙酰胺基)丁酰胺基)-3-苯基丙酰胺基)丙酰胺基)己酰胺;32。

[0186] 4-((S)-2-((S)-2-((S)-2-([1,1′-联苯基]-4-磺酰氨基)-3-甲基丁酰胺基)-3-苯基丙酰胺基)丙酰胺基)-6,6,6-三氟-5-羟基己酰胺;33。

[0187] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-5-甲基-1-苯基-1H-吡唑-4-甲酰胺;34。

[0188] 6,6,6-三氟-5-羟基-4-((S)-2-((S)-2-((S)-3-甲基-2-(2-苯基乙酰胺基)丁酰胺基)-3-苯基丙酰胺基)丙酰胺基)己酰胺;35。

[0189] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-6-苯基烟酰胺;36)

[0190] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-2-(吡啶-4-基)噻唑-4-甲酰胺;37。

[0191] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-4-(吡啶-3-基)苯甲酰胺;38。

[0192] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-4-(羟甲基)苯甲酰胺;39。

[0193] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代

己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-1- 苯基 -1H- 吡唑 -4- 甲酰胺 ;40。

[0194] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4- 吗啉并苯甲酰胺 ;41。

[0195] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4-(吗啉 -4- 羰基) 苯甲酰胺 ;42。

[0196] 6,6,6- 三氟 -5- 羟基 -4-((S)-2-((S)-2-((S)-3- 甲基 -2-(3- 苯基丙酰胺基) 丁酰胺基)-3- 苯基丙酰胺基) 丙酰胺基) 己酰胺 ;43。

[0197] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 烟酰胺 ;44。

[0198] 6,6,6- 三氟 -5- 羟基 -4-((S)-2-((S)-2-((S)-3- 甲基 -2-(2- 苯氧基乙酰胺基) 丁酰胺基)-3- 苯基丙酰胺基) 丙酰胺基) 己酰胺 ;45。

[0199] 4-((6S,9S,12S)-9- 苄基 -6- 异丙基 -12- 甲基 -4,7,10- 三氧代 -1- 苯基 -2- 氧杂 -5,8,11- 三氮杂十三 -13- 酰胺基)-6,6,6- 三氟 -5- 羟基己酰胺 ;46。

[0200] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-1H- 吲哚 -5- 甲酰胺 ;47。

[0201] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-5- 苯基 -1,3,4- 噁二唑 -2- 甲酰胺 ;48。

[0202] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4-(1,3,4- 噁二唑 -2- 基) 苯甲酰胺 ;49。

[0203] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-2- 苯基噻唑 -4- 甲酰胺 ;50。

[0204] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4′ -(甲基磺酰基)-[1,1′ - 联苯基]-4- 甲酰胺 ;51。

[0205] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4′ - 氟 -[1,1′ - 联苯基]-4- 甲酰胺 ;52。

[0206] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 吡嗪 -2- 甲酰胺 ;53。

[0207] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代

己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)苯甲酰胺;54。

[0208] 2S)-N^1-(6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)-2-((S)-2-((S)-2-癸酰胺基-3-甲基丁酰胺基)-3-苯基丙酰胺基)戊二酰胺;55。

[0209] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-4-(甲硫基)-1-氧代丁-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺;56。

[0210] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-4-甲基-1-氧代戊烷-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺;57)

[0211] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-3-甲基-1-氧代丁-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺;58。

[0212] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺;59。

[0213] N-((2S)-1-(((2S)-1-(((2R)-1-(((3S)-6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺;60。

[0214] N-((2S)-1-(((2S)-1-(((2R)-1-(((3R)-6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺;61。

[0215] N-((2S)-1-(((2S)-1-(((2R)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;62。

[0216] N-((2S)-1-(((2S)-1-((2-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-2-氧代乙基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;63。

[0217] N-((2S)-1-(((2S)-1-((1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基甲酰基)环丙基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;64。

[0218] N-((2S)-1-(((2S)-1-(((2R)-1-(((3S)-6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;65。

[0219] N-((2S)-1-(((2S)-1-((1-(((3S)-6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基甲酰基)环丁基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;66。

[0220] N-((2S)-1-(((2S)-1-((1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-2-甲基-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧

代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;67。

[0221] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;68。

[0222] N-((2S)-1-(((2R)-1-(((2R)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;69。

[0223] N-((2S)-1-(((2R)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;70。

[0224] N-((2S)-1-((2-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-2- 氧代乙基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;71。

[0225] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;72。

[0226] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-4-(甲硫基)-1- 氧代丁 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;73。

[0227] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;74。

[0228] N-((5S,8S,11S)-17- 氨基 -8- 苄基 -11- 甲基 -6,9,12,17- 四氧代 -14-(2,2,2- 三氟 -1- 羟基乙基)-2- 硫杂 -7,10,13- 三氮杂十七 -5- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;75。

[0229] N-((2R)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;76。

[0230] N-((1S)-2-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-2- 氧代 -1- 苯基乙基)-[1,1′ - 联苯基]-4- 甲酰胺 ;77。

[0231] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺 ;78。

[0232] N-(2-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-2- 氧代乙基)-[1,1′ - 联苯基]-4- 甲酰胺 ;79。

[0233] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-1- 氧代

丙-2-基)-[1,1′-联苯基]-4-甲酰胺;80。

[0234] N-((5R,8S,11S)-17-氨基-8-苄基-11-甲基-6,9,12,17-四氧代-14-(2,2,2-三氟-1-羟基乙基)-2-硫杂-7,10,13-三氮杂十七-5-基)-[1,1′-联苯基]-4-甲酰胺;81。

[0235] N-((5R,8R,11S)-17-氨基-8-苄基-11-甲基-6,9,12,17-四氧代-14-(2,2,2-三氟-1-羟基乙基)-2-硫杂-7,10,13-三氮杂十七-5-基)-[1,1′-联苯基]-4-甲酰胺;82。

[0236] 2-((S)-2-([1,1′-联苯基]-4-甲酰胺基)-3-甲基丁酰胺基)-N-((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)-2,3-二氢-1H-茚-2-甲酰胺;83。

[0237] N-((2S)-1-(((1S)-2-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-2-氧代-1-苯基乙基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;84。

[0238] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-4-苯基丁-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;85。

[0239] N-((2S)-1-(((2R)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-4-苯基丁-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;86。

[0240] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-3-(4-羟苯基)-1-氧代丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;87。

[0241] 甲酸3-(4-(((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-3-(4-羟苯基)-1-氧代丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)氨基甲酰基)苯基)吡啶-1-鎓;88。

[0242] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-3-(1H-吲哚-3-基)-1-氧代丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺;89。

[0243] N-(1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基甲酰基)环戊基)-[1,1′-联苯基]-4-甲酰胺;90。

[0244] N-(1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基甲酰基)环丙基)-[1,1′-联苯基]-4-甲酰胺;91。

[0245] 以上所列的化合物由数字(以粗体表示)识别。这些数字也是指具体的实例,在实例中以上所列化合物每个都进一步进行描述。如果本文针对本发明化合物所用的结构和名称之间存在差异,则以结构为准。

[0246] 定义:

[0247] 化学基团

[0248] 如本文所用，术语“烷基”是指具有在指定范围内的多个碳原子的单价直链或支链饱和或不饱和脂族烃基。因此，例如，“C1-6 烷基”（或“C1-C6 烷基”）是指己基烷基和戊基烷基异构体以及正、异、仲和叔丁基、正和异丙基、乙基和甲基中的任一者。作为另一个实例，“C1-4 烷基”是指正、异、仲和叔丁基、正和异丙基、乙基和甲基。作为另一个实例，“C1-3 烷基”是指正丙基、异丙基、乙基和甲基。烷基包括不饱和脂族烃，包括炔烃 (R-C ≡ C-R)；和 / 或烯烃 (R-C = C-R)。

[0249] 术语“卤素”（或“卤代”）是指氟、氯、溴和碘（可选地称为氟、氯、溴和碘）。术语“卤代烷基”是指其中一个或多个氢原子已经被替换为卤素（即 F、Cl、Br 和 / 或 I）的如上所定义的烷基。因此，例如，“C1-6 卤代烷基”（或“C1-C6 卤代烷基”）是指具有一个或多个卤素取代基的如上所定义的 C1 至 C6 直链或支链烷基。术语“氟烷基”具有类似的含义，不同的是卤素取代基被限定为氟。合适的氟烷基包括系列 $(CH2)_{0-4}CF_3$（即，三氟甲基、2,2,2- 三氟乙基、3,3,3- 三氟 - 正丙基等）。

[0250] 术语“杂烷基”被赋予本领域中的通常含义，且是指其中一个或多个碳原子被替换为杂原子（例如，氧、氮、硫或其衍生物等）的如本文所述的烷基。杂烷基的实例包括但不限于，烷氧基、烷基取代的氨基、硫醇基如蛋氨酸侧基。多达两个杂原子可以是连续的。当一个前缀，如 C2-6 被用于指杂烷基，碳原子数（在本实例中为 2-6）是指也包括所述杂原子。

[0251] 术语“氨基烷基”是指其中一个或多个氢或碳原子已经被替换为氮或氨基衍生物的如上述所定义的烷基。因此，例如，“C1-6 氨基烷基”（或“C1-C6 氨基烷基”）是指具有一个或多个氨基衍生物（例如，NH、酰胺、双吖丙啶等）的如上所定义的 C1 至 C6 直链或支链烷基。

[0252] 术语“硫代烷基”是指其中一个或多个氢或碳原子已经被替换为硫原子或硫醇衍生物的如上述所定义的烷基。因此，例如，“C1-6 氨烷基”（或“C1-C6 氨基烷基”）是指具有一个或多个硫原子或硫醇衍生物（例如，S、SH 等）的如上所定义的 C1 至 C6 直链或支链烷基。

[0253] 氨基烷基和硫代烷基是术语“杂烷基”或取代的烷基的特定实施例并且由其涵盖，此取决于杂原子取代碳原子或氢原子。

[0254] 术语“环烷基”是指由任选地与另外的 (1-3) 脂族（环烷基）或芳族环体系稠合的饱和 3-8 元环组成的饱和脂环烃，每个另外的环由 3-8 元环组成。它包括但不限于环丙烷、环丁烷、环戊烷和环己烷。

[0255] 术语“杂环基”是指 (i) 含有 1 至 3 个独立地选自 N、O 和 S 的杂原子的 4- 至 7- 元饱和杂环，或 (ii) 为杂二环（例如，苯并环戊基）。本发明范围内的 4- 至 7- 元饱和杂环的实例包括，例如，氮杂环丁烷基、哌啶基、吗啉基、硫代吗啉基、噻唑烷基、异噻唑烷基、噁唑烷基、异噁唑烷基、吡咯烷基、咪唑烷基、哌嗪基、四氢呋喃基、四氢噻吩基、吡唑烷基、六氢嘧啶基、噻嗪烷基、噻氮杂环庚烷基、氮杂环庚烷基、二氮杂环庚烷基、四氢吡喃基、四氢噻喃基和二噁烷基。本发明范围内的 4- 至 7- 元不饱和杂环的实例包括单不饱和的杂环，对应于在前面的句子中列出的饱和杂环其中一个单键被替换为双键（例如，碳 - 碳单键被替换为碳 - 碳双键）。

[0256] 术语“C(O)”指羰基。术语“$S(O)_2$”和“SO_2”各自是指磺酰基。术语“S(O)”是指亚磺酰基。

[0257] 术语“芳基”是指由任选地稠合有另外的 (1-3) 脂族 (环烷基) 或芳环体系的 3-8 元环组成的芳族 (不饱和) 化合物，每个另外的环由 3-8 元环组成。在一个具体的实施方案中，它是指苯基、苯并环戊基或萘基。特别感兴趣的芳基是苯基。术语“杂芳基”是指 (i) 含有 1 至 3 个独立选自 N、O 和 S 杂原子的 3-、4-、5- 或 6- 元杂芳族环，或者 (ii) 选自下列的杂二环 ：喹啉基、异喹啉基和喹喔啉基。合适的 3-、4-、5- 和 6- 元杂芳族环包括，例如，双吖丙啶基、吡啶基 (也称为吡啶基)、吡咯基、二嗪 (例如，吡嗪基、嘧啶基、哒嗪基)、三嗪基、噻吩基、呋喃基、咪唑基、吡唑基、三唑基、噁唑基、异噁唑基、噁二唑基、噁三唑基、噻唑基、异噻唑和噻二唑。特别感兴趣的杂芳基为吡咯基、咪唑基、吡啶基、吡嗪基、喹啉基 (或喹啉基)、异喹啉基 (或异喹啉基) 和喹喔啉基。合适的杂二环包括吲哚基。

[0258] 如本文所用，且除非另有说明，否则术语“烷基”、“卤代烷基”、“氨基烷基”、“环烷基”、“杂环基”、“芳基”、“杂烷基”和“杂芳基”和指定其具体的实施方案的术语 (例如，丁基、氟丙基、氨基丁基、环丙烷、吗啉、苯基、吡唑等) 包括这些基团的取代的 (即在卤代烷基和氨基烷基的情况下，分别地除了它们的卤素和氮取代基) 和未取代的实施方案。因此，例如，术语“苯基”包括未取代的苯基以及氟苯基、羟苯基、甲基磺酰基苯基 (或联苯基)、三氟甲基 - 双吖丙啶 - 苯基、异丙基 - 苯基、三氟羟基 - 苯基。类似地，术语吡唑包括未取代的吡唑以及甲基吡唑。所述一个或多个取代基可以是胺、卤素、羟基、C1-6 氨基烷基、C1-6 杂烷基、C1-6 烷基、C3-8 环烷基、C1-6 卤代烷基、芳基、杂芳基和杂环基 (等)。

[0259] 应理解，上面列出的具体的环不限制在可在本发明中使用的环。这些环仅仅是代表性的。

[0260] 除非在特定情况下明确说明与此相反，否则本文所述的任何各种环状环和环系可被附连到化合物中在任何环原子的其余部分 (即，任何碳原子或任何杂原子)，条件是稳定的化合物产生。

[0261] 除非明确说明与此相反，否则本文引用的所有范围都包括在内。例如，被描述为含有“1 至 4 个杂原子”的杂芳环指所述环可以含有 1、2、3 或 4 个杂原子。还应理解，本文引用的任何范围包括在其范围内所有在该范围内的子范围。因此，例如，被描述为包含“1 至 4 个杂原子”的杂环旨在包括含有 2 至 4 个杂原子、3 或 4 个杂原子、1 至 3 个杂原子、2 或 3 个杂原子、1 或 2 个杂原子、1 个杂原子、2 个杂原子、3 个杂原子和 4 个杂原子的杂环作为其方面。

[0262] 作为另一实例，被描述为任选被“1 至 4 个取代基”取代的芳基或杂芳基旨在包括任选地被 1 至 4 个取代基、2 至 4 个取代基、3 至 4 个取代基、4 个取代基、1 至 3 个取代基、2 至 3 个取代基、3 个取代基、1 至 2 个取代基、2 个取代基和 1 个取代基取代的芳基或杂芳基。

[0263] 当任何变量 (例如，XA 或 XB) 在任何组成或式 I 或在描绘和描述本发明的化合物的任何其它通式中出现多于一次时，其每次出现时的定义独立于在每次其它出现时其定义。此外，取代基和 / 或变量的组合是允许的，只要这样的组合产生稳定的化合物。

[0264] 除非明确说明与此相反，否则由取代基为名的取代允许在环 (例如，环烷基、杂环基、芳基或杂芳基) 的任何原子，条件为这种环取代是化学上允许并产生稳定的化合物。

[0265] 盐、酯、水合物和溶剂合物

[0266] 本发明的化合物包括药理学上可接受的盐和其酯衍生物以及其水合物或溶剂合

物和所参考化合物的所有立体异构形式。本发明化合物和其药理学上可接受的酯可以在必要时形成药理学上可接受的盐。

[0267] 盐

[0268] 术语“药理学上可接受的盐”是指一种本发明化合物可转化的盐，其保留母体化合物的期望生物活性并且不赋予任何不希望的毒理学效应（见例如，Berge，S.M. 等人，1977 J.Pharm.Sci.66:1-19）。这类盐的实例包括酸加成盐和碱加成盐。酸加成盐包括那些衍生自无毒无机酸，如盐酸、硝酸、磷酸、硫酸、氢溴酸、氢碘酸、亚磷酸等，以及无毒有机酸如脂族单和二羧酸、苯基取代的链烷酸、羟基链烷酸、芳香酸、脂族和芳族磺酸等的盐。碱加成盐包括那些衍生自碱土金属，如钠、钾、镁、钙等，以及自无毒有机胺，如N，N′-二苄基乙二胺、N-甲基葡糖胺、氯普鲁卡因、胆碱、二乙醇胺、乙二胺、普鲁卡因等的盐。这样的盐的优选实例包括碱金属盐，如钠盐、钾盐、锂盐、镁盐或钙盐；碱土金属盐如钙盐和镁盐；金属盐，如铝盐、铁盐、锌盐、铜盐、镍盐和钴盐；胺盐如无机盐，包括铵盐；有机盐或铵盐，如叔辛胺盐、二苄胺盐、吗啉盐、葡糖胺盐、苯基甘氨酸烷酯盐、乙二胺盐、N-甲基葡糖胺盐、胍盐、二乙胺盐、三乙胺盐、二环己胺盐、N，N′-二苄基乙二胺盐、氯普鲁卡因盐、普鲁卡因盐、二乙醇胺盐、N-苄基苯乙胺盐、哌嗪盐、四甲基铵盐和三（羟甲基）氨基甲烷盐；无机酸盐如氢卤酸盐如氢氟酸盐、盐酸盐、氢溴酸盐或氢碘酸盐、硝酸盐、高氯酸盐、硫酸盐或磷酸盐；低碳数烷基磺酸盐如甲磺酸盐、三氟甲磺酸盐或乙磺酸盐；芳基磺酸盐如苯磺酸盐或对甲苯磺酸盐等，其对活的有机体是无毒的；有机酸盐如乙酸盐、苹果酸盐、己二酸盐、富马酸盐、琥珀酸盐、柠檬酸盐、藻酸盐、抗坏血酸盐、苯甲酸盐、苯磺酸盐、硫酸氢盐、丁酸盐、樟脑酸盐、樟脑磺酸盐、肉桂酸盐、环戊烷丙酸盐、二葡糖酸盐、十二烷基硫酸盐、乙磺酸盐、葡庚糖酸盐、甘油磷酸盐、半硫酸盐、庚酸盐、己酸盐、盐酸盐、氢溴酸盐、氢碘酸盐、2-羟基乙磺酸盐、衣康酸盐、乳酸盐、马来酸盐、扁桃酸盐、磺酸盐、甲磺酸盐、三氟甲磺酸盐、乙磺酸盐、2-萘磺酸盐、烟酸盐、硝酸盐、草酸盐、扑酸盐、果胶酸盐、过硫酸盐、3-苯基丙酸盐、苦味酸盐、新戊酸盐、丙酸盐、酒石酸盐、硫氰酸盐、甲苯磺酸盐、三氟乙酸盐、十一烷酸盐、酒石酸盐、草酸盐或马来酸盐；和氨基酸盐，如甘氨酸盐、赖氨酸盐、精氨酸盐、鸟氨酸盐、组氨酸盐、谷氨酸盐或天冬氨酸盐。此外，碱性含氮基团可以用诸如以下的试剂季铵化：低碳数烷基卤化物如甲基、乙基、丙基和丁基的氯化物、溴化物和碘化物；二烷基硫酸盐、其中包括二甲基、二乙基和二丁基硫酸盐；和二戊基硫酸盐、长链卤化物如癸基、月桂基、肉豆蔻基和硬脂基氯化物、溴化物和碘化物；芳烷基卤化物，包括苄基和苯乙基溴化物及其它。关于进一步的实例，参见S.M.Berge等人，″ Pharmaceutical Salts，″ J.Pharm.Sci.1977,66,1-19。这类盐可以很容易地通过本领域技术人员使用标准技术形成。

[0269] 与存在于本发明的化合物中的酸性基团形成的盐的优选实例包括金属盐如碱金属盐（例如，钠盐、钾盐和锂盐）、碱土金属盐（例如，钙盐和镁盐）、铝盐和铁盐；胺盐如无机胺盐（例如，铵盐）和有机胺盐（例如，叔辛胺盐、二苄胺盐、吗啉盐、葡糖胺盐、苯基甘氨酸烷酯盐、乙二胺盐、N-甲基葡糖胺盐、胍盐、二乙胺盐、三乙胺盐、二环己胺盐、N，N′-二苄基乙二胺盐、氯普鲁卡因盐、普鲁卡因盐、二乙醇胺盐、N-苄基苯乙胺盐、哌嗪盐、四甲基铵盐和三（羟甲基）氨基甲烷盐；和氨基酸盐如甘氨酸盐、赖氨酸盐、精氨酸盐、鸟氨酸盐、谷氨酸盐和天冬氨酸盐。

[0270] 所有的盐意欲成为本发明范围内的药学上可接受的盐且所有的盐被认为等同于

用于本发明目的的游离形式的相应化合物。

[0271] 酯

[0272] 生理学上/药学上可接受的酯也可适用作活性药剂。术语“药学上可接受的酯”包括本发明化合物的酯，其中羟基（例如在羧酸）已经被转化成相应的酯并且可以作为前药，当前药吸收到温血动物的血液中，可以以释放药物形式并允许药物得到改进的治疗效果的这样的方式裂解。这些酯可以与无机或有机酸如硝酸、硫酸、磷酸、柠檬酸、甲酸、马来酸、乙酸、琥珀酸、酒石酸、甲磺酸、对甲苯磺酸等形成，它们对活的生物体是无毒的。其它实例是与脂族或芳族酸如乙酸或与脂族醇（例如，烷基酯，包括甲基、乙基、丙基、异丙基、丁基、异丁基或戊基酯等）或芳族醇（例如，苄酯）的酯。

[0273] 酯可以从它们相应的酸或盐通过本领域技术人员公知的各种方法，如，例如，通过首先转化酸为酰氯然后使酰氯与合适的醇制备。其它合适的用于制备酯的方法是在 Kemp 和 Vellaccio，1980 中描述。

[0274] 当本发明的酯具有碱性基团如氨基，所述化合物可以通过与酸反应转化为盐，并在酯具有酸性基团如磺基的情况下，所述化合物可以通过将其与碱反应而转化为盐。本发明化合物包括这样的盐。

[0275] 本发明的化合物的盐和酯可以由公知的方法通过采用合适的起始原料或中间体化合物（其是容易得到的和/或在本文中描述的）制备。

[0276] 通常，本发明化合物的期望的盐可以通过在本领域中公知的最终分离和纯化化合物期间在原位制备。例如，所期望的盐可通过使分别在其游离碱或游离酸形式的纯化化合物分别与合适的有机或无机酸，或合适的有机或无机碱反应，并分离由此形成的盐来制备。在碱性化合物的情况下，例如，在合适的溶剂如 THF 中，将游离碱用无水 HCl 处理，和该盐分离为盐酸盐。在酸性化合物的情况下，这些盐可以通过，例如，在合适的溶剂如乙醚中由无水氨处理游离酸和随后分离铵盐。这些方法是常规的，且将是本领域技术人员容易显而易见的。

[0277] 本发明的化合物可以通过各种常规程序，包括使适当酐、羧酸或酰氯与本发明化合物的醇基反应来酯化。使合适的酸酐与醇在碱的存在下反应以促进酰化如 1,8- 双 [二甲基氨基] 萘或 N,N- 二甲氨基吡啶。或者，合适的羧酸可与醇在脱水剂如二环己基碳二亚胺、1-[3- 二甲基氨基丙基]-3- 乙基碳二亚胺或其它用来通过去除水以驱动反应的水可溶脱水剂和任选的酰化催化剂存在下反应。酯化也可以通过使用适当的羧酸在三氟乙酸酐和任选的吡啶存在下进行，或在 N,N- 羰基二咪唑与吡啶存在下进行。酰氯与醇反应可以用酰化催化剂如 4-DMAP 或吡啶进行。

[0278] 本领域技术人员将容易知道如何成功地进行这些以及醇的醚化的其它已知方法。

[0279] 前药和溶剂合物

[0280] 本发明化合物的前药和溶剂合物也设想在本文中。前药的讨论是在 T. Higuchi 和 V. Stella，A.C.S. 论文集丛书的 Pro-drugs as Novel Delivery Systems(1987)14 和在 Bioreversible Carriers in Drug Design，(1987)Edward B. Roche 编辑，美国医药协会和 Pergamon 出版社中提供。术语“前药”是指在体内转化以产生本发明化合物或该化合物的药学上可接受的盐、水合物或溶剂合物的化合物（例如，药物前体）。该转化可通过各种机制（例如，通过代谢或化学过程），诸如例如，通过在血液中水解发生。使用前药的讨论是提

供在 T.Higuchi 和 W.Stella,“Pro-drugs as Novel Delivery Systems,”A.C.S. 论文集丛书的 14 卷和在 Bioreversible Carriers in Drug Design,编辑 Edward B.Roche,美国医药协会和 Pergamon 出版社,1987 中。

[0281] 例如,如果本发明化合物或该化合物的药学上可接受的盐、水合物或溶剂合物含有羧酸官能团,则前药可包括通过用如下基团置换酸基团的氢原子形成的酯:诸如例如,(C1-C8) 烷基、(C2-C12) 烷酰氧基甲基、具有 4 至 9 个碳原子的 1-(烷酰氧基)乙基、具有 5 至 10 个碳原子的 1- 甲基 -1-(烷酰氧基)- 乙基、具有 3 至 6 个碳原子的烷氧基羰氧基甲基、具有 4 至 7 个碳原子的 1-(烷氧基羰氧基)乙基、具有 5 至 8 个碳原子的 1- 甲基 -1-(烷氧基羰氧基)乙基、具有 3 至 9 个碳原子的 N-(烷氧基羰基)氨基甲基、具有 4 至 10 个碳原子的 1-(N-(烷氧基羰基)氨基)乙基、3- 酞基、4- 巴豆酸内酯基、γ- 丁内酯 -4- 基、二 -N,N-(C1-C2) 烷基氨基 (C2-C3) 烷基(如 β- 二甲氨基乙基)、氨基甲酰基 -(C_1-C_2) 烷基、N,N- 二 (C_1-C_2) 烷基氨基甲酰基 -(C_1-C_2) 烷基和哌啶基 -、吡咯烷基 - 或吗啉并 (C_2-C_3) 烷基等。

[0282] 类似地,如果本发明化合物含有醇官能团,则前药可以通过用以下基团置换醇基团的氢原子形成:诸如例如,(C1-C6) 烷酰氧基甲基、1-((C_1-C_6) 烷酰氧基)乙基、1- 甲基 -1-((C_1-C_6) 烷酰氧基)乙基、(C_1-C_6) 烷氧基羰氧基甲基、N-(C_1-C_6) 烷氧基羰基氨基甲基、琥珀酰基、(C_1-C_6) 烷酰基、α- 氨基 (C_1-C_4) 链烷烃基、芳基酰基和 α- 氨酰基或 α- 氨酰基 -α 氨基酰基,其中每个 α- 氨酰基独立地选自天然存在的 L- 氨基酸、P(O)(OH)$_2$、-P(O)(O(C_1-C_6) 烷基)$_2$或糖基(由除去碳水化合物半缩醛形式的羟基产生的自由基)等。

[0283] 如果本发明化合物并入胺官能团,则前药可以通过用以下基团置换胺基团中的氢原子形成:诸如,例如,R- 羰基,RO- 羰基,NRR′ - 羰基,其中 R 和 R′ 各自独立地为 (C_1-C_{10}) 烷基、(C_3-C_7) 环烷基、苄基,或 R- 羰基是天然的 α 氨酰基或天然 α- 氨酰基、-C(OH)C(O)OY1,其中 Y1 为 H、(C_1-C_6) 烷基或苄基、-C(OY_2)Y_3,其中 Y_2是 (C_1-C_4) 烷基且 Y_3是 (C_1-C_6) 烷基、羧基 (C_1-C_6) 烷基、氨基 (C_1-C_4) 烷基或单 -N- 或二 -N,N-(C_1-C_6) 烷基氨基烷基、-C(Y4)Y5,其中 Y_4是 H 或甲基且 Y_5是单 -N- 或二 -N,N-(C_1-C_6) 烷基氨基吗啉并、哌啶 -1- 基或吡咯烷 -1- 基等。

[0284] 本发明的一种或多种化合物可以以未溶剂化以及与药学上可接受的溶剂例如水、乙醇等溶剂化形式存在,和其意图是,本发明涵盖这两种溶剂化和未溶剂化形式。“溶剂合物”是指本发明化合物与一个或多个溶剂分子的物理缔合。此物理缔合涉及不同程度的离子和共价键合,包括氢键合。在某些情况中,溶剂合物能够分离,例如当一个或多个溶剂分子是并入到结晶固体的晶格中时。“溶剂合物”包括溶液相和可分离的溶剂合物。合适的溶剂合物的非限制性实例包括乙醇化物物、甲醇化物等。“水合物”是其中溶剂分子为 H_2O 的溶剂合物。

[0285] 溶剂合物的制备通常是已知的。因此,例如,M.Caira 等人,J.Pharmaceutical Sci.,93(3),601-611(2004) 描述抗真菌剂氟康唑在乙酸乙酯中溶剂合物,以及从水的制备。溶剂合物、半溶剂合物水合物等的类似制备由 E.C.van Tonder 等人,AAPS Pharm Sci Tech.,5(1),文章 12(2004);和 A.L.Bingham 等人,Chem.Commun.,603-604(2001) 描述。典型的非限制性方法涉及在所需量的所需溶剂(有机物或水或其混合物)中在高于环境温度下溶解本发明化合物,并以足以形成晶体的速率冷却溶液,然后通过标准方法分离。分析技

术，诸如，例如红外光谱，表明在作为溶剂合物（或水合物）的晶体中存在溶剂（或水）。

[0286] 水合物

[0287] 如本文所用，术语“药学上可接受的水合物”是指与一个或多个水分子结晶以形成水合形式的本发明化合物。

[0288] 立体异构体、非对映体、对映体、外消旋体、互变异构体

[0289] 本发明化合物具有不对称碳原子 / 手性中心，作为取代基和取代基模式的选择的结果，可以包含另外的手性中心，且因此可作为立体异构体混合物（外消旋物），或作为单独的非对映体或对映体。本发明包括所有这些形式。

[0290] 非对映体（有时称为非对映异构体）是非对映体的立体异构体。当化合物的两种或多种立体异构体具有在一个或多个（但不是全部）等效（相关）立体中心的不同构型，并且不互为镜像时非对映化发生。当两个非对映异构体彼此仅在一个立体中心不同时，它们是差向异构体。每个立构中心产生了两种不同构型，从而产生两种不同的立体异构体。

[0291] 非对映体与对映体的不同之处在于，后者是在所有立构中心不同的立体异构体对，因此彼此为镜像。具有超过一个立构中心的化合物的对映体也是该化合物的非其镜像的其它立体异构体的非对映体。非对映体具有不同的物理性质和不同反应性，不像对映体。本发明的非对映体包括例如番茄碱和 3α - 羟基番茄碱。

[0292] 在一定程度上，取代基和取代基型态提供本发明化合物中互变异构体（例如，酮 - 烯醇互变异构体）的存在，这些化合物的所有互变异构形式，不论是单独存在或以混合物，都在本发明的范围内。杂芳环的一个碳原子上具有羟基取代基的本发明化合物被理解为包括其中只有羟基存在的化合物，其中只有互变异构酮形式（即，氧代取代基）存在的化合物和其中酮和烯醇形式都存在的化合物。

[0293] 对于本说明书来说，“药学上可接受的互变异构体”是指本发明的任何化合物的任何互变异构形式。

[0294] 对映体的纯化和本发明化合物的异构体混合物的分离可以通过本领域中已知的标准技术来完成。

[0295] “稳定”化合物是可被制备和分离的化合物，且其结构和性质保持或可以被导致基本保持不变持续一段时间足以允许出于本文所述目的使用化合物（例如，治疗或预防性施用至受试者）。本发明化合物不限于由式 I 涵盖的稳定的化合物。

[0296] 制备本发明化合物的合成方法在以下通用程序、方案和实例中示出。起始原料是市售的或可以根据在本领域已知或本文所示的程序来制备。本发明化合物可通过以下所示的具体实例来说明。然而，这些具体实例并不被解释为形成被认为是本发明的唯一种类。这些实例进一步说明制备本发明化合物的细节。那些熟练的技术人员将容易理解，以下制备程序的条件和过程的已知变化可用于制备这些化合物。

[0297] 所有的温度都是以摄氏度计。质谱 (MS) 通过电喷雾离子质谱法 (ESI) 在与 Agilent 1100™系列 HPLC 仪器耦合的 Agilent 6120 Quadrapole™ MS 上进行测量。NMR 谱在针对 ^{1}H 为 400MHz 为和针对 ^{19}F 为 376MHz 下被记录在 Varian Mercury 光谱仪上。

[0298] 出于本说明书的目的，下列缩写具有所示含义：

[0299] AcOH ＝乙酸

[0300] Alk ＝烷基

[0301] Ar ＝芳基

[0302] atm ＝大气压

[0303] BINAP ＝ 2,2′ -双（二苯基膦基）-1,1′ -联萘

[0304] Boc ＝叔丁氧基羰基

[0305] n-BuLi ＝正丁基锂

[0306] Cbz ＝羧基苄基

[0307] CH_2Cl_2 ＝二氯甲烷

[0308] DBU ＝ 1,8- 二氮杂双环 [5.4.0] 十一 -7- 烯

[0309] DEAD ＝偶氮二甲酸二乙酯

[0310] DIPEA ＝ N,N- 二异丙基乙胺

[0311] DMAP ＝ 4-（二甲基氨基）吡啶

[0312] DMF ＝ N,N- 二甲基甲酰胺

[0313] DMSO ＝二甲基亚砜

[0314] ESI ＝电喷雾电离

[0315] Et_3N ＝三乙胺

[0316] Et_2O ＝乙醚

[0317] EtOAc 或 EA ＝ 乙酸乙酯

[0318] EtOH ＝乙醇

[0319] h ＝小时

[0320] H_2 ＝氢气

[0321] HATU ＝六氟磷酸 O-(7- 氮杂苯并三唑 -1- 基）-N,N,N′,N′ -四甲基脲

[0322] HCl ＝盐酸

[0323] HPLC ＝高压液相色谱

[0324] iPrOH ＝ 2- 丙醇

[0325] KF ＝氟化钾

[0326] LC-MS ＝液相色谱质谱法

[0327] LiOH ＝氢氧化锂

[0328] MeCN ＝乙腈

[0329] MeMgBr ＝甲基溴化镁

[0330] MeOH ＝甲醇

[0331] MeTHF ＝ 2- 甲基四氢呋喃

[0332] $MgSO_4$ ＝硫酸镁

[0333] min ＝分钟

[0334] MS ＝质谱

[0335] MTBE ＝甲基叔丁基醚

[0336] N_2 ＝氮气

[0337] $NaBH_4$ ＝硼氢化钠

[0338] $NaHCO_3$ ＝碳酸氢钠

[0339] NaOH ＝氢氧化钠

[0340] Na_2SO_4 ＝硫酸钠

[0341] NH_3 ＝氨

[0342] NH_4Cl ＝氯化铵

[0343] NH_4OH ＝氢氧化铵

[0344] NMP ＝ N- 甲基 -2- 吡咯烷酮

[0345] NMR ＝核磁共振光谱

[0346] Moc ＝甲氧基羰基

[0347] P ＝压力

[0348] Pd/C ＝炭载钯

[0349] PG ＝保护基

[0350] Ph ＝苯基

[0351] Pyr ＝吡啶

[0352] rbf ＝圆底烧瓶

[0353] Rf ＝在硅胶上的滞留因子

[0354] rt ＝室温

[0355] TBDMS ＝叔丁基二甲基甲硅烷基

[0356] Ts ＝甲苯 -4- 磺酰基

[0357] TFA ＝三氟乙酸

[0358] TFAA ＝三氟乙酸酐

[0359] TFA-NHS ＝三氟乙酸 N- 羟基琥珀酰亚胺

[0360] THF ＝四氢呋喃

[0361] TLC ＝薄层色谱法

[0362] TMEDA ＝ N,N,N′,N′ - 四甲基乙二胺

[0363] T3P ＝丙基膦酸酐

[0364] 三氟乙酸 N- 羟基琥珀酰亚胺 (TFA-NHS) 的制备描述在以下文献中 :Sohn, C.H. ; Lee, J.E. ;Sweredoski, M.J. ;Graham, R.L.J. ;Smith, G.T. ;Hess, S. ;Czerwieniec, G. ; Loo, J.A. ;Deshaies, R.J. ;Beauchamp, J.L.J. Am. Chem. Soc. 2012, 134, 2672-2680。

[0365] (S)-3- 叔丁氧基羰基 -4-(2- 苄氧基羰基乙基) 噁唑 -5- 酮的制备描述在以下文献中 :Aurelio, L. ;Brownlee, R.T.C. ;Hughes, A.B. ;Sleebs, B.E. Aust. J. Chem. 2000, 53, 425-433。(R)-3- 叔丁氧基羰基 -4-(2- 苄氧基羰基乙基) 噁唑烷 -5- 酮也可以以类似的方式从 N-Boc-D- 谷氨酸 5- 苄酯开始制备。

[0366] 药物组合物

[0367] 在另一个方面,本发明提供了一种组合物,例如,药物组合物,含有与药学上可接受的载体和 / 或赋形剂一起配制的一种本发明化合物或其组合。

[0368] 本发明的药物组合物也可施用在组合治疗中,即,与其它试剂组合。例如,组合治疗可包括至少一种其它降胆固醇剂。可以在组合治疗中使用的治疗剂的实例在下文中更详细地描述。

[0369] 如本文所用,“药学上可接受的载体”或“药学上可接受的赋形剂”包括生理上相容的任何和所有溶剂、分散介质、包衣、抗细菌和抗真菌剂、等渗和吸收延迟剂等。所述载体应

该是适于口服、静脉内、肌内、皮下、肠胃外、脊柱或表皮施用（例如，通过注射或输注）。根据施用途径，活性化合物可以包衣在材料中，以保护免受可能灭活化合物的酸和其它自然条件的作用。

[0370] 载体 / 赋形剂

[0371] 本发明的药物组合物可以包括可药学上可接受的抗氧化剂。药学上可接受的抗氧化剂的实例包括：水溶性抗氧化剂，如抗坏血酸、盐酸半胱氨酸、硫酸氢钠、焦亚硫酸钠、亚硫酸钠等；油溶性抗氧化剂，如抗坏血酸棕榈酸酯、丁基化羟基苯甲醚 (BHA)、丁基化羟基甲苯 (BHT)、卵磷脂、棓酸丙酯、α - 生育酚等；和金属螯合剂，如柠檬酸、乙二胺四乙酸 (EDTA)、山梨醇、酒石酸、磷酸等。

[0372] 合适的本发明药物组合物可以采用的水性和非水性载体的实例包括水、乙醇、多元醇（如甘油、丙二醇、聚乙二醇等）、及其合适的混合物、植物油如橄榄油和可注射的有机酯如油酸乙酯。适当的流动性可以例如通过使用包衣材料，如卵磷脂，在分散体的情况下通过保持所需的粒径，以及通过使用表面活性剂来维持。

[0373] 这些组合物还可含有佐剂，如防腐剂、润湿剂、乳化剂和分散剂。防止微生物的存在可以通过上述的灭菌程序，和通过包含各种抗细菌剂和抗真菌剂，例如对羟基苯甲酸酯、氯丁醇、苯酚山梨酸等两者来确保。它也可能希望包括等渗剂，如糖、氯化钠等在组合物中。此外，可注射药物形式的延长吸收可以通过包含延迟吸收的物质如单硬脂酸铝和明胶来实现。

[0374] 药学上可接受的载体或赋形剂包括无菌水溶液或分散体和用于无菌注射溶液或分散体的临时制备的无菌粉末。用于药学活性物质的此类介质和药剂是本领域已知的。除非任何常规介质或试剂与活性化合物不相容，否则其在本发明药物组合物中的使用是预期的。补充的活性化合物也可以并入组合物。

[0375] 治疗组合物通常在制造和储存的条件下必须是无菌和稳定的。组合物可以配制成溶液、微乳剂、脂质体或适合于高药物浓度的其它有序结构。载体可以是溶剂或分散介质，包含例如水、乙醇、多元醇（例如，甘油、丙二醇和液体聚乙二醇等）及其合适的混合物。适当的流动性可以例如，通过使用包衣如卵磷脂，在分散液的情况下通过保持所需的粒径和通过使用表面活性剂来维持。在许多情况下，人们可以包括等渗剂，例如糖、多元醇如甘露醇、山梨醇或氯化钠在组合物中。

[0376] 可注射组合物的延长吸收可以通过在组合物中包括延缓吸收的试剂，例如，单硬脂酸盐和明胶来实现。

[0377] 无菌可注射溶液可以通过将所需量的活性化合物与一种以上列举成分或其组合（如果需要）并入适当的溶剂中，接着无菌微量过滤来制备。一般地，分散体通过将活性化合物并入到含有基本分散介质和以上列举的所需其它成分的无菌媒剂来制备。在用于制备无菌注射液的无菌粉末的情况中，制备方法是真空干燥和冷冻干燥（冻干），其从先前无菌过滤溶液产生活性成分加上任何另外所需成分的粉末。

[0378] 用于口服施用的药物制剂的实例包括内用的片剂（如未包衣片剂、糖包衣片剂、包衣片剂、肠溶包衣片剂和咀嚼片剂）、施用到口腔的片剂（如经颊制剂、舌下片剂、锭剂和粘合片剂）、粉剂、胶囊（如硬胶囊和软胶囊）、颗粒剂（如包衣颗粒剂、丸剂、锭剂、液体制剂或药用可接受的缓释药物制剂）。能够被口服给药的液体制剂的具体实例是内用的溶液、

摇匀的混合物、悬浮液、乳剂、糖浆剂、干糖浆剂、酏剂、输液和汤和柠檬水。

[0379] 可与载体材料组合以产生单一剂型的活性成分的量将依赖于所治疗受试者和特定的给药模式而变化。可与载体材料组合以产生单一剂型的活性成分的量通常将是产生治疗效果的组合物的量。通常，在百分之百上，该量的范围从约 0.01％至约 99％的活性成分，约 0.1％至约 70％或从约 1％约 30％的活性成分与药学上可接受的载体组合。

[0380] 剂量和给药方案

[0381] 调节剂量方案，以提供最佳的期望反应（例如，治疗反应）。例如，可以施用单次推注，几个分剂量可以随时间施用或剂量可以如治疗情况的紧急程度所示按比例减少或增加。特别有利的是配制成剂量单位形式的肠胃外组合物以易于施用和给药均匀性。此处使用的剂量单位形式是指适合作为单位剂量用于待治疗的受试者的物理上离散的单位；每个单位含有计算与所需的药物载体组合产生期望的治疗效果的预定量的活性化合物。本发明剂量单位形式的规格是由以下决定且直接依赖于以下：活性化合物的独特特性和要实现的特定治疗效果和配制用于治疗个体敏感性的此种活性化合物的领域中固有的局限。

[0382] 对于所述化合物的施用，剂量范围从约 0.0001 至 100mg/kg，更通常为 0.01 至 5mg/kg 宿主体重。例如，剂量可以是 0.3mg/kg 体重、1mg/kg 的体重、3mg/kg 的体重、5mg/kg 体重或 10mg/kg 体重或在 1-10mg/kg 的范围内。示例性的治疗方案需要每天一次、每周、每两周一次、每三周一次、每四周一次、每月一次、每 3 个月一次或每 3 至 6 个月一次施用。

[0383] 在一些方法中，具有不同活性的两种或多种化合物同时施用，在这种情况下施用的每种化合物的剂量落入所示范围内。所述化合物通常多次施用。单剂量之间的间隔可以是，例如，每周、每月、每三个月或每年。间隔也可以是不规则的，如通过测量在患者中化合物的血液水平所指示。在一些方法中，调整剂量以达到约 1-1000 μg/ml 和在一些方法中约 25-300 μg/ml 的化合物的血浆浓度。

[0384] 或者，化合物可以作为持续释放制剂施用，在这种情况下，较不频繁的施用是必需的。剂量和频率取决于化合物在患者中的半衰期而变。施用的剂量和频率可以根据治疗是预防性的还是治疗性而改变。在预防性应用中，相对低的剂量以相对不频繁的间隔施用很长一段时间。一些患者余生继续接受治疗。在治疗性应用中，有时需要相对高的剂量以相对短的间隔，直到疾病的进展减轻或停止，或直至患者显示疾病症状的部分或完全改善。此后，可对患者施用预防性方案。

[0385] 在本发明的药物组合物中活性成分的实际剂量水平可以改变，以便获得活性成分的量，其可有效地达到特定患者、组合物和施用模式的所需治疗反应（例如，降低的血浆 LDL/ 胆固醇水平），而不对患者有毒。选择的剂量水平将取决于多种药物动力学因素，包括使用的本发明的特定组合物中的活性，或其酯、盐或酰胺的活性、施用途径、施用时间、使用的特定化合物的排泄的速率、治疗的持续时间、与所采用的特定组合物组合使用的其它药物、化合物和 / 或材料、所治疗患者的年龄、性别、体重、状况、一般健康和之前的医疗史和在医学领域众所周知的类似因素。

[0386] 本发明化合物的“治疗上有效量”或“有效量”或“治疗有效剂量”可以导致在受试者中降低 LDL-C 的水平、至少一种疾病症状的严重程度的降低（例如，血浆 LDL- 胆固醇的减少，或 LDL- 胆固醇相关病症的症状减少）、增加了无疾病症状期的频率和持续时间或预防由于受试者的疾病痛苦而致的受损或失能。

[0387] 施用途径

[0388] 本发明的组合物可通过使用一个或多个本领域已知的各种方法，一个或多个施用途径来施用。如将被熟练的技术人员所了解，施用途径和 / 或模式将根据所期望的结果而变化。本发明化合物的施用途径包括口服、静脉内、肌内、皮内、腹膜内、皮下、脊髓或其它肠胃外施用途径，例如通过注射或输注。本文所使用的短语“肠胃外施用”是指除肠和局部施用以外的施用模式，通常通过注射，并且包括但不限于，静脉内、肌内、动脉内、鞘内、囊内、眶内、心内、皮内、腹膜内、经气管、皮下、关节内、囊下、蛛网膜下、脊柱内、硬膜外和胸骨内注射和输注。或者，本发明的化合物可以通过非注射途径给药，如局部、表皮或粘膜施用途径，例如鼻内、口服、阴道、直肠、舌下或局部给药。

[0389] 所述活性化合物可以用将保护化合物免于快速释放的载体，如控释制剂，包括植入物、经皮贴剂和微囊化递送系统来制备。可以使用可生物降解的，生物相容的聚合物，如乙烯乙酸乙烯酯、聚酐、聚乙醇酸、胶原、聚原酸酯和聚乳酸。许多制备此类制剂的方法是专利或一般为本领域技术人员所熟知。参见，例如，Sustained and Controlled Release Drug Delivery Systems，J.R.Robinson 编辑，Marcel Dekker，Inc.，纽约，1978。治疗组合物可以用本领域中已知的医疗装置给药。在某些实施方案中，本发明化合物可以配制以确保在体内适当分布。例如，血 - 脑屏障 (BBB) 排除许多高度亲水的化合物。为了确保本发明的治疗化合物穿过 BBB(如果希望)，可以将它们配制在例如脂质体中。脂质体可包含选择性输送到特定的细胞、组织或器官的一个或多个部分，从而增强靶向药物递送 (见，例如，V.V.Ranade，1989 J.Clin.Pharmacol.29 :685)。在一个实施方案中，本发明化合物可以配制为待递送到肝脏 (即，肝细胞)。

[0390] 化合物的功能表征

[0391] 本发明化合物的功能特性可在体外和体内进行测试。例如，可测试化合物抑制 PCSK9 蛋白水解活性、对 LDLR 的 PCSK9 依赖性效应 (例如，LDL-C 的 LDLR 介导的摄取)、PCSK9 依赖性 LDLR 降解 (包括 PCSK9 到 LDLR 的相互作用) 和降低体内 LDL-C 的能力。

[0392] 结合 LDLR 的 PCSK9 可以通过表面等离子共振 (SPR)(使用 BIAcore®)，通过固定 LDLR 到固体支撑并检测结合 LDLR 的可溶 PCSK9 而检测。或者，可固定 PCSK9，并且可检测到 LDLR 结合。PCSK-9/LDLR 结合也可以通过 ELISA(例如，通过检测 PCSK9 结合固定 LDLR)，通过荧光共振能量转移 (FRET)，或噬菌体展示进行分析。为了进行 FRET，可以检测到在溶液中荧光团标记的结合 LDLR 的 PCSK9(参见，例如，美国专利号 5,631,169)。结合 LDLR 的 PCSK9 已经通过免疫共沉淀检测 (Lagace 等人，2006 J.Clin.Inv.116(11) :2995-3005)。例如，为了检查 PCSK9-LDLR 以这种方式结合，将 HepG2 细胞在固醇耗尽的培养基中培养 18 小时。在 0.1mM 氯喹存在下，将纯化的 PCSK9 添加到培养基，将细胞温育 1 小时。细胞在温和的清洁剂 (1％洋地黄，重量 / 体积) 裂解。PCSK9 或 LDLR 是从细胞裂解物免疫沉淀出，通过 SDS-PAGE 分离，且进行免疫印迹来分别检测免疫共沉淀 LDLR 或 PCSK9 的存在 (Lagace 等人，2006，同上)。

[0393] 这些测定法可以用以较高亲和力结合 LDLR 的 PCSK9 的突变体形式 (例如，hPCSK9D374Y，Lagace 等人，2006，同上) 进行。肝细胞在细胞表面上表达 LDLR。加入纯化的 PCSK9 到培养的肝细胞 (例如，HepG2 细胞、ATCC HB-8065、HuH7 细胞、原代人或小鼠肝细胞) 产生 LDLR 表达以剂量和时间依赖性方式降低 (Lagace 等人，2006 同上)。可以测试本

发明化合物增加肝细胞中 LDLR 水平的能力。例如，将 HepG2 细胞在固醇耗尽的培养基（补充有 100U/ml 青霉素、100 μg/ml 硫酸链霉素和 1g/l 葡萄糖，5%（体积 / 体积）新生牛脂蛋白缺乏的血清 (NCLPDS)、10 μM 康帕丁钠和 50 μM 甲羟戊酸钠的 DMEM) 培养 18 小时以诱导 LDLR 表达。将纯化的 PCSK9(5 μg/ml) 加入到培养基中。测定在加入 PCSK9 后的 0、0.5、1、2 和 4 小时收获的细胞的 LDLR 水平 (Lagace 等人，2006，同上）。LDLR 水平可通过流动细胞术、FRET、免疫印迹或其它手段来确定。由细胞（例如，HepG2 细胞、HuH7 细胞）的 LDL-C 的摄取可以通过使用荧光标记的 LDL-C、DiI-LDL(3,3′ - 二 - 十八烷基吲哚羰基青色素低密度脂蛋白）来测量，如由 Stephan 和 Yurachek(1993，J.Lipid Res.34 :325-330) 描述。简言之，将细胞在 37℃具有 DiI-LDL(20-100 μg 蛋白质 /ml) 的培养基温育 2 小时。洗涤细胞，裂解，并使用分光光度计定量内化的 DiI-LDL 的浓度。LDL-C 的摄取可以在与 PCSK9 抑制化合物接触的细胞中来测定（其中 DiI-LDL 存在于细胞培养物时期的之前和 / 或期间）。

[0394] 在肝中过度表达人类 PCSK9 的转基因小鼠相对非转基因小鼠增加血浆 LDL-C 的水平 (Lagace 等人，2006 同上）。另请参见 Maxwell 和 Breslow，2004 Proc.Natl.Acad.Sci.USA，101 :7100，其描述在小鼠使用腺病毒载体的 PCSK9 过度表达。$PCSK9^{-/-}$ 小鼠已经产生 (Rashid 等人，2005 Proc.Natl.Acad.Sci.102(5) :5374-5379)。这些小鼠可以被遗传修饰以表达小鼠或人类 PCSK9 转基因。化合物可以以任何这些模型，或在未遗传修饰的动物中进行测试清除或降低总胆固醇和 / 或 LDL-C 的能力。

[0395] LDL 从血浆清除的动力学可通过注入动物 [^{125}I]- 标记的 LDL，在注射后 0、5、10、15 和 30 分钟获得血液样品，和定量样品中的 [^{125}I]-LDL 来确定 (Rashid 等人，2005 同上）。$PCSK9^{-/-}$ 小鼠的 LDL 清除率相对于野生型小鼠增加 (Rashid 等人，2005 同上）。施用化合物的动物中增加的 LDL 清除表明该试剂抑制体内 PCSK9 活性。

[0396] 响应于与化合物处理降低总血浆胆固醇、血浆甘油三酯和 / 或 LDL-C 指示针对 PCKS9 活性的治疗功效。胆固醇和血脂谱可以通过比色、气 - 液相色谱或使用市售试剂盒的酶方法来确定。

[0397] 确定 PCSK9 活性的方法 / 测定法描述如下。本文所用的术语“PCSK9 活性”和“PCSK9 功能”是指可检测的（直接或间接）可归因于 PCSK9 的酶、生物化学或细胞活性。不局限于此，这些活性包括 PCSK9 对降低在细胞表面 LDLR 的水平，对降低血浆 LDL-C 的效应和 / 或 PCSK9 蛋白酶活性本身（例如，PCSK9 分泌）。

[0398] PCSK9 分泌和加工的体外分析。

[0399] 分泌和 / 或前 PCSK9 至 PCSK9 加工是使用 ELISA 测定法或使用通过 Western 印迹测量在细胞和培养基中的 PCSK9 的生物合成方法进行测试。响应于化合物的存在 PCSK9 分泌下降指示，该化合物抑制 PCSK9 活性。对于统计显著性，每个实验一式三份进行。进行化合物的“剂量依赖性”反应。

[0400] PCSK9 依赖性 LDLR 降解的体外分析。

[0401] 还测试化合物由 PCSK9 对小鼠或人肝细胞系如 HepG2 或 HuH7 抑制 LDLR 增强的降解的能力。该测定法是由新增野生型 (WT)、突变体或嵌合 PCSK9，要么在转染后或直接在存在或不存在所测试化合物下的培养上清液。进行化合物的“剂量依赖性”反应。对于统计显著性，每个“剂量 - 反应”实验针对 4 至 6 个不同剂量一式两份或一式三份进行。

[0402] PCSK9 活性的抑制是通过 LDLR 蛋白表达增加证明和 / 或在细胞表面，由以下证

明：

[0403] • 对于总 LDLR，细胞裂解物的 Western 印迹分析；

[0404] • 对于 LDLR，细胞表面的 FACS 分析；

[0405] • 荧光 DiI-LDL 并入，监测 LDLR 的细胞表面的活性。

[0406] Dil-LDL 荧光摄取试验测定法包括经由 LDLR 内化荧光测定 DiI-LDL 细胞并入（细胞表面 LDLR 活性的量度）。将细胞温育在不同剂量的试验化合物的存在或不存在下的 96 孔格式中 2 小时，然后加入 DiI-LDL，再温育 2 小时。PCSK9 活性的抑制是通过 DiI-LDL 荧光的增加来检测。

[0407] a.WTPCSK9。该测定法包括新增野生型 (WT)PCSK9，或者作为从转染的细胞的条件培养基或纯化，并在所测试化合物的存在或不存在下添加到培养物上清液。常规选择用于细胞外添加 PCSK9 的剂量为 1ug/ml。

[0408] b. 突变体 PCSK9（功能增益）。为了进一步表征测试化合物是否能抑制功能突变的增益的功能，将所述细胞用纯化的突变体蛋白，在存在或不存在不同剂量的试验化合物下培养。纯化的 PCSK9 突变体为 PCSK9-D374Y，例如（表现出对 LDLR 高～ 25 倍的亲和力）或 S127R（显示 PCSK9 的增加稳定性）。常规选择用于细胞外加入 PCSK9 和其功能增益天然突变体 D374Y 的剂量为 1 μg/ml 和 0.2 μg/ml。类似使用其它 PCSK9 突变体。该测定法也可以使用从转染功能增益 PCSK9 突变体的细胞收获的培养基中进行。

[0409] c. 嵌合 PCSK9。用于测量试验化合物对 PCSK9 细胞外途径活性的的活性，测试融合 PCSK9 与细胞表面血管紧张素转化酶 2 的跨膜和胞质结构域的嵌合蛋白质 (PCSK9-ACE2)。或者，融合 PCSK9 与 Lamp-1（其直接通信蛋白质到胞内体 / 溶酶体）的跨膜和胞质结构域的嵌合蛋白 (PCSK9-Lamp1) 被测试用于测量试验化合物对 PCSK9 细胞内途径活性的活性。表达嵌合 PCSK9-ACE2 或 PCSK9-Lamp1 的稳定细胞是可用的且在存在或不存在不同剂量的试验化合物下培养。嵌合蛋白还包括具有 V5 标签的 PCSK9。

[0410] d. 原代人肝细胞。对小鼠和人原代肝细胞测试所述 PCSK9 抑制化合物以测量其对细胞表面 LDLR 的效应。使用小鼠原代肝细胞的优点是，它还测量在野生型或分别表达或缺乏 PCSK9 的基因敲除小鼠的背景下化合物的特异性。也出于相似药物特异性目的使用不再内源性表达 PCSK9 的 HepG2 和 HuH7 细胞（例如，在 shRNA 敲低下）。

[0411] PCSK9 包含在从转染的细胞的条件培养基中。人野生型 PCSK9(PCSK9-WT) 和功能增益 (PCSK9-D374Y) 蛋白质是由 HEK293 细胞或 Huh7 细胞的过度表达产生。简言之，HEK293 或 Huh7 细胞系生长于有 10％胎牛血清 (Invitrogen) 的杜氏改良依格培养基，并在 37℃在 5％ CO2 下保持。根据制造商的协议，将 HEK293 细胞用 jetPRIME™转染 (Polyplus 转染)，且将 Huh7 细胞用 Lipofectamine™2000(Invitrogen) 转染。转染后二十四小时，将细胞洗涤并温育在无血清培养基。含有分泌人类 PCSK9-D374Y 或 PCSK9-WT 蛋白质的条件培养基在 24 小时后收集。在条件培养基中的 PCSK9 蛋白的水平通过酶联免疫吸附试验 (ELISA) 定量，如先前所述 (Dubuc G、Tremblay M、Paré G、Jacques H、Hamelin J、Benjannet S、Boulet L、Genest J、Bernier L、Seidah NG、Davignon J. 2010. A new method for measurement of total plasma PCSK9 :clinical applications J Lipid Res. 51 :140-149.)。

[0412] 稳定表送 PCSK9 的人类 HepG2 细胞。使用 Fugene™ HD 转染试剂 (Roche)，使人类 HepG2 细胞 (ATCC，HB-8065) 转染质粒构建体（在 C 末端标记 V5 的人类 PCSK9)。即，在在

35mm 的培养皿中，使用 HepG2 细胞的 Fugene™ HD 优化协议（对于 7ul 的 Fugene™HD，2ug 的 DNA，按照制造商的说明）转染 HepG2 细胞。转染后 72 小时，将细胞胰蛋白酶化，并转移到含以下选择培养基的 100mm 培养皿：DMEM（高葡萄糖 + 丙酮酸钠）(Wisent)/10％ FBS 和 600ug/ml 的 G418(Wisent)。10 天的选择后，获得表达目的基因的细胞池。另请参见 Seidah，NG、Benjannet，S、Wickham，L、Marcinkiewicz，J、Jasmin，SB、Stifani，S、Baska，A、Prat，A 和 Chretien，M. The secretory proprotein convertase neural apoptosis-regulated convertase 1(NARC-1)：liver regeneration and neuronal differentiation. Proc. Natl. Acad. Sci. U. S. A，100：928-933，2003；Benjannet，S.、Rhainds，D.、Essalmani，R.、Mayne，J.、Wickham，L.、Jin，W.、Asselin，M. C.、Hamelin，J.、varret，M.、Allard，D.、Trillard，M.、Abifadel，M.、Tebon，A.、Attie，A. D.、Rader，D. J.、Boileau，C.、Brissette，L.、Chretien，M.、Prat，A. 和 Seidah，N. G. NARC-1/PCSK9 and its natural mutants：zymogen cleavage and effects on the low density lipoprotein(LDL)receptor and LDL cholesterol. J. Biol. Chem.，279：48865-48875，2004；以及 Benjannet，S.、Rhainds，D.、Hamelin，J.、Nassoury，N. 和 Seidah，N. G. The proprotein convertase PCSK9 is inactivated by furin and/or PC5/6A：Functional consequences of natural mutations and post-translational modifications. J. Biol. Chem.，281：30561-30572，2006。

[0413] PCSK9 分泌测定：通过 ELISA 检测分泌的 PCSK9。在 12 孔板 (Greiner BioOne™) 中，将稳定表达 PCSK9(+V5) 的 HepG2 细胞以 1×10^6 个 / 孔的密度接种在完全培养基中，并在 37℃下温育 20 小时。细胞用 2ml/ 孔的 D-PBS(+ 钙 + 镁)(Wisent) 漂洗一次，随后加入含有以各种浓度 (11、33、100μM) 的抑制化合物或 DMSO 对照 (0.4％最终) 的 0.5ml/ 孔温育培养基 (+0.07％ BSA) 中和隔夜 (24 小时) 培养。收集 24 小时的条件培养基，在 130×g 下离心 5 分钟以除去细胞碎片，将除去的上清液通过 ELISA 进行 PCSK9 定量 (100μl 的 1 ： 30 稀释)。将细胞用冰冷的 D-PBS(+ 钙 + 镁)(Wisent) 洗涤一次，然后用含有蛋白酶抑制剂混合物 (Roche Applied Science) 的 250ul/ 孔的放射免疫沉淀测定缓冲液 (RIPA) (50mM Tris-HCl，pH 7.8，150mM NaCl，1％ Nonident P-40，0.5％脱氧胆酸钠，0.1％ SDS) 在冰上裂解 30 分钟，和在 11，300×g 离心细胞团 5 分钟。除去上清液用于通过 ELISA 量化细胞 PCSK9(1 ： 20 稀释的 100μl) 和通过 Bio-Rad ™ DC 蛋白测定 (Bio-Rad)(一式两份，4μl 细胞裂解物) 进行总蛋白质测定 (Dubuc G、Tremblay M、Paré G、Jacques H、Hamelin J、Benjannet S、Boulet L、Genest J、Bernier L、Seidah NG、Davignon J. 2010. A new method for measurement of total plasma PCSK9：clinical applications J Lipid Res. 51：140-149)。测定培养基 (M) 相对细胞 (C) 的 PCSK9 浓度的比例并降低 M/C ＞ 30％的化合物被认为是活性的。

[0414] 通过 Western 印迹检测。在磷酸盐缓冲盐水 (PBS) 中洗涤细胞 3 次，并在补充有 1× 完全蛋白酶抑制剂混合物 (Roche Applied Science) 的完全 RIPA 缓冲液 (50mM 的 Tris/HCl，pH 8.0，1％ (体积 / 体积) 的 Nonidet P40，0.5％脱氧胆酸钠，150mM 的 NaCl 和 0.1％ (体积 / 体积)SDS) 中裂解。蛋白质由 8％ SDS- 聚丙烯酰胺凝胶电泳分离并印迹在聚偏二氟乙烯 (PVDF，Perkin Elmer) 膜 (GE Healthcare) 上，所述膜在含有 5％脱脂奶粉的 TBS-T(50mM 的 Tris-HCl，pH 7.5，150mM NaCl，0.1％吐温 -20) 阻断 1 小时。然后在 5％脱脂牛奶与多克隆 hPCSK9 抗体 (1 ： 2500)、人 LDLR 抗体 (1 ： 1000，R&D Systems) 或兔

β-肌动蛋白抗体(1 ： 2500，Sigma)任一者中培养膜3小时。合适的辣根过氧化物酶共轭的二级抗体(1 ： 10,000，Sigma)被用于用ECL加试剂盒(GE Healthcare)检测增强的化学发光。

[0415] 荧光激活细胞分选(FACS)定量细胞表面LDLR水平。在37℃下，在存在或不存在添加化合物下，用各种PCSK9构建体培养HuH7细胞4小时或18小时，然后用含0.5％牛血清白蛋白(Sigma)和1g/l葡萄糖的无钙/镁的杜氏磷酸盐缓冲盐水(DPBS)(溶液A)洗涤3次。然后在37℃用500μl的1×Versene™溶液(Invitrogen)将细胞温育5分钟，和层叠在5ml溶液A上。然后将细胞以1000rpm离心5分钟并重新悬浮在含有1 ： 100针对人LDLR的单克隆LDLR抗体C7(mAb-C7，Santa Cruz Biotechnology)的1ml溶液A中40分钟。将细胞用5ml溶液A洗涤一次，离心并在含有1 ： 250的Alexa Fluor 647驴抗小鼠(Molecular Probes)的1ml PBS中重新悬浮20分钟。洗涤细胞，并重新悬浮于300μl的0.2％碘化丙啶(PI)的PBS。然后通过FACS针对PI和Alexa Fluor 647两者使用FACS BD LSR(BD Biosciences)分析活细胞(PI阴性)。。

[0416] 在人Huh7细胞中的LDLR的免疫荧光。将HepG2初始细胞铺于放置在24孔细胞培养板中的聚-L-赖氨酸涂覆(50ug/ml)的圆形显微镜盖玻片1.12mm厚度(Fisherbrand 12CIR#1)。在DMEM完全培养基(10％ FBS)进行接种，并在24小时后，将培养基换为含33.3μM不同抑制剂化合物的培养基(+0.07％ BSA)中(300μl/孔)。将相当于抑制剂化合物体积的DMSO体积也稀释在含有0.07％ BSA的培养基中且用作阴性对照。20小时温育期后，将细胞用PBS洗涤3次，再用3.7％的多聚甲醛固定10分钟。在非透的条件下进行人LDLR的免疫荧光(绿色标签)。用PBS另外洗涤3次后，将细胞用1％ BSA阻断30分钟，接着在4℃下用一级抗体(在1％ BSA中的1 ： 200山羊多克隆抗hLDLR，R&D Systems)温育过夜。在最后用PBS洗涤3次后，在室温下用Alexa fluor标记的二级抗体培养1小时揭示抗原-抗体复合物并安装在ProLong Gold抗淬灭试剂(Molecular Probes，Invitrogen)中。用共聚焦显微镜(Zeiss LSM-710)进行免疫荧光分析。

[0417] PCSK9活性的DiI-LDL摄取细胞基测定法。将HepG2初始细胞和HEK293初始细胞以25,000个细胞/孔的密度，在完全培养基(DMEM高葡萄糖(+丙酮酸钠，对于HepG2细胞)(Wisent)+10％ FBS)中，接种在96孔板(有透明底部的CellBind黑板(Corning；目录号3340))。20小时后，将细胞用无血清DMEM培养基(100μl/孔)洗涤30分钟，除去洗涤培养基，并用含有不同浓度的化合物(11、33、100μM)或DMSO对照(0.4％终)的100μl/孔温育培养基(DMEM高葡萄糖(+丙酮酸钠，对于HepG2)(Wisent)+0.07％ BSA(Sigma-Aldrich))更换。每个条件制备一式三份。在37℃下培养6小时后，将Dil-LDL(Biomedical Technologies(目录号BT-904))以5μg/ml的最终浓度加入到细胞培养基(5μl)，并返回到组织培养箱的细胞再培养18个小时(总共用化合物培养24小时)。用冰冷的D-PBS(Wisent)洗涤2次后(200μl/孔)和吸清最后的洗涤，将板在SpectraMax GeminiEM™平板读数器(Molecular Devices)中进行扫描(底部读)。对于每个孔，将原始Dil-LDL摄取测定为在孔中3个不同点的9个读数的平均荧光强度(RFU)(激发：520nm/em：575nm，截止：550nm)。每个孔中的Dil-LDL摄取通过进行CyQuant™细胞分析(Invitrogen；目录号C7026)对细胞总数校正。即，记录了Dil荧光后，将板在-80℃冷冻过夜。第二天，将板在室温下解冻，根据制造商的方案将细胞裂解，且细胞/孔的数目通过

测量 CyQuant 绿色荧光染料结合至细胞核酸的荧光 (RFU)(激发 :485nm/em :538nm,截止 :515nm) 确定。对于每个条件,Dil-RFU 除以 CyQuant-RFU。校正的 diI-LDL 摄取报告为% DMSO 对照并从一式三份孔获得。

[0418] PCSK9 的转染、生物合成分析、免疫沉淀。用 3×10^5HEK293 细胞进行转染,使用 Effectene™(Qiagen) 和共 0.5μg cDNA。或者,用总共 4μg cDNA 在 Lipofectamine™2000(Invitrogen) 中转染 5×10^5HuH7 或 6×10^5HepG2 细胞。转染后两天,对 HEK293 细胞进行洗涤,然后用任一 250μCi/ml[35S]Met/Cys(PerkinElmer Life Sciences) 温育不同的时间。将细胞在改性 RIPA 缓冲液 (150mM NaCl,50mM Tris-HCl,pH 7.5),1% 的 Nonidet P-40,0.5% 脱氧胆酸钠,0.1% SDS 和蛋白酶抑制剂混合物 (Roche Applied Science) 中裂解,在此之后,制备裂解物和培养基用于免疫沉淀。所使用的抗体是抗 V5 mAb(Invitrogen,1 : 500),和专有的兔抗 PCSK9 31-454(A-03)。免疫沉淀物是通过 SDS-PAGE 在 8% 的 Tricine 凝胶上解析和放射自显影。这些实验至少重复三次。定量是通过使用 ImageQuant™版本 5.2 软件,在 Storm Imager™(Amersham Biosciences) 上进行。生物合成是一种敏感的方法,其允许对测试化合物是否影响内质网中前 PCSK9 至 PCSK9 活化,从而影响活性 PCSK9 络合其前片段到培养基中的分泌。工程化以内源性和稳定地表达 PCSK9(例如 PCSK9-V5)WT 或突变变体的细胞用化合物培养且存在于培养基和细胞裂解物中的不同 PCSK9 形式是通过使用敏感人类 PCSK9 抗体的 Western 印迹进行分析。

[0419] MTT 毒性测定。将过度表达 hPCSK9(+V5) 的 HepG2 稳定细胞以 1×10^5个细胞 / 孔 (100μl) 的密度接种在完全 DMEM(高葡萄糖 + 丙酮酸钠)(Wisent)+10% FBS 培养基中的 96 孔板 (Greiner BioOne) 并温育 20 小时。用 100ul/ 孔的无血清 DMEM 培养基洗涤细胞一次,然后加入含有各种浓度化合物 (11、33、100μM) 或 DMSO 对照 (0.4% 终浓度) 的 100μl/ 孔的 DMEM(高葡萄糖 + 丙酮酸钠)(Wisent)+0.07% 的 BSA(Sigma-Aldrich) 培养基,接着过夜 (24 小时) 温育。MTT 分析 (Promega) 由以下组成 :按照制造商的说明,加入 20μl/ 孔的 MTT 试剂和在 37℃下温育 45 分钟。记录在 490nm 处的吸光度和校正由于非特异性吸光度 (690nm) 的背景。

[0420] 使用化合物的方法

[0421] PCSK9 已经涉及胆固醇体内平衡,因为它似乎在胆固醇生物合成或摄取中具有特定作用。在胆固醇喂养的大鼠的研究中,据报道,PCSK9 以与参与胆固醇生物合成的其它基因类似的方式被下调 (Maxwell 等人,2003 J Lipid Res.44 :2109-2119)。已经发现 PCSK9 表达由他汀类药物以归因于药物的降低胆固醇的效果的方式上调 (Dubuc 等人,2004, Arterioscler Thromb Vasc Biol.24 :1454-1459)。PCSK9 的腺病毒表达导致循环低密度脂蛋白 (LDL) 胆固醇 (LDL-C) 的时间依赖性增加 (Benjannet 等人,2004 J.Biol.Chem.279 :48865-48875),和 PCSK9 基因缺失的小鼠具有增加的肝脏 LDL 受体 (LDLR) 的水平并更迅速从血浆清除 LDL-C(Rashid 等人,2005 同上)。从瞬时转染 PCSK9 的 HepG2 细胞的培养基发现当转移到未转染的 HepG2 细胞时减少细胞表面 LDLR 和 LDL-C 的内化的量 (Cameron 等人,2006 Human Mol.Genet.15 :1551-1558)。此外,加入到 HepG2 细胞的培养基中的纯化的 PCSK9 以剂量和时间依赖性方式降低细胞表面 LDLR 数 (Lagace 等人,2006,同上)。

[0422] 在基因 PCSK9 中的许多突变已与常染色体显性高胆固醇血症 (ADH) 相关联,一种遗传代谢病症,其特征是 LDL-C 颗粒在血浆中显著升高,可能导致过早的心血管功能衰竭

(例如,Abifadel 等人,2003 Nat.Genetics 34 :154-156 ;Tirnms 等人,2004 Hum.Genetics 114 :349-353 ;Leren,2004 Clin.Genet.65 :419-422)。

[0423] PCSK9 的增加的表达或上调与 LDL-C 的增加的血浆水平相关联,且抑制或缺乏 PCSK9 的表达与低 LDL-C 的血浆水平相关联。与 PCSK9 序列变异有关的 LDL-C 的较低水平赋予防止冠心病 (Cohen 等人,2006 N.Engl.J.Med.354 :1264-1272)。

[0424] 本文所述的化合物具有体外和体内治疗用途。例如,这些分子可以施用于培养中的细胞,例如,体外或离体,或有需要的受试者,例如,体内,以治疗、预防或诊断多种与 PCSK9 活性 / 功能相关联的病症。

[0425] 本发明化合物特别适合治疗患有以下或处于以下风险的人类患者 :升高的胆固醇或与升高胆固醇 (例如,LDL 胆固醇) 相关的病症,包括脂质紊乱 (例如,高血脂、高胆固醇血症、黄瘤症)。本发明化合物也可以适用于治疗患有动脉粥样硬化性病症 (例如,动脉粥样硬化)、冠状动脉病、心血管疾病、中风、局部缺血、周围血管疾病的人类患者和预防处于这些疾病危险的患者,例如,由于存在一种或多种危险因素 (例如,高血压、吸烟、糖尿病、肥胖或同型半胱氨酸血症)。

[0426] 本文所用的术语“LDL- 胆固醇相关疾病或病症”是指一种部分由于血流中循环 LDL 胆固醇的高水平的疾病或病症。不被如此限制,LDL- 胆固醇相关的疾病或病症包括高脂血症、高胆固醇血症、黄瘤症和心血管疾病如动脉粥样硬化性病症 (例如,动脉粥样硬化)、冠状动脉疾病、中风、局部缺血和周围血管疾病。

[0427] 如本文所用,术语“治疗 (treat/treating/treatment)”和“预防 (prevent/preventing/prevention)”是指引发期望生物反应,即分别为治疗和预防效果。根据本发明,治疗效果包括减少 / 降低的 LDL- 胆固醇相关疾病或病症的进展或相关联症状的严重程度或完全治愈 LDL- 胆固醇相关疾病或病症和 / 或相关症状。根据本发明,预防效果可包括在施用本发明化合物或组合物后,延迟或下降 LDL- 胆固醇相关疾病或病症和相关症状的发作、其进展,或严重程度 (例如,减少血浆 LDL/ 胆固醇水平)。在一个实施方案中,包含式 (1) 化合物的本发明化合物或组合物治疗或预防受试者的 LDL- 胆固醇相关疾病或病症。本文所述的方法、组合物制剂和用途适合于人类和动物 (包括鸟类),优选哺乳动物。

[0428] 组合治疗

[0429] 当化合物与另一种药物一起施用时,两者可以以任何顺序依序或同时施用 (在同一组合物或在不同的组合物)。在一些实施方案中,将化合物施用给受试者,所述受试者也接受可用于治疗疾病 / 病状的第二药物的治疗 (例如,第二降胆固醇剂)。可与本发明组合施用的活性成分的实例包括但不限于 :改善患者的脂质谱的其它化合物,如 (a) HMG-CoA 还原酶抑制剂,(例如,他汀类,包括洛伐他汀、辛伐他汀、氟伐他汀、罗苏伐他汀、普伐他汀、利伐他汀、阿托伐他汀、伊伐他汀、匹伐他汀、西伐他汀和其它他汀类)。他汀类通过阻断 HMGCoA,胆固醇生物合成中的关键酶来抑制胆固醇合成 ;(b) 胆固醇吸收抑制剂,如甾烷醇酯, β - 谷甾醇,甾醇苷如替奎安 ;和氮杂环丁酮,如依泽替米贝 ;(c) 胆固醇酯转运蛋白 (CETP) 抑制剂 (例如,安塞曲匹或达塞曲匹),现在是在临床试验中,以增加 HDL 和降低 LDL 胆固醇 ;(d) 烟酸和相关化合物,如烟醇、烟酰胺和烟酸或其盐 ;(e) 胆汁酸螯合剂 (消胆胺,考来替泊 (例如,考来替泊盐酸盐),交联葡聚糖的二烷基氨基烷基衍生物,Colestid®, LoCholest®。胆汁酸螯合剂中断胆汁酸从肠道到肝脏的再循环 ;(f) 酰基 CoA :

胆固醇酰基转移酶 (ACAT) 抑制剂,如阿伐麦布和甲亚油酰胺,以及包括选择性 ACAT-1 和 ACAT-2 抑制剂和双重抑制剂 ;(g)PPARγ 激动剂,如吉非贝齐和非诺贝酸衍生物 (贝特类),包括氯贝丁酯、非诺贝特、苯扎贝特、环丙贝特和依托贝特 ;(h) 微粒体甘油三酯转移蛋白 (MTP)/ApoB 分泌抑制剂 ;(i) 抗氧化维生素,如维生素 C 和 E 和 β 胡萝卜素 ;(j) 条拟甲状腺素 ;(k)LDL 受体诱导剂 ;(l) 血小板凝集抑制剂,例如糖蛋白 IIb/IIIa 的血纤维蛋白原受体拮抗剂和阿司匹林 ;(m) 维生素 B 12(也称为氰钴胺素) ;(n) 叶酸或其药学上可接受的盐或酯,如钠盐和甲基葡糖胺盐 ;(o)FXR 和 LXR 配体,包括抑制剂和激动剂 ;(p) 提高 ABCA1 基因表达的试剂 ;(q) 回肠胆汁酸转运体和长链 α , ω - 二羧酸。

[0430] 组合治疗方案可以是相加,或者它可以产生协同结果 (例如,降低胆固醇大于预期的两种药剂的组合使用)。在一些实施方案中,化合物和降胆固醇剂 (例如,他汀类药物、贝特类、依泽替米贝或其组合) 的组合治疗产生协同结果 (例如胆固醇的协同减少)。在一些受试者中,这可以允许减少在降胆固醇剂的剂量以达到所需的胆固醇水平。化合物可以适用于不能耐受另一种降胆固醇剂治疗的受试者,或另一种降胆固醇剂治疗已经产生不充分的结果的受试者 (例如,利用他汀类药物治疗经历不足 LDL-C 降低的受试者)。

[0431] 本文中所描述的化合物可以施用给具有升高胆固醇 (例如,LDL- 胆固醇) 的受试者 (例如,具有 200mg/dl 或更高的总血浆胆固醇水平的人受试者,具有 160mg/dl 或更高的 LDL-C 水平的人受试者)。

[0432] 试剂盒

[0433] 本发明还涵盖包含至少一种本发明化合物的试剂盒。例如,所述试剂盒可包含一种或多种预防和 / 或治疗 LDL- 胆固醇相关疾病或病症的化合物。所述试剂盒可任选地包括一个或更多个对照样品和一个装置 (例如,注射器等)。所述试剂盒可任选地包括一种或多种改善患者的脂质谱的其它活性成分。所述化合物或成分可以包装在合适的容器中。所述试剂盒可以进一步包括使用所述试剂盒的说明 (例如,使用化合物以预防或治疗受试者的低密度脂质胆固醇相关疾病或病症)。

[0434] 对本发明作了充分的描述,本发明进一步由以下实例和权利要求说明,实例和权利要求是说明性的,并不意味着是进一步限制性。本领域技术人员将认识到或能够使用不超过常规实验,以确定,许多本文中所描述的具体程序的等同方案。这些等同方案都是在本发明和权利要求书的范围内。引用在本申请中的所有参考文献的内容,包括授权的专利和公布的专利申请,通过引用并入本文。

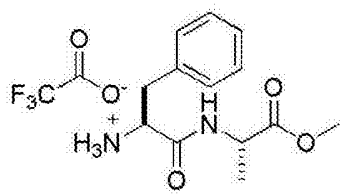
[0435] 实施本发明的方式

[0436] 本发明是通过以下非限制性实例来进一步详细地说明。

[0437] 中间体 1

[0438] 2-((S)-2- 氨基 -3- 苯基丙酰胺基) 丙酸 (S)- 甲基酯三氟乙酸盐

[0439]



[0440] 步骤 1 :在 0 ℃ 下,历时 10 分钟将 DIPEA(46mL,260mmol,3.5 当量) 滴加到

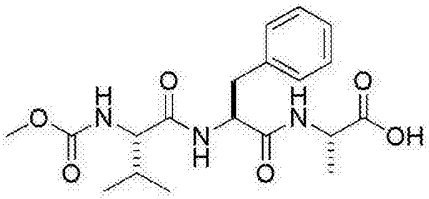
N-Boc-L- 苯基丙氨酸 (20g,74mmol)、L- 丙氨酸甲酯盐酸盐 (12.6g,90.5mmol,1.2 当量) 和 HATU(42.9g,113mmol,1.5 当量) 在 DMF(250mL) 中的搅拌溶液,接着温至室温。在搅拌 18h 后,将反应混合物倒入冷的 $NaHCO_3$饱和溶液并用 1 ： 1EtOAc ：己烷 (2x) 萃取。用水和盐水洗涤合并的有机部分,干燥 ($MgSO_4$)、过滤并在减压下浓缩。在硅胶上用渐增比例的 EtOAc 的己烷溶液洗脱,纯化残余物。

[0441] 步骤 2 :在 0℃下,用 TFA(32.5mL,422mmol,10 当量) 逐滴处理来自步骤 1 的产物 (14.8g,42.2mmol) 在 CH_2Cl_2(141mL) 中的搅拌溶液。随后将反应混合物升温至室温并再搅拌 3h,然后浓缩至干。用 Et_2O 磨碎残余物,且通过过滤来收集固体并在高真空下干燥,得到标题化合物。

[0442] 中间体 2

[0443] (2S)-2-[(2S)-2-[(2S)-2-[(甲氧基羰基) 氨基]-3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酸

[0444]



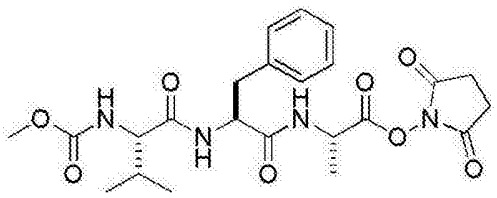
[0445] 步骤 1 :在 0℃下,在搅拌下历时 10 分钟将可力丁 (3.0mL,23mmol,2.5 当量) 滴加到 N- 甲氧基羰基 -L- 缬氨酸 (1.60g,9.13mmol),中间体 1(3.49g,9.59mmol,1.05 当量) 和 HATU(3.82g,10.0mmol,1.1 当量) 在 DMF(30mL) 中的搅拌溶液,在此温度下搅拌 1h,然后在室温下搅拌 18h。然后将混合物再冷却至 0℃并缓慢添加 $NaHCO_3$饱和水溶液 (50mL),接着添加 Et_2O(50mL)。剧烈搅拌混合物 20 分钟,并通过过滤收集固体产物,用单独部分的水和 Et_2O 洗涤,并在抽吸和高真空下干燥。

[0446] 步骤 2 :将 1M LiOH(10.65mL,10.65mmol,1.25 当量) 滴加到步骤 1 产物 (3.47g,8.52mmol) 在 THF(40mL) 和 MeOH(20mL) 的混合物中的搅拌悬浮液中,接着历时几个小时缓慢升温至室温。然后将容器内含物再次冷却至 0℃,滴加另外的 1M LiOH(3.4mL,3.4mmol,0.4 当量),且在室温下再搅拌混合物 2h,然后在 0℃下用 1M HCl 酸化至 pH 4,并用乙酸乙酯 (2x) 萃取。用水、半饱和盐水洗涤合并的有机部分,干燥 ($MgSO_4$),过滤并在减压下浓缩,得到无需进一步纯化即可使用的标题化合物。

[0447] 中间体 3

[0448] (2S)-2-[(2S)-2-[(2S)-2-[(甲氧基羰基) 氨基]-3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酸 2,5- 二氧代吡咯烷 -1- 基酯

[0449]



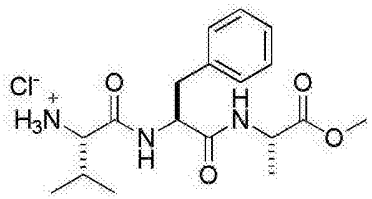
[0450] 在 -78 ℃ 下,将 DIPEA(0.33mL,1.9mmol,3.0 当量) 添加到中间体 2(0.25g,

0.64mmol）和 TFA-NHS(0.40g,1.9mmol,3.0 当量）在 DMF(4.5mL) 中的溶液。然后将反应混合物缓慢升温至 0℃并在此温度下搅拌 2h,然后分配在水和 EtOAc 之间。分离层,并用 EtOAc(2x) 萃取水层。用半饱和盐水洗涤合并的有机层,干燥 ($MgSO_4$),过滤并在减压下浓缩,得到无需进一步纯化即可用于后续反应中的标题化合物。

[0451] 中间体 4

[0452] (2S)-2-[(2S)-2-[(2S)-2- 氨基 -3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酸甲酯盐酸盐

[0453]



[0454] 步骤 1 :在 0℃下,历时 10 分钟将 DIPEA(6.7mL,38mmol,3.5 当量）滴加到中间体 1(4.0g,11mmol)、N-Boc-L- 缬氨酸 (2.62g,12.0mmol,1.1 当量）和 HATU(5.0g,13mmol,1.2 当量）在 DMF(35mL) 中的搅拌溶液。在室温下搅拌过夜后,将容器内含物倒入冰 (100mL)、饱和 $NaHCO_3$ 水溶液 (100mL) 和 Et_2O(200mL) 的混合物中,并搅拌混合物 30 分钟。通过过滤收集固体产物,用水和 1 ： 1Et_2O/ 己烷洗涤并在抽吸和高真空下干燥。

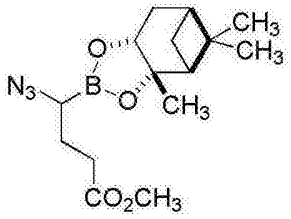
[0455] 步骤 2 :在 0℃下,将 TFA(7.3mL,95mmol,10 当量）滴加到步骤 1 产物 (4.28g,9.52mmol) 在 CH_2Cl_2(30mL) 中的搅拌溶液。然后,将反应容器内含物保持在室温下持续 5h,然后再浓缩至干。用 Et_2O 磨碎残余物并通过过滤收集固体产物且在高真空下干燥。

[0456] 步骤 3 :将 4M HCl 的二噁烷溶液 (14mL,56mmol,5.9 当量）添加到步骤 2 产物 (4.40g,9.52mmol) 在 MeOH(20mL) 中的搅拌悬浮液,在室温下搅拌过夜。在将混合物浓缩至干后,添加 Et_2O 并搅拌 20 分钟并通过过滤收集固体产物且在高真空下干燥,得到标题化合物。

[0457] 中间体 5

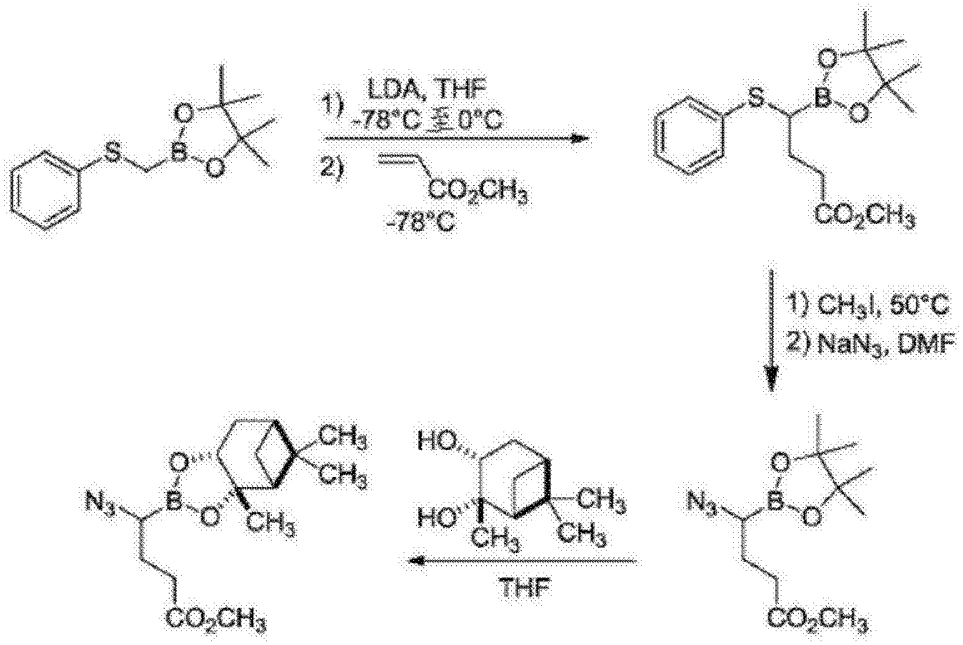
[0458] (4R/S)-4- 叠氮基 -4-[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 丁酸甲酯

[0459]



[0460] 反应顺序 :

[0461]



[0462] 步骤 1：将 DIPEA(6.2mL,44mmol,1.1 当量）和 THF(40mL) 置于用 N_2净化的火焰干燥的 rbf。将温度冷却至 -78℃且历时 2 分钟滴加 n-BuLi(2.5M 的己烷溶液,17mL,42mmol,1.0 当量）到反应容器。然后,将反应混合物升温至 0℃并搅拌 5 分钟,然后冷却回至 -78℃。将 4,4,5,5,- 四甲基 -2- 苯基磺酰基甲基 -1,3,2- 二氧杂硼戊环 (10mL,42mmol) 在 THF(10mL) 中的溶液滴加到反应混合物,接着在 0℃下搅拌。在此温度 1h 后,将反应容器内含物再次冷却到 -78℃且逐滴引入丙烯酸甲酯 (7.6mL,85mmol,2.0 当量）。在 -78℃下 6h 后,通过倒入 200mL 10％柠檬酸水溶液淬灭反应。分离层,且用 Et_2O(3x) 萃取水层。合并有机物,用半饱和盐水洗涤,干燥 ($MgSO_4$),过滤并在减压下浓缩。通过在硅胶上用渐增比例的 EtOAc 的己烷溶液洗脱的快速色谱分离产物。

[0463] 步骤 2：在 50℃下,将步骤 1 产物 (1.80g,5.35mmol)、碘代甲烷 (6.6mL,110mmol,20 当量）和乙腈 (8mL) 一起在 N_2净化的密封管中加热。在此温度下 24h 后,在减压下浓缩容器内含物。将残余物直接用于下一步骤中。

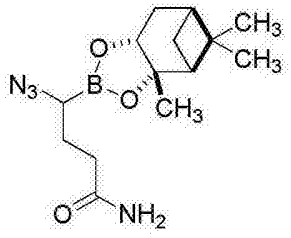
[0464] 步骤 3：将步骤 2 产物 (1.89g,5.35mmol) 溶解于 DMF(11mL) 中,并用叠氮化钠 (0.70g,11mmol,2.0 当量）处理,在室温下搅拌过夜。然后将反应混合物分配在 EtOAc 和水之间。分离层,并用 EtOAc(2x) 萃取水层。用半饱和盐水 (3x) 洗涤合并的有机物,干燥 ($MgSO_4$),过滤并在减压下浓缩。残余物直接用于下一步骤中。

[0465] 步骤 4：在室温下,搅拌步骤 3 产物 (1.43g,5.35mmol) 和 (1S,2S,3R,5S)-(+)- 蒎烷二醇 (1.00g,5.90mmol,1.1 当量）在 THF(21mL) 中的溶液 18h。将反应容器内含物浓缩至干且残余物通过在硅胶上用渐增比例的 EtOAc 的己烷溶液洗脱的快速色谱纯化,得到标题化合物。

[0466] 中间体 6

[0467] (4R/S)-4- 叠氮基 -4-[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 丁酰胺

[0468]

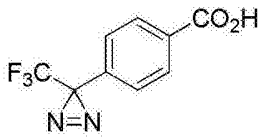


[0469] 在室温下，在 N_2 净化的密封管中，搅拌中间体 5(0.30g，0.94mmol) 与 7.0M NH_3 的 MeOH 溶液 (4mL，30mmol，30 当量)42h。然后在减压下浓缩容器内含物，且残余物通过在硅胶上用渐增比例的 MeOH 的 EtOAc 溶液洗脱的快速色谱纯化，得到标题化合物。

[0470] 中间体 7a

[0471] 4-(3-(三氟甲基)-3H- 双吖丙啶 -3- 基) 苯甲酸

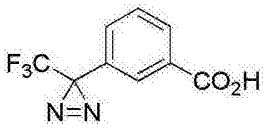
[0472]



[0473] 中间体 7b

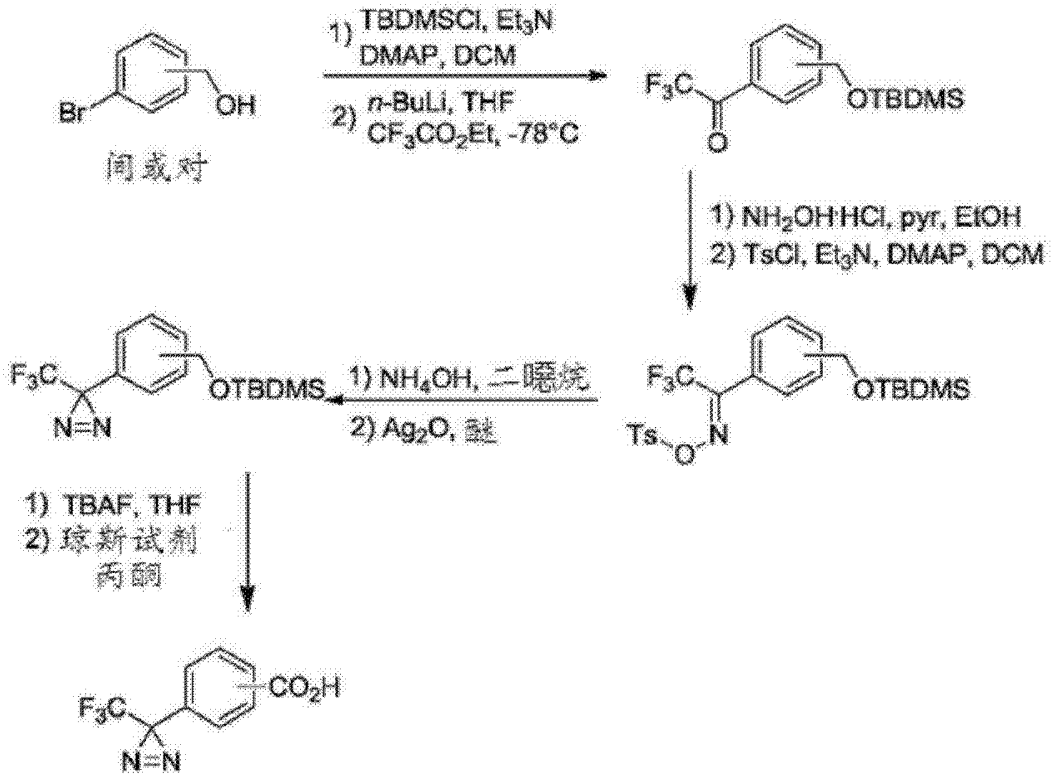
[0474] 3-(3-(三氟甲基)-3H- 双吖丙啶 -3- 基) 苯甲酸

[0475]



[0476] 反应顺序：

[0477]



[0478] 步骤 1 :在 0℃下,以逐份的方式将 Et_3N(11mL,80mmol,1.5 当量) 和 DMAP(0.65g,5.3mmol,0.1 当量) 添加到 4- 溴苄基醇 (以制备 7a) 或 3- 溴苄基醇 (以制备 7b)(10.00g,53.46mmol) 在 CH_2Cl_2(60mL) 中的搅拌溶液,接着添加叔丁基二甲基甲硅烷基氯 (8.86g,58.8mmol,1.1 当量)。然后将反应容器内含物升温至室温持续 3h。添加水,并搅拌混合物直到所有固体已溶解。将混合物转移至分离漏斗并用 CH_2Cl_2(3x) 萃取。用 NH_4Cl 饱和水溶液和水洗涤合并的有机部分,然后干燥 ($MgSO_4$),过滤并在减压下浓缩。残余物直接用于下一步骤中。

[0479] 步骤 2 :在 -78℃下,历时 30 分钟,将 n-BuLi(2.5M 的己烷溶液,24mL,60mmol,1.1 当量) 滴加到步骤 1 产物 (15.89g,52.74mmol) 在 THF(175mL) 中的搅拌溶液。在此温度下搅拌反应 30 分钟,然后历时 30 分钟逐滴引入三氟乙酸乙酯 (7.6mL,63mmol,1.2 当量) 在 THF(30mL) 中的溶液。在 -78℃下,再搅拌反应 90 分钟,然后分配在 EtOAc 和饱和 NH_4Cl 溶液之间。分离相,且用 EtOAc(2x) 萃取水层。用饱和盐水溶液洗涤合并的有机部分,干燥 ($MgSO_4$),过滤并在减压下浓缩。残余物是通过在高真空 (P = 10mmHg) 下蒸馏纯化,收集沸点在 160-170℃之间的馏分。

[0480] 步骤 3 :在回流下,将步骤 2 产物 (8.0g,25mmol) 和羟胺盐酸盐 (1.92g,27.6mmol,1.1 当量) 在吡啶 (12mL) 和 EtOH(6mL) 的混合物中一起加热。3h 后,在减压下移除溶剂且将残余物分配在 EtOAc 和水之间。分离相,并用 EtOAc(2x) 萃取水层。用饱和盐水溶液洗涤合并的有机部分,干燥 ($MgSO_4$),过滤并在减压下浓缩。残余物是在硅胶上用渐增比例的 EtOAc 的己烷溶液纯化。

[0481] 步骤 4 :在 0℃下,以逐份方式将对甲苯磺酰氯 (3.29g,17.2mmol,1.15 当量) 添加到步骤 3 产物 (5.0g,15mmol)、Et_3N(2.5mL,18mmol,1.2 当量) 和 DMAP(0.183g,1.5mmol,0.10 当量) 在 CH_2Cl_2(26mL) 中的搅拌溶液。通过 TLC 判断反应完全后,在减压下浓缩混合物,并将残余物分配在 EtOAc 和水之间。分离层并用另外的水然后用盐水洗涤有机相,然后干燥 ($MgSO_4$),过滤并在减压下浓缩。无需另外的纯化而将残余物用于下一步骤中。

[0482] 步骤 5 :在 10℃下,在厚壁玻璃管中,将浓 NH_4OH(68mL) 添加到步骤 4 产物 (6.85g,14.1mmol) 在二噁烷 (68mL) 中的搅拌溶液。将管封盖,且在室温下搅拌内含物 48h。然后添加水和 EtOAc 并分离层。用 EtOAc(2x) 萃取水层且用饱和盐水溶液洗涤合并的有机部分,干燥 ($MgSO_4$),过滤并在减压下浓缩。残余物是在硅胶上用渐增比例的 EtOAc 的己烷溶液纯化。

[0483] 步骤 6 :在室温下,用 Ag_2O(2.50g,10.9mmol,2 当量) 搅拌步骤 5 产物 (1.8g,5.4mmol) 在 Et_2O(54mL) 中的溶液。4h 后,添加第二部分的 Ag_2O(2.50g,10.9mmol,2 当量),并在室温下搅拌混合物过夜。然后添加己烷 (100mL),并通过硅胶垫过滤混合物。用 9 : 1 己烷 /EtOAc 洗涤垫,并在减压下浓缩滤液。残余物直接用于下一步骤中。

[0484] 步骤 7 :在 0℃下,将 TBAF(1.0M 的 THF 溶液,6.2mL,6.2mmol,1.2 当量) 滴加到步骤 6 产物 (1.70g,5.15mmol) 在 THF(25mL) 中的溶液,接着在室温下搅拌 1h。然后将混合物分配在 EtOAc 和水之间并分离相。用 EtOAc(2x) 萃取水相并用饱和盐水溶液洗涤合并的有机物,干燥 (MgSO4),过滤并在减压下浓缩。残余物是在硅胶上用渐增比例的 EtOAc 的己烷溶液纯化。

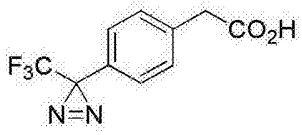
[0485] 步骤 8 :将琼斯试剂 (2.5M,0.56mL,1.4mmol,2.0 当量) 添加到步骤 6 产物 (0.15g,

0.69mmol）在丙酮（7.0mL）中的溶液，在0℃下搅拌1h。然后，用EtOAc稀释混合物，并在分离漏斗中用水洗涤直到水层中颜色不可见。用盐水洗涤有机层，经$MgSO_4$干燥，过滤并在减压下浓缩，得到标题化合物。

[0486] 中间体7c

[0487] 2-(4-(3-(三氟甲基)-3H-双吖丙啶-3-基)苯基)乙酸

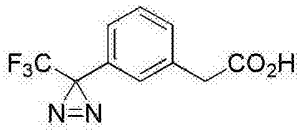
[0488]



[0489] 中间体7d

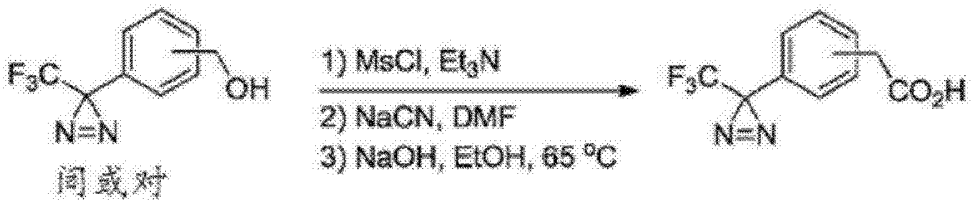
[0490] 2-(3-(3-(三氟甲基)-3H-双吖丙啶-3-基)苯基)乙酸

[0491]



[0492] 反应顺序：

[0493]



[0494] 步骤1：在0℃下，将MsCl(0.22mL，2.8mmol，1.5当量)滴加到在制备中间体7a或7b时步骤7的对-或间-产物(400mg，1.85mmol)和Et_3N(0.52mL，3.7mmol，2当量)在Et_2O(19mL)中的溶液，并在此温度下搅拌15分钟，然后在室温下搅拌。2h后，过滤混合物，用乙醚洗涤固体材料，并真空中浓缩滤液。残余物无需进一步纯化而用于下一步骤中。

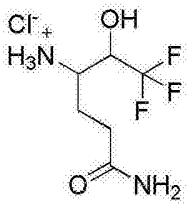
[0495] 步骤2：在室温下，将步骤1产物(535mg，1.82mmol)和NaCN(134mg，2.70mmol，1.5当量)一起在DMF(10mL)中搅拌过夜。随后，将混合物分配在水Et_2O和之间并分离层。用水，然后盐水洗涤有机相，干燥($MgSO_4$)，过滤和浓缩。残余物是通过在硅胶上用1/4EtOAc/己烷洗脱的快速色谱纯化。

[0496] 步骤3：在65℃下，用1MNaOH(4.5mL，5当量)的EtOH(4.5mL)溶液搅拌步骤2产物(200mg，0.89mmol)过夜。在冷却至室温后，通过添加1M HCl(5mL)淬灭反应，并将混合物分配在水和Et_2O之间。分离层并用另外的Et_2O(2x)萃取水相。合并有机物，用盐水洗涤，干燥($MgSO_4$)，过滤和浓缩。残余物是通过在硅胶上用含有1％ AcOH的1/9EtOH/己烷洗脱的快速色谱纯化，得到呈淡黄色固体的标题化合物。

[0497] 中间体8

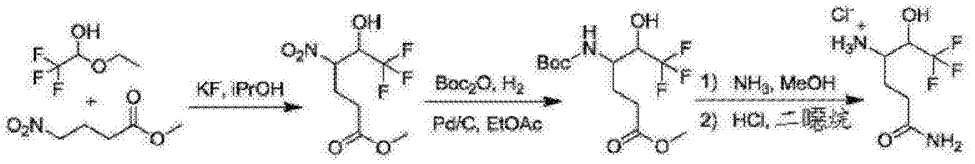
[0498] 4-氨基-6,6,6-三氟-5-羟基己酰胺盐酸盐

[0499]



[0500] 反应顺序：

[0501]



[0502] 步骤 1：将三氟乙醛乙基半缩醛 (90％，15mL，110mmol，1.8 当量）、4- 硝基丁酸甲酯 (8.0mL，62mmol)、氟化钾 (3.65g，62.8mmol) 和 i-PrOH(20mL) 置于密封的反应容器中，并在室温下搅拌混合物 20h。然后，将混合物分配在 EtOAc(200mL) 和 5％ (w/w) 柠檬酸水溶液和盐水的 1 ： 1 混合物 (300mL) 之间并分离层。用另外的盐水 (200mL) 洗涤有机相，并用 EtOAc(200mL) 萃取合并的水层。合并有机层，经 $MgSO_4$ 干燥，过滤和通过在减压下同时保持浴温在 20℃旋转蒸发浓缩。残余物是通过在硅胶上用 20％ EtOAc/ 己烷洗脱的管柱色谱纯化。

[0503] 步骤 2：在真空下，将步骤 1 产物 (13.0g，52.9mmol)、二碳酸二 - 叔丁酯 (15.0g，68.7mmol，1.3 当量）和 10％ Pd/C(4.00g) 的混合物置于隔膜 - 封盖的 rbf 中，并通过注射器引入脱气的 EtOAc(100mL)。然后，将混合物置于经由乳胶气球的 H_2 氛围下，并在超声波浴中使反应容器声波处理 2 分钟，接着在室温下搅拌内含物 24h。通过 TLC 判断反应完全后，将容器用 N_2 净化并添加 CH_2Cl_2。通过硅藻土垫过滤悬浮液，且用 EtOAc 和 CH_2Cl_2 的混合物彻底的洗涤垫。在真空中浓缩滤液，且用 Et_2O/ 庚烷的 1 ： 4 混合物处理残余物。静置 4h 后，通过抽吸过滤收集固体产物，并用少量 1 ： 4Et_2O/ 庚烷洗涤，得到以＞ 10 ： 1 非对映体纯度的所需非对映体。

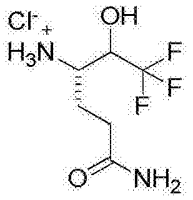
[0504] 步骤 3：在 0℃下，在 N_2 下，在厚壁玻璃管中将 NH_3 在 MeOH 中的溶液 (7.0M，30mL，200mmol，20 当量）添加到步骤 2 产物 (3.0g，9.5mmol)，且用 Teflon 螺帽密封入口，接着在 35℃下加热内含物 5 天。然后，在减压下浓缩混合物，且用 Et_2O 搅拌残余物过夜。通过抽吸过滤收集固体产物并在高真空下干燥。

[0505] 步骤 4：在 0℃下，将 HCl 的 1,4- 二噁烷溶液 (4M，1.2mL，5mmol，5 当量）滴加到步骤 3 产物 (0.30g，1.0mmol) 在 CH_2Cl_2(2mL) 中的悬浮液，接着在室温下搅拌直到通过 LCMS 测定反应为完全 (3h)。然后在减压下将反应混合物浓缩至干，得到标题化合物。在 -15℃下，在 N_2 氛围下储存所述物质，且无需进一步纯化即可用于后续反应中。

[0506] 中间体 9

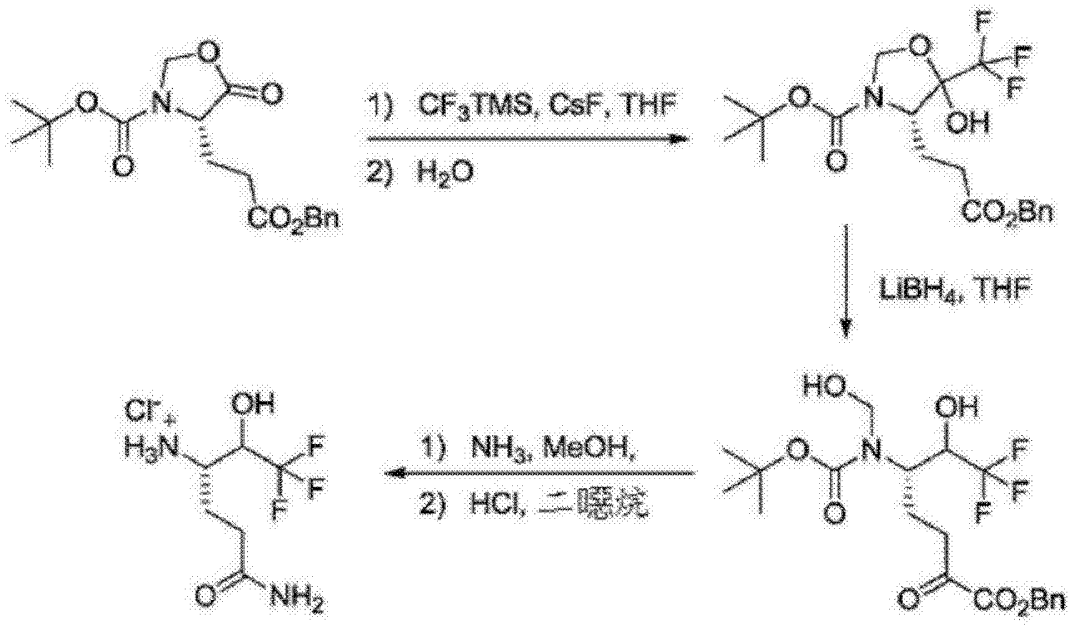
[0507] (4S)-4- 氨基 -6,6,6- 三氟 -5- 羟基己酰胺盐酸盐

[0508]



[0509] 反应顺序：

[0510]



[0511] 步骤 1：在 N_2氛围下，用 CF_3TMS(2.5mL，17mmol，1.2 当量）和 CsF(0.34g，2.2mmol，0.15 当量）处理 (S)-3- 叔丁氧基羰基 -4-(2- 苄氧基羰基乙基）噁唑烷 -5- 酮 (4.97g，14.2mmol) 在无水 THF(30mL) 中的溶液。然后在超声波浴中超声处理反应容器且通过 TLC 监测反应进展。1h 后，添加水 (2.5mL) 且继续超声处理直到通过 TLC 判断中间体甲硅烷基醚的水解已完全 (30 分钟）。添加 EtOAc，且用水和盐水洗涤混合物。用 EtOAc 萃取水相，且合并的有机层经 $Na_2SO_4/MgSO_4$干燥，过滤和在真空下浓缩且残余物直接用于下一步骤中。

[0512] 步骤 2：以足以保持内部温度在 30℃以下的速率将 $LiBH_4$的 THF 溶液 (2.0M，6.5mL，13mmol，1.05 当量）滴加到步骤 1 产物 (5.28g，12.6mmol) 在 THF(50mL) 中的溶液。通过 TLC 判断反应完全后 (5 分钟），将反应容器冷却至 0℃且通过添加冰水淬灭反应。将反应容器内含物分配在 EtOAc 和水之间并分离层。用另外的 EtOAc(2x) 萃取水相且用盐水洗涤合并的有机物，经 $MgSO_4/Na_2SO_4$干燥，过滤和在真空下浓缩，得到呈非对映体的 2.2 ： 1 混合物的产物。通过重复的在硅胶上用渐增比例的 EtOAc(0-30％）的甲苯溶液洗脱的快速色谱分离这些非对映体的混合物。所需非对映体对应于极性更大的化合物且是在反应中产生的较少的异构体。

[0513] 步骤 3：在 0℃下，在 N_2下，在厚壁玻璃管中将 NH_3的 MeOH 溶液 (7.0M，15mL，100mmol，110 当量）添加到步骤 2 产物 (0.38g，0.90mmol) 且用 Teflon 螺帽密封入口，接着在 45℃下加热内含物 48h。然后在减压下浓缩混合物，且将残余用醚 / 庚烷 (2x) 共沸，然后用 Et_2O 磨碎 1h。通过抽吸过滤收集固体产物并在高真空下干燥。

[0514] 步骤 4：在 0℃下，将 HCl 的 1,4- 二噁烷溶液 (4M，0.70mL，2.8mmol，28 当量）滴加

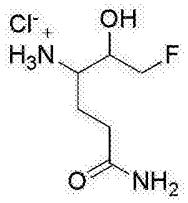
到步骤 3 产物 (30mg,0.10mmol) 在 CH_2Cl_2(0.7mL) 中的悬浮液,接着在室温下搅拌直到通过 LCMS 测定反应已完全 (2h)。然后将反应混合物在减压下浓缩至干,得到标题化合物。无需另外的纯化将所述物质直接用于后续反应中。

[0515] 以上顺序也可用于从 (R)-3- 叔丁氧基羰基 -4-(2- 苄氧基羰基乙基) 噁唑烷 -5- 酮制备 (4R,5S)- 和 (4R,5R)-4- 氨基 -6,6,6- 三氟 -5- 羟基己酰胺盐酸盐。

[0516] 中间体 10

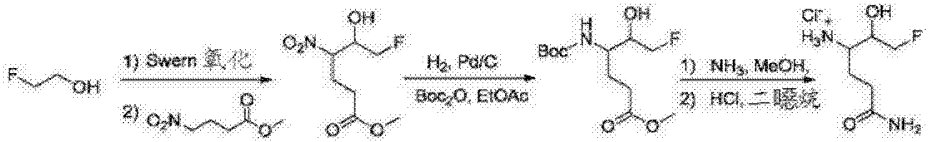
[0517] 4- 氨基 -6- 氟 -5- 羟基己酰胺盐酸盐

[0518]



[0519] 反应顺序 :

[0520]



[0521] 步骤 1 :在 -78℃下,将 DMSO(8.7mL,120mmol,3 当量) 在 CH_2Cl_2(15mL) 中的溶液缓慢添加至草酰氯 (4.2mL,48mmol,1.2 当量) 在 CH_2Cl_2(50mL) 中的溶液,接着在此温度下搅拌 30 分钟。然后,逐滴引入 2- 氟乙醇 (2.4mL,41mmol) 在 CH_2Cl_2(20mL) 中的溶液,且历时 1h 将混合物缓慢升温至 -40℃接着重新冷却到 -78℃,然后再滴加 Et_3N(28.5mL,204mmol,5 当量)。在添加过程中保持必需用手摇晃反应容器来确保均一性的不可溶物质的形成。然后将悬浮液升温至室温,并再搅拌 1.5h,此后添加 4- 硝基丁酸甲酯 (4mL,31.2mmol,0.77 当量) 到反应容器,同时在室温下在水浴中冷却。将混合物储存在 -15℃下过夜,然后在室温下搅拌 2.5h,再将混合物分配在 EtOAc(100mL) 和从 10% 柠檬酸和盐水制得的水溶液之间。用 EtOAc(100mL) 萃取水相且用盐水洗涤合并的有机层,经 $MgSO_4$干燥,过滤和通过在减压下在 20℃的浴温下旋转蒸发浓缩。使残余物经历在硅胶上用渐增比例的 EtOAc(0-50%) 的己烷溶液洗脱的快速色谱,得到呈非对映体混合物的产物。

[0522] 步骤 2 :在真空下,将步骤 1 产物 (4.01g,20.0mmol)、二碳酸二 - 叔丁酯 (5.23g,24.0mmol,1.25 当量) 和 10% Pd/C(1.00g) 置于隔膜封盖的 rbf 中并经由注射器引入脱气的 EtOAc(50mL)。然后将混合物置于经由乳胶气球的 H_2氛围下且使反应容器内含物超声处理 2 分钟,接着在室温下搅拌内含物 24h。然后重复反应容器内含物的超声处理再 2 分钟时间,且在室温下再搅拌混合物 24h。通过 TLC 判断反应完全后,用 N_2净化容器并添加 CH_2Cl_2。通过硅藻土垫过滤悬浮液,且用 EtOAc 和 CH_2Cl_2的混合物彻底洗涤垫。真空中浓缩滤液,且残余物是通过在硅胶上用渐增比例的 EtOAc(10-50%) 的己烷溶液洗脱的快速色谱纯化。主要非对映异构体首先洗脱 (极性较小) 且次要非对映异构体其次洗脱 (极性较大)。通过用 Et_2O/ 己烷磨碎,进一步纯化两种非对映体,得到以 > 10 : 1 非对映体纯度的产物,如通过 ^1H NMR 所测得。

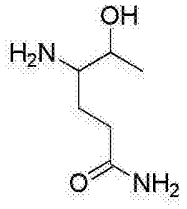
[0523] 步骤 3：在 0℃下，在 N_2下，在厚壁玻璃管中将 NH_3的 MeOH 溶液（7.0M，6.0mL，42mmol，30 当量）添加到步骤 2 中分离的产物（400mg，1.43mmol），且用 Teflon 螺帽密封入口，接着在 40℃下加热内含物 3d。然后将混合物在减压下浓缩至干并用 Et_2O 磨碎残余物。通过抽吸过滤收集固体产物，并在真空下干燥。

[0524] 步骤 4：在 0℃下，将 HCl 的 1，4- 二噁烷溶液（4.0M，1.0mL，4.0mmol，14 当量）滴加到步骤 3 产物（75mg，0.28mmol）在 CH_2Cl_2（1mL）中的悬浮液，接着在室温下搅拌直到通过 LCMS 测定反应已完成（2h）。然后将反应混合物在减压下浓缩至干，得到标题化合物，其无需进一步纯化即可直接用于后续反应中。

[0525] 中间体 11

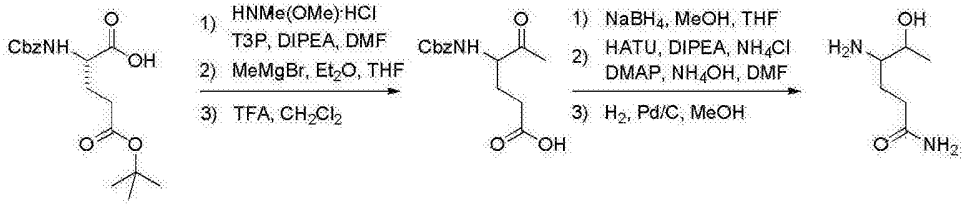
[0526] 4- 氨基 -5- 羟基己酰胺

[0527]



[0528] 反应顺序：

[0529]



[0530] 步骤 1：在 0℃下，将 T3P（50wt. %的 EtOAc 溶液，21mL，35mmol，1.2 当量）和 DIPEA（12.5mL，71.8mmol，2.4 当量）依序添加至 N-Cbz-L- 谷氨酸（10.0g，29.6mmol）和 N，O- 二甲基羟胺盐酸盐（3.46g，35.5mmol，1.2 当量）在 DMF（15mL）中的混合物，接着在室温下搅拌 1h。然后将反应容器内含物倒入冷的 0.5M HCl 水溶液并用 EtOAc（2x）萃取。用冷的 0.5M HCl，然后用盐水洗涤合并的有机物。合并的有机物经 $MgSO_4$干燥并通过硅胶垫过滤。用另外的 EtOAc 洗涤垫，且将滤液浓缩至干。残余物直接用于下一步骤中。

[0531] 步骤 2：在 -78℃下，将 3.0M MeMgBr 的 Et_2O 溶液（34mL，100mmol，3.0 当量）滴加到步骤 1 产物（12.9g，34.0mmol）在 THF（100mL）中的溶液。添加完成后，使混合物升温至室温，并在此温度下搅拌，直到通过 LCMS 测得反应完全（2h）。然后将反应容器内含物倒入冷的 0.5M HCl 水溶液并用 EtOAc（2x）萃取。用冷的 0.5M HCl 水溶液和盐水洗涤合并的有机物，然后经 $MgSO_4$干燥，过滤和在真空下浓缩至干。通过在硅胶上用渐增比例的 EtOAc（25-50％）的己烷溶液洗脱的快速色谱分离酮产物。

[0532] 步骤 3：在 -30℃下，将 TFA（10mL，130mmol，5.4 当量）滴加到步骤 2 产物（8.08g，24.1mmol）在 CH_2Cl_2（2mL）中的搅拌溶液。然后将反应容器内含物缓慢升温至室温并在此温度下搅拌 3h，然后浓缩至干。使含有产物的残余物与甲苯（3x）共沸，在高真空下干燥并直接用于下一步骤中。

[0533] 步骤 4：在 -78℃下，将 $NaBH_4$（170mg，4.44mmol，2.5 当量）和 MeOH（1mL）依序添

加至步骤 3 产物 (500mg,1.79mmol) 在 THF(4mL) 中的溶液,接着升温至 0℃,并在此温度下搅拌 45 分钟。然后将反应容器内含物分配在 10% (w/w) 柠檬酸水溶液和 EtOAc 之间,并分离层。用另外的 EtOAc 萃取水相,并用盐水洗涤合并的有机物,经 Na_2SO_4干燥,过滤,用 DIPEA(0.62mL) 处理,并通过在减压下在 20℃的浴温下旋转蒸发浓缩。在 0℃下,将残余物溶解于 DMF(5mL) 并用 DMAP(22mg,0.18mmol,0.1 当量)、HATU(817mg,2.15mmol,1.2 当量)、DIPEA(1.87mL,10.7mmol,6 当量) 和 NH_4Cl(481mg,9.0mmol,5 当量) 处理,接着升温至室温并搅拌过夜。然后添加浓 NH_4OH 水溶液 (1.8mL,25mmol,14 当量) 和 DMF(5mL),继续在室温下搅拌 3d。然后将反应容器内含物倒入盐水 (50mL) 并用 EtOAc(50mL x 2) 萃取。合并的有机层经 $MgSO_4$干燥,过滤并浓缩至干,和用 Et_2O/EtOAc 的混合物磨碎残余物。通过抽吸过滤收集固体产物并在高真空下干燥。

[0534] 步骤 5 :在高真空下,将步骤 4 产物 (0.27g,0.95mmol)、10% Pd/C(100mg) 的混合物置于橡胶隔膜密封的烧瓶中。然后经由注射器添加脱气的 MeOH(5mL),和将混合物置于由乳胶气球供应的 N_2氛围下。将反应容器浸没于超声波浴中并超声处理 2 分钟。通过由乳胶气球递送 H_2净化 N_2氛围,和在室温下搅拌混合物。5h 后,通过 TLC 分析判断反应完全。将 H_2氛围用 N_2更换,添加 CH_2Cl_2,并通过硅藻土垫过滤悬浮液,随后用 MeOH/CH_2Cl_2的混合物洗涤所述垫。真空中浓缩滤液,并用 MeOH 的 EtOAc 溶液磨碎残余物,得到标题化合物。

[0535] 中间体 12-14 的制备

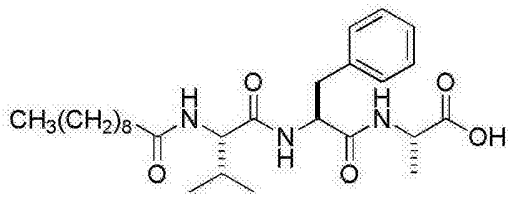
[0536] 反应顺序 :

[0537]

[0538] 中间体 12

[0539] (S)-2-((S)-2-((S)-2- 癸酰胺基 -3- 甲基丁酰胺基)-3- 苯基丙酰胺基) 丙酸

[0540]



[0541] 步骤 1 :在 0℃下,将癸酰氯 (0.73mL,3.6mmol,1.1 当量) 和 Et_3N(1.6mL,12mmol,3.5 当量) 依序滴加到中间体 4(1.25g,3.24mmol) 在 THF(15mL) 中的搅拌悬浮液,接着缓慢升温至室温。在搅拌过夜后,添加另外的 THF(70-80mL) 以促进混合物的搅拌且添加第二份癸酰氯 (0.10mL,0.50mmol,0.15 当量),继续在室温下搅拌直到通过 LCMS 判断反应完全 (24h)。然后将反应容器内含物倒入冷的 0.5M HCl 水溶液、EtOAc 和己烷的混合物。通过重力过滤收集固体产物,用水和 1 : 1Et_2O/ 己烷洗涤,然后在高真空下干燥。

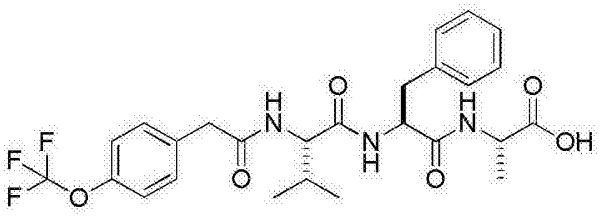
[0542] 步骤 2 :在 0℃下,将 1M LiOH 水溶液 (9mL,9mmol,3 当量) 添加到步骤 1 产物 (1.47g,2.92mmol) 在 MeOH(9mL) 和 THF(18mL) 中的搅拌悬浮液,接着升温至室温。4h 后,

添加另外的 MeOH(18mL) 且在室温下搅拌悬浮液过夜。LCMS 分析表明 24h 后反应未达到完全。将混合物重新冷却至 0℃，并添加另一份 1M LiOH(3mL,3mmol,1 当量)，接着在室温下搅拌第二个 24h 时间。在此时，反应仍未达到完全，这是由于起始酯在反应介质中的低溶解性。必需添加第三份 MeOH 和 1M LiOH(3mL,3mmol,1 当量）以推动反应完全。随后过滤混合物以移除固体，并用 1M HCl 将滤液酸化至 pH 1，接着浓缩至干。将固体残余物悬浮于水中，通过抽吸过滤收集并用水和 Et_2O 洗涤。在抽吸和高真空下干燥固体，提供标题化合物。

[0543] 中间体 13

[0544] (S)-2-((S)-2-((S)-3- 甲基 -2-(2-(4-(三氟甲氧基) 苯基) 乙酰胺基) 丁酰胺基)-3- 苯基丙酰胺基) 丙酸

[0545]



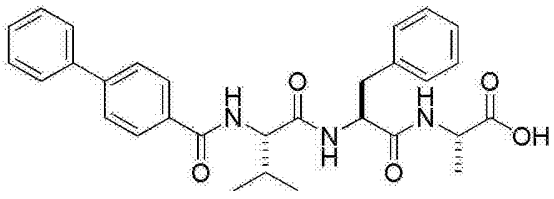
[0546] 步骤 1：在 0℃下，历时 10 分钟，将 DIPEA(0.51mL,2.9mmol,3.5 当量）滴加到中间体 4(0.32g,0.83mmol)、4-(三氟甲氧基) 苯基乙酸 (0.20g,0.91mmol,1.1 当量）和 HATU(0.38g,1.0mmol,1.2 当量）在 DMF(3mL) 中的溶液，接着缓慢升温至室温，并搅拌过夜。然后，用 Et_2O 稀释反应容器内含物并倒入冰和饱和 $NaHCO_3$ 水溶液的混合物中。搅拌此混合物 30 分钟，直到冰已融化且通过抽吸过滤收集固体产物，用水和 Et_2O 洗涤并在高真空下干燥。

[0547] 步骤 2：在 0℃下，将 1M LiOH 水溶液 (3mL,3mmol,4 当量）添加到步骤 1 产物 (0.40g,0.73mmol) 在 MeOH(3mL) 和 THF(6mL) 中的快速搅拌的悬浮液。在此温度 15 分钟后，将反应容器升温至室温，并通过 LC-MS 监测反应进展。2h 后，将混合物冷却至 0℃并通过添加 0.05M HCl 水溶液 (100mL) 酸化至 pH 1，再在室温下搅拌 1h。通过抽吸过滤收集固体产物，用水洗涤和在抽吸和高真空下干燥，得到标题化合物。

[0548] 中间体 14

[0549] (S)-2-((S)-2-((S)-2-([1,1′ - 联苯基]-4- 基甲酰胺基)-3- 甲基丁酰胺基)-3- 苯基丙酰胺基) 丙酸

[0550]

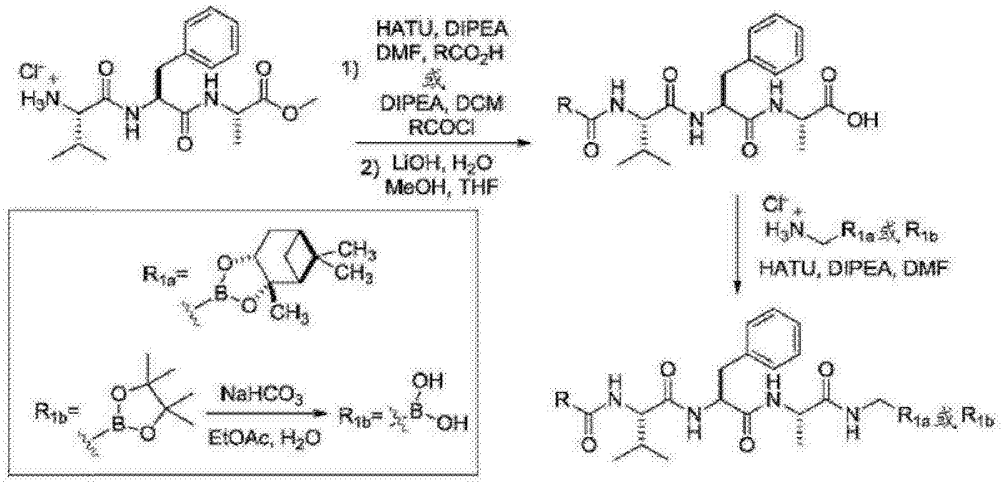


[0551] 步骤 1：在 0℃下，历时 10 分钟，将 DIPEA(0.42mL,2.4mmol,3.5 当量）滴加到中间体 4(0.32g,0.83mmol,1.2 当量）、联苯基 -4- 甲酸 (145mg,0.732mmol) 和 HATU(316mg,0.832mmol,1.2 当量）在 DMF(3mL) 中的溶液，接着在室温下搅拌 3d。然后用 EtOAc 稀释混合物，并倒入冰和饱和 $NaHCO_3$ 水溶液的混合物中，在室温下搅拌直到冰融化 (约 30 分钟)。通过抽吸过滤收集固体产物，依次用水和 EtOAc 洗涤，然后在抽吸和高真空下干燥。

[0552] 步骤 2：在 0℃下，将 1M LiOH 水溶液（3mL，3mmol，5 当量）添加到步骤 1 产物（0.33g，0.62mmol）在 MeOH（3mL）和 THF（6mL）中的悬浮液，接着在室温下快速搅拌直到通过 LCMS 判断反应完全（3h）。然后将反应容器内含物重新冷却至 0℃并通过添加 0.05M HCl 水溶液（100mL）酸化，接着在室温下搅拌 1h。通过抽吸过滤收集固体产物，用水和 Et_2O 洗涤，并在抽吸和高真空下干燥，得到标题化合物。

[0553] 一般顺序 1：

[0554]



[0555] 步骤 1（对于 RCO_2H）：在 0℃下，将 DIPEA（3.5 当量）添加到中间体 4（0.83mmol）、HATU（1.2 当量）和适当酸（1.1 当量）在 DMF（3.0mL）中的搅拌悬浮液，伴随缓慢升温至室温并搅拌过夜。然后添加饱和 $NaHCO_3$ 水溶液和 EtOAc 并通过抽吸过滤收集固体产物，用水和 EtOAc 洗涤并在高真空下。

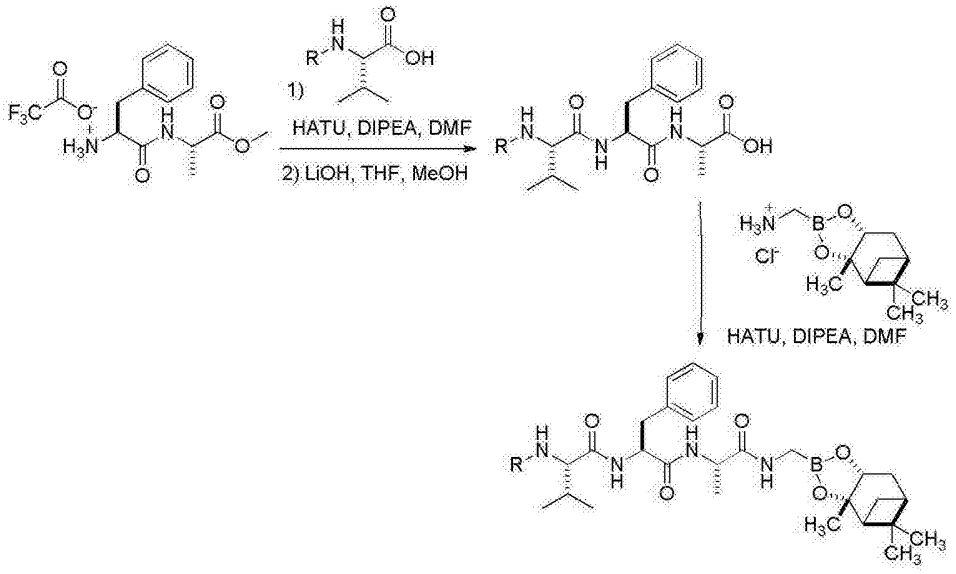
[0556] 步骤 1（对于 RC(O)Cl）：在 0℃下，将适当的酰氯（1.1 当量）逐份添加至中间体 4（1.3mmol）和 DIPEA（2.2 当量）在 CH_2Cl_2（13mL）的搅拌悬浮液，接着缓慢升温至室温并搅拌过夜。添加另外的 CH_2Cl_2 和水至反应容器，且通过抽吸过滤分离固体产物，用 CH_2Cl_2 洗涤，并在高真空下干燥。

[0557] 步骤 2：在 0℃下，将 1M LiOH 水溶液（4 当量）滴加到步骤 1 产物（0.73mmol）在 MeOH（3mL）和 THF（6mL）中的搅拌悬浮液。15 分钟后，使反应升温至室温，并再搅拌 3h。然后添加碎冰，并用 1M HCl 将混合物酸化至 pH 3。再搅拌 1h 后，通过抽吸过滤分离固体产物并在高真空下干燥。通过用 EtOAc（3x）萃取处理，分离在这些条件下无法沉淀的类似物。用盐水洗涤合并的有机物，干燥（Na_2SO_4），过滤和浓缩且残余物直接用于下一步骤中。

[0558] 步骤 3：在 0℃下，将 DIPEA（2.5 当量）添加到步骤 2 产物（0.14mmol）、HATU（1.1 当量）和氨基甲基硼酸频哪醇酯盐酸盐（1.1 当量）或硼 -Gly-(+)- 蒎烷二醇盐酸盐（1.1 当量）在 DMF（1mL）中的搅拌悬浮液，接着缓慢升温至室温，并搅拌过夜。然后添加半饱和 $NaHCO_3$ 水溶液和 EtOAc，且在超声波浴中搅动混合物 1h。通过抽吸过滤分离固体产物并在高真空下干燥。

[0559] 一般顺序 2：

[0560]



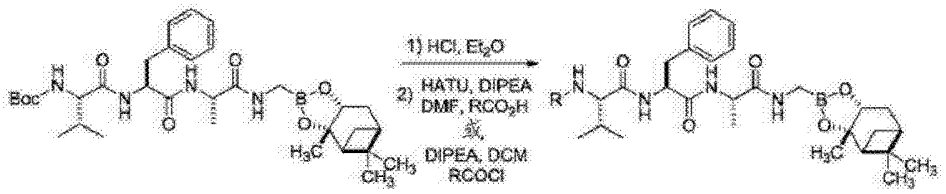
[0561] 步骤 1：在 0℃下，历时 10 分钟，将 DIPEA(38mmol，3.5 当量）滴加到中间体 1(11mmol)、L- 缬氨酸衍生物 (12mmol，1.1 当量）和 HATU(13mmol，1.2 当量）在 DMF(35mL) 中的溶液，接着缓慢升温至室温，并搅拌过夜。然后将混合物倒入碎冰 (100mL)、饱和 $NaHCO_3$ 水溶液 (100mL) 和 Et_2O(200mL) 的混合物中，并搅拌 30 分钟直到冰融化。通过抽吸过滤收集固体产物，用水和 1 ： 1Et_2O/ 己烷洗涤，并在高真空下干燥。

[0562] 步骤 2：在 0℃下，将步骤 1 产物 (3.33mmol) 悬浮于 THF(20mL) 和 MeOH(10mL) 的混合物中并用 1M LiOH(4.3mL，4.3mmol，1.3 当量）处理，接着缓慢升温至室温，并搅拌过夜。然后将混合物重新冷却至 0℃，用 1M HCl 酸化至 pH 4，并用 EtOAc(3x) 萃取。干燥 ($MgSO_4$) 合并的有机物，过滤和浓缩且残余物直接用于下一步骤中。

[0563] 步骤 3：在 0℃下，将 DIPEA(2.0mmol，2.5 当量）滴加到步骤 2 产物 (0.90mmol)、硼 -Gly-(+)- 蒎烷二醇盐酸盐 (1.1mmol，1.2 当量）和 HATU(1.03mmol，1.1 当量）在 DMF(4.5mL) 中的搅拌悬浮液，接着缓慢升温至室温。搅拌过夜后，用半饱和 $NaHCO_3$ 水溶液稀释混合物并用 EtOAc(3x) 萃取。用半饱和盐水 (2x) 洗涤合并的有机物，干燥 ($MgSO_4$)，过滤并在减压下浓缩。用 Et_2O 搅拌残余物并通过过滤收集固体物质且在高真空下干燥，得到最终产物。

[0564] 一般顺序 3

[0565]



[0566] 步骤 1：在 0℃下，用 2M HCl 的 Et_2O 溶液 (20mmol，40 当量）处理具有 R ＝ Boc 的一般顺序 2 的最终产物 (0.51mmol)，在此温度下搅拌 18h，接着在减压下浓缩。残余物直接用于下一步骤中。

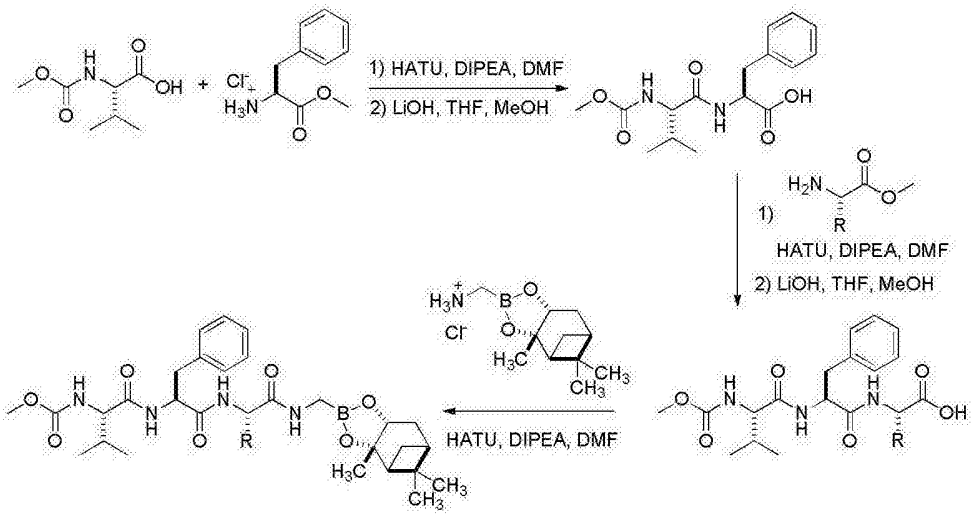
[0567] 步骤 2（对于 RCO_2H）：在 0℃下，将 DIPEA(0.27mmol，3.0 当量）滴加至步骤 1 产物 (0.089mmol)、HATU(0.11mmol，1.2 当量）和适当的羧酸 (1.1 当量）在 DMF(0.89mL) 中的

搅拌悬浮液，接着缓慢升温至室温。搅拌过夜后，添加 EtOAc 和半饱和 $NaHCO_3$ 水溶液并通过抽吸过滤收集固体产物，用水洗涤和在高真空下干燥。

[0568] 步骤 2（对于 RCOCl）：在 0℃下，将 DIPEA(0.13mmol，3.0 当量）添加到步骤 1 产物 (0.042mmol) 在 CH_2Cl_2(0.4mL) 中的搅拌悬浮液，接着逐份引入适当的酰氯 (0.064mmol，1.5 当量）。使反应缓慢升温至室温并搅拌过夜。此后，用 EtOAc 和半饱和 $NaHCO_3$ 稀释反应，并分离层。用 EtOAc(2x) 萃取水层且用半饱和盐水 (2x) 洗涤合并的有机层，干燥 ($MgSO_4$) 和在减压下浓缩。用 Et_2O 磨碎残余物且通过过滤分离固体产物并在高真空下干燥。

[0569] 一般顺序 4

[0570]



[0571] 步骤 1：在 0 ° 下，将 HATU(11.9g，31.4mmol，1.1 当量）和 DIPEA(14.9mL，85.5mmol，3.0 当量）添加到 (S)-2-((甲氧基羰基）氨基)-3- 甲基丁酸 (5.00g，28.5mmol) 和 L- 苯基丙氨酸甲酯盐酸盐 (6.15g，28.5mmol，1.0 当量）在 DMF(100mL) 中的溶液，接着缓慢升温至室温，搅拌过夜。随后用 EtOAc 稀释混合物，并用 10％ HCl 水溶液、饱和 $NaHCO_3$ 水溶液和盐水洗涤。分离有机层，经 $MgSO_4$ 干燥，过滤并在减压下浓缩且残余物是直接用于下一步骤中。

[0572] 步骤 2：在 0℃下，将 1M LiOH 水溶液 (60mL) 添加至步骤 1 产物 (9.58g，28.5mmol) 在 THF(120mL) 和 MeOH(60mL) 中的溶液。在室温下搅拌 3h 后，用 1M HCl 将混合物酸化至 pH 2-3，并用 EtOAc 萃取。分离有机层，用盐水洗涤，经 $MgSO_4$ 干燥，过滤并在减压下浓缩。用 EtOAc 和己烷的混合物磨碎残余物且通过抽吸过滤收集固体产物并在高真空下干燥。

[0573] 步骤 3：在 0℃下，将 HATU(1.12mmol，1.2 当量）和 DIPEA(3.7mmol，4.0 当量）依序添加至步骤 2 产物 (0.93mmol) 和适当的保护的氨基酸 (0.93mmol) 在 DMF(5mL) 中的搅拌混合物，接着缓慢升温至室温，并在此温度下搅拌 3h。然后，将混合物分配在 EtOAc 和 10％ HCl 水溶液之间。分离有机相，并用饱和 $NaHCO_3$ 和盐水洗涤，经 $MgSO_4$ 干燥，过滤和在减压下浓缩。残余物是通过在硅胶上用渐增比例的 MeOH 的 CH_2Cl_2 溶液洗脱的快速色谱纯化。

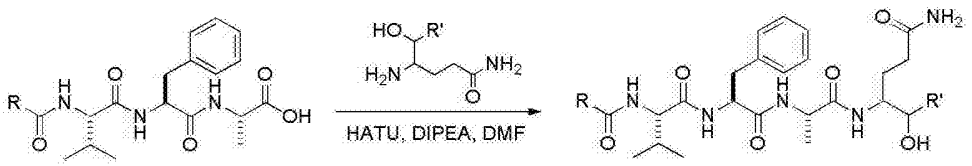
[0574] 步骤 4：在 0℃下，用 1M LiOH 水溶液 (0.95mmol，2.0 当量）处理步骤 3 产物 (0.47mmol) 在 THF(2mL) 和 MeOH(1mL) 中的搅拌悬浮液，缓慢升温至室温并搅拌 3h。然后用 1M HCl 将混合物酸化至 pH 2-3 并用 EtOAc 萃取。分离有机层，用盐水洗涤，经 $MgSO_4$ 干燥，过滤并在减压下浓缩。用 EtOAc 和 Et_2O 的混合物磨碎残余物并通过抽吸过滤分离固体

产物且在高真空下干燥。

[0575] 步骤 5：在 0℃下，用 HATU(0.13mmol，1.2 当量）和 DIPEA(0.44mmol，4.0 当量）处理步骤 4 产物 (0.11mmol) 和硼 -Gly-(+)- 蒎烷二醇盐酸盐 (0.12mmol，1.1 当量）在 DMF(1mL) 中的搅拌悬浮液，接着缓慢升温至室温并搅拌过夜。随后，将反应容器内含物分配在 EtOAc 和半饱和 $NaHCO_3$ 水溶液之间。分离 EtOAc 层，并用另外的 EtOAc(2x) 萃取水层。合并的有机层经 $MgSO_4$ 干燥，过滤并在减压下浓缩。用水磨碎残余物和通过抽吸过滤分离固体产物并在高真空下干燥。

[0576] 一般顺序 5

[0577]



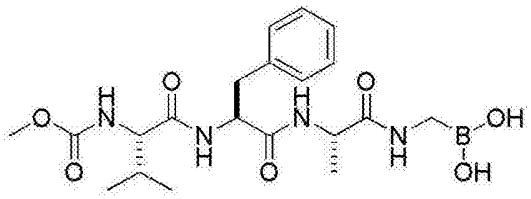
[0578] 在 0℃下，将 DIPEA(3.5 当量）滴加至适当的羧酸（中间体 12、13 或 14）、适当的胺（中间体 8、9、10 或 11，1.2-1.4 当量）和 HATU(1.2-1.3 当量）在 DMF(0.1M) 中的搅拌悬浮液，接着缓慢升温至室温并搅拌过夜。然后用 EtOAc 稀释反应混合物，并用冰冷的半饱和 $NaHCO_3$ 水溶液搅拌。通过过滤分离固体酰胺产物，用 EtOAc 和水洗涤，接着在抽吸和高真空下干燥。当产物可溶于 EtOAc 中时，分离层并用另外的 EtOAc 萃取水相。用 5% LiCl 水溶液和饱和盐水洗涤合并的有机相，然后经 $MgSO_4$ 干燥，过滤并在减压下浓缩。用 Et_2O 磨碎残余物并通过过滤收集固体产物且在高真空下干燥。

[0579] 实例 1 至 24 中所述的化合物上通过实例编号识别。因此，实例 1 中所公开的化合物被指定为化合物 1。

[0580] 实例 1(1)

[0581] {[(2S)-2-[(2S)-2-[(2S)-2-[（甲氧基羰基）氨基]-3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 甲基 } 硼酸

[0582]



[0583] 在 0 ℃下，将 2,4,6- 可力丁 (0.14mL，1.1mmol，2.1 当量）滴加到中间体 2(0.20g，0.51mmol)、HATU(0.194g，0.510mmol) 和氨基甲基硼酸频哪醇酯盐酸盐 (0.099g，0.51mmol) 在 DMF(2.9mL) 中的悬浮液中，同时缓慢升温到室温并搅拌过夜。随后，将容器内含物分配在半饱和的 $NaHCO_3$ 水溶液和 EtOAc 之间。分离有机层，并用 EtOAc(2x) 萃取水相。将合并的有机物干燥 ($MgSO_4$)，过滤并在减压下浓缩。用 EtOAc/Et_2O/ 水的混合物磨碎残余物，并在超声波浴中搅拌 1h。通过抽吸过滤收集固体材料并在高真空下干燥，得到标题化合物。

[0584] ^{1}HNMR（甲 醇 -d4）：δ 7.31-7.18(5H)，4.63(1H)，4.50(1H)，3.80(1H，d)，3.64(3H)，3.20(1H)，2.94(1H)，2.34(2H)，1.92(1H)，1.42(3H)，0.83-0.75(6H)。MS ESI：

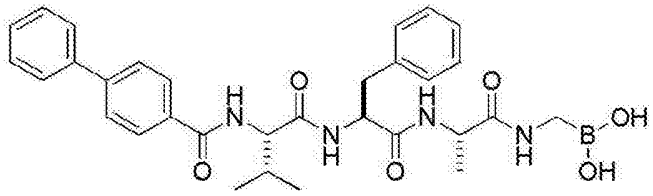
473.3[M+Na]$^+$

[0585] 使用一般顺序 1 制备实例 2-5：

[0586] 实例 2(2)

[0587] {[(2S)-2-[(2S)-2-[(2S)-3- 甲基 -2-[(4- 苯基苯基）甲酰胺基]丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 甲基 } 硼酸

[0588]

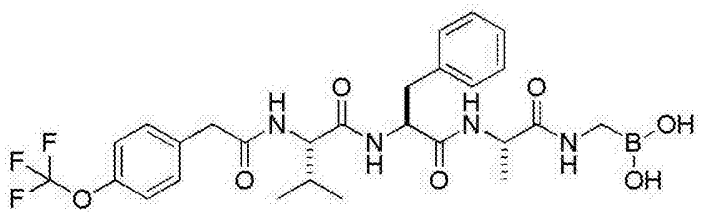


[0589] ^{1}H NMR(甲 醇 -d4)：δ 7.89(2H),7.73(2H),7.68(2H),7.47(2H),7.38(1H),7.28-7.22(2H),7.21-7.13(2H),7.09(1H),4.69(1H),4.39(1H),4.32(1H),3.23(1H),2.93(1H),2.35-2.22(2H),2.13(1H),1.41-1.31(3H),1.00-0.88(6H)。MS ESI：595.4[M+Na]$^+$

[0590] 实例 3(3)

[0591] {[(2S)-2-[(2S)-2-[(2S)-3- 甲基 -2-{2-[4-（三氟甲氧基）苯基] 乙酰胺基 } 丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 甲基 } 硼酸

[0592]

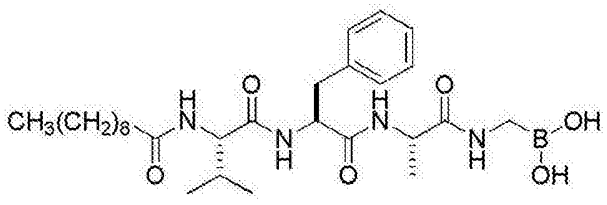


[0593] ^{1}H NMR(DMSO-d_6)：δ 8.26(1H),8.16(1H),8.20-8.12(2H)7.38-7.32(2H),7.30-7.12(7H),4.53(1H),4.30(1H),4.10(1H),3.55(1H),3.45(1H),3.04(1H),2.77(1H),2.27(1H),1.89(1H),1.22-1.18(3H),0.74-0.72(6H)。MS ESI：617.3[M+Na]$^+$

[0594] 实例 4(4)

[0595] {[(2S)-2-[(2S)-2-[(2S)-2- 癸酰胺基 -3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 甲基 } 硼酸

[0596]



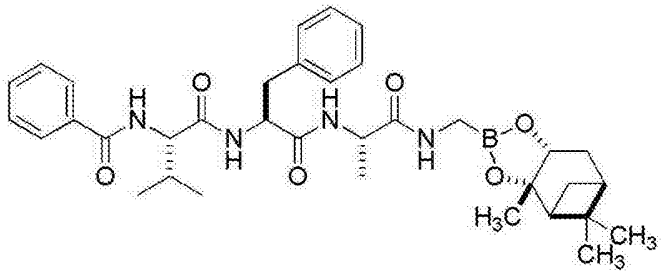
[0597] ^{1}H NMR(甲醇 -d4)：δ 7.29-7.16(5H),4.59(1H),4.48(1H),4.04(1H),3.15(1H),2.94(1H),2.33(2H),2.26-2.14(2H),1.95(1H),1.56(2H),1.40(3H),1.35-1.20(12H),0.91-0.81(9H)。MS ESI：569.4[M+Na]$^+$

[0598] 实例 5(5)

[0599] (2S)-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S,-2,9,9- 三甲

基-3,5-二氧杂-4-硼杂三环[6.1.1.$0^{2,6}$]癸-4-基]甲基}氨基甲酰基)乙基]氨基甲酰基}乙基]-2-(苯基甲酰胺基)丁酰胺

[0600]



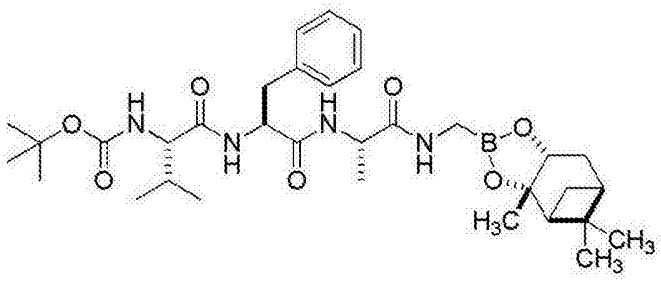
[0601] ^{1}H NMR(DMSO-d_6):δ 8.22-8.16(2H),8.11-8.03(2H),7.84(2H),7.55(1H),7.50-7.45(2H),7.26-7.09(5H),4.57(1H),4.29(1H),4.20(2H),3.05(1H),2.77(1H),2.37(2H),2.22(1H),2.10-1.99(2H),1.86(1H),1.80(1H),1.67(1H),1.28-1.17(10H),0.83(3H),0.79(3H),0.75(3H)。MS ESI:653.5[M+Na]$^+$

[0602] 使用一般顺序2制备实例6和7:

[0603] 实例6(6)

[0604] N-[(1S)-2-甲基-1-{[(1S)-2-苯基-1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9-三甲基-3,5-二氧杂-4-硼杂三环[6.1.1.$0^{2,6}$]癸-4-基]甲基}氨基甲酰基)乙基]氨基甲酰基}乙基]氨基甲酰基}丙基]氨基甲酸叔丁酯

[0605]

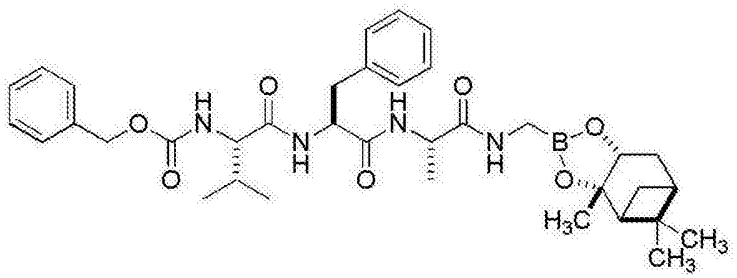


[0606] ^{1}H NMR(DMSO-d_6):δ 8.26(1H),8.08(1H),7.87(1H),7.19-7.16(5H),6.64(1H),4.58(1H),4.29(1H),4.19(1H),3.68(1H),3.02(1H),2.74(1H),2.38(2H),2.22(1H),2.06(1H),1.87(1H),1.84-1.73(2H),1.68(1H),1.36(9H),1.31-1.14(10H),0.79(3H),0.73-0.61(6H)。MS ESI:627.5[M+H]$^+$

[0607] 实例7(7)

[0608] N-[(1S)-2-甲基-1-{[(1S)-2-苯基-1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9-三甲基-3,5-二氧杂-4-硼杂三环[6.1.1.$0^{2,6}$]癸-4-基]甲基}氨基甲酰基)乙基]氨基甲酰基}乙基]氨基甲酰基}丙基]氨基甲酸苄酯

[0609]



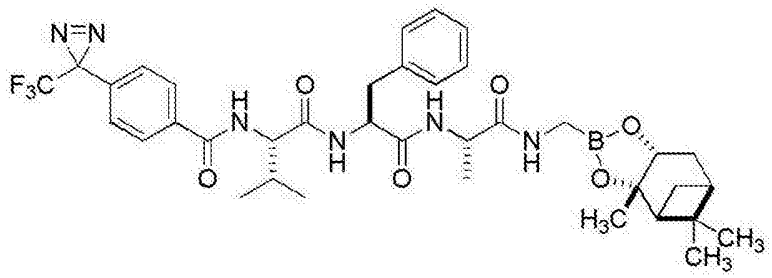
[0610] ^{1}H NMR(DMSO-d6)：δ 8.21(1H)，8.09(1H)，7.98(1H)，7.40-7.29(5H)，7.27-7.12(6H)，5.02(2H)，4.56(1H)，4.29(1H)，4.19(1H)，3.80(1H)，3.04(1H)，2.76(1H)，2.38(2H)，2.22(1H)，2.06(1H)，1.89-1.76(3H)，1.68(1H)，1.30-1.15(10H)，0.79(3H)，0.72(6H)。MS ESI：683.4[M+Na]$^+$

[0611] 使用一般顺序 3 制备实例 8a、8b、8c、8d 和 9：

[0612] 实例 8a(8a)

[0613] (2S)-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.0^{2,6}] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基]-2-({4-[3-(三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 } 甲酰胺基) 丁酰胺

[0614]

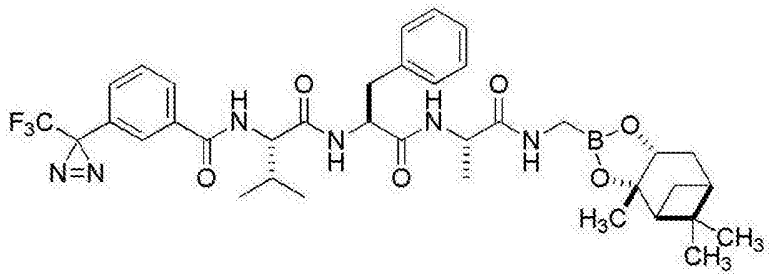


[0615] ^{1}H NMR(DMSO-d$_6$)：δ 8.36(1H)，8.16(1H)，8.10-8.04(2H)，7.93(2H)，7.38(2H)，7.24-7.05(5H)，4.55(1H)，4.32-4.14(3H)，3.03(1H)，2.76(1H)，2.36(2H)，2.20(1H)，2.08-1.96(2H)，1.86(1H)，1.78(1H)，1.66(1H)，1.29-1.13(10H)，0.84-0.72(9H)。MS ESI：739.4[M+H]$^+$

[0616] 实例 8b(8b)

[0617] (2S)-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.0^{2,6}] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基]-2-({3-[3-(三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 } 甲酰胺基) 丁酰胺

[0618]



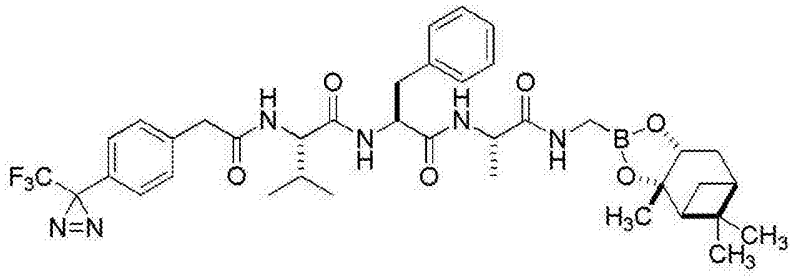
[0619] ^{1}H NMR(DMSO-d$_6$)：δ 8.46(1H)，8.16-8.05(3H)，8.00(1H)，7.65-7.60(2H)，7.55(1H)，7.24-7.19(2H)，7.17-7.05(3H)，4.54(1H)，4.31-4.15(3H)，3.04(1H)，

2.76(1H),2.39-2.34(2H),2.20(1H),2.09-1.97(2H),1.86(1H),1.79(1H),1.67(1H),1.30-1.11(10H),0.86-0.74(9H)。MS ESI:739.4[M+H]$^+$

[0620] 实例 8c(8c)

[0621] (2S)-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.0^{2,6}] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基]-2-(2-{4-[3-(三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 } 乙酰胺基) 丁酰胺

[0622]

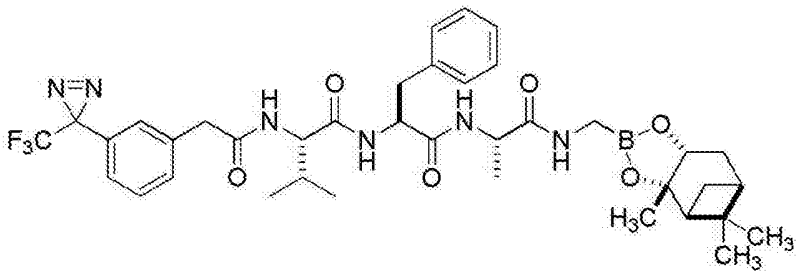


[0623] ^{1}H NMR(DMSO-d_6):δ 8.14-8.04(3H),7.39-7.33(2H,)7.24-7.10(8H),4.51(1H),4.28(1H),4.18(1H)4.09(1H),3.60-3.44(2H),3.01(1H),2.75(1H),2.38-2.34(2H),2.21(1H),2.05(1H),1.92-1.83(2H),1.79(1H),1.66(1H),1.29-1.09(10H),0.81-0.65(9H)。MS ESI:753.5[M+H]$^+$

[0624] 实例 8d(8d)

[0625] (2S)-3- 甲基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.0^{2,6}] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基]-2-(2-{3-[3-(三氟甲基)-3H- 双吖丙啶 -3- 基] 苯基 } 乙酰胺基) 丁酰胺

[0626]

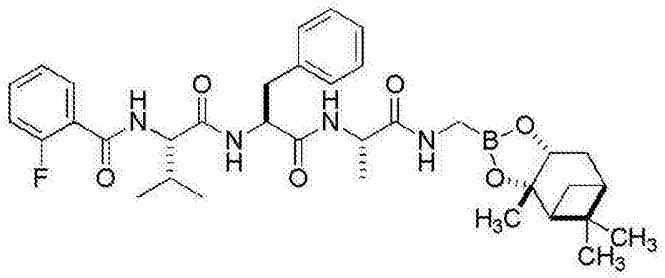


[0627] ^{1}H NMR(DMSO-d_6):δ 8.15-8.03(3H),7.44-7.36(2H),7.23-7.09(8H),4.56-4.42(2H),4.30-4.07(2H),3.63-3.39(2H),3.02(1H),2.76(1H),2.38-2.33(2H),2.20(1H),2.04(1H),1.93-1.82(2H),1.78(1H),1.66(1H),1.28-1.09(10H),0.80-0.75(3H),0.75-0.67(6H)。MS ESI:753.4[M+H]$^+$

[0628] 实例 9(9)

[0629] (2S)-2-[(2- 氟 苯 基) 甲 酰 胺 基]-3- 甲 基 -N-[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.0^{2,6}] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基] 丁酰胺

[0630]



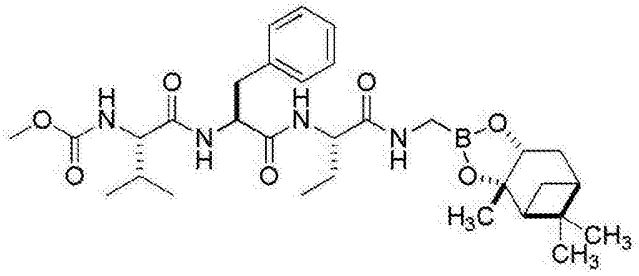
[0631] ^{1}H NMR(DMSO-d_6)：δ 8.25(1H)，8.14-8.08(2H)，8.03(1H)，7.64-7.51(2H)，7.33-7.11(7H)，4.60(1H)，4.30(2H)，4.18(1H)，3.05(1H)，2.75(1H)，2.38(2H)，2.20(1H)，2.10-1.93(2H)，1.87(1H)，1.79(1H)，1.67(1H)，1.29-1.18(10H)，0.82-0.74(9H)。MS ESI：649.4[M+H]$^+$

[0632] 使用一般顺序 4 制备实例 10-14：

[0633] 实例 10(10)

[0634] N-[(1S)-2- 甲基 -1-{[(1S)-2- 苯基 -1-{[(1S)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 丙基] 氨基甲酰基 } 乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯

[0635]

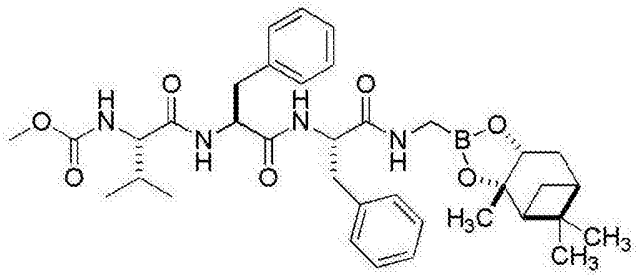


[0636] ^{1}H NMR(DMSO-d_6)：δ 8.11-8.04(2H)，7.98(1H)，7.27-7.21(4H)，7.17(1H)，7.05(1H)，4.58(1H)，4.22-4.14(2H)，3.77(1H)，3.52(3H)，3.02(1H)，2.78(1H)，2.37(2H)，2.22(1H)，2.06(1H)，1.89-1.77(3H)，1.71-1.61(2H)，1.52(1H)，1.31-1.24(4H)，1.23-1.20(3H)，0.85-0.77(6H)，0.74-0.69(6H)。MS ESI：599.4[M+H]$^+$

[0637] 实例 11(11)

[0638] N-[(1S)-2- 甲 基 -1-{[(1S)-2- 苯 基 -1-{[(1S)-2- 苯 基 -1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.$0^{2,6}$] 癸 -4- 基] 甲基 } 氨基甲酰基) 乙基] 氨基甲酰基 } 乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯

[0639]



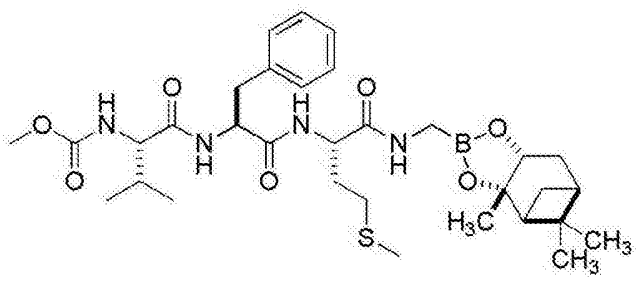
[0640] ^{1}H NMR(DMSO-d_6)：δ 8.23(1H)，8.09(1H)，7.88(1H)，7.29-7.12(10H)，7.03(1H)，4.59-4.45(2H)，4.24(1H)，3.76(1H)，3.53(3H)，3.00-2.68(4H)，2.39(2H)，2.24(1H)，

2.09(1H),1.90(1H),1.86-1.78(2H),1.71(1H),1.34-1.26(4H),1.25-1.20(3H),0.81(3H),0.77-0.62(6H)。MS ESI:661.5[M+H]$^+$

[0641] 实例 12(12)

[0642] N-[(1S)-2- 甲 基 -1-{[(1S)-1-{[(1S)-3-(甲 基 磺 酰 基)-1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.0^{2,6}] 癸 -4- 基] 甲基 } 氨基甲酰基) 丙基] 氨基甲酰基 }-2- 苯基乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯

[0643]

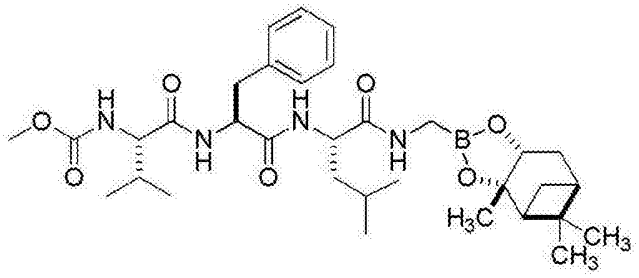


[0644] ^{1}H NMR(DMSO-d$_6$):δ 8.13(1H),8.03(1H),7.95(1H),7.27-7.15(5H),7.06(1H),4.54(1H),4.34(1H),4.22(1H),3.77(1H),3.52(3H),3.02(1H),2.79(1H),2.47-2.32(4H),2.22(1H),2.06(1H),2.01(3H),1.93-1.74(5H),1.69(1H),1.30-1.21(7H),0.79(3H),0.72(6H)。MS ESI:645.4[M+H]$^+$

[0645] 实例 13(13)

[0646] N-[(1S)-2- 甲基 -1-{[(1S)-1-{[(1S)-3- 甲基 -1-({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.0^{2,6}] 癸 -4- 基] 甲基 } 氨基甲酰基) 丁基] 氨基甲酰基 }-2- 苯基乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯

[0647]

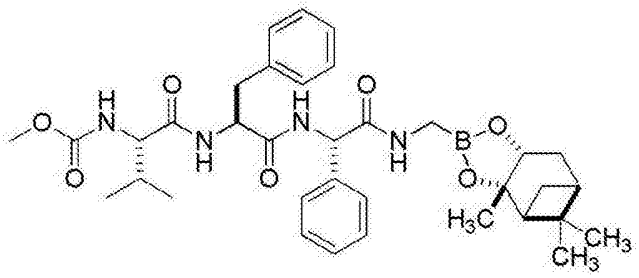


[0648] ^{1}H NMR(DMSO-d$_6$):δ 8.12(1H),8.07(1H,s),8.00(1H),7.26-7.14(5H),7.04(1H),4.56(1H),4.31(1H),4.18(1H),3.79(1H),3.52(3H),3.01(1H),2.78(1H),2.30-2.36(2H),2.21(1H),2.05(1H),1.89-1.76(3H),1.68(1H),1.56(1H),1.48-1.39(2H),1.30-1.24(4H),1.22(3H),0.86(3H),0.85(3H),0.76(3H),0.72(6H)。MS ESI:627.5[M+H]$^+$

[0649] 实例 14(14)

[0650] N-[(1S)-2- 甲 基 -1-{[(1S)-2- 苯 基 -1-{[(S)- 苯 基 ({[(1S,2S,6R,8S)-2,9,9- 三甲基 -3,5- 二氧杂 -4- 硼杂三环 [6.1.1.0^{2,6}] 癸 -4- 基] 甲基 } 氨基甲酰基) 甲基] 氨基甲酰基 } 乙基] 氨基甲酰基 } 丙基] 氨基甲酸甲酯

[0651]

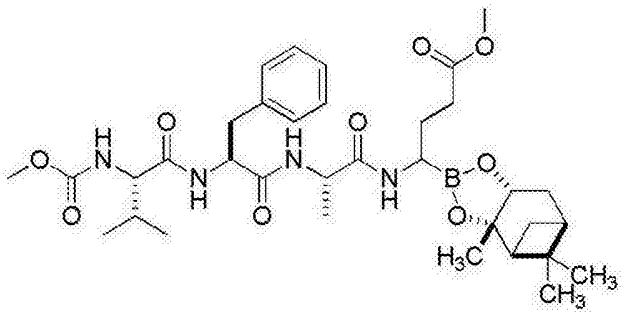


[0652] ^{1}H NMR(DMSO-d_6)：δ 8.66(0.5H)，8.53(0.5H)，8.44(0.5H)，8.32(0.5H)，8.05(0.5H H)，7.97(0.5H)，7.92(1H)，7.42-7.00(10H)，5.47(1H)，4.68(1H)，4.27(1H)，3.77(1H)，3.52(3H)，2.95(1H)，2.76(1H)，2.58-2.42(2H overlapped)，2.23(1H)，2.07(1H)，1.94-1.77(3H)，1.65(1H)，1.32-1.19(7H)，0.79(3H)，0.75-0.63(6H)。MS ESI：647.4[M+H]$^+$

[0653] 实例 15(15)

[0654] (4RS)-4-[(2S)-2-[(2S)-2-[(2S)-2-[(甲氧基羰基)氨基]-3-甲基丁酰胺基]-3-苯基丙酰胺基]-2-苯基乙酰胺基]-4-[(1S,2S,6R,8S)-2,9,9-三甲基-3,5-二氧杂-4-硼杂三环[6.1.1.0^{2,6}]癸-4-基]丁酸甲酯

[0655]



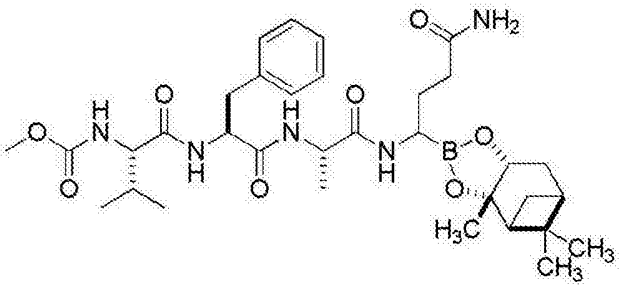
[0656] 将中间体 5(0.027g,0.084mmol)，中间体 3(0.062g,0.13mmol,1.5 当量)和氧化铂(0.002g,0.008mmol,0.1 当量)置于 rbf 中并用隔膜密封。净化(真空,N_2)烧瓶并添加脱气的 THF(2.5mL)。然后在经由乳胶气球提供的 H_2氛围(P = 1atm)下搅拌混合物。18h 后，添加 EtOAc 和 $NaHCO_3$饱和水溶液。分离层并用另外的 EtOAc(2x)萃取水层。合并的有机层经 $MgSO_4$干燥，过滤并在减压下浓缩。残余物是通过在硅胶上用渐增比例的 EtOAc 的己烷溶液洗脱的快速色谱纯化，得到呈非对映体混合物的标题化合物。

[0657] ^{1}H NMR(丙酮-d_6)：δ 7.89(0.5H)，7.77(0.5H)，7.61-7.51(2H)，7.31-7.17(5H)，6.52(0.5H)，6.48(0.5H)，4.58(1H)，4.45(1H)，4.17(1H)，3.87(1H)，3.61-3.55(6H)，3.21(2H)，2.97(1H)，2.68(1H)，2.56-2.32(2H)，2.31-2.17(2H)，1.94-1.69(5H)，1.44(1H)，1.34-1.23(9H)，0.86-0.81(9H)。MS ESI：671.5[M+H]$^+$

[0658] 实例 16(16)

[0659] N-[(1S)-1-{[(1R/S)-1-{[(1S)-1-{[(1S)-3-氨基甲酰基-1-[(1S,2S,6R,8S)-2,9,9-三甲基-3,5-二氧杂-4-硼杂三环[6.1.1.0^{2,6}]癸-4-基]丙基]氨基甲酰基}乙基]氨基甲酰基}-2-苯基乙基]氨基甲酰基}-2-甲基丙基]氨基甲酸甲酯

[0660]



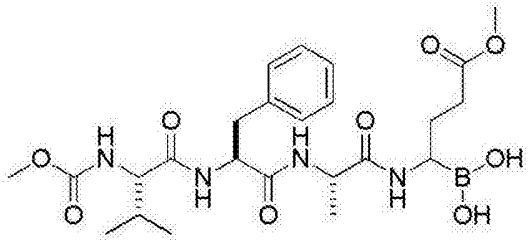
[0661] 将中间体 6(0.12g,0.39mmol)、中间体 3(0.30g,0.61mmol,1.6 当量）和氧化铂(0.009g,0.04mmol,0.1 当量）置于用隔膜密封的 rbf。净化（真空，然后 N_2）烧瓶并添加脱气的 THF(2.5mL)。然后在经由乳胶气球递送的 H_2氛围 (P = 1atm) 下搅拌混合物。18h 后，添加甲醇和硅胶到反应容器并浓缩混合物。将残余物倒入用 EtOAc 预装的硅胶管柱的顶部。在硅胶上用渐增比例的 MeOH 的 EtOAc 溶液洗脱的快速色谱，浓缩含有所需物质的溶离份，用 EtOAc 磨碎残余物，并通过抽吸过滤收集固体产物，接着在高真空下干燥，得到呈非对映体混合物的标题化合物。

[0662] ^{1}H NMR（甲醇 -d4）：δ 7.30-7.18(5H)，4.59(1H)，4.49(1H)，4.18(1H)，3.77(1H)，3.63(3H)，3.20(1H)，2.95(1H)，2.64(1H)，2.37-2.10(4H)，1.97-1.70(6H)，1.42(3H)，1.37-1.31(4H)，1.27(3H)，0.86(3H)，0.82-0.77(6H)。MS ESI：656.5$[M+H]^+$

[0663] 实例 17(17)

[0664] [(1R/S)-4- 甲氧基 -1-[(2S)-2-[(2S)-2-[(2S)-2-[（甲氧基羰基）氨基]-3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基]-4- 氧代丁基] 硼酸

[0665]



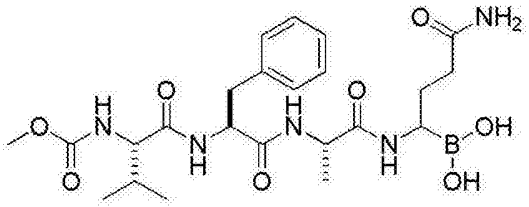
[0666] 在室温下，将实例 15(0.010g,0.015mmol) 与聚合物负载的苯硼酸 (3mmol/g 负载，0.058g) 在 1mL 的 1 ： 1 乙腈 / 水中搅拌 18h。通过过滤移除树脂，并用水和乙腈洗涤。浓缩滤液，添加另外部分的聚合物负载的苯硼酸 (3mmol/g,0.058g) 和乙腈 (5mL)，并在室温下搅拌 18h。通过过滤移除第二部分的树脂，并用水和乙腈洗涤。真空中浓缩滤液以移除乙腈，冷冻干燥残余的水溶液，用 Et_2O 磨碎残余物，得到标题化合物。

[0667] ^{1}HNMR(MeOH-d_4)：δ 7.31-7.17(5H)，4.58(1H)，4.47(1H)，3.80(1H)，3.67-3.61(6H)，3.19(1H)，2.95(1H)，2.58(1H)，2.46-2.39(2H)，1.93(1H)，1.86-1.65(2H)，1.43(3H)，0.84-0.76(6H)。MS ESI：559.3$[M+Na]^+$

[0668] 实例 18(18)

[0669] [(1R/S)-3- 氨基甲酰基 -1-[(2S)-2-[(2S)-2-[(2S)-2-[（甲氧基羰基）氨基]-3- 甲基丁酰胺基]-3- 苯基丙酰胺基] 丙酰胺基] 丙基] 硼酸

[0670]



[0671] 将实例 16 的最终产物的样品 (0.020g,0.030mmol) 悬浮于 Et_2O(2.5mL)、水 (1mL) 和 EtOAc(0.5mL) 的混合物中。添加苯基硼酸 (0.036g,0.30mmol,10 当量) 并在室温下搅拌混合物 18h。分离相,用 EtOAc(5x) 洗涤水层,且弃置收集的有机物。冷冻干燥水层,并用 EtOAc 磨碎残余物,得到标题化合物。

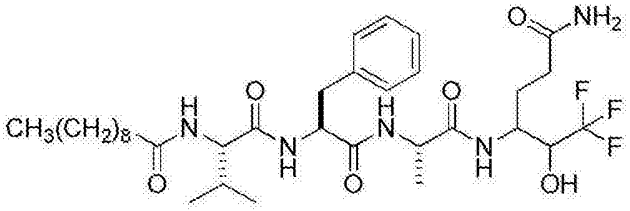
[0672] ^1H NMR(DMSO-d_6+5 % H_2O) :δ 8.08-7.96(2H),7.65(1H),7.37(1H),7.28-7.12(5H),7.04(1H),6.69(1H),4.55(1H),4.30(1H),3.75(1H),3.51(3H),2.99(1H),2.75(1H),2.56(1H),2.02-1.95(2H),1.79(1H),1.69-1.40(2H),1.20-1.12(3H),0.68(6H)。MS ESI :544.3$[M+Na]^+$

[0673] 根据一般顺序 5 制备以下实例 :

[0674] 实例 19(19)

[0675] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺

[0676]



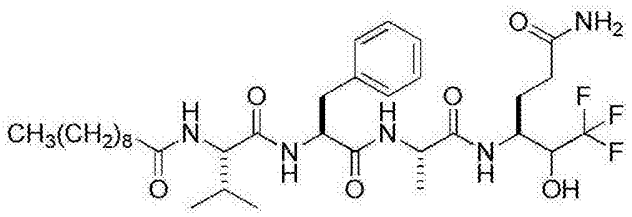
[0677] 从中间体 12(0.10g,0.20mmol)、中间体 8(57mg,0.24mmol,1.2 当量)、HATU(91mg,0.24mmol,1.2 当量) 和 DIPEA(0.12mL,0.69mmol,3.5 当量) 在 DMF(4mL) 中制备成非对映体的混合物。

[0678] ^1H NMR(DMSO-d_6) :δ 8.02-7.87(3H),7.71(1H),7.25-7.12(6H),6.71(1H),4.52(1H),4.21(1H),4.07(1H),3.97-3.77(2H),3.03(1H),2.78(1H),2.20-1.93(4H),1.93-1.81(2H),1.64(1H),1.49-1.38(2H),1.29-1.15(15H),0.85(3H),0.73(6H). ^{19}F NMR(DMSO-d_6) :δ -74.65,-74.72。ESI-MS :672.5$[M+H^+]$

[0679] 实例 20a(20a)

[0680] N-((2S)-1-(((2S)-1-(((2S)-1-(((3S)-6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺

[0681]



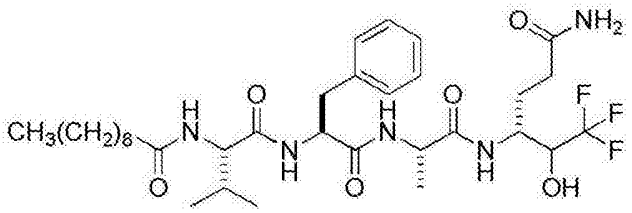
[0682] 从中间体 12(40mg,0.082mmol)、中间体 9(23mg,0.1mmol,1.2 当量)、HATU(38mg,0.1mmol,1.2 当量)和 DIPEA(50μL,0.29mmol,3.5 当量)在 DMF(2mL)中制备。通过溶解于热 EtOH 中,用活性炭处理,并通过硅藻土垫过滤,来纯化固体产物。用另外的热 EtOH 洗涤垫且在减压下浓缩滤液,用含有几滴 MeOH 的 EtOAc 磨碎后,得到呈固体的标题化合物。

[0683] ^{1}H NMR(DMSO-d_6):δ 8.01(1H),7.98(1H),7.92(1H),7.72(1H),7.25-7.12(6H),6.71(1H),6.53(1H),4.52(1H),4.21(1H),4.07(1H),3.93(1H),3.82(1H),3.03(1H),2.78(1H),2.19-1.97(4H),1.97-1.81(2H),1.60(1H),1.49-1.39(2H),1.29-1.16(12H),1.18(3H),0.85(3H),0.73(6H)。^{19}F NMR(DMSO-d_6):δ -74.65。ESI-MS:672.5[M+H$^+$].

[0684] 实例 20b(20b)

[0685] N-((2S)-1-(((2S)-1-(((2S)-1-(((3R)-6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺

[0686]

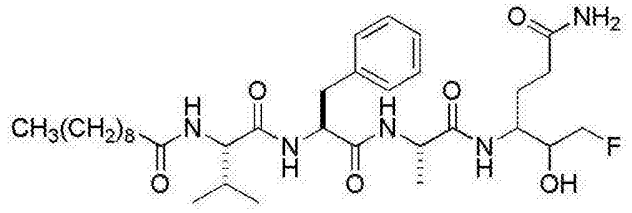


[0687] 从中间体 12 和 ent-中间体 9 针对 20a 制备。

[0688] 实例 21a(21a)、21b(21b)

[0689] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1-氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺

[0690]



[0691] 从中间体 12(100mg,0.20mmol)、中间体 10 的次要非对映异构体(56mg,0.28mmol,1.4 当量)、HATU(100mg,0.26mmol,1.3 当量)和 DIPEA(0.12mL,0.69mmol,3.5 当量)在 DMF(3mL)中制备化合物 21a。将标题化合物通过在硅胶上用渐增比例的 MeOH(5%至 10%)的 CH_2Cl_2 溶液洗脱的快速色谱分离为两种非对映体的混合物。利用中间体 10 的主要非对映异构体,以类似方式制备化合物 21b。

[0692] 化合物 b(参见图 1)为化合物 21a 和 21b 的混合物。

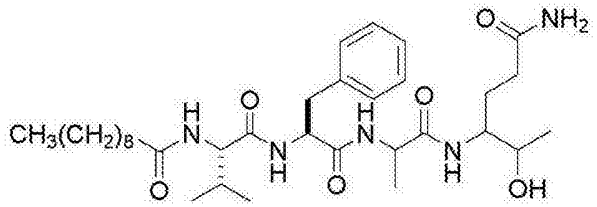
[0693] 21a :^{1}H NMR(DMSO-d_6) :δ 8.01-7.93(2H),7.74-7.62(2H),7.24-7.13(6H),6.69(1H),5.28(1H),4.52(1H, m),4.44-4.03(4H),3.66-3.46(2H),3.03(1H),2.79(1H),2.19-1.82(6H),1.59-1.38(3H),1.30-1.16(15H),0.85(3H),0.74(6H)。^{19}F NMR(DMSO-d6) :δ 3.84,3.18。ESI-MS :636.5[M+H]$^+$

[0694] 21b :^{1}H NMR(DMSO-d_6) :δ 8.04-7.95(2H),7.74-7.67(1H),7.55(05H),7.48(0.5H),7.26-7.12(6H),6.71(1H),4.52(1H),4.37-4.04(4H),3.80-3.67(2H),3.03(1H),2.78(1H),2.20-1.92(4H),1.87(1H),1.76-1.58(2H),1.50-1.40(2H),1.29-1.17(15H),0.85(3H),0.77-0.70(6H)。ESI-MS :636.5[M+H]$^+$

[0695] 实例 22a(22a) 和 22b(2b)

[0696] N-((2S)-1-(((2S)-1-((-1-((6- 氨基 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺

[0697]



[0698] 从中间体 12(100mg,0.204mmol)、中间体 11(40mg,0.27mmol,1.3 当量)、HATU(91mg,0.24mmol,1.2 当量) 和 DIPEA(0.12mL,0.69mmol,3.5 当量) 在 DMF(3mL) 中制备。两种非对映体产物可通过在硅胶上用渐增比例的 MeOH(5％至 10％) 的 CH_2Cl_2 溶液洗脱的快速色谱分离。化合物 22a 首先被洗脱 (极性较小) 非对映体且化合物 22b 上第二被洗脱 (极性较大) 非对映体。在浓缩适当的溶离份并用水和 Et_2O 磨碎残余物后,两种产物被分离为固体。

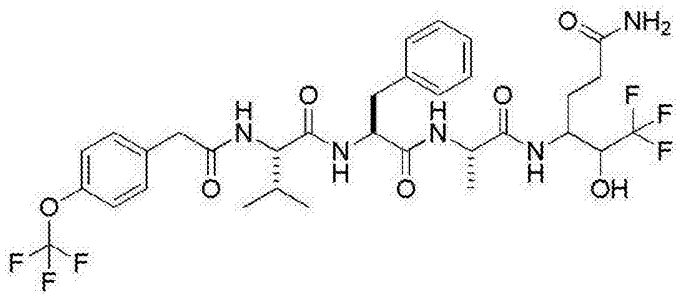
[0699] 22a :^{1}H NMR(DMSO-d_6) :δ 8.15(1H),7.96(1H),7.73(1H),7.45(1H),7.26-7.14(6H),6.69(1H),4.56(1H),4.40(1H),4.16(1H),4.10(1H),3.50-3.41(2H),2.95(1H),2.84(1H),2.20-2.02(2H),2.02-1.82(4H),1.56-1.40(3H),1.30-1.17(12H),1.07(3H),0.99(3H),0.85(3H),0.764(3H),0.748(3H)。ESI-MS :618.5[M+H]$^+$。

[0700] 22b :^{1}H NMR(DMSO-d_6) :δ 8.00(1H),7.93(1H),7.71(1H),7.52(1H),7.25-7.12(6H),6.68(1H),4.63(1H),4.53(1H),4.21(1H),4.07(1H),3.52-3.39(2H),3.03(1H),2.78(1H),2.19-1.81(6H),1.51-1.39(3H),1.29-1.18(15H),0.98(3H),0.85(3H),0.74(6H)。ESI-MS :618.5[M+H]$^+$。

[0701] 实例 23(23)

[0702] 6,6,6- 三氟 -5- 羟基 -4-((S)-2-((S)-2-((S)-3- 甲基 -2-(2-(4-(三氟甲氧基) 苯基) 乙酰胺基) 丁酰胺基)-3- 苯基丙酰胺基) 丙酰胺基) 己酰胺

[0703]



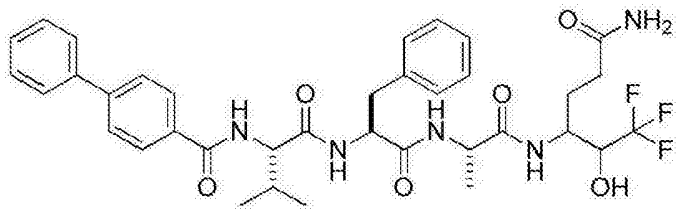
[0704] 从中间体 13(108mg,0.20mmol)、中间体 8(57mg,0.24mmol,1.2 当量)、HATU(91mg,0.24mmol,1.2 当量)、DIPEA(0.12mL,0.69mmol,3.5 当量)在 DMF(2mL)中制备。标题产物通过在硅胶上用渐增比例的 MeOH(5%至 10%)的 CH_2Cl_2溶液洗脱的快速色谱,接着用水和 Et_2O 磨碎,分离为非对映体的混合物。

[0705] ^1H NMR(DMSO-d_6):δ 8.11-8.05(2H),7.99(0.4H),7.92-7.90(1.6H),7.36(2H),7.27(2H),7.24-7.11(6H),6.72(1H),6.50(1H),4.53(1H),4.22(1H),4.10(1H),3.96-3.78(2H),3.57(1H),3.46(1H),3.02(1H),2.77(1H),2.10-1.82(4H),1.64(1H),1.20(1.8H),1.16(1.2H),0.73(6H)。^{19}F NMR(DMSO-d_6):δ -57.2(两种非对映体),-74.6(一种非对映体),-74.71(另一非对映体)。ESI-MS:720.3[M+H$^+$]。

[0706] 实例 24(24)

[0707] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0708]



[0709] 从中间体 14(70mg,0.14mmol)、中间体 8(46mg,0.18mmol,1.3 当量)、HATU(62mg,0.16mmol,1.2 当量)、DIPEA(85μL,0.49mmol,3.5 当量)在 DMF(1.4mL)中制备成非对映体的混合物。

[0710] ^1H NMR(DMSO-d_6):δ 8.27(1H),8.11-8.00(2H),7.97-7.86(3H),7.78(2H),7.74(2H),7.50(2H),7.42(1H),7.26-7.09(6H),6.71(1H),6.52(1H),4.60(1H),4.26-4.19(2H),3.98-3.77(2H),3.05(1H),2.79(1H),2.12-1.82(4H),1.63(1H),1.23(1.5H),1.19(1.5H),0.84(3H,d),0.762(1.5H),0.752(1.5H)。^{19}F NMR(DMSO-d_6):δ -74.64,-74.69。ESI-MS:698.4[M+H$^+$]。

[0711] 方案 1 至 9 和以下内文描述用于制备化合物实例 25-91 的通用实验程序。对湿气、大气中的氧和/或二氧化碳敏感的所有反应是在被预先在分子筛上干燥并通过在高真空下几个冻融循环脱气的溶剂中在无水氮气氛围下进行。在必要时对所述程序的修改是包含在其所适用的具体实例的描述中。最终化合物是分离为米色到无色粉末。除非另有说明,否则从中间体 8 制备的化合物一般分离为非对映体的 1 : 1 混合物。

[0712] 使用 HATU、DIPEA 偶联的胺到羧酸的一般程序:

[0713] 在-20℃下，将DIPEA(3.5当量）滴加到适当的胺（1.2当量）、HATU(1.2当量）和0.2M浓度的适当的羧酸在DMF中的搅拌混合物，接着缓慢升温至0℃并在室温下搅拌过夜。然后用EtOAc稀释反应容器内含物，并用冰冷的半饱和$NaHCO_3$水溶液搅拌。通过过滤分离固体酰胺产物，用水和EtOAc洗涤，并在抽吸和高真空下干燥。在产物可溶于EtOAc中的情况下，分离层并用另外的EtOAc萃取水相。用5-10％ LiCl水溶液或1：1饱和盐水/水洗涤合并的有机物，接着经$MgSO_4$干燥。过滤有机物并在减压下浓缩和用含有0-50％ EtOAc的醚磨碎残余物。通过过滤收集固体产物并在高真空下干燥。在通过磨碎纯化不足以达到＞90-95％的最终纯度的情况下，使分离的物质经历再结晶或通过在硅胶上用渐增比例的EtOAc或MeOH的CH_2Cl_2溶液洗脱的快速色谱。

[0714] 使用氯甲酸异丁酯偶联的胺到羧酸的一般程序：

[0715] (Shieh，Wen-Chung；Carlson，John A.；Shore，Michael E.；Tetrahedron Lett.1999，40，7167-70.)

[0716] 在0℃下，历时1h时间将0.35M氯甲酸异丁酯（1.1当量）的CH_2Cl_2或THF溶液缓慢添加至适当的胺盐酸盐（1.1当量）、N-甲基吗啉（2.2当量）和0.1M浓度的适当的羧酸在CH_2Cl_2或THF中的混合物。继续在此温度下搅拌另外的15分钟，然后通过添加饱和碳酸氢钠水溶液和EtOAc淬灭反应。通过抽吸过滤收集固体酰胺产物，并依序用水、EtOAc和醚洗涤。在抽吸和高真空下干燥，通常得到呈无色到米色粉末的所需化合物。通过萃取性处理程序(EtOAc(2x)，饱和$NaHCO_3$水溶液和NaCl洗涤，Na_2SO_4来干燥有机层，过滤，真空中浓缩）分离可溶性产物。在通过磨碎（通常用醚或用1：1EtOAc/醚）纯化不足以达到＞90-95％的最终纯度的情况下，使产物通过再结晶（在指定溶剂混合物中）或通过在硅胶上用渐增比例的EtOAc或MeOH的CH_2Cl_2溶液或EtOAc的己烷溶液洗脱的快速色谱纯化。

[0717] 用氢氧化锂酯皂化的一般程序：

[0718] 在0℃下，将LiOH水溶液（1M，1.5-2当量）滴加至在MeOH和THF的2：1混合物中的0.1M浓度的适当的甲酯或乙酯。在此温度下15分钟后，将反应升温至室温，并搅拌直到通过LCMS或TLC分析判断反应完全。然后添加碎冰，并用1M HCl将混合物酸化至pH 3-4。再搅拌1h后，通过抽吸过滤分离固体产物并在高真空下干燥。在这些条件下无法沉淀的产物是通过用EtOAc(2x)萃取分离，此后用盐水洗涤合并的有机物，干燥($MgSO_4$)，过滤和浓缩且残余物直接用于下一步骤中。

[0719] 用4M HCl的二噁烷溶液Boc脱保护的一般程序：

[0720] 在0℃下，将4M HCl的1，4-二噁烷溶液（10-20当量）缓慢添加到在CH_2Cl_2中的0.5M浓度的适当的Boc-保护的胺。然后在室温下搅拌所得混合物，且通过LCMS或TLC监测反应进展。当需要时，添加另外的HCl的二噁烷溶液以驱动反应完全。然后将反应混合物在减压下浓缩至干，得到所需脱保护的胺盐酸盐，其无需进一步纯化即可直接用于下一反应中。

[0721] 经由氢解的苄酯或Cbz-脱保护的一般程序：

[0722] 在真空下，在隔膜封盖的rbf中，将会达成0.1-0.2M的基质浓度的测量体积的脱氧的MeOH经由插管添加到适当的Cbz-保护的胺或苄酯和10％ Pd/C(30wt％）。然后将反应容器内含物置于经由乳胶气球的H_2氛围下，并在超声波浴中搅动反应容器2分钟。在室温下搅拌混合物，直到通过LCMS或TLC分析判断反应已完全，随后用N_2净化容器，并用等

体积的CH_2Cl_2稀释反应混合物。通过硅藻土垫过滤悬浮液，并用MeOH和CH_2Cl_2的1 ： 1混合物彻底洗涤垫。浓缩滤液并在高真空下干燥且残余物直接用于下一步骤中。

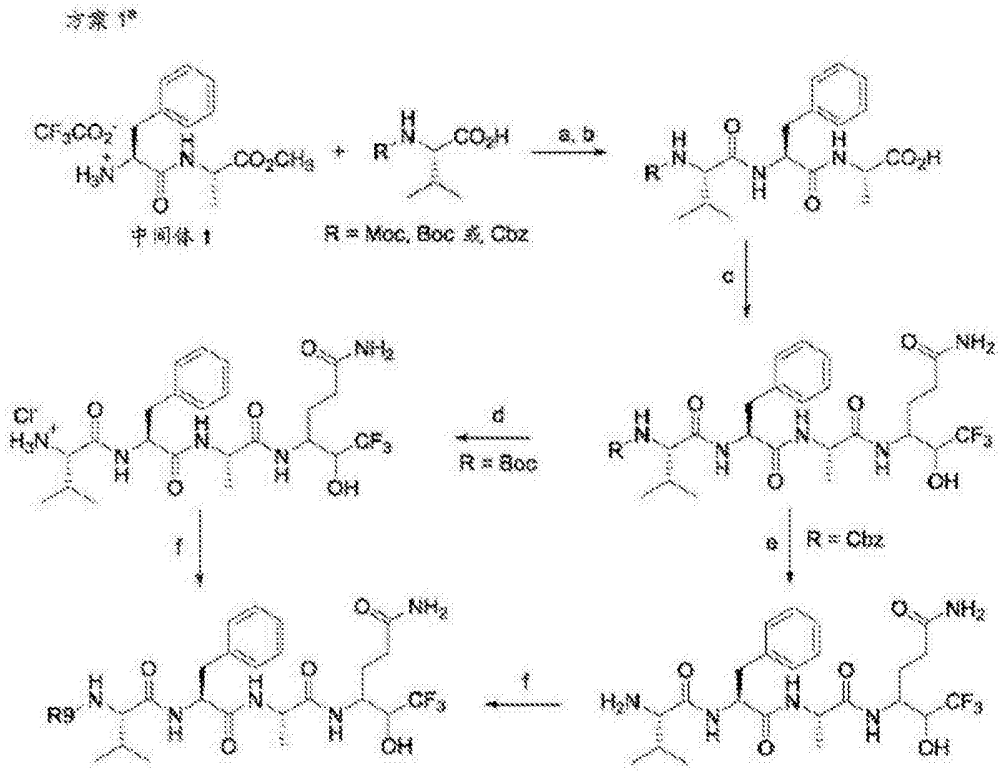
[0723] 使用酰基或磺酰氯引入R9的一般程序：

[0724] 在0℃下，将适当的酰基或磺酰氯（1.1当量）逐份添加至DIPEA(2.2当量）或Et_3N(2.2当量）和0.1-0.2M浓度的适当的胺或胺盐酸盐在CH_2Cl_2中的搅拌混合物，接着缓慢升温至室温，并搅拌直到通过LCMS或TLC分析判断反应已完全。添加另外的CH_2Cl_2和稀HCl水溶液至反应容器，并通过抽吸过滤分离固体产物，用CH_2Cl_2和水洗涤并在高真空下干燥。非固体或可溶性产物是通过用CH_2Cl_2或EtOAc的萃取性处理分离，接着用饱和$NaHCO_3$溶液和盐水洗涤合并的有机萃取物，经$MgSO_4$干燥并在减压下浓缩。用Et_2O磨碎残余物，通常产生固体产物，其通过过滤分离并在高真空下干燥。

[0725] 制备N-酰基氨基酸的一般程序：

[0726] 在室温下，将合适的酰氯（1当量）以一份式添加至含有适当的氨基酸的NaOH水溶液（1M,2当量）、THF和Et_2O的3 ： 2 ： 6混合物，接着快速搅拌过夜。酰化产物是通过用EtOAc(2x)萃取分离，接着用10％ HCl水溶液将混合物调整至pH 2-3。用盐水洗涤合并的有机物，经Na_2SO_4干燥并浓缩至干。使残余物从CH_2Cl_2/Et_2O再结晶，得到以合适纯度的所需N-酰基氨基酸，其可用于后续反应中。

[0727]



[0728] [a]试剂和条件：a)HATU、DIPEA、DMF，-20℃至室温；b)LiOH、H_2O、THF、MeOH，

[0729] 0℃至室温；c)中间体8、9或ent-9、HATU、DIPEA、DMF，-20℃至室温；d)4M HCl的二噁烷溶液或

[0730] 2M HCl的醚溶液，0℃至室温；e)H_2(1atm)、10％ Pd/C、MeOH；f)适当的酰氯或磺酰氯、

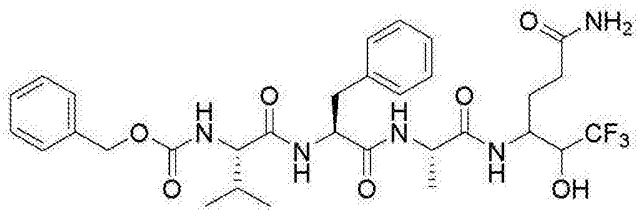
[0731] Et_3N或DIPEA、THF或DMF，0℃至室温或适当的羧酸、HATU、DIPEA，DMF，0℃至室温。

[0732] 根据方案 1 制备的化合物实例：

[0733] 实例 25

[0734] ((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 氨基甲酸苄酯

[0735]

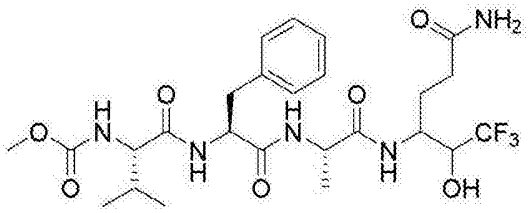


[0736] ^{1}HNMR(DMSO-d_6) ：δ 8.10(0.5H),8.00(1.5H),7.90(1H),7.40-7.08(12H),6.72(1H),6.50(1H),5.00(2H),4.55(1H),4.20(1H),3.99-2.65(3H),3.00(1H),2.72(1H),2.12-1.75(4H),1.60(1H),1.18(3H),0.70(6H) ;^{19}F NMR(DMSO-d_6) ：δ -74.65，-74.69 ;MS ESI :652.4[M+H]$^{+}$

[0737] 实例 26

[0738] ((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 氨基甲酸甲酯

[0739]

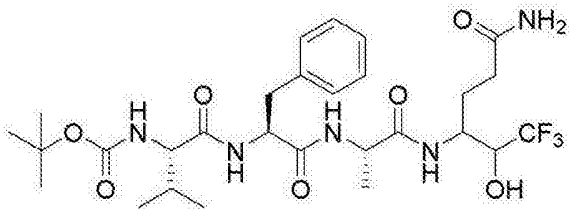


[0740] ^{1}H NMR(DMSO-d_6) ：δ 8.12(0.5H),8.05(0.5H) ;7.94(1H) ;7.63(0.5H),7.51(0.5H) ;7.35-7.12(6H),7.03(1H),6.72(1H),6.56(0.5H),6.52(0.5H),4.56(1H),4.28(1H),4.15-3.98(2H),3.73(1H),3.50(3H),3.00(1H),2.75(1H),2.04(2H),1.82(1H),1.85-1.62(2H),1.20(1.5H),1.17(1.5H),0.72(6H) ;^{19}F NMR(DMSO-d_6) ：δ -75.25，-75.37 ;MS ESI :576.3[M+H]$^{+}$

[0741] 实例 27

[0742] ((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 氨基甲酸叔丁酯

[0743]

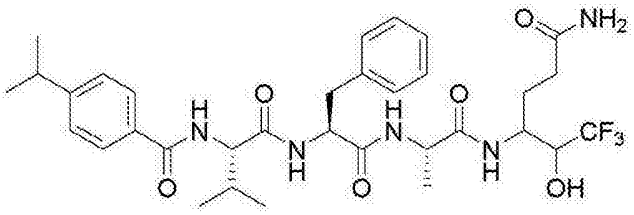


[0744] ^{1}H NMR(DMSO-d_6)：δ 8.12(0.5H)，8.04(0.5H)，7.86(2H)，7.24-7.08(6H)，6.69(1H)，6.62(1H)，6.47(1H)，4.58(1H)，4.20(1H)，3.96-3.75(2H)，3.66(1H)，3.00(1H)，2.73(1H)，2.10-1.80(3H)，1.77(1H)，1.61(1H)，1.38(9H)，1.18(3H)，0.64(6H)；^{19}F NMR(DMSO-d_6)：δ -74.66，-74.69；MS ESI：618.4$[M+H]^+$

[0745] 实例 28

[0746] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4- 异丙基苯甲酰胺

[0747]

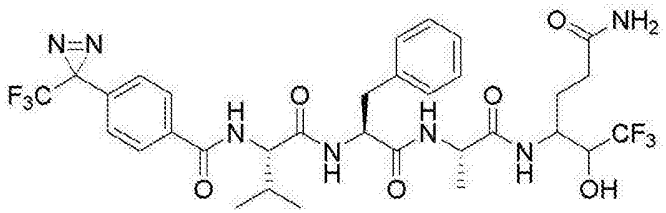


[0748] ^{1}H NMR(DMSO-d_6)：δ 8.14-7.93(3H)，7.84(1H)，7.75(2H)，7.30(2H)，7.25-7.01(6H)，6.70(1H)，6.46(1H)，4.58(1H)，4.25-4.12(2H)，3.98-3.72(2H)，3.02(1H)，2.93(1H)，2.74(1H)，2.11-1.78(4H)，1.60(1H)，1.20(9H)，0.80(3H)，0.70(3H)；^{19}F NMR(DMSO-d_6)：δ -74.65，-74.70；MS ESI：664.4$[M+H]^+$.

[0749] 实例 29

[0750] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4-(3-(三氟甲基)-3H- 双吖丙啶 -3- 基) 苯甲酰胺

[0751]

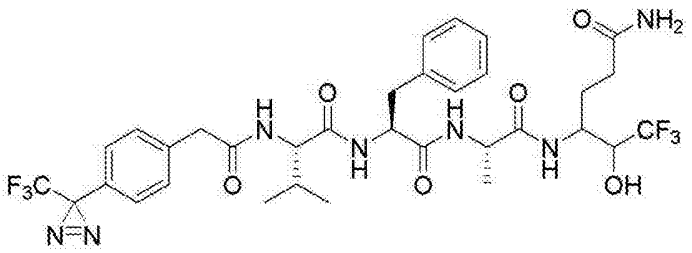


[0752] ^{1}H NMR(DMSO-d_6)：δ 8.38(1H)，8.10-7.80(5H)，7.37(2H)，7.24-7.02(6H)，6.67(1H)，6.45(1H)，4.55(1H)，4.20(2H)，3.97-3.72(2H)，3.01(1H)，2.77(1H)，2.12-1.80(4H)，1.60(1H)，1.20(3H)，0.80(3H)，0.75(3H)；^{19}F NMR(DMSO-d_6)：δ -64.90，-74.66，-74.70；MS ESI：730.4$[M+H]^+$

[0753] 实例 30

[0754] 6,6,6- 三氟 -5- 羟基 -4-((S)-2-((S)-2-((S)-3- 甲基 -2-(2-(4-(3-(三氟甲基)-3H- 双吖丙啶 -3- 基) 苯基) 乙酰胺基) 丁酰胺基)-3- 苯基丙酰胺基) 丙酰胺基) 己酰胺

[0755]

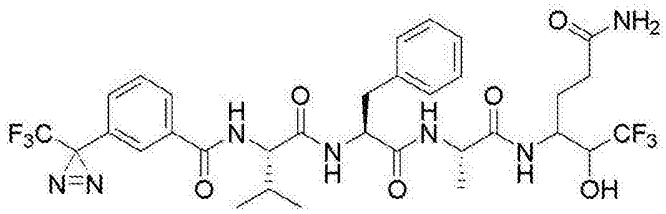


[0756] ^{1}H NMR(DMSO-d_6)：δ 8.08(2H)，7.86(2H)，7.37(2H)，7.30-7.02(8H)，6.68(1H)，6.44(1H)，4.50(1H)，4.20(1H)，4.10(1H)，3.98-3.75(2H)，3.36(1H)，3.24(1H)，3.00(1H)，2.73(1H)，2.12-1.80(4H)，1.60(1H)，1.18(3H)，0.68(6H)；MS ESI：744.4[M+H]$^+$

[0757] 实例 31

[0758] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-3-(3-(三氟甲基)-3H- 双吖丙啶 -3- 基) 苯甲酰胺

[0759]

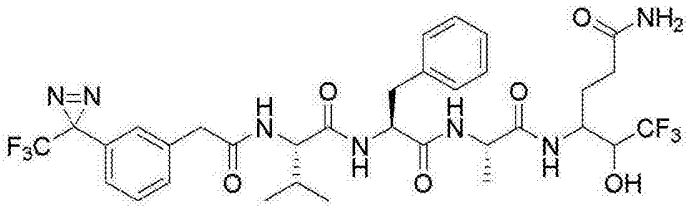


[0760] ^{1}H NMR(DMSO-d_6)：δ 8.46(1H)，8.16(1H)，8.00(1H)，7.98-7.84(2H)，7.60(2H)，7.52(1H)，7.27-7.02(6H)，6.69(1H)，6.47(1H)，4.55(1H)，4.21(2H)，3.97-3.75(2H)，3.01(1H)，2.77(1H)，2.12-1.80(4H)，1.64(1H)，1.18(3H)，0.80(6H)；MS ESI：730.3[M+H]$^+$

[0761] 实例 32

[0762] 6,6,6- 三氟 -5- 羟基 -4-((S)-2-((S)-2-((S)-3- 甲基 -2-(2-(3-(3-(三氟甲基)-3H- 双吖丙啶 -3- 基) 苯基) 乙酰胺基) 丁酰胺基)-3- 苯基丙酰胺基) 丙酰胺基) 己酰胺

[0763]

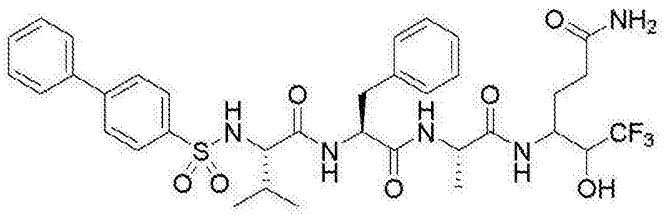


[0764] ^{1}H NMR(DMSO-d_6)：δ 8.20-8.05(2H)，8.00-7.90(2H)，7.40(2H)，7.26-7.08(8H)，6.71(1H)，6.59(1H)，4.51(1H)，4.20(1H)，4.09(1H)，3.98-3.75(2H)，3.60(1H)，3.42(1H)，3.00(1H)，2.75(1H)，2.10-1.80(4H)，1.60(1H)，1.18(3H)，0.70(6H)；MS ESI：744.4[M+H]$^+$

[0765] 实例 33

[0766] 4-((S)-2-((S)-2-((S)-2-([1,1′ - 联苯基]-4- 磺酰氨基)-3- 甲基丁酰胺基)-3- 苯基丙酰胺基) 丙酰胺基)-6,6,6- 三氟 -5- 羟基己酰胺

[0767]



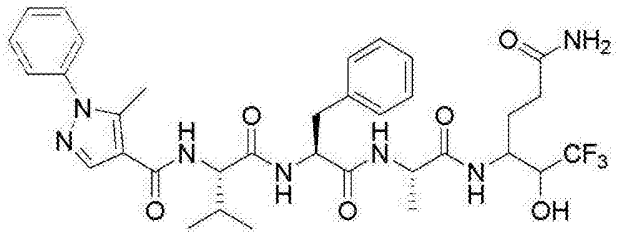
[0768] 利用9 ： 1 CH_2Cl_2 ： MeOH作为有机相进行最终步骤中的萃取处理；通过在硅胶上用2-6％ MeOH/CH_2Cl_2洗脱的快速色谱完成最终纯化。

[0769] ^1H NMR(DMSO-d_6) ：δ 8.10(1H)，7.95(1H)，7.90-7.75(2H)，7.72-7.60(6H)，7.47(2H)，7.40(1H)，7.23-7.07(6H)，6.70(1H)，6.46(1H)，4.30(1H)，4.18(1H)，3.95-3.75(2H)，3.53(1H)，2.89(1H)，2.63(1H)，2.06-1.71(4H)，1.58(1H)，1.13(3H)，0.70(6H)；^{19}F NMR(DMSO-d_6) ：δ -74.66，-74.71；MS ESI ：734.4$[M+H]^+$

[0770] 实例34

[0771] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基）氨基)-1-氧代丙-2-基）氨基)-1-氧代-3-苯基丙-2-基）氨基)-3-甲基-1-氧代丁-2-基)-5-甲基-1-苯基-1H-吡唑-4-甲酰胺

[0772]

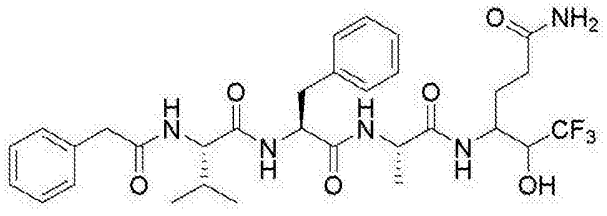


[0773] ^1H NMR(DMSO-d_6) ：δ 8.20(1H)，8.10-7.95(2H)，7.90(1H)，7.82(1H)，7.60-7.40(5H)，7.24-7.05(6H)，6.70(1H)，6.54(1H)，4.57(1H)，4.20(2H)，3.98-3.76(2H)，3.02(1H)，2.80(1H)，2.47(3H)，2.13-1.80(4H)，1.62(1H)，1.20(3H)，0.80(6H)；^{19}F NMR(DMSO-d_6) ：δ -74.64，-74.69；MS ESI ：702.4$[M+H]^+$

[0774] 实例35

[0775] 6,6,6-三氟-5-羟基-4-((S)-2-((S)-2-((S)-3-甲基-2-(2-苯基乙酰胺基）丁酰胺基)-3-苯基丙酰胺基）丙酰胺基）己酰胺

[0776]

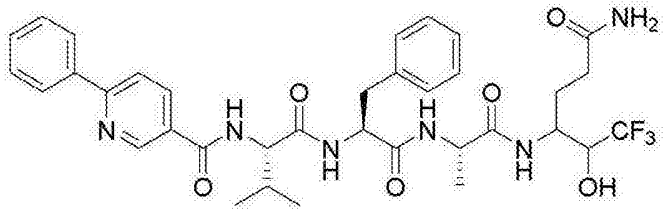


[0777] ^1H NMR(DMSO-d_6) ：δ 8.05(1H)，8.00-7.82(3H)，7.30-7.08(11H)，6.70(1H)，6.50(1H)，4.53(1H)，4.22(1H)，4.10(1H)，3.97-3.76(2H)，3.53(1H)，3.40(1H)，3.01(1H)，2.77(1H)，2.10-1.80(4H)，1.62(1H)，1.20(3H)，0.72(6H)；^{19}F NMR(DMSO-d_6) ：δ -74.64，-74.69；MS ESI ：636.4$[M+H]^+$

[0778] 实例36

[0779] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-6-苯基烟酰胺

[0780]

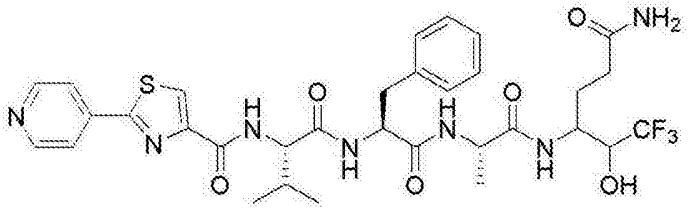


[0781] ^{1}H NMR(DMSO-d_6)：δ 9.07(1H)，8.62(1H)，8.26(1H)，8.20-7.97(5H)，7.90(1H)，7.58-7.42(3H)，7.25-7.02(6H)，6.70(1H)，6.60(1H)，4.57(1H)，4.22(2H)，3.99-3.75(2H)，3.03(1H)，2.80(1H)，2.12-1.80(4H)，1.62(1H)，1.20(3H)，0.80(6H)；MS ESI：699.4[M+H]$^+$

[0782] 实例 37

[0783] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-2-(吡啶-4-基)噻唑-4-甲酰胺

[0784]

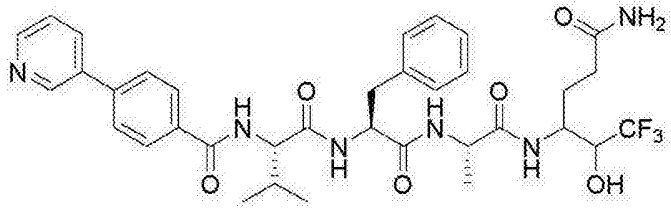


[0785] ^{1}H NMR(DMSO-d_6)：δ 8.76(2H)，8.49(1H)，8.38(1H)，8.15-7.82(5H)，7.11-7.00(6H)，6.70(1H)，6.50(1H)，4.59(1H)，4.33(1H)，4.22(1H)，3.97-3.74(2H)，3.02(1H)，2.77(1H)，2.15-1.80(4H)，1.62(1H)，1.20(3H)，0.80(6H)；^{19}F NMR(DMSO-d_6)：δ -74.64，-74.66；MS ESI：706.3[M+H]$^+$

[0786] 实例 38

[0787] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-4-(吡啶-3-基)苯甲酰胺

[0788]



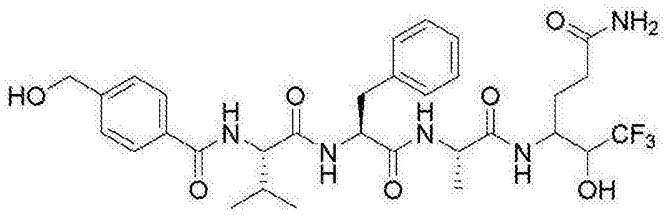
[0789] ^{1}H NMR(DMSO-d_6)：δ 8.97(1H)，8.60(1H)，8.31(1H)，8.20-7.70(8H)，7.50(1H)，7.12-7.00(6H)，6.70(1H)，6.50(1H)，4.60(1H)，4.22(2H)，4.07-3.71(2H)，3.01(1H)，2.79(1H)，2.17-1.78(4H)，1.61(1H)，1.18(3H)，0.79(6H)；^{19}F NMR(DMSO-d_6)：

δ -74.62, -74.65 ;MS ESI :699.4[M+H]$^+$

[0790] 实例 39

[0791] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4-(羟甲基) 苯甲酰胺

[0792]

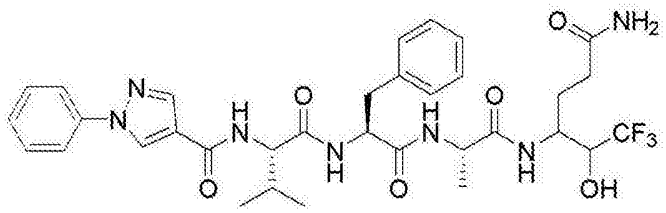


[0793] ^{1}H NMR(DMSO-d_6) : δ 8.12(1H), 8.08(1.5H), 8.0(0.5H), 7.86(1H), 7.80(2H), 7.40(2H), 7.24-7.04(6H), 6.70(1H), 6.50(1H), 5.30(1H), 4.59(1H), 4.56(2H), 4.20(2H), 3.97-3.76(2H), 3.03(1H), 2.75(1H), 2.10-1.80(4H), 1.60(1H), 1.19(3H), 0.80(3H), 0.70(3H) ;^{19}F NMR(DMSO-d_6) : δ -74.64, -74.69 ;MS ESI :652.3[M+H]$^+$

[0794] 实例 40

[0795] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-1- 苯基 -1H- 吡唑 -4- 甲酰胺

[0796]

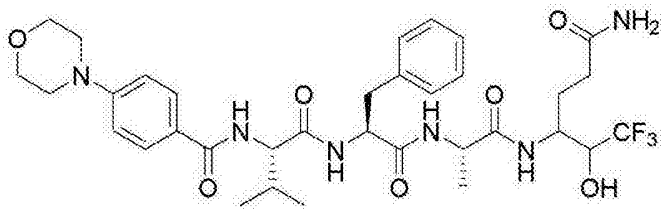


[0797] ^{1}H NMR(DMSO-d_6) : δ 9.04(1H), 8.19(1H), 8.16(1H), 8.00-7.83(3H), 7.82(2H), 7.54(2H), 7.38(1H), 7.23-7.02(6H), 6.71(1H), 6.51(1H), 4.55(1H), 4.23-4.18(2H), 3.98-3.77(2H), 3.04(1H), 2.80(1H), 2.14-1.80(4H), 1.62(1H), 1.20(3H), 0.80(6H) ;^{19}F NMR(DMSO-d_6) : δ -74.65, -74.70 ;MS ESI :688.3[M+H]$^+$

[0798] 实例 41

[0799] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4- 吗啉并苯甲酰胺

[0800]



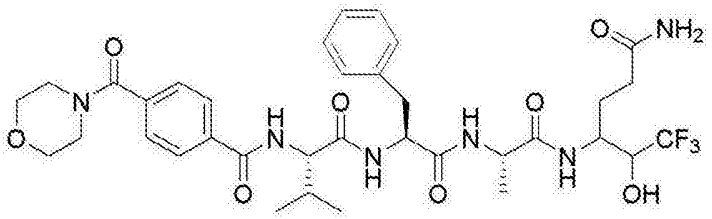
[0801] ^{1}H NMR(DMSO-d_6) : δ 8.08-7.98(2H), 7.98-7.82(2H), 7.76(2H), 7.26-7.06(6H),

6.97(2H),6.70(1H),6.55(1H),4.56(1H),4.25-4.08(2H),3.98-3.78(2H),3.72(4H),3.20(4H),3.02(1H),2.77(1H),2.14-1.80(4H),1.60(1H),1.20(3H),0.80(3H),0.70(3H);^{19}F NMR(DMSO-d_6):δ-74.62,-74.65;MS ESI:707.4[M+H]$^+$

[0802] 实例42

[0803] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-4-(吗啉-4-羰基)苯甲酰胺

[0804]

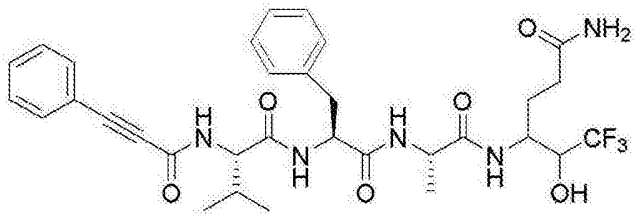


[0805] ^{1}H NMR(DMSO-d_6):δ8.31(1H),8.15-8.10(1.5H),8.01(0.5H),7.97-7.82(3H),7.50(2H),7.27-7.04(6H),6.70(1H),6.52(1H),4.60(1H),4.20(2H),4.00-3.77(2H),3.75-3.42(8H),3.03(1H),2.78(1H),2.15-1.80(4H),1.63(1H),1.20(3H),0.83(3H),0.77(3H);^{19}F NMR(DMSO-d_6):δ-74.64,-74.69;MS ESI:735.4[M+H]$^+$

[0806] 实例43

[0807] 6,6,6-三氟-5-羟基-4-((S)-2-((S)-2-((S)-3-甲基-2-(3-苯基丙酰胺基)丁酰胺基)-3-苯基丙酰胺基)丙酰胺基)己酰胺

[0808]

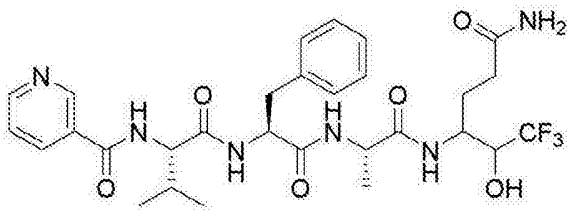


[0809] ^{1}H NMR(DMSO-d_6):δ8.74(1H),8.14(1H),8.06(0.5H),8.00(0.5H),7.92(1H),7.59(2H),7.54-7.40(3H),7.27-7.17(6H),6.70(1H),6.52(1H),4.66(1H),4.31-4.11(2H),3.98-3.72(2H),3.02(1H),2.78(1H),2.12-1.80(4H),1.62(1H),1.18(3H),0.78(6H);^{19}F NMR(DMSO-d_6):δ-74.64,-74.69;MS ESI:646.4[M+H]$^+$

[0810] 实例44

[0811] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)烟酰胺

[0812]

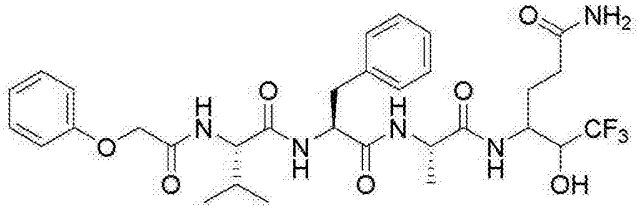


[0813] ^{1}H NMR(DMSO-d_6)：δ 8.99(1H)，8.70(1H)，8.47(1H)，8.17(1H)，8.10(1H)，8.01(0.5H)，7.98(0.5H)，7.90(1H)，7.50(1H)，7.24-7.02(6H)，6.70(1H)，6.48(1H)，4.58(1H)，4.20(2H)，3.97-3.75(2H)，3.03(1H)，2.78(1H)，2.12-1.80(4H)，1.63(1H)，1.20(3H)，0.85(3H)，0.77(3H)；^{19}F NMR(DMSO-d_6)：δ -74.62，-74.68；MS ESI：623.3$[M+H]^+$

[0814] 实例 45

[0815] 6,6,6- 三氟 -5- 羟基 -4-((S)-2-((S)-2-((S)-3- 甲基 -2-(2- 苯氧基乙酰胺基) 丁酰胺基)-3- 苯基丙酰胺基) 丙酰胺基) 己酰胺

[0816]

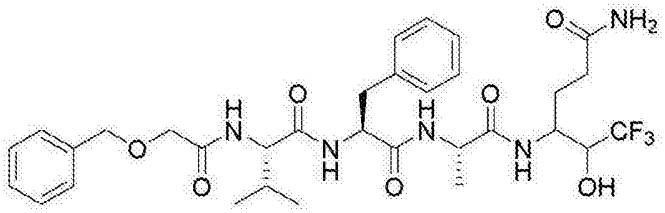


[0817] ^{1}H NMR(DMSO-d_6)：δ 8.20(1H)，8.03(0.5H)，7.98(0.5H)，7.92(1H)，7.72(1H)，7.30-7.06(8H)，6.99-6.84(3H)，6.68(1H)，6.53(1H)，4.60-4.45(3H)，4.27-4.13(2H)，3.98-3.76(2H)，3.03(1H)，2.76(1H)，2.15-1.80(4H)，1.62(1H)，1.20(3H)，0.70(6H)；^{19}F NMR(DMSO-d_6)：δ -74.60，-74.66；MS ESI：652.4$[M+H]^+$

[0818] 实例 46

[0819] 4-((6S,9S,12S)-9- 苄基 -6- 异丙基 -12- 甲基 -4,7,10- 三氧代 -1- 苯基 -2- 氧杂 -5,8,11- 三氮杂十三烷 -13- 酰胺基)-6,6,6- 三氟 -5- 羟基己酰胺

[0820]

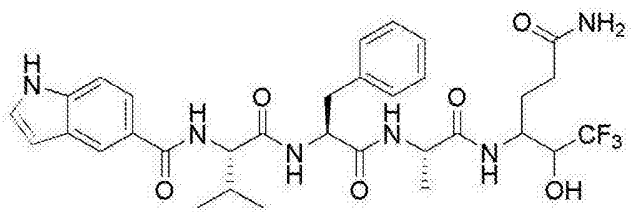


[0821] ^{1}H NMR(DMSO-d_6)：δ 8.23(1H)，8.07(0.5H)，8.00(0.5H)，7.90(1H)，7.42-7.02(12H)，6.70(1H)，6.50(1H)，4.56(1H)，4.50(2H)，4.26-4.11(2H)，3.99-3.72(4H)，3.03(1H)，2.72(1H)，2.12-1.90(4H)，1.61(1H)，1.20(3H)，0.72(6H)；^{19}F NMR(DMSO-d_6)：δ -74.64，-74.69；MS ESI：666.4$[M+H]^+$

[0822] 实例 47

[0823] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-1H- 吲哚 -5- 甲酰胺

[0824]

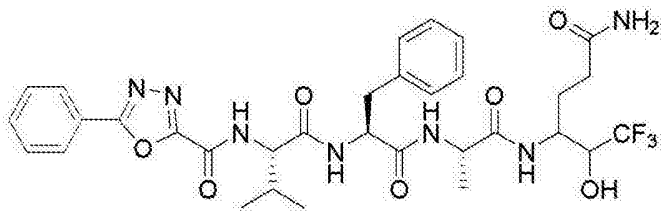


[0825] ^{1}H NMR(DMSO-d_6)：δ 11.31(1H)，8.17-7.95(4H)，7.84(1H)，7.60(1H)，7.40(2H)，7.14-7.01(6H)，6.70(1H)，6.58-6.40(2H)，4.60(1H)，4.24-4.10(2H)，3.98-3.72(2H)，3.02(1H)，2.68(1H)，2.15-1.80(4H)，1.60(1H)，1.20(3H)，0.82(3H)，0.70(3H)；^{19}F NMR(DMSO-d_6)：δ -74.62，-74.69；MS ESI：661.3$[M+H]^+$

[0826] 实例 48

[0827] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-5- 苯基 -1,3,4- 噁二唑 -2- 甲酰胺

[0828]

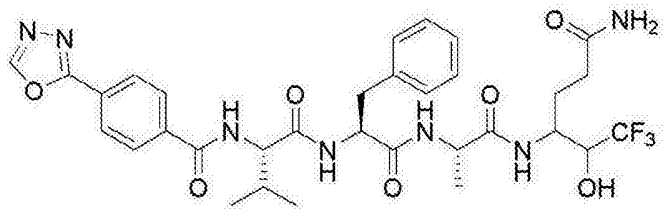


[0829] ^{1}H NMR(DMSO-d_6)：δ 8.87(1H)，8.31(1H)，8.15-8.00(3H)，7.90(1H)，7.70-7.52(3H)，7.27-6.98(6H)，6.70(1H)，6.46(1H)，4.60(1H)，4.23(2H)，3.98-3.72(2H)，3.04(1H)，2.73(1H)，2.14-1.80(4H)，1.60(1H)，1,20(3H)，0.82(3H)，0.73(3H)；^{19}F NMR(DMSO-d_6)：δ -74.67，-74.70；MS ESI：690.3$[M+H]^+$

[0830] 实例 49

[0831] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4-(1,3,4- 噁二唑 -2- 基) 苯甲酰胺

[0832]

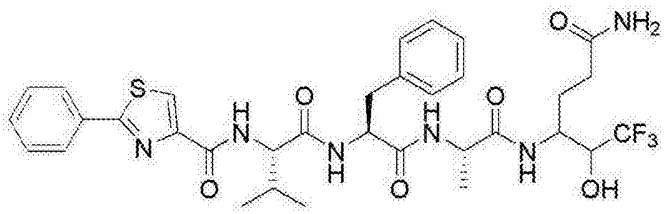


[0833] ^{1}H NMR(DMSO-d_6)：δ 9.40(1H)，8.43(1H)，8.18-7.95(5.5H)，7.96-7.80(1.5H)，7.27-7.00(6H)，6.70(1H)，6.50(1H)，4.58(1H)，4.27-4.14(2H)，3.98-3.75(2H)，3.02(1H)，2.79(1H)，2.14-1.80(4H)，1.60(1H)，1.18(3H)，0.82(3H)，0.76(3H)；^{19}F NMR(DMSO-d_6)：δ -74.63，-74.68；MS ESI：690.4$[M+H]^+$

[0834] 实例 50

[0835] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-2- 苯基噻唑 -4- 甲酰胺

[0836]

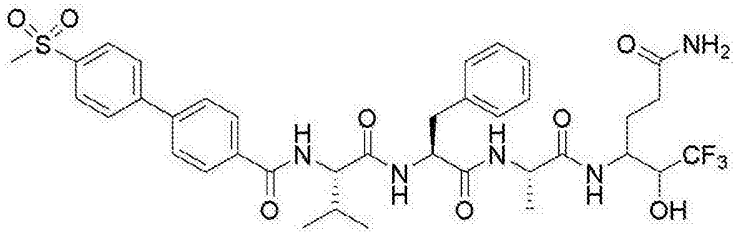


[0837] ^{1}H NMR(DMSO-d_6)：δ 8.38(1H)，8.32(1H)，8.10(0.5H)，8.05(0.5H)，8.00(3H)，7.92(1H)，7.60-7.50(3H)，7.23(2H)，7.15(3H)，7.05(1H)，6.69(1H)，6.50(1H)，4.60(1H)，4,53(1H)，4.21(1H)，3.97-3.72(2H)，3.04(1H)，2.77(1H)，2.14-1.80(4H)，1.60(1H)，1.20(3H)，0.80(6H)；^{19}F NMR(DMSO-d_6)：δ -74.64，-74.68；MS ESI：705.3[M+H]$^+$

[0838] 实例 51

[0839] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4′ -(甲基磺酰基)-[1,1′ - 联苯基]-4- 甲酰胺

[0840]

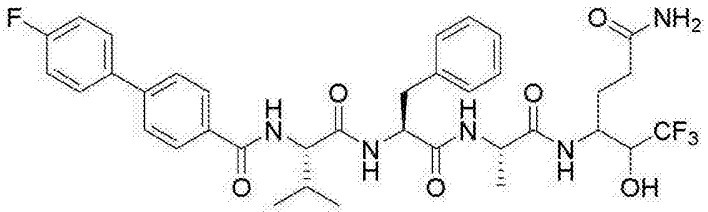


[0841] ^{1}H NMR(DMSO-d_6)：δ 8.33(1H)，8.13-7.93(8H)，7.92-7.80(3H)，7.27-7.04(6H)，6.70(1H)，6.48(1H)，4.60(1H)，4.22(2H)，3.98-3.77(2H)，3.25(3H)，3.04(1H)，2.77(1H)，2.12-1.80(4H)，1.61(1H)，1.20(3H)，0.82(3H)，0.77(3H)；^{19}F NMR(DMSO-d_6)：δ -74.64，-74.69；MS ESI：776.3[M+H]$^+$

[0842] 实例 52

[0843] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-4′ - 氟 -[1,1′ - 联苯基]-4- 甲酰胺

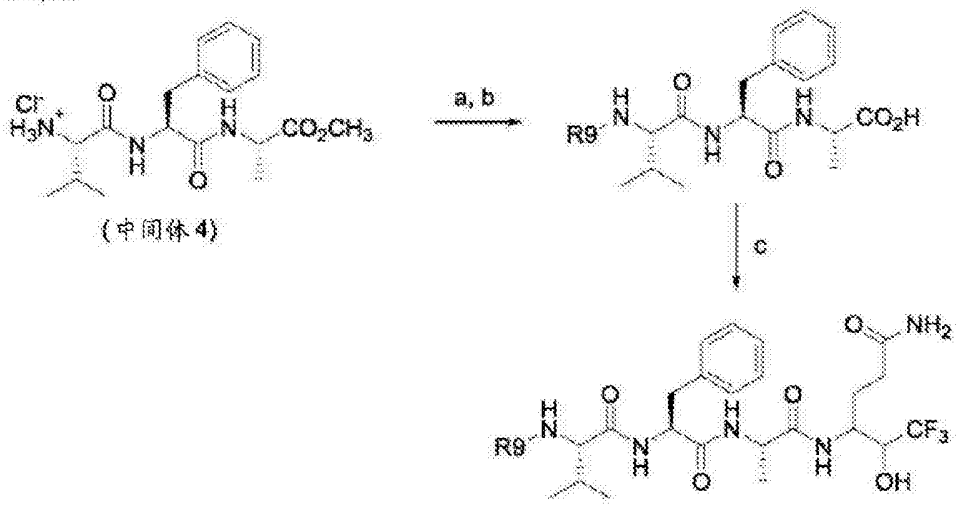
[0844]



[0845] ^{1}H NMR(DMSO-d_6)：δ 8.26(1H)，8.14-8.00(2H)，7.99-7.82(3H)，7.80-7.68(4H)，7.30(2H)，7.26-7.02(6H)，6.70(1H)，6.50(1H)，4.60(1H)，4.30-4.10(2H)，3.99-3.72(2H)，3.03(1H)，2.78(1H)，2.16-1.77(4H)，1.63(1H)，1.20(3H)，0.80(3H)，0.70(3H)；^{19}F NMR(DMSO-d_6)：δ -74.64，-74.69，-115.0；MS ESI：716.3[M+H]$^+$

[0846]

方案 2[a]



(中间体 4)

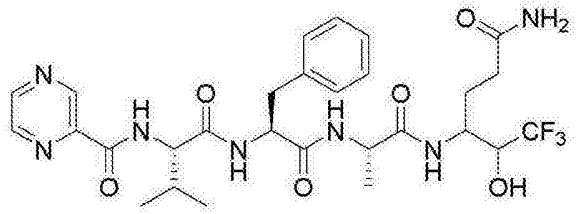
[0847] [a]试剂和条件：a) 适当的酰氯或磺酰氯、Et_3N、THF，0℃至室温或适当的羧酸、HATU、DIPEA、DMF，-20℃至室温；b)LiOH、H_2O、THF、MeOH，0℃至室温；c) 中间体 8、9 或 ent-9、HATU、DIPEA、DMF，-20℃至室温。

[0848] 根据方案 2 制备的化合物实例：

[0849] 实例 53

[0850] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 吡嗪 -2- 甲酰胺

[0851]

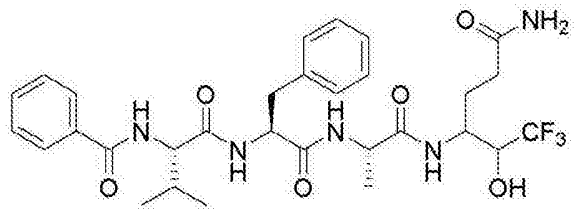


[0852] ^1H NMR(DMSO-d_6)：δ 9.19(1H)，8.90(1H)，8.73(1H)，8.45-8.35(2H)，8.13(0.5H)，8.03(0.5H)，7.90(1H)，7.27-7.10(5H)，7.05(1H)，6.70(1H)，6.50(1H)，4.60(1H)，4.35(1H)，4.20(1H)，3.98-3.72(2H)，3.02(1H)，2.71(1H)，2.14-1.80(4H)，1.60(1H)，1.20(3H)，0.77(6H)；^{19}F NMR(DMSO-d_6)：δ -74.64，-74.68；MS ESI：624.4$[M+H]^+$

[0853] 实例 54

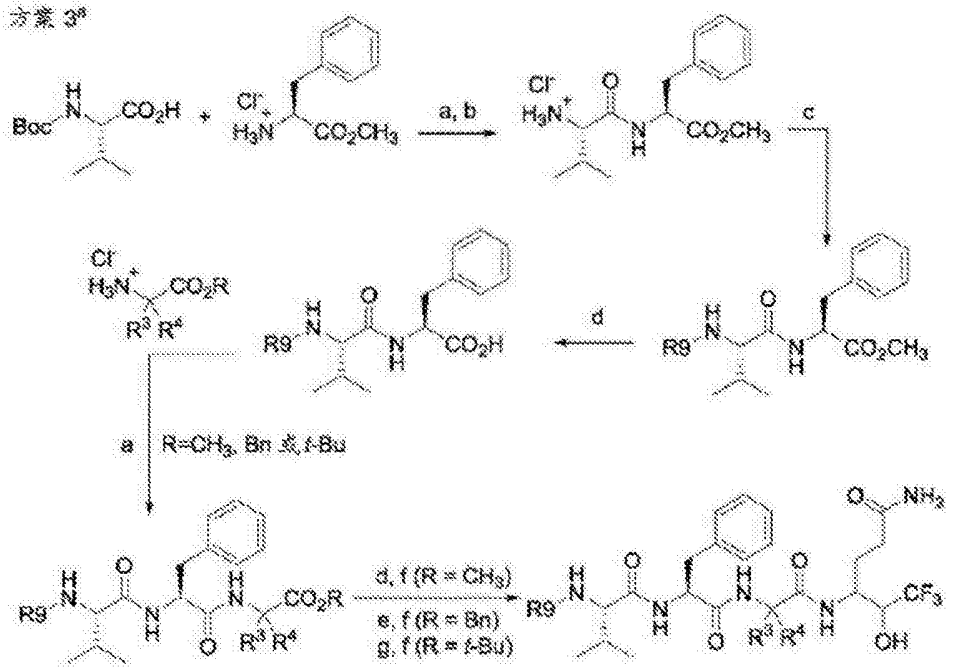
[0854] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 苯甲酰胺

[0855]



[0856] ^{1}H NMR(DMSO-d_6)：δ 8.20(1H)，8.07(1.5H)，8.00(0.5H)，7.88(1H)，7.80(2H)，7.50(1H)，7.45(2H)，7.23-7.02(6H)，6.70(1H)，6.50(1H)，4.56(1H)，4.24-4.12(2H)，3.96-3.76(2H)，3.01(1H)，2.78(1H)，2.10-1.80(4H)，1.61(1H)，1.18(3H)，0.80(3H)，0.72(3H)；^{19}F NMR(DMSO-d_6)：δ -74.65，-74.70；MS ESI：622.4[M+H]$^+$

[0857]



[0858] [a]试剂和条件：a)HATU、DIPEA、DMF，0℃至室温；b)4MHCl 的二噁烷溶液、CH_2Cl_2，0℃至室温；c) 癸酰氯或 4- 苯基苯甲酰氯、Et_3N、THF，0℃至室温；

[0859] d)LiOH、H_2O、THF、MeOH，0℃至室温；e)H_2(1atm)、10％ Pd/C、MeOH；

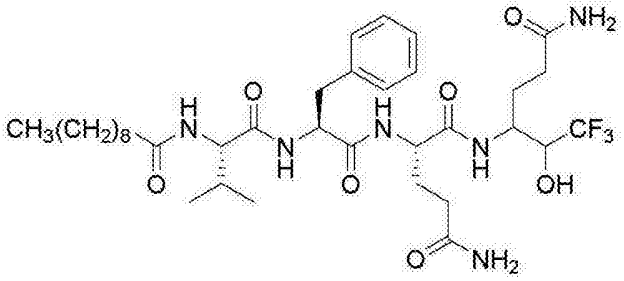
[0860] f) 中间体 8、9 或 ent-9、HATU、DIPEA、DMF，-20℃至室温；g)TFA/CH_2Cl_2(1 ： 1)，0℃至室温。

[0861] 根据方案 3 制备的化合物实例：

[0862] 实例 55

[0863] (2S)-N[1]-(6- 氨基 -1，1，1- 三氟 -2- 羟基 -6- 氧代己 -3- 基)-2-((S)-2-((S)-2- 癸酰胺基 -3- 甲基丁酰胺基)-3- 苯基丙酰胺基) 戊二酰胺

[0864]



[0865] 通过自使用活性炭脱色的乙醇 / 水再结晶来纯化化合物。

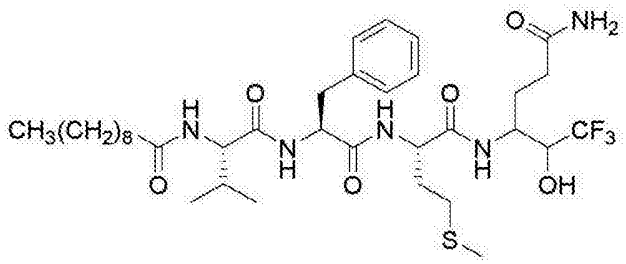
[0866] ^{1}H NMR(DMSO-d_6)：δ 8.01-7.86(3H)，7.71(1H)，7.26-7.05(7H)，6.80-6.64(2H)，6.44(1H)，4.55(1H)，4.20(1H)，4.03(1H)，3.98-3.80(2H)，3.01(1H)，2.80(1H)，2.20-1.94(6H)，1.94-1.55(5H)，1.50-1.30(2H)，1.30-1.10(12H)，0.82(3H)，0.75(6H)；^{19}F

NMR(DMSO-d_6) :δ -74.61, -74.70 ;MS ESI :729.5[M+H]$^+$

[0867] 实例 56

[0868] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-4-(甲硫基)-1- 氧代丁 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺

[0869]

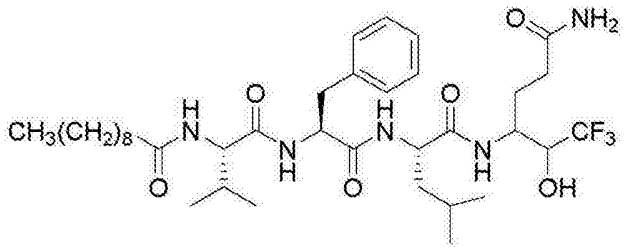


[0870] ^{1}H NMR(DMSO-d_6) :δ 8.10-7.82(3H), 7.75(1H), 7.23-7.08(6H), 6.70(1H), 6.50(1H), 4.52(1H), 4.30(1H), 4.04(1H), 3.98-3.80(2H), 3.00(1H), 2.80(1H), 2.44-2.30(2H), 2.20-1.55(9H), 2.00(3H), 1.52-1.30(2H), 1.30-1.10(12H), 0.81(3H), 0.73(6H) ;MS ESI :732.5[M+H]$^+$

[0871] 实例 57

[0872] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-4- 甲基 -1- 氧代戊烷 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺

[0873]

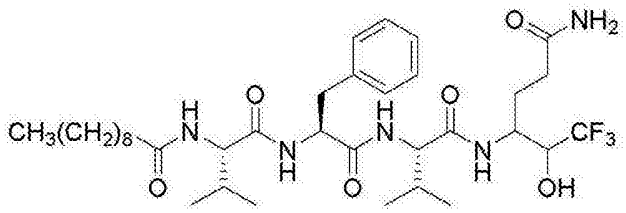


[0874] ^{1}H NMR(DMSO-d_6) :δ 7.98(1H), 7.95(0.5H), 7.83(1.5H), 7.73(1H), 7.23-7.06(6H), 6.70(1H), 6.47(1H), 4.53(1H), 4.44(1H), 4.05(1H), 3.96-3.78(2H), 3.00(1H), 2.80(1H), 2.20-1.80(6H), 1.70-1.33(6H), 1.30-1.10(12H), 0.83(9H), 0.73(6H) ;MS ESI :714.5[M+H]$^+$

[0875] 实例 58

[0876] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 癸酰胺

[0877]

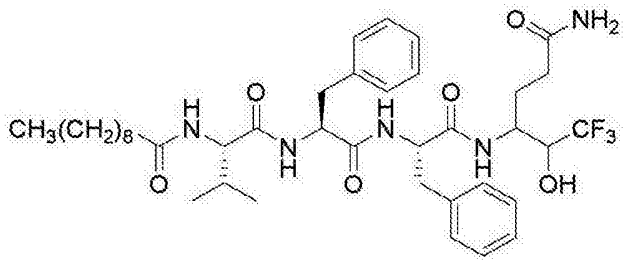


[0878] ^{1}H NMR(DMSO-d_6)：δ 8.04-7.87(2H)，7.79-7.70(2H)，7.23-7.04(6H)，6.70(1H)，6.44(1H)，4.55(1H)，4.19-4.01(2H)，3.90(1H)，3.80(1H)，2.98(1H)，2.78(1H)，2.20-1.76(7H)，1.63(1H)，1.50-1.32(2H)，1.30-1.13(12H)，0.80(9H)，0.75(6H)；^{19}F NMR(DMSO-d_6)：δ -74.61，-74.69；MS ESI：700.5[M+H]$^+$

[0879] 实例 59

[0880] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺

[0881]

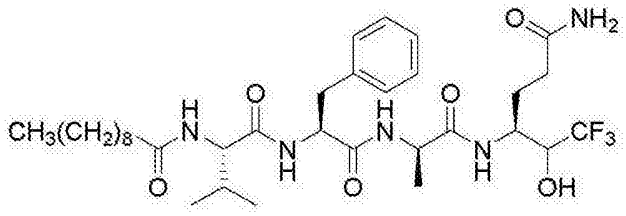


[0882] ^{1}H NMR(DMSO-d_6)：δ 8.03(1H)，8.00(1H)，7.90(1H)，7.70(1H)，7.30-7.07(11H)，6.72(1H)，6.47(1H)，4.60-4.42(2H)，4.05(1H)，4.00-3.74(2H)，3.00-2.82(2H)，2.80-2.62(2H)，2.20-1.97(3H)，1.97-1.73(3H)，1.62(1H)，1.52-1.32(2H)，1.30-1.15(12H)，0.82(3H)，0.70(6H)；MS ESI：748.5[M+H]$^+$

[0883] 实例 60

[0884] N-((2S)-1-(((2S)-1-(((2R)-1-(((3S)-6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺

[0885]



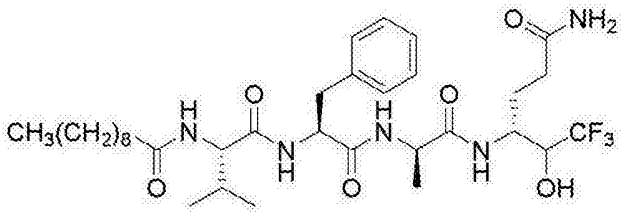
[0886] 单一非对映异构体；使用中间体 9 制备。

[0887] ^{1}H NMR(DMSO-d_6)：δ 8.07(1H)，7.95(1H)，7.78(1H)，7.70(1H)，7.25-7.10(6H)，6.70(1H)，6.40(1H)，4.43(1H)，4.17(1H)，4.09(1H)，3.96-3.79(2H)，2.94(1H)，2.81(1H)，2.20-1.80(6H)，1.68(1H)，1.51-1.38(2H)，1.30-1.14(12H)，1.05(3H)，0.83(3H)，0.74(6H)；^{19}F NMR(DMSO-d_6)：δ -74.67；MS ESI：672.5[M+H]$^+$

[0888] 实例 61

[0889] N-((2S)-1-(((2S)-1-(((2R)-1-(((3R)-6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)癸酰胺

[0890]



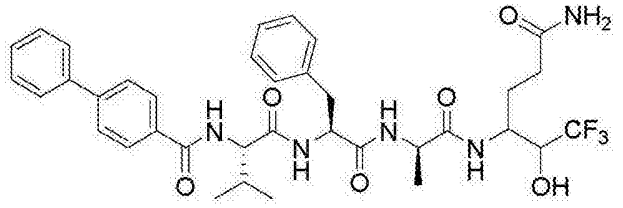
[0891] 单一非对映异构体;使用中间体ent-9制备;通过自EtOAc/EtOH缓慢再结晶纯化。

[0892] ^{1}H NMR(DMSO-d_6):δ 8.10(1H),7.97(1H),7.81(1H),7.64(1H),7.25-7.10(6H),6.66(1H),6.43(1H),4.42(1H),4.18(1H),4.10(1H),3.96-3.77(2H),2.95(1H),2.80(1H),2.20-1.80(6H),1.62(1H),1.44-1.36(2H),1.30-1.14(12H),1.02(3H),0.82(3H),0.74(6H);^{19}F NMR(DMSO-d_6):δ -74.62;MS ESI:672.5[M+H]$^{+}$

[0893] 实例62

[0894] N-((2S)-1-(((2S)-1-(((2R)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0895]

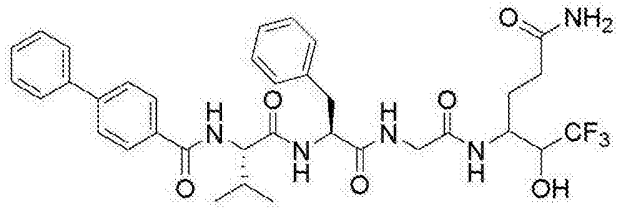


[0896] ^{1}H NMR(DMSO-d_6):δ 8.32-8.16(2H),8.03(1H),7.99-7.89(2H),7.83-7.62(5H),7.55-7.43(2H),7.40(1H),7.24-7.01(6H),6.70(1H),6.42(1H),4.50(1H),4.32-4.12(2H),3.99-3.77(2H),2.96(1H),2.81(1H),2.27-1.78(4H),1.62(1H),1.03(3H),0.81(6H);^{19}F NMR(DMSO-d_6):δ -74.66,-74.69;MS ESI:698.4[M+H]$^{+}$

[0897] 实例63

[0898] N-((2S)-1-(((2S)-1-((2-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-2-氧代乙基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0899]



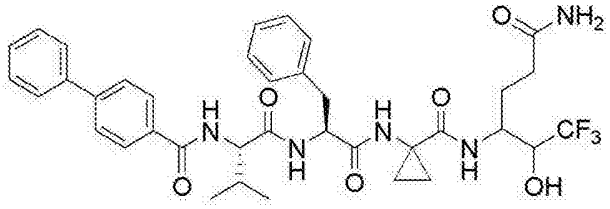
[0900] ^{1}H NMR(DMSO-d_6):δ 8.30-8.20(2H),8.10(1H),7.91(2H),7.84(1H),

7.80-7.64(4H),7.47(2H),7.40(1H),7.25-7.01(6H),6.70(1H),6.50(1H),4.60(1H),4.23(1H),4.00-3.80(2H),3.79-3.59(2H),3.01(1H),2.81(1H),2.16-1.79(4H),1.62(1H),0.83(3H),0.78(3H);^{19}F NMR(DMSO-d_6):δ-74.63,-74.68;MS ESI:684.4[M+H]$^+$

[0901] 实例64

[0902] N-((2S)-1-(((2S)-1-((1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基甲酰基)环丙基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0903]



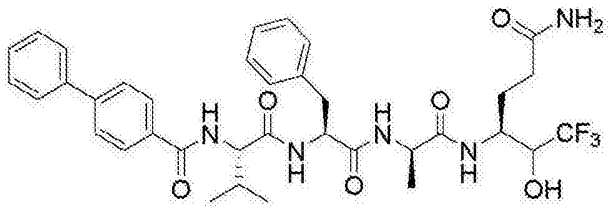
[0904] 分离为非对映异构体的2.4:1混合物。

[0905] ^{1}H NMR(DMSO-d_6):δ8.21(0.7H),8.15(0.3H),8.10(0.3H),8.00(0.7H),7.98(0.3H),7.90(0.7H),7.70-7.58(2H),7.50-7.36(4H),7.18(2H),7.10(2H),6.97-6.75(6H),6.40(0.3H),6.38(0.7H),6.00(1H),4.00(1H),3.90(1H),3.67-3.50(2H),2.70-2.50(2H),1.86-1.30(5H),0.94-0.72(1.7H),0.72-0.48(6.3H),0.30(0.7H),0.13(0.3H),0.02(1H);^{19}F NMR(DMSO-d_6):δ-74.62(次要非对映异构体),-74.84(主要非对映异构体);MS ESI:710.4[M+H]$^+$

[0906] 实例65

[0907] N-((2S)-1-(((2S)-1-(((2R)-1-(((3S)-6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0908]



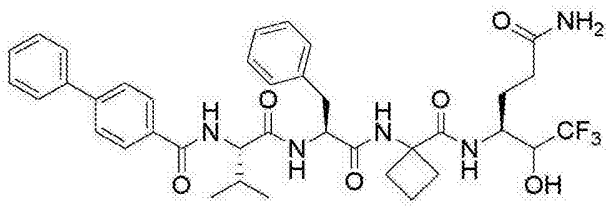
[0909] 单一非对映异构体;使用中间体9制备。

[0910] ^{1}H NMR(DMSO-d_6):δ8.30(1H),8.20(1H),8.03(1H),7.97(2H),7.81(1H),7.76-7.68(4H),7.50(2H),7.40(1H),7.24-7.05(6H),6.70(1H),6.40(1H),4.50(1H),4.33-4.16(2H),3.97-3.79(2H),2,95(1H),2.82(1H),2.10-1.78(4H),1.70(1H),1.05(3H),0.82(3H),0.78(3H);^{19}F NMR(DMSO-d_6):δ-74.66;MS ESI:698.4[M+H]$^+$

[0911] 实例66

[0912] N-((2S)-1-(((2S)-1-((1-(((3S)-6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基甲酰基)环丁基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0913]



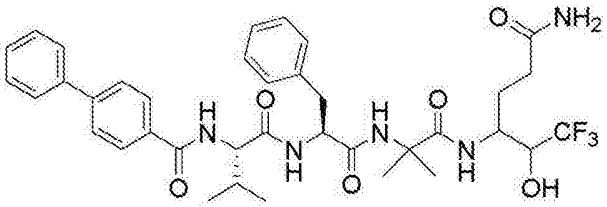
[0914] 单一非对映异构体；使用中间体 9 制备。

[0915] ^{1}H NMR(DMSO-d_6)：δ 8.40(1H)，8.35(2H)，7.97(2H)，7.73(4H)，7.50(2H)，7.40(1H)，7.30-7.12(6H)，7.06(1H)，6.70(1H)，6.36(1H)，4.43(1H)，4.23(1H)，3.92(1H)，3.81(1H)，3.06-2.88(2H)，2.52(1H)，2.24(1H)，2.13-1.53(9H)，0.90(3H)，0.84(3H)；^{19}F NMR(DMSO-d_6)：δ -74.62；MS ESI：724.4[M+H]$^+$

[0916] 实例 67

[0917] N-((2S)-1-(((2S)-1-((1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-2-甲基-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0918]



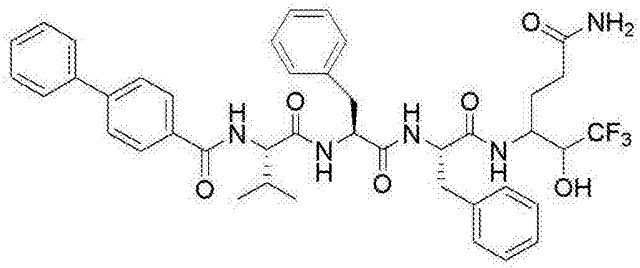
[0919] 通过在硅胶上用 0-4% MeOH 的 EtOAc 溶液洗脱的快速色谱纯化。

[0920] ^{1}H NMR(DMSO-d_6)：δ 8.31-8.18(2H)，7.99-7.88(3H)，7.80-7.64(4H)，7.47(2H)，7.42-7.27(2H)，7.25-7.00(6H)，6.70(1H)，6.36(1H)，4.46(1H)，4.24(1H)，3.98-3.80(2H)，3.01(1H)，2.82(1H)，2.18-1.80(4H)，1.62(1H)，1.32-1.20(6H)，0.95-0.75(6H)；^{19}F NMR(DMSO-d_6)：δ -74.57，-74.67；MS ESI：712.4[M+H]$^+$

[0921] 实例 68

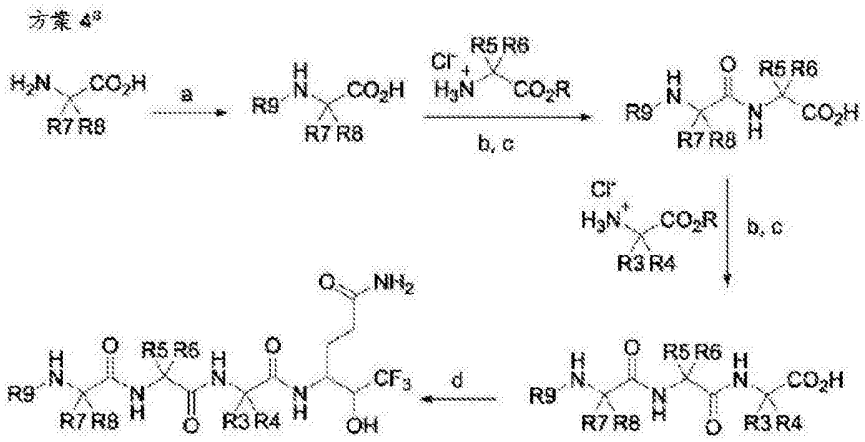
[0922] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0923]



[0924] ^{1}H NMR(DMSO-d_6)：δ 8.27(1H)，8.13(2H)，8.00(1H)，7.94(2H)，7.76(4H)，7.50(2H)，7.42(1H)，7.28-7.02(11H)，6.72(1H)，6.55(1H)，4.62-4.46(2H)，4.23(1H)，3.99-3.77(2H)，3.05-2.88(2H)，2.86-2.63(2H)，2.13-1.74(4H)，1.65(1H)，0.83(3H)，0.74(3H)；^{19}F NMR(DMSO-d_6)：δ -74.69，-74.72；MS ESI：774.4[M+H]$^+$

[0925]



[0926] [a]试剂和条件：a) 适当的酰氯、NaOH、H_2O、THF，0℃至室温；

[0927] b)N- 甲基吗啉、i-BuOCOCl、CH_2Cl_2、0℃或 HATU、DIPEA、DMF，0℃至室温；

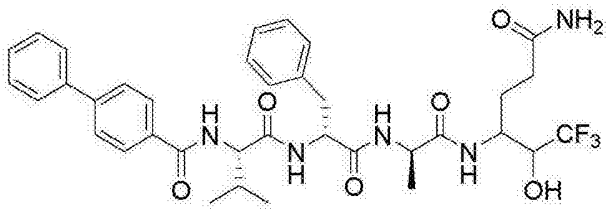
[0928] c)LiOH、H_2O、THF、MeOH，0 ℃ 至 室 温；d) 中 间 体 8、9 或 ent-9、HATU、DIPEA、DMF，-20℃至室温。

[0929] 根据方案 4 制备的化合物实例：

[0930] 实例 69

[0931] N-((2S)-1-(((2R)-1-(((2R)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺

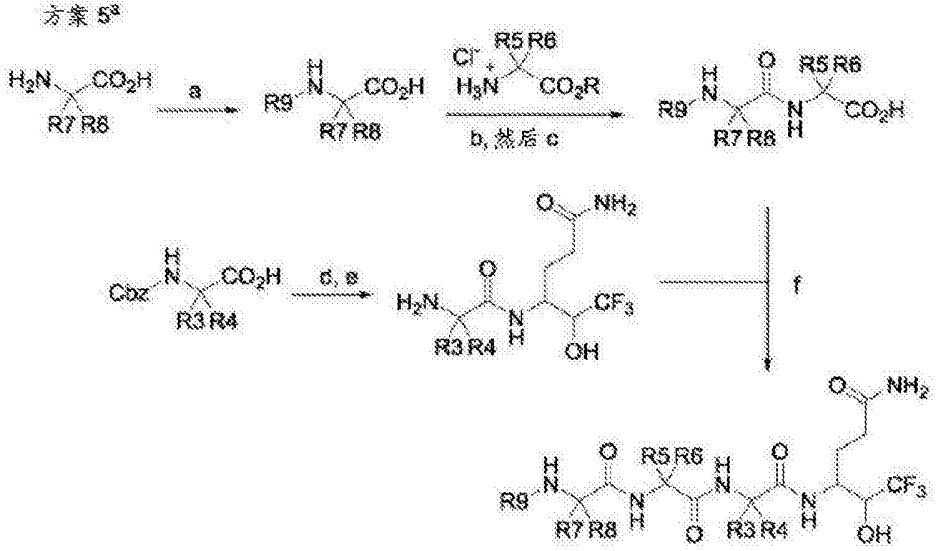
[0932]



[0933] ^1H NMR(DMSO-d_6)：δ 8.42(1H)，8.30(1H)，8.14(1H)，7.95(2H)，7.80(0.5H)，7.73(4.5H)，7.46(2H)，7.40(1H)，7.24(2H)，7.20-7.05(4H)，6.70(1H)，6.45(1H)，4.56(1H)，4.22(1H)，4.17(1H)，3.97-3.74(2H)，3.12(1H)，2.68(1H)，2.10-1.80(4H)，1.60(1H)，1.35-1.20(3H)，0.75(3H)，0.58(3H)；^{19}F NMR(DMSO-d_6)：δ -74.63，-74.75；MS ESI：698.4$[M+H]^+$

[0934]

方案 5[a]



[0935] [a]试剂和条件 :a) 适当的酰氯、NaOH、H_2O、THF,0℃至室温 ;

[0936] b)N- 甲基吗啉、i-BuOCOCl、CH_2Cl_2,0℃或 HATU、DIPEA、DMF,0℃至室温 ;

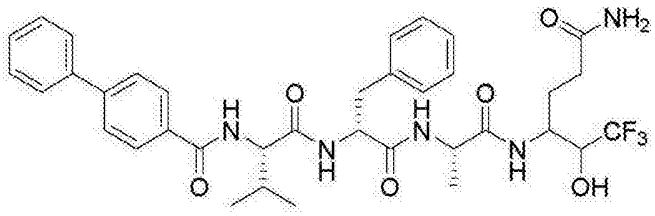
[0937] c)LiOH、H_2O、THF、MeOH,0 ℃ 至 室 温 ;d) 中 间 体 8、9 或 ent-9、HATU、DIPEA、DMF,-20℃至室温 ;e)H_2(1atm)、10% Pd/C、MeOH ;f)HATU、DIPEA、DMF,0℃至室温。

[0938] 根据方案 5 制备的化合物实例 :

[0939] 实例 70

[0940] N-((2S)-1-(((2R)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺

[0941]



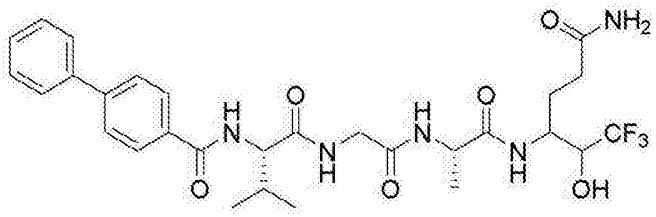
[0942] 分离为非对映异构体的 4 : 1 混合物。

[0943] ^1H NMR(DMSO-d_6) : δ 8.48(1H),8.36(1H),8.05(1H),7.97(2H),7.88(1H),7.76(4H),7.50(2H),7.40(1H),7.23(2H),7.20-7.06(4H),6.70(1H),6.53(1H),4.52(1H),4.22(1H),4.17(1H),3.96(1H),3.83(1H),3.05(1H),2.76(1H),2.18-1.82(4H),1.61(1H),1.18(3H),0.78(3H),0.57(3H) ;^{19}F NMR(DMSO-d_6) : δ -74.63 ;MS ESI :698.4$[M+H]^+$

[0944] 实例 71

[0945] N-((2S)-1-((2-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-2- 氧代乙基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺

[0946]

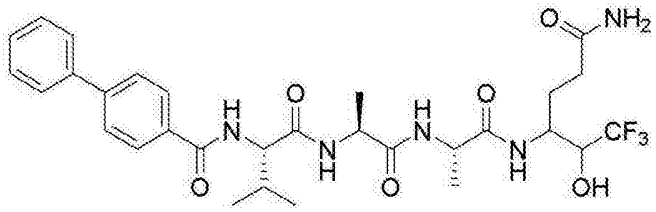


[0947] ^{1}H NMR(DMSO-d_6)：δ 8.44-8.30(2H)，8.00(2H)，7.90(2H)，7.76(4H)，7.48(2H)，7.40(1H)，7.18(1H)，6.68(1H)，6.53(1H)，4.23(2H)，3.99-3.80(2H)，3.76(2H)，2.20-1.80(4H)，1.62(1H)，1.20(3H)，0.97(6H)；^{19}F NMR(DMSO-d_6)：δ -74.64.-74.66；MS ESI：608.4$[M+H]^+$

[0948] 实例 72

[0949] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺

[0950]

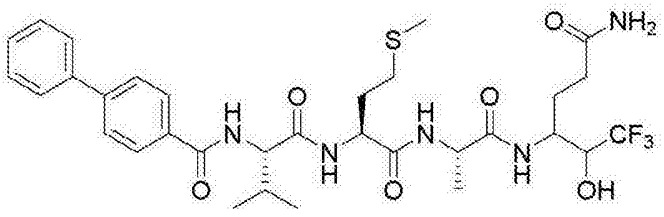


[0951] ^{1}H NMR(DMSO-d_6)：δ 8.32(1H)，8.18(1H)，8.00-7.80(4H)，7.72(4H)，7.48(2H)，7.40(1H)，7.17(1H)，6.68(1H)，6.56(1H)，4.30(2H)，4.20(1H)，3.98-3.73(2H)，2.20-1.80(4H)，1.60(1H)，1.25-1.10(6H)，0.93(6H)；^{19}F NMR(DMSO-d_6)：δ -74.53，-74.54；MS ESI：622.4$[M+H]^+$

[0952] 实例 73

[0953] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-4-(甲硫基)-1- 氧代丁 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺

[0954]



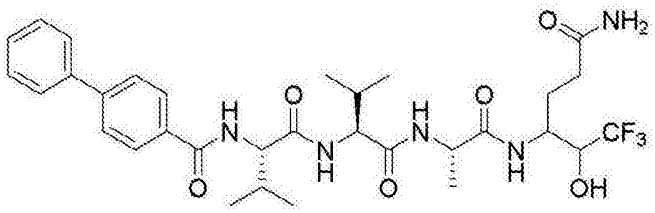
[0955] ^{1}H NMR(DMSO-d_6)：δ 8.39(1H)，8.16(1H)，8.00-7.85(4H)，7.75(4H)，7.47(2H)，7.40(1H)，7.19(0.5H)，7.11(0.5H)，6.70(1H)，4.38(1H)，4.30-4.14(2H)，3.96-3.76(2H)，2.42(2H)，2.20-1.72(6H)，2.00(3H)，1.60(1H)，1.20(3H)，0.94(6H)；^{19}F NMR(DMSO-d_6)：δ -74.63，-74.69；MS ESI：682.4$[M+H]^+$

[0956] 实例 74

[0957] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 氨基)-3- 甲

基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0958]

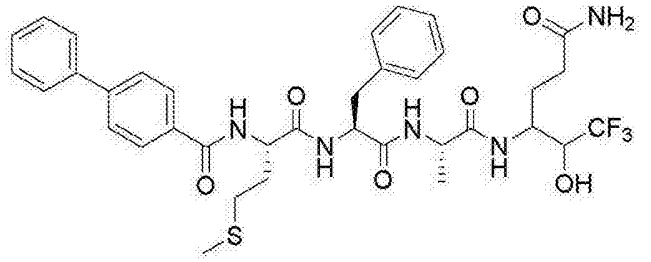


[0959] ^{1}H NMR(DMSO-d_6):δ 8.40(1H),8.03-7.80(5H),7.74(4H),7.46(2H),7.40(1H),7.20(0.5H),7.06(0.5H),6.65(1H),6.47(1H),4.33(1H),4.30-4.10(2H),3.95-3.72(2H),2.21-1.79(5H),1.60(1H),1.23-1.10(3H),0.92(6H),0.83(6H);^{19}F NMR(DMSO-d_6):δ -74.66,-74.70;MS ESI:650.4[M+H]$^+$

[0960] 实例75

[0961] N-((5S,8S,11S)-17-氨基-8-苄基-11-甲基-6,9,12,17-四氧代-14-(2,2,2-三氟-1-羟基乙基)-2-硫杂-7,10,13-三氮杂十七烷-5-基)-[1,1′-联苯基]-4-甲酰胺

[0962]

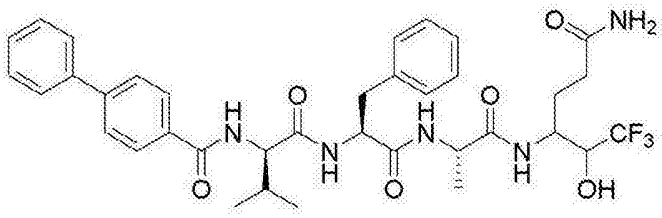


[0963] ^{1}H NMR(DMSO-d_6):δ 8.54(1H),8.03(2H),7.98(2H),7.89(1H),7.75(4H),7.48(2H),7.40(1H),7.23-7.05(6H),6.70(1H),6.48(1H),4.58(1H),4.53(1H),4.20(1H),4.00-3.76(2H),3.03(1H),2.80(1H),2.40(2H),2.15-1.80(5H),2.02(3H),1.62(1H),1.21(3H);^{19}F NMR(DMSO-d_6):δ -74.64,-74.66;MS ESI:730.4[M+H]$^+$

[0964] 实例76

[0965] N-((2R)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[0966]

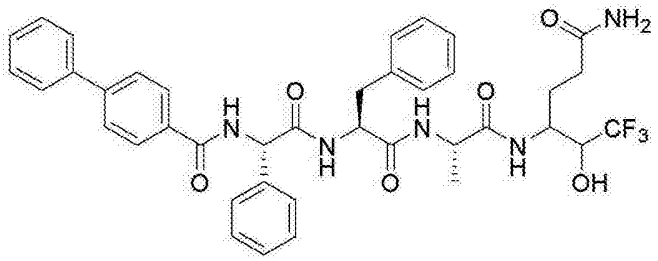


[0967] ^{1}H NMR(DMSO-d_6):δ 8.44(1H),8.31(1H),8.17(1H),7.95(2H),7.87(0.5H),7.80(0.5H),7.77(4H),7.44(2H),7.40(1H),7.24(2H),7.20-7.07(4H),6.70(1H),4.57(1H),4.22(1H),4.18(1H),3.96-3.75(2H),3.13(1H),2.73(1H),2.10-1.80(4H),1.60(1H),1.25(3H),0.77(3H),0.58(3H);^{19}F NMR(DMSO-d_6):δ -74.63,-74.75;MS ESI:698.4[M+H]$^+$

[0968] 实例 77

[0969] N-((1S)-2-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-2- 氧代 -1- 苯基乙基)-[1,1′ - 联苯基]-4- 甲酰胺

[0970]

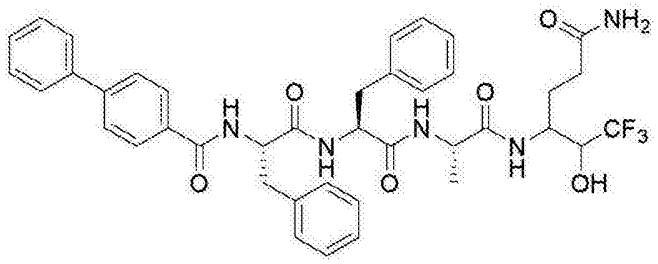


[0971] ^{1}H NMR(DMSO-d_6) :δ 8.81(1H),8.41(1H),8.10-7.92(3H),7.86(1H),7.75(4H),7.50(2H),7.40(1H),7.38-7.00(11H),6.70(1H),6.48(1H),5.63(1H),4.60(1H),4.20(1H),3.98-3.78(2H),3.09(1H),2.82(1H),2.17-1.80(3H),1.61(1H),1.20(3H) ;^{19}F NMR(DMSO-d_6) :δ -74.64,-74.67 ;MS ESI :732.4[M+H]$^+$

[0972] 实例 78

[0973] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨 基 -1,1,1- 三 氟 -2- 羟 基 -6- 氧 代 己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺

[0974]

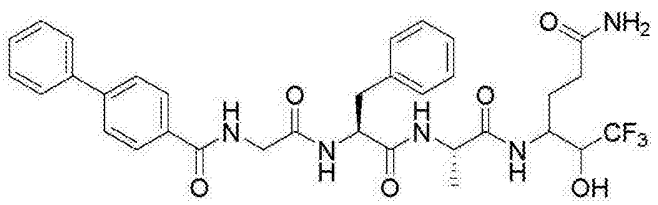


[0975] ^{1}H NMR(DMSO-d_6) :δ 8.60(1H),8.17(2H),7.90(1H),7.84(2H),7.72(4H),7.50(2H),7.40(1H),7.33-7.09(11H),6.70(1H),6.49(1H),4.63(1H),4.58(1H),4.22(1H),4.00-3.78(2H),3.03(2H),2.95(1H),2.82(1H),2.18-1.80(3H),1.61(1H),1.21(3H) ;^{19}F NMR(DMSO-d_6) :δ -74.62,-74.65 ;MS ESI :746.4[M+H]$^+$

[0976] 实例 79

[0977] N-(2-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -3- 苯基丙 -2- 基) 氨基)-2- 氧代乙基)-[1,1′ - 联苯基]-4- 甲酰胺

[0978]

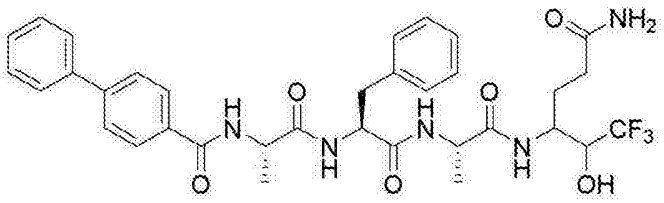


[0979] ^{1}H NMR(DMSO-d_6)：δ 8.79(1H)，8.21-8.12(2H)，7.94(2H)，7.87-7.63(5H)，7.45(2H)，7.40(1H)，7.26-7.07(6H)，6.70(1H)，6.47(1H)，4.57(1H)，4.21(1H)，4.00-3.70(3H)，3.02(1H)，2.80(1H)，2.15-1.80(4H)，1.62(1H)，1.21(3H)；^{19}F NMR(DMSO-d_6)：δ -74.62，-74.68；MS ESI：656.4$[M+H]^+$

[0980] 实例 80

[0981] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基)-1-氧代丙-2-基)-[1,1′-联苯基]-4-甲酰胺

[0982]

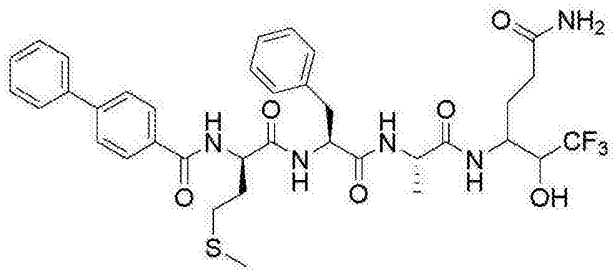


[0983] ^{1}H NMR(DMSO-d_6)：δ 8.57(1H)，8.03-7.95(4H)，7.82(1H)，7.77(4H)，7.50(2H)，7.40(1H)，7.22-7.05(6H)，6.70(1H)，6.50(1H)，4.50(1H)，4.40(1H)，4.20(1H)，4.00-3.77(2H)，3.02(1H)，2.81(1H)，2.16-1.80(3H)，1.61(1H)，2.22(6H)；^{19}F NMR(DMSO-d_6)：δ -74.65，-74.68；MS ESI：670.4$[M+H]^+$

[0984] 实例 81

[0985] N-((5R,8S,11S)-17-氨基-8-苄基-11-甲基-6,9,12,17-四氧代-14-(2,2,2-三氟-1-羟基乙基)-2-硫杂-7,10,13-三氮杂十七烷-5-基)-[1,1′-联苯基]-4-甲酰胺

[0986]

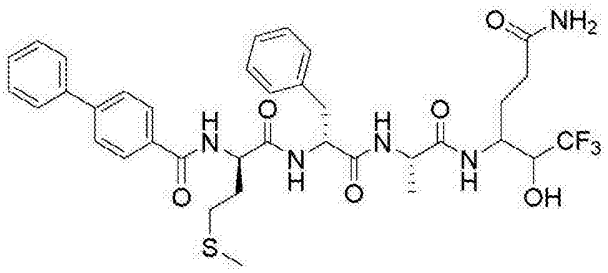


[0987] ^{1}H NMR(DMSO-d_6)：δ 8.52(1H)，8.35(1H)，8.17(1H)，7.96(2H)，7.90(0.5H)，7.72(4.5H)，7.48(2H)，7.40(1H)，7.24-7.04(6H)，6.70(1H)，6.50(1H)，4.59(1H)，4.51(1H)，4.21(1H)，3.98-3.78(2H)，3.10(1H)，2.73(1H)，2.38-2.15(2H)，2.12-1.80(3H)，2,00(3H)，1.80-1.48(3H)，1.24(3H)；^{19}F NMR(DMSO-d_6)：δ -74.62，-74.72；MS ESI：730.4$[M+H]^+$

[0988] 实例 82

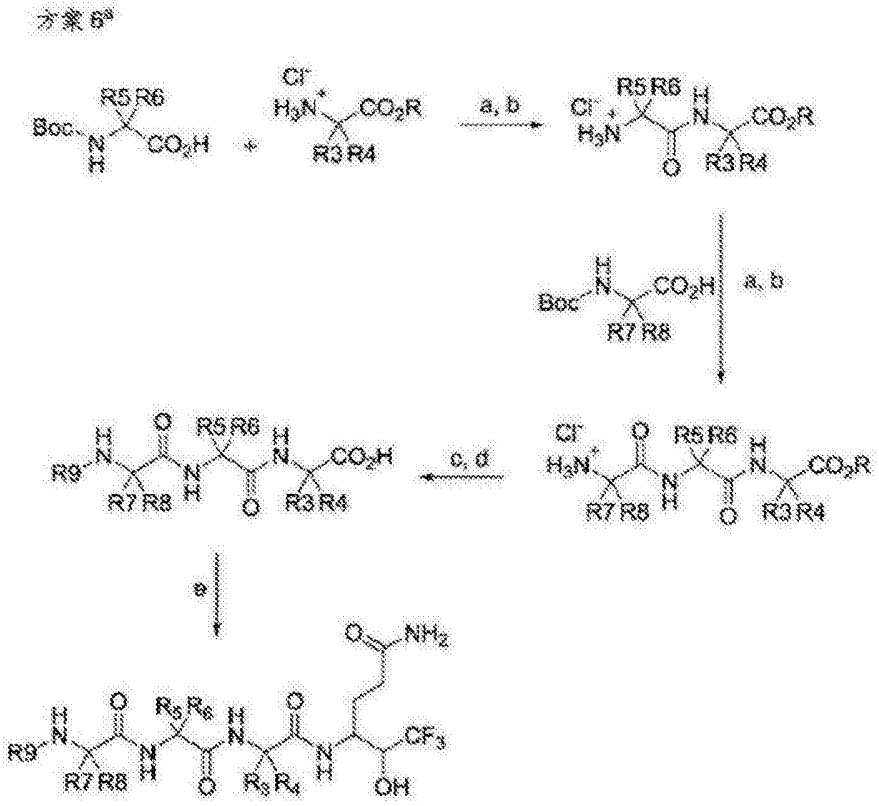
[0989] N-((5R,8R,11S)-17-氨基-8-苄基-11-甲基-6,9,12,17-四氧代-14-(2,2,2-三氟-1-羟基乙基)-2-硫杂-7,10,13-三氮杂十七烷-5-基)-[1,1′-联苯基]-4-甲酰胺

[0990]



[0991] ^{1}H NMR(DMSO-d_6)：δ 8.60(0.5H)，8.56(0.5H)，8.18-8.05(2H)，7.98(2H)，7.85(0.5H)，7.75(4.5H)，7.48(2H)，7.40(1H)，7.23-7.11(6H)，6.77(0.5H)，6.67(0.5H)，6.45(1H)，4.50(2H)，4.20(1H)，3.99-3.78(2H)，2.98(1H)，2.84(1H)，2.44(2H)，2.15-1.52(6H)，2.00(3H)，1.11(3H)；^{19}F NMR(DMSO-d_6)：δ -74.62，-74.65；MS ESI：730.3[M+H]$^+$

[0992]



[0993] [a]试剂和条件：a)HATU、DIPEA、DMF，-20℃至室温；b)4MHCl 的二噁烷溶液、CH_2Cl_2，0℃至室温；c) 适当的酰氯或磺酰氯、Et_3N、THF，0℃至室温或适当的羧酸，HATU、DIPEA、DMF，0℃至室温；d)LiOH、H_2O、THF、MeOH，0℃至室温；

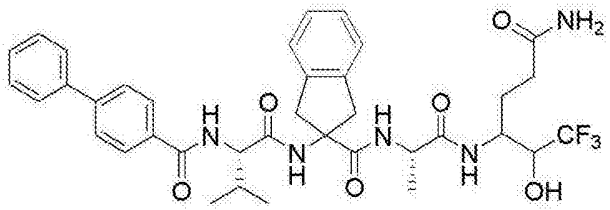
[0994] e) 中间体 8、9 或 ent-9、HATU、DIPEA、DMF，-20℃至室温。

[0995] 根据方案 6 制备的化合物实例：

[0996] 实例 83

[0997] 2-((S)-2-([1,1′-联苯基]-4-甲酰胺基)-3-甲基丁酰胺基)-N-((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)-2,3-二氢-1H-茚-2-甲酰胺

[0998]

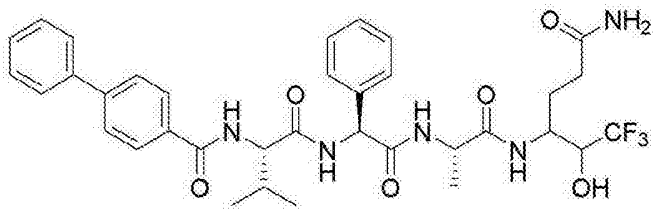


[0999] ^{1}H NMR(DMSO-d_6)：δ 8.72(1H)，8.48(1H)，7.99(2H)，7.75(5H)，7.50(3H)，7.40(1H)，7.22-7.00(5H)，6.70(0.5H)，6.63(0.5H)，6.45(1H)，4.16(1H)，4.01(1H)，3.98-3.73(2H)，3.58(1H)，3.39(1H)，3.24(2H)，2.18-1.80(4H)，1.62(1H)，1.18(3H)，0.94(3H)，0.77(3H)；^{19}F NMR(DMSO-d_6)：δ -74.60，-74.66；MS ESI：710.4[M+H]$^+$

[1000] 实例 84

[1001] N-((2S)-1-(((1S)-2-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-2-氧代-1-苯基乙基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[1002]

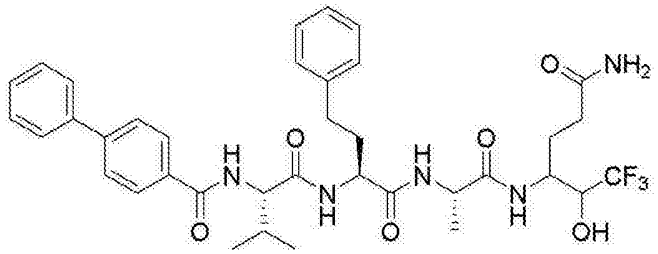


[1003] ^{1}H NMR(DMSO-d_6)：δ 8.42(3H)，7.96(2H)，7.85(0.5H)，7.76(4.5H)，7.50(2H)，7.40(3H)，7.36-7.20(3H)，7.15(0.5H)，7.04(0.5H)，6.69(0.5H)，6.63(0.5H)，6.48(1H)，5.58(1H)，4.40(1H)，4.22(1H)，3.85(1H)，3.75(1H)，2.17(1H)，2.05-1.75(3H)，1.57(1H)，1.20(3H)，0.90(6H)；^{19}F NMR(DMSO-d_6)：δ -74.67，-74.70；MS ESI：684.3[M+H]$^+$

[1004] 实例 85

[1005] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-4-苯基丁-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

[1006]



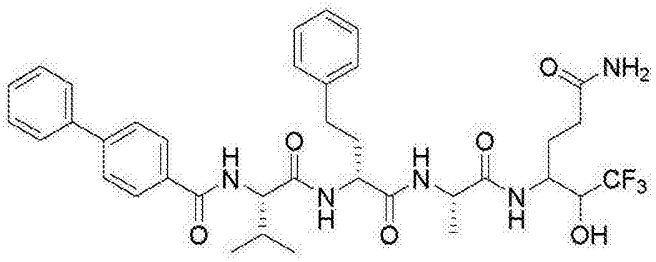
[1007] ^{1}H NMR(DMSO-d_6)：δ 8.60(1H)，8.22(1H)，8.03-7.86(4H)，7.76(4H)，7.50(2H)，7.40(1H)，7.28-7.07(6H)，6.70(1H)，6.60(1H)，4.40-4.15(3H)，4.06-3.72(2H)，2.60(2H)，2.20(1H)，2.15-1.76(5H)，1.61(1H)，1.20(3H)，0.97(6H)；^{19}F NMR(DMSO-d_6)：δ -74.64，-74.71；MS ESI：712.4[M+H]$^+$

[1008] 实例 86

[1009] N-((2S)-1-(((2R)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代

己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-1- 氧代 -4- 苯基丁 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺

[1010]

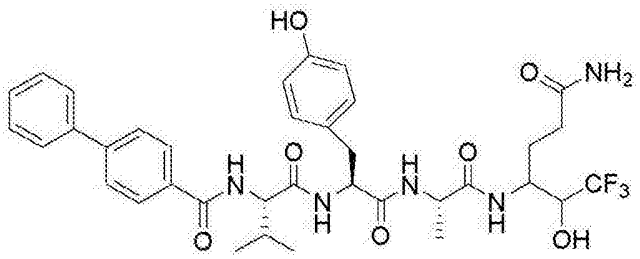


[1011] ^{1}H NMR(DMSO-d_6) : δ 8.57-8.42(2H),8.00(2H),7.93(1H),7.84(1H),7.74(4H),7.49(2H),7.40(1H),7.25(2H),7.16(4H),6.70(1H),6.48(1H),4.22(3H),3.98-3.75(2H),2.61(1H),2.51(1H),2.21-1.72(6H),1.61(1H),1.20(3H),1.00(6H) ; ^{19}F NMR(DMSO-d_6) : δ -74.63,-74.65 ;MS ESI :712.4[M+H]$^{+}$

[1012] 实例 87

[1013] N-((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-3-(4- 羟苯基)-1- 氧代丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基)-[1,1′ - 联苯基]-4- 甲酰胺

[1014]



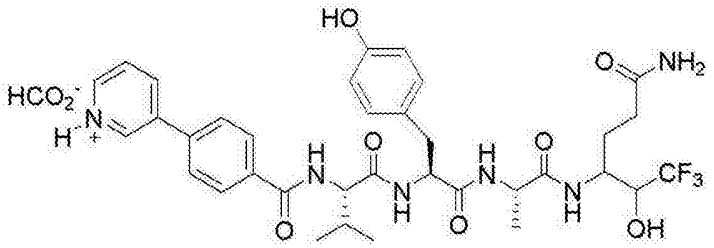
[1015] 通过 RP-HPLC(固定相 :Phenomenex C12 Synergi 4u Max-RP ;流动相 A :0.1% 甲酸的 H_2O 溶液 ;流动相 B :0.1% 甲酸的 CH_3CN 溶液 ;梯度在 A 中的 18-58% B ;运行时间 = 20 分钟) 纯化。

[1016] ^{1}H NMR(DMSO-d_6) : δ 9.12(1H),8.29(1H),8.06-7.82(5H),7.75(4H),7.50(2H),7.40(1H),7.20(1H),7.00(2H),6.69(1H),6.62-6.44(3H),4.50(1H),4.20(2H),4.00-3.78(2H),2.92(1H),2.67(1H),2.18-1.80(4H),1.62(1H),1.20(3H),0.80(6H) ; ^{19}F NMR(DMSO-d_6) : δ -74.64,-74.69 ;MS ESI :714.4[M+H]$^{+}$

[1017] 实例 88

[1018] 甲酸 3-(4-(((2S)-1-(((2S)-1-(((2S)-1-((6- 氨基 -1,1,1- 三氟 -2- 羟基 -6- 氧代己 -3- 基) 氨基)-1- 氧代丙 -2- 基) 氨基)-3-(4- 羟苯基)-1- 氧代丙 -2- 基) 氨基)-3- 甲基 -1- 氧代丁 -2- 基) 氨基甲酰基) 苯基) 吡啶 -1- 鎓

[1019]



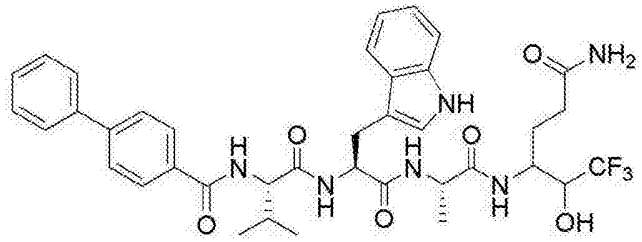
[1020] 通过RP-HPLC(固定相:Phenomenex C12 Synergi 4u Max-RP 8A 50x20mm;流动相A:0.1%甲酸的H_2O溶液;流动相B:0.1%甲酸的CH_3CN溶液;梯度在A中的12-52% B;运行时间=20分钟)纯化。

[1021] ^1H NMR(DMSO-d_6):δ 9.20(1H),8.94(1H),8.60(1H),8.50(1H),8.38(1H),8.14(1H),8.08-7.87(5H),7.83(2H),7.51(1H),7.19(0.5H),7.15(0.5H),7.00(2H),6.70(1H),6.62(1H),6.66(2H),4.48(1H),4.20(2H),3.96-3.74(2H),2.91(1H),2.57(1H),2.11-1.80(4H),1.61(1H),1.19(3H),0.85(3H),0.77(3H);^{19}F NMR(DMSO-d_6):δ -74.64,-74.70;MS ESI:715.4$[M+H]^+$

[1022] 实例89

[1023] N-((2S)-1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代丙-2-基)氨基)-3-(1H-吲哚-3-基)-1-氧代丙-2-基)氨基)-3-甲基-1-氧代丁-2-基)-[1,1′-联苯基]-4-甲酰胺

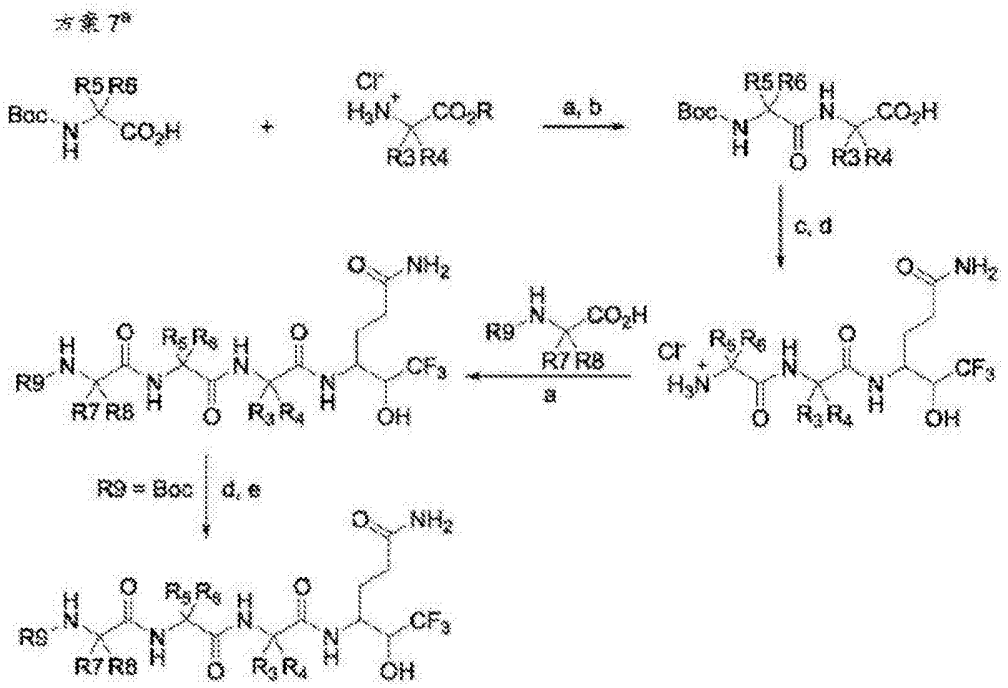
[1024]



[1025] ^1H NMR(DMSO-d_6):δ 10.79(1H),8.28(1H),8.13(1H),8.05-7.80(4H),7.75(4H),7.58(1H),7.50(2H),7.41(1H),7.30(1H),7.18(2H),7.04(1H),6.95(1H),6.73(1H),6.50(1H),4.61(1H),4.30-4.15(2H),3.99-3.77(2H),3.14(1H),2.98(1H),2.15-1.80(4H),1.65(1H),1.30-1.10(3H),0.87(3H),0.80(3H);^{19}F NMR(DMSO-d_6):δ -74.62,-74.65;MS ESI:737.4$[M+H]^+$

[1026] 通过RP-HPLC(固定相:Phenomenex C12 Synergi 4u Max-RP 8A 50x 20mm;流动相A:0.1%甲酸的H_2O溶液;流动相B:0.1%甲酸的CH_3CN溶液;梯度在A中的20-60% B;运行时间=20分钟)纯化。

[1027]



[1028] [a]试剂和条件：a)HATU、DIPEA、DMF，-20℃至室温；b)LiOH、H_2O、THF、MeOH，0℃至室温；

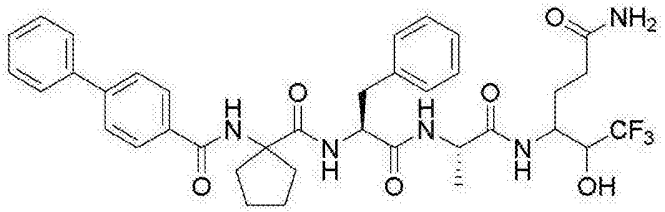
[1029] c)中间体8、9或ent-9、HATU、DIPEA、DMF，0℃至室温；d)4MHCl的二噁烷溶液，CH_2Cl_2，0℃至室温；e)适当的酰氯或磺酰氯、Et_3N、THF，0℃至室温或适当的羧酸、HATU、DIPEA、DMF，0℃至室温。

[1030] 根据方案7制备的化合物实例：

[1031] 实例90

[1032] N-(1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基甲酰基)环戊基)-[1,1′-联苯基]-4-甲酰胺

[1033]

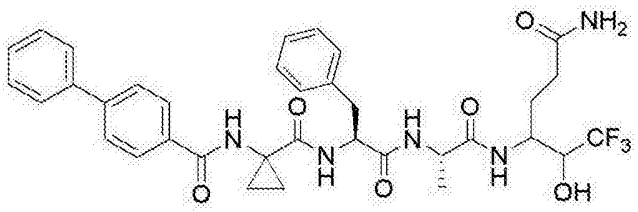


[1034] ^1H NMR(DMSO-d_6)：δ 8.66(0.5H)，8.61(0.5H)，8.00(2H)，7.86-7.60(6H)，7.58-7.36(4H)，7.13(6H)，6.68(1H)，6.45(1H)，4.44(1H)，4.15(1H)，3.99-3.78(2H)，3.10(1H)，2.87(1H)，2.25(1H)，2.17-1.74(5H)，1.73-1.40(6H)，1.23(3H)；^{19}F NMR(DMSO-d_6)：δ -74.62，-74.67；MS ESI：710.4$[M+H]^+$

[1035] 实例91

[1036] N-(1-(((2S)-1-(((2S)-1-((6-氨基-1,1,1-三氟-2-羟基-6-氧代己-3-基)氨基)-1-氧代丙-2-基)氨基)-1-氧代-3-苯基丙-2-基)氨基甲酰基)环丙基)-[1,1′-联苯基]-4-甲酰胺

[1037]



[1038] ^{1}H NMR(DMSO-d_6)：δ 9.10(1H)，8.02-7.90(3H)，7.85-7.66(6H)，7.50(2H)，7.40(1H)，7.12(6H)，6.70(1H)，6.50(1H)，4.53(1H)，4.20(1H)，4.00-3.78(2H)，3.02(1H)，2.90(1H)，2.18-1.79(3H)，1.61(1H)，1.40-1.15(5H)，1.03(1H)，0.93(1H)；^{19}F NMR(DMSO-d_6)：δ -74.63，-74.71；MS ESI：682.3[M+H]$^+$。

[1039] 然后使用生物 PCSK9 基测定法筛选本发明化合物。

[1040] 实例 92

[1041] 初始筛选

[1042] 在不存在 (DMSO) 或存在 10μM、100μM 或渐增浓度 (11、33、100μM) 的试验化合物下培养稳定表达 WT PCSK9(+V5) 的 HepG2 细胞过夜 (24 小时)。收集 24 小时条件培养基，离心，并移除上清液用于通过 ELISA(100μl 的 1 ： 30 稀释) 进行 PCSK9 定量。用含有蛋白酶抑制剂混合物 (Roche Applied Science) 的 250ul/ 孔的放射免疫沉淀测定缓冲液 (RIPA)(50mM Tris-HCl，pH 7.8，150mM NaCl，1％ Nonident P-40，0.5％脱氧胆酸钠，0.1％ SDS) 裂解细胞，并在 11,300xg 下沉淀 5 分钟。移除上清液，用于通过 ELISA(100μl 的 1 ： 20 稀释) 的细胞 PCSK9 定量和通过 Bio-Rad DC 蛋白质测定法 (Bio-Rad) 的总蛋白质测定 (Dubuc G、Tremblay M、Paré G、Jacques H、Hamelin J、Benjannet S、Boulet L、Genest J、Bernier L、Seidah NG、Davignon J.2010.A new method for measurement of total plasma PCSK9：clinical applications.J Lipid Res.51：140-149)。使超过 165 种化合物接受在 100μM 下通过 PCSK9 分泌测定的第一次筛选。

[1043] 确定为在此测定中相对于 DMSO 对照降低 PCSK9- 培养基 / 细胞比至少 30％ (30％抑制) 的某些化合物进一步测试其以剂量依赖性方式抑制 PCSK9 分泌的能力。至少 24 种化合物被确定为以剂量依赖性方式抑制 PCSK9 分泌 (实例 1 至 24 中所述的化合物)。

[1044] 图 1 和表 I 呈现一组精选的试验化合物。图 2 示出了在稳定表达 PCSK9(+V5) 的 HepG2 细胞中剂量依赖性抑制 PCSK9 分泌，以选择试验化合物，即 19、22a 和 23，其在 33.3μM 的剂量下，相对于 DMSO 对照降低 PCSK9- 培养基 / 细胞在 30％与 50％之间。

[1045] 表 I. 在 10μM 的浓度下测试的选定化合物的 PCSK9 分泌

[1046]

实例#	分泌抑制（活性%）a
24	68
29	83
52	79
58	81
59	82
60	73
66	92
70	74
75	100

[1047] 使用以下公式的计算活性%：

[1048]

$$\%\ 活性 = \left\{ 1 - \frac{([PCSK9]_{培养基}/[PCSK9]_{细胞})_{抑制剂}}{([PCSK9]_{培养基}/[PCSK9]_{细胞})_{DMSO}} \right\} X\ 100\ \%$$

[1049] 实例 93

[1050] 细胞毒性分析

[1051] 在初始筛选后，使用MTT毒性试验(3-[4,5-二甲基噻唑-2-基]-2,5-二苯基溴化四唑鎓)，根据制造商方案(Promega)(对于参见图3)或使用细胞活力测定(参见表II)，测试化合物的细胞毒性。

[1052] 对于MTT毒性测定法，将过度表达WT PCSK9(+V5)的HepG2稳定细胞接种于96-孔板(Greiner BioOne)中并培养20小时。简短洗涤后，将细胞在存在渐增浓度(11、33、100μM)的化合物或不存在DMSO(对照)下培养过夜(24小时)。MTT测定法(Promega)由以下组成：按照制造商指示，加入20ul/孔MTT试剂并在37℃下培养45分钟。记录在490nm的吸光度，并针对由于非特异性吸光度(690nm)的背景校正。在33.3μM或更低下具有低毒性的化合物被选为图3中的示例，为化合物19、22a、23、4和17。

[1053] 对于细胞存活力测定法，在存在33μM化合物或在存在DMSO(对照)下测量在实例92中所述的PCSK9分泌测定法期间HepG2细胞的细胞密度。下表II呈现在33.3μM或更低下具有低毒性的化合物。

[1054] 表II：在存在33μM的浓度的选定化合物下的细胞存活力，呈现为在其不存在(DMSO)时所获得的细胞存活力的百分比

[1055]

实例#	细胞活力（%DMSO对照）
59	83
58	93
29	88
60	80

[1056] 选定PCSK9抑制化合物对PCSK9的活性进一步使用各种测定法表征，该测定法包括Western印迹分析和对PCSK9自动处理和分泌的效应的生物合成分析。选定PCSK9

抑制化合物的对 LDLR 降解的效应和活是使用一系列测定法表征，包括免疫荧光测定法、Dil-LDL 摄取测定法和细胞表面 LDLR 的 FACS 分析，以及总 LDLR 的 Western 印迹分析。

[1057] 实例 94

[1058] 免疫荧光测定法

[1059] PCSK9 抑制化合物是通过细胞表面 LDLR 和人转铁蛋白受体作为对照在 HepG2 初始细胞中的免疫荧光来表征。将细胞铺于放置在 24 孔细胞培养板中的聚 -L- 赖氨酸涂覆 (50ug/ml) 的圆形显微镜盖玻片 1.12mm 厚度 (Fisherbrand 12CIR#1)。在接种后第 24h，在不存在 (DMSO) 或在存在 33.3μM 的 PCSK9 抑制剂下培养细胞。20 小时温育期后，将细胞用 3.7％的多聚甲醛固定。在非透的条件下进行人 LDLR 的免疫荧光（绿色标签）。将细胞用 1％ BSA 阻断，接着在 4℃下用一级抗体（在 1％ BSA 中的 1 ： 200 山羊多克隆抗 hLDLR，R&D Systems) 温育过夜。在室温下用 Alexa fluor 标记的二级抗体培养 1 小时揭示抗原 - 抗体复合物并安装在 ProLong Gold 抗淬灭试剂 (Molecular Probes，Invitrogen) 中。用共聚焦显微镜 (Zeiss LSM-710) 进行免疫荧光分析。细胞核用 DAPI 染色（蓝色标签）。LDLR 是用抗 LDLR Ab 染色（绿色标签）。通过在细胞表面增加的 LDLR 检测 PCSK9 活性对 LDLR 降解的抑制，如在图 4 中针对化合物 19 所例示。

[1060] 实例 95

[1061] DiI-LDL 测定法

[1062] PCSK9 抑制化合物也使用如在 Poirier 等人，J.Biol.Chem.284 :28856-28864，2009 中所述的 Dil-LDL 荧光摄取测定法表征。该方法包括在人肝细胞衍生的 HuH7 或 HepG2 细胞系或人胚胎 HEK293 细胞，在存在或不存在化合物下经由 LDLR 内化荧光测定 DiI-LDL 细胞并入（细胞表面 LDLR 活性的量度）。

[1063] 将 HepG2 初始细胞和 HEK293 初始细胞接种在 96 孔板（有透明底部的 CellBind™ 黑板 (Corning；目录号 3340)）。在接种后第 20h，在不存在 (DMSO 或阴性对照）或在存在不同浓度的化合物下培养细胞。每个条件制备一式三份。在 6 小时培养后，将 Dil-LDL(Biomedical Technologies(目录号 BT-904)) 添加到细胞培养基，且细胞返回至组织培养箱再培养 18 小时。将板在 SpectraMax GeminiEM™平板读数器 (Molecular Devices) 中进行扫描（底部读）。对于每个孔，将原始 Dil-LDL 摄取测定为在孔中 3 个不同点的 9 个读数的平均荧光强度 (RFU)(激发 :520nm/ 发射 :575nm，截止 :550nm)。每个孔中的 Dil-LDL 摄取通过按照制造商指示进行 CyQuant™细胞分析 (Invitrogen；目录号 C7026) 对细胞总数校正。校正的 Dil-LDL 摄取报告为％ DMSO 对照并从一式三份孔获得。通过 DiI-LDL 摄取的增加检测 PCSK9 活性对 LDLR 降解的抑制（在图 5A 中针对化合物 19、22a、23、24、21a、21b 在 HepG2 初始细胞中示例）。选择化合物 i 作为阴性对照且化合物 1、17 和 2 显示在该测定法中在 HepG2 细胞中没有可测量的活性。化合物针对 PCSK9 的特异性示例于图 5B 中，其示出了在已知缺乏 PCSK9 表达的 HEK293 细胞中，相同的一组化合物对 Dil-LDL 摄取没有影响。

[1064] 表 III :在 10μM 浓度的选定化合物的存在下，在 HepG2 细胞中的 Dil-LDL 摄取，其呈现为在其不存在 (DMSO) 时所获得的 Dil-LDL 摄取的百分比

[1065]

实例 #	Dil-LDL 摄取 (% DMSO 对照)
24	224 ± 33
29	207 ± 21
52	254 ± 71
58	261 ± 48
59	253 ± 75
60	224 ± 43
66	237 ± 59
70	224 ± 47
75	172 ± 48

[1066] 为了进一步测试这些化合物是否还可以抑制PCSK9的功能突变体D374Y的增益功能，通过使用Dil-LDL荧光摄取测定法分析表达PCSK9-D374Y的细胞。化合物的抑制效果还使用在Dil-LDL摄取测定法中肝脏来源的小鼠细胞系FL-83B(ATCC，CRL-2390)表征。

[1067] 实例96

[1068] Western印迹

[1069] 测试化合物其在人类肝细胞细胞系HepG2中抑制PCSK9的LDLR增强型降解的能力。在不存在或存在试验PCSK9抑制化合物下，将细胞培养24h。在培养后，将细胞在RIPA中裂解。蛋白质通过SDS-聚丙烯酰胺凝胶电泳分离并印迹在聚偏二氟乙烯(PVDF，Perkin Elmer)膜(GE Healthcare)上。然后在存在人LDLR抗体(1 ： 1000，R&D体系)和β-肌动蛋白抗体(1 ： 2500，Sigma)下培养膜。合适的辣根过氧化物酶共轭的二级抗体(1 ： 10,000，Sigma)被用于用ECL加试剂盒(GE Healthcare)检测增强的化学发光。在这些测定法中PCSK9功能的抑制是通过增加的总LDLR/β-肌动蛋白的比率检测，如通过Western印迹分析所测得。使用图像J™软件获得蛋白质带的量化。图5C例示在不存在(Cnt_0)或存在渐增浓度的化合物19下培养24h的HepG2初始细胞中总LDLR的Western印迹分析。将标准化为β-肌动蛋白的总LDLR水平针对每个条件以对照(Cnt)%绘图。

[1070] 实例97

[1071] FACS测定法

[1072] PCSK9抑制化合物阻止PCSK9对细胞表面LDLR的活性的能力还通过使用如在Benjannet S.等人J.Biol.Chem.285:40965-40978,2010中所述的流式细胞术分析进行测量。在HepG2细胞表面的LDLR水平是通过使用抗人类LDLR抗体(1 ： 100,mAb-C7,Santa Cruz Biotechnology)和二级Alexa Fluor 647驴抗小鼠(Molecular Probes)抗体进行测量。然后通过FACS针对PI和Alexa Fluor 647两者使用FACS BD LSR(BD Biosciences)分析活细胞(PI阴性)。细胞表面LDLR是相对于对照(未处理细胞)记录。在这些测定法中PCSK9功能的抑制是通过增减的阳性细胞数而检测，阳性细胞数与在细胞表面的LDLR表达增加相关。

[1073] 更具体地，将人类肝母HepG2细胞(4x 10^5个细胞/孔)接种在12孔板(Greiner BioOne)的完全培养基中，并在37℃下培养20小时，接着在37℃下在无血清培养基中洗涤1小时。接下来，移除洗涤培养基，并将细胞以含有10uM化合物或DMSO对照(0.4%终浓度)的0.9ml/孔培育培养基培养过夜(24小时)。在用缓冲液A冲洗一次后，接着再用

EDTA 溶液 (2.5mM EDTA-2Na) 冲洗，通过在 37℃下在存在新制 EDTA 溶液 (1ml) 下培养 20 分钟，来分离细胞。在细胞分离后的所有步骤是在冰上或 4℃下进行。为了终止分离，将含有细胞转移到含有 3ml 缓冲液 A 的 15ml 管中，并立即以 900rpm(100g)(4℃) 离心 5 分钟。将细胞重新悬浮于 500ul 缓冲液 A 中，连同 1 ： 250 人类 LDLR 抗体一起，并在冰上培养 10 分钟。用 5ml 缓冲液 A 洗涤细胞一次，离心 5 分钟，重新悬浮于含有 1 ： 500Alexa Fluor 647 二级抗体的 500ul 缓冲液 A，并在冰上培养 5 分钟。染色后，用 5ml 缓冲液 A 洗涤细胞一次，离心 5 分钟，并重新悬浮于含有 1.67ug/ml 碘化丙锭的 300ul 缓冲液 A。然后通过针对 Alexa Fluor 647(细胞表面 LDLR) 的 FACS，使用 FACS-CyAn ADP 流式细胞仪 (Beckman Coulter(DAKO)) 和 Summit 软件 (Summit Sonware Inc.) 分析活细胞 (碘化丙锭阴性) 的固定数目 (2,000 个细胞)。

[1074] 材料：

[1075] 人类 HepG2 细胞系 :ATCC，HB-8065

[1076] 接种培养基 :完全培养基 -EMEM(高葡萄糖 + 丙酮酸钠)(Wisent)+10％ FBS

[1077] 洗涤培养基 :无血清培养基 -EMEM(高葡萄糖 + 丙酮酸钠)(Wisent)

[1078] 具有化合物的培养基 :EMEM(高葡萄糖 + 丙酮酸钠)(Wisent)+0.07 ％ BSA(Sigma-Aldrich)

[1079] 缓冲液 A：无钙和镁的 1X D-PBS(Wisent 311-425-CL)+0.5 ％ BSA(Sigma A7409-50ml)+1ug/L 葡萄糖 (Wisent 609-036-EL)。

[1080] 一级抗体 :人 LDLR 抗体，单克隆小鼠 IgG_1 克隆 #472413(MAB2148，R&D Systems)

[1081] 二级抗体 :Alexa Fluor® 647 山羊抗小鼠 IgG(H+L)*2mg/mL*(A21235，Molecular Probes)

[1082] 碘化丙锭 :1mg/ml 溶液 (P4864，Sigma)

[1083] 结果呈现于表 IV 中。

[1084] 表 IV- 在存在和不存在 (DMSO)10μM 浓度的选定化合物下，FACS-LDLR，表示为 LDLR 阳性细胞的百分比

[1085]

实例 #	FACS-LDLR (% DMSO 对照)
24	116 ± 5
29	233 ± 50
52	138 ± 20
58	166 ± 28
59	265 ± 35
60	138 ± 8
66	122 ± 11
70	118 ± 18
75	174 ± 41

[1086] 还在小鼠和人类原代肝细胞上测试 PCSK9 抑制化合物，以测量其对细胞表面 LDLR 的效应。使用小鼠原代肝细胞的优点是，它允许测量分别在野生型或表达或缺乏 PCSK9 的基因敲除小鼠的背景下化合物的特异性。

[1087] 实例 98

[1088] 在动物模型中 PCSK9- 诱导的 LDLR 降解的抑制的表征

[1089] 然后在表达人类 PCSK9 的小鼠模型中使用候选的 PCSK9 抑制化合物，以评估其降低体内血浆胆固醇水平的能力。

[1090] 在过度表达人类 PCSK9 的小鼠中测试化合物。从其自己的启动子构建转基因品系 (Herbert，B.，等人 (2010).Arteriosclerosis，Thrombosis，and Vascular Biology，30(7)：1333-1339)，其携带人类基因组 DNA 中表达人类 PCSK9 的～ 190kb(WT、D374Y 低或 D374Y 高)。进一步交叉产生在 $Pcsk9^{-/-}Ldlr^{+/-}$背景下表达人类 PCSK9 转基因的小鼠品系 (Ldlr 杂合子)。这通过小鼠 PCSK9 基因的内源性表达消除干扰并提高其 LDLc 水平 (图 6)。通过多次回交，在纯的 C57BL/6 背景中获得这些模型小鼠，以用于分析的均一性和再现性。作为特异性的对照，测试在 $Ldlr^{-/-}$背景中的转基因效应。

[1091] 小鼠注射：化合物是静脉内注射到 WT、$Pcsk9^{-/-}$、$Ldlr^{-/-}$和 Pcsk9-Tg 小鼠，以 6 只小鼠 / 基因型 (图 6)。在第一周期间每天且在接下来 2 周每三天测量总胆固醇 (TC)、LDLc 和 PCSK9 水平。血浆中剩余的未络合的 PCSK9 的水平也是通过免疫沉淀，使用先前描述的抗体 (Zaid，A.，等人 (2008).Hepatology，48(2)：646-65) 进行测量。在另一组实验中，在最低 LDLc 的时间点 (注射后 4-7 天) 杀死 6 只小鼠，并分析其 FPLC 血浆脂质谱，以及肝 LDLR 蛋白质。仔细监测任何明显的毒性和 / 或发病率效应。$Pcsk9^{-/-}$、$Ldlr^{-/-}$小鼠的对照允许验证所观察到的效应是 PCSK9- 依赖性的。

[1092] 他汀 +PCSK9 抑制化合物的效应：评估阿托伐他汀和抑制化合物的组合，因为他汀类药物增加 PCSK9 表达，同时降低 LDLR 表达 (Dubuc，G.，等人 (2004).Arteriosclerosis，Thrombosis，and Vascular Biology，24(8)：1454-1459；Lakoski，S.G.，等人 (2009).The Journal of Clinical Endocrinology and Metabolism，94(7)：2537-2543)，并已表明他汀类药物甚至进一步降低 $Pcsk9^{-/-}$小鼠的 LDLc(Rashid，S. 等人 (2005).Proc Natl Acad Sci USA 102(15)：5374-5379)。

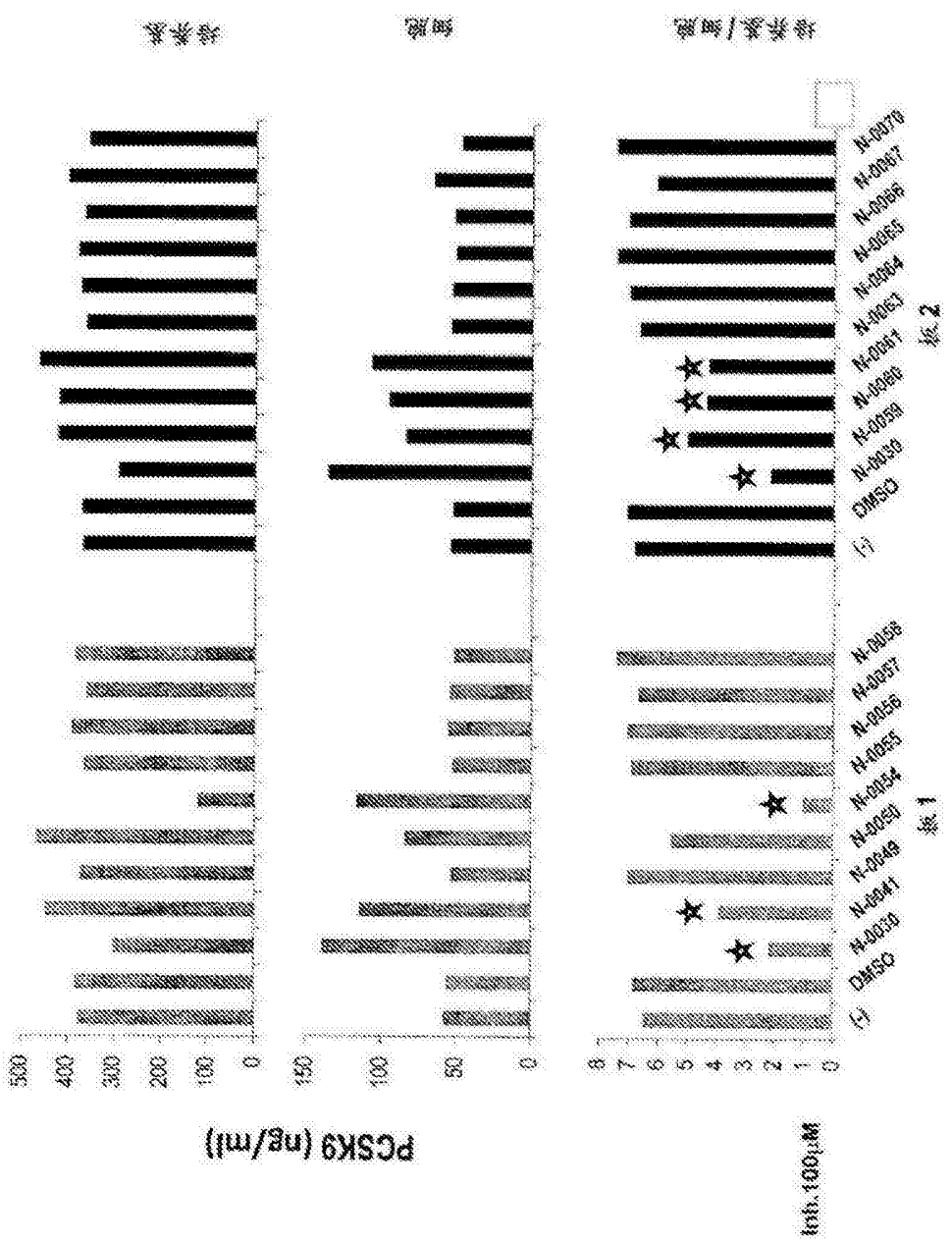
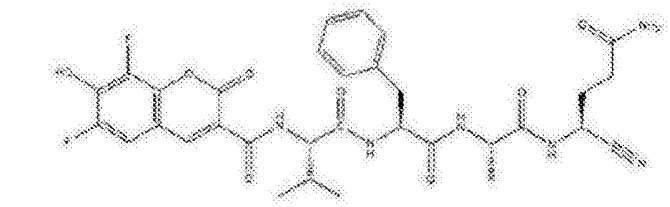
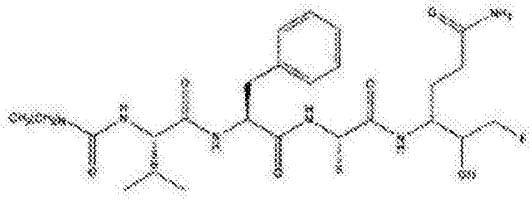

PCSK9 (ng/ml)
Int. 100μM
N-0070
N-0067
N-0066
N-0065
N-0064
N-0063
N-0061
N-0060
N-0059
N-0030
DMSO
(-)
N-0058
N-0057
N-0056
N-0055
N-0054
N-0050
N-0049
N-0041
N-0039
DMSO
(-)
※ 2
※ 1

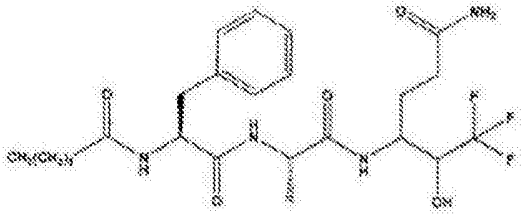
图 1A



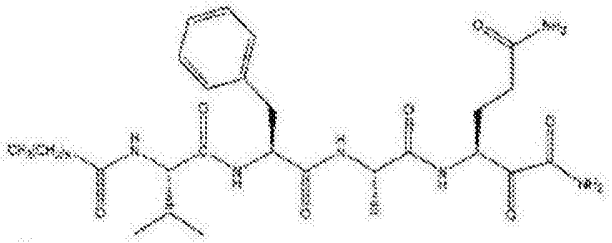
a



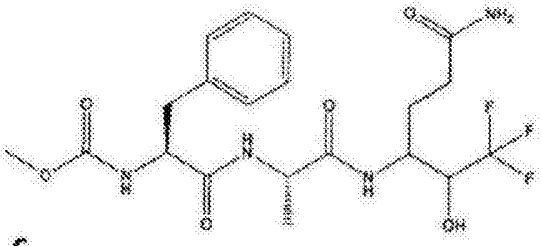
b



c 或 d*

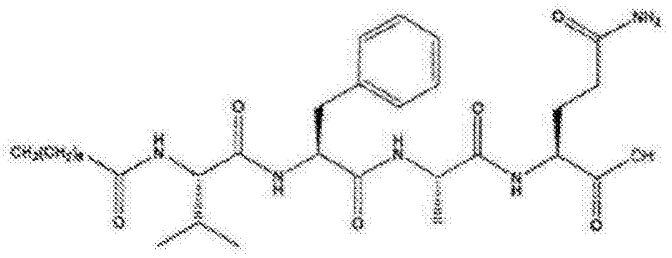


e

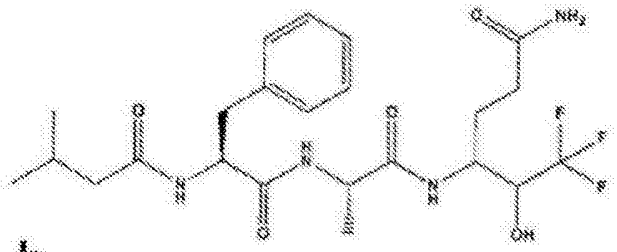


f

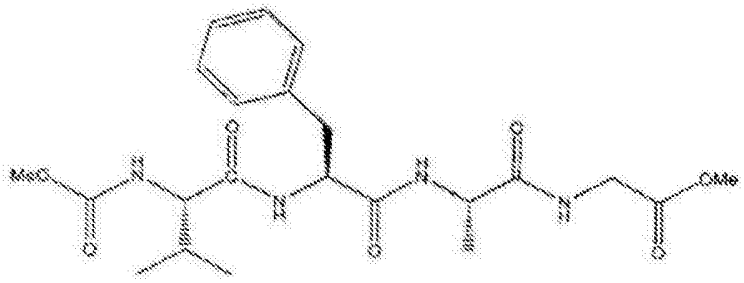
图 1B



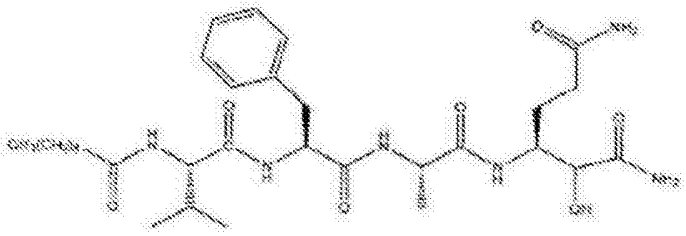
g



h



i



j

图 1B(续)

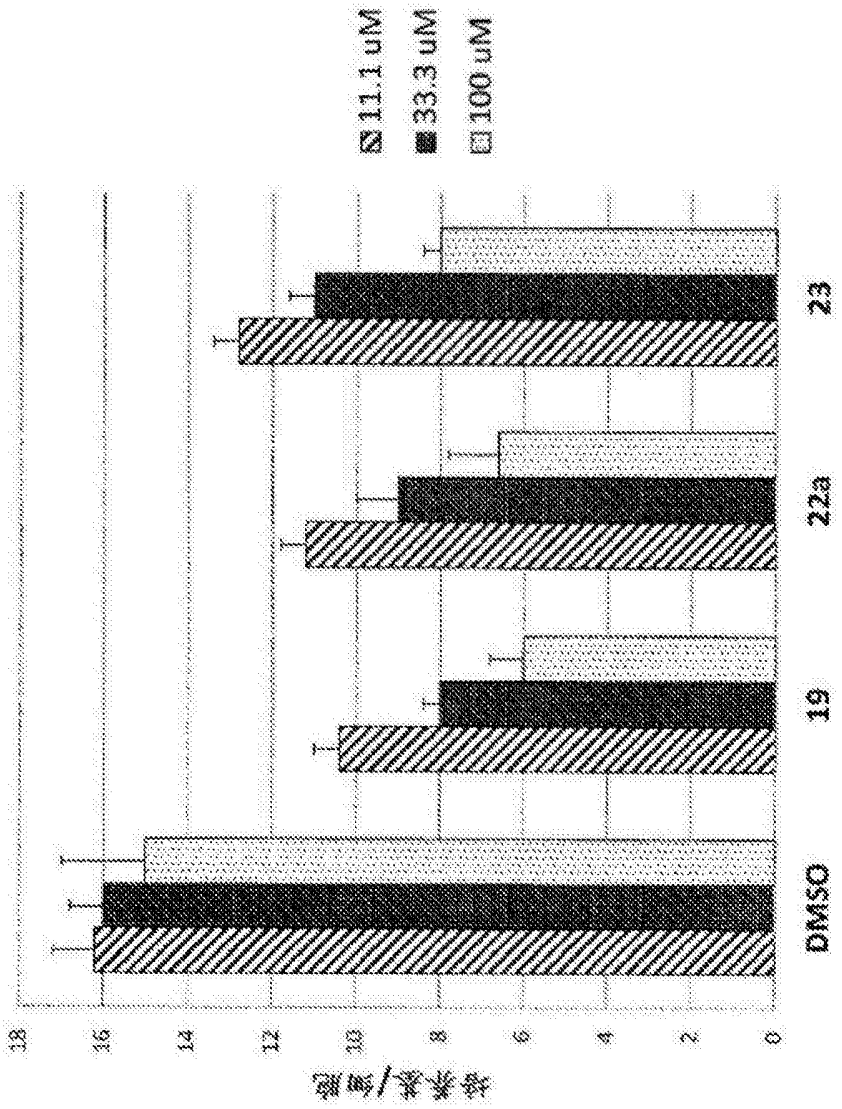


图 2

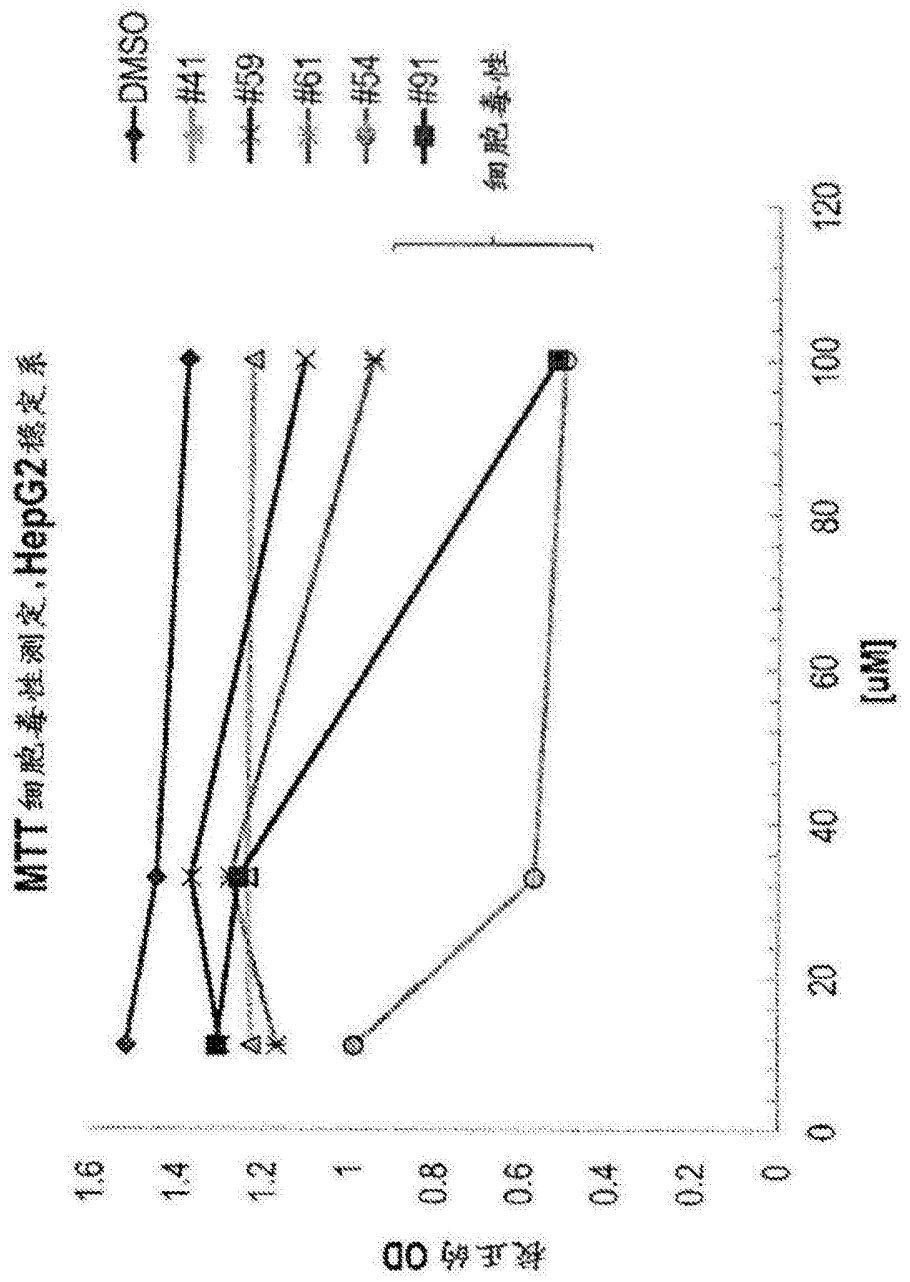

MTT细胞毒性测定，HepG2稳定系
DMSO
#41
#59
#61
#54
#91
细胞毒性
[uM]
0
20
40
60
80
100
120
校正的OD
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6

图 3

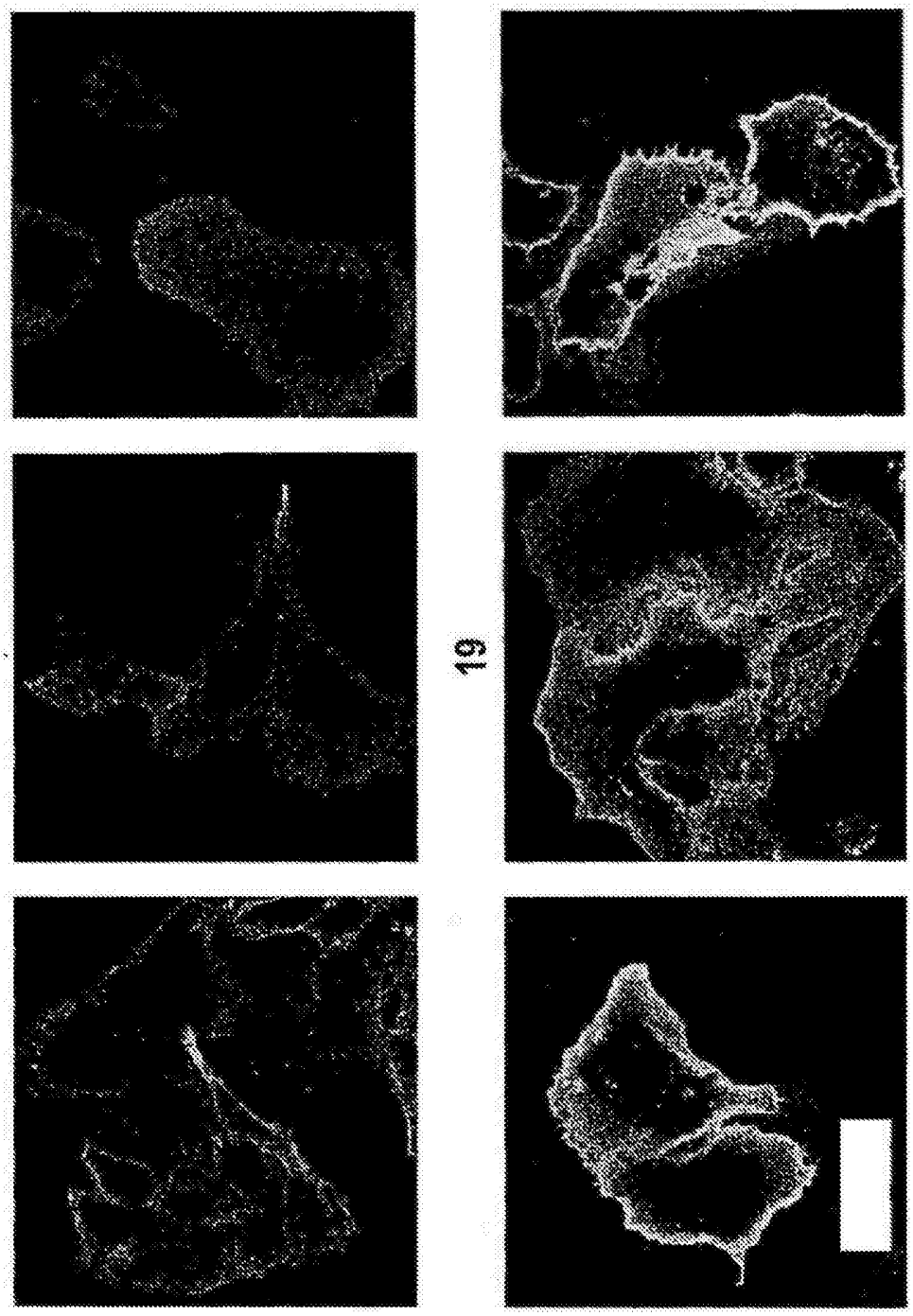


图 4

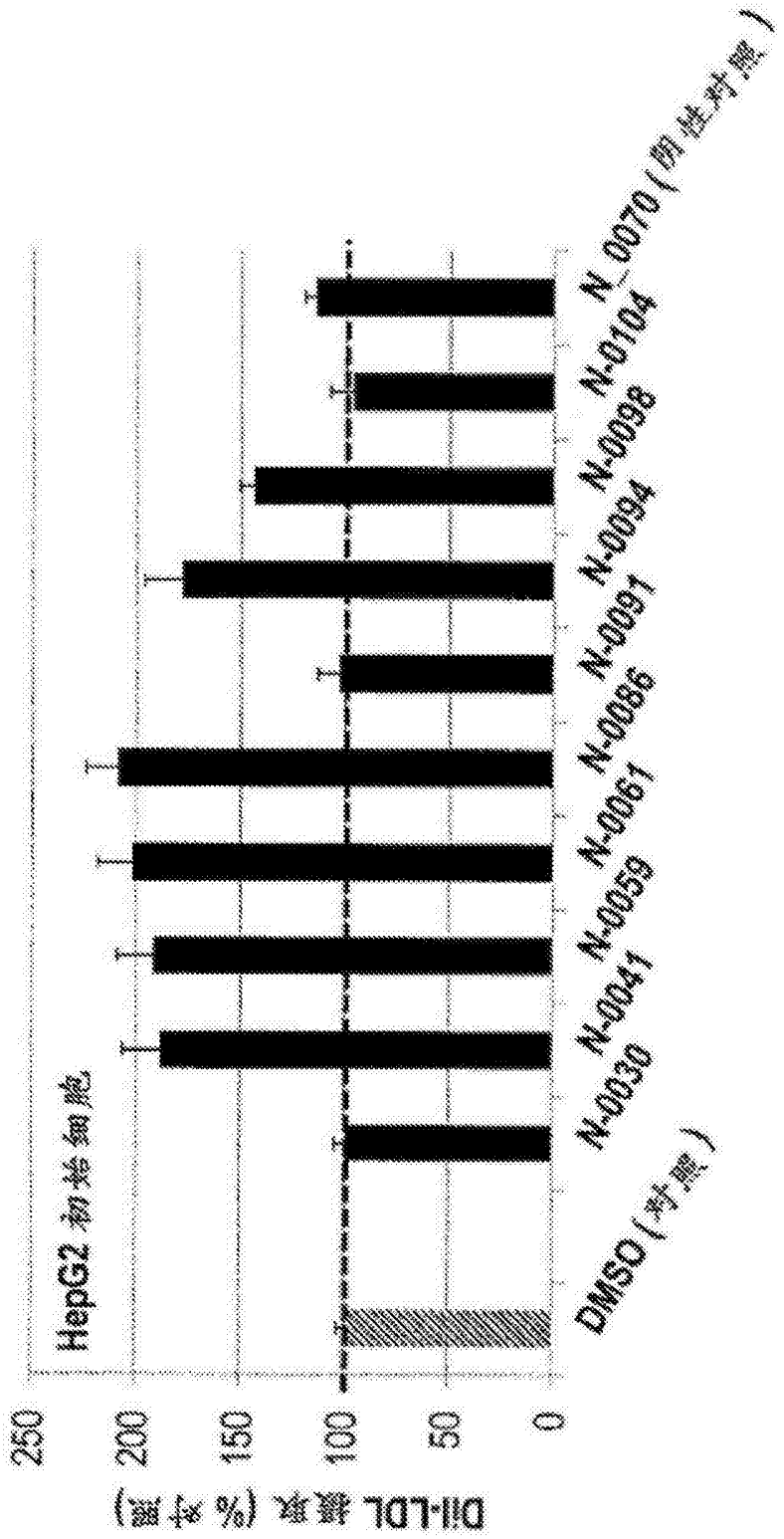

DiI-LDL 摄取 (% 对照)
250
200
150
100
50
0
HepG2 初始细胞
DMSO（对照）
N-0030
N-0041
N-0059
N-0061
N-0086
N-0091
N-0094
N-0098
N-0104
N-0070（阴性对照）

图 5A

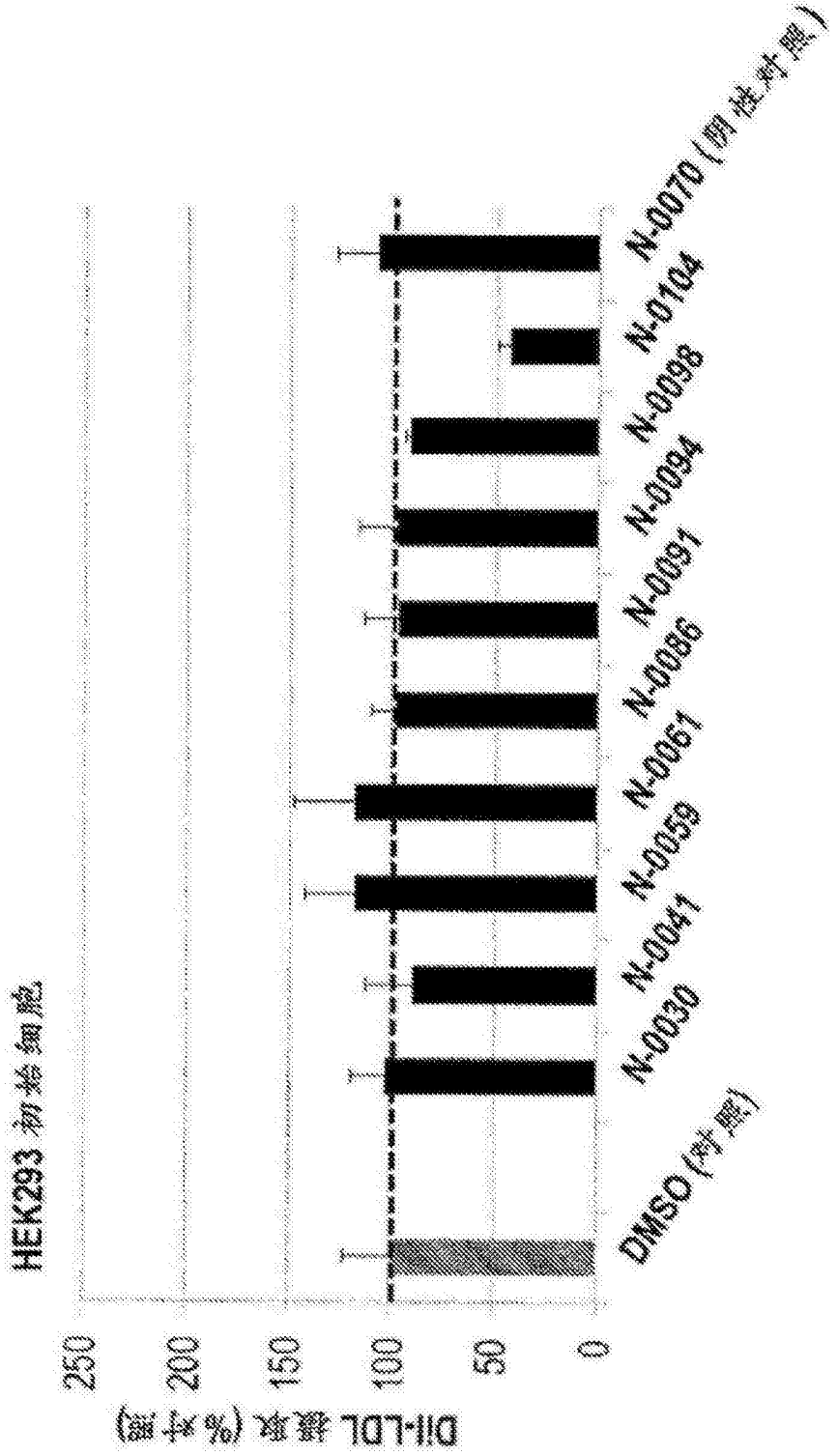

HEK293 初始细胞
Dil-LDL 摄取 (% 对照)
250
200
150
100
50
0
N-0070 (阴性对照)
N-0104
N-0098
N-0094
N-0091
N-0086
N-0061
N-0059
N-0041
N-0030
DMSO (对照)

图 5B

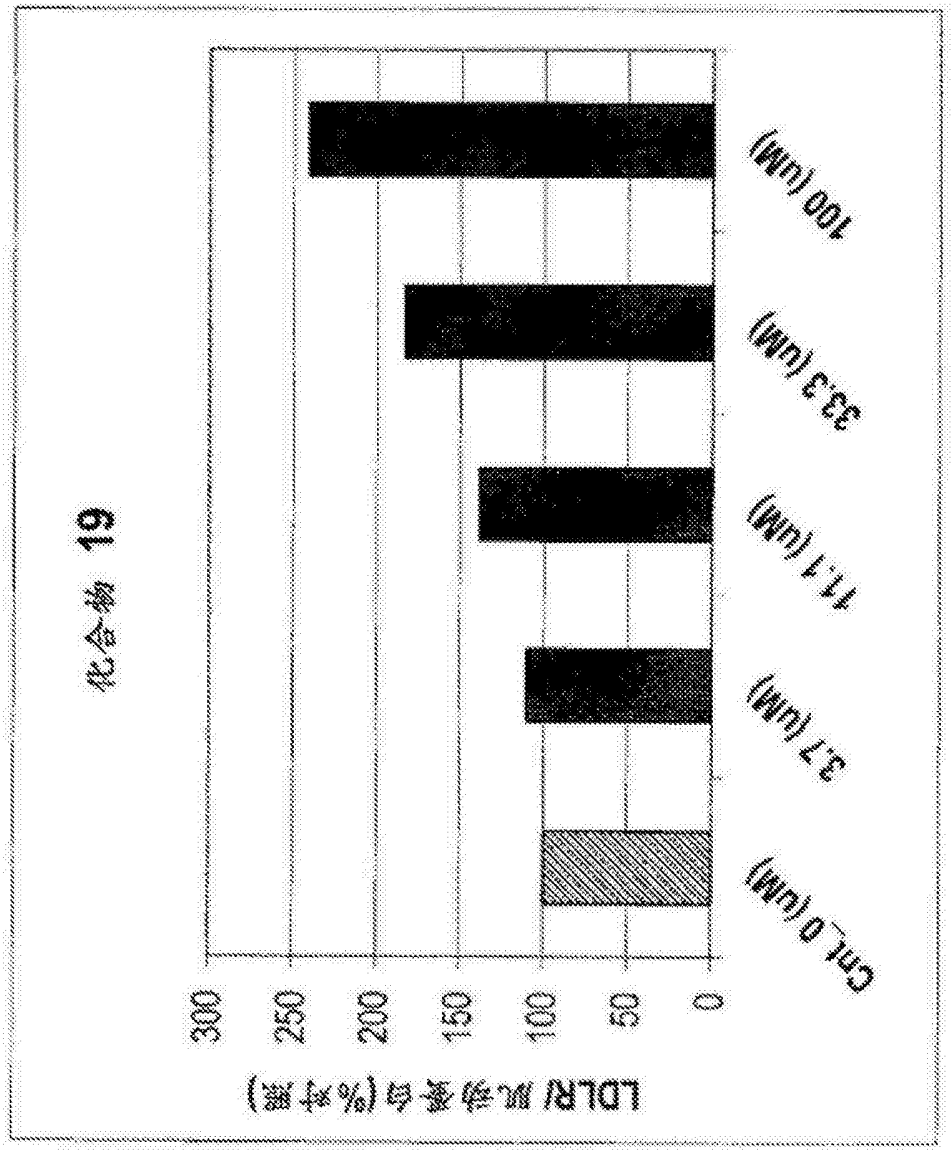

化合物 19
LDLR/ 肌动蛋白(% 对照)
300
250
200
150
100
50
0
Cnt_0 (uM)
3.7 (uM)
11.1 (uM)
33.3 (uM)
100 (uM)

图 5C

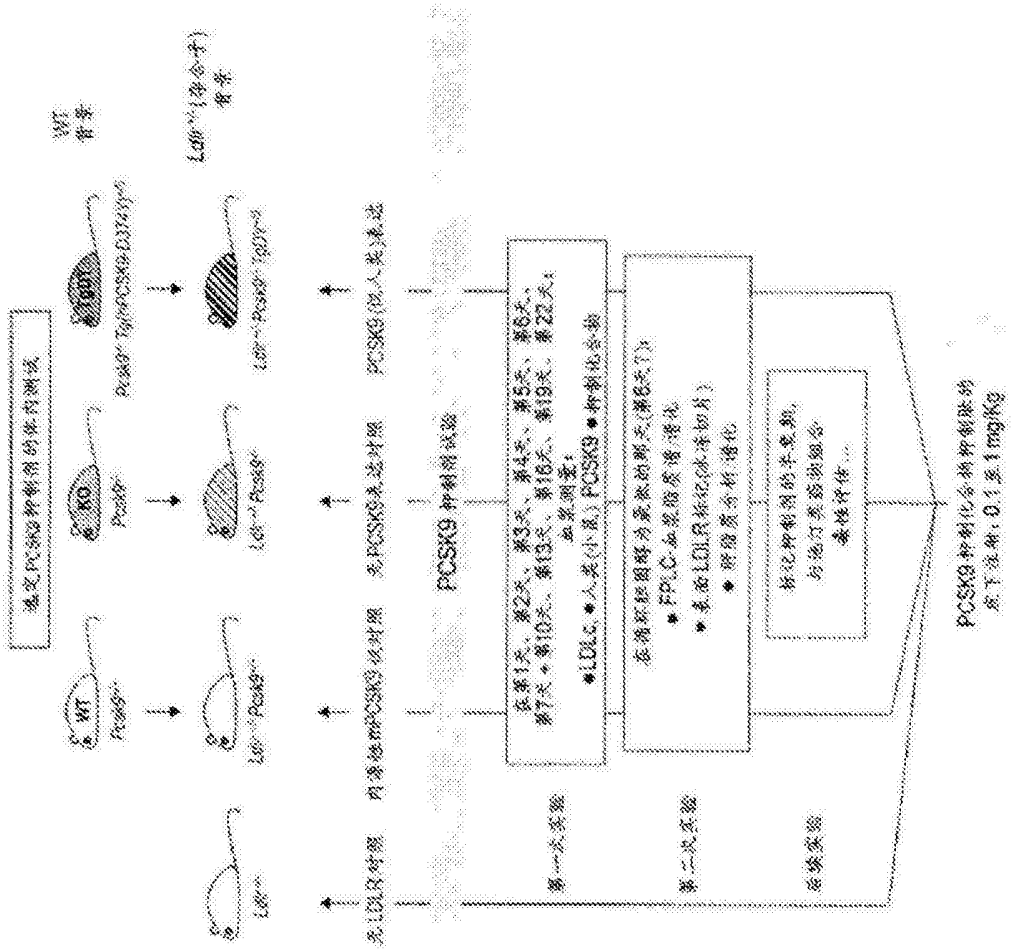


图 6